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ORIGINAL PAPER

New Fluorescent Sensors Based on 1*H*-pyrazolo[3,4-*b*] quinoline Skeleton

Marek Mac · Tomasz Uchacz · Tomasz Wróbel · Andrzej Danel · Ewa Kulig

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Abstract Novel fluorescing dyes 1,3,4-triphenyl-6-(1,4,7, 10-tetraoxa-13-aza-cyclopentadec-13-ylmethyl)-1*H*-pyrazolo [3,4-*b*]quinoline **(K1)** and 2-[(2-hydroxyethyl)-(1,3, 4-triphenyl-1*H*-pyrazolo[3,4-*b*]quinolin-6-ylmethyl)-amino] ethanol **(L1)** have been synthesized and investigated by the means of steady state and time-resolved fluorescence techniques. These compounds act as sensors for the fluorescence detection of small inorganic cations (lithium, sodium, barium, magnesium and calcium) in solvents of different polarities (THF and acetonitrile). The mechanism, which allows application of these compounds as sensors, is an electron transfer from the electro-donative part of molecule to the acceptor part (fluorophore), which is retarded upon complexation of the electro-donative part by inorganic cations. We found that crown ether-containing compound is very sensitive to the addition of any investigated ions but amino alcohol-containing one exhibits better selectivity to the addition of two-valued cations. Two kinds of the complexes (LM^+ and L_2M^+) were found in the investigated systems. In addition, the dyes may be used as fluorescence indicators in solvents of lower polarity like tetrahydrofuran.

M. Mac (✉) · T. Uchacz
Faculty of Chemistry, Jagiellonian University,
Ingardena 3,
30-060 Krakow, Poland
e-mail: mac@chemia.uj.edu.pl

T. Wróbel
Student of Faculty of Chemistry, Jagiellonian University,
Krakow, Poland

A. Danel · E. Kulig
Department of Chemistry, University of Agriculture,
Balicka 122,
31-149 Kraków, Poland

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Introduction

Fluorescent molecular systems, which undergo strong signal changes upon addition of the external substances, are called fluorescent sensors. Such compounds are attractive for fluorescence detection of nonfluorescent analytes, such as metal ions in biological systems. Fluorescent sensors for detection of small inorganic cations such as sodium, potassium, magnesium or calcium were of subject of many investigations [1, 2]. Such cations are involved in many biological processes of fundamental importance for life. Monitoring of these ions occurring in small amounts in blood and urine is of major importance in medicine. Normal concentrations of these cations in blood and urines (data in parentheses) are 143 (125) mM, 5 (65) mM, 1 (4) mM, and 1.5 (4) mM for Na^+, K^+, Mg^{2+} and Ca^{2+}, respectively [1]. Concentration of the lithium cation in plasma is estimated at the level of 0.09–0.15 μmol/l [3]. On the other hand, barium salts are known as toxic agents (classic signs of barium toxicity are repeated profound hypokalemia, cardiac arrhythmias, respiratory failure, prolonged gastrointestinal dysfunction, paralysis, myoclonus, hypertension, and profound lactic acidosis) [4], the maximal concentrations of this cation in serum is in the range of 3–20 μg/dl [5]. Colorimetric determination based on absorptiometric measurements of the complexes formed between some dyes and these cations began to be popular a long time ago, especially using the compounds similar to EDTA. In the last years much attention has been paid to developing fluorescent sensors.

Much of them work on the basis of a simple electron transfer mechanism as follows:

The molecules which contain electron donor and acceptor moieties (EDA systems) strongly interact with each other in the excited state. Thus, in the absence of inorganic cations the fluorescence quenching of the fluorescing part of the system via electron transfer mechanism is observed. However, the recognition moiety (electron donor part of the molecule) can act as a Lewis base in the ground state. Thus, upon addition of the Lewis acid to the EDA system the complex is formed (the electrodonative property of this part of molecule is strongly diminished). In consequence, the quenching of the fluorescence is weaker making the intensity of the fluorescence depending on the concentration of the inorganic cation. This situation is depicted in Scheme 1.

It should be noted that working principle of the indicators might be different from this presented above. There are known sensors with two pendant hydrocarbons groups (like naphthalene or pyrene) containing diazacrown ether between these subunits. Addition of the cations, bounded to the azacrown, change the ratio of the excimer–monomer fluorescence quantum yields [6–8].

