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SYNTHESIS AND APPLICATION OF CHIRAL 18-CROWN-6 ETHERS CONTAINING A PYRIDINE SUBUNIT

PhD Theses

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2012

ABBREVIATIONS

Ac_2O:	acetic anhydride
AcOH:	acetic acid
aq.:	aqueous solution
Bn:	benzyl group
Br-PEA:	1-(4-bromophenyl)ethylamine
CSP:	chiral stationary phase
DHP:	3,4-dihydro-2*H*-pyran
DMF:	*N,N*-dimethylformamide
DMSO:	dimethyl sulfoxide
DPA:	dipicolinic acid (pyridine-2,6-dicarboxylic acid)
Et_2O:	diethyl ether
EtOH:	ethanol
HPLC:	high performance liquid chromatography
IRA-120:	acidic ion-exchange resin
MCPBA:	*m*-chloroperbenzoic acid
MeOH:	methanol
Me_2SO_4:	dimethyl sulfate
1-NEA:	1-(1-naphthyl)ethylamine
2-NEA:	1-(2-naphthyl)ethylamine
NO_2-PEA:	1-(4-nitrophenyl)ethylamine
PAME:	phenylalanine methyl ester
PEA:	1-phenylethylamine
PGME:	phenylglicine methyl ester
PPTS:	pyridinium-*p*-toluenesulfonate
R_S:	chromatographic resolution
TEA:	triethylamine
Tf:	trifluoromethylsulfonyl group
Tf_2O:	trifluoromethanesulfonic anhydride
THF:	tetrahydrofuran
THP:	tetrahydropyranyl group
TMAH:	tetramethylammonium hydroxide
Ts:	tosyl group (*p*-toluenesulfonyl group)
TsCl:	*p*-toluenesulfonyl chloride

1. INTRODUCTION

Development of sensor and selector molecules capable of recognizing and separating the enantiomers of chiral molecules has gained much research interest. The selective complexing ability of sensor and selector molecules is based on the phenomenon of molecular recognition, in which case the host molecule selectively binds a certain type of guest molecule by noncovalent bonding. Molecular recognition is a generally occurring phenomenon in Nature. The best known examples include the metabolism of the single enantiomeric forms of sugars and amino acids in biochemical pathways, the antibody–antigen immune reaction or the specificity of enzymes and natural ionofores. Before 1970s it was belived that (like „*vis vitalis*" theory) molecular recognition is solely a biological phenomenon. However, recent successes of supramolecular chemistry have demonstrated that this biological behaviour can be imitated using small molecules.

The aim of my PhD work was the synthesis and molecular recognition studies of new enantiomerically pure sensor and selector molecules based on pyridino-18-crown-6 ethers and also the preparation of their precursors. Three HPLC columns filled with new pyridino-crown ether–based chiral stationary phases were made, which were applied for chromatographic resolution of four chiral primary amines.

2. LITERATURE BACKGROUND

The basic principles of crown ether chemistry were established by the pioneering work of *Pedersen*, who synthesized the first polyether-type macrocycle as a by-product. Realising its special complex forming ability, he synthesized compounds with related structure, and prepared numerous oxygen-containing macrocycles.[1] Research on the synthesis of macrocycles forming complexes with metal ions soon expanded to the synthesis of macrocycles capable of complex formation with organic cations, anions and neutral molecules.[2]

Enantioselectivity of crown ethers was studied first by *Cram and his co-workers*, who studied the selectivity of bis(binaphthyl)-22-crown-6 ether derivatives toward the enantiomers of protonated primary amines.[3] Since the pioneer work of *Pedersen* and *Cram*, many achiral and enantiomerically pure crown ethers have been synthesized and their metal ion selectivity and enantiomeric discrimination ability have been studied by various methods.

Primary amines, amino acids and their derivatives are very important compounds of biological relevance. Amino acids are the building blocks of proteins, and primary amines are formed during the degradation of amino acids or serve as neurotransmitters. Therefore, the development of synthetic receptors for their enantioselective recognition is of great importance.[4]

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[1] Pedersen, C.S. *J. Am. Chem. Soc.* **1967**, *89*, 2495–2496.; ibid.7017–7036.
[2] Steed, J. W.; Atwood, J. L. *Supramolecular Chemistry* **2009**, Wiley, 2nd ed.
[3] Kyba, E. P.; Siegel, M. G.; Sousa, L. R.; Sogah, G. D. Y.; Cram, D. J. *J. Am. Chem. Soc.* **1973**, *95*, 2691–2692.
[4] Späth, A.; König, B. *Beilstein J. Org. Chem.* **2010**, *6*, Issue 32.

Among the numerous separation techniques, liquid chromatography by chiral stationary phases is proved to be the most precise and most effective one for enantioseparation and for the determination of enantiomeric composition.[5]

Immobilization of enantiopure crown ethers on solid supports (e.g. silica gel, *Merrifield*-type polymer resin, etc.) by covalent bonds results in chiral stationary phases. This technique combines the recyclability of the stationary phase and the selectivity of the macrocycle. Synthesis of selector molecules is expensive and difficult, and they can have toxic effects in biological systems. These disadvantageous properties of crown ethers can be avoided by immobilization with covalent bonds. Immobilized macrocycles can be used as selector molecules for the separation of enantiomers.[6]

Izatt and his co-workers founded that crown ethers containing a pyridine subunit show outstanding complexation properties toward heavy metal ions and protonated primary amines thanks to their aromatic ring and the nitrogen atom.[7]
Studies on complexation of optically active pyridino-crown ethers with the enantiomers of chiral protonated primary aralkyl amines proved that the enantioselectivity is based on three independent interactions: i.) tripodal hydrogen bonding between the pyridine nitrogen and two alternating oxygen atoms of the macroring and the three protons of the ammonium salt, ii.) π–π stacking between the aromatic moieties of the host and the guest, and iii.) steric repulsion between the substituents on the chiral centers of the crown ethers and certain aromatic protons of the ammonium salts. In all cases, tripodal hydrogen bonding between the macrocycles and the ammonium cations involves the pyridine nitrogen atom. The two attaractive interactions pull the host and the guest close and the difference of steric repulsions caused by the different spatial arrangements in the two diastereomeric complexes becomes large resulting in appreciable enantiomeric discrimination. Further general principle is, that the two aromatic rings with the π–π stacking interaction take parallel orientation reaching maximum overlap. Enantioselectivity, which comes from the different complex forming ability of a ligand toward the enantiomers of a selected ammonium salt, is affected by the differences between the steric repulsions. An increase in the size of substituents at the chiral centers usually increases the extent of enantioselectivity since large chiral barriers on macrocyclic compounds cause large steric repulsions. This is in full agreement with the general rule found by *Pirkle and his co-worker* i.e. considerable enantioselectivity of a chiral host molecule toward the enantiomers of a guest molecule can be observed only if there are three independent interactions between the two partners.[8] Optically active macrocycles containing pyridine subunit have become attractive hosts due to their ability for chiral discrimination toward chiral organic ammonium salts, amino acids and their derivatives.

Bradshaw and his co-workers reported the preparation of the first CSP [(*S,S*)-CSP-**25**] containing an optically active pyridino-18-crown-6 ether as chiral selector. They attached an

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[5] a. Subramanian, G. *Chiral Separation Techniques: A Practical Approach*, 3rd ed.; Wiley-VCH:Weinheim, Germany, **2006**.; b. Aboul-Enein, H. Y.; Wainer I. W. *The Impact of Stereochemistry on Drug Development and Use*; Wiley-VCH: New York, NY, USA, **1997**.; c. Ali, I.; Aboul-Enein, H. Y. *Chiral Pollutants: Distribution, Toxicity and Analysis by Chromatography and Capillary Electrophoresis*; Wiley-VCH: Chichester, West Sussex, United Kingdom, **2004**.

