

## ***Supplementary data***

### **Roseabol A, a New Peptaibol from the Fungus *Clonostachys rosea***

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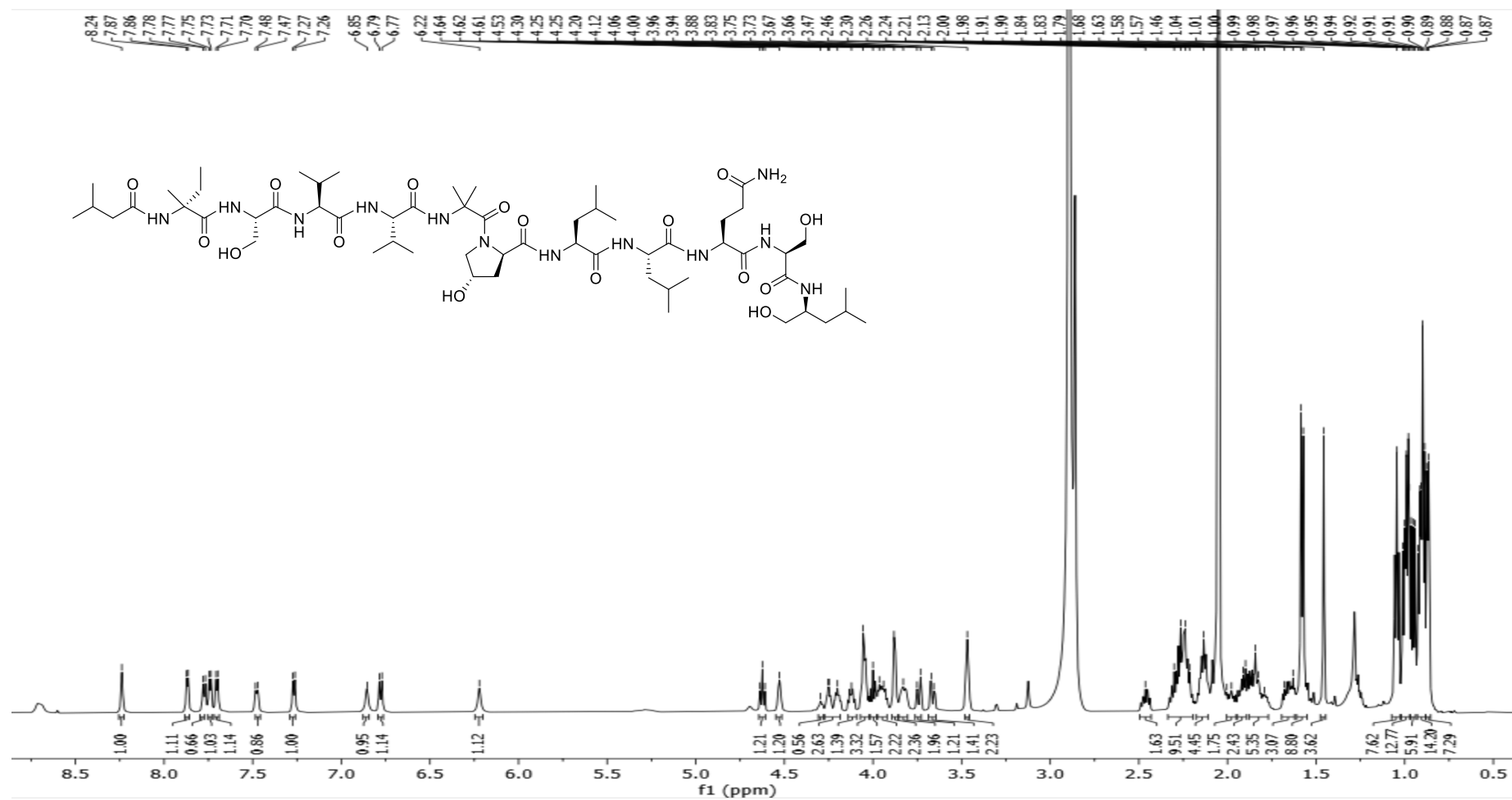
