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Creating Stable Zirconia Phases for Chiral and other HPLC Applications with Novel Chelating Reagents

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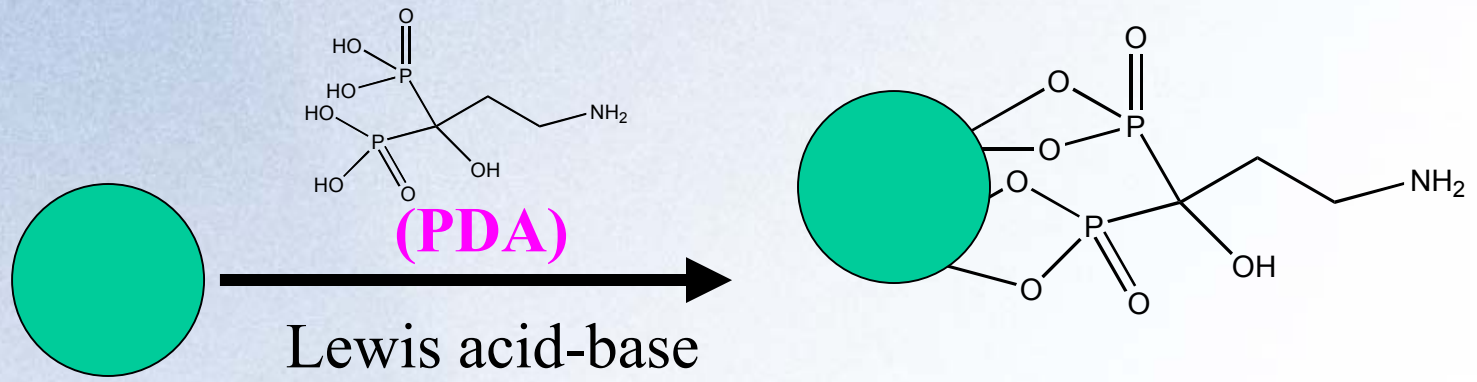
Specialists in High Efficiency, Ultra-Stable Phases for HPLC

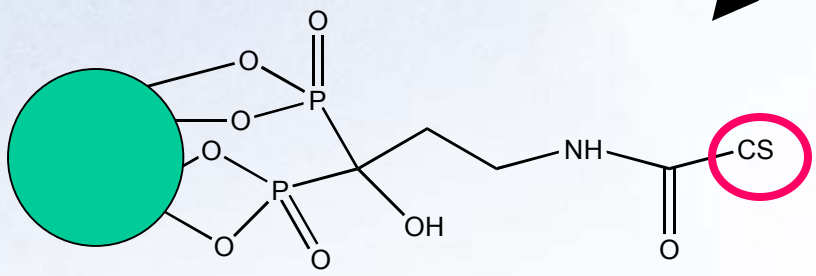
A New Synthetic Approach for Producing Stable Bonded Phases on Zirconia

- 1-Step and 2-Step Synthetic Approaches for Stationary Phase (SP) Attachment, Including Chiral (CSP)
- Chiral Separations on Zirconia Based CSPs
- Effect of Mobile Phase Additives on α, k' and N
- Stability Study
- 1-Step and 2-Step Coating Procedures for Chiral Screening
  - 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 novel possibility of regeneration or use for Chiral Selector Screening.

A 2-Step Synthesis with Bidentate Anchor (PDA)

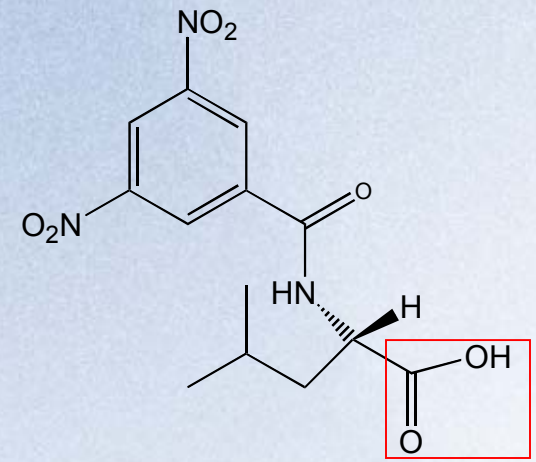


CS-COOH
EEDQ coupling reaction



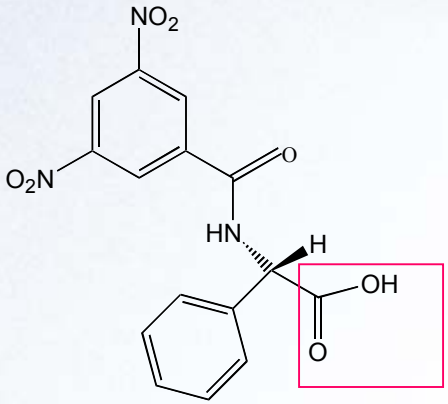
CS = Chiral Selector
This general approach can also be used to anchor any stable stationary phase on Zr or Ti

Chiral Selectors Evaluated[1]



