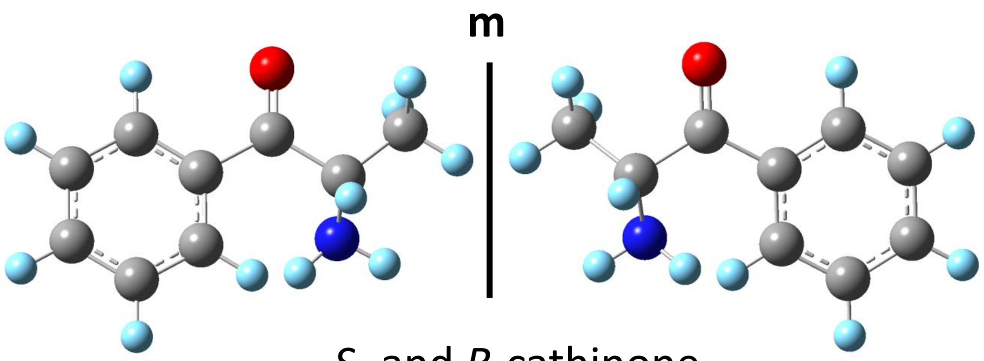
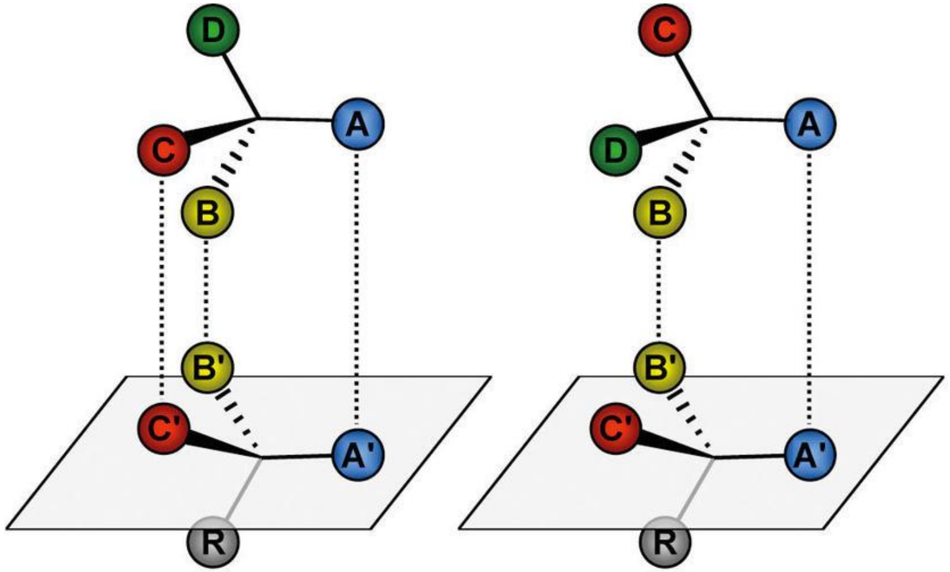
Advances in the design of ion exchange-type chiral stationary phases and understanding of their chiral recognition mechanism

Ina Varfaj, Magdaléna Labíková, Andrea Carotti, Roccaldo Sardella, Jiří Svoboda, Wolfgang Lindner, Leonid Asnin, Michal Kohout

