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Supplementary Information

Lipopeptidomimetics Derived from Teixobactin have Potent Antimicrobial Activity against *Staphylococcus aureus*

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Materials and Methods

General Experimental

All solvents and reagents used in the experiments were purchased from Novabiochem or Sigma Aldrich and were used without purification. All amino acids used in peptides synthesis were either L- or D-configuration (enantiopure) and were Fmoc protected. All amino acids, except Glycine had their side residues protected. DMF was dried over 4 Å molecular sieves and stored under nitrogen.

Solid phase peptide synthesis was performed manually using a polypropylene syringe tube fitted with a polypropylene frit. Solvents were evaporated under reduced pressure on Büchi vacuum rotary evaporators. Peptides were freeze-dried on a Labconco FreeZone2.5 lyophiliser.

Analytical RP-HPLC analyses were preformed on a Gemini^{TM} 5u C18 110Å column (Phenomenex® Inc., Torrance, California, 150 mm × 4.6 mm, 5 µm) with a flow rate of 0.5 mL/min using a linear gradient of solvent A [water (0.1% TFA)] and solvent B [acetonitrile (0.1% TFA)]. Retention times (t_R) from analytical RP-HPLC are reported in minutes. Peptides were purified with a Gemini^{TM} 5u C18 110Å column (Phenomenex® Inc., Torrance, California, 250 mm × 21 mm, 5 µm, C18) using a specified linear gradient of solvent A [water (0.1% TFA)] and solvent B [acetonitrile (0.1% TFA)], with a flow rate of 10.6 mL/min. UV detection wavelengths in analytical HPLC were 214 nm and 260 nm. UV detection wavelength in semi-preparative HPLC was 214 nm.

LCMS analysis was performed on a Waters Xevo Q ToF mass spectrometer coupled to a Water Acquity LC system with Waters Acquity UPLC BEH C18 column (2.1 x 50 mm). Solvent system was 0.1% formic acid in MeCN and 0.1% formic acid in H_2O (deionised). Gradient was 5-100% of 0.1% formic acid in MeCN at 0.6 mL/min. Mass accuracy was accomplished by using a reference lock mass scan once per second. ES cone voltage was 30V. Collision energy was 4 eV. MS acquisition rate was 10 spectra per seconds with m/z range 50-2000 Da.

General Peptide Synthesis Experimental Procedures

Loading of resin

2-Chlorotrityl chloride resin (1.51 mmol/g, 1.32 g, 2 mmol, 1 eq) was added to an oven dried glass filtration vessel. Anhydrous DCM (10 mL) was added, and the resin allowed to swell for 20 minutes. Fmoc-Ala-OH (1.87 g, 6 mmol, 3 eq) was then added, forming a suspension. DIEA (2.09 mL, 12 mmol, 6 eq) was then also added and the vessel flushed with nitrogen. The reaction was shaken overnight at room temperature. Following this, the vessel was drained and the resin washed with DMF (3 x 10 mL), MeOH (3 x 10 mL) and DCM (3 x 10 mL). An Fmoc-loading test was carried out by measuring UV absorption of the Fmoc cleavage product at 300 nm, and found to be 0.75 mmol/g (50% loading, 1 mmol).

Fmoc deprotection

To the loaded resin, 20% piperidine/DMF (10 mL) was added. The vessel was sealed and shaken at room temperature for 20 minutes, after which the solution was drained. The resin was then washed as previously with DMF (3 x 10 mL), MeOH (3 x 10 mL) and DCM (3 x 10 mL). The reaction was monitored by Kaiser Test and repeated if a negative test was observed.

Fmoc-amino acid coupling

The loaded resin was swollen with DMF (10 mL) for 15 minutes before being drained. In a vial, a solution of Fmoc-protected amino acid (3 mmol, 3 eq), HCTU (1.241 g, 3 mmol, 3 eq) and DIEA (1.05 mL, 6 mmol, 6 eq) in DMF (8 mL) was prepared and added to the resin, and shaken at room temperature for one hour, after which the solution was drained. The resin was then washed with DMF (3 x 10 mL), MeOH (3 x 10 mL) and DCM (3 x 10 mL). Reaction was monitored by Kaiser Test and repeated if a positive test was observed.

Acetyl capping

To the loaded resin, DMF (8 mL) was added, followed by acetic anhydride (113 µL, 1.2 mmol, 1.2 eq). The vessel was shaken at room temperature for 30 minutes, before being drained and washed with DMF (3 x 8 mL), MeOH (3 x 8 mL) and DCM (3 x 8 mL).

Esterification

The peptide-bound resin was swollen in DMF (10 mL) for 15 minutes. In a vial, a solution of Fmoc-Ile-OH (1.41 g, 4 mmol, 4 eq) and DMAP (49 mg, 0.4 mmol, 0.4 eq) were dissolved in DMF (8 mL). This solution was added to the resin. DIC (625 µL, 4 mmol, 4 eq) was then added, and the reaction shaken at room temperature for 2 hours. Reaction was monitored by cleaving a small portion of resin with 1% TFA / DCM, and analysed with LCMS.

Splitting of material for analogue synthesis

After deprotection of the Fmoc-Ile residue with piperidine, the resin was split into 0.01 mmol portions for the synthesis of analogues with the various enduracididine substitutions. Each of these portions were handled as a slurry of DCM/DMF and transferred to a plastic SPF tube (5 mL) plugged with a frit. The appropriate Fmoc amino acid was then coupled as described above.

Acid Cleavage

After the completion of all Fmoc-amino acid couplings and the final Fmoc-deprotection, a solution of 1% TFA/DCM (8 mL) was added to the resin. This reaction was shaken at room temperature for 20 mins, before the acidic peptide solution was filtered into a Falcon tube (30 mL). The resin was washed with further additions of DCM (3 x 8 mL), and evaporated under reduced pressure. The residual solution was diluted with H_2O/MeCN (5 mL) and lyophilised to afford the TFA salt of the crude product as an off-white solid.

Macrocyclisation

The crude linear branched peptide Ac-C(Trt)t(IK(Boc)-NH_2)I-OH TFA salt (70 mg, 0.076 mmol, 1 eq) was dissolved in DMF (10 mL, 7.6 mM) and DIEA (80 μL, 0.456 mmol, 6 eq) was added. Separately, in a round bottomed flask (RBF), HATU (58 mg, 0.152 mmol, 2 eq) was dissolved in DMF (20 mL, 7.5 mM) and stirred and room temperature. The peptide/base solution was added dropwise to the HATU solution using a syringe pump over 1 hour. After addition, the reaction was stirred for a further 30 minutes, before evaporating under reduced pressure. The concentrated crude solution was dissolved in MeCN/ H_2O (2 mL), and purified by semi-preparative RP-HPLC (5-100 % solvent B over a 30 minute gradient), to give the purified cyclic peptide as the TFA salt (9.3 mg, 0.010 mmol, 25% yield).

Removal of cysteine trityl protection

A freshly prepared solution of TFA/EDT/TES/DCM (90: 2.5: 2.5: 5) was added to the cyclic peptide and the solution stirred at room temperature for 30 minutes. After this time, the solution was concentrated with a stream of nitrogen and the peptide was then precipitated with ice cold diethyl ether (10 mL).

Prenylation of cysteine thiol

A solution of $Zn(OAc)_2.2H_2O$ (9 mg, 0.04 mmol, 4 eq) in DMF (1 mL) was prepared and added to the free-thiol containing cyclic peptide (1 eq). TFA (5 μL) was then added, and effervescence observed. Finally, geranyl bromide (8 uL, 0.04 mmol, 4 eq) was added and the reaction shaken at room temperature for 30 minutes. After this time, the reaction was concentrated *in vacuo*, diluted with water and lyophilised to dryness. The prenylated cyclic peptide was purified by semi-preparative RP-HPLC (5-100 % solvent B over a 30 minute gradient), to give the final product as the TFA salt.

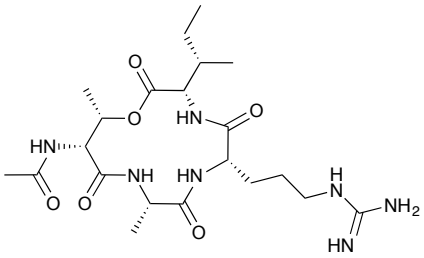
Conversion to HCl salt

The prenylated cyclic peptide was dissolved in aqueous HCl (5mM) and lyophilised to dryness. This was repeated a further two times. The product was analysed by ^{19}F NMR to determine the conversion of TFA to HCl.

Determination of peptide content

Due to the lack of chromophore present in the peptide analogues, the peptide content was determined by ^{1}H NMR. A known volume of peptide in D_2O (or D_2O/MeCN depending on solubility) was added to a known quantity of *para*-nitrophenol in D_2O. The peptide content was calculated by comparing relative integrations of peaks in the 1H NMR spectrum from both compounds respectively.

Figure S1: Structures of cyclic tetradepsipeptides **15** – **17**



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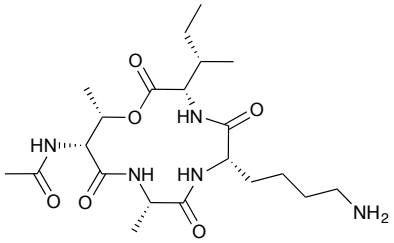

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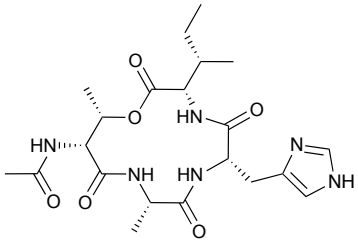

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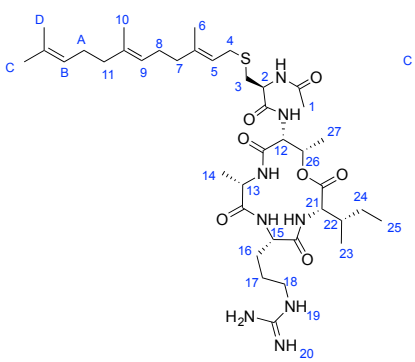
Table S1: Yields of purified Teixobactin analogues

Analogue	Yield (mg)	Yield (%)	Purity (%)	Calc mass	Obs mass
Arg_{10}-farnesylbactin (**1**)	4.3	5	95%	791.4853	791.4873
Lys_{10}-farnesylbactin (**2**)	4.0	5	>99%	763.4792	763.4813
Orn_{10}-farnesylbactin (**3**)	3.0	7	>99%	749.4635	749.4639
Cit_{10}-farnesylbactin (**4**)	4.5	5	98%	792.4694	792.4731
His_{10}-farnesylbactin (**5**)	1.9	4	>99%	772.4431	772.4462
Ala_{10}-farnesylbactin (**6**)	2.6	7	>99%	706.4213	706.4241
Glu_{10}-farnesylbactin (**7**)	3.3	9	>99%	764.4268	764.4266
Arg_{10}-geranylbactin (**8**)	5.2	12	>99%	723.4227	723.4250
Lys_{10}-geranylbactin (**9**)	3.0	7	>99%	695.4166	695.4200
Orn_{10}-geranylbactin (**10**)	4.7	12	>99%	681.4009	681.4003
Cit_{10}-geranylbactin (**11**)	2.8	7	>99%	724.4068	724.4099
His_{10}-geranylbactin (**12**)	1.3	3	>99%	704.3805	704.3825
Ala_{10}-geranylbactin (**13**)	2.4	8	>99%	638.3608	638.3587
Glu_{10}-geranybactin (**14**)	3.0	9	86%	696.3642	696.3663
Cyclic Ac-tARI (**15**)	3.5	14	>99%	484.2884	484.2900
Cyclic Ac-tAKI (**16**)	0.5	2	>99%	456.2822	456.2834
Cyclic Ac-tAHI (**17**)	0.6	2	>99%	465.2462	465.2462

Numbered Compound Structures and NMR Data Tables

Arg_{10}-farnesylbactin (1)



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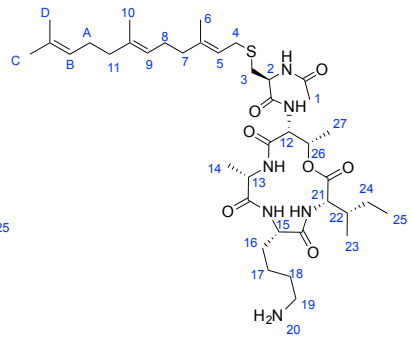

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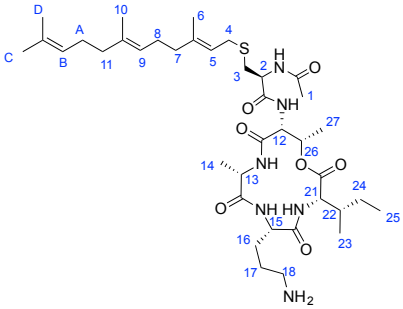

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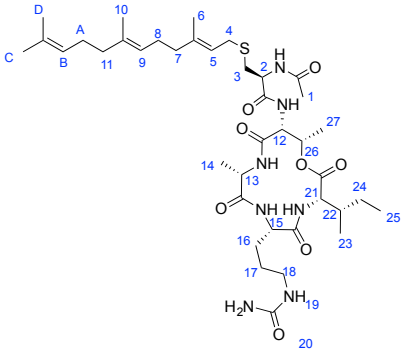

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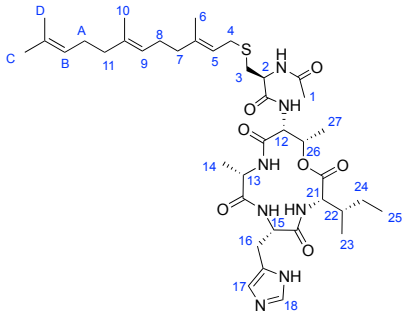

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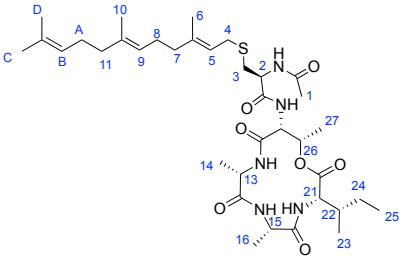

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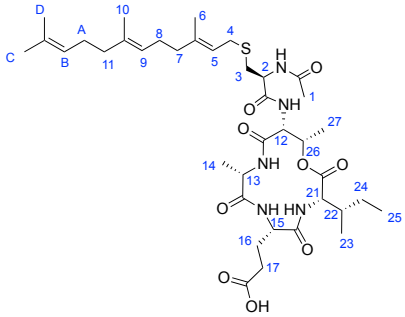

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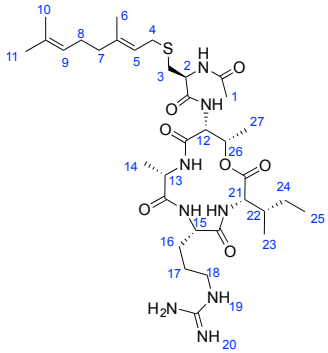

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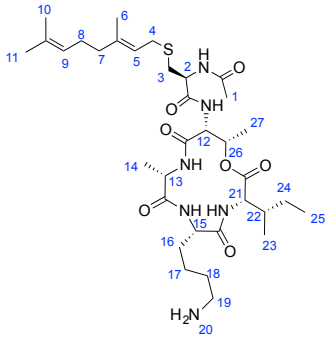
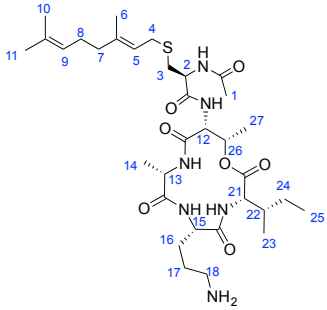


Orn$_{10}$-geranylbactin (10)



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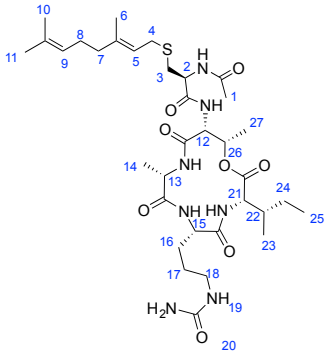

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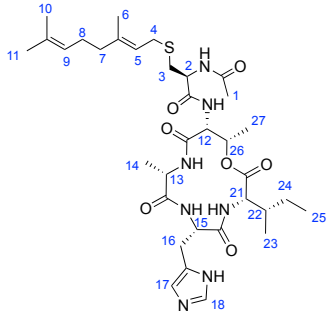

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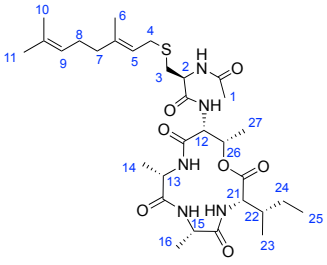
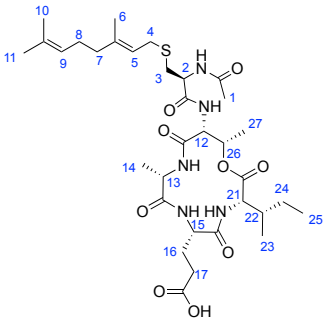


Glu$_{10}$-geranybactin (14)



Compound	1	2	3	4	5	6	7	8	9	10
Arg_{10}-farnesylbactin (**1**)	2.13 (3H s)	4.65 (1H t, *J*=7.5)	3.35-2.98 (1H m) & 2.98-2.90 (1H m)	3.50-3.42 (1H m) and 3.38-3.32 (1H m)	5.29 (1H t, *J*=7.5)	1.75 (3H s)	2.15-2.10 (2H m)	2.02-1.97 (2H m)	5.16-5.09 (1H m)[3]	1.69 (3H s)
Lys_{10}-farnesylbactin (**2**)	2.06 (3H s)	4.76-4.75 (1H m)[7]	2.90-2.80 (2H m)	3.33 (2H qd, *J*=13, 7.5)	5.24 (1H t, *J*=8)	1.70 (3H s)	2.13-2.05 (2H m)	2.08-2.02 (2H m)	5.14-5.07 (1H m) [3]	1.67 (3H s)
Orn_{10}-farnesylbactin (**3**)	not assigned	4.52-4.45 (1H m)[6]	3.04-2.89 (2H m)	3.49-3.39 (1H m) & 3.37-3.30 (1H m)	5.32-5.25 (1H, m)	1.75 (3H s)	2.12-2.04 (2H m)	2.01-1.95 (2H m)	5.16-5.09 (1H m)[3]	1.68 (3H s)
Cit_{10}-farnesylbactin (**4**)	1.98 (3H s)	4.46 (1H t, *J*=7.5)	2.83 (2H d, *J*=7.5)	3.26 (2H d, *J*=8)	5.21 (1H t, *J*=8)	1.66 (3H s)	2.10-2.04 (2H m)	2.05-2.00 (2H m)	5.12-5.05 (1H m) [3]	1.64 (3H s)
His_{10}-farnesylbactin (**5**)	\	\	\	\	\	\	\	\	\	\
Ala_{10}-farnesylbactin (**6**)	not assigned	4.35 (1H t, *J*=8)	3.66-3.50 (1H br m) & 2.85-2.74 (1H m)	3.26-3.15 (1H m) & 3.12-3.03 (1H m)	5.26-5.13 (1H br m)	1.61 3H s)	2.08-2.01 (2H m)	2.00-1.94 (2H m)	5.23-5.14 (1H m) [3]	1.53 (3H s)
Glu_{10}-farnesylbactin (**7**)	not assigned	4.58 (1H t, *J*=7.5)	2.98 (2H br d, *J*=7.5)	3.45-3.36 (2H m)	5.36 (1H t, *J*=7.5)	1.80 (3H s)	2.22-2.15 (2H m)	2.15-2.05 (2H m)	5.27-5.19 (1H m) [3]	1.79 (3H s)
Arg_{10}-geranylbactin (**8**)	2.14 (3H s)	4.61 (1H t, *J*=8)	3.04-2.95 (2H m)	3.40 (2H qd, J=8, 5)	5.34 (1H t, *J*=8)	1.78 (3H, s)	2.24-2.19 (2H m)	2.19-2.15 (2H m)	5.21 (1H t, *J*=7)	1.77 (3H s)
Lys_{10}-geranylbactin (**9**)	2.09 (3H, s)	4.58 (1H t, *J*=7.5)	3.01 (2H t, *J*=6.5)	3.36-3.31 (2H m)	5.29 (1H t, *J*=8.5)	1.71 (3H s)	2.18-2.14 (2H m)	2.14-2.09 (2H m)	5.16 (1H t, *J*=6.5)	1.69 (3H s)
Orn_{10}-geranylbactin (**10**)	2.13 (3H s)	4.61 (1H t, *J*=7.5)	3.03-2.93 (2H m)	3.45-3.36 (2H m)	5.38-5.32 (1H, m)	1.78 (3H s)	2.23-2.19 (2H m)	2.19-2.15 (2H m)	5.21 (1H t, *J*=6.5)	1.77 (3H s)
Cit_{10}-geranylbactin (**11**)	not assigned	4.74-6.64 (1H)[2]	3.14-3.07 (2H m)	3.56-3.47 (2H m)	5.46 (1H t, *J*=8)	1.90 (3H s)	2.35-2.30 (2H m)	2.30-2.26 (2H m)	5.32 (1H t, *J*=6)	1.89 (3H s)
His_{10}-geranylbactin (**12**)	\	\	\	\	\	\	\	\	\	\
Ala_{10}-geranylbactin (**13**)	not assigned	4.43 (1H t, *J*=7.5)	2.83 (2H d, *J*=7)	3.26 (2H qd *J*=7.5, 2.5)	5.21 (1H t, *J*=7)	1.66 (3H s)	2.10-2.04 (2H m)	2.04-2.00 (2H m)	5.07 (1H t, *J*=5.5)	1.64 (3H s)
Glu_{10}-geranybactin (**14**)	not assigned	4.59 (1H t, *J*=8)	3.04-2.96 (2H m)	3.45-3.35 (2H m)	5.34 (1H t, *J*=7.5)	1.78 (3H s)	2.24-2.16 (2H m)	2.24-2.16 (2H m)	5.21 (1H t, *J*=6.5)	1.77 (3H s)

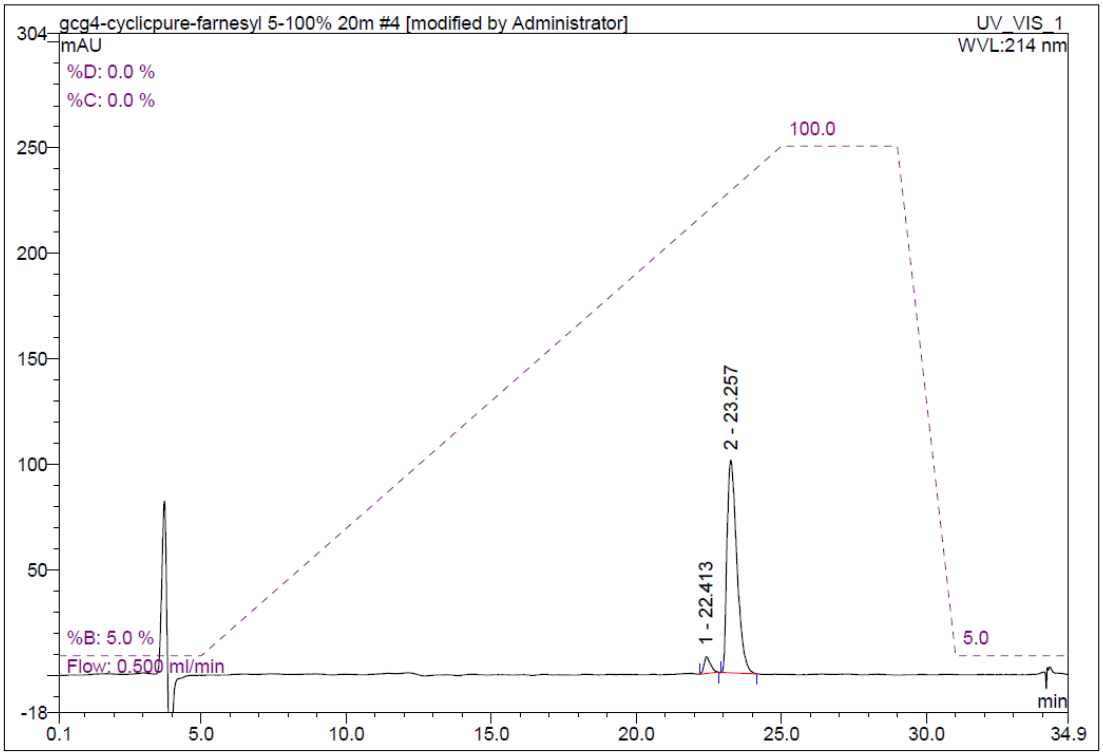
Compound	11	12	13	14	15	16	17	18	19	20
Arg_{10}-farnesylbactin (**1**)	2.12-2.05 (3H m)	not assigned	4.26-4.21 (1H m)	1.56 (3H d, *J*=7)	4.49 (1H t, J=7.5)	2.01-1.94 (1H m) and 1.76-1.66 (1H m)	3.33-3.26 (2H m)	not assigned	not assigned	not assigned
Lys_{10}-farnesylbactin (**2**)	not assigned	4.73 (1H d, *J*=6)	4.09 (1H q, *J*=7.5)	1.45 (3H d, *J*=7)	4.36 (1H dd, *J*=10, 6)[7]		2.91 (2H t, *J*=7.5)	not assigned	not assigned	\
Orn_{10}-farnesylbactin (**3**)	not assigned	4.71-4.68 (1H m)[2]	4.24 (1H q, *J*=7.5)	1.57 (3H d, *J*=7)	4.26-4.19 (1H m)	1.88-1.80 (1H m) and 1.85-1.76 (1H m)	3.07 (2H t, *J*=6.5)		\	\
Cit_{10}-farnesylbactin (**4**)	not assigned	4.72-4.63 (1H m)	4.09 (1H q, J=7)	1.41 (3H d, *J*=7.5)	4.30 (1H dd, *J*=9.5, 6.5)	1.55-1.49 (1H m) and 1.50-1.43 (1H m)	3.05 (2H t, *J*=7)	1.83-1.72 (2H m)	\	\
His_{10}-farnesylbactin (**5**)	\	\	\	\	\	\	\	\	\	\
Ala_{10}-farnesylbactin (**6**)	not assigned	4.76-4.55 (1H br m)	4.13-4.02 (1H m)[2]	1.35 (3H d, *J*=7)	4.32 (1H q, *J*=7.5)	1.35 (3H d, *J*=7)	\	\	\	\
Glu_{10}-farnesylbactin (**7**)	not assigned	4.90-4.75 (1H br m)	4.27-4.23 (1H m) [2]	1.54 (3H d, *J*=7.5)	4.49 (1H dd, *J*=9.5, 6.5)	not assigned	2.48 (2H br t, *J*=7.5)	\	\	\
Arg_{10}-geranylbactin (**8**)	1.70 (3H s)	4.87 (1H m)[2]	4.24 (1H q, J=7.5)	1.55 (3H d, *J*=7)	4.48 (1H dd, *J*=9.5, 7.5)	1.95-1.85 (1H, m) and 1.79-1.74 (1H, m)	3.34-3.25 (2H m)	not assigned	8.01 (2H br s)[1]	8.01 (2H br s)[1]
Lys_{10}-geranylbactin (**9**)	1.63 (3H s)	4.75 (1H m)[2]	4.18 (1H q, *J*=7.5)	1.49 (3H d, *J*=7.5)	4.43 (1H dd, *J*=9.5, 7)	1.95-1.89 (1H m) & 1.90-1.84 (1H m)	3.01 (2H t, *J*=7.5)	not assigned	not assigned	\
Orn_{10}-geranylbactin (**10**)	1.70 (3H s)	4.89 (1H m)[2]	4.24 (1H q, *J*=7.5)	1.55 (3H d, *J*=7.5)	4.49 (1H dd, *J*=9.5, 6)	2.04-1.96 (1H m) & 1.87-1.80 (1H m)	3.09 (2H t, *J*=7.5)	not assigned	\	\
Cit_{10}-geranylbactin (**11**)	1.89 (3H s)	not assigned	4.36 (1H q, *J*=7.5)	1.66 (3H d, *J*=7.5)	4.55 (1H dd, *J*=9.5, 6.5)	2.08-2.01 (1H m) & 1.81-1.73 (1H m)	3.31 (2H t, *J*=7)	\	\	\
His_{10}-geranylbactin (**12**)	\	\	\	\	\	\	\	\	\	\
Ala_{10}-geranylbactin (**13**)	1.57 (3H s)	5.71-4.60 (1H m)	4.16-4.09 (1H m)[5]	1.38 (3H d, *J*=7.5)	4.41-4.34 (1H m)	1.39 (3H d, *J*=7.5)	\	\	\	\
Glu_{10}-geranybactin (**14**)	1.70 (3H s)	4.75 (1H m)[2]	4.25 (1H q, *J*=7.5)	1.54 (3H d, *J*=7.5)	4.48 (1H dd, J=9.5, 6.5)	2.22-2.12 (2H m)	2.50 (2H t, *J*=7.5)	\	\	\

