Supporting Information

Identification of the amino acid labionin and its desulphurised derivative in the type-III lantibiotic LabyA2 by means of GC/MS

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1. Equipment, Solvents and Reagents

General Information:

Dry solvents were purchased from Fisher Scientific-ACROS (Schwerte, Germany). All other reagents were used as obtained (ABCR, Karlsruhe, Germany; Bachem, Bubendorf, Switzerland; Fisher Scientific, Schwerte, Germany; IRIS Biotech, Marktredwitz, Germany; Merck, Darmstadt, Germany; Sigma-Aldrich, Taufkirchen, Germany) unless otherwise noted.

GC-MS analytics:

GC-MS analyses were performed on a GC/MS 5975C (Agilent Technologies, Waldbronn, Germany). Detailed information is described in chapter 3.

HPLC-ESI-MS analytics:

HPLC-ESI-MS coupled measurements were performed on an Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled to an Agilent 1200 HPLC-system (Santa Clara, CA, USA) (column: Eclipse XDD-C18 5µm, 4.6x150mm; solvent A: H_2O + 0.1 % HCOOH; solvent B: MeOH + 0.1 % HCOOH; flow rate 1 ml/min; gradient: 5 % → 100 % B in 25 min) unless otherwise noted.

NMR spectroscopy:

Proton nuclear magnetic resonance (^{1}H-NMR) data were acquired on a Bruker (Karlsruhe, Germany) Avance 400 (400.14 MHz). Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the non-deuterated solvent residual signal. Coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublet; br, broad.

Proton decoupled Carbon-13 nuclear magnetic resonance (^{13}C-NMR) data were acquired on a Bruker Avance 400 (100.62 MHz). Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the undeuterated solvent residual signal.

2. Fermentation and Isolation of Lantibiotics

LabA2 (**6a**) and LabA1 (**24a**) were isolated from *Actinomadura namibiensis* according to procedures described previously.[1]

Nisin (**25**) was purchased from Molekula (Taufkirchen, Germany).

3. Microreactions for GC/MS and LC/MS analytics

General Information for analytical HPLC-ESI-MS

Column: Grom-Sil 120 ODS-5 ST (Grace, United States), length 100 mm, ID 2 mm, 3 µm

Gradient: Solvent A: H_2O + 0.1% HCO_2H

Solvent B: ACN + 0.1% HCO_2H

Flow rate: 0.2 mL / min

0 - 18 min:	Solvent B: 5% → 100%
18 - 25 min:	Solvent B: 100%
25 - 30 min:	Solvent B: 5%

Mass spectrometer:	Exactive Orbitrap (Thermo Fisher Scientific)
HPLC system:	1200 HPLC (Agilent)

Desulphurisation of Lantibiotics

The procedure for desulphurisation was adapted from a protocol by Kawulka et al. (2004):[2]

In brief, $NaBH_4$ (1.25 mg) was added to a suspension of LabA2 (**6a**, 0.5 mg) and $NiCl_2$ (2.37 mg) in MeOH (0.65 mL) and H_2O (dest., 0.318 mL) in a Reacti-Vial, which was then immediately sealed. The reaction was heated to 50 °C for 3 h. The extent of desulphurisation was monitored by LC-MS on an Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to an Agilent 1200 HPLC system (Agilent, Waldbronn, Germany). Therefore, a 100 µL aliquot was removed after 3 hours, acidified by adding 30 µL of TFA and spun for 1 min in a centrifuge. Additional aliquots of $NaBH_4$ and $NiCl_2$ were added portionwise to bring the reaction to completion. The reaction mixture was concentrated after 24 h in a Speedvac (Genevac EZ-2 MK2, Ipswich, United Kingdom) and freeze-dried. For desalting, solid phase extraction on a 1 g C8 column (Macherey & Nagel, Düren, Germany), was used. The column was conditioned with MeOH (1 column volume) and H_2O (1 column volume). Sample was resuspended in H_2O and applied to the column. The column was washed with H_2O (1 column volume), aqueous EDTA (50 mM) (1 column volume), and H_2O (1 column volume). Desulphurised LabA2 (**6b**) was eluted with MeOH (2 column volumes). The fractions were collected and freeze-dried.

LabA1 (**24a**, 0.5 mg) was desulphurised and purified in the same way as described above.

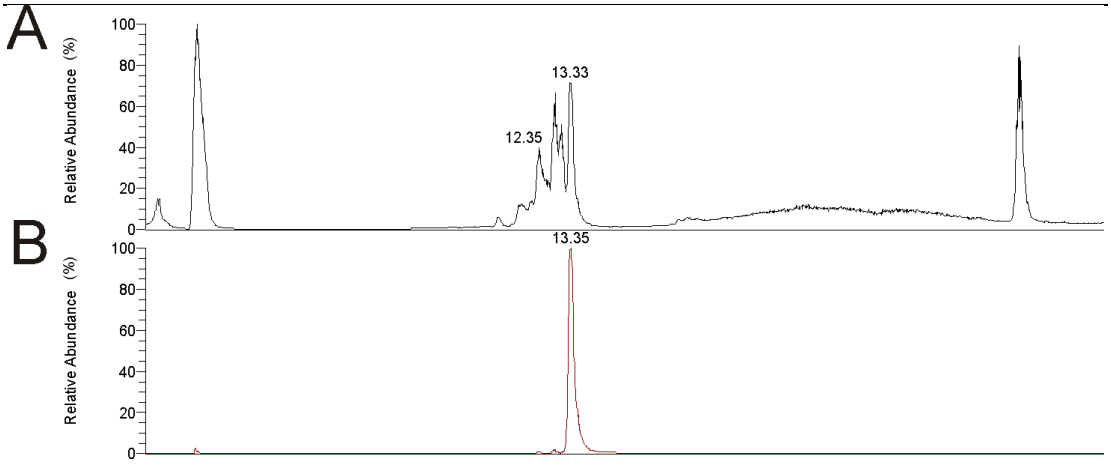


Figure S1: LC-ESI(+)-orbitrap-MS run of LabA2 after desulphurisation (**6b**). (A) Total ion chromatogram (TIC) (B) Extracted ion chromatogram (XIC) of the molecular mass of desulphurised LabA2 (**6b**) [$(M+2H)^{2+}$ = 931 Da] with the retention time R_t= 13.35 min.

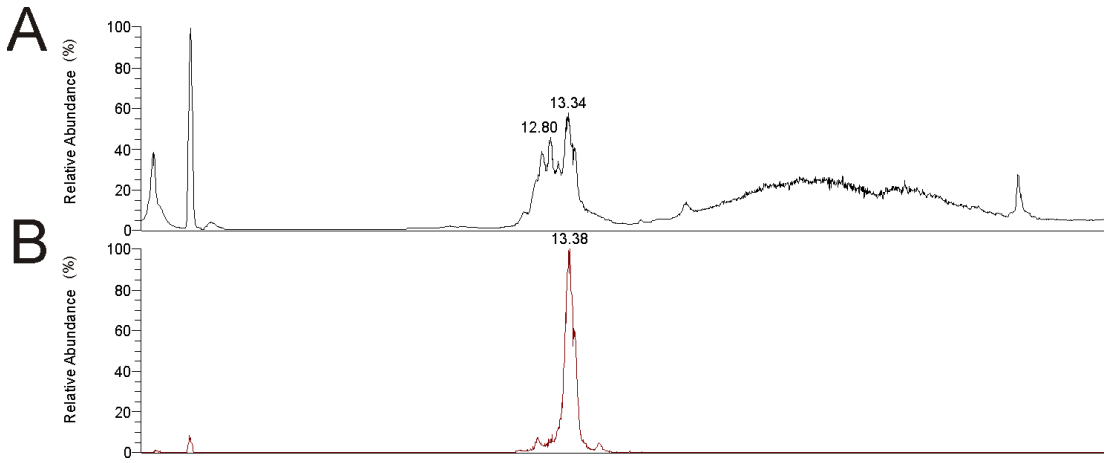


Figure S2: LC-ESI(+)-orbitrap-MS of LabA1 after desulphurisation (**24b**). (A) Total ion chromatogram (TIC) (B) Extracted ion chromatogram (XIC) of the molecular mass of desulphurised LabA1 (**24b**) [$(M+2H)^{2+}$ = 1006 Da] with the retention time R_t= 13.38 min.