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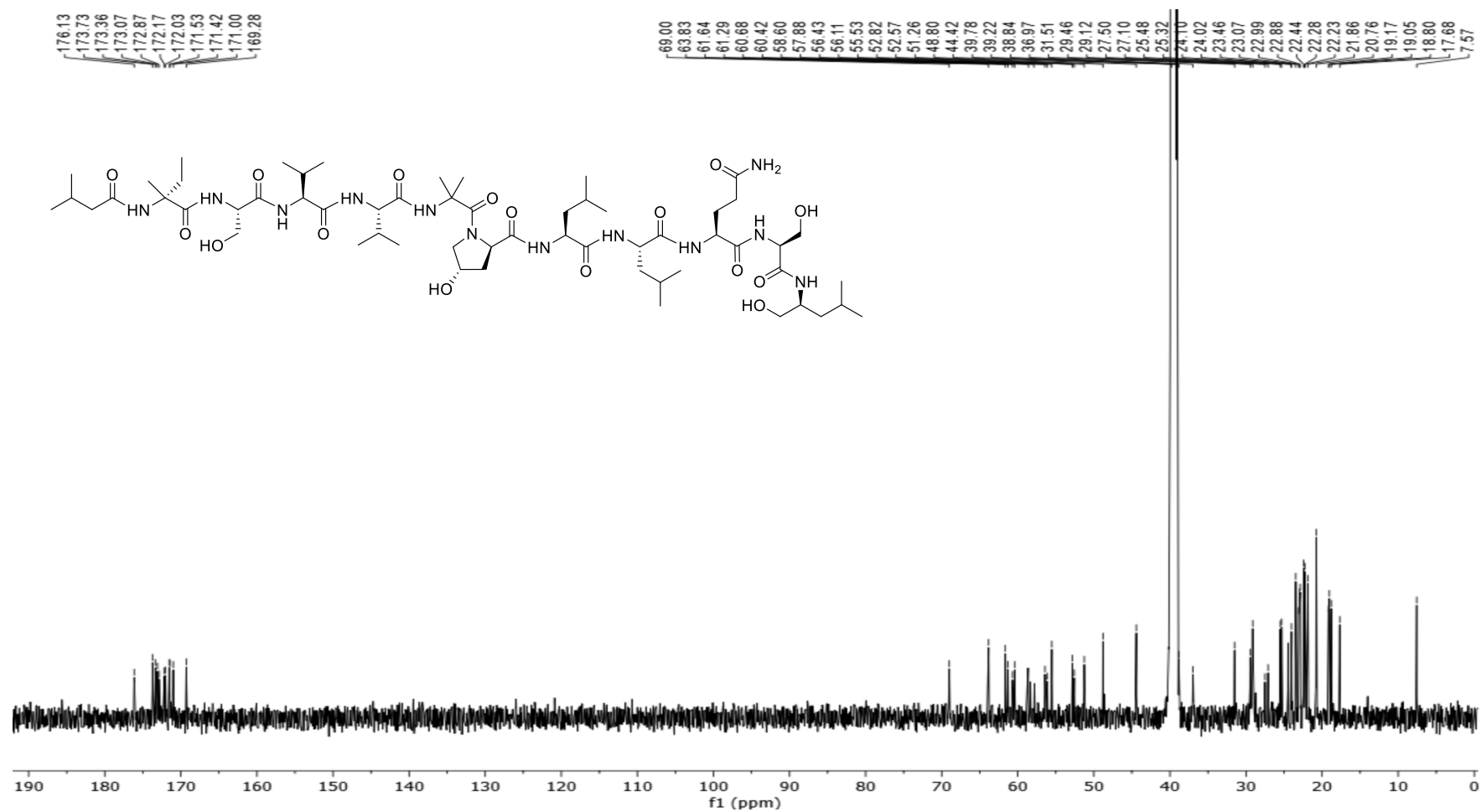
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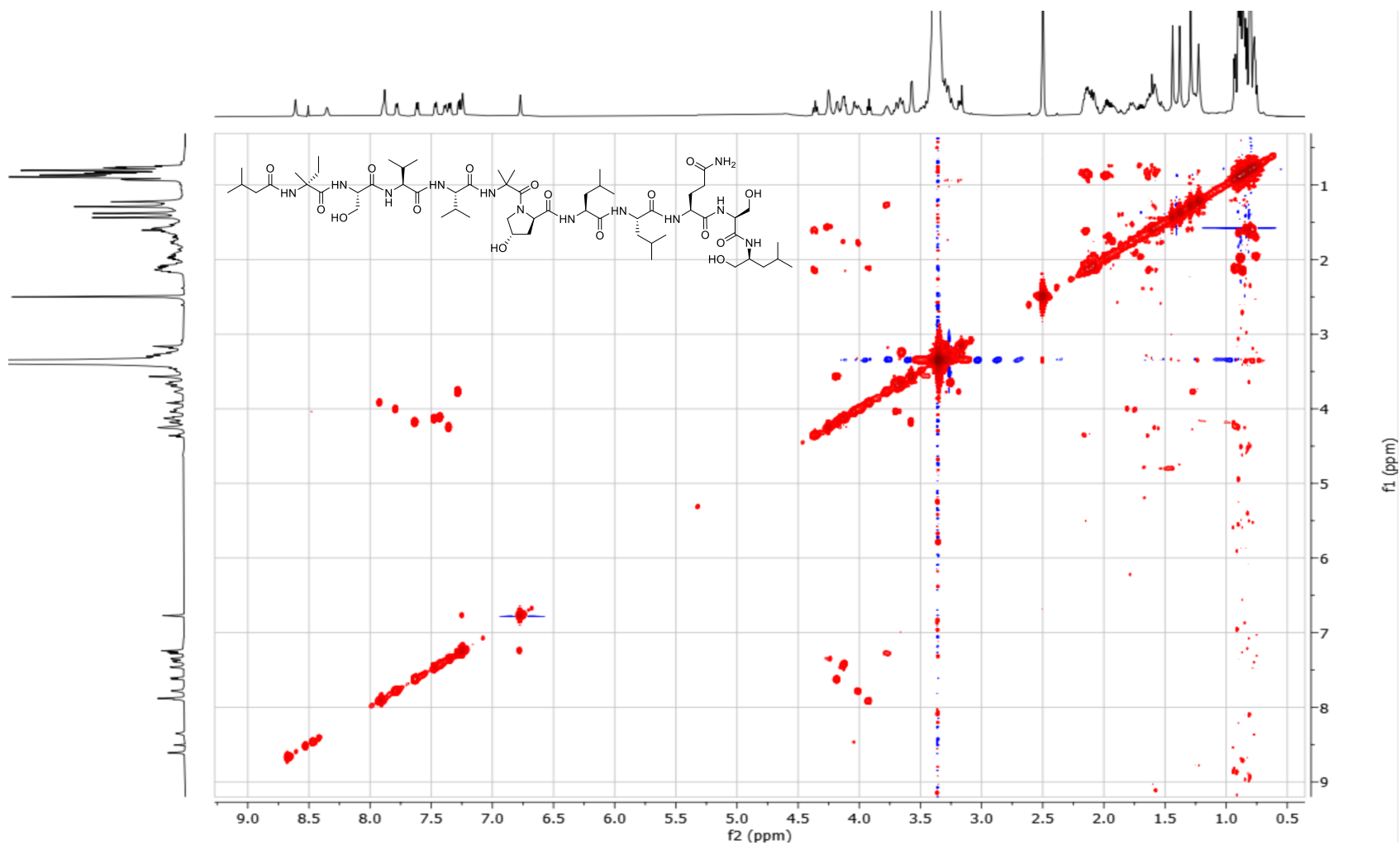


