



Chiral Selector Screening and Regeneration of Novel Brush and Polysaccharide-Type Zirconia Chiral Stationary Phases

PITTCON 2008

Daniel Nowlan¹, Clayton V. McNeff¹, Bingwen Yan¹, Richard A. Henry¹

Yini Wang², Shengxiang Ji², Thomas R. Hoyer²

¹ZirChrom Separations, Inc. 617 Pierce St., Anoka, MN 55303,

²University of Minnesota, 207 Pleasant Street SE, Minneapolis, MN 55455.

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

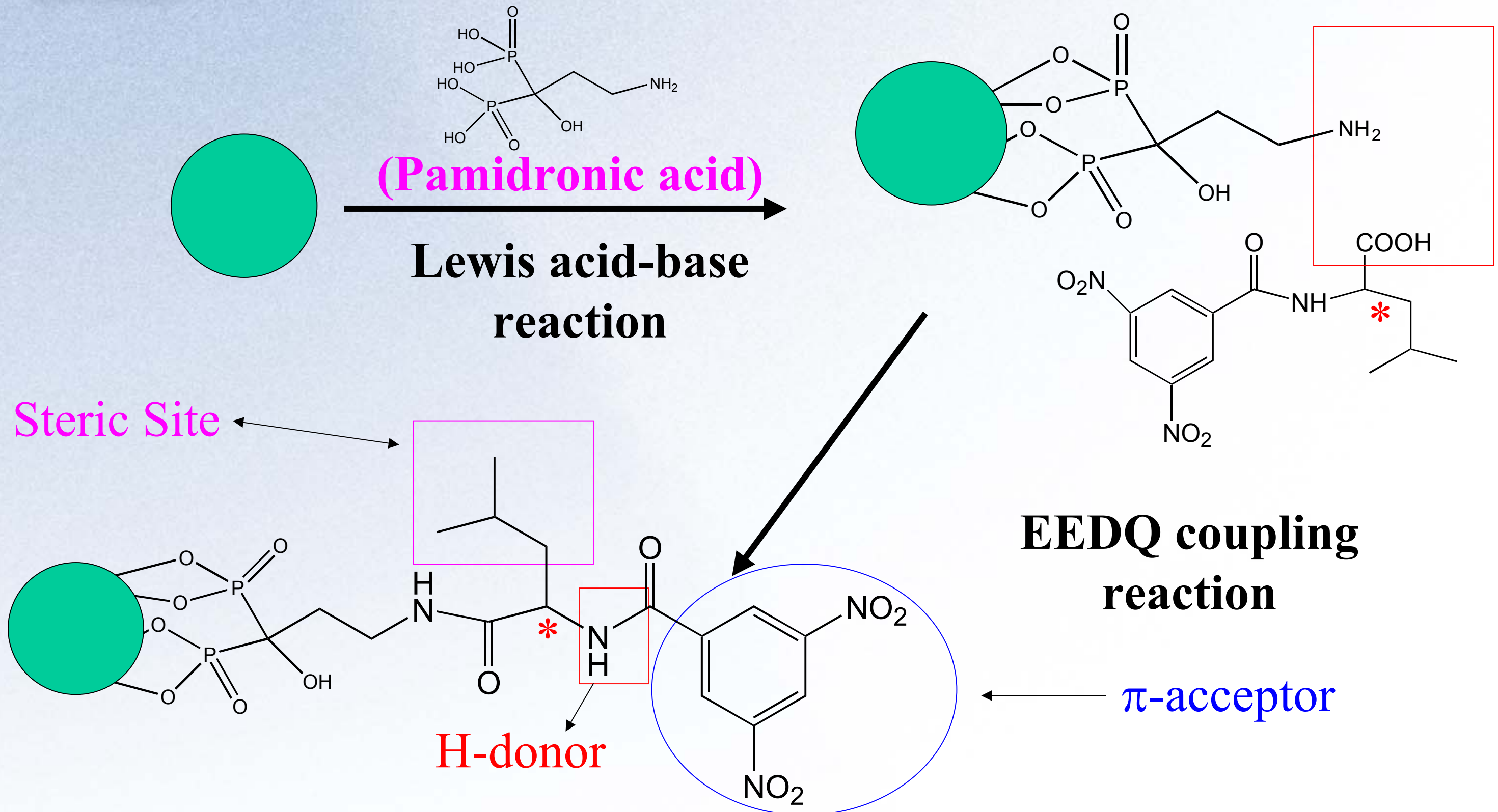


Goal-To Make Zirconia Based Chiral Stationary Phases for Fast Chiral Selector Screening

- Why Zirconia?
- 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.

