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# Development of a potassium ion-selective fluorescent sensor based on 3-styrylated BODIPY

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ABSTRACT

We have developed a red-emitting fluorescent $K^+$ probe, B3TAC, which also shows a wavelength shift upon binding to $K^+$. The probe was synthesized by conjugating a cryptand-based chelator, 2-triazacrypt- and [2,2,3]-1-(2-methoxyethoxy)benzene (TAC), to position 3 of the BODIPY fluorophore through a styryl linker. In water–acetonitrile mixed solvent, it responded to $K^+$ in the physiological concentration range with high selectivity over $Na^+$ and other metal ions. B3TAC is potentially useful for measuring cellular $K^+$ ion concentration, as well as for simple, naked-eye detection of $K^+$ in solution.



Fluorescent probes are useful tools for analysis of physiological events, including ion channel activity, localization of metal ions, and enzyme activity, based on the changes of their optical properties, such as fluorescence intensity and excitation/emission wavelength, as a result of specific interactions with target molecules.[1,2] For example, the role of $Ca^{2+}$ ion as a signaling molecule is now much better understood, following the introduction and application of a variety of $Ca^{2+}$ ion probes with different fluorescence colors and dissociation constants.[2,3] $K^+$ ion, which is the most abundant metal ion in cells, plays essential roles in cardiac and neuronal excitability, cellular ionic homeostasis and cell proliferation.[4,5] Hence, there is a great need for chemical tools that are capable of visualizing intracellular and extracellular $K^+$ concentration, $[K^+]$. But, in contrast to the case of $Ca^{2+}$ probes, there have been a few biological applications of commercially available $K^+$ ion probes incorporating an 18-crown-6 based chelator, such as PBFI,[6] due to their relatively low fluorescence and poor selectivity for $K^+$ over $Na^+$. On the other hand, a $K^+$-selective cryptand-based chelator, 2-triazacryptand [2,2,3]-1-(2-methoxyethoxy)benzene (TAC), was recently synthesized,[7] and several fluorescent $K^+$ probes incorporating TAC have been reported by Verkman et al. [8–10] However, long-wavelength-emitting $K^+$ probes that can be excited at over 550 nm, the optimal region for biological studies in terms of autofluorescence and photo-damage to biological samples, are still rare.[8,9] Furthermore, although these TAC-based probes show good $K^+$ selectivity, their fluorescence properties are controlled by photoinduced electron transfer (PeT),[11] and so the shape of the absorption spectra is insensitive to $[K^+]$. Considering the demand for simple colorimetric detection of molecules in aqueous samples,[12,13] we believe there is a strong need for selective fluorescent $K^+$ indicators that meet the following requirements: (i) excitation at over 550 nm, (ii) significant color change in response to $[K^+]$, and (iii) compatibility with aqueous media (>50% $H_2O$).

Here we report the synthesis and spectroscopic properties of a novel red-emitting, fluorogenic $K^+$ probe, B3TAC, which is also applicable for colorimetric detection of $K^+$ ion. As an ionophore, we selected TAC because of its good selectivity for $K^+$ and fast response to changes of ion concentration.[9] As a chromophore, we adopted 8-phenyl BODIPY because of its sharp absorption and emission spectra, high extinction coefficient and quantum efficiency, and stability to light and chemical reactions.[14] Several BODIPY derivatives bearing ion-recognizing groups at a styryl moiety introduced at position 3 of the chromophore have recently been synthesized.[15–18] In most cases, they have red emission due to the extension of π-conjugation, and they show both fluorescence intensity change and wavelength shift of the absorption/emission spectra, presumably derived from the change of electronic environment as a result of coordination of metal ions to the nitrogen atom linked to the π-system. Those results prompted us to design B3TAC as a novel fluorescent $K^+$ probe that would satisfy the above requirements. Although sensors for $Cu^{2+}$,[15] $Hg^{2+}$,[16] and $F^-$[17] have been developed based on the styryl BODIPY scaffold, $K^+$ has not previously been targeted.

