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The contribution of achiral residues in the laspartomycin family of calcium-dependent lipopeptide antibiotics

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Reagents and general methods

All reagents employed were of American Chemical Society (ACS) grade of higher and were used without further purification unless otherwise stated. Fmoc-Dap(Alloc)-OH, Fmoc-D-*allo*-Thr-OH, Fmoc-D-Pip-OH, Fmoc-Aib-OH and 2-chlorotrityl resin were obtained from Iris Biotech GmbH. D-*allo*-Thr-OH was used without protection of the side chain hydroxyl moiety. All fractions from column chromatography were monitored by thin layer chromatography (TLC) using plates with UV fluorescent indicator (normal SiO_2, Merck 60 F254). Flash chromatography was performed using Merck type 60, 230-400 mesh silica gel.

prep HPLC

Preparative HPLC runs were performed on a BESTA-Technik system with a Dr. Maisch Reprosil Gold 120 C18 column (25 × 250 mm, 10 μm) and equipped with a ECOM Flash UV detector monitoring at 214 nm. The following solvent system, at a flow rate of 12 mL/min, was used: solvent A, 0.1 % TFA in water/acetonitrile 95/5; solvent B, 0.1 % TFA in water/acetonitrile 5/95. Gradient elution was as follows: 70:30 (A/B) for 2min, 70:30 to 0:100 (A/B) over 60min, 0:100 (A/B) for 3min, then reversion back to 70:30 (A/B) over 1min, 70:30 (A/B) for 2min.

HPLC

HPLC analyses were performed on a Shimadzu Prominence-i LC-2030 system with a Dr. Maisch ReproSil Gold 120 C18 column (4.6 × 250 mm, 5 μm) at 30 °C and equipped with a UV detector monitoring 214 nm. The following solvent system, at a flow rate of 1 mL/min, was used: solvent A, 0.1 % TFA in water/acetonitrile 95/5; solvent B, 0.1 % TFA in water/acetonitrile 5/95. Gradient elution was as follows: 95:5 (A/B) for 1 min, 95:5 to 0:100 (A/B) over 25 min, 0:100 (A/B) for 2 min, then reversion back to 95:5 (A/B) over 1min, 95:5 (A/B) 1min.

HRMS

HRMS analyses were performed on a Thermo Scientific Dionex UltiMate 3000 HPLC system with a Phenomenex Kinetex C18 column (2.1 x 150 mm, 2.6 μm) at 35 °C and equipped with a diode array detector. The following solvent system, at a flow rate of 0.3 mL/min, was used: solvent A, 0.1 % formic acid in water; solvent B, 0.1% formic acid in acetonitrile. Gradient elution was as follows: 95:5 (A/B) for 1 min, 95:5 to 5:95 (A/B) over 9 min, 5:95 to 2:98 (A/B) over 1 min, 2:98 (A/B) for 1 min, then reversion back to 95:5 (A/B) over 2 min, 95:5 (A/B) for 1 min. This system was connected to a Bruker micrOTOF-Q II mass spectrometer (ESI ionization) calibrated internally with sodium formate.

Peptide abbreviations

AA	amino acid
Alloc	allyloxycarbonyl
Boc	tert-butyloxycarbonyl
^{t}Bu	tert-butyl
^{t}BuOH	*tert*-butanol
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
DiPEA	N,N-diisopropylethylamine
DMB	2,4-dimethoxybenzyl
DMF	*N,N*-dimethylformamide
Fmoc	Fluorenylmethyloxycarbonyl
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
MTBE	Methyl tert-butyl ether
TIS	triisopropylsilane
Trt	trityl

