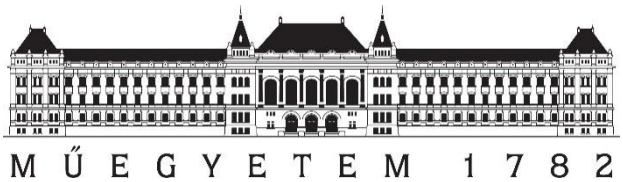

BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY
GEORGE OLAH DOCTORAL SCHOOL

SYNTHESIS AND STUDIES OF ENANTIOPURE FLUORESCENT SENSOR MOLECULES

Summary of PhD thesis

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1. INTRODUCTION

Living organisms are continuously exposed to numerous kinds of molecules and ions, which can have different physiological effects on them. Receptors in living organisms selectively bind some of these species like carboxylates, organic cations as well as various inorganic anions and metal ions by the action of a phenomenon called molecular recognition.

Enantiomeric recognition, which is a special case of molecular recognition, is also an omnipresent and vital phenomenon in Nature since a significant proportion of biologically active molecules contains at least one chiral center. Examples of its action include the anabolism and catabolism of single enantiomeric forms of amino acids. Chiral carboxylic acids, primary amines, amino acids and their derivatives are fundamental molecules of biological relevance. The determination of the enantiomeric composition and the separation of the single enantiomeric forms of chiral organic compounds are highly important tasks in pharmaceutical, pesticide, food and perfume industries, because one enantiomer of a biologically active compound may have different physiological effects.

Fluorescence spectroscopy offers a selective and sensitive detection method with quick response time. Thus, the development of fluorescent chemosensors capable of recognizing molecules, ions or enantiomers selectively has gained much research interest.

Professor Dr. Péter Huszthy's group has been carrying out research on development of heterocycle-based sensor and selector molecules at the Department of Organic Chemistry and Technology, Budapest University of Technology and Economics. I joined this research group in 2011 as an undergraduate student and continued my research activity as a graduate then as a PhD student. My doctoral work was focused on the synthesis and studies of enantiopure fluorescent sensor molecules having potential enantiomeric recognition ability or selective ion sensing properties, which may be suitable for constructing optical sensors. Acridone, 5,5-dioxophenothiazine, benzothiazole and pyrene fluorophores were used as fluorescent signaling units of the receptors. The enantiomeric differentiation of deprotonated carboxylic acids and protonated primary amines and amino acid esters are advantageous, because these molecules exist in their ionic forms under physiological conditions. Therefore, we wished to investigate the complexation properties of the synthesized unreported sensor molecules toward these kinds of chiral ionic guests and in one case, toward metal ions and achiral anions as well. In this summary the compounds are numbered according to the PhD thesis.

2. LITERATURE BACKGROUND

A chiral host molecule and enantiomers of a chiral guest molecule form diastereomeric complexes, which have different physical properties enabling chiral discrimination. *Pirkle and Pochapsky* formulated the conditions of chiral recognition, which is known as the three-point rule.[1] It states that there must be three simultaneous interactions between the chiral host molecule and at least one of the enantiomers of the guest molecule, and that one of these interactions must be stereochemically dependent. Schematically, host **1** is a match for guest **2** but not for guest **3** (*Figure 1.A*). When host **1** and guest **3** interact, they should be capable of two interactions as in *Figure 1.B*, and it is the third interaction C ··· C' that determines

[1] Pirkle, W. H.; Pochapsky, T. C. *Chem. Rev.* **1989**, *89*, 347–362.

the degree of enantioselectivity. This third interaction is the weakest of the three noncovalent ones and, in most cases, is not likely to represent a large energy difference.[2]

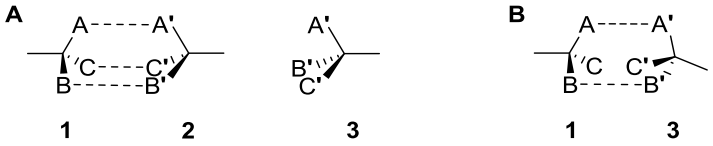

Figure 1. Structure of the complex of host **1** and guest enantiomer **2** and structure of guest enantiomer **3** (**A**), structure of the complex of host **1** and guest enantiomer **3** (**B**)[2]

The most frequently used chiral building blocks in host molecules of anions are amino acid, BINOL, steroid and monosaccharide units, while 1-arylethyl moieties have also been applied[3] as sources of chirality. These sensor molecules often contain urea and thiourea units as receptor parts, which have good hydrogen bond donating ability therefore high affinity toward anions.[4] We had synthesized a chiral bis(thiourea) type 5,5-dioxophenothiazine-based anion sensor and studied its enantiomeric recognition properties by UV–vis spectroscopy.[5]

After the realization of complex forming ability of crown ethers[6] research expanded to the synthesis of enantiopure macrocycles. Enantioselectivity of crown ethers was investigated first by *Cram et al*, who studied the selectivity of bis(binaphthyl)-22-crown-6 ether derivatives toward the enantiomers of protonated primary amines.[7] Since this pioneer work, many enantiopure crown ethers have been synthesized, and their enantiomeric recognition abilities have been studied by various methods. Aza-18-crown-6 ethers were found to be excellent hosts for ammonium ions. Among them crown ethers containing a pyridine subunit showed outstanding complexation properties toward protonated primary amines thanks to their aromatic ring and the nitrogen atom.[8]

A large number of optically active fluorescent sensor molecules have been developed due to the selectivity and sensitivity of fluorescence spectroscopy.[4,9] Many fluorescent cation sensitive sensor molecules have a modular structure consisting of a fluorophore and a receptor unit separated by a short alkylene, most frequently a methylene spacer. They work on the basis of photoinduced electron transfer (PET). In this case the receptor part has electron donor property, it often contains an amino group (e.g., an azacrown ether) and the fluorophore unit plays the role of an electron acceptor. An efficient intramolecular quenching process (PET) causes that the free ligand shows reduced (near-zero) fluorescence in the excited state.

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[2] Atwood, J. L.; Steed, J. W. *Encyclopedia of Supramolecular Chemistry*, Marcel Dekker: New York, NY, 2004.

[3] Hamann, B. C.; Branda, N. R.; Rebek, J., Jr. *Tetrahedron Lett.* **1993**, *34*, 6837–6840; Gunnlaugsson, T.; Davis, A. P.; Hussey, G. M.; Tierney, J.; Glynn, M. *Org. Biomol. Chem.* **2004**, *2*, 1856–1863; Griesbeck, A. G.; Hanft, S.; Miara, Y. D. *Photochem. Photobiol. Sci.* **2010**, *9*, 1385–1390; Trejo-Huizar, K. E.; Ortiz-Rico, R.; Peña-González, M. A.; Hernández-Rodríguez, M. *New J. Chem.* **2013**, *37*, 2610–2613; Zhou, X.-B.; Yip, Y.-W.; Chan, W.-H.; Lee, A. W. M. *Beilstein J. Org. Chem.* **2011**, *7*, 75–81.

[4] Zhang, X.; Yin, J.; Yoon, J. *Chem. Rev.* **2014**, *114*, 4918–4959.

[5] Kormos, A.; Móczár, I.; Pál, D.; Baranyai, P.; Kupai, J.; Tóth, K.; Huszthy, P. *Tetrahedron: Asymmetry* **2013**, *24*, 62–65.

[6] Pedersen, C. J. *J. Am. Chem. Soc.* **1967**, *89*, 2495–2496.; ibid. 7017–7036.

[7] Kyba, E. B.; Koga, K.; Sousa, L. R.; Siegel, M. G.; Cram, D. J. *J. Am. Chem. Soc.* **1973**, *95*, 2692–2693.

[8] Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997**, *97*, 3313–3361.

[9] You, L.; Zha, D.; Anslyn, E. V. *Chem. Rev.* **2015**, *115*, 7840–7892.