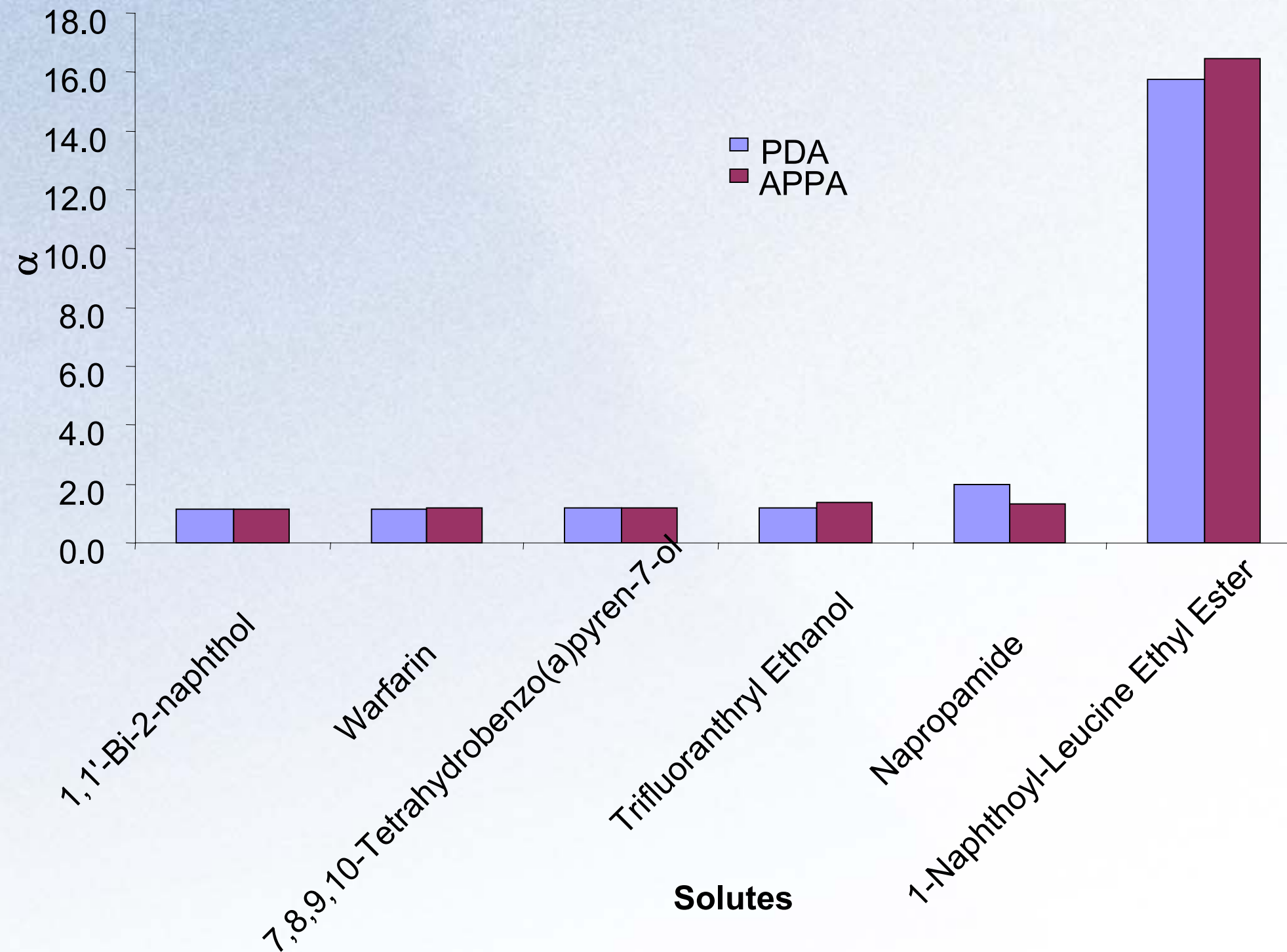


Example of Lewis Acid-Base Modified Zirconia CSPs





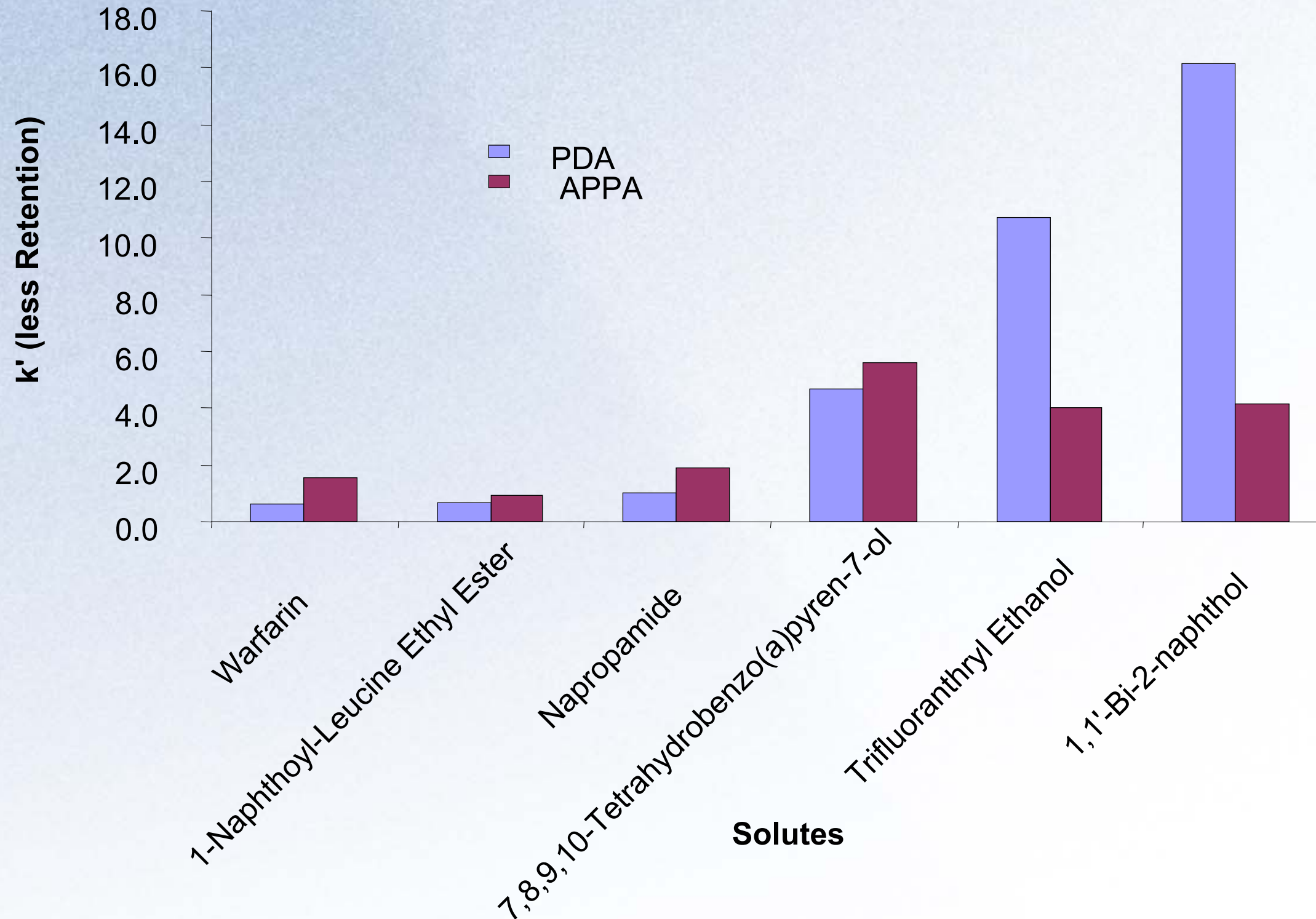
Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



Selectivity for both anchors is very similar.



