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Interaction of porphyrin and sapphyrin macrocycles with nucleobases and nucleosides Spectroscopic, quantum chemical and chromatographic investigation

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Abstract

Novel oligopyrrole macrocycle-based sorbents were prepared via amide covalent bonding of carboxylic acids of porphyrin and sapphyrin derivatives to 3-aminopropyl silica. Covalent attachment of Cu(II)-tetraphenylporphyrin formyl-derivative was performed by amine linkage. Prepared sorbents were characterized by Raman spectroscopy and elemental analysis. Retention behavior of nucleobases and nucleosides was investigated by HPLC. UV–VIS and ^{1}H NMR titrations were used to study the role of oligopyrrol macrocyclic receptors for selective recognition of adenine, cytosine, thymine and uracil and their nucleoside forms. The spectroscopic results were compared with quantum chemical calculations at semiempirical level and with the data obtained by HPLC measurements. Obtained data suggest that an aromatic π–π stacking interactions govern the HPLC separation. A different behavior was found for purine and pyrimidine bases. An introduction of Cu(II) into porphyrin macrocycle led to a dramatic increase of separation efficiency, probably due to an increase of interaction energy.

Keywords: HPLC; Porphyrin; Metalloporphyrin; Separation; Non-covalent interaction

1. Introduction

Studies of molecular interactions contribute to understanding of fundamental intermolecular forces, mimick biological functions and stimulate development of supramolecular devices. In the last decade, attention of many chemists has turned to non-covalent complexes because of their importance in life sciences. Ultraviolet–visible (UV–VIS) and nuclear magnetic resonance (NMR) spectroscopies are traditionally used for investigation of non-covalent complexes based on hydrogen bonds, aromatic π–π, hydrophobic, London dispersive and dipole–dipole interactions of a receptor with a substrate. However, such interactions studied by above-mentioned

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techniques take place in homogenous environment while many biologically important systems involve interactions at interface.

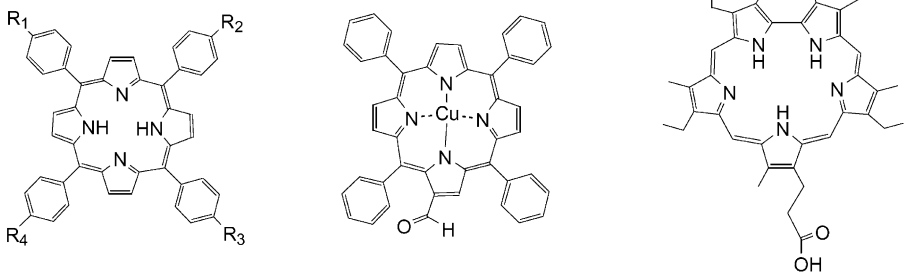
Successful applications of oligopyrrolic macrocycles in medicine stimulate studies of their interactions with biologically important analytes, including nucleobases and nucleosides. Nucleobases and nucleosides are essential parts of nucleic acids and enzyme cofactors, e.g. in the case of defects in purine or pyrimidine metabolisms severe symptoms, such as mental retardation or cardiovascular diseases were observed [1].

Effective high-performance liquid chromatography (HPLC) separation requires high selectivity of the interaction between the stationary phase and the analytes of interest in the mobile phase. A convenient approach which can lead to increasing of the strength and selectivity of such interaction is based on a covalent attachment of a receptor to a solid support [2–4]. The covalent attachment of the receptor opens unique possibility for a deeper mechanistic insight and provides additional information about the heterogeneous interaction processes. This concept is now frequently used and HPLC proved to be a suitable tool for such a purpose [5].

It has been shown that certain porphyrins and metalloporphyrins covalently linked to silica [6] and used as stationary phases in HPLC [7–9], exhibit selectivity towards planar aromatic solutes. Higher retentions of planar versus non-planar aromatic solutes on these sorbents may reflect more extensive π–π overlap of planar aromates with porphyrin macrocycles. Subsequent metallation of immobilized porphyrin macrocycle, which alter electron density, influences the π–π interactions and hence the selectivity [7,9,10].

Stationary phases based on other oligopyrrolic macrocycles have also been prepared. Sapphyrins and calixpyrroles covalently bound to silica were designed for the separation of anionic solutes [11–15]. Where the key mode of the interaction involves a specific chelation between positively charged sapphyrin core and oxoanions, e.g. phosphates [12,16].

Here we present the study of interactions of selected nucleobases (adenine, cytosine, thymine and uracil) and nucleosides (adenosine, cytidine, thymidine and uridine) with tetraphenylporphyrin derivatives (**1**), Cu(II)-3-formyl-5,10,15,20-tetraphenylporphyrin (**2**) and sapphyrin derivative (**3**) (Fig. 1). Interactions were investigated by UV–VIS and ^{1}H NMR titrations in solution and the results were compared with theoretical calculations of geometry and energy and with the chromatographic data obtained by separation on these oligopyrrolic macrocycles covalently attached to silica. The latter technique, which necessitates a covalent attachment of a given selector onto a solid surface, offers a significant advantage in comparison to the UV–VIS and NMR experiments accomplished



1a: $R_1, R_3 = COOCH_2CH_3$; $R_2, R_4 = CH_3$

1b: $R_1 = COOH$; $R_2, R_3, R_4 = CH_3$

1c: $R_1, R_2, R_3, R_4 = CH_3$

1d: $R_1, R_2, R_3, R_4 = SO_3^-Na^+$

2

3

Fig. 1. Structures of oligopyrrolic macrocycles.

in solution since chromatography allows to study also properties of formed complexes otherwise inaccessible in solution because of aggregation, low solubility, etc.

Elemental analysis has been widely used for many years for the confirmation of individual synthetic steps in the process of sorbent preparation. However, it has been demonstrated that a combination of elemental analysis with Raman spectroscopy is a significantly more efficient tool for a sensitive and reliable sorbent characterization [17]. Hence, both techniques were employed in this work [18].