[6] Bradshaw, J. S.; Izatt, R. M. *Acc. Chem. Res.* **1997**, *30*, 338–345.

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

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

enantiopure dimethylpyridino-18-crown-6 ether derivative to ordinary silica gel by covalent bonds (***Figure 1.***),[9] and later applying pure methanol as an eluent they obtained an almost baseline enantioseparation of racemic 1-(1-naphthyl)ethylamine (1-NEA) at atmospheric pressure. The latter research group also attached a diphenylpyridino-18-crown-6 ether with a longer linker to ordinary silica gel by covalent bonds, but this CSP [(*R,R*)-CSP-**26**, see ***Figure 1.***] was less efficient in the enantioseparation of 1-NEA than (*S,S*)-CSP-**25**.[10]

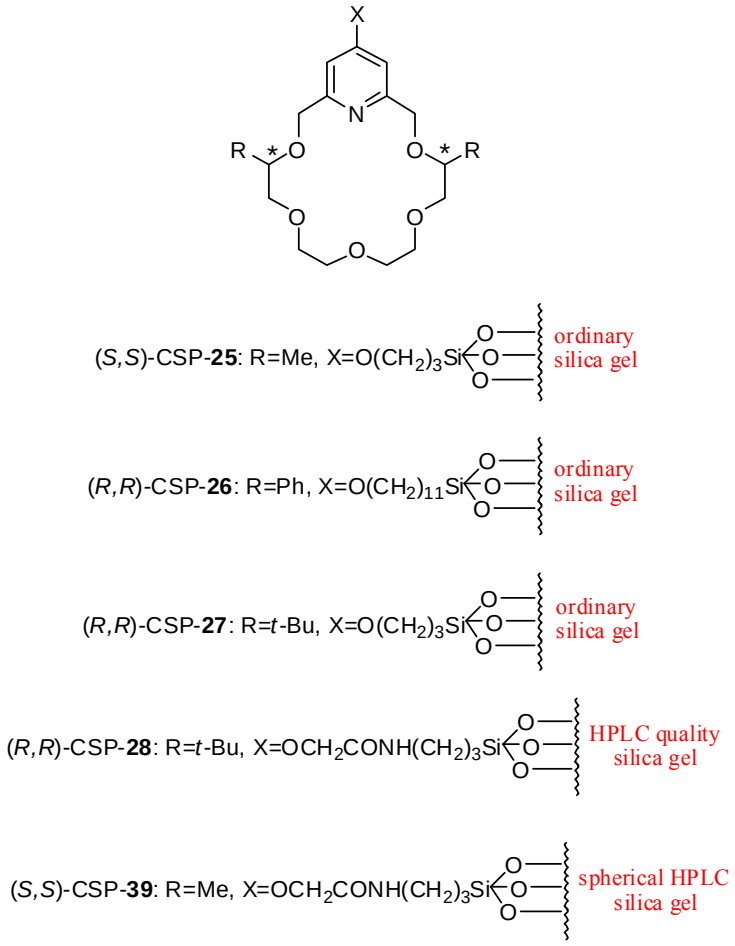


Figure 1. Pyridino-crown ether–based chiral stationary phases

Later on an enantiopure di-*tert*-butylpyridino-18-crown-6 ether derivative was attached to ordinary silica gel by *Köntös and his co-workers*, and the CSP so obtained [(*R,R*)-CSP-**27**, see ***Figure 1.***] separated effectively the enantiomers of racemic 1-NEA_4, 1-phenylethylamine (PEA), phenylalanine methyl ester (PAME) and phenylglycine methyl ester (PGME) at atmospheric pressure.[11] Another optically active di-*tert*-butylpyridino-18-crown-6 ether derivative was immobilized by covalent bonds on HPLC quality silica gel by *Horváth and his co-workers* and the CSP so obtained [(*R,R*)-CSP-**28**, see ***Figure 1.***] separated the enantiomers

[9] Bradshaw, J. S.; Huszthy, P.; Wang, T. M.; Zhu, C. Y.; Nazarenko, A. Y.; Izatt, R. M. *Supramol. Chem.* **1993**, *1*, 267–275.
[10] Huszthy, P.; Bradshaw, J. S.; Bodurov, A. V.; Izatt, R. M. *ACH-Models Chem.* **1994**, *131*, 445–454.
[11] a. Köntös, Z.; Huszthy, P.; Bradshaw, J. S.; Izatt, R. M. *Tetrahedron: Asymmetry* **1999**, *10*, 2087–2099.; b. Köntös, Z.; Huszthy, P.; Bradshaw, J. S.; Izatt, R. M. *Enantiomer* **2000**, *5*, 561–566.

of racemic 1-NEA and PEA under high pressure with great efficiency. However, this CSP was less efficient in the enantioseparation of amino acid derivatives with aromatic side-chains.[12] Finally, an optically active dimethylpyridino-18-crown-6 ether derivative was attached to spherical HPLC quality silica gel by *Farkas and his co-workers* and the CSP so obtained [(*S,S*)-CSP-**39**, see ***Figure 1.***] separated very well the enantiomers of racemic 1-NEA, 1-(2-naphthyl)ethylamine (2-NEA) and the aromatic α-amino acids applying high pressure.[13]

In all the above cases the enantiopure pyridino-18-crown-6 ether derivatives have been attached to silica gel[9–13] through an oxygen atom at position 4 of the pyridine ring. Our aim was to work out a suitable synthetic route to such new pyridino-crown ether–based chiral stationary phases where the selector molecules were attached to the HPLC quality silica gel through a linking unit bonded to the macrocycle through a nitrogen or a carbon atom and to apply these new CSPs for separating the enantiomers of protonated primary aralkylamines.

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 chromatograpy, 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 structure of one of the intermediates was performed by X-ray crystallography in cooperation with *Dr. László Párkányi*.

HPLC columns containing the chiral crown ether selectors were made with the help of a Haskel-type pump. We applied Hitachi-type HPLC system for column-testings.

4. RESULTS

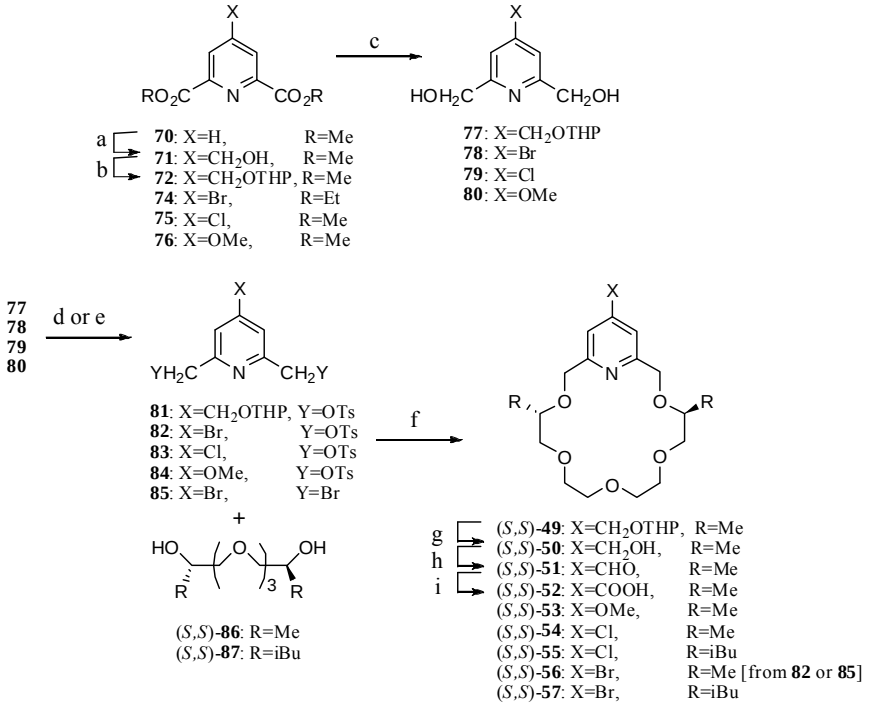
The aim of my PhD work was the synthesis and enantiomer recognition studies of new pyridino-crown ether–based chiral stationary phases where the linking units at position 4 of the pyridine ring are attached through a nitrogen or a carbon atom. I described in my PhD dissertation the synthesis of twenty six new compounds – twenty two enantomerically pure and four achiral ones– and also the preparation of nine reported compounds by a new method. [1–4]