Chirality and chiral entities

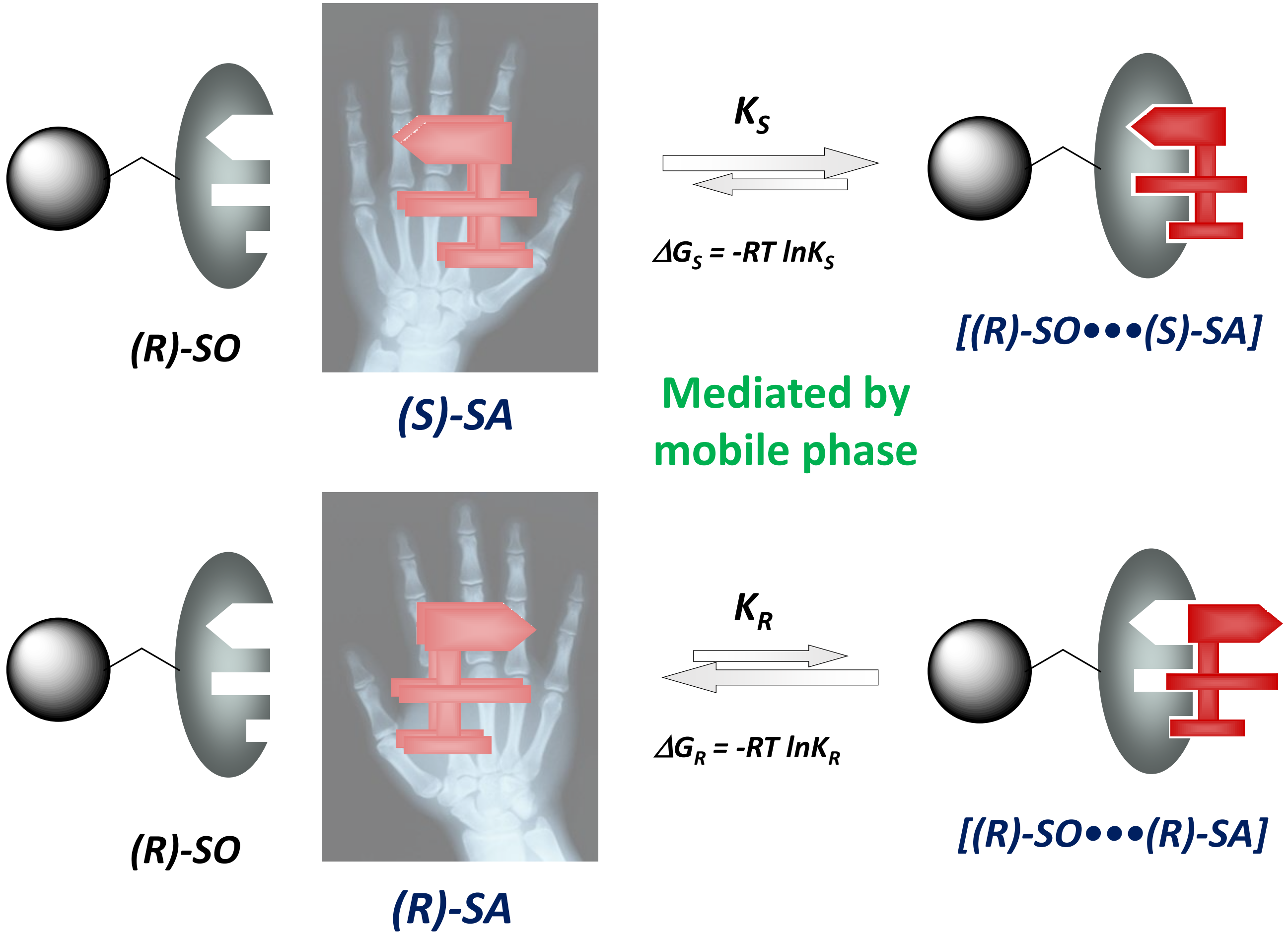


S- and *R*-cathinone



Three-Point-Interaction Model (Dalgliesh)

Enantioselective molecular recognition by chiral selectors



more stable diastereomeric complex

via multiple binding sites

less stable diastereomeric complex

multiple association and dissociation events modulated by chromatographic conditions (mobile phase)

separation factor α

$$K_S > K_R$$

$$\Delta\Delta G_{S,R} = \Delta G_S - \Delta G_s = -RT \ln(K_S / K_R) = -RT\ln\alpha$$

SO = Selector; SA = Selectand

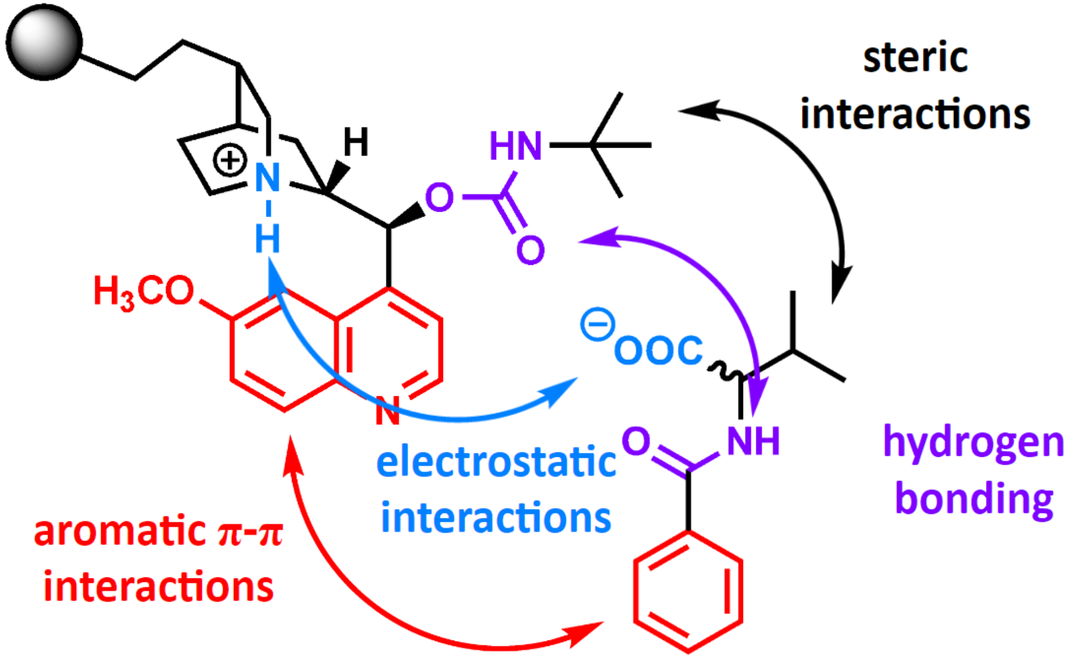
Noncovalent inter- and intramolecular interaction forces

- Electrostatic fit – *functional* complementarity
  - Anion — Cation
  - H-donor — H-acceptor
  - π-acid (e^- poor) — π-base (e^- rich)
  - Dipole (induced) — Dipole (induced)
- Hydrophobic fit – *geometric* match of *hydrophobic* patches (similarity)
- Steric fit – (partial) *size* and *shape* complementarity

Attractive Electrostatic Interactions		[kJ/mol]
Ionic (ion pair) Interactions	**(long range)**	
without H-bond	**non-directed**	**20-30**
via H-bond	directed	**50**
Cation-π Interactions	directed	**4 to 10**
H-bonds (short range)	directed	**4 to 17**
Aryl-Aryl charge transfer (π-π-interactions)	directed	**4 to 17**
Face-to-Face / Face-to-Edge		**2 to 17**
Van der Waals forces		**4**
Hydrophobic Interactions	solvent dependent	

