Synthesis of Stable Zirconia Based Chiral Stationary Phases for Enantiomer Separations and Fast Chiral Selector Screening

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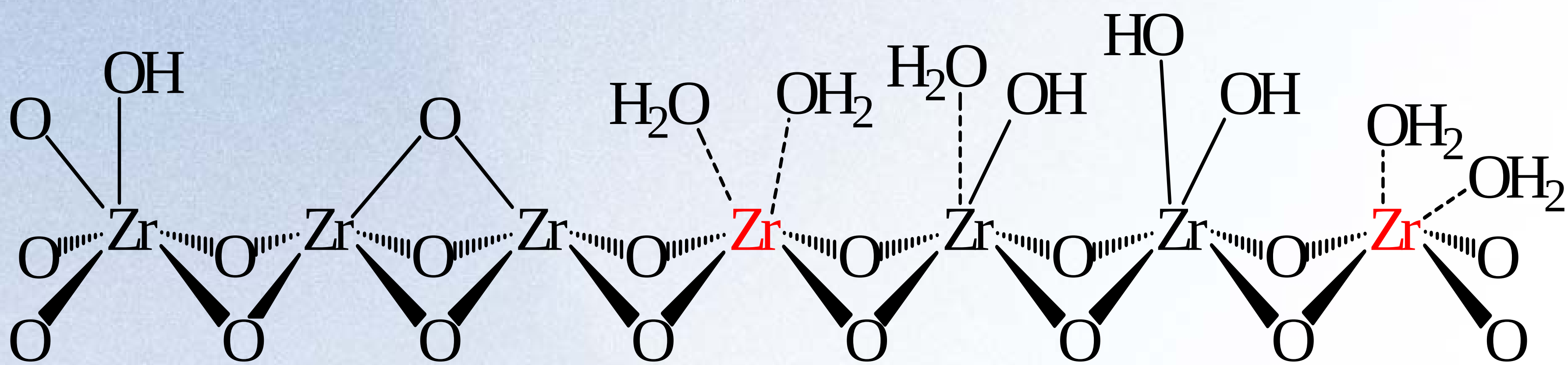
Specialists in High Efficiency, Ultra-Stable Phases for HPLC.

Goal-To Make Zirconia Based Chiral Stationary Phases for Fast Chiral Selector Screening

- Why Zirconia?
- Surface Chemistry
- Synthetic Approach
- Comparison of Zirconia-based CSPs with Commercial Silica-based CSPs
  - Chromatographic Comparison of Different Anchors
  - Chiral Separations on Zirconia Based CSPs
  - Effect of Mobile Phase Additives on α, k' and N
- New approach for Chiral Selector Screening
- Stability Study on New Anchor
- Reproducibility of Zirconia CSPs.
- Conclusion – Careful selection of an anchor group results in a stable CSP that can be stripped off and reattached under high pH condition. This offers the possibility of regeneration or use for Chiral Selector Screening.

***Zirconia* -**
The difference is the surface chemistry.

Surface Chemistry of Zirconia



Brönsted Acid: $ZrOH + {}^{-}OH \rightleftharpoons ZrO^{-} + H_2O$

Brönsted Base: $Zr{-}O{-}Zr + H^{+} \rightleftharpoons Zr{-}O(H^{+}){-}Zr$

Lewis Acid: $\mathbf{Zr^{4+}{:}\ H_2O + R\text{-}COO^{-} \rightleftharpoons Zr^{4+}{:}^{-}OOC\text{-}R + H_2O}$

Other example Lewis bases: RPO_3^{2-} or Catechol

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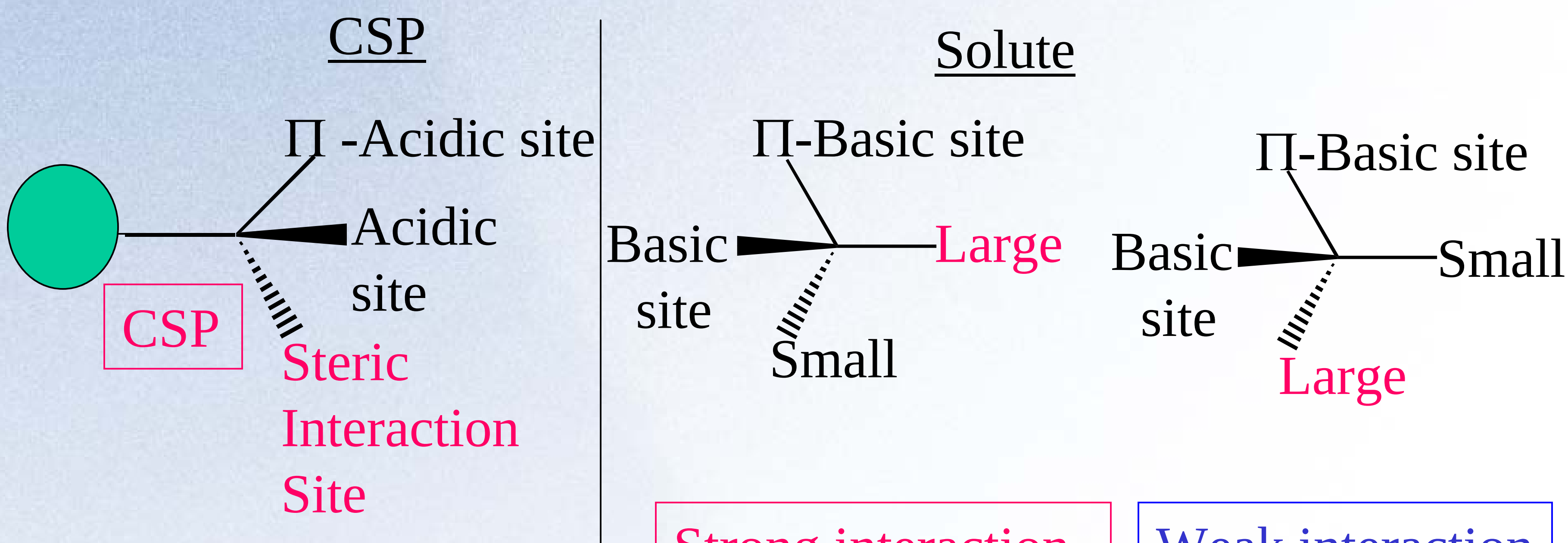
Five Classes of CSPs

1. Pirkle/Brush Type CSPs
2. Polymer Based CSPs
3. Cyclodextrins Based CSPs
4. Protein Based CSPs
5. Ligand exchange CSPs

Three-Point Interactions (Model 1)

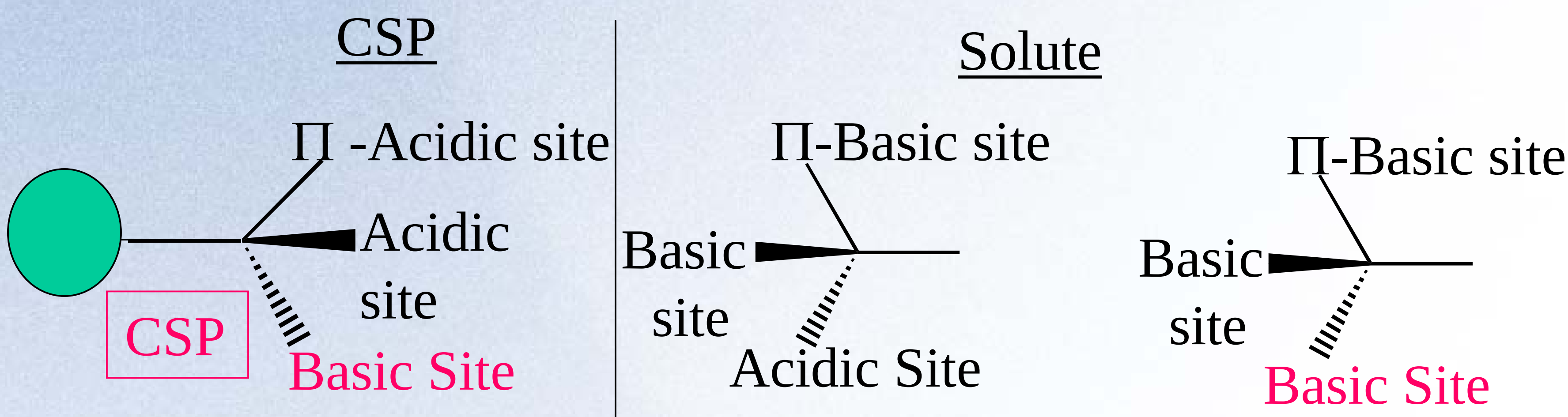


Strong interaction with CSP

Weak interaction with CSP

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

Three-Point Interactions (Model 2)

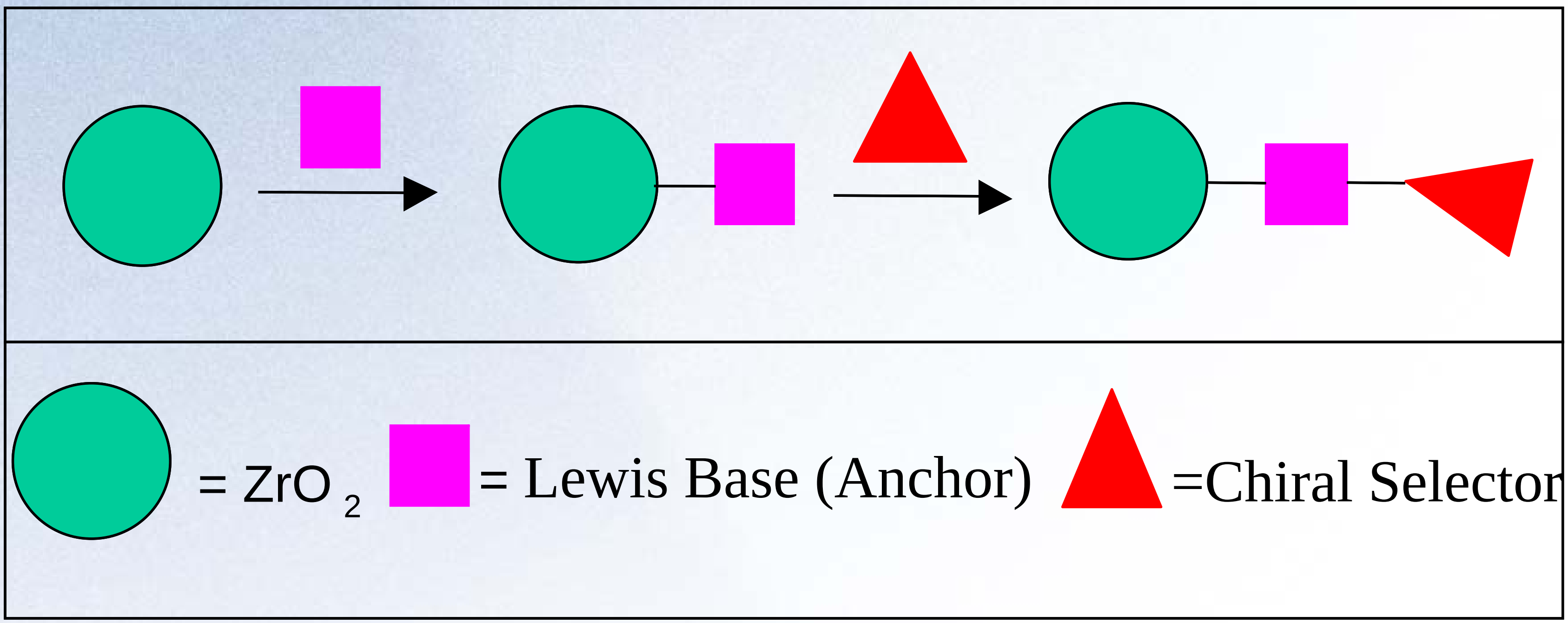


Strong interaction with CSP

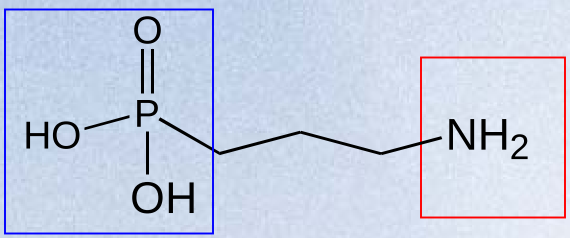
Weak interaction with CSP

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

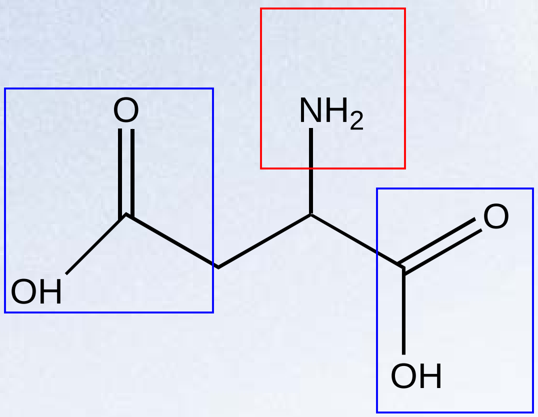
New Way to Attach Chiral Selectors to Zirconia Surface



