Synthesis and spectroscopic characterisation of fluorescent indicators for Na^+ and K^+

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The synthesis and the spectral and cation binding properties of new fluorescent indicators for Na^+, dicaesium 5-[5-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-2-thienyl]-isophthalate (Benzos), and for K^+, dicaesium 5-[5-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-2-thienyl]isophthalate (Benzop), are reported. Both indicators consist of a benzocrown ether with an appropriate cavity for the sodium and the potassium cation, respectively, linked to an aryl thiophene fluorophore. In aqueous solution, the indicators have an absorption maximum at 340 nm and an emission maximum at 430 nm. The chelating abilities of Benzos and Benzop with Na^+, K^+, Li^+, Cs^+, Mg^{2+} and Ca^{2+} were studied by means of their fluorescence properties. Upon cation binding, a change in the fluorescence intensity is observed, whereas the spectral distribution remains unaltered. While Benzos cannot discriminate between Na^+ and other monovalent cations, Benzop shows a three-fold selectivity for K^+ over Na^+.

Introduction

Non-disruptive determination of intracellular cation concentrations, such as those of Na^+, K^+, Ca^{2+} and Mg^{2+}, inside living cells has, within a decade, become of great importance in cell physiology and clinical medicine.[1] Nearly all cells maintain a large difference in ion concentrations between their interiors (90–130 mM for K^+, 5–30 mM for Na^+) and the extracellular environment (1–10 mM for K^+, 100–300 mM for Na^+).[2] In the past, the most commonly used techniques for measuring intracellular sodium and potassium were flame photometry,[3] ion selective electrodes,[4] atomic absorption spectroscopy[5] and nuclear magnetic resonance.[6] Properly designed fluorescent probes have proven to be the most suitable tools for sensing such concentrations, because they are non-invasive, sensitive, and their response is fast and highly localised.[7]

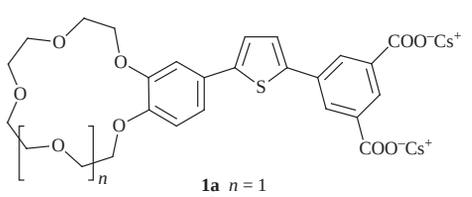
Successful indicators must fulfil a series of criteria in addition to the chemically obvious ones of sensitivity and specificity.[8] The major requirements for an indicator are: (i) an affinity that matches normal cytosolic concentrations for the ion in question (for K^+, 50 mM < K_d < 150 mM; for Na^+, 0 mM < K_d < 50 mM, where K_d is the ground-state dissociation constant), (ii) a selectivity for the desired ion over other ions present inside the cytosol, and (iii) an easily detectable change in the excitation and/or emission spectrum upon binding.

Although there has been a continuous development of fluorescent indicator technology, particularly for the determination of intracellular Ca^{2+},[9] the indicators SBFI and PBFI (sodium- and potassium-binding benzofuran isophthalate) for cytosolic Na^+ and K^+, respectively, developed in 1989 by Tsien and coworkers,[10] are still the most frequently used.

To be useful in biological systems, synthetic cation receptors must be able to bind the desired cation selectively in an aqueous medium. Since a clear relationship exists between the cation diameter and the cavity size of the crown compound[11] on the one hand, and the stability constants of the appropriate complexes,[12] on the other hand, an 18-crown-6 derivative has been proposed as a selective ionophore for K^+. Although 15-crown-5 compounds as such are known to have a limited selectivity for Na^+ *vs.* other cations in aqueous solutions,[13] an Na^+ indicator based on a 15-crown-5 derivative was developed, in accordance with the Na^+ indicators previously reported.[14]

Based on promising spectral properties of a model compound, methyl 4-(4-methyl-5-phenyl-2-thienyl)benzoate,[15] for which the product of the molar extinction coefficient, ε, and the quantum yield of fluorescence, φ_F, exceeds 10^3 M^{-1} cm^{-1}, a thiophene-containing unit was chosen as the fluorophore. The thiophene group is linked to a phenyl ring, substituted with two carboxylate functionalities to ensure water solubility.

In this paper, we present the synthesis of new fluorescent indicators for Na^+, dicaesium 5-[5-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-2-thienyl]isophthalate (Benzos **1a**), and for K^+, dicaesium 5-[5-



1a $n = 1$
1b $n = 2$

(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-2-thienyl]isophthalate (Benzop **1b**).

Besides the synthesis of the indicators for Na^+ and K^+, the spectral and cation binding properties of the indicators are reported. From absorption and steady-state fluorescence measurements, the position of the spectra, the molar extinction coefficient, ε, the quantum yield of fluorescence, φ_F, of both the free and saturated forms of the indicators, the dissociation constant, K_d, in the ground state of the formed host : guest complexes, and the selectivity *vs.* other ions were determined.

Theory

Determination of K_d from fluorimetric titration

The expression of the fluorescence signal as a function of the ion concentration has been derived by Kowalczyk *et al.*[16] for the case of a 1 : 1 complex between a fluorescent indicator and a cation. For a consecutive, multiple binding model, this expres-

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sion can be expanded by taking into account the formation of an n:1 ion:indicator complex. Consider a system consisting of species ***0*** that can undergo a reversible reaction with ion X (determined by the dissociation constant K_{d1}) to form species ***1***, which in turn can react with another equivalent of X (determined by the dissociation constant K_{d2}), to yield species ***2***, *etc.* [eqns. (1.1)–(1.*n*)]. Species ***0*** represents the free form of a fluorescent indicator, species ***1*** the 1:1 complex with a cation X and species ***n*** the n:1 ion:indicator complex. The global reaction is determined by the composite dissociation constant K_d [eqn. (2)] (Scheme 1).

$$\boldsymbol{0} + \mathrm{X} \rightleftharpoons \boldsymbol{1} \quad K_{d1} = \frac{[\boldsymbol{0}]\,[\mathrm{X}]}{[\boldsymbol{1}]} \tag{1.1}$$

$$\boldsymbol{1} + \mathrm{X} \rightleftharpoons \boldsymbol{2} \quad K_{d2} = \frac{[\boldsymbol{1}]\,[\mathrm{X}]}{[\boldsymbol{2}]} \tag{1.2}$$

$$\vdots \quad \vdots \quad \vdots$$

$$(\boldsymbol{n}-\boldsymbol{1}) + \mathrm{X} \rightleftharpoons \boldsymbol{n} \quad K_{dn} = \frac{[(\boldsymbol{n}-\boldsymbol{1})]\,[\mathrm{X}]}{[\boldsymbol{n}]} \tag{1.n}$$

$$\boldsymbol{0} + n\mathrm{X} \rightleftharpoons \boldsymbol{n} \quad K_d = \frac{[\boldsymbol{0}]\,[\mathrm{X}]^n}{[\boldsymbol{n}]} = \prod_{i=1}^{n} K_{di} \tag{2}$$

Scheme 1

If one assumes that the intermediate complexes [species ***1***–(***n*** − ***1***)] are present in concentrations as low as to make only a negligible contribution to the fluorescence intensity, the total fluorescence signal due to excitation at λ_{ex} and observed at λ_{em} can be expressed by eqn. (3), where $\varepsilon_0(\lambda_{ex})$ and $\varepsilon_n(\lambda_{ex})$ denote the

$$F(\lambda_{ex}, \lambda_{em}, [\mathrm{X}]) = \{a_0(\lambda_{em})\varepsilon_0(\lambda_{ex})[\boldsymbol{0}] + a_n(\lambda_{em})\varepsilon_n(\lambda_{ex})[\boldsymbol{n}]\}\,\psi(\lambda_{ex}, \lambda_{em}) \tag{3}$$

molar extinction coefficients for species ***0*** and ***n***, respectively. ψ is a proportionality factor dependent on the impinging flux of photons at λ_{ex} and on instrumental properties of the fluorimeter.