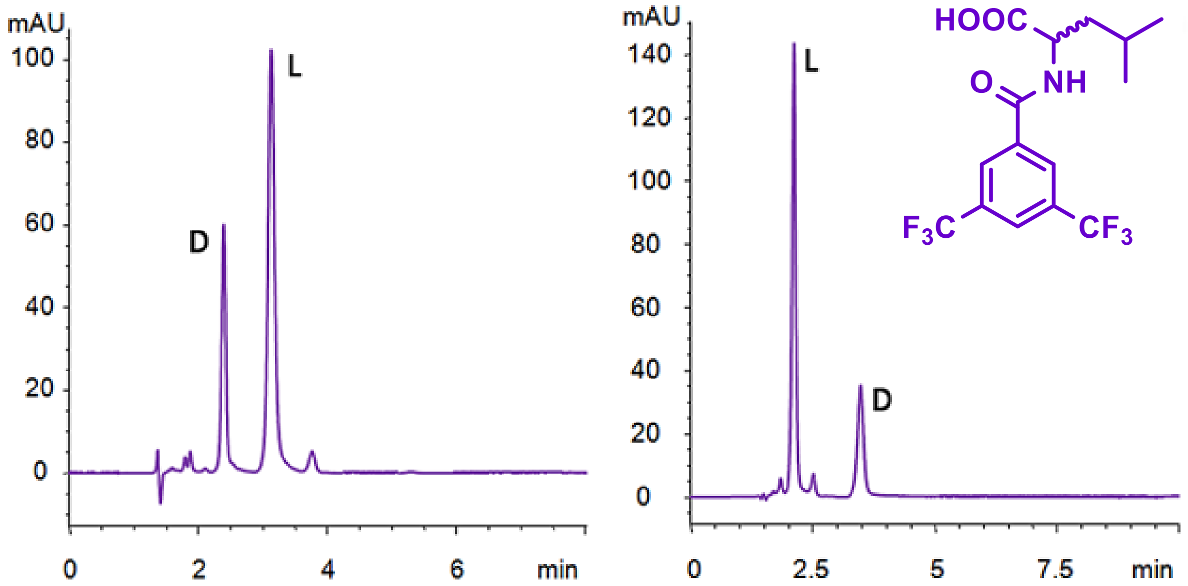
Compiled by W. Lindner from H. Kubinyi, QSAR: Hansch Analysis and Related Aproaches, VCH, 1993

Chiral recognition by Cinchona-based ion exchangers



- Charged stationary phase
- Charged analyte
- Weakly acidic mobile phase
- Solvent – polar organic with appropriate buffer, acetic acid + ammonium acetate

Reversal of the elution order of enantiomers on changing quinine to quinidine



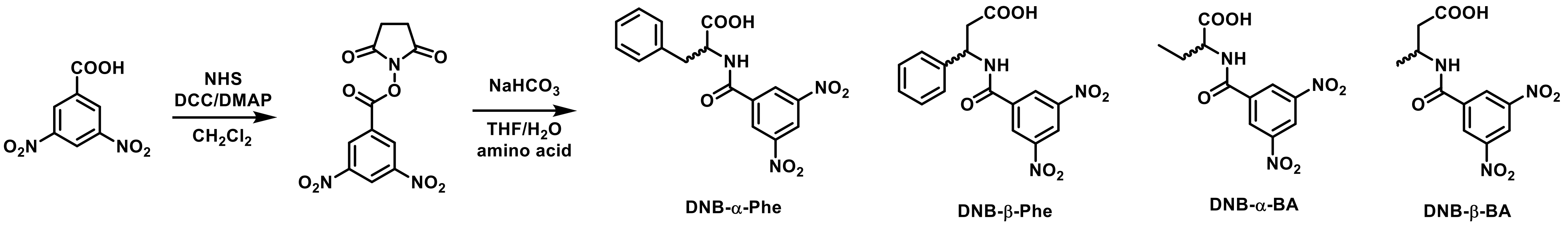
Data were obtained at structurally modified chiral stationary phases, column dimensions 150×4 mm, 5 μm, mobile phase: MeOH/AcOH/$AcONH_4$ (98/2/0.5, v/v/w), flow rate 1 ml min^{-1}, room temperature, detection wavelength λ=254 nm.

M. Kohout, et al. J. Sep. Sci. 2018, 41, 1355.

The case of β-amino acids

Analytes

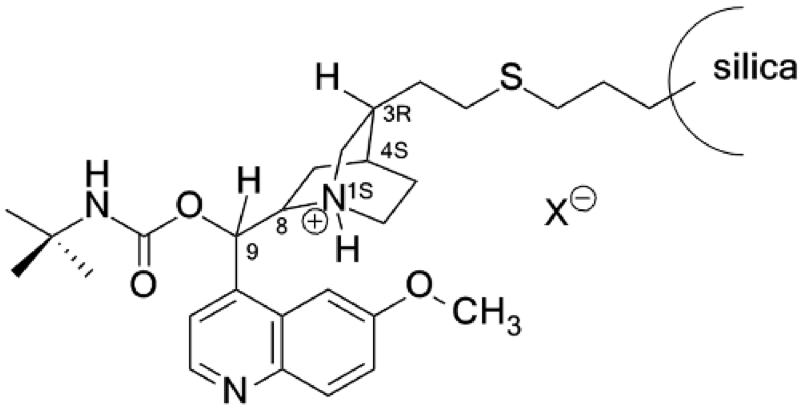
A series of α- and β-amino acids in racemic and enantiomerically pure form