Minimum inhibitory concentration data

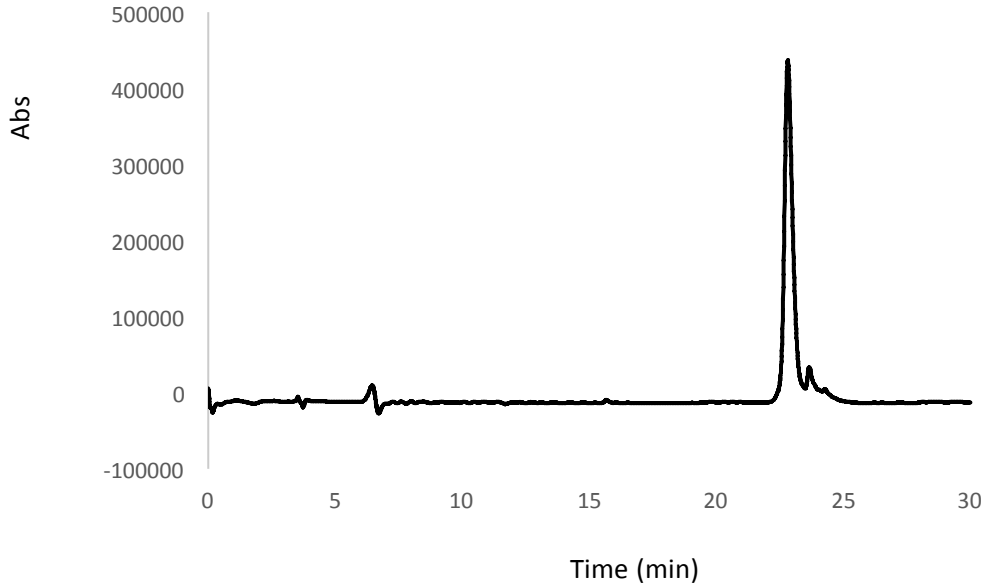
Minimum inhibitory concentrations (MICs) were determined by broth microdilution according to CLSI (Clinical & Laboratory Standards Institute) guidelines.[1] Blood agar plates (obtained from VWR) were inoculated with glycerol stocks of MRSA USA300 and *S. simulans* 22 followed by incubation for 16 hours at 37°C and 30°C respectively. Cation adjusted Mueller-Hinton broth (MHB) containing 10 mg.L^{-1} Mg^{2+} was inoculated with individual colonies of *S. aureus* and Methicillin-resistant *S. aureus* (MRSA USA300), and incubated for 16 hours at 220 RPM. The peptides were dissolved in MHB (10 mg.L^{-1} Mg^{2+}) and serially diluted on polypropylene microtiter plates with a volume of 50 µL per well. Inoculated MHB (2x105 CFU.mL^{-1}) containing 10 mg.L^{-1} Mg^{2+} and varying concentrations of Ca^{2+} (0-10 mM) was added to reach a total volume of 100 µL per well. The microtiter plates were sealed with an adhesive membrane and after 16 hours of incubation at 37°C or 30°C and 220 RPM the wells were visually inspected for bacterial growth. All reported MIC values result from two or more measurements

	MRSA USA300				***S. simulans 22***			
Compound	**0 mM**	**2.5 mM**	**5 mM**	**10 mM**	**0 mM**	**2.5 mM**	**5 mM**	**10 mM**
1(Lasp C)	≥128	8	4	2	≥128	4	4	2
2	≥128	16	16	16	≥128	32	32	16
3	≥128	64	32	32	≥128	64	64	64
4	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128
5	≥128	64	64	64	32	32	32	32
6	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128
7	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128
8	64	64	64	64	32	32	16	16
9	≥128	16	8	4	≥128	16	8	4
10	≥128	16	8	2	≥128	16	8	4
11	≥128	64	32	16	≥128	≥128	64	32
12	64	16	8	1	≥128	16	8	4

