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Novel, Stable Zirconia-based Chiral Stationary Phases for Enantiomer Separations

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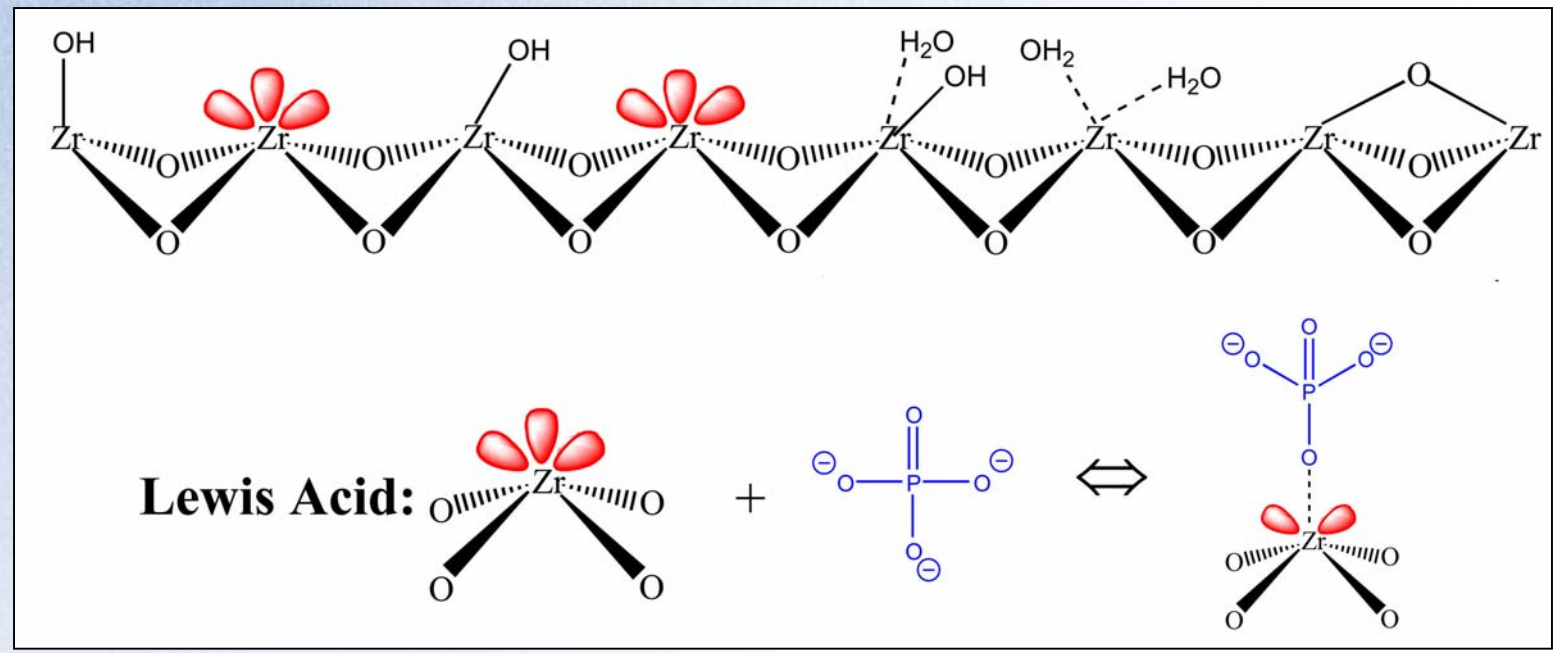
Surface Chemistry of Zirconia



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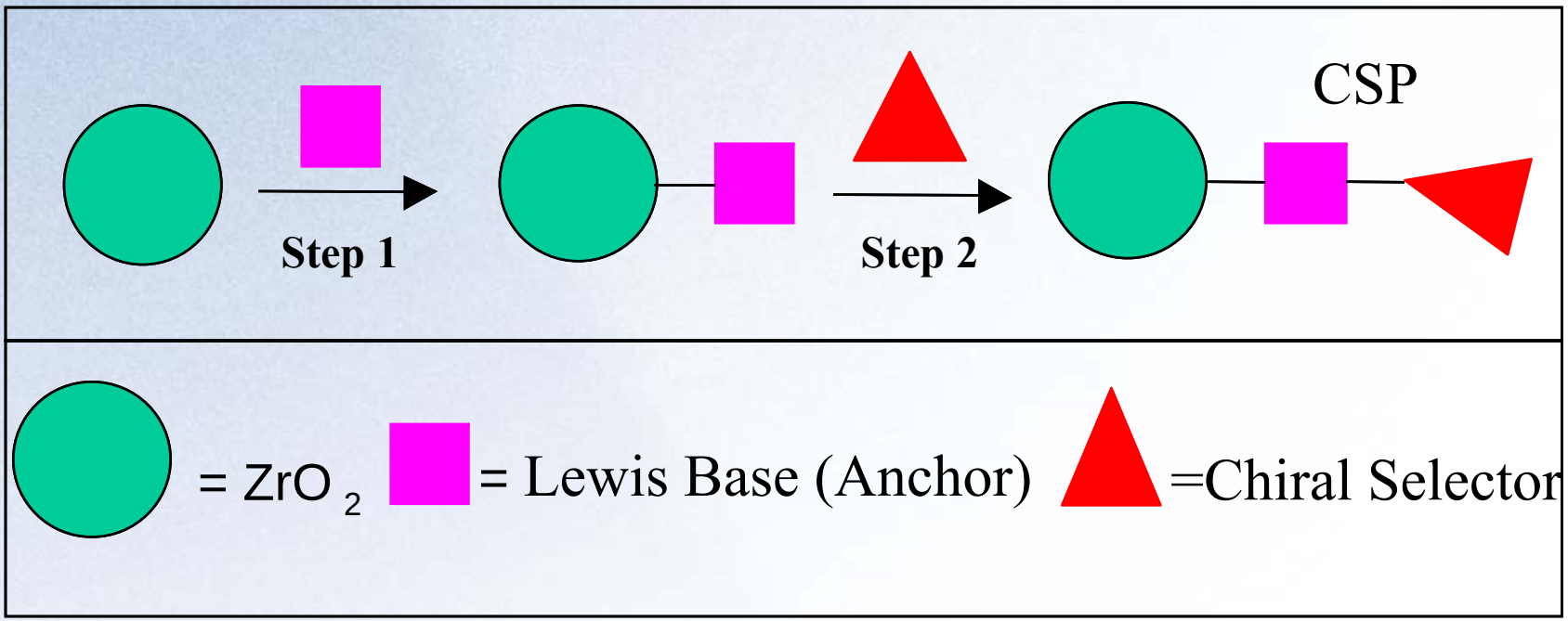
A Novel Approach to Attaching Chiral Selectors[1] to Zirconia[2]



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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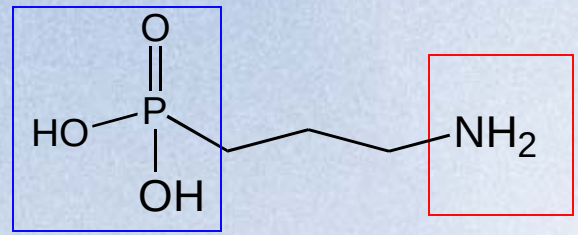
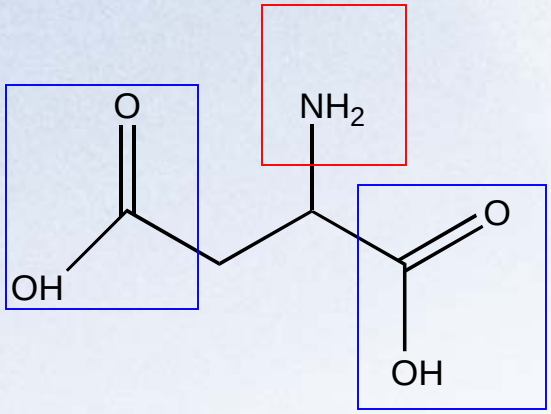
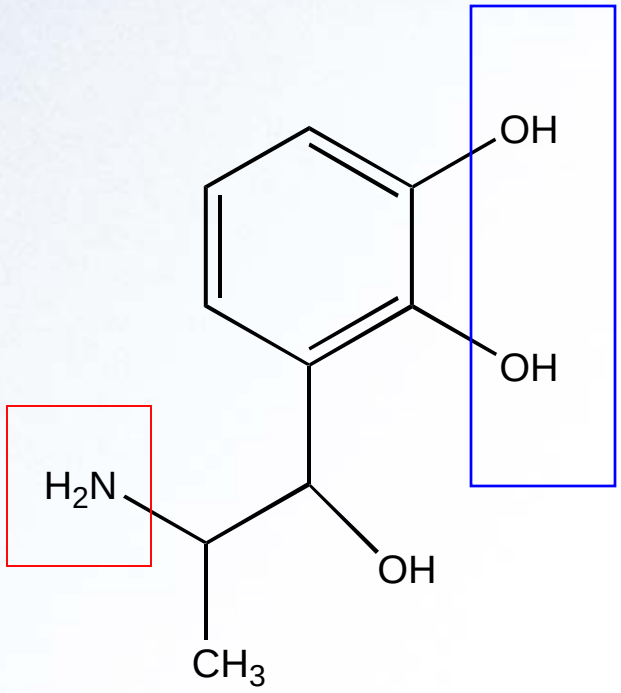


APPA (Aminopropylphosphonic acid)



