Supporting Information

Metastable hydrogels from aromatic dipeptides

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1. Formation of the FF and FF-NH_2 hydrogels

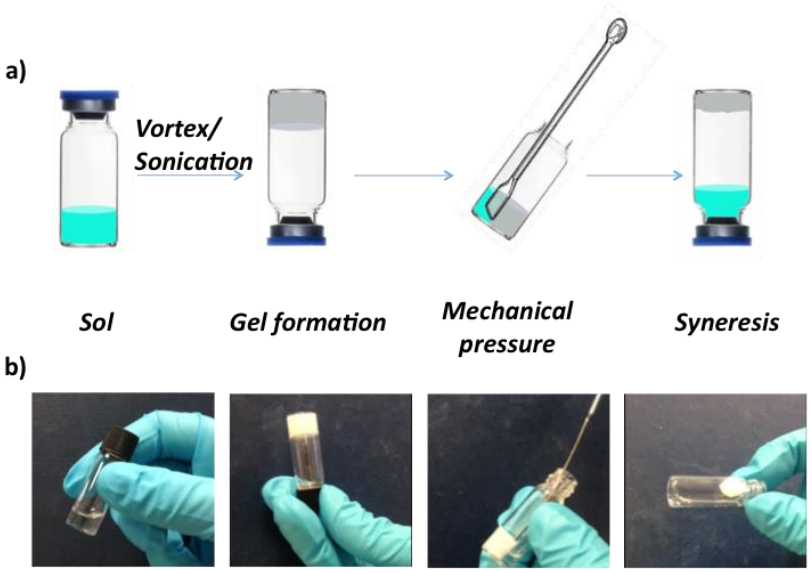

Figure S1 a) Schematic of the gelation process for FF and FF-NH_2. The opaque gel forms upon vortexing and sonication of the dipeptide solution. Mechanical touching with a spatula causes the rapid syneresis (seconds) of the gel which collapses to approximately 40% of its original volume. (b) Screenshots to illustrate the process. The full video is also available.

2. Characterisation of the control sample FY

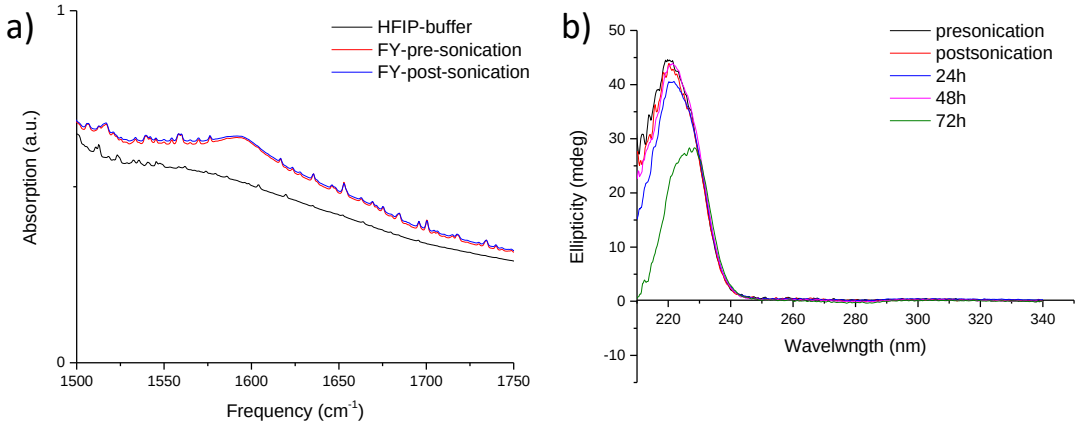

Figure S2 FTIR spectra of the reference dipeptide FY. The spectrum of the solvent (8% HFIP in pH8 buffer) is provided in black, the spectra of the FY solution before and after sonication are reported in red and blue respectively. No difference is observed in the spectrum upon sonication and no peaks associated with a H-bonded network are shown in the spectrum. CD spectra of the reference peptide FY. This molecule is chiral giving rise to a peak around 220nm but no substantial difference is observed upon sonication. After 72 h the intensity of the spectrum decreases, probably because of the formation of aggregates in the sample.

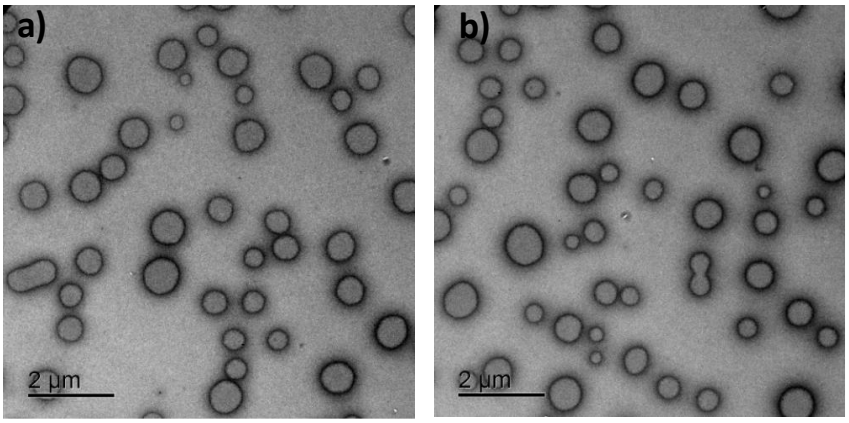

Figure S3 TEM images of the reference dipeptide FY before (a) and after (sonication). Sonication does not affect the molecular organization of this sample as opposed to the effect on the dipeptides FF and FF-NH2.