Compound	21	22	23	24	25	26	27	A	B	C	D
Arg_{10}-farnesylbactin (**1**)	4.28 (1H d, J=9)	1.96-1.89 (1H m)	0.99-0.92 (3H m)[4]	1.59-1.52 (1H m) and 1.28-1.22 (1H m)	0.99-0.92 (3H m)[4]	5.67-5.60 (1H m)	1.38 (3H d, J=6)	2.10-2.05 (2H m)	5.16-5.09 (1H m)[3]	1.61 (3H s)	1.61 (3H s)
Lys_{10}-farnesylbactin (**2**)	4.14 (1H d, *J*=9)	1.93-1.85 (1H m)	0.89 (3H d, *J*=7)	1.55-1.47 (1H m) and 1.25-1.15 (1H m)	0.87 (3H t, *J*=7)	5.45 (1H qd), *J*=6.5, 2	1.23 (3H d, *J*=6.5)	not assigned	5.14-5.07 (1H m)[3]	1.60 (3H s)	1.60 (3H s)
Orn_{10}-farnesylbactin (**3**)	4.27 (1H d, *J*=9)	1.82-1.74 (1H m)	1.00-0.92 (3H m) [11]	1.63-1.57 (1H m) & 1.28-1.22 (1H m)	1.00-0.92 (3H m) [11]	5.64 (1H qd, *J*=7, 2)	1.38 (3H d, *J*=6)	not assigned	5.16-5.09 (1H m)[3]	1.61 (3H s)	1.61 (3H s)
Cit_{10}-farnesylbactin (**4**)	4.15 (1H d, *J*=9)	1.83-1.74 (1H m)	0.86 (3H d, *J*=7)	1.51-1.42 (1H m) and 1.18-1.11 (1H m)	0.86 (3H t, *J*=7)	5.49 (1H qd, *J*=6.5, 2.5)	1.24 (3H d, *J*=6.5)	not assigned			
His_{10}-farnesylbactin (**5**)	\	\	\	\	\	\	\	\	\	\	\
Ala_{10}-farnesylbactin (**6**)	4.13-4.05 (1H m)[2]	1.75-1.63 (1H m)	0.83-0.77 (3H m)	1.48-1.36 (1H m) & 1.12-1.01 (1H m)	0.86-0.79 (3H m)	5.51-5.41 (1H m)	1.21 (3H d, *J*=5.5)	not assigned	not assigned	not assigned	not assigned
Glu_{10}-farnesylbactin (**7**)	4.31 (1H d, *J*=9)	1.94-1.85 (1H m)	1.04-0.97 (3H m) [4]	1.64-1.56 (1H m) & 1.33-1.23 (1H m)	1.04-0.97 (3H m) [4]	5.65 (1H qd, *J*=6.5, 2)	1.40 (3H d, *J*=6.5)	not assigned	5.27-5.19 (1H m) [3]	1.72 (3H s)	1.72 (3H s)
Arg_{10}-geranylbactin (**8**)	4.30 (1H d, J=8.5)	1.95-1.85 (1H, m)	0.98 (3H d, J=7.5)	1.61-1.52 (1H m) and 1.30-1.23 (1H m)	0.99 (3H t, *J*=6.5)	5.65 (1H qd, *J*=6.5, 2.5)	1.41 (3H d, J=6.5)	\	\	\	\
Lys_{10}-geranylbactin (**9**)	4.23 (1H d, *J*=9)	1.89-1.84 (1H m)	0.92 (3H d, *J*=7)	1.55-1.50 (1H m) & 1.24-1.17 (1H m)	0.90 (3H t, *J*=7.5)	5.60 (1H qd, *J*=6.5, 2.5)	1.35 (3H d, *J*=6.5)	\	\	\	\
Orn_{10}-geranylbactin (**10**)	4.30 (1H d, *J*=9)	1.97-1.87 (1H m)	0.99 (3H d, *J*=7)	1.62-1.52 (1H m) & 1.30-1.23 (1H m)	0.98 (3H t, *J*=7.5)	5.64 (1H qd, *J*=6.5, 2)	1.41 (3H d, *J*=6.5)	\	\	\	\
Cit_{10}-geranylbactin (**11**)	4.42 (1H d, *J*=8.5)	2.01.193 (1H m)	1.13-1.08 (3H m)	1.72-1.64 (1H m) & 1.42 -1.34 (1H m)	1.13-1.08 (3H m)	5.77 (1H qd, *J*=6.5, 2.5)	1.52 (3H d, *J*=6.5)	\	\	\	\
His_{10}-geranylbactin (**12**)	\	\	\	\	\	\	\	\	\	\	\
Ala_{10}-geranylbactin (**13**)	4.16-4.10 (1H m)[5]	1.79-1.72 (1H m)	0.90-0.82 (3H m)	1.52-1.42 (1H m) & 1.15-1.10 (1H m)	0.90-0.82 (3H m)[4]	5.49 (1H qd, *J*=6.5, 2.5)	1.25 (3H d, *J*=6.5)	\	\	\	\
Glu_{10}-geranybactin (**14**)	4.32 (1H d, *J*=8.5)	1.90-1.82 (1H m)	1.01-0.96 (3H m)[4]	1.60-1.53 (1H m)and 1.31-1.22 (1H m)	1.01-0.96 (3H m)[4]	5.66 (1H qd, *J*=6.5, 2.5)	1.41 (3H d, J=6.5)	\	\	\	\

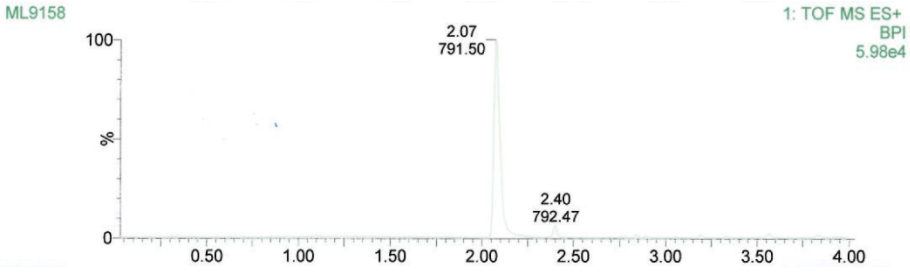
1 Due to exchange 19 and 20 appear as one peak
2 Obscured by water peak
3 Overlap 9 and B
4 Overlap between 23 and 25
5 Overlap with 21 and 13
6 Overlap 2 and 15
7 Overlap with 12 and 2

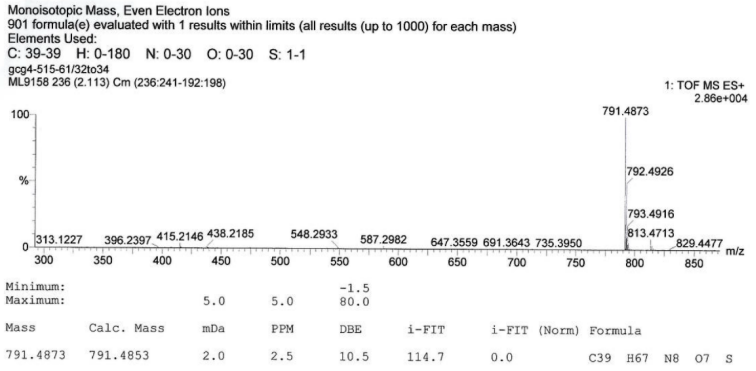
RP-HPLC, MS and NMR spectra of Teixobactin lipopeptidomimetic Analogues

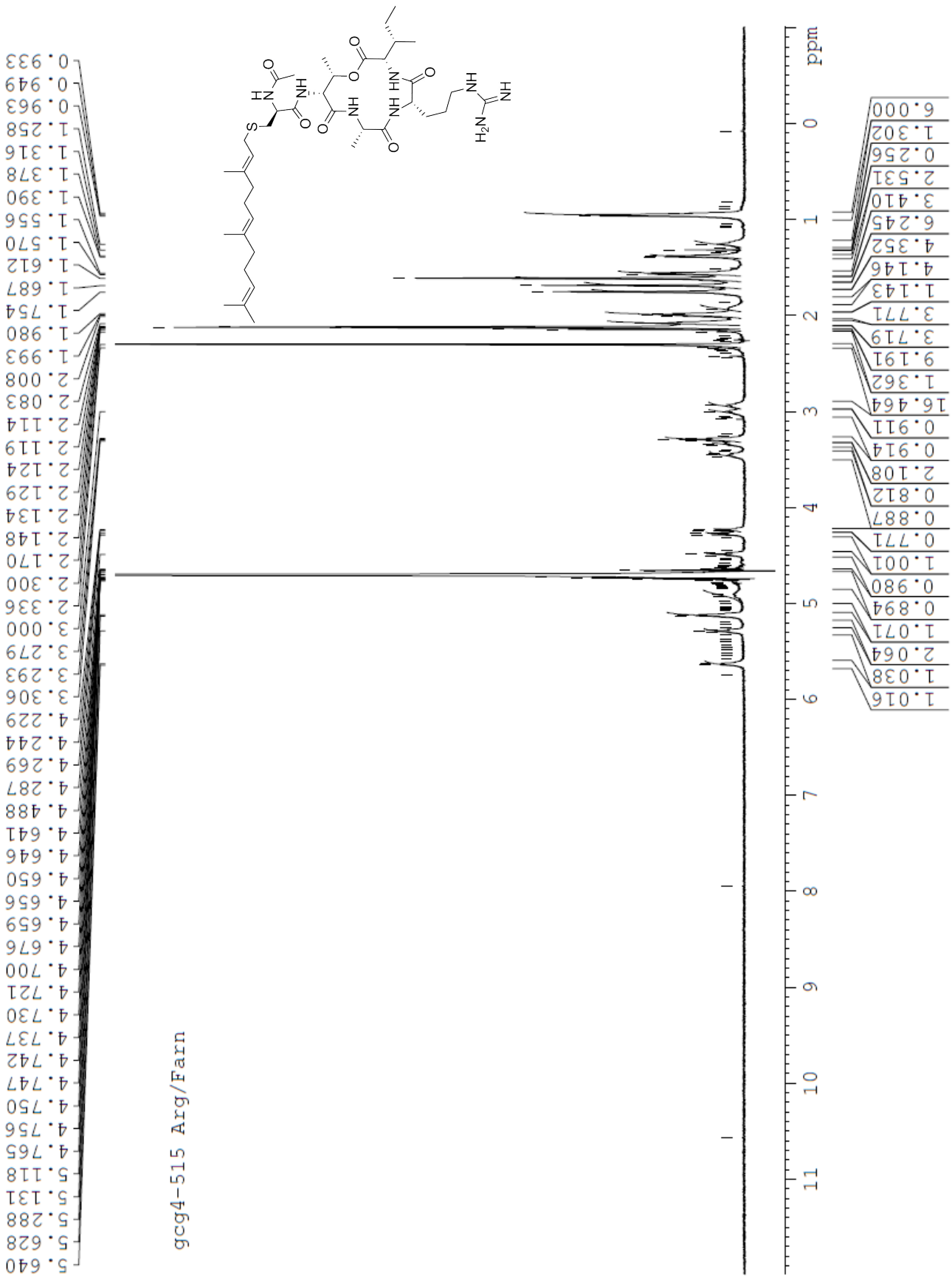
Arg_{10}-farnesylbactin (1)

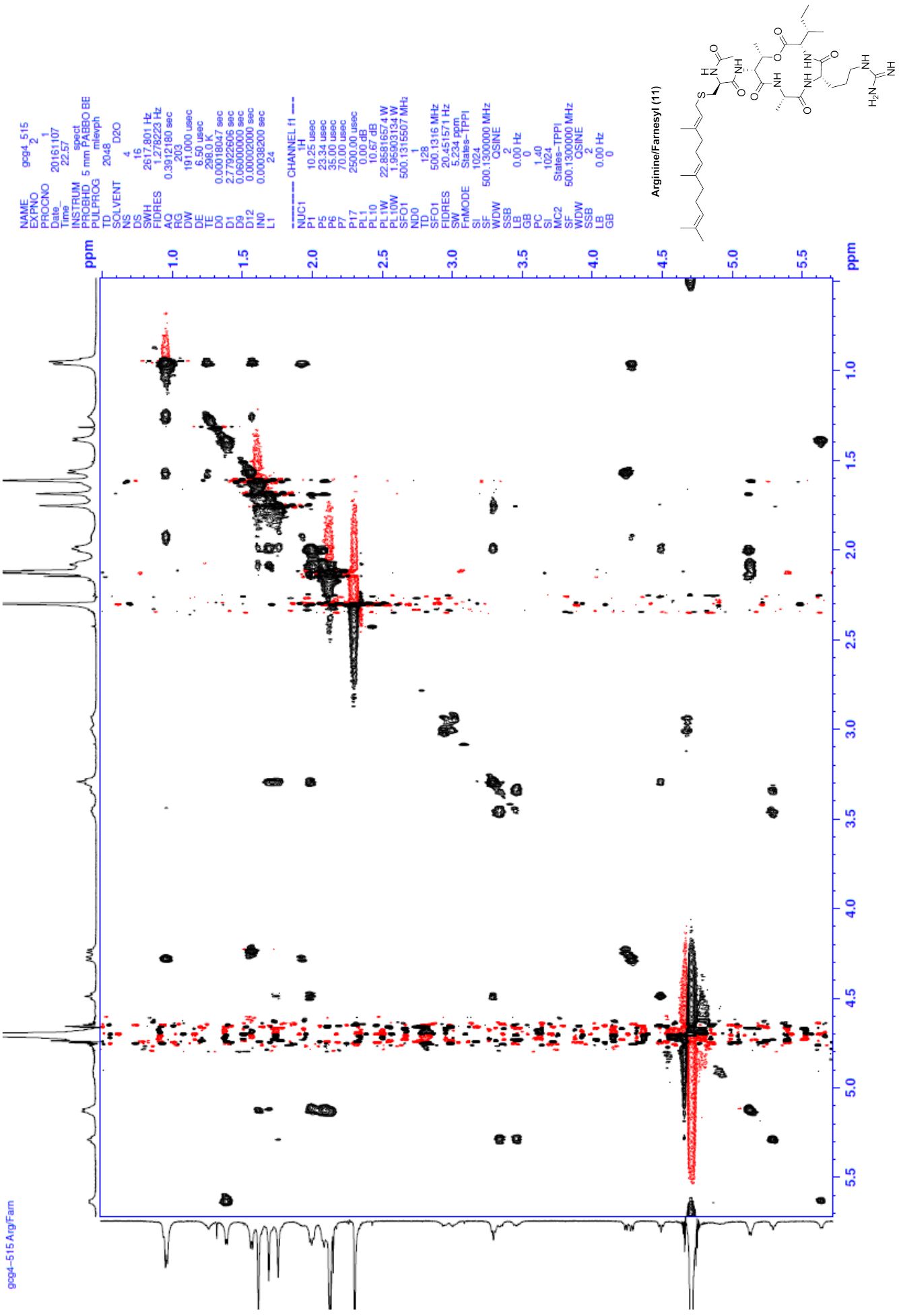


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	22.41	n.a.	7.996	2.021	4.83	n.a.	BMB
2	23.26	n.a.	100.728	39.822	95.17	n.a.	BMB
Total:			108.724	41.843	100.00	0.000	

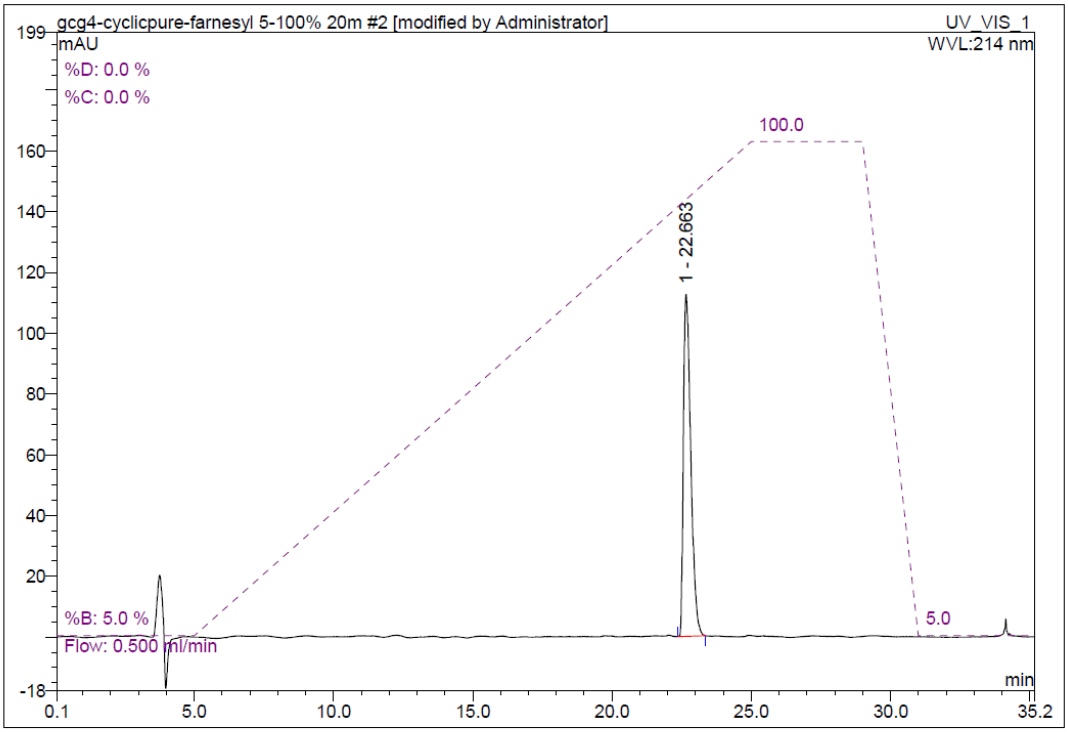




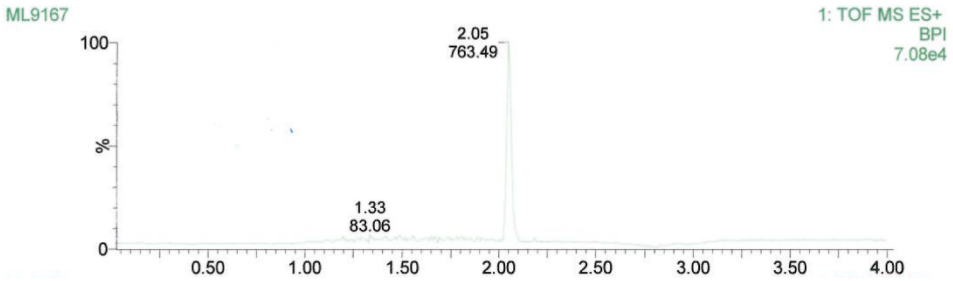

gcg4-515 Arg/Farn
ppm


Arginine/Farnesyl (11)
gog4-515 Arg/Farn
ppm

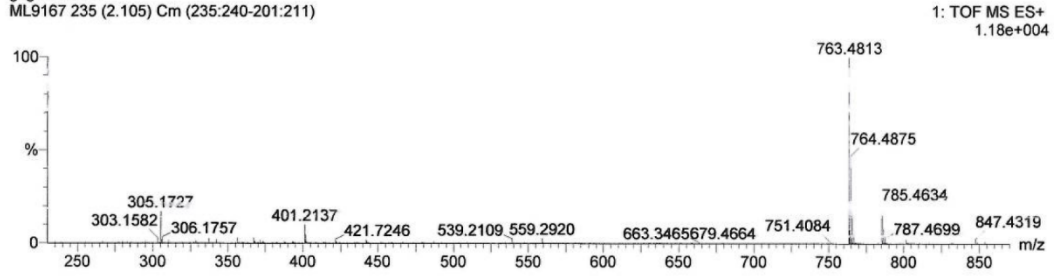
Lys$_{10}$-farnesylbactin (2)



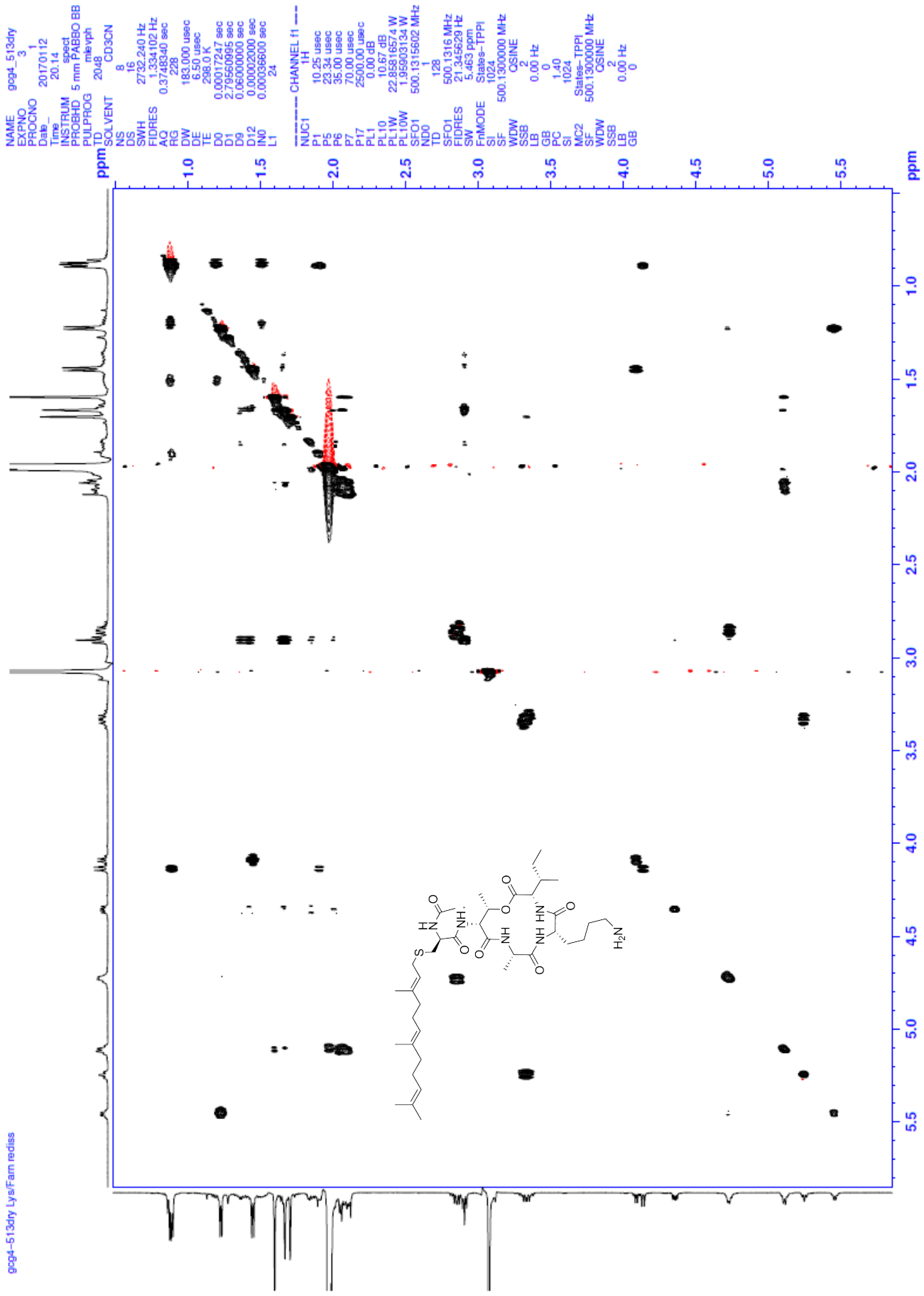
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	22.66	n.a.	112.547	34.597	100.00	n.a.	BMB
Total:			112.547	34.597	100.00	0.000	