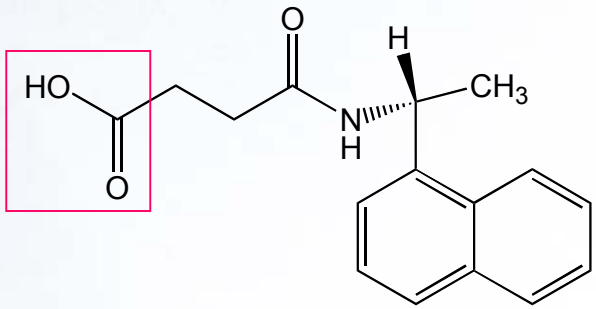
(S)-DNB-L-Leucine

[(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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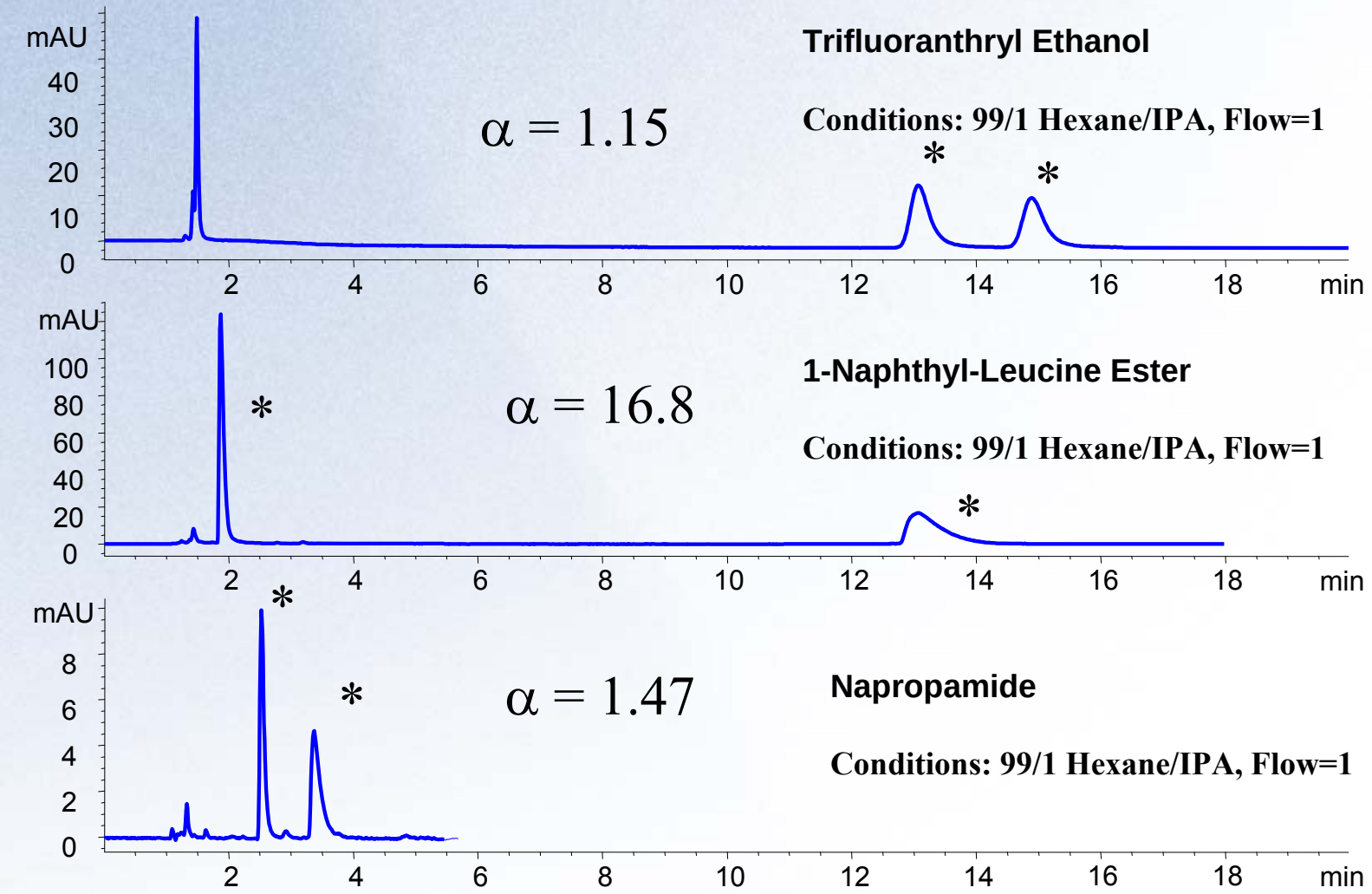
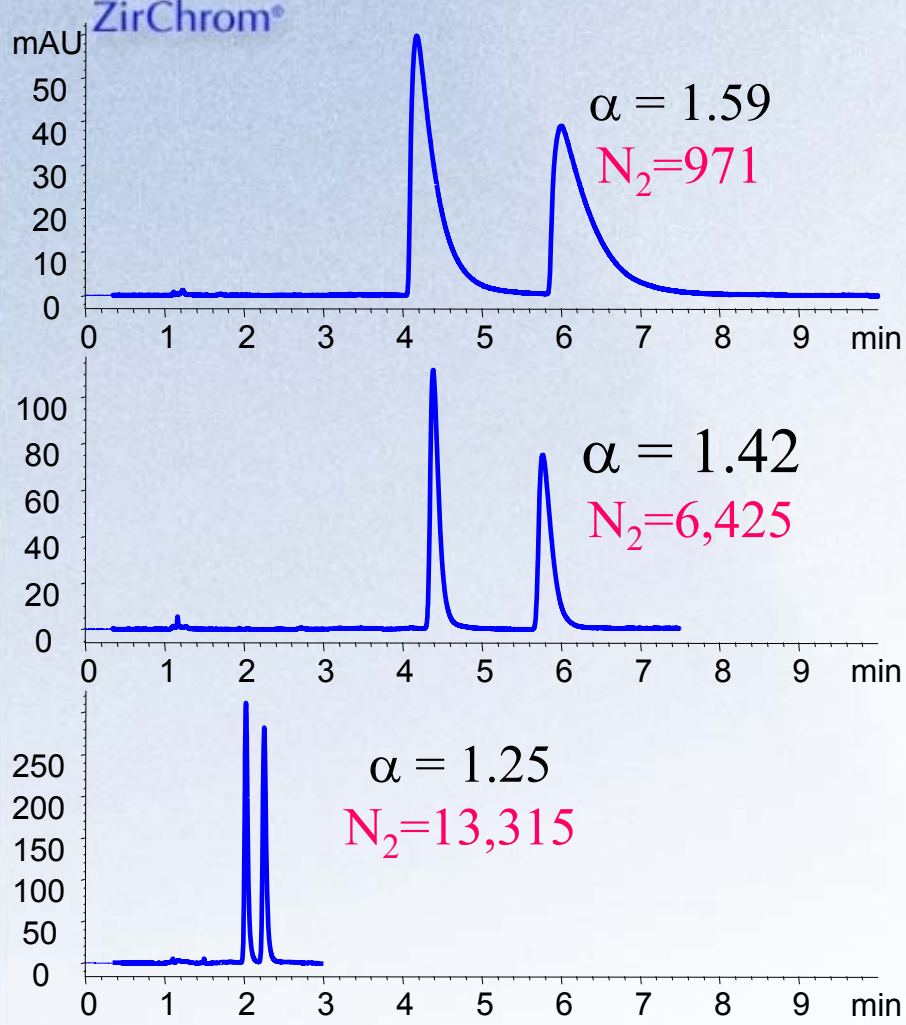


Methanol Effect on Zr (S)-NESA



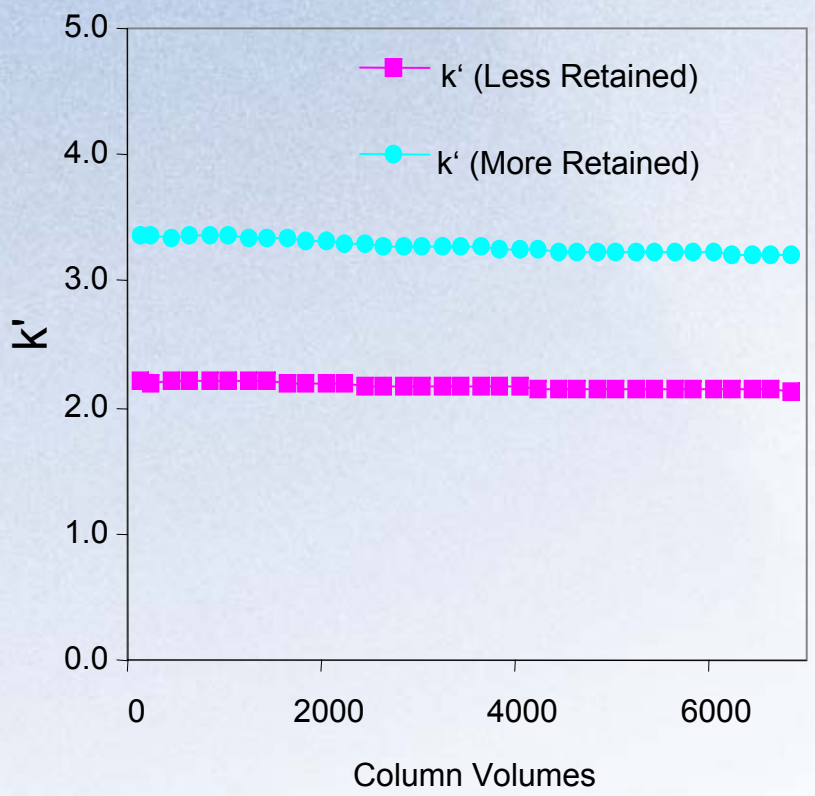
Sample: (R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate

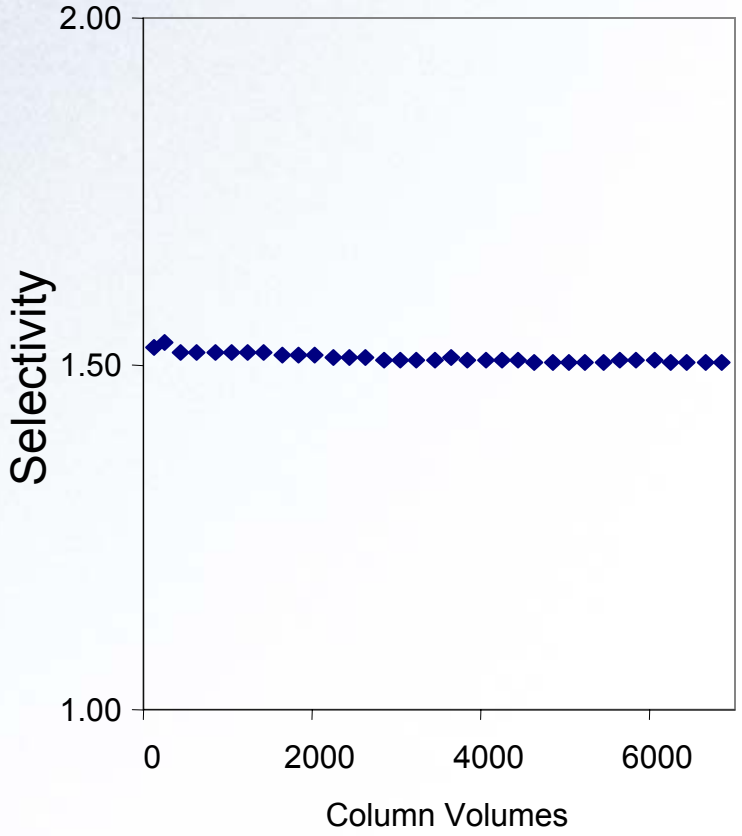
Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.