**Figure S2.**  $^1\text{H}$  NMR (600 MHz,  $\text{Acetone-}d_6$ ) spectrum of **1**

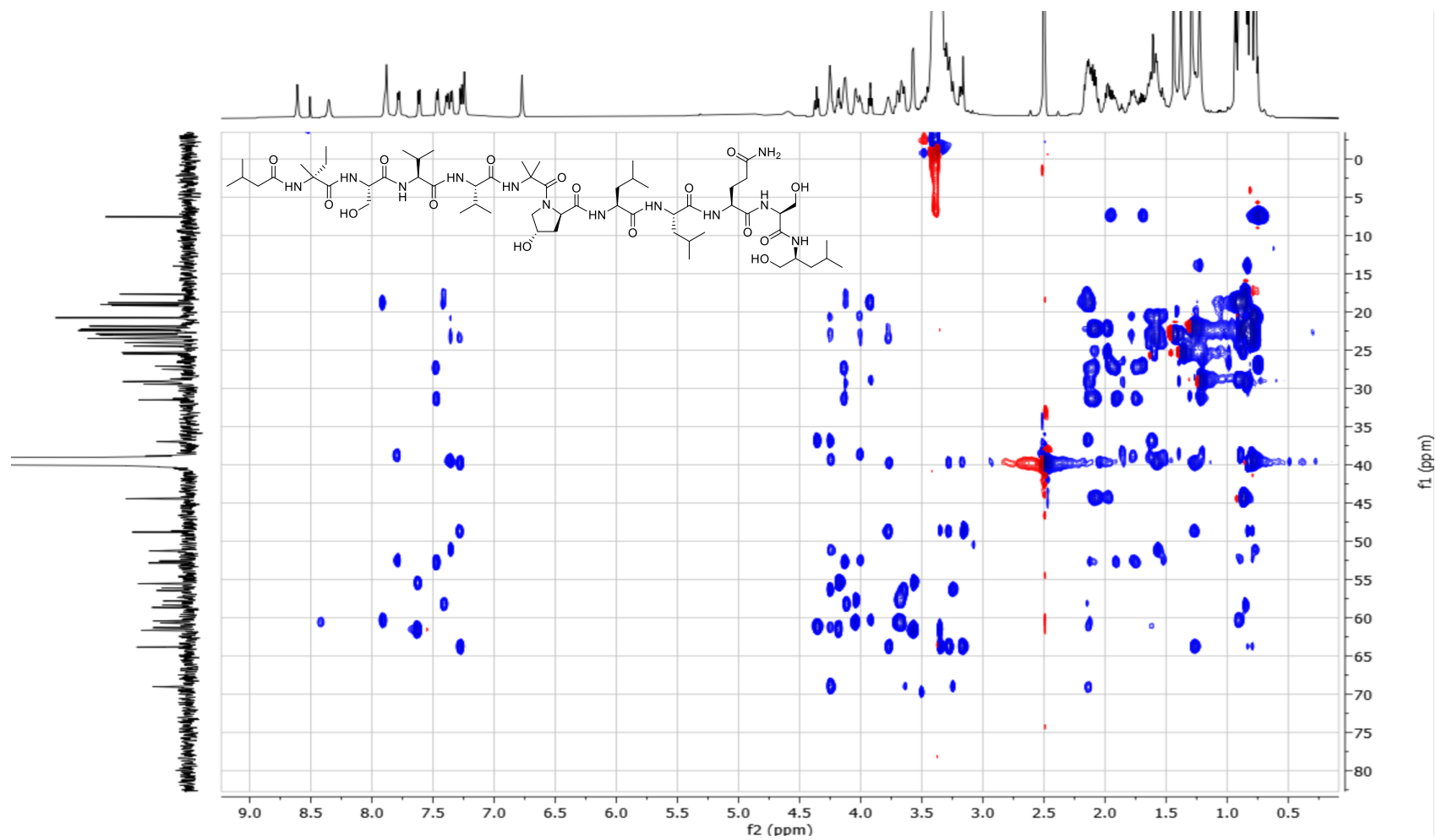


**Figure S3.**  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO}-d_6$ ) spectrum of **1**



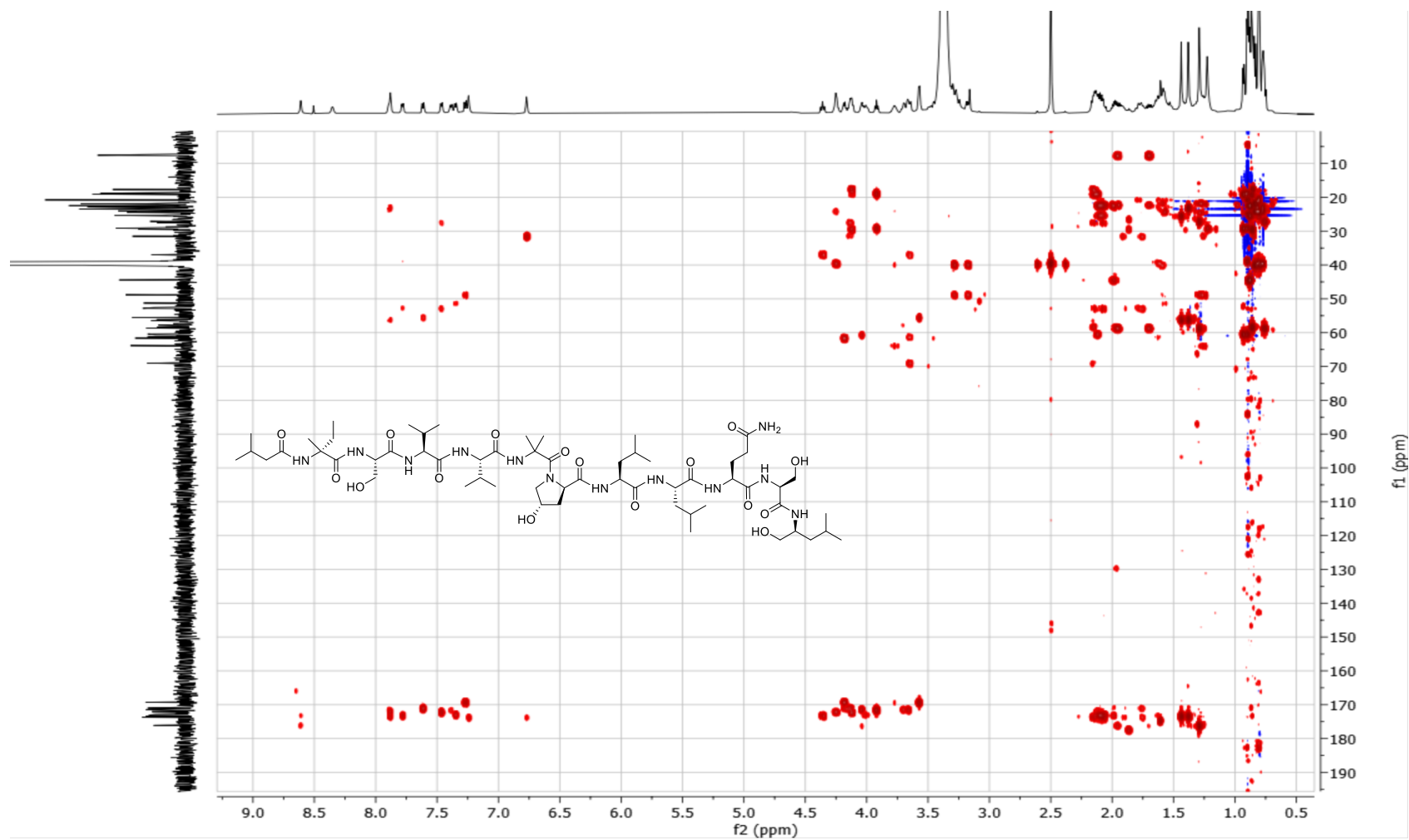


**Figure S5.** COSY NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of **1**

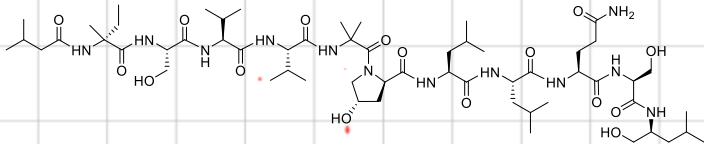


**Figure S6.** HSQC-TOCSY NMR (600 MHz, DMSO- $d_6$ ) spectrum of **1**





**Figure S7.** HMBC NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of **1**



**Figure S8.** ROESY NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of **1**

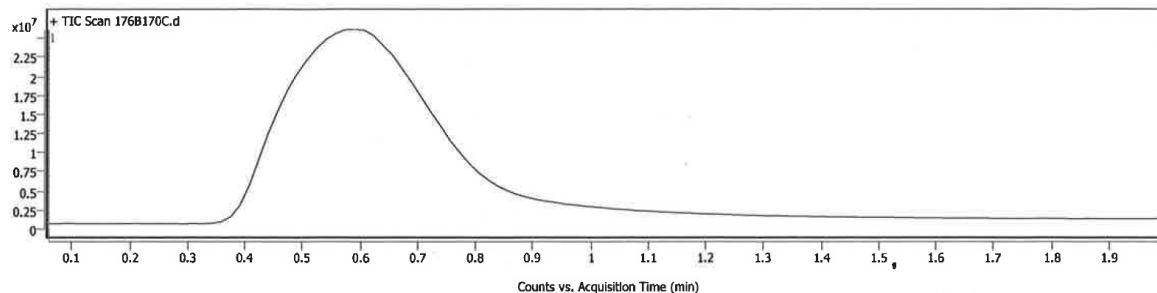
