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Coumaryl crown ether based chemosensors: selective detection of saxitoxin in the presence of sodium and potassium ions

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Abstract—Two novel fluorescent chemosensors in which an aza-crown is linked to 4-coumaryl fluorophores by a methylene spacer have been synthesized for sensing saxitoxin. Fluorescence enhancement was observed upon binding of the dicationic toxin molecule, whereas several metal ions produced no effect.

1. Introduction

Chemosensors, which apply the principle of photoinduced electron transfer, are called PET chemosensors.[1,2] Different fluorophores were successfully used previously for the construction of such sensor devices.[3] For this purpose coumarins were also successfully employed.[4–6] In our earlier work[7] an anthracylmethyl-crown sensor, originally developed by de Silva for the detection of sodium and potassium in methanol,[8] was proven to bind saxitoxin (Fig. 1), a marine toxin produced by dinoflagellates and several blue–green algae species.[9–14] The effect of saxitoxin consumption is better known as paralytic shellfish poisoning (PSP) and its most severe symptom is respiratory paralysis.[15] In the anthracylmethyl-aza-crown system, saxitoxin binds to the aza-crown part in a 1:1 stoichiometry.[7] The sensing of the toxin is believed to work through photoinduced electron transfer.

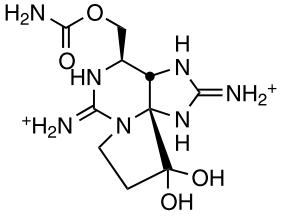

Figure 1. Structure of saxitoxin.

The long-term plan for our project is to develop an optical fiber based fluorescence sensor that detects saxitoxin. This sensor would consist of a monolayer of the fluorophore molecules covalently bound on the fiber surface. For this, the monolayer properties of the fluorophore have been determined. We have examined the monolayer of an amphiphilic anthracylmethyl-aza-crown derivative at the air–water interface; however, fluorescence could not be detected at neutral pH. This was probably due to fluorescence quenching caused by aggregation of the fluorophores.[15] Therefore, our need was to develop new aza-crown containing PET pH sensors that have high quantum efficiency and show fluorescence at the air–water interface even in the presence of aggregates.

We have recently introduced a new coumarin amphiphile that was studied at the air–water interface.[16] Although this amphiphile also tended to form aggregates, fluorescence was present all the time. This prompted us to synthesize new coumarin PET-crown sensors that later could be modified into amphiphiles if they show sensitivity for saxitoxin. Coumarins generally show good spectral features such as large Stokes shifts (70–100 nm) and high quantum yields.

2. Results and discussion

We have synthesized two novel coumaryl-aza-crown derivatives that differ in substituent at the 7th position of the coumarin (Fig. 2). 4-Bromomethyl-7-methoxy-coumarin is a commercially available derivatizing agent often used to label amino acids. Alkylation of the

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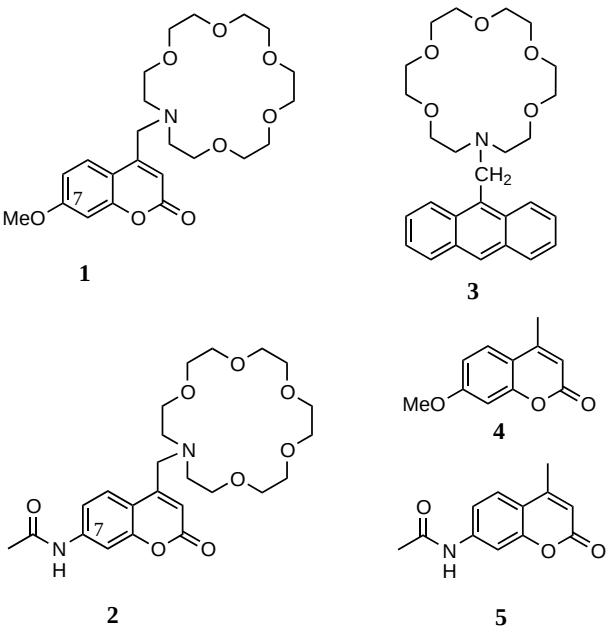

Figure 2. Structure of the fluorophores.

aza-crown gave the methoxycoumaryl-aza-crown derivative **1**.[17]

The synthesis of the acetamido species started with the acetylation of the amino group of 3-aminophenol **6**. A Pechmann condensation of the resulting acetamidophenol **7**, with commercially available ethyl-4-chloroacetoacetate gave 4-chloromethyl-7-acetamidocoumarin **8**.[18] Alkylation of 1-aza-18-crown-6 with **8** afforded **2**[19] in 30% yield (Fig. 3).

