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New conjugate of bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate with naphthalimide as a fluorescent sensor for calcium cations

Marina A. Zakharko,*[a] Pavel A. Panchenko,[a,b] Pavel A. Ignatov,[b] Yurii V. Fedorov[a] and Olga A. Fedorova[a,b]

[a] *A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 119991 Moscow, Russian Federation. E-mail: Marina_Zr@mail.ru*

[b] *D. I. Mendeleev University of Chemical Technology of Russia, 125047 Moscow, Russian Federation*

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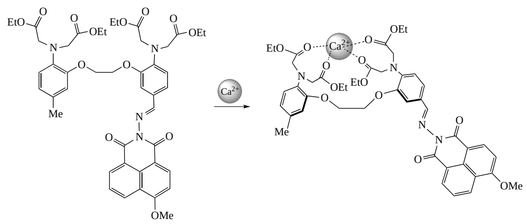
New 4-methoxy-1,8-naphthalimide derivative equipped with bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate receptor moiety at the imide nitrogen atom showed a fluorescent response to Ca^{2+} cations due to the formation of complexes of 1 : 1 ligand–metal composition.



Keywords: sensor, calcium cation, fluorescence, 1,8-naphthalimide, photoinduced electron transfer.

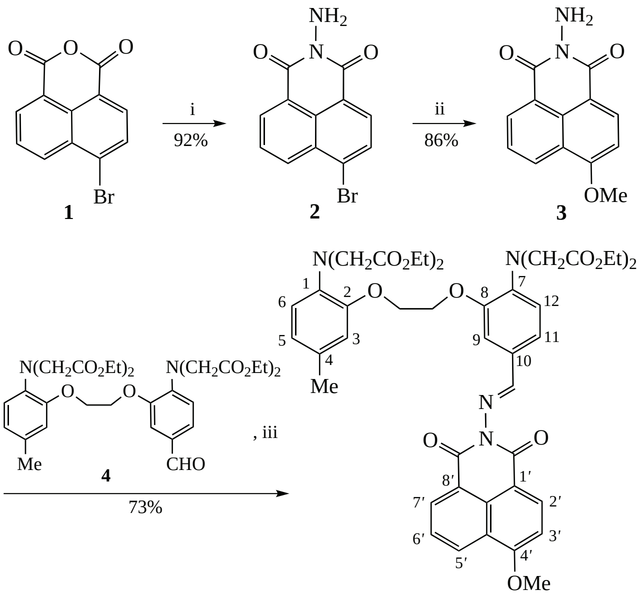
The development of highly selective and sensitive sensors for Ca^{2+} cations is an urgent goal in supramolecular chemistry because they are in high demand in medical and biological chemistry. Calcium cations are involved in blood coagulation processes and regulate muscle contractions and transmission of nerve impulses. Therefore, a disturbance of calcium metabolism can serve as an indicator of serious diseases.[1–3] Fast and highly accurate determination of ion concentration under physiological conditions can be achieved by fluorescence spectroscopy.[4]

Luminophores based on 1,8-naphthalimide are widely used as optical platforms in the development of fluorescent chemosensors for *in vitro* detection of metal cations,[5–9] anions[10,11] and small molecules.[13–15] The optical sensors based on the intramolecular photoinduced electron transfer (PET) from a donor group in the receptor moiety of the sensor to the naphthalimide core are of great practical interest. The PET process after the photoexcitation significantly decreases the fluorescence quantum yield of the system. Binding of an electron-rich functional group involved in the PET process with a metal cation reduces the electron transfer efficiency and produces an optical signal resulting in fluorescence buildup (‘turn-on’ signal). This type of response is preferable to ‘turn-off’ (quenching the sensor fluorescence upon binding with the analyte) because ‘turn-on’ sensors are usually characterized by high optical response and signal-to-noise ratios.

A derivative of 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), one of the best-known chelators for Ca^{2+} ions, was used as the receptor moiety of the sensor. BAPTA binds calcium ions in aqueous solutions with high efficiency, it is insensitive to the medium acidity in the physiological pH range[16–24] and has high selectivity for the binding of Ca^{2+} ions in comparison with Mg^{2+}: the $\lg K_{(L)\cdot Ca^{2+}}/\lg K_{(L)\cdot Mg^{2+}}$ value is 5.2.[16] The presence of biologically active ions such as Zn^{2+}, Mg^{2+} as well as Mn^{2+} does not interfere with the detection of calcium.[19] 4-Methoxy substituted naphthalimide was selected as a fluorophore. It has a relatively strong electron-deficient nature in excited state, which makes fluorescence switching by the PET mechanism more contrast in comparison with 4-amino derivatives often used in the development of sensor devices.[5,6]

The synthesis of compound BAPTA-NI (Scheme 1) included the preparation of Schiff base from 1-amino-4-methoxy-1,8-naphthalimide **3** and formyl derivative **4** at the key step. Compounds **2–4** were obtained as reported[19,25–27] from 4-bromonaphthalic anhydride **1** and 5-methyl-2-nitrophenol, respectively (for details, see Online Supplementary Materials).

