



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

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MN 55455.

**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  $\alpha$ ,  $k'$  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.



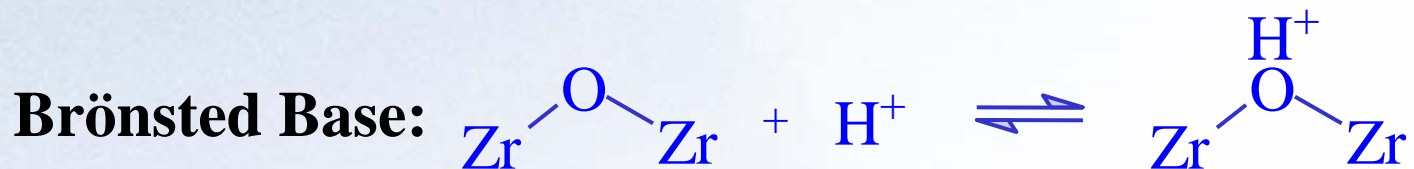
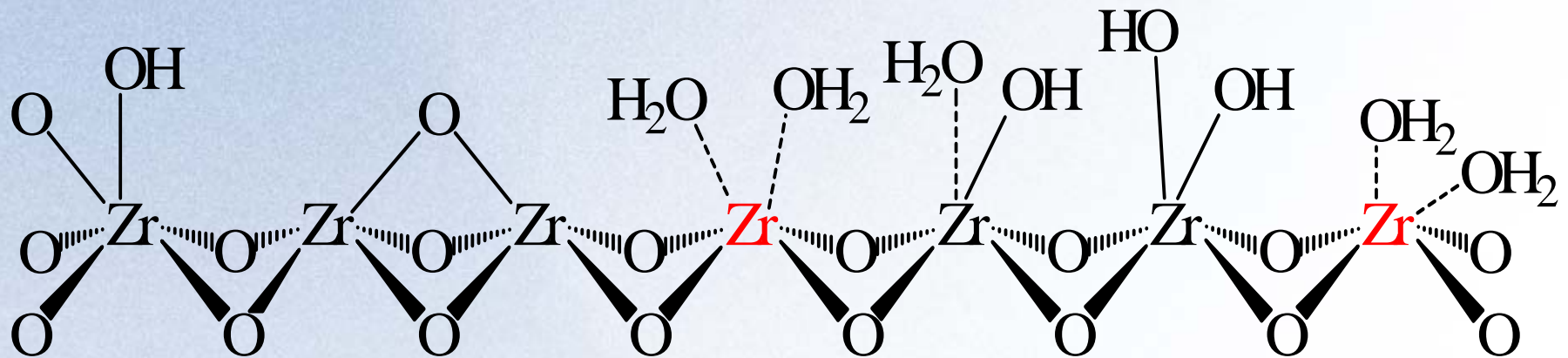
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*Zirconia* -  
The difference is the  
surface chemistry.



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# Surface Chemistry of Zirconia



**Other example Lewis bases:**  $\text{RPO}_3^{2-}$  or Catechol

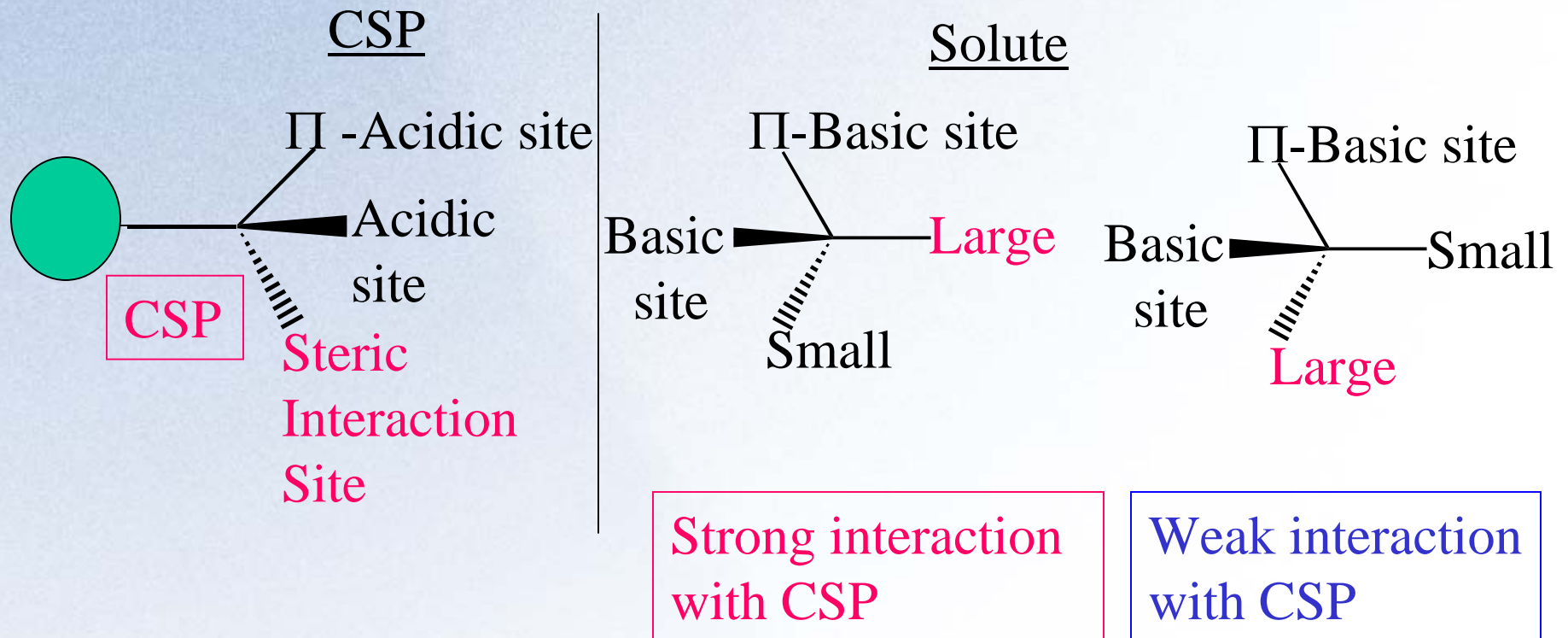


# Five Classes of CSPs

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



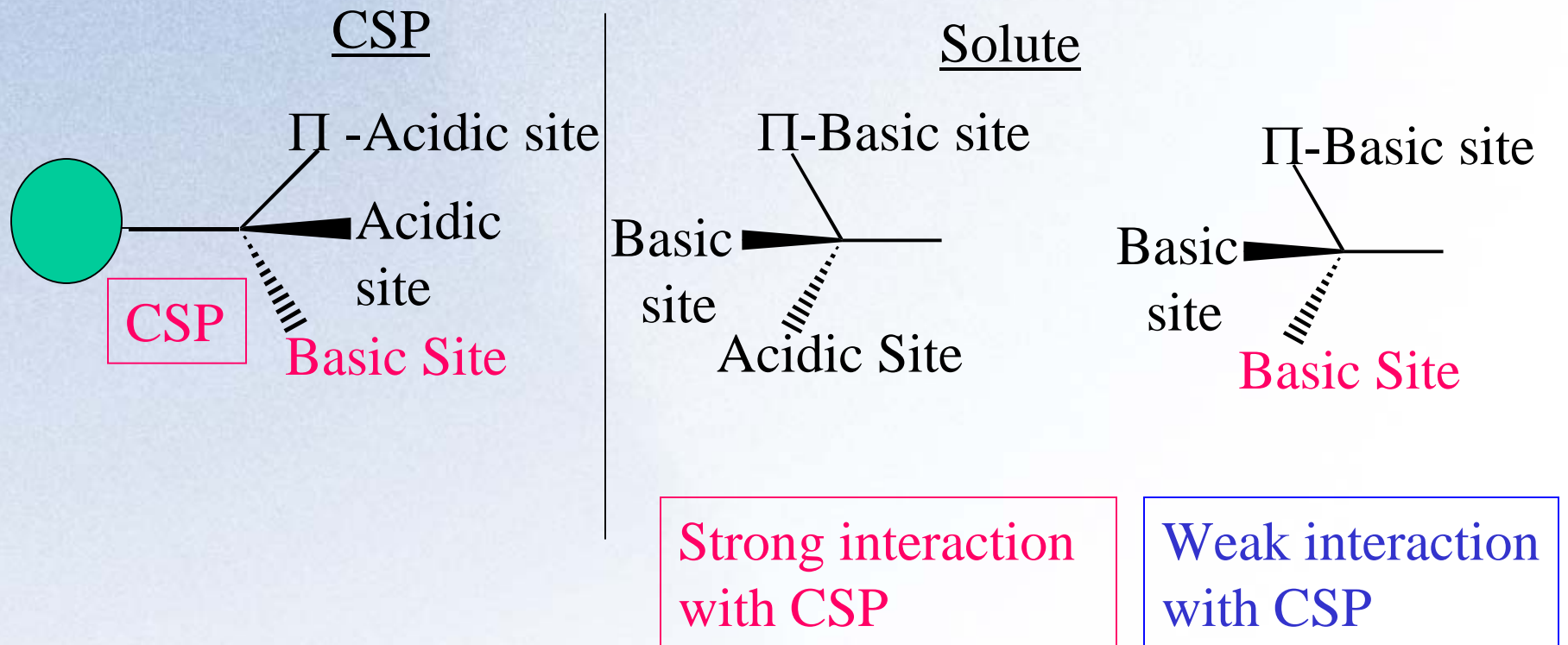
# Three-Point Interactions (Model 1)



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



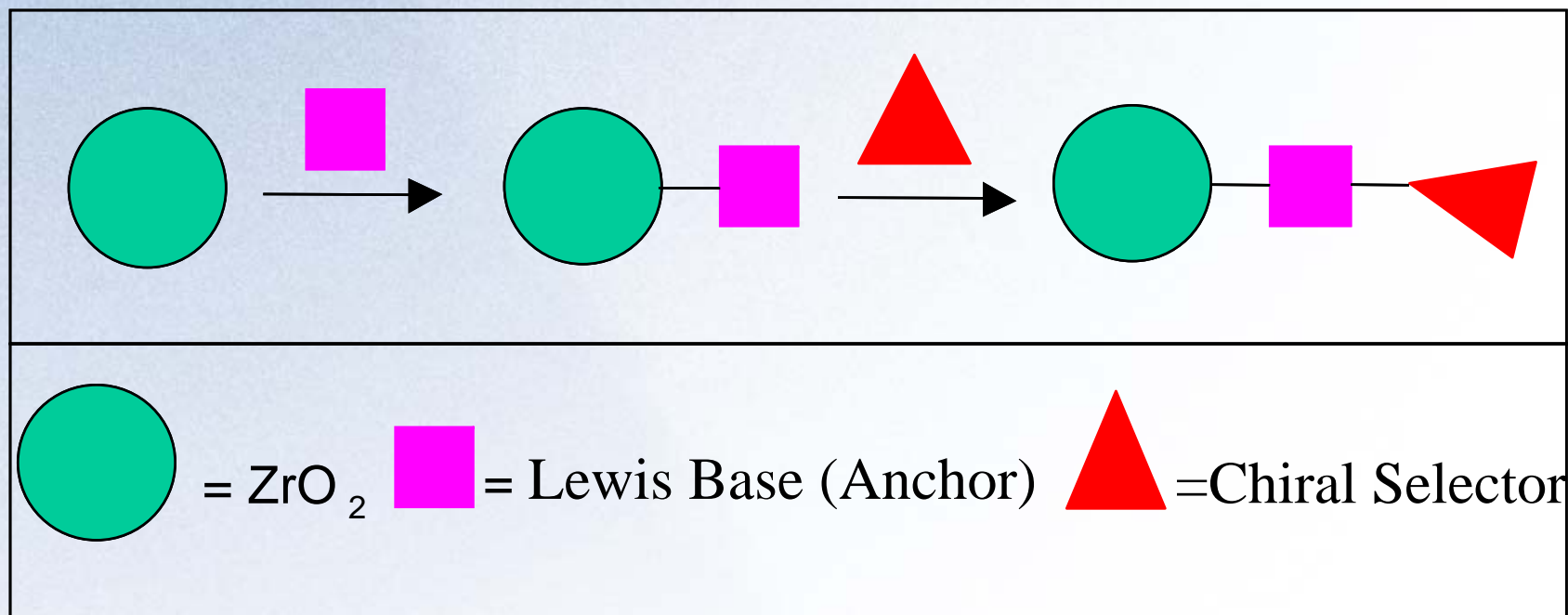
# Three-Point Interactions (Model 2)