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

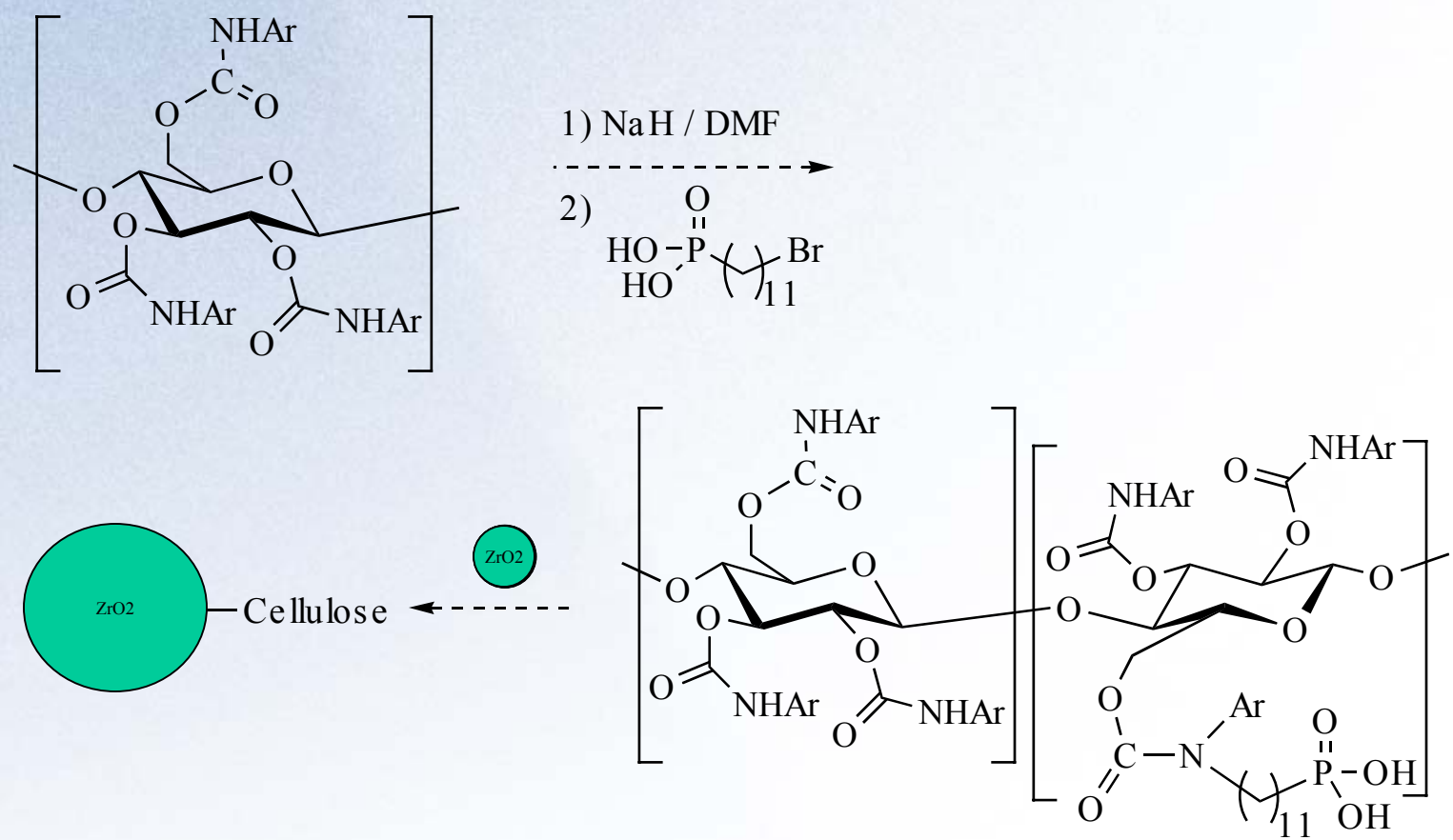
Stability of Zr-(S)-NESA at pH 2



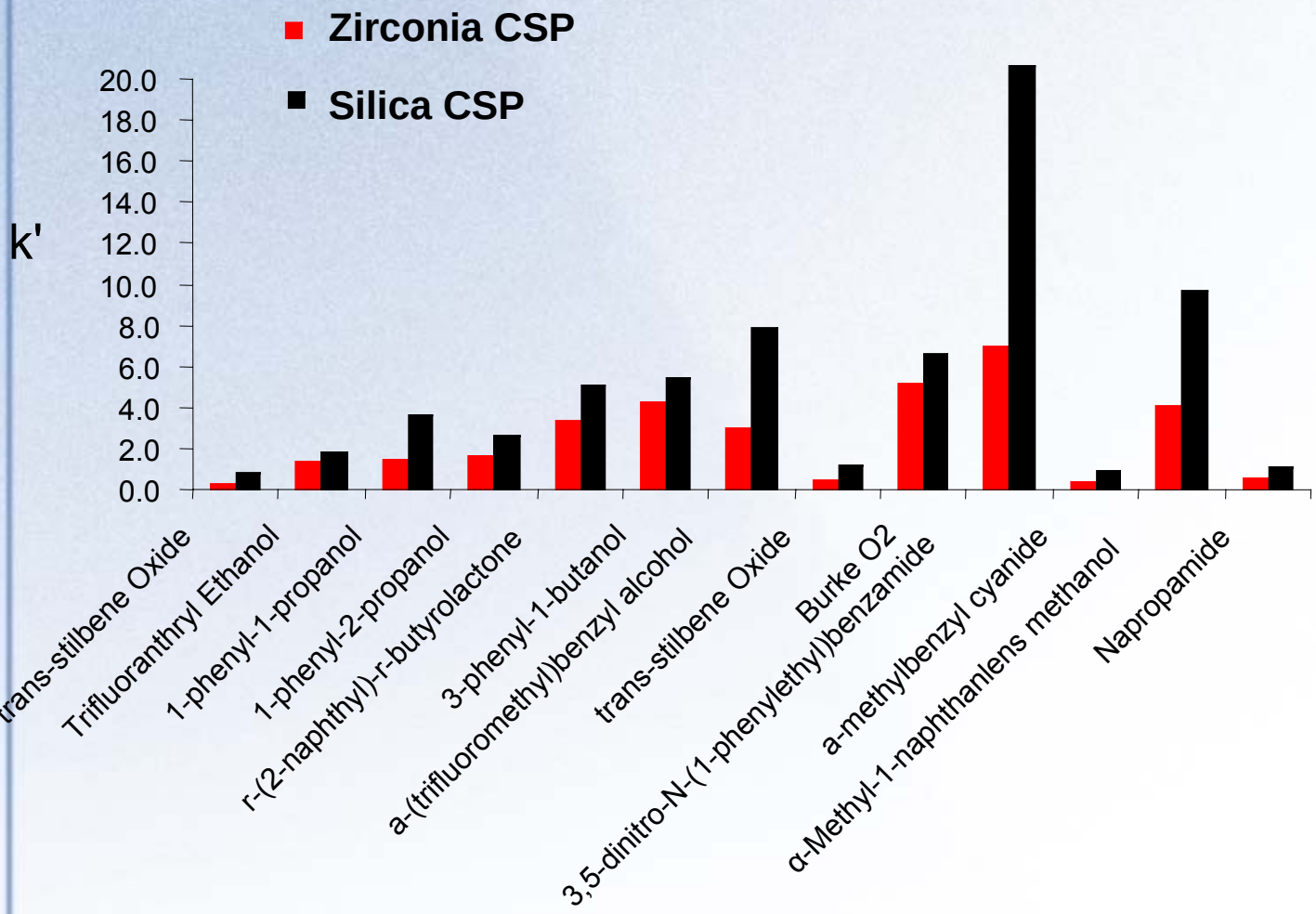


Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

Phosphonate Modified Cellulose-Based CSP on Zirconia



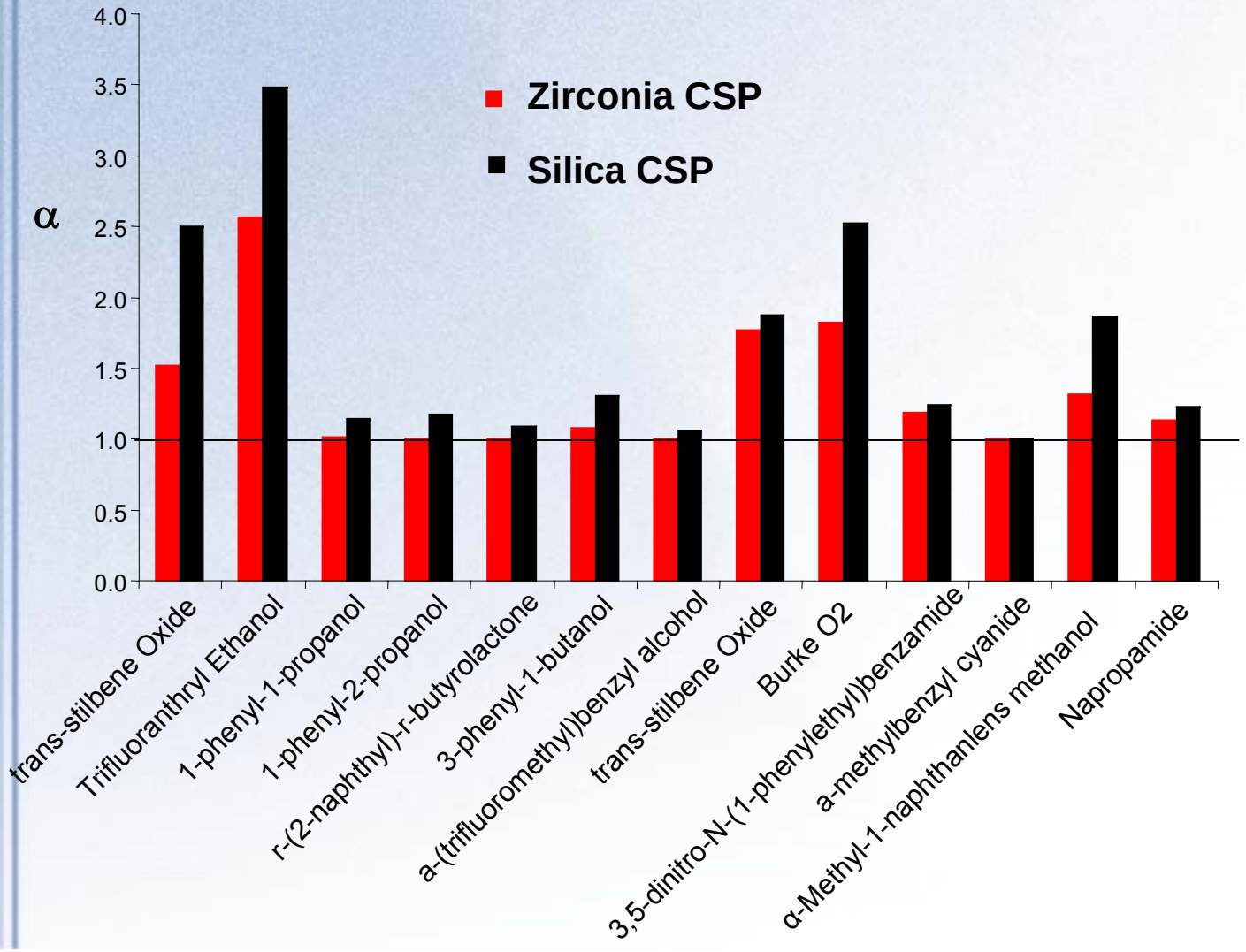
Retention Comparison Between Undecylphosphonate Cellulosic Zirconia and a Commercial CSP



Comments:

Zirconia phase has less retention compared to the commercial silica-based column likely due to lower loading. Future studies will look at increased stationary phase loading.

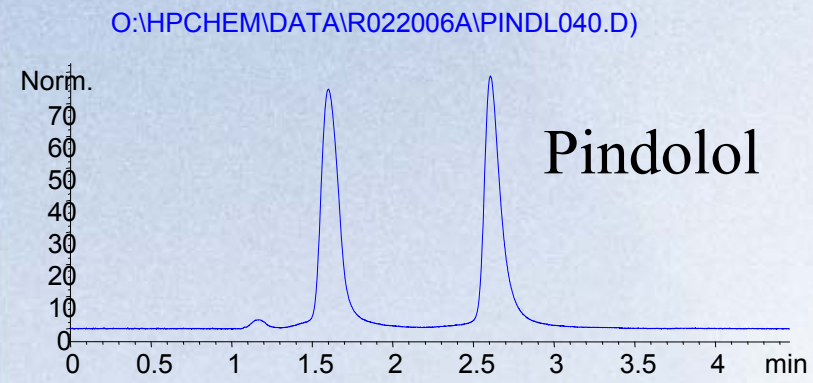
Selectivity Comparison Between Undecylphosphonate Modified Cellulosic Zirconia and a Commercial CSP

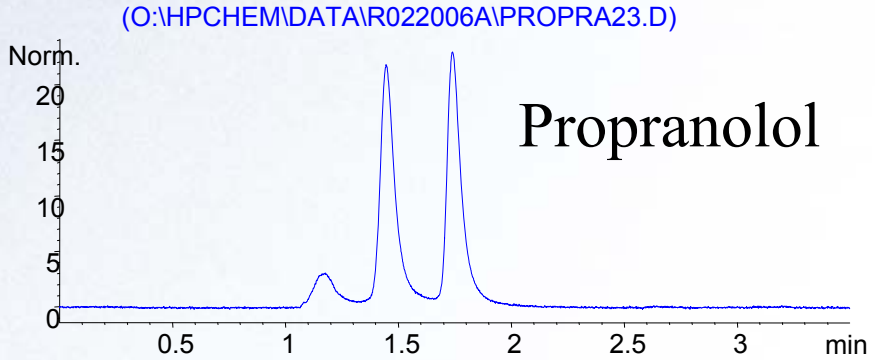


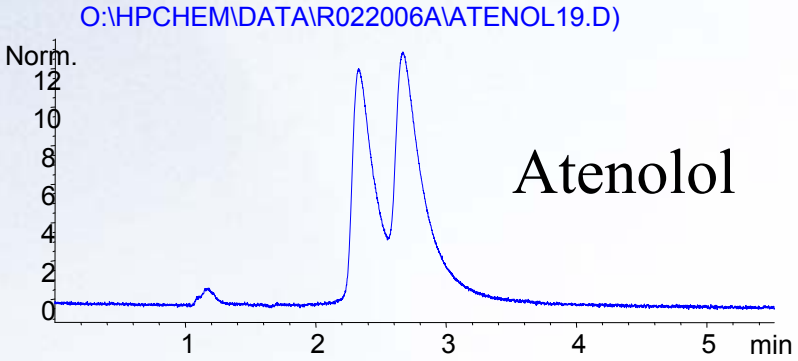
Comments:

Undecylphosphonate modified cellulosic zirconia has comparable selectivity to the commercial silica-based column.