The synthetic route to B3TAC is depicted in Scheme 1. B3TAC was obtained by Knoevenagel-type condensation of TAC-aldehyde (**1**) and 8-phenyl-1,3,5,7-tetramethyl BODIPY (**2**) in a Dean–Stark apparatus with 15% yield. The purity of B3TAC was confirmed by $^1$H-NMR, HRMS and HPLC (Supplementary data).

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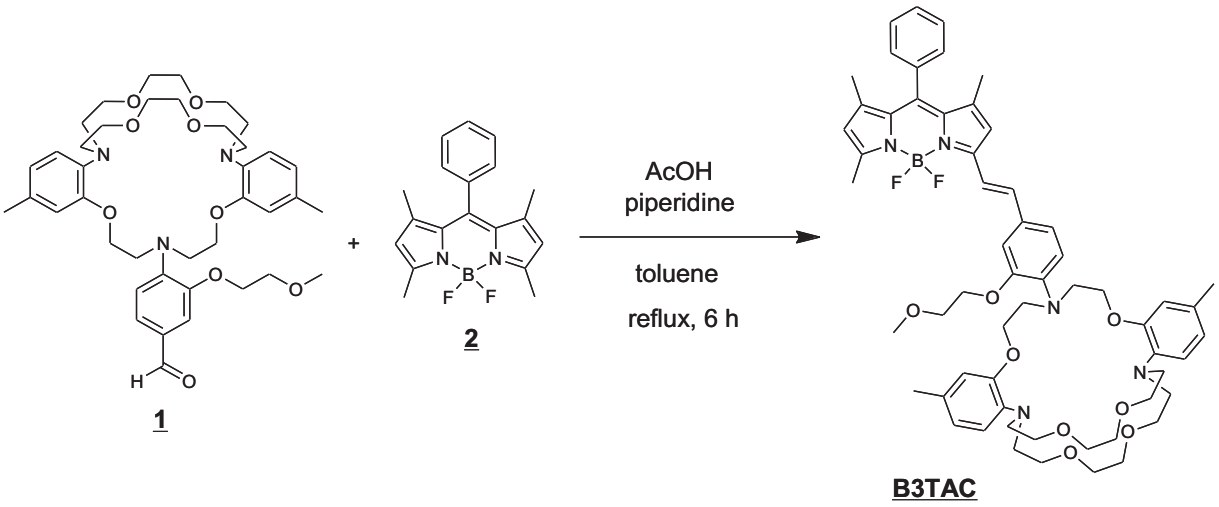


