Macrocyclic Chiral Stationary Phases

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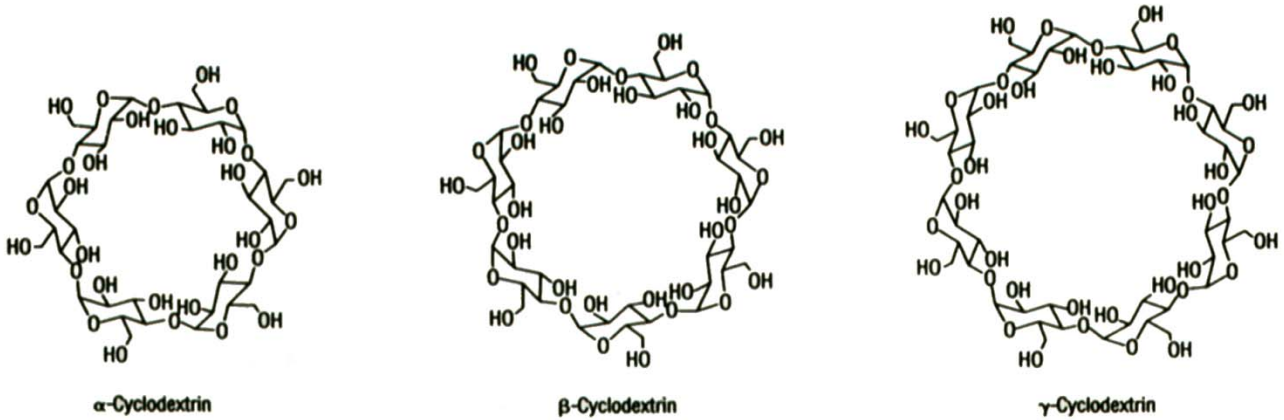
Macrocycles in Separations Today

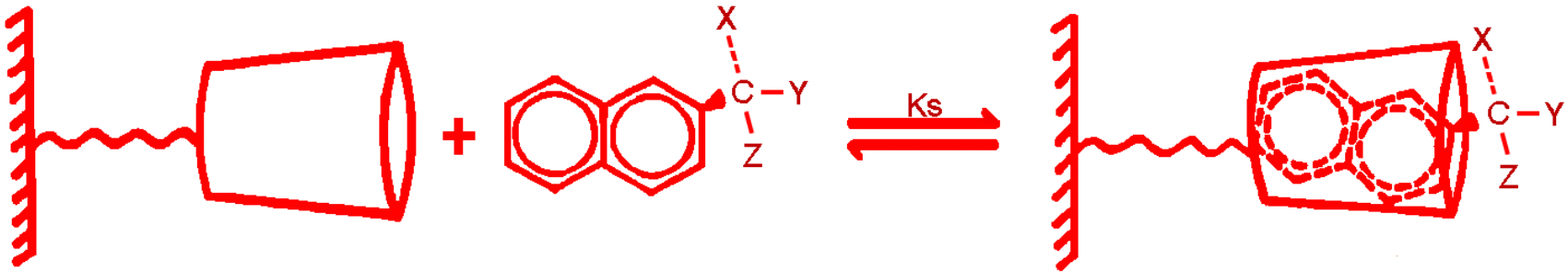
1. >95% of all GC chiral separations are done on cyclodextrin derivative stationary phases.
2. >90% of all CE chiral separations are done with cyclodextrin-based chiral selectors.
3. CDs are the dominant water-based NMR chiral shift reagents.
4. However, today CDs are used for only about 5-10% of LC chiral separations.
5. Conversely, macrocyclic glycopeptides have the broadest selectivity and have superseded all other CSPs for the separation of chiral amino acids and many other compounds. They are the dominant macrocycle used for chiral LC separations.
6. Cyclofructans are the newest and least developed chiral macrocycle. They will have huge future impact.

D.W. Armstrong, Journal of Chromatographic Science, Vol. 22, September, 1984, pg. 412.



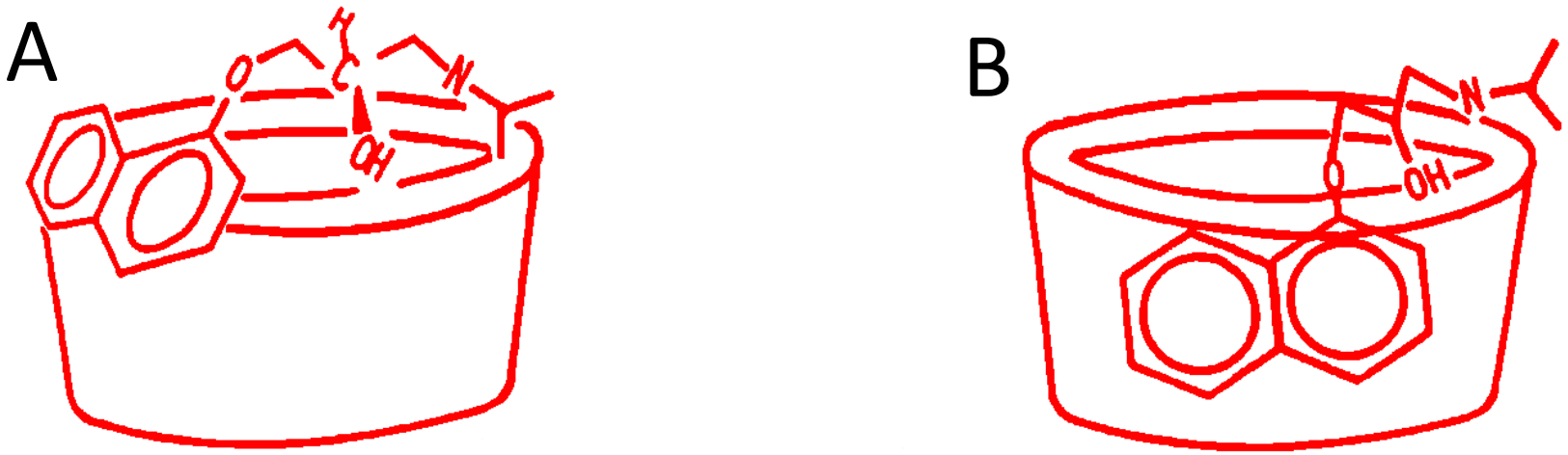
Structural diagrams of α-, β-, and γ-cyclodextrin (55–61).

Schematic showing the structure and relative size of the three most common cyclodextrin molecules. (A) τ-cyclodextrin (or cyclooctaamylose). (B) β-cyclodextrin (or cycloheptaamylose), and (C) α-cyclodextrin (or cyclohexaamylose).



A schematic of cyclodextrin bonded to a silica gel support and reversibly forming an inclusion complex with a chiral molecule. Neither the linkage nor the cyclodextrin contain nitrogen (e.g., amines or amides) in any form.

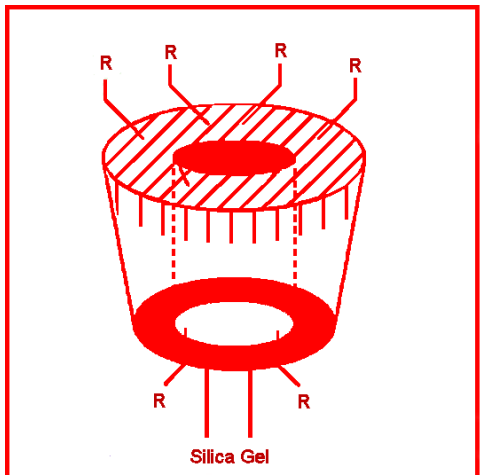
Two different chiral recognition mechanisms for Beta-Cyclodextrin

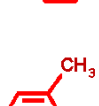


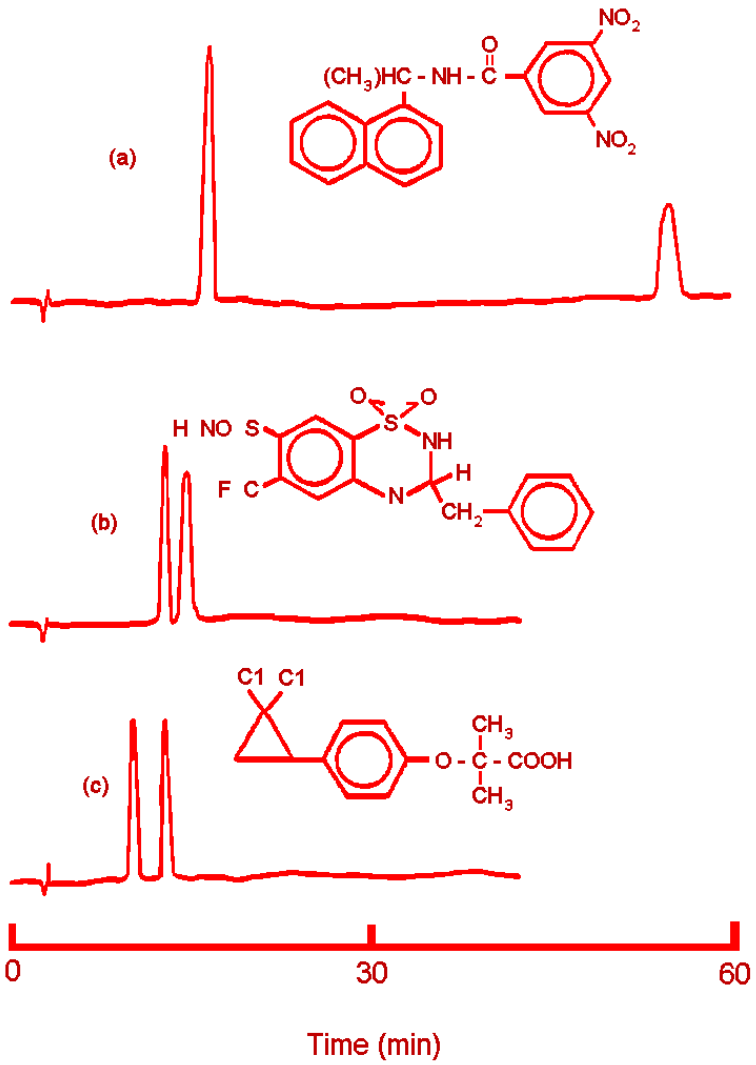
Simplified schematics illustrating two different enantioselective retention mechanisms for the native β-cyclodextrin/propranolol system. Case "A" is the polar organic mode where acetonitrile occupies the hydrophobic cavity and the analyte is retained via a combination of hydrogen bonding and dipolar interactions at the mouth of the cyclodextrin. Steric interactions also can contribute to chiral recognition. In case "B", (the reversed phase mode) retention is mainly due to hydrophobic inclusion complexation, while enantioselectivity also requires hydrogen bonding and steric interactions at the mouth of the cyclodextrin cavity.

Summary of Derivatives of CYCLOBOND 1 2000

Bonded Derivatized Cyclodextrins



R =	CYCLOBOND I 2000 SUFFIX
$-COCH_3$	AC** (acetylated)
$-CH_2\underset{*}{C}H(OH)CH_3$	SP or RSP (hydroxypropyl ether)
$-CONH\underset{*}{C}H(CH_3)$ 	RN or SN (naphthylethyl carbamate)
$-CONH$	DMP (3,5-dimethylphenyl carbamate)



The separation of three racemates using an (S)-NEC-β-cyclodextrin column, including the (a) normal phase separation of the 3,5-dinitrobenzoyl derivative of racemic 1-(1naphthyl)ethylamine, (b) reversed phase separation of racemic bendroflumethiazide, and (c) separation of the enantiomers of ciprofibrate. Column dimensions: 25 cm x 4.6 mm; mobile phase (a): 70:30 (v/v) hexane-isopropyl alcohol; (b): 30:70 (v/v) acetonitrile 1 vol % triethylammonium acetate in water; (c): 80:20:1 (v/v/v) acetonitrile – ethanol-acetic acid. Flow rate: 1.0 mL/min.

Remember:

The separation of enantiomers of hydrophobic molecules with little functionality is often best done with Cyclodextrin-based stationary phases

J. Chromatogr. A 666 (1994) 445-448

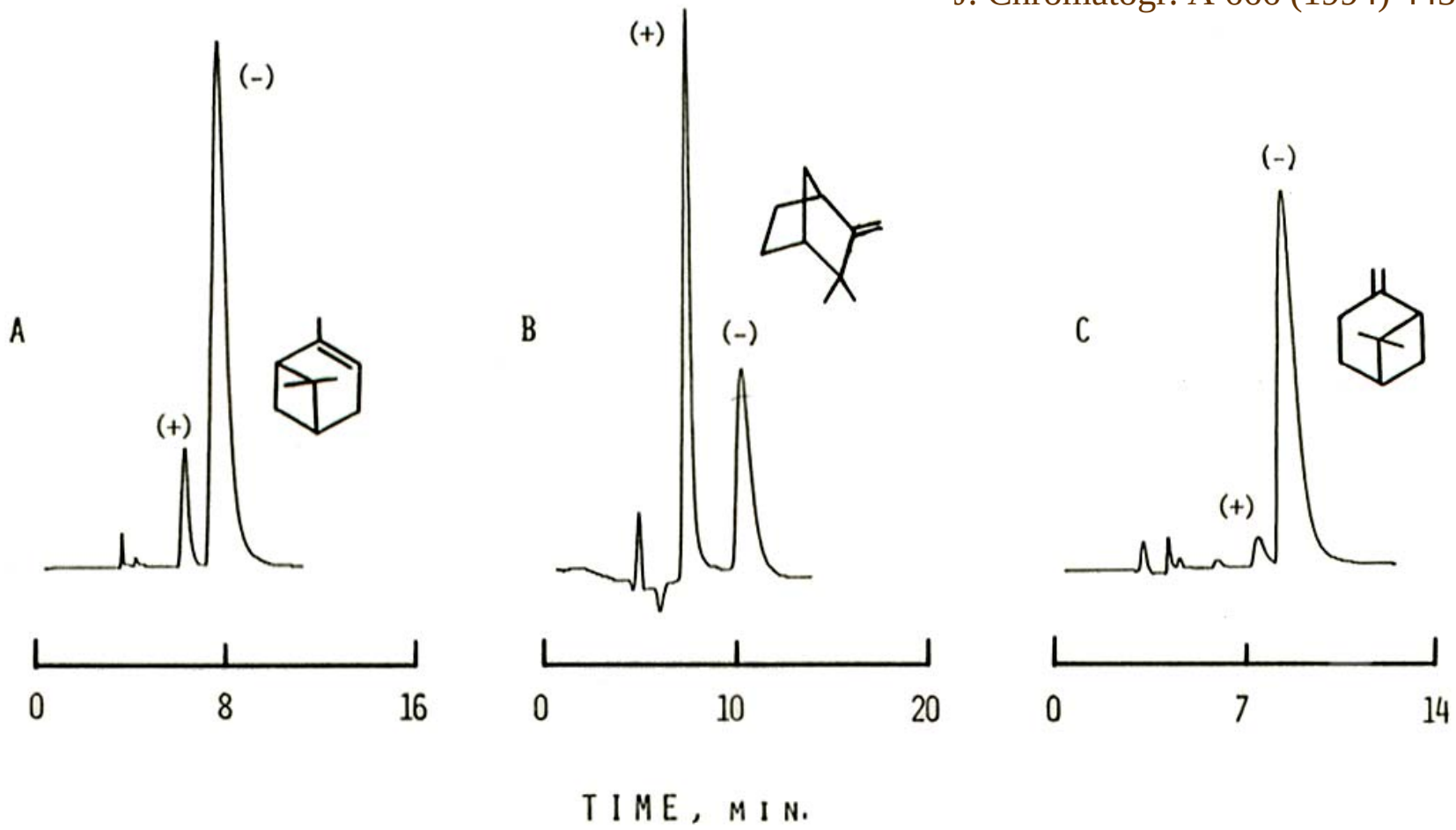
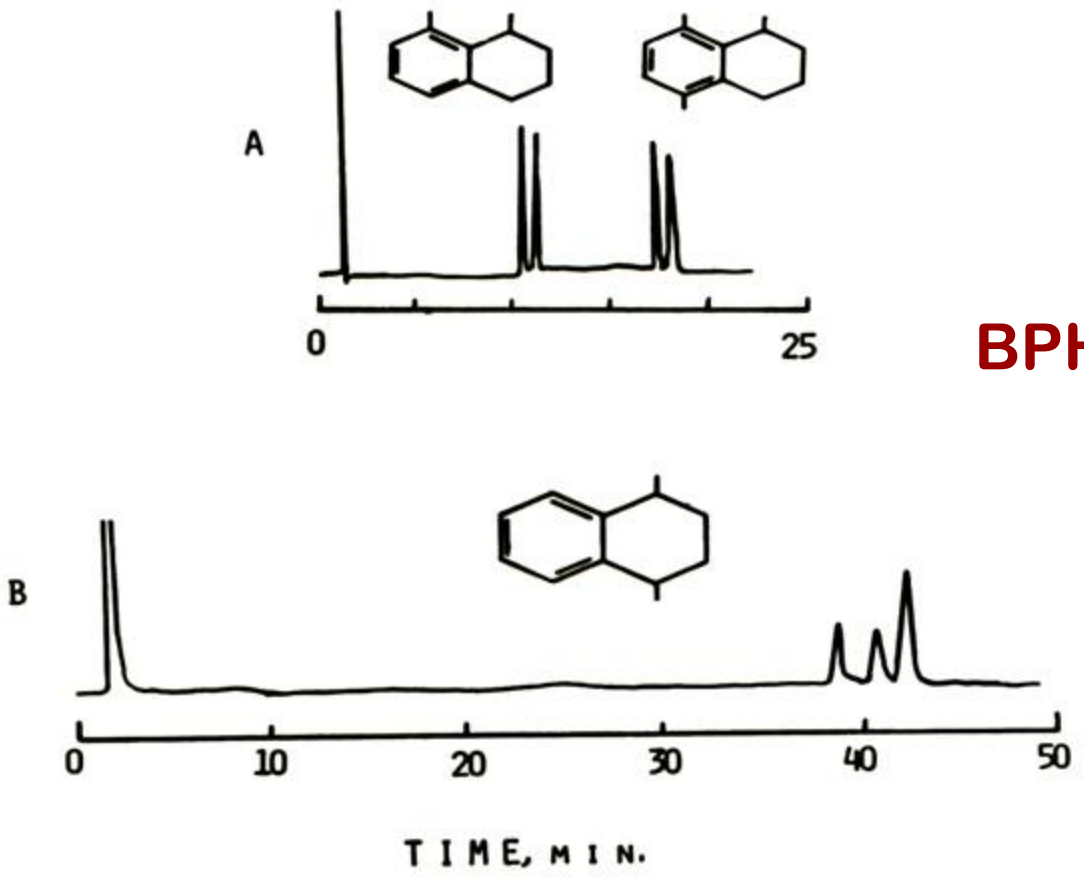
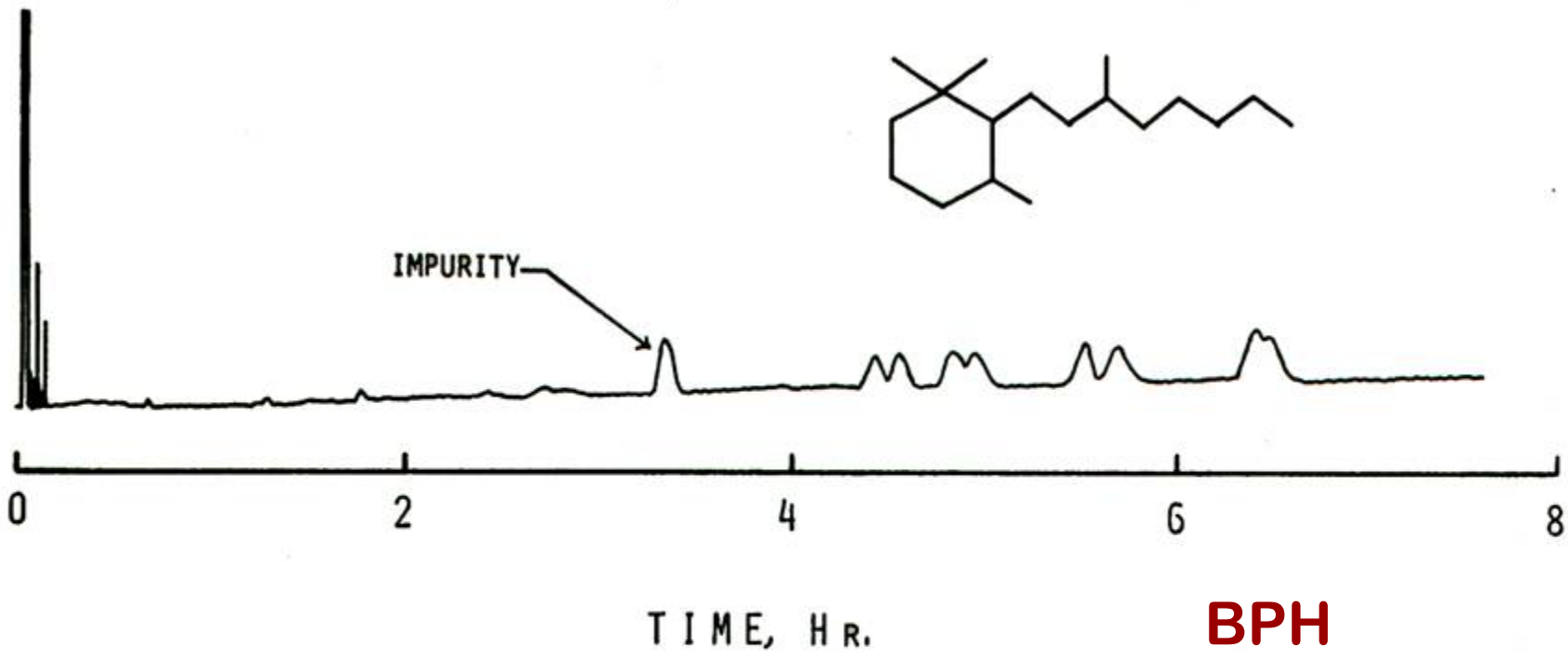


Figure 3. Representative chromatograms showing the retention order and enantiomeric purity of commercial samples of (A) (-)-α-pinene (B) (+)-camphene and (C) (-)-β-pinene. Experimental conditions were the same as those indicated for Figure 2D.



BPH

Chromatogram A shows the resolution of racemic 1,8-dimethyltetralin and 1,5,8-trimethyltetralin. Chromatogram B shows the separation of the three steroisomers (one pair of enantiomers) of 1,4-dimethyltetralin. Both separations were done on a 10 M B-PH capillary GC column at 110°C for A and 80°C for B.