3. Scanning electron microscopy images of FF and FF-NH_2 samples

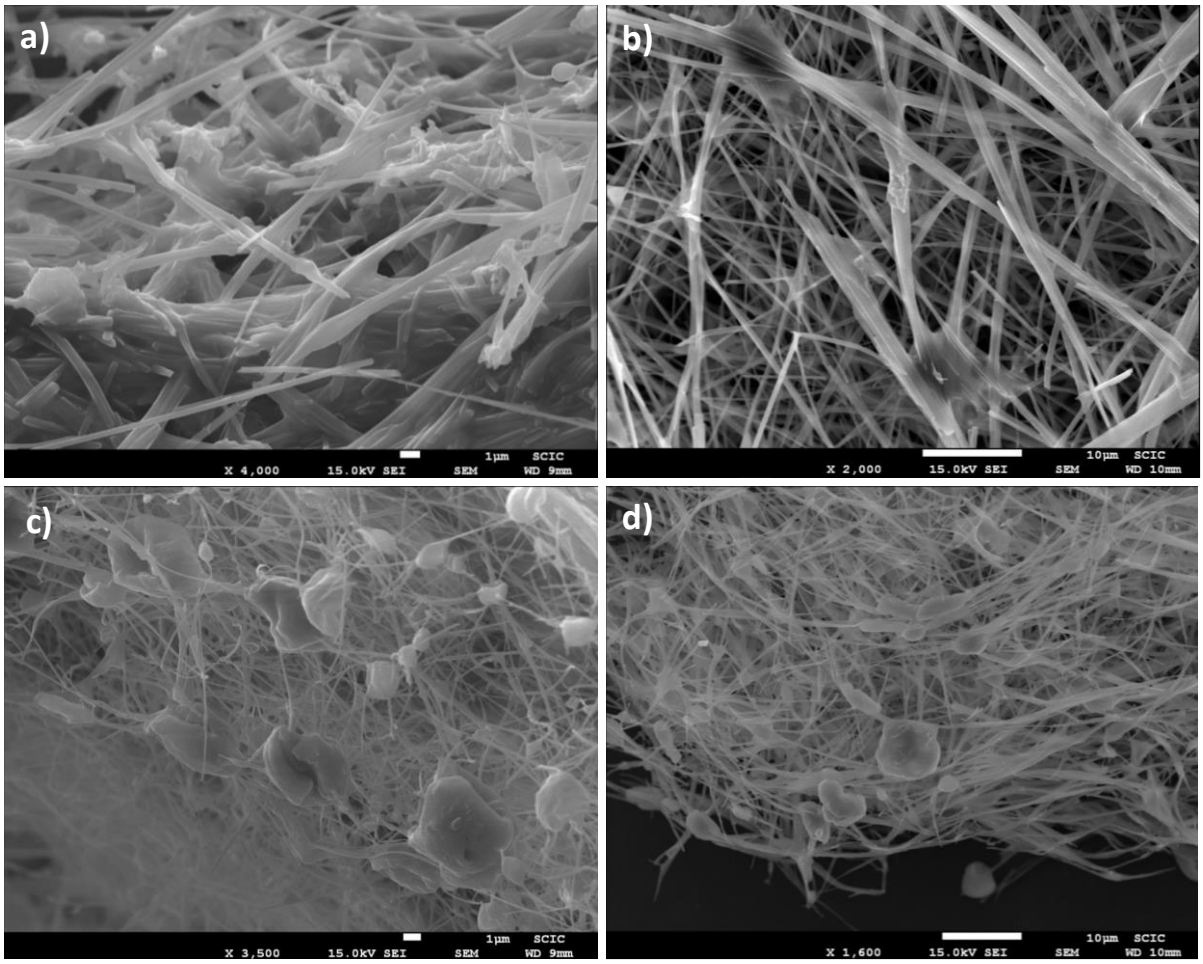

Figure S4 SEM images of the gels before and after syneresis. a) FF gel before syneresis; b) FF gel after syneresis; c) $FFNH_2$ gel before syneresis; d) $FFNH_2$ gel after syneresis. For both FF and $FFNH_2$ gels, the morphology of the fibrous network does not change upon syneresis.

4. Transmission electron microscopy of the samples after heat cooling cycles

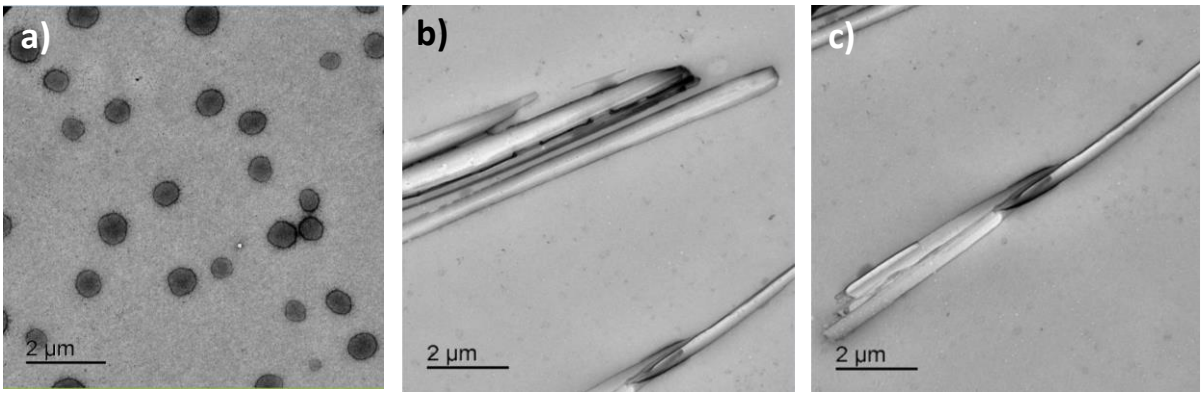

Figure S5 TEM images of FY (a), FF (b) and FF-NH_2 (c) after several heat/cool cycles. Upon annealing both FF and FF-NH_2 samples show exclusively the presence of tubular structures, confirming that this is the most thermodynamically stable state, whereas the reference sample FY is not affected by the heat/cool process.

5. Rheology measurements

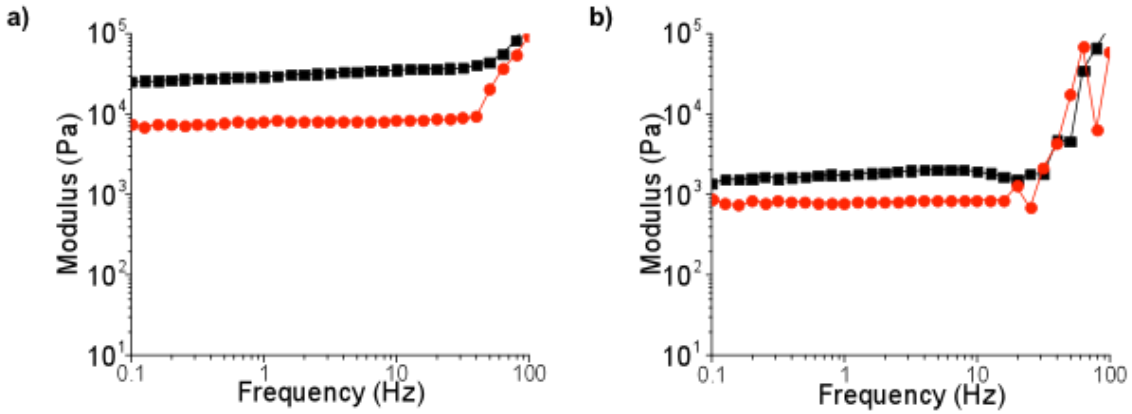

Figure S6 Linear viscoelastic diagrams of FF-NH_2 (a) and FF (b) gels. For both sample the storage moduls G' (black line) is greater than the loss modulus G" (red line), indicating a gel like-behaviour.

6. ANS binding assay

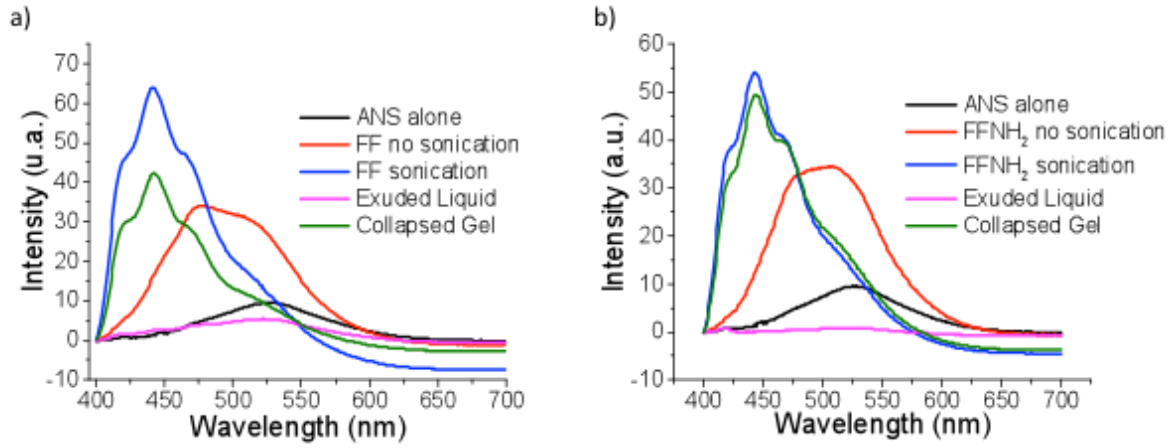

Figure S7 ANS binding fluorescence assay on FF (a) and FF-NH_2 (b) gels. For both systems, the assay shows a significant increase of fluorescence and a blue shift of the maximum, both before and after sonication. The assay also highlights the hydrophobic nature of the nanofiber network.