It has been established that in many cases the indicators undergo 1/1 and 2/1 (ligand/metal ion) complexation [9–11]. Thus, the following equilibria should be concerned in the description of the overall complexation process:

$$M^+ + L \overset{K_1}{\rightleftharpoons} ML^+$$

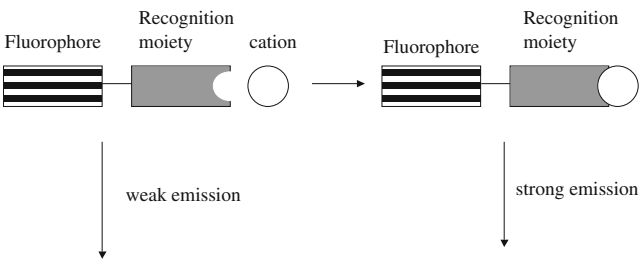
$$M^+ + 2L \overset{K_2}{\rightleftharpoons} ML_2{}^+$$

and

$$ML_2{}^+ + M^+ \overset{K}{\rightleftharpoons} 2ML^+$$

On the other hand, these types of fluorescent molecular sensors can also be used as YES logic gate. The working principle of YES logic gate is the same as EDA fluorescent sensor. The YES logic gate has a single input and a single output—when the input (cation) is 0 the output (fluorescence) is 0, and when the input is 1 the output is 1. Such an operation is so trivial that it is not considered in an electronic context, where it simply represents an electronic conductor. However, it is not trivial in more general context of input and output. On a molecular basis, a lot of examples of YES-type behavior are known and are used for detection of small particles in biological systems, but have not been recognized from a logical viewpoint [12].

In our paper we would like to present the fluorometric data of two compounds being derivatives of 1,3,4-triphenyl-1H-pyrazolo[3,4-*b*]quinoline (PQ).



Scheme 1 Principle of work of the EDA cation fluorescence indicators

Experimental

The synthesis of PQ sensors

Both sensors L1 and K1 can be easily prepared from cheap starting materials in 3–4 steps. Thus, 6-methyl-1,3,4-triphenyl-1H-pyrazolo[3,4-*b*]quinoline **1** was prepared in the three-component reaction by heating a mixture of p-toluidine, benzaldehyde and 2,5-diphenyl-2,4-dihydropyrazol-3-one according to the procedure developed in our laboratory [13]. The resulting compound was brominated with NBS in the presence of catalytic amount of AIBN. Bromomethyl derivative **2** reacts with commercially available 1,4,7,10-tetraoxa-13-aza-cyclopentadecane and diethanolamine yielding **3 (K1)** and **4 (L1)** respectively (Scheme 2).

Experimental

Melting points were determined on a Mel-Temp Apparatus II (capillary). NMR spectra were recorded on a Varian (Mercury) spectrometer at 25 °C.

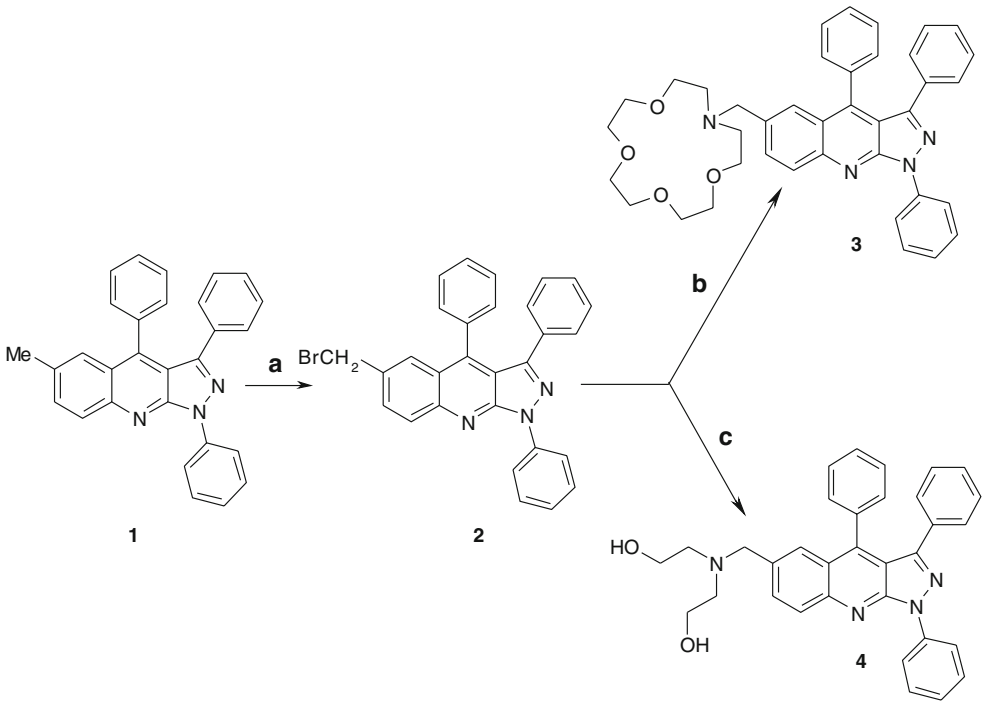
6-Methyl-1,3,4-triphenyl-1*H*-pyrazolo[3,4-*b*]quinoline 1

Equimolar amounts (0.02 mol) of p-toluidine, benzaldehyde and 2,5-diphenyl-2,4-dihydropyrazol-3-one were heated together in diethylene glycol (10 mL) at 190 °C for 2 h. After cooling the reaction mixture was diluted with ethanol (20 mL) and boiled. The yellow crystalline precipitate was filtered off, dried and recrystallized from toluene/hexane (3:1).

Yellow crystals, yield 33%, mp 208–210 °C.

