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Synthesis and Characterisation of New Fluorescent Na^+ and K^+ Indicators

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Abstract: The synthesis of new fluorescent indicators for Na^+, Benz-15-5 or Diaz-15-5, and for K^+, Benz-18-6 or Diaz-18-6, respectively, is reported. They consist of a benzocrown or a diazacrown ether linked to a pyrazoline fluorophore substituted with carboxylate groups to ensure water solubility. They were obtained via reaction of an α,β-unsaturated ketone with an aryl hydrazine. The fluorescence intensity of Benz-15-5 is nearly insensitive to Na^+. Benz-18-6 displays a four-fold selectivity for K^+ over Na^+, with a dissociation constant of 3.6 mM for the K^+:indicator complex. Diaz-15-5 and Diaz-18-6 have only a weak affinity for monovalent cations, so that they only can be used for the determination of high Na^+ and K^+ concentrations.

INTRODUCTION

Fluctuations in intracellular cation concentration, like those of Ca^{2+}, K^+, Na^+... have important regulatory functions in many biological processes.[1] Evaluation of the role of these cations requires quantitative measurement of cytosolic free cation concentrations with high spatial and temporal resolution. Because of their inherently interesting properties, fluorescent ion indicators are the ideal tool for monitoring intracellular ion concentrations and their variations. Such indicators consist of a metal chelating group linked to a fluorescent dye which undergoes changes in fluorescence intensity and/or in the wavelength position of the spectra upon cation binding.[2]

In this paper we report on the synthesis and the spectral and cation binding properties in aqueous solution of new Na^+ and K^+ fluorescent indicators Benz-15-5 **1** or Diaz-15-5 **3** and Benz-18-6 **2** or Diaz-18-6 **4** (Figure 1). These indicators consist of a crown or diazacrown ether of appropriate size (benzo- or diaza-15-crown-5 as Na^+ chelator and benzo- or diaza-18-crown-6 as K^+ chelator) linked to a pyrazoline moiety (a fluorophore with potentially useful spectroscopic properties). An aryl substituent with polar carboxylate groups was attached to make them water-soluble and membrane-impermeable. Steady-state fluorescence measurements were used to study their ion binding properties in aqueous solution. The cells can be loaded conveniently with the indicator by incubating them with its membrane-permeable non-polar derivative rather than by microinjection or other membrane disruptive techniques.[3] Therefore, the carboxylates were converted to acetoxymethyl esters, which can diffuse through the plasma membrane of cells.[3]

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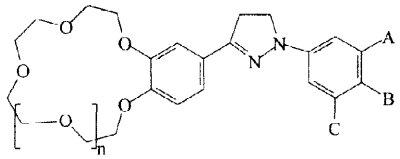
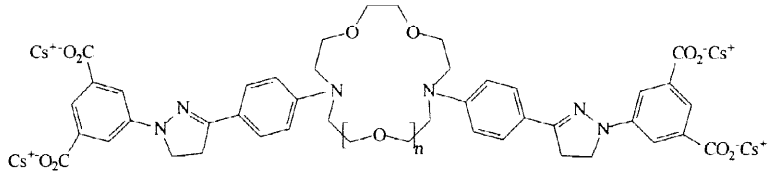


1 n = 1 A = C = H Benz-15-5
B = $CO_2^-Cs^+$
2 n = 2 A = C = $CO_2^-Cs^+$ Benz-18-6
B = H



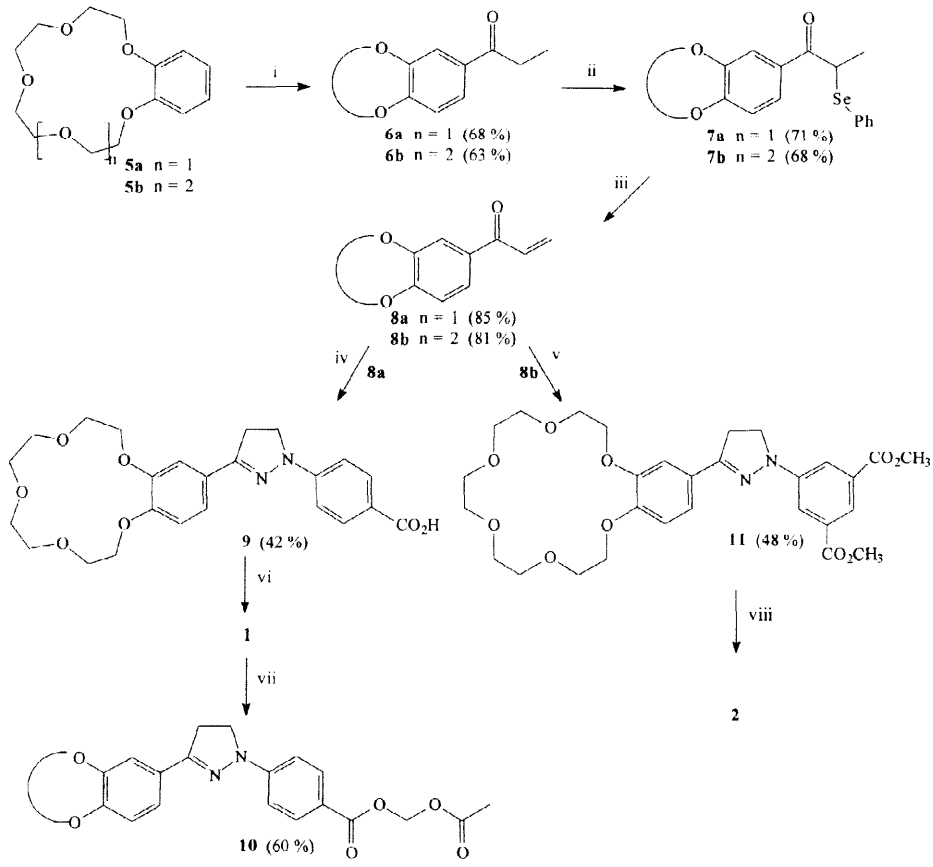
3 n = 1 Diaz-15-5
4 n = 2 Diaz-18-6

Figure 1

RESULTS AND DISCUSSION

Synthesis:

Since the relative size of the cation and the cavity of the crown ether seem to be the most important factors determining complex formation and selectivity, we have chosen benzo-15-crown-5 and benzo-18-crown-6 as complexing agents for Na^+ and K^+, respectively. From these ionophores the pyrazoline fluorophore was gradually built up. It is known from the literature that an α,β-unsaturated carbonyl group on a benzene nucleus is the most suitable substituent to generate the pyrazoline moiety, and therefore we elaborated the synthesis of the α,β-unsaturated ketones **8**. We acylated the 4-position of benzo-15-crown-5 **5a** and benzo-18-crown-6 **5b** with propanoic anhydride/polyphosphoric acid (PPA) in propanoic acid at 60 °C to obtain compounds **6** (Scheme 1).[4] Transformation of ketones **6** to the α-seleno derivatives **7**[5] followed by oxidation with sodium periodate gave the corresponding selenoxides, which underwent a *syn* elimination to yield the α,β-unsaturated ketones.[6] Finally, condensation of α,β-unsaturated ketone **8a** with the commercially available 4-hydrazinobenzoic acid in acetic acid at room temperature provided compound **9**,[7] which was converted into the caesium salt **1** (Benz-15-5) by reflux in methanol in the presence of 10 equivalents caesium hydroxide, according to the method described by Minta and Tsien.[8] After reaction of the caesium salt **1** with an excess acetoxymethyl bromide[9] in dimethylformamide (Scheme 1) the membrane-permeable ester **10** was obtained.[3a,10] Addition of the prepared dimethyl 5-hydrazinoisophthalate to the α,β-unsaturated ketone **8b** in acetic acid at room temperature yielded compound **11**. The required hydrazine was obtained from the corresponding 5-aminoisophthalic acid by conversion of the amine function to a diazonium salt with sodium nitrite under acidic conditions followed by *in situ* reduction with sodium sulphite.[11] The diester **11** was converted into the corresponding caesium salt **2** (Benz-18-6) via the method used for **1**.

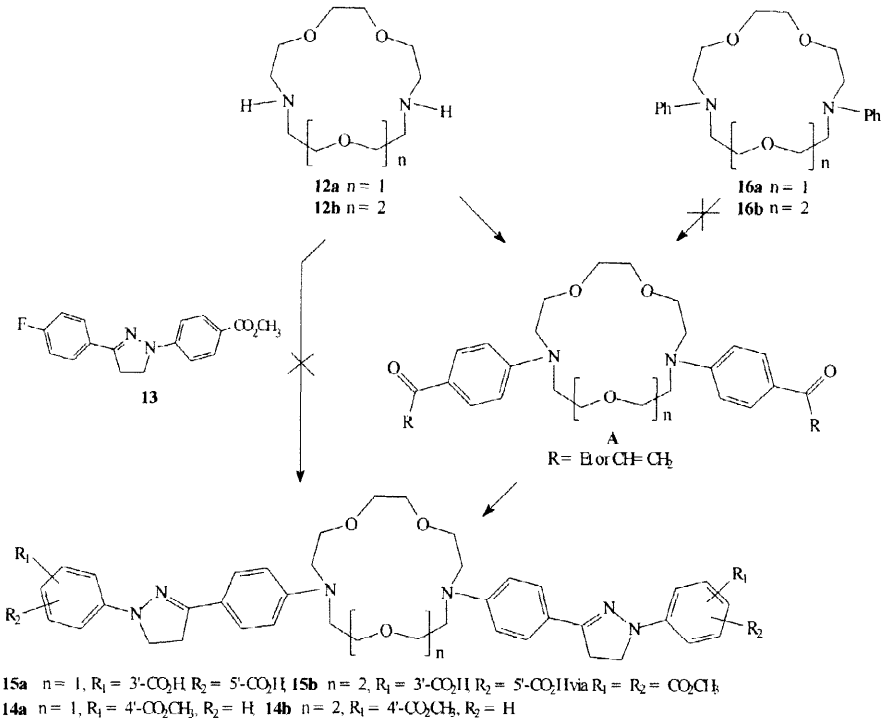


i: propanoic anhydride, PPA, propanoic acid, 60°C, 12 h; ii: 1.2 eq. PhSeCl, EtOAc, rt, 3 d; iii: $NaIO_4$, $NaHCO_3$, MeOH/H_2O, rt, 2 h; iv: 1.2 eq. 4-hydrazinobenzoic acid, acetic acid, rt, 2 d; v: 1.2 eq. dimethyl hydrazinoisophthalate, acetic acid, rt, 8 d; vi: 1 eq. Cs_2CO_3, THF/H_2O; vii: excess $BrCH_2OCOCH_3$, dry DMF, rt, 12 h; viii: 10 eq. CsOH, MeOH, overnight reflux.

Scheme 1

Since the use of aniline type nitrogens to link chelating groups to fluorochromes has proven to be highly successful in the design of K^+ and Na^+ indicators,[12] we used diazacrown ethers (7,13-diaza-1,4,10,-trioxacyclopentadecane for Na^+ and 7,16-diaza-1,4,10,13,-tetraoxacyclooctadecane for K^+) as chelators, with both sp^3-hybridised nitrogens connected to a pyrazoline fluorophore. With these two-armed diazacrown ethers a three-dimensional binding can be achieved with retained flexibility and binding dynamics. In this way, we expected to enhance the cation binding ability as well as the selectivity.[13,14]

At first we tried in several experiments to attach the synthesised fluorophore **13** to the diazacrown ethers **12** (Scheme 2). However, generation of **14** via this method was not successful. Alternatively, the synthesis of α,β-unsaturated ketones of type **A** (Scheme 2) with a diazacrown ether moiety is the key step to yield compounds such as **15**. To obtain the required diketones we explored three different routes. The first and most straightforward method, acylation of 7,16-diphenyldiaza-4,7,13,16-tetraoxacyclooctadecane **16b** with acryloyl chloride or propanoyl chloride failed to give compounds of type **A**.

