SUPPORTING INFORMATION

Chemical Synthesis of Peptidoglycan Mimetic – Disaccharide-Tetrapeptide Conjugate and its Hydrolysis with Bacteriophage T5, RB43 and RB49 L-Alanyl-D-Glutamate Peptidases

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1 NMR spectra and HPLC chromatograms

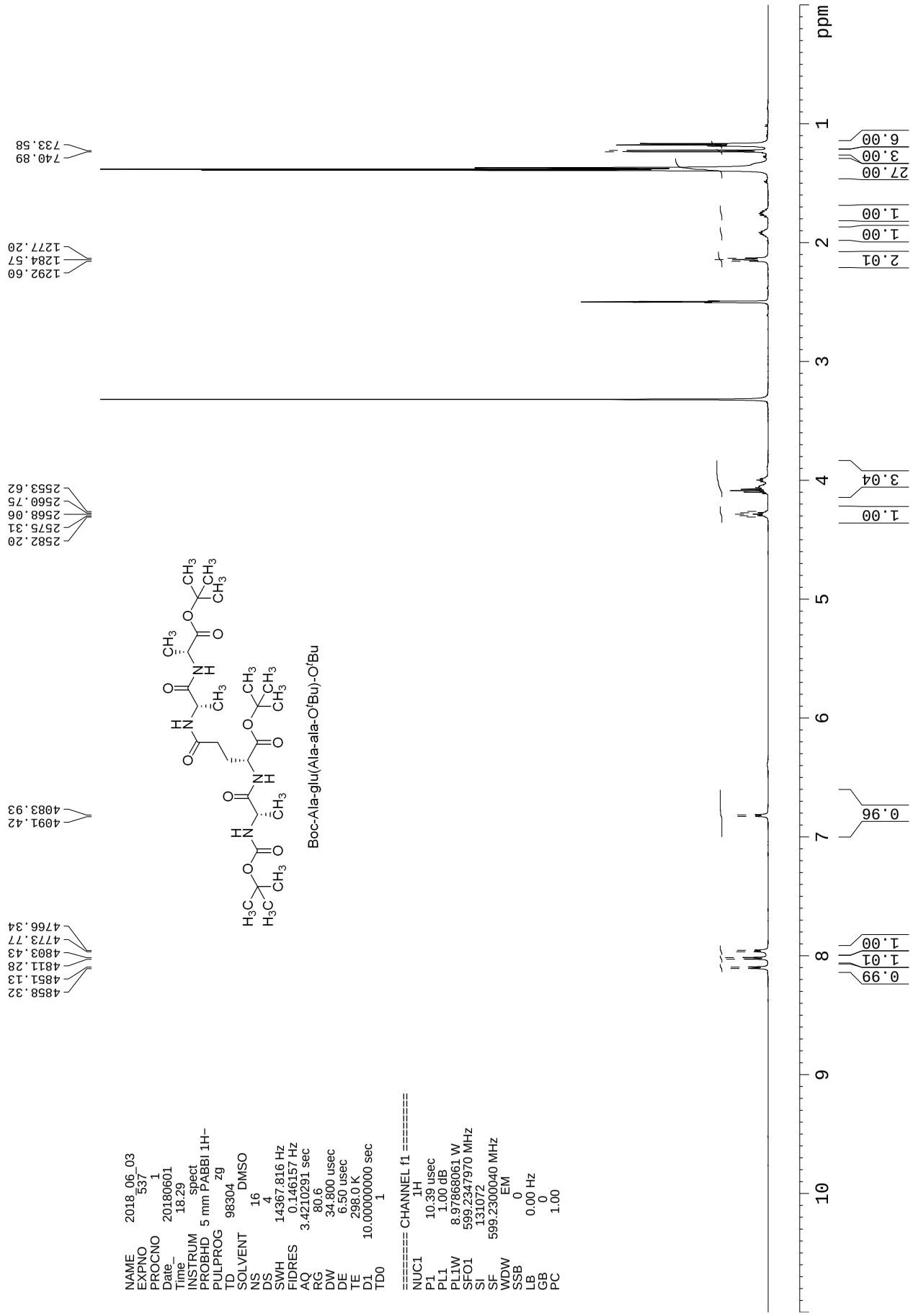


Figure 1: N_α-tert-butoxycarbonyl-L-alanyl-α-tert-butyl-D-glutamyl-L-alanyl-D-alanine tert butyl ester (**4**), ^{1}H NMR spectrum.