HPLC and HRMS data

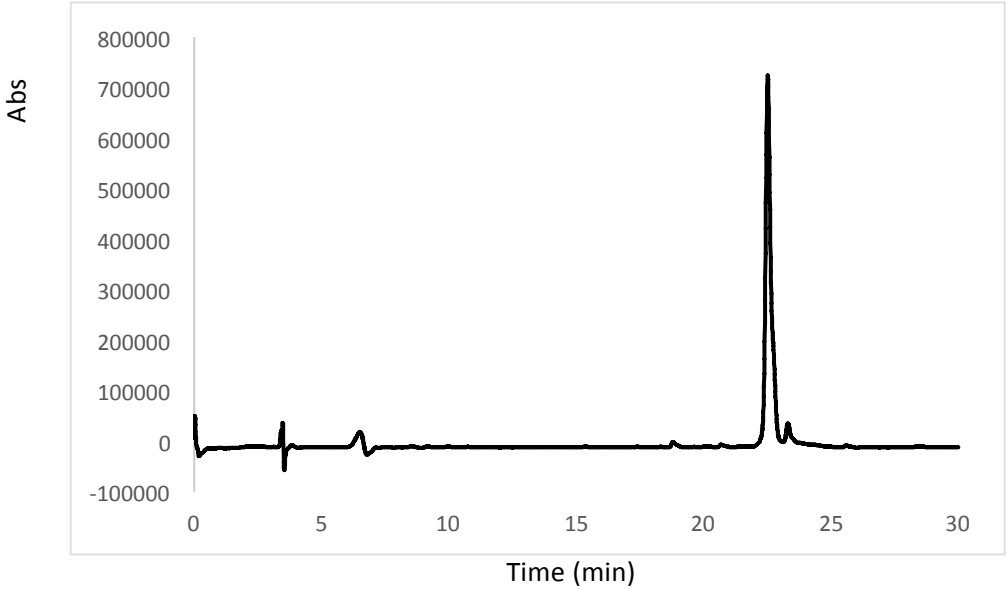
4-Aib **(2)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{59}H_{94}N_{12}O_{19}$	1274.6758	1275.6836	638.3458	1275.6865



6-Aib **(3)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{59}H_{94}N_{12}O_{19}$	1274.6758	1275.6836	638.3458	1275.6867



8-Aib **(4)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
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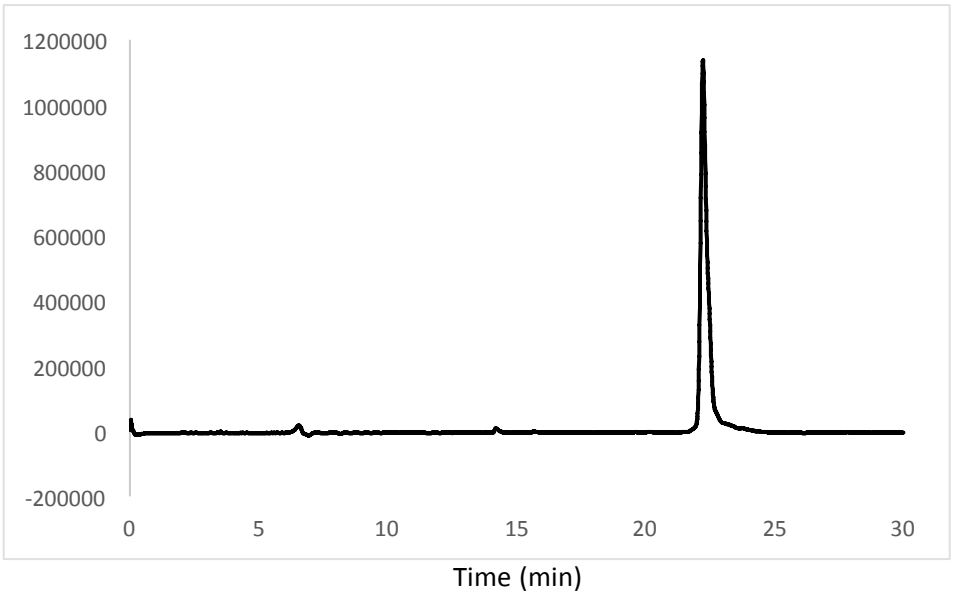
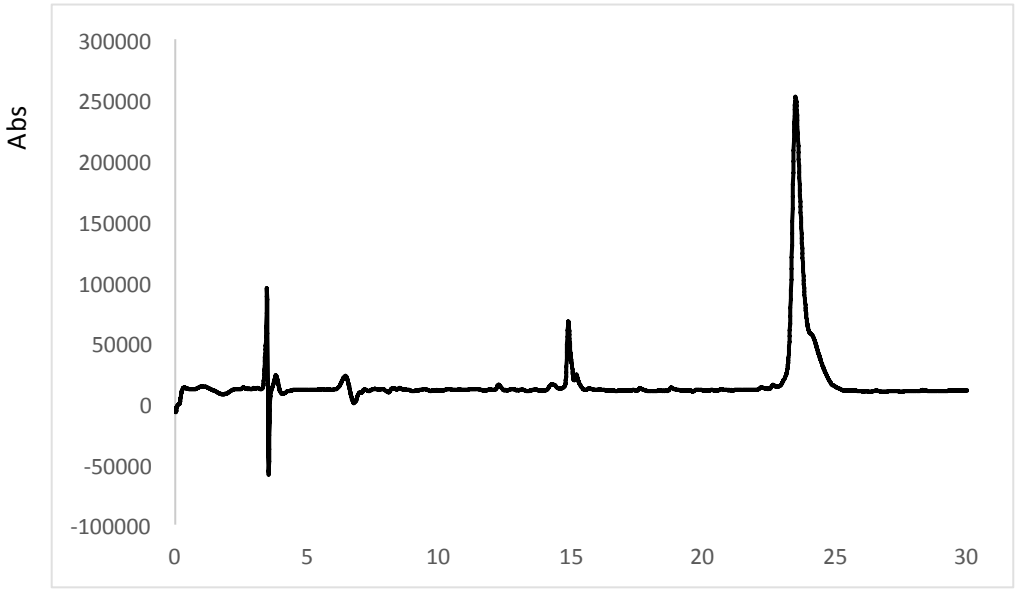