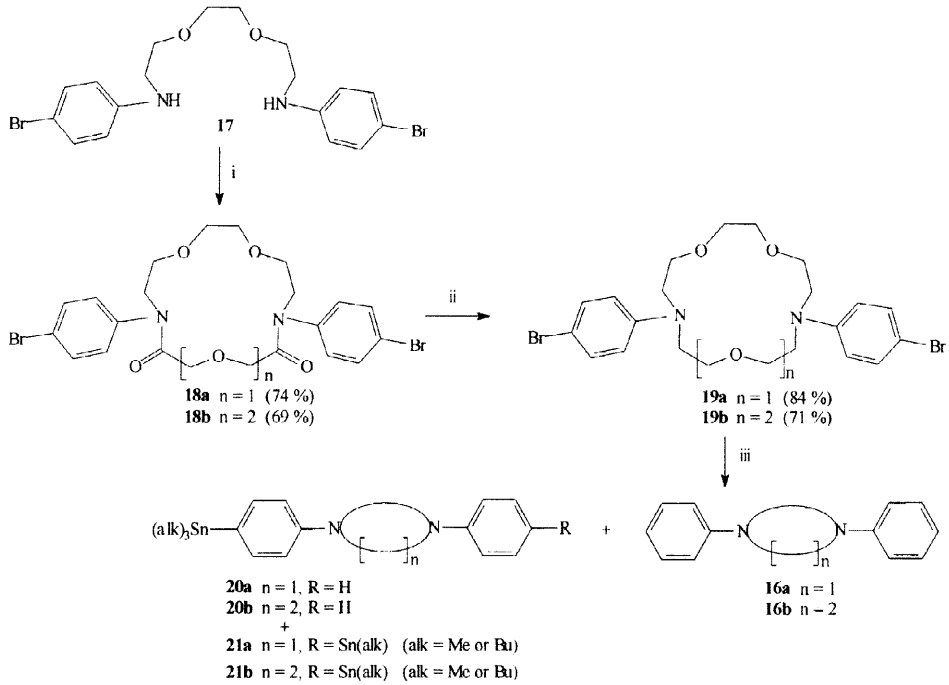


Scheme 2

Then, we tried to introduce an acyl group via a palladium catalysed coupling reaction (Scheme 3) using an acid chloride and the bistrialkyltin (alk = Me or Bu) derivative of the diazacrown ether **19**.[15] The latter was generated by reduction of the amide **18** obtained from the reaction of the diacid dichloride[16] with diamine **17**. Formation of the organotin compound was realised by metallation of the prepared di-*p*-bromophenyldiazacrown ether **19** with *tert*-BuLi, followed by transmetallation with trialkyltinchloride. However, this reaction repeatedly provided a mixture of three products: the diphenyldiazacrown ether **16**, the monotrialkyltin compound **20** and the ditrialkyltin compound **21**, which were too unstable to separate. Treatment of the crude unstable mixture with propanoyl chloride or acryloyl chloride did not give rise to the expected diacylated products.

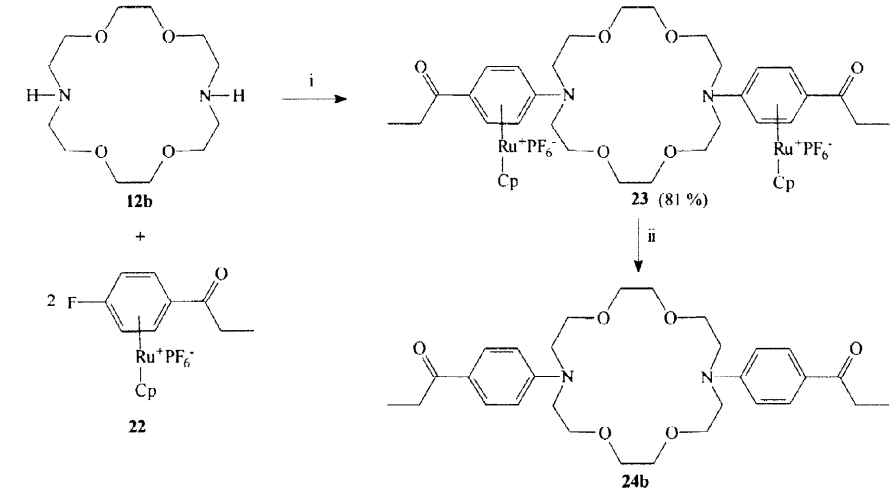
Finally, we studied two possibilities to catalyse the nucleophilic aromatic substitution of the diazacrown ether **12** with *p*-fluoropropiophenone: (a) activation via an arylhalide-RuCp-complex (Scheme 4) and (b) base catalysed substitution. The diketone product should then later be converted to a bis α,β-unsaturated ketone of type **A** (see formula **28**).

a) Formation of **23** was realised by reaction of the synthesised *p*-fluoropropiophenone-RuCp-complex **22**[17] and diazacrown ether **12b** in DMF in the presence of Na_2CO_3 at 50 °C (Scheme 4). Subsequent decomplexation was carried out by irradiation (254 nm; 350 nm) in the presence of suitable donor ligands. The diketone **24b** could be prepared in this way under relatively mild and neutral conditions. However, the yields of this time consuming method were very low. Direct connection of the RuCp-complex of fluorophore **13** with the diazacrown ether could not be realised due to complex formation of the other more electron rich aromatic ring.[18]



i: diglycoldiacid dichloride or triglycoldiacid dichloride, toluene, high dilution conditions;
ii: $Me_2S.BH_3$, THF; iii: a) dry THF, -78 °C, 2.2 eq. *tert*-BuLi, 1 h, b) 2 eq. $(alk)_3SnCl$, -20 °C, 2 h, c) rt, 12 h

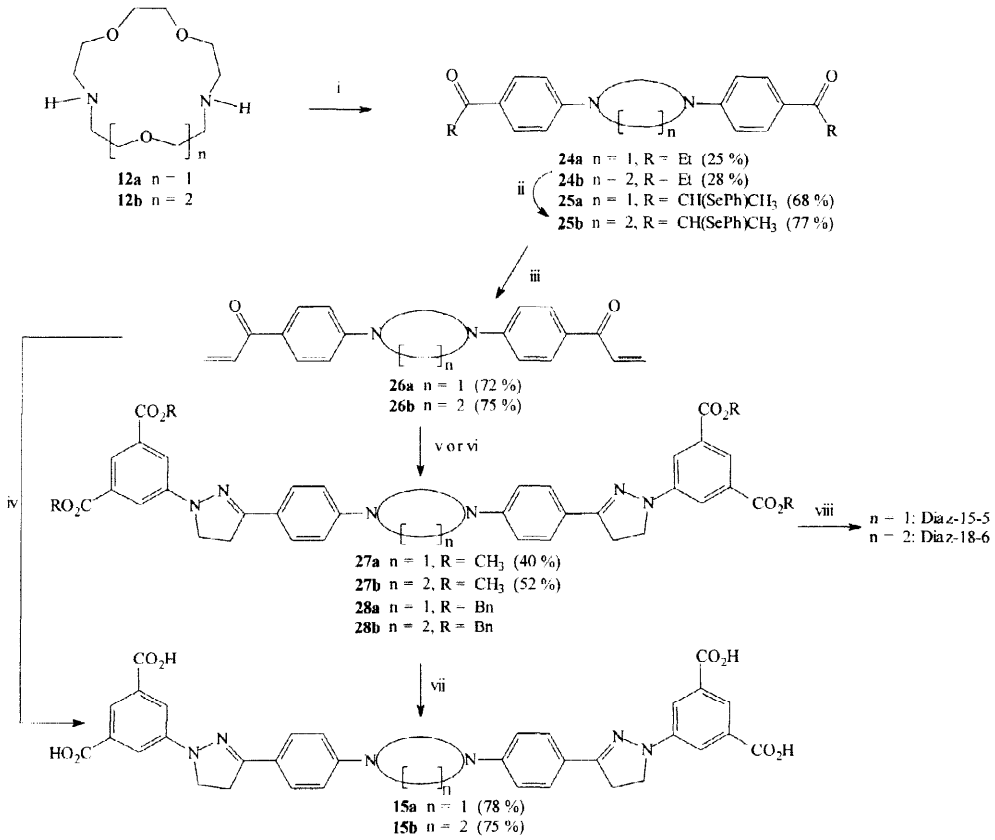
Scheme 3



i: 6 eq. Na_2CO_3, DMF, 50 °C, 20 h; ii: CH_3CN, hv

Scheme 4

b)A more satisfactory route is the introduction of the propiophenone moiety on the diazacrown ether via a base catalysed nucleophilic substitution on *p*-fluoropropiophenone. Many experiments failed, but heating of the crown ether **12** in an excess of *p*-fluoropropiophenone as solvent at 150 °C using 2.5 equivalents $Bu_4N^+F^-.3H_2O$ as base was rather successful (Scheme 5).[19]



i: 2.5 eq. $Bu_4N^+F^-.3H_2O$, excess p-fluoropropiophenone, 150 °C, 12 h; ii: a) LDA (2M, THF/heptane), dry THF, -78 °C, b) 3.7 eq. PhSeCl, 0 °C, 2 h; iii: 4.6 eq. $NaIO_4$, 2.4 eq. $NaHCO_3$, THF, MeOH/H_2O, rt; iv: 2.4 eq. 5-hydrazinoisophthalic acid, acetic acid, rt; v: dimethyl 5-hydrazinoisophthalate or dibenzyl 5-hydrazinoisophthalate **29**, acetic acid, rt, 11 d; vi: 3 eq. dimethyl 5-hydrazinoisophthalate or dibenzyl 5-hydrazinoisophthalate **29**, 3 drops $HCl_{conc.}$, *ter*-BuOH, reflux, 1 h; vii: a) 8 eq. $KOSi(CH_3)_3$ or $NaOSi(CH_3)_3$, dry THF, reflux, 12 h, b) neutralisation with 0.1 N HCl; viii: excess CsOH, MeOH, reflux

Scheme 5

Further, we transformed the acyl group of compounds **24** via selenides **25** to α,β-unsaturated ketones **26**. The ketone enolate, obtained by deprotonation of the ketone using lithium diisopropylamide, was reacted with phenylselenyl chloride (Scheme 5).[6] Subsequent oxidation of **25** with $NaIO_4$ and *in situ* selenoxide elimination gave rise to compounds **26**. Treatment of the α,β-unsaturated ketones **26** with 5-hydrazinoisophthalic acid[7] (step iv of Scheme 5) can lead to water soluble indicators **15** containing four carboxylic groups. The required hydrazine was obtained from the corresponding 5-aminoisophthalic acid by conversion of the amine function to a diazonium salt with sodium nitrite under acidic conditions followed by *in situ* reduction with sodium sulphite.[11] After cyclisation of **26b** with 5-hydrazinoisophthalic acid, formation of the desired compound **15b** was indeed observed. However, due to its high polarity it was not possible to purify and isolate the product. To overcome this problem we tried to prepare the less polar tetramethyl or tetrabenzyl ester that could be purified by column chromatography before hydrolysis or deprotection to the final product. The tetrabenzyl esters **28** seemed to be most interesting because their deprotection by hydrogenation could easily deliver the tetraacid. However, reflux of **26** with the dibenzyl 5-hydrazinoisophthalate in *tert*-BuOH/HCl or CH_3CO_2H gave rather low yields of the corresponding tetrabenzyl esters **28**. The preparation of the tetramethyl esters **27a** and **27b** could be performed by reaction of **26** either with dimethyl 5-hydrazinoisophthalate in acetic acid at room

temperature or by reflux in *tert*-BuOH under acidic conditions. Finally, the purified tetramethyl esters **27** were deprotected by treating them with $KOSiMe_3$ or $NaOSiMe_3$ under inert atmosphere followed by acidic work-up to give the desired compounds **15**.[20] Since the free acids **15a,b** showed limited solubility in aqueous solution at physiological pH (7.0), we preferred to use the method of Minta and Tsien[8] to prepare directly their caesium salts Diaz-15-5 **3** and Diaz-18-6 **4** from the tetramethyl esters **27a,b**.