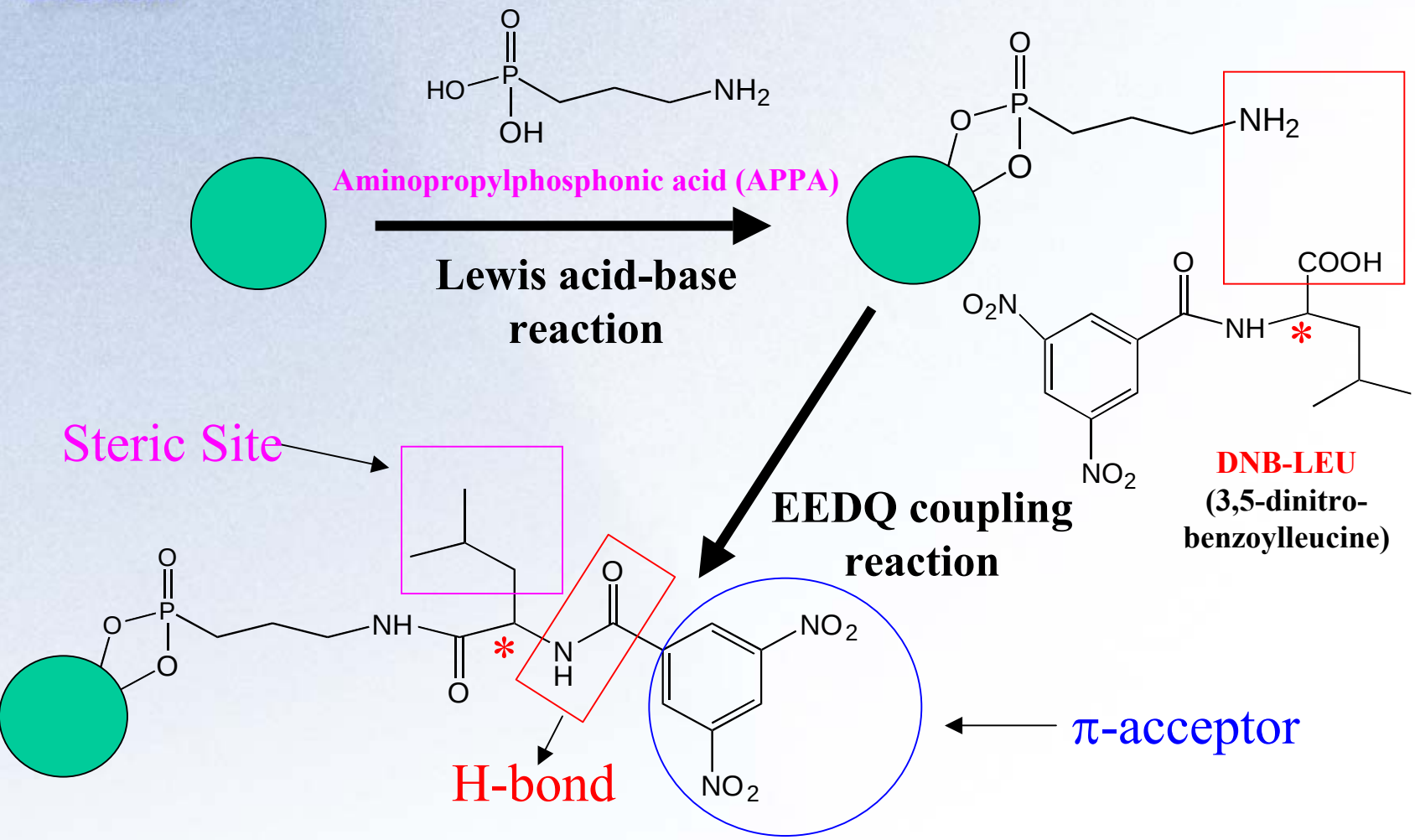
ASPA (Aspartic acid)



DHNP (3,4-Dihydroxynorephedrine)

Phase I Anchors

Zirconia CSP 2-Step Synthesis



Stability of Zirconia DNB-L-LEU with Different Anchors

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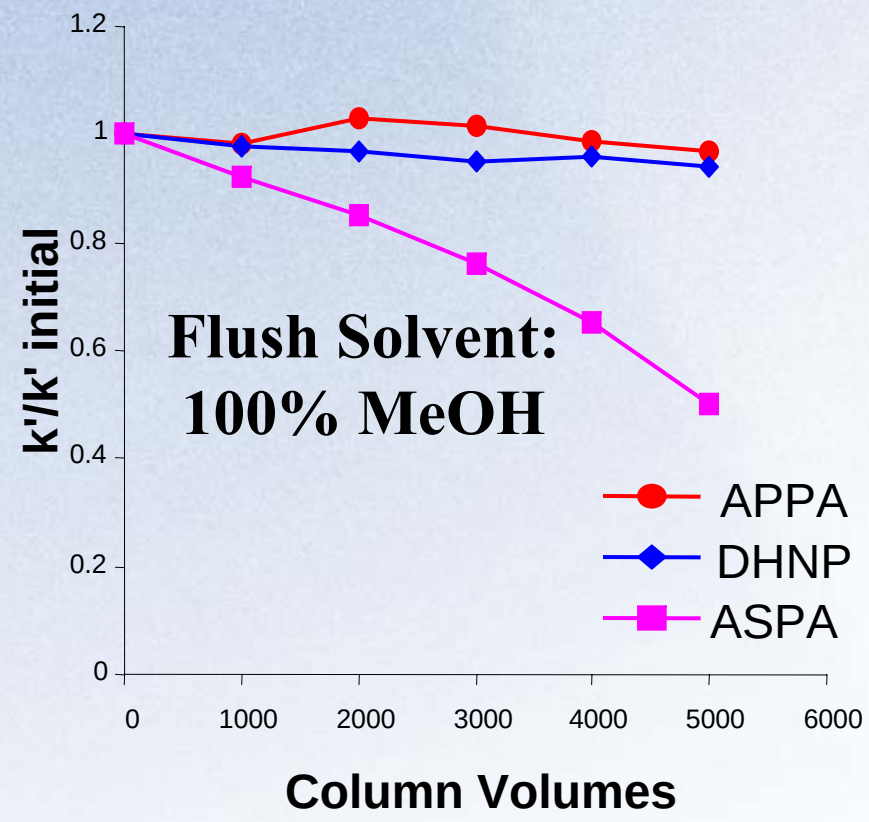
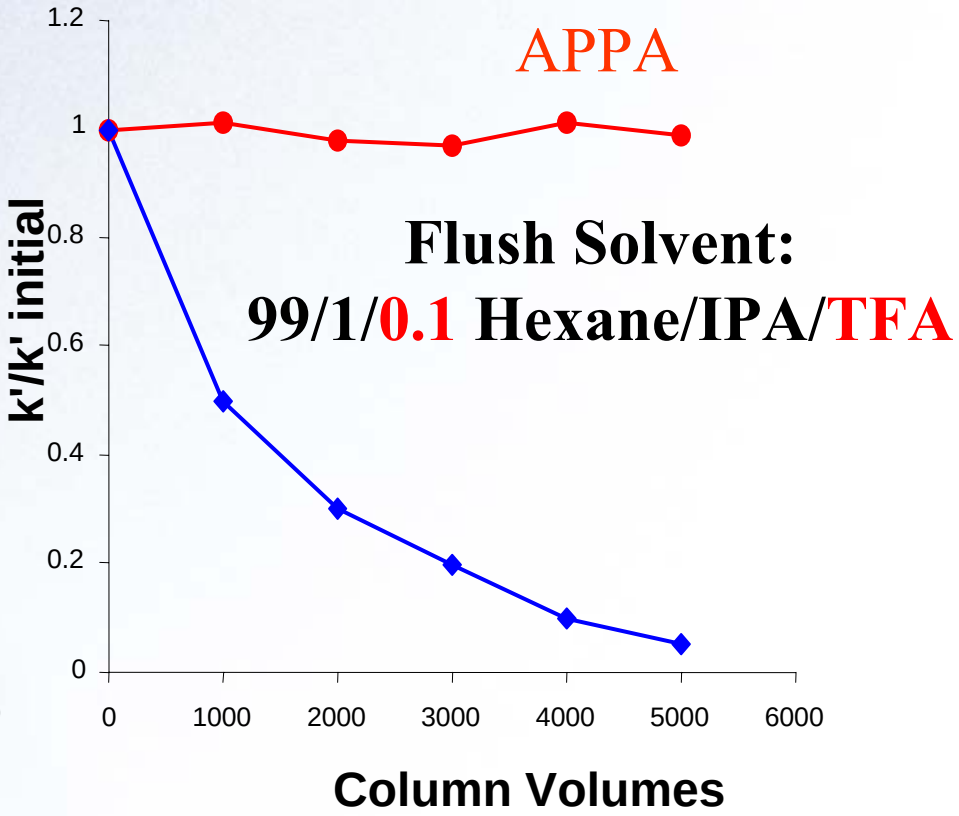
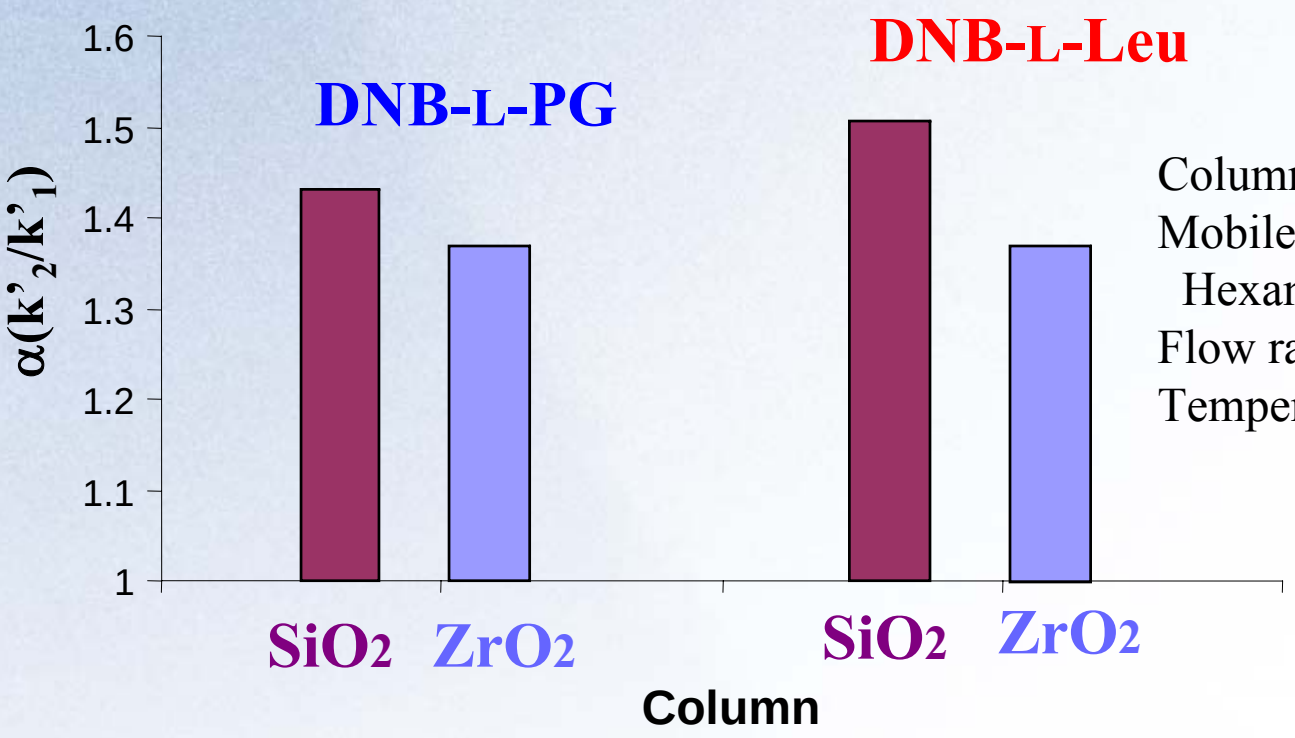