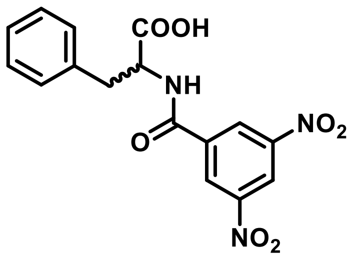
Stationary phases

CSP 1: Chiralpak® QN-AX (1*S*, 3*R*, 4*S*, 8*S*, 9*R*)
CSP 2: Chiralpak® QD-AX (1*S*, 3*R*, 4*S*, 8*R*, 9*S*)

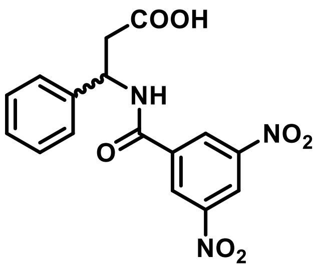
Chiral weak anion exchangers in experimental design
(selectors immobilized on 3 μm silica particles)



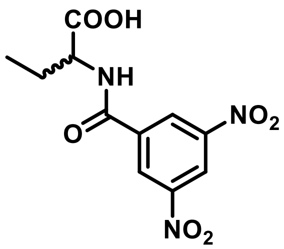
The case of β-amino acids



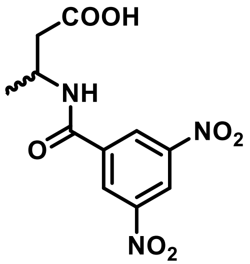
DNB-α-Phe



DNB-β-Phe

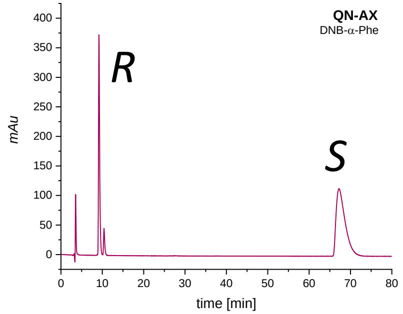


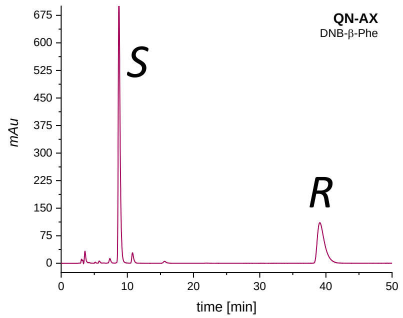
DNB-α-BA

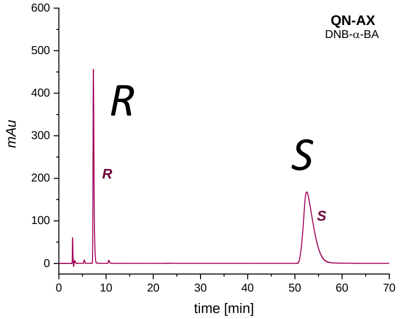


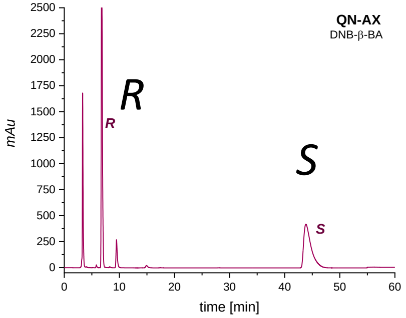