7. Thioflavin T (ThT) retention in the gels

UV-Vis spectroscopy was employed to monitor the release/ retention of ThT by the gels upon syneresis. Reference solutions at different ThT concentration were prepared dissolving ThT in pH8 100 mM sodium phosphate buffer. The UV-Vis absorption of the ThT buffered solutions was measured for ThT concentrations ranging between 10^{-3} and 10^{-5} M (lowest detectable concentration with the instrument). The dipeptide gels containing ThT were formed dissolving 5 mg of FF or FF-NH_2 in 80 µl of HFIP and then diluiting up to 1 ml with the buffered ThT solution 10-3 M. The samples were vortexed and sonicated and syneresis of the gels was triggered by mechanical compression of the gels. The exuded liquid was collected and the UV-Vis absorption was measured. No absorbance peak was detected for the liquids exuded both from FF and FF-NH_2 gels, indicating that the exuded liquids contain less than 10^{-5} M ThT. More than 99% of the fluorophore is hence retained in the collapsed gel. The absorption of the collapsed gel or of the gel before syneresis cannot be evaluated since absorbance values increase significantly upon ThT interaction with the fibres overcoming the saturation limit of the instrument.

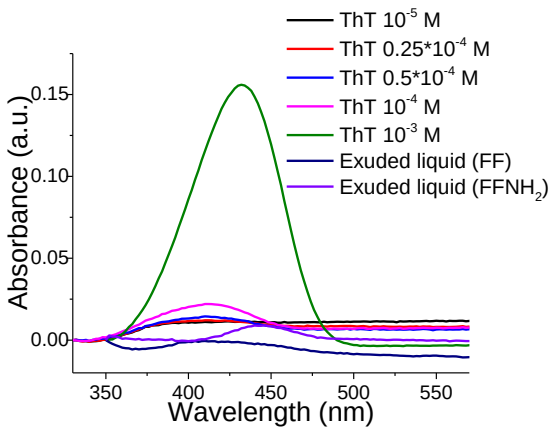

Figure S8 UV-Vis absorbance of ThT solutions and of the liquid exuded from the ThT containg gels. For both the liquid exuded from FF and FF-NH_2 gels upon syneresis, the characteristic ThT absorption peak around 415 nm is not visible.

8. Preparation of the samples

The samples were prepared by dissolving 5mg of dipeptide (FF-NH_2, FF-OH or FY-OH) into 80 µl of HFIP and then adding 920 µl of sodium phosphate buffer (pH ranging from 5 to 9). The samples were briefly vortexed and then sonicated several times for 10 s (VWR ultrasonic cleaner, 45 Hz). To prepare the free-standing gels, the peptide solutions were transferred into a 8-well chamber slide (Lab Tek) prior to sonication. The chamber slides were wrapped in Parafilm ® to avoid water infiltration into the wells during sonication.

9. Transmission Electron Microscopy (TEM)

Gels were prepared according to the procedure described in section 2 and left overnight. TEM images were captured using a FEI Tecnai T20 transmission electron microscope operating at 200kV. Carbon-coated copper grids (200 mesh) were glow discharged in air for 30 s. The support film was touched onto the gel surface for 3 seconds and blotted down using filter paper. Negative stain (20 µL, 1 % aq. Methylamine vanadate obtained from Nanovan, Nanoprobes) was applied and the mixture blotted again with filter paper to remove excess. Each sample was allowed to dry afterwards for 2-3 minutes in a dust-free environment prior to TEM imaging. The dried grids with the samples were then imaged and the images were saved using Gatan Digital Micrograph software.

10. Scanning Electron Microscopy (SEM)

Gels were prepared according to the procedure described in section 2 and left overnight. Two different sets of samples were imaged with two different microscopes. For the first set of samples (Fig. S2 a, b, c), solvent was expelled by touching the gels with a spatula causing collapse. The

collapsed gel was collected and placed onto an aluminium SEM pin stub and left overnight to air dry then Au/Pd coated before viewing on the SEM. The samples were imaged with a JEOL 6400 SEM running at 10kV. The images were captured using Olympus Scandium software. The second set of samples were freeze dried before (Fig. S3 a and c) or after syneresis (Fig. S2 d, S3 b and d) and placed on top of an aluminium specimen mount stub and sputtered with Pt before imaging. The samples were imaged with a JEOL 7001F microscope equipped with a digital camera.

11. UV-Vis absorption spectroscopy

UV-Vis absorption spectra were recorded in Jasco V-660 spectrophotometer. Samples for the measurement were prepared in PMMA UV-grade cuvettes (Fisher Scientific). The spectra were measured with a bandwidth of 5 nm, with a medium response and a scan speed of 40 nm/min

12. Infrared Spectroscopy (IR)

The gels were prepared according to the procedure described in section 2, but using deuterated buffers and left overnight. Samples were transferred in a standard IR transmission cell (Harrick Scientific) between two 2 mm CaF_2 windows, separated by a polytetrafluoroethylene (PFTE) spacer of 50 µm thickness. Spectra were recorded on a Bruker Vertex 70 spectrometer by averaging 25 scans at a spectral resolution of 1 cm^{-1}. Spectra were corrected for absorption from the buffer with a blank sample 8% HFIP in phosphate buffer.

13. Circular Dichroism (CD)

Samples were monitored by CD spectroscopy on a Jasco J-810 spectrometer equipped with a temperature control; spectra were recorded at 25 °C. Samples were prepared dissolving 1.25 mg of FF-NH_2 or 2.5 mg of FF in 80 µl of HFIP and then adding 920 µl of buffer. The samples were vortexed and sonicated and immediately loaded into a 0.2 mm quartz cover slip cuvette before gelation occurred. The spectra were measured at 50 $nmmin^{-1}$ with a 0.5 nm step size and a 1.0 nm bandwidth, between 200 and 350 nm. The measurements were repeated after 24, 48 and 72 h.

14. Rheology

Dynamic frequency sweep experiments were carried out on a strain-controlled rheometer (Kinexus rotational rheometer from Malvern) using a parallel-plate geometry (20 mm) with a 1 mm gap. An integrated temperature controller was used to maintain the temperature of the sample stage at 25°C. To ensure the measurements were made in the linear viscoelastic regime, an amplitude sweep was performed and the results showed no variation in elastic modulus (G') and viscous modulus (G")

up to a strain of 0.06%. The gels were prepared according to the procedure previously described. The dynamic modulus of the gels was measured as a frequency function, where the frequency sweeps were carried out between 1 and 100 Hz. The measurements were repeated at least three times to ensure reproducibility.

15. Fluorescence

PMMA UV grade cells with a path length of 10 mm were used for the study. Fluorescence emission spectra were measured on a Jasco FP-6500 spectrofluorimeter with light measured orthogonally to the excitation light, at a scanning speed of 500 nm min^{-1}. The excitation wavelength was 255 nm, and the spectra were measured between 265 and 500nm, with a bandwidth of 5 nm, a medium sensitivity and 0.5 nm data pitch.

16. ANS binding

8-Anilino-1-naphthalenesulfonic acid (ANS) (Sigma Aldrich, UK) was dissolved in pH 8 100 mM sodium phosphate buffer to a final concentration of 100 uM. 2.5 mg of the dipeptides FF or $FFNH_2$ were dissolved in 80 ul of HFIP and then diluited up to 1 ml with the ASN buffered solution. Two set of samples were prepared. The first set was not sonicated. The second set was sonicated inducing the gelation of the samples. Fluorescence emission was measured for all the samples as prepared and then after 24, 48 and 72 h. Fluorescence was monitored for the ANS buffered solution as well. Fluorescence emission spectra were measured on a Jasco FP-6500 spectrofluorimeter with light measured orthogonally to the excitation light, at a scanning speed of 500 nm min^{-1}. The excitation wavelength was 365 nm, and the spectra were measured between 400 and 700nm, with a bandwidth of 5 nm, a low sensitivity and 0.5 nm data pitch.

17. Primary characterisation of the compounds

Reversed-phase High Performance Liquid Chromatography (HPLC)

A Dionex P680 HPLC system was used to analyze the dipeptides. Aliquots of 30 µL were injected with a flow rate of 1 mL min–1 into a Macherey-Nagel C-18 column (250 mm of length and 4.6 mm of internal diameter) containing silica particles (diameter: 5 µm, pores diameter: 10 nm). The gradient used was a linear exchange between water/acetonitrile (95:5, 0.1% TFA) at 4 min to water/acetonitrile (5:95, 0.1% TFA) at 35 min. This concentration was kept constant until 40 min when the gradient was decreased to 20% (v/v) acetonitrile in water at 42 min. The intensity of each

identified peak was determined by UV detection at 255 nm. The estimated purity from HPLC integrated peak areas was 99.15%, 98.88% and 99.34% respectively for $FFNH_2$, FF and FY.

