Supporting Information

Synthesis and structural characterization of the individual diastereoisomers of a cross-stapled alkene-bridged nisin DE-ring mimic

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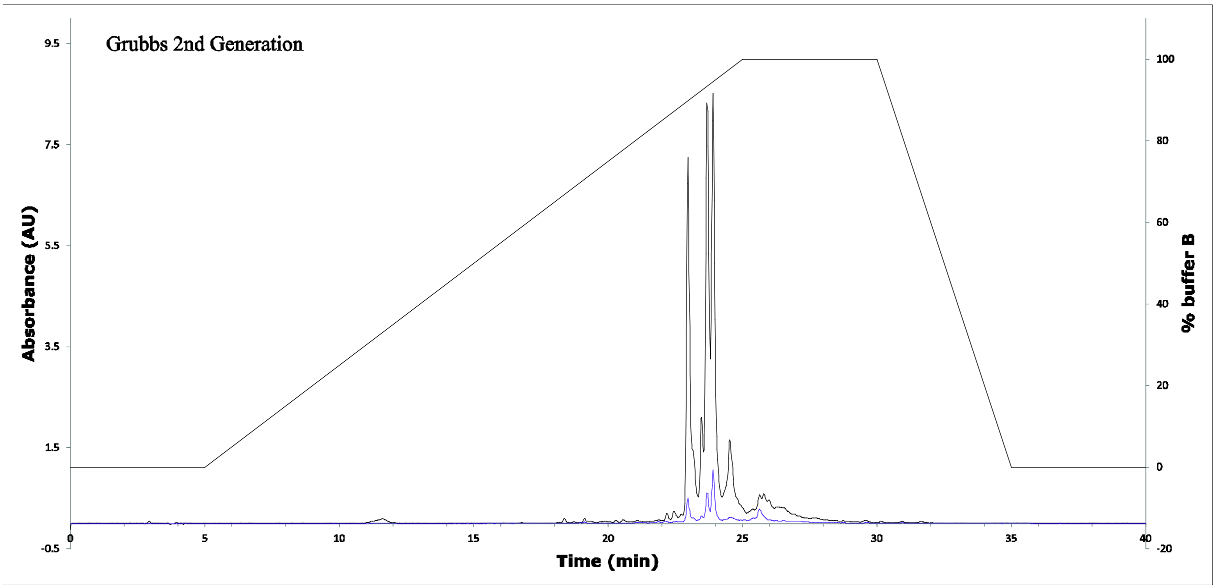
Contents

1. HPLC chromatograms of RCM reaction mixtures.



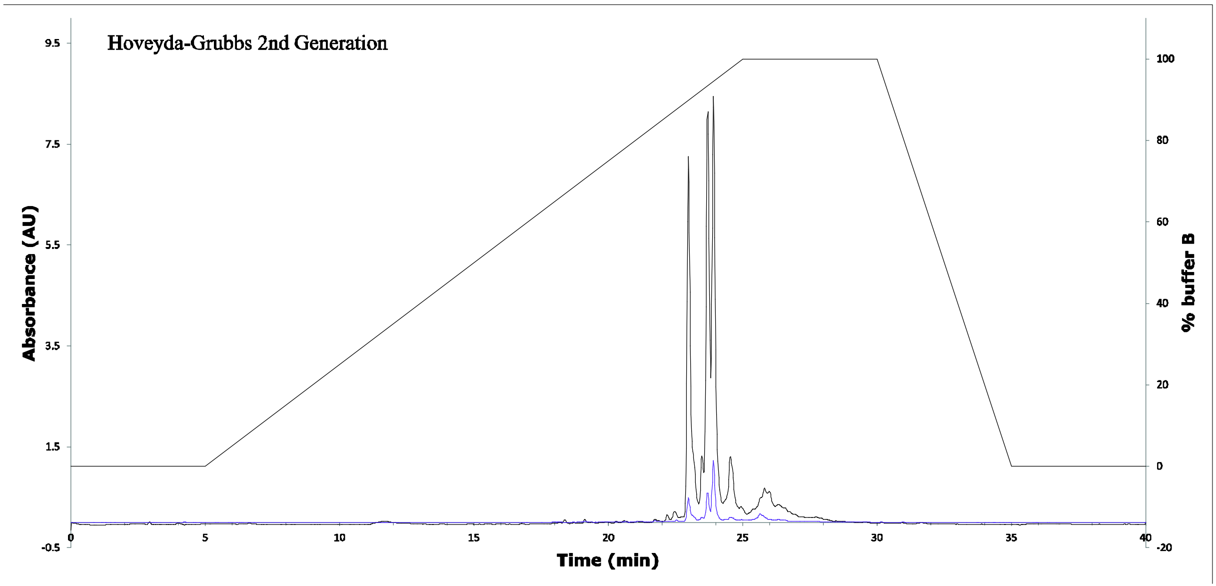
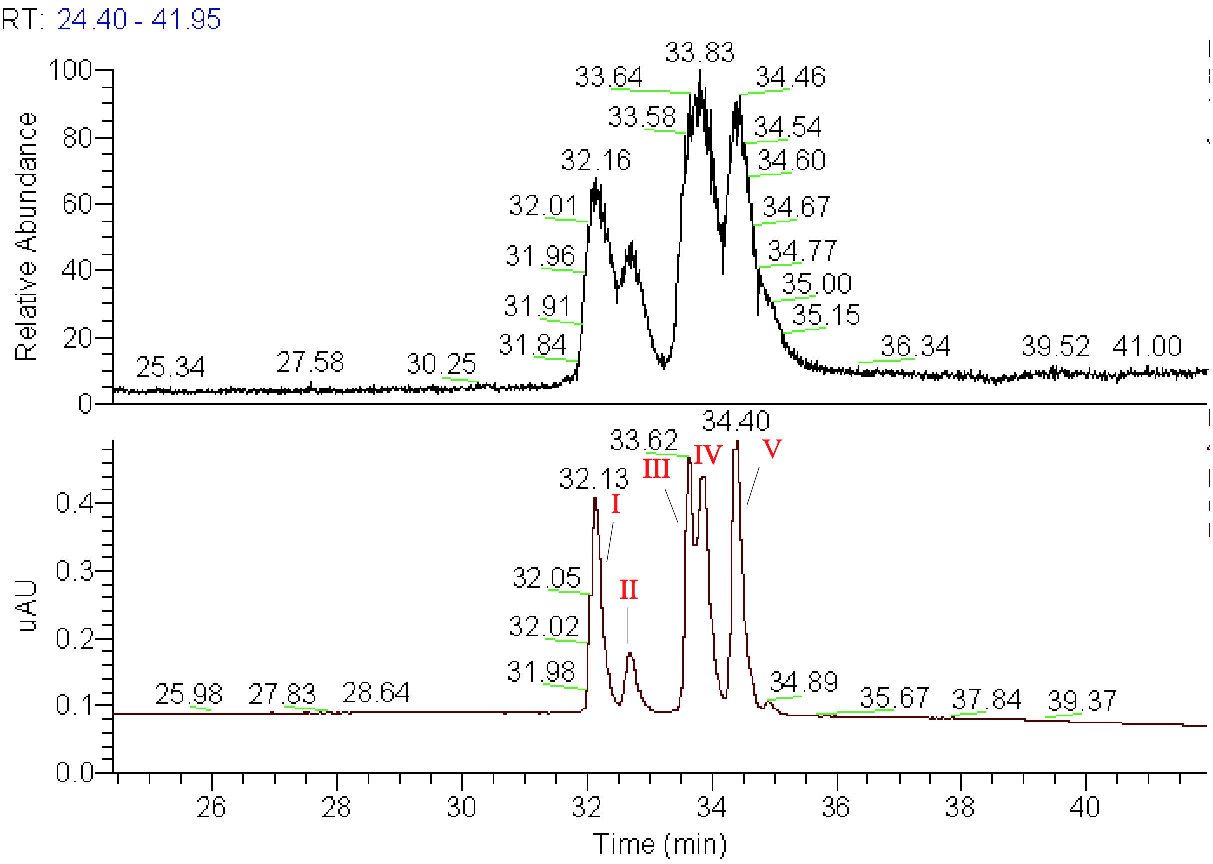
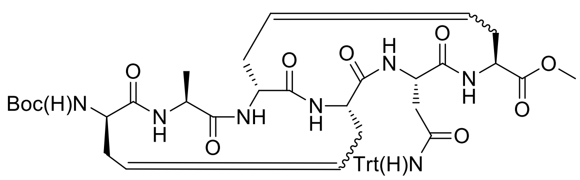
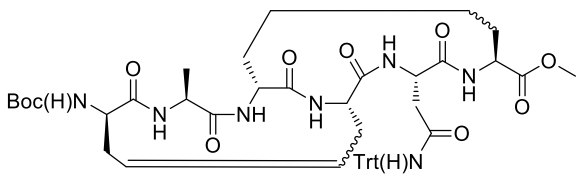


Figure SI 1: HPLC chromatograms of the RCM reactions mixtures of bicyclo[1-4/3-6]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2**) using Grubbs 2nd generation and Hoveyda-Grubbs 2nd generation catalyst.

2. LC-MS data hydrogenation DE-Ring 2.







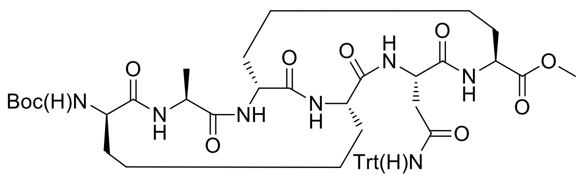


Figure SI 2: A zoom-in of the LC-MS data of the hydrogenation reaction mixture starting with bicyclo[1-4/3-6]-Boc-D-Alg^1-Ala^2-D-Alg^3-Alg^4-Asn(Trt)^5-Alg^6-OMe (**2**) shows five peaks, I, II, III, IV and V respectively. The chemical structures represent the expected product and reaction intermediates.

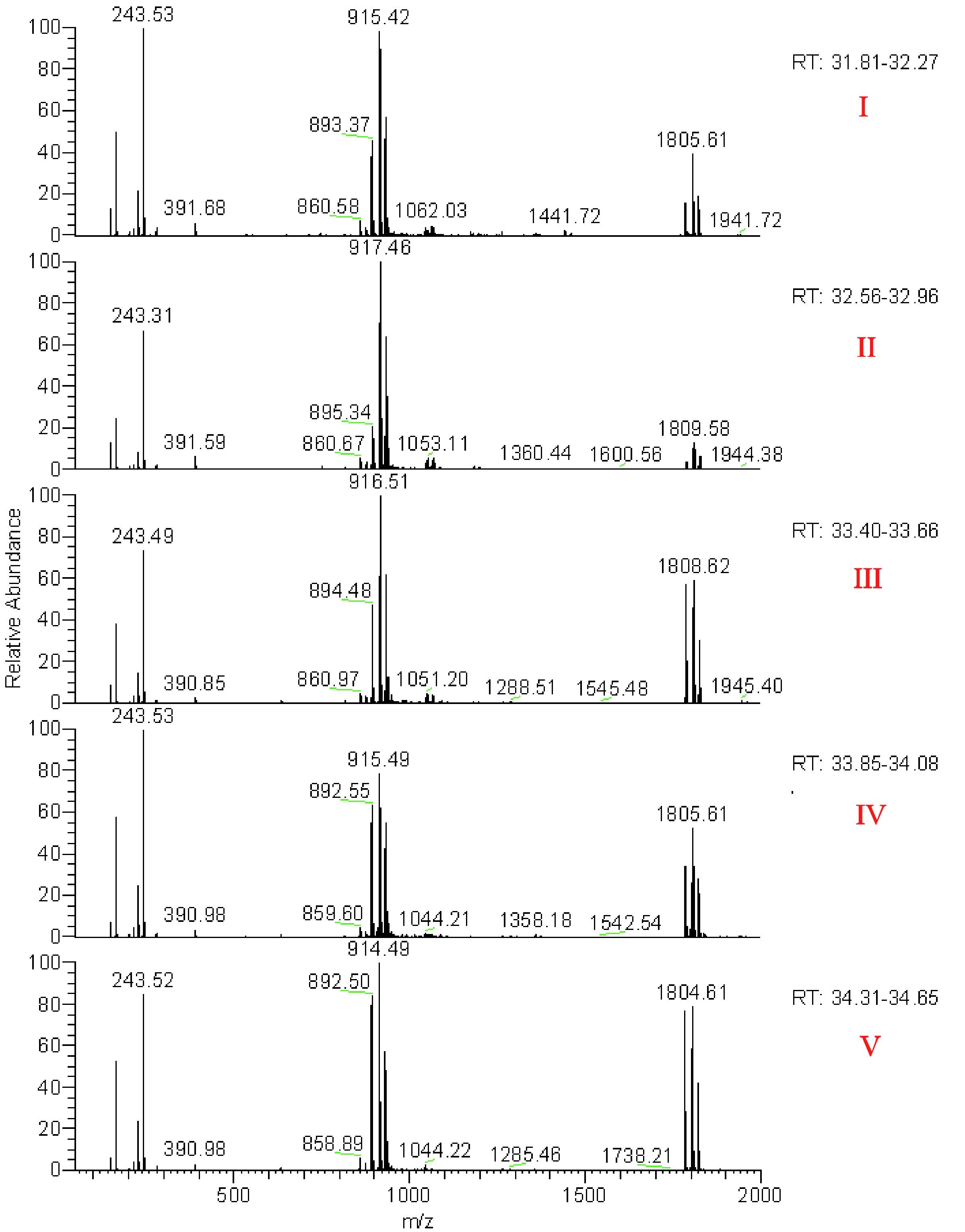


Figure SI 3: MS spectra derived from the observed five peaks (I, II, III, IV and V) from the LC-MS data of the hydrogenation reaction mixture starting with bicyclo[1-4/3-6]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2**).

3. Copies of ^{1}H, TOCSY, ROESY and HSQC NMR spectra of bicyclic peptide 2a, 2b, 2c and 2d.

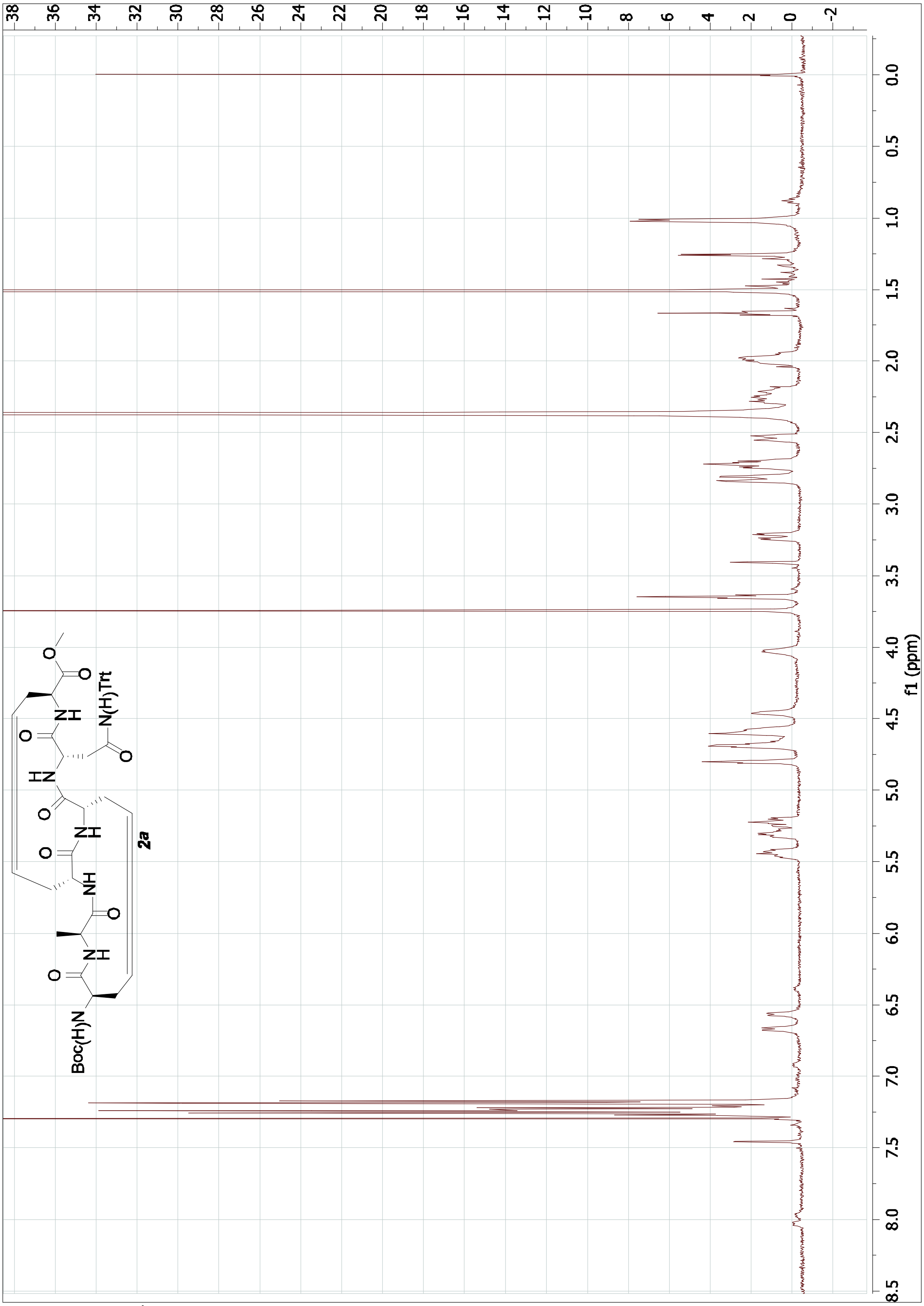


Figure SI 4: ^{1}H NMR spectrum of bicyclo[$Z^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^1-Ala^2-D-Alg^3-Alg^4-Asn(Trt)^5-Alg^6-OMe (**2a**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

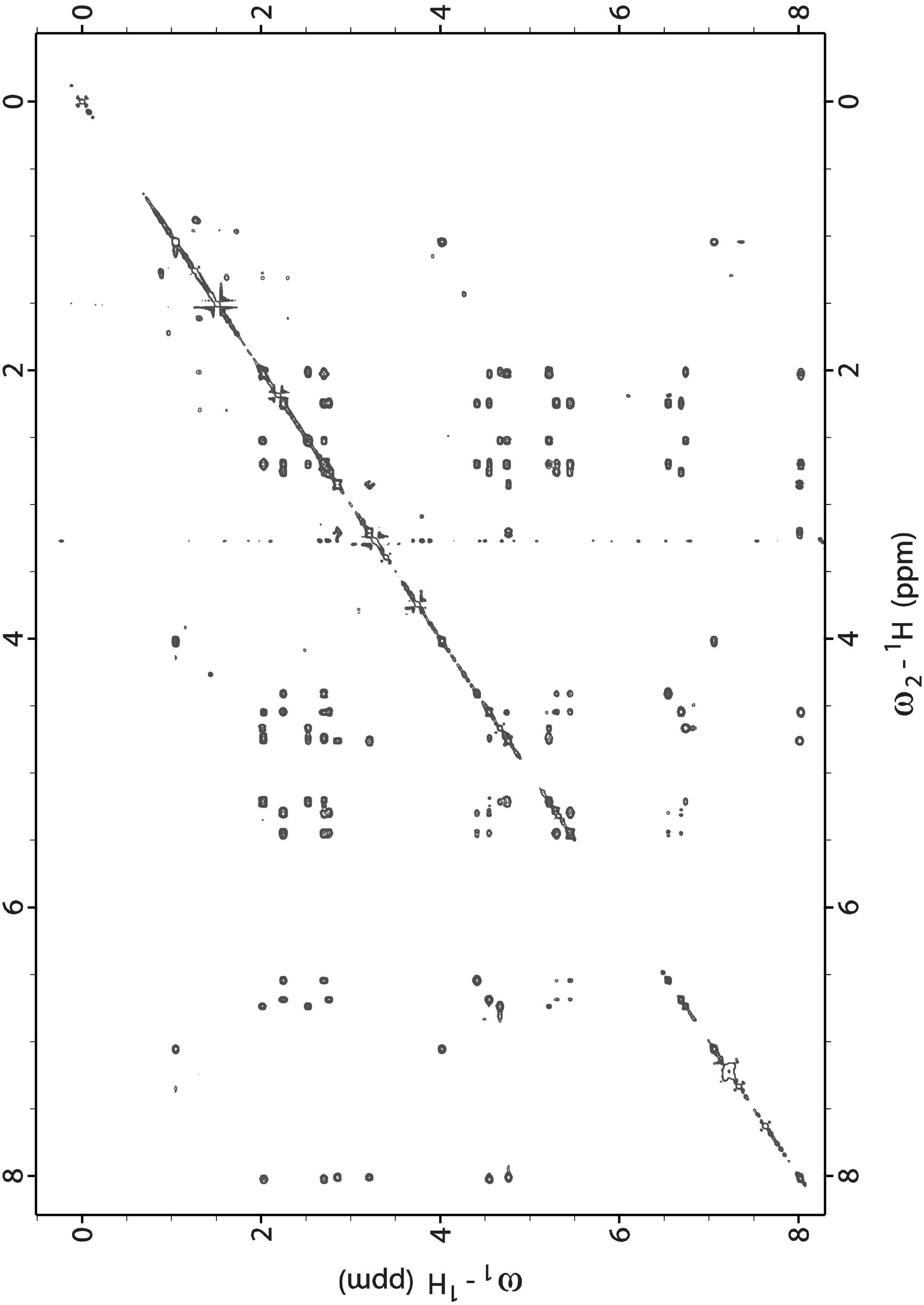


Figure SI 5: ^{1}H-TOCSY NMR spectrum of bicyclo[$Z^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2a**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

