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Substituent dependent fluorescence response of diazacrown-based PET sensors

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Dedicated to Professor Kálmán Medzihradszky on the occasion of his 80th birthday

ABSTRACT

The sensing ability of three 1,10-diaza-18-crown-6 based sensors bearing a coumarin fluorophore was studied and compared with the analogous monoaza-18-crown-6 sensor. Coordination experiments between hosts and organic ammonium salts with varying steric demand were studied using fluorescence and ^{1}H NMR spectroscopy. We have found that the fluorescent signal generated on complexation was greatly influenced by the structure of the sensor. Surprisingly, in two cases, we observed the decrease of fluorescence on complexation, which was attributed to changes in the conformational dynamics of the sensors on complexation.

1. Introduction

A large number of today's fluorescent chemosensory systems exploit the photoinduced electron transfer (PET) phenomenon.[1] Guest binding to the receptor unit changes the redox potential of an adjacent donor site, which reverses its ability to quench the fluorescence of the fluorophore part resulting in measurable fluorescence enhancement (OFF–ON systems). The generality and efficiency of this switching process led to the common use of PET sensors in numerous fields from simple ion sensing to logic gates.[2]

The effect of guest binding on the PET process is mostly rationalized by the guest's coordinative interaction with the donor site. There are certain cases for common PET sensors, however, where the formation of secondary interactions between the guest and the donor site in the host does not give a satisfactory explanation for the observed change of fluorescence.[3] This suggests that in these sensors change in the conformational dynamics of the receptor part might also play a significant role in signal generation, which has been studied for some unique structures.[4] The role of steric perturbation and conformational constraints at binding site has rarely been investigated in detail so far.[5]

In our recent report, we have introduced a new sensor design, which used the influence of conformational dynamics on the PET process (Fig. 1).[6a] These new sensors gave comparable or even greater signals in the presence of guest molecules with the salient feature of their increased sensitivity toward the steric demand of guests. These experiments have pointed out that the conformational mobility of the donor site's surroundings has a profound effect on its signaling potential.[6] Our continuing efforts at understanding signal generation in common sensor types to track down the effects of conformational dynamics on their signal generation process are presented herein.

2. Results and discussion

Four 18-crown-6 based sensors were selected for the present study: **1**[3b] contains an azacrown host unit and an attached coumarin fluorophore, while **2a**–**c** have a 1,10-diazacrown core with either two coumarin units (**2a**)[7] or pendant coumaryl and benzyl (**2b**) or *tert*-butoxycarbonyl-methyl groups (**2c**) (Fig. 2). According to the classical working hypothesis, the electron-donating nitrogen atoms of the azacrown moieties quench the luminescence of the attached coumarins as long as they are not perturbed by any

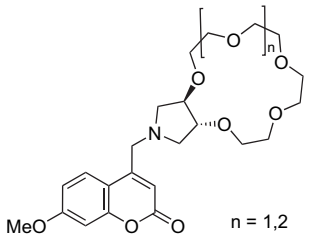

Figure. 1. Sensors where signal generation are invoked by changes in the conformational dynamics of the molecule.

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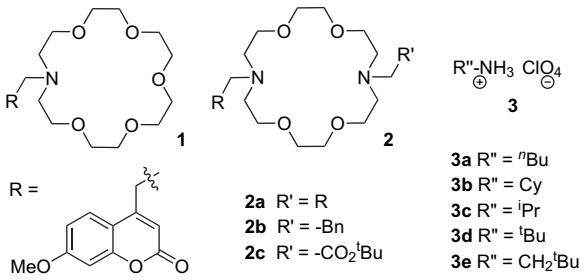

Figure. 2. The studied PET sensors and guests.

secondary interaction. On complexation, hydrogen bonding or protonation, the redox potential of the donor nitrogen is increased, weakening its donating capability that leads to an increase in the fluorescence intensity.

