## Cation-dependent spectral properties of fluorescent complexon based on 1,8-naphthalimide with PET mechanism of optical response\*

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Complexation of 4-amino-1,8-naphthalimide derivative containing an aza-15-crown-5 ether receptor unit in the *N*-aryl substituent at the imide nitrogen atom of the naphthalimide core with alkaline earth metal cations was studied using optical and NMR spectroscopy. The binding of the cations with crown ether receptors was accompanied by signifacant fluorescence enhancement of naphthalimide moiety caused by the inhibition of the photoinduced electron transfer (PET) process. An increase in the cation radii on going from  $Mg^{2+}$  to  $Ca^{2+}$  to  $Ba^{2+}$  leads to a decrease in the complex stability and an increase in the quantum yield of its fluorescence.

**Key words:** 1,8-naphthalimide, metal cation, crown ether, fluorescence, photoinduced electron transfer, sensor.

The design of metal cation optical chemosensors is one of the most actively developing fields of supramolecular chemistry. In recent years, increased attention was directed at fluorescent chemosensors, whose molecules contain a receptor selectively reacting with a substrate and a signal fluorophore fragment capable of changing the intensity and/or position of a spectrum emission maximum as a result of complexation.<sup>1</sup> The great interest to the fluorometric measurements method is due to the relative feasibility of the experiment in combination with high sensitivity to detected substrates.<sup>2</sup>

Naphthalic acid imide (1,8-naphthalimide) derivatives are a practically important class of organic luminophores, which are used in different fields of science and technology, such as optical bleaching,<sup>3</sup> luminescent flaw detection,<sup>4</sup> solar energy conversion,<sup>5</sup> development of antitumor agents<sup>6</sup> and electroluminescent devices.<sup>7</sup> A wide variety of photophysical properties of the naphthalimide fluorophore together with relative simplicity of carrying out the synthetic operations directed towards the modification of molecular structure make this type of compounds promising for the use as a photoactive component of fluorescent sensors for cations.<sup>8</sup>

In the present work, we studied in detail the optical effects occuring upon the complexation of 4-amino-1,8naphthalimide derivative containing an aza-15-crown-5 ether receptor in the N-aryl substituent (ANI) with alkaline earth metal cations. In the framework of systematic studies of the sensor properties of 1,8-naphthalimide crown-containing derivatives carried out in our laborato $ry,^{9-13}$  it was shown that the introduction of electronreleasing receptor fragments in the molecules provides the conditions for the realization of the photoinduced electron transfer (PET) process from the N-aryl substituent to the naphthalimide moiety in the excited state. The possibility of application of such systems as fluorescent sensors is due to the possibility of switching of the emission signal intensity between the "OFF" (switched off) and "ON" (turned on) states upon binding cations with crown ether substituents.



Experimental

The synthesis of naphthalimide derivative **ANI** was described in our recent work.<sup>13</sup> Anhydrous calcium, magnesium, and bari-

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um perchlorates used in the work were dried *in vacuo* at 240 °C to a constant weight. Optical studies were carried out in acetonitrile (Aldrich, spectrophotometric grade, the content of water <0.005%).

Electron absorption spectra were measured on a two-channel Varian-Cary 5G spectrophotometer, fluorescence spectra were recorded on a FluoroLog-3-221 spectrofluorimeter at  $20\pm1$  °C. The fluorescence was detected at a right angle relative to the excitation beam. Fluorescence spectra were corrected with respect to the sensitivity of the photoelectron multiplier used.

Quantum yields of fluorescence were calculated using formula (1):<sup>14</sup>

$$\varphi_i^{\text{fl}} = \varphi_0^{\text{fl}} \frac{S_i (1 - 10^{-D_0}) n_i^2}{S_0 (1 - 10^{-D_i}) n_0^2},\tag{1}$$

where  $\varphi_i^{\text{fl}}$  and  $\varphi_0^{\text{fl}}$  are the quantum yields of fluorescence of the analyzed solution and the standard, respectively,  $D_i$  and  $D_0$  are the absorptions of the analyzed solution and the standard, respectively,  $S_i$  and  $S_0$  are the areas under the fluorescence spectra curves of the analyzed solution and the standard, respectively,  $n_i$  and  $n_0$  are the refractive indices of the solvents of the studied compound and the standard compound. Coumarin 481 in acetonitrile ( $\varphi^{\text{fl}} = 0.08$ )<sup>15</sup> was used as the standard for the calculation of quantum yields.