**Scheme 1.** Synthesis of B3TAC.

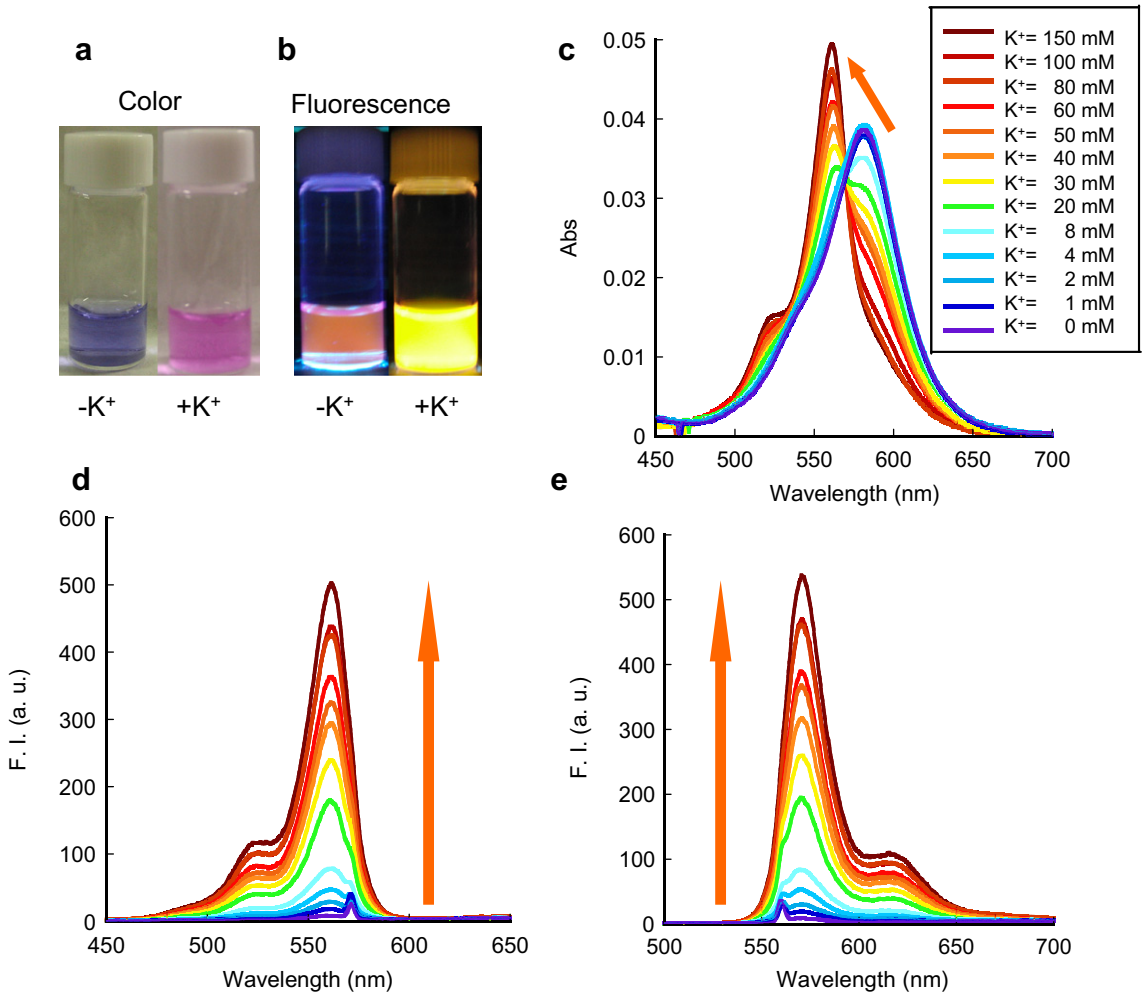


**Figure 1.** Optical properties of B3TAC. Change in (a) color and, (b) fluorescence (excitation at 365 nm) of a MeCN solution containing B3TAC with or without $K^+$, (c) Absorption, (d) excitation ($\lambda_{em}$ = 571 nm) and, (e) emission ($\lambda_{ex}$ = 560 nm) spectra of 1 μM B3TAC in 5 mM HEPES (pH 7.0)/MeCN = 60:40 containing the indicated concentration of $K^+$ as the $ClO_4^-$ salt. Ionic strength was maintained at 150 mM by addition of $NaClO_4$. The mixture contained 2% DMSO as a cosolvent.

First, we preliminarily evaluated the response of B3TAC to $K^+$ ion in a glass sample vial, and confirmed that, in acetonitrile, B3TAC showed almost no fluorescence in the absence of $K^+$ ion, but emitted strong yellow to orange fluorescence when excess $KClO_4$ was added (Fig. 1b). As expected, a significant color change of the sample from blue to pink was also observed (Fig. 1a). We next examined whether B3TAC worked in aqueous environment (Fig. S1). As the percentage of 5 mM HEPES buffer (pH 7.0) in

**Table 1**
Optical properties of B3TAC in 5 mM HEPES (pH 7.0)/MeCN = 60:40 containing the indicated concentrations of $Na^+$ and $K^+$

| $K^+/Na^+$ (mM) | $Abs_{max}$ (nm) | $Ex_{max}$ (nm) | $Em_{max}$ (nm) | $\varepsilon$ ($M^{-1}$ $cm^{-1}$) | $\varphi_{FL}$ |
|---|---|---|---|---|---|
| Buffer only | 578 | 560 | 568 | $7.3 \times 10^4$ | 0.022 |
| Buffer + 150 mM $Na^+$ | 581 | 559 | 569 | $7.4 \times 10^4$ | 0.018 |
| Buffer + 150 mM $K^+$ | 561 | 562 | 570 | $8.9 \times 10^4$ | 0.39 |

acetonitrile was increased up to 70%, little change of the absorption spectrum was observed, although the fluorescence intensity was decreased to some extent. However, when the percentage of HEPES buffer was 80% or more, the absorption spectrum was broadened and the fluorescence disappeared completely with a concomitant increase of scattered light. This is considered to be due to aggregation of B3TAC, because of its hydrophobic nature and poor solubility in pure water. Hence, subsequent experiments were performed in a mixed aqueous media containing 60% HEPES buffer, in which the probe should behave as individual molecules.