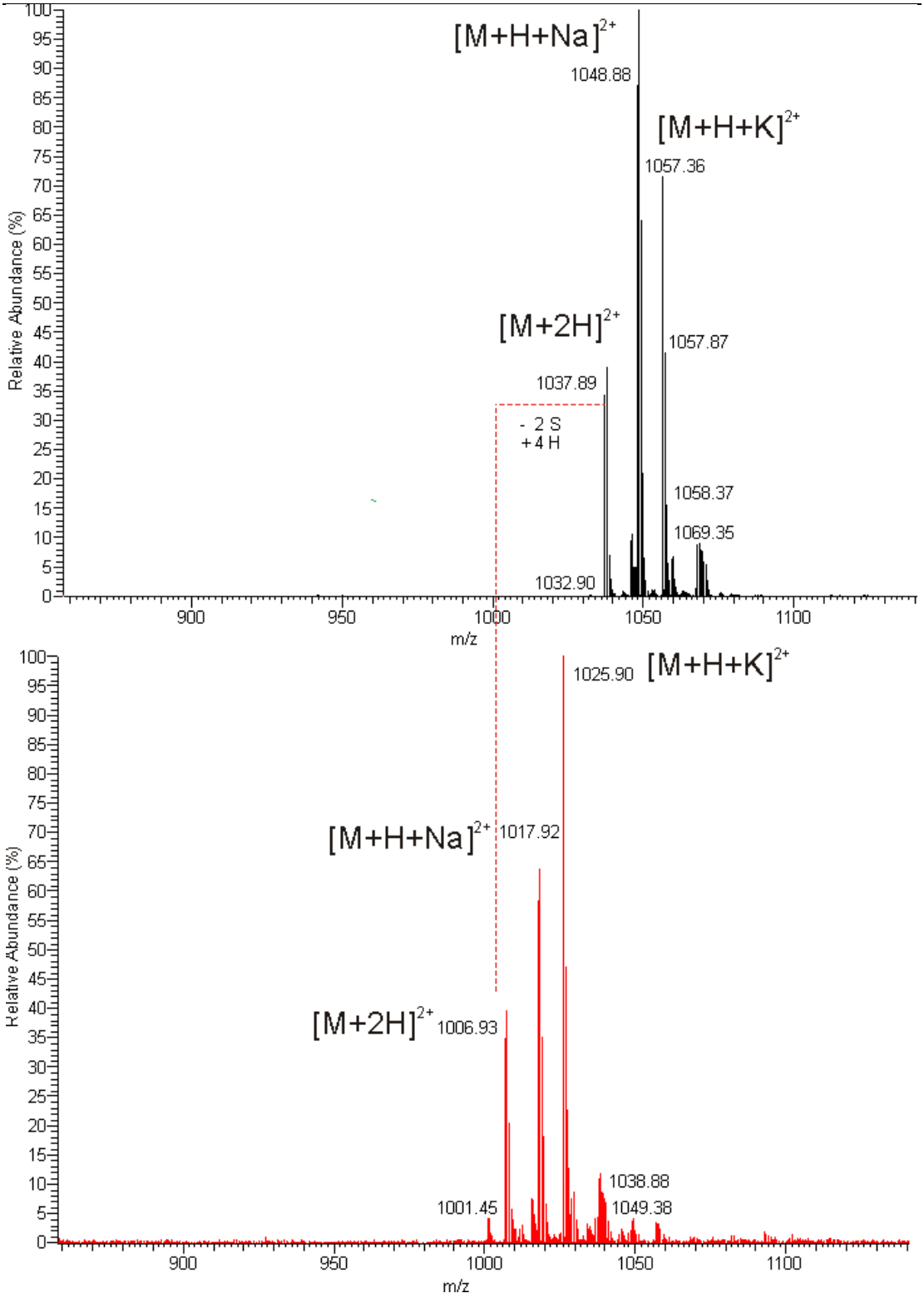


Figure S3: HR-ESI(+)-orbitrap mass spectra of LabA1 before desulphurisation (black, **24a**) [$(M+2H)^{2+}$ = 1006 Da] and after desulphurisation (red, **24b**) [$(M+2H)^{2+}$ = 1037 Da].

Total hydrolysis, derivatisation and GC/MS investigations

Total hydrolyses of LabA2 (**6a**, 0.5 mg) and desulpho-LabA2 (**6b**, 0.5 mg), respectively, were performed at 110 °C in aqueous 6 M hydrochloric acid solution (200 µl) (Sigma Aldrich, grade “for amino acid analysis”) under vacuum for 24 h in glass-ampoules. After 24 h, the hydrochloric acid was removed in a gentle stream of nitrogen.

For GC/MS analysis hydrolysates were transformed into their *N*-trifluoroacetyl/ethyl ester derivatives: To dry hydrolysate a total volume of 200 µl of 2M ethanolic HCl-solution, generated from acetylchloride in abs. ethanol (1:4, v:v) in a Reacti-Vial was added. The samples were heated for 30 min at 110 °C and reagents were removed at 110 °C in a gentle stream of nitrogen. Acetylation was performed by adding 100 µL dichloromethane and 50 µL trifluoroacetic anhydride (TFAA) to the samples. The mixtures were heated again for 10 min at 110 °C. Excess of reagent was removed at ambient temperature in a stream of nitrogen. Resulting residues were dissolved in 50 µL anhydrous toluene and portions of 2 µL were subjected to GC/MS (5975C, Agilent Technologies) using the parameters given in Table S1.

LabA1 (**24a**, 0.5 mg) desulpho-LabA1 (**24b**, 0.5 mg) and Nisin (**25**, 0.5 mg) were treated in the same way.

Amino acid standard α-methyl-2,4-diaminoglutaric acid (Dmg, **+/-22a/b**) was synthesised according to procedures outlined in chapter 4. GC-MS derivatisation was done as described above.

Amino acids were identified by prominent mass spectral fragments in positive chemical ionisation-(PCI)-mode compiled in Table S2.

Table S1: Parameters for GC/MS analysis.

Column	5% Phenyl methyl siloxan (Agilent 190913-433), 325 °C, 30 m, 250 µm x 0.25 µm
Temperature program	70 °C (2 min isothermal), 300 °C, 5 °C/min (1 min isothermal)
Scan	Full-Scan, 50-800 *m/z*
Injection	2 µL, split less injection
Heater	300 °C
MSD-Transfer line	280 °C
Pressure	11.052 psi
Flow	1.2 mL/min, 40.3 cm/s with He as carrier gas
PCI-Mode	Energy: 91 eV, MS Source: 300 °C, MS Quad: 150 °C, collision gas: methane with flow rate 18%

Table S2: Prominent molecular ions and mass spectral fragments obtained under GC-PCI-MS-conditions of the *N*-trifluoroacetyl/ethylester derivatives of the amino acids. Characteristic retention times (R_t) are indicated.

Three letter code	Prominent mass spectral fragments ions under PCI-MS-conditions (in amu)	$[M+H]^+$ under PCI-MS-conditions (in amu)	R_t (in min)
Ala	140, 168	214	6.3
Gly	126, 154	200	6.7
Thr	152, 180, 266	340	8.6
Leu	182, 210	256	10.8
Asp	212, 240	286	15.1
Glu	226, 254	300	18.3
Phe	216, 244	290	19.4
Trp	351, 379	425	28.8
Pro	166, 194	240	13.6
Val	168, 196	242	8.9
$(Cys)_2$	*n.d.*	*n.d.*	*n.d.*
Dmg	**238, 351, 379**	**425**	**20.9**
Lab	**520, 554, 594, 622**	**668**	**36.3**
Lan	**270, 383, 411**	**457**	**28.4**
MeLan	284, 397, 425	471	27.5

n.d. = not detected

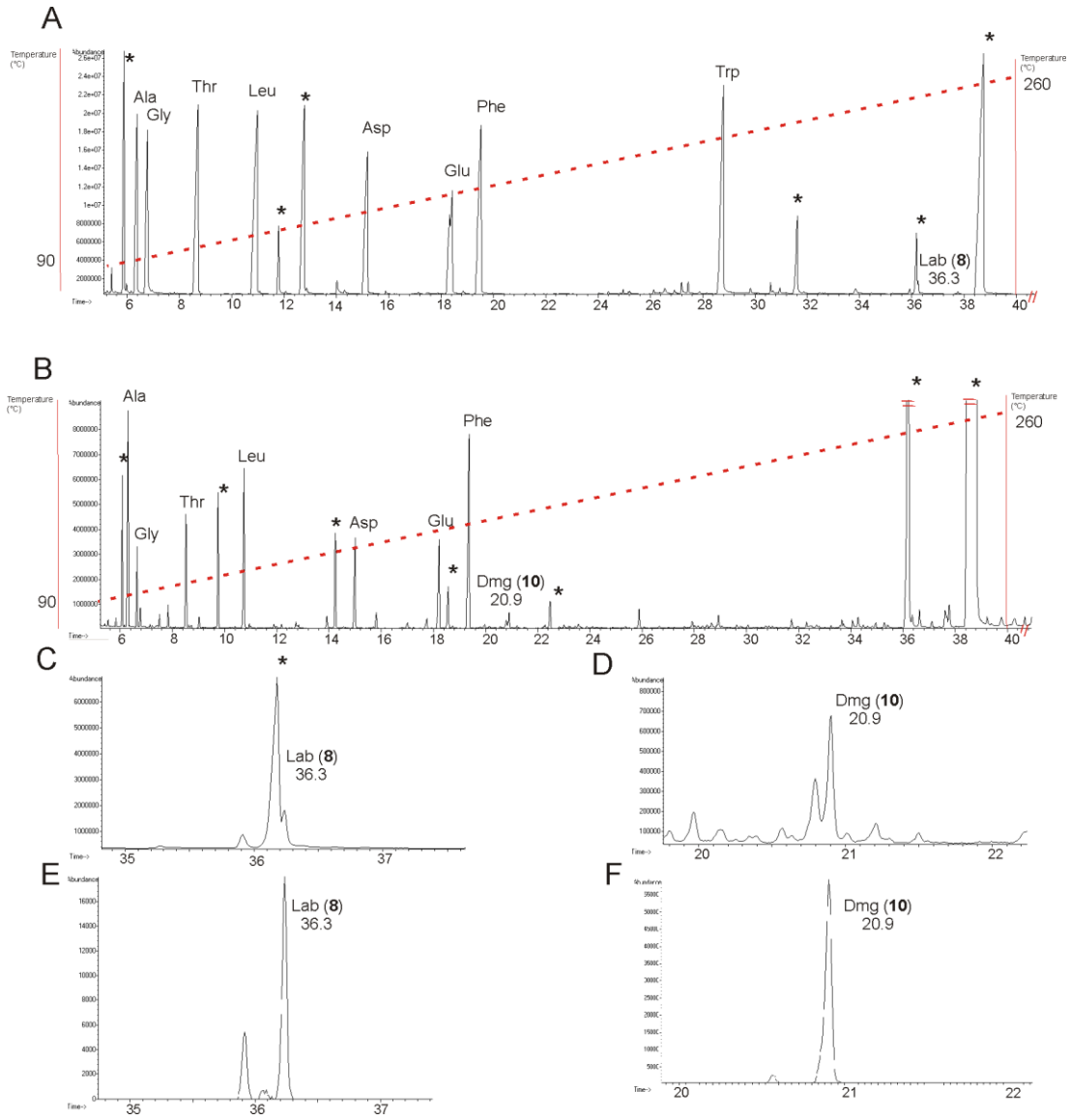


Figure S4: GC-MS analysis of amino acid compositions after total hydrolysis (6N HCl, 24 h) and derivatisation to the corresponding *N*-trifluoracetyl/ethylesters.

(A) LabA2 (**6a**). $(Cys)_2$ was not detected.

(B) desulpho-LabA2 (**6b**). $(Cys)_2$ and Trp were not detected.

(C) Enlarged section of (A) shows the retention behaviour of Lab (**8**) at R_t = 36.3 min.

(D) Enlarged section of (B) shows the retention behaviour of Dmg (**10**) at R_t = 20.9 min.

(E) XIC $[M+H]^+$ = 668 Da for Lab (**8**).

(F) XIC $[M+H]^+$ = 425 Da for Dmg (**10**).

Peaks marked with (*) could not be assigned.

