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# A highly selective fluorescent molecular sensor for potassium based on a calix[4]bisazacrown bearing boron-dipyrromethene fluorophores

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The synthesis of a ditopic fluorescent sensor for cations associating a 1,3 alternate calix[4]bisazacrown-5 as an ionophore and substituted boron-dipyrromethene dyes as fluorophores is reported. Photophysical studies revealed that in medium and high polarity solvents an efficient charge transfer (CT) reaction occurs in the excited state leading to a dual emission and a strong quenching of fluorescence. This CT process is either totally suppressed by amino group protonation or hampered by cation complexation; in both cases, a strong fluorescence enhancement is observed. This ''switching-on'' of the emission is more pronounced when two cations are coordinated. The stability constants of complexes with sodium, potassium, caesium, calcium and barium cations were measured in acetonitrile and ethanol. The system shows a high sensitivity and selectivity for potassium over other metal ions. A test under physiological conditions was successfully achieved.

## Introduction

The increasing demand of sensors for application in environmental science, food technology, medical diagnosis has currently stimulated considerable research devoted to the design and synthesis of abiotic molecular devices capable of signaling a guest molecule or ion. Among the numerous analytical tools, fluorescent molecular sensors offer several distinct advantages in terms of sensitivity, selectivity, time response and spatial resolution.[1] Typical modular construction of such a system is based on a link between a fluorophore and a recognition subunit (ionophore) leading to a fluoroionophore. Photophysical sensing processes are varied and can be for example Photoinduced Electron Transfer (PET), Photoinduced Charge Transfer (PCT), Energy Transfer, or excimer formation.[2] Target substrates of particular interest are alkali and alkaline-earth metal ions like $K^+$, $Na^+$, and $Ca^{2+}$ since their concentration fluctuations play a major regulatory role in many biological processes.[3] A current challenging issue is the specific detection of $K^+$: the main difficulty lies in interference and/or competing reactions with $Na^+$ present in much larger concentrations (the average physiological concentrations of $Na^+$ and $K^+$ are 165 mM and 5 mM, respectively).[3] $K^+$ *vs.* $Na^+$ selectivity can be achieved by synthesis of concave receptors having a suitable size and capable of forming specific and stable interactions with its guest cation such as sandwich crown-ether complexes,[4] cryptands[5] or calixarenes.[6] In particular, 1,3 alternate calix[4]crown-5 or azacrown-5 ligands appear to be good candidates since they showed a very high affinity for $K^+$.[7] A $K^+$ selective fluoroionophore has been designed by using a naphthalene connected to a calix[4]-crown-5.[8] Kim *et al.*[9] reported a 1,3 alternate calix[4]azacrown-5 with an appended nitrophenol chromophore; this system used as a cation-extractant showed a high selectivity towards $K^+$ *versus* $Na^+$. The same authors[10] designed another sensor with a 1,3 alternate calix[4]arene bearing a crown-5 ether and an azacrown-5 ether at the rims, and also a pyrene fluorophore was connected to the nitrogen atom leading to a PET sensing process: $K^+/Na^+$ selectivity was perfectly maintained. This kind of specific receptor combined with a fluorophore that can be excited at higher wavelengths can offer a better alternative sensor for biological applications. Boron-dipyrromethene (BDP) is a good candidate as a fluorophore since it fulfils the photophysical requirements: high molar absorption coefficient, high fluorescence quantum yield, good photostability, long wavelength of excitation ($\sim$500 nm).[11] Kollmannsberger *et al.*[12] reported an azacrown-5 substituted boron-dipyrromethene; despite its lack of selectivity this fluoroionophore showed an extremely high fluorescence enhancement in the presence of alkali and alkaline-earth metal ions.

The present paper describes the synthesis of a new fluorescent molecular sensor **1** Calix-Bodipy. Spectroscopic properties, protonation effects and binding ability are reported. Suitable potassium recognition in the presence of interfering cations is demonstrated. A test under physiological conditions is described.

## Experimental

### General procedures

$^1H$ NMR spectra were recorded at room temperature on a Bruker AC300 spectrometer using tetramethylsilane as reference; chemical shifts are reported in ppm. Elemental analyses were performed at the Institut de Chimie des Substances Naturelles (France). All chemicals were of reagent grade and were used without further purification unless otherwise noted. Toluene, tetrahydrofuran (THF), dimethylformamide (DMF), dichloromethane and acetonitrile, received from Aldrich or SDS, were distilled prior to use following standard methods.

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### Synthesis

**4-{Bis-[2-(2-hydroxyethoxy)ethyl]amino}benzaldehyde 3.** 2-(2-{[2-(2-Hydroxyethoxy)ethyl]phenylamino}ethoxy)ethanol (14 g, 52 mmol) and hexamethylenetetramine (10.4 g, 74.2 mmol, 1.4 eq) in 12 ml of absolute ethanol was refluxed for 1 h under argon. 45 ml of mixture glacial acetic acid–formic acid (50 : 50 v/v) was slowly added to the mixture and the solution was refluxed for 3 h. 100 ml of HCl 0.5 M were slowly added to the mixture and the solution was stirred for 30 min. The solution was neutralized with $K_2CO_3$ and extracted with dichloromethane. The combined organic phases were dried ($MgSO_4$) and concentrated under vacuum. The residue was purified by silica gel chromatography (dichloromethane–acetone 80 : 20) to give 6.1 g (39%) of an oil. $\delta_H$ (300 MHz, $CDCl_3$) 2.48 (br s, 2 H), 3.56 (t, $J = 3$ Hz, 4 H), 3.70 (m, 12 H), 6.74 (d, $J = 8.8$ Hz, 2 H), 7.70 (d, $J = 8.8$ Hz, 2 H), 9.70 (s, 1 H). $^{13}$C NMR ($CDCl_3$, 100 MHz): 51.2 ($CH_2$), 61.6 ($CH_2$), 68.5 ($CH_2$), 72.6 ($CH_2$), 111.2 (CHAr), 125.5 (CqAr), 132.1 (CHAr), 152.7 (CqAr), 190.1 (CHO).

