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Enantiomer Separations and Fast Chiral Selector Screening

……on Stable Zirconia-Based Chiral Stationary Phases

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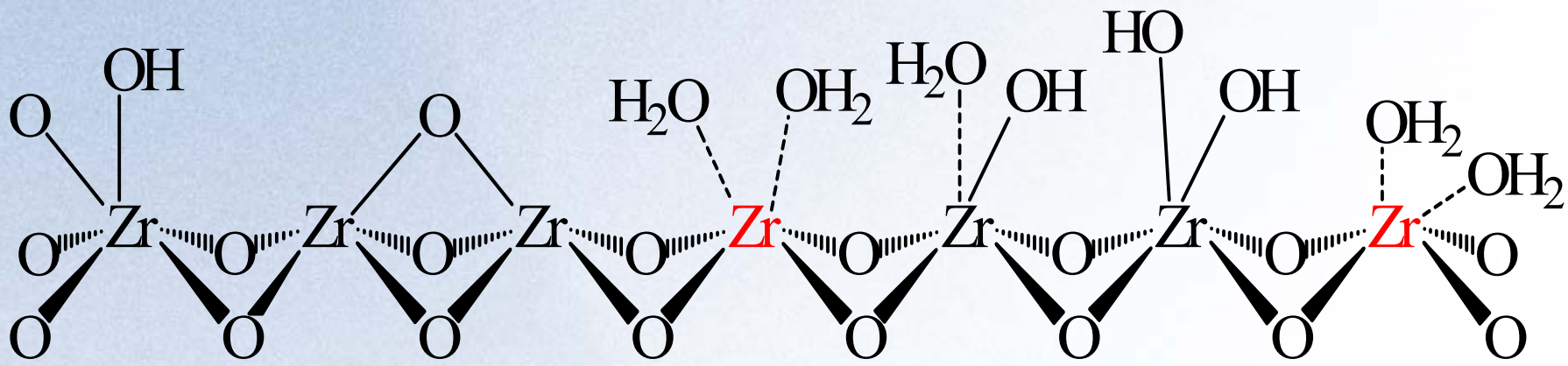
Specialists in High Efficiency, Ultra-Stable Phases for HPLC

Surface Chemistry of Zirconia



Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid: $Zr^{4+}: H_2O + RPO_3^{2-} \rightleftharpoons Zr^{4+}: RPO_3^{2-} + H_2O$

Other Lewis base examples: PO_4^{3-}, RCO_2^-, Catechol

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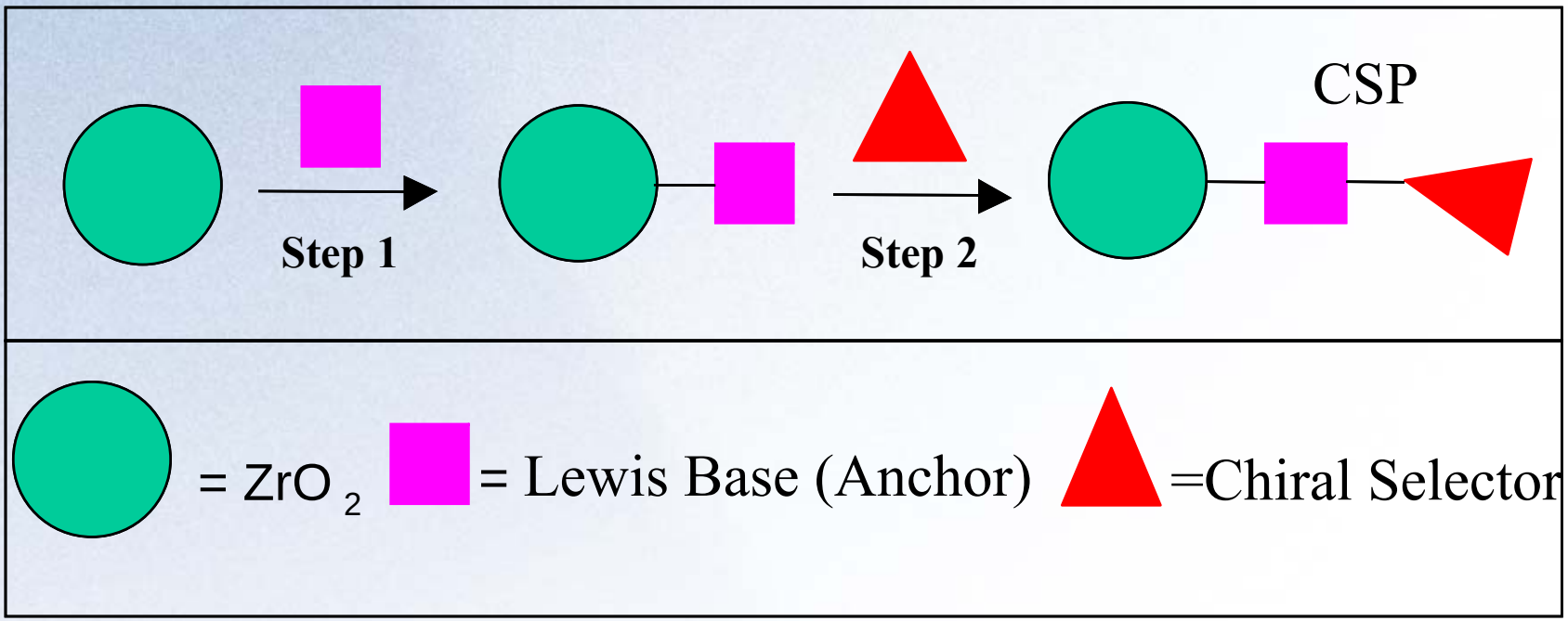
A Novel Approach to Attaching Chiral Selectors[1] to Zirconia[2]



1. William H. Pirkle, et. al., J. Chromatogr., 316 (1984) 585.

2. Phase I SBIR Grant (NIH).

Three Anchor Groups Tested

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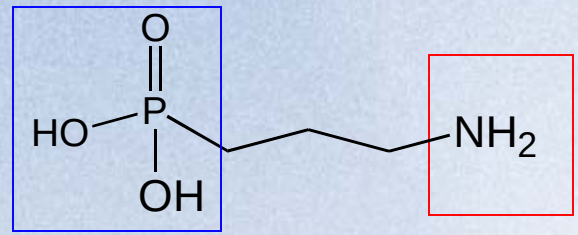
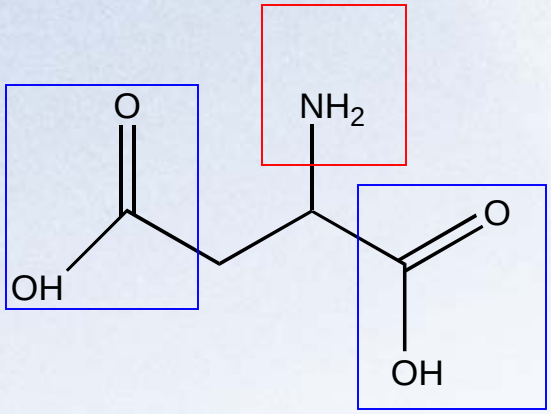
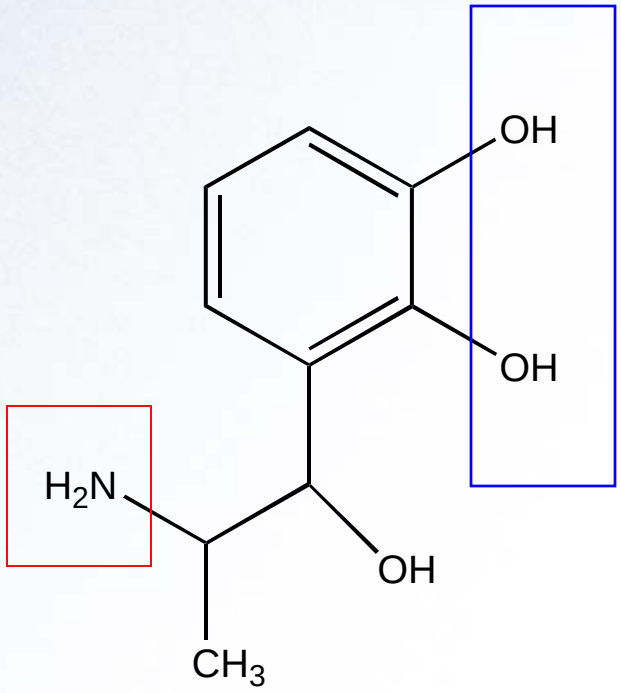


APPA (Aminopropylphosphonic acid)



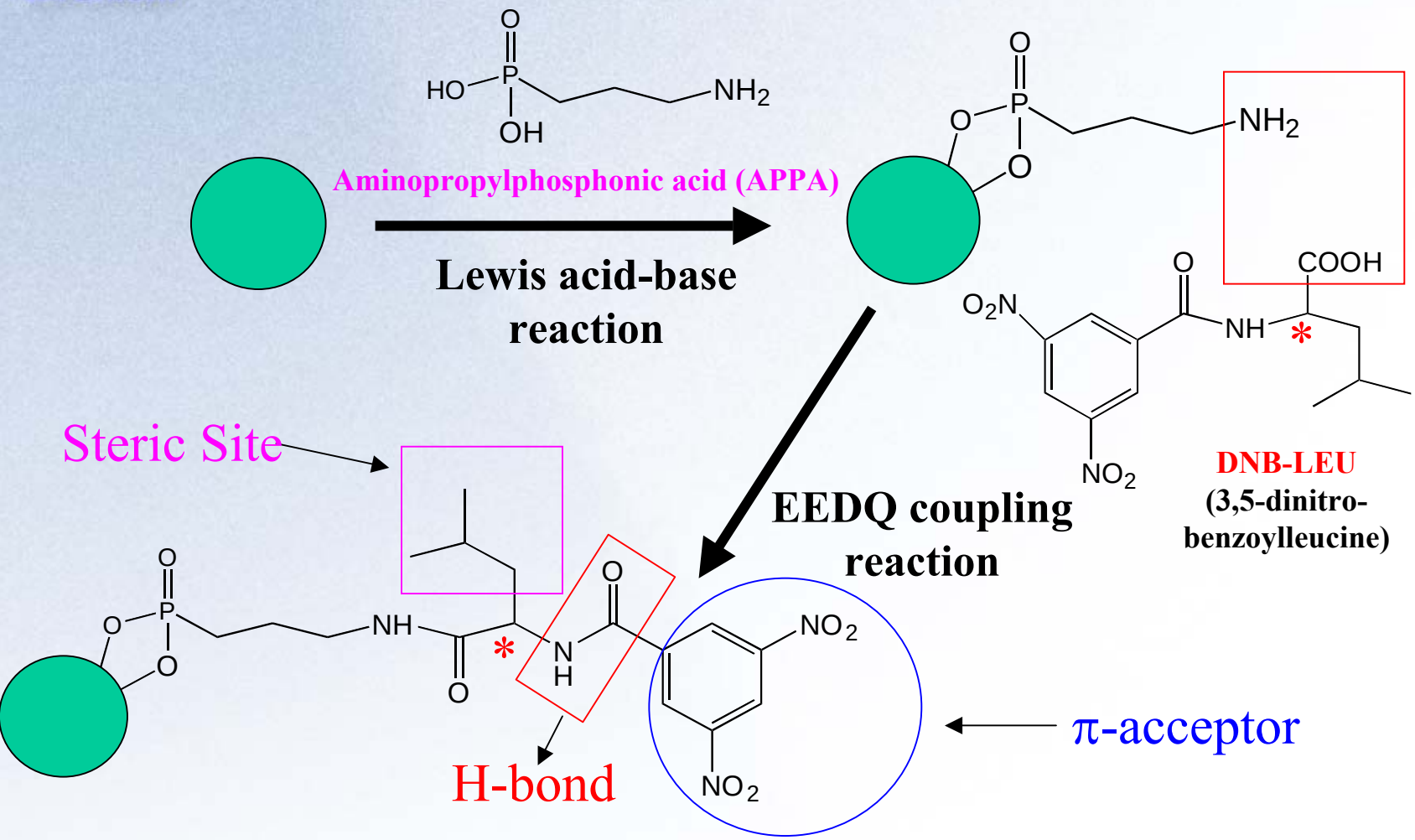
ASPA (Aspartic acid)



DHNP (3,4-Dihydroxynorephedrine)

