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High Na^+ and K^+-induced fluorescence enhancement of a π-conjugated phenylaza-18-crown-6-triazol-substituted coumarin fluoroionophore†

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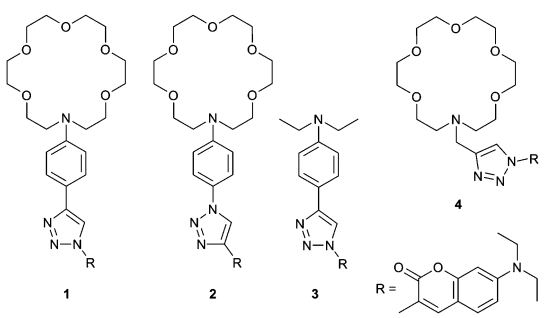
The new π-conjugated 1,2,3-triazol-1,4-diyl fluoroionophore 1 generated *via* Cu(I) catalyzed [3 + 2] cycloaddition shows high fluorescence enhancement factors (FEF) in the presence of Na^+ (FEF = 58) and K^+ (FEF = 27) in MeCN and high selectivity towards K^+ under simulated physiological conditions (160 mM K^+ or Na^+, respectively) with a FEF of 2.5 for K^+.

Recently 1,2,3-triazol-1,4-diyl fluoroionophores for Zn^{2+},[1] Ni^{2+},[2] Cu^{2+},[3] Hg^{2+},[4] Ag^+,[4] and Al^{3+},[5] were generated by Cu(I) catalyzed reaction between an azide and an alkyne (CuAAC). In these fluoroionophores the 1,2,3-triazol-1,4-diyls serve, in addition to the conventional function as covalent linkers, as both chelating ligand of the metal ions and as electronic transmitter of a coordination event to the fluorophore.[1a] Only Zn^{2+} and Al^{3+} could be detected by fluorescent enhancement, whereas the other metal ions were analysed through fluorescence quenching. In these known 1,2,3-triazol-1,4-diyl fluoroionophores, no electronically conjugated signal transduction chain: ionophore-1,2,3-triazole-fluorophore is used. Either receptor and 1,2,3-triazole are connected through a deconjugated linker[1,2] or the triazole and the fluorophoric group are deconjugated.[3–5]

In a systematic investigation Diederich *et al.* showed the capacity of the 1,2,3-triazol-diyls as active π-linkers in Charge Transfer (CT) chromophores.[6] Such push–pull chromophores are only weakly or even nonfluorescent. 1(4-Butoxyphenyl)-4-pyridyl-1,2,3-triazole displays turn-on fluorescence upon addition of metal cations.[7]

We found that in the simple CuAAC-generated fluoroionophore **1**, the electronic conjugation of the *N*-phenylaza-18-crown-6 ether and the 7-diethylaminocoumarin fluorophore through a 1,2,3-triazol-1,4-diyl π-linker results in a perfect signal transduction chain for the sensing of Na^+ and K^+. In MeCN high cation-induced FEFs were obtained for **1** (FEF_{Na^+}: 58; FEF_{K^+}: 27). The signal transduction in **1** also works nicely under simulated physiological conditions. In the presence of 160 mM K^+ a FEF of 2.5 was observed, whereas the same concentration range of Na^+ resulted in an almost negligible fluorescence enhancement. We assume that compound **1** is a PET-fluoroionophore with a virtual spacer between the anilinotriazole electron donor unit and the coumarin electron acceptor moiety. The constitutional isomer of **1**, the 1,2,3-triazole-fluoroionophore **2** shows smaller cation induced FEFs than **1**, suggesting that in the presented conjugated sensors the substitution on the 1,2,3-triazole ring has a basic influence on the quality of the fluorionophore.

