Synthesis of Stable Brush-Type Zirconia Based Chiral Stationary Phases for Enantiomer Separations

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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

Lewis acid-base reaction

(Pamidronic acid)

Steric Site

H-donor

π-acceptor

EEDQ coupling reaction
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)

Trifluoranthryl Ethanol
Conditions: 99/1 Hexane/IPA, Flow=1 ml/min
\[ \alpha = 1.15 \]

1-Naphthyl-Leucine Ester
Conditions: 99/1 Hexane/IPA, Flow=1 ml/min
\[ \alpha = 16.8 \]

Napropamide
Conditions: 99/1 Hexane/IPA, Flow=1 ml/min
\[ \alpha = 1.47 \]
Chiral Separations on Zr (S)-NESA (pi-donor phase)

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

\[ \alpha = 2.18 \]

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

(R/S)-(3,5-dinitrobenzoyl)-α-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate

\[ \alpha = 1.28 \]

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

(R/S)-(3,5-dinitrobenzoyl)-phenylglycine

\[ \alpha = 1.65 \]

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-\(\alpha\)-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA

\[ \alpha = 1.59 \quad N_2 = 971 \]

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

\[ \alpha = 1.42 \quad N_2 = 6,425 \]

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

\[ \alpha = 1.25 \quad N_2 = 13,315 \]

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

Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide
Stability of Zr-(S)-DNB-Leu at pH 8

Probe solutes:(R/S)-2, 2, 2-trifluoro-1-(9-anthryl)ethanol
2-Step Online Zirconia CSP Synthesis for Chiral Screening

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

(Pamidronic acid)

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

3) Chromatographic Test

4) CSP Column Stripping (1M NaOH for 2 hrs at 60°C, water and 1M HNO₃ finish)

ZrO₂-PDA-CS

ZrO₂-PDA

ZrO₂-PDA-CS

CS (Chiral Selector)
Stripping of (S)-PG CSP

Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, flow rate=1 ml/min, ambient temperature, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D (100 × 4.6 mm, 3 μm, Running HPLC coated on PHASE110805A, batch#: 52-132). Solute: (1) 1,3,5-Tri-t-butylbenzene, (2) (S)-2,2,2-Trifluoro-1-(9-anthryl) ethanol, (3) (R)-2,2,2-Trifluoro-1-(9-anthryl) ethanol (5 μl injection)
Changes During (S)-PG Stripping

1- Original column
2- Column flushed with 15/85 ACN/pH 12 NH₄OH 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 °C, then flushed with 10 column volumes of water, 10 column volumes of 1 M nitric acid, and 10 column volumes of water
Changing (S) to (R)-Phenylglycine CSP on Same Zr Column

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)

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
Conclusions

• Five new CSPs were attached to zirconia using the PDA anchor, including:
  \( \pi\text{–}\text{acceptors} \): Zr (S)-Leu, Zr (R)-PG, and Zr (S)-PG
  \( \pi\text{–}\text{donors} \): Zr (R)-NESA, Zr (S)-NESA
• The new Zirconia-based CSPs were found to be fairly stable in reversed-phase mobile phase from pH 2 to pH 8.
• Zirconia based CSPs have the potential to regenerate the chiral stationary phase online.
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Thanks *very much* for listening!

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