1,1′-Binaphthyl-Substituted Macrocycles as Receptors for Saccharide Recognition

Oleksandr Rusin,[a] Kamil Lang,[b] and Vladimír Král*[a]

Abstract: The preparation of receptors for saccharide recognition in a natural environment has been an unmet goal for a long time. We present herein the synthesis and binding properties of (*R*,*S*)-1,1′-binaphthyl-substituted macrocycles as receptors for saccharide recognition in water/acetonitrile (1:1) and in DMSO. Porphyrin and metalloporphyrin macrocycles with two to four 1,1′-binaphthyl substituents and multiple hydroxy groups generate a binding site for saccharides that incorporates hydrogen-bonding hydroxy groups together with the aromatic hydrophobic pocket. The specificity for di- and trisaccharides is governed by the cavity size. The mechanism of binding has been studied by ^{1}H NMR spectroscopy and the role of H-bonding and CH–π interactions has been evaluated; the ability to bind saccharides has been demonstrated by the surface plasmon resonance (SPR) technique. The application of these macrocyclic receptors to sensor development is also presented.

Keywords: biaryls · carbohydrates · macrocycles · molecular recognition · receptors · sensors · surface plasmon resonance

Introduction

The role of oligosaccharides in biological regulation has attracted a great deal of attention in recent years.[1–4] Modern biomedical science ascribes more significant roles to saccharides than merely acting as energy pool and structural components. Currently, it is known that oligosaccharides participate in many essential processes in living organisms, for example, cell–cell recognition, infection of cells by pathogens, immune response, distribution and reactivity of proteins within cells, and membrane transport. The presence of carbohydrates on cell and protein surfaces is suggestive of their previously unrecognized importance. Moreover, complex carbohydrates, with their unique multiply linked monomers and branched structures, contain more information in a short sequence than any other biological oligomers. Among all biological molecules, carbohydrates, in a short sequence, display the largest number of ligand structures suitable for binding with proteins in molecular recognition systems.

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The mechanism of interaction of saccharides with their receptors is rather complex. X-ray studies of protein–saccharide complexes have clearly demonstrated multipoint binding through cooperation of van der Waals forces, coordination, and hydrogen bonds.[1] From the viewpoint of host–guest chemistry, saccharides can be classified as "chemical chameleons". They are highly hydrophilic, noncharged, nonfluorescent compounds that exist in various cyclic forms in aqueous solution. Recently, the role of hydrogen bonds in the saccharide–receptor interaction has been very intensively studied. These bonds are especially effective in nonpolar media.[5] On the other hand, in aqueous solution, hydrogen bonds between a saccharide and its receptor are often significantly disrupted due to strong competition with water molecules. Moreover, the recognition process can be further obscured by self-aggregation and solvation effects of the saccharide molecules. For these reasons, successful applications of synthetic receptors for saccharides operating in aqueous systems are relatively scarce.

Porphyrins are suitable for the design of receptors for saccharide recognition in aqueous media; when appropriately substituted they can offer a hydrophobic pocket with binding sides together with extraordinary photophysical properties. Porphyrins are naturally occurring compounds with extraordinary properties. They exhibit characteristic sharp and intense absorption maxima in the visible region of their electronic spectra (Soret band) and a strong fluorescence that can be exploited for analytical purposes.[6] The introduction of appropriate substituents at *meso* positions of the porphyrin core facilitates the creation of three-dimensional "cage" and

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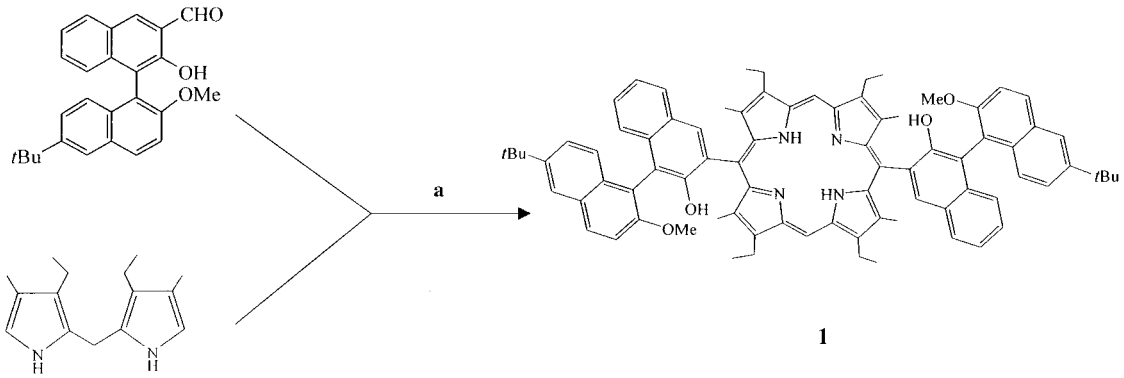
"cleft" structures that can be effectively employed for substrate entrapment.[7] Sufficient solubility can be achieved by the introduction of water-solubilizing groups at the porphyrin periphery. Water-soluble porphyrins have recently been extensively studied, mainly due to their possible medico-biological applications.[8, 9] New synthetic procedures for porphyrins and novel applications thereof offer great promise in the intriguing field of modern molecular recognition chemistry.

Results and Discussion

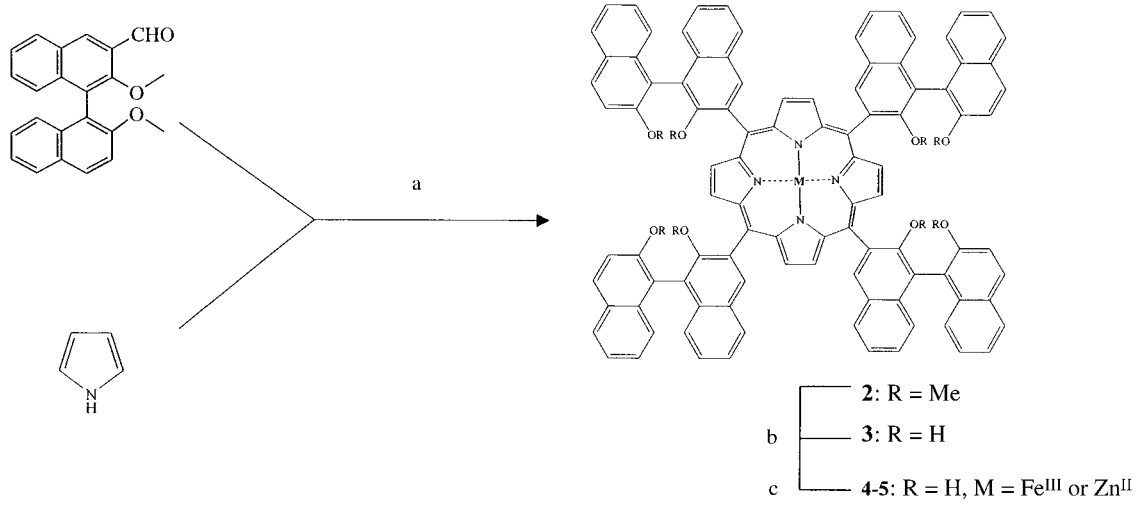
Elegant receptors for saccharide binding based on non-covalent interactions have recently been reported by Ogoshi and co-workers,[10–12] Bonar-Law and co-workers,[13–17] Diederich and co-workers,[18–23] Davis and co-workers,[5, 24–27] as well as other authors.[5, 28–31] These receptors, based on various macrocyclic systems such as porphyrins, calixresorcinols, and cyclophanes, possess different recognition and signalling units. On the other hand, very efficient receptors based on boronic acid binding groups have been reported by Shinkai and co-workers.[32–43] Nevertheless, there are only a few systems that can operate in aqueous environments.[5, 32–46]