The CuAAC of the ethinyl-functionalized *N*-phenylaza-18-crown-6 ether[8] with 3-azido-7-diethylaminocoumarin[9] afforded 1,2,3-triazole-fluoroionophore **1**. The triazole-isomer **2** was obtained by the reaction of the azido-functionalized *N*-phenylaza-18-crown-6 ether[10] with 3-ethinyl-7-diethylaminocoumarin.[11] Dye **3**, in which the crown has been replaced by a diethylamino group was synthesised in order to represent a reference compound for **1** (Scheme 1). A further reference compound for **1** is compound **4**, in which the *p*-phenylene linker between aza-18-crown-6 and the triazole ring is replaced by a deconjugated methylene group. Compound **4** was obtained by the reaction of *N*-propargylaza-18-crown-6 ether[12] with 3-azido-7-diethylaminocoumarin. The new 1,4-disubstituted 1,2,3-triazoles **1–4** are stable in solutions of MeCN and DMSO at room temperature for many weeks. UV-irradiation over a period of several hours did not show any decomposition of the fluoroionophores **1** and **2**.



Scheme 1 Conjugated fluoroionophores **1** and **2** with 1,2,3-triazol-1,4-diyl as the π-linker, and reference compounds **3** and **4**.

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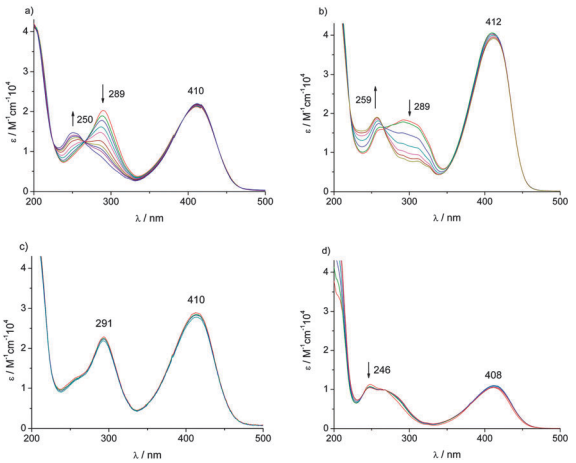

Fig. 1 UV/VIS absorption spectra of **1–4** in MeCN in the presence of increasing concentrations of $NaPF_6$: (a) **1** +0.00–2.40 mM, (b) **2** +0.00–2.80 mM, (c) **3** +0.00–2.80 mM, (d) **4** +0.00–4.60 mM.

The UV/Vis absorption spectra of the 3-(anilino-1,2,3-triazolyl) substituted 7-diethylaminocoumarins **1–4** in MeCN show the typical long-wavelength coumarin CT absorption band between λ_{max} = 408 and 412 nm (Fig. 1a–d), which are similar to that of 3-(2-methylbenzimidazol-1-yl)-7-diethylaminocoumarin at λ_{max} = 407 nm.[13]

The 1,2,3-triazol-1,4-diyl constitutional isomerism in the case of fluoroionophores **1** and **2** has only a small effect on the λ_{max} of the long-wavelength CT absorption. However, the intensity of this CT band is higher for **2** (ε = 40 050 M^{-1} cm^{-1}) than for **1** (ε = 21 250 M^{-1} cm^{-1}). This might be caused by the more efficient conjugation between the triazole and coumarin unit in **2** through the sp^2-hybridized carbon atom C(4) of the triazole, whereas in **1** the coumarin unit is connected to the triazole ring at N(1).

In the UV/Vis-spectra of **1** and **2** we observe next to the long-wavelength CT transition a second short-wavelength CT transition at λ_{max} = 289 and 294 nm, respectively (Fig. 1a and b). The CT nature of these absorption bands is confirmed by the reduction in intensity in the presence of Na^+ (Fig. 1a and b) or K^+ (Fig. S1 and S2, ESI†), respectively. The coordination of Na^+ or K^+ in the cavity of the azacrown reduces the electron donor character of the anilino moiety. Thus the CT towards the conjugated electron poor triazole (acceptor) is energetically shifted upwards. We observed such a CT absorption band around 290 nm also in the UV/Vis absorption spectrum of a 1,2,3-triazol-fluoroionophore in which the conjugated *N*-phenylaza-18-crown ether-1,2,3-triazol-1-yl unit is connected to a 9-anthracenyl moiety by a methylene-spacer. Addition of Na^+ leads to a complete disappearance of the CT absorption band at λ_{max} = 289 and 294 nm in the spectra of **1** and **2**, respectively (Fig. 1a and b), whereas the presence of K^+ reduces the intensity of this CT absorption band to a lesser extent (Fig. S1 and S2, ESI†). This can be explained by the larger charge density of Na^+. The complexation of Na^+ or K^+ by the azacrown of **1** or **2** has only very little influence on the long-wavelength CT absorption at λ_{max} = 410 or 412 nm.