Phase I Anchors

Zirconia CSP 2-Step Synthesis



Enantiomer Separations on Zirconia DNB-L-LEU

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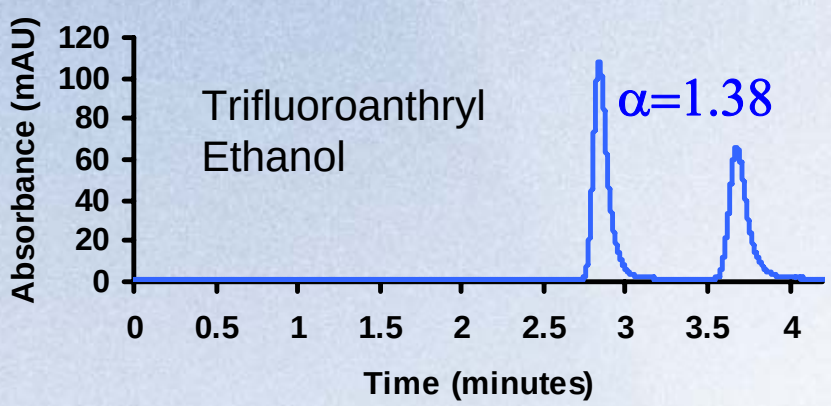
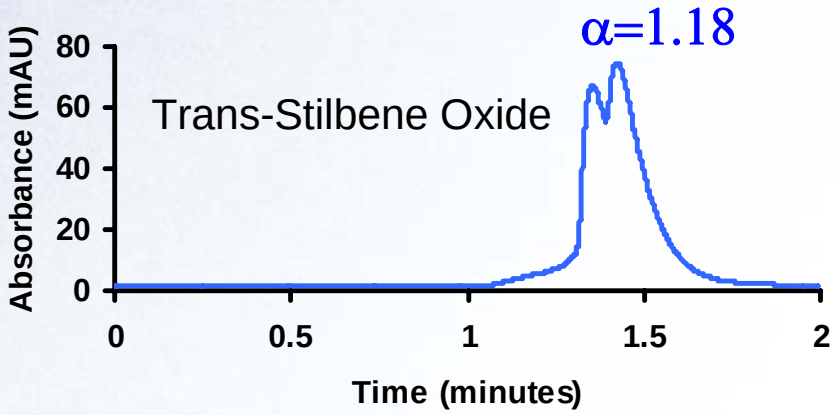
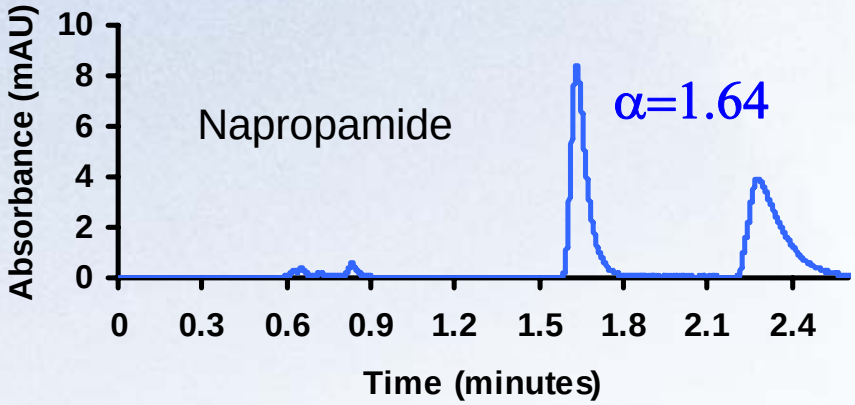
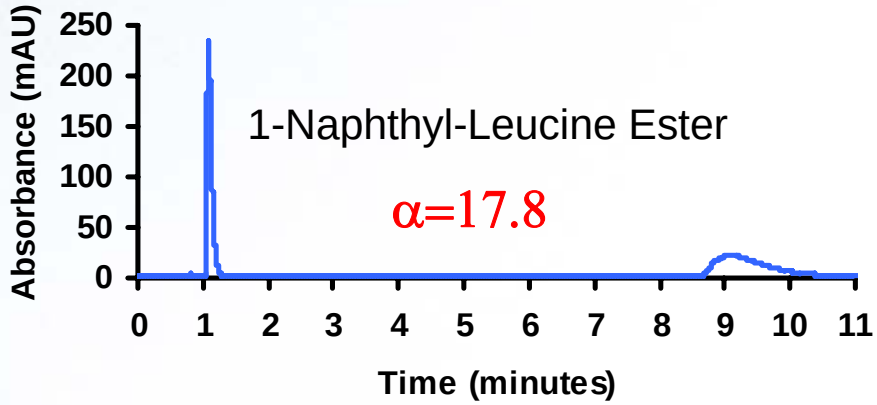


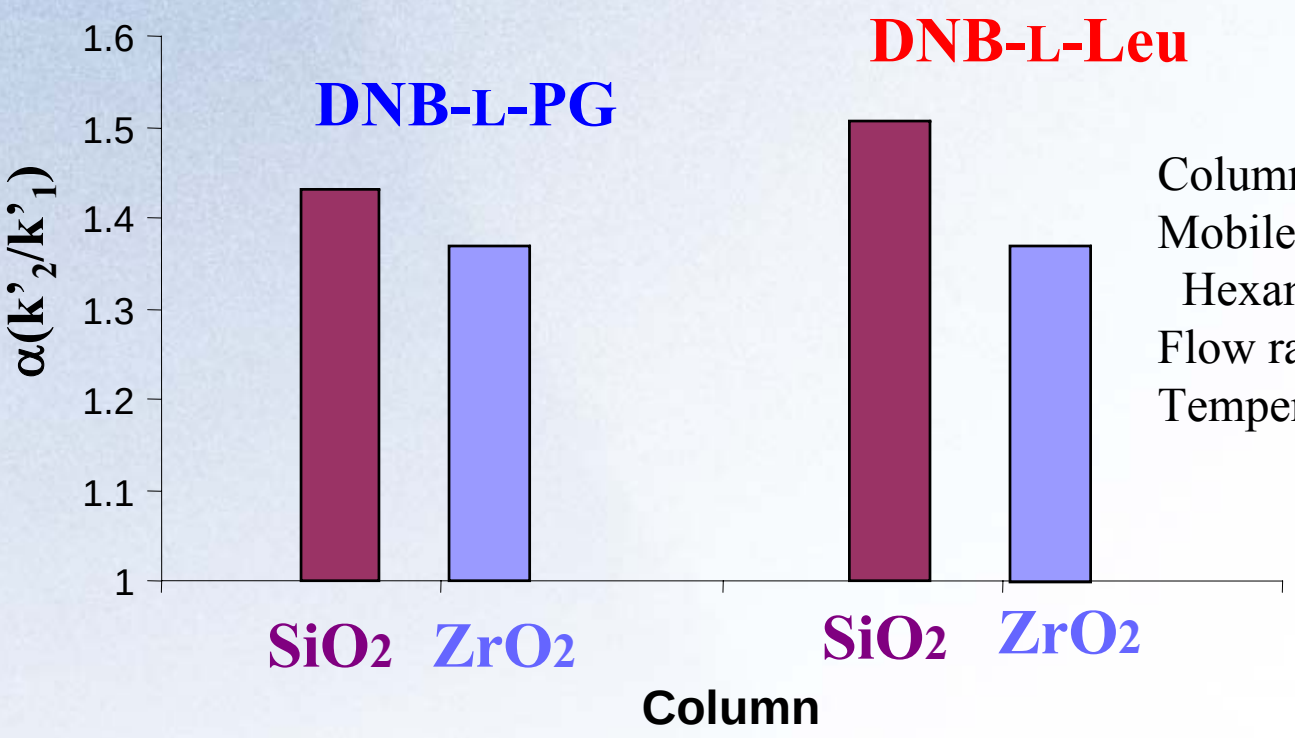






Selected chromatograms of chiral compounds on Zirconia DNB-L-LEU anchored with APPA. Chromatographic conditions: 50x4.6mm, 3µm, 99/1 Hexane/IPA.

Selectivity Comparison of Zirconia and Silica CSPs



Column: 50x4.6mm, 3µm
Mobile phase: 99/1
Hexane/IPA
Flow rate: 1 ml/min
Temperature: 30 °C

Zirconia CSPs (APPA anchor) compare favorably to Silica CSPs with trifluoroanthryl ethanol .

Comparison of DNB-L-LEU Zirconia and Silica CSP Selectivity

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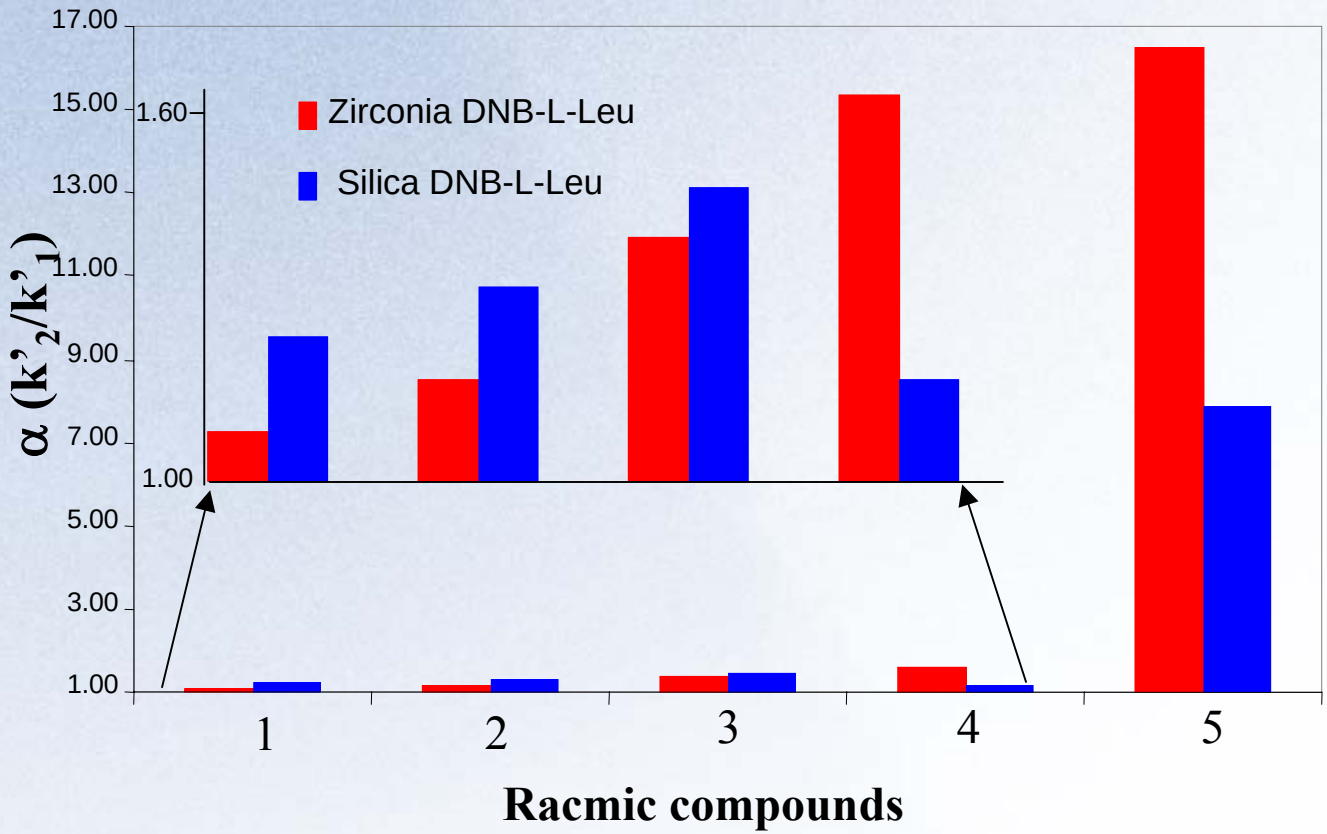

1 trans-stibene oxide
2 1,1'-bi-2-naphthol
3 trifluoranthyl ethanol
4 napropamide
5 1-naphthyl leucine ester

Mobile phase:
99/1Hexane/IPA
Flow rate: 1 ml/min;
Temperature: 30 °C

Zirconia CSP (APPA anchor) shows enhanced separation for napropamide and 1-naphthyl leucine ester.

Stability of Zirconia DNB-L-LEU with Different Anchors

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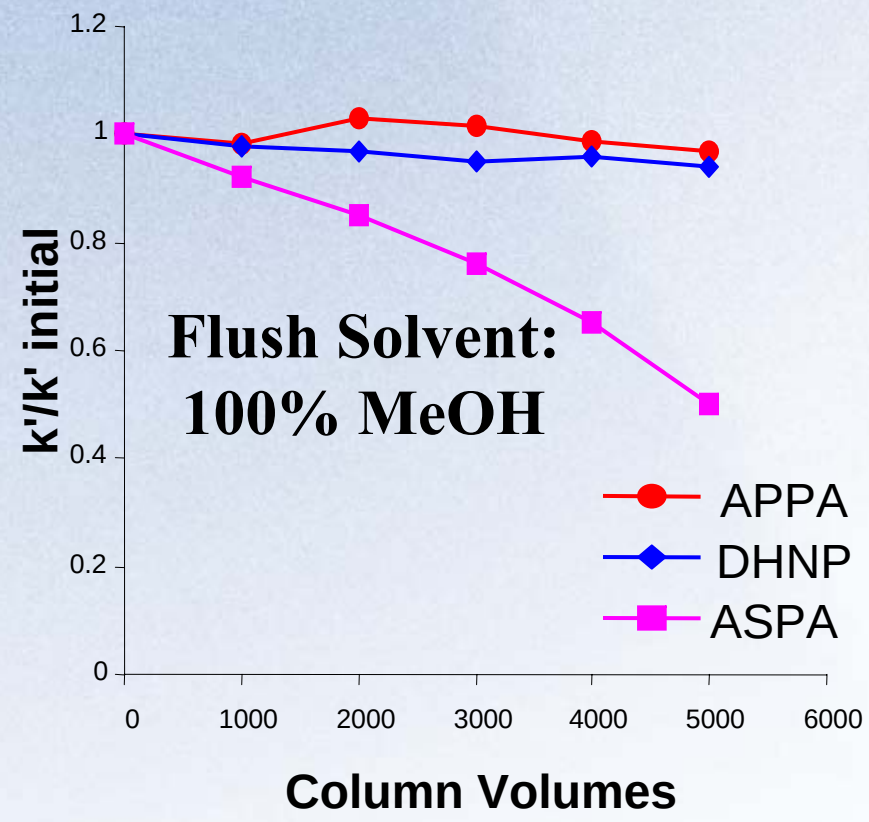

