

Utilization of Macrocyclic Glycopeptide Stationary Phases for the Separation of Peptides

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Peptide Analogs

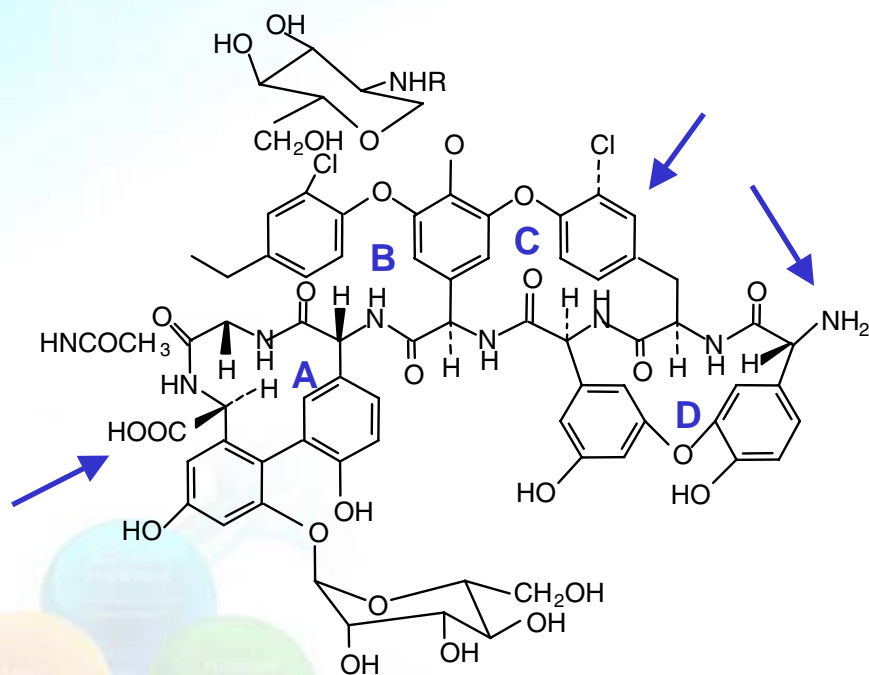
- Closely related peptides with few amino acid substitutions or that possess chiral substitutions
- Why the interest?
 - peptide therapeutics
 - synthetic peptides
 - unusual amino acids

Separation of Peptide Analogs

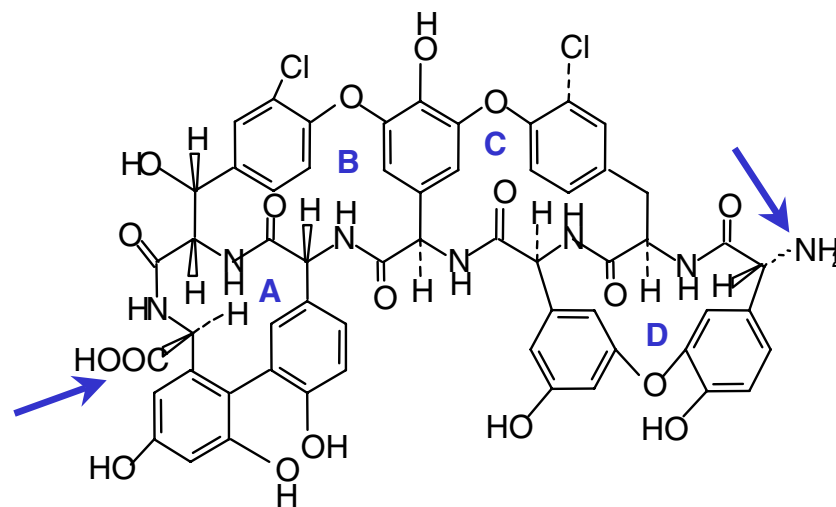
- Current LC Methods
 - RPC: generally done with ion-pairing reagents
 - IEX: generally requires elevated levels of salts
- Astec Chirobiotic phases
 - use water/acetonitrile mobile phases with buffers/salts that are MS compatible
 - provide unique selectivity from RP and IEX methods
- Acknowledgements/References
 - J.T. Lee
 - D.W. Armstrong
 - Zhang, Soukup, & Armstrong. J Chrom A, 1053 (2004) 89-99.