2. Experimental

2.1. Chemicals

Nucleobases (adenine, cytosine, thymine, and uracil) and nucleosides (adenosine, cytidine, uridine and thymidine) were used as purchased from Sigma. Deuterium oxide (D_2O), dimethylsulfoxide, deuterated dimethylsulfoxide (DMSO-d_6), methanol (MeOH), *N,N′*-dimethylcarbodiimide, trimetylsilylimidazole, 1-hydroxybenzotriazol, and 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS) were purchased from Aldrich. All other chemicals were of 95% purity or higher. Ethylester of dicarboxyphenyl-substituted porphyrin, 5,15-bis(4-carboxyphenyl)-10,20-bis(4-methylphenyl)porphyrin ethylester (**1a**), monocarboxyphenyl-substituted porphyrin, 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl)porphyrin (**1b**), and 5,10,15,20-tetrakis (4-methylphenyl)porphyrin (**1c**) were synthesized according to the method published previously [19,20]. About 5,10,15,20-tetrakis(4-sulphonatophenyl)porphyrin tetrasodium salt (**1d**) was purchased from Aldrich.

Synthesis of Cu(II)-3-formyl-5,10,15,20-tetraphenylporphyrin (**2**) was based on formylation of Cu(II)-5,10,15,20-tetraphenylporphyrin with formylating reagent prepared from phosphorus oxychloride and dimethylformamide [21].

Synthesis of the monocarboxylic acid derivative of sapphyrin, 3,8,17,22-tetraethyl-12-(2-carboxyethyl)-2,7,13,18,23-pentamethylsapphyrin (**3**), followed a previously published procedure [22,23].

2.2. Sorbents

All HPLC stationary phases prepared and tested in this work were derived from aminopropyl silica (SGX NH_2, Tessek Ltd., Czech Republic) by an appropriate modification. Physico-chemical characteristics of the starting 3-aminopropyl silica were as follows: particle diameter 7 μm, mean pore size 8 nm, pore volume 1.0 ml g^{-1}, specific surface 500 m^2 g^{-1}, 5.20% carbon, this carbon loading corresponds with 3.15 μmol NH_2 groups per square meter of silica.

2.2.1. Preparation of Si-sorbent

Free residual silanol groups on the surface of the starting 3-aminopropyl silica were silanized in a solution of trimethylsilylimidazole as silylating agent in dry 1,2-dichloroethane at 70°C for 8 h.

2.2.2. Preparation of *1b*-sorbent and *3*-sorbent

Detailed description of synthetic route used for immobilization of **1b** and **3** was published elsewhere [14]. About 2.0 g of 3-aminopropyl silica was suspended in a solution of dry dichloromethane, dry pyridine, and 4-dimethylaminopyridine. The macrocyclic carboxylic acid (0.05 mmol) was dissolved in dry dichloromethane and activated with *N,N′*-diisopropylcarbodiimide and 1-hydroxybenzotriazole at room temperature for 90 min. The latter solution was slowly added to the 3-aminopropyl silica slurry referred above. The resulting reaction mixture was stirred at room temperature for 4 days. Then the silica was filtered off, washed with dichloromethane, methanol, water, methanol, and dichloromethane, and dried under high vacuum. Sorbents based on porphyrin and sapphyrin where marked as **1b**-sorbent and **3**-sorbent, respectively.

Samples of **1b**-sorbent and **3**-sorbent were subjected to the elemental analysis giving 7.35 and 8.23% carbon, respectively, it corresponds with the calculated selector coverage (according to ref. [24]) ∼0.077 μmol m^{-2} and ∼0.131 μmol m^{-2} for the respective sorbents.

2.2.3. Preparation of *2*-sorbent

The attachment of the selector was based on reaction of formyl group of **2** with aminogroup of 3-aminopropyl silica. The initially formed imine was

reduced by sodium borohydride giving the amine linkage.

3-Aminopropyl silica (3 g) was added to the solution of **2** (17 mg, 0.071 mmol) in dry acetonitrile (30 ml). Reaction mixture was slurred at room temperature for 1 day. Then sodium borohydride (100 mg, 2.6 mmol) was slowly added and the resulting mixture was slurred for next 25 h, filtered through steel filter, washed with chloroform, methanol, water, methanol, and chloroform (20 ml of each solvent) and dried in vacuum. The result of the elemental analysis of **2**-sorbent gave 7.29% carbon corresponding to the selector coverage 0.080 μmol m^{-2} silica.

The procedure described above was also used for the preparation of **2**-sorbent 2 and **2**-sorbent 3 with 8 mg and 40 mg of **2** reacted, respectively. The elemental analysis of carbon contents showed 6.86% for **2**-sorbent 2, corresponding to the selector coverage 0.063 μmol m^{-2} silica, and 8.73% for **2**-sorbent 3, corresponding to the selector coverage 0.137 μmol m^{-2} silica.

Free residual silanol groups on all synthesized sorbents were finally protected using the same protocol as mentioned above (see preparation of Si-sorbent).

2.3. Methods

2.3.1. UV–VIS spectrophotometry

All experiments were carried out on Cary 400 Scan, (Varian Ltd, Australia) at room temperature. Two solvents were used: dimethylsulfoxide (DMSO) and a mixture of DMSO–water (1:1, v/v). UV–VIS titration was performed with constant concentration of the macrocycle (usually in the range $(1–6) \times 10^{-6}$ mol l^{-1}) by adding the solution of nucleobase or nucleoside. Association isotherms for all compounds studied were used for the estimation of association constants from the Scott's plot [25].

2.3.2. ^1H NMR spectroscopy

Varian NMR spectrometer, model Gemini 300HC was used, with working frequency 300.075 MHz, deuterium lock, temperature 298 K and 5 mm NMR tubes for all measurements. One-dimensional ^1H NMR spectra were acquired using a 45° pulse (10 μs), spectral width 6000 Hz, acquisition time 1.779 s, and typically with 64 accumulations. Deuterium oxide was used as a solvent and all spectra were referenced to DSS.

2.3.3. Raman spectroscopy

Raman spectra were collected using a Fourier-transform near-infrared (FT-NIR) spectrometer Equinox 55/S (Bruker, Germany) equipped with an FT Raman module FRA 106/S (Bruker). The focused laser beam (250 mW) of Nd:YAG laser (1064 nm, Coherent) irradiated the samples of sorbents in glass vials placed on motorized *X-Y-Z* sample stage. Defocused laser beam with reduced laser power (50 mW) was used to measure spectra of pure macrocyclic receptors. Scattered light was collected in the backscattering geometry. Interferograms were obtained with a quartz beamsplitter and a Ge detector (liquid N_2 cooled). Typically 128 of accumulated interferograms were processed by the Fourier transformation with Blackman–Harris 4-term apodization and a zerofilling factor of 8 in order to obtain individual FT Raman spectra with a 4 cm^{-1} resolution.

