Electronic Supplementary Material (ESI) for RSC Advances

The importance of hydrophobic moieties in ice recrystallization inhibitors

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Supporting Information

(i) General Experimental

All anhydrous reactions were performed in flame-dried or oven-dried glassware under a positive pressure of dry argon or nitrogen. Air or moisture-sensitive reagents and anhydrous solvents were transferred with oven-dried syringes or cannulae. All flash chromatography was performed with EMD Silica gel 60 (230-400 mesh). All solution phase reactions were monitored using analytical thin layer chromatography (TLC) with 0.2 mm pre-coated silica gel aluminum plates 60 F254 (E. Merck). Components were visualized by illumination with a short-wavelength (254 nm) ultraviolet light and/or staining (ceric ammonium molybdate, potassium permanganate, or phosphomolybdate stain solution).

All solvents used for anhydrous reactions were distilled. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone under nitrogen. Dichloromethane, acetonitrile, triethylamine, benzene and diisopropylethylamine (DIPEA) were distilled from calcium hydride. *N,N*-dimethylformamide (DMF) was stored over activated 4Å molecular sieves under argon.

^{1}H (300, 400, or 500 MHz) and ^{13}C NMR (75, 100 or 125 MHz) spectra were recorded at ambient temperature on a Bruker Avance 300, Bruker AM 360, Bruker Avance 400 or Bruker Avance 500 spectrometer. Deuterated chloroform ($CDCl_3$), methanol (CD_3OD), or deuterium oxide (D_2O) were used as NMR solvents, unless otherwise stated. Chemical shifts are reported in ppm downfield from TMS and corrected using the solvent residual peak or TMS as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. Low resolution mass spectrometry (LRMS) was performed on a Micromass Quatro-LC Electrospray spectrometer with a pump rate of 20 µL/min using electrospray ionization (ESI) or a Voyager DE-Pro matrix-assisted desorption ionization-time of flight (MALDI-TOF), (Applied Biosystem, Foster City, CA) mass spectrometer operated in the reflectron/positive-ion mode with DHB in 20% EtOH/H_2O as the MALDI matrix. High resolution mass spectrometry (HRMS) data was acquired on Applied Biosystems/Sciex QStar (Concord, ON). Samples in CH_2Cl_2/MeOH 1:1 were mixed with Agilent ES tuning mix for internal calibration, and infused into the mass spectrometer at 5 µL/min.

(ii) Assessing Ice Recrystallization Inhibition (IRI) Activity

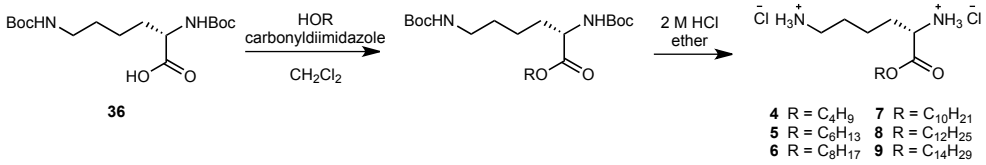
Sample analysis for IRI activity was performed using the "splat cooling" method as previously described.[1] In this method, the analyte was dissolved in phosphate buffered saline (PBS) solution and a 10 *µL* droplet of this solution was dropped from a micropipette through a two meter high plastic tube (10 cm in diameter) onto a block of polished aluminum precooled to approximately -80 °C. The droplet froze instantly on the polished aluminum block and was approximately 1 cm in diameter and 20 *µm* thick. This wafer was then carefully removed from the surface of the block and transferred to a cryostage held at -6.4 °C for annealing. After a period of 30 min, the wafer was photographed between crossed polarizing filters using a digital camera (Nikon CoolPix 5000) fitted to the microscope. A total of three images were taken from each wafer. During flash freezing, ice crystals spontaneously nucleated from the supercooled solution. These initial crystals were relatively homogeneous in size and quite small. During the annealing cycle, recrystallization occurred, resulting in a dramatic increase in ice crystal size. A quantitative measure of the difference in recrystallization inhibition of two compounds X and Y is the difference in the dynamics of the ice crystal size distribution. Image analysis of the ice wafers was performed using a novel domain recognition software (DRS) program.[2] This processing employed the Microsoft Windows Graphical User Interface to allow a user to visually demarcate and store the vertices of ice domains in a digital micrograph. The data was then used to calculate the domain areas. All data was plotted and analyzed using Microsoft Excel. The mean grain (or ice crystal) size (MGS) of the sample was compared to the MGS of the control PBS solution for that same day of testing. IRI activity is reported as the percentage of the MGS (% MGS) relative to the PBS control, and the % MGS for each sample was plotted along with its standard error of the mean. Large percentages represent a large MGS, which is indicative of poor IRI activity.

(iii) Assessing Thermal Hysteresis (TH)

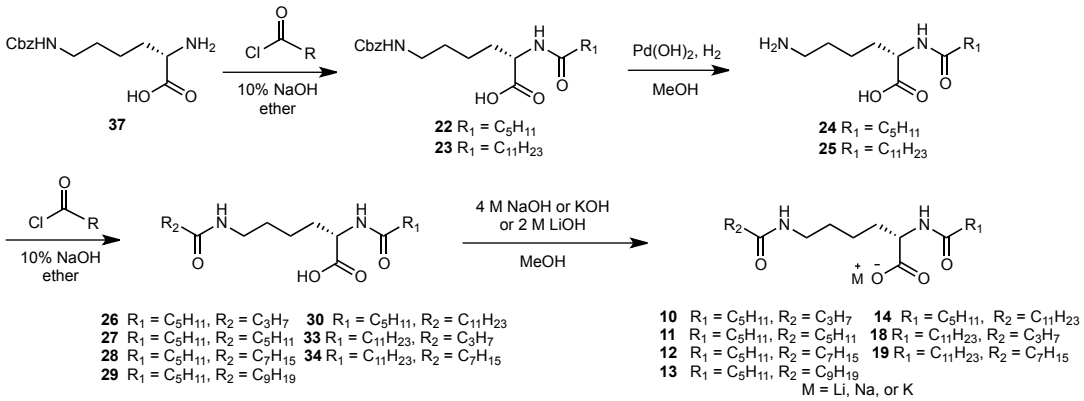
Nanoliter osmometry was performed using a Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, NY), as described by Chakrabartty and Hew.[3] All of the measurements were performed in doubly distilled water. Ice crystal morphology was observed through a Leitz compound microscope equipped with an Olympus 20× (infinity-corrected) objective, a Leitz Periplan 32X photo eyepiece, and a Hitachi KPM2U CCD camera connected to a Toshiba MV13K1 TV/VCR system. Still images were captured directly using a Nikon CoolPix digital camera.

