Chiral stationary phases based on *Cinchona* alkaloids and dipeptides Design, synthesis and application in chiral separation

Anežka Kuncová,[1] Jiří Svoboda,[1] Jiří Tůma,[1] Leonid Asnin,[2] Kevin Schug,[3] Michal Kohout[1]

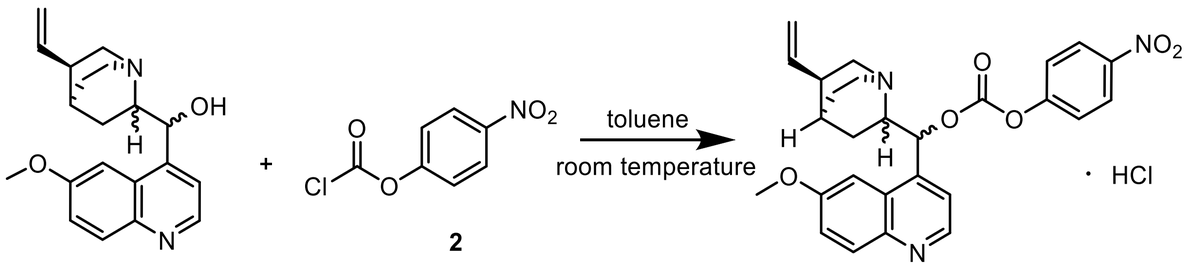
[1]*Department of Organic Chemistry, University of Chemistry and Technology Prague, Prague*
[2]*Department of Chemistry and Biotechnology, Perm National Research Polytechnic University, Perm*
[3]*Department of Chemistry and Biochemistry, College of Sciences, UT Arlington, Arlington*