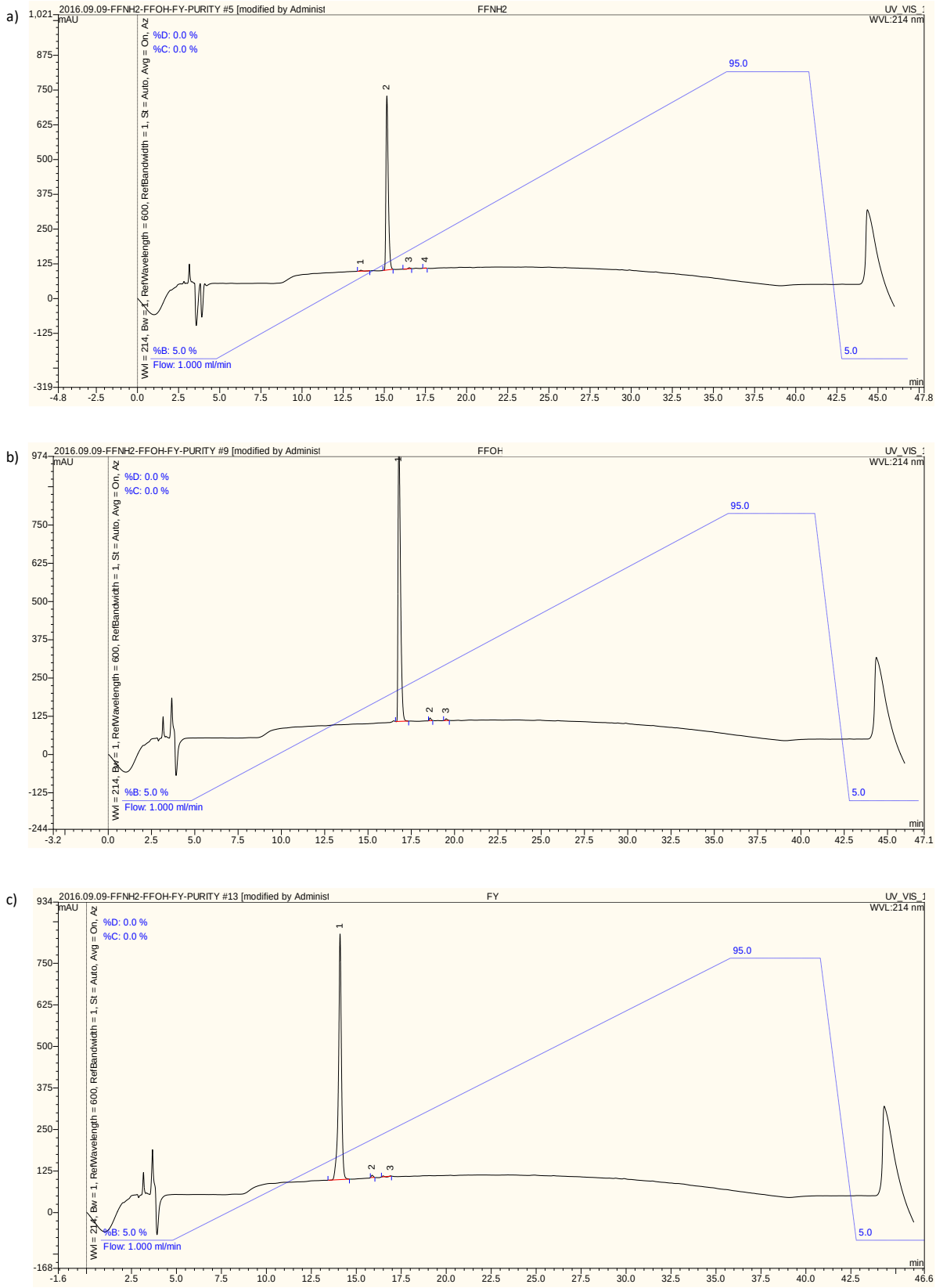

Figure S9 HPLC traces of the dipeptides $FFNH_2$ (a), FF (b) and FY (c).

Nuclear Magnetic Resonance (NMR) Spectroscopy

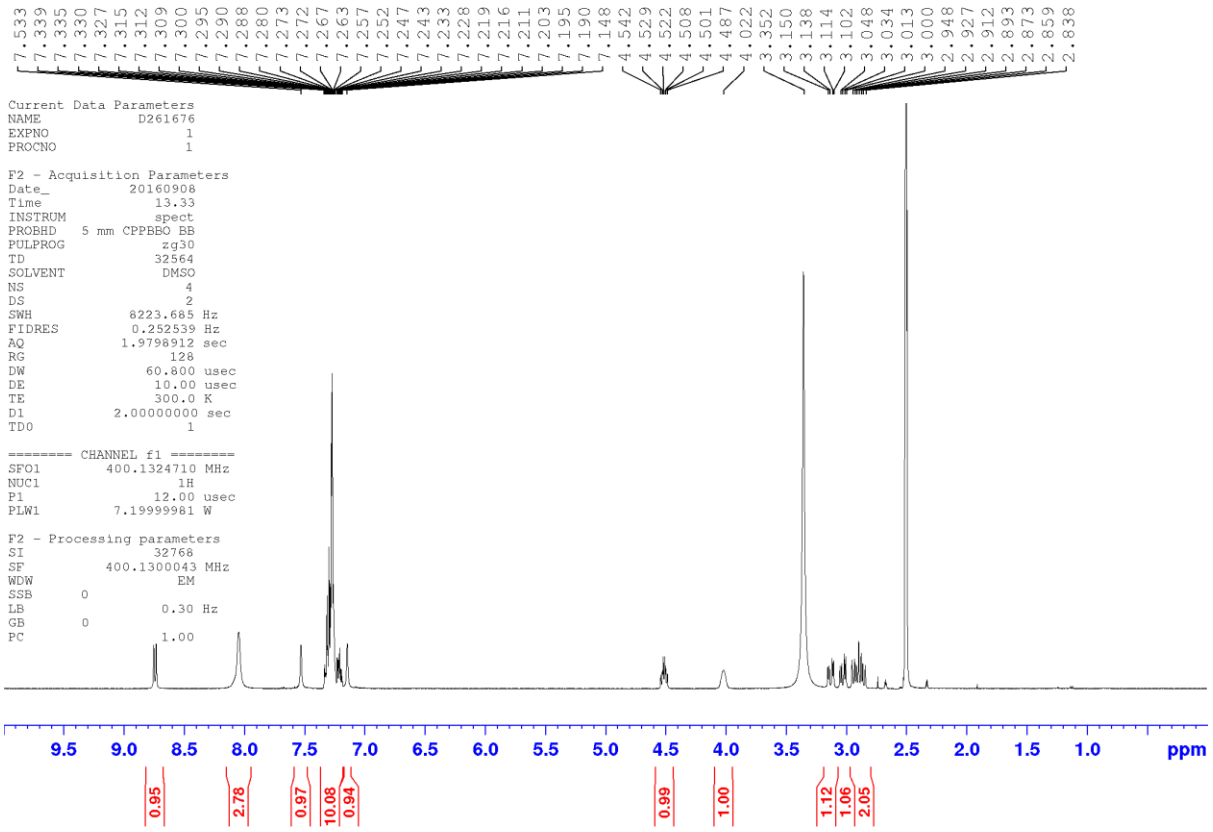

Figure S10 ^{1}H NMR of FFNH$_2$ (400 MHz, (CD$_3$)$_2$SO). 8.75 (1 H, d), 8.1 (2 H, s), 7.5 (1 H, s), 7.2-7.35 (10 H, m), 7.15 (1 H, s), 4.5 (1 H, m), 4 (1 H, s), 3.14 (1 H, dd), 3.01 (1 H, dd), 2.9 (2 H, 2dd). The intense peak around 3.4 ppm is due to residual H$_2$O in the solvent.

