A Highly Potassium-Selective Ratiometric Fluorescent Indicator Based on BODIPY Azacrown Ether Excitable with Visible Light
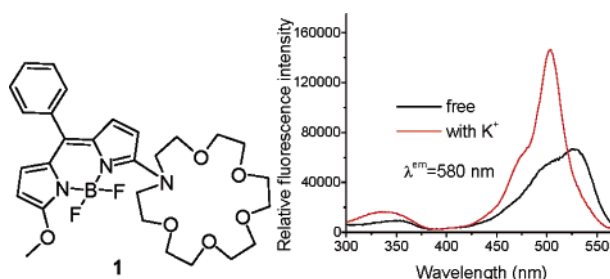
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ABSTRACT

A potassium-selective fluorescent BODIPY-linked azacrown ether chemosensor has been synthesized using novel substitution reactions of 3,5-dichloroBODIPY. The indicator absorbs and emits light in the visible wavelength range. The dissociation constant K_d for the complex between K⁺ and the probe is 0.5 mM in acetonitrile. Quantum chemical calculations show that the experimental hypsochromic shifts in absorption and fluorescence upon potassium binding are due to complexation, which induces a large conformational change of the sensor.

Fluoroionophores, which produce quantifiable fluorescence changes upon complexation with suitable guest ions, are widely exploited in analytical and life sciences.[1−3] SBFI[2,4,5] and PBFI[2,5,6] are commercial fluorescent indicators for sodium and potassium, respectively, based on benzofuranyl fluorophores absorbing light in the UV region and linked to a diazacrown ether chelator. The cavity size of the crown ether provides selectivity for K⁺ versus Na⁺ (or vice versa). There is still a strong demand for new indicators with improved properties, especially *ratiometric* probes that can be excited in the *visible* wavelength region.

This work aims at the synthesis and the fluorimetric characterization of the new fluorescent sensor **1** (Scheme 1) with high selectivity for potassium over other alkali metal ions. Compound **1** absorbs and emits light in the visible spectrum, with aza-18-crown-6 as K⁺ chelator linked via its N atom at position 3 to BODIPY[7] (4,4-difluoro-4-bora-3a,-4a-diaza-*s*-indacene or boron dipyrromethene) as fluorophore. BODIPY is a well-known fluorophore with many valuable qualities, including elevated (photo)chemical stability and relatively high fluorescence quantum yields and absorption

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Scheme 1. Synthesis of **1**
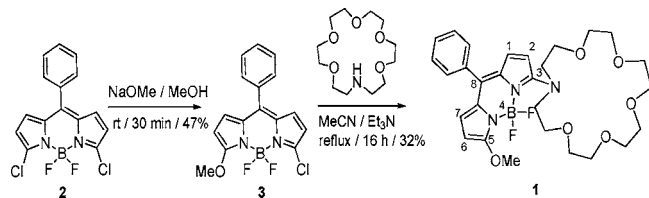
coefficients.[8] Furthermore, BODIPY dyes can be optically excited with visible light, show narrow absorption and emission bands with high peak intensities, and are amenable to structural modification so that spectral shifts in the absorption and emission bands can be generated by introducing the appropriate substituent pattern.

In the past, the photophysical properties of an azacrown-substituted BODIPY dye that forms 1:1 complexes with various alkali and alkanine-earth metal ions have been studied.[9] In these complexes, coordination of the cation to the nitrogen donor atom of the azacrown inhibits the intramolecular charge transfer (ICT) process to the BODIPY acceptor, leading to a huge cation-induced fluorescence enhancement with no spectral shift. The same research group linked the BODIPY fluorophore directly to a benzocrown electron donor, resulting in a fast ICT and leading to strong fluorescence quenching.[10] Upon binding of Na$^+$ and K$^+$ to the benzocrown receptor, the electron-donating properties of the latter are reduced, thereby partly suppressing the ICT and resulting in large fluorescence enhancement factors without changes in the shape of the absorption and emission spectra. By combining the biaryl boron dipyrromethene fluorophore with an oligoethyleneglycol bridge acting as binding site for metal cations, Yamada et al.[11] made a sodium-selective fluorescent indicator. A modest Na$^+$-induced fluorescence enhancement with no significant spectral shift was observed.

