New Classes of Chiral Selectors for LC, CE, and GC

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Chiral Selectors to be Discussed

1) V_2 and T_2
2) Diaminocyclohexylacrylamide (P-CAP)
3) Boron-Complex
4) Chiral Ionic Liquids

Common Chiral Selectors

- Marcrocyclic Chiral Selectors
  - Cyclodextrins and their derivatives
  - Glycopeptides
  - Chiral crown ethers
- Polymeric Chiral Selectors
  - Derivatized carbohydrates
  - Proteins
  - Synthetic polymers
- π- π Association Chiral Selectors
  - π-Electron acceptor
  - π-Electron donor
  - Combination of π-Electron acceptor and donor
- Ligand Exchange Chiral Selectors
- Miscellaneous and Hybrid Chiral Selectors

Armstrong, D.W.; Zhang, B. *Analytical Chemistry*, **2001**, *73*, 557A-561A

CHIROBIOTIC V2 AND T2

Extensions of the CHIROBIOTIC V and T created by changing the position of several linkages and the chain length used to anchor them. These changes have enhanced the selectivity and capacity of these phases in the polar organic and polar ionic modes only. They are, therefore, investigated in the optimization step when selectivity has been observed in these modes.

Anal. Chem., 68, 2501 (1996).

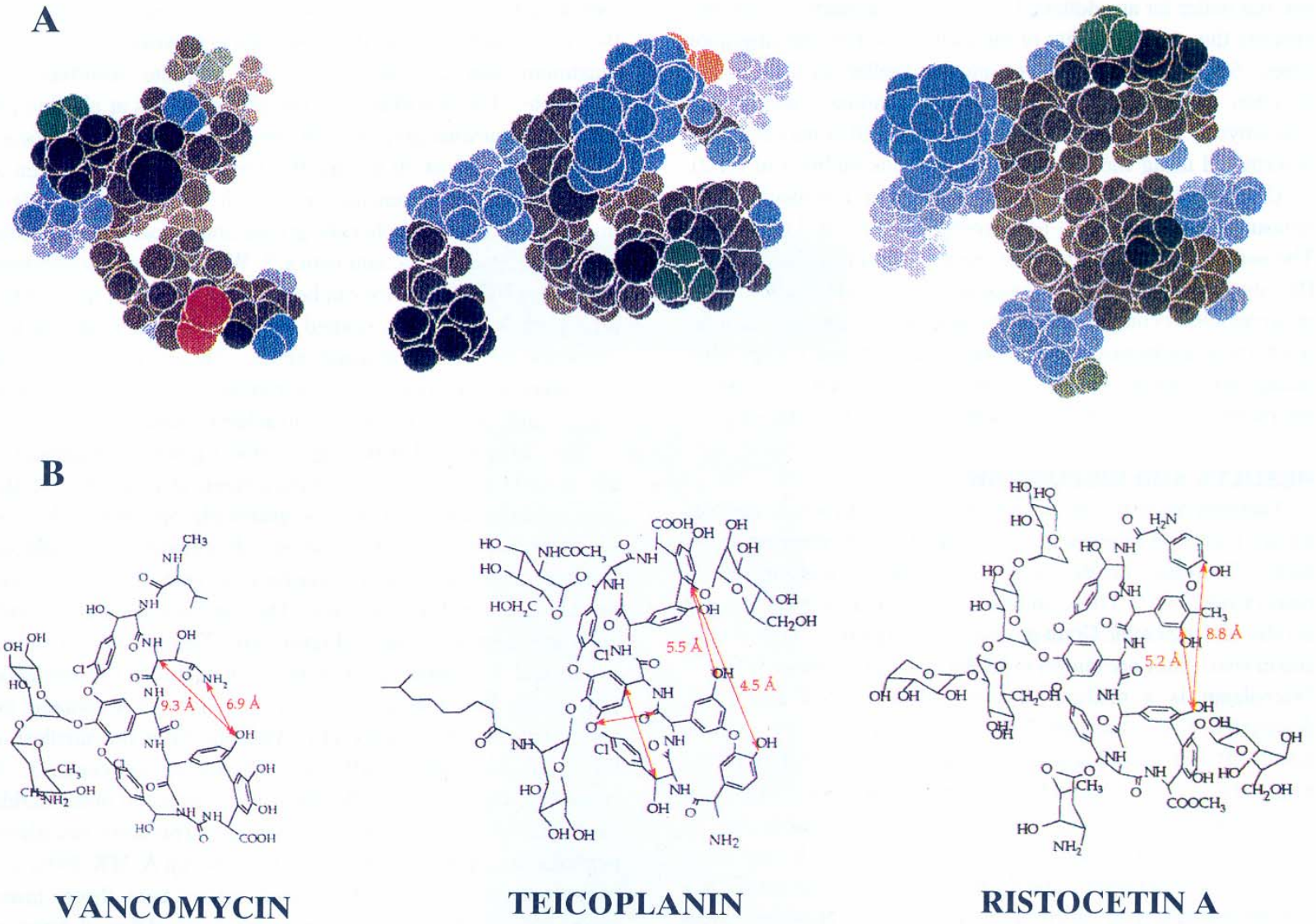


Figure 1. Structures of the macrocyclic antibiotics vancomycin, teicoplanin, and ristocetin A showing a profile view of the aglycon "basket" using (A) space-filling molecular models produced through energy minimization and (B) stick figures. The colored atoms in part A denote the hydrophilic moieties, while the black portion designates the more hydrophobic regions. Red represents carboxylate groups, green are ammonium groups, and blue are hydroxyls. Black regions include the aromatic rings, connecting carbons, and amido linkages.

CHIROBIOTIC V2 and T2

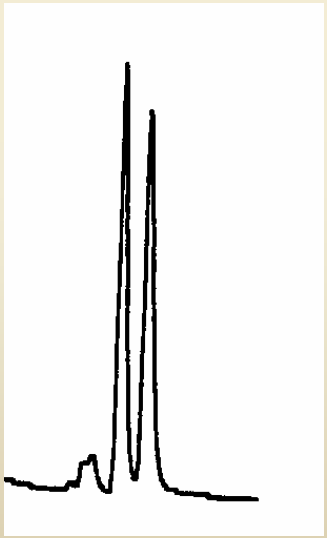
Example of Improved Resolution and Capacity for a Basic Compound

Analytical
CHIROBIOTIC V
250x4.6mm

Peak 1 – 10.51 min.

Peak 2 – 11.53 min.

α - 1.13



100/0.2/0.1: MeOH/HOAc/TEA

0.9 mL/min.

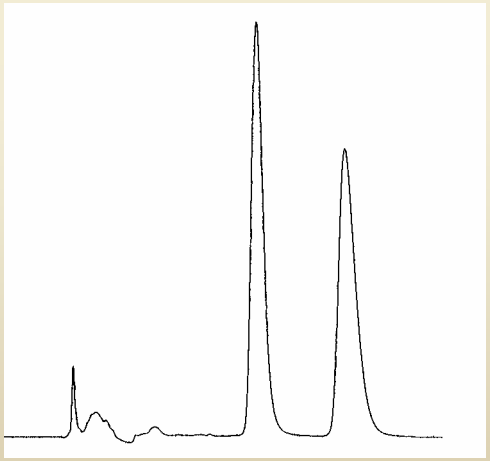
10 μg "on column"

Analytical
CHIROBIOTIC V2
250x4.6mm

Peak 1 – 8.83 min.

Peak 2 – 11.76 min.

α - 1.50



100/0.5/0.5: MeOH/HOAc/TEA

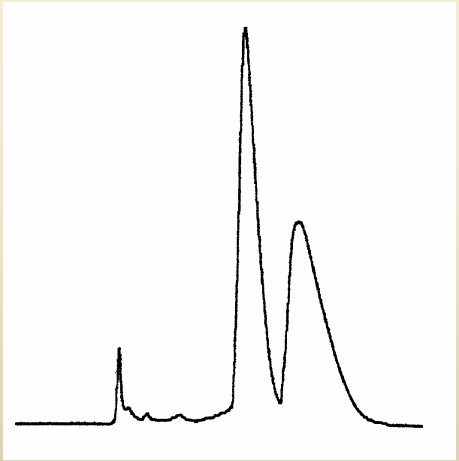
1.0 mL/min.

50 μg "on column"

Preparative
CHIROBIOTIC V2
250x21.2mm

Peak 1 – 10.22 min.

Peak 2 – 12.54 min.



100/0.5/0.5: MeOH/HOAc/TEA

15.0 mL/min.

140 mg "on column"

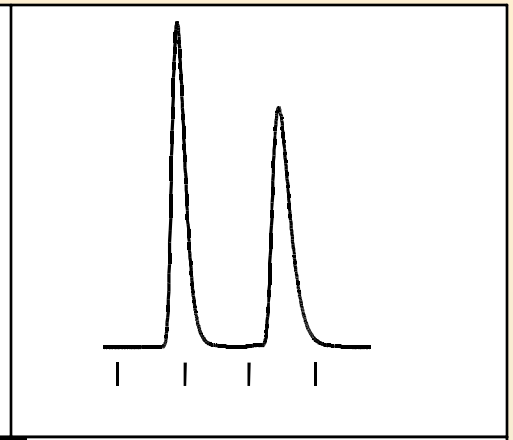
Load Study

Example of V over V2 in Polar Ionic Mode: Tolperisone

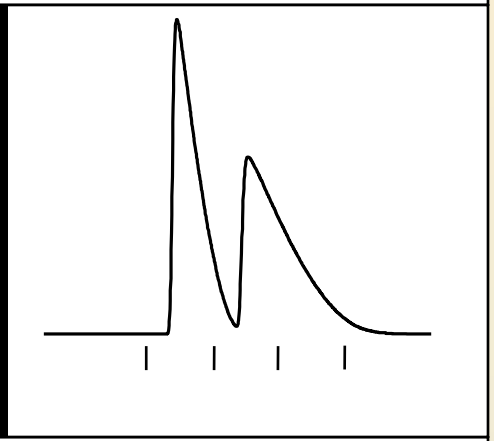
Solubility: >30mg/mL in MeOH
Column: CHIROBIOTIC V2
Mobile Phase: 100/0.1w%, MeOH/NH_4TFA
Selectivity: 1.33

Analytical: 250x4.6mm, 5µM
Load: 2mg/mL x 5µL (10 µg)
Flow Rate: 1 mL/min
UV: 230nm

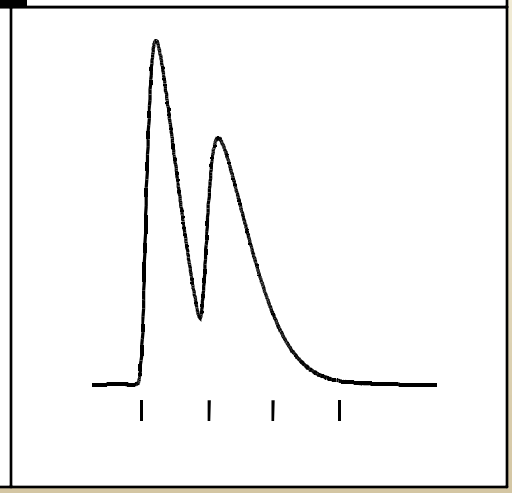
Peak 1: 8.80 min
Peak 2: 10.69 min



Analytical: 250x4.6mm, 5µM
Load: 30mg/mL x 0.03 mL (0.9 mg)
Flow Rate: 1mL/min
UV: 265 nm