BPH

Gas chromatographic separation for the stereoisomers of 1,1,3-trimethyl-2(3-methyloctyl)cyclohexane on a 20M β-PH column, Temp. = 70°C. The split ratio was 100 to 1.

Also, remember:

It is not widely appreciated that Cyclodextrin-based stationary phases are unsurpassed at separating structural isomers, geometrical isomers and organic sulfonates and sulfates.

They also are the most orthogonal reversed phase stationary phases to C18/C8 types.

Macrocyclic Glycopeptides: CHIROBIOTIC COLUMNS

Anal. Chem., 68, 2501 (1996)

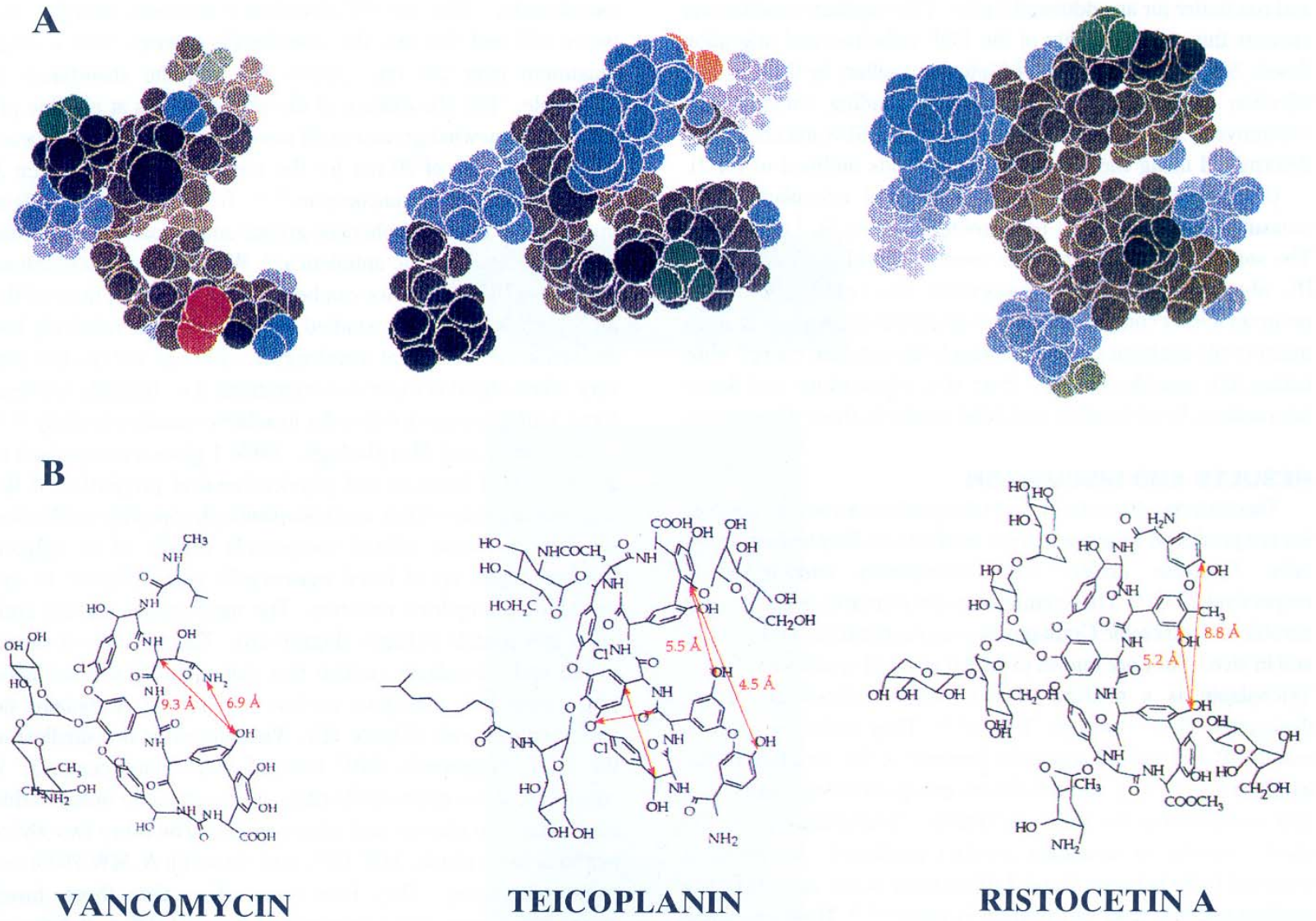


Figure 1. Structures of the macrocyclic antibiotics vancomycin, teicoplanin, and ristocetin A showing a profile view of the aglycon "basket" using (A) space-filling molecular models produced through energy minimization and (B) stick figures. The colored atoms in part A denote the hydrophilic moieties, 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.

Anal. Chem. 66 (1994) 1473

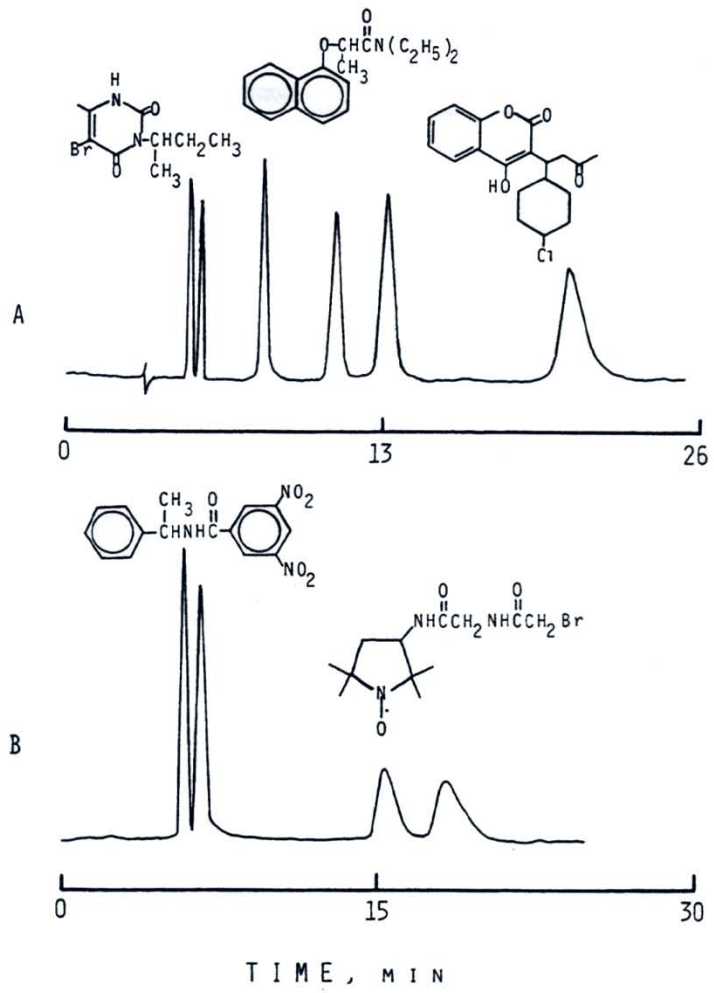


Figure 2. (A) Reversed-phase enantiomeric separation of (from left to right) bromocil, devrinol, and coumachlor. The column was a 25 cm × 0.44 cm (i.d.) vancomycin CSP (5-μm silica gel). The mobile phase consisted of 10:90 acetonitrile–1% pH 7 triethylammonium acetate buffer (by volume). The flow rate was 1.0 mL/min at ambient temperature (22 °C). UV absorbance detection (254 nm) was used. (B) Normal-phase enantiomeric separation of (from left to right) *N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine and 3-[2-(2-bromoacetamido)-acetamido]-PROXYL on the same column as in (A). The mobile phase consisted of 50:50 2-propanol–hexane (by volume). The flow rate was 1.0 mL/min at ambient temperature (22 °C). UV absorbance detection (254 nm) was used.

J. Chromatogr. A 731 (1996) 123-137

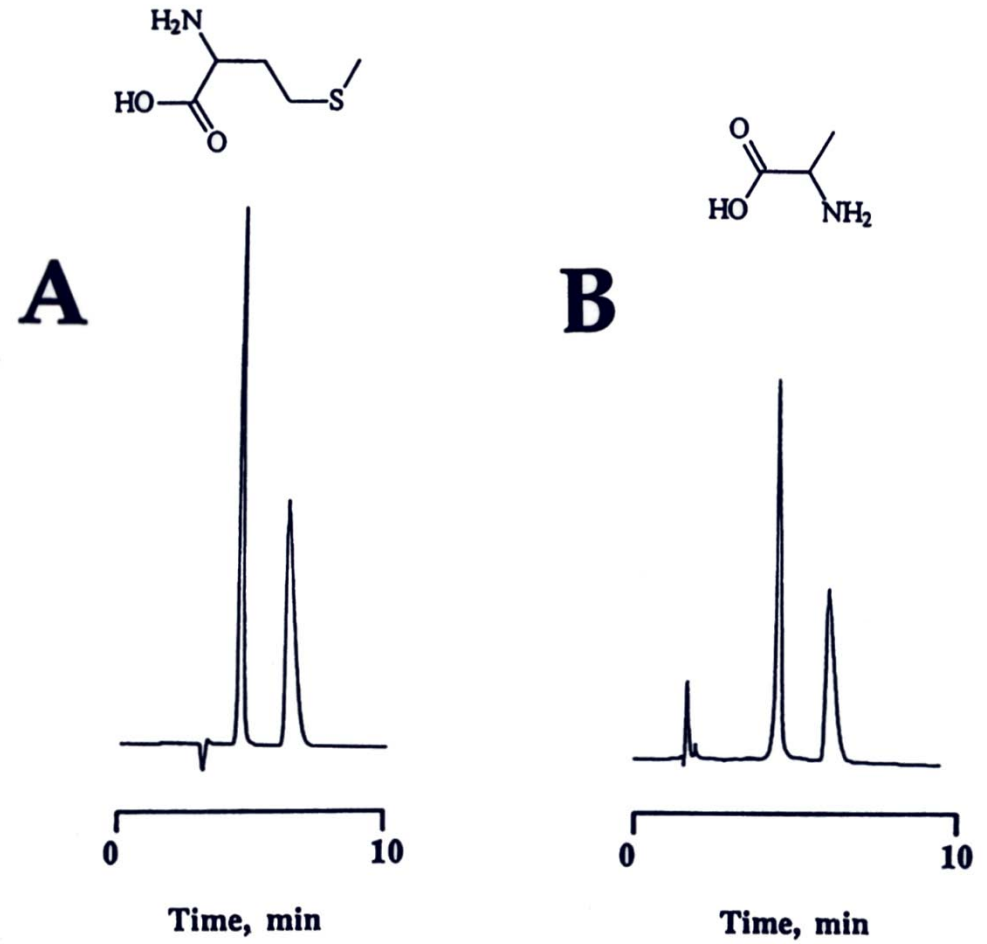


Fig. 2. Reversed-phase enantiomeric separations of naturally occurring amino acids. (A) methionine; (B) alanine. Mobile phase: methanol–water (60–40, v/v); flow-rate, 1 ml/min. Column: 25 cm × 0.46 cm I.D., Chirobiotic T (5-μm silica particle bonded with teicoplanin). Injection volume: 20 μl; injection mass: ~20 μg; UV detection at 215 nm.

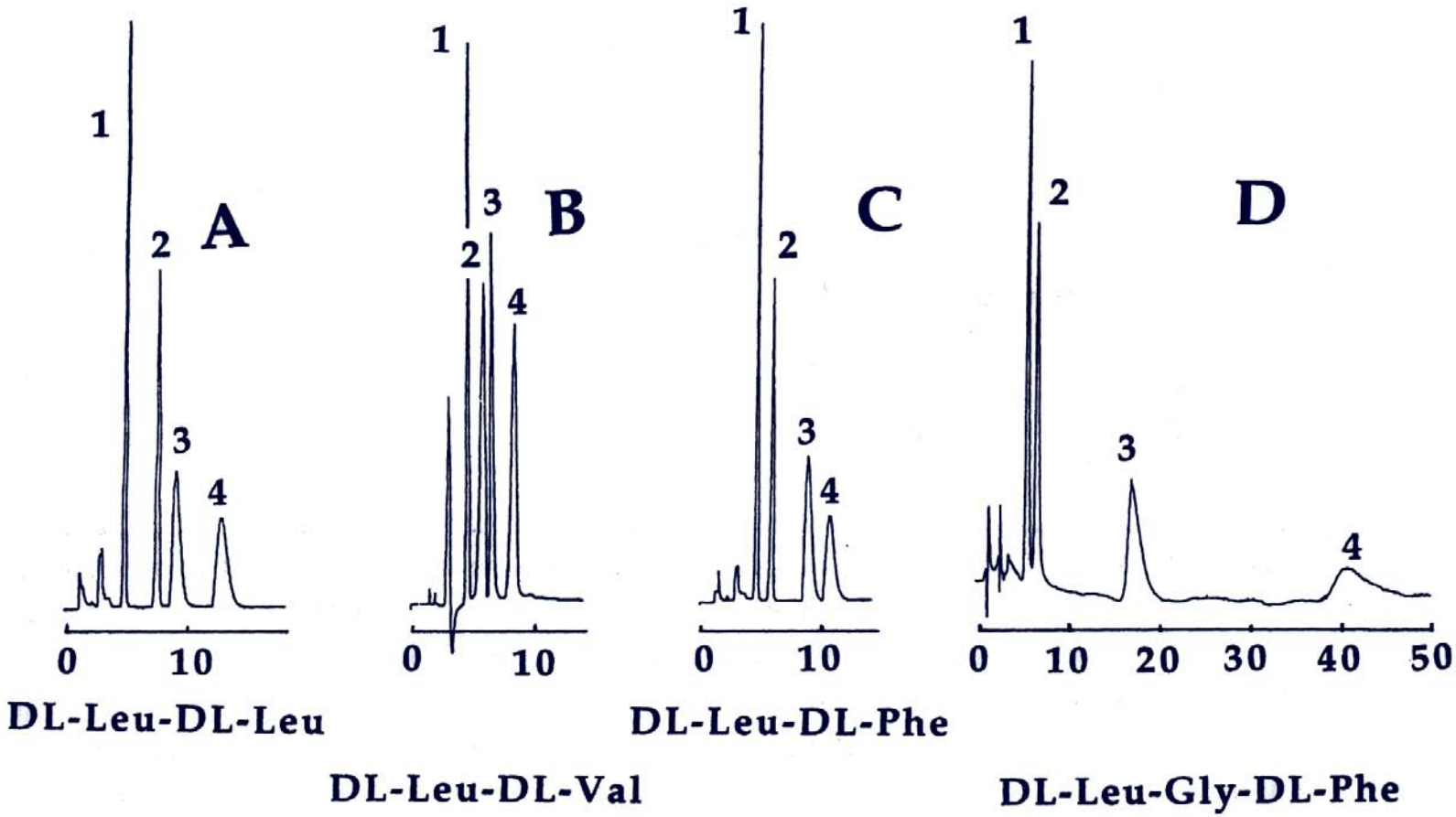
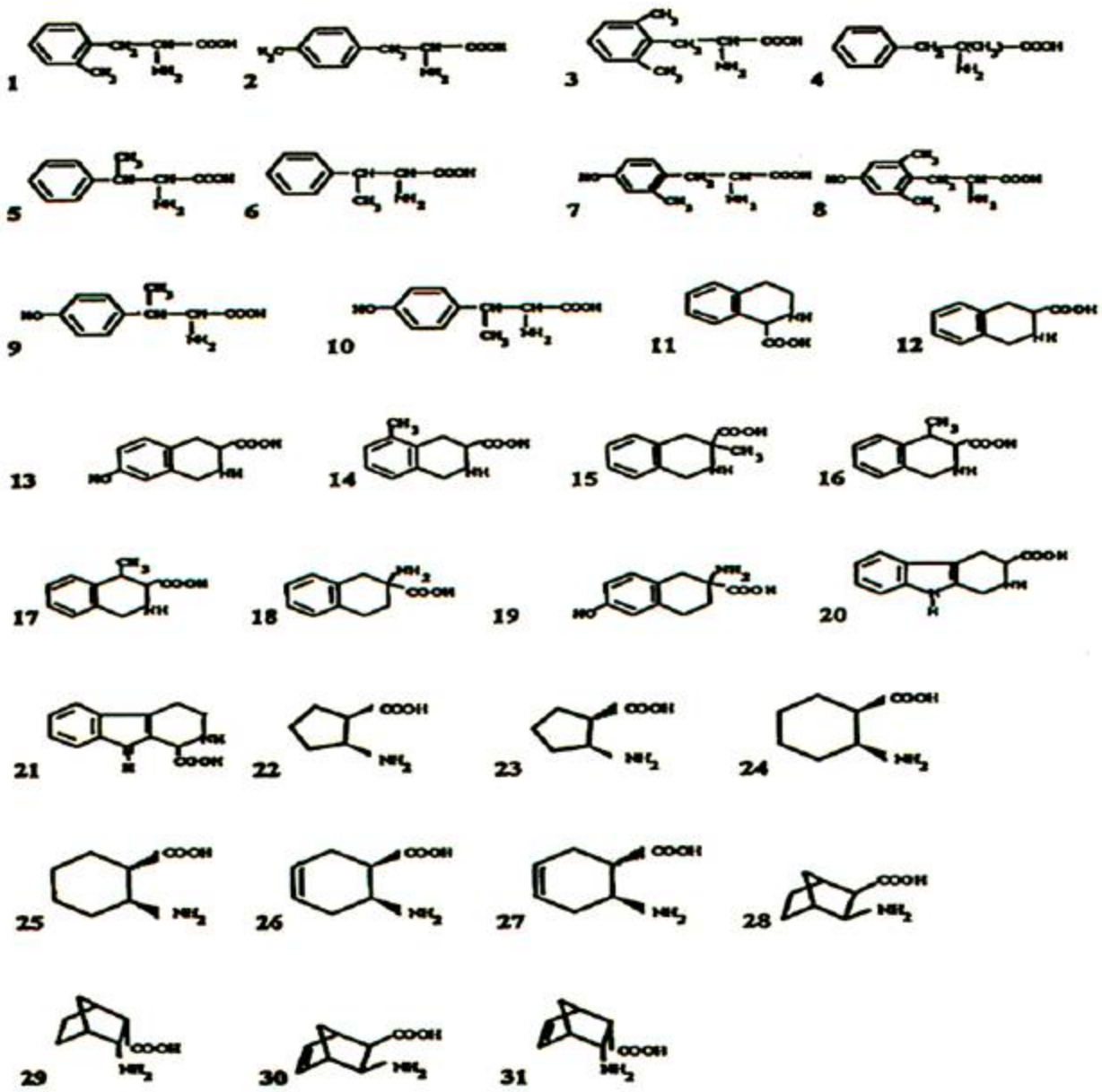


Fig. 7. Dipeptide and tripeptide separations. Operating conditions: see Fig. 6 legend.



J. Chromatogr. A 793 (1998) 283-296

Principle of Complementary Separations

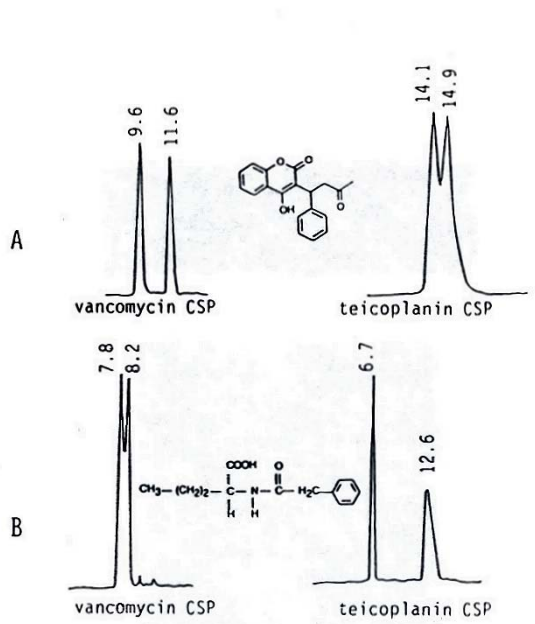


Figure 3. Chromatograms illustrating that the "principle of complementary separations" for the macrocyclic antibiotic CSPs applies to a variety of different compounds. Part "A" shows the separation of racemic warfarin on a vancomycin vs a teicoplanin CSP. In both cases the mobile phases consisted of 10:90, acetonitrile: 1% triethylammonium acetate buffer, pH 4.1; and a flow rate of 1.0 ml/min. Part "B" shows the separation of racemic N-CBZ-norvaline. In both cases the mobile phase consisted of 20:80, methanol: triethylammonium acetate buffer, pH 4.1; and a flow rate of 1.0 ml/min.