To obtain new triethoxysilyl derivatives where the linking units at position 4 of the pyridine ring are attached through a nitrogen or a carbon atom we needed to prepare pyridino-crown ethers substituted with halogen atoms, trifluormethylsulfonyloxy, cyano, hydroxymethyl, formyl or alkoxy groups. [1–4]
For the synthesis of enantiopure pyridino-crown ethers two synthetic pathways were investigated:

[12] Horváth, G.; Huszthy, P.; Szarvas, S.; Szókán, G.; Redd, J. T.; Bradshaw, J. S.; Izatt, R. M. *Ind. Eng. Chem. Res.* **2000**, *39*, 3576–3581.
[13] Farkas, V.; Tóth, T.; Orosz, G.; Huszthy, P.; Hollósi, M. *Tetrahedron: Asymmetry* **2006**, *17*, 1883–1889.

1) An easily transformable substituent was introduced into position 4 of the pyridine ring before macrocyclization, and a solution of enantiopure dialkyl-substituted tetraethylene glycol dialkoxide was reacted with the pyridine precursor performing a *Williamson*-type ether-forming macrocyclization.

Pyridine diesters substituted with a hydroxymethyl group (**71**),or a halogen atom (**74**, **75**) or a methoxy group (**76**) at position 4 of the pyridine ring were synthesized from commercially available and relatively cheap starting materials (like acetone, diethyl oxalate, sodium, ethanol) by easier and environmentally friendlier ways than published methods in the literature. [2, 3] Dipicolinic acid (DPA) dimethyl ester **70** was subjected to a regioselective hydroxymethylation applying a modified *Fenton*-type reaction to give 4-hydroxymethyl-DPA dimethyl ester **71** (see ***Scheme 1***.). Instead of sulfuric acid in water we used concentrated (70% in water) perchloric acid, and we also changed iron sulfate to iron perchlorate resulting in a higher yield. Tetrahydropyranyl (THP) protecting group for the hydroxymethyl moiety (**72**) seemed to be advantageous, because of its resistance to the highly basic conditions needed during the *Williamson*-type ether-forming macrocyclization. [3] Pyridine diesters substituted at position 4 of the pyridine ring were reduced to diols **77**–**80** with sodium tetrahydridoborate, and then contrary to the published method[14], they were isolated. Chloro-diol **79** and methoxy-diol **80** were purified by recrystallization instead of the time-consuming continous flow extraction. [2]



Scheme 1. Synthesis of enantiomerically pure pyridino-crown ethers with macrocyclization
(a: $Fe(ClO_4)_2$, H_2O_2, MeOH, $HClO_4$; b: DHP, PPTS, CH_2Cl_2; c: $NaBH_4$, EtOH; d: TsCl, 40% aq. KOH/ CH_2Cl_2; e: PBr_3, Et_2O; f: NaH, THF; g: IRA-120 (H^+), MeOH; h: $(COCl)_2$, DMSO, TEA, CH_2Cl_2; i: HCOOH, H_2O_2)

Diols **77**–**80** were transformed to pyridine derivatives bearing good leaving groups on benzylic-type methylene groups at positions 2 and 6 of the pyridine ring. We obtained ditosylates **81**–**84** and bisbromomethyl derivative **85**, which were reacted with the dialkoxides

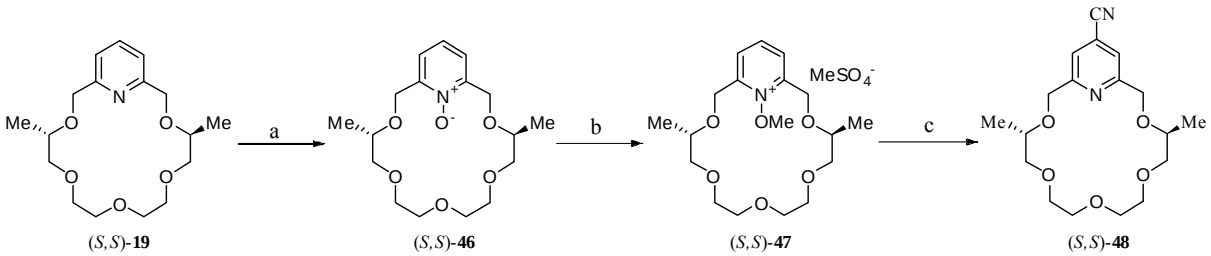
[14] Horváth, G.; Rusa, C.; Köntös, Z.; Gerencsér, J.; Huszthy, P. *Synth. Commun.* **1999**, *29*, 3719–3731.

prepared from the corresponding enantiopure dialkyl-substituted tetraethylene glycols [(*S,S*)-**86**, or (*S,S*)-**87**] to give the pyridino-crown ethers substituted with THP-protected hydroxymethyl-group [(*S,S*)-**49**], methoxy group [(*S,S*)-**53**], or halogen atom [(*S,S*)-**54**–(*S,S*)-**57**]. Pyridino-crown ether substituted with bromine atom at position 4 of the pyridine ring and bearing methyl groups at the chiral centers [(*S,S*)-**56**] was prepared both from bromo ditosylate **82** and from triboromo derivative **85**. Because of the better yield when starting from the ditosylate, it is better to convert diols to ditosylates. It was also found that the macrocyclization gives better yield, if the tetraethylene glycols are substituted with isobutyl groups at their chiral centers (***Scheme 1.***).[2, 3]

THP-protected pyridino-crown ether (*S,S*)-**49** was converted to hydroxymethyl-substituted derivative (*S,S*)-**50** by deblocking the THP protecting group using an ion-exchange resin (H^+ form) in methanol. The latter gave by *Swern* oxidation the formyl-substituted [(*S,S*)-**51**], then by further oxidation the carboxy-substituted [(*S,S*)-**52**] pyridino-crown ether derivatives. Carboxylic acid (*S,S*)-**52** is a precursor of a new chiral stationary phase. [3]

2) In our other synthetic pathway pyridino-crown ether (*S,S*)-**19** substituted with methyl groups at the chiral centers and pyridono-crown ether substituted with methyl [(*S,S*)-**88**] or isobutyl [(*S,S*)-**89**] groups at the chiral centers were prepared by published methods, and then these macrocycles were substituted with easily tranformable functional groups at position 4 of the pyridine ring (***Scheme 2.***, ***3.***). Applying this synthetic pathway pyridino-crown ethers substituted with halogen atom at position 4 of the pyridine ring were prepared with better yields, than if we introduce halogen atom before the macrocyclization step. [2, 4]

