SYNTHESIS AND MOLECULAR RECOGNITION
STUDIES OF ACRIDONO- AND ACRIDINO-
CROWN ETHER-BASED SENSOR AND
SELECTOR MOLECULES

Summary of PhD thesis

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1. INTRODUCTION

Development of sensor and selector molecules for the analysis of metal ions and for selective recognition and separation of the enantiomers of chiral molecules is of great interest due to their potential applications in pharmaceutical and food industries as well as in environmental chemistry. The complexing ability of these sensors and selectors is based on molecular recognition. The action of this phenomenon can be best described as a selective discrimination between two or more molecules by which specific interaction ordered structures, complexes are formed. Good examples for the action of molecular recognition are the double-helix formation of DNA, antigen-antibody interactions of immunological responses and the selective metal ion binding and transport of natural ionophores across biological membranes. Complexes formed by the action of molecular recognition are held together by non-covalent forces emerging between stereoelectronically complementary groups. Previously, molecular recognition was considered as a biological phenomenon, but findings of the past few decades clearly proved that it can be brought about by relatively simple synthetic molecules, such as crown ethers.

My PhD research was focused on the synthesis and molecular recognition studies of new achiral and enantiomerically pure sensor and selector molecules and their precursors based on acridono- and acridino-18-crown-6 and 21-crown-7 ethers. The metal ion selectivity and enantiomeric selectivity of the new ligands were studied using UV-vis and fluorescence spectroscopies. Furthermore, four new HPLC columns filled with acridino-crown ether-based chiral stationary phases were prepared. Their chromatographic enantioseparation ability for the mixtures of enantiomers of protonated primary amino compounds containing an aromatic moiety was demonstrated. Our additional aim was to substitute the acridine ring with an aromatic unit at position 9 in order to increase the degree of selectivity of complexation.

2. LITERATURE BACKGROUND

The concept of molecular recognition had been devised by Emil Fischer in 1894 by explaining the specific action of an enzyme with a single substrate by the “Lock and Key” analogy. The introduction of the notion of supramolecular chemistry can be attributed to Jean-Marie Lehn; in his view, it may be defined as “chemistry beyond the molecule”. The ever-expanding field of supramolecular chemistry deals with the formation, the properties and the applications of molecular associations held together by non-covalent forces. Since Charles J. Pedersen, a pioneer among the researchers of this field, reported the preparation and evaluation of the first synthetic host molecules; macrocycles called crown ethers, this area of research has gained much attention. The Nobel Prize in chemistry in 1987 was awarded jointly to Donald J. Cram, Jean-Marie Lehn and Charles J. Pedersen "for their development and use of molecules with structure-specific interactions of high selectivity", indicating the importance of this area of research. Furthermore, in 2016, the awarding of the Nobel Prize in chemistry to Jean-Pierre Sauvage, Sir J. Fraser Stoddart and Bernard L. Feringa for “the design and
production of molecular machines” clearly indicates that this field of chemistry is still flourishing.

The preparation of the first enantiopure chiral crown ether was accomplished by Stoddart and Szarek;\(^5\) a carbohydrate-based macrocycle (1, Figure 1) was synthesized starting from D-(−)-ribose. Cram and co-workers synthesized macrocyclic compounds with axial chirality, bis(1,1′-binaphthyl)-22-crown-6 ethers (S,S)-2, Figure 1), and performed the first enantiomeric recognition study of crown ethers.\(^6\)

![Figure 1. The first chiral crown ether](image)

It was found that beside hydrogen bonding, other weak interactions such as \(\pi-\pi\) interaction\(^7\) and cation-\(\pi\) interaction\(^8\) interaction are of great significance for the action of molecular and enantiomeric recognition. Extensive modifications of the crown ether structure have been made in the search for new functions. Several examples are known of crown ethers incorporating heterocycles in the macro-ring systems. These compounds often have multiple complexation centers, which may influence the rigidity of the macrocycle, and are expected to have increased selectivity and complex formation properties. A large number of these macrocycle contain an \(N\)-heterocyclic (e.g. pyridine\(^9\), pyrimidine, triazine or triazole) subunit. The study of their enantiomeric recognition properties toward chiral organic ammonium salts has also been accomplished using calorimetric titration, chromatography, circular dichroism spectroscopy, NMR spectroscopy and electrochemical methods.\(^10\)