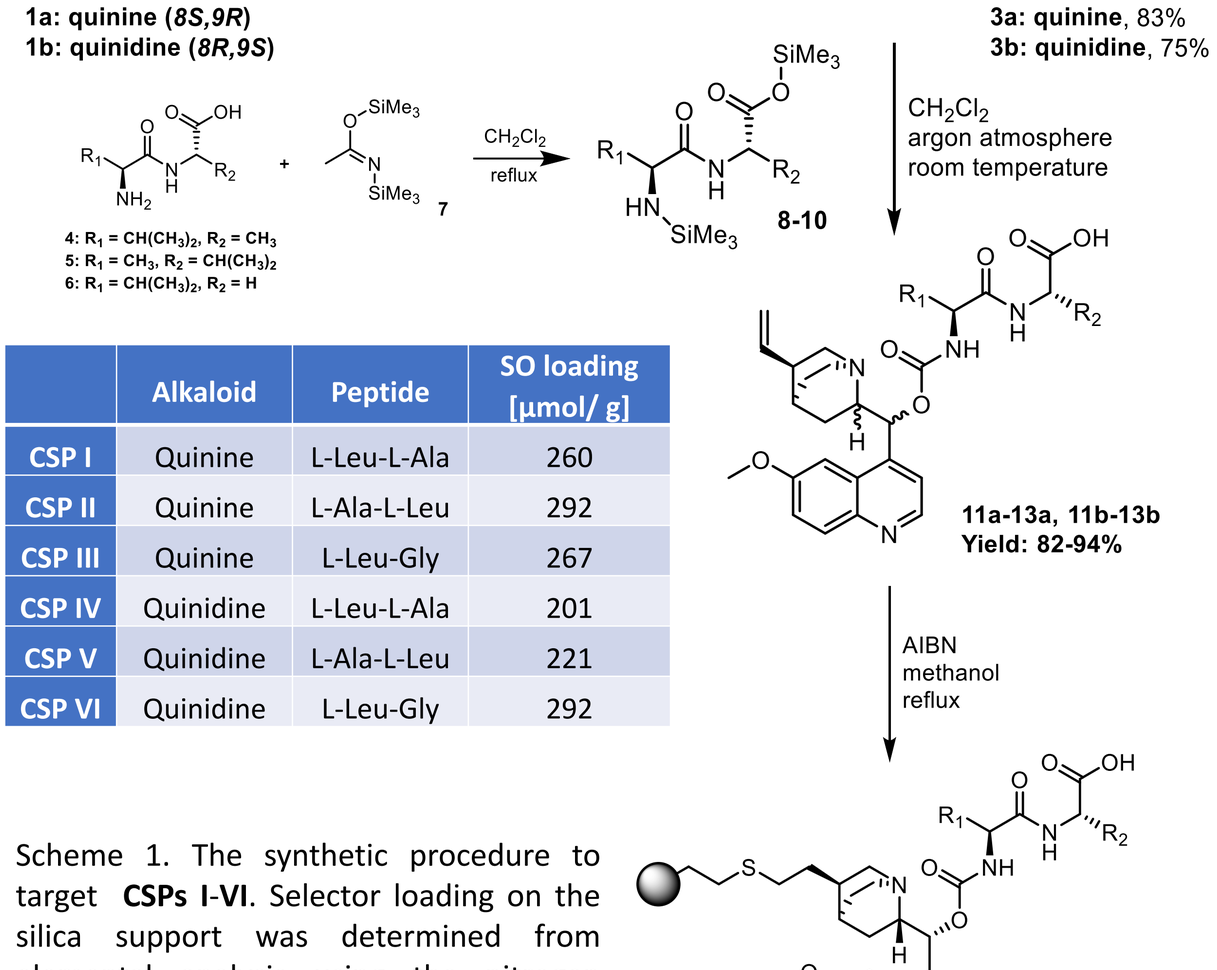
Introduction

Chiral separation of polar substances like native amino acids and peptides represent a challenging chromatographic task. Due to the increasing number of peptidic pharmaceuticals, particularly, short antimicrobial peptides, this challenge is nowadays more frequently accepted. However, from the plethora of available chiral stationary phases, only few show required enantioselectivity towards ionized analytes,[1] and some limitations in the chiral resolution of peptides by commercial chiral zwitterion ion exchangers have already been documented.[2] Therefore, we have introduced a dipeptide unit into the structure of zwitterionic *Cinchona*-based selector with the aim to achieve higher similarity between the selector and the peptide analytes.

Synthesis

The procedure started from the appropriate *Cinchona* alkaloid, which was first activated by 4-nitrophenylchloroformate. Such active ester was subsequently added to a solution of trimethylsilyl-protected dipeptide in anhydrous dichloromethane. The reaction mixture was stirred in an inert argon atmosphere for two days and then decomposed with anhydrous methanol. The crude product was purified by column chromatography, characterized by spectroscopic methods and high-resolution mass spectrometry. The obtained chiral selectors were immobilized on 3-mercaptopropyl-modified silica by means of a radical reaction.





	Alkaloid	Peptide	SO loading [µmol/ g]
CSP I	Quinine	L-Leu-L-Ala	260
CSP II	Quinine	L-Ala-L-Leu	292
CSP III	Quinine	L-Leu-Gly	267
CSP IV	Quinidine	L-Leu-L-Ala	201
CSP V	Quinidine	L-Ala-L-Leu	221
CSP VI	Quinidine	L-Leu-Gly	292

Scheme 1. The synthetic procedure to target **CSPs I-VI**. Selector loading on the silica support was determined from elemental analysis using the nitrogen content.

The prepared CSPs were slurry packed into analytical columns (150×3 mm, id) and evaluated using HPLC Shimadzu LCMS-8030 instrument.

Conclusions

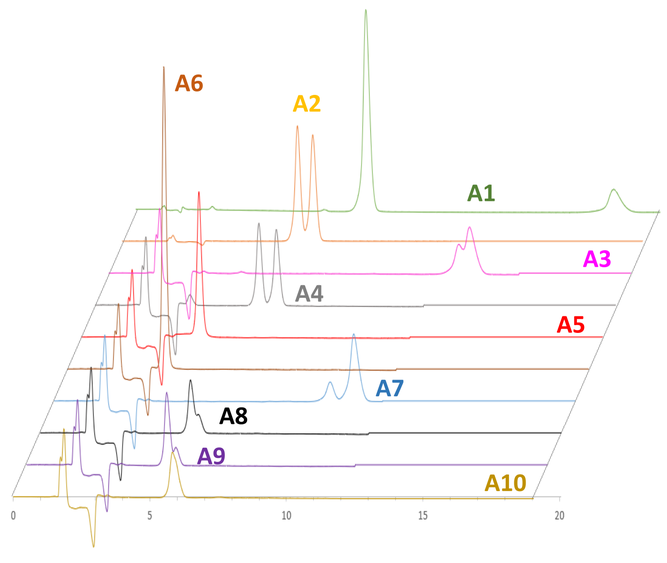
- We have elaborated a **facile synthesis** towards a new class of chiral zwitterion ion exhangers.
- We have confirmed that the **driving force for chiral resolution** is the presence of the *Cinchona* scaffold.
- We have proven the **cooperative action of the *Cinchona* alkaloid and peptide** residue within the selector in chiral resolution of dipeptide stereoisomers.

Acknowledgement

The work was supported by Czech Science Foundation (project No. 21-31139J). The support by Chromservis s.r.o. in column packing is gratefully acknowledged.

