Facile t-BOC and FMOC Amino Acid Chiral Separations by HPLC

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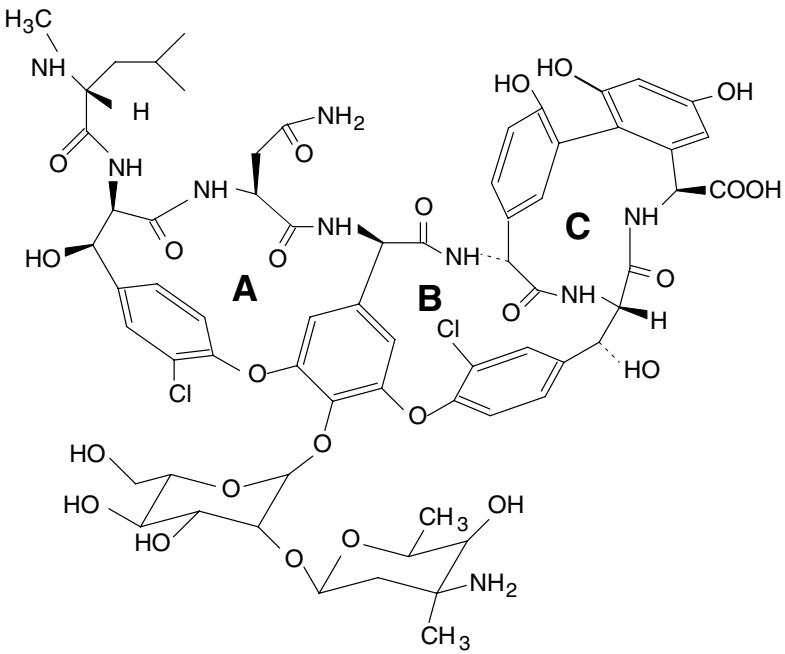
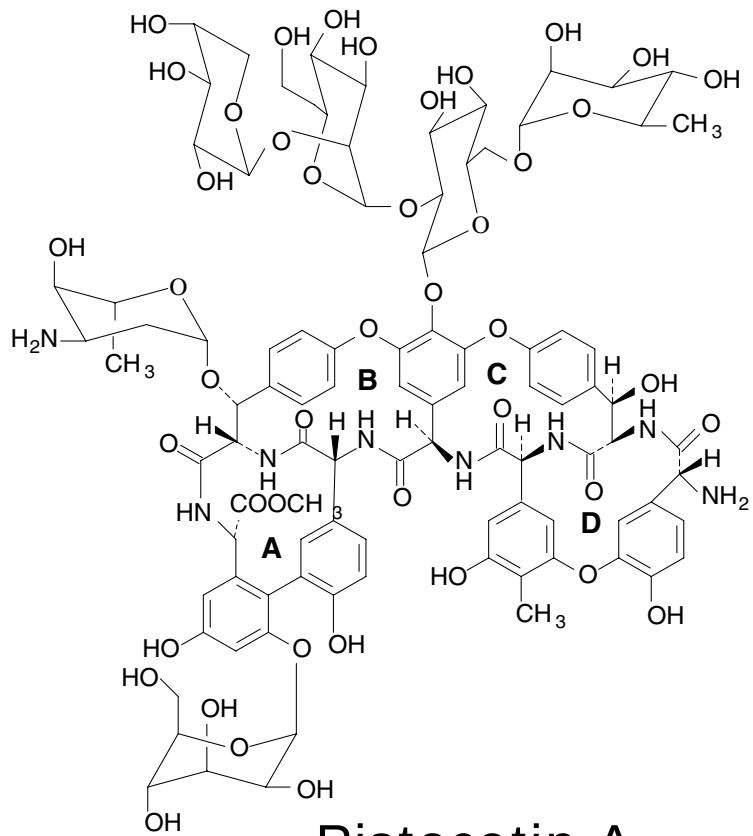
Abstract