Compounds **1** and **2** showed absorption maximum at 323 and 327 nm and emission maximum at 419 and 412 nm, respectively, in pure water. Both compounds displayed pH sensitive fluorescence intensity in an on–off fashion (Fig. 4). This means that the PET causes fluorescence quenching only when unprotonated. Neither of the corresponding parent fluorophores **4**, **5** showed any significant pH dependency reflected in their fluorescence intensity. pH Dependent fluorescence intensity measurements were used to determine the pK_a values for the two fluorophores. As expected they were found to be similar, 5.8 and 6.0 for **1** and **2**, respectively.

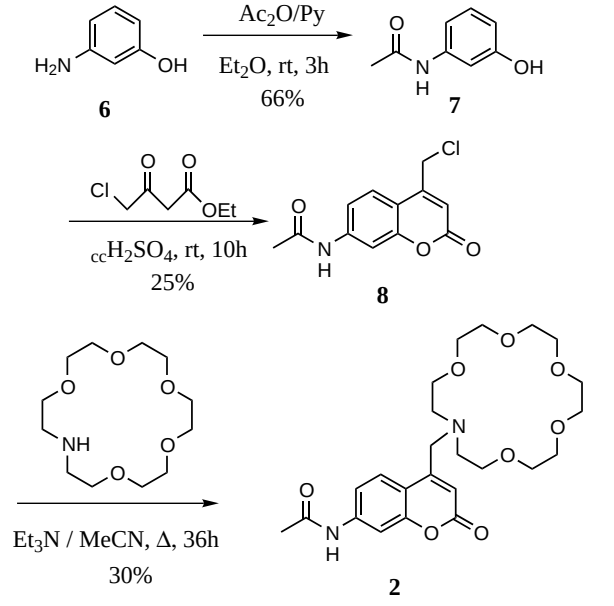

Figure 3. Synthesis of chemosensor **2**.

In aqueous solutions the addition of Na^+, K^+ and Ca^{2+} ions did not show any relevant influence on the fluorescence intensities. The data in Fig. 5, shown in log scale on the abscissa for clarity, reveal random noise in response to increasing cation concentrations. The lack of response of these chemosensors to alkali and alkali earth cations is relevant to future applications, where

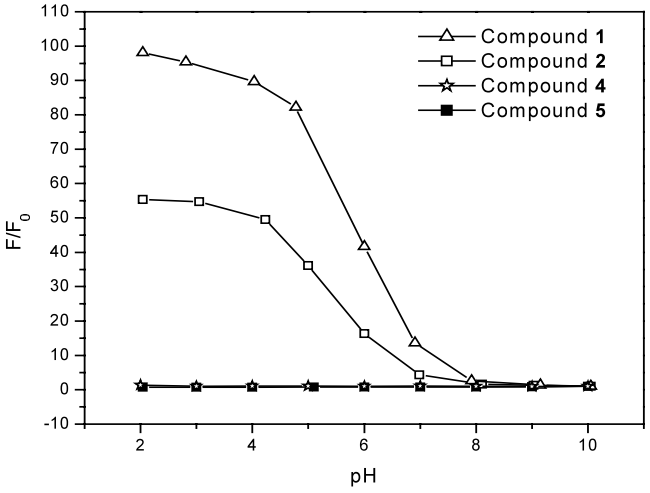

Figure 4. Normalized fluorescence intensity versus pH for compounds **1**, **2**, **4** and **5**. F_0 represents the lowest intensity, e.g. at pH 10.0.

