SUPPORTING INFORMATION AND FIGURES

Title

Quantitative analysis of protein lipidation and acyl-CoAs reveals substrate preferences of the S-acylation machinery

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General Methods. NMR experiments were carried out on a Varian Mercury 400 instrument (400 MHz for ^{1}H and 101 MHz for ^{13}C) for the characterization of the FAH molecules and on a Bruker Avance III HD spectrometer (operating at 500MHz for peptide characterization). Chemical shifts (δ) are reported in part per million (ppm) referenced to the residual solvent. Signal characterization is described using the following abbreviations: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), p (pentuplet), m (multiplet), br (broad signal) and app (apparent). Spin-spin coupling constants (*J*) are reported in Hertz (Hz).

High-resolution mass spectra (HRMS) of synthetic compounds were recorded on a Acquity UPLC system coupled to an LCT Premier orthogonal accelerated time–of–flight mass spectrometer (Waters) using electrospray ionization (ESI) technique and an Acquity UPLC® BEH C18 1.7 µm, 2.1x100 mm column; flow rate: 0.3 mL/min, mobile phase: water with 20 mM formic acid (Solvent B) and acetonitrile with 20 mM formic acid (Solvent A); linear gradient from 10% to 100% of A over 8 minutes.
Cells were lysed by passing them through a Branson Ultrasonics Sonifier™ SFX150 Cell Disruptor. Absorbance measurements were performed on a BioTek Synergy H1 microplate reader at room temperature using 96 well microplates.
Samples from lipid extractions were evaporated using a SAVANT SPD131DDA SpeedVac concentrator coupled to a ThermoScientific OFP400 Vacuum Pump and a SAVANT RVT5105 Refrigerated Vapor Trap.

Experimental models. Three eight-week-old SWISS CD-1 male mice (Janvier-Labs) were used for the study. The animals were housed in the Research and Development Center animal facility (CID-CSIC) under a 12 h light–dark cycle in an environmentally controlled room with free access to water and food. Mice were euthanized via cervical dislocation. All tissues were weighed, rapidly frozen in liquid nitrogen, and stored at −80 °C for further analyses.

Cell culture and reagents. The human melanoma cell line A375, the human lung adenocarcinoma cell line A549 and the human embryonic kidney cells HEK293 were obtained from ATCC. The human colon adenocarcinoma cell line HT29 and the hepatocarcinoma cells HepG2 were obtained from Celltec UB (Barcelona, Spain). Cell lines were maintained at 37 °C in 5% CO2 in Dulbecco's Modified Eagle Medium with high glucose, L-glutamine and sodium bicarbonate without sodium pyruvate, supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin

Monitoring FAH formation from peptides by LC-MS assay

Peptides were dissolved in H_2O_{dd} at a concentration of 1 mM. A 4 M and a 1.4 M concentration solution of HA in H_2O_{dd} were prepared and its pH value was adjusted to 7 with a 1 M NaOH solution. C19 FAH was used as an internal standard for proper quantification. 200 µL of a mixture of peptides (**1** or **2** and **3**) at a 15 µM concentration each, **HA** at 0.7M or 2 M final concentration and C19 FAH at 1.6 µM were mixed in buffer (25 mM Tris·HCl 150 mM NaCl) at different pH depending on the experiment (6.5, 7.0, 7.5). Reactions were shaken at room temperature for the indicated times (2h, overnight), and then the reaction was stopped by evaporating the solvent under a stream of nitrogen and stored at -20 °C. Before LC-HRMS analysis, samples were dissolved in 200 µL of UPLC-grade methanol.

Samples were analyzed by liquid chromatography coupled with a high-resolution mass spectrometer (LC-HRMS) using an Acquity Ultra High-performance Liquid Chromatography (UHPLC) System (Waters, USA) connected to a time-of-flight (TOF; LCT Premier XE) detector controlled with Waters Micromass MassLynx v.4.1 software, and equipped with an Acquity UHPLC BEH C8 column (1.7 µm particle size, 100 mm × 2.11 mm, Waters). Samples were introduced via a cooled autosampler with a temperature set to 10 °C. A flow rate of 0.3 mL/min was applied, the column was thermostated at 30 °C and an injection volume of 8 µL was used. The mobile phase was 1 mM ammonium formate and 0.2% formic acid in methanol (Solvent A) and 2 mM ammonium formate and 0.2% formic acid in water (Solvent B). Gradient elution started at 65% Solvent A, and it was increased to 99% over 15 min, held for 2 min at 99%, and then returned to 65% Solvent A over 3 min. The acquisition range of the TOF detector was *m/z* 50 to 1.800, the capillary voltage was set to 3.0 kV, the desolvation temperature was 350°C, and the desolvation gas flow rate was 600 liters/h. The peak area of each FAHs in each sample and standard was quantified using the MassLynx software. The concentration of FAHs was calculated in relation to the C19 FAH internal standard spiked into the sample (320 pmols).

Monitoring peptide thioester cleavage by HPLC

Peptides were dissolved in buffer (100 mM Tris·HCl, NaCl 250 mM, pH 7.4) at a 4 mM concentration. A 4 M and a 1.4 M concentration solution of HA in H_2O_{dd} were prepared and its pH value was adjusted to 7 with a 1 M solution of NaOH.

100 µL of a mixture of peptides (**1** or **2** and **3**) at 2 mM concentration each, were mixed with 100 µL of HA solution and shaken at room temperature. The peptide **3**, bearing an amide bonded fatty acid was used as an internal reference to assess the amount of

remaining peptide. Aliquots of 20 µL were taken at given times, diluted with 20 µL of a 0.5 M TFA solution and subsequently analyzed by HPLC. The progress of the reaction was measured by assessing the area of the peak corresponding to peptides **1** or **2** in comparison with the area of the peak corresponding to peptide **3**, which was used as an internal reference. Separations were performed on an Analytical RP-HPLC Hewlett Packard 1100 equipped with an automatic injector and hyphenated to a G1315A photodiode array detector. Separations were carried out in a Phenomenex Aeris Peptide 3.6 µm XB-C18, 150 x 4.60 mm column. Runs were carried out at room temperature using a linear gradient of 0 to 70% of buffer B (I with 0.036% TFA) in buffer A (H_2O_{dd} with 0.045% TFA) during 30 min (unless otherwise stated) at a flow rate of 1 mL/min. UV detection was done at 220, 247, and 280 nm. Chromatograms were analyzed using the Agilent OpenLab Data Analysis software. Retention times observed for peptide **1**: 23.6 min; peptide **2**: 22.8 min; and peptide **3**: 21.9 min.

Monitoring acyl-CoA thioester cleavage by HPLC

Peptide **3** and oleoyl-CoA (sodium salt) were dissolved in buffer (100 mM Tris·HCl, NaCl 250 mM, pH 7.4) at 4 mM concentration. A 4 M concentration solution of HA in buffer was prepared and its pH value was adjusted to 7.4 with a 10 M solution of NaOH.

100 µL of the 4 mM peptide **3** solution and 100 µL of the 4 mM oleoyl-CoA solution were mixed with 200 µL of the 4 M HA solution and shaken at room temperature in a glass vial. The peptide **3**, bearing an amide-bound fatty acid was used as an internal reference to assess the amount of remaining oleoyl-CoA. Aliquots of 20 µL were taken at given times, diluted with 20 µL of a 1 M TFA solution and subsequently analyzed by HPLC. The advance of the reaction was measured by assessing the area of the peak corresponding to oleoyl-CoA in comparison with the area of the peak corresponding to peptide **3**. Separations were performed on an Analytical RP-HPLC Hewlett Packard 1100 equipped with an automatic injector and hyphenated to a G1315A photodiode array. Separation of the compounds was carried out using a Waters Symmetry300^{TM} C4 5 µM, 4.6x150 mm column and a linear gradient from 0 to 70% of buffer B (I with 0.045% TFA) in buffer A (H_2O_{dd} with 0.036% TFA) during 30 min at a flow rate of 1 mL/min. UV detection was carried out at 220 and 280 nm. Chromatograms were analyzed using the Agilent OpenLab Data Analysis software. Retention times observed for oleoyl-CoA: 3.5 min; peptide **3**: 20.4 min.

Monitoring FAH formation from oleoyl-CoA by LC-MS assay

Oleoyl-CoA (1mg) was dissolved in buffer (25 mM Tris·HCl, 150 mM NaCl, pH 7.4) at a concentration of 1 mM, then, a 30 µM solution was prepared from that one. A 2 M concentration solution of HA in buffer was also prepared and its pH value was adjusted to 7.4 with 10 M NaOH solution. C19 FAH was used as an internal standard for proper quantification. 50 µL of either oleoyl-CoA at a 15 µM concentration or buffer (for the blanks) were mixed with 35 µL of the 2 M HA solution and 16 µL of a 10 µM solution of C19 FAH (160 pmol) in glass vials. Reactions were left shaking at room temperature for 2h or overnight. Then, samples were dried in a SpeedVac concentrator and stored at -20°C until analysis.

Before injection in the mass spectrometer, the dried samples were resuspended in 300 µL of UPLC-grade methanol. Samples were analyzed by liquid chromatography coupled with a high-resolution mass spectrometer (LC-HRMS) using an Acquity Premier Ultra High-performance Liquid Chromatography (UPLC) System (Waters, USA) connected to an ESI Quadrupol-Time-of-Flight (Q-TOF) Cyclic IMS controlled with Waters Micromass MassLynx v 4.2 SCN 016 Software, and equipped with an Acquity™ Premier BEH C18 column (1.7 µm particle size, 50 mm × 2.1 mm, Waters). Samples were introduced via a cooled autosampler with a temperature set to 10 °C. A flow rate of 0.3 mL/min was applied, the column was thermostated at 30 °C and an injection volume of 8 µL was used. The mobile phase was 1 mM ammonium formate and 0.2% formic acid in methanol (Solvent B) and 2 mM ammonium formate and 0.2% formic acid in water (Solvent A). Gradient elution started at 65% Solvent B, and it was increased to 99% over 15 min, held for 2 min at 99%, and then returned to 65% Solvent B over 3 min. The acquisition range of the TOF detector was *m/z* 50 to 1.200, the capillary voltage was set to 3.0 kV, the desolvation temperature was 250°C, and the desolvation gas flow rate was 800 liters/h. Total thioester cleavage was assessed in both 2h and overnight reactions by checking the inexistence of the starting material (oleoyl-CoA) at retention time 8.22’ and m/z 1032.3675.

Western blotting against cysteine string protein (CSP)

After protein precipitation, samples were incubated overnight at room temperature with end-over-end rotation with either lysis buffer or a 2 M HA solution. Afterwards 4x Laemmli buffer with mercaptoethanol (10%) was added and the samples were boiled for 5 min at 95 °C. Equal amounts of protein (35 µg) were loaded and separated on 12 % polyacrylamide/SDS gels using a TRIS/Glycine/SDS Running Buffer at a constant voltage of 90 V for 2 hours at room temperature. Afterward, the gels were transferred onto nitrocellulose membranes using a Trans-Blot® TurboTM Transfer System from Bio-

Rad (1.3 A, 25 V, 7 min). Following blocking with a 3 % BSA solution in 0.1% TBS-Tween for 45 min, the membranes were incubated overnight at 4 °C with the primary antibody, either anti-CSP (1:1000) or anti-β-actin (1:5000). After washing, the corresponding anti-rabbit (1:5000) or anti-mouse (1:10.000) horseradish peroxidase (HRP)-conjugated secondary antibody was added and shaken for two hours at room temperature. Finally, the protein was visualized using enhanced chemiluminescence (ECL) and imaged in a LI-COR C-DiGit® Blot Scanner. Band intensities were quantified using the LI-COR Image Studio Lite Software.

Treatment of cells with Linoleic acid

HT29 cells were seeded into T75 flasks and grown to a confluence of approximately 90%. A 5 mM stock solution of Linoleic acid was prepared by adding the fatty acid to a solution of 40 µL ethanol and 300 µL NaOH 0.1 M. The solution was heated at 60°C for 10 minutes and mixed from time to time, until the fatty acid was completely dissolved. After removal of the growing media by aspiration, cells were washed with 2 mL of pre-warmed DMEM. Then the media was aspirated, and 9 mL of new media were transferred to each flask. After 15 min incubation at 37 °C, 1 mL of DMEM media containing either 5% BSA alone, or 5% BSA together with different concentrations of linoleic acid was transferred to the flasks to obtain a final fatty acid concentration of 5, 20 or 50 µM. Cells were incubated for 4 h at 37 °C, then the media was aspirated, 2 mL of DMEM were added and cells were harvested by scrapping. The cell suspension was centrifuged for 5 min at 300 rpm, the supernatant was aspirated and the cell pellet was resuspended in 2 mL PBS. After a new centrifugation step, the cell pellet was resuspended in 500 µL lysis buffer (50 mM Tris·HCl, 150 mM NaCl, pH 7.2) containing protease inhibitors. Cells were lysed by sonication at 0°C (30 cycles, 5 seconds sonication followed by 5 seconds resting period, set at 50% power). Proteome was precipitated following the protocol stated above. The precipitated proteome was resuspended in lysis buffer and total protein concentration was determined using the BCA assay. After quantification, 600 µg portions of protein were distributed into 1.5 mL microcentrifuge tubes and treated with HA at a final concentration of 2 M overnight at room temperature. Lipid extraction and LC-MS analysis were performed as stated above.

Statistical analysis

Comparison between two means has been carried out with the unpaired two-tailed *t*-test and statistical differences are marked with asterisks. Unless otherwise indicated, statistical significance was calculated using Prism version 8.

Synthesis of Peptides

Peptides were synthesized by manual stepwise solid phase peptide synthesis (SPPS) on a Fmoc-Rink amide resin (loading of 0.74 mmol/g, 0.5 g scale) placed in a polypropylene syringe. First, the resin was swollen with CH_2Cl_2 and then with dimethylformamide (DMF). Next, the Fmoc protecting group was removed using a 20% piperidine solution in DMF (2 x 1 min, 1 x 10 min) and the excess was washed away with clean DMF. Then, the first amino acid: Fmoc-L-Lys(Boc)-OH (937 mg, 2 mmol, 5.4 eq) was preactivated with a 0.5 M solution of OxymaPure in DMF (4 mL, 2 mmol, 5.4 eq) and N, N'-Diisopropylcarbodiimide (DIC) (340 µL, 2.2 mmol, 5.9 eq) for 3 minutes and then N,N'-Diisopropylethylamine (DIPEA) was added (174 µL, 1 mmol, 2.7 eq) and the solution was added to the resin. The resulting mixture was gently stirred for 45 minutes. Unreacted amine groups were capped with acetic anhydride (Ac_2O) (1 mL) and DIPEA (0.5 mL) in DMF for 5 minutes. Proper incorporation of the amino acid was checked in all the cases using the Kaiser test.[14] Fmoc removal was performed as previously described, and the second amino acid: Fmoc-L-Lys(Boc)-OH (937 mg, 2 mmol, 5.4 eq) was also incorporated using the same methodology. Following Fmoc removal, the rest of the peptide chain was elongated, sequentially incorporating: Fmoc-L-Ile-OH (707 mg, 2 mmol, 5.4 eq), Fmoc-L-Lys(Boc)-OH (937 mg, 2 mmol, 5.4 eq) and Fmoc-L-Val-OH (679 mg, 2 mmol, 5.4 eq).

