Optical sensing of sulfate by polymethinium salt receptors: colorimetric sensor for heparin†

Tomáš Bříza,*[ab] Zdeněk Kejík,[a] Ivana Císařová,[c] Jarmila Králová,[d] Pavel Martásek[b] and Vladimír Král*[ae]

Received (in Austin, TX, USA) 29th November 2007, Accepted 31st January 2008
First published as an Advance Article on the web 20th February 2008
DOI: 10.1039/b718492a

Polymethinium salts based on substituted malondialdehyde have been prepared; the salt 9 with PEG substitution showed high selectivity for sulfate anions and heparin in aqueous medium at physiological condition; intracellular imaging of heparin-rich subcellular compartments was achieved with our polymethinium novel receptor 9 for cancer cell lines.

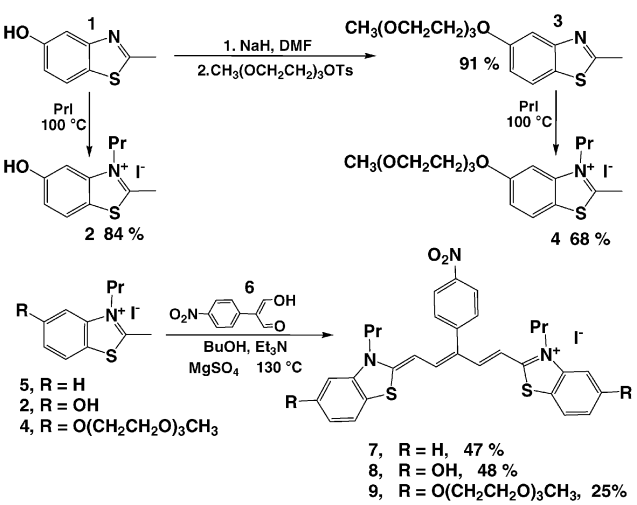
Anions and anionic biopolymers play a fundamental role in a wide range of chemical and biological processes. Development of receptors, which are designed for these analytes is an important branch of modern chemistry.[1–7] One of the major challenges in supramolecular chemistry is the design of receptors for selective anion recognition. Nature has developed selective protein receptors even for structurally very similar biologically important anions, *e.g.*, including phosphate and sulfate binding proteins.[8]

The design of synthetic receptors for selective sulfate over phosphate recognition in aqueous media has been a significant challenge. In the case of polysulfates, such as heparin, two types of optically-responsive synthetic receptors have been studied. One of these is using cationic boronic sensors.[9–11] Limitations of the boronic acid sensors include pH dependent operation modes. Another reported method involves the colorimetric detection of anionic polymers,[12] mainly DNA[13] by cationic heteroaromatic systems—cyanine dyes. This allows for the preparation of sensors with larger extinction coefficients that can exhibit significant spectral changes in the visible region.[14] We intend to prepare new types of these salts as receptors and to explore their properties as optical (colorimetric) sensors.[11] Our goal was the preparation of a receptor, which can display a significant spectral change in the visible spectral region for the determination of sulfate and polysulfate even in presence of phosphate anions under physiological conditions. Recently, we reported the synthesis of chromophoric trimethinium 1,1′-binaphthyls with benzothiazolium units;[15] now we test them as sensors for recognition sulfate over phosphate. We have observed affinity of the binaphthyls for sulfate ($\log K = 4.7$) and for phosphate ($\log K = 3.2$) in 1 : 1 complexation. This study has showed promising potential of polymethinium compounds for sulfate/phosphate recognition. Therefore we decided to test other methinium systems for selective anion recognition. The synthetic protocol of new polymethinium sensors with benzothiazolium units is described in Scheme 1.

The structure of polymethinium chromophoric receptor **7** was determined by 1D and 2D NMR spectroscopy and single-crystal X-ray analysis (Fig. 1).‡

Initial study revealed that salt **7** (Scheme 1) exhibits a strong interaction with sulfate anion, but no significant spectral change in the presence of chloride, fluoride, nitrate, acetate and phosphate was observed as shown in Fig. 2. Because of the low water solubility of receptor **7**, mixed solvent was used in order to avoid aggregation effects. Multiple binding modes were determined in the case of sulfate anion. For sulfate anion and salt **7** the 2 : 2 and 1 : 2 complexes had K values of 2×10^{14} and 5×10^{11}, respectively; values of K of other tested anions were below 10.

In the next synthetic step, in order to increase water solubility, the salt **8** substituted with two hydroxy groups on benzothiazolium unit was prepared (Scheme 1). However, this synthetic



Scheme 1 Synthetic strategy for the preparation of receptors **7–9**.