A single crystal was prepared from chloropyridino-crown ether (*S,S*)-**54** by repeated crystallizations using a mixture of heptane and dichloromethane. X-ray analysis proved that no water molecule is complexed in the crystalline form of macrocycle (*S,S*)-**54**, although several dimethyl-substituted pyridino-crown ethers prepared from (*S,S*)-**54** form complexes with one molecule of water. [2]

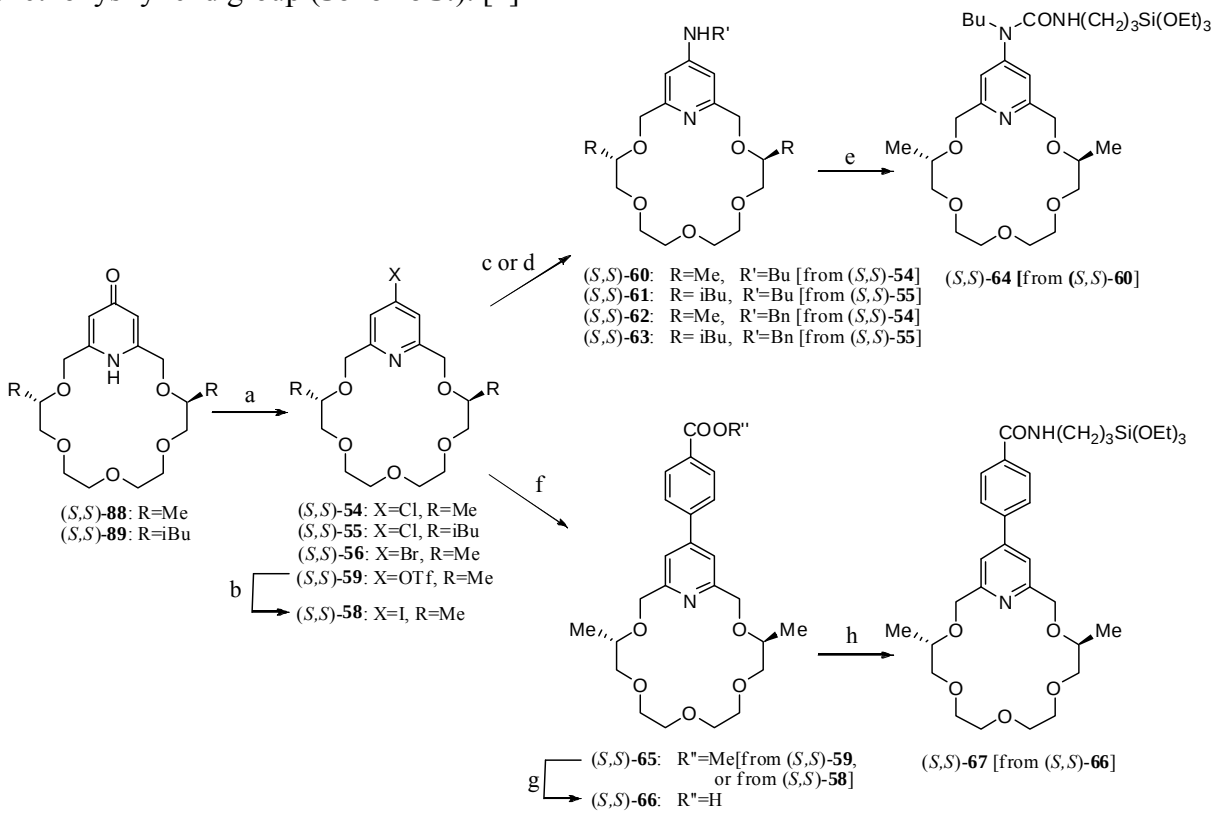


Scheme 2. Synthesis of pyridino-crown ether containing cyano group at position 4 of the pyridine ring (a: MCPBA, CH_2Cl_2; b: Me_2SO_4; c: NaCN, $MeOH/H_2O$)

Pyridino-crown ether (*S,S*)-**19** was oxidized to *N*-oxide (*S,S*)-**46**, then the latter was transformed to an *N*-methoxy-derivative (*S,S*)-**47**, which can be attacked by a nucleophile at position 4 of the pyridine ring. The nucleophile this time was the cyanide anion rendering nitrile (*S,S*)-**48** (***Scheme 2.***). [1]

Pyridino-crown ethers (*S,S*)-**54**–(*S,S*)-**56** substituted with halogen atoms were synthesized from dimethyl- [(*S,S*)-**88**] and diisobutyl- [(*S,S*)-**89**] substituted pyridono-crown ethers. Chloro derivatives (*S,S*)-**54** and (*S,S*)-**55** were treated with butylamine, and benzylamine, respectively in a sealed tube to obtain butylamino- [(*S,S*)-**60**, (*S,S*)-**61**], and benzylamino- [(*S,S*)-**62**, (*S,S*)-**63**] substituted macrocycles, respectively. Secondary amines (*S,S*)-**60**–(*S,S*)-**63** are the precursors of pyridino-crown ether–based new chiral stationary phases. We gained

the butylamino-derivative (*S,S*)-**60** with the best yield, so we reacted it with 3-(triethoxysilyl)propyl isocyanate to obtain selector molecule (*S,S*)-**64** containing a triethoxysilyl end group (***Scheme 3.***). [4]