Monoisotopic Mass, Even Electron Ions
880 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 39-39 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-513-60
ML9167 235 (2.105) Cm (235:240-201:211)



Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
763.4813	763.4792	2.1	2.8	9.5	96.0	0.0	C39 H67 N6 O7 S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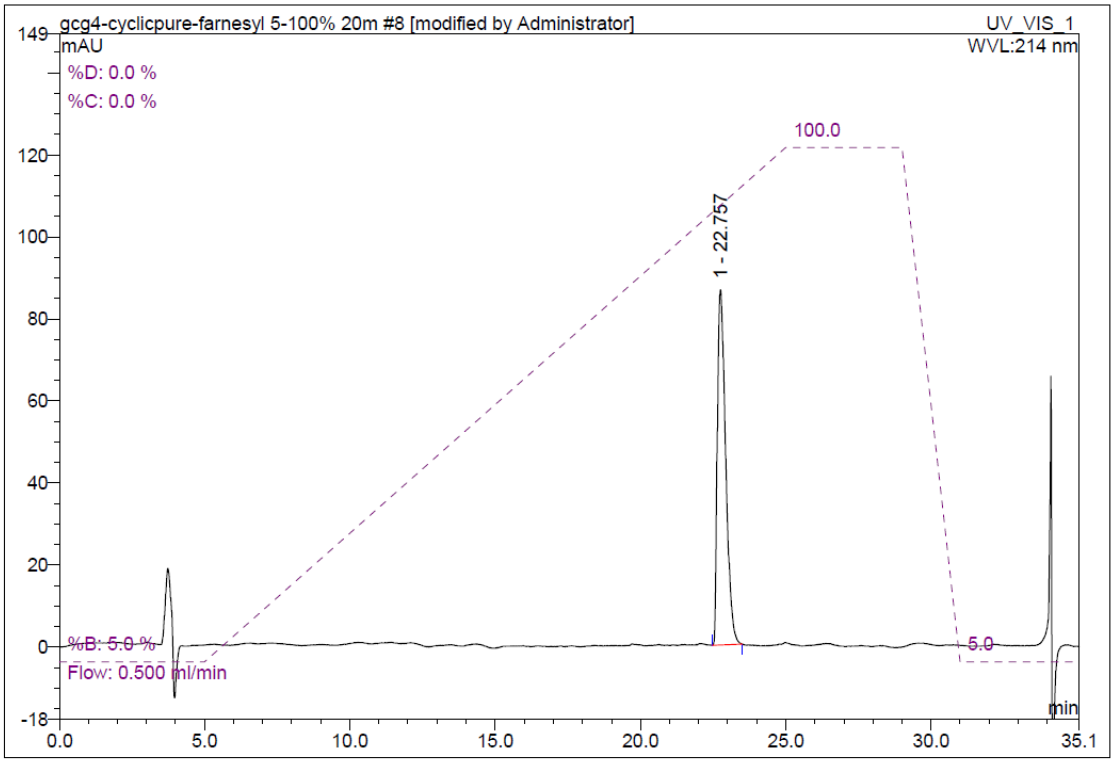
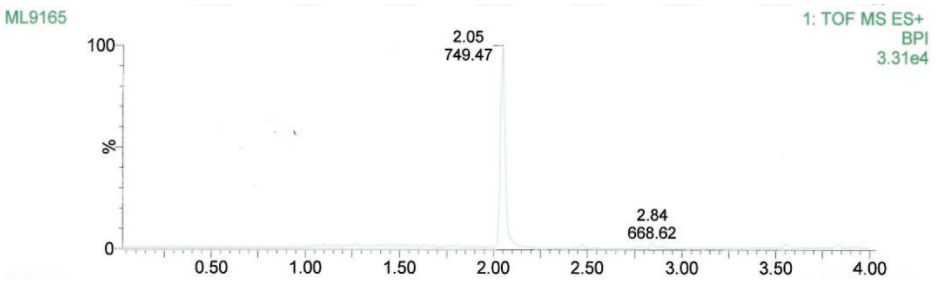
EXPNO 3
PROCNO 1
Date_ 20170112
Time 20.14
INSTRUM spect
PROBHD 5 mm PABBO BB
PULPROG mlevph
TD 2048
SOLVENT CD3CN
NS 8
DS 16
SWH 2732.240 Hz
FIDRES 1.334102 Hz
AQ 0.3748340 sec
RG 228
DW 183.000 usec
DE 6.50 usec
TE 298.0 K
D0 0.00017247 sec
D1 2.79560995 sec
D9 0.06000000 sec
D12 0.00002000 sec
IN0 0.00036600 sec
L1 24
CHANNEL f1
1H
NUC1
P1 10.25 usec
P5 23.34 usec
P6 35.00 usec
P7 70.00 usec
P17 2500.00 usec
PL1 0.00 dB
PL10 10.67 dB
PL1W 22.85816574 W
PL10W 1.95903134 W
SFO1 500.1315602 MHz
ND0 1
TD 128
SFO1 500.1316 MHz
FIDRES 21.345629 Hz
SW 5.463 ppm
FnMODE States-TPPI
SI 1024
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 States-TPPI
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
ppm
gog4-513dry Lys/Fam redise

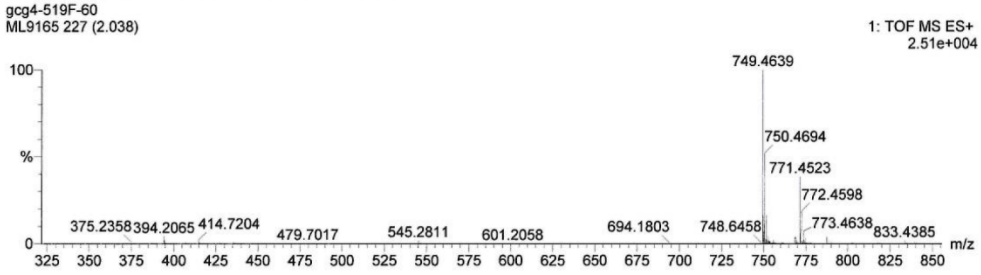
Orn_{10}-farnesylbactin (3)



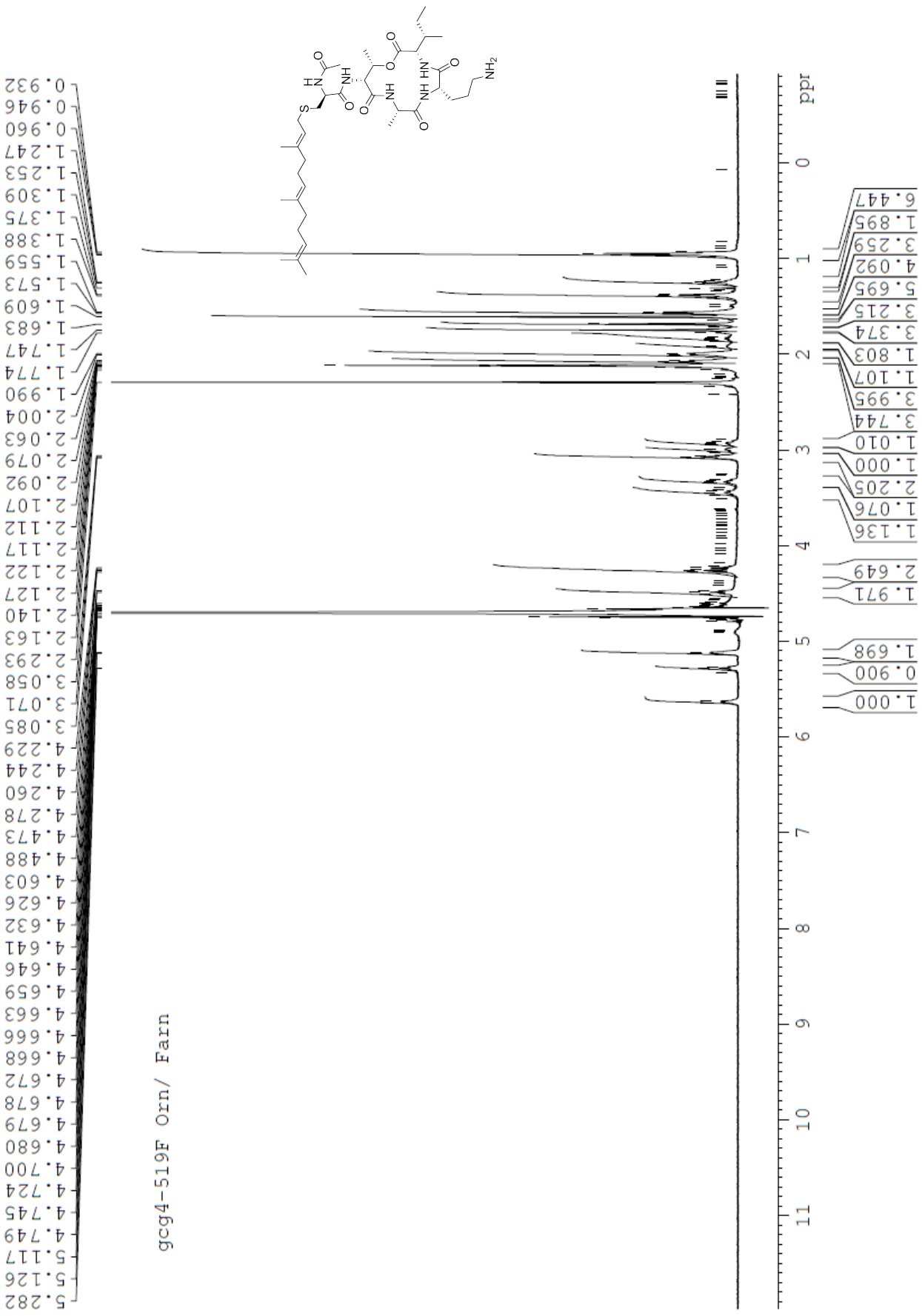
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	22.76	n.a.	86.629	28.680	100.00	n.a.	BMB
Total:			86.629	28.680	100.00	0.000	

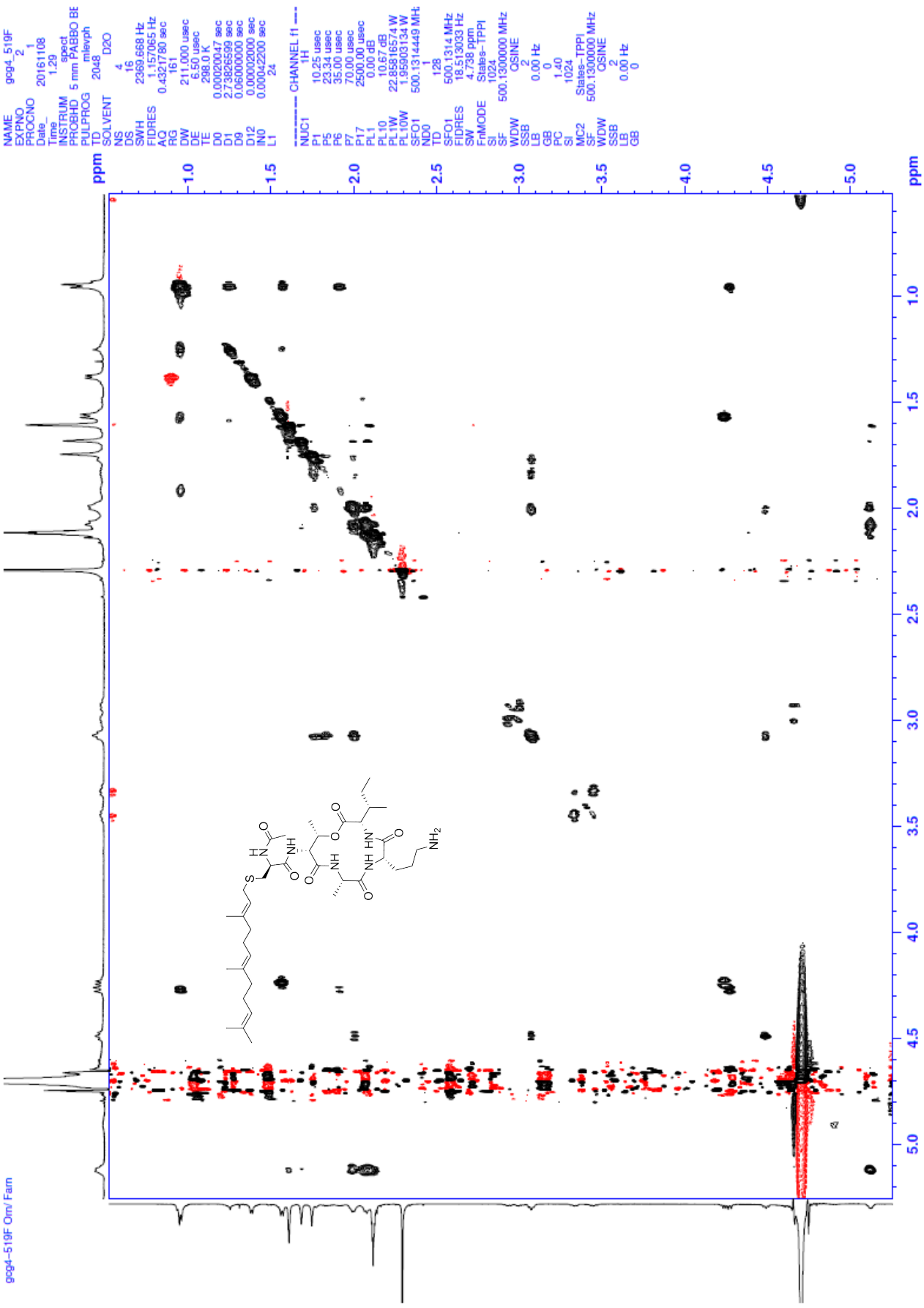


Monoisotopic Mass, Even Electron Ions
869 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 38-38 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-519F-60
ML9165 227 (2.038)



Minimum:				-1.5					
Maximum:		5.0	5.0	80.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula		
749.4639	749.4635	0.4	0.5	9.5	117.8	0.0	C38	H65	N6 O7 S


gcg4-519F Orn/ Farn
ppm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PROCNO 1
Date_ 20161108
Time 1.29
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PULPROG mlevph
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SOLVENT D2O
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FIDRES 1.157065 Hz
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RG 161
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DE 6.50 usec
TE 298.0 K
D0 0.00020047 sec
D1 2.73826599 sec
D9 0.06000000 sec
D12 0.00002000 sec
IN0 0.00042200 sec
L1 24
CHANNEL f1
1H
NUC1
P1 10.25 usec
P5 23.34 usec
P6 35.00 usec
P7 70.00 usec
P17 2500.00 usec
PL1 0.00 dB
PL10 10.67 dB
PL1W 22.85816574 W
PL10W 1.95903134 W
SFO1 500.1314449 MHz
ND0 1
TD 128
SFO1 500.1314 MHz
FIDRES 18.513033 Hz
SW 4.738 ppm
FnMODE States-TPPI
SI 1024
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 States-TPPI
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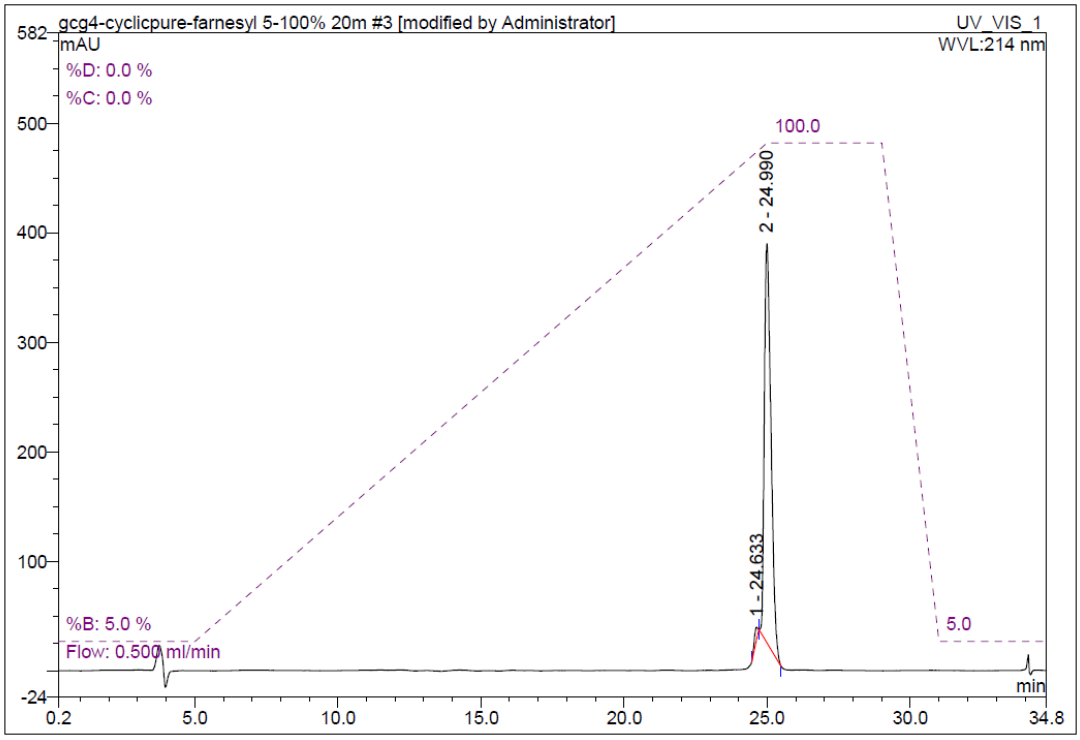
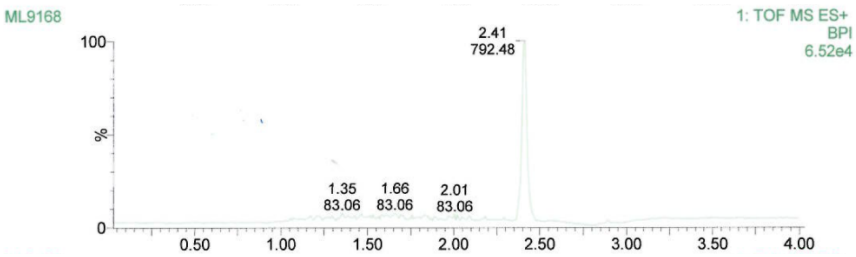
SSB 2
LB 0.00 Hz
GB 0
ppm
gcg4-519F Om/ Fam

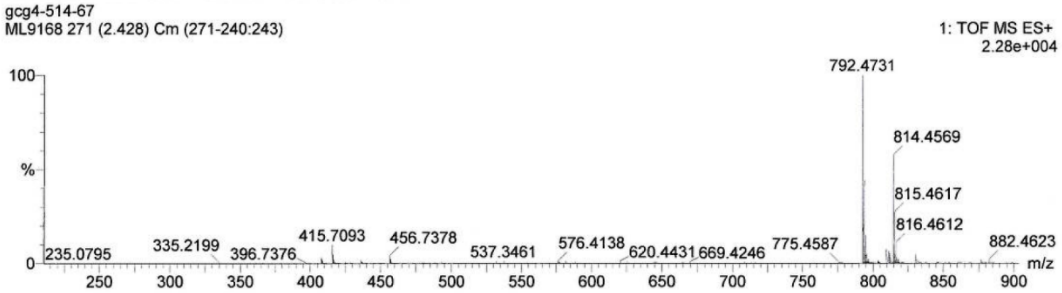
Cit_{10}-farnesylbactin (4)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	24.63	n.a.	11.829	1.907	1.92	n.a.	BMb
2	24.99	n.a.	364.246	97.555	98.08	n.a.	bMB
Total:			376.075	99.462	100.00	0.000	



Monoisotopic Mass, Even Electron Ions
902 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 39-39 H: 0-180 N: 0-30 O: 0-30 S: 1-1



Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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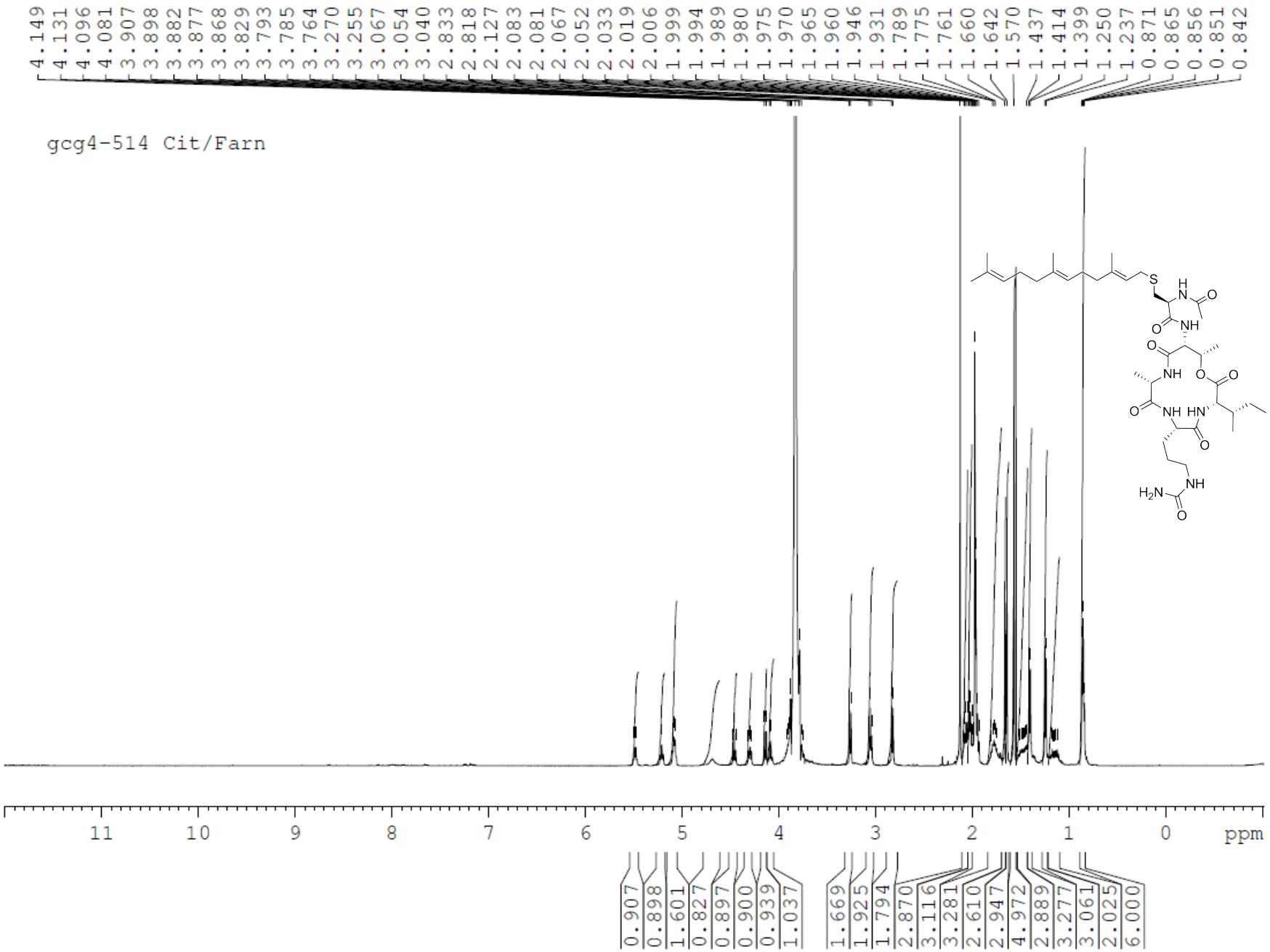
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3.898
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3.829
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3.764
3.270
3.255
3.067
3.054
3.040
2.833
2.818
2.127
2.083
2.081
2.067
2.052
2.033
2.019
2.006
1.999
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1.789
1.775
1.761
1.660
1.642
1.570
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1.250
1.237
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0.865
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0.851
0.842
gcg4-514 Cit/Farn
11
10
9
8
7
6
5
4
3
2
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0
ppm
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0.897
0.900
0.939
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1.925
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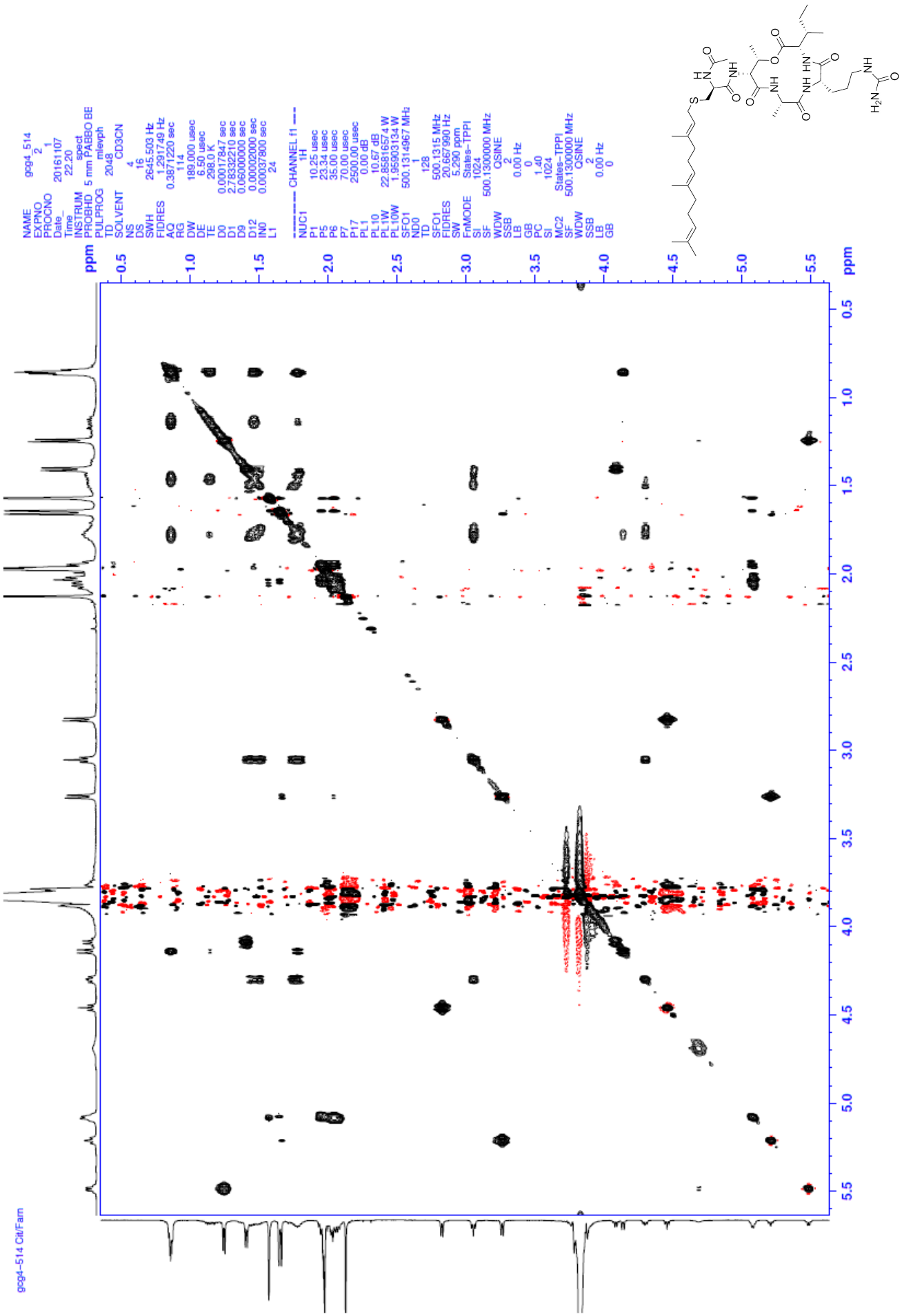
6.000


