ANALYTICAL APPLICATION OF OLIGOPYRROLE MACROCYCLES

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Progress of modern analytical chemistry is closely related with advancement in other fields such as organic chemistry and biochemistry. Successful solution of current scientific problems is inconceivable without close cooperation of different chemical disciplines. As an example of such hot and very intricate theme research in the field of molecular recognition of biologically active compounds can serve, where numerous methods of analytical chemistry, organic chemistry and biochemistry can suitably be utilized, elaborated and brought into consonance. This multidisciplinary overlap logically leads to the advent of new scientific fields with their own tools, methodologies and subjects of exploration – bioanalytical chemistry and nanotechnology. This review covers different aspects of analytical application of oligopyrrole macrocycles (mainly porphyrins and sapphyrins). These compounds are widely used in analytical chemistry due to their outstanding optical properties. In our contribution oligopyrrole macrocycles are considered as signaling and structural parts of chemical receptors and selectors in various applications. Introduction of different moieties into *meso*-position of macrocyclic rings allows to obtain *e.g.*, sterically well-organized receptors for recognition of biologically important analytes, new chromatographic materials, and powerful tools in electrochemical research. Finally, future trends in the field are outlined briefly.
Keywords: Pyrrols; Oligopyrroles; Macrocycles; Porphyrins; Sapphyrins; Analytical applications; Chromatographic separations; Porphyrin receptors; Saccharide recognition; DNA binding; Spectroscopy; Self-assembled monolayers; Ion-selective electrodes; Electronic nose and tongue.

1. INTRODUCTION

Porphyrins, often called “pigments of life”, are a very important group of conjugated tetrapyrrole macrocycles studied mostly for their biochemical application, enzyme mimics and technological applications (*e.g.* nanotechnology). While the most application of oligopyrrole macrocycles was devoted to above mentioned areas, there is also great potential for analytical applications, *i.e.* searching for new receptor systems for development and construction of chemical and biochemical sensors.

Properties of porphyrins[1,2] and other oligopyrrole marocycles[3] have been reported from different point of view. The focus of this article is to show how variety of remarkable spectroscopic and electrochemical properties of oligopyrrole macrocycle can be utilized in novel methodology of contemporary analytical chemistry. Some important properties of these compounds are mentioned in the following text.

The bare tetrapyrrole nucleus is known as porphin and the porphyrins are formally derived from this structure by substitution of some or all of the peripheral positions with a variety of side-chains. The IUPAC system is shown in Fig. 1 and this system will be used in further text.

Both major tautomeric forms have delocalization pathways with opposite pyrrole NH groups. The macrocycle is planar but chelation with a large metal ion can cause some buckling of the ring.[1] Porphyrins and their metal complexes are highly-melting, highly-colored compounds. The brilliant red color of many porphyrins and derivatives is manifested in their electronic absorption spectra. The major feature of these spectra, the so-called Soret band found around 410 nm and possessing a molar absorption coefficient sometimes as large as $4 \cdot 10^5$ l mol^{-1} cm^{-1}, and the satelite peaks (Q bands) with lower molar absorption coefficients, these peaks are known to vary with the peripheral substituents.

While oligopyrrole macrocycles exhibit excellent photophysical properties allowing easy monitoring of binding process by a number of methods (see text below) selective binding properties of core unsubstituted macrocycle usually does not show remarkable specificity. Such property can be introduced by proper peripheral substitution which opens the whole area of highly specific elegant receptors with several unique features:

1. An approximately planar structure gives a facile design of receptors having a geometrically well-defined binding pocket consisting of an aromatic porphyrin core and recognition groups at periphery.

2. Chemical properties of porphyrin systems could be varied during the metal complexes are formed; complexes with most metals in the periodic table have been prepared and characterized[4].

3. Porphyrin unit is a strong chromophore allowing the ultraviolet-visible spectroscopy (UV-VIS), circular dichroism, fluorescence and other optical methods to be used to probe the intermolecular interactions. The intense Soret band is a characteristic of the 18π-electron delocalization pathway present in the porphyrin nucleus; if the conjugated pathway is interrupted,

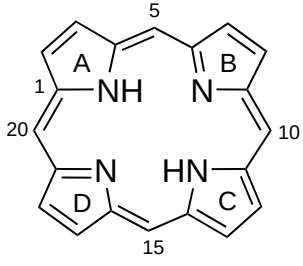


FIG. 1
Structure of porphyrin

the Soret band disappears. Chelation of porphyrins with metal ions usually results in an increase of the Soret absorption and simplification of the satellite peaks to give only two.

4. The aromaticity of the porphyrin macrocycle has been largely investigated using nuclear magnetic resonance spectroscopy. The ring current due to the large delocalization pathway in the porphyrin nucleus has been used to investigate aggregation and variety of other phenomena. Because of deshielding, the *meso*-protons (protons at positions 5, 10, 15 and 20; see Fig. 1) appear at 10–12 ppm while the shielded N–H resonances occur between –2 and –4 ppm. Measurements of chemical shifts in ^{1}H and ^{13}C NMR spectra have been complicated by the concentration dependence of the chemical shifts, due largely to aggregation of the molecules into layers in solution. When one porphyrin molecule closely approaches another in solution the ring current of one has the effect of causing an upfield shift of protons or carbons in the substituents of another.

5. Porphyrins and metalloporphyrins can also be reduced, either reversibly or irreversibly. Reversible reduction can be best carried out electrochemically to give mono- and dianions or the change of coordination sphere of metal incorporated in porphyrin core.

6. Spectroscopic and binding properties can be modified by increasing number of pyrrole units, so called expanded porphyrin family[2].

7. Aromaticity of oligopyrrole macrocycles (with different number of pyrrole units, usually from four to eight) can be modified by conjugation stoppers – *i.e.* the family of calixphyrins[5–7] or fully stopped calixpyrroles[8–10].

Exceptional binding properties of substituted porphyrins play a key role in their analytical applications. The unsubstituted porphyrin itself offers several modes of interaction including H-bonding, aromatic π–π interaction, Coulombic interaction (after protonation), hydrophobic interaction and after metallation axial ligand binding. Appropriate subsequent peripheral modification of the basic skeleton can lead to smart chemical structures with perfectly tuned interaction abilities highly specific for a given analyte, *i.e.* receptors well suited for the construction of chemical selectors and sensors.

In this review, we would like to describe the basic features of oligopyrrole macrocycles in terms of molecular recognition properties and their analytical application, including application for saccharide recognition, chromatographic separations, formation of self-assembled monolayers, chiral recognition, porphyrin based electrochemical sensors (*e.g.* ion-selective electrodes) and finally application for development of electronic nose and

tongue will be mentioned. We have focused on applications of oligopyrrole macrocycles, which are currently under investigation in our laboratory.

2. PORPHYRIN-BASED RECEPTORS FOR BIOLOGICALLY IMPORTANT ANALYTES

2.1. Porphyrin-Based Receptors for Saccharide Recognition

2.1.1. Introduction

Significance of oligosaccharides in biological regulation has attracted a great deal of interest in recent years[11–14]. Modern biomedical science has defined the role of saccharides in living organisms. It has been found that saccharides are not only a necessary source of energy and structural components, but they also mediate cell-cell recognition events and participate in many other processes like infection of cells by pathogens, many aspects of immune response, distribution and reactivity of proteins within cells and membrane transport. Moreover, saccharides, as unique multilinkage molecules capable of creating branched structures, contain more information in a short sequence than any other biological mono- or oligomer. The information potential of oligosaccharides is greater than that of proteins and nucleic acids of equivalent molecular weight, and thus their presence on cell surfaces and in many proteins suggests their significance which previously has remained unexplored. Among the other biologically important molecules, carbohydrates, in a given short sequence, display the largest number of ligand structures capable of binding with proteins in molecular recognition systems.

Current effort to describe the interactions of saccharides with their natural receptors still bring more intriguing questions than clear answers, mainly due to their intrinsically complex mechanism. Imitation of these processes by application of synthetic receptors leads to the fast development of new drug delivery systems, specific cell markers, regulators of membrane transport for biomolecules, carbohydrate sensors and other applications in chemistry and biomedicine.

In this part of the review, we evaluate porphyrins as potential candidates for recognition of saccharides in organic and aqueous media.

2.1.2. Porphyrin Molecule as a Model of Receptor

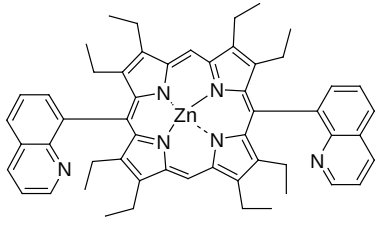
It is well known that the interaction between a synthetic receptor and a saccharide is often accompanied by many specific problems. From the viewpoint of the host-guest chemistry, saccharides are classified as "bad" or "chameleon-like" guests. They are highly hydrophilic, electroneutral, non-fluorescent compounds existing in different cyclic forms in solution. The X-ray studies of protein–saccharide complexes clearly demonstrated their multivalent binding with a substrate by cooperation of van der Waals forces, coordination interactions and a network of hydrogen bonds with hydrophobic binding sites. Recently, numerous synthetic receptors have been developed for saccharide recognition. The interactions between sacharides and receptors have been studied mainly in non-polar organic media. It has been found that under such conditions hydrogen bonds play a very important role. In contrast, it is reasonable to expect a strong perturbation of hydrogen bonds between saccharides and receptors in aqueous media as a consequence of the presence of water molecules. Probably for this reason, there have been published only a few papers devoted to water-soluble synthetic receptors intended for selective recognition of saccharides in aqueous media. Especially in this context porphyrins can be considered as a prospective starting material for the design of ligands for saccharide recognition. Among potential host molecules, porphyrins belong to the class of naturally occurring compounds with unique optical properties. Porphyrins exhibit characteristic sharp and intense absorption maxima in the visible region of spectra (Soret bands) and also intense fluorescence; both of these properties are very advantageous for analytical applications[15]. The introduction of suitable substituents in *meso*-positions of the planar porphyrin core allows to obtain three-dimensional cage, cavity and cleft structures, which are effective for substrate entrapping[16]. Solubility of porphyrins in different solvents can be tuned by the introduction of appropriate functional groups in the porphyrin periphery.

Water-soluble porphyrins have been recently studied, mainly due to their possible medico-biological applications[17,18].

Also the use of porphyrins and their derivatives for molecular recognition of saccharides is a very promising approach in modern bioorganic chemistry. Here we discuss some aspects of the application of porphyrins as major constituents in "saccharide recognition devices".

2.1.3. Porphyrin-Saccharide Non-Covalent Interactions

As mentioned above, hydrogen bonding is especially effective in non-polar organic media. Under such conditions, molecules of solvent do not compete strongly with substrate for binding sites. Mizutani *et al.*[19] has proposed functionalized porphyrins **1**–**4** for the recognition of alkyl pyranosides in $CHCl_3$ (ref.[20]). These porphyrin hosts contain complexed zinc ion as Lewis acid in the cavity formed by two bulky substituents. In the binding site of hosts **1**, **2**, two quinoline nitrogen atoms act as hydrogen bonding acceptors. In the binding sites of receptors **3** and **4**, two phenolic hydroxy groups form hydrogen bonds with guests and act as hydrogen bonding donors. Receptors **1**–**4** were tested with series of octyl pyranosides. UV-VIS and ^1H NMR investigations revealed that receptor **1** is able to recognize *trans*-1,2-dihydroxy (hydroxymethyl) grouping of the pyranoside through the



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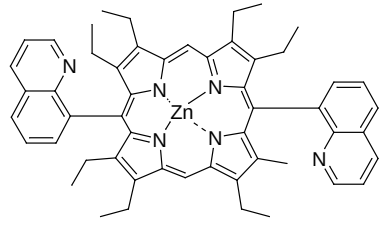

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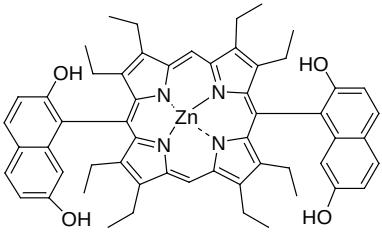

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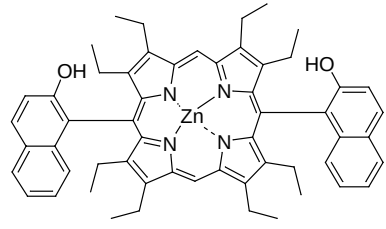


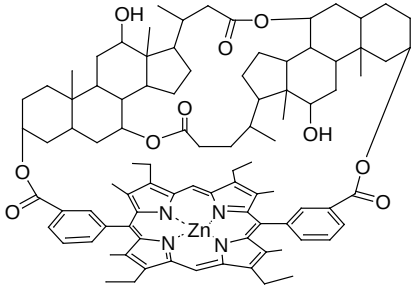
4

zinc site and two quinoline sites. In receptor **2**, only one quinoline nitrogen and zinc preferably interact with ligands containing 1,2-*trans*-dihydroxy grouping. Receptors **3**, **4** showed weaker interactions with substrates than compounds **1**, **2**. However, they also formed a hydrogen-bonding network with octyl pyranosides. Circular dichroism (CD) spectros-

copy demonstrated the ability of receptor **4** to distinguish the α- and β-anomers of the octyl galactopyranosides.

Steroid-capped cleft-like porphyrin **5** was introduced for complexation of various organic soluble pyranoside derivatives[21]. Binding selectivity of receptor **5** was related to the strength of intermolecular hydrogen bonding with a given substrate. Pyranosides were bound in the order: mannose > glucose > galactose. Moreover, enantioselectivity was observed for the L- and D-enantiomers of α-glucopyranoside with a stronger interaction with the natural isomer. The ^{1}H NMR investigations allow to propose the binding models of alkyl pyranosides with receptor **5**. The binding of the pyranoside ring with the receptor is mediated by 3-, 4- and 6-OH groups of a saccharide. Finally, the participation of the central metal ion of the metallated porphyrin in the binding with pyranosides was also confirmed.

(*R*,*S*)-1,1′-tetrakis(1,1′-binaphthyl)-substituted porphyrins **6**, **7** have been designed for the saccharide recognition as well[22]. Recently, the optically active 1,1′-binaphthyl-derived "clefts" have been successfully used for the



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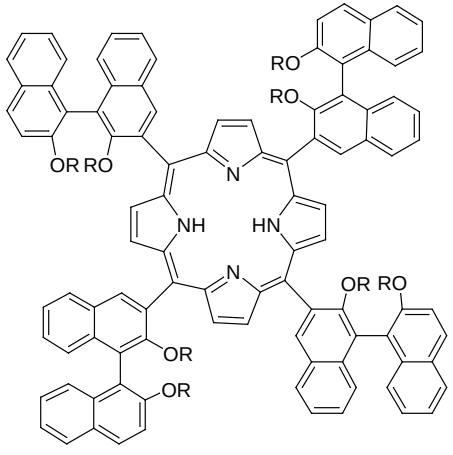


6, R = Me
7, R = H; **7a**, Zn
7b, Fe

enantioselective recognition of amino acids and saccharides[23,24]. The 2,2′-binaphthyl substituents are inefficient in locking dihedral angles and corresponding receptors usually do not display high degrees of recognition[23]. Design of receptors **6**, **7** is based on multiple H-bonding between a saccharide and corresponding macrocycle. Steric interactions between the *ortho*-substituents in the binaphthyl rings and the pyrrole β-hydrogens re-

sult in a high-energy barrier for the rotation around the porphyrin–binaphthyl bonds. As a result, each *ortho*-substituent is essentially fixed on one side of the porphyrin plane at room temperature. This leads to the formation of four atropisomers, which correspond to the four ways of distribution of the substituents above and below the porphyrin plane. One of these atropisomers (α,β,α,β) was used for a complexation study. The binding site of **7** contains eight phenolic hydroxy groups, which can form hydrogen bonds with guests. The binding pocket of porphyrins **6**, **7** formed by the bulky binaphthyl substituents creates suitable conditions for binding of oligosaccharides in a similar way as in lectins. The structural differences of both porphyrin receptors determine their complexation properties. Compound **6** is insoluble in water, whereas **7** is soluble in water rich solvent (water–methanol; 95 : 5, v/v), which consequently allows the determination of binding constants for saccharides. Both porphyrins **6** and **7** show strong absorption at 427 nm facilitating monitoring of the binding process. The binding constants for both hosts can be determined by corresponding UV-VIS titration experiments (Fig. 2). Receptor **6** (R = Me) shows low binding affinity for unmodified saccharides. However, substrates such as α- or β-D-methyl glucopyranoside are able to interact more strongly with the host **6** than non-substituted glucose with a remarkable selectivity for β-anomer (Fig. 3). Saccharide derivatives with octyl and 4-nitrophenyl substituents, namely octyl α-D- and octyl β-D-glucopyranoside and galactopyranoside showed higher selectivity for the α-anomer. The decisive

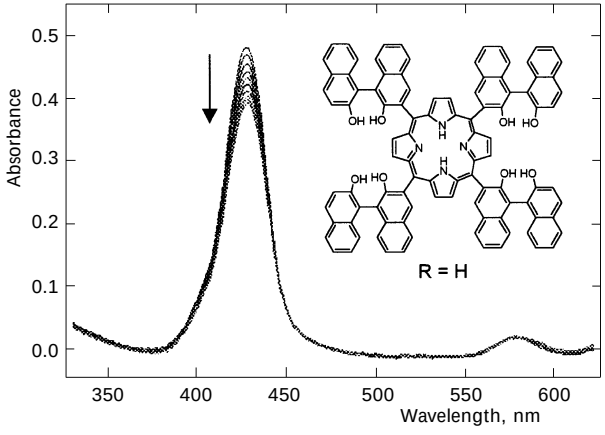


FIG. 2
Typical UV-VIS spectral changes of **7** in water media; λ_{max} = 427 nm. Interaction of **7** with D-glucose

role in the host–guest interaction apparently plays hydrophobic interaction between the alkyl (or aryl) chain of the substrates and the naphthyl rings of receptor **6**. A different tendency was observed for **7**. This receptor (R = H) easily interacts with unmodified saccharides in water (water–methanol; 95 : 5, v/v) and shows a significant preference to oligosaccharides over monosaccharides. The results obtained by UV-VIS titration of **7** with different saccharide species were confirmed also by fluorescence spectroscopy (Fig. 4). The observed values of the association constants (K_a) increased from α-D-glucose to disaccharides and then decreased for maltotriose. ^{1}H NMR investigation of octyl α- and β-D-glucopyranoside interaction with **6** revealed the downfield shifts of the macrocyclic aromatic proton signals. The contribution of saccharide OH groups to the complex formation is also observed in IR experiments. A red shift of OH absorption was observed in the complexation of β-D-glucopyranoside with **6**. A weak red shift was found also for CO absorption.