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



# New Way to Attach Chiral Selectors to Zirconia Surface

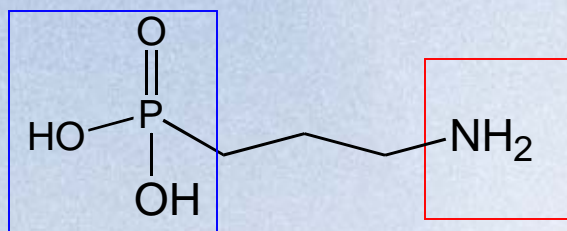




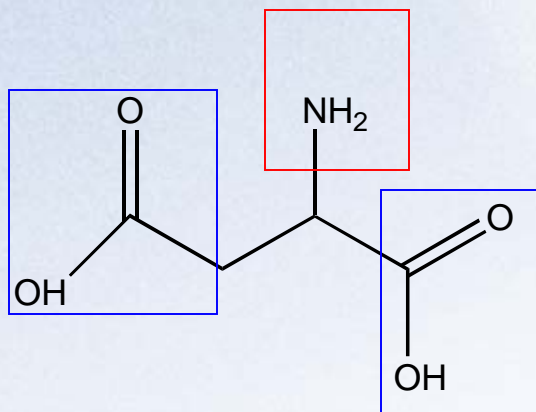


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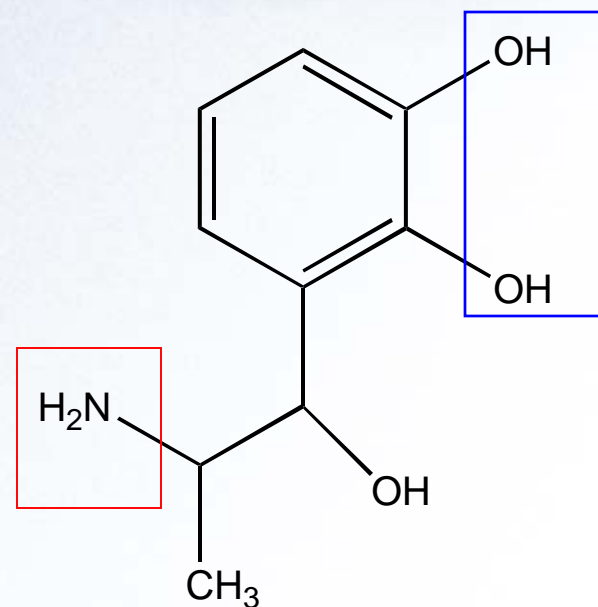
# Three Anchors in This Study



**APPA** (Aminopropylphosphonic acid)



**ASPA** (Aspartic acid)



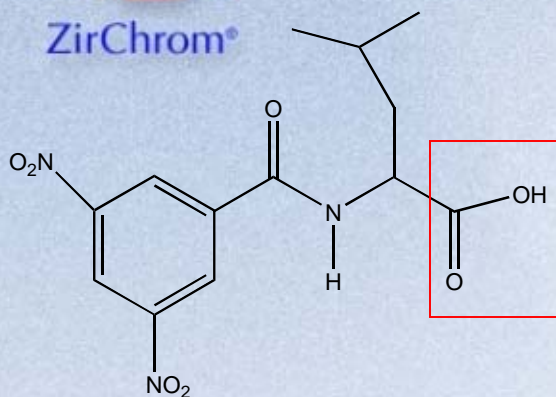
**DHNP** (3,4-Dihydroxynorephedrine)

## Phase I Anchors

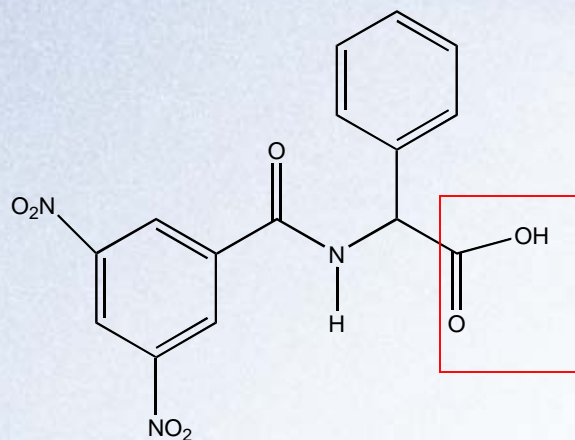


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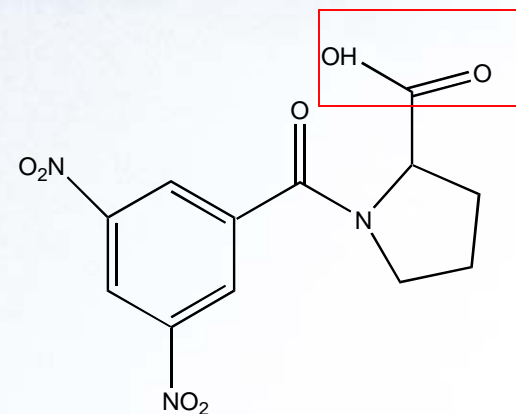
# Chiral Selectors in This Study



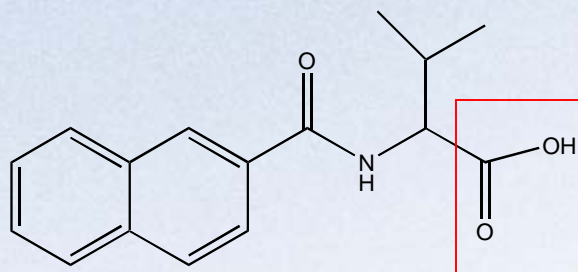
**DNB-LEU** (3,5-dinitrobenzoyl-leucine)



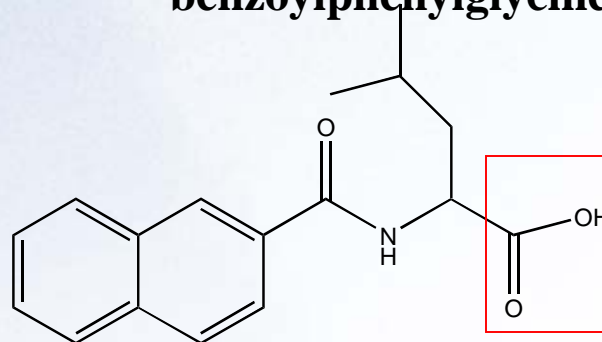
**DNB-PG** (3,5-dinitrobenzoyl-phenylglycine)



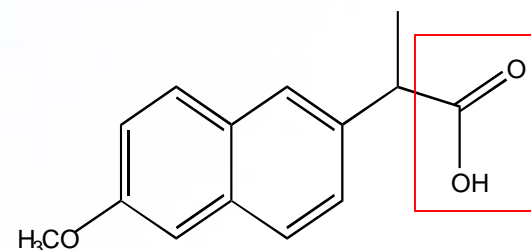
**DNB-PRO** (3,5-dinitrobenzoyl-proline)