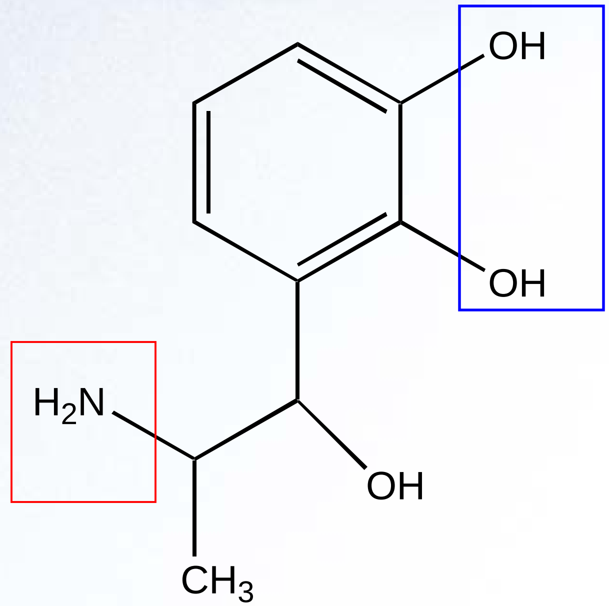
Three Anchors in This Study



APPA (Aminopropylphosphonic acid)



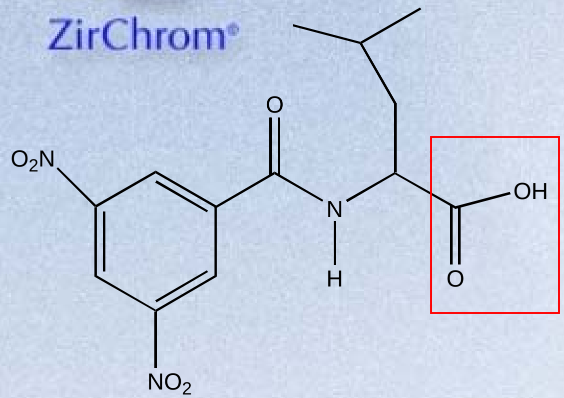
ASPA (Aspartic acid)



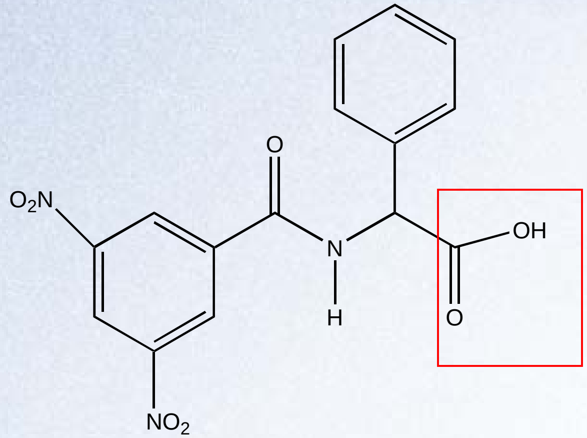
DHNP (3,4-Dihydroxynorephedrine)

Phase I Anchors

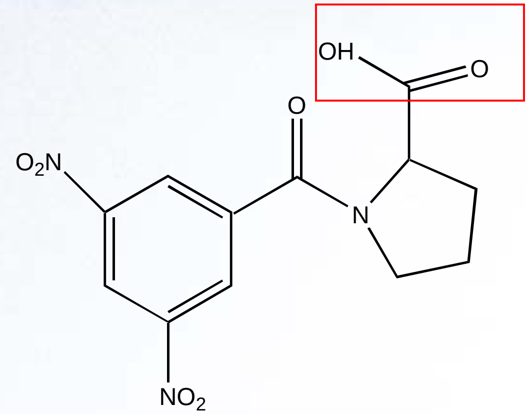
Chiral Selectors in This Study



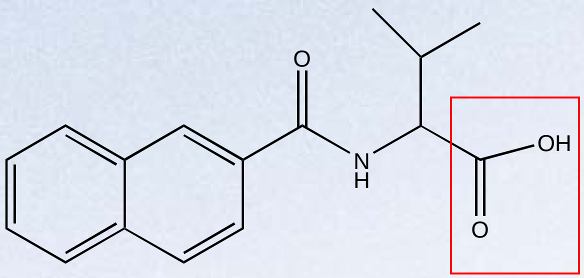
DNB-LEU (3,5-dinitro-benzoylLeucine)



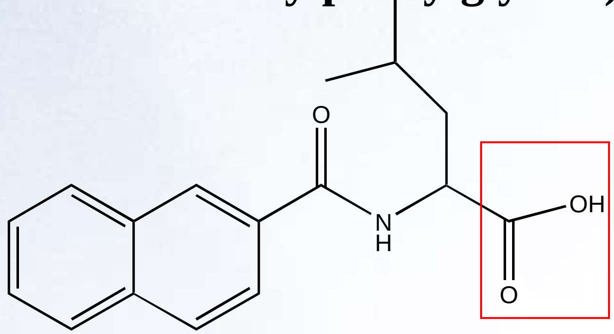
DNB-PG (3,5-dinitro-benzoylphenylglycine)



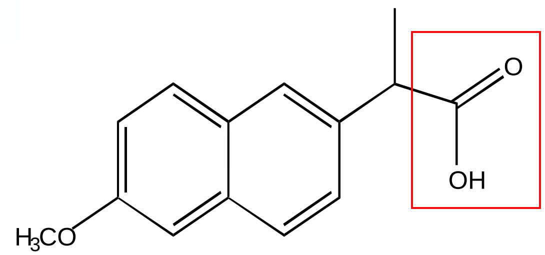
DNB-PRO (3,5-dinitro-benzoylproline)



NAP-VAL (Naphthoyl-valine)

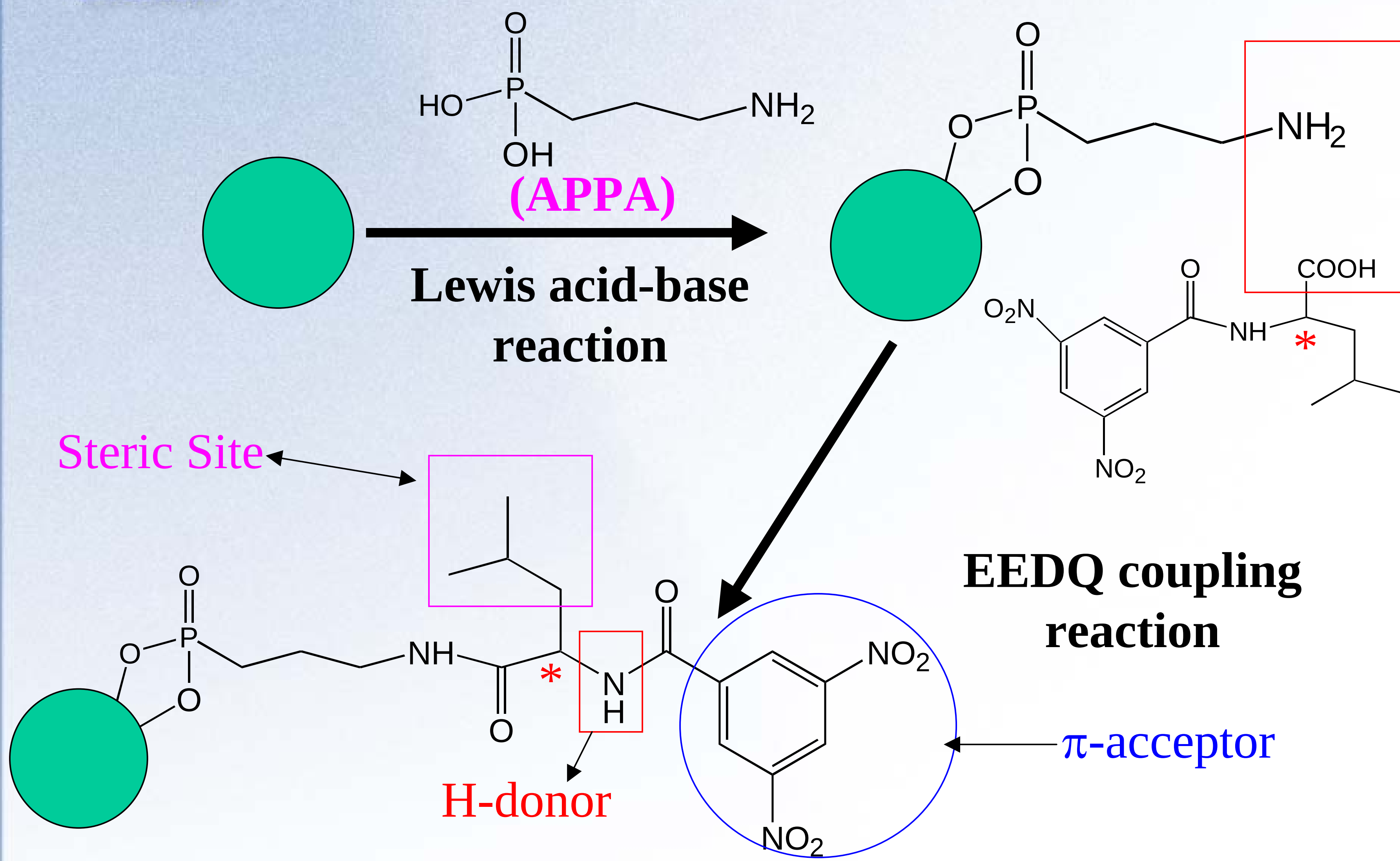


2-NAP-LEU (2-Naphthoyl-leucine),



NAP (naproxen)

Example of Lewis Acid-Base Modified Zirconia CSPs

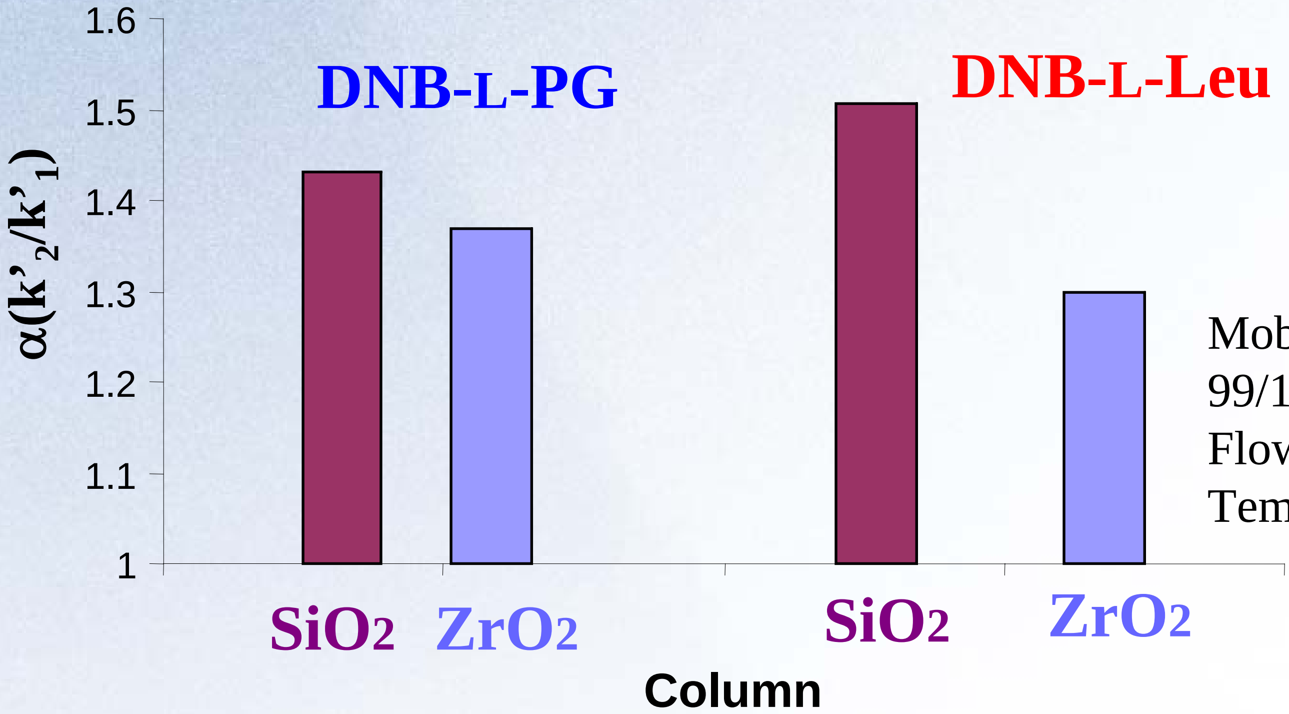


List of Zirconia and Silica CSPs Studied

Column	CSP	Anchor
Z1	DNB-Leu	APPA
Z2	DNB-Leu	Aspartic acid
Z3	DNB-Leu	DHNP
Z4	DNB-PG	APPA
Z5	DNB-PG	Aspartic acid
Z6	DNB-PG	DHNP
Z7	DNB-Pro	DHNP
Z8	NAP-Leu	APPA
Z9	NAP-Val	DHNP
Z10	Naproxen	APPA
R1	DNB-PG	--
R2	DNB-Leu	--