**Ditosylate of 4-{bis-[2-(2-hydroxyethoxy)ethyl]amino}benzaldehyde 4.** A mixture of **3** (2.30 g, 7.75 mmol), *p*-toluenesulfonyl chloride (2.97 g, 15.5 mmol) in dichloromethane (60 ml) was cooled at 0 °C. Then, triethylamine (2.17 ml, 16 mmol) was added dropwise After 24 h, the reaction mixture was neutralized to pH ~1 by addition of 1 M HCl. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was chromatographed on a silica column with a 90 : 10 mixture of dichloromethane–acetone as eluent to give pure ditosylate **4** (2.9 g; 61%) as a pale yellow oil. $\delta_H$ (300 MHz, $CDCl_3$) 2.42 (s, 6 H), 3.62 (m, 12 H), 4.12 (t, $J = 3.3$ Hz, 4 H), 6.67 (d, $J = 9$ Hz, 2 H), 7.31 (d, $J = 8.1$ Hz, 4 H), 7.68 (d, $J = 9$ Hz, 2 H), 7.75 (d, $J = 8$. Hz, 4H), 9.72 (s, 1 H). $\delta_C$ ($CDCl_3$, 100 MHz) 21.5 ($CH_3$Ph), 51.0 ($CH_2$), 68.5 ($CH_2$), 68.6 ($CH_2$), 69.1 ($CH_2$), 111.0 ($CH_2$), 125.4 (2 CHAr), 127.8 (CHAr), 129.8 (CHAr), 132.0 (CHAr), 132.8 (CqAr), 144.9 (CqAr), 152.4 (CqAr), 190.1 (CHO). Anal. Calc. for $C_{29}H_{35}NO_9S_2$. $H_2O$: C 55.84; H 5.98; N 2.25; found: C, 55.62; H, 5.44; N, 2.21%.

**Compound 5.** Calix[4]arene (1.24 g; 2.9 mmol) and $K_2CO_3$ (4.04 g; 29.3 mmol) were stirred in acetonitrile (170 ml) at room temperature for 1 h. Then, ditosylate **4** (1.77 g; 2.93 mmol) dissolved in 10 ml of acetonitrile) was added and the reaction mixture was refluxed for 7 days. Another portion (1.77 g; 2.93 mmol) of **2** dissolved in 10 ml of $CH_3CN$ and (4.04 g; 29.3 mmol) of $K_2CO_3$ was added to the solution mixture. After refluxing the solution for 7 days, the solution was cooled to room temperature and the solvents were removed under reduced pressure. The organic layer was washed with water, dried over $Na_2SO_4$, filtered and evaporated. Chromatography on silica column with a 95 : 5 mixture of dichloromethane–acetone as eluent followed by precipitation with methanol gave product **5** (0.58 g; 21%) as a beige solid. $\delta_H$ (300 MHz, $CDCl_3$) 3.54 (m, 24 H), 3.72 (m, 16 H), 6.67 (t, $J = 6$ Hz, 4 H), 6.77 (d, $J = 8$ Hz, 2 H), 7.10 (d, $J = 6$ Hz, 8 H), 7.79 (d, $J = 8$ Hz, 4 H), 9.77 (s, 2H). $\delta_C$ ($CDCl_3$, 100 MHz) 37.8 ($CH_2$), 51.1 ($CH_2$), 68.4 ($CH_2$), 70.5 ($CH_2$), 71.2 ($CH_2$), 111.8 (CHAr), 121.8 (CHAr), 125.4 (CqAr), 130.3 (CHAr), 132.2 (CHAr), 133.6 (CqAr), 152.4 (CqAr), 157.0 (CqAr), 190.0 (CHO). Anal. Calc. for $C_{58}H_{62}N_2O_{10} + CH_2Cl_2$: C 68.66; H 6.25; N 2.71. Found: C, 68.78; H, 6.41; N, 2.58%.

**Compound 1.** 130 mg of compound **5** (0.137 mmol) and 52 mg of dimethylpyrrole were dissolved in 50 ml of dichloromethane under argon atmosphere. After addition of one drop of trifluoroacetic acid, the reaction mixture was left for 2 h at room temperature. 57 mg of dichlorodicyanobenzoquinone were added to the mixture and the solution was stirred for 30 min at room temperature. Then, 1.5 ml of *N*-ethyl-*N*,*N*-diisopropylamine and 1.5 ml of $BF_3 \cdot Et_2O$ were added to the mixture. After 30 min of stirring at room temperature, the reaction mixture was washed with water, dried over $Na_2SO_4$ and chromatographed (eluent $CH_2Cl_2$–MeOH 90 : 10) followed with precipitation in MeOH give 44 mg of pure compound **1** (yield 23%). $\delta_H$ (300 MHz, $CDCl_3$) 1.56 (s, 12 H), 2.56 (s, 12 H), 3.41 (t, $J = 6$ Hz, 8 H), 3.58 (t, $J = 6$ Hz, 8 H), 3.72 (s, 8 H), 3.8 (m, 16 H), 5.97 (s, 4 H), 6.60 (t, $J = 6$ Hz, 4 H), 6.84 (d, $J = 9$ Hz, 4 H), 7.14 (d, $J = 9$ Hz, 12 H). $\delta_C$ ($CDCl_3$, 100 MHz) 14.5 ($CH_3$), 14.7 ($CH_3$), 37.5 ($CH_2$), 51.4 ($CH_2$), 69.1 ($CH_2$), 71.2 ($CH_2$), 72.5 ($CH_2$), 77.1 (CHAr), 111.4 (CHAr), 120.7 (CHAr), 121.5 (CHAr), 121.9 (CqAr), 129.2 (CHAr), 130.8 (CHAr), 132.1 (CqAr), 133.5 (CqAr), 142.9 (CqAr), 143.0 (CqAr), 147.7 (CqAr), 154.7 (CqAr), 157.1 (CqAr). ESI: *m*/*z* calculated for $C_{82}H_{88}B_2F_4N_6O_8$: 1383.2, found 1383. Anal. Calc. for $C_{82}H_{88}B_2F_4N_6O_8 \cdot H_2O$: C 70.29; H 6.47; N 6.00. Found: C, 69.72; H, 6.54; N, 5.73%.