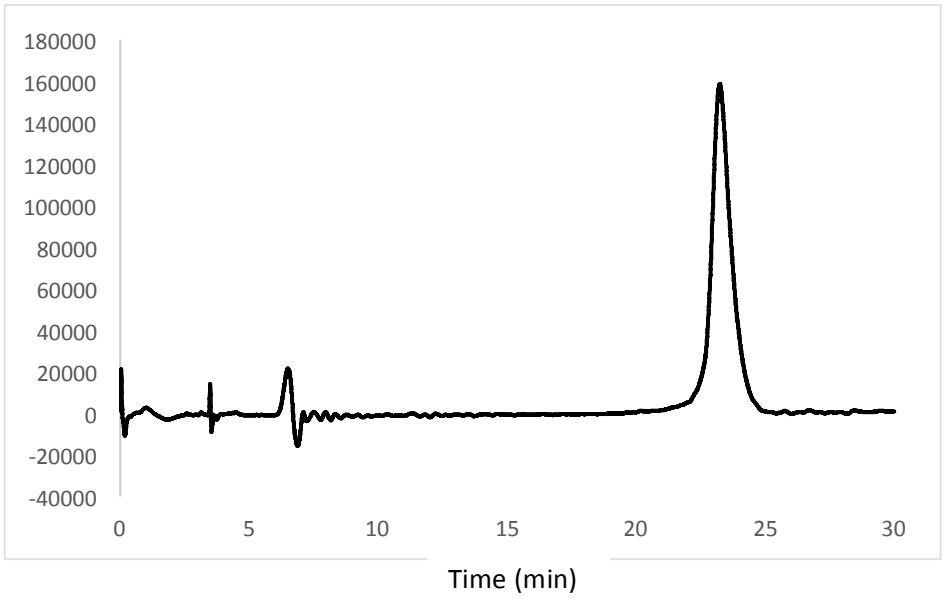
4,6-Aib **(5)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{61}H_{98}N_{12}O_{19}$	1302.7071	1303.7149	652.3614	1303.7168