Assuming that the rate of ion binding in the excited state is negligible, the coefficients $a_0(\lambda_{em})$ and $a_n(\lambda_{em})$ are constant. F [eqn. (3)] can be rewritten as a function of the product of the dissociation constants, $K_d = \prod_{i=1}^{n} K_{di}$, the analytical concentration of the fluorescent indicator, C_A, and the ion concentration, [X] [eqn. (4)]. The extreme values of F([X]) are

$$F([\mathrm{X}]) = \left(\frac{a_0\varepsilon_0 K_d + a_n\varepsilon_n[\mathrm{X}]^n}{K_d + [\mathrm{X}]^n}\right) C_A\psi \tag{4}$$

determined by eqns. (5a) and (5b), where F_{min} corresponds to

$$F_{min} = F([\mathrm{X}]\rightarrow 0) = a_0\varepsilon_0 C_A\psi \tag{5a}$$

$$F_{max} = F([\mathrm{X}]\rightarrow \infty) = a_n\varepsilon_n C_A\psi \tag{5b}$$

the fluorescent signal of the free form of the indicator and F_{max} is the fluorescence signal of the saturated form of the indicator. With these values, eqn. (4) can be rearranged to give eqn. (6).

$$F = \frac{[\mathrm{X}]^n F_{max} + K_d F_{min}}{K_d + [\mathrm{X}]^n} \tag{6}$$

Eqn. (6) can be linearised in the form of a Hill plot [eqn. (7)].

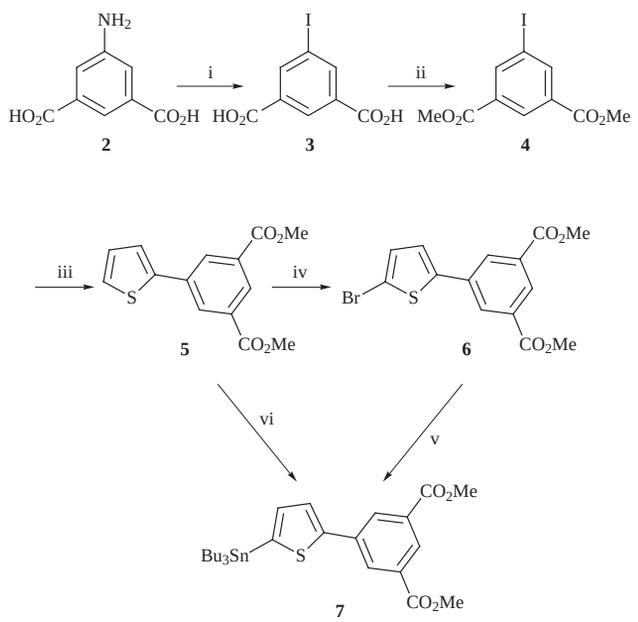
$$\log\frac{F - F_{min}}{F_{max} - F} = n\log[\mathrm{X}] - \log K_d \tag{7}$$

To determine K_d and n *via* a Hill plot, the expression on the left hand side of eqn. (7) is plotted as a function of log [X]. From the slope of this linear graph, a value for n can be derived, while the intersection with the abscissa corresponds to (log K_d)/n. In some cases, it is difficult to obtain reliable values for the extreme values F_{min} and F_{max} from the experimental fluorescence titration. Therefore, fitting eqn. (6) to the fluorescence data F may yield values for F_{min}, F_{max}, K_d and n.

Results

Synthesis

The synthesis of both indicators **1a** and **1b** was carried out following a convergent route (Scheme 2); in addition, for **1b**, a non-convergent reaction path (Scheme 4) was developed. In both approaches, the main difficulty was to find an appropriate method for the coupling of highly substituted (hetero)aromatics. Thanks to important advances in the field of organotransition metal chemistry,[17] several methods for carbon–carbon coupling have been developed, among which palladium-catalysed organotin reactions are the most general and powerful ones.



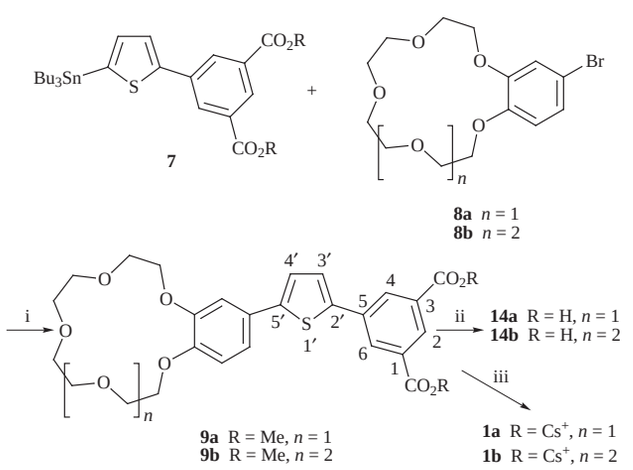
Scheme 2 *Reagents and conditions*: i: (a) 1 equiv. $NaNO_2$, HCl, 0 °C, (b) 3.3 equiv. KI; ii: (a) $SOCl_2$, reflux, (b) MeOH; iii: 2-(tributylstannyl)thiophene, 2 mol% $(MeCN)_2PdCl_2$, DMF, air, rt; iv: Br_2, acetic acid, reflux; v: 1.5 equiv. bis(tributyltin), 1 mol% $Pd(PPh_3)_4$, toluene, reflux; vi: (a) 2,2,6,6-tetramethylpiperidine, Bu^{n}Li, THF, 0 °C, (b) **5**, tributyltin chloride, THF, 2 h at −78 °C, rt overnight

In the convergent route, the fluorophore synthon **7** was synthesised independently (Scheme 2), after which it was attached to the respective ionophores in a palladium-catalysed cross-coupling reaction (Scheme 3). In the non-convergent route (Scheme 4), the indicator **9b** was gradually built up. The key steps remained the carbon–carbon couplings, but they were performed at different stages in the reaction sequence.

Convergent route. *Design of the fluorophore.*—The fluorophore building block dimethyl 5-(5-tributylstannyl-2-thienyl)-isophthalate **7** (Scheme 2) was synthesised starting from 5-aminoisophthalic acid **2**, which was converted into 5-iodoisophthalic acid **3** by diazotization,[18] followed by a Sandmeyer-type reaction.[19] The corresponding ester **4**[20] was then coupled at room temperature to 2-(tributylstannyl)thiophene in a cross-coupling reaction under air in DMF, catalysed by 2 mol% of the ligandless catalyst bis(acetonitrile)dichloropalladium(II)[21] to give compound **5** in high yields. The conversion of dimethyl 5-(2-thienyl)isophthalate **5** to its stannyl derivative **7** was achieved *via* two different routes.

In a first attempt, compound **5** was brominated[22] and subsequently converted into its stannyl analogue **7** by overnight reflux in anhydrous toluene under argon in the presence of 1.5 equiv. bis(tributyltin) and 1 mol% tetrakis(triphenylphosphine)palladium[23] (step v of Scheme 2). The purification was tedious, however, and, moreover, the yields were very low. Therefore, the tin compound **7** was alternatively prepared from compound **5** *via* a lithium intermediate (step vi of Scheme 2). A sterically hindered base, lithium 2,2,6,6-tetramethylpiperidine,[24] prepared *in situ*, was used to lithiate the 5-position of the thiophene nucleus in compound **5** without reaction on the ester functionalities. Addition at −78 °C of this lithium derivative in anhydrous THF to a mixture of compound **5** and tributyltin chloride gave the desired fluorophore building block **7** in moderate yields.