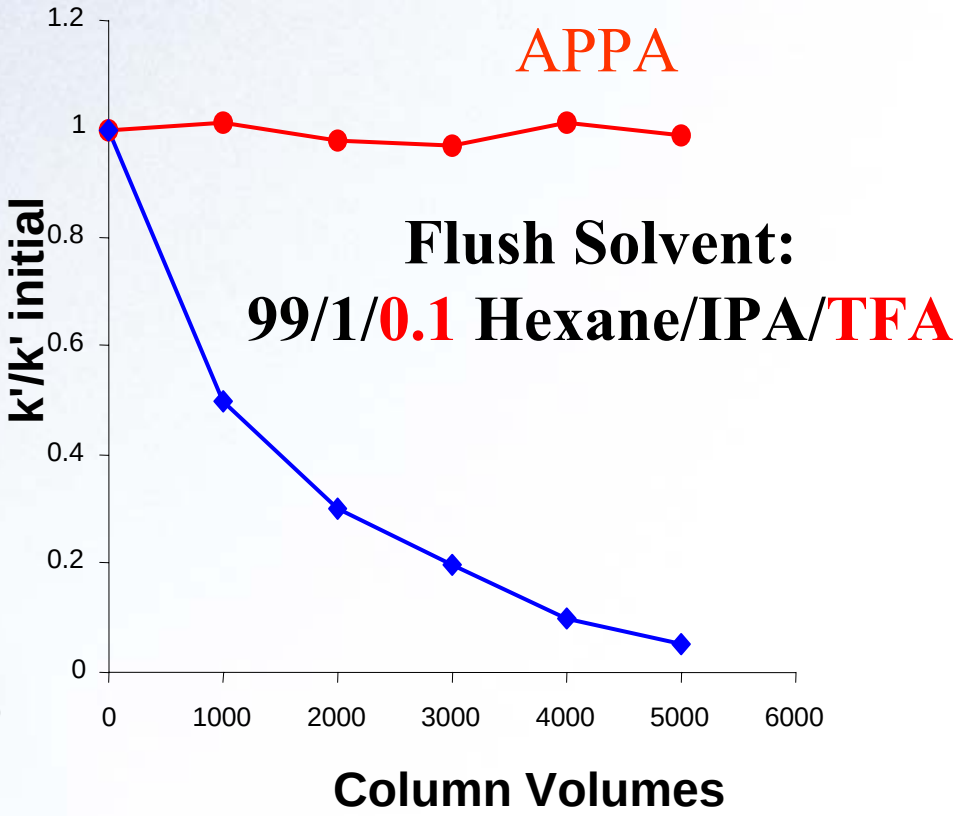

Test solute: trifluoranthryl ethanol (retention factor ratio for less retained isomer)

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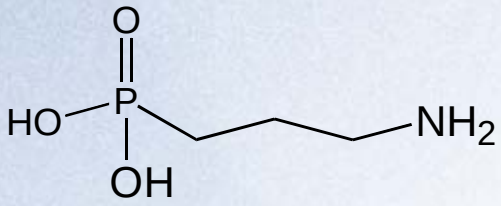
Interaction Strength of Lewis Bases with Zirconia[1]

Interaction Strength	Lewis Base (L)
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
	Nitrate
	Chloride
Weakest	Water

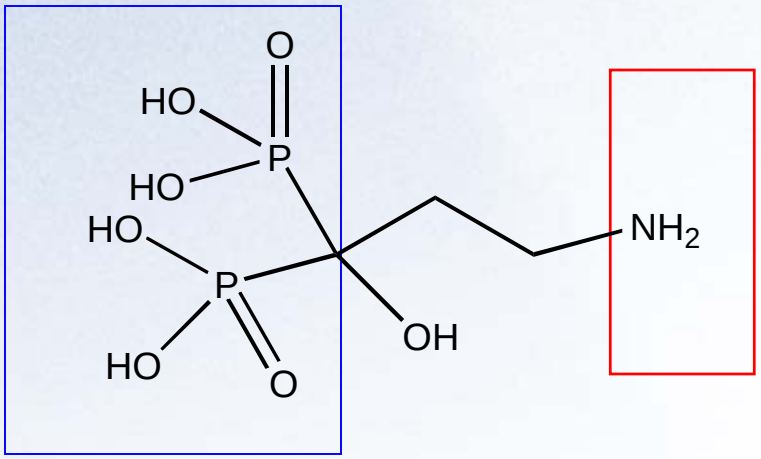
Small Lewis bases with high electron density and low polarizability interact more strongly with Zr atoms.

1. J.A. Blackwell and P.W. Carr, "Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide," Anal. Chem. 64, 863-73 (1992).

A Bidentate Phosphonate Anchor– the Key to Improved Stability[1]



Aminopropylphosphonic acid (APPA)

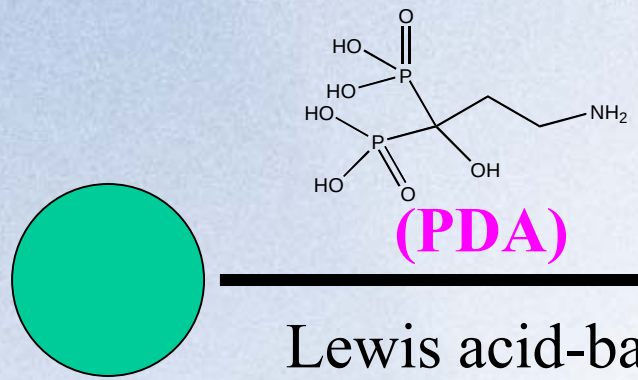


Pamidronic acid (PDA)[1]

(Phase II Anchor)

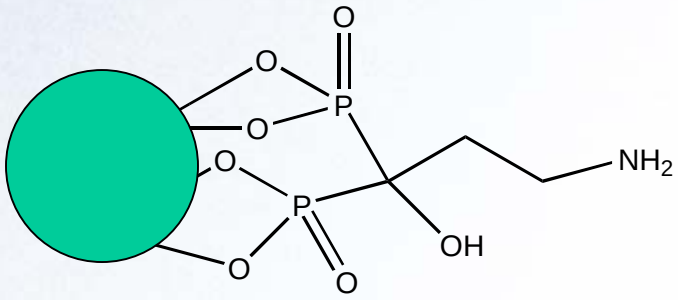
1. Phase II SBIR (NIH).

Zirconia CSP 2-Step Synthesis with Bidentate Anchor (PDA)

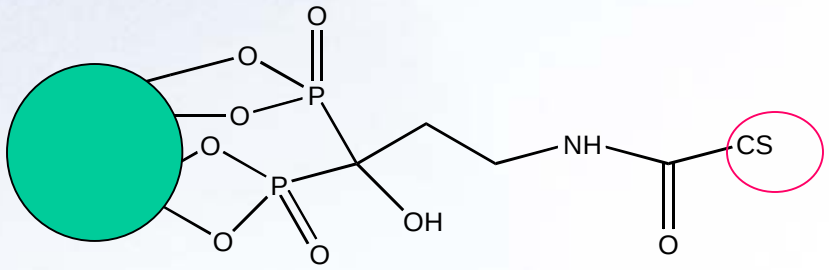


(PDA)

Lewis acid-base reaction

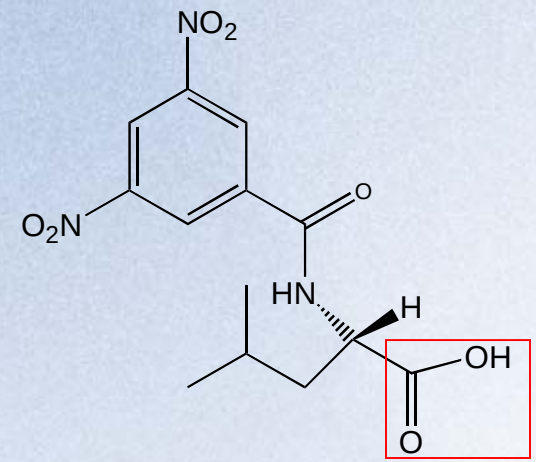


CS-COOH
EEDQ coupling reaction



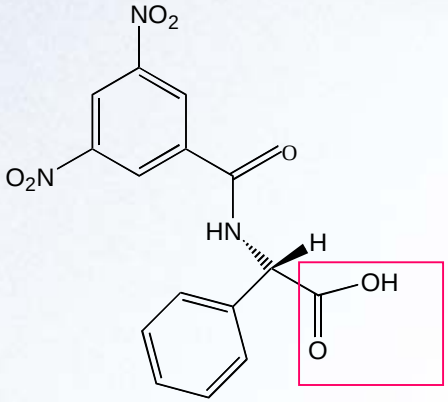
CS = Chiral Selector

Chiral Selectors Evaluated[1]



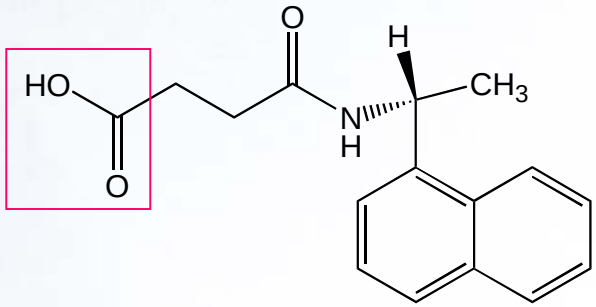
(S)-DNB-L-Leucine

[(S)-Leu]



(S)-DNB-L-Phenylglycine

[(S)-PG]

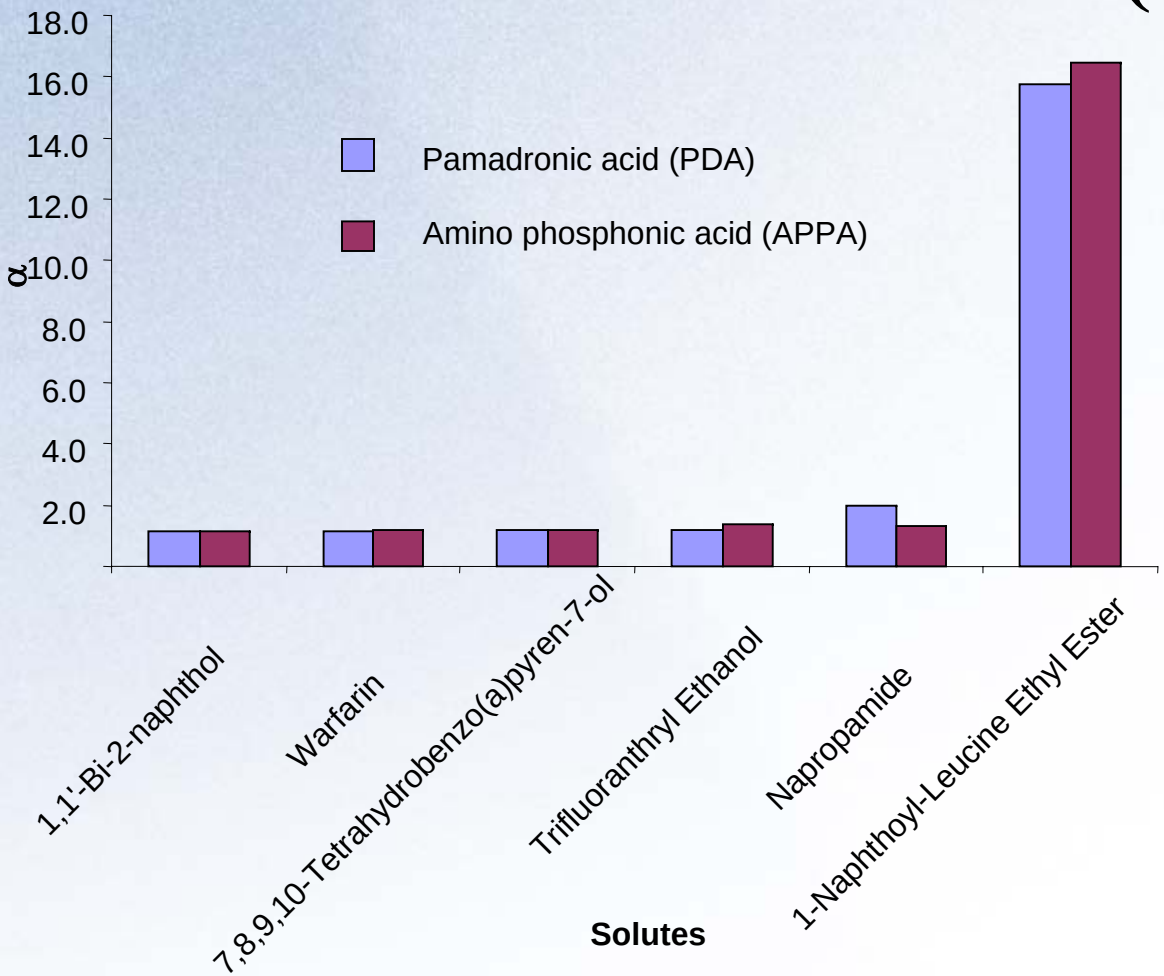


(S)-N-[1-(1-naphthyl)ethyl]succinamic acid

[(S)-NESA]

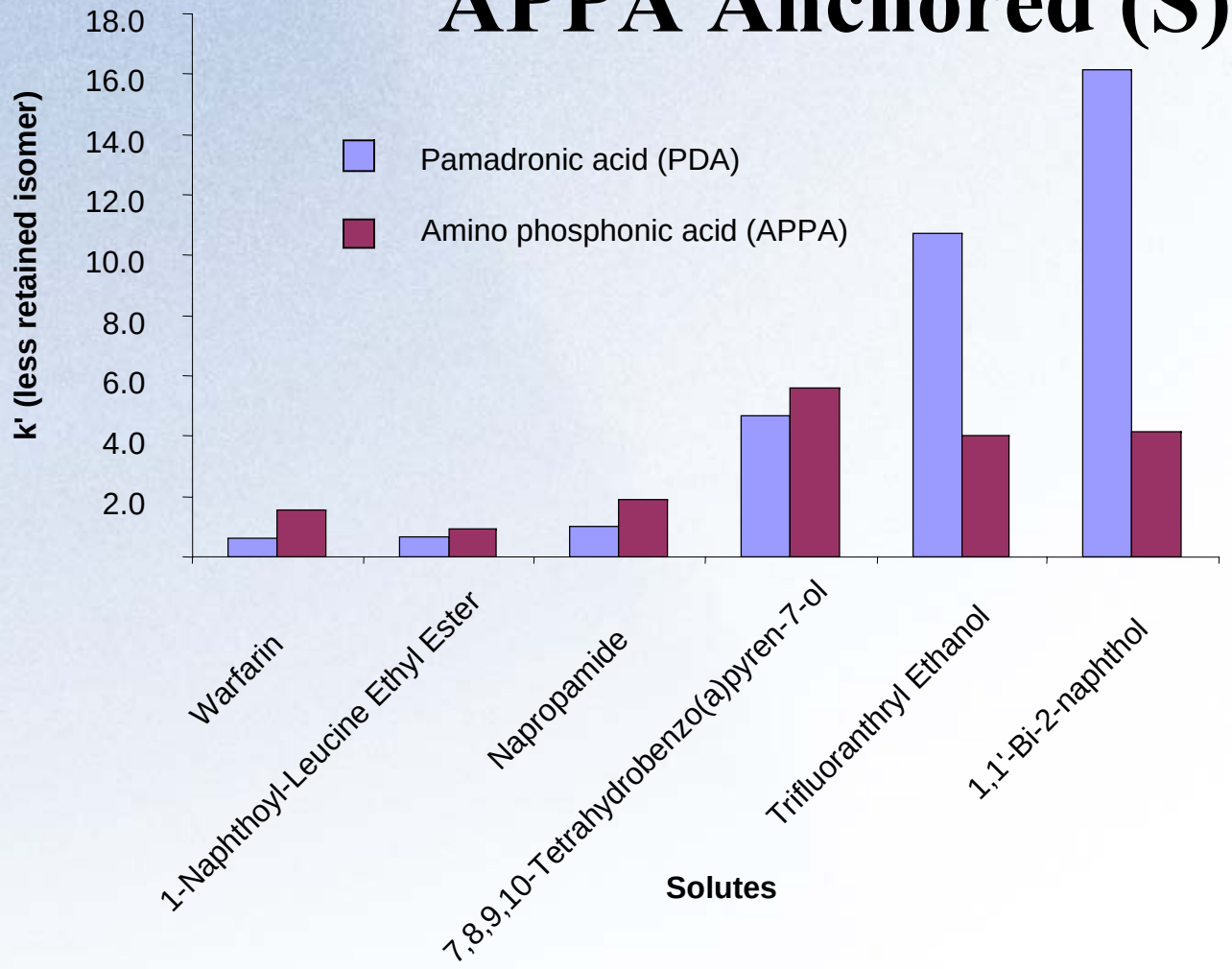
1. Phase II SBIR (NIH)

Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



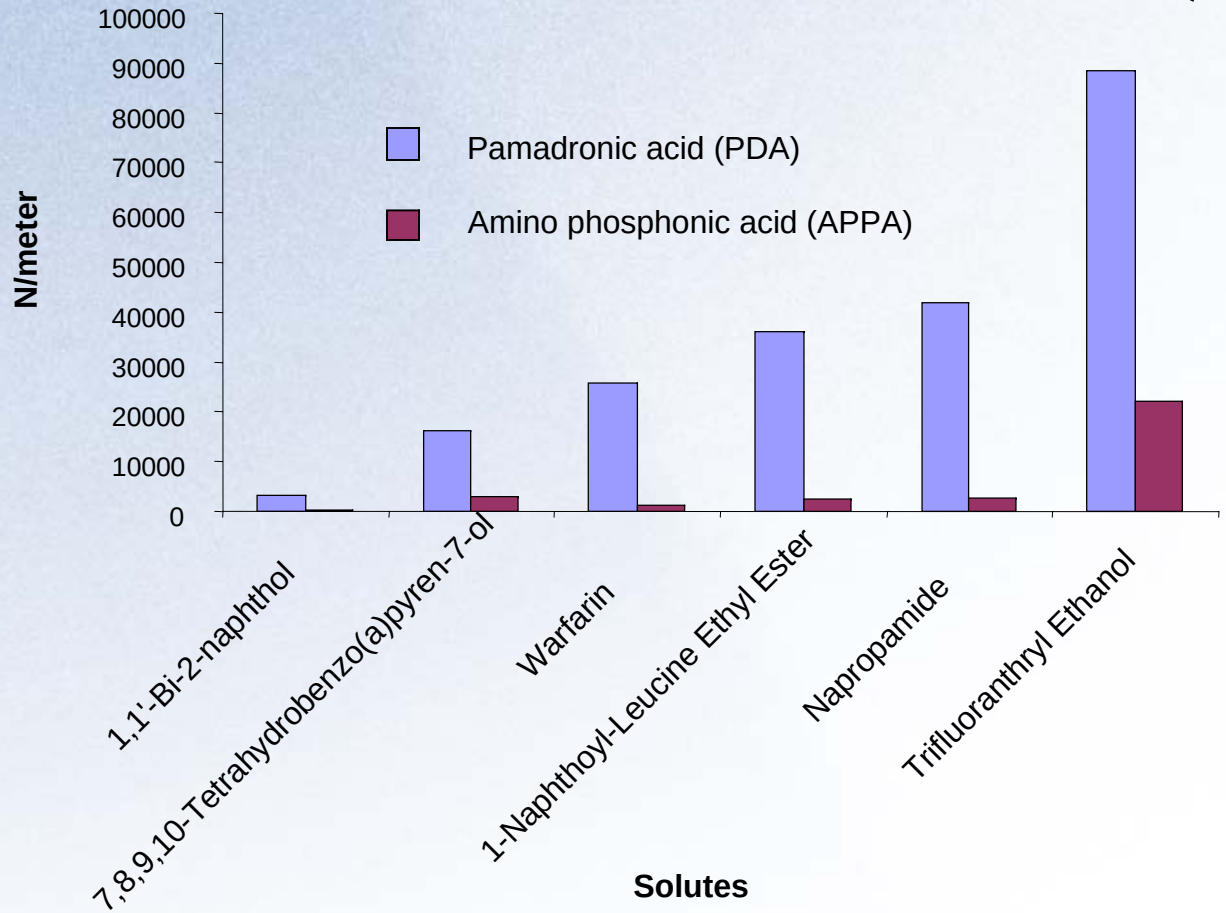
Selectivity for the two anchors is similar.

Retention Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



Retention for the two anchors is different.

Efficiency Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



Efficiency on PDA anchored Zr (S)-Leu is much better.

Chiral Separation on Zr (S)-Leu (π-acceptor phase)

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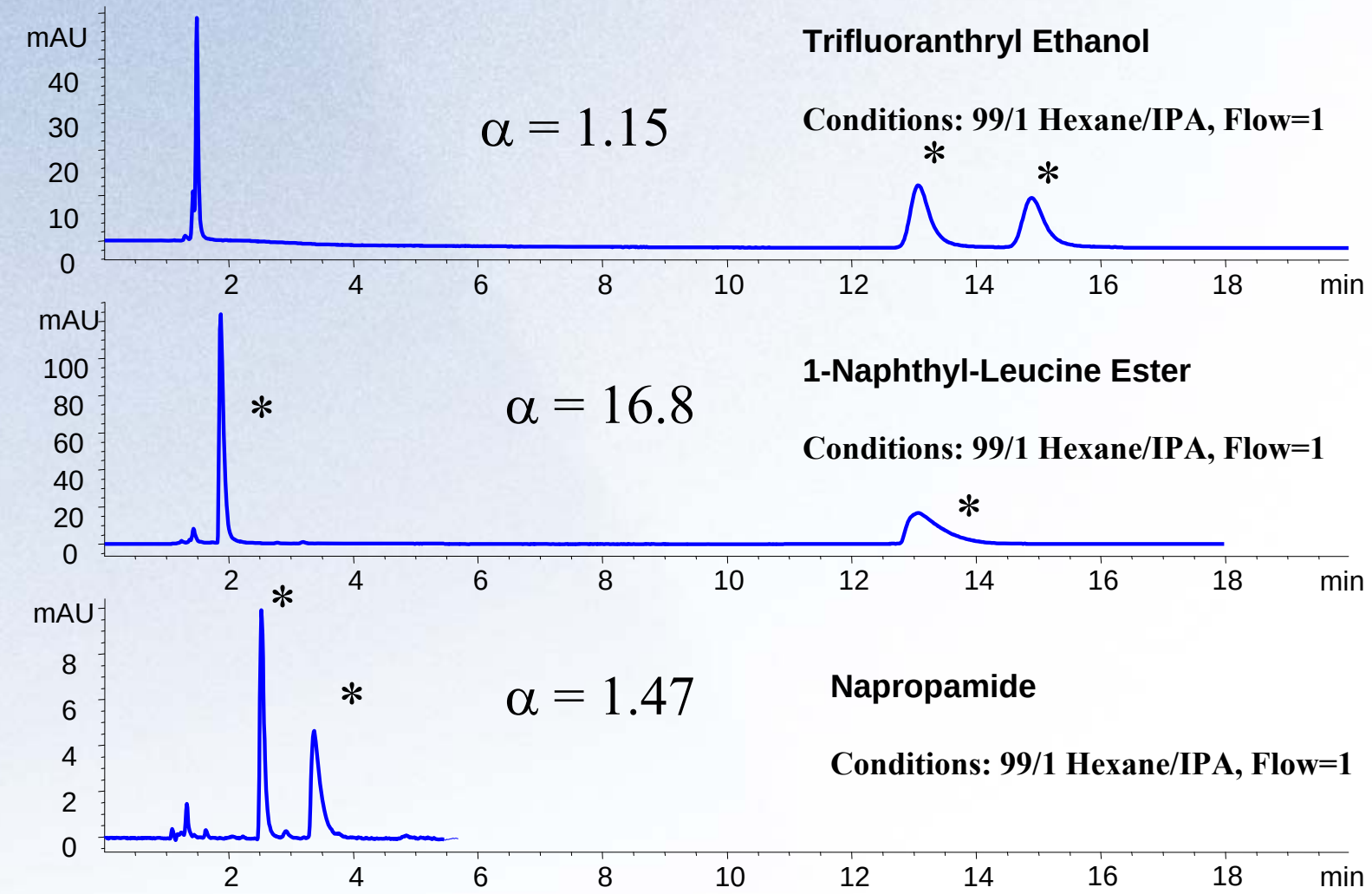

Chiral Separation on Zr (R)-PG (π-acceptor phase)

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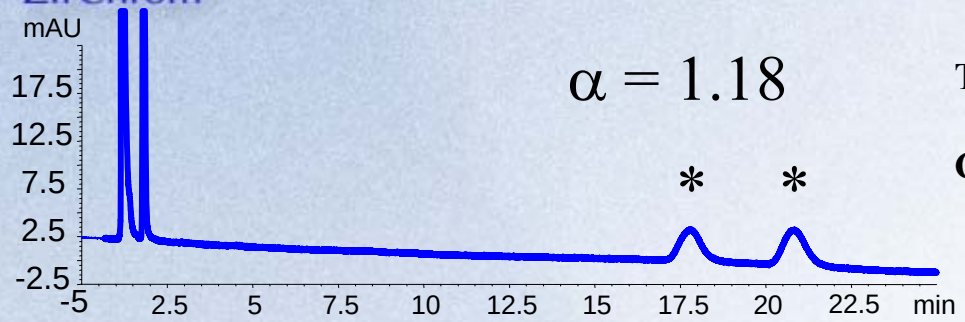
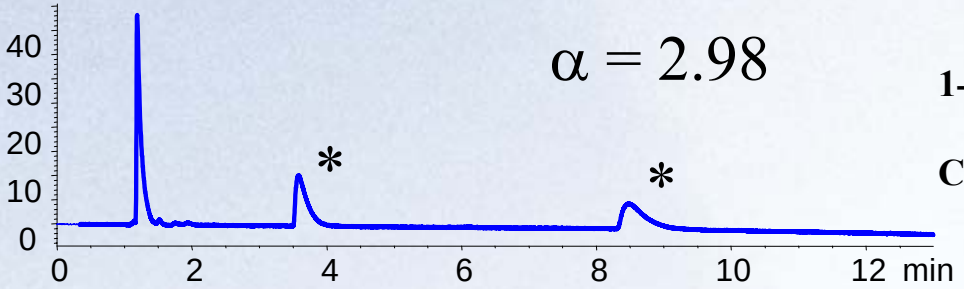


$\alpha = 1.18$

Trifluoranthryl Ethanol

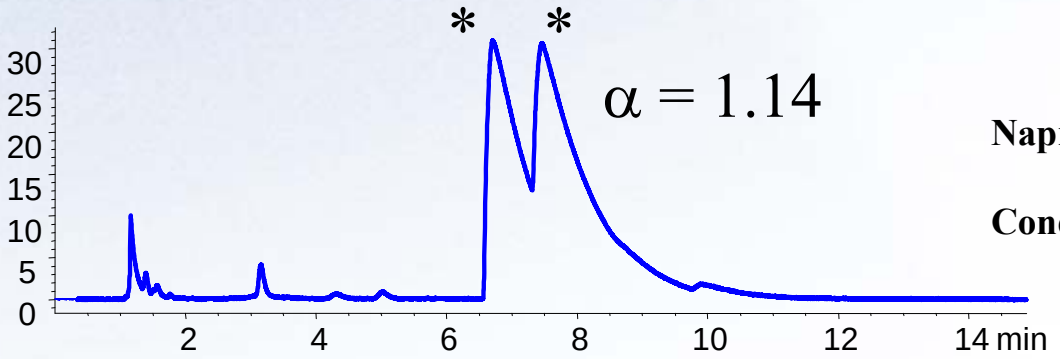
Conditions: 99/1 Hexane/IPA, Flow=1



$\alpha = 2.98$

1-Naphthyl-Leucine Ester

Conditions: 99/1 Hexane/IPA, Flow=1



$\alpha = 1.14$

Napropamide

Conditions: 99/1 Hexane/IPA, Flow=1

Chiral Separations on Zr (S)-NESA (pi-donor phase)