**NAP-VAL** (Naphthoyl-valine)



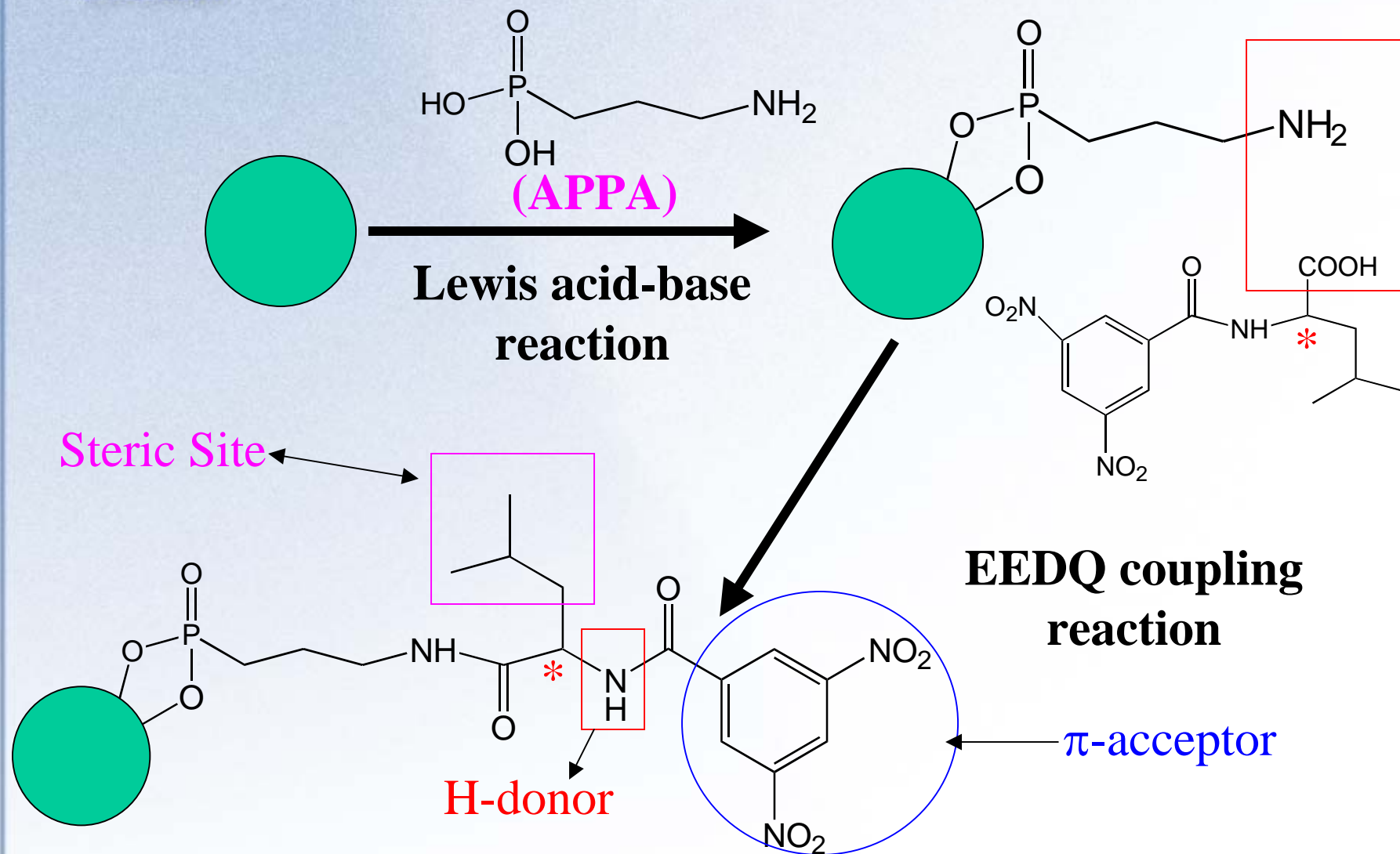
**2-NAP-LEU** (2-Naphthoyl-leucine),



**NAP** (naproxen)



# Example of Lewis Acid-Base Modified Zirconia CSPs





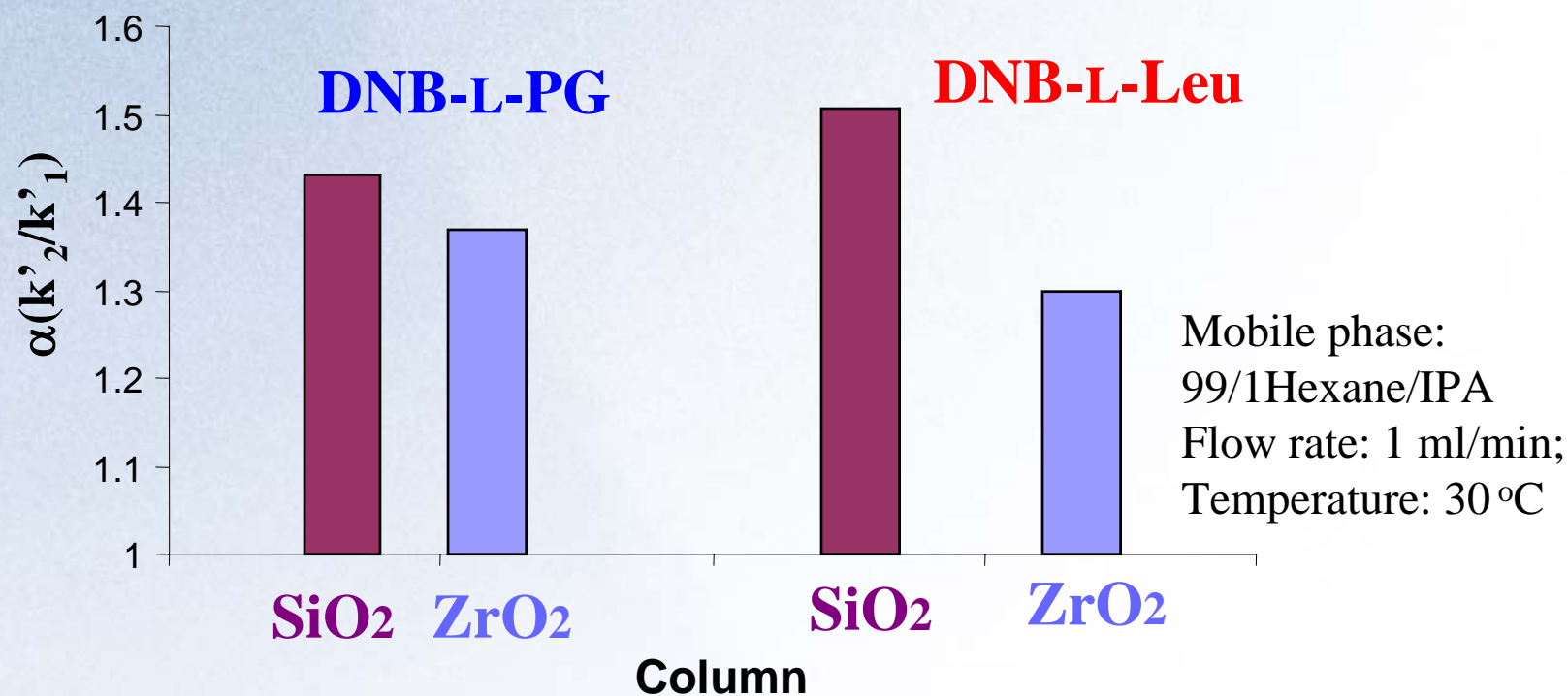
# List of Zirconia and Silica CSPs Studied

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

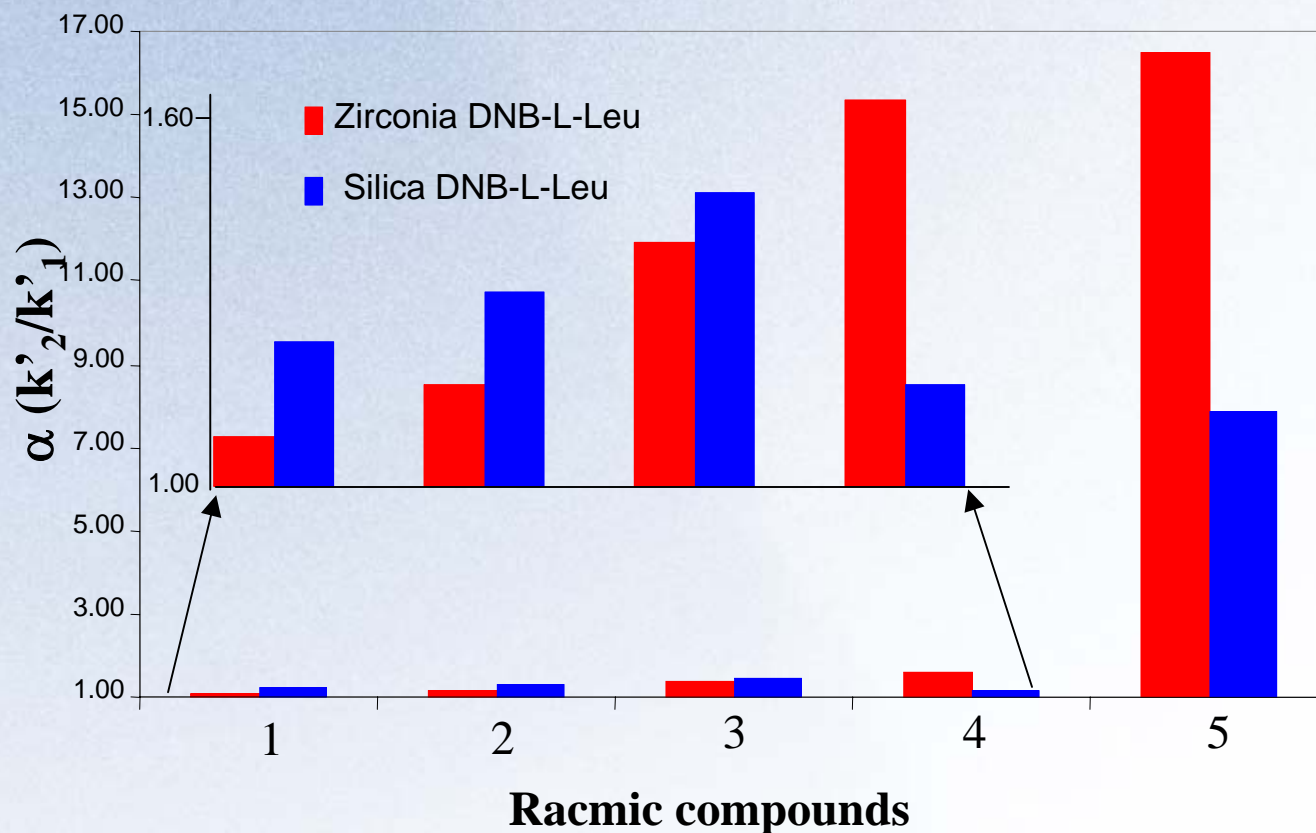


**Probe solute: Trifluoroanthryl ethanol**

**Conclusion: Zirconia based CSPs compared favorably.**



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



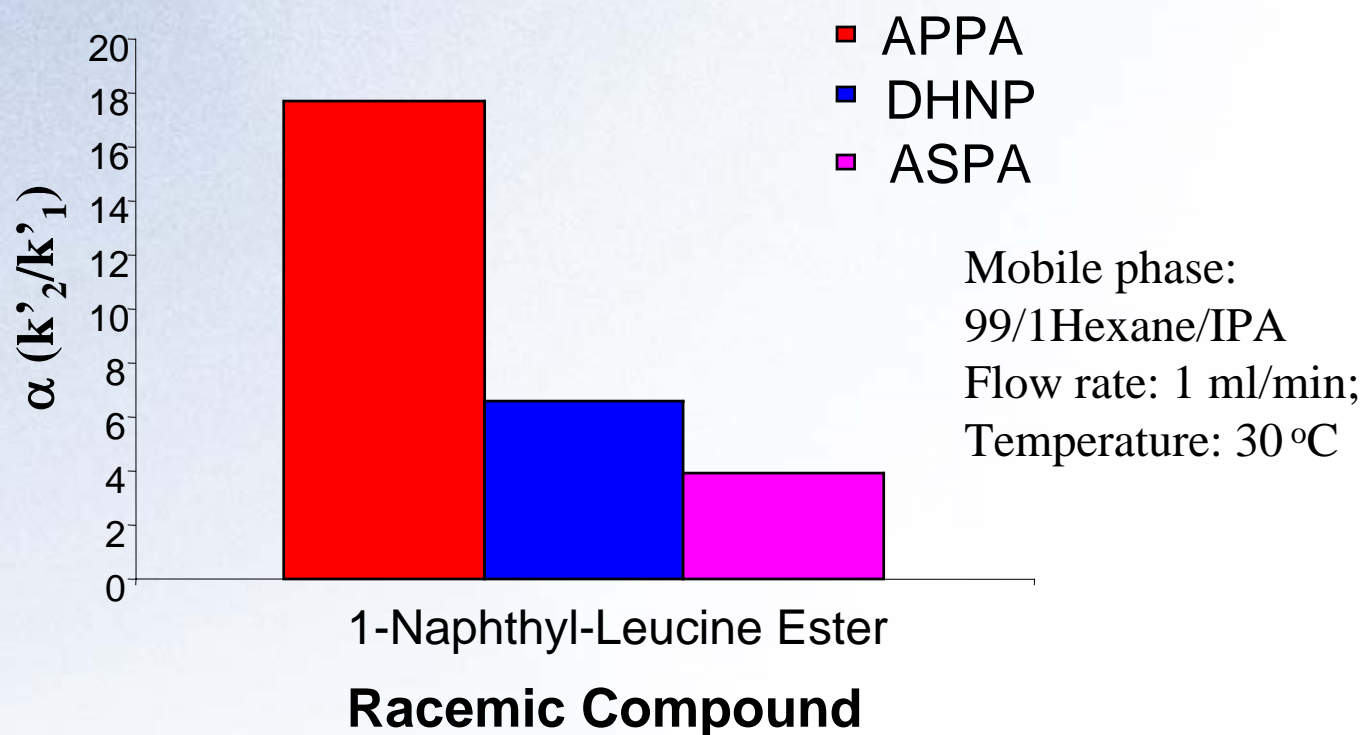
- 1 trans-stibene oxide
- 2 1,1'-bi-2-naphthol
- 3 trifluoranthyl ethanol
- 4 napropamide
- 5 1-naphthyl leucine ester

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.



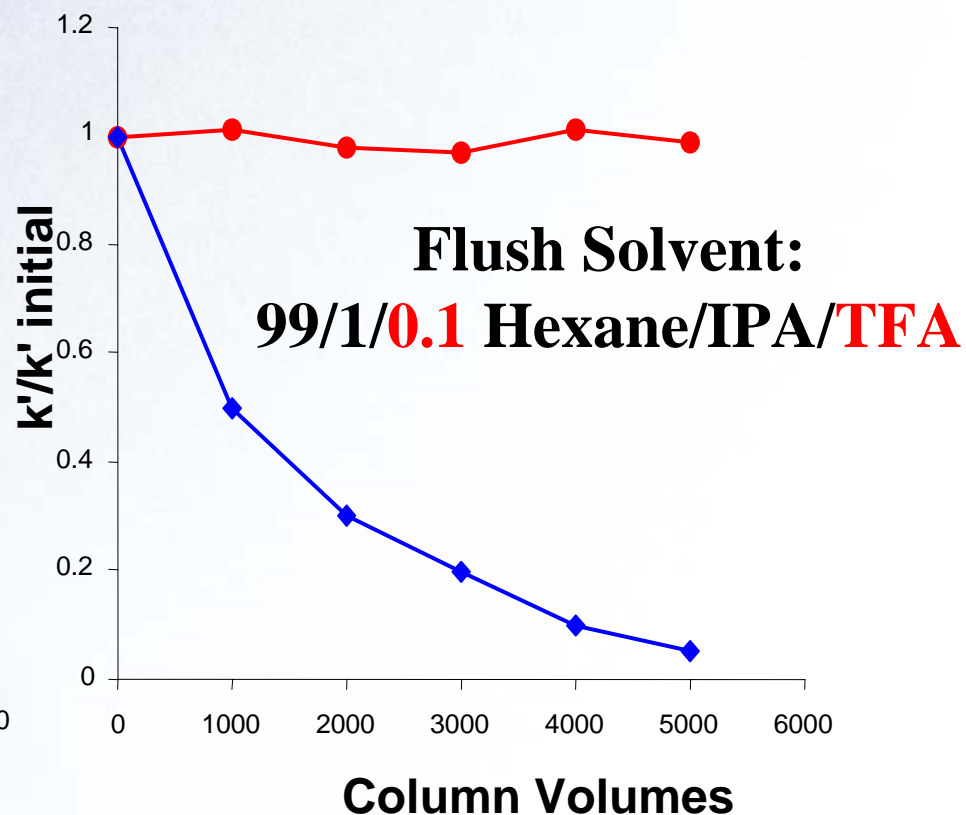
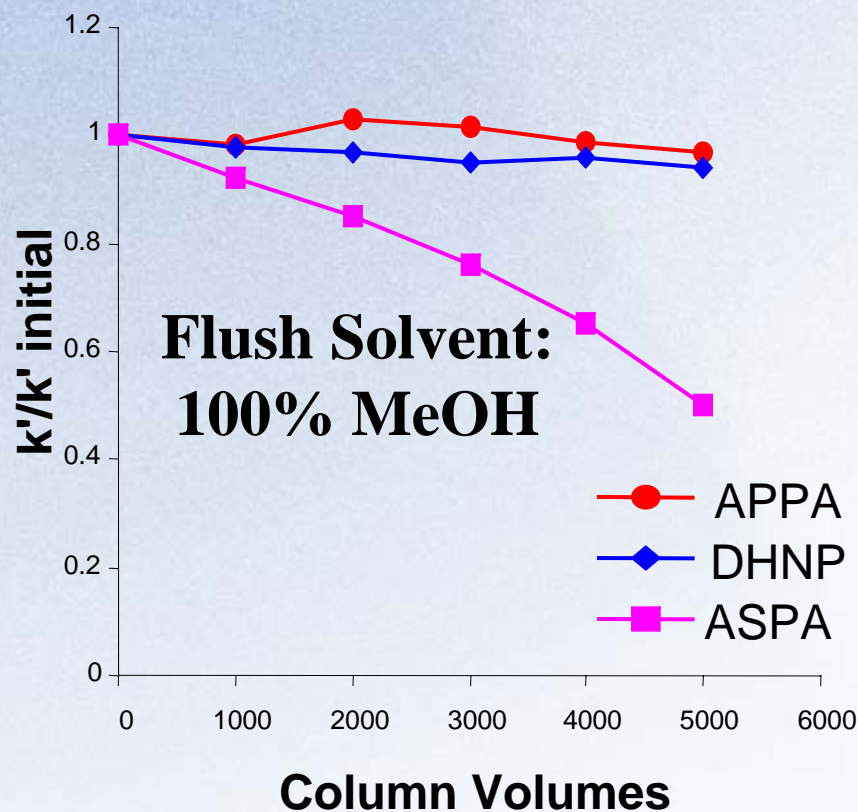
# Chromatographic Comparison of Differently-Anchored Zirconia-based DNB-L-LEU