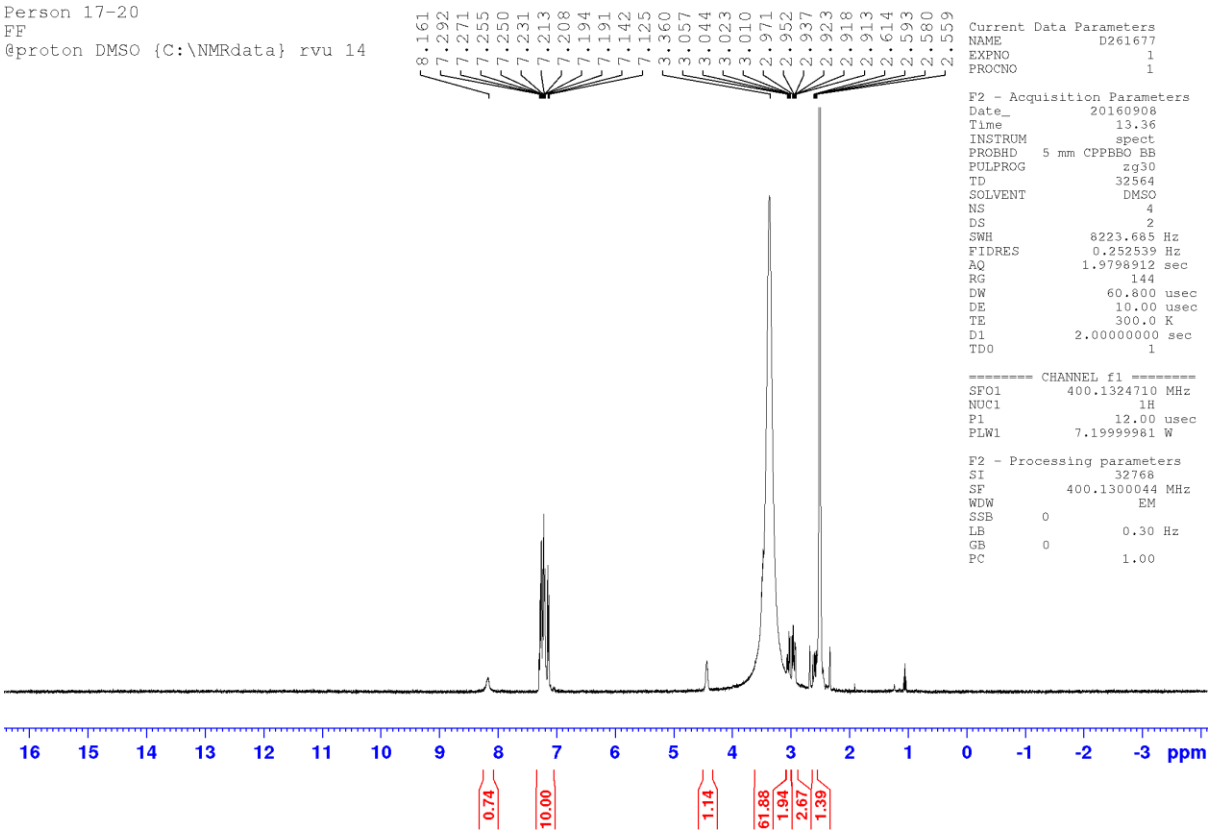

Figure S11 ^{1}H NMR of FF (400 MHz, $(CD_3)_2SO$). 8.2 (1 H, s), 7.2 (10 H, m), 4.5 (1 H, s), 3.02 (1 H, dd), 2.95 (1 H, dd), 2.6 (2 H, 2dd). The intense peak around 3.4 ppm is due to residual H_2O in the solvent.

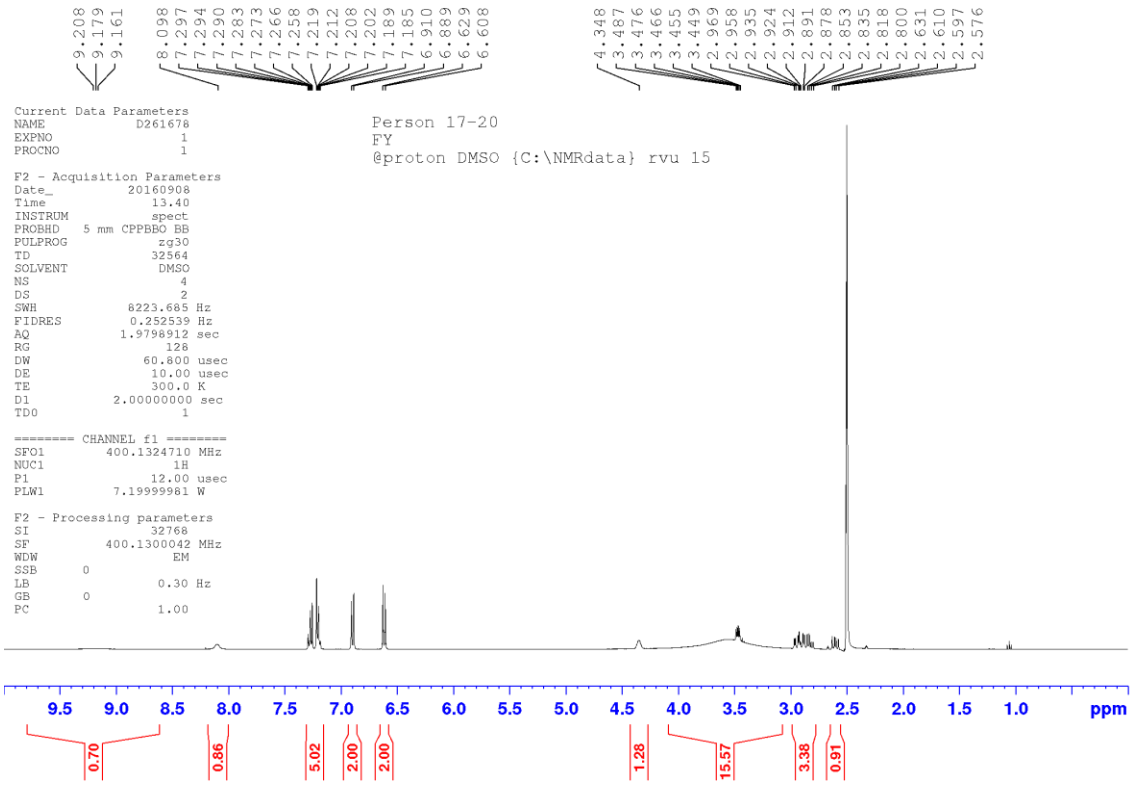

Figure S12 ^{1}H NMR of FY (400 MHz, $(CD_3)_2SO$). 8.1 (1 H, s), 6.9 (2 H, dd), 7.2-7.3, (5 H, m), 6.6 (2 H, dd), 4.3 (1 H, s), 2.9 (3 H, m), 2.6 (1 H, m).

Liquid Chromatography – Mass Spectroscopy (LCMS)

An Agilent LCMS 6130 single quad dual source mass spec was used to analyse the dipeptides. Aliquots of 10 µL were injected with a flow rate of 1 mL min^{-1} into a Agilent Poroshell 120 EC-C18 4.6mm x75mm x 2.7um. The gradient used was a linear exchange between water/acetonitrile (95:5, 5 mM ammonium acetate) at 1.48 min to water/acetonitrile (0:100, 5 mM ammonium acetate) at 8.5 min. This concentration was kept constant until 13.5 min when the gradient was decreased to 5% (v/v) acetonitrile in water at 16.5 min. The intensity of each identified peak was determined by UV detection at 254 nm. The results of the positive and negative scans are reported for the three dipeptides.