# Analysis Report



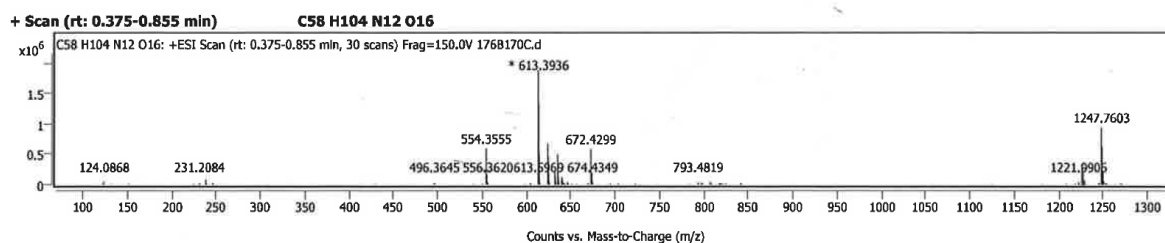
## Sample Information

<b>Name</b>	1768170C	<b>Data File Path</b>	C:\Users\bokeschh\Desktop\020620\1768170C.d
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<b>Instrument</b>	Instrument 1	<b>Method Path (Acq)</b>	C:\Users\admin\Desktop\methods\FIA_SM_LowFlow.m
<b>MS Type</b>	QTOF	<b>Version (Acq SW)</b>	6200 series TOF/6500 series Q-TOF B.09.00 (B9044.1 SP1)
<b>Inj. Vol. (ul)</b>	1	<b>IRM Status</b>	Success
<b>Position</b>	Vial 1	<b>Method Path (DA)</b>	
<b>Plate Pos.</b>		<b>Target Source Path</b>	
<b>Operator</b>		<b>Result Summary</b>	

