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?
- Synthetic Approach
  - Surface Chemistry
  - Building a zirconia-based CSP
  - Proof of concept
- Chiral Separations on Zirconia Based CSPs
- Effect of Mobile Phase Additives on α, k' and N
- Stability Study
- 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.

Surface Chemistry of Zirconia

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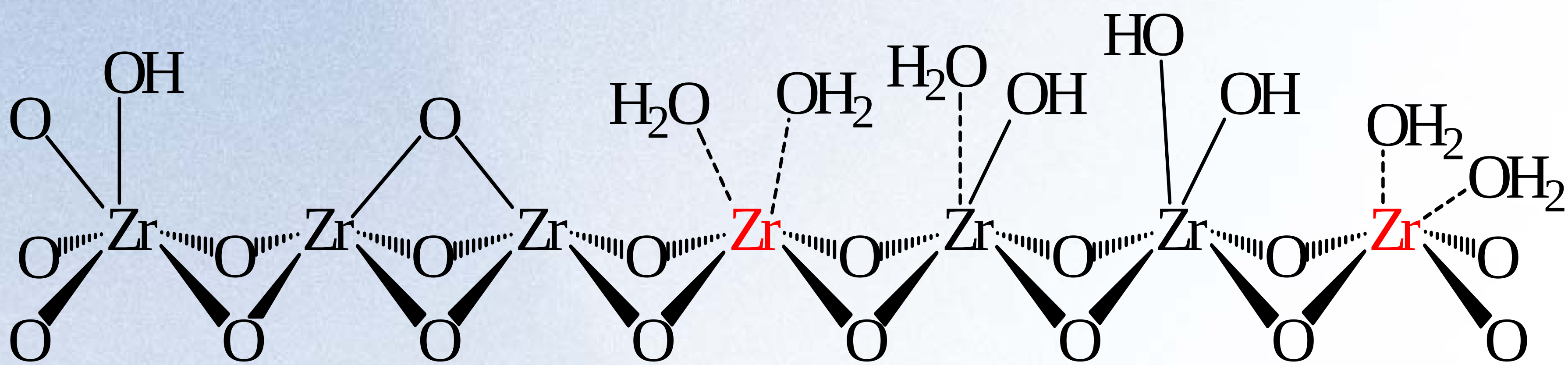


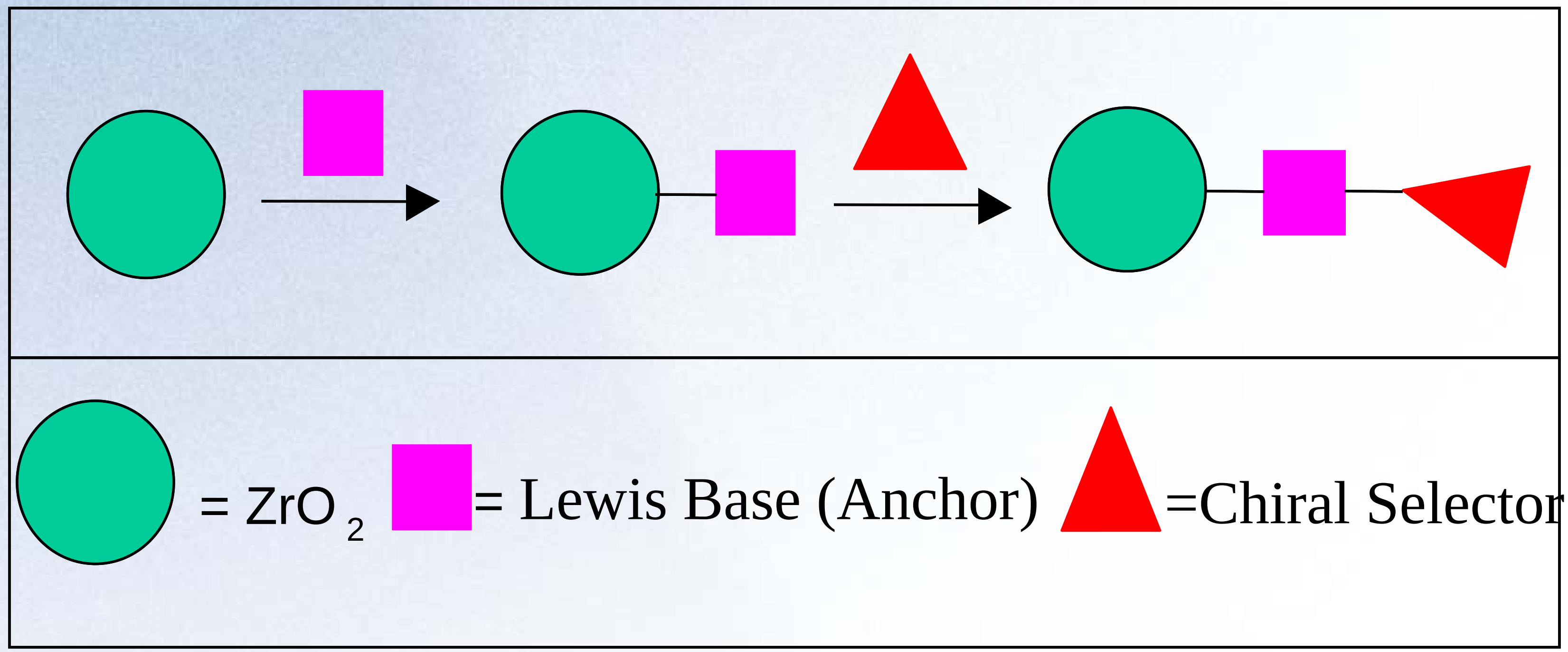
Brönsted Acid: $ZrOH + {}^{-}OH \rightleftharpoons ZrO^{-} + H_2O$

