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Fast Screening of Chiral Stationary Phases for Chiral Separations on Zirconia

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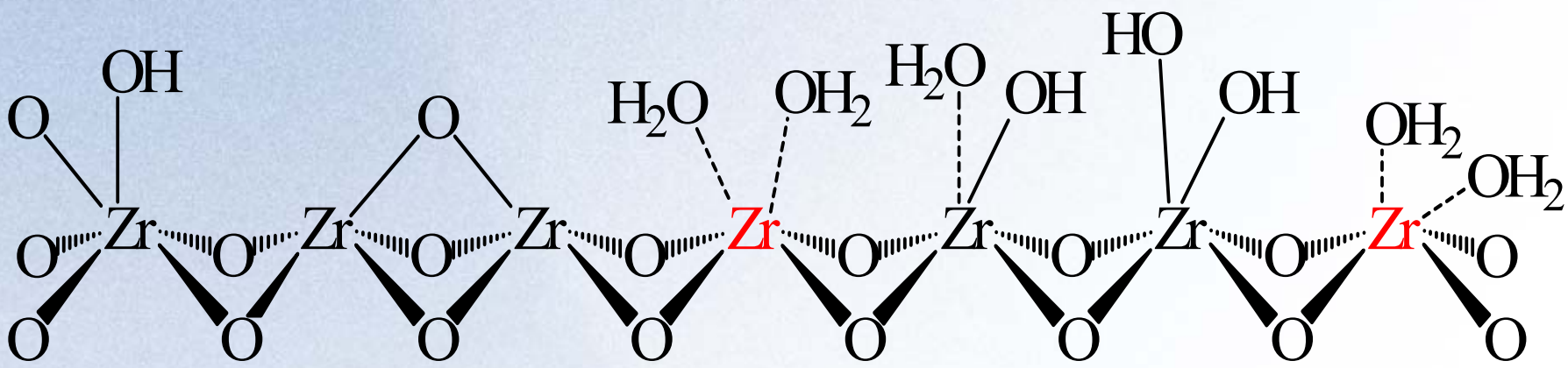
Specialists in High Efficiency, Ultra-Stable Phases for HPLC.

EAS 2004

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

- Why Zirconia?
- Synthetic Approach
- Zirconia-based vs Silica-based CSPs
- Chromatographic Comparison of Different Anchors
- Stability Study
- Example Separations on Zirconia Based CSPs
- Use for Fast Chiral Selector Screening
- **Conclusions – Zirconia Based CSPs Have Comparable Chromatographic Performance Compared to Silica Based CSPs. Fast Chiral Selector Screening is Possible.**

Surface Chemistry of Zirconia



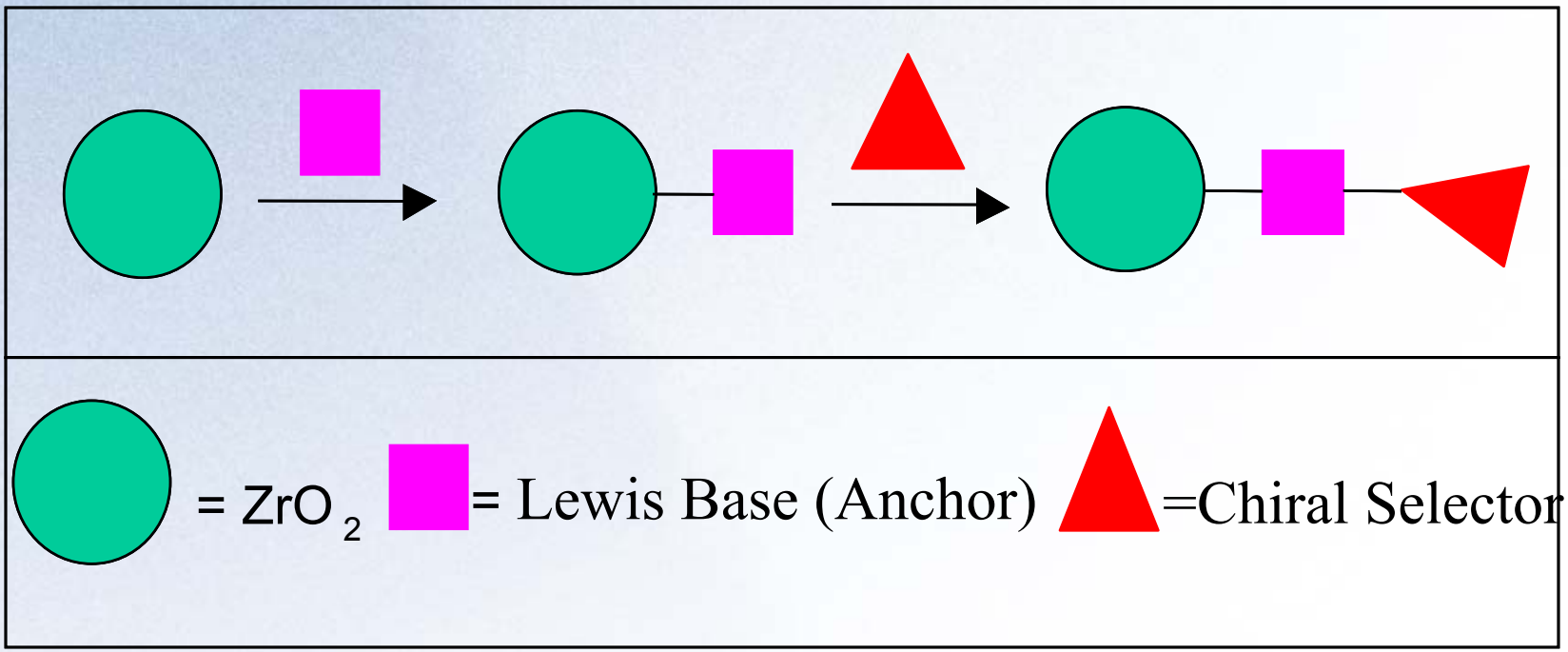
Brönsted Acid: $ZrOH + {}^{-}OH \rightleftharpoons ZrO^{-} + H_2O$

