# Supplementary material

# Optical sensing of sulphate by polymethinium salt receptors: Colorimetric sensor for heparin

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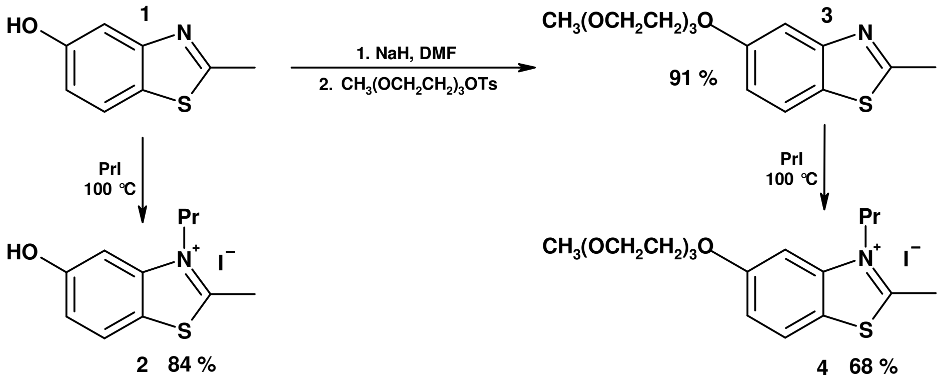
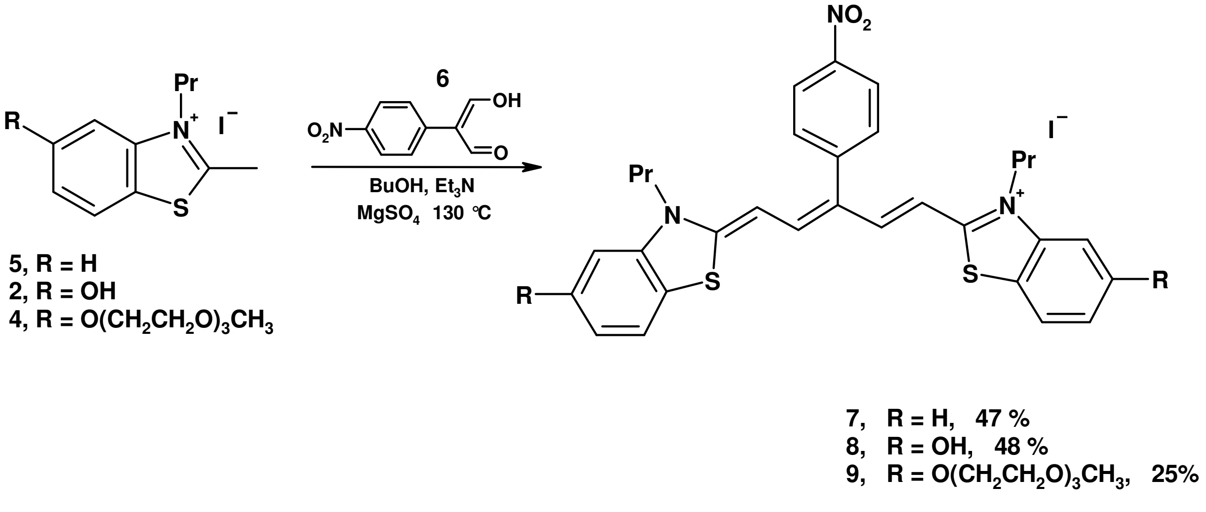
## Synthetic strategy

Our syntetic strategy was based on our preliminary results.[Bříza, T.; Kejík, Z.; Vašek, P.; Králová, J.; Martásek, P.; Císařová, I.; Král, V. *Org. Lett.* **2005**, *17*, 3661-3664] We have used derivatives of benzothiazolium iodide **2**, **4** and **5**, which were reacted with *para*-nitrophenylmalondialdehyde **6**.

In the first step, there was necessary to prepare the salts **2** and **4** 5-hydroxy-2-methyl benzothiazole was reacted with propyl iodide under 100 °C for 3 days and corresponding iodonium salt **2** was obtained in yield 84 %. In case of salt **4** at first, the hydroxybenzothiazole **1** was substituted with triethylene glycol chain by means of triethyleneglycol tosylate in presence of sodium hydride in dimethylformamide. The substitution afforded product **3** in high yield 91 %. Benzothiazole **3** was then treated with propyl iodide at 100 °C to obtain salt **4** in 68 % yield.

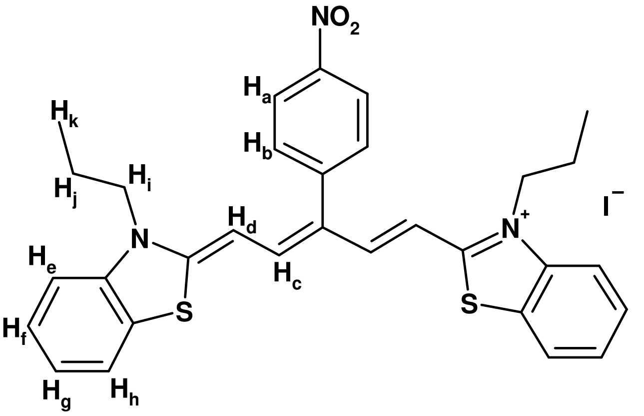


In comparison with the method of synthesis of chromophoric binaphthyls[Bříza, T.; Kejík, Z.; Vašek, P.; Králová, J.; Martásek, P.; Císařová, I.; Král, V. *Org. Lett.* **2005**, *17*, 3661-3664.], we slightly modified the conditions of the reaction. Instead of mixture of n-BuOH/benzene 10:3, we used only n-BuOH as a rection medium. Furthermore the reaction was carried out in presence of trace of triethylamine. The reaction mixture was heated to 120 °C for 18 h. Methinium salts **7** - **9** were formed in moderate yields.



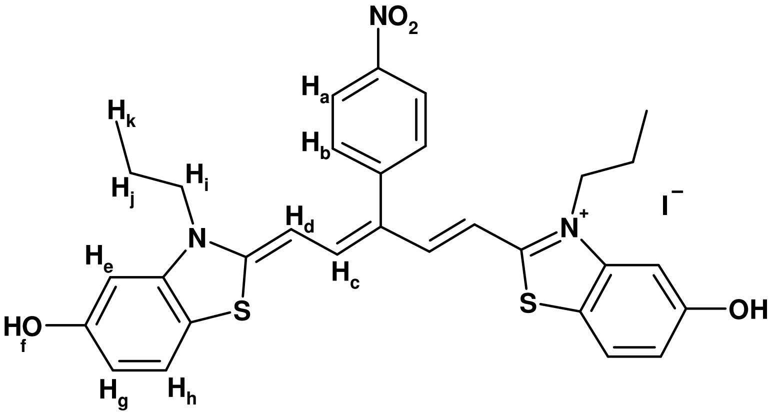
Carefull NMR analysis confirmed the same geometry of the compound **7** in solution as well as in solit state. The analysis was based on NOE experiment. The assignment of individual hydrogens was accomplished by means of $^1$H-NMR, $^{13}$C-NMR, COSY, and NOE experiment. Assignment of hydrogens according $^1$H NMR spectra.

Compound **7**



$H_a$ 8.38 (d, 2H, $^3J_{HH}$ = 8.8 Hz)
$H_b$ 7.62 (d, 2H, $^3J_{HH}$ = 8.8 Hz)
$H_c$ 8.00 (d, 2H, $^3J_{HH}$ = 13.8 Hz)
$H_d$ 5.88 (d, 2H, $^3J_{HH}$ = 13.8 Hz)
$H_e$ 7.75 (d, 2H, $^3J_{HH}$ = 8.5 Hz)
$H_f$ 7.54 (t, 2H, $^3J_{HH}$ = 7.4 Hz)
$H_g$ 7.41 (t, 2H, $^3J_{HH}$ = 7.4 Hz)
$H_h$ 8.05 (d, 2H, $^3J_{HH}$ = 7.4 Hz)
$H_i$ 4.15 (t, 4H, $^3J_{HH}$ = 6.9 Hz)
$H_j$ 1.63 (q, 4H, $^3J_{HH}$ = 7.2 Hz)
$H_k$ 0.79 (t, 6H, $^3J_{HH}$ = 7.2 Hz)

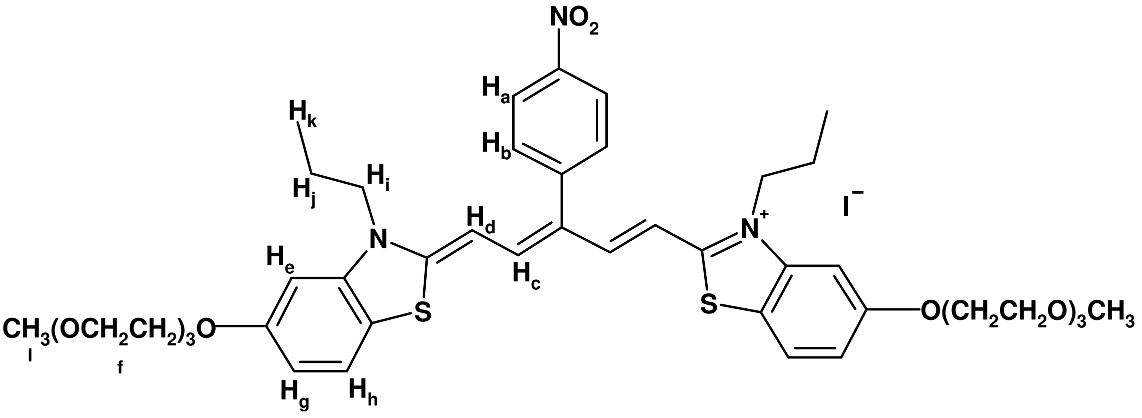
Compound **8**



$H_a$ 8.36 (d, 2H, $^3J_{HH}$ = 8.8 Hz)
$H_b$ 7.61 (d, 2H, $^3J_{HH}$ = 8.8 Hz)
$H_c$ 7.90 (d, 2H, $^3J_{HH}$ = 13.8 Hz)
$H_d$ 5.85 (d, 2H, $^3J_{HH}$ = 14.1 Hz)
$H_e$ 7.04 (d, 2H, $^4J_{HH}$ = 1.8 Hz)
$H_f$ 10.16 (bs, 2H)
$H_g$ 6.88 (dd, 2H, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.1 Hz)

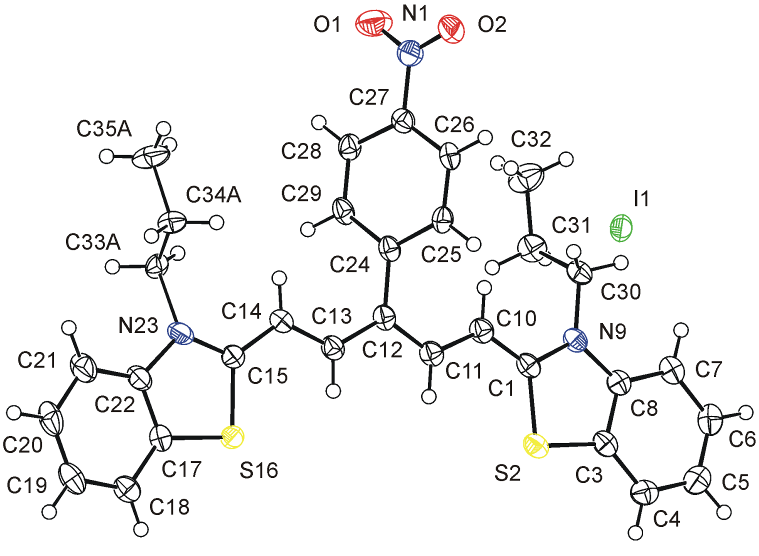
$H_h$ 7.78 (d, 2H, $^3J_{HH} = 8.5$ Hz)
$H_i$ 4.04 (t, 4H, $^3J_{HH} = 7.3$ Hz)
$H_j$ 1.61 (q, 4H, $^3J_{HH} = 7.0$ Hz)
$H_k$ 0.79 (t, 6H, $^3J_{HH} = 7.3$ Hz)

