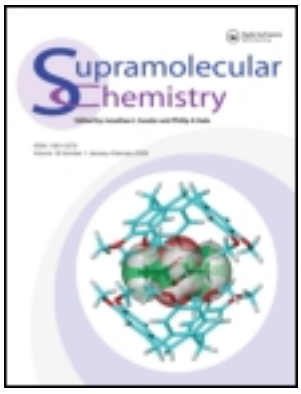

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Synthesis and cation binding properties of fluorescent calix[4]arene derivatives bearing tryptophan units at the lower rim

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The fluorescent peptidocalixarenes, 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(*O*-methyl-L-tryptophanylcarbonylmethoxy)calix[4]arene (**1**) and 5,11,17,23-tetra-*tert*-butyl-25,27-di(*O*-methyl)-26,28-bis(*O*-methyl-L-tryptophanylcarbonylmethoxy)calix[4]arene (**2**), were prepared by introducing tryptophan subunits at a lower calixarene rim. Coordination abilities of **1** and **2** towards Eu(III) and alkali metal cations were studied by spectrophotometric, spectrofluorimetric, conductometric and potentiometric titrations in acetonitrile at 25°C. Rather strong complexation was observed for smaller alkali metal cations Li^+ and Na^+ ($\log K_{\mathbf{Li1}} > 6$, $\log K_{\mathbf{Li2}} > 6$, $\log K_{\mathbf{Na1}} = 8.25$, $\log K_{\mathbf{Na2}} = 6.94$), and moderate for K^+ ($\log K_{\mathbf{K1}} = 5.09$, $\log K_{\mathbf{K2}} = 4.09$). Larger Rb^+ and Cs^+ cations did not fit in the ion binding site of **1** so no complexation was detected, whereas the more flexible ligand **2** accommodated Rb^+ cation ($\log K_{\mathbf{Rb2}} = 3.44$). The fluorescence of **1** ($\lambda_{ex} = 280$ nm, $\lambda_{em} = 340$ nm) was remarkably quenched by Eu(III). Stability constant of 1:1 (Eu^{3+}:**1**) complex determined spectrofluorimetrically amounted to $\log K_{\mathbf{Eu1}} = 6.16$.

Keywords: calixarenes; alkali metal cations; Eu(III); stability constants; fluorescence

1. Introduction

Calixarenes have received a great deal of attention as host molecules in supramolecular chemistry (*1*). An important feature of these compounds is their synthetic flexibility. Chemical modification of lower or upper calixarene rims by introducing groups with different binding abilities enables them to form inclusion complexes with a wide variety of guest species. Depending on the appended groups and the number of repeating phenolic units (usually four or six), which defines a macrocycle cavity size, functionalised calixarenes are suitable receptors for alkali (*1g, 2*), alkaline-earth (*1g, 2c–e, 3*), transition and heavy metal cations (*1g, 2d, 4*), anions (*5*), neutral (*6*) and chiral molecules (*7*). Complex formation was investigated using different spectroscopic techniques including NMR, UV–vis, vibrational and luminescence spectroscopy (*8*). Due to its high sensitivity, spectrofluorimetry received a considerable attention. Fluorimetric determination of cations based on calixarene derivatives with anthracene, naphthalene, pyrene and dansyl groups as fluorophore units are well compiled in the recent review articles (*9*). Two-armed calix[4]arene derivatives bearing tryptophan units were proposed as enantioselective fluorescent sensors for chiral carboxylates (*10*).

Calix[4]arene derivatives with oxygen donor atoms, such as calixarene esters, ketones, amides (including peptidocalixarenes), can selectively bind alkali and alkaline-earth cations (*2d, 11*). Recently, we have reported on the cation binding affinities of the calix[4]arene tetra(*O*-[*N*-acetyl-D-phenylglycine methyl ester]) derivative which was shown to be an efficient binder for Li^+ and Na^+ cations in acetonitrile (*12*). In view of the fact that tryptophan is a fluorescent amino acid, in the present paper, we report on the synthesis and binding abilities of two novel fluorescent calix[4]arene derivatives containing two and four tryptophan subunits at the lower rim. The binding properties of these compounds towards Eu(III) and alkali metal cations were examined by spectroscopic and electrochemical methods.

2. Results and discussion

2.1 Synthesis

The synthesis of peptidocalixarenes **1** and **2** in four reaction steps is shown in Scheme 1. The stepwise route (*11b, 13*) via the calix[4]arene tetraacetic acid, its chloride and L-tryptophan methyl ester hydrochloride (Et_3N, CH_2Cl_2) gave **1** in 55.0% yield. Calix[4]arene 1,3-*O*Me derivative **2** with two 2,4-*O*-(CH_2COTrpCOOMe) strands was prepared from a 1,3-dimethyl-2,4-(CH_2COOEt) derivative and its acid chloride and coupled with L-tryptophan methyl ester hydrochloride. Compound **2** was obtained in 63.5% yield. Calixarene derivatives were characterised by spectroscopic methods and high-resolution mass spectrometry. The ^{1}H