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

RPO_3^{2-} or Catechol

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

New Way to Attach Chiral Selectors to Zirconia Surface



Three Anchors Studied

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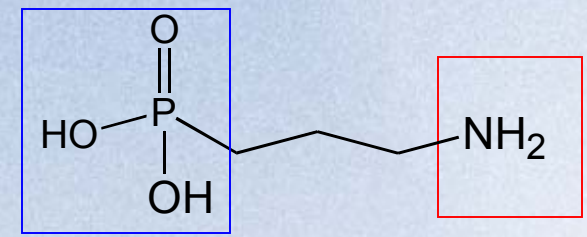
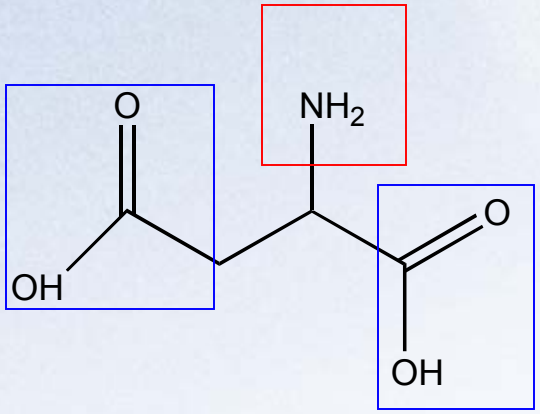
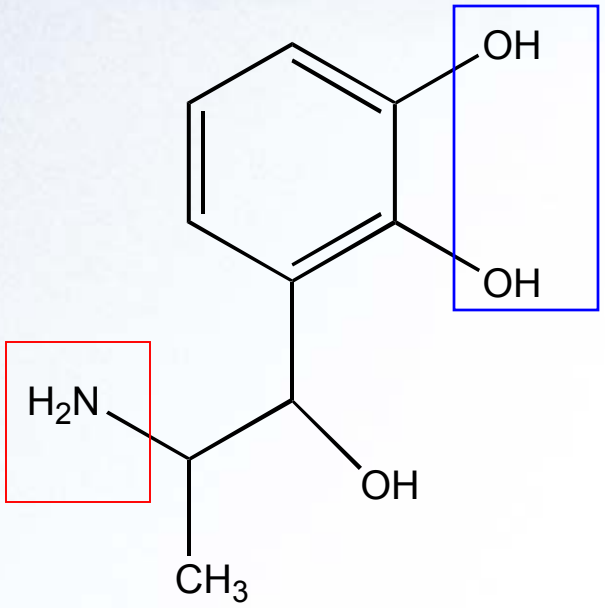


1) APPA (Aminopropylphosphonic acid)



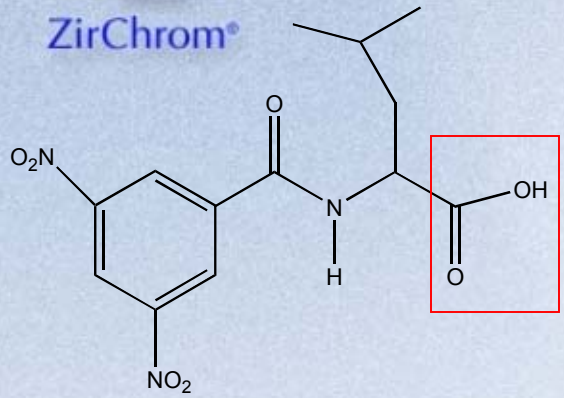
3) ASPA (Aspartic acid)