β-Blocker Separations on Undecylphosphonate Modified Cellulosic Zirconia







LC Conditions: Agilent 1100 with chemstation, flow rate 0.5 mL/min., UV 254, mobile phase = 35/65 heptane/IPA with 100 mM ammonium acetate, Temperature = ambient, column dimension 10 cm x 4.6 mm id, 3 micron particles.

Mobile Phase Additive Effect on the Separation of Enantiomers on Undecylphosphonic Acid Modified Cellulosic Zirconia

Ion Strength/ Selectivity	Ammonium Actate in IPA (mM)			
	200	100	80	40
Pindolol	2.87	2.10	1.79	1.30
Propranolol	1.55	1.53	1.35	1.10
Atenolol	1.26	1.12	1.09	1.00
Nadolol	1.00	1.00	1.00	1.00

Decreasing ammonium acetate decreases the enantioselectivity.

LC Conditions: Agilent 1100 with chemstation, flow rate 0.5 mL/min., UV 254, mobile phase = 100% IPA with specified concentration of ammonium acetate, Temperature = ambient, column dimension 10 cm x 4.6 mm id, 3 micron particles.

2-Step Online Zirconia CSP Synthesis for Chiral Screening

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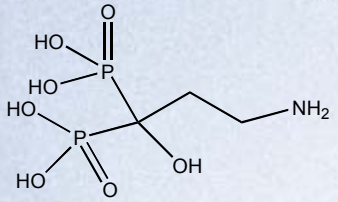
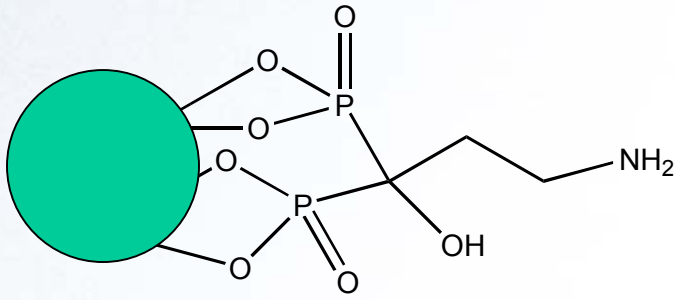


(Pamidronic acid)

ZrO_2

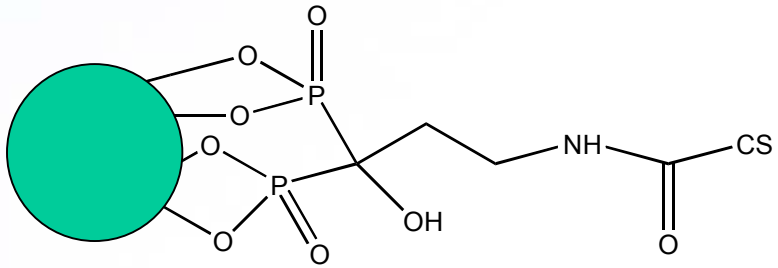
1) Lewis Acid-base Anchor Attachment (10 mg/mL aq. PDA, recycle for 16 hrs at 1 mL/min and 60°C)

ZrO_2-PDA



2) EEDQ Coupling Reaction (CS reagent in THF for 16 hrs at 30°C)

ZrO_2-PDA-CS



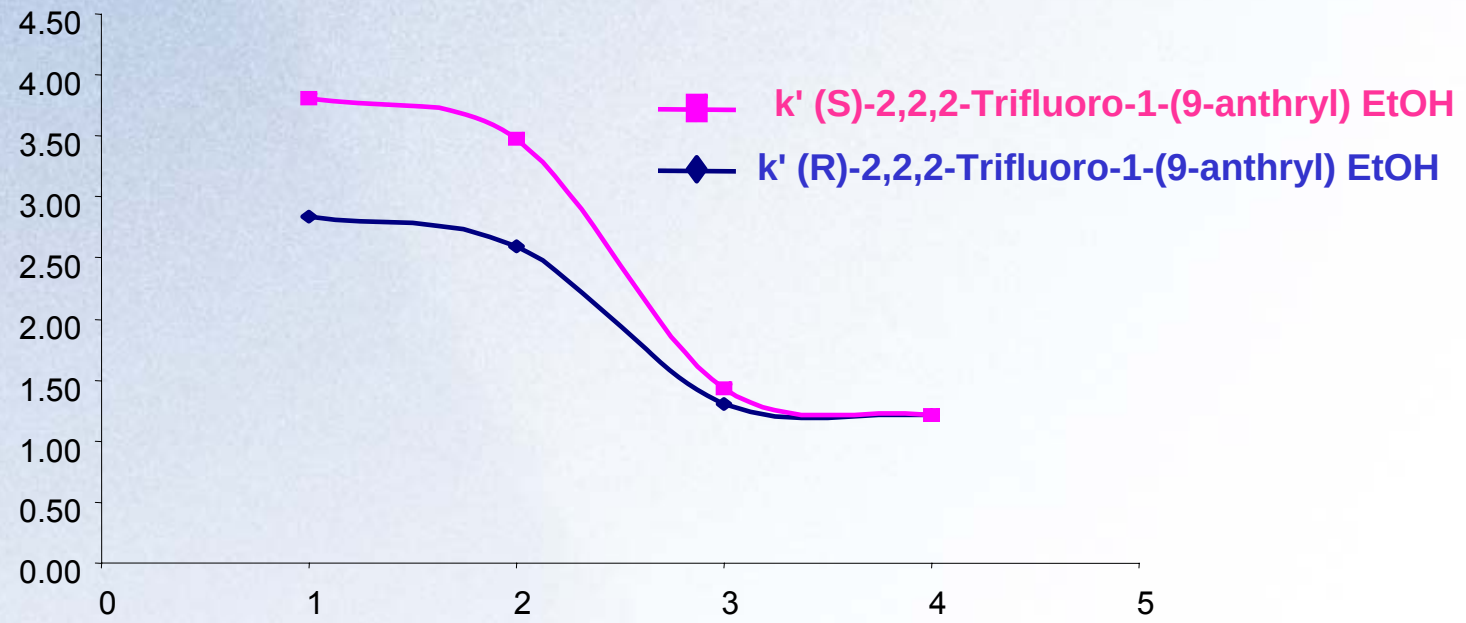
CS (Chiral Selector)

3) Chromatographic Test

ZrO_2-PDA-CS

4) CSP Column Stripping (1M NaOH for 2 hrs at 60°C, water and 1M HNO_3 finish)

Changes During (S)-PG Stripping



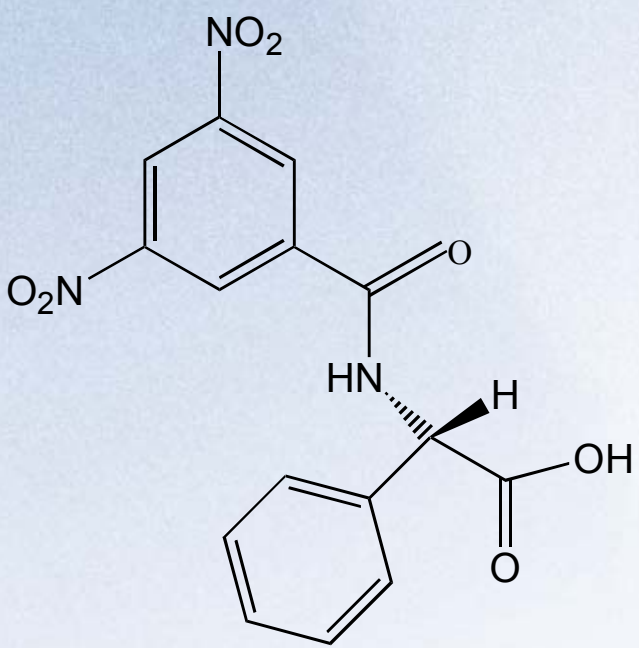
1- Original column.