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

RPO_3^{2-} or Catechol

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

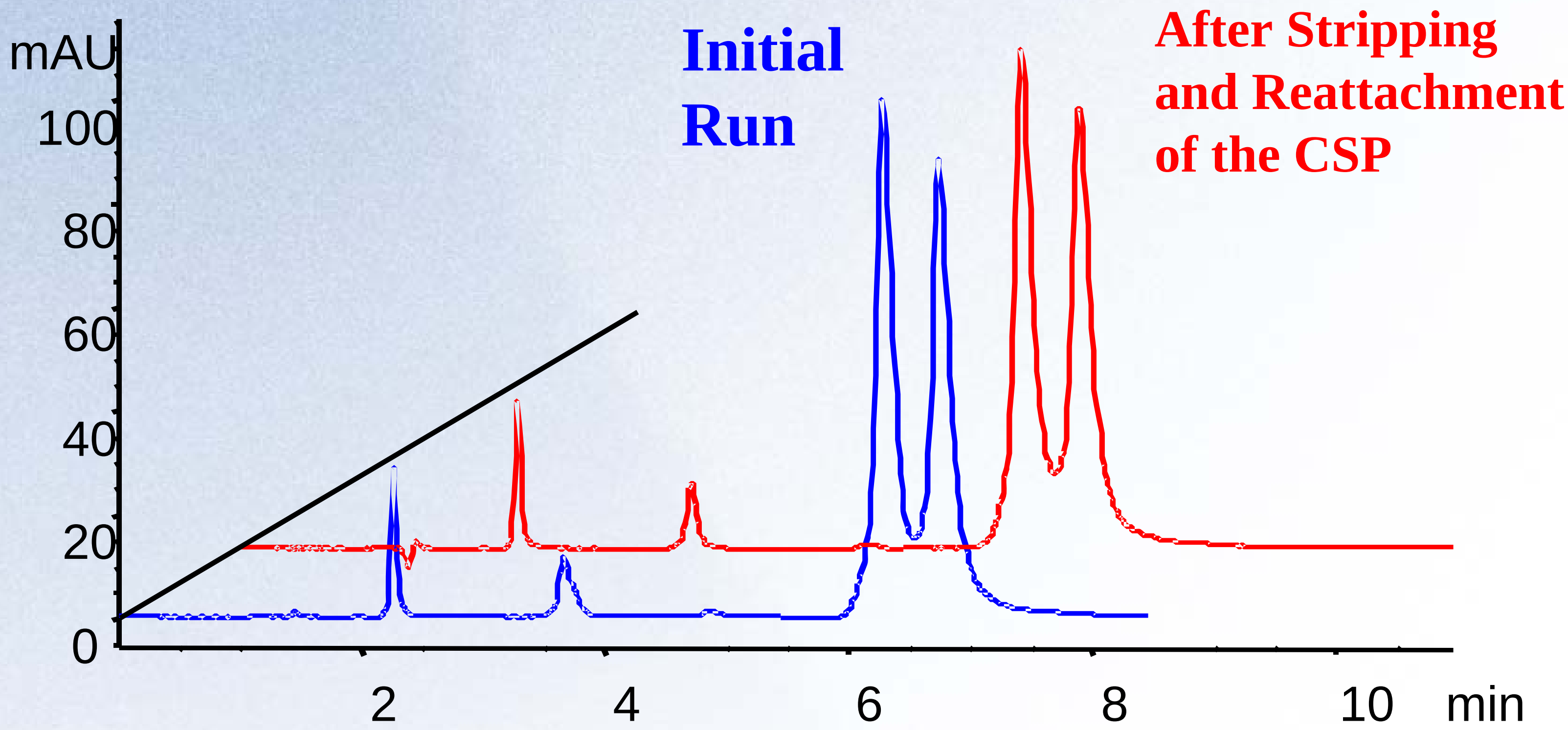
New Way to Attach Chiral Selectors to Zirconia Surface



Example Attachment and De-attachment Cycle

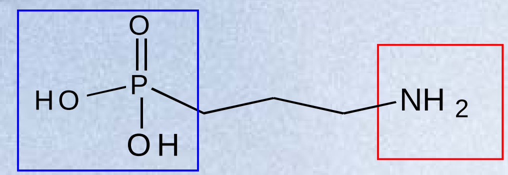
- Pass a solution of 20 mM N-(4-nitrobenzoyl)-L-glutamic acid (CSP) in tetrahydrofuran for 10 minutes at a column temperature of 60°C and a flow rate of 1 mL/min .
- Flushed column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
- Separate a racemate solution of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol.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

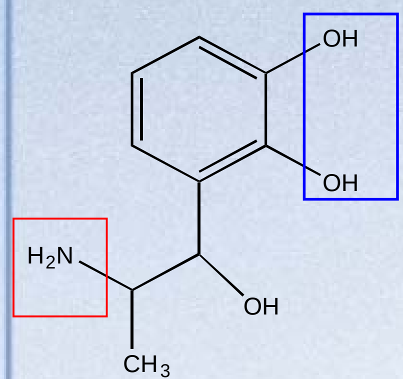
Proof of Concept

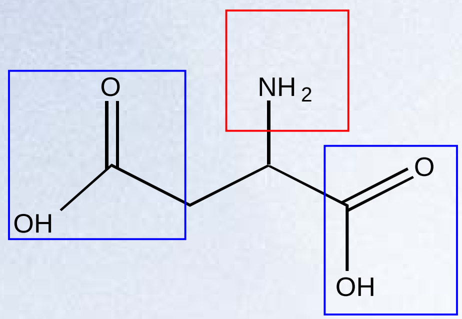


Comparison between the initial and final separation of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol leucine ester during a single CSP screening cycle.
Chromatographic conditions: mobile phase: 99/1 hexane/IPA; flow rate: 1 ml/min; temperature: 30 °C, solute concentration = 1mg/mL, 5 microliter injection.

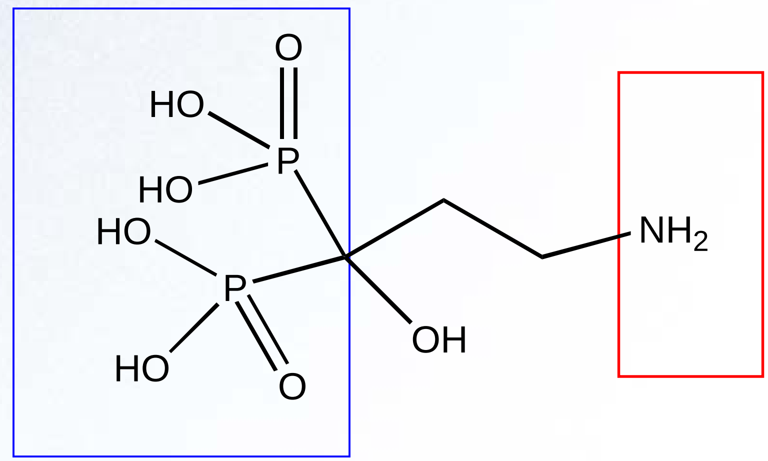
Anchors







1) **APPA** **(Aminopropylphosphonic acid)**
2) **DHNP** **(3,4-Dihydroxynorephedrine)**
3) **ASPA** **(Aspartic acid)**