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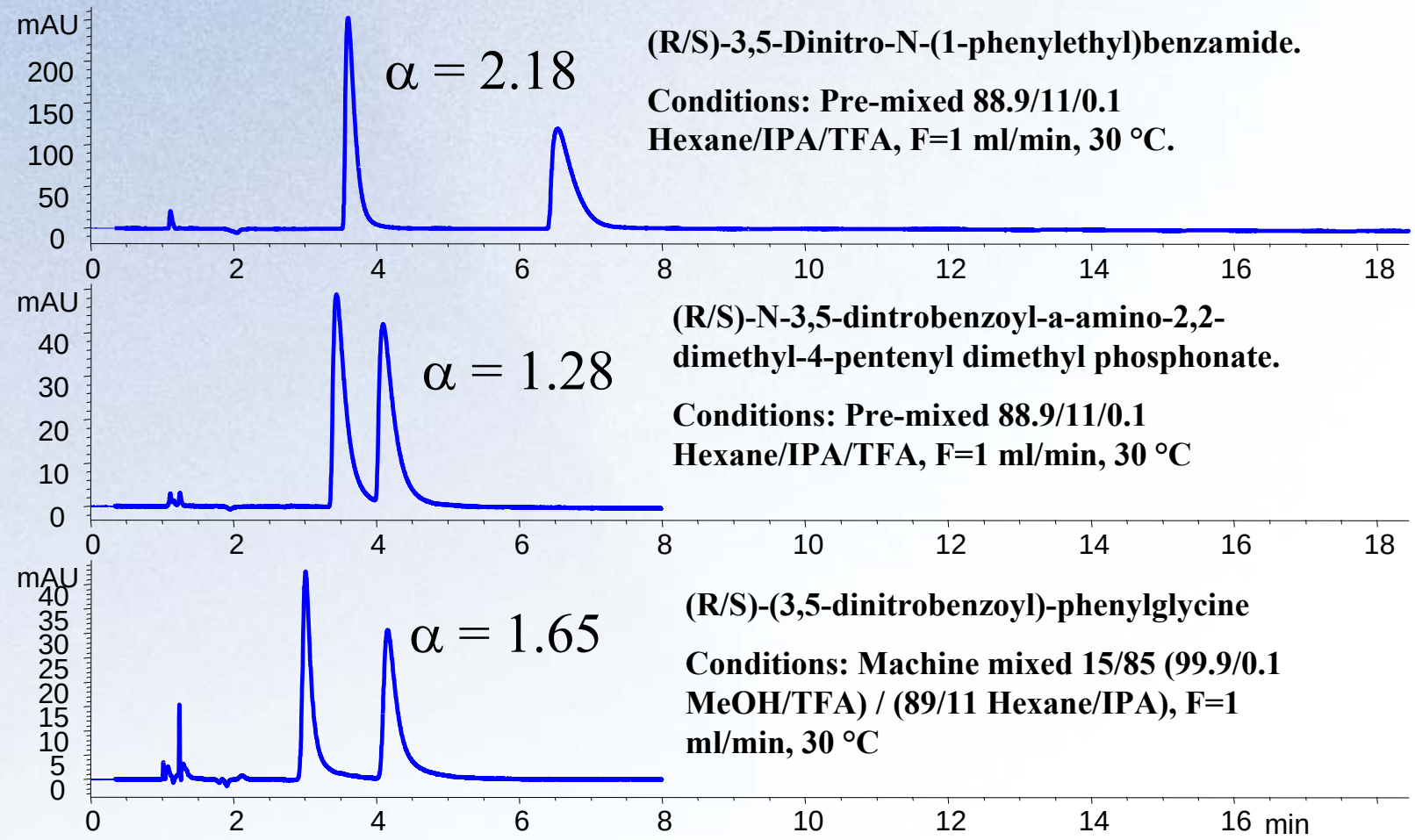


(R/S)-3,5-Dinitro-N-(1-phenylethyl)benzamide.

Conditions: Pre-mixed 88.9/11/0.1 Hexane/IPA/TFA, F=1 ml/min, 30 °C.

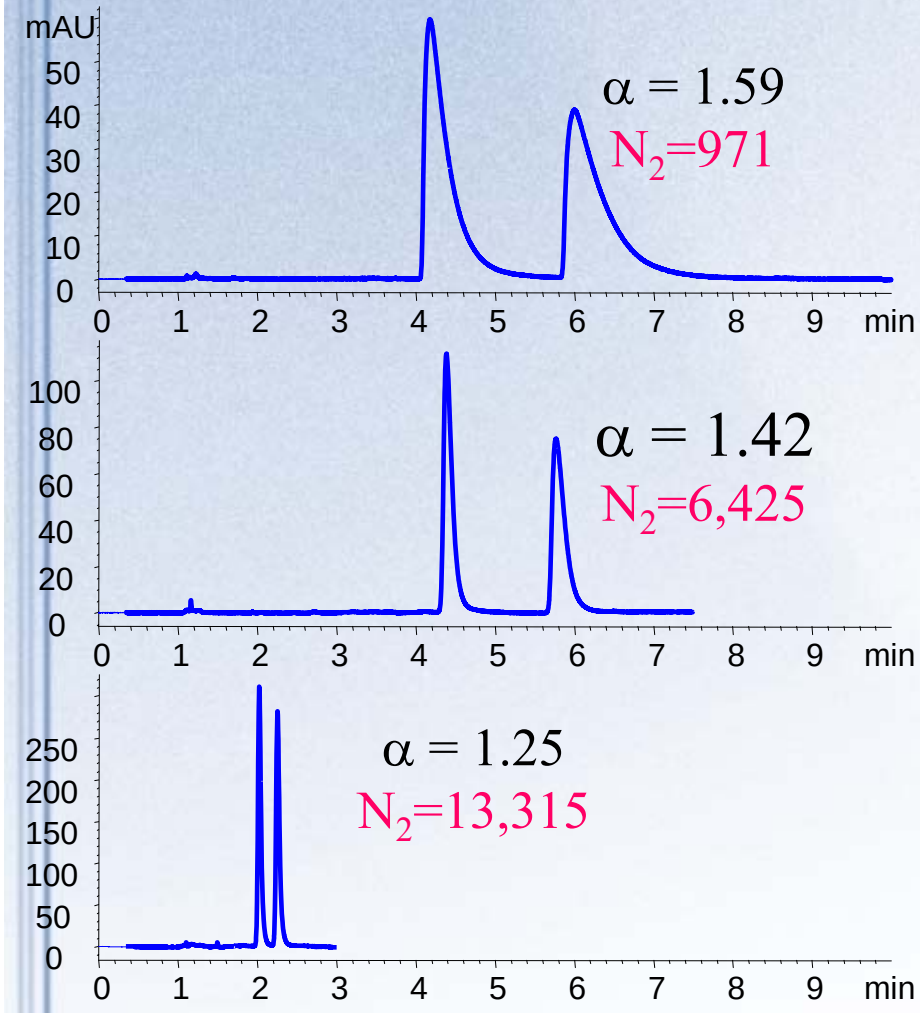
(R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate.

Conditions: Pre-mixed 88.9/11/0.1 Hexane/IPA/TFA, F=1 ml/min, 30 °C

(R/S)-(3,5-dinitrobenzoyl)-phenylglycine

Conditions: Machine mixed 15/85 (99.9/0.1 MeOH/TFA) / (89/11 Hexane/IPA), F=1 ml/min, 30 °C

Methanol Effect on Zr (S)-NESA



Sample: (R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate

Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.

Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

Conditions: 80 / 10 / 10 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

Stability of Zr-(S)-NESA at pH 2

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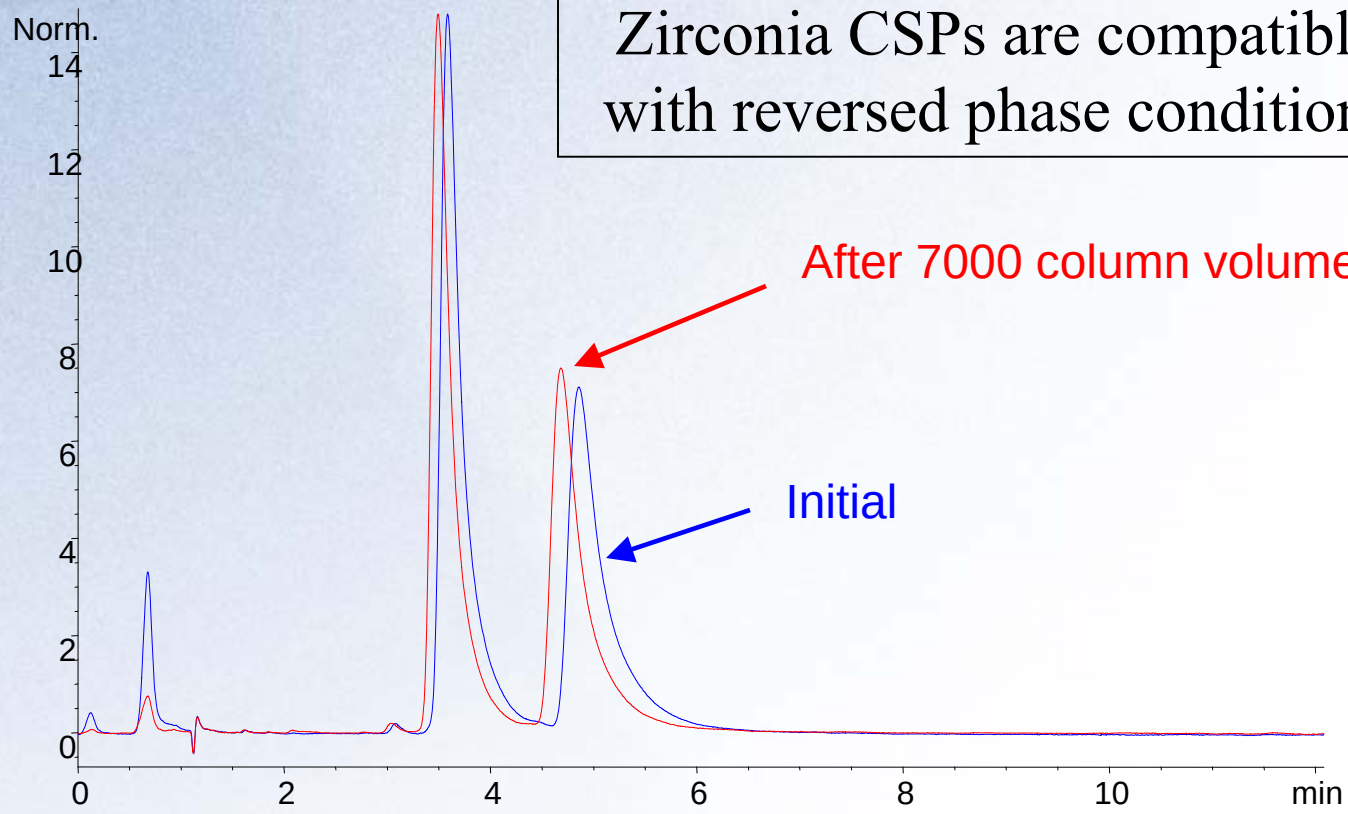
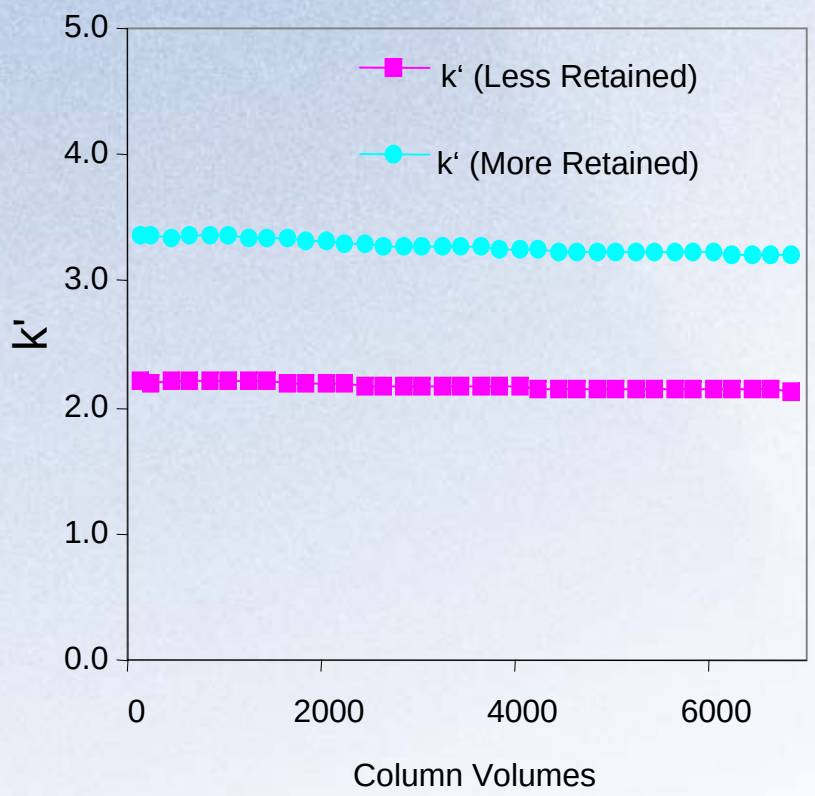
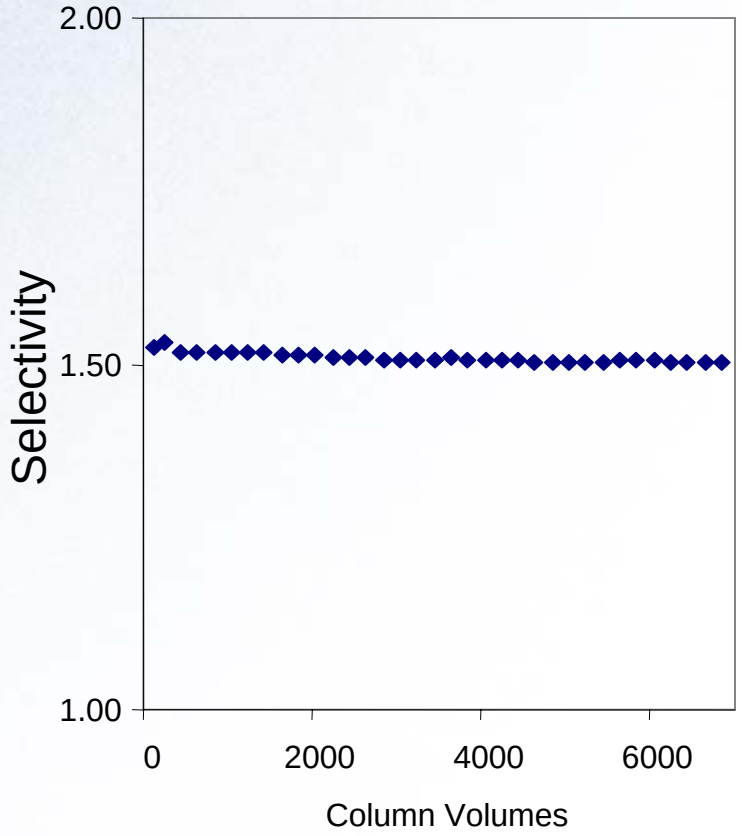
Zirconia CSPs are compatible with reversed phase conditions



Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

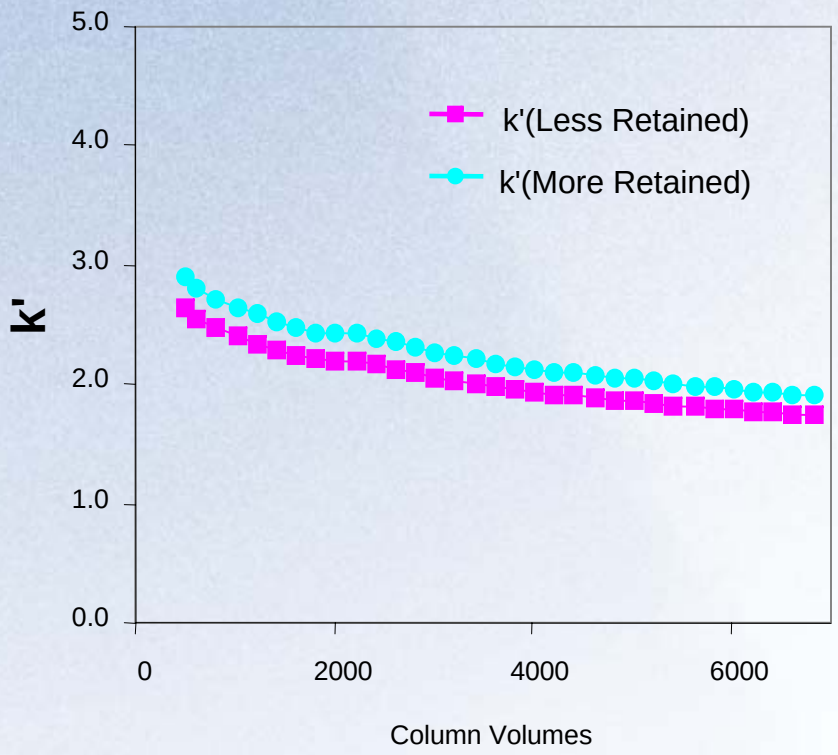
Stability of Zr-(S)-NESA at pH 2

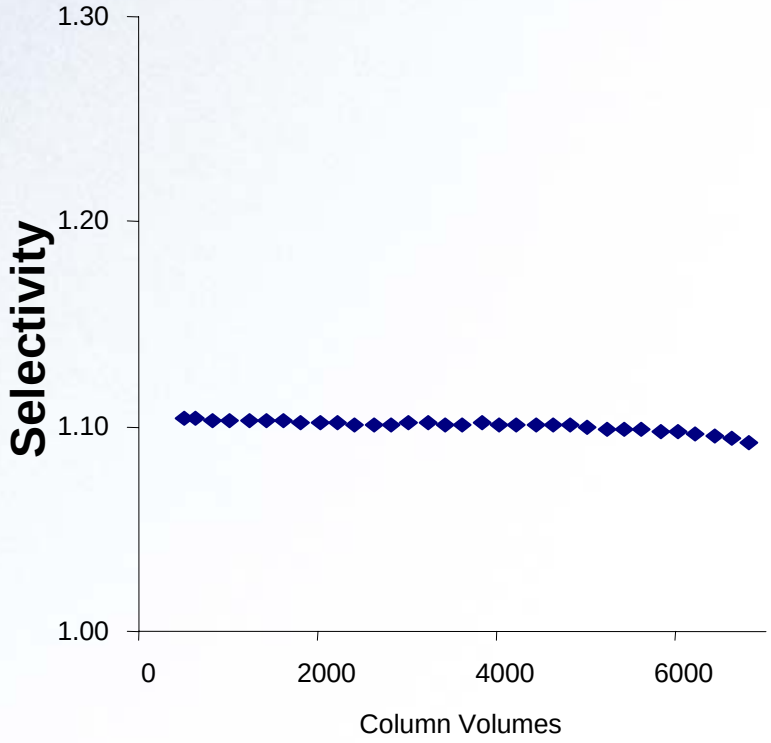




Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

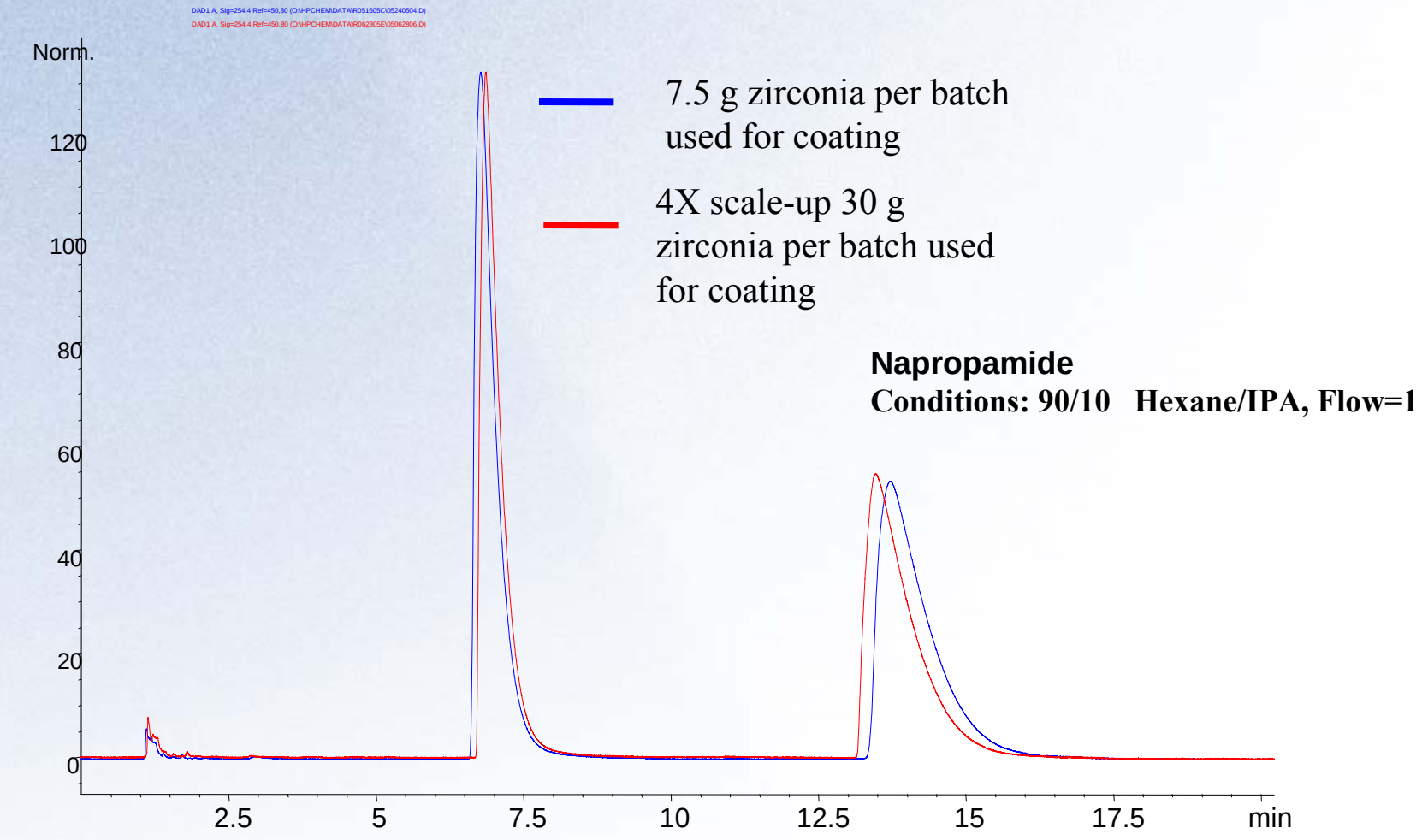
Stability of Zr-(S)-DNB-Leu at pH 8



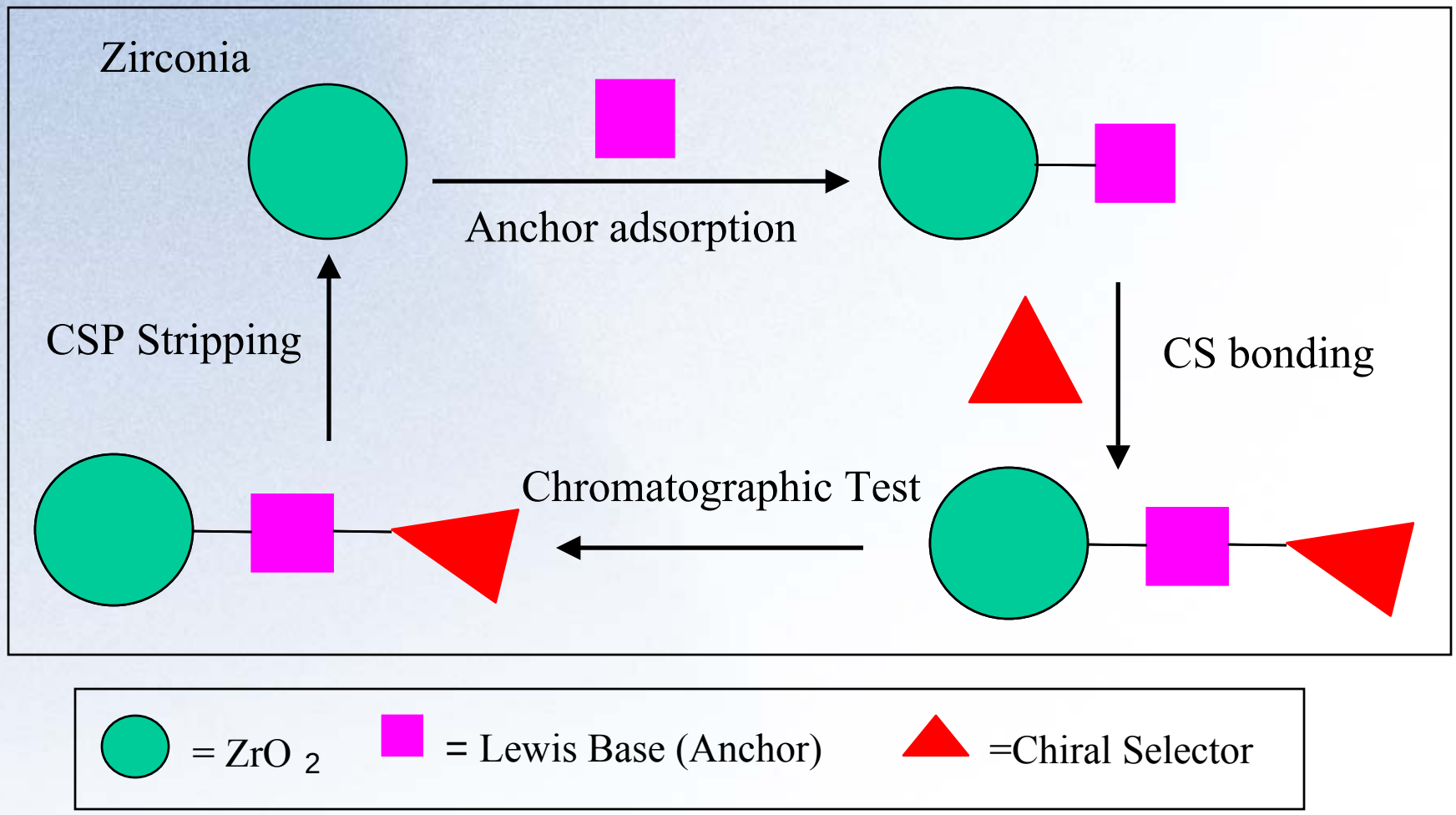


Column ID: ZrCSP032805A, Mobile phase: 15/85 ACN/5 mM ammonium hydrogencarbonate pH 8.0, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-2, 2, 2-trifluoro-1-(9-anthryl)ethanol

Production Scale-up of Zr (S)-NESA

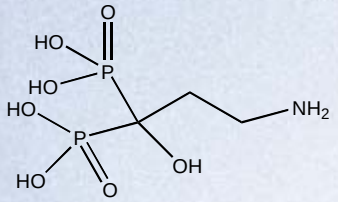


2-Step Synthesis of Zirconia CSPs for Chiral Selector Screening



2-Step Online Zirconia CSP Synthesis for Chiral Screening

ZirChrom®

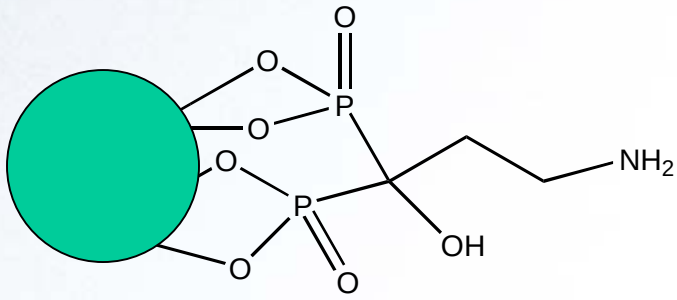


(Pamidronic acid)

ZrO_2

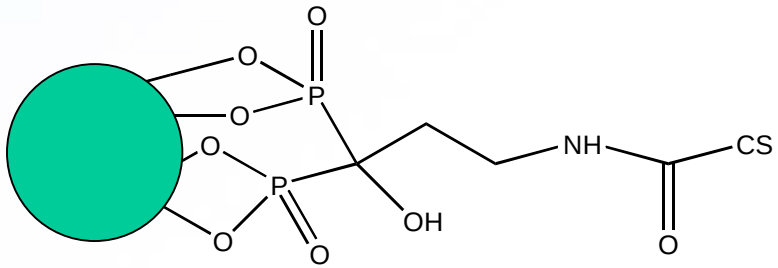
1) Lewis Acid-base Anchor Attachment (10 mg/mL aq. PDA, recycle for 16 hrs at 1 mL/min and 60°C)

ZrO_2-PDA



2) EEDQ Coupling Reaction (CS reagent in THF for 16 hrs at 30°C)