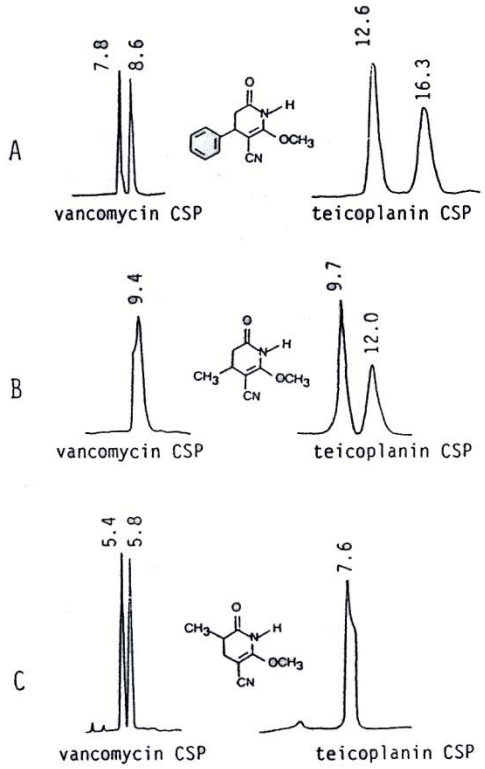
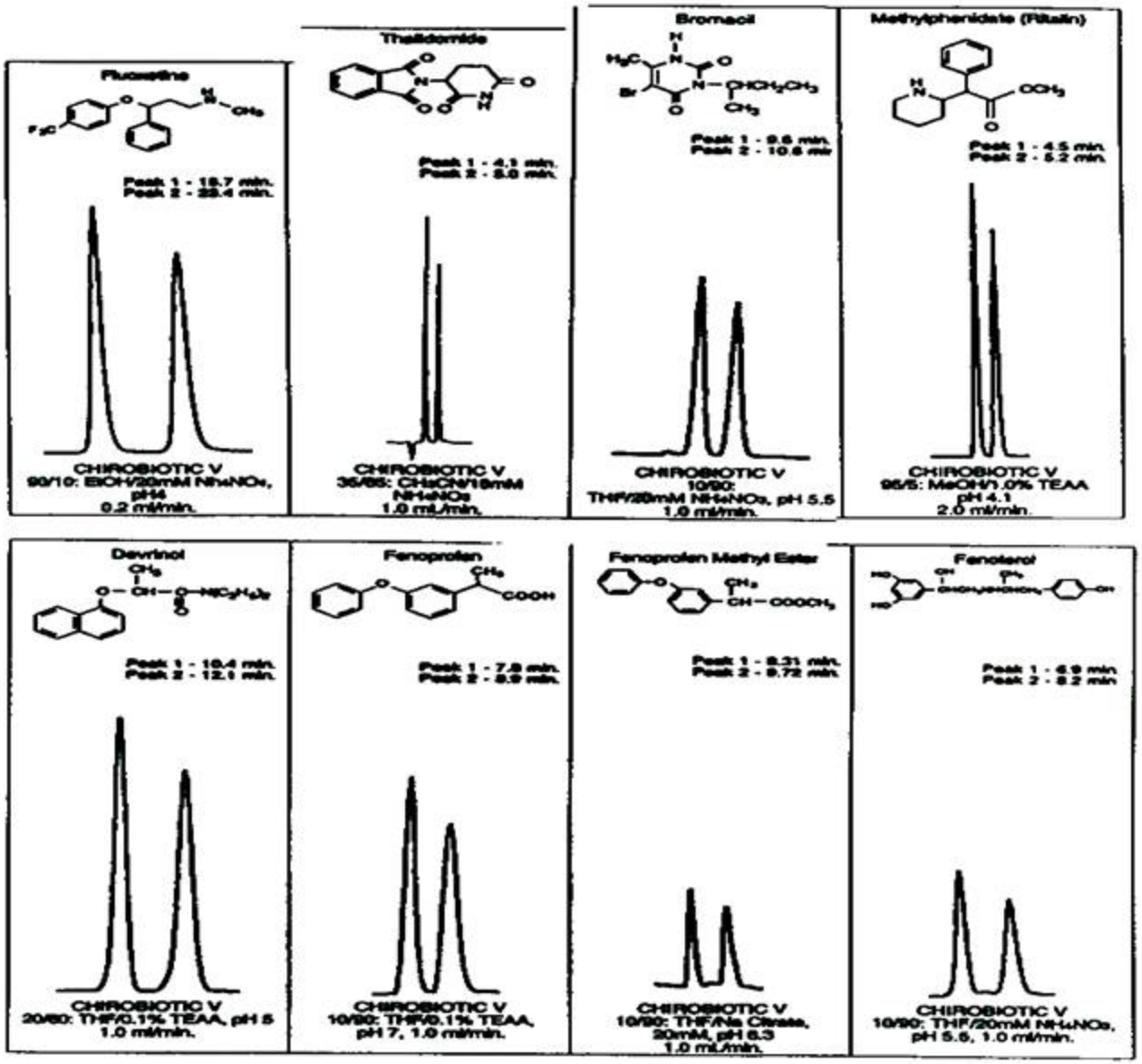
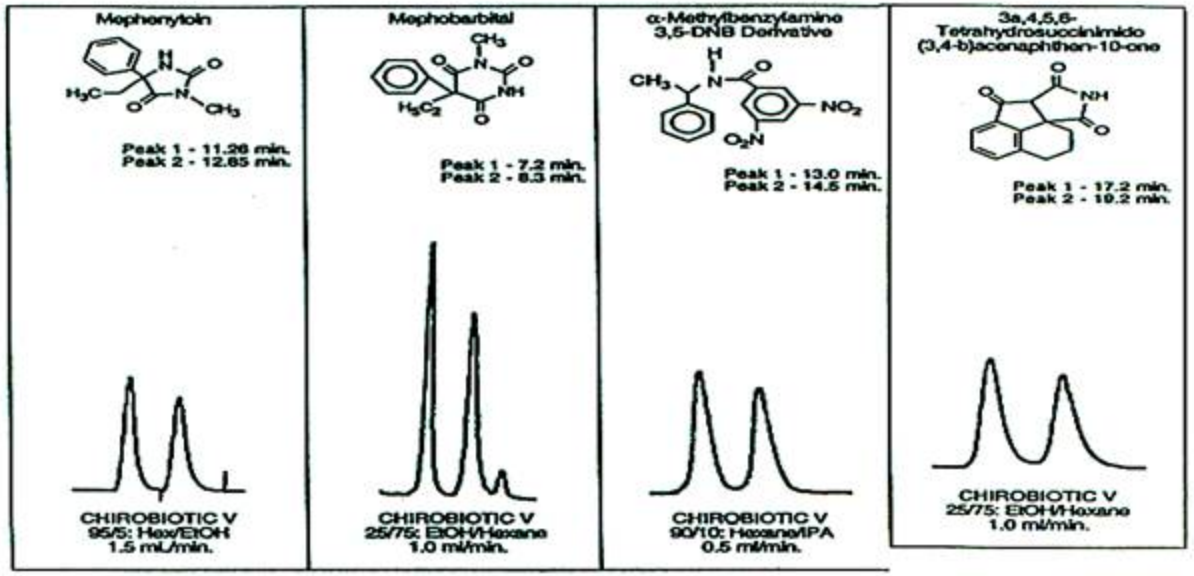


Figure 2. Enantiomeric separations of three racemic substituted pyridones that illustrate the complementary enantioselectivities of the two related antibiotic chiral stationary phases. All three racemates (A, B and C) were separated under identical conditions (i.e., mobile phase = 10:90, methanol: 1% triethylammonium acetate buffer, pH 4.1 at a flow rate of 1.0 ml/min). In each case the columns consisted of either a 25 cm x 0.44 cm (i.d.) vancomycin or teicoplanin bonded phase material (5 μ silica support).

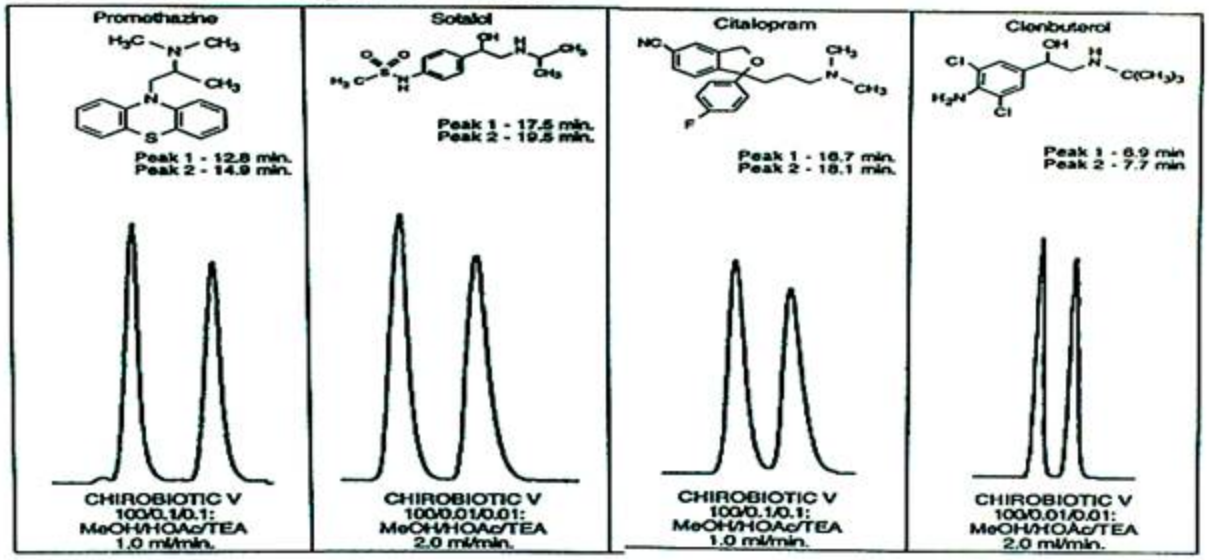
Reversed Phase Mode



Normal Phase Mode



New Polar Organic Mode



Polar Organic Mode (For Cyclodextrins)

Composition: ACN + MeOH + HOAc + TEA
Dominant interactions: Hydrogen bonding, dipole-dipole interaction

New Polar Organic Mode (For Macrocyclic Glycopeptides)

Composition: MeOH + HOAc + TEA
Dominant interactions: Ionic interaction, hydrogen bonding

Remember:

- Enantiomeric separations done in the Polar Organic Mode are most amenable to LC-MS formats.

- Most reversed phase separations can be altered for LC-MS as well.

Simultaneous separation of all 19 amino acids

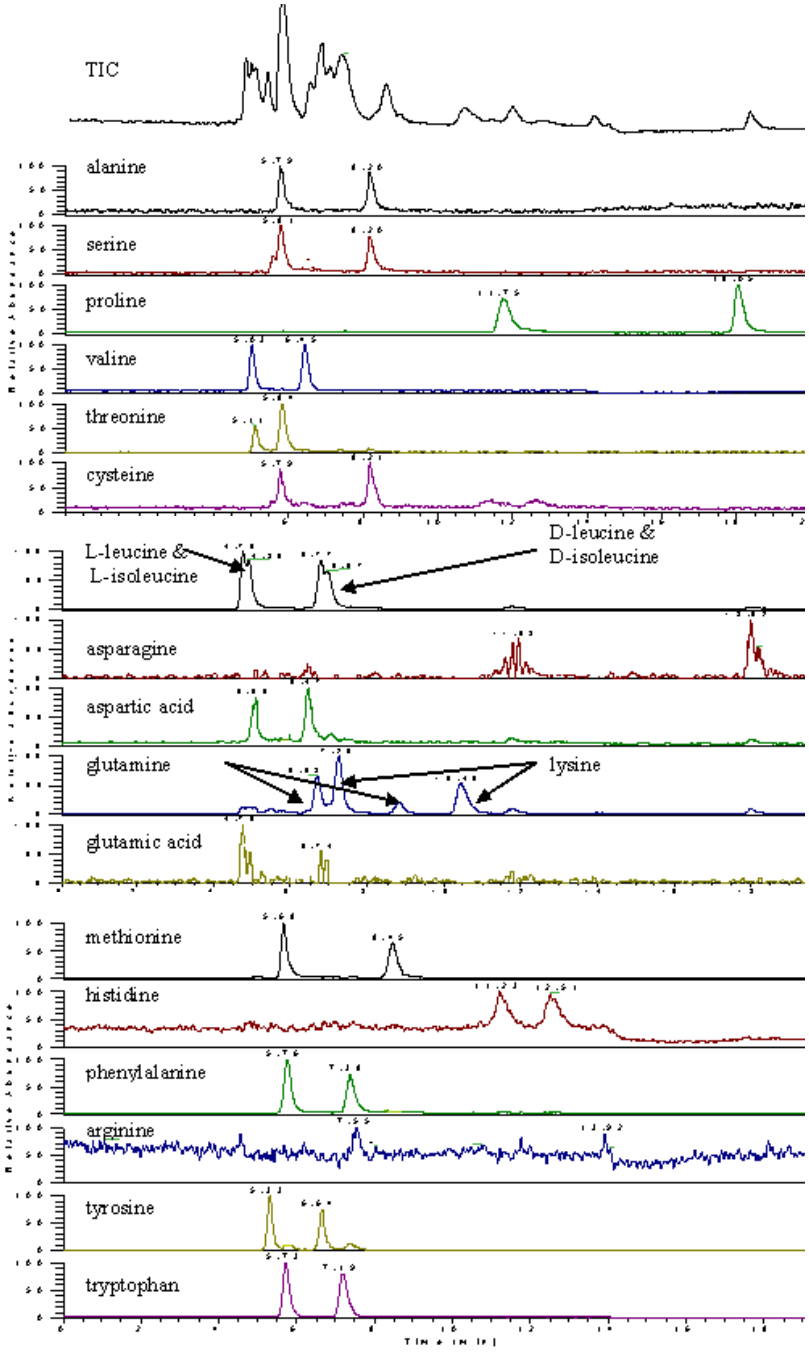
Step gradient: 80:20 A:B 0 – 7 min then 20:80 A:B 7- 20 min

A: 1.0% NH_4TFA in MeOH

B: 0.1 % formic acid in H_2O

Chirobiotic T LC-APCI-MS

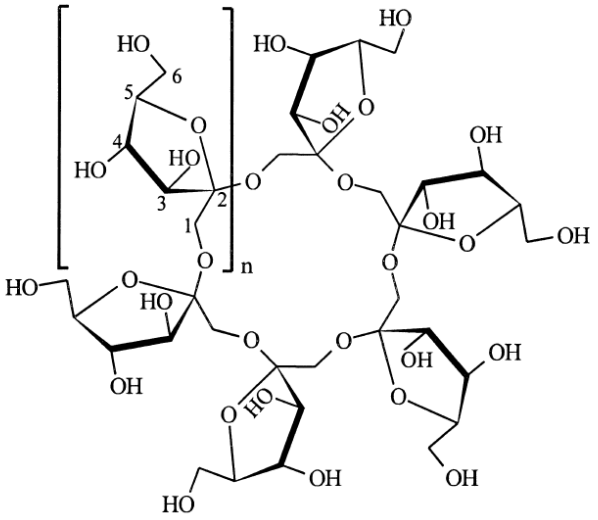
Full Scan Mode m/z range : 87.0-207.0

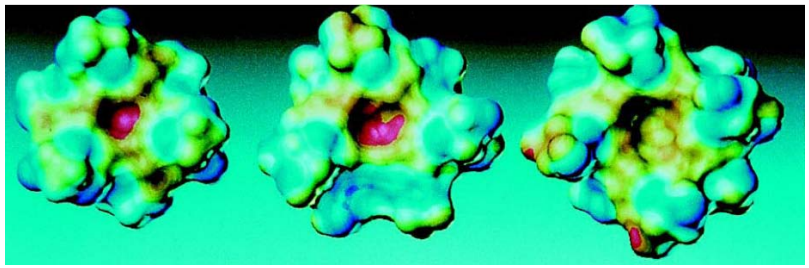


Desai MJ and Armstrong DW. (2004) *J. Mass Spectrom.* 36, 177-187.

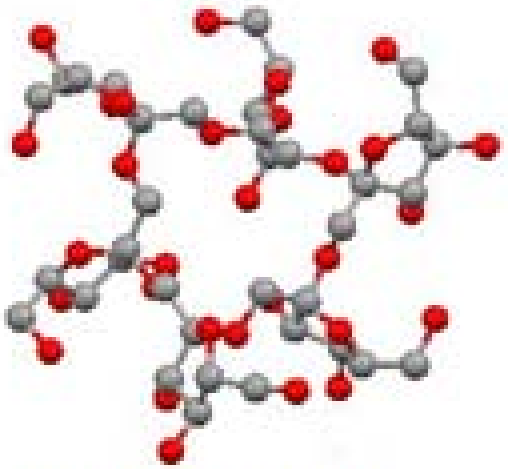
Cyclofructans (CFs)

- Cyclic oligosaccharide
- Fructosyltransferase, inulin
- Beta-(2-1) linked D-fructofuranose
- 6,7,8 fructofuranose units
- Crown ether skeleton
- UV transparent
- Solubility: >1.2g/ml
- No health hazardous