2.3.4. Quantum chemical calculations

Gaussian program package [26] was used for the estimation of the binding energy and complex geometry, using the PM3 model. The calculations were carried on personal computer (2 × 700 MHz CPU, 528 MB RAM).

2.3.5. HPLC

The HPLC system consisted of a Picron LC 4000 ternary pump (Picron, Czech Republic), an Ingos six-way injection valve (Ingos, Czech Republic) with a 20 μl sample loop and a Picron LCD 4000 variable-wavelength UV–VIS detector. Bases of nucleic acids and nucleosides were detected at 260 nm. DataAPEX 3.1 integration software (DataAPEX, Czech Republic) was used for data processing.

All modified silica columns were packed by the slurry method. Each of the prepared stationary phases (about 1.0 g) was slurried in 35 ml of 2-propanol. After thorough sonication, the slurry was transferred into the packing assembly of a high-performance liquid chromatography column packing system (Academy of Sciences, Czech Republic). The sorbents were packed into 80 mm × 4.0 mm stainless steel column blanks (Tessek, Czech Republic) by the down fill technique with 500 ml of acetone under pressure

60 MPa. Column dead times t_0 were estimated from a dip on chromatograms after injection of pure solvent (at 0.28 ml min^{-1}) and were as follows: Si-sorbent (1.6 min), **1b**-sorbent (2.0 min), **2**-sorbent (1.7 min), **2**-sorbent 2 (1.6 min), **2**-sorbent 3 (1.7 min), **3**-sorbent (1.5 min).

Sample solutions (0.5 mg ml^{-1}) were prepared by dissolving pure substances in the mobile phase. Full loop technique was used for injection. The mobile phase consisted of a mixture methanol/phosphate buffer (1:1, v/v). Phosphate buffer was prepared by dissolving sodium dihydrogenphosphate in deionized water to reach the final concentration 10 mmol l^{-1}, pH was adjusted at 5.5 with a solution of sodium hydroxide. Buffer solution was filtered through 0.45 μm membrane filter before mixing with methanol. Finally, the mobile phase was degassed by sonication.

3. Results and discussion

3.1. UV–VIS study

In order to achieve the nucleobase- and nucleoside-recognition it is necessary to endow the receptor molecules with binding sites capable of selective interaction with the nucleobase or the sugar moiety. Such interaction may be mediated by hydrogen bonding with sites capable of forming complementary hydrogen bonding patterns and/or by stacking with a π-system of the host compound.

The association constants for each nucleobase and nucleoside with macrocycles can be determined by UV–VIS spectroscopy because of the unique absorption properties of the oligopyrrolic macrocyclic ring. Because of the above-mentioned necessity of homogenous environment, the macrocycles used in the UV-study were not exactly of the same structure as those immobilized on silica in HPLC experiments. The structures **1a**, **2**, **3** are shown in Fig. 1.

As can be seen from measurements of association constants of the selected nucleobases with the oligopyrrolic compound **3** in DMSO (Table 1) the highest constant was obtained for cytosine, followed by thymine and adenine, and the smallest interaction was obtained for uracil. Absorption spectra and the binding isotherm measured during titration of **3** with cytosine are given in Fig. 2.

Titration of **3** with nucleosides in DMSO indicated only interaction with cytidine because of the weak response for the other nucleosides. This can be explained by the spatial fitting of cytosine into the saphyrin core, even thought it is bearing hydrophilic and bulky sugar moiety. The association constant of cytidine with **3** was more than 13 times lower than that for cytosine.

Completely different situation was observed during UV–VIS experiments carried out in more polar DMSO–water (1:1 v/v) solution. Here, the aromatic π–π interactions become stronger. It is evident from Table 1 that the introduction of Cu(II) (compound **2**) plays an important role in nucleobase or nucleoside interactions with the macrocycle. The highest association constant was achieved for adenine, this is in a good correlation with the HPLC results (see below). Cytosine, uracil and thymine had comparable association constants. Significantly lower

Table 1
Association constants (l mol^{-1}) for oligopyrrolic receptors with nucleobases and nucleosides in DMSO or DMSO–water (1:1 v/v) mixture

	Solvent			
	DMSO	DMSO–water		
	Receptor **3**	Receptor **1a**	Receptor **2**	Receptor **3**
Adenine	2.5×10^4	[a]	1.0×10^6	[a]
Thymine	5.6×10^4	1.4×10^4	4.1×10^5	[a]
Cytosine	4.1×10^5	[a]	3.5×10^5	1.3×10^3
Uracil	2.6×10^3	7.6×10^3	1.6×10^5	[a]
Adenosine	[a]	[a]	3.7×10^4	[a]
Thymidine	[a]	[a]	2.7×10^4	4.5×10^4
Cytidine	3.0×10^4	[a]	7.8×10^4	[a]
Uridine	[a]	7.7×10^5	7.2×10^4	4.4×10^4

[a] Possible aggregation, complicated evaluation of 1:1 complexes.