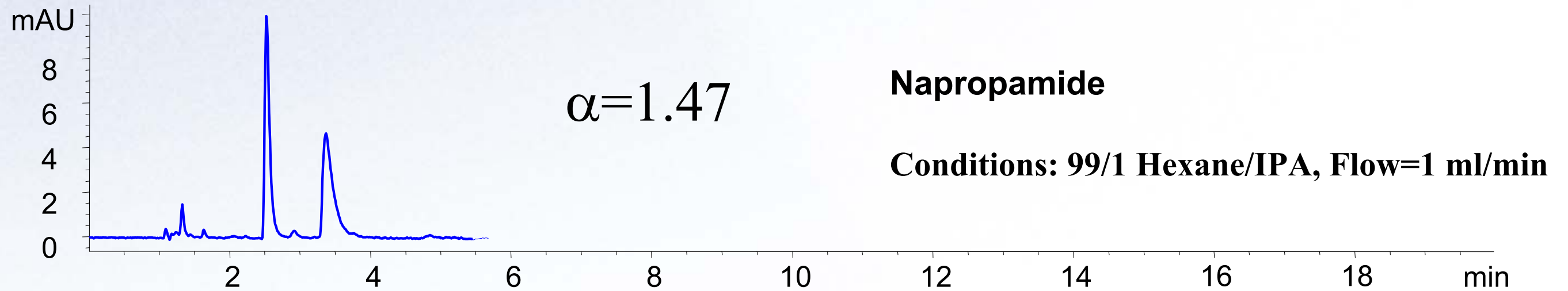
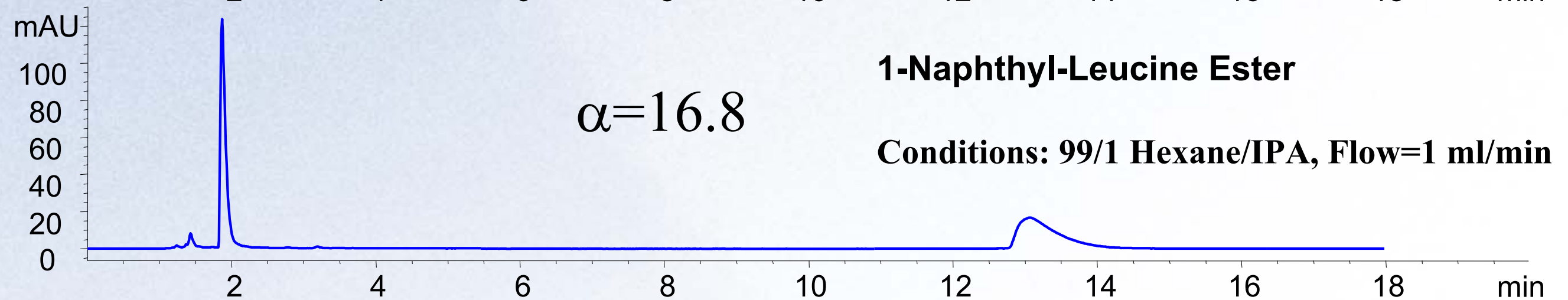
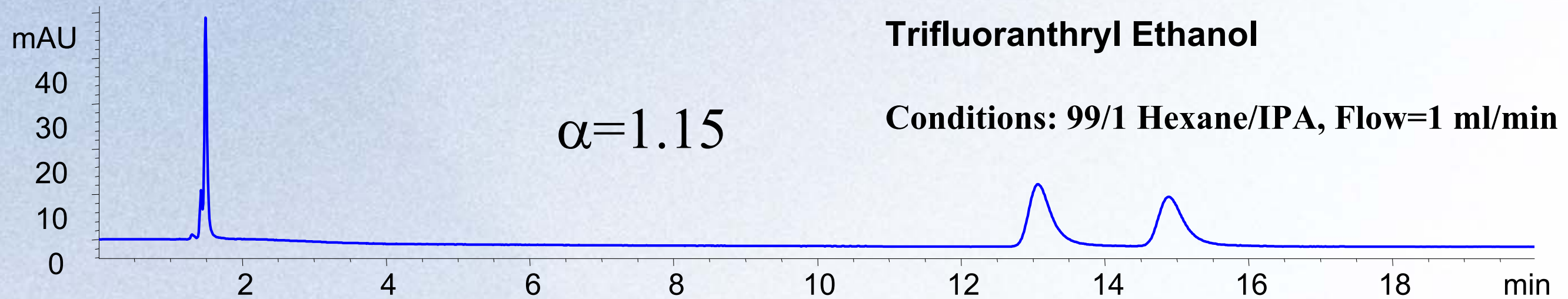
Retention Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



Retention for both anchors is different.



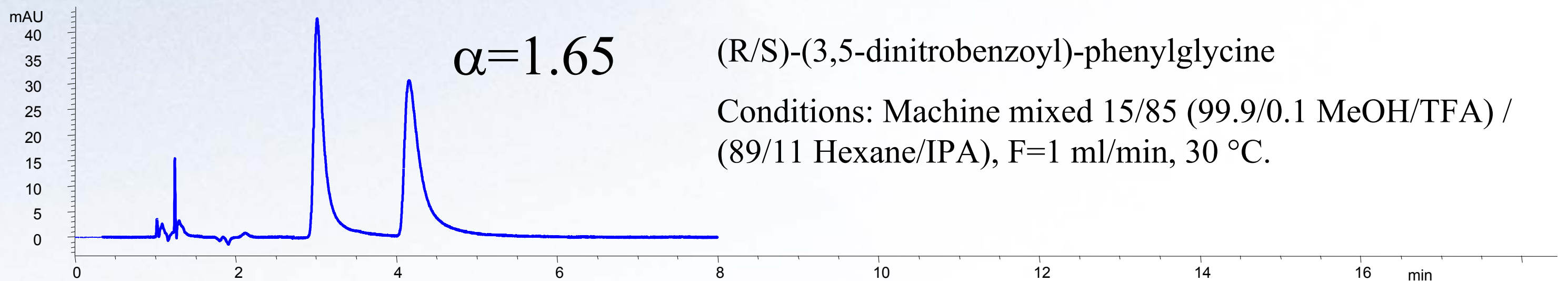
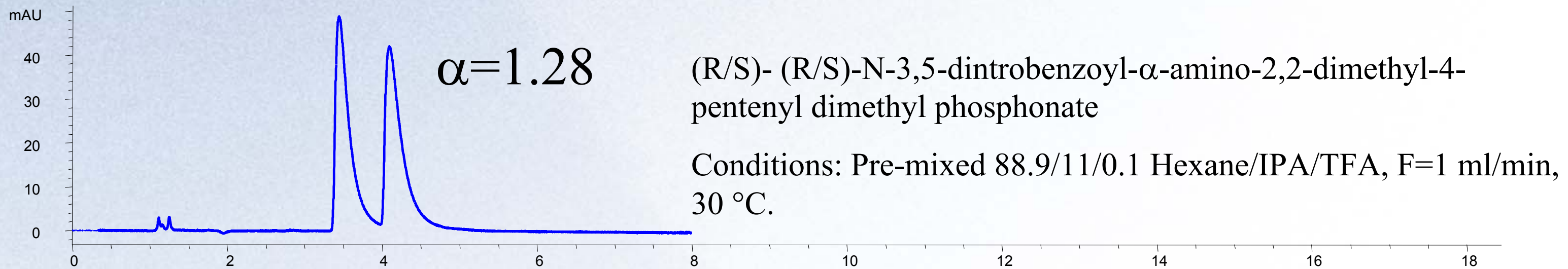
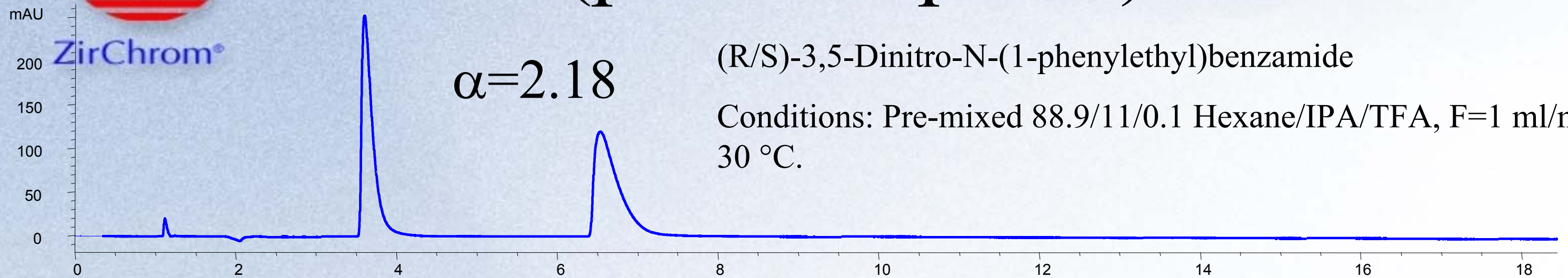
Chiral Separation on Zr (S)-Leu (pi-acceptor phase)