DNB-β-BA

CSP 1
QN-AX

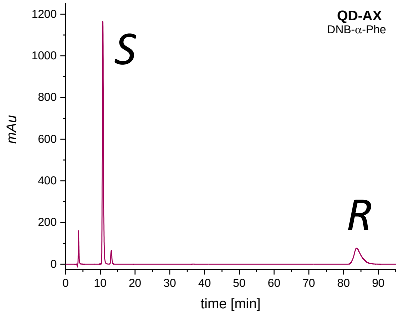


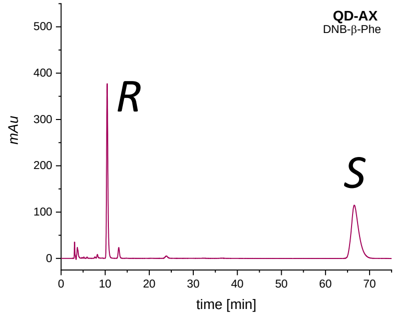


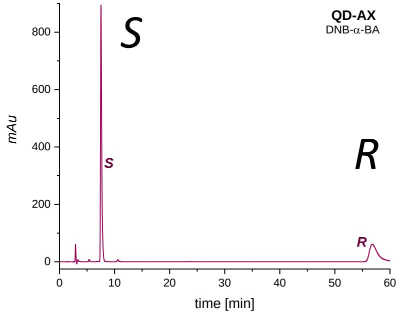


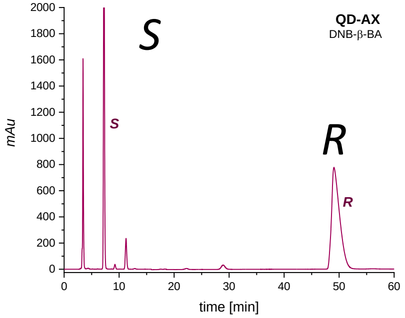


CSP 2
QD-AX





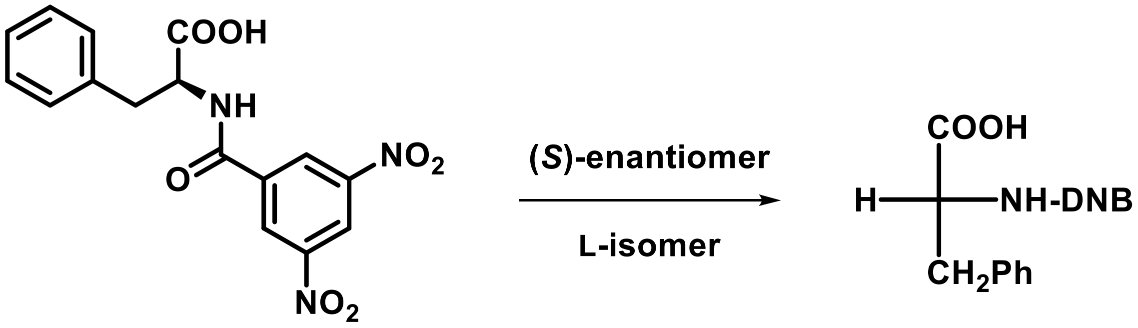




Nomenclature-based reversal of elution order

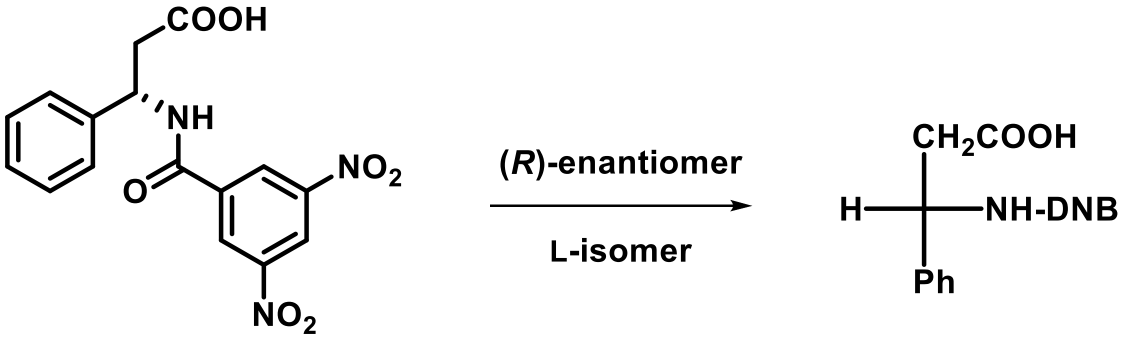
Apparent reversal of elution order

Cahn-Ingold-Prelog rule



DNB-α-Phe

Fischer projection



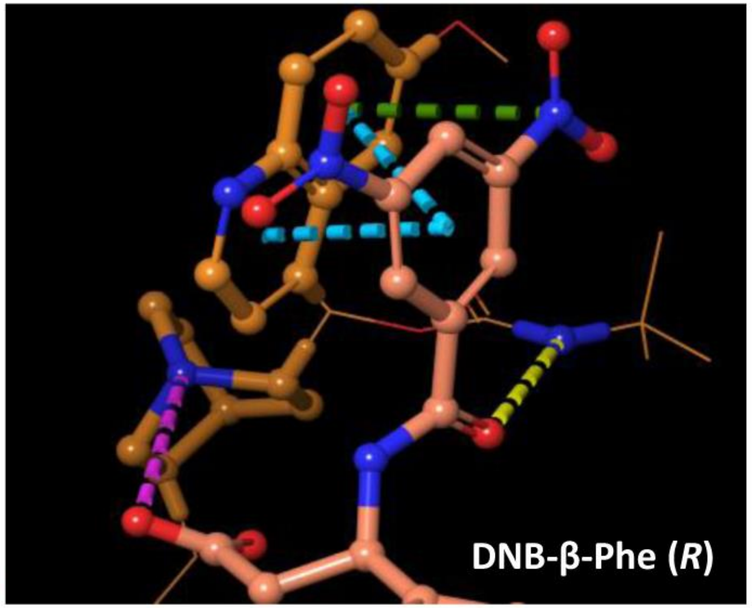
DNB-β-Phe

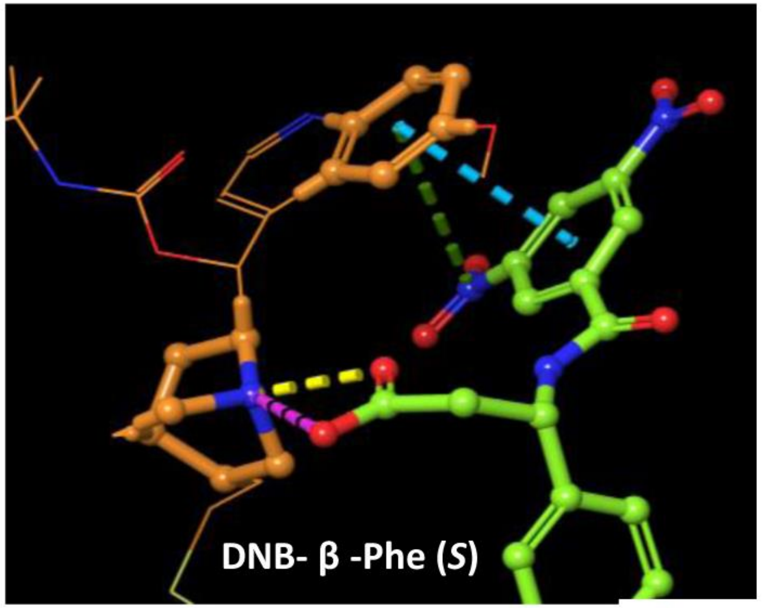
I. Varfaj, M. Labíková, et al. Anal. Chim. Acta 2024, 1314, 342791