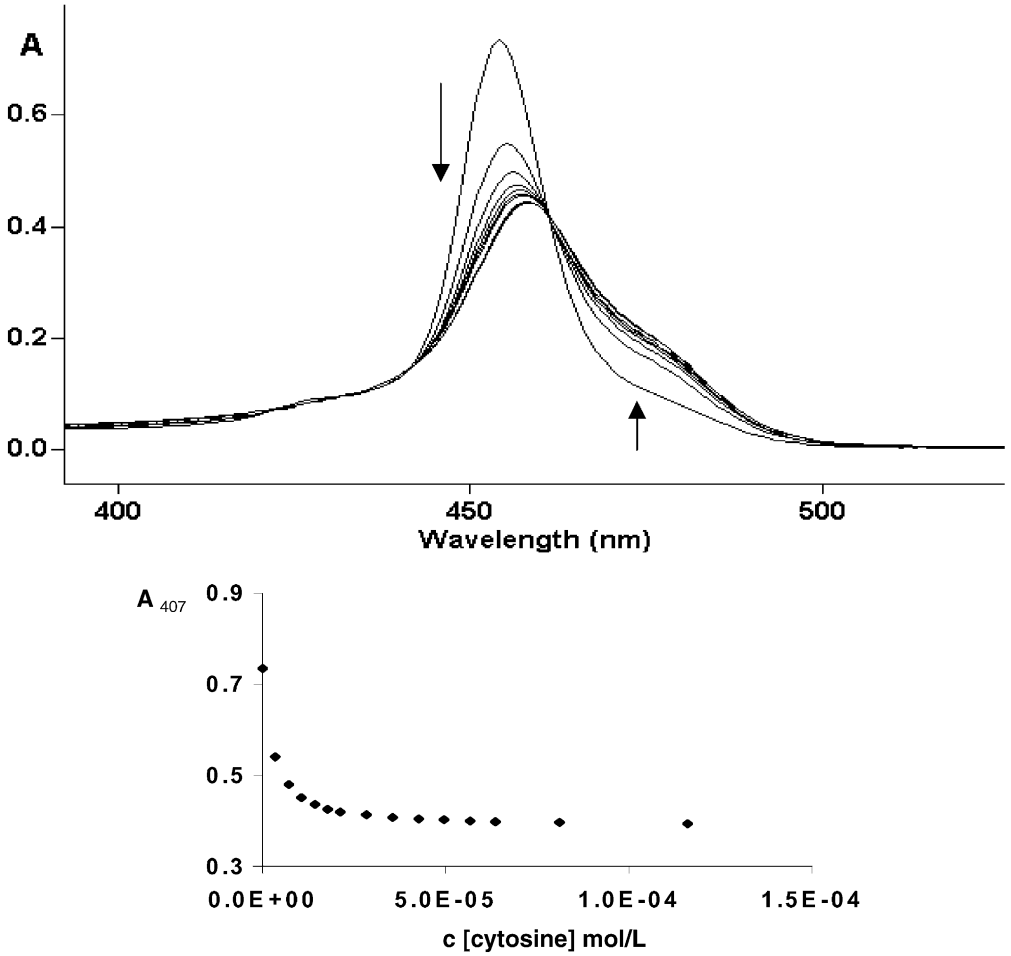


Fig. 2. Absorption spectra of **3** (5.3×10^{-6} mol l^{-1}) with cytosine ($c = 0$–1.2×10^{-4} mol l^{-1}) in DMSO at 25°C (top). Binding isotherm obtained by addition of cytosine to **3** in DMSO at 407 nm.

values of association constants were obtained for nucleosides.

UV–VIS experiments in DMSO–water (1:1 v/v) solution carried out with the receptor **1a** indicate some selectivity toward uridine, modest selectivity for thymine and uracil. Uridine seems to fit into the cavity of **1a** in a suitable conformation. On the other hand, purine-like structures, adenine and adenosine seem to be too large and sterically unsuitable to interact with **1a**.

For the interaction of receptor **3** with the tested biomolecules in the mixed solution, the situation is similar as in the case of **1a**. We obtained measurable constants only for uridine and thymidine, having approximately the same values and for cytosine with one order of magnitude lower constant.

3.2. Calculations

In order to estimate complex geometry as well as approximate energy involved in the complexations the PM3 method [24,27] was used. In spite of the approximations involved in the PM3 model, we suppose that the calculations relatively faithfully describe the forces involved in molecular interactions and provide the rough estimate of the energy. The more accurate

Table 2
Calculated energies (kJ) and corresponding association constants[a] calculated for **1c**-nucleobase complexes by the PM3 method

	ΔE	K
1c·Adenine	−19.6	3.0×10^3
1c·Thymine	−18.0	1.5×10^3
1c·Cytosine	−25.1	2.8×10^4
1c·Uracil	−14.2	3.3×10^2

[a] $K = \exp(\Delta E/(RT))$; $T = 295\,\mathrm{K}$; $R = 8.314\,\mathrm{J\,K^{-1}\,mol^{-1}}$.

ab initio computations would require also an appropriate solvent model [28] and are currently inaccessible for systems of this size with our computational equipment.

Geometry of **1c** (receptor), nucleobases (substrates) and receptor–substrate complexes were optimized by energy minimization. Because we expected that the π–π interaction would be the most favorable interaction, the starting geometry was co-planar orientation of aromatic core of porphyrin and nucleobases. Stabilization energy of the complexes (ΔE) were estimated from electronic energy without vibrational correction, which we consider to be minor because of the similarity of vibrational modes in complexes and separated ligands. The energy and corresponding association constant are listed in Table 2.

While the values presented in Table 2 are probably too inaccurate for reliable prediction of relative base activities, the order of stabilization energy agrees well with the values of experimental association constants (c.f. $\Delta E \sim -RT \ln K$) listed in Table 1.

3.3. NMR titration

In order to find detailed mechanism of interaction between nucleobases and/or nucleosides (substrates) on the one side and porphyrin receptor on the other side, we performed ^{1}H NMR studies. Our goal was to determine the complexation-induced shift (CIS) of substrates caused by the porphyrin ring current. If sandwich type complexes are formed we should observe the shift of at least some analyte proton signals to higher fields.

Experiments with receptor **1a** were carried out in DMSO where no CIS was observed. Therefore, we used deuterium oxide as a solvent that possesses higher polarity than DMSO and consequently the π–π interactions should play a more important role.

This was confirmed by the experiment. Compound **1d** was chosen as a model of the tetraphenylporphyrin receptor for its high water solubility and complex formation with adenosine as a model substrate. The results of ^{1}H NMR study in D_2O suggest a strong interaction leading to a significant up field shift of protons of adenosine (Ado) over 200 Hz (spectra A–C in Fig. 3). Quantitative evaluation of NMR titration experiment gave the stability constant for the complex about $3.6 \times 10^2\,\mathrm{l\,mol^{-1}}$ using EQNMR software [29].

3.4. Sorbent synthesis

Several classes of novel sorbents were prepared, characterized and tested. Porphyrin- (**1b**-sorbent) and sapphyrin- (**3**-sorbent) derivatized silica (Fig. 4) were synthesized by the reaction of corresponding carboxyl macrocyclic derivatives, activated with diisopropylcarbodiimide, and the amine group of the aminopropyl silica [14]. Cu(II)-tetraphenylporphyrin modified silica, **2**-sorbent, was synthesized by coupling the aldehyde group on β-pyrrole of metalloporphyrin with the amino group of the sorbent. Primarily formed imine on the aminopropylated silica was reduced in the next step by sodium borohydride giving amino linkage (Fig. 5). Finally, residual silanol groups of all sorbents were protected with the silylating agent.