gcg4_514
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mlevph
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CD3CN
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1.291749 Hz
0.3871220 sec
114
189.000 usec
6.50 usec
298.0 K
0.000017847 sec
2.783322210 sec
0.060000000 sec
0.000002000 sec
0.000037800 sec
24
CHANNEL f1
1H
10.25 usec
23.34 usec
35.00 usec
70.00 usec
2500.00 usec
0.00 dB
10.67 dB
22.858165?4 W
1.959803134 W
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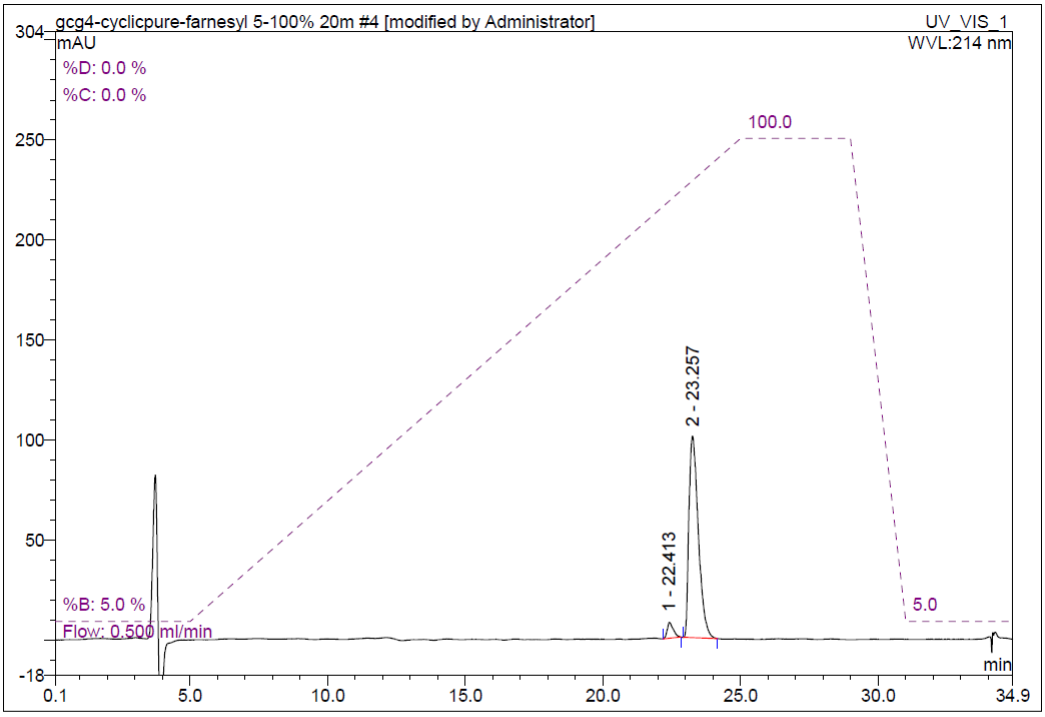
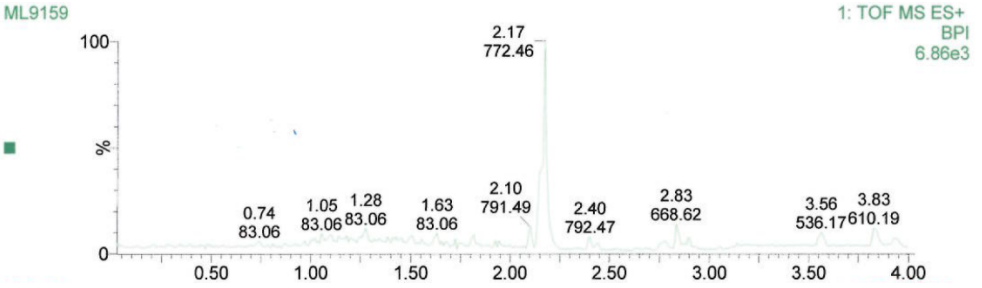
1
128
500.1315 MHz
20.667990 Hz
5.290 ppm
1024
States-TPPI
500.1300000 MHz
QSINE
2
0.00 Hz
0
1.40
1024
States-TPPI
500.1300000 MHz
QSINE
2
0.00 Hz
0
ppm
gcg4-514 Cit/Farn

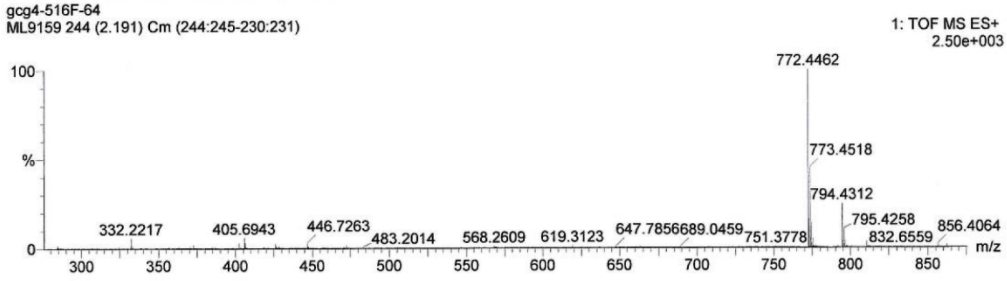
His_{10}-farnesylbactin (5)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	22.41	n.a.	7.996	2.021	4.83	n.a.	BMB
2	23.26	n.a.	100.728	39.822	95.17	n.a.	BMB
Total:			108.724	41.843	100.00	0.000	

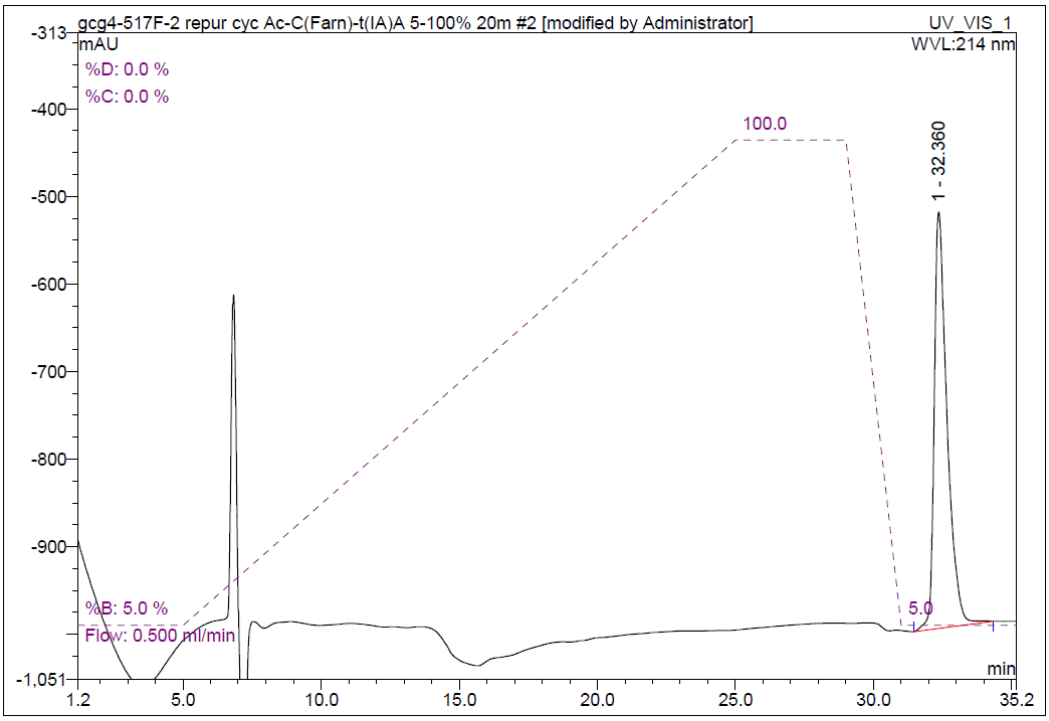


Monoisotopic Mass, Even Electron Ions
888 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 39-39 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-516F-64
ML9159 244 (2.191) Cm (244:245-230:231)

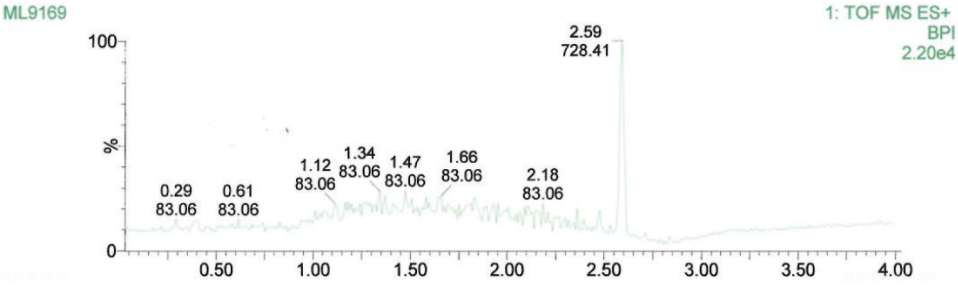


Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
772.4462	772.4431	3.1	4.0	12.5	63.7	0.0	C39 H62 N7 O7 S

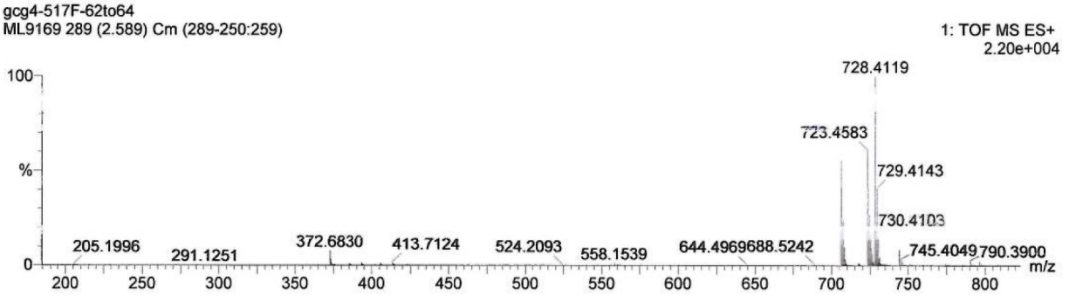
Ala_{10}-farnesylbactin (6)



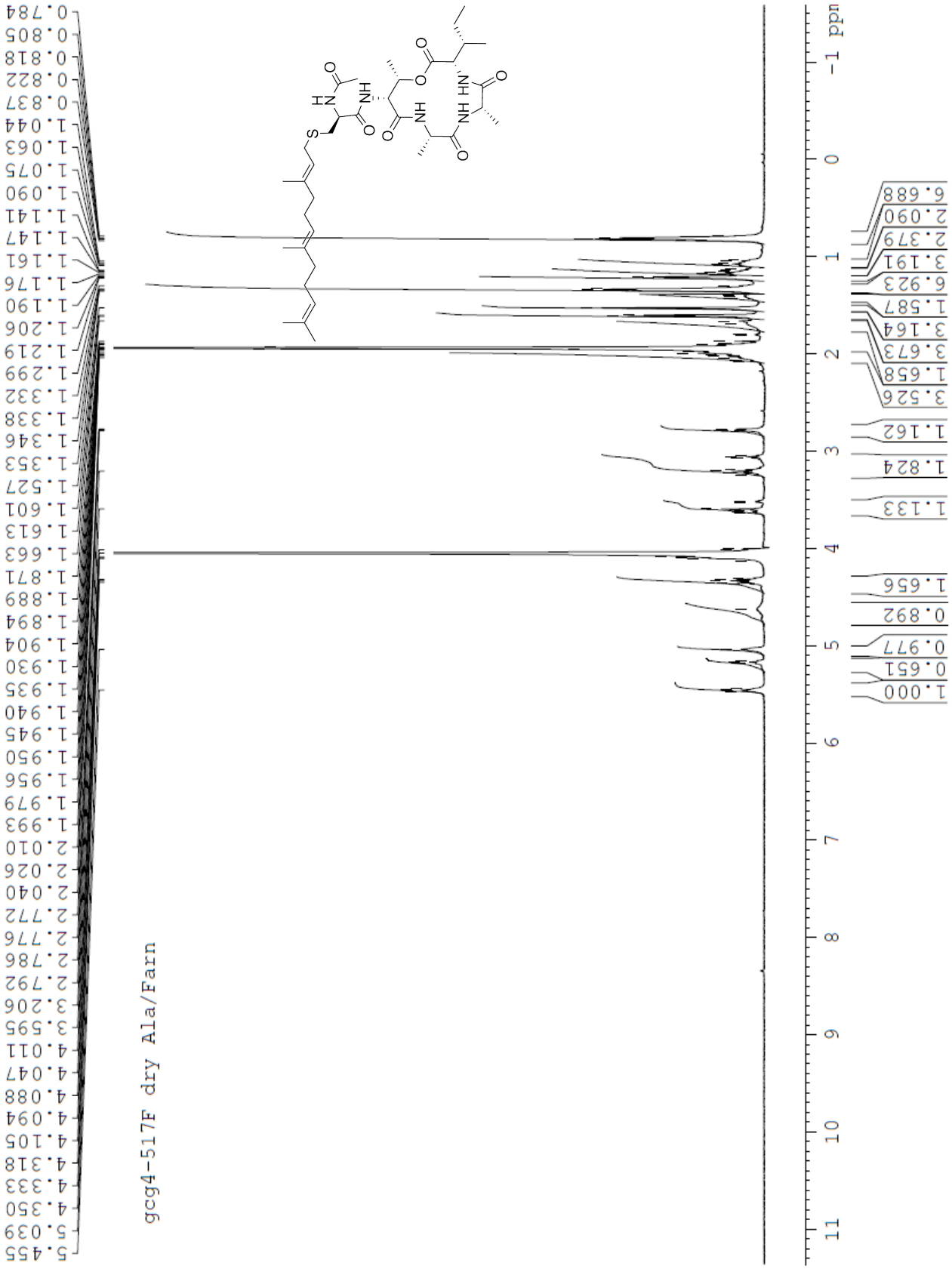
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	32.36	n.a.	475.432	257.147	100.00	n.a.	BMB
Total:			475.432	257.147	100.00	0.000	

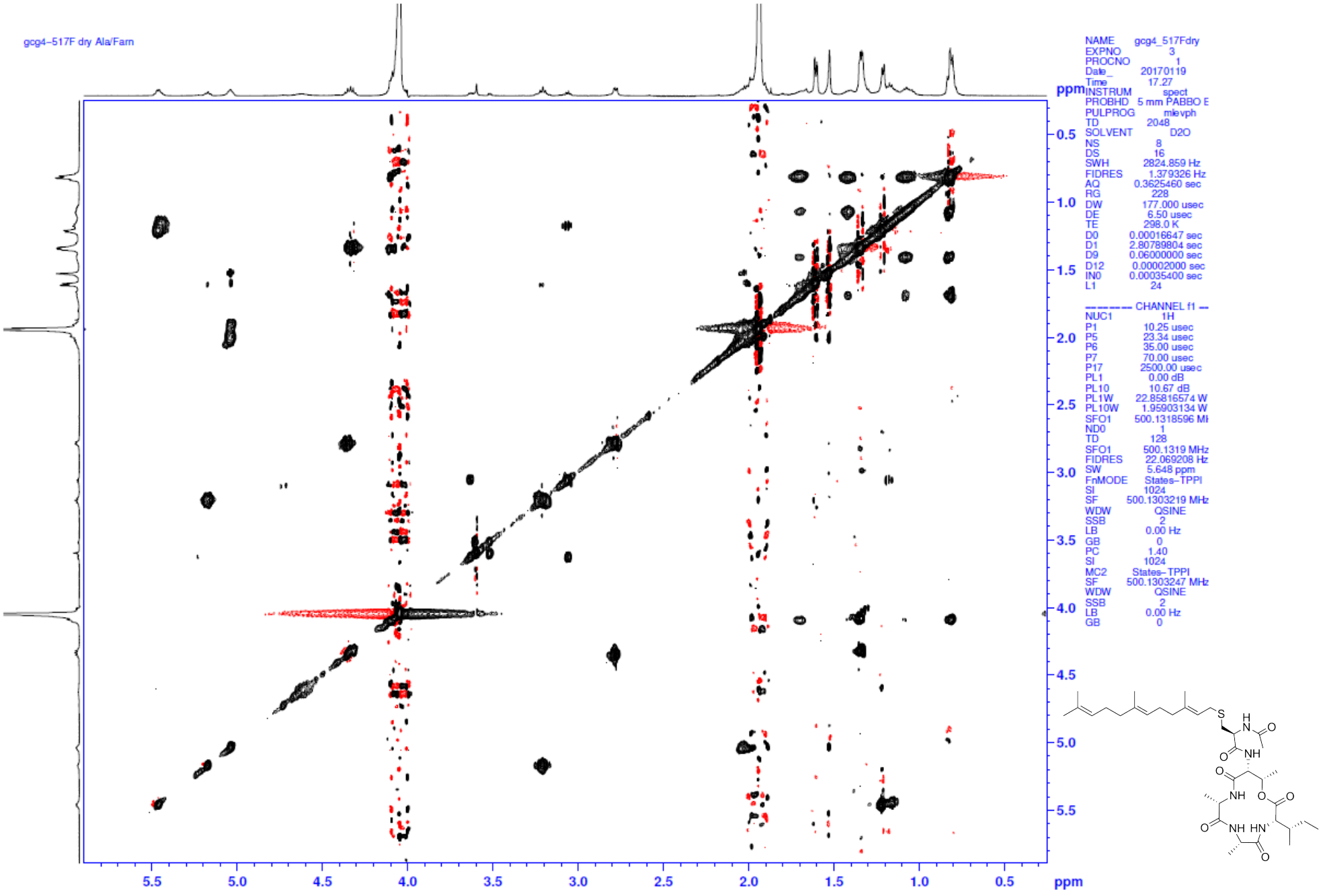


Monoisotopic Mass, Even Electron Ions
826 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 36-36 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-517F-62to64
ML9169 289 (2.589) Cm (289-250:259)

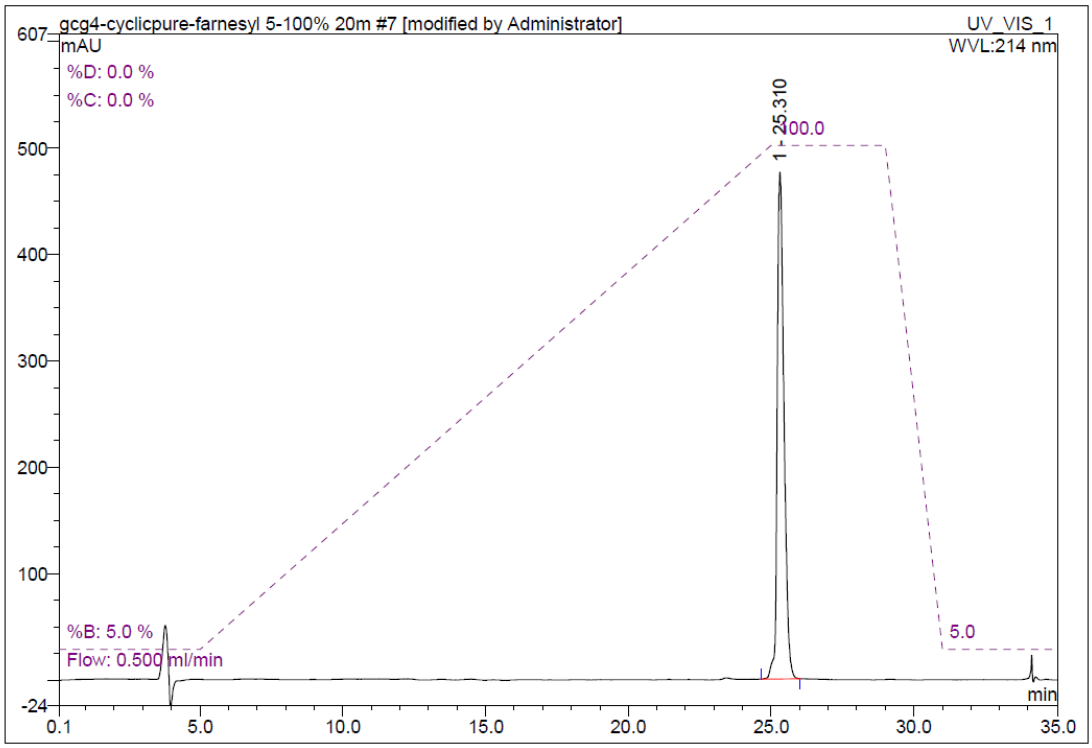


Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
706.4241	706.4213	2.8	4.0	9.5	102.1	0.0	C36 H60 N5 O7 S

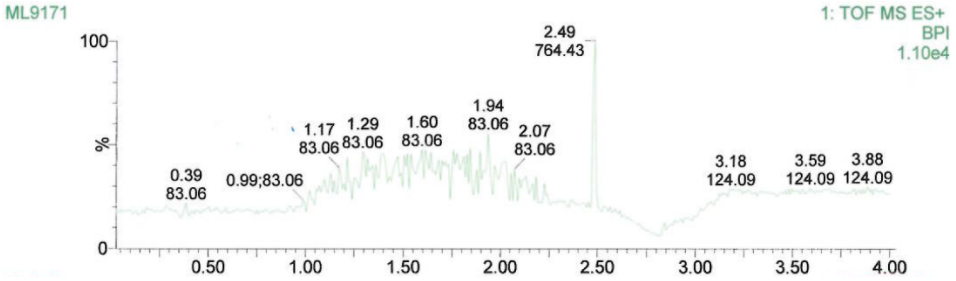

gcg4-517F dry Ala/Farn
ppm
11
10
9
8
7
6
5
4
3
2
1
0
−1
1.000
0.651
0.977
0.892
1.656
1.133
1.824
1.162
3.526
1.658
3.673
3.164
1.587
6.923
3.191
2.379
2.090
6.688
5.455
5.039
4.350
4.333
4.318
4.105
4.094
4.088
4.047
4.011
3.595
3.206
2.792
2.786
2.776
2.772
2.040
2.026
2.010
1.993
1.979
1.956
1.950
1.945
1.940
1.935
1.930
1.904
1.894
1.889
1.871
1.663
1.613
1.601
1.527
1.353
1.346
1.338
1.332
1.299
1.219
1.206
1.190
1.176
1.161
1.147
1.141
1.090
1.075
1.063
1.044
0.837
0.822
0.818
0.805
0.784


gcg4-517F dry Ala/Farn
ppm
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
NAME gcg4_517Fdry
EXPNO 3
PROCNO 1
Date_ 20170119
Time 17.27
INSTRUM spect
PROBHD 5 mm PABBO E
PULPROG mlevph
TD 2048
SOLVENT D2O
NS 8
DS 16
SWH 2824.859 Hz
FIDRES 1.379326 Hz
AQ 0.3625460 sec
RG 228
DW 177.000 usec
DE 6.50 usec
TE 298.0 K
D0 0.00016647 sec
D1 2.80789804 sec
D9 0.06000000 sec
D12 0.00002000 sec
IN0 0.00035400 sec
L1 24
CHANNEL f1
NUC1 1H
P1 10.25 usec
P5 23.34 usec
P6 35.00 usec
P7 70.00 usec
P17 2500.00 usec
PL1 0.00 dB
PL10 10.67 dB
PL1W 22.85816574 W
PL10W 1.95903134 W
SFO1 500.1318596 MH
ND0 1
TD 128
SFO1 500.1319 MHz
FIDRES 22.069208 Hz
SW 5.648 ppm
FnMODE States-TPPI
SI 1024
SF 500.1303219 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 States-TPPI
SF 500.1303247 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
S
N
H
O
NH
NH
O
O
NH
HN
O