Macrocyclic glycopeptide-based chiral stationary phases (CSPs) have become more popular due to their multimodal capability, broad selectivity and ruggedness. Owing to their unique chiral ionic character, the enantiomer resolution of a wide variety of acids, bases and amphoteric racemates has been an easier task. Recently, with the advances of genomics/proteomics a huge demand for amino acids and peptides analysis has evolved. Specifically, the chiral analysis of N-blocked amino acids, i.e. t-BOC and FMOC, has become essential. There are three types of macrocyclic stationary phases suitable for this type of analysis. The unique phenomenon of complementary separations among these chiral stationary phases (vancomycin, teicoplanin, ristocetin A) renders them very effective tools for separating a wide variety of amino acids and N-blocked amino acids. A large number of t-BOC and FMOC amino acids have been tested, and baseline resolution is easily achieved for every racemate tested. In some cases, the selectivity/resolution can be greater than 5. The method development protocol and optimization methods are very straightforward in two mobile phase types, the polar organic phase and the reversed phase mode. The polar organic and reversed phase systems with the use of volatile buffers like ammonium trifluoroacetate and/or ammonium acetate are very compatible with LC/MS and LC/MS/MS platforms. The chiral recognition mechanisms involved in this type of chromatography will be proposed along with the rationale for optimization.

Proposed Structures of Glycopeptide CSPs

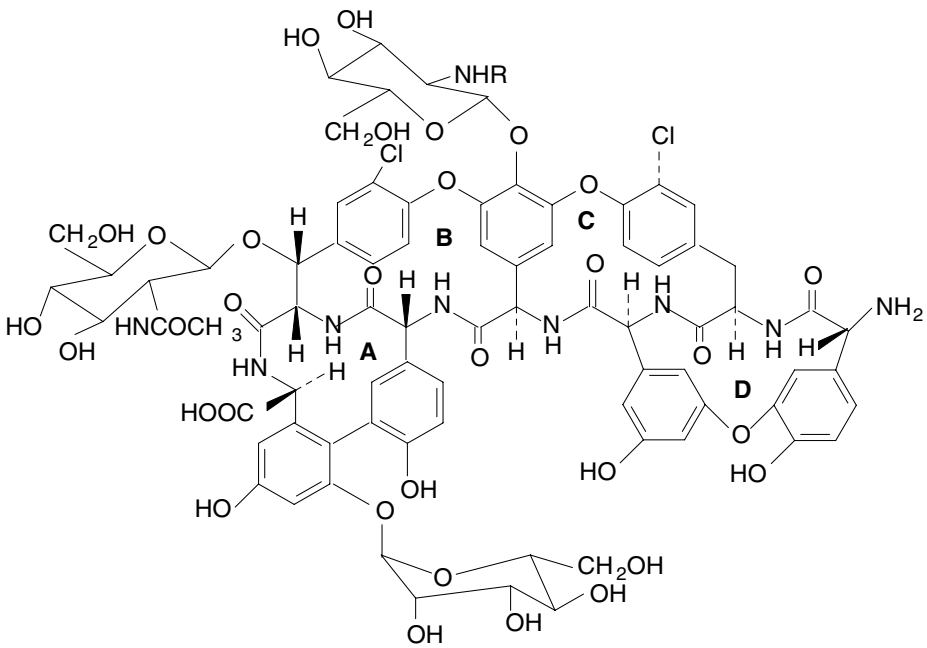


Vancomycin



Ristocetin A

Proposed Structures of Glycopeptide CSPs



Teicoplanin

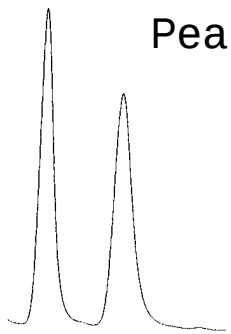
N-FMOC (9-Fluorenylmethyl Chlorophormate) Amino Acids