XRD measurements have established that,\(^11\) in good agreement with the “three-point rule” described by Pirkle and Pochapsky,\(^12\) enantiomeric discrimination abilities of macrocycles containing an \(N\)-heterocyclic subunit (such as pyridine or acridine) are based on three separate interactions (at least one of them being stereochemically dependent); a tripod-like hydrogen bonding involving the nitrogen atom and two alternate oxygen atoms of the \(N\)-heterocyclic macrocycle host and three protons of the ammonium salt guest, \(\pi-\pi\) interaction between the heteroaromatic ring of the ligand and the aromatic group of the ammonium salt, steric repulsion between the groups at the chiral centers of the host molecule and some groups of the guest molecule.

The strength of cation–π and π–π interactions can be enhanced by incorporating a heterocyclic unit containing a more extended aromatic system into the macroring, such as an acridine or acridone unit. These tricyclic ring systems also make the crown ether framework more rigid, which may further improve selectivity. Furthermore, due to their chromogenic and fluorogenic properties, their complexing abilities can be studied by sensitive photophysical methods such as UV-vis and fluorescence spectroscopies. For these reasons, over the past decade the preparation and study of several acridino- and acridono-crown ether-based sensors have been accomplished.\(^{13}\)

Apart from being applied as sensors, crown ethers can also be used as selectors for enantioseparation of chiral organic compounds. Among the numerous separation techniques, liquid chromatography by chiral stationary phases (CSPs) has been proven to be the most precise and most effective method for the above-mentioned tasks.\(^{14}\)

Several \(N\)-heterocycle-based CSPs have been prepared by the attachment of a chiral selector by covalent bonds to a solid support. They can be classified according to the applied solid support; selectors have been bound to ordinary silica gel,\(^{15}\) to Merrifield resin,\(^{16}\) to HPLC quality silica gel\(^{17}\) and to spherical HPLC quality silica gel.\(^{18}\)

Before starting my PhD work, the synthesis of an acridino-crown ether-based CSP had been described only in one article.\(^{19}\) Lakatos and co-workers reported the preparation of a macrocycle containing a terminal double bond, \((R,R)-50\), which was attached to \(\gamma\)-mercaptpropyl-functionalized spherical HPLC quality silica gel yielding chiral stationary phase \((R,R)-\text{CSP-51 (Scheme 1)}\).

Taking all of the earlier mentioned advantages into consideration it could be expected that CSPs containing an acridine unit were likely to show a higher degree of enantiomeric recognition compared to their pyridino-analogaues, therefore their preparation was of interest.

3. EXPERIMENTAL METHODS

During the synthesis of the compounds the well-established methods of preparative organic chemistry were used. The progress of reactions was followed by thin layer chromatography. The crude products were purified by column chromatography, preparative thin layer chromatography, recrystallization, or distillation.

Purity of the compounds was determined by thin layer chromatography, measuring melting points and optical rotations. Structures of the products were determined using IR, $^1$H- and $^{13}$C-NMR, MS spectroscopies and elemental analysis.

Determination of the crystal structures of three crystalline complexes was performed by X-ray crystallography in cooperation with Ibolya Leveles and Prof. Beáta G. Vértessy. HPLC columns containing the chiral crown ether selectors were filled with the help of a Haskel-type pump. We applied Hitachi-type HPLC system for column-testing.

Complexation properties of the synthesized ligands for metal ions and the enantiomers of optically active protonated amines and α-amino acid esters were studied using UV-vis, and fluorescence spectroscopic methods. Complex stability constants were determined by the Benesi-Hildebrand method or by global nonlinear regression analysis using ReactLab Equilibria™ program based on the data of UV-vis and fluorescence spectroscopic titrations.