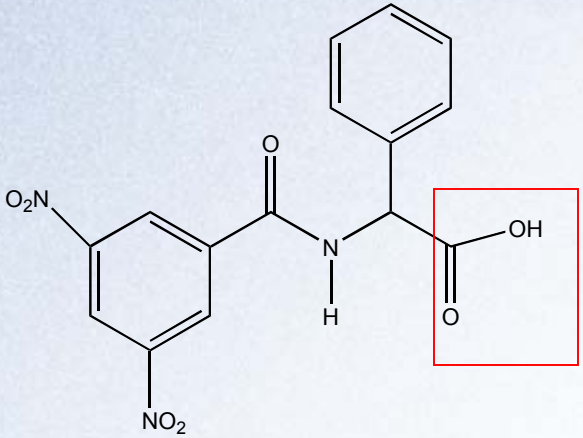
2) DHNP (3,4-Dihydroxynorephedrine)

Anchors should have two function groups: (1) A group anchoring to zirconia surface, and (2) A group bonding to Chiral selector.

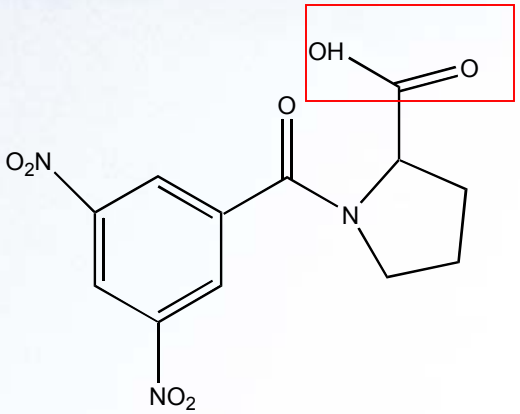
Chiral Selectors in This Study



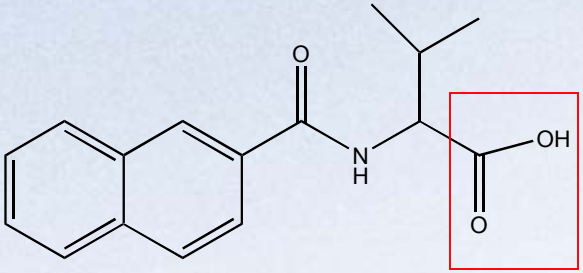
DNB-LEU (3,5-dinitro-benzoylLeucine)



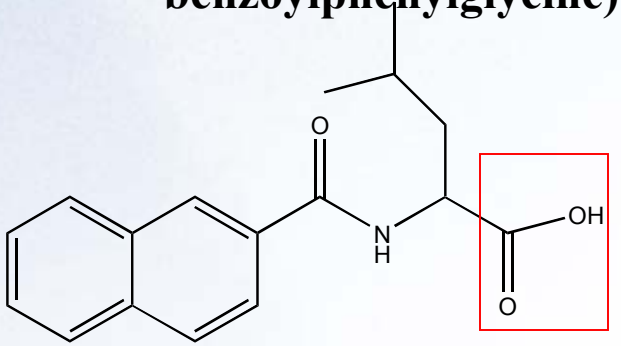
DNB-PG (3,5-dinitro-benzoylphenylglycine)



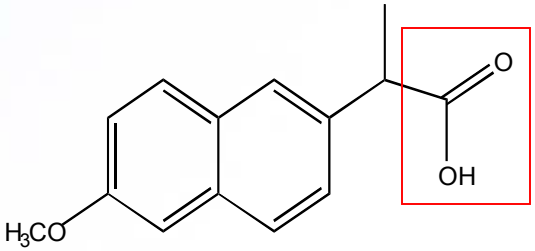
DNB-PRO (3,5-dinitro-benzoylproline)



NAP-VAL (Naphthoyl-valine)