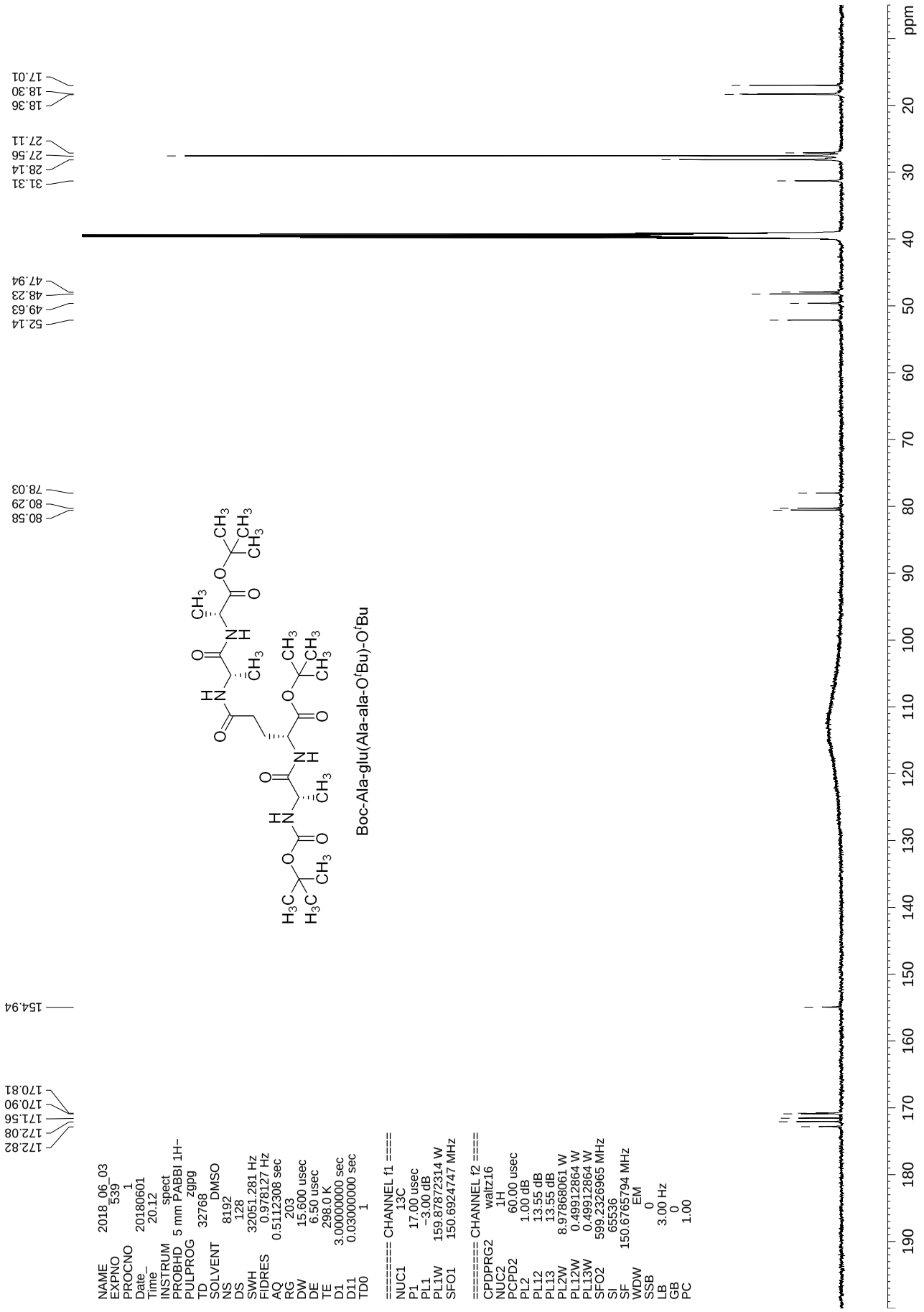


Figure 2: N$_\alpha$-tert-butoxycarbonyl-L-alanyl-α-tert-butyl-D-glutamyl-L-alanyl-D-alanine tert butyl ester (**4**), $\{^1H\}^{13}C$ NMR spectrum.