(iv) Experimental Data

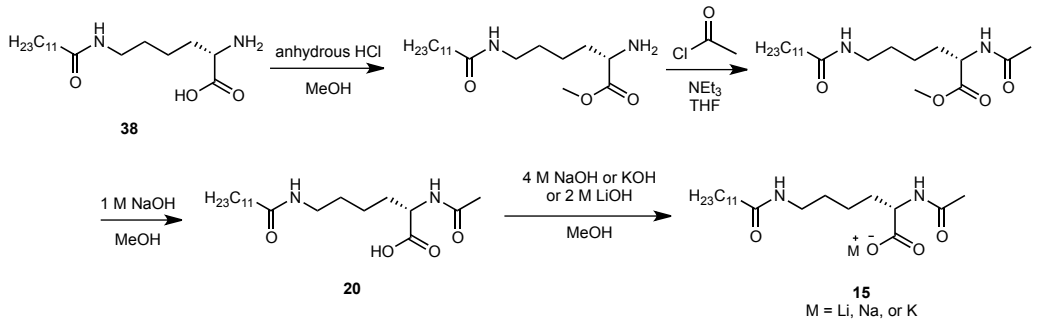
(a) Reaction Schemes for Compounds 4 – 21



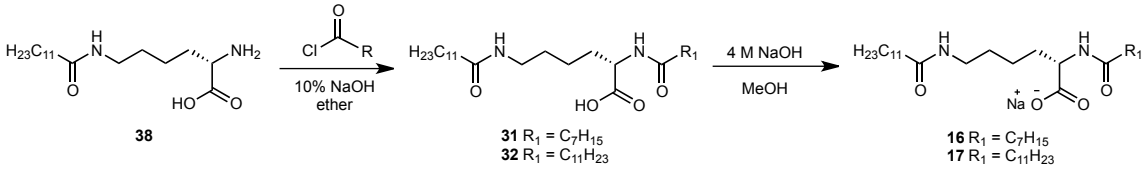
Scheme 1 Synthesis of L-lysine alkyl ester dihydrochloride derivatives **4 - 9**



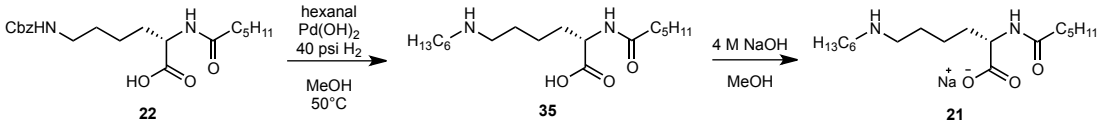
Scheme 2 Synthesis of lithium, sodium, or potassium L-lysinate derivatives **10** – **14**, **18**, **19**



Scheme 3 Synthesis of lithium, sodium, or potassium L-lysinate derivatives **15**[4]



Scheme 4 Synthesis of sodium L-lysinate derivative **16**, **17**



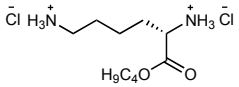
Scheme 5 Synthesis of sodium L-lysinate derivative **21**

(b) L-Lysine alkyl ester dihydrochloride derivatives (4-9)

General Procedure

N^{α},N^{ε}-Bis(*t*-butyloxycarbonyl)-L-lysine and carbonyldiimidazole (1.1 eq) were dissolved in anhydrous DCM (final concentration of 0.1 M) and the mixture was stirred at room temperature for 45 min. Alcohol (1.1 eq) was added and the mixture was stirred overnight. The solvent was evaporated under reduced pressure and the residue was purified via silica column chromatography (8:2 petroleum ether: EtOAc) to yield N^{α},N^{ε}-bis(*t*-butyloxycarbonyl)-L-lysine alkyl ester as a yellowish oil. The oil was suspended in 2 M HCl in anhydrous Et_2O (final concentration of 0.1 M) and stirred at room temperature for 2 hrs after which the residue was concentrated under reduced pressure. The oil was washed with Et_2O x 4 and residual Et_2O was removed under reduced pressure to yield L-lysine alkyl ester dihydrochloride as a yellow oil.

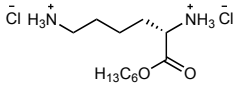
L-Lysine butyl ester dihydrochloride (4)



The procedure above was used to give compound **4** in an isolated yield of 62 %.

^{1}H NMR (400 MHz, *D_2O*) δ 4.26 (td, *J* = 6.5, 1.8 Hz, 2H), 4.13 (t, *J* = 6.4 Hz, 1H), 2.98 (t, *J* = 7.7 Hz, 2H), 1.97 (m, 2H), 1.67 (m, 4H), 1.49 (m, 2H), 1.35 (m, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (400 MHz, *D_2O*) δ 170.11, 67.12, 52.60, 38.92, 29.62, 29.22, 26.21, 21.42, 18.36, 12.76. **ESI-MS** *m/z* calcd for $C_{10}H_{22}N_2O_2$ $[M+H]^+$: 203.18. Found: 203.26.

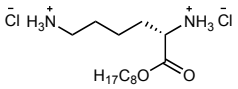
L-Lysine hexyl ester dihydrochloride (5)



The procedure above was used to give compound **5** in an isolated yield of 58 %.

^{1}H NMR (500 MHz, *D_2O*) δ 4.23 (td, *J* = 6.3, 4.0 Hz, 2H), 4.10 (t, *J* = 6.3 Hz, 1H), 2.96 (t, *J* = 7.7 Hz, 2H), 1.95 (m, 2H), 1.66 (m, 4H), 1.49 (m, 1H), 1.43 (m, 1H), 1.32 (m, 2H), 1.25 (m, 4H), 0.82 (t, *J* = 6.4, 3H). **^{13}C NMR** (400 MHz, *D_2O*) δ 170.10, 67.39, 52.57, 38.89, 30.45, 29.21, 27.46, 26.18, 24.58, 21.77, 21.38, 13.17. **ESI-MS** *m/z* calcd for $C_{12}H_{26}N_2O_2$ $[M+H]^+$: 231.21. Found: 231.24.

