

# Novel porphyrin based receptors for saccharide recognition in water

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## Abstract

A binding study of the porphyrin phosphonates (**1**, **2**), porphyrin–oligopeptide conjugates (**3–5**) and meso-tetrakis-(*p*-sulfonato-phenyl) porphyrin (**7**) to saccharides and alkyl pyranosides showed strong interaction in water and DMSO. The <sup>1</sup>H, <sup>31</sup>P NMR, IR-spectroscopy and SPR technique were used for the study of mechanisms of complexation and potential using of receptors as sensor part; participant of hydrogen bonds in porphyrin–saccharide complexes formation was demonstrated. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Porphyrin phosphonates; Porphyrin–oligopeptide conjugates; Binaphthyl compounds; Saccharide recognition

## 1. Introduction

The problem of biomolecules recognition is one of the interesting topics of modern chemistry, biology and medicine. Numerous host systems have been described in the recent literature that utilize sterically well-organized receptors for the recognition of biologically important substrates [1]. The design of such systems is based on the combination of different covalent and non-covalent binding modes. Great efforts have been focused on the preparation of water-soluble porphyrins, where sufficient solubility was achieved by the introduction of water-solubilizing groups on the porphyrin periphery. Water-soluble porphyrins have been extensively studied, mainly due to their possible medicobiological applications [2,3]. Most of described derivatives possess positively charged groups. On the other hand, there are only a few examples of negatively charged porphyrins, which are prepared mainly by the introduction of carboxylate or sulfonate groups into the porphyrin periphery. The use of phosphonate group thus came as a new alternative toward known anionic groups. Phosphonates allow the polarity and solubility ‘tuning’ by the hydrolysis of their esters to hydrophilic compounds soluble in water. For this reason can be tested their biological properties.

The P=O group is widespread in nature in numerous biomolecules. It plays an important role in non-covalent bonding of proteins or other specific ligands to their substrates. This group is a strong hydrogen bond acceptor. There are several examples of synthetic receptors containing P=O groups concerning of the sugar recognition [4].

The interaction between saccharides and their natural ligands, e.g. lectins is realized by the formation of hydrogen bonds between hydroxy groups of saccharide and amide or carboxylic groups in binding site of the receptor proteins [5]. In cooperation with other recognition structures of sugar-specific proteins, carboxylic group participates in entrapping and orientation of the saccharide molecule into the binding site.

The sulfonate group is also an effective proton acceptor. Recently, porphyrin sulfonates have been intensively investigated for the photodynamic therapy of cancer [6], radiological imaging [7], self-assembly modeling and other applications. Tetrasulfonate derivatives of resorcinol cyclic tetramer showed the expressed complexation with saccharides in water [4].

We present complexation studies of 4-(2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-1,5,9-(*p*-phosphonomethyl phenyl)–porphyrin-5-yl) benzylphosphonate (**1**, **2**) [8] and porphyrin-peptide conjugates (**3–5**) in comparison with porphyrin monocarboxylate (**6**) and the meso-tetrakis-(*p*-sulfonato-phenyl) porphyrin (**7**). These compounds are designed as artificial receptors for the saccharide recognition and can serve as recognition and signaling units of chemical sensors.

## 2. Experimental

Fig. 1 shows the structures of the hosts (**1–7**) possessed proton donor/acceptor groups. The host (**1**) showed the absorption at 403 nm in DMSO, the host (**2**) at 430 nm in H<sub>2</sub>O. The planar surface of the porphyrin macrocycle favors stacking interactions in solution, which leads to the formation