Results and discussion

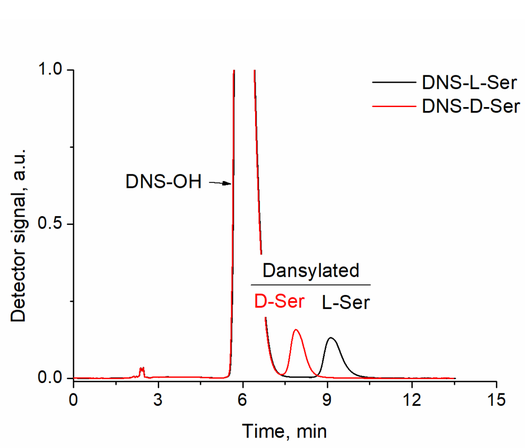
The chiral resolution power of the new CSPs was tested using diverse analytes, ranging from acids and bases to zwitterions.

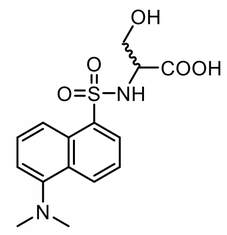


Label	Analyte
A1	*N*-DNB-DL-α-Phenylglycine
A2	DL-Phenylmercapturic acid
A3	*N*-Cbz-DL-Phenylglycine
A4	*N*-Acetyl-S-benzyl-DL-Cysteine
A5	DL-Mandelic acid
A6	DL-p-Hydroxyphenyllactic acid
A7	*N*-Cbz-DL-Phenylalanine
A8	*N*-Boc-DL-Phenylalanine
A9	*N*-Cbz-DL-Valine
A10	*N*-Cbz-DL-Leucine

Figure 1. Mobile phase conditions screening with **CSP V**; mobile phase: MeOH-H_2O 50/50 (v/v) + 50 mM FA + 25 mM TEA; temperature, 40 °C; flow rate 0.5 ml/min; injection volume 10 µl.

The resolution of *N*-protected amino acids is not surprising, since they are strongly interacting with the *Cinchona* residue. Similarly to previous studies,[3] the 3,5-dinitrobenzoyl protecting group contributes significantly to enantioselectivity (Figure 1). ´The new CSPs can be utilized also in analysis of pre-column derivatized amino acids (Figure 2).





Chiral resolution of *N*-protected amino acids is facilitated by the *Cinchona* unit of chiral selectors.

Figure 2. Analysis of dansyl-serine on **CSP IV**; mobile phase: MeOH-H_2O 20/80 (v/v) 0.2 mM ammonium acetate; room temperature; flow rate 0.5 ml/min; detection wavelength 254 nm.

Chiral resolution of model dipeptides in practically non-buffered reversed phase conditions (Figure 3) documents the cooperative action of both parts of the selector: alkaloid and peptide.

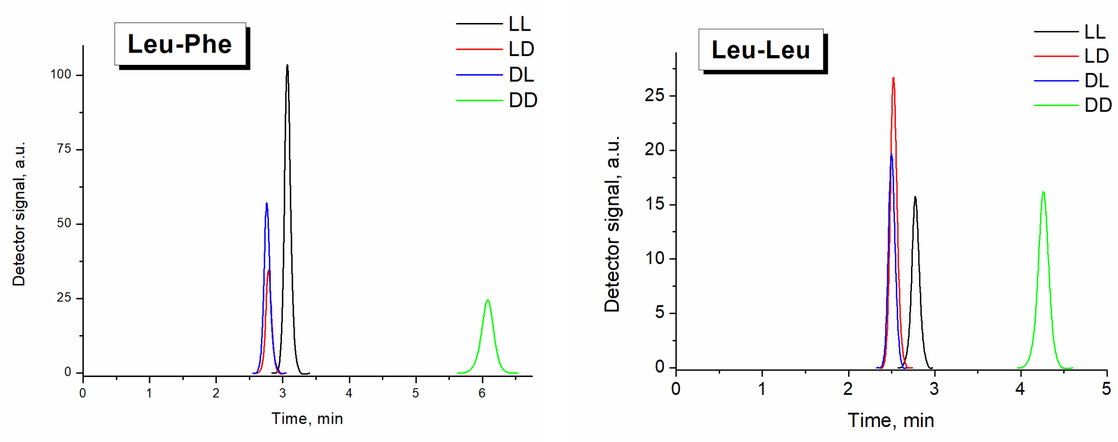


Figure 3. Mobile phase conditions screening with **CSP IV**; mobile phase: MeOH-H_2O 20/80 (v/v) 0.2 mM ammonium acetate; room temperature; flow rate 0.5 ml/min; detection wavelength 254 nm.

References

1. Lämmerhofer, M. *Anal. Bioanal. Chem.* **2014,** *406*, 6095.
2. Wernisch, S.; Lindner, W. *J. Chromatogr. A* **2012,** *1269*, 297.
3. Kohout, M.; Wernisch, S.; Tůma, J.; Hettegger, H.; Pícha, J.; Lindner, W. *J. Sep. Sci.* **2018**, *41*, 1355.
4. Kuncová, A.; Svoboda, J.; Tůma, J.; Asnin, L.; Schug, K.; Kohout M., **2023**.