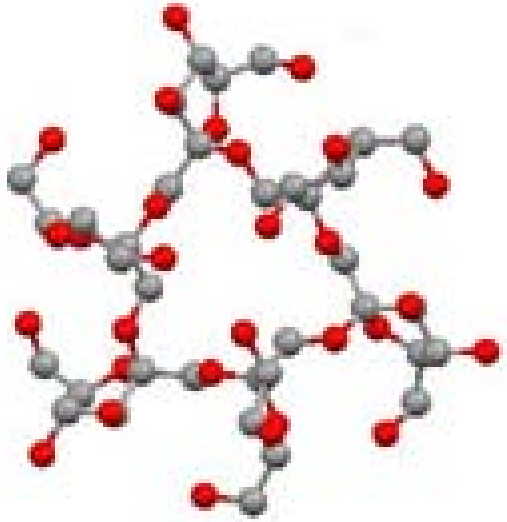




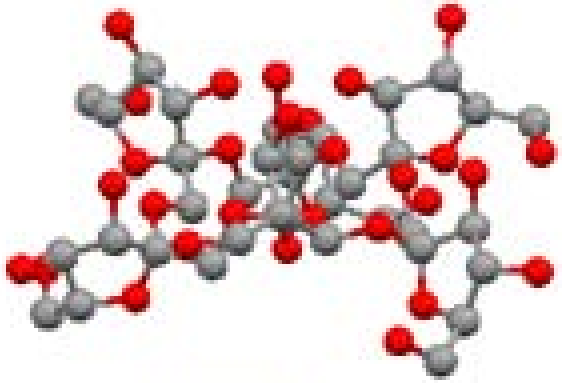
Cyclofructan Crystal Structure



Hydrophobic Face

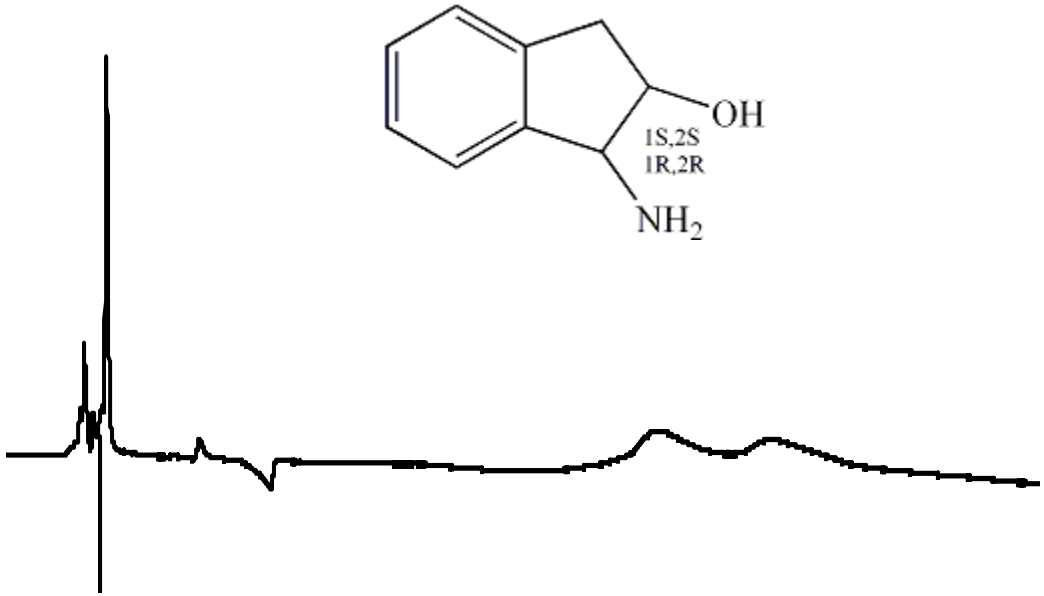


Hydrophilic Face



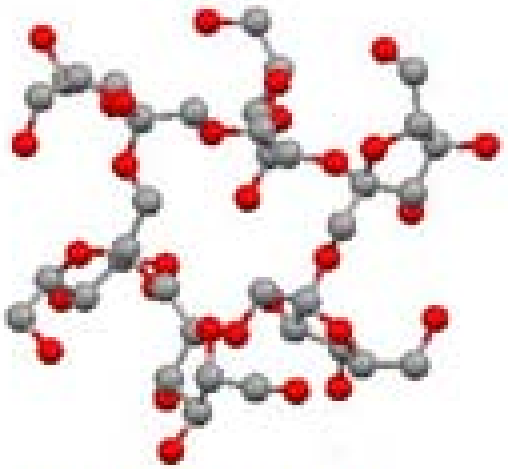
Side View

Native CF6

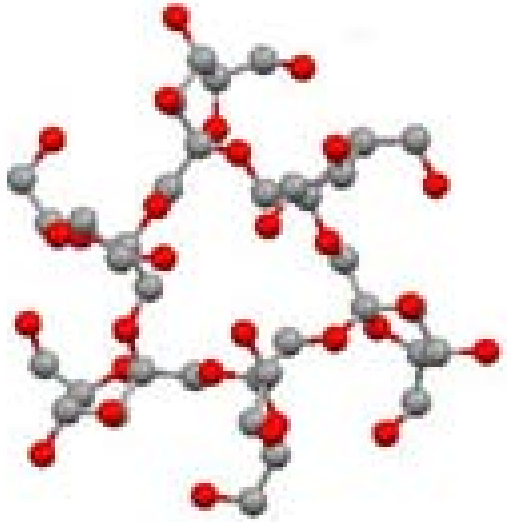


Native CF6
Acetonitrile/methanol/AA/TEA
90/10/0.3/0.2

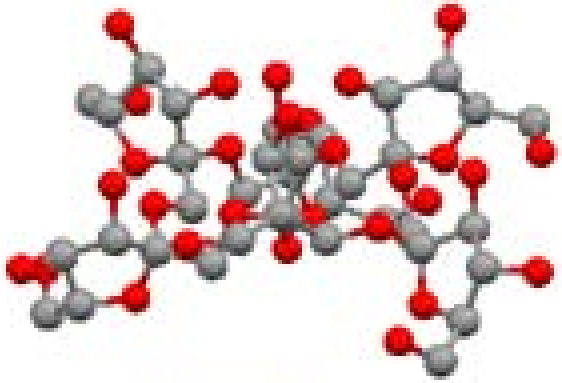
Cyclofructan Crystal Structure



Hydrophobic Face

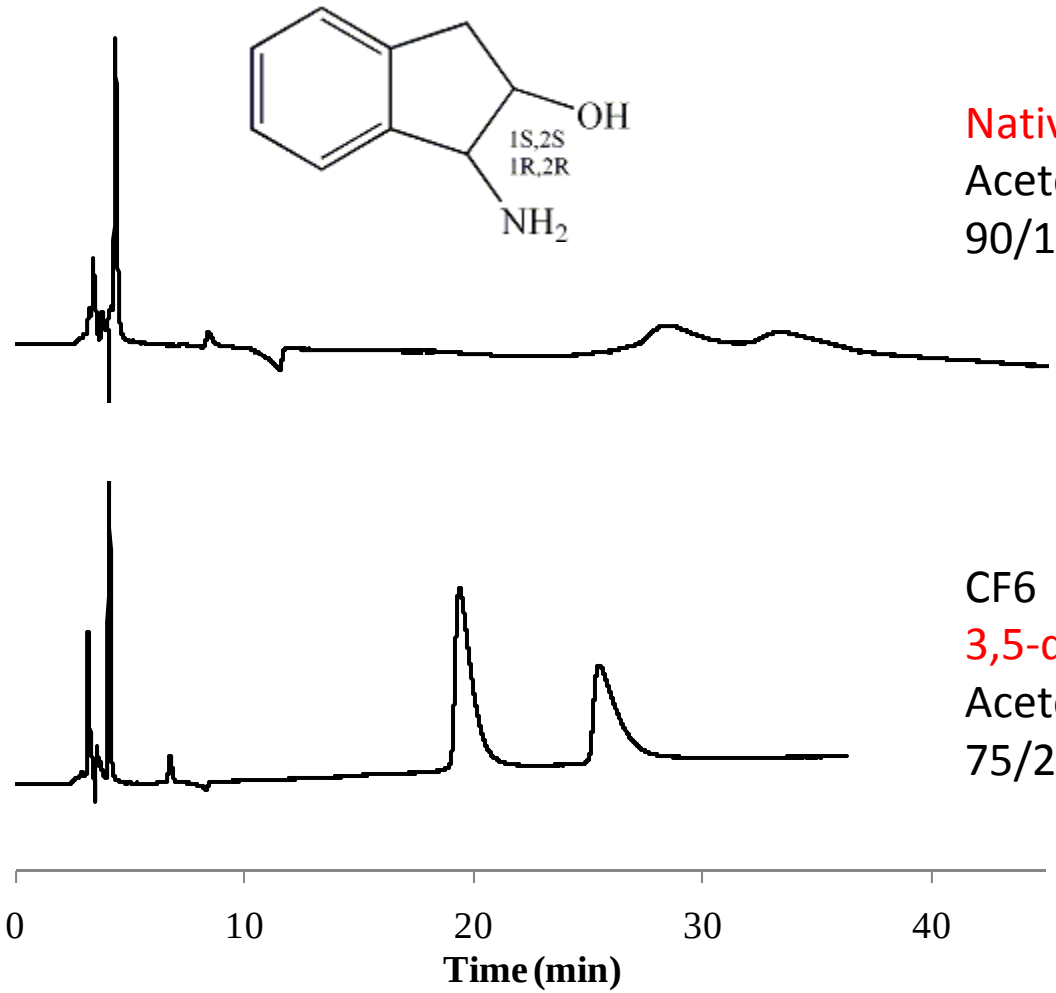


Hydrophilic Face



Side View

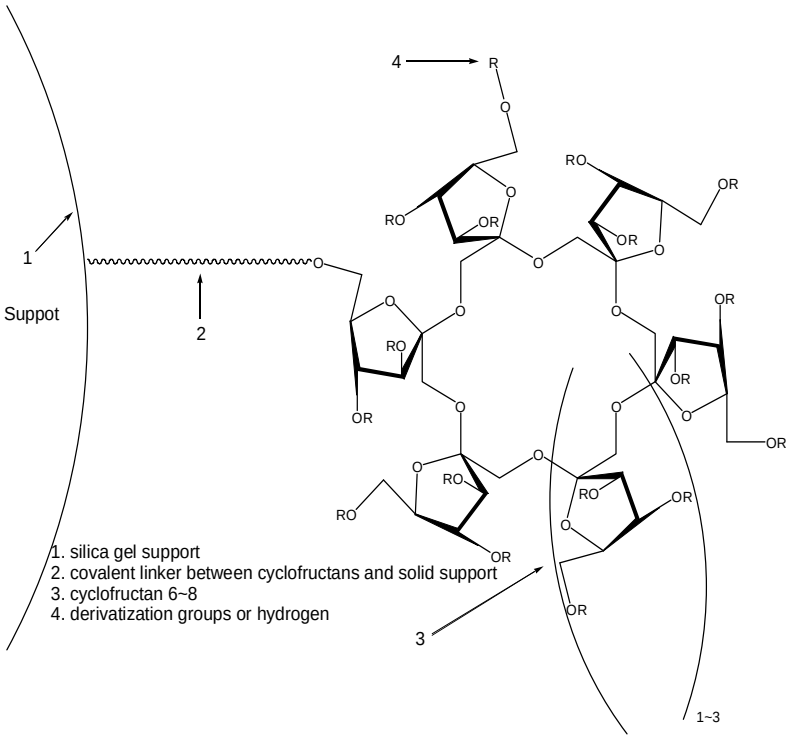
Native vs. Derivatized CF6



Native CF6
Acetonitrile/methanol/AA/TEA
90/10/0.3/0.2

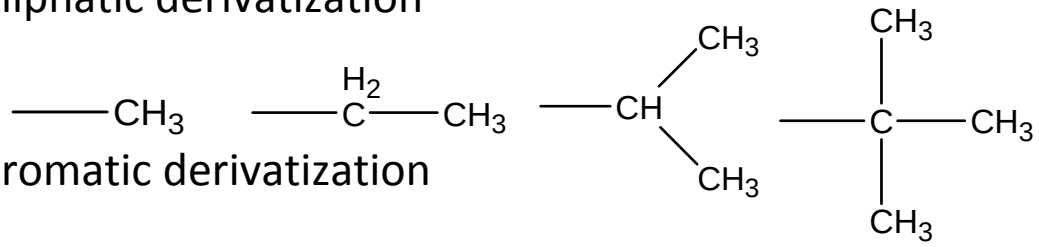
CF6
3,5-dimethylphenyl carbamate
Acetonitrile/methanol/AA/TEA
75/25/0.3/0.2

Structure of Chemically-Bonded CF CSPs

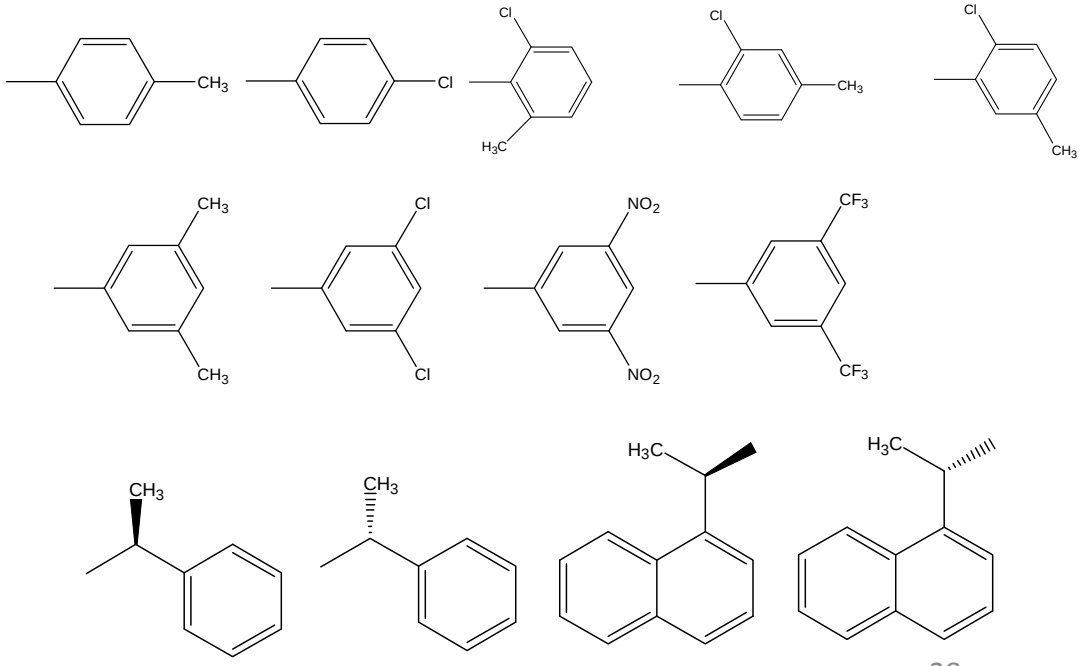


Diverse derivatization groups

- Aliphatic derivatization



- Aromatic derivatization



A Closer Examination of the Separation of Enantiomers of Primary Amine Containing Compounds

119 Randomly chosen compounds were evaluated on the:

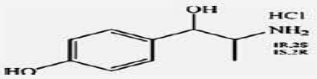
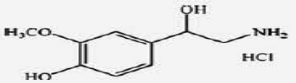
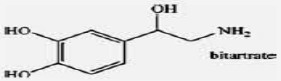
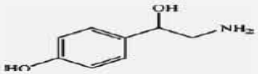
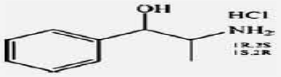
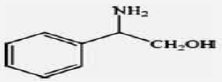
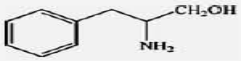
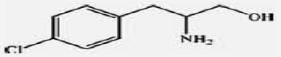
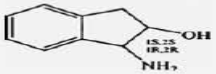
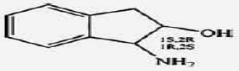
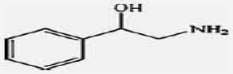
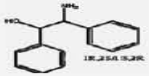
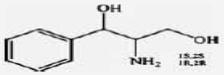
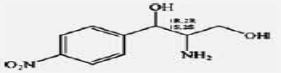
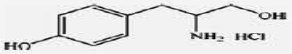
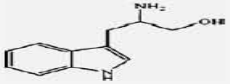
1) **LARIHC CF6-P** CSP (isopropyl derivative)

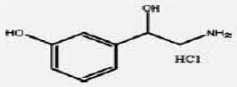
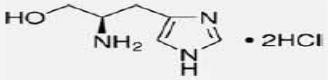
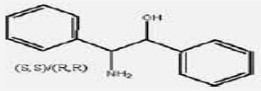
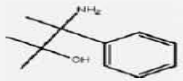
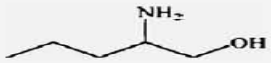
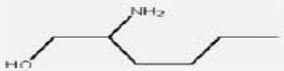
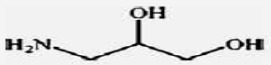
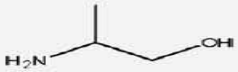
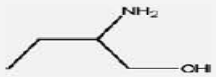
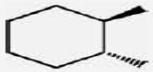
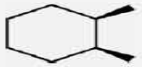
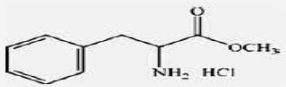
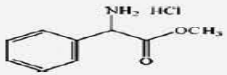
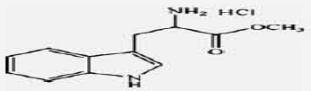
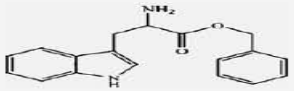
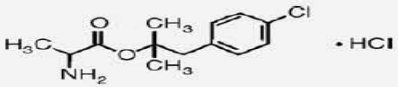
and

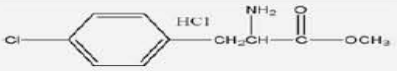
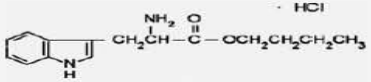
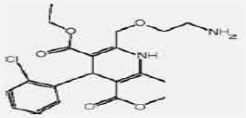
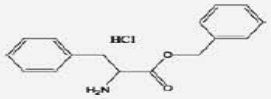
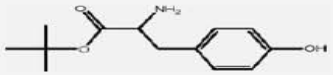
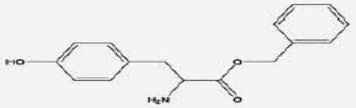
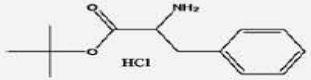
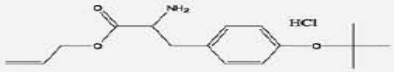
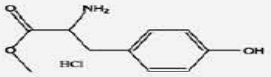
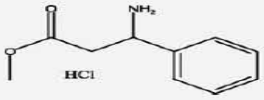
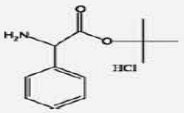
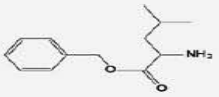
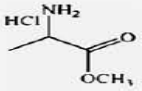
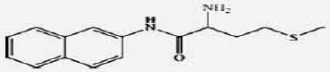
2) **LARIHC CF6-M** CSP (methyl derivative)

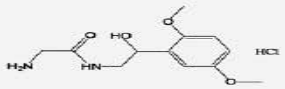
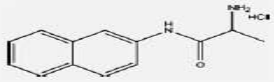
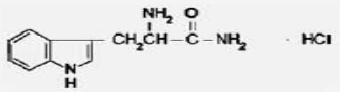
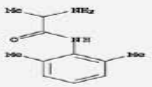
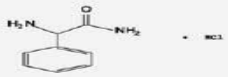
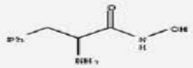
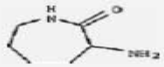
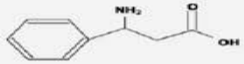
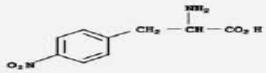
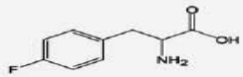
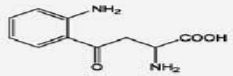
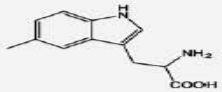
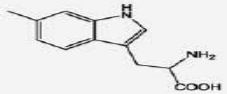
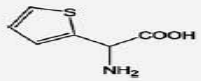
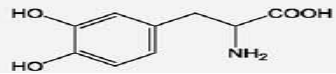
J. Chromatogr., 1217 (2010) 4904-4918

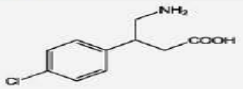
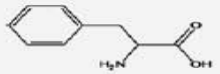
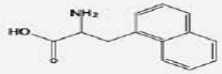
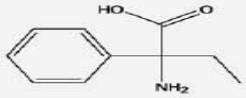
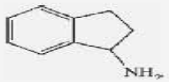
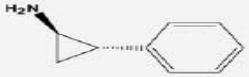
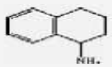
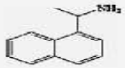
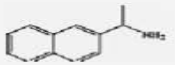
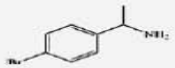
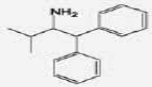
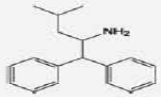
Chromatographic data of racemic primary amine-containing compounds separated on the IP-CF6 stationary phases in the optimized condition

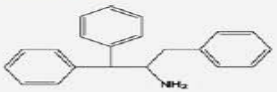
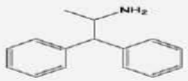
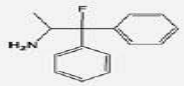
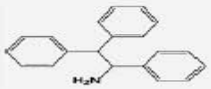
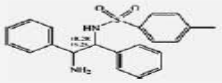
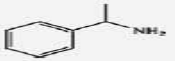
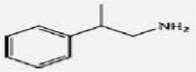
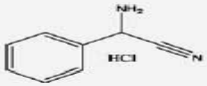
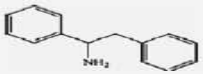
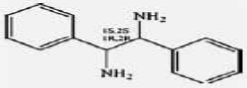
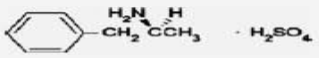
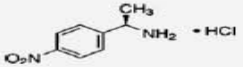
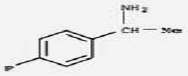
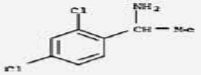
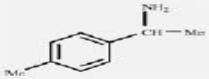
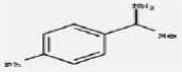
	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
Group A: amino alcohols						
1	(±)-α-(1-Aminoethyl)-4-hydroxybenzyl alcohol hydrochloride		1.95	1.09	1.6	50A50M0.3AA0.2T
2	Normetanephrine hydrochloride		1.27	1.17	2.2	30A70M0.3AA0.2T
3	Norepinephrine L-bitartrate hydrate		7.28	1.13	1.9	60A40M0.3AA0.2T
4	Octopamine hydrochloride		1.24	1.15	1.9	30A70M0.3AA0.2T
5	Phenylpropanolamine hydrochloride		1.44	1.10	1.7	40A60M0.3AA0.2T
6	2-Phenylglycinol		1.49	1.08	1.5	50A50M0.3AA0.2T
7	2-Amino-3-phenyl-1-propanol		0.86	1.15	1.8	30A70M0.3AA0.2T
8	4-Chlorophenyl alaninol		0.90	1.14	1.7	30A70M0.3AA0.2T
9	*Trans*-1-Amino-2-indanol		0.92	1.29	3.5	30A70M0.3AA0.2T
10	*Cis*-1-Amino-2-indanol		1.14	1.13	1.7	40A60M0.3AA0.2T
11	2-Amino-1-phenylethanol		1.26	1.17	1.8	30A70M0.3AA0.2T
12	2-Amino-1,2-diphenylethanol		0.49	1.27	2.5	30A70M0.3AA0.2T
13	2-Amino-1-phenyl-1,3-propanediol		3.41	1.10	1.8	60A40M0.3AA0.2T0C
14	2-Amino-1-(4-nitrophenyl)-1,3-propanediol		1.20	1.14	1.9	50A50M0.3AA0.2T
15	Tyrosinol hydrochloride		1.00	1.12	1.5	30A70M0.3AA0.2T
16	Tryptophanol		0.80	1.16	1.9	30A70M0.3AA0.2T

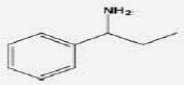
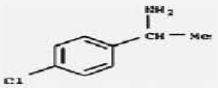
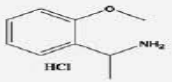
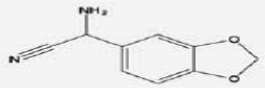
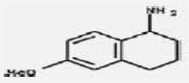
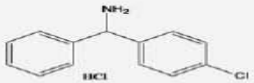
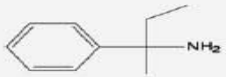
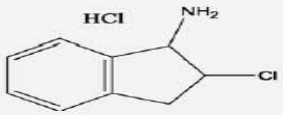
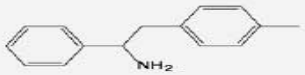
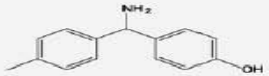
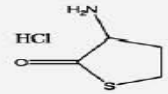
	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
17	Norphenylephrine hydrochloride		1.22	1.15	1.8	30A70M0.3AA0.2T
18	Histidinol dihydrochloride		20.59	1.12	1.5	60A40M0.3AA0.2T0C
19	(R,R/S,S) 2-Amino-1,2-diphenylethanol		2.34	1.06	1.0	60A40M0.3AA0.2T0C
20	3-Amino-2-methyl-3-phenyl-butan-2-ol		1.42	1.13	2.1	60A40M0.3AA0.2T
21	2-Amino-1-pentanol		4.32	1.09	1.5	70A30M1.2AA0.8T[b]
22	2-Amino-1-hexanol		6.08	1.09	1.5	70A30M0.3AA0.2T[c]
23	3-Amino-1,2-propanediol		1.57	1.06	0.8	30A70M0.3AA0.2T[c]
24	2-Amino-1-propanol		3.91	1.11	1.5	60A40M0.3AA0.2T[c]
25	2-Amino-1-butanol		2.63	1.05	0.6	60A40M0.3AA0.2T[c]
26	*Trans*-2-amino-cyclohexanol		6.58	1.11	1.5	80A20M1.2AA0.8T[b]
27	*Cis*-2-amino-cyclohexanol		6.03	1.03	0.5	80A20M1.2AA0.8T[c]
Group B: amino acid esters						
1	Phenylalanine methyl ester hydrochloride		9.93	1.13	2.4	70H30E0.1TFA
2	2-Phenylglycine methyl ester hydrochloride		13.66	1.06	1.0	70H30E0.1TFA
3	Tryptophan methyl ester hydrochloride		3.05	1.23	1.5	60H40E0.1TFA
4	Tryptophan benzyl ester		9.47	1.17	2.6	70H30E0.1TFA
5	Alaproclate hydrochloride		8.43	1.10	1.5	70H30E0.1TFA

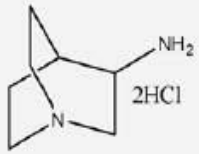
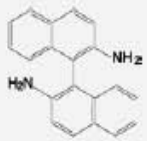
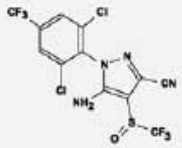
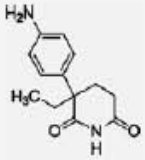
	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
6	4-Chloro-DL-phenylalanine methyl ester hydrochloride		10.46	1.08	1.5	70H30E0.1TFA
7	Tryptophan butyl ester hydrochloride		7.39	1.16	1.6	70H30E0.1TFA
8	Amlodipine		5.22	1.11	1.8	60A40M0.3AA0.2T0C
9	Phenylalanine benzyl ester hydrochloride		8.88	1.06	0.8	70H30E0.1TFA
10	Tyrosine tert-butyl ester		18.37	1.14	1.7	80H20E0.1TFA
11	Tyrosine benzylester		11.49	1.13	0.9	70H30E0.1TFA
12	Phenylalanine tert-butyl ester hydrochloride		0.61	1.04	0.4	95A5M0.3AA0.2T
13	Tert-Butyl-tyrosine allyl ester hydrochloride		15.40	1.09	1.5	80H20E0.1TFA
14	Tyrosine methyl ester hydrochloride		14.41	1.13	1.1	70H30E0.1TFA
15	Methyl 3-phenyl-3-amino-propionate hydrochloride		1.14	1.12	1.7	60A40M0.3AA0.2T
16	Phenylglycine tert-butyl ester hydrochloride		13.98	1.04	0.7	80H20E0.1TFA
17	DL-Leucine benzyl ester		26.59	1.12	1.8	80H20E0.1TFA0C
18	Alanine methyl ester hydrochloride		2.70	1.04	0.7	60A40M0.3AA0.2Tb
Group C: amino amides						
1	DL-Methionine β-naphthylamide		9.40	1.23	3.4	70H30E0.1TFA

	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
2	Midodrine hydrochloride		5.32	1.03	0.7	60A40M0.3AA0.2T
3	Alanine-β-naphthylamide hydrochloride		4.09	1.02	0.6	75A25M0.3AA0.2T
4	Tryptophanamide hydrochloride		8.27	1.12	2.1	75A25M0.3AA0.2TOC
5	Tocainide		4.07	1.08	1.7	75A25M0.3AA0.2T
6	Phenylglycinamide hydrochloride		3.86	1.02	0.5	75A25M0.3AA0.2T
7	Phenylalanine hydroxamate		6.67	1.15	0.8	60H40E0.1TFA
8	3-Aminohexahydro-2-azepinone		2.01	1.48	2.5	50A50M0.3AA0.2T[c]
Group D: amino acids						
1	3-Amino-3-phenylpropionic acid		6.41	1.08	1.1	60A40M0.3AA0.2T0C
2	4-Nitro-DL-phenylalanine		15.35	1.14	1.5	75A25M0.3AA0.2T0C[d]
3	p-Fluorophenylalanine		11.17	1.15	1.5	75A25M0.3AA0.2T0C
4	Kynurenine		9.80	1.03	0.7	75A25M0.3AA0.2T
5	5-Methyl-tryptophan		9.17	1.14	1.5	75A25M0.3AA0.2T0C
6	6-Methyl-tryptophan		9.87	1.18	1.5	75A25M0.3AA0.2T0C
7	α-Amino-2-thienylacetic acid		10.28	1.04	0.7	75A25M0.3AA0.2T
8	3,4-Dihydroxy-phenylalanine		2.89	1.11	1.5	60A40M0.3AA0.2T0C