Synthesis of Peptide Ac-Gly-Cys(Palm)-Val-Lys-Ile-Lys-Lys-NH_2 (1)

0.2 g of the resin containing the fragment Val-Lys-Ile-Lys-Lys were taken to continue the synthesis of peptide **1**. First, we completed the desired sequence by incorporating Fmoc-Cys(*t*-Buthio)-OH (432 mg, 1 mmol, 6.8 eq) and Fmoc-L-Gly-OH. The *N*-terminus was finally acetylated by treatment with Ac_2O/DIPEA/DMF (1:0.5:5, v/v/v, 6.5 mL, 2 x 5 min). The S-^{t}Butyl protecting group of the Cys(S-^{t}Butyl) side chain was then removed by treatment with a solution containing mercaptoethanol/1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU)/N-Methyl-2-pyrrolidone (NMP) (2:1:7, v/v/v, 5 mL, 2 x 1 min, 1 x overnight). A solution of CH_2Cl_2 and DIPEA (192 µL, 1.1 mmol, 7.4 eq) containing palmitoyl chloride (303 µL, 1 mmol, 6.8 eq) was then added to the resin that was shaken for 1 h at room temperature. To cleave the peptide from the solid support, the resin was transferred into a 15 mL polypropylene tube and 5 mL of a solution containing trifluoroacetic acid (TFA)/Triisopropylsilane (TIS)/H_2O_{dd} (95:2.5:2.5) were added. The resulting mixture was left at room temperature with rotary stirring for

90 min. Afterwards, the solution was filtered and the filtrate was concentrated under a stream of nitrogen almost to dryness. Ice-cold diethyl ether (Et_2O) (40 mL) was added to precipitate the peptide and the mixture was centrifuged (6000 rpm, 10 min, 4 °C). The supernatant was then decanted and the solid was dissolved in 20 mL of a H_2O/ACN (1:1) solution and freeze-dried. The peptide was purified using a semi-preparative HPLC system (Waters PrepLC system furnished with a Waters 1525 Binary HPLC Pump and a Waters 2489 UV/Visible Detector) on an XBridge Prep BEH130 C18 5 µm OBD 19 x 50 mm column. A linear gradient of H_2O/TFA 0.1% (Solvent A) and acetonitrile (CAN)/TFA 0.05% (Solvent B), from 0% to 70% of B over 30 min at a flow rate of 16 mL/min was used. UV monitoring was done at 220 nm and 280 nm. After lyophilization, the pure peptide was obtained as a powder, 23.9 mg (15% yield). HPLC analysis (Waters Symmetry300^{TM} C4 5 µM, 4.6x150 mm): retention time (t_R) = 6.5 min, purity > 99%.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.17 (t, *J* = 5.8 Hz, 1H, NH(Gly)), 8.12 (d, *J* = 8.2 Hz, 1H, NH(Cys)), 8.09 (d, *J* = 7.9 Hz, 1H, NH(Lys)), 8.00 (d, *J* = 7.8 Hz, 1H, NH(Lys)), 7.83 (d, *J* = 8.0 Hz, 1H, NH(Lys)), 7.78 – 7.65 (m, 8H, NH(Val), NH(Ile), 3xNH_2(Lys)), 7.36 (s, 1H, -NH_2), 7.04 (s, 1H, -NH_2), 4.45 (td, *J* = 8.6, 5.0 Hz, 1H, Hα(Cys)), 4.24 (m, 2H, 2xHα(Lys)), 4.20 – 4.09 (m, 3H, Hα(Lys), Hα(Ile), Hα(Val)), 3.72 (dd, *J* = 16.6, 6.0 Hz, 1H, Hα(Gly)), 3.63 (dd, *J* = 16.6, 5.6 Hz, 1H, Hα(Gly)), 3.25 (dd, *J* = 13.5, 4.9 Hz, 1H, Hβ(Cys)), 3.03 (dd, *J* = 13.5, 9.0 Hz, 1H, Hβ(Cys)), 2.74 (t, *J* = 7.2 Hz, 8H, 4x-CH_2-(Lys)), 2.55 (t, *J* = 7.4 Hz, 2H, -CH_2-CO-), 1.97 (h, *J* = 6.8 Hz, 1H, Hβ(Val)), 1.85 (s, 3H, CH_3-CO-), 1.74 – 1.68 (m, 1H, Hβ(Ile)), 1.68 – 1.59 (m, 4H, -CH_2-(Lys), -CH_2-(Ile)), 1.58 – 1.46 (m, 14H, 6x-CH_2-(Lys), -CH_2-CH_2-CO-), 1.23 (s, 26H, -CH_2-(Lys), 12x-CH_2-(Palm)), 0.88 – 0.76 (m, 15H, -CH_3(Palm), 2x-CH_3(Val), 2x-CH_3(Ile)). HR-MS: *m/z* calculated for $C_{52}H_{100}N_{11}O_9S$: 1054.7426 $[M+H]^+$, found: 1054.7428 $[M+H]^+$.

Synthesis of Peptide Ac-Gly-Ser(Palm)-Val-Lys-Ile-Lys-Lys-NH_2 (2)

The synthesis was continued with 0.2 g of the peptidyl-resin containing the fragment Val-Lys-Ile-Lys-Lys. First, we incorporated the remaining sequence. To do this, Fmoc-Ser-OH*H_2O (327 mg, 1 mmol, 6.8 eq) was preactivated for 3 minutes with a 0.5 M solution of OxymaPure in DMF (2 mL, 1 mmol, 6.8 eq) containing DIC (136 µL, 1.1 mmol, 7.4 eq) and then DIPEA (88 µL, 0.5 mmol, 3.4 eq) was added and the resin was shaken with this mixture for 45 minutes at room temperature. Since the hydroxyl group of the last amino acid incorporated was not protected, no DIPEA was added in the following steps. Therefore, the solution of preactivated Fmoc-L-Gly-OH (297 mg, 1 mmol, 6.8 eq) with OxymaPure and DIC and was added to the resin and shaken for 45 minutes. Finally, the *N*-terminus was acetylated by treatment with preactivated acetic

acid (57 µL, 1 mmol, 6.8 eq) using a 0.5 M solution of OxymaPure in DMF (2 mL, 1 mmol, 6.8 eq) for 45 minutes. Kaiser tests were performed to monitor coupling efficiency. A solution of CH_2Cl_2 and DIPEA (192 µL, 1.1 mmol, 7.4 eq) containing palmitoyl chloride (303 µL, 1 mmol, 6.8 eq) was then added to the resin that was shaken for 2 h. The resin was washed with CH_2Cl_2 and the acylation reaction was repeated. To cleave the peptide from the solid support, the resin was transferred into a 15 mL polypropylene tube and 5 mL of a solution containing TFA/TIS/H_2O_{dd} (95:2.5:2.5) were added. The mixture was left at room temperature with rotary stirring rotation for 90 min. Afterwards, the solution was filtered and the filtrate was concentrated under a stream of nitrogen almost to dryness. Ice-cold Et_2O (40 mL) was added to precipitate the peptide and the mixture was centrifuged (6000 g, 10 min, 4 °C). The supernatant was then decanted and the solid dissolved in 20 mL of an H_2O/ACN (1:1) solution and freeze-dried. The peptide was purified using a semi-preparative HPLC system as stated above. After lyophilization, the pure peptide **2** was obtained as a powder, 18.2 mg (12% yield). HPLC analysis (Waters Symmetry300^{TM} C4 5 µM, 4.6x150 mm): retention time (t_R) = 6.4 min, purity > 99%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, *J* = 8.0 Hz, 1H, NH(Ser)), 8.16 (t, *J* = 5.8 Hz, 1H, NH(Gly)), 8.08 (d, *J* = 7.9 Hz, 1H, NH(Lys)), 8.00 (d, *J* = 7.8 Hz, 1H, NH(Lys)), 7.82 (d, *J* = 8.3 Hz, 2H, NH(Lys), NH(Val)), 7.71 (s, 6H, 3xNH_2(Lys)), 7.67 (d, *J* = 8.7 Hz, 1H, NH(Ile)), 7.36 (s, 1H, -NH_2), 7.04 (s, 1H, -NH_2), 4.62 (td, *J* = 7.5, 4.6 Hz, 1H, Hα(Ser)), 4.29 – 4.20 (m, 2H, 2xHα(Lys)), 4.19 – 4.08 (m, 5H, Hα(Lys), Hα(Ile), Hα(Val), 2xHβ(Ser)), 3.75 (dd, *J* = 16.6, 5.9 Hz, 1H, Hα(Gly)), 3.69 (dd, *J* = 16.5, 5.7 Hz, 1H, Hα(Gly)), 2.75 (m, 8H, 4x-CH_2-(Lys)), 2.25 (t, *J* = 7.4 Hz, 2H, -CH_2-CO-), 2.02 – 1.92 (m, *J* = 6.8 Hz, 1H, Hβ(Val)), 1.85 (s, 3H, CH_3-CO-), 1.73 – 1.59 (m, 5H, Hβ(Ile), -CH_2-(Lys), -CH_2-(Ile)), 1.51 (m, 14H, 6x-CH_2-(Lys), -CH_2-CH_2-CO-), 1.30 (m, 26H, -CH_2-(Lys), 12x-CH_2-(Palm)), 0.88 – 0.76 (m, 15H, -CH_3(Palm), 2x-CH_3(Val), 2x-CH_3(Ile)). HR-MS: *m/z* calculated for $C_{52}H_{100}N_{11}O_{10}$: 1038.7655 $[M+H]^+$, found: 1038.7656 $[M+H]^+$.

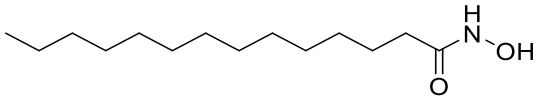
Synthesis of Peptide Palm-Gly-Ser-Val-Lys-Ile-Lys-Lys-NH_2 (3)

The synthesis was continued with 0.1 g of the peptidyl-resin containing the peptide fragment Val-Lys-Ile-Lys-Lys. First, we completed the desired sequence by incorporating Fmoc-Ser(*t*-Bu)-OH (192 mg, 0.5 mmol, 6.8 eq) preactivated for 3 minutes with a 0.5 M solution of OxymaPure in DMF (1 mL, 0.5 mmol, 6.8 eq) and DIC (68 µL, 0.55 mmol, 7.4 eq), and the posterior addition of DIPEA (44 µL, 0.25 mmol, 3.4 eq) to the mixture, leaving it to gently stir for 45 minutes at room temperature. Then, the last amino acid: Fmoc-L-Gly-OH was incorporated using the same procedure. After Fmoc deprotection, a solution containing palmitic acid (128 mg, 0.5 mmol, 6.8 eq), 0.5

M OxymaPure in DMF (1 mL, 0.5 mmol, 6.8 eq) and DIC (68 µL, 0.55 mmol, 7.4 eq) was poured into the syringe and DIPEA (44 µL, 0.25 mmol, 3.4 eq) was finally added. The mixture was shaken for an additional 45 minutes at room temperature. To cleave the peptide from the solid support, the resin was transferred into a 15 mL polypropylene tube and 5 mL of a solution containing TFA/TIS/H_2O_{dd} (95:2.5:2.5) was added, then the mixture was left at room temperature with rotary stirring for 90 min. Afterwards, the solution was filtered and the filtrate was concentrated under a stream of nitrogen almost to dryness. Ice-cold Et_2O (40 mL) was added to precipitate the peptide and the mixture was centrifuged (6000 g, 10 min, 4 °C). The supernatant was then decanted and the solid dissolved in 20 mL of an H_2O/ACN (1:1) solution and freeze-dried. The peptide was purified using a semi-preparative HPLC as stated above. After lyophilization, the pure peptide **3** was obtained as a powder, 29.9 mg (41% yield). HPLC analysis (Waters Symmetry300™ C4 5 µM, 4.6x150 mm): retention time (t_R) = 6.1 min, purity = 97%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (t, *J* = 5.8 Hz, 1H, NH(Gly)), 8.02 (d, *J* = 7.9 Hz, 1H, NH(Lys)), 7.98 (d, *J* = 7.7 Hz, 1H, NH(Lys)), 7.90 (d, *J* = 7.6 Hz, 1H, NH(Ser)), 7.81 (d, *J* = 8.0 Hz, 1H, NH(Lys)), 7.78 (d, *J* = 8.3 Hz, 1H, NH(Val)), 7.69 (s, 6H, 3xNH_2(Lys)), 7.62 (d, *J* = 8.5 Hz, 1H, NH(Ile)), 7.34 (s, 1H, -NH_2), 7.04 (s, 1H, -NH_2), 5.04 (t, *J* = 5.5 Hz, 1H, -OH(Ser)), 4.37 (dt, *J* = 7.6, 5.6 Hz, 1H, Hα(Ser)), 4.22 (m, 2H, 2x Hα(Lys)), 4.15 (m, 3H, Hα(Lys), Hα(Ile), Hα(Val)), 3.72 (dd, *J* = 5.7, 3.4 Hz, 2H, 2x Hα(Gly)), 3.64 – 3.50 (m, 2H, 2x Hβ(Ser)), 2.75 (m, 8H, 4x-CH_2-(Lys)), 2.12 (t, *J* = 7.5 Hz, 2H, -CH_2-CO-), 2.01 (h, *J* = 6.7 Hz, 1H, Hβ(Val)), 1.72 (m, 1H, Hβ(Ile)), 1.65 (m, 4H, -CH_2-(Lys), -CH_2-(Ile)), 1.57 – 1.41 (m, 14H, 6x-CH_2-(Lys), -CH_2-CH_2-CO-), 1.24 (m, 26H, -CH_2-(Lys), 12x-CH_2-(Palm)), 0.89 – 0.76 (m, 15H, -CH_3(Palm), 2x-CH_3(Val), 2x-CH_3(Ile)). HR-MS: *m/z* calculated for $C_{50}H_{98}N_{11}O_9$: 996.7549 $[M+H]^+$, found: 996.7569 $[M+H]^+$.

Synthesis of Fatty acid hydroxamates

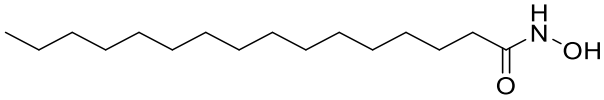
Synthesis of C14 FAH



In a 10 mL round bottom flask, 313 µL of T3P® solution (50 % in ethyl acetate (EtOAc), 527 µmol, 1.2 eq) were diluted with 2 mL of acetonitrile. To the mixture, triethylamine (TEA) (245 µL, 1.76 mmol, 4.0 eq) and myristic acid (99 mg, 435 µmol, 1 eq) were added. The solution was stirred for 30 min at room temperature. Then, $NH_2OH{\cdot}HCl$ (61 mg, 881 µmol, 2.0 eq) was added and the reaction was stirred overnight at room

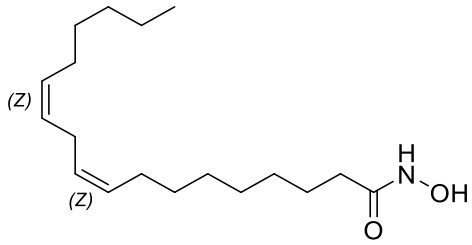
temperature. After this time, the reaction mixture was diluted with 30 mL of EtOAc and washed three times with brine (10 mL). The resulting organic phase was dried (Na_2SO_4), filtered and evaporated. The crude was purified by column chromatography on silica gel (50 % EtOAc in hexane), yielding 46.4 mg (191 µmol, 44 %) of the desired product as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (bs, 2H, -NH, -OH), 2.05 (t, *J* = 7.5 Hz, 2H, -CH_2-CO-), 1.55 (p, *J* = 7.1 Hz, 2H, -CH_2-CH_2-CO-), 1.31 – 1.13 (m, 20H, -CH_2-), 0.83 (t, *J* = 6.7 Hz, 3H, -CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 33.0, 32.0, 29.72, 29.69, 29.66, 29.53, 29.39, 29.34, 29.22, 25.5, 22.7, 14.1, HR-MS: *m/z* calculated for $C_{14}H_{30}NO_2$: 244.2277 $[M+H]^+$, found: 244.2266 $[M+H]^+$.