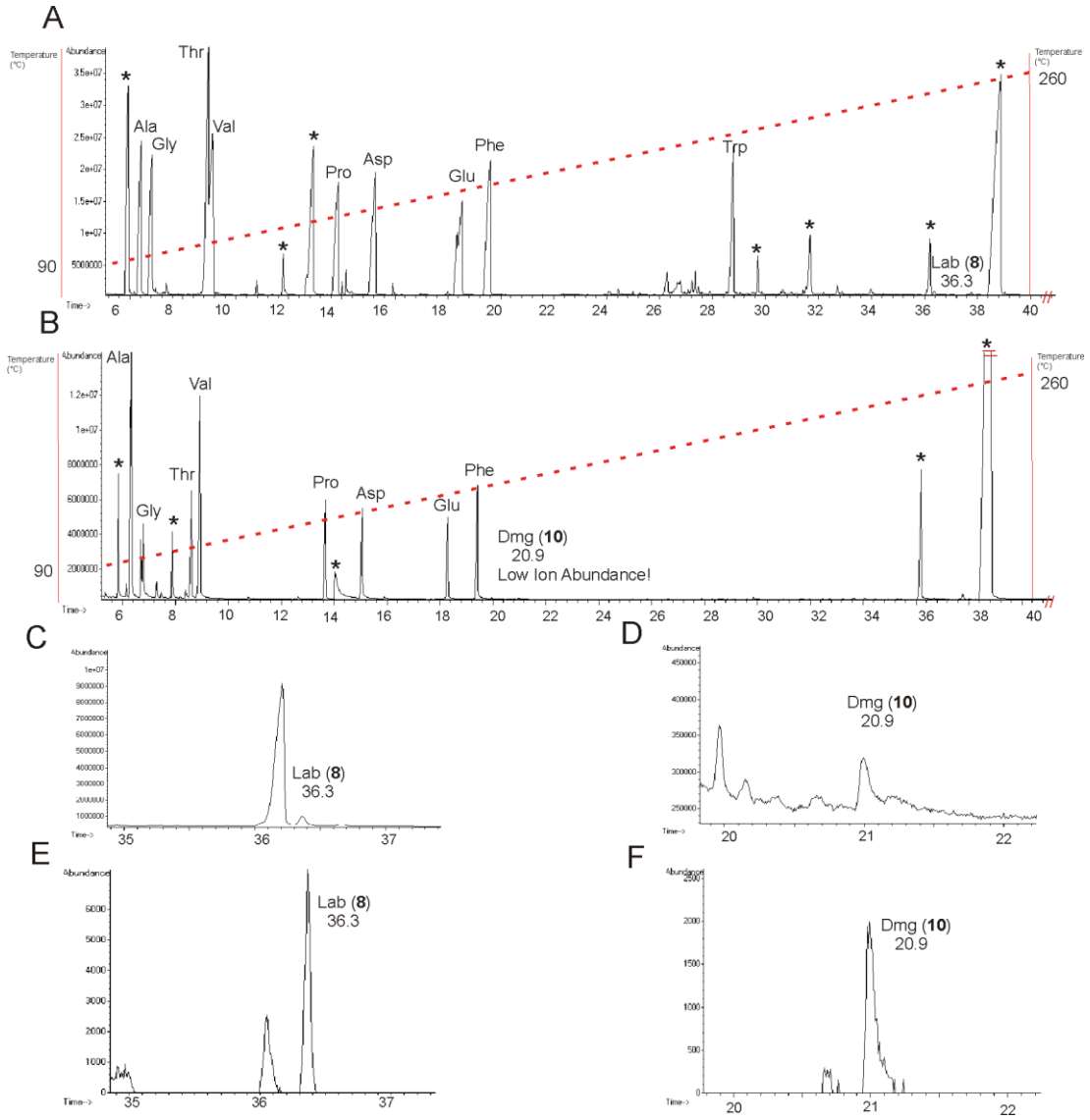


Figure S5. GC-MS analysis of amino acid compositions after total hydrolysis (6N HCl, 24 h) and derivatisation to the corresponding *N*-trifluoracetyl/ethylesters.

(A) LabA1 (**24a**). $(Cys)_2$ was not detected.

(B) LabA1 after desulphurisation (**24b**). $(Cys)_2$ and Trp were not detected.

(C) Enlarged section of (A) shows the retention behaviour of Lab (**8**) at R_t = 36.3 min.

(D) Enlarged section of shows the retention behaviour of Dmg (**10**) at R_t = 20.9 min.

(E) XIC $[M+H]^+$ 668 Da for Lab (**8**).

(F) XIC $[M+H]^+$ 425 Da for Dmg (**10**).

Peaks marked with (*) could not be assigned.

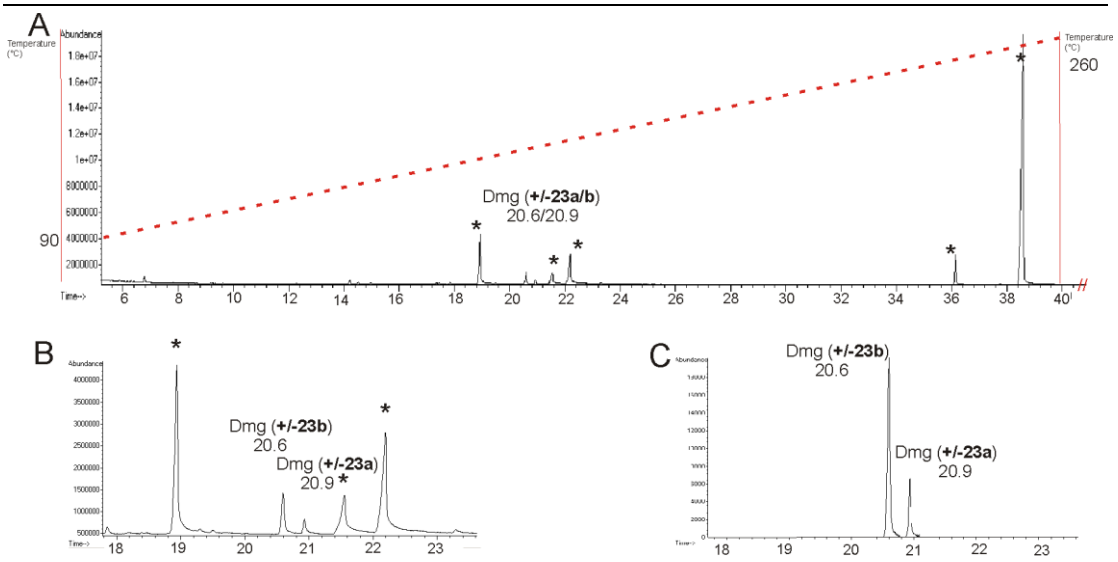


Figure S6: GC-MS chromatograms of synthetic α-methyl-2,4-diaminoglutaric acid (Dmg, **+/-22a/b**) standard after derivatisation to the corresponding *N*-trifluoracetyl/ethylesters. (A) Dmg (**+/-23a/b**) (B) Enlarged section of (A) shows the GC-chromatogram of Dmg diastereomers at R_t = 20.6 min (**+/-23b**) and R_t = 20.9 min (**+/-23a**) (C) XIC $[M+H]^+$ = 425 Da for Dmg (**+/-23a/b**). Peaks marked with (*) could not be assigned.

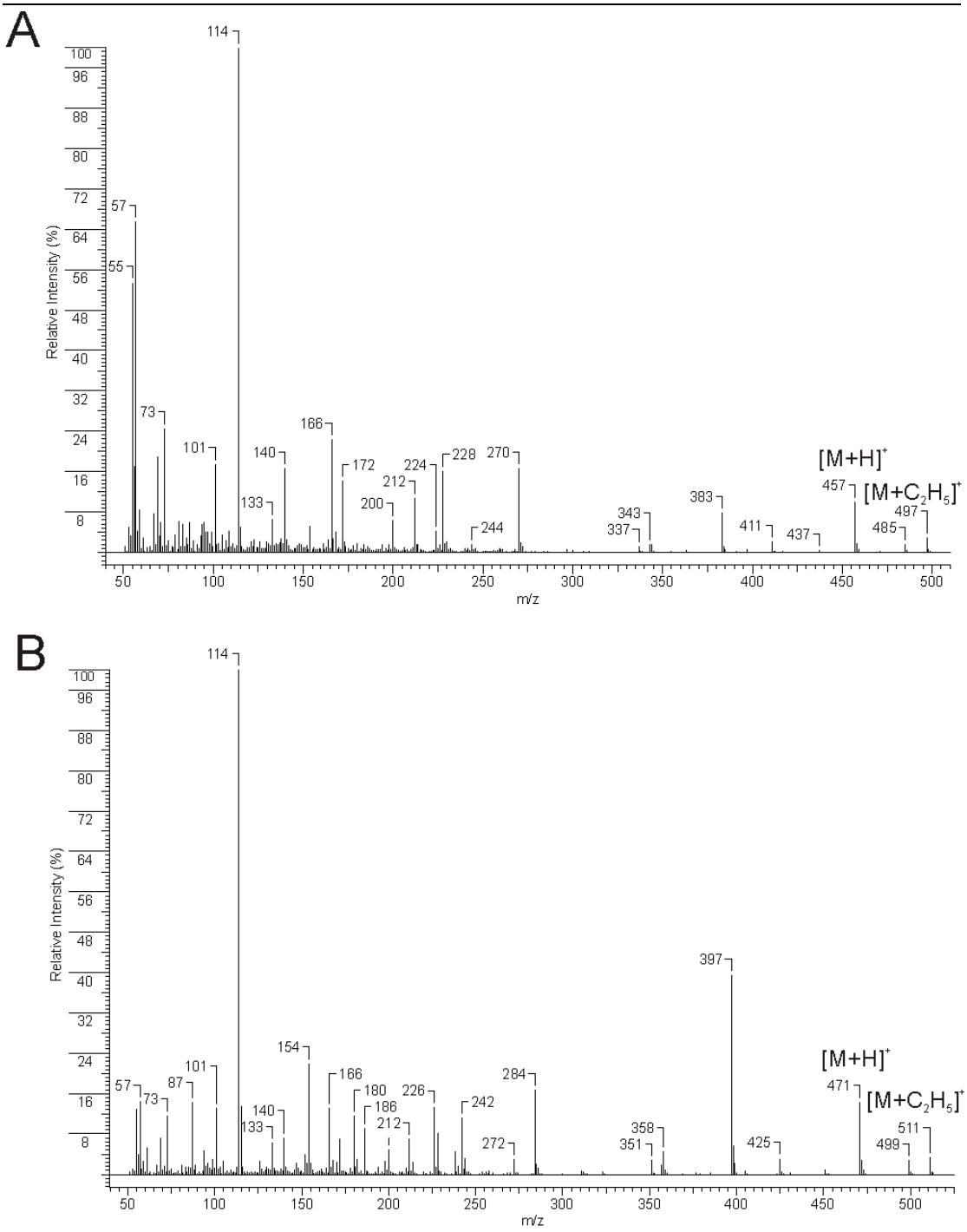


Figure S6: PCI mass spectra of *N*-trifluoracetyl/ethylesters of (A) Lan (**1**, R_t = 28.4 min). (B) MeLan (**2**, R_t = 27.5 min) from hydrolysis, derivatisation and GC-MS analysis of Nisin (**25**).