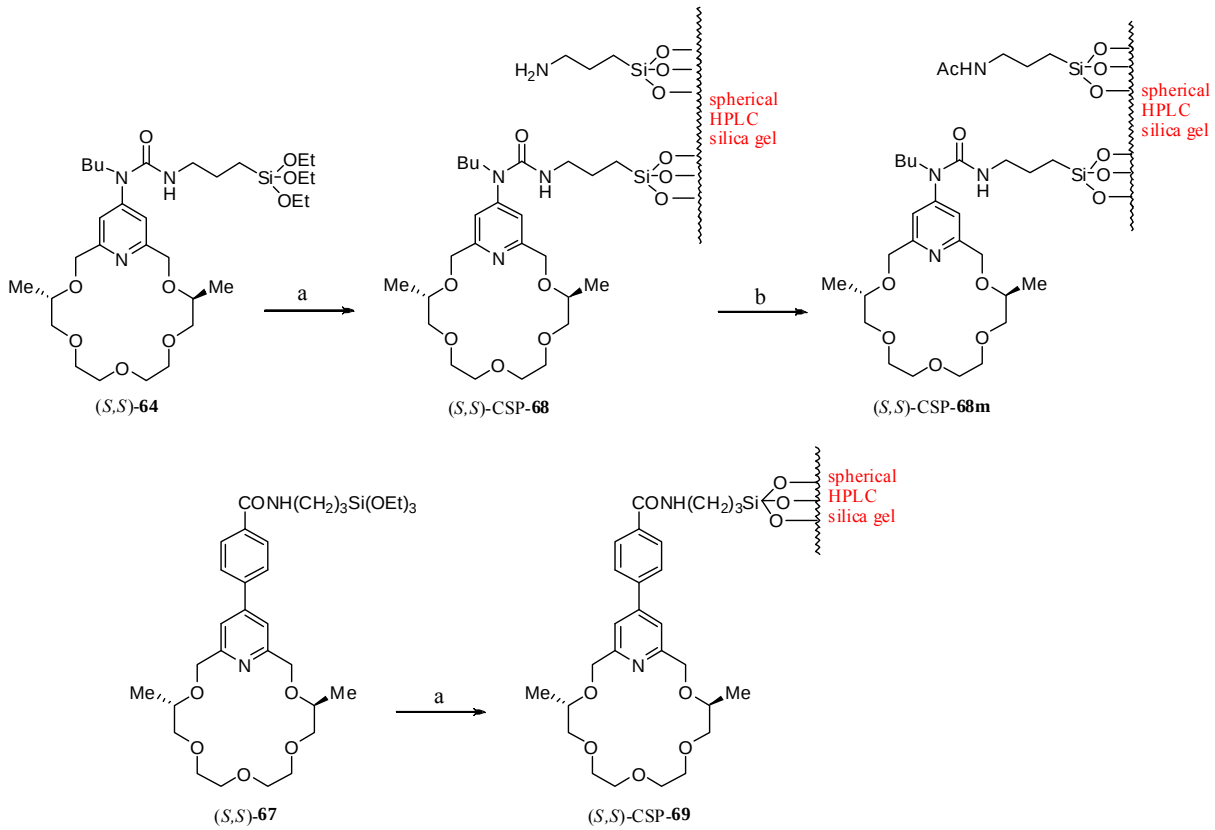


Scheme 3. Synthesis of macrocycles containing a triethoxysilyl end group
(a: $SOCl_2$, $CHCl_3$, cat. DMF; b: NaI, 30% aq. HCl, CH_3CN; c: $BuNH_2$; d: $BnNH_2$; e: $(EtO)_3Si(CH_2)_3NCO$; f: $MeOOC(C_6H_4)B(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, KBr, dioxane; g: 25% aq. TMAH, MeOH, then AcOH; h: $SOCl_2$, then $(EtO)_3Si(CH_2)_3NH_2$, TEA, THF)

To obtain a new pyridino-crown ether derivative where the linking unit at position 4 of the pyridine ring is attached through a carbon atom, we needed to apply a carbon–carbon bond formation reaction. Carrying out this, pyridono-crown ether (*S,S*)-**88** was first converted to triflate (*S,S*)-**59**. The latter triflate and the iodide (*S,S*)-**58** prepared from the triflate served as appropriate electrophiles for *Suzuki* reaction. Ester (*S,S*)-**65** could be synthesized from iodide (*S,S*)-**58** with a better yield than starting from triflate (*S,S*)-**59**, but the overall yield was better in the last case. Carboxylic acid (*S,S*)-**66** was prepared by the hydrolysis of ester (*S,S*)-**65**. This carboxylic acid was converted to the corresponding acyl chloride by reacting it with thionyl chloride, and then this acyl chloride was treated with 3-(triethoxysilyl)propyl amine to obtain amide (*S,S*)-**67** containing a triethoxysilyl end group (***Scheme 3.***).

We attached triethoxysilyl derivatives (*S,S*)-**64** and (*S,S*)-**67**, respectively, to spherical HPLC silica gel by covalent bonds to obtain pyridino-crown ether–based chiral stationary phases (*S,S*)-CSP-**68** and (*S,S*)-CSP-**69**, respectively (***Scheme 4.***).

During the immobilization of selector molecule (*S,S*)-**64** to silica gel, as a result of the long and harsh conditions a part of the urea units of the attached pyridino-crown ether was hydrolyzed, thus we obtained a chiral stationary phase containing 3-aminopropylsilyl groups bonded to silica gel [(*S,S*)-CSP-**68**]. We pumped a mixture of acetic anhydride and triethylamine in dimethylformamide through the column to give modified chiral stationary phase (*S,S*)-CSP-**68m** (***Scheme 4.***). [4]



Scheme 4. Preparation of the new chiral stationary phases
(a: spherical HPLC silica gel, toluene; b: Ac_2O; TEA, DMF)

HPLC Columns packed with (*S,S*)-CSP-**68** and its modified form [(*S,S*)-CSP-**68m**] and also with (*S,S*)-CSP-**69**, respectively, were used for separating the enantiomers of four different guest molecules (1-NEA, 2-NEA, Br-PEA, NO_2-PEA). It was found that in all cases the (*S*)-enantiomers eluted with shorter retention times than those of their antipodes, so all of the chiral stationary phases showed heterochiral preferency. The most retained analyte among our model compounds was 1-NEA, and the enantioselectivity achieved was the highest (***Table 1.***, ***Figure 2.***).

Only the enantiomers of 1-NEA could be separated using chiral stationary phase (*S,S*)-CSP-**68** with sufficient resolution factor (R_s>1,5), but in case of other analytes the 3-aminopropylsilyl groups bonded to silica gel caused peak-broadening (***Figure 2.a***, ***4.a***, ***5.a***).

The modified CSP [(*S,S*)-CSP-**68m**] showed better enantioseparation factors for the mixtures of enantiomers of 1-NEA, Br-PEA and NO_2-PEA than (*S,S*)-CSP-**68** and it separated the mixtures of enantiomers of 1-NEA more effectively than the reported pyridino-crown ether–based CSPs [(*R,R*)-CSP-**28**, (*S,S*)-CSP-**39**] (***Table 1.***, ***Figure 2.b***). Applying (*S,S*)-CSP-**68m** we obtained an almost baseline separation of the mixtures of enantiomers of 2-NEA, but this CSP showed worse enantioseparation than the reported chiral stationary phase (*S,S*)-CSP-**39**. (***Figure 3.a***)

Chiral stationary phase (*S,S*)-CSP-**69** showed the best enantiomer separating ability for the mixtures of enantiomers of amine compounds among the pyridino-crown ether–based CSPs ever synthesized (***Table 1.***, ***Figure 2.c***, ***3.b***, ***4.c***, ***5.c***). The extremely high enantioselectivity is probably due to the strong π–π interaction of the extended π-system of the aryl-substituted pyridine unit. [4]

Table 1. Comparison of chromatographic data for the separation of the mixtures of enantiomers of protonated primary aralkylamines on the reported CSPs [(*R,R*)-CSP-**28**, (*S,S*)-CSP-**39**], and on the new CSPs [(*S,S*)-CSP-**68**, (*S,S*)-CSP-**68m**, (*S,S*)-CSP-**69**].

Chiral stationary phase	Analyte	*t (S)* [min]	*t (R)* [min]	*α*	R_S	eluent
(*R,R*)-CSP-**28**	1-NEA	4.48	6.02	1.52	1.54	A
(*S,S*)-CSP-**39**	1-NEA	4.77	8.65	2.12	2.73	B
(*S,S*)-CSP-**68**	1-NEA	4.61	6.22	1.67	1.67	C
(*S,S*)-CSP-**68m**	1-NEA	7.40	11.47	1.78	4.54	C
(*S,S*)-CSP-**69**	1-NEA	16.22	37.79	2.49	9.20	D
(*S,S*)-CSP-**39**	2-NEA	4.74	7.00	1.66	1.97	B
(*S,S*)-CSP-**68m**	2-NEA	8.35	10.92	1.42	1.60	C
(*S,S*)-CSP-**69**	2-NEA	15.79	25.07	1.66	4.53	D
(*S,S*)-CSP-**68**	Br-PEA	3.61	4.23	1.44	0.85	C
(*S,S*)-CSP-**68m**	Br-PEA	4.97	6.23	1.46	1.00	C
(*S,S*)-CSP-**69**	Br-PEA	15.91	23.09	1.51	3.58	D
(*S,S*)-CSP-**68**	NO_2-PEA	2.70	2.91	1.40	0.61	C
(*S,S*)-CSP-**68m**	NO_2-PEA	3.02	3.30	1.35	0.95	C
(*S,S*)-CSP-**69**	NO_2-PEA	16.12	21.86	1.40	3.30	D

A: Isocratic elution: in a 9:1 mixture of dichloromethane–methanol (flow rate: 1.0 mL/min).
B: Gradient elution: 5–0% methanol + 1% triethylamine (1.2 mL/min).
C: Isocratic elution: 0.05% formic acid and 0.2% triethylamine in acetonitrile–methanol (7:3) (1.0 mL/min).
D: Isocratic elution: 0.2% formic acid and 0.1% triethylamine in acetonitrile–methanol (1:4) (1.0 mL/min).