Column: CHIROBIOTIC T in Reversed Phase Mode

Peak 1: 6.77

Peak 2: 11.57

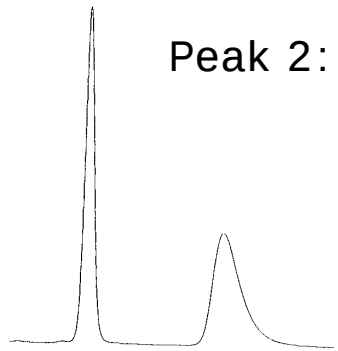
Alanine



Peak 1: 5.55

Peak 2: 6.73

Aspartic Acid



Peak 1: 7.87

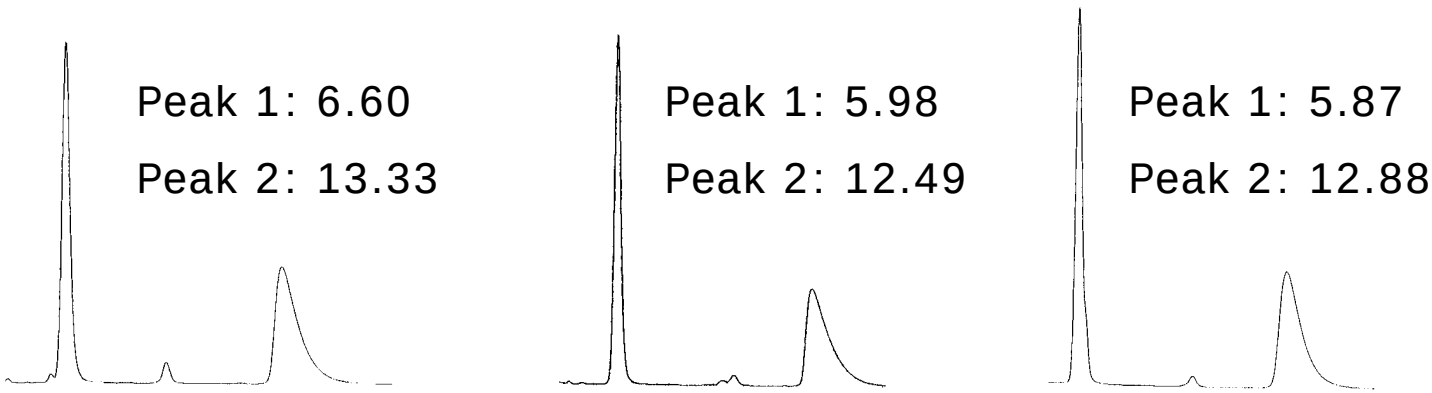
Peak 2: 10.30

Glutamic Acid

Mobile Phase: 40/60: MeOH/0.1% TEAA, pH=4.1

N-FMOC (9-Fluorenylmethyl Chloroformate) Amino Acids

Column: CHIROBIOTIC T in Reversed Phase Mode

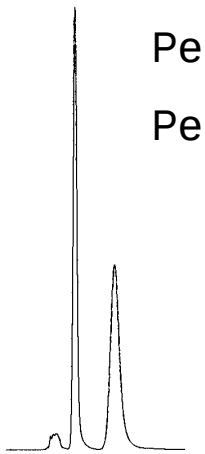


Norleucine Norvaline Methionine

Mobile Phase: 40/60: MeOH/0.1% TEAA, pH=4.1

N-FMOC (9-Fluorenethylmethyl Chloroformate) Amino Acids

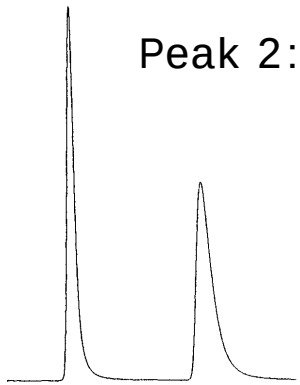
Column: CHIROBIOTIC R in Polar Organic Mode



Peak 1: 2.95

Peak 2: 4.40

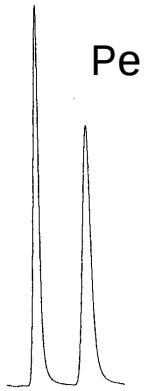
Alanine



Peak 1: 8.11

Peak 2: 13.66

Glutamine



Peak 1: 4.05

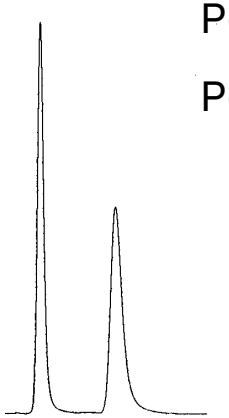
Peak 2: 5.16

Isoleucine

Mobile Phase: 100/0.1%: MeOH/NH_4TFA (LC/MS Compatible)

N-FMOC (9-Fluorenethylmethyl Chloroformate) Amino Acids

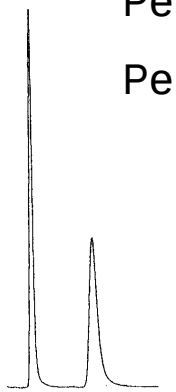
Column: CHIROBIOTIC R in Polar Organic Mode