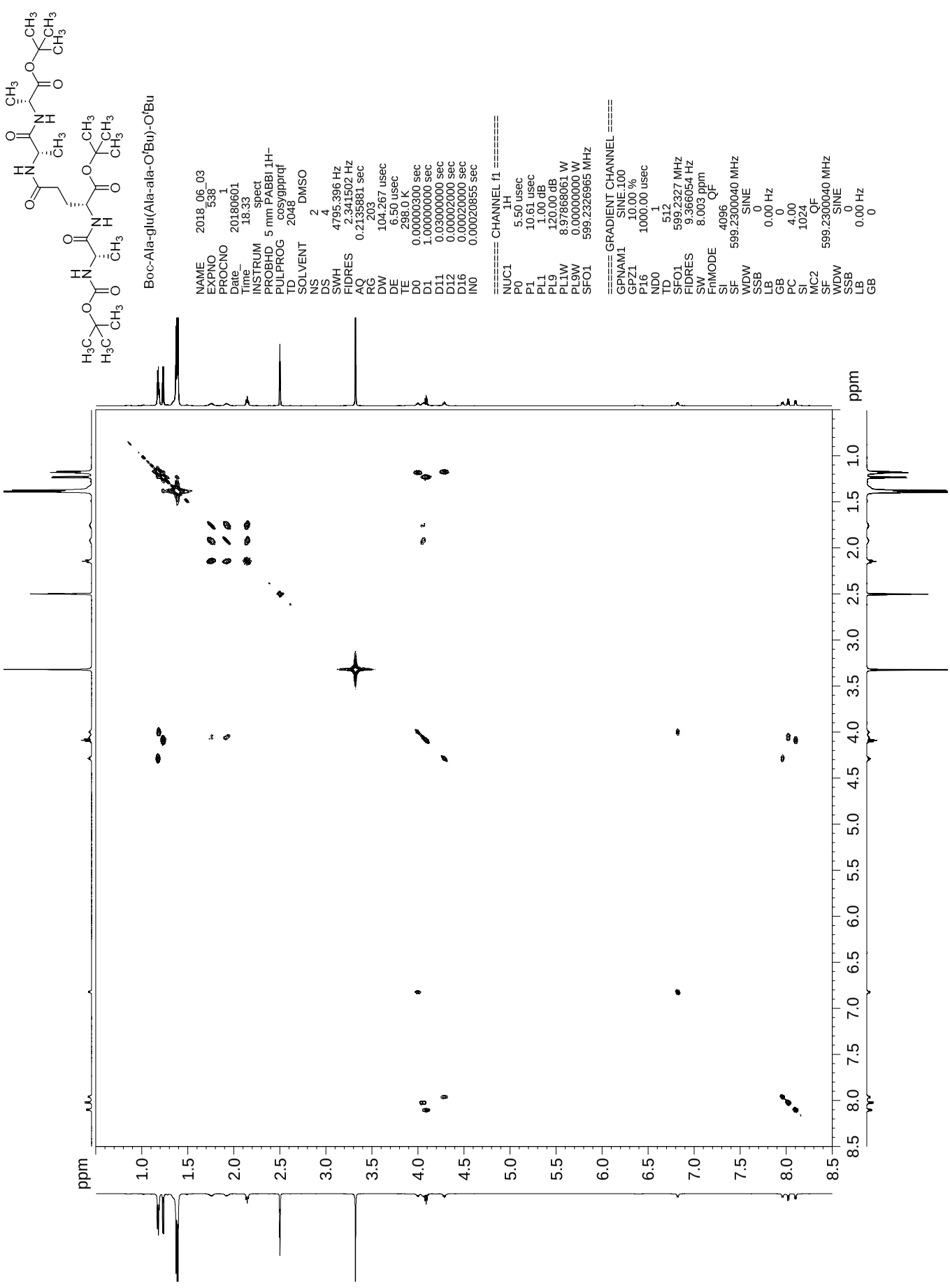


Figure 3: N$_\alpha$-tert-butoxycarbonyl-L-alanyl-α-tert-butyl-D-glutamyl-L-alanyl-D-alanine tert butyl ester (**4**), 2D COSY NMR spectrum