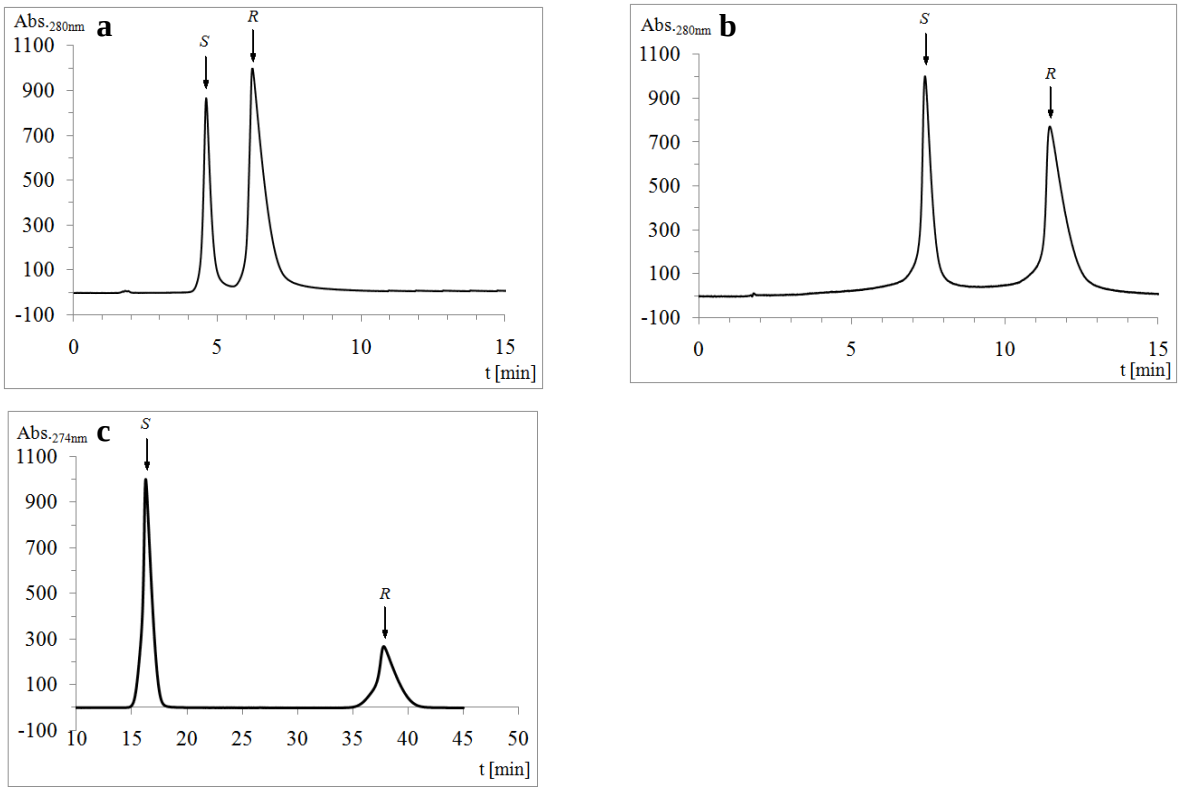


Figure 2. Chromatograms for enantioseparations of mixtures of enantiomers of 1-NEA using a) (*S,S*)-CSP-**68** with eluent C; b) (*S,S*)-CSP-**68m** with eluent C; c) (*S,S*)-CSP-**69** with eluent D.

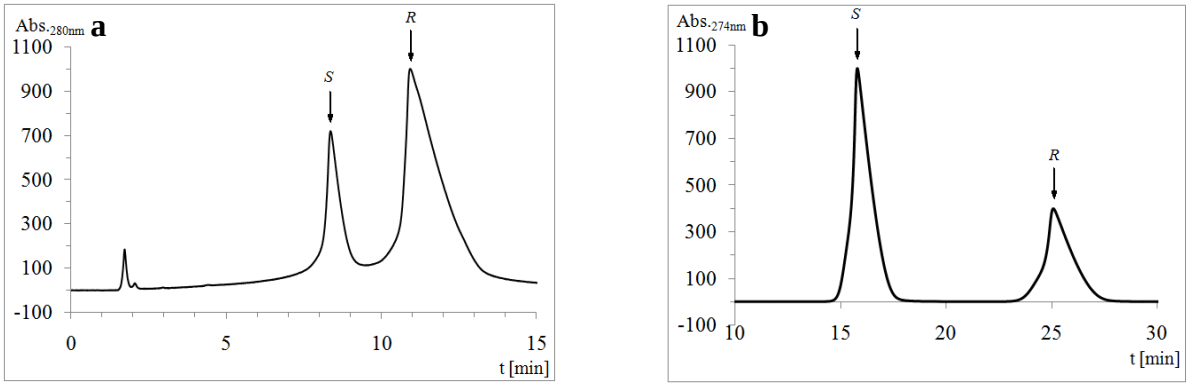


Figure 3. Chromatograms for enantioseparations of mixtures of enantiomers of 2-NEA using a) (*S,S*)-CSP-**68m** with eluent C; b) (*S,S*)-CSP-**69** with eluent D.