FT Raman spectroscopy was used to verify individual steps of the synthesis. Firstly, FT Raman spectra were measured for both non-derivatized silica and 3-aminopropyl silica. Typical bands of $–CH_2–$ groups and $–NH_2$ group and broad features of silica itself were identified (in detail ref. [17]). The FT Raman spectra of derivatized silica with macrocyclic compounds exhibit new bands in comparison with the spectrum of the initial 3-aminopropyl silica. The bands of macrocyclic compounds covalently bonded to the silica surface after subtraction of the spectrum of 3-aminopropyl silica are well distinguished as demonstrated in Fig. 6A and B. Fig. 6 shows the characteristic features of **2** (1601, 1573, 1504, 1375, 1239 1078, 1003 $\mathrm{cm^{-1}}$). The same bands of reduced intensity are observed after the silylation step (Fig. 6C). The decrease of band intensities for the derivatization agent after silylation is typical not only for the macrocyclic compounds studied in this work but also for other compounds, e.g. cloprostenol,

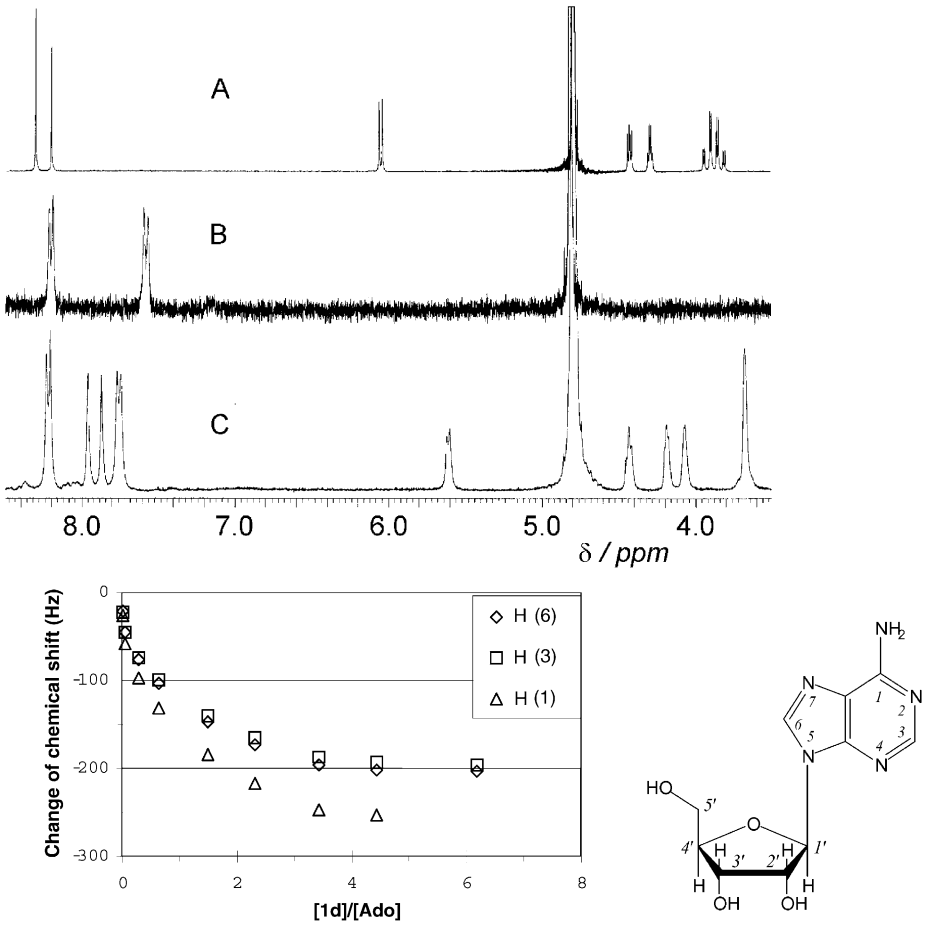


Fig. 3. ^{1}H NMR spectra of Ado (A), **1d** (B), and **1d**–Ado mixture ([**1d**]/[Ado] = 0.6) (C) in D_2O. At the bottom is the dependence of complex-induced chemical shift of three different hydrogens (for their descriptions see structure on the side) on the **1d**–Ado molar ratio. (Spectrum C signal assignment (chemical-shift values δ are in (ppm) units: 8.21 (8H; d; $J_{AB} = 7.7$ Hz; **1d**-H(Ar)), 7.96 (1H; s; Ado-H(6)), 7.87 (1H; s; Ado-H(3)), 7.75 (8H; d; $J_{BA} = 7.7$ Hz; **1d**-H(Ar)), 5.61 (1H; d; Ado-H(1′), 4.43 (1H; m; Ado-H(2′)), 4.19 (1H; m; Ado-H(3′)), 4.07 (1H; m; Ado-H(4′)), 3.68 (1H; bs; Ado-H(5′))).

binaphtholcarboxylic acid, etc. [17]. Partial replacement of derivatization agent by trimethylsilyl groups is thus expected. Spectra of the samples after the protection of free silanol groups by trimethylsilyl moieties were compared with those obtained before protection step and with spectra of silylated 3-aminopropyl silica. The change of spectral pattern with overall increased intensity in the range 3000–2850 cm^{-1} is clearly observed (Fig. 7). Spectra of all silylated samples (Fig. 7C and D) exhibit typical bands of $–CH_3$ groups (2964, 2903 cm^{-1}) overlapping the bands of $–CH_2–$ groups of aminopropyl chains observed prior to silylation (Fig. 7A and B). The characteristic bands of trimethylsilyl groups can be finally used for the evaluation of the efficiency of the protection step [17].

3.5. Separation of nucleobases

Chromatographic experiments with **1b**-sorbent, **2**-sorbent, and **3**-sorbent were performed under reverse-phase separation conditions. Mobile phase consisted of a mixture methanol/phosphate buffer (pH = 5.5, 10 mmol l^{-1}) (1:1, v/v). Retention factors (k) of nucleobases adenine, cytosine, thymine,

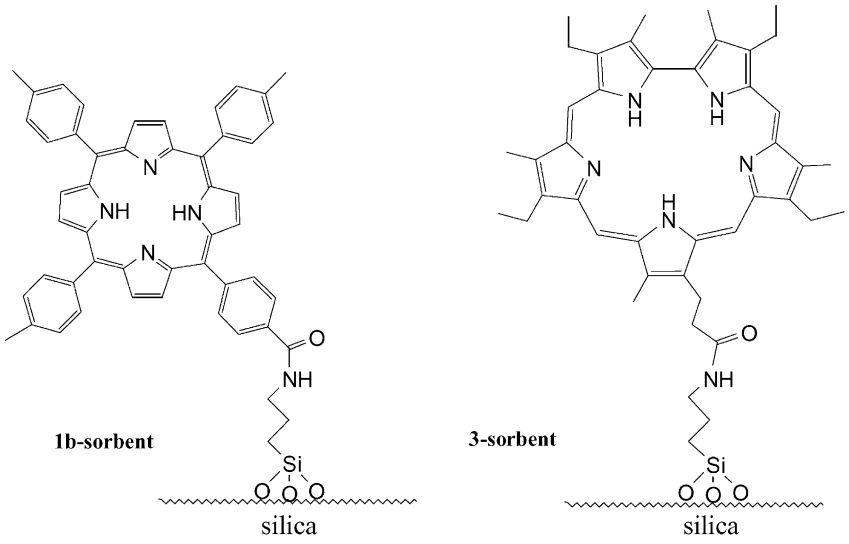


Fig. 4. Structures of **1b**-sorbent and **3**-sorbent.