Palladium-catalysed cross-coupling reaction.—Both bromo substituted crown compounds (**8a** for Na^+ and **8b** for K^+, Scheme 3) are commercially available. The fluorescent indicators Benzos **9a** for sodium and Benzop **9b** for potassium were obtained in good yields by refluxing a mixture of 1.2 equiv. of the fluorophore building block **7** and 1 equiv. of the appropriate crown compound **8a** or **8b** in anhydrous toluene under nitrogen atmosphere in the presence of 1 mol% tetrakis(triphenylphosphine)palladium (Scheme 3). It is noteworthy that when 10 mol% of the catalyst was used, the yield decreased significantly. From analysis of the crude reaction mixture, a significant amount of a side product, originating from the cross-coupling reaction of the tin compound **7** with a phenyl ring of the triphenylphosphine ligand, was detected.



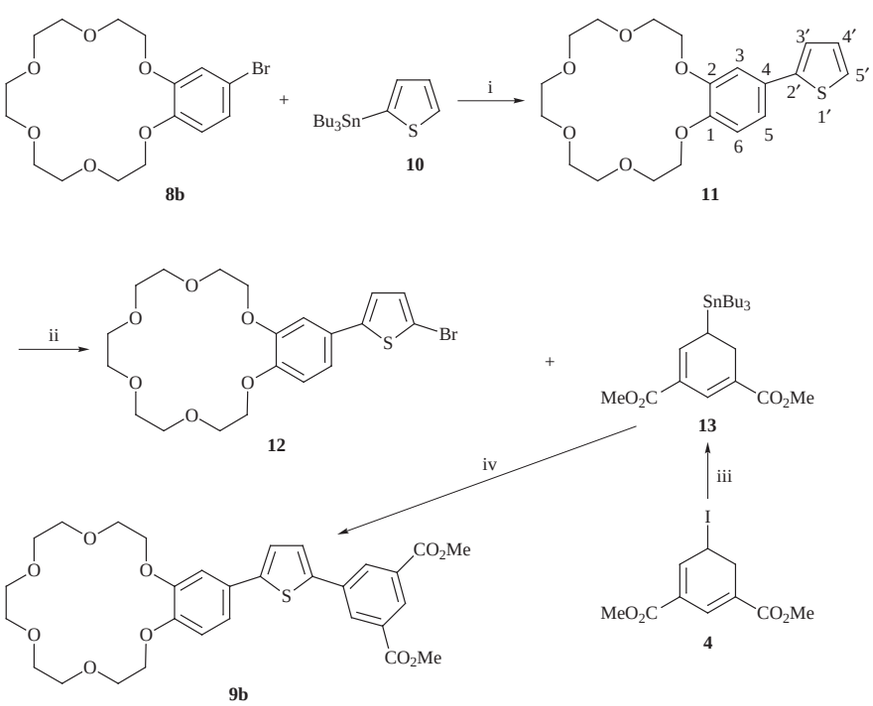
Scheme 3 *Reagents and conditions*: i: 1 mol% $Pd(PPh_3)_4$, toluene, 15 h reflux; ii: $KOSiMe_3$, THF, overnight reflux; iii: 10 equiv. CsOH, MeOH, overnight reflux

Non-convergent route. In the non-convergent approach to synthesis of **9b** (Scheme 4), 2-(tributylstannyl)thiophene **10** was initially attached to the crown compound **8b** in a cross-coupling reaction similar to the one described above. The fluorophore was built up gradually from compound **11**. The thiophene nucleus was brominated in the 2-position, as was done for compound **6** (Scheme 2). The indicator **9b** was then obtained alternatively by the palladium-catalysed coupling of the synthons **12** and **13**. The latter was generated by reaction of dimethyl 5-iodoisophthalate **4** with bis(tributyltin) in conditions similar to those described for the preparation of **7** from the brominated precursor.

Conversion to the water soluble form. The final reaction step comprises the conversion of the esters **9a**,**b** into the corresponding water soluble forms, because the measurements are to be performed in aqueous medium. The free acids **14a**,**b** were obtained by refluxing compounds **9a**,**b** overnight in dry THF with 2.5 equiv. potassium trimethylsilanolate.[25] Since the free acids **14a**,**b** showed a limited solubility in aqueous solution at physiological pH (7.05), we preferred to use the method of Minta and Tsien[26] to prepare directly their caesium salts. The methyl esters **9a**,**b** were converted into the corresponding caesium salts by reflux in methanol with a large excess of caesium hydroxide and were used as such for the fluorescence measurements. Comparison of the UV spectra of the esters in methanol and the caesium salts in water indicates that the fluorophore structure remains the same.

Steady-state fluorescence

Steady-state fluorescence and absorption measurements were performed on the caesium salts of the indicators in an aqueous medium at 20 °C and pH 7.05. The indicator concentrations



Scheme 4 *Reagents and conditions*: i: 10 mol% $Pd(PPh_3)_4$, toluene, 15 h reflux; ii: Br_2, acetic acid, reflux; iii: 1.5 equiv. bis(tributyltin), 1 mol% $Pd(PPh_3)_4$, toluene, reflux; iv: 1 mol% $Pd(PPh_3)_4$, toluene, 15 h reflux

Table 1 Binding properties of Benzos with the respective cations determined from fluorimetric Hill plots [eqn. (7)]. The dissociation constants were measured with 5 μM of the indicator and 10^{-2} M MOPS at a pH 7.05 buffered by (5–6) × 10^{-3} M tetramethylammonium hydroxide. The fluorescence emission spectra were measured as a function of the cation concentration. No correction was made for the change in ionic strength of the solution.

Ion	log K_d	K_d/mM	K_s^a/M^{-1}	n stoichiometry	φ_F relative to the free form	Saturation value/M
Li^+	−1.5 ± 0.3	32.8	30.5	1.1 ± 0.1	Decrease	0.5
Na^+	−1.7 ± 0.1	18.9	52.9	1.11 ± 0.05	Increase	0.1
K^+	−1.8 ± 0.2	17.8	56.2	1.3 ± 0.1	Increase	0.1
Cs^+	−2.06 ± 0.07	8.7	114.9	0.80 ± 0.02	Decrease	0.04
Mg^{2+}	−2.1 ± 0.2	7.6	131.6	1.04 ± 0.07	Decrease	0.05
Ca^{2+}	−1.9 ± 0.2	12.9	77.5	1.10 ± 0.09	Decrease	0.08

[a] K_s (= K_d^{-1}) represents the stability constant of the ion : indicator complex.

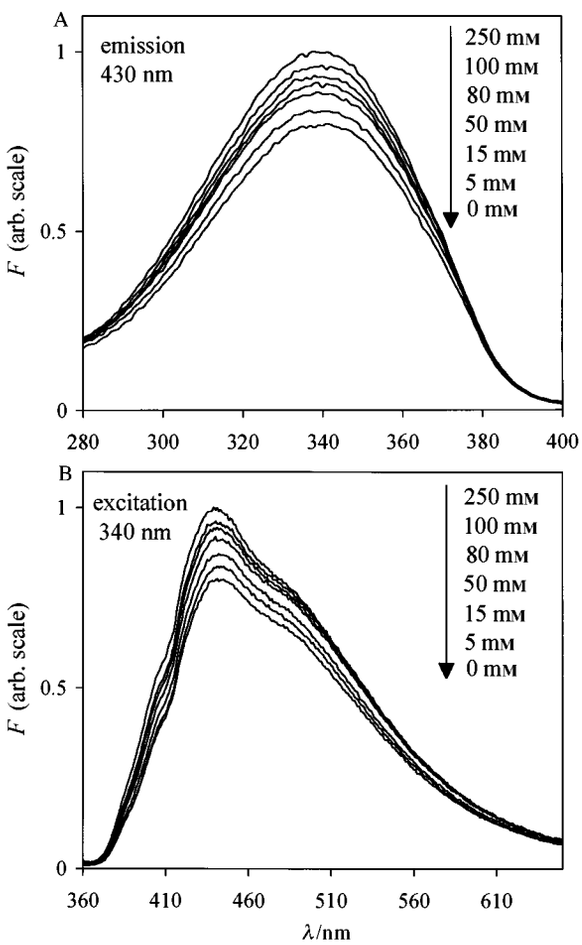


Fig. 1 Fluorescence excitation (A) and emission (B) spectra of Benzos as a function of [Na^+] at 20 °C and pH 7.05