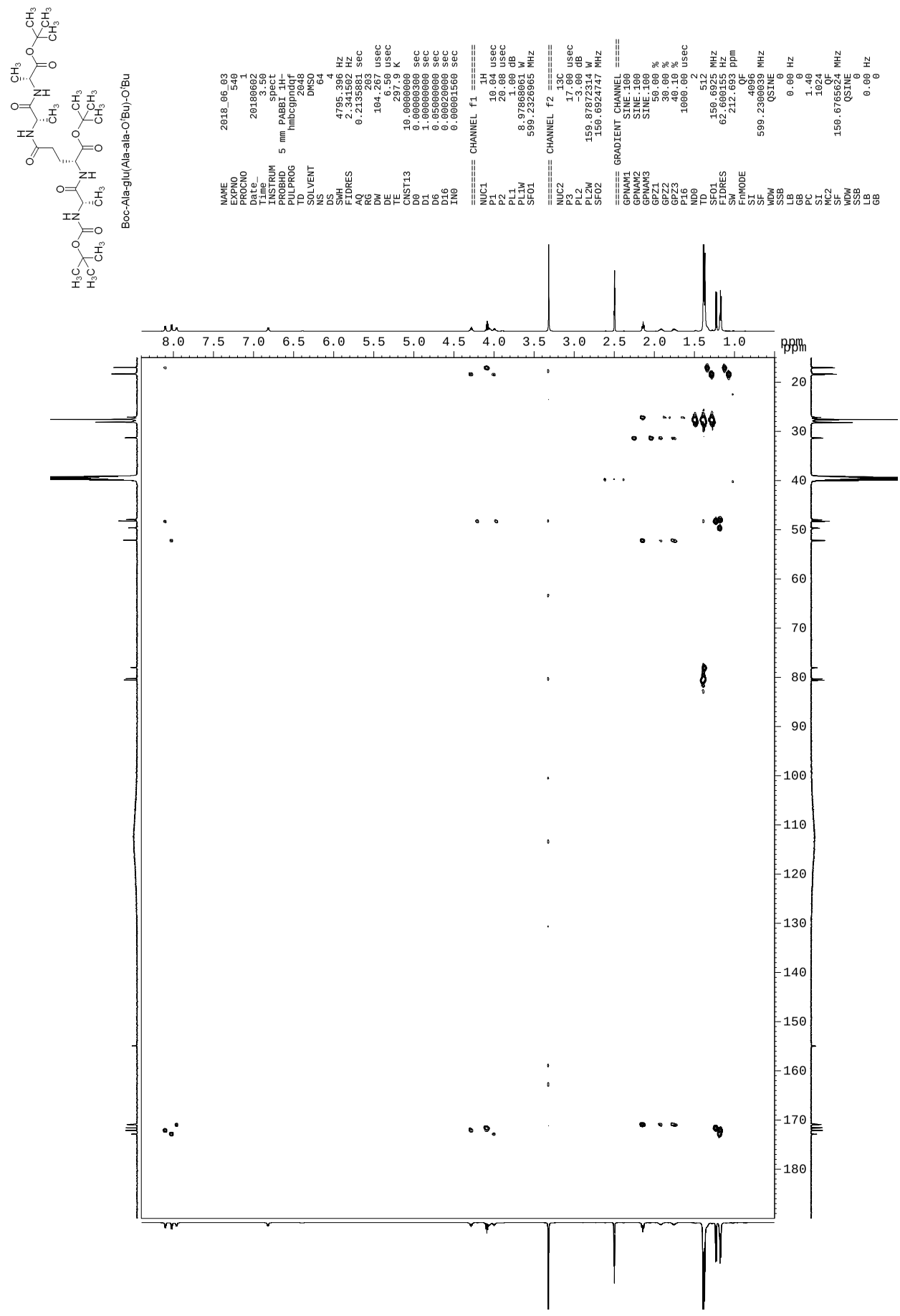
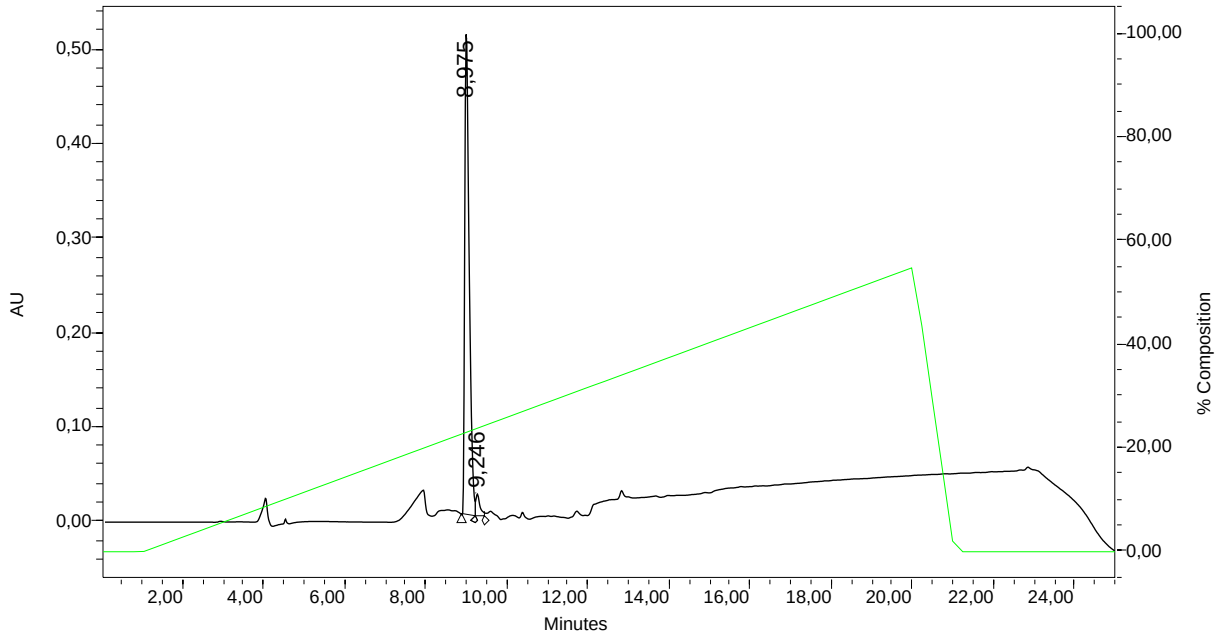