To examine the strength of the complexation between selected guests and sensors, we conducted a series of ^{1}H NMR spectroscopic experiments. The stoichiometry of the complexes between *n*-butylammonium perchlorate (**3a**) and **1** or **2a** (Fig. 3, inset) was established as 1:1 by continuous variation method (Job's plot).[8]

We also determined the stability constants for selected sensor (**1** and **2a**,**b**)–guest (**3a**,**d**,**e**) complexes. Among these complexes, medium-strength binder hosts (**2a**,**b**) were studied by direct chemical shift titrations with the corresponding guest in CH_2Cl_2/$CDCl_3$/CD_3OD 90/9/1 v/v/v%. Figure 3 shows the representative results[9]

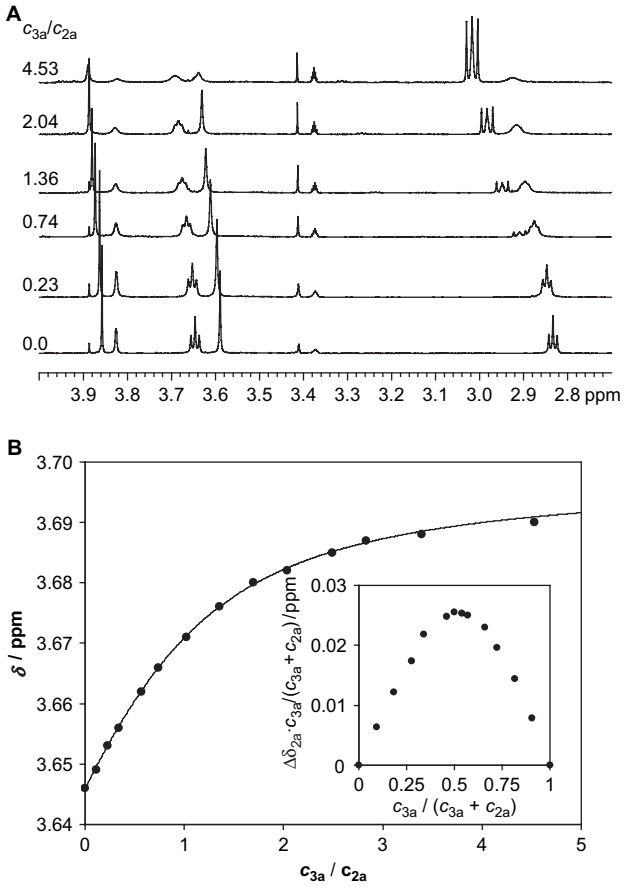

Figure 3. (A) ^{1}H NMR (600 MHz) partial spectral series obtained in the titration of 0.5 mM **2a** with 3.8 mM **3a** (CH_2Cl_2/$CDCl_3$/CD_3OD 90/9/1, 25 °C). (B) Chemical shift of crown O–CH_2 of **2a** upon titration with **3a**. Inset: Job's plot for the same chemical shift versus guest mole fraction (c_{2a}+c_{3a}=1 mM).

Table 1
Stability constants (log *K*) of complexes (uncertainties in parenthesis are estimated standard deviations of the last significant unit)

	1	2a	2b
3a	6.0 (1)	3.53 (3)	4.54 (2)
3d	4.62 (3)	2.75 (8)	4.53 (3)
3e	5.23 (8)	2.84 (8)	5.06 (5)

and the measured binding constants are collected in Table 1. Since log K>5 values are accessible only by competition titrations,[10] in experiments including the high-affinity host **1**, the host was added to the corresponding ammonium complex of a medium-strength binder host (e.g., **2a**).

The NMR titrations show that, of the studied sensors, **1** has the highest affinity toward the ammonium salts, and **2a** the lowest. In the case of **2b**, the determined stability constants lie between those of **1** and **2a**, although for ammonium salts of increased steric demand the experimental values are closer to **1**. It is more difficult to establish a trend in the binding strength of the different ammonium salts with the same sensor, although we might state that **3d** has a smaller binding constant in each case than the other salts.