Analogous experiments with receptor **7** indicated more pronounced changes in the spectra. Aromatic proton signals of receptor **7** show downfield shifts simultaneously with broadening of signals of phenolic protons after addition of a glucopyranoside into solution. Saccharide CH pro-

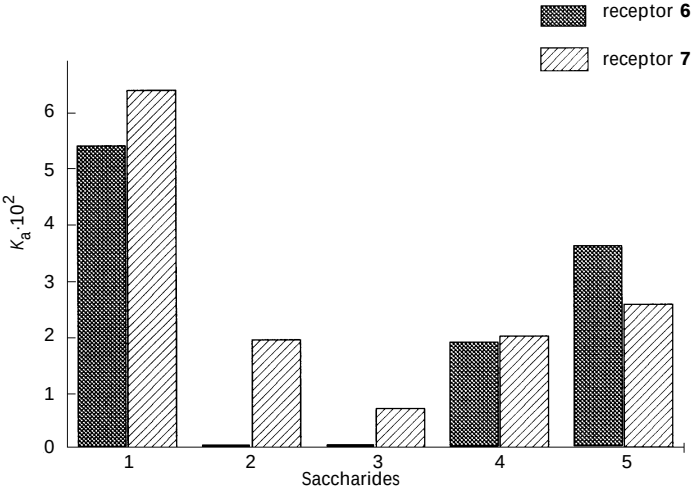


FIG. 3
Association constants for binding of saccharides with receptors **6**, **7** in DMSO (UV-VIS titration). 1 Octyl α-D-glucopyranoside, 2 octyl β-D-glucopyranoside, 3 methyl α-D-glucopyranoside, 4 methyl β-D-glucopyranoside, 5 4-nitrophenyl β-D-galactopyranoside

ton signals show a significant upfield shift. The IR spectroscopic investigation revealed an intense broadening of OH absorption of the receptor and the red shift of the saccharide OH signals. A characteristic blue shift was observed in CH resonance of β-D-glucopyranoside. These facts indicate a strong interaction of receptor **7** with saccharides through hydrogen bonds.

An introduction of metal ions into the receptor **7** dramatically changes its binding properties. Cooperation of H-bonds with coordination interactions through Zn(II) (**7a**) or Fe(III) (**7b**) ions in binding to particular saccharides leads to increased receptor specificity to oligomeric analogues (unpublished results).

Sensitivity of porphyrin-based receptors to saccharides can also be enhanced using other strategies. As demonstrated above, usually, a binding site of a receptor must be structurally pre-organized (*e.g.* hydrophobic cavity formed) to provide appropriate hydrogen donors or acceptors groups. Hydroxy groups in cooperation with metal ions usually play a very important role in the binding process. Other structure components of the receptor can also be included, *e.g.* cleft-like structures with anionic groups, which are known as strong hydrogen bond acceptors. A recent study evalu-

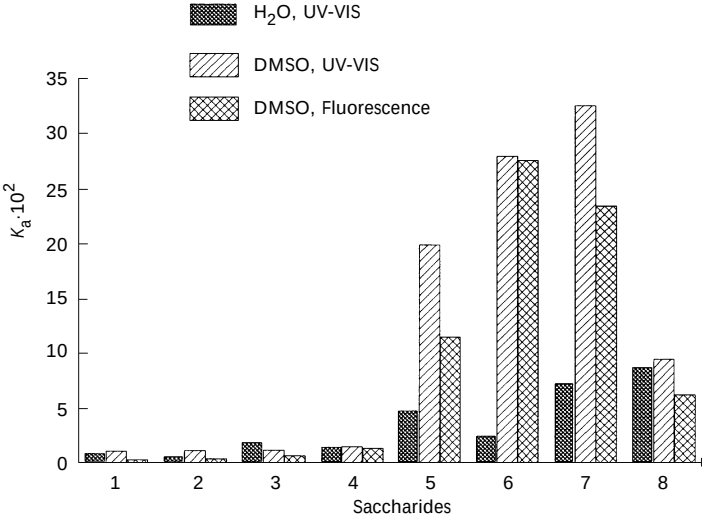
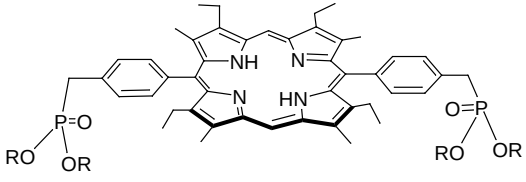


FIG. 4

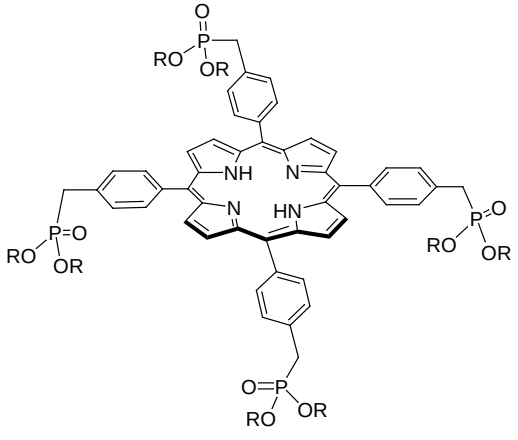
Association constants for binding of saccharides with receptor **7** (UV-VIS titration). 1 D-Galactose, 2 D-glucose, 3 D-fructose, 4 D-ribose, 5 D-trehalose, 6 α-D-lactose, 7 β-D-lactose, 8 maltotriose

ated some macrocyclic phosphonates with respect to their ability to bind hydroxy compounds[25].

Porphyrin phosphonates **8**–**10** are a novel group of compounds with interesting binding properties, where P=O groups, which are known to be strong hydrogen bond acceptors, play a vital role. Design of receptors **8**–**10** leads to combination of UV and fluorescence signaling unit (porphyrin moiety) with two or four binding sites (phosphonate groups). Complexation properties of the phosphonated porphyrins have been described recently[26]. Mechanism of their interaction includes the formation of hydrogen bonds between vicinal diols of the pyranose ring with two oxygen atoms of the corresponding phosphonate group[24,27,28]. Our studies confirmed validity of this concept.



8, R = C_2H_5
9, R = H



10, R = H

Figure 5 summarizes the association constants calculated for receptors **8**–**10**. UV-VIS spectroscopic method indicates preferable binding of **8** to octyl α-D-glucopyranoside over octyl β-D-galactopyranoside. UV-VIS studies of interaction of **9** and **10** with mono- and disaccharides showed a stronger binding to trehalose, D-maltose and α-lactose (Figs 5 and 6). Additional information on the saccharide binding mode in solution came from ^{1}H and ^{31}P NMR spectra. A model complexation experiment of octyl α-D-glucopyranoside with the receptor **8** in equimolar ratio in $CDCl_3$ showed broadening and downfield shift of OH saccharide signals. A downfield chemical shift in ^{31}P NMR spectra of complex of **8** with octyl α-D-glucopyranoside under the same conditions was observed as well.

Interaction of D-glucose with **9** (equimolar ratio) in DMSO-d_6 caused broadening of proton signals corresponding to glycosidic OH and the CH-1. Signals of saccharide CH protons were insignificantly influenced by complexation. The participation of phosphonate group in saccharide complexation was demonstrated by ^{31}P NMR spectroscopy. The downfield chemical shift of P=O group was observed for **9**–glucose complex (1 : 1) in water.

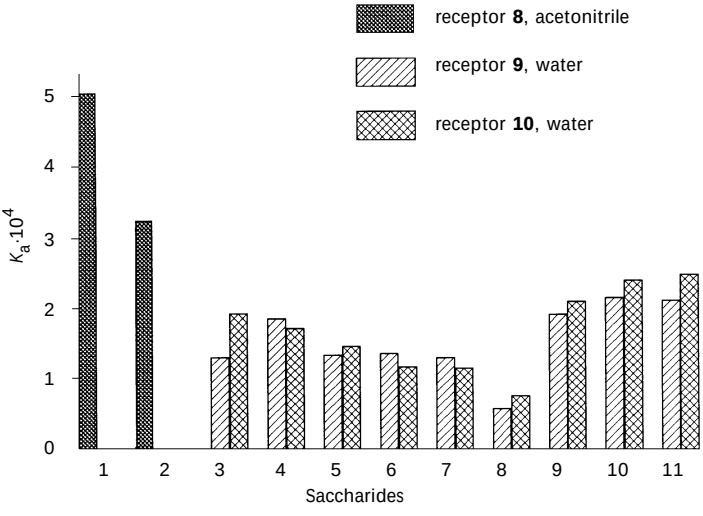


FIG. 5

Association constants for binding of saccharides with porphyrin phosphonates **8**–**10** in water followed by UV-VIS titration. 1 Octyl α-D-glucopyranoside, 2 octyl α-D-galactopyranoside, 3 D-galactose, 4 D-glucose, 5 D-arabinose, 6 D-mannose, 7 D-fructose, 8 D-ribose, 9 trehalose, 10 maltose, 11 lactose

Interaction of saccharides with porphyrin phosphonates can be also monitored by infrared spectroscopy (IR). A red shift of saccharide OH absorption together with the shift of CO absorption for **9**–octyl α-D-glucopyranoside complex was found. The interaction of **9** with D-glucose is also accompanied by the shift of absorption of saccharide OH bonds, and the shift and broadening of the the signal of C–O bonds. Broadening of absorption band for P=O groups was observed simultaneously with the shift of absorption for simple P–O bond.

All spectroscopic data prove that hydrogen bonds between phosphonate groups and vicinal diol segments of saccharides play a decisive role in phosphonated porphyrin–saccharide complexation. Receptor **8** showed a stronger interaction with alkyl glucopyranoside than with galactopyranoside. Porphyrin phosphonates **9** and **10** tend to bind to oligosaccharides especially to D-maltose and α-D-lactose.

We have already above illustrated the effectiveness of open cavity and cleft receptors for saccharide binding in aqueous media with selected model compounds, 1,1′-binaphthyl-substituted porphyrins and porphyrin phosphonates. Another strategy, which is often employed for the design of receptors, consists in the preparation of completely closed cavities or molecular cages. Recent papers describe water-soluble sandwich type porphyrin structures applied to electron transfer studies of photosynthetic

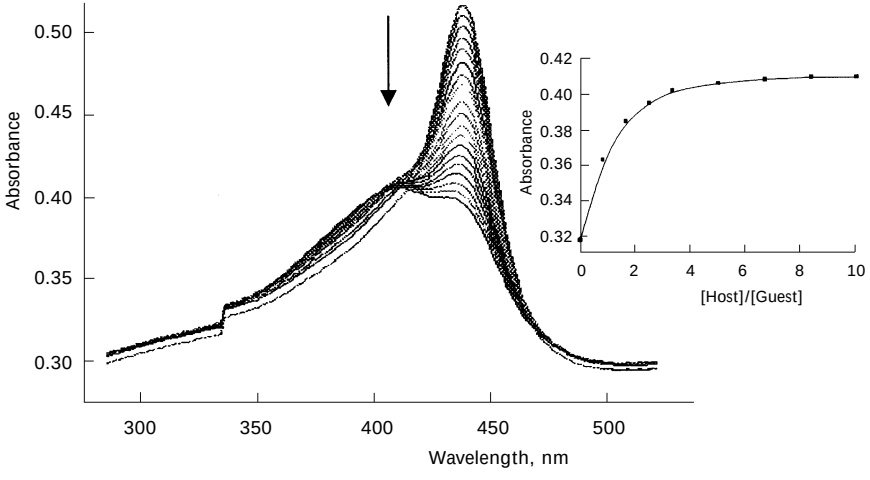
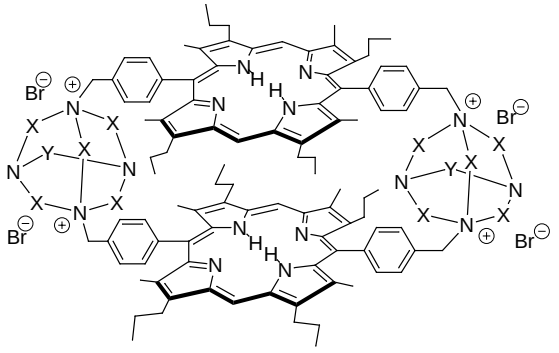


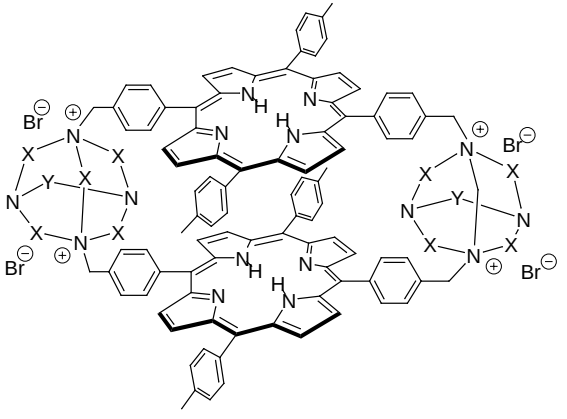
FIG. 6

A typical UV-VIS titration experiment. Spectral UV-VIS changes upon the incremental addition of α-D-glucose to **9** in water; λ_{max} = 428 nm

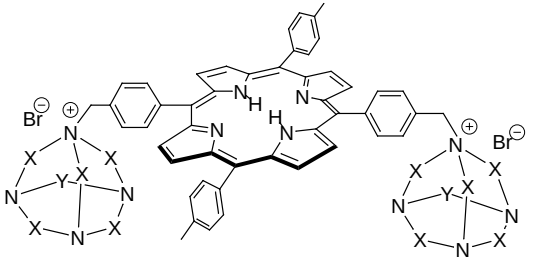
and artificial enzymatic models[29–31]. Cycloporphyrins **11**, **12** and their linear analogue **13** used as molecular devices for saccharide entrapping are



11, X = $(CH_2)_6$; Y = $CO(CH_2)_4CO$



12, X = $(CH_2)_6$; Y = $CO(CH_2)_4CO$



13, X = $(CH_2)_6$; Y = $CO(CH_2)_4CO$

presented here. The binding studies were focused on specific aspects of carbohydrate recognition such as binding in polar solvents, preferably in water, and facile detection of the binding events. We have recently described the synthesis and applications of porphyrin "tetac" conjugates for the recognition of certain saccharide species[32], which also showed interesting binding properties to phosphorylated saccharides (unpublished results).

Porphyrin–cryptand systems **11**, **12** combining di- and tetraphenyl porphyrin building blocks with cryptand are water-soluble receptors with a well defined hydrophobic cavity, which contains precisely spatially oriented binding sites. The main idea behind the combination of such building blocks is the creation of several binding modes in receptor molecules **11**, **12**, which are important for successful binding of saccharides in highly competitive environment. The porphyrin units creating hydrophobic cavity (where non-covalent binding forces can take place) are combined with macrobicyclic cryptand, which has not only well described anion binding properties, but also possesses amide binding sites oriented into the cavity, which participate in the binding process.

Receptor systems **11** and **12** are designed to generate cooperative binding modes based on Coulombic attraction, hydrogen bonding (C=O···OH), CH–π and π–π interactions. The combination of different non-covalent binding modes leads to strong complexation properties of receptors **11** and **12** for saccharides, even in highly competitive media such as water.

The capability of the water-soluble hosts **11**–**13** to bind saccharides in highly competitive environment was proven by optical spectroscopy (Fig. 7). Figure 8 summarizes the association constants calculated for mono-, oligosaccharides and alkyl glycopyranosides. The results indicate a strong binding with an important selectivity feature: the association constants increase from mono- through di- (trehalose, lactose) to trisaccharides. Trisaccharides are the most preferred substrates for cyclic receptors **11** and **12** with well defined cavities, while this trend was not observed, as expected, for linear receptor **13** without a well defined cavity. The alkyl substituent of alkyl α-D-glucopyranoside increases the value of the association constant in comparison with glucose. Interestingly, the novel receptors are able to discriminate between α- and β-anomers by a factor of 11. These results allow to make the conclusion that the recognition process is governed by the substrate geometry and size. The chemical nature of monosaccharides and configuration of the OH-1 group turn out to be important factors for a selective binding of saccharides to macrocycles **11** and **12**. This selectivity is also governed by a receptor "rigidity". Circular dichroism spectroscopy allows to distinguish between inter- and intramolecular types of host–guest com-

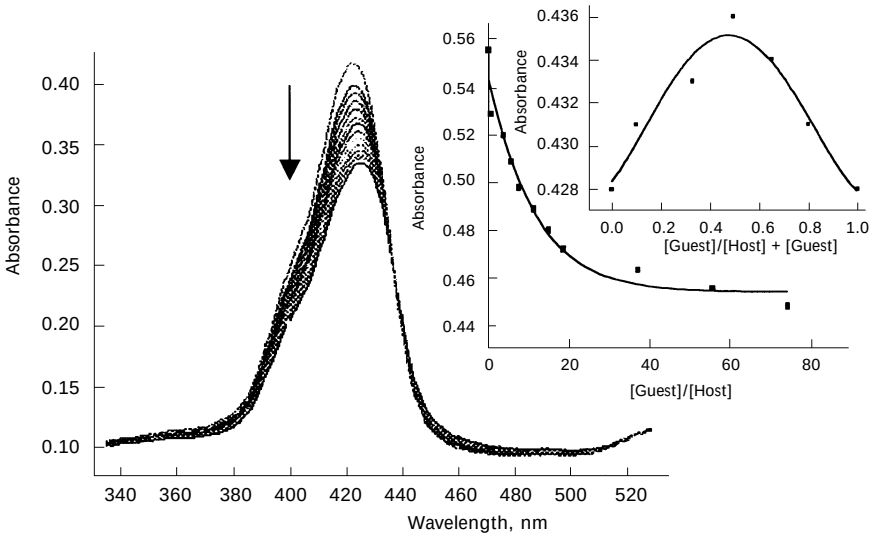


FIG. 7

Typical spectral UV-VIS changes upon the incremental addition of glucose to receptor **13** in water at room temperature; $2.4 \cdot 10^{-6}$ mol l^{-1} **13** in H_2O–5% MeOH; λ_{max} = 420 nm

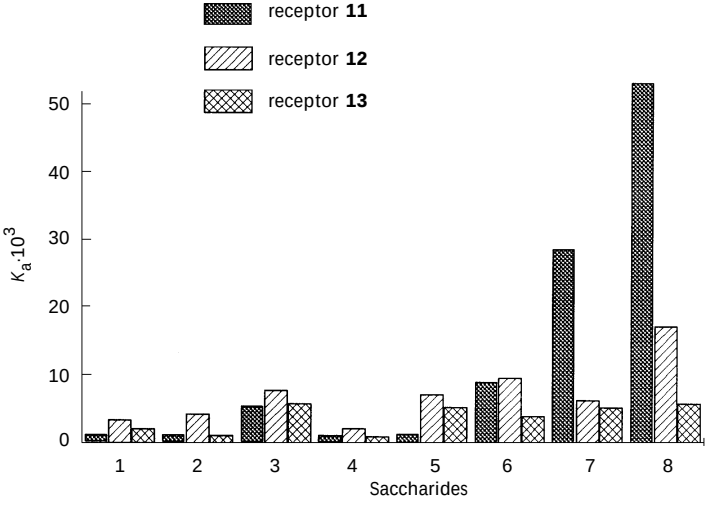


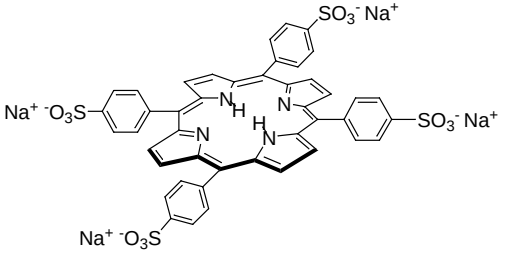
FIG. 8

Association constants for binding of saccharides with receptors **11**–**13** in water media (UV-VIS titration). 1 D-Galactose, 2 D-glucose, 3 methyl α-D-glucopyranoside, 4 methyl β-D-glucopyranoside, 5 octyl α-D-glucopyranoside, 6 trehalose, 7 lactose, 8 maltotriose

plexes. For more flexible **13**–saccharide complex, the induced Soret signal (induced CD) was observed. This fact indicates a chiral intermolecular type of complexation. A very weak induced CD response was obtained for more rigid complexes **11** or **12**–saccharide, indicating the inclusion type of complexation.

Complexation studies based on IR spectroscopy (KBr) showed that as a consequence of the interaction of **11** with glucose, a shift of signal of the macrocyclic amide group is observed. This fact indicates the binding mode to saccharide *via* carbonyl groups of the host, which was predicted from the result of molecular modeling. ^{1}H NMR spectroscopy in DMSO-d_6 revealed the broadening of signals of glucose hydroxy groups as a result of the complexation with macrocyclic receptor **11**. Contribution of CH (guest)–π (host) interactions in the complex formation is expected but experimentally difficult to prove because of overlap of corresponding host and guest signals in ^{1}H NMR spectrum of the complexes.