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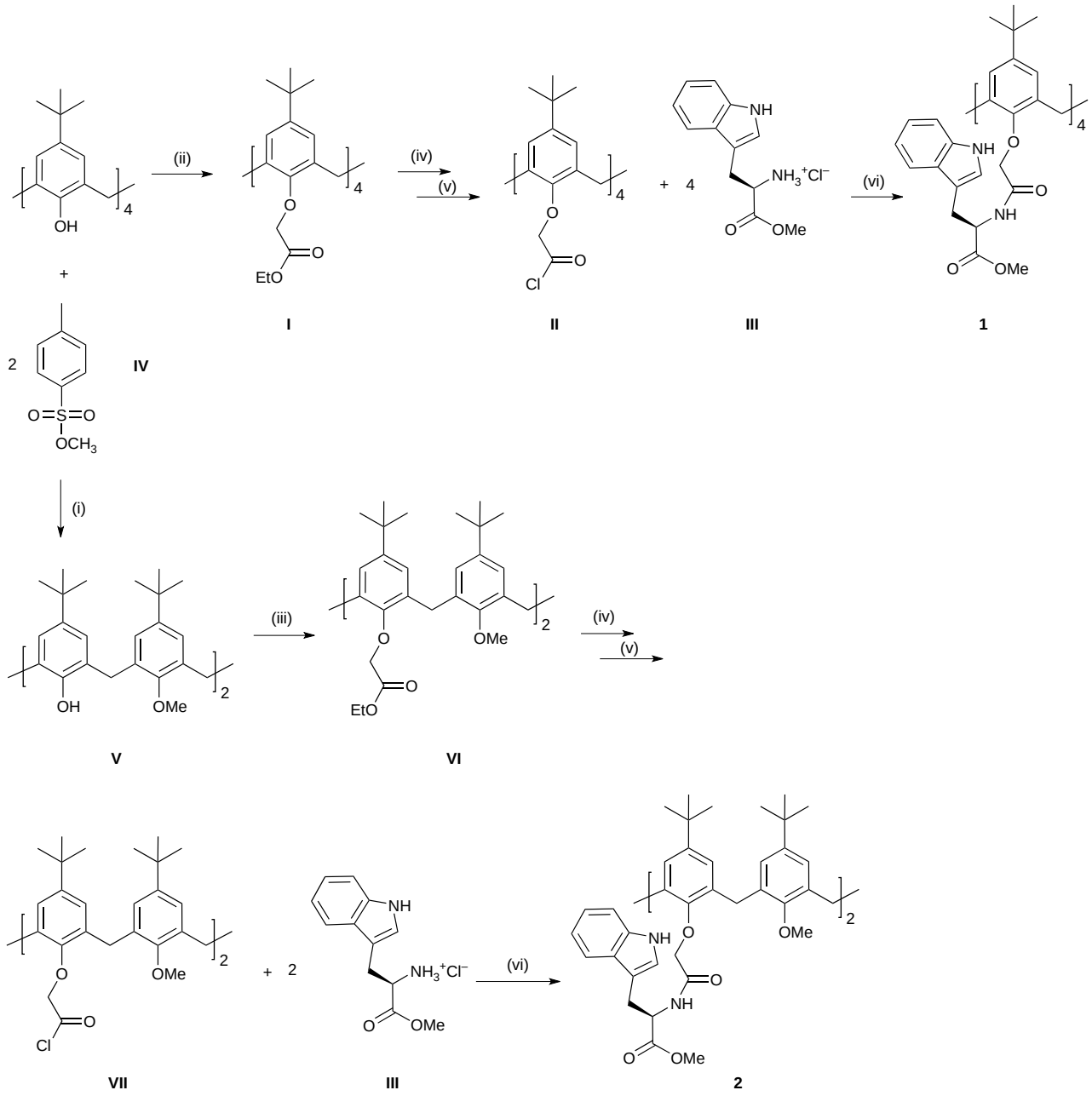

Scheme 1. Syntheses of compounds **1** and **2**. Reagents and conditions: (i) K_2CO_3, MeCN, Δ; (ii) Ethyl bromoacetate, K_2CO_3, acetone, Δ; (iii) Ethyl bromoacetate, NaH, THF (Ar), 0°C → Δ; (iv) NaOH, EtOH/H_2O; (v) $SOCl_2$ and (vi) Et_3N, CH_2Cl_2 (Ar), 0°C → r.t.

NMR spectrum of **1** in $CHCl_3$ showed two singlets due to *tert*-butyl groups ($\delta = 1.05$ ppm) and calixarene aromatic protons ($\delta = 6.63$ ppm), a pattern characteristic of *cone* conformation and C_4 symmetry of tetrasubstituted calix[4]arene (*10, 12*). Four sets of doublets due to the bridging methylene protons ($\delta = 2.87$ and 4.21 ppm) and $ArOCH_2$ protons ($\delta = 4.31$ and 4.46 ppm) indicated the structural rigidity and possible intramolecular hydrogen bonding interactions (*13*). A rather high chemical shift of NH protons ($\delta = 7.37$ ppm) also indicated the presence of intramolecular NH···O=C hydrogen bonds (*11b*). A complete assignment of signals corresponding to indole ring protons (see Section 3) was enabled by 2D (COSY and NOESY) ^{1}H NMR spectra. Contrary to **1**, in the chloroform solution at room temperature, disubstituted calix[4]arene **2** existed as a mixture of all possible conformational isomers. This was concluded on the basis of the much more complex ^{1}H NMR spectrum which showed the splitting of signals

(*4c*). Obviously, two —OCH_3 groups at a lower rim were not bulky enough to prevent conformational interconversion by rotation through the calixarene cavity.

2.2 Cation complexation studies

The hypochromic effect on the UV spectrum of the acetonitrile solution of **1** was observed upon addition of $LiClO_4$, $NaClO_4$, $KClO_4$ and $Eu(NO_3)_3$ (Figures 1(a) and 2(a)). In addition, a neat isosbestic point at 266 nm appeared in the case of the titration with Eu(III) (Figure 2(a)). In the titrations of **1** with lithium, sodium and europium cations, a linear relationship of absorbance vs. the amount of cation added was observed up to the ratio $n(\text{cation})/n(\mathbf{1}) \approx 1$, followed by a break in the titration curve (Figures 1(b) and 2(b)). These findings revealed a formation of 1:1 complexes and rather strong complexation (corresponding stability constants could only be estimated, Table 1). It should be noted that since the