2- Column flushed with 15/85 ACN/pH 12 NH_4OH for 10 column volumes, then 10 column volumes of water, 10 column volumes of 1.0 M nitric acid, and 10 column volumes of water.

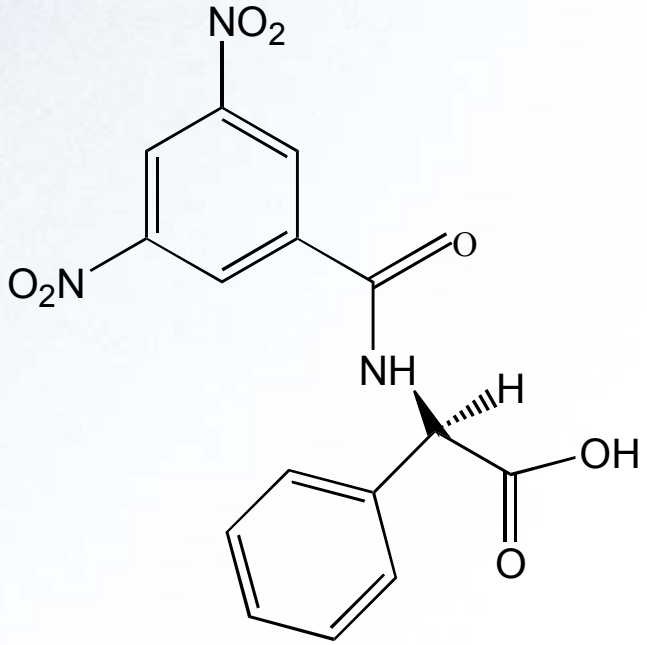
3- Column then flushed with 50 column volumes of 20/80 ACN/ 1 M NaOH, then 10 column volumes of water, 10 column volumes of 1 M nitric acid and 10 column volumes of water.

4- Column then flushed with 20/80 ACN/ 1 M NaOH for 50 column volumes at 60 ^oC, then flushed with 10 column volumes of water, 10 column volumes of 1 M nitric acid, and 10 column volumes of water.

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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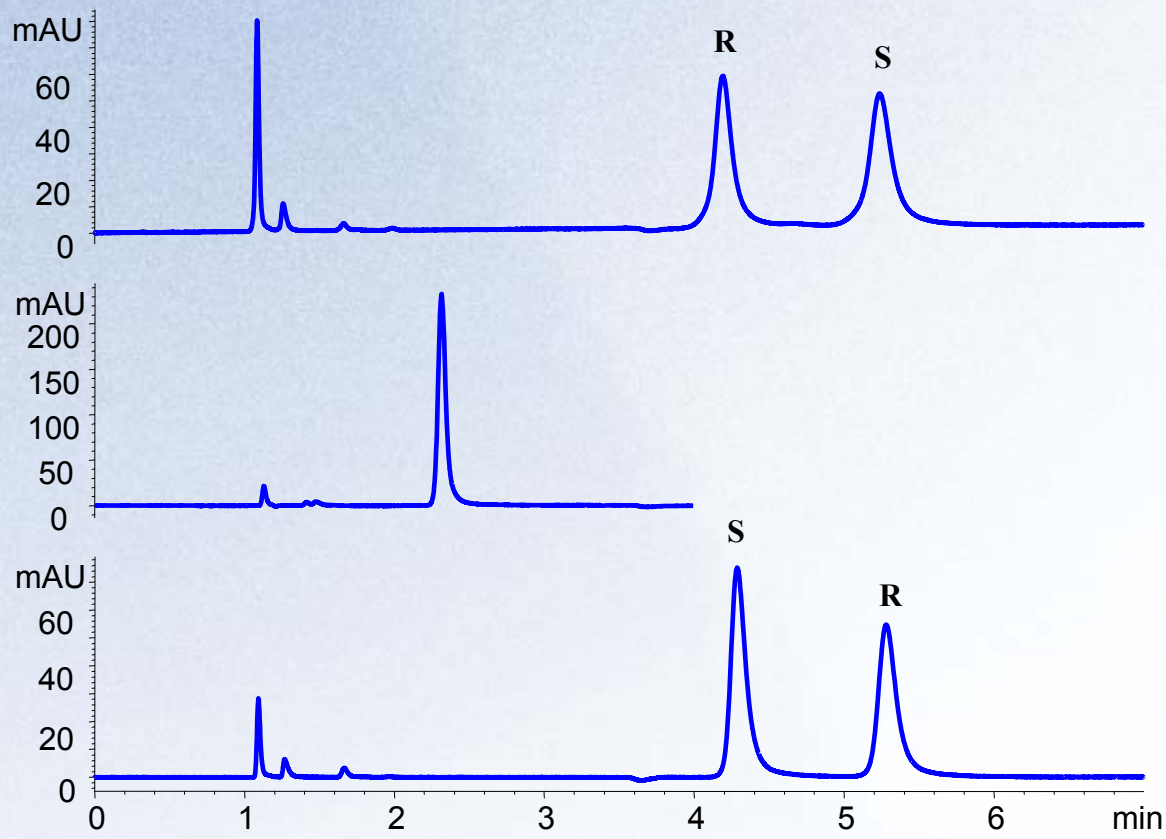