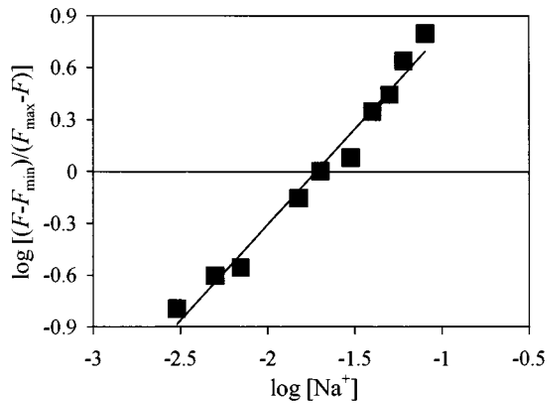


Fig. 2 Hill plot for the complex formation between Na^+ and Benzos calculated from fluorescence emission spectra recorded for an extended series of the solutions used in Fig. 1

were of the order of 5 × 10^{-6} M, yielding an absorbance per cm path length of approximately 0.1 at the absorption maximum. A physiological pH was obtained by buffering the separate solutions with 10^{-2} M MOPS (3-morpholinopropanesulfonic acid), adjusted with tetramethylammonium hydroxide.

Benzos. The absorption spectra of Benzos at various concentrations of the different cations show no pronounced change in absorbance. The absorption maximum is situated at 340 nm and the molar extinction coefficients at 340 nm of the free and the Na^+ saturated forms of the indicator are (28.3 ± 0.5) × 10^3 M^{-1} cm^{-1} and (29.1 ± 0.4) × 10^3 M^{-1} cm^{-1}, respectively.

The fluorescence excitation and emission spectra of Benzos with Na^+ are shown in Fig. 1. The excitation maximum is situated at 340 nm and the emission maximum at 430 nm. An increase in the fluorescence intensity is observed upon increasing the concentration of Na^+, while the position of the maxima remains constant. The indicator is saturated at 100 mM Na^+, where the fluorescence intensity reaches a plateau value. At sodium concentrations above 250 mM, a second slight increase in the fluorescence intensity occurs, which might be attributed to an excited-state reaction.[16]

The ground-state dissociation constant, K_d, of the Na^+ : indicator complex was determined from a Hill plot using fluorescence measurements at λ_{ex} = 340 nm, λ_{em} = 430 nm, at 20 °C and pH 7.05. The Hill plot for the binding of Na^+ by Benzos, which is given in Fig. 2, yields a value of 18.9 mM for K_d and a slope of 1.11 ± 0.05, indicating a 1 : 1 stoichiometry for the Na^+ : indicator complex.

The fluorescence quantum yield, φ_F, is 0.009 for the free form and 0.012 for the Na^+ bound form of Benzos.

The selectivity of Benzos *vs.* other cations, like K^+, Li^+, Cs^+, Mg^{2+} and Ca^{2+}, can be calculated from the ratio of their respective K_d values (Table 1). From these values, it is clear that the indicator shows a non-selective behaviour towards all monovalent cations. Upon binding of Mg^{2+} and Ca^{2+}, the indicator shows a particularly strong decrease in fluorescence intensity. The characteristics of Benzos are summarised in Table 1, while Fig. 3 shows the relative fluorescence responses of the indicator towards the respective cations at the same cation concentration ([X] = 30 mM). The ionic strength was not kept constant for all solutions.

Benzop. For Benzop, the measurements were done using the same conditions as for Benzos. No shift in the absorption maximum at 340 nm is observed upon cation binding, nor a pronounced change in absorbance (figure not shown). The molar extinction coefficient at 340 nm for the free form of the indicator, ε_0(340 nm), is (31 ± 1) × 10^3 M^{-1} cm^{-1}, while the correspondng value, ε_n(340 nm), for the K^+ : indicator complex is (29.1 ± 0.6) × 10^3 M^{-1} cm^{-1}.

The fluorescence excitation and emission spectra of Benzop with increasing concentrations of K^+ are represented in Fig. 4. Analogous to Benzos, no wavelength shift occurs upon complex formation, but the spectral response on cation binding of Benzop is more pronounced, as can be seen from Fig. 5, where the relative fluorescence responses of the indicator towards the cations studied are given.

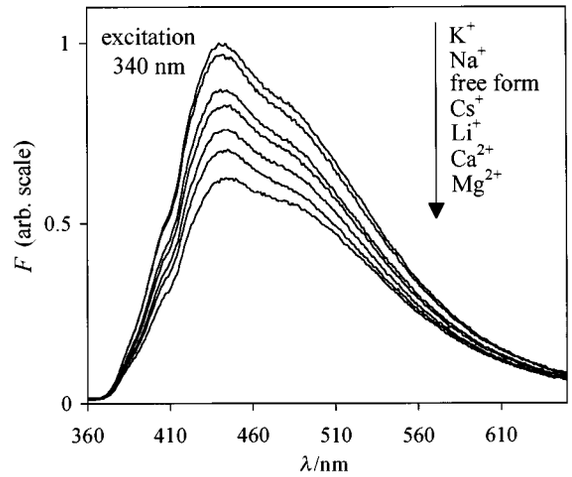


Fig. 3 Plot of the relative fluorescence responses F of Benzos upon binding the respective cations X. All displayed spectra (except the free form) are recorded at [X] = 30 mM. The ionic strength was not kept the same for all solutions. The other experimental conditions are the same as in Fig. 1.

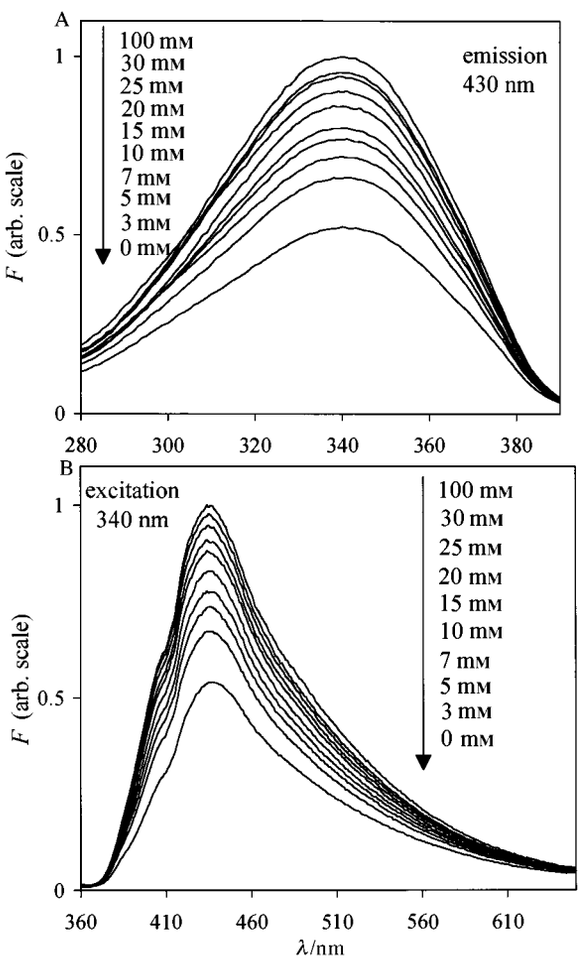


Fig. 4 Fluorescence excitation (A) and emission (B) spectra of Benzop as a function of [K^+]. The experimental conditions are as in Fig. 1.