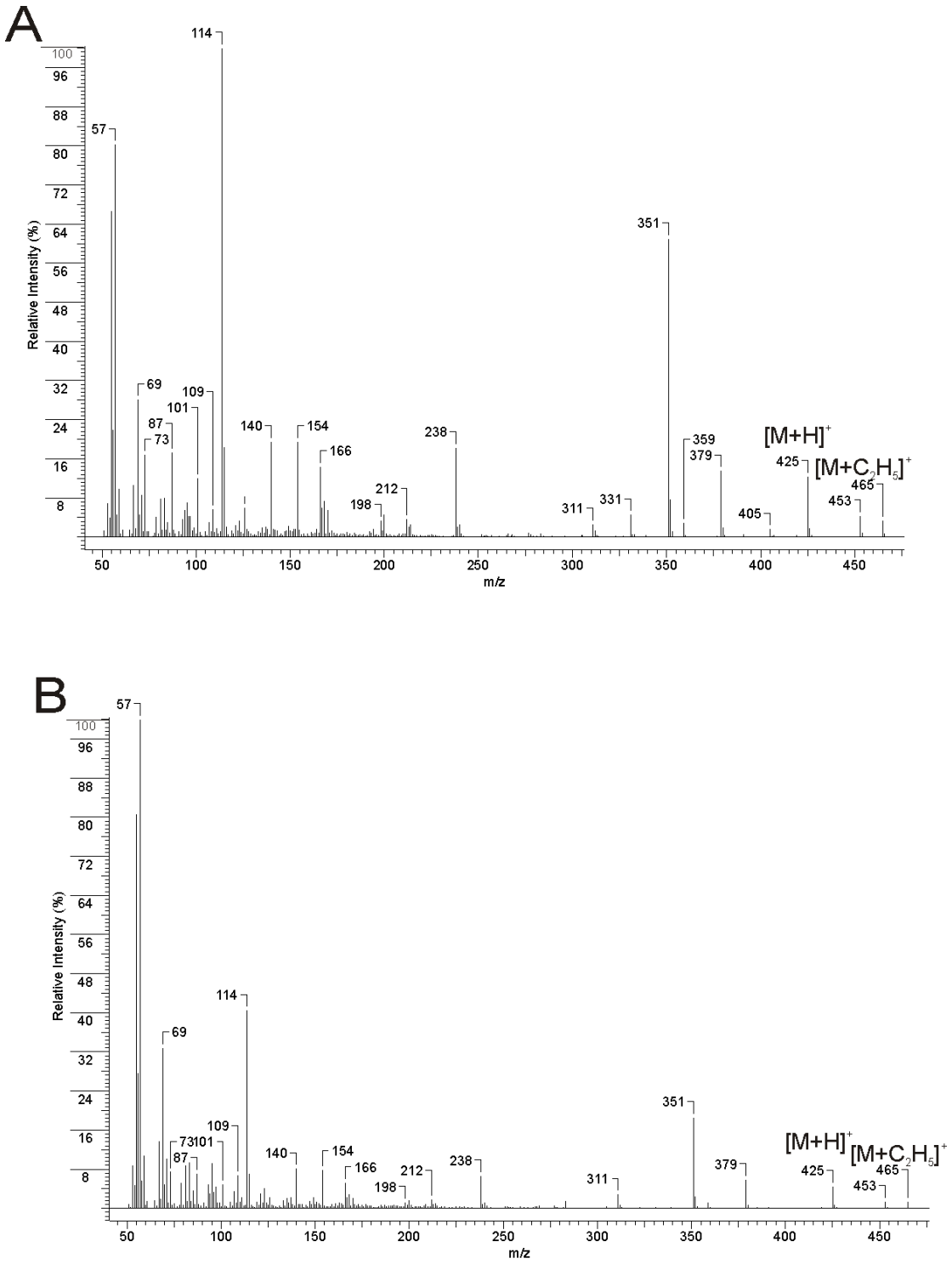


Figure S8: PCI mass spectra of synthetic Dmg diastereomers (**+/-23a/b**; $(M+H)^+$ = 425 amu) after *N*-trifluoracetyl/ethylester derivatisation (A) **+/-23b** R_t = 20.6 min (B) **+/-23a** R_t = 20.9 min.

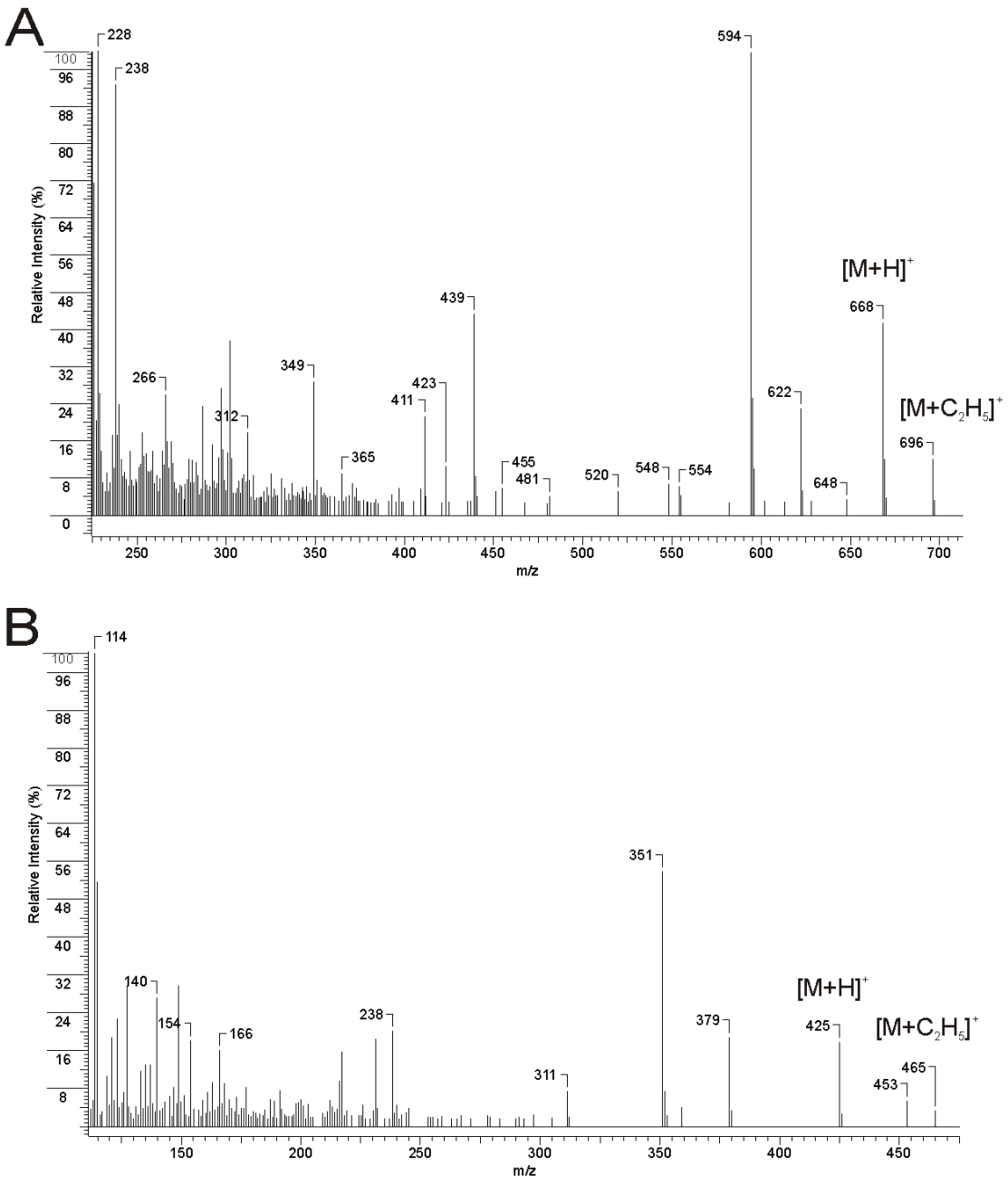


Figure S9: PCI mass spectra of *N*-trifluoracetyl/ethylesters of (A) Lab (**8**, R_t = 36.3 min) and (B) Dmg (**10**, R_t = 20.9 min) detected in hydrolysates before and after desulphurisation of LabA1 (**24a/b**).

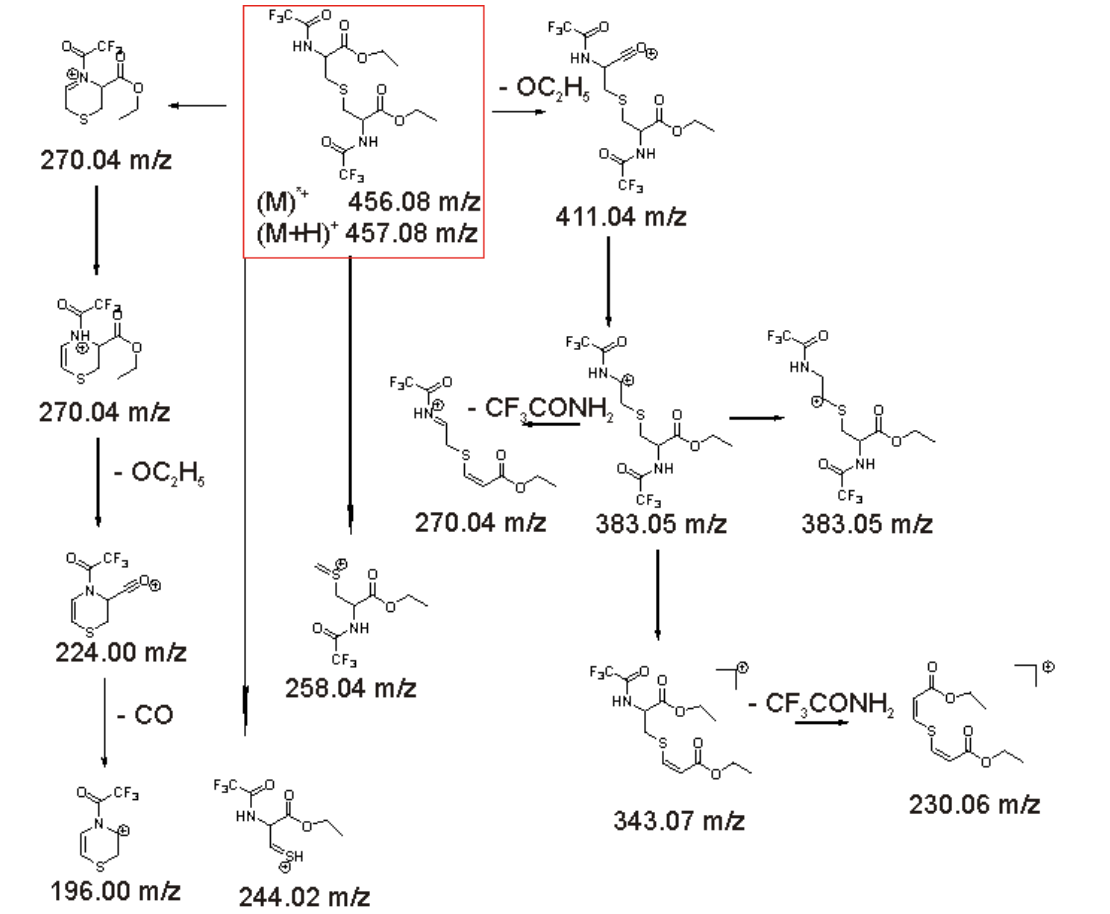


Figure S10: Characteristic fragment ions series for *N*-trifluoroacetyl/ethylesters of the amino acid Lan (**1**).

