Synthesis of Stable Brush-type and Cellulose-type Zirconia-based Chiral Stationary Phases for Enantiomer Separations

Eastern Analytical Symposium 2007

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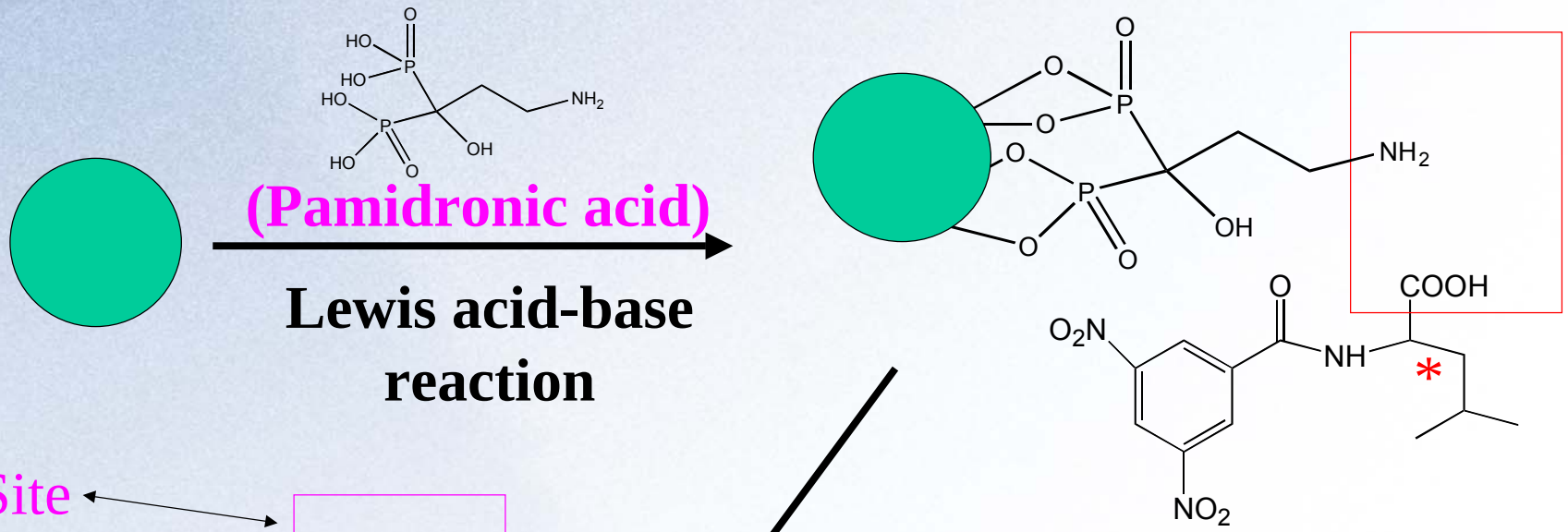
Specialists in High Efficiency, Ultra-Stable Phases for HPLC

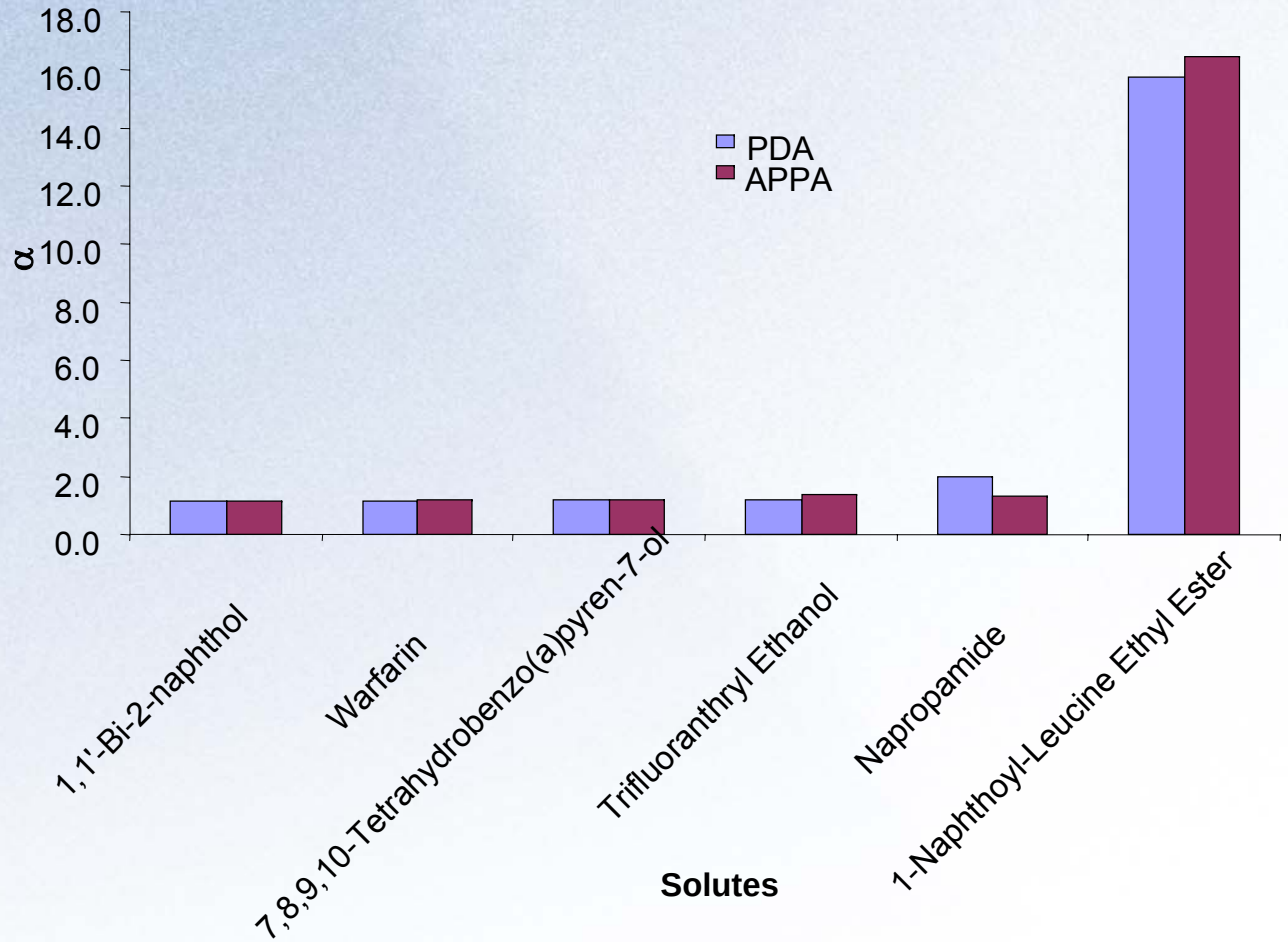
Goal-To Make Zirconia Based Chiral Stationary Phases for Fast Chiral Selector Screening