The UV/Vis absorption spectrum of the reference compound **3** in MeCN is barely changed in the presence of Na^+ (Fig. 1c) or K^+ (Fig. S3, ESI†). This result proves the coordination of Na^+ and K^+ in the cavity of the aza-18-crown-6 ether of **1** and **2** in MeCN.

Table 1 Photophysical properties of **1–4** in MeCN

Ligand	**1**	**2**	**3**	**4**
λ_{em}/nm[a]	484	470	493	480
λ_{ex}/nm[b]	410	412	410	408
Φ_f(ligand)[c]	0.008	0.03	0.017	0.56
Φ_f(ligand + Na^+)[d]	0.62	0.79	0.018	0.55
Φ_f(ligand + K^+)[d]	0.23	0.40	0.016	0.58

[a] Emission maxima. [b] Excitation maxima. [c] Fluorescence quantum yields (Φ_f). [d] Φ_f were determined in the presence of 40 mM of $NaPF_6$ or 40 mM KPF_6, respectively.

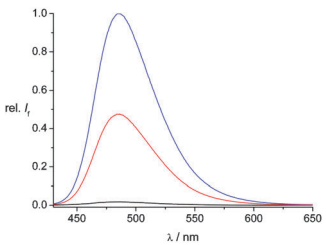

Fig. 2 Fluorescence spectra of **1** in MeCN (black) upon addition of 0–2.5 mM $NaPF_6$ (blue) and 0–1.16 mM KPF_6 (red), respectively.

The fluorescence quantum yield of **1** in MeCN is extremely low (Φ_f(**1**) = 0.008, Table 1). The fluorescence spectra of **1** in the presence of Na^+ and K^+, respectively (Fig. 2) show the impressive fluorescence enhancement upon coordination of the alkali metal ions. High FEFs could be determined for Na^+ (FEF = 58) and for K^+ (FEF = 27).[14]

The B3LYP/6-31G*-optimized geometry of **1** (Fig. S10, ESI†) shows that the π-electron system of **1** is twisted at the coumarin–triazole link (6°) owing to the steric hindrance caused by the carbonyl unit. The anilino moiety is nearly coplanar with the triazole ring. As found in many twisted systems this twisting should result in a virtual spacer.[15a] Thus, the well-delocalized planar anilinotriazole unit serves as a good PET donor and the coumarin serves as a PET acceptor. The planarity of the anilino-triazole ensures a low oxidation potential. Hence, a very small fluorescence quantum yield is found. Complexation of Na^+ or K^+ by the azacrown increases the oxidation potential of the anilinotriazole and the fluorescence switch-on happens by the stoppage of the PET. Also, in the UV/Vis-spectra, the lowest energy coumarin absorption is essentially unchanged upon Na^+ or K^+ addition, which typically characterises fluorescent PET systems. Only the higher energy anilinotriazole absorption is blue-shifted, owing to the removal of the anilino electron pair by binding to Na^+ or K^+. Similar blue-shifts of higher-energy bands combined with unchanged lower-energy bands are known from the UV/Vis-spectra of PET sensors.[15b,c]

The fluorescence quantum yield of the constitutional isomer **2** in MeCN is higher than that of **1** (Φ_f(**2**) = 0.03, Table 1). The fluorescence of **2** is also enhanced in the presence of increasing concentrations of Na^+ and K^+ (ESI†, Fig. S7 and S8, respectively). However the observed FEFs for Na^+ (FEF = 17) and K^+ (FEF = 13) are significantly lower than for the isomer **1**. The B3LYP/6-31G*-optimized geometry of **2** revealed that in **2** the anilino unit is twisted against the triazole ring with a torsion

angle of about 24° (Fig. S10, ESI†). The poorer planarity of the anlinotriazole unit in **2** results in a higher oxidation potential. Therefore, free **2** shows a stronger fluorescence than **1** and lower FEF values are found.

The reference compound **4** consists of a poorer PET donor due to the aliphatic amine which is electronically separated from the triazole. Hence **4** has a high quantum yield (Φ_f(**4**) = 0.56, Table 1) and is therefore less affected by the presence of Na^+ and K^+.