The sulfonate group is also known as an effective proton acceptor. Recently, porphyrin sulfonates have been intensively investigated for biomedical applications such as photodynamic therapy of cancer[33] and radiological imaging[34]. Tetrasulfonate derivatives of resorcinol cyclic tetramer showed a pronounced complexation with saccharides in water[15,25]. Complexation studies of *meso*-tetrakis(4-sulfonatophenyl)porphyrin **14** in comparison with porphyrin monocarboxylate **15** were carried out. Interaction of receptor **14** with carbohydrates in water can be easily monitored by UV-VIS spec-



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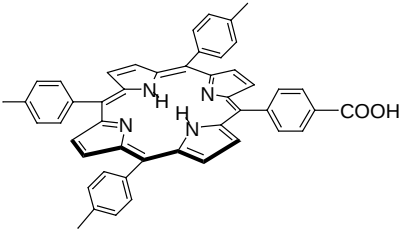

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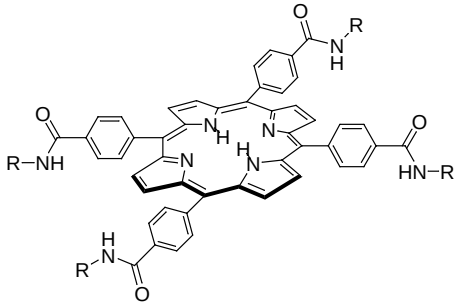
troscopy (Fig. 9). Intensity changes of a Soret band and a red shift of its maxima accompanied the addition of saccharide species to the solution. The ^{1}H NMR monitoring of the host–guest interaction in DMSO-d_6 showed a strong shift of CH and OH protons of the saccharide. The investigation of **14**–α-D-glucose complex by Raman spectroscopy also showed a significant shift of CH and CO absorption which indicates the interaction.

Several porphyrin-oligopeptide conjugates of **16**–**18** were tested for saccharide recognition (Fig. 10). Porphyrin-oligopeptide conjugates have been recently intensively studied with the focus on their possible catalytic



16, R = Lys-Ala-Ala-OMe, OH
17, R = Lys-Ala-Asp-OMe, OH
18, R = Lys-Leu-Asp-OMe, OH

activity[35], electro- and photochemical properties[36,37], oxygen sensing[38] and interactions with other molecules[39–42]. The interaction of saccharides with polypeptides is realized in natural saccharide receptors such as lectins. This fact was utilized in design of a set of water-soluble porphyrin-peptide conjugates for the saccharide recognition.

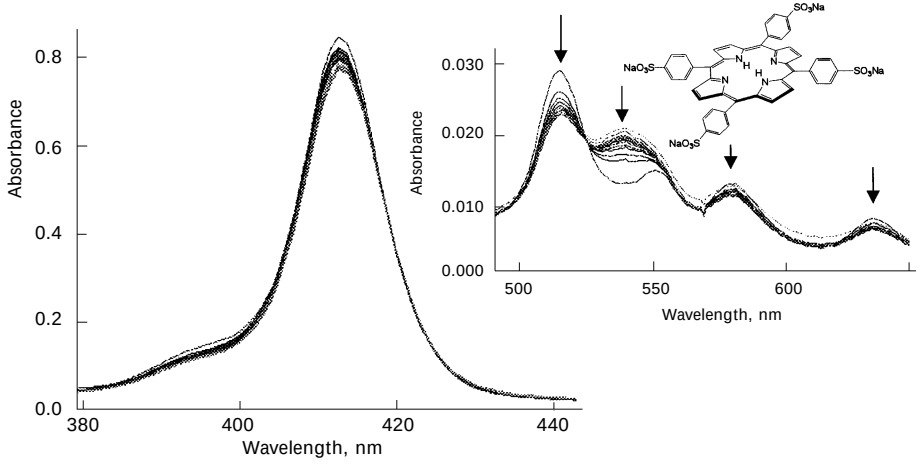


FIG. 9

UV-VIS spectral changes of the receptor **14**; interaction of **14** with D-glucose; λ_{max} = 413 nm. A: changes of Q-bands in spectra

All porphyrin-oligopeptide receptors show higher specificity to di- and trisaccharides than monosaccharides with the only exception of β-lactose (Fig. 11). The association constants calculated for Asp-containing **18** are

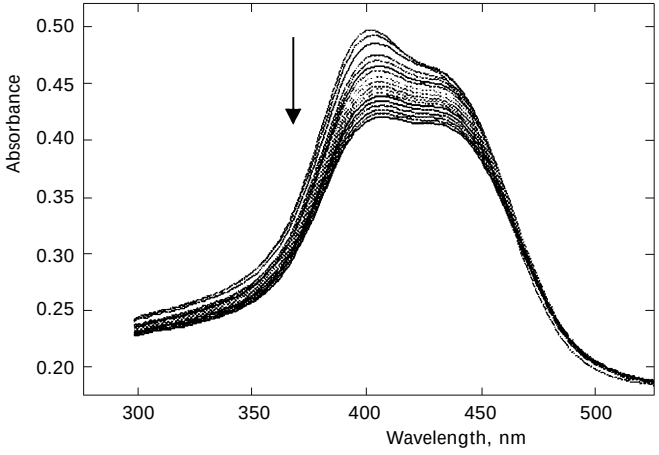


FIG. 10
Interaction of **16** with D-glucose indicated by typical UV-VIS spectral changes in aqueous media; λ_{max} = 403 nm

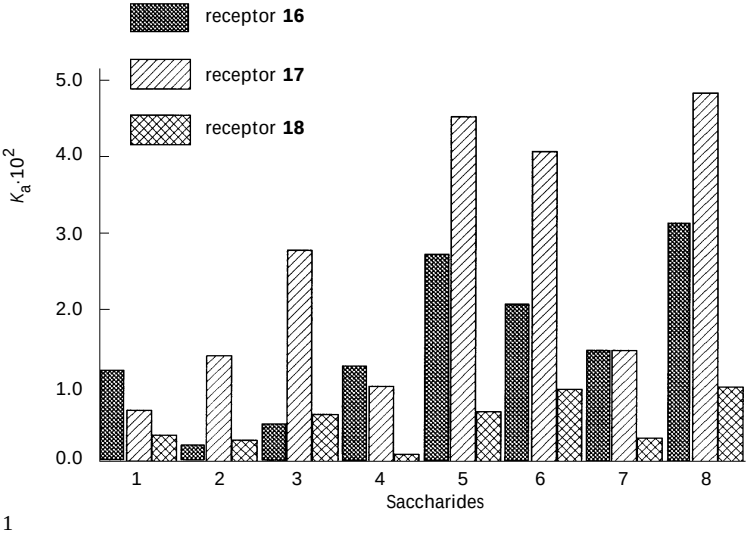
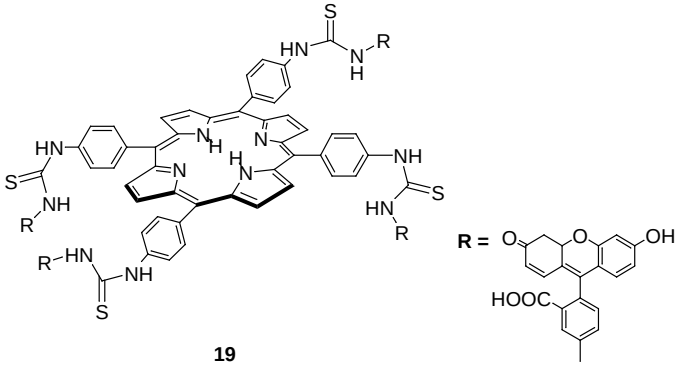


FIG. 11
Association constants for binding of saccharides with receptors **16**–**18** in water media (UV-VIS titration). 1 D-Galactose, 2 D-glucose, 3 D-fructose, 4 D-ribose, 5 trehalose, 6 α-lactose, 7 β-lactose, 8 maltotriose

lower than those for **16** and **17**. The IR spectroscopy proved an involvement of saccharide OH and carboxylate group of the receptor in the interaction. The described porphyrins were also tested using SPR technique[43]. The results are shown in Fig. 12. The differences in the shape of SPR-curves reflect the ability of receptors **16** and **17** to bind α-D-glucose from its concentrated water solution (3 mol l^{-1}). Even more convincing results were obtained for immobilized receptor **16**.

Introduction of fluorescein subunits in the porphyrin periphery *via* thiourea bridges allows to obtain a receptor **19** with interesting binding



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properties. No changes were found by UV-VIS spectroscopy after addition of saccharides to a solution of receptor **19** in aqueous media. However, sig-

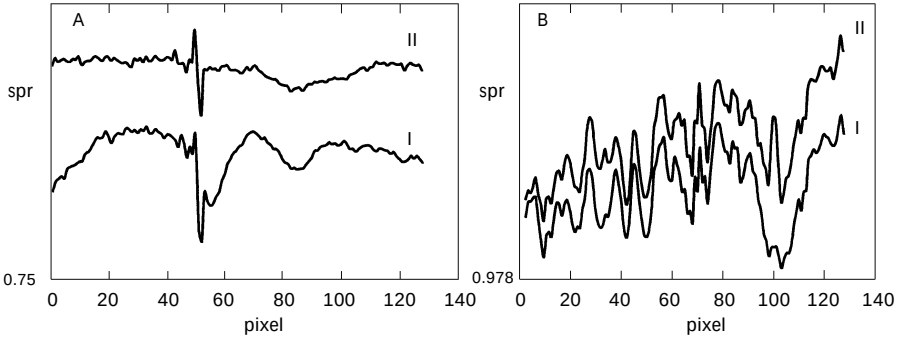


FIG. 12

Interaction of the porphyrin peptide conjugates with D-glucose on the gold surface monitored SPR. A plot reflects light intensity *versus* pixel (SPR-curves) for starting compound (curve I receptor **4**) and for complex (curve II receptor **4**–glucose): A conjugate **16**; B conjugate **17**

nificant changes were observed in its fluorescence spectra (Fig. 13). The fluorescence maximum of **19** at 514 nm is continuously increasing with a gradual addition of a saccharide. Selectivity to certain saccharides is summarized in Fig. 14. Receptor **19** is able to distinguish certain saccharide species, namely D-galactose from D-glucose and D-fucose from L-fucose.

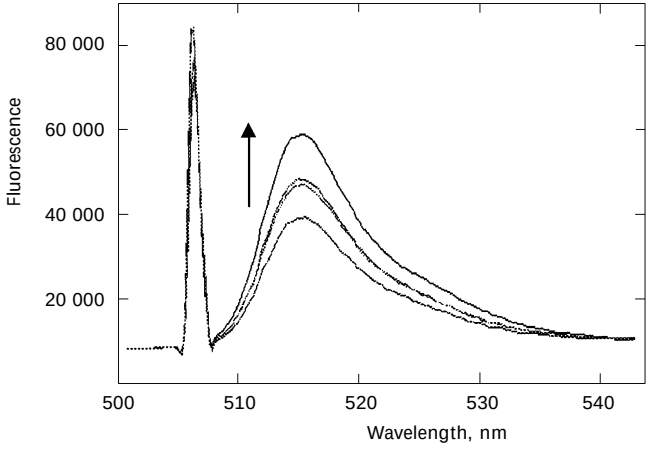


FIG. 13
Fluorescence spectral changes of **19** in aqueous media; λ_{max} = 514 nm. Interaction of **19** with D-glucose

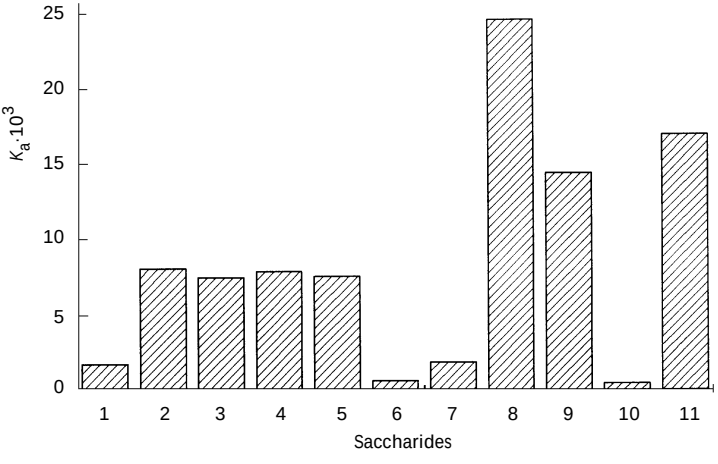


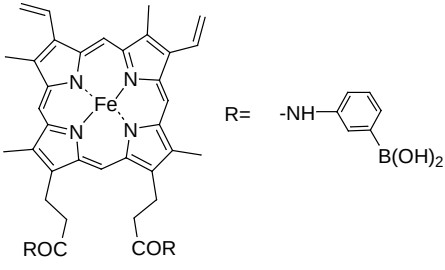
FIG. 14
Interaction of the receptors **19** with saccharides. 1 D-Galactose, 2 D-glucose, 3 fructose, 4 ribose, 5 mannose, 6 D-fucose, 7 L-fucose, 8 trehalose, 9 α-lactose, 10 β-lactose, 11 maltotriose

Oligosaccharides tend to bind more strongly than monosaccharides. In addition, selective binding of α-D-lactose was observed.

2.1.4. Porphyrin–Saccharide Interactions – Covalent Binding

This part of the review deals with water-soluble porphyrins bearing boronic acid groupings. In 1954 Kuivila *et al.* noticed that boronic acid solubilized saccharides and polyols by the formation of cyclic esters[44]. The efficiency of boronic acid-containing receptors is based on the formation of covalent bonds between a boronic acid and corresponding diol. Boronic acids form bonds with 1,2- or 1,3-diols to generate five- or six-membered cyclic esters in nonaqueous or aqueous alkaline media[45]. Rigid vicinal *cis*-diols of saccharides form stable cyclic esters. Selectivity of phenylboronic acid to saccharides was studied by Lorand and Edwards[46]. Porphyrinboronic acid receptors showed outstanding optical properties, which allow their effective use for saccharide recognition in water.

Phenylboronic acid derivative of myoglobin **20** showed a slight change in UV-VIS spectrum when D-fructose was added to the solution[47,48]. Saccharide-sensing receptor **21** was also proposed for saccharide recognition[49]. UV-VIS and fluorescence spectroscopy are suitable techniques for



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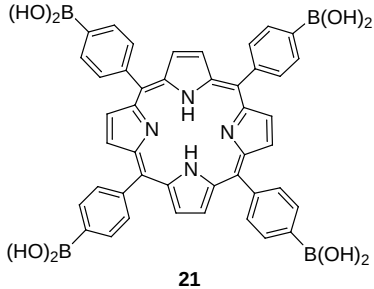

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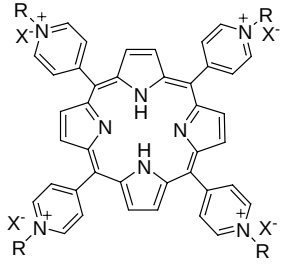
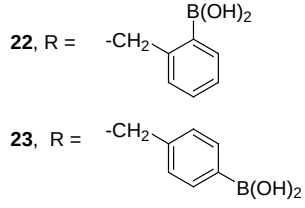
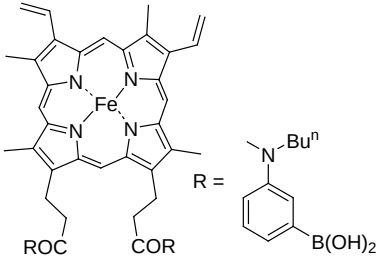
the indication of complex formation. Receptors **22**, **23** were employed for monitoring the complexation-decomplexation equilibrium between the receptors and aromatic disulfonates[50]. Addition of D-fructose leads to the dissociation of the complexes with aromatic guests. This method was proposed for controlling the efficiency of photoinduced electron transfer which imitates the incipient stage of photosynthesis. The ability of phenylboronic acid to bind with saccharides was also used for the control

of porphyrin-DNA binding properties[51]. Addition of D-fructose or D-glucose to complex **23**–DNA was accompanied by dissociation of the complex which was confirmed by changes in its CD spectrum.

Receptors **22**, **23** displayed selectivity to D-glucose and xylose[50]. These macrocycles gave specific exciton-coupling bands in CD spectroscopy only in the presence of the above mentioned saccharides. The ability of receptors **20**, **24**–**26** to form helical structures with the saccharides in aqueous media was described earlier[52]. Spectroscopic, light-scattering, DSC and electron microphotographic studies proved the formation of twisted fibrous aggregates. These studies were proposed to mimic the morphological







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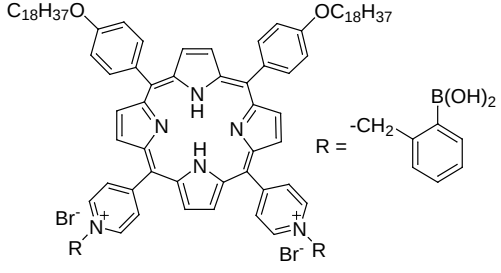

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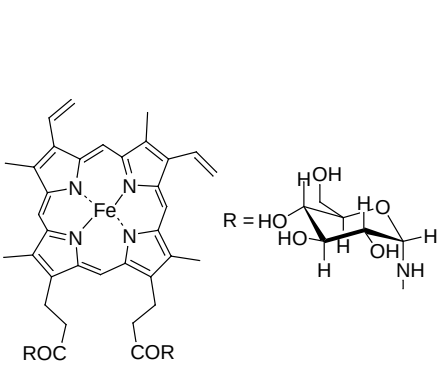
functions of certain cell membranes, the surface of which is covered by saccharides. In this system the boronic acid groups play a crucial role in composing the hydrophilic moiety of an amphiphile. Saccharides bound to the boronic acid sites introduce chiral receptors to the aggregate. Receptor **27** was proposed as a photoinduced electron-transfer (PET) sensor[53]. Fluorescence intensity of this compound dramatically depends on the concentration of a saccharide in solution. The authors proposed this model as an

"on-off type" PET sensor, which is sensitive to 1,2-diols. An interesting example of porphyrin–boronic acid saccharide receptor is Ce(IV) bis(porphyrinate) double decker scaffold **28** described by the Shinkai group[54].



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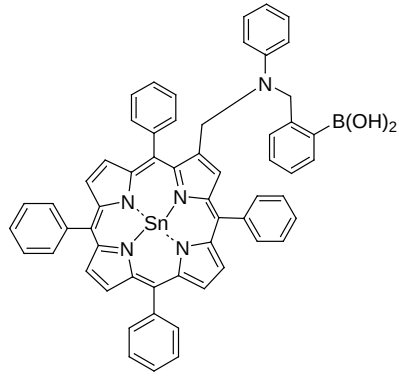

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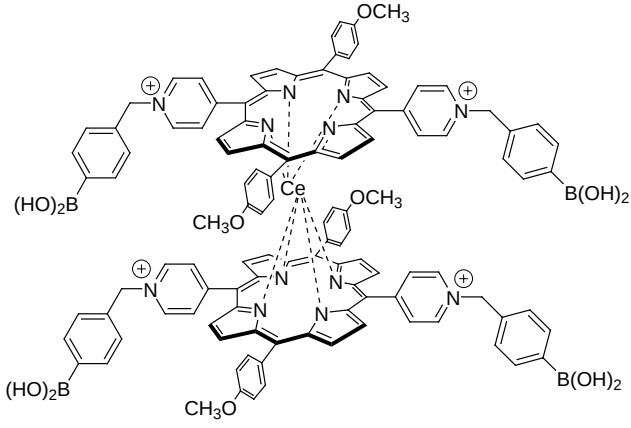

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2.2. Interaction of Novel Cationic Oligopyrrole Macrocycles with DNA and Nucleotides

Molecular recognition of DNA is one of the most fundamental processes in nature and analyzing the essence of interaction of small molecules with DNA continues to be an important area of research. The binding of cationic porphyrins and some expanded porphyrins to DNA is of considerable inter-

est in view of cancer research, gene technology, antiviral agents, and selective cleavage of RNA (refs[55–58]). Ligand-induced changes of DNA conformation could serve for specific probing of DNA structure. The development in this area is based upon detailed understanding of binding mechanism.