- Why Zirconia?
- Synthetic Approach
  - Building a zirconia-based CSP
  - Proof of concept
- Chiral Separations on Zirconia Based CSPs
- Stability Study
- Column Regeneration
- 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.

Example of Lewis Acid-Base Modified Zirconia CSPs

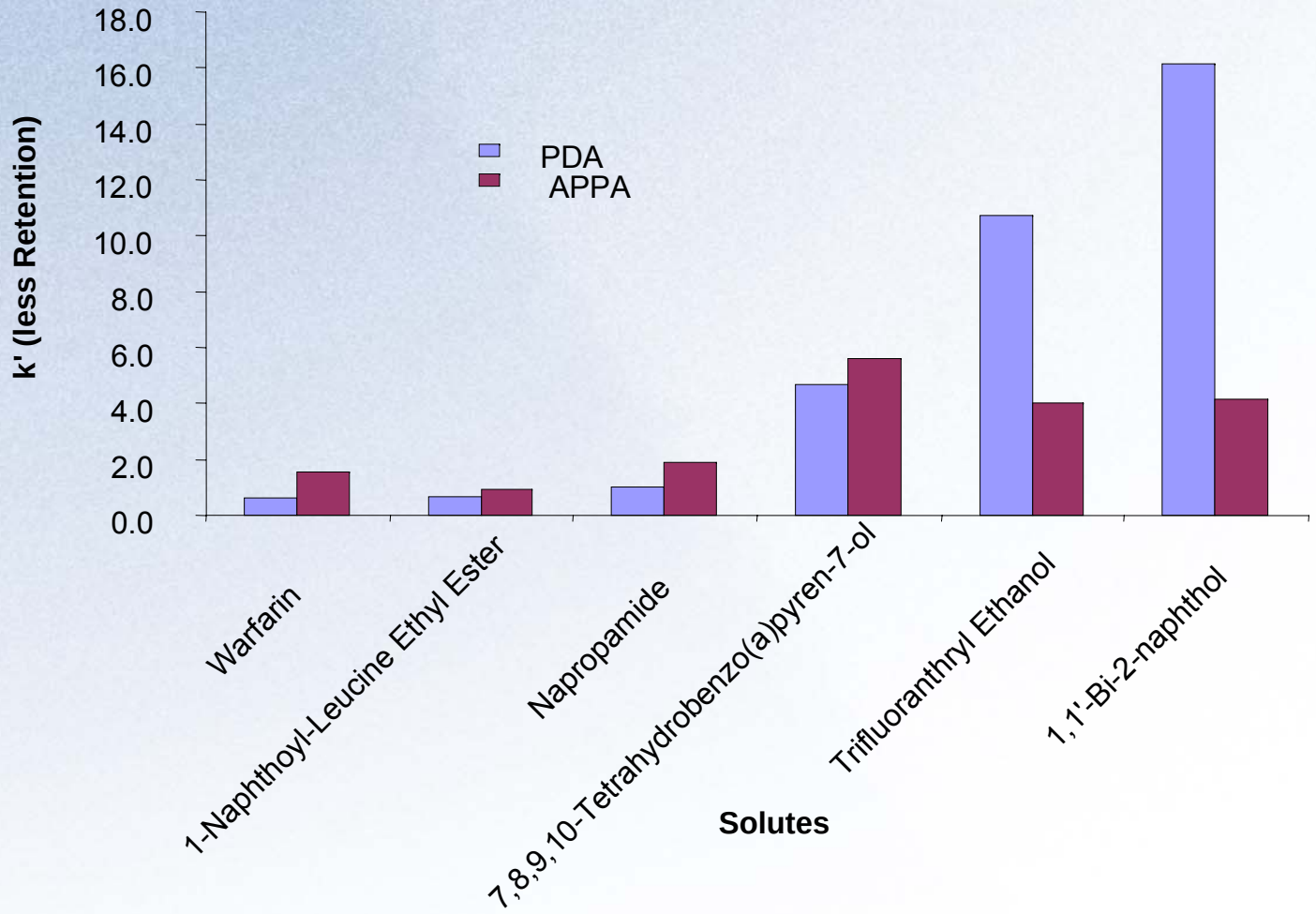


Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



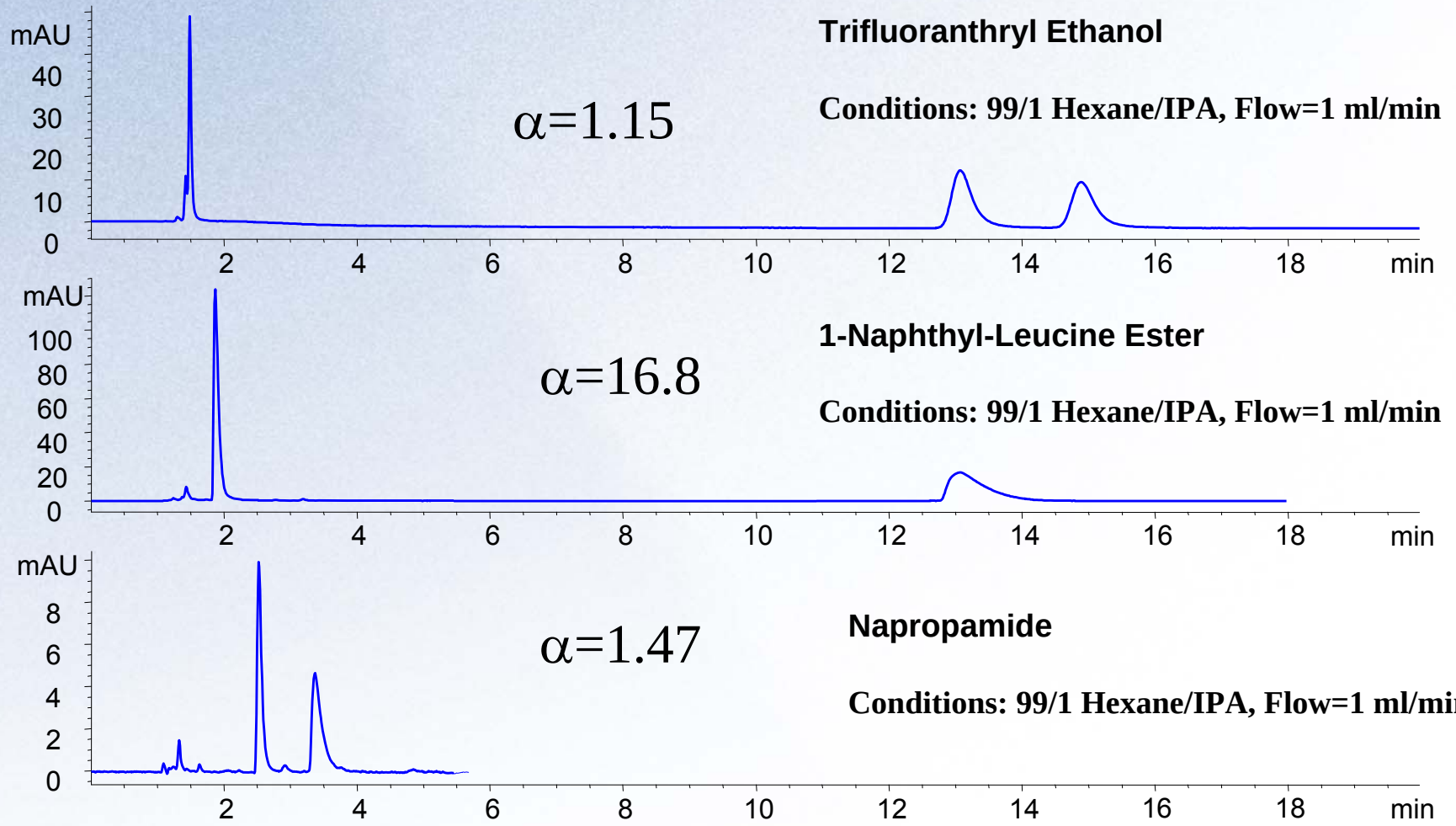
Selectivity for both anchors is very similar.

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

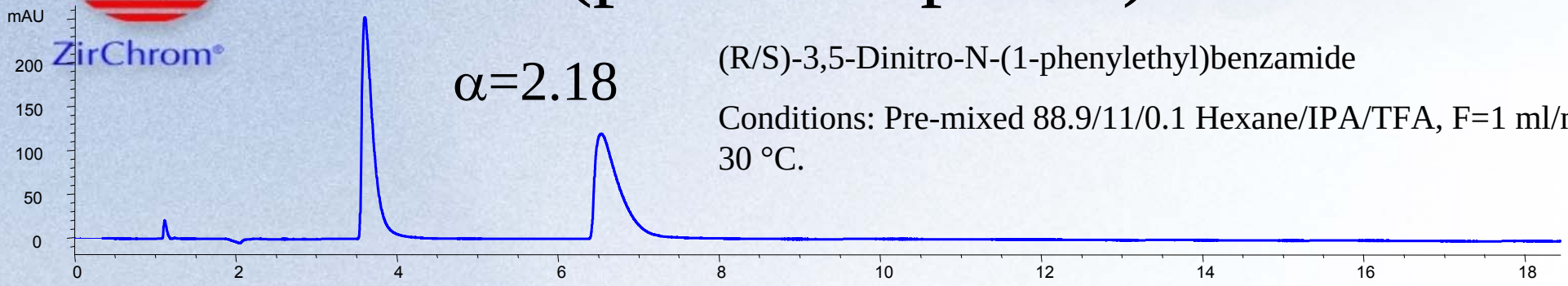


Retention for both anchors is different.