Recently, we reported the synthesis of porphyrin-based receptors for recognition of saccharides in highly competitive media: tetrakis(1,1′-binaphthyl)porphyrins, porphyrin phosphonates, and porphyrin–cryptand cyclic systems for inclusion-type carbohydrate complexation in aqueous media.[47–52] Similar types of receptors, such as 1,1′-binaphthyl-substituted calixresorcinols, have also been examined as potential saccharide sensors.[48, 53] The naphthyl and binaphthyl structural motifs were used in the design of saccharide binding receptors in the recent studies of Ogoshi and co-workers and Diederich and co-workers.[10–12, 18, 19, 22, 23] These receptors were developed for the complexation of glycopyranosides in organic media. Herein, we discuss the complexation properties of (*R*,*S*)-1,1′-bis(binaphthyl)- and (*R*,*S*)-tetrakis(1,1′-binaphthyl)-substituted porphyrins and metalloporphyrins with saccharides.

Receptors **1** and **3**–**7** were designed to facilitate saccharide coordination through a combination of hydrophobic interactions and multiple H-bonding contacts (Schemes 1 and 2). The binding sites of porphyrins **1** and **3** contain two and eight phenolic hydroxy groups, respectively, which are capable of forming hydrogen bonds with guests. In addition to eight phenolic hydroxy groups, the analogous porphyrins **4** and **5** incorporate complexed Fe^{III} and Zn^{II} ions, respectively. All



Scheme 1. Synthesis of porphyrin **1**: a) propionic acid, reflux 4 h.



Scheme 2. Synthesis of porphyrins **2**–**5**: a) propionic acid, reflux 4 h; b) BBr_3 (10 equiv), 1 day, room temperature; c) Fe^{III} acetylacetonate or $ZnCl_2$/acetonitrile; reflux 2 h.

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the modified porphyrins possess a hydrophobic pocket of bulky aryl substituents. This is the basis for a binding of oligosaccharides akin to that of lectins.

The (*R*,*S*)-1,1′-binaphthyl-substituted resorcinol molecules **6** and **7** are cup-shaped structures with the smaller polyhydroxylic lower rim and bulky binaphthyls on the upper part providing a deep cavity. These groups, together with the other aromatic moieties, play an essential role in the complexation of saccharides.

Steric hindrance between the *ortho* substituents on the binaphthyl rings and the pyrrole β-hydrogen atoms results in a high energy barrier for rotation about the porphyrin–binaphthyl bonds through a coplanar conformation. As a result, each individual *ortho* substituent is fixed on one side of the porphyrin plane. This leads to the existence of two (for porphyrin **1**) or four (for porphyrins **2** and **3**) atropisomers with different orientations of the substituents with respect to the porphyrin plane, which correspond to the four possible mutual arrangements of the substituents.

In the first step, a statistical mixture of all atropisomers was synthesized. This mixture was then separated by liquid column chromatography or HPLC. The single binding cavity provides complexation with 1:1 stoichiometry, and for this reason the (*R*,*S*)-α,α-atropisomer (porphyrin **1**) and (*R*,*S*)-$\alpha,\alpha,\alpha,\alpha$-atropisomer (porphyrins **2**–**5**) were used for the complexation studies (Figure 1). The atropisomers were

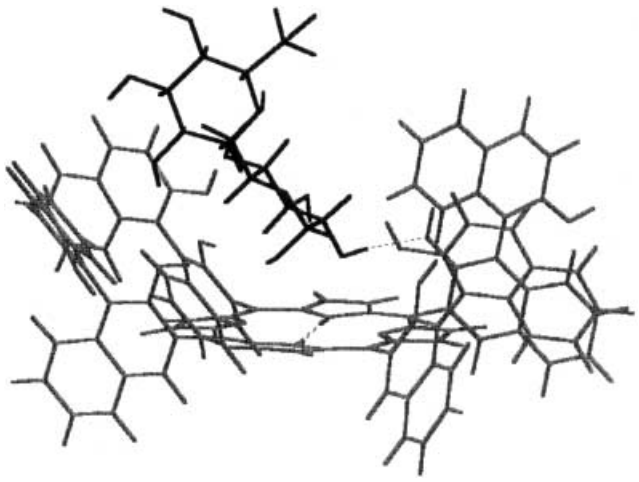

Figure 1. Optimized structure of the complex of **3** with D-glucose.

characterized on the basis of chromatographic and NMR analyses of similar systems.[54–59] With R_f values based on their expected polarities, the most polar $\alpha,\alpha,\alpha,\alpha$-atropisomer (or α,α-isomer) is the slowest moving. The $\alpha,\alpha,\alpha,\alpha$-atropisomer exhibited two singlet β-pyrrolic resonances, as was expected, whereas the $\alpha,\alpha,\alpha,\alpha$-isomer exhibited two doublets.

The decision to use a racemic mixture of 1,1′-binaphthyl aldehydes in the synthesis can be rationalized by the fact that racemization of the 1,1′-binaphthyls occurs in acidic media, especially at elevated temperatures. During our experiments, we observed a total racemization of optically pure (*R*)- and (*S*)-2,2′-dihydroxy-1,1′-binaphthyl-3-carbaldehydes under macrocyclization conditions in the presence of HCl at room temperature over 12 h. This was also the case for similar compounds such as (*R*)- and (*S*)-2,2′-dihydroxy-1,1′-binaphthyl-3,3-dicarboxylates. Not surprisingly, this process occurs more rapidly in our synthesis, performed according to Rothemund's protocol using boiling propionic acid. Therefore, we used (*R*,*S*)-tetrakis(1,1′-binaphthyl) aldehydes as starting compounds.

Whereas porphyrins **1** and **2** are insoluble in water, derivatives **3** and **4** are soluble in water/acetonitrile (1:1, v/v). The binding constants of various saccharides were determined by UV/Vis titration experiments in DMSO (for hosts **1**–**3**) and in water/acetonitrile (for hosts **3** and **4**) at room temperature (Figure 2). Fluorescence emission spectroscopy could also be utilized for this purpose (Figure 3).