Test solute: trifluoranthryl ethanol (retention factor ratio for less retained isomer)

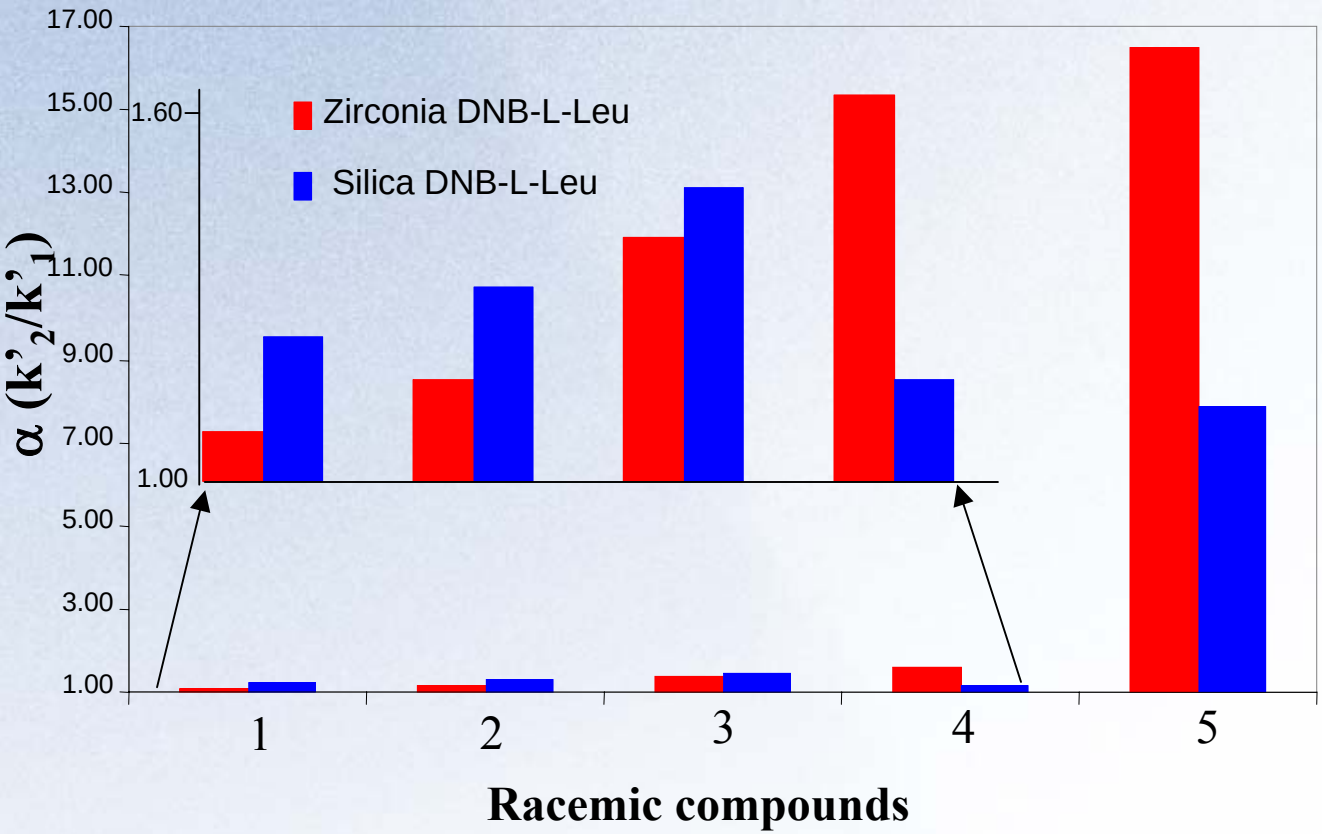
Selectivity Comparison of Zirconia and Silica CSPs



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

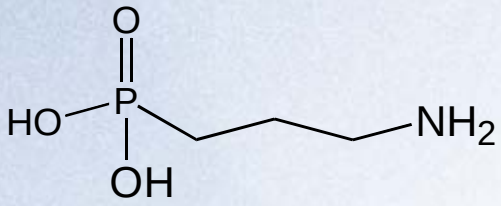


1 trans-stibene oxide
2 1,1'-bi-2-naphthol
3 trifluoranthyl ethanol
4 napropamide
5 1-naphthyl leucine ester

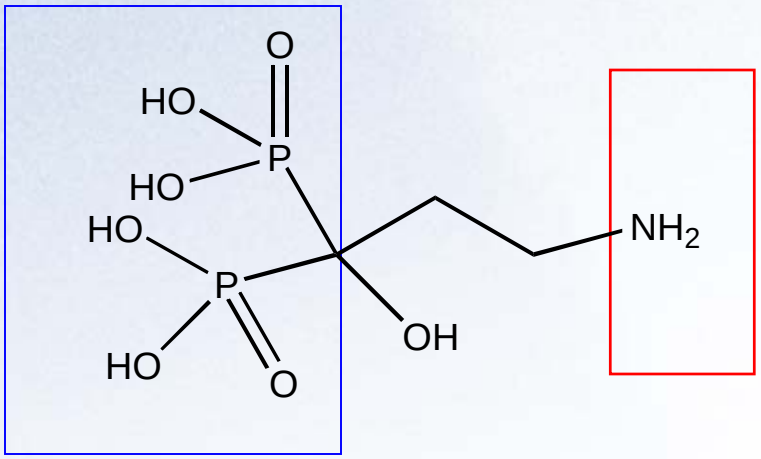
Mobile phase:
99/1Hexane/IPA
Flow rate: 1 ml/min;
Temperature: 30 °C

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

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



Aminopropylphosphonic acid (APPA)

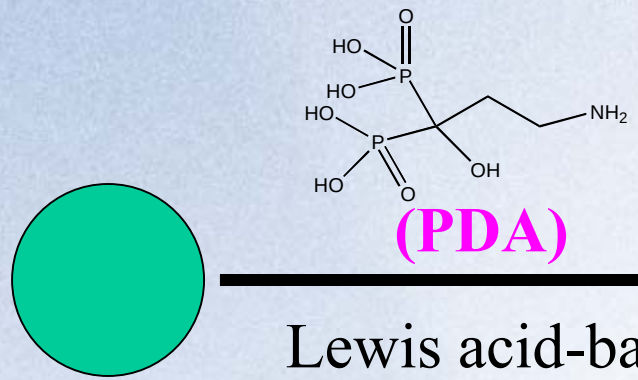


Pamidronic acid (PDA)[1]

(Phase II Anchor)

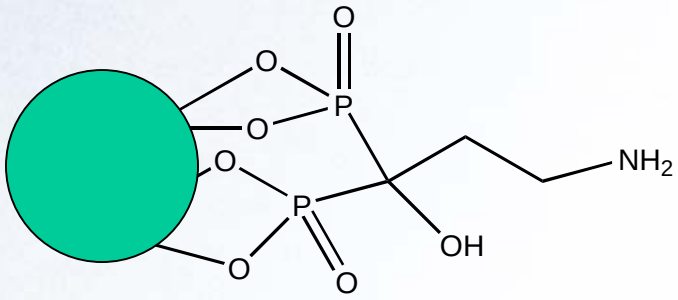
1. Phase II SBIR (NIH).

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

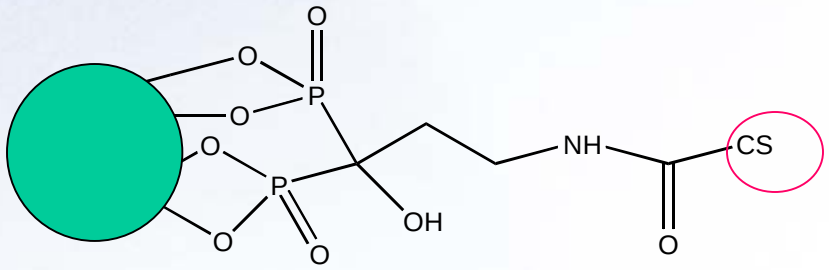


(PDA)

