Supplementary Materials for

Genomic and chemical decryption of the Bacteroidetes phylum for its potential to biosynthesize natural products

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- R-script for chemotype-barcoding matrix
- Figs. S1 to S39
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- Synthesis of β-hydroxyamino acids (Phe, Ile, Asp)

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1. R-script for chemotype-barcodeing matrix

```r
library(readr)
library(reshape)
library(ggplot2)

setwd("E:/Stephan")  # Working directory
experiment<-"2020-12-16_chitinophaga_v1"  # Experiment name
project<(paste(c(experiment,""), collapse=""))

presencetable.gesamt<as.matrix(read_tsv(paste(c(project,".txt"), collapse=""), col_names=TRUE, col_types = NULL, na = c("","NA"), trim_ws = FALSE, skip = 0, n_max = Inf, progress = show_progress(), skip_empty_rows = TRUE))  # load project file

#presencetable.transformed<-t(presencetable.gesamt)
n<-ncol(presencetable.gesamt)
presencetable.ges<-presencetable.gesamt[,2:n]
names<-presencetable.gesamt[,1]
rownames(presencetable.ges)<-names
melt.data<-melt(presencetable.ges)

colnames(melt.data)<c("Bucket","Condition","Value")
png(filename=paste(c(project,"_test.png"), collapse=""), width = 50*400,
height = 50*400,
res = 1000,
pointsize = 5)
qplot(data=melt.data,
x=Bucket,
y=Condition,
fill=factor(Value),
#   geom="tile")+scale_fill_manual(values=c("more than one"="black", "R2A"="green", "5065"="blue", "3018"="orange","3021"="pink","5294"="brown","empty"="white", "isolated"="red" ))
geom="tile")+scale_fill_manual(values=c("A - more than one"="black", "F - R2A"="green", "D - 5065"="blue", "B - 3018"="orange","E - 3021"="pink","C - 5294"="brown","G - empty"="white", "H - isolated"="red" ))
dev.off()
```
2. Supplementary Figures 1 to 39

Fig. S1. Gene cluster families (GCF) of the elansolids and pinensins color coded by PFAM domains. a, Reference biosynthetic gene cluster of the elansolids deposited at MIBiG and two further identical BGCs identified on genomes of different strains. b, Reference biosynthetic gene cluster of the pinensins deposited at MIBiG and six modified derivatives found on genomes of different strains are depicted with the PFAM domains color coded and the gene cluster family shown to the right. BiG-SCAPE results with a cutoff of 0.6. c, Clustal W alignment (111) of the pinensin core peptide with those found in its GCF. No core peptide was found on the contig of *Chitinophaga* sp. Mgbs1 (RIAR01000029.1.region001).
Fig. S2 Distribution of non-unique/unique buckets revealed by metabolomics analysis shown as 2D-scatterplot. RT, retention time in seconds; m/z, mass to charge ratio; CH$_3$CN, acetonitrile.
Fig. S3 (A) Analytics (extracted ion chromatograms and MS/MS fragments) of chitinopeptin A with m/z 603.6680 [M+3H]$^{3+}$ (red) and chitinopeptin B with m/z 598.6661 [M+3H]$^{3+}$ (green), (B) and chitinopeptin C1+C2 with m/z 628.0074 [M+3H]$^{3+}$ (purple) and chitinopeptin D1+D2 with m/z 623.3356 [M+3H]$^{3+}$ (blue).
Fig. S4 $^1$H NMR spectrum (700 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin A.

Fig. S5 $^{13}$C NMR spectrum (175 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin A.
Fig. S6 COSY NMR spectrum (700 MHz, H₂O/CD₃CN 1:1) of chitinopeptin A.

Fig. S7 HMBC NMR spectrum (700 MHz, H₂O/CD₃CN 1:1) of chitinopeptin A.
Fig. S8 TOCSY NMR spectrum (700 MHz, H₂O/CD₃CN 1:1) of chitinopeptin A.

Fig. S9 HSQC NMR spectrum (700 MHz, H₂O/CD₃CN 1:1) of chitinopeptin A.
Fig. S10 ROESY NMR spectrum (700 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin A.

Fig. S11 $^1$H NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin B.
Fig. S12 $^{13}$C NMR spectrum (125 MHz, D$_2$O/CD$_3$CN 1:1) of chitinopeptin B.

Fig. S13 COSY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin B.
Fig. S14 HSQC NMR spectrum (500 MHz, D2O/CD3CN 1:1) of chitinopeptin B.

Fig. S15 HMBC NMR spectrum (500 MHz, D2O/CD3CN 1:1) of chitinopeptin B.
Fig. S16 TOCSY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin B.

Fig. S17 ROESY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin B.
Fig. S18 $^1$H NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin C1 and C2 5/4 mixture.

Fig. S19 $^{13}$C NMR spectrum (125 MHz, D$_2$O/CD$_3$CN 1:1) of chitinopeptin C1 and C2 5/4 mixture.
Fig. S20 COSY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin C1 and C2 5/4 mixture.

Fig. S21 HSQC NMR spectrum (500 MHz, D$_2$O/CD$_3$CN 1:1) of chitinopeptin C1 and C2 5/4.
Fig. S22 HMBC NMR spectrum (500 MHz, D$_2$O/CD$_3$CN 1:1) of chitinopeptin C1 and C2 5/4.

Fig. S23 TOCSY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin C1 and C2 5/4 mixture.
Fig. S24 ROESY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin C1 and C2 5/4 mixture.

Fig. S25 $^1$H NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin D1 and D2 mixture.
Fig. S26 $^{13}$C NMR spectrum (125 MHz, D$_2$O/CD$_3$CN 1:1) of chitinopeptin D1 and D2.

Fig. S27 COSY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin D1 and D2.
Fig. S28 HSQC NMR spectrum (500 MHz, D$_2$O/CD$_3$CN 1:1) of chitinopeptin D1 and D2.

Fig. S29 HMBC NMR spectrum (500 MHz, D$_2$O/CD$_3$CN 1:1) of chitinopeptin D1 and D2.
**Fig. S30** COSY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin D1 and D2.

**Fig. S31** ROESY NMR spectrum (500 MHz, H$_2$O/CD$_3$CN 1:1) of chitinopeptin D1 and D2.
Fig. S32 Comparison of the Marfey derivatization products of the chitinopeptin A-D DCI hydrolysates and commercially available amino acid standards derivatized with L-FDVA. (A) Commercially available L-amino acid standards. (B) Commercially available D-amino acid standards. (C) DCI hydrolysate of chitinopeptin A. (D) DCI hydrolysate of chitinopeptin B. (E) DCI hydrolysate of chitinopeptin C1+C2. (F) DCI hydrolysate of chitinopeptin D1+D2.
Fig. 33 Chiral HPLC of $2R,3S$-Ile and $2R,3R$-Ile standards and chitinopeptin samples derivatized with L-FDVA. Chiral HPLC conditions: Chiralpak IC; hexane:isopropyl alcohol:formic acid 75:25:0.2. (A) chitinopeptin D1+D2, (B) $2R,3R$-Ile, (C) $2R,3S$-Ile, (D) mixture of chitinopeptin A and B, (E) chitinopeptin C1+C2
Fig. S34 C18-RP UHPLC-MS extracted ion chromatograms of β-hydroxyaspartic acids derivatized with L-FDVA at m/z 430.1205 [M+H]+ within synthesized β-hydroxyaspartic acid standards and chitinopeptin A-D samples. (A) (2R,3R)-3-hydroxyaspartic acid, (B) (2S,3S)-3-hydroxyaspartic acid, (C) chitinopeptin A, (D) chitinopeptin B, (E) chitinopeptin C1+C2, (F) chitinopeptin D1+D2
Fig. S35 C18-RP UHPLC-MS extracted ion chromatograms of β-hydroxyisoleucines derivatized with L-FDVA at \( m/z \) 428.1776 \([\text{M+H}]^+\) within synthesized β-hydroxyisoleucine standards and chitinopeptin A-D samples. (A) (2S,3R)- and (2R,3S)-3-hydroxyisoleucine, (B) (2S,3S)- and (2R,3R)-3-hydroxyisoleucine, (C) (2S,3R)-3-hydroxyisoleucine, (D) (2S,3S)-3-hydroxyisoleucine, (E) chitinopeptin A, (F) chitinopeptin B, (G) chitinopeptin C1+C2, (H) chitinopeptin D1+D2
Fig. S36 C18-RP UHPLC-MS extracted ion chromatograms of β-hydroxyphenylalanines derivatized with L-FDVA at m/z 462.1619 [M+H]^+ within synthesized β-hydroxyphenylalanine standards and chitinopeptin A-D samples. (A) (2S,3S)- and (2R,3R)-3-hydroxyphenylalanine, (B) (2S,3R)- and (2R,3S)-3-hydroxyphenylalanine, (C) (2S,3S)-3-hydroxyphenylalanine, (D) (2S,3R)-3-hydroxyphenylalanine, (E) chitinopeptin A, (F) chitinopeptin B, (G) chitinopeptin C1+C2, (H) chitinopeptin D1+D2
Fig. S37 Iron chelating properties of chitinopeptin A-D. Fe(III)Citrate was added in excess to pure compounds.
**Fig. S38** Production of chitinopeptin A and B in medium 3018 after 7 days of cultivation in 24 well plate cultivation.
Fig. S 39 Analytics of putatively cyclic lipodepsipeptides produced by *C. niastensis* DSM 24859 with m/z of 680.9817 [M+3H]^{3+} and 685.6545 [M+3H]^{3+}. (A) overlaid Base Peak Chromatogram (BPC, grey) and Extracted Ion Chromatogram (EIC) of m/z 680.9817 [M+3H]^3+ (red) and 685.6545 [M+3H]^3+ (green). (B) Zoom into the chromatogram. (C) Isotope pattern of both derivatives.
3. Supplementary Tables 1 to 7

Tab. S1 Strain list of all *Chitinophaga* strains used for the chemical barcoding matrix and bioactivity-guided NP discovery process.

| Phylum     | Class         | Order         | Family          | Genus     | Species   | Strain     |
|------------|---------------|---------------|-----------------|-----------|-----------|------------|
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | alhagiae  | KCTC62518  |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | arvensicola | DSM3695     |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | barathri   | KCTC42472   |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | coeni      | KCTC62265   |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | cymbioli   | KCTC23738   |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | dinghuensis | DSM29821    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | eiseniae   | DSM22224    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | filiformis | DSM527      |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | flavo      | KCTC62435   |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | ginsengisgetis | DSM18108 |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | ginsengisoli | DSM18107   |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | japonensis | DSM13484    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | jiangningensis | DSM27406 |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | niabensis | DSM24787    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | niastensis | DSM24859    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | pinensis   | DSM2589     |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | pinensis   | DSM2588     |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | rupis      | DSM21039    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | sancti     | DSM784      |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | sedimenti | KCTC52590   |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | silvisoli | KCTC62860   |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | skermanii | DSM23857    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | sp.       | DSM18078    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | terrae     | DSM23920    |
| Bacteroidetes | Chitinophagia | Chitinophagales | Chitinophagaceae | Chitinophaga | varians   | KCTC52926   |


Tab. S2 | $^1$H-NMR spectroscopic data of chitinopeptins in a mixture of H$_2$O and CD$_3$CN in a ratio of 1:1. Chitinopeptin A: 700.13 MHz, 299 K; chitinopeptins B-D: 500.30 MHz, 300 K; $^1$H-chemical shifts are referenced to sodium-3-(trimethylsilyl)propionate-2,2,3,3-d$_4$. The chitinopeptin D sample was a mixture of at least four derivatives with chitinopeptin D1 being the major component. Only the relevant part (Val$_{10}$-Dabs) of chitinopeptin D2 was assigned due to the signal overlap.