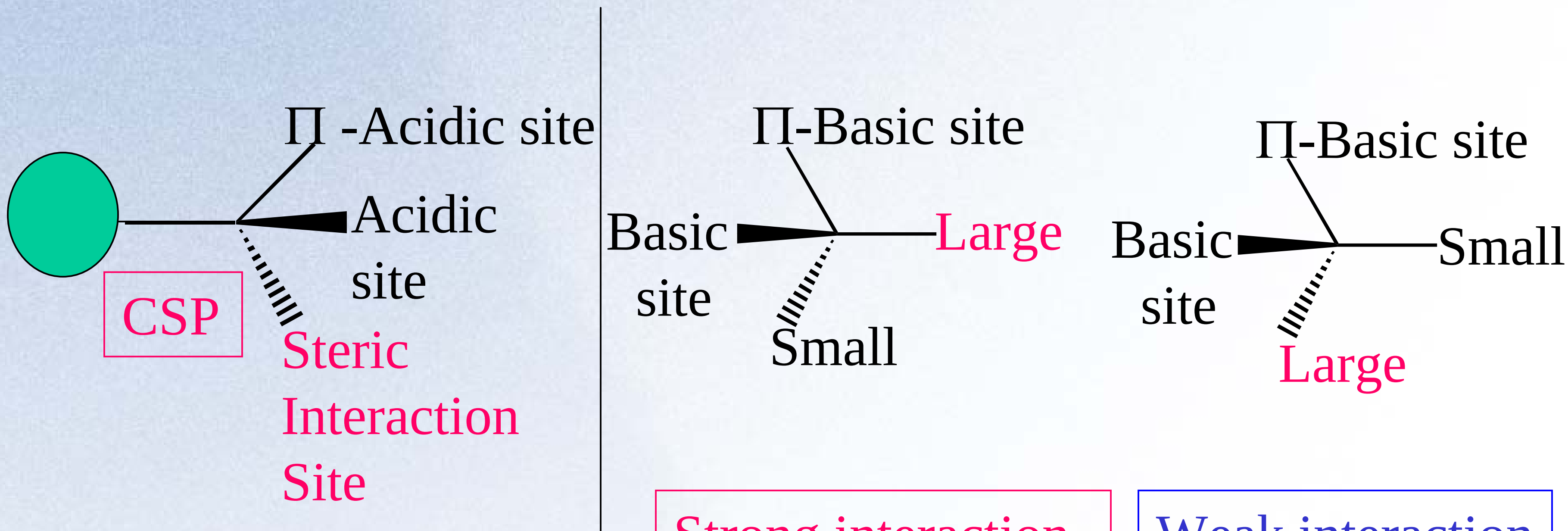


PDA **(Pamidronic acid)**

Phase I Anchors

Phase II Anchor

Three-Point Interactions

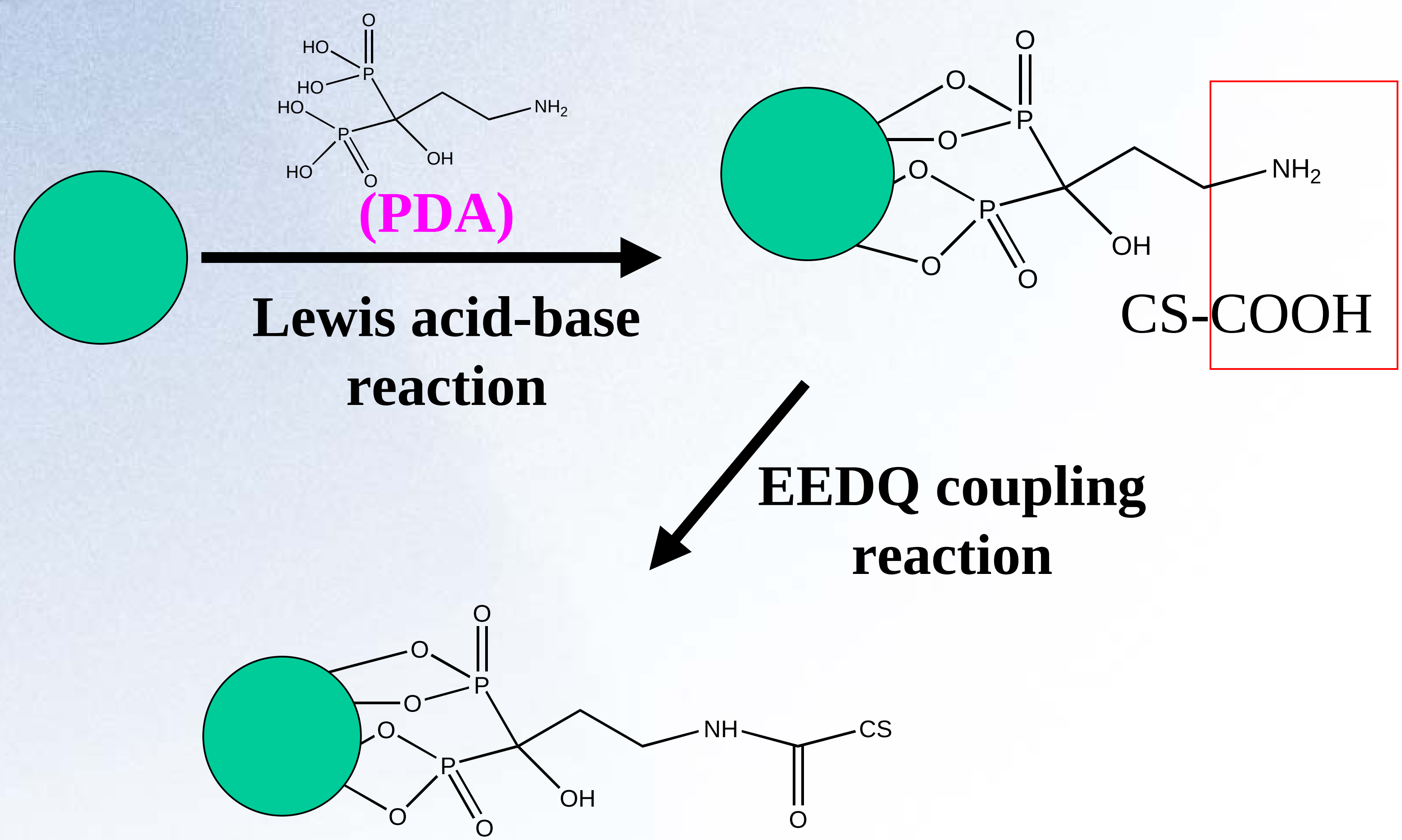


Strong interaction with CSP

Weak interaction with CSP

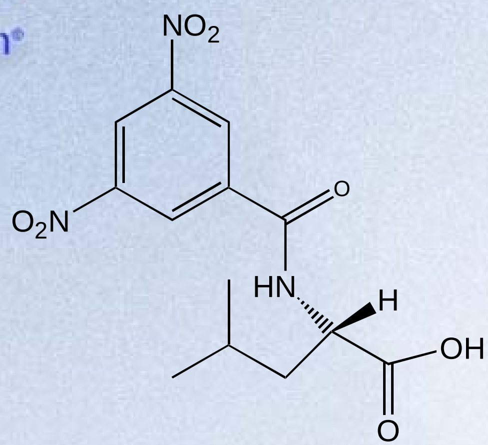
William H. Pirkle et al., J. Chromatogr., 316 (1984) 585