FY: predicted MW = 328.36; experimental (negative scan) = 327.0; experimental (positive scan) = 329.1;

FF: predicted MW = 312.36; experimental (negative scan) = 311.1; experimental (positive scan) = 313.1;

FF-NH_2 predicted MW = 311.38; experimental (negative scan) = 310.1; experimental (positive scan) = 312.0

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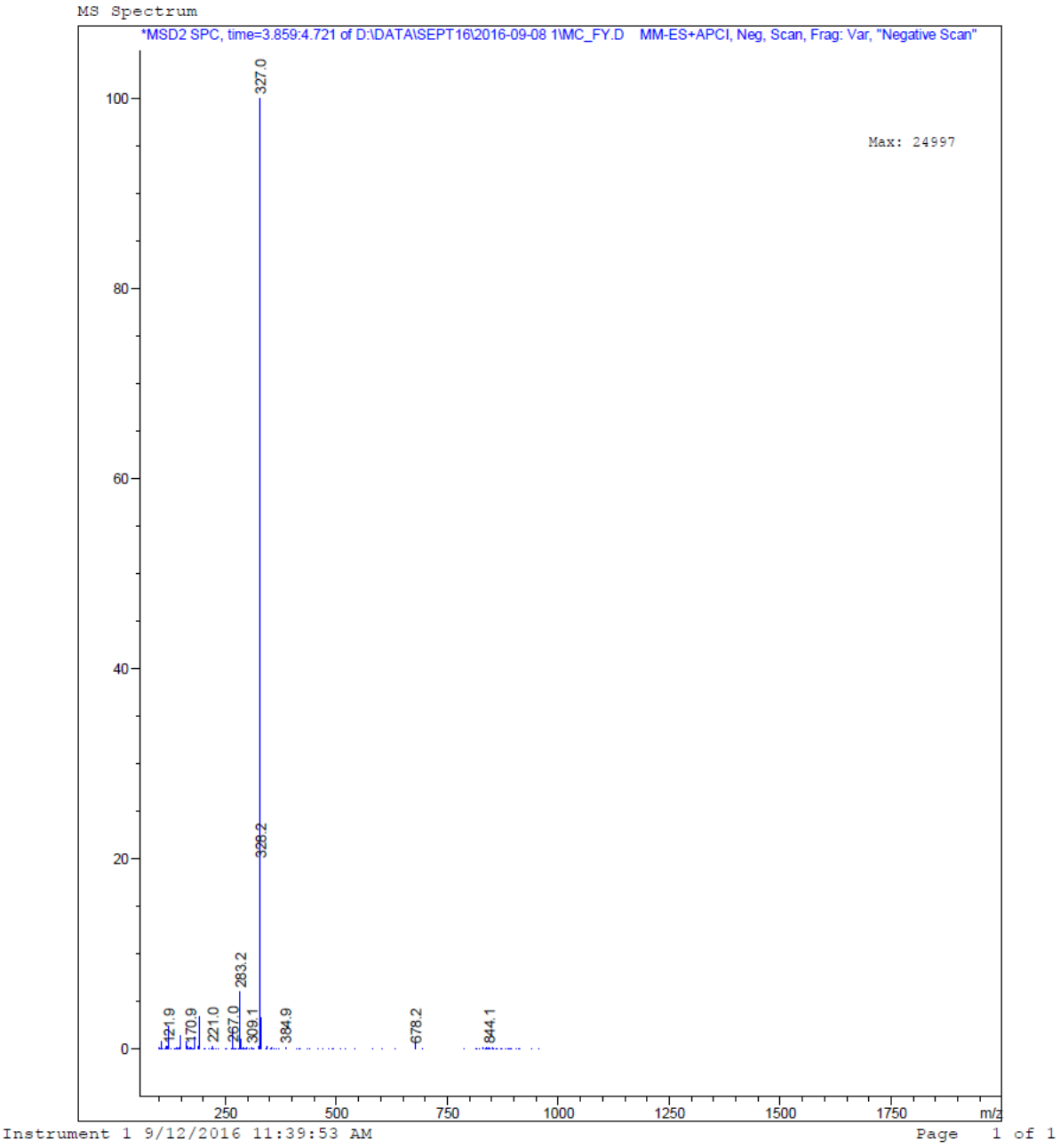

Figure S13 Negative scan for FY

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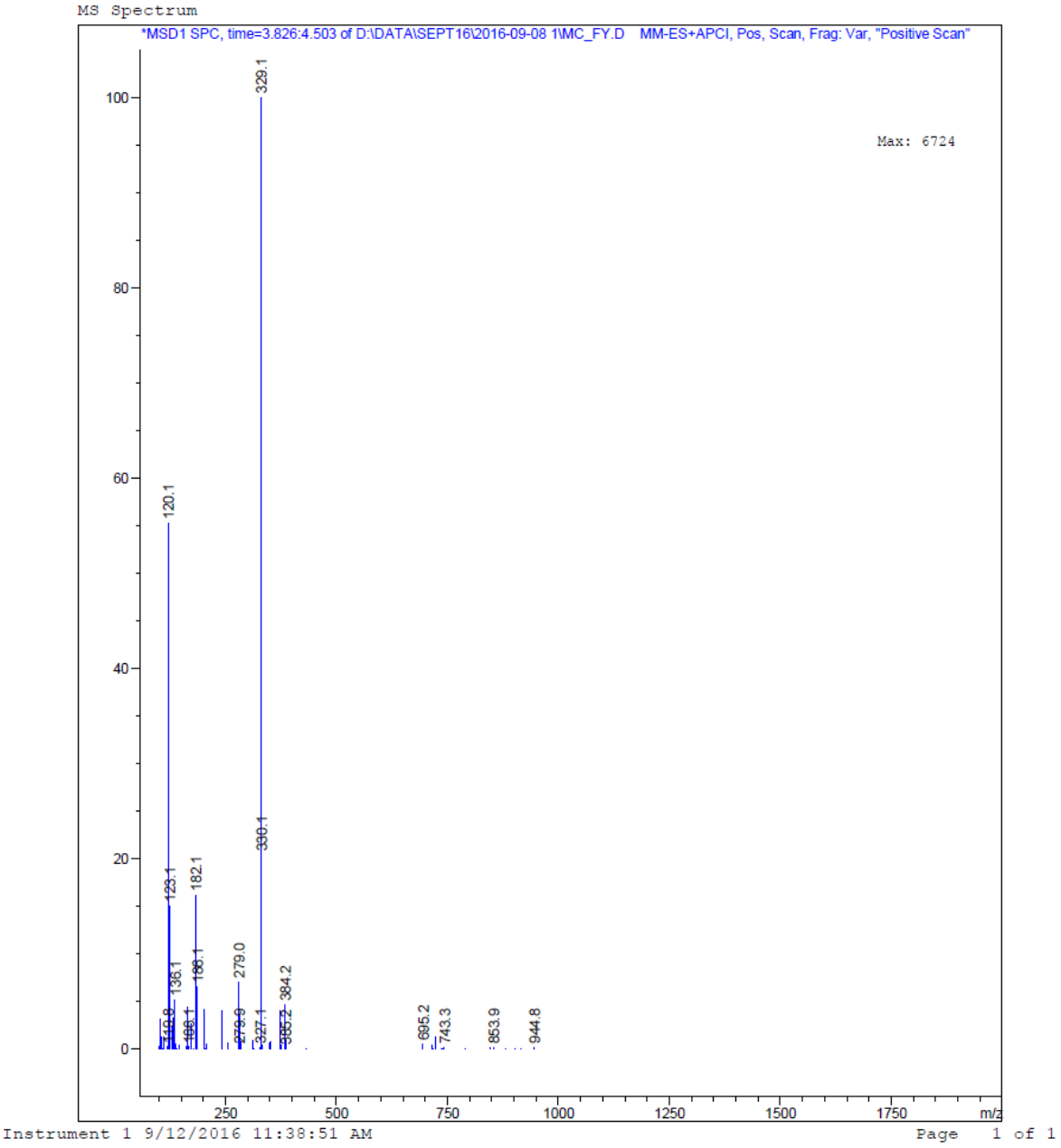