### Spectroscopic measurements

UV/Vis absorption spectra were recorded on a Varian Cary5E spectrophotometer. Corrected emission spectra were obtained on a Jobin-Yvon Spex Fluoromax spectrofluorimeter. Coumarin 540 in ethanol ($\phi_f$ 0.78) and DCM in methanol ($\phi_f$ 0.43)[12] were used as fluorescence standards. The complexation constants were determined by global analysis of the evolution of all absorption and/or emission spectra by using the Specfit Global Analysis System V3.0 for 32-bit Windows system. This software uses singular value decomposition and non-linear regression modelling by the Levenberg–Marquardt method.[13]

Fluorescence intensity decays were obtained by the single-photon timing method with picosecond laser excitation using a Spectra-Physics set-up composed of a Titanium-Sapphire Tsunami laser pumped by an argon ion laser, a pulse detector, and doubling (LBO) and tripling (BBO) crystals. Light pulses were selected by optoacoustic crystals at a repetition rate of 4 MHz. Fluorescence photons were detected through an interferential filter by means of a Hamamatsu MCP R3809U photomultiplier connected to a Becker & Hickl electronic board (SPC 630). Data were analysed by a nonlinear least-squares method using Globals software (Globals Unlimited, University of Illinois at Urbana-Champaign, Laboratory of Fluorescence Dynamics).

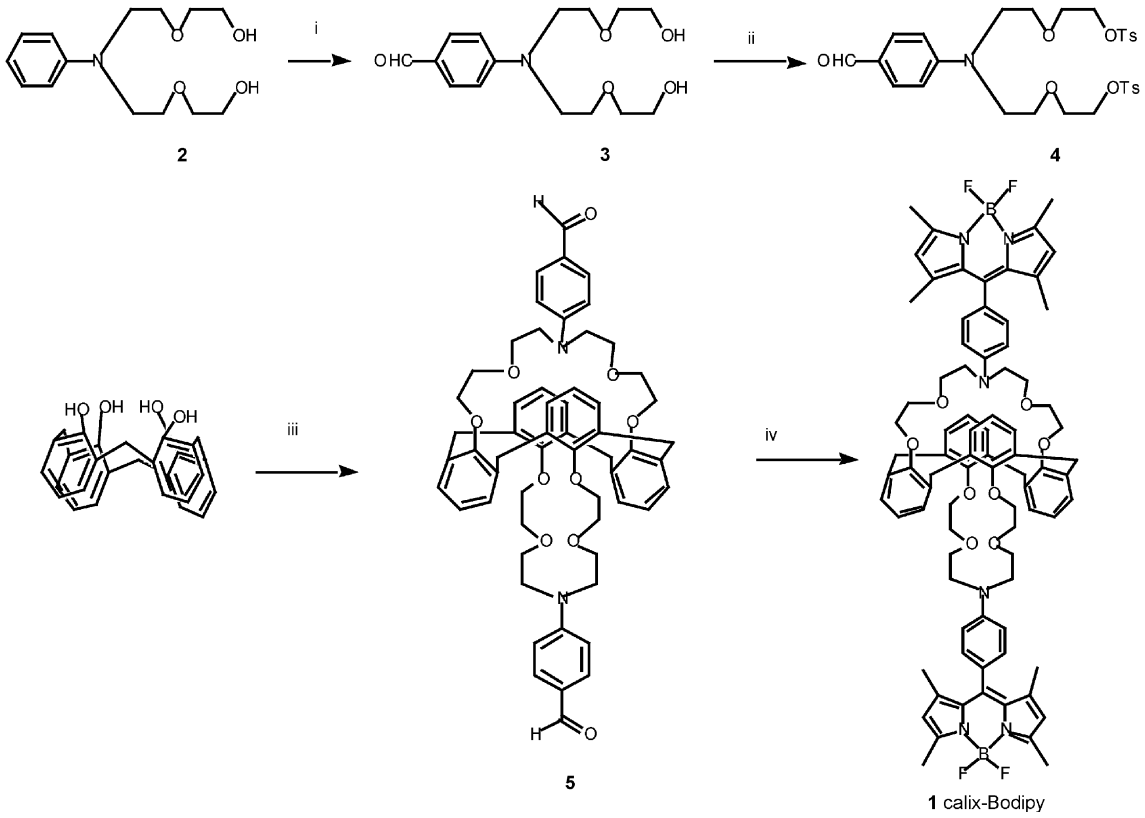
## Result and discussion

### Synthesis

The synthesis of the fluoroionophore **1** was performed according to Scheme 1. The aldehyde **3** was obtained according to the procedure described by Duff[14] by reacting the dialcohol **2** with hexamethylenetetramine in EtOH with a 39% yield. Ditosylate **4** is synthesized by reaction of **3** with toluene-*p*-sulfonyl chloride in the presence of triethylamine in $CH_2Cl_2$ in 76% yield. The biscalixcrown **5** is obtained in 21% yield by condensing calix[4]arene with 2 eq of ditosylate **4** and 10 eq of $K_2CO_3$ in $CH_3CN$ thanks to our the previously reported procedure for similar compounds.[15] The fluoroionophore **1** was synthesized according to the procedure described by Kollmannsberger[12] in 23% yield from 2,4-dimethylpyrrole and biscalixcrown **5**, followed by oxidation *in situ* with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and further reaction with boron trifluoride etherate in the presence of ethyldiisopropylamine.

### Photophysical properties

Fig. 1 depicts the absorption and fluorescence spectra of **1** in several solvents of different polarity and Table 1 summarizes the spectroscopic data.




**Scheme 1** *Reagents and conditions*: i hexamethylenetetramine, HCl, ii, TsCl, $NEt_3$, iii, **4**, $K_2CO_3$, iv dimethylpyrrole TFA, DDQ, $NEtPr^i_2$–$BF_3 \cdot Et_2O$.