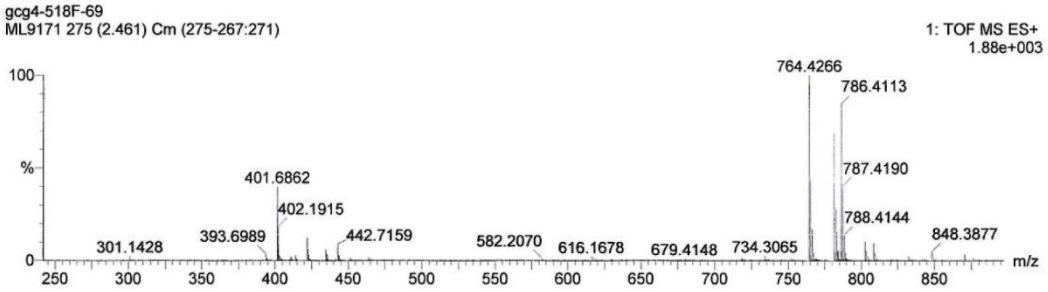
Glu_{10}-farnesylbactin (7)



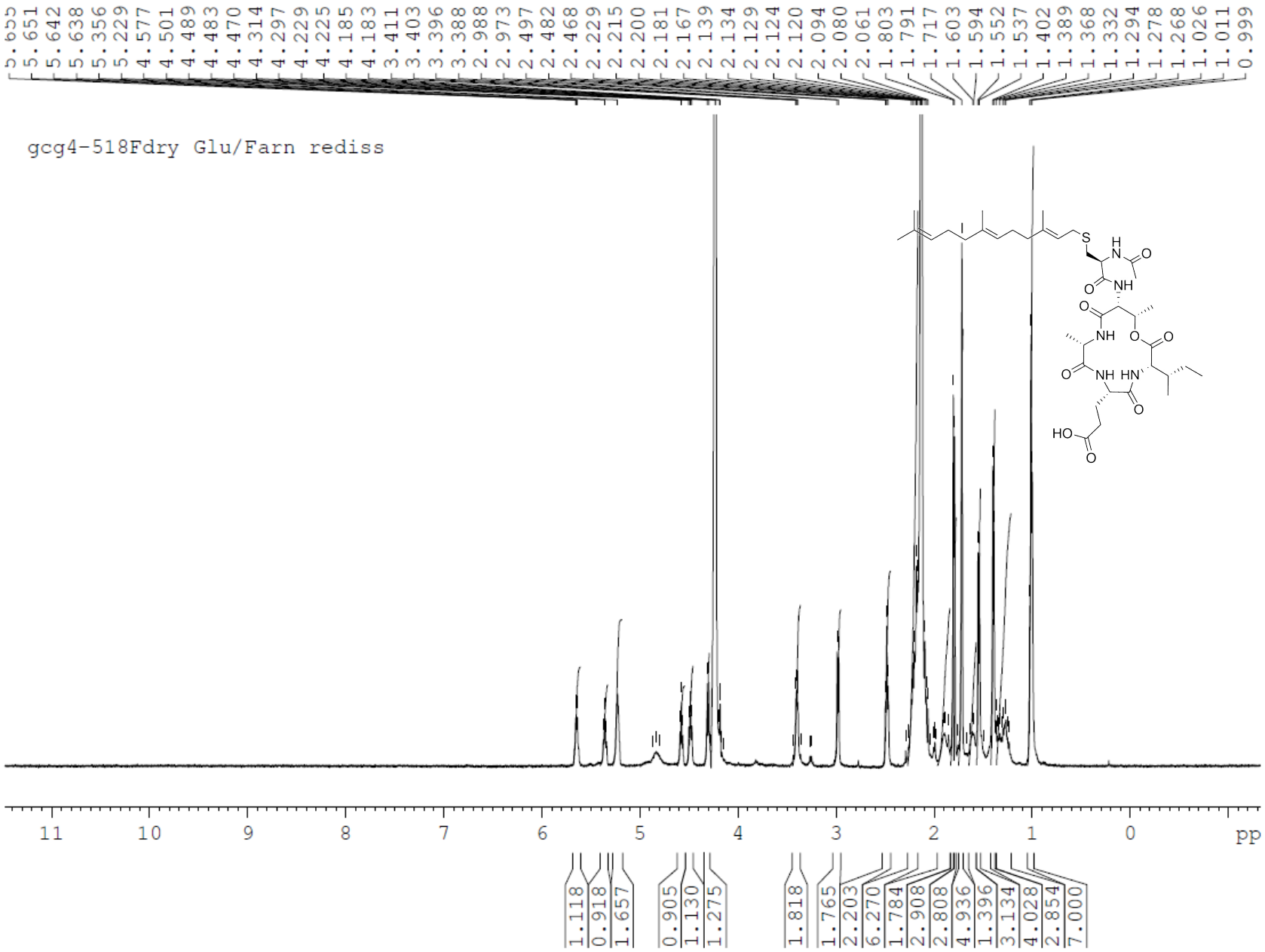
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	25.31	n.a.	476.311	130.641	100.00	n.a.	BMB
Total:			476.311	130.641	100.00	0.000	

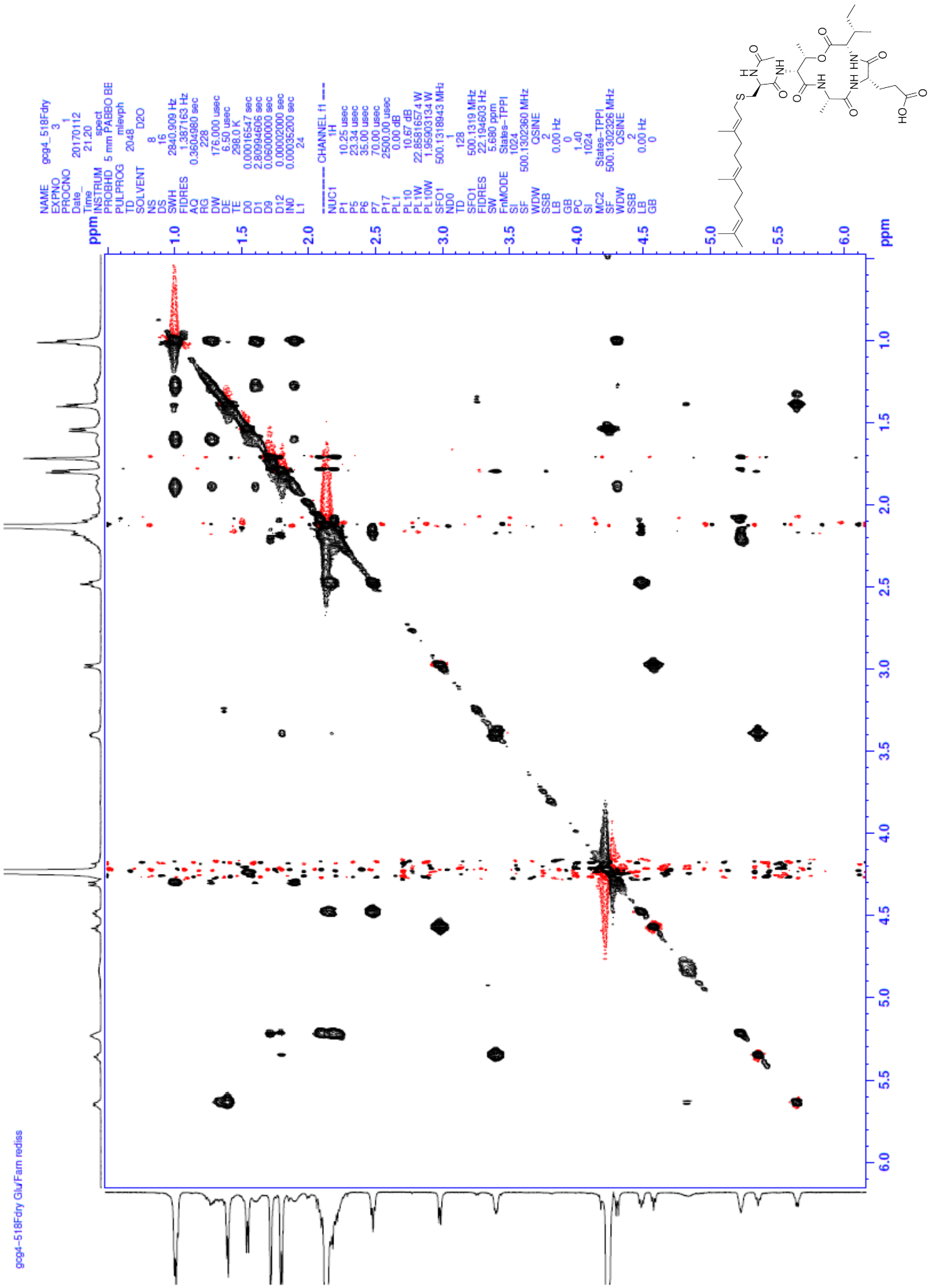


Monoisotopic Mass, Even Electron Ions
881 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 38-38 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-518F-69
ML9171 275 (2.461) Cm (275-267:271)

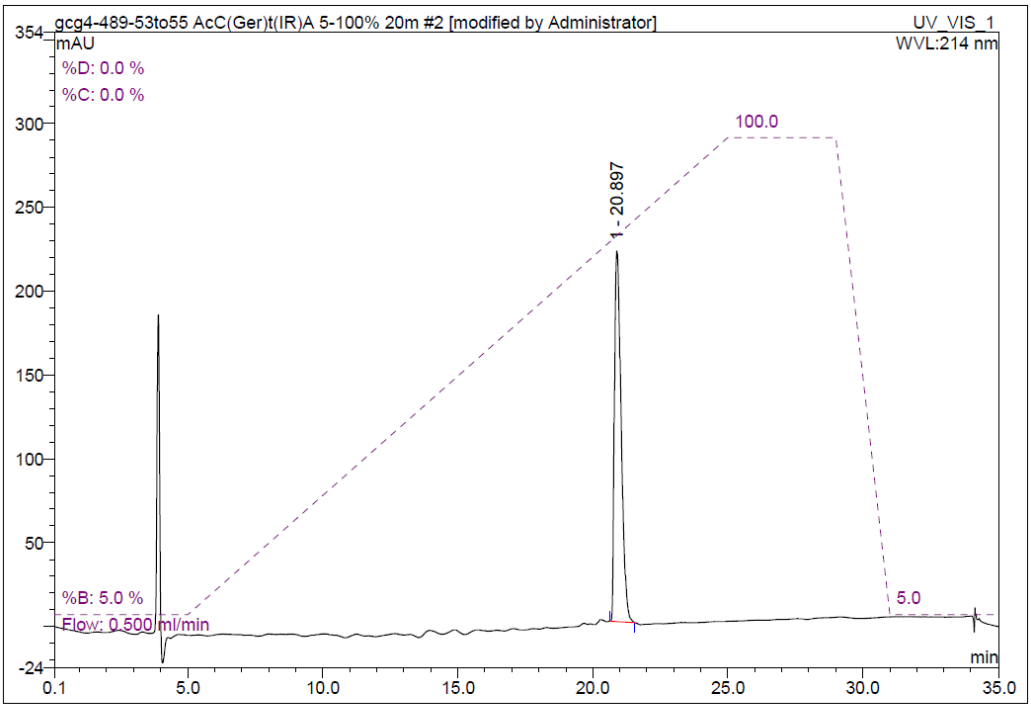


Minimum:				-1.5					
Maximum:		5.0	10.0	80.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula		
764.4266	764.4268	-0.2	-0.3	10.5	43.2	0.0	C38	H62	N5	O9	S

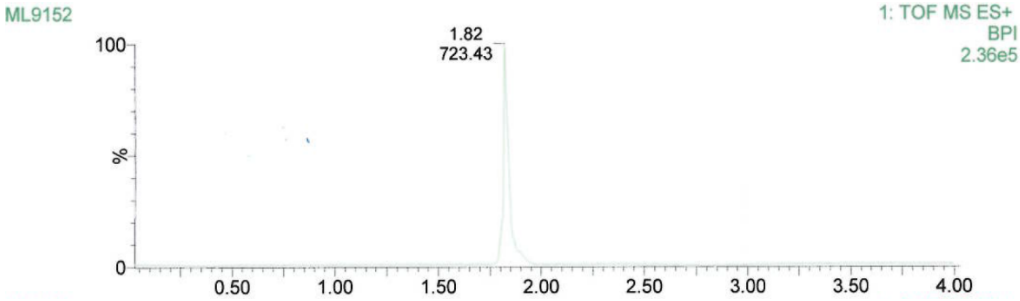

5.655
5.651
5.642
5.638
5.356
5.229
4.577
4.501
4.489
4.483
4.470
4.314
4.297
4.229
4.225
4.185
4.183
3.411
3.403
3.396
3.388
2.988
2.973
2.497
2.482
2.468
2.229
2.215
2.200
2.181
2.167
2.139
2.134
2.129
2.124
2.120
2.094
2.080
2.061
1.803
1.791
1.717
1.603
1.594
1.552
1.537
1.402
1.389
1.368
1.332
1.294
1.278
1.268
1.026
1.011
0.999
gcg4-518Fdry Glu/Farn rediss
11
10
9
8
7
6
5
4
3
2
1
0
pp
1.118
0.918
1.657
0.905
1.130
1.275
1.818
1.765
2.203
6.270
1.784
2.908
2.808
4.936
1.396
3.134
4.028
2.854
7.000


gcg4--518Fdry Glu/Farn rediss
ppm
NAME gcg4_518Fdry
EXPNO 3
PROCNO 1
Date_ 20170112
Time 21.20
INSTRUM spect
PROBHD 5 mm PABBO BE
PULPROG mlevph
TD 2048
SOLVENT D2O
NS 8
DS 16
SWH 2840.909 Hz
FIDRES 1.387163 Hz
AQ 0.3604980 sec
RG 228
DW 176.000 usec
DE 6.50 usec
TE 298.0 K
D0 0.00016547 sec
D1 2.80994606 sec
D9 0.06000000 sec
D12 0.00002000 sec
IN0 0.00035200 sec
L1 24
CHANNEL f1
NUC1 1H
P1 10.25 usec
P5 23.34 usec
P6 35.00 usec
P7 70.00 usec
P17 2500.00 usec
PL1 0.00 dB
PL10 10.67 dB
PL1W 22.8581657 W
PL10W 1.95903134 W
SFO1 500.1318943 MHz
ND0 1
TD 128
SFO1 500.1319 MHz
FIDRES 22.194603 Hz
SW 5.680 ppm
FnMODE States-TPPI
SI 1024
SF 500.1302360 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 States-TPPI
SF 500.1302326 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
ppm

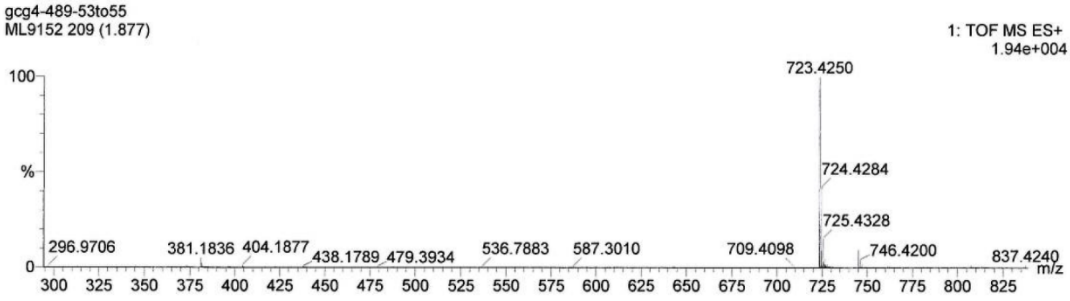
Arg_{10}-geranylbactin (8)



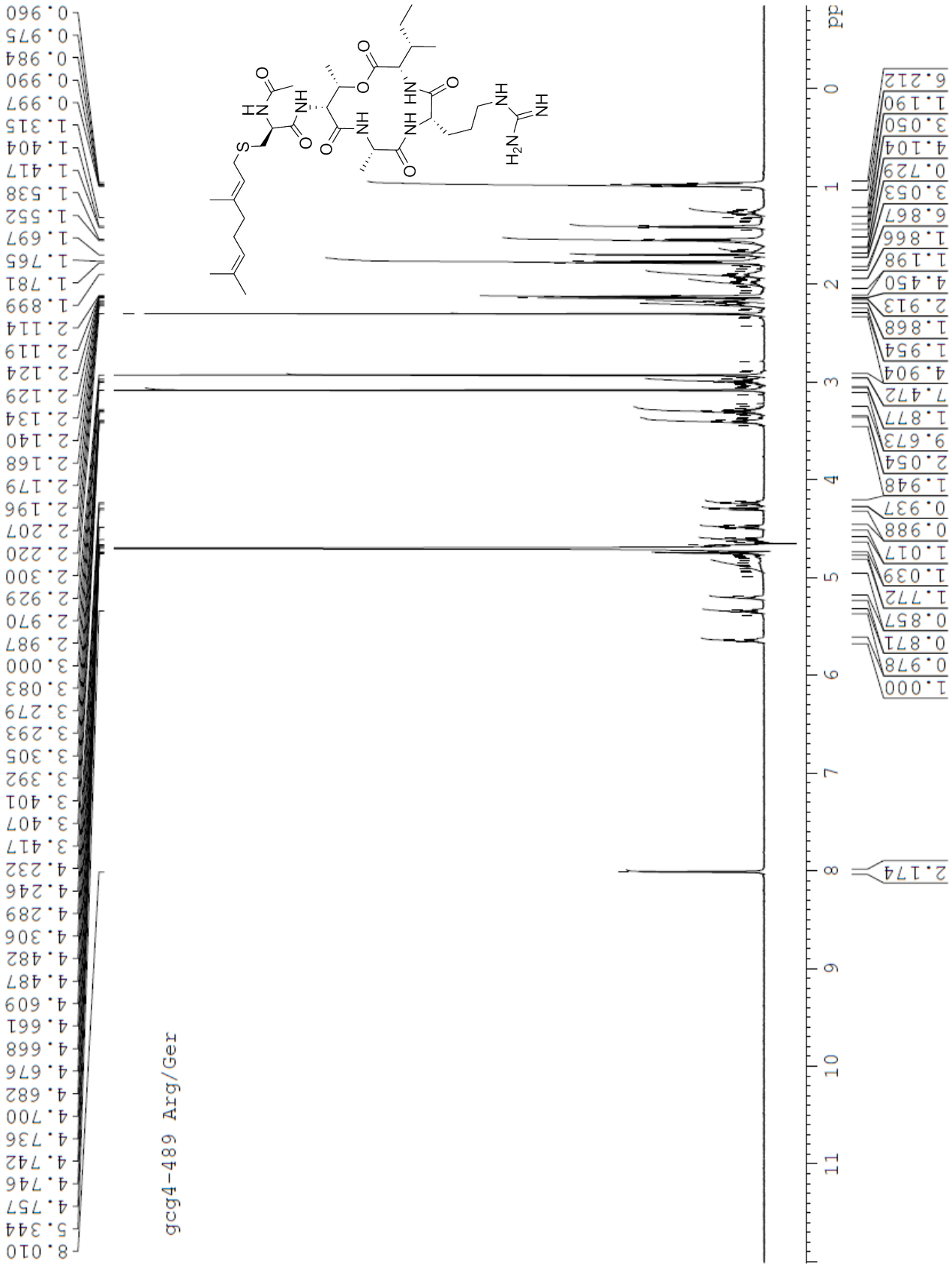
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	20.90	n.a.	221.102	64.018	100.00	n.a.	BMB
Total:			221.102	64.018	100.00	0.000	

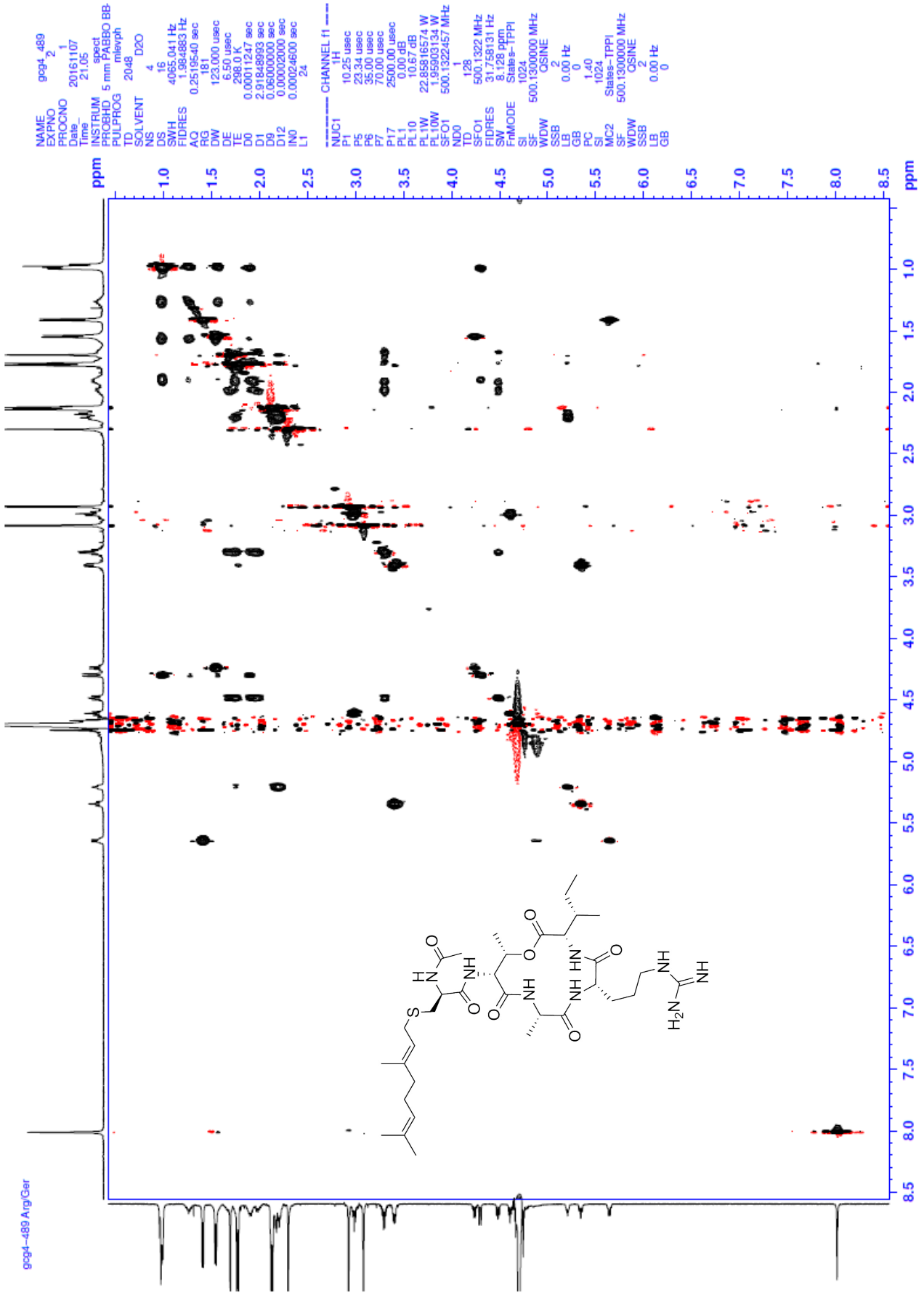


Monoisotopic Mass, Even Electron Ions
843 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 34-34 H: 0-180 N: 0-30 O: 0-30 S: 1-1

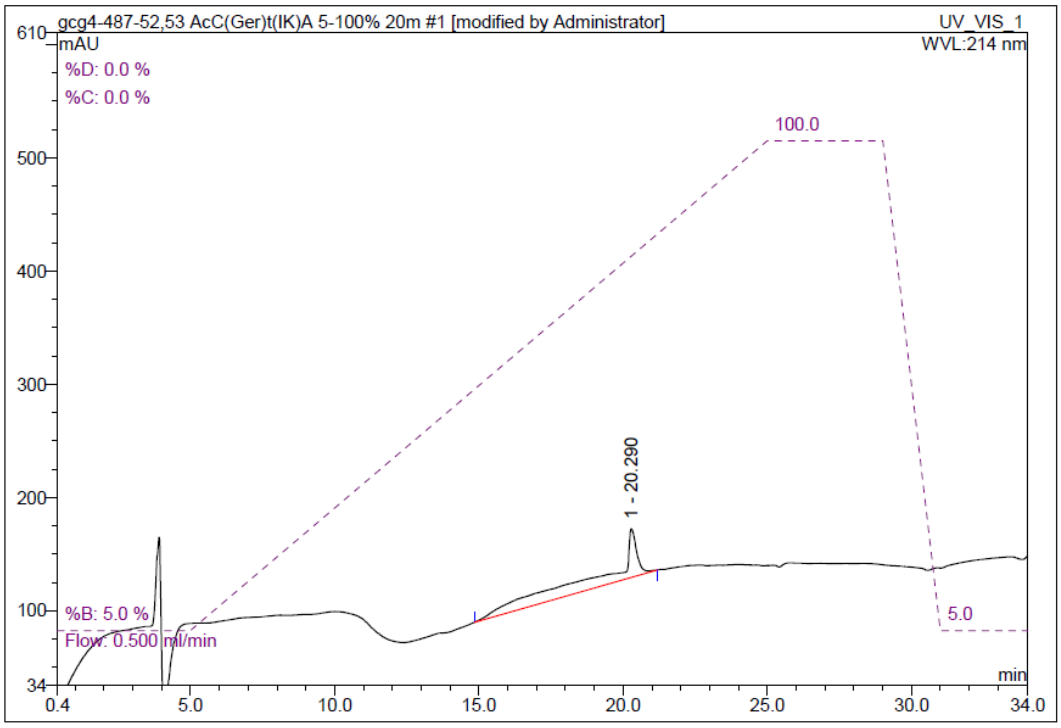


Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**i-FIT**	**i-FIT (Norm)**	**Formula**
723.4250	723.4227	2.3	3.2	9.5	99.1	0.0	C34 H59 N8 O7 S