Figure S14 Positive scan for FY

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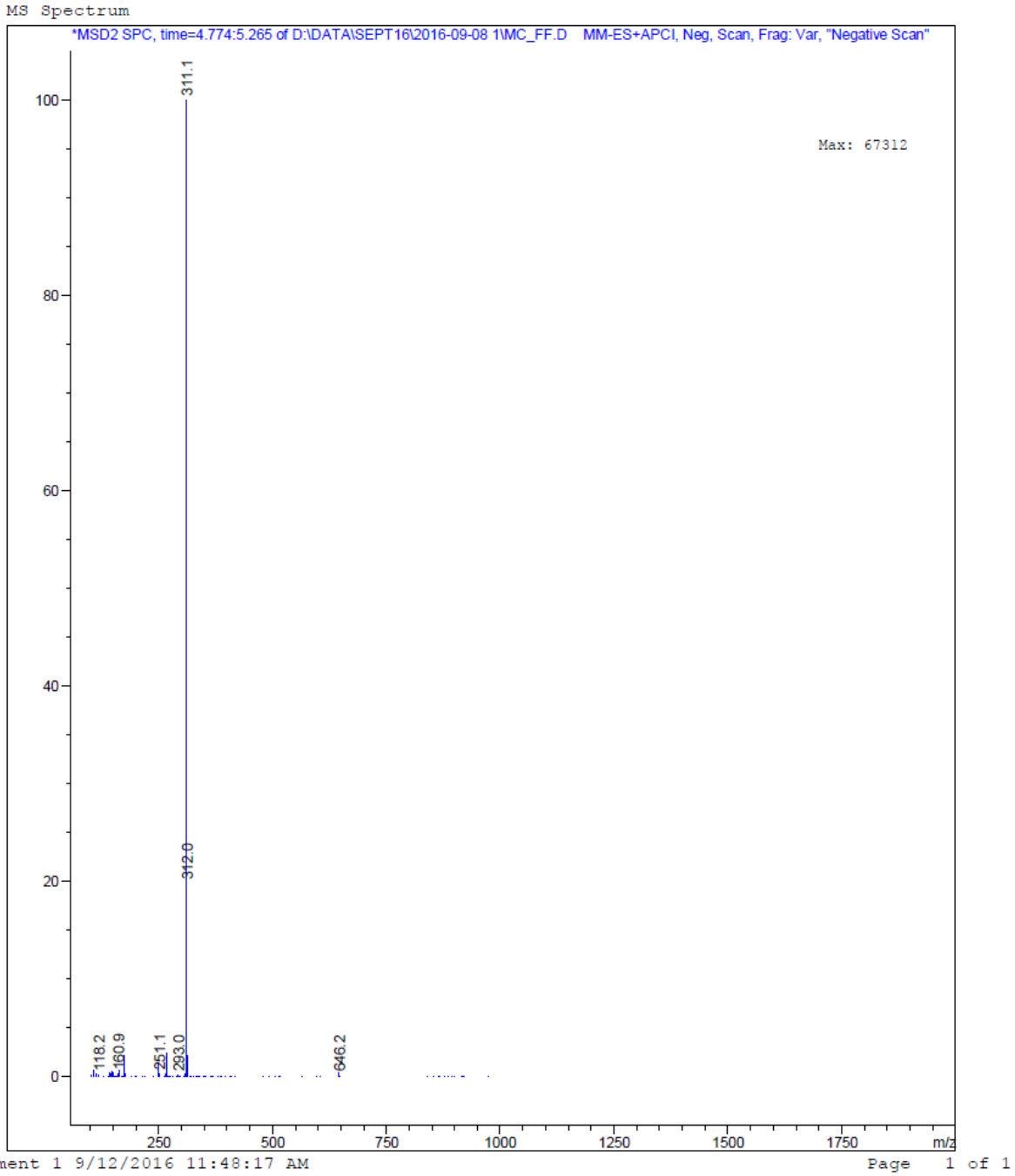


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Figure S15 Negative scan for FF

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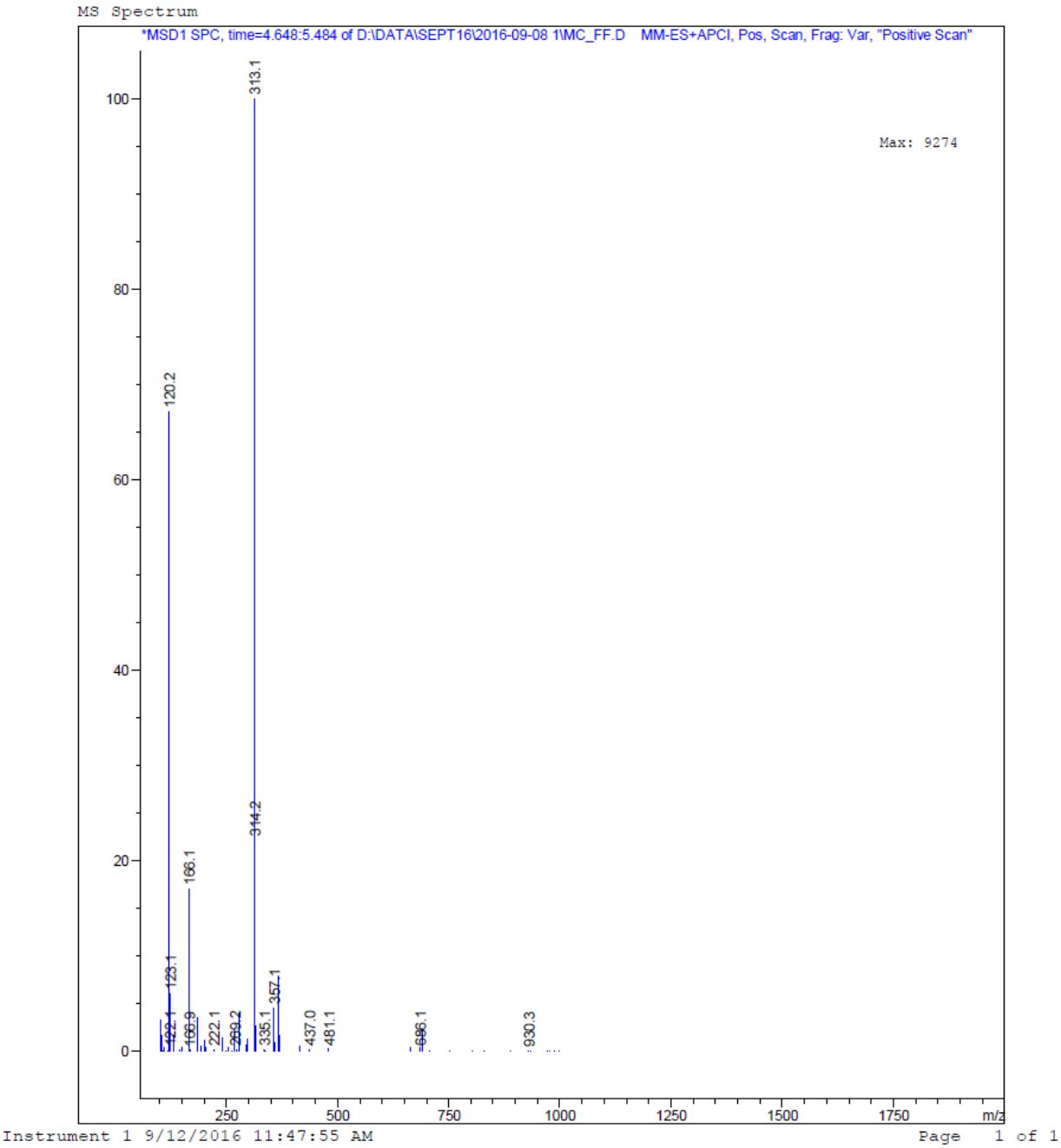