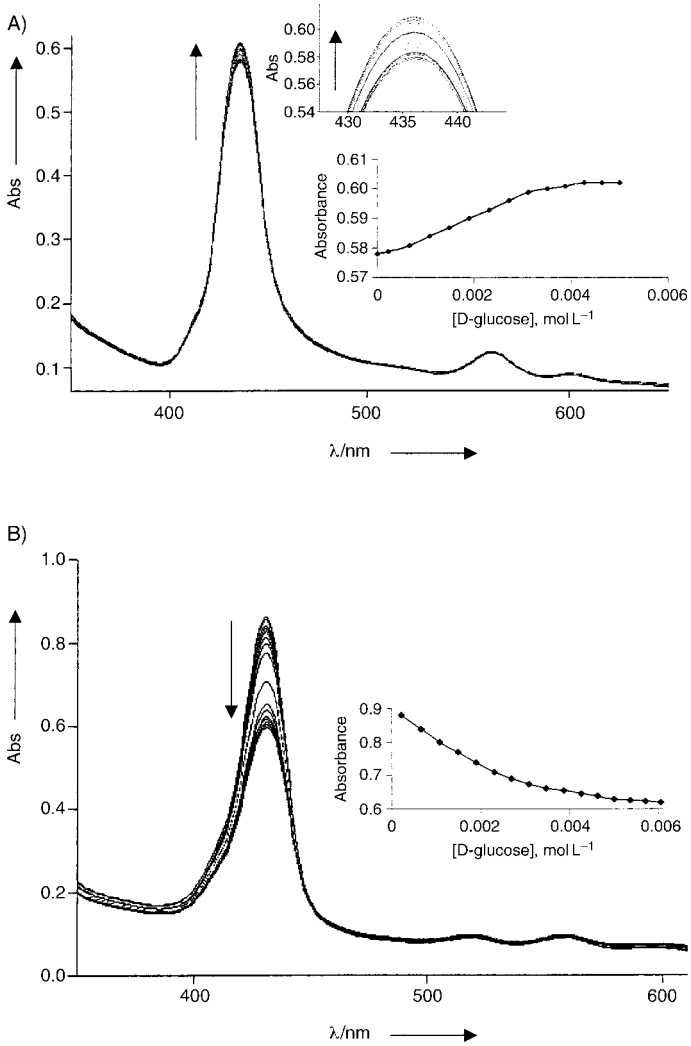

Figure 2. UV/vis spectral changes upon incremental addition of D-glucose to **3** (A) and **4** (B) in aqueous medium.

Dilution experiments in the water/acetonitrile mixture (1:1) revealed a linear dependence of absorbance on the concentration ratio of the saccharide over a broad concentration range ($0-10^{-4}$ mol L^{-1}) for receptors **3** and **4**. The working concentration of macrocycles in the binding study was 6.2×10^{-6} mol L^{-1}. At this concentration, the aggregation effect of host molecules **3** and **4** is minimized.

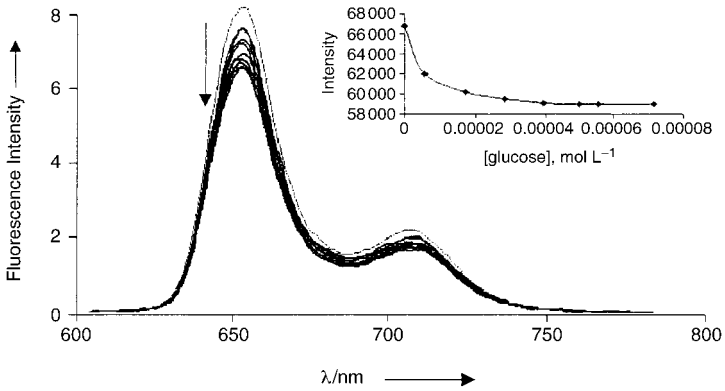

Figure 3. Changes in the fluorescence spectrum upon incremental addition of D-glucose to **3** in aqueous medium.

A crucial question regarding the interpretation of the UV/Vis spectra was whether the observed changes corresponded to receptor aggregates caused by saccharides or whether they could be attributed to a well-defined binding process in which the saccharide is oriented above the porphyrin plane such that multiple H-bonding takes place for efficient recognition and binding. To answer this question, we performed resonance light-scattering (RLS) measurements; the saccharide was added to a solution of the receptor and it was ascertained whether aggregation occurred. The porphyrin receptors were spectroscopically characterized in acetonitrile and DMSO up to concentrations of 6.2×10^{-6} mol L^{-1} (see Experimental Section). The absorbances in the Soret region (around 420 nm) and in the visible region (500–650 nm) were found to obey the Beer law, indicating that the receptors are in the monomeric form. Upon addition of distilled water (> 50%, v/v), the Soret band shows a strong hypochromism concomitant with a large broadening. Significant influence of the water content on the absorption spectra suggests that the porphyrins extensively aggregate. This is not a desirable process because aggregates are structurally undefined species sensitive to many parameters (ageing, mixing, etc.) and are non-fluorescent. As aggregation precludes use of the receptors for the specific probing of saccharides, we combined UV/Vis and resonance light-scattering (RLS) spectroscopies to select a solvent in which the porphyrins are predominantly in the monomeric form. Light scattering can be enhanced when the molar absorption and size of the species are sufficiently great and when strong exciton coupling exists among the chromophores.[60, 61] Because the amount of scattered light is directly proportional to the volume of the particles and monomeric molecules and small oligomers show no enhanced scattering, this method is particularly useful. The formation of extended aggregates of the receptor **3** was confirmed by intense RLS profiles, as recorded in 50% DMSO and 25% acetonitrile (v/v). The peaks of the RLS profiles are centered near the absorption bands of the aggregate at about 440 nm; a slight red-shift is caused by self-absorption of scattered light near the Soret maxima. It emerged from these results that aqueous acetonitrile (50%, v/v) is a good solvent for studying the complexation phenomena because the receptors **3** and **4** remain in predominantly monomeric form. The addition a saccharide does not induce aggregation. Similarly, dilution experiments in water/acetonitrile (1:1) revealed a linear dependence of the absorbance on the concentration ratio of the saccharide over a broad concentration range ($0-10^{-4}$ mol L^{-1}) for receptors **3** and **4**. As the working concentration of the receptors was 6.2×10^{-6} mol L^{-1}, aggregation of **3** and **4** was minimized.