Prep: 250x21.2mm, 5µM
Flow Rate: 20 mL/min
Load: 30mg/mL x 0.7mL (21 mg)
UV: 265 nm
Throughput: 1.8 mg/g CSP/hr



P-CAP™

New Normal Phase CSP

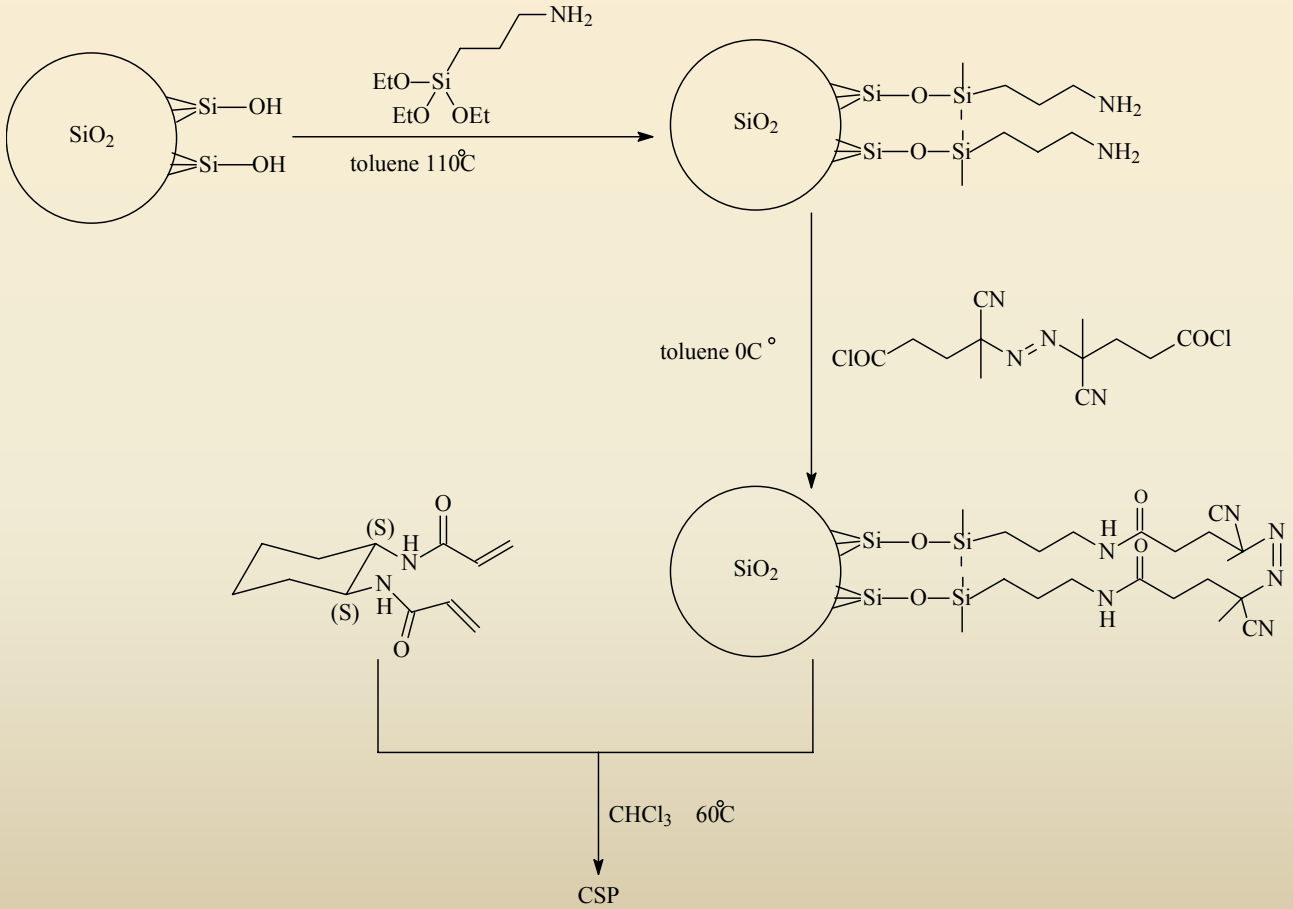
P-CAP

- New bonded polymeric chiral stationary phase
- *No* solvent limitations
- Reversible elution order as R,R and S,S configurations
- High efficiency – thin, ordered layer bonded to the silica surface gives fast kinetics
- Derivatization has little effect on selectivity

Synthesis of DACH-ACR

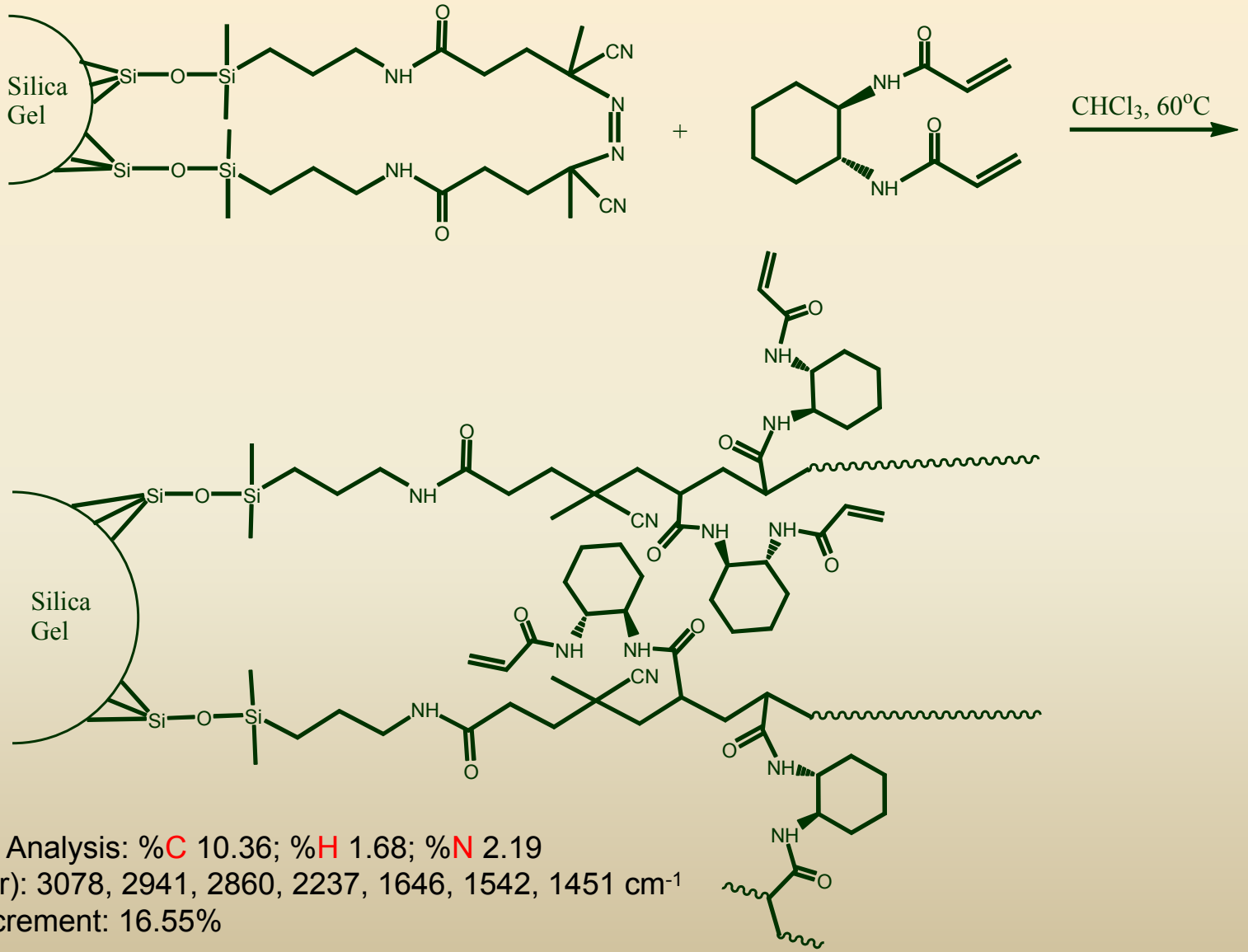
DIPEA = Diisopropyl Ethyl Amine

Synthesis of the new CSP



F. Gasparrini, D. Misiti, C. Villani, Italian Patent, RM2002A000155

Free Radical Polymerization on Functionalized Silica Gel



Elemental Analysis: %C 10.36; %H 1.68; %N 2.19
FT-IR (KBr): 3078, 2941, 2860, 2237, 1646, 1542, 1451 cm^{-1}
Weight Increment: 16.55%

Features of new CSP

- Absence of aromatic structures, no π-π interactions
- Retention and selectivity depend on hydrogen bond interactions, dipole-dipole interactions and steric interactions

Mobile Phase Modes and Solvents

1. Polar-organic mode: (acetonitrile + methanol + additive)

2. Normal Phase mode
   a. hexane + isopropanol
   b. methylene chloride + alcohol

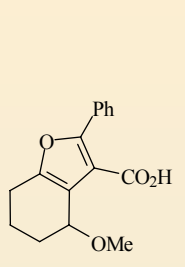
P-CAP: Solvent Compatibility

- Universal compatibility : all organic solvents
- Can add R_3N, R_2NH, HOAc, $R_3NH^+Ac^-$ for ionizable compounds
- *No* solvent memory effect
- Carbon dioxide with polar modifier for SFC (sub- and super-critical)
- Salts and/or addition of HOAc increase efficiency

P-CAP: Solvent Compatibility

- High efficiency in DCM/MeOH
- Selectivity not greatly affected by mobile phase; the same separation can also be achieved in ACN/MeOH (adding NH_4OAc or HOAc for MS detection)
- Can also be run in heptane/EtOH, hexane/DCM, acetone

Types of Compounds Separated on the P-CAP CSP



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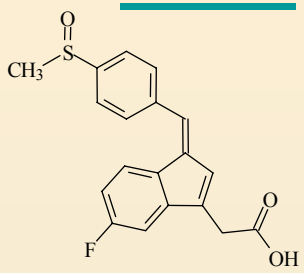

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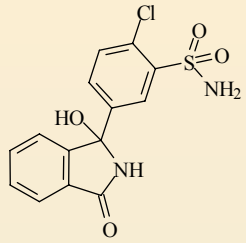

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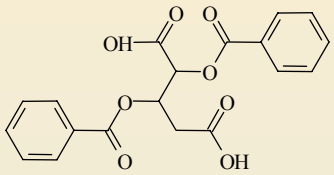

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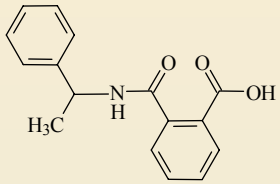

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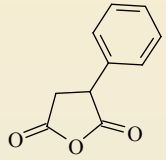