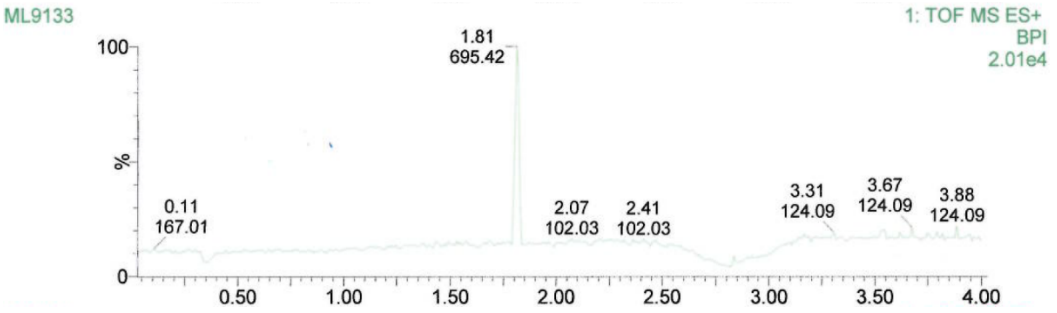

gcg4-489 Arg/Ger
ppm


gcg4_489
2
1
20161107
21.05
spect
5 mm PABBO BB
mlevph
2048
D2O
4
16
4065.041 Hz
1.984883 Hz
0.251954 sec
181
123.000 usec
6.50 usec
298.0 K
0.000011247 sec
2.918488993 sec
0.060000000 sec
0.000002000 sec
0.000024600 sec
24
CHANNEL f1
1H
10.25 usec
23.34 usec
35.00 usec
70.00 usec
2500.00 usec
0.00 dB
10.67 dB
22.858816574 W
1.959403134 W
500.1322457 MHz
1
128
500.1322 MHz
31.758131 Hz
8.128 ppm
States-TPPI
1024
500.1300000 MHz
QSINE
2
0.00 Hz
0
1.40
1024
States-TPPI
500.1300000 MHz
QSINE
2
0.00 Hz
0
ppm
gcg4-489 Arg/Ger

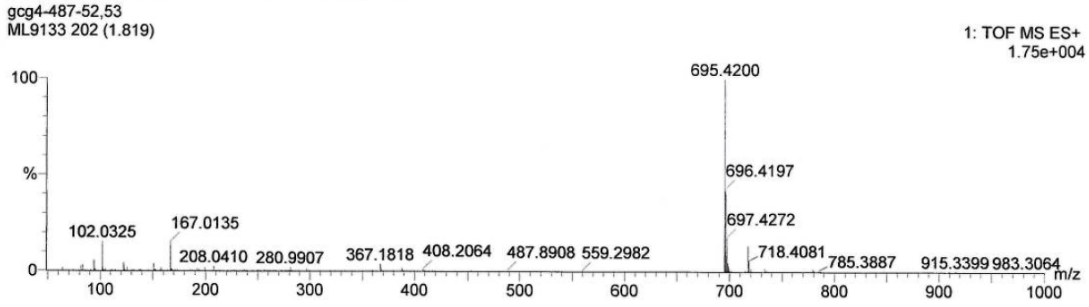
Lys_{10}-geranylbactin (9)



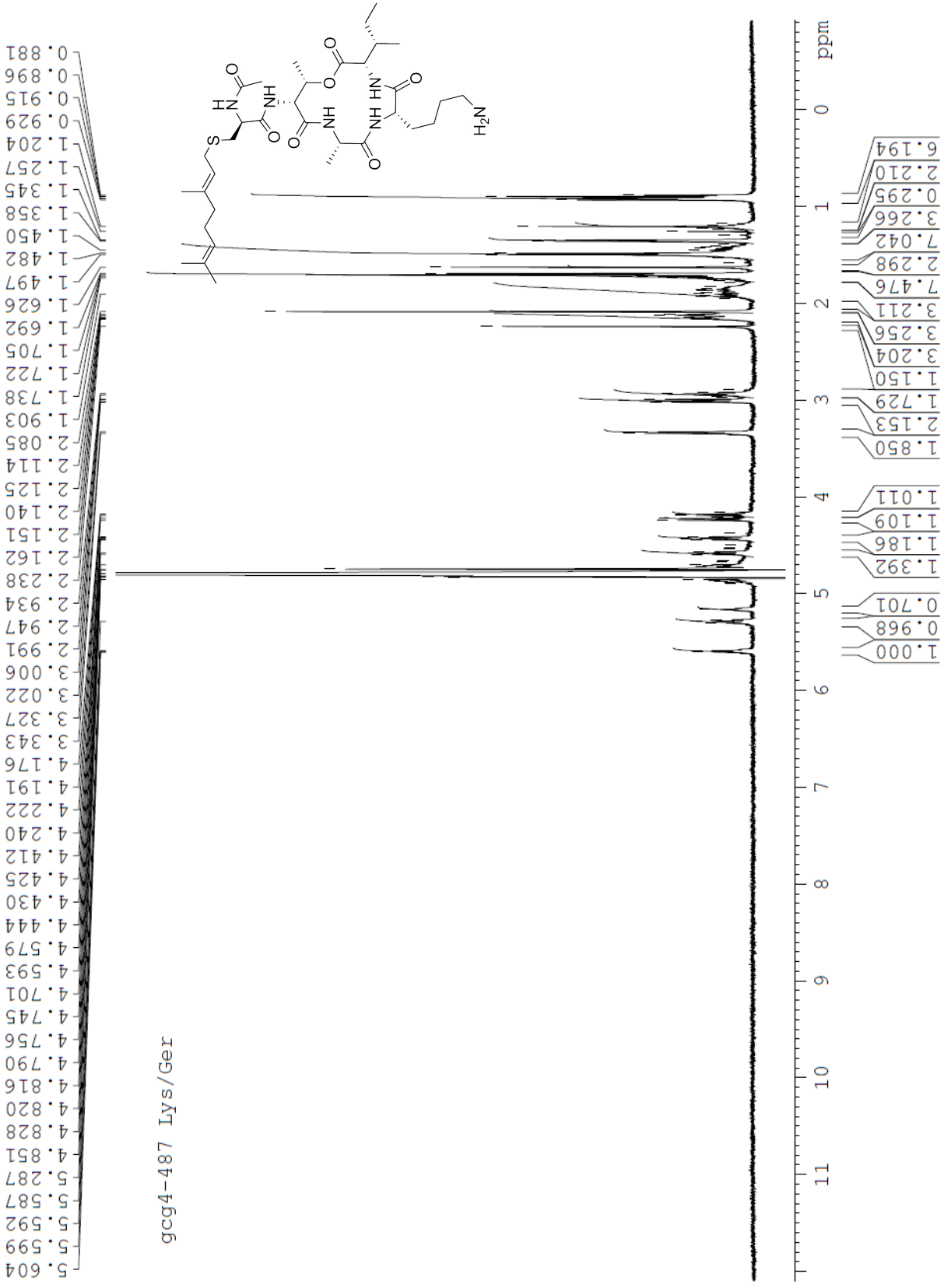
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	20.29	n.a.	43.006	56.436	100.00	n.a.	BMB
Total:			43.006	56.436	100.00	0.000	



Monoisotopic Mass, Even Electron Ions
816 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 34-34 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-487-52,53
ML9133 202 (1.819)



Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
695.4200	695.4166	3.4	4.9	8.5	107.7	0.0	C34 H59 N6 O7 S


gcg4-487 Lys/Ger
ppm
0 1 2 3 4 5 6 7 8 9 10 11
6.194
2.210
0.295
3.266
7.042
2.298
7.476
3.211
3.256
3.204
1.150
1.729
2.153
1.850
1.011
1.109
1.186
1.392
0.701
0.968
1.000
0.881
0.896
0.915
0.929
1.204
1.257
1.345
1.358
1.450
1.482
1.497
1.626
1.692
1.705
1.722
1.738
1.903
2.085
2.114
2.125
2.140
2.151
2.162
2.238
2.934
2.947
2.991
3.006
3.022
3.327
3.343
4.176
4.191
4.222
4.240
4.412
4.425
4.430
4.444
4.579
4.593
4.701
4.745
4.756
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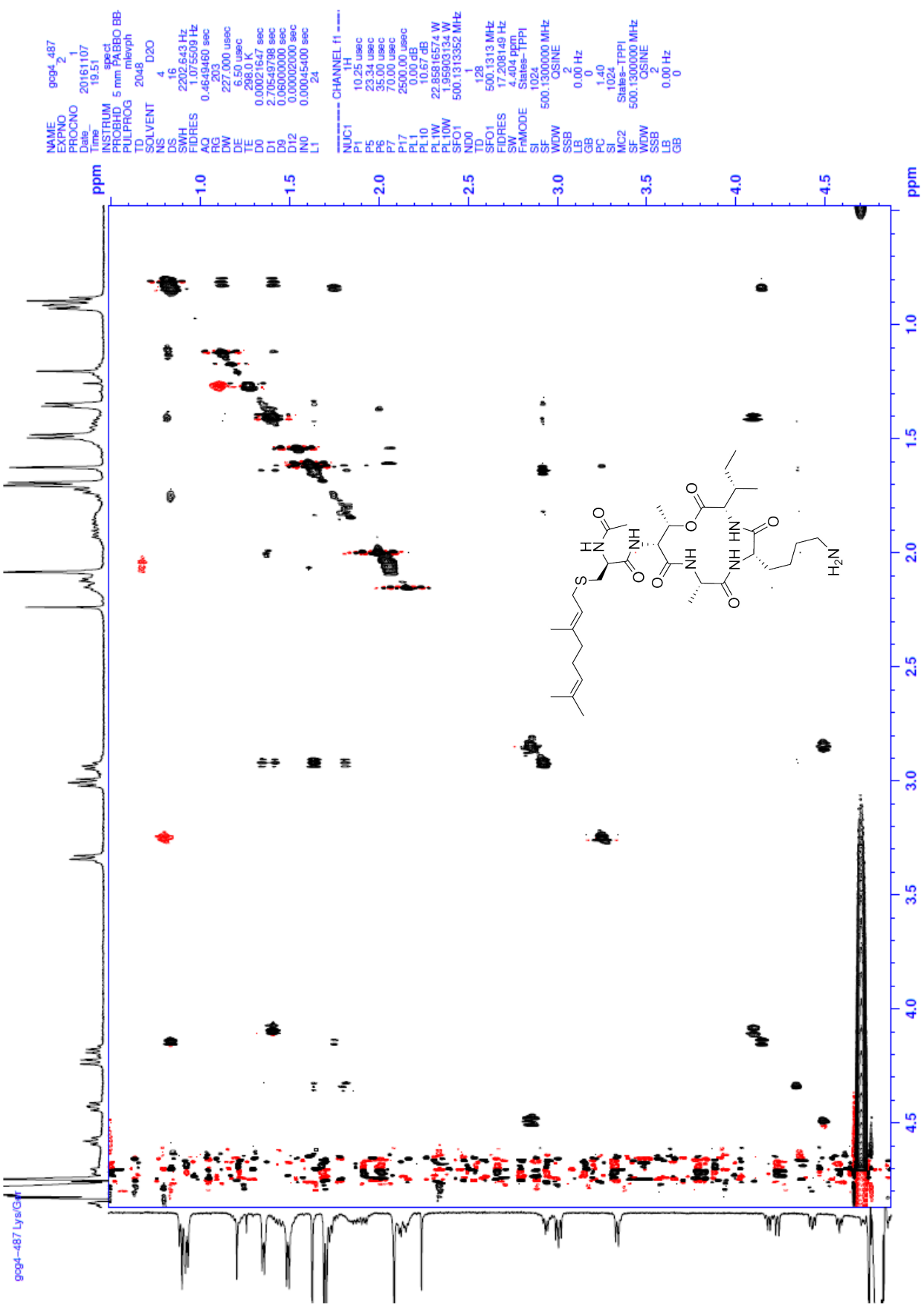
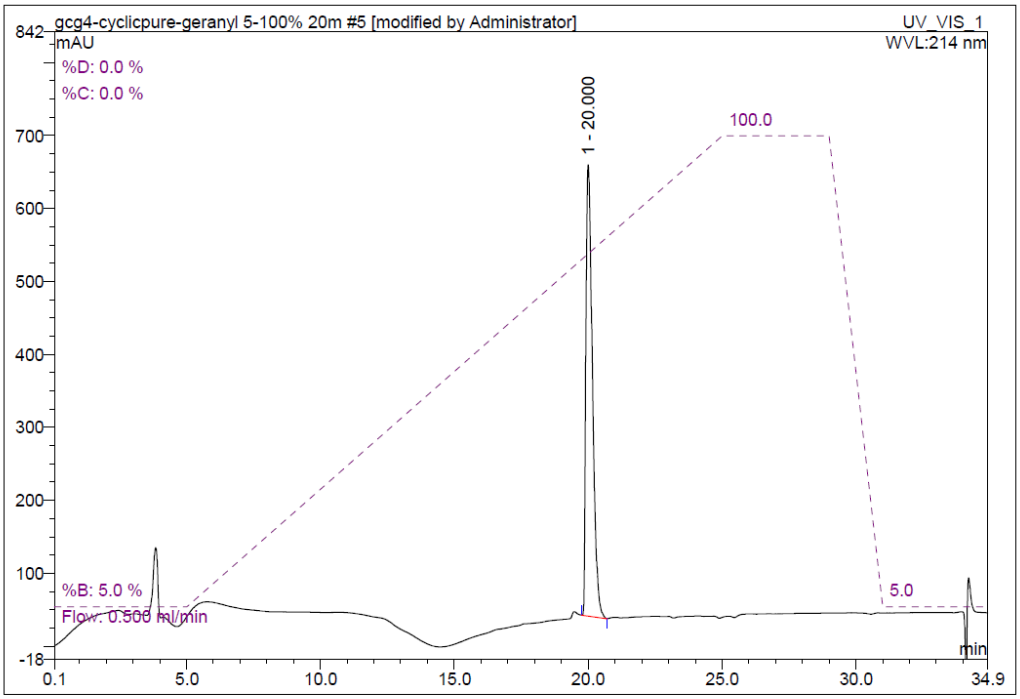
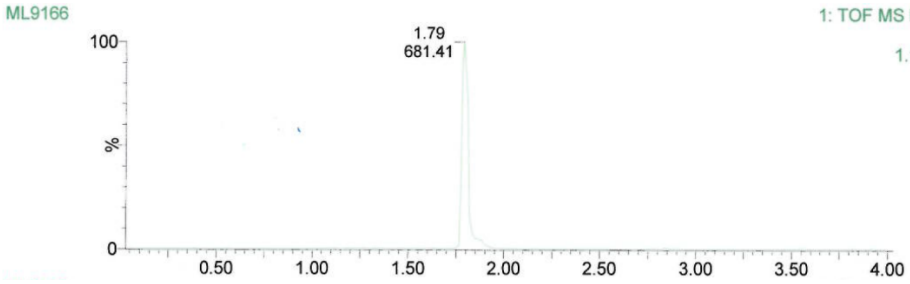
4.816
4.820
4.828
4.851
5.287
5.587
5.592
5.599
5.604


NAME gog4_487
EXPNO 2
PROCNO 1
Date_ 20161107
Time 19.51
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG mlevph
TD 2048
SOLVENT D2O
NS 4
DS 16
SWH 2202.643 Hz
FIDRES 1.075509 Hz
AQ 0.4649460 sec
RG 203
DW 227.000 usec
DE 6.50 usec
TE 298.0 K
D0 0.00021647 sec
D1 2.70549798 sec
D9 0.06000000 sec
D12 0.00002000 sec
IN0 0.00045400 sec
L1 24
CHANNEL f1
NUC1 1H
P1 10.25 usec
P5 23.34 usec
P6 35.00 usec
P7 70.00 usec
P17 2500.00 usec
PL1 0.00 dB
PL10 10.67 dB
PL1W 22.85816574 W
PL10W 1.95903134 W
SFO1 500.1313352 MHz
ND0 1
TD 128
SFO1 500.1313 MHz
FIDRES 17.208149 Hz
SW 4.404 ppm
FnMODE States-TPPI
SI 1024
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 States-TPPI
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
gog4-487 Lys/Gen

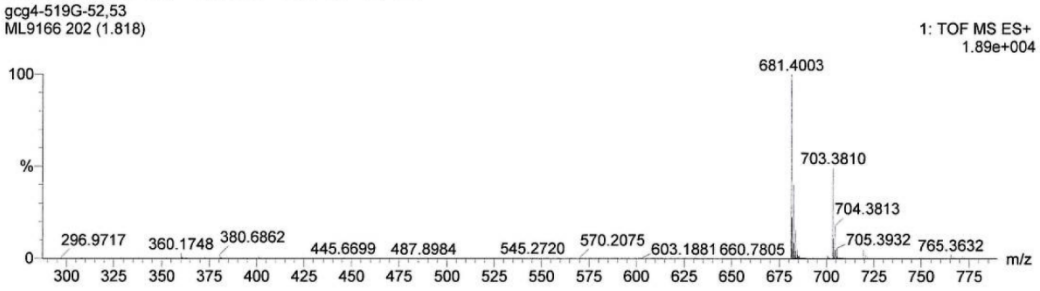
Orn$_{10}$-geranylbactin (10)



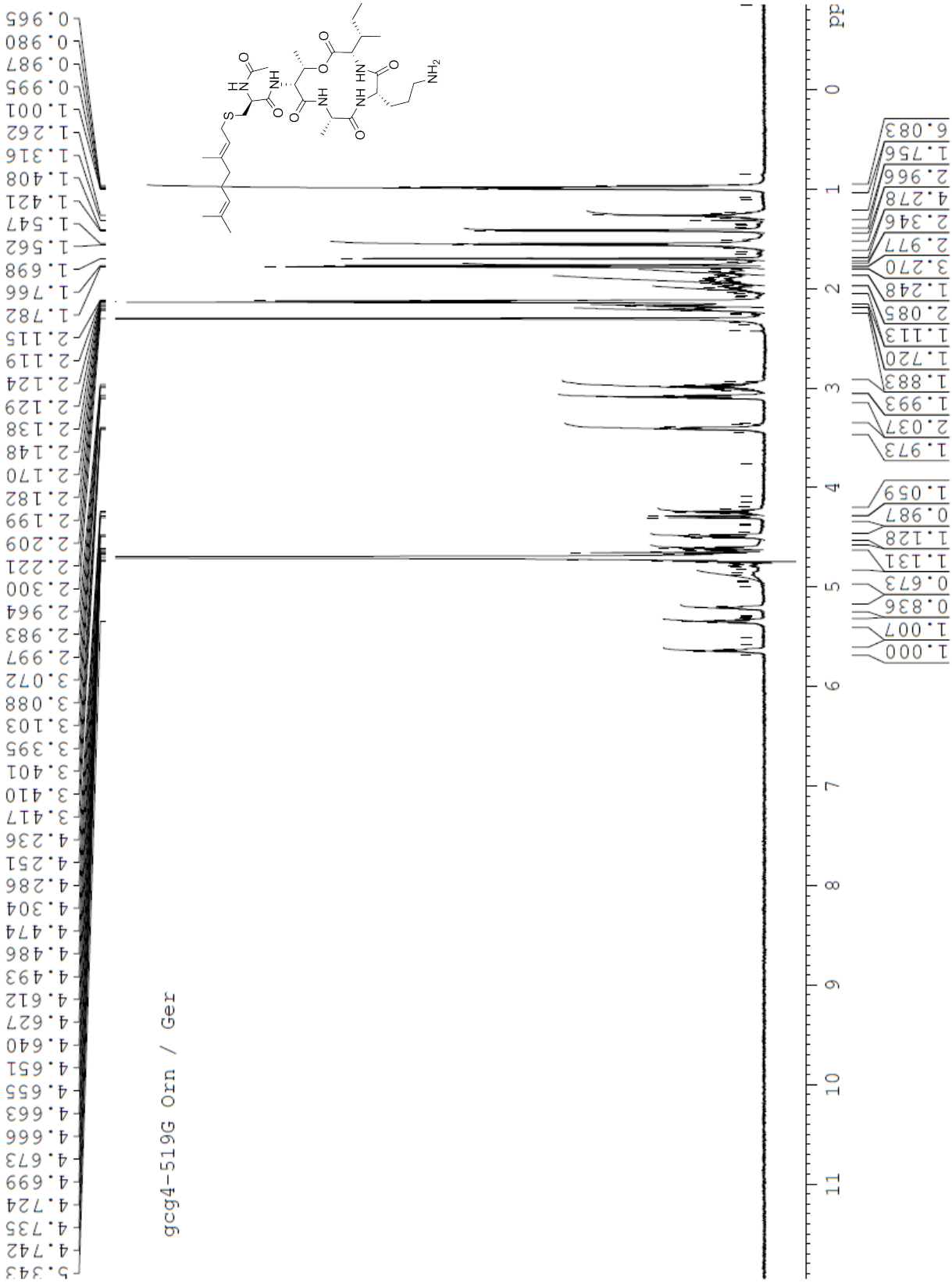
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	20.00	n.a.	618.695	174.948	100.00	n.a.	BMB*
Total:			618.695	174.948	100.00	0.000	

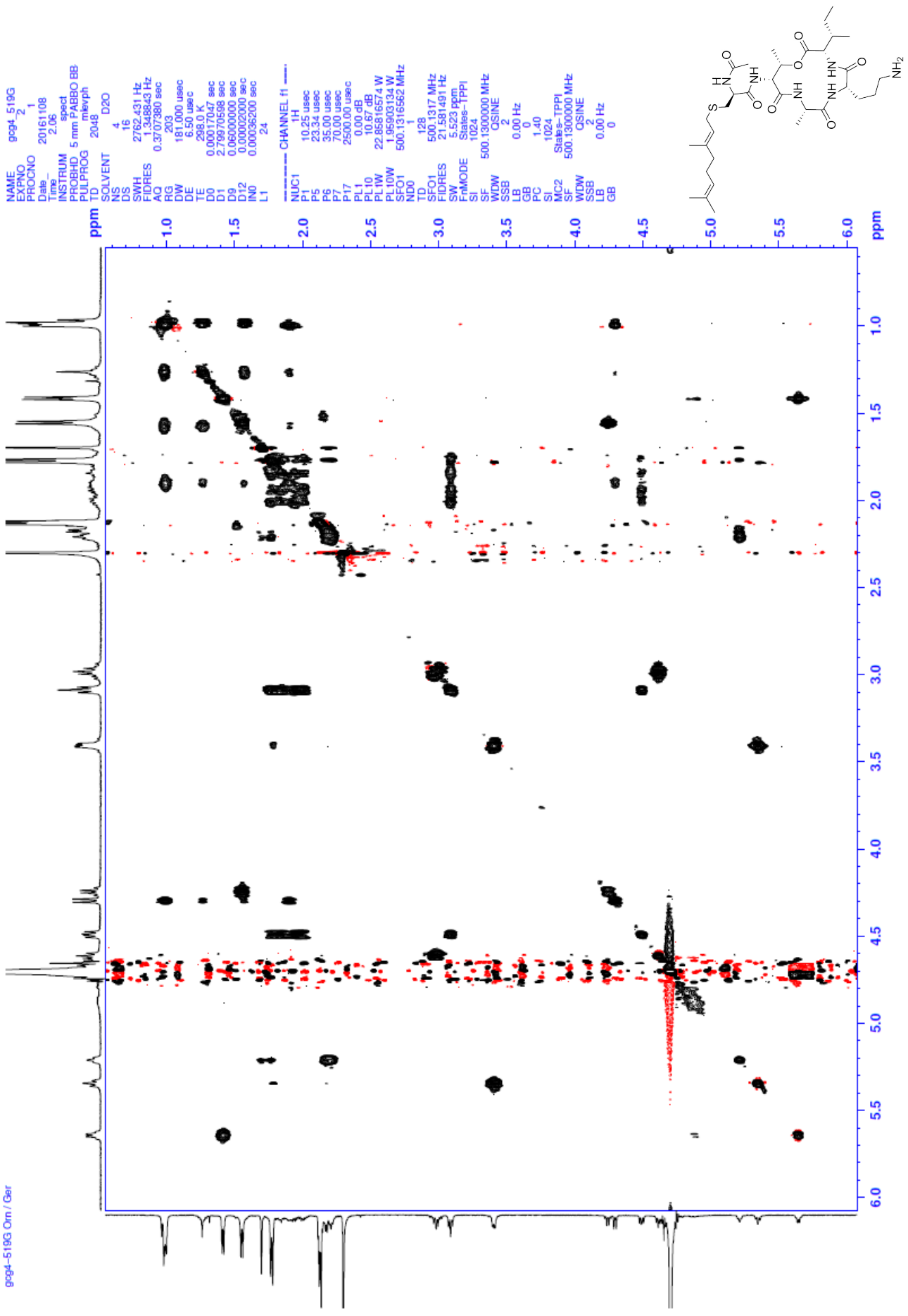


Monoisotopic Mass, Even Electron Ions
799 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 33-33 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-519G-52,53
ML9166 202 (1.818)

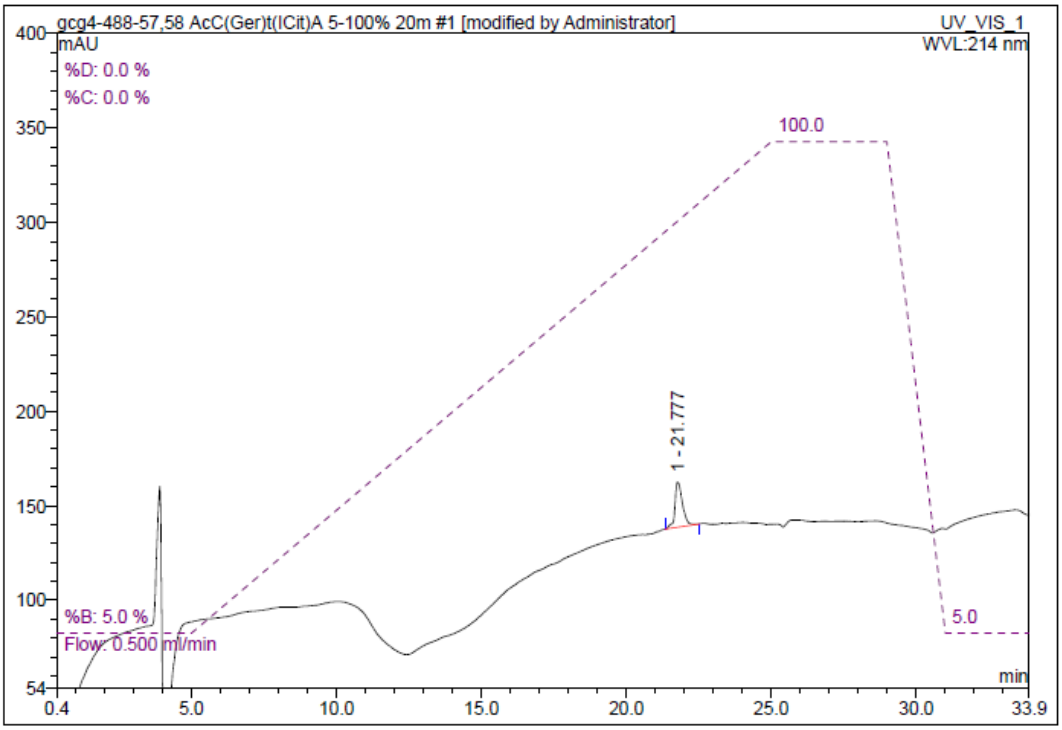


Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
681.4003	681.4009	-0.6	-0.9	8.5	109.0	0.0	C33 H57 N6 O7 S