By means of a Hill plot (Fig. 6), the ground-state dissociation constants, K_d, for the respective ion : indicator complexes were determined. In the absence of Na^+, a K_d value of 5.54 mM for the K^+ : indicator complex is obtained and a slope of 0.96 ± 0.05, indicating a 1 : 1 stoichiometry. The indicator is saturated with K^+ at 25 mM. In solutions with a total Na^+ and K^+ concentration of 135 mM, the K_d value for the K^+ : Benzop complex equals 26.9 mM.

The fluorescence quantum yield, φ_F, of the free form of

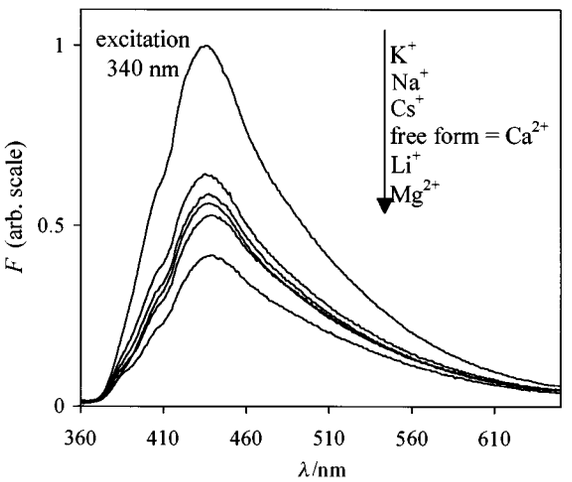


Fig. 5 Plot of the relative fluorescence responses F of Benzop upon binding the respective cations X. All displayed spectra (except the free form) are recorded at [X] = 30 mM. The ionic strength was not kept the same for all solutions. The other experimental conditions are the same as in Fig. 4.

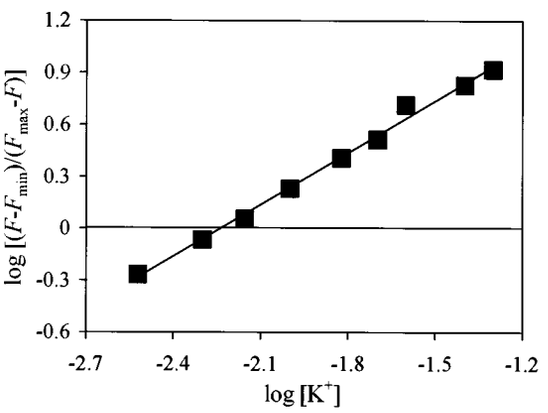


Fig. 6 Hill plot for the complex formation between K^+ and Benzop calculated from fluorescence emission spectra recorded for an extended series of the solutions used in Fig. 4

Benzop is 0.005; for the K^+ saturated form, φ_F equals 0.008. The binding properties of Benzop are summarised in Table 2.

Discussion

Examination of the data obtained for Benzos clearly shows that the indicator can hardly discriminate between Na^+ and the other monovalent cations. The non-selective complexing abilities of the indicator are consistent with earlier findings[27] that the stability constants, K_s, for the complex formation in aqueous solution of Na^+ and K^+ with benzo-15-crown-5 are nearly the same.

Expansion of the crown ether not only increases the K^+ affinity but also slightly decreases the Na^+ affinity. The potassium indicator Benzop shows a selectivity of 3 : 1 for K^+ over Na^+, this is two times better than the selectivity of PBFI for K^+ *vs.* Na^+. The K_d of the K^+ : Benzop complex is strongly dependent on whether Na^+ is present, with a value of 5.54 mM in the absence of Na^+ and 26.9 mM in solutions with [Na^+] + [K^+] = 135 mM, approximating physiological ionic strength. A similar dependence of the K_d value for the K^+ : PBFI complex on the presence of Na^+ was reported:[10] a value of 5.1 mM was found for K_d in Na^+ free solutions, and 44 mM in solutions with combined Na^+ and K^+ concentrations of 135 mM. Since the indicator has an affinity for both Na^+ and K^+, theoretically a competitive binding model should be used in solutions containing Na^+ as well as K^+.

Since both indicators, Benzos and Benzop, only show a

Table 2 Binding properties of Benzop with the respective cations determined from fluorimetric titrations. The experimental conditions were the same as in Table 1. A reliable K_d value for Ca^{2+} : Benzop could not be estimated from fluorescence measurements because no measurable change in intensity was observed as a function of $[Ca^{2+}]$. In the fitting according to eqn. (6), F_{max}, F_{min}, K_d, and n are adjustable parameters.

Ion	log K_d	K_d/mM	K_s[a]/M^{-1}	n stoichiometry	φ_F relative to the free form	Saturation value/ M	Calculated *via* eqn.
Li^+	−1.4 ± 0.1	39.8	25.1	1.03 ± 0.09	Decrease	0.2	(7)
Na^+	−1.7 ± 0.2	18.3	54.6	1.22 ± 0.08	Increase	0.03	(6)
K^+	−2.3 ± 0.1	5.54	180.5	0.96 ± 0.05	Increase	0.025	(6)
Cs^+	−1.9 ± 0.2	13.6	73.5	0.96 ± 0.07	Increase	0.07	(7)
Mg^{2+}	−2.5 ± 0.1	3.01	332.2	1.00 ± 0.04	Decrease	0.014	(6)
$[Na^+] + [K^+] = 0.1$ M	−1.6 ± 0.1	26.9	37.2	1.13 ± 0.04	Increase	0.06	(7)

[a] K_s (= K_d^{-1}) represents the stability constant of the ion : indicator complex.

change in intensity upon cation binding and no corresponding spectral shifts, they do not allow one to perform ratiometric measurements at dual wavelengths. It is not yet understood why the fluorescence intensity of the ion : indicator complex increases for one ion and decreases for another ion relative to that of the ion-free indicator (Tables 1 and 2, Figs. 3 and 5). Benzop and Benzos both have reasonable molar extinction coefficients, but rather low fluorescence quantum yields. While the spectral response for Benzos is only minor (the fluorescence intensity increases by one third between the free form and 10 mM Na^+), a more pronounced response was observed for Benzop in the presence of K^+: the intensity increases by a factor of 2 between 0 and 25 mM K^+.

Experimental

Materials and methods

When required, solvents and reagents were dried prior to use. Tetrahydrofuran (THF) and toluene were distilled over sodium. 4-Bromobenzo-15-crown-5 was purchased from Acros and 4-bromobenzo-18-crown-6 from Aldrich. Both compounds were used without further purification. ^{1}H NMR spectra were recorded on a Bruker WM 250 or a Bruker AMX 400 instrument. The spectra were measured using deuteriochloroform ($CDCl_3$) or deuterated DMSO as solvent, the ^{1}H and ^{13}C chemical shifts are reported in ppm relative to tetramethylsilane or the deuterated solvent as an internal reference and the coupling constants J are given in Hz. Mass spectra were run using a Kratos MS50TC instrument and a DS90 data system. IR spectra were recorded on a Perkin-Elmer 1720 Fourier transform spectrometer. Melting points were determined with a Reichert Thermovar apparatus and are uncorrected.

The absorption measurements were performed on a Perkin-Elmer Lambda 6 UV–VIS spectrophotometer. Corrected steady-state excitation and emission spectra were recorded on a SPEX Fluorolog 212. Fluorescence quantum yields of the free and saturated forms of the indicators were determined using quinine sulfate in 0.05 M sulfuric acid as reference. The quantum yield of the reference was taken to be 0.54.[28] The samples were measured in Milli-Q water. No correction for the refractive index was necessary.