We next performed a titration experiment with B3TAC and $K^+$ in aqueous 5 mM HEPES (pH 7.0)/MeCN = 60:40 by increasing the concentration of $KClO_4$ under the condition that the total ionic strength was maintained at 150 mM with $NaClO_4$ (Figs. 1c–e and S2). In the absence of $K^+$, a rather broad and structureless absorption spectrum was observed with $\lambda_{max}$ = 581 nm, and the probe emitted very low fluorescence ($\lambda_{ex}$ = 560 nm).[19] In the presence of 150 mM $K^+$, however, the absorption spectrum became sharp, with a 20 nm blue shift of the peak to 561 nm, and the fluorescence intensity was elevated dramatically, by approximately 100-fold.[20] As shown in Figure 1c, the shape of the absorption spectrum gradually changed in a [$K^+$]-dependent manner with a clear isosbestic point at 570 nm. The results indicated the conversion of $K^+$-free B3TAC to a $K^+$-bound form, probably the 1:1 complex.[9] Notably, B3TAC was capable of detecting 1 mM $K^+$ even in the presence of a high concentration of $Na^+$. The photophysical properties of B3TAC and its $K^+$ complex are summarized in Table 1. Using cresyl violet ($\varphi_{FL}$ = 0.54 in methanol) as a standard,[21] the quantum efficiency ($\varphi_{FL}$) of B3TAC was determined to be 0.022 in buffer, 0.018 at 150 mM $Na^+$ and 0.39 at 150 mM $K^+$, but when the absorption wavelength change is taken into account, the effective activation of fluorescence intensity is much larger (Fig. 1e). High concentrations of $Na^+$ caused a small (ca. 3 nm) red shift of the absorption peak and a slight decrease of quantum efficiency compared to the buffer-only condition. Also, the fluorescence intensity of B3TAC in the presence of 80 mM $K^+$ was decreased to some extent when >100 mM $Na^+$ was added (Fig. S3). Although the mechanism remains to be examined, excess $Na^+$ might compete with $K^+$ and fix B3TAC in the $K^+$-free 'quenched' conformation. From a plot of fluorescence intensity versus [$K^+$], the apparent dissociation constant ($K_{Dapp}$) was calculated to be 53 mM[22] (Fig. S4). Since extracellular $K^+$ concentration is reported to be about 5 mM, while intracellular $K^+$ concentration is around 150 mM,[23] this value of $K_{Dapp}$ means that the probe is suitable for measuring physiological $K^+$ concentrations.

We also examined the selectivity of B3TAC for $K^+$ (Fig. 2). Compared to the fluorescence intensity in 5 mM HEPES (pH 7.0)/MeCN = 60:40, B3TAC showed no increase in fluorescence in the presence of $Na^+$ (at 150 mM), but showed slightly increased fluorescence with other metal ions such as $Li^+$, $Mg^{2+}$, $Ca^{2+}$ (at 50 mM), $Zn^{2+}$, $Fe^{3+}$ and $Cu^{2+}$ (at 100 μM). Importantly, however, B3TAC recognized $K^+$ even in the presence of these ions, and the fluorescence intensity of the $K^+$-added sample was not greatly affected by the co-presence of the other ions. On the other hand, strong fluorescence was observed in the presence of $Rb^+$ or $Cs^+$ (at 50 mM), which are alkali metal ions with a larger radius than $K^+$; these ions are known to be chelated by TAC.[8] For biological applications, however, this should not be a problem because the predominant metal ions in extracellular and intracellular fluids are $Na^+$ and $K^+$, respectively, and $Rb^+$ and $Cs^+$ are not present at relevant concentrations. The pH dependence of the probe was also examined, and it was confirmed that the effect of pH is negligible in the physiological range (Fig. S6).