4. RESULTS

4.1. Synthesis of the new CSPs

Macrocyclization is the key step in the synthesis of crown ethers. These bimolecular nucleophilic substitution (S$_2$N$_2$) type reactions were carried out in the presence of a cheap base, K$_2$CO$_3$, in MeCN. Ring closure of formamide 60$^{19}$ and diisobutylo- or dimethyl-substituted tetra- or pentaethylene glycol di(p-toluenesulphonate) (S,S)-61a, (S,S)-61b, (S,S)-61c, and (S,S)-61d afforded macrocycles (R,R)-62a, (S,S)-62b, (S,S)-62c and (R,R)-62d (Scheme 2) [P1,P2]. In the cases of secondary ditosylates [(S,S)-61a, (S,S)-61b] chiral centers are affected, thus reactions proceed with inversion of configuration. For primary ditosylates [(S,S)-61b, (S,S)-61c] the chiral centers are not involved in the reaction, thus the configuration remains unchanged. In order to avoid racemization, and to ensure total inversion of configuration the reaction temperature was kept at 50 °C. Tetraethylene glycol ditosylates (S,S)-61a$^{20}$ and (S,S)-61b$^{21}$, and pentaethylene glycol ditosylate (S,S)-61c$^{22}$ were obtained as described in the literature. Chiral pentaethylene glycol ditosylate (S,S)-61d was prepared by the reaction of the appropriate diol$^{23}$ and tosyl chloride (TsCl).

Removal of the formyl protecting group from the nitrogen atom of macrocycle (R,R)-62a was carried out by using potassium hydroxide in aqueous ethanol. In the case of ligands (S,S)-62b, (S,S)-62c and (R,R)-62d, because of the strong potassium cation

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complexation tendency of macrocycles (S,S)-64b, (S,S)-64c and (R,R)-64d we refrained from the use of any alkali metal hydroxide, so we removed the formyl group with hydrochloric acid in aqueous methanol obtaining the secondary amines in very good yields (Scheme 2) [P1, P2]. Acylation of the secondary amino group of the appropriate amines with oxalyl chloride in toluene, followed by a Friedel-Crafts type reaction in the presence of catalytic amount of iodine in bromobenzene gave isatin derivatives (R,R)-67a, (S,S)-67b, (S,S)-67c and (R,R)-67d, respectively. The new acridino-18-crown-6 and acridino-21-crown-7 ether carboxylic acids (R,R)-68a, (S,S)-68b, (S,S)-68c, (R,R)-68d and (R,R)-68e were prepared from new isatins (R,R)-67a, (S,S)-67b, (S,S)-67c, (R,R)-67d and the reported one [(R,R)-67e] using aqueous tetramethylammonium hydroxide (Scheme 2).

Scheme 2. Synthesis of enantiopure crown ethers containing a 9-acridinecarboxylic acid unit

Carboxylic acids (R,R)-68a and (R,R)-68e were transformed to triethoxysilyl derivatives (R,R)-69a and (R,R)-69b containing an amide functional group by addition of (3-aminopropyl)triethoxysilane [P1]. Acylations of (3-aminopropyl)triethoxysilane (TSPA) were carried out using dicyclohexylcarbodiimide (DCC) in dichloromethane. The crown ether derivatives containing triethoxysilyl end groups were heated under mechanical stirring without purification with spherical HPLC quality silica gel in toluene to obtain (R,R)-CSP-52a and (R,R)-CSP-52b (Scheme 3 A). This method is known as the traditional batch process. It is generally accepted that the most reliable packing method for HPLC columns is the low viscosity slurry-pack technique. In order to challenge this, it became our intention to develop a novel method for the preparation of chiral stationary phases [P3]. We applied flow chemistry in the multistep-synthesis of an acridino-18-crown-6 ether-based CSP.

Chiral stationary phase \((R,R)\)-CSP-52c was prepared starting from chiral acridino-crown ether \((R,R)\)-68e as outlined in Scheme 3. To carboxylic acid \((R,R)\)-68e, TSPA and DCC were added, the solution was continuously recirculated through an HPLC column containing blank silica gel (SP-70), at elevated pressure and temperature, until the immobilization of the selector molecule on the HPLC quality silica gel by covalent bonds was finished (Scheme 3 B) [P3].