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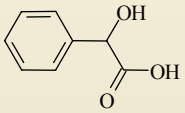

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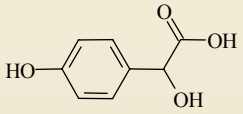

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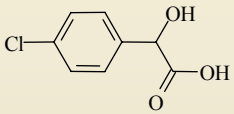

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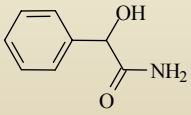

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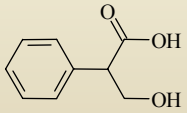

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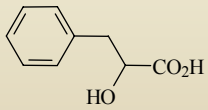

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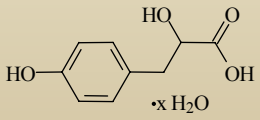

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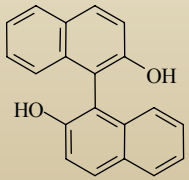
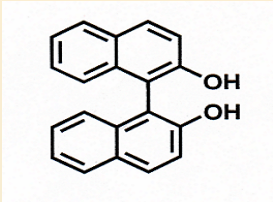


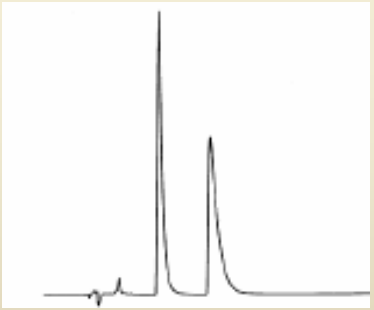
14

P-CAP: Mobile Phase Versatility

Separation of 1,1'-Bi-2-naphthol
Column: P-CAP (250x4.6 mm, 5μm)

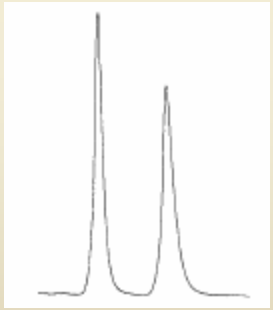


Eluent: 100% Acetone
Flow: 1.0 mL/min
Detection: UV, 330nm



Peak 1: 7.85 min
Peak 2: 11.42 min

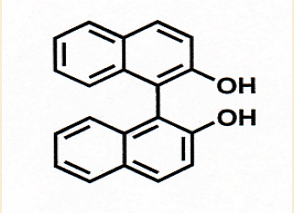
Eluent: ACN/MeOH/NH_4Ac, 95/5/10mM
Flow: 1.0 mL/min
Detection: UV, 254nm



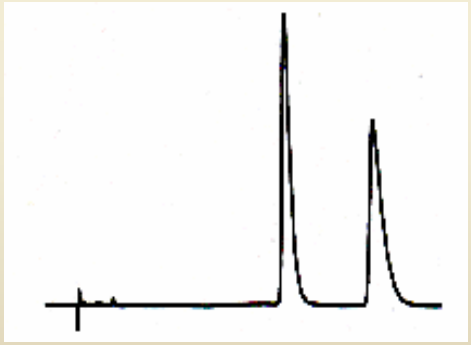
Peak 1: 7.91 min
Peak 2: 9.51 min

P-CAP: Mobile Phase Versatility

Separation of 1,1'-Bi-2-naphthol
Column: P-CAP (250x4.6 mm, 5μm)

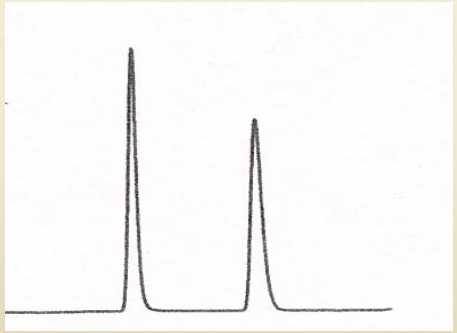


Eluent: n-Hexane/EtOH, 79/21
Flow: 1.0 mL/min
Detection: UV, 254nm



Peak 1: 18 min
Peak 2: 24.5 min

Eluent: DCM/MeOH, 95/5
Flow: 1.0 mL/min
Detection: UV, 254nm



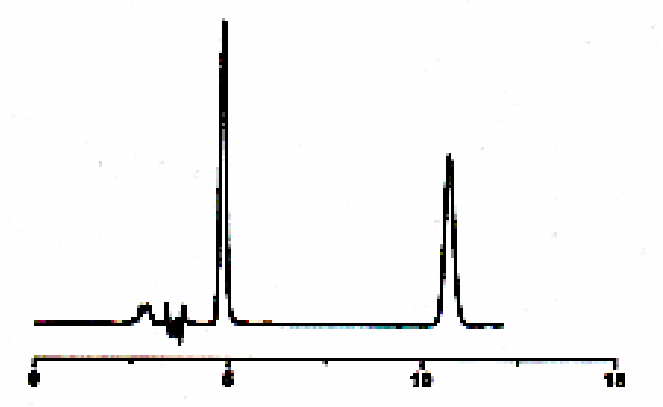
Peak 1: 6.15 min
Peak 2: 8.26 min

P-CAP: Mechanism

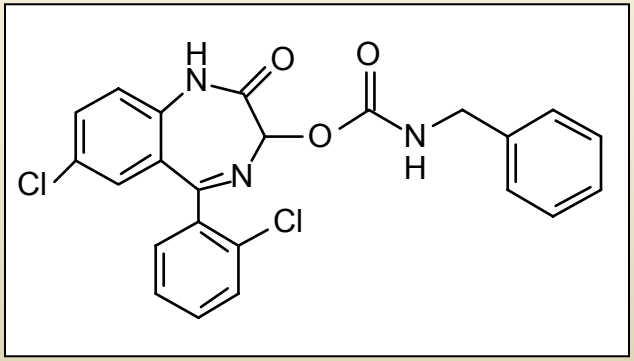
- Operates via hydrogen donor and acceptor sites
- Compounds with two or more functional groups work best
- Highest selectivity obtained if these are in a juxta position

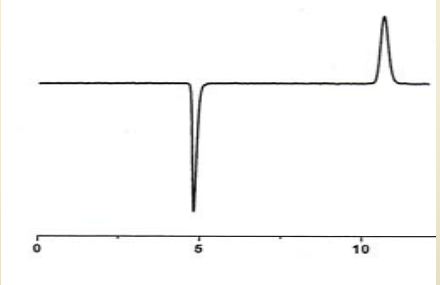
P-CAP: Reversal of Elution Order

Sample: Lorazepam analogue

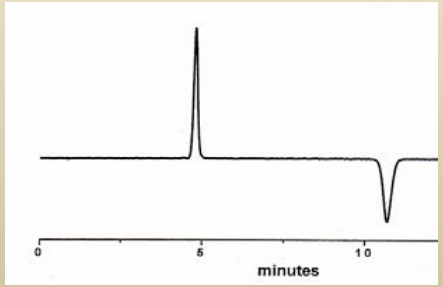


Eluent: DCM/MeOH, 95/5
Flow: 1.0 mL/min
Detection: UV, 254nm,
Polarimeter (Chiralyser)





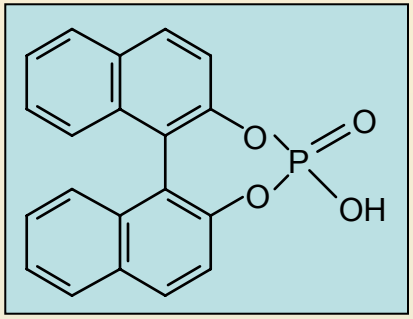
Column: R,R P-CAP
(250x4.6 mm, 5μm)

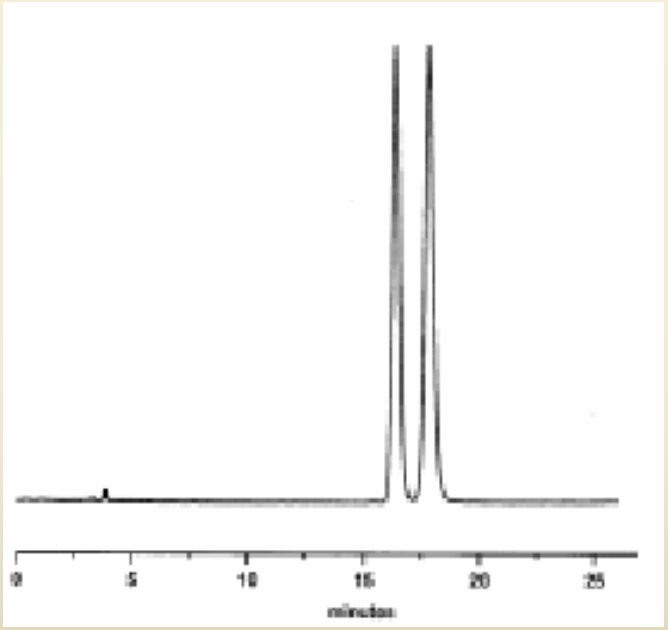


Column: S,S P-CAP
(250x4.6 mm, 5μm)

P-CAP: Polar Organic Mode

Sample: 1,1’-binaphthalenyl-2,2’-diol phosphonate





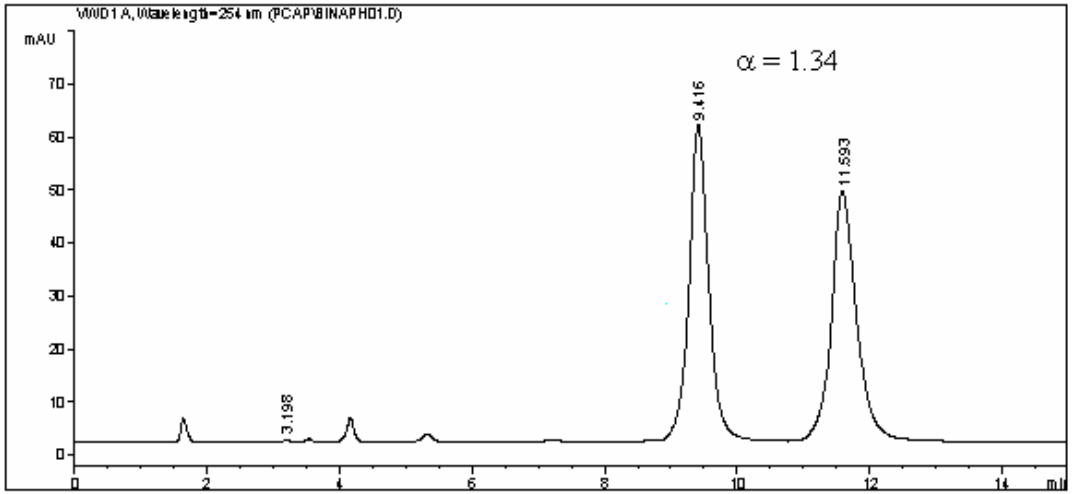
Eluent: ACN/MeOH, 70/30 v/v, 20mM NH_4Ac