Stability constants of complexes were determined by spectrofluorometric titration.<sup>16</sup> A solution of metal salt in acetonitrile with a known concentration was added in small portions  $(5-20 \,\mu\text{L})$ to a solution of the corresponding ligand in the same solvent (also with a known concentration), each time recording the fluorescence spectrum. The titration was considered complete when the fluorescence spectrum stopped changing upon the addition of the next portion of the titrant. The set of data obtained was used for the calculation of stability constants of the complexes using the SPECFIT 32 Version 3.0.37 program. In the calculations, a possibility of the formation of complexes according to Eqs (2) and (3) was taken into account.

L + M 
$$\leftarrow K_{11}$$
 LM,  $K_{11} = [LM]/([L][M])$  (2)

2 L + M 
$$\stackrel{K_{21}}{\longleftarrow}$$
 L<sub>2</sub>M,  $K_{21} = [L_2M]/([L]^2[M])$  (3)

It was found that the experimental data agree with the theoretical data when only Eq. (2) is taken into account, while no formation of 2 : 1 complexes was detected.

To determine quantum yields of fluorescence of complexes, the solutions containing  $\geq$ 95% of the ligand bound in a complex were used. The necessary excess of metal perchlorate was calculated by the SPECFIT 32 Version 3.0.37 program, using the known stability constants of the complexes.

Quantum chemical calculations were carried out in the MOPAC 2009 program complex, using the semiempirical method PM6.<sup>17</sup> The configurational interaction included eight highest occupied and eight lowest unoccupied molecular orbitals (HOMO and LUMO, respectively). The iterative procedure was applied until the difference in energy of molecule for two neighboring iterations became <0.01 kcal mol<sup>-1</sup>. The effect of the solvent nature was included according to the COSMO model (Conductorlike Screening Model) incorporated into the MOPAC 2009. For calculations it was assumed that the solvent had a dielectric permittivity  $\varepsilon$  equal to 40 and a refractive index *n* such that  $n^2 = 2$ .

<sup>1</sup>H NMR spectra were recorded on an Avance 400 spectrometer (Bruker, 400.13 MHz) for solutions of the samples in deuterated acetonitrile at 31 °C. Chemical shifts for <sup>1</sup>H nuclei were determined with an accuracy of 0.01 ppm, using residual signals of the solvent as a reference and recalculating to the internal standard (Me<sub>4</sub>Si), spin-spin coupling constants were measured with an accuracy of 0.1 Hz. To obtain the NMR spectra of the complexes, we used solutions of ligand **ANI** containing 5–10-fold excess of metal perchlorate. The <sup>1</sup>H NMR spectra of free ligand **ANI** and its complexes with Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Ba<sup>2+</sup> cations are given below.

Free ligand ANI.  $C_{\rm L} = 3.0 \cdot 10^{-3} \text{ mol } \text{L}^{-1}$ . <sup>1</sup>H NMR,  $\delta$ : 3.57–3.66 (m, 16 H, CH<sub>2</sub>); 3.74–3.79 (m, 4 H, CH<sub>2</sub>); 5.98 (br.s, 2 H, NH<sub>2</sub>); 6.77 (d, 2 H, H(11), H(13), <sup>3</sup>*J* = 8.9 Hz); 6.93 (d, 1 H, H(3), <sup>3</sup>*J* = 8.3 Hz); 7.04 (d, 2 H, H(10), H(14), <sup>3</sup>*J* = 8.9 Hz); 7.69 (dd, 1 H, H(6), <sup>3</sup>*J*<sub>1</sub> = 7.3 Hz, <sup>3</sup>*J*<sub>2</sub> = 8.3 Hz); 8.27 (d, 1 H, H(2), <sup>3</sup>*J* = 8.3 Hz); 8.35 (d, 1 H, H(7), <sup>3</sup>*J* = 8.3 Hz); 8.49 (d, 1 H, H(5), <sup>3</sup>*J* = 7.3 Hz).