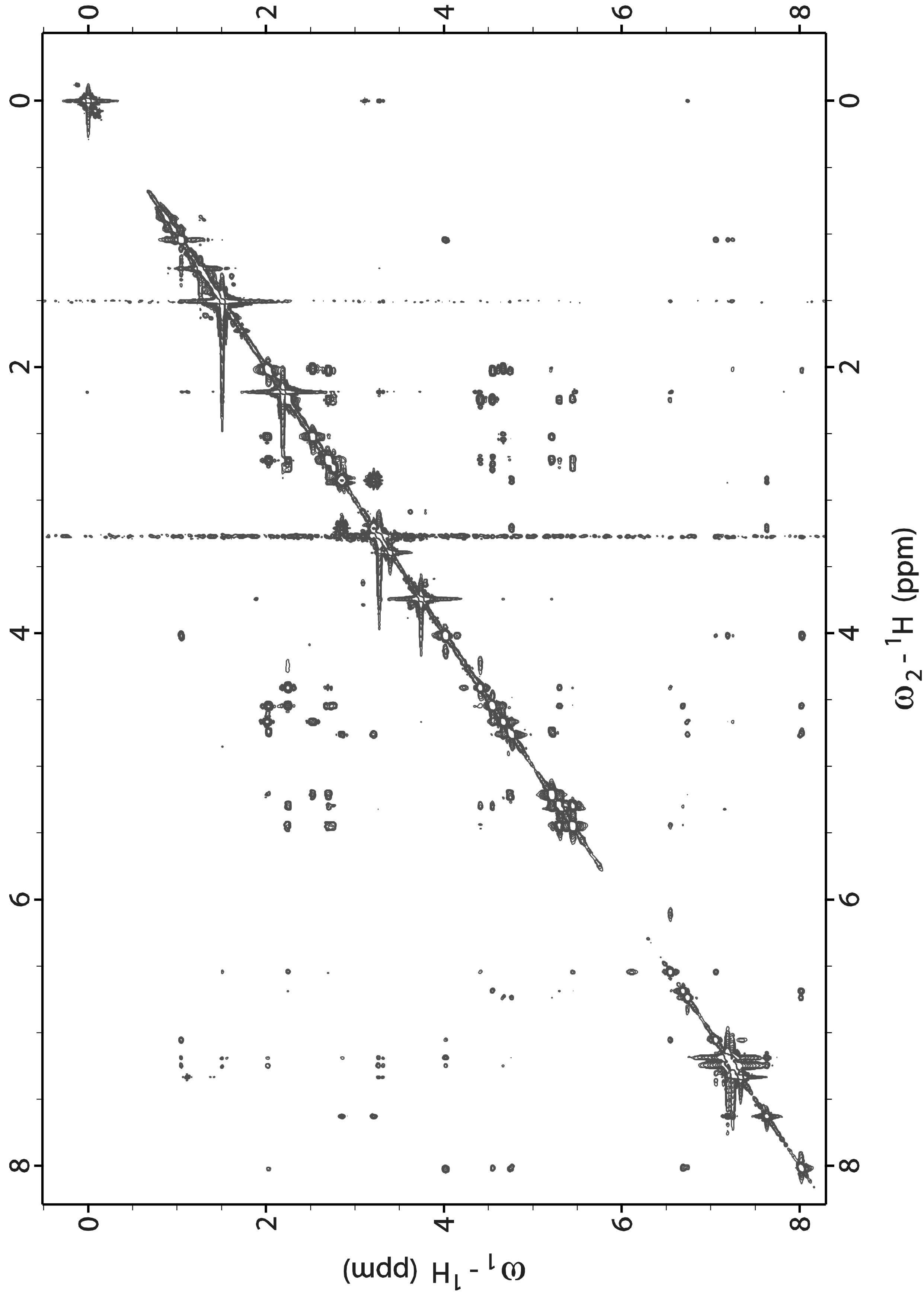


Figure SI 6: ^{1}H-ROESY NMR spectrum of bicyclo[$Z^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2a**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

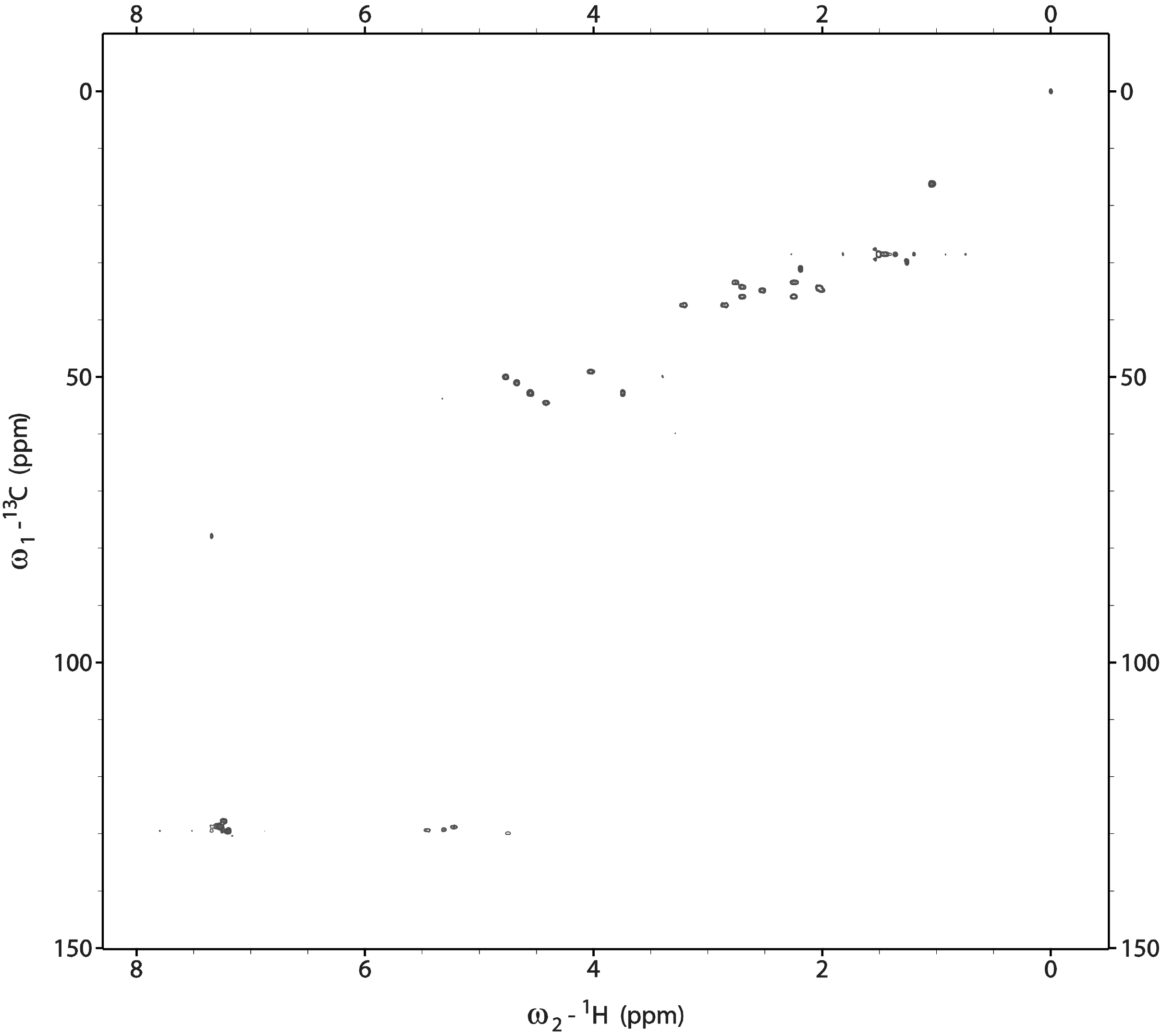


Figure SI 7: ^{1}H/^{13}C-HSQC NMR spectrum of bicyclo[$Z^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^1-Ala^2-D-Alg^3-Alg^4-Asn(Trt)^5-Alg^6-OMe (**2a**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

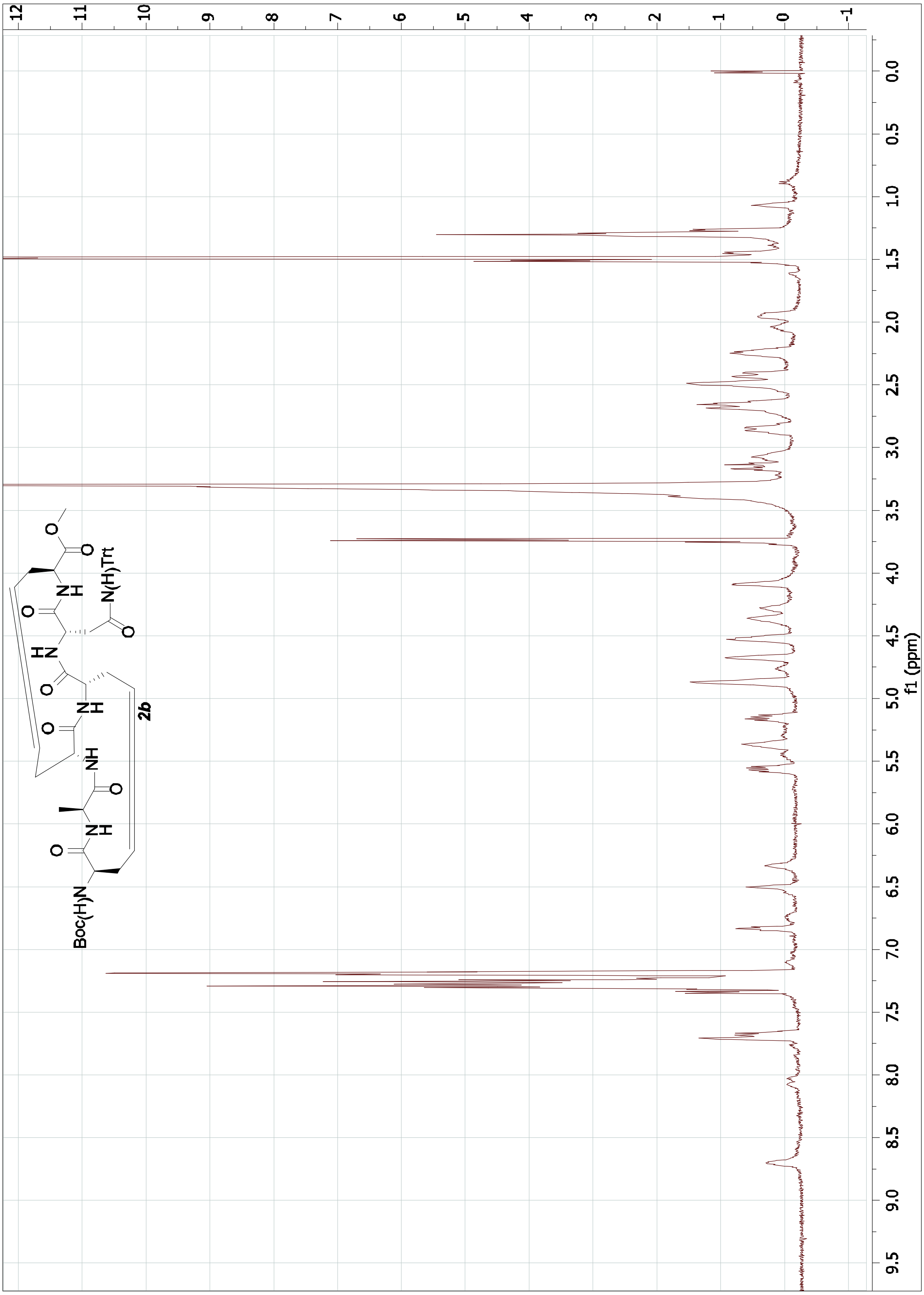


Figure SI 8: ^{1}H NMR spectrum of bicyclo[$Z^{1\text{-}4}$/$E^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2b**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

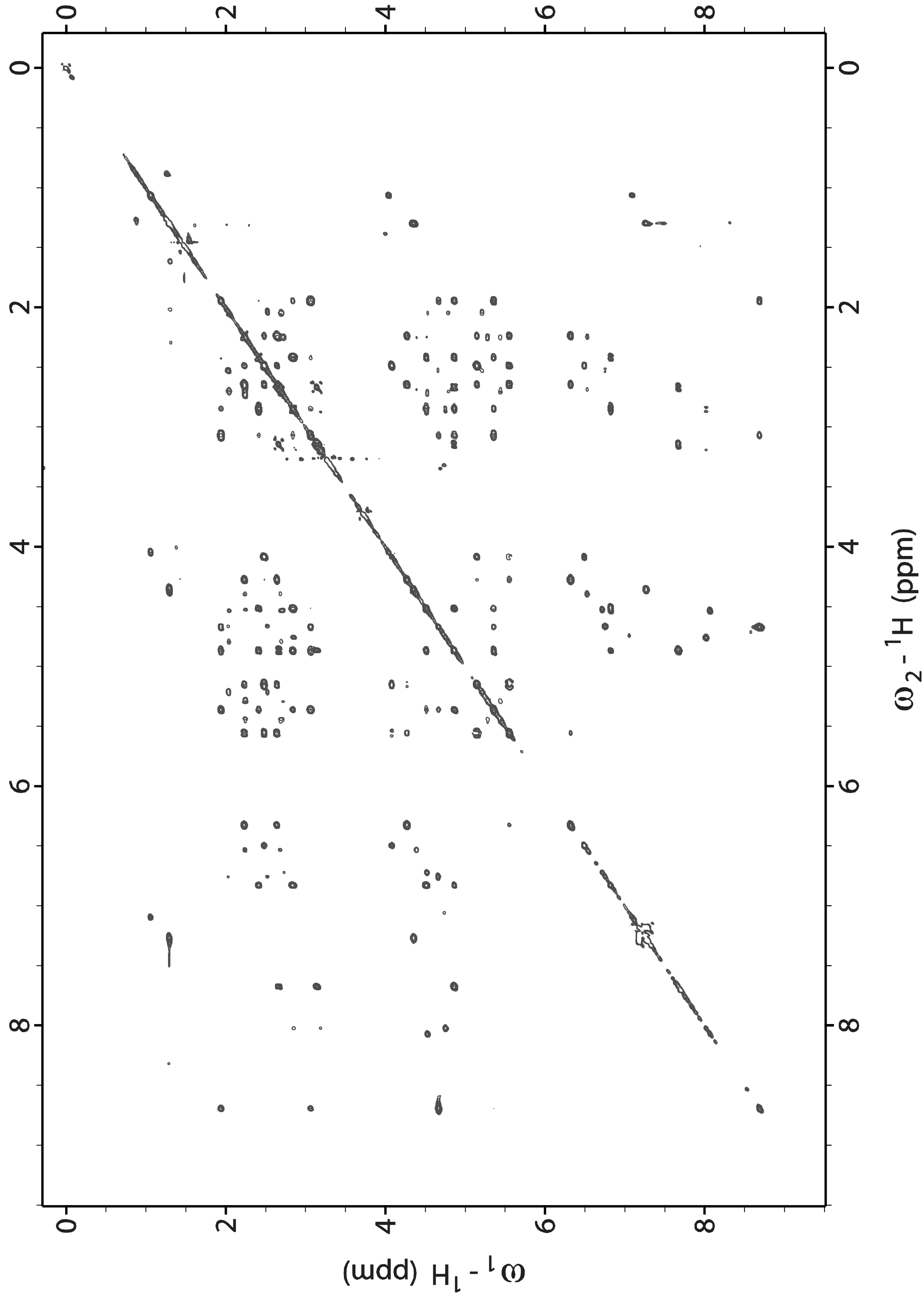


Figure SI 9: ^{1}H-TOCSY NMR spectrum of bicyclo[$Z^{1\text{-}4}$/$E^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2b**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

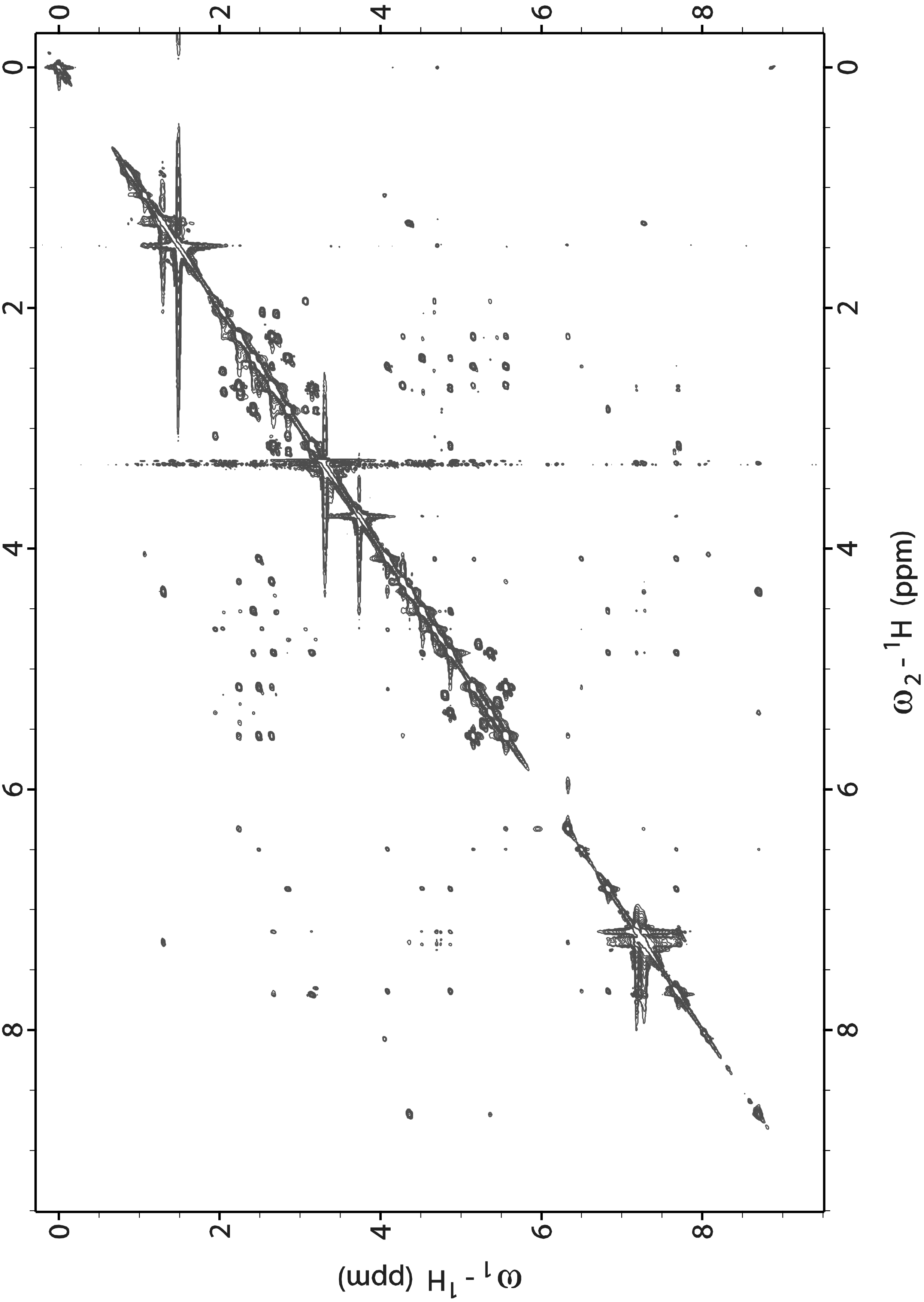


Figure SI 10: ^{1}H-ROESY NMR spectrum of bicyclo[$Z^{1\text{-}4}$/$E^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2b**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

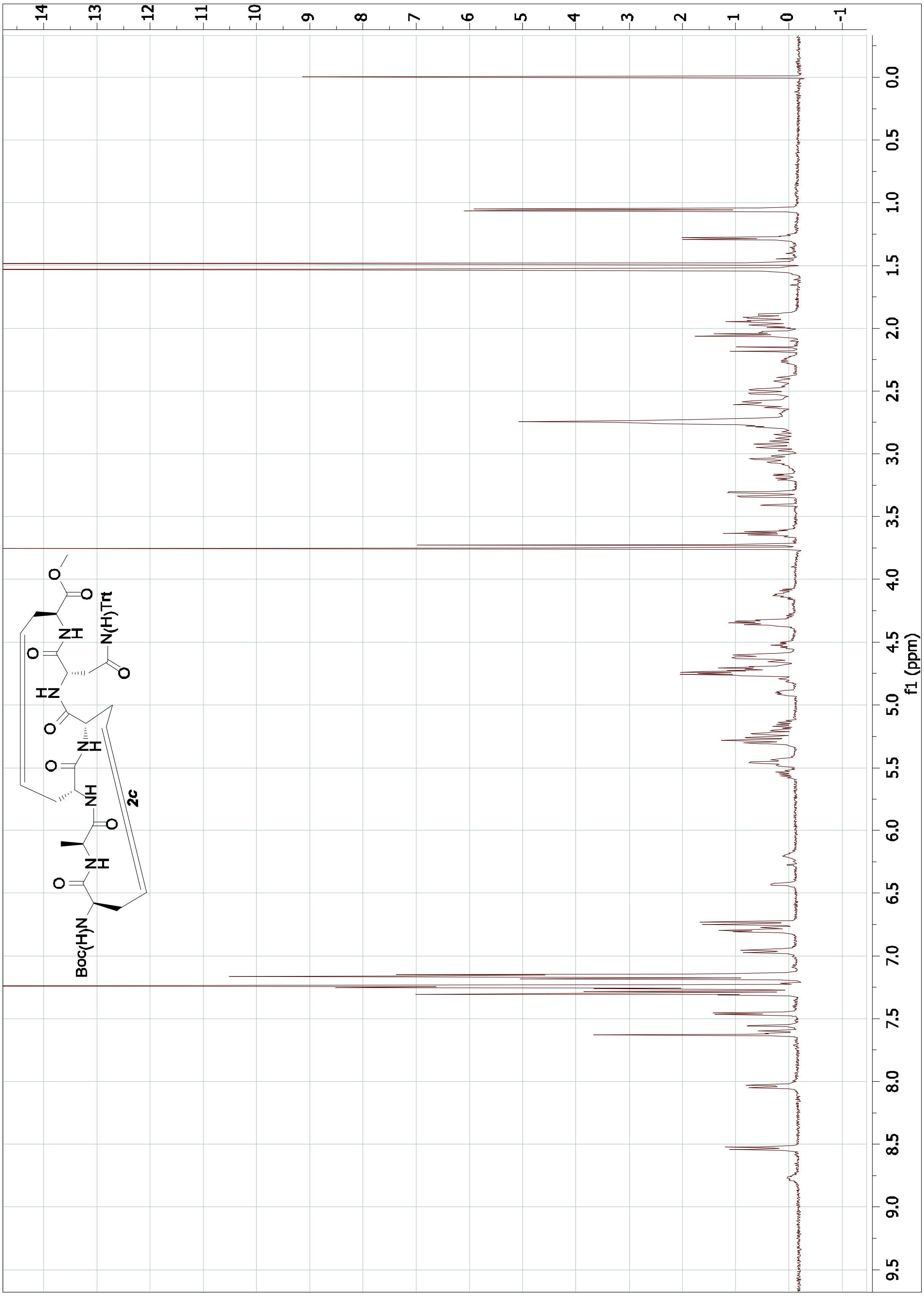


Figure SI 11: ^{1}H NMR spectrum of bicyclo[$E^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2c**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