Synthesis of C16 FAH



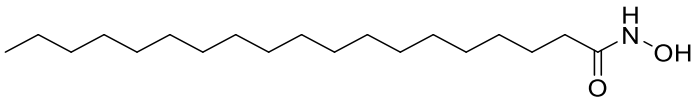
In a 10 mL round bottom flask, 280 µL of a T3P® solution (50 % in EtOAc, 470 µmol, 1.4 eq) were diluted with 2 mL of acetonitrile. To the mixture, TEA (220 µL, 1.58 mmol, 4.7 eq) and palmitic acid (101 mg, 337 µmol, 1 eq) were added. The solution was stirred for 30 min at room temperature. Then, $NH_2OH{\cdot}HCl$ (55 mg, 789 µmol, 2.3 eq) was added and the reaction was stirred overnight at room temperature. After this time, 30 mL of ethyl acetate were added and the organic phase was washed three times with brine (10 mL). The resulting organic phase was dried (Na_2SO_4), filtered and evaporated. The crude was purified by column chromatography on silica gel (1-10 % methanol (MeOH) in dichloromethane (CH_2Cl_2)) obtaining 46.2 mg (170 µmol, 44 %) of the desired product as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (bs, 2H, -NH, -OH), 2.07 (t, *J* = 7.5 Hz, 2H, -CH_2-CO-), 1.58 (p, *J* = 7.3 Hz, 2H, -CH_2-CH_2-CO-), 1.31 – 1.16 (m, 24H, -CH_2-), 0.84 (t, *J* = 6.4 Hz, 3H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 49.6, 33.1, 32.0, 29.8, 29.72, 29.68, 29.55, 29.43, 29.36, 29.25, 25.5, 22.8, 14.2; HR-MS: *m/z* calculated for $C_{16}H_{34}NO_2$: 272.2589 $[M+H]^+$, found: 272.2588 $[M+H]^+$.

Synthesis of C18:2 FAH



In a 10 mL round bottom flask, 115 µL of T3P® solution (50 % in EtOAc, 194 µmol, 1.2 eq) were diluted with 0.8 mL of acetonitrile. To the mixture, TEA (90 µL, 646 µmol, 4.0 eq) and linoleic acid (50 µL, 161 µmol, 1 eq) were added. The solution was stirred for 30 min at room temperature. Then, $NH_2OH{\cdot}HCl$ (23.6 mg, 340 µmol, 2.1 eq) was added and the reaction was stirred overnight at room temperature. After this time, 20 mL of ethyl acetate were added and the organic phase was washed three times with brine (5 mL). The resulting organic phase was dried (Na_2SO_4), filtered and evaporated. The crude was purified by column chromatography on silica gel (3 % MeOH in CH_2Cl_2) obtaining 8 mg (27 µmol, 17 %) of the desired product as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 – 5.28 (m, 4H, -C<u>H</u>=C<u>H</u>-), 2.77 (t, *J* = 6.3 Hz, 2H, -<u>CH_2</u>-CH=), 2.41 – 2.26 (m, 2H, -<u>CH_2</u>-CH_2-CH=), 2.18 – 2.09 (m, 2H, -<u>CH_2</u>-CH_2-CH=), 2.09 – 1.95 (m, 2H, -<u>CH_2</u>-CO-), 1.75 – 1.51 (m, 2H, -<u>CH_2</u>-CH_2-CO-), 1.45 – 1.21 (m, 14H, -<u>CH_2</u>-), 0.88 (t, *J* = 6.6 Hz, 3H, -<u>CH_3</u>); HR-MS: *m/z* calculated for $C_{18}H_{34}NO_2$: 296.2589 $[M+H]^+$, found: 296.2589 $[M+H]^+$.

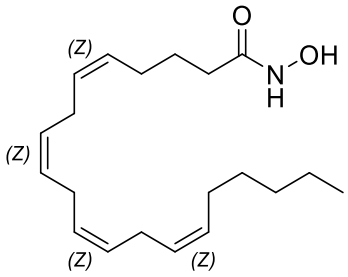
Synthesis of C19 FAH



In a 10 mL round bottom flask, 246 µL of T3P® solution (50 % in EtOAc, 414 µmol, 1.2 eq) were diluted with 2 mL of acetonitrile. To the mixture, TEA (192 µL, 1.38 mmol, 4.1 eq) and nonadecanoic acid (100 mg, 336 µmol, 1 eq) were added. The solution was stirred for 30 min at room temperature. Then, $NH_2OH{\cdot}HCl$ (49 mg, 711 µmol, 2.1 eq)

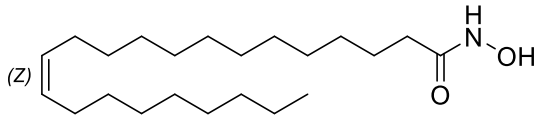
was added and the reaction was stirred overnight at room temperature. After this time, 30 mL of ethyl acetate were added and the organic phase was washed three times with brine (10 mL). The resulting organic phase was dried (Na_2SO_4), filtered and evaporated. The crude was purified by column chromatography on silica gel (1-10 % MeOH in CH_2Cl_2) obtaining 46.4 mg (148 µmol, 44 %) of the desired product as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (bs, 2H, -NH, -OH), 2.07 (t, *J* = 7.6 Hz, 2H, -CH_2-CO-), 1.58 (p, *J* = 7.2 Hz, 2H, -CH_2-CH_2-CO-), 1.22 (m, 30H, -CH_2-), 0.85 (t, *J* = 6.7 Hz, 3H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 33.0, 32.0, 29.78, 29.73, 29.69, 29.56, 29.44, 29.26, 25.5, 22.8, 14.2; HR-MS: *m/z* calculated for $C_{19}H_{40}NO_2$: 314.3059 $[M+H]^+$, found: 314.3056 $[M+H]^+$.

Synthesis of C20:4 FAH



In a 1.5 mL polypropylene tube, 0.3 mL of acetonitrile are added. Then, 49 µL of T3P® solution (50 % in EtOAc, 82 µmol, 1.4 eq), TEA (34 µL, 244 µmol, 4.0 eq) and arachidonic acid (20 µL, 61 µmol, 1 eq) were added. The solution was stirred for 30 min at room temperature. Then, $NH_2OH{\cdot}HCl$ (9.3 mg, 133 µmol, 2.2 eq) was added and the reaction was stirred overnight at room temperature. After this time, 10 mL of ethyl acetate were added and the organic phase was washed three times with brine (4 mL). The resulting organic phase was dried (Na_2SO_4), filtered and evaporated. The crude was purified by column chromatography on silica gel (3 % MeOH in CH_2Cl_2) obtaining 2.5 mg (7.8 µmol, 13 %) of the desired product as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 – 5.28 (m, 8H, -CH=CH-), 2.88 – 2.79 (m, 10H, -CH_2-CH=), 2.14 (dq, *J* = 14.2, 7.3 Hz, 2H, -CH_2-CH_2-CH=), 2.06 (m, 2H, -CH_2-CO-), 1.64 – 1.49 (m, 2H, -CH_2-CH_2-CO-), 1.48 – 1.23 (m, 6H, -CH_2-), 0.96 – 0.81 (m, 3H, -CH_3); HR-MS: *m/z* calculated for $C_{20}H_{34}NO_2$: 320.2589 $[M+H]^+$, found: 320.2587 $[M+H]^+$.

Synthesis of C22:1 FAH



In a 10 mL round bottom flask, 228 µL of T3P® solution (50 % in EtOAc, 383 µmol, 1.2 eq) were diluted with 1.5 mL of acetonitrile. To the mixture, TEA (178 µL, 1.28 mmol, 4.0 eq) and erucic acid (90 %, 120 mg, 319 µmol, 1 eq) were added. The solution was stirred for 30 min at room temperature. Then, $NH_2OH \cdot HCl$ (45 mg, 638 µmol, 2.0 eq) was added and the reaction was stirred overnight at room temperature. After this time, the mixture was diluted with 30 mL of EtOAc and washed three times with brine (10 mL). The resulting organic phase was dried (Na_2SO_4), filtered and evaporated. The crude was purified by column chromatography on silica gel (1-5 % MeOH in CH_2Cl_2) obtaining 71.1 mg (201 µmol, 63 %) of the desired product as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (t, *J* = 4.9 Hz, 2H, -C<u>H</u>=C<u>H</u>-), 2.14 (t, *J* = 7.5 Hz, 2H, -<u>CH_2</u>-CO-), 2.06 – 1.96 (m, 4H, -<u>CH_2</u>-CH=), 1.63 (p, *J* = 7.2 Hz, 2H, -<u>CH_2</u>-CH_2-CO-), 1.40 – 1.18 (m, 24H, -<u>CH_2</u>-), 0.88 (t, *J* = 6.7 Hz, 3H, -<u>CH_3</u>); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.74, 130.08, 130.02, 33.17, 32.06, 29.93, 29.86, 29.77, 29.75, 29.71, 29.68, 29.61, 29.48, 29.41, 29.28, 27.37, 25.47, 22.84, 14.27; HR-MS: *m/z* calculated for $C_{22}H_{44}NO_2$: 354.3372 $[M+H]^+$, found: 354.3376 $[M+H]^+$.

Supplementary Figures

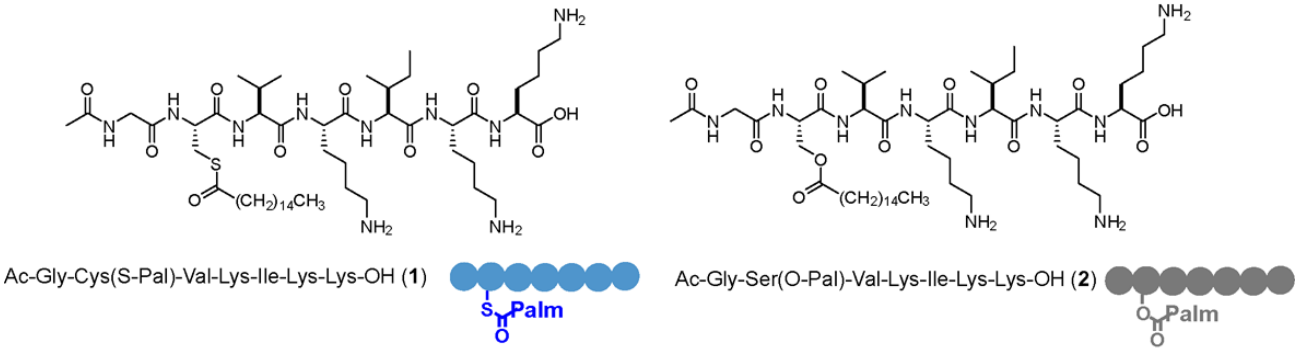


Figure S1. Structure of peptides **1** and **2**.

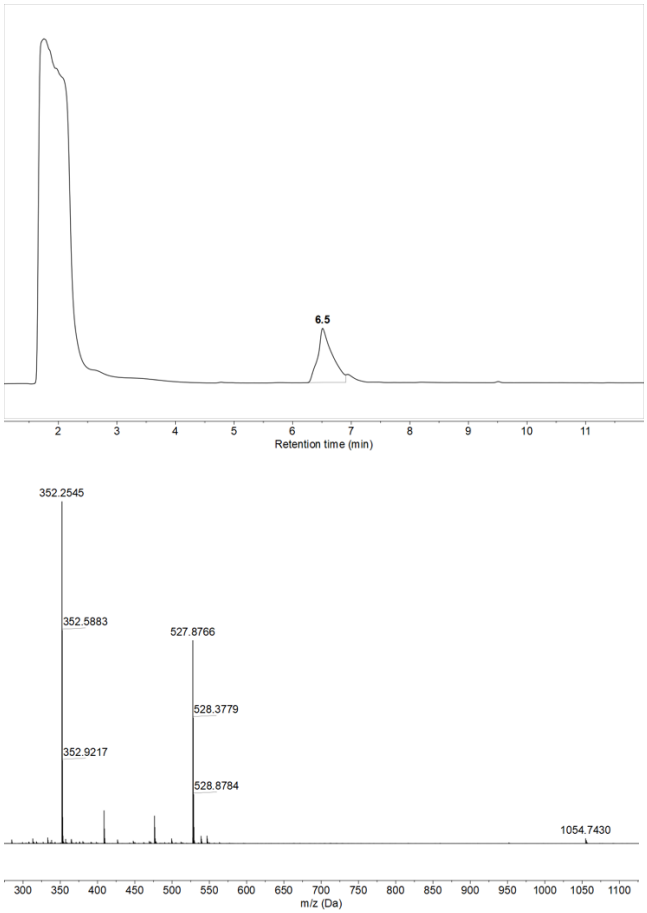


Figure S2. Reverse Phase (RP)-HPLC chromatogram and mass spectra of the purified peptide (**1**)

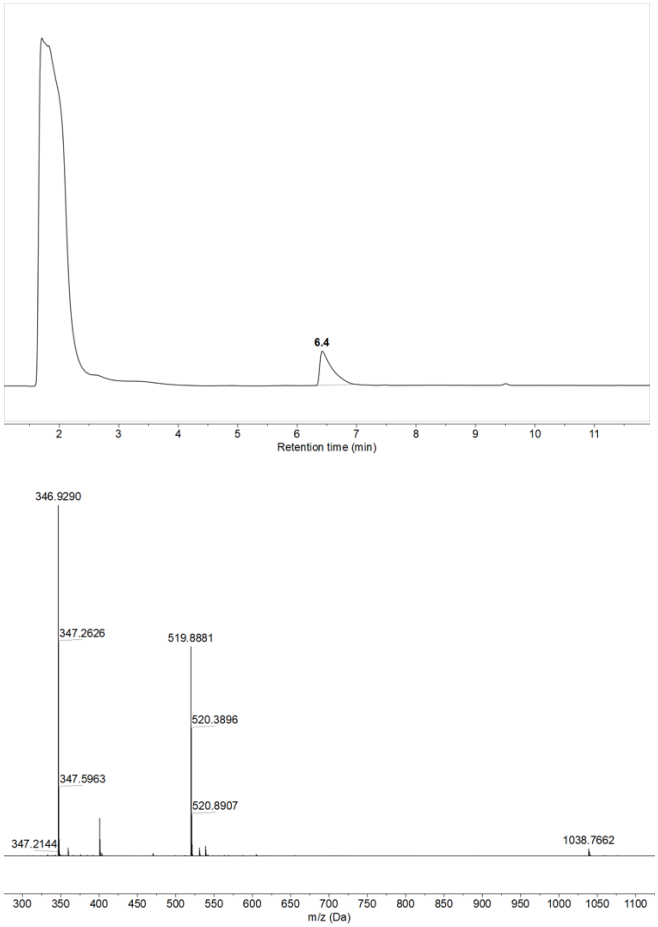


Figure S3. RP-HPLC chromatogram and mass spectra of the purified peptide (**2**)

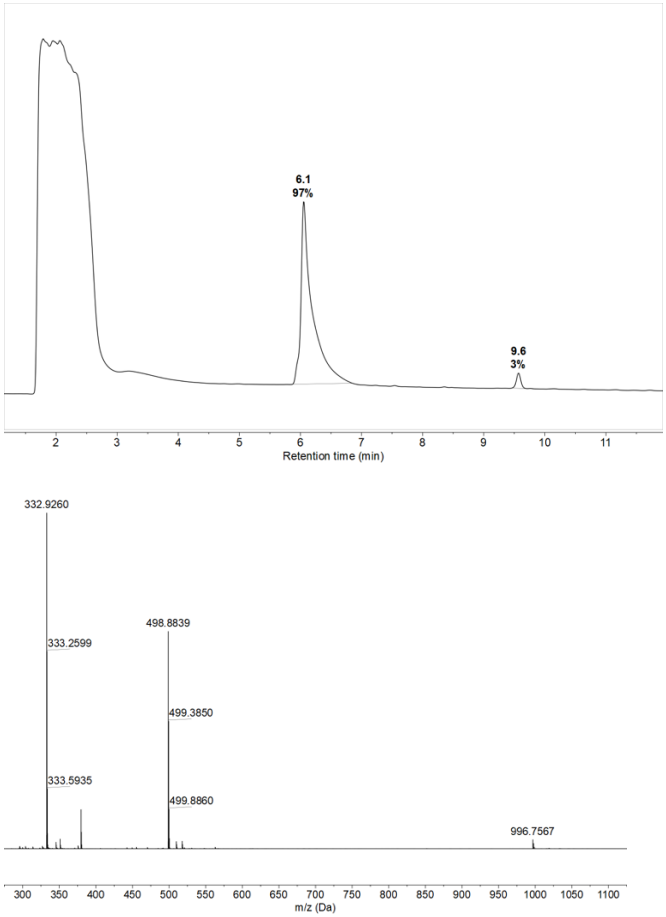


Figure S4. RP-HPLC chromatogram and mass spectra of the purified peptide (**3**)

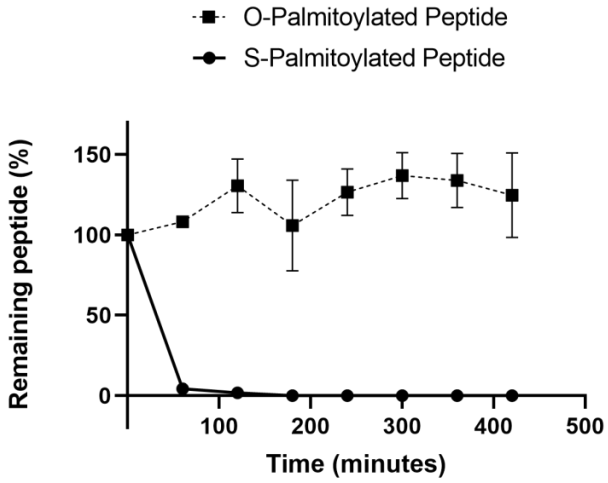


Figure S5. HPLC monitoring of the thioester/ester bond hydrolysis of peptides **1** and **2** at room temperature under the presence of a 2 M solution of NH_2OH.