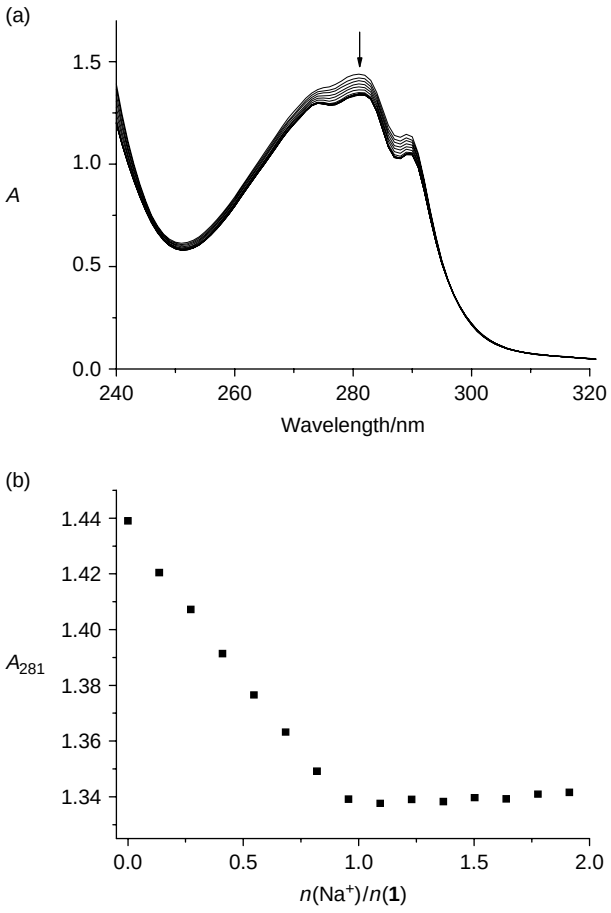

Figure 1. (a) Spectrophotometric titration of **1** ($c = 6.04 \times 10^{-5}$ mol dm^{-3}) with $NaClO_4$ in acetonitrile. $l = 1$ cm; $\vartheta = (25.0 \pm 0.1)$°C; $c(Na^+) = 0$ (top curve)–1.02×10^{-4} mol dm^{-3} (bottom curve); the spectra are corrected for dilution. (b) Dependence of absorbance at 281 nm on the $n(NaClO_4)/n(\mathbf{1})$ ratio.

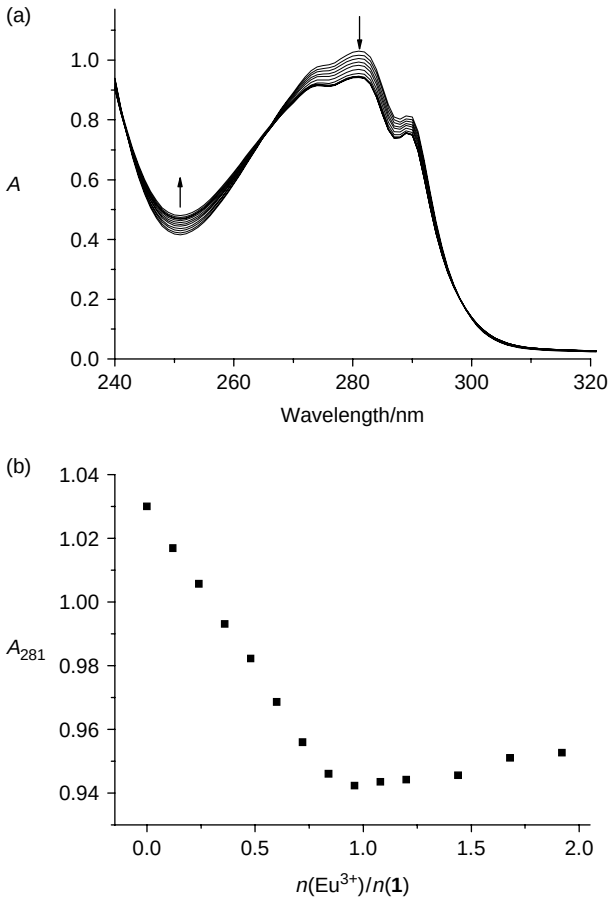

Figure 2. (a) Spectrophotometric titration of **1** ($c = 4.2 \times 10^{-5}$ mol dm^{-3}) with $Eu(NO_3)_3$ in acetonitrile. $l = 1$ cm; $\vartheta = (25.0 \pm 0.1)$°C; $c(Eu^{3+}) = 0$ (top curve)–5.38×10^{-5} mol dm^{-3} (bottom curve); the spectra are corrected for dilution. (b) Dependence of absorbance at 281 nm on the $n(Eu(NO_3)_3)/n(\mathbf{1})$ ratio.

$Eu(NO_3)_3$ absorbed in the same spectral region as a $Eu\mathbf{1}^{3+}$ complex, the increase in absorbance after the break in the titration curve was recorded (Figure 2(b)).

Although observable, the absorbance changes in the titration of **1** with $KClO_4$ were insufficient to enable their reliable quantitative processing. The addition of $RbNO_3$ and $CsNO_3$ into the ligand solution did not cause any significant changes in its UV spectrum, indicating that under conditions used no observable complexation took place.

The results of spectrophotometric titrations of **2** with lithium and sodium cations were similar to those of **1**. A rather strong complexation and formation of 1:1 complexes were observed (Table 1). The spectral changes recorded in the titrations of **2** with K^+ and Rb^+ were too small for the quantitative analysis, although they definitely indicated the complexation of these cations with ligand **2**. Almost no change in the UV spectrum of **2** was observed upon addition of $CsNO_3$ into the acetonitrile ligand

Table 1. Stability constants of complexes of europium and alkali cations with **1** and **2** in acetonitrile at 25°C.

	$\log(K/\text{mol}^{-1}\,\text{dm}^3) \pm \sigma$	
Cation	**1**	**2**
Li^+	>6[a]	>6[a]
Na^+	8.25 ± 0.01[b]	6.94 ± 0.01
K^+	5.09 ± 0.03[c]	4.09 ± 0.02
Rb^+	–[d]	3.44 ± 0.03
Cs^+	–[d]	–[d]
Eu^{3+}	6.16 ± 0.02[e]	–[d]

[a] Estimated by spectrophotometry and conductometry.
[b] Potentiometric determination.
[c] Conductometric determination.
[d] Addition of Rb^+, Cs^+ and Eu^{3+} salts into the calixarene solution had no significant effect on the absorbance and emission of **1** and **2**. Likewise, in conductometric titrations of Rb^+ and Cs^+ solutions, no significant changes in conductivity were observed.
[e] Spectrofluorimetric determination.

solution. Besides, contrary to Eu**1**$^+$, the formation of the Eu**2**$^+$ complex was not detected.

Excited at 280 nm, both calixarene derivatives emitted at 340 nm (Figure 3(a)). The fluorescence was associated with the tryptophan subunits of the investigated compounds. Thus, the shapes of the excitation and emission spectra of both compounds were basically the same. The tetrasubstituted calixarene **1** emitted strongly whereas compound **2**, bearing only two tryptophan subunits, emitted moderately under the same experimental conditions. The influence of europium and alkali metal cations on the emission spectra of **1** and **2** was examined at fixed molar ratio, n(metal ion):n(calixarene) = 5. The addition of Rb^+ and Cs^+ salts did not show any significant effect on the ligands emission. The other alkali metal cations, i.e. Li^+, Na^+ and K^+, affected the calixarene fluorescence, but the changes in the intensity were less than 15%. The addition of europium nitrate into the acetonitrile solution of **1** resulted in fluorescence quenching of almost 90%. For this reason, the complexation of europium was studied in more details, and the spectrofluorimetric titration of **1** with $Eu(NO_3)_3$ in acetonitrile was carried out (Figure 4). By processing the fluorimetric data, the stability constant of 1:1 Eu**1**$^{3+}$ complex was obtained (Table 1). The addition of Eu(III) nitrate into the acetonitrile solution of **2** did not cause any significant effect on the calixarene emission spectrum, indicating that no complexation took place under the experimental conditions used. This result was in accordance with the one obtained by UV–vis spectrometry.