**Different anchors show different selectivity.**



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

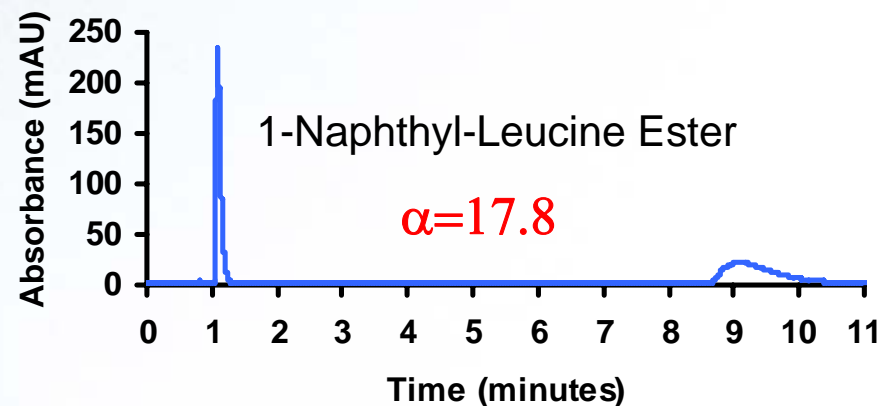
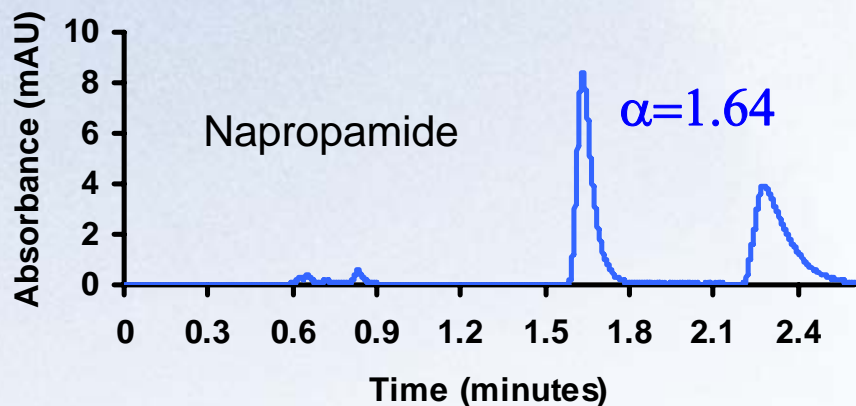
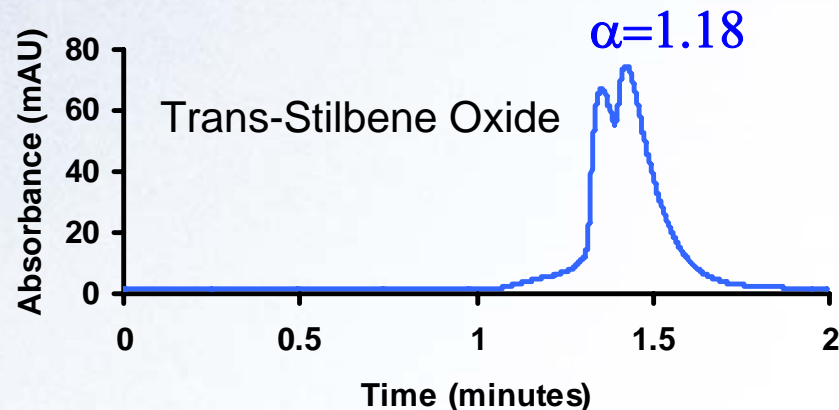
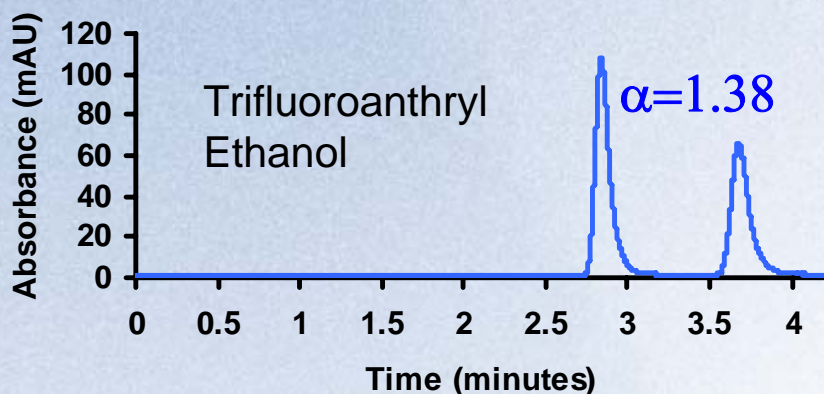


Test solute: trifluoranthryl ethanol. Note that the retention factor ratio is for the less retained isomer.





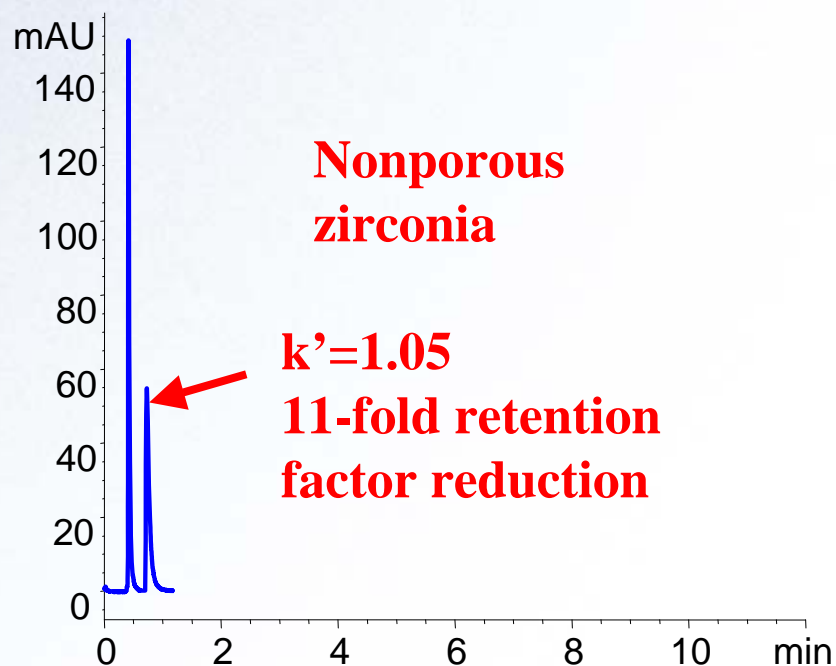
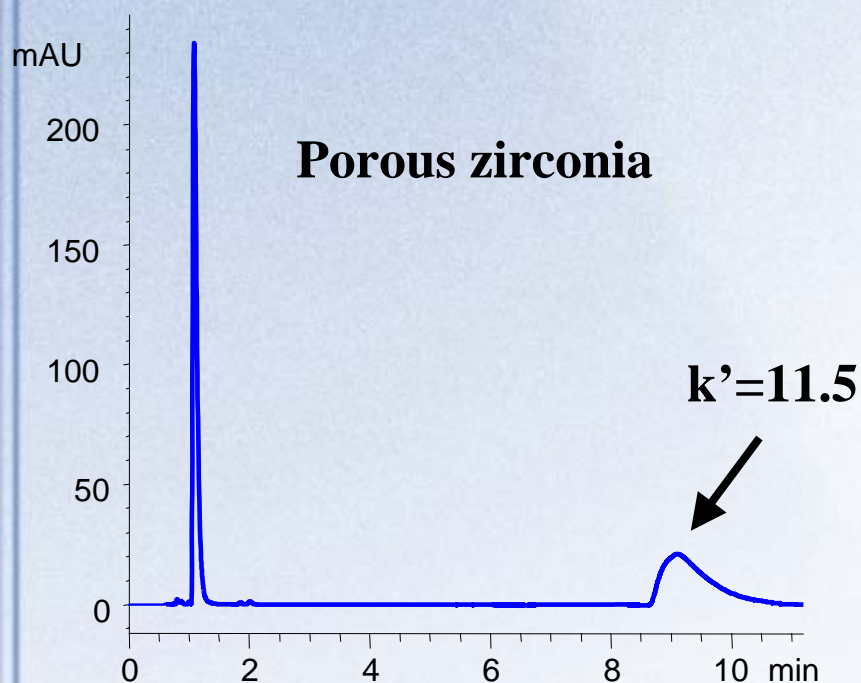
# Enantiomer Separations on DNB-L-LEU Modified Zirconia Phase Anchored via APPA



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



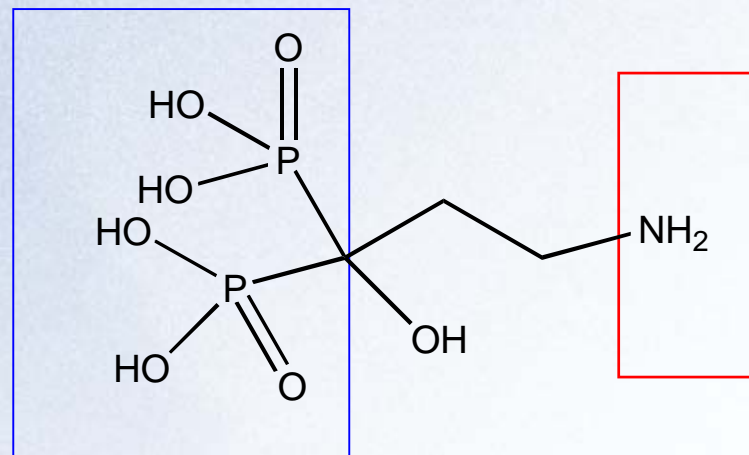
# Fast Chiral Separation on Nonporous Zirconia-based DNB-L-Leu