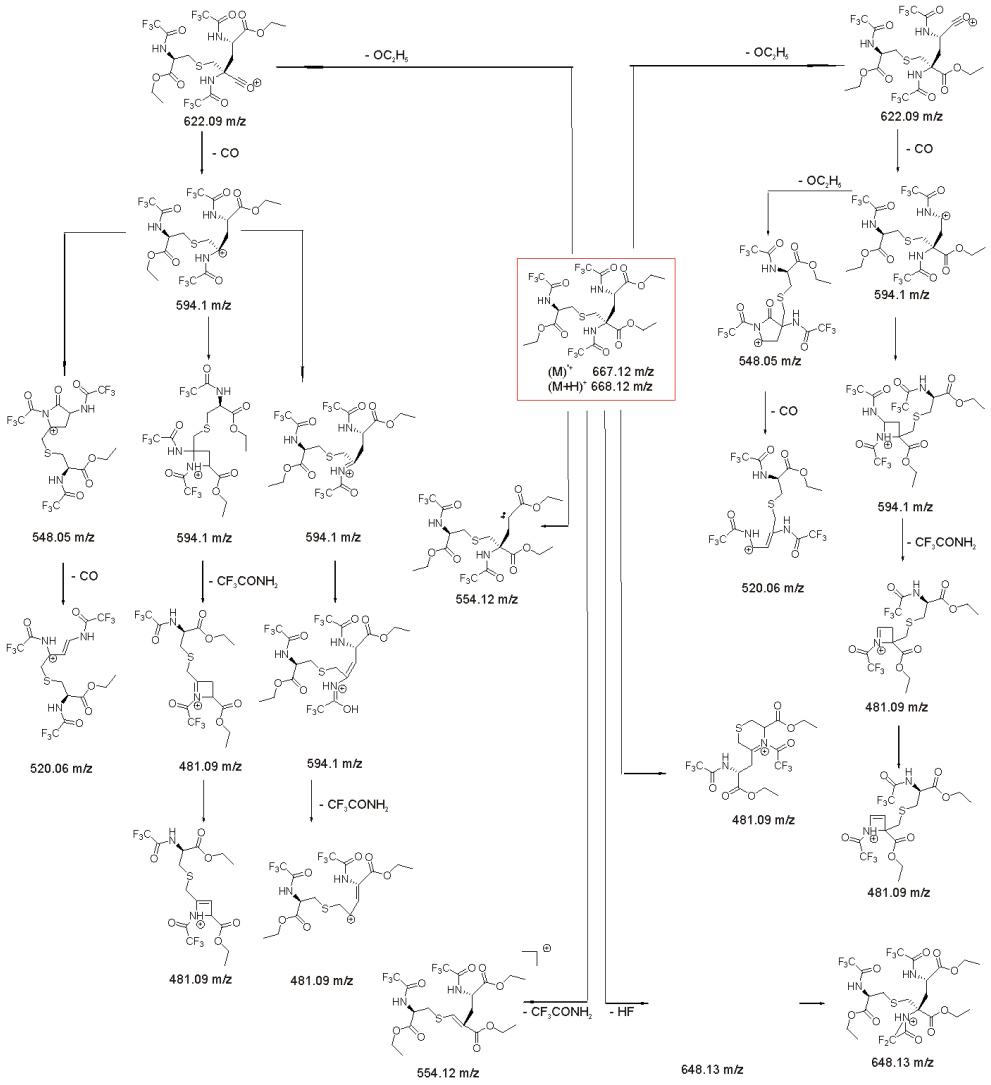


Figure S11: Characteristic fragment ions series for *N*-trifluoroacetyl/ethylesters of the amino acid Lab (**8**).

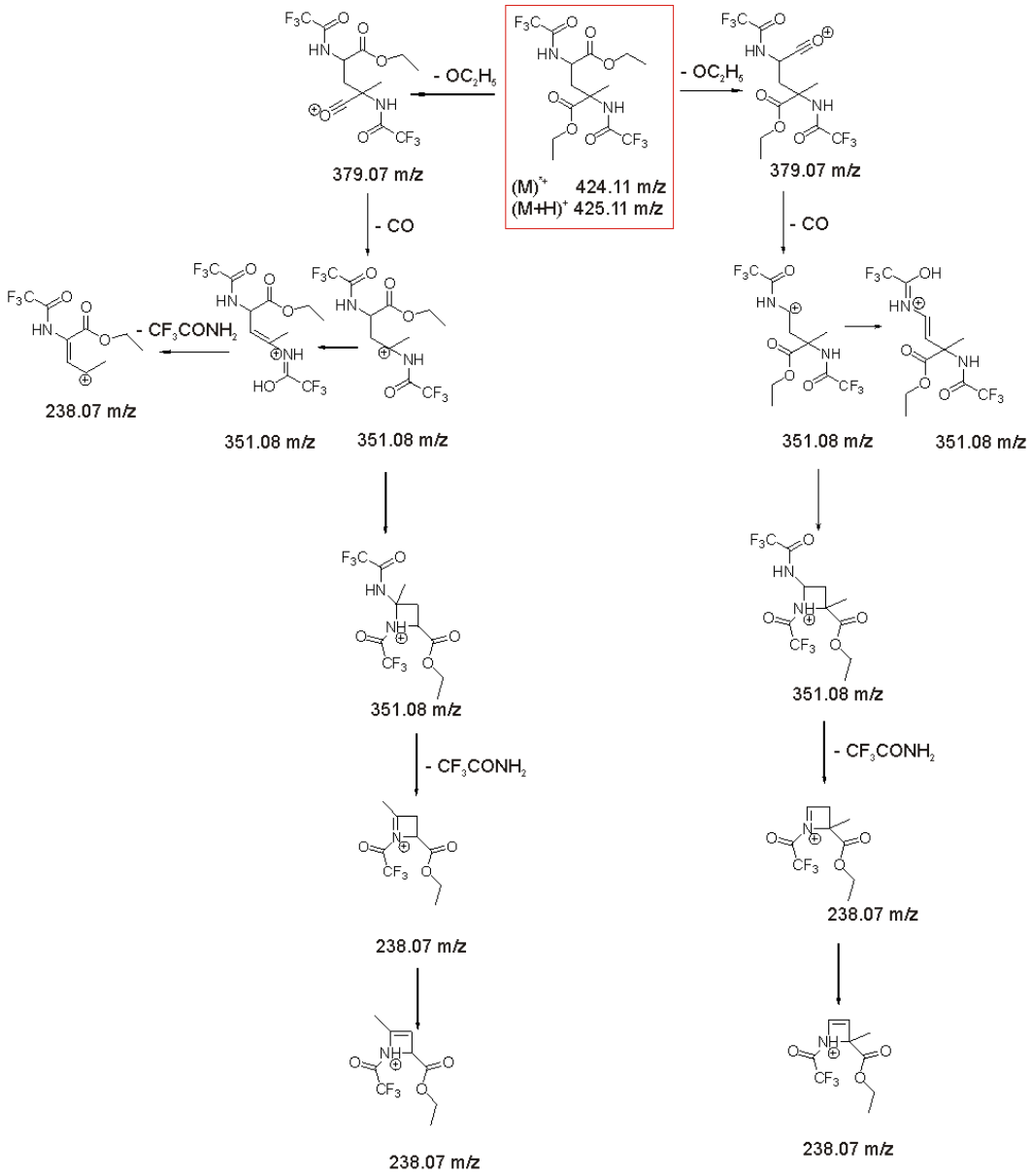


Figure S12: Characteristic fragment ions series for *N*-trifluoroacetyl/ethylesters of the amino acid Dmg (**10, +/-23a/b**).

4. Synthesis of α-Methyl-2,4-Diaminoglutaric Acid (Dmg, +/-23a/b)

General Information:

Flash chromatography was performed with Davisil® silica gel (GRACE Davison, Hollerhecke, Germany; 40-63 µm grade) or using the CombiFlash Rf System with prepacked columns (Teledyne ISCO, Lincoln, NE, USA).

Analytical thin-layer chromatography was performed with commercial TLC aluminium sheets (Merck, Darmstadt, Germany, TLC Silica gel 60 F_{254}). Compounds were visualised by UV-light at λ= 254 nm and/or by dipping the plates in potassium permanganate-, ninhydrin-, or 2,6-dichlorophenolindophenol-solution followed by heating.

Staining solutions:

Permanganate reagent:

3 g $KMnO_4$, 20 g K_2CO_3, 300 ml H_2O, 5 ml 5 % NaOH-solution

Ninhydrine-reagent:

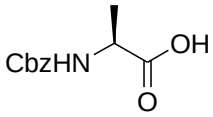
0.3 g ninhydrine, 100 ml n-BuOH

2,6-Dichlorophenolindophenol-reagent:

0.38 g 2,6-dichlorophenolindolphenol (Na-salt), 480 ml EtOH

Chiral HPLC-analyses were performed on a *Merck-Hitachi* LaChrom system with a CHIRALPAK OD-H column using *n*-PrOH and *n*-hexane as eluents (flow rate 1 ml/min).

Cbz-L-Alanine (12)



L-Alanine **11** (7 g, 78.57 mmol) was dissolved in saturated $NaHCO_3$-solution (320 ml) benzylchloroformate (16.8 ml, 117.7 mmol) was added. The reaction mixture was stirred at room temperature. After 16 h the mixture was washed with diethylether (3x250 ml). The aquatic layer was acidulated with aqueous 5% HCl und extracted with EtOAc (5x250 ml). The organic layer was dried and concentrated to give 17.19 g (yield 98%) of the Cbz-L-Ala-OH.