Compound **9**



$H_a$ 8.38 (d, 2H, $^3J_{HH} = 8.8$ Hz)
$H_b$ 7.62 (d, 2H, $^3J_{HH} = 8.8$ Hz)
$H_c$ 7.95 (d, 2H, $^3J_{HH} = 14.4$ Hz)
$H_d$ 5.86 (d, 2H, $^3J_{HH} = 14.1$ Hz)
$H_e$ 7.35 (d, 2H, $^4J_{HH} = 2.1$ Hz)
$H_f$ 4.22 (t, 4H, $^3J_{HH} = 3.8$ Hz), 3.76 (t, 4H, $^3J_{HH} = 4.4$ Hz), 3.62 – 3.32 (m, 8H)
$H_g$ 7.06 (dd, 2H, $^3J_{HH} = 8.8$ Hz, $^4J_{HH} = 1.8$ Hz)
$H_h$ 7.90 (d, 2H, $^3J_{HH} = 9.1$ Hz)
$H_i$ 4.14 (t, 4H)
$H_j$ 1.63 (sextet, 4H, $^3J_{HH} = 7.3$ Hz)
$H_k$ 0.80 (t, 6H, $^3J_{HH} = 7.6$ Hz)
$H_l$ 3.22 (s, 6H)

## X-ray structure of salt 7



Overall view on of methinium salt **7**. The displacement ellipsoids are drawn on 50% probability level. Selected bond distances (Å) and angles (°): S(2)-C(1) 1.739(2), S(2)-C(3) 1.746(2), C(3)-C(8) 1.402(3), N(9)-C(8) 1.396(3), N(9)-C(1) 1.356(3), C(1)-C(10) 1.394(3), C(10)-C(11) 1.380(3), C(11)-C(12) 1.405(3), C(12)-C(13) 1.401(3), C(13)-C(14) 1.379(3), C(14)-C(15) 1.398(3), C(12)-C(24) 1.489(3); C(1)-C(10)-C(11) 126.11(19), C(10)-C(11)-(C12) 125.09(19), C(11)-C(12)-C(13) 118.56(18), C(12)-C(13)-C(14) 127.63(19), C(13)-C(14)-C(15) 122.23(19), C(11)-C(12)-C(24) 119.85(18), C(13)-C(12)-C(24) 121.59(18)

Crystal data for **7** : $C_{31}H_{30}N_3O_2S_2^+$, $I^-$ , M=667.60, triclinic, P -1 (No 2), $a$ = 10.1420(2) Å, $b$ = 10.3469(2)Å, $c$ = 15.9680(3)Å, $\alpha$ = 95.4235(13), $\beta$ = 108.3581(9), $\gamma$ = 108.4221(11) °, Z = 2, $D_x$ = 1.505 Mg $m^{-3}$. An green crystal of dimensions 0.18x0.20x0.50mm was mounted on glass capillary with epoxy glue and measured at Nonius KappaCCD diffractometer by monochromatized MoKα radiation ( λ = 0.71073 Å) at 150(2)K. The numerical correction on absorption was carried out (μ = 1.261 $mm^{-1}$ , $T_{min}$ = 0.569, $T_{max}$ = 0.875); a total of 24946 measured reflections ($\theta_{max}$= 27.5°), from which 6771 were unique ($R_{int}$=0.070) , 6262 observed according to the $I > 2\sigma(I)$ criterion. The structure was solved by direct methods (SIR92[16]) and refined by full matrix least squares based on $F^2$ (SHELXL97[17]). The hydrogen atoms were fixed into idealised positions (riding model) and assigned temperature factors either $H_{iso}(H)$ = 1.2 Ueq(pivot atom) or $H_{iso}(H)$ = 1.5 $U_{eq}$(pivot atom) for methyl moiety. The refinement converged ($\Delta/\sigma_{max}$=0.000) to R = 0.0274 for observed reflections and wR2 = 0.0666, GOF = 1.04 for 383 parameters and all 6771 reflections. The final difference map displayed no peaks of chemical significance ($\Delta\rho_{max}$ = 0.95, $\Delta\rho_{min}$ -0.93 e.Å$^{-3}$).

## Experimental part:

Compound **2**

A presure tube was charged with 5-hydroxy-2-methylbenzothiazole (500 mg, 3.03 mmol) and propyliodide (5 mL). The mixture was heated to 100 °C for 3 days. After this time, the mixture was evaporated to dryness. Solid rest was then washed with hexane and recrystalized from ethanol. The product was obtained as a white-grey crystaline powder (650 mg, 84 %).

**$^1$H-NMR** (DMSO) δ: 10.65 (s, 1H), 8.21 (d, 1H, $^3J_{HH}$ = 8.8 Hz), 7.55 (d, 1H, $^4J_{HH}$ = 1.65 Hz), 7.26 (dd, 1H, $^3J_{HH}$ = 9.1 Hz, $^4J_{HH}$ = 1.9 Hz), 4.57 (t, 2H, $^3J_{HH}$ = 7.7 Hz), 3.16 (s, 3H), 1.85 (2H, q, $^3J_{HH}$ = 7.7 Hz), 0.99 (t, 3H, $^3J_{HH}$ = 7.4 Hz); **$^{13}$C-NMR** (DMSO) δ: 176.3, 159.0, 142.4, 125.3, 119.0, 118.0, 101.8, 50.2, 21.0, 10.8. **Elementary analysis:** For $C_{11}H_{14}NOSI$ calculated: H 4.21%, C 39.42%, found: H 4.53 %, C 39.17%.

Compound **3**

A flask was charged with 5-hydroxy-2-methylbenzothiazole (500 mg, 3.03 mmol), dimethylformamide (15 mL) and sodium hydride (120 mg, 60 % suspension in mineral oil, 3.03 mmol). The mixture was stirred for 15 minutes at laboratory temperature and then triethyleneglycol mesylate (700 mg, 2.89 mmol) was added to the mixture. Then the mixture was heated to 80 °C for 5 hours and then stirred at laboratory temperature for 2 days. The mixture was extracted between water and dichloromethane and organic portion was dried with anhydrous magnesium sulphate. Product was separated by means of column chromatography on silica gel (10×5 cm, eluent – diethylether). The product **3** was obtained as a colorless viscous oil (820 mg, 91 %).

**$^1$H-NMR** ($CDCl_3$) δ: 7.58 (d, 1H, $^3J_{HH}$ = 8.8 Hz), 7.37 (d, 1H, $^4J_{HH}$ = 2.3 Hz), 6.95 (dd, 1H, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.6 Hz), 4.13 (m, 2H), 3.83 (m, 2H), 3.68 (m, 2H), 3.65-3.57 (m, 4H), 3.48 (m, 2H), 3.30 (s, 3H); **$^{13}$C-NMR** ($CDCl_3$) δ: 168.0, 157.8, 154.4, 127.4, 121.4, 115.0, 105.9, 71.8, 70.7, 70.5, 70.4, 69.6, 67.7, 58.9, 20.0; **IR** (KBr) 2926, 2880, 1671, 1603, 1559, 1519, 1450, 1323, 1272, 1251, 1170, 1128, 1100. **Elementary analysis:** $C_{15}H_{21}NO_4S$ calculated: H 6.80 %, C 57.86%, found: H 6.34 %, C 57. 98%.

Compound **4**

A presure tube was charged with glycol derivative **3** (688 mg, 2.21 mmol) and propyliodide (5 mL). The mixture was heated to 100 °C for 3 days. After this time, the

mixture was evaporated to dryness. Solid rest was then washed with hexane and dried. The product was obtained as a slightly yellow powder (652 mg, 68 %).
**$^{1}$H-NMR** ($CDCl_3$) δ: 8.18 (d, 1H, $^{3}J_{HH}$ = 9.1 Hz), 7.49 (d, 1H, $^{4}J_{HH}$ = 2.4 Hz), 7.25 (dd, 1H, $^{3}J_{HH}$ = 9.1 Hz, $^{4}J_{HH}$ = 2.3 Hz), 4.85 (t, 2H, $^{3}J_{HH}$ = 7.3 Hz), 4.35 (m, 2H), 3.90 (m, 2H), 3.78-3.62 (m, 4H), 3.53 (m, 2H), 3.33 (s, 3H), 3.36 (s, 3H), 1.98 (sextet, 2H, $^{3}J_{HH}$ = 7.3 Hz), 1.09 (t, 3H, $^{3}J_{HH}$ = 7.3 Hz); **$^{13}$C-NMR** ($CDCl_3$) δ: 174.3, 160.7, 142.5, 125.1, 120.5, 118.9, 100.5, 71.8, 70.7, 70.5, 70.4, 69.5, 69.3, 59.0, 52.4, 22.0, 19.5, 11.3; **IR** ($CDCl_3$) 2966, 2875, 1605, 1483, 1445, 1356, 1284, 1233, 1184, 1100, 1060. **Elementary analysis:** $C_{18}H_{28}NO_4SI$ calculated: H 5.86 %, C 44.91%, found: H 5.41 %, C 45. 23%.

Compound **7**

A flask was charged with dry butanol (40 mL), nitrophenylmalondialdehyde **6** (90 mg, 0.47 mmol), 2-methyl-3-propyl-benzothiazolium iodide **5** (330 mg, 1.03 mmol), triethylamine (1 drop) and anhydrous magnesium sulphate (300 mg). The mixture was heated to 120 °C overnight. After cooling to laboratory temperature, the mixture was extracted with water to remove magnesium sulphate and then filtered. Obtained solid was dissolved in dichloromethane (40 mL) and methanol (15 ml) under heating. The solution was let to crystalize. Crystalization afforded metallic shiny red-green crystals of compound **7** (145 mg, 47%).
**$^{1}$H-NMR** (DMSO) δ: 8.38 (d, 2H, $^{3}J_{HH}$ = 8.8 Hz), 8.05 (d, 2H, $^{3}J_{HH}$ = 7.4 Hz), 8.00 (d, 2H, $^{3}J_{HH}$ = 13.8 Hz), 7.75 (d, 2H, $^{3}J_{HH}$ = 8.5 Hz), 7.60 (d, 2H, $^{3}J_{HH}$ = 8.8 Hz), 7.54 (t, 2H, $^{3}J_{HH}$ = 7.4 Hz), 7.41 (t, 2H, $^{3}J_{HH}$ = 7.4 Hz), 5.88 (d, 2H, $^{3}J_{HH}$ = 13.8 Hz), 4.15 (t, 4H, $^{3}J_{HH}$ = 6.9 Hz), 1.63 (sextet, 4H, $^{3}J_{HH}$ = 7.2 Hz), 0.79 (t, 6H, $^{3}J_{HH}$ = 7.2 Hz); **$^{13}$C-NMR** (DMSO) δ: 164.7, 149.2, 146.6, 143.5, 141.4, 131.3, 128.1, 127.0, 125.31, 125.29, 124.2, 123.2, 113.7, 98.0, 47.4, 20.7, 10.9; **HRMS:** For $C_{31}H_{30}N_3O_2S_2I$ calculated: 540.1779, found: 540.1789; **UV/vis** (30% MeOH/70%$H_2O$): $\lambda_{max}$ = 665 nm; **IR** (KBr) 2927, 1631, 1534, 1512, 1453, 1337, 1314, 1212, 1189, 1153, 1122, 1040, 1014, 816, 756; **Elementary analysis:** For $C_{31}H_{30}N_3O_2S_2I$ calculated: H 4.53 %, C 55.77%, found: H 4.56 %, C 55. 76%.