2-Step Load (S)-PG CS
k'(less) = 2.84
k'(more) = 3.81
α = 1.34

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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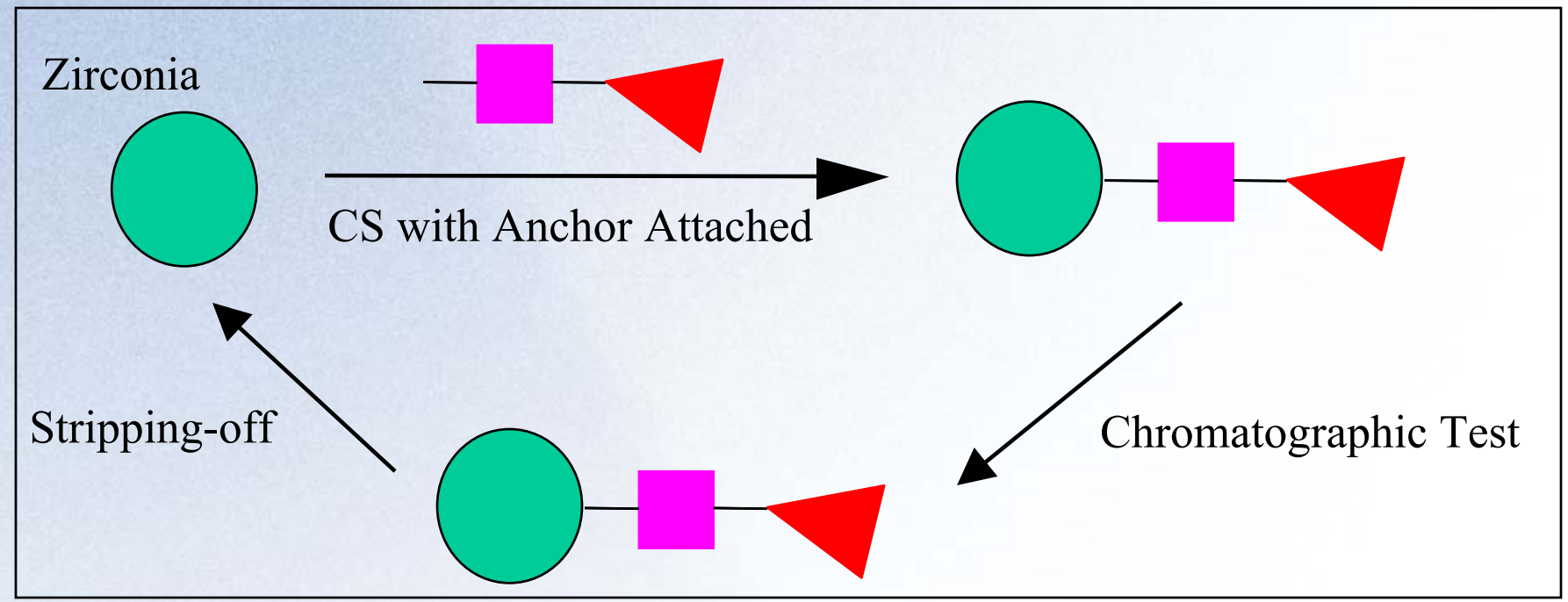
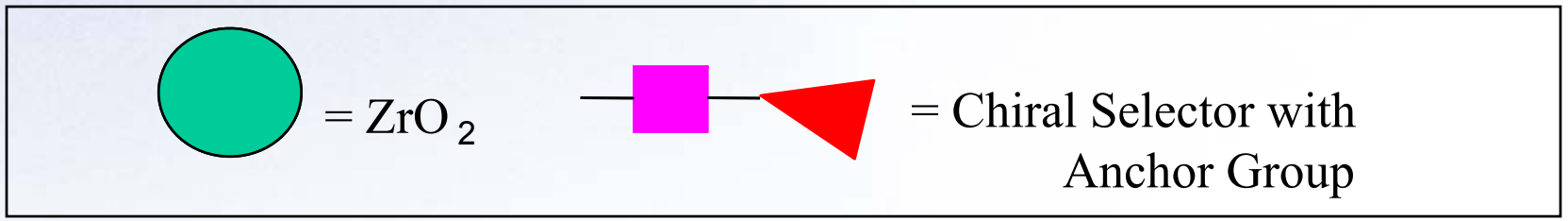
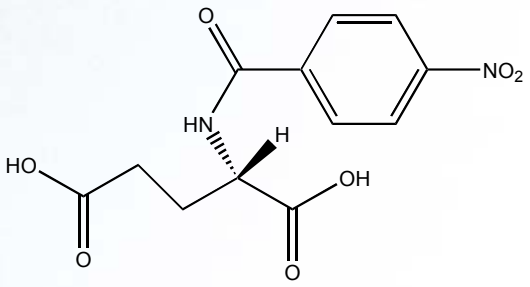
1-Step Synthesis of Zirconia CSPs for Fast Chiral Screening





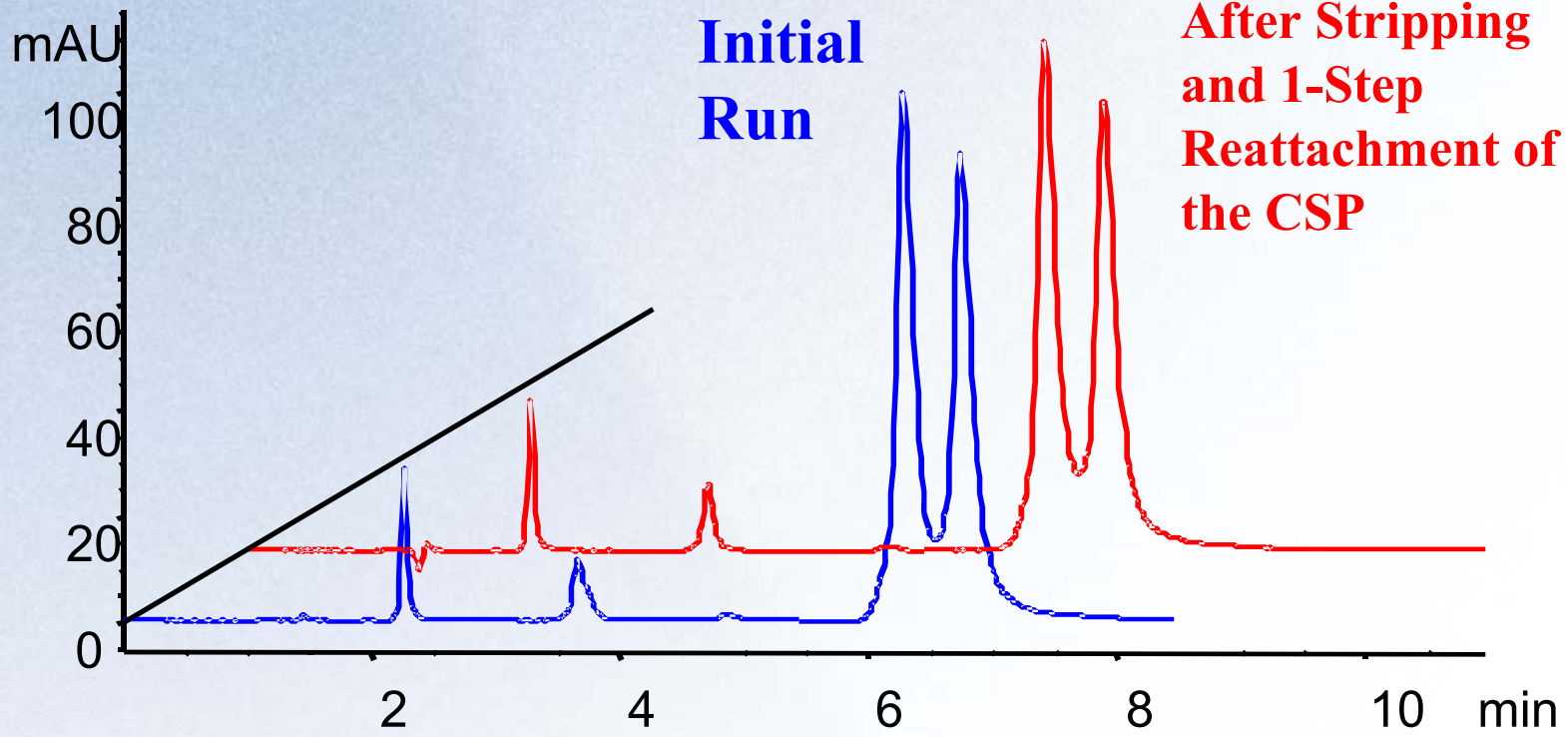
Example 1-Step Attachment and Detachment Cycle

- 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 .
- Flush column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
- Separate a racemic 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