Flow: 1.0 mL/min

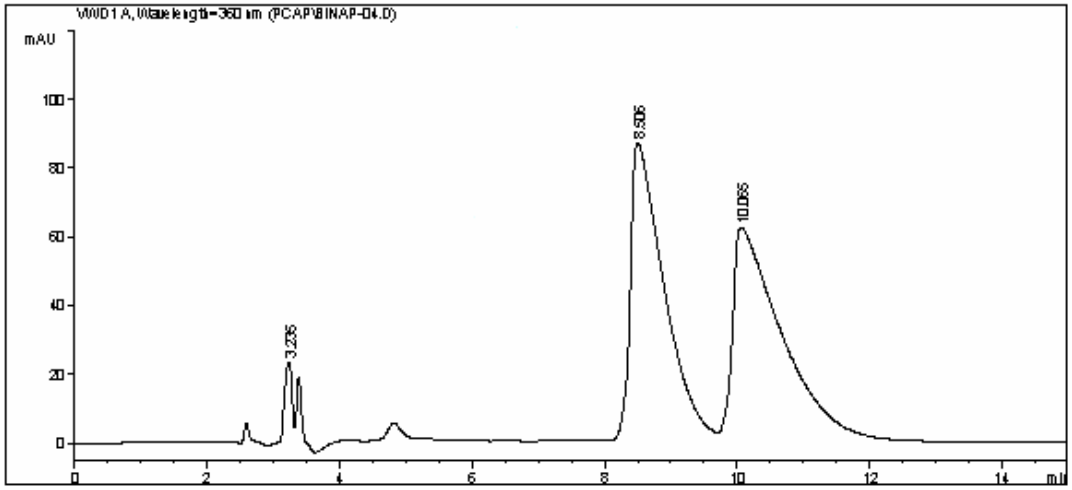
Detection: UV, 254nm

Loading Study of 1,1'-Binaphthol in Normal Phase Mode

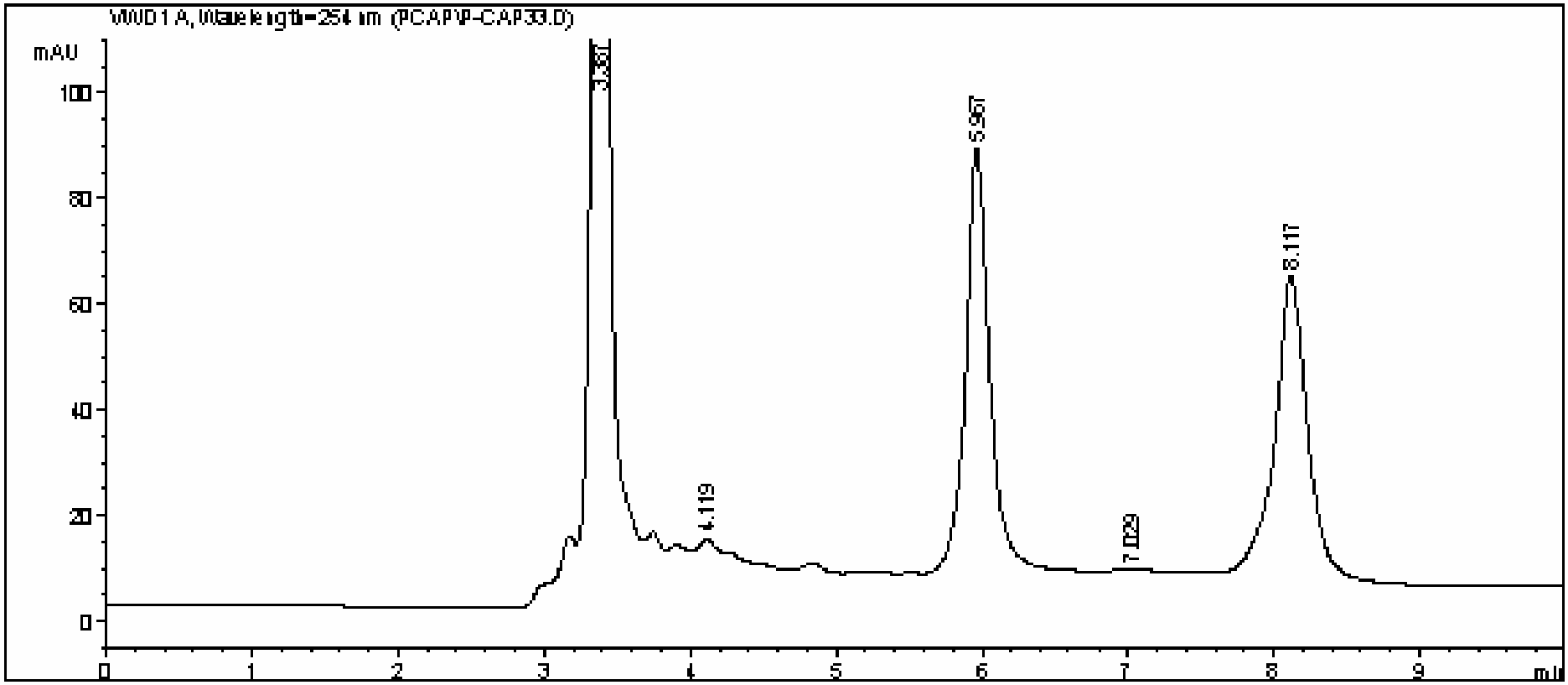
Column: (R,R) P-CAP (250x4.6 mm)
Mobile Phase: EtOH/Heptane 50/50
Flow Rate: 1.0 ml/min
Detection: UV@254 nm
Temperature: 25 °C
Sample: 1,1'-Binaphthol
Sample loading: 1µg



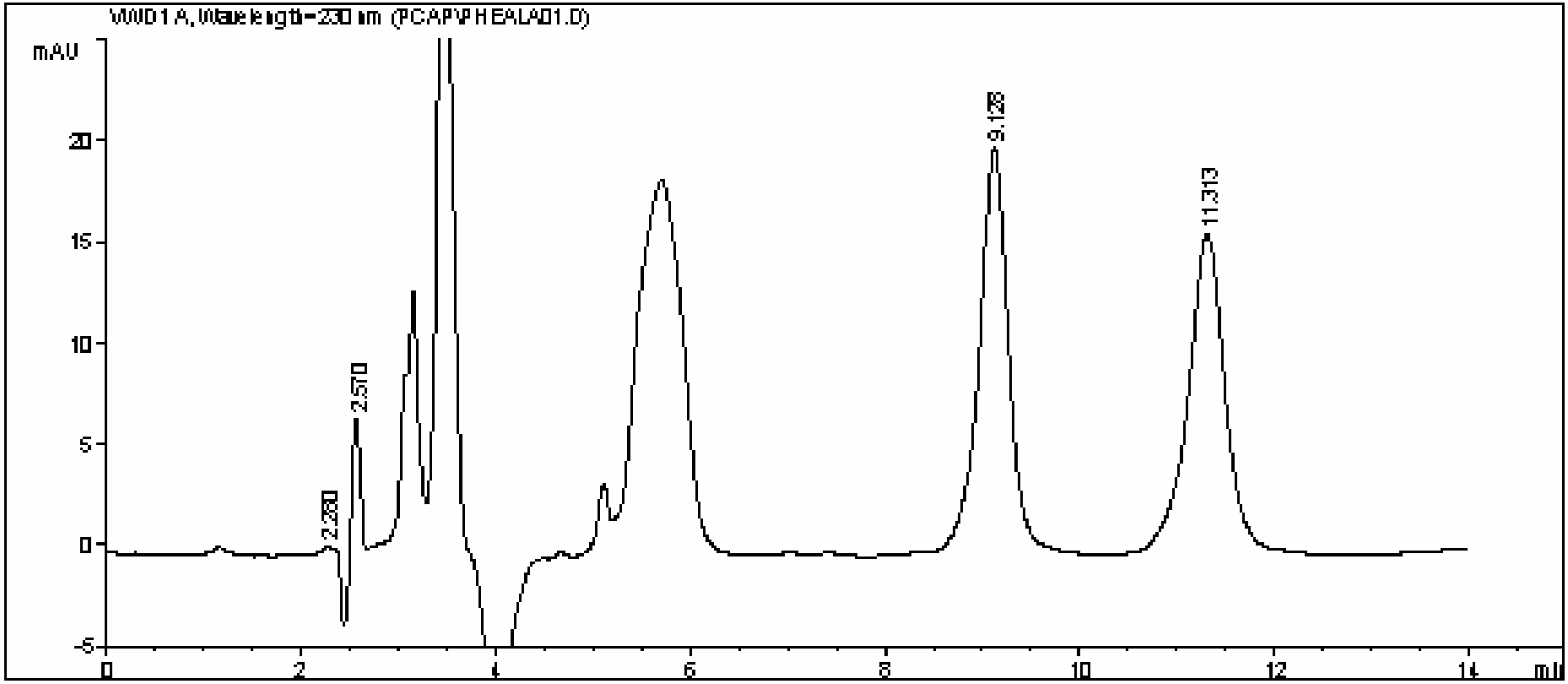
Sample loading: **1000 µg**



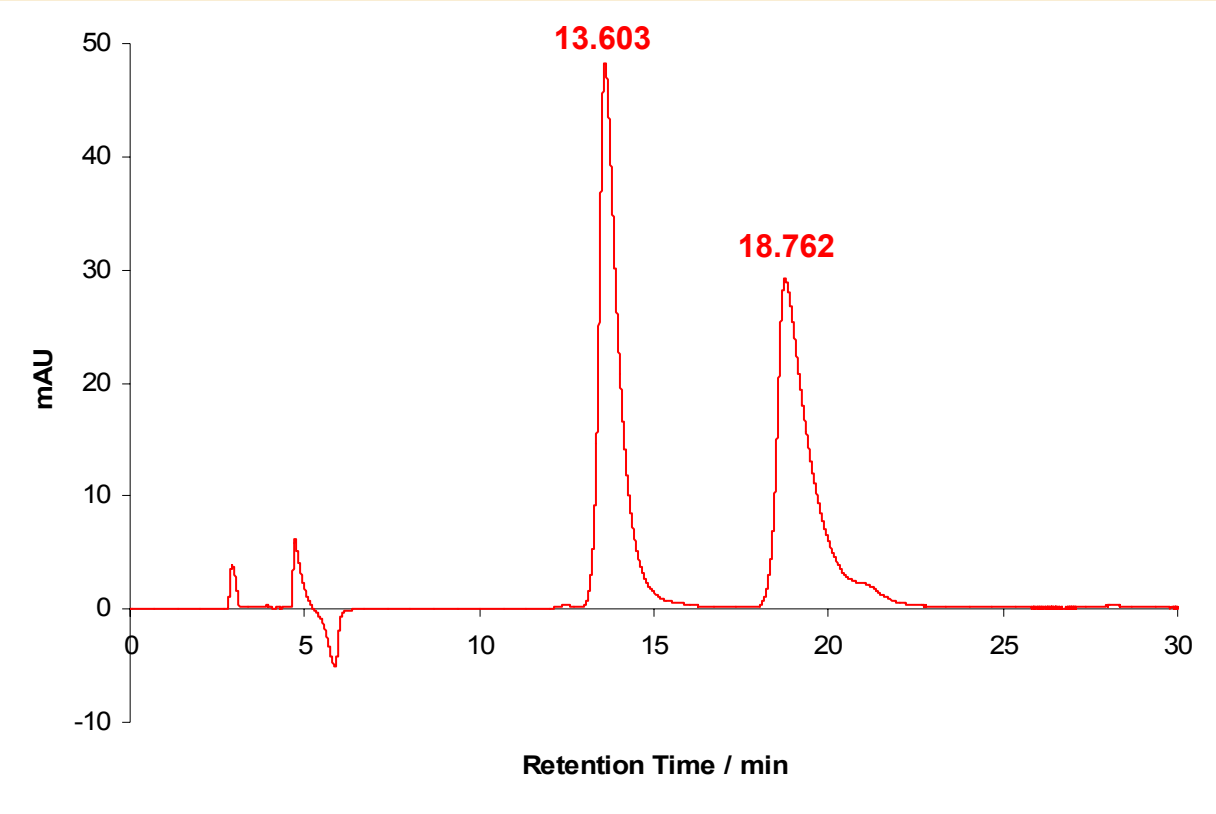
Column: (R,R) P-CAP (250x4.6 mm)
Mobile Phase: ACN/MeOH/NH_4OAc 70/30/20 mM
Flow Rate: 1.0 ml/min
Detection: UV@254 nm
Temperature: 25 °C
Sample: Lorazepam