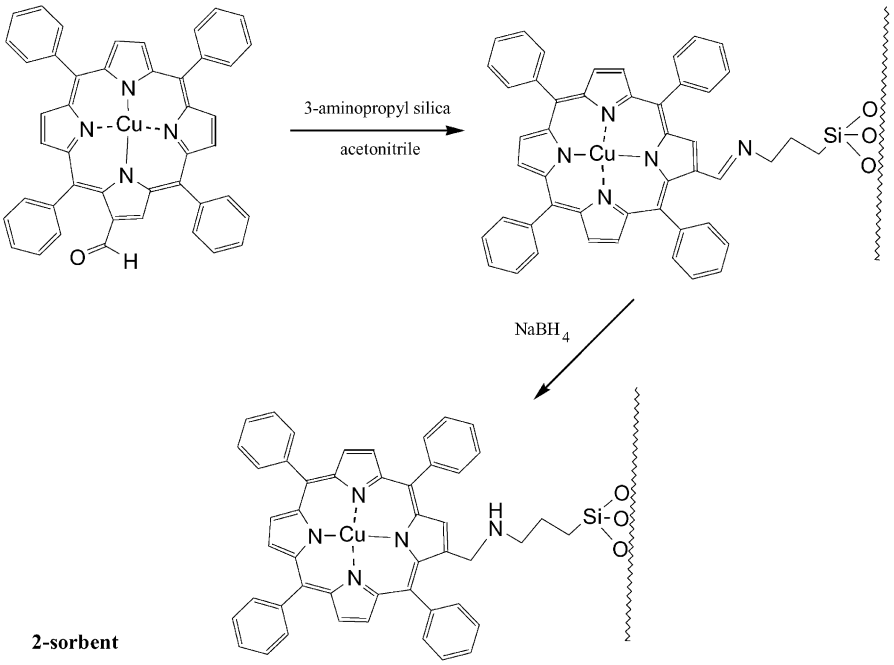


Fig. 5. Synthetic strategy of preparation of **2**-sorbent.

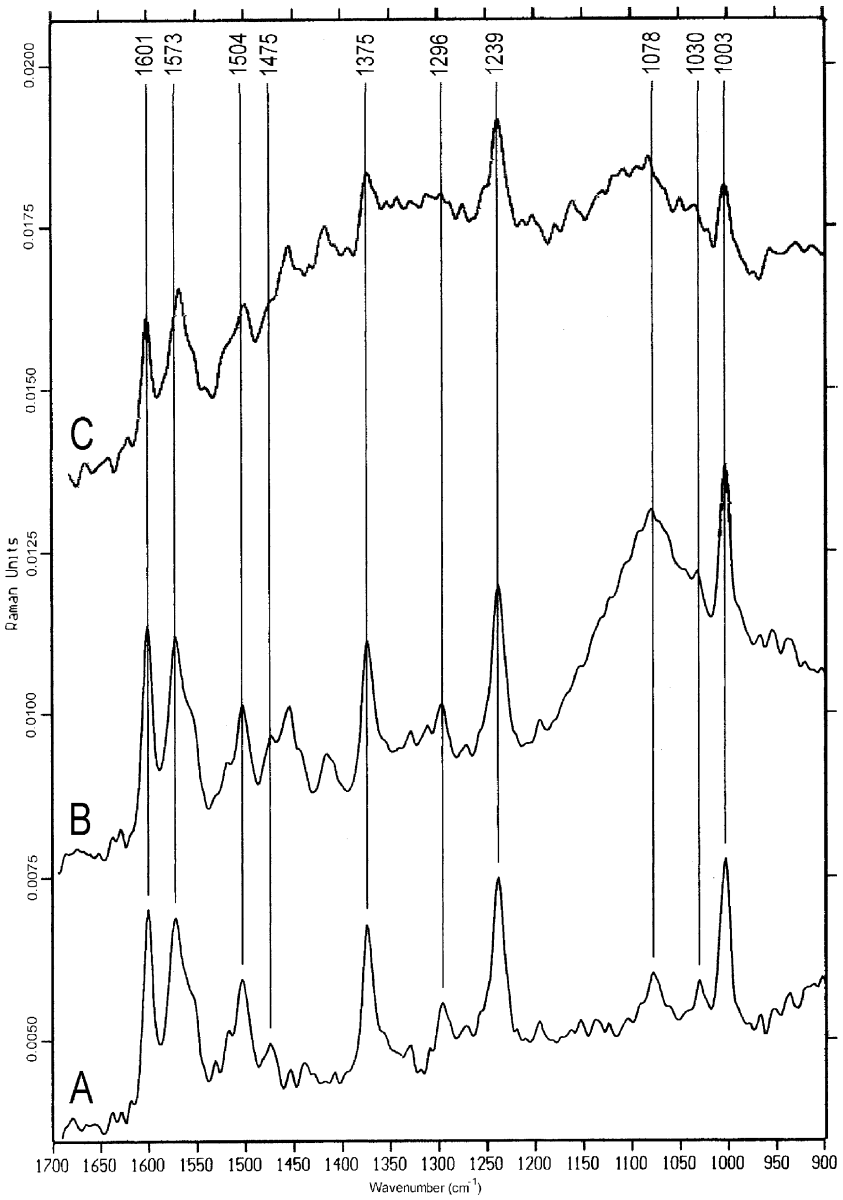


Fig. 6. FT Raman spectra of **2**-sorbent in the range of characteristic bands of modification agent: (A) subtraction of spectrum of **2**-sorbent and initial 3-aminopropyl silica (showing characteristic bands of **2**); (B) **2**-sorbent before silylation; (C) **2**-sorbent after silylation.

and uracil are presented in Fig. 8. Guanine exhibits extremely long retention and it was excluded from the study. Results on silylated 3-aminopropyl silica (**Si**-sorbent) are shown for comparison.