To establish the efficiency of the PET process in the studied sensors (that also corresponds to the highest available signal on complexation), the fluorescence enhancements (FEs) observed on their protonation using excess HBF_4 were examined. We compared the fluorescence of the sensor molecules and their protonated forms, where the PET process is completely blocked (Table 2). Sensors bearing a diazacrown moiety have all shown increased signaling potential, compared to **1**, regardless of the number of the fluorophore units. The fluorescence enhancements in the **2** series were 3–4 times higher than that of **1**. The lower signaling ability of **1** originates in the less efficient fluorescence quenching by PET in its non-protonated form (compared to **2a**–**c**). This finding is in agreement with the earlier observations on phenanthridinyl,[11] and pyrenylmethyl[12] substituted mono- and diazacrown systems. The rationale for this peculiarity might either invoke the self-quenching of the fluorescence in **2a**–**c** having *syn*-aligned chromophores or explain the differences on the basis of restricted conformational mobility around the donor sites in **2a**–**c**. The *syn*-alignment of the coumarin units is unlikely since by measuring the fluorescence of **2a** at various concentrations we observed no sign of excimer formation.[13] Moreover, the X-ray structure[14] of **2a** (Fig. 4) also indicates the preferential trans-alignment of the fluorophores in the solid phase. The molecule has an inversion center, which is mirrored by the space group symmetry having half of the molecule in the asymmetric unit.

Since the conformational mobility of the donor site's surroundings has a profound effect on its fluorescence quenching, we presume that restricting the conformational freedom of the crown moiety statistically increases the occurrence of such conformers, where the donor site is more efficient in quenching the fluorescence. The decreased signaling potential of **1** is the result of the fact that one bulky substituent causes less constraint on the conformational mobility of the 18-membered ring system than two of them (as in **2a**–**c**).

Next, we conducted a series of coordination experiments with different organic ammonium salts including *n*-butyl, cyclohexyl, isopropyl, *tert*-butyl, and neopentylammonium perchlorates (**3a**–**e**, respectively). The ammonium salts were selected on the basis of

Table 2
Fluorescence enhancement observed on blocking the PET process in **1** and **2a**–**c** by protonation of the sensor molecules (see Supplementary data for experimental details)

Sensor	1	2a	2b	2c
FE	38	135	149	169

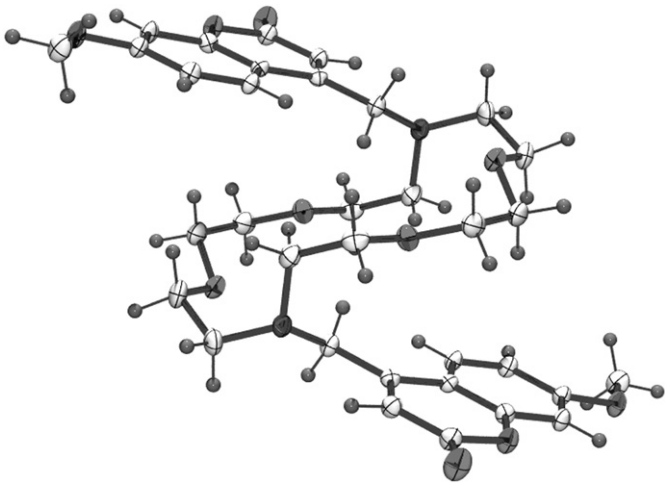

Figure 4. X-ray structure of **2a**.

their similar binding modes but various steric demands. The measured fluorescence enhancements (FEs) for the different host–guest combinations are summarized in Figure 5. Comparing sensors **1** and **2a**, the measured FE values with guests **3a**–**e** show a similar pattern, the values in the **2a** series being significantly higher. Bearing in mind that the binding constants for **1** are 2 orders of magnitude higher than that for **2a**, and that the binding of the ammonium salts proceeds in a similar manner, the increased signaling potential of **2a** is striking.