^{1}H NMR(300 MHz, $CDCl_3$, δppm): 8.60(d, J = 8.6 Hz, 2H, 2,6-H_{1Ph}); 8.17(d, J = 8.4 Hz, 1H, 8-H); 7.64–7.56(m, 4H); 7.59(t, J = 7.9 Hz, 2H,); 7.35–7.04(m, 11H); 2.45(s, 3H, 6-Me).

Scheme 2 **a** NBS/CCl_4/AIBN; **b** K_2CO_3/AcCN/aza-crown; **c** K_2CO_3/AcCN/diethanoloamine



6-Bromomethyl-1,3,4-triphenyl-1*H*-pyrazolo[3,4-*b*] quinoline 2

Pyrazoloquinoline **1** (0.01 mol) was heated with NBS (0.012 mol) in CCl_4 (20 mL) for 12 h. The hot solution was filtered to remove insoluble residues and evaporated. The precipitate was crystallized from toluene/hexane (4:1).

Yellow powder, 89%, mp 183–5 °C (dec).

^{1}H NMR(300 MHz, $CDCl_3$, δppm): 8.58(d, *J* = 8.7 Hz, 2H); 8.23(*J* = 8.9 Hz,1H,8-H); 7.85(d, *J* = 1.6 Hz, 1H, 5-H); 7.81(dd, *J* = 8.9 Hz; 1.6 Hz, 1H, 7-H); 7.58(t, J = 7.1 Hz, 2H); 7.38–7.03(m, 11H); 4.59(s, 2H).

^{13}C NMR(300 MHz, $CDCl_3$): 150.51; 148.33; 146.89; 144.79; 139.81; 134.18; 133.49; 132.48; 131.55; 130.39; 130.17; 129.11; 129.07; 128.61; 128.08; 127.71; 127.58; 126.67; 125.63; 123.08; 121.04; 115.32; 34.09.

1,3,4-Triphenyl-6-(1,4,7,10-tetraoxa-13-aza-cyclopentadec-13-ylmethyl)-1*H*-pyrazolo[3,4-*b*] quinoline 3 (K1)

$BrCH_2$PQ **2** (1 mmol), 1,4,7,10 –tetraoxa-13-aza-cyclopentadecane (1 mmol) and anhydrous K_2CO_3 (1.5 mmol) were heated in acetonitrile (10 mL) at 80 °C for 5 h. After this time the solid was filtered off and the residue was subjected to column chromatography (silica gel, Merck 60, 70–230 mesh, using toluene as eluent at the beginning, next a mixture toluene/ethyl acetate 3:1 and ethyl acetate at the end of elution).

Yellow crystals, yield 25%, mp133 °C.

^{1}H NMR(300 MHz, $CDCl_3$, δppm): 8.56(d, J = 8.7 Hz, 2H); 8.14(d, J = 8.8 Hz, 1H, 8-H); 7.84(dd, J = 8.8 Hz,1.8 Hz; 1H, 7-H); 7.67(d, J = 1.2 Hz, 1H, 7-H); 7.53(t, J = 7.9 Hz, 2H); 7.30–6.99(m, 11H); 3.71(s, 2H); 3.62–3.53(m, 16H); 2.74(t, 4H).

2-[(2-Hydroxyethyl)-(1,3,4-triphenyl-1*H*-pyrazolo[3,4-*b*] quinolin-6-ylmethyl)-amino]etanol 4 (L1)

$BrCH_2$PQ **2** (1 mmol), diethanoloamine (1 mmol) and anhydrous K_2CO_3 (1.5 mmol) were heated in acetonitrile (10 mL) at 80 °C for 5 h. After this time the solid was filtered off and the solution was cooled. The yellow crystalline precipitate was filtered off and subjected to column chromatography (silica gel, Merck 60, 70–230 mesh, using toluene as eluent at the beginning, next a mixture toluene/ethyl acetate 3:1 and ethyl acetate at the end of elution).

Yellow crystals, mp 143 °C, yield 65%.

^{1}H NMR(300 MHz, $CDCl_3$, δppm): 8.62(d, J = 8.7 Hz, 2H); 8.25(d, J = 8.5 Hz, 1H,8-H); 7.83–7.80(d + s, 2H, 5-H,7-H); 7.61(t, J = 7.9 Hz, 2H); 7.64–7.07(m, 11H); 3.83 (s,2H); 3.62(t,4H); 2.74(t,4H), 2.1(b, 2H, OH).

Fluorescence

The solvents: cyclohexane (CHX), dibutyl ether (DBE), ethyl acetate (EtAc), tetrahydrofuran (THF), glycerol

triacetate (GTA), dichloromethane ($MeCl_2$), aliphatic alcohols (from methanol to octanol), acetonitrile (ACN), dimethylformamide (DMF), propylene carbonate (PC) and dimethyl sulfoxide (DMSO) were of spectroscopic grade and were used as received (all from Aldrich). All the solvents did not show any traces of fluorescence. For fluorescence measurements the solutions of the dyes were degassed using multiple freeze-pump-thaw cycles. The sample concentration of the dyes for spectroscopic measurements was ca. 10^{-5} M (this corresponds to absorbance's of ca. 0.2–0.3 at the excitation wavelengths used in the fluorescence investigations). Lithium, sodium, barium, magnesium and calcium (tetrahydrate) perchlorate (Aldrich) were used as received. In an independent measurement it was found that a small addition of deionised water couldn't influence significantly the fluorescence of the acetonitrilic solution of the dyes (K1 and L1).