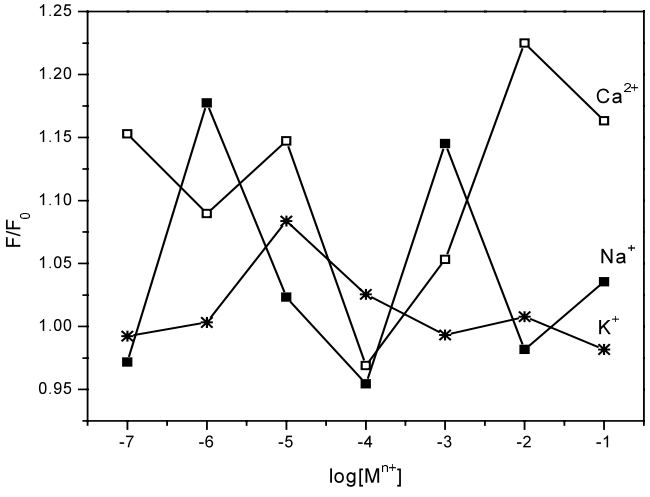

Figure 5. Normalized fluorescence intensity of compound **2** in the presence of metal ions (10^{-3} M Me_4NOH in water was used as solvent). F_0 represents fluorescence intensity in the absence of metal ions.

we hope to analyze for saxitoxin in environmental samples.

The lack of sensitivity of these sensors to these metal cations permitted us to use sodium and potassium containing buffered aqueous solution (phosphate buffer, pH 7.4, [NaCl] = 0.137 M, [KCl] = 0.0027 M) for the experiments when the sensors were titrated with saxitoxin, using 328 nm as excitation wavelength for all coumarin derivatives and 366 nm for the anthracene derivative. Keeping the fluorophore concentration at a constant value (10^{-6} M) 10^{-4} to 10^{-7} M saxitoxin concentrations were examined using the dilution methods described elsewhere.[7] Fig. 6 shows the F/F_0 curves for the two new compounds along with anthracyl-aza-crown (**3**) when titrated with saxitoxin.

Measuring the fluorescence intensity while titrating with saxitoxin gave us saturation type of curves. Fitting a theoretical equation to the measurement points made it possible to determine binding constants.[20] The equation that was used is:

$$F = \{(1+cK_b[STX])/(1+K_b[STX]\}F_0$$

where F_0 represents the fluorescence intensity when saxitoxin is absent, F in the presence of the toxin, K_b the binding constant and c being a constant related to the ratio of the quantum efficiencies of the free and bound fluorophores. Fig. 7 shows an example of compound **1** titrated with saxitoxin. The continuous line represents the fitted theoretical equation.

The two coumaryl crowns **1** and **2**, as well as the anthracylmethyl-crown **3**, gave similar binding constants (Table 1). This is expected considering that the aza-crown moiety common to all three species is responsible for the binding. Noteworthy is the fact that these binding constants are one order of magnitude higher than those observed when 80:20 EtOH/water was used as solvent.[7]

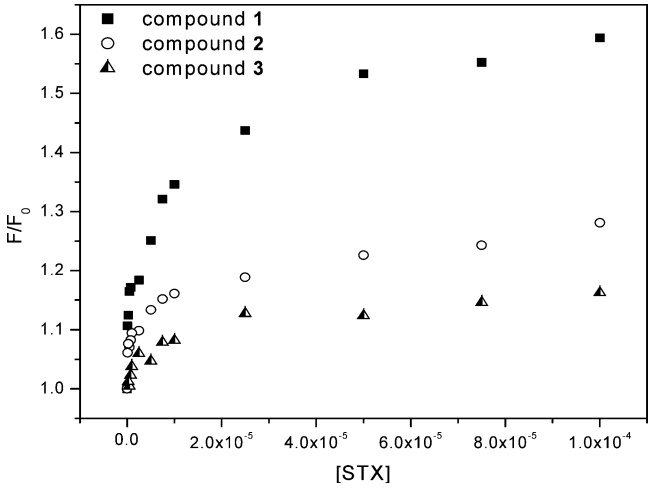

Figure 6. Normalized fluorescence intensity against concentration of saxitoxin (STX) for compounds **1**, **2** and **3**. F_0 represents the fluorescence intensity in the absence of STX.