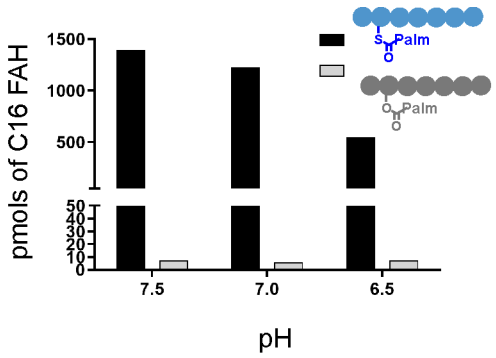


Figure S6. LC-MS monitoring of C16 FAH formation upon an overnight treatment of peptides **1** and **2** with a 2 M concentration of NH_2OH.

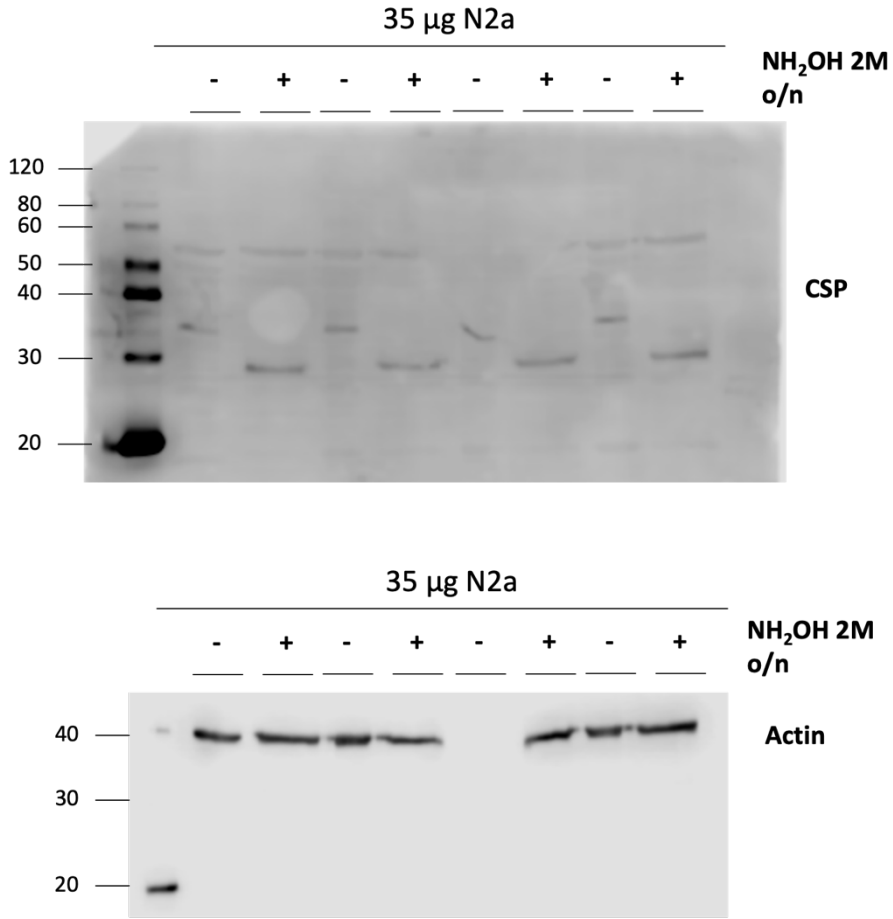


Figure S7.35 µg of proteins from N2a cell lysates (untreated or treated with 2M NH_2OH overnight) were subjected to Western blotting and probed against Cysteine String Protein (CSP) or Actin. The mass shift shows the depalmitoylation of CSP by NH_2OH. The positions of the molecular weight markers are shown on the left side.

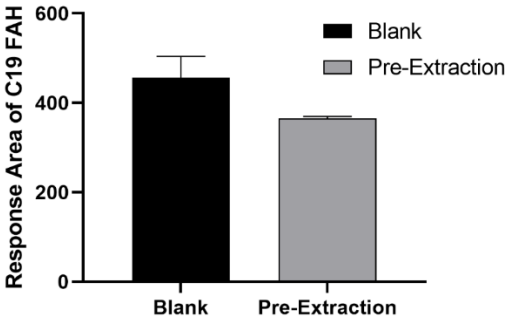
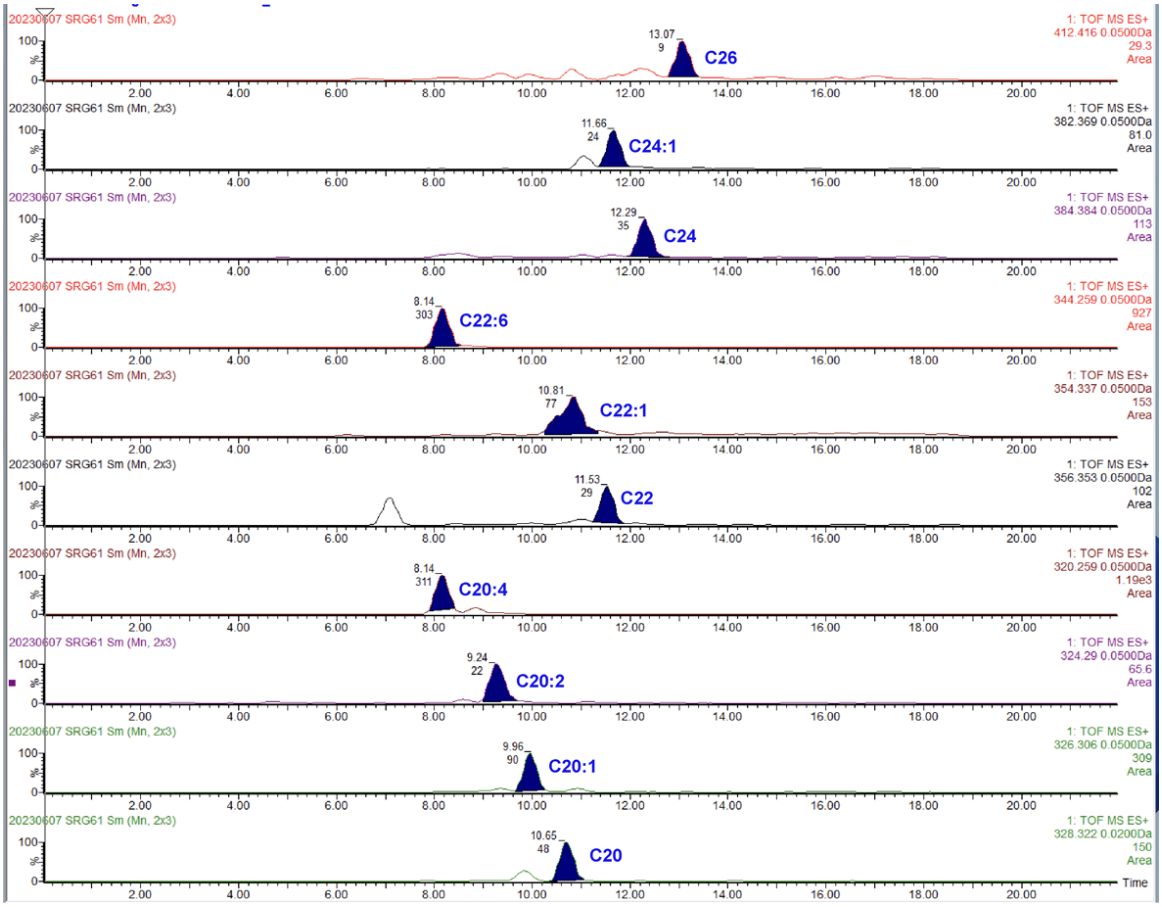


Figure S8. The recovery of the internal standard was measured by comparing the peak areas obtained with the C19 FAH spiked pre-extraction with the one present in a matrix blank. The percentage of recovery was established at 80% (±4.9, SEM, n = 3)



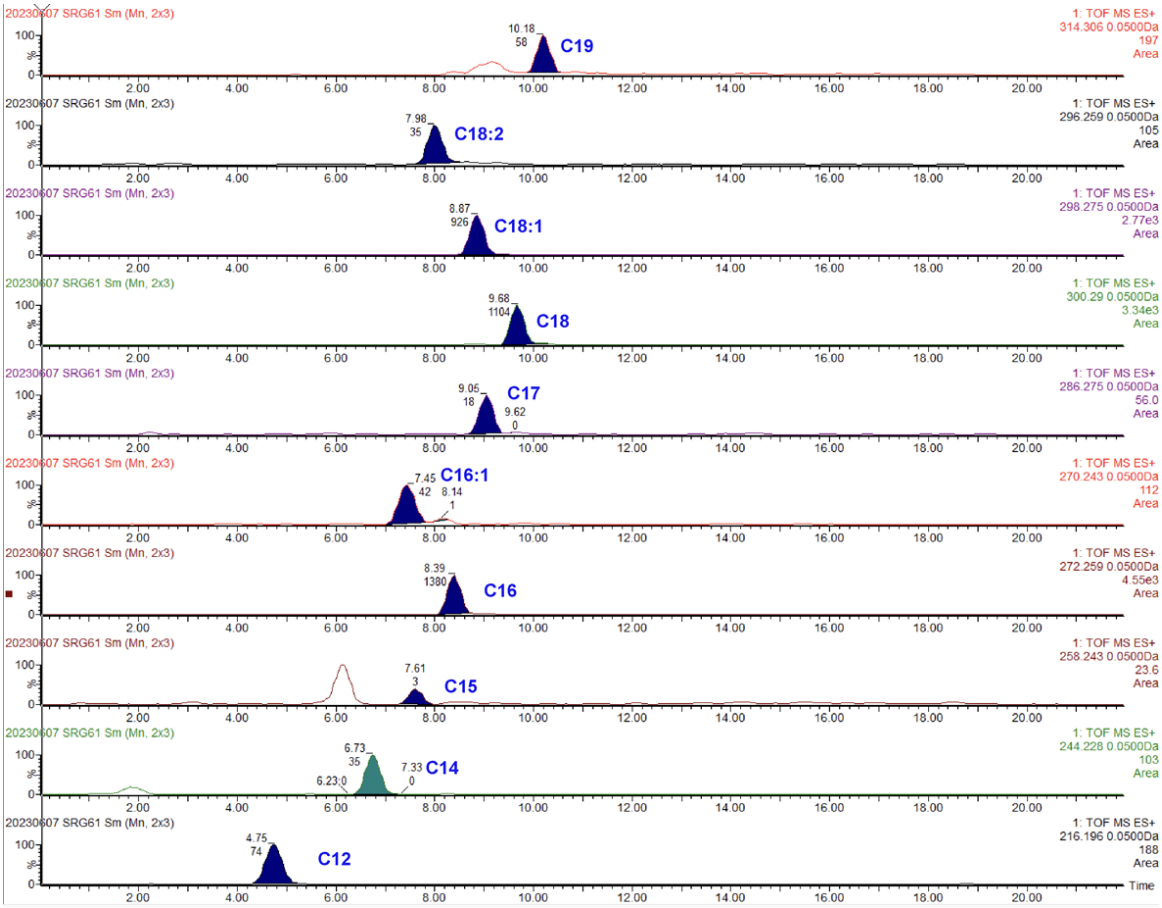


Figure S9. Representative extracted ion chromatograms of different fatty acid hydroxamates (FAH). Peak annotation displays the retention time and the peak area and, on the right, the calculated m/z $[M+H]^+$ for each species is indicated.

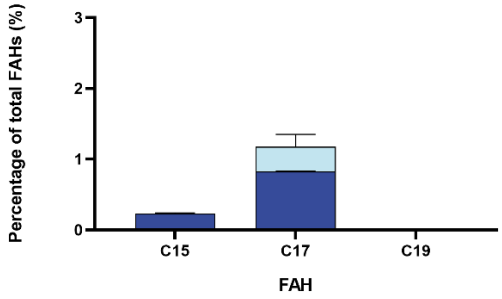


Figure S10. Abundance of FAHs derived from odd-chain fatty acids detected in the proteome of A375 cells. Percentages are calculated from the sum of the peak areas observed in an experiment performed in the absence of the C19 FAH internal standard. All panels display mean with error bars representing standard deviation.

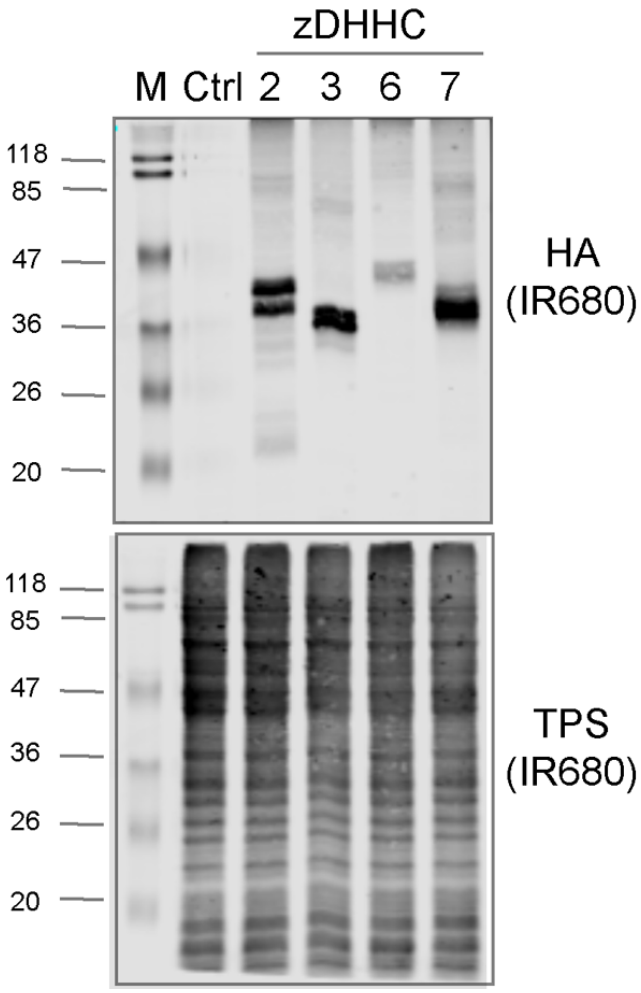


Figure S11. HEK293T cells were transfected with the HA-tagged zDHHCs or pEF-BOS-HA (referred to as control (Ctrl) in the figure). Representative images show zDHHC (top; IR680) and total protein stain (TPS) (bottom panel; IR680). The positions of the molecular weight markers are shown on the left side.