In order to study the influence of the position of the chiral centers on the degree of enantiomeric recognition a new chiral stationary phase \((S,S)\)-CSP-52d was prepared from carboxylic acid \((S,S)\)-68b. The latter carboxylic acid was first reacted with thionyl chloride with a catalytic amount of dimethylformamide. The resulting acid chloride, due to its unstable nature, was then reacted with TSPA in dichloromethane. Crown ether \((S,S)\)-71 was heated without purification with spherical HPLC quality silica gel in toluene to obtain \((S,S)\)-CSP-52d (Scheme 3 C) [P2]. This method was faster and it gave better yield than the procedure described for the synthesis of \((R,R)\)-CSP-52b.

![Scheme 3. Preparation of CSPs](image)

The packing of the 150×4 mm stainless steel HPLC columns with the modified spherical HPLC quality silica gel containing the appropriate selector compound

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[(R,R)-CSP-52a, (R,R)-CSP-52b or (S,S)-CSP-52d] was performed by applying a Haskel-pump at 500 bar using the slurry-pack method [P1,P2].

4.2. Separation studies of the CSPs

The studies of the newly prepared chiral stationary phases containing acridino-18-crown-6 ether selectors for their enantiomeric separation ability of the mixtures of enantiomers of protonated primary amino compounds containing an aromatic moiety [1-(1-naphthyl)ethylamine (1-NEA), 1-(2-naphthyl)ethylamine (2-NEA), 1-(4-bromophenyl)ethylamine (Br-PEA), 1-(4-nitrophenyl)ethylamine (NO₂-PEA)] were performed by HPLC (Table 1) [P1–P3]. In all cases isocratic elution was applied with flow rates of 1.0 mL/min.

Table 1. Chromatographic data for the separation of the mixtures of enantiomers of protonated primary aralkylamines on the new CSPs

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CSP</th>
<th>t (R) [min]</th>
<th>t (S) [min]</th>
<th>α</th>
<th>Rs</th>
<th>Solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-NEA</td>
<td>(R,R)-CSP-52a</td>
<td>1.96</td>
<td>3.49</td>
<td>1.78</td>
<td>2.46</td>
<td>B</td>
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<tr>
<td></td>
<td>(R,R)-CSP-52b</td>
<td>6.91</td>
<td>12.87</td>
<td>1.86</td>
<td>2.53</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>(S,S)-CSP-52c</td>
<td>11.02</td>
<td>13.51</td>
<td>1.23</td>
<td>1.20</td>
<td>D</td>
</tr>
<tr>
<td>2-NEA</td>
<td>(R,R)-CSP-52a</td>
<td>5.19</td>
<td>9.95</td>
<td>1.92</td>
<td>2.06</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>(R,R)-CSP-52b</td>
<td>1.75</td>
<td>2.74</td>
<td>1.57</td>
<td>1.55</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>(R,R)-CSP-52c</td>
<td>6.98</td>
<td>13.23</td>
<td>1.89</td>
<td>3.41</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>(S,S)-CSP-52d</td>
<td>9.47</td>
<td>10.05</td>
<td>1.06</td>
<td>0.34</td>
<td>E</td>
</tr>
<tr>
<td>Br-PEA</td>
<td>(R,R)-CSP-52a</td>
<td>5.42</td>
<td>12.47</td>
<td>2.30</td>
<td>2.94</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>(R,R)-CSP-52b</td>
<td>2.06</td>
<td>4.23</td>
<td>2.05</td>
<td>2.31</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>(R,R)-CSP-52c</td>
<td>4.83</td>
<td>7.54</td>
<td>1.56</td>
<td>1.36</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>(S,S)-CSP-52d</td>
<td>7.62</td>
<td>7.84</td>
<td>2.05</td>
<td>&lt;0.2</td>
<td>E</td>
</tr>
</tbody>
</table>