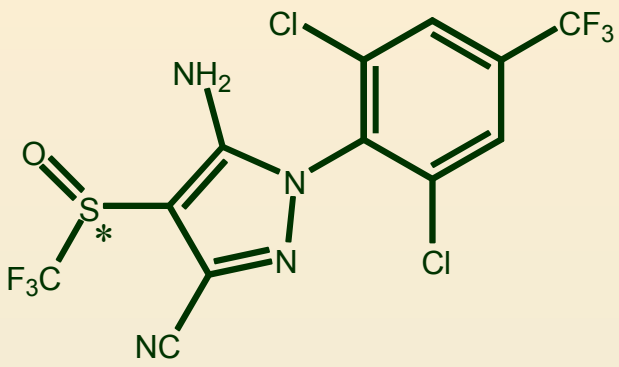


Column: (R,R) P-CAP (250x4.6 mm)
Mobile Phase: ACN/MeOH/TFA 95/5/0.1
Flow Rate: 1.0 ml/min
Detection: UV@254 nm
Temperature: 25 °C
Sample: FMOC-phenylalanine



Normal Phase Chiral Separation



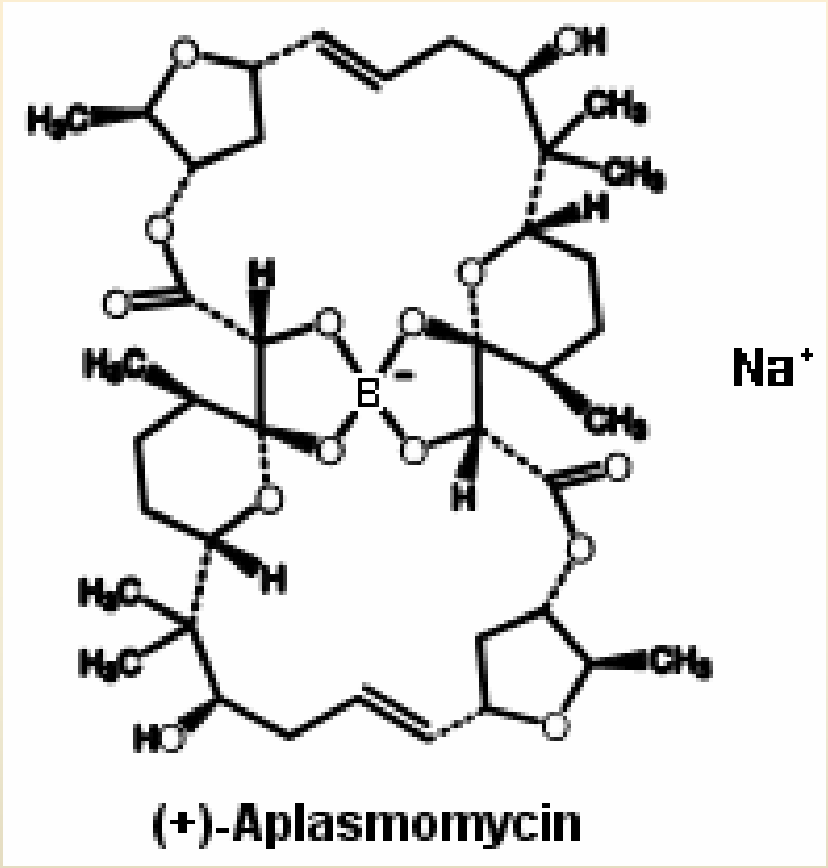


Fipronil

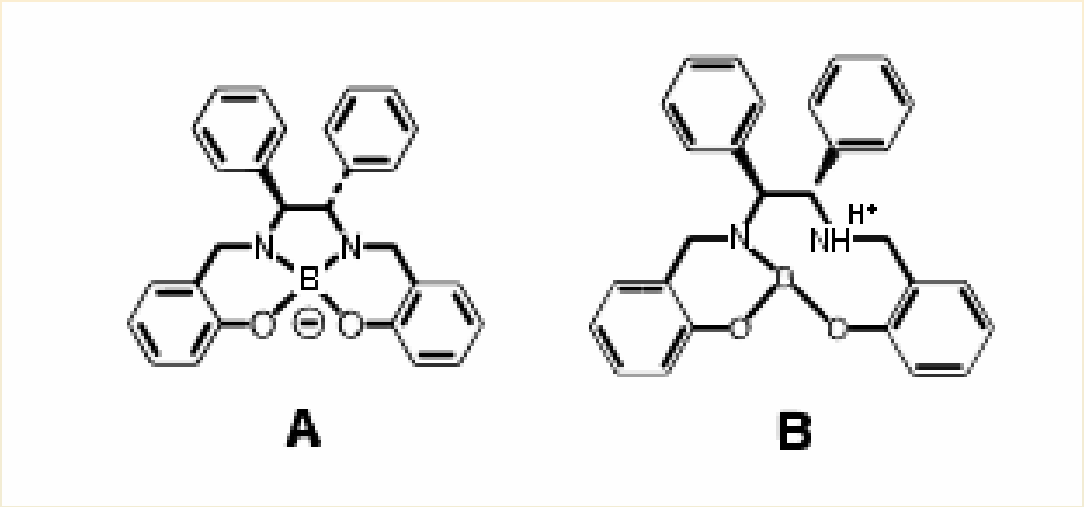
Column	**RR-P-CAP**
Flow Rate	**1 ml/min**
Mobile Phase (v/v)	**Heptane/IPA/TFA 80/20/0.1**
Selectivity α	**1.48**
Resolution R_s	**4.21**
UV Detector Wavelength	**254 nm**

Conclusions for P-CAP

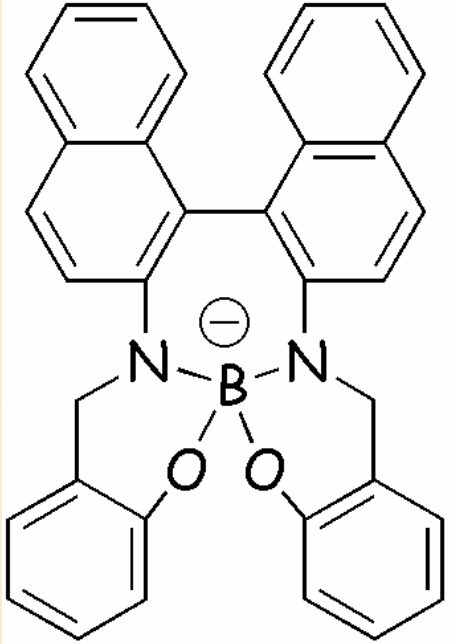
- New normal phase, high capacity CSP
- No solvent limitations, DCM/MeOH – most efficient
- Reversible elution order with R,R and S,S formats



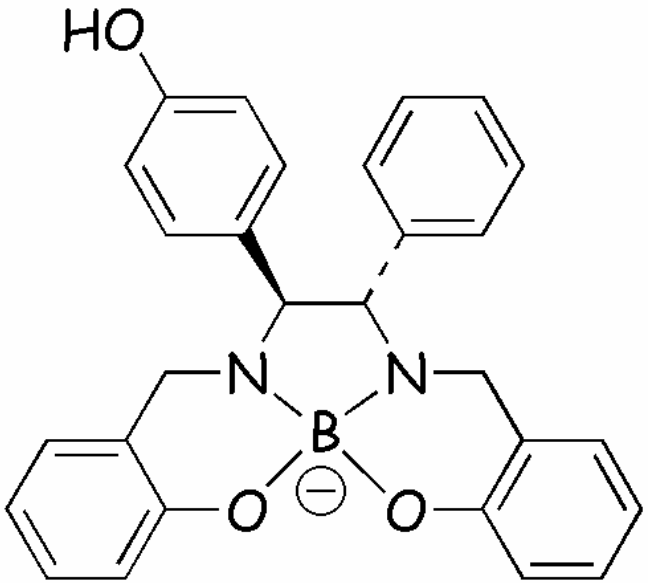
(+)-Aplasmomycin



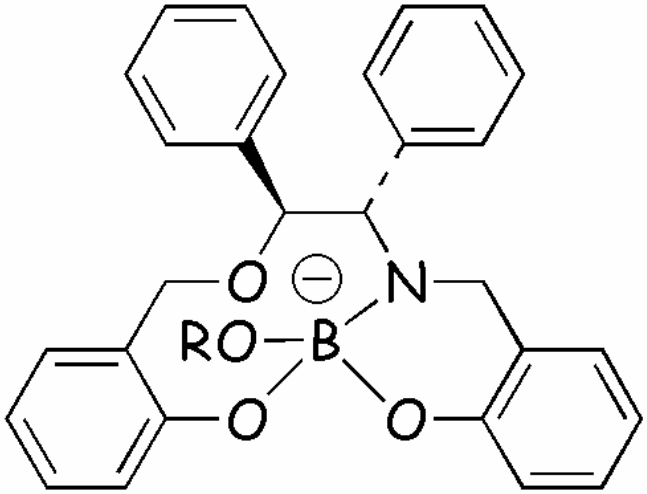
Structures of (R,R)-organoboron-1 showing both its (A) anionic and B cationic forms.