Although the mechanism of fluorescence quenching in the absence of $K^+$ remains to be fully investigated, B3TAC is different from previously reported TAC-based sensors[7–10] in that the change of fluorescence intensity is accompanied with a distinct color change. This type of blue shift of the absorption spectrum in response to the coordination of ions is a general phenomenon of amine-containing BODIPYs and other fluorophores, and has been attributed to the suppression of internal charge transfer (ICT).[3,16,24] However, in contrast to these typical ICT-based probes, the shape of the excitation spectrum of B3TAC was not influenced by [$K^+$] (Figs. 1d and S2). In other words, the unbound form of

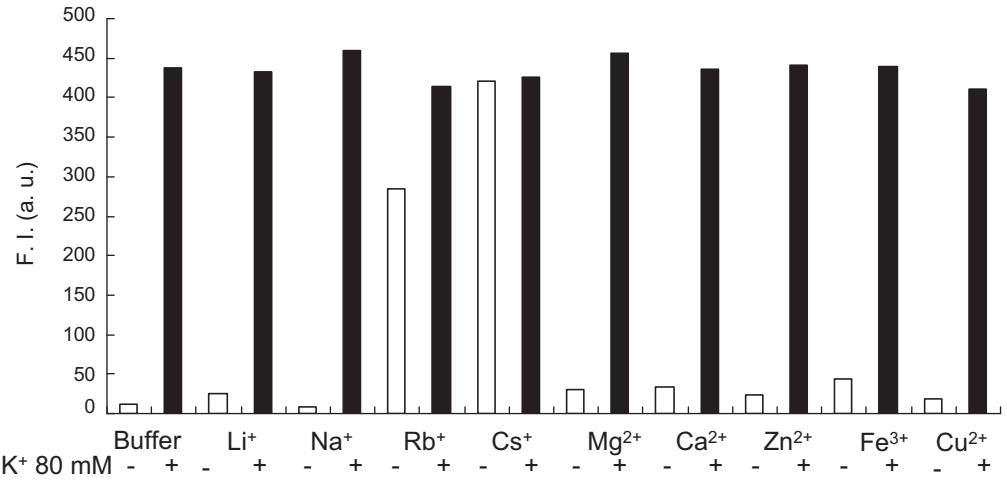


**Figure 2.** Ion selectivity of B3TAC. Emission spectra were measured with 1 μM B3TAC dissolved in 5 mM HEPES (pH 7.0)/MeCN = 60:40 containing 2% DMSO as a co-solvent ($\lambda_{ex}$ = 560 nm). Concentrations were as follows: $Li^+$, $Rb^+$, $Cs^+$, $Mg^{2+}$ and $Ca^{2+}$ 50 mM, $Na^+$ 150 mM, $Zn^{2+}$, $Fe^{3+}$ and $Cu^{2+}$ 100 μM, each as the $ClO_4^-$ salt.

B3TAC appears to be quenched very efficiently by charge transfer from the fluorophore-linked amine of TAC, although some contribution of PeT involving the other phenyl rings in the chelator can not be excluded.

In conclusion, we have developed a novel fluorescent probe for $K^+$, B3TAC, which possesses many desirable properties for biological applications, including high selectivity for $K^+$, suitability for colorimetric detection, long excitation wavelength, solubility in aqueous media, and large fluorescence increase in response to $K^+$. This probe was able to sense physiological concentrations of $K^+$ with high values of signal-to-noise ratio, enabling quantification of potassium concentration in blood or cell lysate in the presence of some organic solvent to improve the solubility. A change of $K_{Dapp}$ value in pure water cannot be ruled out, but we believe that improving the water solubility of B3TAC by appropriate modification of the fluorophore in the future should allow for intracellular or extracellular [$K^+$] measurement in terms of fluorescence change in the red wavelength region even in the absence of organic solvent. Furthermore, to our knowledge, B3TAC is the first colorimetric $K^+$ sensor that allows for naked-eye detection of the ion in aqueous media. With these properties, B3TAC should serve as a potentially useful tool to measure [$K^+$] in solution.

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## Supplementary data

Supplementary data (detailed synthetic procedure and characterization of B3TAC, methods of optical measurements, Figs. S1–S6) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.056.

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