**1** shows a narrow absorption band at 500 nm with a shoulder at the short wavelength side (Fig. 1) characteristic of the BDP chromophore.[11] This band is insensitive to the polarity of the solvent since it is slightly blue shifted when going from hexane to acetonitrile. The molar absorption coefficient at the maximum wavelength (140 000 L $mol^{-1}$ $cm^{-1}$ in acetonitrile) is twice as large as the value of a typical BDP connected to a phenyl group,[16] suggesting that the two chromophores do not interact in the ground state. No charge transfer band is observed at long wavelengths. In the UV-region, a sharp and intense band is located around 260 nm (Fig. 2); this band could correspond to a transition centered on the *N*-alkyl aniline moieties.[17] In contrast to the absorption spectrum, the fluorescence spectrum is strongly solvent dependent (Fig. 1). In non-polar solvents, the highly radiative locally excited state (LE) exhibits a fluorescence band of mirror image shape, weakly Stokes-shifted. In solvents more polar than hexane, the fluorescence is strongly quenched: the fluorescence quantum yield drops from 0.27 in hexane to 0.003 in acetonitrile. Moreover, a second fluorescent band appears at 630 nm. This broad and unstructured band is well developed in solvents of medium polarity like tetrahydrofuran but completely disappears in polar solvents. The red emission is attributed to a species having a strong charge transfer (CT) character and formed by a fast reaction in the excited state.[12] The driving force for the formation of this CT-state can be estimated from the Rehm–Weller equation[18] ($\Delta G_{eT} = E_{ox} - E_{red} - \Delta E_{00}$) neglecting the Coulomb part of the stabilization energy. With the assumption of electron transfer from the *N*,*N* dialkyl anilino group ($E_{ox}$ = 0.77 V *vs.* SCE)[19] to the BDP moiety ($E_{red}$ = −1.15 V *vs.* SCE)[20] and with a zero–zero energy transition equal to 2.47 eV in acetonitrile, the estimated driving force is strongly exergonic: $\Delta G_{eT}$ = −0.55 eV.

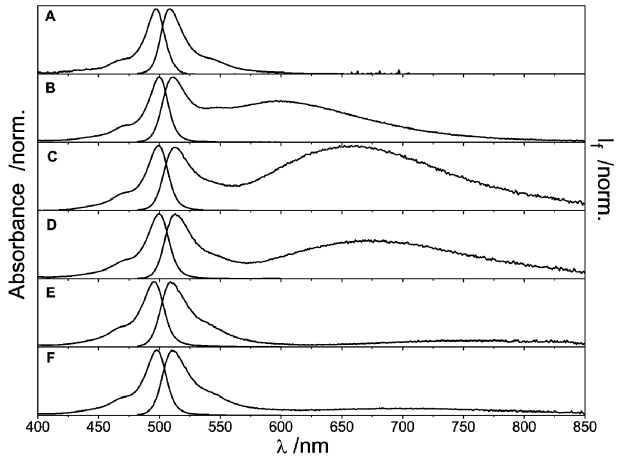



**Fig. 1** Absorption and fluorescence spectra of **1** in hexane (A), 1,4 dioxane (B), tetrahydrofuran (C), dichloromethane (D), acetonitrile (E) and ethanol (F).

Time-resolved fluorescence measurements were performed in non-polar and polar solvents. In hexane the fluorescence decay profile is mono-exponential (2.20 ns) yielding a radiative rate constant of $0.13 \times 10^9$ $s^{-1}$ in good accordance with analogous fluorophores.[12,21] In polar solvents like acetonitrile, the fluorescence decay profile is more intricate since a sum of three exponentials was required for a satisfactory fit. The fast component (13 ps) with a relative amplitude of 0.83 corresponds to the lifetime of the CT species. The slow component (2.80 ns) is attributed to the lifetime of the LE state. This latter value is markedly shorter than the lifetime of a phenyl substituted BDP (3.2 ns);[12] this is consistent with a precursor–successor mechanism where a first excited emitting species leads to a second one during a reaction in the excited state. Observation of a rise time in the spectral region where the CT species fluoresces is expected; however, this very short rise time could not be accurately measured because of the limitations of our experimental setup. Besides, a third component whose relative amplitude is smaller than 0.05 has a corresponding lifetime of 165 ps. This intermediate decay time was also

**Table 1** Absorption and emission properties of **1** in different solvents

| Solvent | $\lambda_{abs}$/nm | $\lambda_{em}$ (LE)/nm | $\lambda_{em}$ (CT)/nm | $\phi_{CT}/\phi_{LE}$[a] | $\phi_F$[b] | $\tau_1$/ps[c] | $\tau_2$/ps[c] | $\tau_3$/ns[c] | $\chi_\rho^2$ |
|---|---|---|---|---|---|---|---|---|---|
| Hexane | 498 | 509 | — | 0 | 0.28 | — | — | 2.20 ± 0.03 | 1.15 |
| Dioxane | 499 | 512 | 596 | 3.98 | 0.052 | | | | |
| $CHCl_3$ | 502 | 514 | 609 | 2.28 | 0.043 | | | | |
| THF | 499 | 512 | 658 | 5.34 | 0.019 | <10 | 272 ± 20 | 2.9 ± 0.1 | 1.36 |
| $CH_2Cl_2$ | 500 | 513 | 672 | 3.03 | 0.014 | | | | |
| MeCN | 496 | 509 | — | 0 | 0.003 | ~10 | 165 ± 40 | 2.8 ± 0.1 | 1.15 |
| EtOH | 498 | 512 | — | 0 | 0.006 | ~10 | 212 ± 30 | 3.1 ± 0.2 | 1.12 |
| MeOH | 497 | 509 | — | 0 | 0.008 | | | | |

[a] The CT band is deconvoluted from the global fluorescence spectrum by using a Gaussian function. [b] Error: 5–10%, [c] $\lambda_{ex}$: 483 nm, $\lambda_{em}$ = 515 nm.

observed by Kollmannsberger *et al.*[12] The authors suggested the existence of a double minimum in the potential surface of the excited CT state as a function of the twist angle between the donor and acceptor group.

### Protonation effects

The fast reaction from LE state to the CT state can be suppressed by protonation of the amino group. Addition of trifluoroacetic acid to a solution of **1** in ethanol shifts the BDP absorption band by 3 nm whereas the band located at 260 nm disappears completely (Fig. 2). A concomitant drastic enhancement of fluorescence intensity is observed (inset of Fig. 2); the fluorescence quantum yield rises from 0.003 to 0.67. The fluorescence decay profile of the protonated species is monoexponential ($\tau_1$ = 4.04 ns) and leads to a radiative rate constant of $0.17 \times 10^9$ $s^{-1}$, *i.e.* similar to the value calculated in hexane.