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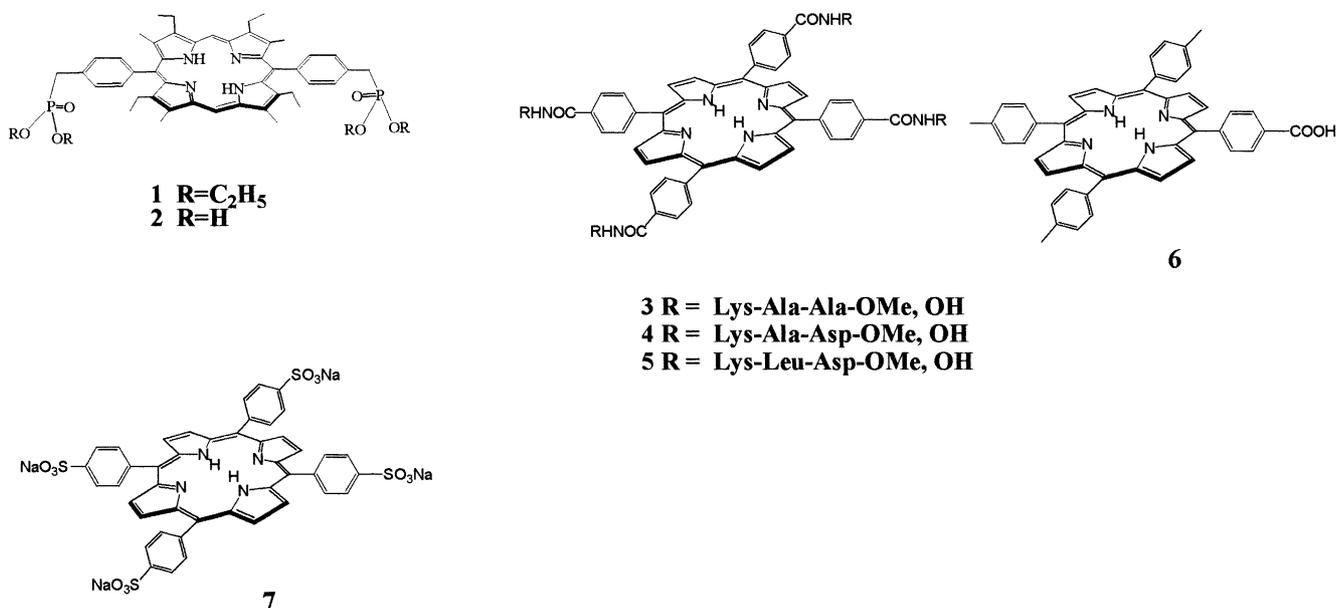


Fig. 1. Structures of water soluble receptors (1–7).

aggregates [9]. The polar groups (sulfonic, carboxylic, amino, phosphonic or ammonium groups) which are attached around the porphyrin core can exhibit a strong intermolecular aggregation with other porphyrin molecules in water in the absence or the presence of inorganic salts [10,11]. Binding to macromolecules in the solution also evolves the aggregation of some water-soluble porphyrins. The formation of the aggregates changes the optical properties of porphyrins. This can be easily observed by the UV–VIS or fluorescence spectroscopy [12]. Beer's law deviation and the increase in the width of absorption bands indicated the formation of the porphyrin aggregates at high concentration. Dilution of the (1–7) aggregate solutions results in disaggregation. In the present work we carried out optical measurements at the porphyrin concentration  $6.15 \times 10^{-6}$  mol/l, where no deviation from Beer's law was observed.

### 2.1. Preparations

The preparation of porphyrin phosphonates and monocarboxylate (1, 2) and (6) was described elsewhere [9]. Meso-tetrakis(*p*-sulfonato-phenyl) porphyrin (7) was obtained from Fluka.

The preparation of porphyrin-peptide conjugates (3–5). A mixture of *p*-tetracarboxy phenyl porphyrin (0.1 g, 0.126 mM) and oxalyl chloride (0.127 g, 0.63 mM) in dry CH<sub>2</sub>Cl was stirred for 1 h, then the solvent was evaporated. The obtained acylchloride was redissolved in dry CHCl<sub>3</sub>. The protected peptides H<sub>2</sub>N–Lys–Ala–Ala, H<sub>2</sub>N–Lys–Ala–Asp or H<sub>2</sub>N–Lys–Leu–Asp (0.63 mM) in dry CHCl<sub>3</sub> with excess of triethylamine were added dropwise. The reaction mixture was stirred for 48 h, the solvent was evaporated and conjugate was isolated by column chromatography on silica gel (CH<sub>2</sub>Cl:MeOH, 9:1) and deprotected. The yield was 70%.