Synthesis

5-Iodoisophthalic acid 3. The diazonium salt of **2** was prepared according to the procedure described by Delzenne and Laridon.[18] For the Sandmeyer-type reaction, the conditions reported by Grahl[19] were followed, yielding 5-iodoisophthalic acid **3** (80%); mp 285–287 °C (lit.,[19] 288–289 °C); ν_{max}(KBr)/cm^{-1} 3000–2700 (OH), 1700 (CO), 1560 (Ar); δ_H([$^2H_6$]DMSO) 8.4 (3 H, s, 2-, 4-, 6-H); δ_C([$^2H_6$]DMSO) 94.65 (CI), 129.07 (2-C), 133.06 (1-, 3-C), 141.37 (4-, 6-CH), 165.18 (CO); *m*/*z* 292 (M^+, 35%), 247 ($M^+ - CO_2H$, 5), 165 ($M^+ - I$, 22), 127 (I^+, 31), 74 ($C_6H_2^+$, 78), 45 (CO_2H^+, 100); Found: 291.9237, Calc. for $C_8H_5IO_4$: 291.9232.

Dimethyl 5-iodoisophthalate 4. 5-Iodoisophthalic acid **3** (24 mmol) was esterified by reflux in thionyl chloride followed by addition of methanol. Purification by column chromatography (SiO_2; $CHCl_3$) yielded **4** as white crystals (54%); mp 102–103 °C (lit.,[20] 100–102 °C); ν_{max}(KBr)/cm^{-1} 3080–3000 (CH_3), 1730 (CO), 1570 (Ar); δ_H($CDCl_3$) 3.9 (6 H, s, CH_3), 8.5 (2 H, d, J 1.5, 4-, 6-H), 8.58 (1 H, t, J 1.5, 2-H); δ_C($CDCl_3$) 52.57 (CH_3), 93.4 (CI), 129.74 (2-C), 132.11 (1-, 3-C), 142.3 (4-, 6-CH), 164.8 (CO); *m*/*z* 320 (M^+, 100%), 289 ($M^+ - CH_3O$, 14), 277 ($M^+ - CO_2$, 16); Found: 319.9543, Calc. for $C_{10}H_9IO_4$: 391.9545.

Dimethyl 5-(2-thienyl)isophthalate 5. Compound **5** was prepared from compound **4** according to the procedure described by Bumagin and Bumagina.[21] To a mixture of compound **4** (5 g, 15.6 mmol) and 2-(tributylstannyl)thiophene (5.77 g, 15.6 mmol) in anhydrous DMF (50 cm^3), $(MeCN)_2PdCl_2$ (81 mg, 0.31 mmol, 2%) was added. After stirring overnight at room temperature, water (300 cm^3) was added to the reaction mixture, which was then extracted with chloroform (4 × 100 cm^3). The combined organic layers were washed with water (100 cm^3), a saturated potassium fluoride solution and again with water, and then dried over $MgSO_4$. After evaporation, the residue was purified by column chromatography (SiO_2; diethyl ether–hexane, 1 : 1). A white powder was obtained (3.87 g, 90%); mp 133 °C; ν_{max}(KBr)/cm^{-1} 2955 (CH_3, CH), 1720 (CO), 1560 (Ar); δ_H($CDCl_3$) 3.95 (6 H, s, CH_3), 7.11 (1 H, dd, J 3.65 and J 5.1, 4′-H), 7.34 (1 H, dd, J 1.1 and J 5.1, 5′-H), 7.43 (1 H, dd, J 1.1 and J 3.65, 3′-H), 8.42 (2 H, d, J 1.5, 4-, 6-H), 8.55 (1 H, t, J 1.5, 2-H), δ_C($CDCl_3$) 52.4 (CH_3), 124.48 (3′-C), 126.09 (5′-C), 128.28 (4′-C), 129.13 (2-C), 130.66 (4-, 6-C), 131.3 (1-, 3-C), 135.26 (5-C), 142 (2′-C), 165.93 (CO); *m*/*z* 276 (M^+, 100%), 245 ($M^+ - CH_3O$, 87), 217 ($M^+ - CO_2Me$, 44), 202 [$M^+ - C(OMe)_2$, 51]; Found: 276.0458, Calc. for $C_{14}H_{12}O_4S$: 276.0456.

Dimethyl 5-(5-bromo-2-thienyl)isophthalate 6. Compound **6** was prepared from compound **5** according to the procedure proposed by Gjøs and Gronowitz.[22] To a stirred solution of **5** (2.6 g, 9.41 mmol) in acetic acid (18 cm^3) was added drop-wise a solution of bromine (1.5 g, 9.39 mmol) in acetic acid (15 cm^3). After complete addition, the mixture was refluxed for 5 h, cooled down to room temperature and then poured into water (80 cm^3). After standing overnight, a precipitate was obtained which was purified by column chromatography (SiO_2; diethyl ether–hexane, 2 : 1). A white powder was obtained (1.93 g, 58%); mp 158–161 °C; ν_{max}(KBr)/cm^{-1} 2950 (CH_3, CH), 1720 (CO), 1560 (Ar), 750 (CBr); δ_H($CDCl_3$) 3.97 (6 H, s, CH_3), 7.06 and 7.19 (2 H, 2 × d, J 3.93, 3′- and 4′-H), 8.32 (2 H, d, J 1.5, 4-, 6-H), 8.56 (1 H, t, J 1.5, 2-H); δ_C($CDCl_3$) 52.55 (CH_3), 113.01 (CBr), 124.69 (3′-C), 129.49 (2-C), 130.7 (4-, 6-C), 131.15 (4′-C), 131.6 (1-, 3-C), 134.41 (5-C), 143.37 (2′-C), 165.79 (CO); *m*/*z* 354 (M^+, 100%), 323 ($M^+ - CH_3O$, 47), 295 ($M^+ - CO_2Me$, 15); Found: 353.9564, Calc. for $C_{14}H_{11}BrO_4S$: 353.9561.

Dimethyl 5-(5-tributylstannyl-2-thienyl)isophthalate 7. *Method A.*—In a three-necked flask equipped with a septum and a stopcock, 2,2,6,6-tetramethylpiperidine (3.36 g, 24 mmol) was dissolved in anhydrous THF (50 cm^3). After cooling to 0 °C, a solution of *n*-butyllithium (15 cm^3, 24 mmol) was added *via* a syringe. The slightly yellow mixture was stirred at room

temperature under argon for 1 h. This solution, containing the lithiated anion, was then added dropwise to a mixture of the thiophene **5** (5.52 g, 20 mmol) in THF (120 cm^3) and tributyltin chloride (7.82 g, 24 mmol) at −78 °C. The mixture was stirred for 2 h at −78 °C and then overnight at room temperature. Subsequently, it was poured into glacial brine (200 cm^3) and after addition of diethyl ether (100 cm^3) was stirred for 15 min at room temperature. The aqueous phase was extracted with diethyl ether, whereupon the combined organic layers were washed with brine. After evaporation, the crude product was purified by column chromatography (Al_2O_3; hexane–AcOEt, 98 : 2), which yielded a reddish oil (4.66 g, 41%).

Method B.—The procedure proposed here is based on the method described by Azizian *et al.*[23] To a solution of bis-(tributyltin) (2.26 g, 3.9 mmol, 1.3 equiv.) in anhydrous toluene (10 cm^3) under argon, the brominated compound **6** (1.065 g, 3 mmol) and $Pd(PPh_3)_4$ (33.45 mg, 1 mol%) were added. After refluxing for 48 h, the mixture was filtered and the filtrate was evaporated. The yellow residue was purified by column chromatography (Al_2O_3; hexane–EtOAc, 98 : 2); a colourless oil was obtained (370 mg, 21%); $\nu_{max}(CCl_4)/cm^{-1}$ 2960, 2930, 2875 (CH_3, CH_2, CH), 1730 (CO), 1600 (Ar); $\delta_H(CDCl_3)$ 0.9–1.13 (9 H, m, CH_3) 1.16–1.26 (6 H, m, CH_2), 1.3–1.42 (6 H, m, CH_2), 1.59–1.76 (6 H, m, CH_2), 4 (6 H, s, CH_3), 7.2 and 7.6 (2 H, 2 × d, *J* 3.3, 3′- and 4′-H), 8.46 (2 H, s, 4-, 6-H), 8.53 (1 H, s, 2-H); $\delta_C(CDCl_3)$ 10.92 (CH_2Sn), 13.62 (CH_3), 27.23 (CH_2), 28.94 (CH_2), 52.36 (CH_3), 125.6 (3′-C), 128.77 (4′-C), 130.66 (4-, 6-C), 131.22 (5′-C), 135.48 (1-, 3-C), 136.61 (2-C), 136.8 (5-C), 147.54 (2′-C), 166.08 (CO); *m*/*z* 566 (M^+, 20%), 509 (M^+ − butyl, 39), 290 [$Sn(Bu)_3^+$, 69], 276 [M^+ − $Sn(Bu)_3$, 60], 57 ($butyl^+$, 100); Found: 566.1507, Calc. for $C_{26}H_{38}O_4SSn$: 566.1512.