Proposed Structure of Macrocyclic Glycopeptide CSPs

Chirobiotic T/T2
(Teicoplanin)



Chirobiotic TAG
(Teicoplanin Aglycone)



→ Key interaction sites; A, B, C and D are cavities

Peptide Analogs Examined

- tripeptide
 - (DL)-Ala-(DL)-Leu-Gly (dl-A-dl-LG)
- enkephalins
- enkephalinamides

Experimental Strategy

- Chirobiotic Phases

- variables to screen:
 - stationary phase (T, T2, TAG)
 - buffer strength
 - volume fraction of acetonitrile
- ammonium formate buffer, pH 4 ($\frac{w}{w}$ pH) ; titrated with formic acid
- 5 μ , 25 cm columns
- isocratic elution

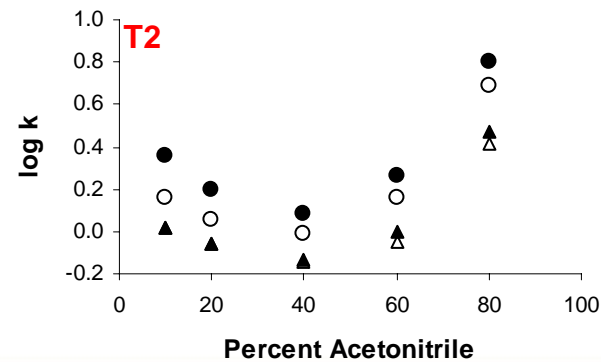
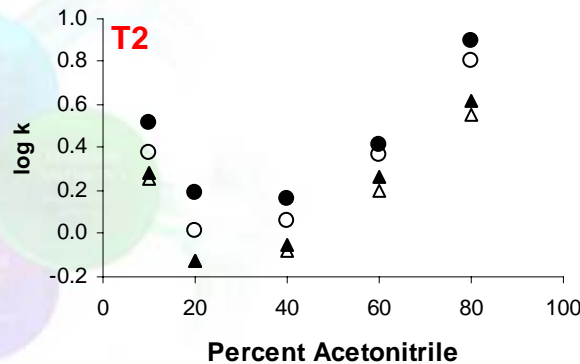
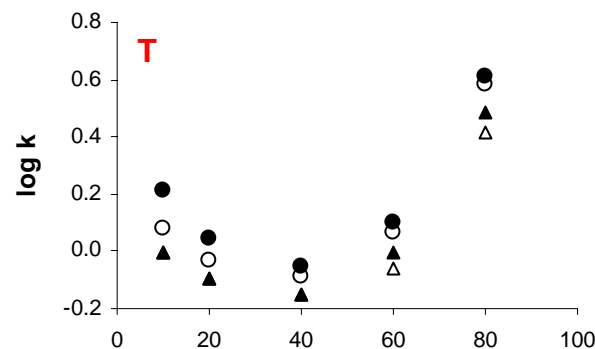
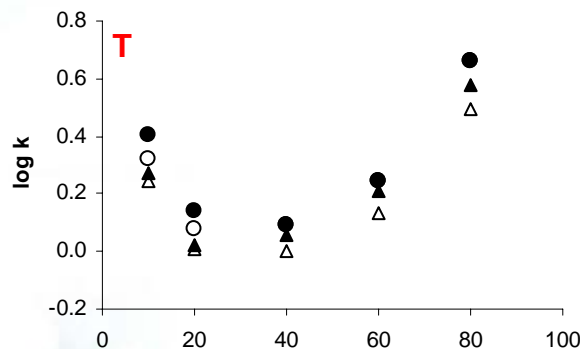
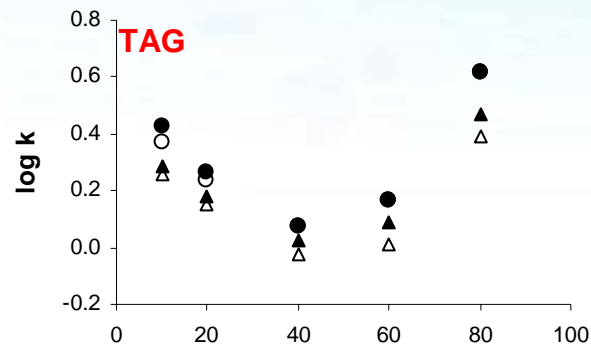
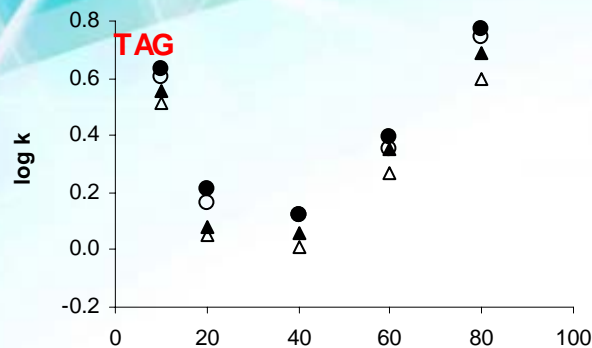
- RP Phase