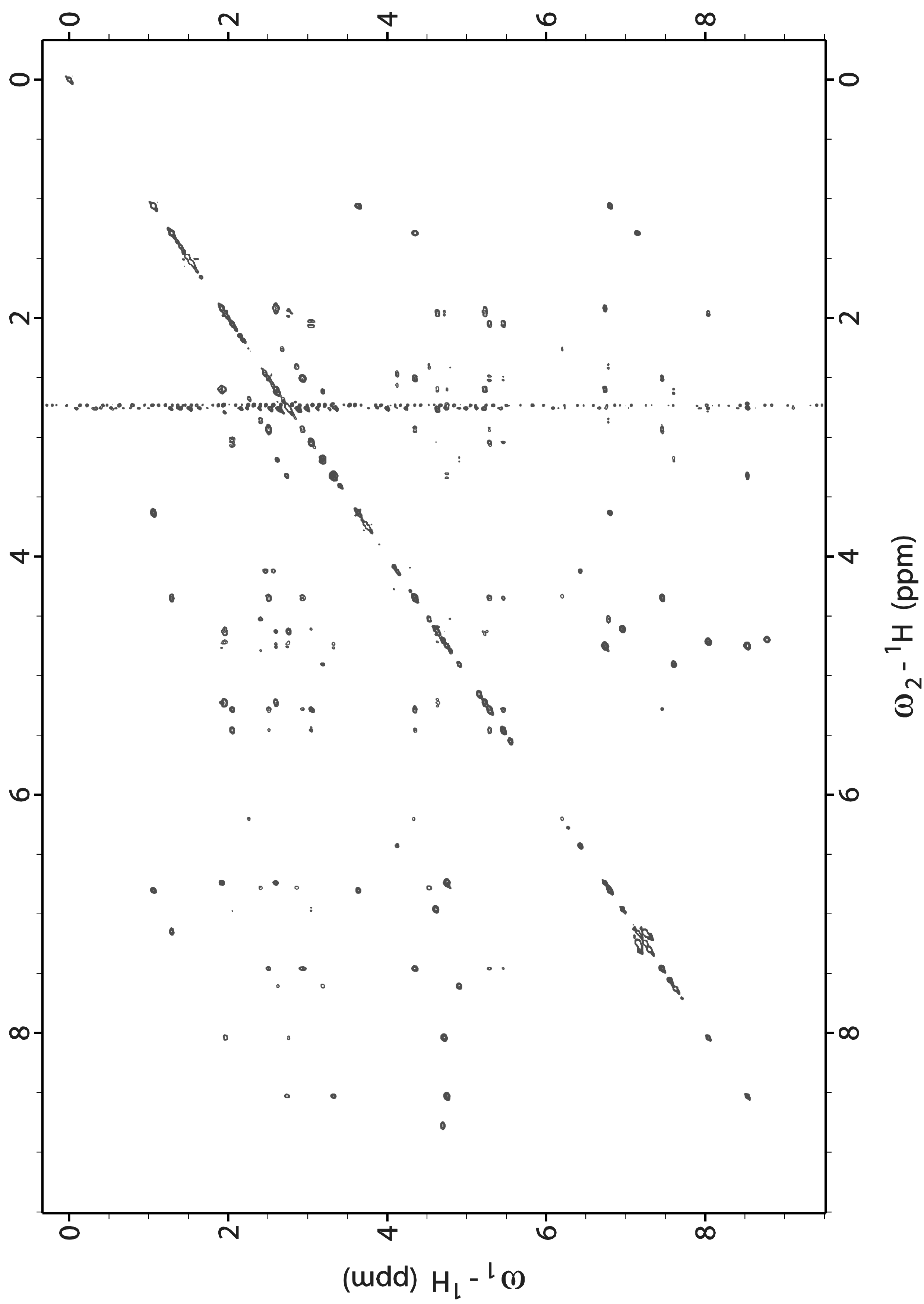


Figure SI 12: ^{1}H-TOCSY HSQC NMR spectrum of bicyclo[$E^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2c**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

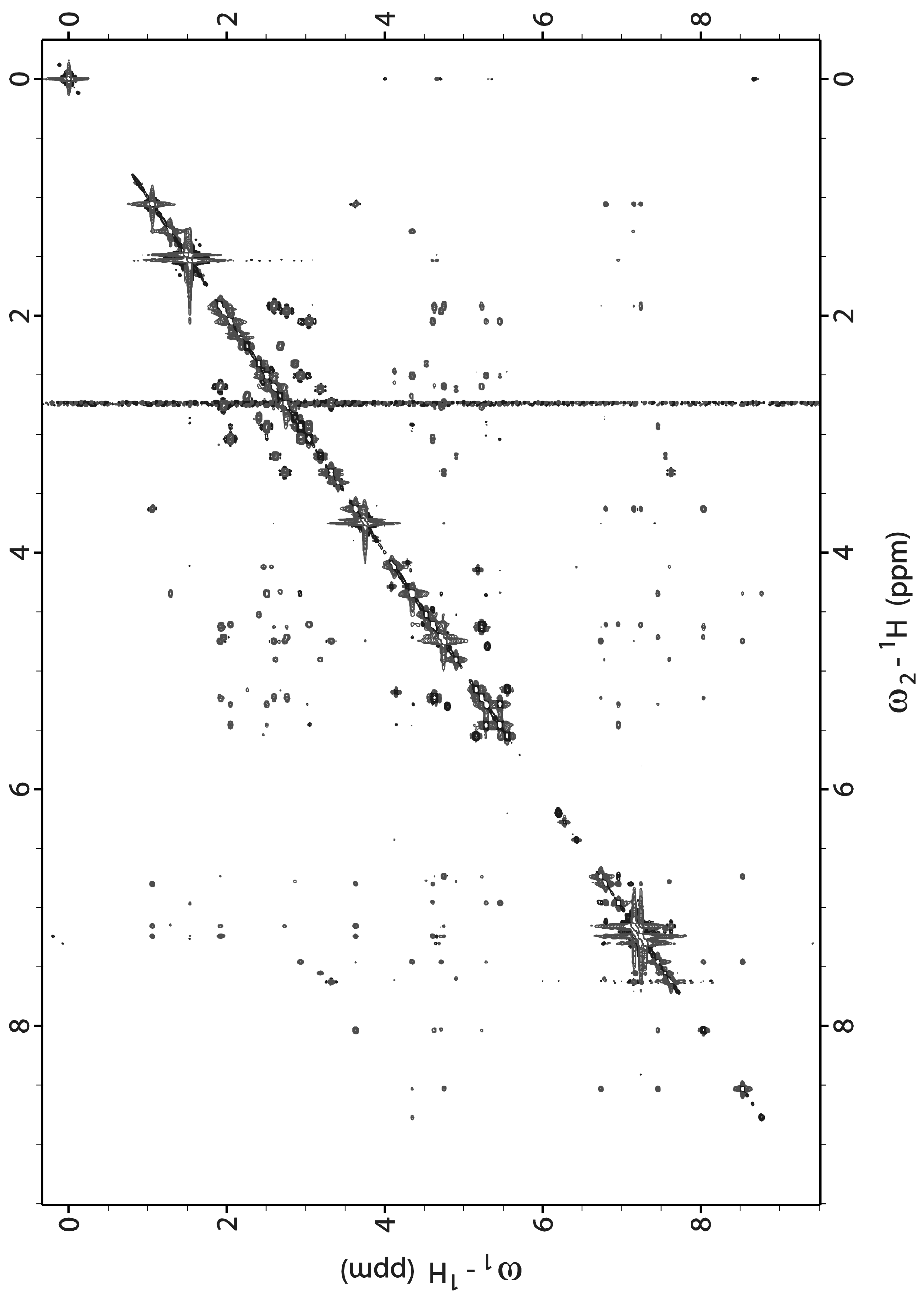


Figure SI 13: ^{1}H-ROESY HSQC NMR spectrum of bicyclo[$E^{1\text{-}4}/Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2c**) (500 MHz, $CDCl_3/CD_3OH$ 95:5 v/v, T = 298 K).

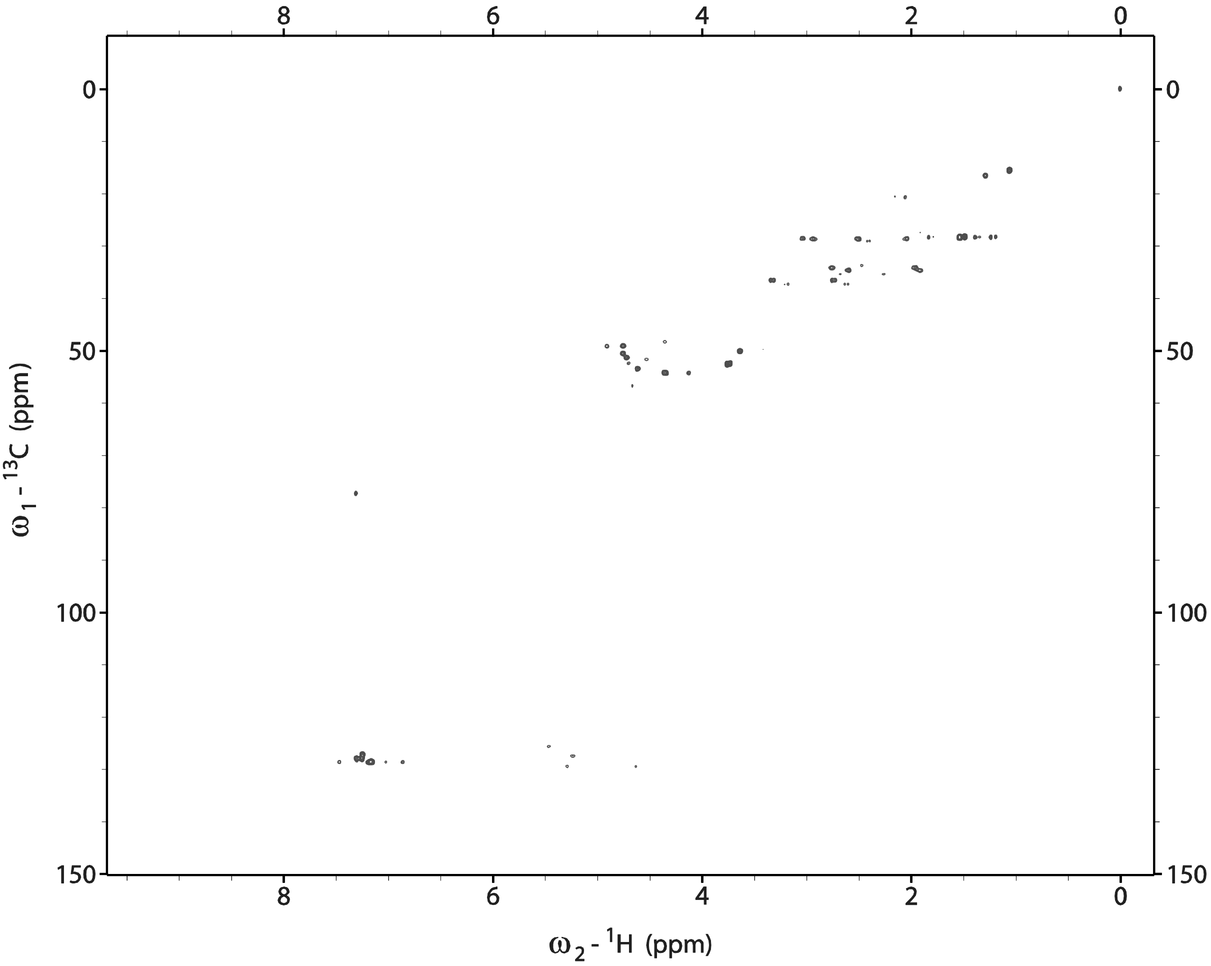


Figure SI 14: ^{1}H/^{13}C-HSQC NMR spectrum of bicyclo[$E^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2c**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

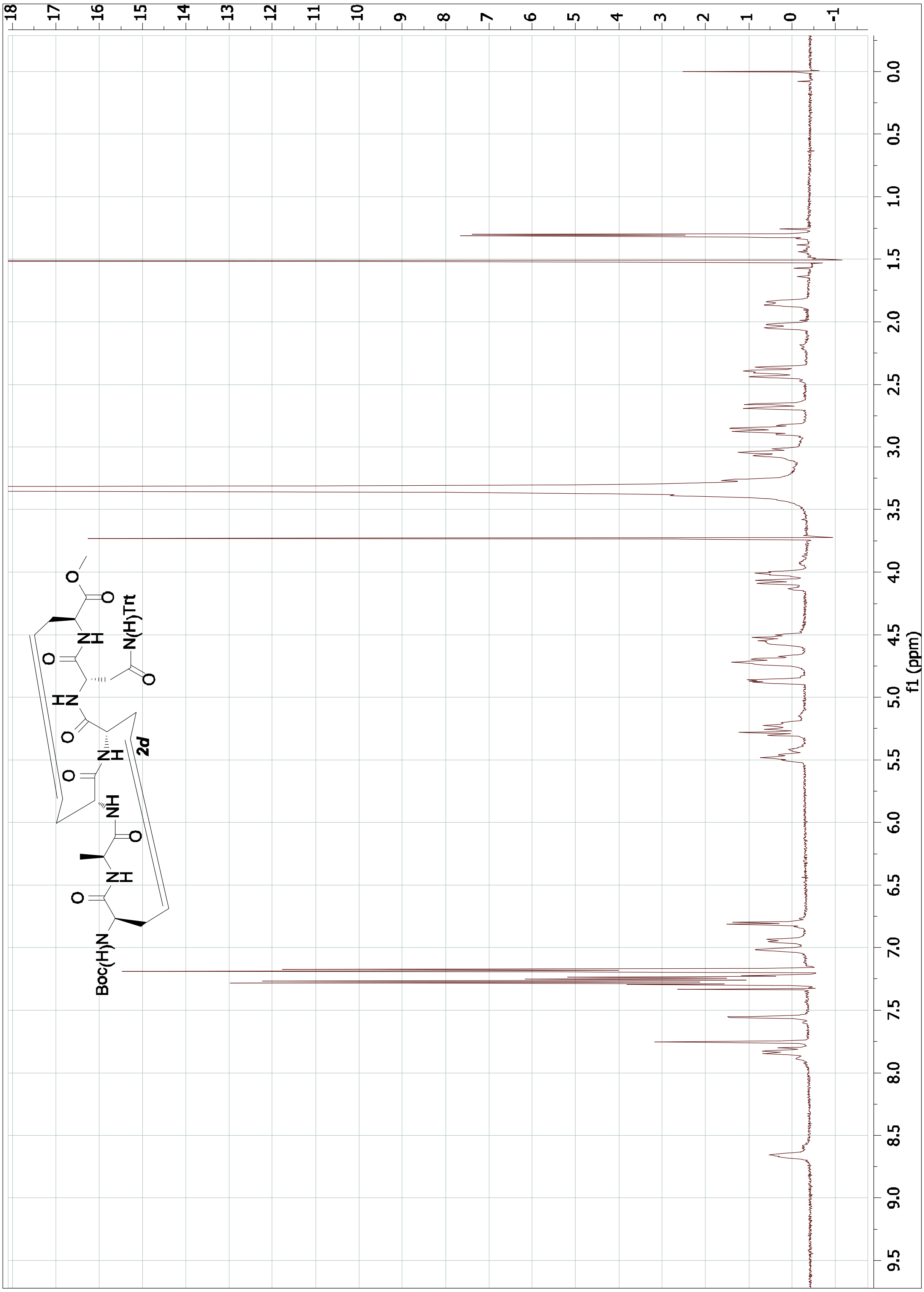


Figure SI 15: ^{1}H NMR spectrum of bicyclo[$E^{1\text{-}4}$/$E^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2d**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

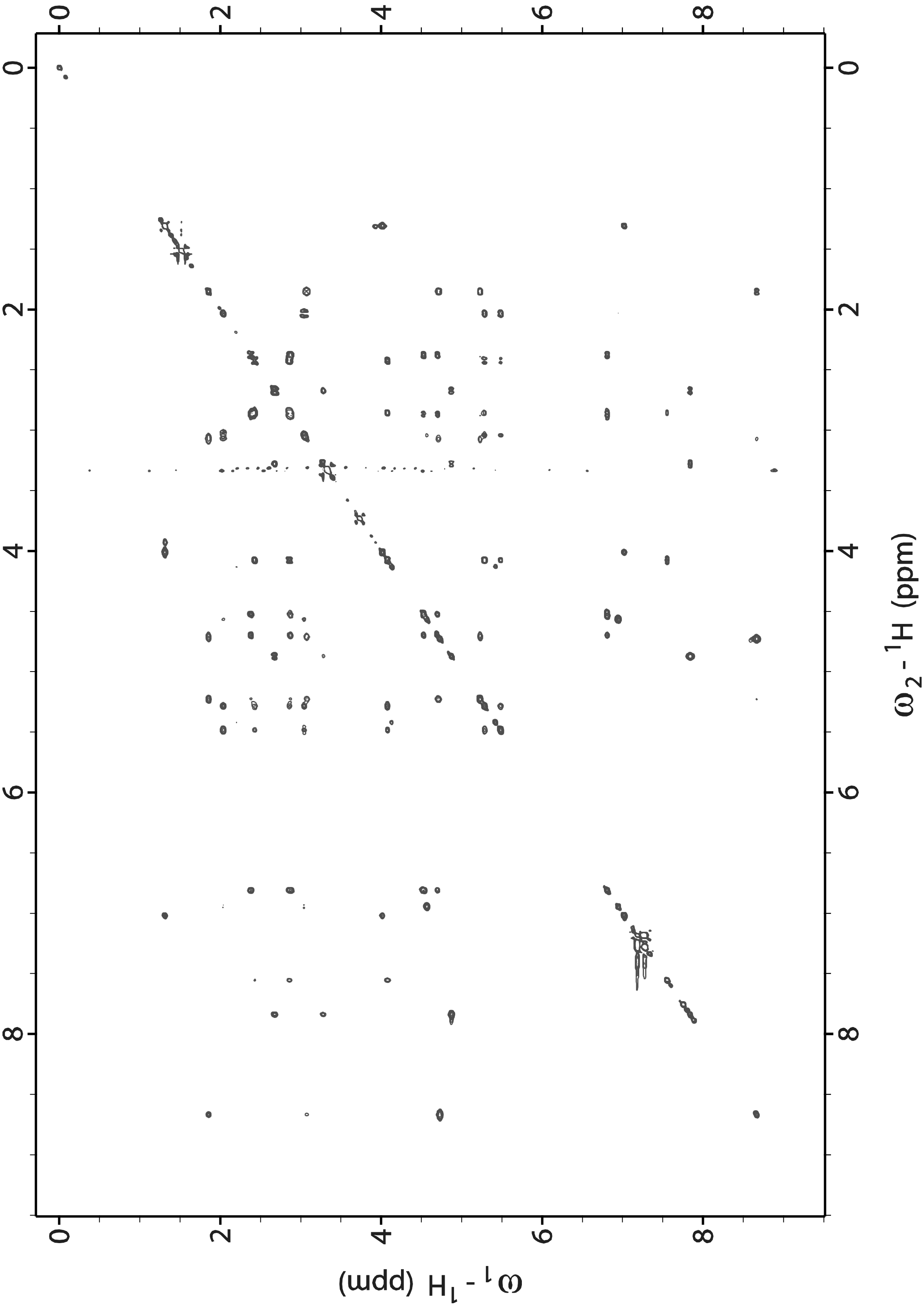


Figure SI 16: ^{1}H-TOCSY NMR spectrum of bicyclo[$E^{1\text{-}4}$/$E^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2d**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

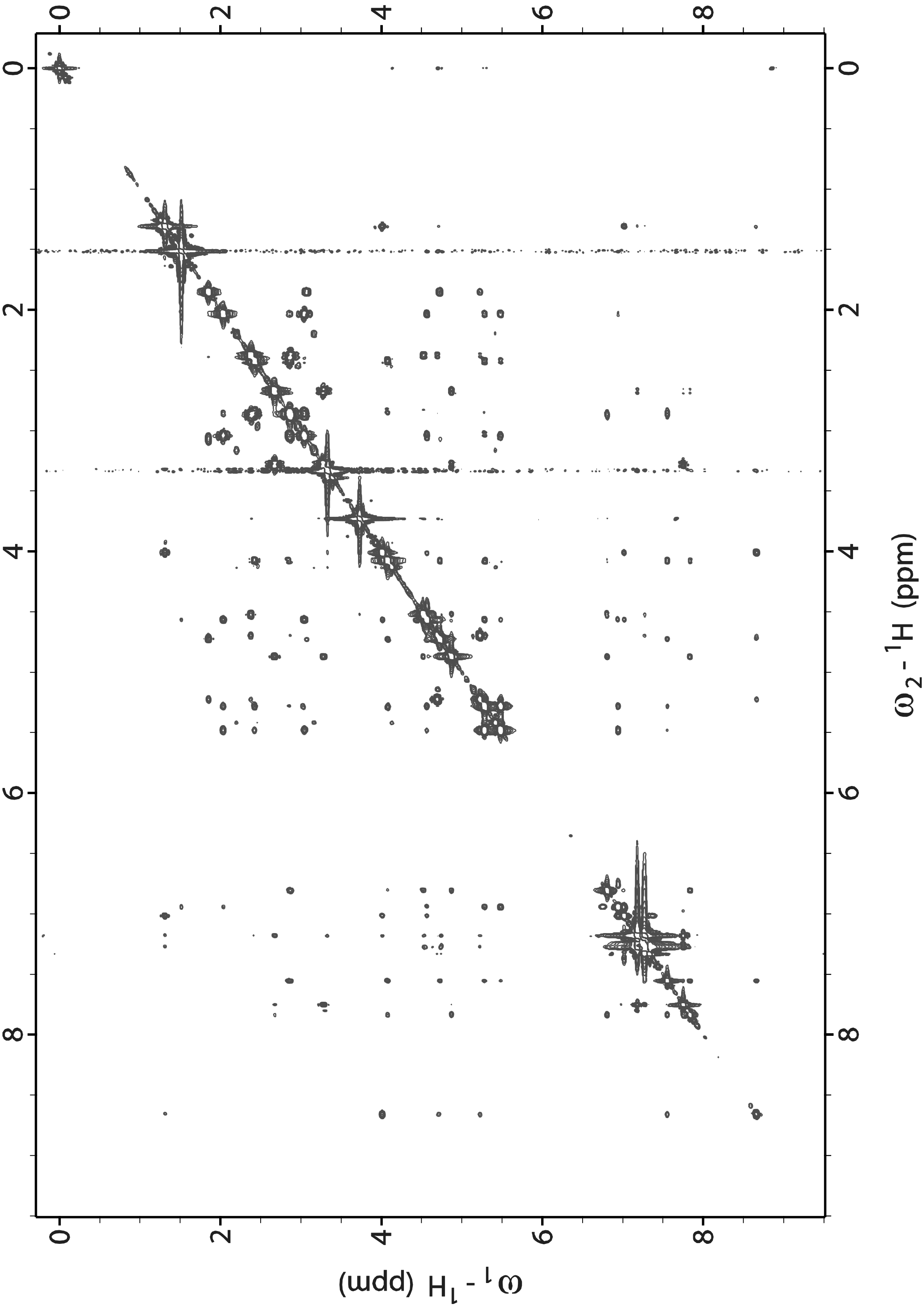


Figure SI 17: ^{1}H-ROESY NMR spectrum of bicyclo[$E^{1\text{-}4}$/$E^{3\text{-}6}$]-Boc-D-Alg^1-Ala^2-D-Alg^3-Alg^4-Asn(Trt)^5-Alg^6-OMe (**2d**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