The important role of hydrogen bonds in the formation of the porphyrin–saccharide complex is confirmed by the data summarized in Table 1. While porphyrin **2** (R = Me) interacts with D-glucose only weakly ($K_a < 10$ M^{-1} in DMSO), values of the association constants for this saccharide with porphyrins **1** (R = Me/OH) and **3** (R = H) are more impressive. Substrates such as α- or β-D-octyl (or phenyl) glucopyranoside interact more strongly with all the hosts studied. The differences in the selectivity for alkyl α- and β-glucopyranosides reflect the increasing contribution of hydrophobic CH–π interactions, which favor the octyl derivative more than the methyl derivative. On the other hand, the selectivity for the anomers may be governed not only by the geometries of the host and guest, but also by the nature of the solvent. For this reason, we obtained presumably different specificities of the receptors for α- and β-anomers in DMSO as a result of competitive binding in different environments.

Table 1. Association constants for the binding of saccharide derivatives with receptors **1**–**3** in DMSO (from UV/Vis titrations).[a]

Saccharide	Association constant (K_a) [M^{-1}]		
	1	**2**	**3**
α-D-glucose	40	< 10	100
octyl α-D-glucopyranoside	600	500	650
octyl β-D-glucopyranoside	< 10	< 10	110
methyl α-D-glucopyranoside	< 10	< 10	70
methyl β-D-glucopyranoside	< 10	255	160
p-nitrophenyl galacto-β-pyranoside	400	390	298

[a] Procedure used for UV/Vis determination of the association constants: A 6.15×10^{-6} M solution of the macrocycle in DMSO was placed in a 1 cm^2 quartz cuvette. A known amount of a given saccharide was added in small increments (0–100 equivalents; the concentration of the saccharide solution was the same as that of the receptor solution). Absorbance changes were measured at the absorption maxima at room temperature; the resulting data were evaluated using least-squares curve fitting. K_a values were calculated for 1:1 complexes. The reproducibility of the K_a determination was ±10% in triplicate runs.

Hydrophobic interactions between the alkyl (or aryl) chains of the substrates and the naphthyl rings of hosts **1**–**3** are apparently important in the complex formation. Nevertheless, the number of hydroxy groups on the binaphthyl moieties of porphyrin **3** led to different affinities for the saccharide guests. Water-soluble porphyrin **3** displays a significant preference for unmodified di- and trisaccharides over monosaccharides in the aforementioned water/acetonitrile mixture, which indicates saccharide binding within the receptor cavity. Higher oligosaccharides (tetroses to hexoses) were also tested, but were found to exhibit lower binding affinities. Taken together, our results represent evidence for saccharide binding within the cavity formed by the binaphthol-modified porphyrin macrocycles, where the crucial binding forces are H-bonding and CH–π interactions. The association constants of saccharides with receptor **3** in aqueous media are given in Table 2.

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Table 2. Association constants for the binding of saccharides by receptors **3** and **4** in aqueous medium (water/acetonitrile, 1:1, v/v) (from UV/Vis titrations).[a]

Saccharide	Association constants K_a [M^{-1}]	
	3	**4**
α-D-glucose	60	110
galactose	50	100
methyl-α-D-glucoside	20	50
methyl-β-D-glucoside	<10	20
L-fucose	75	100
maltose	150	230
maltotriose	110	180
maltotetraose	85	100
maltopentaose	70	100

[a] Changes in absorbance were measured at 427 nm for **3** and at 430 nm for **4**. UV/Vis measurements of receptor–saccharide complexes were performed using a 1 cm^2 quartz cuvette containing a 6.15×10^{-6} mol L^{-1} solution of macrocycle **3** or **4** in H_2O/acetonitrile (1:1, v/v). The saccharide was added in aliquots of a stock solution (0–1000 equivalents). Absorbance changes at the position of the Soret band were measured at room temperature and the data were evaluated by means of least-squares curve fitting. The apparent K_a values were calculated for 1:1 complexes. The reproducibility of apparent K_a determinations was $\pm 15\,\%$ in triplicate runs.

Introduction of a metal ion into the porphyrin core often amplifies the binding capability of porphyrin-based receptors.[7] Porphyrin **3** was metalated with a series of metal cations including Zn^{II}, Co^{II}, Mn^{II}, Cu^{II}, and Fe^{III} to test the influence of a core metal cation on saccharide binding affinity. Our studies showed that only the Fe^{III} complex (receptor **4**) can be solubilized in the water/acetonitrile. Receptor **4** showed significant binding affinity towards saccharide guests. Strong aggregation was observed for the other metalated receptors in this series in aqueous media. Therefore, only receptor **4** was studied. After the addition of saccharide to receptor **4**, distinct changes in the intensity of the Soret band were observed. Receptor **4** interacts with saccharides in aqueous solution more strongly than its metal-free analogue **3**, and displays a preference for disaccharides over monosaccharides. The association constants calculated for various guests are summarized in Table 2. It was found that the corresponding glycosides showed a decrease of K_a. Comparison of the association constants calculated for D-glucose and α- or β-glucopyranosides illustrates this fact. The position of the methyl group at C-1 also influences the selectivity, with a preference for the β-anomer. On the other hand, substitution of hydrogen by a methyl group at positions other than C-1 was found to have only a weak influence on the selectivity; for instance, the K_a values for D-glucose and D-fucose are very similar. It is known that complexation of saccharides in water demands participation of the hydroxyls at the C-1, C-2, and C-3 positions.[1, 5] Substitution (protection) of some of these positions effectively blocks complexation.[1] We assume that the presence of a methyl group at the C-1 position sterically hinders the optimal binding geometry of a saccharide–receptor complex in aqueous media, which, in turn, leads to only weak complexation with methyl glucopyranosides.

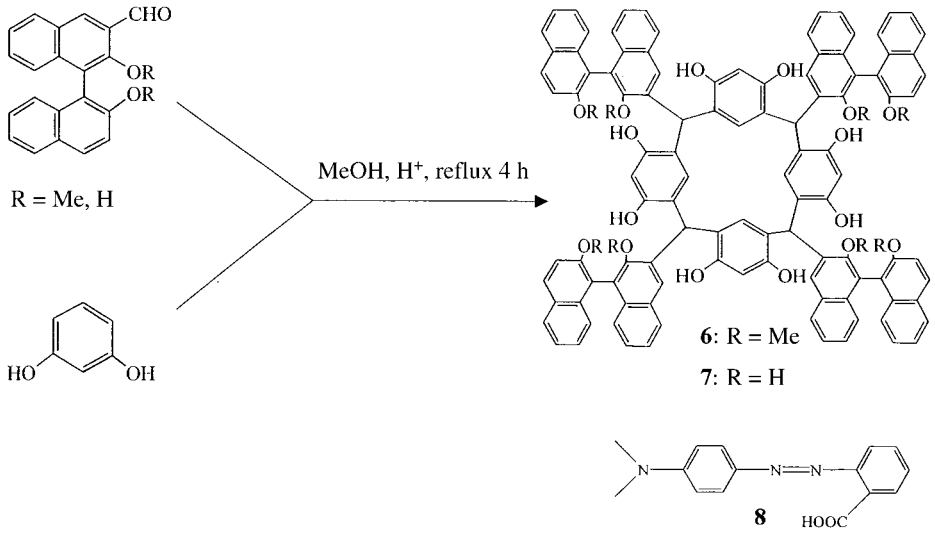
To understand the binding mechanism and geometry of the complex formed, we performed a ^1H NMR titration of the receptor **3** with D-glucose in [D_6]DMSO. This solvent was chosen because at NMR concentrations no aggregation of the receptor was observed; this is in contrast to aqueous media, in which aggregation was observed at millimolar concentrations. Our NMR data indicated a 1:1 binding mode with saccharide, with several different binding modes being involved in this type of complexation, as described below. A marked broadening and upfield shift for the 1-H (from $\delta = 6.21$ to $\delta = 6.00$), 6-H (from $\delta = 4.89$ to $\delta = 4.28$), and OH signals of D-glucose was observed. We also observed chemical shift changes for the aromatic proton signal of the receptors (from $\delta = 9.54$ to 9.49), indicating H-binding and CH–aromatic interactions. ^1H NMR titration of receptor **3** with D-glucose in [D_6]DMSO showed an association constant of $K_a = 30\,M^{-1}$.