### Complexation studies

Metal binding abilities of **1** were investigated in acetonitrile and ethanol by addition of thiocyanate salts of $Na^+$, $K^+$, perchlorate salts of $Ca^{2+}$, $Ba^{2+}$ and caesium acetate. The absorption spectrum of **1** does not change significantly in the presence of $Na^+$, $Cs^+$, $Ca^{2+}$, $Ba^{2+}$ (from 0 to 0.1 mol $L^{-1}$) whereas increasing the concentration of $K^+$ leads to a progressive decrease of the band at 260 nm. An isosbestic point is then maintained at 251 nm, which confirms the existence of two interconvertible species in the ground state. The complexation-induced effects on fluorescence are similar to those observed when amino groups are protonated and correspond to an enhancement of the LE emission band (Fig. 3). Fluorimetric titration was performed by analysing the evolution of the whole emission spectrum by means of the SPECFIT program (using global analysis with 180 wavelengths). The constants $K_{11}$ and $K_{21}$ of the successive equilibria are defined as:

$$M + L \rightleftharpoons ML \quad K_{11} = \frac{[ML]}{[M][L]} \tag{1}$$

$$M + ML \rightleftharpoons M_2L \quad K_{21} = \frac{[M_2L]}{[ML][L]} \tag{2}$$

The global equilibrium for the formation of the complex $M_2L$ is:

$$2M + L \rightleftharpoons M_2L \quad \beta_{21} = \frac{[M_2L]}{[ML][L]} \tag{3}$$

The values of $K_{11}$ and $K_{21}$ for $K^+$ are reported in Table 2. In contrast to the high value of $K_{11}$ for $K^+$ (log $K_{11}$ = 4.32), the values for $Cs^+$, $Ca^{2+}$, $Ba^{2+}$ were found to be very small (log $K_{11}$ < 0.5). $K_{11}$ is slightly higher for $Na^+$ (log $K_{11}$ = 1.32). A typical fit of the fluorescence intensity evolution in the presence of $K^+$ is presented in the inset of Fig. 3.

It should be noted that the value of $K_{11}$ for $K^+$ is of the same order as those previously reported for calix[4]crown-5 ethers.[7,9,10] The selectivity towards potassium ion with respect to the other cations, expressed as the ratio of the stability

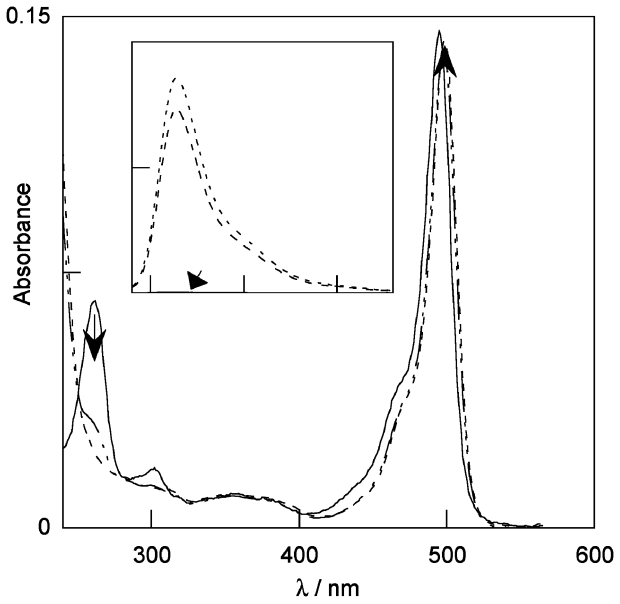



**Fig. 2** Evolution of the absorption spectrum of **1** upon addition of trifluoroacetic acid (solvent: ethanol). Inset: evolution of the fluorescence spectrum of **1** by addition of trifluoroacetic acid ($\lambda_{exc}$ = 475 nm; solvent: ethanol).

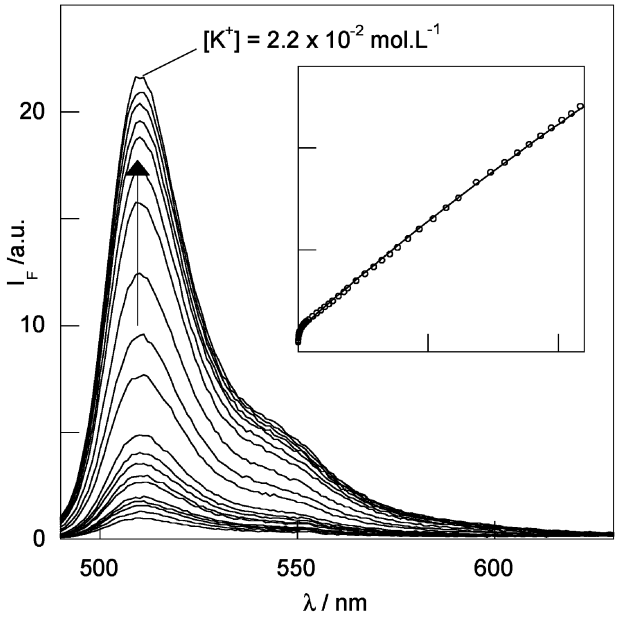



**Fig. 3** Fluorescence spectra of **1** ($6.9 \times 10^{-7}$ mol $L^{-1}$) with increasing concentration of $K^+$ in ethanol ($\lambda_{exc}$ = 475 nm). Inset: titration curve at 510 nm.