Z1-Z10 **zirconia** based CSPs, R1, R2-commercialized **silica** based CSPs

Chromatographic Comparison of Zirconia- and Silica-CSPs

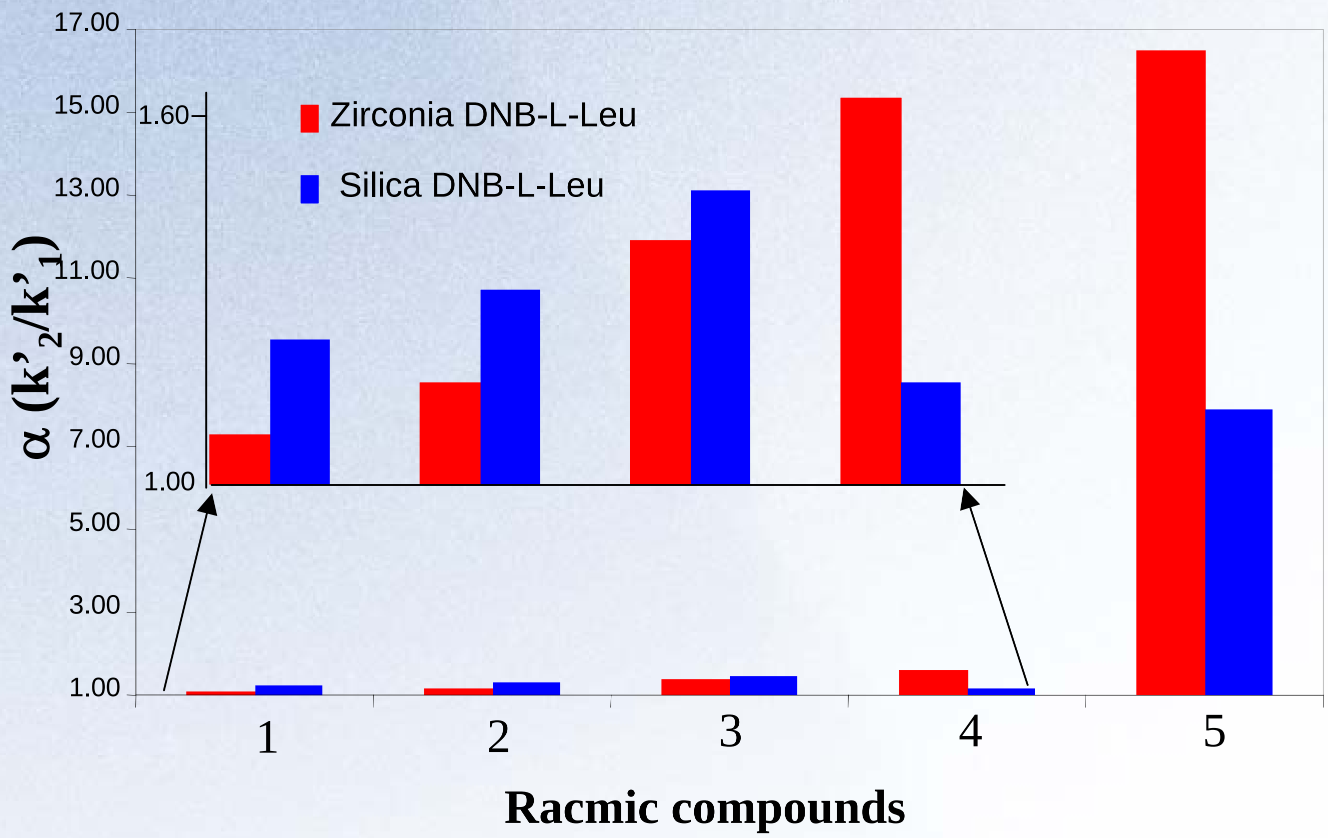


Mobile phase:
99/1Hexane/IPA
Flow rate: 1 ml/min;
Temperature: 30 °C

Probe solute: Trifluoroanthryl ethanol

Conclusion: Zirconia based CSPs compared favorably.

Direct Comparison of DNB-L-LEU Zirconia and Silica Based CSPs

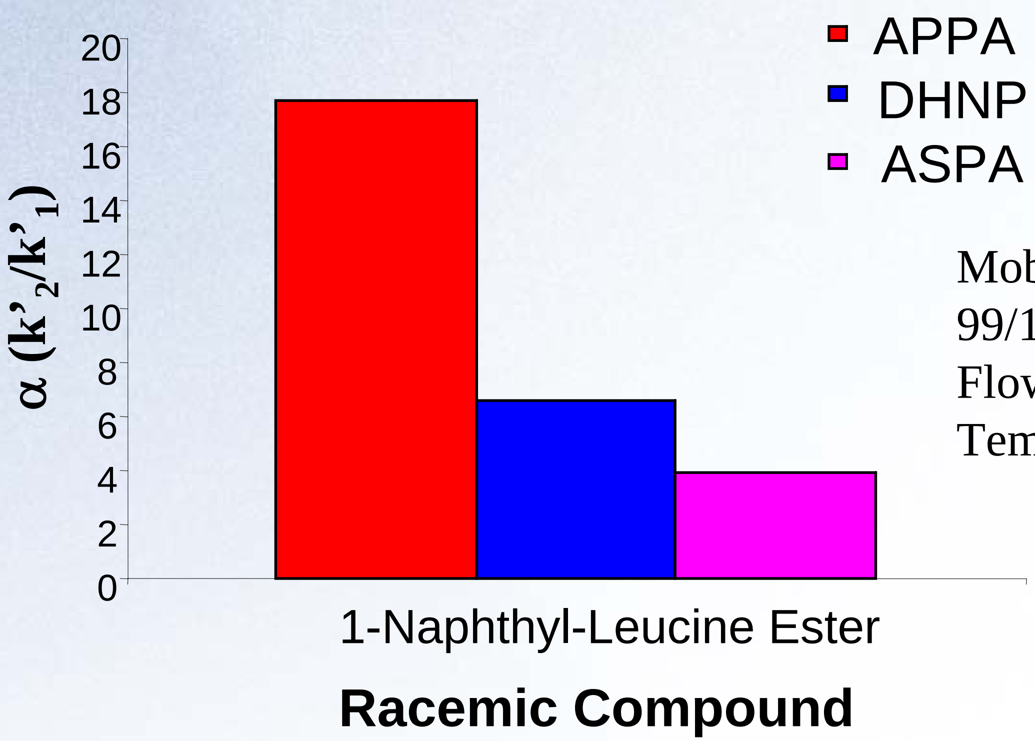


1 trans-stibene oxide
2 1,1'-bi-2-naphthol
3 trifluoranthyl ethanol
4 napropamide
5 1-naphthyl leucine ester

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

Much better separations for napropamide and 1-naphthyl leucine ester are obtained on zirconia-based CSPs.

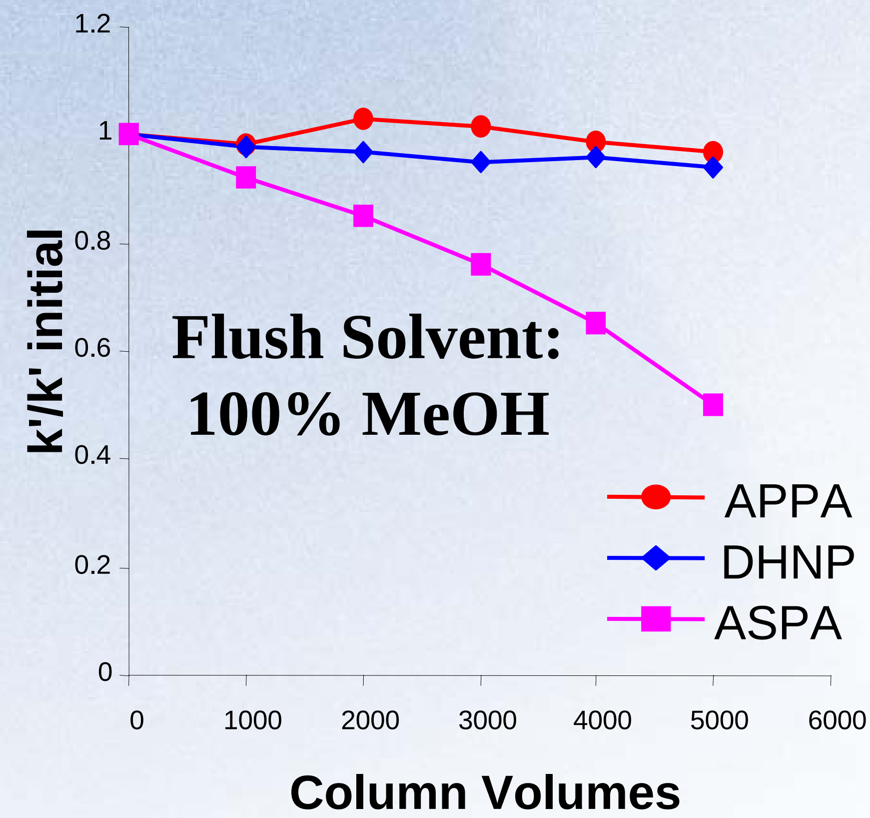
Chromatographic Comparison of Differently-Anchored Zirconia-based DNB-L-LEU

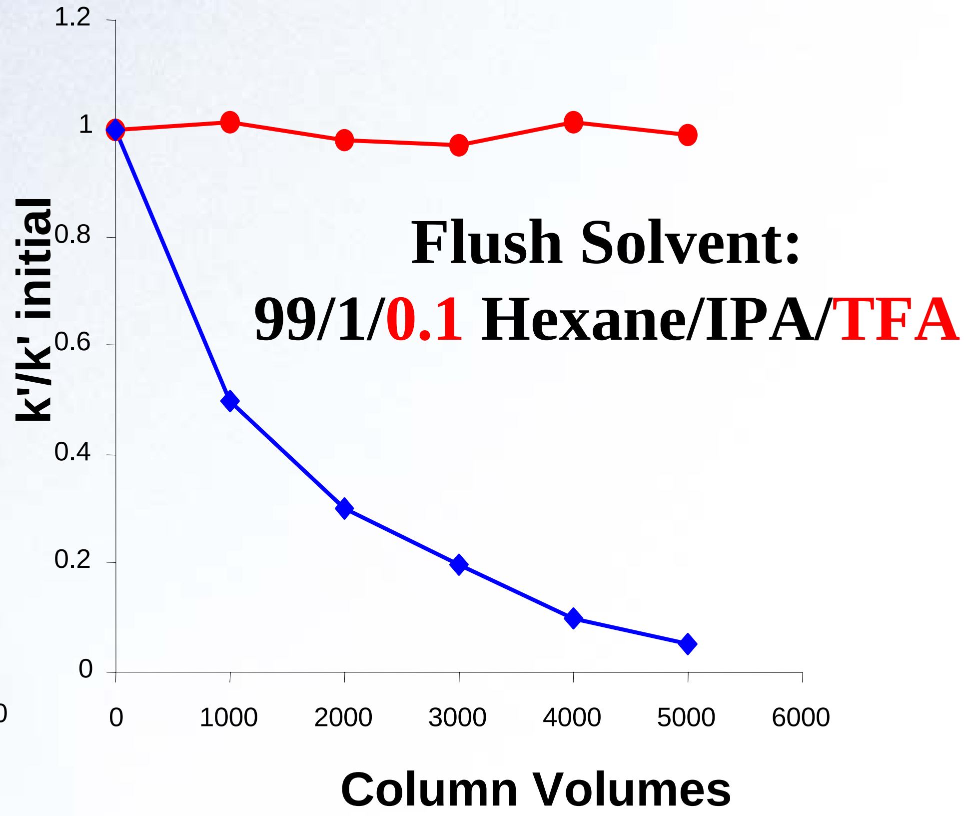


Mobile phase: 99/1Hexane/IPA
Flow rate: 1 ml/min;
Temperature: 30 ^{o}C

Different anchors show different selectivity.