To synthesize our target molecule **1** (Scheme 1), the 3,5-dichloroBODIPY compound **2** was first prepared according to a previously reported procedure.[12,13] One chlorine atom of **2** was subsequently substituted by a methoxy group upon addition of sodium methoxide in methanol to yield compound

3. The second chlorine atom of the isolated compound **3** was then substituted by aza-18-crown-6 in refluxing acetonitrile with triethylamine as base. These novel nucleophilic addition−elimination substitution reactions of the 3,5-dichloro-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene core turn out to be a very successful approach for preparing a variety of symmetric and asymmetric BODIPY compounds with substitution patterns that are difficult to realize otherwise. The scope of these novel substitution reactions of the iminium chloride functions in BODIPY analogues will be described in detail elsewhere.

After full characterization of the chemical structure by NMR and MS spectroscopy, the absorption and fluorescence spectra of **1** were recorded in acetonitrile. The ion-free fluoroionophore **1** shows an absorption band with a maximum at 529 nm and a fluorescence emission band with a maximum at 565 nm with a low fluorescence quantum yield (0.006). The changes in optical properties induced by addition of Li$^+$, Na$^+$, K$^+$, and Cs$^+$ perchlorates to acetonitrile solutions of **1** are portrayed in Figure 1a. Whereas the absorption and fluorescence spectra of **1** are hardly altered in the presence of Li$^+$, Na$^+$, and Cs$^+$ ions, much more pronounced changes are observed upon addition of K$^+$ ions: (i) The lowest-energy absorption band of the indicator shifts hypsochromically by about 20 nm (from 529 to 505 nm) when K$^+$ ions are added to the acetonitrile solution. The relative contributions of the 505/529 nm signals change with varying [K$^+$] and show an isosbestic point at 512 nm (Figure 1b). (ii) Similar changes are observed in the fluorescence excitation spectra (Figure 1c). The maximum of the fluorescence intensity shifts from around 529 nm in the absence of ions to 504 nm when K$^+$ ions are added to the solution and is accompanied by a pseudoemissive point at 520 nm. The fluorescent signal at 504 nm increases significantly and simultaneously the fluorescence at 529 nm decreases with increasing [K$^+$]. (iii) The maximum of the fluorescence emission band shifts from 565 nm in ion-free acetonitrile to 520 nm in the presence of potassium and is accompanied by a significant increase in intensity (Figure 1d). The low ϕ_f value for uncomplexed **1** can be attributed to an efficient quenching via an excited-state ICT process from the nitrogen atom of the azacrown moiety to the strongly electron-deficient BODIPY acceptor. Upon binding of K$^+$, the electron-donating properties of the amine are reduced, partially suppressing the ICT and causing an enhancement in the fluorophore's fluorescence intensity. The highest fluorescence quantum yield value (0.04 for excitation at 488 nm) is found above 6 mM potassium.

To gain insight into the mechanisms of the changes in optical properties of **1** in the presence of K$^+$, we combined force field simulations (using the universal force field, UFF)[14] of the molecular conformation with a semiempirical INDO/SCI (intermediate neglect of differential overlap/ single configuration interaction)[15] description of its electronic excited states. The ground-state potential energy curve of **1** with and