Figure S16 Positive scan for FF

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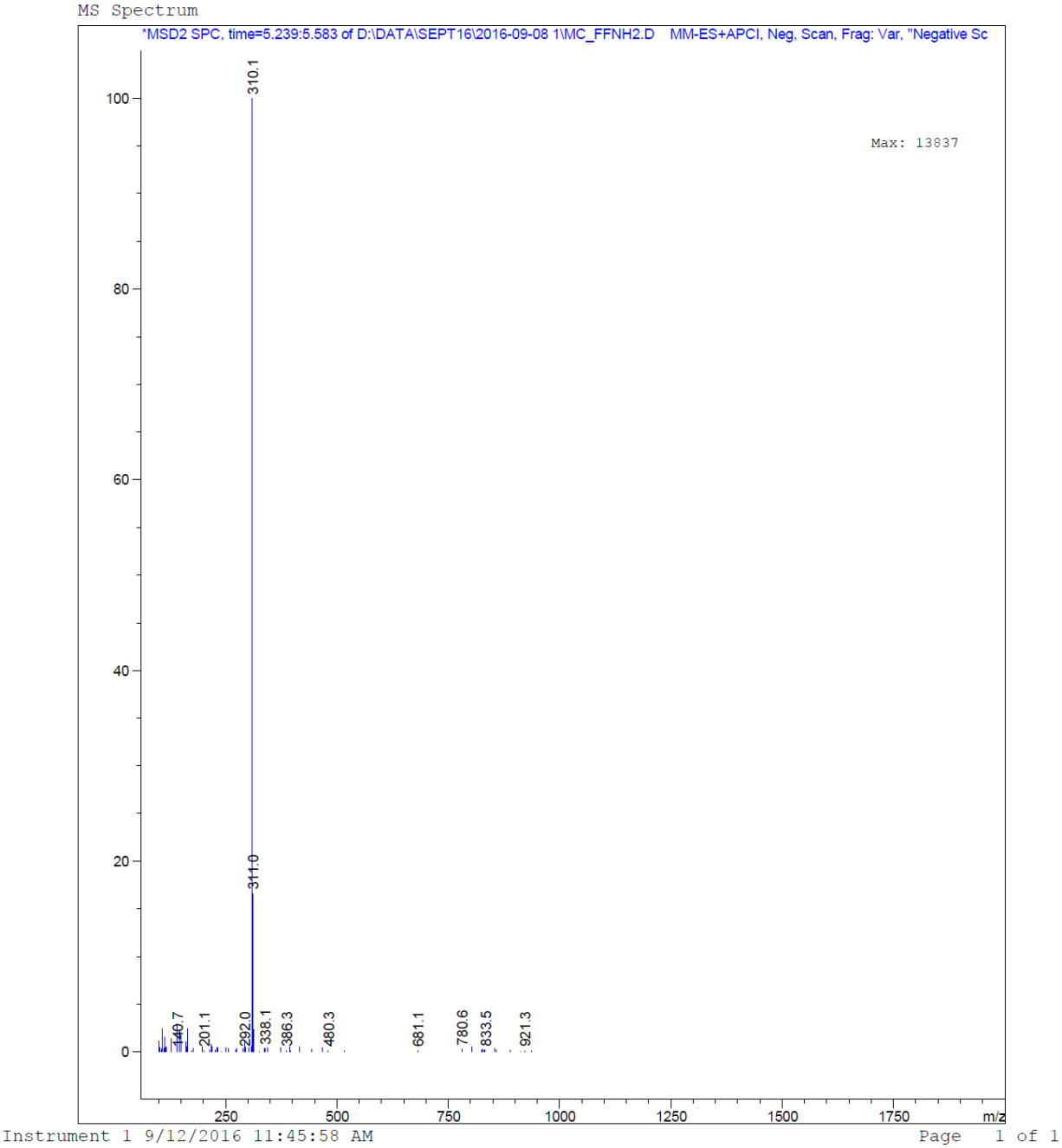


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Figure S17 Negative scan of $FFNH_2$

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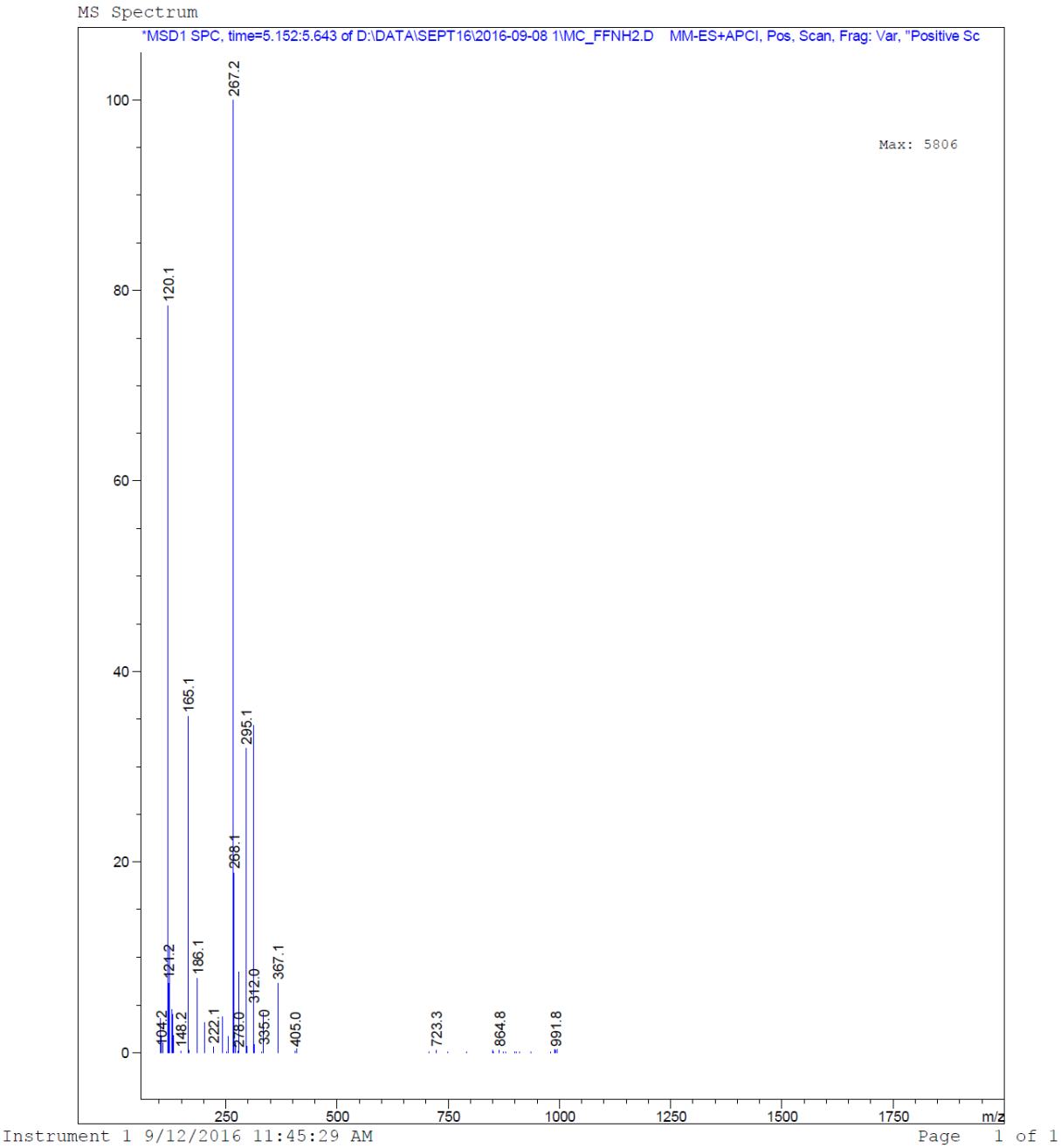

Figure S18 Positive scan for FF-NH_2