	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
9	Baclofen		22.59	1.04	0.7	75A25M0.3AA0.2T
10	Thyroxine		8.56	1.02	0.8	75A25M0.3AA0.2T
11	Phenylalanine		11.94	1.13	1.5	75A25M0.3AA0.2T0C
12	3-(1-Naphthyl)alanine		9.83	1.19	1.8	75A25M0.3AA0.2T0C
13	2-Amino-2-phenylbutyric acid		1.87	1.15	1.8	60A40M0.3AA0.2T
14	Arginine		4.86	1.15	0.7	50H50E0.1TFA
Group E: Others						
1	1-Aminoindan		1.03	1.16	2.3	30A70M0.3AA0.2T
2	p-Chloroamphetamine HCl		5.54	1.02	0.7	60A40M0.3AA0.2T
3	*Trans*-2-Phenylcyclopropyl-amine		8.15	1.06	1.5	60A40M0.3AA0.2T0C
4	1,2,3,4-Tetrahydro-1-naphthylamine		0.72	1.17	1.6	30A70M0.3AA0.2T
5	1-(1-Naphthyl)ethylamine		0.86	1.24	2.9	30A70M0.3AA0.2T
6	1-(2-naphthyl)ethylamine		0.90	1.18	2.5	30A70M0.3AA0.2T
7	1-(4-Borophenyl)-ethylamine		1.03	1.18	2.4	30A70M0.3AA0.2T
8	2-Amino-3-methyl-1,1-diphenylbutane		10.98	1.10	1.7	90H10E0.1TFA0C
9	2-Amino-4-methyl-1,1-diphenylpentane		8.43	1.08	1.5	90H10E0.1TFA0C

	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
10	1-Benzyl-2,2-diphenylethyl-amine		3.25	1.09	1.5	80H20E0.1TFA
11	1,1-Diphenyl-2-aminopropane		1.39	1.10	1.8	60A40M0.3AA0.2T
12	1,1-Diphenyl-1-fluoro-2-aminopropane		11.63	1.07	1.5	90H10I0.1TFA
13	1,2,2-Triphenylethylamine		0.65	1.22	2.2	75A25M0.3AA0.2T
14	N-p-Tosyl-1,2-diphenylethyl-enediamine		1.66	1.48	1.6	60H40E0.1TFA
15	α-Methylbenzylamine		0.86	1.17	1.8	30A70M0.3AA0.2T
16	β-Methylphenethylamine		8.86	1.03	0.6	75A25M0.3AA0.2T
17	2-Phenylglycinonitrile hydrochloride		8.98	1.09	1.7	70H30E0.1TFA
18	1,2-Diphenylethylamine		2.20	1.19	2.0	60A40M0.3AA0.2T
19	1,2-Diphenylethylenediamine		5.48	1.14	1.5	75A25M0.3AA0.2TOC[d]
20	Amphetamine sulfate salt		4.45	1.03	0.7	60A40M0.3AA0.2T
21	α-Methyl-4-nitrobenzylamine hydrochloride		1.26	1.13	1.9	30A70M0.3AA0.2T
22	4-Fluoro-α-methylbenzylamine		1.43	1.21	1.6	30A70M0.3AA0.2T0C
23	2,4-Dichloro-α-phenethylamine		0.82	1.32	2.6	30A70M0.3AA0.2T
24	1-(4-Methylphenyl)-ethylamine		1.24	1.22	1.7	30A70M0.3AA0.2T0C
25	1-(1,1′-Biphenyl-4-yl)-ethylamine		0.97	1.17	2.2	30A70M0.3AA0.2T

	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
26	α-Ethylbenzylamine		0.94	1.19	2.8	30A70M0.3AA0.2T
27	4-Methoxy-α-methylbenzylamine		1.14	1.11	1.5	30A70M0.3AA0.2T
28	1-(4-Chlorophenyl)ethylamine		1.30	1.15	1.9	30A70M0.3AA0.2T
29	1-(2-Methoxyphenyl)-ethanamine hydrochloride		0.84	1.17	2.4	30A70M0.3AA0.2T
30	Amino(1,3-benzodioxol-5-yl)-acetonitrile		2.79	1.09	1.0	60H40E0.1TFA
31	6-Methoxy-1,2,3,4-tetra-hydro-1-naphthalenylamine		0.97	1.18	2.4	30A70M0.3AA0.2T
32	4-Chlorobenzhydrylamine hydrochloride		2.29	1.35	1.5	60H40E0.1TFA
33	1-Methyl-1-phenyl-propyl-amine		0.92	1.18	2.5	30A70M0.3AA0.2T
34	2-Chloro-indan-1-ylamine hydrochloride[e]		0.58 2.89	1.33 1.07	3.6 1.5	30A70M0.3AA0.2T 60A40M0.3AA0.2T
35	4-Methyl-alpha-phenylphen-ethylamine		0.77	1.25	3.0	30A70M0.3AA0.2T
36	4-(Amino-p-tolyl-methyl)-phenol		6.31	1.16	1.8	60H40E0.1TFA
37	DL-Homocysteine thiolactone hydrochloride		13.25	1.14	1.1	60H40E0.1TFA
38	Cyclohexylethylamine		6.22	1.11	1.7	70A30M0.3AA0.2T[c]
39	*endo*-2-Amino norbornane hydrochloride		0.97	1.29	1.9	30A70M0.3AA0.2T[f]

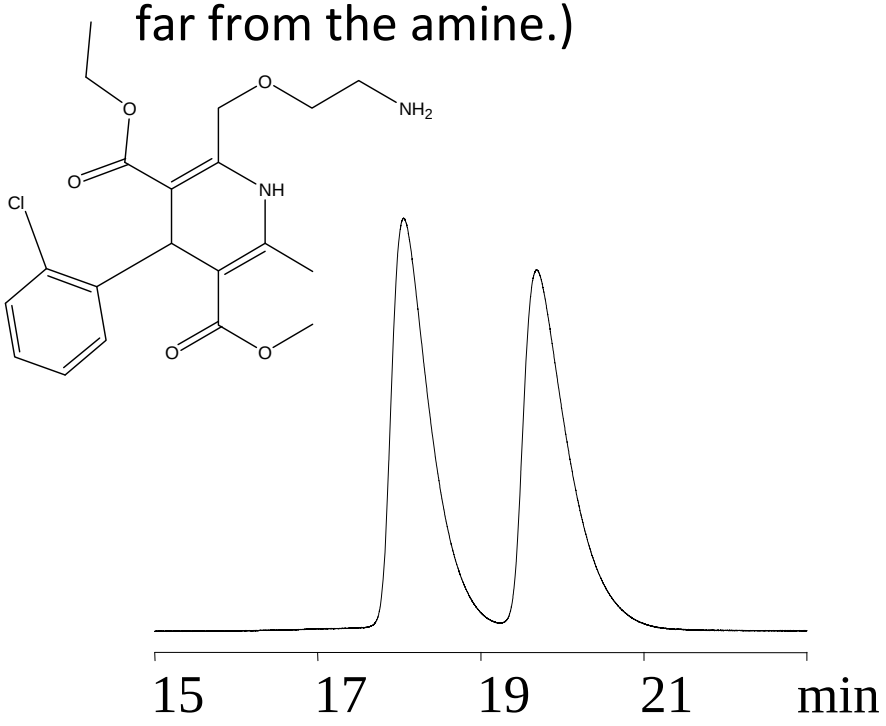
	Compound name	Structure	k_1	α	R_s	Mobile phase[a]
40	*exo*-2-Amino norbornane		3.69	1.10	1.5	70A30M1.2AA0.8T[b]
41	3-Aminoquinuclidine dihydrochloride		5.05	1.10	1.2	80A20M0.1TFA0.1T[c]
42	2,2′-Diamino-1,1'-binaphthalene		2.15	1.16	3.0	80H20E
43	Fipronil		2.83	1.08	1.5	90H10E0.1TFA
44	Aminoglutethimide		2.41	1.06	0.9	60H40E0.1TFA

[a] *Abbreviations*: A, acetonitrile; M, methanol; AA, acetic acid; T, triethylamine; H, heptane; E, ethanol; TFA, trifluoroacetic acid; 0C: the column temperature is set at 0 °C.
[b] Detected with the refractive index detector and the column flow rate was 0.5 mL/min.
[c] Detected with the refractive index detector and the column flow rate was 1 mL/min.
[d] The flow rate was 0.5 mL/min.
[e] This analyte consists two pairs of enantiomers.
[f] Detected with post-column fluorescence derivatization. See Section 2 for details.

Typical separations

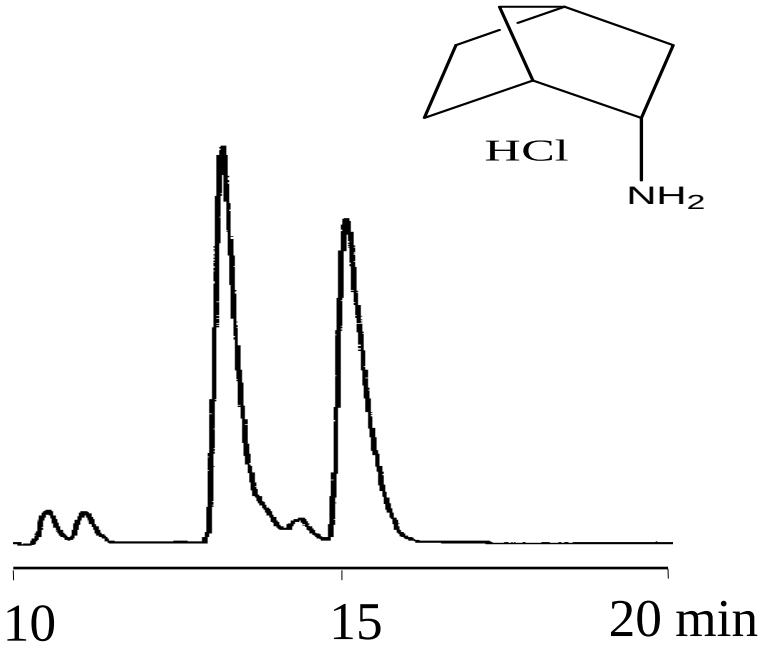
1°amine + ester

(Note that the chiral center is far from the amine.)



60A40M0.3AA0.2TEA (0 °C)

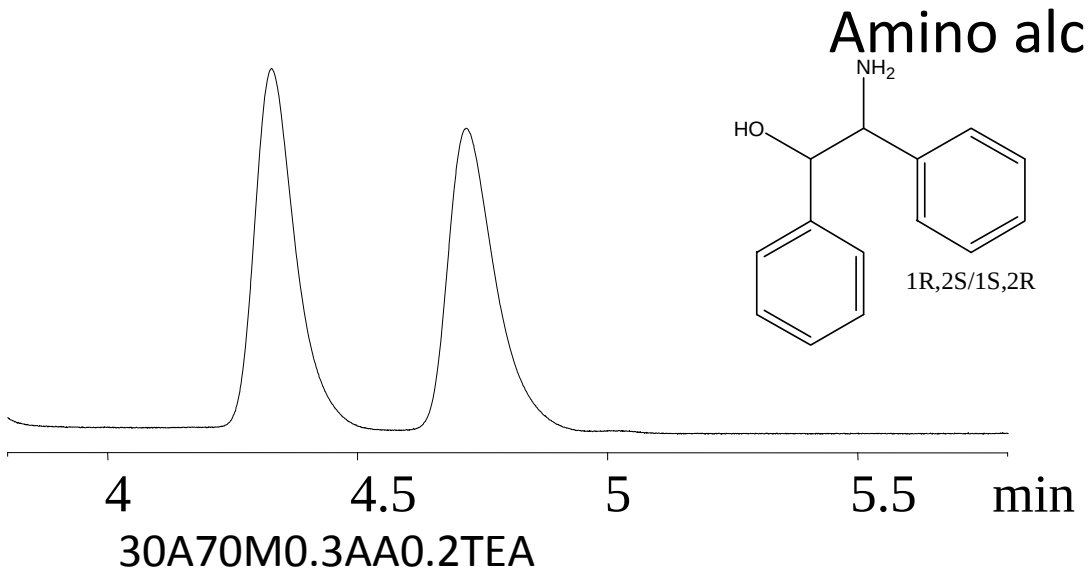
1°amine without UV chromatophore



30A70M0.3AA0.2TEA (Post column derivatization, 0.5mL/min)

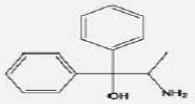
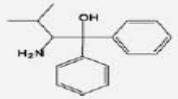
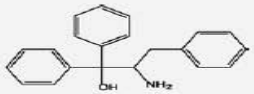
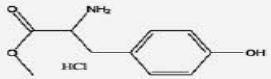
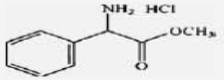
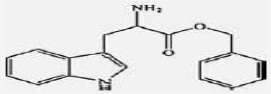
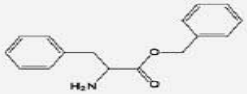
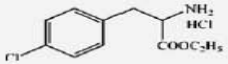
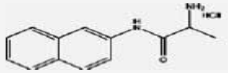
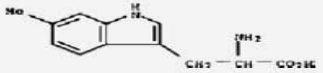
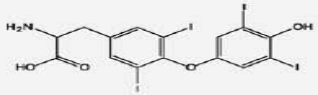
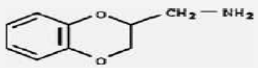
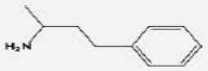
Larihc CF6-P

- This CSP showed enantioselectivity toward 93% of all tested primary amines.
- This CSP usually works more effectively in the polar organic mode (less retention & sharper peaks).



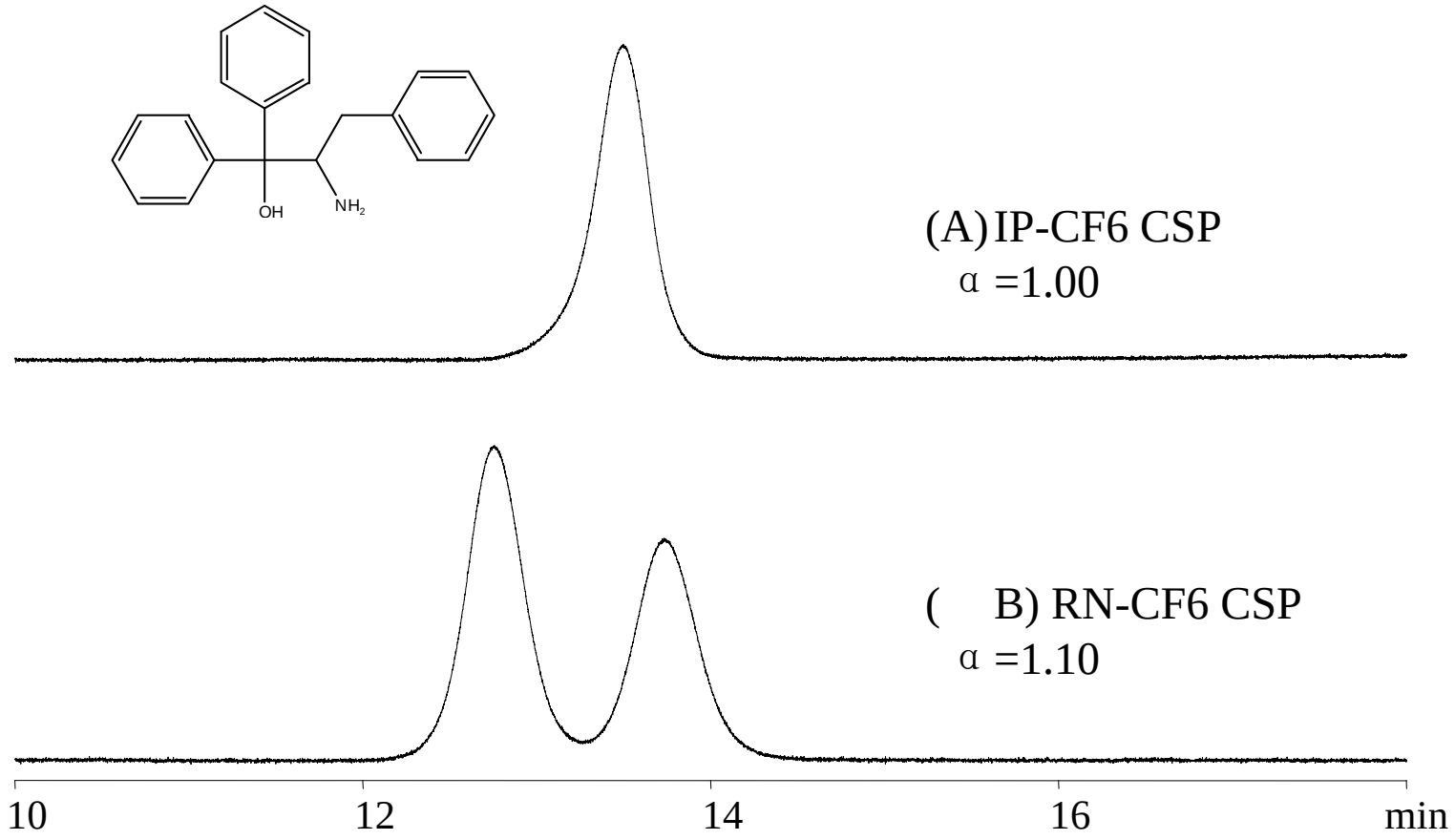
J. Chromatogr. 1217 (2010) 4904-4918

Better resolution of a few racemic primary amines can be obtained with the RN-CF6 column

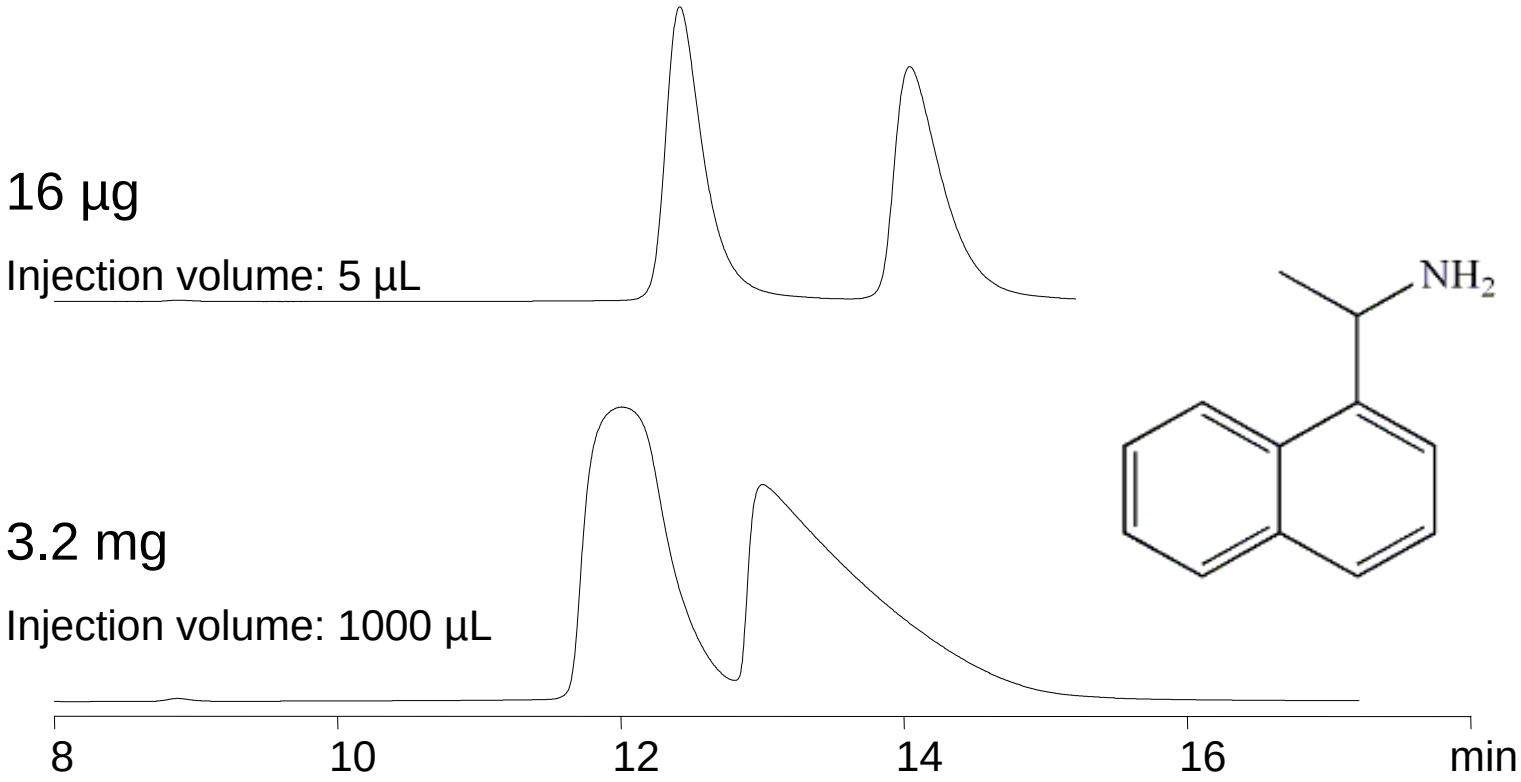
Compound name		Structure	k_1	α	R_s	Mobile phase[a]
1	2-Amino-1,1-diphenyl-1-propanol[b]		5.23	1.11	1.5	80H20E0.1TFA
2	2-Amino-3-methyl-1,1-diphenyl-1-butanol[b]		9.00	1.06	1.5	90H10E0.1TFA[c]
3	2-Amino-1,1,3-triphenyl-1-propanol[b]		3.40	1.10	1.5	80H20E0.1TFA
4	DL-Tyrosine methyl ester hydrochloride		5.04	1.11	1.7	70H30E0.1TFA
5	2-Phenylglycine methyl ester hydrochloride		11.31	1.06	1.5	80H20E0.1TFA[c]
6	Tryptophan benzyl ester		11.30	1.07	1.8	80H20E0.1TFA[c]
7	Phenylalanine benzyl ester hydrochloride		7.53	1.07	1.0	80H20E0.1TFA
8	Chlorophenylalanine ethyl ester hydrochloride[b]		16.14	1.04	0.7	90H10E0.1TFA
9	DL-Alanine-β-naphthylamide hydrochloride		10.03	1.10	1.5	80H20E0.1TFA
10	6-Methyl-DL-tryptophan		13.96	1.16	1.6	80H20E0.1TFA
11	DL-Thyroxine		1.07	1.34	1.6	60H40E0.1TFA
12	2-Aminomethyl-1,4-benzodioxane[b]		5.28	1.05	0.7	60H40E0.1TFA
13	1-Methyl-3-phenylpropyl-amine[b]		9.76	1.05	0.4	85H15E0.1TFA

[a] *Abbreviations*: H, heptanes; E, ethanol; TFA, trifluoroacetic acid.
[b] Enantiomers of the analyte were not separated by the IP-CF6 CSP.
[c] The flow rate was 0.5 mL/min.