Compound **8**

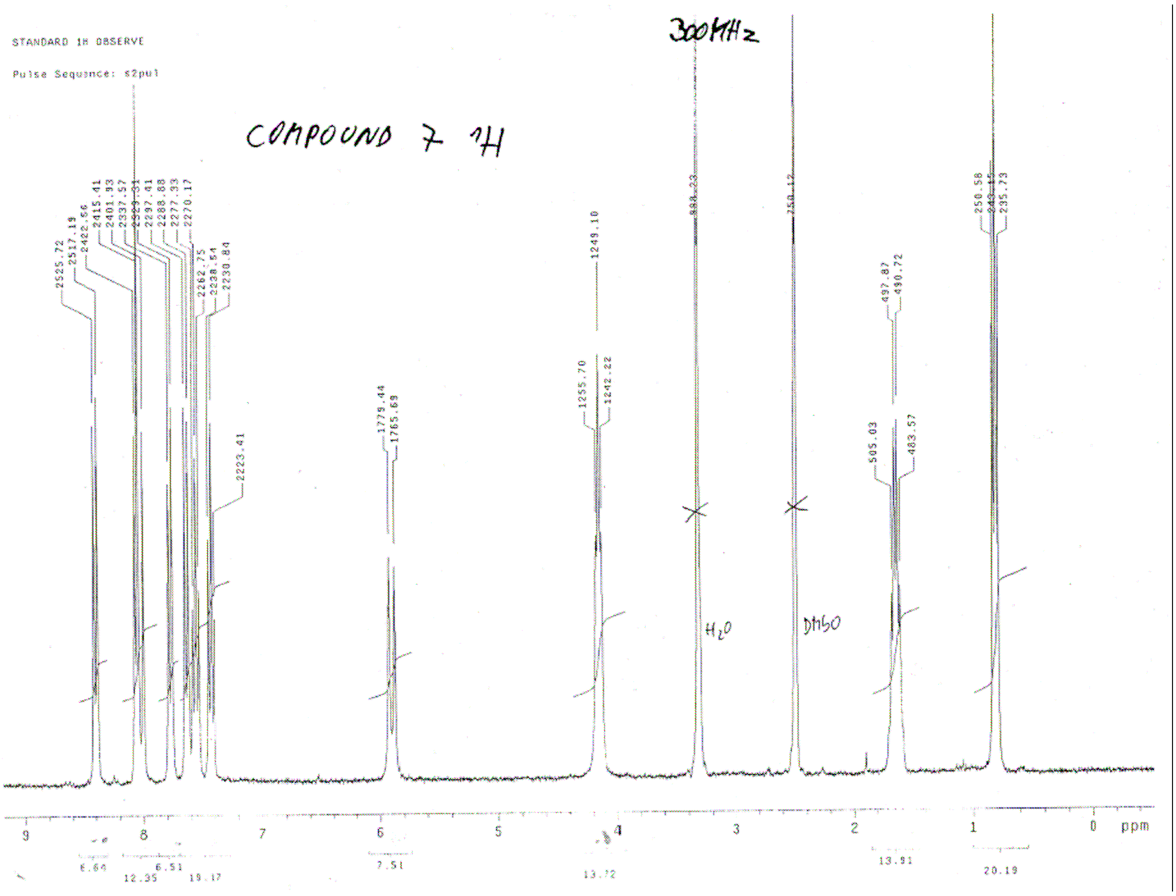
A flask was charged with dry butanol (40 mL), nitrophenylmalondialdehyde **6** (30 mg, 0.16 mmol), 5-hydroxy-2-methyl-3-propyl-benzothiazolium iodide **2** (110 mg, 0.33 mmol), triethylamine (1 drop) and anhydrous magnesium sulphate (100 mg). The mixture was heated to 120 °C overnight. After cooling to laboratory temperature, the mixture was extracted with water to remove magnesium sulphate and then filtered. Obtained solid was washed with methanol (5 mL), dichloromethane (10 mL). Product **8** was obtained in form of green metallic shiny small needles (52 mg, 48%).
**$^{1}$H-NMR** (DMSO) δ: 10.16 (bs, 2H), 8.36 (d, 2H, $^{3}J_{HH}$ = 8.8 Hz), 7.90 (d, 2H, $^{3}J_{HH}$ = 13.8 Hz), 7.78 (d, 2H, $^{3}J_{HH}$ = 8.5 Hz), 7.61 (d, 2H, $^{3}J_{HH}$ = 8.8 Hz), 7.04 (d, 2H, $^{4}J_{HH}$ = 1.8 Hz), 6.88 (dd, 2H, $^{3}J_{HH}$ = 8.8 Hz, $^{4}J_{HH}$ = 2.1 Hz), 5.85 (d, 2H, $^{3}J_{HH}$ = 14.1 Hz), 4.04 (t, 4H, $^{3}J_{HH}$ = 7.3 Hz), 1.61 (sextet, 4H, $^{3}J_{HH}$ = 7.0 Hz), 0.79 (t, 6H, $^{3}J_{HH}$ = 7.3 Hz); **$^{13}$C-NMR** (DMSO) δ: 165.4, 158.2, 148.3, 146.5, 143.7, 142.8, 131.3, 126.5, 124.2, 123.6, 114.8, 114.0, 100.2, 97.9, 47.4, 20.6, 10.9; **HRMS:** For $C_{31}H_{30}N_3O_4S_2.I$ calculated: 572.1678, found: 572.1699; **UV/vis** (30%MeOH/2%DMSO/68%$H_2O$): $\lambda_{max}$ = 662 nm; **IR** (KBr) 2964, 1602, 1530, 1512, 1437, 1346, 1332, 1197, 1159, 1123, 1104, 1038, 1017, 940; **Elementary analysis:** For $C_{31}H_{30}N_3O_4S_2.I$ calculated: H 4.32 %, C 53.22%, found: 4.78 H %, C 53.81 %.

Compound **9**

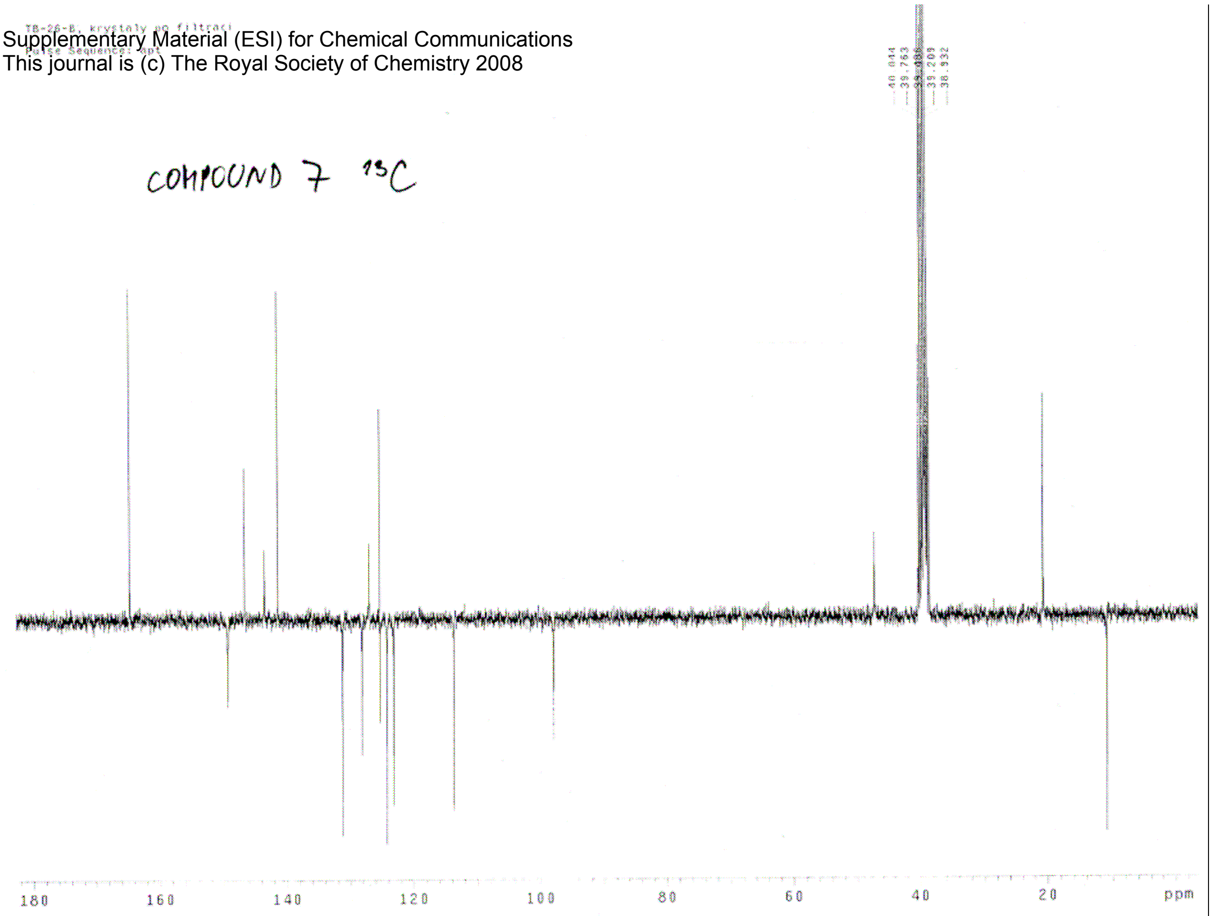
A flask was charged with dry butanol (40 mL), nitrophenylmalondialdehyde **6** (30 mg, 0.16 mmol), glycol-benzothiazolium salt **4** (150 mg, 0.31 mmol), triethylamine (1 drop) and anhydrous magnesium sulphate (100 mg). The mixture was heated to 120 °C overnight. After cooling to laboratory temperature, the mixture was extracted with water to remove magnesium sulphate and then filtered. Obtained dark solid was washed diethylethere (5 mL). The solid was dissolved in dichloromethane and slowly crystalyzed. The dark green crystals were sonicated with diethylether, decanted and dryied. This procedure was repeated two times. Product **9** was obtained in form of green metallic shiny amorphous solid (67 mg, 25 %).

**$^1$H-NMR** (DMSO) δ: 8.38 (d, 2H, $^3J_{HH}$ = 8.8 Hz), 7.95 (d, 2H, $^3J_{HH}$ = 14.4 Hz), 7.90 (d, 2H, $^3J_{HH}$ = 9.1 Hz), 7.62 (d, 2H, $^3J_{HH}$ = 8.8 Hz), 7.35 (d, 2H, $^4J_{HH}$ = 2.1 Hz), 7.06 (dd, 2H, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 1.8 Hz), 5.86 (d, 2H, $^3J_{HH}$ = 14.1 Hz), 4.22 (t, 4H, $^3J_{HH}$ = 3.8 Hz), 4.14 (t, 4H), 3.76 (t, 4H, $^3J_{HH}$ = 4.4 Hz), 3.62 – 3.32 (m, 8H), 3.22 (s, 6H), 1.63 (sextet, 4H, $^3J_{HH}$ = 7.3 Hz), 0.80 (t, 6H, $^3J_{HH}$ = 7.6 Hz); **$^{13}$C-NMR** (DMSO) δ: 165.4, 159.2, 148.4, 146.6, 143.6, 142.8, 131.4, 126.9, 124.2, 123.7, 116.6, 113.5, 99.5, 98.1, 71.2, 69.9, 69.8, 69.6, 68.8, 68.1, 58.0, 47.3, 20.7, 10.8; **HRMS:** For $C_{45}H_{58}N_3O_{10}S_2$.I calculated: 864.3564, found: 864.3561; **UV/vis** (13%PEG-400/2%MeOH/85%$H_2O$): $\lambda_{max}$ = 663 nm; **IR** (KBr) 2921, 2852, 1633, 1601, 1575, 1533, 1471, 1344, 1281, 1235, 1206, 1127, 1105, 1053, 965