We further investigated the influence of Na^+ and K^+ on the fluorescence of **1** under simulated physiological conditions. The ligand was exposed to aqueous solutions in the physiological interesting concentration range of 0–160 mM Na^+ or K^+, respectively. Additionally, the solutions contained 2 mM Ca^{2+} and 2 mM Mg^{2+}. The pH was adjusted to 7.2 with 10 mM Tris and a constant ionic strength of 180 mM was maintained with choline chloride. Under these conditions, the fluorescence of **1** (λ_{ex} = 424 nm, λ_{em} = 500 nm, Φ_f = 0.07) is hardly increased in the presence of Na^+ whereas increasing concentrations of K^+ resulted in a modest fluorescence enhancement (Fig. 3a). In the presence of 160 mM K^+ a FEF of 2.5 is observed with very little variations in the FEF values (Fig. 3b). It is noteworthy, that the presence of physiological important extracellular cations, such as Mg^{2+} and Ca^{2+} does not affect the signal response.

In comparison, the FEF of **1** for K^+ in MeCN is clearly smaller than the FEF in water under simulated physiological conditions. This can be explained by the significantly smaller stability constants of the K^+ complex with the *N*-phenylaza-18-crown-6 in water [lg *K* (H_2O) > 0.5; lg *K* (MeCN) = 3.95 ± 0.08]. The fact that the fluorescence of **1** in water is exclusively enhanced in the presence of K^+ and not by Na^+ can be rationalized by the stronger hydration enthalpy of Na^+. However, the fluorescence enhancement of **1** in the presence of K^+ under simulated physiological conditions shows that the signaling transduction chain in this fluoroionophore works well in water.

The dissociation constant (K_d) of **1** + K^+ amounts to ~260 mM in solutions which approximates the physiological ionic strength. To measure intracellular or extracellular concentrations of K^+ (K_d around 140 or 4 mM) tuning of probe **1** towards a higher complex stability while maintaining the selectivity will be necessary.

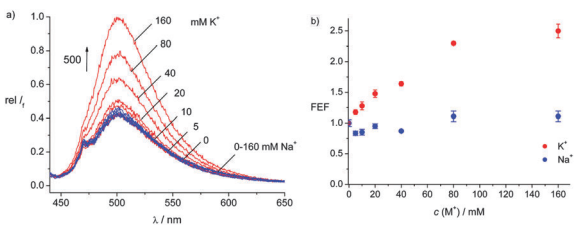

Fig. 3 Fluorescence measurements of **1** (λ_{ex} = 424 nm) under simulated physiological conditions (ESI†). (a) Fluorescence spectra in the presence of 0–160 mM Na^+ (blue) and K^+(red), respectively and (b) the corresponding FEF with error bars in the presence of 0–160 mM K^+ or Na^+.

Recently He *et al.*[16a] and the Verkman group[16b] designed fluorescence switch-on PET sensors with high K^+/Na^+ selectivity and sensitivity for physiological K^+ in the concentration range of 0–40 mM. In these K^+ fluoroionophores a [3.2.2]-cryptand represents the ionophore. A drawback though, is the very expensive synthetic procedure.[16c]

To investigate the pH-sensitivity of **1**, the fluorescence intensity was measured in water at different pH values. The resulting pK_a of **1** is near 4.5 (Fig. S9, ESI†) meaning that **1** is less pH-sensitive than the *o*-methoxyphenylaza-15-crown-5 Na^+-fluoroionophore (pK_a ≈ 5.5) of He *et al.*[16]

In summary, we have shown for the first time that an electronically conjugated 1,2,3-triazole-fluoroionophore consisting of the signaling transduction chain: triazole-fluorophore works as an effective sensor for Na^+ and K^+ in acetonitrile with high cation-induced FEFs. Under simulated physiological conditions **1** selectively detects K^+ with the modest FEF being limited by the rather simple receptor unit. The efficient signaling transduction chain in the fluoroionophore **1** should be a promising design concept for generating novel highly sensitive fluoroionophores for Na^+, K^+, Mg^+ and Ca^+ with interesting potential for biological applications. Further studies towards the fine tuning of the receptor unit are currently under investigation in our laboratories.‡

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