| Pos. | Chitinopeptin A | Chitinopeptin B | Chitinopeptin C1 | Chitinopeptin C2 | Chitinopeptin D1 | Chitinopeptin D2 |
|------|----------------|----------------|----------------|----------------|----------------|----------------|
|      | δ$_u$, mult. [J (Hz)] | δ$_u$, mult. [J (Hz)] | δ$_u$, mult. [J (Hz)] | δ$_u$, mult. [J (Hz)] | δ$_u$, mult. [J (Hz)] | δ$_u$, mult. [J (Hz)] |
| FA   | - | - | - | - | - | - |
| 1    | 3.22, m | 3.23, m | 2.44/2.35, m | 2.42/2.34, m | 2.41/2.35, m | - |
| 2-Me| 1.26, m | 1.26, m | - | - | - | - |
| 3    | 3.27, m | 3.26, m | 3.98, b | 3.96, b | 3.96, m | - |
| 4    | 1.61/1.51, m | 1.61/1.52, m | 1.44, m | 1.44, m | 1.44, m | - |
| 5    | 1.34/1.27, m | 1.33/1.27, m | 1.38/1.29, m/b | 1.38/1.29, m/b | 1.38/1.28, m | - |
| 6    | 1.27/1.21, m | 1.29/1.24, m | 1.28, b | 1.28, b | 1.28, m | - |
| 7    | 1.27, m | 1.27, m | 1.29, b | 1.29, b | 1.29, m | - |
| 8    | 1.17, m | 1.27, m | 1.18, b | 1.18, b | 1.18, b | - |
| 9    | 1.52, m | 1.30, m | 1.53, m | 1.53, m | 1.53, b | - |
| 10   | 0.88, m | 0.90, m | 0.88, d (6.7) | 0.88, d (6.7) | 0.88, m | - |
| 11   | 0.88, m | - | 0.88, d (6.7) | 0.88, d (6.7) | 0.89, m | - |
| Dab0 | NH | 8.37, d (8.1) | 8.345, b | 8.34, b | - | - |
|      | a | 5.24, dd (7.2/15.1) | 5.12, dd (7.5/13.8) | 5.11, b | - | - |
|      | β | 3.37/3.20, m | 3.36/3.22, m | 3.36/3.20, m | - | - |
| NMe-Val1 | NH | 3.09, s | 3.09, s | 3.01, s | 3.05, s | 3.06, s | - |
|      | a | 4.67, d (11.1) | 4.67, d (11.3) | 4.56, d (10.9) | 4.55, d (10.9) | 4.55, d (6.1) | - |
|      | β | 2.26, m | 2.26, m | 2.23, m | 2.24, m | 2.24, m | - |
|      | γ | 1.02, d (6.7) | 1.02, d (6.7) | 0.96, b | 0.98, d (3.1) | 0.97, m | - |
|      | δ | 0.88, m | 0.88, m | 0.85, m | 0.85, m | 0.85, m | - |
| Thr2 | NH | 8.35, d (7.5) | 8.35, d (5.4) | 8.37, b | 8.36, b | 8.37, b | - |
|      | a | 4.77, m | 4.76, m | 4.63, m | 4.73, m | 4.78, m | - |
|      | β | 5.23, m | 5.23, m | 5.31, m | 5.30, m | 5.30, m | - |
|      | γ | 1.17, d (6.5) | 1.17, d (6.6) | 1.31, d (5.5) | 1.24, d (7.5) | 1.23, m | - |
| Ala3 | NH | 8.22 (b) | 8.22, d (4.6) | 7.91, d (7.5) | 8.02 | 8.05, d (5.9) | - |
|      | a | 4.39, m | 4.39, m | 4.41, b | 4.415 | 4.40, b | - |
|      | β | 1.33, d (7.0) | 1.33, d (7.0) | 1.33, d (5.1) | 1.34 | 1.34, d (9.4) | - |
|      | γ | - | - | - | - | - | - |
|      | δ | - | - | - | - | - | - |
| β-OH-Asp4 | NH | 8.13, d (8.9) | 8.13, d (9.3) | 8.02, b | 8.07, b | 8.06, b | - |
|      | a | 4.83, m | 4.83, m | 4.96, m | 4.895, m | 4.89, b | - |
|      | β | 4.50, m | 4.51, b | 4.42, d (7.0) | 4.48, b | 4.46, b | - |
|      | γ | - | - | - | - | - | - |
|      | δ | - | - | - | - | - | - |
| β-OH-Phe5 | NH | 8.34, 3 (6.8) | 8.34, 2 (3.4) | 8.38, b | 8.315, d (5.8) | 8.39, b | - |
|      | a | 4.72, m | 4.72, m | 4.68, m | 4.69, m | 4.70, m | - |
|      | β | 5.05, d (9.5) | 5.05, d (9.4) | 4.99, d (8.3) | 4.95, d (8.8) | 4.98, d (9.0) | - |
|      | γ | - | - | - | - | - | - |
|      | δ | - | - | - | - | - | - |
| Ile6 | NH | 7.88, d (8.1) | 7.88, d (7.8) | 7.99, d (7.7) | 7.98, d (7.9) | 7.97, b | - |
|      | a | 4.12, d (4.5) | 4.11, m | 4.14, m | 4.13, m | 4.13, m | - |
|      | β | 1.75, m | 1.75, m | 1.77, m | 1.77, m | 1.76, m | - |
|      | β-Me | 0.63, d (7.2) | 0.62, d (7.1) | 0.57, d (7.5) | 0.59, d (7.8) | 0.59, b | - |
|      | γ | 0.54/0.49, m/m | 0.54/0.49, m/m | 0.69/0.48, m/m | 0.60/0.48, m/m | 0.59/0.50, m/m | - |
|      | δ | 0.56, m | 0.56, m | 0.67, t (6.4) | 0.58, m | 0.59, t (7.5) | - |
|      | δ' | - | - | - | - | - | - |
|       | Chitinopeptin A | Chitinopeptin B | Chitinopeptin C1 | Chitinopeptin C2 | Chitinopeptin D1 | Chitinopeptin D2 |
|-------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|
|       | δ<sub>a</sub> mult. [J (Hz)] | δ<sub>β</sub> mult. [J (Hz)] | δ<sub>α</sub> mult. [J (Hz)] | δ<sub>γ</sub> mult. [J (Hz)] | δ<sub>δ</sub> mult. [J (Hz)] | δ<sub>ε</sub> mult. [J (Hz)] |
| Ser7  |                 |                 |                 |                 |                 |                 |
| NH    | 7.77, d (6.1)   | 7.77, d (6.1)   | 8.12, d (6.6)   | 7.92, d (8.0)   | 7.90, b         |                 |
| α     | 4.28, m         | 4.29, m         | 4.32, b         | 4.32, b         | 4.32, b         |                 |
| β     | 3.93/3.84, d/d  | 3.92/3.85, d/d  | 3.87, dd        | 3.92/3.86, b/b  | 3.90/3.85, d/b  |                 |
| C'    | -               | -               | -               | -               | -               |                 |
| Lys8  |                 |                 |                 |                 |                 |                 |
| NH    | 8.65, d (8.1)   | 8.65, d (7.9)   | 8.095, b        | 8.64, d (7.2)   | 8.59, d (7.6)   |                 |
| α     | 4.30, m         | 4.38, m         | 4.36, m         | 4.33, m         |                 |                 |
| β     | 1.93/1.78, m/m  | 1.93/1.77, m/m  | 1.85/1.64, m/m  | 1.90/1.79, m/m  | 1.88/1.78, m/m  |                 |
| γ     | 1.44/1.38, m/m  | 1.41, m         | 1.35, m         | 1.42, m         | 1.41, m         |                 |
| δ     | 1.64, m         | 1.64, m         | 1.64, m         | 1.64, m         | 1.63, m         |                 |
| ε     | 2.95, m         | 2.95, m         | 2.93, m         | 2.94, m         | 2.93, m         |                 |
| C'    | -               | -               | -               | -               | -               |                 |
| β-NH<sub>2</sub>-Ala9/β-Dab9/Dab9 |                 |                 |                 |                 |                 |                 |
| NH    | 8.92, d (8.3)   | 8.90, d (8.5)   | 8.17, d (5.5)   | 8.84, d (8.4)   | 8.93, d (8.3)   | 3.72/3.59       |
| α     | 4.79, m         | 4.79 m          | 3.72/3.56, m    | 4.82, m         | 4.82, m         | 4.23            |
| β     | 3.38/3.34, d/d  | 3.36, m         | 4.24, m         | 3.37, m         | 3.37, m         |                 |
| C'    | -               | -               | -               | -               | -               |                 |
| Leu/ile/Val10 |                 |                 |                 |                 |                 |                 |
| NH    | 7.94, d (7.3)   | 7.94, d (6.7)   | 8.71, d (6.9)   | 7.68, d (7.6)   | 7.73, d (7.9)   | 8.74, d (5.8)   |
| α     | 4.37, m         | 4.37, m         | 4.21, m         | 4.34, m         | 4.15, m         | 4.01, m         |
| β     | 1.57, m         | 1.57, m         | 1.88, m         | 1.89, m         | 2.04, (a)       | 2.03, (a)       |
| γ     | 1.56, m         | 1.56, m         | 1.36/1.17, m/m  | 1.34/1.15, m/m  | 0.89, d (1.6)   | 0.97, b         |
| γ'    | -               | -               | 0.93, m         | 0.83, m         | 0.88, d (1.6)   | 0.90, b         |
| δ     | 0.91, d (6.3)   | 0.90, d (6.5)   | 0.89, m         | 0.89, m         |                 |                 |
| δ'    | 0.87, m         | 0.88, m         | -               | -               | -               |                 |
| C'    | -               | -               | -               | -               | -               |                 |
| β-OH-Asp11 |                 |                 |                 |                 |                 |                 |
| NH    | 7.97, d (8.8)   | 7.98, d (8.5)   | 7.97, d (8.0)   | 7.98, d (8.0)   | 7.97, b         |                 |
| α     | 4.41, b         | 4.43, b         | 4.52, b         | 4.45, b         | 4.46, b         |                 |
| β     | -               | -               | -               | -               | -               |                 |
| C'    | -               | -               | -               | -               | -               |                 |
| Leu12 |                 |                 |                 |                 |                 |                 |
| NH    | 8.14, b         | 8.15, d (7.9)   | 8.05, d (8.0)   | 8.10, d (7.6)   | 8.02, b         |                 |
| α     | 4.42, b         | 4.42, b         | 4.45, b         | 4.466, b        | 4.47, b         |                 |
| β     | 1.64, m         | 1.65, m         | 1.67, m         | 1.65, m         | 1.65, m         |                 |
| γ     | 1.64, m         | 1.65, m         | 1.67, m         | 1.65, m         | 1.67, m         |                 |
| δ     | 0.93, d (6.3)   | 0.93, d (6.3)   | 0.94, b         | 0.927, d (2.1)  | 0.93, b         |                 |
| δ'    | 0.88, m         | 0.88, m         | 0.88, b         | 0.88, b         | 0.87, b         |                 |
| C'    | -               | -               | -               | -               | -               |                 |
| β-OH-Asp13 |                 |                 |                 |                 |                 |                 |
| NH    | 7.68, d (5.9)   | 7.69, d (8.9)   | 7.78, d (8.6)   | 7.71, b         | 7.71, d (9.3)   |                 |
| α     | 4.84, m         | 4.85, m         | 4.79, m         | 4.82, m         | 4.82, m         |                 |
| β     | 4.33, b         | 4.33, m         | 4.36, m         | 4.36, m         | 4.36, m         |                 |
| γ     | -               | -               | -               | -               | -               |                 |
| δ     | 0.93, d (6.3)   | 0.93, d (6.3)   | 0.94, b         | 0.927, d (2.1)  | 0.93, b         |                 |
| C'    | -               | -               | -               | -               | -               |                 |
| β-OH-ile14 |                 |                 |                 |                 |                 |                 |
| NH    | 7.88, d (8.3)   | 7.88, d (8.9)   | 7.83, d (8.5)   | 7.89, d (9.3)   | 7.86, d (8.6)   |                 |
| α     | 4.40, b         | 4.41, b         | 4.42, b         | 4.41, b         | 4.40, b         |                 |
| β     | -               | -               | -               | -               | -               |                 |
| β-Me  | 1.25, s         | 1.25, s         | 1.21, s         | 1.23, s         | 1.23, s         |                 |
| γ     | 1.58/1.53, m    | 1.58/1.54, m    | 1.55, m         | 1.57, m         | 1.56, m         |                 |
| δ     | 0.85, m         | 0.86, m         | 0.85, m         | 0.86, m         | 0.86, m         |                 |
| C'    | -               | -               | -               | -               | -               |                 |

<sup>a</sup>Abbreviations: b = broad signal; a = <sup>1</sup>H-signal below solvent signal; n.a. = not assigned
Tab. S3 ¹³C-NMR spectroscopic data of chitinopeptins A-D in a mixture of D₂O and CD₃CN in a ratio of 1:1. Chitinopeptin A: 176.05 MHz, 299 K; chitinopeptins B-D: 125.82 MHz, 300 K. ¹³C-chemical shifts were referenced to the solvent signal (CD₃CN, ¹³C: 1.30 ppm). The chitinopeptin D sample was a mixture of at least four derivatives with chitinopeptin D1 being the major component. Only the relevant part (Val₁₀-Dabs₉) of chitinopeptin D2 was assigned due to the signal overlap.²

| Pos. | Chitinopeptin A Δ₀, mult. | Chitinopeptin B Δ₀, mult. | Chitinopeptin C1 Δ₀, mult. | Chitinopeptin C2 Δ₀, mult. | Chitinopeptin D1 Δ₀, mult. | Chitinopeptin D2 Δ₀, mult. |
|------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| FA   | 177.81, C                 | 177.79, C                 | 174.27, C                 | 174.35, C                 | n.a.                      |                           |
|      | 36.08, CH                 | 36.05, CH                 | 43.85, CH                 | 43.80, CH                 | 43.80, CH                 |                           |
|      | 14.77, CH₃                | 14.76, CH₃                |                          |                           |                           |                           |
| 2-Me | 55.85, CH                 | 55.60, CH                 | 69.19, CH                 | 69.18, CH                 | 69.19                     |                           |
|      | 32.37, CH₃                | ~32.2 (b), CH₃            | 37.49, CH₃                | 37.49, CH₃                | 37.48, CH                 |                           |
| 3    | 26.29, CH                 | 26.25, CH                 | 26.02, CH                 | 25.99, CH                 | 25.99, CH                 | 30.28, CH                 |
|      | 30.11, CH₃                | 29.79, CH₃                | 30.28, CH                 | 30.28, CH                 |                           |                           |
| 7    | 27.56, CH                 | 29.46, CH                 | 27.83, CH                 | 27.83, CH                 | 27.83, CH                 |                           |
| 8    | 39.49, CH₃                | 32.52, CH₃                | 39.52, CH₃                | 39.52, CH₃                | 39.52, CH                 |                           |
|      | 28.37, CH                 | 23.09, CH                 | 28.47, CH                 | 28.47, CH                 | 28.47, CH                 |                           |
|      | 22.84, CH₃                | 14.31, CH₃                | 22.88, CH₃                | 22.88, CH₃                | 22.88, CH₃                |                           |
| 11   | 22.81, CH₃                |                          | 22.88, CH₃                | 22.88, CH₃                | 22.88, CH₃                |                           |
| Dab0 | NH                        |                           |                           |                           |                           |                           |
|      | α                         | 48.52, CH                 | 48.67, CH                 | 48.68, CH                 |                           |                           |
|      | β                         | 41.02, CH₃                | 41.07, CH₃                | 41.04, CH₃                |                           |                           |
|      | C                         | 171.75, C                 | 171.76, C                 | 171.76, C                 |                           |                           |
| N-Me-Val1 | NMe                  | 31.64, CH₃                | 31.65, CH₃                | 31.44, CH₃                | 31.60, CH₃                | 31.65, CH₃                |
|      | a                         | 63.22, CH                 | 63.21, CH                 | 63.88, CH                 | 64.14, CH                 | 64.17, CH                 |
|      | β                         | 26.70, CH                 | 26.71, CH                 | 26.98, CH                 | 26.90, CH                 | 26.93, CH                 |
|      | γ                         | 19.74, CH₃                | 19.73, CH₃                | 19.51, CH                 | 19.60, CH                 | 19.53, CH                 |
|      | β                         | 19.30, CH                 | 18.28, CH                 | 18.06, CH                 | 18.23, CH                 | 18.09, CH                 |
|      | C                         | 172.16, C                 | 172.11, C                 | 171.90, C                 | 172.17, C                 | 171.76, C                 |
| Thr2 | NH                        |                           |                           |                           |                           |                           |
|      | α                         | ~56.4 (b), CH             | ~56.3 (b), CH             | 57.14, CH                 | 56.56, CH                 | 56.34, CH                 |
|      | β                         | 73.06, CH                 | 73.03, CH                 | 71.96, CH                 | 72.21, CH                 | 72.10, CH                 |
|      | γ                         | 15.40, CH                 | 15.44, CH₃                | 16.54, CH                 | 15.90, CH                 | 15.75, CH                 |
|      | C                         | 169.65, C                 | 169.57, C                 | 169.91, C                 | 169.88, C                 | 169.90, C                 |
| Ala₃ | NH                        |                           |                           |                           |                           |                           |
|      | α                         | 51.12, CH                 | 50.98, CH                 | 50.03, CH                 | 50.48, CH                 | 50.55, CH                 |
|      | β                         | 17.90, CH₃                | 17.86, CH                 | 18.93, CH₃                | 18.34, CH₃                | 18.20, CH₃                |
|      | C                         | 175.19, C                 | 175.10, C                 | 174.50, C                 | 174.97, C                 | 175.00, C                 |
| β-OH-Asp4 | NH                  | 57.36, CH                 | 57.24, CH                 | 56.59, CH                 | 56.86, CH                 | 56.84, CH                 |
|      | β                         | 72.56, CH                 | 72.45, CH                 | 72.85, CH                 | 72.00, CH                 | 72.44, CH                 |
|      | γ                         | 176.69, C                 | 176.7 (b), C              | 177.11, C                 | 176.76, C                 | n.a.                      |
|      | C                         | 173.27, C                 | 173.20, C                 | 172.47, C                 | 172.95, C                 | n.a.                      |
| β-OH-Phe5 | NH                  | 61.62, CH                 | 61.50, CH                 | 61.26, CH                 | 61.64, CH                 | 61.53, CH                 |
|      | β                         | 73.78, CH                 | 73.66, CH                 | 73.91, CH                 | 73.91, CH                 | 73.95, CH                 |
|      | γ                         | 140.39, C                 | 140.34, CH                | 140.32, C                 | 140.17, C                 | 140.17, C                 |
|      | δ                         | 128.23, CH                | 128.25, CH                | 128.05, CH                | 128.26, CH                | 128.25, C                 |
|      | ε                         | 129.49, CH                | 129.11, CH                | 129.58, CH                | 129.54, CH                | ~129.5 (a), CH            |
|      | ζ                         | 129.38, CH                | 129.42, CH                | 129.42, CH                | 129.53, CH                | ~129.5 (a), CH            |
|      | C                         | 171.29, C                 | 171.24, CH                | 171.58, C                 | 171.65, C                 | 171.57, C                 |
| Ile6 | NH                        |                           |                           |                           |                           |                           |
|      | α                         | 57.48, CH                 | 57.39, CH                 | 57.89, CH                 | 57.47, CH                 | 57.49, CH                 |
|      | β-Me                      | 36.57, CH                 | 36.55, CH                 | 36.70, CH                 | 36.50, CH                 | 36.55, CH                 |
|      | γ                         | 14.46, CH₃                | 14.45, CH₃                | 14.58, CH₃                | 14.42, CH₃                | 14.45, CH₃                |
|      | δ                         | 26.21, CH₃                | 26.22, CH₃                | 26.12, CH₃                | 26.12, CH₃                | 26.13, CH₃                |
|      | C₉                        | 174.31, C                 | 174.25, C                 | 174.17, C                 | 174.12, C                 | 174.11, C                 |
| Position | Chitinopeptin A | Chitinopeptin B | Chitinopeptin C1 | Chitinopeptin C2 | Chitinopeptin D1 | Chitinopeptin D2 |
|----------|----------------|----------------|----------------|----------------|----------------|----------------|
| Ser7     |                |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 56.71, CH      | 56.60, CH      | 57.02, CH      | 56.98, CH      | 56.98, CH      | 56.98, CH      |
| β        | 62.55, CH      | 62.40, CH      | 62.09, CH      | 62.40, CH      | 62.36, CH      | 62.36, CH      |
| C'       | 173.16, C      | 173.10, C      | 172.77, C      | 173.09, C      | 173.09, C      | 173.09, C      |
| Lys8     |                |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 54.75, CH      | 54.64, CH      | 53.98, CH      | 54.93, CH      | 55.03          | 55.03          |
| β        | 31.26, CH      | 31.20, (CH)    | 31.07, CH      | 31.18, CH      | 31.16, CH      | 31.16, CH      |
| γ        | 22.97, CH      | 22.97, CH      | 22.92, CH      | 22.95, CH      | 22.97, CH      | 22.97, CH      |
| δ        | 27.06, CH      | 26.91, CH      | 26.88, CH      | 26.98, CH      | 26.98, CH      | 26.98, CH      |
| ε        | 40.31, CH      | 40.02, CH      | 39.91, CH      | 40.04, CH      | 39.93, CH      | 39.93, CH      |
| C'       | 175.32, C      | 175.25, C      | 174.85, C      | 175.46, C      | 175.48, C      | 175.48, C      |
| β-NH₂-Ala9/β-Dab9/Dab9 |        |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 51.28, CH      | 51.03, CH      | 41.07, CH      | 51.05, CH      | 50.99, CH      | 50.99, CH      |
| β        | 40.76, CH      | 40.48, CH      | 53.70, CH      | 40.34, CH      | 40.31, CH      | 53.67          |
| C'       | 170.76, C      | 170.67, C      | 168.96, C      | 170.78, C      | 170.71, C      | 169.0 (a)      |
| Leu1/Leu/Val10 |               |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 53.64, CH      | 53.54, CH      | 59.84, CH      | 58.62, CH      | 60.52, CH      | 61.7 (a)       |
| β        | 40.57, CH      | 40.52, CH      | 36.61, CH      | 37.43, CH      | 31.00, CH      | 30.3 (a)       |
| γ        | 25.18, CH      | 25.20, CH      | 15.33, CH      | 14.97, CH      | 19.30, CH      | 19.3 (a)       |
| δ        | 23.12, CH      | 23.12, CH      | 11.68, CH      | 11.79, CH      | 11.79, CH      |                |
| ε        | 21.82, CH      | 21.80, CH      |                |                |                |                |
| C'       | 174.57, C      | 174.49, C      | 174.02, C      | 173.47, C      | 173.55, C      | 174.17          |
| β-OH-Asp11 |                |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 57.18, CH      | 57.05, CH      | 57.42, CH      | 57.20, CH      | 57.37, CH      | 57.37, CH      |
| β        | 72.06, CH      | 71.94, CH      | 72.29, CH      | 72.48, CH      | 72.10, CH      | 72.10, CH      |
| γ        | 176.80, C      | ~176.8 (b), C | 176.68, C      | 176.76, C      | n.a.           |                |
| C'       | 172.33, C      | 172.25, C      | 172.05, C      | 172.29, C      | n.a.           |                |
| Leu12    |                |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 53.23, CH      | 53.11, CH      | 53.14, CH      | 52.91, CH      | ~53.0 (a), CH  |                |
| β        | 40.37, CH      | 40.33, CH      | 40.71, CH      | 40.46, CH      | 40.57, CH      |                |
| γ        | 25.18, CH      | 25.18, CH      | 25.23, CH      | 25.14, CH      | 25.14, CH      | 25.14, CH      |
| δ        | 23.34, CH      | 23.34, CH      | 23.37, CH      | 23.37, CH      | 23.36, CH      | 23.36, CH      |
| ε        | 21.52, CH      | 21.51, CH      | 21.58, CH      | 21.49, CH      | 21.53, CH      | 21.53, CH      |
| C'       | 174.55, C      | 174.49, C      | 174.66, C      | 174.58, C      | n.a.           |                |
| β-OH-Asp13 |               |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 56.71, CH      | 56.60, CH      | 56.86, CH      | 56.81, CH      | ~56.8 (a), CH  |                |
| β        | 72.88, CH      | 72.77, CH      | 72.51, CH      | 72.68, CH      | 72.7 (a), CH   |                |
| γ        | 176.82, C      | ~176.8 (b), C | 176.88, C      | 176.88, C      | n.a.           |                |
| C'       | 171.94, C      | 171.87, C      | 171.84, C      | 171.98, C      | n.a.           |                |
| β-OH-Ile14 |               |                |                |                |                |                |
| NH       |                |                |                |                |                |                |
| α        | 60.68, CH      | 60.59, CH      | 60.04, CH      | 60.29, CH      | 60.35, CH      |                |
| β        | 74.68, C       | 74.61, C       | 74.72, C       | 74.63, C       | 74.58, C       |                |
| β-Me     | 23.34, CH      | 23.28, CH      | 23.25, CH      | 23.28, CH      | 23.26, CH      |                |
| γ        | 32.69, CH      | 32.65, CH      | 32.61, CH      | 32.64, CH      | 32.64, CH      |                |
| δ        | 8.41, CH       | 8.39, CH       | 8.35, CH       | 8.35, CH       | 8.35, CH       |                |