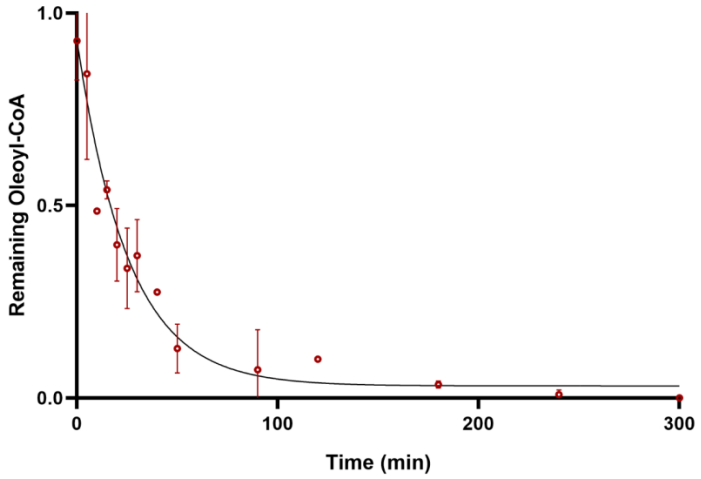


Figure S12. Monitoring oleoyl-CoA thioester cleavage by HPLC. Oleoyl-CoA thioester was treated with a 2 M solution of NH_2OH. At the given times, aliquots were taken and the amount of remaining acyl-CoA was measured by HPLC (n=3).

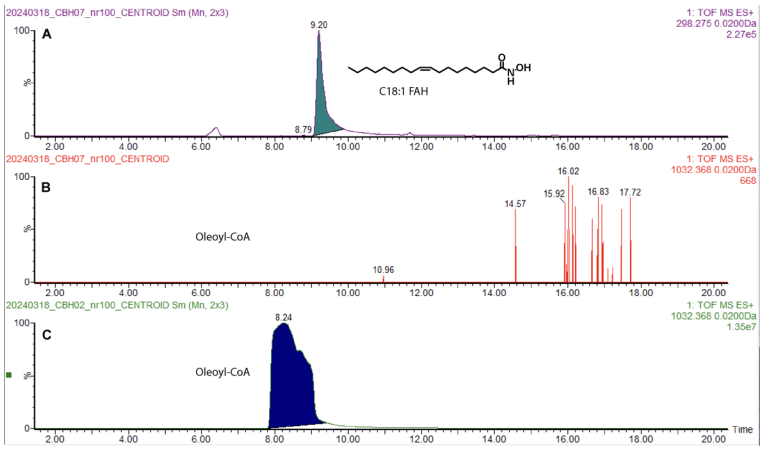


Figure S13. A) Extracted ion chromatogram of m/z 298.2746 displays a peak at retention time 9.20 that corresponds to the C18:1 FAH (*m/z* calculated for $C_{18}H_{35}NO_2$: 298.2746 $[M+H]^+$; and B) extracted ion chromatogram of m/z 1032.3684 of a sample of oleoyl-CoA treated with a 0.7 M solution of NH_2OH for 2 h (oleoyl-CoA, *m/z* calculated for $C_{39}H_{68}N_7O_{17}P_3S$: 1032.3684 $[M+H]^+$). C) Extracted ion chromatogram m/z 1032.3684 of a control sample of oleoyl-CoA displays a peak at retention time 8.24. The absence of detectable oleoyl-CoA in the B chromatogram, together with the

formation of C18:1 FAH (in A) confirms the complete cleavage of the oleoyl-CoA. Similar results were obtained after overnight treatment (n=3).

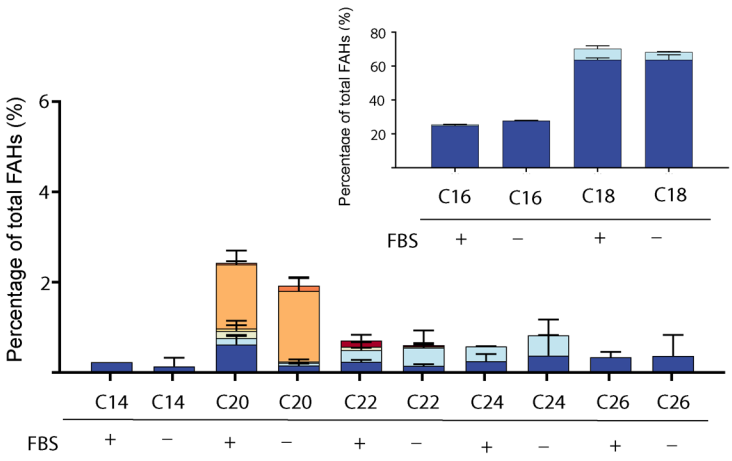
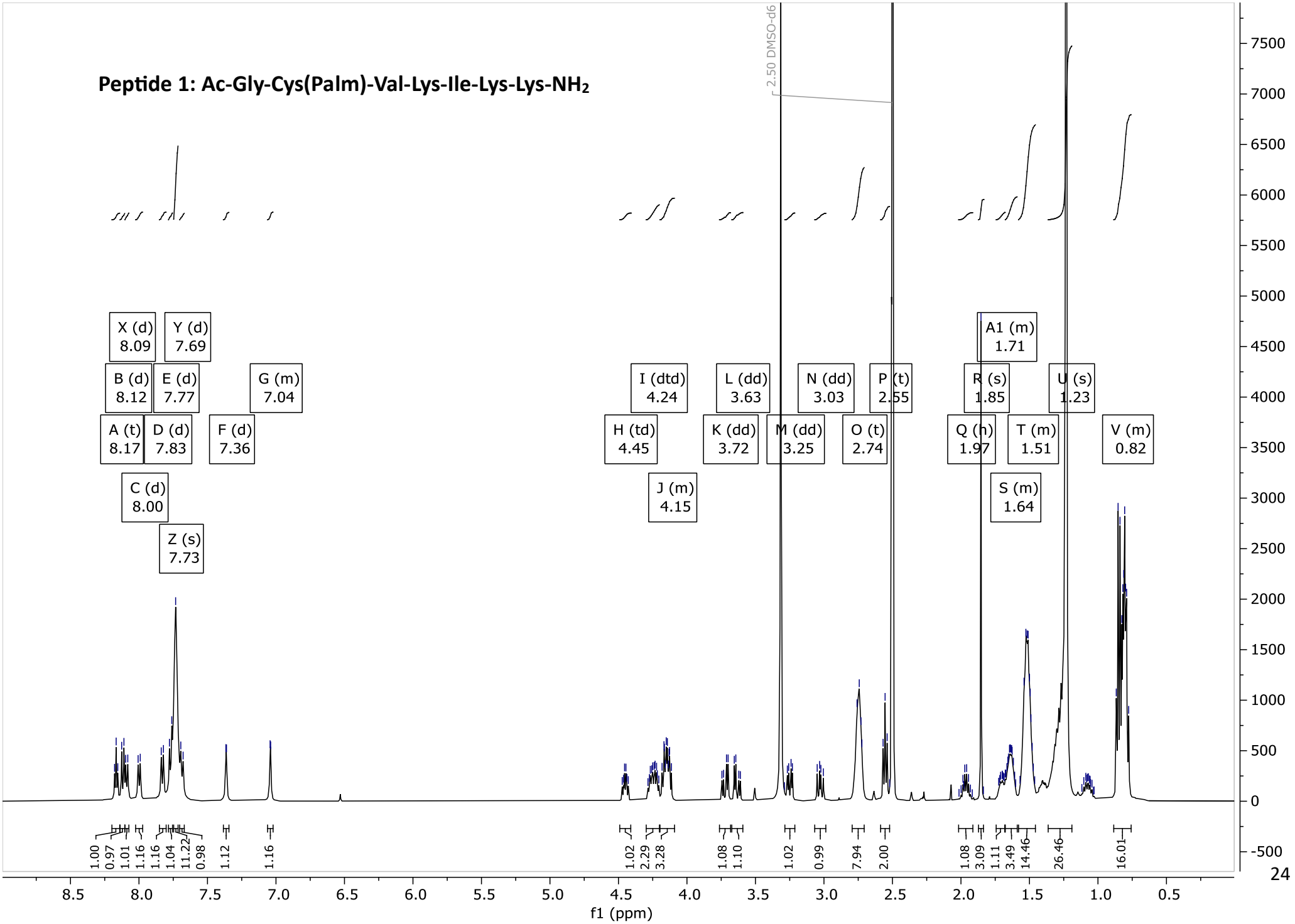
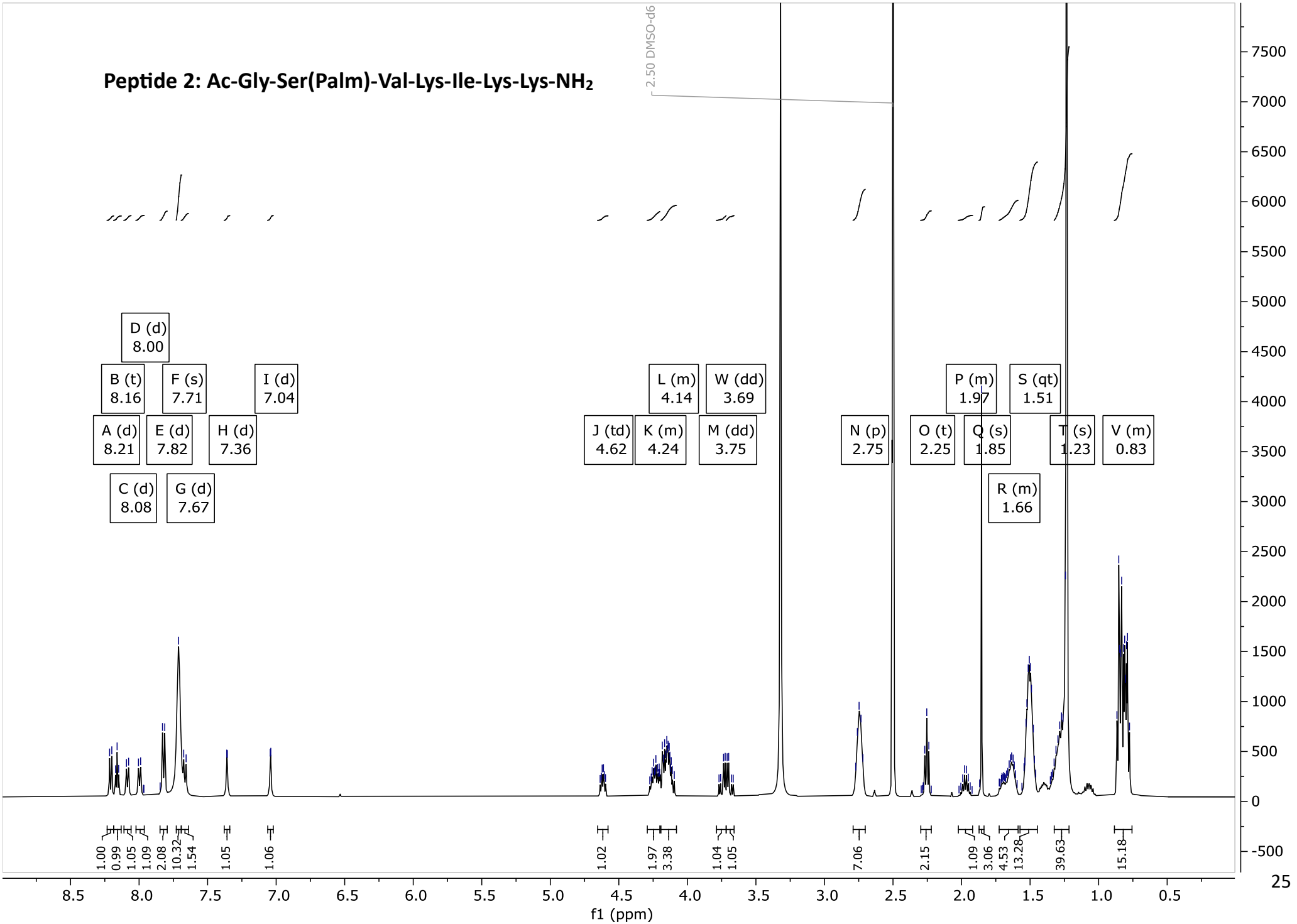
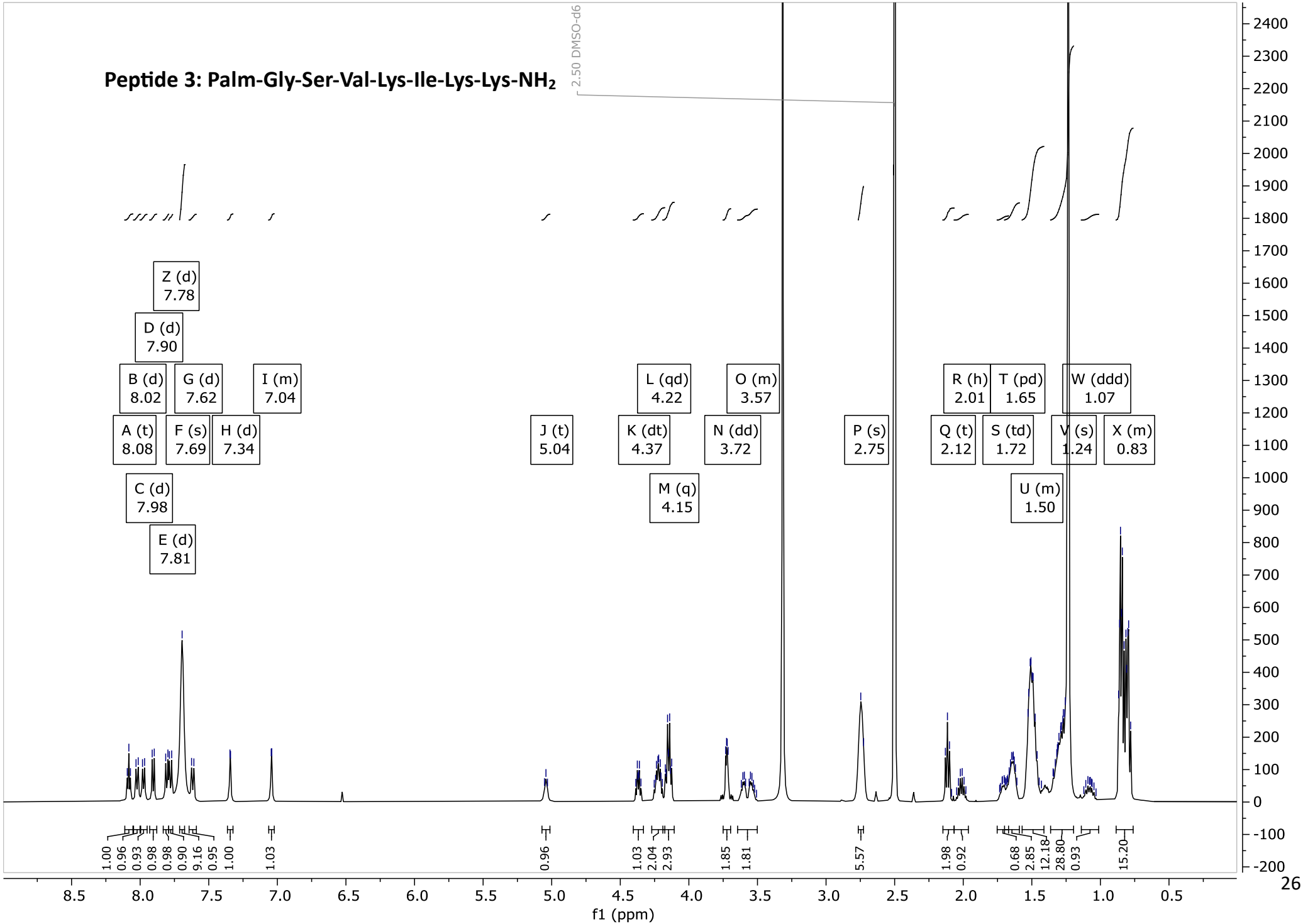