Peak 1: 4.20

Peak 2: 5.61

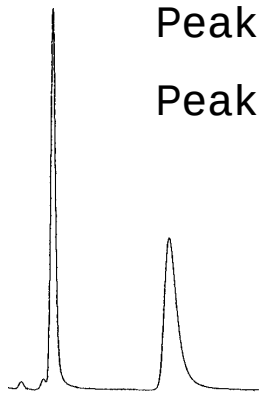
Norleucine



Peak 1: 4.51

Peak 2: 7.17

Norvaline



Peak 1: 5.4

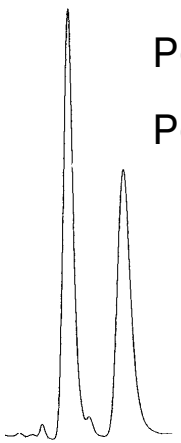
Peak 2: 9.90

Methionine

Mobile Phase: 100/0.1%: MeOH/NH_4TFA (LC/MS Compatible)

N-FMOC (9-Fluorenylmethyl Chloroformate) Amino Acids

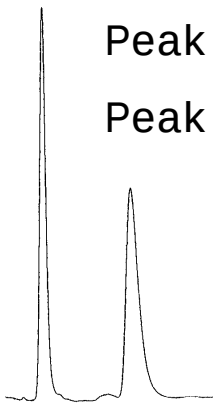
Column: CHIROBIOTIC R in Reversed Phase Mode



Peak 1: 7.66

Peak 2: 9.95

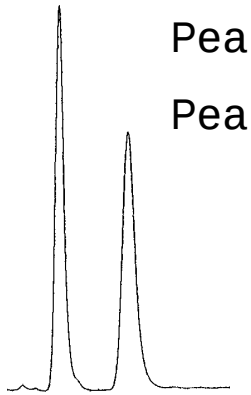
Asparagine



Peak 1: 5.79

Peak 2: 9.87

Glutamine



Peak 1: 6.22

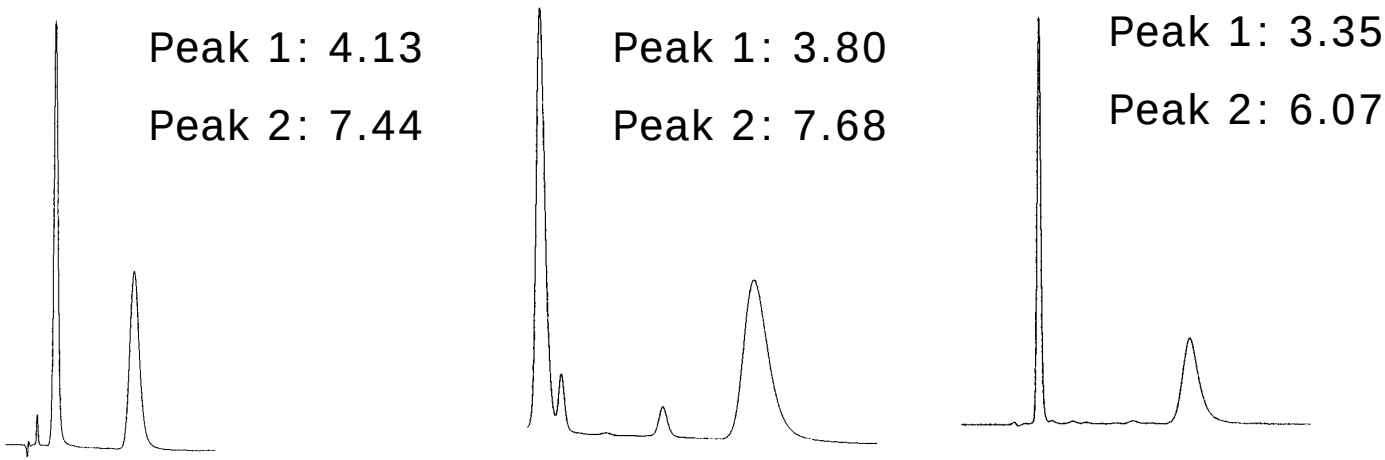
Peak 2: 8.78

Serine

Mobile Phase: 30/70: MeOH/20mM NH_4OAc (LC/MS Compatible)