Conjugate (3): <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>, δ<sub>H</sub> ppm), 8.85 (s, β-pyrrole); 8.19–8.05 (m, phenyl, *o*- and *m*-); 4.8–4.25 (m, methylen); 1.23 (s, methyl); –2.91 (s, NH-porphyrin). FAB MS: for C<sub>96</sub>H<sub>118</sub>N<sub>20</sub>O<sub>20</sub> *m/z* calculated 1872.09; found 1874.

Conjugate (4): <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), 8.86 (β-pyrrole); 8.2 (m, phenyl, *o*- and *m*-); 5.16–3.31 (m, methylen); 1.31 (m, methyl); –2.85 (s, NH-porphyrin). FAB MS: for C<sub>100</sub>H<sub>122</sub>N<sub>24</sub>O<sub>24</sub> *m/z* calculated 2044.19; found 2045.

Conjugate (5): <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), 9.25–7.85 (m, phenyl, β-pyrrole); 5.00–2.50 (m, methylen); 1.29 (m, methyl); –2.90 (s, NH-porphyrin). For C<sub>106</sub>H<sub>131</sub>N<sub>19</sub>O<sub>26</sub> *m/z* calculated 2087.29; found 2088.

### 2.2. Apparatus and procedures

Absorption spectra were recorded with of 'Cary 400 scan' spectrometer. <sup>1</sup>H and <sup>31</sup>P NMR spectra were measured using a 500 MHz 'Bruker' spectrometer. IR spectra were obtained on FTIR spectrometer 'Nicolet 210'. Raman spectra were recorded on 'Bruker EQUINOX 55/S' spectrometer. Surface plasmon resonance (SPR) measurements were carried out on 'Spreeta' miniature integrated liquid sensing system (Texas Instruments Inc.). The *K<sub>a</sub>* values were determined by the least squares curve fitting for 1:1 complexes. The mixture methanol/water, 95:5 and DMSO were used as solvents, respectively.

## 3. Results and discussion

### 3.1. Porphyrin phosphonates (1, 2)

Binding studies of the receptors were carried out in DMSO for (1) and in methanol/water 95:5 mixture for

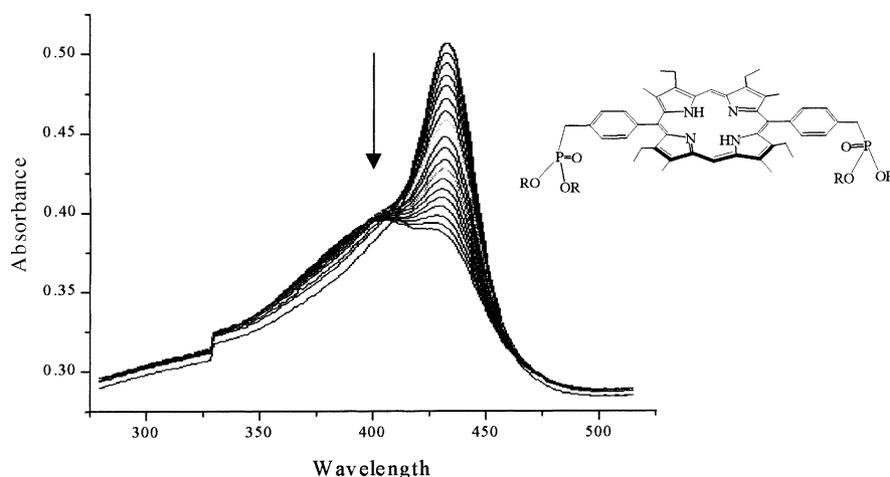


Fig. 2. Typical UV–VIS spectral changes of (2); interaction of (2) with  $\alpha$ -D-glucose;  $\lambda_{\text{max}} = 430$  nm.