In order to examine the findings obtained by spectrometric methods, conductometric titrations of alkali metal cations in acetonitrile solutions with calixarene derivatives were also performed. For Li^+ and Na^+ perchlorate solutions, an almost linear decrease in molar conductivities upon addition of **1** was observed. A break in the titration curve was recorded at the molar ratio

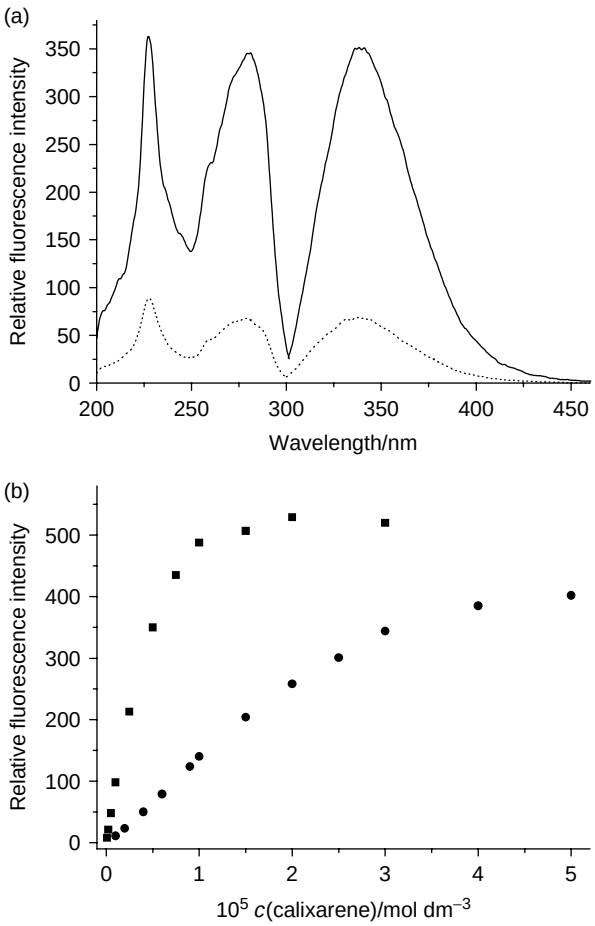

Figure 3. (a) Excitation and emission spectra of **1** (—) and **2** (---) in acetonitrile. $c(\mathbf{1}) = c(\mathbf{2}) = 5 \times 10^{-6}$ mol dm^{-3}. (b) Dependence of relative fluorescence intensity on the concentration of **1** (■) and **2** (•). $\lambda_{ex} = 280$ nm; $\lambda_{em} = 340$ nm; slit width = 4 nm.

n(**1**)/n(M$^+$) ≈ 1 (Figure 5). A decrease in molar conductivity was due to a lower electric mobility of the larger M**1**$^+$ complex compared to the free cation, and was also observed for K^+ (Figure 6). The latter data were analysed by non-linear least-squares regression, and the calculated stability constant is given in Table 1. During the titrations of acetonitrile solutions of $RbNO_3$ and $CsNO_3$ with **1**, almost no changes in molar conductivities were observed.

The addition of **2** in the Li^+ and Na^+ perchlorate solutions revealed the formation of 1:1 complexes. A linear decrease in molar conductivities and a break in the titration curves at the molar ratio n(**2**)/n(M$^+$) ≈ 1 were recorded (Figure 7(a) and (b)). Changes in molar conductivity upon addition of **2** in $KClO_4$ and $RbNO_3$ solutions are displayed in Figure 8(a) and (b). By processing these data in the same manner as for **1**, stability constants of the K**2**$^+$ and Rb**2**$^+$ complexes were calculated (Table 1). No changes in molar

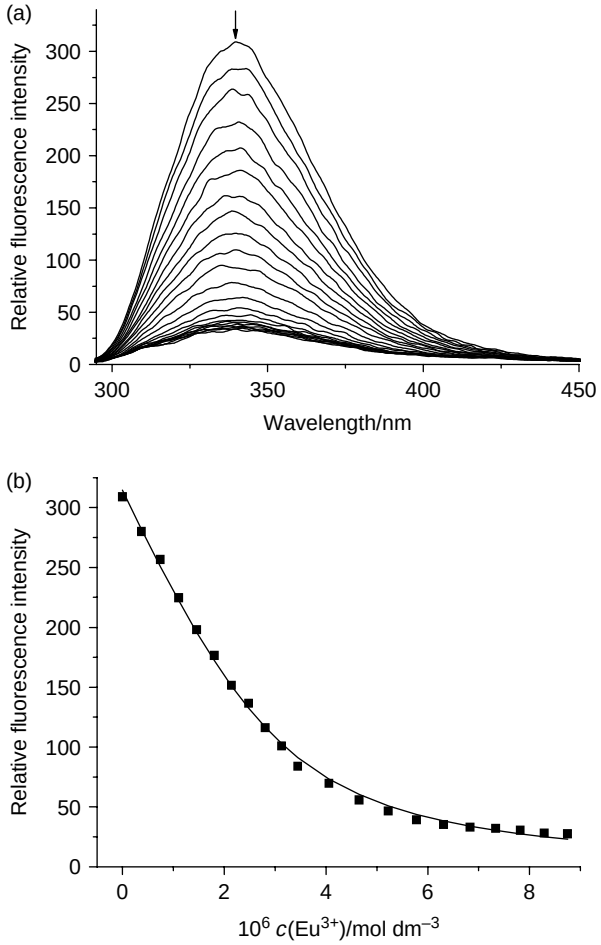

Figure 4. (a) Spectrofluorimetric titration of **1** ($c(\mathbf{1}) = 3.2 \times 10^{-6}$ mol dm^{-3}) with $Eu(NO_3)_3$ in acetonitrile. $c(Eu^{3+}) = 0$ (top curve)–8.75×10^{-6} mol dm^{-3} (bottom curve). (b) Dependence of relative fluorescence intensity on the Eu^{3+} concentration. $\lambda_{ex} = 280$ nm; $\lambda_{em} = 340$ nm; slit width = 4 nm; ■ experimental; — calculated.