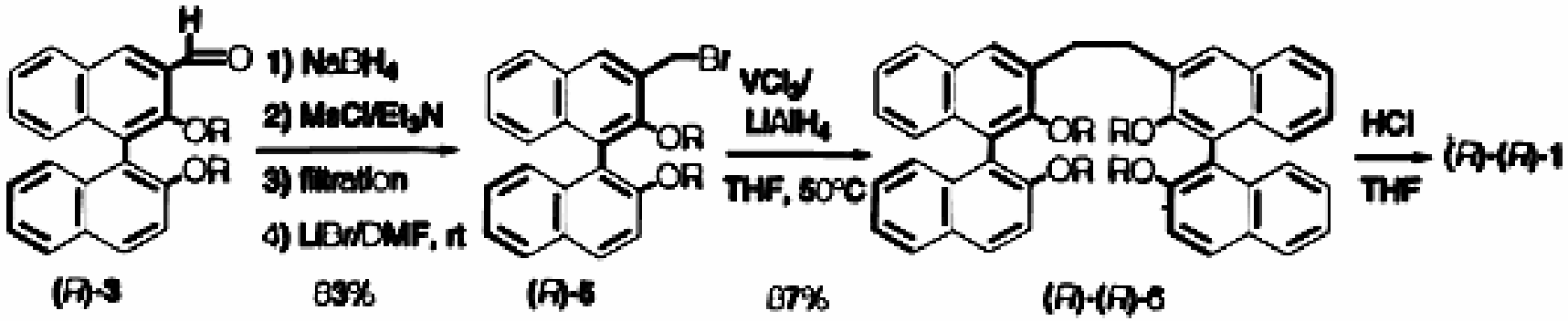
1



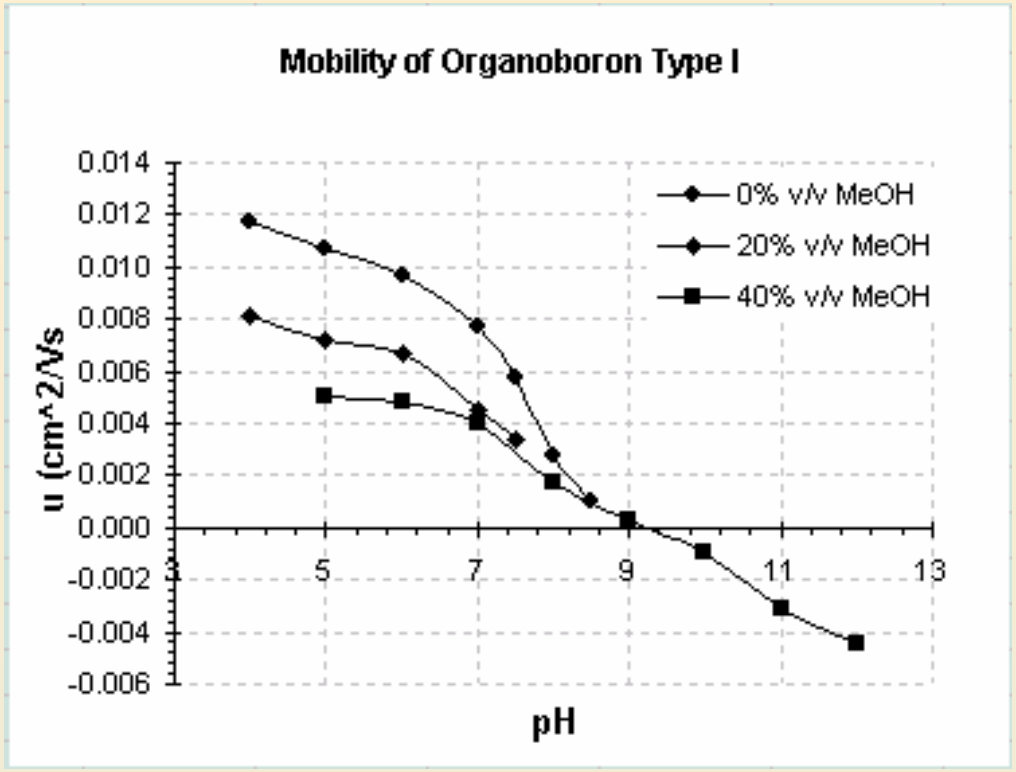
2



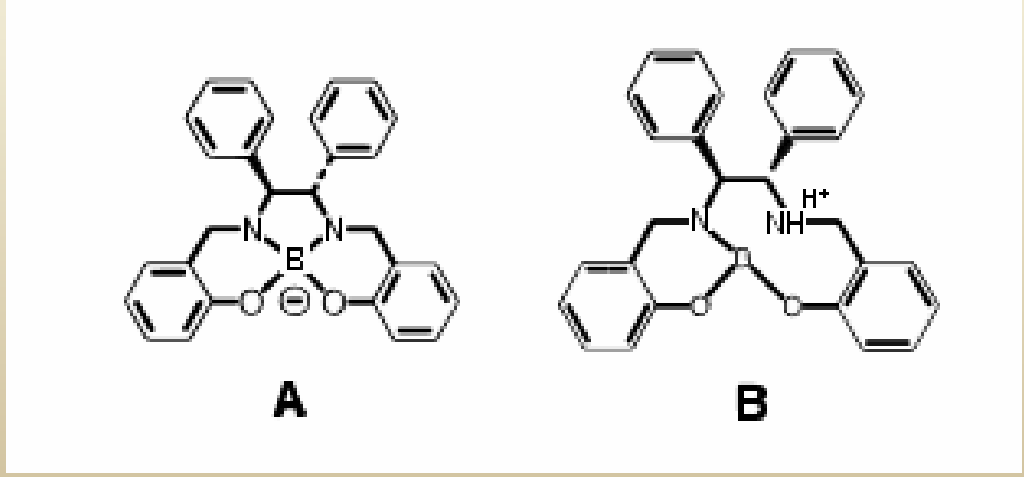
3

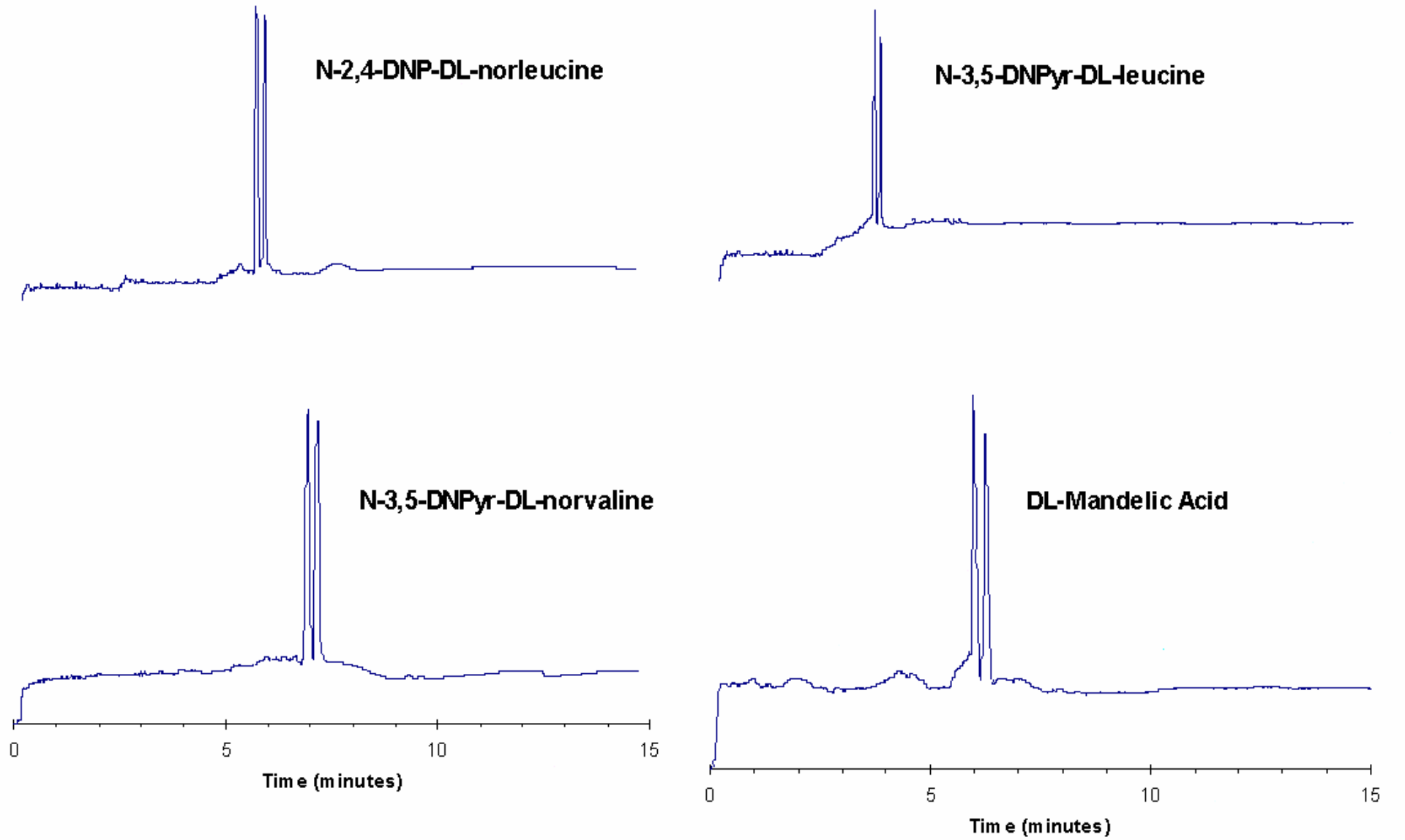

H
O
OR
OR
1) NaBH4
2) MsCl/Et3N
3) filtration
4) LiBr/DMF, rt
Br
OR
OR
VCl3/
LiAlH4
THF, 50°C
OR
RO
OR
RO
HCl
THF
(R)-(R)-1
(R)-3
83%
(R)-6
87%
(R)-(R)-6

Scheme 2.

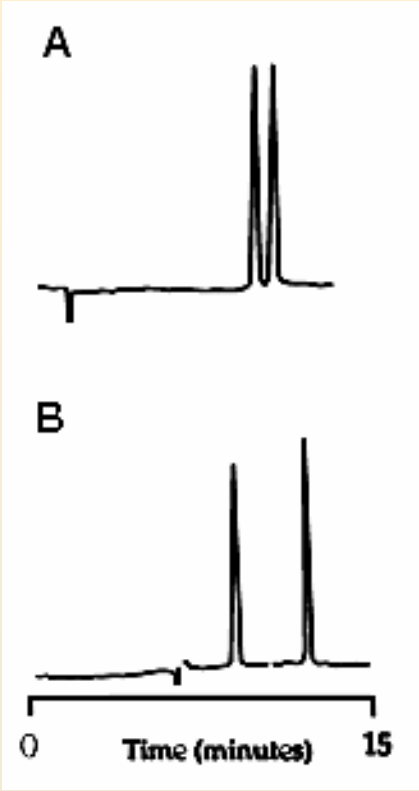


Electrophoretic mobility of organoboron-1 vs pH in the presence and absence of MeOH organic modifier.





CE enantioseparation of three racemic N-blocked amino acids and racemic mandelic acid. Experimental conditions are identical to Figure 5 except the pH=9.2.



CE separation of (A) (R,S)-1phenyl-propanol and (B) (R,S)-methamphetamine using 1 mM organoboron-1 in 80:20, buffer:MeOH. A 40 cm x 0.1mm (i.d.) capillary was used and UV 254 detection. The pH = 6.0.