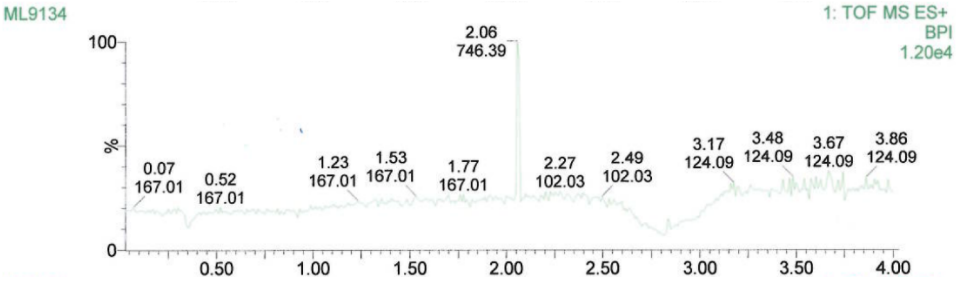

gcg4-519G Orn / Ger
ppm


gcg4_519G
2
1
20161108
2.06
spect
5 mm PABBO BB
mkvph
2048
D2O
4
16
2762.431 Hz
1.348843 Hz
0.3707380 sec
203
181.000 usec
6.50 usec
298.0 K
0.000017047 sec
2.799970598 sec
0.060000000 sec
0.000002000 sec
0.000362200 sec
24
CHANNEL f1
1H
10.25 usec
23.34 usec
35.00 usec
70.00 usec
2500.00 usec
0.00 dB
10.67 dB
22.85816574 W
1.959003134 W
500.1316562 MHz
1
128
500.1317 MHz
21.5814491 Hz
5.523 ppm
States-TPPI
1024
500.1300000 MHz
QSINE
2
0.00 Hz
0
1.40
1024
States-TPPI
500.1300000 MHz
QSINE
2
0.00 Hz
0
ppm
gcg4-519G Orn / Ger

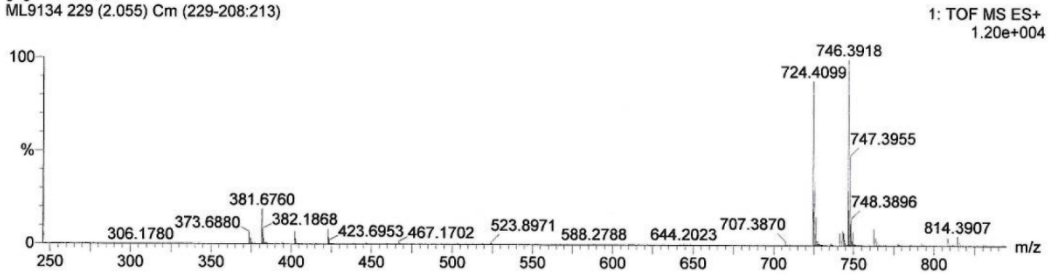
Cit$_{10}$-geranylbactin (11)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	21.78	n.a.	23.878	7.154	100.00	n.a.	BMB*
Total:			23.878	7.154	100.00	0.000	

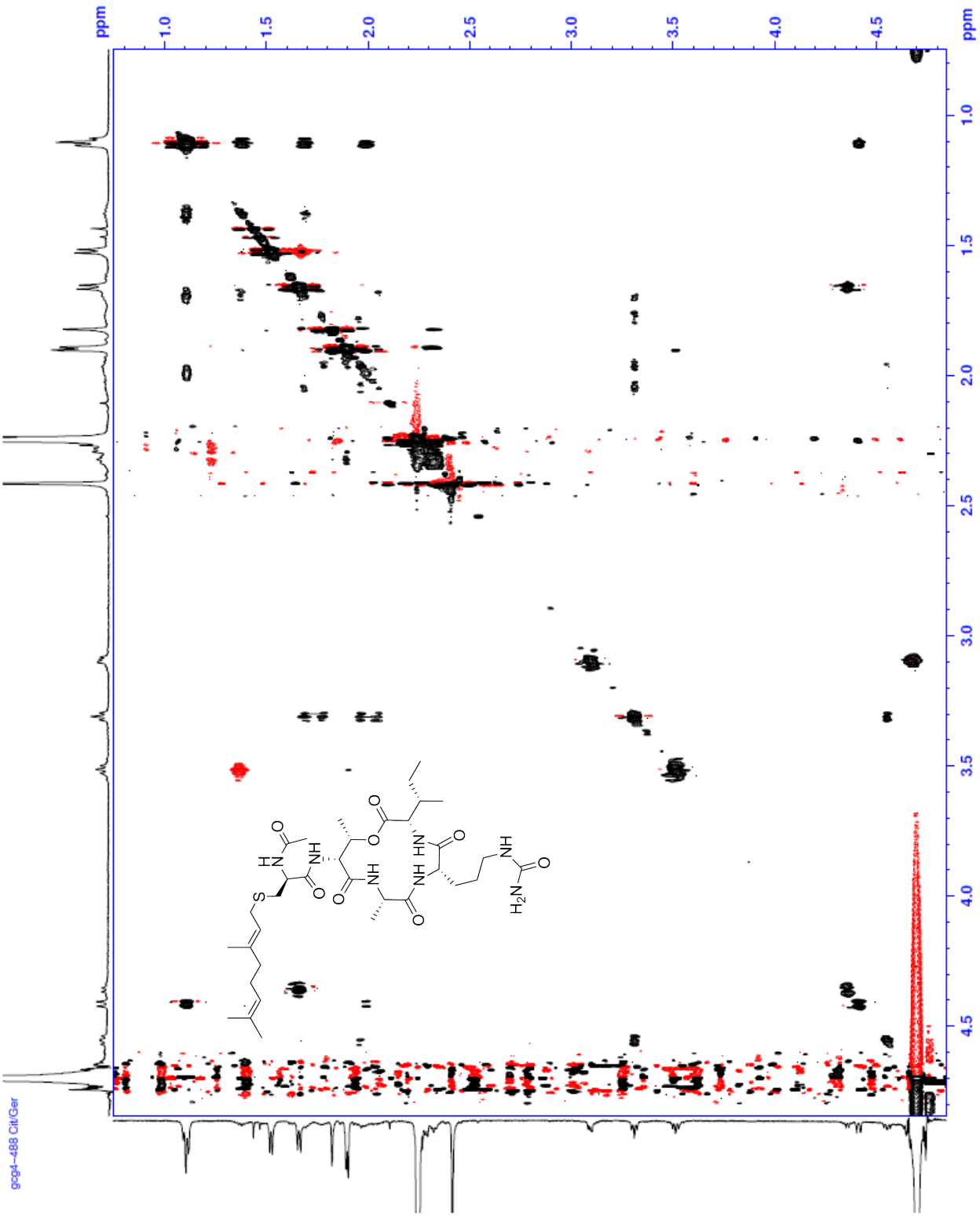


Monoisotopic Mass, Even Electron Ions
844 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 34-34 H: 0-180 N: 0-30 O: 0-30 S: 1-1
gcg4-488-57,58
ML9134 229 (2.055) Cm (229-208:213)

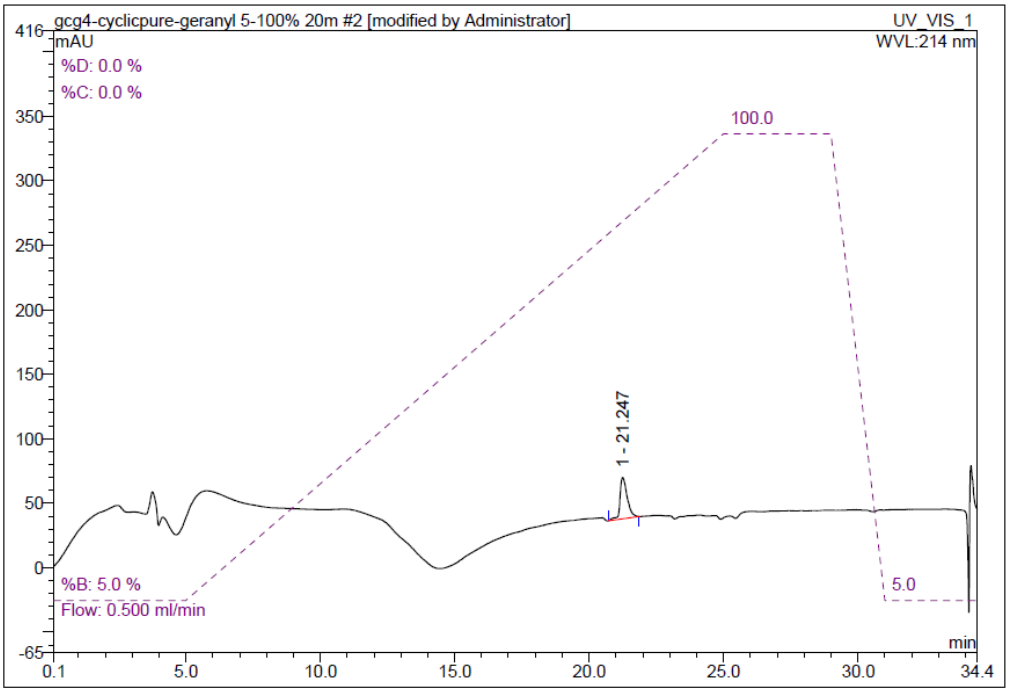


Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
724.4099	724.4068	3.1	4.3	9.5	94.1	0.0	C34 H58 N7 O8 S

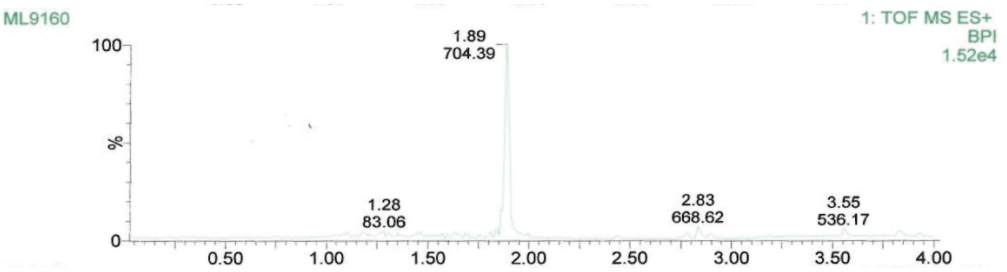
gcg4-488 Cit/Ger
1.104
1.115
1.436
1.468
1.517
1.530
1.653
1.668
1.695
1.823
1.892
1.902
1.966
2.107
2.236
2.241
2.246
2.250
2.255
2.262
2.269
2.281
2.293
2.312
2.323
2.337
2.415
3.082
3.091
3.099
3.104
3.297
3.310
3.324
3.499
3.513
3.528
4.352
4.405
4.423
4.550
4.556
4.569
4.634
4.638
4.643
4.646
4.650
4.656
4.662
4.666
4.698
4.723
4.725
4.732
0.887
0.948
0.805
1.841
1.033
1.097
1.914
1.880
1.752
2.056
1.855
0.623
1.049
2.062
2.670
2.703
2.529
1.062
1.335
3.244
3.186
1.125
6.000
p
0
1
2
3
4
5
6
7
8
9
10
11


NAME gcg4_488
EXPNO 2
PROCNO 1
Date_ 20161107
Time_ 20.28
INSTRUM spect
PROBHD 5 mm PABBO BB
PULPROG mlevph
TD 2048
SOLVENT D2O
NS 4
DS 16
SWH 2049.180 Hz
FIDRES 1.000576 Hz
AQ 0.4997620 sec
RG 203
DW 244.000 usec
DE 6.50 usec
TE 298.0 K
D0 0.00023347 sec
D1 2.67068195 sec
D9 0.06000000 sec
D12 0.00002000 sec
IN0 0.00048800 sec
L1 24
CHANNEL f1
NUC1 1H
P1 10.25 usec
P5 23.34 usec
P6 35.00 usec
P7 70.00 usec
P17 2500.00 usec
PL1 0.00 dB
PL10 10.67 dB
PL1W 22.85816574 W
PL10W 1.95903134 W
SFO1 500.1313979 MHz
ND0 1
TD 128
SFO1 500.1314 MHz
FIDRES 16.009222 Hz
SW 4.097 ppm
FnMODE States-TPPI
SI 1024
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 States-TPPI
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
gcg4-488 Cit/Ger

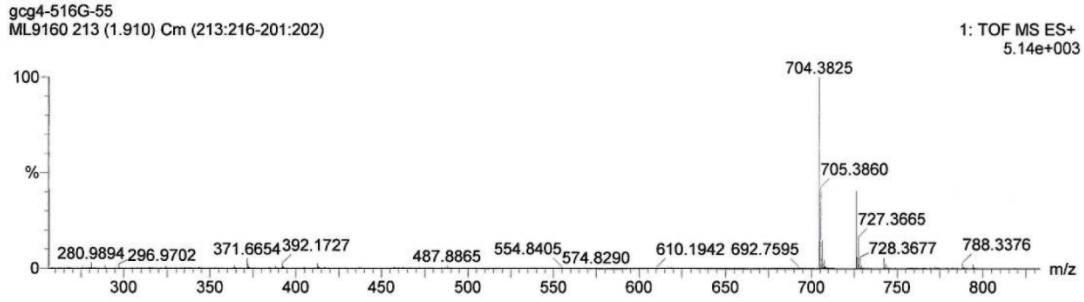
His_{10}-geranylbactin (12)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	21.25	n.a.	31.985	9.964	100.00	n.a.	BMB
Total:			31.985	9.964	100.00	0.000	

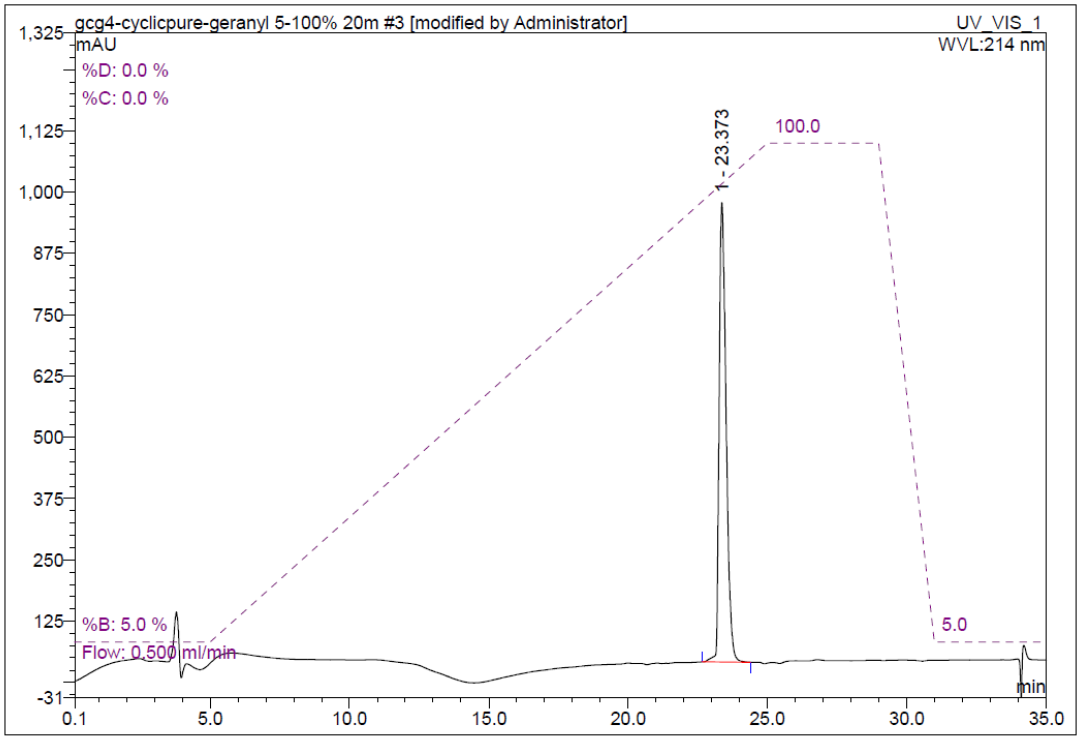


Monoisotopic Mass, Even Electron Ions
821 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 34-34 H: 0-180 N: 0-30 O: 0-30 S: 1-1

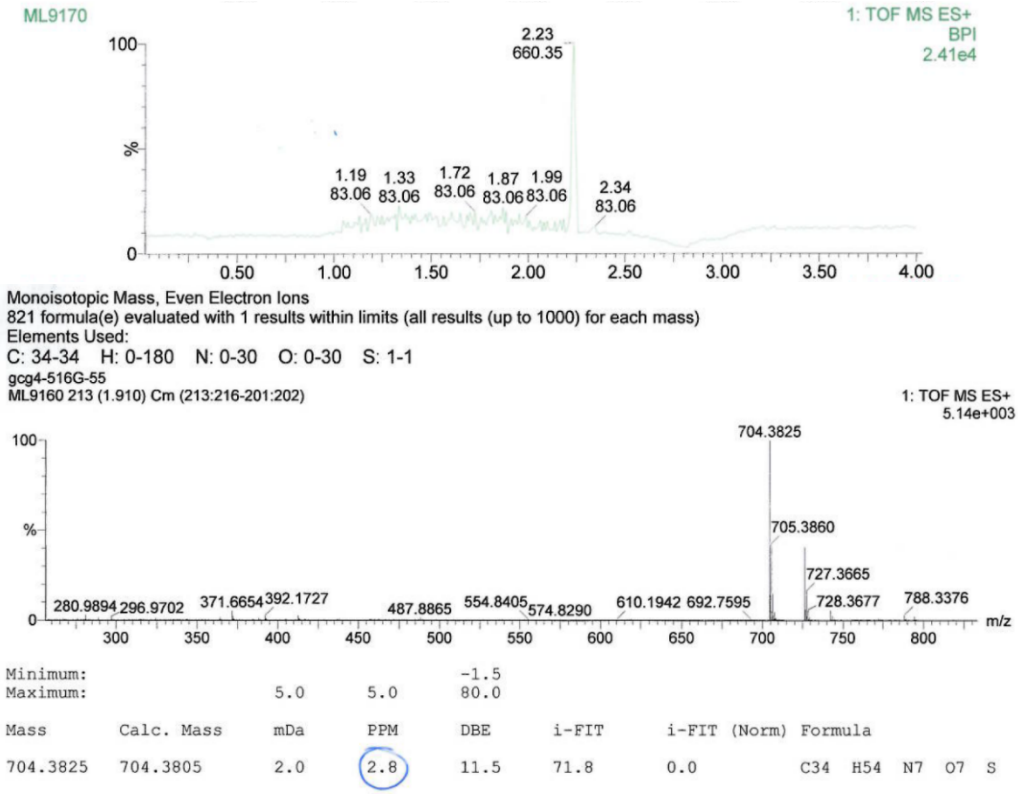


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
704.3825	704.3805	2.0	2.8	11.5	71.8	0.0	C34 H54 N7 O7 S	

Ala_{10}-geranylbactin (13)

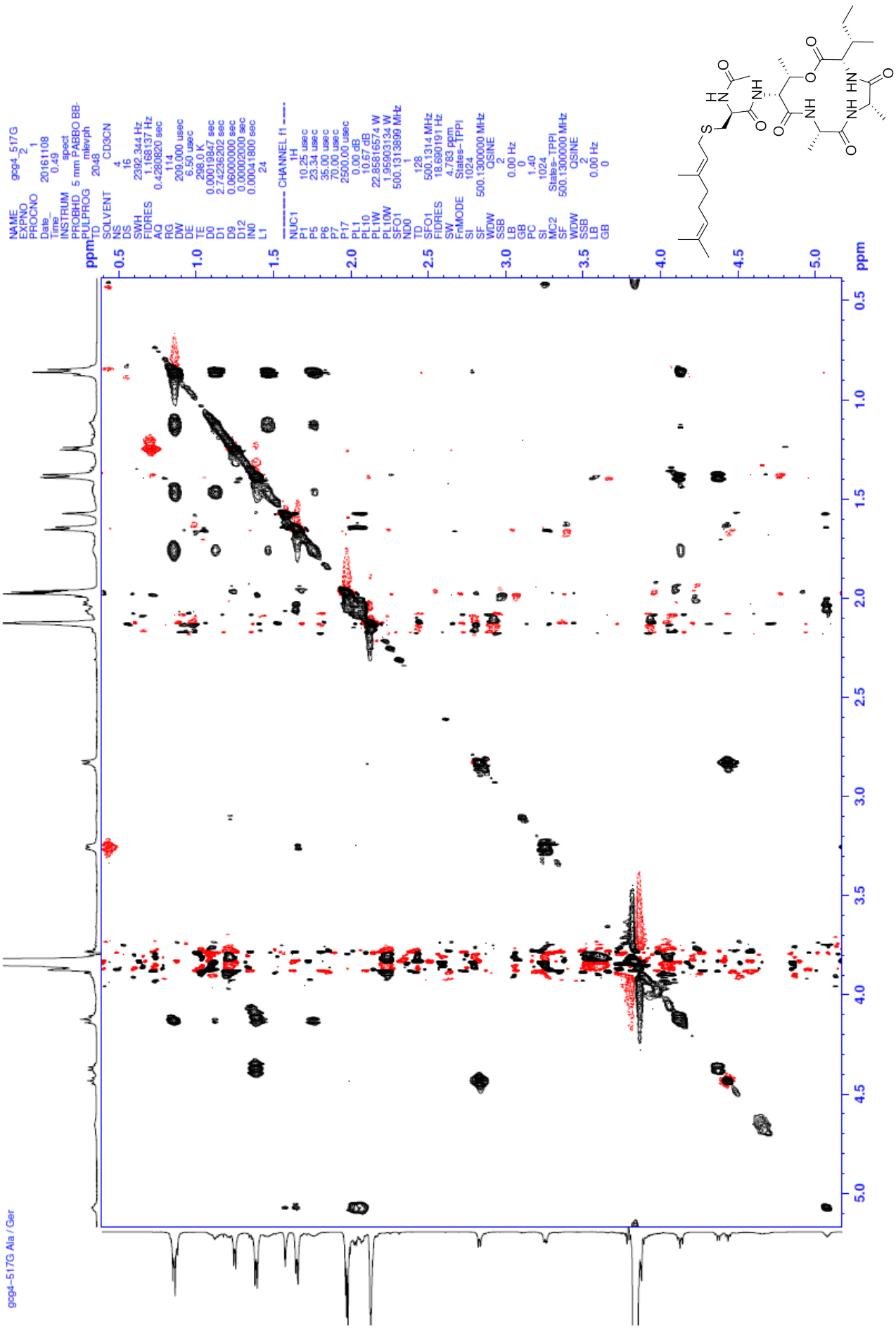


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	23.37	n.a.	937.542	273.184	100.00	n.a.	BMB
Total:			937.542	273.184	100.00	0.000	

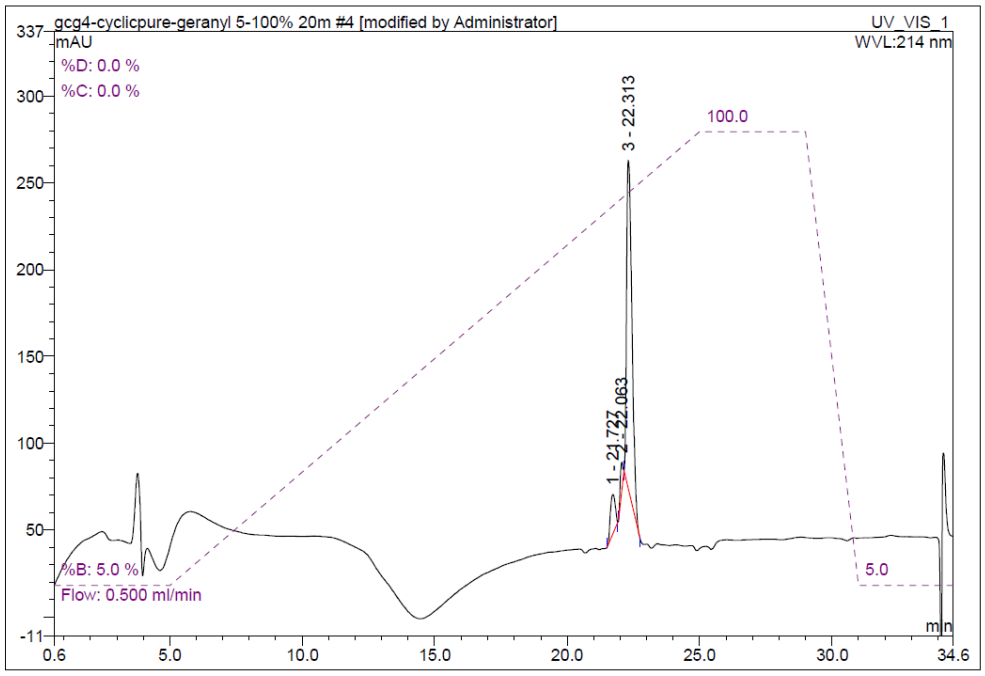


Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
704.3825	704.3805	2.0	2.8	11.5	71.8	0.0	C34 H54 N7 O7 S