(2). Alkyl and aryl pyranosides were tested for the complexation with the water insoluble receptor (1). Several mono- and oligosaccharides were used for the complexation with the water soluble receptor (2). The host (1) showed the Soret maximum in UV–VIS region at 403 nm in DMSO; the host (2) at 430 nm in water (Fig. 2). The measurements indicated preferable binding of (1) to the long or bulky side chain substituted saccharides in comparison to octyl–methyl analogues (Table 1). It indicates the influence of hydrophobic CH– $\pi$  and  $\pi$ – $\pi$  interactions.

The water soluble host (2) showed strong interaction with different saccharides (Table 1). The receptor (2) also displayed stronger binding to  $\alpha$ -D-glucose and D-fructose. The values of association constants calculated for di and trisaccharides are slightly higher than for majority of monosaccharides. Recently, a binding mode between macrocyclic phosphonates and saccharide species has been proposed [7].

The mechanism of interaction includes the formation of hydrogen bonds between vicdiol of pyranose cycle and the two oxygen atoms of corresponding phosphonate group [4]. We suppose that decisive role in porphyrin–saccharide complex formation between (1) and (2) and vicinal diol segment of saccharide play to hydrogen bonds. The  $^1\text{H}$ ,  $^{31}\text{P}$  NMR and IR investigations clearly indicated an involvement of phosphonate and saccharide hydroxylic groups in the process of the complex formation.

### 3.2. Porphyrin-peptide conjugates (3–5)

We examined several peptide sequences attached to the porphyrin core as a recognition element for saccharides binding. Porphyrin-peptide conjugates (3–5) were tested in the above described water/methanol system. The addition of saccharide to the macrocycle solution was accompanied

Table 1

Association constants for binding of saccharides with receptors in DMSO (1) and water (2–7, UV–VIS titration)<sup>a</sup>

Saccharide	Association constant ( $K_a$ ), $10^2$ ( $\text{M}^{-1}$ )					
	(1)	(2)	(3)	(4)	(5)	(7)
Octyl $\alpha$ -D-glucopyranoside	51.11					
Octyl $\beta$ -D-glucopyranoside	48.90					
Methyl $\alpha$ -D-glucopyranoside	11.50					
Methyl $\beta$ -D-glucopyranoside	10.90					
<i>p</i> -Nitrophenyl galacto- $\beta$ -pyranoside	60.00					
D-Galactose		135.00	1.20	0.68	0.35	1.35
$\alpha$ -D-Glucose		190.00	0.22	1.40	0.29	1.20
D-Fructose		233.00	0.51	2.78	0.63	1.00
D-Ribose		60.00	1.26	1.00	0.11	1.00
D-Trehalose		194.00	2.74	4.53	0.67	2.00
$\alpha$ -D-Lactose		213.00	2.09	4.08	0.97	2.13
$\beta$ -D-Lactose		210.10	1.48	1.48	0.33	2.20
Maltotriose		200.20	3.15	4.85	1.00	2.00

<sup>a</sup> The formation and UV–VIS estimation constants of sugar-receptor complexes. In a 1 cm square quartz cuvette was placed  $6.15 \times 10^{-6}$  M solution of macrocycle in DMSO or  $\text{H}_2\text{O}$  contained 5% of MeOH (v/v). A known amount of saccharide was added in increments (0–100 equivalents; the solution contained the same concentration of receptor as in cuvette). The absorbance changes were measured at the absorption maxima (room temperature), and data were then evaluated with the aid of the least squares curve fitting. The  $K_a$  calculated for 1:1 complexes. The reproducibility of the  $K_a$  values was  $\pm 10\%$  in triplicate runs.