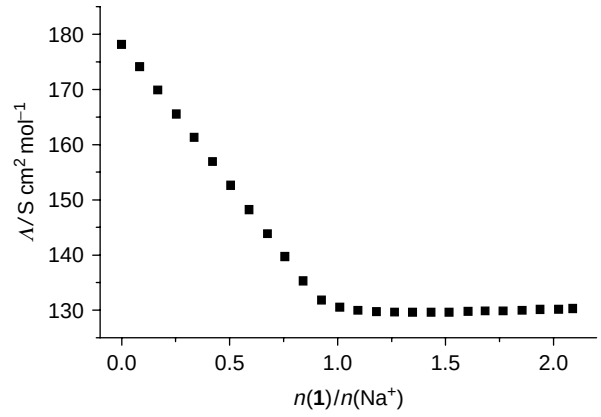

Figure 5. Conductometric titration of $NaClO_4$ with **1** in acetonitrile; $c(Na^+) = 2.01 \times 10^{-4}$ mol dm^{-3}; $\vartheta = (25.0 \pm 0.1)$°C.

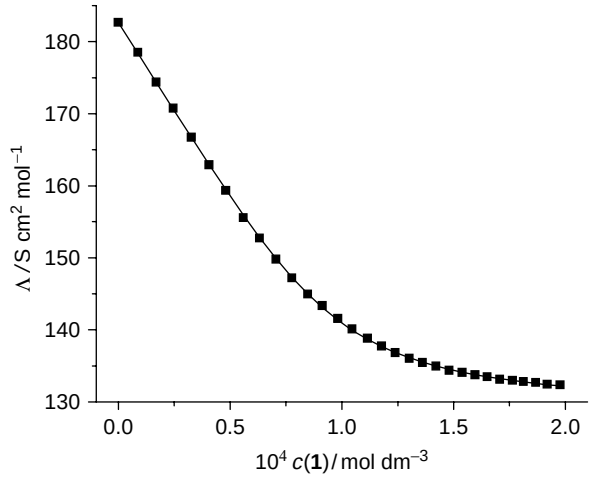

Figure 6. Conductometric titration of $KClO_4$ with **1** in acetonitrile. $c(K^+) = 1.02 \times 10^{-4}$ mol dm^{-3}; $c(\mathbf{1}) = 8.4 \times 10^{-4}$ mol dm^{-3}; $\vartheta = (25.0 \pm 0.1)$°C; ■ experimental; — calculated.

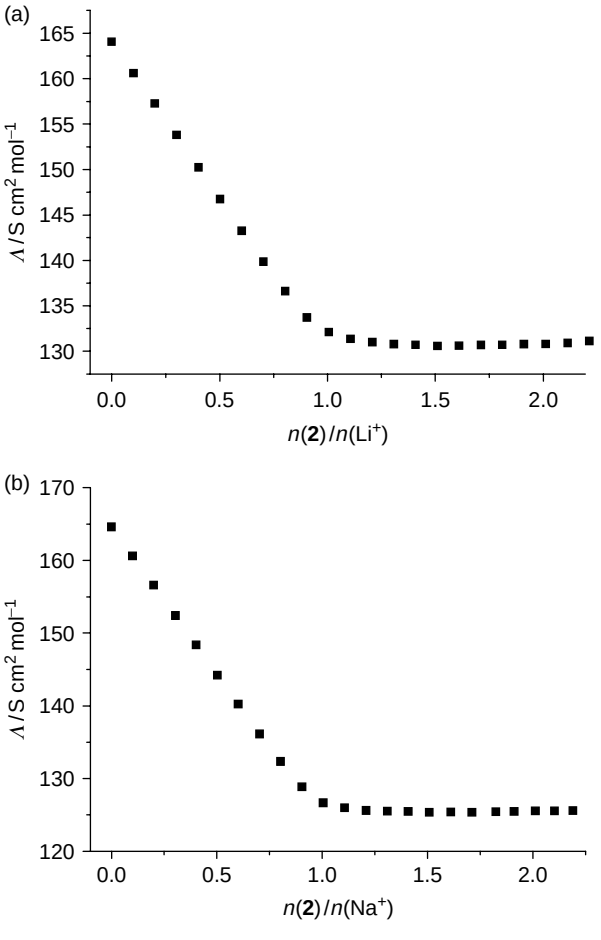

Figure 7. Conductometric titration of (a) $LiClO_4$ and (b) $NaClO_4$ with **2** in acetonitrile. $c(Li^+) = c(Na^+) = 2.02 \times 10^{-4}$ mol dm^{-3}; $\vartheta = (25.0 \pm 0.1)$°C.