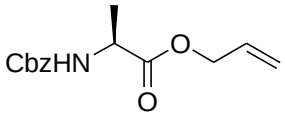
^{1}H-NMR (DMSO-d_6): 1.22 (d, J=7.39 Hz, 3H), 3.99 (dt, J_1=14.4 Hz, J_2=7.4 Hz, 1H), 5.1 (s, 2H), 7.3 (m, 5H)

^{13}C-NMR (DMSO-d_6): 17.4, 49.9, 65.42, 127.83, 128.42, 137.11, 155.92, 174.47

Rf 0.47 (9:2; $CHCl_3$:MeOH)

MS (EI): 223.0847 $[M]^{\bullet+}$

Cbz-L-Alanine-allylester (13)



Cbz-L-Ala-OH **12** (5 g, 22.4 mmol) was dissolved in DMF (400 ml). Cesium carbonate (15 g, 46.03 mmol) and allyl bromide (2.14 ml, 24,7 mmol) were added and stirred at room temperature. After 16 h the mixture was diluted with EtOAc (300 ml) and washed with brine (5x 300 ml). The organic layer was dried and concentrated to yield 5.77 g (98%) of desired Cbz-L-Ala-OAll.

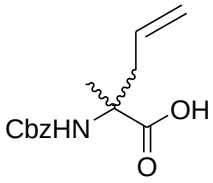
^{1}H-NMR (DMSO-d_6): 1.42 (d, *J*=7.39 Hz, 3H), 4.41 (dt, J_1=14.67 Hz, J_2=7.32 Hz, 1H), 4.57 (d, *J*=4.30 Hz, 2H), 5.02 (s, 2H), 5.19 (d. *J*=10.86 Hz, 2H), 5.29 (d, *J*=17.33 Hz, 2H), 5.86 (ddd, J_1=22.23 Hz, J_2=10.61 Hz, J_3=5.44 Hz, 1H), 7.33 (m, 5H), 7.73 (d, *J*=6.98 Hz, 1H),

^{13}C-NMR ($CDCl_3$): 18.68, 49.64, 65.92, 66.89, 118.72, 128.08, 128.14, 128.50, 131.50 136.23, 155.54, 174.64

Rf 0.45 (3:1, hexane-EtOAc)

MS (EI): 263.1165 $[M]^{\bullet+}$

rac-Cbz-α-Allyl-Alanine (14)



Diisopropylamine (3.5 ml, 25 mmol) was dissolved in THF (23 ml) and *n*-butyllithium-solution (1.6 M, 15.7 ml) was added at -30 °C, to prepare a solution of LDA. The mixture was stirred at this temperature for 1 h, then the temperature was decreased to - 78°C. Cbz-L-Ala-OAll **13** (3 g, 11.4 mmol) dissolved in THF (17 ml) was added, followed by $ZnCl_2$ (1.8 g, 13.2 mmol) in THF (17 ml). The mixture was stirred for 4 h at -78°C and then allowed to warm up to room temperature for 12 h. Then the mixture was diluted with EtOAc (100 ml) and washed with aqueous 5% HCl. The organic layer was dried und concentrated. The crude product was purified by column chromatography (Hexane - EtOAc; 2:1) to give an oil (2.5 g, yield 86%).

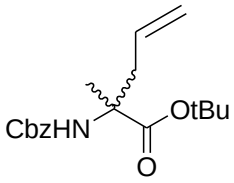
^{1}H-NMR (MeOD-d_4): 1.44 (s, 3H), 2.59 (dd, J_1= 13.57 Hz, J_2= 8.06 Hz, 1H), 2.68 (dd, J_1= 13.40, Hz, J_2= 7.23, Hz, 1H), 5.06 (m, 4H), 5.72 (m, 1H), 7.29(m, 6H)

^{13}C-NMR (MeOD-d_4): 23.23, 41.94, 59.92, 67.27, 119.35, 128.73, 128.92, 129.41, 133.86, 138.28, 157.14, 177.27

Rf 0.54 (9:1, $CHCl_3$:MeOH)

MS (EI): 263.1157 $[M]^{\bullet+}$

rac-Cbz-α-Allyl-Alanine-tert-butylester (15)



Acid **14** (2.21 g, 5.57 mmol) was dissolved in DCM (14 ml) a solution of *tert*-butyl-trichloroacetimidate (3.66 g, 16.75 mmol) in hexane (18 ml) and borontrifluoride diethyletherate (0.169 ml, 1.37 mmol) was added. The mixture was stirred for 12 h and then filtrated. The solvent was removed in the vacuum and the crude product was purified by column chromatography (pentane-EtOAc; 5:1) to give 1.61 g (yield 60%) of the product.

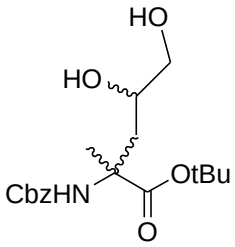
^{1}H-NMR (DMSO-d_6): 1.24 (s, 3H), 1.31 (s, 9H), 2.37 (dd, J_1= 13.70 Hz, J_2= 7.66 Hz, 1H), 2.53 (m, 1H), 5.02 (m, 4H), 7.31(m, 6H), 7.51 (s, 1H)

^{13}C-NMR ($CDCl_3$): 23.28, 27.83, 41.00, 59.57, 66.18, 82.00, 119.04, 127.93, 127.96, 128.43, 136.65, 154.52, 172.73

Rf 0.66 (3:1, hexane-EtOAc)

MS (EI): 319.1788 $[M]^{\bullet+}$

rac-Cbz-2-Amino-tert-buty-4,5-dihydroxy-2-methyl-pentanoate(16)



tert-Butyl-ester **15** (1.78 g, 5.57 mmol) was dissolved in water/*tert*-butanol (1:1, 26 ml). AD-Mix β (7.84 g) was added and the reaction mixture was stirred for 12 h at room temperature. The reaction was quenched by the addition of Na_2SO_3 and the mixture was extracted with EtOAc (3x10 ml). The organic layer was dried and concentrated and the crude product was purified by column chromatography (Hex-EtOAc; 1:1) to give 1.2 g (yield 61%) of a colourless oil.

^{1}H-NMR ($CDCl_3$): 1.42 (s, 3,6H); 1.44 (s, 5.2H); 1.54 (s, 1.8H), 1.58 (s, 1.3H), 1.84 (d, J= 14.24 Hz, 0.56H), 1.98 (dd, J_1= 14.37 Hz, J_2= 10.34 Hz, 1H), 2.08 (m, 0.74H); 2.22 (d, J= 14.37 Hz, 0.72H), 3.39 (dd, J_1= 10.95 Hz, J_2= 7.46 Hz, 1H), 3.52 (m, 1 H), 3.79 (m, 1H), 5.045 (m, 2H), 6.16 (s, 0.35H), 6.20 (s, 0.53H), 7.31 (m, 5H)

^{13}C-NMR ($CDCl_3$): 24.06, 27.77, 39.22, 40.53, 58.37, 59.51, 66.37, 66.83, 66.91, 68.77, 69.40, 82.32, 82.39, 128.02, 128.07, 128.10, 128.50, 136.57, 154.68, 173.19, 173.70

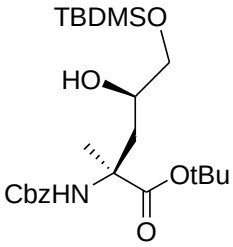
Rf 0.22 (1:1; hexane-EtOAc)

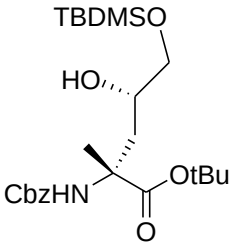
MS (ESI): 354.1914 $[M+H]^+$

General procedure for the synthesis of *Cbz-2-Amino-tert-buty-5-tert-butylsilylether-4-hydroxy-2-methyl-pentanoate (+/-17a/b)*

Diol **16** (1 eq.) was dissolved in DMF (6.25 ml/mmol), *tert*-butyldimethylsilylchloride (1.05 eq.) and imidazole (1.1 eq) were added. The mixture was stirred for 4 h. The reaction was quenched by addition of water and the mixture was diluted with EtOAc (45 ml/mmol). The organic layer was washed with brine (4x), dried and concentrated. The crude product was purified and the corresponding diastereomers ***+/-17a*** and ***+/-17b*** were separated by column chromatography (hexane-EtOAc, 9:1).

(2*S*,4*R*)/ (2*R*,4*S*)-*Cbz-2-Amino-tert-buty-5-tert-butylsilylether-4-hydroxy-2-methyl-pentanoate (+/-17a)*





Yield 41 % (921 mg)

de = 98 %

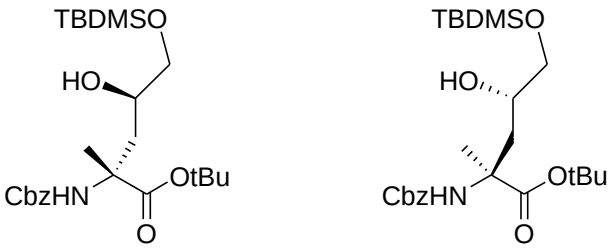
^{1}H-NMR ($CDCl_3$): 0.04 (d, *J*= 0.94 Hz, 6H), 0.88 (s, 9H), 1.44 (s, 9H), 1.55 (s, 3H), 1.97 (dd, J_1= 14.24 Hz, J_2= 10.34 Hz, 1H), 2.14 (d, *J*= 14.80 Hz, 1H), 2.31 (d, *J*= 3.90 Hz, 1H), 3.36 (dd, J_1= 9.87 Hz, J_2= 7.05 Hz, 1H), 3.56 (dd, J_1= 10.21 Hz, J_2= 2.96 Hz, 1H), 3.73 (ddd, J_1= 9.74 Hz, J_2= 6.78 Hz, J_3= 2.96 Hz, 1H), 5.07 (dd, J_1= 16.25 Hz, J_2= 12.48 Hz, 2H), 6.32 (s,1H), 7.29 (m, 5H)

^{13}C-NMR ($CDCl_3$): -5.46, -5.38, 18.23, 23.66, 25.84, 27.75, 39.17, 58.36, 66.07, 67.02, 68.40, 81.79, 86.60, 127.84, 127.42, 136.77, 154.53, 172.52

Rf 0.57 (5:1; hexane-EtOAc)