[a] *Department of Analytical Chemistry, Institute of Chemical Technology in Prague, Technická 5, Prague 6, 16628, Czech Republic. E-mail: ftor@seznam.cz; Fax: +420 224 310 352; Tel: +420 224 310 352*
[b] *First Faculty of Medicine, Charles University in Prague, Kateřinská 32, Prague 2, 12108, Czech Republic*
[c] *Department of Inorganic Chemistry, Charles University in Prague, Hlavova 8, Prague 2, 12108, Czech Republic*
[d] *Institute of Molecular Genetics, Academy of Sciences, Flemingovo sq. 2, 166 10 Prague 6, Czech Republic*
[e] *Zentiva R & D, U Kabelovny 130, 10237 Prague 10, Czech Republic. E-mail: vladimir.kral@vscht.cz*

† Electronic supplementary information (ESI) available: Syntheses of compounds **7–9** and details of binding studies. See DOI: 10.1039/b718492a

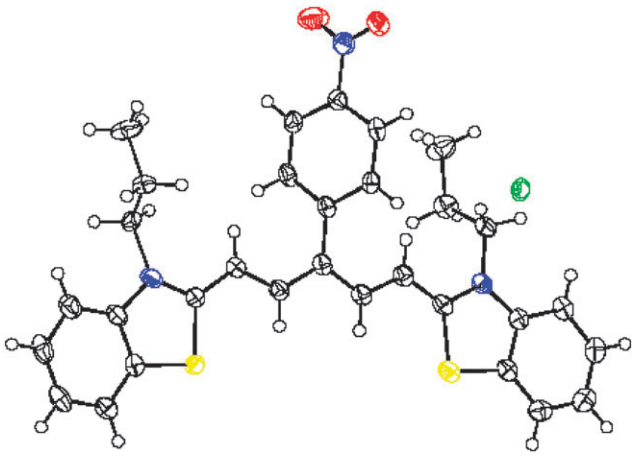


Fig. 1 Single-crystal X-ray structure of methinium salt **7**.

modification of the basic skeleton did not lead to the desired properties; the salt **8** showed very strong precipitation in aqueous medium, probably due to self-aggregation of individual molecules by means of π–π stacking and ion pairing.

For generation of a receptor with potential to be applied under physiological conditions, we appended triethyleneglycol chains to the benzothiazolium unit. Thus we have prepared salt **9** (Scheme 1), which was sufficiently soluble in aqueous medium and showed strong interactions with sulfate anion, higly selective over other anions, including chloride, fluoride and nitrate. The salt **9** was studied in a mixture of water–PEG (n = 9) where PEG was added as a cosolvent.[16] The notable sulfate selectivity led us to use our novel receptor for the colorimetric sensing of biopolymers with sulfate moieties. We chose heparin as a model substrate. We have found that compound **9** showed significant spectral changes in the presence of heparin (hypsochromic shift from 663 to 485 nm) (Fig. 3).

UV-Vis study with other anionic polysaccharides, such as pectin, polygalacturonic acid and also 2-sulfoglucosamine showed, in comparison to heparin, relatively weak interactions (Fig. 4). K values for **9** with a series of anionic polysaccharides are summarized in Table 1.

The next question was whether our receptor **9** could be applied to the *in vivo* detection of sulfonated biopolymers, namely cancer markers.

The main obstacle for molecular recognition of cancer biomarkers and/or cancer cells is the relatively weak binding

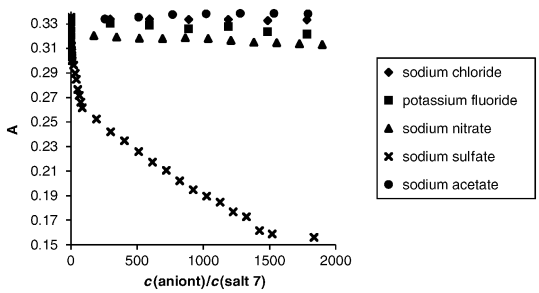


Fig. 2 Selectivity study of salt **7** for simple anions. Concentration of **7** = 2.2×10^{-6} M in a solution of 68% H_2O–30% MeOH–2% DMSO, pH = 5.53, medium = 1 mM phosphate buffer, wavelength = 663 nm.

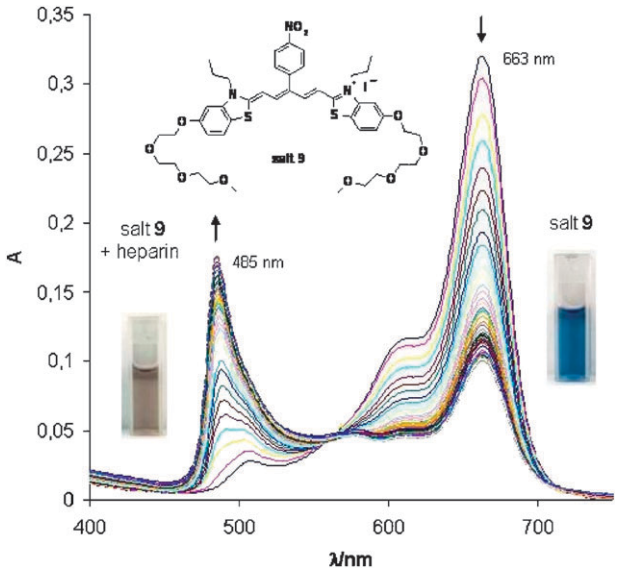


Fig. 3 Titration of **9** ($c = 4.6 \times 10^{-6}$ M) with heparin in a solution of 85% H_2O–15% PEG (n = 9), 1 mM phosphate buffer pH = 5.53; no. of equivalents are from 0 to 100.