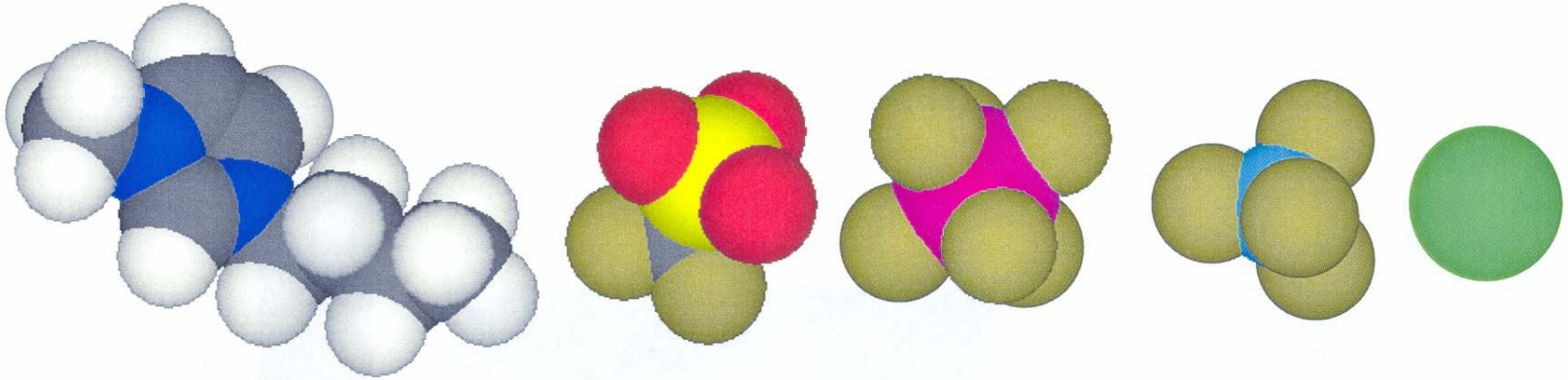
What is a Room Temperature Ionic Liquid?

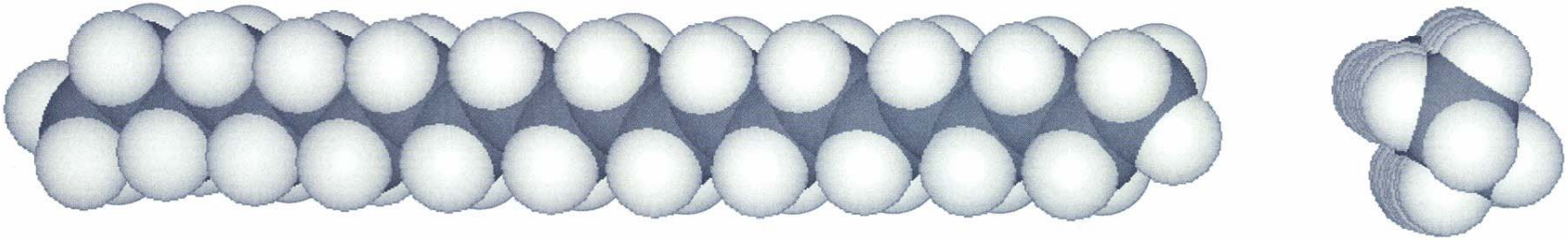
- Liquid salt consisting of at least one organic component (cation or anion)
- Room temperature ionic liquid (RTIL) if melting point is below room temperature
- Properties:
  - Negligible vapor pressure
  - High thermal stability (~250-400°C)
  - High viscosity
  - Hydrophobic or hydrophilic
  - Capable of undergoing multiple solvation interactions

Ethyl ammonium nitrate $(EtNH^{+}_{3})(NO^{-}_{3})$, which has a melting point of 12°C, was first described in 1914.

P. Walden, *Bull. Acad. Imper. Sci.* (St. Petersburg) 1800 (1914).

Size Comparison (Space Filling Model)





Effect of the nature of the anion on physicochemical properties of 1-butyl-3-methyl imidazolium salts and of the cation of the bis(triflyl)amides salts (20°C).

gegenion	m.p. °C	d g/cm³	N	Viscosity cP (20°C)	Conductivity S/m
1-butyl-3-methyl imidazolium salts					
BF_4^-	-82 (g)	1.17	1.429	233	0.17
PF_6^-	-8	1.36	1.411	312	0.14
Cl^-	65	1.10*	solid	solid	solid
CF_3COO^-	~-40 (g)	1.21	1.449	73	0.32
$CF_3SO_3^-$	16	1.29	1.438	90	0.37
$(CF_3SO_2)N^-$	-4	1.43	1.427	52	0.39
$C_3F_7COO^-$	~-40 (g)	1.33	1.414	182	0.10
$C_4F_9SO_3^-$	20	1.47	1.405	373	0.045
bis-(trifluoromethyl sulfonyl) amide salts					
1-methyl-3-methyl imidazolium	22	1.56	1.422	44	0.84
1-ethyl-3-methyl imidazolium	-3	1.52	1.423	34	0.88
1-ethyl-3-ethyl imidazolium	14	1.45	1.426	35	0.85
1-butyl-3-methyl imidazolium	-4	1.43	1.427	52	0.39
1-isobutyl-3-methyl imidazolium	~-30 (g)	1.43	1.429	83	0.26
1-butyl-3-ethyl imidazolium	~-30 (g)	-	1.428	-	-
1-methoxyethyl-3-methylimidazolium	~-30 (g)	1.50	1.429	54	0.42
1-methyl-2-methyl-3-ethylimidazolium	20	1.51	1.430	88	0.32
1-trifluoroethyl-3-methyl imidazolium	~-30 (g)	1.66	1.409	248	0.10
1-ethyl-3-ethyl-4-methyl imidazolium	-22	1.43	1.430	36	0.62
1-methyl-3-ethyl-4-methyl imidazolium	-3	1.47	1.427	37	0.66

Data from Refs 1, 12-15.

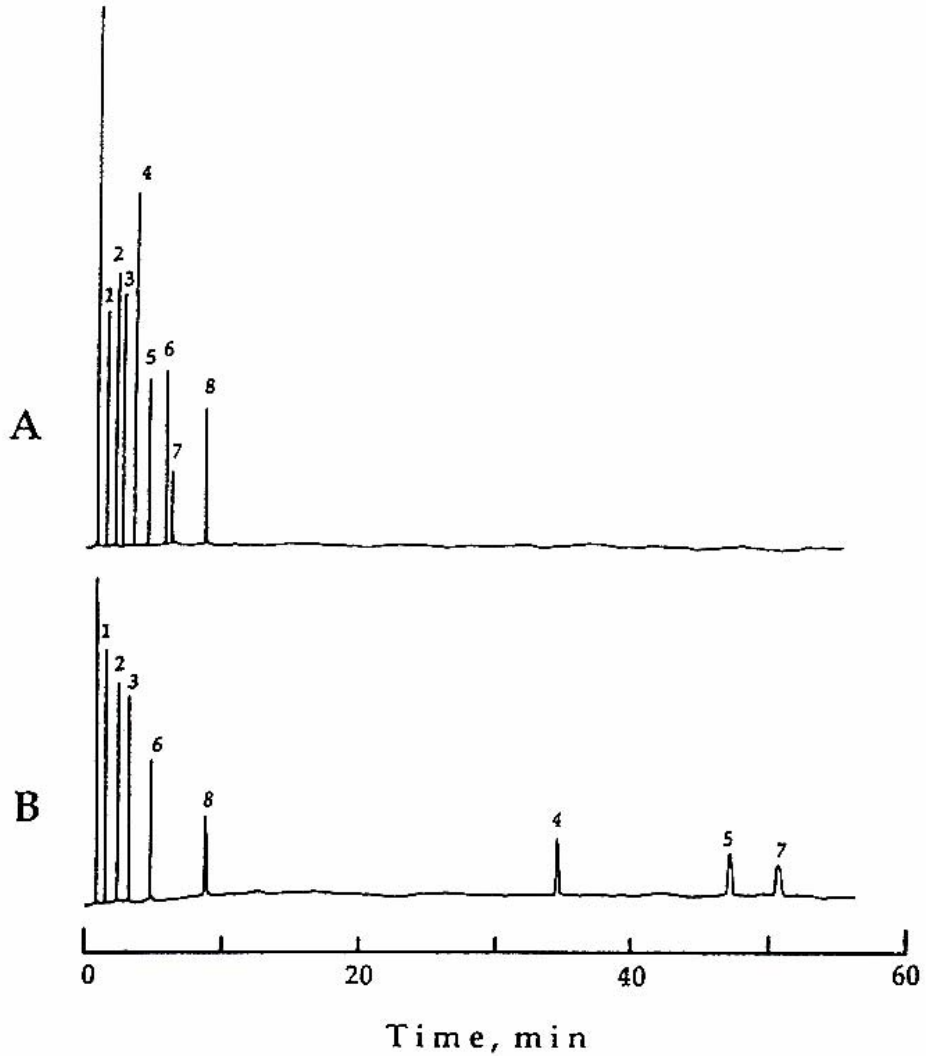
* supercooled liquid at 25°C

(g) glass transition, ~ approximate value (+/- 10°C)

Uses of RTILs

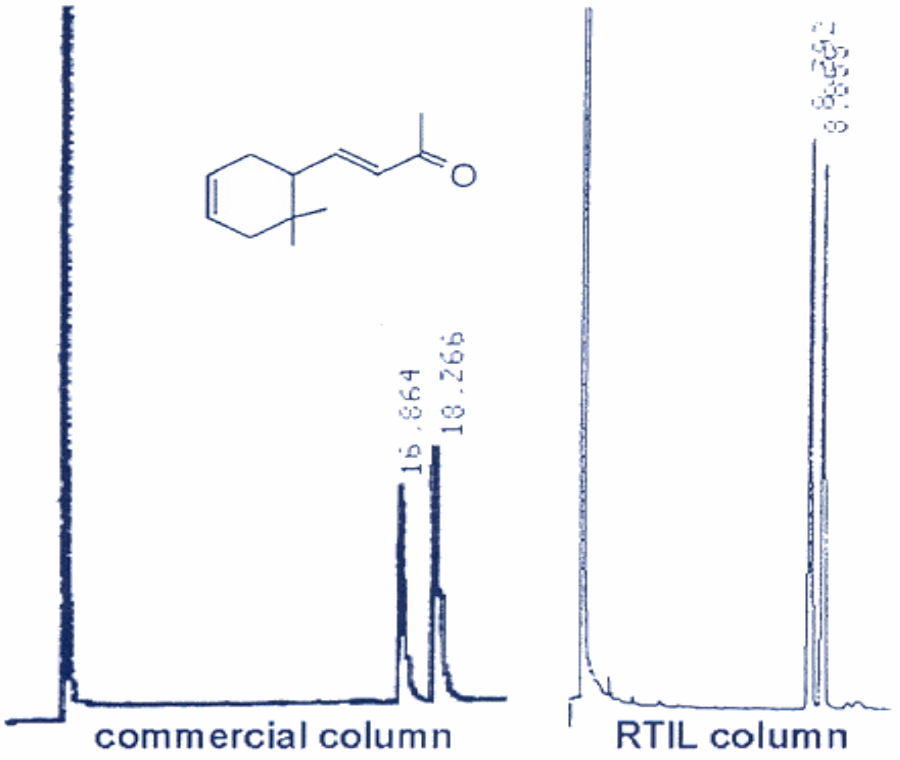
- Novel solvents in organic synthesis and liquid-liquid extraction
- Mobile phase additives in HPLC
- Run buffer additives in CE
- Matrixes in Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectrometry
- Stationary phases in gas-liquid chromatography

Anal. Chem., 71 (1999) 3873-3876.