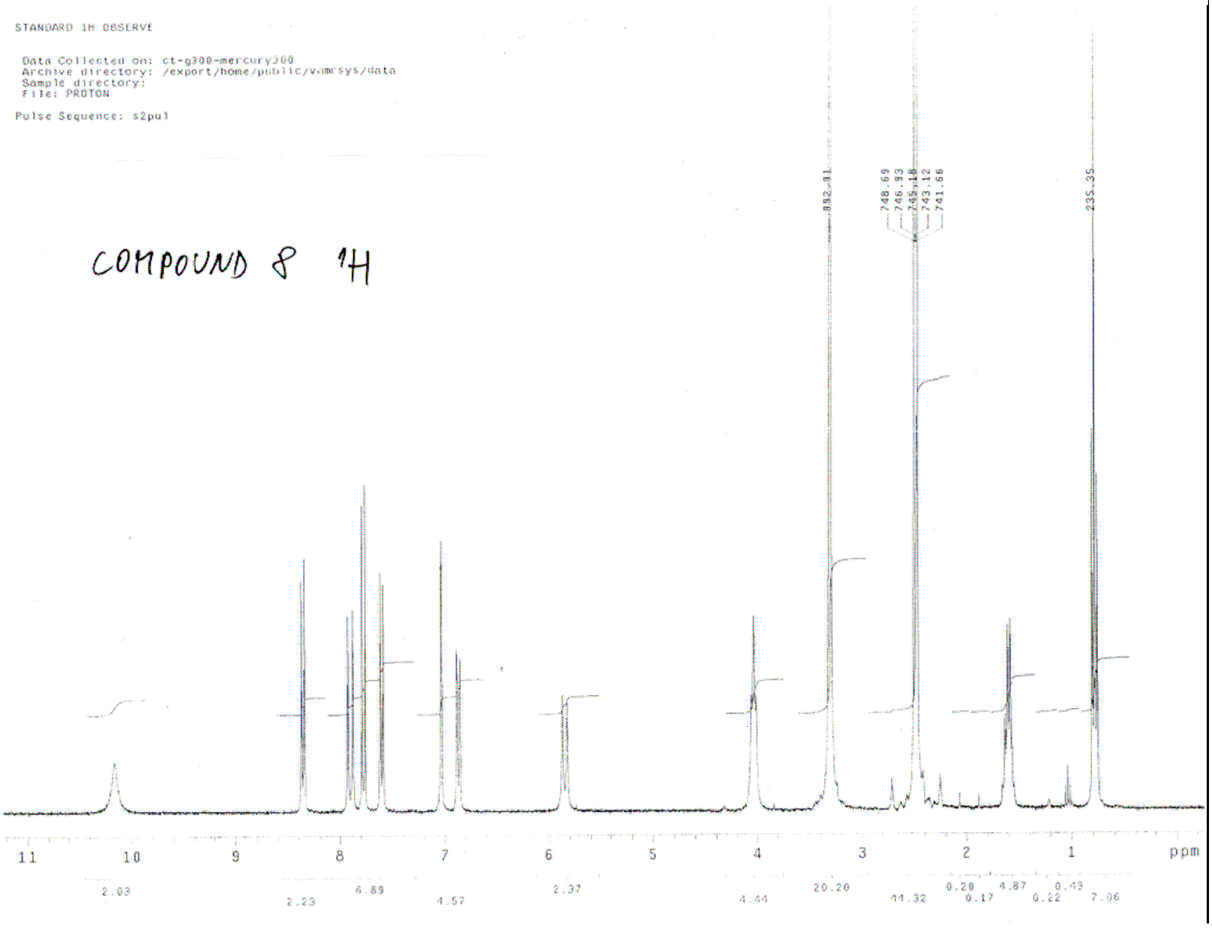
Scanned NMR spectra of compound **7 – 9**

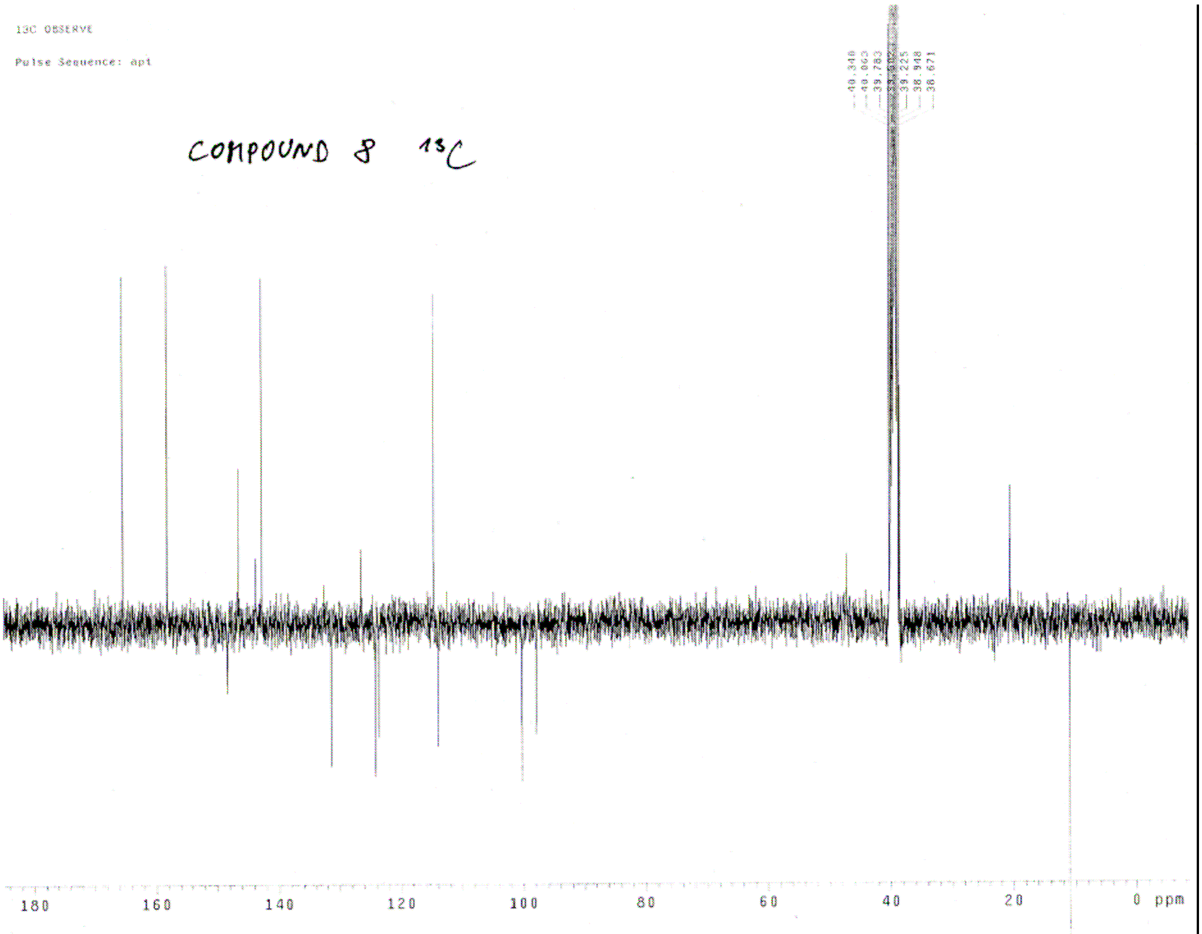


COMPOUND 7 $^{13}C$



COMPOUND 8 $^{1}H$

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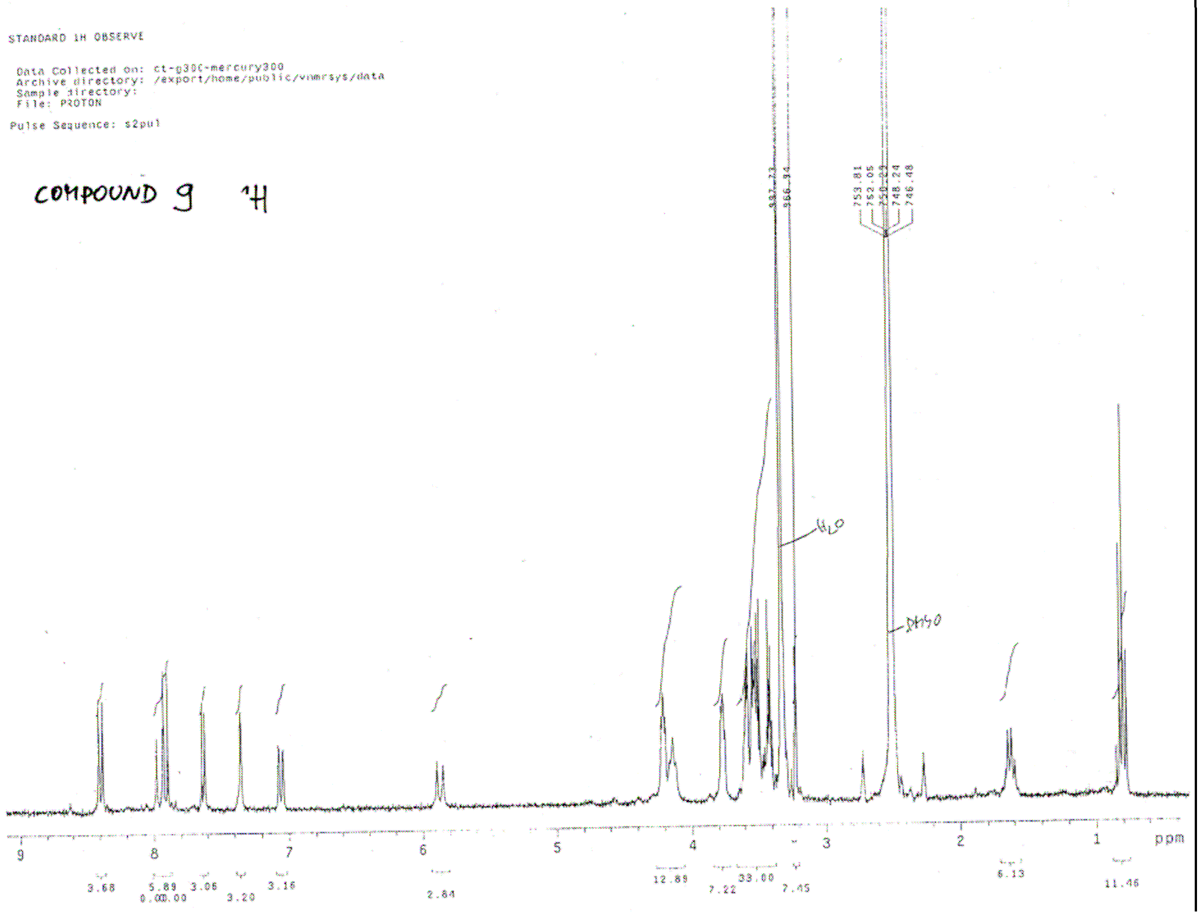
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966.34
753.81
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DMSO
9
8
7
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3
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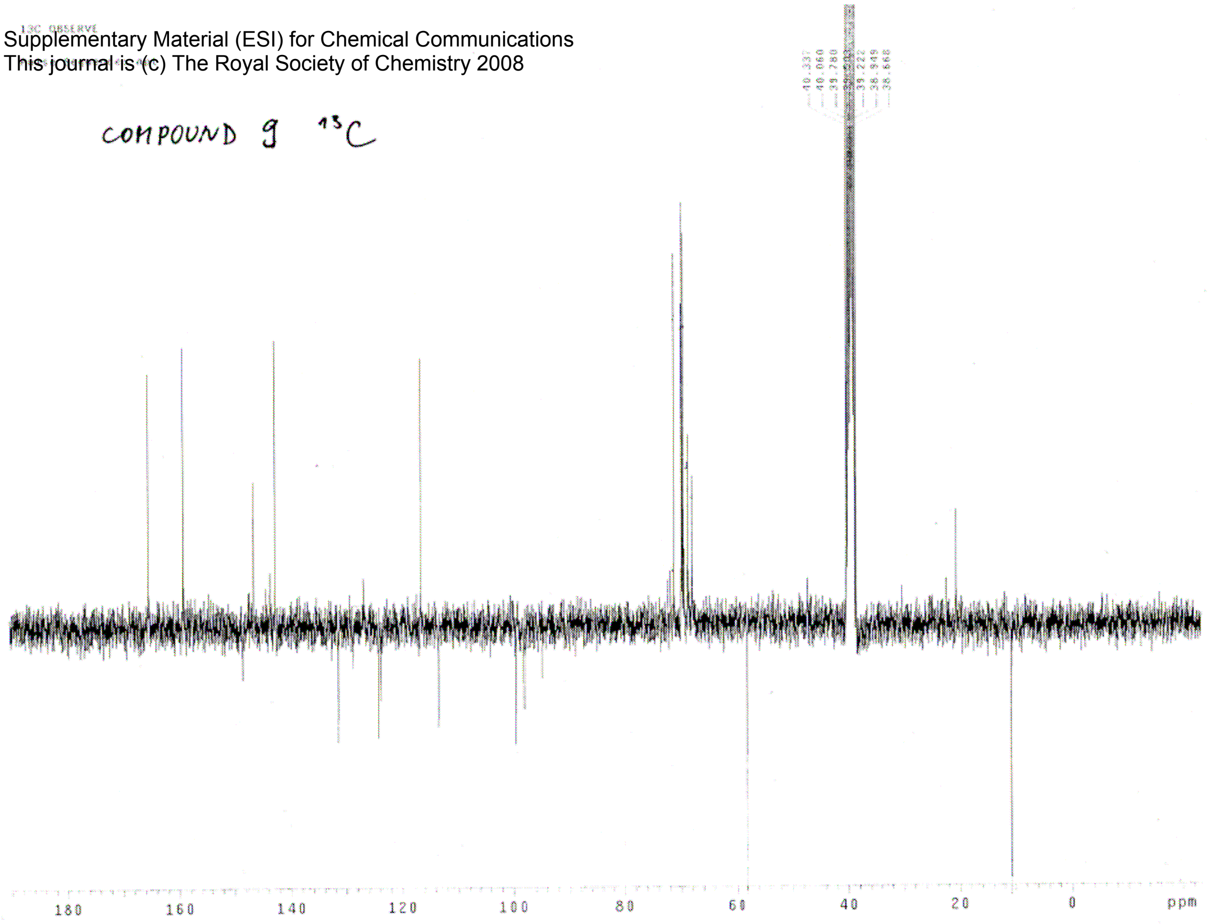
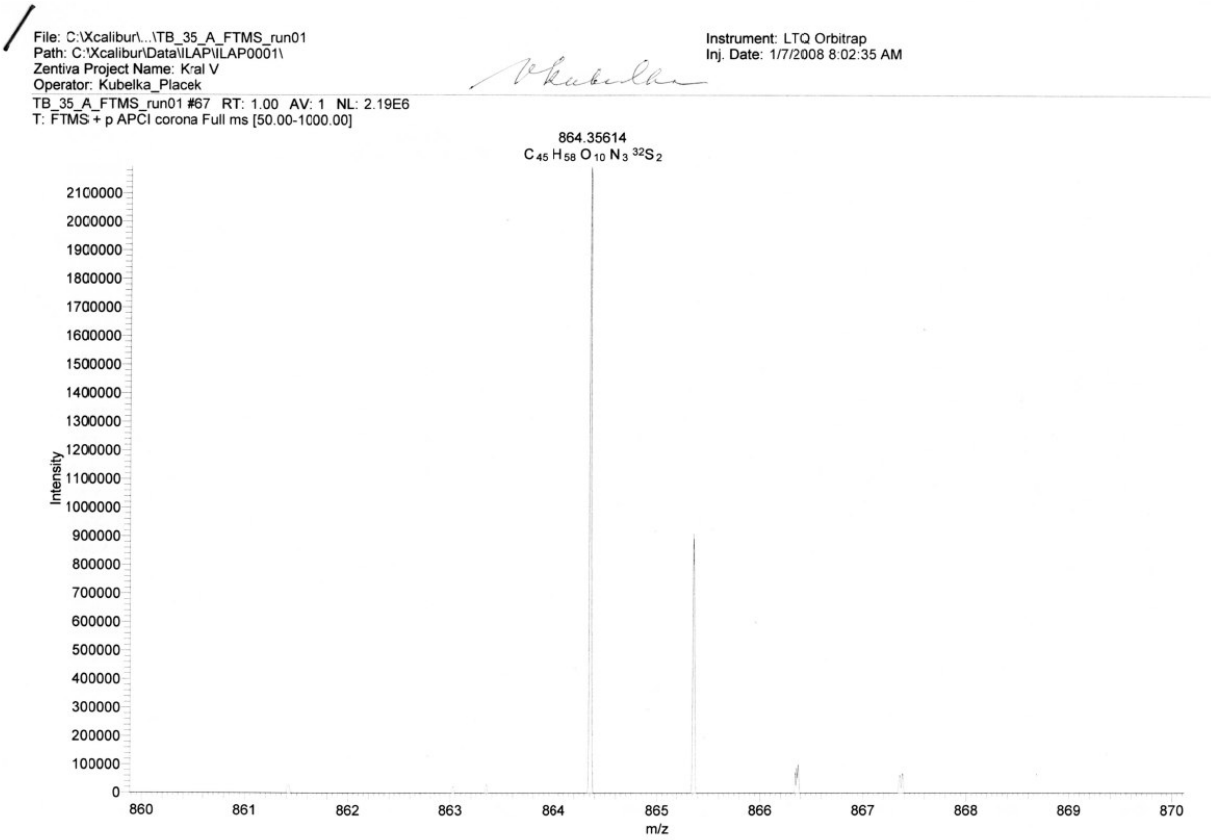
COMPOUND 9 $^{13}C$