As can be seen in Fig. 8, the behavior of adenine is different from that of other bases since it is the most retained nucleobase on all sorbents including the **Si**-sorbent. However, presence of macrocycle bonded on the surface of the stationary phase brought higher selectivity between purine and pyrimidine moieties. Pyrimidine bases were retained only slightly on both **Si**-sorbent and **1b**-sorbent. They were eluted close to

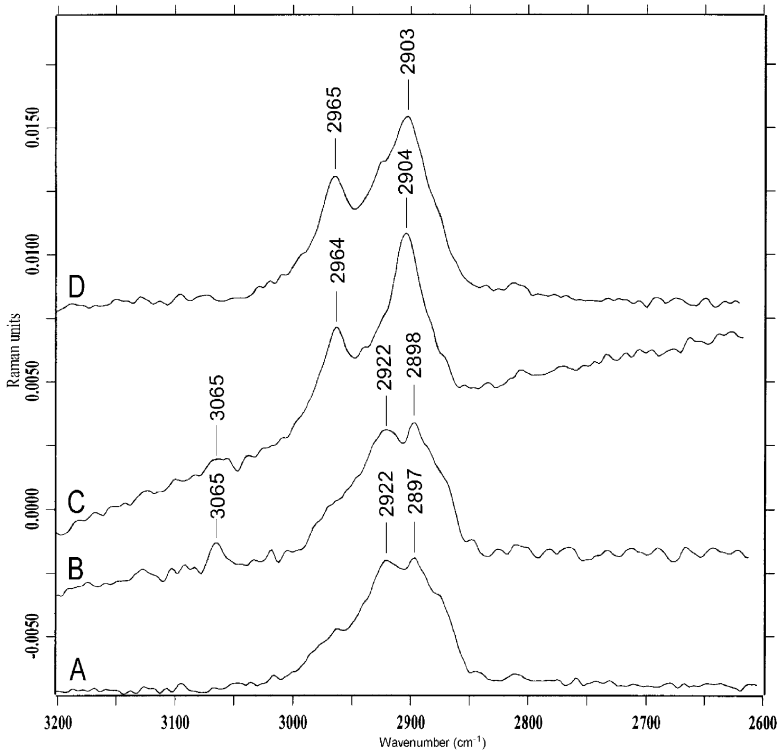


Fig. 7. Comparison of FT Raman spectra of silylated and non-silylated sorbents in the range of stretching C–H vibrations: (A) 3-aminopropyl silica; (B) **2**-sorbent before silylation; (C) **2**-sorbent after silylation; (D) 3-aminopropylated silica after silylation.

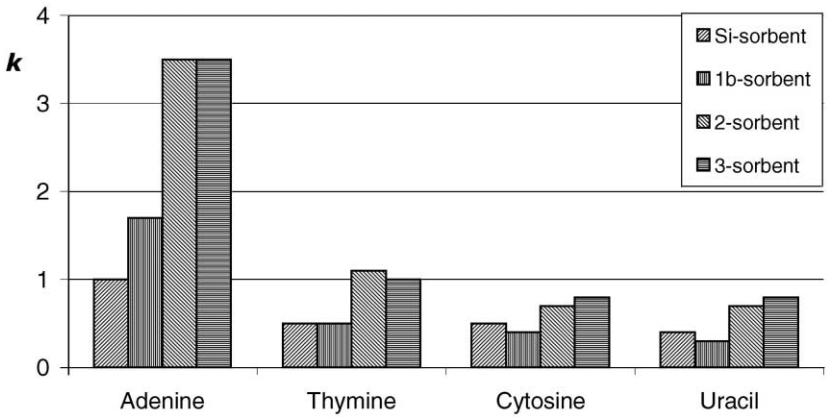


Fig. 8. The dependence of retention factors (k) of nucleobases on the type of stationary phase. ($k = (t_R - t_0)/t_0$, where t_R is retention time and t_0 is the dead time of column).

the dead time on the columns. In the case of **2**-sorbent and **3**-sorbent, higher retention times were measured and a higher binding interaction is supposed. Modification of the structure of the immobilized macrocycle leads to the extension of interaction of pyrimidine bases with relatively weak aromatic character. We assume that the interaction of nucleobases with **2**-sorbent is strengthened by a donation of d_{z^2} electrons of the copper in **2**-sorbent into the π-antibonding orbital of nucleobases [6,30]. In the case of **3**-sorbent, sapphyrin aromatic core has 22-π electrons in contrast to 18-π electrons of porphyrin core [31,32] and the large aromatic core of sapphyrin can contribute to higher retention of pyrimidine bases [33].

Chromatogram of nucleobases on **1b**-sorbent is presented in Fig. 9A. Incomplete separation has been achieved under given chromatographic conditions. The number of theoretical plates (N) was calculated by following equation:

$$N = 5.545\left(\frac{t_R}{w_h}\right)^2$$

where t_R is retention time and w_h is peak width at its half height. The number of theoretical plates per column usually varied in the range 100–400.

3.6. Separation of nucleosides

Introduction of ribose moiety (or in thymidine structure deoxyribose) makes the nucleosides more hydrophilic. This weakens the hydrophobic interaction with stationary phases and contributes to retention that can be extended by hydrogen bonding interaction of sugar moieties. Chromatogram of separation of nucleosides on **1b**-sorbent is shown in Fig. 9B. Results of all the studied analytes on all sorbents except of **2**-sorbent (Fig. 10) are in agreement with such model.

The nucleosides adenosine, thymidine and cytidine have higher retention than corresponding nucleobases on **2**-sorbent. Behavior of porphyrins in chromatographic system is completely changed after the introduction of Cu(II) into porphyrin core [34]. This could be related to previously mentioned d^9 electronic configuration of Cu(II) which prefers square planar orientation of coordinated ligand. As a consequence π-coordination with planar aromatic substrates is more effective [8,30].