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



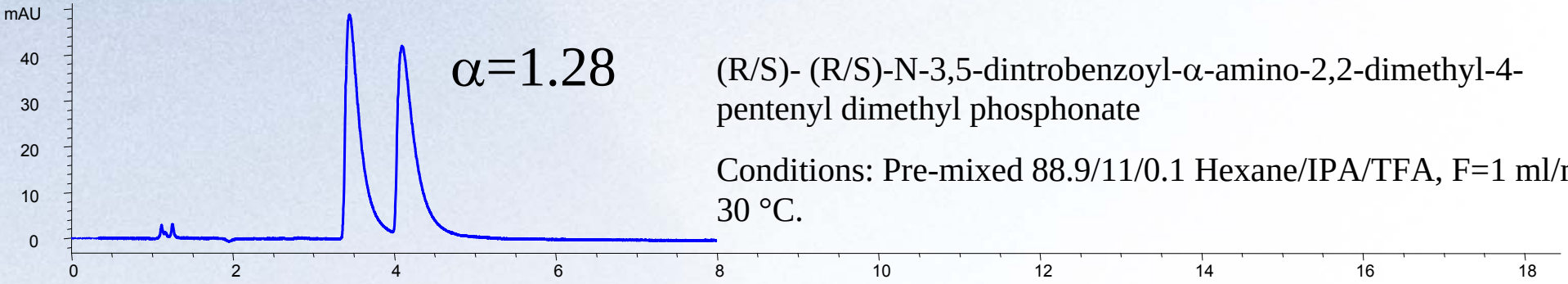
Chiral Separations on Zr (S)-NESA (pi-donor phase)



α=2.18

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

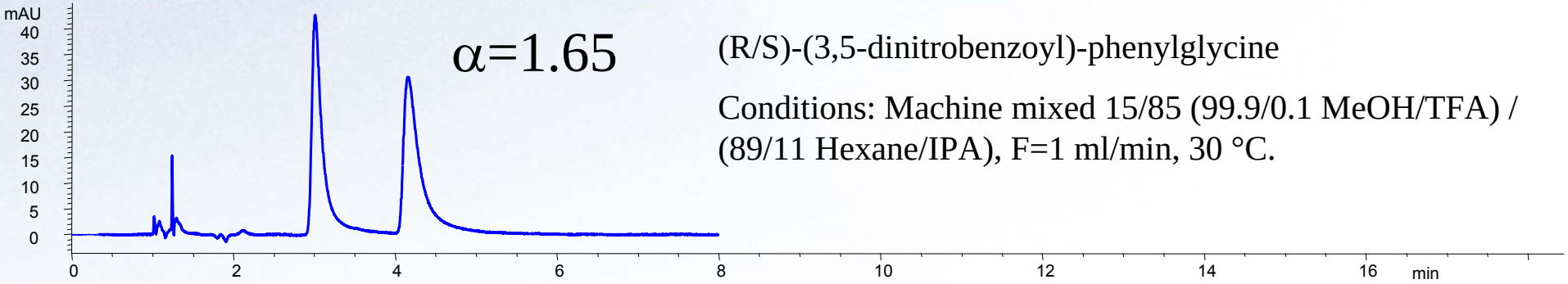
Conditions: Pre-mixed 88.9/11/0.1 Hexane/IPA/TFA, F=1 ml/min, 30 °C.



α=1.28

(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.