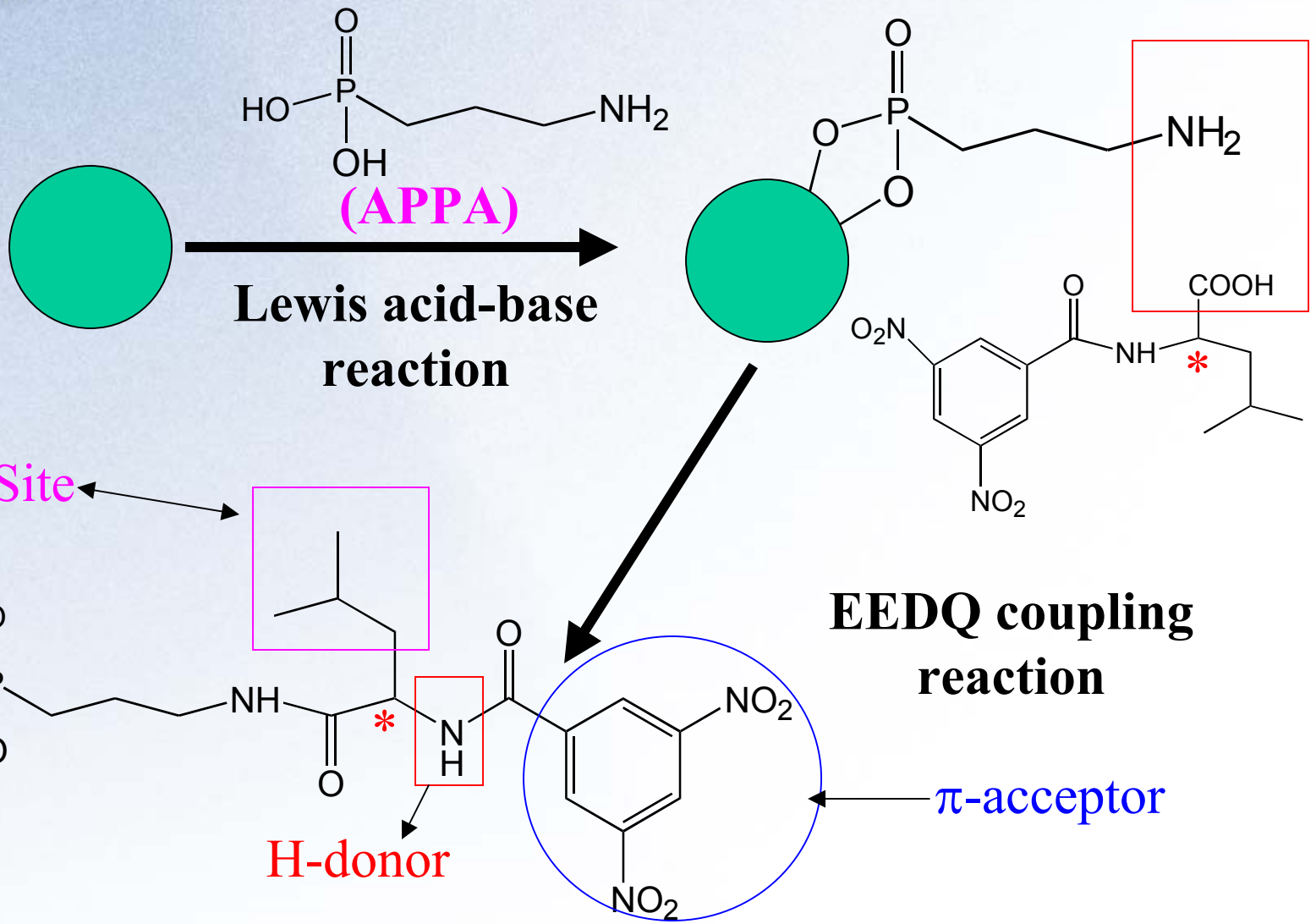


2-NAP-LEU (2-Naphthoyl-leucine),



NAP (naproxen)