Lewis acid-base reaction

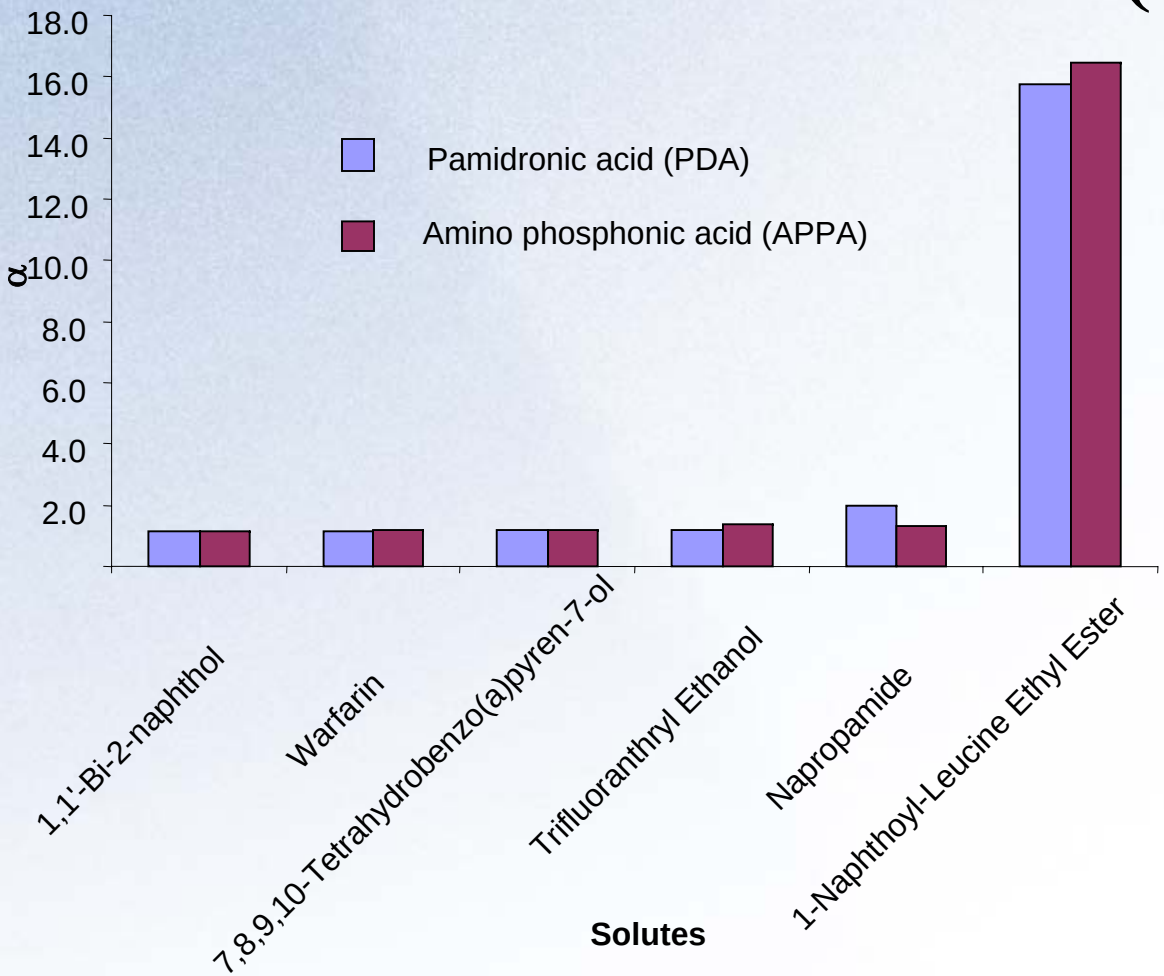


CS-COOH
EEDQ coupling reaction



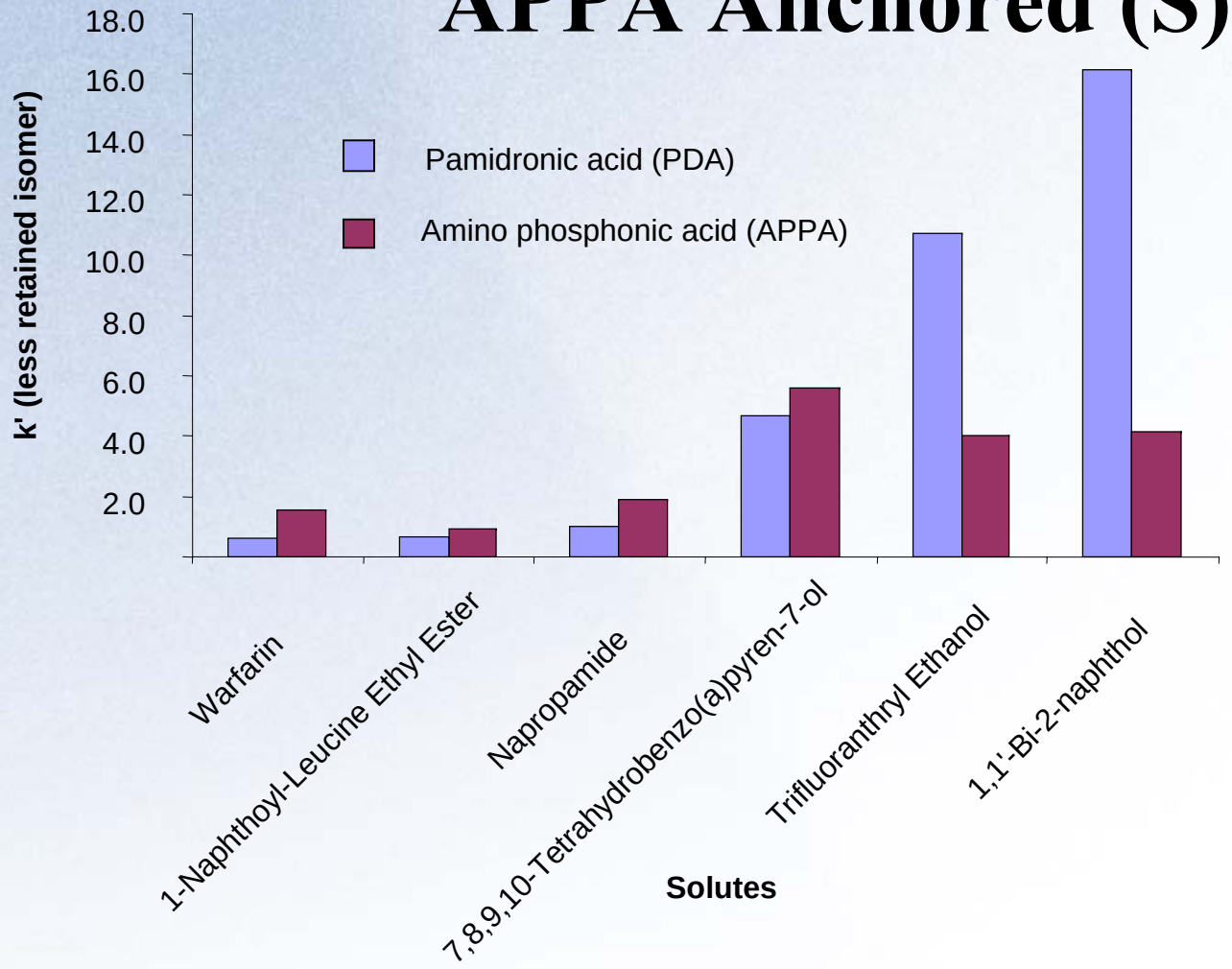
CS = Chiral Selector

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



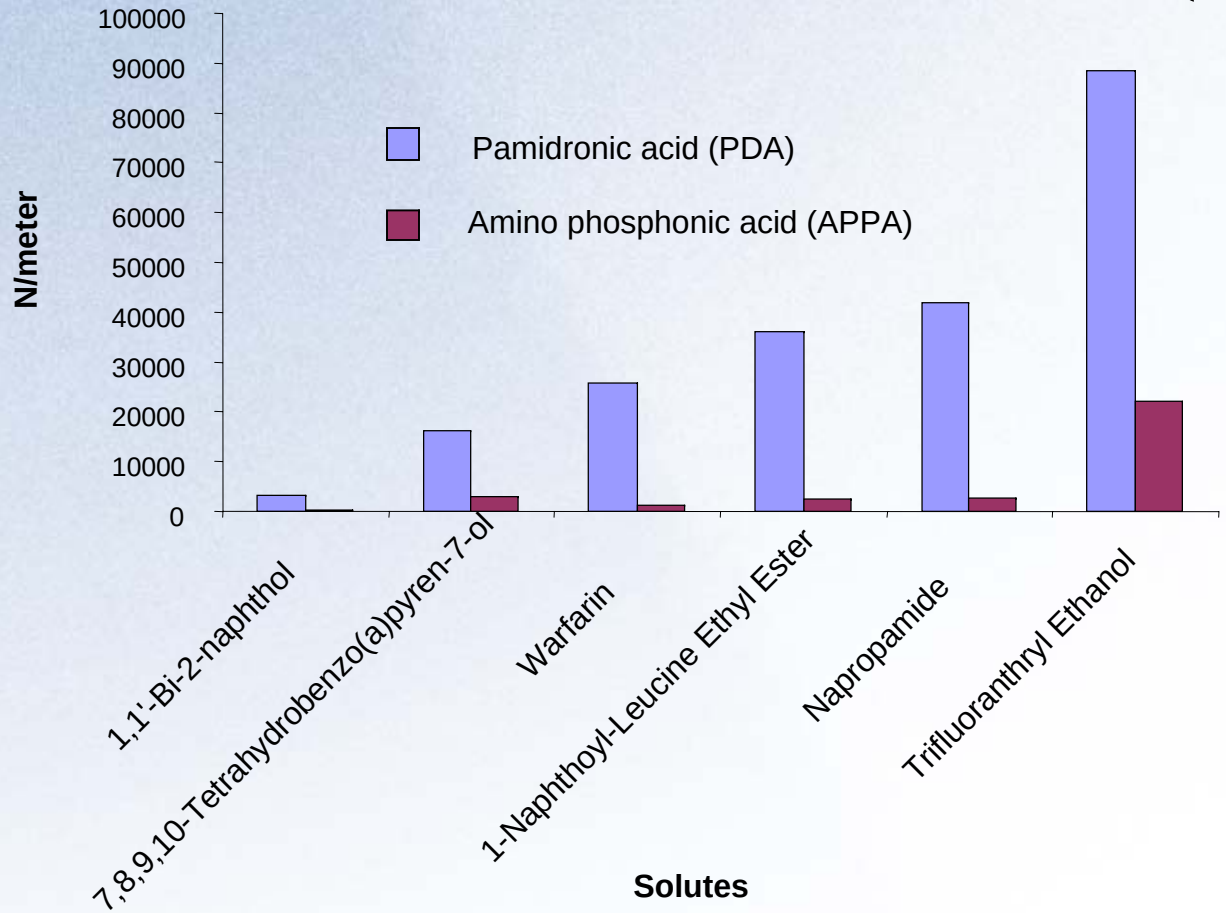
Selectivity for the two anchors is similar.