We were interested not only in the thermodynamics of the binding process, but also in the kinetics. The fact that some saccharides change their structure with time (mutarotation) has been largely ignored in binding studies to date. Thus, we examined the binding preferences of individual forms of saccharides with our receptors. Monitoring this complexation by ^1H NMR spectroscopy in D_2O/[D_4]methanol (9:1, v/v) showed an upfield shift (0.1 ppm) of the relevant CH proton signals of the saccharide as a consequence of a weak interaction between porphyrin **3** and the linear form of D-glucose. This effect is more pronounced for complexation with the cyclic form of D-glucose, which results in a strong upfield shift (2 ppm) of the relevant CH proton signals (the glucose CH protons resonate at $\delta = 1.88$, 1.96, and 2.15). This is also indicative of a binding mechanism in aqueous media in which CH–π interactions play a major role. Unfortunately, due to exchange with the deuterated solvent, it is impossible to evaluate the contribution of H-bonding. Nevertheless, the importance of both hydrogen bonds and CH–π interactions in the complexation of saccharides and 1,1′-binaphthyl-substituted receptors has recently been demonstrated by ^1H NMR spectroscopy in [D_3]acetonitrile.[53]

An analogous series of ^1H NMR experiments in [D]chloroform/[D_4]methanol (3:1, v/v) was performed with octyl α-D-glucopyranoside (a cyclic saccharide derivative). These experiments provided valuable information about the complexation phenomena. The aromatic proton signals of receptor **3** underwent a downfield shift of 0.3 ppm, while the signals of phenolic hydroxyls were broadened. The signals of the hydroxyls of the alkyl glucopyranoside were also shifted downfield (by 0.5 ppm). These facts are indicative of complexation between octyl α-D-glucopyranoside and **3** through hydrogen bonds between the 1,1′-binaphthylic hydroxyls and those of the saccharide. The participation of hydrogen bonds in complexation was further confirmed by IR spectroscopy. These measurements showed a marked broadening of the absorptions of the phenolic hydroxy groups of receptor **3**, as well as a red shift of the absorptions of the saccharide hydroxy groups from 3354 cm^{-1} to 3339 cm^{-1}. Additionally, a blue shift from 895 cm^{-1} to 906 cm^{-1} was observed for the α-D-glucopyranoside C–H absorption.

Metalated tetrakis-(1,1′-binaphthyl)-substituted porphyrins can also be employed as chemical sensors, and for comparison a Zn^{II} metalated porphyrin (receptor **5**) was tested. Its ability to bind saccharides was demonstrated by the surface plasmon resonance (SPR) technique. This technique is often used for monitoring lectin–saccharide interactions, immunochemical

reactions, and for the detection of organic compounds.[62–67] The SPR detection principle relies on the change in refractive index and the corresponding shift in the SPR signal that is induced by the binding of the analyte to the sensor surface. Receptor **5** was physically adsorbed on the gold surface of the optical chip and incubated for 30 min in an aqueous solution of D-glucose (3 M) at room temperature. This sample was then irradiated with polarized light. A photodiode records the intensity of the light beams reflected from the gold surface and the surface coated with the receptor–saccharide complex. The differences in the values of refractive index obtained from the chip can be monitored as SPR curves; the results are summarized in Figure 4. Significant changes were found in the SPR spectra as a result of the saccharide complexation. This effect can therefore be conveniently employed for saccharide sensing. Control experiments with tetraphenylporphyrin showed no change in the SPR curves before and after treatment with a D-glucose solution.



Scheme 3. Synthesis of macrocycles **6** and **7**.

Recently, sterically well-defined polycyclic structures **6** and **7** with (*R*,*S*)-1,1′-binaphthyl subunits were used for the visual recognition of saccharides in aqueous media (Scheme 3).[51, 53] Similar calixresorcinol-based hosts have appeared in recent papers.[30, 70–75] We observed that the colorimetric indicator methyl red (**8**) binds to the receptors **6** and **7**. This conclusion was based on a ^{1}H NMR study, which revealed shifts of the aromatic proton signals of **6**, **7**, and **8**, as well as shifts of the proton signals of the dimethylamino group of **8**. We also observed a broadening of the ^{1}H NMR signals of the resorcinol OH protons (for receptor **6**) and of the CH_3 proton signals of **8** in [D_3]acetonitrile. This suggests that the lower rim of the receptor is exposed to the solvent. These data indicate that the receptor **6** forms a sandwich-type complex in acetonitrile and methanol. For the receptor **7**, the aromatic hydroxy groups of the (*R*,*S*)-1,1′-binaphthylic moieties were seen to be involved in complexation with **8** as shifts of their aromatic proton signals of about 0.5 ppm were observed in [D_3]acetonitrile.

The complex **7**:**8**, initially formed in acetonitrile, is readily soluble in water. The shifts of the aromatic proton signals of **7** and **8** by about 0.2 ppm in the system D_2O/[D_3]acetonitrile (5:1, v/v) indicate that the receptor **7** preferentially forms an inclusion-type complex with **8** in water.

Studies of the interaction of receptors **6** and **7** with methyl red **8** showed the formation of weak complexes with K_a values of 60 and 80 M^{-1}, respectively, in [D_3]acetonitrile. We subsequently employed the complexes of **6** or **7** with **8** in studies of selective saccharide binding, in which binding resulted in a color change. Job plots obtained from UV/Vis and ^{1}H NMR spectroscopic measurements in acetonitrile and/or methanol were indicative of 1:1 stoichiometries for the complexes **6**:**8** and **7**:**8**. Due to the poor solubility of **8** and of the receptors **6** and **7** in pure water, methanol-containing solutions (up to 1%, v/v) were used for the initial complex formation (the receptor **6** or **7** with **8**).