**Table 2** Stability constants of the complexes of **1** with $K^+$ in acetonitrile, in ethanol and in a mixture EtOH–$H_2O$ (75 : 25 v/v)

| Solvent | Acetonitrile | Ethanol | EtOH–$H_2O$ (75 : 25 v/v)) |
|---|---|---|---|
| Log $K_{11}$ | 4.32 ± 0.04 | 4.47 ± 0.14 | 2.4 ± 0.10 |
| Log $K_{21}$ | 1.73 ± 0.04 | 0.78 ± 0.14 | 1.25 ± 0.15 |
| Log $\beta_{21}$ | 6.05 ± 0.04 | 5.26 ± 0.14 | 3.5 ± 0.1 |

constants, is higher than 1000 and this very high value is attributed to the size fit of the calix[4] azacrown-5 ionophore[7,9,10] and to a preferential cation π-interaction of $K^+$ with the phenyl moieties of the 1,3 alternate calix[4]arene.[6]

Furthermore a second step of complexation is observed for $K^+$ at higher cation concentrations (millimolar domain), and is attributed to the [2 : 1] complex formation (metal : ligand). The fluorescence intensity increases more gradually, and the derived association constant ($K_{21}$) has a value of 1.73 and 0.78 (logarithmic scale) in acetonitrile and ethanol, respectively. These constants which are more than two orders of magnitude smaller than $K_{11}$ show that the complexation of the second cation is more difficult. The ratio $K_{21}/K_{11}$ (~0.002), which is far below the statistical value of 1/4 observed if the two binding sites were independent and equivalent,[22] is evidence for an anticooperative process. The molecular structures of the complexes [1 : 1] and [2 : 1] optimized by the AM1 semi-empirical method[23] indicate that the average distance between $K^+$ and the nitrogen atom decreased by 1.28 Å, from 4.28 Å to 3.00 Å, when two $K^+$ are coordinated. Electrostatic repulsion between the two cations can be here invoked.[24] The extrapolated fluorescence spectra of the [1 : 1] and [2 : 1] complexes in acetonitrile are displayed in Fig. 4. The fluorescence quantum yield of the [2 : 1] complex ($\phi_{21}$) is 10 times higher than that of the [1 : 1] complex ($\phi_{11}$). This result suggests a more efficient inhibition of the excited CT process in the [2 : 1] complex, and this is consistent with the calculated structural data since the proximity of potassium ions reduces the electron-donating character of the amino group. The intensity of the LE band is therefore enhanced.

The selectivity of $K^+$ is even better in ethanol (polar protic solvent) since no significant fluorescence change was observed upon addition of a large excess of $Na^+$, $Cs^+$, $Ca^{2+}$, $Ba^{2+}$ ($c_M$ > 0.1 mol $L^{-1}$). Moreover, the extrapolated fluorescence spectra of each complex indicate that the fluorescence quantum yield of the [2 : 1] complex is 75 and 154 times more emissive than that of the [1 : 1] complex and **1**, respectively. The species distribution profiles as a function of the concentration of $K^+$ were calculated for **1** and for its relevant complexes (Fig. 5). With an initial free ligand concentration of $6.8 \times 10^7$ mol $L^{-1}$, the [1 : 1] complex outnumbers the other species in the millimolar domain (98.5% of the species). However, the [2 : 1] complex also starts being formed and because of its high relative fluorescence quantum yield, a slight increase of its concentration leads to a high enhancement of the fluorescence signal. Thus, above 1 mmol $L^{-1}$ of $K^+$, the total fluorescence intensity is mainly governed by the fluorescence intensity contribution of the [2 : 1] complex, as displayed in Fig. 6.

It is now of interest to examine the practical use of this new fluoroionophore **1** for the determination of $K^+$ in aqueous solutions. It turned out that **1** cannot be used in pure water

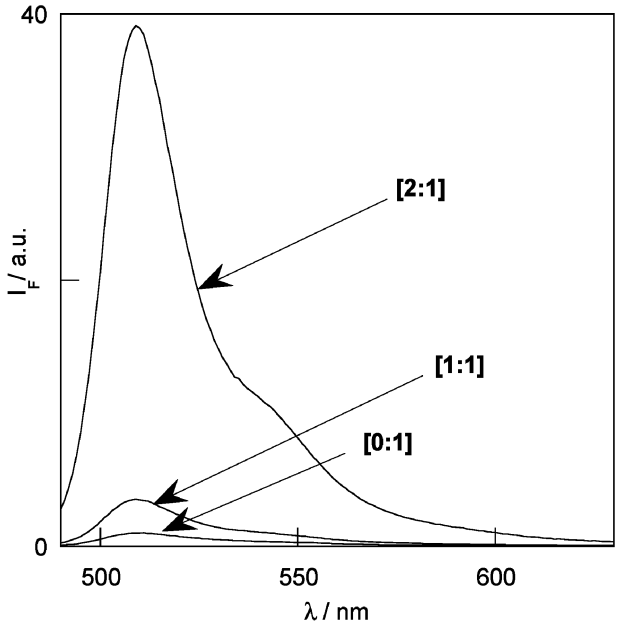



**Fig. 4** Extrapolated fluorescence spectra of **1** and its [1 : 1] and [2 : 1] complexes with $K^+$ in ethanol.

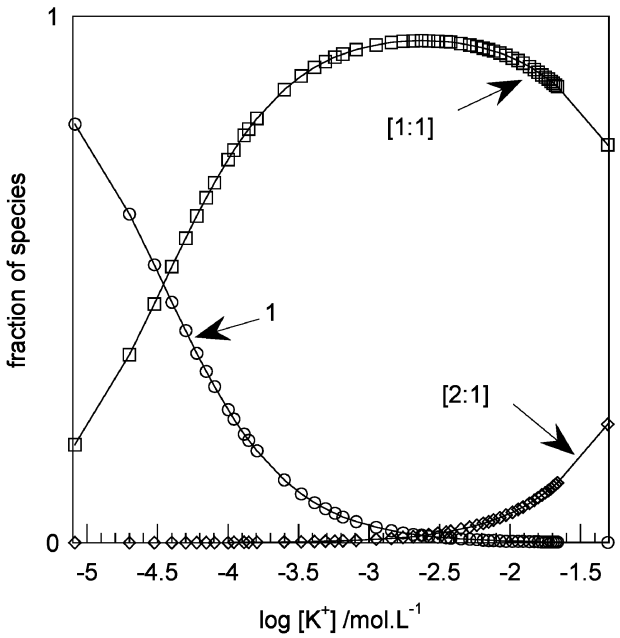



**Fig. 5** Concentration profiles of **1**, and its [1 : 1] and [2 : 1] complexes with $K^+$ in ethanol ([1] = $6.8\ 10^{-7}$ mol $L^{-1}$).