**Complex (ANI)·Mg<sup>2+</sup>.**  $C_{\rm L} = 3.0 \cdot 10^{-3} \text{ mol } {\rm L}^{-1}$ . The ratio M : L = 5 : 1. <sup>1</sup>H NMR, 8: 3.43-3.61 (m, 4 H, CH<sub>2</sub>); 3.68-4.00 (m, 16 H, CH<sub>2</sub>); 5.98 (br.s, 2 H, NH<sub>2</sub>); 6.94 (d, 1 H, H(3), <sup>3</sup>J = 8.3 Hz); 7.14-7.52 (br.s, 4 H, H(10), H(14), H(11), H(13)); 7.70 (dd, 1 H, H(6), <sup>3</sup>J<sub>1</sub> = 7.3 Hz, <sup>3</sup>J<sub>2</sub> = 8.3 Hz); 8.28 (d, 1 H, H(2), <sup>3</sup>J = 8.4 Hz); 8.38 (d, 1 H, H(7), <sup>3</sup>J = 8.4 Hz); 8.51 (d, 1 H, H(5), <sup>3</sup>J = 7.3 Hz).

**Complex (ANI)** • Ca<sup>2+</sup>.  $C_L = 3.0 \cdot 10^{-3} \text{ mol } L^{-1}$ . The ratio M : L = 8 : 1. <sup>1</sup>H NMR,  $\delta$ : 3.39–3.49 (m, 4 H, CH<sub>2</sub>); 3.82–3.98 (m, 16 H, CH<sub>2</sub>); 6.04 (br.s, 2 H, NH<sub>2</sub>); 6.92 (d, 1 H, H(3), <sup>3</sup>J = 8.3 Hz); 7.37 (d, 2 H, H(11), H(13), <sup>3</sup>J = 8.9 Hz); 7.48 (d, 2 H, H(10), H(14), <sup>3</sup>J = 8.9 Hz); 7.72 (dd, 1 H, H(6), <sup>3</sup>J<sub>1</sub> = 7.3 Hz, <sup>3</sup>J<sub>2</sub> = 8.3 Hz); 8.31 (d, 1 H, H(2), <sup>3</sup>J = 8.4 Hz); 8.38 (d, 1 H, H(7), <sup>3</sup>J = 8.4 Hz); 8.54 (d, 1 H, H(5), <sup>3</sup>J = 7.3 Hz).

**Complex (ANI)·Ba<sup>2+</sup>.**  $C_{\rm L} = 3.0 \cdot 10^{-3} \text{ mol } {\rm L}^{-1}$ . The ratio M : L = 10 : 1. <sup>1</sup>H NMR, 8: 3.46–3.54 (m, 4 H, CH<sub>2</sub>); 3.81–3.92 (m, 16 H, CH<sub>2</sub>); 6.08 (br.s, 2 H, NH<sub>2</sub>); 6.95 (d, 1 H, H(3), <sup>3</sup>J = 8.3 Hz); 7.33 (s, 4 H, H(10), H(14), H(11), H(13)); 7.71 (dd, 1 H, H(6), <sup>3</sup>J<sub>1</sub> = 7.3 Hz, <sup>3</sup>J<sub>2</sub> = 8.3 Hz); 8.32 (d, 1 H, H(2), <sup>3</sup>J = 8.4 Hz); 8.41 (d, 1 H, H(7), <sup>3</sup>J = 8.4 Hz); 8.52 (d, 1 H, H(5), <sup>3</sup>J = 7.3 Hz).

## **Results and Discussion**

The complexation of **ANI** was studied in an acetonitril solution, using anhydrous magnesium, calcium, and barium perchlorates. The practically complete absence of nucleophilic properties in perchlorate anion made it possible to avoid complications in the complexation scheme with organic fluoroionophore due to undesirable coordination of the anion with metal cations. The choice of the doublecharged cations of alkaline earth metals was made in order to achieve the formation of more stable complexes in dilute acetonitrile solutions, which is convenient when studying cation-induced optical effects. The spectral characteristics of free ligand **ANI** and its complexes with the indicated cations are given in Table 1. The absorption and fluorescence spectra of **ANI** are shown in Fig. 1.

The long-wavelength band in the absorption spectrum of **ANI** (see Fig. 1) is related to the charge transfer from the electron-releasing amino group at position 4 of the

Com- pound	$\lambda_{\max}^{abs}/nm$ (loge)	$\lambda_{\max}^{fl}(\lambda_{ex})$ /nm	$\phi^{fl}$	logK	<b>R</b> <sup>a</sup> /Å
$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	415 (4.17) 420 (4.02) 420 (4.16) 420 (4.04) 420 (4.12)	518 (420) 520 (420) 520 (420) 520 (420) 520 (420) 520 (420)	0.0024 0.31 0.43 0.50 0.53	5.30±0.03 4.94±0.02 4.08±0.02 Not found <sup>b</sup>	0.85—1.10 0.74 0.99 1.73 —