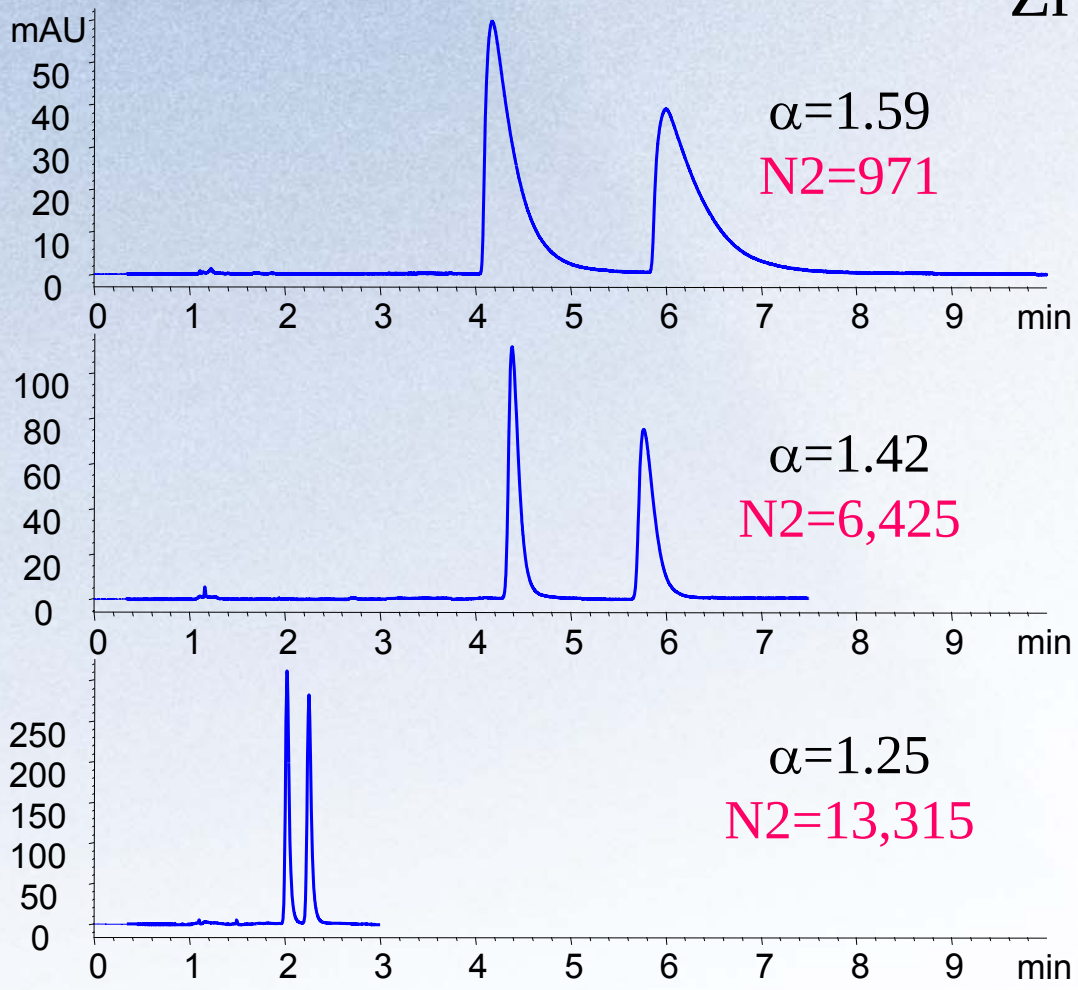


α=1.65

(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



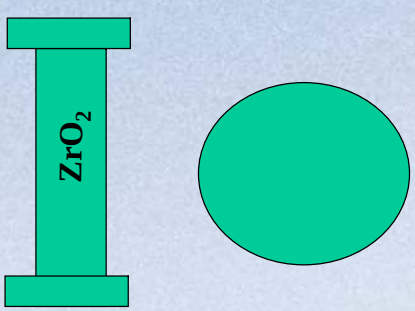
Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.

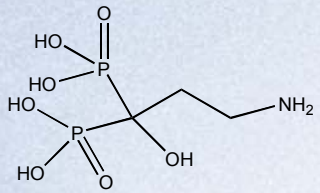
Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / **MeOH** / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

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

2-Step Online Zirconia CSP Synthesis for Chiral Screening

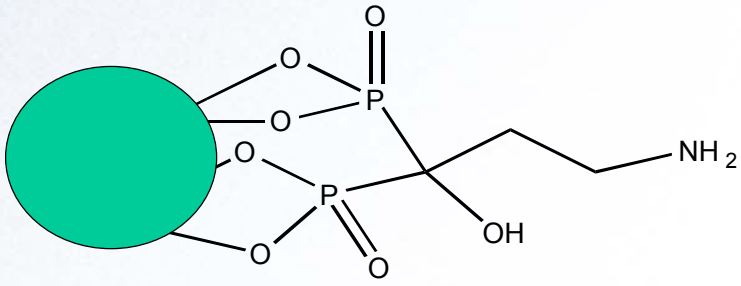
ZirChrom®



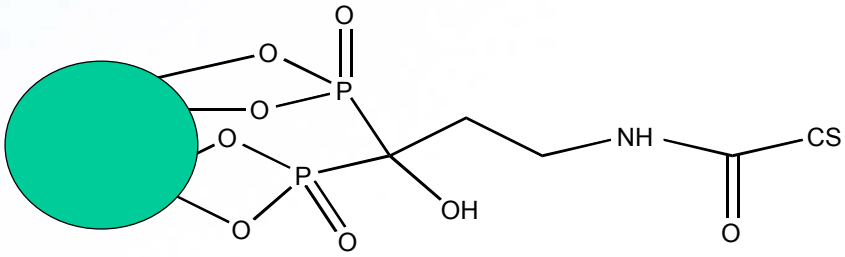


(Pamidronic acid)

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

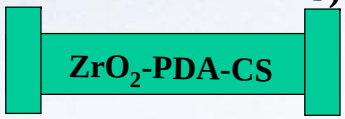


2) EEDQ Coupling Reaction (CS reagent in THF overnight (16 hrs) at 30°C)



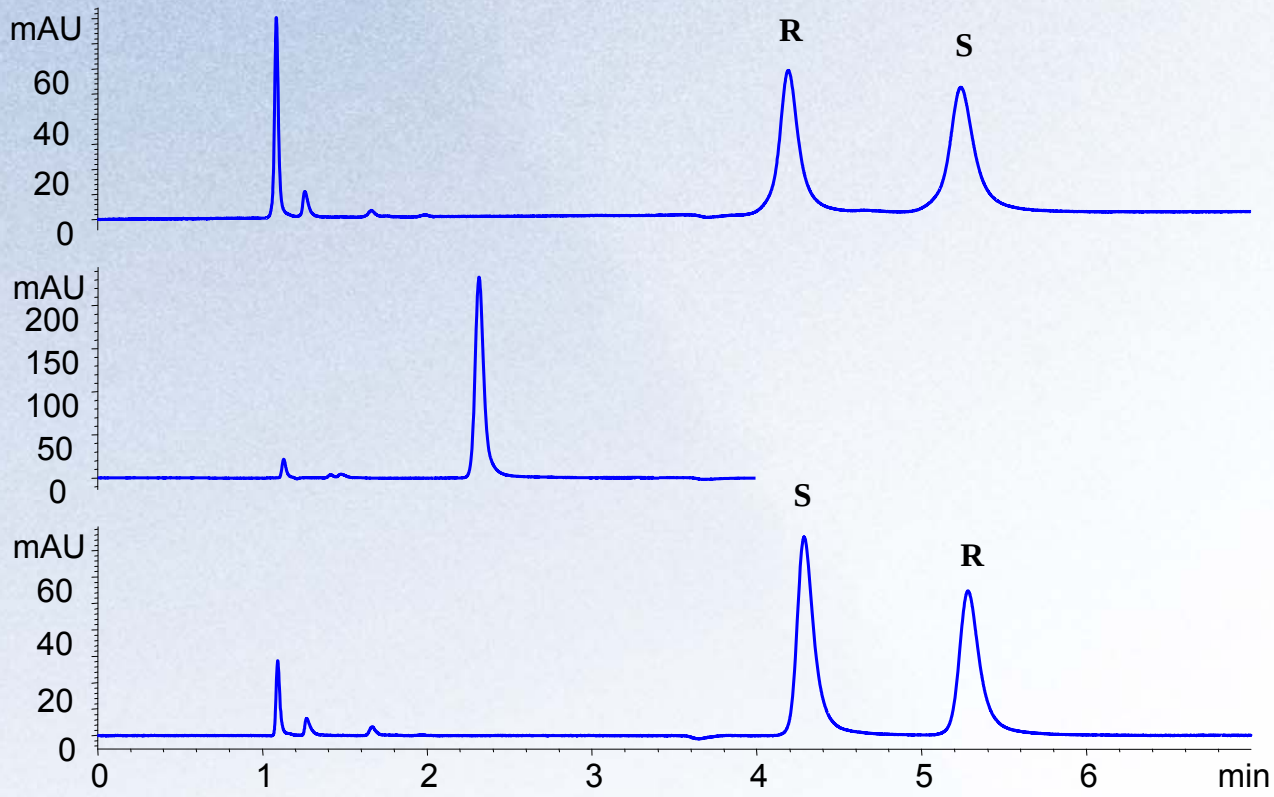
CS (Chiral Selector)