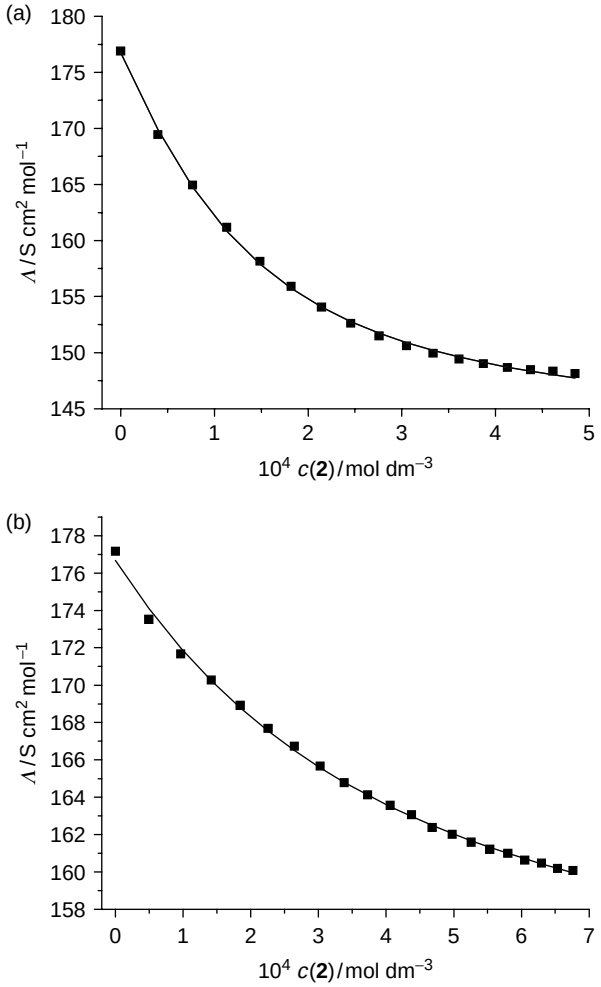

Figure 8. Conductometric titration of (a) $KClO_4$ and (b) $RbNO_3$ with **2** in acetonitrile. $c(K^+) = 1.01 \times 10^{-4}$ mol dm^{-3}; $c(Rb^+) = 1.02 \times 10^{-4}$ mol dm^{-3}; c(**2**) $= 2.0 \times 10^{-3}$ mol dm^{-3}; $\vartheta = (25.0 \pm 0.1)$°C; ■ experimental; — calculated.

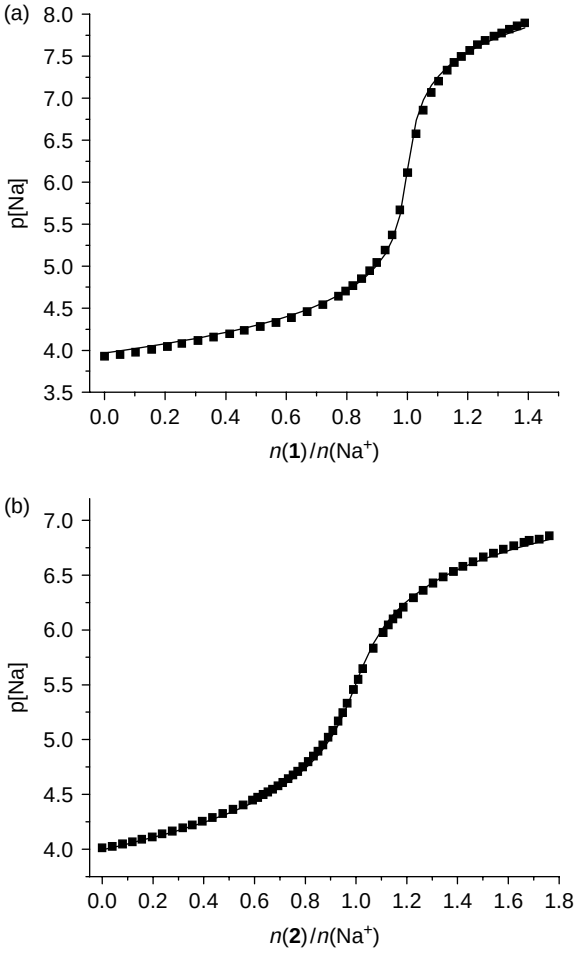

Figure 9. Potentiometric titration of $NaClO_4$ with (a) **1** and (b) **2** in acetonitrile. $V_0 = 30.3$ cm^3; $I_c = 0.01$ mol dm^{-3} (Et_4NClO_4); $\vartheta = (25.0 \pm 0.1)$°C; (a) c(**1**) $= 8.363 \times 10^{-4}$ mol dm^{-3}, $c_0(Na^+) = 1.07 \times 10^{-4}$ mol dm^{-3}; (b) c(**2**) $= 6.06 \times 10^{-4}$ mol dm^{-3}, $c_0(Na^+) = 1.01 \times 10^{-4}$ mol dm^{-3}. ■ experimental; — calculated.

conductivity were observed during the titration of $CsNO_3$ in the acetonitrile solution with **2**.

The stability constants of the Na**1**$^+$ and Na**2**$^+$ complexes in acetonitrile were too high for spectrophotometric and conductometric determination. For this reason, direct potentiometry using a sodium-selective glass electrode was applied. Potentiometric titrations of $NaClO_4$ with **1** and **2** are shown in Figure 9(a) and (b), respectively. A steep p[Na] jump occurred at the 1:1 n(**1**):$n(Na^+)$ and n(**2**):$n(Na^+)$ ratios, which was in accordance with the results obtained by the other techniques. However, the p[Na] jump was steeper in the case of ligand **1**, indicating the larger stability of the Na**1**$^+$ complex compared to Na**2**$^+$. The stability constants of these complexes obtained by processing potentiometric data using Hyperquad program are given in Table 1.

By comparison with the analogous peptidocalix[4]arenes (*11b, 12–14*), the cation binding site of **1** is expected to comprise four ether and four amide carbonyl oxygen atoms of the substituents at the lower rim. Accordingly, the binding site of compound **2** possesses two carbonyl oxygen atoms less, but, on the other hand, it is more flexible.

The above considerations are reflected in the results presented in Table 1. As can be seen, the affinity of **1** towards Na^+ and K^+ cations is considerably larger than that of **2**, the difference being more pronounced in the case of sodium. This is expected because both cations prefer coordination with eight oxygen atoms (four carbonyl and four ether), but Na^+ fits better than K^+ into the binding site formed by these atoms (as is the case of ligand **1**). This leads to the greater difference between the stability constants of Na**1**$^+$ and Na**2**$^+$ complexes compared to K**1**$^+$ and K**2**$^+$ (Table 1). As a consequence, the Na^+/K^+ selectivity of **1** expressed as the K_{Na1}/K_{K1} ratio is about

1400, whereas the corresponding selectivity of **2** is lower and amounts to approximately 700. Since no complexation of Rb^+ and Cs^+ ions with **1** was observed, it can be concluded that these (larger) cations are not compatible with the ion binding site of the investigated tetrasubstituted calix[4]arene. However, the binding site of the 1,3 disubstituted ligand **2** is flexible enough to accommodate the Rb^+ cation (Table 1).