*aAbbreviations: b = broad signal; a = 13C-signal below solvent signal; n.a. = not assigned*
Tab. S4 Bioactivity screening data of chitinopeptins A-D.*

| Organism and genotype | MIC (µg/mL) | Iron Derivatives | Chitinopeptin | Reference data |
|-----------------------|-------------|------------------|---------------|----------------|
|                        |             | A | B | C1+C2 | D1+D2 | Rifamicin | Tetracycline | Gentamycin/ Isoniazid |
| **E. coli ATCC 35218** | >64         | n.d. | >64 | n.d. | >64 | n.d. | 4 | 4 | 0.125 |
| **E. coli ATCC 25922 ΔTolC** | >64 | n.d. | >64 | n.d. | >64 | n.d. | 2 | 0.5 | 0.25 |
| **E. coli MG1655** | >256 | n.d. | n.d. | n.d. | n.d. | n.d. | 16 | 0.5 | 0.5 |
| **P. aeruginosa ATCC 27853** | >64 | n.d. | >64 | n.d. | >64 | n.d. | 32 | 64 | 1 |
| **P. aeruginosa PAC750** | >64 | n.d. | n.d. | n.d. | n.d. | n.d. | 16 | 0.25 | 0.125 |
| **K. pneumoniae ATCC 13883** | >64 | n.d. | n.d. | n.d. | n.d. | n.d. | 8 | 2 | ≤0.031 |
| **M. catarrhalis ATCC 25238** | 2 | 4 | 2 | n.d. | 2 | n.d. | ≤0.031 | 1 | 0.125 |
| **A. baumannii ATCC 19606** | 16 | n.d. | 64 | n.d. | 16 | n.d. | 2 | 16 | 16 |
| **B. subtilis DSM 10** | 4 | 8 | 4 | n.d. | 4 | n.d. | ≤0.031 | 4 | ≤0.031 |
| **S. aureus ATCC 25923** | >64 | n.d. | >64 | n.d. | >64 | n.d. | ≤0.031 | 1 | 0.063 |
| **M. luteus DSM 20030** | 32 | n.d. | 32 | n.d. | 32 | n.d. | ≤0.031 | 0.5 | ≤0.031 |
| **L. monocytogenes DSM 20600** | >64 | n.d. | n.d. | n.d. | n.d. | n.d. | ≤0.031 | 0.5 | ≤0.031 |
| **M. smegmatis ATCC 607** | >64 | n.d. | >64 | n.d. | >64 | n.d. | 8 | 0.5 | 1/2 |
| **C. albicans FH2173** | 4 | 32 | 8 | n.d. | 16 | n.d. | 2<sup>a</sup> | 4<sup>d</sup> | 16<sup>d</sup> |
| **A. flavus ATCC 9170** | >64 | n.d. | >64 | n.d. | 64 | n.d. | 2<sup>a</sup> | 16-8<sup>a</sup> | 16<sup>a</sup> |
| **Z. tritici MUCL45407** | 16 | 32-16 | 16 | n.d. | 16-8 | n.d. | 0.5<sup>c</sup> | 0.031<sup>c</sup> | 0.5<sup>c</sup> |
| **F. oxysporum ATCC 7601** | >64 | n.d. | >64 | n.d. | >64 | n.d. | >64<sup>d</sup> | 4<sup>a</sup> | 4<sup>d</sup> |

*Abbreviation: n.d. = not determined
Tab. S5 Partial Clustal W alignment (111) of the proposed dinuclear binding motif (His-X-His-X-Asp-His) by Makris et al. (2010) (79) in all three diiron-monoxygenases with other homologs in NRPS antibiotic biosynthetic pathways.

| Species                        | Antibiotic      | Protein-ID         | Consensus sequence |
|--------------------------------|-----------------|--------------------|--------------------|
| Chitinophaga eiseniae          | Chitinopeptin A-B | SKA37886.1         | H^{303}-N-H-Q-D-H  |
| Chitinophaga flavae            | Chitinopeptin C-D | RBL90130.1         | H^{303}-N-H-Q-D-H  |
| Chitinophaga oryziterrae       | /               | MVT41610.1         | H^{301}-N-H-Q-D-H  |
| Chitinophaga nistansis         | /               | PSL45608.1         | H^{301}-N-H-Q-D-H  |
| Streptomyces venezuelae        | Chloramphenicol  | CAE54208.1         | H^{305}-N-H-Q-D-H  |
| Actinoplanes teichomycetius    | Teicoplanin      | CAE53366.1         | H^{301}-G-H-S-D-H  |
| Nonomuraea sp.                 | M40926          | CAD91223.1         | H^{301}-G-H-S-D-H  |
| Streptomyces toyacaensis       | M47934          | AAM80528.1         | H^{301}-G-H-S-D-H  |
| Burkholderia pyrocinia         | Occidiofungin    | KFL51884.1         | H^{305}-S-H-H-D-H  |
| Lysobacter sp.                 | Lysobactin       | AEH59101.1         | H^{303}-P-H-Q-D-H  |
| Burkholderia thailandensis     | Bactobolin       | ABC35075.1         | H^{305}-S-H-H-D-H  |
### Tab. S6 Manual BLASTp search results between chitinopeptin enzymes no. 5+6 and reported enzymes to L-Dap biosynthesis.

| Organism                        | Related products | Homologous enzymes   | Protein-ID                  | Identities (enzyme no. 5/ no. 6) | Positives (enzyme no. 5/ no. 6) | Ref. |
|---------------------------------|------------------|----------------------|----------------------------|----------------------------------|---------------------------------|-----|
| Staphylococcus aureus           | Staphylococcus   | Zwittermycin         | Zwa5A/Zwa5B, ACM79805/ACM79806 | 48/48/52/49/52                  | 49/49/52/50/51                  |     |
| Bacillus thuringiensis          | Z. W. B.        | Capresoporin         | CmnB/CmnK, ABR67745/ABR67754 | 33/32/24/33/23                  | 49/49/50/51/49                  |     |
| Streptomyces sp.                | Viomycin         | VioB/VioK, ABR52482/ABR52501 | 30/26/24/30/23                | 47/47/49/49/49                  | 50/50/42/42/42                  |     |
| Amycolatopsis japonicum         | [S,S]‐EDDS       | AesC/AesA, AIG74590/AIG74588 | 23/24/24/25/24                | 46/46/50/50/51                  | 85/85/85/85/85                  |     |
| Streptomyces sp.                | VIHA             | MA_5143a_00503/00505 | -                           | 23/24/25/24/24                  | 46/45/45/45/45                  |     |
Tab. S7 Comparison of the amino acid prediction based on Stachelhaus code (88) and the assembled amino acids verified by NMR and Marfey’s analysis. Amino acids marked in grey are most likely all L-Dap based on the same Stachelhaus code.

| Chitinopeptin A & B | C. flava | C. oryziterrae | C. niastensis |
|---------------------|---------|----------------|--------------|
| NMR predicted       | Stachelhaus predicted | NMR predicted | Stachelhaus predicted | NMR predicted | Stachelhaus predicted |
| 2S                  | Val (nMT) | DAPWIGTTRK     | Val (nMT)    | DAPWIGTTRK     | Val (nMT)    | DAPWIGTTRK     |
| 2R,3R Thr           | Thr (E)  | DFWNGMVHK      | Thr (E)      | DFWNGMVHK      | Thr (E)      | DFWNGMVHK      |
| 2R Ala              | Ser (E)  | DWHSLIDK       | Ser (E)      | DWHSLIDK       | Ser (E)      | DWHSLIDK       |
| 2S,3S β-OH-Asp (Hya) | Asp     | DLTKGHGK       | Asp          | DLTKGHGK       | Asp          | DLTKGHGK       |
|                    | -        | -              | -            | -              | -            | -              |
| 2S,3R β-OH-Phe      | Phe      | DAYVAAVVK      | Phe          | DAYVAAVVK      | Phe          | DAYVAAVVK      |
| 2S                  | Ile (E)  | DAPFGLITFK     | Ile (E)      | DAPFGLITFK     | Ile (E)      | DAPFGLITFK     |
| 2S                  | Ser      | DWHSLIDK       | Ser          | DWHSLIDK       | Ser          | DWHSLIDK       |
| 2S                  | Lys      | DAYVEDGEVK     | Lys          | DAYVEDGEVK     | Lys          | DAYVEDGEVK     |
| 2S                  | Leu      | DAWFLGNVK      | Leu          | DAWFLGNVK      | Leu          | DAWFLGNVK      |
| 2S,3S β-OH-Asp (Hya) | Asp     | DLTKGHGK       | Asp          | DLTKGHGK       | Asp          | DLTKGHGK       |
| 2S                  | Val      | DAWFLGNVK      | Val          | DAWFLGNVK      | Val          | DAWFLGNVK      |
| 2S,3S β-OH-Lys      | Lys      | DAWFLGNVK      | Lys          | DAWFLGNVK      | Lys          | DAWFLGNVK      |
| 2S                  | Tyr      | DAYVEDGEVK     | Tyro (E)     | DAYVEDGEVK     | Tyro (E)     | DAYVEDGEVK     |
| 2S                  | Dap      | DAWFLGNVK      | Dap          | DAWFLGNVK      | Dap          | DAWFLGNVK      |
|                    | -        | -              | -            | -              | -            | -              |

Chitinopeptin A & B: NMR predicted.
C. flava: Predicted Stachelhaus code.
C. oryziterrae: Predicted Stachelhaus code.
C. niastensis: Predicted Stachelhaus code.
4. Synthesis of β-hydroxyamino acids (Phe, Ile, Asp)

4.1. Synthetic procedures and compound characterization

4.1.1. General Methods

All chemicals and solvents/anhydrous solvents were commercially supplied and used without further purification. Reactions were monitored using thin layer chromatography (TLC) or using Agilent 1100 series LCMS with UV detection at 254 nm and a low resonance electrospray mode (ESI). TLC was performed on pre-coated silica gel glass plates (Merck TLC Silica gel 60 F254) and compounds were detected under UV light (254 nm) and/or by staining with an aqueous solution of Phosphomolybdic acid, Cerium(IV) sulfate and H₂SO₄ followed by heating with a heat gun. Products were purified by using an automated flash column chromatography system (puriFlash® XS520Plus from Intechim) equipped with PF-15SIHC flash columns of different sizes from Interchim (eluants are given in parentheses). Alternatively, purification was performed by HPLC with a Waters AutoPurification HPLC/MS system using RP-18 columns with H₂O/TFA 0.1% as mobile phase A and acetonitrile as mobile phase B. The product containing fractions were collected and freeze-dried. NMR spectra were recorded on a Bruker AVANCE II WB spectrometer (400 MHz), a AVANCE III HD spectrometer (400 MHz) or a AVANCE III spectrometer (500 MHz) equipped with a TCI CryoProbe with CDCl₃, D₂O or DMSO-d₆ as solvent with chemical shifts (δ) quoted in parts per million (ppm) and referenced to the solvent signal (δ¹H/¹³C: CDCl₃ 7.26/77.16, δ¹H: D₂O 4.79, δ¹H/¹³C: DMSO-d₆ 2.50 / 39.52 ppm) or TSPA (δ¹H/¹³C: 0.00/0.00) as external standard. Assignment was confirmed based on COSY, ROESY, HSQC and HMBC correlations. High resolution mass spectrometry was performed on a maXis II (Bruker) ESI TOF MS equipped with 1290 UPLC (Agilent) with DAD and ELSD. Specific rotation was measured by a polarimeter (P 3000 series) from Krüss.
4.1.2. Preparation of the β-hydroxyaspartic acids

All four stereoisomers of β-hydroxyaspartic acid were obtained according to a modified procedure described by Breuning et al. Commercial available (−)-dibenyl D-tartrate or (+)-dibenyl L-tartrate were chosen as starting material since the benzyl esters allow for accurate ee-determination by chiral HPLC (due to their UV absorption at a detection wavelength of 207 nm) and traceless deprotection accompanied by azide reduction via catalytic hydrogenation as a last step. While the anti-isomers are directly accessible, the syn-isomers were obtained by selective base-induced epimerization of the azido-intermediates and separation of the two isomers by HPLC (Scheme S1). The high measured ee values of the products SI2, SI4, ent-SI2 and ent-SI4 proved a selective epimerization at the azido-bearing 2-position.

Scheme S1: Preparation of the β-hydroxyaspartic acids, exemplarily shown for one diastereomeric series.

Conditions: a) SOCl₂, DCM; b) NaN₃, DMF; c) DBU, THF, 40 °C; d) H₂, Pd/C; 1 M HClaq, 1,4-dioxane.

Breuning, A., Vicik, R. & Schirmeister, T. An improved synthesis of aziridine-2,3-dicarboxylates via azido alcohols—epimerization studies. *Tetrahedron: Asymmetry* **14**, 3301–3312; 10.1016/j.tetasy.2003.09.015 (2003).
Dibenzyl (2S,3R)-2-azido-3-hydroxysuccinate (SI2):

SOCl₂ (0.389 mL, 5.45 mmol, 1.20 eq.) was added to a solution of (+)-dibenzyl L–tartrate (SI1, 1.50 g, 4.54 mmol, 1.00 eq.) in anhydrous DCM (20 mL) at room temperature. A catalytic amount of anhydrous DMF (1 drop) was added and the reaction mixture was stirred for 7 h, until LC-MS indicated complete conversion. The reaction mixture was concentrated in vacuo and crude cyclic sulfite was used for the next step without further purification.