3) Chromatographic Test



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

Changing (S) to (R)-Phenylglycine CSP on Same Zr Column



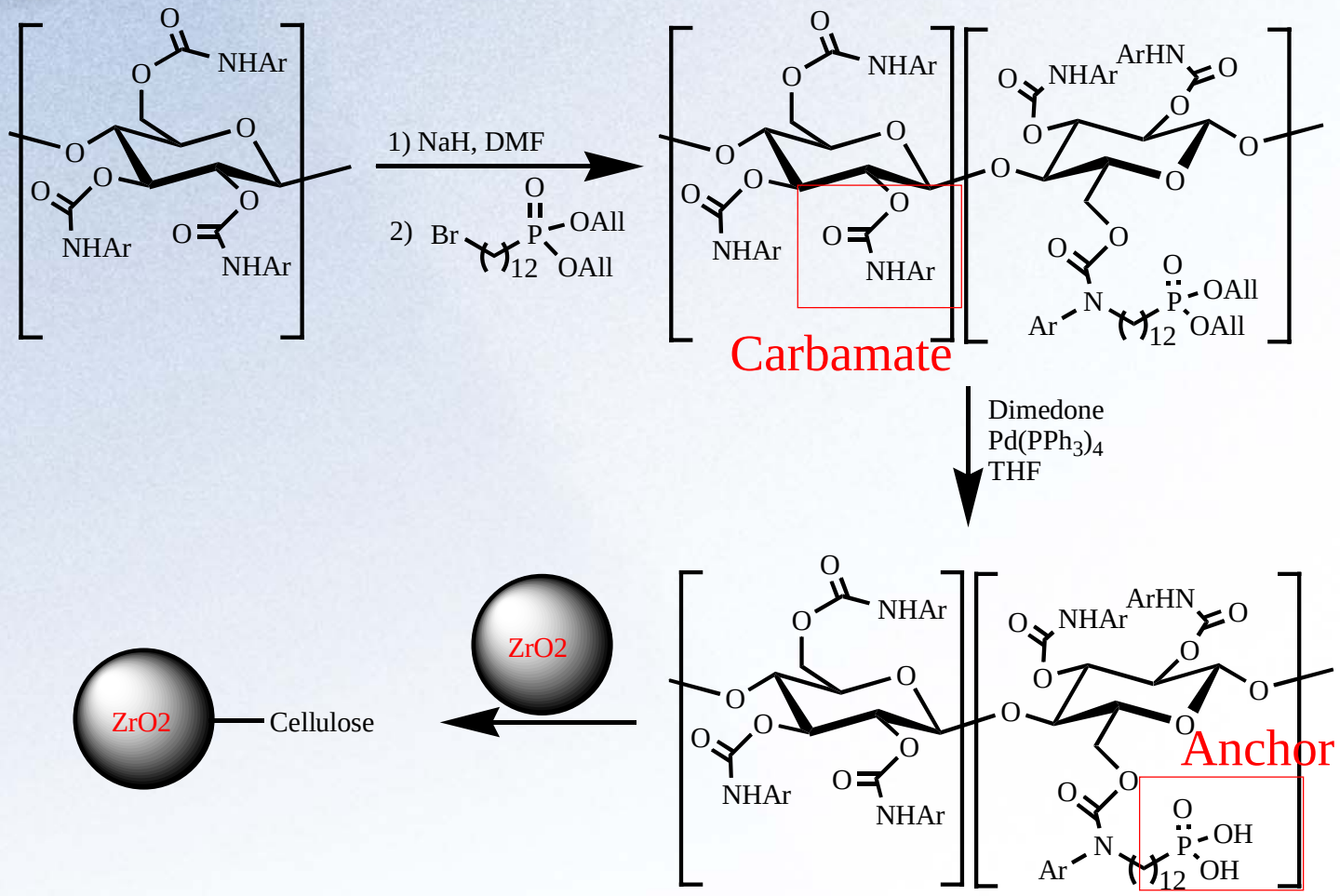
2-Step Load (S)-PG CS
k'(less) = 2.84
k'(more) = 3.81
α = 1.34

Strip (S)-PG CS
No separation.