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



# Anchor Group – the Key to Chemical Stability

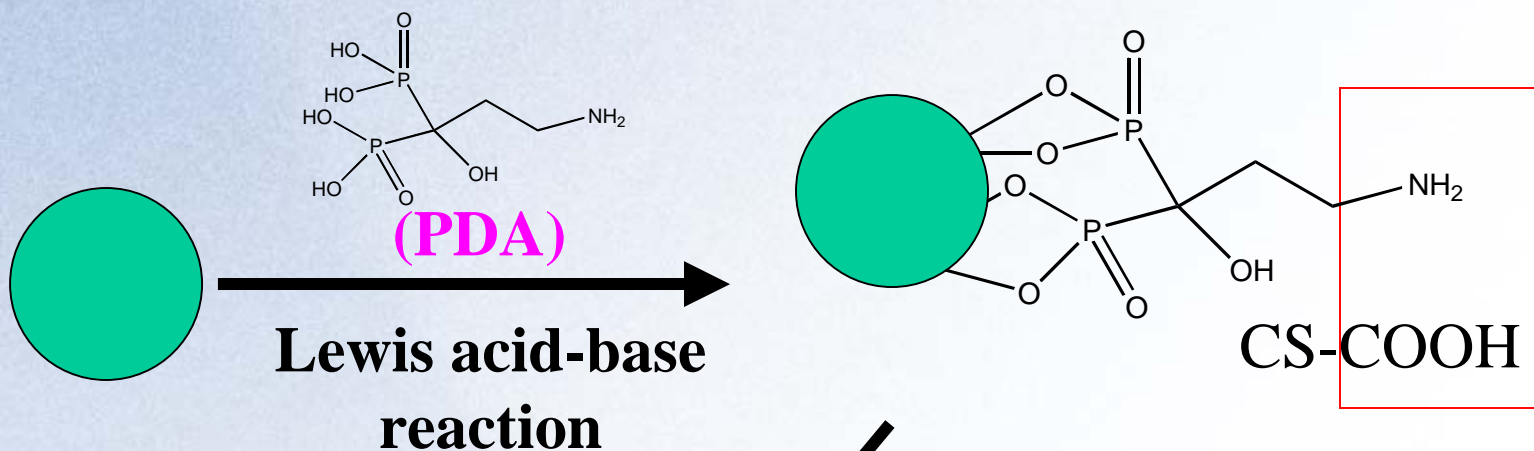


**PDA** (Pamidronic acid)

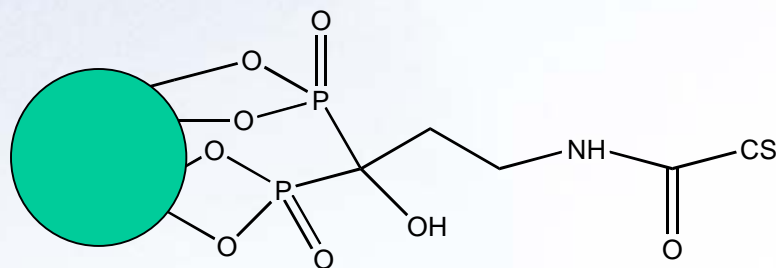
**Phase II Anchor**



# Example of Lewis Acid-Base Modified Zirconia CSPs



**EEDQ coupling reaction**

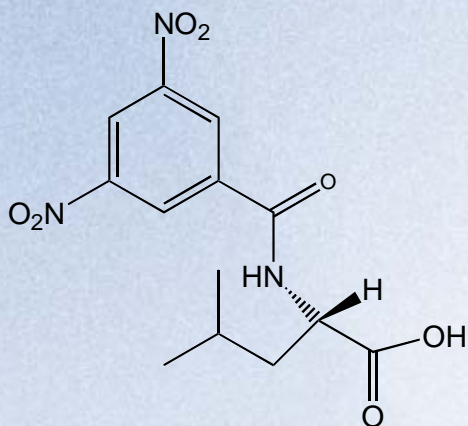


**CS = Chiral Selector**

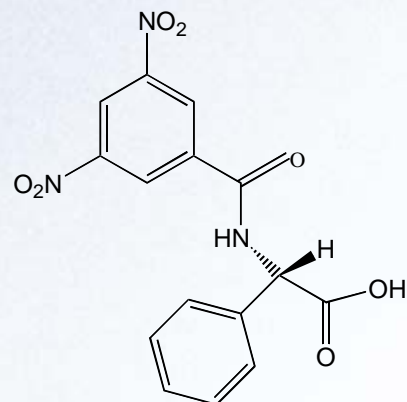


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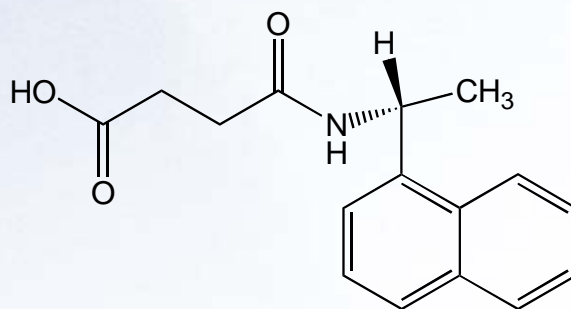
# Chiral Selectors



(S)-DNB-L-Leucine ((S)-Leu)



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

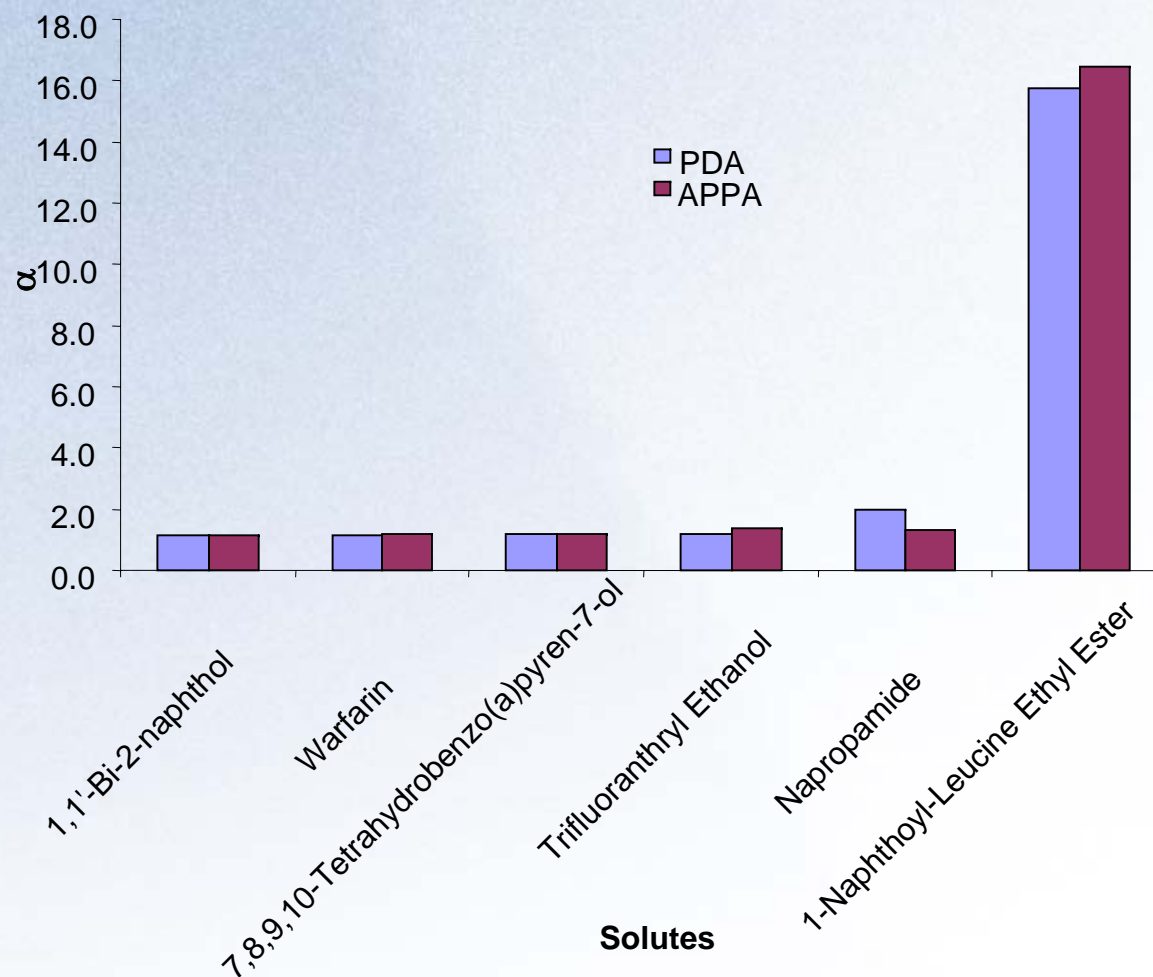


(S)-N-[1-(1-naphthyl)ethyl]succinamic acid ((S)-NESA)





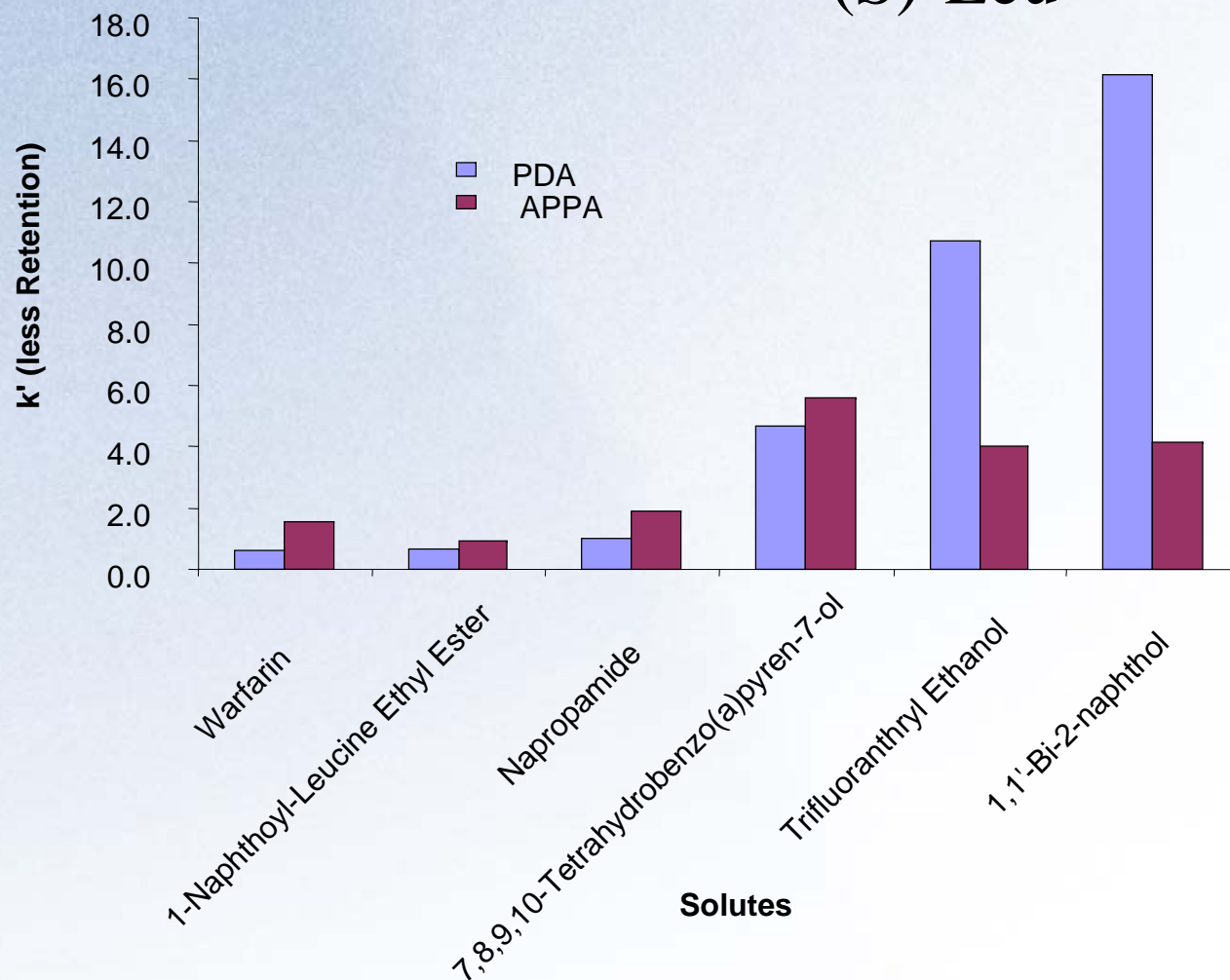
## Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