MS (ESI): 468.2767 $[M+H]^+$

(2R,4R)/ (2S,4S)-Cbz-2-Amino-tert-buty-5-tert-butylsilylether-4-hydroxy-2-methyl-pentanoate (+/-17b)



Yield 27 % (606 mg)

de = 98 %

^{1}H-NMR ($CDCl_3$): 0.04 (d, *J*= 0.94 Hz, 6H), 0.88 (s, 9H), 1.42 (s, 9H), 1.61 (s, 3H), 1.82 (dd, J_1= 14.78 Hz, J_2= 2.15 Hz, 1H), 1.88 (dd, J_1= 14.50 Hz, J_2= 9.34 Hz, 1H), 2.66 (s), 3.35 (dd, J_1= 9.81 Hz, J_2= 3.76 Hz, 1H), 3.77 (dd, J_1= 14.78 Hz, J_2= 2.15 Hz, 1H), 5.07 (s, 2H), 6.40 (s,1H), 7.32 (m, 5H)

^{13}C-NMR ($CDCl_3$): -5.38, 18.27, 24.73. 25.85, 27.88, 40.68, 59.66, 66.25, 67.28, 69.30, 81.71, 86.60, 127.91, 128.07, 128.42, 136.81, 155.52, 172.96

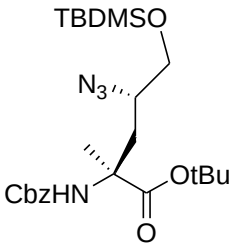
Rf 0.45 (5:1; hexane-EtOAc)

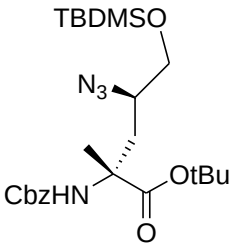
MS (ESI): 468.2767 $[M+H]^+$

General procedure for the synthesis of *Cbz-2-Amino-4-azido-tert-butyl -5-tert-butylsilylether-2-methyl-pentanoate (+/-18a/b)*

TBDMS-ether **17** (1 eq.) was dissolved in dry THF (10 ml/mmol), PPh_3 (2.5 eq.) was added. The mixture was cooled down and DEAD (7.5 eq.) and DPPA (2.5 eq.) were slowly added at -78 °C. The reaction mixture was stirred at -78 °C for 12 h, then the solvent was removed. The crude product was purified by column chromatography (hexane: EtOAc; 14:1).

(2S,4S)/ (2R,4R)-Cbz-2-Amino-4-azido-tert-butyl-5-tert-butylsilylether-2-methyl-pentanoate (+/-18a)





Yield 49 % (156 mg)

de = 97 %

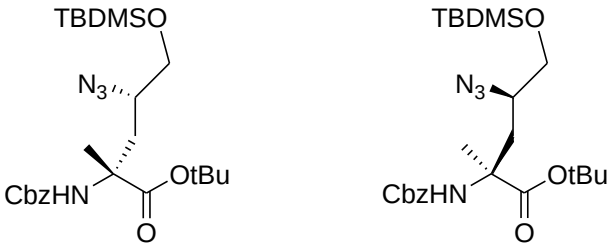
^{1}H-NMR ($CDCl_3$): 0.06 (d, *J*= 1.48 Hz, 6H), 0.90 (s, 9H), 1.44 (s, 9H), 1.56 (s, 3H), 1.93 (dd, J_1= 14.84 Hz, J_2= 3.02 Hz, 1H), 2.05 (m, 1H), 3.39 (m, 1H), 3.58 (dd, J_1= 10.2 Hz, J_2= 7.39 Hz, 1H), 3.64(dd, J_1= 10.2 Hz, J_2= 4.43 Hz, 1H), 5.08 (dd, J_1= 21.9 Hz, J_2= 12.2 Hz, 2H), 5.79 (s, 1H), 7.33 (m, 5H)

^{13}C-NMR ($CDCl_3$): -5.57, -5.54, 18.20, 24.28, 25.76, 27.81, 36.83, 56.51, 59.14, 60.62, 66.42, 66.95, 82.32, 128.02, 128.05, 128.46, 136.59, 154.92, 172.58

Rf 0.44 (5:1; hexane-EtOAc)

MS (ESI): 493.2830 $[M+H]^+$, 515.2646 $[M+Na]^+$

(2R,4S)/ (2S,4R)-Cbz-2-Amino-4-azido-tert-butyl-5-tert-butylsilylether-2-methyl-pentanoate (+/-18b)



Yield 68 % (188 mg)

de = 92 %

^{1}H-NMR ($CDCl_3$): 0.06 (d, *J*= 1.88 Hz, 6H), 0.90 (s, 9H), 1.54 (s, 9H), 1.57 (s, 3H), 1.87 (dd, J_1= 14.37 Hz, J_2= 10.61 Hz, 1H), 2.36 (d, *J*= 14.10 Hz, 1H), 3.36 (m, 1H), 3.54 (dd, J_1= 10.34 Hz, J_2= 7.66 Hz, 1H), 3.69 (d, *J*= 8.19 Hz, 1H), 5.07 (s, 2H), 6.14 (s, 1H), 7.32 (m, 5H)

^{13}C-NMR ($CDCl_3$): -5.58, -5.54, 18.20, 24.40, 25.78, 27.65, 36.63, 58.31, 60.28, 66.27, 82.66, 127.86, 128.05, 128.51, 136.61, 154.30, 172.61

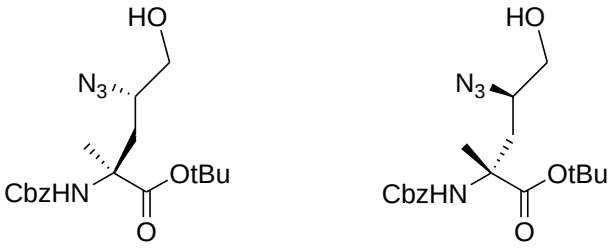
Rf 0.55 (5:1; hexane-EtOAc)

MS (ESI): 493.2823 $[M+H]^+$, 515.2639 $[M+Na]^+$

General procedure for the synthesis of *Cbz-2-Amino-4-azido-tert-butyl -5-hydroxy-2-methyl-pentanoate* (*+/-19a/b*)

At 0 °C a TBAF-solution (1 M in THF, 1.2 eq.) was added to a solution of azide **18** (1 eq.) in THF (7.4 ml/ mmol). The mixture was stirred at 0 °C. After 4 h the reaction was quenched by addition of saturated NH_4Cl-solution followed by extraction with EtOAc (3x). The organic layer was dried and the solvent was removed. The crude product was purified by column chromatography (hexane-EtOAc, 3:1).

(*2S,4S*)/ (*2R,4R*)-*Cbz-2-Amino-4-azido-tert-butyl-5-hydroxy-2-methyl-pentanoate* (*+/-19a*)



Yield 70 % (128 mg)

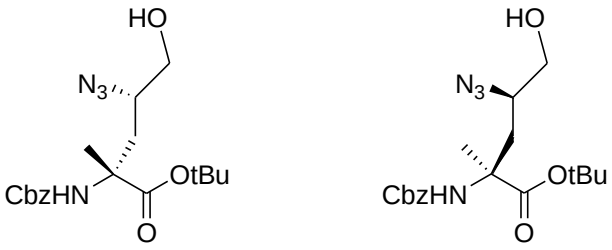
^{1}H-NMR ($CDCl_3$): 1.45 (s, 9H), 1.56 (s, 3H), 2.06 (dd, J_1= 14.84 Hz, J_2= 3.96 Hz, 1H), 2.27 (dd, J_1= 14.71 Hz, J_2= 7.72 Hz, 1H), 3.43 (m,1H), 3.54 (dd, J_1= 11.30 Hz, J_2= 7.11 Hz, 1H), 3.62 (dd, J_1= 11.15 Hz, J_2= 4.23 Hz, 1H), 5.08 (dd, J_1= 21.85 Hz, J_2= 12.28 Hz, 2H), 5.78 (s, 1H), 7.33 (m, 5H)

^{13}C-NMR ($CDCl_3$): 24.60, 27.78, 36.47, 58.98, 61.13, 65.49, 66.56, 82.80, 128.10, 128.49, 136.45, 154.89, 172.63

Rf 0.55 (1:1; hexane-EtOAc)

MS (ESI): 379.1965 $[M+H]^+$, 401.1778 $[M+Na]^+$

(2R,4S)/ (2S,4R)-Cbz-2-Amino-4-azido-tert-butyl-5-hydroxy-2-methyl-pentanoate (+/-19b)



Yield 80 % (154 mg)

^{1}H-NMR ($CDCl_3$): 1.48 (s, 9H), 1.53 (s, 3H), 1.93 (dd, J_1= 14.31 Hz, J_2= 10.41 Hz, 1H), 2.47 (d, J= 15.04 Hz, 1H), 3.38 (ddd, J_1= 12.69 Hz, J_2= 7.39 Hz, J_3= 3.39 Hz,1H), 3.54 (m, 1H), 3.67 (d, J= 11.69 Hz, 1H), 5.05 (s, 2H), 6.16 (s, 1H), 7.33 (m, 5H)

^{13}C-NMR ($CDCl_3$): 24.46, 27.61, 36.66, 58.30, 60.92, 65.73, 66.41, 82.89, 127.96, 128.10, 136.45, 154.41, 172.79

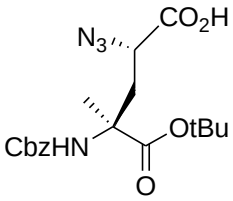
Rf 0.66 (1:1; hexane-EtOAc)

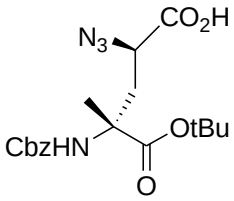
MS (ESI): 379.1970 $[M+H]^+$, 401.1786 $[M+Na]^+$

General procedure for the synthesis of *Cbz-4-Amino-2-azido-4-(tert-Butyloxycarbonyl)-pentanoic acid (+/-20a/b)*

A solution of the azidoalcohol **19** (1 eq.) in acetone (5 ml/mmol) was treated with 5% $NaHCO_3$ (2.5 ml/mmol) and KBr (0.1 eq.). At 0°C TEMPO (1.1 eq.) was added to this mixture followed by NaOCl (2.5 ml/mmol). The mixture was stirred at 0 °C. After 1 h the reaction was quenched by addition of 5% HCl and the mixture was extracted with EtOAc (3x). The organic layer was dried and then the solvent was removed to yield the desired acid.