A: 0.06 % HCOOH and 0.03 % triethylamine in a 3:7 mixture of MeCN/MEOH
B: 0.04 % HCOOH and 0.02 % triethylamine in a 1:4 mixture of MeCN/MEOH
C: 0.12 % HCOOH and 0.08 % diethylamine TEA in a 1:4 mixture of MeOH/MeCN
D: 1:4 mixture of MeCN/25 mmol/L aqueous ammonium acetate
E: 1:4 mixture of MeCN/40 mmol/L aqueous ammonium acetate

It was found for the studied compounds that in all cases the (R)-enantiomer eluted with a shorter retention time than that of its antipode. In the cases of (R,R)-CSP-52a, (R,R)-CSP-52b and (R,R)-CSP-52c this order of elution represents the higher stability of heterochiral complexes [(R,R)-crown ether-(S)-ammonium salt] compared to that of homochiral complexes [(R,R)-crown ether-(R)-ammonium salt]. This behavior is in full agreement with our earlier observations using CSPs containing N-heterocycle-based macrocyclic chiral selectors attached to silica gel.¹⁴,¹⁹ However, in the case of (S,S)-
CSP-52d the order of elution indicated the higher stability of homochiral complexes. This behavior can be attributed to the altered position of the chiral centers; by placing the chiral centers one carbon atom farther away from the acridine unit, steric repulsion, one of the main factors which influences the enantiomeric recognition, between the host and guest molecules becomes less significant. Other secondary interactions such as hydrogen-bonding and π–π interaction, take over the determining role during the enantiomeric separation.

Comparing the two chiral stationary phases (R,R)-CSP-52a and (R,R)-CSP-52b; (R,R)-CSP-52a containing isobutyl groups at the chiral centers of its selector showed better separation and resolution factors for all studied analytes (Table 1). This can be attributed to the increased bulkiness of the isobutyl group compared to that of the methyl group. For all the studied analytes highest selectivity (α values) was observed in the case of the diisobutyl-substituted CSP [(R,R)-CSP-52a]; highest resolution factors (R5 values) were achieved by the CSP prepared by the continuous recirculation (flow) method [(R,R)-CSP-52c]. Also, the latter CSP gave longer retention times, better separation and better resolution factors for the enantiomers of protonated 1-NEA, 2-NEA and Br-PEA than the dimethyl-substituted acridino-crown ether-based CSP [(R,R)-CSP-52b] prepared by traditional batch process.

4.3. XRD study of diastereomeric complexes

With the purpose of identifying and understanding better the noncovalent secondary interactions governing the enantiomeric recognition of crown ether-based sensor and selector molecules containing an acridine unit, suitable single crystals for X-ray analysis were prepared from the reported dimethyl-substituted acridino-crown ether (R,R)-19c, a parent compound of the selectors of the CSPs, and the enantiomers of 1-NEA (Figure 2). We found that the heterochiral complex [(R,R)-19c–(S)-1-NEAH+ ClO4−] is more stable than the homochiral one [(R,R)-19c–(R)-1-NEAH+ ClO4−] (Figure 2) [P4]. Strong hydrogen bonds formed in both diastereomers between the protons of the ammonium group of 1-NEAH+ ClO4− and the appropriate N and O atoms of the crown ether host. The most significant difference between the diastereomers is the presence (in the case of the heterochiral complex) or the absence (in the case of the homochiral complex) of π–π interaction.

![Figure 2. (A) Host and guest molecules studied by XRD, (B) heterochiral complex, (C) homochiral complex](image-url)
4.4. Synthesis of sensors containing an acridone unit

The synthesis of new acridono-18-crown-6 ether type sensor 53 and its reported analogue 35a\textsuperscript{26} was carried out as outlined in Scheme 4 [P5]. The reaction of aniline derivative 73\textsuperscript{27} with formic acid gave formamide 74 in excellent yield. The latter was treated with 2-bromoanisole derivative 75\textsuperscript{28} in Dowtherm\textsuperscript{8} A using copper powder and cuprous oxide as catalysts in the presence of potassium carbonate, then the unreported formamide derivative 76 was demethylated with anhydrous aluminium chloride in chlorobenzene to obtain formamide 72. Macrocyclization of formamide 72 and ditosylate 77\textsuperscript{29} was performed in the presence of potassium carbonate in acetonitrile. Removal of the formyl protecting group from the resulted crown ether 78 gave secondary amine 79. Acylation of the amino group of crown ether 79 with oxalyl chloride in toluene, followed by a Friedel-Crafts type acylation in the presence of a catalytic amount of iodine in bromobenzene gave isatin derivative 81a. Carboxylic acids 82a and 82b were obtained from isatins 81a and 81b\textsuperscript{19} using aqueous tetramethylammonium hydroxide. The decarboxylation of the latter carboxylic acids was accomplished using sulfuric acid, resulting in the formation of 53 and 35a\textsuperscript{26}.