## Sample Chromatograms



## Sample Spectra



## Spectrum Identification Table

Best ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	MFG	C58 H104 N12 O16	(M+H)+	1225.7773	0.29		99.30			99.30	

MassHunter Qual 10.0  
(End of Report)

Figure S9. HRESI-MS data for 1

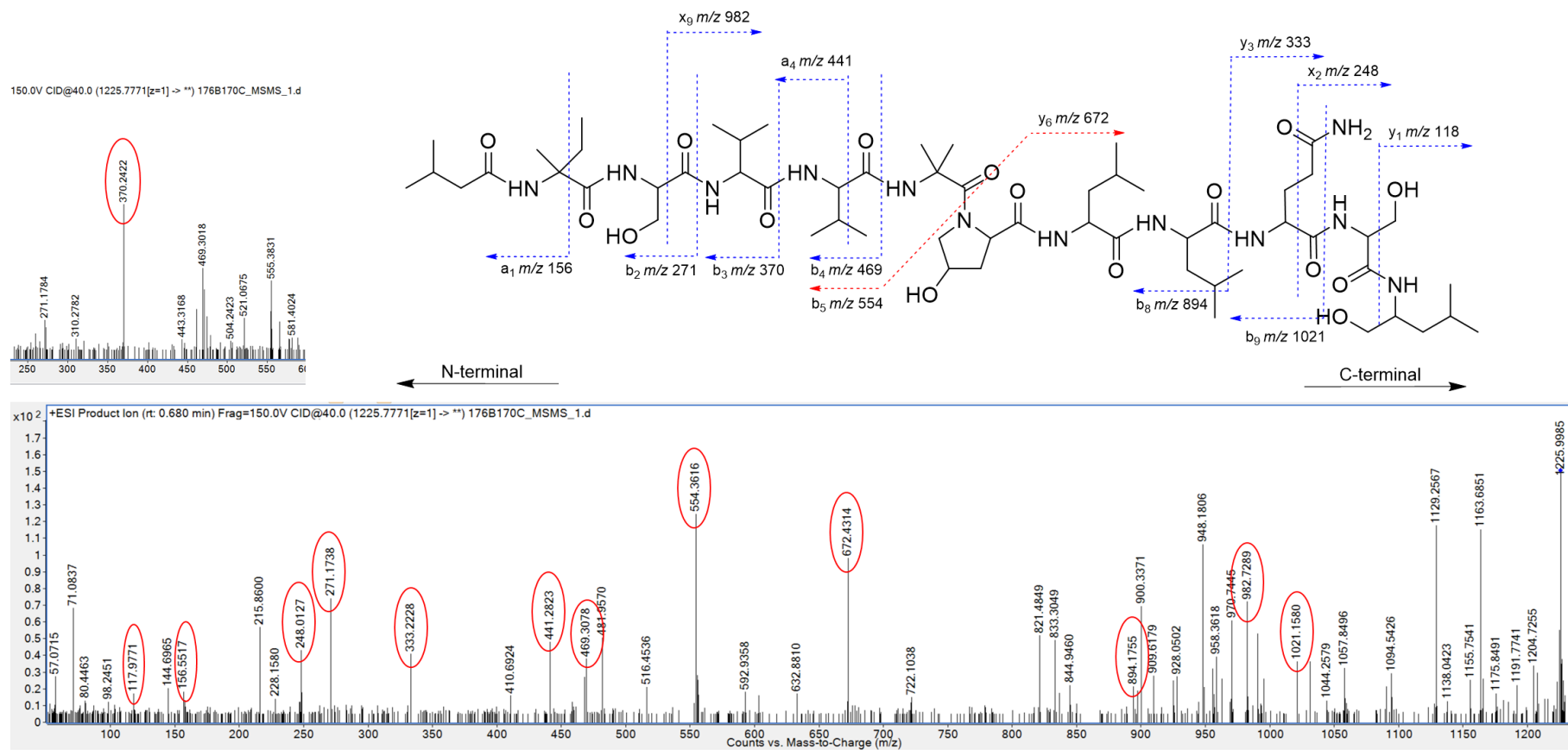
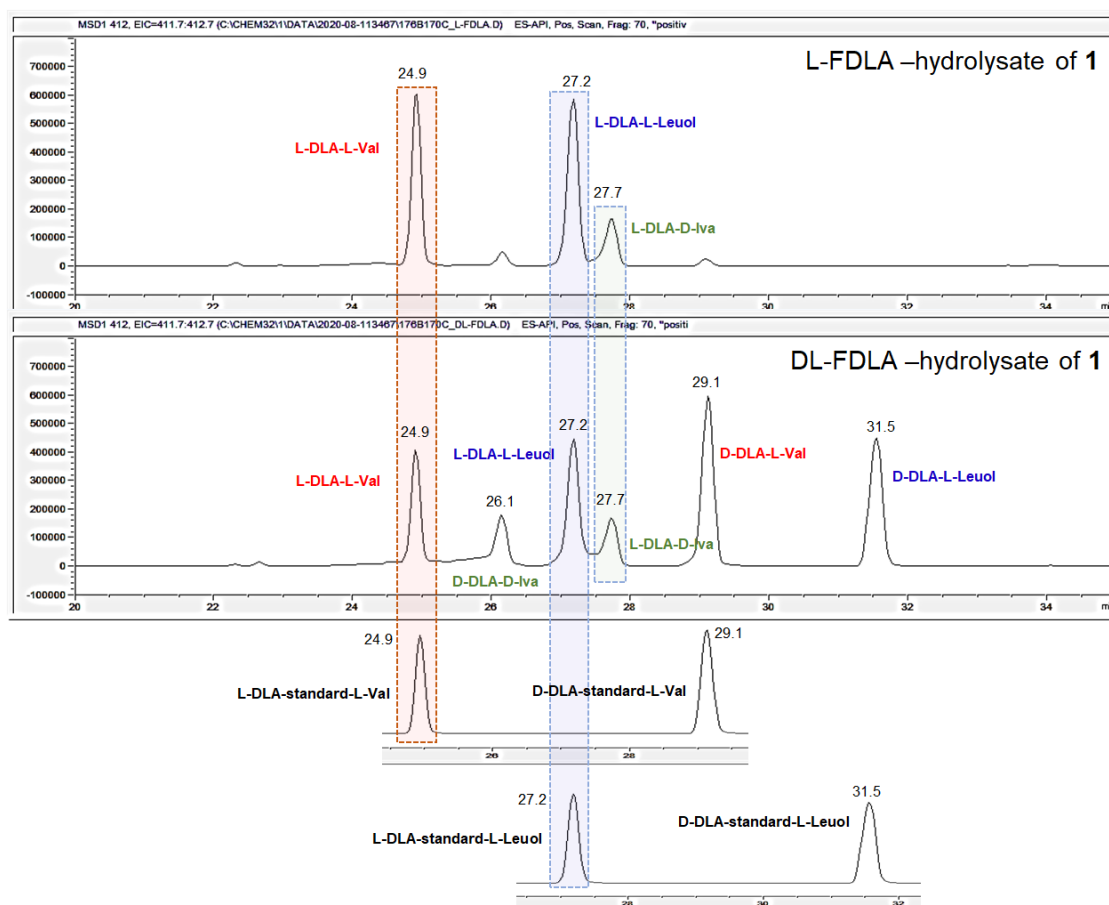
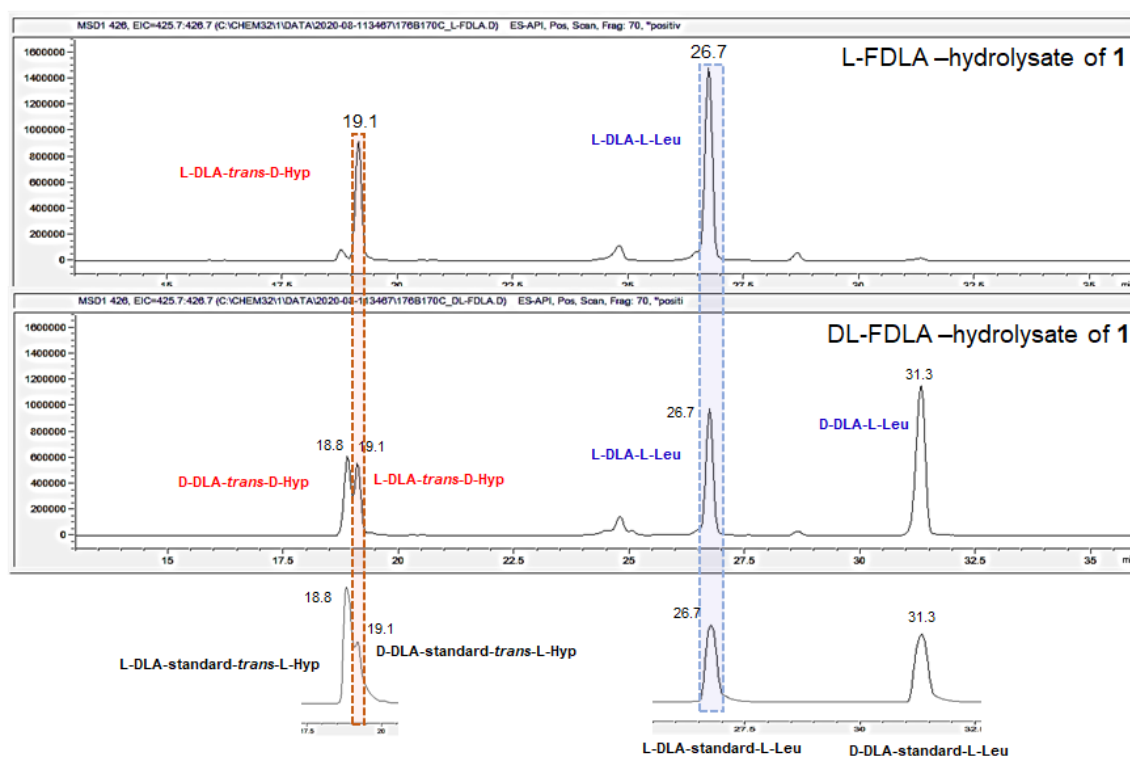