(*2S,4S*)/ (*2R,4R*)-*Cbz-4-Amino-2-azido-4-(tert-Butyloxycarbonyl)-pentanoic acid* (*+/-20a*)





Yield 95 % (135 mg)

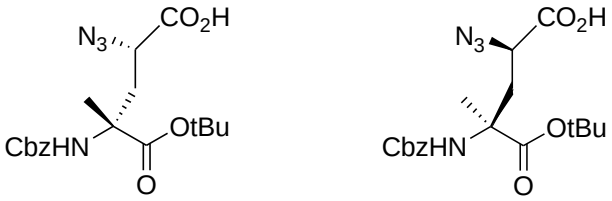
^{1}H-NMR (DMSO-d_6): 1.32 (s, 9H), 1.35 (s, 3H), 1.85 (dd, J_1= 14.71 Hz, J_2= 9.47 Hz, 1H), 2.44 (dd, J_1= 14.64 Hz, J_2= 2.96 Hz, 1H), 3.91 (dd, J_1= 9.40 Hz, J_2= 2.96 Hz, 1H), 5.00 (d, J= 1.75 Hz, 2H), 7.31 (m, 5H), 7.69 (s, 1H)

^{13}C-NMR (DMSO-d_6): 22.67, 27.31, 36.85, 57.76, 58.11, 65.14, 80.36, 127.73, 127.78, 128.29, 154.83, 171.75, 172.06

Rf 0.34 (9:1; $CHCl_3$-MeOH)

MS (ESI): 393.1765 $[M+H]^+$, 415.1581 $[M+Na]^+$

(2S,4R)/ (2R,4S)-Cbz-4-Amino-2-azido-4-(tert-Butyloxycarbonyl)-pentanoic acid (+/-20b)



Yield 91 % (146 mg)

^{1}H-NMR (DMSO-d_6): 1.32 (s, 3H), 1.33 (s, 9H), 2.18 (dd, J_1= 14.84 Hz, J_2= 9.34 Hz, 1H), 2.28 (dd, J_1= 14.95 Hz, J_2= 2.95 Hz, 1H), 3.90 (dd, J_1= 8.93 Hz, J_2= 2.89 Hz, 1H), 5.00 (d, J= 3.49 Hz, 2H), 7.31 (m, 5H), 7.54 (s, 1H)

^{13}C-NMR (DMSO-d_6): 22.89, 27.41, 36.36, 57.67, 57.94, 65.23, 80.70, 127.79, 127.86, 128.36, 154.89, 171.93, 172.26

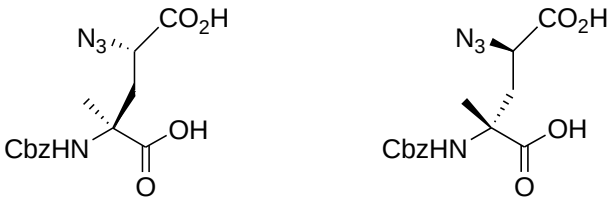
Rf 0.37 (9:1; $CHCl_3$-MeOH)

MS (ESI): 393.1762 $[M+H]^+$, 415.1577 $[M+Na]^+$

General procedure for the synthesis of *Cbz-2-Amino-4-azido-2-methyl-pentandioic acid* (*+/-21a/b*)

The azidoacid **20** (1 eq.) was dissolved in TFA (7.7 ml/mmol) and triethylsilane (5 eq) was added. After 5 h the TFA was removed in vacuum. The residue was dissolved in EtOAc (30 ml /mmol) and extracted with K_2CO_3-Solution (3x). The aquatic layer was acidulated and extracted with EtOAc (4x). The organic layer was dried and the solvent was removed to give the desired diacid.

(*2S,4S*)/ (*2R,4R*)-*Cbz-2-Amino-4-azido-2-methyl-pentandioic acid* (*+/-21a*)



Yield 80 % (78 mg)

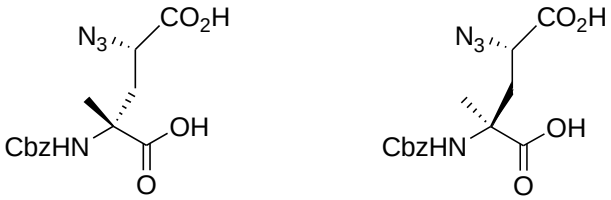
^{1}H-NMR (DMSO-d_6): 1.37 (s, 3H), 2.26 (dd, J_1= 14.91 Hz, J_2= 9.54 Hz, 1H), 2.53 (m, 1H), 3.90 (dd, J_1= 9.20 Hz, J_2= 2.62 Hz, 1H), 5.00 (s, 2H), 7.31 (m, 5H), 7.65 (s, 1H)

^{13}C-NMR (DMSO-d_6): 22.89, 36.42, 57.12, 57.15, 65.19, 127.59, 127.77, 128.32, 136.96, 154.88, 171.79, 174.74

Rf 0.42 (EtOAc-nBuOH-AcOH-H_2O; 2:1:1:1)

MS (ESI): 335.0979 $[M-H]^-$

(2R,4S)/ (2S,4R)-Cbz-2-Amino-4-azido-2-methyl-pentandioic acid (+/-21b)



Yield 67 % (112 mg)

^{1}H-NMR (DMSO-d_6): 1.36 (s, 3H), 2.26 (dd, J_1= 14.91 Hz, J_2= 9.54 Hz, 1H), 2.35 (dd, J_1= 14.95 Hz, J_2= 2.82 Hz, 1H), 3.91 (dd, J_1= 9.40 Hz, J_2= 2.96 Hz, 1H), 5.00 (dd, J_1= 16.55 Hz, J_2= 12.76 Hz, 2H), 7.31 (m, 5H), 7.39 (s, 1H)

^{13}C-NMR (DMSO-d_6): 22.87, 36.00, 56.85, 57.88, 65.19, 127.56, 127.71, 128.25, 136.92, 154.72, 171.81, 174.81

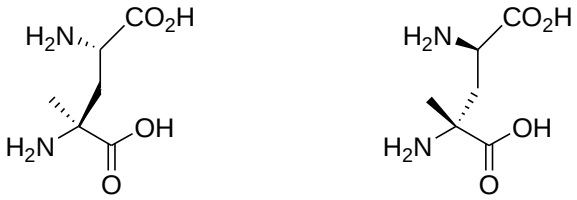
Rf 0.53 (EtOAc-nBuOH-AcOH-H_2O; 2:1:1:1)

MS (ESI): 335.0978 $[M-H]^-$

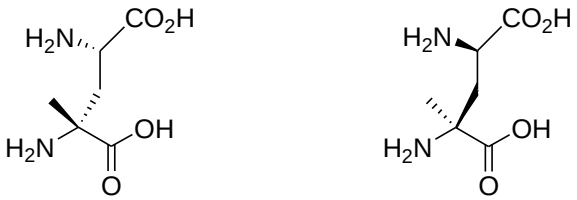
General procedure for the synthesis of *2,4-Diamino-2-methyl-pentandioic acid* (*+/-22a/b*)

The diacid **21** (1 eq.) was dissolved in THF (34 ml/mmol) and Pd/C (0,5 mass-eq) was added. The reaction mixture was stirred for 16 h under hydrogen atmosphere (1 bar). Then the reaction mixture was filtrated and the solvent was removed in vacuum. The crude product was directly derivatised for GC/MS analysis as described in chapter 3.

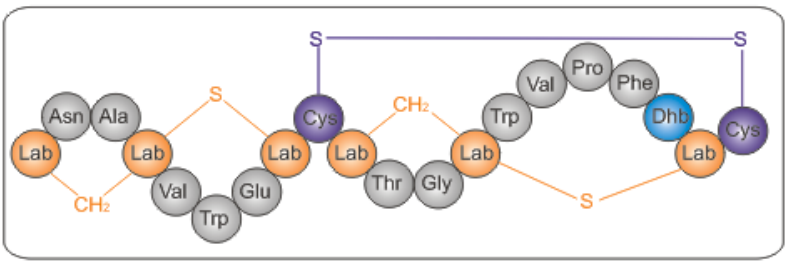
(*2S,4S*)/ (*2R,4R*)-*Cbz-2-amino-4-azido-2-methyl-pentandioic acid* (*+/-22a*)



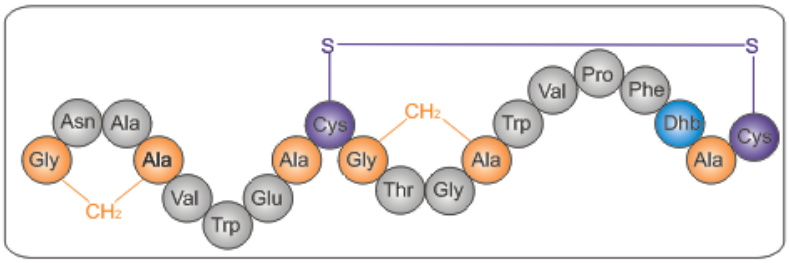
(*2R,4S*)/ (*2S, 4R*)-*Cbz-2-amino-4-azido-2-methyl-pentandioic acid (+/-22b)*



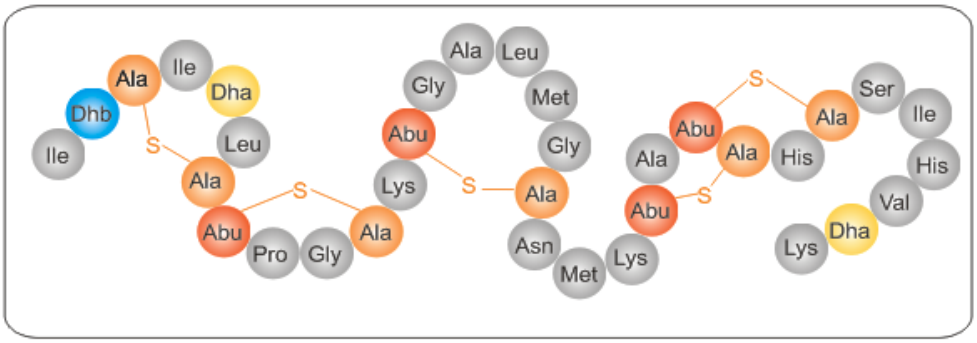
5. Structures of LabA1 and Nisin



24a



24b



25

Figure S13: Structures of LabA1 (**24a**), desulpho-LabA1 (**24b**) and Nisin (**25**).

6. References

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2 E.K. Kawulka, T. Sprules, C.M. Diper, R.M. Whittal, T.R. McKay, P. Mercier, P. Zuber, and J.C. Vederas, *Biochemistry*. 2004, **43**, 3385.