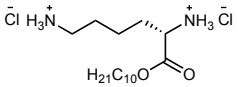
L-Lysine octyl ester dihydrochloride (6)



The procedure above was used to give compound **6** in an isolated yield of 70 %.

^{1}H NMR (400 MHz, D_2O) δ 4.27 (td, J = 6.3, 3.2 Hz, 2H), 4.14 (t, J = 6.4 Hz, 1H), 3.00 (t, J = 7.9 Hz, 2H), 1.99 (m, 2H), 1.70 (m, 4H), 1.50 (m, 2H), 1.14-1.21 (m, 10H), 0.85 (t, J = 6.1 Hz, 3H). **^{13}C NMR** (400 MHz, D_2O) δ 170.10, 67.38, 52.57, 38.90, 30.96, 29.21, 28.23, 28.12, 27.49, 26.19, 24.89, 21.91, 21.39, 13.31. **ESI-MS** *m/z* calcd for $C_{14}H_{30}N_2O_2$ $[M+H]^+$: 259.41. Found: 259.34.

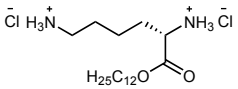
L-Lysine decyl ester dihydrochloride (7)



The procedure above was used to give compound **7** in an isolated yield of 72 %.

^{1}H NMR (400 MHz, D_2O) δ 4.26 (td, J = 6.2, 2.2 Hz, 2H), 4.14 (s, 1H), 3.00 (t, J = 7.6 Hz, 2H), 1.98 (m, 2H), 1.70 (m, 4H), 1.50 (m, 2H), 1.38-1.20 (m, 14H), 0.84 (t, J = 6.0 Hz, 3H). **^{13}C NMR** (400 MHz, D_2O) δ 169.92, 67.01, 52.56, 48.81, 31.47, 29.26, 29.07, 29.03, 28.84, 28.65, 27.73, 26.20, 25.16, 22.24, 21.47, 13.57. **ESI-MS** *m/z* calcd for $C_{16}H_{34}N_2O_2$ $[M+H]^+$: 287.27. Found: 287.35.

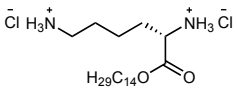
L-Lysine dodecyl ester dihydrochloride (8)



N^{α},N^{ε}-Bis(*t*-butyloxycarbonyl)-L-lysine (0.25 g, 0.71 mmol) and carbonyldiimidazole (0.13 g, 0.78 mmol) were dissolved in anhydrous DCM (7.1 mL) and the mixture was stirred at room temperature for 45 min. Dodecanol (0.19 mL, 0.85 mmol) was added and the mixture was stirred overnight. The solvent was evaporated under reduced pressure and the residue was purified via silica thin layer chromatography (toluene x 3 elution) to yield N^{α},N^{ε}-bis(*t*-butyloxycarbonyl)-L-lysine dodecyl ester (0.22 g, 0.41 mmol, 58%) as a yellowish oil. The oil (0.023 g, 0.047 mmol) was suspended in 2 M HCl in anhydrous Et_2O (0.5 mL) and stirred at room temperature for 2 hrs after which the residue was concentrated under reduced pressure. The oil was washed with Et_2O x 4 and residual Et_2O was removed under reduced pressure to yield L-lysine dodecyl ester dihydrochloride (**8**) as a yellow oil (0.017 g, 0.043 mmol, 96 %).

^{1}H NMR (500 MHz, D_2O) δ 4.23 (m, 2H), 4.12 (t, J = 6.4 Hz, 1H), 2.97 (t, J = 7.7, 2H), 1.95 (m, 2H), 1.67 (m, 4H), 1.47 (m, 2H), 1.36-1.18 (m, 18H), 0.82 (t, J = 6.9 Hz, 3H). **^{13}C NMR** (400 MHz, D_2O) δ 170.08, 67.32, 52.61, 38.94, 31.28, 29.54, 29.28, 28.89, 28.80, 28.72, 28.62, 28.35, 27.62, 26.24, 25.02, 22.10, 21.47, 13.46. **ESI-MS** *m/z* calcd for $C_{18}H_{38}N_2O_2$ $[M+H]^+$: 315.30. Found: 315.38.

L-Lysine tetradecy ester dihydrochloride (9)



N^{α},N^{ε}-Bis(*t*-butyloxycarbonyl)-L-lysine (0.21 g, 0.60 mmol) and carbonyldiimidazole (0.11 g, 0.66 mmol) were dissolved in anhydrous DCM (6.0 mL) and the mixture was stirred at room temperature for 45 min. Tetradecanol (0.16, 0.72 mmol) was added and the mixture was stirred overnight. The solvent was evaporated under reduced pressure and the residue was purified via silica thin layer chromatography (toluene x 3 elution) to yield N^{α},N^{ε}-bis(*t*-butyloxycarbonyl)-L-lysine tetradecyl ester (0.20 g, 0.37 mmol, 61%) as a yellowish oil. The oil (0.20 g, 0.37 mmol) was suspended in 2 M HCl in anhydrous Et_2O (0.5 mL) and stirred at room temperature for 2 hrs after which the residue was concentrated under reduced pressure. The oil was washed with Et_2O x 4 and residual Et_2O was removed under reduced pressure to yield L-lysine tetradecyl ester dihydrochloride (**9**) as a yellow oil (0.15 g, 0.36 mmol, 98 %).

^{1}H NMR (500 MHz, *D_2O*) δ 4.23 (m, 2H), 4.11 (t, *J* = 6.4 Hz, 1H), 2.97 (t, *J* = 7.6 Hz, 2H), 1.96 (m, 2H), 1.67 (m, 4H), 1.47 (m, 2H), 1.36-1.17 (m, 22H), 0.82 (t, *J* = 6.4 Hz, 3H). **^{13}C NMR** (400 MHz, *D_2O*) δ 169.96, 67.02, 52.64, 38.97, 31.72, 29.55, 29.55, 29.48, 29.46, 29.37, 29.37, 29.37, 29.17, 28.96, 27.93, 26.30, 25.39, 22.45, 21.60, 13.75. **ESI-MS** *m/z* calcd for $C_{20}H_{42}N_2O_2$ $[M+H]^+$: 343.32. Found: 343.41.