Spectral properties:

To study the *in vitro* photophysical properties of the indicators, steady-state fluorescence measurements were carried out in aqueous solution at physiological pH (7.0). The indicator concentrations were in the order of 3-8 × 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 3-5 × 10^{-3} M MOPS (3-[N-morpholino]propane sulphonic acid), adjusted with tetramethylammonium hydroxide. In Table 1 the spectroscopic properties of the indicators **1-4** are summarised. The excitation maxima of **1**, **2** and **4** are located in a wavelength region where most tissue autofluorescence will be avoided. The corresponding maximum of **3** is, however, situated at wavelengths too short to completely rule out autofluorescence. Although the fluorescence quantum yields, ϕ_F, of Benz-15-5 in the absence and presence of Na^+ are rather high, the increase in fluorescence intensity upon Na^+ binding is too small to be of practical use. In comparison with Benz-15-5, Benz-18-6 has a rather low fluorescence efficiency in the absence and presence of K^+, but the relative increase is much larger. Figures 2 and 3 depict the fluorescence emission and excitation spectra of Benz-18-6 for K^+ concentrations ranging from zero to 40 mM. Upon binding K^+, a significant increase of the fluorescence intensity is observed without a concomitant change in the position of the maxima. The other indicators **1**, **3** and **4** have similar structureless excitation and emission spectra. The very low fluorescence quantum yields of Diaz-15-5 and Diaz-18-6 limit their applicability.

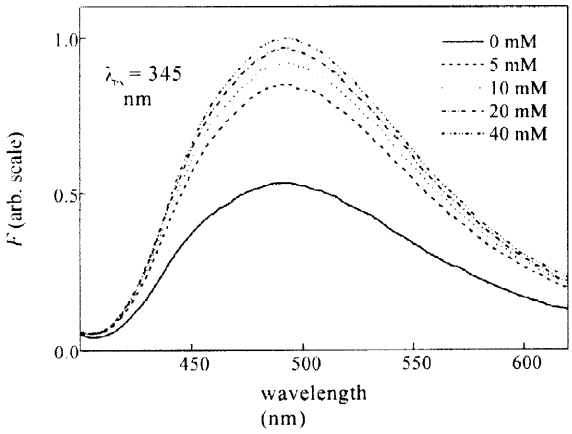


Figure 2: Emission spectra of Benz-18-6 as a function of the indicated [K^+].

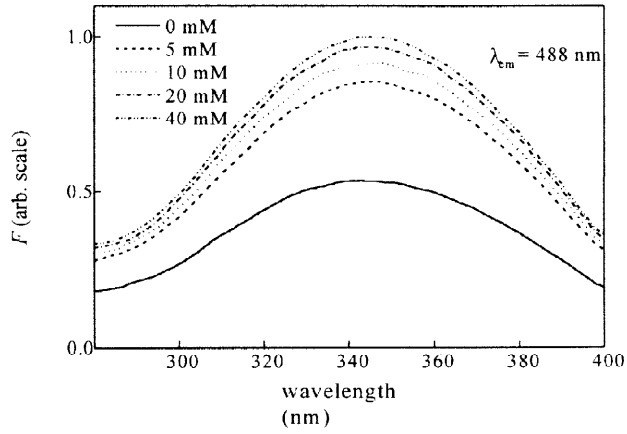


Figure 3: Excitation spectra of Benz-18-6 as a function of the indicated [K^+].

Table 1: Spectroscopic properties of the indicators ***1-4****. For receptors* ***1*** *and* ***3*** *the bound substrate is* Na^+, *whilst for* ***2*** *and* ***4*** *it is* K^+.

Indicator	Absorption λ_{max} (nm)		Emission λ_{max} (nm)		ϕ_F		$\varepsilon_{max} \times 10^{-3}$ (M^{-1} cm^{-1})	
	free	bound	free	bound	free	bound	free	bound
Benz-15-5 **1**	368	368	485	485	0.47	0.52	15.7 ± 0.2	15.9 ± 0.4
Benz-18-6 **2**	345	345	488	488	0.008	0.031	12.5 ± 0.2	12.2 ± 0.2
Diaz-15-5 **3**	325	317	440	430	0.0015	0.0006	39 ± 1	38.7 ± 0.7
Diaz-18-6 **4**	350	350	485	485	0.0019	0.0001	47.3 ± 0.2	46.5 ± 0.5

Only Diaz-15-5 shows a hypsochromic wavelength shift in both the excitation and emission maxima upon ion binding. However, since the shift is rather small, this indicator cannot be used for ratiometric measurements. The fluorescence intensity of Diaz-18-6 decreases with increasing $[K^+]$ and $[Na^+]$, whereas Li^+ causes an increase.

The ground-state dissociation constants, K_d, of the complexes between each fluorescent indicator and various monovalent cations X were determined from fluorimetric titrations as a function of [X]. We assume a consecutive, multiple binding model with stoichiometry n as depicted in Scheme 6.[21]

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

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

$$\vdots \qquad\qquad \vdots \qquad\qquad \vdots$$

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

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

Scheme 6

In this scheme ***0*** denotes the free form of the indicator, while ***n*** (***1***, ***2***, ... , ***n***) corresponds to the indicator bound to n ions. The global reaction (eq 2) is described by the composite dissociation constant K_d. The fluorescence signal F at [X] is given by[22]

$$F = \frac{[X]^n F_{max} + K_d F_{min}}{K_d + [X]^n} \tag{3}$$

where F_{min} is the fluorescence signal of the free form of the indicator and F_{max} corresponds to the fluorescence signal of the fully complexed form (i.e., bound to n ions). Fitting eq 3 to the steady-state fluorescence data F measured as a function of [X] yields values for F_{min}, F_{max}, K_d, and n.

According to this procedure, the dissociation constants of compounds **1-4** with various cations were calculated and are summarised in Table 2.

Table 2: Values of the dissociation constants K_d of ***1-4*** *complexes with various cations determined by fitting eq 3 to the fluorescence data with F_{min}, F_{max}, K_d and n as adjustable parameters.*

Indicator	Cation	log K_d	K_d (mM)	*n*
Benz-15-5	K^+	-2.1 ± 0.2	7.9	1.09 ±0.07
Benz-18-6	Na^+	-1.8 ± 0.3	14.5	1.01 ± 0.1
	K^+	-2.4 ± 0.1	3.6	1.07 ± 0.06
	Li^+	-0.91 ± 0.04	123	1.01 ± 0.06
	Mg^{2+}	-0.78 ± 0.09	166	0.99 ± 0.04
Diaz-15-5	Na^+	-0.84 ± 0.01	140	1.33 ± 0.01
	K^+	-0.6 ± 0.1	270	1.3 ± 0.1
Diaz-18-6	Na^+	-0.15 ± 0.04	700	1.23 ± 0.07
	K^+	-0.40 ± 0.05	400	1.07 ± 0.06
	Li^+	0.065 ± 0.09	1200	1.46 ± 0.02

The K_d of Diaz-15-5:Li^+ and of the Na^+, Li^+ and Mg^{2+} complexes of Benz-15-5 could not be determined by fluorimetric titration because the fluorescence intensity of the respective indicators was insensitive to these ions. While Benz-15-5 is nearly insensitive to changes in ion concentration (except for K^+), Benz-18-6 shows a selective behaviour for K^+ *vs.* Na^+, Li^+ and Mg^{2+}, as can be seen from the ratio of the respective K_d values (Table 2). Benz-18-6 binds K^+ with a dissociation constant of 3.6 mM, which is slightly lower than the K_d of PBFI:K^+.[23] For Diaz-18-6 the K_d values increase in the order $K^+ < Na^+ < Li^+$. A K_d value of 400 mM was found for the Diaz-18-8:K^+ complex, while the affinity of the indicator towards Na^+ is approximately half that for K^+. Table 2 clearly shows that Diaz-15-5 and Diaz-18-6 only have a weak affinity for the respective cations in contrast to the commercially available indicators SBFI and PBFI.[23] Apparently, the pyrazoline fluorophore in Diaz-15-5 and Diaz-18-6 is more electron withdrawing than the benzofuran fluorophores in PBFI and SBFI. As a consequence, the nitrogen lone pairs are less available for complex formation with monovalent cations. The absence of axial donor substituents, such as aromatic ether oxygens as in SBFI and PBFI,[1] may also contribute to the poor cation affinities. Since the dissociation constants for Diaz-15-5:Na^+ and Diaz-18-8:K^+ exceed the respective intracellular [Na^+] (5-30 mM) and [K^+] (90-130 mM) levels, respectively, the indicators cannot be used to determine intracellular [Na^+] and [K^+]. The K_d value for Diaz-15-5:Na^+ lies in the extracellular [Na^+] region (120 mM < K_d < 450 mM).

CONCLUSION

The synthesis of the target indicators **1-4** involves key intermediates **6** or **24**. Acylation of benzo-15-crown-5 or benzo-18-crown-6 directly provided **6**. Research of some methods for synthesising intermediate **24** illustrated that a base catalysed nucleophilic aromatic substitution of *p*-fluoropropiophenone on 7,13-diaza-

1,4,10-trioxacyclopentadecane **12a** or 7,16-diaza-1,4,10,13-tetraoxacyclooctadecane **12b** with tetrabutylammonium fluoride trihydrate as base at 150 °C gives the best results. Condensation of the α,β-unsaturated ketones **8** or **26**, obtained from the (di)ketones **6** or **24** via the (di)selenides **9** or **25**, with an aryl hydrazine gave target compounds **9**, **11** or **27**, **28**. Treatment with CsOH (after deprotection) yielded the indicators **15**. Steady-state fluorescence measurements reveal that despite a large fluorescence quantum yield, Benz-15-5 is nearly insensitive to changes in Na^+ concentration. Benz-18-6 has a rather low fluorescence efficiency in the absence and presence of K^+, but the relative increase is much larger. The dissociation constant of the Benz-18-6:K^+ complex is found to be 3.6 mM, indicating a four-fold selectivity for K^+ over Na^+. Diaz-15-5 and Diaz-18-6 have only a weak affinity for monovalent cations, so that they can only be used for the determination of high Na^+ and K^+ concentrations. Although the selectivity of Diaz-15-5 for Na^+ *vs.* K^+ is sufficient, fluorimetric determinations of [Na^+] with this indicator are of limited usefulness because of the negligible change in fluorescence intensity upon ion binding. Furthermore, the hypsochromic shift is too small to allow reliable ratiometric measurements. In contrast to Diaz-15-5, Diaz-18-6 shows a more significant intensity change upon cation binding. Both indicators have reasonable molar extinction coefficients, but very low fluorescence quantum yields, which limits their applicability.