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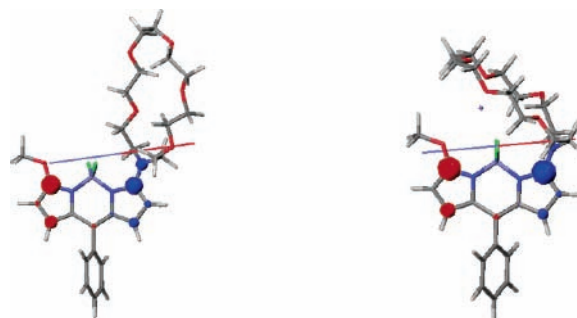
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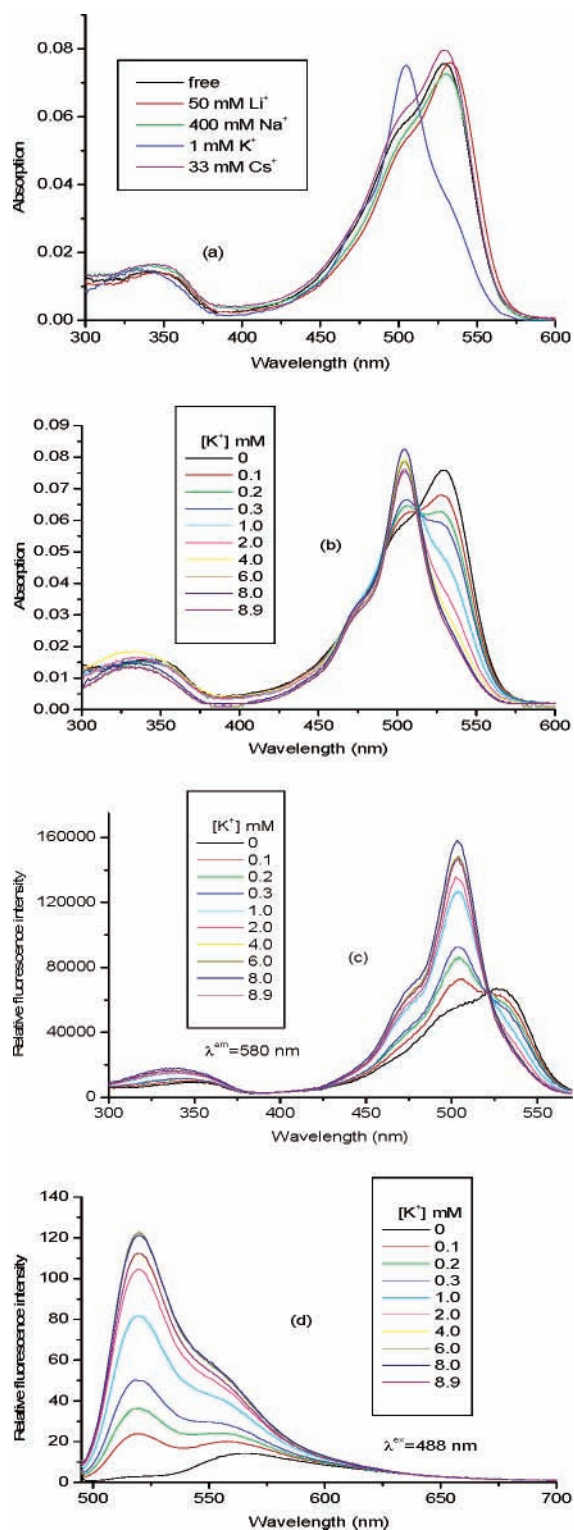
Figure 1. (a) Response of absorption spectra of **1** to added alkali perchlorate salt. (b) Absorption spectra of **1** as a function of [K$^+$] and (c) corresponding fluorescence excitation spectra ($\lambda^{em} = 560$ nm) and (d) corresponding fluorescence emission spectra ($\lambda^{ex} = 488$ nm). All spectra are recorded in acetonitrile solution.

Figure 2. Calculated conformations of **1** in the absence (left) and presence (right) of a K$^+$ ion. The atomic transition densities (red and blue dots) and the transition dipole moments (red and blue lines) computed for the optical transition are also shown. Note the smaller contribution on the N atom of the azacrown ring when a K$^+$ ion is coordinated.

tions. Strikingly, the addition of K$^+$ induces a marked reorganization of the molecule, with the ion-including azacrown ether ring "folding" onto the BODIPY core. This structural rearrangement allows for the potassium cation to be coordinated not only to the nitrogen and oxygen atoms of the azacrown ring but also to the oxygen of the methoxy group in position 5 as well as to the fluorine atoms bound to boron.

Such a conformational change increases the torsion angle around the chemical bond between the carbon atom in position 3 of the conjugated backbone and the N atom to which the azacrown ring is linked, thereby driving the lone pair of the nitrogen atom out of the conjugated π-system. As a consequence of the resulting reduced delocalization and electron-donating effect, the simulated INDO/SCI absorption spectrum of the molecule is shifted to the blue (from 515 to 490 nm) when a complex between K$^+$ and the azacrown ring is formed. The weaker electronic coupling between the nitrogen atom of the ion-including azacrown ether ring and the BODIPY core also clearly shows up in the transition density distributions computed for the lowest optical transition (these charges provide a local map for the electronic transition dipole moment): a smaller contribution on the N-linkage site is indeed calculated in the puckered conformation after K$^+$ complexation with respect to the ion-free dye **1**.