Figure S10. HR ESI-MS/MS fragmentations for **1**

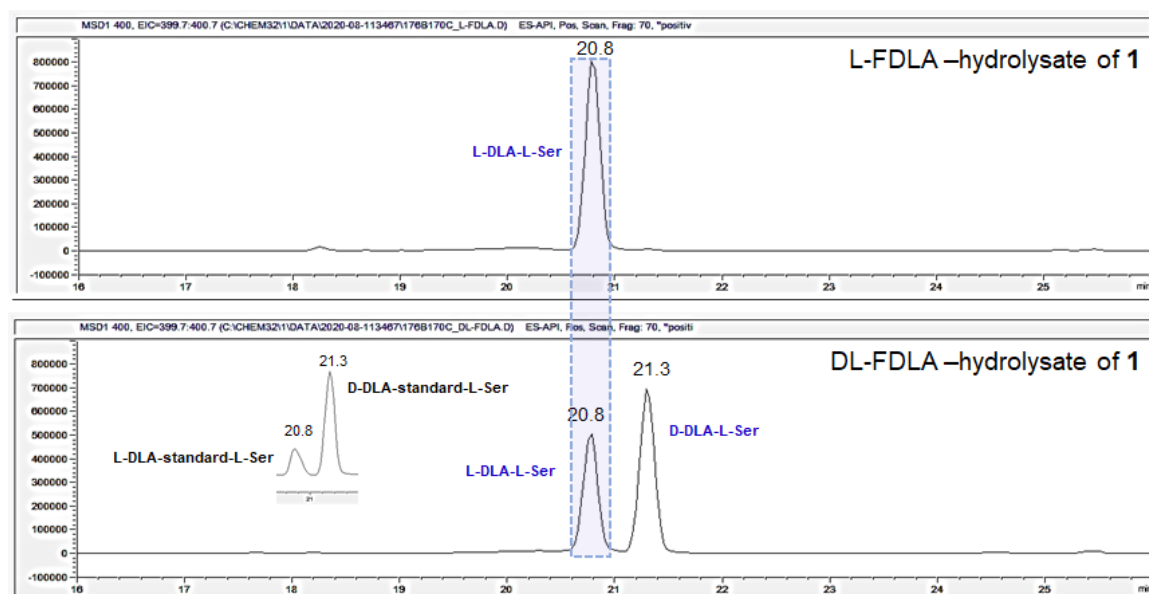
## Advanced Marfey's analysis of **1**



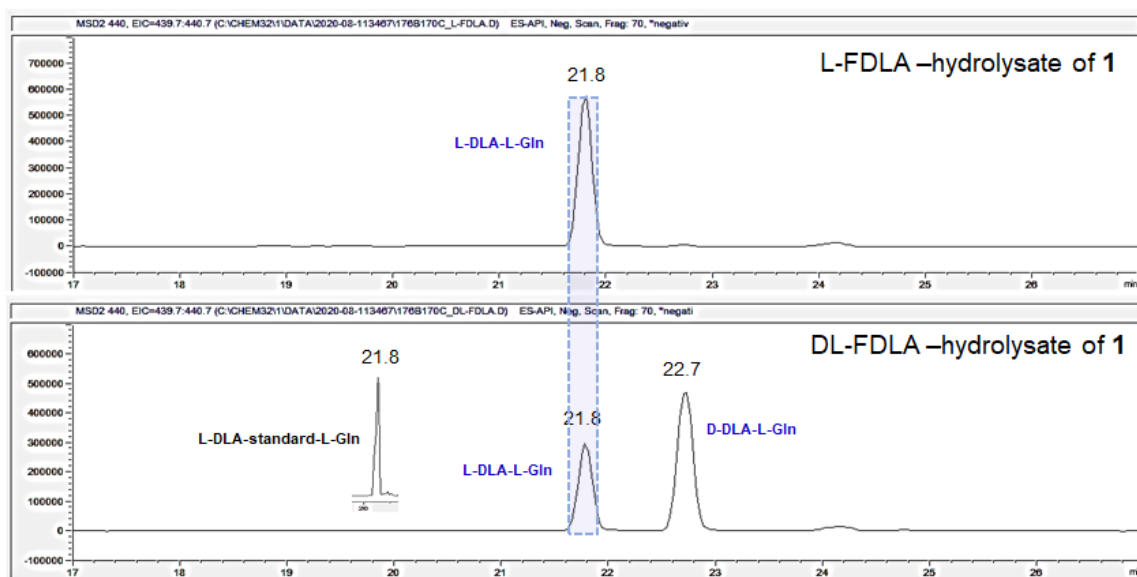
**Figure S11.** Ion chromatograms of the L-FDLA derivatives (top) and the D/L-FDLA derivatives (middle) of the hydrolysis product of **1** and the D/L-FDLA derivatives of the standard L-Val and L-Leuol (bottom) in positive ion mode monitoring at  $m/z$  412 for Iva, Val, and Leuol residues.



**Figure S12.** Ion chromatograms of the L-FDLA derivatives (top) and the D/L-FDLA derivatives (middle) of the hydrolysis product of **1** and the D/L-FDLA derivatives of the standard *trans*-L-Hyp and L-Leu (bottom) in positive ion mode monitoring at  $m/z$  426 for *trans*-Hyp and Leu residues.