HRMS spectrum of compound **9**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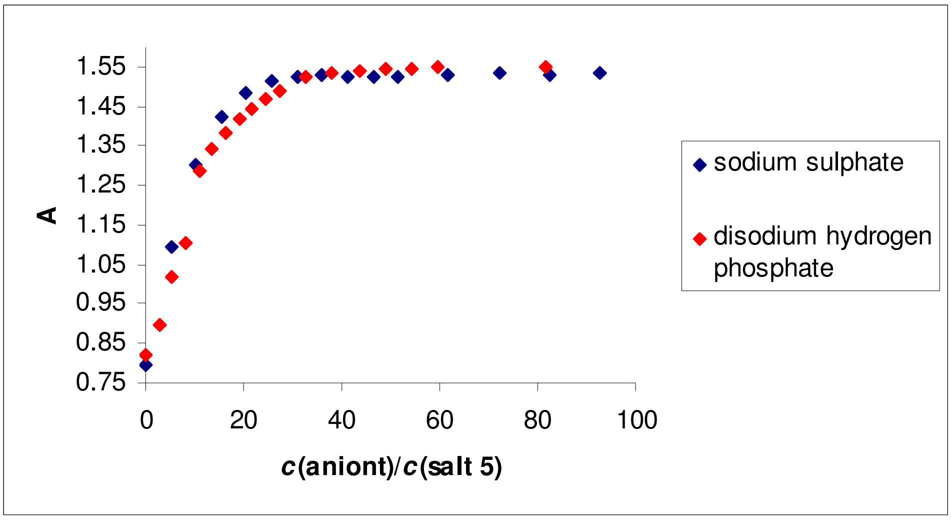
## Titrations

Preliminary study of sulphate over phosphate selectivity for salt **5,** binaphtyls and **7** was made at 30%MeOH, 2%DMSO, 68% $H_2O$(v/v). Studies of anion and anionic saccharides with salt **7** were made at 1mM phosphate buffer 30%MeOH, 2%DMSO, 68% $H_2O$ (v/v). Studies of anion and anionic saccharides with salt **9** were made at 1mM phosphate buffer 15%PEG(n=9), 85% $H_2O$ (v/v).

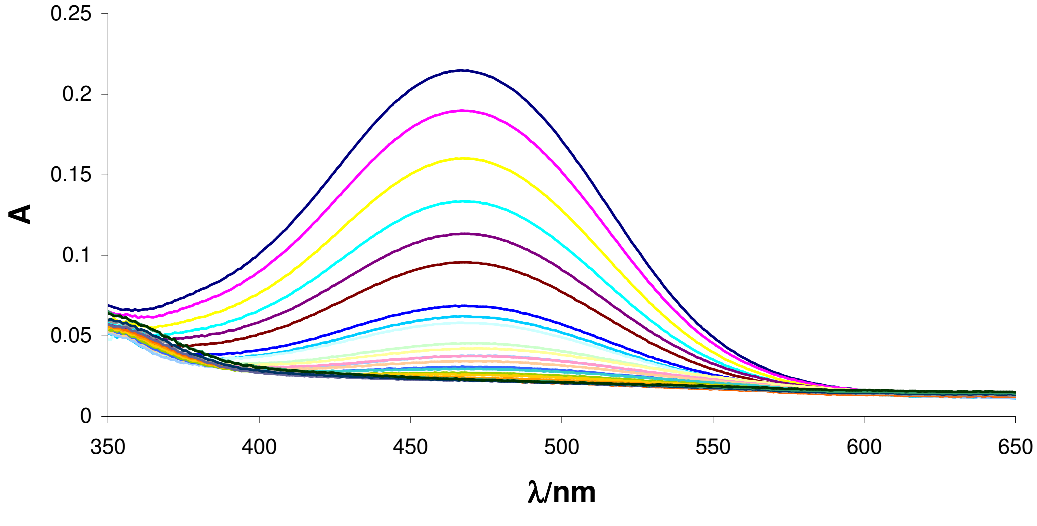
For calculation of binding constants, nonlinear regresion by Letagroup Spefo 2005 have been utilized. For binding study we selected these anionts(chloride, floride, sulphate, acetate and nitrate anion) and following aninonic saccharides (pectin, polygalacturic acid, 2-aminoglucososulphate). In case of nitrate anion, precipitation was observed at pH 6.22 and 7.35. Ks of complexes of salts **7-9** and polysaccharides were calculated using a polysaccharide concentration defined by the concentration of each basic saccharide unit.

Salt **5** (2-methyl-3-propyl-benzothiazolium iodide)
At first we tested phosphate versus sulphate selectivity of benzothiazolium unit. Selectivity of benzothiazolium unit for sulphate was observed. Ks of salt **5** with phosphate and with sulphate were $4\times10^6$ ad $1.3\times10^7$ for complex - salt **5** : anion (1:2) respectively.

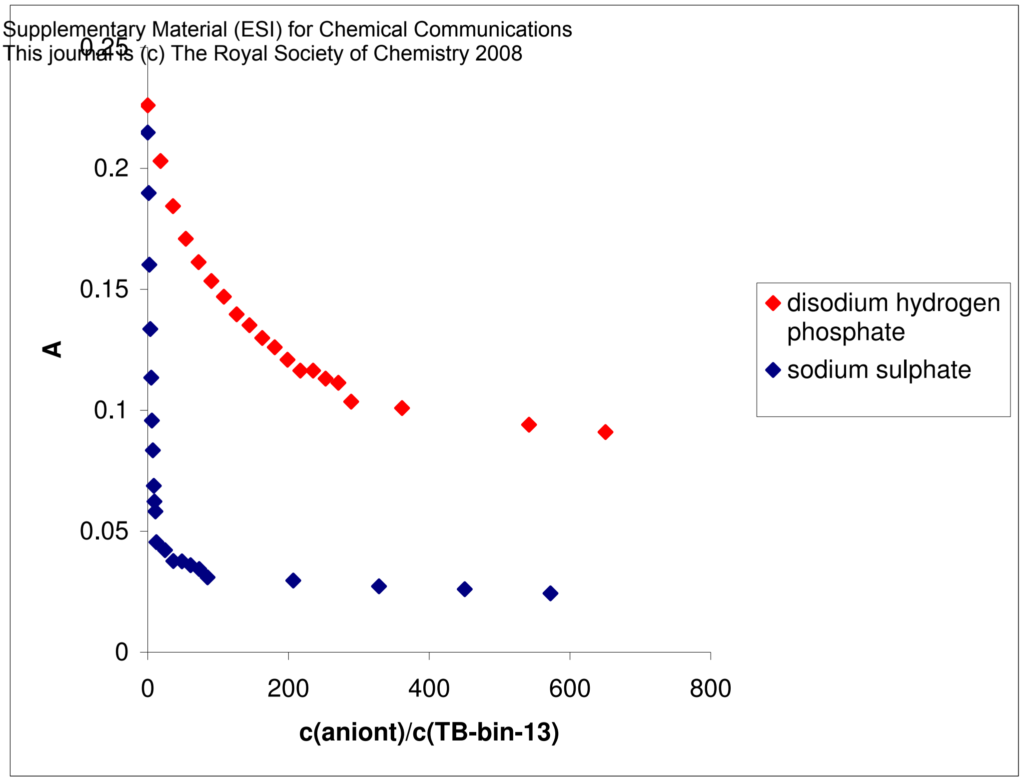


Titration of the salt **5** with anions. Concentration of the salt **5** = $1.3\times10^{-4}$ M in solution of 68% $H_2O$/30% MeOH/2% DMSO, walve length = 277 nm

Titration of binaphtyls with sulphate and phosphate

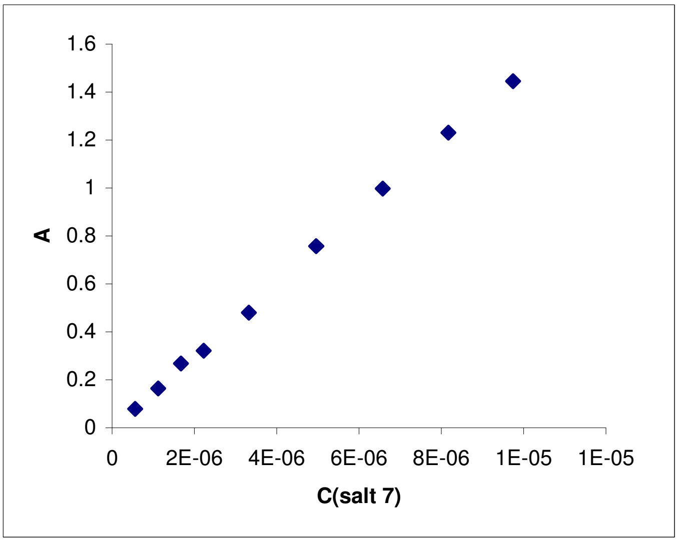


Titration of the binaptyl salt with sulfate. Concentration of the binaptyl salt = $6.1\times10^{-6}$ M in solution of 68% $H_2O$/30% MeOH/2% DMSO, walve length = 467 nm.