Comparison of the fluorescence quantum yields of BAPTA-NI ($\varphi^{fl} = 0.0022$) and *N*-ethyl-4-methoxynaphthalimide ($\varphi^{fl} = 0.64$[28]) in water allows us to assume that the fluorescence



BAPTA-NI

Scheme 1 *Reagents and conditions*: i, N_2H_4, EtOH, Δ; ii, MeONa, MeOH, Δ; iii, AcOH, EtOH, Δ.

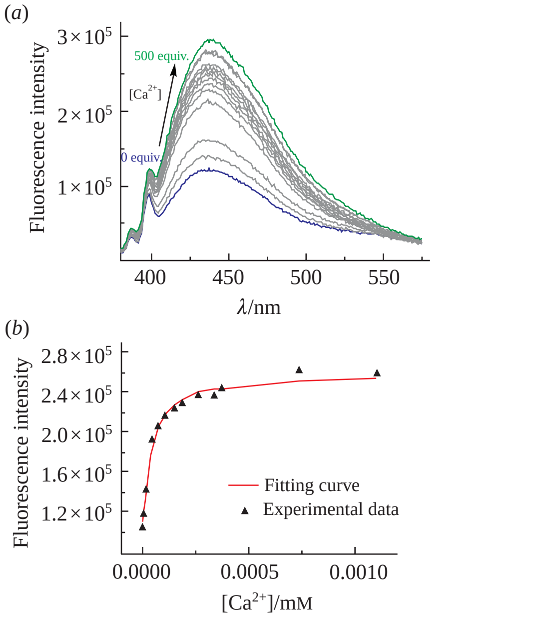


Figure 1 (*a*) Changes in the fluorescence spectrum of BAPTA-NI in acetonitrile (4×10^{-6} M) upon addition of calcium perchlorate. The excitation wavelength was 355 nm; (*b*) the dependence of fluorescence intensity at 434 nm on the concentration of Ca^{2+}.

of the naphthalimide chromophore in the sensor is efficiently quenched due to the electron transfer process in the system. Binding of BAPTA-NI to a calcium cation that decreases the electron-donating nature of the nitrogen atoms in the receptor group should be accompanied by a decrease in the probability of the PET process and fluorescence buildup.

The complexation of the sensor with Ca^{2+} cations was studied by spectrofluorimetric titration. Fluorescence spectra were recorded during gradual addition of calcium perchlorate to a BAPTA-NI solution in acetonitrile. The long-wavelength band in the BAPTA-NI electronic absorption spectrum located at about 355 nm (see Online Supplementary Materials, Figure S8) is due to the photoexcited charge transfer from the naphthalimide 4-positioned methoxy donor group to the acceptor carbonyl groups. It was found that raising the concentration of calcium cations caused insignificant changes in the absorption spectra of the sensor, whereas a spectral response (a fluorescence buildup upon binding to Ca^{2+}) was observed in BAPTA-NI fluorescence spectra (Figure 1).

The experimental data on the dependence of fluorescence intensity on the concentration of Ca^{2+} ions agree most perfectly [see Figure 1(*b*)] with the theoretical calculation of the complexation constant, taking into account the formation of one type of complex in the solution with 1:1 (ligand/metal) composition. The logarithm of the complex stability constant was calculated to be 4.31 ± 0.05. The (L)·Ca^{2+} complex demonstrates a 2.5 times more intense fluorescence at 435 nm than the free ligand. The detection limit for Ca^{2+} in acetonitrile was calculated to be 7×10^{-7} M (see Online Supplementary Materials). BAPTA-NI also exhibits a spectral response to calcium cations in aqueous solutions (Figure S9), since the fluorescence quantum yields for methoxy naphthalimides do not change significantly on moving from acetonitrile to water. The logarithm of stability constant for the BAPTA-NI and Ca^{2+} complex in water was calculated to be 5.4 ± 0.1 (Table S1). There was a good linearity at micro molar concentration levels between fluorescent intensity data at 471 nm and concentrations of Ca^{2+} in water to 3.6×10^{-6} M indicating that the probe BAPTA-NI can detect quantitatively relevant concentrations of Ca^{2+}. The detection limit for Ca^{2+} was calculated to be close to that in acetonitrile and amounted to 4×10^{-7} M (for detailed calculations, see Online Supplementary Materials).