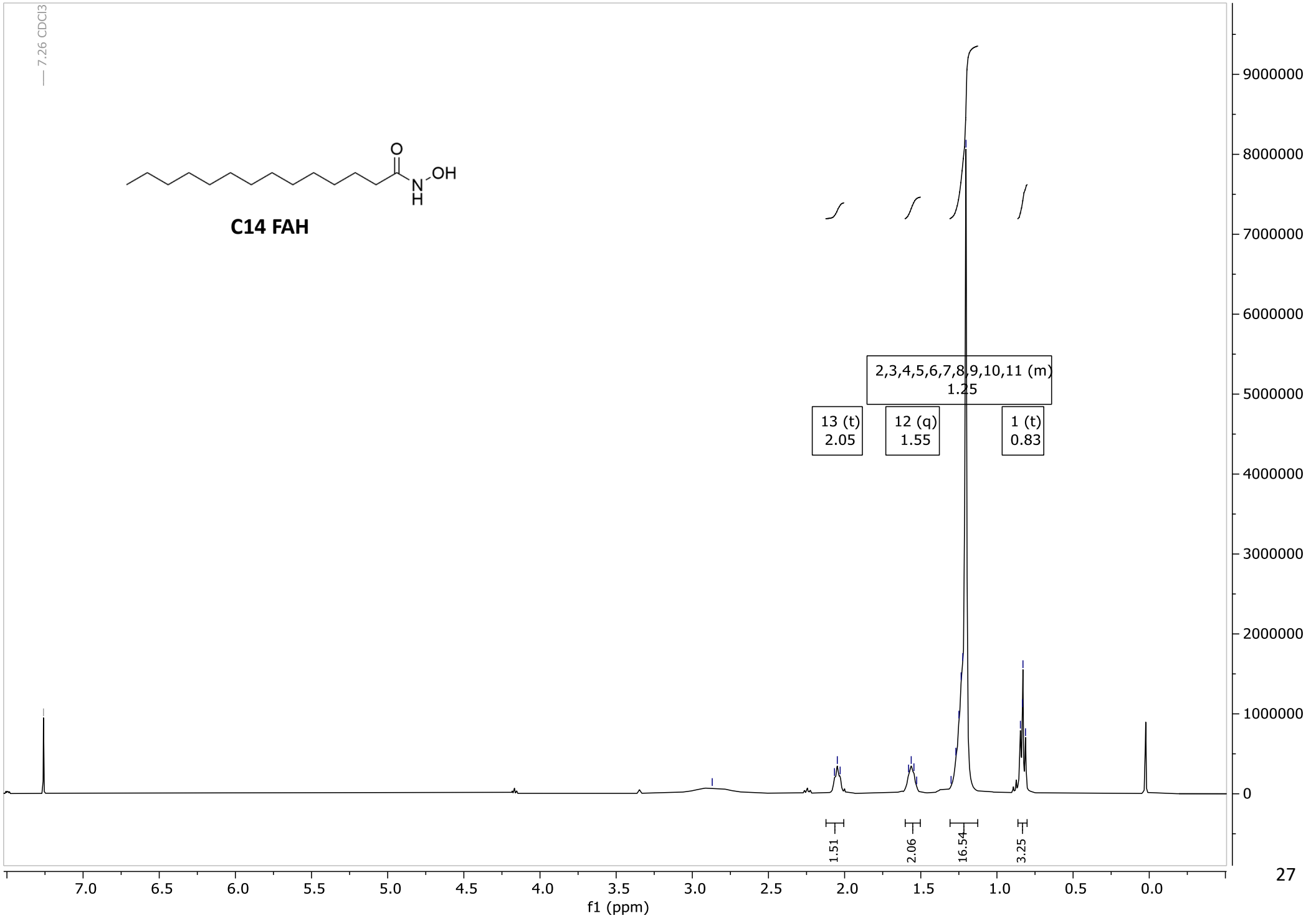
Figure S14. Effect of serum withdrawal on the composition of the S-acylome, expressed as a percentage of total FAHs detected.

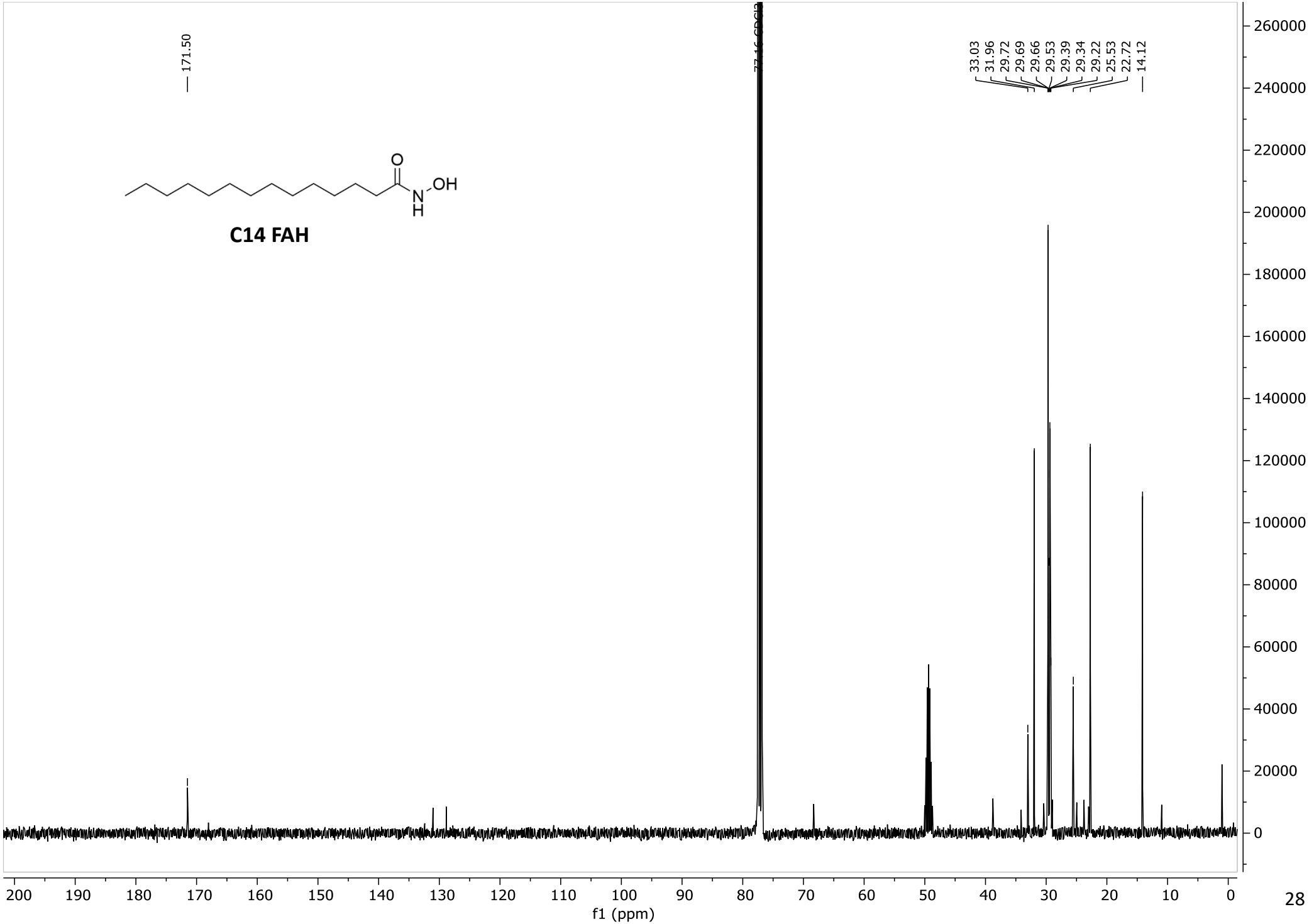
Figure S15. NMR of all peptides and FAHs.

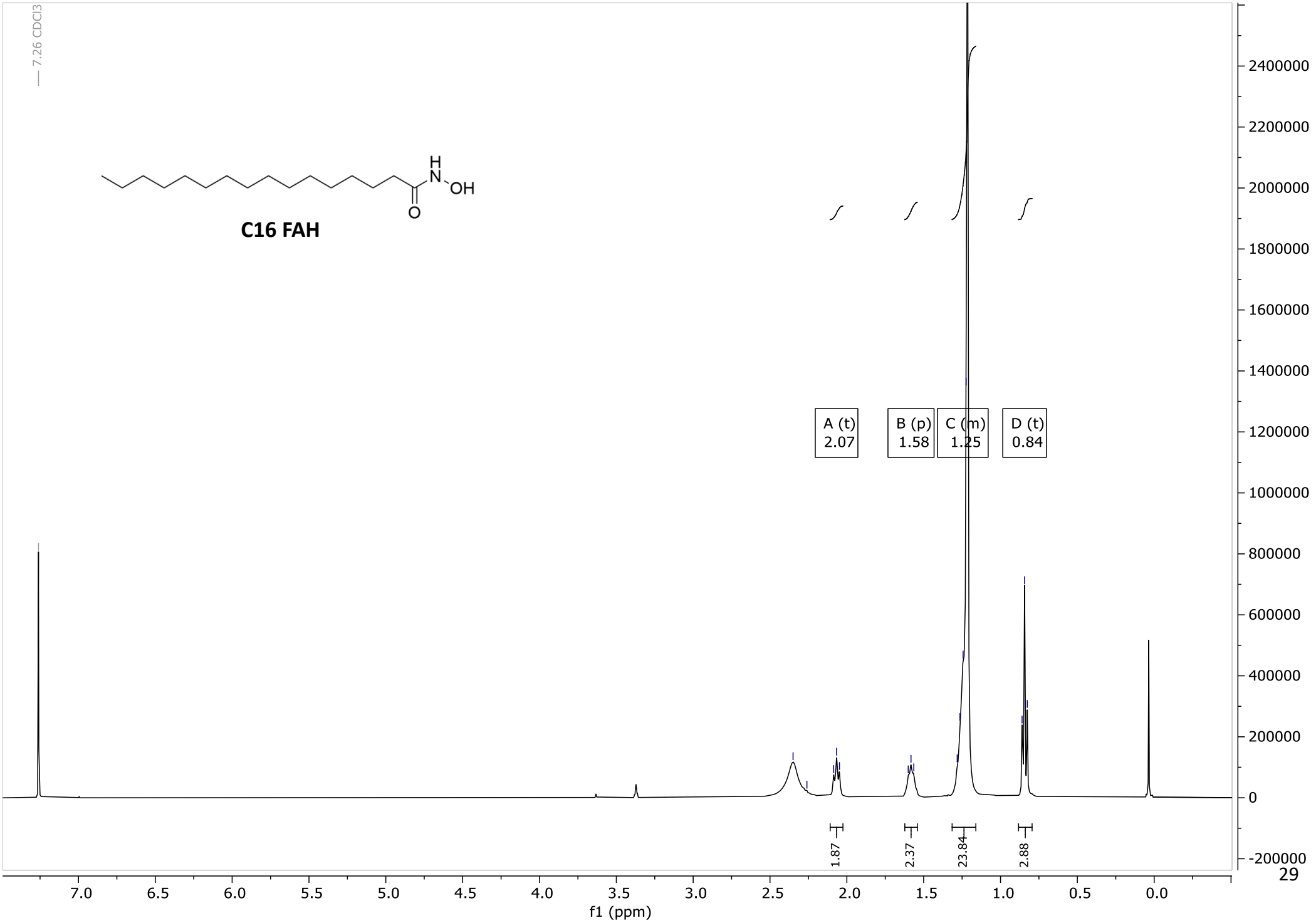

Peptide 1: Ac-Gly-Cys(Palm)-Val-Lys-Ile-Lys-Lys-NH2
2.50 DMSO-d6
X (d) 8.09
Y (d) 7.69
B (d) 8.12
E (d) 7.77
G (m) 7.04
A (t) 8.17
D (d) 7.83
F (d) 7.36
C (d) 8.00
Z (s) 7.73
I (dtd) 4.24
L (dd) 3.63
N (dd) 3.03
P (t) 2.55
H (td) 4.45
K (dd) 3.72
M (dd) 3.25
O (t) 2.74
J (m) 4.15
A1 (m) 1.71
R (s) 1.85
U (s) 1.23
Q (h) 1.97
T (m) 1.51
V (m) 0.82
S (m) 1.64
1.00 0.97 1.01 1.16 1.16 1.04 11.22 0.98 1.12 1.16
1.02 2.29 3.28 1.08 1.10 1.02 0.99 7.94 2.00 1.08 3.09 1.11 3.49 14.46 26.46 16.01
f1 (ppm)

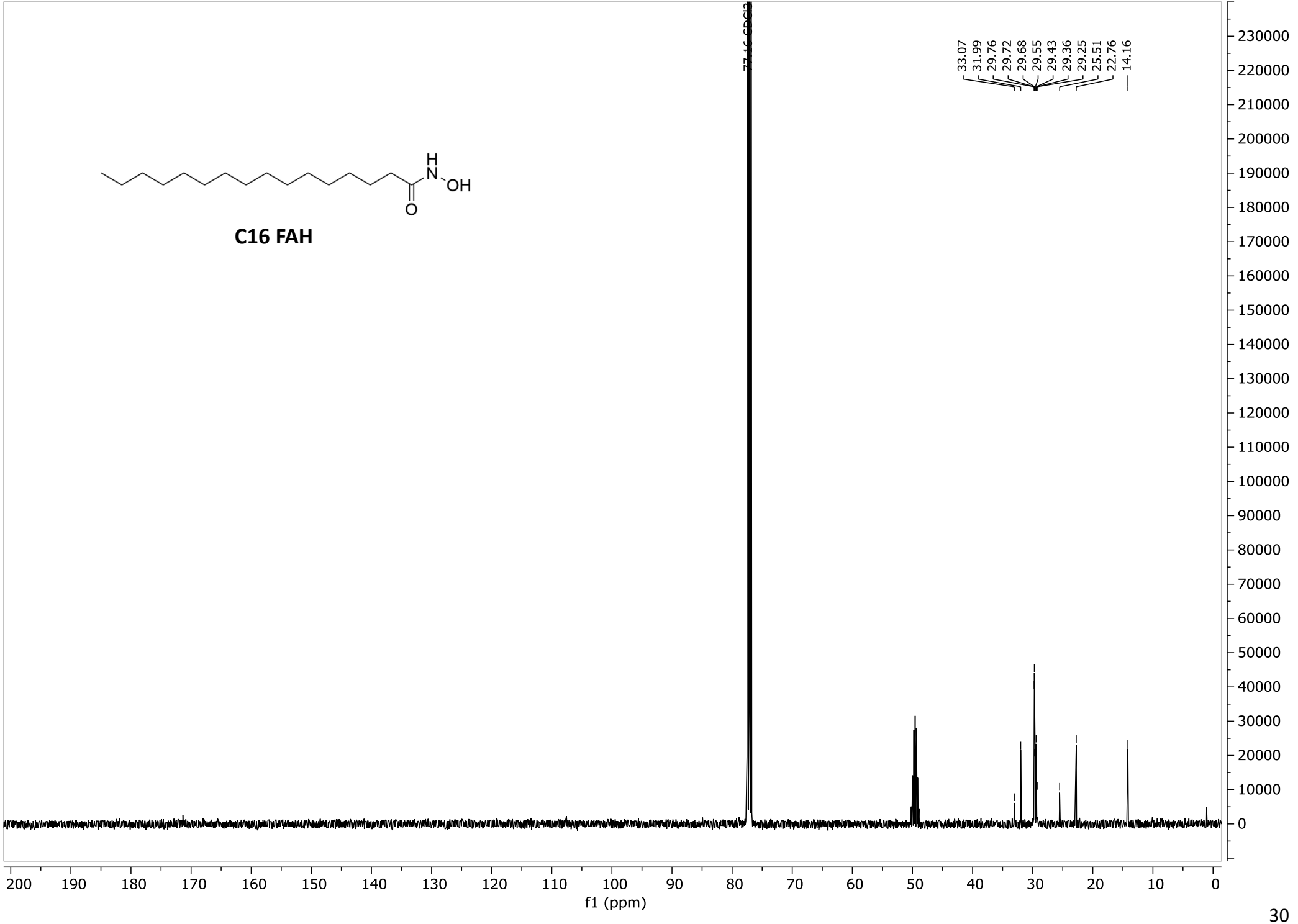

Peptide 2: Ac-Gly-Ser(Palm)-Val-Lys-Ile-Lys-Lys-NH2
2.50 DMSO-d6
D (d) 8.00
B (t) 8.16
F (s) 7.71
I (d) 7.04
A (d) 8.21
E (d) 7.82
H (d) 7.36
C (d) 8.08
G (d) 7.67
L (m) 4.14
W (dd) 3.69
J (td) 4.62
K (m) 4.24
M (dd) 3.75
N (p) 2.75
P (m) 1.97
S (qt) 1.51
O (t) 2.25
Q (s) 1.85
T (s) 1.23
V (m) 0.83
R (m) 1.66
1.00
0.99
1.05
1.09
2.08
10.32
1.54
1.05
1.06
1.02
1.97
3.38
1.04
1.05
7.06
2.15
1.09
3.06
4.53
13.28
39.63
15.18
f1 (ppm)