![Scheme 4. Synthesis of the sensors 35a and 53](image)

4.5. Study of the complexing properties of 35a and 53 by UV-vis spectroscopy and XRD

The cation recognition abilities of sensor molecules 35a and 53 toward the perchlorate salts of nine metal ions (Ag\textsuperscript{+}, Ca\textsuperscript{2+}, Cd\textsuperscript{2+}, K\textsuperscript{+}, Mg\textsuperscript{2+}, Na\textsuperscript{+}, Ni\textsuperscript{2+}, Pb\textsuperscript{2+}, Zn\textsuperscript{2+}) were studied in acetonitrile [P5]. Upon addition of a tenfold excess of these metal ions the UV-vis spectra of the tetramethyl-substituted ligand 53 did not show any changes.

This can be attributed to the lack of complexation or low stabilities of the complexes. Szalay and coworkers suggested that in the cases of analogous chiral acridono-crown ethers during complexation the ligands shifted to the hydroxyacridine tautomeric form. Therefore, we assumed that the substitution of the acridone moiety with electron-donating methyl groups, in the case of 53, hindered the formation of the 9-hydroxyacridine tautomeric form, which presumably favors complexation. Additionally, conformational changes, due to the presence of the additional methyl groups, can also be the cause of the absence of complexation. The complexation ability of the parent compound 35a was also studied; in this case among the studied metal ions only Pb^{2+} gave rise to significant spectral changes, namely a bathochromic shift of the absorption spectra (Figure 3 A). In order to determine the stoichiometry and stability constant (K_s) of the lead(II)–ligand complex UV titration was performed (Figure 3 B). UV-vis spectra were measured at 13 different Pb^{2+} to 35a ratios. We could assume the formation of a complex with 1:1 ligand to metal ion ratio because upon being treated with Pb^{2+} the UV-vis enhancement of the sensor followed the Benesi-Hildebrand equation. The logK value determined by this method was 3.64.

In order to further study the complex of 35a and lead(II), suitable single crystals for X-ray analysis were obtained and measured [P6]. In the crystal, a complex consisting of two monomers was found (Figure 4). One monomer of the heterodimer formed a complex with the lead(II) ion. In the other monomer, a complexed water molecule was found in the cavity of the macrocycle.

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**Figure 3.** Study of complexation by UV-vis spectroscopy

**Figure 4.** Structure of the heterodimer containing two monomers. Note that one monomer coordinates to a lead(II) ion, whereas the other monomer binds a water molecule

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The acridine ring substituted with an aromatic unit at position 9 may further increase the degree of complexation. Therefore, our attention turned toward the preparation of 9-phenylacridino-18-crown-6 ether type sensors \(54a\) and \((R,R)-54b\) (Scheme 5) [P7]. The novel sensors were synthesized starting from 4,5-dimethoxyacridin-9(10H)-one \(86\) which was transformed to 9-chloro-4,5-dimethoxyacridine \(85\) using phosphoryl chloride. Introduction of the phenyl group obtaining \(84\) was accomplished by modifying the reaction conditions of the Kharasch reaction. The \(o\)-demethylation of \(84\) gave 9-phenylacridine-4,5-diol \(83\) in good yield; the key intermediate for the macrocyclization. The new 9-phenylacridino-18-crown-6 ethers \([54a\) and \((R,R)-54b]\) were prepared by the macrocyclization reaction of \(83\) and ditosylate \(77\) or \((S,S)-87\), in the presence of a weak base, potassium carbonate in DMF.