N-FMOC (9-Fluorenethylmethyl Chloroformate) Amino Acids

Column: CHIROBIOTIC R in Reversed Phase Mode



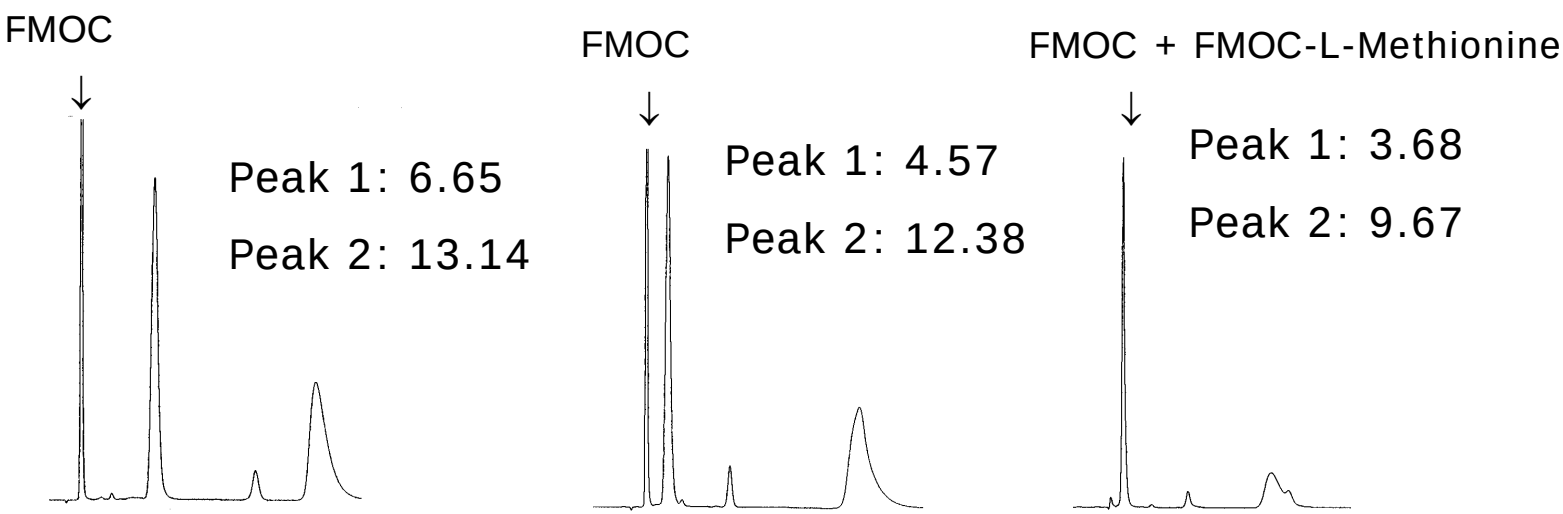
Alanine Methionine Phenylalanine

Mobile Phase: 50/50: MeOH/20mM NH_4OAc (LC/MS Compatible)