Example of Lewis Acid-Base Modified Zirconia CSPs

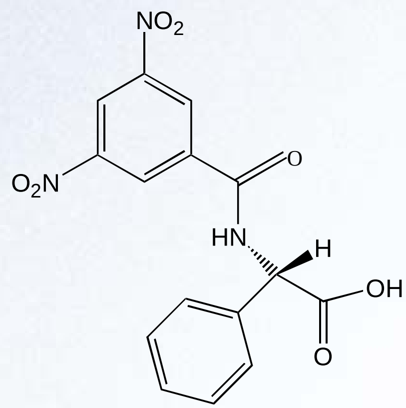


CS = Chiral Selector

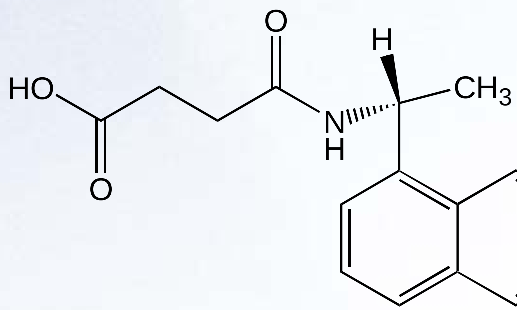
Chiral Selectors



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

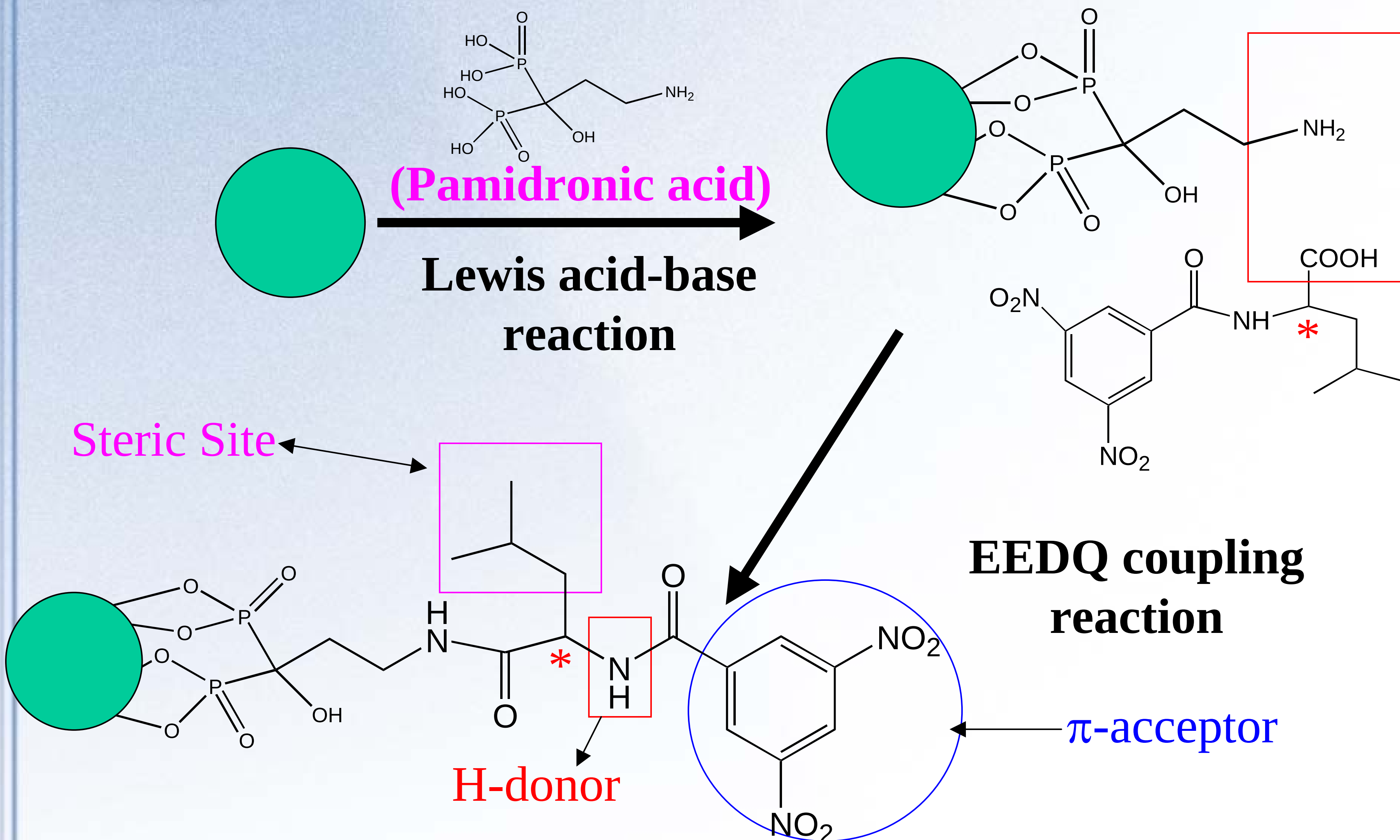


(S)-DNB-L-Phenylglycine ((S)-PG)

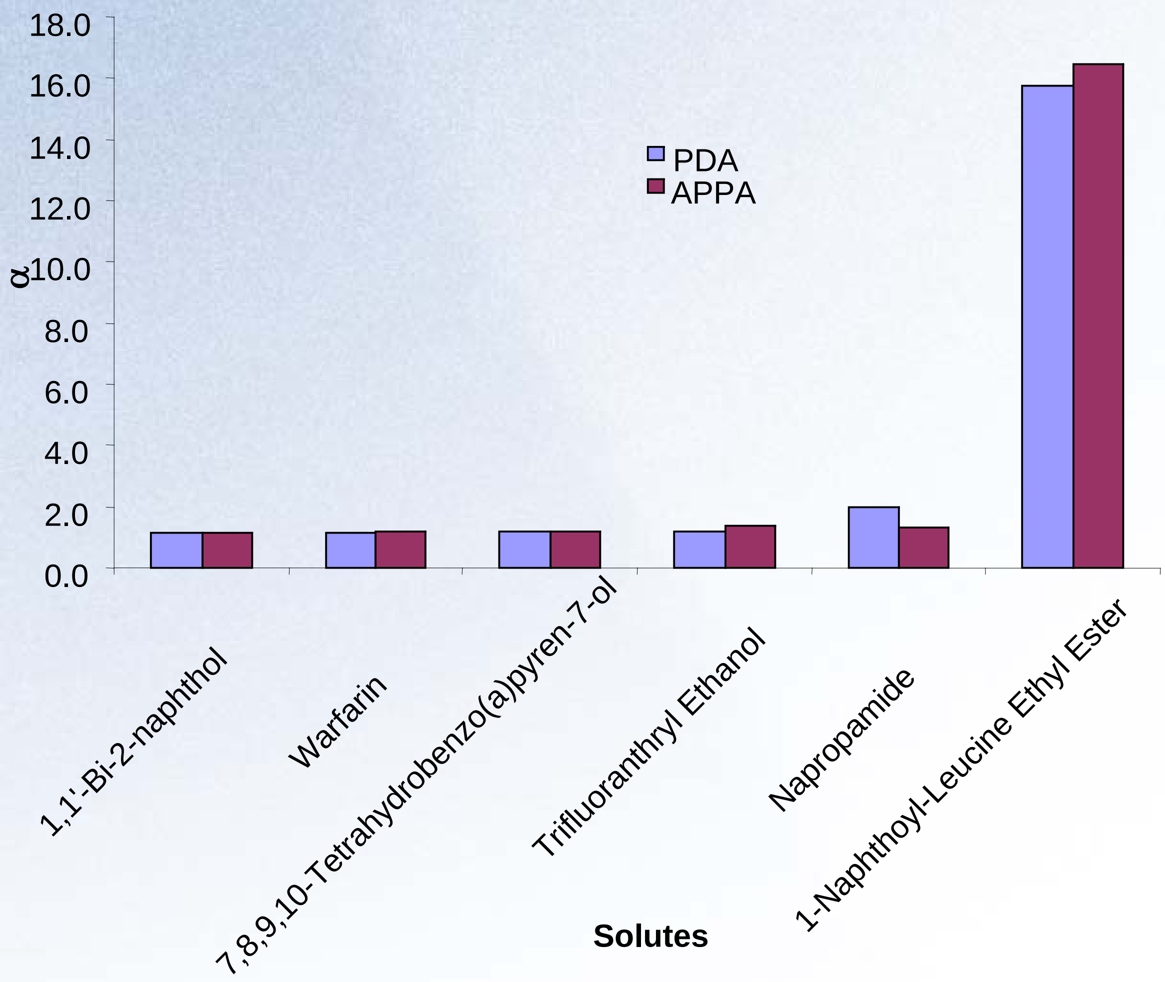


(S)-N-[1-(1-naphthyl)ethyl]succinamic acid ((S)-NESA)