**Selectivity for both anchors is very similar.**



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

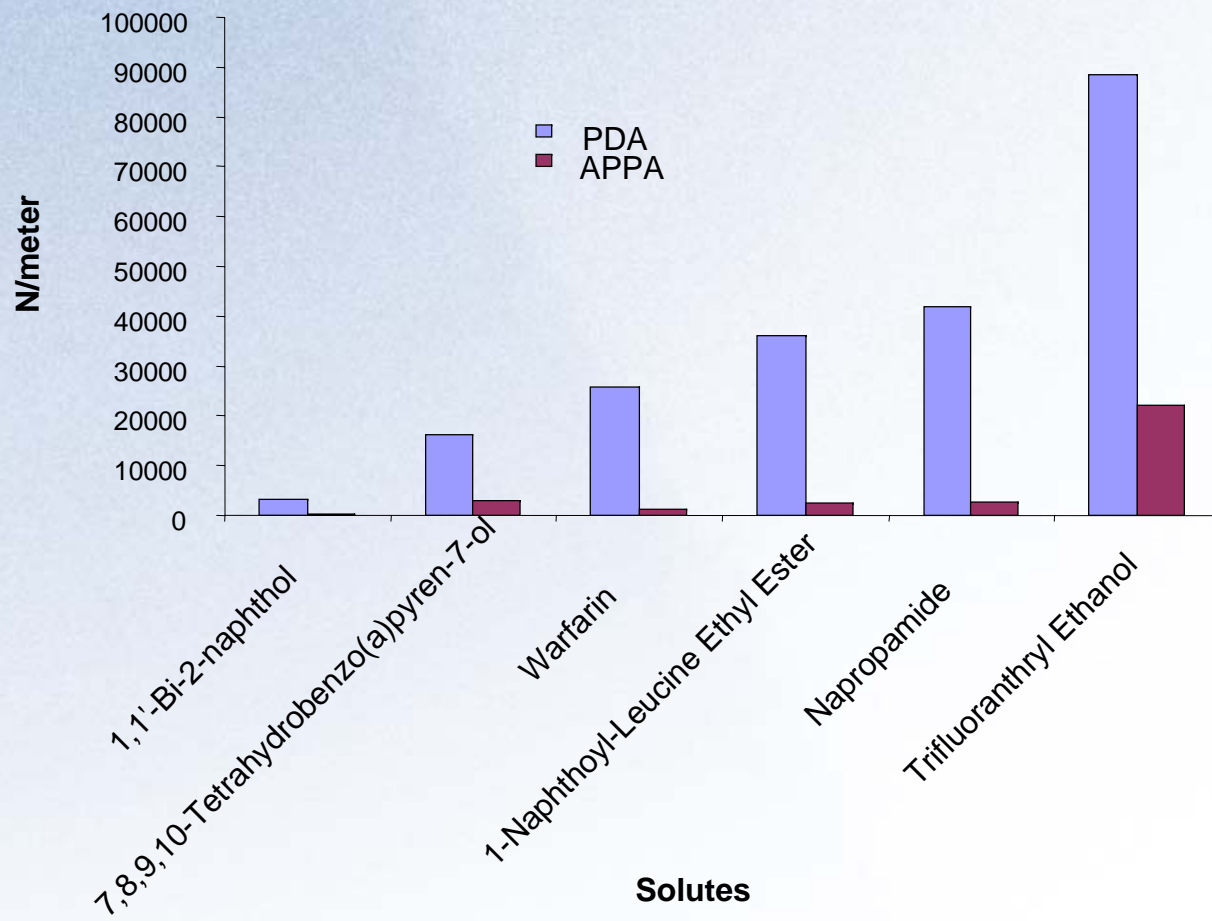


**Retention for both anchors is different.**





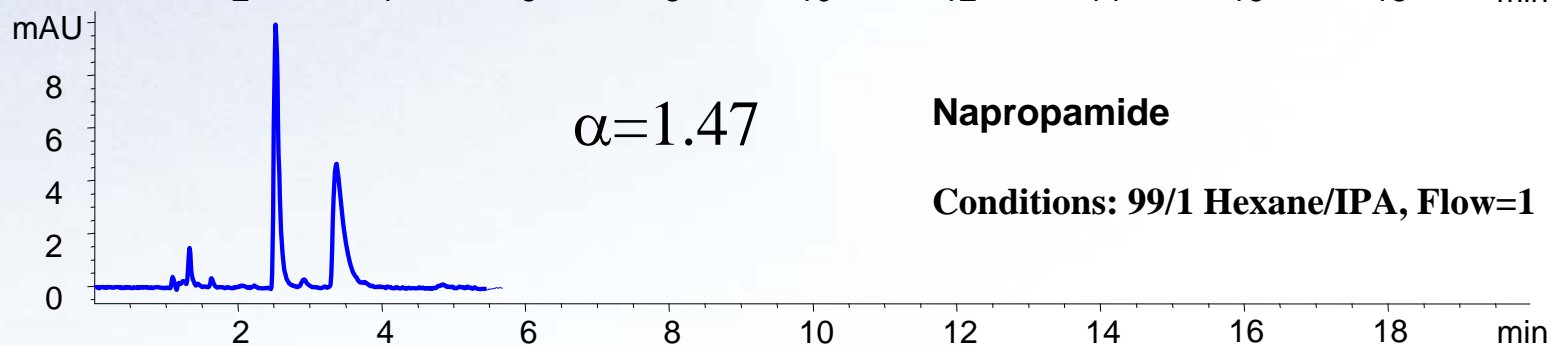
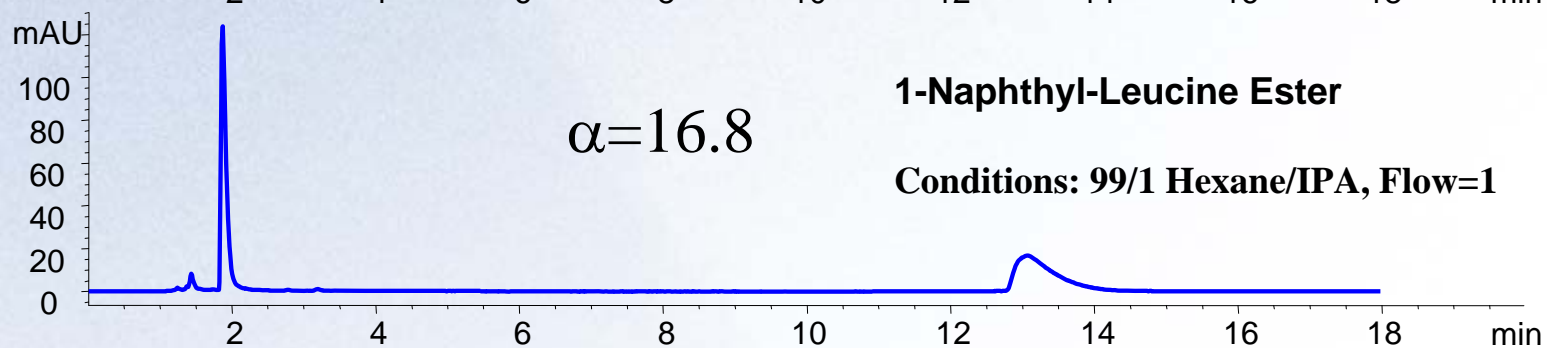
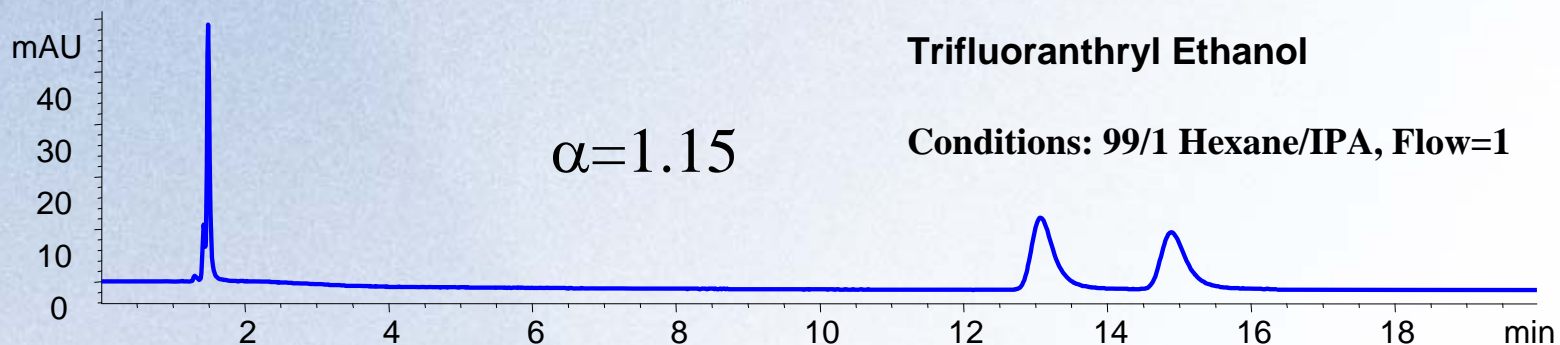
## Efficiency Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



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



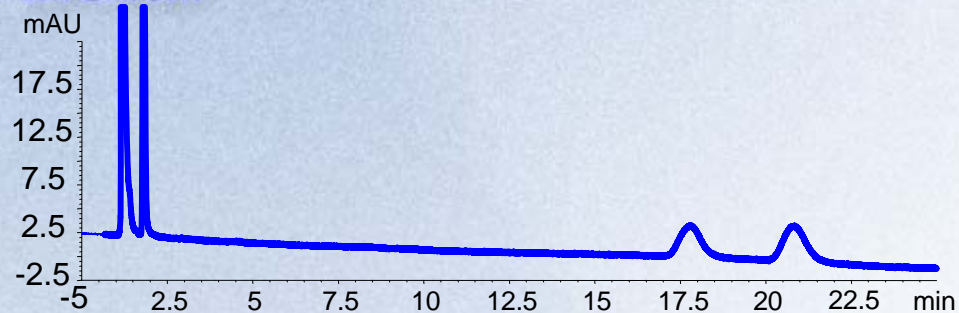
# Chiral Separation on Zr (S)-Leu ( $\pi$ -acceptor phase)





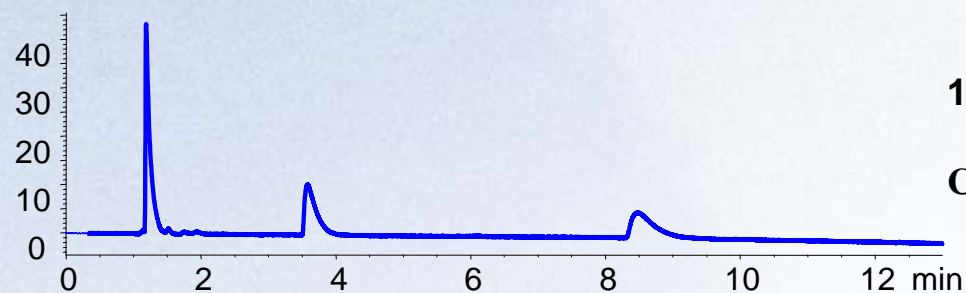
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# Chiral Separation on Zr (R)-PG ( $\pi$ -acceptor phase)