Fluorescence measurements were performed on a home-built spectrofluorimeter and a time-correlated single photon counting setup. For time-resolved fluorescence measurements a picosecond diode laser (λ = 400 nm, 70 ps pulse duration) (IBH-UK) was used as the excitation source. For steady-state fluorescence measurements a 365 or 405-nm line of medium-pressure mercury lamp was used. The fluorescence quantum yield measurements were carried out with quinine sulphate in water (Φ_{fl} = 0.55) [14] as an actinometer. The measurements of the fluorescence intensity upon addition of the perchlorates were done without degassing of the samples.

The equilibrium constants (K_1) for the complexation of the ligand by alkali cations are calculated by fitting the Eq. 1 to the relative fluorescence intensities in the presence of different concentration (c_M) of perchlorates [15].

$$\Phi(c_M) = \Phi(0) + \frac{\Phi(\infty) - \Phi(0)}{2c_L}\left[c_L + c_M + 1/K_1 - \sqrt{(c_L + c_M + 1/K)^2 - 4c_L c_M}\right] \quad (1)$$

where $\Phi(0)$, $\Phi(\infty)$ and $\Phi(c_M)$ is the fluorescence intensity of the dye alone, with the large concentration of the salt and with the c_M concentration of the salt, respectively, c_L indicates the analytical concentration of the ligand.

For 2/1 complexation where the binding constant K_2 is defined by the Eq. 2:

$$K_2 = \frac{[ML_2^+]}{[L]^2[M^+]} \quad (2)$$

The Eq. 1 can be converted to a more complex one:

$$\Phi(c_M) = \Phi_0 + \frac{2x}{c_L}(\Phi_\infty - \Phi_0) \quad (3)$$

Where x denotes the concentration of the complex ML_2^+. This quantity can be obtained by numerical solving of the equation, using own Fortran procedures:

$$ax^3 + bx^2 + cx + d = 0 \quad (3a)$$

With the following parameters:

$$\begin{aligned} a &= -4K_2 \\ b &= 4K_2(c_L + c_M) \\ c &= -(K_2 {c_L}^2 + 4K_2 c_L c_M + 1) \\ d &= K_2 {c_L}^2 c_M \end{aligned} \quad (3b)$$

The values of the binding constants K_1 and K_2 were obtained from fluorescence intensities using nonlinear least-square fitting coded in FORTRAN.

Results

Absorption spectra of the dyes are rather insensitive to solvent polarity. The addition of the perchlorates only influences the absorption in the UV region of the spectra. The influence is rather small as pointed out in Fig. 1.

Fluorescence quantum yields of the dyes strongly depend on solvent polarity. In solvents of low polarities the fluorescence quantum yields are very high and decrease with increasing solvent polarity, even though the position of the maximum is almost not influenced by polarity of the solvent.

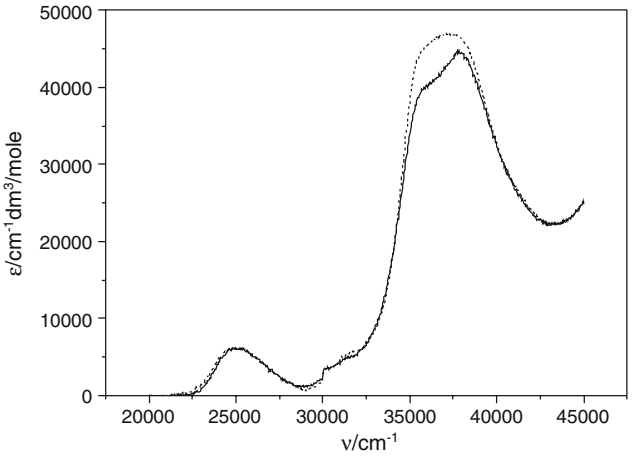


Fig. 1 Absorption spectra of K1 in acetonitrile without (*solid*) and with 0.01 M $LiClO_4$ (*dotted*)

The fluorescence decay functions are monoexponential in solvents of low polarities and clearly biexponential in solvents of dielectric constants greater that 7 (i.e. more polar than THF) i.e. $I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$. The analysis of the decay functions in solvents of larger polarities gives the long- and short-time characteristics, i.e. fluorescence lifetimes τ_1 and τ_2 and corresponding amplitudes A_1 and A_2. It should be noted that the fluorescence decay time τ_1 is weakly solvent polarity dependent (ca. 17 ns) (Fig. 2).

Fluorescence quantum yields and the fluorescence lifetimes (τ_2) of the dyes as solvent polarity function are presented in Fig. 3.

Upon addition of perchlorates to the acetonitrile solutions of dyes L1 and K1 the fluorescence significantly increases. At the same time, the amplitude A_2 of the fluorescence decay functions decreases. The fluorescence decay function becomes single-exponential in the presence of perchlorates exceeding 10^{-5} M (except of the compound L1 with sodium perchlorate). The dependence of the fluorescence quantum yield on salt concentration is presented in Fig. 4.