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



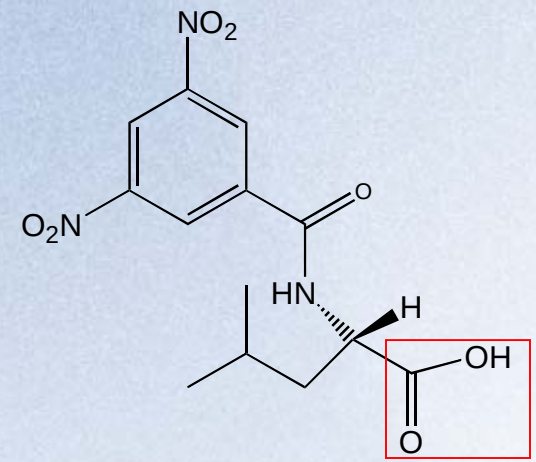
Retention for the two anchors is different.

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



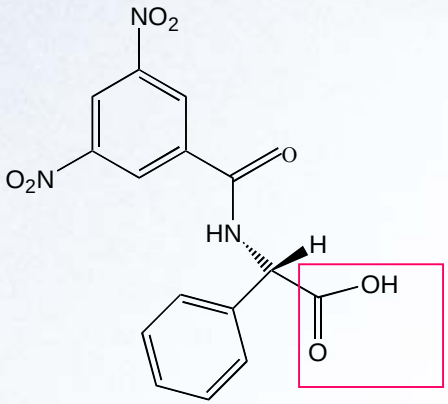
Efficiency on PDA anchored Zr (S)-Leu is much better.

Chiral Selectors Evaluated[1]



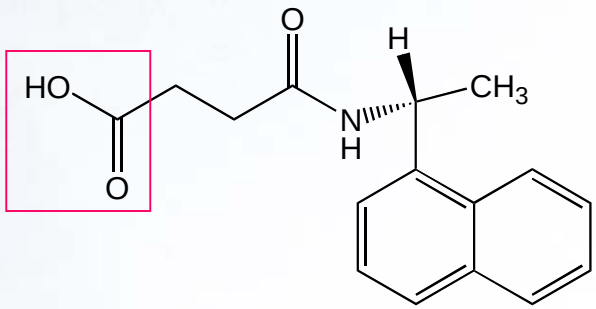
(S)-DNB-L-Leucine

[(S)-Leu]



(S)-DNB-L-Phenylglycine

[(S)-PG]



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

[(S)-NESA]

1. Phase II SBIR (NIH)

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

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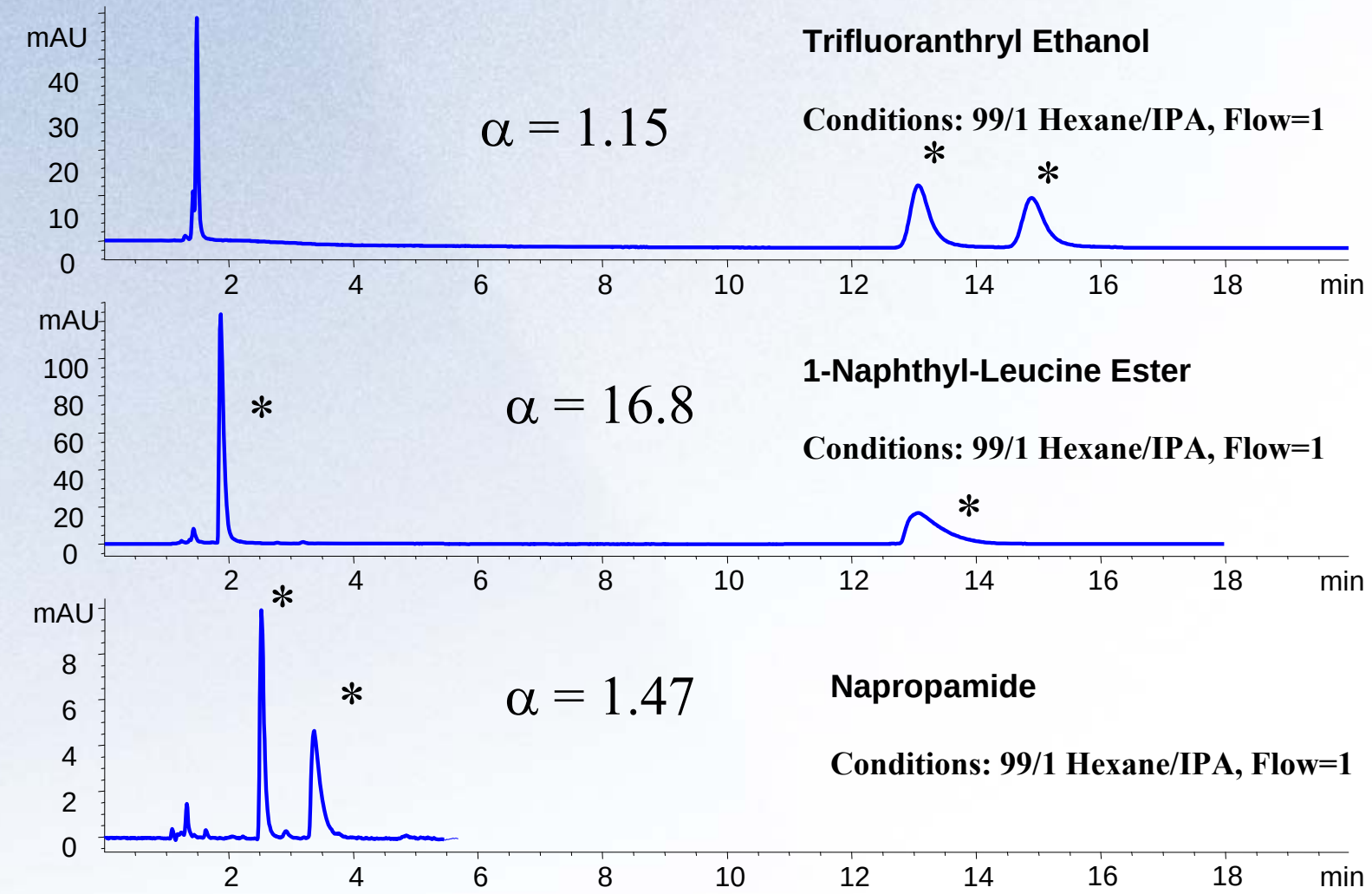

Chiral Separation on Zr (R)-PG (π-acceptor phase)

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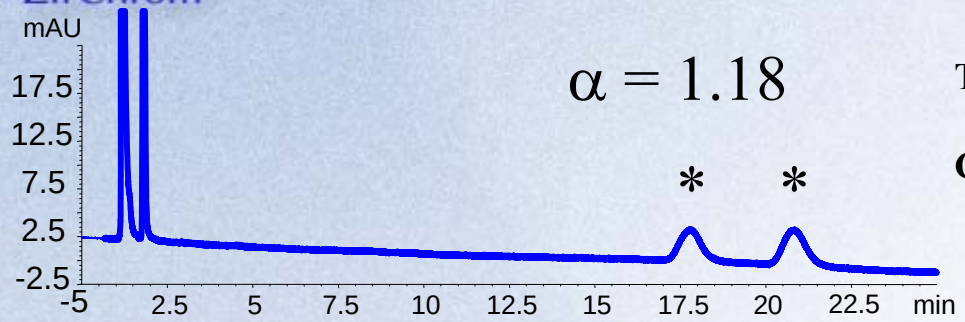
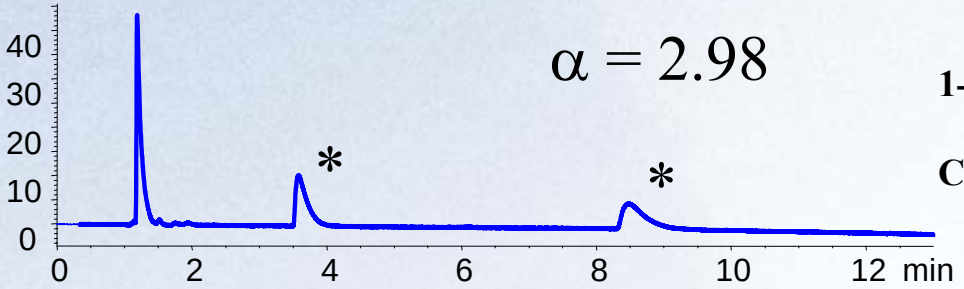


$\alpha = 1.18$

Trifluoranthryl Ethanol

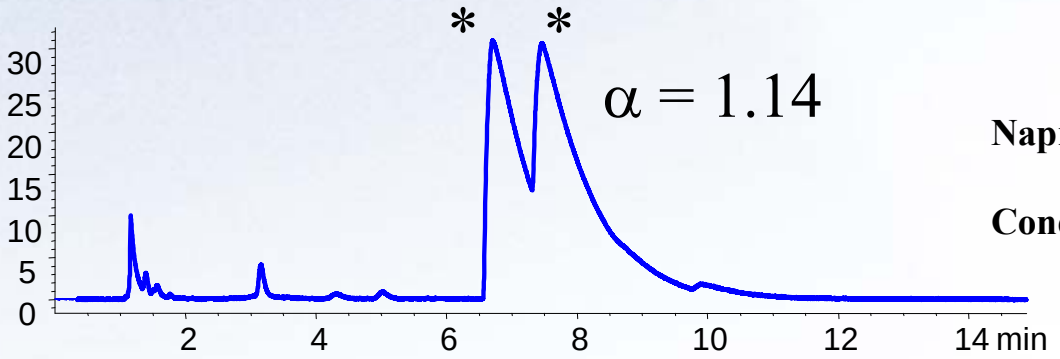
Conditions: 99/1 Hexane/IPA, Flow=1



$\alpha = 2.98$

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 (π -donor phase)