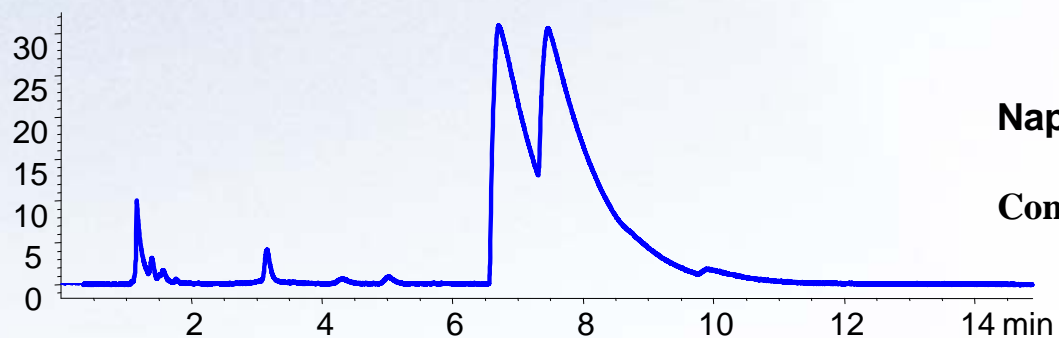
**Trifluoranthryl Ethanol**

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



**1-Naphthyl-Leucine Ester**

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

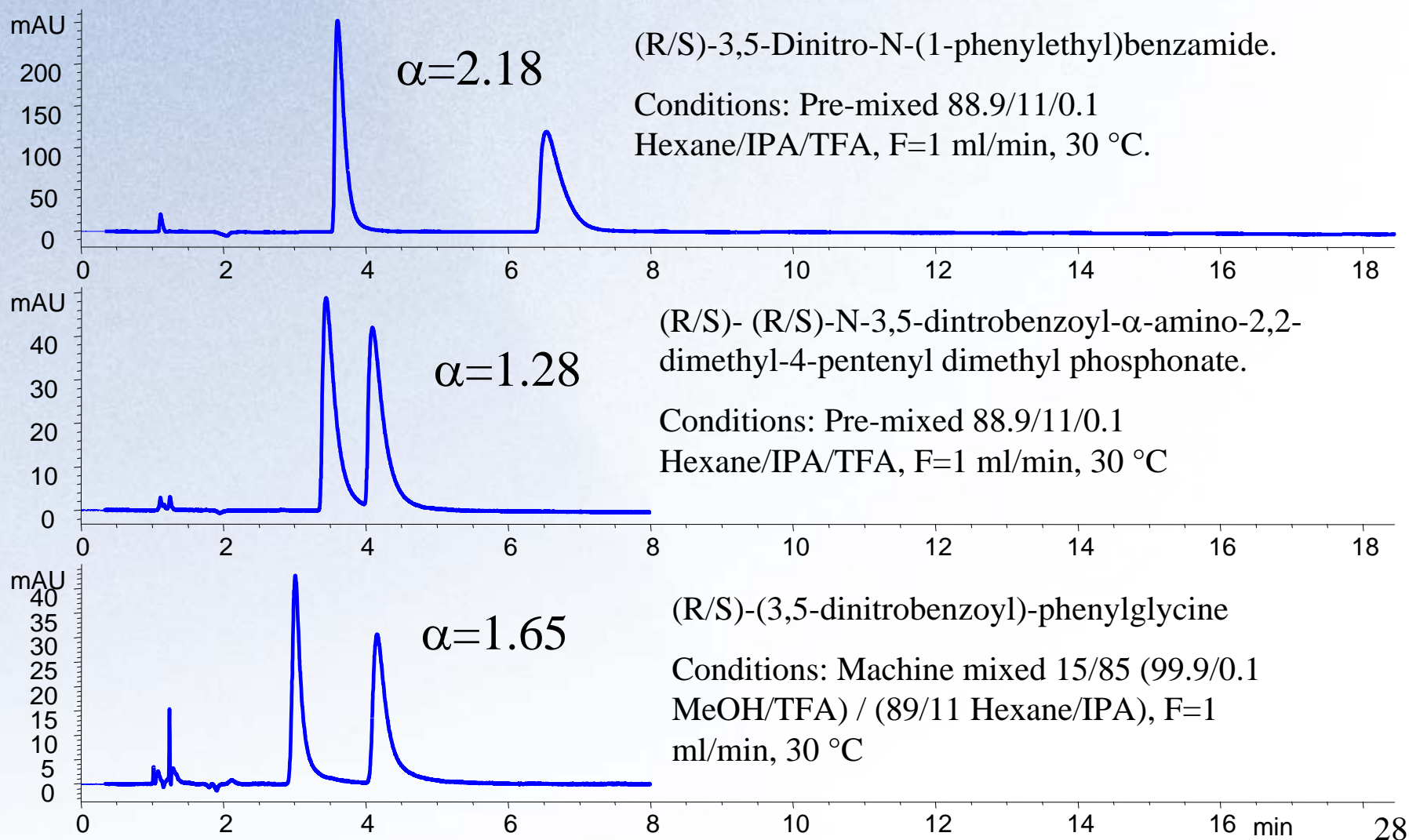


**Napropamide**

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



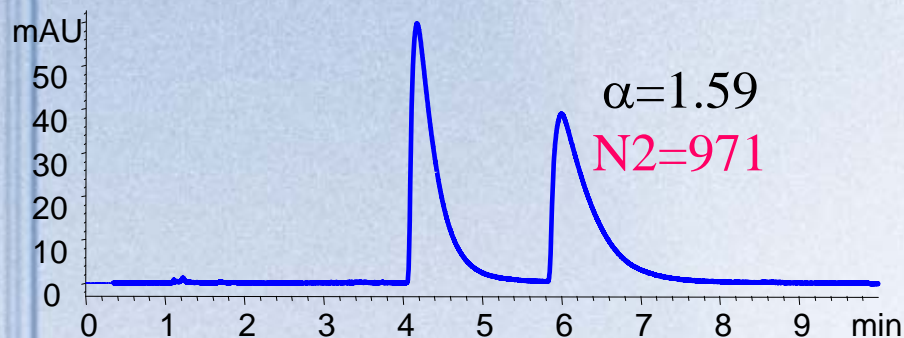
# Chiral Separations on Zr (S)-NESA (pi-donor phase)



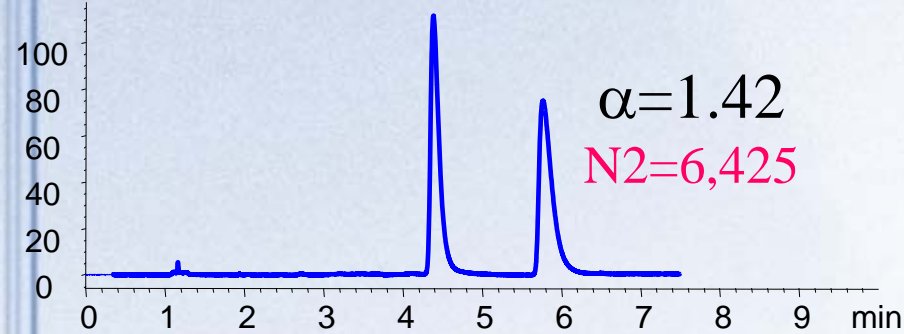


# Mobile Phase Effect of adding MeOH on Separation of (R/S)-N-3,5-dinitrobenzoyl- $\alpha$ -amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA

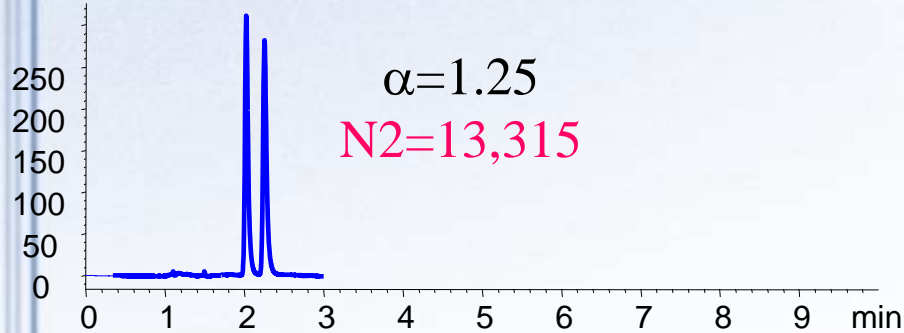
Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.



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



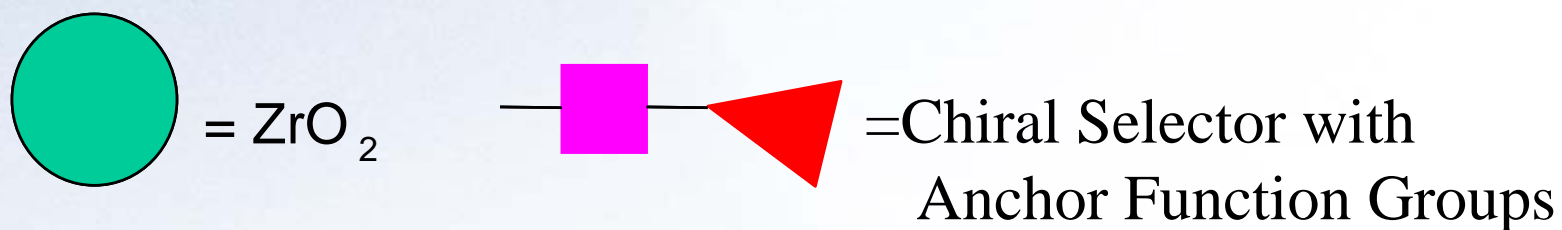
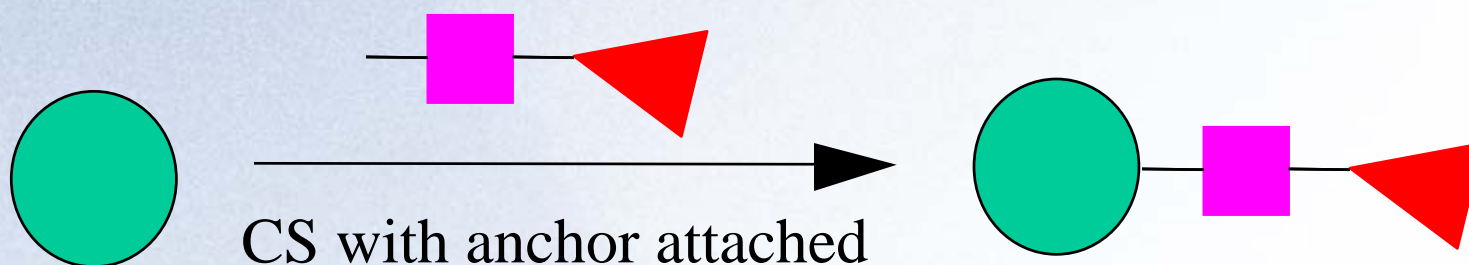
Conditions: 80 / 10 / 10 (99/1 Hexane/IPA) / **MeOH** / (70/30 Hexane/IPA), F=1 ml/min, 30 °C





# New Way to Synthesize Zirconia Based CSPs for Chiral Screening

## Phase II Reaction Scheme: One-Step Modification



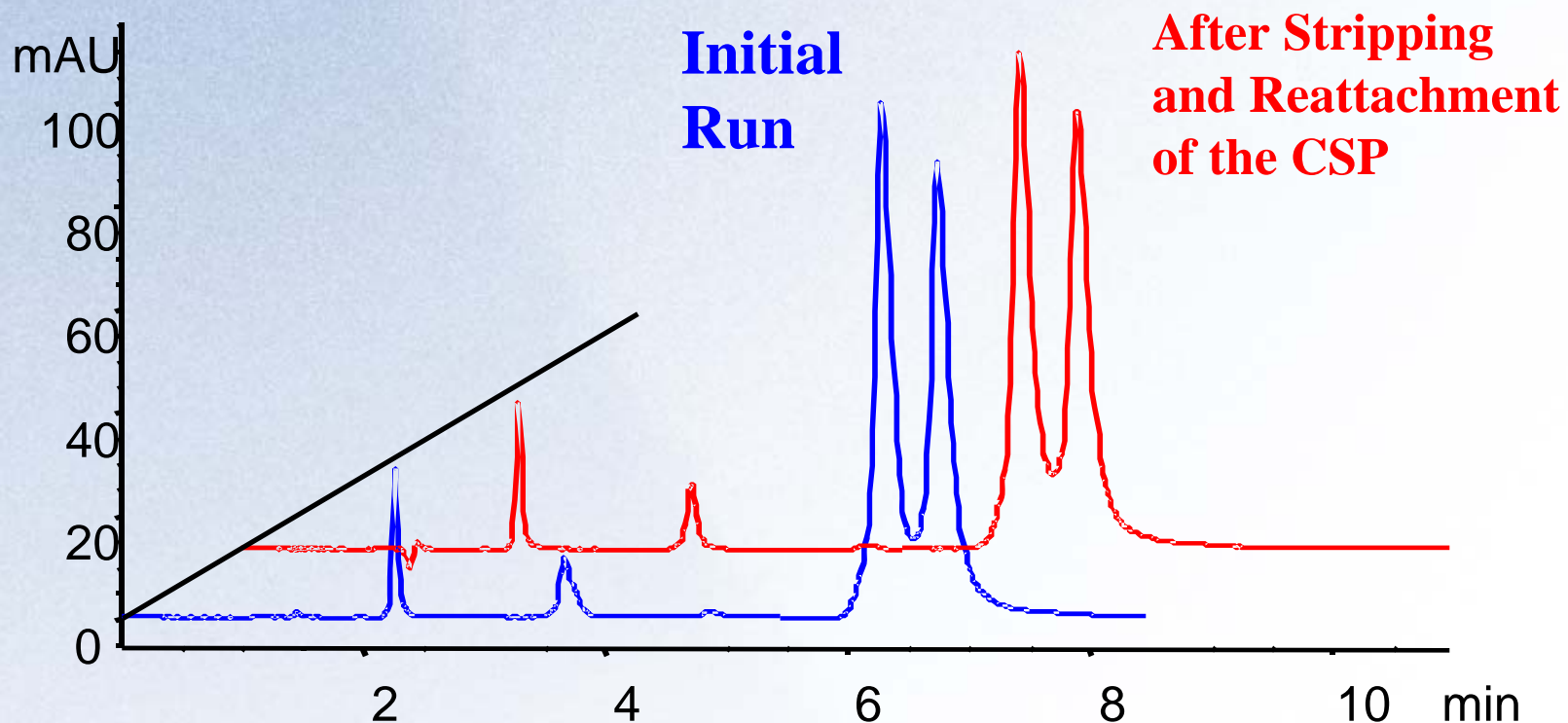


# Example Attachment and De-attachment Cycle

- 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 ( $\pm$ )-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 ( $\pm$ )-2,2,2-trifluoro-1-(9-anthyl)ethanol leucine ester during a single CSP screening cycle.