For the compound L1 the intensity of the dye changes due to added salts is presented in Fig. 5.

Figure 6 presents the dependence of the relative fluorescence of L1 in the presence of $Mg(ClO_4)_2$ in acetonitrile. It has been found that the complex of this dye with Mg^{2+} is 2/1 type. For other used salts the 1/1 complexation was observed.

The equilibrium constants calculated using Eqs. 1 and 2 are collected in Table 1.

We performed the measurements also in tetrahydrofuran (THF). In this case only 1/1 complexation in all investigated systems was observed.

The relative fluorescence intensities with the best fits as functions of the analytical salts concentration to the Eq. 1 are presented in Fig. 7.

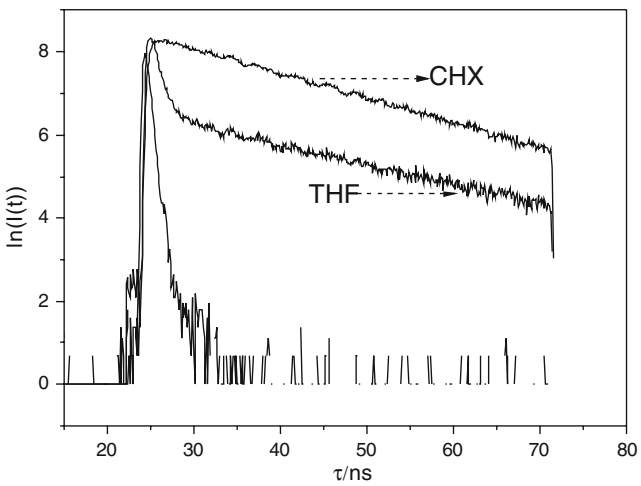


Fig. 2 Fluorescence decay functions of K1 in CHX and THF measured at 450 nm. The pulse profile (λ_{max} = 400 nm) is also depicted

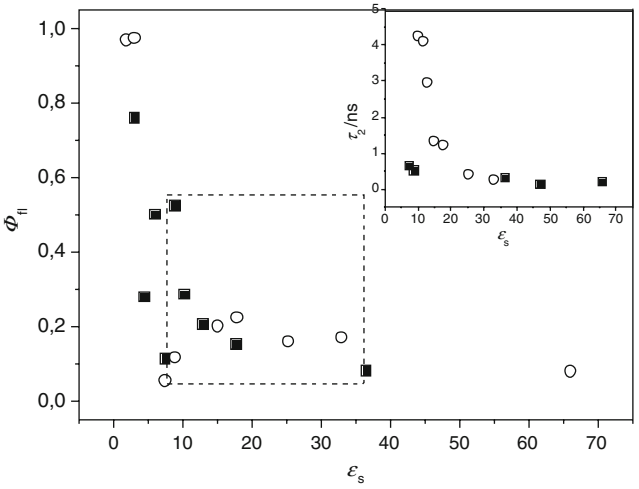


Fig. 3 Solvent polarity dependence of the fluorescence quantum yields of the dyes L1(*black squares*) and K1 (*open circles*). The data in alcohols are marked in the *dashed box*. *Inset*—the dependence of the fluorescence lifetimes (τ_2) for K1 on solvent polarity. *Open circles*—the data in alcohols

The binding constants are presented in Table 2. For L1 with monovalent cations no complexation has been observed up to 0.5 M concentration of the salts. In all cases the 1/1 complexes have been detected.

Discussion

The studied molecules do not show significant solvent polarity influence on the ground state absorption and the

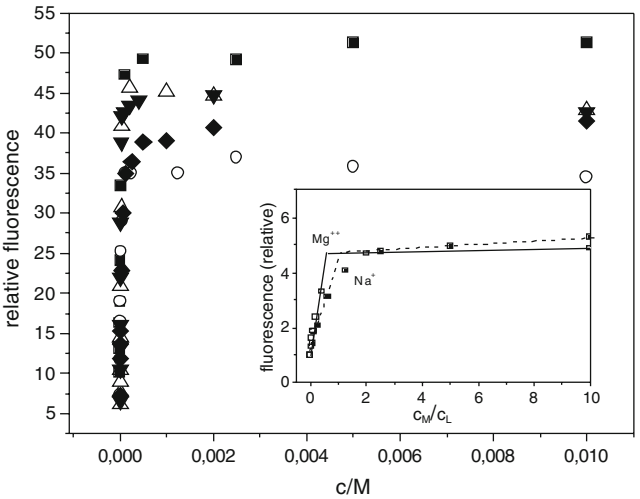


Fig. 4 Salt concentration dependence of the relative fluorescence quantum yield of K1 upon addition of lithium (*open circles*), sodium (*black diamonds*), calcium (*open triangles*), barium (*black triangles*) and magnesium perchlorates (*black squares*) in acetonitrile. Dependence of the relative fluorescence of the complex K1-Na^+ and K1-Mg^{2+} on the ratio of salt to dye concentration in ACN (*inset*)