Dimethyl 5-[5-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-2-thienyl]isophthalate 9a. To a solution of compound **7** (745 mg, 1.32 mmol, 1.2 equiv.) in anhydrous toluene (20 cm^3), 4-bromobenzo-15-crown-5 **8a** (382 mg, 1.1 mmol) and $Pd(PPh_3)_4$ (13 mg, 1%) were added. The mixture was refluxed for 15 h under nitrogen. After evaporation, the yellow residue was purified by column chromatography (Al_2O_3; $CHCl_3$–hexane, 70 : 30, then $CHCl_3$–MeOH, 98 : 2). A yellow powder was obtained which was recrystallised from hot CH_2Cl_2 followed by addition of cold hexane (490 mg, 82%); mp 145–146 °C; ν_{max}(KBr)/cm^{-1} 2950–2870 (CH_3, CH_2, CH), 1730 (CO), 1600, 1560, 1510 (Ar), 1150 (C-O); $\delta_H(CDCl_3)$ (Scheme 3) 3.62 (8 H, s, CH_2), 3.77–3.79 (4 H, m, CH_2), 3.91 (6 H, s, CH_3), 4.06–4.16 (4 H, m, CH_2OAr), 6.97 (1 H, d, *J* 8.25, Ar-H), 7.21 (1 H, dd, *J* 1.95 and *J* 8.25, Ar-H), 7.26 (1 H, d, *J* 1.95, Ar-H), 7.48 and 7.69 (2 H, 2 × d, *J* 3.8, 3′- and 4′-H), 8.3 (2 H, d, *J* 1.45, 4-, 6-H), 8.33 (1 H, t, *J* 1.45, 2-H); $\delta_C(CDCl_3)$ 52.44 (CH_3), 71.13, 70.53, 69.58, 69.32, 69.12 (CH_2O), 112.02 and 114.26 (Ar-CH), 119.01 (3′-C), 123.33 (4′-C), 125.28 (Ar-CH), 127.44 (Ar-C), 128.87, 130.08, 131.27 (1-, 2-, 3-, 4-, 6-C), 135.18 (5′-C), 140.06 (5-C), 145.06 (2′-C), 149.36 and 149.4 (ArC-O), 165.93 (CO); *m*/*z* 542 (M^+, 90%), 454 [M^+ − $(CH_2CH_2O)_2$, 21], 410 [M^+ − $(CH_2CH_2O)_3$, 100]; Found: 542.1607, Calc. for $C_{28}H_{30}O_9S$: 542.1610.

Dimethyl 5-[5-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-2-thienyl]isophthalate 9b. *Method A.*—Compound **9b** was prepared from 4-bromobenzo-18-crown-6 **8b** (586 mg, 1.5 mmol) and compound **7** (1.02 g, 1.8 mmol, 1.2 equiv.) as described for **9a**, and isolated as a yellow powder (700 mg, 79%).

Method B.—Following the same procedure, starting from **12** (310 mg, 0.65 mmol), **13** (376 mg, 0.78 mmol, 1.2 equiv.) and $Pd(PPh_3)_4$ (7 mg, 1%) in toluene (10 cm^3), **9b** was obtained in 80% yield (240 mg); mp 166–167 °C; ν_{max}(KBr)/cm^{-1} 2950, 2900, 2860 (CH_3, CH_2, CH), 1730 (CO), 1600, 1560, 1520 (Ar), 1150, 1120 (C-O); $\delta_H(CDCl_3)$ 3.7 (4 H, s, CH_2), 3.72–3.81 (8 H, m, CH_2), 3.93–3.97 (4 H, m, CH_2), 3.98 (6 H, s, CH_3), 4.19–4.25 (4 H, m, CH_2), 6.9 (1 H, d, *J* 8.25, Ar-H), 7.16 (1 H, s, Ar-H), 7.2 (1 H, d, *J* 8.25, Ar-H), 7.22 (1 H, d, *J* 3.8, 4′-H), 7.4 (1 H, d, *J* 3.8, 3′-H), 8.43 (2 H, d, *J* 1.5, 4-, 6-H), 8.55 (1 H, t, *J* 1.5, 2-H); $\delta_C(CDCl_3)$ 52.44 (CH_3), 70.93, 70.83, 69.89, 69.47, 69.32 (CH_2O), 112.32 and 114.55 (Ar-CH), 119.09 (3′-C), 123.35 (4′-C), 125.32 (Ar-CH), 127.54 (Ar-C), 128.93, 130.2, 131.32 (1-, 2-, 3-, 4-, 6-C), 135.25 (5′-C), 140.16 (5-C), 145.11 (2′-C), 149.28 and 149.33 (ArCO), 166 (CO); *m*/*z* 586 (M^+, 57%), 498 [M^+ − $(CH_2CH_2O)_2$, 9], 454 [M^+ − $(CH_2CH_2O)_3$, 6], 410 [M^+ − $(CH_2CH_2O)_4$, 100]; Found: 586.1871, Calc. for $C_{30}H_{34}O_{10}S$: 586.1872.

18-(2-Thienyl)-2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecine 11. To a solution of 2-(tributylstannyl)thiophene (1.21 g, 3.25 mmol, 1.3 equiv.) in anhydrous toluene (15 cm^3), 4-bromobenzo-18-crown-6 (1 g, 2.5 mmol) and $Pd(PPh_3)_4$ (278 mg, 10%) were added. The mixture was refluxed for 15 h under nitrogen and then evaporated. The black residue was purified by column chromatography (Al_2O_3; hexane–CH_2Cl_2, 70 : 30 followed by CH_2Cl_2–MeOH, 99 : 1). The obtained white powder was recrystallised from hot CH_2Cl_2 followed by addition of cold hexane (770 mg, 89%); mp 84–85 °C; ν_{max}(KBr)/cm^{-1} 2880 (CH_2, CH), 1600, 1580, 1500 (Ar), 1120 (C-O); $\delta_H(CDCl_3)$ (Scheme 4) 3.69 (4 H, s, CH_2), 3.72–3.81 (8 H, m, CH_2), 3.92–3.97 (4 H, m, CH_2), 4.17–4.25 (4 H, m, CH_2), 6.9 (1 H, d, *J* 8, Ar-H), 7.05 (1 H, dd, *J* 3.6 and *J* 5, 4′-H), 7.1 (1 H, d, *J* 8, Ar-H), 7.15 (1 H, s, Ar-H), 7.2 (1 H, d, *J* 5, 5′-H), 7.23 (1 H, d, *J* 3.6, 3′-H); $\delta_C(CDCl_3)$ 70.9, 70.82, 69.72, 69.47, 69.35 (CH_2O), 112 and 114.65 (Ar-CH), 119.27 (3′-C), 122.33 (Ar-CH), 124.02 (5′-C), 127.88 (4′-C), 128.18 (Ar-C), 144.37, 148.89, 149.25, (Ar-CO and 2′-C); *m*/*z* 394 (M^+, 100%), 350 [M^+ − (CH_2CH_2O), 7], 306 [M^+ − $(CH_2CH_2O)_2$, 65], 262 [M^+ − $(CH_2CH_2O)_3$, 52], 218 [M^+ − $(CH_2CH_2O)_4$, 65]; Found: 394.1453, Calc. $C_{20}H_{26}O_6S$: 394.1450.