Titration of the binaptyl salt with anionts. Concentration of the binaptyl salt = $6.1\times10^{-6}$ M in solution of 68% $H_2O$/30% MeOH/2% DMSO,  walve length = 467 nm

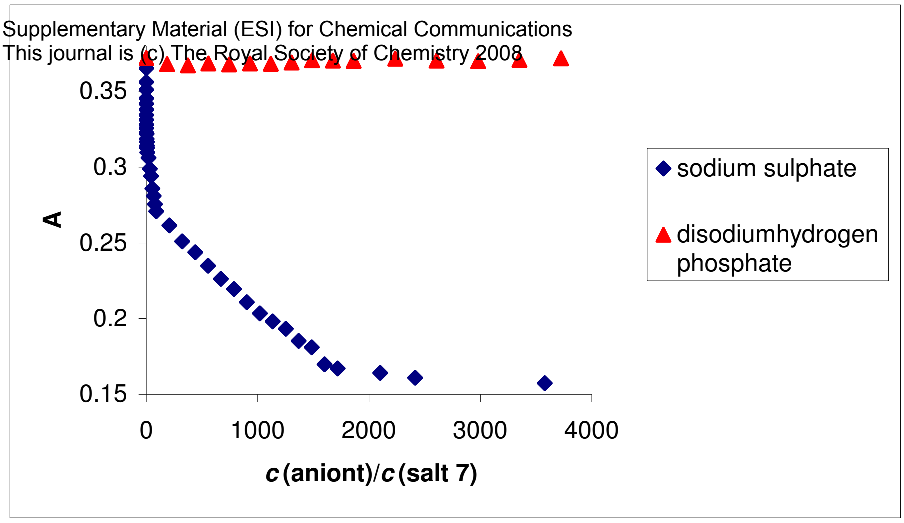
## Dependence of A versus concentration of salt 7



68% $H_2O$/30% MeOH/2% DMSO,  walve length = 663 nm

## Selectivity sulphate versus phosphate

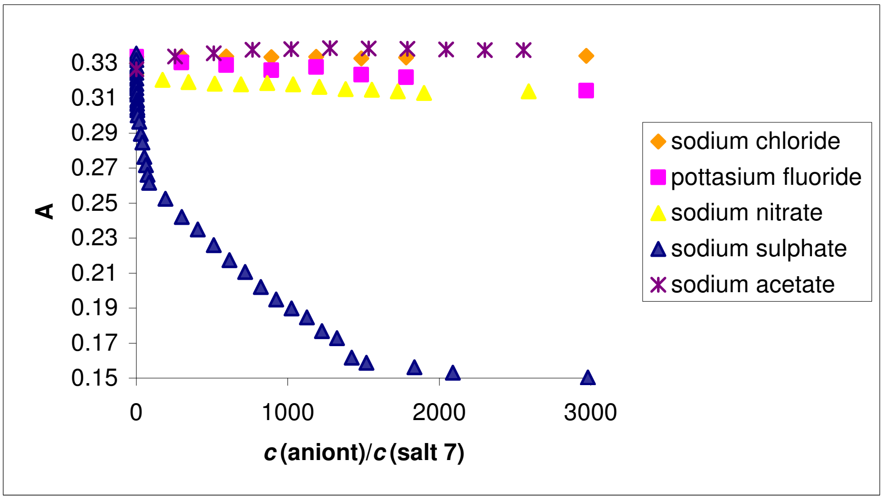
Salt **7**



Titration of the salt **7** with anions. Concentration of the salt **7** = 2.4×10$^{-6}$ M in solution of 68% $H_2O$/30% MeOH/2% DMSO, walve length = 663 nm.

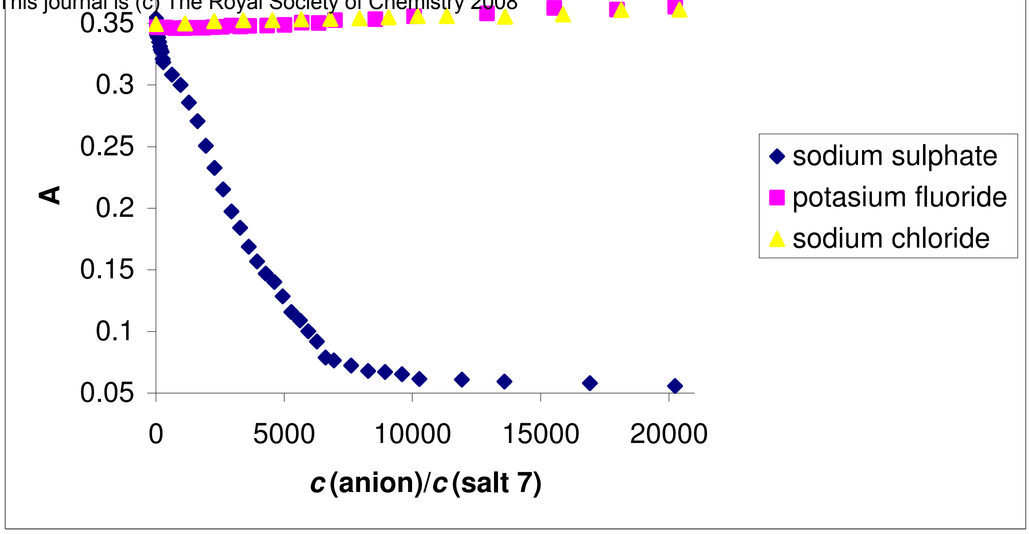
## Titrations of selected anions with salt 7 at various pH

### pH 5.53



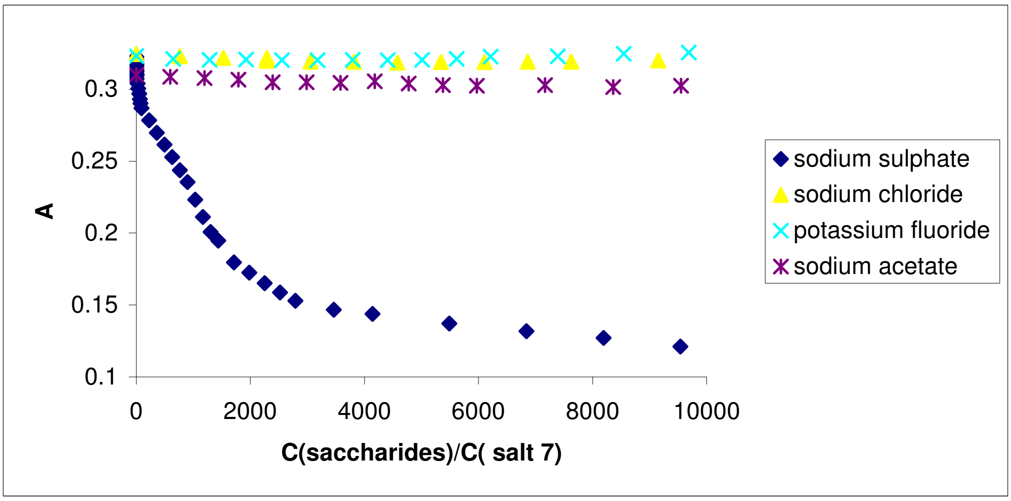
Titration of the salt **7** with anions. Concentration of the salt **7** = 2.2×10$^{-6}$ M in solution of 68% $H_2O$/30% MeOH/2% DMSO, pH = 5.53, medium = 1mM phosphate buffer, walve length = 663 nm.

### pH 6.22



Titration of the salt **7** with anions. Concentration of the salt **7** = 2.2×10$^{-6}$ M in solution of 30%MeOH, 2%DMSO, pH = 6.22, medium = 1mM phosphate buffer, walve length = 663 nm.

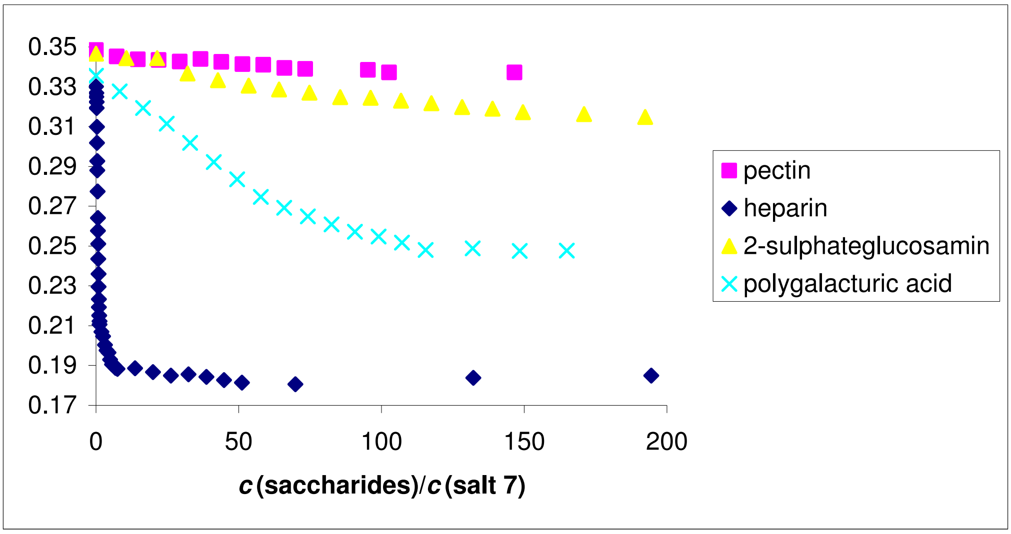
### pH 7.35



Titration of the salt **7** with anions. Concentration of the salt **7** = 2.2×10$^{-6}$ M in solution of 30%MeOH, 2%DMSO, pH = 7.35, medium = 1mM phosphate buffer, walve length = 663 nm.

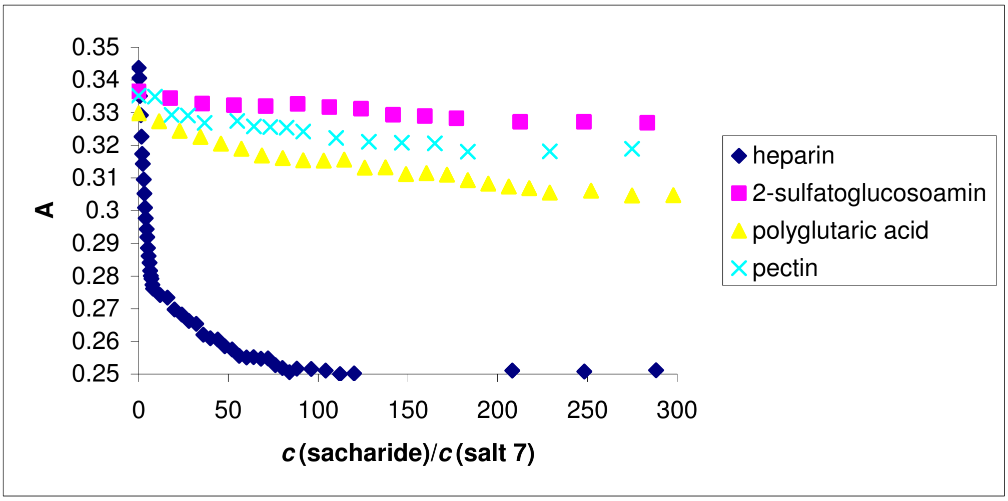
## Titrations of selected aninonic saccharides with salt 7 at various pH

## pH 5.53



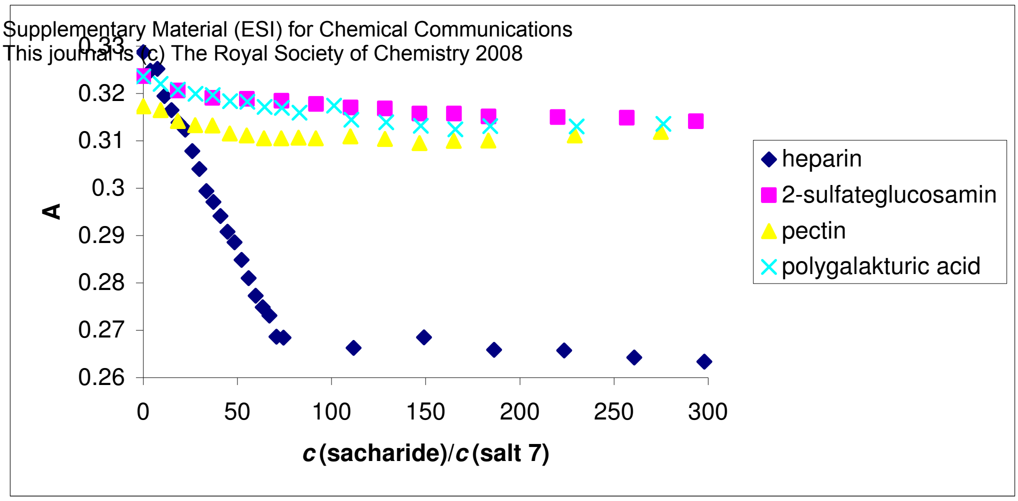
Titration of the salt **7** with acidic sacharides. Concentration of the salt **7** = $2.2\times10^{-6}$ M in solution of 68% $H_2O$/30% MeOH/2% DMSO, pH = 5.53, medium = 1mM phosphate buffer, walve length = 663 nm.