EXPERIMENTAL SECTION

IR spectra were recorded as solids in KBr pellets on a Perkin Elmer 297 grating IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker WM 250 or a Bruker AMX 400 instrument. The ^{1}H and ^{13}C chemical shifts are reported in ppm relative to tetramethyl silane as an internal reference. *J* values are recorded in Hz. Mass spectra were run on a Kratos MS50 instrument and a DS90 data system. Exact mass measurements were performed at a resolution of 10000. Analytical thin layer chromatography was performed using plates with silica (Alugram Sil G/UV_{254} of Macherey-Nagel) or alumina (Fluka). Column chromatography was carried out using 70-230 mesh silica gel 60 (Macherey-Nagel) or neutral aluminum oxide (Riedel-de Haën). Absorption spectra were recorded at room temperature on a Perkin Elmer Lambda 6 UV/VIS spectrophotometer. Fluorescence spectra were taken at room temperature on a SPEX Fluorolog model 1691 and were fully corrected. Fluorescence quantum yields of the free and saturated forms of the indicators were determined using quinine sulphate in 0.05 M sulphuric acid as reference. The quantum yield of the reference was taken to be 0.54.[24] The samples were measured in Milli-Q water. No correction for the refractive index was necessary.

Acylation of the benzocrown ethers 5a-5b: Generation of ketones 6a-6b

A mixture of benzo-15-crown-5 **5a** (10 mmol) or benzo-18-crown-6 **5b** (6.4 mmol), propanoic anhydride (6.8 ml or 4.3 ml), polyphosphoric acid (PPA, 15 ml) in propanoic acid (130 ml or 80 ml) was stirred for 12 hours at 60 °C. The mixture was allowed to cool to room temperature, subsequently poured into water (200 ml) and extracted with chloroform (3 × 100 ml). The combined organic layers were washed with a saturated sodium bicarbonate solution until carbon dioxide evolution had ceased, subsequently with water (100 ml), and dried over $MgSO_4$. After evaporation of the solvent the yellow-brown oil was extracted with hot petroleum ether (40-60°C) (4 × 200 ml). The extraction residue after evaporation was purified by column chromatography

(Al_2O_3 with EtOAc/$CHCl_3$: 80/20) and recrystallised from hexane or EtOAc/hexane.

1-(2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-1-propanone (6a)

yield: 68 % as white needles; mp: 86 - 87.5 °C (hexane); IR (KBr) cm^{-1}: 2919 (m) and 2860 (m), 1673 (s), 1455 (m), 1129 (s); ^1H NMR ($CDCl_3$) δ: 7.56 (dd, ^oJ = 8 Hz, ^mJ = 2 Hz, 1H, ArH-16), 7.51 (d, ^mJ = 2 Hz, 1H, ArH-14), 6.84 (d, ^oJ = 8 Hz, 1H, ArH-17), 4.18 - 3.77 (m, 16H, -OCH_2CH_2O-), 2.94 (q, ^3J = 5 Hz, 2H, -$CO\underline{CH_2}CH_3$), 1.20 (t, ^3J = 5 Hz, 3H, -$COCH_2\underline{CH_3}$); ^{13}C NMR ($CDCl_3$) δ: 199.5 (CO), 153.3 and 148.9 (ArC-17a and ArC-13a), 130.4 (ArC-15), 122.8 (ArC-16), 112.9 (ArC-14), 111.9 (ArC-17), 71.2 - 68.7 (-OCH_2CH_2O-), 31.3 (C-2), 8.5 (C-3); MS [m/z (%)]: 324 (6) M^+, 207 (6), 163 (100), 135 (11), 107 (9), 57 (27); HRMS: calcd for $C_{17}H_{24}O_6$: 324.1573; found: 324.1578

1-(2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-1-propanone (6b)

yield: 63 % as white needles; mp: 72.5 - 73.6 °C (EtOAc/hexane); IR (KBr) cm^{-1}: 2930 (m) and 2870 (m), 1668 (s), 1456 (m), 1120 (s); ^1H NMR ($CDCl_3$) δ: 7.57 (dd, ^oJ = 8 Hz, ^mJ = 2 Hz, 1H, ArH-19), 7.53 (d, ^mJ = 2 Hz, 1H, ArH-17), 6.87 (d, ^oJ = 8 Hz, 1H, ArH-20), 4.23 - 3.69 (m, 20H, -OCH_2CH_2O-), 2.94 (q, ^3J = 5 Hz, 2H, -$CO\underline{CH_2}CH_3$), 1.21 (t, ^3J = 5 Hz, 3H, -$COCH_2\underline{CH_3}$); ^{13}C NMR ($CDCl_3$) δ: 199.4 (CO), 153.2 (ArC-20a), 148.7 (ArC-16a), 130.3 (ArC-18), 122.8 (ArC-19), 113.0 (ArC-17), 112.1 (ArC-20), 71.0 - 69.0 (-OCH_2CH_2O-), 31.3 (C-2), 8.5 (C-3); MS [m/z (%)]: 368 (31) M^+, 339 (2), 207 (12), 163 (100), 135 (8), 107 (5), 57 (11); HRMS: calcd for $C_{19}H_{28}O_7$: 368.1835; found: 368.1834

Introduction of the phenylselenyl group: Preparation of compounds 7

To a stirred solution of **6a** (3 mmol) or **6b** (3.7 mmol) in EtOAc (30 ml) was added 1.2 equivalents of PhSeCl. The resulting red-orange solution was stirred until it had turned pale yellow (2 days). Then water (20 ml) was added. The organic phase was dried over $MgSO_4$, and the solvent removed. Purification by column chromatography (alumina with EtOAc) and recrystallisation from EtOH gave the selenides **7a** and **7b**.

1-(2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-2-(phenylseleno)-1-propanone (7a)

yield: 71 % as colourless crystals; mp: 97.8 - 99.4 °C (EtOH); IR (KBr) cm^{-1}: 2921 (s) and 2893 (s) and 2867 (s), 1712 (s), 1449 (s), 1127 (s), 759 (s) and 695 (m); ^1H NMR ($CDCl_3$) δ: 7.68 - 7.22 (m, 7H, ArH-16, ArH-14, PhH), 6.79 (d, ^oJ = 8 Hz, 1H, ArH-17), 4.67 (q, ^3J = 6 Hz, 1H, -$CO\underline{CH}CH_3$), 4.18 - 3.77 (m, 16H, -OCH_2CH_2O-), 1.64 (d, ^3J = 6 Hz, 3H, -$COCH\underline{CH_3}$); ^{13}C NMR ($CDCl_3$) δ: 195.4 (CO), 153.4 (ArC-17a), 148.8 (ArC-13a), 136.3 (*o*-C_{PhSe}), 129.0 (*m*-C_{PhSe}), 128.9 (*i*-C_{PhSe}), 128.7 (*p*-C_{PhSe}), 127.9 (ArC-15), 123.0 (ArC-16), 113.5 (ArC-14), 111.8 (ArC-17), 71.2 - 68.7 (-OCH_2CH_2O-), 39.8 (C-2), 17.6 (C-3); MS [m/z (%)]: 480 (4) M^+, 295 (49), 207 (12), 163 (100), 135 (15), 107 (14); HRMS: calcd for $C_{23}H_{28}O_6Se$: 480.1051; found: 480.1057

1-(2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-2-(phenylseleno)-1-propanone (7b)

yield: 68 % as colourless crystals; mp: 100.6 - 101.9 °C (EtOH); IR (KBr) cm^{-1}: 2927 (m) and 2884 (m), 1705 (s), 1429 (s), 1125 (s), 745 (s) and 694 (m); ^1H NMR ($CDCl_3$) δ: 7.52 - 7.19 (m, 7H, ArH-19, ArH-17, PhH), 6.78 (d, ^oJ = 8 Hz, 1H, ArH-20), 4.64 (q, ^3J = 6 Hz, 1H, -$CO\underline{CH}CH_3$), 4.27 - 3.62 (m, 20H, -OCH_2CH_2O-), 1.64 (d, ^3J = 6 Hz, 3H, -$COCH\underline{CH_3}$); ^{13}C NMR ($CDCl_3$) δ: 195.4 (CO), 153.2 (ArC-20a), 148.9 (ArC-16a), 136.3 (*o*-C_{PhSe}), 129.0 (*m*-C_{PhSe}), 128.9 (*i*-C_{PhSe}), 128.7 (*p*-C_{PhSe}), 127.6 (ArC-18), 123.0 (ArC-19), 113.5 (ArC-17), 111.9 (ArC-20), 71.0 - 69.0 (-OCH_2CH_2O-), 39.7 (C-2), 17.7 (C-3); MS [m/z (%)]: 524 (21) M^+, 339

(100), 207 (8), 163 (100), 135 (12), 107 (8); HRMS: calcd for $C_{25}H_{32}O_7Se$: 524.1313; found: 524.1321

Generation of the α,β-unsaturated ketones 8 by oxidation of selenides 7

To a solution of selenide **7** (2.09 mmol) in methanol (40 ml) was added water (7 ml), 1.2 equivalents $NaHCO_3$ and 2.3 equivalents $NaIO_4$ under vigorous stirring. After 30 minutes at room temperature, the reaction mixture was poured into a mixture of 15 % ether/pentane (40 ml) and saturated $NaHCO_3$ solution (40 ml). The organic layer was washed with water and brine. After evaporation of the solvent, the residue was purified by column chromatography (alumina with EtOAc).

1-(2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-2-propen-1-one (8a)

yield: 85 % as a yellow powder; mp: 62.5 - 64 °C; IR (KBr) cm^{-1}: 2932 (s) and 2873 (s), 1662 (s), 1592 (s), 1454 (m), 1129 (s); ^1H NMR ($CDCl_3$) δ: 7.58 (dd, ^oJ = 8 Hz, ^mJ = 2 Hz, 1H, ArH-16), 7.54 (d, ^mJ = 2 Hz, 1H, ArH-14), 7.17 (dd, ^{tr}J = 17 Hz, ^{cis}J = 10 Hz, 1H, H-2), 6.88 (d, ^oJ = 8 Hz, 1H, ArH-17), 6.42 (dd, ^{tr}J = 17 Hz, ^{gem}J = 2 Hz, 1H, H-3tr), 5.86 (dd, ^{cis}J = 10 Hz, ^{gem}J = 2 Hz, 1H, H-3cis), 4.18 - 3.77 (m, 16H, $-OCH_2CH_2O-$); ^{13}C NMR ($CDCl_3$) δ: 189.1 (CO), 153.6 (ArC-17a), 149.0 (ArC-13a), 132.0 (C-2), 130.5 (ArC-15), 129.1 (C-3), 123.7 (ArC-16), 113.4 (ArC-14), 111.8 (ArC-17), 71.2 - 68.9 ($-OCH_2CH_2O-$); MS [m/z (%)]: 322 (12) $M^{+\cdot}$, 207 (2), 190 (20), 163 (100), 135 (11), 107 (9), 55 (46); HRMS: calcd for $C_{17}H_{22}O_6$: 322.1416; found: 322.1417