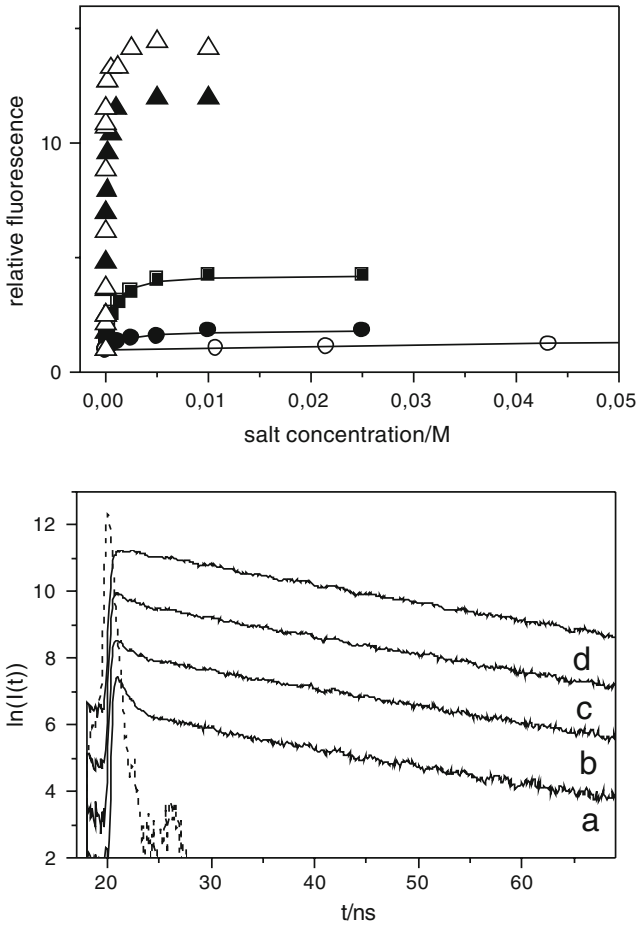


Fig. 5 Dependence of the relative fluorescence intensity of L1 on the molar concentration of magnesium (*open triangles*), calcium (*black triangles*) barium (*black squares*), lithium (*black circles*) and sodium (*open circles*) perchlorates (*top*). The correlation lines represent the predictions given by Eq. 1. Fluorescence decay functions for compound L1 in the absence (**a**) and in the presence of 1.6×10^{-5} M (**b**), 3.2×10^{-5} M (**c**) and 0.17 M $Ca(ClO_4)_2$. The excitation profile is also presented (*bottom*)

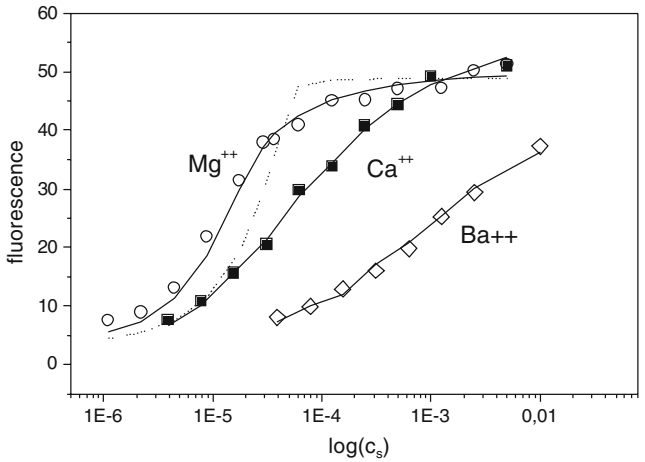


Fig. 6 Dependence of the relative fluorescence quantum yield of L1 on logarithm of the salt concentration of magnesium (*open circles*), calcium (*black squares*) and barium (*open triangles*) perchlorates in ACN. Broken line represents the best fit to the Eq. 1, and *solid line* represents the fit according to the Scheme 2 (2/1 complexation—Eqs. 2–3) for magnesium salt. For other systems the correlation (*solid*) lines are calculated by Eq. 1

position of the maximum of fluorescence. However, the recognition moiety (amino alcohol and crown ether) influences significantly the fate of the fluorescing state of the fluorophore. In the excited singlet state the fluorescence quenching occurs, as can be judged from the solvent polarity dependence of the fluorescence decay functions and quantum yields. In solvents of lowest polarity the quenching is hardly observed but in THF the long-lived fluorescence component occurs only in trace (cf. Fig. 2). This behaviour is also observed for aprotic solvents of larger polarity. The long fluorescence lifetime (τ_1) is not dependent on solvent polarity (it scatters around the value of 17 ns). Similar data were observed for the parent molecule i.e. 1,3,4-triphenyl-1*H*-pyrazolo[3,4-*b*]quinoline (PQ) [18]. Fluorescence quantum yields clearly depend on solvent polarity as can be judged from Fig. 3. They decrease with increasing solvent polarity. This is consistent with the previous observation, namely that the solvent polarity influences fluorescence quenching. Increasing solvent polarity decreases the energy of the charge transfer pair, which is formed in monomolecular charge transfer process, resulting in more efficient fluorescence quenching in polar solvents. Some increment in the fluorescence quantum yield is observed in alcohols, the effect is more significant for compound L1 than K1. The effect is certainly caused by the ground state interaction between the lone pair at the nitrogen atom and hydrogen of the hydroxyl group of the alcohols. Thus, the electron, which may be transferred in the excited singlet state to the pyrazoloquinoline skeleton (PQ), is now engaged in the hydrogen bonding and the electron donative property of the recognition moiety is significantly lowered. This is also reflected in much longer fluorescence decay in higher alcohols (cf. Fig. 3—inset).