In contrast, coordination of a cation increases the fluorescence intensity to a great extent (off–on characteristic). Controllable characteristics of an optical phenomenon such as fluorescence can serve as a basis for designing not only sensor molecules, but also molecular logic gates usable for constructing molecular devices having potential for information processing.[10]

Fluorescent sensor molecules for metal ion analysis have great importance due to their potential applications in many areas such as medical diagnosis and monitoring contamination by heavy metals.[10,11] Anions also play an important role in many chemical and biological processes. The molecular recognition properties of certain cation and / or anion sensing receptors can be exploited for mimicking Boolean logic operations such as AND, OR, XOR, NAND, NOR, XNOR, IMPLICATION (IMP) and INHIBIT (INH) gates upon applying appropriate chemicals as inputs and detecting emission intensity or absorbance as an output. Moreover, various reported sensor molecules are able to perform more complex logic operations by integrating logic gates. The obtained combinational molecular logic circuits include half-adder, half-subtractor and comparator integrated logic functions.[12]

3. EXPERIMENTAL METHODS

During the syntheses preparative organic chemical methods were used. The progress of reactions and the purity of products were checked by thin layer chromatography. The crude products were purified by recrystallization, by distillation under atmospheric or reduced pressure, by column or layer chromatography and by trituration. New compounds were characterized by their physical data (melting point, optical rotation), spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, MS) and elemental analysis. Determination of the crystal structure of compound (*S*,*S*)-**65** was performed by X-ray crystallography in cooperation with *Dr. Beáta G. Vértessy and Ibolya Leveles*. Diffraction data were collected at 109 K.

The complexation properties of the new receptor molecules were studied for the enantiomers of chiral salts, different anions and metal ions in acetonitrile (unless otherwise noted) by UV–vis and fluorescence spectroscopies. The equilibrium constants were determined by global nonlinear regression analysis using SPECFIT/32^{TM} program based on the data of fluorescence spectroscopic titrations in cooperation with *Dr. Péter Baranyai*. The degrees of enantiomeric discrimination were calculated according to $\Delta \log K = \log K_{(R)} - \log K_{(S)}$.

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[10] de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.

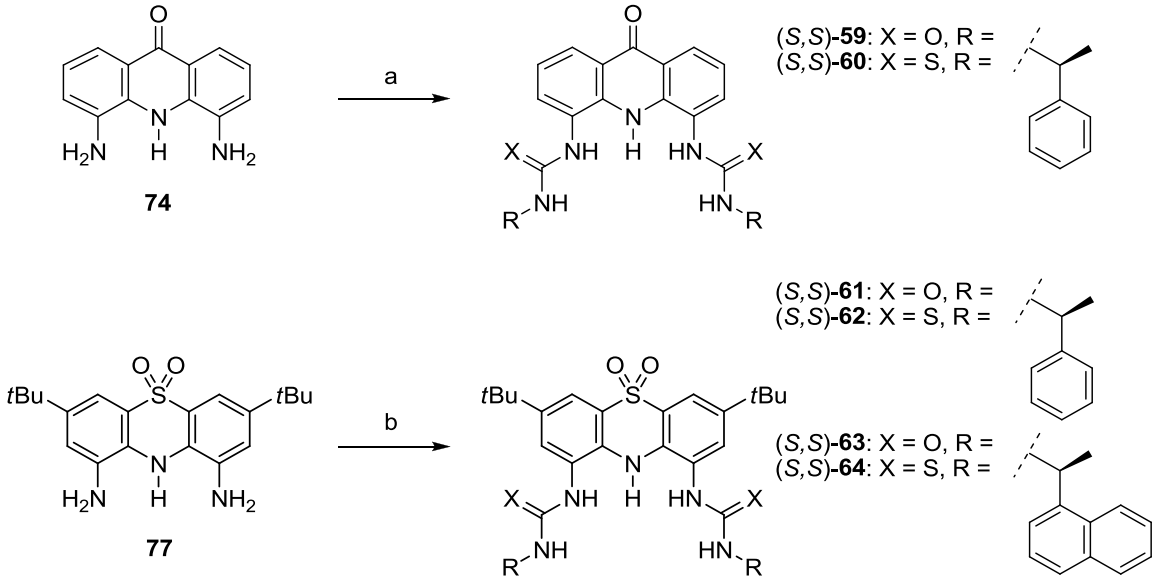
[11] Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3–40; Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588; Jeong, Y.; Yoon, J. *Inorg. Chim. Acta* **2012**, *381*, 2–14; Formica, M.; Fusi, V.; Giorgi, L.; Micheloni, M. *Coord. Chem. Rev.* **2012**, *256*, 170–192; Wong, J. K.-H.; Todd, M. H.; Rutledge, P. J. *Molecules* **2017**, *22*, 200; Wu, D.; Sedgwick, A. C.; Gunnlaugsson, T.; Akkaya, E. U.; Yoon, J.; James, T. D. *Chem. Soc. Rev.* **2017**, *46*, 7105–7123; Chowdhury, S.; Rooj, B.; Dutta, A.; Mandal, U. *J. Fluoresc.* **2018**, *28*, 999–1021.

[12] Madhuprasad; Bhat, M. P.; Jung, H.-Y.; Losic, D.; Kurkuri, M. D. *Chem. Eur. J.* **2016**, *22*, 6148–6178.

4. RESULTS

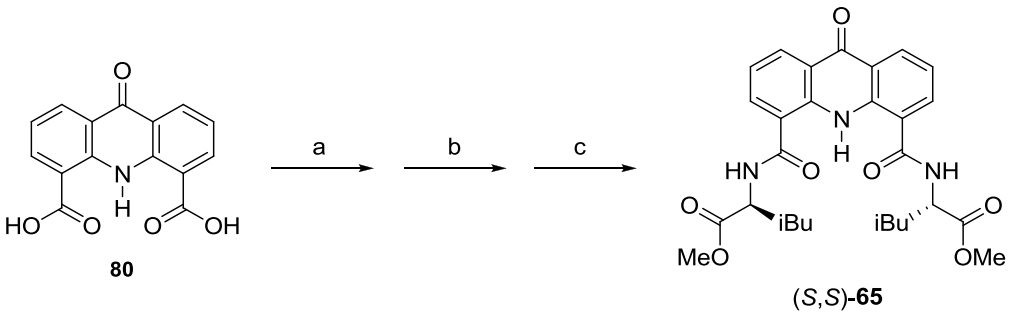
4.1. Synthesis of the new compounds

Diamines **74** and **77** were acylated with the appropriate isocyanates and isothiocyanates to yield bis(urea)s (*S*,*S*)-**59**, (*S*,*S*)-**61** and (*S*,*S*)-**63** and bis(thiourea)s (*S*,*S*)-**60**, (*S*,*S*)-**62** and (*S*,*S*)-**64** (*Scheme 1*) [1, 2]. Diamine **74** was prepared from the appropriate dinitro compound by catalytic hydrogenation as a more efficient method than the ones published in the literature [1].