1-(2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-2-propen-1-one (8b)

yield: 81 % as a yellow oil; IR (KBr) cm^{-1}: 2926 (s) and 2869 (s), 1653 (s), 1592 (s), 1432 (m), 1126 (s); ^1H NMR ($CDCl_3$) δ: 7.59 (dd, ^oJ = 8 Hz, ^mJ = 2 Hz, 1H, ArH-19), 7.56 (d, ^mJ = 2 Hz, 1H, ArH-17), 7.19 (dd, ^{tr}J = 17 Hz, ^{cis}J = 10 Hz, 1H, H-2), 6.90 (d, ^oJ = 8 Hz, 1H, ArH-20), 6.43 (dd, ^{tr}J = 17 Hz, ^{gem}J = 2 Hz, 1H, H-3tr), 5.86 (dd, ^{cis}J = 10 Hz, ^{gem}J = 2 Hz, 1H, H-3cis), 4.28 - 3.66 (m, 20H, $-OCH_2CH_2O-$); ^{13}C NMR ($CDCl_3$) δ: 189.0 (CO), 153.4 (ArC-20a), 148.9 (ArC-16a), 132.0 (C-2), 130.4 (ArC-18), 129.1 (C-3), 123.6 (ArC-19), 113.1 (ArC-17), 111.8 (ArC-20), 70.9 - 68.8 ($-OCH_2CH_2O-$); MS [m/z (%)]: 366 (8) $M^{+\cdot}$, 339 (6), 190 (18), 163 (100), 135 (8), 135 (8), 107 (5); HRMS: calcd for $C_{19}H_{26}O_7$: 366.1679; found: 366.1675

Preparation of the caesium salt 1 and the acetoxymethyl esters 10

4-[3-(2,3,5,6,8,9,11,12-Octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]benzoic acid (9) and the caesium salt (1)

A mixture of **8a** (0.62 mmol) and 1.2 equivalents of 4-hydrazinobenzoic acid in acetic acid (10 ml) was stirred at room temperature for 2 days. The desired pyrazoline precipitated completely on addition of water, was filtered and washed with water. Compound **9** was converted into the caesium salt **1** by overnight reflux in methanol in the presence of 10 equivalents caesium hydroxide.[8]

yield of **9**: 42 % as a yellow powder; mp: 218.6 - 220.2 °C; IR (KBr) cm^{-1}: 2922 (w), 1664 (m), 1601 (s) and 1518 (s), 1428 (m), 1132 (s), 834 (w); ^1H NMR (DMSO-d_6) δ: 12.1 (br.s, 1H, $-CO_2H$), 7.84 (d, ^oJ = 8 Hz, 2H, ArH-2, ArH-6), 7.38 (d, ^mJ = 2 Hz, 1H, ArH-14'), 7.22 (dd, ^oJ = 8 Hz, ^mJ = 2 Hz, 1H, ArH-16'), 7.08 (d, ^oJ = 8 Hz, 2H, ArH-3, ArH-5), 6.99 (d, ^oJ = 8 Hz, 1H, ArH-17'), 4.11 - 3.63 (m, 16H, $-OCH_2CH_2O-$), 3.92 (t, ^3J = 10 Hz, 2H, pyrH-5), 3.32 (t, ^3J = 10 Hz, 2H, pyrH-4); ^{13}C NMR (DMSO-d_6) δ: 167.5 (CO_2H), 152.0 (ArC-17'a), 149.9 (ArC-4), 148.5 (ArC-13'a), 148.2 (pyrC-3), 131.0 (ArC-2), 125.0 (ArC-15'), 119.9 (ArC-16'), 119.4 (ArC-1), 113.0 (ArC-14'), 111.5 (ArC-3), 110.6 (ArC-17'), 70.5 - 68.3 ($-OCH_2CH_2O-$), 47.2 (pyrC-5), 31.9 (pyrC-4); MS [m/z (%)]: 456 (7) M^+, 412 (1), 324 (5), 175 (13), 163 (20), 43 (100); HRMS: calcd for

$C_{24}H_{28}N_2O_7$: 456.1897; found: 456.1904

(Acetoxy)methyl 4-[3-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecin-15-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]benzoate (10)

To a solution of acid **9** (0.328 mmol) in THF/water:10/1 (10 ml) was added 1 equivalent of Cs_2CO_3. After stirring for 15 minutes, the solvent was removed and dry dimethylformamide was added followed by evaporation to dryness (2 ×), providing the caesium salt **1**. To a solution of the caesium salt in dry dimethylformamide (10 ml) an excess of acetoxymethylbromide[9] was added. The suspension was stirred under inert atmosphere at room temperature. After completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by column chromatography (alumina with EtOAc) and recrystallised from EtOAc/hexane.

yield: 60 % as a yellow powder; mp: 124.6 - 126.2 °C (EtOAc/hexane); IR (KBr) cm^{-1}: 2932 (m) and 2875 (m), 1756 (s), 1719 (s), 1603 (s) and 1514 (s), 1444 (m), 1272 (s), 1138 (s), 843 (m); ^1H NMR ($CDCl_3$) δ: 8.00 (d, ^oJ = 8 Hz, 2H, ArH-2, ArH-6), 7.43 (d, ^mJ = 2 Hz, 1H, ArH-14'), 7.13 (dd, ^oJ = 8 Hz, ^mJ = 2 Hz, 1H, ArH-16'), 7.05 (d, ^oJ = 8 Hz, 2H, ArH-3, ArH-5), 6.85 (d, ^oJ = 8 Hz, 1H, ArH-17'), 5.97 (s, 2H, $-OCH_2O-$), 4.26 - 3.77 (m, 18H, $-OCH_2CH_2O-$, pyrH-5), 3.28 (t, ^3J = 10 Hz, 2H, pyrH-4), 2.13 (s, 3H, $-COCH_3$); ^{13}C NMR ($CDCl_3$) δ: 169.9 (-<u>CO</u>CH$_3$), 165.3 (-<u>CO</u>Ph), 151.4 (ArC-17'a), 150.6 (ArC-4), 149.2 (ArC-13'a), 148.9 (pyrC-3), 131.7 (ArC-2), 125.5 (ArC-15'), 120.0 (ArC-16'), 117.8 (ArC-1), 113.0 (ArC-14'), 111.6 (ArC-3), 111.2 (ArC-17'), 79.3 ($-OCH_2O-$), 71.1 - 68.8 ($-OCH_2CH_2O-$), 47.1 (pyrC-5), 32.2 (pyrC-4), 20.8 ($-COCH_3$); MS [m/z (%)]: 528 (69) M^+, 456 (14), 439 (13), 368 (14), 324 (59), 175 (7), 117 (8), 73 (14), 43 (100); HRMS: calcd for $C_{27}H_{32}N_2O_9$: 528.2108; found: 528.2108

Preparation of the caesium salt 2 of dimethyl 5-[3-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclo-octadecin-18-yl)-4,5-dihydro-1H-pyrazol-1-yl]isophthalate (11)

Synthesis of dimethyl 5-hydrazinoisophthalate[11]

A solution of 1.04 equivalents sodium nitrite in water (20 ml) was added over 15 minutes to a stirred slurry of 0.11 mmol 5-aminoisophthalic acid in 4.8 M HCl solution (100 ml) at 0 °C. Stirring was continued for 1 hour at 0-5 °C and the slurry was then added to a solution of 4.4 equivalents sodium sulphite in water (250 ml) at 2 °C. The resulting homogeneous solution was heated at 50-60 °C for 45 minutes. Concentrated HCl (13 ml) was added and the reaction mixture was heated further at 90 °C for 1 hour. After cooling to room temperature another portion of concentrated HCl (110 ml) was added. The solid was isolated by filtration and washed with acidified water and hexane. A NaOH solution of the white solid was acidified with glacial acetic acid to yield a thick slurry, which was filtered and washed. The 5-hydrazinoisophthalic acid, isolated in this way, was dissolved in MeOH (400 ml) containing H_2SO_4 (50 ml). After refluxing for 2 hours, the solvent was evaporated until 100 ml was left in the round bottomed flask and ice (50 g) was added. The reaction mixture was cooled to -10 °C and treated with a 30 % NaOH solution. The filtered precipitate was redissolved in water and the free hydrazine was obtained after gradually adding NaOH. The solid was isolated by filtration, washed with water and hexane, followed by recrystallisation from MeOH.

yield: 34 % as yellow needles; mp: 183 - 184 °C (MeOH); IR (KBr) cm^{-1}: 3354 (s) and 3304 (s), 1729 (s), 1288 (s), 889 (s); ^1H NMR (DMSO-d_6) δ: 7.73 (t, ^mJ = 2 Hz, 1H, ArH-2), 7.62 (d, ^mJ = 2 Hz, 2H, ArH-4, ArH-6), 4.02 (br.s, 3H, $-NHNH_2$), 3.86 (s, 6H, $-COOCH_3$); ^{13}C NMR (DMSO-d_6) δ: 166.1 (CO), 153.0 (ArC-5), 130.5 (ArC-1), 117.5 (ArC-2), 115.8 (ArC-4), 52.1 ($-OCH_3$); MS [m/z (%)]: 224 (100) M^+, 209 (11), 193 (34), 165 (23), 59 (12); HRMS: calcd for $C_{10}H_{12}N_2O_4$: 224.0797; found: 224.0798

Synthesis of dimethyl 5-[3-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin-18-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]isophthalate (11) and its salt (2)

The α,β-unsaturated ketone **8b** (1.5 mmol) was dissolved in acetic acid (15 ml) together with 1.2 equivalents dimethyl 5-hydrazinoisophthalate. This reaction mixture was stirred for 8 days at room temperature. Subsequently, water (15 ml) was added followed by extraction with $CHCl_3$ (3 × 15 ml). The combined organic layers were dried ($MgSO_4$), and after removal of the solvent, the residue was purified by column chromatography (silica with EtOAc/$CHCl_3$: 90/10).

The salt **2** was obtained by overnight reflux in methanol in the presence of 10 equivalents caesium hydroxide.

yield of **11**: 48 % as a yellow powder; mp: 123.6 - 124.2 °C; IR (KBr) cm^{-1}: 2869 (m) ν_{CH}, 1721 (s) $\nu_{C=O}$, 1598 (s) $\nu_{C=N}$, 1439 (s) ν_{CH}, 1124 (s) ν_{COC}, 861 (w) ν_{paraPh}; ^{1}H NMR ($CDCl_3$) δ: 8.14 (t, ^mJ = 2 Hz, 1H, ArH-2), 7.93 (d, ^mJ = 2 Hz, 2H, ArH-4, ArH-6), 7.5 (dd, ^oJ = 8 Hz, ^mJ = 2 Hz, 1H, ArH-19'), 7.46 (d, ^mJ = 2 Hz, 1H, ArH-17'), 6.88 (d, ^oJ = 8 Hz, 1H, ArH-20'), 4.04 - 3.69 (m, 22H, -OCH_2CH_2O-, pyrH-5), 3.96 (s, 6H, -OCH_3), 3.30 (t, ^3J = 10 Hz, 2H, pyrH-4); ^{13}C NMR ($CDCl_3$) δ: 166.1 (CO), 152.1 (ArC-20'a), 148.3 (pyrC-3), 148.2 (ArC-16'a), 145.9 (ArC-5), 131.4 (ArC-1), 125.1 (ArC-18'), 120.7 (ArC-2), 119.9 (ArC-19'), 117.1 (ArC-4), 113.1 (ArC-17'), 110.5 (ArC-20'), 70.6 - 68.4 (-OCH_2CH_2O-), 52.2 (-OCH_3), 47.9 (pyrC-5), 32.2 (pyrC-4); MS [m/z (%)]: 572 (100) M^+, 541 (2), 396 (53), 368 (14); HRMS: calcd for $C_{29}H_{36}N_2O_{10}$: 572.2370; found: 572.2375

Preparation of the fluorophore methyl 4-[3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]benzoate (13)

Procedure A. To a stirred solution of 1-(4-fluorophenyl)-2-propen-1-one (6.67 mmol) and 1.1 equivalents methyl 4-hydrazinobenzoate in 10 ml *tert*-BuOH, were added three drops of $HCl_{conc.}$. The solution was refluxed for 1 hour, evaporated to dryness and the residue re-dissolved in CH_2Cl_2/water and made alkaline with Cs_2CO_3; the organic layers were separated and evaporated. The residue was purified by column chromatography (silica with CH_2Cl_2/hexane: 90/10).