## pH 6.22



Titration of the salt **7** with anions. Concentration of the salt **7** = $2.2\times10^{-6}$ M in solution of 30%MeOH, 2%DMSO, pH = 6.22, medium = 1mM phosphate buffer, walve length = 663 nm

## pH 7.35

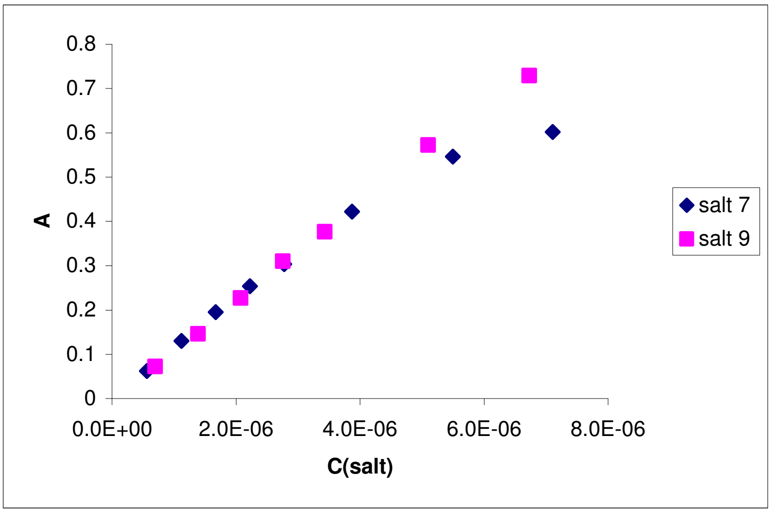


Titration of the salt **7** with anions. Concentration of the salt **7** = 2.2×$10^{-6}$ M in solution of 30%MeOH, 2%DMSO, pH = 7.35, medium = 1mM phosphate buffer, walve length = 663 nm.

## Table: Binding constants of salt 7 in various pH

| analytes | Complex - salt **7** : analyt | 5.53 | 6.22 | 7.35 |
|---|---|---|---|---|
| **Heparin** | 1 : 1 | 8 | 5.2 | 4 |
| | 2 : 2 | 15 | - | - |
| | 1 : 2 | 13 | - | - |
| **Polygalacturic acid** | 1 : 2 | 7.2 | - | - |
| **Sodium sulfate** | 2 : 1 | 11.7 | 7.8 | 7.7 |
| | 2 : 2 | 14.3 | 10.1 | 10.4 |

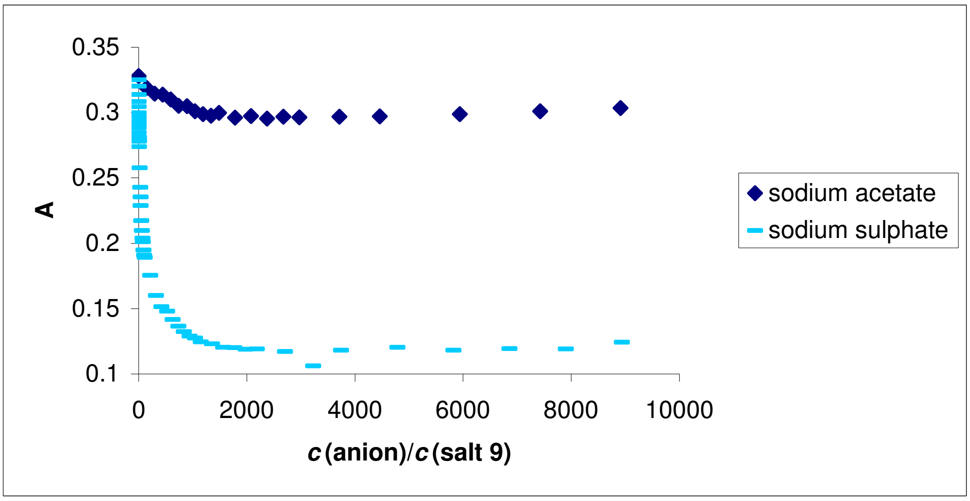
### Dependence of A versus concentration of salt 7 and 9



85% $H_2O$/15% PEG n=9 , walve length = 663nm

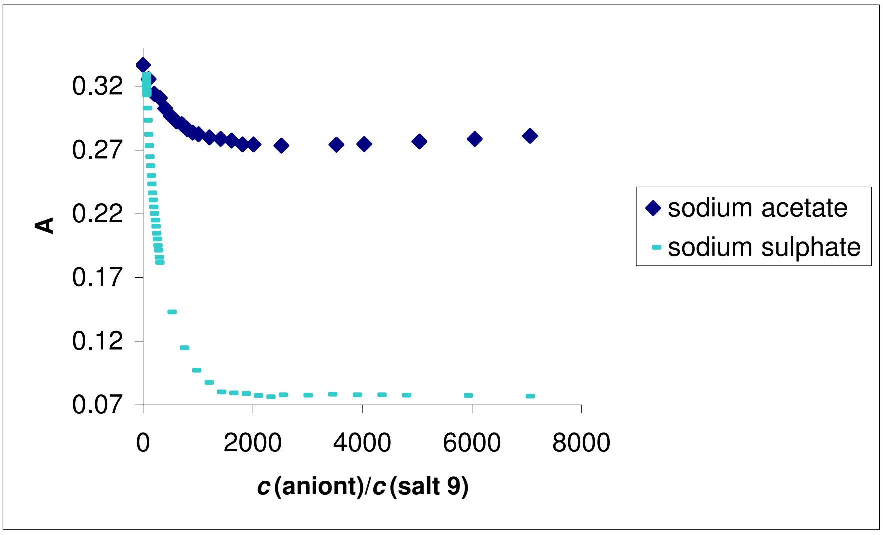
# Titrations of selected anions with salt 9 at various pH

## pH 5.53



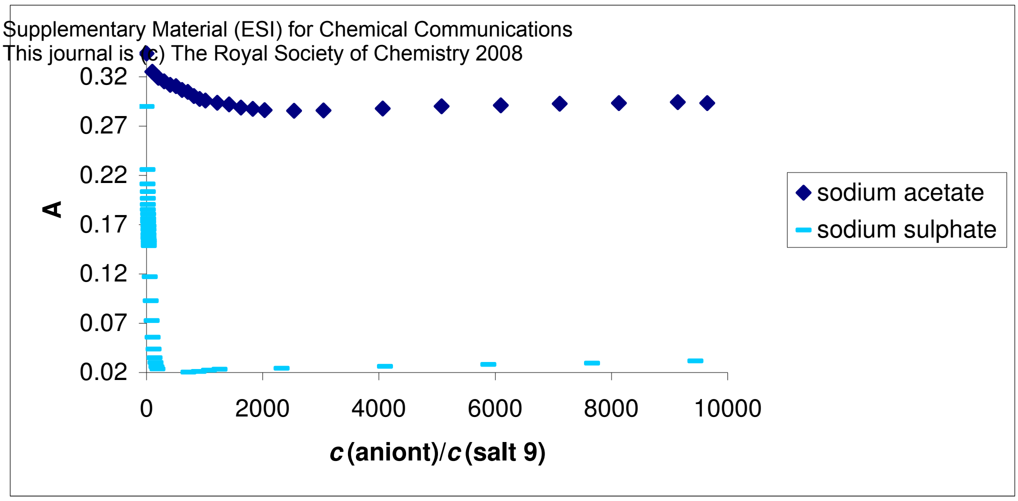
Titration curves of salt **9** with anionts. Concentration of the salt **9** = 4.6×10$^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 5.53, medium = 1mM phosphate buffer, walve length = 663 nm.

## pH 6.22



Titration curves of salt **9** with anionts. Concentration of the salt **9** = 4.6×10$^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 6.22, medium = 1mM phosphate buffer, walve length = 663 nm.

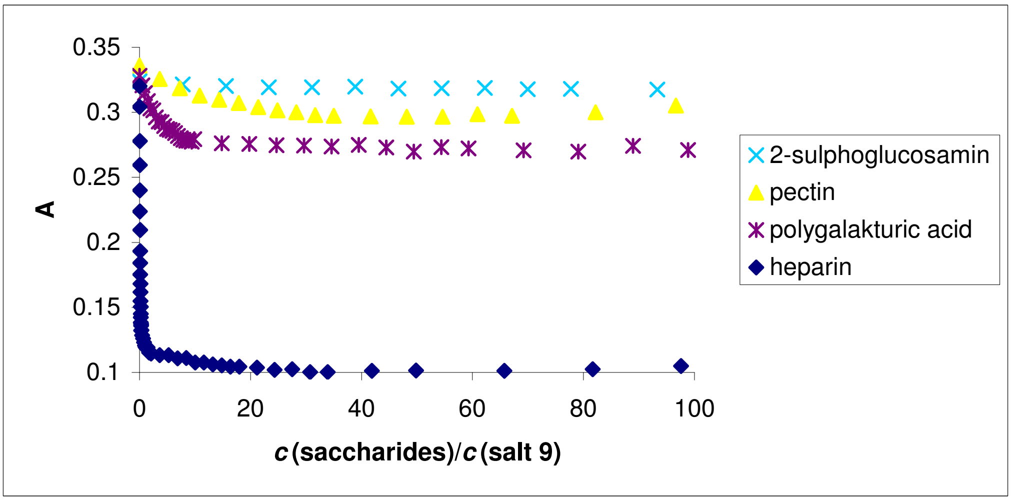
## pH 7.35



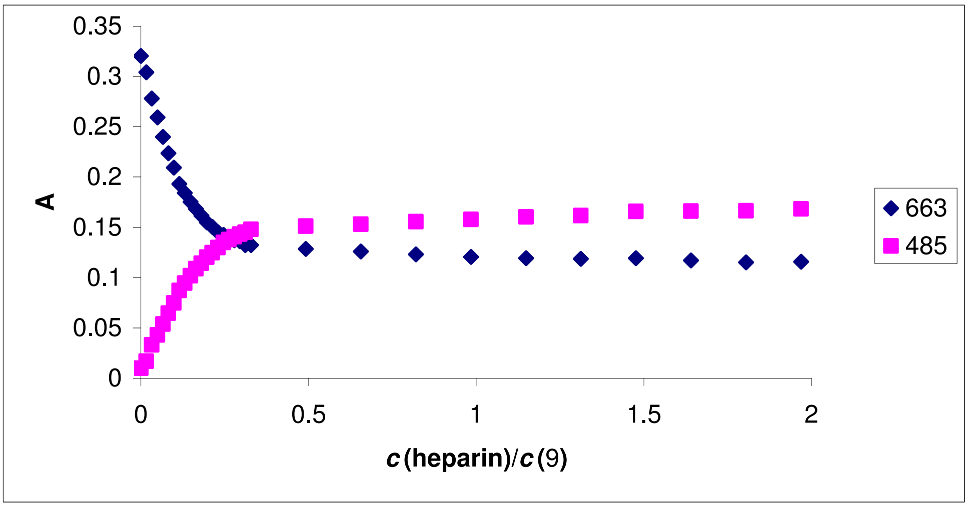
Titration curves of salt **9** with anionts. Concentration of the salt **9** = 4.6×10$^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 7.35, medium = 1mM phosphate buffer, walve length = 663 nm.