Scheme 1. Synthesis of the new anion sensors (a: R–N=C=X, DMF; b: R–N=C=X, pyridine)

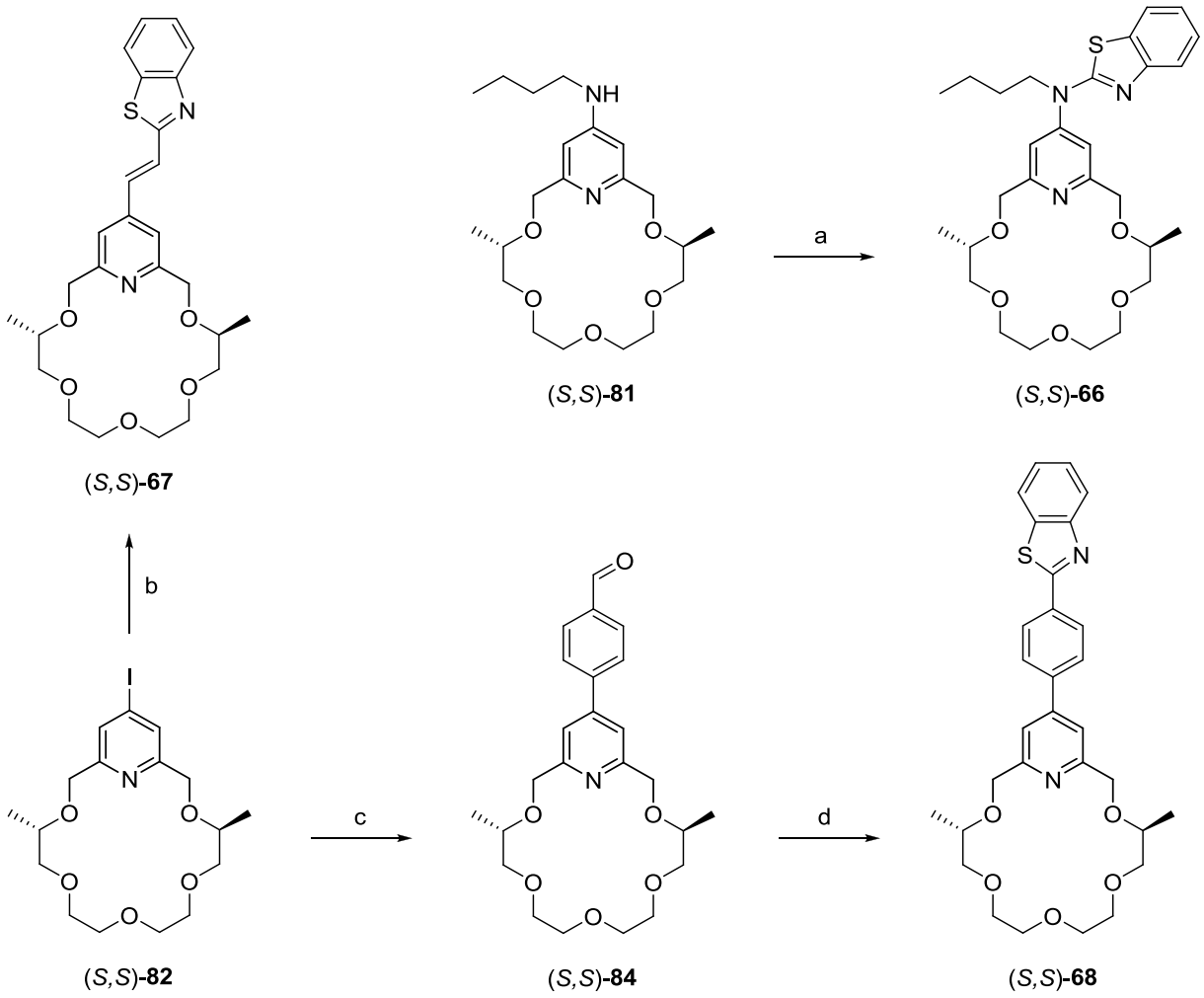
Acridone dicarboxylic acid **80** was treated with thionyl chloride in the presence of catalytic amount of DMF, then the crude product was reacted with (*S*)-leucine methyl ester hydrogen chloride in THF using triethylamine as a base. Hydrolysis of the second crude product gave acridone derivative (*S*,*S*)-**65** (*Scheme 2*) [3].



Scheme 2. Synthesis of new sensor molecule (*S*,*S*)-**65** [a: $SOCl_2$, cat. DMF; b: (*S*)-leucine methyl ester hydrogen chloride, Et_3N, THF; c: THF–AcOH–H_2O 8:1:1]

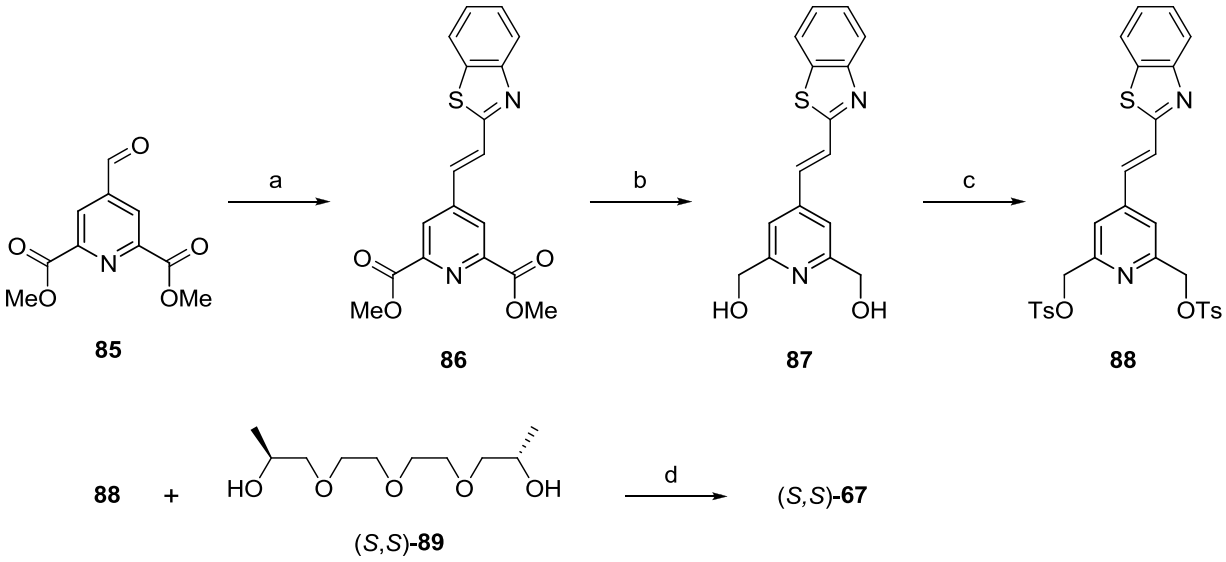
The synthesis of new pyridino-crown ether derivatives (*S*,*S*)-**66**–(*S*,*S*)-**68** containing a benzothiazole unit was carried out as outlined in *Schemes 3 and 4* [4].

Enantiopure butylamino-pyridino-crown ether (*S*,*S*)-**81** was reacted with 2-chlorobenzothiazole in the mixture of THF and DMF using sodium hydride as a strong base to yield sensor molecule (*S*,*S*)-**66**. We also utilized carbon–carbon bond forming reactions to obtain new fluorescent pyridino-18-crown-6 ether derivatives whereby the linking units are attached at position 4 of the pyridine ring. Iodopyridino-crown ether (*S*,*S*)-**82** was reacted with 2-vinylbenzothiazole in a *Heck* reaction to furnish ligand (*S*,*S*)-**67**. Iodo derivative (*S*,*S*)-**82** was converted into aldehyde (*S*,*S*)-**84** in a *Suzuki–Miyaura* cross-coupling reaction. Reacting aldehyde (*S*,*S*)-**84** with 2-aminobenzenethiol gave ligand (*S*,*S*)-**68** (*Scheme 3*).



Scheme 3. Synthesis of new pyridino-crown ether-based sensor molecules (*S*,*S*)-**66**–(*S*,*S*)-**68** [a: 2-chlorobenzothiazole, NaH, THF, DMF; b: 2-vinylbenzothiazole, $Pd(OAc)_2$, K_2CO_3, DMF; c: 4-formylphenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, KBr, dioxane–H_2O 6:1; d: 2-aminobenzenethiol, EtOH]

Starting from formylpyridine diester **85** we worked out another route for the synthesis of pyridino-crown ether (*S*,*S*)-**67** (*Scheme 4*). Heating formyl derivative **85** with 2-methylbenzothiazole in a mixture of acetic acid and acetic anhydride produced diester **86** containing a vinylene unit. The latter was reacted with sodium borohydride in the presence of calcium chloride using methanol as a solvent to selectively obtain diol **87**, which was tosylated to give ditosylate **88**. Sensor molecule (*S*,*S*)-**67** was prepared by *Williamson* type ether synthesis by reacting ditosylate **88** and enantiomerically pure dimethyl-substituted tetraethylene glycol (*S*,*S*)-**89**.