Procedure B. A mixture of 1-(4-fluorophenyl)-2-propen-1-one (1.66 mmol) and 1.1 equivalents methyl 4-hydrazinobenzoate in 10 ml acetic acid was stirred under nitrogen for 20 hours at room temperature. After extraction with CH_2Cl_2 (3 × 50 ml) the combined organic layers were dried over $MgSO_4$ and the solvent was removed. The crude product was purified by column chromatography (silica with CH_2Cl_2/hexane: 90/10).

yield: 70 % (A), 65 % (B) as colourless needles; mp: 139.5 - 142 °C; IR (KBr) cm^{-1}: 1716 (s), 1604 (s) and 1511 (s), 1284 (s), 840 (m); ^{1}H NMR ($CDCl_3$) δ: 7.96 (d, ^oJ = 9 Hz, 2H, ArH-2, ArH-6), 7.71 (dd, $^oJ_{HH}$ = 9 Hz, $^mJ_{HF}$ = 5.25 Hz, 2H, ArH-2', ArH-6'), 7.09 (t, $^oJ_{HH}$ = 9 Hz, $^oJ_{HF}$ = 9 Hz, 2H, ArH-3', ArH-5'), 7.06 (d, ^oJ = 9 Hz, 2H, ArH-3, ArH-5), 3.94 (t, ^3J = 10.5 Hz, 2H, pyrH-5), 3.87 (s, 3H, -CO_2CH_3), 3.27 (t, ^3J = 10.5 Hz, 2H, pyrH-4); ^{13}C NMR ($CDCl_3$) δ: 167.3 (CO), 162.3 (d, $^1J_{CF}$ = 245 Hz, ArC-4'), 149.9 (pyrC-3), 148.4 (ArC-4), 131.3 (ArC-2), 128.7 (d, $^4J_{CF}$ = 3 Hz, ArC-1'), 127.7 (d, $^3J_{CF}$ = 9 Hz, ArC-2'), 119.9 (ArC-1), 115.7 (d, $^2J_{CF}$ = 20 Hz, ArC-3'), 111.7 (ArC-3), 51.6 (-OCH_3), 47.4 (pyrC-5), 32.1 (pyrC-4); MS [m/z (%)]: 298 (100) M^+, 267 (33), 239 (5), 149 (12); HRMS: calcd for $C_{17}H_{15}FN_2O_2$: 298.1118; found: 298.1122

Preparation of 4,4'-dibromophenyl diazacrown ethers 19

Synthesis of diamine 17

A mixture of triethyleneglycol di-*p*-tosylate (6.55 mmol), 2.2 equivalents *p*-bromoaniline and 3 ml Et_3N in dry toluene was refluxed under nitrogen for 20 hours. The mixture was allowed to warm up to room temperature, the solvent was removed under reduced pressure and the residue was purified by column chromatography (silica with $CHCl_3$/EtOAc: 100/0 → 50/50).

1,2-Bis[2-(4-bromoanilino)ethoxy]ethane (17)

yield: 72 %; IR (NaCl) cm^{-1}: 3396 (w), 2867 (m), 1460 (w), 1136 (s), 814 (s); ^1H NMR ($CDCl_3$) δ: 7.23 (d, ^oJ = 10 Hz, 4H, ArH-3, ArH-5), 6.46 (d, ^oJ = 10 Hz, 4H, ArH-2, ArH-6), 4.01 (br.s, 2H, -NH), 3.67 - 3.46 (m, 8H, $-CH_2O-$), 3.24 (t, ^3J = 4 Hz, 4H, $-CH_2NH-$); ^{13}C NMR ($CDCl_3$) δ: 147.1 (ArC-1), 131.8 (ArC-3), 114.6 (ArC-2), 109.1 (ArC-4), 70.2 (C-4), 69.4 (C-2), 43.5 (CNH); MS [m/z (%)]: 456 (3) M^+, 298 (2) M, 259 (77), 184 (99), 155 (5); HRMS: calcd for $C_{18}H_{22}Br_2N_2O_2$: 456.0048; found: 456.0044

Synthesis of cyclic diamides 18

Two separate solutions of diamine **17** (2.19 mmol), 0.5 ml dry pyridine in 50 ml dry toluene and the corresponding diacid dichloride[16] (1.2 equivalents) in 70 ml dry toluene were placed in two dropping funnels. The two solutions were added simultaneously and dropwise to 100 ml of dry toluene stirred under inert atmosphere. After 12 hours of stirring at room temperature, the mixture was filtered and the filtrate was evaporated. The residue was purified by column chromatography (silica with MeOH/$CHCl_3$: 1/99).

7,13-Bis(4-bromophenyl)-1,4,10-trioxa-7,13-diazacyclopentadecane-8,12-dione (18a)

yield: 74 % as white needles; mp: 146 - 147 °C; IR (KBr) cm^{-1}: 2870 (m), 1670 (s), 1437 (m), 1102 (s), 838 (w); ^1H NMR ($CDCl_3$) δ: 7.57 (d, ^oJ = 8 Hz, 4H, ArH-3, ArH-5), 7.28 (d, ^oJ = 8 Hz, 4H, ArH-2, ArH-6), 4.20 (s, 4H, $-CH_2CO-$), 3.85 (s, 4H, $-CH_2N-$), 3.60 - 3.45 (m, 8H, $-CH_2O-$); ^{13}C NMR ($CDCl_3$) δ: 169.1 (CO), 139.7 (ArC-1), 132.4 (ArC-3), 130.9 (ArC-2), 121.9 (ArC-4), 71.1 (C-9), 70.4 (C-3), 67.3 (C-5), 49.4 (C-6); MS [m/z (%)]: 554 (5) $M^{+\cdot}$, 475 (3), 358 (44), 213 (72), 197 (94), 183 (78), 169 (27), 155 (30); HRMS: calcd for $C_{22}H_{24}Br_2N_2O_5$: 554.0052; found: 554.0042

7,16-Bis(4-bromophenyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-8,15-dione (18b)

yield: 69 % as white needles; mp: 144.5 - 145.5 °C; ^1H NMR ($CDCl_3$) δ: 7.60 (d, ^oJ = 8 Hz, 4H, ArH-3, ArH-5), 7.30 (d, ^oJ = 8 Hz, 4H, ArH-2, ArH-6), 3.93 (s, 4H, $-OCH_2CO-$), 3.89 (s, 4H, $-CH_2N-$), 3.62 - 3.43 (m, 12H, $-OCH_2-$); ^{13}C NMR ($CDCl_3$) δ: 168.9 (CO), 139.4 (ArC-1), 132.6 (ArC-3), 130.5 (ArC-2), 122.2 (ArC-4), 71.0 (C-9), 70.4 (C-3), 70.1 (C-11); 67.3 (C-5), 47.9 (C-6); MS [m/z (%)]: 598 (5) $M^{+\cdot}$, 519 (4), 402 (37), 213 (21), 197 (61), 183 (65), 169 (21), 155 (21); HRMS: calcd for $C_{24}H_{28}Br_2N_2O_6$: 598.0314; found: 598.0322

Synthesis of 4-dibromophenyl diazacrown ethers 19

A 1 M solution of borane.methyl sulfide complex (4.86 equivalents) was added dropwise via a syringe to a solution of diamide **18** in anhydrous tetrahydrofuran. The reaction mixture was refluxed under nitrogen for 4 hours. After cooling, the excess diborane was destroyed by dropwise addition of water followed by evaporation (2 ×). Treatment of the residue with $CHCl_3$, subsequent filtration and condensation of the filtrate gave a crude product, which was purified by column chromatography (silica with $CHCl_3$/EtOAc: 85/15)

7,13-Bis(4-bromophenyl)-1,4,10-trioxa-7,13-diazacyclopentadecane (19a)

yield: 84 % as white needles; mp: 114.5 - 115 °C; IR (KBr) cm^{-1}: 2865 (s), 1450 (m), 1113 (s), 806 (s); ^1H NMR ($CDCl_3$) δ: 7.23 (d, ^oJ = 9 Hz, 4H, ArH-3, ArH-5), 6.56 (d, ^oJ = 9 Hz, 4H, ArH-2, ArH-6), 3.74 - 3.49 (m, 20H, $-CH_2-$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.3 (ArC-1), 131.8 (ArC-3), 114.0 (ArC-2), 108.1 (ArC-4), 70.8 (C-3), 69.8 (C-9), 68.9 (C-5), 52.1 (C-8), 52.0 (C-6); MS [m/z (%)]: 526 (11) M^+, 447 (38), 368 (2), 255 (39), 228 (100), 197 (49), 183 (28), 169 (25), 155 (14); HRMS: calcd for $C_{22}H_{28}Br_2N_2O_3$: 526.0467; found: 526.0462

7,16-Bis(4-bromophenyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (19b)

yield: 71 % as white needles; mp: 111.8 - 113.1 °C ($CHCl_3$); IR (KBr) cm^{-1}: 2862 (m), 1446 (w), 1108 (s), 800

(s); ^{1}H NMR ($CDCl_3$) δ: 7.24 (d, ^oJ = 10 Hz, 4H, ArH-3, ArH-5), 6.53 (d, ^oJ = 10 Hz, 4H, ArH-2, ArH-6), 3.70 - 3.52 (m, 24H, -CH_2-); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ: 146.9 (ArC-1), 131.9 (ArC-3), 113.4 (ArC-2), 107.9 (ArC-4), 71.0 (C-3), 69.0 (C-5), 51.4 (C-6); MS [m/z (%)]: 570 (49) $M^{+\cdot}$, 491 (25), 412 (9), 255 (40), 242 (69), 197 (40), 183 (50), 169 (28), 155 (15); HRMS: calcd for $C_{24}H_{28}Br_2N_2O_6$: 570.0729; found: 570.0725

Preparation of the aryl-RuCp-complex 7,16-bis{[η^6-4-(1-oxopropyl)phenyl](η^5-cyclopentadienyl)-ruthenium}-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane hexafluorophosphate (23)

Synthesis of RuCp-complex 22

A solution of $[CpRu(CH_3CN)_3]PF_6$ (1.72 mmol) prepared according to the procedure of Zelonka and Baird[7a] and 1 equivalent *p*-fluoropropiophenone in 15 ml 1,2-dichloroethane was refluxed under nitrogen for 3.5 hours. After cooling of the reaction mixture, 10 ml of dry dimethylformamide was added and 1,2-dichloroethane was removed. The obtained RuCp-complex **22** was too unstable to purify.