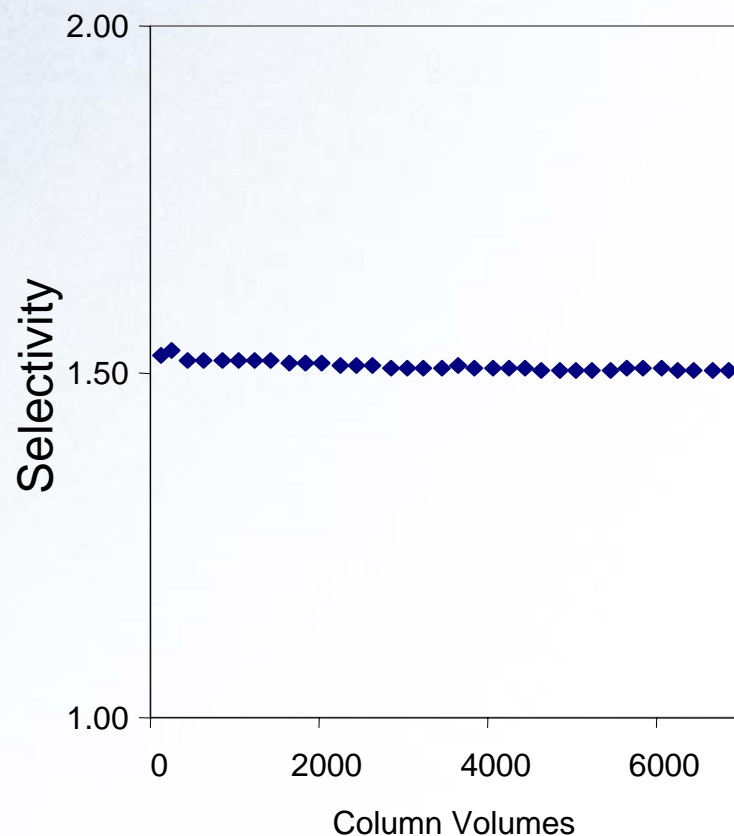
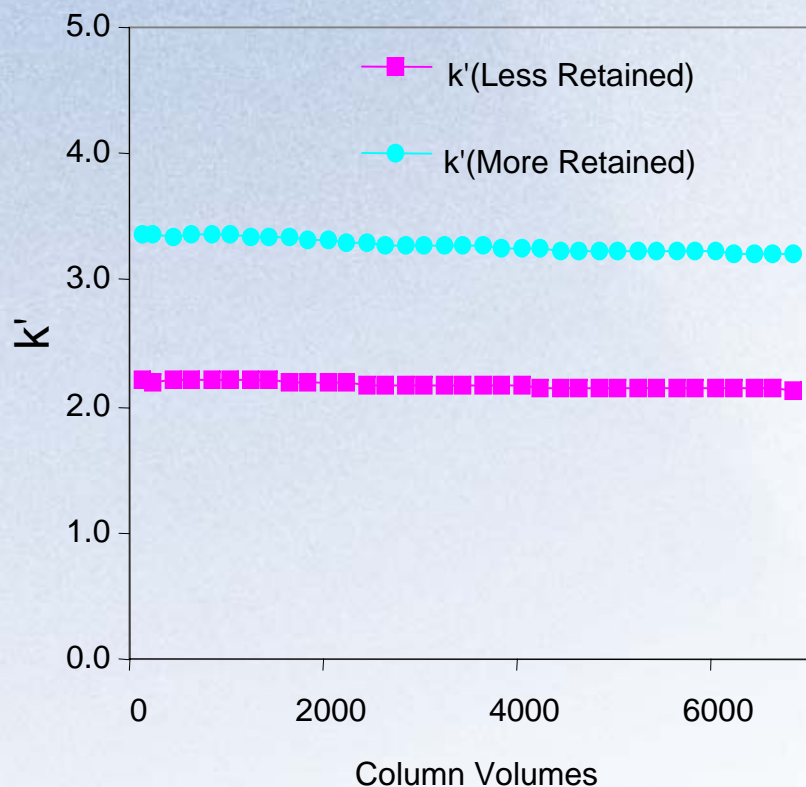
Chromatographic conditions: mobile phase: 99/1 hexane/IPA; flow rate: 1 ml/min; temperature: 30 °C, solute concentration = 1mg/mL, 5 microliter injection.





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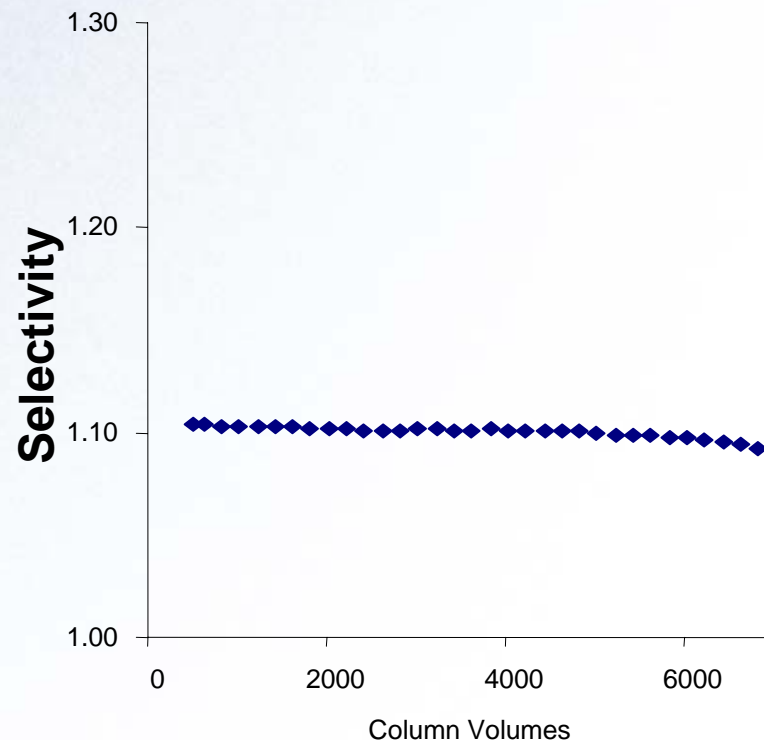
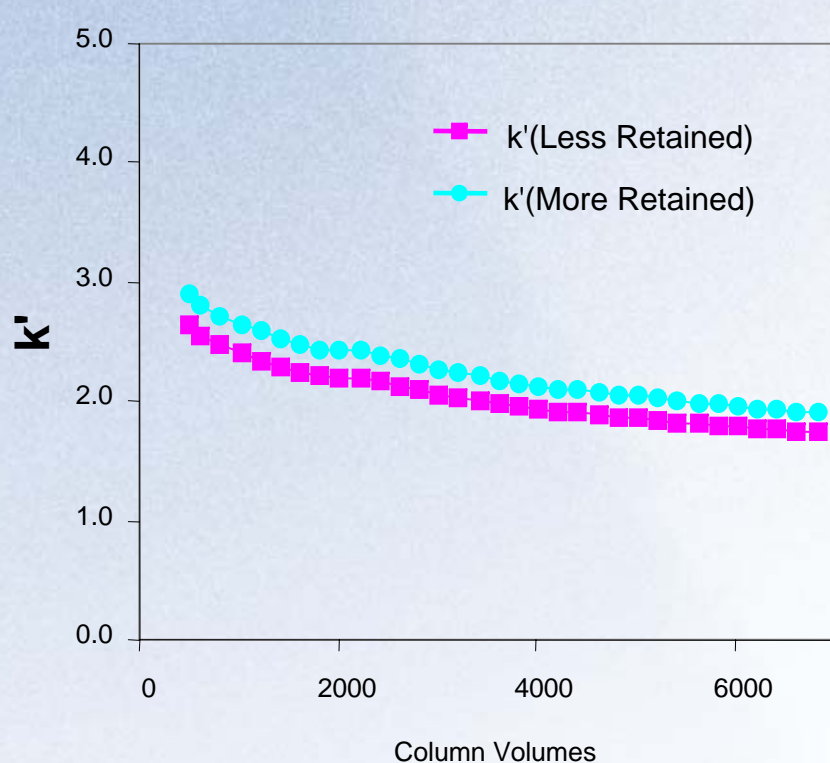
# Stability of Zr-(S)-NESA at pH 2



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.



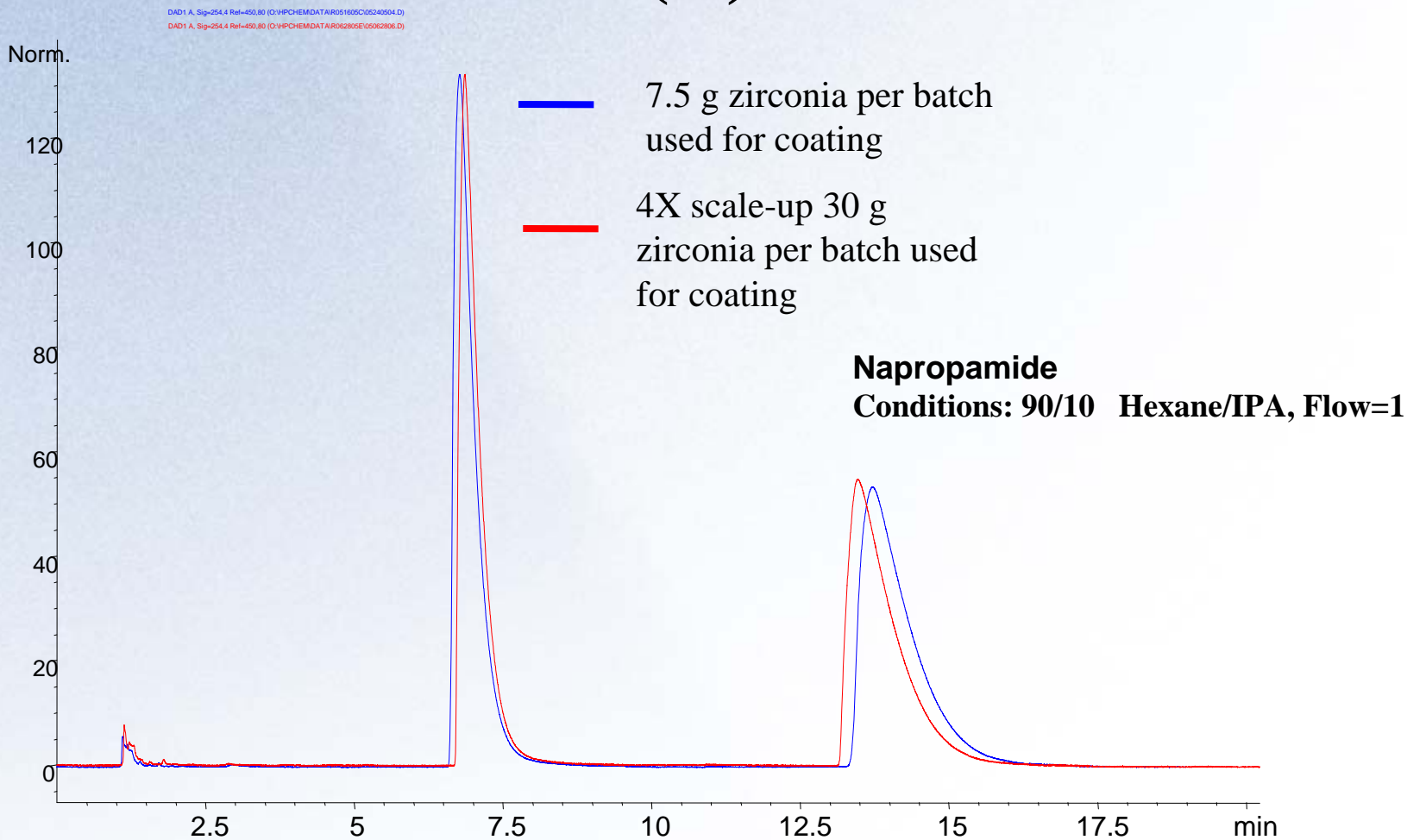
# Stability of Zr-(S)-DNB-Leu at pH 8



Column ID: ZrCSP032805A, Mobile phase: 15/85 ACN/5 mM ammonium hydrogencarbonate pH 8, Temperature: 30 °C. 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





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## 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.



# Conclusions

- Five new CSPs were attached to zirconia using the PDA anchor, including:
  - $\pi$ -acceptors:* Zr (S)-Leu, Zr (R)-PG, and Zr (S)-PG
  - $\pi$ -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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