pH Effect

Column: CHIROBIOTIC T

FMOC Methionine



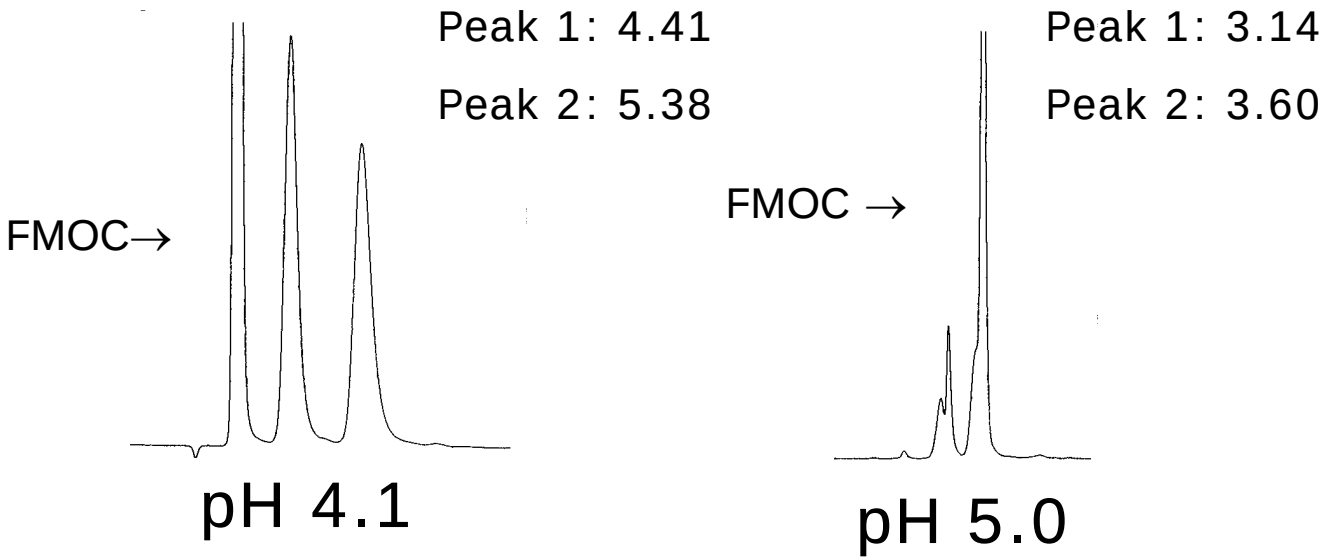
pH 4.1 pH 5.0 pH 5.5

Mobile phase: 40/60: MeOH/20mM NH_4OAc

pH Effect

Column:CHIROBIOTIC T

FMOC Aspartic acid



pH 4.1 pH 5.0

Mobile Phase: 40/60: MeOH/20mM NH_4OAc

CHIROBIOTIC T
(Reversed Phase Mode)

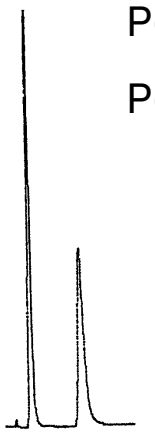
LC→LC/MS Compatible Mobile Phase

Example: FMOC Alanine

Mobile Phase: MeOH/Buffer*, 40/60

Peak 1: 6.77

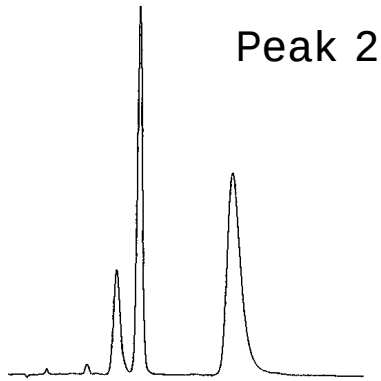
Peak 2: 11.57



*Buffer: 0.1% TEAA, pH=4.1

Peak 1: 6.56

Peak 2: 9.41



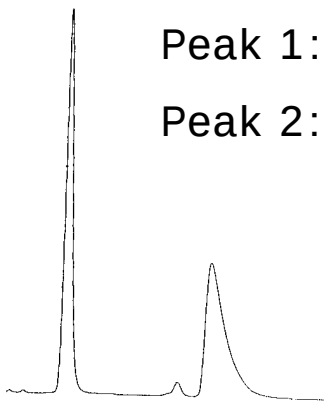
*Buffer: 20mM NH_4OAc, pH=4.1

CHIROBIOTIC T
(Reversed Phase Mode)