Recently it has been shown[59–62] that sapphyrins (pentapyrrole macrocycles with a bipyrrole unit) and some other expanded porphyrins can effectively bind anions. X-Ray diffraction in the solid state as well as NMR studies in solution revealed that diprotonated sapphyrin is chelated to the phosphate anion *via* Coulombic attractive forces and H-bonding. This phosphate chelation is responsible for binding of water-soluble sapphyrins to the anionic phosphodiester backbone of DNA. Nucleobase substituted sapphyrins were found to be efficient models for membrane transport of nucleotides at neutral pH.

Three types of cationic porphyrin binding with DNA have been described so far in the literature: intercalation, outside (groove) binding and outside binding with self-stacking leading to long-range porphyrin structures on the DNA exterior[55,57,63,64]. Special attention has been paid to porphyrins bearing pyridinium or ammonium groups[55,57,63,65,66] such as *meso*-tetrakis(4-*N*-methylpyridyl)porphyrin (TMPyP). Intercalative binding of free-base TMPyP and corresponding planar metal derivatives leads to a large red shift (>10 nm) and to an extensive hypochromicity of the Soret band[55,67]. The Soret region is usually very sensitive to factors like solvent, concentration, aggregation, ionic strength, binding of small molecules, whereas the visible (Q) bands are less affected. Binding of porphyrins is usually complicated by their aggregation in aqueous solutions especially when they contain bulky hydrophobic peripher substituents and/or the concentration of porphyrins in solutin is high. According to Vergeldt *et al.*[68] TMPyP is monomeric up to 10^{-3} mol l^{-1}. Recently some porphyrins bearing phosphonium residues have been found to have a strong aggregation power exhibiting π–π stacking interaction in water[69]. On the other hand, adsorption of porphyrins to cell walls is expected to complicate optical spectroscopy measurements especially at very low concentration (<10^{-7} mol l^{-1}).

The porphyrin–DNA complexes can be highly stabilized by electrostatic interactions between positively charged substituents on the porphyrin periphery and negatively charged phosphate oxygen atoms of DNA. When such porphyrin is excited, photosensitized cleavage of DNA and nucleotides can be initiated by electron and/or energy transfer between excited porphyrin and an adjacent base pair. Because the triplets of TMPyP bound to DNA have longer lifetime than the free triplets in solution[70], effective

electron and/or energy transfer under binding conditions can occur. In the presence of oxygen, the triplet states are quenched, thus forming singlet oxygen in the close vicinity of DNA. The target for singlet oxygen attack is believed to be guanine residuum[71–73] as the rate constants are greater than 10^6 l mol^{-1} s^{-1} considerably exceeding the reactivity of adenine, cytidine and thymine bases[62].

Meso-tetraphenylporphyrin (TPP) is known to be a good sensitizer of singlet oxygen with high quantum yields[74] (0.34–0.87). We have developed a general synthetic strategy for the preparation of tetrasubstituted TPP with positively charged ammonium, pyridinium, phosphonium and sulfonium groups. The synthetic protocol is based on the alkylation of tetrakis-(4-bromomethyl)porphyrin. The cationic centers are separated from the phenyl ring by a methylene bridge and thus have minimal influence on electron density of the porphyrin moiety. Therefore, these porphyrins are supposed to have similar photophysical properties as TPP, however, different binding affinity modulated by substituents. Moreover, the presence of charged groups improves the solubility in polar media (methanol, dimethyl sulfoxide, H_2O).

We have reported recently[75] on the self-aggregation behavior of novel cationic porphyrins **29**–**34** (Fig. 15), their basicity and the stability of porphyrin-nucleotide and porphyrin-DNA complexes. The results were compared with the behavior of cationic *meso*-tetrakis(4-*N*-methylpyridyl)porphyrin (TMPyP) and anionic *meso*-tetrakis(4-sulfonatophenyl)-porphyrin (TPPS). The aim of this study was to find new efficient porphyrin type sensitizers suitable for biological applications.

2.2.1. Design and Synthesis of Porphyrins

We have developed a general strategy for easy access to novel porphyrins. The synthesis is based on the introduction of bromomethyl groups to form tetrakis(bromomethylphenyl)porphyrins followed by the quarternization reaction with a variety of acceptors – phosphines, sulfides, amines, thiourea – thus, furnishing the corresponding positively charged porphyrin-phosphonium, -sulfonium, -ammonium, -isothiouronium derivatives. The general methodology is shown in Fig. 15. The first step comprises heating of porphyrin bromomethyl derivatives (60–80 °C for 12 h) dissolved or suspended in the acceptor either in the form of liquid (sulfides, alkyl phosphines, amines, *etc.*) or in melts (arylphosphines). The work-up is relatively simple: the product precipitates and it is filtered off after the addition of a nonpolar solvent solubilizing the unreacted acceptor and partially re-

acted porphyrin. The yields were above 90%. Porphyrins **29**–**34** are soluble in water, DMSO and methanol.

Flexibility of this approach opens a route to incorporation of other atoms, such as selenium, arsen, *etc.* Behavior of the positively charged groups, modulating steric properties, ability to form highly ordered molecular aggregates, binding modes and/or affinity to biologically important polyanionic species is a compelling driving force for detailed study of physicochemical properties (Fig. 16).

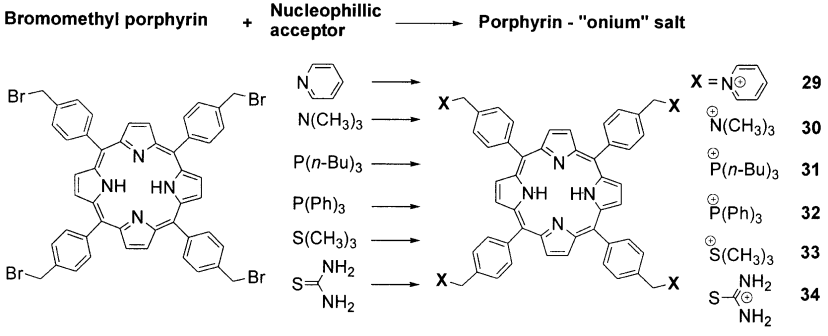


FIG. 15

Molecular structures of cationic *meso*-tetraphenylporphyrins bearing substituents with nitrogen (**29**, **30**, **34**), phosphorus (**31**, **32**) and sulphur (**33**, **34**) atoms

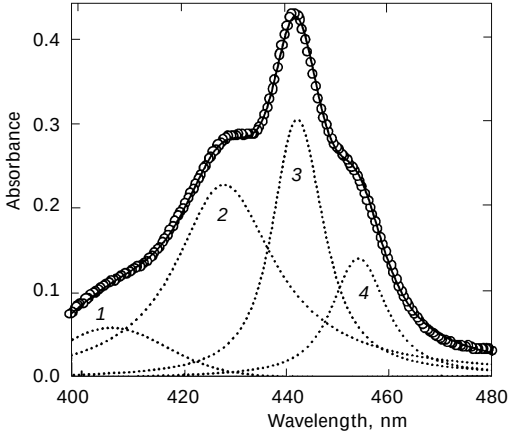


FIG. 16

Soret band of 2.7 μmol l^{-1} **32** in water at pH 3.1. Superposition of four Voigtians: H-aggregate (1), monomer (2), J-aggregate (3), protonated porphyrin (4)

2.2.2. Interaction of Porphyrins with DNA and Mononucleotides in Water and Phosphate Buffer

The spectral properties of porphyrins, titrated with aliquots of double-stranded DNA, were investigated in the range of 250–800 nm as a function of r_0 (the ratio of total concentration of porphyrin to that of dsDNA in base pairs). While spectra in the visible region above 500 nm did not exhibit considerable changes, the Soret maximum rendered features allowing specification of the extent of porphyrin binding. It should be recalled that porphyrins **29** and **30** are predominantly monomeric in phosphate buffer immediately after mixing. Thus, spectral perturbations upon addition of dsDNA arise only from association of **29** and **30** with the DNA matrix (Figs 17 and 18). This is indicated by a red shift of the Soret maximum to 421 nm ($\varepsilon_{421} = 2.0 \times 10^5$ l mol^{-1} cm^{-1} for P_1 and $\varepsilon_{421} = 2.2 \times 10^5$ l mol^{-1} cm^{-1} for P_2) and by an extensive (≈50%) hypochromicity. The significant hypochromicity suggests that the porphyrin π-electrons were perturbed considerably after association with DNA. Spectral changes show a single isosbestic point typical for a simple equilibrium between free (unbound) and bound porphyrin (Figs 17 and 18). Respective forms are discernible due to their different diffusion properties that are manifested by quenching of the excited triplet states by oxygen. Transient T-T spectra of **29** and **30** have typical broad absorption maxima at 450 nm, not affected by DNA. In

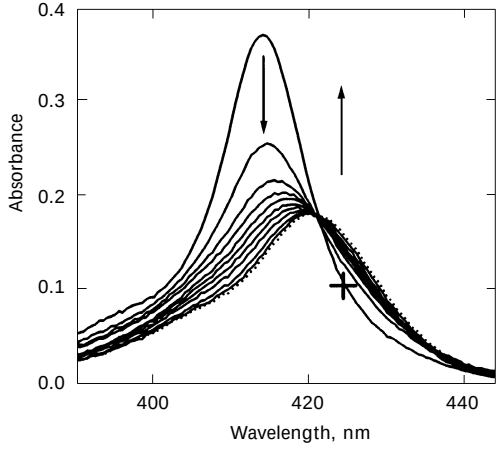


FIG. 17

Spectral titration of 1.5 μmol l^{-1} **29** with DNA. Arrows designate changes in the Soret band, r_0 down to 0.018 (dotted line), the cross indicates the isosbestic point with J-aggregate; 20 mmol l^{-1} phosphate buffer, pH 7.0, 100 mmol l^{-1} NaCl, corrected for dilution

air-saturated solution, the triplet states are quenched by oxygen monoexponentially with lifetime of 1.5 μs (Fig. 19, curve *1*). At r_0 = 0.13, both free and bound porphyrins are indicated by two component quenching of the excited triplets with lifetimes of 1.5 and 18 μs (Fig. 19, curve *2*). At higher DNA concentrations (r_0 = 0.02, Figure 19, curve *3*) even three dis-

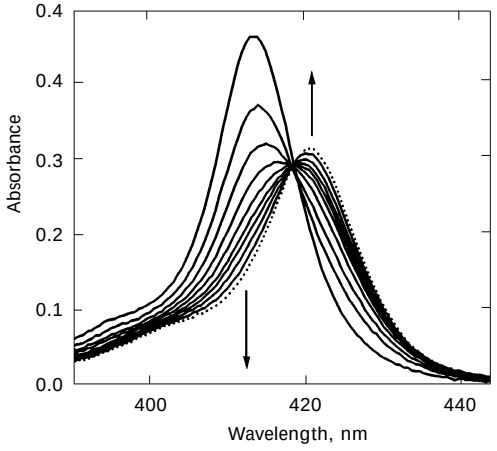


FIG. 18

Spectral titration of 1.4 μmol l^{-1} **30** with DNA. Arrows designate changes in the Soret band, r_0 down to 0.025 (dotted line); 20 mmol l^{-1} phosphate buffer, pH 7.0, 100 mmol l^{-1} NaCl, corrected for dilution

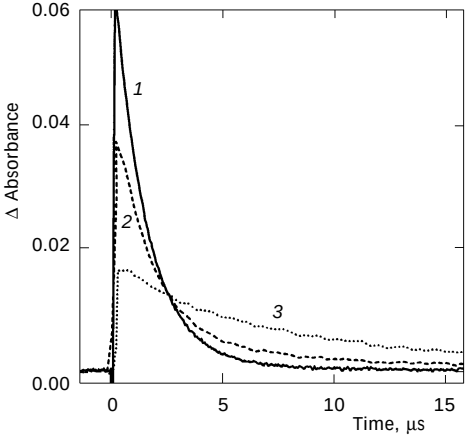


FIG. 19

Quenching of the transient absorption of the **29** triplet states by oxygen: no DNA (*1*); DNA, r_0 = 0.13 (*2*); DNA, r_0 = 0.02 (*3*). Excitation wavelength 412 nm, monitored at 450 nm; 2.8 μmol l^{-1} of **29**, 20 mmol l^{-1} phosphate buffer, pH 7.0, 100 mmol l^{-1} NaCl, corrected for dilution

tinct porphyrin excited triplets were apparent – free, and two bound components with lifetimes of 7.7 and 30 μs, probably given by the triplets in different DNA microenvironments.

Spectral features of TMPyP bound to DNA have been already reported[55,57]. We found similar bathochromic shift of the Soret band from 424 nm (ε_{424} = 2.3 × 10^5 l mol^{-1} cm^{-1}) to 435 nm (ε_{435} = 1.6 × 10^5 l mol^{-1} cm^{-1}) concomitant with a large hypochromicity (not shown). The complexity of oxygen quenching of the excited states of TMPyP in the presence of DNA has been described recently[70].

Based on a correlation with Beer's law (*vide supra*), the addition of DNA (Fig. 20, curve *2*) as well as of buffer (Fig. 20, curve *3*) to **31**–**34** does not perturb the absorption spectra (Fig. 20, curve *1*) due to extensive self-stacking. The Soret bands are broad regardless DNA presence indicating considerable amount of J-aggregates. The experimental support stems from transient T-T spectra. It is well known[76] that the quantum yield of the triplet states Φ_T decreases dramatically when aggregation occurs. Assuming similar absorption coefficients of the triplet states in buffer, dimethylsulfoxide and methanol, porphyrins **31**–**34** have two orders of magnitude lower Φ_T in buffer when compared with the organic solvents, where **31**–**34** are predominantly monomeric. Summing up, self-aggregation of **31**–**34** is a dominant process in aqueous solutions causing that aggregates do not dissociate or dissociate very slowly upon the addition of DNA. However, association of **31**–**34** with the surface of DNA cannot be excluded as a result of

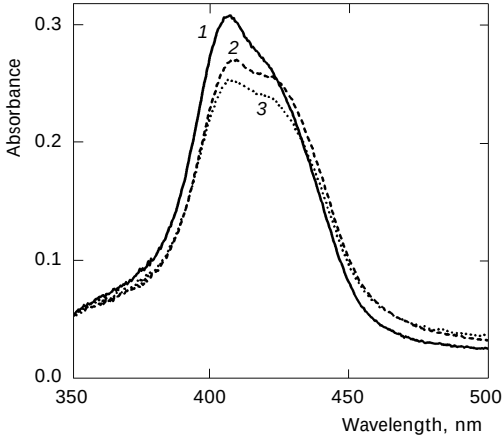


FIG. 20

Soret region of 2.8 μM **34**: no DNA (*1*); DNA, r_0 = 0.035 (*2*); addition of phosphate buffer (*3*). 20 mmol l^{-1} phosphate buffer, pH 7.0, 100 mmol l^{-1} NaCl, corrected for dilution

hydrophobic interactions and Coulombic attraction between DNA backbone phosphates and aggregates bearing localized positive charges. TPPS, the only anionic tetravalent porphyrin used, does not exhibit any sign of interaction with DNA (nor nucleotides).

Absorption data of TMPyP **29** and **30** were analyzed according to the McGhee–von Hippel model for binding of noninteracting ligands to a lattice of binding residues. Independent experiments reveal solid information on the free porphyrin concentration r for different values of r_0. The binding constants K_a and the exclusion parameters n were obtained[75] from the linear limiting part of the binding isotherms at low r according to Pasternack *et al.*[57]

TMPyP is an intercalator at GC sites with the apparent binding constant[57] K_a of 7.7×10^5 l mol^{-1}. Moreover, outside binding, predominantly at AT sites, occurs when r_0 and/or ionic strength increase[55,57]. Our conditions of low TMPyP loading, where intercalation is supposed to prevail, afford the well comparable value of $K_a = 1.3 \times 10^6$ l mol^{-1} as well as the number of unavailable DNA residues. Compounds **29** and **30** have surprisingly apparent binding constants of the same order[75].

Studies of photoinduced processes within DNA by optical methods are rendered difficult due to a variety of low quantum yield products. Hence the reactivity of individual bases cannot be distinguished. To assess respective nucleotides we ran spectrophotometric titrations of **29**–**34** with AMP, GMP, CMP and TMP (up to 1 mmol l^{-1} as limited by nucleotide solubility) in buffer. Absorption and fluorescence emission spectra of **31**–**34** visualize porphyrins that are highly aggregated rather than nucleotide–porphyrin complexes. Such complexes cannot be, however, excluded since all porphyrins bear high positive charge. Under the same conditions, TMPyP forms stacking-type complexes – the binding constants up to 10^3 l mol^{-1} depend on a nucleotide[56,77]. Stronger complexes are formed with double ring purine bases. Pyrimidines afford weaker complexes because of a comparatively lower π–π overlap.

2.2.3. Binding Modes: Induced CD Spectra and Molecular Biology Study

Circular dichroism spectroscopy (Fig. 21) was applied to ascertain the effect of the stereogenic DNA environment around accommodated cationic porphyrins[75]. Binding modes are controlled by porphyrin shape, charge, and sequence of DNA as documented by extensive research on free base and metal complexes[57] of TMPyP. Intercalative and external groove binding

modes have been associated with negative and positive induced Soret CD bands, respectively. Compounds **29**–**34** are achiral and hence no CD spectra were displayed in buffer in the absence of DNA. Porphyrins **29** and **30** give similar circular dichroic features upon the addition of DNA and display sensitivity to porphyrin loading r_0 (Fig. 21). At high values of $r_0 > 0.3$, a single positive peak centered at 424 nm is observed. With further addition of DNA ($0.015 > r_0 > 0.3$) a new positive signal appears at 418 nm. The intensity of this peak is enhanced with decreasing porphyrin/DNA values and eventually at $r_0 < 0.03$ only the short wavelength peak is present, with $\Delta\varepsilon$ of 23.5 l mol^{-1} cm^{-1} for **29** and 12.2 l mol^{-1} cm^{-1} for **30**, respectively. Low intensity negative peaks at 430 and 427 nm are observed under extreme porphyrin/DNA dilution ($r_0 = 0.002$). The appearance of two positive bands at low r_0 suggests high binding affinity of **29** and **30** and a number of outside binding configurations on the calf thymus DNA exterior at both GC (42%) and AT sequences. Evidently, the binding preference depends upon porphyrin loading. This has been observed recently for MnTMPyP where

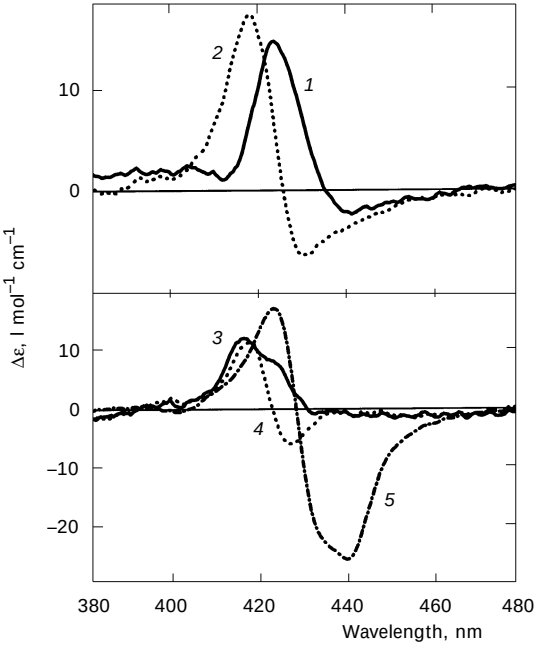


FIG. 21

Induced circular dichroism spectra of **29** (2.7 μmol l^{-1}), **30** (1.6 μmol l^{-1}) and **32** (4.1 μmol l^{-1}) with DNA. **29**, $r_0 = 0.346$ (*1*); $r_0 = 0.003$ (*2*). **30**, $r_0 = 0.078$ (*3*); $r_0 = 0.002$ (*4*). **32**, $r_0 = 0.005$ (*5*). 20 mmol l^{-1} phosphate buffer, pH 7.0, 100 mmol l^{-1} NaCl, corrected for dilution

the shorter and the longer wavelength peaks are assigned to the minor and to the major groove binding modes[78]. Compounds **29** and **30** do not display a conservative CD spectrum, that excludes the presence of long range assemblies of stacked porphyrin units arranged into helical domains on DNA. In accordance with it, having employed the resonance light scattering method[79] we did not find any enhancement of scattered light intensity. However, induced CD spectra at $r_0 = 0.002$ looks like exciton splitting with a few porphyrin units in close contact. Strong porphyrin based signal of **32** (Fig. 21, curve *5*) is commonly attributed to highly ordered outside stacking binding mode originally proposed by Fiel[55]. Under conditions of low $r_0 < 0.1$ and of low ionic strength, intercalation of porphyrins into calf thymus DNA induces predominantly a negative CD band in the Soret region[57,63]. Thus, CD spectra brought no evidence for porphyrin intercalation. Biochemical study corroborates this conclusion[80]. Topoisomerase I assay was used to probe the nature of interaction with selected porphyrins **29**, **33** and **34**. This enzyme can detect the unwinding of the dsDNA helix induced by a small molecule, a classic proof of DNA intercalation. No unwinding of supercoiled pBR322 DNA was found.