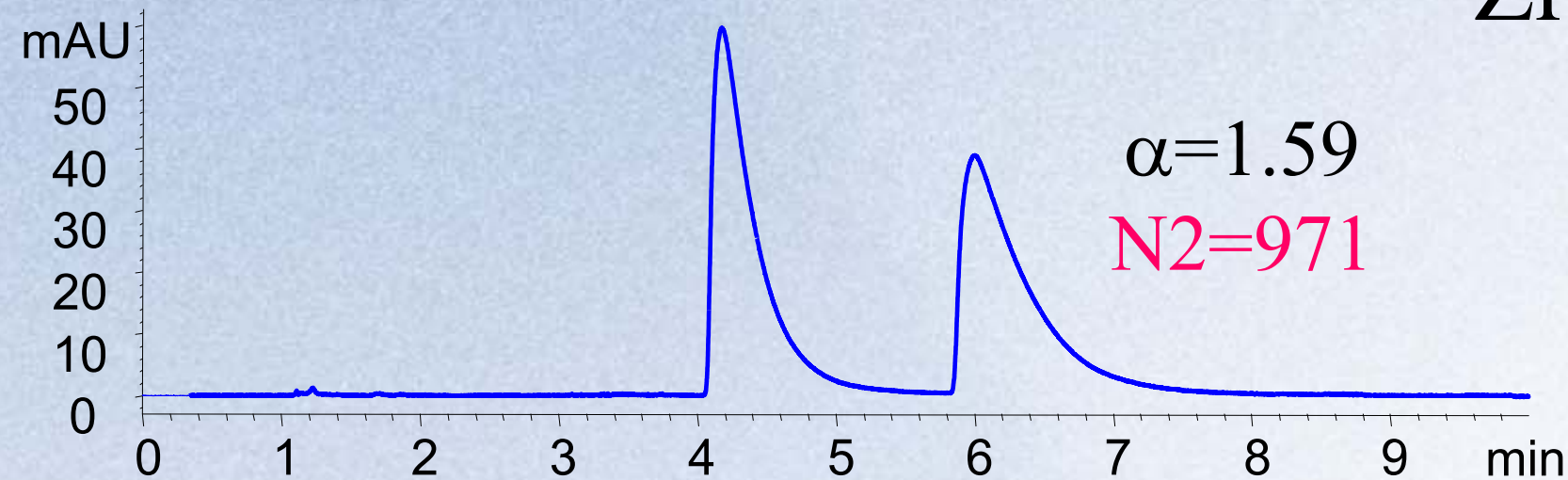
ZirChrom®

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

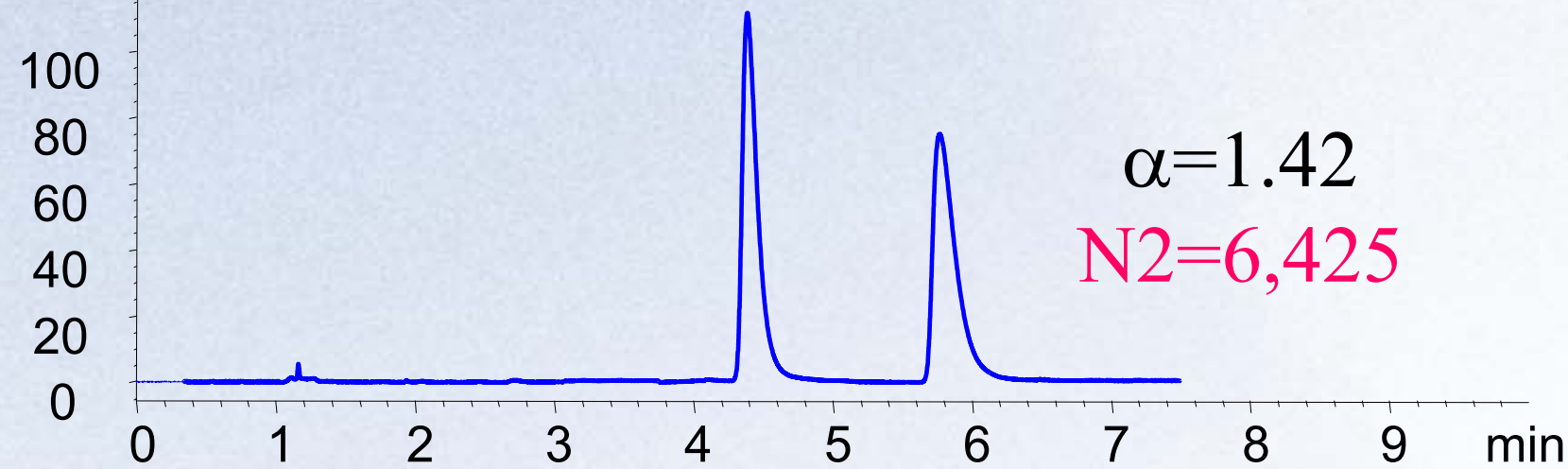




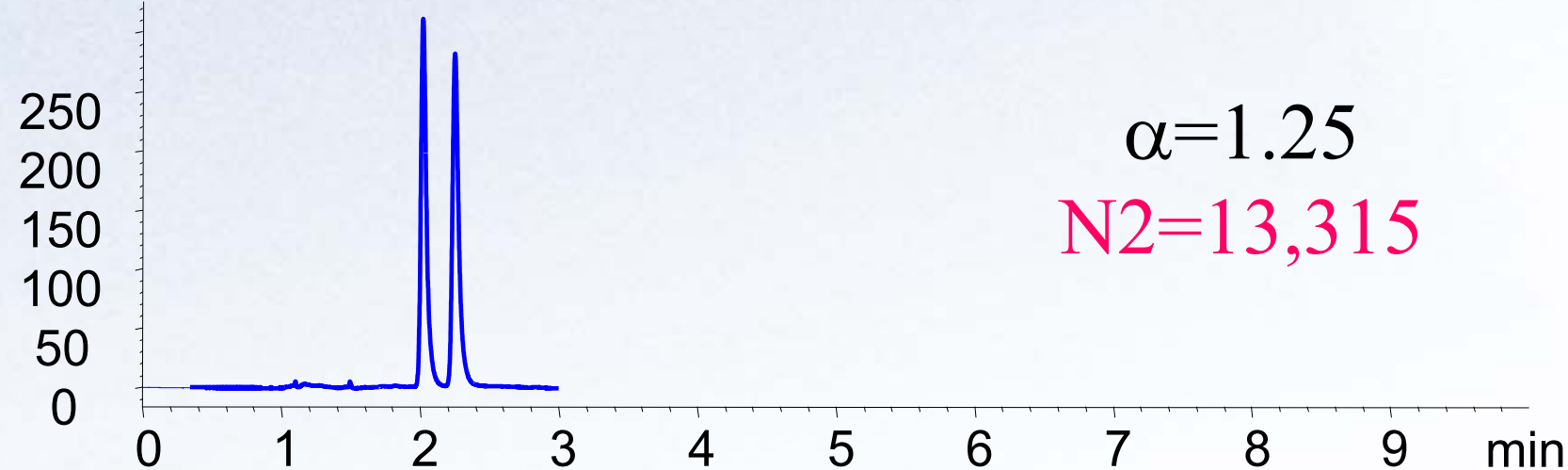
Mobile Phase Effect of adding MeOH on Separation of (R/S)-N-3,5-dinitrobenzoyl- α -amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA



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



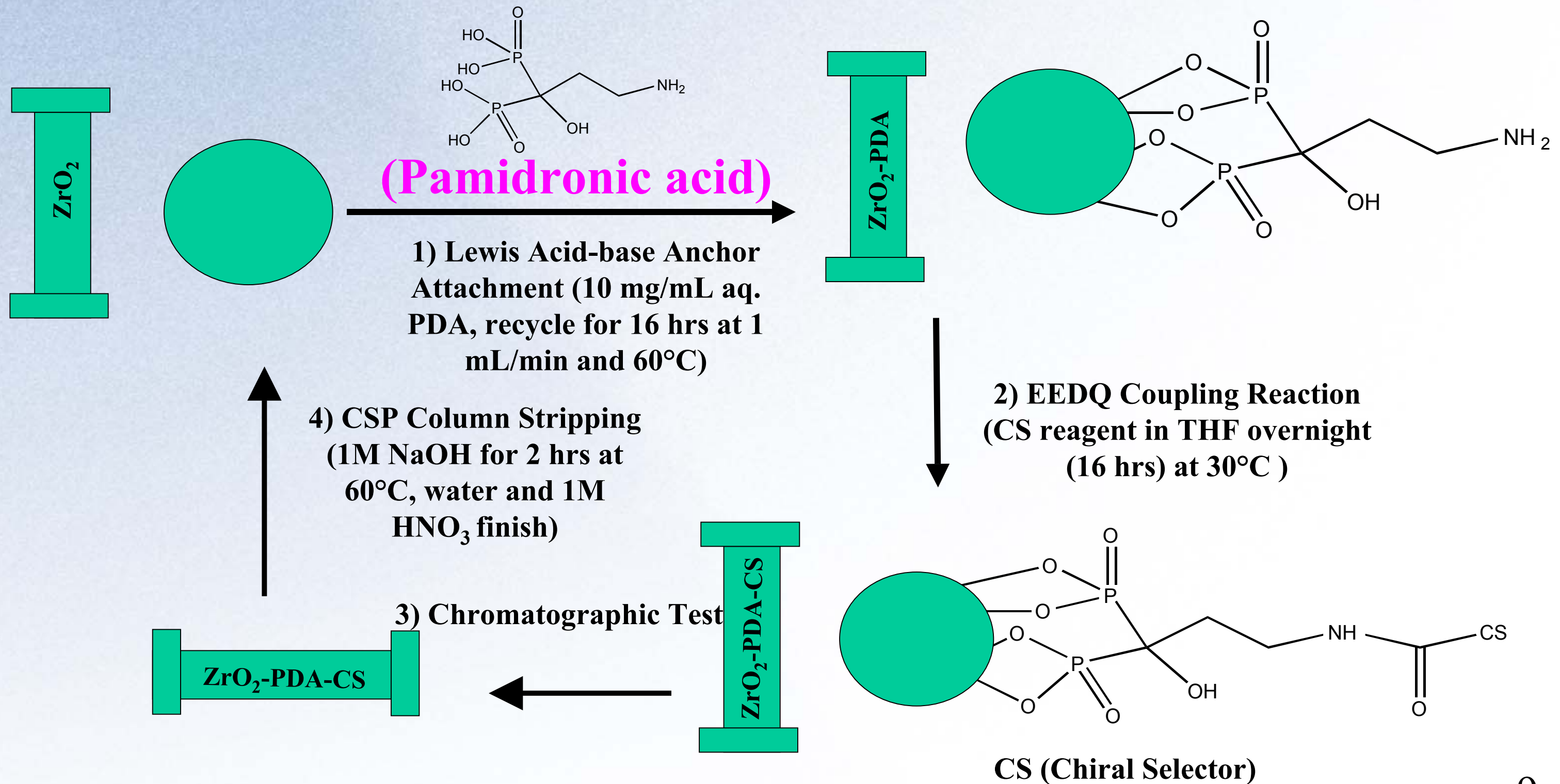
Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / **MeOH** / (70/30 Hexane/IPA), F=1 ml/min, 30 °C