The crude product was dissolved in anhydrous DMF (4 mL), NaN₃ (0.590 g, 9.08 mmol, 2.00 eq.) was added and the reaction mixture was stirred overnight at room temperature, until LC-MS indicated complete conversion. DCM (10 mL) and H₂O (3 mL) were added and the reaction mixture was allowed to stir for 2 h. Ethyl acetate (50 mL) was added and the mixture was washed with saturated aqueous NaCl (2 x 20 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (0–50% ethyl acetate in n-heptane) to give SI2 (1.04 g, 2.92 mmol, 64% over two steps) as colorless solid.

¹H-NMR (CDCl₃, 400 MHz): 7.38–7.31 (m, 6H, aryl-H), 7.31–7.25 (m, 4H, aryl-H), 5.13 (dd, 1H, J = 11.9 Hz, CH₂), 5.08 (s, 2H, CH₂), 5.02 (dd, 1H, J = 11.9 Hz, CH₂), 4.68 (d, 1H, J = 2.7 Hz, CH), 4.37 (d, 1H, J = 2.7 Hz, CH), 3.15 (s, br, 1H, O-H); ¹³C-NMR (CDCl₃, 100 MHz): 170.7 (CO), 166.9 (CO), 134.6 (aryl-C₂), 134.4 (aryl-C₂), 129.0 (aryl-C), 128.9 (aryl-C), 128.9 (aryl-C), 128.8 (aryl-C), 128.8 (aryl-C), 72.2 (CH), 68.6 (CH₂), 68.3 (CH₂), 64.5 (CH); HRMS (ESI) m/z calcd. for C₁₈H₁₇N₃O₅Na+: (M+Na)⁺, 378.1060; found: 378.1067 (M+Na)⁺; Rₜ (n-heptane/ethyl acetate 2:1): 0.37; Chiral HPLC (Chiralpak AS-H/122, 250x4.6 mm; EtOH:MeOH 1:1): SI2 (Rₜ = 3.9 min) : ent-SI2 (Rₜ = 4.3 min) 100 : 0 (> 99% ee); Specific rotation [α]D²⁰.₆ = + 19.8 (c = 1.11; CHCl₃).  

Dibenzyl (2S,3R)-2-azido-3-hydroxysuccinate (SI2) and dibenzyl (2R,3R)-2-azido-3-hydroxysuccinate (SI4):

SI2 (0.300 g, 0.844 mmol, 1.00 eq.) was dissolved in anhydrous THF (10 mL), DBU (0.382 mL, 2.53 mmol, 3.00 eq.) was added and the reaction mixture was stirred at 40 °C. After 1 h the epimerization was stopped by addition of ethyl acetate (50 mL) and 1 M aqueous HCl (10 mL). The layers were separated, the organic layer was washed with saturated aqueous NaCl (10 mL), dried over MgSO₄, filtered and concentrated
under reduced pressure. The crude product was purified via HPLC (RP-18, 15 min, 50-80% MeCN in H₂O/TFA 0.1%) to yield SI2 (0.065 g, 0.18 mmol, 22%) as colorless solid and SI4 (0.092 g, 0.26 mmol, 31%) as colorless oil.

**Dibenzyl (2R,3R)-2-azido-3-hydroxysuccinate (SI4)**

\[
\text{BnO} \quad \text{O} \quad \text{OH} \quad \text{O} \quad \text{Bn} \\
\text{SI4}
\]

\( ^1\text{H-NMR} \) (CDCl₃, 400 MHz): 7.42–7.32 (m, 10H, aryl-H), 5.28 (s, 2H, \( \text{CH}_2 \)), 5.27 (d, 2H, \( J = 2.1 \text{ Hz} \), \( \text{CH}_2 \)), 4.81 (d, 1H, \( J = 2.3 \text{ Hz} \), \( \text{CH} \)), 4.25 (d, 1H, \( J = 2.3 \text{ Hz} \), \( \text{CH} \)), 3.20 (s, br, 1H, \( \text{OH} \)); \( ^{13}\text{C-NMR} \) (CDCl₃, 100 MHz): 171.1 (C=O), 167.4 (C=O), 134.8 (aryl-C₆), 134.6 (aryl-C₆), 129.1 (aryl-C), 128.9 (aryl-C), 128.8 (aryl-C), 128.7 (aryl-C), 128.6 (aryl-C), 72.2 (CH), 68.6 (CH₂), 68.3 (CH₂), 63.5 (CH); \( \text{HRMS (ESI)} \) m/z calcd. for \( \text{C}_{18}\text{H}_{17}\text{N}_{3}\text{O}_{5}\text{Na} \): (M+Na)\(^{+}\), 378.1060; found: 378.1065 (M+Na)\(^{+}\); \( R_f \) (n-heptane/ethyl acetate 2:1): 0.38; \( \text{Chiral HPLC} \) (Chiralpak AS-H/148, 250x4.6 mm; EtOH:MeOH 1:1): SI4 (Rt = 6.8 min) : \( \text{ent-SI4} \) (Rt = 5.5 min) 99.3 : 0.7 (99% ee); \( \text{Specific rotation} \) \( [\alpha]_D^{20.6} = +59.9 \) (c = 0.87; CHCl₃).

**Dibenzyl (2R,3S)-2-azido-3-hydroxysuccinate (ent-SI2) and dibenzyl (2S,3S)-2-azido-3-hydroxysuccinate (ent-SI4):**

SOCl₂ (0.389 mL, 5.45 mmol, 1.20 eq.) was added to a solution of (−)-dibenzyl d-tartrate (ent-SI1, 1.50 g, 4.54 mmol, 1.00 eq.) in anhydrous DCM (20 mL) at room temperature. A catalytic amount of anhydrous DMF (1 drop) was added and the reaction mixture was stirred for 7 h, until LC-MS indicated complete conversion. The reaction mixture was concentrated in vacuo and crude cyclic sulfite was used for the next step without further purification.

The crude product was dissolved in anhydrous DCM (4 mL), NaN₃ (0.590 g, 9.08 mmol, 2.00 eq.) was added and the reaction mixture was stirred overnight at room temperature, until LC-MS indicated complete conversion. DCM (10 mL) and H₂O (3 mL) were added and the reaction mixture was allowed to stir for 2 h. Ethyl acetate (50 mL) was added and the mixture was washed with saturated aqueous NaCl (2 x 20 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product (1.62 g, 4.54 mmol), quant.) was divided in two parts: 400 mg (1.13 mmol) were used for the epimerization (see below), the rest (1.22 g, 3.41 mmol) was purified via HPLC (RP-18, 15 min, 55-75% MeCN in H₂O/TFA 0.1%) to yield \( \text{ent-SI2} \) (0.470 g, 1.32 mmol, 39% over two steps) as colorless solid.
Crude \textit{ent-SI2} (0.400 g, 1.13 mmol, 1.00 eq.) was dissolved in anhydrous THF (10 mL), DBU (0.509 mL, 3.38 mmol, 3.00 eq.) was added and the reaction mixture was stirred at 40 °C. After 1 h the epimerization was stopped by addition of ethyl acetate (50 mL) and 1 M aqueous HCl (10 mL). The layers were separated, the organic layer was washed with saturated aqueous NaCl (10 mL), dried over MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified \textit{via} HPLC (RP-18, 15 min, 60-70% MeCN in H$_2$O/TFA 0.1%) to yield \textit{ent-SI2} (0.077 g, 0.22 mmol, 19% over three steps) as colorless solid and \textit{ent-SI4} (0.115 g, 0.324 mmol, 29% over three steps) as colorless oil.
Dibenzyl (2R,3S)-2-azido-3-hydroxysuccinate (ent-SI2)

The NMR data are identical to the ones reported for SI2.

HRMS (ESI) m/z calcd. for C_{18}H_{17}N_{3}O_{5}Na: (M+Na)^+; 378.1060; found: 378.1066 (M+Na)^+; \textbf{R}_{f} (n-heptane/ethyl acetate 2:1): 0.37; \textbf{Chiral HPLC} (Chiralpak AS-H/122, 250x4.6 mm; EtOH:MeOH 1:1): \textbf{ent-SI2} (Rt = 4.3 min) : SI2 (Rt = 3.9 min): 99.5 : 0.50 (99% ee); \textbf{Specific rotation} \([\alpha]_{D}^{20.6} = -19.3\) (c = 1.34; CHCl3).

Dibenzyl (2S,3S)-2-azido-3-hydroxysuccinate (ent-SI4)

The NMR data are identical to the ones reported for SI4.

HRMS (ESI) m/z calcd. for C_{18}H_{17}N_{3}O_{5}Na: (M+Na)^+; 378.1060; found: 378.1065 (M+Na)^+; \textbf{R}_{f} (n-heptane/ethyl acetate 2:1): 0.38; \textbf{Chiral HPLC} (Chiralpak AS-H/148, 250x4.6 mm; EtOH:MeOH 1:1): \textbf{ent-SI4} (Rt = 5.5 min) : SI4 (Rt = 6.8 min) 99.7 : 0.3 (99% ee); \textbf{Specific rotation} \([\alpha]_{D}^{20.6} = -67.9\) (c = 0.68; CHCl3).

\textbf{General procedure for hydrogenation:}

The acid SI2, \textbf{ent-SI2}, SI4 or \textbf{ent-SI4} (0.1–0.2 mmol) was dissolved in 1,4-dioxane (5 mL) and 1 M aqueous HCl (1 mL), Pd/C (10 %, 0.05 eq.) was added and the reaction mixture was hydrogenated at 4 bar pressure in an autoclave at room temperature. After reaction overnight, LC-MS indicated complete hydrogenation to SI3, \textbf{ent-SI3}, SI5 or \textbf{ent-SI5}, respectively. The reaction mixture was filtered, diluted with H2O (20 mL) and freeze-dried to yield the desired product (HCl salt, quant.) as colourless solid.

(2S,3R)-2-Amino-3-hydroxysuccinic acid ((2S,3R)-3-hydroxyaspartic acid) (SI3):

\(\text{H-NMR} (\text{D}_{2}O, 400 \text{ MHz}): 4.73 (\text{d}, 1\text{H}, J = 2.7 \text{ Hz, CH}); 4.59 (\text{d}, 1\text{H}, J = 2.7 \text{ Hz, CH}); \text{^{13}C-NMR} (\text{D}_{2}O, 100 \text{ MHz}): 176.1 (\text{COOH}), 71.7 (\text{CH}), 58.5 (\text{CH}); \text{HRMS (ESI)} m/z calcd. for C_{4}H_{8}NO_{5}: (M+H)^+; 150.0397; found: 150.0397 (M+H)^+; \textbf{Specific rotation} \([\alpha]_{D}^{20.8} = +46.6\) (c = 1.03; H2O).
(2R,3S)-2-Amino-3-hydroxysuccinic acid ((2R,3S)-3-hydroxyaspartic acid) (ent-SI3):

The NMR data are identical to the ones reported for SI3.

HRMS (ESI) m/z calcd. for C₄H₈NO₅: (M+H)⁺, 150.0397; found: 150.0397 (M+H)⁺; Specific rotation [α]²⁰⁺ = −44.3 (c = 0.65; H₂O).

(2R,3R)-2-Amino-3-hydroxysuccinic acid ((2R,3R)-3-hydroxyaspartic acid) (SI5):

¹H-NMR (D₂O, 400 MHz): 4.86 (d, 1H, J = 2.4 Hz, CH), 4.40 (d, 1H, J = 2.4 Hz, CH); ¹³C-NMR (D₂O, 100 MHz): 71.5 (CH), 58.4 (CH); HRMS (ESI) m/z calcd. for C₄H₈NO₅: (M+H)⁺, 150.0397; found: 150.0398 (M+H)⁺; Specific rotation [α]²⁰⁺ = +9.7 (c = 0.51; H₂O).

(2S,3S)-2-Amino-3-hydroxysuccinic acid ((2S,3S)-3-hydroxyaspartic acid) (ent-SI5):

The NMR data are identical to the ones reported for SI5.

HRMS (ESI) m/z calcd. for C₄H₈NO₅: (M+H)⁺, 150.0397; found: 150.0397 (M+H)⁺; Specific rotation [α]²⁰⁺ = −8.0 (c = 0.63; H₂O).
4.1.3. Preparation of the β-hydroxyphenylalanines

The L-isomers of the β-hydroxyphenylalanines and β-hydroxyisoleucines were synthesized by diastereoselective addition of Grignard reagents to orthoester protected L-serine aldehyde SI7, initially described by Blaskovich and Lajoie\(^2\). This synthetic pathway offers several advantages for our envisaged access to reference samples of these β-hydroxyamino acids:

- High \textit{threo}-selectivities are reported for the addition of Grignard reagents to aldehyde SI7 and ketones SI18 and SI25 (Scheme S2 and Scheme S3).
- The addition products are bearing the oxidation stage of the desired amino acids and only protecting group manipulations are required to obtain the reference samples. In contrast to the commonly used synthetic strategy for β-hydroxyamino acids starting from Garner’s Aldehyde\(^3,4\), no protection of the secondary alcohol and no further oxidation step are required.
- The Cbz-group allows for accurate ee-determination by chiral HPLC (due to their UV absorption at a detection wavelength of 207 nm) and convenient deprotection via catalytic hydrogenation or under acidic conditions at the last step.
- The epimerization of aldehyde SI7 via simple flash chromatography on silica exhibits a practical access to racemic reference samples which are required for the ee-determination by chiral HPLC and to cover the corresponding \(R\)-series of the β-hydroxyamino acids.

\[ \text{Scheme S2. Preparation of \(\beta\)-hydroxyphenylalanines.}^a \]

\(^2\) Blaskovich, M. A. \textit{et al.} Stereoselective Synthesis of \textit{Threo} and \textit{Erythro} \(\beta\)-Hydroxy and \(\beta\)-Disubstituted-\(\beta\)-Hydroxy \(\alpha\)-Amino Acids. \textit{J. Org. Chem.} \textbf{63}, 3631–3646 (1998).

\(^3\) Garner, P. Stereocontrolled addition to a penallic acid equivalent: an asymmetric of \textit{threo-\(\beta\)-hydroxy-L-glutamic acid.} \textit{Tetrahedron Lett.} \textbf{25}, 5855–5858 (1984).

\(^4\) Passiniemi, M. & Koskinen A. M. P. Garner’s aldehyde as a versatile intermediate in the synthesis of enantiopure natural products. \textit{Beilstein J. Org. Chem.} \textbf{9}, 2641–2659 (2013).
Conditions: a) (COCl)$_2$, DMSO, DCM, $-78 \, ^\circ C$, 15 min; SI6, $-78 \, ^\circ C$, 90 min; DIPEA, $-78 \, ^\circ C$ to 0 $^\circ C$, 30 min; SI7 used as a crude; rac-SI7 purified by flash column chromatography; b) PhMgBr, THF, rt; c) 1 M HCl$_{aq}$, 1,4-dioxane; d) LiOH, MeCN/H$_2$O 4:1.

The synthesis of SI7 and rac-SI7 was performed in close analogy to a literature known procedure from SI6$^5$. To obtain both diastereomers in one reaction and in sufficient quantities, the addition of PhMgBr was performed at room temperature (Scheme S2). Under these conditions, a selectivity of 84:16 for SI8:SI9 is described$^2$. In our hands, complete separation of SI8 and SI9 via flash chromatography was difficult on 1 g-scale. Therefore, the mixture of SI8 and SI9 was directly treated by aqueous HCl to achieve orthoester hydrolysis and resulting products SI10 and SI11 were separated by preparative HPLC. Previous orthoester hydrolysis is required since the OBO-orthoesters are unstable to acidic HPLC conditions. Contrary to Ref. 2, we performed a saponification of SI10 and SI11 to Cbz-protected amino acids SI12 and SI14 to obtain UV-active compounds for ee-determination. Careful monitoring of the base equivalents and the reaction progress is required since an excess of LiOH resulted in formation of cyclic carbamates SI13 and SI15, respectively.$^6$ However, we were able to suppress this side reaction to an acceptable level. For the Marfey-protocol, both the ester SI10 and SI11 and their corresponding acids SI12 and SI14 were suitable because the acidic conditions cleave the Cbz-proteting group and the ester. In our synthesis, we achieved acceptable ees for the S-series (79-83%) and the epimerization of SI7 via flash chromatography was successful (ees from 0-6% for the rac-series).

**Benzyl (S)-(1-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)-2-oxoethyl)carbamate (SI7):**

SI7 was synthesized according to a slightly modified literature-known procedure$^5$: The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of (COCl)$_2$ (0.441 mL, 5.11 mmol, 1.70 eq.) in anhydrous DCM (20 mL) anhydrous DMSO

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$^5$ Rose, N. G. W. et al. Preparation of 1-[N-Benzylxocarbonyl-(1S)-1-amino-2-oxoethyl]-4-methyl-2,6,7-trioxabicyclo-[2.2.2]octane. *Organic Syntheses, Coll. Vol. 10*, 73 (2004); *Vol. 79*, 216 (2002).