Complexation in acetonitrile was also confirmed by the results of an NMR experiment with titration of the BAPTA-NI sensor with calcium perchlorate (Figure 2). As a result of rapid chemical exchange between the free ligand and the calcium complex in the presence of 0.5 equiv. of Ca^{2+} in the BAPTA-NI solution, only one signal set is observed in the ^{1}H NMR spectrum in which the chemical shifts are weighted averages between the signals of the (L)·Ca^{2+} complex and the free ligand. Addition of calcium perchlorate to a BAPTA-NI solution in CD_3CN causes a downfield shift of the proton signals for the sensor's receptor moiety, while the signals for the naphthalimide residue undergo nearly no shift. This fact can be explained by the coordination of the calcium cation exclusively with the sensor receptor moiety.

To confirm the mechanism of optical response generation by the BAPTA-NI sensor, quantum-chemical calculations of the energy of the boundary orbitals of the BAPTA-NI molecule and in the (L)·Ca^{2+} complex were performed by the PM6 method using the MOPAC software package. It can be concluded from the data on the optimized complex geometry that coordination of the cation by the molecule receptor moiety occurs through the oxygen atoms of the ester groups. The interatomic distance between Ca^{2+} in the complex and the oxygen atoms of the ester

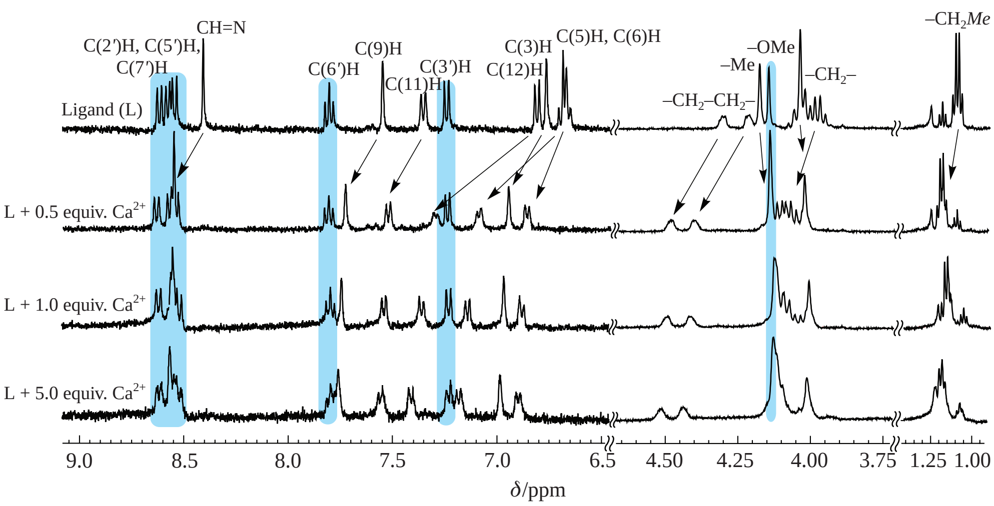


Figure 2 ^{1}H NMR spectra of BAPTA-NI in CD_3CN in the presence and in the absence of calcium perchlorate. The atom numbering in BAPTA-NI used in the assignment of signals is shown in Scheme 1. The arrows indicate the shifts of proton signals upon transition from the free ligand to the (L)·Ca^{2+} complex. The signals of the naphthalimide nucleus protons that do not undergo shifts on addition of calcium perchlorate to the solution are marked in blue.

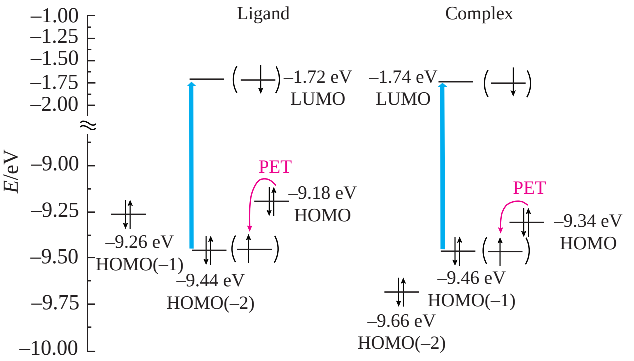


Figure 3 Energy diagram of the MO levels of BAPTA-NI (Ligand) and its complex with calcium cation (Complex). The blue arrow indicates the long-wavelength electron transition.