N-(4-nitrobenzoyl)-L-glutamic acid

Glutamic Acid Proof of Concept



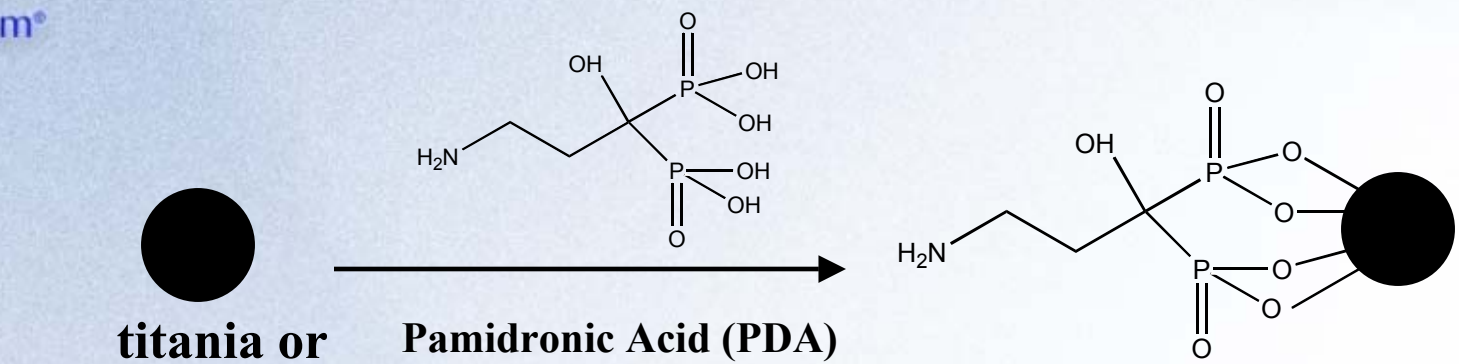
Comparison between the initial and final separation of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol leucine ester during a single CSP screening cycle. Chromatographic conditions: mobile phase: 99/1 hexane/IPA; flow rate: 1 ml/min; temperature: 30 °C, solute concentration = 1mg/mL, 5 µL injection.

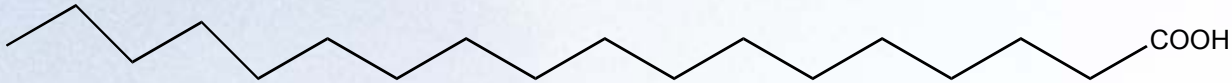
Conclusions

- Five new CSPs were attached to zirconia using Lewis acid-base chemistry:
  - ***π–acceptors***: Zr (S)-Leu, Zr (R)-PG, and Zr (S)-PG
  - ***π–donors***: Zr (R)-NESA, Zr (S)-NESA
- A novel cellulosic based CSP on zirconia was also successfully synthesized and compared to an analogous silica-base CSP.
- Zirconia CSPs are reproducible, stable and have comparable chromatographic performance to commercial silica-based CSPs for the chiral compounds tested.
- Mobile phase additives (ammonium acetate) had a positive effect on efficiency, retention, and selectivity under NP conditions for the Undecylphosphonic Acid Modified Cellulosic Zirconia Column.
- ***Attachment of different chiral selectors has been demonstrated on zirconia using a single column and multiple chiral selectors. Attachment has been demonstrated by both 2-step and 1-step methods.***

Acknowledgement: ***National Institutes of Health Grant*** (Phase II SBIR) 2R44HL070334-02A2.

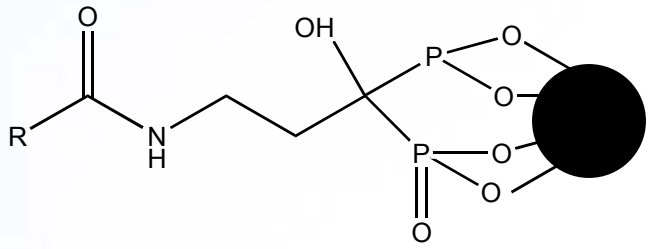
Zr-C18 Synthesis



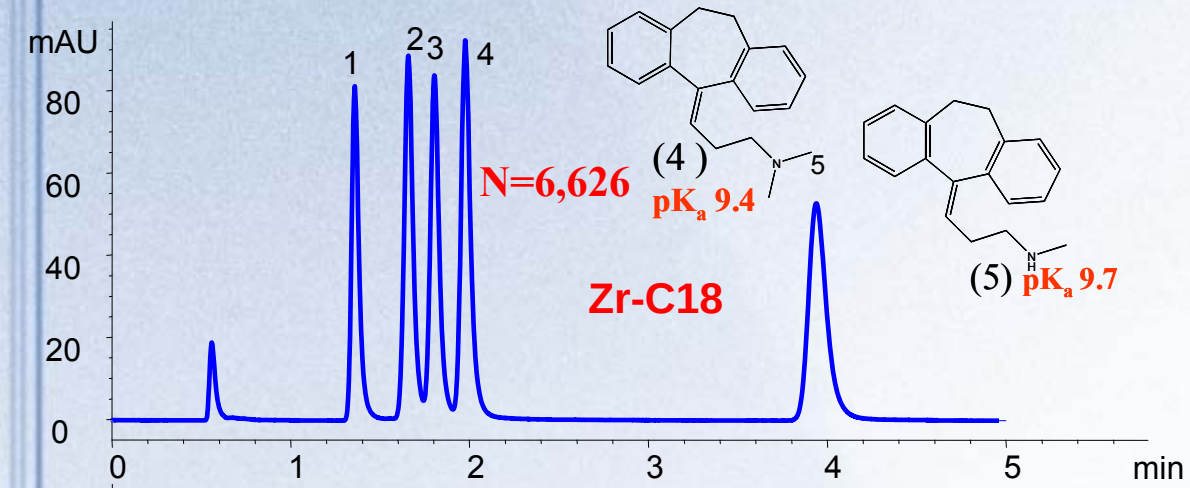


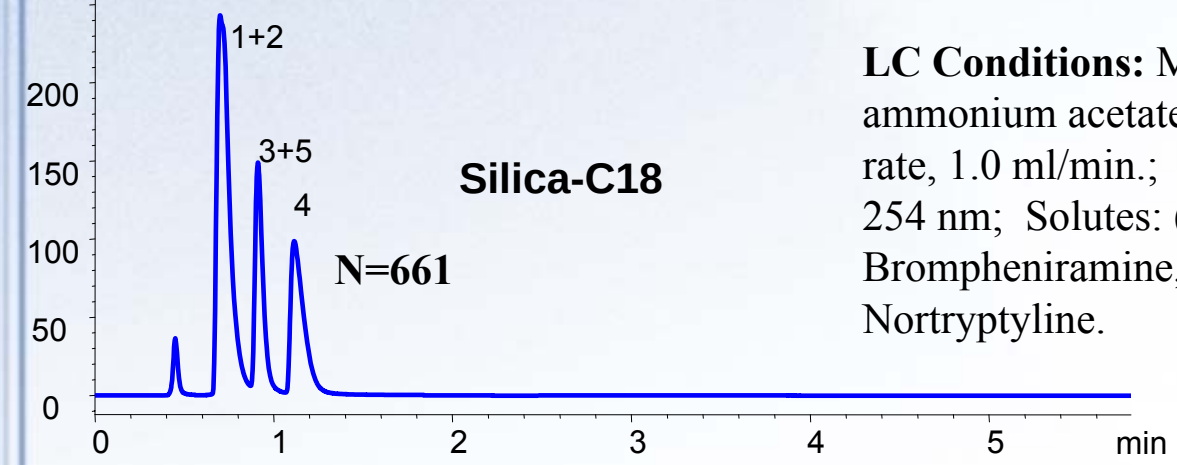
EEDQ

$R = -(CH_2)_{16}CH_3$



Separation Comparison of Basic Pharmaceuticals on Zr-C18 and Silica C18





Compounds elute according to IEX, not RP interactions at near neutral pHs.

LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume, 5 ul; 35 ºC; UV @ 254 nm; Solutes: (1) Methapyrilene, (2) Brompheniramine, (3) Doxepin, (4) Amitriptyline, (5) Nortryptyline.