The ground-state dissociation constants K_d of the complex between K$^+$ and **1** were determined in acetonitrile solution by *direct* fluorimetric titration as a function of [K$^+$] using the fluorescence excitation or emission spectra. Nonlinear fitting of eq 1[16] to the steady-state fluorescence data F recorded as a function of [K$^+$] yields values of K_d, the

without a potassium ion inside the azacrown ether ring was first explored by running UFF molecular dynamics simula-

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fluorescence signals F_{min} and F_{max} at minimal and maximal [K$^+$], respectively (corresponding to the free and K$^+$ bound forms of the probe, respectively), and n (the number of potassium ions bound per probe).

$$F = \frac{F_{max}[\text{K}^+]^n + F_{min}K_d}{K_d + [\text{K}^+]^n} \quad (1)$$

An average K_d value of 0.50 mM was obtained by direct fluorimetric titration, which agrees with the value ($K_d = 0.60$ mM) obtained from absorbance measurements.

Since large spectral shifts are observed in the fluorescence excitation and emission spectra, the *ratiometric* method (eq 2) can be used to estimate values of K_d. The fluorimetric ratiometric method can be performed in two ways. One can either measure the ratio $R = F(\lambda^{em}, \lambda_1^{ex})/F(\lambda^{em}, \lambda_2^{ex})$ at a common emission wavelength and two different excitation wavelengths or the ratio $R = F(\lambda_1^{em}, \lambda^{ex})/F(\lambda_2^{em}, \lambda^{ex})$ at a common excitation wavelength and two different emission wavelengths. R_{min} and R_{max} correspond to the ratios at minimal and maximal [K$^+$]; $\xi = F_{min}(\lambda^{em}, \lambda_2^{ex})/F_{max}(\lambda^{em}, \lambda_2^{ex})$ for the excitation ratiometric method and $\xi = F_{min}(\lambda_2^{em}, \lambda^{ex})/F_{max}(\lambda_2^{em}, \lambda^{ex})$ for the emission ratiometric method.[16] The average K_d value from ratiometric excitation and emission data estimated on the basis of eq 2 is 0.46 mM, in good agreement with that obtained by the direct titration method. Figure 3 is a representative example of the fit of eq 2 to the ratiometric fluorescence excitation data.

$$R = \frac{R_{max}[\text{K}^+]^n + R_{min}K_d}{K_d\xi + [\text{K}^+]^n} \quad (2)$$

To conclude, we have used novel substitution reactions of the iminium chloride functions of the 3,5-dichloro-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene core to synthesize a fluorescent indicator with a high selectivity for K$^+$ over other
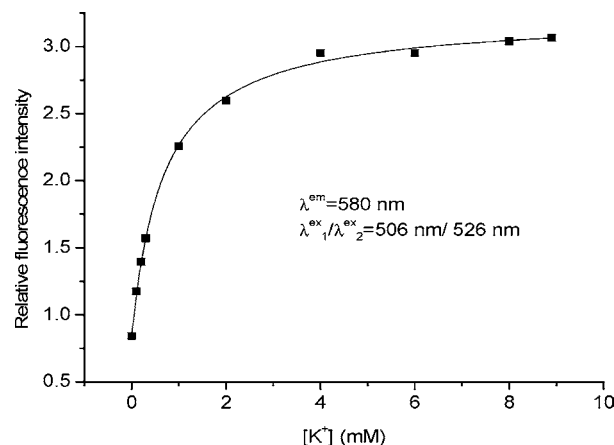
Figure 3. The solid line shows the best fit of eq 2 using the ratiometric method with $n = 1$ to the fluorimetric titration data of **1** obtained from the excitation spectra of Figure 1c.

alkali metal ions. The probe absorbs and emits light in the visible spectral region and shows a large wavelength shift and a considerable conformational change upon K$^+$ binding.

Supporting Information Available: All experimental procedures and spectroscopic characterization of compounds **1**, **2** and **3**; fluorescence excitation and emission spectra; fluorimetric titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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