(c) General Procedure for synthesis of di-alkyl lysine derivatives (20 – 34)

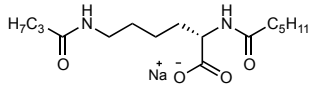
*General Procedure for synthesis of salts (**10** – **19**, **21**)*

Sodium Salts:
The di-alkyl L-lysine was dissolved in MeOH (final concentration of 0.1 M) and 4 M NaOH (1 eq) was added. The mixture was stirred at room temperature for 30 min after which the solvent was evaporated under reduced pressure. The residue was dissolved in H_2O and filtered through a Celite pad and dried by lyophilisation to yield a white powder or a yellowish oil. All yields were > 95 %.

Lithium Salts:
The above procedure was used except a 2 M LiOH solution was used.

Potassium Salts:
The above procedure was used except a 4 M KOH solution was used.

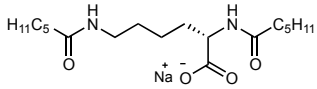
Sodium N^{α}-hexanoyl-N^{ε}-butanoyl-L-lysinate (10)



The procedure above was used from the di-alkyl L-lysine **26.**

^{1}H NMR (300 MHz, *D_2O*) δ 4.00 (dd, *J* =9.0, 4.2 Hz, 1 H), 3.04 (t, *J* = 6.7 Hz, 2 H), 2.14 (td, *J* = 4.1, 7.4 Hz, 2 H), 2.06 (t, *J* = 7.3 Hz, 2H), 1.66 (m, 1H), 1.53-1.24 (m, 7H), 1.24-1.02 (m, 6H), 0.76 (t, *J* = 7.10 Hz, 3H), 0.85 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (300 MHz, *D_2O*) δ 179.39, 176.80, 176.61, 54.80, 38.97, 37.66, 35.64, 31.16, 30.39, 27.80, 25.00, 22.61, 21.56, 18.99, 13.18, 12.66. **ESI-MS** *m/z* calcd for $C_{16}H_{29}N_2O_4^-$ $[M]^-$: 313.21. Found: 313.17.

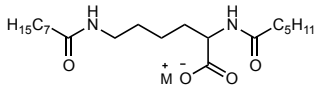
Sodium N^{α},N^{ε}-bis(hexanoyl)-L-lysinate (11)



The procedure above was used from the di-alkyl L-lysine **27.**

^{1}H NMR (500 MHz, *D_2O)* δ 4.10 (dd, *J* = 9.2, 4.4 Hz, 1H), 3.13 (t, *J* = 6.8 Hz, 2H), 2.24 (m, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 1.76 (m, 1H), 1.68-1.41 (m, 7H), 1.38-1.17 (m, 11H), 0.83 (t, *J* = 7.0 Hz, 3H), 0.82 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (300 MHz, *D_2O*) d 179.33, 177.03, 176.56, 54.85, 39.03, 35.77, 35.73, 31.30, 30.48, 30.41, 27.87, 25.10, 25.06, 22.65, 21.63, 21.60, 13.20, 13.17. **ESI-MS** *m/z* calcd for $C_{20}H_{37}N_2O_4^-$ $[M]^-$: 341.24. Found: 341.25.

Lithium, Sodium, or Potassium N^{α}-hexanoyl-N^{ε}-octanoyl-L-lysinate (12)

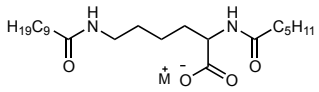


The procedures above were used from the di-alkyl L-lysine **28.**

^{1}H NMR (500 MHz, *D_2O*) δ 4.11 (dd, *J* = 9.3, 3.9 Hz, 1H), 3.14 (bt, *J* = 6.5 Hz, 2H), 2.24 (m, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 1.78 (m, 1H), 1.71-1.40 (m, 7H), 1.40-1.12 (m, 14H), 0.83 (t, *J* = 6.7 Hz, 3H), 0.81 (t, *J* = 7.0 Hz, 3H).**^{13}C NMR** (400 MHz, *D_2O*) d 179.33, 175.96, 175.93, 54.81, 38.95, 36.02, 35.93, 31.45, 31.42,

30.85, 28.74, 28.65, 28.14, 25.74, 25.34, 22.76, 22.29, 21.96, 13.62, 13.52. **ESI-MS** *m/z* calcd for $C_{20}H_{37}N_2O_4^-$ $[M]^-$: 369.28. Found: 369.25.

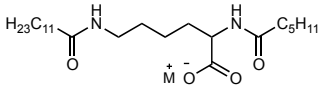
Lithium, Sodium, or Potassium N^{α}-hexanoyl-N^{ε}-decanoyl-L-lysinate (13)



The procedures above were used from the di-alkyl L-lysine **29**.

^{1}H NMR (500 MHz, D_2O) δ 4.13 (bdd, J = 9.2, 4.1 Hz, 1H), 3.15 (m, 1H), 2.26 (m, 2H), 2.17 (bt, 2H), 1.79 (m, 1H), 1.57-1.25 (m, 7H), 1.26-0.96 (m, 19H), 0.85 (bt, 3H), 0.81 (bt, 3H). **^{13}C NMR** (500 MHz, D_2O) δ179.21, 175.54, 175.32, 54.68, 38.83, 36.07, 35.91, 31.77, 31.36, 30.97, 29.50, 29.35, 29.22, 29.13, 29.19, 25.88, 25.39, 22.75, 22.46, 22.06, 13.69, 13.63. **ESI-MS** *m/z* calcd for $C_{22}H_{41}N_2O_4^-$ $[M]^-$: 397.31. Found: 397.24.

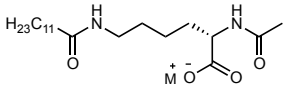
Lithium, Sodium, or Potassium N^{α}-hexanoyl-N^{ε}-dodecanoyl-L-lysinate (14)



The procedures above were used from the di-alkyl L-lysine **30**.

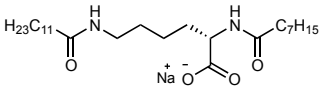
^{1}H NMR (400 MHz, D_2O) δ 4.15 (dd, J = 9.4, 3.8 Hz, 1H), 3.16 (m, 2H), 2.28 (m, 2H), 2.19 (t, J = 7.4 Hz, 2H), 1.80 (m, 1H), 1.73-1.42 (m, 7H), 1.42-0.12 (m, 22H), 0.87 (t, J = 6.9 Hz, 3H), 0.83 (t, J = 6.9 Hz, 3H). **^{13}C NMR** (500 MHz, D_2O) δ179.27, 175.26, 174.96, 54.64, 38.82, 36.17, 36.07, 31.94, 31.77, 31.42, 30.00, 29.90, 29.79, 29.50, 29.03, 29.01, 28.26, 26.08, 25.93, 22.80, 22.57, 22.55, 13.92, 13.67. **ESI-MS** *m/z* calcd for $C_{24}H_{45}N_2O_4^-$ $[M]^-$: 425.34. Found: 425.33.