ZrO_2-PDA-CS



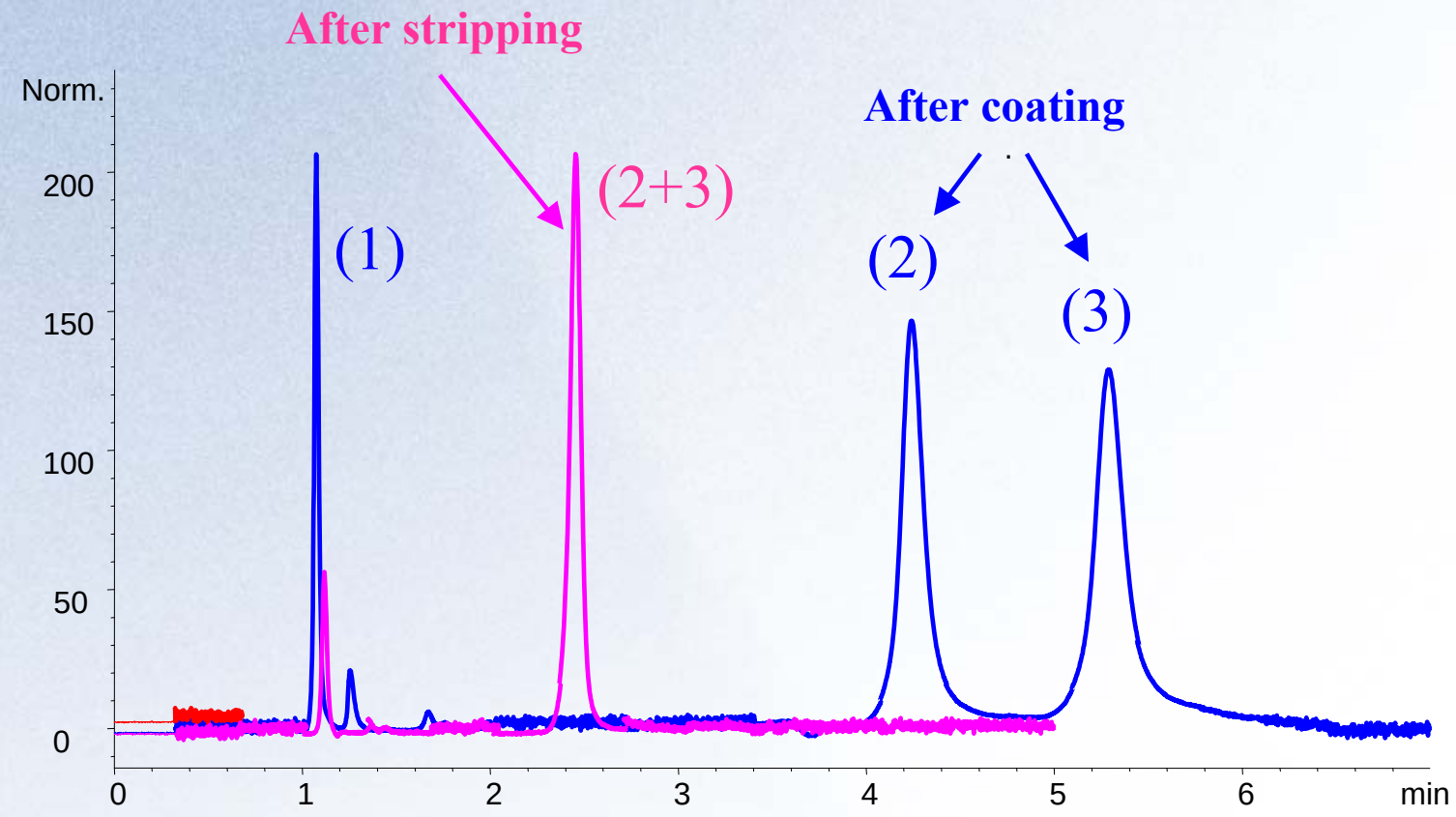
CS (Chiral Selector)

3) Chromatographic Test

ZrO_2-PDA-CS

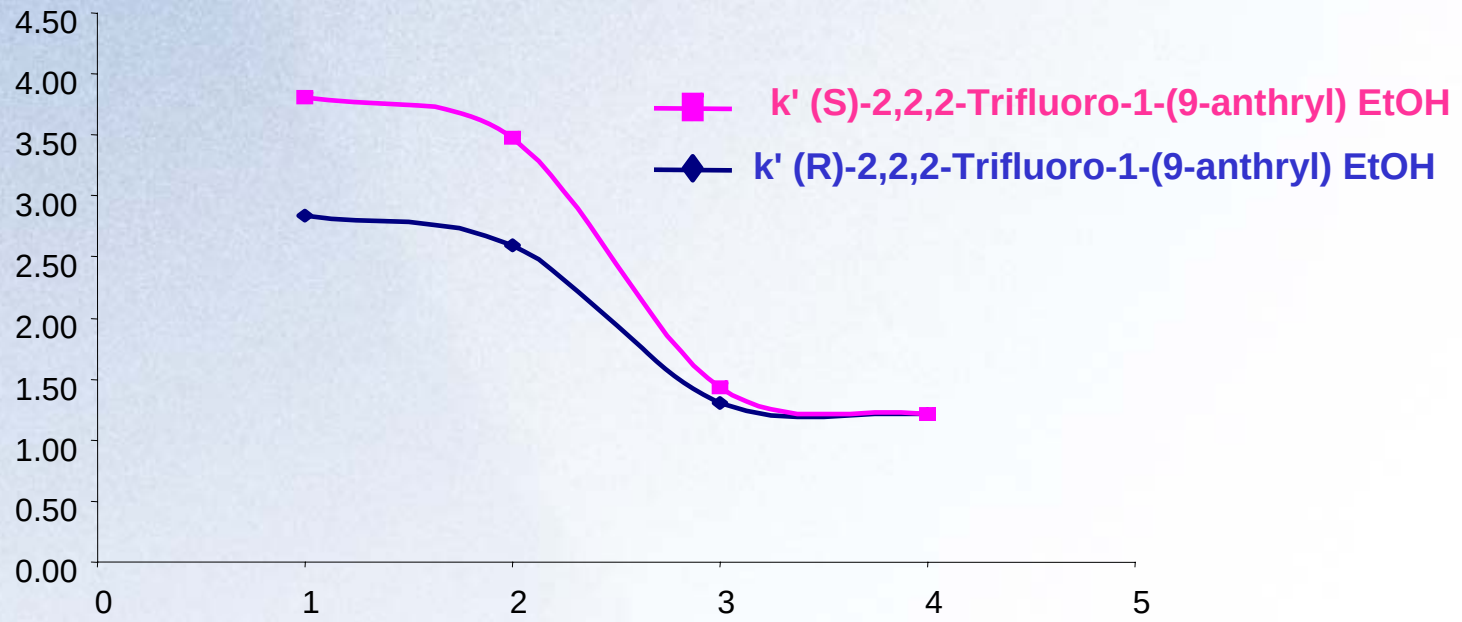
4) CSP Column Stripping (1M NaOH for 2 hrs at 60°C, water and 1M HNO_3 finish)

Stripping of (S)-PG CSP



Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, flow rate=1 ml/min, ambient temperature, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D and ZirChrom PDA-(R)-PG, S/N RPG020806A (100 × 4.6 mm, 3 µm, Running HPLC coated on PHASE110805A, batch#: 52-132). Solute: (1) 1,3,5-Tri-t-butyl-benzene, (2) (S)-2,2,2-Trifluoro-1-(9-anthryl) ethanol , (3) (R)-2,2,2-Trifluoro-1-(9-anthryl) ethanol 5 µl injection.

Changes During (S)-PG Stripping



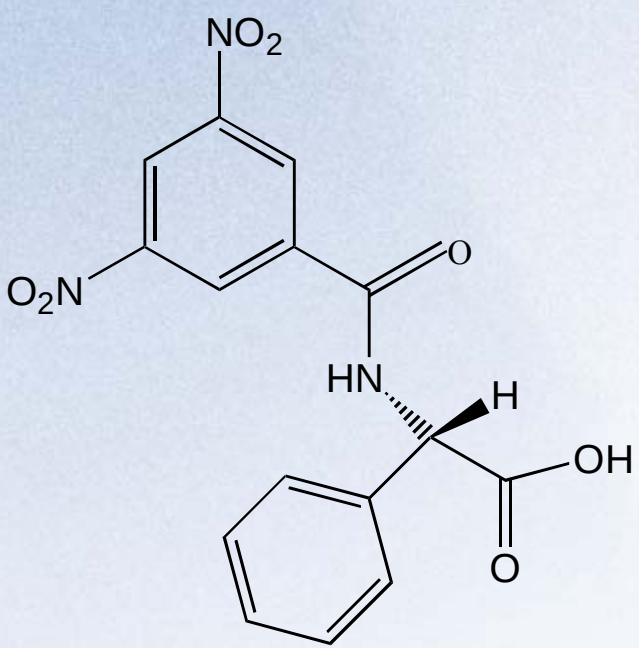
1- Original column.

2- Column flushed with 15/85 ACN/pH 12 NH_4OH for 10 column volumes, then 10 column volumes of water, 10 column volumes of 1.0 M nitric acid, and 10 column volumes of water.

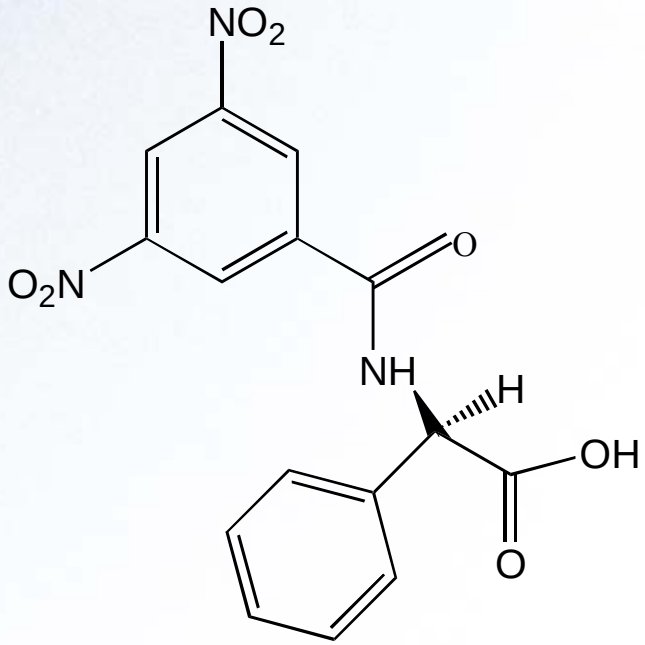
3- Column then flushed with 50 column volumes of 20/80 ACN/ 1 M NaOH, then 10 column volumes of water, 10 column volumes of 1 M nitric acid and 10 column volumes of water.

4- Column then flushed with 20/80 ACN/ 1 M NaOH for 50 column volumes at 60 ^oC, then flushed with 10 column volumes of water, 10 column volumes of 1 M nitric acid, and 10 column volumes of water.

Changing Chiral Selectors



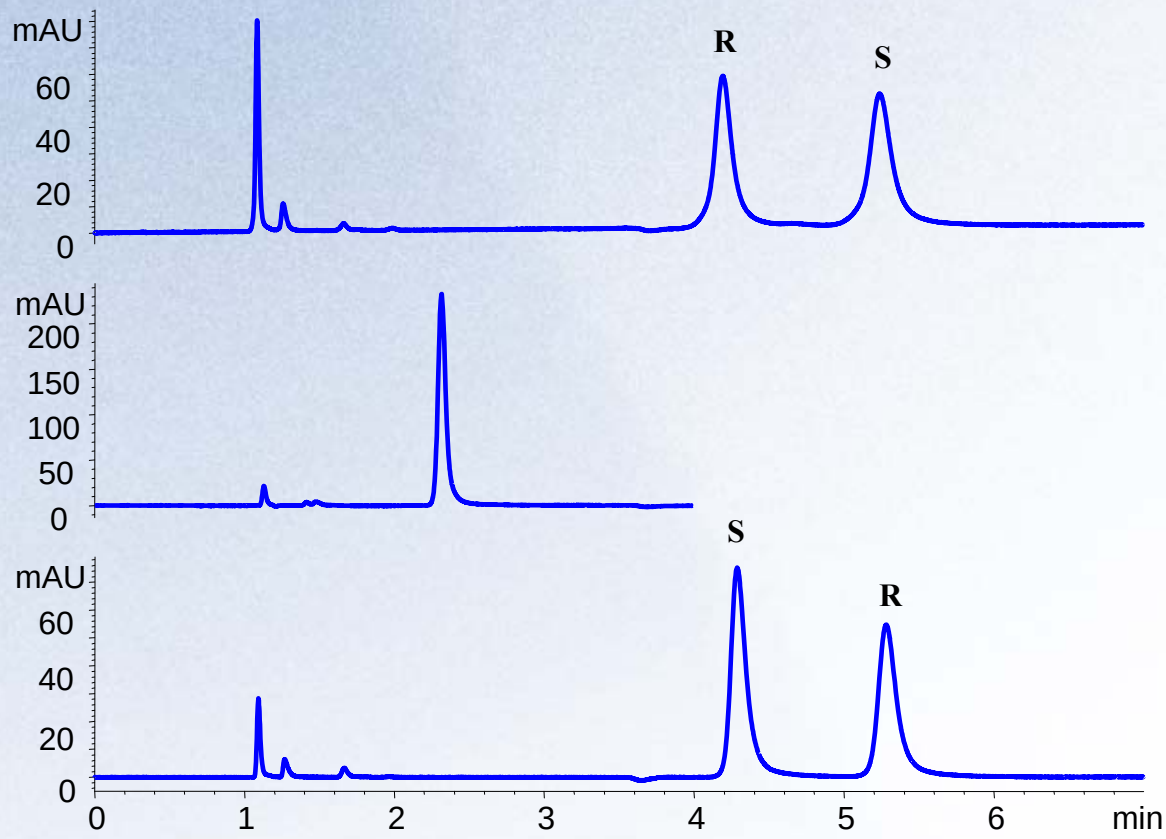
(S)-DNB-L-Phenylglycine (S-PG)



(R)-DNB-L-Phenylglycine (R-PG)