LC→LC/MS Compatible Mobile Phase

Example: FMOC Leucine

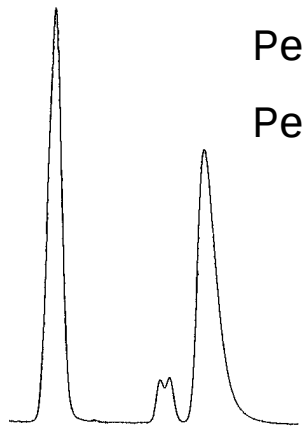
MeOH/ Buffer*, 40/60



Peak 1: 6.10

Peak 2: 10.58

*Buffer: 0.1% TEAA, pH=4.1



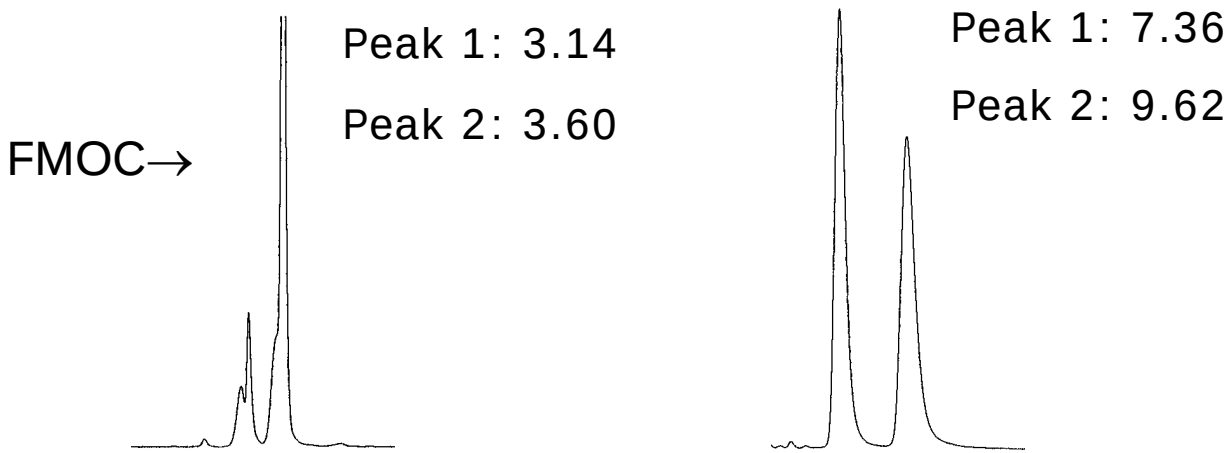
Peak 1: 6.97

Peak 2: 11.88

*Buffer: 20mM NH_4OAc, pH=4.1

CHIROBIOTIC T vs R

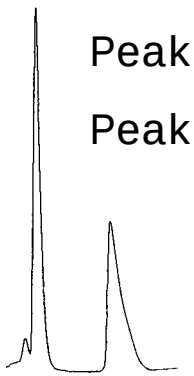
FMOC Aspartic acid



Mobile phase: 40/60: MeOH/20mM NH_4OAc, pH=5.0

N-t-BOC Amino Acids

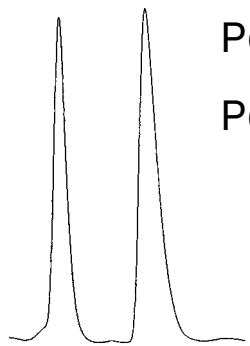
Column: CHIROBIOTIC R



Peak 1: 6.26

Peak 2: 8.74

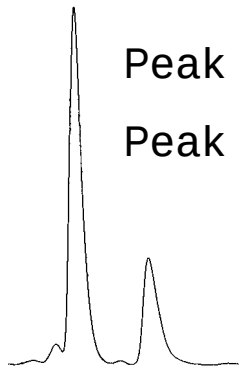
Alanine



Peak 1: 7.79

Peak 2: 8.32

Asparagine



Peak 1: 6.13

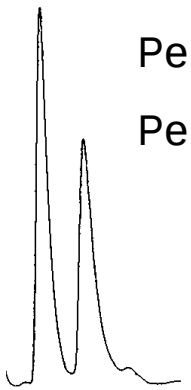
Peak 2: 7.55

Glutamine

Mobile Phase: 20/80: MeOH/0.1 % TEAA, pH=4.1

N-t-BOC Amino Acids

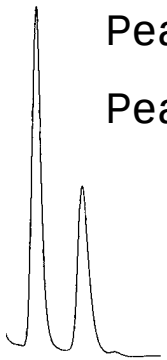
Column:CHIROBIOTIC R



Peak 1: 8.01

Peak 2: 9.24

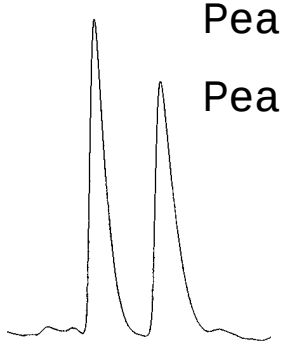
Isoleucine



Peak 1: 5.64

Peak 2: 6.42

Serine



Peak 1: 7.33

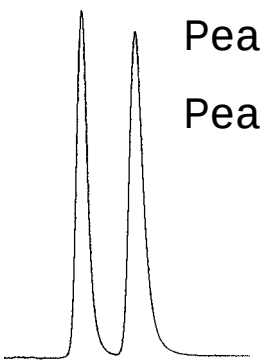
Peak 2: 8.43

Valine

Mobile Phase: 20/80: MeOH/0.1% TEAA, pH=4.1

N-t-BOC Amino Acids

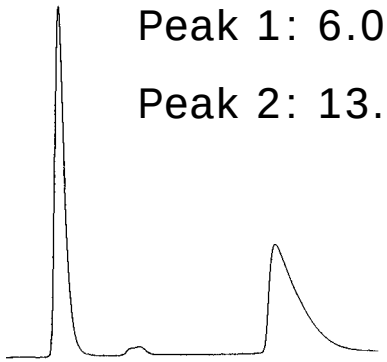
Column: CHIROBIOTIC R



Peak 1: 5.43

Peak 2: 6.32

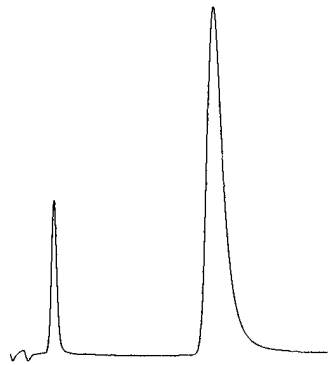
Histidine



Peak 1: 6.06

Peak 2: 13.24

Phenylalanine



Peak 1: 4.84

Peak 2: 10.12