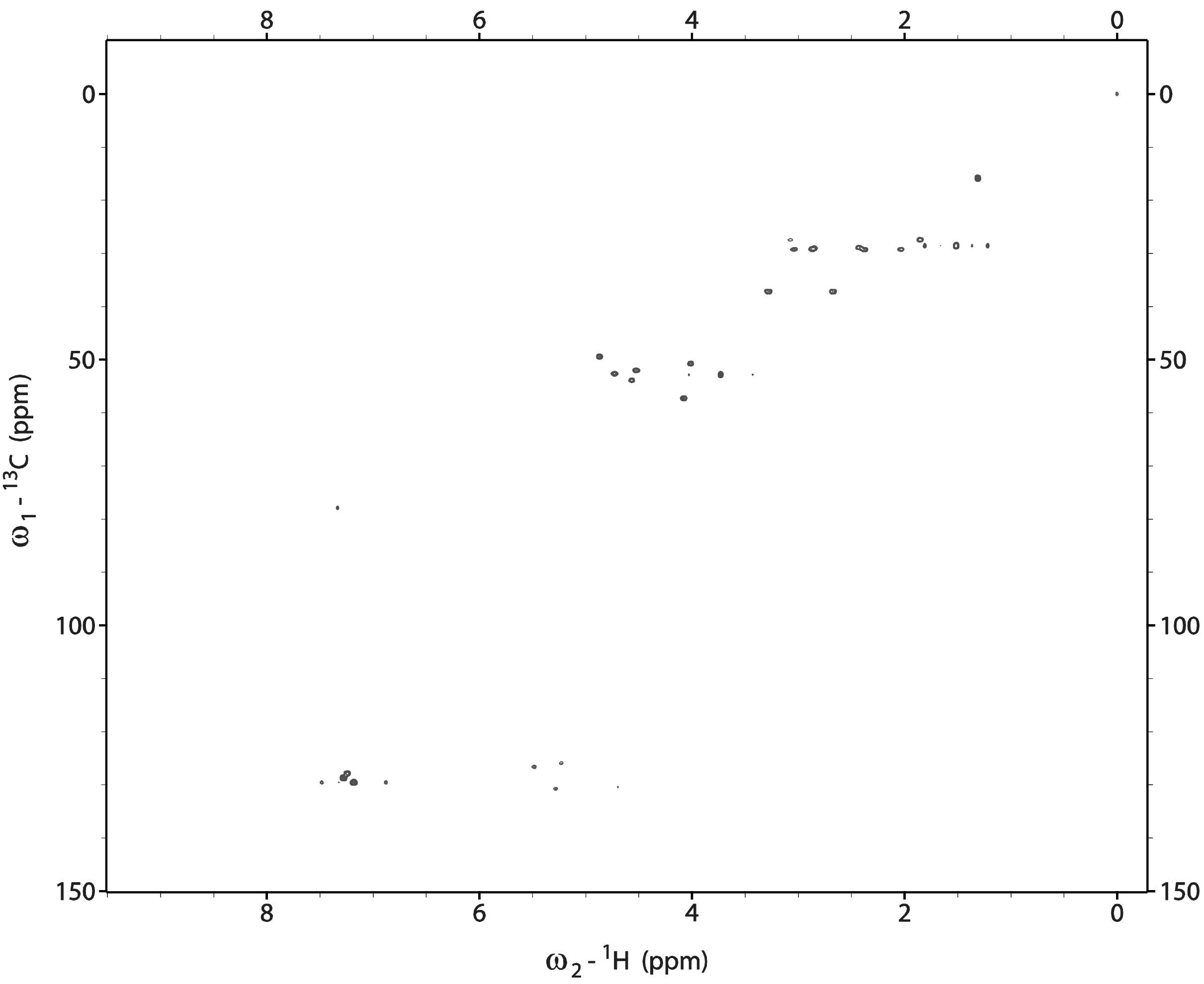


Figure SI 18: ^{1}H/^{13}C-HSQC NMR spectrum of bicyclo[E^{1-4}/E^{3-6}]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2d**) (500 MHz, $CDCl_3$/CD_3OH 95:5 v/v, T = 298 K).

4. Copies of ^{1}H, ^{1}H COSY and ^{13}C (APT) NMR spectra of compounds 7, 9-11.

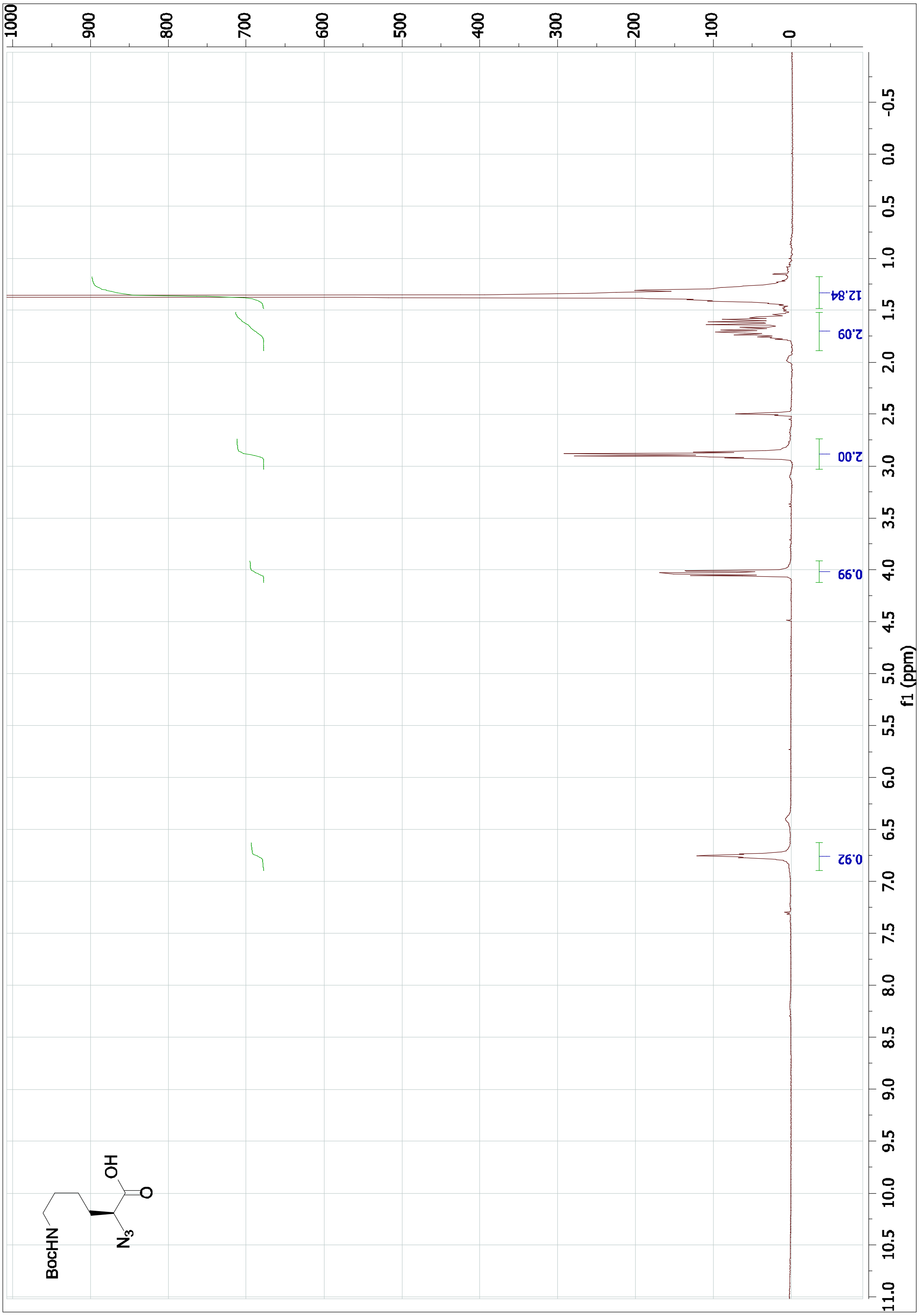


Figure SI 19: ^{1}H NMR spectrum of N_3-Lys(Boc)-OH (**7**) (300 MHz, DMSO-d_6, T = 298 K).

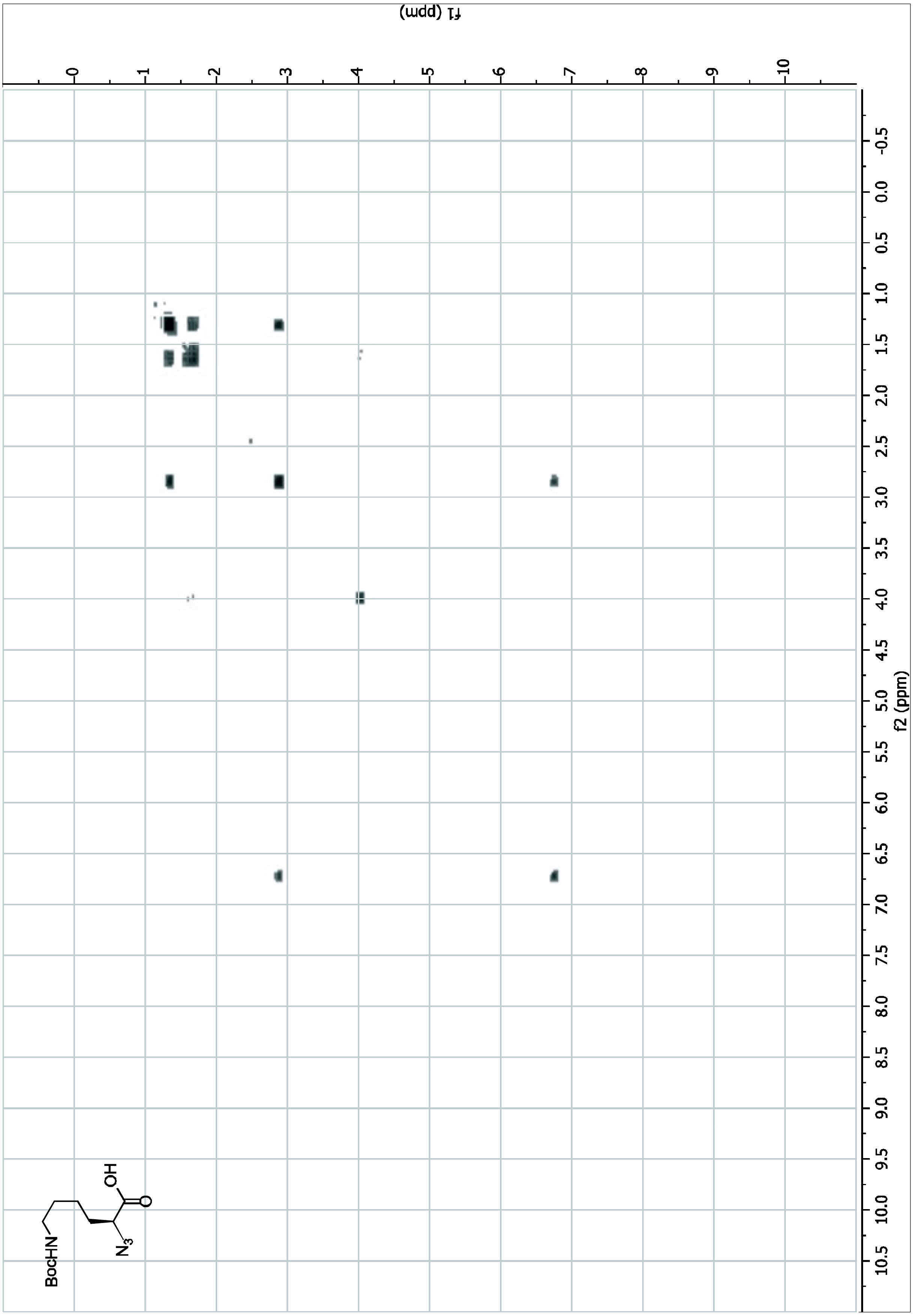


Figure SI 20: ^{1}H-COSY NMR spectrum of N_3-Lys(Boc)-OH (**7**) (300 MHz, DMSO-d_6, T = 298 K).

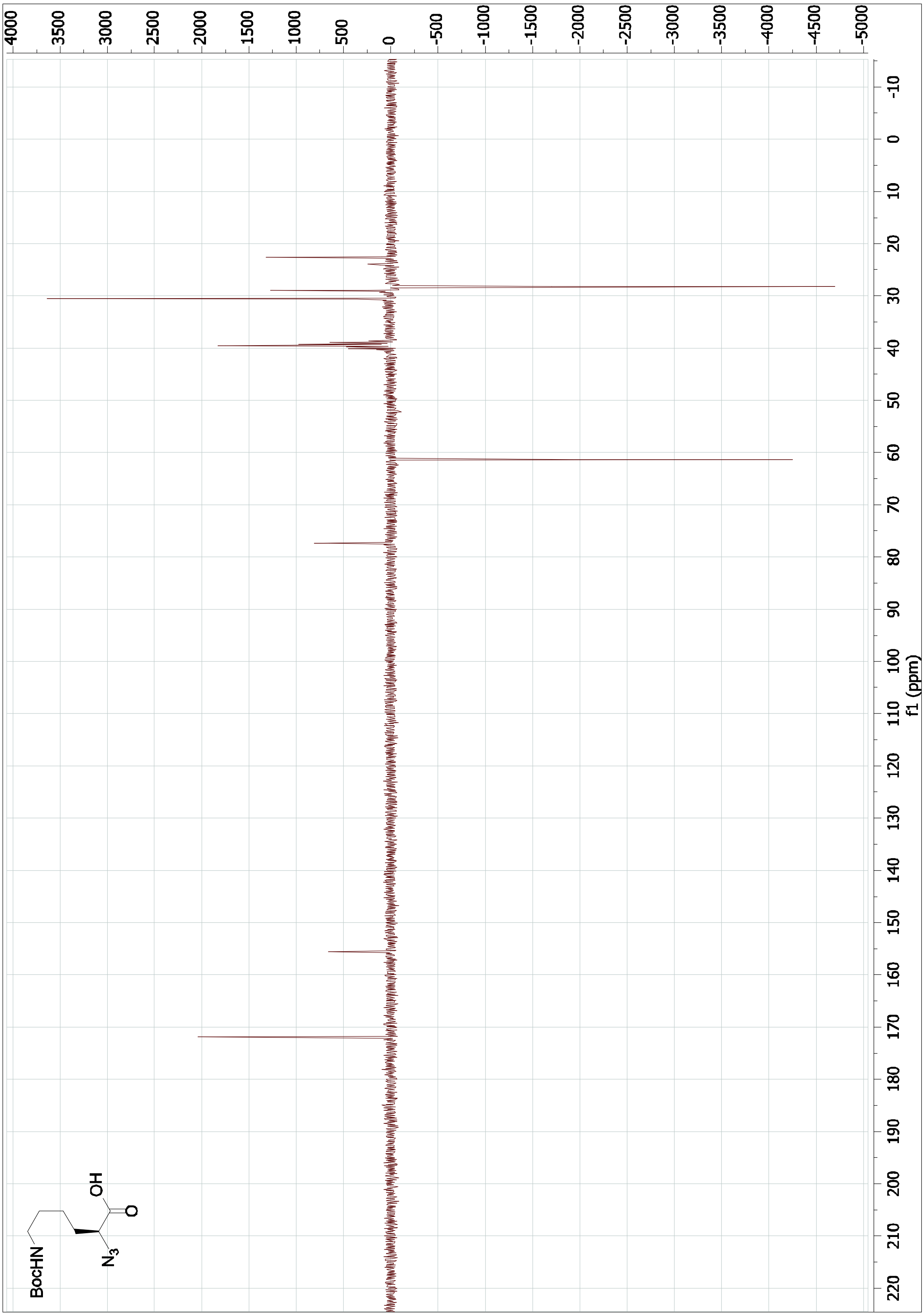


Figure SI 21: ^{13}C (APT) NMR spectrum of N_3-Lys(Boc)-OH (**7**) (75.5 MHz, DMSO-d_6, T = 298 K).

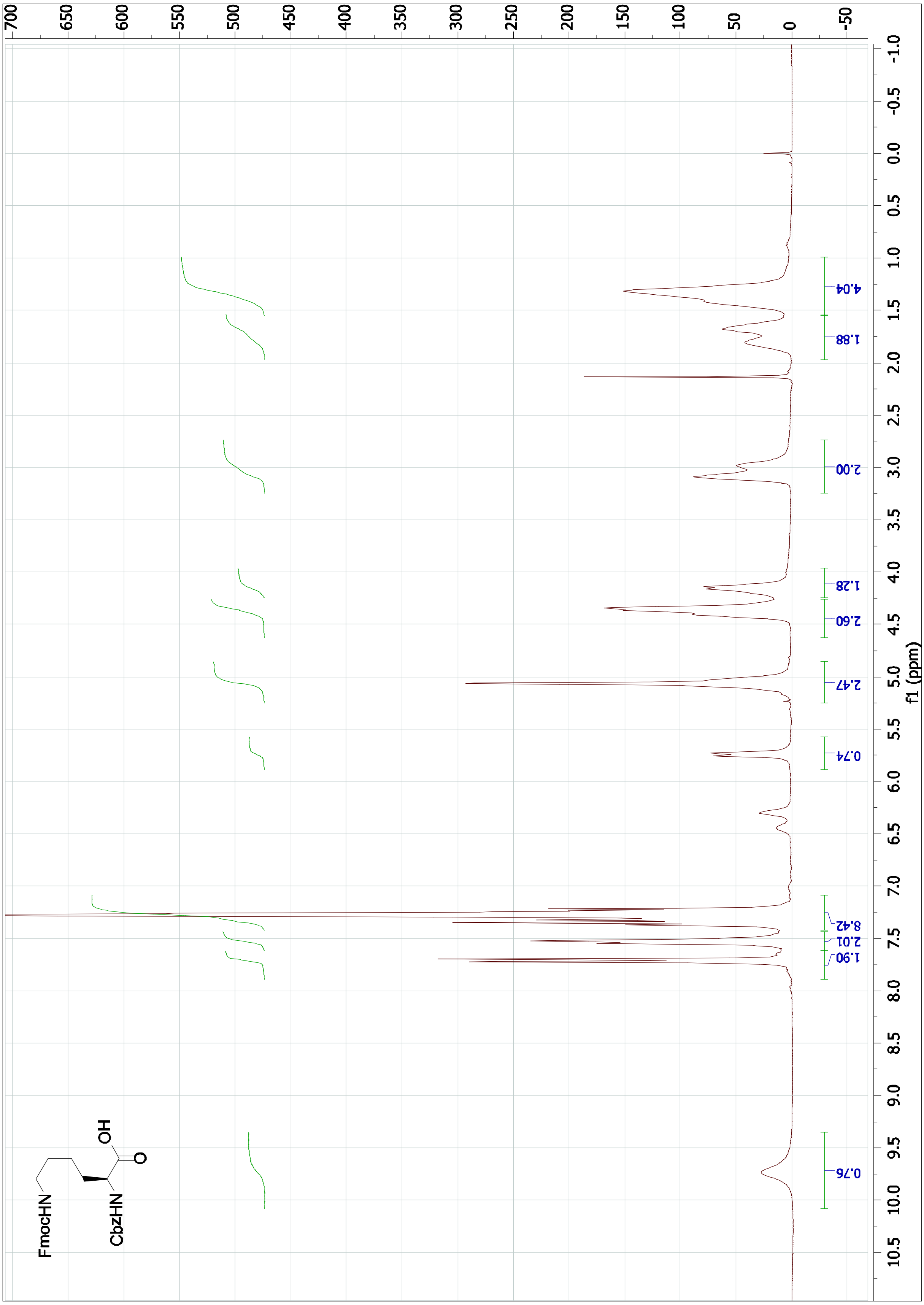


Figure SI 22: ^{1}H NMR spectrum of Cbz-Lys(Fmoc)-OH (**9**) (300 MHz, $CDCl_3$, T = 298 K).