Stability Comparison of Differently-Anchored Zirconia-Based DNB-L-LEU





Test solute: trifluoranthryl ethanol. Note that the retention factor ratio is for the less retained isomer.

Enantiomer Separations on DNB-L-LEU Modified Zirconia Phase Anchored via APPA

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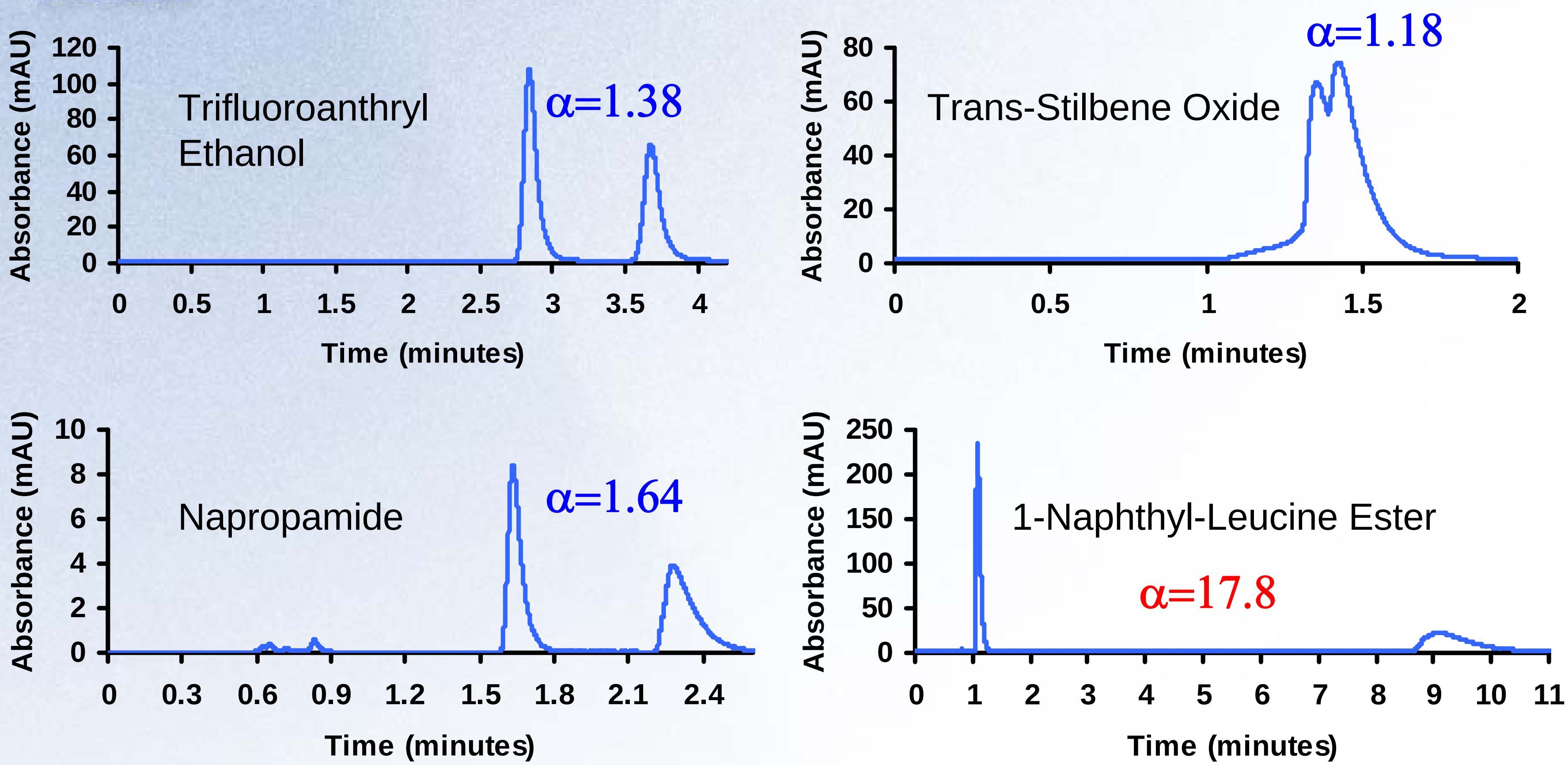
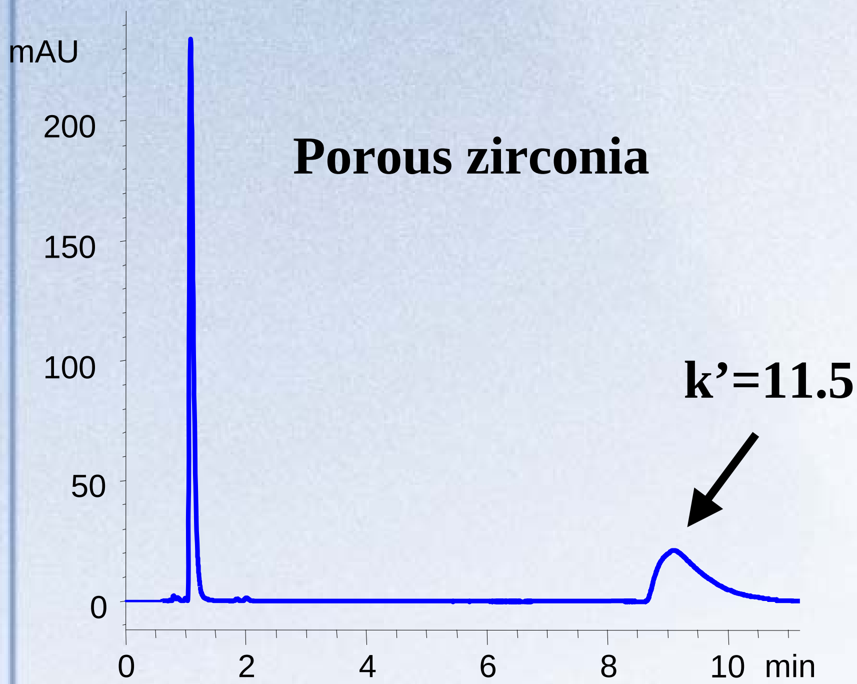
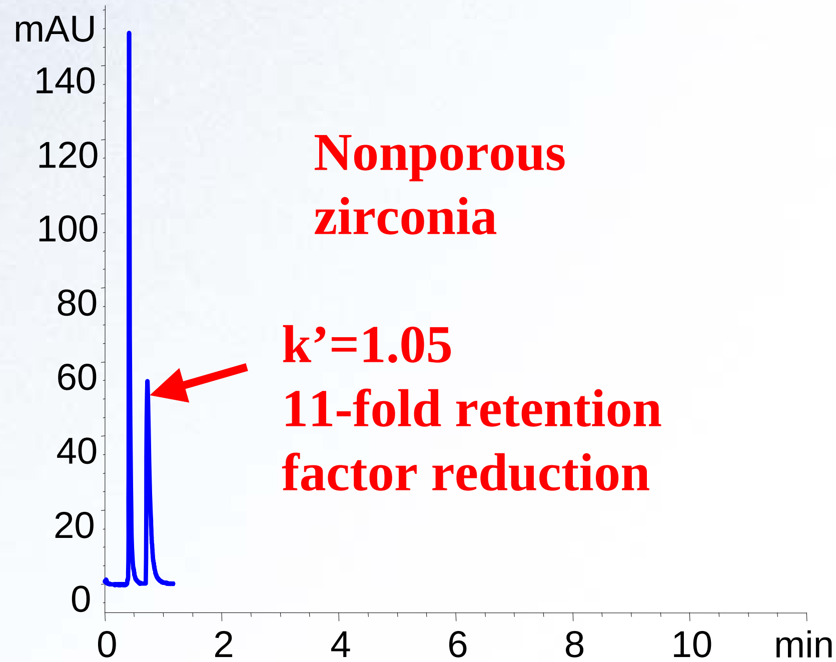


Selected Chromatograms of Chiral Compounds on Zirconia Based DNB-L-LEU Anchored with APPA. Chromatographic conditions: mobile phase 99/1 Hexane/IPA.

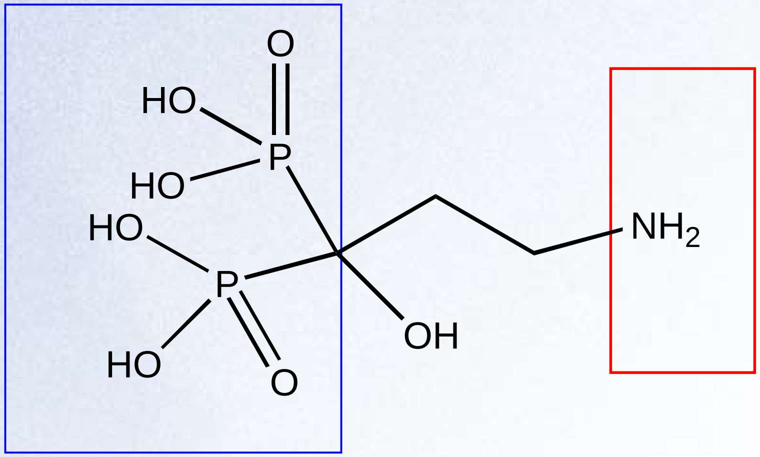
Fast Chiral Separation on Nonporous Zirconia-based DNB-L-Leu





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

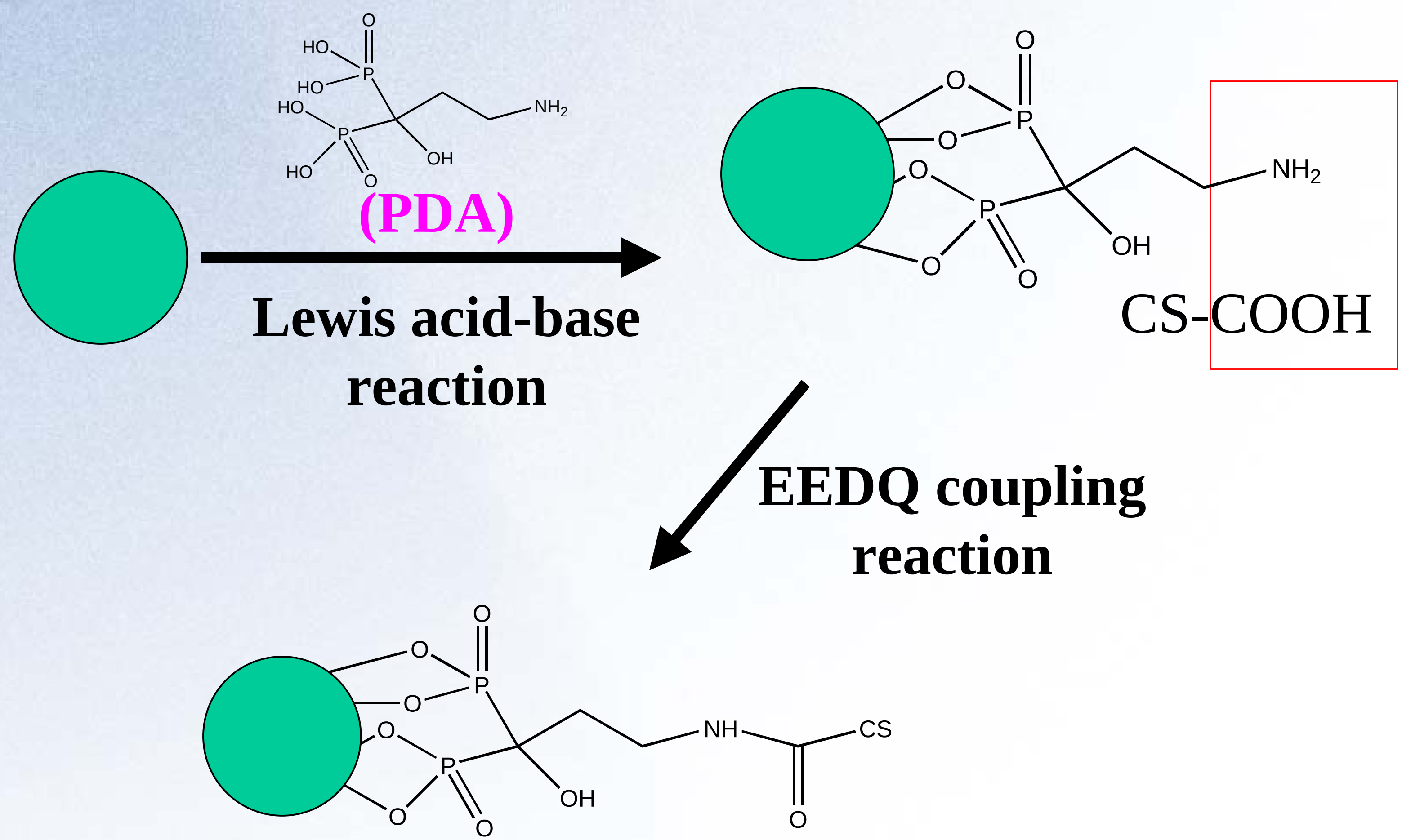
Anchor Group – the Key to Chemical Stability