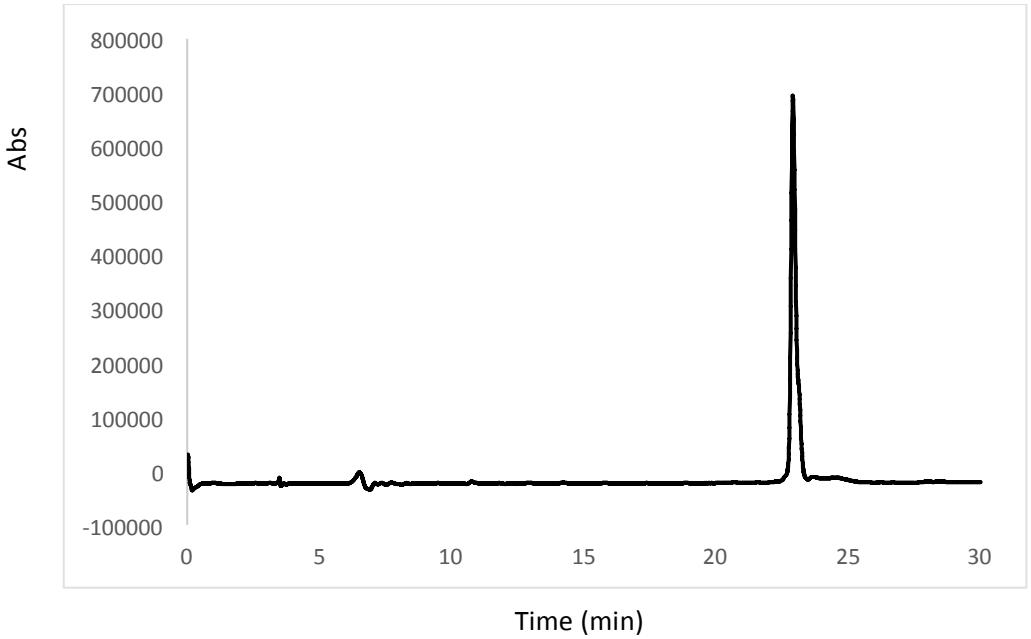
4,8-Aib **(6)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{61}H_{98}N_{12}O_{19}$	1302.7071	1303.7149	652.3614	1303.7158



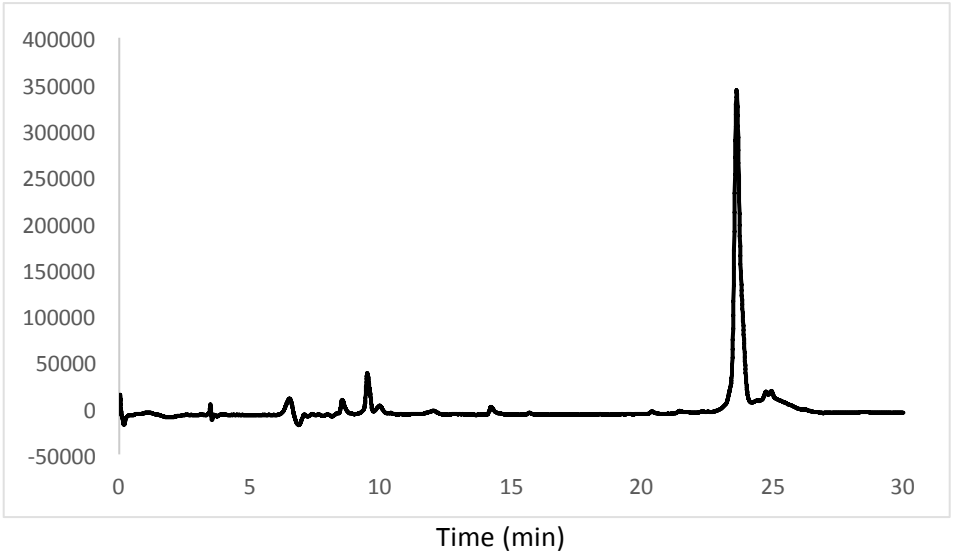
6,8-Aib **(7)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{61}H_{98}N_{12}O_{19}$	1302.7071	1303.7149	652.3614	1303.7169