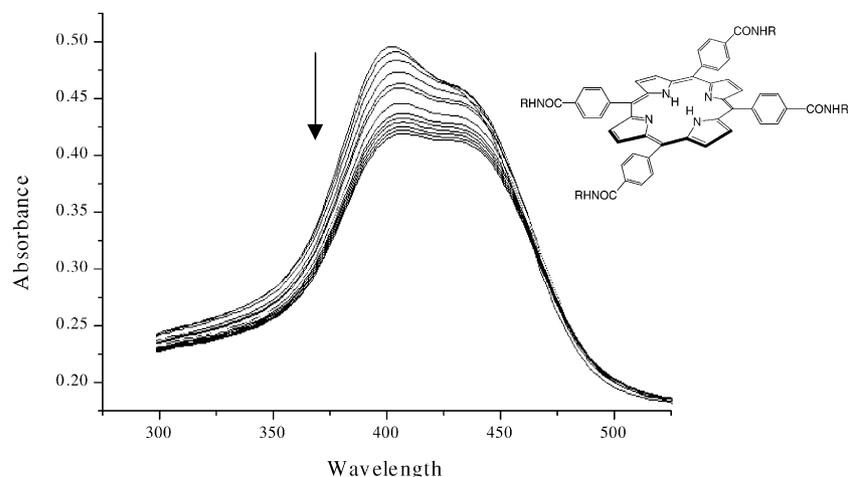


Fig. 3. Typical UV–VIS spectral changes of (3–5) in water;  $\lambda_{\max} = 403$  nm. Interaction of (3) with  $\alpha$ -D-glucose.

with change of Soret maximum intensity (Fig. 3). The maximum in UV–VIS region for (3) is at 403 nm, for (4) at 406 nm, and for (5) at 407 nm. All three receptors display higher specificity to di and trisaccharides except for  $\beta$ -D-lactose (Table 1). The interaction of (3–5) with saccharides was investigated also by  $^1\text{H}$  NMR spectroscopy in  $\text{DMSO-d}_6$  and  $\text{CDCl}_3$ . This method allow to determine the shift of saccharide OH group proton signals caused by complexation. The association constants calculated for Asp-contained (5) are lower than that for (3) and (4). Nevertheless, (5) is able to extract  $\alpha$ -D-lactose from aqueous media to  $\text{CHCl}_3$ . This fact was confirmed by  $^1\text{H}$  NMR investigation where splitting and broadening of signals of amino, amido and CH– groups of peptide tails were monitored. Comparative  $^1\text{H}$  NMR investigation of complexation of the monocarboxylic porphyrin derivate (6) [8] to  $\alpha$ -D-octylglucopyranoside showed broadening and shifting of OH proton signals. The IR spectroscopy also clearly showed involvement of saccharide OH– and carboxylate group of receptor in the interaction process.

The ability of receptors (3, 4) to bind saccharides has been employed by SPR technique. Receptors (3, 4) were adsorbed on the gold surface of the optical sensor and used for determination of binding of the  $\alpha$ -D-glucose in water solution (3–5 M). The differences in the refractive index values due the sensor surface (so-called SPR curves) with adsorbed free receptor and receptor–saccharide complex were monitored. The results are summarized on Figs. 5 and 6. We found significant changes in SPR spectra as a result of saccharide complexation. This effect indicates interaction and can serve for saccharide sensing. Control experiments with tetraphenyl porphyrin (TPP) showed no changes in the SPR curves sensor before and after treatment of glucose solution (Fig. 7).

### 3.3. Porphyrin sulfonate (7)

Interaction of the receptor (7) with carbohydrates in water can be easily monitored by UV–VIS spectroscopy (Fig. 4).