Chromatograms comparing the retention and separation of eight compounds on the same size GC columns (15 m × 0.25-mm i.d.) and under identical conditions (isothermal @ 100 °C and a pressure of 9 psi). Column A is a commercial DB-5 column, and Column B utilizes the ionic liquid [BuMIm][PF_6] as the stationary phase. The test compounds are: 1, butyl acetate; 2, *n*-heptanol, 3, *p*-dichlorobenzene, 4, *o*-cresol, 5, 2,5-dimethylphenol, 6, *n*-dodecane, 7, 4-chloroaniline, and 8, *n*-tridecane.

Chromatographia, 53 (2001) 63-68.



Chromatogram of α-ionone on the commercial B-PM column (left) and the RTIL containing BPM column (right). Experimental conditions: 100 °C isotherm, helium carrier gas, 0.07 MPa (10 psi) inlet pressure, gas average velocity = 44 cm.s^{-1}, FID detection. Commercial column: Chiraldex B-PM, 20 m, 250 µm i. d., 0.25 µm film thickness, dead time 46 s; RTIL column: [BuMI][Cl] with 26.4% BPM CD, 10 m, 250 µm i. d., 0.25 µm film thickness, dead time 23 s.

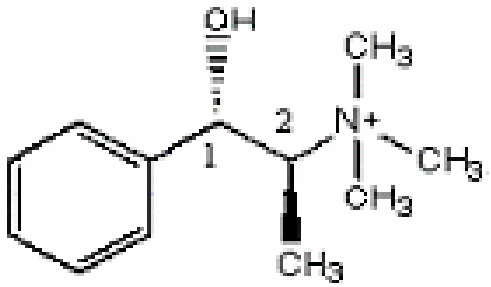
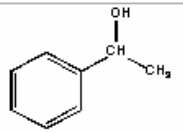
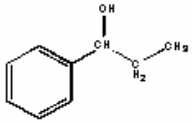
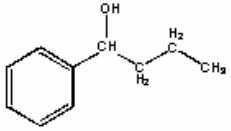
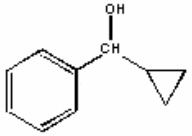
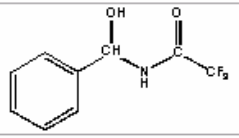
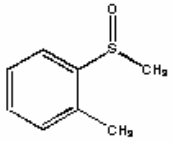
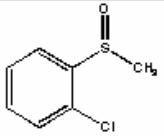
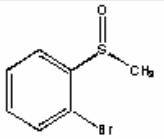
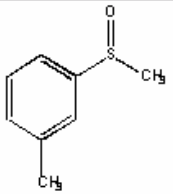
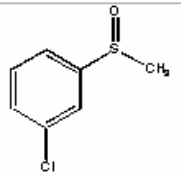
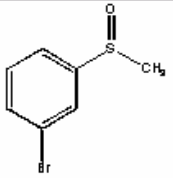
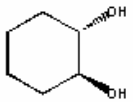
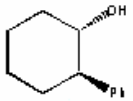


Figure 2. Structure of (1R,2S)-(-)-N,N-dimethylephedrinium ions

Table 1 Separation of 14 compounds on (1*S*, 2*R*)-(+)-*N*, *N*-dimethylephedrinium-bis(trifluoromethanesulfon)imidate column* * Column length: 8 meters, flow rate: 1 ml/min

#	Compound	Structure	T(°C)	k_1	α
1	*sec*-Phenethyl alcohol		120	7.64	1.11
2	1-Phenyl-1-propanol		120	10.1	1.11
3	1-Phenyl-1-butanol		120	15.3	1.07
4	α-Cyclopropylbenzyl alcohol		100	37.4	1.03
5	α-Phenylethylamine (TFA derivative)		100	84.1	1.02
6	β-Pinene Oxide		100	12.3	1.03
7	*m*-Methylphenylmethyl sulfoxide		140	73.3	1.03
8	*m*-Chlorophenylmethyl sulfoxide		140	35.4	1.02
9	*m*-Bromophenylmethyl sulfoxide		140	59.2	1.02
10	*p*-Methylphenylmethyl sulfoxide		120	241	1.01
11	*p*-Chlorophenylmethyl sulfoxide		120	196	1.01
12	*p*-Bromophenylmethyl sulfoxide		120	374	1.01
13	*trans*-1,2-Cyclohexandiol		120	21.4	1.10
14	*trans*-2-Phenyl-1-cyclohexanol		100	100	1.02

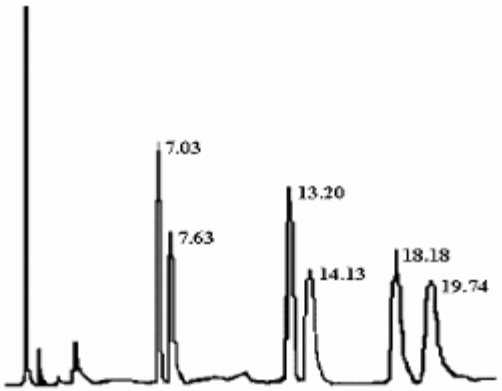
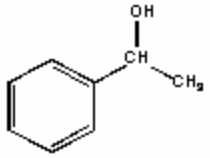
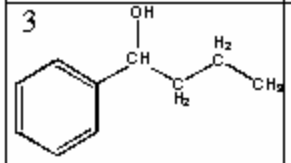
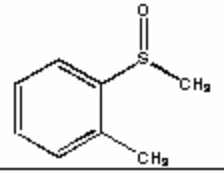
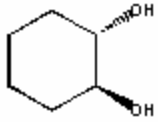
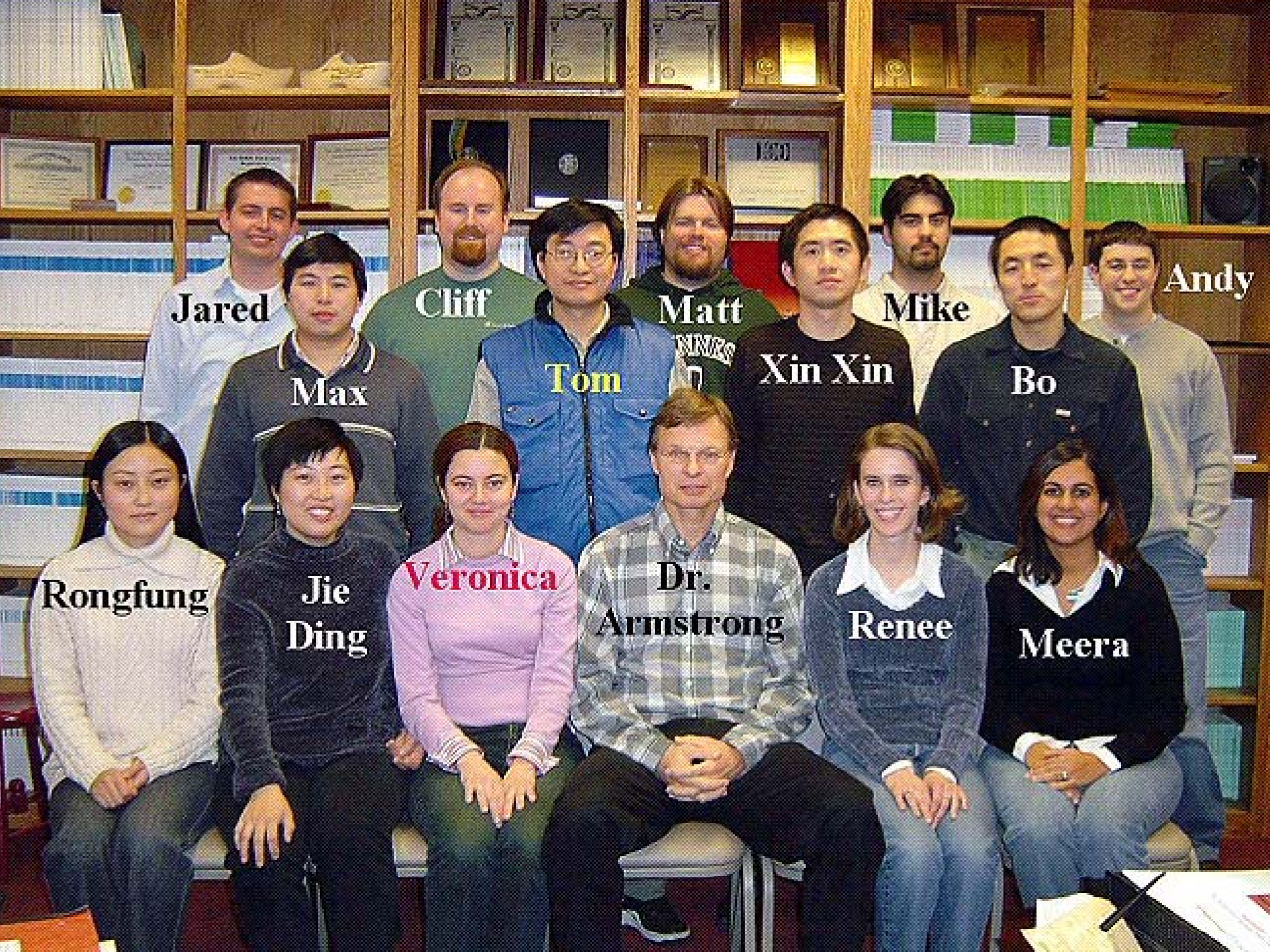


Figure 1. GC chromatogram showing the enantiomeric separation of (from left to right) sec-phenylethyl alcohol, 1-phenyl-1-butanol and trans-1,2-cycloheanediol. Chromatographic conditions: column = 8m long x 250 μm(i.d.) fused silica capillary coated with (1S,2R)-(+)-N,N-dimethylephedrinium triflate. Temp = 120°C, He flow rate = 1.0 ml/min, split ratio = 100:1, FID.

Table 2 Elution order for selected compounds on different chiral ionic liquid stationary phases

	(1*S*,2*R*)-(+)-*N*,*N*-dimethylephedrinium-bis(trifluoromethanesulfon)imidate	(1*R*,2*S*)-(-)-*N*,*N*-dimethylephedrinium-bis(trifluoromethanesulfon)imidate	(1*S*,2*S*)-(+)-*N*,*N*-dimethylephedrinium-bis(trifluoromethanesulfon)imidate
1 	(S), (R)	(R), (S)	Not separated
3 	(S), (R)	(R), (S)	Not separated
7 	(R), (S)	(S), (R)	(R), (S)
13 	(1R, 2S), (1S,2R)	(1S, 2R), (1R, 2S)	Not separated


Jared
Cliff
Matt
Mike
Andy
Max
Tom
Xin Xin
Bo
Rongfung
Jie Ding
Veronica
Dr. Armstrong
Renee
Meera