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

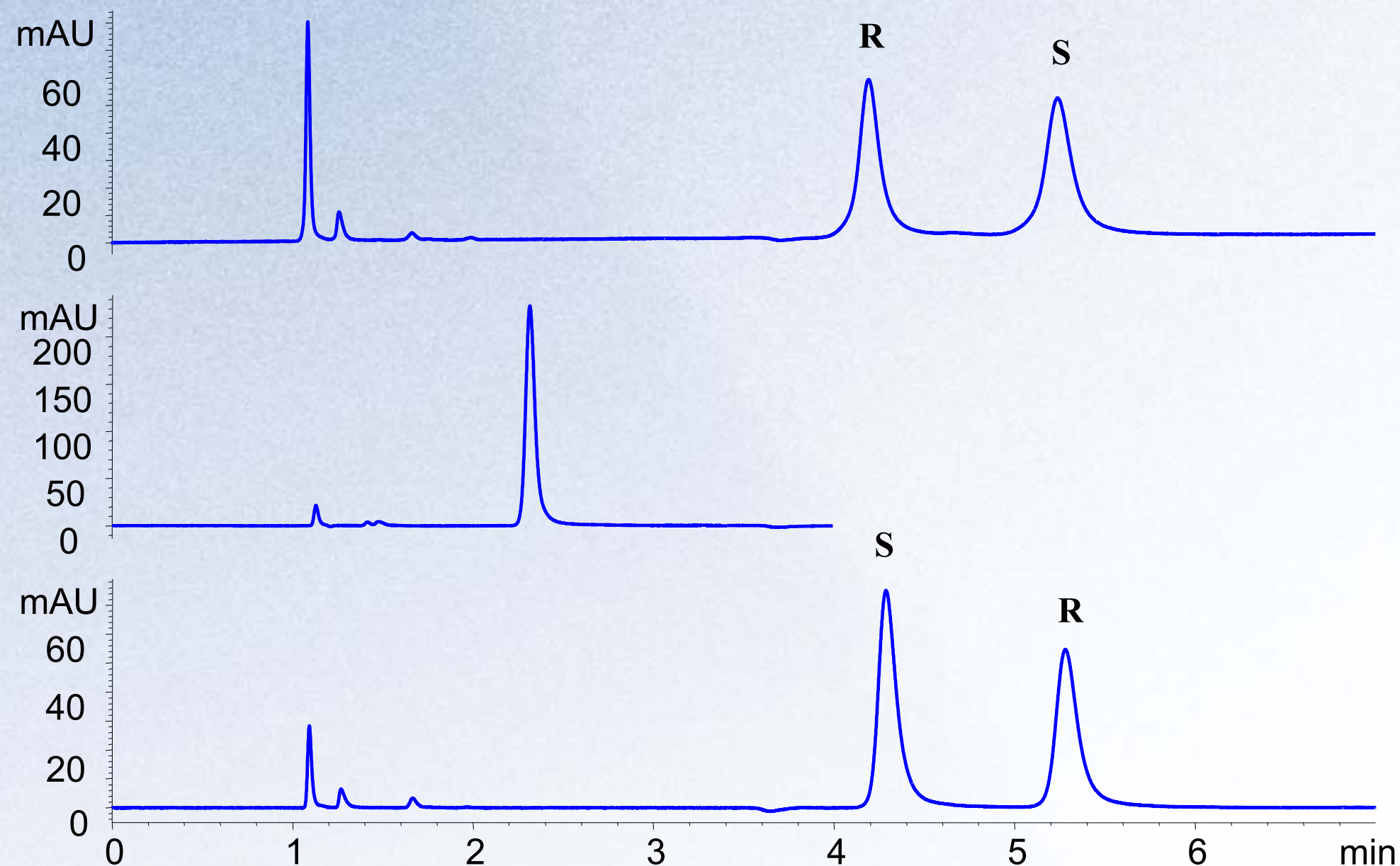


2-Step Online Zirconia CSP Synthesis for Chiral Screening





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



2-Step Load (S)-PG CS

$$k'(\text{less}) = 2.84$$

$$k'(\text{more}) = 3.81$$

$$\alpha = 1.34$$

Strip (S)-PG CS

No separation.

2-Step Load (R)-PG CS

$$k'(\text{less}) = 2.92$$

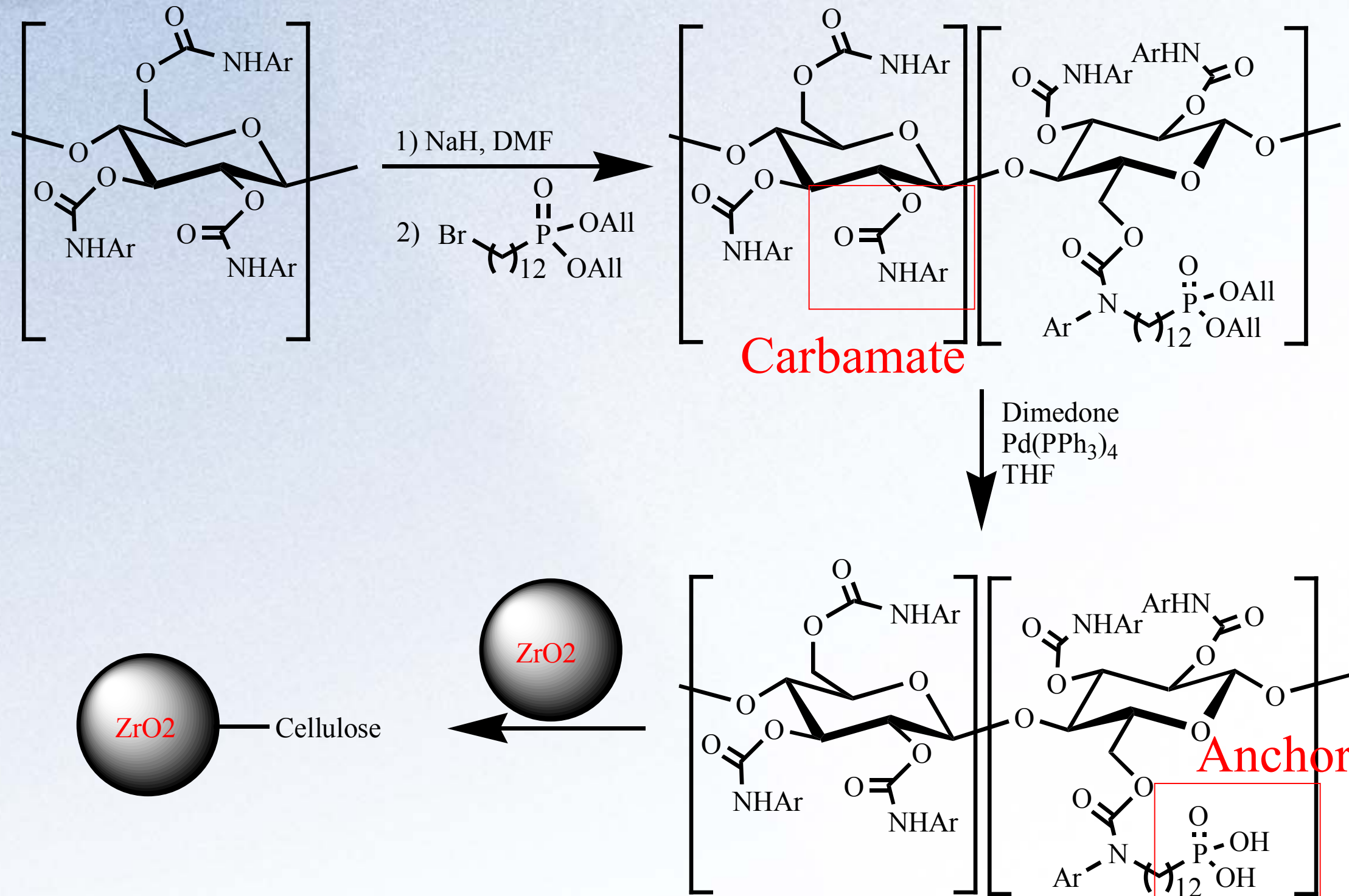
$$k'(\text{more}) = 3.83$$

$$\alpha = 1.34$$

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-butylbenzene, (R orS)-2,2,2-Trifluoro-1-(9-anthryl) EtOH (5 μl injection)