Compound **1** binds Eu(III) rather strongly, whereas the complexation of Eu(III) with **2** cannot be observed (Table 1). This finding could be accounted for by considering that europium prefers higher coordination numbers (the most common are 8 and 9 (*15*)) which can be achieved in the complex Eu**1**$^{3+}$, while compound **2** offers only six coordinating atoms (see above). Although quite high, the stability constant of Eu**1**$^{3+}$ is more than two orders of magnitude lower than that of the Na**1**$^+$ complex. This could seem surprising since ionic radii of Eu^{3+} and Na^+ are similar (107 and 118 pm for coordination number 8, respectively (*16*)), and the europium cation is much more charged. Therefore, apart from the electronic structure of the cations examined, one would expect $K_{\mathrm{Eu}\mathbf{1}}$ to be even larger than $K_{\mathrm{Na}\mathbf{1}}$. The observed difference in complex stabilities can be partly explained by taking into account that Eu^{3+} with higher charge number and similar ionic radius is more strongly solvated than Na^+. Therefore, the replacement of solvent molecules by the coordination sites of the ligand during the complexation process is less favourable in the case of reaction of **1** with Eu(III).

The most attractive feature of the tetrasubstituted calix[4]arene tryptophan derivative **1** is its prominent fluorescence which is significantly altered by the presence of the europium cation (Figure 4), making such system interesting as a potential fluorimetric sensor for Eu(III).

3. Experimental

3.1 General

Melting points were determined with a Kofler apparatus. Optical rotations were measured on an AA-10 automatic polarimeter (Optical Activity Ltd, Ramsey, UK). IR spectra were acquired with a Bruker Vector 22 Fourier Transform spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with Bruker Avance 300 MHz. COSY and NOESY spectra were recorded to enable a complete assignment of signals. Mass spectra were obtained on a MALDI-TOF/TOF analyser (Applied Biosystems, Inc., Foster City, CA, USA) equipped with a 200 Hz, 355 nm Nd:YAG laser. The spectra were recorded in the CHCA MALDI matrix by averaging 1600 laser shots covering a mass range of *m/z* 700–2500. The ions from trypsin autolysis were used for internal calibration in positive ion mode (*m/z* 842.5094 and 2211.1040). UV spectra were obtained with a Varian Cary 5 double-beam spectrometer. Fluorescence spectra were measured on a Perkin-Elmer LS55B spectrofluorimeter. Conductometric measurements were performed using a Metrohm 712 conductometer. A Metrohm 713 pH meter was used for potentiometric measurements. A sodium-selective glass electrode (Metrohm) was used as the indicator electrode and Ag/AgCl (Metrohm) filled with 0.01 mol dm^{-3} Et_4NCl as the reference electrode. Commercially available reagents were used as received. The salts used for titrations in acetonitrile (Merck, Uvasol) were $LiClO_4$, $NaClO_4$, $KClO_4$, $RbNO_3$, $CsNO_3$ and $Eu(NO_3)_3{\cdot}5H_2O$ (Merck, p.a.). Compounds **I** (*17*), **II** (*18*), **III** (*19*), **IV** (*20*), **V** (*21*), **VI** and **VII** (*22*) used in the synthesis of **1** and **2** (Scheme 1) were prepared according to the procedures described in the literature.

3.2 Synthesis of 5,11,17,23-tetra-tert-butyl-25,26,27,28-tetrakis(O-methyl-L-tryptophanylcarbonylmethoxy)calix[4]arene (1)

L-Tryptophane methyl ester hydrochloride (0.98 g, 3.85 mmol) was suspended in dry CH_2Cl_2 (40 cm^3) and cooled (−10°C). After the addition of triethylamine (1.1 cm^3, 7.88 mmol), the mixture was stirred for 30 min under argon. Acid chloride of calix[4]arene tetraacetic acid (the cone conformer; 834.0 mg, 0.873 mmol) in dry CH_2Cl_2 (40 cm^3) was added at once by a syringe. After 1 h, the mixture was warmed up to room temperature, stirred for additional 18 h and then extracted with NaOH ($c = 1$ mol dm^{-3}), water, HCl ($c = 1$ mol dm^{-3}), brine and water. The organic layer was dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the product was recrystallised from CH_2Cl_2–light petroleum to give **1** (890 mg, 55%). $R_f = 0.53$ (MeOH–CH_2Cl_2 = 10:90); Mp 138–141°C; $[\alpha]_D^{25} = +22°$ ($\gamma = 1$ g dm^{-3}, CH_2Cl_2); IR (KBr) ν_{max} (cm^{-1}) = 3417 (NH), 1740 (COOMe), 1668 (CONH, amide I), 1538 (CONH, amide II), 1195 (COC); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta_H = 1.05$ (36H, s, *t*-Bu), 2.87 (4H, d, $J = 13.17$, $ArCH_{2eq}Ar$), 3.29 (8H, d, $J = 2.28$, CH_2-ArTrp), 3.52 (12H, s, $COOCH_3$), 4.21 (4H, d, $J = 12.99$, $ArCH_{2ax}Ar$), 4.31 (4H, d, $J = 14.19$, $OCH_{2A}CO$), 4.46 (4H, d, $J = 14.19$, $OCH_{2B}CO$), 4.87 (4H, d, $J = 6.42$, NC∗HCO), 6.63 (8H, s, ArH), 6.86 (4H, d, $J = 1.14$, ArTrp-H2), 7.04 (4H, t, ArTrp-H5), 7.14 (4H, t, ArTrp-H6), 7.24 (4H, d, $J = 8.04$, ArTrp-H7), 7.37 (4H, d, $J = 7.02$, CONH), 7.47 (4H, d, $J = 7.68$, ArTrp-H4), 8.38 (4H, s, ArNHTrp) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta_C = 27.18$ (CH_2-Trp), 30.86 (Ar-CH_2-Ar), 31.38 ($C(CH_3)_3$), 33.84 ($C(CH_3)_3$), 52.32 (∗CH), 52.97 ($COOCH_3$), 73.55 (OCH_2CO), 109.94 (ArTrp-C3), 111.37 (ArTrp-C7), 118.38 (ArTrp-C4), 119.47 (ArTrp-C6), 121.97 (ArTrp-C5), 123.33 (ArTrp-C2), 125.45 (*ortho*-Ar-C), 125.66 (*ortho*-Ar-C), 127.50

(ArTrp-C3a), 132.57 (*meta*-Ar-C), 132.87 (*meta*-Ar-C), 136.07 (ArTrp-C7a), 145.24 (Ar-C-*t*-Bu), 152.57 (*para*-Ar-C), 169.89 (CONH), 172.70 (*C*OOMe) ppm. HR-MS: 1703.8030 $(M + Na)^+$ (calcd for $C_{100}H_{112}N_8O_{16}Na$ 1703.8089).