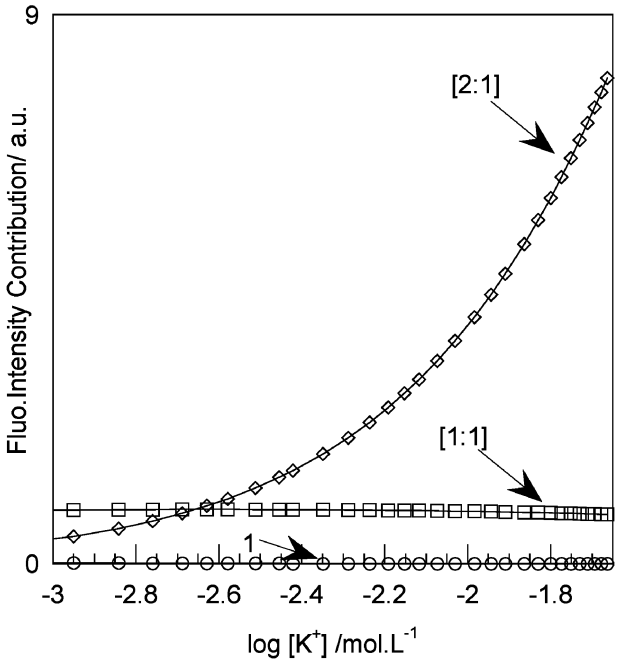



**Fig. 6** Relative fluorescence intensity of each species as a function of potassium concentration in ethanol ([**1**] = $6.8 \times 10^{-7}$ mol $L^{-1}$).

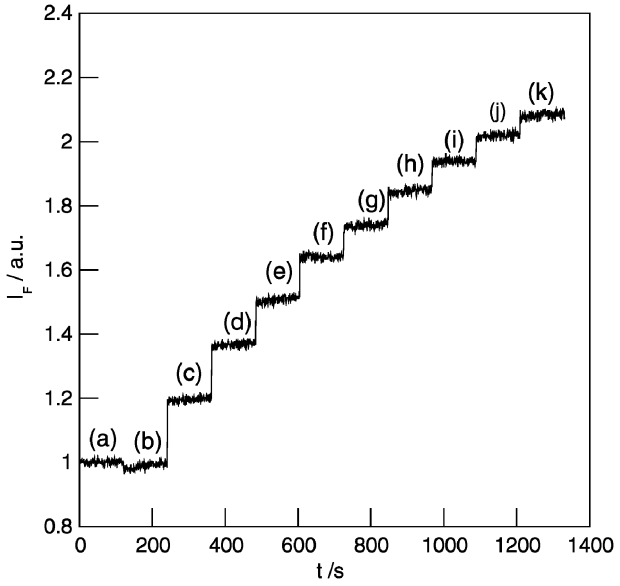



**Fig. 7** Fluorescence intensity response of **1** ($6 \times 10^{-7}$ mol $L^{-1}$) in water–ethanol (1 : 3, v : v), $\lambda_{exc} = 475$ nm, $\lambda_{em} = 515$ nm. Potassium, sodium concentration are ($Na^+/K^+$ in $10^{-3}$ mol $L^{-1}$): (a) 0/0; (b) 44/0; (c) 44/0.52; (d) 44/1.05; (e) 44/1.57; (f) 44/2.1; (g) 44/2.62; (h) 44/3.15; (i) 44/3.67; (j) 44/4.2; (k) 44/4.72.

because of self-aggregation, as previously reported,[25] that alters the photophysical properties. However, a mixture EtOH –$H_2O$ (3 : 1 v/v)) was found to be still suitable, in spite of the decrease in affinity for $K^+$. It should be noticed that in contrast to the previously system described by Kim *et al.*[9,10] with a tertiary amine linked to a calix[4]azacrown-5 our system composed of a dialkylaniline with a boron-dipyrromethene as an acceptor group in the *para* position exhibits a much lower $pK_a$,[21c] which enables application under neutral conditions. The titration curve remains consistent with the formation of two successive [1 : 1] and [2 : 1] complexes (log $K_{11} = 2.48$ and log $K_{21} = 1.25$).

Then, a test of the analytical performance of **1** for the determination of $K^+$ in extracellular conditions (serum or whole blood) was achieved. In such conditions, the concentration of $K^+$ is about 5 mM, and that of $Na^+$ about 150 mM. Thus, considering a biological sample that is diluted with an ethanol solution (containing 1 at the appropriate concentration) by a factor of 4, the question arises as to whether one can detect about 1 mM of $K^+$ in the presence of about 40 mM of $Na^+$. Fig. 7 shows that this is indeed possible: increasing the concentration of $Na^+$ from 0 to 44 mM has almost no effect on the fluorescence intensity, whereas addition of $K^+$ from 0 to 5 mM in the presence of 44 mM $Na^+$ leads to a quasi-linear increase in fluorescence intensity. This demonstrates the sensitivity and selectivity of **1** towards $K^+$ at physiological concentrations.

## Conclusion

The synthesis of a new fluoroionophore associating a 1,3 alternate calix[4] azacrown-5 and a boron–dipyrromethene as a fluorophore is reported. The ionophore, which possesses two binding sites whose size perfectly fits the potassium cation, exhibits an excellent $K^+/Na^+$ selectivity in acetonitrile, ethanol, and ethanol–water mixtures. Cation coordination to the amino group blocks the efficient CT mechanism, which leads to a strong fluorescence enhancement of the LE band. The enhancement is drastically larger with the [2 : 1] species (Fluorescence Enhancement Factor = 154 in ethanol); this result is consistent with a closer proximity between the cation and the nitrogen atom of the aniline moiety. Fluorescence signal amplification is all the more improved as the [2 : 1] complex is produced which is the case in the millimolar range of $K^+$ concentration. Thus, the high $K^+/Na^+$ selectivity under physiological conditions combined with its good sensitivity make this system a suitable $K^+$-sensor for analytical applications.