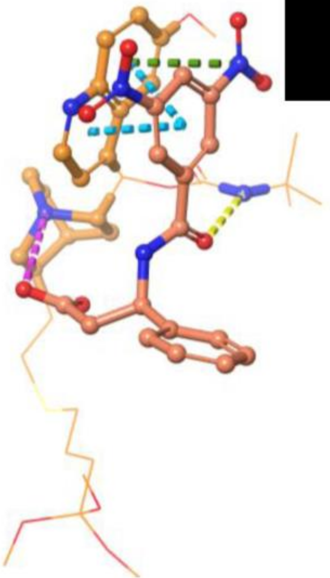
Understanging chiral recognition mechanism

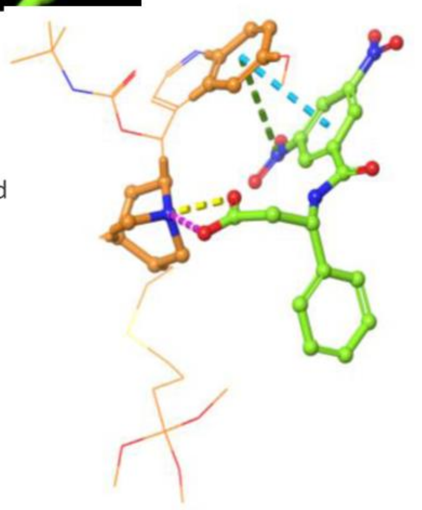
EEO: S < R

Molecular dynamics run:
300 ns, 1000 frames







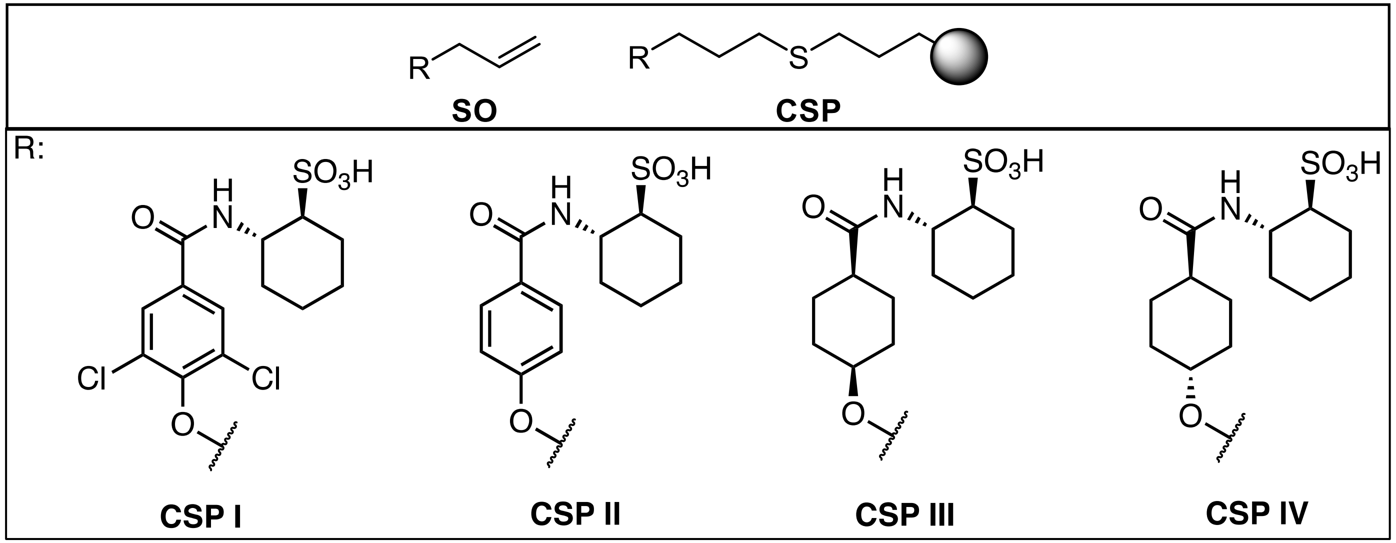


QN-AX	DNB_beta-PheR		DNB_beta-PheS	
Interaction	**Moiety**		**Moiety**	
	SO	SA	SO	SA
Aromatic Hbond				
H-Bond (HB)	OCONH	O-NH	Quinuclidine-N+	COO-
Salt Bridge (SB)	Quinuclidine-N+	COO-	Quinuclidine-N+	COO-
Π - Cation	Ring Quin	$ON^{+}O$	Ring Quin	ONO-
Π-Π Stacking	Ring Quin	DNB-ring	Ring Quin	DNB-ring
	Ring Quin	DNB-ring		

- Aromatic Hbond
- H-Bond (HB)
- Salt Bridge (SB)
- Π - Cation

Chiral strong cation exchangers lacking π–π-interactions

Is chiral recognition power reduced or lost on diminishing the π–π-interactions?