These observations may be useful in designing these molecules as fluorescence indicators of small inorganic cations.

The change of the first absorption band upon addition of the salts is not significant. Only small influence in the deep UV-region is observed (cf. Fig. 1). Addition of small amounts (up to 4×10^{-5} M) of perchlorates (lithium, sodium, calcium, barium and magnesium) to the solution of K1 in acetonitrile causes a ca. 8-fold increase of the fluorescence intensity. It means that even a small concentration of the cations is efficient to complexate the dye and this complex is stable in the excited singlet state. It means a large sensitivity of this dye on the presence of *any* investigated cation. Thus, this dye cannot be used as a

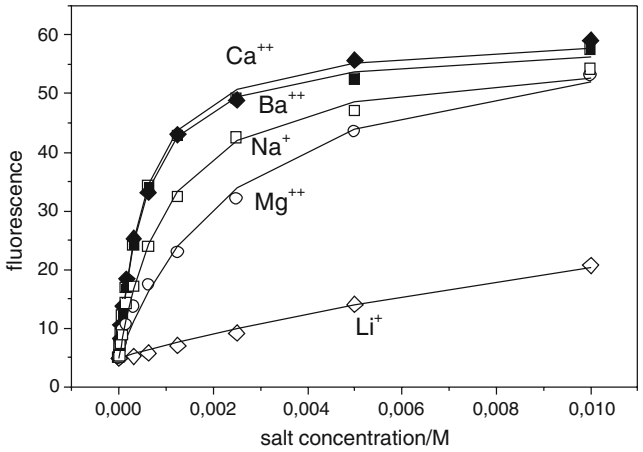


Fig. 7 Dependence of the relative fluorescence quantum yield of K1 on the salt concentration of lithium (*open triangles*), sodium (*open squares*), magnesium (*open circles*), calcium (*black diamonds*) and barium (*black squares*) perchlorates in THF. *Solid lines* represent the best fit to the Eq. 1

fluorescing indicator for the cations in acetonitrile. An interesting behaviour has been observed upon addition of the salts concentrations which many times exceed the concentration at which the maximal fluorescence quantum yield has been reached. Upon successive addition of the salt a decrease of the fluorescence quantum yield has been observed. This effect is most pronounced with $LiClO_4$, as presented in Fig. 8.

Most probably the initially formed 2/1 complex undergoes consecutive complexation with the excess of the lithium cations to form 1/1 complex as it has been mentioned in Introduction. We measured also the fluorescence decay function of K1 with the addition of $Mg(ClO_4)_2$ and $LiClO_4$ at higher salt concentration. We found that at higher concentration the fluorescence lifetime is significantly decreased (12.1 ns) compared to this at micromolar salt concentration (15.5 ns). Similar (although not so pronounced) effect was observed with the addition of magnesium perchlorate. At higher salt concentration the lifetime of the complex was smaller (19.5 ns) in comparison to that with the addition of the micromolar salt concentration (21.5 ns).

Table 1 Binding constants i.e. equilibrium constants between different inorganic cations and the compounds K1 and L1 in acetonitrile determined from fluorimetric titration

Cation (X)	Radius (Å) [16]	Charge density of cation [17]	K_1(L1M)/M^{-1} or K_2(L1$_2$M)/M^{-2}	K_2(K1$_2$M)/M^{-2}
Li^+	0.59	1.47	589	1.4×10^8
Na^+	1.02	1.03	16.8	1.1×10^9
Ba^{++}	1.34	1.49	1,632	2.9×10^9
Ca^{++}	1.0	2.02	2.32×10^4	1.5×10^9
Mg^{++}	0.66	3.03	$K_2=1.5\times10^9$	2.6×10^9

Table 2 Binding constants i.e. equilibrium constants between different inorganic cations and the compounds K1 and L1 in THF determined from fluorimetric titration

Cation (X)	Radius (Å)	Charge density of cation	K_1(L1X)/M^{-1}	K_1(K1X)/M^{-1}
Li^+	0.59	1.47	No complexation	44.4
Na^+	1.02	1.03	No complexation	955
Ba^{++}	1.34	1.49	0.5	1.93×10^3
Ca^{++}	1.0	2.02	644	1.92×10^3
Mg^{++}	0.66	3.03	546	383.2