PDA (Pamidronic acid)

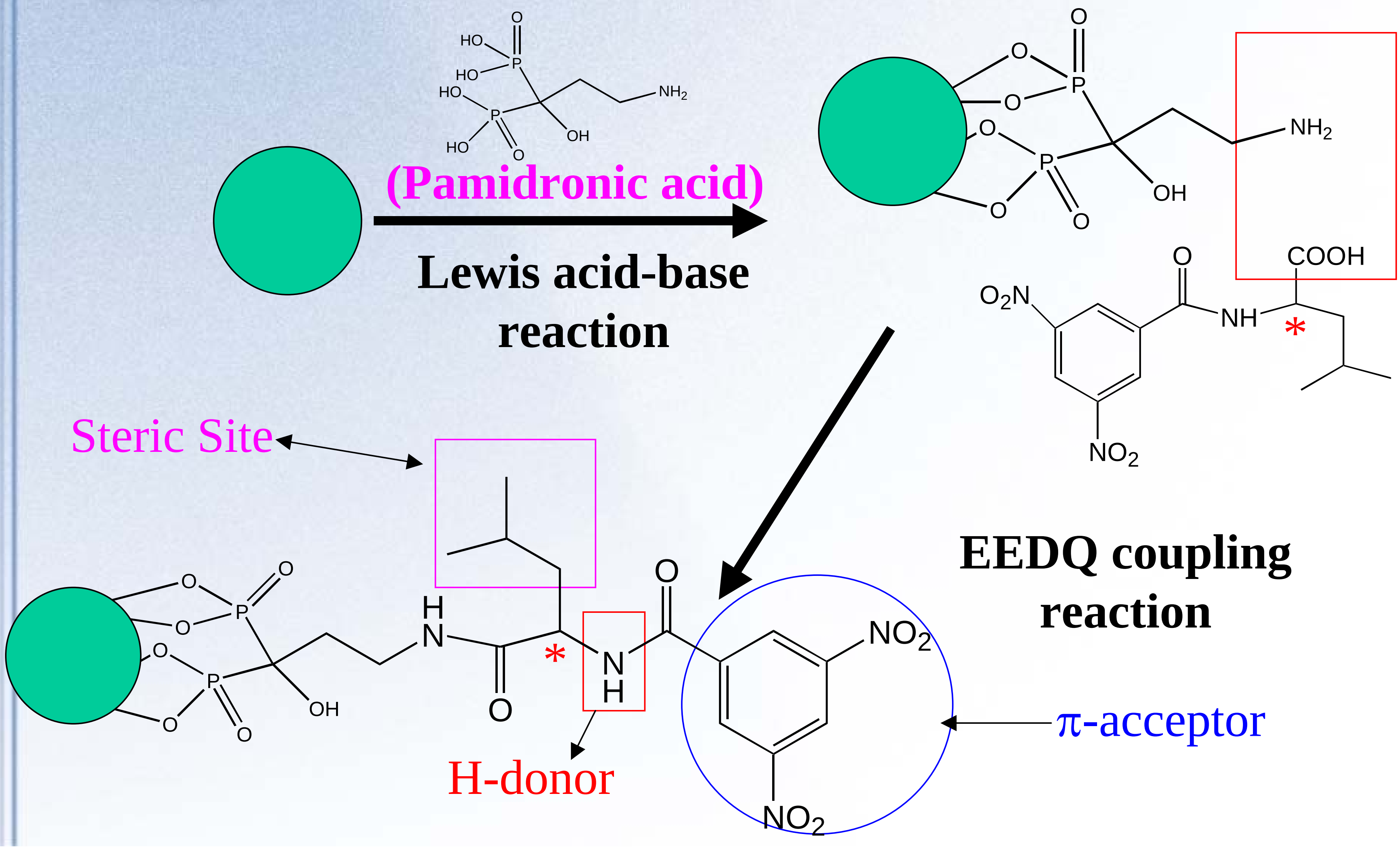
Phase II Anchor

Example of Lewis Acid-Base Modified Zirconia CSPs

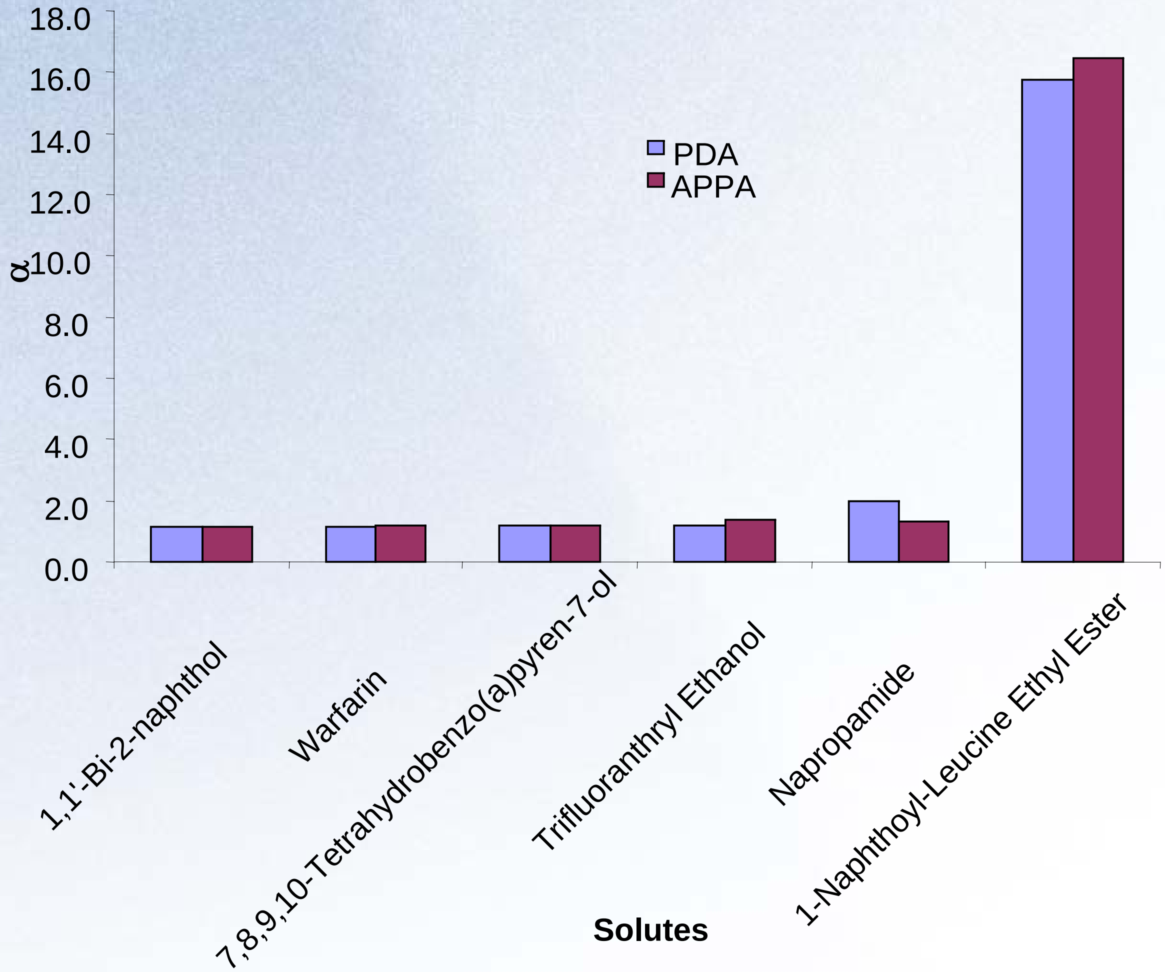


CS = Chiral Selector

Example of Lewis Acid-Base Modified Zirconia CSPs

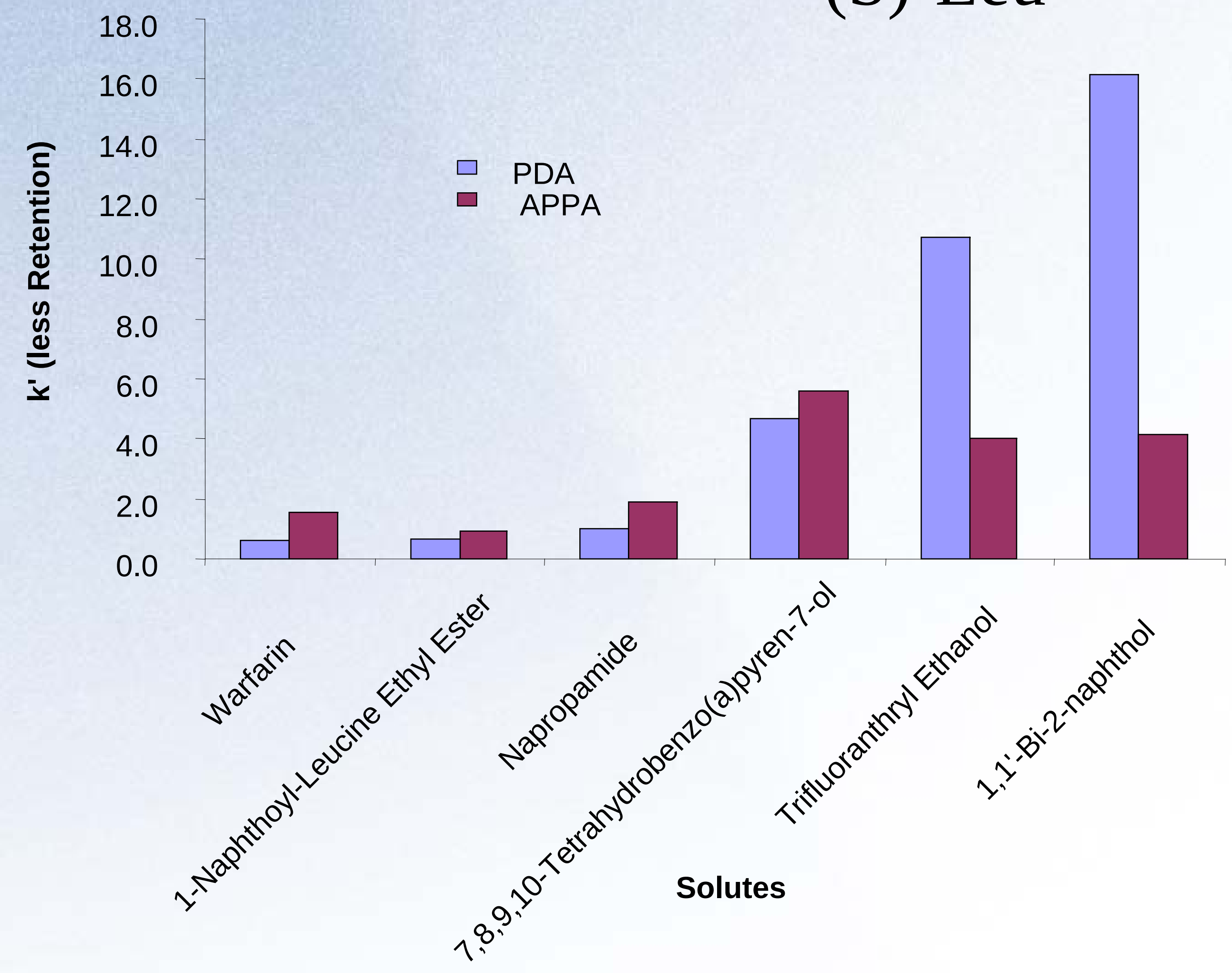


Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



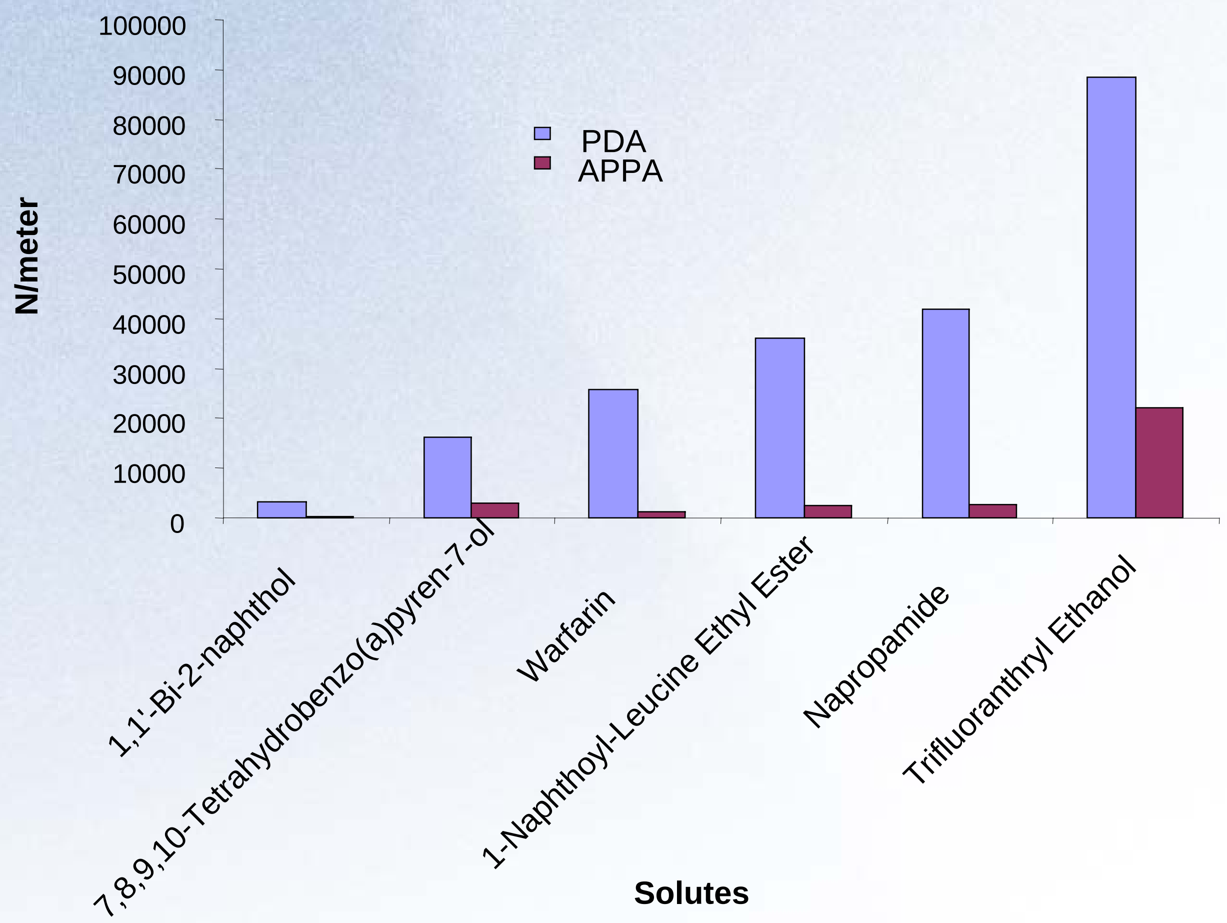