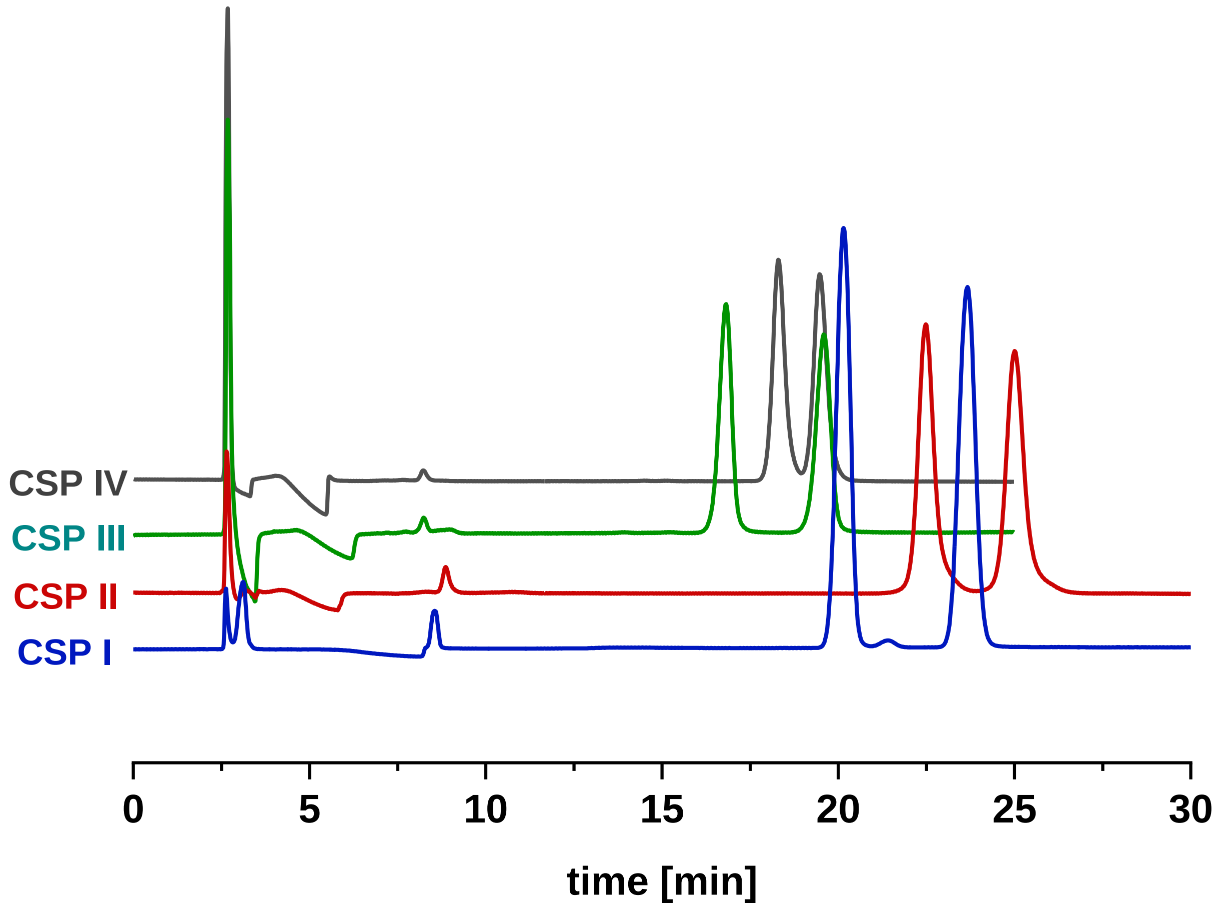
- Removal of substituents shall affect the π–π-stacking ability
- No π–π-interactions for cyclohexane rings
- Is there a chromatographic difference using *cis*- and *trans*-cyclohexane