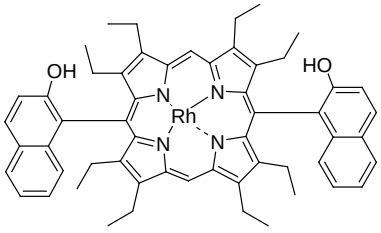
CD and biochemical experiments are in good agreement with the McGhee–von Hippel analysis of the porphyrin-DNA binding isotherms. The apparent binding constants of **29** and **34** are comparable to that of TMPyP for intercalation[75]. However, as evidenced above, high values of K_a are not indicative of intercalation and only confirm that outside-binding porphyrins **29** and **30** exhibit large affinity to DNA (ref.[63]). In contrast to TMPyP, the number of DNA base pairs (n), covered by **29** and **30**, is much higher and corroborates that they exhibit a different binding mode than TMPyP. Intuitively, large pendant groups in the *meso*-positions sterically block intercalation. The size of substituents increases in the order: TMPyP ($n = 2.7$) < **30** ($n = 6.2$) < **29** ($n = 8.1$). Porphyrins **29** and **30** have a diameter larger than 1.5 nm. As proposed by Fiel *et al.*[55], Coulombic interactions with the backbone phosphate groups could result in a face-on arrangement in the major groove where the plane of the bound porphyrin is parallel to the helix axes. Assuming only this type of interaction and that adjacent bases are separated by 0.34 nm (B-DNA), the porphyrin diameter corresponds to about 5 bases along the helix axis and removes equivalent number of base pairs by physical coverage. The values of $n = 8.1$ and 6.2 for **29** and **30** indicate that about 8 and 6 base pairs are made unavailable. This can by partly explained by steric hindrance of bulky groups combined with a face-on orientation of the porphyrin moiety on the DNA exterior.

Interaction between highly hydrophobic porphyrins **31**–**34** and DNA cannot be followed by spectrophotometric method. On the other side, CD is much more sensitive to microenvironment and shows that **32** is bound externally as stacked units on the surface exterior of DNA. To conclude, porphyrins **29**–**34** do not intercalate but bind to the surface of DNA helices.

2.3. Porphyrin-Based Receptors: Model Hosts for Amino Acids Recognition

Numerous porphyrin-based amino acid receptors were designed mainly for action in non-polar solvents similarly as mentioned saccharide receptors. The recognition ability of porphyrins toward amino acid esters was described firstly for the Rh(III)-porphyrin complex **35** (refs[81,82]). Later, other authors observed enantiomeric recognition of methyl esters of amino acids with Zn-porphyrins **36**–**40** (refs[83–88]). UV-VIS titration experiments in chloroform with doubly bridged α,α,β,β-atropoisomer of **36** and various types of amino acid methyl esters indicated the formation of strong 1 : 1 complexes as well as for hosts **37**–**40**. For the receptors of this type the recognition mechanism is based on non-covalent interactions and accomplished by a cooperative action of metal ion as a strong coordination site and hydrogen bonds between C=O and NH_2 groups of the guest and corresponding hydrogen donated groups of the host. The coordination of the amine group with metal ion of the porphyrin and hydrogen bonding were confirmed by ^{1}H NMR spectroscopy[85–88]. Chiral recognition stems from the steric interaction between the metal and the residual group of the amino acid. The hydrogen bonding interactions fix the guest and, consequently, enantioselection results[89]. The Zn atom binds amino group and for this reason the Zn-porphyrins differentiate between amino acid esters and zwitterions. These porphyrins exhibit significant enantiomeric recognition toward numerous amino acids[85–88]. Porphyrin **41** exhibited high enentioselectivity for carboxylate anions of A-protected amino acids[90]. In this case central Zn metal axially binds an acetate anion. Chiral recognition of amino acids with **41** was tested by solvent extraction of Na-salts of A-protected amino acids from aqueous solution into a liquid phase containing $CHCl_3$ (refs[89,90]). In most cases, the (+)- and (–)-**41** preferentially bound the L- and D-substrates, respectively[89].

Gadolinium(III)-porphyrin **42** was designed as strong chelator for unprotected amino acids[57,91]. This water-insoluble receptor was able to extract specifically L-phenylalanine from its aqueous solution into dichloromethane phase that was proved by UV-VIS and CD spectroscopy. The presence of Gd(III) ion is absolutely necessary for binding. When Zn(II)-



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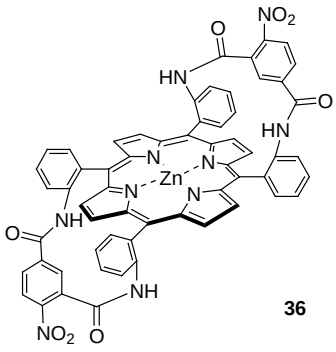

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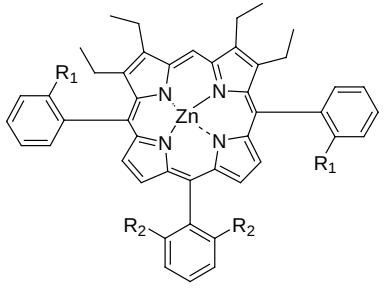
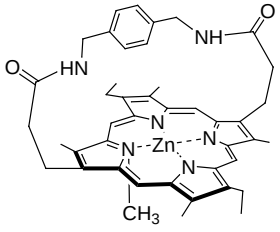


37, R_1 = OH; R_2 = CH_2COOCH_3
38, R_1 = OH; R_2 = H
39, R_1 = H; R_2 = CH_2COOCH_3
40, R_1 = H; R_2 = H



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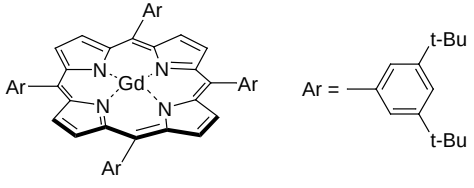

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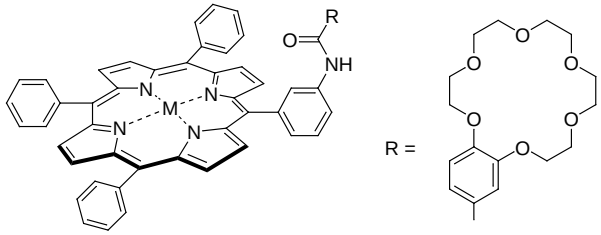
porphyrin **42** was employed instead of gadolinium-porphyrin **42**, L-phenylalanine was rarely extracted from the aqueous phase. Among other 19 natural chiral α-L-amino acids, 16 amino acids gave CD signal at the Soret band region with different intensivity. Other lanthanide complex of porphyrin **43** with covalently attached benzo-18-crown-6 moiety was designed for synergistic binding of zwitterionic amino acids[92]. In this system the benzo-18-crown-6 coordinates to the NH_3^+ moiety and lanthanide-porphyrinate part binds the COO^- moiety of amino acid. Lanthanide-porphyrinate-crown ether conjugates also afforded an efficient extraction

of several amino acids from their aqueous solutions into dichloromethane phase. The chirality-specific CD signals were observed *via* complexation of the conjugates with L-amino acids.



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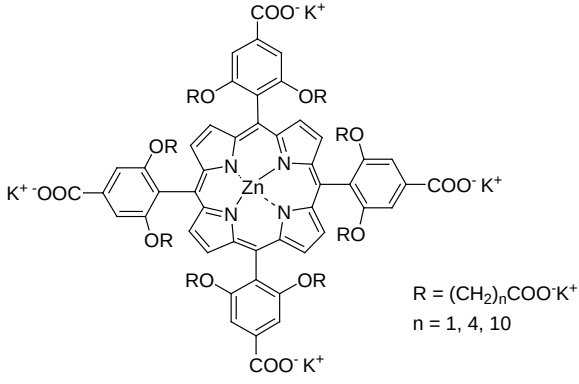

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A water-soluble porphyrin receptor for amino acids and oligopeptides in water was proposed by Mizutani group[93]. The porphyrin receptor has the Zn-tetrakis(carboxyphenyl)porphyrin as a structural unit, and eight ω-carboxyalkoxy groups at the *ortho*-positions of the phenyl groups. Receptor **44** binds amino acids with 1 : 1 and 1 : 2 stoichiometry with various selectivity. The binding event can be monitored by UV-VIS spectroscopy.

3. APPLICATIONS OF CHIRAL PORPHYRINS

Oligopyrrole macrocycles have received increased attention in chemistry in the last years because they have been successfully utilized for the exploration of mechanisms of various biologically important reactions, such as

photosynthesis[94], photodynamic therapy[95], development of receptors for molecular recognition[96] and chiral catalysts for asymmetric synthesis[97]. Chiral recognition of asymmetric compounds is one of the most important subjects not only in the field of supramolecular chemistry but also in biomedical applications.

Large aromatic ring systems such as porphyrins are in-plane polarized, therefore intrinsically achiral[98], show characteristic π–π^* absorption bands in the UV-VIS region. Chirality is induced by the introduction of appropriate substituents in the periphery of the ring, or by substitution leading to nonplanarity of the ring. The induced rotatory strength can be estimated on the basis of a coupled-oscillator model, which provides information on the local environment of the chromophore. Many spectroscopic properties can be derived from the symmetry rules for the D_{2h} symmetry of the porphyrin skeleton, which often adopts even higher D_{4h} symmetry in its metal complexes. Perturbations of this symmetry by the interaction with a chiral environment may lead to optical activity. Therefore the using of the chiroptical spectroscopic techniques is just described in this chapter.

3.1. Chiroptical Spectroscopy: Electronic and Vibrational Circular Dichroism

Circular dichroism (CD) spectroscopy, measured as differential absorption of left and right circularly polarized light by chiral molecules, can be used as a chiroptical method for monitoring chiral reactions. The difference in absorption appears around the absorption maxima in CD spectrum (Cotton effect) and intensity of the Cotton effect depends on coupling of chromophores and their absorption coefficients. The origin of the heme Cotton effect in myoglobin and hemoglobin was first investigated by Hsu *et al.*[99]

Electronic circular dichroism[100] (ECD) in UV-VIS and electronic magnetic circular dichroism (MCD) spectroscopy in the same spectral region when a molecule is placed in magnetic field play an increasing role in monitoring molecular properties and chiral interactions of porphyrins[101–103]. Advances in MCD technique of heme proteins and iron porphyrins were summarized by Dawson *et al.*[104,105]. Also the magnetic vibrational circular dichroism (MVCD) spectroscopy was used to describe the structure of metalloporphyrins[106], and most recently, the vibrational circular dichroism[107] (VCD) in the mid-IR spectral region was applied to study of peptide–porphyrin systems in aqueous solution[108,109]. A considerable progress in applications of all branches of CD spectroscopy is recently also supported by a substantial advance in the field of instrumentation and theoretical computation.

The utilization and application of ECD spectroscopy in the studies of multifaceted properties of porphyrin and metalloporphyrin systems have been reviewed in the last years[110,111]. The propensity to undergo π,π-stacking and facile incorporation of various metals make the porphyrins one of the most attractive and sensitive chromophores used in CD spectroscopy[112]. The sharp intense Soret band with higher intensity compared to the Q bands, whose position depends on the substituent groups in the periphery, is localized around 400 nm in the near UV spectral region and is especially useful in ECD studies because it reflects with high sensitivity the identity of guests and binding modes of host/guest complexation. Porphyrins offer extensive possibilities for studying stereochemistry of chiral porphyrin assemblies with small as well as large biological molecules (proteins, amino and nucleic acids). Huang *et al.*[110] stimulated the progress and application of ECD and induced CD (ICD) in the area of recognition and determination of absolute configuration of biologically important chiral molecules using porphyrins. The correlation between sign of the bisignate CD feature (*i.e.* CD couplet) and stereochemistry can be accounted for the stereoselective intramolecular π,π-stacking of the two porphyrin molecules; this can be utilized to determine the absolute configuration of compounds containing a single stereogenic center[113], for example, in coordination of amines with zinc-porphyrins[114].

3.2. CD of Chiral Porphyrins

Chirality can be introduced to porphyrin molecules in several ways: by nonplanarity leading to molecular asymmetry, or chiral substituent groups, which leads to atropisomerism[115]. When two or more chirally oriented chromophores are close to each other[116], their excited states couple and such a situation is known as exciton coupling[111] characteristic by intense bisignate CD (CD couplet). The sign of the CD band depends on the angle between electric transition moments of chromophores and its intensity on the distance between coupled substituents.

An advance in the chemistry of chiral porphyrins and chiral metalloporphyrins and their applications in enantioselective reactions includes (i) synthetic methodologies for asymmetric catalysis by chiral porphyrins[117–119], (ii) asymmetric reactions mediated by metalloporphyrins (*e.g.* with zinc, iron, gadolinium) and free porphyrin bases[120], (iii) enantioselective recognition of specified chiral substrates[121] as a amino acid derivatives[90] and (iv) study of Pt(II)- and Pd(II)-porphyrin assemblies with phosphines[122] using chiral metalloporphyrin-based receptors. Chiral

strapped porphyrins and metalloporphyrins were applied to enantioselective reactions and recognition of amino acid derivatives[85], helix-sense selective recognition of poly(glutamic acid)[123] and enantioselective reconstitution with apocytochrome b562 (refs[124,125]) studied by CD spectroscopy. The bisporphyrin modified by Tröger's base showing a negative exciton couplet in Soret band, indicating that two porphyrins form a left-handed twist, was used for the recognition of L-histidine with high enantioselectivity[126]. Optically active porphyrin dimers provide almost identical UV-VIS spectra as monoporphyrins; however, the electronic interaction between two porphyrin molecules was clearly evident from the bisignate CD in Soret band arising from exciton coupling of the two porphyrins[127].

The use of chiral Zn(II)-metalloporphyrin derivative for molecular recognition of chiral amines by spectrophotometric titration has been described recently[128]. A selective binding of (*S*)-1-(1-naphthyl)ethylamine with the binding ratio K_S/K_R = 2.4 was clearly observed. A higher affinity to α,ω-diamines containing more than five carbon atoms was shown for chiral binaphthyl-linked zinc-porphyrin dimers in comparison to their short diamine analogues[129]. Strong bisignate CD pattern arising from exciton coupling of two porphyrins indicates that only longer diamines form intramolecular bridges between two zinc-porphyrins through ditopic interactions.

Geometries of phthalocyanines with two and four optically active binaphthyl units were characterized by Kobayshi *et al.*[130]. The binaphthyl substituents induced CD in the in-plane polarized Q and Soret bands of phthalocyanines. (*R*)- and (*S*)-binaphthyls show a positive and negative CD for these bands, respectively. Conformation of enantiomers of chiral phosphorus porphyrins[131], which displayed different CD spectra in basic and acid media due to a conformational change of the porphyrin ring and diastereomeric mandelate complexes of tetraphenylporphyrins[132,133] with molecular asymmetry, was studied by ECD spectroscopy.

3.3. CD of Achiral Porphyrins Interacting with Chiral Substances

Various artificial porphyrin receptors have been synthesized for the molecular recognition and determination of absolute configuration of biologically important chiral guests such as amino acids[85,90,91,126,134–136], polypeptides and proteins[137–145], nucleic acids[57,63,143,144,146,147] and carbohydrates[19,52,148–150]. In these cases the porphyrin chromophores are achiral and the rotatory strength can only arise when the perturber has an electric transition moment, which is skewed with respect to the moment of the

ring system. A different induction channel is opened when the perturber itself exhibits a strong circular dichroism, which can be directly transferred to the ring system by a CD stealing mechanism.

The exciton coupling analysis was used for conformational studies of saccharides[52,151,152], pentols[153], hydroxycarboxylic acids[154–156] and steroids[157]. Mizutani *et al.*[136] observed induced CD of zinc porphyrins with naphthyl substituents in the case of recognition of α-amino acids (Val, Leu) esters through a two-point fixation mechanism: amine–zinc coordination and ester carbonyl–naphthol hydrogen bonding. Similar systems with gadolinium[91] and magnesium[135,158] metalloporphyrins were used for recognition of among others, L-histidine, L-proline, L-serine, L-trypthophan where the sign of ICD in Soret region selectively reflects the absolute configuration of a given amino acid. The zinc porphyrins were also used for binding of saccharides[19]; at that, ICD in the Soret band region due to complexation with saccharides displayed a characteristic pattern for each studied saccharide. The ICD was caused probably by the perturbation of the Soret transition of the porphyrin and sensitively reflected the porphyrin–saccharide interaction modes. The boronic acid derivatives of zinc-porphyrin sensitively recognize monosaccharides, D-glucose, D-fucose, D-arabinose[149,159], D-lactulose[150], stereoisomers of fructose, xylose and threitol[52]. The sign of exciton CD couplet can be correlated with the angle between the two porphyrin molecules in the complexes depending on the absolute configuration of a monosaccharide.

The nonempirical method utilizing zinc porphyrin tweezers as chromophoric hosts for determining the absolute configuration of primary amines was described by Huang *et al.*[114,160]. Absolute stereochemistry of the monoamines can be determined from the sign of the CD couplet of the amine–porphyrin complexes. Bis(Fe(III)-porphyrin) tweezers were used for the selective and sensitive detection of saccharides[161] (glucose, galactose) by CD spectroscopy, which also enables determination of these saccharides in concentration of about 10^{-5} mol l^{-1}. The tweezer approach is very sensitive technique for configurational assignment of diamines and can be extended to other classes of substances containing one amino group (*e.g.* amino acids, amino alcohols). The absolute stereochemistry of the monoamines, amino acids and amino alcohols can be determined from the sign of the circular dichroic couplet of the complexes[114].