## Titrations of selected anionic sacharides with salt 9 at various pH

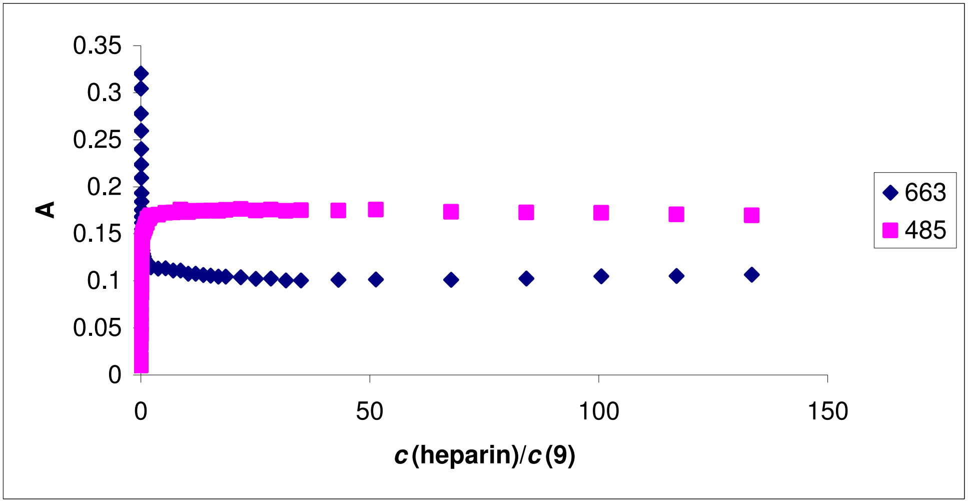
### pH 5.53



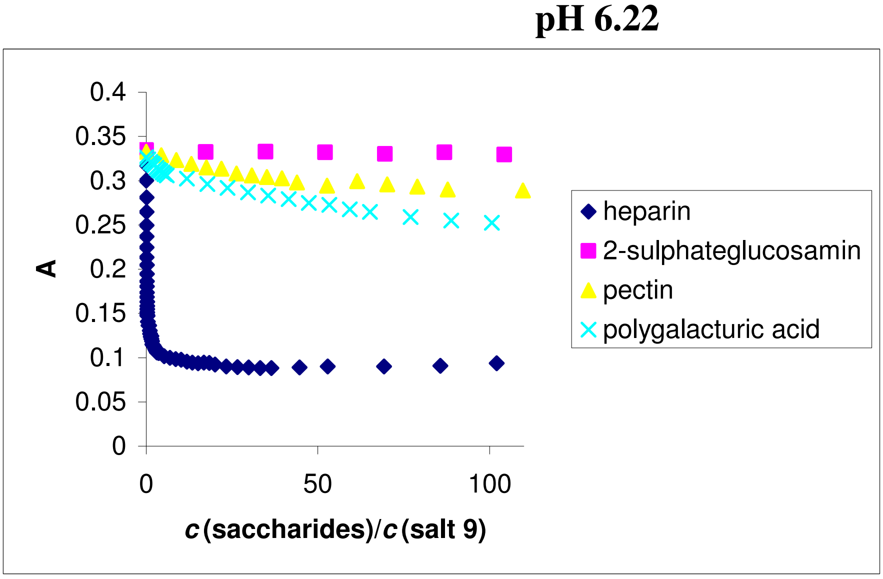
Titration of the salt **9** with acidic polysacharides. Concentration of the salt **9** = 4.6×10$^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 5.53, medium = 1mM phosphate buffer, walve length = 663 nm.



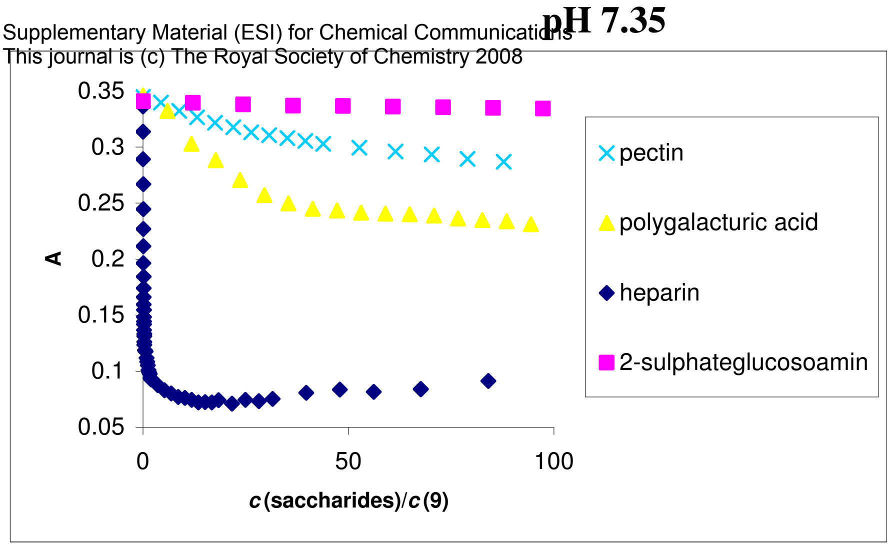
Titration curves of the salt **9** with heparin . Concentration of the salt **9** = 4.6×10$^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 5.53, medium = 1mM phosphate buffer



Titration of curves the salt **9** with heparin . Concentration of the salt **9** = 4.6×10$^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 5.53, medium = 1mM phosphate buffer

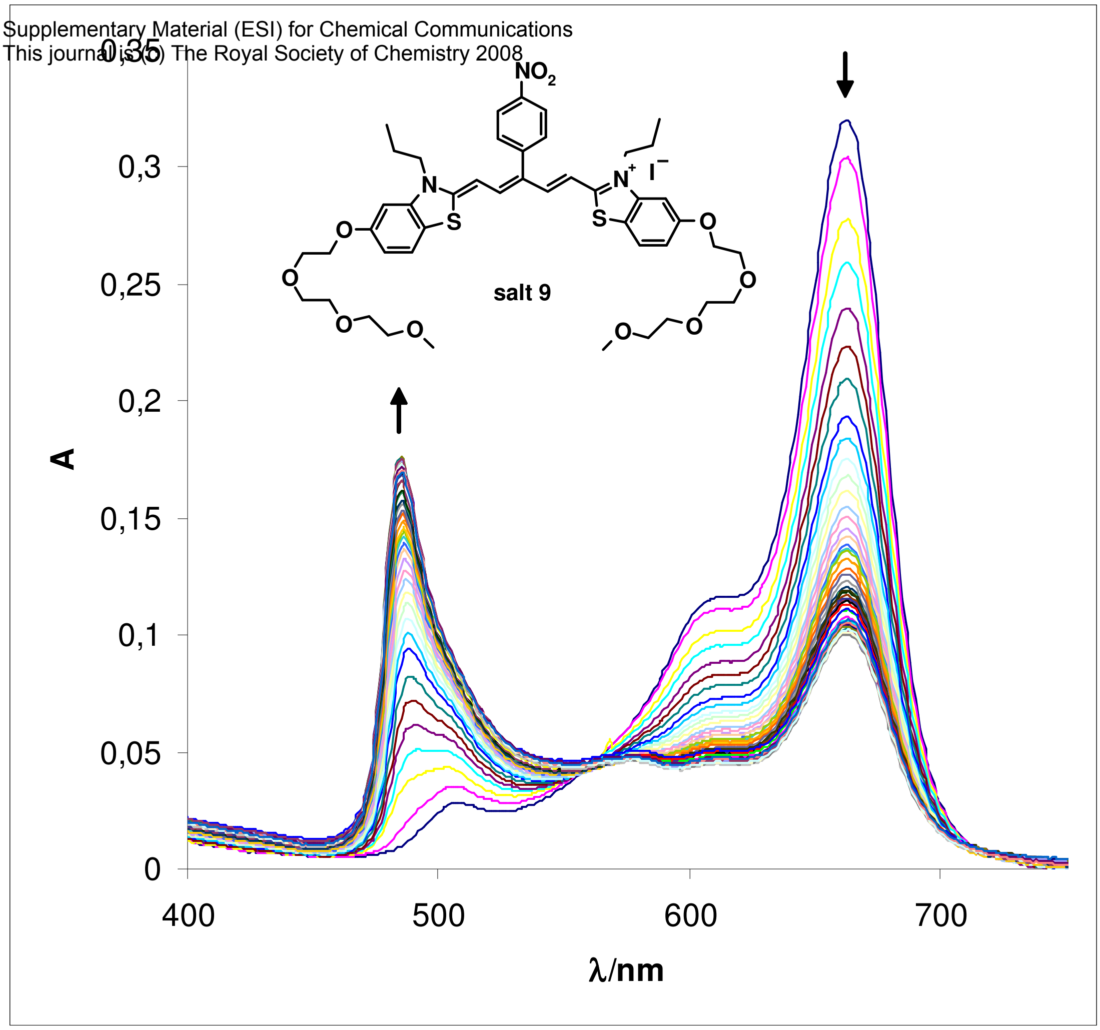


Titration of the salt **9** with acidic polysacharides. Concentration of the salt **9** = 5.519×10$^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 6.22 medium = 1mM phosphate buffer, walve length = 663 nm



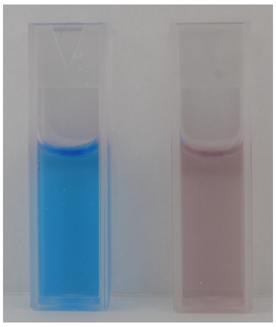
Titration of the salt **9** with acidic polysacharides. Concentration of the salt **9** = $4.6 \times 10^{-6}$ M in solution of 85% $H_2O$/15% PEG n=9, pH = 7.35 medium = 1mM phosphate buffer, walve length = 663 nm

Spectral changes of receptor **9** in presence of Heparin



Titration of salt **9**(c = 4.6×10-6mol/l) by heparin in solution of 85% $H_2O$/15% PEG n=9, 1mM phosphate buffer pH = 5.53, number of eqivalents are from 0 to 140.

Color change of receptor **9** after adding of solution of Heparin



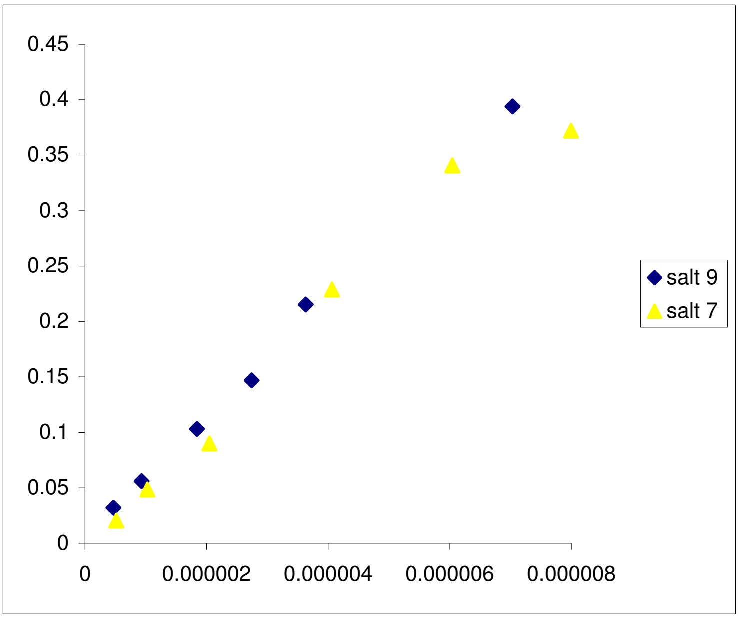
**Blue solution** – salt **9** in solution of 85% $H_2O$/15% PEG 400, pH = 5.53, medium = 1mM phosphate buffer Concentration of **9** is 1.4×10-5M.
**Violet solution** – salt **9** + Heparin in solution of 85% $H_2O$/15% PEG 400, pH = 5.53, medium = 1mM phosphate buffer Concentration of **9** and Heparin are 1.4×10-5M and 5×10-6M respectively.

**Table: Log Ks values and stechiometry of salt 9 in various pH**

| Analyt | Stechiometry of 9 and Analyt | 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 |
| **Polygacturic acid** | 1:1 | 4.8 | 3.6 | 4 |
| **Sulphate anion** | 2:1 | 11.7 | 7.8 | 3.8 |
| | 2:2 | 14.7 | 11.3 | 13.5 |

## Dependence of A versus concentration of salt 7 and 9 for water



2% DMSO, 98%$H_20$, walve length = 665nm for salt **7** and 669nm for salt **9**