3.7. The influence of surface coverage

The values of surface coverage for the macrocycle-based sorbents are generally rather low in comparison with the results published elsewhere [9,30]. This can be explained by the several reasons, e.g. pore size of the used aminopropyl silica (8 nm), size of the macrocycles, incomplete conversion of reactants in sorbent preparation. It is probable that the use of wide-pore silica could lead to higher amount of bonded macrocycles. As a consequence of low coverage, the resolution of the studied analytes on our sorbents was

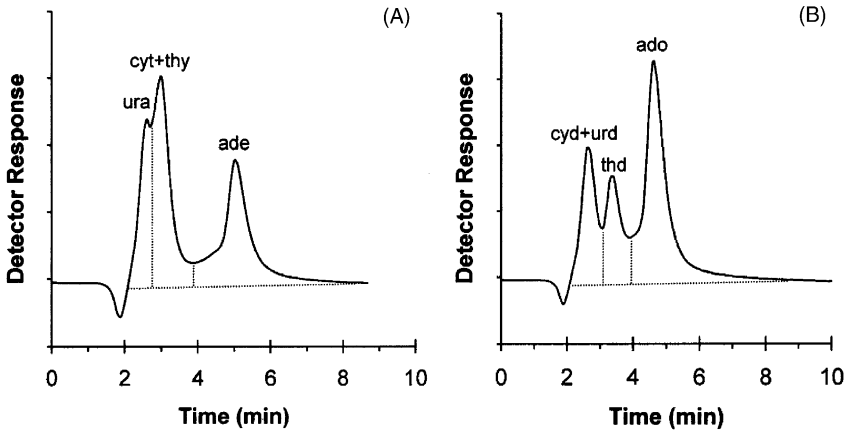


Fig. 9. Separation of: (A) nucleobases uracil (ura), cytosine (cyt), thymine (thy), and adenine (ade); (B) nucleosides cytidine (cyd), uridine (urd), thymidine (thd), and adenosine (ado) on **1b**-sorbent. For separation conditions see Section 2.

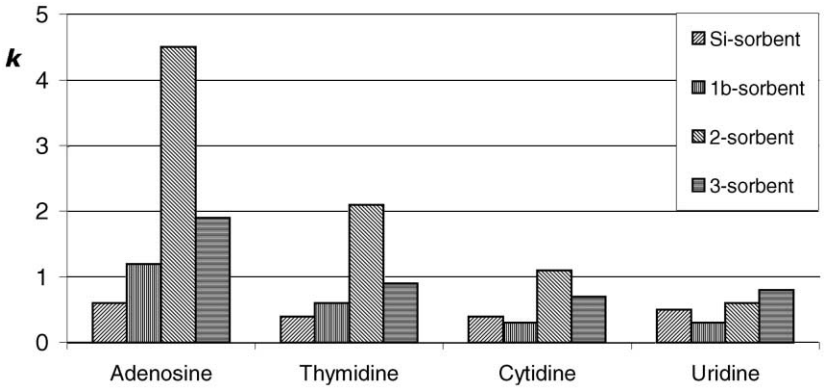


Fig. 10. The dependence of retention factors (k) of nucleosides on the type of stationary phase.

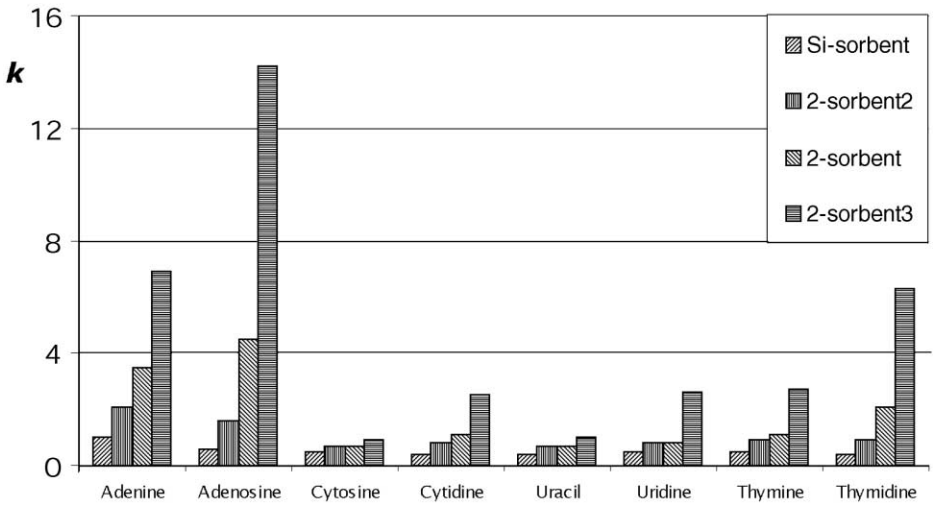


Fig. 11. The dependence of retention factors (k) of nucleobases and nucleosides on the amount of bonded **2** (**2**-sorbent 2 8 mg, **2**-sorbent 17 mg, **2**-sorbent 3 40 mg).

incomplete, however, evaluation of interaction forces was still feasible.

The retention of an analyte is mediated by two kinds of forces: (a) non-specific interactions with silica surface, mainly with residual aminopropyl and silanol groups, and (b) by specific interactions with bonded molecules of receptors. An increase of the amount of attached receptor resulted in higher retention of all nucleobases and nucleosides except cytosine and uracil (Fig. 11). The results confirmed that the retention of the studied compounds was affected by interaction with **2** covalently linked onto the surface of the silica.

4. Conclusions

Interactions of the oligopyrrolic macrocyclic receptors with nucleobases and nucleosides were tested in solution and on a solid–liquid interface. UV–VIS

titration was used for the study of interactions between porphyrin, metalloporphyrin and sapphyrin receptors and nucleobase/nucleoside substrates. Proposed π–π sandwich complex formation as the main interaction mode was confirmed by quantum chemical calculations and ^{1}H NMR experiment. Novel receptor-based sorbents were prepared and tested for their affinity to nucleobases and nucleosides. Different separation behavior for purine and pyrimidine bases was observed.

An important advantage of the porphyrin-based sorbents consists in the opportunity to introduce variety of metals into the receptor molecule, which alternates electron density of the macrocycle and leads to different binding energy and geometry; in some cases an axial ligand binding mode can be introduced. As these sorbents are easily available, they could potentially represent a low-cost alternative to conventional reversed-phase stationary phases and enable separation of the purine and pyrimidine heterocycles.

The increase in retention for nucleoside substrates on Cu(II)-tetraphenylporphyrin-based stationary phase can be attributed to the change of electron distri- bution in porphyrin core as well as to the influence of the sugar moiety and its interaction with stationary phase.

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