Selectivity for both anchors is very similar.

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



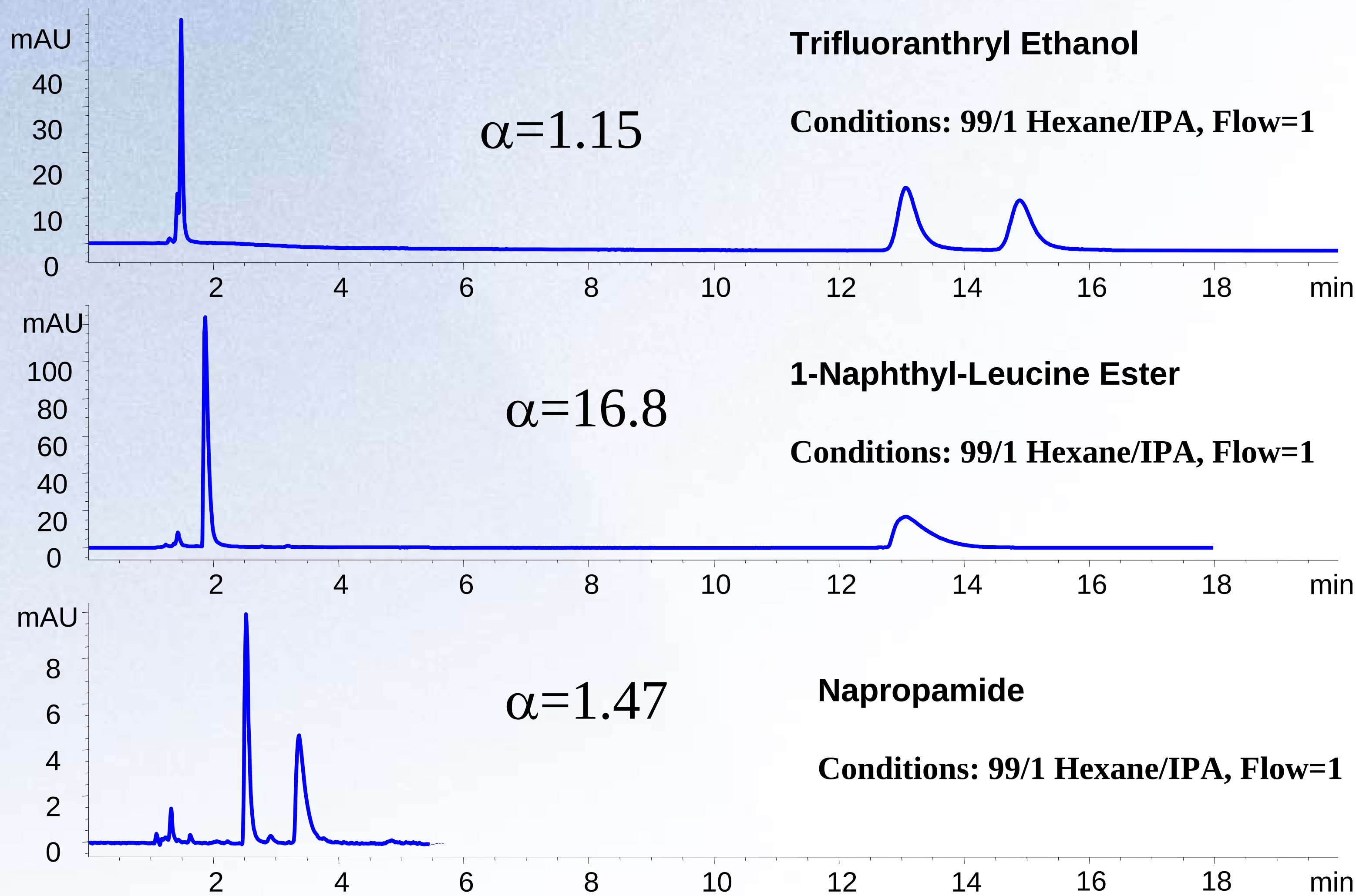
Retention for both anchors is different.

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

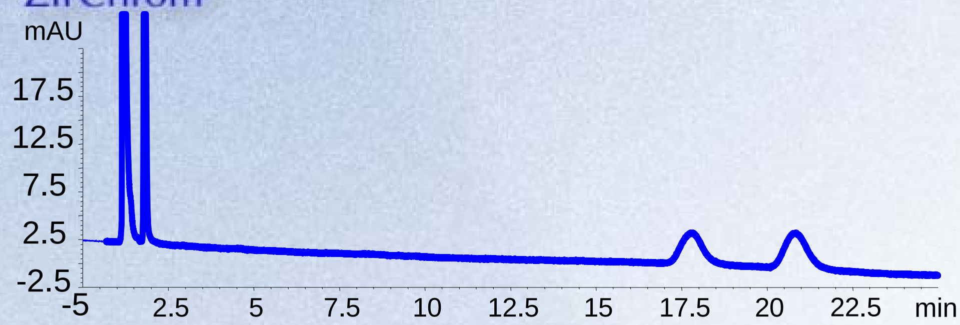


Efficiency on PDA anchored Zr (S)-Leu is much better than on APPA anchored Zr (S)-Leu.