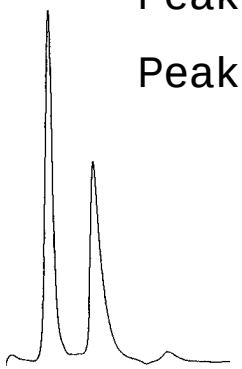
Tryptophan

Mobile Phase: 20/80: MeOH/0.1% TEAA, pH=6.0

N-t-BOC Amino Acids

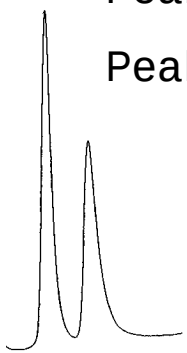
Column: CHIROBIOTIC T

Peak 1: 4.36
Peak 2: 5.10



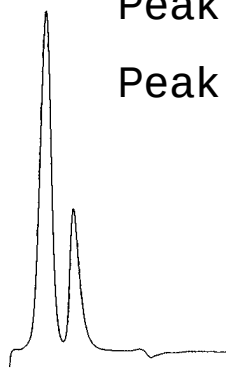
Alanine

Peak 1: 8.66
Peak 2: 10.08



Phenylalanine

Peak 1: 4.23
Peak 2: 4.69

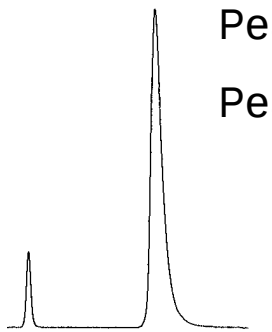


Glutamine

Mobile Phase: 10/90: MeOH/0.1% TEAA, pH=4.1

N-t-BOC Amino Acids

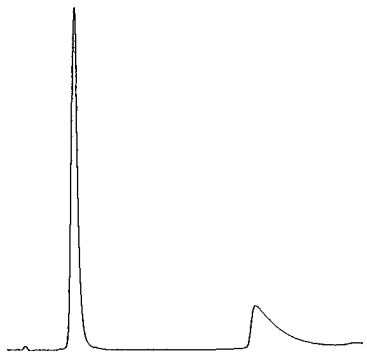
Column: CHIROBIOTIC T



Peak 1: 4.43

Peak 2: 8.62

Methionine



Peak 1: 4.87

Peak 2: 10.94

Phenylglycine

Mobile Phase: 20/80: MeOH/0.1% TEAA, pH=4.1

Conclusions

1. Of the three macrocyclic glycopeptides (CSPs) investigated, CHIROBIOTIC T (Teicoplanin) and CHIROBIOTIC R (Ristocetin A) are the best choices for FMOC and t-BOC amino acids.
2. For FMOC amino acids, both reversed phase and polar organic mode work pretty well in most cases on CHIROBIOTIC R while reversed phase is the best choice for CHIROBIOTIC T.
3. For t-BOC amino acids, reversed phase mode is the viable choice for both CHIROBIOTIC T and R.
4. Both reversed phase and polar organic phase systems can be converted to LC/MS platforms.
5. It appears that carboxylate group of the analytes is the key interaction site with chiral amino group of the CSPs.