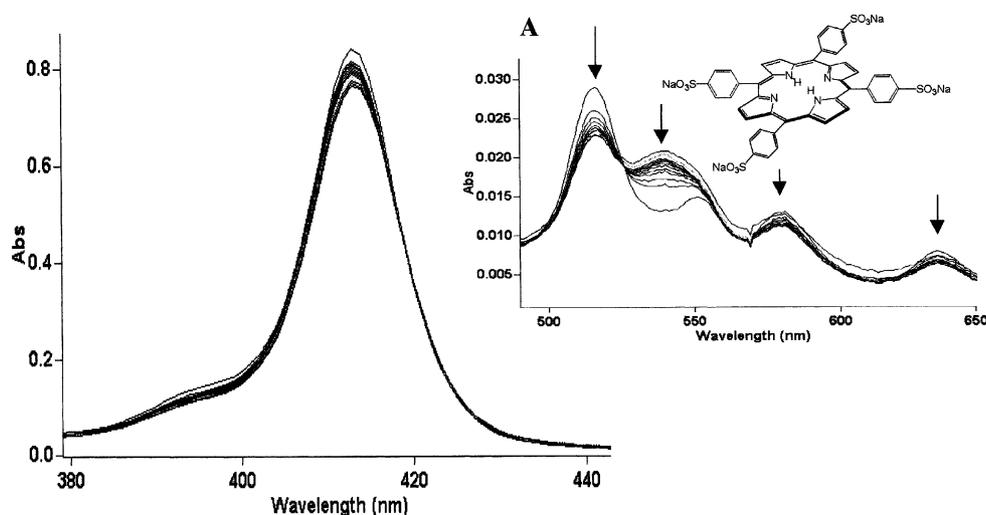


Fig. 4. UV–VIS spectral changes of the receptor (7); interaction of the (7) with  $\alpha$ -D-glucose;  $\lambda_{\max} = 413$  nm. A: spectral changes at Q-bands.