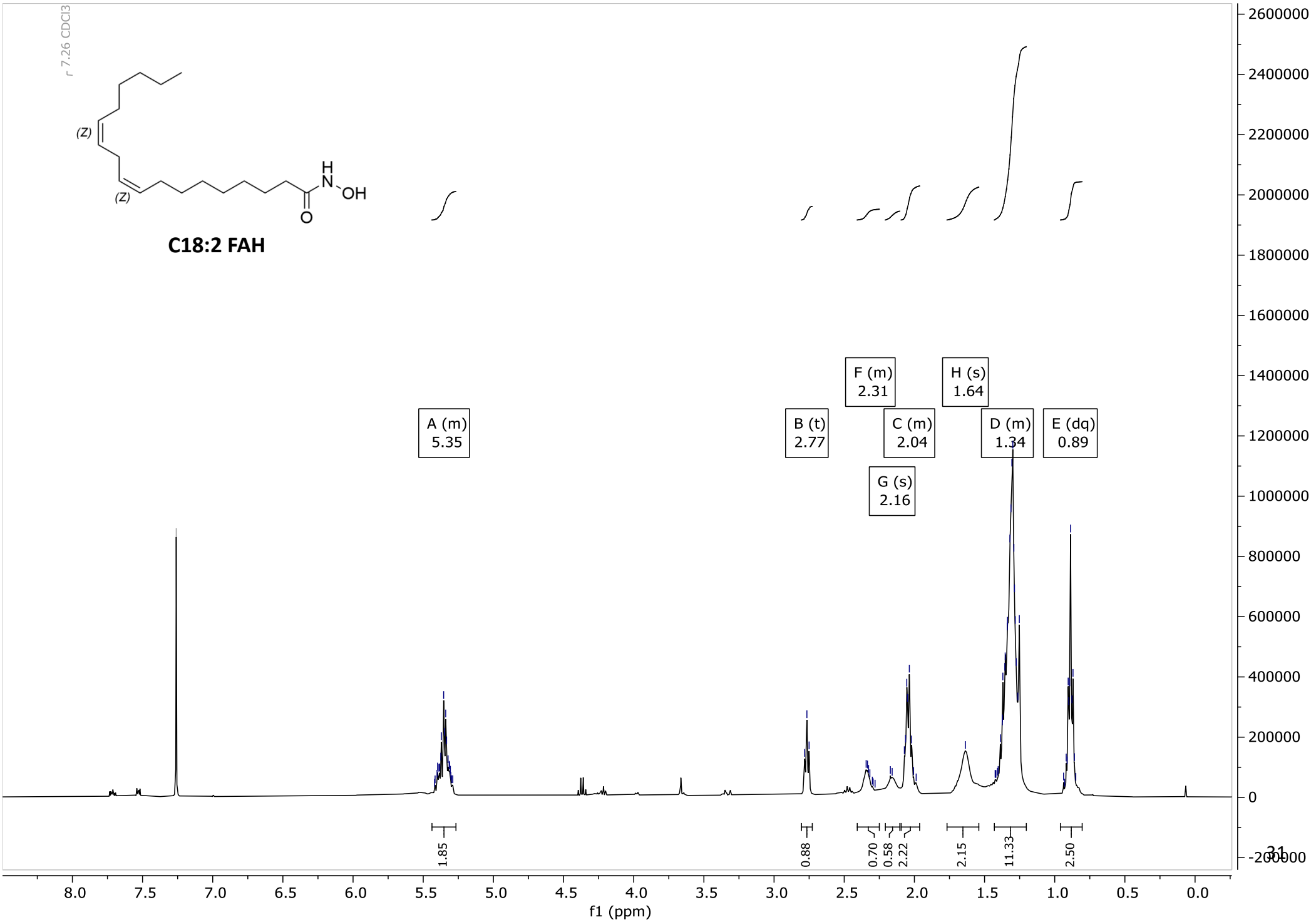

Peptide 3: Palm-Gly-Ser-Val-Lys-Ile-Lys-Lys-NH2
2.50 DMSO-d6
Z (d) 7.78
D (d) 7.90
B (d) 8.02
G (d) 7.62
I (m) 7.04
A (t) 8.08
F (s) 7.69
H (d) 7.34
C (d) 7.98
E (d) 7.81
J (t) 5.04
L (qd) 4.22
K (dt) 4.37
M (q) 4.15
O (m) 3.57
N (dd) 3.72
P (s) 2.75
R (h) 2.01
T (pd) 1.65
W (ddd) 1.07
Q (t) 2.12
S (td) 1.72
V (s) 1.24
X (m) 0.83
U (m) 1.50
1.00 0.96 0.93 0.98 0.98 0.90 9.16 0.95 1.00 1.03 0.96 1.03 2.04 2.93 1.85 1.81 5.57 1.98 0.92 0.68 2.85 12.18 28.80 0.93 15.20
f1 (ppm)

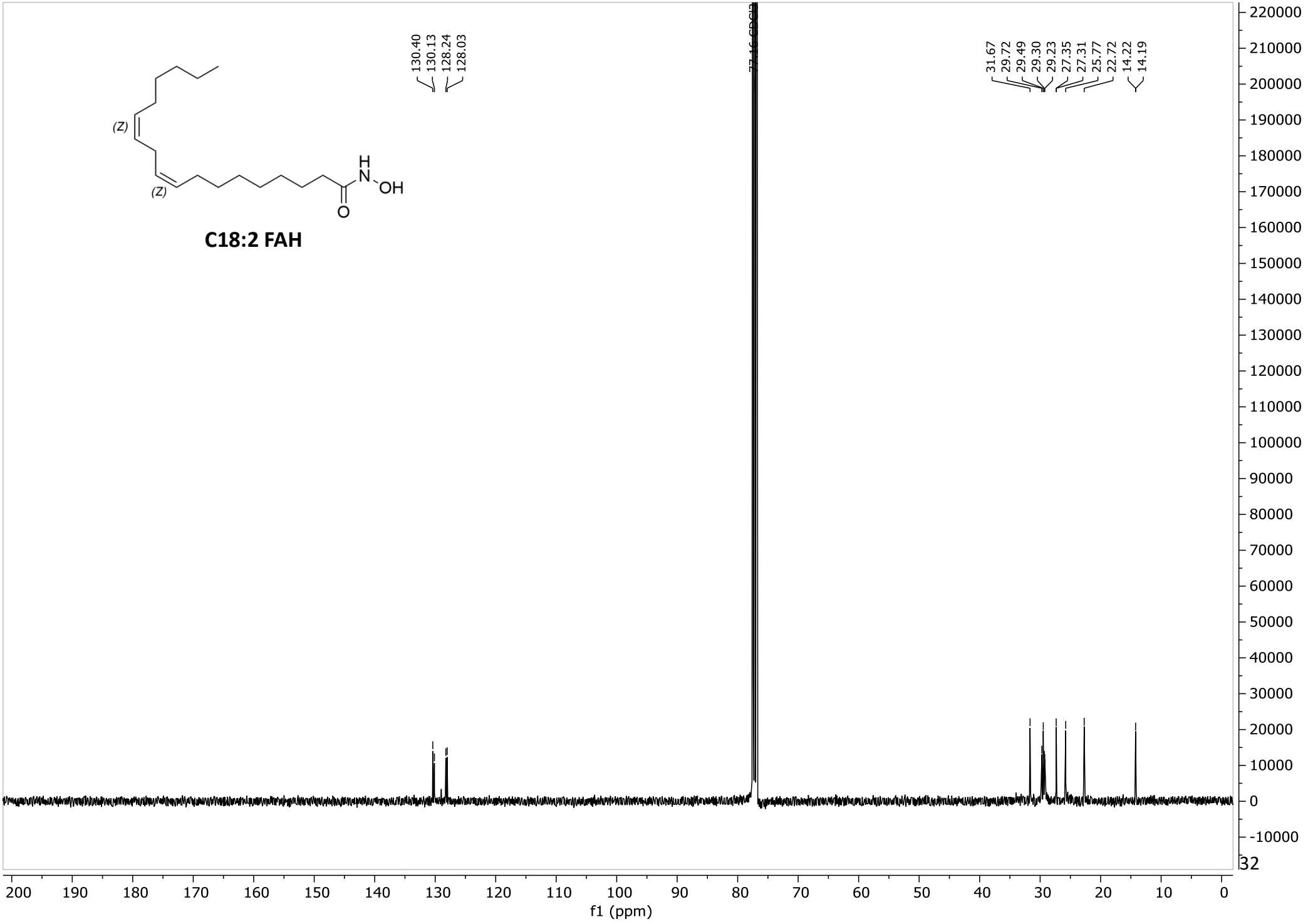

— 7.26 CDCl3
O
N
H
OH
C14 FAH
2,3,4,5,6,7,8,9,10,11 (m)
1.25
13 (t)
2.05
12 (q)
1.55
1 (t)
0.83
1.51
2.06
16.54
3.25
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

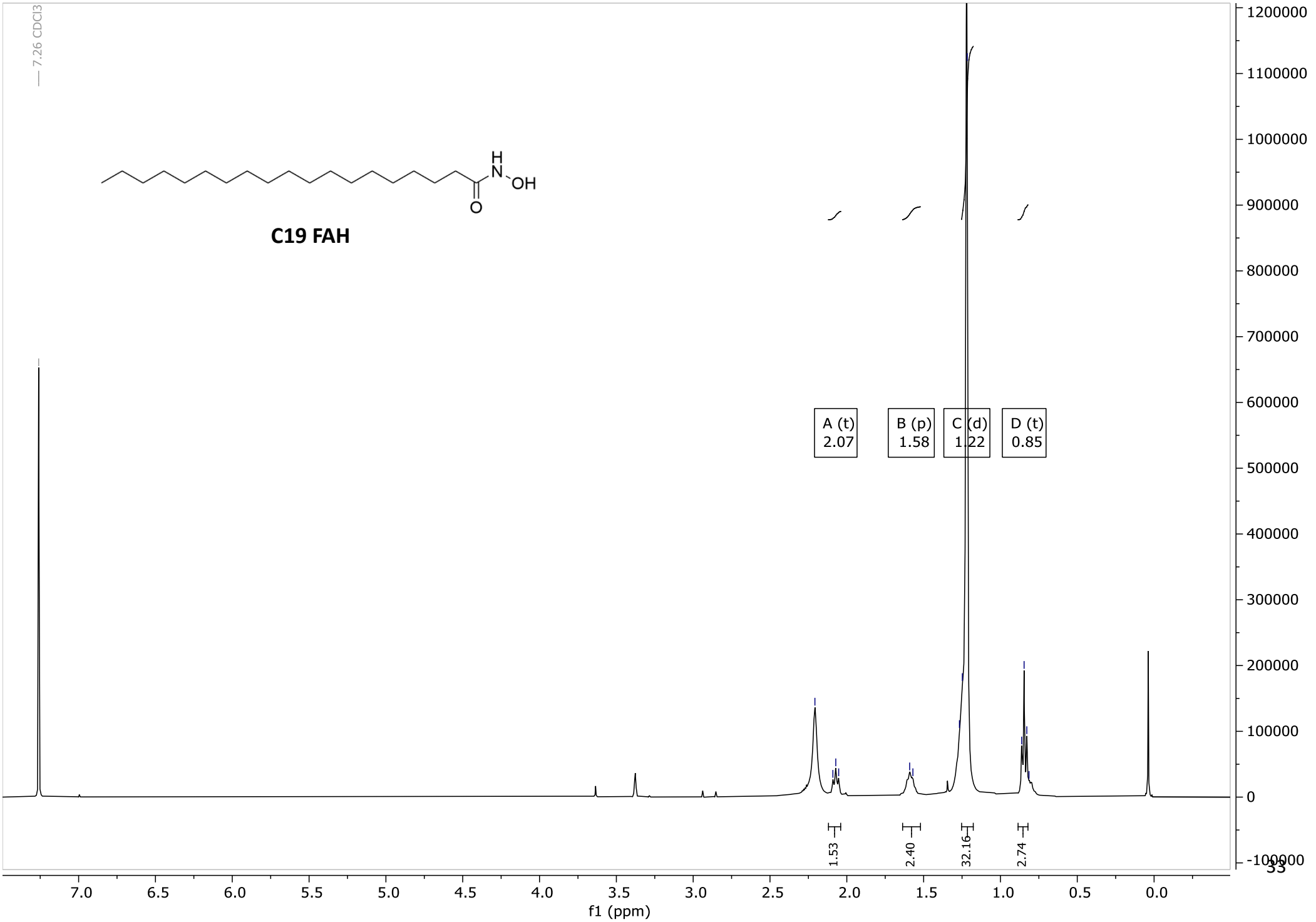

171.50
77.16 CDCl3
33.03
31.96
29.72
29.69
29.66
29.53
29.39
29.34
29.22
25.53
22.72
14.12
O
N
H
OH
C14 FAH
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