2-Step Load (R)-PG CS
k'(less) = 2.92
k'(more) = 3.83
α = 1.34

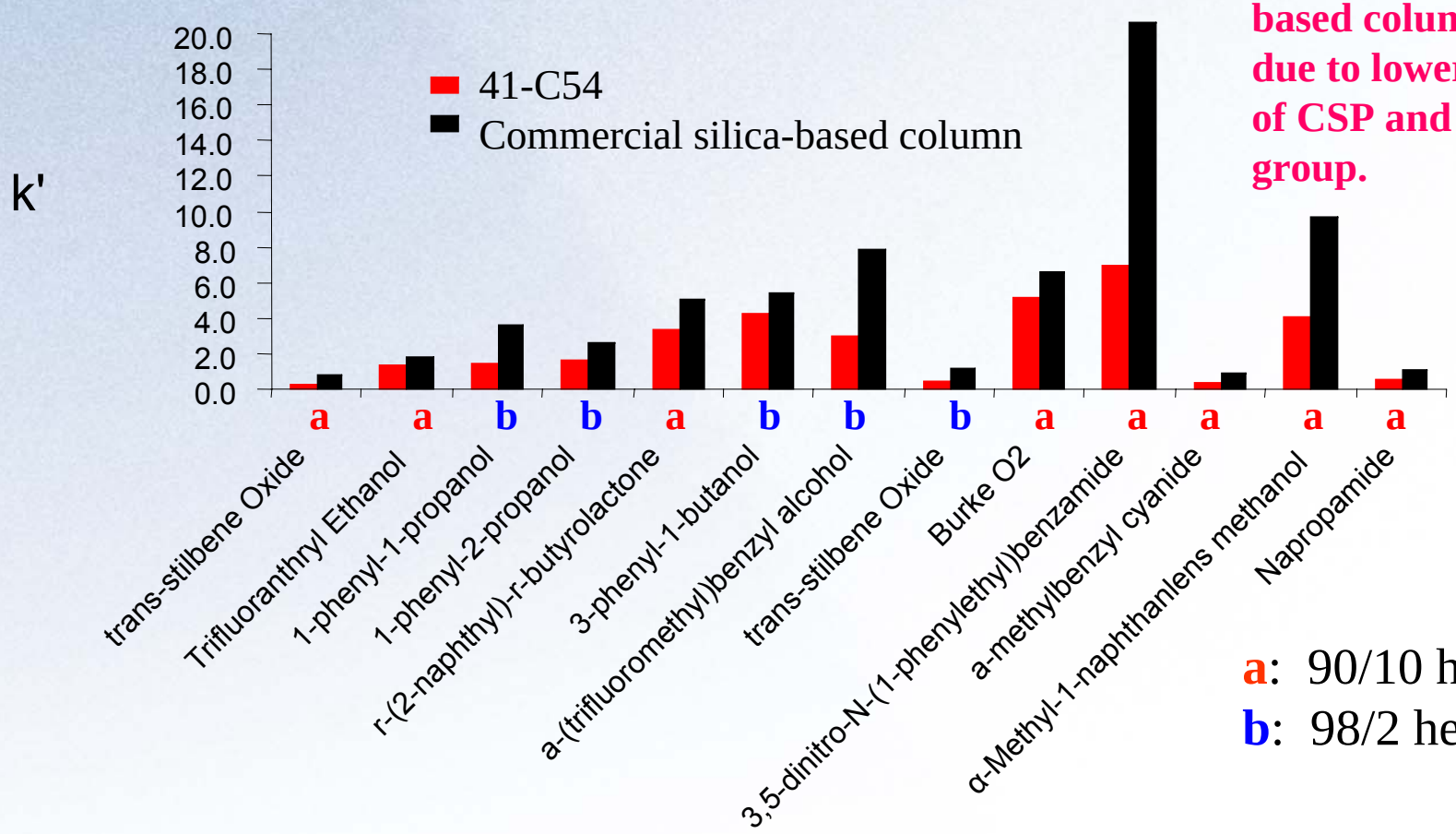
Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, F=1 ml/min, 30 °C, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D and ZirChrom PDA-(R)-PG, S/N RPG020806A (100 × 4.6 mm, 3 µm, Running HPLC coated on PHASE110805A, batch#: 52-132). Solute: 1,3,5-Tri-t-butyl-benzene, (R orS)-2,2,2-Trifluoro-1-(9-anthryl) EtOH (5 µl injection)

Phosphonate Modified Cellulose Based CSP on Zirconia



Retention Comparison Between Alkylphenyl Modified Cellulosic CSPs and Commercial Silica CSPs

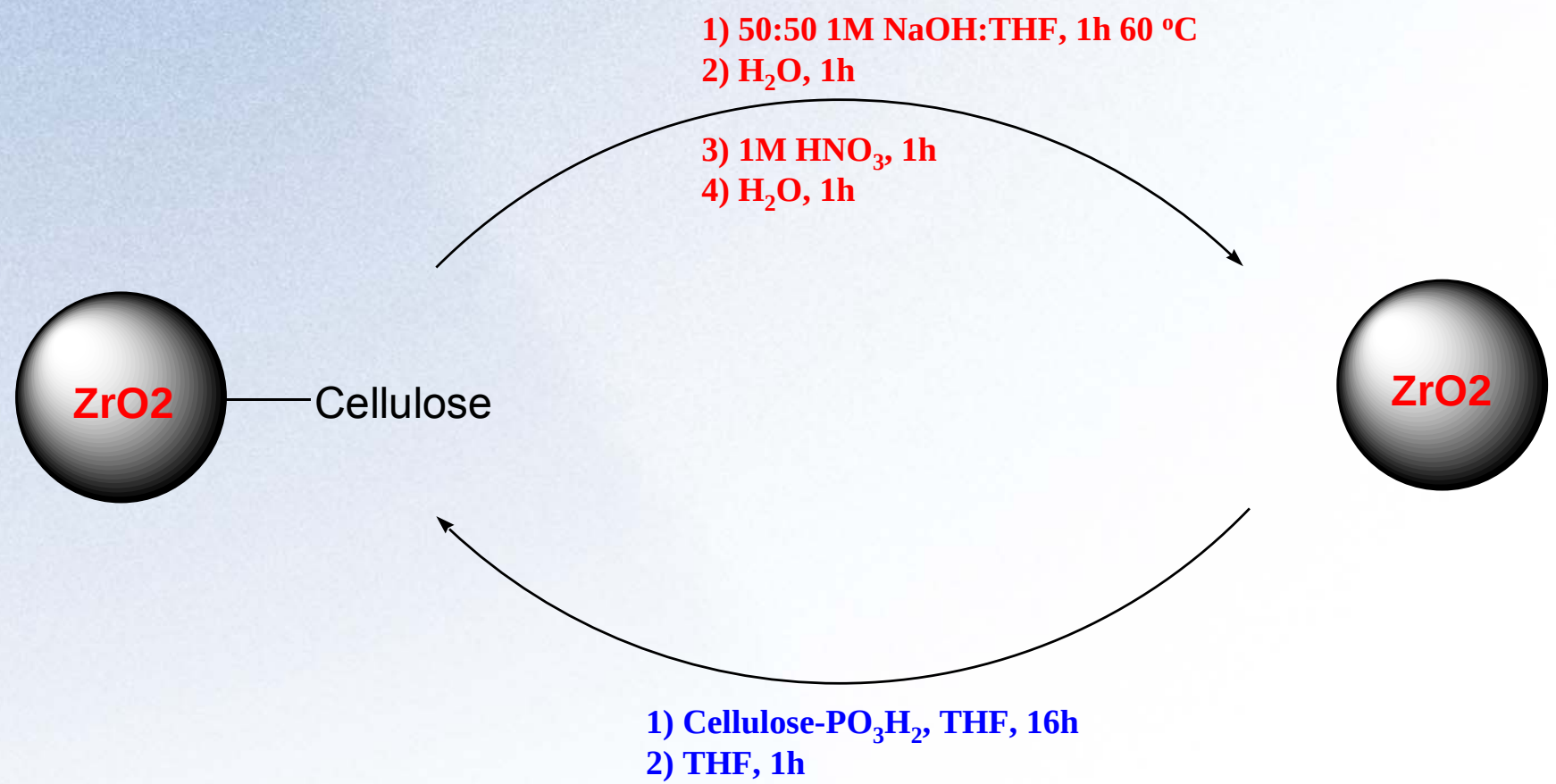
41-C54, J04-175, 3,5-dimethylphenyl, -$C_{11}H_{22}PO_3H$
Commercial Silica CSP column