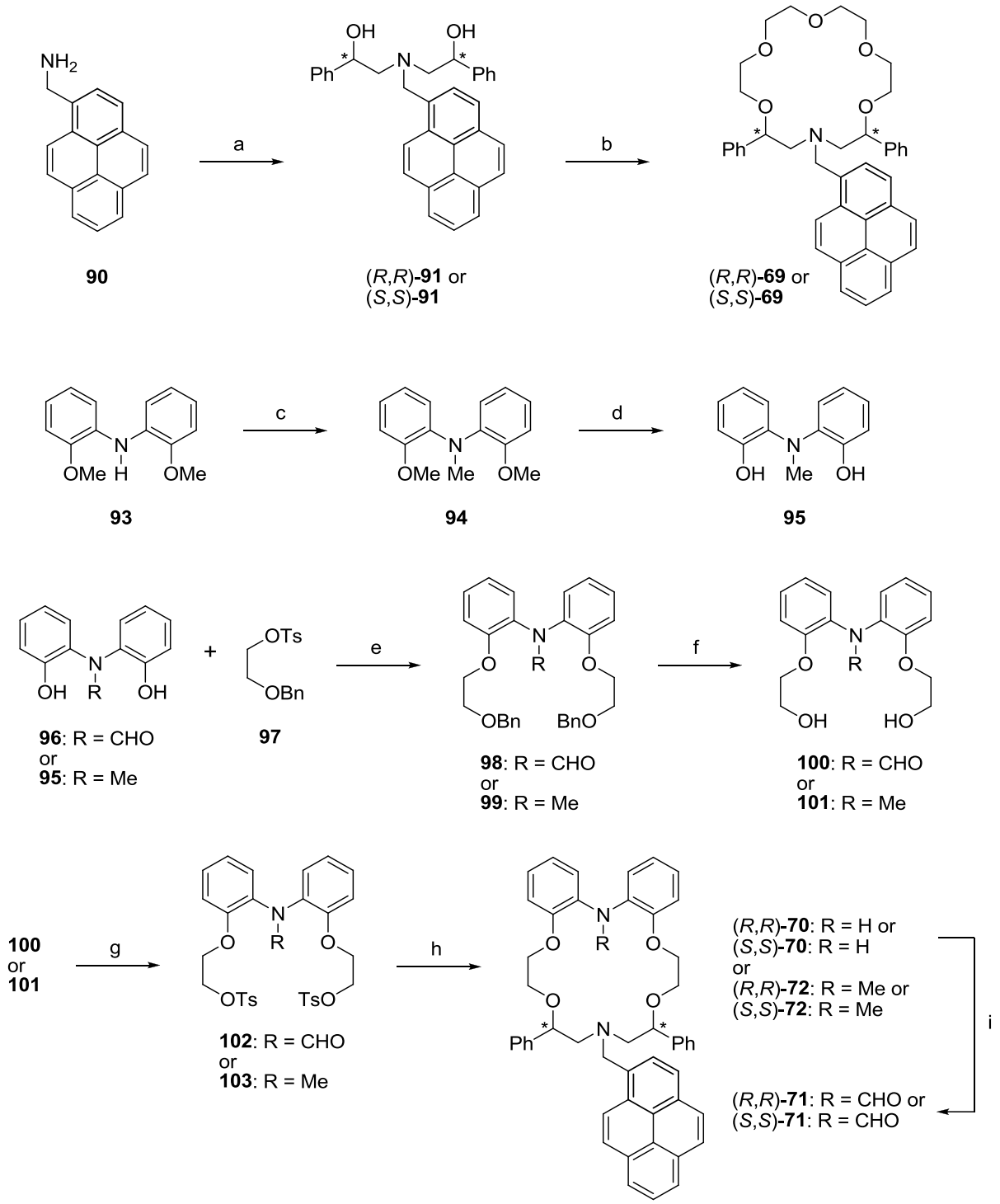
Scheme 4. Alternative synthesis of sensor molecule (*S*,*S*)-**67** (a: 2-methylbenzothiazole, AcOH, Ac_2O; b: $NaBH_4$, $CaCl_2$, MeOH; c: TsCl, aq. KOH, CH_2Cl_2; d: NaH, THF)

Eight new aza- and diazacrown ether based ligands containing a pyrene fluorescent signaling unit [(*R*,*R*)-**69**–(*R*,*R*)-**72** and (*S*,*S*)-**69**–(*S*,*S*)-**72**, *Scheme 5*] were prepared [5].

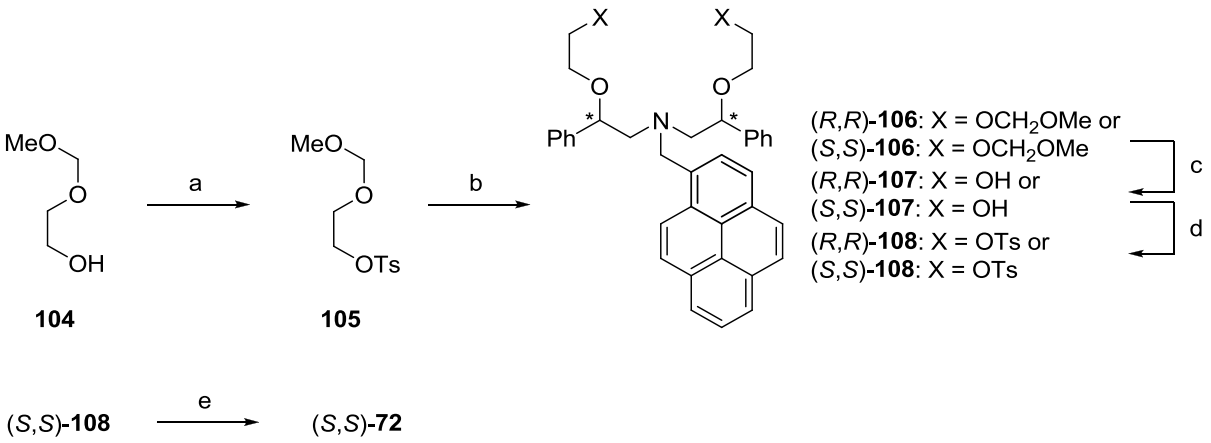
Pyren-1-ylmethanamine (**90**) was heated with (*R*)-phenyloxirane or (*S*)-phenyloxirane in methanol in a sealed tube to give enantiopure aminodiol (*R*,*R*)-**91** or (*S*,*S*)-**91**, which ones and tetraethylene glycol ditosylate were used in macrocyclization reactions to yield azacrown ether derivatives (*R*,*R*)-**69** and (*S*,*S*)-**69**.

Bis(2-methoxyphenyl)amine (**93**) was alkylated with methyl iodide to obtain *N*-methylated amine **94**. For the synthesis of diphenol **95**, selective *O*-demethylation of amine **94** was carried out with anhydrous aluminium chloride in chlorobenzene. Diphenol derivatives **96** and **95** were reacted with benzyl protected ethylene glycol tosylate (**97**) to furnish formamide derivative **98** and tertiary amine **99**, respectively. Catalytic hydrogenolysis of *O*-benzyl protected derivatives **98** and **99** gave diols **100** and **101**, which were transformed to ditosylates **102** and **103**. The macrocyclization reactions of ditosylates **102** and **103** with aminodiol (*R*,*R*)-**91** or (*S*,*S*)-**91** were carried out in DMF in the presence of sodium hydride. The reactions of **102** rendered (*R*,*R*)-**70** and (*S*,*S*)-**70** due to deformylation. Sensor molecules (*R*,*R*)-**72** and (*S*,*S*)-**72** were prepared from **103**. Formylation of (*R*,*R*)-**70** and (*S*,*S*)-**70** using a mixture of formic acid and acetic anhydride gave (*R*,*R*)-**71** and (*S*,*S*)-**71** successfully (*Scheme 5*).