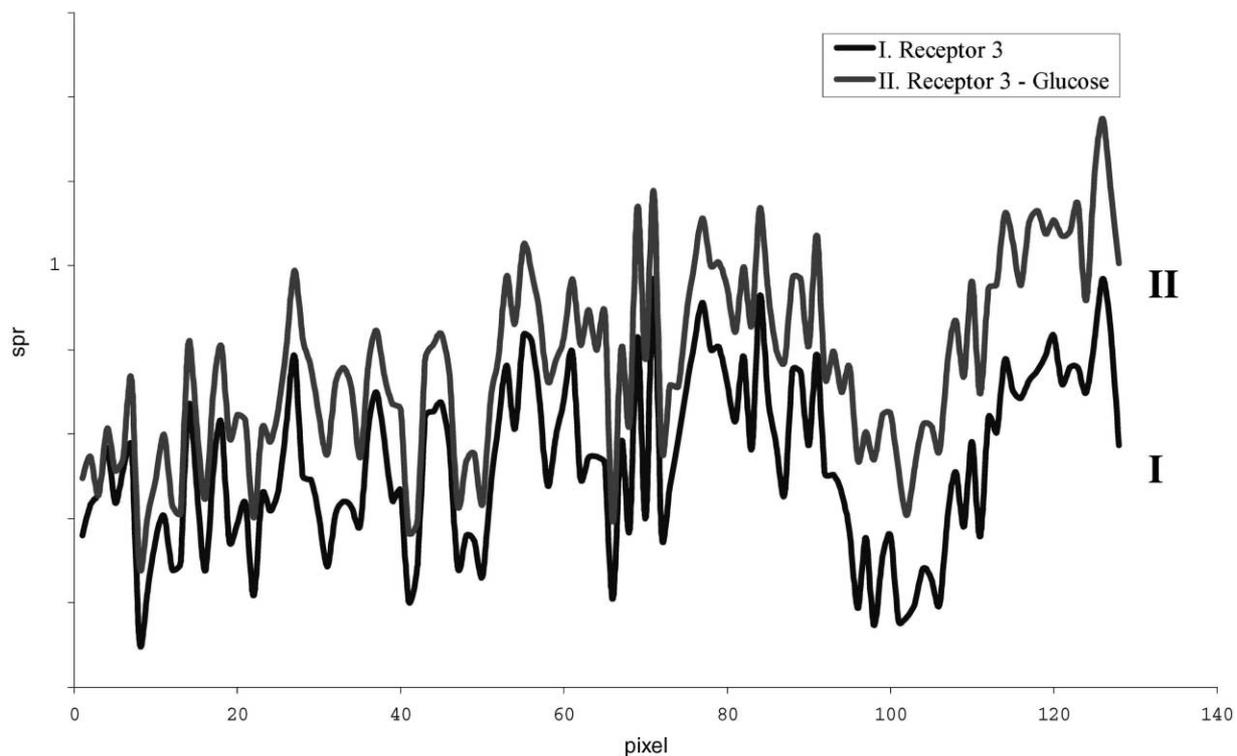


Fig. 5. Interaction of the receptor (3) with  $\alpha$ -D-glucose on the gold surface monitored by surface plasmon resonance technique. A plot of reflects light intensity versus pixel (SPR curves) for starting compound (3) (curve I) and for complex (curve II).

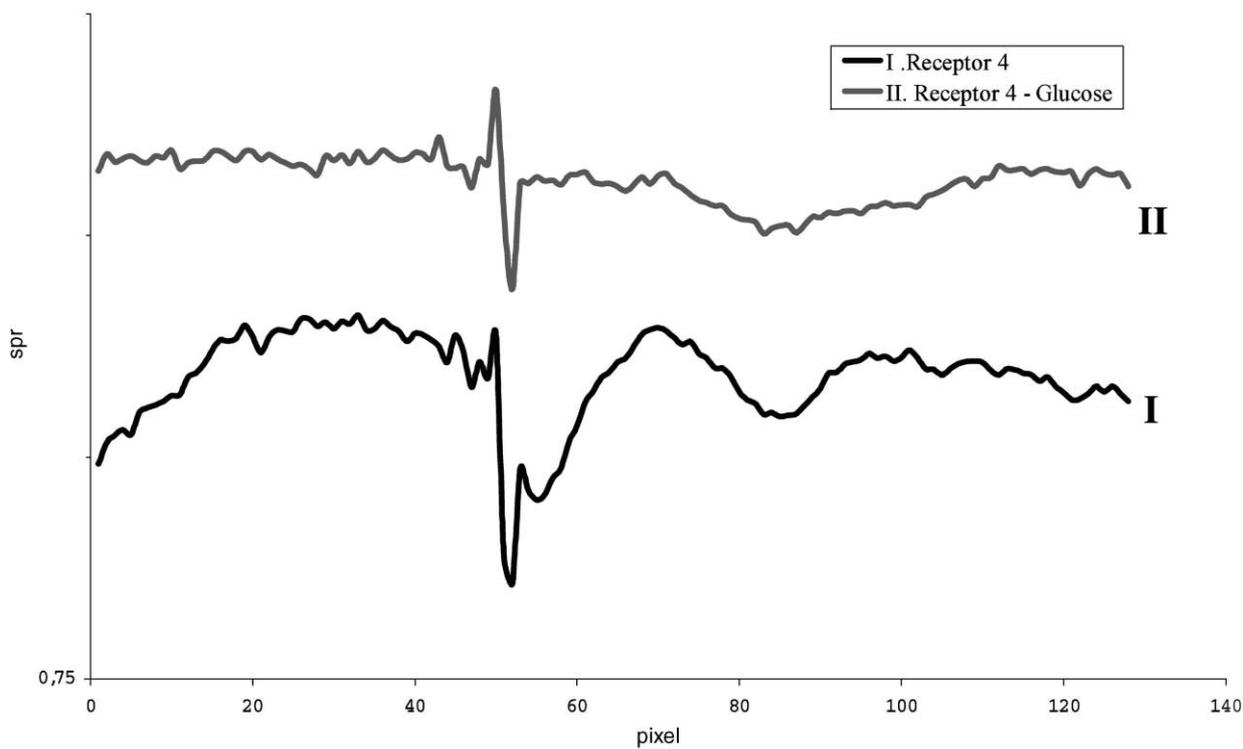


Fig. 6. Interaction of the receptor 4 with  $\alpha$ -D-glucose on the gold surface monitored by surface plasmon resonance technique. A plot of reflects light intensity versus pixel (SPR curves) for starting compound 4 (curve I) and for complex (curve II).