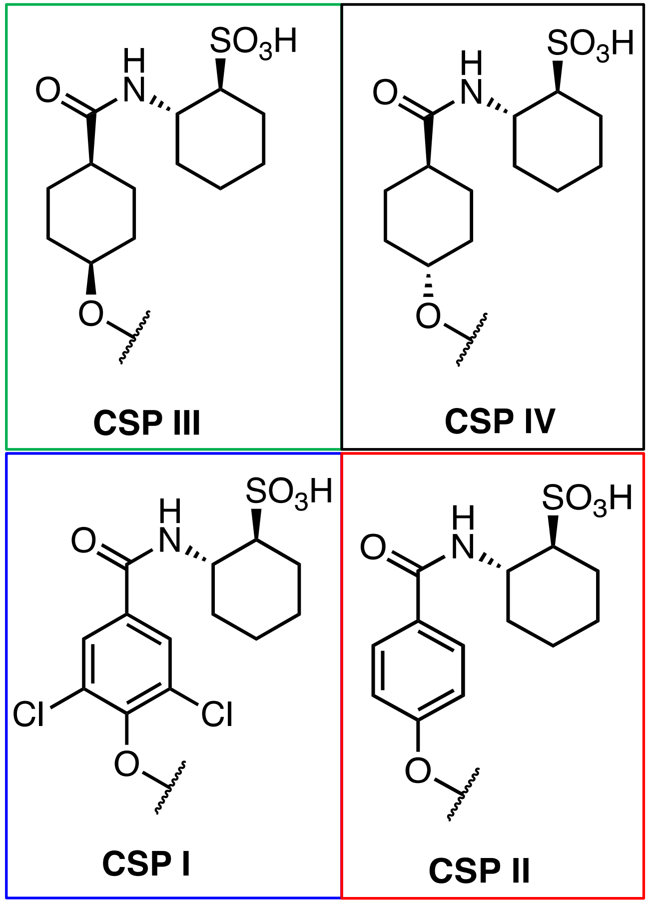
Presented today at poster no. 9

M. Labíková, et al. J. Chromatogr. A 2024, 1719, 464729

Chiral strong cation exchangers lacking π–π-interactions

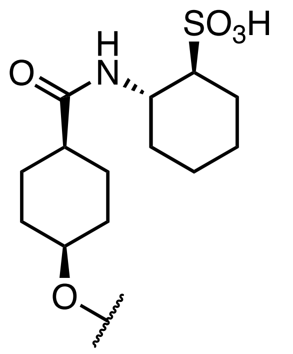
Life is full of surprises…



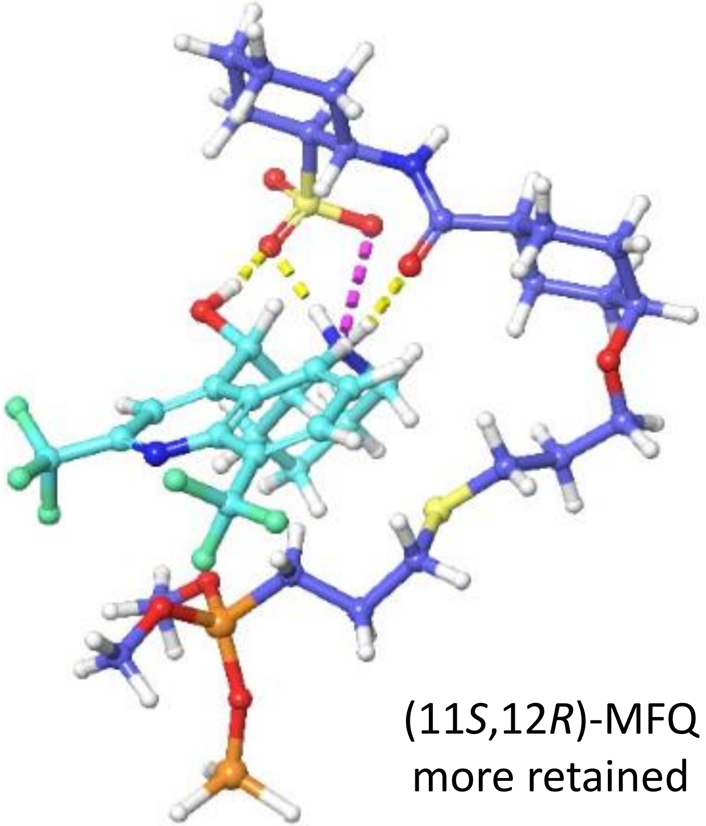


Chiral strong cation exchangers lacking π–π-interactions

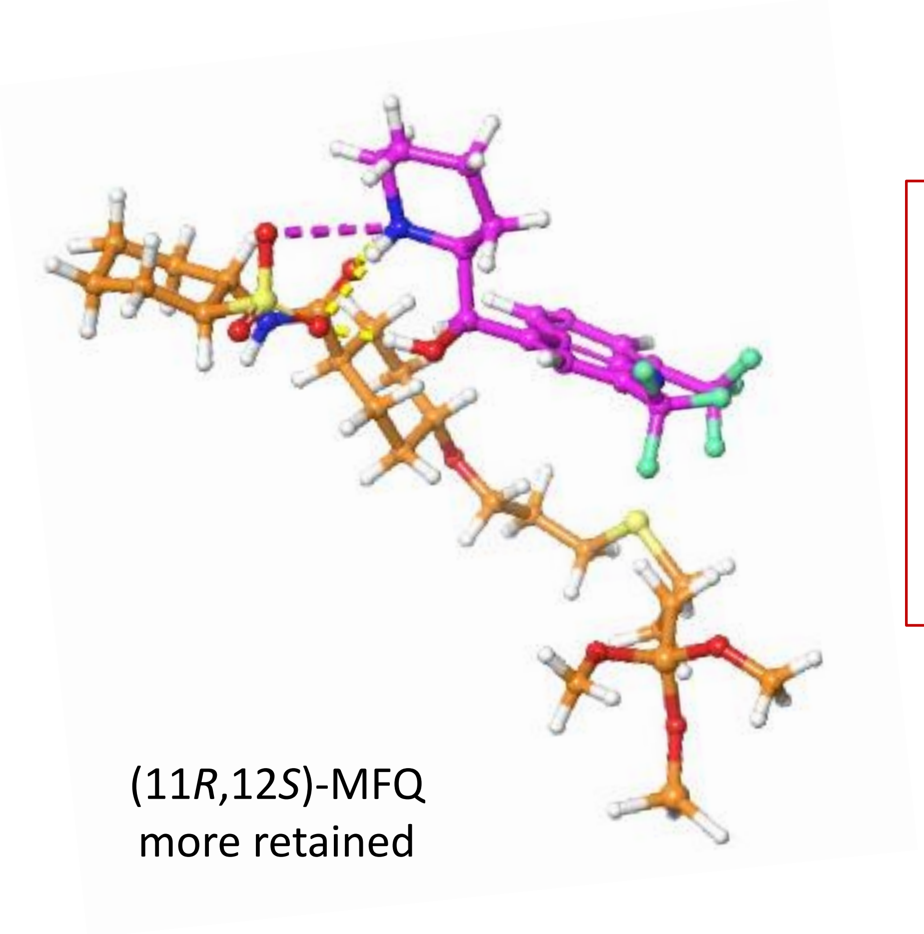
Molecular structure – chromatographic properties relationships



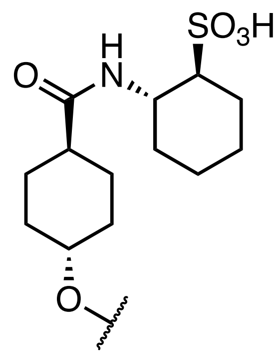
CSP III



(11*S*,12*R*)-MFQ
more retained



(11*R*,12*S*)-MFQ
more retained



CSP IV

Conclusions

- There are still many aspects of chiral recognition on the molecular level that we do not understand

- Understanding the chiral recognition mechanism can help to design tailor-made selectors to resolve specific chiral substances

- Even rather simple chiral entities can behave completely different than we envision

- Molecular dynamic simulations can help to understand this behaviour at nanoscale

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