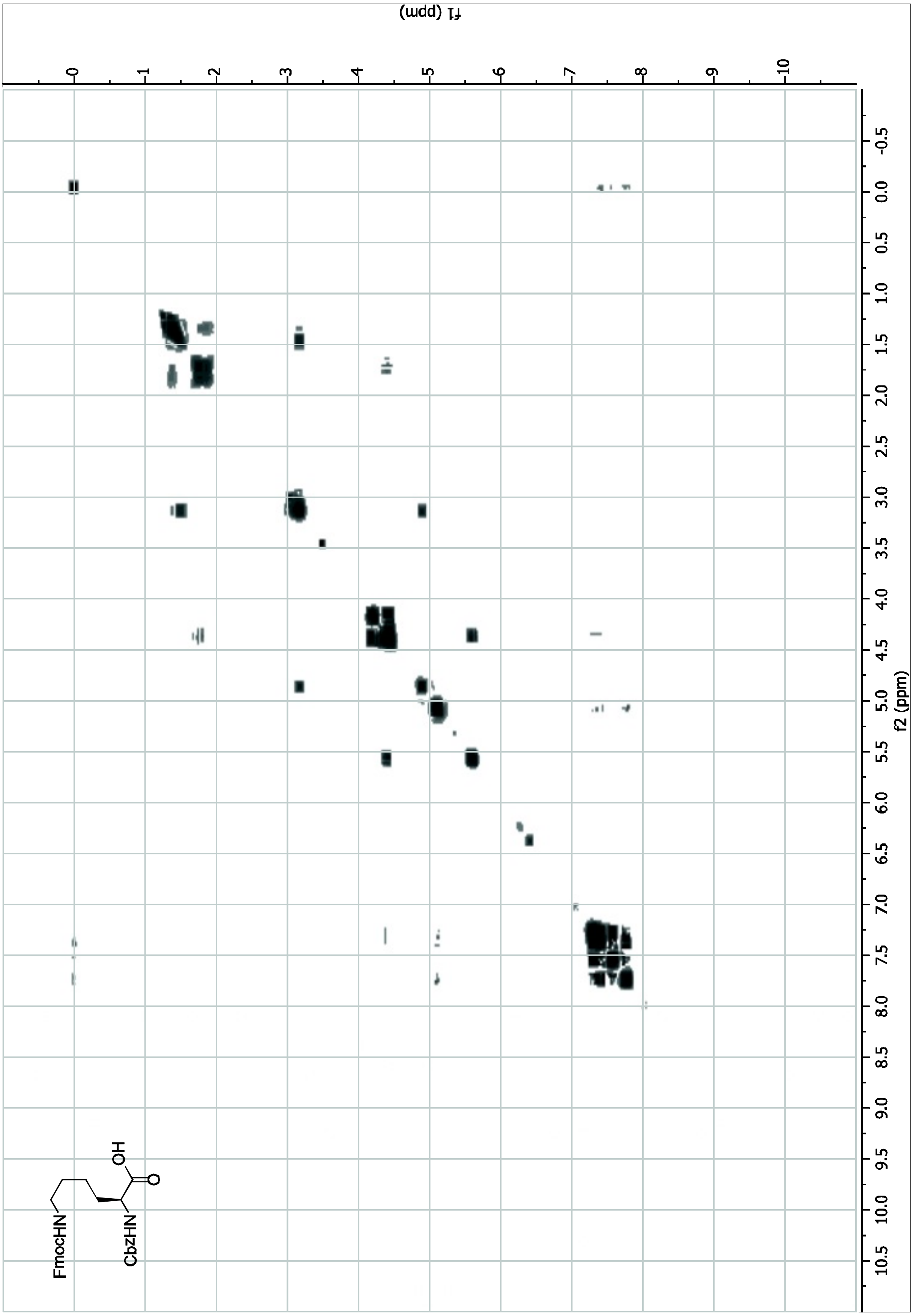


Figure SI 23: ^{1}H-COSY NMR spectrum of Cbz-Lys(Fmoc)-OH (**9**) (300 MHz, $CDCl_3$, T = 298 K).

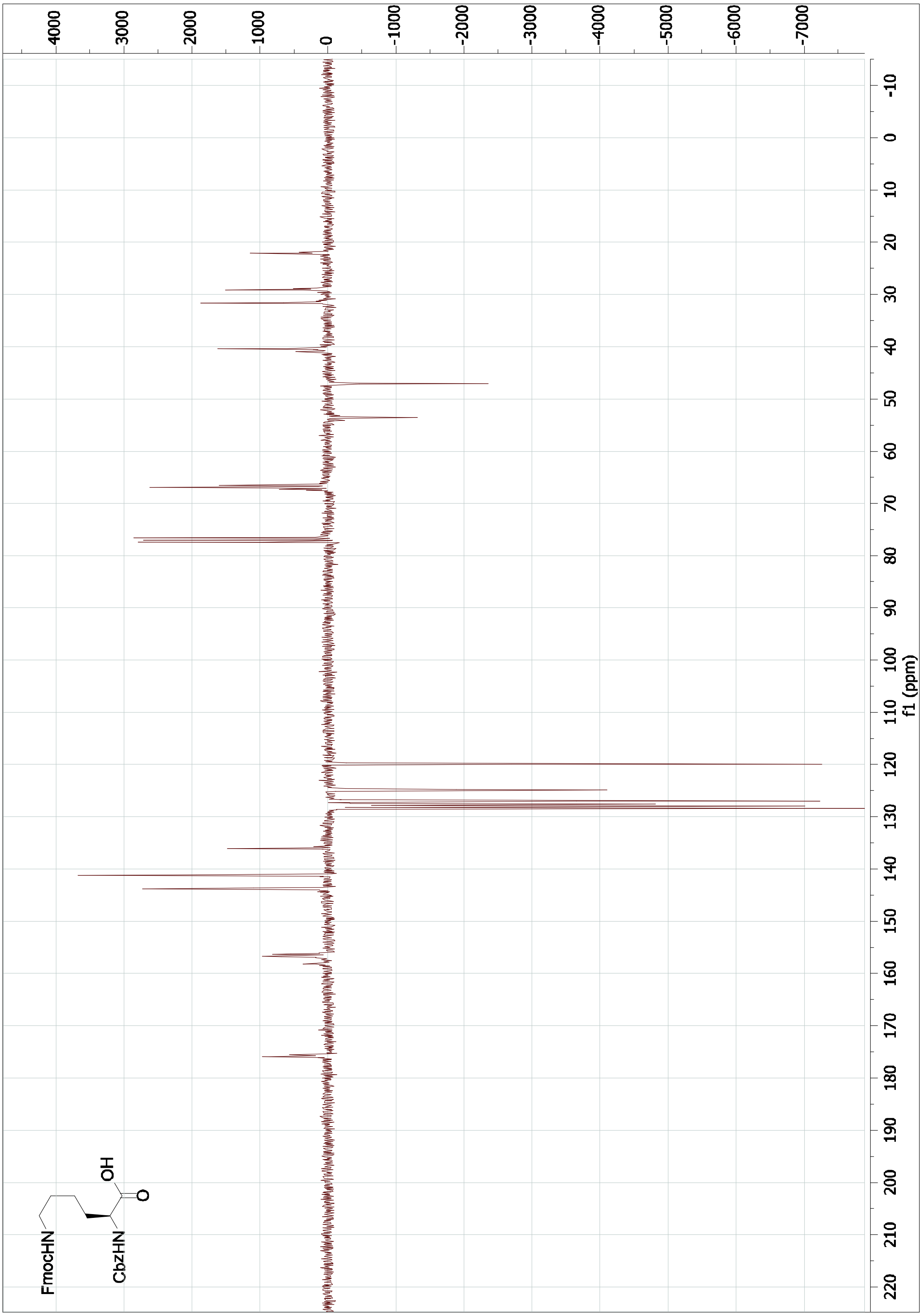


Figure SI 24: ^{13}C (APT) NMR spectrum of Cbz-Lys(Fmoc)-OH (**9**) (75.5 MHz, $CDCl_3$, T = 298 K).

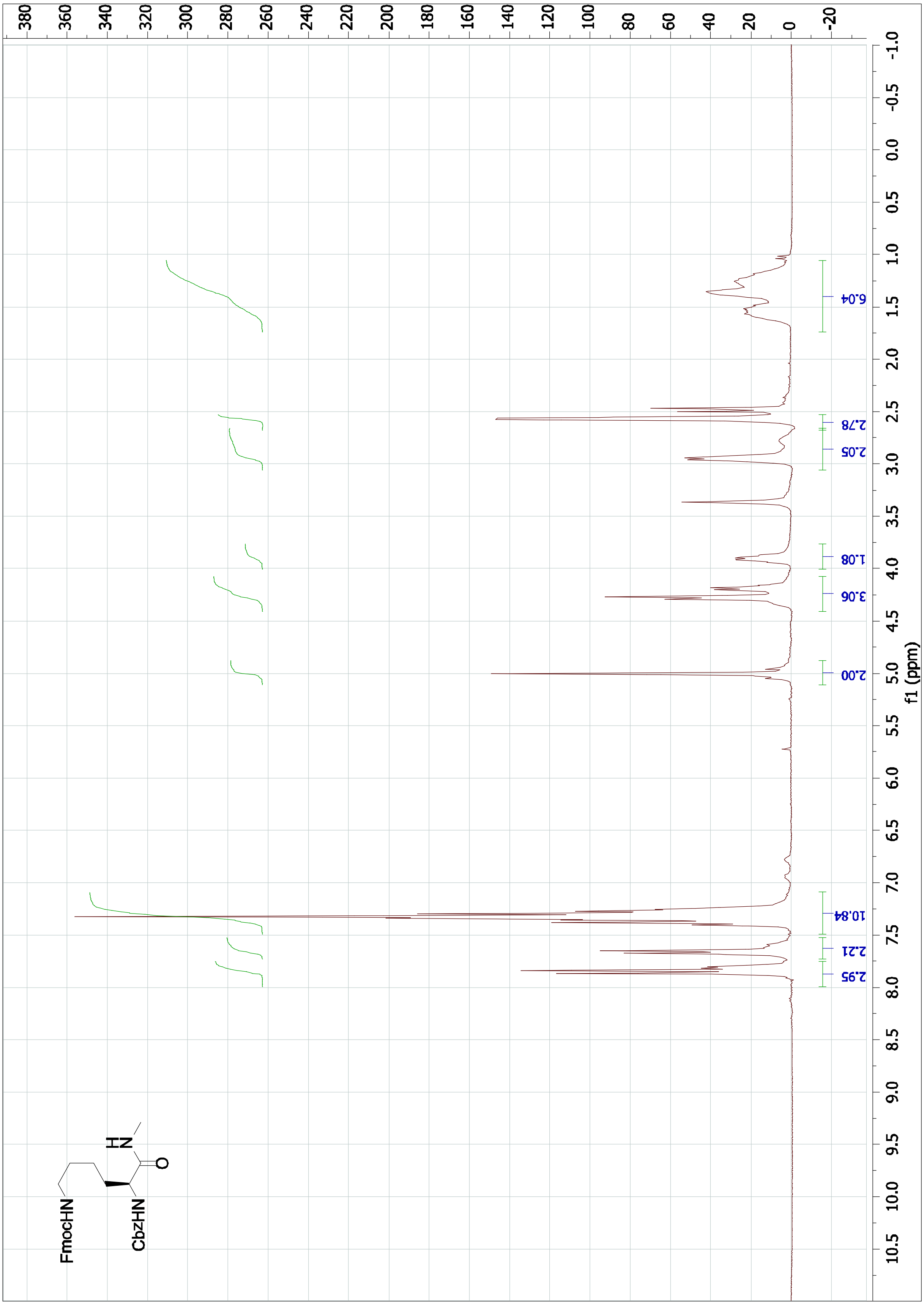


Figure SI 25: ^{1}H NMR spectrum of Cbz-Lys(Fmoc)-NHMe (**10**) (300 MHz, DMSO-d$_6$, T = 298 K).

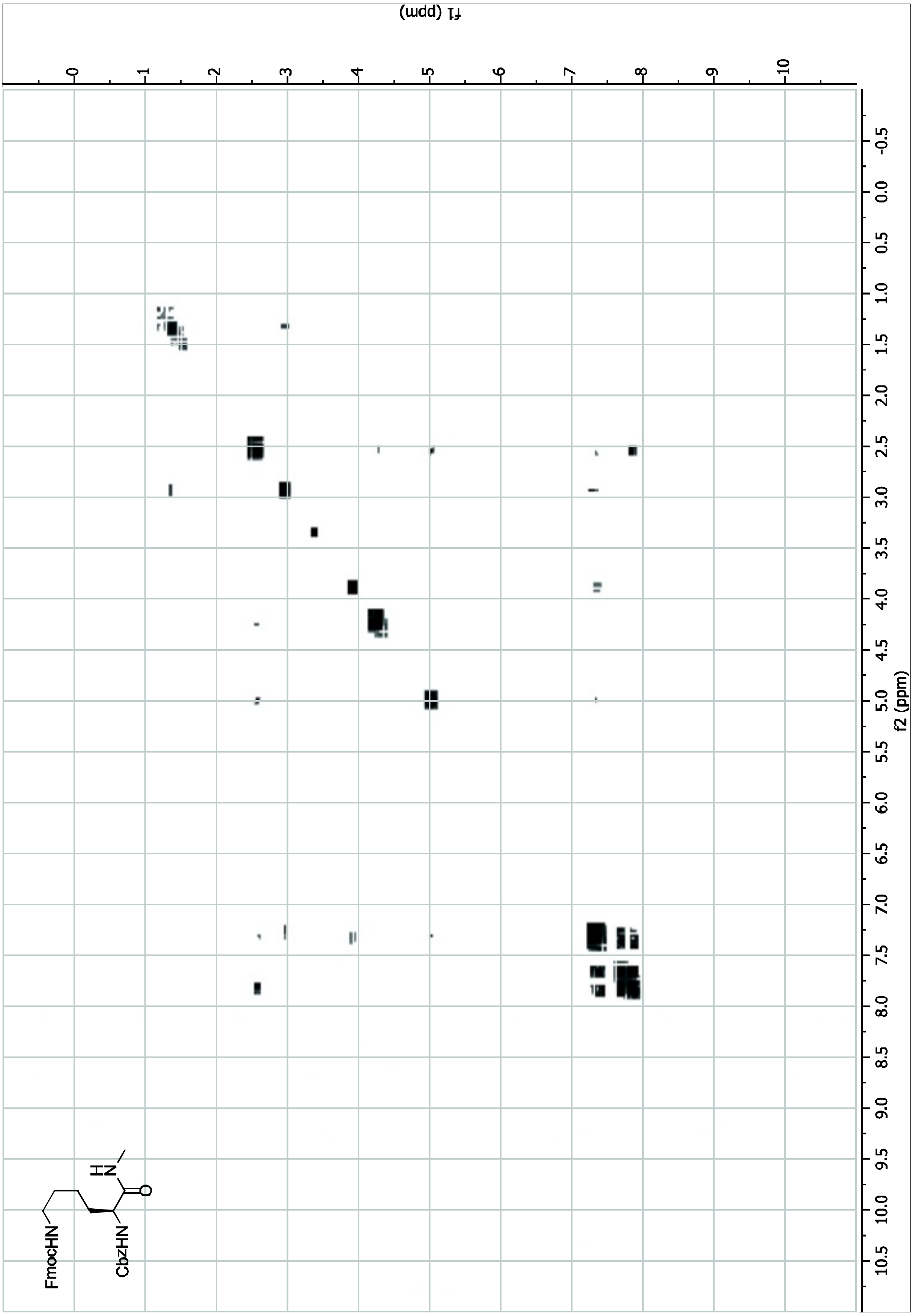


Figure SI 26: ^{1}H-COSY NMR spectrum of Cbz-Lys(Fmoc)-NHMe (**10**) (300 MHz, DMSO-d_6, T = 298 K).

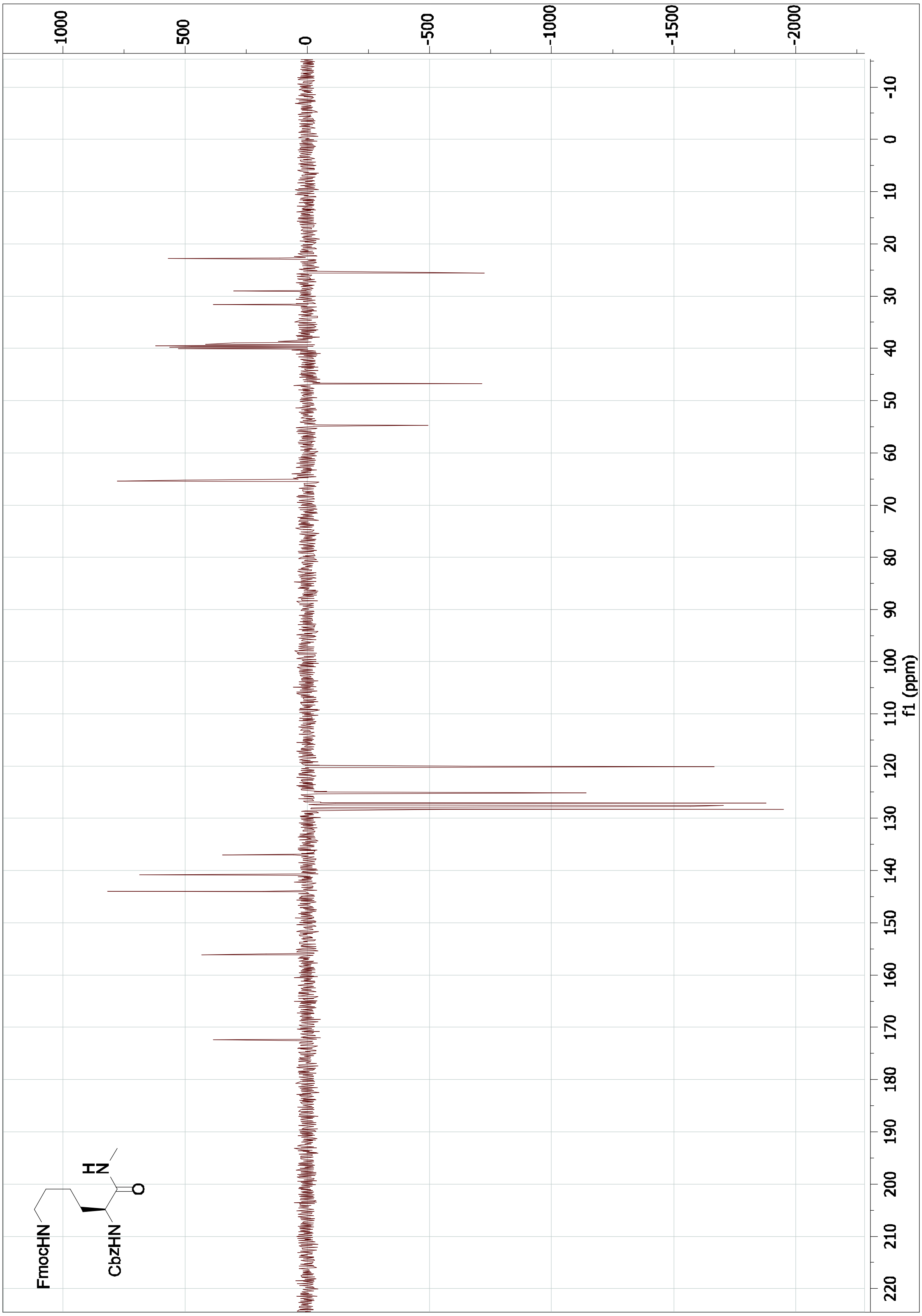


Figure SI 27: ^{13}C (APT) NMR spectrum of Cbz-Lys(Fmoc)-NHMe (**10**) (75.5 MHz, DMSO-d_6, T = 298 K).

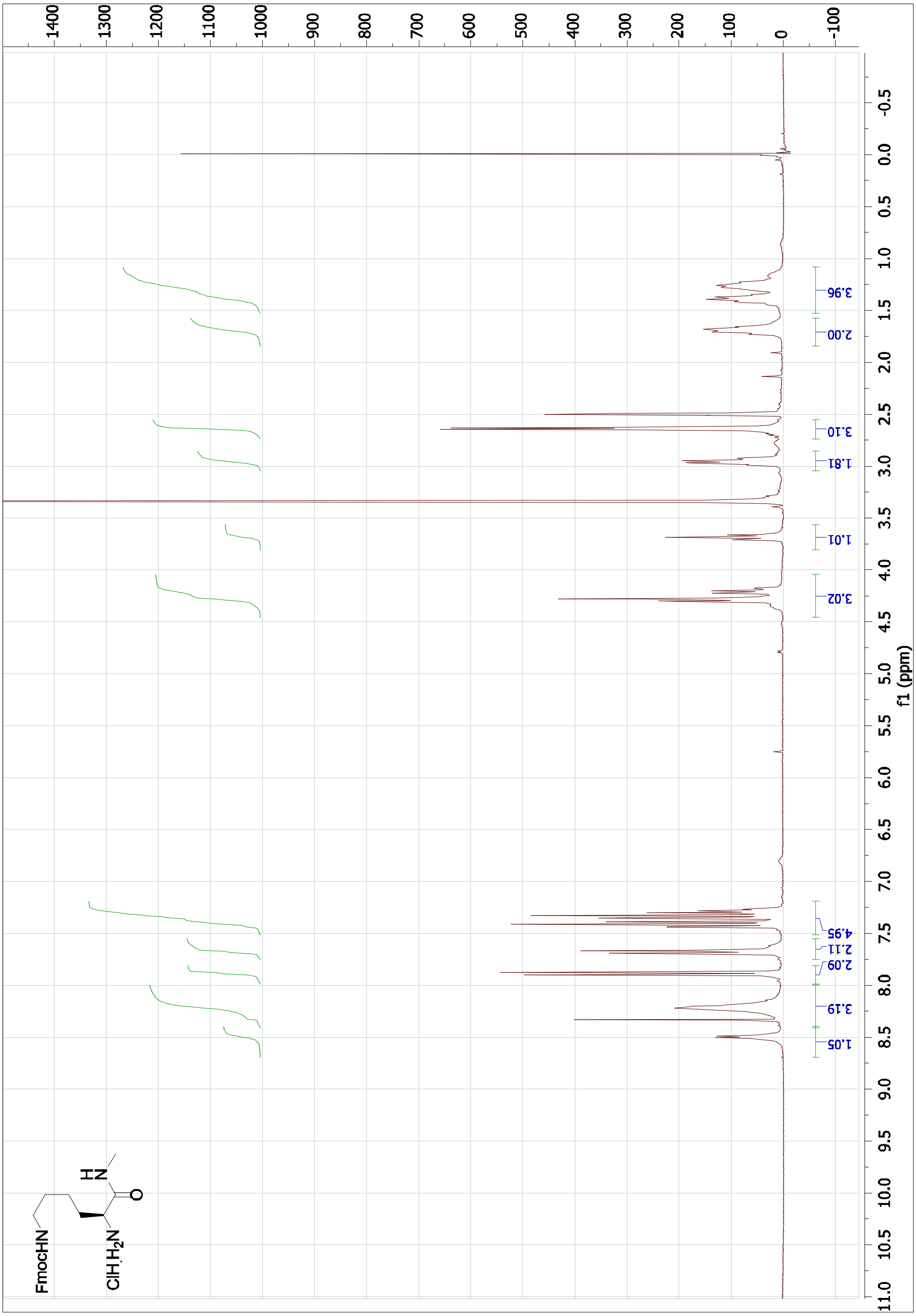


Figure SI 28: ^{1}H NMR spectrum of HCl·H-Lys(Fmoc)-NHMe (**11**) (300 MHz, DMSO-d$_6$, T = 298 K).