**Table 1.** Spectral characteristics and stability constants of ligand ANI and its complexes with  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Ba^{2+}$ , and  $H^+$  cations in acetonitrile at 273 K

<sup>a</sup> Metal ionic radii<sup>18</sup> and aza-15-crown-5 ether cavity radius.<sup>19</sup>

<sup>b</sup> In the case of spectrofluorometric titration of ligand **ANI** with perchloric acid in acetonitrile, the fluorescence intensity reached the maximum value after the addition of 1.2 equiv. of  $HClO_4$  (with the concentration of ligand **ANI** being  $5.0 \cdot 10^{-6}$  mol L<sup>-1</sup>). Most likely, the stability constant of complex (**ANI**)  $\cdot$  H<sup>+</sup> lies above  $10^7$  and thus cannot be determined by our procedure.

naphthalene core to the carbonyl groups of the dicarboximide fragment. The low quantum yield of fluorescence of **ANI** can be explained by the existence of the PET-interaction between the *N*-phenylazacrown ether group and the naphthalimide chromophore, which competes in the excited state with the relaxation emission channel.<sup>13</sup> As the PM6 calculation of the **ANI** molecule shows, the local HOMO of the *N*-aryl substituent is higher in energy than the singly occupied orbital of the 4-aminonaphthalimide moiety (Fig. 2). This makes the electron transfer process thermodinamically favorable, which makes the level of the **ANI** fluorescence low.

The binding of the metal cations by the ANI crowncontaining derivative was confirmed by the optical spectroscopy and NMR spectroscopy data. A comaprison of the <sup>1</sup>H NMR spectra of ANI in acetonitrile-d<sub>3</sub> in the presence and in the absence of the metal perchlorates (Fig. 3) unambiguously indicates that the coordination of the cation in all cases occurs at the crown ether fragment, resulting in the downfield shift of the signals for the protons of



Fig. 1. Absorption (1) and fluorescence spectra (2) of ANI in acetonitrile. Concentration of ANI 2.5  $\cdot$  10<sup>-6</sup> mol L<sup>-1</sup>. Excitation wavelength  $\lambda_{ex} = 420$  nm.

the benzene ring and the azacrown ether ring. The positions of proton signals of the naphthalimide moiety remain practically the same.

The complexation does not lead to any considerable changes in the position and intensity of the long-wavelength band in the absorption spectrum (see Table 1), which agrees with the quantum chemical calculation data, that indicate the absence of the conjugation between the *N*-aryl core and the naphthalimide moiety because of their noncoplanar spatial arrangement (see Fig. 2). A small bathochromic shift (5 nm, see Table 1) is caused by the indirect influence of complexation due to the weakening



**Fig. 2.** Energy levels and electron density distribution in the frontier molecular orbitals of compound **ANI**. The local MO of the *N*-aryl group is marked with a circle. The orbitals in parentheses correspond to the photoexcited naphthalimide fragment. The grey vertical arrow shows the long wavelength electron transition with the charge transfer to the naphthalimide chromophore. The dihedral angle formed by the planes of the naphthalimide moiety and the benzene ring is  $41.3^{\circ}$ .



**Fig. 3.** <sup>1</sup>H NMR spectra of **ANI** and its complexes with alkaline earth metal cations in CD<sub>3</sub>CN: free ligand **ANI** (*a*), (**ANI**)  $\cdot$  Ca<sup>2+</sup> (*b*), (**ANI**)  $\cdot$  Mg<sup>2+</sup> (*c*), (**ANI**)  $\cdot$  Ba<sup>2+</sup> (*d*). The numbering scheme for carbon atoms of the naphthalimide core and the *N*-aryl fragment used in the Figure for the designation of proton signals is shown in the **ANI** formula (see above).

of the +I-effect of the *N*-aryl ring, which leads to a slight increase in the electronegativity of the imide nitrogen atom.

The addition of metal perchlorates causes a considerable increase in the intensity of the fluorescence spectrum bands, which is explained by the suppression of the PET-process, with the position of the emission maximum remaining practically unchanged. Figure 4 exemplifies how the fluorescence spectrum changes upon the gradual addition of calcium perchlorate to the solution of **ANI** in acetonitrile. Similar changes were also observed in the case of other cations (H<sup>+</sup>, Mg<sup>2+</sup>, and Ba<sup>2+</sup>). The extent of fluorescence enhancement (FE) observed in the presence of 10 equiv. of the cations in solution is shown as histogram in Fig. 5.