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



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

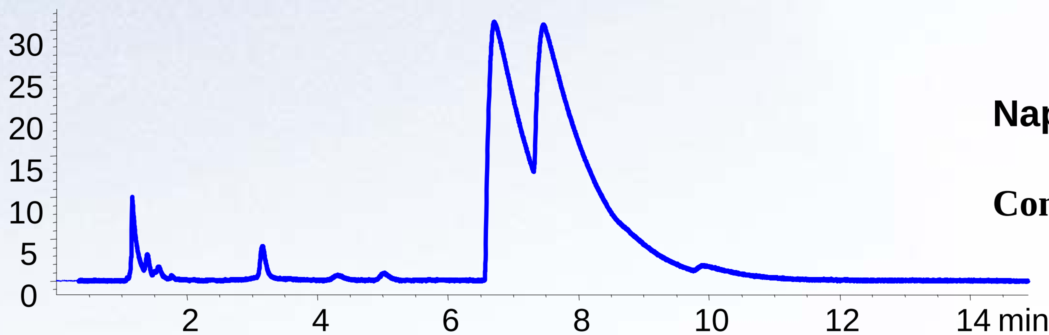


Trifluoranthryl Ethanol

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

1-Naphthyl-Leucine Ester

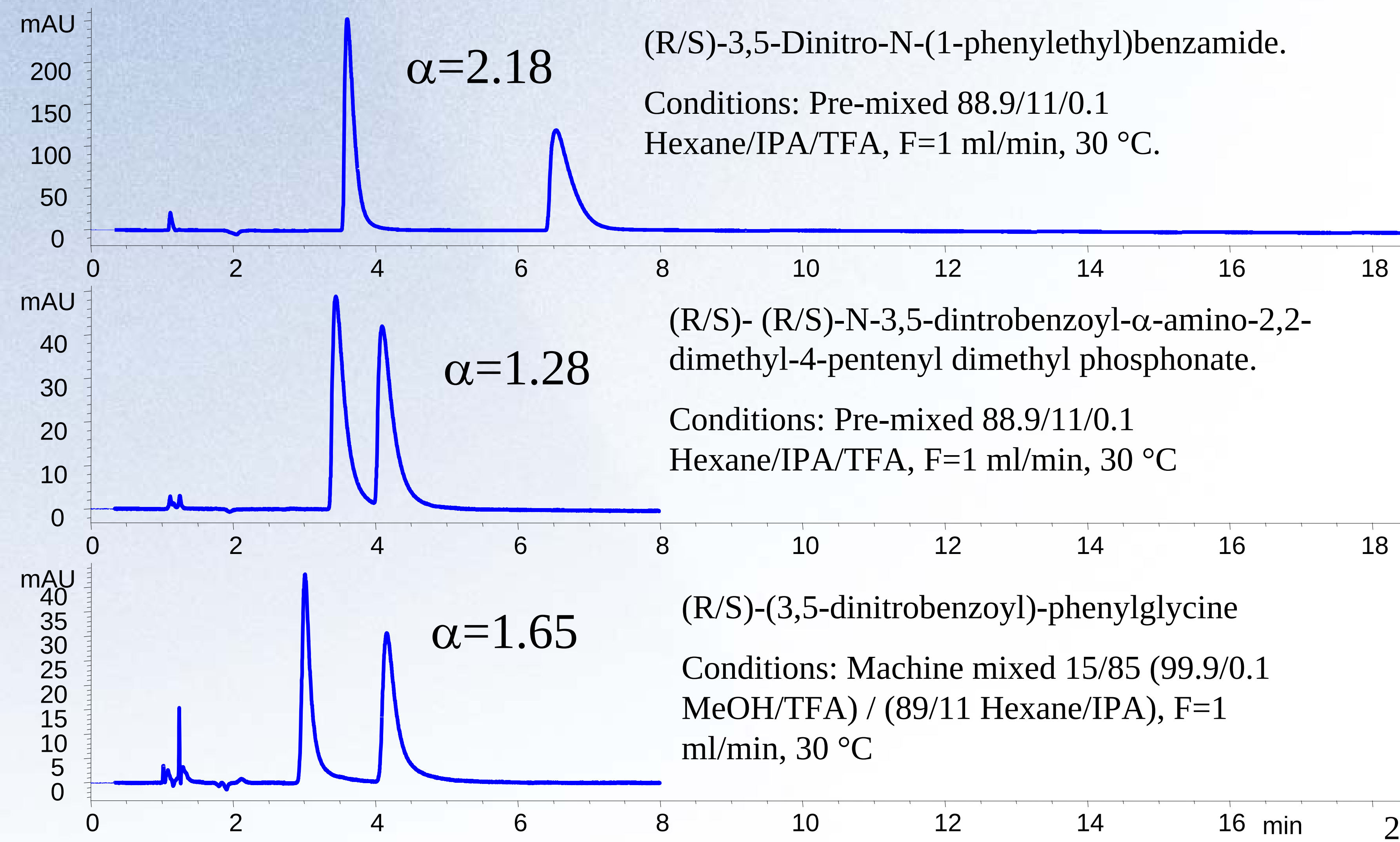
Conditions: 99/1 Hexane/IPA, Flow=1



Napropamide

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

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



(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.

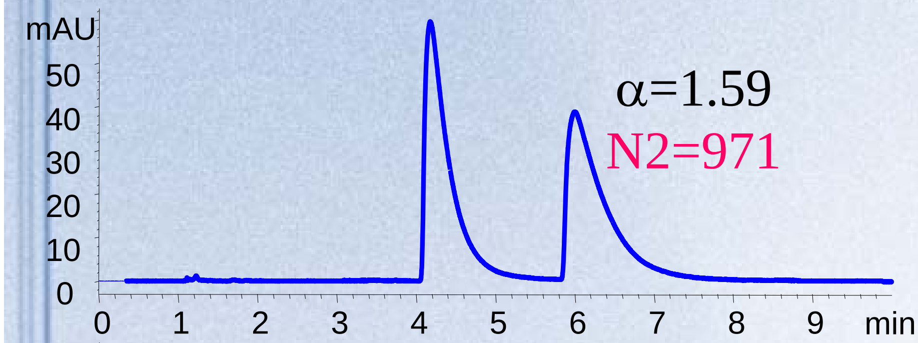
(R/S)- (R/S)-N-3,5-dintrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate.

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

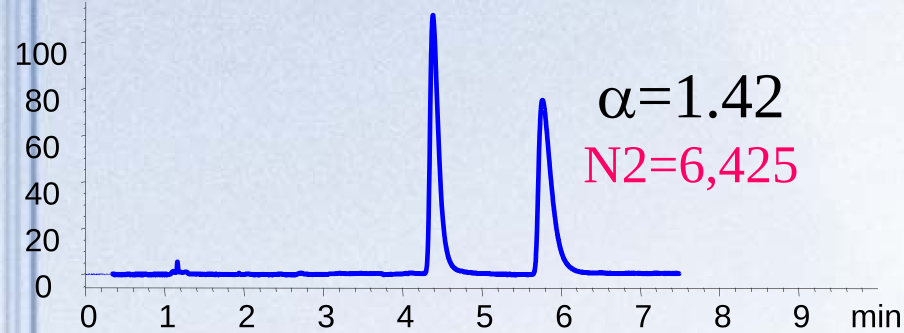
(R/S)-(3,5-dinitrobenzoyl)-phenylglycine

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

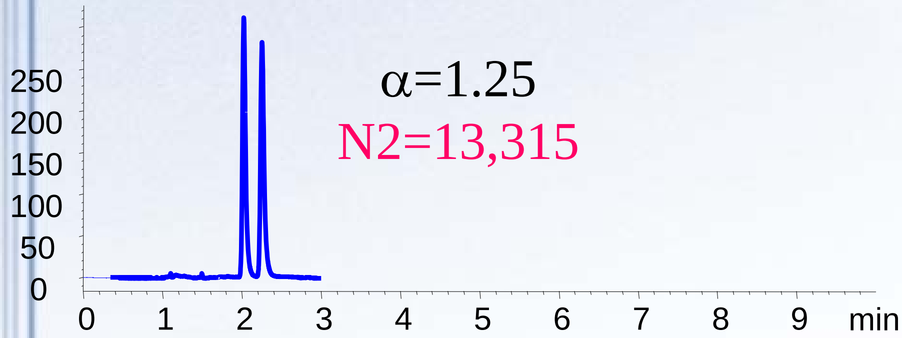
Mobile Phase Effect of adding MeOH on Separation of (R/S)-N-3,5-dintrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA



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



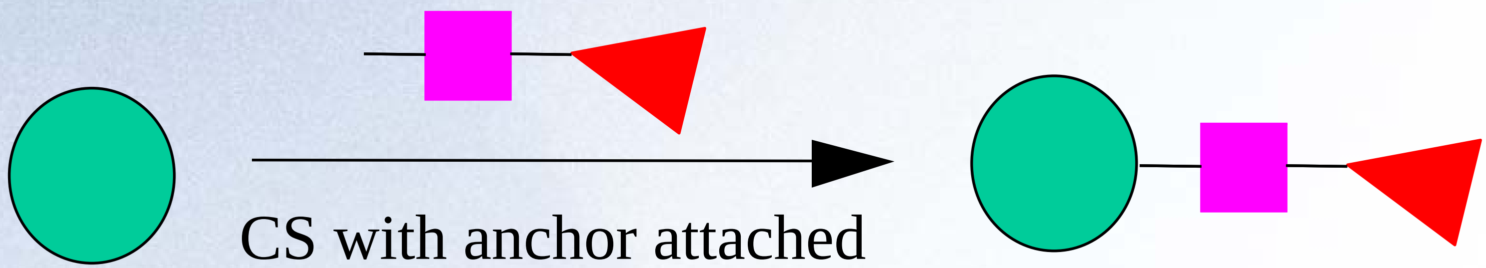
Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / **MeOH** / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

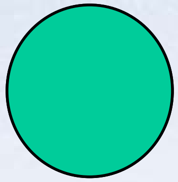


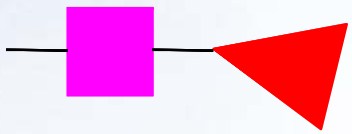
Conditions: 80 / 10 / 10 (99/1 Hexane/IPA) / **MeOH** / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

New Way to Synthesize Zirconia Based CSPs for Chiral Screening

Phase II Reaction Scheme: One-Step Modification



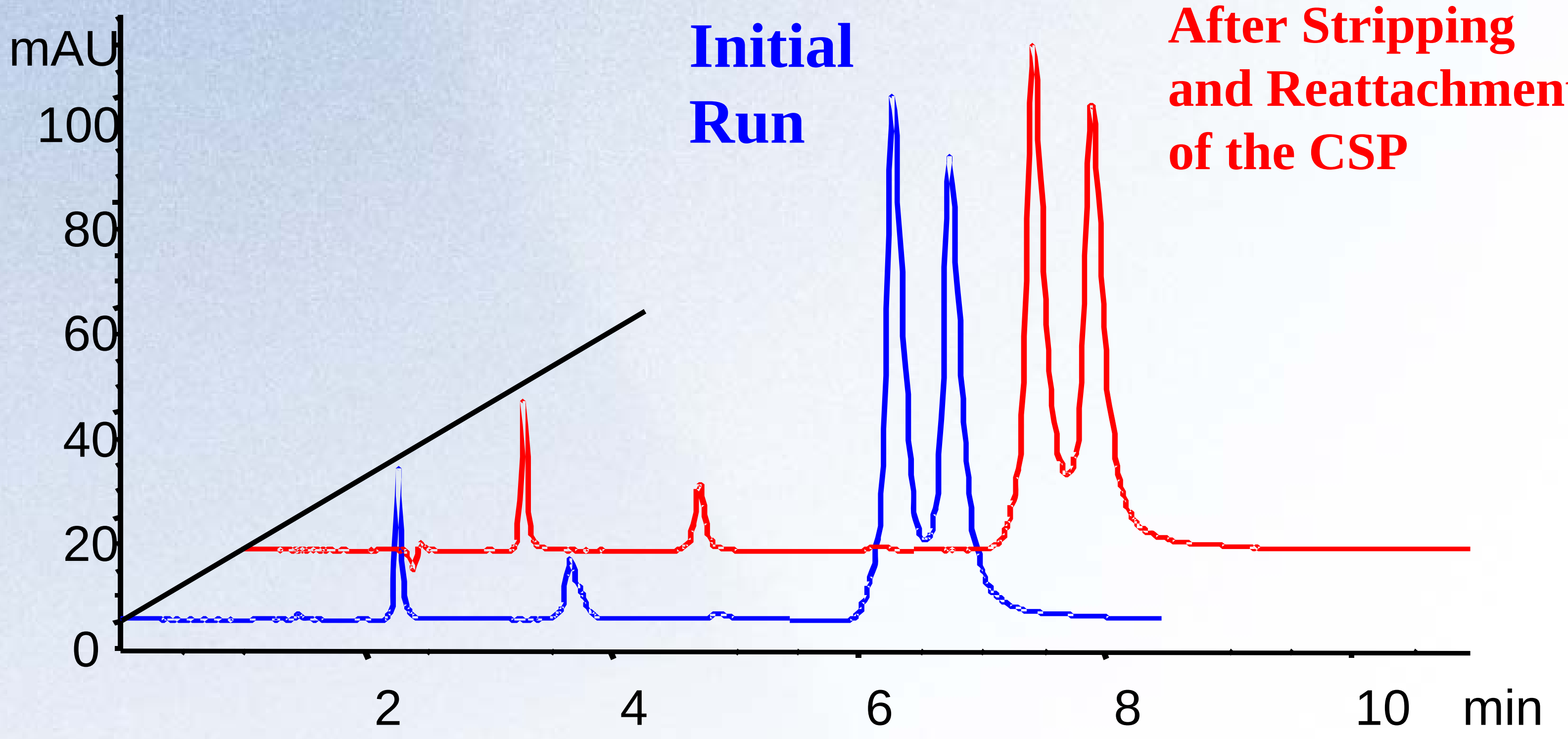
 = ZrO_2

 =Chiral Selector with Anchor Function Groups

Example Attachment and De-attachment Cycle

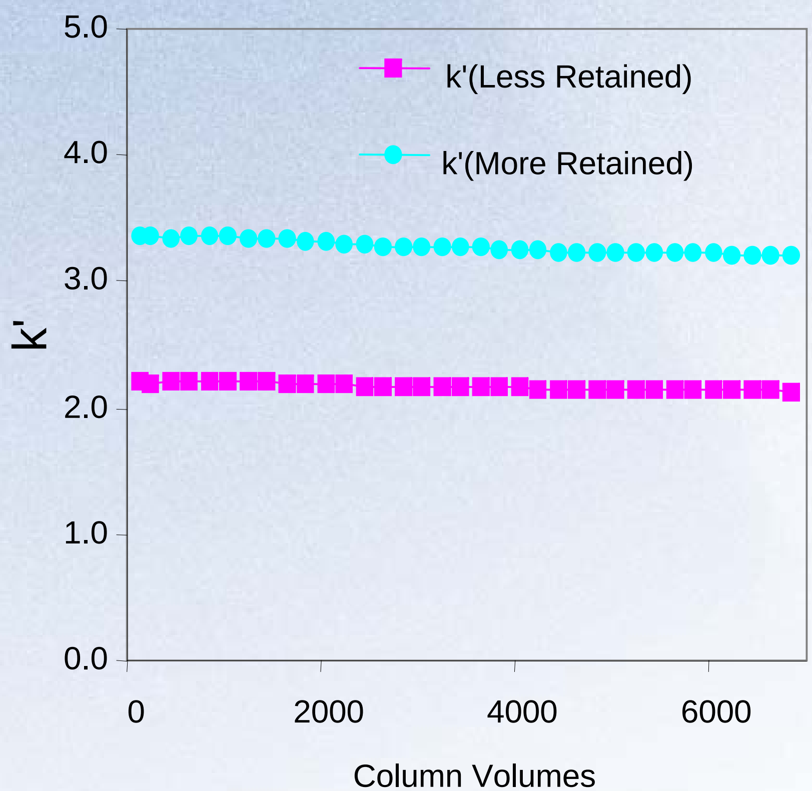
- Pass a solution of 20 mM N-(4-nitrobenzoyl)-L-glutamic acid (CSP) in tetrahydrofuran for 10 minutes at a column temperature of 60°C and a flow rate of 1 mL/min .
- Flushed column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
- Separate a racemate solution of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol.
- 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