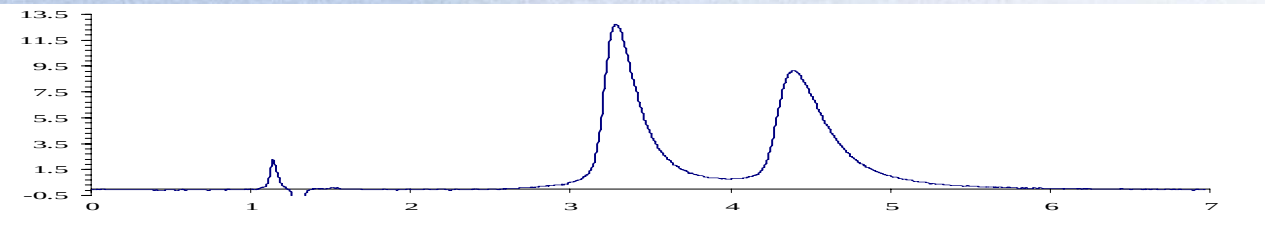
New phase has less retention than commercial Silica-based column likely due to lower loading of CSP and anchor group.

a: 90/10 hexane/IPA
b: 98/2 hexane/IPA

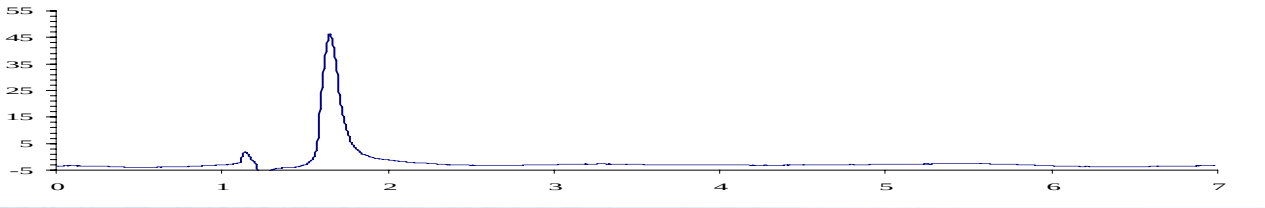
Cellulose Phase Regeneration



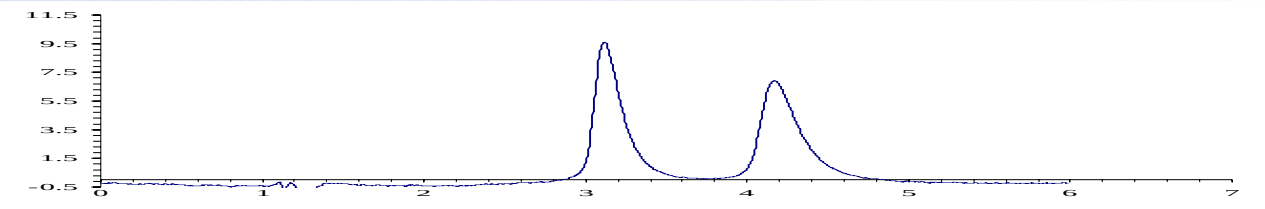
Cellulose Phase Regeneration



Original Cellulose
k'(less) = 1.73
k'(more) = 2.67
α = 1.54



Remove Cellulose
No separation.



Reload Cellulose
k'(less) = 1.59
k'(more) = 2.47
α = 1.55

Pre-mixed 90/10 Hexane/IPA, F=1 ml/min, rm °C, 254 nm, Column: ZirChrom-CelluloZe, S/N R020907W (100 × 4.6 mm, 5 µm, batch 67-C46). Solute: a-Burke, 10 µl injection.

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
- Polysaccharide based CSPs were attached to zirconia using a phosphonate anchor.
- Zirconia based CSPs can be regenerated online allowing for fast screening of chiral phases with only one column.
- Acknowledgement: ***National Institutes of Health Grant*** (Phase II SBIR) 2R44HL070334-02A2.

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