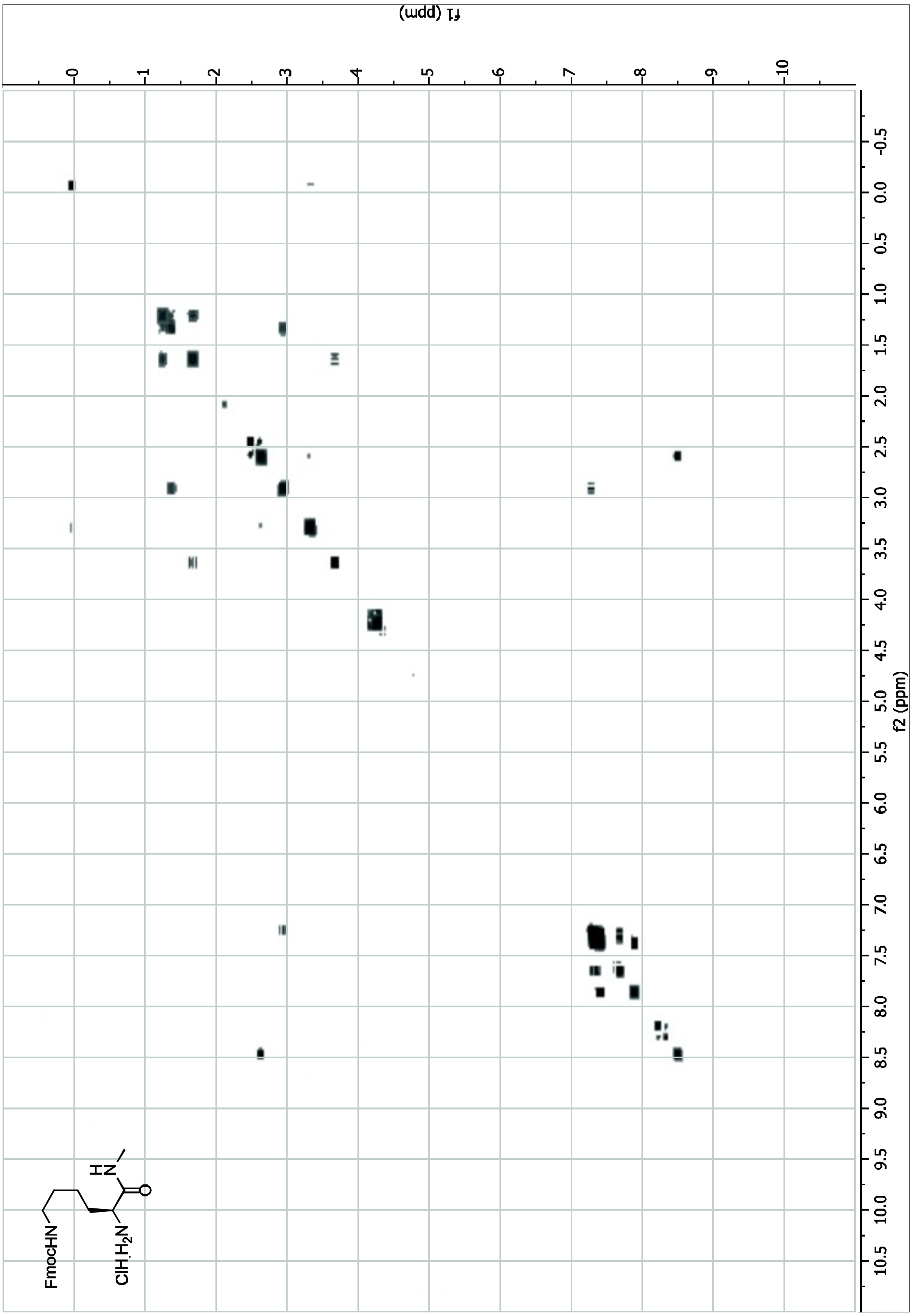


Figure SI 29: ^{1}H-COSY NMR spectrum of HCl·H-Lys(Fmoc)-NHMe (**11**) (300 MHz, DMSO-d$_6$, T = 298 K).

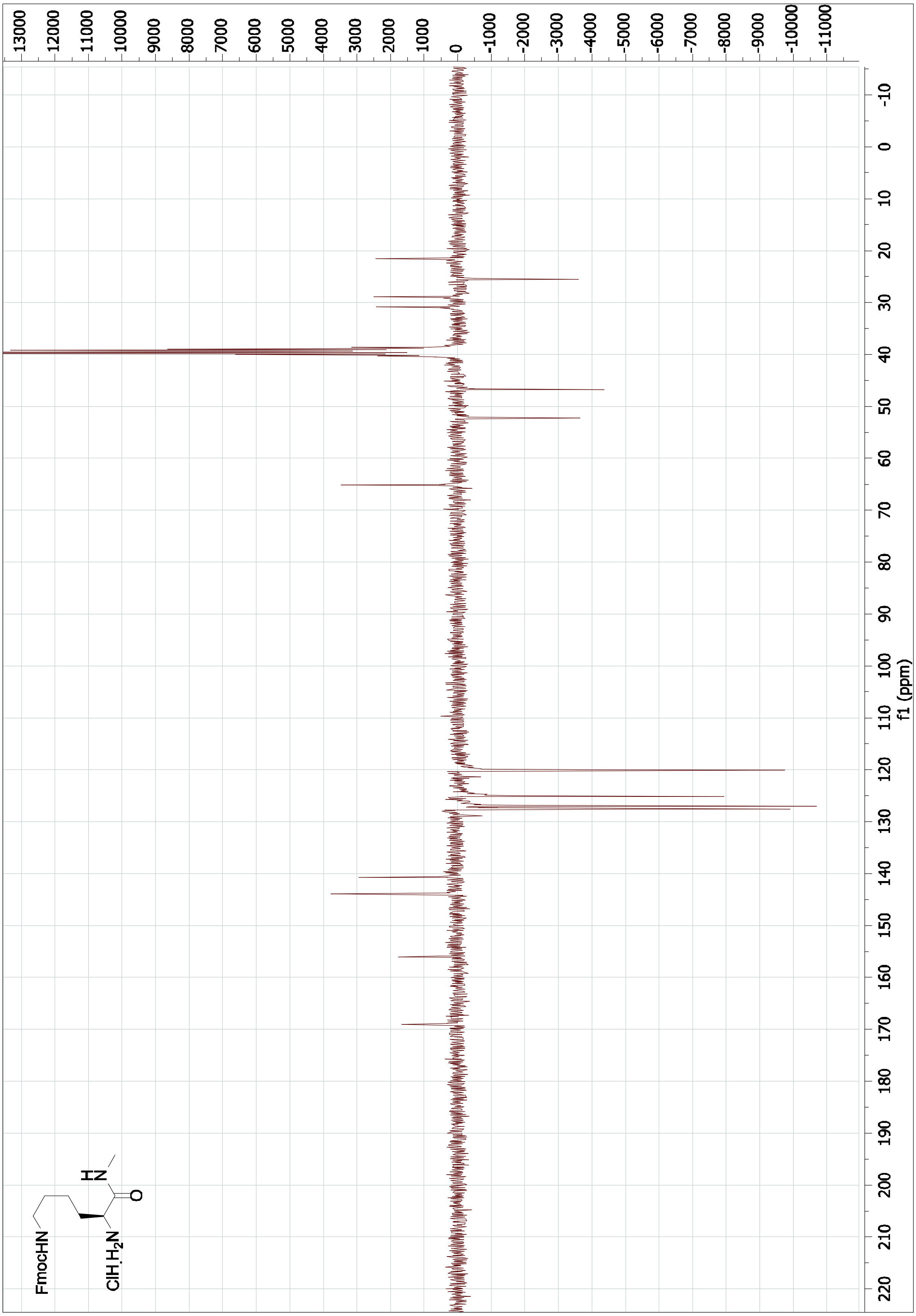
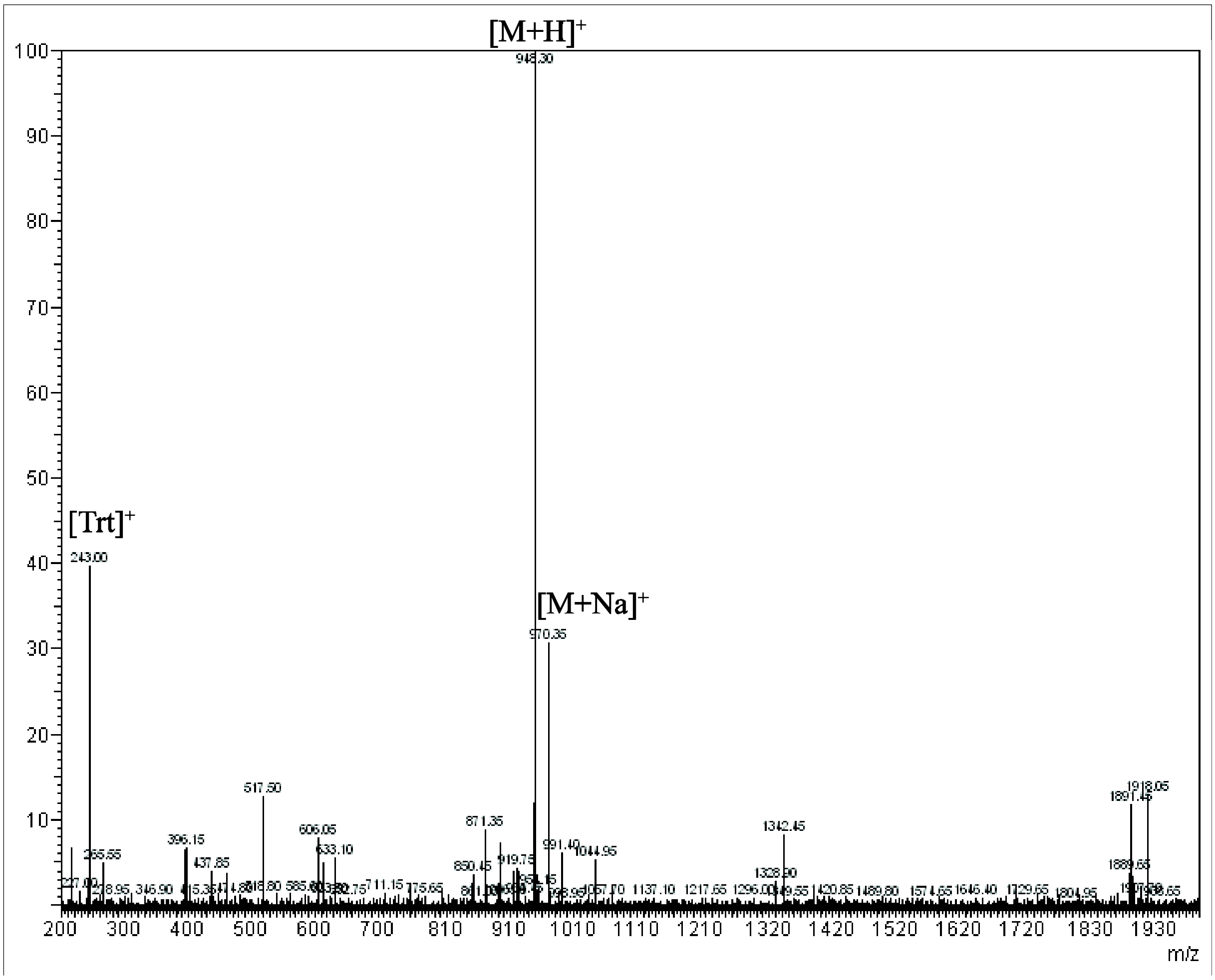


Figure SI 30: ^{13}C (APT) NMR spectrum of HCl·H-Lys(Fmoc)-NHMe (**11**) (75.5 MHz, DMSO-d_6, T = 298 K).

5. Copies of ESI-MS spectra and HPLC chromatograms of peptides 1, 2a-d, 3-5, 13.



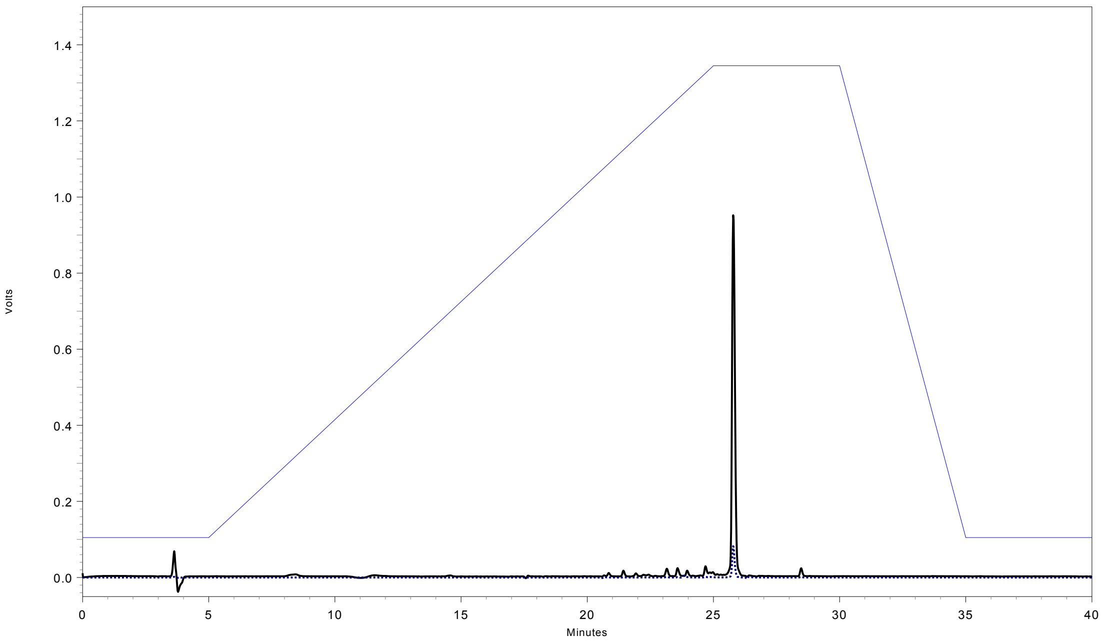
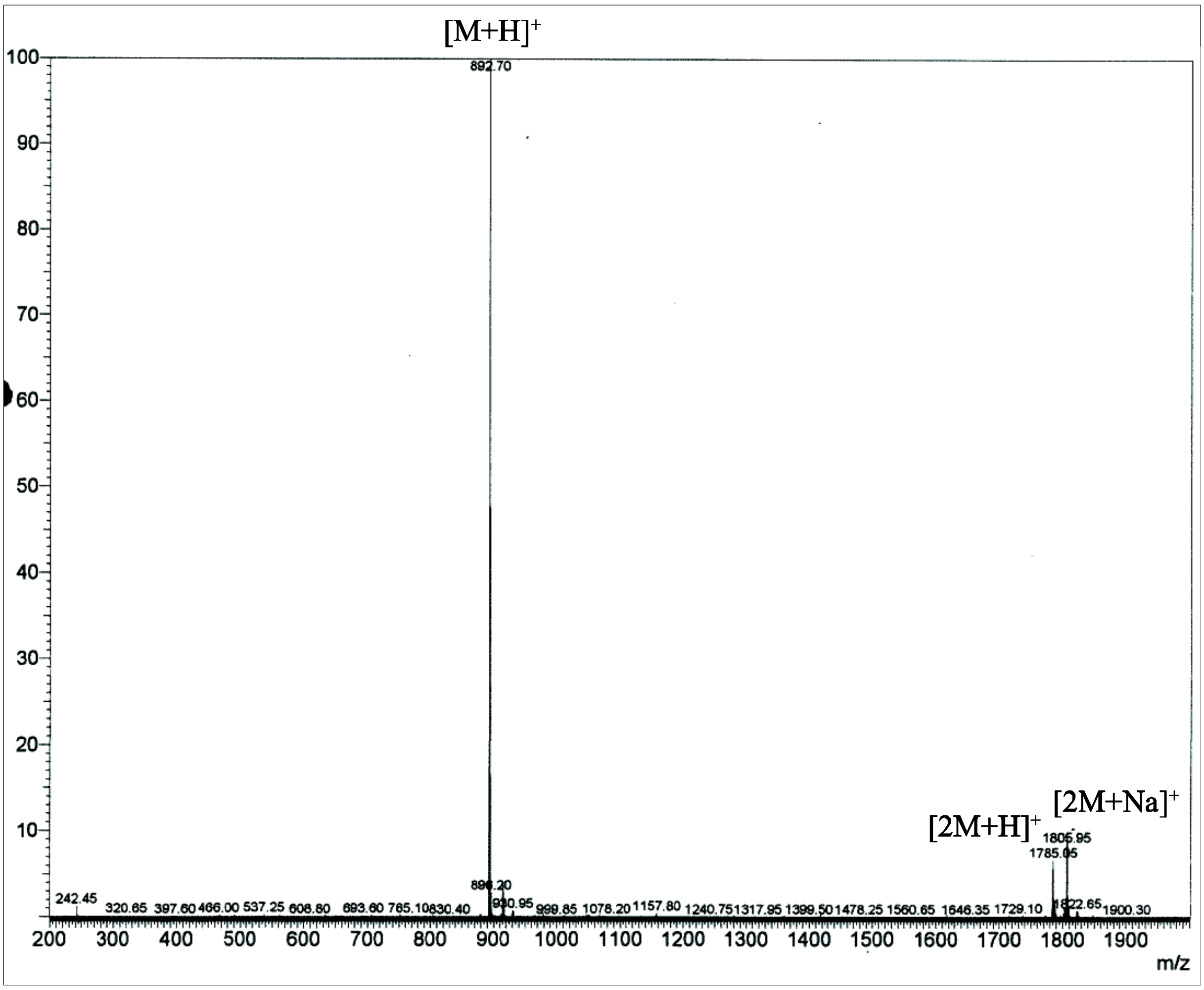


Figure SI 30: ESI-MS spectrum and HPLC chromatogram of Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**1**).



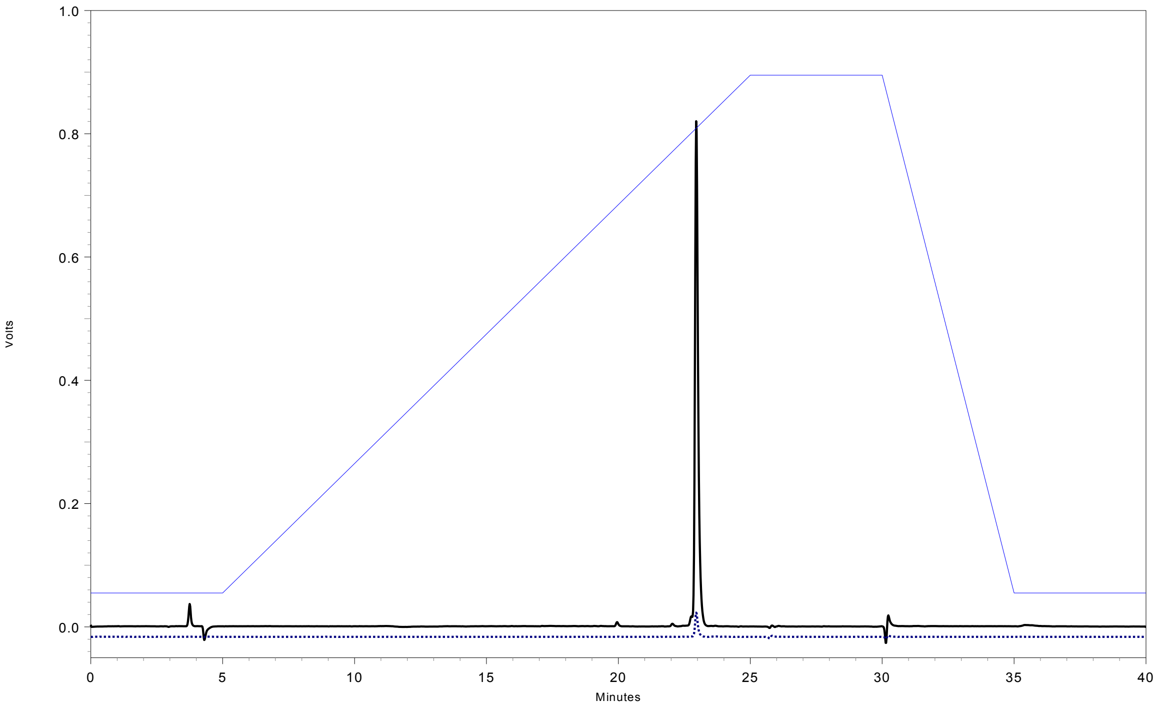
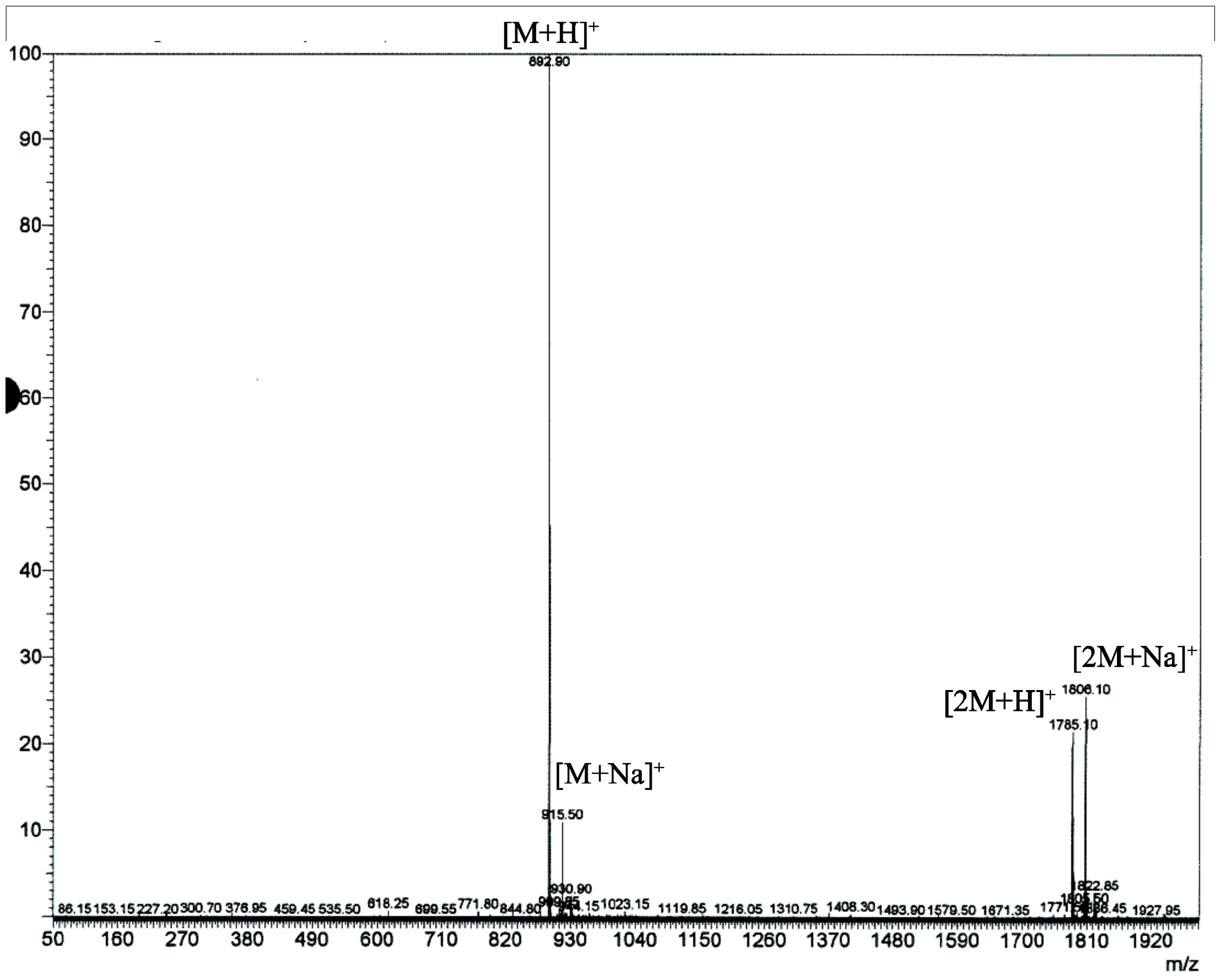


Figure SI 31: ESI-MS spectrum and HPLC chromatogram of bicyclo[$Z^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2a**).