Example of Lewis Acid-Base Modified Zirconia CSPs

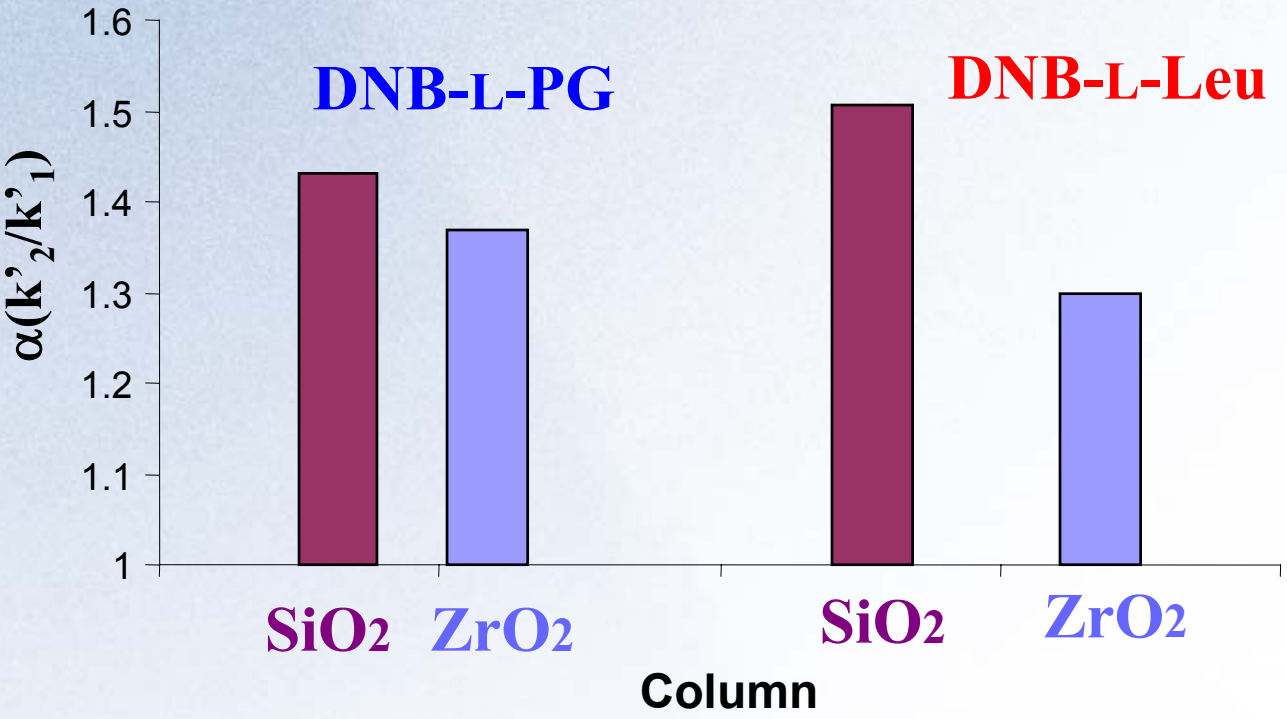


List of Zirconia and Silica CSPs Studied

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

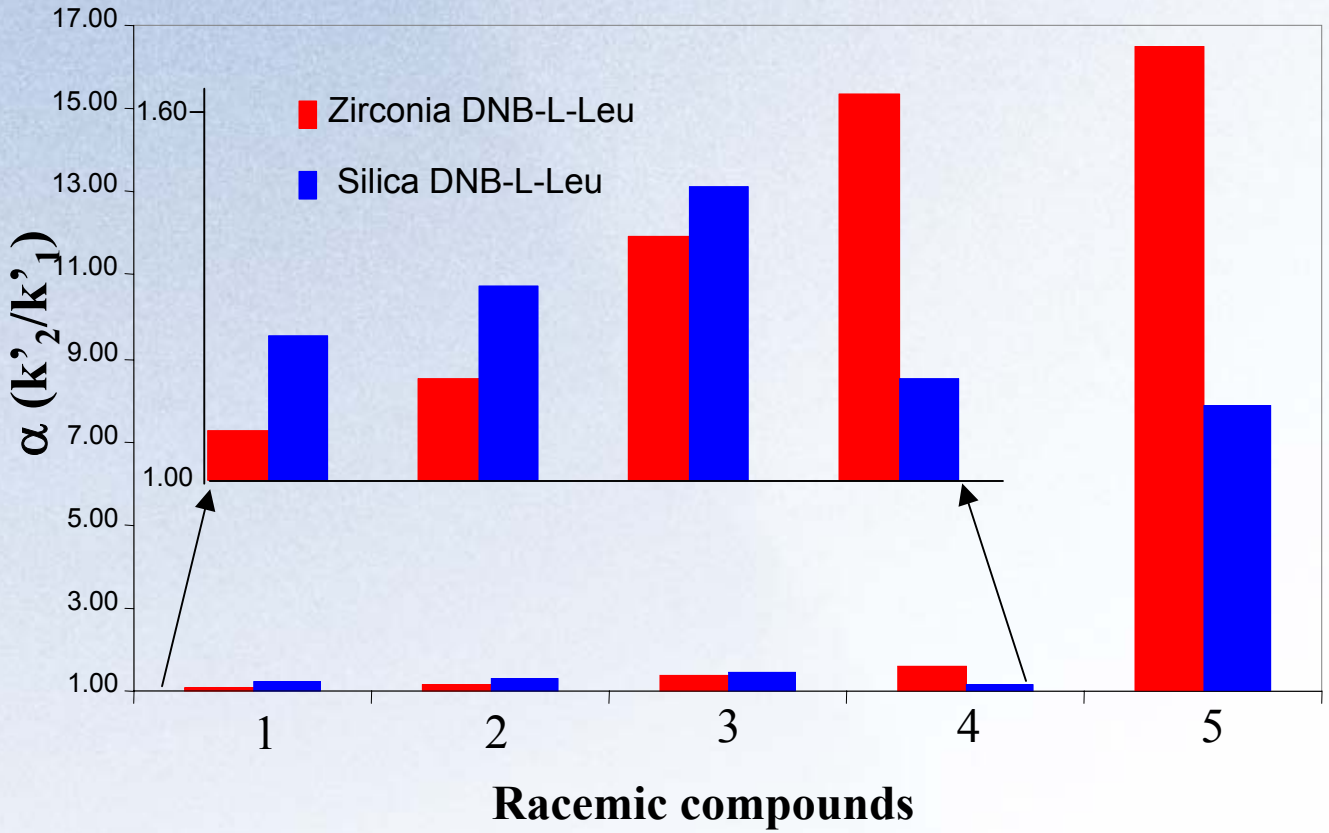
Chromatographic Comparison of Zirconia- and Silica-CSPs



Probe solute: Trifluoroanthryl ethanol

Conclusion: Zirconia based CSPs performed quite well.