We worked out another route for the synthesis of diazacrown ether (*S*,*S*)-**72** through new enantiopure precursors (*Scheme 6*), which can be useful for the preparation of other enantioselective fluorescent sensor molecules as well. Methoxymethyl (MOM)-protected ethylene glycol **104** was tosylated to obtain tosylate **105**. Reaction of the latter with enantiopure aminodiol (*R*,*R*)-**91** or (*S*,*S*)-**91** (*Scheme 5*) gave MOM ethers (*R*,*R*)-**106** and (*S*,*S*)-**106**. The removal of the MOM protecting groups of (*R*,*R*)-**106** and (*S*,*S*)-**106** by aqueous hydrochloric acid furnished diols (*R*,*R*)-**107** and (*S*,*S*)-**107**, which were transformed to ditosylates (*R*,*R*)-**108** and (*S*,*S*)-**108**. *O*-Alkylation of diphenol derivative **95** (*Scheme 5*) with (*S*,*S*)-**108** yielded macrocycle (*S*,*S*)-**72**.



Scheme 5. Synthesis of the new sensor molecules (*R*,*R*)-**69**–(*R*,*R*)-**72** and (*S*,*S*)-**69**–(*S*,*S*)-**72**
[a: (*R*)-phenyloxirane or (*S*)-phenyloxirane, MeOH; b: tetraethylene glycol ditosylate, NaH, THF; c: MeI, NaH, THF; d: $AlCl_3$, chlorobenzene; e: K_2CO_3, MeCN; f: H_2, Pd/C, MeOH; g: TsCl, CH_2Cl_2, R = CHO: Et_3N, R = Me: aq. KOH; h: (*R*,*R*)-**91** or (*S*,*S*)-**91**, NaH, DMF; i: HCOOH, Ac_2O, iPr_2O]



Scheme 6. Synthesis of new precursors [a: TsCl, aq. KOH, CH_2Cl_2; b: (*R*,*R*)-**91** or (*S*,*S*)-**91**, NaH, THF, DMF; c: aq. HCl, THF; d: TsCl, aq. KOH, CH_2Cl_2; e: **95**, K_2CO_3, MeCN]

4.2. Studies on the sensor molecules

The enantiomeric recognition abilities of receptors (*S*,*S*)-**59**–(*S*,*S*)-**65** were studied toward the enantiomers of tetrabutylammonium salts of mandelic acid (Man), *tert*-butoxycarbonyl-protected phenylglycine (Boc-Phg), *tert*-butoxycarbonyl-protected phenylalanine (Boc-Phe) and *tert*-butoxycarbonyl-protected alanine (Boc-Ala) [1–3].

In the cases of anion sensors (*S*,*S*)-**59** and (*S*,*S*)-**60** the fluorescence titration spectra showed emission decreases upon addition of carboxylates (in acetonitrile–DMSO 99:1), which could be fitted satisfactorily by assuming 1:1 complex formation. Based on these results, receptor (*S*,*S*)-**59** had an appreciable enantiomeric recognition ability toward (*S*)-Boc-Ala over its (*R*)-isomer (Δ log K = –0.56) (*Figure 2*). However, in the cases of other chiral carboxylates (Man, Boc-Phg and Boc-Phe) containing a phenyl or benzyl group at their stereogenic centers, receptor (*S*,*S*)-**59** showed practically no enantiomeric discrimination. This can be attributed to an additional π–π interaction between the phenyl or benzyl moiety and one of the urea carbonyl units beside the hydrogen-bonded interactions between the carboxylate and the NH groups. Presumably, the complexes with the enantiomers of Boc-Ala containing an aliphatic and less bulky methyl group had a different structure relative to the complexes with Man, Boc-Phg and Boc-Phe. The change of the urea moieties to thiourea ones had a significant effect on enantiomeric recognition. Namely, receptor (*S*,*S*)-**60** showed moderate selectivity toward the enantiomers of most chiral anions studied (the only exception was Boc-Phe) [1].

Upon addition of the carboxylates to the solutions of receptors (*S*,*S*)-**63** and (*S*,*S*)-**64** the fluorescence spectra revealed increases and decreases, respectively, which could be fitted satisfactorily by assuming 1:1 complex formation. In most cases, receptors (*S*,*S*)-**63** and (*S*,*S*)-**64** showed slight or no enantiomeric recognition abilities toward the enantiomers of chiral carboxylates. However, in the case of bis(thiourea) receptor (*S*,*S*)-**64** and the enantiomers of Boc-Phg, moderate enantioselectivity could be observed (Δ log K = 0.24), which was the highest among the others. In the cases of receptor molecules (*S*,*S*)-**61** and (*S*,*S*)-**62**, the slight absorption and fluorescence spectral changes upon addition of the chiral carboxylates did not allow the accurate determination of the stability constants of complexes [2].

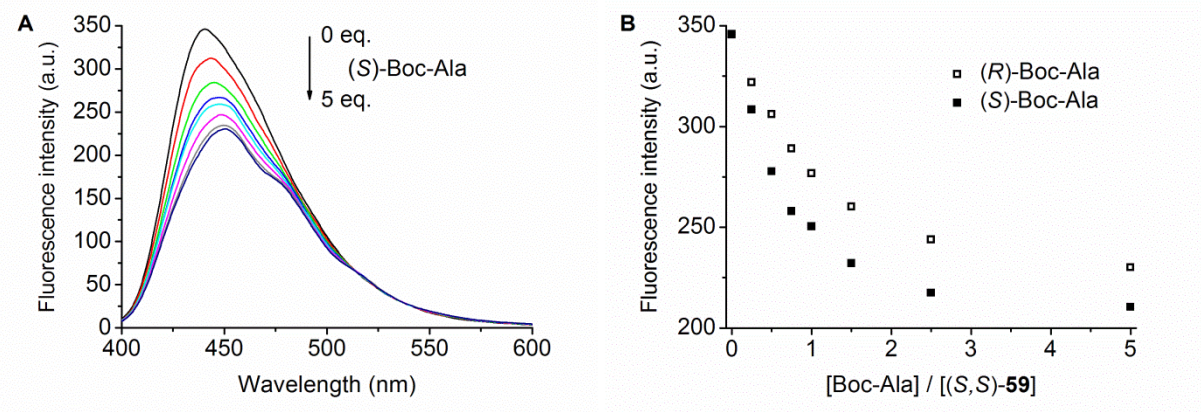


Figure 2. Series of fluorescence emission spectra upon titration of (*S*,*S*)-**59** (2 μM) with (*S*)-Boc-Ala (0, 0.25, 0.5, 0.75, 1, 1.5, 2.5, 5 eq.) (**A**), titration curves with (*R*)-Boc-Ala and (*S*)-Boc-Ala (0–5 eq.) at 440 nm (**B**)

The crystal structure of acridone derivative (*S*,*S*)-**65** was determined by X-ray crystallography. *Figure 3.A* shows that NH···O type intramolecular bifurcated H-bond was formed between the acridone NH and the amide oxygens. Isobutyl and methoxycarbonyl groups of receptor (*S*,*S*)-**65** revealed flexibility in the crystal (*Figure 3.A*) [3].

The emission spectrum of receptor (*S*,*S*)-**65** exhibited two bands: one at 427 nm, which arises from the unchanged sensor molecule, and a much weaker and broad band at 586 nm (*Figure 3.B*), which can be explained by an excited state intramolecular proton transfer (ESIPT)[13] from the acridone NH to the amide oxygens. The latter process is promoted by the intramolecular bifurcated H-bond between the acridone NH and the amide oxygens in the ground state (*Figure 3.A*).

In the case of receptor (*S*,*S*)-**65**, none of the chiral carboxylates induced spectral changes characteristic to complexation. Addition of Boc-Ala and Boc-Phe accompanied with the formation of a small amount of deprotonated receptor (*Figure 3.B*).