The crude product **22** dissolved in dry dimethylformamide was treated with 1 equivalent $NaHCO_3$ and 0.57 mmol diazacrown ether **12b**. The mixture was stirred under nitrogen for 20 hours at 50 °C. After evaporation to dryness and purification by column chromatography (alumina with $CHCl_3/CH_3CN$: 50/50 → 0/100), product **23** was crystallised from CH_2Cl_2/ether.

yield: 81 % as yellow crystals; mp: 214.9 - 216.2 °C; IR (KBr) cm^{-1}: 2925 (w), 1700 (m); ^{1}H NMR (DMSO-d_6) δ: 6.35 (d, ^oJ = 5 Hz, 4H, ArH-2, ArH-6), 5.88 (d, ^oJ = 5 Hz, 4H, ArH-3, ArH-5), 5.11 (s, 10H, Cp), 3.63 - 3.48 (m, 24H, -CH_2), 2.86 (q, ^3J = 5 Hz, 4H, -CO$\underline{CH_2}CH_3$), 1.07 (t, ^3J = 5 Hz, 6H, -$COCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6) δ: 201.2 (CO), 130.9 (ArC-4), 88.8 (ArC-1), 84.5 (ArC-2), 81.1 (Cp), 71.9 (C-3'), 69.4 (ArC-3), 68.5 (C-5'), 53.0 (C-6'), 32.6 (C-2), 8.3 (C-3)

Generation of Diketones 24

A mixture of the diazacrown ether **12a** (4.58 mmol) or **12b** (3.82 mmol), 2.5 equivalents tetrabutylammonium fluoride trihydrate and an excess of *p*-fluoropropiophenone was heated and stirred at 150 °C for 12 hours. The reaction mixture was then cooled to room temperature followed by purification by column chromatography (silica with EtOAc/$CHCl_3$: 30/70 or silica EtOAc/$CHCl_3$: 50/50)

7,13-Bis[4-(1-oxopropyl)phenyl]-1,4,10,-trioxa-7,13-diazacyclopentadecane (24a)

yield: 25 % as a white powder; mp: 104 - 105 °C; IR (KBr) cm^{-1}: 2969 (m) and 2935 (m) and 2875 (m), 1664 (s), 1472 (w), 1131 (s) and 1106 (s), 795 (m); ^{1}H NMR ($CDCl_3$) δ: 7.81 (d, ^oJ = 9 Hz, 4H, ArH-2, ArH-6), 6.67 (d, ^oJ = 9 Hz, 4H, ArH-3, ArH-5), 3.80 - 3.58 (m, 20H, -CH_2-), 2.88 (q, ^3J = 7 Hz, 4H, -CO$\underline{CH_2}CH_3$), 1.19 (t, ^3J = 7 Hz, 6H, -$COCH_2\underline{CH_3}$); ^{13}C NMR ($CDCl_3$) δ: 198.9 (CO), 151.6 (ArC-4), 130.1 (ArC-2), 125.1 (ArC-1), 110.9 (ArC-3), 70.7 (C-3'), 69.6 (C-9'), 68.8 (C-5'), 52.0 (-CH_2N-), 30.9 (C-2), 8.7 (C-3); MS [m/z (%)]: 482 (35) $M^{+\cdot}$, 453 (23), 206 (100), 132 (37); HRMS: calcd for $C_{28}H_{38}N_2O_5$: 482.2781; found: 482.2783

7,16-Bis[4-(1-oxopropyl)phenyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (24b)

yield: 28 % as a white powder; mp: 120 - 120.5 °C; IR (KBr) cm^{-1}: 2950 (m) and 2888 (m) and 2866 (m), 1654 (s), 1450 (m), 1112 (s), 797 (s); ^{1}H NMR ($CDCl_3$) δ: 7.85 (d, ^oJ = 10 Hz, 4H, ArH-2, ArH-6), 6.63 (d, ^oJ = 10 Hz, 4H, ArH-3, ArH-5), 3.83 - 3.57 (m, 24H, -CH_2-), 2.90 (q, ^3J = 7.5 Hz, 4H, -CO$\underline{CH_2}CH_3$), 1.22 (t, ^3J = 7.5 Hz, 6H, -$COCH_2\underline{CH_3}$); ^{13}C NMR ($CDCl_3$) δ: 198.9 (CO), 151.3 (ArC-4), 130.4 (ArC-2), 125.1 (ArC-1), 110.4 (ArC-3), 71.0 (C-3'), 68.8 (C-5'), 51.3 (-CH_2N-), 30.9 (C-2), 8.8 (C-3); MS [m/z (%)]: 526 (100), 497 (18), 250 (77), 132 (60); HRMS: calcd for $C_{30}H_{42}N_2O_6$: 526.3043; found 526.3048

Conversion of diketones 24 into Diselenides 25

Into a three-necked round-bottomed flask was added 10 ml dry tetrahydrofuran and 0.34 ml or 1.1 ml LDA (2 M solution in tetrahydrofuran/heptane) under nitrogen. The flask was cooled to -78 °C and 0.21 mmol **24a** or 0.68 mmol **24b** in 4 ml dry tetrahydrofuran was added dropwise followed by stirring for 1 hour at -78 °C. After addition of 3.7 equivalents PhSeCl, the reaction mixture was allowed to warm to 0 °C for 2 hours. Then 1 ml 0.5 M HCl-solution, 100 ml CH_2Cl_2 and a saturated K_2CO_3 solution were added, and the organic layer was separated. After removal of the solvent, the crude reaction product was purified by column chromatography (silica with EtOAc/$CHCl_3$: 30/70 (**25a**) or 60/40 (**25b**).

7,13-Bis[4-(1-oxo-2-phenylselenylpropyl)phenyl]-1,4,10-trioxa-7,13-diazacyclopentadecane (25a)

yield: 68 % as a yellow oil; IR (NaCl) cm^{-1}: 2919 (m) and 2862 (m) and 2963 (m), 1771 (m), 1448 (m), 1122 (s) and 1100 (s), 831 (m), 761 (s) and 693 (s); ^1H NMR ($CDCl_3$) δ: 7.78 (d, ^oJ = 9 Hz, 4H, ArH-2, ArH-6), 7.51 (d, ^oJ = 7.5 Hz, 4H, H^o-SePh), 7.32 - 7.24 (m, 6H, H^m and H^p-SePh), 6.64 (d, ^oJ = 9 Hz, 4H, ArH-3, ArH-5), 4.66 (q, ^3J = 6 Hz, 2H, -CO<u>CH</u>CH_3), 3.80 - 3.58 (m, 20H, -CH_2-), 1.62 (d, ^3J = 6 Hz, 6H, -COCH<u>CH_3</u>); ^{13}C NMR ($CDCl_3$) δ: 195.0 (CO), 151.8 (ArC-4), 136.2 (o-C_{PhSe}), 130.7 (ArC-2), 128.9 (m-C_{PhSe}), 128.5 (p-C_{PhSe}), 127.8 (i-C_{PhSe}), 123.4 (ArC-1), 110.0 (ArC-3), 70.8 (C-3'), 69.8 (C-9'), 68.8 (C-5'), 52.1 (C-8'), 51.9 (C-6'), 39.6 (C-2), 17.8 (C-3); MS [m/z (%)]: 794 (2) $M^{+\cdot}$, 609 (11), 453 (95), 206 (100), 157 (66), 132 (87); HRMS: calcd for $C_{40}H_{46}N_2O_5Se_2$: 794.1737; found: 794.1728

7,16-Bis[4-(1-oxo-2-phenylselenylpropyl)phenyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (25b)

yield: 77 % as a yellow oil; IR (NaCl) cm^{-1}: 2962 (m) and 2918 (m) and 2862 (m), 1771 (s), 1450 (m), 1118 (s), 830 (s), 761 (s) and 694 (s); ^1H NMR ($CDCl_3$) δ: 7.80 (d, ^oJ = 9 Hz, 4H, ArH-2, ArH-6), 7.51 (d, ^oJ = 7.5 Hz, 4H, H^o-SePh), 7.32 - 7.24 (m, 6H, H^m and H^p-SePh), 6.61 (d, ^oJ = 9 Hz, 4H, ArH-3, ArH-5), 4.66 (q, ^3J = 6 Hz, 2H, -CO<u>CH</u>CH_3), 3.72 - 3.64 (m, 24H, -CH_2-), 1.61 (d, ^3J = 6 Hz, -COCH<u>CH_3</u>); ^{13}C NMR ($CDCl_3$) δ: 194.9 (CO), 151.4 (ArC-4), 136.2 (o-C_{PhSe}), 130.9 (ArC-2), 128.9 (m-C_{PhSe}), 128.5 (p-C_{PhSe}), 127.8 (i-C_{PhSe}), 123.3 (ArC-1), 110.4 (ArC-3), 71.0 (C-3'), 68.8 (C-5'), 51.3 (C-6'), 39.6 (C-2), 17.8 (C-3); MS [m/z (%)]: 838 (4) $M^{+\cdot}$, 653 (27), 497 (81), 250 (47), 157 (67), 132 (100)

Generation of bis-α,β-unsaturated ketones 26

To a solution of 0.44 mmol **25a** or 0.53 mmol **25b** in 10 ml of tetrahydrofuran was added 7 ml of methanol, 3 ml of water, 2.4 equivalents $NaHCO_3$ and 4.6 equivalents $NaIO_4$ under vigorous stirring. After 30 min at room temperature, the reaction mixture was poured into 20 ml of 15 % ether/pentane and 20 ml saturated $NaHCO_3$ solution, and subsequently extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with water and NaCl solution. After evaporation of the solvent, the residue was purified by column chromatography (silica with EtOAc or silica with EtOAc/$CHCl_3$: 80/20).

7,13-Bis[4-(1-oxo-2-propenyl)phenyl]-1,4,10-trioxa-7,13-diazacyclopentadecane (26a)

yield: 72 % as a yellow oil; IR (NaCl) cm^{-1}: 2963 (m) and 2861 (m) and 2851 (m), 1651 (s), 1589 (s), 1450 (m), 1122 (m) and 1102 (m), 999 (s) and 962 (s) , 841 (s); ^1H NMR ($CDCl_3$) δ: 7.78 (d, ^oJ = 9 Hz, 4H, ArH-2, ArH-6), 7.12 (dd, ^{tr}J = 17 Hz, ^{cis}J = 10 Hz, 2H, H-2), 6.64 (d, ^oJ = 9 Hz, 4H, ArH-3, ArH-5), 6.62 (dd, ^{tr}J = 17 Hz, ^{gem}J = 2 Hz, 2H, H-3tr), 5.72 (dd, ^{cis}J = 10 Hz, ^{gem}J = 2 Hz, 2H, H-3cis), 3.80 - 3.46 (m, 20H, -CH_2-); ^{13}C NMR ($CDCl_3$) δ: 187.5 (CO), 151.6 (ArC-4), 131.9 (C-2), 130.7 (ArC-2), 127.4 (C-3), 125.1 (ArC-1), 111.0 (ArC-3), 70.4 (C-3'), 69.2 (C-9'), 68.7 (C-5'), 51.8 (C-8'), 51.7 (C-6'); MS [m/z (%)]: 478 (12) $M^{+\cdot}$, 204 (49), 174 (28), 132 (52); HRMS: calcd for $C_{28}H_{34}N_2O_5$: 478.2468; found 478.2467

7,16-Bis[4-(1-oxo-2-propenyl)phenyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (26b)

yield: 75 % as a yellow oil; IR (NaCl) cm^{-1}: 2918 (m) and 2863 (m), 1652 (s), 1582 (s), 1455 (m), 1118 (s), 1000 (s) and 963 (s), 837 (s); ^1H NMR ($CDCl_3$) δ: 7.89 (d, ^oJ = 8 Hz, 4H, ArH-2, ArH-6), 7.18 (dd, ^{tr}J = 16 Hz, ^{cis}J = 10 Hz, 2H, H-2), 6.66 (d, ^oJ = 8 Hz, 4H, ArH-3, ArH-5), 6.40 (dd, ^{tr}J = 16 Hz, ^{gem}J = 2 Hz, 2H, H-3tr), 5.78 (dd, ^{cis}J = 10 Hz, ^{gem}J = 2 Hz, 2H, H-3cis), 3.74 - 3.64 (m, 24 H, $-CH_2-$); ^{13}C NMR ($CDCl_3$) δ: 188.0 (CO), 151.6 (ArC-4), 132.1 (C-2), 131.2 (ArC-2), 127.9 (C-3), 125.3 (ArC-1), 110.6 (ArC-3), 71.0 (C-3'), 68.8 (C-5'), 51.3 (C-6'); MS [m/z (%)]: 522 (27) M^+, 248 (22), 174 (16), 132 (16); HRMS: calcd for $C_{30}H_{38}N_2O_6$: 522.2730; found: 522.2726