S Lithium, Sodium, or Potassium N^{α}-ethanoyl-N^{ε}-dodecanoyl-L-lysinate (15)



Compounds were prepared as previously described from **20**.[4] All spectral data was consistent with that reported in the literature.[4]

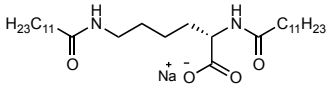
Sodium N^{α}-octanoyl-N^{ε}-dodecanoyl-L-lysinate (16)



The procedure above was used from the di-alkyl L-lysine **31**.

^{1}H NMR (500 MHz, D_2O) δ 4.14 (bd, J = 8.5 Hz, 1H), 3.15 (m, 2H), 2.27 (bt, 2H), 2.18 (m, 2H), 1.80 (m, 1H), 1.79 (s, 1H), 1.57-1.26 (m, 7H), 1.26-0.92 (m, 26H), 0.85 (t, J = 6.8 Hz, 3H), 0.80 (t, J = 6.7 Hz, 3H). **^{13}C NMR** (126 MHz, D_2O) δ 179.27, 175.26, 174.96, 54.64, 38.82, 36.17, 36.07, 31.94, 31.77, 31.42, 30.00, 30.00, 29.90, 29.79, 29.50, 29.50, 29.03, 29.01, 28.26, 26.08, 25.93, 22.80, 22.57, 22.55, 13.92, another 13? **ESI-MS** *m/z* calcd for $C_{26}H_{49}N_2O_4^-$ $[M]^-$: 453.37. Found: 453.37.

Sodium N^{α},N^{ε}-bis(dodecanoyl)-L-lysinate (17)

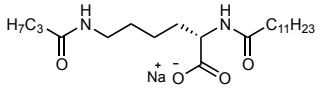


The procedure above was used from the di-alkyl L-lysine **32**.

^{1}H NMR (300 MHz, D_2O + 10 % CD_3OD) δ 4.18 (dd, J = 7.7, 4.7 Hz, 1H), 3.06 (t, J = 6.8 Hz, 2H), 2.14 (t, J = 7.5 Hz, 2H), 2.07 (t, J = 7.5 Hz, 2H), 1.74 (m, 1H), 1.68-1.36 (m, 7H), 1.36-1.10 (m, 34H), 0.85 (t, J =

6.7 Hz, 3H), 0.81 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR** (300 MHz, D_2O + 10 % CD_3OD) δ 179.19, 176.23, 175.40, 55.99, 40.35, 37.48, 37.25, 33.87, 33.14, 30.84, 30.82, 30.82, 30.81, 30.80, 30.75, 30.71, 30.58, 30.55, 30.55, 30.54, 30.52, 30.47, 30.43, 30.12, 27.19, 27.12, 24.24, 23.80, 14.50, 14.50. **ESI-MS** *m/z* calcd for $C_{30}H_{57}N_2O_4^-$ [M]$^-$: 509.43. Found: 509.42.

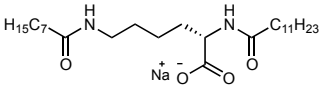
Sodium N^{α}-dodecanoyl-N^{ε}-butanoyl-L-lysinate (18)



The procedure above was used from the di-alkyl L-lysine **33**.

^{1}H NMR (500 MHz, D_2O) δ ppm 4.14 (dd, *J* = 8.8, 3.8 Hz, 1H), 3.14 (m, 2H), 2.25 (t, *J* = 7.4 Hz, 2H), 1.77 (m, 1H), 1.55-1.24 (m, 7H), 1.25-0.96 (m, 18H), 0.87 (t, *J* = 7.4 Hz, 3H), 0.82 (t, *J* = 6.6 Hz, 3H). **^{13}C NMR** (300 MHz, D_2O) δ ppm 179.15, 175.59, 175.22, 54.66, 38.87, 37.83, 36.06, 31.81, 31.47, 29.67, 29.61, 29.61, 29.38, 29.28, 29.06, 28.22, 25.86, 22.74, 22.45, 19.14, 13.77, 13.11. **ESI-MS** *m/z* calcd for $C_{22}H_{41}N_2O_4^-$ [M]$^-$: 397.31. Found: 397.34.

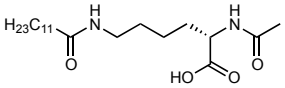
Sodium N^{α}-dodecanoyl-N^{ε}-octanoyl-L-lysinate (19)



The procedure above was used from the di-alkyl L-lysine **34**.

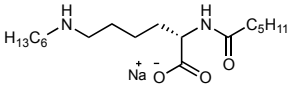
^{1}H NMR (500 MHz, D_2O) δ 4.13 (dd, *J* = 8.7, 3.3 Hz, 1H), 3.15 (m, 2H), 2.26 (m, 2H), 2.18 (t , *J* = 7.4 Hz, 2H), 1.79 (m, 1H), 1.70-1.40 (m, 7H), 1.40-1.10 (m, 26H), 0.85 (t, *J* = 7.0 Hz, 3H), 0.82 (t, *J* = 6.6 Hz, 3H). **^{13}C NMR** (500 MHz, D_2O) δ 179.25, 175.30, 175.10, 54.62, 38.75, 36.16, 36.07, 31.93, 31.77, 31.40, 29.97, 29.91, 29.85, 29.72, 29.50, 29.33, 29.05, 29.03, 28.22, 26.05 25.90, 22.70, 22.58, 22.55, 13.88, 13.77. **ESI-MS** *m/z* calcd for $C_{26}H_{49}N_2O_4^-$ [M]$^-$: 453.37. Found: 453.43.

N^{α}-ethanoyl-N^{ε}-dodecanoyl-L-lysine (20)



Compound was prepared as previously described.[4] All spectral data was consistent with that reported in the literature.[4]

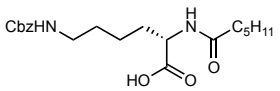
Sodium N^{α}-hexanoyl-N^{ε}-hexyl-L-lysinate (21)



The procedure above was used from the di-alkyl L-lysine **35**.