The starting solutions were prepared by the addition of water to an equimolar mixture of **6** or **7** with **8** in methanol (the final methanol concentration in water was 1%). Under

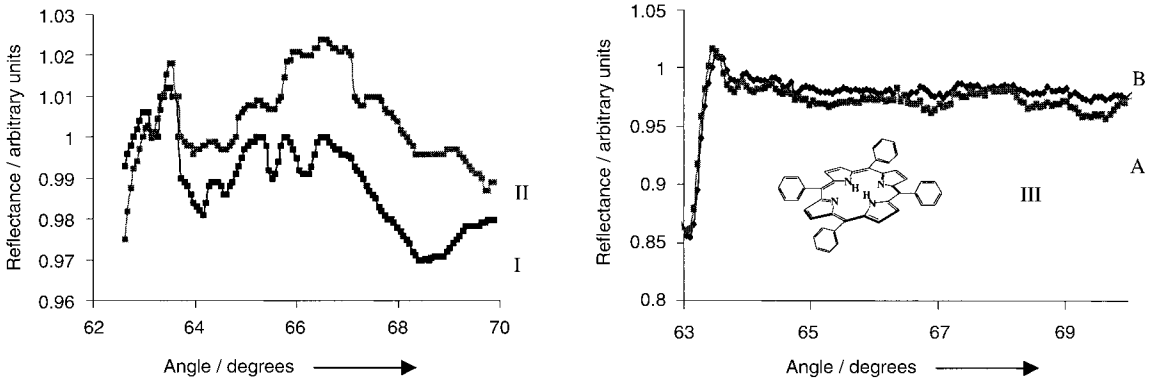


Figure 4. Interaction of 1,1′-binaphthyl-substituted porphyrin **5** with D-glucose on a gold surface as monitored by the surface plasmon resonance technique. The plot shows reflected light intensity versus angle (SPR curves) for the starting compound (curve I) and for the complex (curve II). Control experiments (curve III) with tetraphenylporphyrin showed no change in the SPR curves before (A) and after treatment (B) with a D-glucose solution.

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these conditions, orange (for **6**:**8**) and violet (for **7**:**8**) complexes in water at pH 6.0 were formed. No pH changes during titration were observed. Both receptors show intense absorbances in the UV range, and **7** additionally shows low-intensity absorbance bands at 513 and 422 nm. The complex **6**:**8** exhibits three intense absorption maxima at 436, 519, and 547 nm, while the complex **7**:**8** exhibits retains the band at 513 nm. All these bands are suitable for the visual detection of analytes.

The formation of stoichiometric complexes should be studied in a concentration range in which complexation is unaffected by aggregation effects. In our case, a linear dependence of the absorption on the concentration of the complex **7**:**8** was observed over the concentration range 0–2.5×10^{-4} mol dm^{-3}. For this reason, all measurements were carried out at 1.2×10^{-4} mol dm^{-3}. A gradual increase in the saccharide concentration in solutions of the non-covalent receptors **6**:**8** and **7**:**8** was accompanied by colour changes from orange to yellow (for **6**:**8**) and from violet to yellow (for **7**:**8**), along with changes in the intensities of the maxima at the aforementioned wavelengths (Figure 5). Isosbestic points were found at 470 nm for the receptor **6**:**8**, and at 467 nm for the receptor **7**:**8**. This suggests that the macrocycle (**6** or **7**)–saccharide complexes have 1:1 ratios. Parallel experiments were carried out with octyl-α-D-glucopyranoside and the aforementioned receptor–indicator complexes by ^{1}H NMR spectroscopy in [D$_3$]acetonitrile. In both cases, we observed strong shifts of the resorcinol and saccharide OH signals, indicating a competition of the saccharide guest with the indicator for binding sites on the complexes **6**:**8** and **7**:**8**.

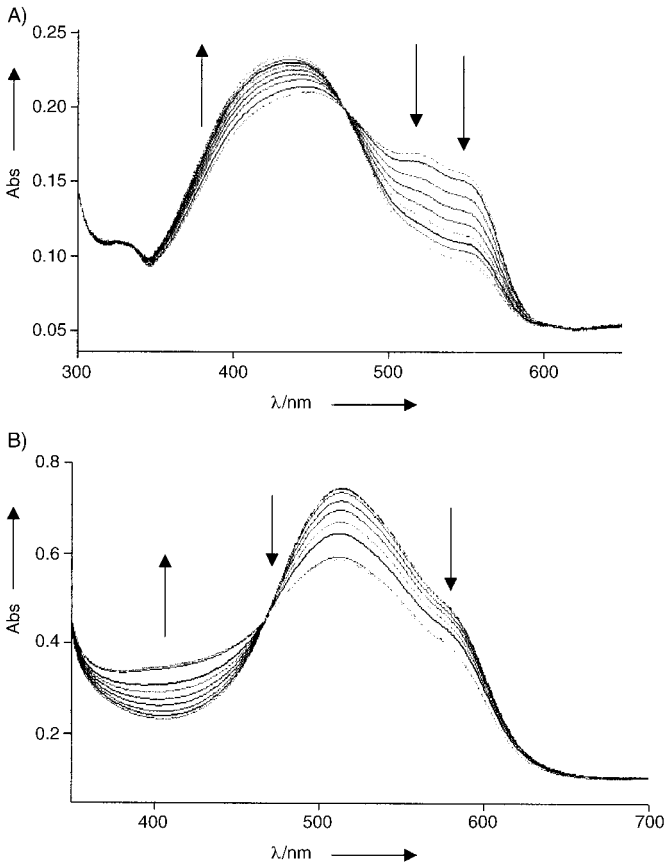

Figure 5. Interaction of complexes [**6**:**8**] (A) and [**7**:**8**] (B) with D-glucose in aqueous media followed by UV/Vis titration: for A) at $\lambda_{max} = 436$, 519, and 547 nm; for B) at $\lambda_{max} = 513$ nm.

Table 3. Association constants for binding of saccharides and other selected compounds to receptor complexes [(**6** or **7**)-methyl red] in aqueous medium, as followed by UV/Vis titration.[a]

Test compound	Association constant (K_a) [10^2 M^{-1}]	
	6	**7**
methyl-α-D-glucopyranoside	11.9	15.0
D(+)-galactose	9.0	9.5
D(−)-fructose	7.7	7.5
D(−)-ribose	8.1	7.3
L-sorbitol	1.4	1.9
L-gulonic acid γ-lactone	1.0	1.6
D-glucose	9.9	11.0
D(+)-maltose	45.2	45.5
maltotriose	39.9	40.2
maltotetraose	28.4	40.0
α-D-lactose	18.7	35.3
β-D-lactose	16.4	33.0

[a] 1.19×10^{-4} mol L^{-3} solution of receptor (**6** or **7**)-methyl red (1:1) in methanol/water (0.5% methanol, v/v) was placed in a 1 cm^2 quartz cuvette. A known amount of a given saccharide was added in increments (0–50 equivalents; concentration of the receptor in the cuvette kept constant). The absorbance changes at 436 nm (for **6**) and at 513 nm (for **7**) were measured at room temperature and the data were evaluated with the aid of least-squares curve fitting. The apparent K_a values were calculated for 1:1 complexes. The reproducibility of the K_a values was ±10% in triplicate runs.