- 5 μ C18, 15 cm column
- gradient elution
- TFA

Screening of Chirobiotic Teicoplanin Phases for Resolution of (DL)-Ala-(DL)-Leu-Gly

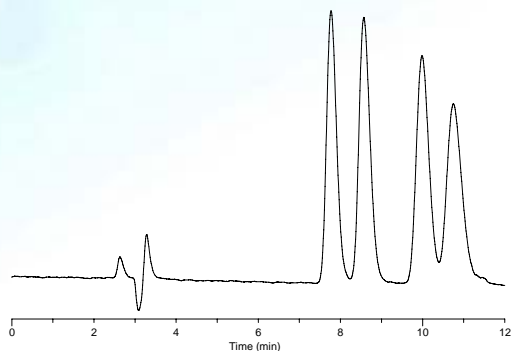
2 mM buffer

10 mM buffer

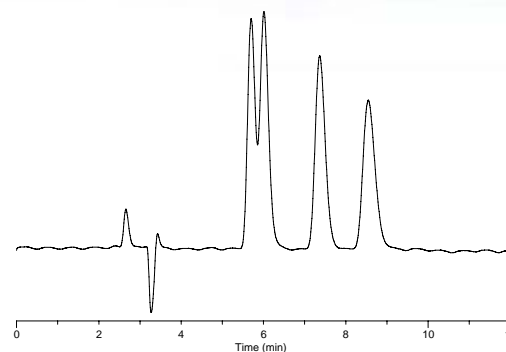


Comparisons of ionic strength in resolution of tripeptide

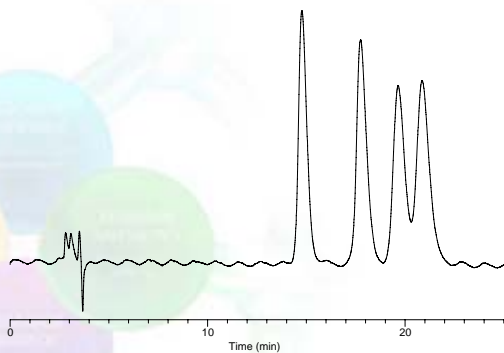
T2 2 mM buffer, 60% MeCN



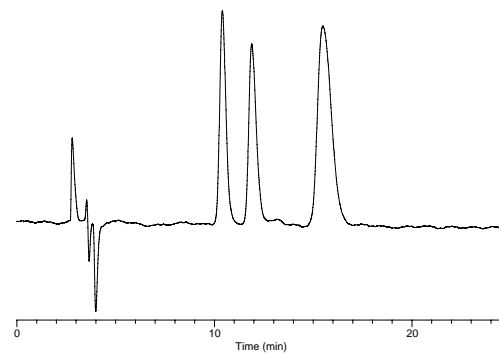
T2 10 mM buffer, 60% MeCN



TAG 2 mM buffer, 80% MeCN