Figure 4: N_{α}-tert-butoxycarbonyl-L-alanyl-α-tert-butyl-D-glutamyl-L-alanyl-D-alanine tert butyl ester (**4**), 2D HMBC NMR spectrum

Project Name: testing_peptides
Reported by User: System

SAMPLE INFORMATION

Sample Name:	Ag(Ag)	Acquired By:	System
Sample Type:	Standard	Date Acquired:	15.06.2018 14:29:03
Vial:	1	Acq. Method:	gradient1_0_55_25min
Injection #:	4	Date Processed:	24.03.2020 14:09:31
Injection Volume:	50,00 ul	Channel Name:	2487Channel 1
Run Time:	25,00 Minutes	Channel Desc.:	226
Column Type:		Sample Set Name:	



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	8,975	3904417	95,40	515390	95,57
2	9,246	188377	4,60	23872	4,43

Report Method: Gradient Overlay Report ASC Printed 14:09:41 24.03.2020 Page: 1 of 1

Figure 5: HPLC analysis of L-Alanyl-γ-D-glutamyl-L-alanyl-D-alanine trifluoroacetate (**5**)

Project Name: testing_peptides
Reported by User: System

SAMPLE INFORMATION

Sample Name:	ppt2 C18, 6% AcCN	Acquired By:	System
Sample Type:	Standard	Date Acquired:	22.01.2019 13:37:41
Vial:	1	Acq. Method:	grad_3_2m_50_226_280
Injection #:	7	Date Processed:	24.03.2020 13:33:52
Injection Volume:	50,00 ul	Channel Name:	2487Channel 1
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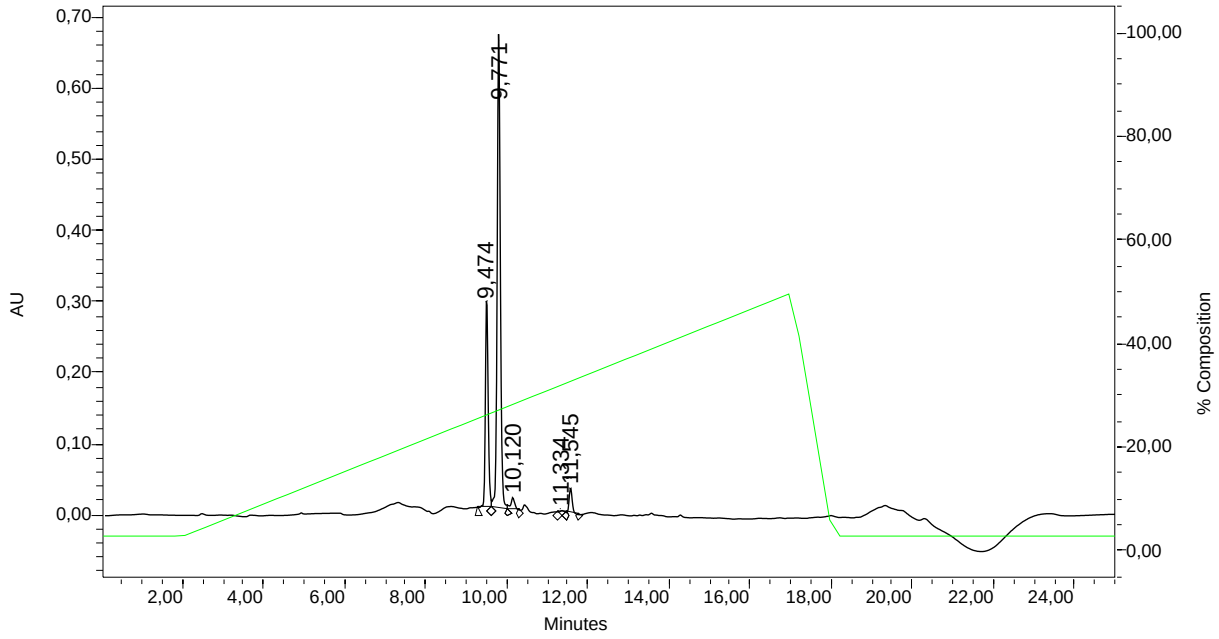
Column Type:		Sample Set Name:	