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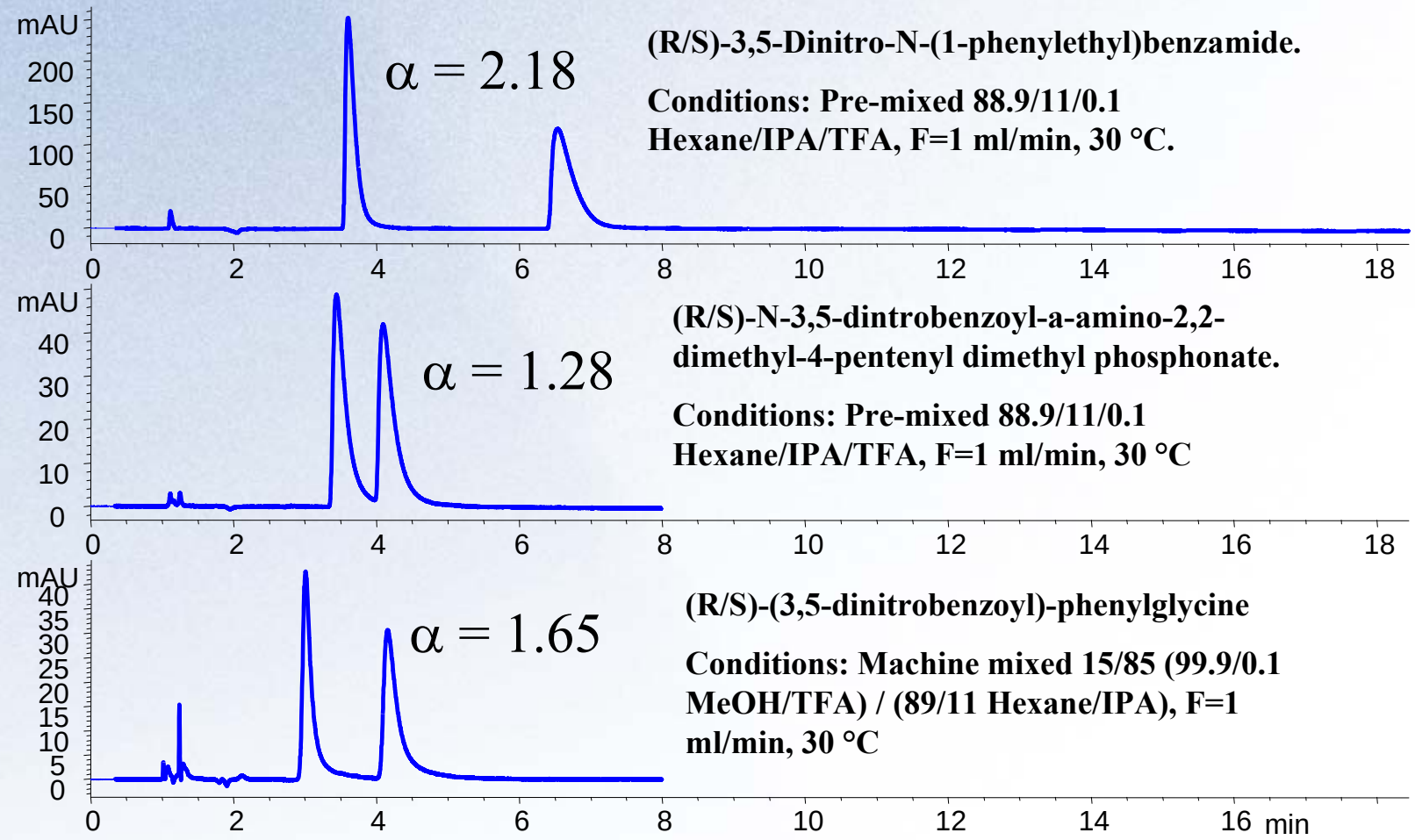


(R/S)-3,5-Dinitro-N-(1-phenylethyl)benzamide.

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

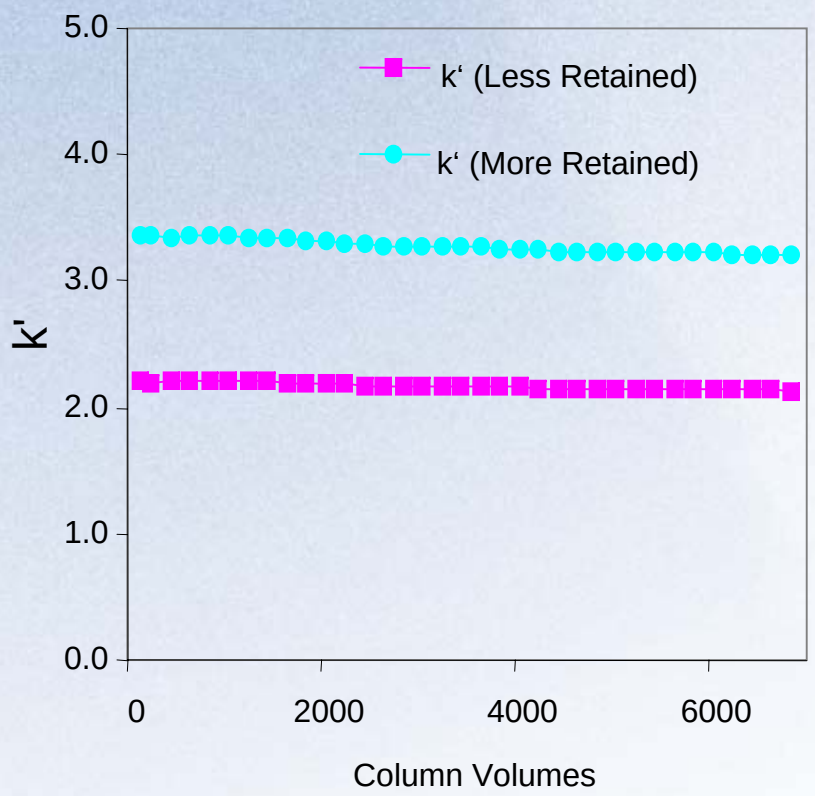
(R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate.

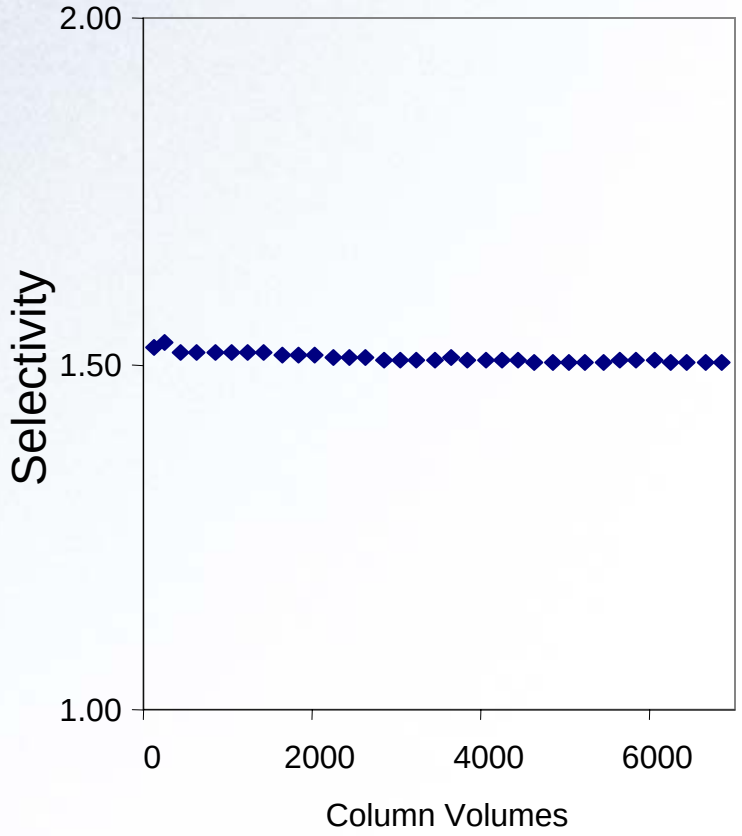
Conditions: Pre-mixed 88.9/11/0.1 Hexane/IPA/TFA, F=1 ml/min, 30 °C