The similar pattern in the enhancement values observed for **1** and **2a** can be rationalized on the basis of the proton-donating abilities (acidity) of guests **3a**–**d**. The order of the FE values for both sensors correlates with the proton-donating abilities of **3a**–**d**, since the stronger the proton-donating ability of the ammonium ion is, the more it depletes the electron density of the donor nitrogen atom. Although the literature data on the acidity of **3e** show some variation, the measured FE values for **3e** in the **1** and **2a** series are most likely in line with the hydrogen-bond donor ability of the neopentylammonium moiety.

The fact that **2a** shows increased FE values points to the existence of a signal amplification process, which might originate in the conformational control of the electron transfer process, an area little studied so far in related systems.[5,15] Based on the measured complex stabilities, we can state that the relative amount of complexed sensors for **2a** is lower than that for **1** for a given guest, therefore, the higher signal for **2a** cannot originate solely in secondary host–guest interactions. We presume that guest binding in **2a** invokes a more drastic change of the conformational dynamics of the crown ether unit, and this decreases the fluorescence quenching ability of the ring nitrogen atom.

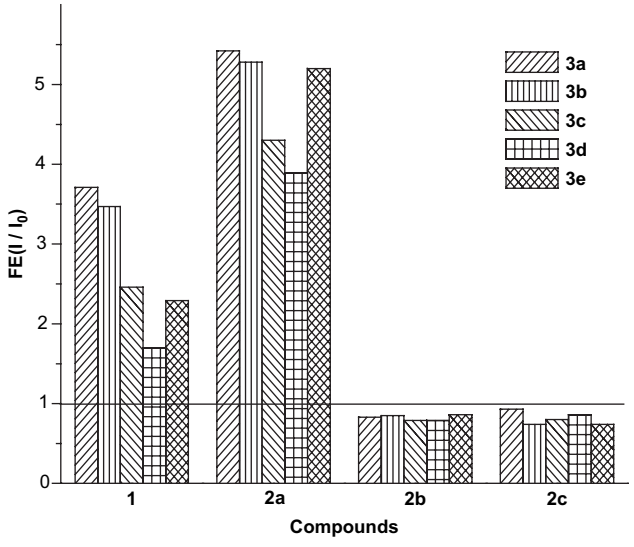

Figure 5. Enhancement of the fluorescent signal of sensors **1** and **2a**–**c** on binding ammonium salts **3a**–**e** in 1% MeOH in CH_2Cl_2.

In **1**, the guest can approach the macrocycle without encountering steric hindrance of the chromophore, therefore, conformational changes in this sensor are mainly induced by the crown ether–guest coordinative interaction.[16] Accepting that in its preferred conformation the fluorophore units in **2a** are located on the opposite sides of the azacrown unit, sterically hindering the approach of the guest from both sides, it is evident that conformational changes on guest binding have to be more significant than that for **1** since the movement of at least one of the coumarins is a prerequisite for guest capturing. The change in the conformational characteristics of the macrocycle can lead to a significant decrease in the efficiency of the PET process and a highly increased fluorescence signal.[6] Superposition of this dynamic effect on the effect of the secondary interactions (that are similar for both sensors) results in an increased FE.

Further evidence of the different measures of guest induced structural alterations in **1** and **2a** is provided by comparing the changes in the ^{1}H NMR chemical shifts of selected nuclei (Table 3). Evidently, the guests have a more profound effect on the crown frame in the case of **2a**, which is in agreement with the results of the fluorescence measurements.

To extend our study, the complex formation between sensors **2b** and **2c** and ammonium salts **3a**–**e** was also examined in detail. Since the signaling potentials of **2b** and **2c** are similar to **2a** (see Table 2), we expected a similar complexation behavior. Interestingly, upon complexation of ammonium salts **3a**–**e** the fluorescence of **2b** and **2c** showed a moderate but persistent decrease (Fig. 5). The observation that such a PET sensor gives a fluorescence decrease (quenching) upon guest binding is quite unprecedented and could not be rationalized on the basis of secondary interactions. Consideration of the structural differences between **2b**,**c** and **2a** led to the assumption that in **2b** and **2c** the ring nitrogen atom next to the coumarin unit does not participate in the complexation.