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	9,474	1449587	26,46	291774	28,94
2	9,771	3722905	67,96	662888	65,74
3	10,120	103782	1,89	16464	1,63
4	11,334	16751	0,31	2644	0,26
5	11,545	184791	3,37	34560	3,43

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Figure 6: HPLC analysis of N-Acetylglucosaminyl-β-(1-4)-N-acetylmuramoyl-L-Alanyl-γ-D-glutamyl-L-alanyl-D-alanine (**1**). Note: compound **1** posesses an anomeric center and hence it exists as a mixture of diastereomers: peaks at 9.47 and 9.77 min.

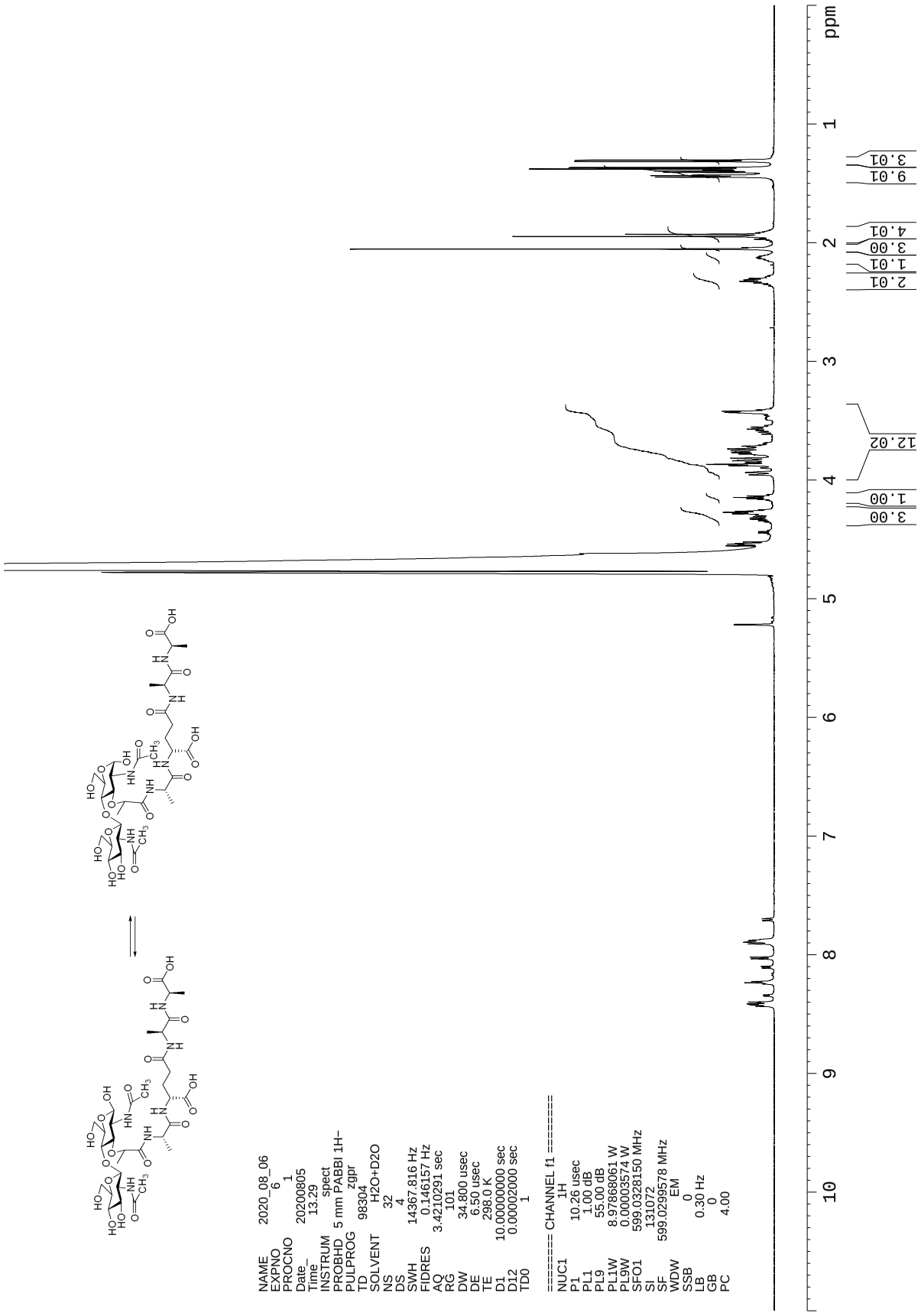

Figure 7: N-Acetylglucosaminyl-β-(1-4)-N-acetylmuramoyl-L-Alanyl-γ-D-glutamyl-L-alanyl-D-alanine (**1**). ^{1}H NMR spectrum. Note: compound **1** posesses an anomeric center and hence it exists as a mixture of diastereomers. Therefore, only partial integration of proton signals was made. At the same time, the result of integration does support the fact that compound **1** exists as an adduct of one molecule of disaccharide and one molecule of tetrapeptide. For complete signal assignment, that also supports the nature of the linkage between the disachraride unit and the tetrapeptide, see manuscript text. Also, no impurities signals with normalized areas larger than 5 % except residual solvent are observed, hence compound **1** does attain purity requirments.