3.3 *Synthesis of 5,11,17,23-tetra-*tert*-butyl-25,27-di(*O*-methyl)-26,28-bis(*O*-methyl-*L*-tryptophanylcarbonylmethoxy)calix[4]arene (2)*

Triethylamine (0.68 cm^3, 4.9 mmol) was added to the cooled (−10°C) solution of L-tryptophane methyl ester hydrochloride (668.4 mg, 2.62 mmol) in dry CH_2Cl_2 (50 cm^3) and stirred for 30 min under argon. Upon addition of **VII** (940 mg, 1.093 mmol) in dry CH_2Cl_2 (20 cm^3), the ice-cooled mixture was stirred for several hours, and then for additional 15 h at room temperature. After filtration, the filtrate was extracted with NaOH ($c = 1\,mol\,dm^{-3}$), water, HCl ($c = 1\,mol\,dm^{-3}$), brine and water. After drying with anhydrous $MgSO_4$ and evaporation of the organic solvent, the residue was recrystallised from the MeOH–H_2O mixture to give **2** (850 mg, 63.5%). $R_f = 0.30$ (MeOH–CH_2Cl_2 = 5:95); Mp 130–132°C; $[\alpha]_D^{25} = +25°$ ($\gamma = 1\,g\,dm^{-3}$, CH_2Cl_2); IR (KBr) ν_{max} (cm^{-1}) = 3416 (NH), 1745 (COOMe), 1681 (CONH, amide I), 1519 (CONH, amide II), 1208 (COC); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta_H = 0.79$ (18H, s, *t*-Bu), 1.35 (18H, s, *t*-Bu), 2.95–4.44 (28H, m, br m, CH_{eq}, CH_{ax}, OCH_A, OCH_B, OCH_3, $OOCH_3$, CH_2-ArTrp), 5.13 (2H, q, NC∗HCO), 6.34–6.48 (8H, m, br m, Ar-H), 6.88–7.60 (12H, m, ArTrp-H, CH_2-ArTrp), 8.17 (2H, s, ArTrp-NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta_C = 27.50$ (CH_2-Trp), 31.02 (C(CH_3)$_3$), 31.72 (C(CH_3)$_3$), 31.07 (Ar-CH_2-Ar), 33.58 (*C*(CH_3)$_3$), 34.18 (*C*(CH_3)$_3$), 52.43 (∗CH), 52.83 (COO*C*H_3), 60.09 (ArO*C*H_3), 74.06 (O*C*H_2CO), 110.16 (ArTrp-C3), 111.30 (ArTrp-C7), 118.46 (ArTrp-C4), 119.77 (ArTrp-C6), 122.26 (ArTrp-C5), 122.54 (ArTrp-C2), 124.66, 124.76, 125.66, 125.76 (*ortho*-Ar-C), 127.71 (ArTrp-C3a), 130.99, 131.03, 135.29, 135.30 (*meta*-Ar-C), 136.04 (ArTrp-C7a), 145.09, 145,57 (Ar-C-*t*-Bu), 151.70, 155.09 (*para*-Ar-C), 169.15 (CONH), 172.46 (*C*OOMe) ppm. HR-MS: 1215.6353 $(M + Na)^+$ (calcd for $C_{74}H_{88}N_4O_{10}Na$ 1215.6393).

3.4 *Spectrophotometry and spectrofluorimetry*

UV titrations of **1** and **2** in acetonitrile ($V = 2.0\,cm^3$, $c_{\mathbf{1}} = 6 \times 10^{-5}\,mol\,dm^{-3}$, $c_{\mathbf{2}} = 1 \times 10^{-4}\,mol\,dm^{-3}$) were performed at (25.0 ± 0.1)°C by the stepwise addition of Eu(III) or the alkali salt solution ($c = 1 \times 10^{-3}\,mol\,dm^{-3}$) directly into the measuring quartz cell ($l = 1\,cm$).

The fluorimetric titration was carried out by the stepwise addition of Eu(III) nitrate ($c = 3.8 \times 10^{-5}\,mol\,dm^{-3}$) to the calixarene solution ($V = 2.0\,cm^3$, $c_{\mathbf{1}} = 3.2 \times 10^{-6}\,mol\,dm^{-3}$) placed in the measuring quartz cell.

The obtained spectrometric data were processed using SPECFIT program (*23*).

The influence of alkali metal cations on the emission spectra of **1** and **2** was studied at fixed molar ratio, $n(M^+)/n(\text{calixarene}) = 5$ ($c_{\mathbf{1}} = 4 \times 10^{-6}\,mol\,dm^{-3}$, $c_{\mathbf{2}} = 2 \times 10^{-5}\,mol\,dm^{-3}$, $\lambda_{ex} = 280\,nm$, $\lambda_{em} = 340\,nm$, slit width = 4 nm).

3.5 *Conductometry*

Conductometric titrations were carried out in acetonitrile at (25.0 ± 0.1)°C. The cell constant (0.849 cm^{-1}) was determined using 0.1 $mol\,dm^{-3}$ aqueous KCl solution. The alkali salt solution ($V_0 = 20.0\,cm^3$, $c_0 \approx 1 \times 10^{-4}\,mol\,dm^{-3}$ or $2 \times 10^{-4}\,mol\,dm^{-3}$) was titrated with the ligand solution ($c \approx 1 \times 10^{-3}\,mol\,dm^{-3}$ or $2 \times 10^{-3}\,mol\,dm^{-3}$) in a closed, thermostated titration vessel. The measured conductivities were corrected for the conductivity of the solvent.

3.6 *Potentiometry*

The stability constants of Na**1**$^+$ and Na**2**$^+$ complexes in acetonitrile were determined at (25.0 ± 0.1)°C by potentiometric titration of 30.3 cm^3 $NaClO_4$ solution ($c_0 \approx 1 \times 10^{-4}\,mol\,dm^{-3}$) with the solution of **1** or **2** ($c \approx 1 \times 10^{-3}\,mol\,dm^{-3}$) in a thermostated titration vessel. The ionic strength of all solutions was set to 0.01 $mol\,dm^{-3}$ by Et_4NClO_4. The cell was calibrated by the incremental addition of $NaClO_4$ solution (0.01 $mol\,dm^{-3}$) to 30.0 cm^3 of 0.01 $mol\,dm^{-3}$ solution of Et_4NClO_4. The potentiometric data were analysed using HYPERQUAD program (*24*).

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