The reason behind this is that the benzylic- and the *tert*-butoxycarbonyl-methyl-amine moieties are significantly more basic than the coumarylmethyl-amine moiety.[17] Assuming that guest binding in **2b** and **2c** occurs preferentially to the more basic nitrogen, this directs guest coordination opposite to the fluorophore's side, which leads only to the conformational changes in the macrocycle and no direct secondary interaction between the guest and the coumarylmethyl nitrogen. This means that the changed redox properties of the said nitrogen atom are the result of changes in the conformational dynamics of its environment. The observed changes in fluorescence therefore stem from the altered conformational dynamics of the sensor. Such spatial arrangements might become favorable around the coumarylmethyl nitrogen on guest binding (e.g., an arrangement of atoms similar to that of pyrrolidine) that lead to a more efficient fluorescence quenching by PET.[6]

Table 3
Maximal changes of ^{1}H NMR chemical shifts ($\Delta\delta$) of **1** and **2a** in the presence of 5 equiv of guests added (the studied signals are that of N–CH_2)

	1	2a
3a	0.052	0.112
3e	0.061	0.228

The foundation of this reasoning is the hypothesis that the guests do differentiate the two nitrogen atoms in **2b** and **2c**. To provide experimental support for this, **2c** (where the various *N*-CH_2 signals could be differentiated) was titrated with acid and the process was monitored by ^{1}H NMR. On the addition of acid, the signal next to the ester unit showed a more significant change than the signal of the methylene group next to the coumarin unit. It is evident from this experiment that the protonation in this system occurs preferentially next to the *tert*-butoxycarbonyl-methyl moiety, supporting our assumption (for more details see Supplementary data).

3. Conclusions

In summary, the present study—directed at comparing the fluorescent signal generation in different diazacrown-based sensors—revealed that the changes in the conformational mobility of these sensors induced by guest binding have a profound effect on their signaling. We demonstrated that the diazacrown ether based sensor with two coumarin fluorophore units gives an increased fluorescent signal on the complexation of alkylammonium ions compared with the appropriate monoazacrown ether based sensor. Very surprisingly, studying the other two similar sensor molecules we observed signal weakening on sensing, a behavior quite unique so far. The origin of the altered signaling (i.e., increase in some cases and decrease in others) was linked to the changes in the sensors' conformational dynamics on complexation.

Moreover, these results confirmed that effects derived from conformational dynamics are present in sensors of very simple design and are not a peculiarity coupled with complex frameworks. Exploiting the effect of conformational mobility on signal generation, one might be able to devise sensors that have enhanced sensitivity toward the steric demand of the guests, a possibility the investigation of which is underway in our laboratory.

4. Experimental

4.1. General

Unless otherwise indicated, all starting materials were obtained from commercial suppliers (Aldrich, Fisher, Merck) and were used without further purification. Analytical thin-layer chromatography (TLC) was performed on Polygram SIL G/UV 254 pre-coated plastic TLC plates with 0.25 mm silica gel from Macherey–Nagel & Co. Silica gel column chromatography was carried out with Flash silica gel (0.040–0.063 mm) from Merck. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-250 spectrometer. Chemical shifts (δ) are presented in parts per million using TMS as internal standard. Coupling constants (J) are reported in hertz (Hz). Splitting patterns are designated as s (singlet), d (doublet), t (triplet), m (multiplet), and dd (doublet doublet). IR spectra were obtained on a Bruker IFS55 spectrometer on a single-reflection diamond ATR unit. High-resolution mass spectrometric analyses were performed on a Bruker MicroTOF-Q equipment using electrospray ionization by the Laboratory for Mass Spectrometry, Department of Applied Chemistry, University of Debrecen, Debrecen, Hungary.