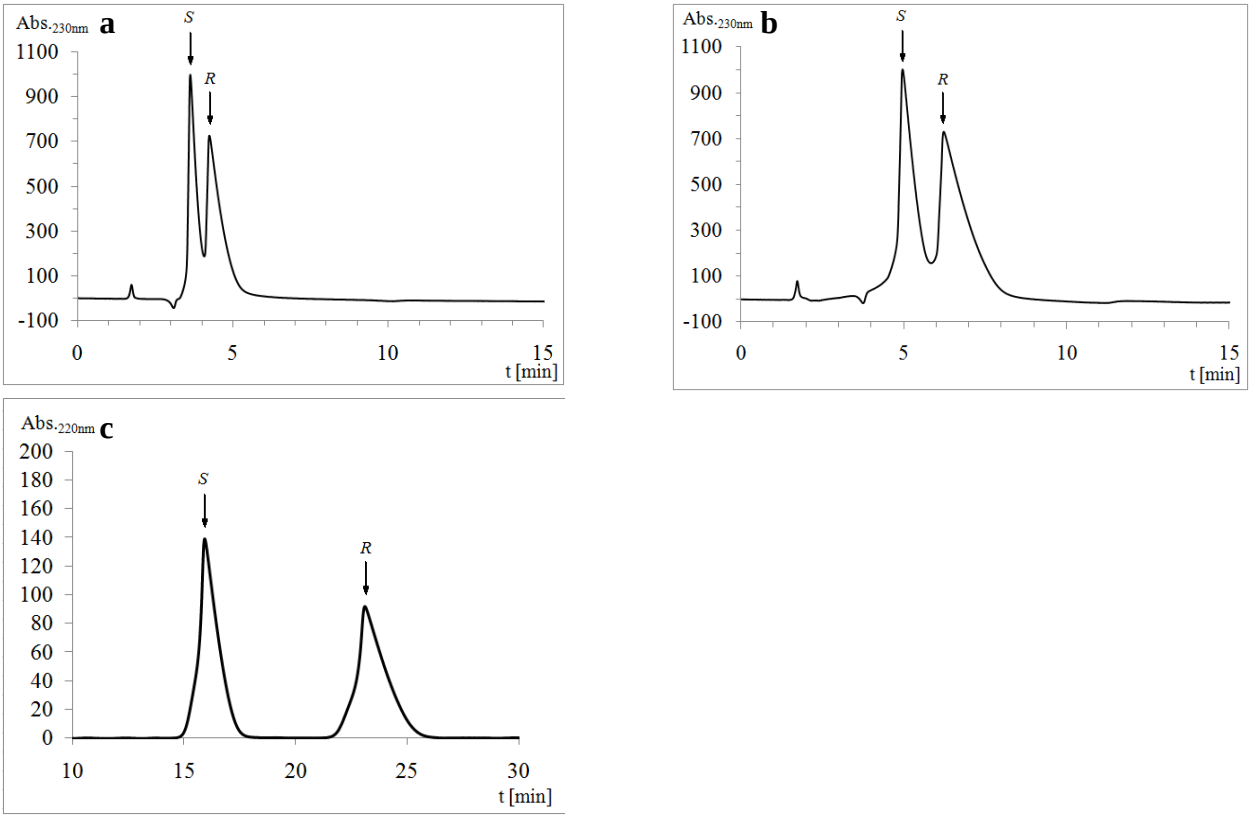


Figure 4. Chromatograms for enantioseparations of mixtures of enantiomers of Br-PEA using a) (*S,S*)-CSP-**68** with eluent C; b) (*S,S*)-CSP-**68m** with eluent C; c) (*S,S*)-CSP-**69** with eluent D.

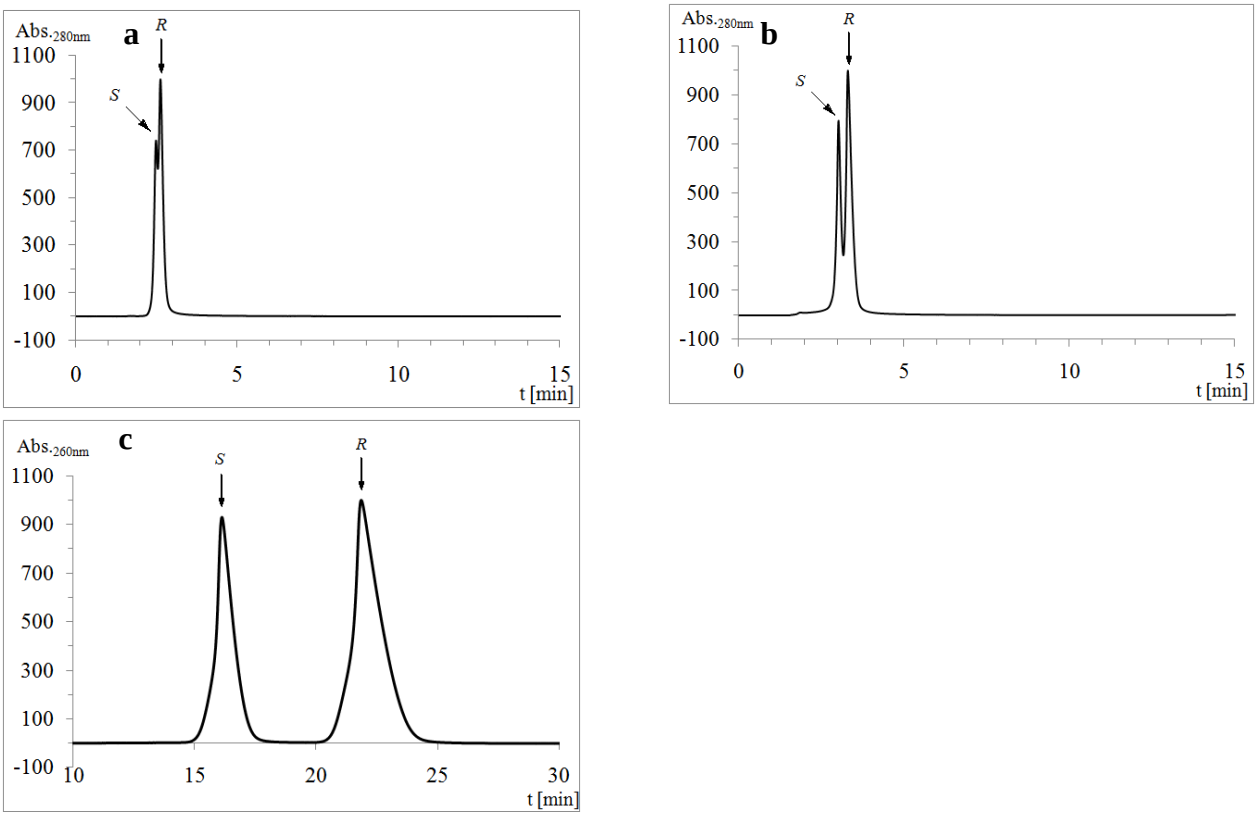


Figure 5. Chromatograms for enantioseparations of mixtures of enantiomers of NO_2-PEA using a) (*S,S*)-CSP-**68** with eluent C; b) (*S,S*)-CSP-**68m** with eluent C; c) (*S,S*)-CSP-**69** with eluent D.

We can conclude that all the three new chiral stationary phases [(*S,S*)-CSP-**68**, (*S,S*)-CSP-**68m**, (*S,S*)-CSP-**69**] are suitable for enantiomeric separation of protonated primary aralkylamines. Furthermore, chiral stationary phases (*S,S*)-CSP-**68m** and (*S,S*)-CSP-**69** showed better enantioseparating factors than the reported chiral stationary phases. [4]

5. THESES

1. Twenty two new 18-crown-6 ethers containing a pyridine subunit and their four unpublished precursors were prepared. [1–4]
2. We worked out a synthetic method for introduction the butylamino, and the benzylamino group, respectively, into the position 4 of the pyridine ring starting from pyridino-crown ethers substituted with a chlorine atom with almost quantitative yields. [4]
3. A new chiral stationary phase based on a pyridino-crown ether where the linking unit at position 4 of the pyridine ring is attached through a nitrogen atom has been prepared. We proved that this CSP separated the mixtures of enantiomers of protonated primary aralkylamines efficiently, and it shows heterochiral preferency. [4]
4. We proved that pumping acetic anhydride through the HPLC column is an appropriate method for acetylating the 3-aminopropylsilyl groups bonded to silica gel during the immobilization of the triethoxysilyl derivative, thus the peak-broadening effect of these groups can be avoided obtaining a modified chiral stationary phase with better enantioseparation factors. [4]
5. A new chiral stationary phase based on a pyridino-crown ether where the linking unit at position 4 of the pyridine ring is attached through an aryl moiety has been prepared. This CSP showed the best enantioseparation factors for the mixtures of enantiomers of protonated primary aralkylamines among all the pyridino-crown ether–based CSPs, and its heterochiral preferency was also proved. [4]
6. As precursors for new chiral stationary phases, pyridino-crown ethers substituted with cyano or carboxylic groups at position 4 of the pyridine ring were synthesized. [1, 3]
7. We proved that we can gain pyridino-crown ethers substituted by a halogen atom with better yields, if we introduce the halogen atom into position 4 of the pyridine ring before macrocyclization than if we do this after the macrocyclization step. [2]
8. It was found that the macrocyclization gives a better yield if the tetraethylene glycols are substituted with isobutyl groups at their chiral centers, and the pyridino precursor with a tosylate leaving group than the bisbromomethyl derivative. [2]
9. We proved that a *Fenton*-type reaction, which is an appropriate method for regioselective hydroxymethylation, gave a better yield if we use iron perchlorate in concentrated perchloric acid, instead of iron sulfate in 30% aqueous sulfuric acid as reported earlier. [3]

6. POSSIBLE APPLICATIONS

The three new chiral stationary phases [(*S,S*)-CSP-**68**, (*S,S*)-CSP-**68m**, (*S,S*)-CSP-**69**] can be used for enantiomeric separation of protonated primary aralkylamines. Pyridino-crown ethers substituted with cyano [(*S,S*)-**48**], or carboxylic [(*S,S*)-**52**], or butylamino [(*S,S*)-**61**] or benzylamino [(*S,S*)-**62**, (*S,S*)-**63**] group at position 4 of the pyridine ring are useful precursors for new chiral stationary phases.