Example of Lewis Acid-Base Modified Zirconia CSPs

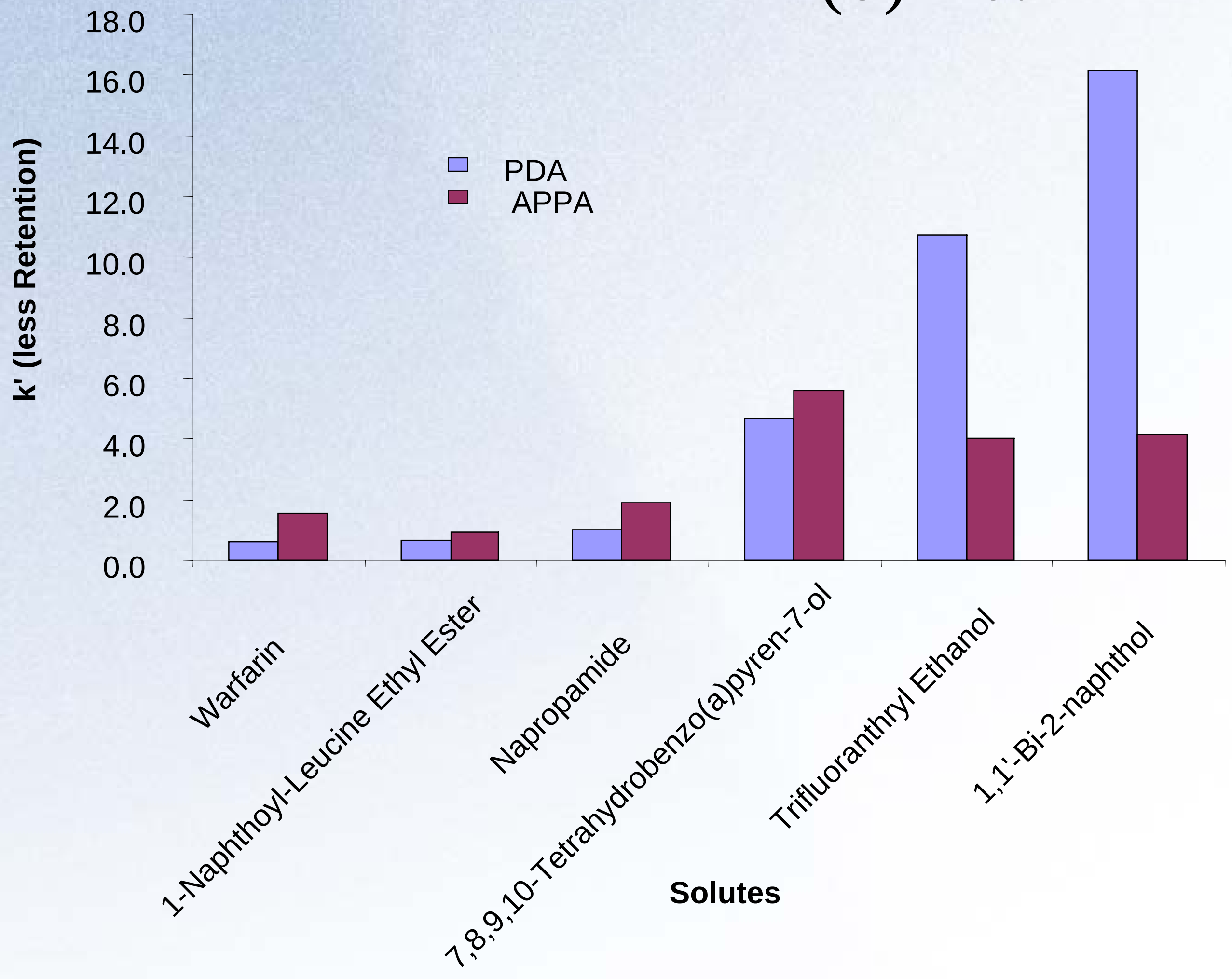


Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



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.

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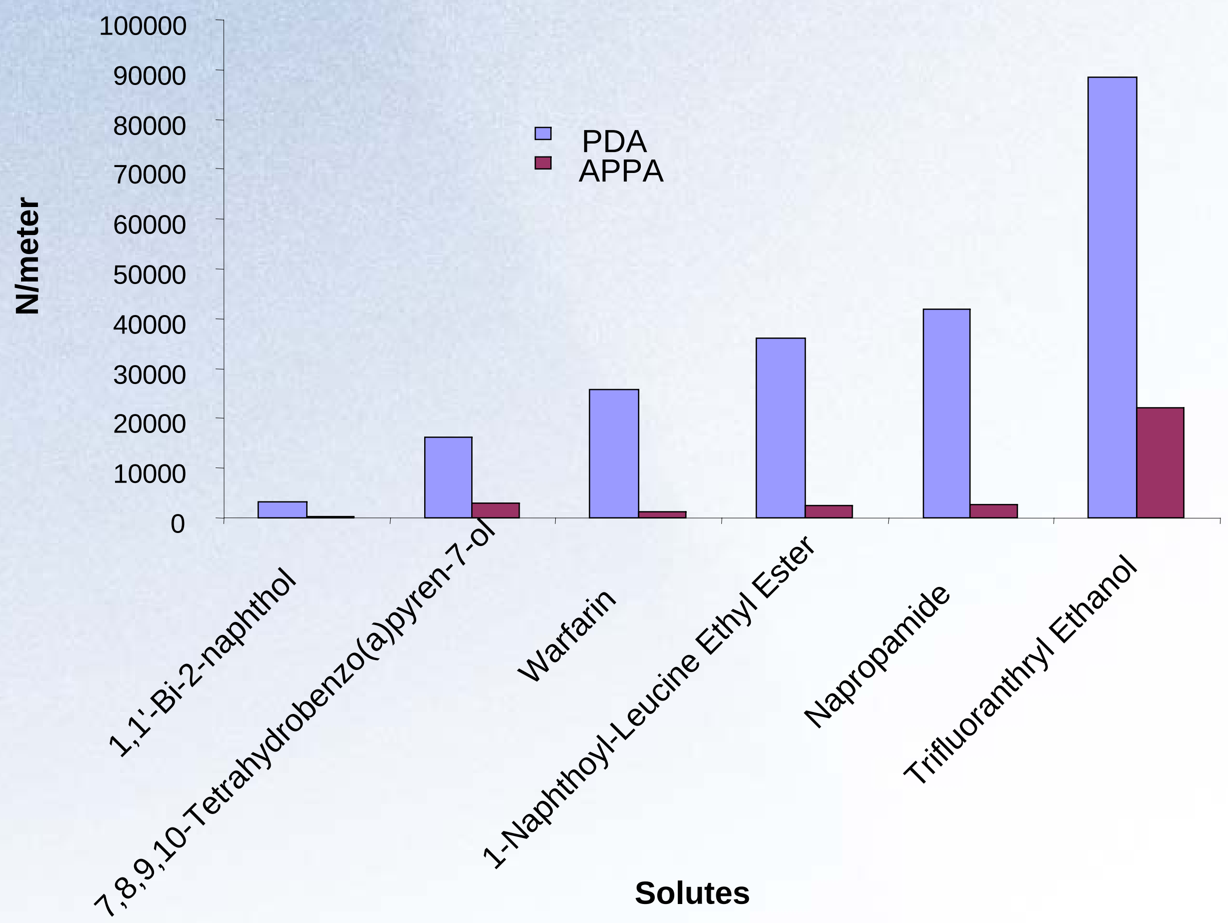
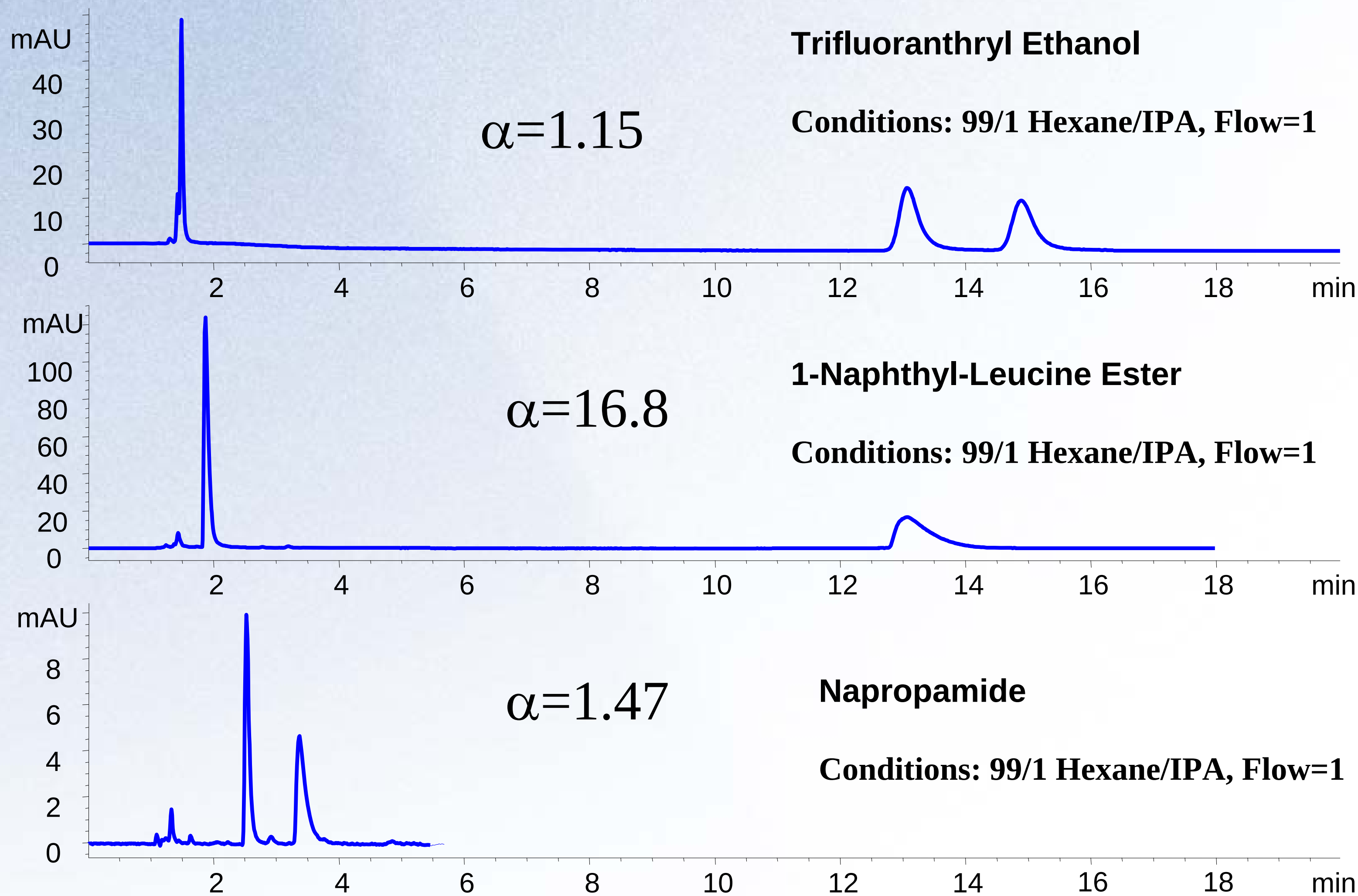
Efficiency Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