4.2. Synthesis of sensors 2b and 2c

4.2.1. Synthesis of *2b*

4-(Chloromethyl)-7-methoxycoumarin (0.360 g, 1.60 mmol) and 1-benzyl-1,10-diaza-18-crown-6[18] (0.360 g, 1.02 mmol) were refluxed in CH_2Cl_2 (20 mL) in the presence of triethylamine (0.42 mL) for 12 h. The reaction mixture was allowed to cool to rt and concentrated in vacuo. The product was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 50:1 v/v%) to give 0.130 g **2b** as a yellow oil (24%). R_f=0.20 (10% MeOH in CH_2Cl_2). IR (neat) ν=1610, 1715, 2860, 3059 cm^{-1}. ^{1}H NMR ($CDCl_3$ vs TMS) δ=2.72–2.87 (8H, m, 4×–$NCH_{2(crown)}$), 3.46–3.70 (18H, m, 8×$CH_{2(crown)}$+CH_2–), 3.77 (2+3H, s, CH_2–, CH_3O–), 6.43 (1H, s, $CH_{C3(coumarin)}$), 6.70–6.79 (2H, m, $CH_{C6,C8(coumarin)}$), 7.10–7.30 (5H, m, Ar), 7.69 (1H, d, J=8.8 Hz, $CH_{C5(coumarin)}$). ^{13}C NMR ($CDCl_3$ vs TMS) δ=53.3, 53.5, 54.3, 55.5, 56.4, 59.5, 69.4, 69.5, 69.7, 70.5, 100.5, 111.3, 111.9, 112.3, 125.6, 126.9, 128.0, 128.9, 138.6, 153.8, 155.3, 161.4, 162.2. HRMS calcd for $C_{30}H_{41}N_2O_7$: 541.2914 $(M+H)^+$, found: 541.2953.

4.2.2. Synthesis of 1-[(7-methoxy)-4-ylmethylcoumarin]-1,10-diaza-18-crown-6

A mixture of 1,10-diazacrown-6 (0.300 g, 1.14 mmol), cesium carbonate (0.441 g, 2.28 mmol), and potassium iodide (0.010 g, 0.06 mmol) in dry THF (20 mL) was heated to reflux and a solution of 4-(chloromethyl)-7-methoxycoumarin (0.272 g, 1.21 mmol) in THF (40 mL) was added over 2.5 h. After the addition was complete, the reaction mixture was refluxed for 18 h. The reaction mixture was allowed to cool to rt and then it was filtered through a pad of Celite. The filtrate was concentrated in vacuo. Product was purified on silica gel using acetone and acetone–triethylamine (10:1 v/v%) as eluent to give the title compound as a yellow oil, 0.130 g (25%). R_f=0.11 (10% MeOH in CH_2Cl_2). IR (neat) ν=1608, 1713, 2864, 3380 cm^{-1}. ^{1}H NMR ($CDCl_3$ vs TMS) δ=2.71 (4H, t, J=4.7 Hz, 2×$NCH_{2(crown)}$), 2.78 (4H, t, J=5.4 Hz, 2×$NCH_{2(crown)}$), 3.42–3.60 (17H, m, 8×$CH_{2(crown)}$+NH–), 3.76 (3H, s, CH_3O–), 3.80 (2H, s, CH_2–), 6.37 (1H, s, $CH_{C3(coumarin)}$), 6.69 (1H, d, J=2.4 Hz, $CH_{C8(coumarin)}$), 6.69 (1H, dd, J=2.5, 8.9 Hz, $CH_{C6(coumarin)}$), 7.74 (1H, d, J=8.9 Hz, $CH_{C5(coumarin)}$). ^{13}C NMR ($CDCl_3$ vs TMS) δ=49.4, 53.8, 55.4, 56.3, 69.9, 70.1, 70.7, 100.4, 111.2, 111.8, 112.3, 125.8, 153.9, 155.3, 161.4, 162.1. HRMS calcd for $C_{23}H_{35}N_2O_7$: 451.2439 $(M+H)^+$, found: 451.2446.