of synthetic ligands to biological receptors. The advantage of our colorimetic molecular probe **9**, is, that can recognize the unique molecular signatures of cancer cells (localized on the surface, or on the subcellular structure)—sulfonated saccharides with very high selectivity and efficiency (based on very high binding constant under physiological conditions, see Table 1).

We have observed relatively fast into-cell transport of probe **9** for mammary gland tumor tissue cell culture; and because of high lysosome levels of polysulfated polysaccharides[17] for this cancer cell line, we used these cells as a model for an

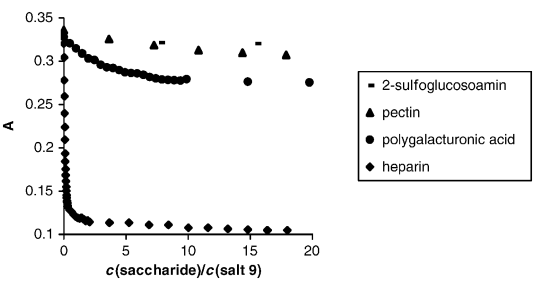


Fig. 4 Titration curves of the salt **9** with acid saccharides. Concentration of the salt **9** = 4.6×10^{-6} M in a solution of 85% H_2O–15% PEG (n = 9), pH = 5.53, 1 mM phosphate buffer, wavelength = 663 nm.

Table 1 Log K values (error <15%) and stoichiometry of salt **9** at various pH values

Analyte	Analyte : **9** stoichiometry	pH 5.53	pH 6.22	pH 7.35
Heparin	1 : 2	10.7	10.7	10.8
	1 : 3	18.4	18.1	17.8
Polygalacturonic acid	1 : 1	4.8	3.6	4
Sulfate anion	2 : 2	14.7	11.3	13.5
	1 : 2	11.7	7.8	3.8

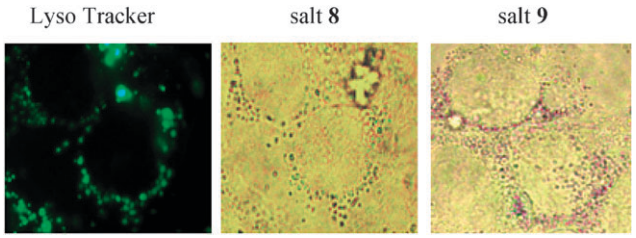


Fig. 5 Partial co-localization of salt **8** and **9**, compared with staining with commercial lysosome specific probe LysoTracker Green.

intracellular distribution study.[18] Indeed, lysosome localization of the polymethinium salt **9** was observed. Intracellular lysosome imaging is summarized on Fig. 5.

In conclusion, we have developed highly selective sulfate receptors (based on a substituted polymethine scaffold) and demonstrated their application as optical sensors for heparin. These colorimetric sensors can recognize heparin over other acidic polysaccharides such as pectin and polygalacturonic acid in the presence of high phosphate concentration, as well as other anions present under physiological conditions. Intracellular localization of novel receptors opens the possibility for imaging of highly sulfonated compartments as has been demonstrated on lysosome imaging for cancer cells.

This work was supported by grants from MŠMT of Czech Republic-MŠMT 1M 6837805002, LC 512, KAN200200651, MSM0021620857 and Grant Agency of the Czech Republic No. 203/06/1038.

Notes and references

‡ *Crystal data* for **7**: $C_{31}H_{30}IN_3O_2S_2$, M = 667.60, triclinic, space group $P\bar{1}$, a = 10.1420(2), b = 10.3469(2), c = 15.9680(3) Å, α = 95.4235(13), β = 108.3581(9), γ = 108.4221(11)°, V = 1473.42(5) Å^3, Z = 2, D_c = 1.505 g cm^{-3}, μ = 1.261 mm^{-1}, T = 150(0) K, 6771 reflections, 6262 with $I > 2\sigma(I)$, R(all) = 0.0308, $R(I > 2\sigma(I))$ = 0.0274, wR(all) = 0.0666, $wR(I > 2\sigma(I))$ = 0.0645. CCDC 671040. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b718492a

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18 4T1 cells were sequentially loaded with 1 μM TB-30 and then with 500 nM LysoTracker Green (Molecular Probes). Staining with methinium salt (violet) was monitored in regular light and green fluorescence of LysoTracker using a bandpass filter BP450-490 for excitation and a long pass filter LP 515 for emission.