groups is 2.27–2.30 Å, while the distances to the nitrogen atoms of the amino groups are 2.89 Å and 2.95 Å [for N–C(1) and N–C(7), respectively], as well as 2.71Å [O–C(8)] and 2.63 Å [O–C(9)] to the oxygen atoms of the ethylene glycol bridge. Based on the calculation results, an energy diagram was built (Figure 3). It demonstrates the processes that occur during the photoexcitation of a free sensor molecule and its complex. Figure 4 shows the electron density distribution over the boundary orbitals in the molecules of the BAPTA-NI sensor and the calcium complex.

As one can see from the molecular orbital diagram, the long-wavelength electronic transition in the sensor molecule occurs from the HOMO to the LUMO located on the naphthalimide moiety. In this case, photoinduced electron transfer to a single-occupied HOMO of the molecule from the HOMO(–1) orbital located on the receptor site is possible in the system. Thus, the PET process is a probable relaxation channel of the excited state. Binding of the ligand with the calcium cation results in a significant decrease in the energy of the orbitals localized on the receptor arene rings. However, the orbital belonging to the naphthalimide chromophore still remains the HOMO(–1) and the electron transfer process is still possible in the molecule.

In conclusion, BAPTA-NI, a new derivative of 4-methoxy-1,8-naphthalimide equipped with bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate receptor moiety for the binding of calcium cations, was obtained. The observed fluorescence buildup upon Ca^{2+} binding is due to the suppression of the PET process in the free ligand. Using the optical and NMR spectroscopy methods, the formation of one type of complex with a composition of 1 : 1 was established, so BAPTA-NI may be qualified as a fluorescent PET chemosensor for the Ca^{2+} cation.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.05.024.

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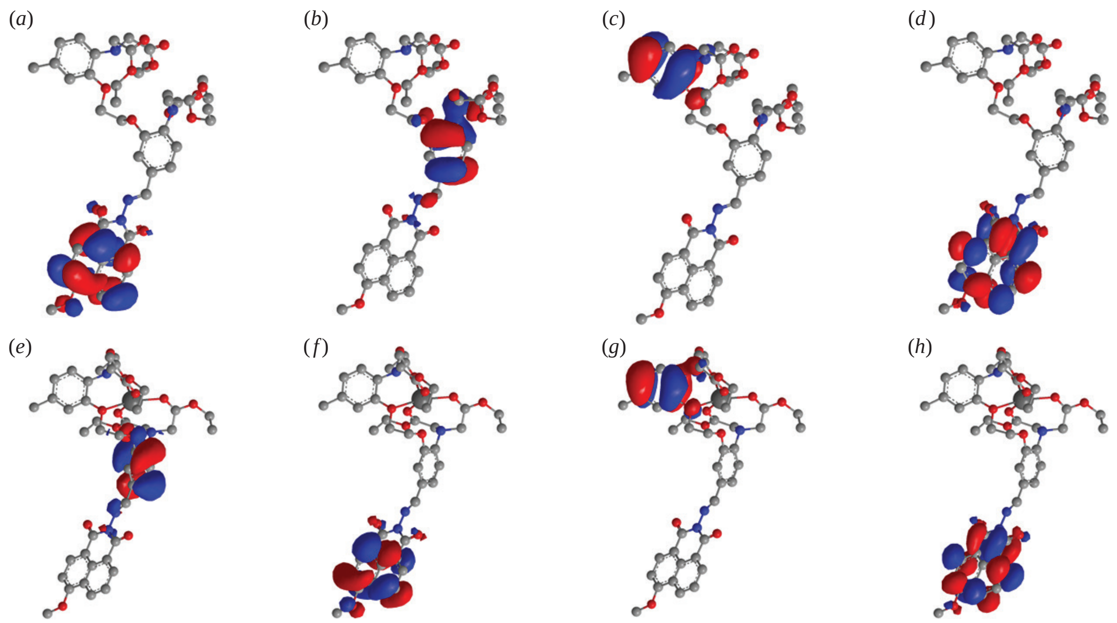

Figure 4 Electron density distribution over the boundary orbitals of (*a*)–(*d*) BAPTA-NI (Ligand) and (*e*)–(*h*) its complex with calcium cation (L)·Ca^{2+}; (*a*), (*e*) HOMO(–2); (*b*), (*f*) HOMO(–1); (*c*), (*g*) HOMO; (*d*), (*h*) LUMO.

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