18-(5-Bromo-2-thienyl)-2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecine 12. This compound was prepared as described for compound **6** starting from compound **11** (394 mg, 1 mmol). A yellow oil was obtained (280 mg, 59%); $\nu_{max}(CHCl_3)/cm^{-1}$ 2930–2880 (CH_2, CH), 1600, 1580, 1500 (Ar), 1120 (C-O); $\delta_H(CDCl_3)$ 3.69 (4 H, s, CH_2), 3.7–3.79 (8 H, m, CH_2), 3.9–3.96 (4 H, m, CH_2), 4.13–4.24 (4 H, m, CH_2), 6.44 (1 H, s, Ar-H), 6.68 and 6.73 (2 H, 2 × d, *J* 8.45, Ar-H), 6.88 and 7.36 (2 H, 2 × d, *J* 3.9, 3′-, 4′-H); $\delta_C(CDCl_3)$ 69.22, 69.38, 70.75, 70.87 (CH_2O), 112.16 (Ar-CH), 114.53 (CBr), 117.24 (Ar-CH), 119.39 (3′-C), 127.87 (Ar-CH), 130.66 (Ar-CH), 133.47 (4′-C), 148.14, 149.14, 149.46 (ArC-O and 2′-C); *m*/*z* 472 (M^+, 32%), 384 [M^+ − $(CH_2CH_2O)_2$, 13], 340 [M^+ − $(CH_2CH_2O)_3$, 11], 296 [M^+ − $(CH_2CH_2O)_4$, 13], 176 [$(CH_2CH_2O)_4^+$, 20], 132 [$(CH_2CH_2O)_3^+$, 26], 86 (CH_2CH_2-OCH=CHO^+, 100); Found: 472.0549, Calc. for $C_{20}H_{25}BrO_6S$: 472.0555.

Dimethyl 5-(tributylstannyl)isophthalate 13. Compound **13** was prepared as described in *method B* for compound **7** starting from compound **4** (960 mg, 3 mmol). A colourless oil was obtained (650 mg, 45%); $\nu_{max}(CCl_4)/cm^{-1}$ 2960, 2930, 2870 (CH_2, CH_3), 1730 (CO); $\delta_H(CDCl_3)$ 0.95 (9 H, t, *J* 7.1, CH_3), 1.15–1.21 (6 H, m, CH_2), 1.35–1.47 (6 H, m, CH_2), 1.57–1.69 (6 H, m, CH_2Sn), 4.01 (6 H, s, CH_3), 8.3 (2 H, d, *J* 1.5, 4-, 6-H), 8.6 (1 H, t, *J* 1.5, 2-H); $\delta_C(CDCl_3)$ 9.76 (CH_2Sn), 13.55 (CH_3), 27.22–28.95 (CH_2), 52.16 (CH_3), 129.59 (CH_2), 130.3 (1-, 3-C), 141.44 (4-, 6-CH), 143.4 (CSn), 166.7 (CO); *m*/*z* 484 (M^+, 28%), 426 (M^+ − butyl, 30), 290 ($SnBu_3^+$, 100); Found: 484.1632, Calc. for $C_{22}H_{36}O_4Sn$: 484.1635.

5-[5-(2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-2-thienyl]isophthalic acid 14a. The free acid **14a** was prepared from **9a** following the procedure by Laganis and Chenard[25] (90%); mp 215–217 °C; ν_{max}(KBr)/cm^{-1} 3000 (OH), 2900–2870 (CH_2, CH), 1720 (CO), 1600–1515 (Ar), 1100–1130 (C-O); $\delta_H([^2H_6]DMSO)$ 3.62 (8 H, s, CH_2), 3.76 (4 H, br s, CH_2), 4.06–4.11 (4 H, m, CH_2), 6.94 (1 H, d, *J* 8.35, 10′-H), 7.22 (1 H, d, *J* 8.35, 11′-H), 7.26 (1 H, s, 7′-H), 7.45 and 7.5 (2 H, 2 × d, *J* 3.4, 3′-H and 4′-H), 8.32 (2 H, s, 4-, 6-H), 8.37

(1 H, s, 2-H); δ_C([$^2H_6$]DMSO) 69.87, 69.18, 68.29, 68.14 (CH_2O), 110.74 (7′-C), 113.89 (10′-C), 118.19 (3′-C), 124.24 (4′-C), 125.65 (Ar-CH), 126.57 (6′-C), 128.38, 128.65, 133.8 (1-, 2-, 3-, 4-, 6-C), 133.9 (5′-C), 139.9 (5-C), 143.8, 148.26, 148.53 (ArC-O and 2′-C), 166.87 (CO); *m*/*z* 514 (M^+, 16), 470 [$M^+ - (CH_2CH_2O)$, 12], 382 [$M^+ - (CH_2CH_2O)_3$, 43], 338 [$M^+ - (CH_2CH_2O)_4$, 22], 45 (CO_2H^+, 100); Found: 514.1300, Calc. for $C_{26}H_{26}O_9S$: 514.1297.

5-[5-(2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-2-thienyl]isophthalic acid 14b. The free acid **14b** was prepared from **9b** as described for **14a** (90%); mp 266–267 °C; ν_{max}(KBr)/cm^{-1} 3000 (OH), 2920–2880 (CH_2, CH), 1700 (CO), 1560–1520 (Ar), 1110 (C-O); δ_H([$^2H_6$]DMSO) 3.52 (4 H, s, CH_2), 3.56–3.59 (8 H, m, CH_2), 3.77–3.91 (4 H, m, CH_2), 4.1–4.19 (4 H, m, CH_2), 7.23 (1 H, d, *J* 8.5, Ar-H), 7.26 (1 H, s, Ar-H), 7.49–7.68 (2 H, 2 × d, *J* 3.5, 3′-H and 4′-H), 7.98 (1 H, d, *J* 8.5, Ar-H), 8.36 (3 H, s, 2-, 4-, 6-H); δ_C([$^2H_6$]DMSO) 68.14, 68.2, 69.64, 69.74, 69.84 (CH_2O), 110.2 (Ar-CH), 113.34 (Ar-CH), 118.04 (Ar-CH), 124.3 (4′-C), 126.01 (1-, 3-C), 126.21 (3′-C), 128.46 (2-C), 128.85 (4-, 6-C), 132.8 (Ar-C), 134.31 (5-C), 139.26 (5′-C), 144.06, 148.26, 148.46 (ArC-O and 2′-C), 166.52 (CO); *m*/*z* 558 (M^+, 21), 514 [$M^+ - (CH_2CH_2O)$, 8], 470 [$M^+ - (CH_2CH_2O)_2$, 5], 426 [$M^+ - (CH_2CH_2O)_3$, 5], 382 [$M^+ - (CH_2CH_2O)_4$, 83], 45 (CO_2H^+, 100); Found: 558.1566, Calc. for $C_{28}H_{30}O_{10}S$: 558.1559.

Dicaesium 5-[5-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-2-thienyl]isophthalate 1a. The caesium salt **1a** was prepared from **9a** according to the procedure described by Minta and Tsien.[26] A solution of **9a** (5.4 mg, 1×10^{-5} mol) and anhydrous caesium hydroxide (17 mg, 1×10^{-4} mol, 10 equiv.) in methanol (3 cm^3) was refluxed overnight. After evaporation of the methanol, the product was dissolved in water (100 cm^3) and used as such for the fluorescence measurements.

Dicaesium 5-[5-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-2-thienyl]isophthalate 1b. The caesium salt **1b** was prepared as described for **1a** starting from **9b** (5.9 mg, 1×10^{-5} mol), dissolved in water (100 cm^3) and used as such for the fluorescence measurements.

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