![Scheme 5. Synthesis of 9-phenylacridino-18-crown-6 ethers 54a and (R,R)-54b](image)

4.7. Study of the complexing properties of 54a and (R,R)-54b by UV-vis and fluorescence spectroscopies

The complexation ability of achiral sensor \(54a\) toward the perchlorate salts of nine metal ions \((\text{Ag}^+, \text{Ca}^{2+}, \text{Cd}^{2+}, \text{K}^+, \text{Mg}^{2+}, \text{Na}^+, \text{Ni}^{2+}, \text{Pb}^{2+}, \text{Zn}^{2+})\) and two ammonium ions (ammonium perchlorate and benzylammonium perchlorate) was first studied by UV-vis spectroscopy in acetonitrile [P7]. The UV-vis spectra of ligand \(54a\) did not show any changes upon addition of a twentyfold excess of \(\text{Ca}^{2+}, \text{K}^+, \text{Mg}^{2+}, \text{Na}^+\) and benzylammonium ions. However, a bathochromic shift of the absorption spectra was observed in the cases of \(\text{Ag}^+, \text{Cd}^{2+}, \text{Ni}^{2+}, \text{Pb}^{2+}, \text{Zn}^{2+}\) and \(\text{NH}_4^+\). Fluorescence titration was performed in order to determine the stoichiometry and stability constants \((K_s)\) of the complexes. As an example the titration of \(54a\) with \(\text{Ag}^+\) ions is shown in Figure 5. With the exception of \(\text{Cd}^{2+}\), in all cases the fluorescence emission spectra showed a
decrease upon addition of the salts, which means that the fluorescence was quenched in the complexes. In the case of Cd$^{2+}$ a bathochromic shift of the spectra was observed. The log$K$ values determined by global nonlinear regression analysis using ReactLab Equilibria$^{\text{TM}}$ program are shown in Table 2.

![Figure 5](image-url) Fluorescence emission series of spectra upon titration of 54a (10 µM) on increasing addition of Ag$^+$ (0–400 equiv.) (A), and of (R,R)-54b (20 µM) with PAMEH$^+$ ClO$_4^-$ [B: (R)-PAMEH$^+$ ClO$_4^-$, 0–125 equiv, C: (S)-PAMEH$^+$ ClO$_4^-$, 0–125 equiv] in MeCN, $\lambda_{ex} = 380$ nm

Table 2. Stability constants for 1:1 stoichiometric complexes of 54a with Ag$^+$, Cd$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$ and NH$_4^+$ in MeCN, $\lambda_{ex} = 380$ nm

<table>
<thead>
<tr>
<th>Ion</th>
<th>Ag$^+$</th>
<th>Cd$^{2+}$</th>
<th>Ni$^{2+}$</th>
<th>Pb$^{2+}$</th>
<th>Zn$^{2+}$</th>
<th>NH$_4^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>log$K$</td>
<td>2.43</td>
<td>4.86</td>
<td>2.48</td>
<td>5.07</td>
<td>4.32</td>
<td>5.73</td>
</tr>
</tbody>
</table>

The enantiomeric discrimination ability of the chiral dimethyl-substituted 9-phenylacridinino-18-crown-6 ether sensor (R,R)-54b was studied toward the enantiomers of the hydrogen perchlorate salts of PEA, 1-NEA, PGME and PAME using UV-vis and fluorescence spectroscopies [P7]. The absorbances of ligand (R,R)-54b were essentially unchanged upon titration with the enantiomers of the salts. However, the fluorescence emission spectra showed a relatively large decrease upon addition of the guest molecules (an example of the enantiomers of PAME is shown in Figure 5). This means that the fluorescence was significantly quenched in the complexes. The latter fluorescence changes were used to determine the stability constants of the complexes and the degree of enantiomeric differentiation ($\Delta$log$K = \log K_{(S)} - \log K_{(R)}$) (Table 3). All of the titration series of the spectra could be fitted satisfactorily using a complex form with 1:1 stoichiometry.