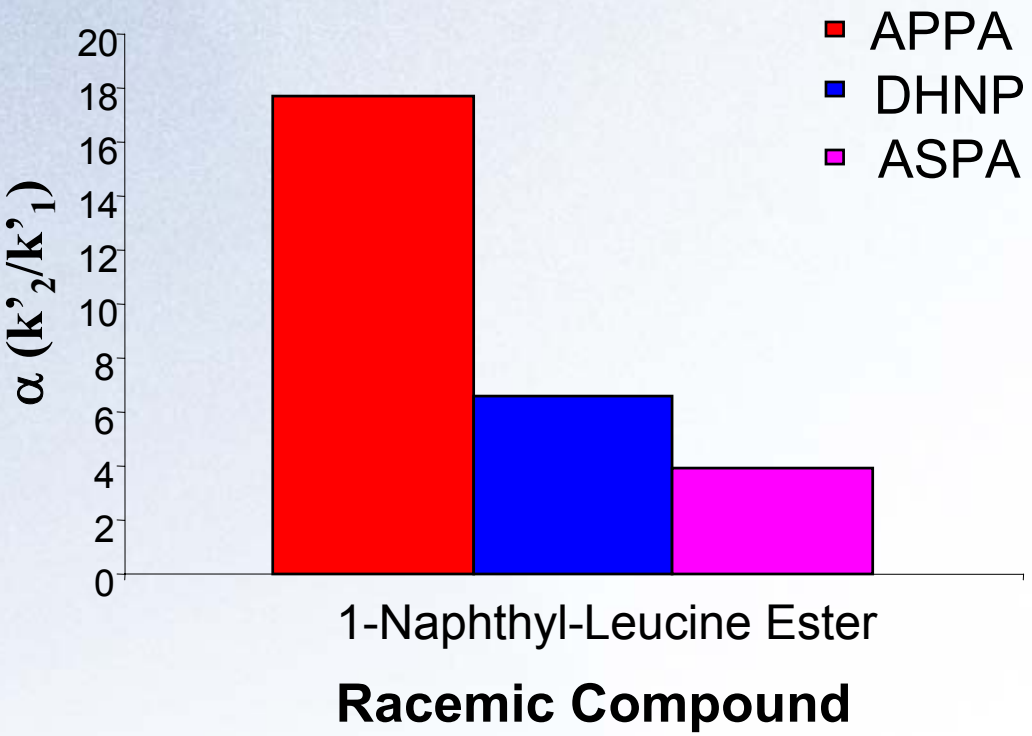
Direct Comparison of DNB-L-LEU Zirconia and Silica Based CSPs



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

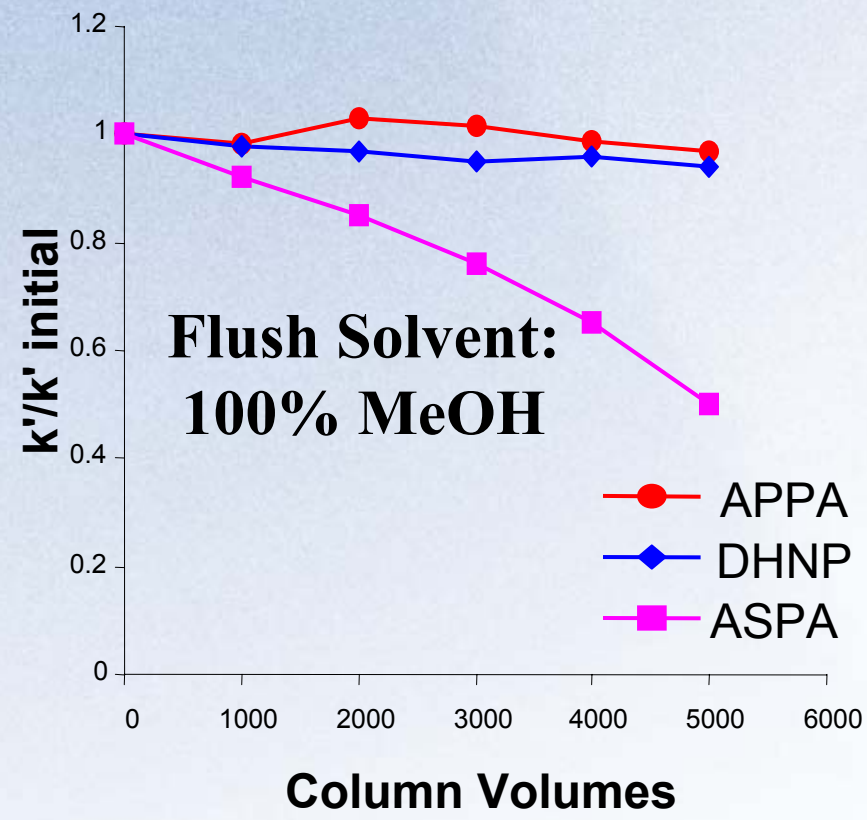
Much better separations for napropamide and 1-naphthyl leucine ester are obtained on zirconia-based CSPs.