(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

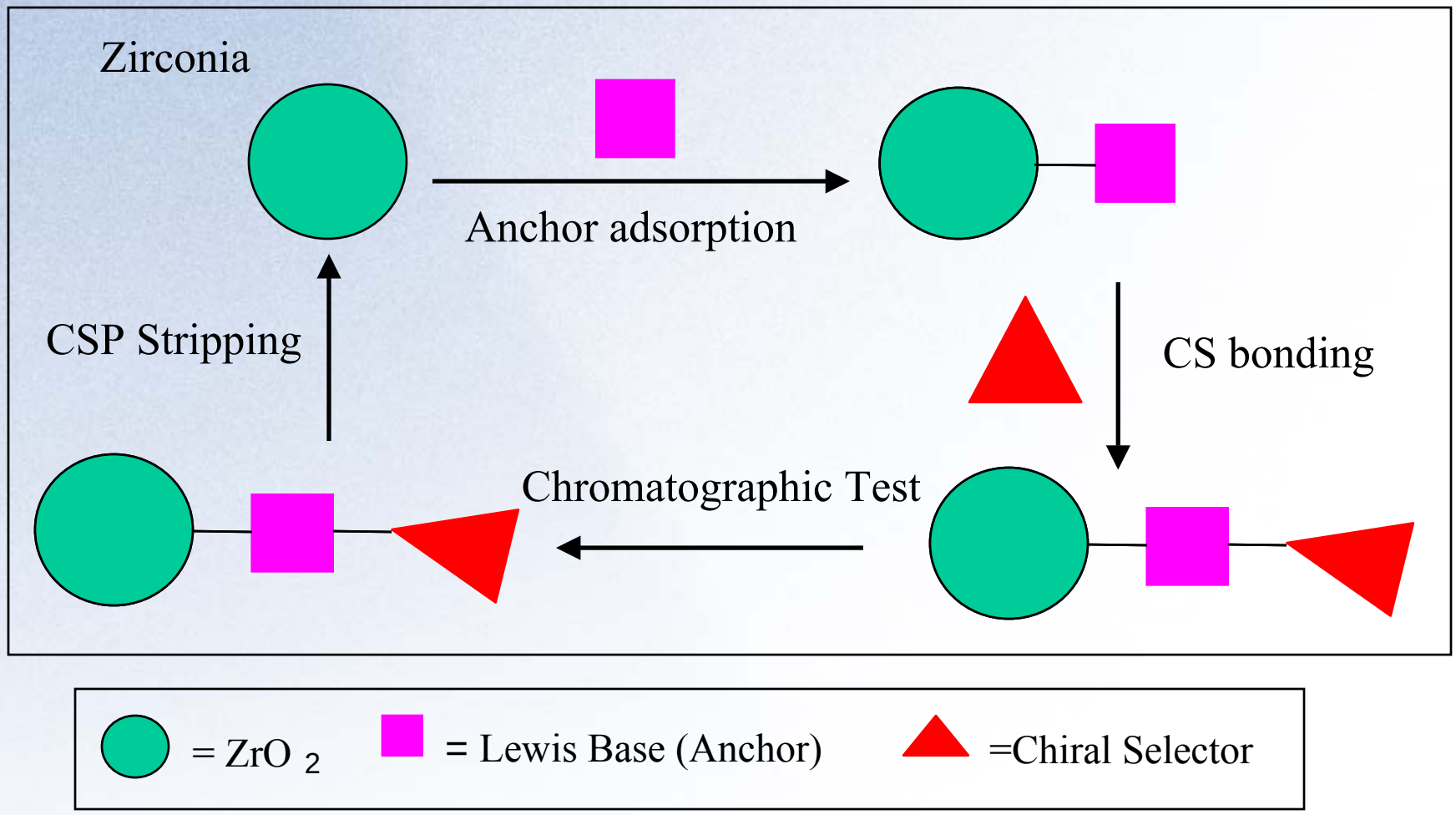
Stability of Zr-(S)-NESA at pH 2





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.

2-Step Synthesis of Zirconia CSPs for Chiral Selector Screening



2-Step Online Zirconia CSP Synthesis for Chiral Screening

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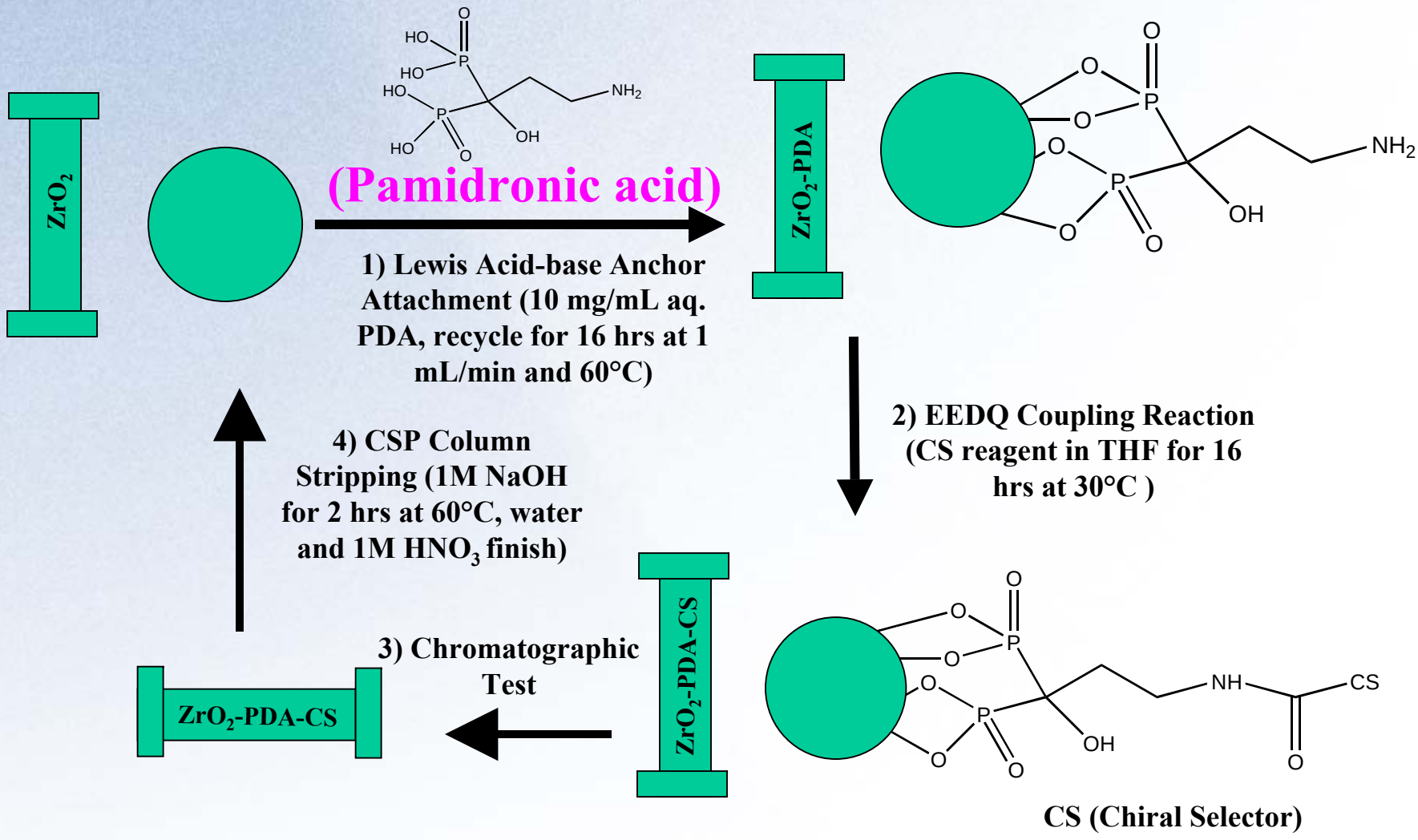
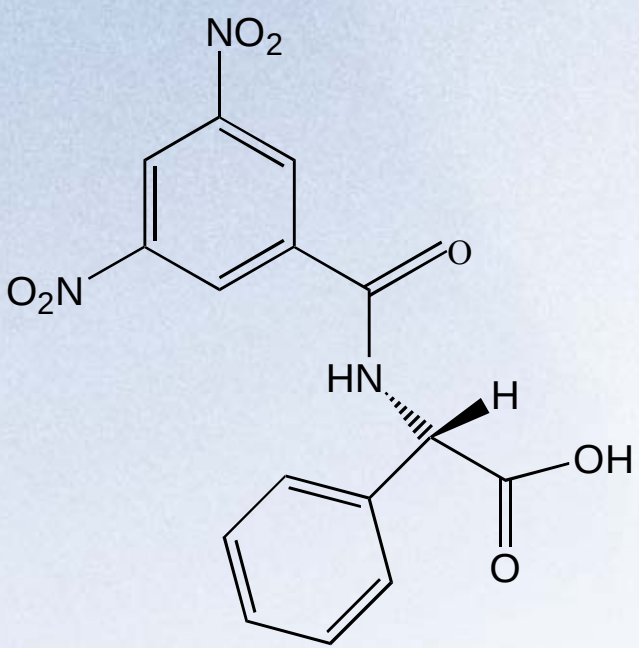
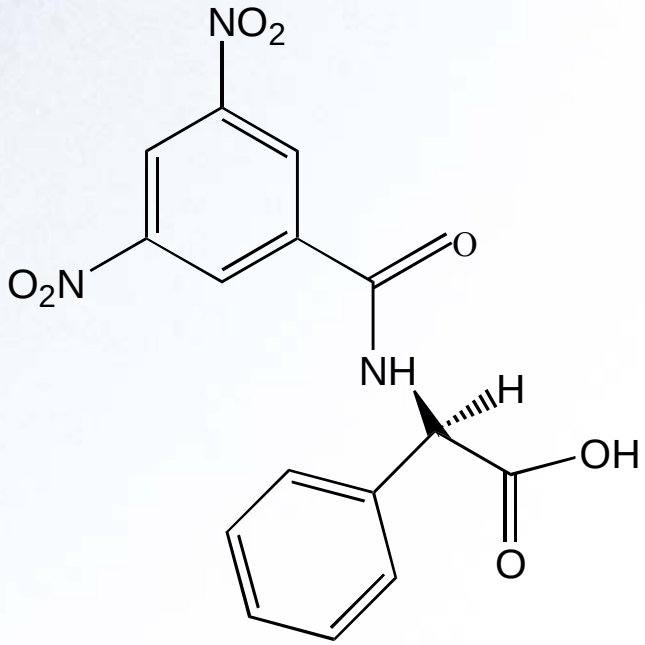