Borovkov *et al.* studied achiral syn-folded conformer of ethane-bridged bis(Zn(II)-porphyrin)[162], which subsequently transforms into the chiral extended anti form in the presence of the achiral alcohols[163] and enantiopure alcohols or amines[164] upon lowering temperature from 293 to 183 K. The

mechanism of the supramolecular chirality induction is based upon the formation of right- or left-handed screw diastereomers of the anti form.

For chiral recognition of the racemic phosphine derivatives[165] can be used Ru(II)-porphyrin leading to the formation of one of several possible diastereomers with high enantioselectivity.

The inclusion complexation of α-, β- and γ-cyclodextrin and their derivatives with *meso*-tetrakis(4-sulfonatophenyl)porphyrin (TPPS) in aqueous alkaline solutions was studied by spectroscopic methods[166,167], among others by ECD. α- and β-Cyclodextrin form 1 : 1 and 2 : 1 host/guest inclusion complexes with TPPS. γ-Cyclodextrin forms 1 : 1 complex with TPPS. An induced CD spectrum of TPPS in the presence of α- or β-cyclodextrin exhibits negative sign, whereas that in the presence of γ-cyclodextrin exhibits positive sign, indicating different inclusion modes of α-, β- or γ-cyclodextrin.

Relationship between the molecular components of basic building blocks of the photosynthetic apparatus – porphyrin–protein complexes – is assumed to ensure their proper biological functions, especially for pigments involved in primary photophysical processes of photosynthesis[94,168,169]. Interactions between cationic or anionic porphyrins and polypeptide templates with opposite charges have been extensively investigated for their possible applications in biomedicine and biotechnology (*e.g.* photodynamic therapy of cancer[95]). Analogous systems are important as models for DNA and protein recognition by porphyrins[144] and sapphyrins[170], one of the most important processes in nature.

Spectroscopic characterization of metalloporphyrin dimers containing an amino acid bridge, as a model of porphyrin/protein complexes[171], and characterization of configuration of chiral diamines[148], amino acids and alcohols through complexation with porphyrins was published[114]. Self-assembly of porphyrins on polypeptides and nucleic acids[143], metalloporphyrin–polynucleotide interactions[172] and supramolecular aggregates of porphyrins were used for determination of DNA[144]. Novel cationic *meso*-tetraphenylporphyrins (*para*-substituted with $-CH_2(\text{pyridinio})^+$ and $-CH_2N^+(CH_3)_3$ groups) form stable complexes with calf thymus DNA and nucleotides, association constant about 10^6 l mol^{-1} for DNA and about 10^3–10^4 l mol^{-1} for some nucleotides was found[75]. Also in study of B-DNA binding with Co(III)-, Pt(II)-, Cu(II)- and V(II)-tetrapyridylporphyrins was used the ECD and MCD spectroscopy as a powerful tool for description of orientation porphyrin molecules in the major groove of DNA[173,174].

Nezu *et al.*[141,142] reported studies of interactions of anionic TPPS with cationic poly(L-lysine) (PL) and tetravalent cationic *meso*-tetrakis-(1-methyl-4-pyridyl)porphyrin (TMPyP) tetratosylate with poly(L-glutamic

acid) (PLGA) in aqueous solutions using ECD spectroscopy. Additionally, several spectroscopic measurements of poly- and oligopeptide complexations with various porphyrin derivatives were described[94,144,145,168]. All these studies demonstrated that water-soluble porphyrin derivatives were bound to the α-helical[145], β-form[142] and randomly coiled[141] polypeptides in aqueous solution simply electrostatically. Induced CD of porphyrins in the Soret region was observed due to this interaction and the induction of tetraphenylporphyrin optical activity in ECD of the Soret band indicates large changes in the structure of electronic levels of porphyrin bound to oligopeptides[134,168]. It was proposed[142,145] that TMPyP ions bind to the α-helical PLGA as a monomer, while TPPS ions were in pairs on the α-helical PL, *i.e.* two ions bind consecutively and dissymmetrically. Therefore the mode of interaction of TPPS molecules with the random coil form of PL differs from that in the PLGA–TMPyP system[145].

While the interaction effects in porphyrins were studied in detail[94,141,142,144,145,168], their influence on the peptide matrices was less obvious and has been investigated mostly in the last decade[175,176]. VCD spectroscopy and *ab initio* computational analysis were used for conformational study of oligopeptide complexes with TPPS (refs[108,109]). Although the induced chirality of the peptide–porphyrin complexes can interfere with the VCD of peptides, the magnitude and frequency range of possible VCD signal of the TPP chromophore was simulated and no significant contribution to the VCD signal in the amide I′ from the TPP residue was found in theoretical and experimental spectra (Fig. 22, spectrum *4*). Conformational dependence of vibrational spectra was compared for absorption, Raman and VCD intensities. It may be expected that the TPP moiety can be used as a probe in the mid- and lower-frequency range in VCD experiment and, preferably, in Raman optical activity studies of peptide–porphyrin complexes. Thus, possible peptide conformational changes occurring during the complexation can be monitored directly in the amide I′ region. Moreover, a comparison between the simulated and experimental spectra suggests possible conformational transition of the peptide chain during the complex formation in dependence on the length of peptide chain, which is a crucial parameter for conformation of peptide–porphyrin aggregates[109]. While the conformation of PL containing hundreds of amino acid residues in the chain was preserved in the presence of TPPS, in the case of oligopeptides L-lysyl-L-alanyl-L-alanine (KAA), (L-lysyl-L-alanyl-L-alanine)$_2$ (KAA)$_2$ and PL containing up to tens of residues, the observed spectral changes of VCD pattern in the amide I′ were interpreted as a partial conformational transition of peptides[108,109] from a random coil (Fig. 22, spectrum *1*) to the

β-sheet structure (Fig. 22, spectrum *3*). For PLGA–TMPyP systems[108], the conformation change was not observed in aqueous solution for various lengths of PLGA chain (Fig. 23).

4. OLIGOPYRROLE MACROCYCLES AS MOLECULAR BUILDING BLOCKS OF SELF-ASSEMBLED MONOLAYERS

4.1. Introduction

So far, molecular recognition has been mostly studied in solution. However, very important recognition and assembling processes in nature take place on interfaces. Artificial preparation of monolayers of various molecules (receptors, hosts) on suitable surfaces creates appropriate starting conditions for detailed studies of important recognition and assembling processes taking place on the interfaces. The immobilized molecules are able to interact more or less selectively with different substrates (guests, analytes) present in solution. Thus, the preparation of such monolayers opens many interesting alternatives for development of very sensitive and selective devices, such as sensors, ion-selective electrodes, based on molecu-

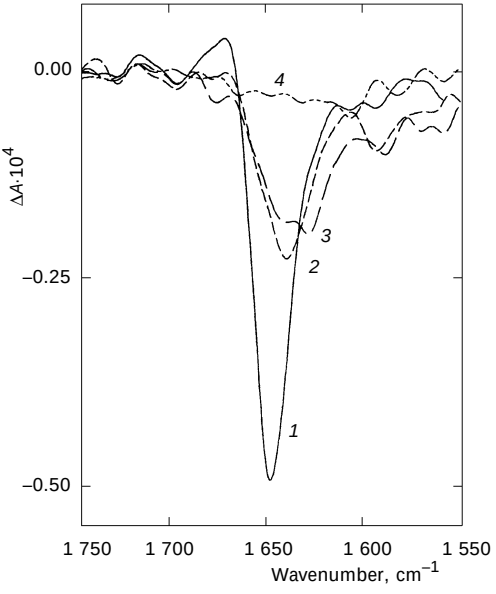


FIG. 22

VCD spectra of KAA with TPPS (c_{KAA} = 0.14 mol l^{-1}) in the amide I′ region. c_{KAA}/c_{TPPS}: pure KAA (*1*); 2 (*2*); 1 (*3*); pure TPPS (*4*)

lar recognition processes localized on a specific interface. There are several approaches to modeling and studying interface recognition processes. Preparation of well-ordered monolayer films formed by adsorption of long-chain alkanethiols from solution on the gold surface is a well-established procedure[177,178]. The resulting self-assembled monolayers (SAMs) provide a useful tool to study molecular recognition events because of their relatively simple characterization and facile chemical modification.

This part of the review is mainly focused on the synthesis, characterization and applications of porphyrin and metalloporphyrin monolayers formed on solid surfaces.

4.2. Preparation of Monolayers

Generally, there are two strategies established for the preparation of functional SAMs chemisorbed on solid support:

1. One-step approach, *i.e.* spontaneous adsorption of a compound containing a group(s) capable of binding to the surface.

2. Two-step and/or multistep approach, *i.e.* spontaneous adsorption of bifunctional molecule (spacer) in the first step followed by either a direct

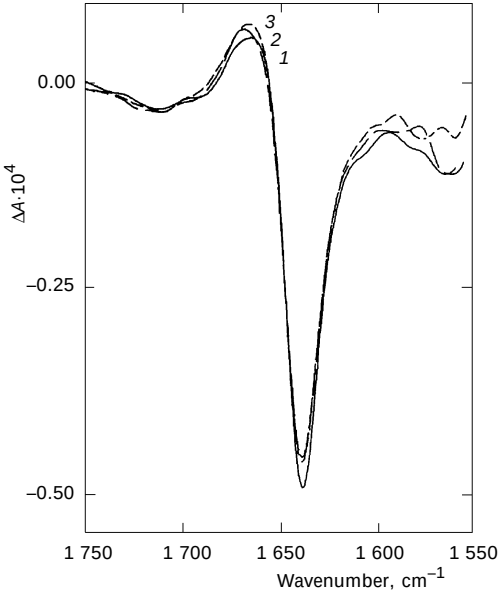


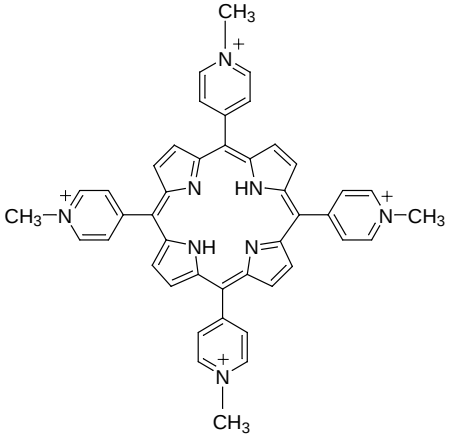
FIG. 23

VCD spectra of PLGA (M_W = 54 400) with TMPyP (c_{PLGA} = 0.50 mol l^{-1}) in the amide I′ region. c_{PLGA}/c_{TMPyP}: pure PLGA (*1*); 50 (*2*); 14 (*3*)

derivatization reaction leading to the desired monolayer or one or more consecutive reactions finally giving the functional SAM attached to the spacer monolayer.

4.3. Monolayers of Porphyrins

Spontaneous adsorption of a cationic porphyrin **45** on the silica surface is one of the simplest methods of immobilization of porphyrin on the solid surface[179]. For molecular imaging, porphyrin derivatives **46**, **47** were pre-



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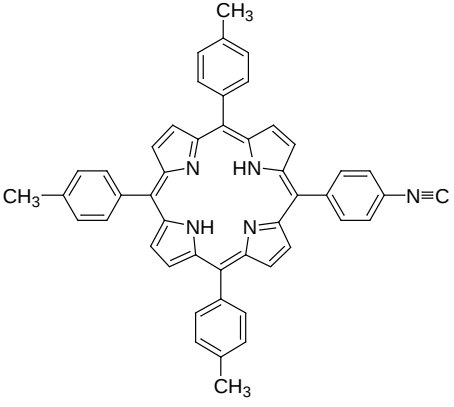

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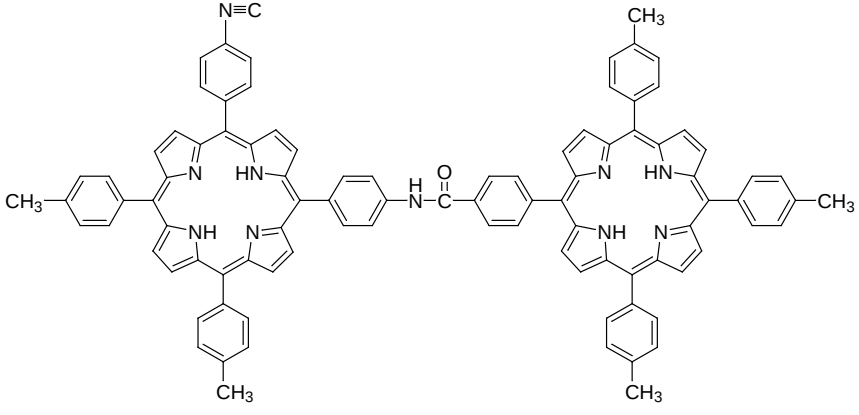


47

pared with pendant isocyano groups, which react with gold metal to fix the macrocycle on the gold surface[180]. SAMs of porphyrins were prepared by self-assembling thiol or disulfide derivatives[181,182]. Porphyrins with disulfide-containing straps possess greater air stability than their dithiol analogues previously prepared[183]. There are two ways leading to a sulfur-derived porphyrin. The first approach is based on the derivatization of a substituted porphyrin with a thiol or protected thiol[184–186], the other is the application of an aldehyde precursor in porphyrin synthesis, which contains thiol unit (ref.[181] and references therein). The attachment mode of a given macrocycle, *i.e.* the number and location of side chains introduced in order to anchor the macrocycle to the substrate, governs the structure and properties of the resulting SAM.

Many of the prepared organized SAMs derived from porphyrin macrocycles were used for photochemical studies[187–189]. In these works, the effect of the chain length of a linker, *i.e.* the molecule connecting thiol group to the porphyrin moiety, was discussed. Systems of porphyrin-ferrocene-thiol linked molecules were used for the construction of a stable and efficient photoconversion device where uphill photoinduced electron transfer takes place[190]. A similar system containing fullerene unit has been recently published[191,192].

Another method to obtain monolayers of oriented porphyrins exploits self-assembly on a pretreated substrate. Successful preparation of a covalently bonded self-assembled porphyrin monolayer was performed on surface-modified fused quartz, silicon substrate having a native oxide layer and gold layer (Fig. 24). This procedure was employed for the synthesis of tetra(4-pyridyl)porphyrin monolayer **48** (ref.[193]). Similarly, the formation of covalently linked SAMs of natural vinyl- or 2-hydroxyethyl-substituted protoporphyrin (**49**) and hematoporphyrin on thiolated silanized quartz

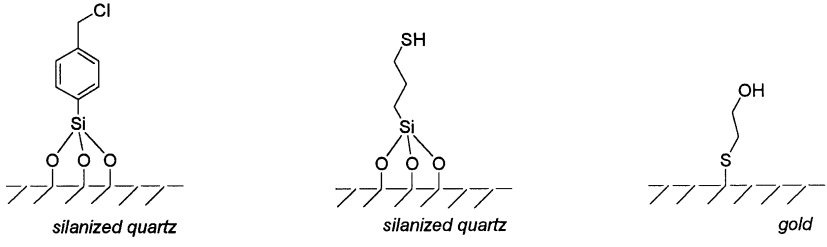
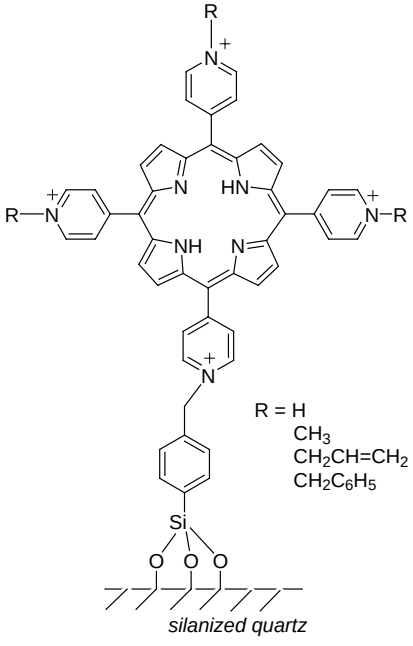


FIG. 24
Functionalization of surface for derivatization in the next step

was published[194,195]. Reportedly, the reactions used allow the monolayer formation in aqueous media at neutral pH and room temperature.

In our approach, we used the two-step synthetic strategy where the adsorption of 2-sulfanylethanol on gold surface is followed by the reaction of free hydroxy groups with mono- and dicarboxylic acid of tetraphenylporphyrin and sapphyrin (Fig. 25)[196]. The resulting monolayers were characterized by spectroscopic and electrochemical methods and the functionalities of SAMs containing porphyrin derivatives were tested by a study of interaction with aromatic compounds.



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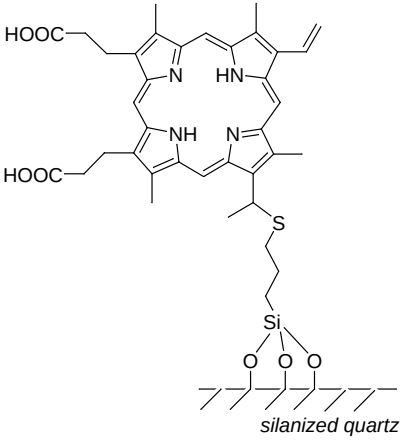

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Despite the fact that the covalent binding of carboxyl derivatives of porphyrins to amino silica gel cannot be considered exactly a self-assembly process, such modification also leads to well defined organized monolayers. This technique is mainly used for the preparation of porphyrin-modified sorbents for liquid chromatography[197–200] and is discussed in the HPLC part of this review in more detail.