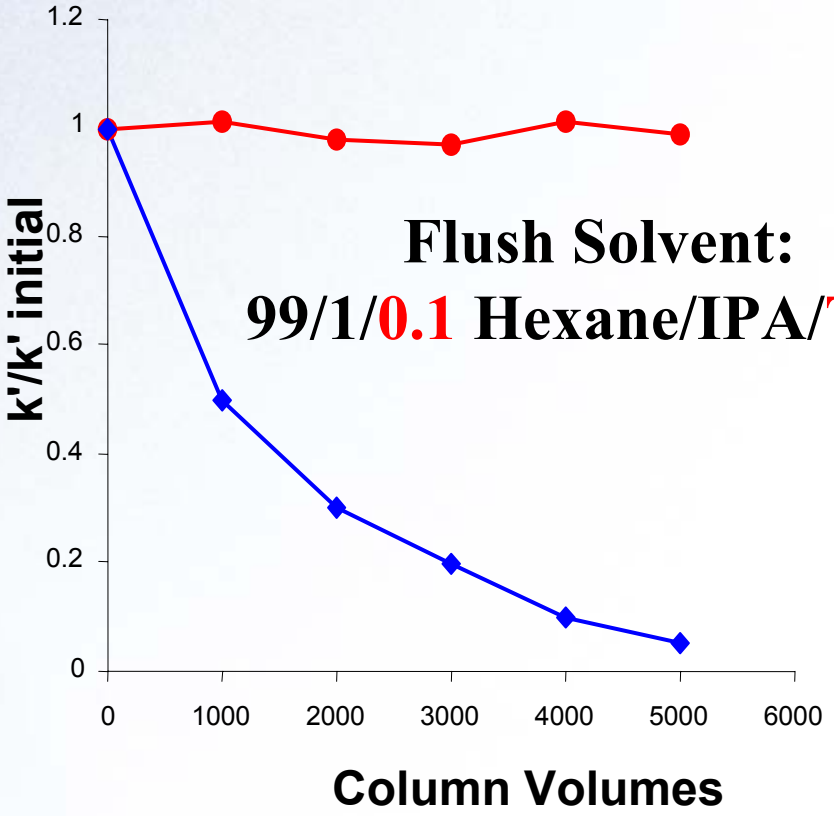
Chromatographic Comparison of Differently Anchored Zirconia-based DNB-L-LEU



Different anchors show different selectivity.

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

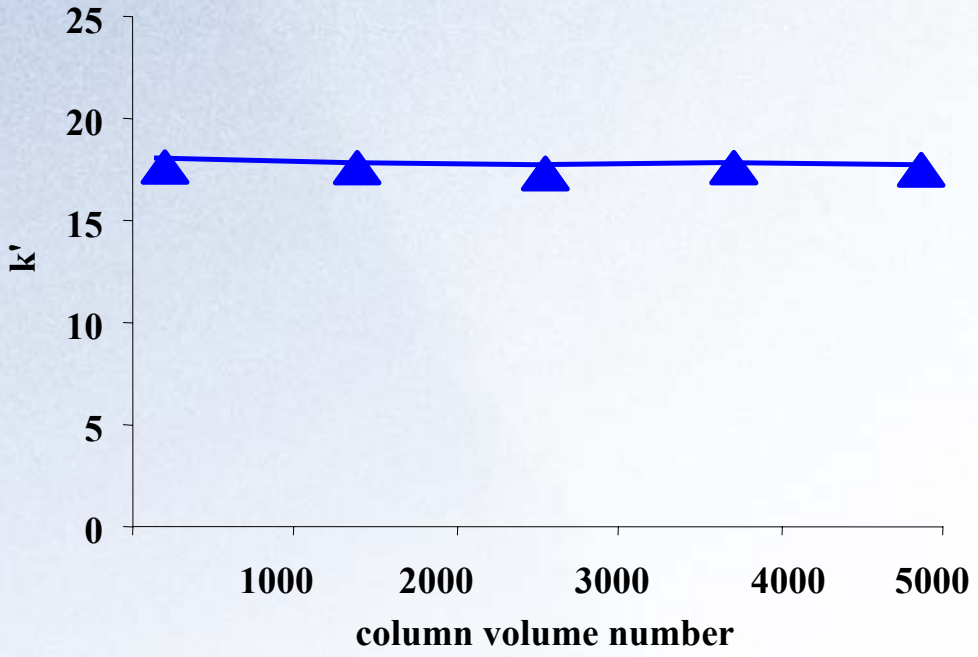




Test solute: trifluoranthryl ethanol. Note that the retention factor ratio is for the less retained isomer.