Changing Chiral Selectors



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



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

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

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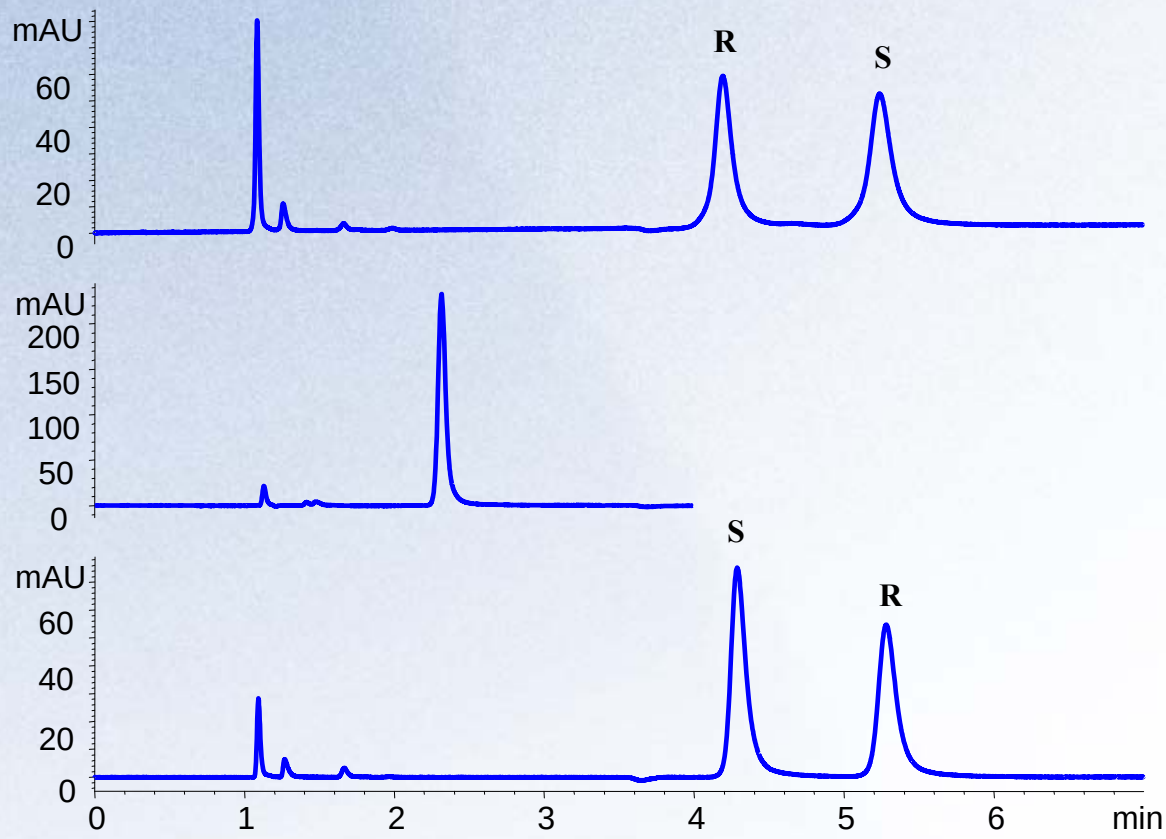

2-Step Load (S)-PG CS
k'(less) = 2.84
k'(more) = 3.81
α = 1.34

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.

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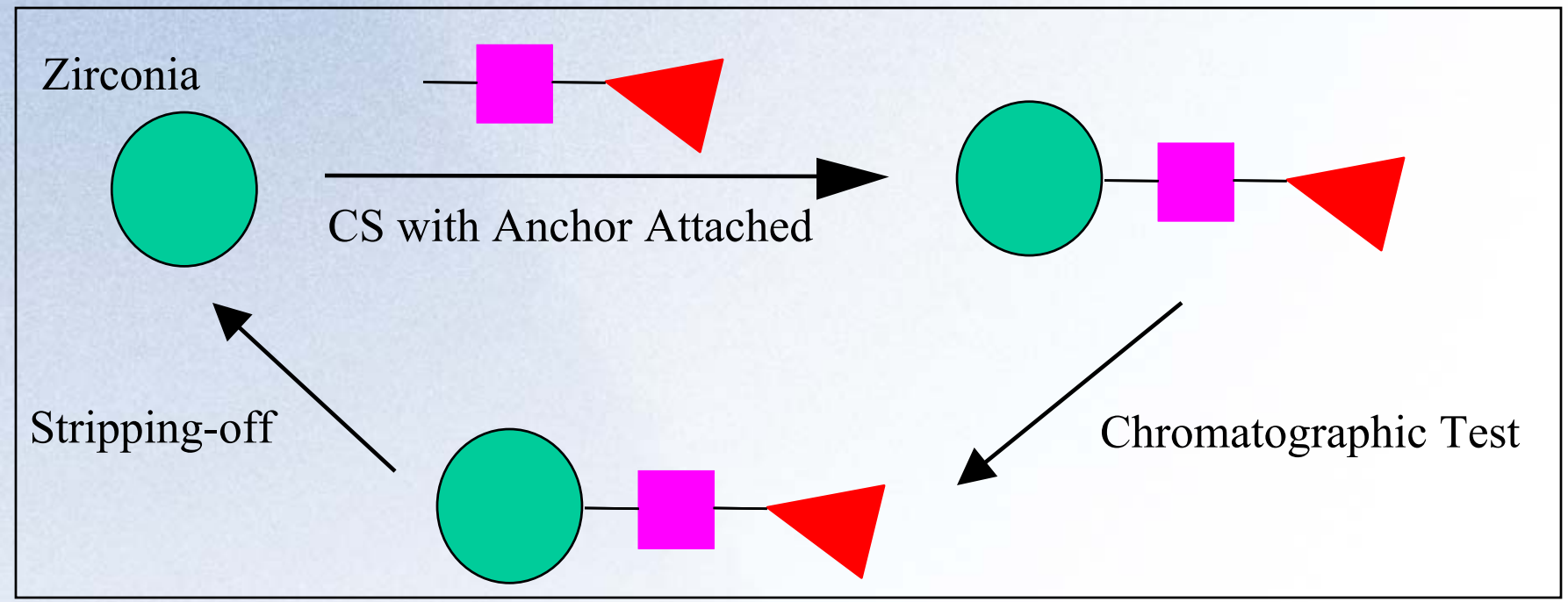
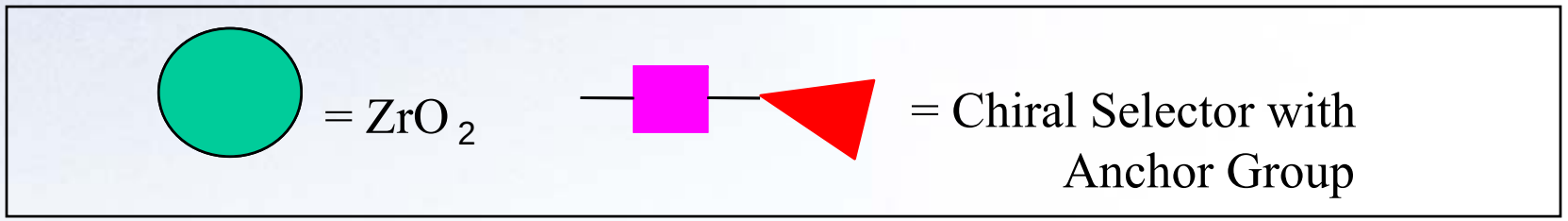
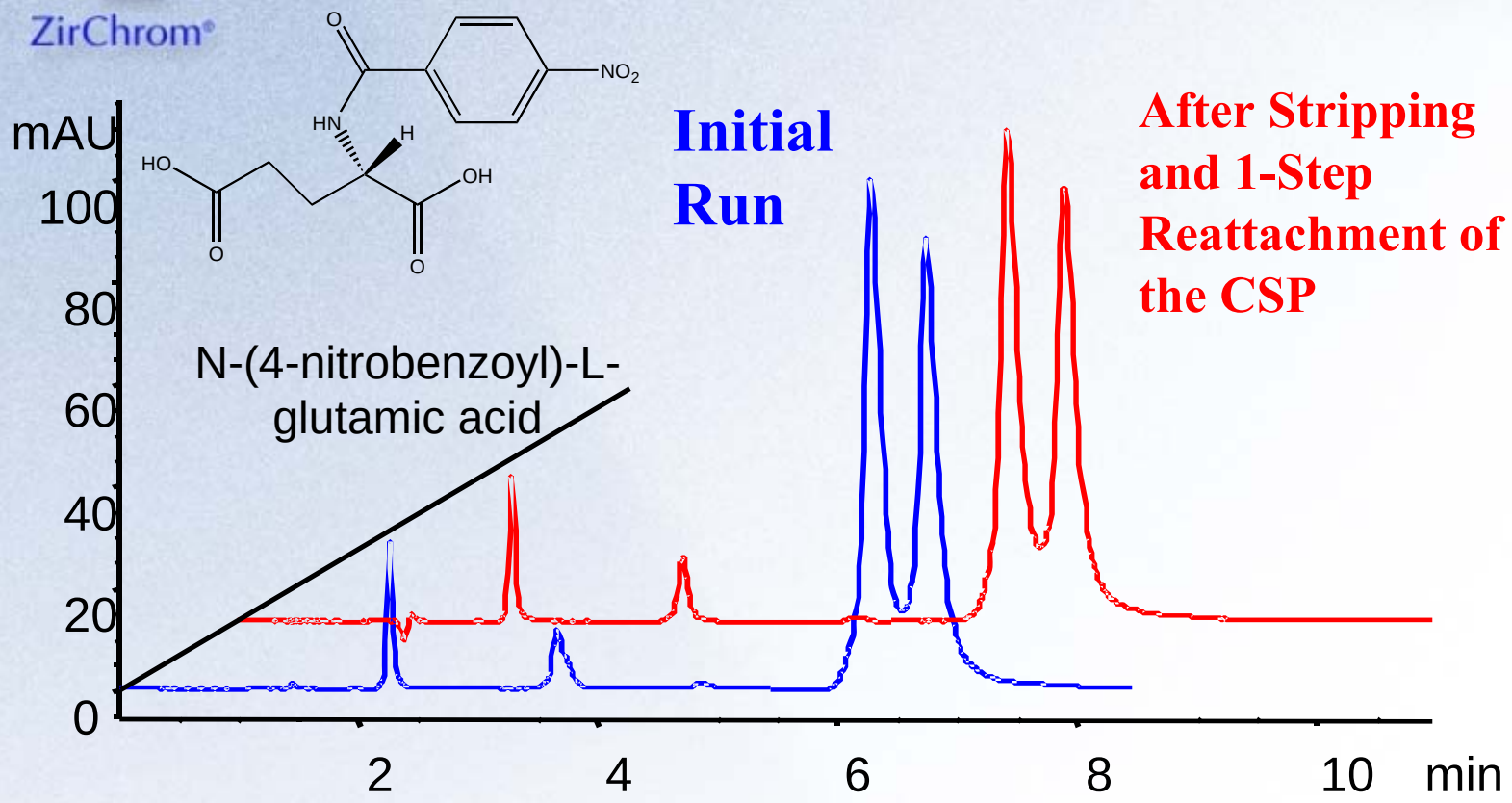
1-Step Synthesis of Zirconia CSPs for Fast Chiral Screening





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.
- ***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.

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Thanks *very much* for listening!

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www.zirchrom.com