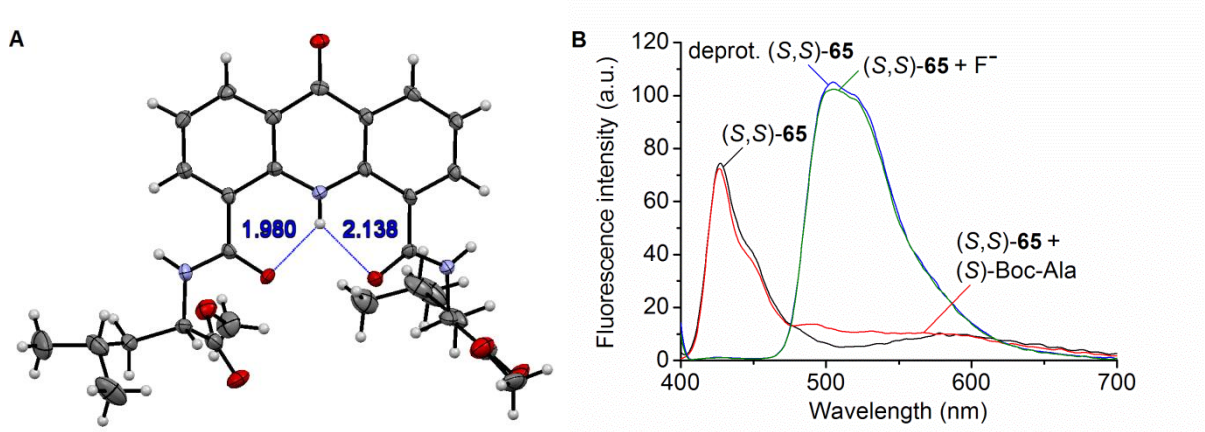


Figure 3. ORTEP style diagram of the X-ray structure of (*S*,*S*)-**65**, intramolecular bifurcated H-bond is indicated by numbered lines, distances are given in Å (**A**). Fluorescence emission spectra of (*S*,*S*)-**65** alone (20 μM) and in the presence of 50 eq. of DBU (deprotonated form), 4 eq. of F^- and 200 eq. of (*S*)-Boc-Ala (**B**).

[13] Chen, C.-L.; Chen, Y.-T.; Demchenko, A. P.; Chou, P.-T. *Nat. Rev. Chem.* **2018**, *2*, 131–143.

The recognition ability of receptor (*S*,*S*)-**65** toward achiral anions, viz., F^-, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, HSO_4^-, $H_2PO_4^-$ and AcO^- was also examined using their tetrabutylammonium salts. Absorption and fluorescence spectral changes upon addition of these anions also did not show complexation. However, F^-, AcO^- and $H_2PO_4^-$ caused deprotonation of the sensor molecule, which was accompanied with large bathochromic shifts in the absorption and emission spectra. Among the three anions, F^- had the largest effect (*Figures 3.B and 4.A*); thus, in the presence of 4 eq. of anions, selective absorption and fluorescence responses to F^- could be observed. The stoichiometry of the deprotonation was determined as 1:2 receptor to anion ratio for F^-, supporting the formation of $[HF_2]^-$, and as 1:1 ratio for AcO^- and $H_2PO_4^-$ [3].

Because of the cation coordinating ability of ester and amide carbonyl oxygens in receptor (*S*,*S*)-**65**, we also investigated its complexation properties with cations, viz., Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Ag^+, Cd^{2+}, Hg^{2+} and Pb^{2+} using the perchlorate salts of these metal ions. Hg^{2+} caused significant bathochromic shifts in the absorption spectrum providing a selective response, which can be attributed to the conversion of the acridone unit to its 9-hydroxyacridine tautomeric form upon complexation. Large fluorescence enhancements were induced by Ca^{2+} and Hg^{2+} with slight and significant bathochromic shifts, respectively (*Figure 4.B*). Selective fluorescence responses to Ca^{2+} and Hg^{2+} could be obtained by detection the emission intensities at 425 or 550 nm in the former or the latter case. The fluorescence titration series of spectra were fitted satisfactorily by assuming 1:1 complexation, except for Ca^{2+}, in which case formation of a 1:2 receptor–cation complex at large excess of Ca^{2+} could also be detected [3].

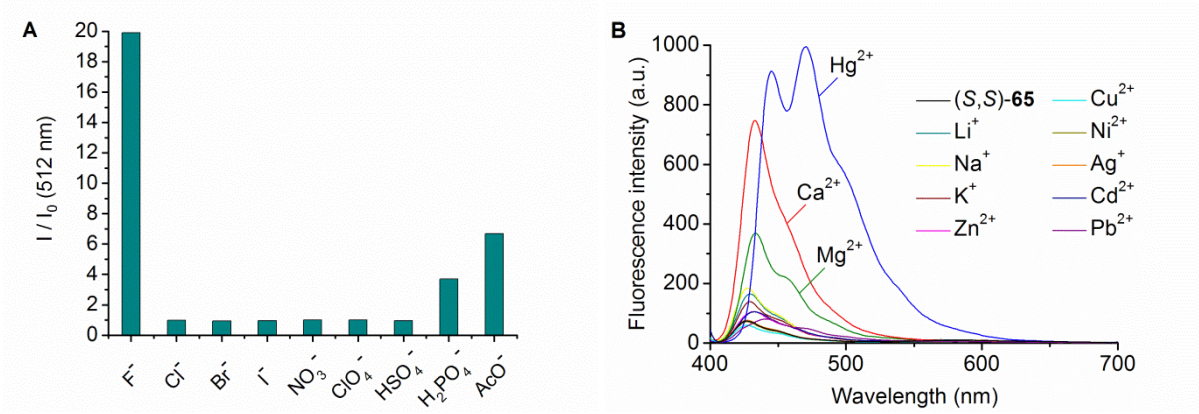


Figure 4. Fluorescence enhancement at 512 nm for (*S*,*S*)-**65** (20 μM) with 4 eq. of anions (**A**), fluorescence emission spectra of (*S*,*S*)-**65** alone (20 μM) and in the presence of 400 eq. of cations (**B**)

Based on the aforementioned optical responses of sensor molecule (*S*,*S*)-**65** toward anionic and cationic guests, we established double input / single output logic gates with the use of F^- and Ca^{2+} as chemical inputs and exploiting their reaction. The addition of 80 eq. of F^- and 40 eq. of Ca^{2+} provided the two inputs (Inputs 1 and 2), while the detection of the intensities of fluorescence emission at 425, 550, and 483 nm and in the absorbances at 310 and 475 nm furnished the output signals (Outputs 1–5), which led to IMP, INH, XOR and XNOR logic gates. Combinations of the particular molecular logic gates rendered molecular logic circuits, namely, a complementary IMP / INH circuit, a half-subtractor (XOR / INH) and a comparator (XNOR / INH), which could be operated by a single molecule and set of inputs. Using negative logic mode for one of the outputs, all the three complex logic functions could be performed using fluorescence spectroscopy [3].

The recognition abilities of crown ethers (*S*,*S*)-**66**–(*S*,*S*)-**72** and (*R*,*R*)-**69**–(*R*,*R*)-**72** were studied toward the enantiomers of hydrogen perchlorate salts of 1-phenylethylamine (PhEt), 1-(1-naphthalen-1-yl)ethylamine (NapEt), phenylglycine methyl ester (PhgOMe) and phenylalanine methyl ester (PheOMe) [4, 5].