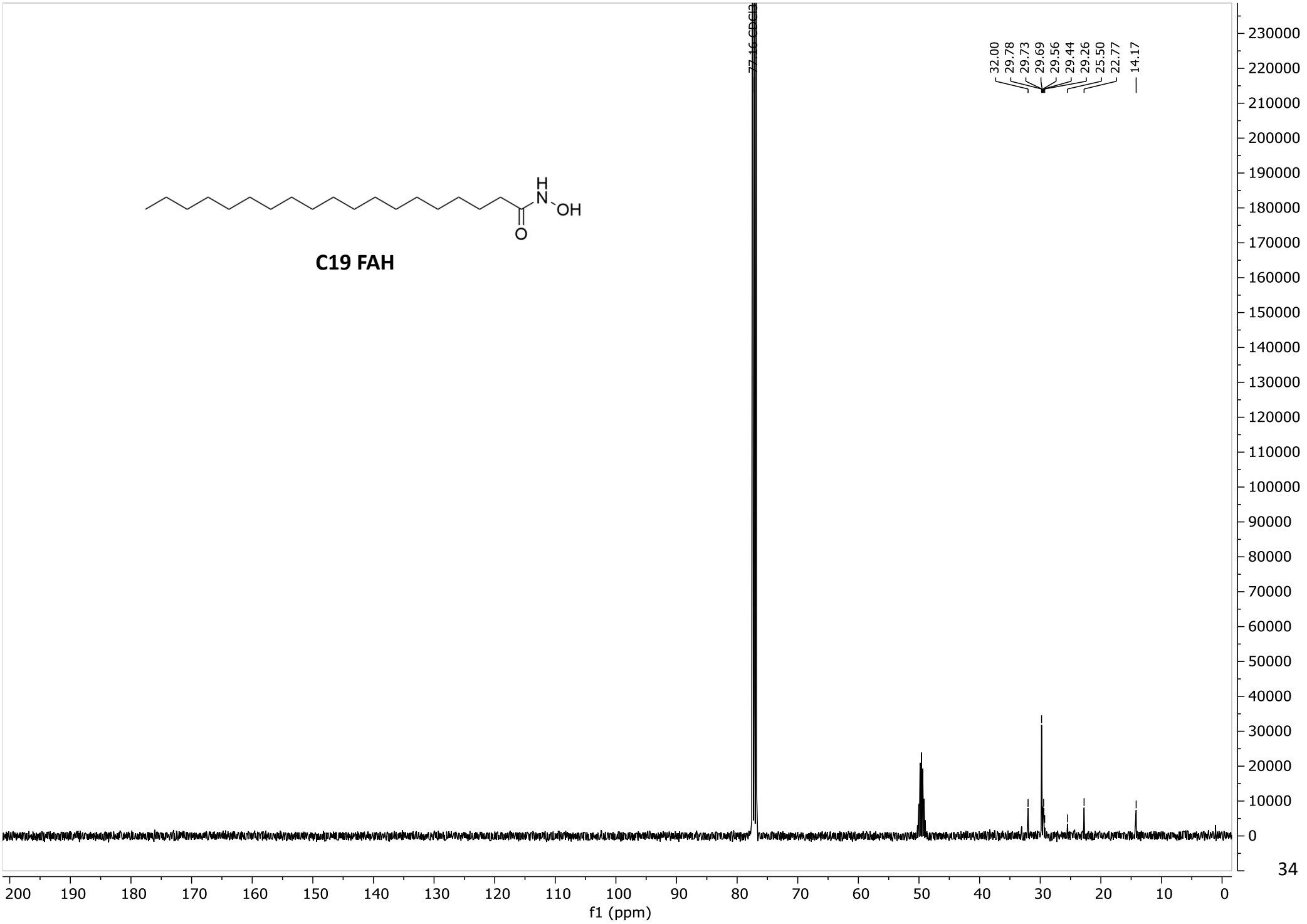

— 7.26 CDCl3
C16 FAH
A (t)
2.07
B (p)
1.58
C (m)
1.25
D (t)
0.84
1.87
2.37
23.84
2.88
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

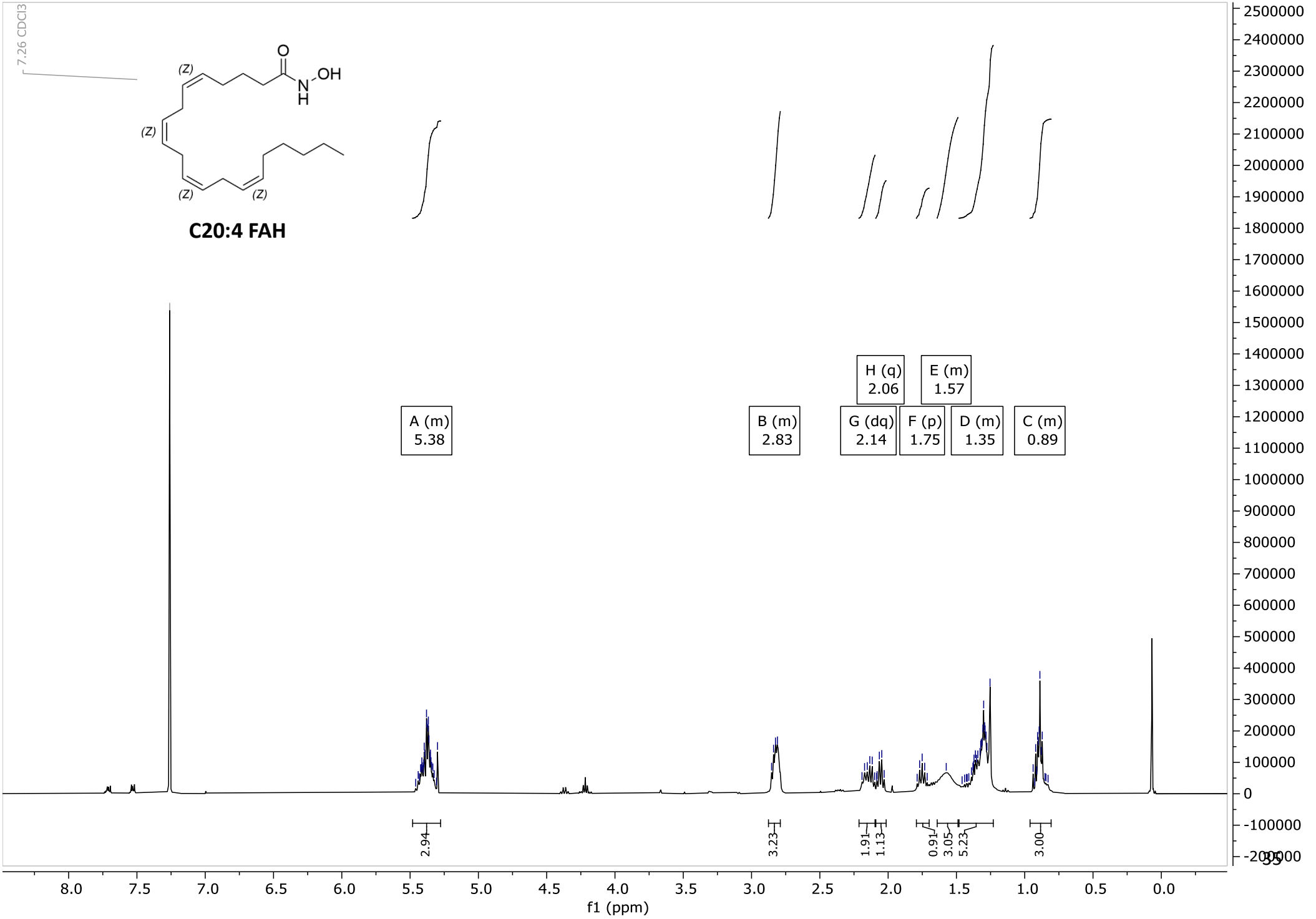

C16 FAH
77.16 CDCl3
33.07
31.99
29.76
29.72
29.68
29.55
29.43
29.36
29.25
25.51
22.76
14.16
f1 (ppm)

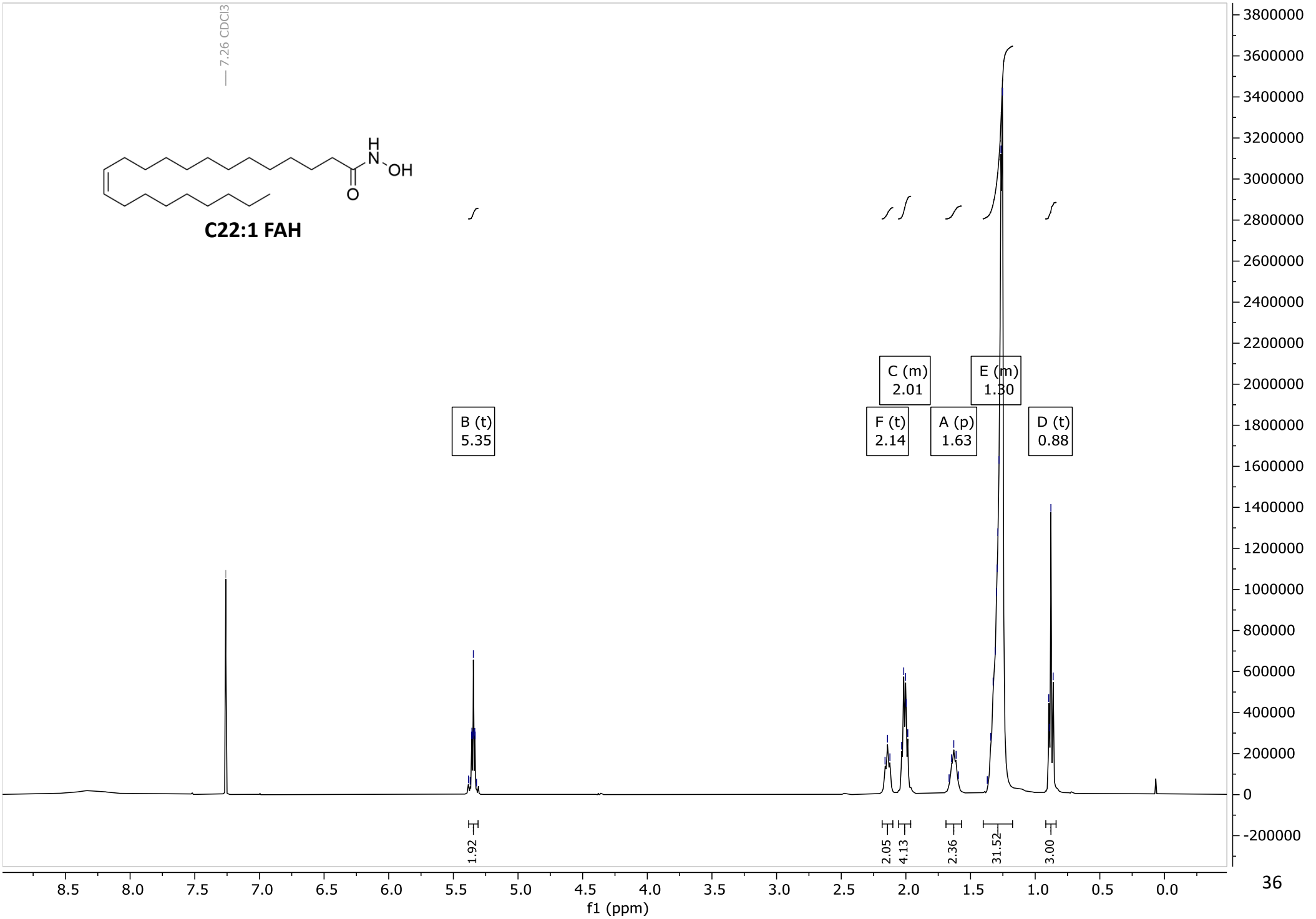

7.26 CDCl3
(Z)
(Z)
H
N
OH
O
C18:2 FAH
A (m)
5.35
B (t)
2.77
F (m)
2.31
G (s)
2.16
C (m)
2.04
H (s)
1.64
D (m)
1.34
E (dq)
0.89
1.85
0.88
0.70
0.58
2.22
2.15
11.33
2.50
f1 (ppm)

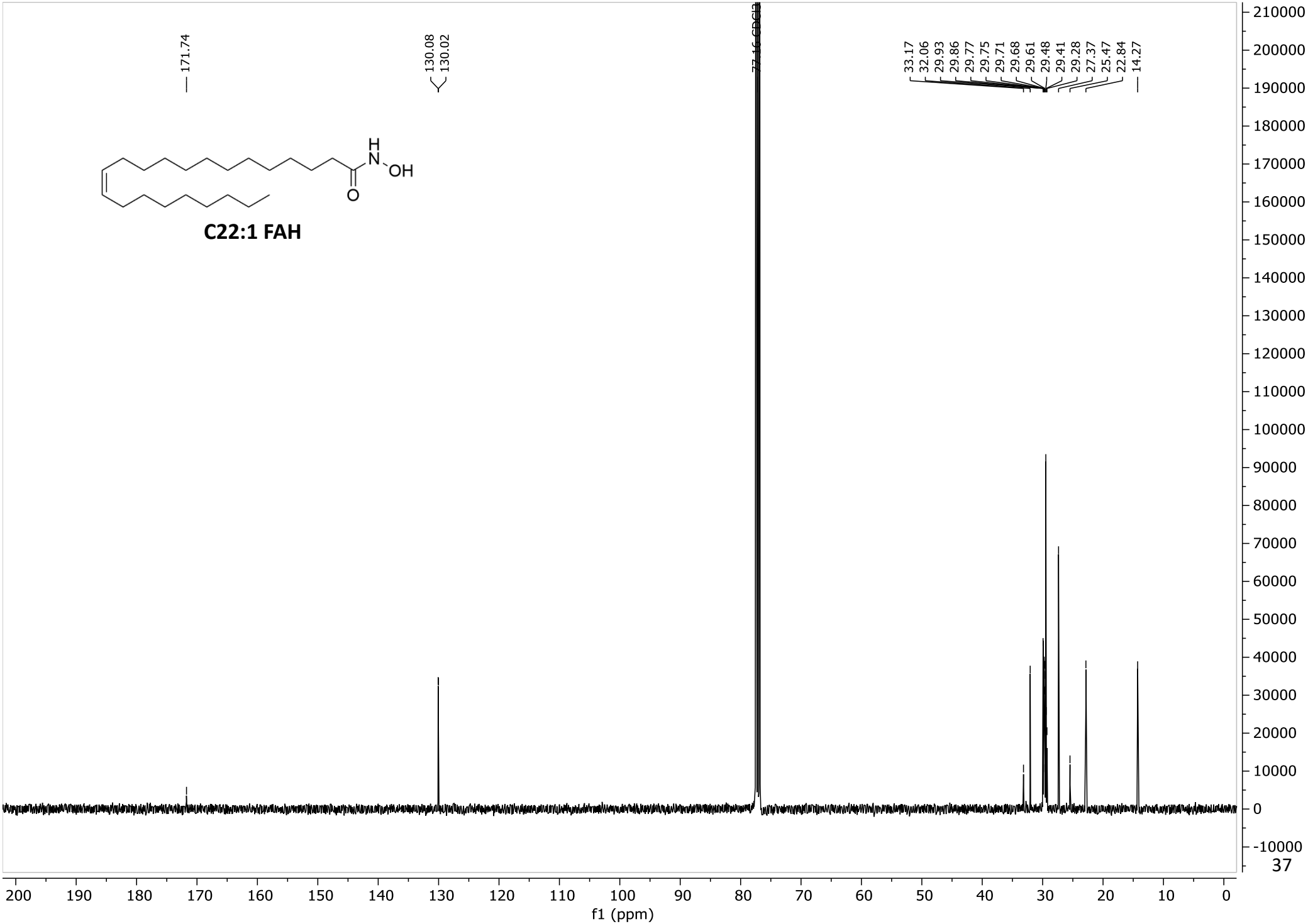

130.40
130.13
128.24
128.03
77.16 CDCl3
31.67
29.72
29.49
29.30
29.23
27.35
27.31
25.77
22.72
14.22
14.19
(Z)
(Z)
H
N
OH
O
C18:2 FAH
220000
210000
200000
190000
180000
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.26 CDCl3
C19 FAH
A (t)
2.07
B (p)
1.58
C (d)
1.22
D (t)
0.85
1.53
2.40
32.16
2.74
f1 (ppm)


77.16 CDCl3
32.00
29.78
29.73
29.69
29.56
29.44
29.26
25.50
22.77
14.17
H
N
OH
O
C19 FAH
230000
220000
210000
200000
190000
180000
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.26 CDCl3
(Z)
(Z)
(Z)
(Z)
O
N
H
OH
C20:4 FAH
A (m)
5.38
B (m)
2.83
H (q)
2.06
E (m)
1.57
G (dq)
2.14
F (p)
1.75
D (m)
1.35
C (m)
0.89
2.94
3.23
1.91
1.13
0.91
3.05
5.23
3.00
f1 (ppm)


7.26 CDCl3
C22:1 FAH
B (t)
5.35
C (m)
2.01
F (t)
2.14
A (p)
1.63
E (m)
1.30
D (t)
0.88
1.92
2.05
4.13
2.36
31.52
3.00
f1 (ppm)


171.74
130.08
130.02
77.16 CDCl3
33.17
32.06
29.93
29.86
29.77
29.75
29.71
29.68
29.61
29.48
29.41
29.28
27.37
25.47
22.84
14.27
H
N
OH
O
C22:1 FAH
f1 (ppm)