$^6$ The carbamates were only isolated for the racemic series for characterization.
(0.683 mL, 9.62 mmol, 3.20 eq.) was added carefully at −78 °C. After stirring for 15 min, alcohol SI6 (0.972 g, 3.01 mmol, 1.00 eq.) dissolved in anhydrous DCM (20 mL) was added. The reaction mixture was stirred for 1.5 h at −78 °C. Then DIPEA (3.30 mL, 18.7 mmol, 6.23 eq.) was added and the solution was stirred for 30 min at −78 °C and for further 30 min without cooling bath. A mixture of cooled toluene/saturated aqueous NH4Cl (4:1, 250 mL, pre-cooled to 0 °C) was added to the reaction mixture. The layers were separated and the organic layer was washed with saturated aqueous NH4Cl (3 x 50 mL, pre-cooled to 0 °C), with saturated aqueous NaHCO3 (50 mL, pre-cooled to 0 °C), and with saturated aqueous NaCl (50 mL, pre-cooled to 0 °C). The organic layer was dried over MgSO4, filtered, and concentrated under reduced pressure to yield crude aldehyde SI7 (1.00 g, max. 3.01 mmol, quant.) as slightly yellow oil which was used in the next stage without further purification.

**Rac-SI7** was synthesized starting from SI6 in a similar manner with an additional purification step via chromatography, which led to full epimerization of the stereocenter7: The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of (COCl)2 (1.22 mL, 14.0 mmol, 1.70 eq.) in anhydrous DCM (60 mL) anhydrous DMSO (2.09 mL, 30.0 mmol, 3.60 eq.) was added carefully at −78 °C. After stirring for 15 min, alcohol SI6 (2.65 g, 8.21 mmol, 1.00 eq.) dissolved in anhydrous DCM (60 mL) was added. The reaction mixture was stirred for 1.5 h at −78 °C. Then DIPEA (9.00 mL, 51.2 mmol, 6.23 eq.) was added and the solution was stirred for 30 min at −78 °C and for further 30 min without cooling bath. Saturated aqueous NH4Cl (100 mL) and DCM (100 mL) were added to the reaction mixture, the layers were separated and the organic layer was washed with saturated aqueous NH4Cl (50 mL), with saturated aqueous NaHCO3 (50 mL), and with saturated aqueous NaCl (50 mL). The organic layer was dried over MgSO4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography8 (100% DCM, then DCM : EtOAc 4:1) to give rac-SI7 (2.06 g, 6.40 mmol, 78 %) as a colorless solid and was directly used for the next step.

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7 The epimerization of the stereocenter of OBO-protected serinals such as SI7 via chromatography is a known phenomenon and was described by Blascovich and Lajoie (Blaskovich, M. A. & Lajoie, G. A. Synthesis of a chiral serine aldehyde equivalent and its conversion to chiral .alpha.-amino acid derivatives. J. Am. Chem. Soc. 115, 5021–5030 (1993).) In our case, the purification via column chromatography resulted in measured ee-values of 0–6% for the rac-series.

8 Conditioning of the silica column was performed with a mixture of DCM and NEt3 (98:2) to neutralize the slightly acidic silica and to prevent partial orthoester hydrolysis during chromatography.
$R_t$ ($n$-heptane/ethyl acetate 1:3): 0.65
3-Hydroxy-2-(hydroxymethyl)-2-methylpropyl (2S,3R)-2-(((benzyl oxy)carbonyl)amino)-3-hydroxy-3-phenylpropanoate (SI10) and 3-Hydroxy-2-(hydroxymethyl)-2-methylpropyl (2S,3S)-2-(((benzyl oxy)carbonyl)amino)-3-hydroxy-3-phenylpropanoate (SI11): The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of aldehyde SI7 (0.965 g, 3.00 mmol, 1.00 eq.) in anhydrous THF (30 mL) was added PhMgBr (1 M in THF, 12.0 mL, 12.0 mmol, 4.00 eq.) at room temperature. After 90 min, TLC indicated full conversion of the starting material (Rf of SI8/SI9 (n-heptane/ethyl acetate 1:3): 0.60). Saturated aqueous NH₄Cl (20 mL) and EtOAc (100 mL) were added to the reaction mixture, the layers were separated and the organic layer was washed with saturated aqueous NaCl (20 mL). The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude mixture of SI8/SI9 was dissolved in 1,4-dioxane (20 mL) and aqueous HCl (1 N, 1 mL) and the reaction mixture was stirred for 30 min until full hydrolysis of the orthoester was observed by TLC. All volatiles were removed under reduced pressure and the residue was purified via HPLC (RP-18, 15 min, 35-45% MeCN in H₂O/TFA 0.1%) to yield SI10 (0.549 g, 1.32 mmol, 44% over two steps) and SI11 (0.124 g, 0.297 mmol, 10% over two steps) as colorless oils. For SI10: ¹H-NMR (DMSO-d₆, 400 MHz): 7.42–7.19 (m, 11H, NH, aryl-H), 5.69 (d, 1H, J = 6.2 Hz, CH-OH), 5.11 (dd, 1H, J = 6.1, 3.9 Hz, CH-OH), 4.95 (s, 2H, Ph-CH₂), 4.47 (m, 2H, CH₂-OH), 4.40 (dd, 1H, J = 9.3, 3.7 Hz, CH-NH), 3.96 (d, 1H, J = 10.5 Hz, CH₂), 3.90 (d, 1H, J = 10.5 Hz, CH₂), 3.30–3.21 (m, 4H, CH₂-OH), 0.77 (s, 3H, CH₃); ¹³C-NMR (DMSO-d₆, 100 MHz): 170.4 (CO), 156.2 (CO-NH), 141.6 (aryl-C₆), 136.9 (aryl-C₆), 128.3 (aryl-C₆), 127.9 (aryl-C₆), 127.7 (aryl-C₆), 127.3 (aryl-C₆), 127.2 (aryl-C₆), 126.1 (aryl-C₆), 72.3 (CH-OH), 67.1 (CH₂), 65.4 (Ph-CH₂), 63.5 (CH₂-OH), 60.5 (CH-NH), 40.7 (C₃-CH₃), 16.3 (CH₃); HRMS (ESI) m/z calcd. for C₂₂H₂₇NO₇Na: (M+Na)⁺, 440.1680; found: 440.1685 (M+Na)⁺; Rf (n-heptane/ethyl acetate 1:3): 0.25; Chiral HPLC (Chiralpak ID/174, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI10 (Rt = 8.6 min) : ent-SI10 (Rt = 5.6 min) 89.3 : 10.7 (79% ee); Specific rotation [α]D²⁰₀ = −35.1 (c = 0.29; MeCN).
For **SI11**: $^1$H-NMR (DMSO-d$_6$, 400 MHz): 7.64 (d, 1H, $J = 9.2$ Hz, NH), 7.42–7.37 (m, 2H, aryl-H), 7.35–7.23 (m, 6H, aryl-H), 7.21–7.10 (m, 2H, aryl-H), 5.76 (d, 1H, $J = 4.7$ Hz, CH-OH), 4.93 (d, 1H, $J = 13.4$ Hz, Ph-CH$_2$), 4.76 (dd, 1H, $J = 8.3$, 4.2 Hz, CH-OH), 4.42 (s, br, 2H, CH$_2$-O), 4.24 (t, 1H, $J = 8.9$ Hz, C-H-NH), 3.96 (d, 1H, $J = 10.6$ Hz, CH$_2$), 3.91 (d, 1H, $J = 10.6$ Hz, CH$_2$), 3.26 (s, 4H, CH$_2$-OH), 0.75 (s, 3H, CH$_3$); $^{13}$C-NMR (DMSO-d$_6$, 100 MHz): 170.9 (CO), 155.5 (CO-NH), 141.9 (aryl-C), 136.8 (aryl-C), 128.3 (aryl-C), 127.8 (aryl-C), 127.7 (aryl-C), 127.4 (aryl-C), 127.4 (aryl-C), 126.8 (aryl-C), 72.3 (CH-OH), 66.7 (CH$_2$), 65.3 (Ph-CH$_2$), 63.5 (CH$_2$-OH), 60.5 (CH-NH), 40.7 (C$_q$-CH$_3$), 16.3 (CH$_3$); HRMS (ESI) m/z calcd. for C$_{22}$H$_{27}$NO$_7$Na: (M+Na)$^+$, 440.1680; found: 440.1685 (M+Na)$^+$; $R_f$ (n-heptane/ethyl acetate 1:3): 0.25; Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): **SI11** (Rt = 13.7 min) : ent-SI11 (Rt = 8.5 min) 91.6 : 8.4 (83% ee); Specific rotation $\left[\alpha\right]_{D}^{19.9} = + 6.2$ (c = 0.32; MeCN).

**Rac-SI10** (0.482 g, 1.16 mmol, 54% over two steps) and **rac-SI11** (0.118 g, 0.283 mmol, 13% over two steps) were synthesized from **rac-SI7** (0.685 g, 2.13 mmol, 1.00 eq.) in analogous manner. The NMR data are identical to the ones reported for SI10 and SI11.

For **rac-SI10**: $R_f$ (n-heptane/ethyl acetate 1:3): 0.25; Chiral HPLC (Chiralpak ID/174, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): **SI10** (Rt = 8.6 min) : ent-SI10 (Rt = 5.6 min) 52.3 : 47.7 (5% ee).

For **rac-SI11**: $R_f$ (n-heptane/ethyl acetate 1:3): 0.25; Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): **SI11** (Rt = 14.0 min) : ent-SI11 (Rt = 8.5 min) 53.2 : 46.8 (6% ee).
(2S,3R)-2-(((Benzyloxy)carbonyl)amino)-3-hydroxy-3-phenylpropanoic acid (SI12):

To a solution of SI10 (0.509 g, 1.22 mmol, 1.00 eq.) in MeCN/H2O (4:1, 20.4 mL/5.1 mL) was added LiOH (1 N in H2O, 1.22 mL, 1.22 mmol, 1.00 eq.) at room temperature. After 20 min, another portion of LiOH (1 N in H2O, 0.61 mL, 0.61 mmol, 0.50 eq.) was added to reach full conversion of starting material after 30 min monitored by LC-MS. The reaction was stopped by addition of acetic acid (0.1 mL). All volatiles were removed under reduced pressure and the residue was purified via HPLC (RP-18, 15 min, 10-55% MeCN in H2O/TFA 0.1%) to yield SI12 (0.270 g, 0.856 mmol, 70%) as a colorless solid.

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\text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6, 400 MHz): 12.81 (s, br, 1H, COO\textsubscript{H}), 7.45–7.19 (m, 10H, aryl-\textit{H}), 7.08 (d, 1H, J = 9.5 Hz, NH\textsubscript{H}), 5.68 (s, br, 1H, OH\textsubscript{H}), 5.16 (s, br., 1H, CH-OH), 4.96 (d, 1H, J = 13.5 Hz, Ph-CH\textsubscript{2}), 4.93 (d, 1H, J = 13.5 Hz, Ph-CH\textsubscript{2}), 4.33 (dd, 1H, J = 9.5, 3.4 Hz, CH-NH); \textsuperscript{13}C-NMR (DMSO-\textit{d}_6, 100 MHz): 171.9 (COO\textsubscript{H}), 156.2 (CO-NH), 142.1 (aryl-C\textsubscript{q}), 137.0 (aryl-C\textsubscript{q}), 128.3 (aryl-C), 127.8 (aryl-C), 127.7 (aryl-C), 127.3 (aryl-C), 127.1 (aryl-C), 126.2 (aryl-C), 72.3 (CH-OH), 65.3 (Ph-CH\textsubscript{2}), 60.4 (CH-NH); HRMS (ESI) m/z calcd. for C\textsubscript{17}H\textsubscript{17}NO\textsubscript{5}Na: (M+Na\textsuperscript{+}) 338.0999; found: 338.1000 (M+Na\textsuperscript{+}); Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI12 (Rt = 6.8 min) : ent-SI12 (Rt = 5.5 min) 89.7 : 10.3 (79% ee); Specific rotation \([\alpha]_{\text{D}}^{19.9} = -23.0 \) (c = 0.65; MeCN).
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Rac-SI12 (0.210 g, 0.666 mmol, 63%) and rac-SI13 (0.055 g, 0.27 mmol, 25%) were synthesized from rac-SI10 (0.442 g, 1.06 mmol, 1.00 eq.) in analogous manner. The NMR data of rac-SI12 are identical to the ones reported for SI12. Rac-SI12 and rac-SI13 were obtained as colorless solids.

For rac-SI12: Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI12 (Rt = 6.8 min) : ent-SI12 (Rt = 5.5 min) 52.8 : 47.2 (6% ee).

(4S,5R)-2-Oxo-5-phenyloxazolidine-4-carboxylic acid (rac-SI13):

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\text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6, 500 MHz): 13.45 (s, br., 1H, COO\textsubscript{H}), 8.36 (s, br., 1H, NH\textsubscript{H}), 7.53–7.26 (m, 5H, aryl-\textit{H}), 5.57 (d, 1H, J = 4.9 Hz, O-CH\textsubscript{H}), 4.23 (dd, 1H, J = 4.9, 0.7 Hz, NH-CH\textsubscript{H}); \textsuperscript{13}C-NMR (DMSO-\textit{d}_6, 125 MHz): 171.8 (COOH), 157.7 (CO), 139.0 (aryl-C\textsubscript{q}), 128.8 (aryl-C), 128.8 (aryl-C), 125.8 (aryl-C),
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\footnote{The formation of SI13 was observed via LC-MS, but was not isolated for the S-series.}
78.4 (O-CH), 60.6 (NH-CH); **HRMS (ESI)** m/z calcd. for C_{10}H_{9}NO_{4}Na: (M+Na)^{+}, 230.0424; found: 230.0425 (M+Na)^{+}. 
(2S,3S)-2-(((Benzyloxy)carbonyl)amino)-3-hydroxy-3-phenylpropanoic acid (SI14):

To a solution of SI11 (0.083 g, 0.20 mmol, 1.00 eq.) in MeCN/H$_2$O (4:1, 3.2 mL/0.8 mL) was added LiOH (1 N in H$_2$O, 0.20 mL, 0.20 mmol, 1.00 eq.) at room temperature. After 20 min, another portion of LiOH (1 N in H$_2$O, 0.10 mL, 0.10 mmol, 0.50 eq.) was added to reach full conversion of starting material after 30 min monitored by LC-MS. The reaction was stopped by addition of acetic acid (0.1 mL). All volatiles were removed under reduced pressure and the residue was purified via HPLC (RP-18, 15 min, 10-55% MeCN in H$_2$O/TFA 0.1%) to yield SI14 (0.043 g, 0.14 mmol, 69%) as a colorless solid.

$^1$H-NMR (DMSO-d$_6$, 400 MHz): ~12.51 (s, v. br, 1H, COOH), 7.48 (d, 1H, J = 9.3 Hz, NH), 7.42–7.36 (m, 2H, aryl-H), 7.35–7.23 (m, 6H, aryl-H), 7.21–7.14 (m, 2H, aryl-H), ~5.74 (s, v. br, 1H, OH), 4.93 (d, 1H, J = 13.3 Hz, Ph-CH$_2$), 4.90 (d, 1H, J = 13.4 Hz, Ph-CH$_2$), 4.73 (d, 1H, J = 8.5 Hz, CH-CH), 4.15 (t, 1H, J = 8.8 Hz, CH-NH); $^{13}$C-NMR (DMSO-d$_6$, 100 MHz): 172.3 (C=O), 155.5 (C=O-NH), 142.2 (aryl-C$_q$), 136.9 (aryl-C$_q$), 128.3 (aryl-C), 127.7 (aryl-C), 127.4 (aryl-C), 127.3 (aryl-C), 126.9 (aryl-C), 125.4 (CH-CH), 65.2 (Ph-CH$_2$), 60.4 (CH-NH); HRMS (ESI) m/z calcd. for C$_{17}$H$_{17}$NO$_5$Na: (M+Na)$^+$, 338.0999; found: 338.0998 (M+Na)$^+$; Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI14 (Rt = 5.2 min) : ent-SI14 (Rt = 6.2 min) 90.9 : 9.1 (82% ee); Specific rotation $[\alpha]_D^{20.0} = +32.3$ (c = 0.31; MeCN).

**Rac-SI14**: (0.053 g, 0.17 mmol, 89%) and rac-SI15 (0.002 g, 0.01 mmol, 5%) were synthesized from rac-SI11 (0.079 g, 0.19 mmol, 1.00 eq.) in analogous manner. The NMR data of rac-SI14 are identical to the ones reported for SI14. Rac-SI14 and rac-SI15 were obtained as colorless solids.

For rac-SI14: Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI14 (Rt = 5.2 min) : ent-SI14 (Rt = 6.2 min) 50.2 : 49.8 (0% ee).