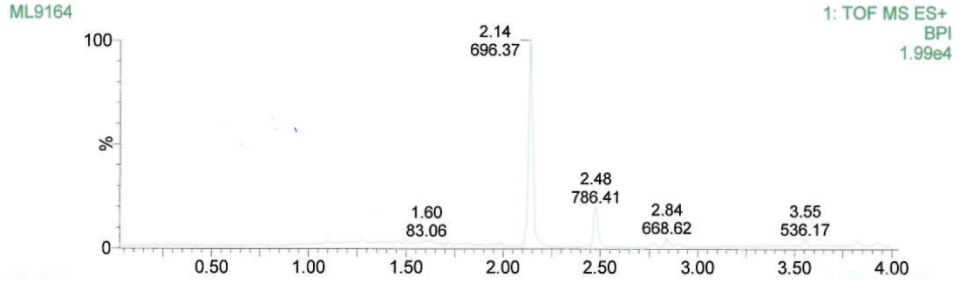
gcg4-517G Ala / Ger
ppm
0 1 2 3 4 5 6 7 8 9 10 11
0.831
0.848
0.862
0.877
1.112
1.117
1.123
1.220
1.241
1.254
1.343
1.357
1.378
1.393
1.574
1.643
1.657
1.960
1.965
1.970
1.975
1.980
1.994
2.007
2.021
2.034
2.053
2.065
2.075
2.078
2.081
2.123
2.127
2.821
2.835
3.245
3.250
3.260
3.265
3.779
3.782
3.785
3.799
3.831
3.871
3.875
3.888
3.896
3.904
4.107
4.122
4.140
4.364
4.378
4.433
1.962
2.145
13.415
2.067
2.189
9.990
1.179
6.286
2.645
1.754
7.380
3.846
3.227
7.852
2.476
1.195
1.048
1.041
0.859
1.040
1.186


gcg4-517G Ala / Ger
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

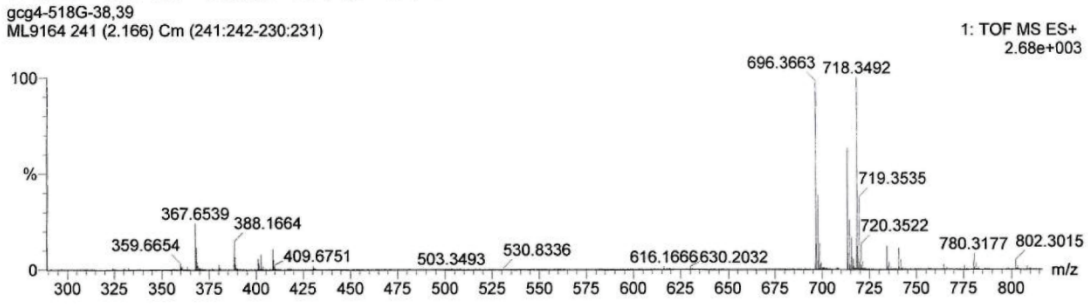
Glu_{10}-geranybactin (14)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	21.73	n.a.	22.725	4.892	9.50	n.a.	BMB
2	22.06	n.a.	15.595	1.877	3.64	n.a.	BMb
3	22.31	n.a.	190.040	44.735	86.86	n.a.	bMB
Total:			228.360	51.503	100.00	0.000	

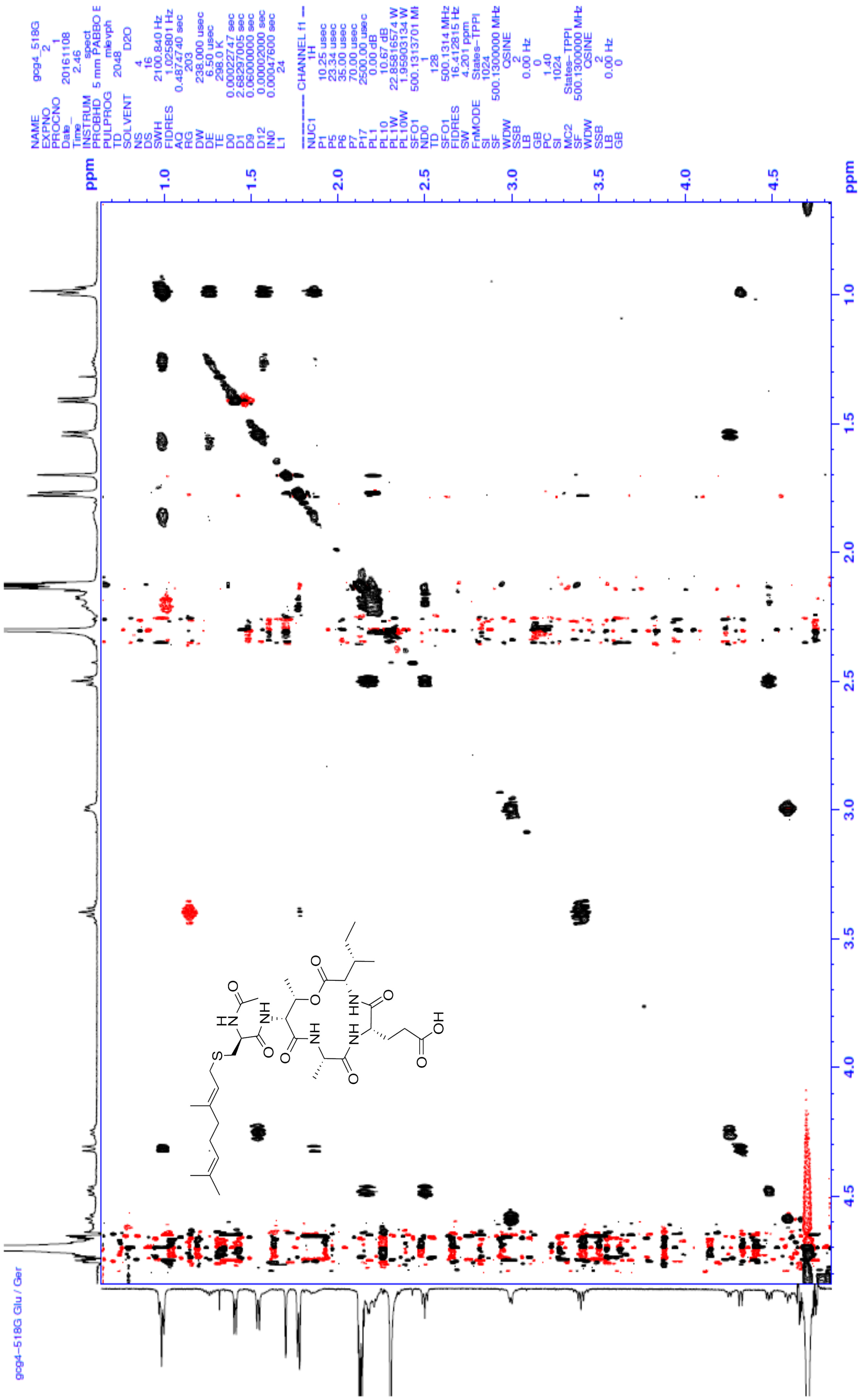


Monoisotopic Mass, Even Electron Ions
814 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 33-33 H: 0-180 N: 0-30 O: 0-30 S: 1-1

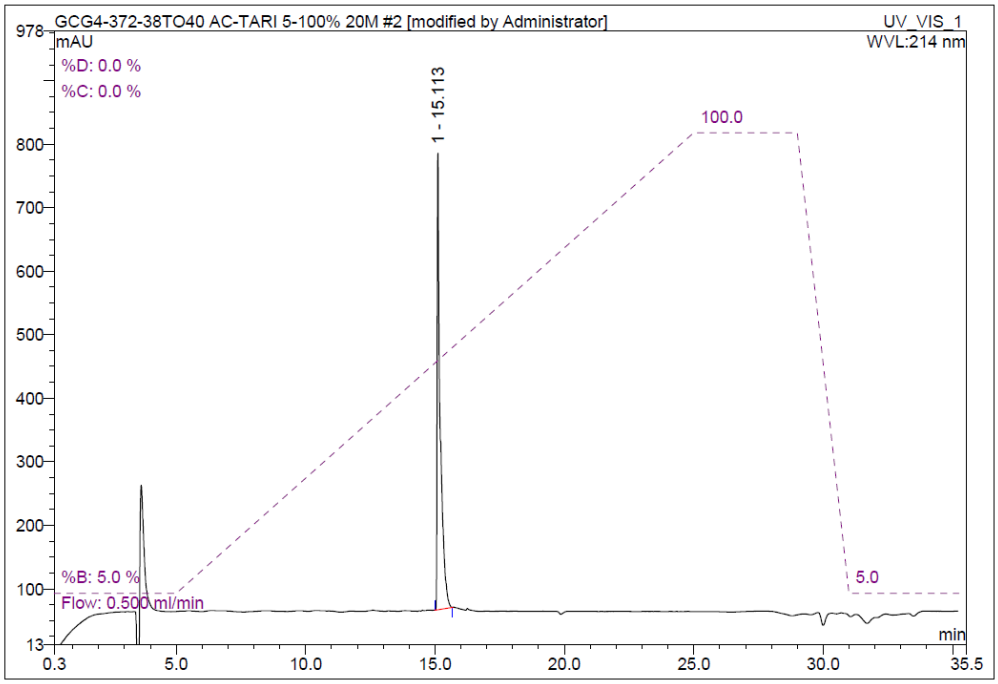


Minimum:				-1.5			
Maximum:		5.0	5.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
696.3663	696.3642	2.1	3.0	9.5	57.0	0.0	C33 H54 N5 O9 S

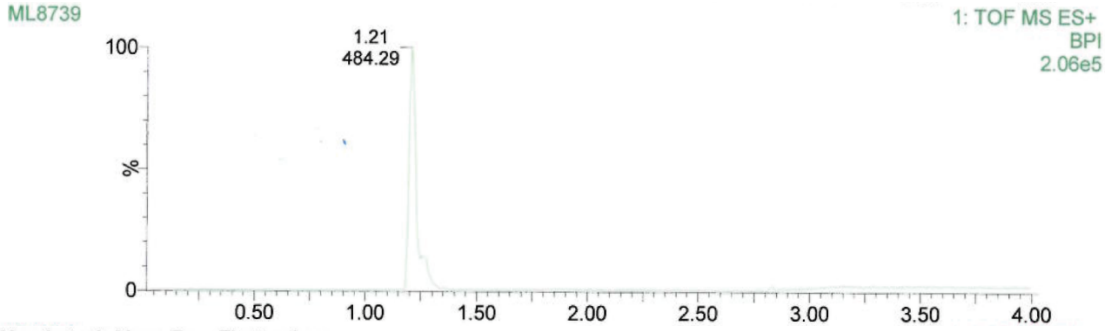
gcg4-518G Glu / Ger
ppm
0.971
0.986
1.000
1.319
1.402
1.416
1.534
1.548
1.700
1.768
1.780
2.117
2.122
2.127
2.132
2.137
2.141
2.150
2.163
2.173
2.181
2.190
2.209
2.219
2.302
2.486
2.500
2.515
2.989
3.003
3.381
3.396
3.412
4.306
4.324
4.463
4.476
4.482
4.495
4.587
4.604
4.639
4.654
4.658
4.666
4.672
4.679
4.700
4.724
4.725
4.726
4.731
4.736
4.744
4.749
4.753
1.050
1.014
0.894
1.774
1.414
1.213
1.182
2.162
1.886
2.000
2.588
2.842
1.031
6.000
2.778
0.835
3.346
3.096
1.109
6.504


gcg4-518G Glu / Ger
NAME gcg4_518G
EXPNO 2
PROCNO 1
Date_ 20161108
Time 2.46
INSTRUM spect
PROBHD 5 mm PABBO B
PULPROG mlevph
TD 2048
SOLVENT D2O
NS 4
DS 16
SWH 2100.840 Hz
FIDRES 1.025801 Hz
AQ 0.487440 sec
RG 203
DW 238.000 usec
DE 6.50 usec
TE 298.0 K
D0 0.00022747 sec
D1 2.68297005 sec
D9 0.06000000 sec
D12 0.00002000 sec
IN0 0.00047600 sec
L1 24
======== CHANNEL f1 ========
NUC1 1H
P1 10.25 usec
P5 23.34 usec
P6 35.00 usec
P7 70.00 usec
P17 2500.00 usec
PL1 0.00 dB
PL10 10.67 dB
PL1W 22.85816574 W
PL10W 1.95903134 W
SFO1 500.1313701 MHz
ND0 1
TD 128
SFO1 500.1314 MHz
FIDRES 16.412815 Hz
SW 4.201 ppm
FnMODE States-TPPI
SI 1024
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 States-TPPI
SF 500.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
ppm

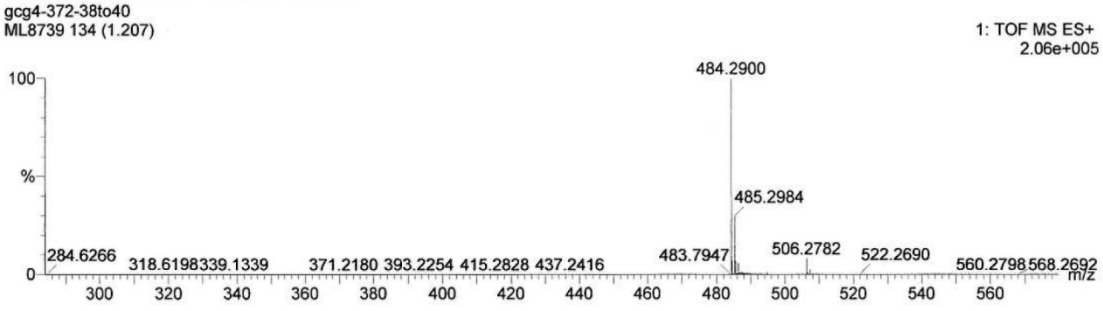
Cyclic Ac-tARI (15)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	15.11	n.a.	718.369	98.279	100.00	n.a.	BMB
Total:			718.369	98.279	100.00	0.000	



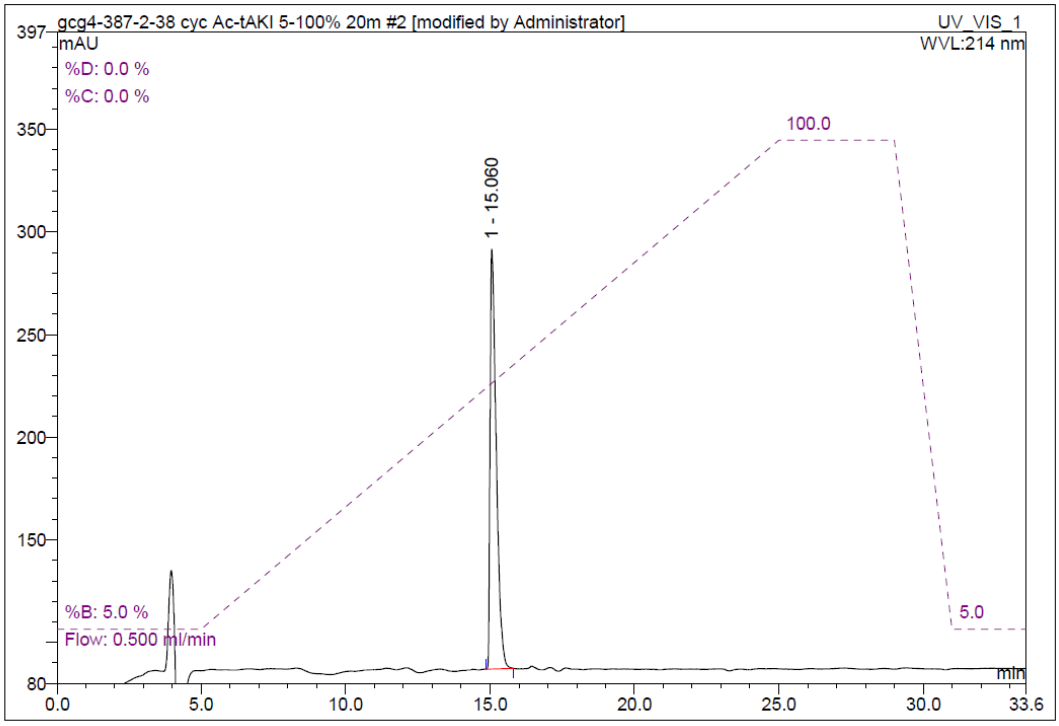
Monoisotopic Mass, Even Electron Ions
541 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 21-21 H: 0-250 N: 0-30 O: 0-30
gcg4-372-38to40
ML8739 134 (1.207)



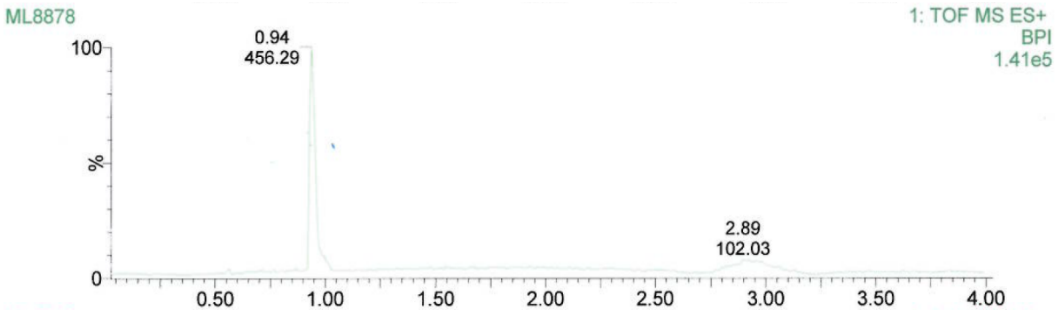
Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
484.2900	484.2884	1.6	3.3	6.5	174.9	0.0	C21 H38 N7 O6

gcg4-372 pure cyc Ac-tARI
ppm
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
1.691
1.000
2.130
2.904
0.988
2.094
1.157
1.036
3.969
2.949
1.113
6.141
0.883
0.902
0.920
0.937
1.188
1.206
1.222
1.242
1.287
1.303
1.464
1.482
1.499
1.509
1.614
1.621
1.631
1.638
1.655
1.696
1.706
1.720
1.809
1.820
1.833
1.843
1.856
1.864
1.879
1.885
1.905
1.920
1.944
2.250
2.850
2.890
3.046
3.241
3.259
3.275
3.290
3.307
4.235
4.252
4.259
4.411
4.431
4.452
4.789
4.836
5.573
5.579
5.590
5.596

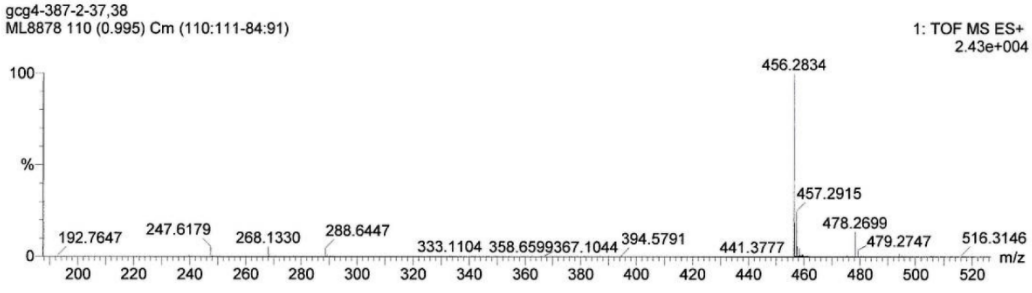
Cyclic Ac-tAKI (16)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	15.06	n.a.	204.544	50.621	100.00	n.a.	BMB
Total:			204.544	50.621	100.00	0.000	

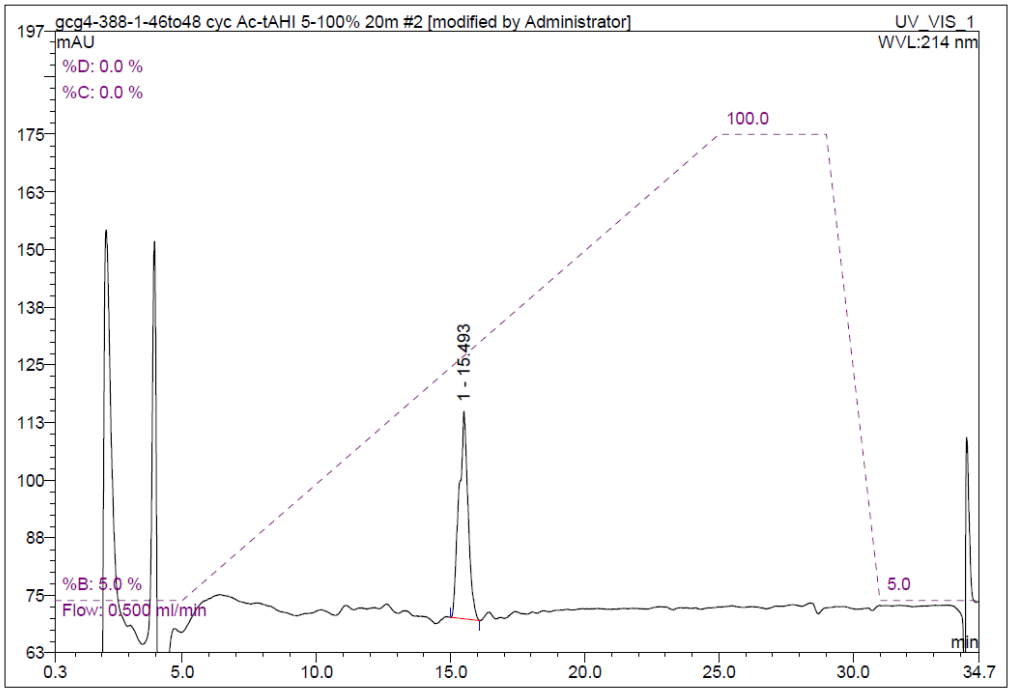


Monoisotopic Mass, Even Electron Ions
420 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 21-21 H: 0-250 N: 0-20 O: 0-30
gcg4-387-2-37,38
ML8878 110 (0.995) Cm (110:111-84:91)

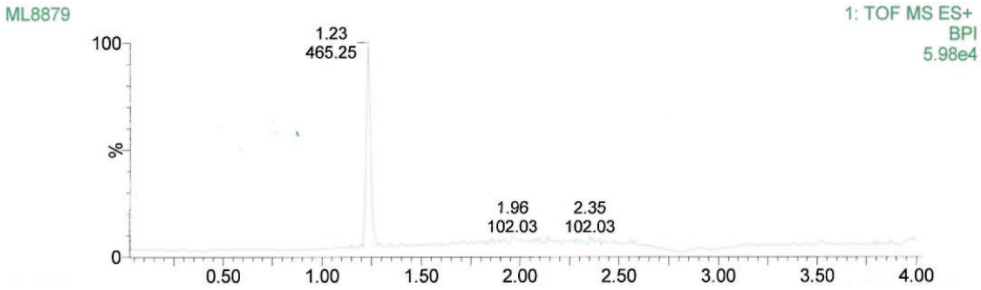


Minimum:				-1.5				
Maximum:		5.0	20.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
456.2834	456.2822	1.2	2.6	5.5	194.1	0.0	C21 H38 N5 O6	

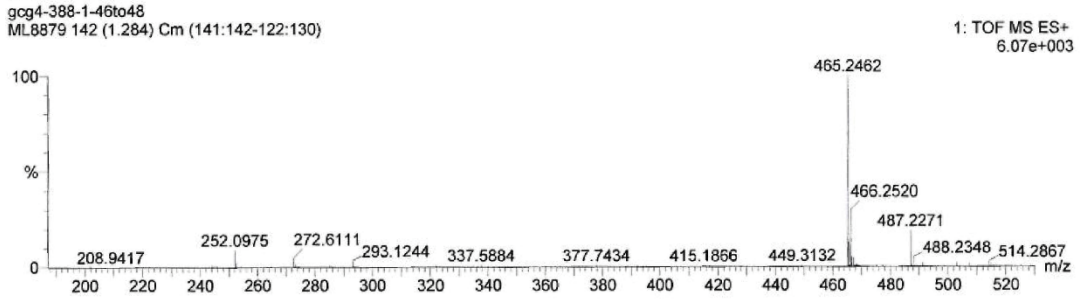
Cyclic Ac-tAHI (17)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	15.49	n.a.	44.920	17.648	100.00	n.a.	BMB
Total:			44.920	17.648	100.00	0.000	



Monoisotopic Mass, Even Electron Ions
434 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 21-21 H: 0-250 N: 0-20 O: 0-30
gcg4-388-1-46to48
ML8879 142 (1.284) Cm (141:142-122:130)



Minimum:				-1.5			
Maximum:		5.0	20.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
465.2462	465.2462	0.0	0.0	8.5	121.4	0.0	C21 H33 N6 O6

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays

Bacterial strains
Two laboratory reference strains were used for the antimicrobial susceptibility testing. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 strains were retrieved from the -80°C frozen stock in the microbial genetics laboratory (University Of Leicester).

Microdilution method for susceptibility testing to antimicrobials
Initial preparation of Mueller–Hinton broth (MHB) medium containing double concentration of the maximum concentration of the antimicrobial compound to be tested was made (128 µg/ml was made to start with 64 µg/ml). 200 µl was aliquoted into the first column of a 96 well plate and two-fold serial dilutions of the compound were performed.

MIC and MBC Assays
The Minimum inhibitory concentration (MIC) of Teixobactin and its analogues was performed as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines using the broth microdilution method.[1] Briefly, each strain was streaked for a single colony on Mueller–Hinton agar plates (Oxoid Ltd., Basingstoke, UK) and incubated at 37°C for 24 hours. Next day, a starting inocula of 1×10^5 CFU/ml of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 strains were aliquoted into 96-well plates containing serial dilutions of the Teixobactin or analogues in the range 64 to 0.125 µg/ml using Mueller-Hinton broth (Oxoid Ltd., Basingstoke, UK). Results were read with a Jenway (6705UV/Vis.) spectrophotometer after 18 hours of incubation at 37 ˚C. Minimum bactericidal concentration (MBC) was determined by subculturing 10 µl from each well without visible bacterial growth onto Mueller–Hinton agar plates (Oxoid Ltd., Basingstoke, UK). After 24 h of incubation at 37°C, the dilution yielding three colonies or less was scored as the MBC.

Reference: CLSI. Methods for Dilution Antimicrobial Susceptibility Testing For Bacteria That Grow Aerobically. Approved Standard M7-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2009