Addition of the enantiomers of PhEt and NapEt to sensor molecules (*S*,*S*)-**66**–(*S*,*S*)-**68** resulted in significant fluorescence quenching. However, the titrations of ligand (*S*,*S*)-**68** with PhgOMe and PheOMe showed fluorescence quenching with the simultaneous appearance of a new emission band (*Figure 5.A*). The latter spectral shape refers to the protonated form of ligand (*S*,*S*)-**68**. Since the absorption titration series of spectra exhibited negligible extent of protonation beside the complexation in the ground state, the protonation of ligand (*S*,*S*)-**68** occured only in the excited state. It can be assumed that the formation of the protonated ligand took place from the hydrogen-bonded complexes, because the fluorescence titration series of spectra could be fitted satisfactorily for 1:1 complex formation. The latter phenomenon (proton transfer in the complex upon excitation) was also observed in the cases of ligand (*S*,*S*)-**67** and PhgOMe and PheOMe. The sensor molecules revealed moderate or appreciable enantiomeric recognition abilities toward the enantiomers of PhEt, NapEt and PhgOMe. Greater degrees of enantiomeric recognition were experienced in the cases of pyridino-crown ethers (*S*,*S*)-**67** and (*S*,*S*)-**68** with PhEt and NapEt (Δ log *K* between 0.28 and 0.35) [4].

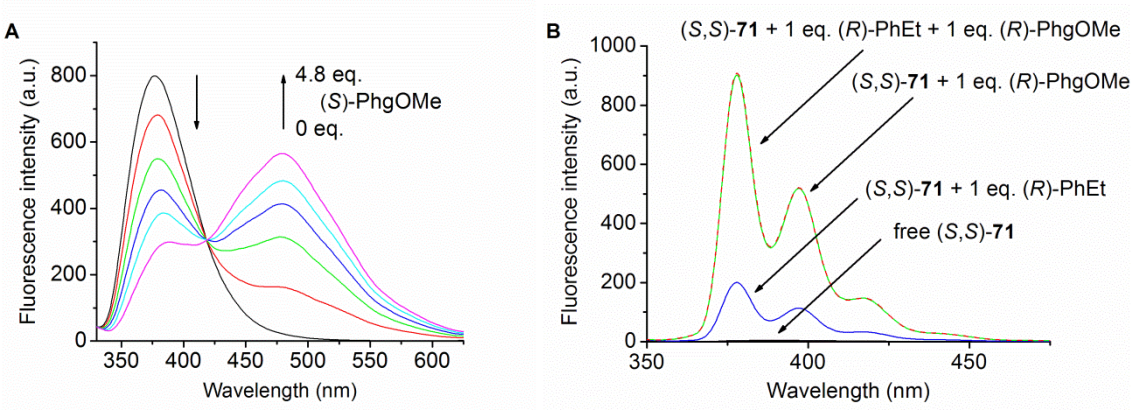


Figure 5. Series of fluorescence emission spectra upon titration of (*S*,*S*)-**68** (5 μM) with (*S*)-PhgOMe (0, 0.4, 0.8, 1.4, 2.4, 4.8 eq.) (**A**), fluorescence emission spectra of (*S*,*S*)-**71** alone (20 μM) and in the presence of 1 eq. of (*R*)-PhEt, 1 eq. of (*R*)-PhgOMe and 1 eq. of (*R*)-PhEt + 1 eq. of (*R*)-PhgOMe together (**B**)

The addition of PhEt, NapEt, PhgOMe and PheOMe to sensor molecules (*R*,*R*)-**69**–(*R*,*R*)-**72** and (*S*,*S*)-**69**–(*S*,*S*)-**72** resulted in large fluorescence enhancement (*Figure 5.B*). In all cases, restoration of the pyrene fluorescence with emission bands at 378, 397 and 417 nm could be observed, because complexation of the ammonium guest enantiomers inhibited the PET quenching directed from the (trialkylamine type) nitrogen to the pyrene unit. The titration series of spectra could be fitted satisfactorily for 1:1 complex formation. The sensor molecules revealed practically no enantiomeric recognition ability; however, a kind of molecular recognition ability was found. It was observed that the stabilities of PhgOMe and PheOMe complexes are higher than those of PhEt and NapEt ones. Diazacrown ethers (*R*,*R*)-**71** and (*S*,*S*)-**71** formed significantly more stable complexes with protonated amino acid esters (PhgOMe and PheOMe, log *K* between 6.25 and 6.51) than with protonated primary amines (PhEt and NapEt, log *K* between 3.79 and 4.09), which make them suitable for selective sensing of the former type species (*Figure 5.B*) [5].

5. THESES

1. Two new acridone- and four new 5,5-dioxophenothiazine-based bis(urea) and bis(thiourea) type enantiopure fluorescent receptor molecules containing (*S*)-1-arylethyl moieties were synthesized. We proved that four of these molecules could be applied as anion sensors for the recognition of the enantiomers of biologically important deprotonated carboxylic acid derivatives. We demonstrated that the difference of urea and thiourea units had a great effect on the degree of enantiomeric recognition [1, 2].

2. The newly synthesized fluorescent sensor molecule containing an acridone moiety, as well as amide and ester groups showed selectivity toward F^-, Ca^{2+} and Hg^{2+}. While F^- caused deprotonation of the sensor molecule, the latter forms different structures with Ca^{2+} and Hg^{2+}. Using the optical responses of the sensor molecule for F^- and Ca^{2+}, we established double chemical input / single optical output molecular logic gates [3].

3. Three new enantiopure pyridino-18-crown-6 ether-based sensor molecules containing a benzothiazole fluorophore, and four unreported pyridine derivative precursors were prepared. Using fluorescence spectroscopy, we demonstrated that these crown ethers had moderate or appreciable enantiomeric recognition abilities toward the enantiomers of protonated primary amines. In the cases of two crown ethers and selected protonated amino acid esters, we proved that the formation of protonated macrocycles took place in the excited state [4].

4. We synthesized eight new enantiomerically pure aza- and diaza-18-crown-6 ethers containing a pyrene fluorophore and their unreported precursors. We proved that these crown ethers formed H-bonded complexes with protonated primary amines and amino acid esters, which resulted in large fluorescence enhancement due to the inhibition of the PET process. We proved that the diazacrown ether containing an *N*-formyl group formed significantly more stable complexes with the studied protonated amino acid esters than with the protonated primary amines [5].

6. POSSIBLE APPLICATIONS

Anion sensor (*S*,*S*)-**59** has an appreciable enantiomeric recognition ability toward Boc-Ala. Receptor (*S*,*S*)-**65** has considerable selectivity toward F^-, Ca^{2+} and Hg^{2+} ions. Pyridino-crown ether-based ligands (*S*,*S*)-**66**–(*S*,*S*)-**68** render appreciable (in one case moderate) enantiomeric recognition abilities toward PhEt and NapEt and moderate selectivity toward the enantiomers of PhgOMe. Ligands (*R*,*R*)-**71** and (*S*,*S*)-**71** selectively bind protonated amino acid esters. These fluorescent receptors may be good candidates for incorporating them into polymeric membranes with the aim of constructing optical sensors. There is a great demand for the application of optical sensors in fields such as pharmaceutical industry, food industry, environmental protection, medical analysis, cell biology and process control.

7. PUBLICATIONS

7.1. Publications related to the PhD thesis

[1] Pál, D.; Móczár, I.; Kormos, A.; Baranyai, P.; Óvári, L.; Huszthy, P.: Synthesis and enantiomeric recognition studies of optically active acridone bis(urea) and bis(thiourea) derivatives, *Tetrahedron: Asymmetry* **2015**, *26*, 1335–1340.
DOI: 10.1016/j.tetasy.2015.10.004, [IF: 2.108, independent citations: 6]

[2] Pál, D.; Móczár, I.; Kormos, A.; Baranyai, P.; Huszthy, P.: Synthesis and enantiomeric recognition studies of optically active 5,5-dioxophenothiazine bis(urea) and bis(thiourea) derivatives, *Tetrahedron: Asymmetry* **2016**, *27*, 918–922.
DOI: 10.1016/j.tetasy.2016.08.002, [IF: 2.126, independent citations: 4]

[3] Pál, D.; Baranyai, P.; Leveles, I.; Vértessy, B. G.; Huszthy, P.; Móczár, I.: An acridone-based fluorescent chemosensor for cationic and anionic species, and its application for molecular logic operations, *ChemistrySelect* **2019**, *4*, 11936–11943.
DOI: 10.1002/slct.201903483, [IF(2018): 1.716]