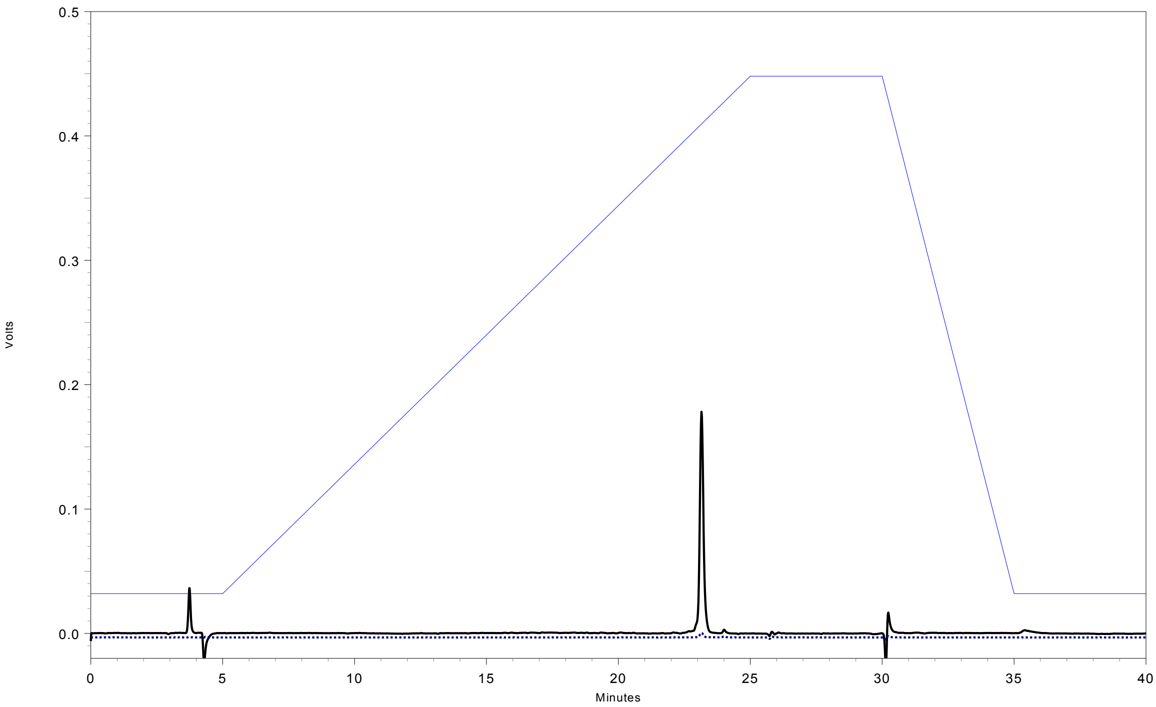
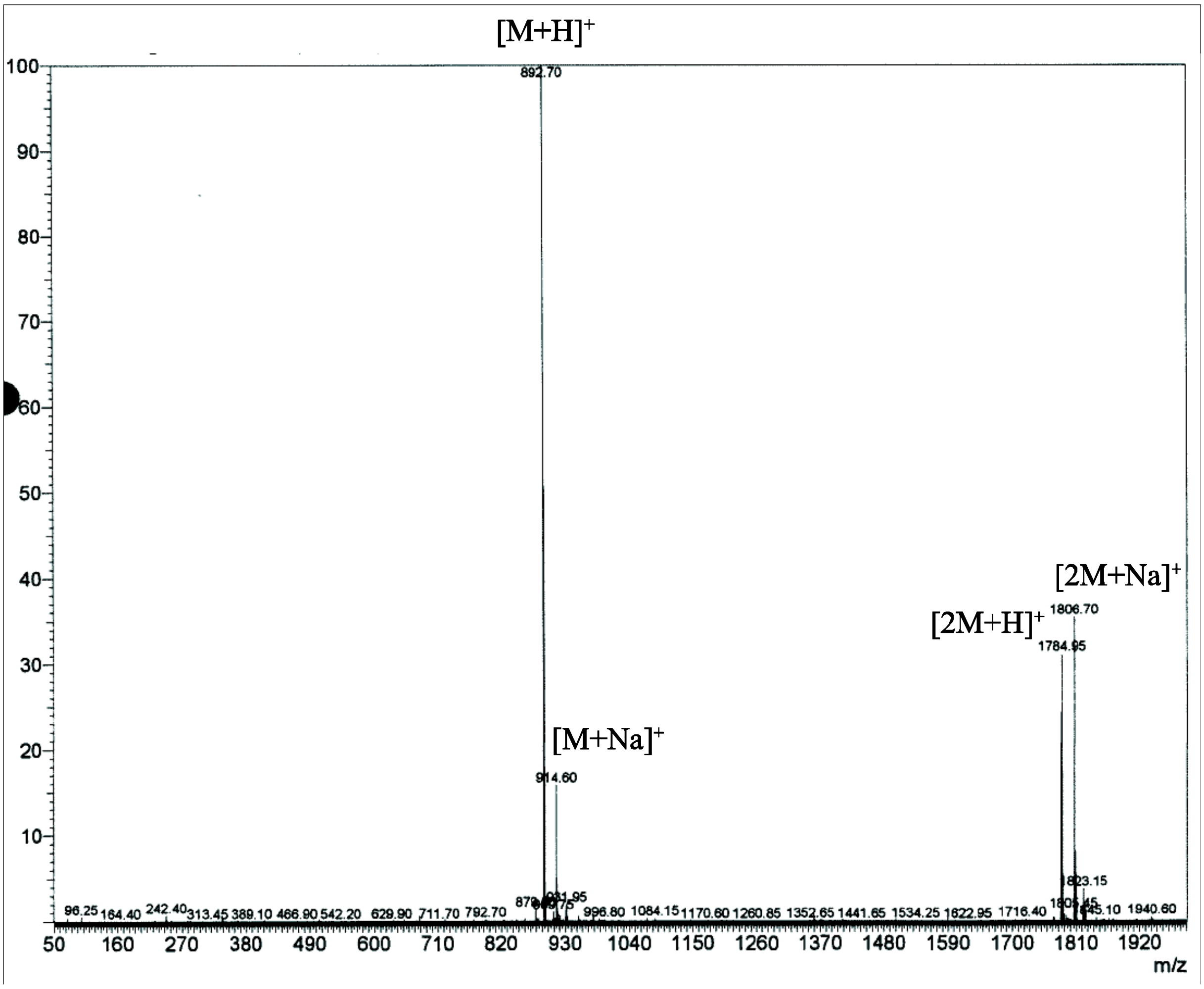


Figure SI 32: ESI-MS spectrum and HPLC chromatogram of bicyclo[$Z^{1\text{-}4}$/$E^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2b**).



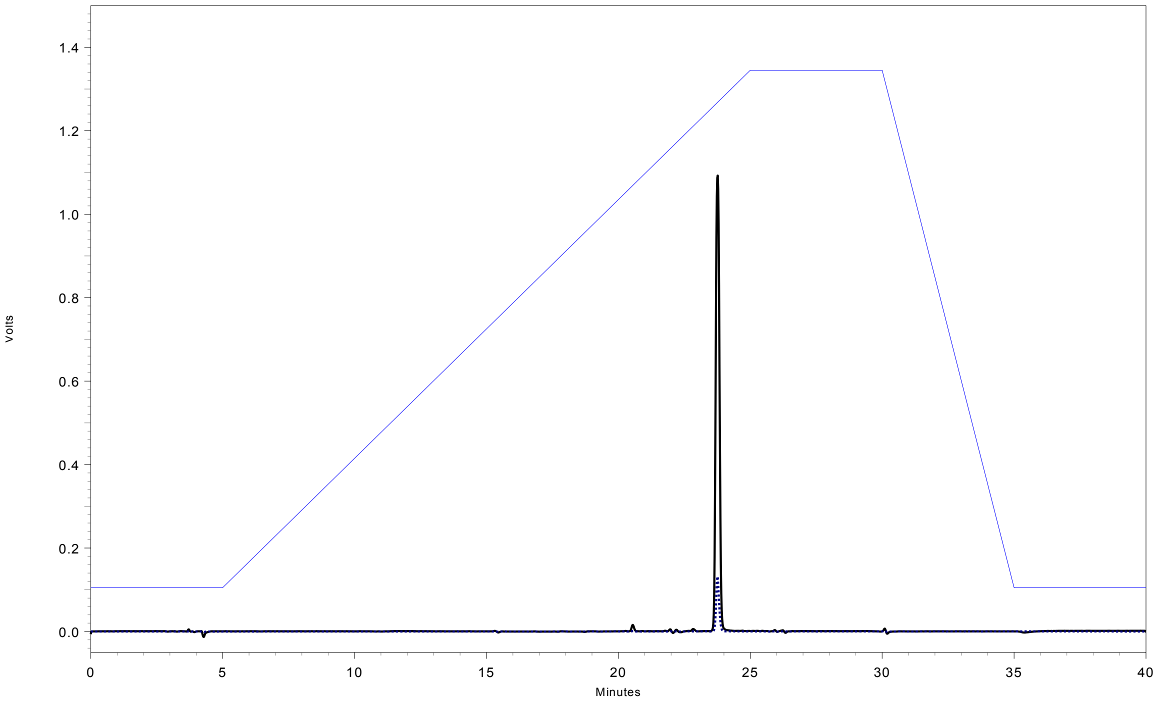
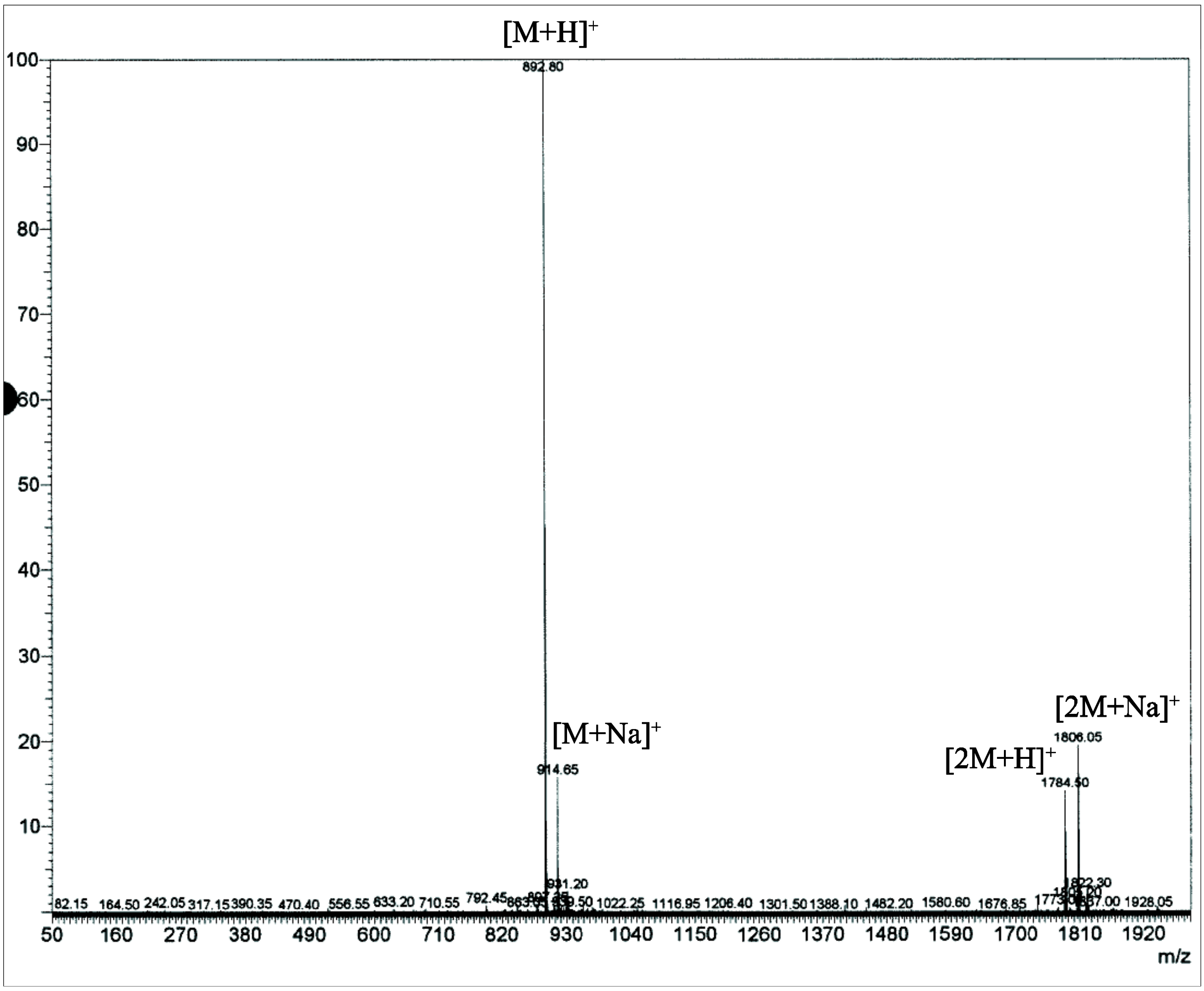


Figure SI 33: ESI-MS spectrum and HPLC chromatogram of bicyclo[$E^{1\text{-}4}$/$Z^{3\text{-}6}$]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2c**).



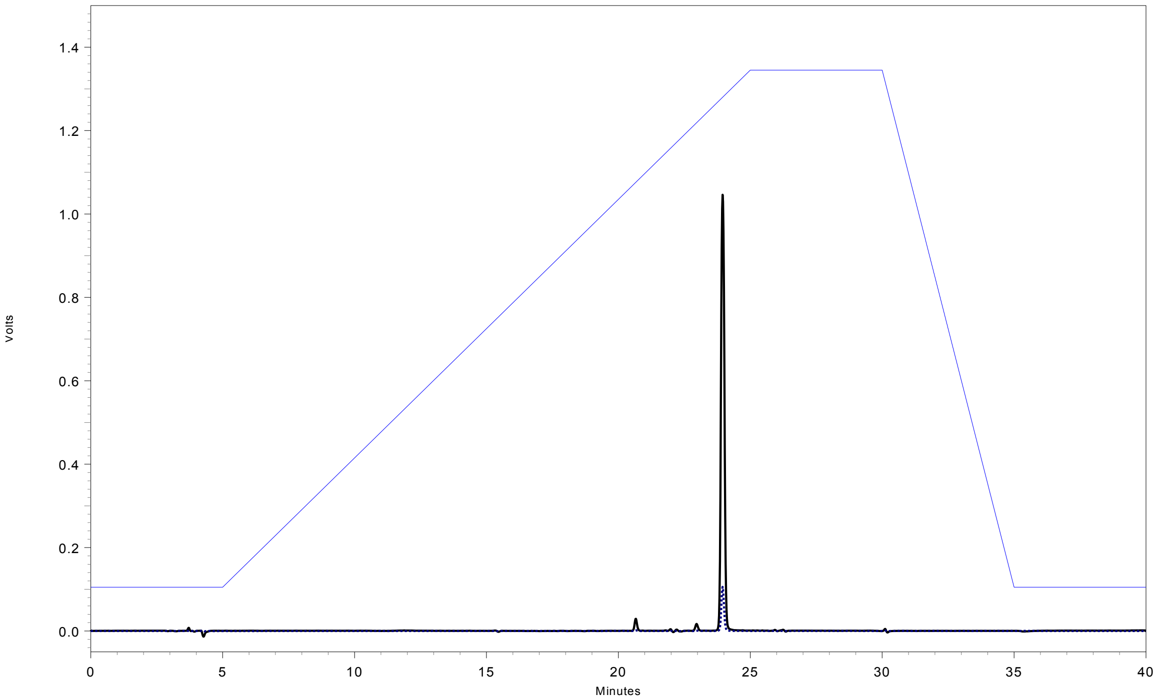


Figure SI 34: ESI-MS spectrum and HPLC chromatogram of bicyclo[E^{1-4}/Z^{3-6}]-Boc-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn(Trt)^{5}-Alg^{6}-OMe (**2d**).

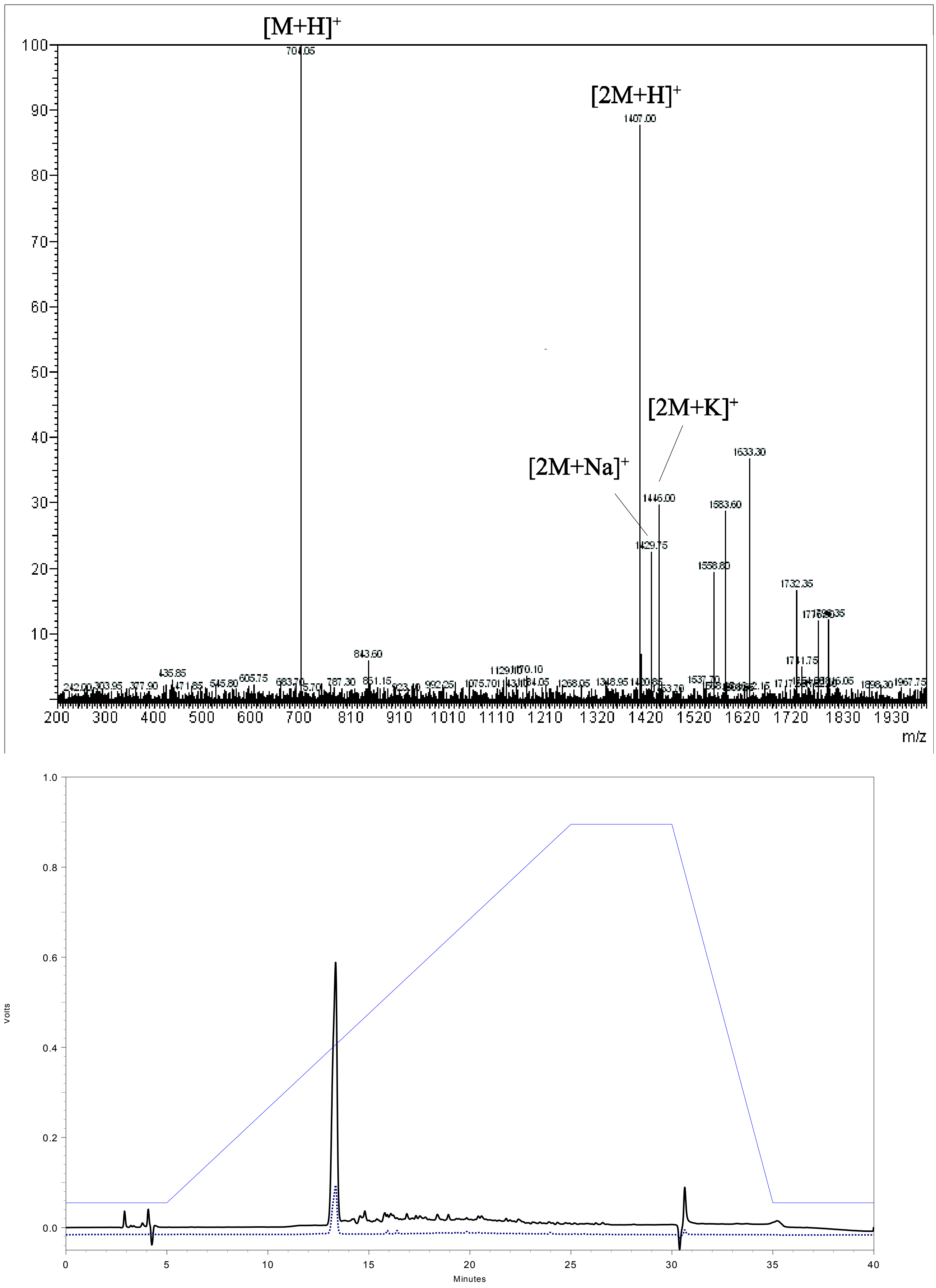
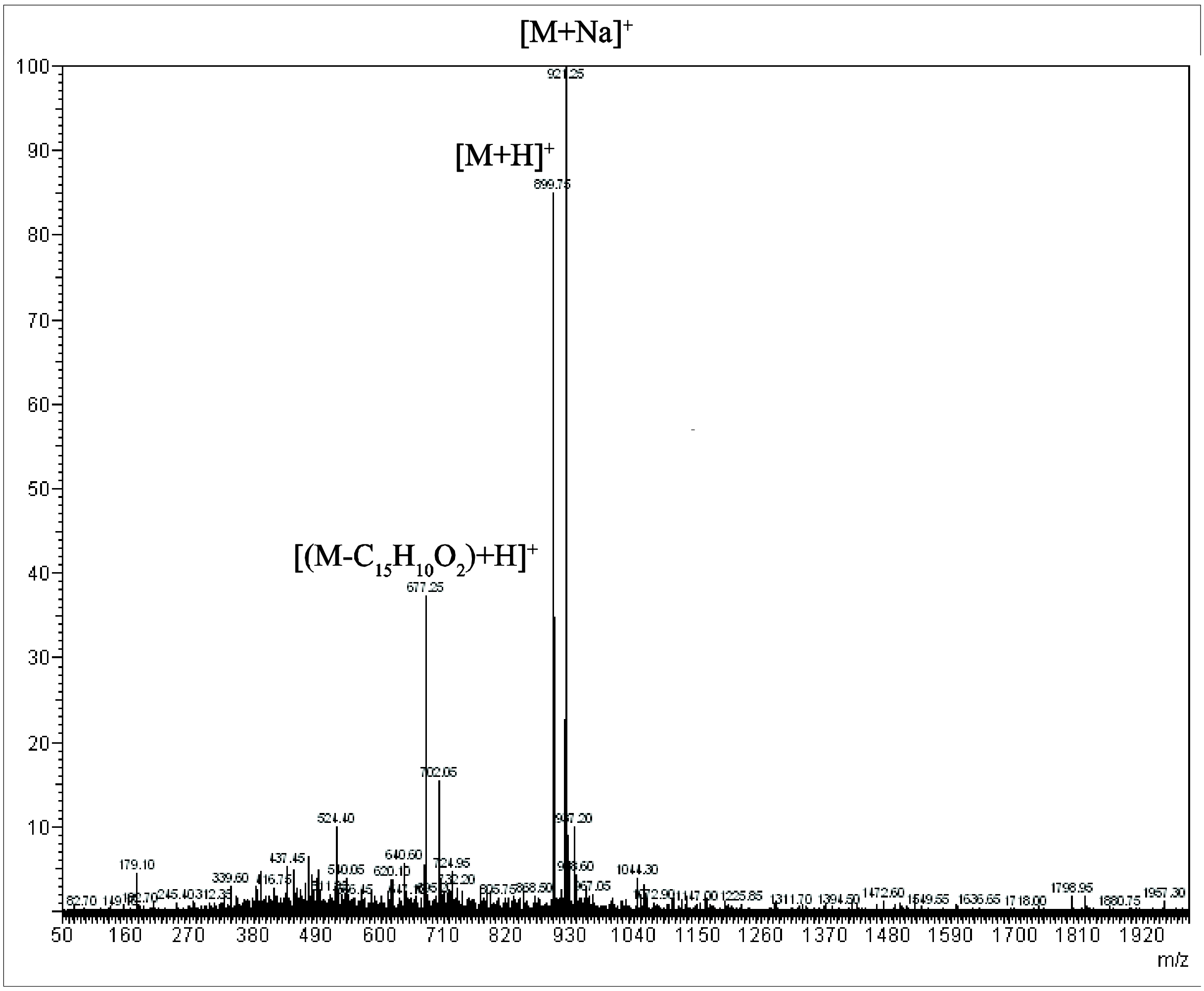


Figure SI 35: ESI-MS spectrum (after TFA deprotection step) and HPLC chromatogram of bicyclo[1-4/3-6]-N_3-Lys(Boc)-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn^{5}-Alg^{6}-OMe (**3**).