4.2.3. Synthesis of *2c*

A mixture of 1-[(7-methoxy)-4-ylmethylcoumarin]-1,10-diaza-18-crown-6 (0.110 g, 0.24 mmol) and triethylamine (0.69 mL) in dry toluene (3 mL) was heated to reflux and then a solution of *tert*-butyl chloroacetate (0.051 g, 0.34 mmol) in toluene (3 mL) was added dropwise over 3 min. Catalytic amount of KI (10 mg, 0.06 mmol) was added to the reaction mixture and was refluxed for 18 h. After cooling to rt, the mixture was filtered and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with water (2×10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and then purified on silica gel (acetone then acetone/Et_3N 9:1 v/v%) to give **2c** (80 mg, 58%) as a yellow oil. R_f=0.18 (10% MeOH in CH_2Cl_2). IR (neat) ν=1610, 1717, 2865 cm^{-1}. ^{1}H NMR ($CDCl_3$ vs TMS) δ=1.43 (9H, s, 3×CH_3), 2.84 (4H, t, J=5.5 Hz, 2×–$NCH_{2(crown)}$), 2.94 (4H, t, J=5.5 Hz, 2×–$NCH_{2(crown)}$), 3.36 (2H, s, CH_2–), 3.52–3.65 (16H, m, 8×$CH_{2(crown)}$), 3.82 (2H, s, CH_2–);, 3.84 (3H, s, CH_3O–);, 6.45 (1H, s, $CH_{C3(coumarin)}$), 6.76–6.84 (2H, m, $CH_{C6,C8(coumarin)}$), 7.76 (1H, d, J=8.5 Hz, $CH_{C5(coumarin)}$). ^{13}C NMR ($CDCl_3$ vs TMS) δ=28.1, 54.0, 54.4, 55.6, 56.6, 57.0, 69.9, 70.1, 70.6, 70.7, 80.7, 100.6, 111.5, 112.0, 112.4, 125.8, 153.9, 155.5, 161.5, 162.3, 170.9. HRMS calcd for $C_{29}H_{45}N_2O_9$: 565.3125 $(M+H)^+$, found: 565.3141.

4.2.4. Crystallographic characterization of *2a*

Compound **2a** was prepared by the literature method.[7] Single crystals were grown by very slow evaporation of ethanol solution. Colorless block (0.3×0.26×0.2 mm) crystals of $C_{17}H_{21}N_1O_5$, M=319.35, triclinic, a=9.3744(10) Å, b=9.851(3) Å, c=10.528(4) Å, α=64.24(1), β=66.06(1), γ=69.81(1), V=783.3(4) Å^3, Z=2, space group: $P\bar{1}$ (no. 2), ρ_{calcd}=1.354 g cm^{-3}. Data were collected at 293(1) K, Enraf Nonius MACH3 diffractometer, Mo Kα radiation λ=0.71073 Å, ω–2θ motion, θ_{max}=25.3°, 3141 measured, 1587 reflections were unique with $I>2\sigma(I)$, decay: 1%. The structure was

solved using the SIR-92 software[19] and refined on F^2 using SHELX-97 program,[20] publication material was prepared with the WINGX-97 suite,[21] $R(F)$=0.057 and $wR(F^2)$=0.137 for 2844 reflections, 209 parameters. Residual electron density: 0.19/−0.16 e/Å^3. Non-hydrogen atoms were refined anisotropic, while hydrogen atoms were placed into geometric positions. Orientation of methyl groups was refined using a riding model. Additional crystallographic information is provided in the deposited CIF.

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Supplementary data

Supplementary data available: NMR spectra of compounds, details of ^{1}H NMR titrations, and fluorescence studies. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2008.05.006.

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