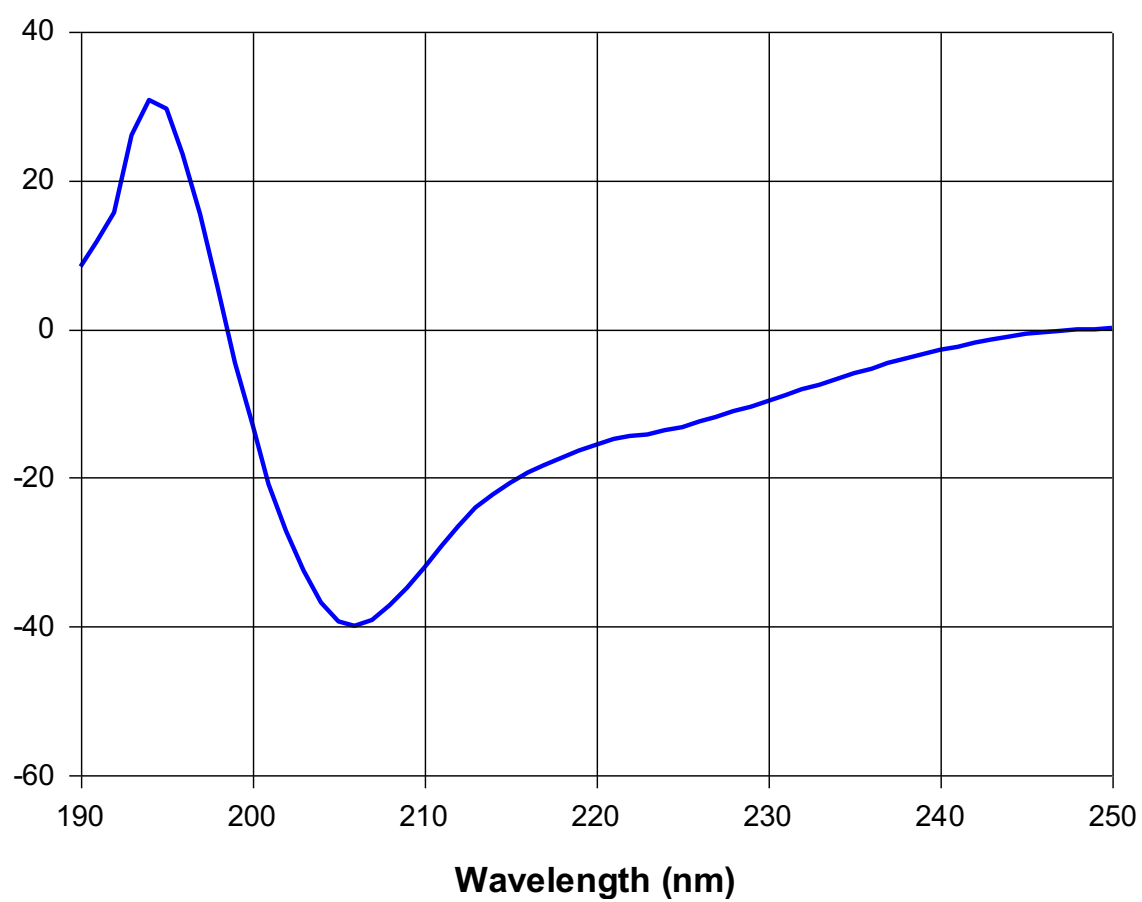
**Figure S13.** Ion chromatograms of the L-FDLA derivatives (top) and the D/L-FDLA derivatives (bottom) of the hydrolysis product of **1** and the D/L-FDLA derivatives of the standard L-Ser in positive ion mode monitoring at  $m/z$  400 for Ser residue.

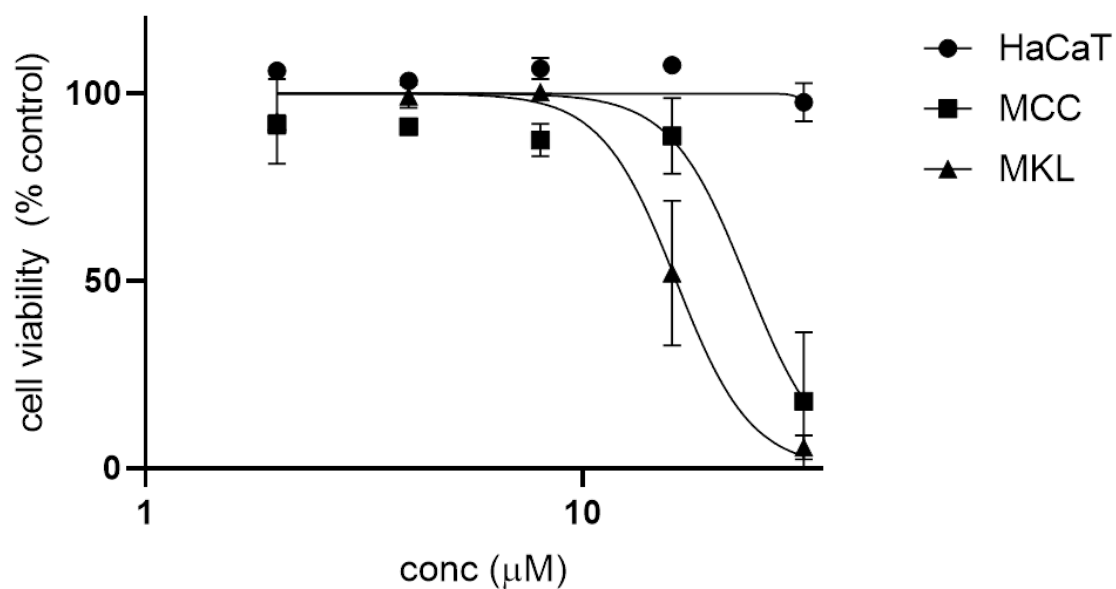


**Figure S14.** Ion chromatograms of the L-FDLA derivatives (top) and the D/L-FDLA derivatives (bottom) of the hydrolysis product of **1** and the D/L-FDLA derivatives of the standard L-Gln in negative ion mode monitoring at  $m/z$  440 for Gln residue.



**Figure S15.** ECD spectrum of roseabol A (**1**)





**Figure S16.** Merkel cell carcinoma cytotoxic activity for compound **2** assessed against the MKL-1 (solid triangles) and MCC26 (open circles) cell lines, with HaCaT cells (solid circles) as a noncancerous control. Bortezomib was the positive control with an  $IC_{50}$  of  $13.1 \pm 5.8$  nM for the three cell lines. Samples were tested in duplicate and the compound purity was assessed as  $> 90\%$  by HPLC, NMR, and MS analysis.