Phosphonate Modified Cellulose Based CSP on Zirconia

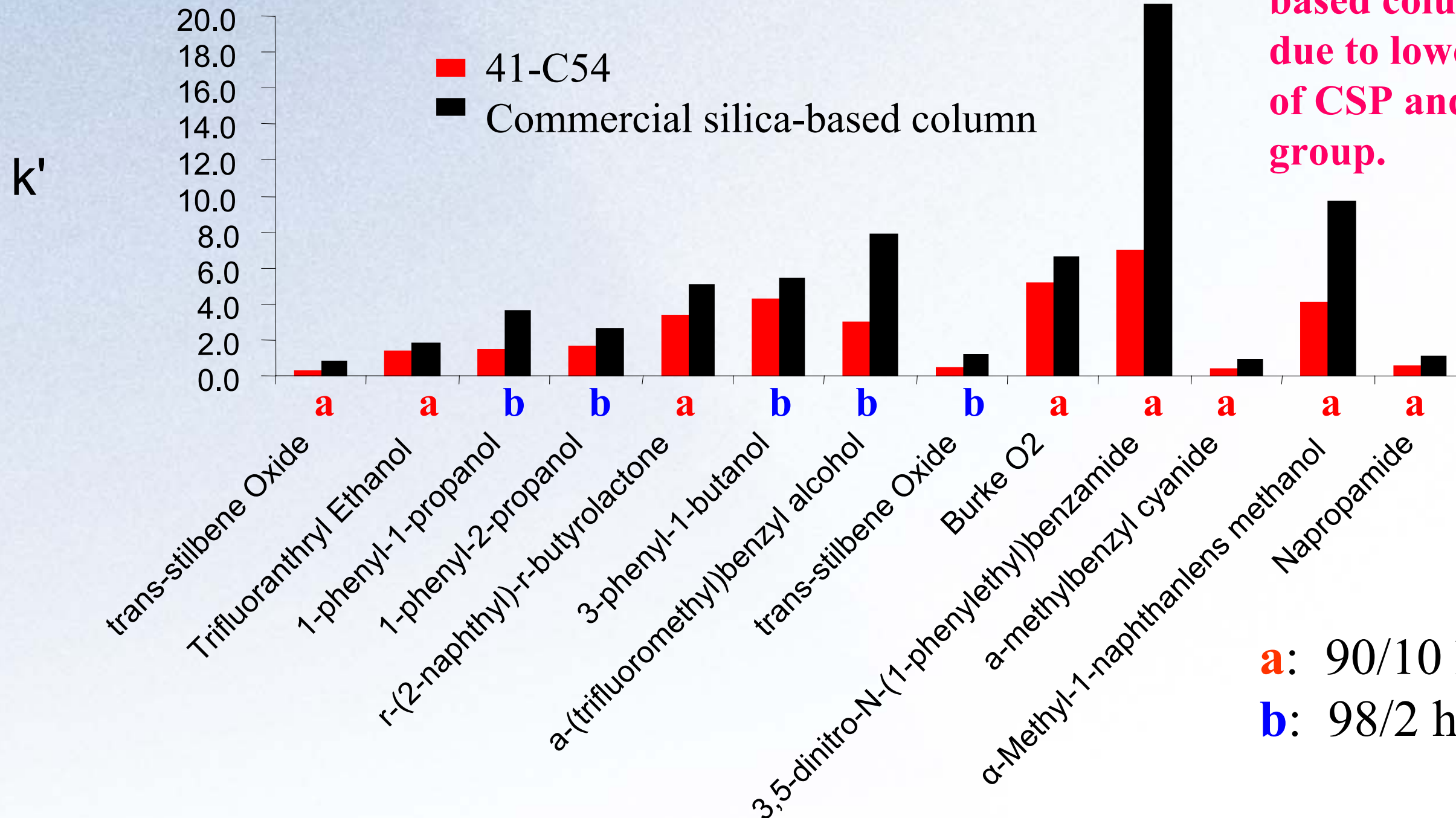




Retention Comparison Between Alkylphenyl Modified Cellulosic CSPs and Commercial Silica CSPs

41-C54, J04-175, 3,5-dimethylphenyl, -C₁₁H₂₂PO₃H
Commercial Silica CSP column

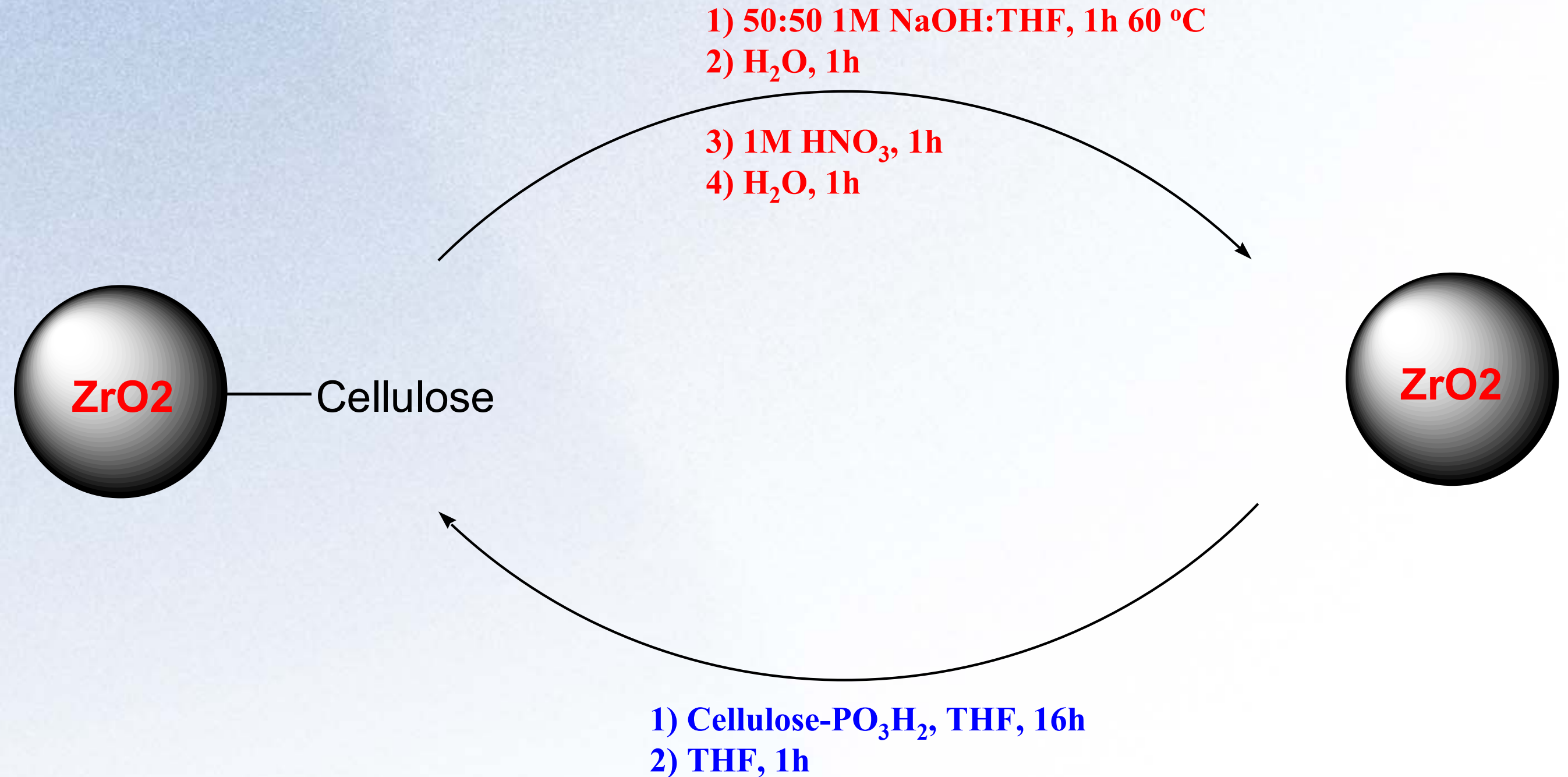
New phase has less retention than commercial Silica-based column likely due to lower loading of CSP and anchor group.