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$^{10}$ The formation of SI15 was observed via LC-MS, but was not isolated for the S-series.
(4S,5S)-2-Oxo-5-phenyloxazolidine-4-carboxylic acid (rac-SI15):

$^1$H-NMR (DMSO-$d_6$, 500 MHz): 12.71 (s, br., 1H, COOH), 8.15 (s, 1H, NH), 7.43–7.33 (m, 5H, aryl-H), 5.85 (d, 1H, $J$ = 8.9 Hz, O-CH), 4.59 (d, 1H, $J$ = 8.9 Hz, NH-CH); $^{13}$C-NMR (DMSO-$d_6$, 125 MHz): 170.5 (COOH), 158.4 (CO), 135.2 (aryl-C$_3$), 128.6 (aryl-C), 128.1 (aryl-C), 126.4 (aryl-C), 77.9 (O-CH), 59.3 (NH-CH); HRMS (ESI) $m/z$ calcd. for C$_{10}$H$_{10}$NO$_4$: (M+H)$^+$, 208.0604; found: 208.0604 (M+H)$^+$. 
4.1.4. Preparation of the β-hydroxyisoleucines

The synthesis of the β-hydroxyisoleucines was performed in close analogy to the synthesis of the β-hydroxyphenylalanines (see chapter 4.1.3). Addition of MeMgBr and EtMgBr, respectively, to aldehyde SI7 was performed at room temperature and the crude mixture of the diastereomers SI16/SI17 and SI23/SI24 was subjected to Swern oxidation to yield crude ketones SI19 and SI26 (Scheme S3). High diastereoselectivities > 10:1 were achieved at the addition of MeMgBr and EtMgBr, respectively, to ketones SI19 and SI26. The reactions, however, turned out to be slow and therefore were performed at room temperature. For the racemic series, an additional equivalent of Grignard reagent was added after 90 min, which led to a significant improvement of the isolated yields compared to the previously performed S-series. Saponifications of SI20 and SI27 were accompanied with faster formation of the cyclic carbamates SI22 and SI29 compared to the β-hydroxyphenylalanine series (see chapter 4.1.3) but sufficient amounts of target products SI21 and SI28 were obtained. Noteworthy, the formation of cyclic carbamate SI22 is significantly quicker than SI29, which resulted in different diastereomeric ratios of the isolated products SI21 and SI28. Neither at the stage of the ester SI20 and SI27 nor at the stage of the free carboxylic acids SI21 and SI28, separation of the diastereomers was possible. However, the samples were sufficiently diastereomer-enriched to be used for Marfey’s protocol. The ratio of the diastereomers was distinguished by 1H-NMR and chiral HPLC.
Scheme S3. Preparation of β-hydroxyisoleucines.a

a Conditions: a) MeMgBr, THF, rt; b) (COCl)₂, DMSO, DCM, −78 °C, 15 min; SI16/SI17 or SI23/SI24 or the corresponding racemic mixtures, −78 °C, 90 min; DIPEA, −78 °C to 0 °C, 30 min; SI18, rac-SI18, SI25 or rac-SI25 used as a crude; c) EtMgBr, THF, rt; d) 1 M HCl aq, 1,4-dioxane; e) LiOH, MeCN/H₂O 4:1.

3-Hydroxy-2-((hydroxymethyl)-2-methylpropyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-hydroxy-3-methylpenta-oate (SI20):

The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of aldehyde SI7 (0.830 g, 2.58 mmol, 1.00 eq.) in anhydrous THF (30 mL) was added MeMgBr (3 M in Et₂O, 3.44 mL, 10.3 mmol, 4.00 eq.) at room temperature. After 90 min, TLC indicated full conversion of the starting material (Rf of SI16/SI17 (n-heptane/ethyl acetate 1:3): 0.48). Saturated aqueous NH₄Cl (20 mL) and EtOAc (100 mL) were added to the reaction mixture, the layers were separated and the organic layer was washed with saturated aqueous NaCl (20 mL). The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude
mixture of **SI16/SI17** (0.801 g, 2.37 mmol, 92%) was used for the oxidation without further purification.

The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of (COCl)$_2$ (0.307 mL, 3.56 mmol, 1.50 eq.) in anhydrous DCM (20 mL) anhydrous DMSO (0.533 mL, 7.50 mmol, 3.16 eq.) was added carefully at −78 °C. After stirring for 15 min, the crude mixture of **SI16/SI17** (0.801 g, 2.37 mmol, 1.00 eq.) dissolved in anhydrous DCM (20 mL) was added. The reaction mixture was stirred for 1.5 h at −78 °C. Then DIPEA (2.30 mL, 13.1 mmol, 5.50 eq.) was added and the solution was stirred for 30 min at −78 °C and for further 30 min without cooling bath. A mixture of cooled toluene/saturated aqueous NH$_4$Cl (4:1, 250 mL, pre-cooled to 0 °C) was added to the reaction mixture. The layers were separated and the organic layer was washed with saturated aqueous NH$_4$Cl (3 x 50 mL, pre-cooled to 0 °C), with saturated aqueous NaHCO$_3$ (50 mL, pre-cooled to 0 °C), and with saturated aqueous NaCl (50 mL, pre-cooled to 0 °C). The organic layer was dried over MgSO$_4$, filtered, and concentrated under reduced pressure to yield crude ketone **SI18** (0.854 g, max. 2.37 mmol, quant., $R_f$ (n-heptane/ethyl acetate 1:3): 0.64) as slightly yellow oil which was used in the next stage without further purification.

The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of ketone **SI18** (0.854 g, max. 2.37 mmol, 1.00 eq.) in anhydrous THF (30 mL) was added EtMgBr (1 M in THF, 9.49 mL, 9.49 mmol, 4.00 eq.) at room temperature. After 90 min, TLC indicated full conversion of the starting material ($R_f$ of **SI19** (n-heptane/ethyl acetate 1:3): 0.61). Saturated aqueous NH$_4$Cl (20 mL) and EtOAc (100 mL) were added to the reaction mixture, the layers were separated and the organic layer was washed with saturated aqueous NaCl (20 mL). The organic layer was dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude product of **SI19** was dissolved in 1,4-dioxane (20 mL) and aqueous HCl (1 N, 1 mL) and the reaction mixture was stirred for 30 min until full hydrolysis of the orthoester was observed by TLC. All volatiles were removed under reduced pressure and the residue was purified via HPLC (RP-18, 15 min, 30-40% MeCN in H$_2$O/TFA 0.1%) to yield **SI20** (0.126 g, 0.329 mmol, 13% over four steps)$^{11}$ as a colorless oil.

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$^{11}$ 14:1 $dr$ and >10:1 $dr$ were determined for **SI20:SI27** by chirale HPLC and $^1$H-NMR analysis.
**1H-NMR** (DMSO-\(d_6\), 400 MHz): 7.42–7.26 (m, 5H, aryl-\(H\)), 7.21 (d, 1H, \(J = 8.8\) Hz, NH), 5.04 (s, 2H, Ph-CH\(_2\)), 4.07 (d, 1H, \(J = 8.8\) Hz, NH), ~4.06 (s, v. br, 3H, OH), 3.90 (s, 2H, CH\(_2\)), 3.33–3.21 (m, 4H, CH\(_2\)-OH), 1.48 (m, 2H, CH\(_2\)-CH\(_3\)), 1.11 (s, 3H, CH\(_3\)), 0.85–0.76 (m, 6H, CH\(_2\)-CH\(_3\)), CH\(_3\)).

**13C-NMR** (DMSO-\(d_6\), 100 MHz): 170.8 (CO), 156.1 (CO-NH), 136.8 (aryl-C\(_q\)), 128.3 (aryl-C), 127.8 (aryl-C), 127.7 (aryl-C), 72.4 (C\(_q\)-OH), 67.0 (CH\(_2\)), 65.7 (Ph-CH\(_2\)), 63.5/63.5 (CH\(_2\)-OH), 61.7 (CH-NH), 40.5 (C\(_q\)-CH\(_3\)), 31.9 (C\(_q\)-CH\(_3\)), 23.1 (CH\(_3\)), 16.5 (CH\(_3\)), 8.0 (CH\(_2\)-CH\(_3\)); **HRMS** (ESI) m/z calcd. for \(C_{19}H_{29}NO_7\)Na: (M+Na)** = 406.1836; found: 406.1837 (M+Na)**.

**Chiral HPLC** (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI20 (Rt = 13.2 min): **ent-SI20** (Rt = 8.2 min) 89.5 : 3.9 (92% ee); **Specific rotation** \(\alpha\)\(_D\)\(_{20}\) = −21.1 (c = 0.07; MeCN).

**Rac-SI20** (0.300 g, 0.783 mmol, 37% over four steps)\(^{12}\) was synthesized from **rac-SI7** (0.685 g, 2.13 mmol, 1.00 eq.) in analogous manner. In the conversion of **rac-SI18** to **rac-SI19**, an additional portion of EtMgBr (1 M in THF, 1.00 eq.) was added after 90 min and the reaction mixture was stirred for 60 min after addition. This modification resulted in a significantly improved overall yield. The NMR data are identical to the ones reported for **SI20**.

For **rac-SI20**: **Chiral HPLC** (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI20 (Rt = 13.5 min): **ent-SI20** (Rt = 8.2 min) 47.9 : 44.6 (4% ee).

**3-Hydroxy-2-(hydroxymethyl)-2-methylpropyl** (2S,3S)-**2-(((benzyloxy)carbonyl)amino)-3-hydroxy-3-methylpenta-noate** (SI27):

The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of aldehyde **SI7** (0.830 g, 2.58 mmol, 1.00 eq.) in anhydrous THF (30 mL) was added EtMgBr (1 M in THF, 10.3 mL, 10.3 mmol, 4.00 eq.) at room temperature. After 90 min, TLC indicated full conversion of the starting material (R\(_f\) of SI23/SI24 (n-heptane/ethyl acetate 1:3): 0.58). Saturated aqueous NH\(_4\)Cl (20 mL) and EtOAc (100 mL) were added to the reaction mixture, the layers were separated and the organic layer was washed with saturated aqueous NaCl (20 mL). The organic layer was dried over MgSO\(_4\), filtered, and concentrated under reduced pressure. The crude

\(^{12}\) 12:1 \(dr\) and >10:1 \(dr\) were determined for **SI20:SI27** by chiral HPLC and **1H-NMR** analysis.
mixture of SI23/SI24 (0.813 g, 2.31 mmol, 90%) was used for the oxidation without further purification.

The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of (COCl)$_2$ (0.300 mL, 3.47 mmol, 1.50 eq.) in anhydrous DCM (20 mL) anhydrous DMSO (0.509 mL, 7.17 mmol, 3.10 eq.) was added carefully at −78 °C. After stirring for 15 min, the crude mixture of SI23/SI24 (0.813 g, 2.31 mmol, 1.00 eq.) dissolved in anhydrous DCM (20 mL) was added. The reaction mixture was stirred for 1.5 h at −78 °C. Then DIPEA (2.24 mL, 12.7 mmol, 5.50 eq.) was added and the solution was stirred for 30 min at −78 °C and for further 30 min without cooling bath. A mixture of cooled toluene/saturated aqueous NH$_4$Cl (4:1, 250 mL, pre-cooled to 0 °C) was added to the reaction mixture. The layers were separated and the organic layer was washed with saturated aqueous NH$_4$Cl (3 x 50 mL, pre-cooled to 0 °C), with saturated aqueous NaHCO$_3$ (50 mL, pre-cooled to 0 °C), and with saturated aqueous NaCl (50 mL, pre-cooled to 0 °C). The organic layer was dried over MgSO$_4$, filtered, and concentrated under reduced pressure to yield crude ketone SI25 (0.808 g, 2.31 mmol, quant., R$_f$ (n-heptane/ethyl acetate 1:3): 0.68) as slightly yellow oil which was used in the next stage without further purification.

The reaction was carried out in moisture-free glassware under inert atmosphere. To a solution of ketone SI25 (0.808 g, max. 2.31 mmol, 1.00 eq.) in anhydrous THF (30 mL) was added MeMgBr (3 M in Et$_2$O, 3.08 mL, 9.25 mmol, 4.00 eq.) at room temperature. After 90 min, TLC indicated full conversion of the starting material (R$_f$ of SI26 (n-heptane/ethyl acetate 1:3): 0.61). Saturated aqueous NH$_4$Cl (20 mL) and EtOAc (100 mL) were added to the reaction mixture, the layers were separated and the organic layer was washed with saturated aqueous NaCl (20 mL). The organic layer was dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude product of SI26 was dissolved in 1,4-dioxane (20 mL) and aqueous HCl (1 N, 1 mL) and the reaction mixture was stirred for 30 min until full hydrolysis of the orthoester was observed by TLC. All volatiles were removed under reduced pressure and the residue was purified via HPLC (RP-18, 15 min, 30-40% MeCN in H$_2$O/TFA 0.1%) to yield SI27 (0.218 g, 0.569 mmol, 22% over four steps)$^{13}$ as a colorless oil.

$^{13}$ 32:1 $dr$ and >10:1 $dr$ were determined for SI27:SI20 by chiral HPLC and $^1$H-NMR analysis.
H-NMR (DMSO-d$_6$, 400 MHz): 7.42–7.26 (m, 6H, aryl-H, NH), 5.04 (s, 2H, Ph-CH$_2$), 4.54 (s, 1H, C$_{q}$-OH), 4.45 (t, 2H, $J = 5.2$ Hz, CH$_{2}$-OH), 4.10 (d, 1H, $J = 9.2$ Hz, NH-CH$_3$), 3.93 (d, 1H, $J = 10.7$ Hz, C$_{$_{2}$}H$_3$), 3.87 (d, 1H, $J = 10.7$ Hz, CH$_3$), 3.31–3.21 (m, 4H, C$_{$_{2}$}H$_2$-OH), 1.56–1.39 (m, 2H, C$_{$_{2}$}H$_2$-CH$_3$), 0.86 (s, 3H, C$_{$_{3}$}H$_3$), 0.84 (t, 3H, $J = 7.4$ Hz, CH$_2$-CH$_3$), 0.80 (s, 3H, CH$_3$); $^{13}$C-NMR (DMSO-d$_6$, 100 MHz): 170.8 (CO), 156.1 (CO-NH), 136.9 (aryl-C$_{_{q}}$), 128.4 (aryl-C), 127.9 (aryl-C), 127.7 (aryl-C), 72.7 (C$_{_{q}}$-OH), 67.0 (C$_{_{2}}$H$_2$), 65.6 (Ph-CH$_2$), 63.6 (CH$_2$-OH), 61.6 (CH-NH), 40.5 (C$_{$_{2}$}CH$_3$), 31.6 (CH$_2$-CH$_3$), 23.1 (CH$_3$), 16.5 (CH$_3$), 7.9 (CH$_2$-CH$_3$); HRMS (ESI) m/z calcd. for C$_{19}$H$_{29}$NO$_7$Na: (M+Na)$^+$, 406.1836; found: 406.1839 (M+Na)$^+$; Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI27 (Rt = 22.9 min) : ent-SI27 (Rt = 6.9 min) 91.7 :5.2 (89% ee); Specific rotation $[\alpha]^D_{20.0} = -11.6$ (c = 0.35; MeCN).

Rac-SI27 (0.337 g, 0.879 mmol, 41% over four steps)$^{14}$ was synthesized from rac-SI17 (0.685 g, 2.13 mmol, 1.00 eq.) in analogous manner. In the conversion of rac-SI25 to rac-SI26, an additional portion of MeMgBr (3 M in Et$_2$O, 1.00 eq.) was added after 90 min and the reaction mixture was stirred for 60 min after addition. This modification resulted in a significantly improved overall yield. The NMR data are identical to the ones reported for SI27.

For rac-SI27: Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH:MeOH 5:1:1 + 0.1% TFA): SI27 (Rt = 23.0 min) : ent-SI27 (Rt = 6.9 min) 51.4 :45.7 (6% ee).

(2S,3R)-2-(((Benzyloxy)carbonyl)amino)-3-hydroxy-3-methylpentanoic acid (SI21):

To a solution of SI20 (0.084 g, 0.22 mmol, 1.00 eq.) in MeCN/H$_2$O (4:1, 3.2 mL/0.8 mL) was added LiOH (1 N in H$_2$O, 0.22 mL, 0.22 mmol, 1.00 eq.) at room temperature. After 20 min, another portion of LiOH (1 N in H$_2$O, 0.11 mL, 0.11 mmol, 0.50 eq.) was added to reach full conversion of starting material after 30 min monitored by LC-MS. The reaction was stopped by addition of acetic acid (0.1 mL). All volatiles were removed under reduced pressure.