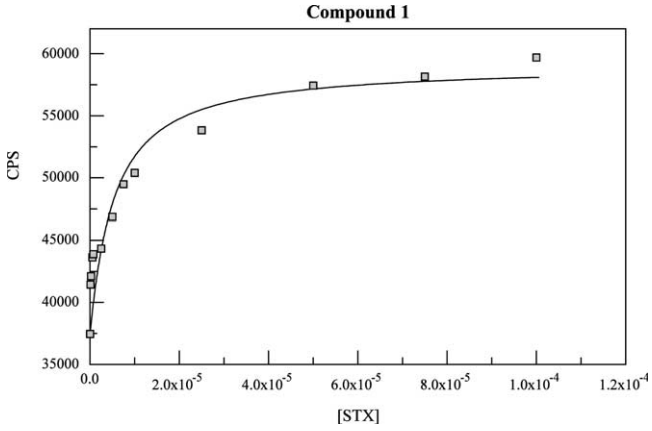

Figure 7. Fluorescence intensity of compound **1** with increasing concentrations of STX (dots). The continuous line represents a fitted equation that was used to calculate K_b.

Table 1. Binding constants and correlation coefficients for the chemosensors

Compound	K_b (M^{-1})	Correlation coefficient
1	$1.35 \pm 0.72 \times 10^5$	0.96
2	$4.15 \pm 0.12 \times 10^5$	0.92
3	$1.25 \pm 0.22 \times 10^5$	0.98

In summary, two coumaryl PET sensor molecules were able to detect saxitoxin in water, selectively over sodium and potassium ions. The presence of calcium ions also caused only random effects in the fluorescence signal. Amphiphile derivatives of the coumaryl crowns will be tested for monolayer properties in the near future.

Acknowledgements

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17. ^{1}H NMR (CD_3OD versus TMS) $\delta=2.85$ (4H, t, $J=4.82$ Hz), 3.5–3.7 (20H, m), 3.81 (2H, s), 3.92 (3H, s), 6.43 (1H, s), 6.97 (1H, d, $J=2.63$ Hz), 7.03 (1H, dd, $J=2.63$ Hz, $J=8.77$ Hz), 7.77 (1H, d, $J=8.77$ Hz); ^{13}C NMR ($CDCl_3$ versus TMS) $\delta=56.6$, 58.1, 68.8, 70.9, 71.0, 71.2, 101.9, 112.9, 113.6, 114.4, 126.9, 156.0, 157.1, 163.1, 164.6; HRMS: (HM^+) calcd for $C_{23}H_{34}NO_8^+$, 452.2284; found, 452.2284.
18. ^{1}H NMR ($(CD_3)_2SO$ versus TMS) $\delta=2.11$ (3H, s), 4.98 (2H, s), 6.54 (1H, s), 7.47 (1H, dd, $J=1.75$ Hz, $J=8.77$ Hz), 7.78 (1H, d, $J=8.77$ Hz), 7.81 (1H, d, $J=1.75$ Hz), 10.43 (1H, s); ^{13}C NMR ($(CD_3)_2SO$) $\delta=24.1$, 41.1, 105.5, 112.0, 112.9, 114.9, 125.6, 142.8, 150.4, 154.0, 159.8, 169.1; HRMS: (HM^+) calcd for $C_{12}H_{11}ClNO_3^+$, 252.0427; found, 252.0427.
19. ^{1}H NMR (CD_3OD versus TMS) $\delta=2.16$ (3H, s), 2.84 (4H, t, $J=5.26$ Hz), 3.55–3.70 (20H, m), 3.95 (2H, s), 6.62 (1H, s), 7.43 (1H, dd, $J=2.19$ Hz, $J=8.77$ Hz), 7.75 (1H, d, $J=2.19$ Hz), 7.94 (1H, d, $J=8.77$ Hz); ^{13}C NMR (CD_3OD versus TMS) $\delta=24.1$, 55.9, 57.4, 71.0, 71.4, 71.7, 71.9, 107.7, 113.3, 116.0, 116.8, 127.1, 143.5, 155.7, 156.7, 163.6, 172.0; HRMS: (HM^+) calcd for $C_{24}H_{35}N_2O_8^+$, 479.2393; found, 479.2393.
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