A set of spectral curves obtained for different excesses of metal cation in solution was used for the calculation of stability constants of 1 : 1 complexes formed (see Experimental). It is seen from Table 1 that the complex (ANI)  $\cdot$  Mg<sup>2+</sup> possesses the highest stability among the complexes of Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Ba<sup>2+</sup> cations, which is due to the efficient coordination of the hard Mg<sup>2+</sup> cation with the oxygen-containing part of the crown ether receptor. Note that the Ca<sup>2+</sup> cation, which is characterized by the better geometrical correspondance to the crown ether cavity size of aza-15-crown-5 ether (see Table 1), but bearing a smaller charge density as compared to  $Mg^{2+}$ , forms



Fig. 4. Spectrofluorometric titration of ANI with calcium perchlorate in acetonitrile. Concentration of ligand ANI  $C_{\rm L} = 5.0 \cdot 10^{-6}$  mol L<sup>-1</sup>. Excitation wavelength  $\lambda_{\rm ex} = 420$  nm. In the insert: the dependence of fluorescence intensity at the maximum point on the number of equivalents of calcium perchlorate in solution (q).



**Fig. 5.** Extent of fluorescence enhancement (FE) of ligand **ANI** upon the addition to its solution in acetonitrile of 10 equiv. of the cation. The FE value was calculated as the ratio of fluorescence intensity at 520 nm after addition of the cation to the intensity at 520 nm before addition of the cation.

slightly less stable complex. The binding of the  $Ba^{2+}$  cations led to the formation of complex (ANI)  $\cdot Ba^{2+}$  possessing the lowest stability constant, which is explained by the high value of the ionic radius and a low charge density of  $Ba^{2+}$ .

The order of changes in the quantum yields of fluorescence for complexes of ANI with metal cations differs from the order of changes in the stability constant: the value  $\phi^{fl}$  increases as the metal cation radius grows (see Table 1). The regularities observed can be explained by the fact that the larger cation forms a coordination bond with the nitrogen atom of the azacrown ether ring more efficiently, thus facilitating the disinvolvement of the lone electron pair (LEP) of the heteroatom from the conjugation with the benzene ring. It is obvious that the weakening of the  $n-\pi$ -conjugation would lead to a decrease in the electron-releasing properties of the N-aryl fragment and, therefore, to a weakening of the PET-interaction in the complex. In the case of the  $Ba^{2+}$  cation, the LEP on the nitrogen atom is practically completely involved in the formation of the bond with the cation, since the quantum yield of fluorescence of the barium complex (0.50) is almost the same as the quantum yield of the protonated form of the ligand (0.53),\* in which the LEP of the heteroatom is completely localized in the N–H  $\sigma$ -bond.

A considerable degree of involvement of the LEP on the nitrogen atom of the azacrown ether receptor in the coordination of the  $Ba^{2+}$  cation is also reflected in the <sup>1</sup>H NMR spectra (see Fig. 3). A decrease in the electronreleasing properties of the macrocycle nitrogen atom in  $(ANI) \cdot Ba^{2+}$  is so significant, that its +M-effect becomes similar to that of the imide nitrogen atom in the naphthalimide moiety. As a result, the chemical shifts for the protons of the benzene ring at positions C(10), C(14) and C(11), C(13) become identical, and the singlet observed in the spectrum corresponds to four protons: H(10), H(11), H(13), and H(14).

In conclusion, we showed that the binding of alkaline earth metal cations with crown-containing 4-amino derivative of 1,8-naphthalimide **ANI** takes place due to the coordination at the crown ether receptor and is accompanied by a considerable increase in the intensity of fluorescence of the naphthalimide chromophore. The optical effect observed upon the complexation depends on both the stability of the complex and the quantum yield of its fluorescence. A decrease in complex stabilities in the order of metal cations  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Ba^{2+}$  with a simultaneous increase in the quantum yield leads to the fact that the largest changes in the fluorescence spectrum (with the same excess of cations in solution) is caused by the addition of calcium perchlorate.

The regularities considered above are important for the development of fluorescent sensors for cationic analysis based on naphthalimide crown-containing derivatives. From the data reported in this work, it follows that the development of a sensor molecule with the desired sensitivity and selectivity of the optical response is possible only in the case of a combination of a high value binding constant with a possibility of an efficient interaction of the receptor electron-releasing group involved in electron transfer to the photoexcited fluorophore with the analyzed cation.

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<sup>\*</sup> The difference in the quantum yields of fluorescence of the protonated form and the **ANI** barium complex lies within the measurement error, which usually is 10-15%.

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