The association constants are summarized in Table 3. Both receptors **6**:**8** and **7**:**8** bind oligosaccharides more strongly than monosaccharides. Control experiments on the binding of saccharides to methyl red gave very low association constants (below 20 mol^{-1}). Control experiments also indicated that binding between saccharides and free **8** does not occur. Association constants of methyl-α-D-glucopyranoside with the receptors **6**:**8** and **7**:**8** were found to be higher than those for unmodified monosaccharide analogues. Two saccharide-like guests, namely L-sorbitol and L-gulonic acid γ-lactone, were also tested. Despite their similarity to saccharides, both receptors were able to distinguish these guests from other saccharides (association constants were lower than for monosaccharides).

Conclusion

We have presented several 1,1′-binaphthyl-substituted porphyrins, metalloporphyrins, and calixresorcinols as novel receptors for saccharides in highly competitive environments (DMSO, water/acetonitrile (1:1, v/v), and water/methanol (9.5:0.5, v/v)). The versatility of our approach and the possibility of applying it to other macrocycles, such as calixresorcinols, offers great potential for sensor development with this synthetic methodology.

We have studied the mechanism of saccharide binding, and found that in protic (aqueous) media it is predicated on CH–π interactions and a hydrophobic effect, whereas in polar organic solvents binding is facilitated through effective hydrogen bonding. We are continuing to test these novel receptors for their saccharide sensor potential.

Experimental Section

Methods: Absorption spectra were measured on a Cary 400 Scan spectrophotometer. Cells containing the samples were equilibrated at room temperature for 5 min. Absorbance titrations were conducted with concentrated stock solutions of a saccharide. The concentration of the receptor was 6.2 μM (**1**–**4**) or 0.12 mM (**6,7**). Saccharide binding was studied in DMSO (**1**–**3**), water/acetonitrile (1:1, v/v) (**3**, **4**), and water/methanol (95:0.5, v/v) (**6**, **7**). The binding constants K_a for the receptor–saccharide complexes were determined from the absorbance changes at the Soret (for porphyrins) and other (for calixresorcinol–methyl red complexes) maxima using the Benesi–Hildebrand equation assuming a 1:1 stoichiometry and that the saccharide concentration is always significantly larger than the receptor concentration. The stoichiometry was confirmed by the Job method of continuous variations. The solutions of the receptor and the saccharide were mixed to a standard volume with varying molar ratios of the two components. Absorbance differences were taken at the Soret band of the porphyrin receptors; 427 nm for **1** and **2**, and at 420 nm for **3** and **4**. The intercepts of linear least-squares fits to the left- and right-hand portions of the Job plots gave the binding stoichiometry. The fluorescence spectra were recorded on a FluoroMax-2 spectrophotometer. Resonance light-scattering experiments (RLS) were conducted by taking simultaneous scans of the excitation and emission monochromators over the range 350–700 nm on a Perkin-Elmer LS 50B luminescence spectrometer. Surface plasmon resonance was measured using a Spreeta spectrometer.

Preparation of 1: The synthesis of *cis*- and *trans*-(*R*,*S*)-5,15-bis(1,1′-binaphthyl-2-methoxy-2′-hydroxy-6′-*tert*-butyl)-3,7,13,17-tetraethyl-2,8,12,18-tetramethylporphyrin (**1**) was carried out according to a known methodology for the preparation of porphyrins (Rothemund protocol). Thus, bis(3-ethyl-4-methylpyrrol-2-yl)methane was cyclized with 2-methoxy-2′-hydroxy-6′-*tert*-butyl-3-formyl-1,1′-binaphthol (Scheme 1).[72, 73] A mixture of bis(3-ethyl-4-methylpyrrol-2-yl)methane (0.5 g, 2.17 mmol) and (*R*,*S*)-2-methoxy-2′-hydroxy-6-*tert*-butyl-3-formyl-1,1′-binaphthol (0.75 g, 2.17 mmol) was refluxed in propionic acid (1 L) for 5 h. The reaction mixture was then concentrated to dryness and the dry residue was redissolved in dichloromethane. The resulting solution was washed with saturated aqueous sodium hydrogen carbonate solution, and then separated by chromatography on a silica gel column eluting with petroleum ether/chloroform (1:9). Two isomers were isolated. For further investigations, the (*R*,*S*)-α,α-isomer was used. The yield of porphyrin **1** was 10%. The isomers were characterized on the basis of chromatographic and spectroscopic (NMR) analyses of similar systems.[54–59]

For the (*R*,*S*)-*cis*-conformer **1**: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 10.15$ (s, 2H; CH), 8.22–6.85 (m, 20H; Ar), 6.18 (br s, 2H; OH), 3.95 (q, 8H; CH_3CH_2), 3.72 (s, 6H; OCH_3), 3.65 (q, 12H; CH_3), 1.81 (t, 12H; CH_3CH_2), 1.31 (s, 18H, *tert*-butyl), −2.05 (br s, 2H; NH); MS (MALDI/TOF): *m*/*z*: 1188 (calcd. for $C_{82}H_{82}N_4O_4$: 1187.55); elemental analysis calcd (%) for $C_{82}H_{82}N_4O_4$: C 82.93, H 6.96, N 4.72; found: C 82.52, H 7.05, N 4.49; UV/Vis (dichloromethane): $\lambda_{max} = 625$, 574, 537, 505, 410 nm; $\varepsilon_{410} = 950\,000\ mol^{-1}dm^3cm^{-1}$.

Preparation of 2: The synthesis of (*R*,*S*)-5,10,15,20-tetrakis(1,1′-binaphthyl-2,2′-dimethoxy)porphyrin (**2**) (Scheme 2) was also carried out according to the Rothemund protocol by cyclotetramerization of protected 1,1′-binaphthyl-3-carbaldehyde with pyrrole. A mixture of (*R*,*S*)-2,2′-dihydroxy-3-formyl-1,1′-binaphthol (0.2 g, 5.8 mmol) and pyrrole (0.04 g, 5.8 mmol) was refluxed in propionic acid (1 L) for 5 h. The solvent was then removed, the product was redissolved in dichloromethane, and the resulting solution was washed with saturated aqueous sodium hydrogen carbonate solution. The organic phase was collected, dried, and separated by chromatography on silica gel eluting with petroleum ether/dichloromethane (1:9). Four atropisomers were isolated in an overall yield of 17%; these were characterized as the (*R*,*S*)-α,α,α,α, (*R*,*S*)-α,α,α,β, (*R*,*S*)-α,α,β,β, and (*R*,*S*)-α,β,α,β-isomers. With R_f values based on their expected polarities, the most polar α,α,α,α-atropisomer was the slowest moving.[63] This isomer was collected in 4% yield and used for the complexation study. The isomers were characterized on the basis of NMR analyses of similar systems.[54–59]

For the (*R*,*S*)-α,β,α,β-conformer **2**: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 9.13$ (m, 4H; β-pyrrole), 8.71 (m, 4H; β-pyrrole), 8.61 (s, 4H; Ar), 8.08–7.26 (m, 40H; Ar), 4.01 (m, 12H; OCH_3), 2.64 (s, 12H; OCH_3), −2.42 (s, 2H; NH).