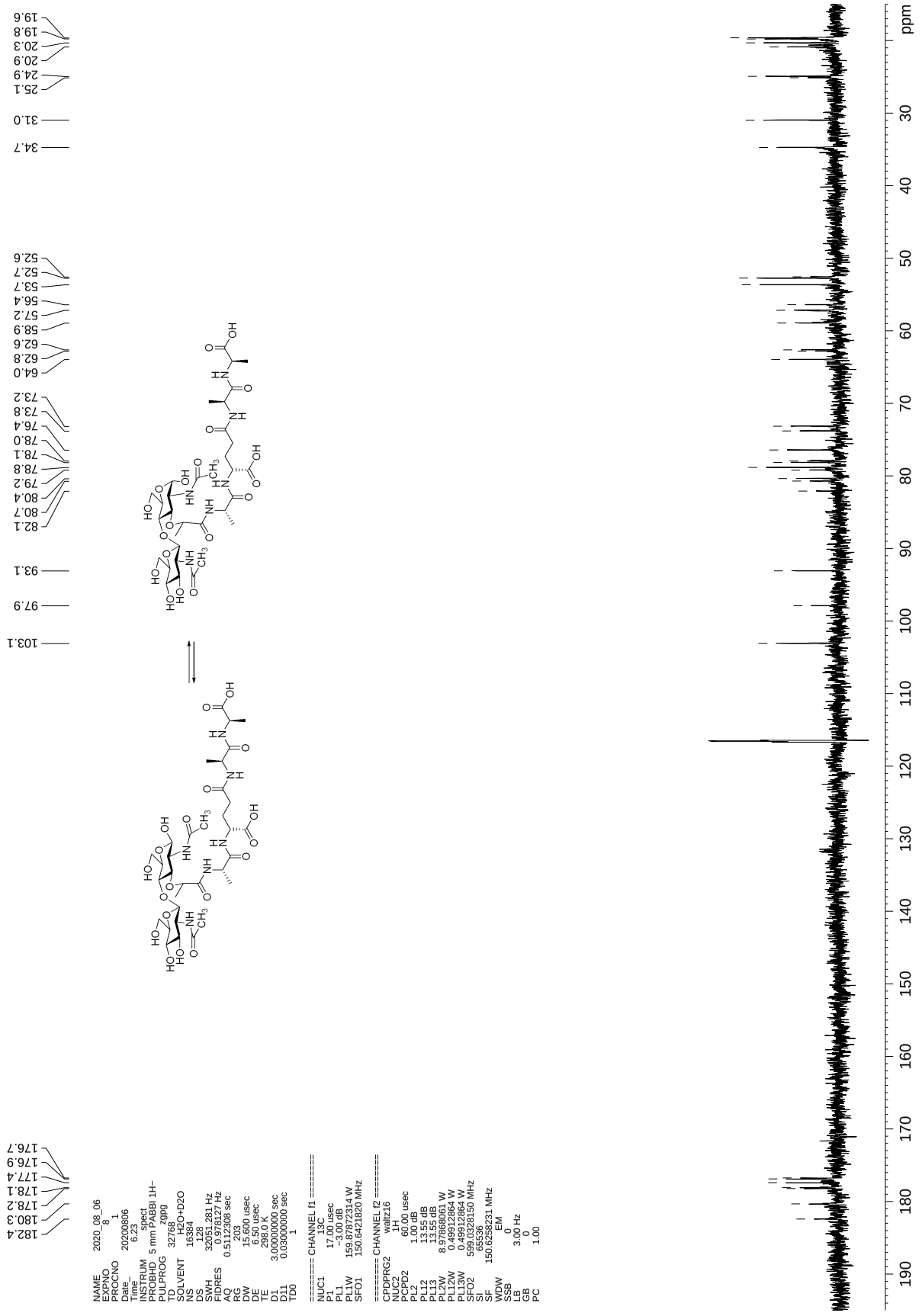

Figure 8: N-Acetylglucosaminyl-β-(1-4)-N-acetylmuramoyl-L-Alanyl-γ-D-glutamyl-L-alanyl-D-alanine (**1**). ^{13}C NMR spectrum. For complete signal