Changing (S) to (R)-Phenylglycine CSP on Same Zr Column

ZirChrom®



2-Step Load (S)-PG CS
k'(less) = 2.84
k'(more) = 3.81
α = 1.34

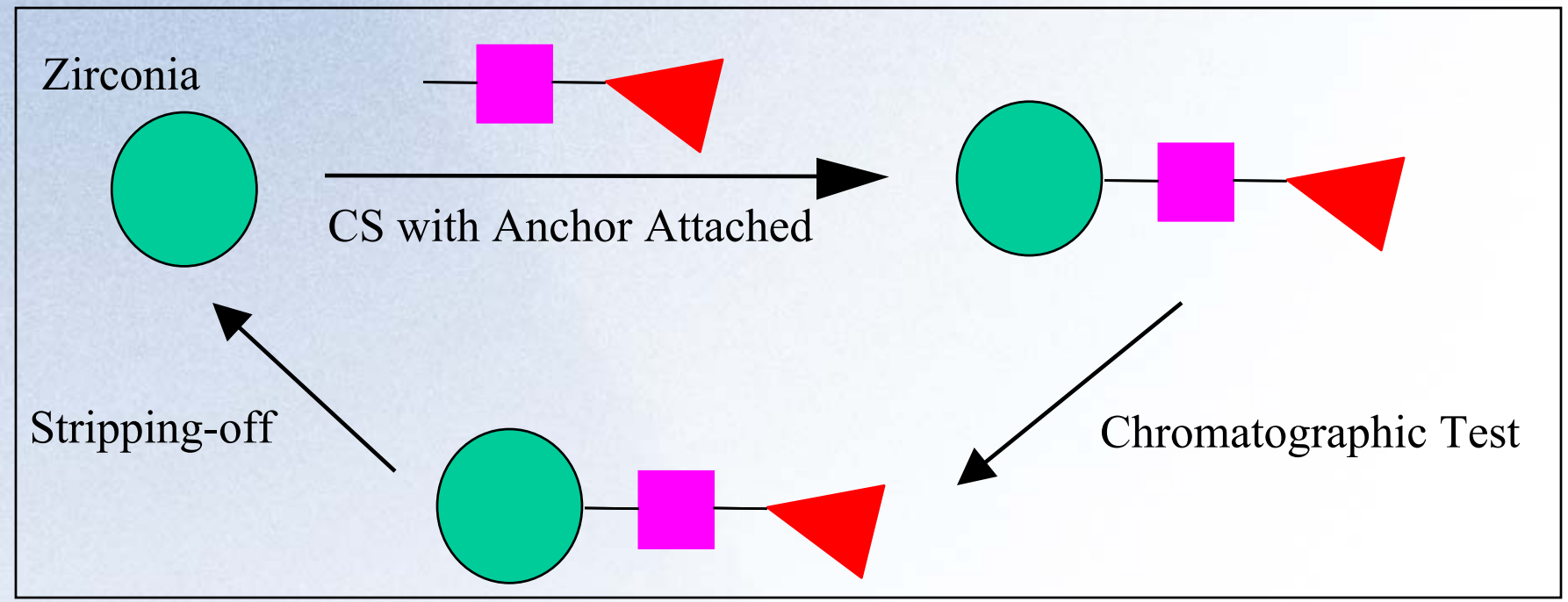
Strip (S)-PG CS
No separation.

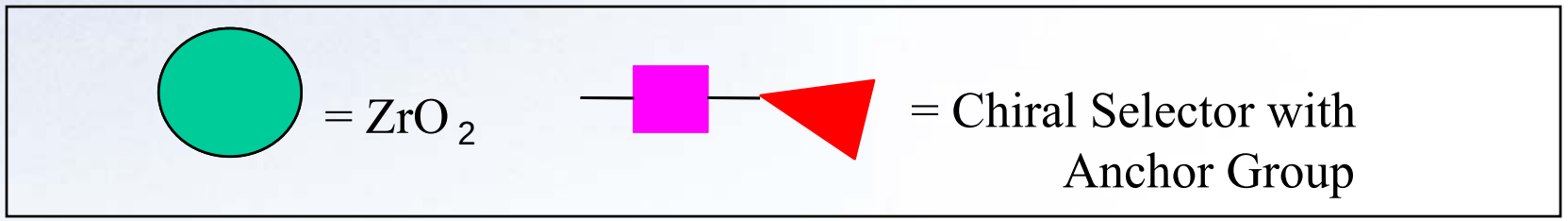
2-Step Load (R)-PG CS
k'(less) = 2.92
k'(more) = 3.83
α = 1.34

Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, F=1 ml/min, rm °C, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D and ZirChrom PDA-(R)-PG, S/N RPG020806A (100 × 4.6 mm, 3 µm, Running HPLC coated on PHASE110805A, batch#: 52-132). Solute: 1,3,5-Tri-t-butyl-benzene, (R orS)-2,2,2-Trifluoro-1-(9-anthryl) EtOH. 5 µl injection.

ZirChrom®

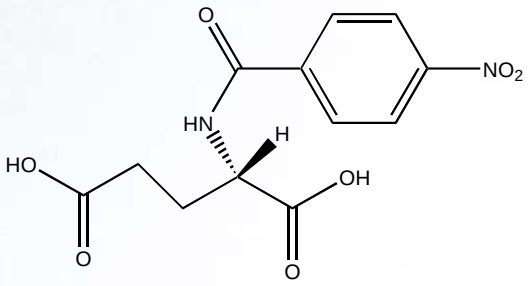
1-Step Synthesis of Zirconia CSPs for Fast Chiral Screening





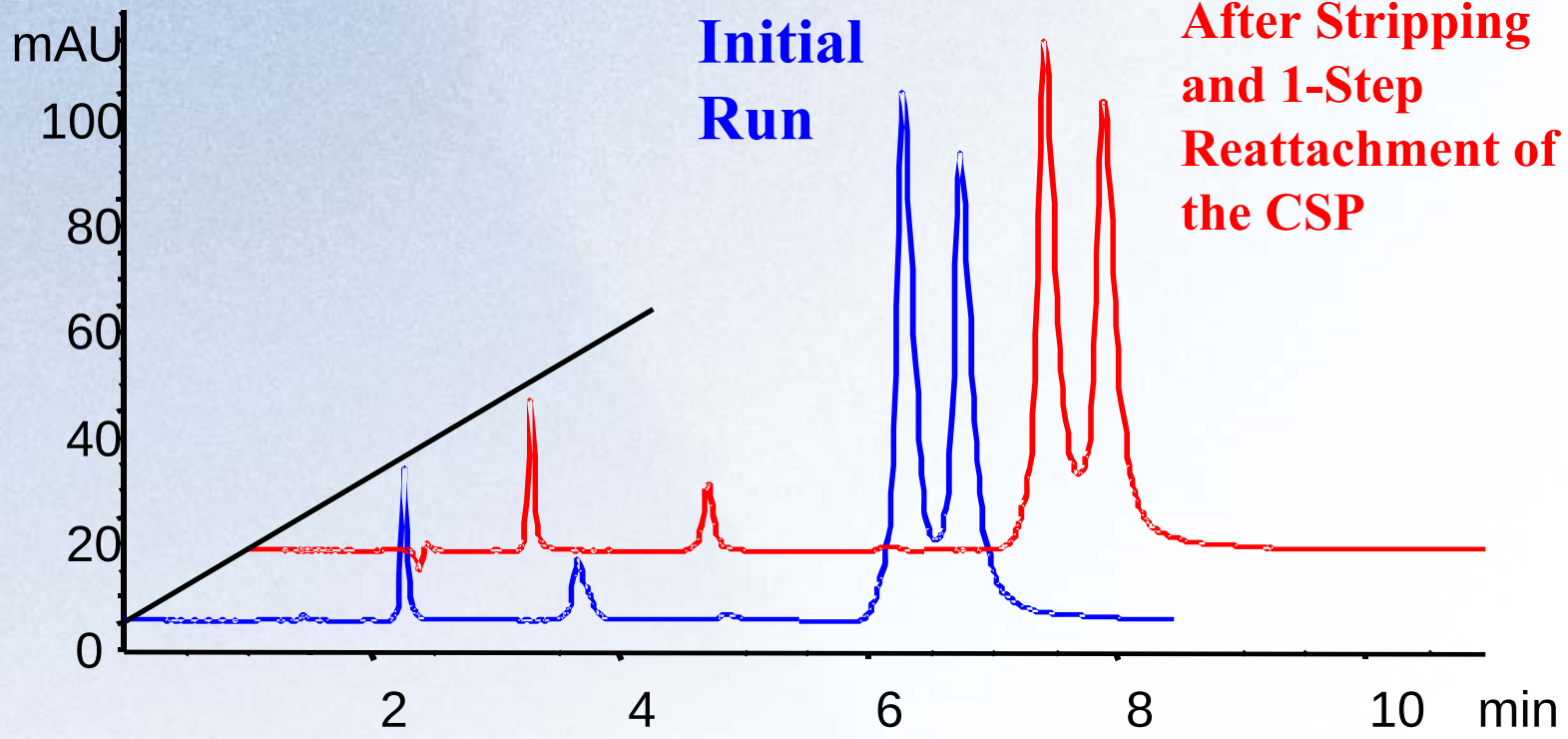
Example 1-Step Attachment and Detachment Cycle

- Pass a solution of 20 mM N-(4-nitrobenzoyl)-L-glutamic acid (CSP) in tetrahydrofuran for 10 minutes at a column temperature of 60°C and a flow rate of 1 mL/min .
- Flush column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
- Separate a racemic solution of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol.
- Strip the CSP by flushing the column with a 50 mM solution of tetramethylammonium hydroxide solution (pH 12) for 20 minutes at 60°C using a flow rate of 1 mL/min.
- Repeat procedure using the same CSP



N-(4-nitrobenzoyl)-L-glutamic acid

Glutamic Acid Proof of Concept



Comparison between the initial and final separation of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol leucine ester during a single CSP screening cycle. Chromatographic conditions: mobile phase: 99/1 hexane/IPA; flow rate: 1 ml/min; temperature: 30 °C, solute concentration = 1mg/mL, 5 µL injection.

Conclusions

- Five new CSPs were attached to zirconia using PDA:

  π–acceptors: Zr (S)-Leu, Zr (R)-PG, and Zr (S)-PG

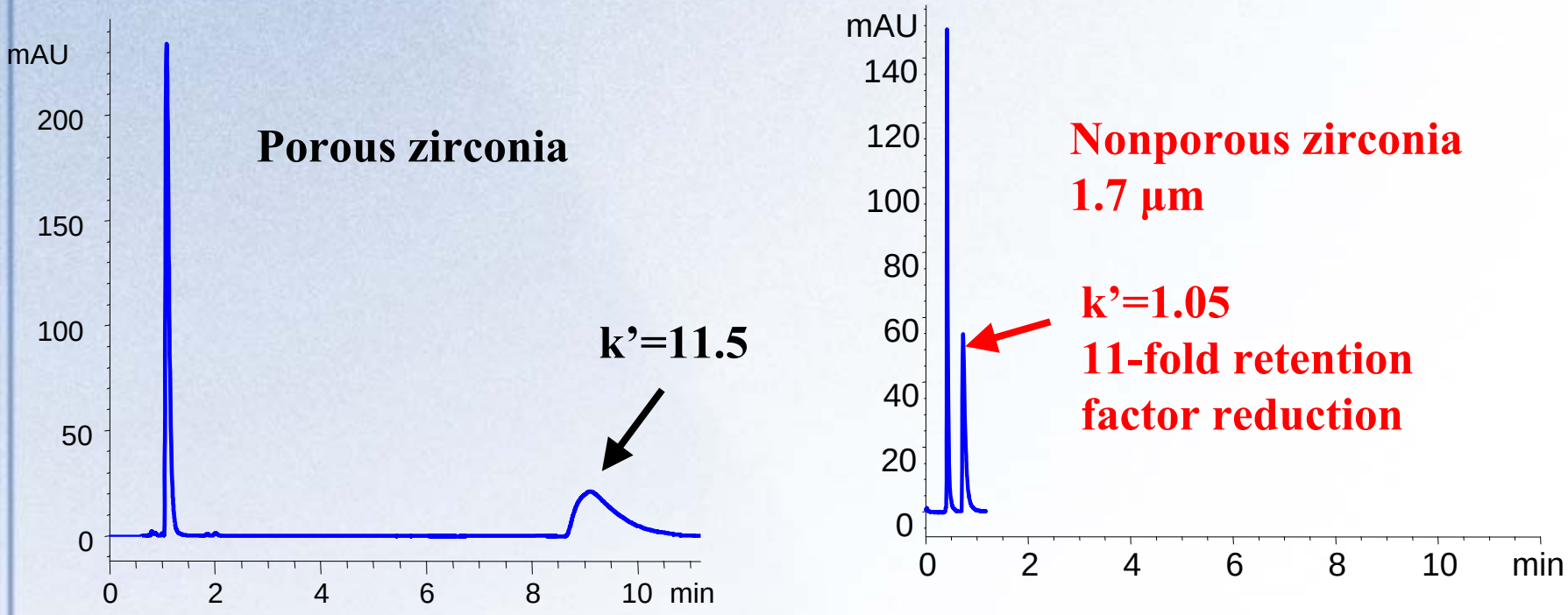
  π–donors: Zr (R)-NESA, Zr (S)-NESA
- Zirconia CSPs are reproducible, stable and have comparable chromatographic performance to commercial silica CSPs for range of chiral compounds.
- Methanol in the mobile phase had a positive effect on efficiency, retention, and selectivity under NP conditions.
- ***Fast user screening of chiral selectors is possible by changing the CSP online using a single, rugged zirconia column; both 2-step and 1-step methods have been developed for ambient and near-ambient conditions.***

Acknowledgement: ***National Institutes of Health Grant*** (Phase II SBIR) 2R44HL070334-02A2.

Future Studies and Development

- Expanded number and type of Zirconia CSPs.
- Zirconia CSP with both pi-donor and pi-acceptor functions.
- Cellulosic Zirconia CSPs.
- Titania CSPs
- Reagents and procedures for fast user screening of various chiral selectors using a single, rugged zirconia column.

Fast Chiral Separation on Nonporous Zirconia DNB-L-Leu



Chiral compounds on nonporous and porous zirconia DNB-L-Leu anchored with APPA. Chromatographic conditions: mobile phase 99/1Hexane/IPA, probe solute: (±)1-naphthyl leucine ester.

Thanks *very much* for listening!

Request copies of the talk at Booth 2214

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