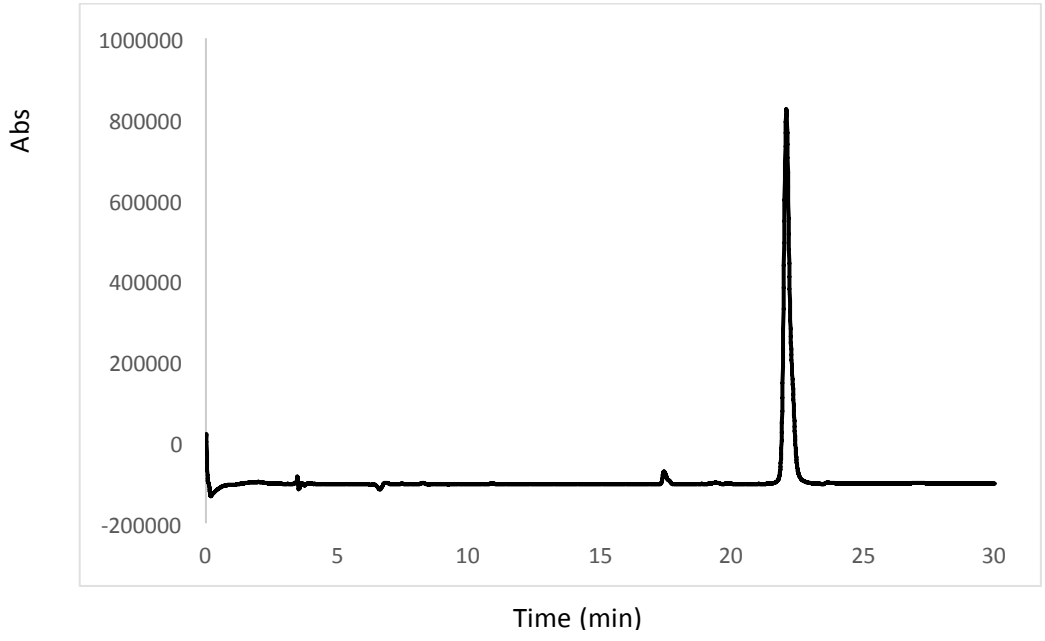
4,6,8-Aib **(8)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{63}H_{102}N_{12}O_{19}$	1330.7384	1331.7462	666.3771	1331.7493



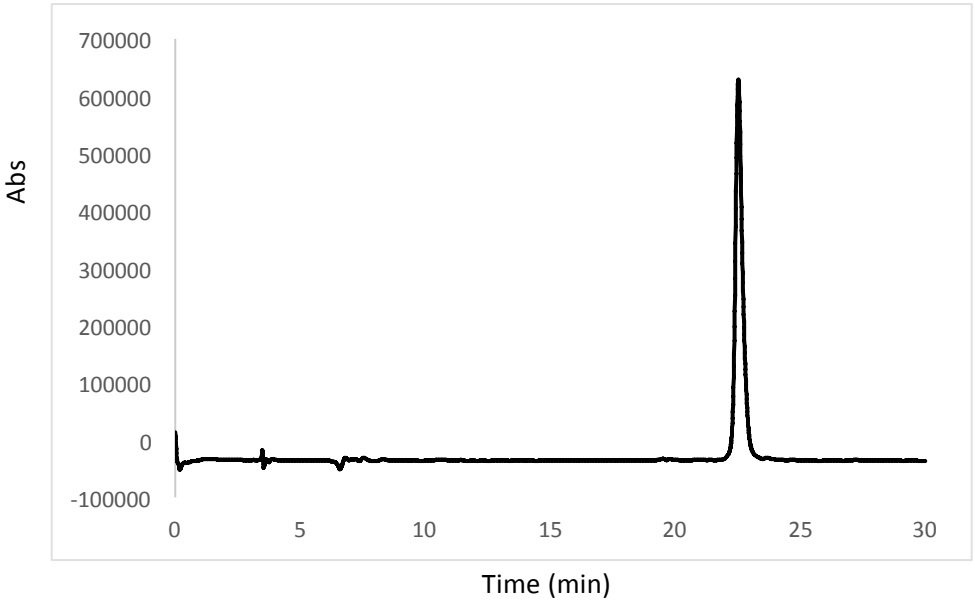
4L-Ala **(9)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{58}H_{92}N_{12}O_{19}$	1260.6602	1261.6680	631.3379	1261.6700