[4] Pál, D.; Móczár, I.; Szemenyei, B.; Marczona, D.; Kocsis, I.; Prikler, G.; Vezse, P.; Baranyai, P.; Huszthy, P.: Pyridino-18-crown-6 ether type chemosensors containing a benzothiazole fluorophore unit: Synthesis and enantiomeric recognition studies, *Tetrahedron* **2019**, *75*, 2900–2909.
DOI: 10.1016/j.tet.2019.04.024, [IF(2018): 2.377]

[5] Pál, D.; Gede, M.; Móczár, I.; Baranyai, P.; Bagi, P.; Huszthy, P.: Synthesis and complexation studies of optically active aza- and diazacrown ethers containing a pyrene fluorophore unit, *Period. Polytech. Chem. Eng.* **2020**, *64*, 20–36.
DOI: 10.3311/PPch.14467, [IF(2018): 1.382]

7.2. Other publications

6. Kormos, A.; Móczár, I.; Pál, D.; Baranyai, P.; Holczbauer, T.; Palló, A.; Tóth, K.; Huszthy, P.: Unique fluoride anion complexation in basic media by 5,5-dioxophenothiazine bis(phenylurea) and bis(phenylthiourea), *Tetrahedron* **2013**, *69*, 8142–8146.
DOI: 10.1016/j.tet.2013.07.051, [IF: 2.817, independent citations: 3]

7. Kormos, A.; Móczár, I.; Pál, D.; Baranyai, P.; Kupai, J.; Tóth, K.; Huszthy, P.: Synthesis and enantiomeric recognition studies of a novel 5,5-dioxophenothiazine-1,9 bis(thiourea) containing glucopyranosyl groups, *Tetrahedron: Asymmetry* **2013**, *24*, 62–65.
DOI: 10.1016/j.tetasy.2012.11.020, [IF: 2.165, independent citations: 14]

8. Szemenyei, B.; Móczár, I.; Pál, D.; Kocsis, I.; Baranyai, P.; Huszthy, P.: Synthesis and enantiomeric recognition studies of optically active pyridino-crown ethers containing an anthracene fluorophore unit, *Chirality* **2016**, *28*, 562–568.
DOI: 10.1002/chir.22614, [IF: 1.956, independent citations: 2]

9. Márton A.; Szabó-Szentjóbi H.; Pál D.; Dargó G.; Balogh Gy. T.; Tóth T.; Huszthy P.: Koronaéter származékok előállítása P-C kötés kialakításával. In: *Fiatal Kémikusok Fóruma Konferencia Kiadvány*, 49–55. (Debrecen, April 3–5, 2019)
ISBN: 978-615-6018-00-7

10. Szabó-Szentjóbi, H.; Márton, A.; Pál, D.; Dargó, G.; Szigetvári, Á.; Szántay, C.; Balogh, G. T.; Tóth, T.; Huszthy, P.: Synthesis, fluorescence and NMR spectroscopic studies of a

novel phosphinoxido-18-crown-6 ether containing an anthracene fluorophore unit, *Period. Polytech. Chem. Eng.* **2020**, *64*, 37–45.
DOI: 10.3311/PPch.14646, [IF(2018): 1.382]

7.3. Presentations related to the topics of the PhD thesis

1. Kormos A.; Móczár I.; **Pál D.**; Baranyai P.; Tóth K.; Huszthy P.: Fentiazin egységet tartalmazó anionszenzorok szintézise és molekuláris felismerőképességük vizsgálata (*XXXV. Kémiai Előadói Napok*, Szeged, October 29–31, 2012; ISBN: 978-963-315-099-3, pp. 65–66.)
2. Kormos, A.; **Móczár, I.**; Pál, D.; Baranyai, P.; Holczbauer, T.; Palló, A.; Tóth, K.; Huszthy, P.: Unique Fluoride Complexation in Basic Media by Urea and Thiourea Derivatives of Phenothiazine-5,5-dioxide (*8th International Symposium on Macrocyclic and Supramolecular Chemistry*, Arlington, Virginia, USA, July 7–11, 2013) (poster)
3. **Pál D.**; Móczár I.; Kormos A.; Baranyai P.; Óvári L.; Huszthy P.: Akridon fluorofort tartalmazó enantiomertiszta anionszenzorok előállítása és vizsgálata (*MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése*, Balatonszemes, May 21–23, 2014)
4. **Szemenyei B.**; Móczár I.; Kocsis I.; Pál D.; Baranyai P.; Huszthy P.: Antracén fluorofort tartalmazó enantiomertiszta koronaéterek előállítása és vizsgálata (*MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése*, Balatonszemes, May 27–29, 2015)
5. **Móczár, I.**; Szemenyei, B.; Kocsis, I.; Pál, D.; Baranyai, P.; Huszthy, P.: Synthesis and enantiomeric recognition studies of pyridino-crown ethers containing an anthracene fluorophore unit (*10th International Symposium on Macrocyclic and Supramolecular Chemistry*, Strasbourg, France, June 28–July 2, 2015) (poster)
6. **Pál D.**; Móczár I.; Kormos A.; Baranyai P.; Huszthy P.: 5,5-Dioxofentiazin egységet tartalmazó enantiomertiszta anionszenzorok előállítása és vizsgálata (*MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése*, Balatonszemes, May 18–20, 2016)
7. **Pál D.**; Móczár I.; Kormos A.; Baranyai P.; Óvári L.; Huszthy P.: Heterociklus egységet tartalmazó optikailag aktív anionszenzorok előállítása és vizsgálata (*14th Conference of George Oláh Doctoral School*, Budapest, February 2, 2017)
8. **Pál, D.**; Móczár, I.; Kormos, A.; Baranyai, P.; Huszthy, P.: Chiral anion sensors containing a 5,5-dioxophenothiazine unit (*19th JCF-Frühjahrssymposium*, Mainz, Germany, March 29–April 1, 2017) (poster)
9. **Pál, D.**; Móczár, I.; Marczona, D.; Baranyai, P.; Huszthy, P.: Synthesis and Enantiomeric Recognition Studies of a Pyridino-18-crown-6 Ether Containing a Benzothiazole Unit (*25th Croatian Meeting of Chemists and Chemical Engineers*, Poreč, Croatia, April 19–22, 2017) (poster)
10. **Pál D.**; Móczár I.; Marczona D.; Baranyai P.; Huszthy P.: Benztiazol egységet tartalmazó piridino-18-korona-6-éter előállítása és enantiomerfelismerő-képességének vizsgálata (*MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése*, Balatonszemes, May 15–17, 2017)
11. **Gede M.**; Pál D.; Móczár I.; Huszthy P.: Pirén fluorofort tartalmazó enantiomertiszta diaza-18-korona-6-éterek előállítása (*MTA Szteroid- és Terpenoidkémiai Munkabizottság előadóülése*, Szeged, November 27, 2017)

12. **Szemenyei B.**; Pál D.; Móczár I.; Huszthy P.: Néhány piridin egységet tartalmazó új koronaéter szintézise és felhasználási lehetőségeik szenzor-, illetve szelektormolekulaként (*MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése*, Balatonszemes, June 6–8, 2018)

7.4. Other presentations

13. **Szabó-Szentjóbi H.**; Márton A.; Pál D.; Tóth T.; Huszthy P.: Synthesis and Studies of Crown Ether Based Fluorescent Sensor Molecules (*XXIV. Nemzetközi Vegyész-konferencia*, Szovátafürdő, Romania, October 24–27, 2018)
14. **Márton A.**; Szabó-Szentjóbi H.; Pál D.; Dargó G.; Balogh Gy. T.; Tóth T.; Huszthy P.: Fluoreszcens, foszforatomot tartalmazó 18-korona-6-éterek előállítása és vizsgálata (*MTA Szteroid- és Terpenoidkémiai Munkabizottság előadóülése*, Szeged, November 26, 2018)