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

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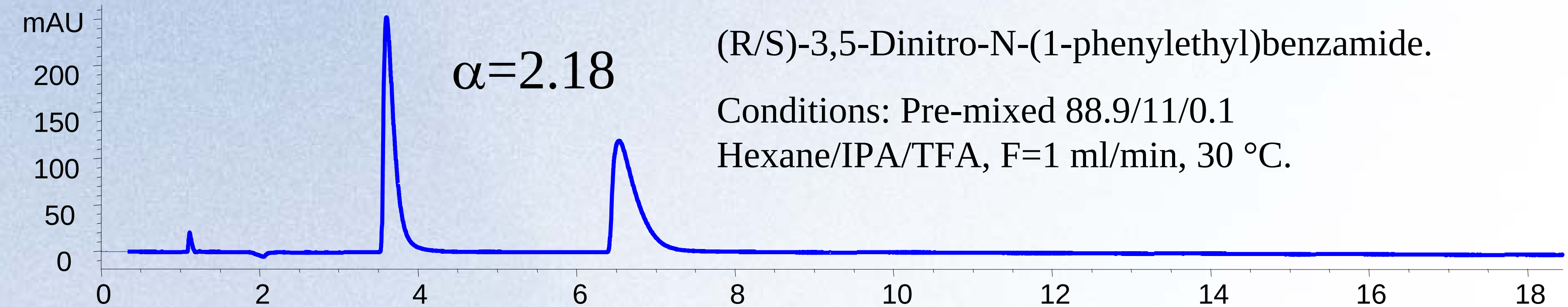
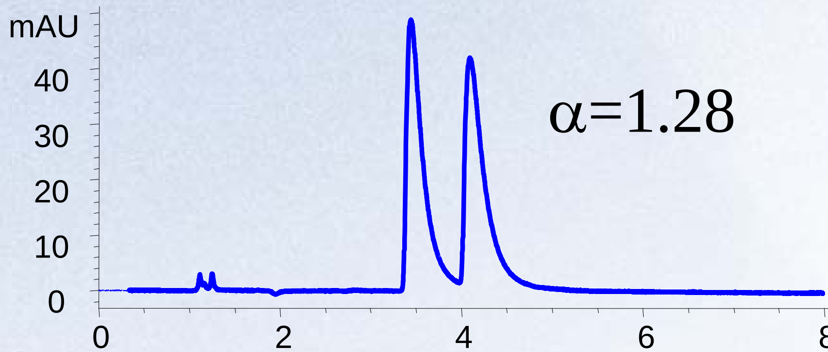


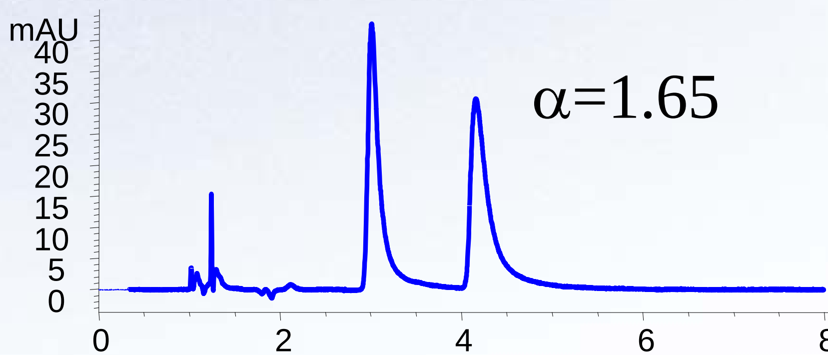
(R/S)-3,5-Dinitro-N-(1-phenylethyl)benzamide.