## References


1 (*a*) *Chemosensors of Ion and Molecular Recognition*, ed. J.-P. Desvergne and A. W. Czarnik, NATO ASI Series, Kluwer Academic, Dordrecht, 1997, vol. C492; (*b*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (*c*) B. Valeur and I. Leray, *Coord. Chem. Rev.*, 2000, **205**, 3–40.
2 B. Valeur, *Molecular Fluorescence. Principles and Applications*, Wiley-VCH, Weiheim, 2002.
3 A. Minta and R. Y. Tsien, *J. Biol. Chem.*, 1989, **264**, 19449.
4 W. S. Xia, R. H. Schmehl and C. J. Li, *J. Am. Chem. Soc.*, 1999, **121**, 5599.
5 (*a*) D. B. Lehn, J. P. Sauvage and J. Blanzat, *Tetrahedron*, 1973, **29**, 1629; (*b*) A. P. de Silva, H. Q. N. Gunaratne and K. R. A. S. Sandanayake, *Tetrahedron Lett.*, 1990, **31**, 5193; (*c*) R. Crossley, Z. Goolamali and P. G. Sammes, *J. Chem. Soc., Perkin Trans. 2*, 1994, 1615; (*d*) H. He, M. A. Mortellaro, M. J. P. Leiner, R. J. Fraatz and J. K. Tusa, *J. Am. Chem. Soc.*, 2003, **125**, 1468.
6 (*a*) C. D. Gutsche, *Calixarenes Revisited*, The Royal Society of Chemistry, Cambridge, 1998; (*b*) *Calixarenes 2001*, ed. Z. Asfari, V. Böhmer, J. Harrowfield and J. Vicens, Kluwer Academic, Dordrecht, 2001.
7 (*a*) A. Casnati, A. Pochini, R. Ungaro, C. Bocchi, F. Ugozzoli, R. J. M. Egberink, H. Strujik, R. Lugtenberg, F. de Jong and D. N. Reinhoudt, *Chem. Eur. J.*, 1996, **2**, 436; (*b*) F. Arnaud-Neu, Z. Asfari, B. Souley and J. Vicens, *New J. Chem.*, 1996, **20**, 453; (*c*) J. S. Kim, W. K. Lee, D. Y. Ra, Y. I. Lee, W. K. Choi, K. W. Lee and W. Z. Oh, *Microchem. J.*, 1998, **59**, 464; (*d*) K. N. Koh, K. Araki, S. Shinkai, Z. Asfari and J. Vicens, *Tetrahedron Lett.*, 1995, **36**, 6095; (*e*) S. K. Kim, S. H. Lee, J. Y. Lee, J. Y. Lee, R. A. Bartsch and J. S. Kim, *J. Am. Chem. Soc.*, 2004, **126**, 16499.
8 J. H. Bu, Q. Y. Zheng, C. F. Chen and Z. T. Huang, *Org. Lett.*, 2004, **6**, 3301.
9 J. S. Kim, O. J. Shon, J. W. Ko, M. H. Cho, I. Y. Yu and J. Vicens, *J. Org. Chem.*, 2000, **65**, 2386.
10 J. S. Kim, O. J. Shon, J. A. Rim, S. K. Kim and J. Yoon, *J. Org. Chem.*, 2002, **67**, 2348.
11 (*a*) E. Vos de Wal, J. Pardoen, J. A. Van Koeveringe and J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas*, 1977, **96**, 306; (*b*) J. Karolin, L. B.-A. Johansson, L. Strandberg and T. Ny, *J. Am. Chem. Soc.*, 1994, **116**, 7801; (*c*) T. Werner, C. Huber, S. Heinl, M. Kollmannsberg, J. Daub and O. S. Wolfbeis, *Fresenius' J. Anal. Chem.*, 1997, **359**, 150.
12 M. Kollmannsberger, K. Rurack, U. Resch-Genger and J. Daub, *J. Phys. Chem. A*, 1998, **102**, 10211.
13 H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1985, **32**, 95–101.
14 J. C. Duff, *J. Chem. Soc.*, 1945, **276**.
15 I. Leray, Z. Asfari, J. Vicens and B. Valeur, *J. Chem. Soc., Perkin. Trans. 2*, 2002, 1429.
16 P. Toele, H. Zhang, C. Trieflinger, J. Daub and M. Glasbeek, *Chem. Phys. Lett.*, 2003, **368**, 66.
17 (*a*) M. Ballester and J. Riera, *Spectrochim. Acta A*, 1967, **23**, 51533; (*b*) S. Kapelle, W. Rettig and R. Lapouyade, *Chem. Phys. Lett.*, 2001, **348**, 416; (*c*) J. P. Malval, J. P. Morand, R. Lapouyade, W. Rettig, G. Jonusauskas, J. Oberlé, C. Trieflinger and J. Daub, *Photochem. Photbiol. Sci.*, 2004, **3**, 939.
18 D. Rehm and A. Weller, *Isr. J. Chem.*, 1970, **8**, 259.
19 D. R. Worall, S. L. Williams, F. Wilkinson, J. E. Crossley, H. Bouas-Laurent and J. P. Desvergne, *J. Phys. Chem.*, 1999, **109**, 9255.
20 R. Y. Lai and A. J. Bard, *J. Phys. Chem. B*, 2003, **107**, 5036.
21 (*a*) M. Kollmannsberger, K. Rurack, U. Resch-Genger, W. Rettig and J. Daub, *Chem. Phys. Lett.*, 2000, **329**, 363; (*b*) K. Rurack, M. Kollmannsberger, U. Resch-Genger and J. Daub, *J. Am. Chem. Soc.*, 2000, **122**, 968; (*c*) K. Rurack, M. Kollmannsbergerb and J. Daub, *New J. Chem.*, 2001, **25**, 289.
22 K. A. Connors, *Binding constants. The measurement of Molecular Complex Stability*, John Wiley & Sons, New York, 1987.
23 HYPERCHEM, v. 7.03, Hypercube, Inc., Gainesville, FL, 2002.
24 I. Leray, Z. Asfari, J. Vicens and B. Valeur, *J. Fluoresc.*, 2004, **14**, 451.
25 F. Bergström, I. Mikhalyov, P. Häggöf, R. Wortmann, T. Ny and L. B. A. Johansson, *J. Am. Chem. Soc.*, 2001, **124**, 196.