^{1}H NMR (500 MHz, D_2O) δ 4.13 (dd, *J* = 8.5, 4.3 Hz, 1H), 2.71 (m, 3H), 2.25 (m, 2H), 2.18 (t *J* = 7.4 Hz, 2H), 1.79 (m, 1H), 1.70-1.40 (m, 7H), 1.42-1.14 (m, 13H), 0.84 (t, *J* = 6.9 Hz, 3H), 0.83 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (500 MHz, D_2O) δ 179.21, 176.59, 54.66, 48.00, 47.72, 35.65, 31.23, 30.66, 30.42, 27.02, 26.61, 25.76, 25.02, 22.61, 21.76, 21.61, 13.20, 13.17. **ESI-MS** *m/z* calcd for $C_{18}H_{35}N_2O_3^-$ [M]$^-$: 327.27. Found: 327.21.

N^{α}-Hexanoyl-N^{ε}-carboxybenzyl-L-lysine (22)

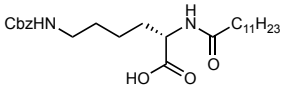


N^{α}-Carboxybenzyl-L-lysine **37** (0.61 g, 2.18 mmol) was dissolved in 10 % NaOH (3 parts) and ethyl ether (2 parts) was added (final concentration of 0.1 M). The mixture was cooled to 0 °C and hexanoyl chloride (0.46 mL, 3.26 mmol, 1.5 eq) was added slowly to the ether layer with no mixing. The biphasic solution

was vigorously stirred at 0 °C for 4 hrs and then at room temperature for 18 hrs. The ether was evaporated under a stream of air and the solution was carefully acidified with concentrated HCl to ca. pH = 1. The mixture was extracted with DCM five times. The combined organic phases were washed with brine, dried with $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by silica column chromatography (3:7 Pet Ether: EtOAc + 1 % AcOH) to yield white product (0.73 g, 1.94 mmol, 89 %).

^{1}H NMR (300 MHz, *$CDCl_3$)* δ 10.22 (bs, 1H), 7.26 (m, 5H), 6.58 (d, *J* = 7.09 Hz, 1H), 5.01 (bs, 2H), 4.51 (m, 1H), 3.11 (dt, *J* = 6.4, 6.2 Hz, 2H), 2.15 (t, *J* = 7.6 Hz, 2H), 1.80 (m, 1H), 1.68 (m, 1H), 1.61-1.11 (m, 11H), 0.80 (t, *J* = 6.84 Hz, 3H). **^{13}C NMR** (400 MHz, *$CDCl_3$*) δ 174.90, 174.47, 156.91, 136.35, 128.51, 128.13, 127.97, 67.28, 66.73, 52.98, 40.30, 36.26, 31.32, 29.39, 25.27, 22.30, 22.12, 13.90. **ESI-MS** *m/z* calcd for $C_{20}H_{30}N_2O_5$ $[M+H]^+$: 379.23. Found: 379.33.

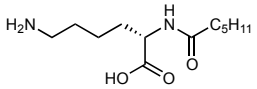
N^{α}-Dodecanoyl-N^{ε}-carboxybenzyl-L-lysine (23)



N^{α}-Carboxybenzyl-L-lysine **37** (0.31 g, 1.09 mmol) was dissolved in 10 % NaOH (3 parts) and ethyl ether (2 parts) was added (final concentration of 0.1 M). The mixture was cooled to 0 °C and dodecanoyl chloride (0.38 mL, 1.64 mmol, 1.5 eq) was added slowly to the ether layer with no mixing. The biphasic solution was vigorously stirred at 0 °C for 4 hrs and then at room temperature for 18 hrs. The ether was evaporated under a stream of air and the solution was carefully acidified with concentrated HCl to ca. pH = 1. The mixture was extracted with DCM five times. The combined organic phases were washed with brine, dried with $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by silica column chromatography (3:7 Pet Ether: EtOAc + 1 % AcOH) to yield white product (0.73 g, 1.94 mmol, 89 %).

^{1}H NMR (300 MHz, *$(CD_3)_2SO$*) δ 7.78 (d, *J* = 7.8 Hz, 1H), 7.73 (m, 5H), 7.04 (bs, 1H), 5.02 (s, 2H), 4.16 (td, *J* = 8.4, 5,1 Hz, 1H), 2.99 (dd, *J* = 13.0, 6.7 Hz, 2H), 2.11 (dt, *J* = 7.2, 6.9 Hz, 2H), 1.68 (m, 1H), 1.57 (m, 1H), 1.49 (m, 2H), 1.42 (m, 2H), 1.35-1.20 (m, 18H), 0.86 (t, *J* = 6.89 Hz, 3H). **^{13}C NMR** (400 MHz, *$CDCl_3$*) δ 174.99, 174.51, 156.95, 136.44, 128.53, 128.14, 128.00, 127.91, 66.74, 52.14, 40.45, 36.37, 31.93, 31.59, 31.45, 29.66, 29.65, 29.56, 29.37, 29.37, 29.30, 25.70, 22.70, 22.24, 22.05, 14.14. **ESI-MS** *m/z* calcd for $C_{26}H_{42}N_2O_5$ $[M+H]^+$: 363.32. Found: 463.47.

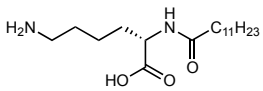
N^{α}-Hexanoyl-L-lysine (24)



N^{α}-Hexanoyl-N^{ε}-carboxybenzyl-L-lysine (**22**) (0.053 g, 0.14 mmol) was dissolved in MeOH (final concentration of 0.1 M) and $Pd(OH)_2$ on carbon (5 mg) was added. H_2 atmosphere was introduced into the flask and the mixture was stirred for 16 hours at room temperature after which the solution was filtered through a Celite pad and concentrated under reduced pressure to yield a white powder (**24**) (0.034 g, mmol 98 %).

^{1}H NMR (300 MHz, *D_2O*) δ 7.71 (d, *J* = 7.7 Hz, 1H), 4.03 (m, 1H), 2.84 (t, *J* = 7.6 Hz, 2H), 2.13 (t, *J* = 7.5 Hz, 2H), 1.74-1.34 (m, 6H), 1.36-1.04 (m, 6H), 0.72 (t, *J* = 6.8 Hz, 3H). **^{13}C NMR** (500 MHz, *D_2O*) d 178.97, 176.65, 54.60, 39.13, 35.61, 30.99, 30.41, 26.22, 24.96, 22.04, 21.57, 13.12. **ESI-MS** *m/z* calcd for $C_{12}H_{24}N_2O_3$ $[M+H]^+$: 245.19. Found: 245.24.