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



(R/S)- (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

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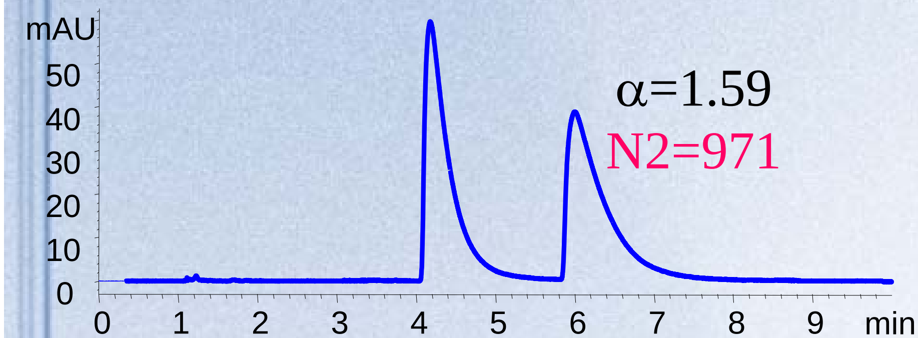
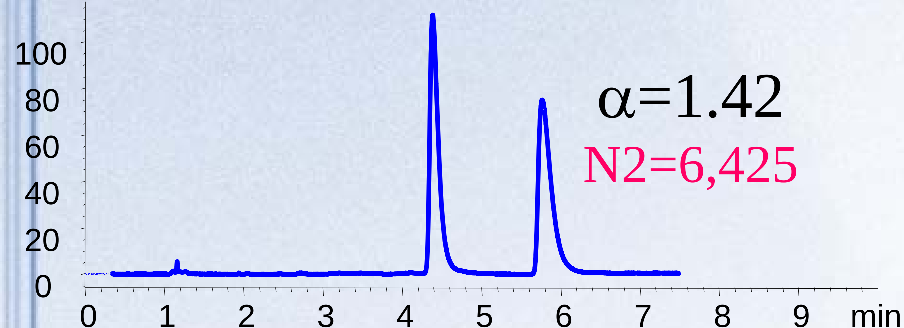
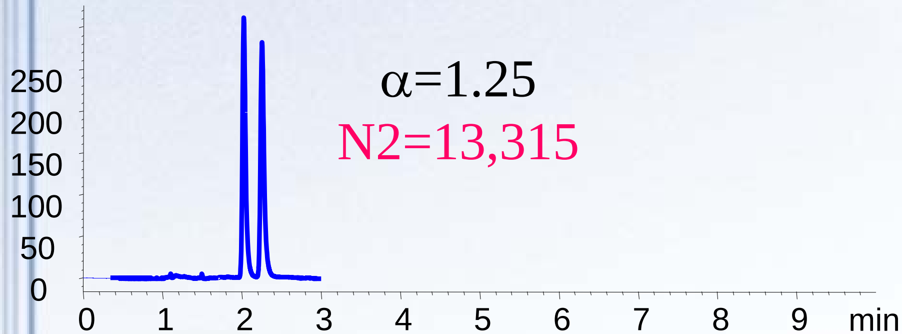
Mobile Phase Effect of adding MeOH on Separation of (R/S)-N-3,5-dintrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA



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

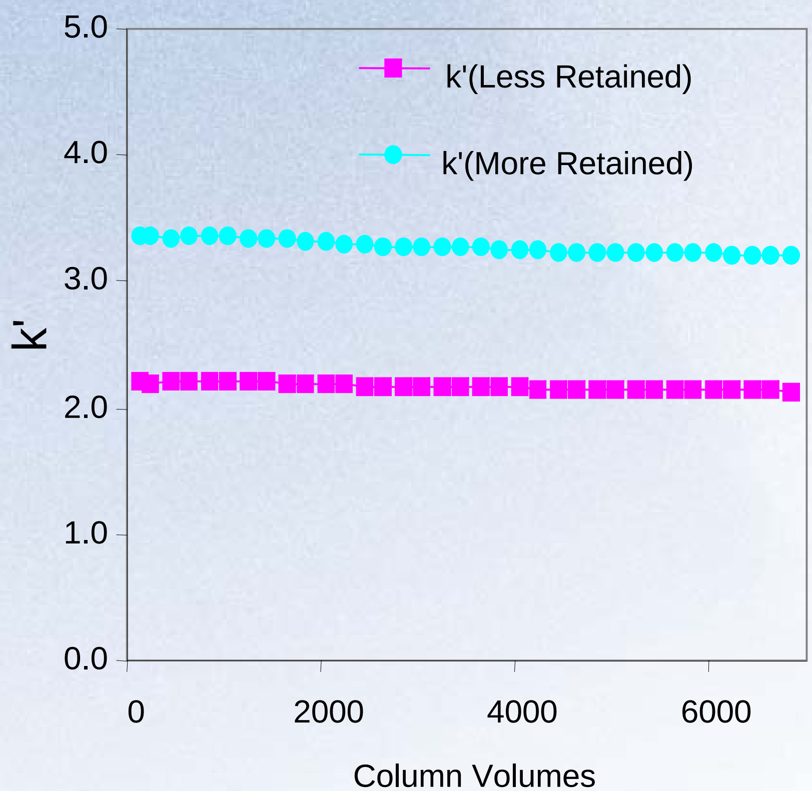


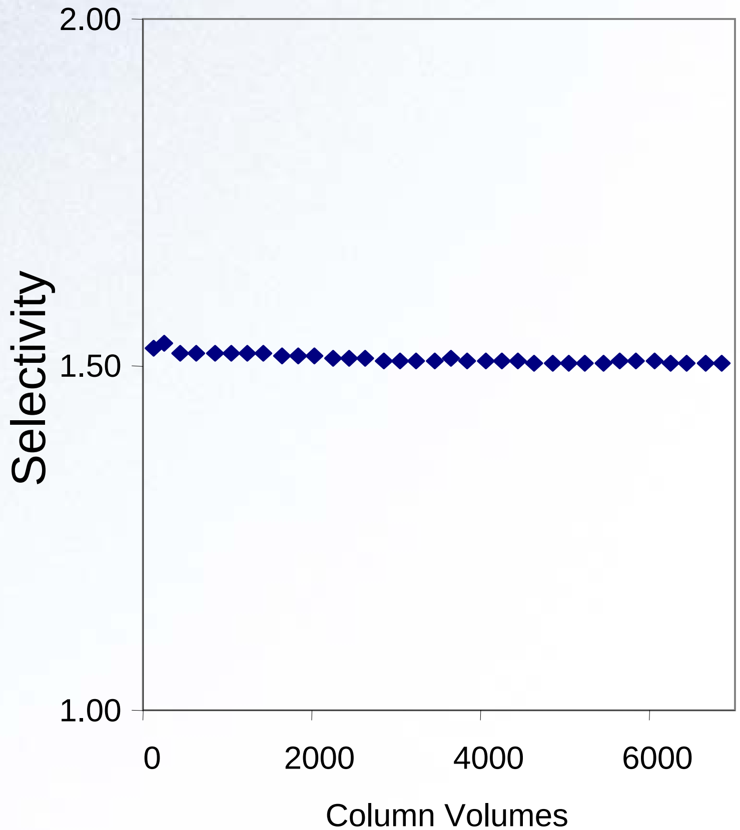
Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / **MeOH** / (70/30 Hexane/IPA), F=1 ml/min, 30 °C