4.4. Monolayers of Metalloporphyrins

The introduction of various metal ions into porphyrin core, *i.e.* metallation of porphyrins, strongly affects and changes porphyrin properties. A new binding site capable of axial coordination could be employed in the construction of SAMs. Axial coordination was used for the formation of monolayers of metalloporphyrins where the orientation of the porphyrin

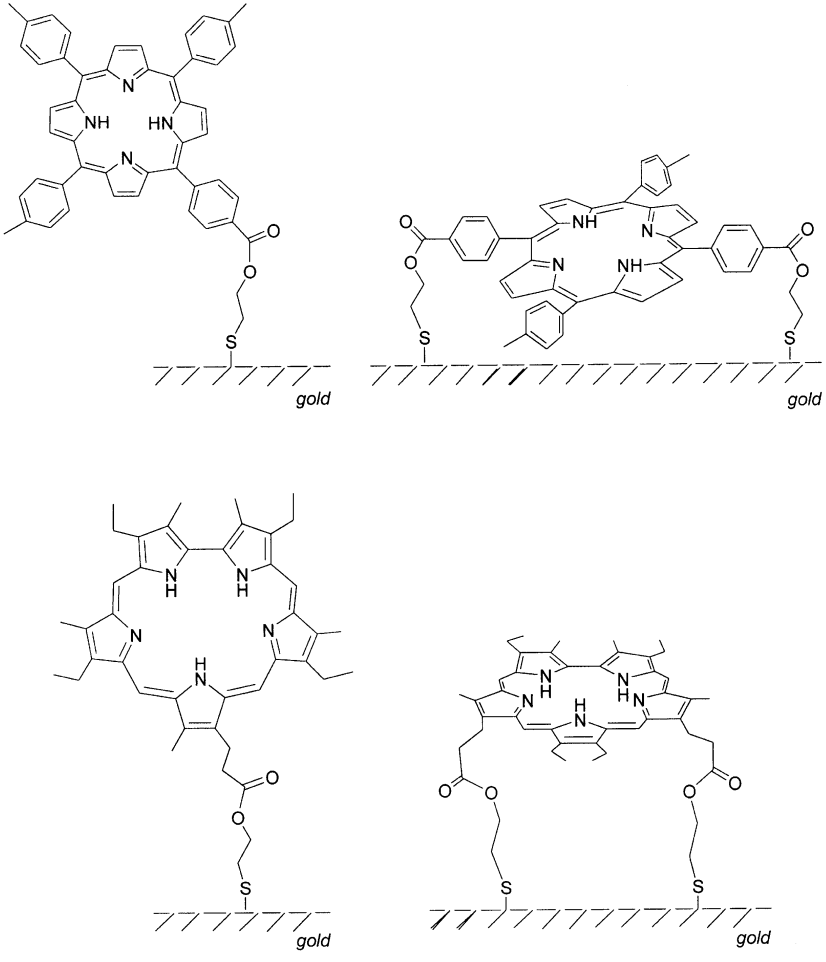
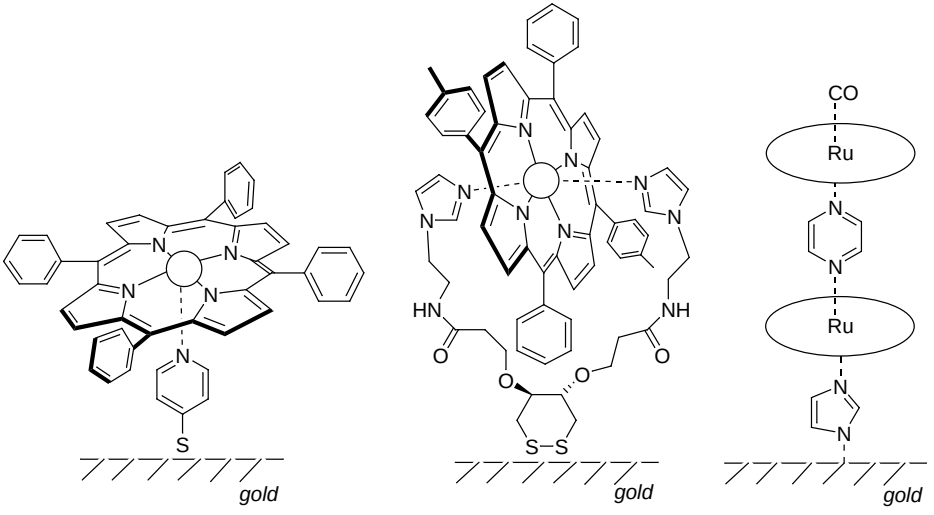


FIG. 25
Mono- and difunctional porphyrin and sapphyrin derivatives immobilized on gold surface

core *versus* the surface varies. The preparation of SAM of pyridine-4-thiol[201,202], 4-aminobenzene-1-thiol[202] and coordination of metalloporphyrin gives coplanar conformation of the surface and the porphyrin core (**50**). On the other hand, the synthesis of SAM of bis-imidazole derivative[202,203] and the coordination in the next step results in perpendicular orientation of the porphyrin core (**51**). The construction of SAMs of different pyridine derivatives allowed the formation of the monolayer of a metalloporphyrin coordinated to the nitrogen of pyridine ring of various orientations[204]. The introduction of Ru(II) ion in the porphyrin core enables the formation of organized multilayers (**52**). On the basis of this metalloporphyrin, stacked multilayers bound to an electrode *via* specific axial ligation to SAM were prepared[205,206].



50 **51** **52**

Coordination of amino group to Zn(II) ion was used for the formation of a porphyrin monolayer film by axial ligation of Zn(II)-protoporphyrin IX to an amino-terminated silanized glass surface[207]. However, porphyrins are not only able to coordinate metal ions. The phosphorus center was introduced by refluxing tetraphenylporphyrin with phosphorus oxychloride[208]. The axial chloride ligands on phosphorus were replaced by 2-sulfanylethoxy group. This derivative forms monolayer with coplanar orientation of the porphyrin core.

Monolayers of metalloporphyrins with different orientation to the surface, where metal center is free for interaction with analytes, were prepared by self-assembly of thiol derivatives and their interesting distinct behavior was studied[185,186,209] (Fig. 26). Organization of porphyrin molecules on the surface, which results from different techniques of anchoring a given macrocycle or from various substituents in porphyrin core, strongly affects the behavior of the prepared monolayer. The influence of the substituents of the porphyrin core was demonstrated in the case of various Co(III)-porphyrins adsorbed on graphite electrodes[205].

First studies of the preparation, structure and stability of SAMs showed that SAMs could be deposited on the solid surface also by UV irradiation[210] or by heating in organic solvents[177]. Besides, a post-assembly insertion of metal ions into thiol-derivatized porphyrin monolayers on gold was published and no damage of the monolayer was observed during the process which included refluxing in organic solvents[211].

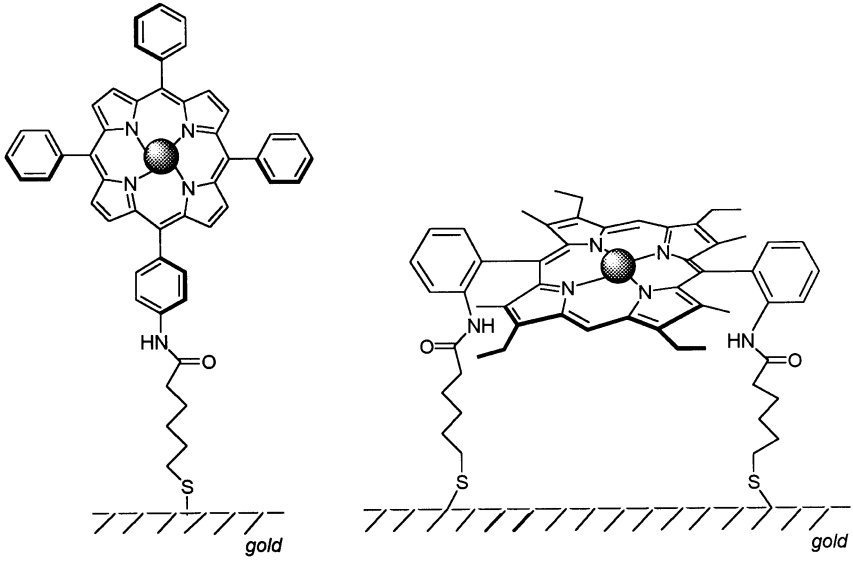


FIG. 26
Different orientation of porphyrin cores controlles by a number of thiol groups in structure of porphyrin derivative

4.5. Methods of Analysis

There are many spectroscopic methods used for analysis of monolayers (for a detailed survey, see *e.g.* ref.[178]). Vibration spectra of SAM can be measured by surface-enhanced Raman scaterring spectroscopy (SERS) or infrared reflection absorption spectroscopy. SERS is a very effective technique for structure determination of layers fixed on metal surfaces[210,212,213] (*e.g.* Au, Ag, Cu). The interaction of excitation laser beam with surface of noble metals is accompanied by excitation of surface plasmons. Surface plasmon waves cause an enormous (usually $\approx 10^6$-fold) enhancement of the Raman scaterring efficiency for molecules located near the surface[214,215].

Infrared reflection absorption spectroscopy (IRRAS) provides the same type of information as common infrared spectroscopy. IRRAS using linear polarized light (especially *p*-polarized) at grazing incidence is a widely used method of studying thin films deposited on metallic substrates[216]. Currently, polarization modulation of the incident electromagnetic field is used to increase the sensitivity and the in situ experimental ability of IRRAS (ref.[217]).

After the formation of a porphyrin monolayer on glass micas, UV-VIS and fluorescent spectra can be measured. Several approaches are used to obtain SAM on a transparent support. First, self-assembling of thiol derivatives on thin gold layer is used. A gold layer is still transparent up to ≈60 nm thickness, hence UV-VIS spectra can be measured[202,209,211]. In this technique, problems come from absorption of a bare gold layer, which gives a relative high background. The other approach is based on thiol-silanized quartz slides, which can be derivatized to form porphyrin monolayers[194]. In this case, the absorption of monolayer is of the order of 10^{-3}.

Surface plasmon resonance (SPR) method is a surface-sensitive spectroscopic technique, which can be used to characterize variety of ultrathin films[43]. This method is often used for the construction of sensing and multisensing devices[218]. The application of macrocyclic receptors to binding of neutral analytes using SPR method is, of course, feasible[219].

There are also many different electrochemical techniques successfully applied to investigation of structure properties of monolayers[178], of which cyclic voltammetry is most commonly used in the case of metal surface monolayers. Using this method, desorption-absorption analysis of SAMs can be made and reducing-oxidative properties studied[220–223].

5. HPLC APPLICATIONS OF OLIGOPYRROLE MACROCYCLES

Complexation abilities of oligopyrrole compounds have been utilized in many high-performance liquid chromatography (HPLC) applications. Complexes of porphyrins with metals can also be easily separated by HPLC and detected in the UV-VIS region. These topics have been recently reviewed in detail[15,224,225].

Oligopyrrole compounds have been immobilized on silica matrixes by physical sorption or covalent linking. Resulting chromatographic phases have been successfully used for separation of many different analytes under normal- or reverse-phase conditions. Oligopyrrole phases provide more types of interactions (*e.g.*, hydrophobic, π–π, dipole–dipole, Coulombic, *etc.*) than common chromatographic phases. Assertion of the above mentioned interactions is driven by the shape of a receptor, type of central ion, present substituents and many other parameters.

Meyerhoff and Kibey[198,199] used immobilized TPP **53** and metallated TPP for HPLC separations. They showed that porphyrins and metalloporphyrins covalently linked to a silica matrix exhibit high selectivity to planar aromatic solutes present in the mobile phase. Higher retention of planar *versus* non-planar aromatic solutes on these sorbents may reflect a more extensive π–π overlap of planar aromatics with porphyrin macrocycles. Metallation of an immobilized porphyrin macrocycle alters the electron density and, consequently, influences the π–π interactions and selectivity of separation[198–200].

Meyerhoff and Chen[200] studied retention of polycyclic aromatic hydrocarbons (PAHs) on metallated protoporphyrin (ProP) phases. They studied effects of the metal ion and the amount of the immobilized receptor on the retention and resolution of selected analytes. High retention factors and better selectivities were obtained for Cu(II)-ProP than for the other metallated ProP. Electron configuration with Cu(II) prefers square-planar ligation. The authors assume that this fact affects stronger interaction of planar *versus* non-planar PAHs. In addition large polycyclic aromatic hydrocarbons were separated on Cu(II)-phthalocyanine modified anion-exchange resin[226]. Various PAHs were separated on aminopropyl silica modified with metallated phthalocyanines[227].

Other π-electron-rich analytes, fullerenes, were also successfully chromatographed. Meyerhoff *et al.*[228,229] separated C_{60}- and C_{70}-fullerenes on TPP, Zn(II)-TPP and In(III)-TPP stationary phases, respectively. Resolution varied significantly with changes in the mobile phase composition and in the metal ions incorporated in the porphyrin macrocycle. Meyerhoff *et al.*

used a novel immobilization method for the preparation of TPP phases with high selectivity for fullerenes ($\alpha_{C70/C60}$ = 7, in toluene). 5-(*p*-Hydroxyphenyl)-10,15,20-triphenylporphyrin was immobilized *via* reaction with glycidoxypropyltrimethoxysilane[230]. Also Guiochon and co-workers[231] were interested in the separation of C_{60}- and C_{70}-fullerenes on the TPP phases. The authors optimized their semipreparative process using 1-methylnaphthalene as the mobile phase. For the same purpose, Gumanov and Korsounskii[232] prepared sorbents based on TPP and Al(III)-TPP. The latter stationary phase was successfully used for large-scale separations.

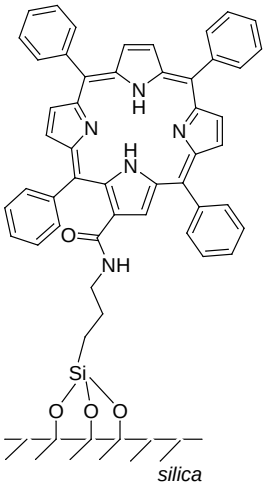
Immobilized metalloporphyrins are used not only in the reverse-phase (RP) HPLC mode, but also in the ion-exchange chromatographic (IEC) arrangement. TPP and metallated TPP phases have been used for the separation of aromatic sulfonates and aromatic heterocycles[199]. Meyerhoff and co-workers separated peptides and amino acids on TPP- and ProP-modified silica[233,234]. Aromatic amino acids and peptides containing aromatic acids were retained more strongly than aliphatic amino acids. Undoubtedly, the π–π overlap contributes to the retention of aromatic analytes.

Biesaga *et al.*[235] separated mixtures of dipeptides and tripeptides containing tyrosine. As a stationary phase, aminopropyl silica with covalently linked TPP was used. The effect of metallation of porphyrin with Cu(II) and Zn(II) on retention was investigated. The observed separation is based on a mixed mechanism involving π–π and hydrophobic interactions as well as complex formation between immobilized metal ions and peptides.

Stationary phases based on other oligopyrrole macrocycles have been also prepared. By Sessler's group, calixpyrroles and sapphyrins covalently bound to silica were intentionally designed for separation of anionic solutes[197,236–242]. Calix[4]pyrroles immobilized on silica (**54**, **55**) were successfully used for the separation of various inorganic and organic anions, amino acids, oligonucleotides and polyfluorinated biphenyls[240].

In the case of sapphyrin linked to aminopropyl silica (**56**), the key mode of interaction involves specific chelation of the positively charged sapphyrin core with oxoanions, *e.g.* phosphates[239,242]. Cytosine substituted sapphyrin immobilized on silica (**57**) was used for the separation of mono-, di- and triphosphate nucleotides[238].

In our group, the retention behavior of nucleobases and nucleosides on porphyrin-, metalloporphyrin- and sapphyrin-modified chromatographic stationary phases was studied[239]. Sorbents were characterized by Raman spectroscopy and elemental analysis. UV-VIS and ^{1}H NMR titration experiments were employed to study the role of macrocyclic receptors in selective recognition of adenine, cytosine, thymine and uracil, and related nucleo-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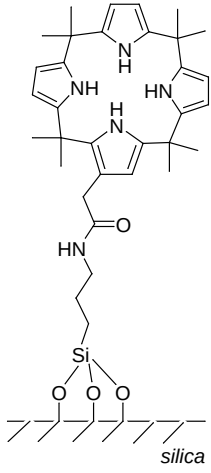
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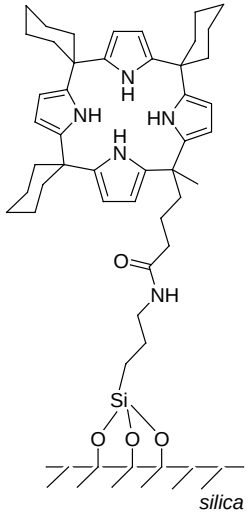
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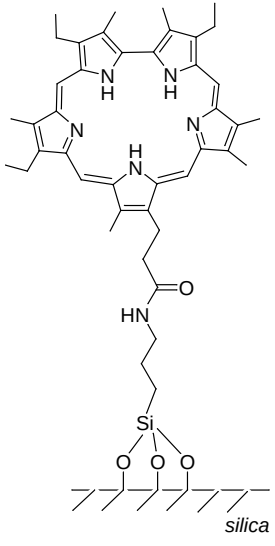
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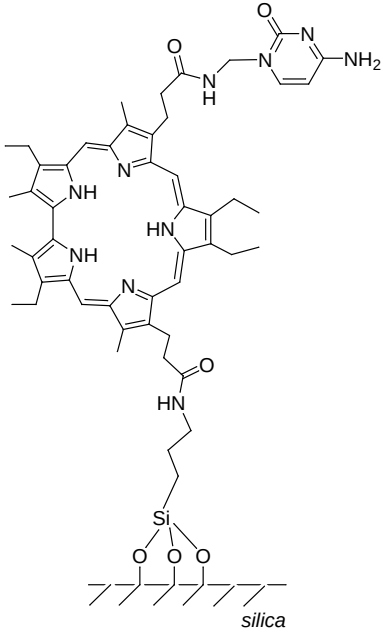
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sides. Using metalloporphyrins and sapphyrins as modifiers of chromatographic phases led to an increase of retention of analytes. This phenomenon can be explained by the fact that sapphyrin aromatic core contains more π-electrons than porphyrin (22 *vs* 18). Retention of aromatic compounds on metaloporphyrin phases was discussed elsewhere[235]. The most retained analyt from the above mentioned was adenine. The strongest π–π interactions were expected, because adenine contains the biggest aromatic moiety.

Chromatographic stationary phases based on oligopyrrole macrocycles have been found usable for separation of neutral and charged aromatic compounds, metal ions and inorganic and organic anions. Application of even more complex macrocyclic structures immobilized or covalently linked to a suitable supporting stationary phase is envisaged in the near future. Those phases will provide higher specifity for target molecules including chiral recognition.

6. ELECTROANALYTICAL CHEMISTRY

6.1. Introduction

The development of new and more efficient methods of real-time monitoring of chemical and biochemical analytes by means of sensor technique ranks among the most significant challenges faced by modern science. The nature of the component used to generate the diagnostic signal is fundamental for the overall performance of any chemical sensor. It defines characteristics of a device, namely its selectivity, sensitivity, lifetime and response time. The role of a sensing agent in a chemical sensor is to provide a transduction mechanism, which enables an analytical signal to be obtained. In electrochemistry, potentiometric and/or voltametric-amperometric methods were used to study of interactions of oligopyrrole macrocycles.

Voltammetric techniques generally display good concentration sensitivity but low selectivity. However, selectivity of electrodes can be improved by their chemical modification. Modification process can be realized *via* mechanically prepared layer of porphyrin, chemically bonded porphyrin or electrochemically deposited porphyrin layer. Conductivity of a porphyrin layer on the electrode surface is high because translation of charge *via* system of π-electrons is facile. Significant role in electron transfer plays a central metal atom in porphyrin skeleton[243–246]. Prepared electrodes show

catalytic and redox properties, which can be exploited to increase selectivity of their voltammetric-amperometric response.

Oligopyrrole macrocycles have been also developed as prospective selective sensing agents for potentiometric sensors, more popularly known as ion-selective electrodes (ISEs). ISEs belong to important tools in analytical chemistry. Among the other things their development has significantly stimulated progress in the field of molecular recognition. In supramolecular chemistry, the analogy between abiotic synthetic molecules and biological receptors has often been seen in the selective recognition of a given guest molecule only. However, this recognition process is only the first step in the cascade of events triggered by biological receptors. ISEs provide a more active means of studying molecular recognition phenomena. Therefore ISEs can serve as a new important tool for shaping our thinking in the field of biomimetic receptors. In this part of the review, we will concentrate on molecular recognition phenomena involving oligopyrrole macrocycles for construction of selective electrodes, specifically those based on polymeric matrix liquid membranes and electropolymerized film electrodes.

There are two generalized modes of signal transduction that may be induced by host-guest complexation on the membrane surface: namely, the membrane potential change and the membrane permeability change. Interestingly, in the case of ion-selective electrodes mostly the induced membrane potential change has been used as a mode of signal transduction. In the case of membrane potential changes, there are several principles established for discrimination of organic substrates by host-guest complexation at membrane surfaces[247–249]. The first principle (1) involves potentiometric discrimination based on hydrogen bonding, the second (2) charged group interaction with specific functional groups present in substrates and the third principle (3) involves potentiometric discrimination based on shape recognition arising from steric interaction between targeted substrates and receptor. Additional sources of discrimination include those based on lipophilicity[250–252], chirality[253–255] and axial ligand binding[256].

A number of ISEs based on polymeric matrix liquid membranes have been investigated. Many of them showed high selectivity to particular target substances and are now commercially available[257–263]. In most cases an organic liquid membrane contains a hydrophobic host molecule generally supported by a polyvinyl chloride (PVC) matrix. This combination produces a liquid membrane/aqueous phase interface, where the charge distribution is modulated by guest binding.