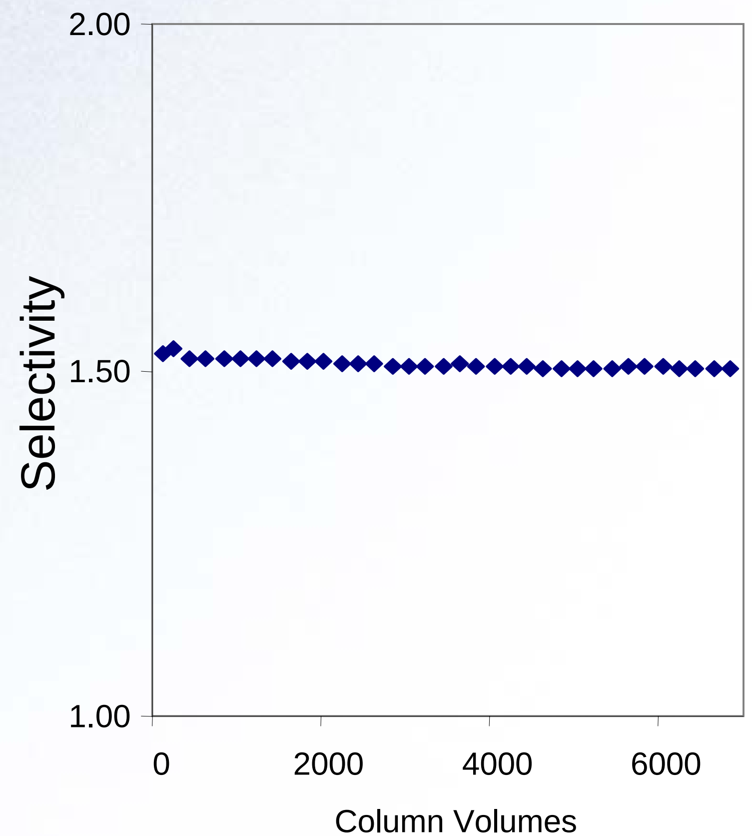
Proof of Concept



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 microliter injection.

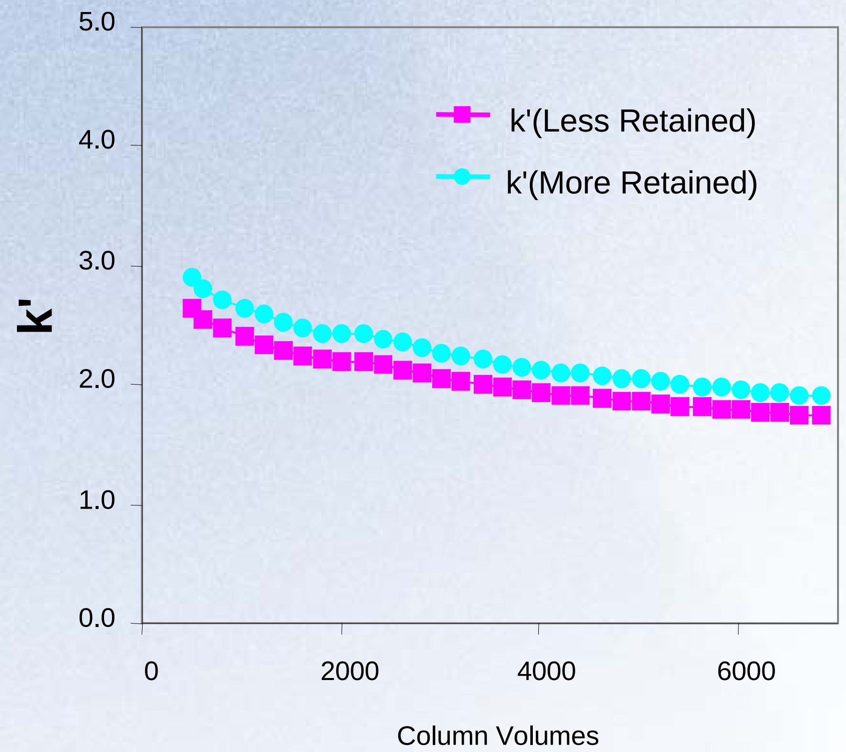
Stability of Zr-(S)-NESA at pH 2

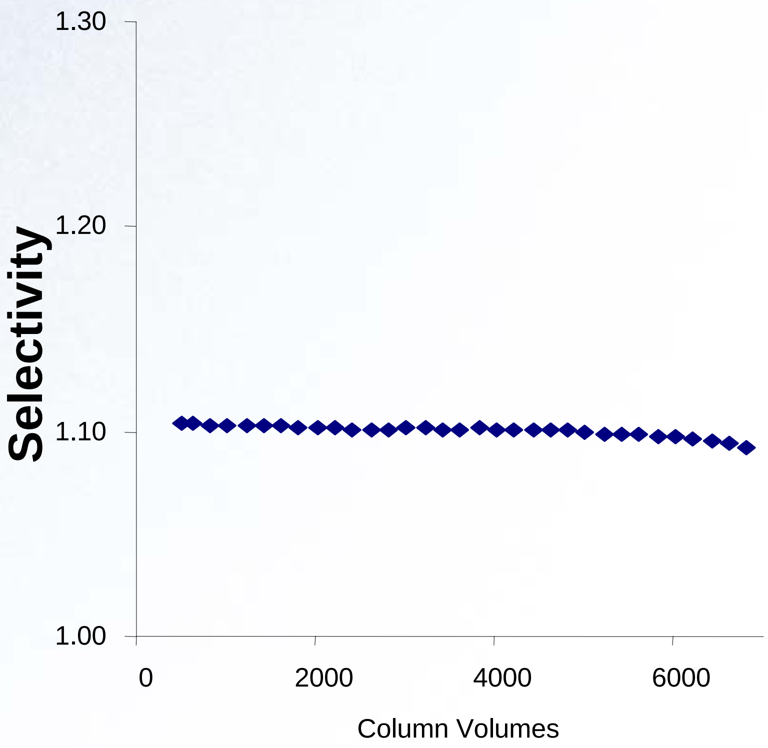




Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 ^oC. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

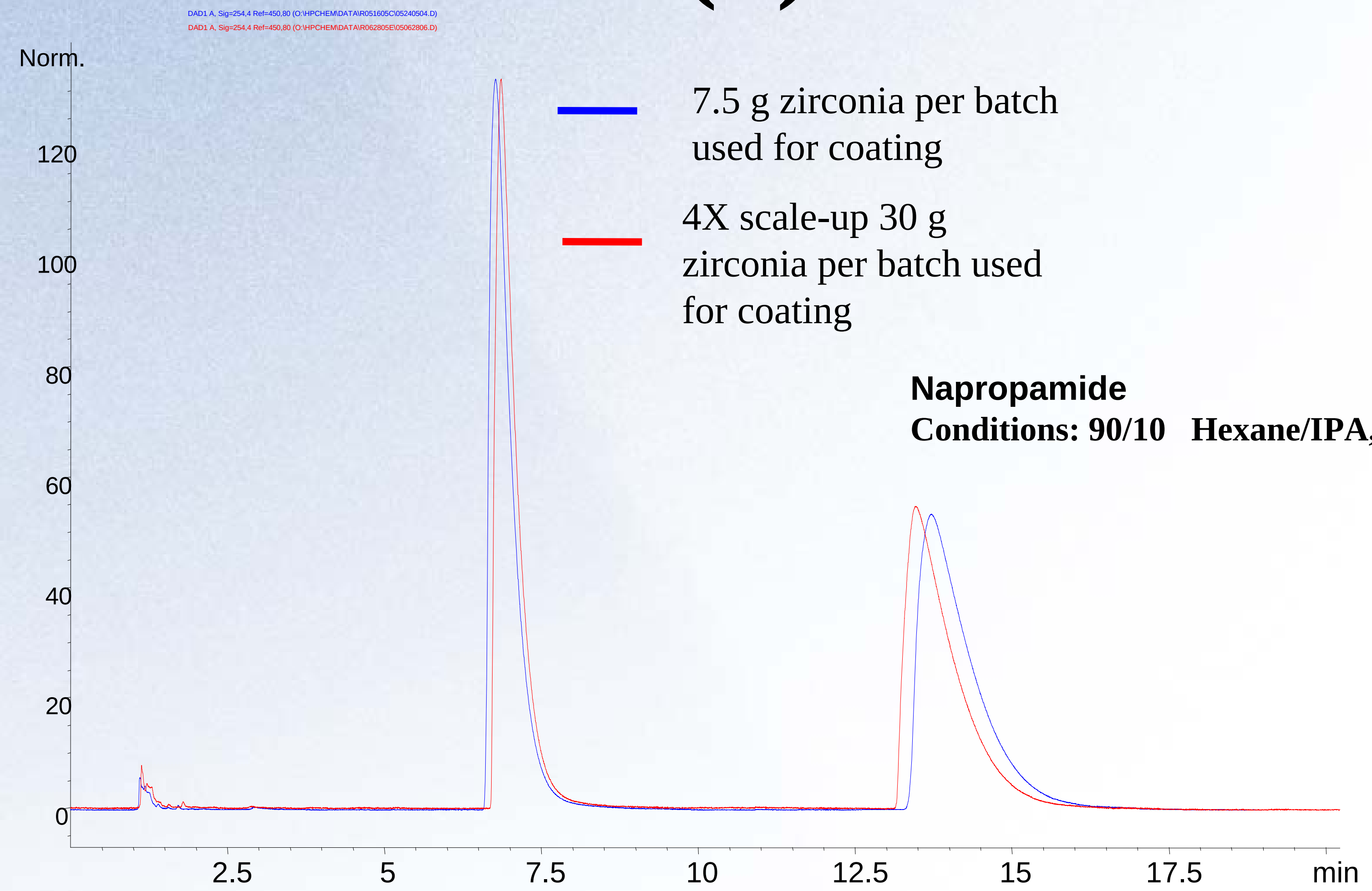
Stability of Zr-(S)-DNB-Leu at pH 8





Column ID: ZrCSP032805A, Mobile phase: 15/85 ACN/5 mM ammonium hydrogencarbonate pH 8, Temperature: 30 ºC. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-2, 2, 2-trifluoro-1-(9-anthryl)ethanol

Scale-up the Production of Zr (S)-NESA



Napropamide
Conditions: 90/10 Hexane/IPA, Flow=1

Future Work

- Demonstrate Attachment and Reattachment to zirconia with Different Chiral Selectors.
- Develop a CSP With a Function of Both pi-donor and pi-acceptor.
- Cellulose Based Zr CSPs.
- Expand the number and type of Zirconia Based CSPs.

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Conclusions

- Five new CSPs were attached to zirconia using the PDA anchor, including:

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

  π–donors: Zr (R)-NESA, Zr (S)-NESA
- Zirconia based CSPs have ***comparable*** chromatographic performance compared to the commercial silica based CSPs for a wide range of chiral compounds.
- Fast Separation can be achieved on ***nonporous*** zirconia based CSPs.
- Small amounts of ***methanol in the mobile phase had a positive effect*** on efficiency, retention, and selectivity.
- The new Zirconia-based CSPs were found to be ***stable*** in reversed-phase mobile phase from pH 2 to pH 8.
- ***The CSP synthesis is very reproducible***.
- ***Chiral selector screening is possible on the new zirconia-based CSPs***.
- Acknowledgement: ***National Institutes of Health Grant*** (Phase II SBIR) 2R44HL070334-02A2.

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