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

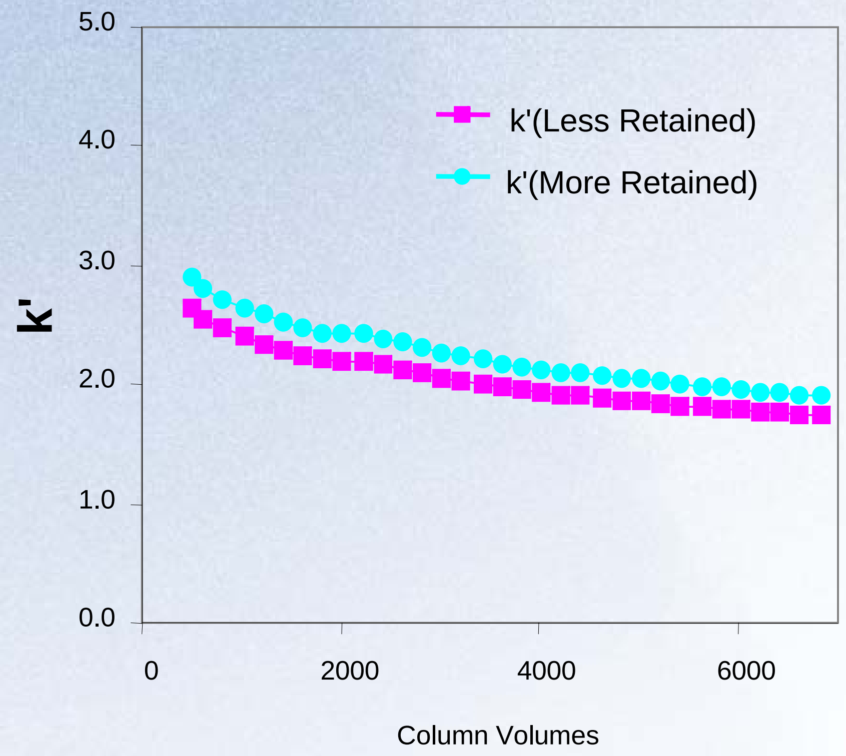
Stability of Zr-(S)-NESA at pH 2

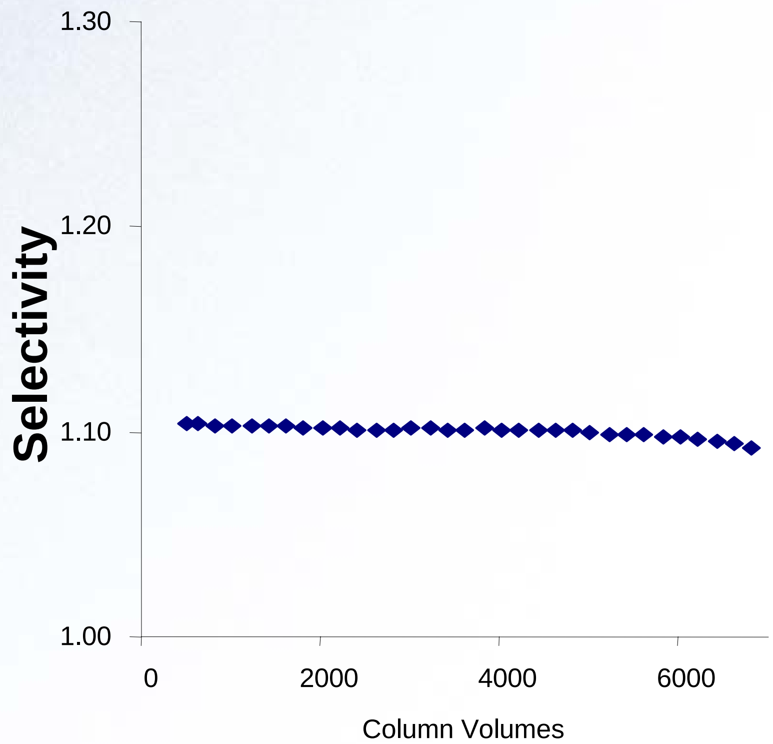




Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 ^{o}C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

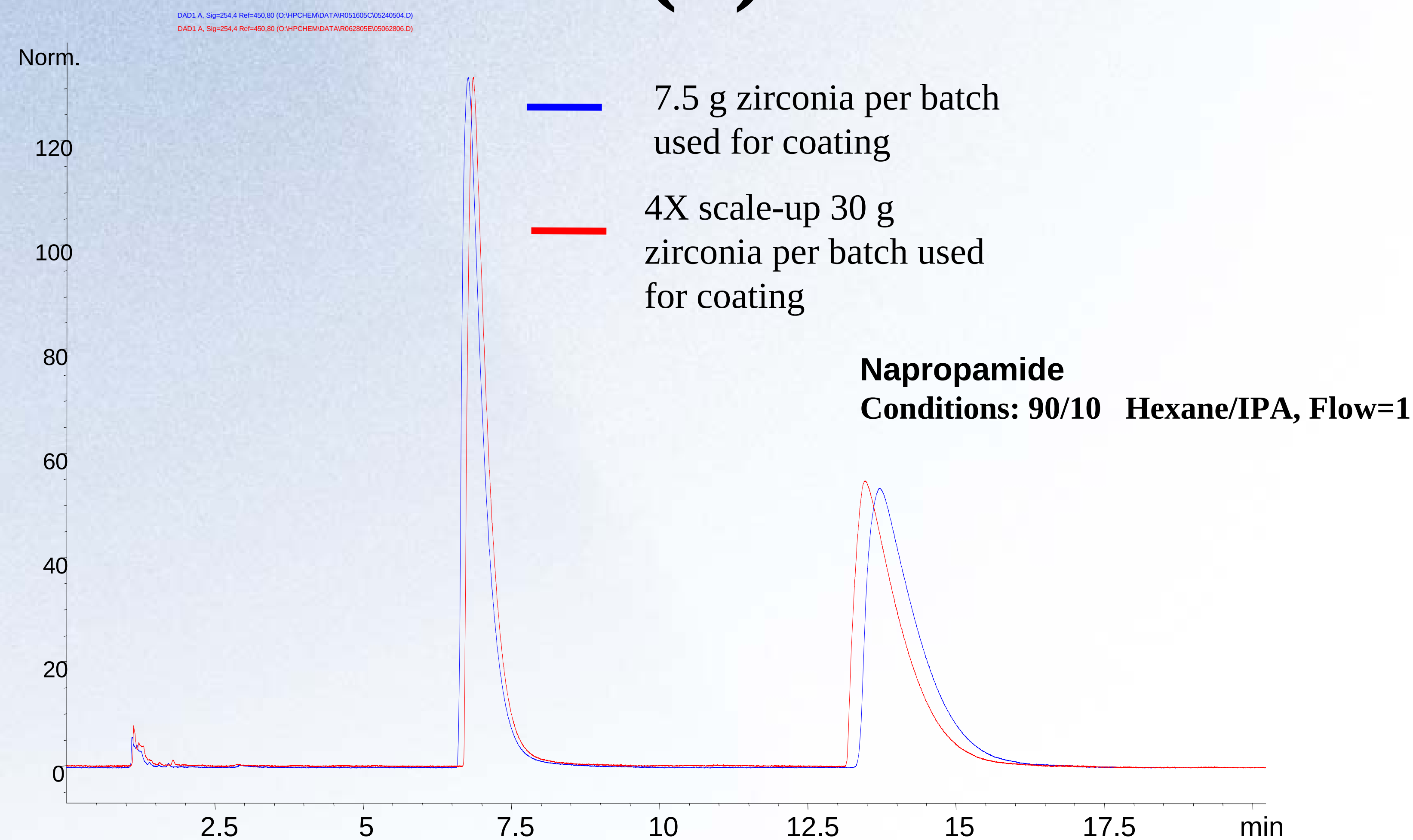
Stability of Zr-(S)-DNB-Leu at pH 8





Column ID: ZrCSP032805A, Mobile phase: 15/85 ACN/5 mM ammonium hydrogencarbonate pH 8.0, Temperature: 30 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



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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
- Small amounts of methanol in the mobile phase had a large effect on efficiency, retention, and selectivity.
- The new Zirconia-based CSPs were found to be ***fairly stable*** in reversed-phase mobile phase from pH 2 to pH 8.
- ***The CSP synthesis is reproducible***.
- ***Chiral selector screening is possible on the new zirconia-based CSPs***.
- Acknowledgement: ***National Institutes of Health Grant*** (Phase II SBIR) 2R44HL070334-02A2.

Thanks *very much* for listening!

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