Preparation of the tetramethyl esters 27a and 27b

Synthesis of dibenzyl 5-hydrazinoisophthalate 29

In a three necked flask, a mixture of dimethyl 5-hydrazinoisophthalate (26.79 mmol), 4.8 equivalents benzyl alcohol, 5 g *tert*-BuOK and molecular sieves 3 Å (30 g) in 200 ml benzene was refluxed under nitrogen for 21 hours. The mixture was cooled to room temperature, then aq. NH_4Cl (2 g/4 ml water) was added under nitrogen. The benzene layer was separated and the residue was extracted with EtOAc (p.a.). The combined organic layers were evaporated and the residue was washed with hexane (p.a., 3×100 ml) to afford a yellow solid product **29**, which was re-crystallised from MeOH.

yield: 18 % as a yellow powder; mp: 121 - 122 °C; IR (KBr) cm^{-1}: 3389 (m), 1698 (s), 1264 (s), 886 (s), 750 (s) and 694 (s); ^1H NMR (DMSO-d_6) δ: 7.75 (t, ^mJ = 2 Hz, 1H, ArH-2), 7.65 (d, ^mJ = 2 Hz, 2H, ArH-4, ArH-6), 7.46 - 7.36 (m, 10H, Ph), 5.35 (s, 4H, $-OCH_2Ph$), 3.36 (br.s, 3H, $-NHNH_2$), ^{13}C NMR (DMSO-d_6) δ: 165.4 (CO), 153.1 (ArC-5), 136.0 (i-C_{Ph}), 130.4 (ArC-1), 128.5 (m-C_{Ph}), 128.1 (p-C_{Ph}), 128.0 (o-C_{Ph}), 117.3 (ArC-2), 115.8 (ArC-4), 66.2 ($-CH_2-$); MS [m/z (%)]: 376 (98) M^+, 269 (19), 91 (100); HRMS: calcd for $C_{22}H_{20}N_2O_4$: 376.1423; found: 376.1426

Synthesis of tetramethyl esters 27a and 27b

Procedure A. To a stirred solution of 0.52 mmol **26a** or 0.479 mmol **26b** and 3 equivalents dimethyl 5-hydrazinoisophthalate in 20 ml *tert*-BuOH, were added a few drops of $HCl_{conc.}$. After reflux for 1 hour, the solution was evaporated to dryness and the residue was re-dissolved in 100 ml CH_2Cl_2 and 10 ml water. After basifying with Cs_2CO_3, the organic layer was separated and evaporated. The residue containing **27a** or **27b** was purified by column chromatography (alumina with EtOAc/$CHCl_3$: 5/95 or 20/80) and re-crystallised from CH_2Cl_2/hexane.

Procedure B. A mixture of **26a** (0.96 mmol) or **26b** (0.67 mmol) and 2.4 equivalents of dimethyl 5-hydrazinoisophthalate in acetic acid (10 ml) was stirred at room temperature for 11 days. Removal of the solvent and purification by column chromatography and recrystallisation as mentioned above gave the tetramethyl ester **27a** or **27b**.

7,13-Bis{4-[1-(3,5-dimethoxycarbonyl)phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl}-1,4,10-trioxa-7,13-diazacyclopentadecane (27a)

yield: 30 % (A), 40 % (B) as a yellow powder; mp: 190 - 191 °C (CH_2Cl_2 - hexane); IR (KBr) cm^{-1}: 2949 (w) and 2862 (w), 1724 (s), 1601 (s) and 1522 (m), 1438 (m), 1115 (m) and 1101 (m), 862 (w), 816 (w); ^1H NMR ($CDCl_3$) δ: 8.08 (t, ^mJ = 2 Hz, 2H, ArH-2), 7.87 (d, ^mJ = 2 Hz, 4H, ArH-4, ArH-6), 7.58 (d, ^oJ = 9 Hz, 4H, ArH-2', ArH-6'), 6.71 (d, ^oJ = 9 Hz, 4H, ArH-3', ArH-5'), 3.94 (s, 12H, $-CO_2CH_3$), 3.85 (t, ^3J = 10 Hz, 4H, pyrH-5), 3.66 - 3.60 (m, 20H, $-CH_2-$), 3.25 (t, ^3J = 10 Hz, 4H, pyrH-4); ^{13}C NMR ($CDCl_3$) δ: 166.8 (CO),

151.2 (ArC-4'), 148.8 (pyrC-3), 146.3 (ArC-5), 131.2 (ArC-1), 127.4 (ArC-2'), 120.4 (ArC-1'), 120.0 (ArC-2), 117.3 (ArC-4), 112.0 (ArC-3'), 70.7 (C-3'), 69.8 (C-9'), 69.0 (C-5'), 52.2 (-OCH_3), 52.1 (C-6', C-8'), 47.8 (pyrC-5), 32.4 (pyrC-4); MS [m/z (%)]: 890 (38) M^+, 655 (67), 467 (16), 432 (82), 408 (100); HRMS: calcd for $C_{48}H_{54}N_6O_{11}$: 890.3851; found: 890.3850

7,16-Bis{4-[1-(3,5-dimethoxycarbonyl)phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl}-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (27b)

yield: 35 % (A), 52 % (B) as a yellow powder; mp: 146.5 - 148 °C (CH_2Cl_2 - hexane); IR (KBr) cm^{-1}: 2863 (w), 1718 (s), 1602 (s) and 1522 (m), 1438 (m), 1134 (s), 862 (w), 816 (m); ^1H NMR ($CDCl_3$) δ: 8.07 (t, ^mJ = 1.5 Hz, 2H, ArH-2), 7.87 (d, ^mJ = 1.5 Hz, 4H, ArH-4, ArH-6), 7.60 (d, ^oJ = 9 Hz, 4H, ArH-2', ArH-6'), 6.67 (d, ^oJ = 9 Hz, 4H, ArH-3', ArH-5'), 3.94 (s, 12H, -CO_2CH_3), 3.85 (t, ^3J = 10 Hz, 4H, pyrH-5), 3.73 - 3.63 (m, 24H, -CH_2-), 3.23 (t, ^3J = 10 Hz, 4H, pyrH-4); ^{13}C NMR ($CDCl_3$) δ: 166.8 (CO), 151.1 (ArC-4'), 148.5 (pyrC-3), 146.3 (ArC-5), 131.2 (ArC-1), 127.5 (ArC-2'), 120.2 (ArC-1'), 120.0 (ArC-2), 117.4 (ArC-4), 111.4 (ArC-3'), 71.1 (C-3'), 69.0 (C-5'), 52.2 (-OCH_3), 51.4 (C-6'), 47.8 (pyrC-5), 32.4 (pyrC-4); MS [m/z (%)]: 934 (100) M^+, 699 (13), 467 (29); HRMS: calcd for $C_{50}H_{58}N_6O_{12}$: 934.4113; found: 934.4106

Conversion of the tetramethyl esters 27a or 27b into the tetraacids 15a or 15b

Potassium trimethylsilanolate (8 equivalents) was added to a stirred slurry of **27a** (0.112 mmol) in dry tetrahydrofuran. Similarly sodium trimethylsilanolate was added to **27b** (0.107 mmol). The reaction mixture was refluxed under inert atmosphere for 12 hours. After cooling, the white solid was filtered under nitrogen and washed with tetrahydrofuran. The precipitate was dissolved in water and subsequently neutralised by gradually adding 0.1 N HCl solution. The resulting tetraacids **15** were isolated by filtration, washed with water and dried under vacuum.

7,13-Bis{4-[1-(3,5-dicarboxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl}-1,4,10-trioxa-7,13-diazacyclopentadecane (15a)

yield: 78 % as a yellow powder; mp: 195.6 - 196.8 °C; ^1H NMR ($CDCl_3$) δ: 13.18 (br.s, 4H, -CO_2H), 7.86 (t, ^mJ = 1.5 Hz, 2H, ArH-2), 7.72 (d, ^mJ = 1.5 Hz, 4H, ArH-4, ArH-6), 7.54 (d, ^oJ = 8 Hz, 4H, ArH-2', ArH-6'), 6.70 (d, ^oJ = 8 Hz, 4H, ArH-3', ArH-5'), 3.87 (t, ^3J = 10 Hz, 4H, pyrH-5), 3.71 - 3.60 (m, 20H, -CH_2-), 3.26 (t, ^3J = 10 Hz, 4H, pyrH-4); ^{13}C NMR ($CDCl_3$) δ: 166.9 (CO), 151.7 (ArC-4'), 148.5 (pyrC-3), 146.2 (ArC-5), 131.8 (ArC-1), 127.4 (ArC-2'), 120.3 (ArC-1'), 119.1 (ArC-2), 116.4 (ArC-4), 111.6 (ArC-3'), 70.0 (C-3'), 69.0 (C-9'), 68.3 (C-5'), 52.1 (-OCH_3), 51.0 (C-6', C-8'), 47.3 (pyrC-5), 32.1 (pyrC-4); HRMS: calcd for $C_{44}H_{47}N_6O_{11}$: 835.3303; found: 835.3295

7,16-Bis{4-[1-(3,5-dicarboxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl}-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (15b)

yield: 75 %; mp as a yellow powder: 203.5 - 204.5 °C; ^1H NMR ($CDCl_3$) δ: 13.12 (br.s, 4H, -CO_2H), 7.86 (t, ^mJ = 1.5 Hz, 2H, ArH-2), 7.74 (d, ^mJ = 1.5 Hz, 4H, ArH-4, ArH-6), 7.55 (d, ^oJ = 8 Hz, 4H, ArH-2', ArH-6'), 6.73 (d, ^oJ = 8 Hz, 4H, ArH-3', ArH-5'), 3.86 (t, ^3J = 10 Hz, 4H, pyrH-5), 3.62 - 3.57 (m, 24H, -CH_2-), 3.26 (t, ^3J = 10 Hz, 4H, pyrH-4); ^{13}C NMR ($CDCl_3$) δ: 167.0 (CO_2H), 151.8 (ArC-4'), 148.4 (pyrC-3), 146.1 (ArC-5), 131.9 (ArC-1), 127.4 (ArC-2'), 120.2 (ArC-1'), 119.1 (ArC-2), 116.4 (ArC-4), 111.1 (ArC-3'), 70.2 (C-3'), 68.2 (C-5'), 50.4 (C-6'), 47.4 (pyrC-5), 32.1 (pyrC-4); HRMS: calcd for $C_{46}H_{51}N_6O_{12}$: 879.3565; found: 879.3554

Conversion of the tetramethyl esters 27a or 27b into the tetracaesium salts Diaz-15-5 or Diaz-18-6

The caesium salts **Diaz-15-5** and **Diaz-18-6** were prepared from **27a-b** according to the procedure described by Minta and Tsien.[8] A solution of **27a-b** (1×10^{-5} mol) and anhydrous caesium hydroxide (17 mg, 1×10^{-4} mol, 10 equivalents) 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.

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