98% of all chiral primary amine containing compounds were separated on the LARIHC CF6-P + LARIHC CF6-RN Columns. An example of a separation on the aromatic functionalized CF6-RN column is shown below.

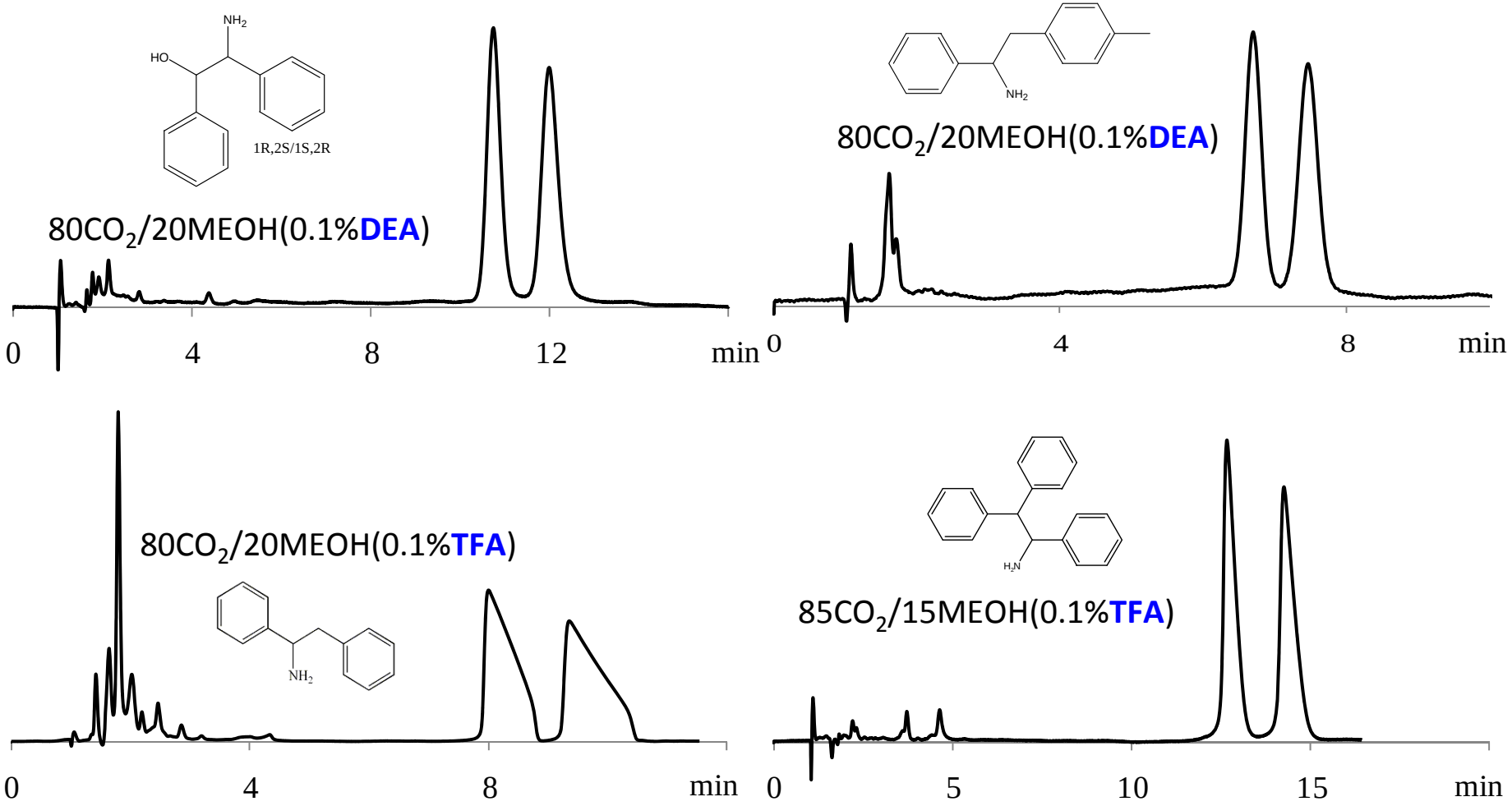


Good loading capacity



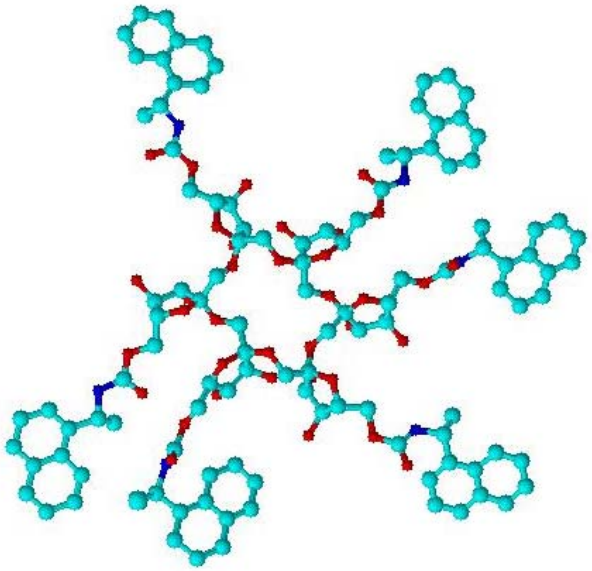
Column: 0.46 cm× 25 cm
MP: 60A40M0.3AA0.2TEA
Analyte concentration: 3.2 mg/mL

Excellent performance in SFC

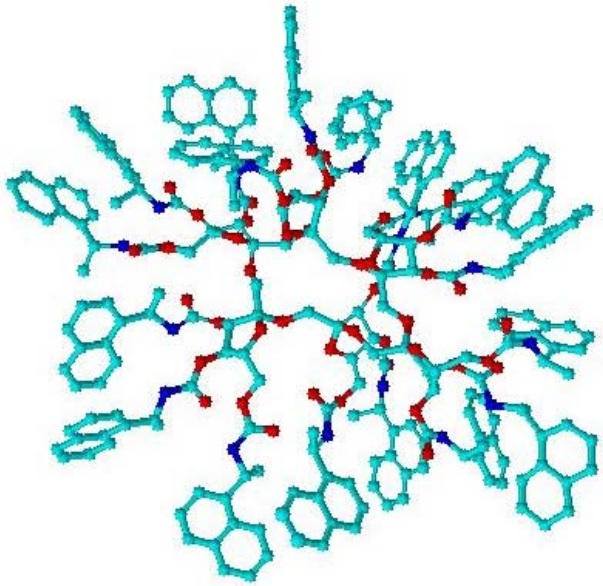


- Either basic additives or acidic additives can work.

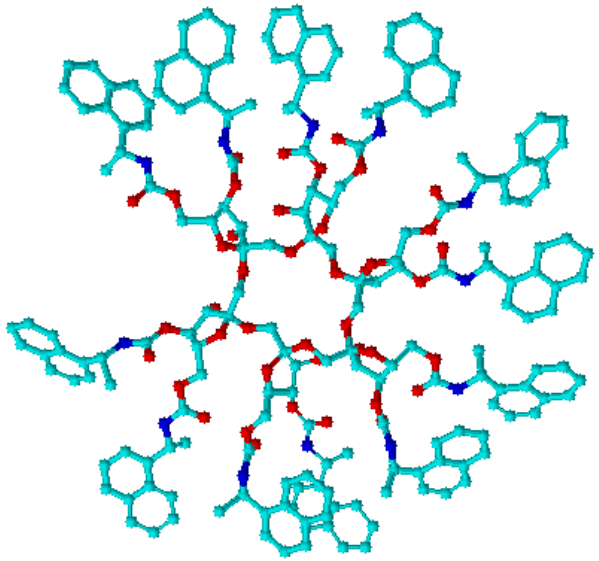
3D Optimized Cyclofructan RN Derivative (6, 12, 18)



RN 6 Hydrophobic Face

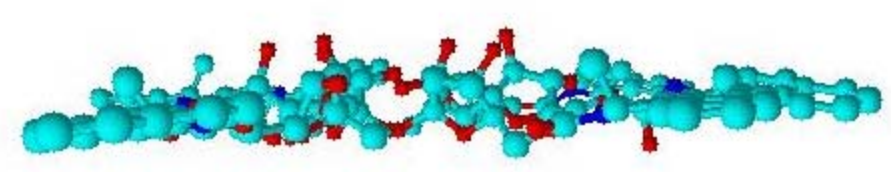


RN 18 Hydrophilic Face

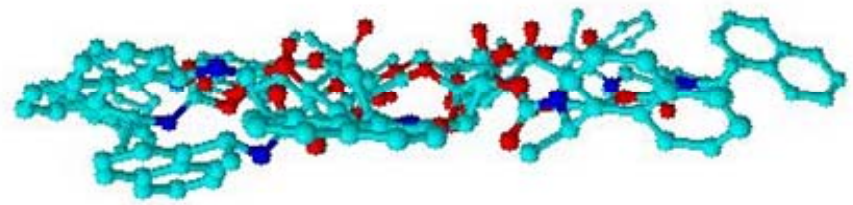


RN 12 Hydrophilic Face

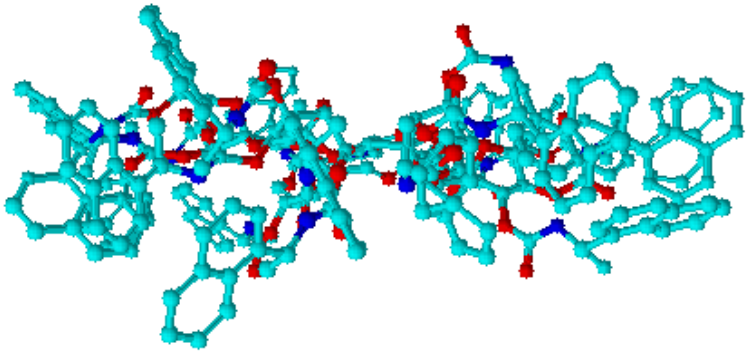
3D Optimized Cyclofructan RN Derivative (6, 12, 18)



RN 6 Side View

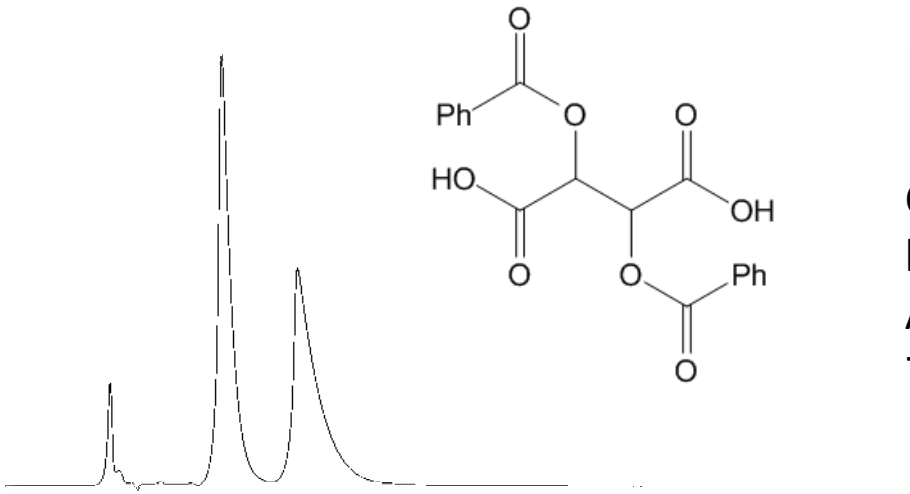


RN 12 Side View

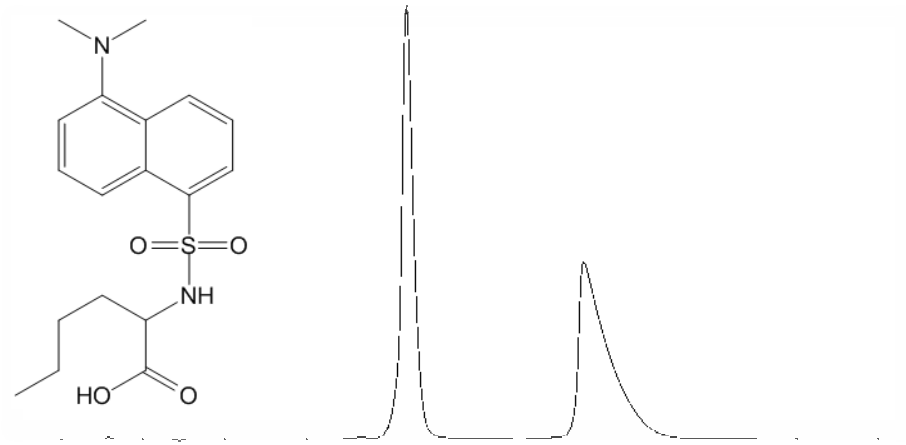


RN 18 Side View

Separation of Chiral Acids



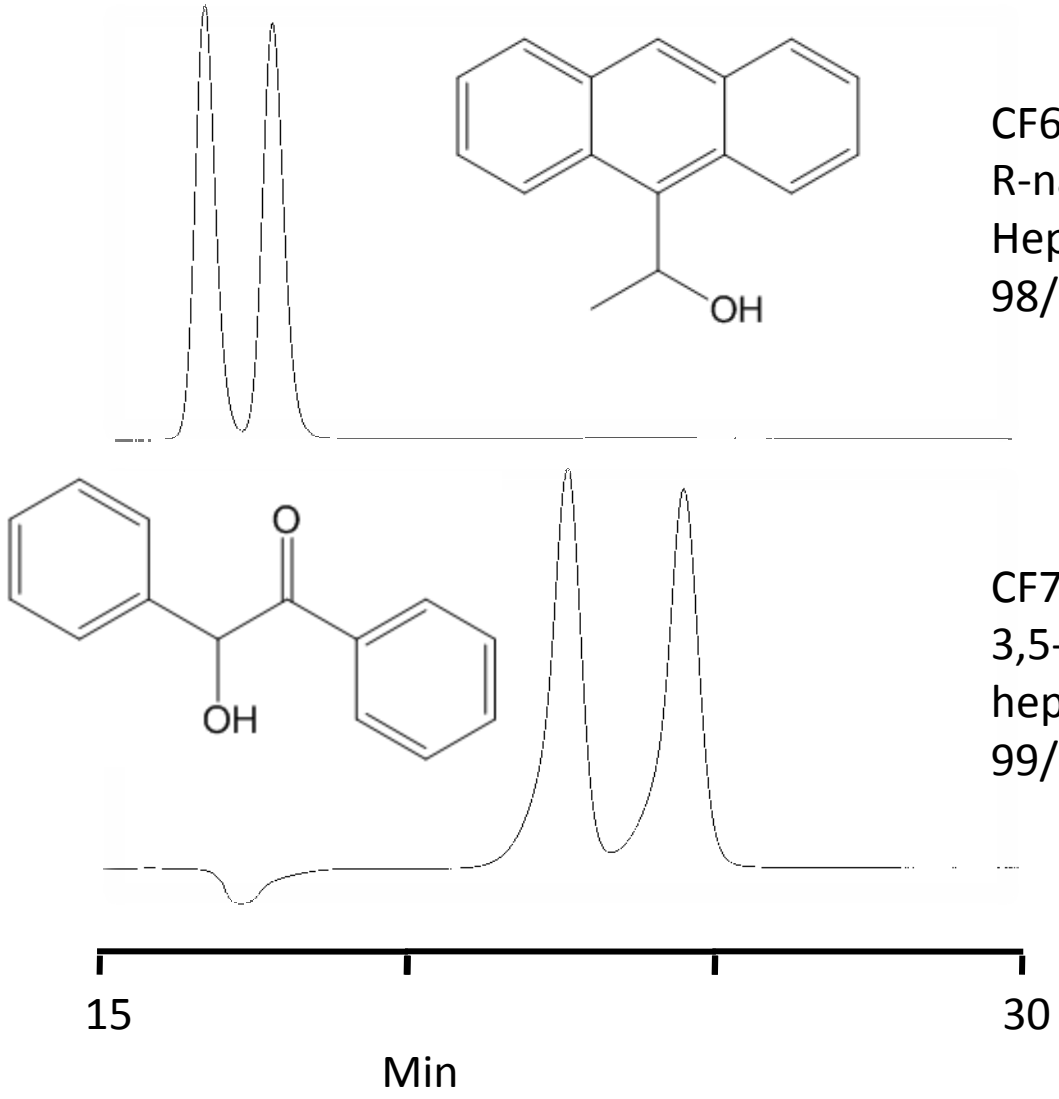
CF6
bis(trifluoromethylphenly carbamate
Acetonitrile/methanol/AA/TEA
75/25/0.3/0.2



CF7
3,5-dimethylphenyl carbamate
heptane/ethanol/TFA
80/20/0.1

0 Min 20

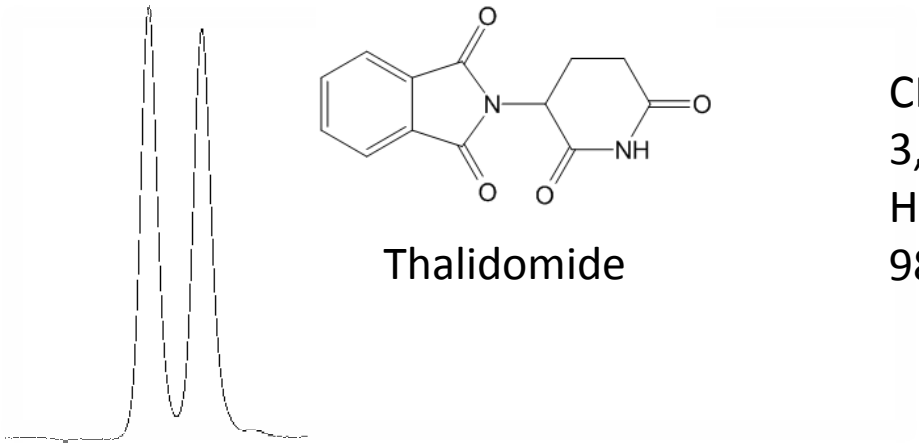
Separation of Chiral Alcohols



CF6
R-naphthylethyl carbamate
Heptane/isopropanol/TFA
98/2/0.1

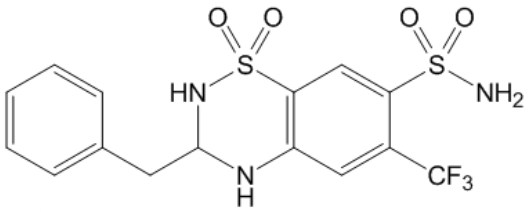
CF7
3,5-dimethylphenyl carbamate
heptane/ethanol/TFA
99/1/0.1

Separation of Chiral Pharmaceutical Compounds



Thalidomide

CF6
3,5-dimethylphenyl carbamate
Heptane/isopropanol/TFA
98/2/0.1

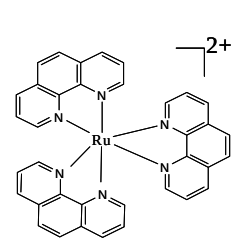


Bendroflumethiazide

CF7
R-naphthylethyl carbamate
heptane/ethanol/TFA
99/1/0.1

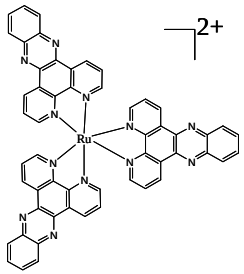
Separation of Ru tris(diimine) Complexes