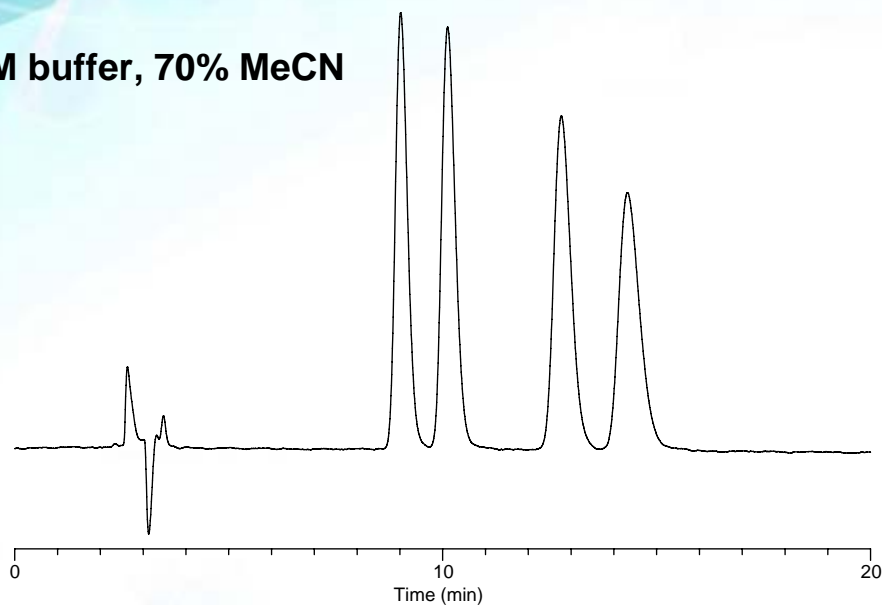


TAG 10 mM buffer, 80% MeCN

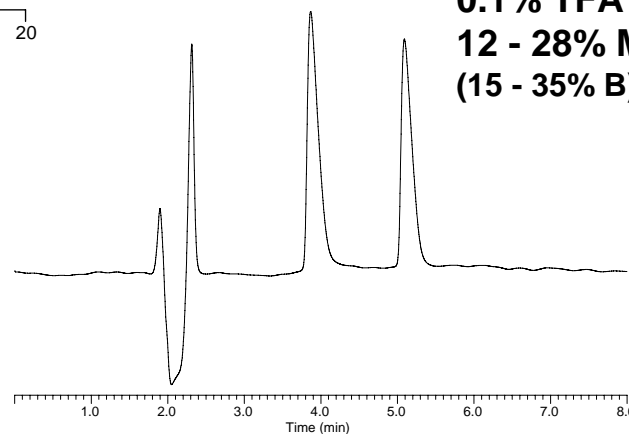


Optimized separation of (DL)-Ala-(DL)-Leu-Gly on Chirobiotic T2

2 mM buffer, 70% MeCN



5 μ C18
0.1% TFA
12 - 28% MeCN in 8 min
(15 - 35% B)



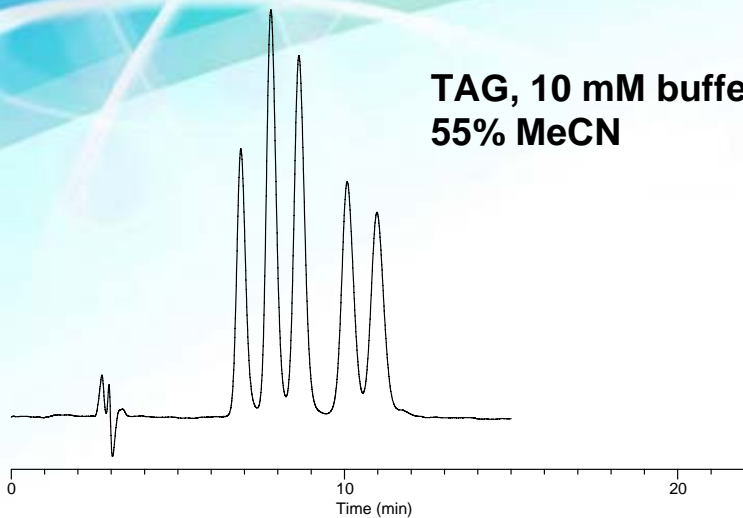
Enkephalins

- (a) YGGFM
- (b) YGGFL
- (c) Y-dA-GFL
- (d) Y-dA-GF-dL
- (e) Y-dA-GF-dM

- single amino acid substitutions
 - a \rightarrow b
 - d \rightarrow e
- single chiral substitutions
 - c \rightarrow d