For the (*R*,*S*)-α,α,β,β-conformer **2**: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 9.15$ (m, 4H; β-pyrrole), 8.71 (m, 4H; β-pyrrole), 8.60 (m, 4H; Ar), 8.09–7.29 (m, 40H; Ar), 4.02 (m, 12H; OCH_3), 2.64 (s, 12H; OCH_3), −2.42 (s, 2H; NH).

For the (*R*,*S*)-α,α,α,β-conformer **2**: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 9.17$ (q, 8H; β-pyrrole), 8.69 (s, 4H; Ar), 8.11–7.11 (m, 40H; Ar), 4.03 (m, 12H; OCH_3), 2.67 (m, 12H; OCH_3), −2.43 (s, 2H; NH).

For the (*R*,*S*)-α,α,α,α-conformer **2**: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 9.14$ (m, 8H; β-pyrrole), 8.61 (m, 4H; Ar), 8.08–7.11 (m, 40H; Ar), 3.98 (s, 12H; OCH_3), 2.68 (s, 12H; OCH_3), −2.44 (s, 2H; NH); MS (FAB positive): *m*/*z*: 1560.8 (MH), calcd for $C_{108}H_{78}N_4O_8$: 1559.8; elemental analysis calcd (%) for $C_{108}H_{78}N_4O_8$: C 83.16, H 5.04, N 3.59; found: C 82.92, H 5.20, N 3.34; UV/Vis for all atropisomers (chloroform): $\lambda_{max} = 647$, 593, 554, 517, 427 nm; $\varepsilon_{427} = 144\,000\ mol^{-1}dm^3cm^{-1}$.

Preparation of 3: Porphyrin **3** was obtained by treating **2** with boron tribromide (9 equiv) in dry dichloromethane at room temperature for 24 h. The reaction mixture was subsequently washed with sodium hydrogen carbonate solution and the organic phase was collected and separated by column chromatography on silica gel eluting with dichloromethane/methanol (3:1). The deprotected product **3** was isolated in 80% yield.

For the (*R*,*S*)-α,β,α,β-conformer **3**: ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 9.53$ (s, 8H; β-pyrrole), 8.73 (q, 4H; Ar), 8.08–7.39 (m, 40H; Ar), 5.42–5.29 (m, 8H; Ar-OH), −2.4 (s, 2H; NH).

For the (*R*,*S*)-α,α,α,α-conformer **3**: ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 9.54$ (d, 8H; β-pyrrole), 8.72 (d, 4H; Ar), 8.08–7.39 (m, 40H; Ar), 5.40 (m, 4H; Ar-OH), 5.29–5.24 (m, 4H; Ar-OH), −2.4 (s, 2H; NH); MS (FAB positive): *m*/*z*: 1448.6 (MH); calcd for $C_{100}H_{62}N_4O_8$: 1447.6; elemental analysis: calcd (%) for $C_{100}H_{62}N_4O_8$: C 82.97, H 4.32, N 3.87; found: C 82.59, H 4.43, N 3.64; UV/Vis (DMSO): $\lambda_{max} = 622$, 597, 556, 427 nm; $\varepsilon_{427} = 172\,000\ mol^{-1}dm^3cm^{-1}$.

Preparation of 4 and 5: Porphyrins **4** and **5** were obtained by refluxing **3** with iron(III) acetylacetonate in acetonitrile (molar ratio 1:2), and with $ZnCl_2$ in methanol, respectively. After removal of the solvent, the metallated porphyrins were dissolved in methanol/dichloromethane (1:4) and purified by column chromatography on silica gel eluting with methanol/dichloromethane (1:9). Yields: 90%. MS of **4** (MALDI/TOF): *m*/*z*: 1501 ([M^+]; calcd for $C_{100}H_{60}N_4O_8Fe$: 1501.4); UV/Vis (methanol): $\lambda_{max} = 622$, 598, 556, 427 nm; $\varepsilon_{427} = 180\,000\ mol^{-1}dm^3cm^{-1}$; MS of **5** (MALDI/TOF): *m*/*z* 1511 ([M^+]; calcd for $C_{100}H_{60}N_4O_8Zn$: 1510.9); UV/Vis (methanol): $\lambda_{max} = 622$, 598, 556, 427 nm; $\varepsilon_{427} = 177\,000\ mol^{-1}dm^3cm^{-1}$.

Preparation of 6 and 7: A mixture of resorcinol (0.07 g, 0.6 mmol) with either (*R*,*S*)-2,2′-dimethoxy-3-formyl-1,1′-binaphthol or (*R*,*S*)-2,2′-dihydroxy-3-formyl-1,1′-binaphthol (0.2 g, 0.6 mmol) in methanol (100 mL) containing HCl as a catalyst was refluxed for 4 h. The reaction mixture was subsequently washed with water and filtered. The solid product was dissolved in methanol, dried, and separated by column chromatography eluting with methanol/dichloromethane (10:90, v/v). The yields of **6** and **7** were 55% and 85%, respectively.

For **6**: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.67$ (s, 4H; OH), 7.99–6.78 (m, 44H; Ar), 6.34 (s, 4H; Ar), 6.2 (d, 4H; Ar), 5.62 (s, 4H; OH), 5.30 (s, 4H; CH), 3.81 (d, 12H; OCH_3), 3.05 (d, 12H; OCH_3), 2.06 (d, 12H; CH_3), 1.7 (s, 12H; CH_3). MS of **6** (FAB positive): *m*/*z*: 1738.5 (calcd. for $C_{111}H_{88}O_{16}$: 1737.6). UV/vis (methanol): $\lambda_{max} = 335$, 325, 282, 235 nm.

For **7:** ^{1}H NMR (300 MHz, [D$_3$]acetonitrile/$CDCl_3$, 1:1, 25 °C, TMS): $\delta = 7.77–5.87$ (m, 44H; Ar; 8H; OH), 5.45 (s, 8H; OH), 5.2 (s, 4H; CH); MS of **7** (FAB positive): *m*/*z*: 1626 (calcd. for $C_{108}H_{72}O_{16}$: 1625.5); UV/Vis (methanol): $\lambda_{max} = 513$, 422, 282, 235 nm.

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