$[Ru(phen)_3](Cl_2)$



k_1=0.883, α=1.51, Rs=4.4

$[Ru(dppz)_3](Cl_2)$

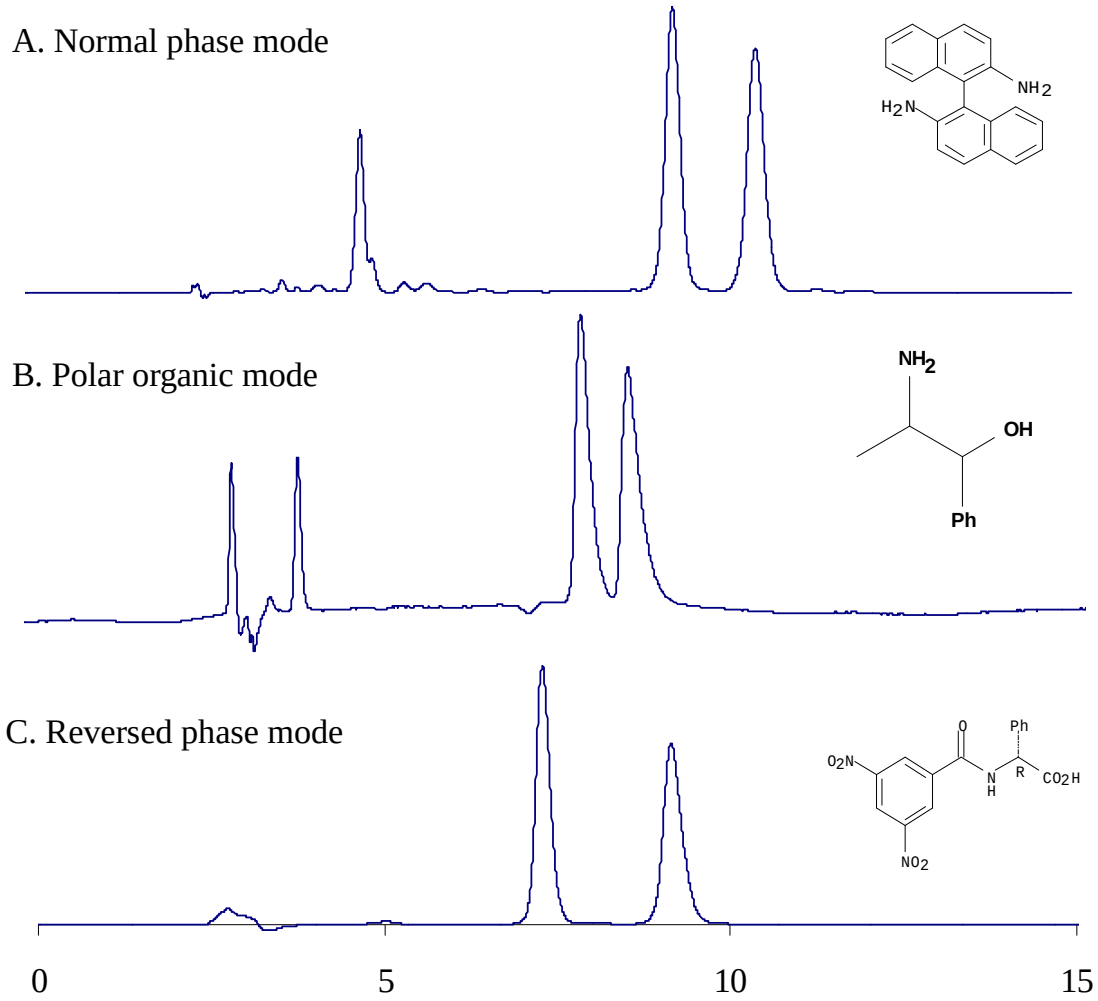


k_1=1.27, α=2.81, Rs=12.1

RNCF6 column

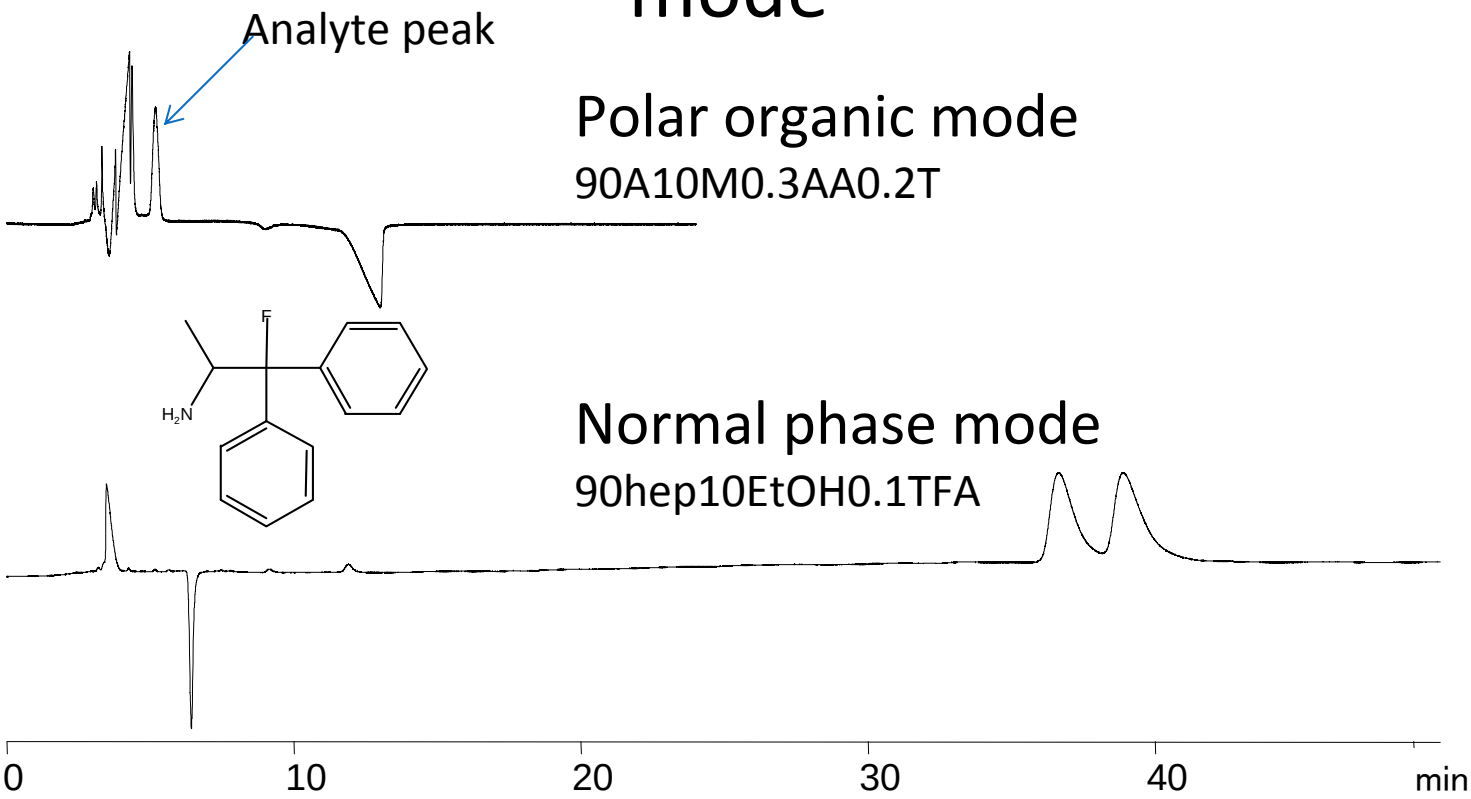
60methanol/40acetonitrile/25 mM NH_4NO_3

CF-based CSPs can be used alternately in polar organic, reversed-phase and normal phase solvents without damage.



Mobile phase: (A) 70% heptane/30% ethanol: (B) 60% acetonitrile/40% methanol/0.3% acetic acid/0.2% triethylamine. CSP: CF6-RN

Polar organic mode *vs* Normal phase mode



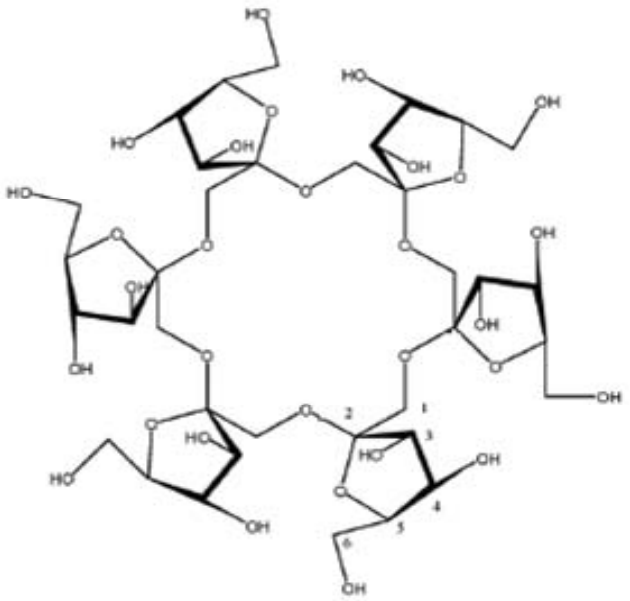
•When short retention is observed in the polar organic mode, the normal phase mode should be tested.

CF6 and CF6-Sulfonate as HILIC Stationary Phases FRULIC-N and FRULIC-C

HILIC = Hydrophilic Interaction Chromatography

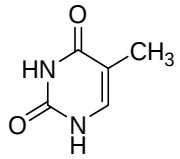
The best way to separate polar molecules

Cyclofructan 6

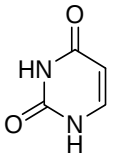


J. Chromatogr. A, 1218 (2011) 270-279.

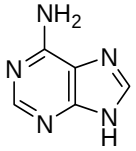
Nucleic acid bases:



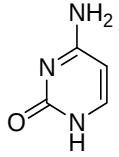
Thymine



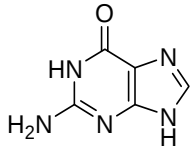
Uracil



Adenine

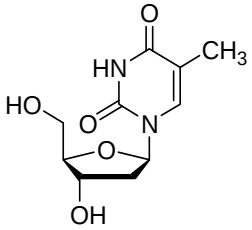


Cytosine



Guanine

Nucleosides:



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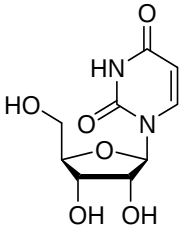

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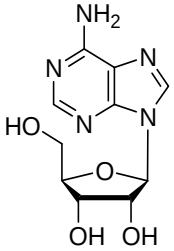

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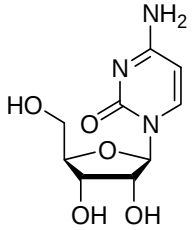

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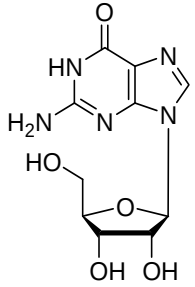
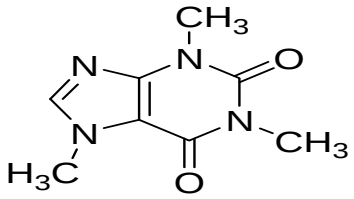
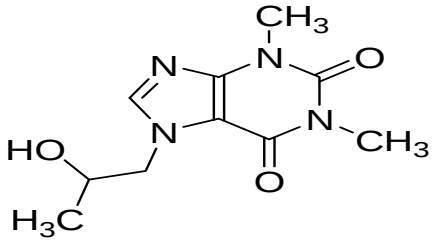


Guanosine

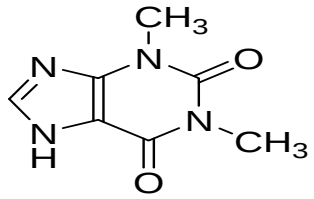
Xanthines



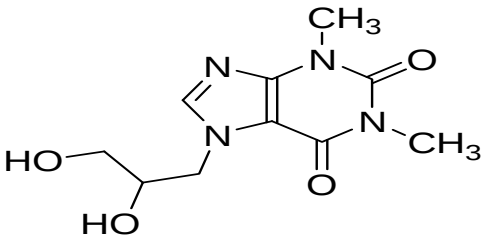
Caffeine



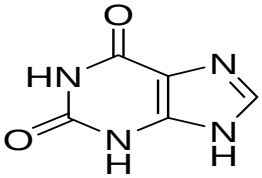
7-β-Hydroxypropyl Theophylline



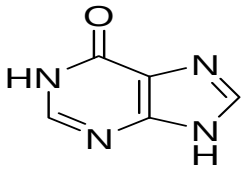
Theophylline



Dyphylline

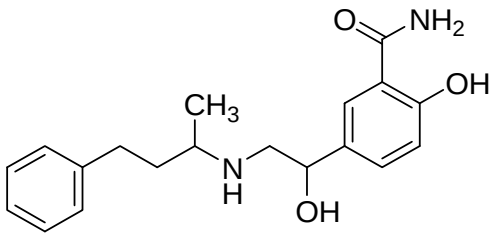


Xanthine

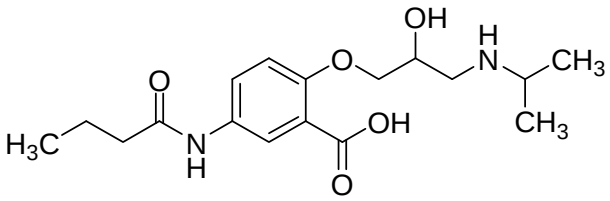


Hypoxanthine

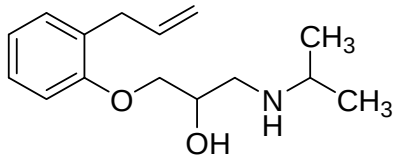
β-blockers:



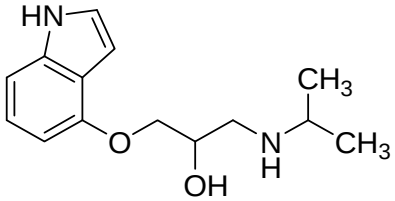
Labetalol



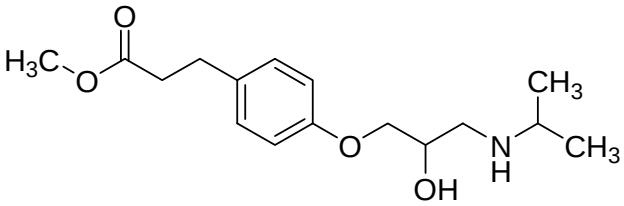
Acebutolol



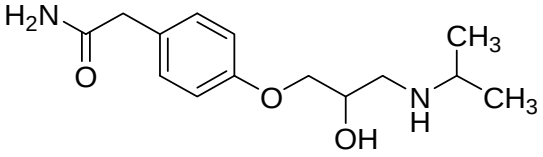
Alprenolol



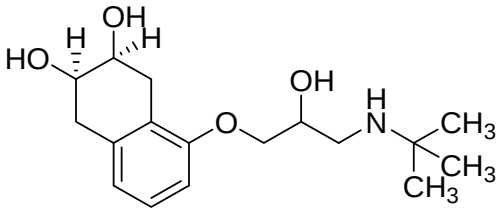
Pindolol



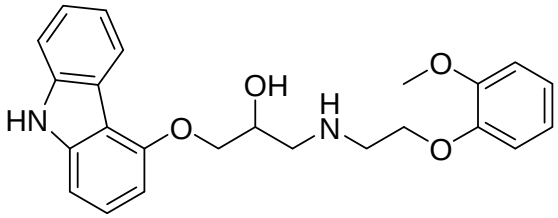
Esmolol



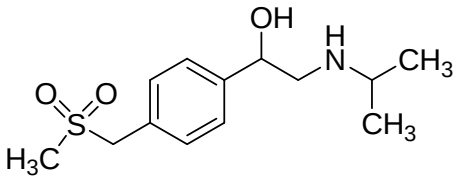
Atenolol



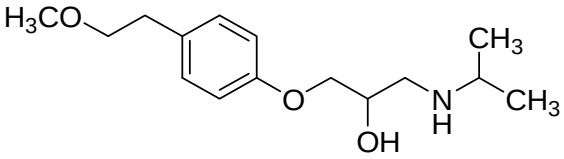
Nadolol



Carvedilol

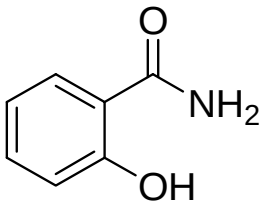


Sotalol

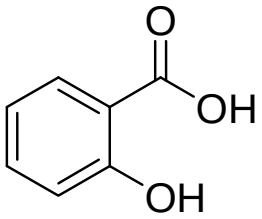


Metoprolol

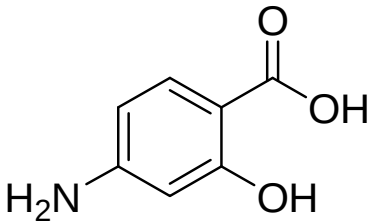
Salicylic acid and derivatives



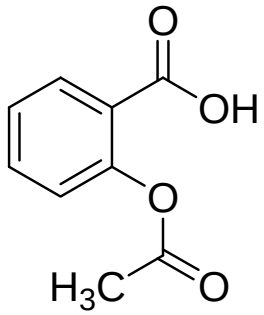
Salicylamide



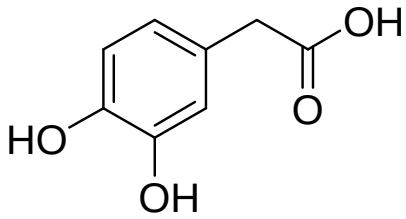
Salicylic acid



4-Aminosalicylic acid

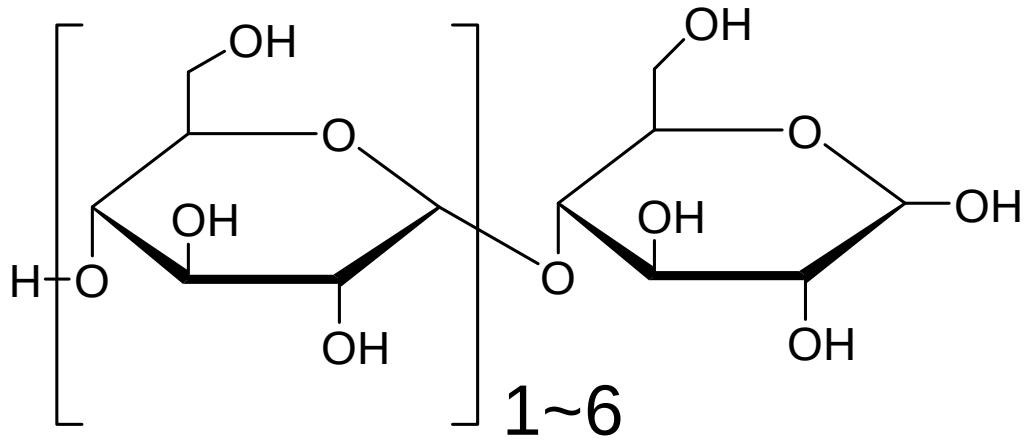


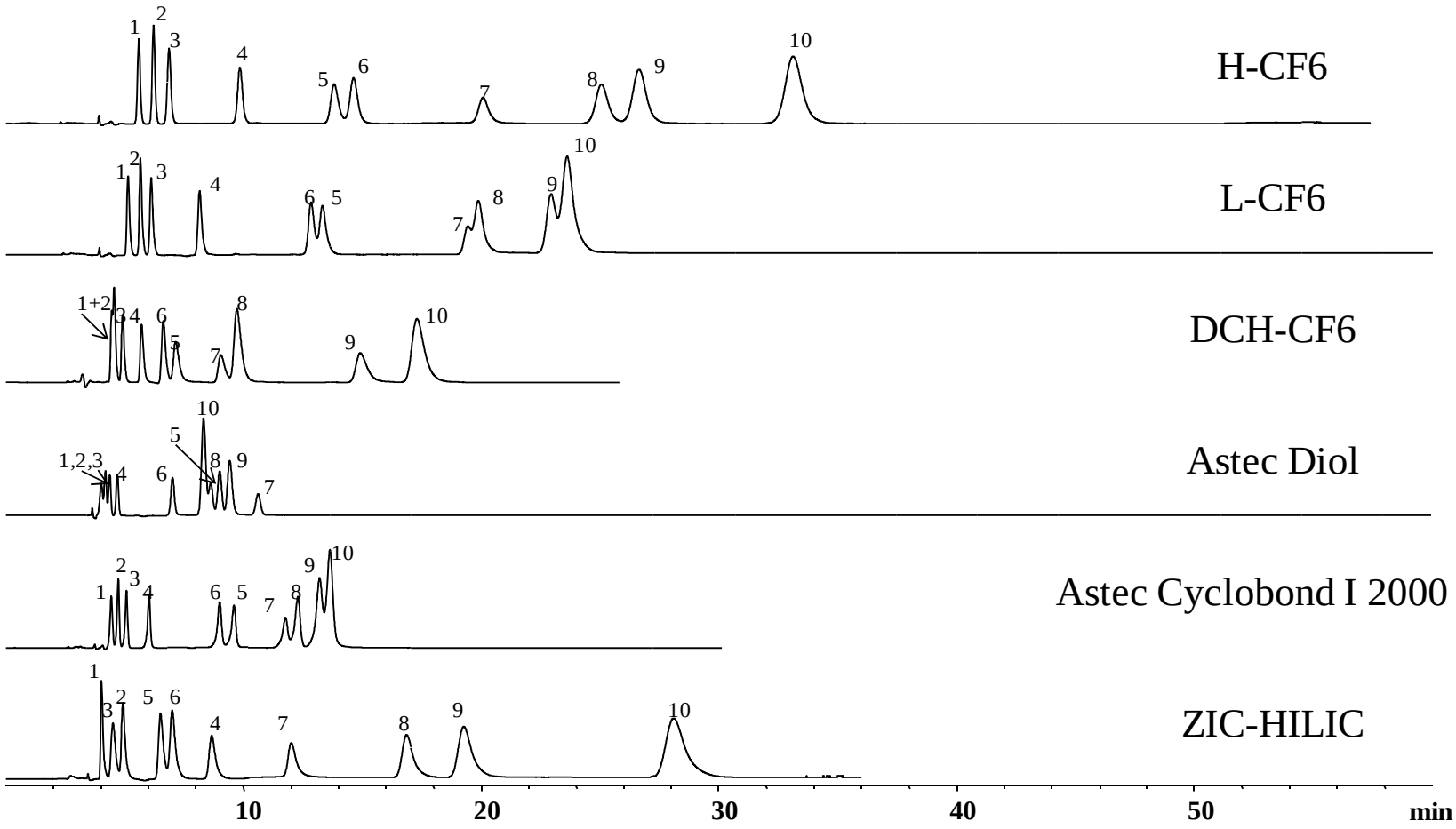
Acetylsalicylic acid



3,4-Dihydroxyphenyl acetic acid

Maltooligosaccharides

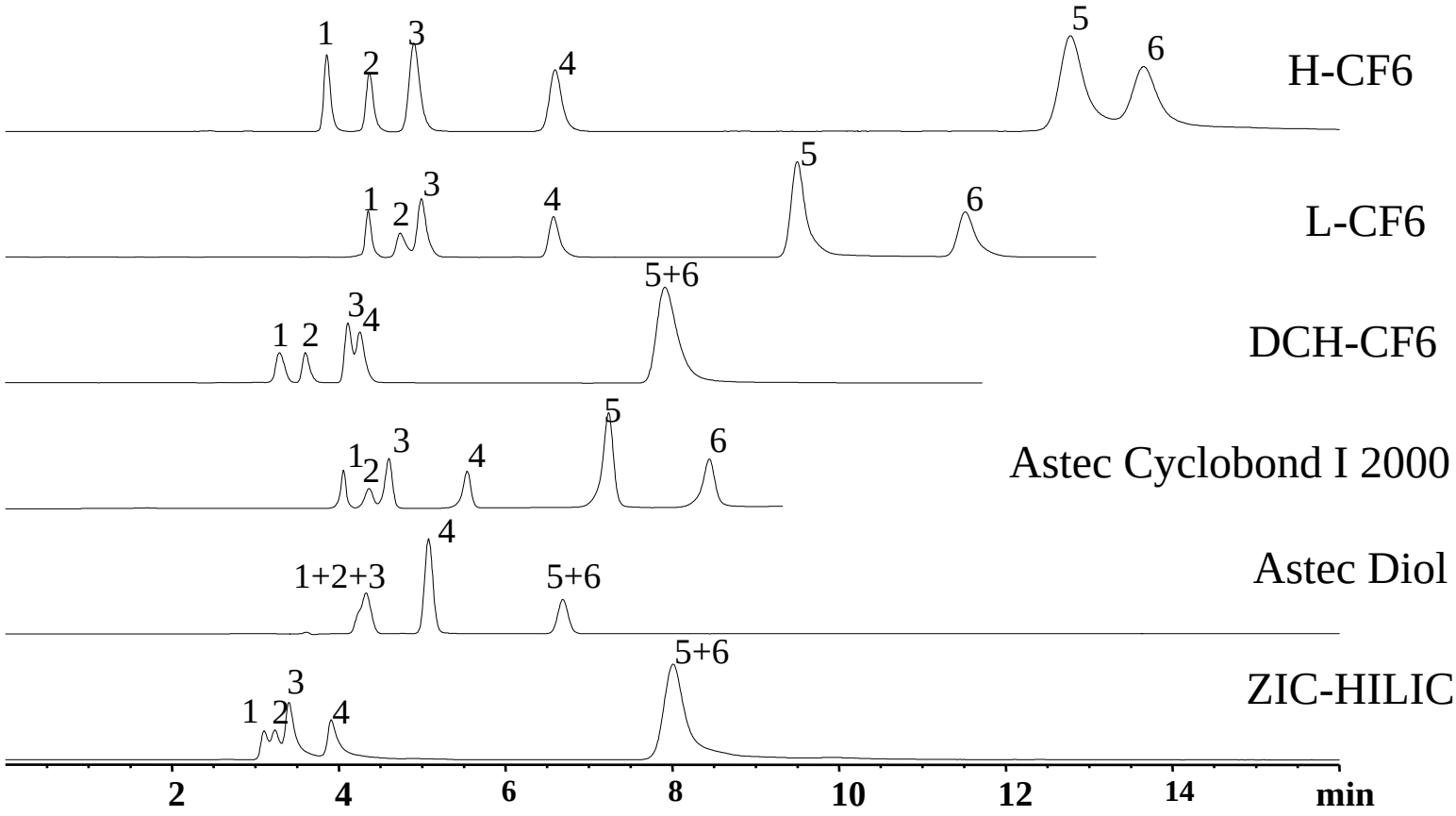


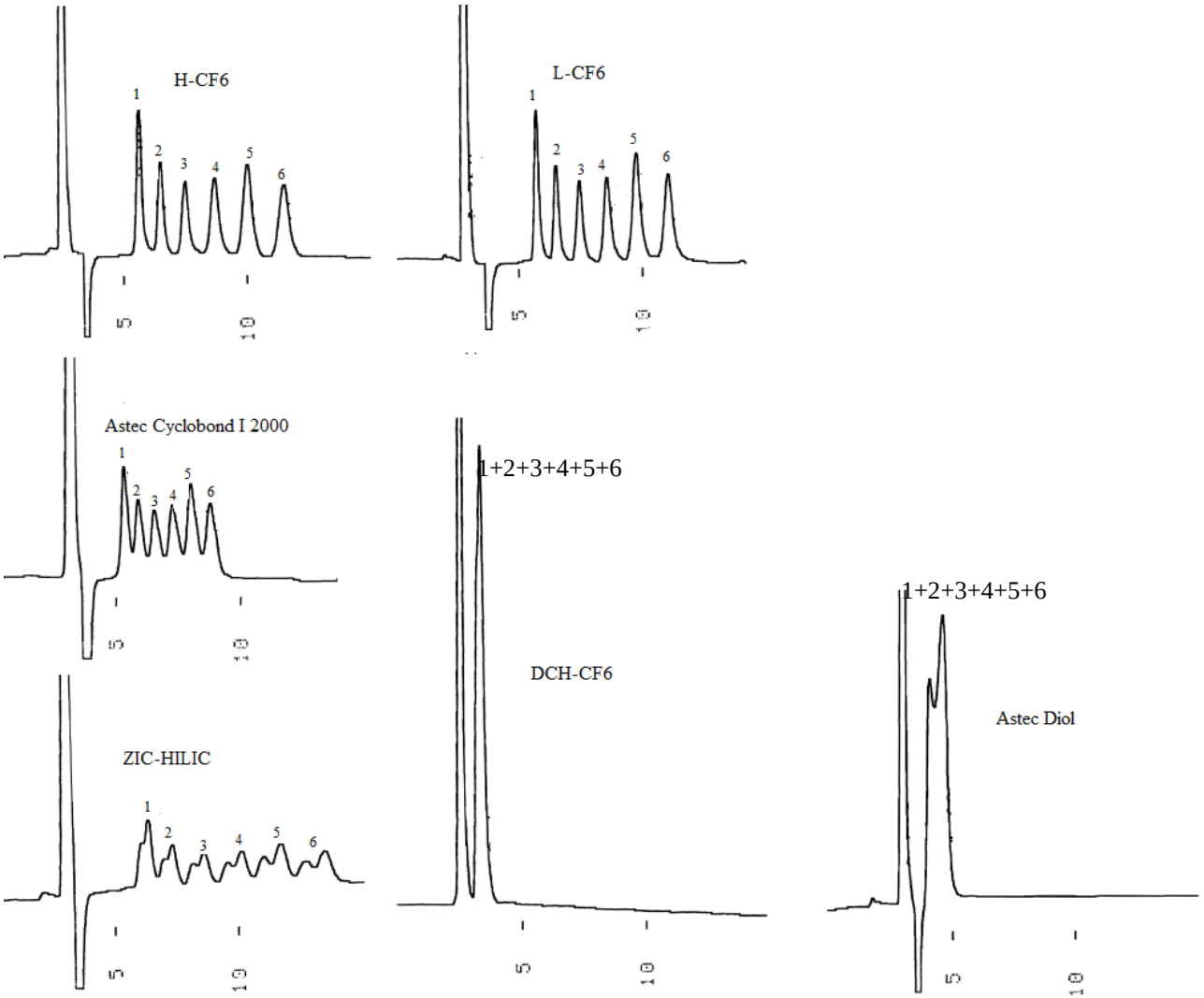


Separation of nucleic acid bases and nucleosides on six compared columns. Mobile phase: acetonitrile/20 mM ammonium acetate, pH = 4.1, 90/10 (*v/v*); flow rate: 1.0 mL/min; UV detection: 254 nm. Compounds: 1. Thymine; 2. Uracil; 3. Thymidine; 4. Uridine; 5. Adenine; 6. Adenosine; 7. Cytosine; 8. Guanine; 9. Cytidine; 10. Guanosine.

Separation of xanthines on the six compared stationary phases.

Mobile phase: acetonitrile/20 mM ammonium acetate buffer, pH = 4.1, 90/10 (*v/v*); flow rate: 1.0 mL/min; UV detection: 254 nm. Compounds: 1. Caffiene; 2. 7- β -Hydroxypropyl Theophylline; 3. Theophylline; 4. Dyphilline; 5. Hypoxanthine; 6. Xanthine.





Separation of maltooligosaccharides on the six compared columns.

Mobile phase: acetonitrile/water 65/35 (*v/v*); flow rate: 1.0 mL/min; RI detection.

Compounds: 1. Maltose; 2. Maltotriose; 3. Maltotetraose; 4. Maltopentaose; 5. Maltohexaose; 6. Maltoheptaose.

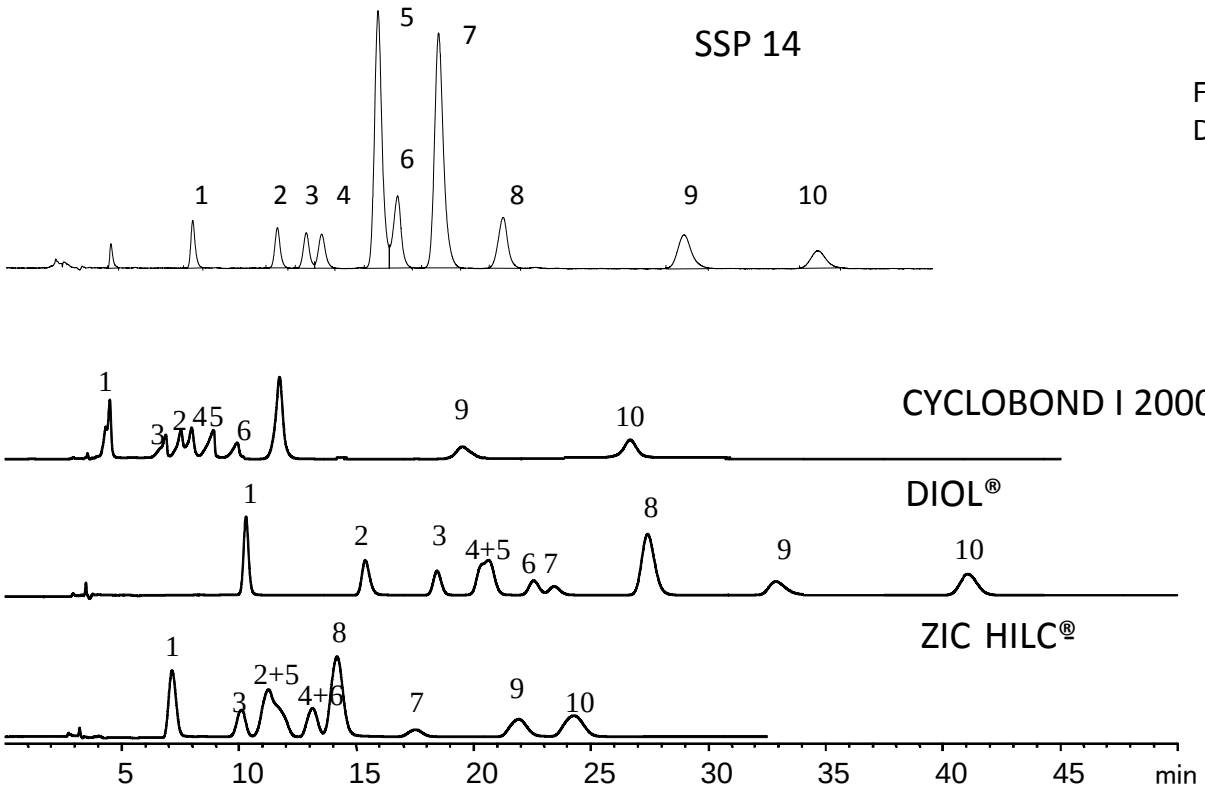
HILIC-sulfonate derivative separation of beta-blockers

Conditions

MP :ACN/20 mM Ammonium acetate buffer pH = 4.1 (70/30,v/v)

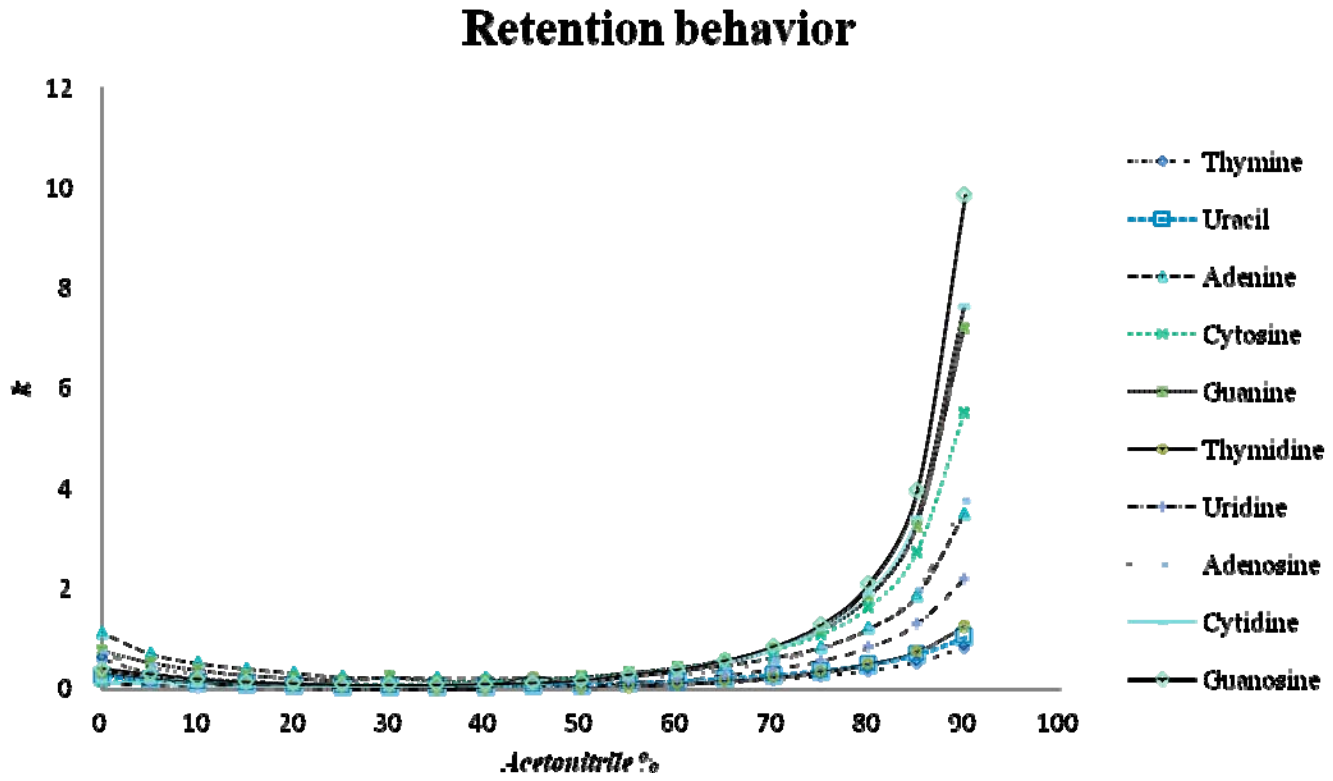
Flow rate : 1.0 ml/min

Detection : UV detection at 254 nm



MP : ACN/20 Mm Ammonium acetate buffer pH = 4.1 (90/10v/v)

1. Carvedilol; 2. Alprenolol; 3. Esmolol; 4. Labetalol; 5. Pindolol;
6. Metoprolol ; 7. Acebutolol; 8. Sotalol; 9. Nadolol; 10. Atenolol



Effect of acetonitrile content on the retention (*k*) of ten tested nucleic acid bases and nucleosides on the H-CF6 column.

Mobile phase: acetonitrile and 20 mM ammonium acetate, pH = 4.1. Flow rate: 1.0 mL/min. UV detection at 254 nm.


JANUARY 4, 2010
C&EN
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SMART GRID
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ACS president on the year of chemistry P.2

CHROMATOGRAPHERS' CLARION CALL

PACIFICHEM NEWS: To expand the menu of chiral selectors for separations, the expertise of synthetic organic chemists would be tremendously useful

A. MAUREEN ROUHI, C&EN WASHINGTON

IF CHRISTOPHER WELCH had his way, the powerhouses of organic synthesis would be seriously devoting part of their time to the design and synthesis of enantiomer-selective molecules for use as stationary phases in chiral chromatographic separations. For this reason, at the 6th International Chemical Congress of Pacific Basin Societies, or Pacifichem 2010, the separations guru at Merck & Co. intentionally included the symposium "Interface between Organic Synthesis & Chromatography" in the technical program for organic chemistry instead of analytical chemistry.

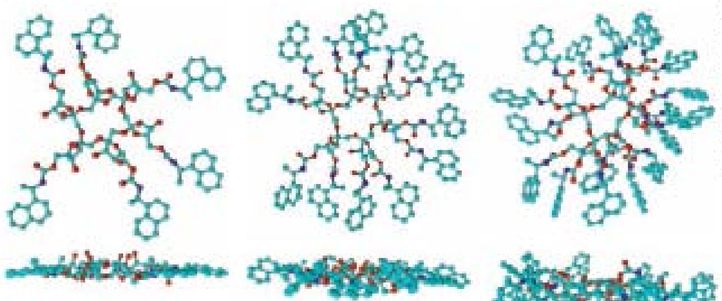
According to several symposium speakers, current chiral selectors, although versatile, aren't sufficient to analyze and purify the ever-increasing number of valuable chiral molecules. The stalwarts of chiral separation called for renewed R&D vigor and urged synthetic chemists to participate—not only to add their expertise to the design and synthesis of chiral selectors but also to partake of the fruits of R&D by applying chiral chromatography to their synthetic work. Meanwhile, a new class of carbohydrate-derived chiral selectors—the cyclofructans—seems poised for chiral chromatographic stardom.

"When a chromatographer talks to synthetic chemists about the details of separation studies, eyes tend to glaze over," Welch told C&EN. "Similarly, chromatographers are often uninterested in the details of organic synthesis. And yet, new chromatographic stationary phases have to come from somewhere—and that is organic synthesis. But the field has received little attention from the real masters of molecule making."

The experts of synthetic organic chemistry could take the field beyond the point that the average synthetic chemist, as Welch describes himself, is able to. "What molecules I make are geared by my knowledge of organic chemistry, which may be a little bit old-fashioned," Welch explained. "But if you can involve the real synthetic powerhouses, they can make things that were unsynthesizable a few years ago."

Furthermore, microscale chromatography is opening up new opportunities for chiral separations, Welch said. "One of the great things about using microcolumns for analysis is the very small amount of stationary phase needed—less than 1% of what is needed in a conventional column," he explained. "This means that chromatographic stationary phases that would be prohibitively expensive to use in conventional columns could be cost-effective in microscale formats. This opens up some very interesting opportunities for a future in which complex and sophisticated specialty stationary phases could be used as powerful analytical tools."

Among the speakers in the symposium



MODULATING ACCESS These three-dimensional optimized structures (facial views on top and side views on bottom) show the effects of aromatic substitution on a cyclofructan 6 derivative. When lightly derivatized (left views), the molecule is in a relaxed, almost planar conformation, and its core is accessible. When the degree of substitution increases from 6 to 12 (center) to 18 (right), the structure becomes more intricate, and its crown-ether core becomes less accessible, as steric bulk and repulsion of substituent groups increase. C is light blue, N is dark blue, and O is red.

were several who had developed commercially successful chiral selectors. Welch invented the Whelk-O chiral stationary phase while working on his Ph.D. Yoshio Okamoto, an emeritus professor at Nagoya University, in Japan, and a professor at Harbin Engineering University, in China, is well-known for cellulose- and amylose-based chiral stationary phases commercialized by the Japanese company Daicel Chemical Industries under the trade names Chiralcel and Chiralpak. "Chiralcel OD and Chiralpak AD are the superstars of the chiral-stationary-phase world," according to Welch.

Daniel W. Armstrong, a professor of chemistry and biochemistry at the University of Texas, Arlington, recently founded the start-up company AZYP to commercialize new chiral stationary phases just a few years after Sigma-Aldrich bought Advanced Separations Technologies (Astec), a company he cofounded to bring chiral selectors based on cyclodextrins and macrocyclic glycopeptides to market. And Myung Ho Hyun, a chemistry professor at Pusan National University, in South Korea, is the inventor of Chirosil, a crown-ether-based chiral stationary phase that has been commercialized by RStech Corp., in South Korea, and by Regis Technologies, in the U.S. In addition to crown-ether-based chiral stationary phases, Hyun is also developing ligand-exchange chiral selectors and chiral sensors.

These four chemists are all trained in organic synthesis, and their intellectual products represent a rich variety of chiral selectors, yet all say the field needs to advance. "A universal column or chiral stationary phase that can resolve every kind of racemic compound is not possible," Hyun told C&EN.

"You always like to have an influx of talent no matter what area; the more people you have involved, the faster and further it will advance," Armstrong added.

"There remains a need for new phases with special selectivity or other properties," Welch said.

For large-scale production of commercially valuable molecules, such as chiral drugs, Okamoto said, "we need a diverse library" of stationary phases to get the most efficient preparative separation possible. "If we have really nice columns," Okamoto contended, synthetic chemists might not always need to make single enantiomers; even a racemate would be okay.

"Some pharmaceuticals are manufactured with the help of chromatographic purification because it is cost-effective," Welch added, explaining that all large-scale chiral chromatography involves taking the undesired enantiomer, racemizing, and rechromatographing it until all the material comes through as the desired enantiomer. "Chromatography can enable some things that are really difficult or impossible," he said. "It is an extremely strong synthetic tool and needs to be part of the synthetic toolbox."

And even if asymmetric synthesis turns out to be a more economical production route than chiral chromatography, "you still need the analytical chiral separation for quality control," Armstrong told C&EN. "You need analysis every step of the way, from discovery to the very end, regardless of the route you take." Thus, there remains a great need for new chiral stationary phases with substantial impact on enantiomeric separations, he explained.

CYCLOFRUCTANS ARE set to have that kind of impact, Armstrong argued. He is so convinced of their worth that he has invested his own money in the start-up AZYP to commercialize the new class of chiral stationary phases.

Cyclofructans are macrocyclic oligosaccharides consisting of six or more β(2→1)-linked D-fructofuranose units around a crown-ether skeleton. They are produced inexpensively by bacterial fermentation of inulin—which are plant polysaccharides built largely from fructose—or by incubation of inulin with the enzyme cycloinulo-oligosaccharide fructanotransferase. The compounds have been known since 1989 and have been used in various applications, such as in a food additive to reduce bitterness, but it was Armstrong's group that first laid claim to them as chiral selectors. AZYP now sells four cyclofructan chiral stationary phases under the brand name Larihc (chiral spelled backward).

"Cyclofructans are unique in that they are almost universal for chiral primary amines and separate them in organic solvents or in supercritical fluids," Armstrong said at the symposium. That's important because the only other way to separate primary amines is in acidic water. Pharma companies—the major users of chiral stationary phases—would love to avoid water and to use organic solvents or supercritical fluids instead, both of which are a lot easier to remove, he said.

Armstrong believes that the cyclofructan's selectivity for primary chiral amines is based on the crown-ether core, which is blocked in the natural 6-fructose cyclofructan, but opens and becomes accessible to guests when various hydroxyl groups are derivatized. Alkyl groups in the hydroxyl positions yield chiral stationary phases that favor primary chiral amines, whereas aromatic groups yield ones that are broadly selective for non-primary-amine compounds, such as secondary amines, acids, and neutral molecules. His group's work has convinced Armstrong that with cyclofructans it might be possible to determine which chiral stationary phase will work for a given compound without the need for screening.

As for the clamor for more people to design and prepare chiral selectors, China could provide the answer. "Right now, many groups in China are devoting research programs to designing chiral stationary phases," Armstrong told C&EN. Okamoto himself has a research group in China; the latest work he presented at the symposium—a new amylose-based chiral selector that is derivatized at positions that had been difficult to reach before—is one that he developed there. Indeed, a search of the Chemical Abstracts Service database reveals a surge in patent and literature output on chiral selectors from China during the period 2000–09. ■

CHIRAL LEAP

In the past decade, China led the countries that publish most on chiral selectors

	2000–09	1990–99
China	[illegible]	[illegible]
U.S.	[illegible]	[illegible]
Japan	[illegible]	[illegible]
Italy	[illegible]	[illegible]
South Korea	[illegible]	77
France	[illegible]	[illegible]

NOTE: Data include the sum of journal and patent publications attributed to a country for the indicated periods. **SOURCE:** Chemical Abstracts Service

"New chromatographic stationary phases have to come from somewhere—and that is organic synthesis."