Carboxylic acids (*S,S*)-**52** and (*S,S*)-**66**, respectively, can be suitable chiral selectors for a cyclodextrin–based capillary electrophoretic method for enantioseparation of 1-(1-aminoarylmethyl)-2-naphthol and 2-(1-aminoarylmethyl)-1-naphthol derivatives and other protonated primary aralkylamines.

7. PUBLICATIONS

7.1. Publications related to the PhD thesis

1. Tóth, T.; Huszthy, P.; **Kupai, J.**; Nyitrai, J.: Synthesis of new enantiopure dimethyl-substituted pyridino-18-crown-6 ether-type macrocycles containing different substituents at position 4 of the pyridine ring for enantiomeric recognition studies, *Arkivoc* **2008**, *iii*, 66–79. (IF: 1.377)

2. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.; Párkányi, L.: Synthesis of new enantiopure dimethyl- and diisobutyl-substituted pyridino-18-crown-6 ethers containing a halogen atom or a methoxy group at position 4 of the pyridine ring for enantiomeric recognition studies, *Arkivoc* **2011**, *ix*, 77–93. [IF(2010): 1.096]

3. **Kupai, J.**; Huszthy, P.; Katz, M.; Tóth, T.: Synthesis of new enantiopure dimethyl-substituted pyridino-18-crown-6 ethers containing a hydroxymethyl, a formyl, or a carboxyl group at position 4 of the pyridine ring for enantiomeric recognition studies, *Arkivoc* **2012**, *v*, 134–145. [IF(2010): 1.096]

4. **Kupai, J.**; Lévai, S.; Antal, K.; Balogh, G. T.; Tóth, T.; Huszthy, P.: Preparation of pyridino-crown ether–based new chiral stationary phases and preliminary studies on their enantiomer separating ability for chiral protonated primary aralkylamines, *Tetrahedron:Asymmetry* **2012**, *23*, 415–427. [IF(2010): 2,484]

7.2. Proceedings related to the PhD thesis

5. **Kupai, J.**; Huszthy, P.; Székely, K.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise. In: *XIV. Nemzetközi Vegyészkonferencia*, Ed Majdik, K., ISSN 1843-6293, p. 77–80. (2008) (IF:0)

7.3. Other publications

6. Ilisz, I.; Iványi, R.; Pataj, Z.; **Kupai, J.**; Huszthy, P.; Szatmári, I.; Fülöp, F.; Péter, A.: CE Enantioseparation of Betti Bases with Cyclodextrins and Crown Ether as Chiral Selectors, *Chromatographia* **2010**, *71*, S115–S119. (IF: 1.075; citations: 2)

7. Székely, G.; Csordás, B.; Farkas, V.; **Kupai, J.**; Pogány, P.; Sánta, Z.; Szakács, Z.; Tóth, T.; Hollósi, M.; Nyitrai, J.; Huszthy, P.: Synthesis and Preliminary Structural and Binding Characterization of Novel Enantiopure Crown Ethers Containing an Alkyl Diarylphosphinate or a Proton-Ionizable Diarylphosphinic Acid Unit, *Eur. J. Org. Chem.* **2012**, in press, DOI: 10.1002/ejoc.201101769. [IF(2010): 3.206]

7.4. Presentations related to the topics of the PhD thesis

1. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise, Oláh György Doktori Iskola V. Doktoráns Konferenciája, Budapest, 2008. february 8., poster

2. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise, MTA Heterociklusos Kémiai Munkabizottsági Előadóülés, Balatonszemes, 2008. may 21–23.

3. **Kupai, J.**; Huszthy, P.; Székely, K.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise, XIV. Nemzetközi Vegyészkonferencia, Kolozsvár, 2008. november 13–15.

4. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise, Oláh György Doktori Iskola VI. Doktoráns Konferenciája, Budapest, 2009. february 4., poszter

5. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise, MTA Heterociklusos Kémiai Munkabizottsági Előadóülés, Balatonszemes, 2009. may 20–22.

6. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise, XV. Nemzetközi Vegyészkonferencia, Marosvásárhely, 2009. november 12–15.

7. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.: A piridingyűrű 4-es helyzetében szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise enatiomer-felismerés tanulmányozása céljából, Oláh György Doktori Iskola VII. Doktoráns Konferenciája, Budapest, 2010. february 4.

8. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.; Ilisz, I.; Iványi, R.; Pataj, Z.; Szatmári, I.; Fülöp, F.; Péter, A.; Hollósi, M.; Farkas, V.; Csordás, B.: Piridino-18-korona-6 éter alapú szenzor- és szelektormolekulák előállítása és vizsgálata, MTA Heterociklusos Kémiai Munkabizottsági Előadóülés, Balatonszemes, 2010. may 19–21.

9. **J. Kupai**; P. Huszthy; K. Székely; T. Tóth: Synthesis of new enantiopure pyridino-18-crown-6 ethers containing different substituents at position 4 of the pyridine ring for enantiomeric recognition studies, 12th Belgian Organic Synthesis Symposium (BOSSXII), Namur, Belgium, 2010. july 11–16., poster

10. **Kupai, J.**; Huszthy, P.; Székely, K.; Tóth, T.; Katz, M.: A piridingyűrű 4-es helyzetében hidroximetil-, formil- és karboxilcsoporttal szubsztituált új enantiomertiszta piridino-18-korona-6 éterek szintézise, XVI. Nemzetközi Vegyészkonferencia, Kolozsvár, 2010. november 11–14.

11. **J. Kupai**; K. Székely; P. Huszthy; S. Lévai; Gy. T. Balogh; T. Tóth; G. Varga: Enantioseparation of protonated primary aralkylamines and amino acid derivatives containing an aromatic moiety on a pyridino-crown ether–based new chiral stationary phase, 4th European Conference on Chemistry for Life Sciences , (4ECCLS), Eötvös Loránd University, Budapest, 2011. august 31–september 3., poster

12. **Kupai, J.** A piridingyűrű 4-es helyzetében szubsztituált új enantiomertista piridino-18-korona-6-éterek szintézise enantiomer-felismerés tanulmányozása céljából, I. BME Doktorandusz Konferencia, Budapest, 2011. november 25.

7.5. Other presentations

Ilisz, I.; **Kupai, J.**; Huszthy, P.; Iványi, R.; Pataj, Z.; Szatmári, I.; Fülöp, F.; Péter ,A.: Capillary Electrophoretic Enantioseparation of Betti Bases with Cyclodextrins and a Crown Ether as Chiral Selectors, 8th Balaton Symposium on High-Performance Separation Methods, Siófok, 2009. september 2–4., poster

J. Kupai; P. Huszthy; K. Székely; T. Tóth: Synthesis of new enantiopure pyridino-18-crown-6 ether derivatives, 5th International Symposium on Macrocyclic & Supramolecular Chemistry (ISCMSC), Nara, Japan, 2010. june 6–10., poster