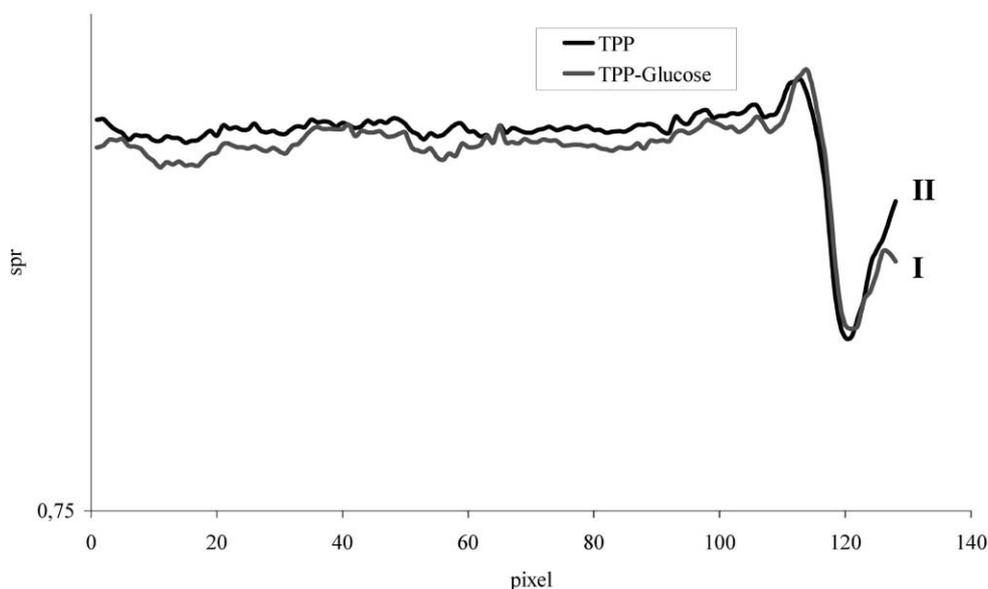


Fig. 7. Control experiment. Treatment of tetraphenyl porphyrin (TPP) by  $\alpha$ -D-glucose on the gold surface (no interaction). A plot of reflects light intensity versus pixel (SPR curves) for TPP before (curve I) and after treatment (curve II).

The addition of saccharide species was accompanied by the intensity changes of Soret band (at 413 nm) and the red shift of the maxima. The  $^1\text{H}$  NMR monitoring of host–guest interaction in  $\text{DMSO-d}_6$  showed the down shift of saccharide CH– and OH protons. The investigation by Raman spectroscopy of 7- $\alpha$ -D-glucose complexes also showed a significant shift CH– and CO– resonances that indicates the interaction.

#### 4. Conclusions

Several water soluble porphyrins were proposed as suitable receptors for the recognition of neutral biomolecules. Different anionic groups as phosphonate and sulfonate provide strong hydrogen donating/accepting effect and are able to form effective bonds with saccharides in water. Porphyrin-peptide conjugates also form complexes with saccharides, where carboxylic and amido groups are important for binding. Spectroscopic methods allow to observe the binding mode of the complex formation. Binding effect can be monitored with optical SPR sensor.

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*Vladimír Král* received his MS degree from Charles University, Prague, in 1974. He obtained his PhD degree under the supervision of Professor Z. Arnold in the Czech Academy of Sciences, Prague. After graduation, he worked as research scientist at the Institute of Organic Chemistry and Biochemistry in Prague. His postdoctoral experience have been proceed in the Academy of Science, Russia (Professor L.A. Yanovskaya, Moscow, 1988–1989), University of Kuopio, Finlandia (Professors P. Kauranen and Laatikainen, Kuopio, 1983), Max Planck Institute, BRD (Professor H.A. Staab, Heidelberg, 1988–1989), University of Texas at Austin (Professor J.L. Sessler, 1992–1995). At present, he is an associated professor at the Institute of Chemical Technology, Prague. His research interest focused on the synthesis of the macrocyclic compounds and chemical sensors development.

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