assignment see manuscript text.

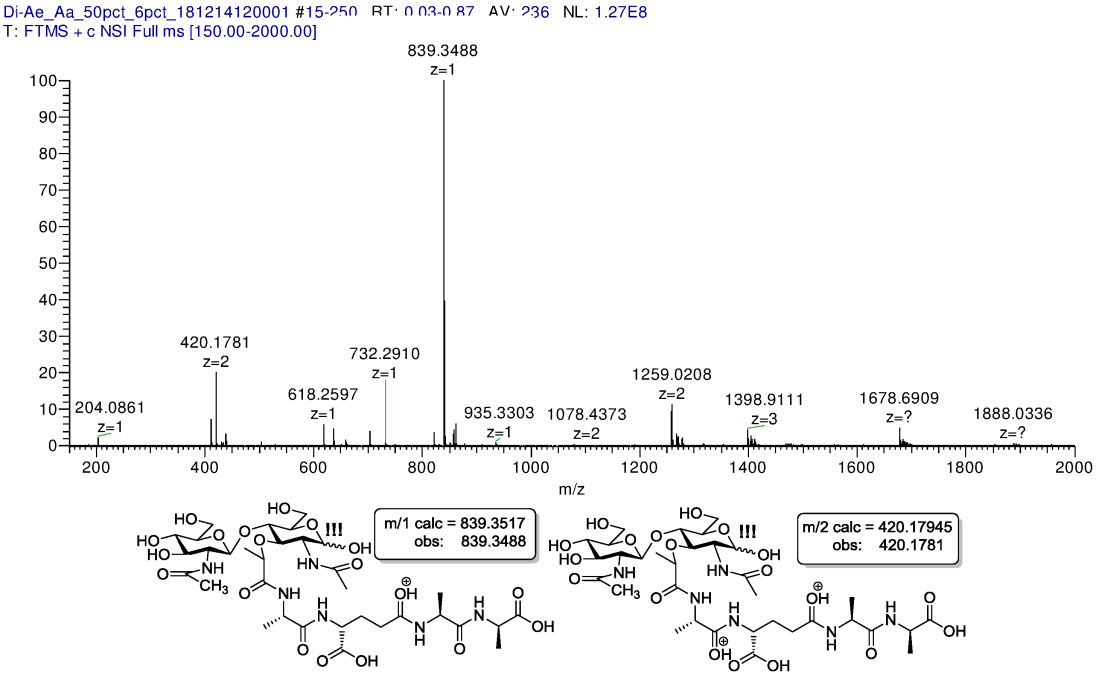

Figure 9: N-Acetylglucosaminyl-β-(1-4)-N-acetylmuramoyl-L-Alanyl-γ-D-glutamyl-L-alanyl-D-alanine (**1**). HRMS spectrum.