N^{α}-Dodecanoyl-L-lysine (25)



N^{α}-Dodecanoyl-N^{ε}-carboxybenzyl-L-lysine (**23**) (0.12 g, 0.25 mmol) was dissolved in MeOH (final concentration of 0.1 M) and $Pd(OH)_2$ on carbon (5 mg) was added. H_2 atmosphere was introduced into the flask and the mixture was stirred for 16 hours at room temperature after which the solution was filtered

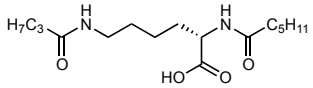
through a Celite pad and concentrated under reduced pressure to yield a white powder (**25**) (0.079 g, 0.21 mmol, 96 %).

^{1}H NMR (300 MHz, D_2O) δ 4.19 (bt, J = 6.3 Hz, 1H), 2.84 (t, J = 7.5 Hz, 2H), 2.15 (t, J = 7.5 Hz, 2H), 1.74 (m, 1H), 1.77-1.51(m, 5H), 1.51-1.17 (m, 19H), 0.81 (t, J = 6.6 Hz, 3H). **^{13}C NMR** (500 MHz, $(CD_3)_2SO$) δ 175.31, 172.57, 52.27, 37.52, 34.33, 30.24, 30.10, 27.79, 27.76, 27.68, 27.52, 27.49, 27.41, 25.02, 24.02, 20.75, 20.47, 11.45. **ESI-MS** *m/z* calcd for $C_{18}H_{36}N_2O_3$ $[M+H]^+$: 329.28. Found: 329.38.

*General procedure for di-alkyl lysine derivatives (**26 - 34**)*

N^{α}-Alkyl-L-lysine (**22**) was dissolved in 10 % NaOH (3 parts) and ethyl ether (2 parts) was added (final concentration of 0.1 M). The mixture was cooled to 0 °C and alkyl acyl chloride (1.5 eq) was added slowly to the ether layer with no mixing. The biphasic solution was vigorously stirred at 0 °C for 4 hrs and then at room temperature for 18 hrs. The ether was evaporated under a stream of air and the solution was carefully acidified with concentrated HCl to ca. pH = 1. The white precipitate was filtered, washed with water then dried. If the product did not precipitate, the mixture was extracted with DCM five times. The combined organic phases were washed with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by silica column chromatography (2:8 Pet Ether:EtOAc + 1 % AcOH to 9:1 EtOAc:MeOH + 1 % AcOH) to yield white product.

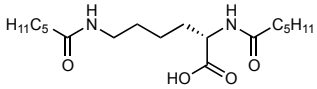
N^{α}-Hexanoyl-N^{ε}-butanoyl-L-lysine (26)



The procedure above was used with N^{α}-hexanoyl-L-lysine (**22**) (0.033 g, 0.13 mmol) and butryl chloride (20 µL, 0.20 mmol) to yield **26** (0.014 g, 0.045 mmol, 34 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (d, J = 7.2 Hz, 1H), 6.06 (t, J = 4.1, 1H), 4.55 (td, J = 7.2, 5.0, 5.0 Hz, 1H), 3.33 (m, 1H), 3.20 (m, 1H), 2.25 (t, J = 7.3 Hz, 2H), 2.18 (t, J = 7.5 Hz, 2H), 1.83 (m, 2H), 1.64 (m, 4H), 1.53 (m, 2H), 1.44-1.19 (m, 6H), 0.93 (t, J = 7.4, 7.4 Hz, 3H), 0.88 (t, J = 7.4, 7.4 Hz, 3H). **^{13}C NMR** (400 MHz, $CDCl_3$) δ 174.92, 174.37, 174.30, 52.21, 38.89, 38.39, 36.26, 31.46, 31.34, 28.76, 25.33, 22.29, 22.27, 19.19, 13.88, 13.66. **ESI-MS** *m/z* calcd for $C_{16}H_{30}N_2O_4$ $[M+H]^+$: 315.23. Found: 315.25.

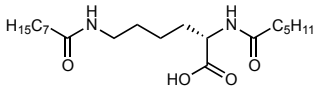
N^{α},N^{ε}-Bis(hexanoyl)-L-lysine (27)



The procedure above was used with N^{α}-hexanoyl-L-lysine (**22**) (0.033 g, 0.14 mmol) and hexanoyl chloride (28 µL, 0.20 mmol) to yield **27** (0.034 g, 0.10 mmol, 74 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81 (d, J = 6.2 Hz, 1H), 6.03 (t, J = 4.8, 1H), 4.54 (m, 1H), 3.31 (s, 1H), 3.20 (s, 1H), 2.25 (t, J = 7.5, 7.5 Hz, 4H), 2.19 (t, J = 7.5, 7.5 Hz, 2H), 1.83 (m, 2H), 1.57 (m, 3H), 1.30 (m, 11H), 0.89 (t, J = 6.2, 6.2 Hz, 3H), 0.89 (t, J = 6.2, 6.2 Hz, 3H). **^{13}C NMR** (400 MHz, $CDCl_3$) d 174.63, 174.59, 174.33, 53.53, 50.69, 38.93, 36.63, 36.37, 31.48, 31.44, 31.41, 28.88, 25.52, 25.39, 22.37, 22.21, 13.94, 13.94. **ESI-MS** *m/z* calcd for $C_{18}H_{34}N_2O_4$ $[M+H]^+$: 343.26. Found: 343.3.

N^{α}-Hexanoyl-N^{ε}-octanoyl-L-lysine (28)

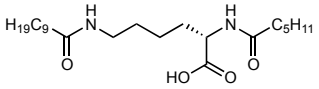


The procedure above was used with N^{α}-hexanoyl-L-lysine (**22**) (0.026 g, 0.11 mmol) and octanoyl chloride (28 µL, 0.62 mmol) to yield **28** (0.032 g, 0.086 mmol, 80 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (d, J = 6.5 Hz, 1H), 5.82 (m, 1H), 4.52 (td, J = 7.3, 4.8, 4.8 Hz, 1H), 3.36 (m, 1H), 3.18 (m, 1H), 2.35 (t, J = 7.5 Hz, 1H), 2.27 (td, J = 7.1, 1.1 Hz, 2H), 2.19 (t, J = 7.6 Hz, 2H),

1.90 (m, 1H), 1.81 (m, 1H), 1.63 (m, 4H), 1.53 (m, 2H), 1.45-1.23 (m, 13H), 0.89 (t, *J* = 6.7, 3H), 0.87 (t, *J* = 6.1, 3H). **^{13}C NMR** (400 MHz, *CDCl$_3$*)d 174.59, 174.59, 174.32, 52.13, 38.93, 36.68, 36.37, 31.70, 35.50, 31.41, 29.28, 29.10, 28.93, 25.86, 25.39, 22.61, 22.37, 22.23, 14.07, 13.95. **ESI-MS** *m/z* calcd for $C_{20}H_{38}N_2O_4$ $[M+H]^+$: 371.29. Found: 371.32.