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

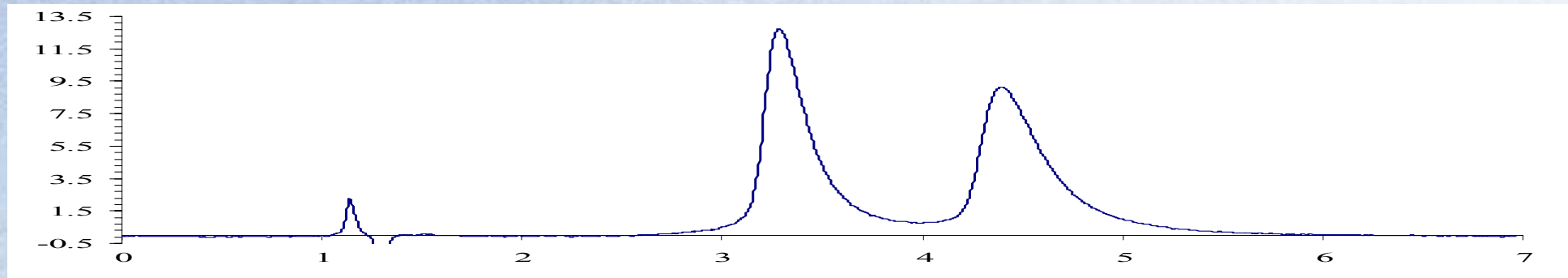


Cellulose Phase Regeneration





Cellulose Phase Regeneration

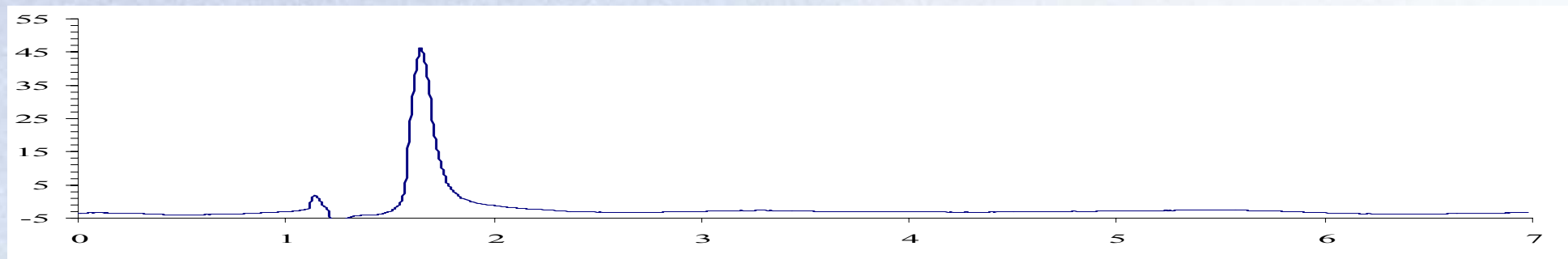


Original Cellulose

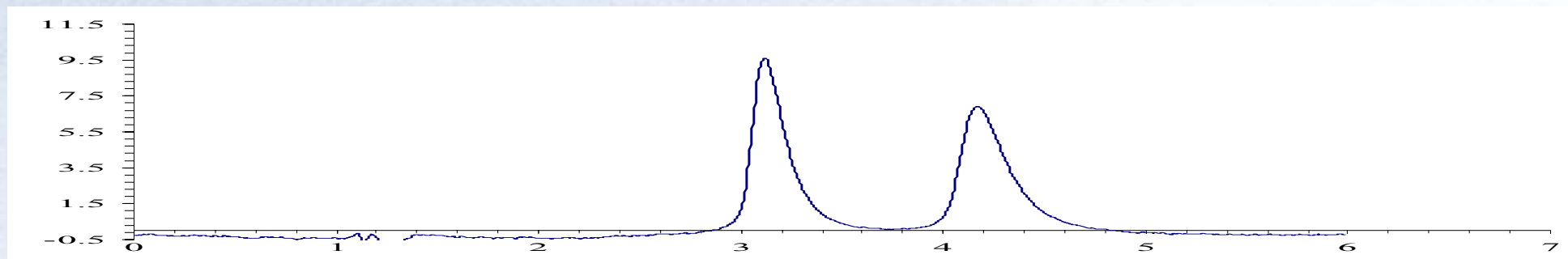
$$k'(\text{less}) = 1.73$$

$$k'(\text{more}) = 2.67$$

$$\alpha = 1.54$$



Remove Cellulose
No separation.



Reload Cellulose

$$k'(\text{less}) = 1.59$$

$$k'(\text{more}) = 2.47$$

$$\alpha = 1.55$$

Pre-mixed 90/10 Hexane/IPA, F=1 ml/min, 25 °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.*



Thanks *very much*
for listening!

Visit Us at Booth 2702



For more information and web access to the free
Buffer Wizard: www.zirchrom.com