The compound L1 shows larger sensitivity with respect to the cation, meaning that the ratio of the binding constant between Ca^{2+} and Na^+ containing complexes exceeds 1,300. Also the difference between the binding constants with barium and calcium exceeds factor 14 (see Table 1). A small amount of calcium perchlorate (ca. 0.001 M) forms a stable complex with the dye causing an increase of the fluorescence intensity of the factor ca. 6. The effect is a little bit smaller upon addition of barium perchlorate, much smaller in the presence of lithium salt and almost not observed upon addition of sodium cation. Magnesium cation, which has the

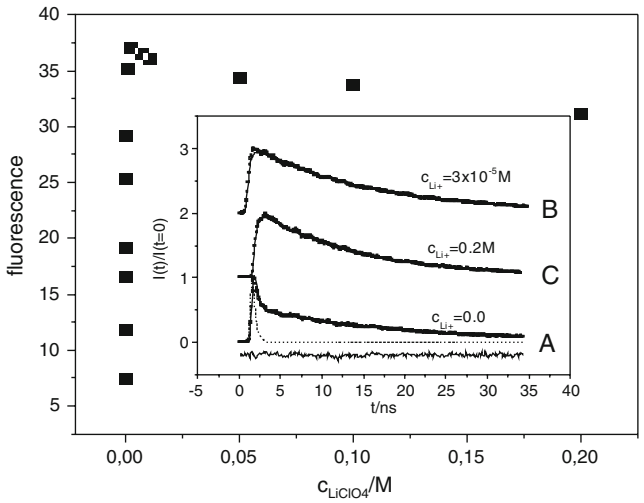


Fig. 8 Dependence of relative fluorescence of K1 on the concentration of lithium perchlorate in ACN. *Inset*—Fluorescence decay functions of K1 without LiClO4 (*A*) and with addition of the salt (*B*) and (*C*) and their fits to the biexponential (*A*) and monoexponential decay function (*B* and *C*). The residuals for decay function *A* are also plotted. The decay functions are vertically shifted for clarity of the picture. The decay parameters are as follows: (*A*) τ_1=17.0 ns (A_1=0.07), τ_2=0.17 ns (A_2=0.37), (*B*) τ_1=14.5 ns, (*C*) τ_1=12.1 ns

largest charge density from the cations, investigated here forms a 2/1 complex in acetonitrile.

The binding constants (L1M in acetonitrile) correlate well with the charge density of the cations, which are engaged in the complex. The larger value of the charge density implies the larger value of the binding constant of the complex. Similar trends of the binding constants with ion size (or ion charge density) were reported in several papers [6–8, 19]. However, our compounds show a larger increase of the fluorescence intensity upon addition of the salts than those proposed by other authors [6–12]. The reason may be a relatively large fluorescence lifetime of the parent compound 1,3,4-triphenyl-1*H*-pyrazolo[3,4-*b*]quinoline (PQ) (up to 20 ns, hardly depending on solvent polarity), thus the efficient fluorescence quenching by the recognition moiety occurs, making the fluorescence of the uncomplexed dye low efficient. The complexation changes the fluorescence properties a lot, and in the presence of small amounts of the salt an intensive green-colored fluorescence appears. The electron from the lone pair of the nitrogen atom of the recognition unit is more or less engaged in the relatively strong binding with the cation, which is preserved in the excited singlet state.

From Tables 1 and 2 we can recognize that complexation in less polar tetrahydrofurane (THF) is less efficient compared to that in strongly polar acetonitrile. Moreover, only 1/1 complexes have been detected in all investigated systems. The reason as such behavior may lie in less dissociation of the perchlorates in less polar solvents. Perchlorates are easily soluble even in solvents of lower polarities and they occur in the form of ionic pairs and triple ions [20]. The dissociation constant of the ionic pair (K_d) is strongly dependent on solvent polarity and may be expressed by Fuoss equation:

$$K_d = \frac{3}{4\pi N_A a^3} \exp\left(z_A z_B e^2/\varepsilon_s akT\right) \quad (4)$$

where parameter a is the distance of closest approach of the ions, z_A and z_B are charges of the ions and ε_s is the dielectric constant of the solvent. This linear dependence of the dissociation constant of lithium perchlorate on inverse of dielectric constant was presented in our previous paper [21]. Similar situation is thus expected for other perchlorates. Lower concentration of the free cations in THF is thus responsible for smaller equilibrium constant for complexation of investigated dyes with cations in THF. Moreover, the perchlorates of divalence cations (Me^{2+} = Ba^{2+}, Ca^{2+} and Mg^{2+}) exist in THF rather as $MeClO_4^+$ cations [22], therefore the 1/1 complexation seems to be rationalized in less polar solvents, as it is observed in the investigated systems.

Conclusions

Novel fluorescing cation indicators were synthesized and investigated by means of fluorescence spectroscopy. The stability of the fluorescing complexes (K1X and L1X) was determined by fluorescence titration. The crown-containing system (K1) shows the large sensitivity towards the presence of small cation concentration, which means that even micromolar ion concentration of the small inorganic cations causes a 8-fold increase of the fluorescence quantum yield. Better selectivity is exhibited by the chelate-type system (L1), which is very sensitive to the presence of two-valued ions (Mg^{2+},Ca^{2+} and Ba^{2+}). These systems may be applicative in practice. In less polar THF better selectivity was observed in both compounds.

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