Stability of Zirconia-based DNB-L-LEU

Retention Factor Stability for S-Napthylleucine ester



Zirconia APPA-DNB-Leucine

<3% Decrease

Flush Solvent: 49.5/49.5/1 Hexane/IPA/TFA

Zirconia-based CSP is a very stable CSP.

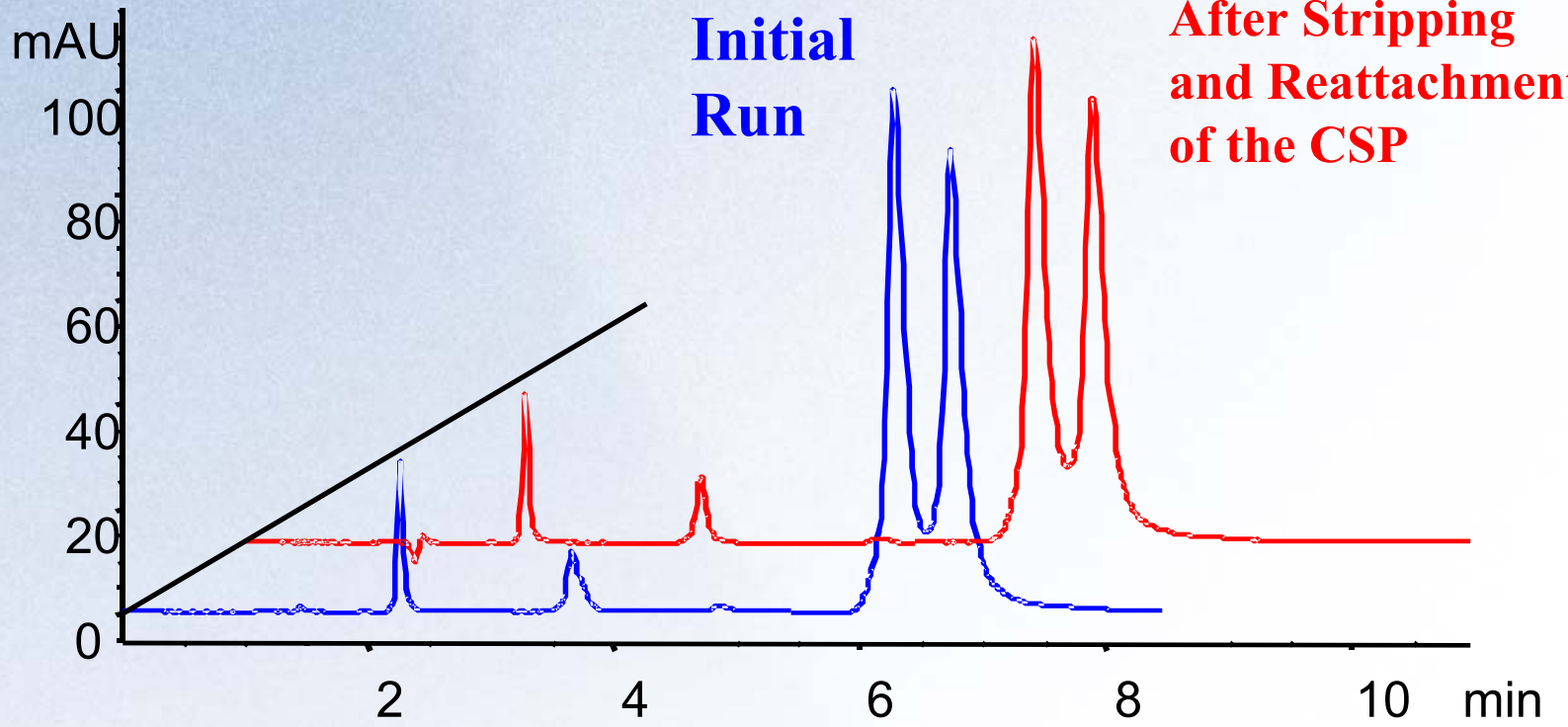
Novel Chiral Selector Screening Method

- Attached a CSP to a bare zirconia column in-situ.
- Flush the column to remove unbound CSP.
- Screen chiral target compound.
- Strip off CSP using basic conditions.
- Repeat Cycle until desired resolution is achieved using different CSPs.

Example 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 .
- 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.

Conclusions

- ***Flexible*** attachment chemistry.
- APPA is the best anchor in terms of column stability.
- Zirconia based CSPs have ***comparable chromatographic performance compared to the commercial silica*** based CSPs for a wide range of chiral compounds.
- Phase II testing will involve ***new anchors and different CSPs*** with more optimization of screening techniques.
- Acknowledgement: National Institutes of Health Grant (Phase I) R43 HL070334-01.

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