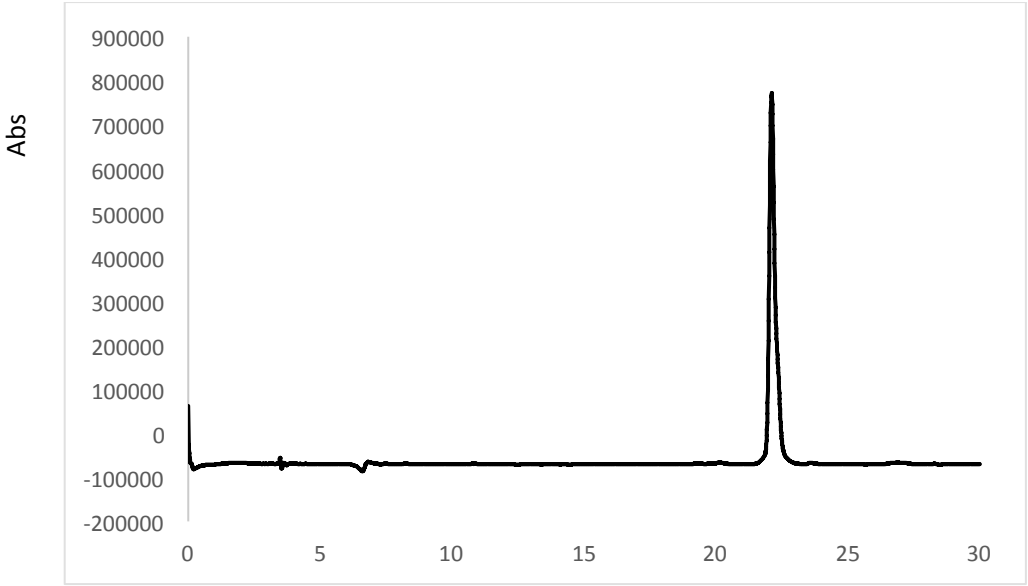
4D-Ala **(10)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{58}H_{92}N_{12}O_{19}$	1260.6602	1261.6680	631.3379	1261.6699



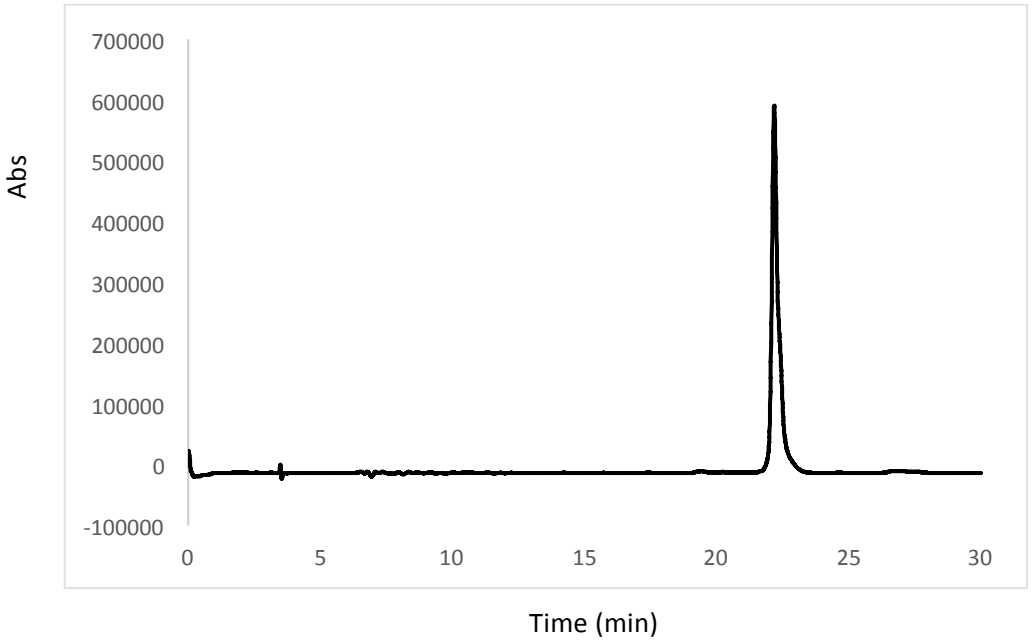
6L-Ala **(11)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{58}H_{92}N_{12}O_{19}$	1260.6602	1261.6680	631.3379	1261.6702



6D-Ala **(12)**

Chemical formula	Exact mass	Exact M+H	Exact (M+H)/2	data
$C_{58}H_{92}N_{12}O_{19}$	1260.6602	1261.6680	631.3379	1261.6720



References

1 Clinical Laboratory Standard Institute, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically ; Approved Standard — Ninth Edition Performance Standards for Antimicrobial Susceptibility Testing ; Twenty-Second Informational Supplement*, 2012, vol. 32.