Table 3. Stability constants and selectivity of the complexes of (R,R)-54b with the enantiomers of protonated PEA, 1-NEA, PGME and PAME in MeCN, $\lambda_{ex} = 380$ nm

<table>
<thead>
<tr>
<th>Guest</th>
<th>log$K$</th>
<th>$\Delta$log$K$</th>
<th>Guest</th>
<th>log$K$</th>
<th>$\Delta$log$K$</th>
</tr>
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<td>(R)-PEA</td>
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<td></td>
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<tr>
<td>(R)-1-NEA</td>
<td>4.78</td>
<td>+0.46</td>
<td>(R)-PAME</td>
<td>3.87</td>
<td>+0.11</td>
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<tr>
<td>(S)-1-NEA</td>
<td>5.24</td>
<td></td>
<td>(S)-PAME</td>
<td>3.98</td>
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5. THESES

1. Three new chiral stationary phases based on dimethyl- or diisobutyl-substituted acridino-crown ethers where the linking unit at position 9 of the acridine ring is attached through an amide group were prepared. We demonstrated that these CSPs separated the mixtures of enantiomers of primary aralkylamines efficiently. We proved that the bulkiness and the position of the alkyl groups at the chiral centers have significant effect on the degree of enantiomeric recognition [P1–P3].

2. We elaborated a new and effective continuous recirculation method for the preparation of a dimethyl-substituted acridino-18-crown-6 ether-based chiral stationary phase [P3].

3. We synthesized four new enantiopure dimethyl-substituted acridino-18-crown-6 and acridino-21-crown-7 ether type macrocycles containing a carboxyl group at position 9 of the acridine ring and measured their pKa values by the UV-pH titration method. We identified the neutral forms of these acids as zwitterionic and demonstrated that flexible and zwitterionic systems still pose a challenge for pKa predictor algorithms [P2].

4. We prepared suitable crystals for X-ray analysis from a dimethyl-substituted acridino-18-crown-6 ether, the parent compound of the selectors of the CSPs, and the enantiomers of 1-NEA. We found that the heterochiral complex is more stable than the homochiral one, due to the presence of an intermolecular π–π interaction between the naphthyl unit of 1-NEA and the acridine moiety in the former complex and the absence of it in the latter [P4].

5. We prepared a reported acridono-18-crown-6 ether-based sensor via a new synthetic route and proved by UV-vis spectroscopy that it selectively binds lead(II) ions [P5]. We studied this complex by X-ray diffraction and proved that the host shifts to the 9-hydroxyacridine tautomeric form upon lead(II) complexation. The presence of π–π and cation–π interactions was confirmed [P6].

6. We worked out a new synthetic method for introduction of the phenyl group into position 9 of the acridine unit by a C-C coupling reaction starting from acridino-derivatives substituted with a chlorine atom [P7].

7. Novel 9-phenylacridino-18-crown-6 ether type sensor molecules were prepared. Studying the complex formation properties of these sensors with organic and metal ions, as well as perchlorate salts of primary amines and α-amino acid esters, we observed fluorescence quenching. The enantiopure acridino-crown ether-based sensor showed heterochiral selectivity toward the enantiomers of protonated primary organic aralkylamines [P7].
6. POSSIBLE APPLICATIONS

Experiments are in progress to prepare derivatives of sensors 35a and 54a, which contain lipophilic side chains in order to prepare ion-selective electrodes. Thus, apart from the detection of ions, the measurement of their quantity may also be possible.

Our other objectives include the preparation of new chiral stationary phases from (S,S)-68c and (R,R)-68d by flow chemistry method.

Also our further aim is to synthesize an analogue of (R,R)-54b which contains a carboxylic group at position 4 of the phenyl group; a functional group suitable for attachment through a spacer to silica gel with covalent bonds in order to produce a new CSP.

7. PUBLICATIONS

Publications that form the basis of this PhD thesis:


molecules. *Periodica Polytechnica Chemical Engineering* 2017, online available, DOI: 10.3311/PPch.11277. [IF: 0.557 (2016)].

**Further articles:**


**Conference proceedings (Hungarian):**


**Oral presentations (in Hungarian):**


**Poster presentations (in English):**