Selectivity of anions on ISEs is usually exclusively driven by their lipophilicity, such selectivity pattern is called Hofmeister series[264,265]. ISEs

with non-Hofmeister behavior for anion binding are of special interest. An important class of molecules that has been employed as active membrane components in liquid/polymeric anion-selective electrodes[266–272] is represented by metallated porphyrins. Metalloporphyrin-based devices display anion selectivity that differs significantly from selectivity of conventional anion-selective, ion-exchange electrodes derived from quaternary ammonium salts[273]. Exact explanation of mechanism of anion response for the porphyrin-derived electrodes is still a subject of debate. Moreover, the mechanism could vary depending on the nature of the porphyrin species doped into membrane[274]. Nonetheless, several reports indicate that anion selectivity patterns are dictated by the relative strength of interaction of anions as axial ligands with the metal center of the metalloporphyrin[267,273], as well as by structural influences from the surrounding porphyrin macrocycle[275–278].

6.2. Voltammetry

Tetraphenylporphyrin surface modified glassy carbon electrode was used for anodic stripping voltammetry of heavy metals[279]. Better sensitivity to copper was found in comparison to bare electrode. The influence of layer thickness, pH and accumulation time was also investigated. Malinski *et al.* described the use of demetallated polymerized pophyrin film for preconcentration and determination of Ni (ref.[271]). This method was also applied for the study of Ni behavior in a single cell and a comparison with another methods was provided[280,281].

Catalytic properties of metalloporphyrins and their ability to detect nitric oxide in low concentration were described. Nitric oxide is a small molecule with a very important role in living organism. Malinski *et al.* descibed exploitation of polymerized film of Ni(II)-tetrakis(3-methoxy-4-hydroxyphenyl)porphyrin (NiTMHPP) prepared on carbon electrode for the detection of nitric oxide[272]. Synthesized polymer was covered with Nafion layer. This polyanion worked as a filter for interfering anions, such as nitrites. Influence of polymerized film thickness[282] and the application of some other “filtering” materials than Nafion were studied[283]. Cizsewski *et al.* found catalytic properties also for demetallated porphyrin film[284]. Electrodes based on porhyrins with Fe as a central atom *e.g.*, hemoglobin[285], hemin, hematin, Fe(III)-*meso*-tetrakis(*N*-methyl-4-pyridyl)porphyrin (FeTMPyP) and FeTPPS (ref.[243]), and Ni as a central atom, poly(Ni-phthalocyanine) with structure similar to porhyrins[286], were employed for the determination of nitric oxide. A critical comparison with some other

electrodes designated for the detection of nitric oxide was given[287,288]. On the contrary, Lantoine *et al.* did not find catalytic effect of porphyrin film[289]. Nevertheless, polymerized porphyrin electrodes were studied as sensors for nitric oxide [290] and microelectrodes on carbon fiber were successfully used for the determination of nitric oxide in living organisms, biological materials and single cells[287,288,291–294].

A sensor based on Co-porphyrin was described for simple analysis of halogenated hydrocarbons in water without preliminary oxygen elimination[263]. Electropolymerized films derived from protoporphyrins with Ni, Co and Cu as central ion were presented as suitable sensors for the detection of phenols[295]. Interaction of phenol with porphyrin film *via* phenolic hydroxy group was confirmed by spectroscopy. An electrode modified with poly(NiTMHPP) was used for the determination of methanol in solution[244]. The kinetics of formaldehyde oxidation was studied with the same electrode[296]. Sugawara *et al.* used a graphite paste electrode modified with CuTPP for sugar determination by cyclic voltammetry[297].

Finally, porphyrins have been also used for the indirect determination of DNA. Qu *et al.* tested complexation of TMAP with DNA by means π–π interactions and he found that the resulting complex was electrochemically inactive[298]. This property was utilized for the determination of low quantity of DNA in solution. Cyclic voltammogram of Cu- or Ni-porphyrin solution was measured. Decrease of porphyrin peak after the addition of DNA corresponds with the amount of DNA[299,300].

6.3. Amperometry

Electrodes modified with porphyrin can be employed as selective amperometric detectors. Carbon electrode modified with zinc and cobalt metalloporphyrins was used as a detector in flow injection analysis (FIA) measurement of sulfites and nitrites[301]. Araki *et al.* used graphite electrode modified with Co(II)-*meso*-tetrakis(4-pyridyl)porphyrin (CoTPyP) as a selective sensor for the same purpose[302].

Wu *et al.* electropolymerized Fe(III)-tetra(3-methoxy-4-hydroxyphenyl)-porphyrin (FeTMHPP) on the surface of Pt electrode and he used the resulting electrode as an oxygen sensor[246]. Similar oxygen sensors were prepared by making a thin polymeric film of β-cyclodextrin on platinum surface. Hydrophobic peripherally substituted porphyrins, CoTMPyP, CoTPP and CoTPPS were incorporated into cyclodextrin cavity. The prepared electrodes were used for oxygen detection[245]. Yuasa *et al.* used colloidal Co-porphyrins

covered with poly(vinyl alcohol) or poly(2-vinylpyridin) as oxygen sensors[303].

Electrodes for the determination of biologically important analytes can be prepared by combination of enzymes with an oxygen sensor. Ti(IV)-porphyrin was used for the detection of hydrogen peroxide generated from oxalate in column modified with immobilized oxalate-oxidase in FIA measurement[304]. Dong and Kuwana prepared amperometric sensor of glucose based on a mixture of CoTMPyP with Nafion covered with glucose-oxidase layer[305]. Similarly, acetylcholine was determined by this electrode covered by acetylcholin-esterase[306]. Oyama *et al.* described a glucose electrode based on poly(Co-*meso*-tetrakis(*o*-aminophenyl)porphyrin) (poly-(CoTAPP)) covered with glucose-oxidase[307].

Guerra *et al.* fabricated a hydrazine sensor based on carbon paste electrode with CuTPP as modifier[308,309]. A glassy carbon electrode covered with Fe(III)-TPP for the detection of herbicide Propanil was described by Priyantha *et al.*[310]. Huang *et al.* used glassy carbon electrode modified by NiTAPP for the determination of acetaminophen[311].

Duong described the application of Zn(II) protoporhyrin for dopamine determination[312]. It was found that undesirable interference of ascorbic acid in the dopamine measurement could be significantly suppressed using a graphite electrode covered with electropolymerized film of protoporphyrin. Angnes used μ-*meso*-tetrakis(4-pyridyl)porphyrinate-Co(III)-tetrakis[bis(bipyridine)(chloro)-Ru(II)] complex for the detection of dopamine and NADH (ref.[313]). Kang *et al.* employed a similar structure of electropolymerized tetraaminophthalocyanatonickel(II) for the same purpose[314].

6.4. Potentiometry

Membrane electrodes doped with porphyrins or metalloporhyrins as active components show good selectivity to ions. Schulthess *et al.* determined changes in selectivity pattern for a membrane doped with vitamin B_{12} derivative possessing chemical structure similar to porphyrin[315]. This was the first electrode with selectivity pattern different from the Hofmeister series. Amman *et al.* tested other lipophilic amines including vitamin B_{12} derivatives and Co-porphyrins with various axial ligands and he found significant differences in selectivity patterns[266], the influence of plasticizers and metalloporphyrin concentration in membrane on the electrode response and selectivity was studied as well[316]. Hodinar and Yo described PVC membrane containing Co(III)-porphyrins with nitrite as axial ligand. They tested

the influence of pH and composition of membrane on response for nitrites[270,317].

The influence of a charge of a central ion in porphyrin on the properties of a prepared electrode and principles of ion exchange reactions have been studied[318]. Yoon *et al.* used MnTPP, MnEOP, InTPP and InOEP as modifiers of membranes designated for the determination of chlorides in blood serum[319]. Steinle *et al.* described ion selective electrodes based on Ga, In and Tl porphyrins in silicone rubber matrix[320]. Ga-porphyrins showed a significant response to fluorides whereas In- and Tl-porphyrin electrodes were preferable for the detection of chlorides.

Different systems have been applied for various anions. Gao *et al.* used PVC membrane electrode modified with $(FeTPP)_2O$ for selective determination of thiocyanates and he studied transfer of thiocyanates through membrane by AC impedance measurement[321]. Chaniotakis *et al.* applied Sn(IV)-TPP to the determination of salicylates in human urine[269]. Antonisse *et al.* analyzed fluorides and nitrates by CoTPP in siloxane membrane electrode[322]. Sun *et al.* described silver electrode modified by aminothiol linked $TPPS_4$ for the determination of iodine in sea-grass and compared this method with spectrophotometric determination[323]. Amemiya described a PVC membrane electrode with TPP modified by urea (**58**) for the detection of acetates by means of hydrogen bonding[324]. Amini *et al.* determined histidine using a Mn(III)-TPP modified PVC membrane electrode[325].

Gupta *et al.* applied TPP and TMPP modified PVC membrane electrode for the detection of Ni(II) (ref.[326]). Jain *et al.* compared TPP and two different tetraazamacrocycles PVC membrane electrodes to the determination of Co(II) cations[327].

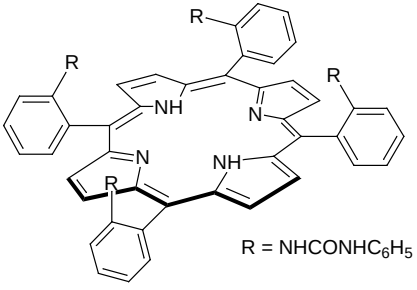
Ito *et al.* used lipophilic amines including sapphyrin as modifiers of PVC membrane and he found significant response of this electrode to other neutral phenols[328]. Lin *et al.* prepared electrodes with sapphyrin **59**, rubyrin **60** and rosarin **61**, and he tested the electrodes for the determination of different organic anions[329].

In our group, we tested a PVC membrane electrode modified by *meso*-octamethylcalix[4]pyrrole for the detection of various halogenides and we also described the influence of pH on the response of the electrode[330]. At low pH the ISE displays strong anionic response to bromides and chlorides, in contrary, at high pH the electrode displays cationic response to these anions. Park *et al.* used ISE based on calix[4]pyrroles for the detection of Tl(I) cations[331].

Daunert *et al.* studied changes in selectivity pattern for an electrode based on Pt wire covered with electropolymerized CoDAPP (ref.[332]), Kliza *et al.* de-

tected iodides using SnTPP and TPP (ref.[333]). Yuan *et al.* described the application of a electropolymerized TAPP electrode as a pH sensor[334].

Volf *et al.* prepared an eletropolymerized DAPP electrode with selectivity to iodides and thiocyanates[335] and an electrode based on electropolymerized CoDAPP as a sensor for cysteine with detection limit 10^{-6} mol l^{-1} and selectivity coefficients relative to other aminoacids 10^{-2}–10^{-3} (ref.[336]). Here, the interaction between the central cobalt atom and sulfur of cystein controls the response of the electrode.



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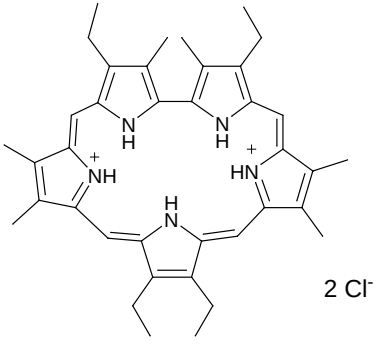

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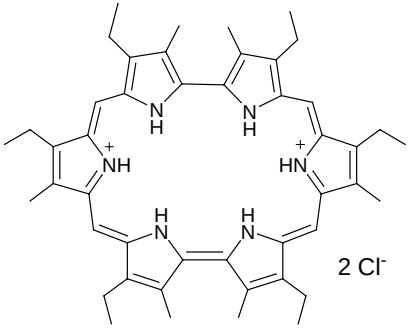

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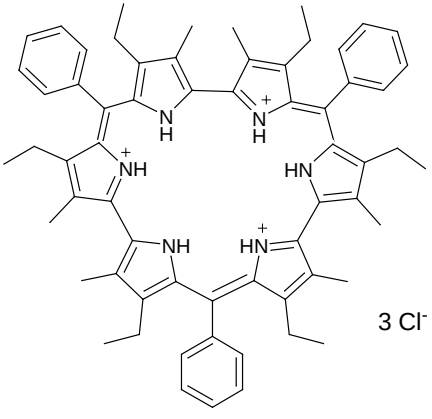

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7. CONCLUSIONS AND FUTURE TRENDS

In this review we selected and described the most important aspects of analytical applications of oligopyrrole macrocycles which have been reported so far. In this chapter we would like to give a brief outline of some future trends and analytical applications. Presently, it seems that there are two main areas of a great importance and interest; the first one is related with synthetic methodology for novel types of porphyrinoid receptors and goal-directed modification of existing oligopyrrole macrocycles, the second one is synonymous with nanotechnology and miniaturization of analytical devices and chemical sensors.

7.1. Novel Oligopyrrole Macrocycles

Besides porphyrins[337] and sapphyrins[338] widely discussed in the preceding parts, there are also some new members of a large family of oligopyrroles. Firstly, porphyrin isomers (where connection of four pyrrole units was altered going from original methine bridge in porphin to variety of combinations keeping the overall number of carbons constant) (Fig. 27) developed mainly by Vogel and Sessler can bring novel interesting applications to this area.

Structure of porphyrin can be modified by coupling with other heterocycles, *e.g.* thiophene, furan and even with larger heterocycles (see *e.g.* ref.[339] and references therein). Another area has been opened by the synthesis of more-than-four-membered pyrrole macrocycles: thus, sapphyrin or pentaphyrin (five-membered), rubyrin and hexaphyrin (six-membered) or even giant oligopyrrole macrocycles have been prepared[340]. Alternative ways of modification of a porphyrin motif have been associated with the synthesis of inverted (or *N*-confused) porphyrins which are largely studied in Kyoto's group[341–344] or modification of aromaticity of a macrocycle either by enlargement (*e.g.* refs[345,346]) or restriction (calixphyrins[7] or calixpyrroles[8,9]). All above mentioned synthetic methodologies lead to macrocycles with novel interesting spectroscopic properties, which will be examined for analytical applications in the near future[347,348]. For instance, a well-known anion-binding ability of sapphyrin[349] has been recently used for solvent extraction and fluorometric determination of fluorides at ppb level[350].

Despite interesting binding properties of oligopyrrole macrocycles, the specifity of the interaction between a receptor and an analyte of interest is still often rather limited. One possibility how to overcome this problem is

to combine several macrocycles in proper topology in order to get specific recognition properties for a given substrate. Beside synthetic methodology reported to date for construction of such macrocyclic arrays, there are several examples already known systems where analytical application is indicated, mainly for separation science and specific receptors for sensor application. Thus, variation of connecting units and number of macrocycles create tremendous potential, which can further extend known properties and use of individual macrocycles. Several following examples will probably represent leading trends in the future . Oligomers of porphyrins can be based on covalent[346,351–354], non-covalent[355] and coordination chemistry[356–359]. However, not only porphyrin macrocycles can be used in this way. Also expanded porphyrin a sapphyrin dimer[360] and oligomers[361] have been synthesized and multitopic interaction with anions have been studied.

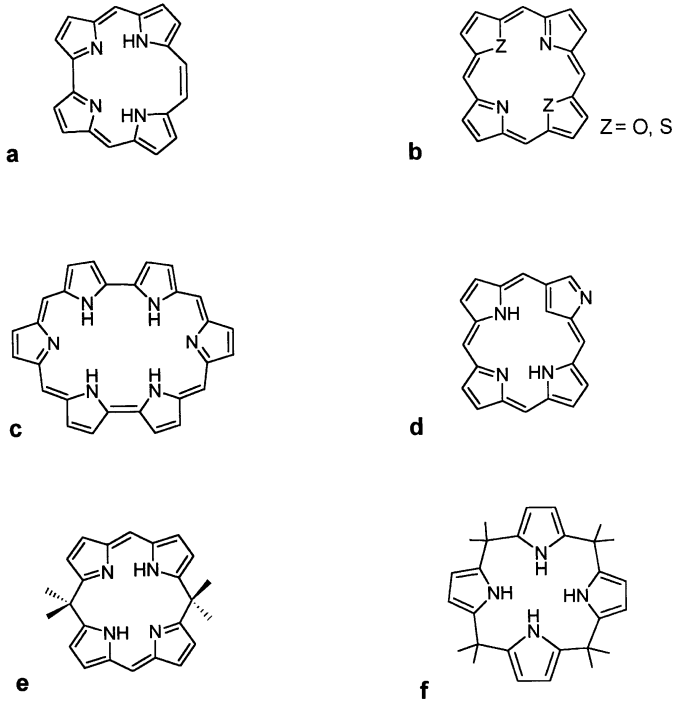

FIG. 27
Structures of some novel derivatives of oligopyrrols; porphyrin isomer (**a**), core modified porphyrin (**b**), rubyrin (**c**), inverted porphyrin (**d**), calix[4]phyrin (**e**), calix[4]pyrrole (**f**)

7.2. Electronic Noses and Tongues

Recent advent of electronic noses and electronic tongues has brought a new way of thinking and an innovative approach to the field of analyses of complex samples[362,363]. Classical analytical methodology is based on the separation and determination of individual components present in a given sample. On the other hand, the application of electronic noses and tongues employs an array of bio- or chemical sensors (not necessarily highly selective) that give, after an appropriate mathematical data processing of primary sensor responses, rather global information on features or quality of the sample[364]. The transformation of the primary data (receptor signals) to meaningful qualitative and quantitative results is considered to be the most difficult part of the analytical process. However, this difficulty is largely outweighed by the fact that this novel methodology does not require the application of highly selective receptors to obtain analytically relevant outputs, which has been imperative in classical methodology.

Gas sensors arrays, *i.e.* electronic noses, are by far more studied than their wet chemical counterparts, electronic tongues[365]. Electronic noses have already been established for qualitative analysis in various fields of analytical chemistry.

Presently, it appears that analyses of multicomponent mixtures with sensor arrays working in gaseous environment or in solution open a new area also for the application of oligopyrrole macrocycles. Many oligopyrrole macrocycles, often easily accessible, are currently being tested for these novel applications even if they have severe drawbacks in terms of their limited selectivity. In this context it is important to recall that low and even non-selective receptors can be successfully exploited in arrays, under the condition that each sensor element has different sensitivity to the specific analyte in a mixture[366,367].

Recently, porphyrins, matalloporphyrins, metallophtalocyanines and their derivatives have been proposed as sensitive elements for mass variation based transducers for the detection of volatile compounds[363,366]. Main feature of such sensors has been shown to be the dependence of the sensing properties on the nature of both the central metal and peripheral substituents of the macrocyclic complex. While the broad selectivity of these sensors is generally related to weak interactions such as van der Waals forces and hydrogen bonding, when metal complexes are used as sensing elements an additional term, due to the coordination of analytes, should be taken into account. Despite the interesting and promising applications, sys-

tematic studies of fundamentals of the sensing mechanism are scarce and findings differ considerably[366].

Feasibility of using metalloporphyrins as sensitive substances for liquid analysis has been proposed in the past[367]. Metalloporphyrins, such as CuTPP, ZnTPP, CoTPP and RhTPP, were incorporated into a polymeric membrane and their sensing abilities tested under standard potenciometric conditions. The sensors were found to be sensitive to diethylamine in low concentrations.

As the pH of aqueous solutions depends on the presence of amines that behave as bases, it was considered reasonable to check also pH sensitivity of the porphyrin-PVC sensors. This idea was proven to be valid and the sensors displayed H^+ sensitivity in the concentration range from pH 2 to 9 (ref.[367]).

The cooperative utilization of an electronic nose and an electronic tongue can improve the classification performance. This has been shown in two different experiments[367]. The first one was aimed at clinical contest, such as the measurement of urine samples, while the other was concerned with the food analysis being devoted to the analysis of various kinds of milks.

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