$^{14}$ 33:1 $dr$ and >10:1 $dr$ were determined for SI27:SI20 by chiral HPLC and $^1$H-NMR analysis.
and the residue was purified via HPLC (RP-18, 15 min, 5-55% MeCN in H₂O/TFA 0.1%) to yield \textbf{SI21} (0.021 g, 0.075 mmol, 34%)\(^\text{15}\) as a colorless oil.\(^\text{16}\)

\(^1\)H-NMR (DMSO-d\(_6\), 400 MHz): 7.42–7.26 (m, 5H, ary1-H), 7.03 (d, 1H, J = 8.8 Hz, NH), 5.04 (s, 2H, Ph-CH\(_2\)), 3.96 (d, 1H, J = 9.0 Hz, NH-CH\(_2\)), 1.56–1.34 (m, 2H, CH\(_2\)-CH\(_3\)), 1.11 (s, 3H, CH\(_3\)), 0.80 (t, 3H, J = 7.5 Hz, CH\(_2\)-CH\(_3\))); \(^{13}\)C-NMR (DMSO-d\(_6\), 100 MHz): 172.3 (COOH), 156.2 (CO-NH), 137.0 (aryl-C\(_q\)), 128.4 (aryl-C), 127.8 (aryl-C), 127.7 (aryl-C), 72.4 (C\(_q\)-OH), 65.6 (Ph-CH\(_2\)), 61.4 (CH-NH), 32.0 (CH\(_2\)-CH\(_3\)), 23.1 (CH\(_3\)), 8.0 (CH\(_2\)-CH\(_3\)); HRMS (ESI) m/z calcd. for \(C_{14}H_{19}NO_5\)Na: (M+Na)\(^+\), 304.1155; found: 304.1154 (M+Na)\(^+\); Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH 10:1 + 0.1% TFA): \textbf{SI21} (Rt = 16.7 min) : ent-\textbf{SI21} (Rt = 29.3 min) 75.2 : 2.6 (93% ee); Specific rotation \([\alpha]_D^{19.1} = 0\) (c = 0.19; MeCN).\(^\text{17}\)

\textbf{Rac-SI21} (0.072 g, 0.26 mmol, 38%)\(^\text{18}\) and \textbf{rac-SI22} (0.038 g, 0.22 mmol, 32%) were synthesized from \textbf{rac-SI20} (0.260 g, 0.678 mmol, 1.00 eq.) in analogous manner. The NMR data of \textbf{rac-SI21} are identical to the ones reported for \textbf{SI21}. \textbf{Rac-SI21} was obtained as colorless oil and \textbf{rac-SI22} as colorless solid.

For \textbf{rac-SI21}: Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH 10:1 + 0.1% TFA): \textbf{SI21} (Rt = 16.7 min) : ent-\textbf{SI21} (Rt = 28.9 min) 43.0 : 43.0 (0% ee).

\((4S,5R)-5\)-Ethyl-5-methyl-2-oxooxazolidine-4-carboxylic acid (ent-SI22):

\(^1\)H-NMR (DMSO-d\(_6\), 400 MHz): 13.23 (s, br, 1H, COOH), 7.89 (s, 1H, NH), 4.03 (d, 1H, J = 0.6 Hz, CH\(_2\)), 1.72 (q, 2H, J = 7.5 Hz, CH\(_2\)), 1.24 (s, 3H, CH\(_3\)), 0.91 (t, 3H, J = 7.5 Hz, CH\(_2\)-CH\(_3\))); \(^{13}\)C-NMR (DMSO-d\(_6\), 100 MHz): 171.3 (COOH), 157.4 (CO-NH), 82.7 (O-C\(_q\)), 61.2 (CH-NH), 33.2 (CH\(_2\)-CH\(_3\)), 20.7 (CH\(_3\)), 7.5 (CH\(_2\)-CH\(_3\)); HRMS (ESI) m/z calcd. for \(C_{7}H_{12}NO_4\) (M+H)\(^+\), 174.0761; found: 174.0761 (M+H)\(^+\).

\((2S,3S)-2-(((Benzyloxy)carbonyl)amino)-3-hydroxy-3-methylpentanoic acid (SI28):

\(^{15}\) 3.5:1 \(dr\) and 3:1 \(dr\) were determined for \textbf{SI21}:\textbf{SI28} by chirale HPLC and \(^1\)H-NMR analysis.

\(^{16}\) The formation of \textbf{SI22} was observed via LC-MS, but was not isolated for the S-series.

\(^{17}\) Since the sample is a mixture of diastereomers and the measured rotation was close to 0, no specific rotation was determined.

\(^{18}\) 6:1 \(dr\) and >5:1 \(dr\) were determined for \textbf{SI21}:\textbf{SI28} by chirale HPLC and \(^1\)H-NMR analysis.
To a solution of **SI27** (0.178 g, 0.464 mmol, 1.00 eq.) in MeCN/H$_2$O (4:1, 7.2 mL/1.8 mL) was added LiOH (1 N in H$_2$O, 0.46 mL, 0.46 mmol, 1.00 eq.) at room temperature. After 20 min, another portion of LiOH (1 N in H$_2$O, 0.23 mL, 0.23 mmol, 0.50 eq.) was added to reach full conversion of starting material after 30 min monitored by LC-MS. The reaction was stopped by addition of acetic acid (0.1 mL). All volatiles were removed under reduced pressure and the residue was purified via HPLC (RP-18, 15 min, 10-50% MeCN in H$_2$O/TFA 0.1%) to yield **SI28** (0.051 g, 0.18 mmol, 39%)$^{19}$ as a colorless oil.$^{20}$

$^1$H-NMR (DMSO-d$_6$, 400 MHz): 7.42–7.27 (m, 5H, aryl-H), 7.18 (d, 1H, $J$ = 9.2 Hz, NH$_2$), 5.05 (s, 2H, Ph-CH$_2$), 4.01 (d, 1H, $J$ = 9.2 Hz, NH-CH$_2$), 1.58–1.40 (m, 2H, CH$_2$-CH$_3$), 1.09 (s, 3H, CH$_3$), 0.84 (t, 3H, $J$ = 7.4 Hz, CH$_2$-CH$_3$); $^{13}$C-NMR (DMSO-d$_6$, 100 MHz): 172.3 (COOH), 156.1 (CO-NH), 137.0 (aryl-C$_q$), 128.3 (aryl-C$_q$), 127.8 (aryl-C$_q$), 127.7 (aryl-C), 72.6 (C$_q$-OH), 65.5 (Ph-CH$_2$), 61.2 (CH-NH), 31.5 (CH$_2$-CH$_3$), 23.1 (CH$_3$), 7.8 (CH$_2$-CH$_3$); HRMS (ESI) m/z calcd. for C$_{14}$H$_{19}$NO$_5$Na: (M+Na)$^+$, 304.1155; found: 304.1157 (M+Na)$^+$; Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; n-heptane:EtOH 10:1 + 0.1% TFA): **SI28** (Rt = 20.6 min) : **ent-SI28** (Rt = 27.2 min) 94.2 : 5.1 (90% ee); Specific rotation $[\alpha]_D^{19,9} = +18.5$ (c = 0.05; MeCN).

(4S,5S)-5-Ethyl-5-methyl-2-oxooxazolidine-4-carboxylic acid (SI29):

$^1$H-NMR (DMSO-d$_6$, 400 MHz): 13.24 (s, br, 1H, COOH), 7.88 (s, 1H, NH$_2$), 4.05 (d, 1H, $J$ = 0.6 Hz, CH$_2$), 1.73 (q, 2H, $J$ = 7.5 Hz, CH$_2$), 1.24 (s, 3H, CH$_3$), 0.91 (t, 3H, $J$ = 7.5 Hz, CH$_2$-CH$_3$); $^{13}$C-NMR (DMSO-d$_6$, 100 MHz): 171.0 (COOH), 157.4 (CO-NH), 82.7 (O-C$_q$), 63.4 (CH-NH), 28.3 (CH$_2$-CH$_3$), 24.3 (CH$_3$), 7.7 (CH$_2$-CH$_3$); HRMS (ESI) m/z calcd. for C$_7$H$_{12}$NO$_4$: (M+H)$^+$, 174.0761; found: 174.0762 (M+H)$^+$.

**Rac-SI28** (0.132 g, 0.469 mmol, 61%)$^{21}$ and **rac-SI29** (0.021 g, 0.12 mmol, 16%) were synthesized from **rac-SI27** (0.297 g, 0.775 mmol, 1.00 eq.) in analogous manner. The NMR data of **rac-SI28** are identical to the ones reported for **SI28**. **Rac-SI28** was obtained as colorless oil and **rac-SI29** as colorless solid.

$^{19}$ 138:1 $dr$ and >100:1 $dr$ were determined for **SI28:SI21** by chirale HPLC and $^1$H-NMR analysis.

$^{20}$ The formation of **SI29** was observed via LC-MS, but was not isolated for the S-series.

$^{21}$ 33:1 $dr$ and >50:1 $dr$ were determined for **SI28:SI21** by chirale HPLC and $^1$H-NMR analysis.
For **rac-SI28**: Chiral HPLC (Chiralpak IF/181, 250x4.6 mm; \( n \)-heptane:EtOH 10:1 + 0.1% TFA): SI28 (Rt = 20.8 min) : **ent-SI28** (Rt = 26.8 min) 48.4 : 48.7 (0% ee).
4.2. NMR spectra of synthetic β-hydroxyamino acid intermediates
DMSO-\textsubscript{d6}, 400 MHz

\textbf{rac-SI22}

DMSO-\textsubscript{d6}, 100 MHz
4.3. Determination of the enantiomeric excesses (ee) of synthetic β-hydroxyamino acid intermediates

Chiral Separation
Library, SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Hohst, G 936, Room 007
D-65926 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30782

Sample Information:
Customer: Dr. Rivera
Analyst: K. Rahm-Hotze
Batch Ref No: FF.ASMJ00012.1
Sample Name: FF.ASMJ00012.1
Racemate:
Lab Journal:
Comment:

Separation Information:
ARCI/Poeuerlein/Poeuerlein_2021
HPLC-System: LC_30
Flow rate: 1.0 ml/min
Temperature: 30°C
HPLC Column: Chiralpak AS-H/122, 250x4.6 mm
Eluent: B(OH)/MeOH 1:1

Results

|     |     |     |     |
|-----|-----|-----|-----|
| RT  | Area| % Area| Height |
| 1   | 3.05| 100.00 | 173881 |

Current Date: 4/13/2021
Date Acquired: 4/13/2021 3:29:30 PM CEST
Vial: 47
Inj Vol: 5.00 uL

1 of 2
**Chiral Separation**

**Sample Information:**
- **Customer:** Dr. Röverlein
- **Analyst:** K. Rahn-Hölze
- **Batch Ref No:** FF.ASMJ00019.1
- **Sample Name:** FF.ASMJ00019.1
- **Racemate:**
- **Lab Journal:**
- **Comment:**

**Separation Information:**
- **HPLC-System:** LC_30
- **Flowrate:** 1.0 ml/min
- **Temperature:** 30 °C
- **HPLC Column:** Chiralpak AS-H/122, 250x4.6 mm
- **Eluent:** B2OH:MeOH 1:1

---

**Results**

| RT  | Area       | % Area | Height |
|-----|------------|--------|--------|
| 3.531 | 65663     | 0.00   | 9794   |
| 4.27 | 1380266   | 96.60  | 1508163|

**Current Date:** 4/13/2021  
**Date Acquired:** 4/13/2021 3:42:32 PM CST  
**Visi:** 40  
**Inj Vol:** 2.00 µL  

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**Graph:**
- Peak at 3.531 minutes, batch_ref_no FF.ASMJ00019.1
- Empower Project: APCI/Poevelmei/Poevelmei_2021
- 207.0 nm, Inj ID 1322, Res ID 1327
Sample Information:
Customer: Dr. Peverlein
Batch Ref No: FF.ASM0019.1
Sample Name: FF.ASM0019.1
Comment:

Spectrum Index Plot

Batch_Ref_No: FF.ASM0019.1  Project Name: APCI/Peverlein/Peverlein_2021

Current Date: 4/13/2021  Vial: 48
Date Acquired: 4/13/2021 3:42:32 PM CEST  Inj Vol: 2.00 uL
# Chiral Separation

**Library:** SM, Chiral & Peptide Purification  
**R&D / IDD in vitro Biology & HT Chemistry**  
**Industriepark Höchst, G 636, Room 007**  
**D-65926 Frankfurt**  
Dr. Schaffrath  
Tel.: +49-(0)69-305-30782  

## Sample Information:
- **Customer:** Dr. Röverlein
- **Analyst:** K. Rahn-Hotze
- **Batch Ref No:** FF.ASMJ00014.2
- **Sample Name:** FF.ASMJ00014.2
- **Racemate**
- **Lab Journal**
- **Comment**

## Separation Information:
- **HPLC System:** LC_30
- **Flowrate:** 1.0 ml/min
- **Temperature:** 30°C
- **HPLC Column:** Chiralpak AD-H/148, 250x4.6 mm
- **Eluent:** EtOH:MeOH 1:1

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### Results

| RT | Area | % Area | Height |
|----|------|--------|--------|
| 1  | 5.46 | 34946  | 0.71   |
| 2  | 6.05 | 419444 | 59.29  |

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**Current Date:** 4/13/2021  
**Date Acquired:** 4/13/2021 2:28:50 PM CEST  
**Val:** 43  
**Inj Vol:** 5.00 uL  
**Page:** 1 of 2
Sample Information:
Customer: Dr. Pöverlein
Batch Ref No: FF.ASMJ00014.2
Sample Name: FF.ASMJ00014.2
Comment:

Spectrum Index Plot

Batch_Ref_No FF.ASMJ00014.2 Project Name: APO/Poeverlein/Poeverlein_2021

Current Date: 4/13/2021 Vial: 43
Date Acquired: 4/13/2021 2:28:50 PM CEST Inj Vol: 5.00 uL
Sample Information:
Customer: Dr. Föverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00021.2
Sample Name: FF.ASMJ00021.2
Racemate:
Lab Journal:
Comment:

Separation Information:
APCI/Poeverlein/Poeverlein_2021
HPLC-System: LC_30
Flowrate: 1.0 ml/min
Temperature: 30°C
HPLC Column: Chiralpak AD-H/148, 250x4.6 mm
Eluent: EOH:MeOH 1:1

Results:

| RT  | Area  | % Area | Height |
|-----|-------|--------|--------|
| 1   | 5.05  | 100.00 | 142200 |
| 2   | 8.65  | 50.84  | 4696   |

Current Date: 4/13/2021
Date Acquired: 4/13/2021 2:38:46 PM CEST
Inj Vol: 5.00 uL
Chiral Separation

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00075.2
Sample Name: FF.ASMJ00075.2
Racemat: No
Lab Journal: No
Comment: 

Separation Information:
APC/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flow rate: 1.0 mL/min
Temperature: 30°C
HPLC Column: Chiralpak ID'174, 250x4.6 mm
Eluent: Hep:EtOH:MeOH 5:1:1 + 0.1% TFA

Results

| RT   | Area   | %Area | Height |
|------|--------|-------|--------|
| 5.64 | 187787 | 100%  | 151838 |
| 8.06 | 1519738 | 88.32 | 790002 |

Batch_Ref_No: FF.ASMJ00075.2
Empower Project: APC/Poeverlein/Poeverlein_2021

205.0nm Inj ID 1607; Res ID 1695

Current Date: 5/19/2021
Date Acquired: 5/18/2021 3:34:03 PM CEST
Vial: 32
Inj Vol: 2.00 uL
Chiral Separation

Library, SM, Chiral & Peptide Purification
R&D/IDD in vitro Biology & HT Chemistry
Industriepark Hochst, G 838, Room 007
D-65926 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30762

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00072.2
Sample Name: FF.ASMJ00072.2
Racemat:
Lab Journal:
Comment:

Separation Information:
APC/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flowrate: 1.0 mL/min
Temperature: 30 °C
HPLC Column: Chiralpak ID174, 250x4.6 mm
Eluent: Hep:EtOH:MeOH 5:1:1 + 0.1% TFA

Results:

| RT  | Area     | % Area | Height |
|-----|----------|--------|--------|
| 1.42| 46024444 | 47.71  | 496270 |
| 2.42| 59239982 | 52.29  | 302304 |

205.0nm Inj ID 1561; Res ID 1568
Batch_Ref_No FF.ASMJ00072.2
Empower Project: APC/Poeverlein/Poeverlein_2021

Current Date: 5/19/2021
Date Acquired: 5/18/2021 10:18:44 AM
Vial: 26
Inj/Vol: 5.00 uL

1 of 2
### Chiral Separation

Library: SM, Chiral & Peptide Purification  
R&D / IDD in vitro Biology & HT Chemistry  
Industriepark Höchst, G 836, Room 007  
D-65926 Frankfurt  
Dr. Schaffrath  Tel.: +49-(0)69-303-30782

---

#### Sample Information:
- **Customer:** Dr. Riverlein  
- **Analyst:** K. Rahn-Hotze  
- **Batch Ref No:** FF.ASMJ00075.1  
- **Sample Name:** FF.ASMJ00075.1  
- **Racemat:**  
- **Lab Journal:**  
- **Comment:**