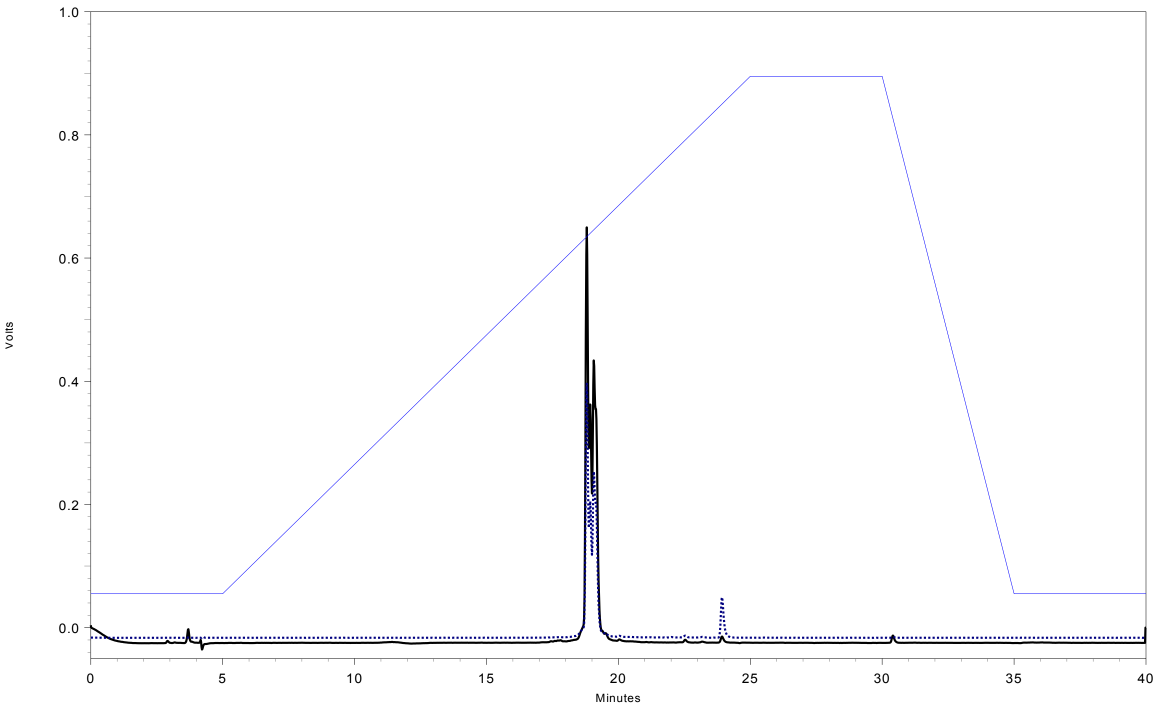
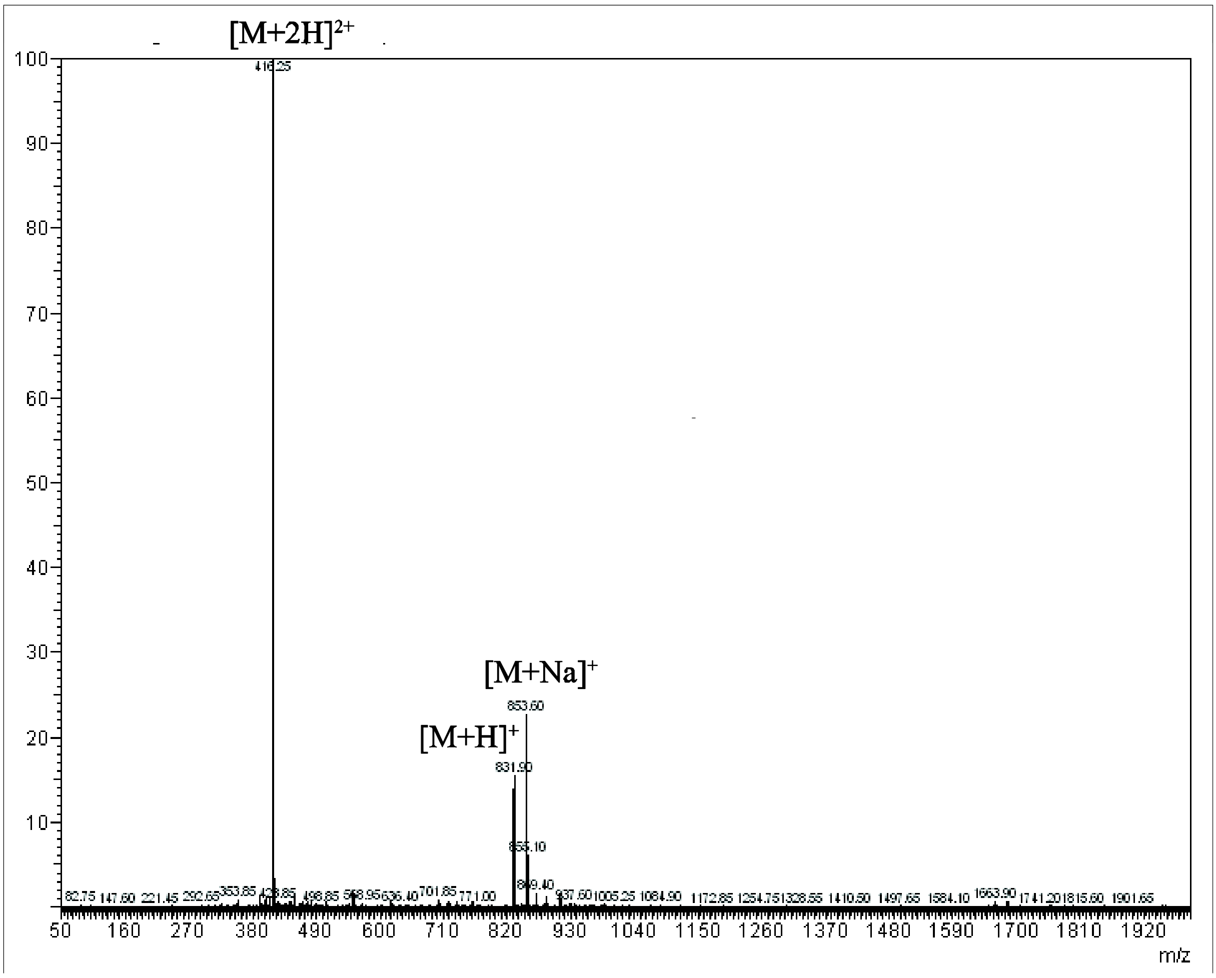


Figure SI 36: ESI-MS spectrum and HPLC chromatogram of bicyclo[1-4/3-6]-H-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn^{5}-Alg^{6}-Lys(Fmoc)-NHMe·TFA (**4**).



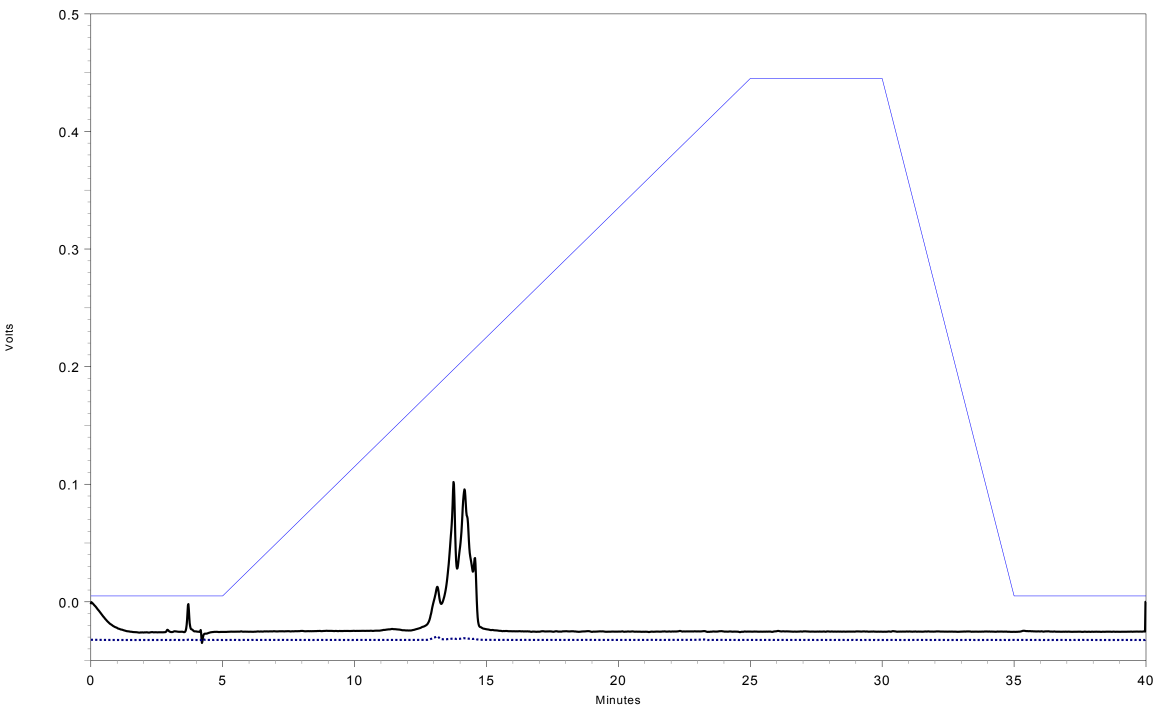
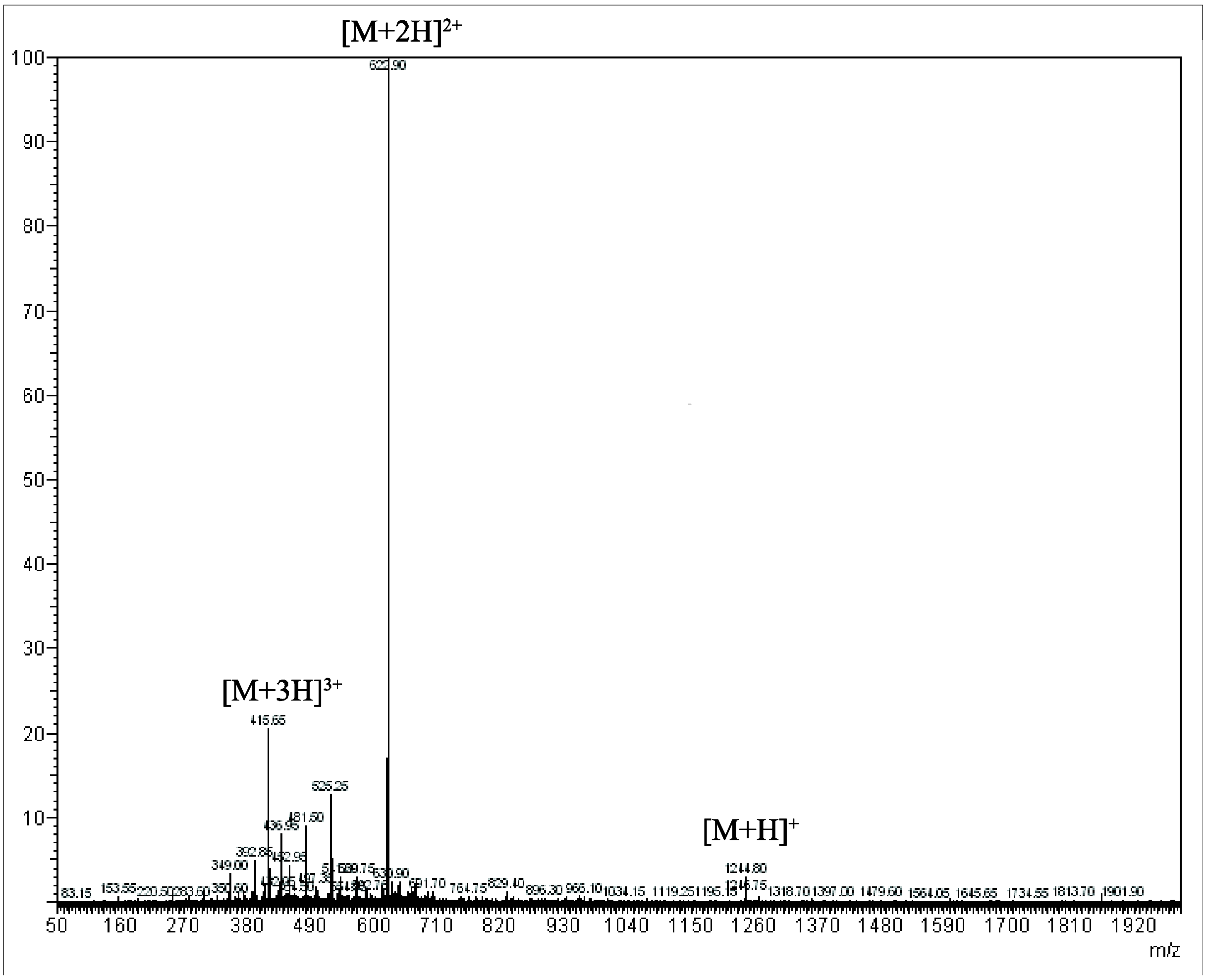


Figure SI 37: ESI-MS spectrum and HPLC chromatogram of bicyclo[1-4/3-6]-N_3-Lys-D-Alg^{1}-Ala^{2}-D-Alg^{3}-Alg^{4}-Asn^{5}-Alg^{6}-Lys-NHMe·2TFA (**5**).



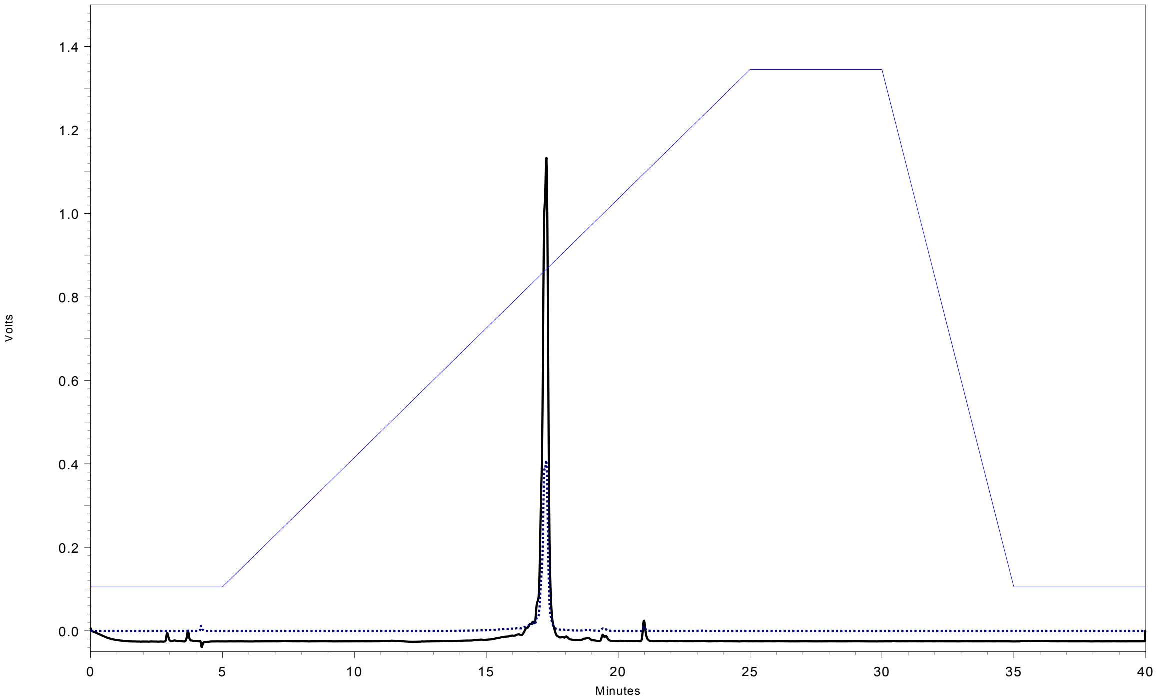


Figure SI 38: ESI-MS spectrum and HPLC chromatogram of "click" compound **13**.

6. Vesicle leakage experiments

Carboxyfluorescein (CF) loaded large unilamellar vesicles (LUVs) were prepared and used in a model membrane leakage experiment according to a literature procedure (*Biochemistry* **1997**, *36*, 6968-6976). The LUVs consisted of an equimolar amount of the zwitterionic lipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and the anionic lipid 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG). The peptide-induced leakage of CF from the vesicles was monitored by measuring the increase in fluorescence intensity at 515 nm (excitation at 492 nm) on a SPF 500 C spectrophotometer (SLM instruments Inc., USA) at 20 °C. A solution (1.0 mL) of CF-loaded vesicles (20 μM final concentration) in buffer (10 mM Tris/HCl pH = 7.0, 100 mM NaCl) was added to a quartz cuvette and fluorescence was measured (A_0). After 20 s, a buffer solution (25 μL) containing the peptide of interest (stock: 1 mM; final: 25 μM) was added and peptide-induced membrane leakage was followed during 60 s (A_{60}), after which a buffer solution (10 μL) of Triton-X (stock: 20%; final: 0.2%) was added to induce total leakage of the vesicles (A_{Total}). The % of peptide-induced membrane leakage was calculated by: $((A_{60} - A_0)/(A_{Total} - A_0)) \times 100\%$. All measurements were performed in duplo. In case of anoplin (H-Gly-Leu-Leu-Lys-Arg-Ile-Lys-Thr-Leu-Leu-NH_2), the final concentration was 43 μM (50 μg/mL) and in case of nisin, the final concentration was 1 nM.

7. Growth inhibition assay

Bacillus subtilis was used for determination of antimicrobial activity The minimal inhibitory concentration (MIC) of each peptide was determined using a broth microtitre dilution assay adapted from a literature procedure as previously described by Hancock.[1] Peptide stock solutions were prepared at a concentration of 100 to 1000 µM peptide in 0.2% bovine serum albumin (BSA) and 0.01% acetic acid. Serial three-fold solutions of peptide were made in 0.2% BSA and 0.01% acetic acid. To each well was added, 50 µL of the test bacterium in tryptic soya broth to a final concentration of 2 × 10^{6} CFU/mL and 50 µL of the peptide with different concentrations. After incubation for 24 h at 37 °C at 120 rpm in a Certomat incubator, the OD at 630 nm was measured. The MIC (expressed in µM) of each peptide was read as the lowest concentration of peptide that was able to inhibit visible bacterial growth. All measurements were performed in duplicate.

[1]The MIC determination assay was performed according to the protocol of R. E. W. Hancock. For further information see: 'Hancock Laboratory Methods', Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada. http://www.cmdr.ubc.ca/bobh/methods.htm [07-08-2013, date last accessed].

Data:

MIC in Bacillus subtilis

[Plate: M 630]										Nisin	DE fragments
		Nisin			DE dicarbo **5**			DE native(22-31)		conc in uM	
A	0.055	0.049	0.048	**0.595**	**0.519**	**0.041**	**0.622**	**0.471**	**0.042**	50	**500**
B	0.041	0.042	0.041	0.561	0.603	0.041	0.653	0.666	0.043	16.7	167
C	0.041	0.042	0.039	0.495	0.512	0.041	0.448	0.437	0.04	5.56	56
D	0.051	0.04	0.039	0.554	0.527	0.048	0.444	0.596	0.039	1.85	19
E	0.043	0.042	0.042	0.446	0.503	0.04	0.497	0.473	0.04	0.62	6.2
F	**0.041**	**0.04**	**0.04**	0.504	0.547	0.041	0.484	0.436	0.041	**0.21**	2.1
G	0.263	0.154	0.042	0.633	0.529	0.041	0.403	0.485	0.04	0.07	0.7
H	0.511	0.457	0.04	0.498	0.57	0.04	0.567	0.526	0.047	0.0	0.0