Enkephalins

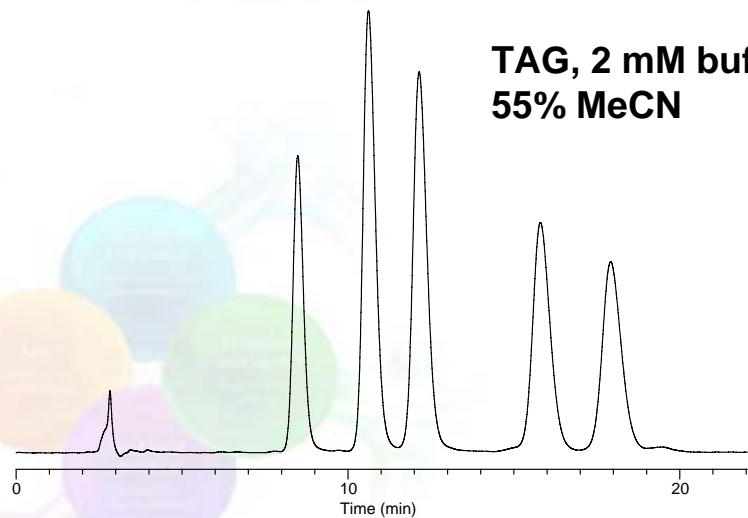
**TAG, 10 mM buffer
55% MeCN**



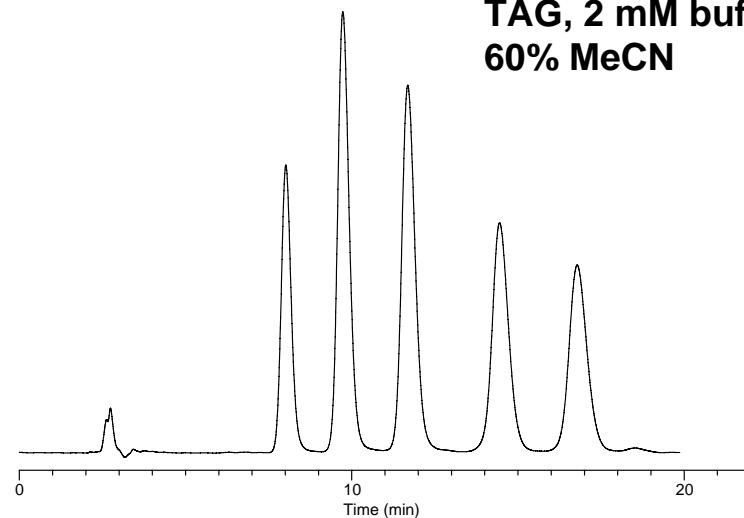
Elution order:

- 1. Y-dA-GF-dM**
- 2. Y-dA-GF-dL**
- 3. YGGFM**
- 4. Y-dA-GFL**
- 5. YGGFL**

**TAG, 2 mM buffer
55% MeCN**

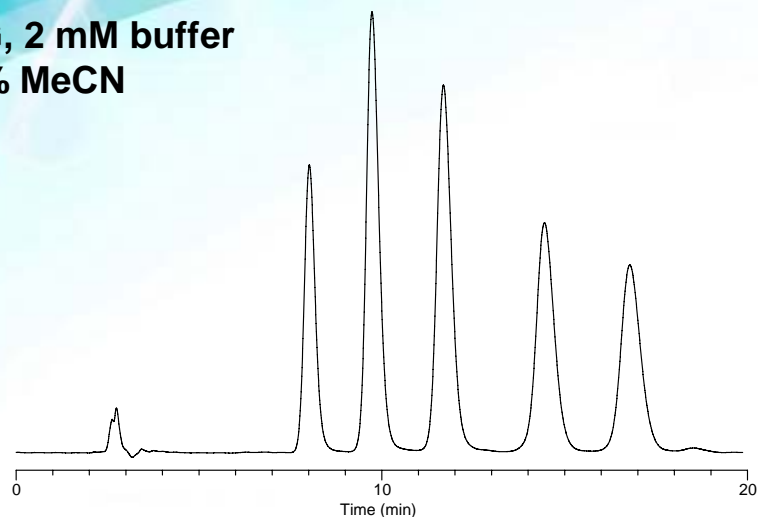


**TAG, 2 mM buffer
60% MeCN**

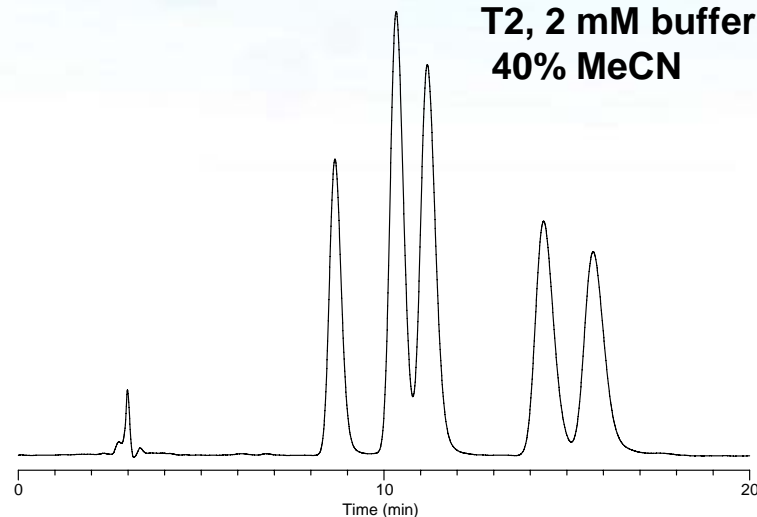


Enkephalins

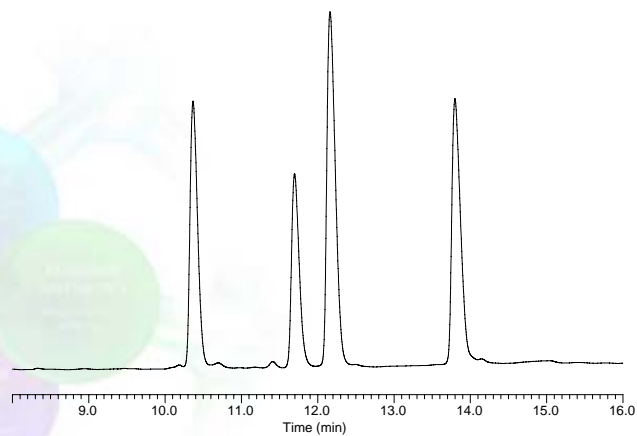
**TAG, 2 mM buffer
60% MeCN**



**T2, 2 mM buffer
40% MeCN**



**5 μ C18
0.1% TFA
12 - 80% MeCN in 34 min
(15 - 100% B)**



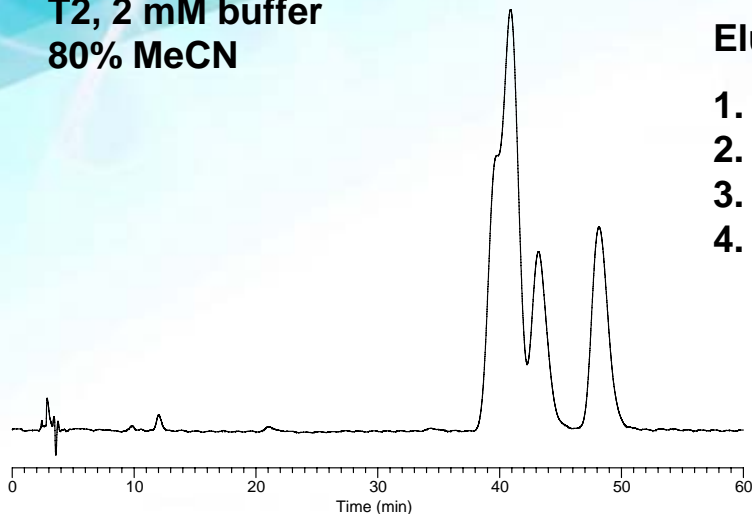
Enkephalinamides

- (a) **YGGFL**-amide
- (b) **YGGFM**-amide
- (c) **YAGFM**-amide
- (d) **Y-dA-GFM**-amide
- (e) **Y-dA-GF-dM**-amide

- single amino acid substitutions
 - a → b
 - b → c
- single chiral substitutions
 - c → d
 - d → e

Enkephalinamides

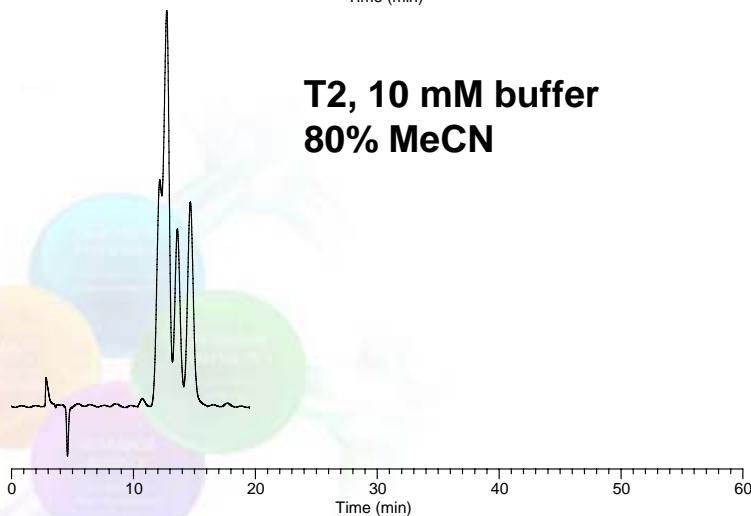
**T2, 2 mM buffer
80% MeCN**



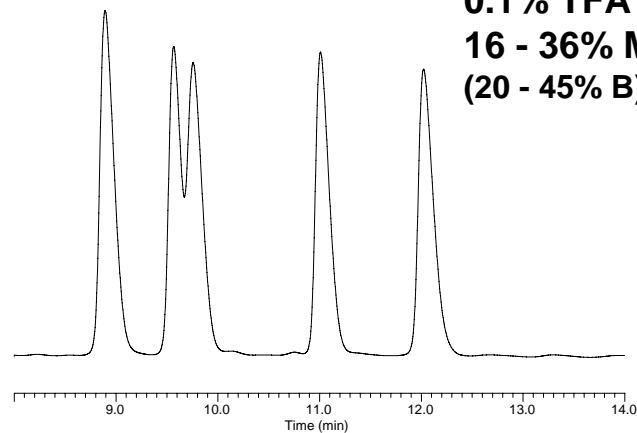
Elution order:

- 1. Y-dA-GF-dM**
- 2. YGGFL, Y-dA-GFM**
- 3. YAGFM**
- 4. YGGFM**

**T2, 10 mM buffer
80% MeCN**



**5 μ C18
0.1% TFA
16 - 36% MeCN in 20 min
(20 - 45% B)**



Conclusions

- Macrocyclic glycopeptide CSPs can provide resolution of peptide stereoisomers that can be achieved under isocratic MS-friendly conditions.
- Retention of peptides by the macrocyclic glycopeptide CSPs, under mixtures of buffered aqueous and organic mobile phases, exhibits both reversed-phase and aqueous-normal phase behavior.
- Substitutions with D amino acids confer reduced retention.
- Ionic interactions of the peptides with the macrocyclic glycopeptide CSPs is critical for resolving analogs.
- The Chirobiotic T/T2/TAG phases provide complementary selectivities.
- The potential for resolution of chiral peptide analogues by traditional RPC needs to be further explored.