#### Separation Information:
- **APC/Poeverlein/Poeverlein_2021**  
- **HPLC-System:** LC_03  
- **Flow rate:** 1.0 ml/min  
- **Temperature:** 30 °C  
- **HPLC Column:** Chiralpak IF181, 250x4.6 mm  
- **Eluent:** Hep:EtOH:MeOH 5:1:1 + 0.1% TFA

---

![Chromatogram](image)

**Batch Ref No:** FF.ASMJ00075.1  
**Empower Project:** APC/Poeverlein/Poeverlein_2021

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### Results

| RT   | Area  | % Area | Height |
|------|-------|--------|--------|
| 8.46 | 24988800 | 8.36 | 112677 |
| 13.74 | 27238981 | 91.62 | 759090 |

---

**Current Date:** 5/19/2021  
**Date Acquired:** 5/18/2021 2:30:24 PM CEST  
**Vial:** 29  
**Inj Vol:** 5.00 uL
Chiral Separation
Library: SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Höchst, G 936, Room 007
D-65926 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30782

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00072.1
Sample Name: FF.ASMJ00072.1
Racemate: 
Lab Journal: 
Comment: 

Separation Information:
APC/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flowrate: 1.0 ml/min
Temperature: 30° C
HPLC Column: Chiralpak IF/181, 250x4.6 mm
Eluent: Hep.BOH:MeOH 5:1:1 + 0.1% TFA

Results:
| RT  | Area  | % Area | Height |
|-----|-------|--------|--------|
| 1   | 8.40  | 2005204| 46.77  |
| 2   | 13.96 | 4357885| 53.23  |

Current Date: 5/19/2021
Date Acquired: 5/18/2021 11:04:49 AM CEST
Vial: 25
Inj Vol: 5.00 uL

205.0nm; Inj ID 1573; Res ID 1575
Batch_Ref_No FF.ASMJ00072.1
Empower Project: APC/Poeverlein/Poeverlein_2021

ent-SI11
 SI11
Sample Information:
Customer: Dr. Roverlein
Batch Ref No: FF.ASMJ00072.1
Sample Name: FF.ASMJ00072.1
Comment:

Spectrum Index Plot

Batch_Ref_No: FF.ASMJ00072.1  Project Name: APO/Poeverlein/Poeverlein_2021

Current Date: 5/19/2021  Vial: 25
Date Acquired: 5/18/2021  11:04:49 AM CEST  Inj Vol: 5.00 uL
Chiral Separation
Library: SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Höchst, G 836, Room 007
D-65926 Frankfurt
Dr. Schaffrath. Tel.: +49-(0)69-305-30782

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00095.1
Sample Name: FF.ASMJ00095.1
Racemat: 
Lab Journal:
Comment:

Separation Information:
APCI/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flow rate: 1.0 mL/min
Temperature: 30°C
HPLC Column: Chiralpak IF™181, 250x4,6 mm
Eluent: Hep.BOH:MeOH 5:1:1 + 0.1% TFA

Results

| RT | Area | % Area | Height |
|----|------|--------|--------|
| 1  | 5.47 | 141542 | 18972  |
| 2  | 6.94 | 1230490| 89.72  |

Batch_Ref_No: FF.ASMJ00095.1
Empower Project: APCI/Poeverlein/Poeverlein_2021

Current Date: 5/19/2021
Date Acquired: 5/18/2021 2:57:07 PM CEST
Vial: 31
Inj Vol: 5.00 uL

210.0nm: Inj ID 1594; Res ID 1598
Chiral Separation
Library, SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Höchst, G-9336, Room 007
D-65926 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30782

Sample Information:
- Customer: Dr. Riverlein
- Analyst: K. Rahn-Hotze
- Batch Ref No: FF.ASMJ00091.1
- Sample Name: FF.ASMJ00091.1
- Racemate:
- Lab Journal:
- Comment:

Separation Information:
- APC:PoeverleinPoeverlein_2021
- HPLC-System: LC_03
- Flowrate: 1.0 ml/min
- Temperature: 30°C
- HPLC Column: Chiralpak IF181, 250x4.6 mm
- Eluent: Hep:BuOH:MeOH 5:1:1 + 0.1% TFA

Results

|   | RT  | Area | % Area | Height |
|---|-----|------|--------|--------|
| 1 | 5.46| 16853237| 47.19 | 1508030 |
| 2 | 6.60| 18991425| 52.81 | 1179035 |

Batch_Ref_No FF.ASMJ00091.1
Empower Project: APC:PoeverleinPoeverlein_2021

Current Date: 5/18/2021
Date Acquired: 5/18/2021 9:54:32 AM CEST
Vial: 28
Inj Vol: 5.00 µL

1 of 2
Chiral Separation
Library: SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Höchst, G 836, Room 007
D-65926 Frankfurt
Dr. Schaffrath, Tel.: +49-0-69-305-30782

Sample Information:
Customer
Analyst
Batch Ref No
Sample Name
Racemat
Lab Journal
Comment

Separation Information:
APC/Poeverlein/Poeverlein_2021
HPLC-System LC_03
Flowrate 1.0 ml/min
Temperature 30°C
HPLC Column Chiralpak IF181, 250x4.6 mm
Eluent Hep:EtOH:MeOH 5:1:1 + 0.1% TFA

Results

| RT  | Area | % Area | Height |
|-----|------|--------|--------|
| 5.24| 1139482 | 90.60 | 112016 |
| 6.24| 1135961 | 9.40  | 91036  |

Current Date 5/19/2021
Date Acquired 5/18/2021 2:47:15 PM CEST
Vial 30
Inj Vol 5.00 uL
Sample Information:
Customer: Dr. Poverlein
Batch Ref No: FF.ASMJ00094.1
Sample Name: FF.ASMJ00094.1
Comment:

Spectrum Index Plot

Batch_Ref_No FF.ASMJ00094.1  Project Name: APO/Poverlein/Poverlein_2021

Current Date: 5/19/2021  Vial: 30
Date Acquired: 5/18/2021 2:47:15 PM CEST  Inj Vol: 5.00 uL
Chiral Separation
Library, SM, Chiral & Peptide Purification
R&D / I&D in vitro Biology & HT Chemistry
Industriepark Höchst, G 838, Room 007
D-65926 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30782

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00090.1
Sample Name: FF.ASMJ00090.1
Racemat: 
Lab Journal: 
Comment: 

Separation Information:
APC/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flowrate: 1.0 ml/min
Temperature: 30°C
HPLC Column: Chiralpak IF/181, 250x4.6 mm
Eluent: Hep:BuOH:MeOH 5:1:1 + 0.1% TFA

Results

| RT | Area | % Area | Height |
|----|------|--------|--------|
| 1  | 5.22 | 478660 | 50.20  |
| 2  | 6.21 | 472572 | 46.80  |

Batch_Ref_No: FF.ASMJ00090.1
Empower Project: APC/Poeverlein/Poeverlein_2021

Current Date: 5/19/2021
Date Acquired: 5/18/2021 11:37:51 AM CEST
Vial: 27
Inj Vol: 5.00 uL
**Sample Information:**
Customer: Dr. Poverlein
Batch Ref No: FF.ASMJ00090.1
Sample Name: FF.ASMJ00090.1
Comment:

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**Spectrum Index Plot**

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Batch_Ref_No: FF.ASMJ00090.1 Project Name: APC/Poeverlein/Poeverlein_2021

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Current Date: 5/19/2021
Date Acquired: 5/18/2021 11:37:51 AM CEST
Vial: 27
Inj Vol: 5.00 uL
Chiral Separation

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00088.1
Sample Name: FF.ASMJ00088.1
Lab Journal
Comment

Separation Information:
APCI_Poeverlein_Poeverlein_2021
HPLC-System: LC_03
Flowrate: 1.0 ml/min
Temperature: 30°C
HPLC Column: Chiralpak IF181, 250x4.6 mm
Eluent: Hep/CH3OH:MeOH 5:1:1 + 0.1% TFA

Results

| RT  | Area     | % Area | Height |
|-----|----------|--------|--------|
| 1   | 6.99     | 108888 | 0.00   | 13440  |
| 2   | 8.21     | 1103333| 3.93   | 76159  |
| 3   | 13.33    | 2635768| 60.52  | 677889 |
| 4   | 23.90    | 1671610| 5.96   | 33362  |

Current Date: 5/19/2021
Date Acquired: 5/19/2021 9:06:21 AM CEST
Inj Vol: 5.00 µl
**Chiral Separation**

Library: SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Höchst, G 136, Room 007
D-65925 Frankfurt
Dr. Schaffrath, Tel.: +49-(0)69-305-30762

**Sample Information:**
- **Customer:** Dr. Riverlein
- **Analyst:** K. Rahn-Hotze
- **Batch Ref No:** FF.ASMJ00079.1
- **Sample Name:** FF.ASMJ00079.1
- **Racemat:**
- **Lab Journal:**
- **Comment:**

**Separation Information:**
- **Method:** APC/Poeverlein/Poeverlein,2021
- **HPLC-System:** LC_03
- **Flowrate:** 1.0 ml/min
- **Temperature:** 30°C
- **HPLC Column:** Chiralpak IF181, 250x4.6 mm
- **Eluent:** H2O:MeOH 5:1.1 + 0.1% TFA

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![Chiral separation chromatogram](image)

Batch_Ref_No FF.ASMJ00079.1  Empower Project: APC/Poeverlein/Poeverlein_2021

**Results**

| RT  | Area  | %Area | Height |
|-----|-------|-------|--------|
| 1   | 6.69  | 3.05  | 40224  |
| 2   | 8.17  | 44.55 | 45079  |
| 3   | 13.51 | 47.52 | 23804  |
| 4   | 23.96 | 3.68  | 11880  |

**Current Date:** 5/19/2021  **Vial:** 35  **1 of 2**

**Date Acquired:** 5/19/2021 6:04:37 AM CEST  **Inj Vol:** 5.00 µL

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Sample Information:
Customer: Dr. Poeverlein
Batch Ref No: FF.ASMJ00079.1
Sample Name: FF.ASMJ00079.1
Comment:

Spectrum Index Plot

Batch_Ref_No: FF.ASMJ00079.1  Project Name: APCI/Poeverlein/Poeverlein_2021

Current Date: 5/19/2021  Date Acquired: 5/19/2021 8:04:37 AM CEST
Vial: 35  Inj Vol: 5.00 uL

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Chiral Separation

Library, SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Hochst, G 338, Room 007
D-65926 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30762

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00089.1
Sample Name: FF.ASMJ00089.1
Racemat
Lab Journal
Comment

Separation Information:
APC/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flowrate: 1.0 mL/min
Temperature: 30° C
HPLC Column: Chiralpak IF/181, 250x4.6 mm
Eluent: Hep:EtlOH:MeOH 5:1:1 + 0.1% TFA

Results
| RT   | Area  | % Area | Height |
|------|-------|--------|--------|
| 1    | 6.86  | 743487 | 5.24   |
| 2    | 8.20  | 57550  | 0.40   |
| 3    | 13.58 | 370482 | 2.02   |
| 4    | 22.91 | 1300659| 9.73   |

Current Date: 5/19/2021
Date Acquired: 5/19/2021 11:12:46 AM CEST
Vial: 38
Inj Vol: 3.00 uL
Chiral Separation

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00080.1
Sample Name: FF.ASMJ00080.1
Racemat:
Lab Journal:
Comment:

Separation Information:
APCI/Poeverlein/Poeverlein_2021
HPLC-System: C_03
Flowrate: 0.5 mL/min
Temperature: 30 °C
HPLC Column: Chiralpak IF181, 250x4.6 mm
Eluent: Hep:BIOH:MeOH 5:1:1 + 0.1% TFA

Results:

| RT  | Area  | % Area | Height |
|-----|-------|--------|--------|
| 1   | 6.90  | 9952190| 97.72  |
| 2   | 8.21  | 244351 | 1.44   |
| 3   | 13.71 | 336811 | 1.00   |
| 4   | 23.03 | 10300761| 0.12   |

Current Date: 5/19/2021
Date Acquired: 5/19/2021 8:35:28 AM CEST
Vial: 36
Inj Vol: 5.00 mL
Sample Information:
Customer: Dr. Poverlein
Batch Ref No: FF.ASMJ00080.1
Sample Name: FF.ASMJ00080.1
Comment:

Spectrum Index Plot

Batch Ref No: FF.ASMJ00080.1  Project Name: APO/Poverlein/Poverlein_2021

Current Date: 5/19/2021  
Date Acquired: 5/19/2021 8:35:28 A.M. CEST
Chiral Separation
Library: SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Hochel, G836, Room 007
D-65926 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30782

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00096.1
Sample Name: FF.ASMJ00096.1
Racemate:
Lab Journal:
Comment:

Separation Information:
APC/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flowrate: 1.0 mL/min
Temperature: 30°C
HPLC Column: Chiralpak IF181, 250x4.6 mm
Eluent: Hep.BOH 10.1 + 0.1% TFA

Results:

| RT  | Area   | % Area | Height |
|-----|--------|--------|--------|
| 1   | 16.05s | 764233 | 75.21  |
| 2   | 20.09s | 200091 | 20.07  |
| 3   | 27.32s | 210036 | 2.16   |
| 4   | 29.64s | 200046 | 2.66   |

Batch_Ref_No: FF.ASMJ00096.1
Empower Project: APC/Poeverlein/Poeverlein_2021

Current Date: 6/8/2021
Date Acquired: 6/8/2021 10:08:03 AM CEST
Vial: 44
Inj Vol: 5.00uL

1 of 2
Chiral Separation
Library: SM, Chiral & Peptide Purification
RID / IID in vitro Biology & HT Chemistry
Industriepark Höchst, G 836, Room 007
D-65925 Frankfurt
Dr. Schaffrath Tel.: +49-(0)69-305-30782

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hötze
Batch Ref No: FF.ASMJ00092.1
Sample Name: FF.ASMJ00092.1
Racemat
Lab Journal
Comment

Separation Information:
APC|Poeverlein|Poeverlein_2021
HPLC-System LC_03
Flowrate 1.0 mL/min
Temperature 30° C
HPLC Column Chiralpak IF181, 250x4.6 mm
Eluent Hep:HOH 10:1 + 0.1% TFA

Results

| RT  | Area | % Area  | Height |
|-----|------|---------|--------|
| 1   | 16.72| 467600  | 42.90  |
| 2   | 21.02| 403025  | 6.08   |
| 3   | 27.16| 767986  | 6.44   |
| 4   | 28.92| 489496  | 6.03   |

Batch_Ref_No FF.ASMJ00092.1 Empower Project: APC|Poeverlein|Poeverlein_2021

Current Date 6/8/2021 Vial 42
Date Acquired 6/8/2021 9:00:19 AM CEST Inj Vol 5.00 uL
Chiral Separation

Sample Information:
- Customer: Dr. Riverlein
- Analyst: K. Rahn-Hotze
- Batch Ref No: FF.ASMJ00097.1
- Sample Name: FF.ASMJ00097.1
- Racemate
- Lab Journal
- Comment

Separation Information:
- APC/Poeverlein/Poeverlein_2021
- HPLC-System: LC_03
- Flowrate: 1.0 ml/min
- Temperature: 30°C
- HPLC Column: Chiralpak IF181, 250x4.6 mm
- Eluent: HEP:HOH 10:1 + 0.1% TFA

Results

| RT  | Area  | % Area | Height |
|-----|-------|--------|--------|
| 1   | 1680  | 0.59   | 617    |
| 2   | 2096  | 94.16  | 65830  |
| 3   | 2718  | 5.12   | 39117  |
| 4   | 2594  | 0.13   | 730    |

Current Date: 8/8/2021
Date Acquired: 6/8/2021 10:41:54 AM CEST
Chiral Separation

Library, SM, Chiral & Peptide Purification
R&D / IDD in vitro Biology & HT Chemistry
Industriepark Hochst, G 838, Room D07
D-65926 Frankfurt
Dr. Schaffarth Tel.: +49-(0)69-303-30782

Sample Information:
Customer: Dr. Riverlein
Analyst: K. Rahn-Hotze
Batch Ref No: FF.ASMJ00093.1
Sample Name: FF.ASMJ00093.1

Separation Information:
AFC/Poeverlein/Poeverlein_2021
HPLC-System: LC_03
Flow rate: 1.0 ml/min
Temperature: 30°C
HPLC Column: Chiralpak IF/181, 250x4.6 mm
Eluent: Hep:EtOH 10:1 + 0.1% TFA

Results

| RT    | Area  | % Area | Height |
|-------|-------|--------|--------|
| 1     | 16.51 | 289523 | 2.11   |
| 2     | 20.82 | 902283 | 48.36  |
| 3     | 26.77 | 904285 | 48.67  |
| 4     | 28.25 | 115446 | 0.65   |

Current Date: 6/8/2021
Date Acquired: 6/9/2021 11:17:59 AM CEST
Vial: 43
Inj Vol: 15.00 µL