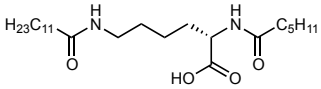
N^{α}-Hexanoyl-N^{ε}-decanoyl-L-lysine (29)



The procedure above was used with N^{α}-hexanoyl-L-lysine (**22**) (0.022 g, 0.090 mmol) decanoyl chloride (28 µL, 0.13 mmol) toyield **29** (0.027 g, 0.67 mmol, 75 %).

^{1}H NMR (500 MHz, *CDCl$_3$*) δ 6.83 (bd, *J* = 5.2 Hz, 1H), 6.18 (bs, 1H), 4.45 (bd, *J* = 4.8 Hz, 1H), 3.57 (q, *J* = 7.2, 7.2, 7.2 Hz, 2H), 3.21 (m, 2H), 2.22 (t, *J* = 7.7, 7.7 Hz, 2H), 2.16 (t, *J* =7.7, 7.7, 2H), 1.86 (m, 1H), 1.71 (m, 1H), 1.67-1.44 (m, 6H), 1.44-1.18 (m, 21H), 0.87 (t, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR** (400 MHz, *CDCl$_3$*) d 173.69, 173.41, 173.37, 57.94, 39.18, 36.78, 36.73, 31.88, 31.51, 29.51, 29.43, 29.31, 28.61, 25.93, 25.48, 22.68, 22.54, 22.42, 14.12, 13.99, 8.10, 8.10. **ESI-MS** *m/z* calcd for $C_{22}H_{42}N_2O_4$ $[M+H]^+$: 399.32. Found: 399.36.

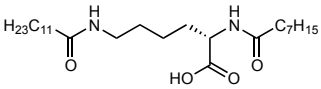
N^{α}-Hexanoyl-N^{ε}-dodecanoyl-L-lysine (30)



The procedure above was used with N^{α}-hexanoyl-L-lysine (**22**) (0.032 g, 0.13 mmol) and dodecanoyl chloride (45 µL, 0.20 mmol) to yield **28** (0.049 g, 0.11 mmol, 87 %).

^{1}H NMR (300 MHz, *CDCl$_3$*) δ 7.11 (bs, 1H), 6.63 (bs, 1H), 4.37 (bs, 1H), 3.16 (bs, 2H), 2.18 (m, 4H), 1.79 (m, 1H), 1.71-1.40 (m, 7H), 1.40-1.15 (m, 22H), 0.86 (bt, *J* = 5.88, 5.88 Hz, 6H). **^{13}C NMR** (300 MHz, *CDCl$_3$*) δ 174.29, 174.13, 174.13, 38.93, 36.64, 36.28, 31.87, 31.47, 29.65, 29.61, 29.61, 29.61, 29.57, 29.43, 29.43, 29.43, 29.32, 29.32, 25.91, 25.44, 22.64, 22.38, 14.06, 13.94. **ESI-MS** *m/z* calcd for $C_{24}H_{46}N_2O_4$ $[M+H]^+$: 427.36. Found: 427.54.

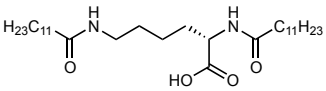
N^{α}-Octanoyl-N^{ε}-dodecanoyl-L-lysine (31)



The procedure above was used with N^{ε}-dodecanoyl-L-lysine (**38**) (0.034 g, 0.10 mmol) and octanoyl chloride (26 µL, 0.15 mmol) to yield **31** (0.029 g, 0.065 mmol, 63 %).

^{1}H NMR (500 MHz, *CDCl$_3$*) δ 6.79 (d, *J* = 6.7 Hz, 1H), 5.89 (m, 1H), 4.53 (s, 1H), 3.35 (m, 1H), 3.20 (m, 1H), 2.26 (m, 2H), 2.19 (m, 2H), 1.90 (m, 1H), 1.81 (m, 1H), 1.71-1.47 (m, 6H), 1.46-1.20 (m, 26H), 0.87 (t, *J* = 6.6 Hz, 6H), 0.87 (t, *J* = 6.6 Hz, 6H). **^{13}C NMR** (400 MHz, *CDCl$_3$*) δ 174.60, 174.51, 174.29, 52.10, 38.83, 36.69, 36.40, 31.87, 31.68, 31.39, 29.66, 29.62, 29.59, 29.51, 29.36, 29.31, 29.27, 28.99, 28.86, 25.83, 25.68, 22.64, 22.58, 22.11, 14.08, 14.03. **ESI-MS** *m/z* calcd for $C_{26}H_{50}N_2O_4$ $[M+H]^+$: 455.39. Found: 455.44.

N^{α},N^{ε}-Bis(dodecanoyl)-L-lysine (32)

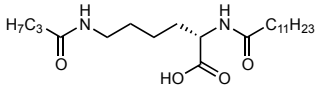


The procedure above was used with N^{ε}-dodecanoyl-L-lysine (**38**) (33 mg, 0.10 mmol) and dodecanoyl chloride (35 µL, 0.15 mmol) to yield **32** (0.045 g, 0.088 mmol, 87 %).

^{1}H NMR (500 MHz, *CDCl$_3$*) δ 6.83 (d, *J* = 7.1 Hz, 1H), 5.84 (t, *J* = 5.9 Hz, 1H), 4.51 (m, 1H), 3.36 (m, 1H), 3.18 (m, 1H), 2.26 (dt, *J* = 7.3, 2.19 Hz, 2H), 2.18 (m, 2H), 1.89 (m, 1H), 1.81 (m, 1H), 1.62 (m, 4H),

1.53 (m, 2H), 1.45-1.19 (m, 34H), 0.87 (t, *J* = 7.0 Hz, 6H). **^{13}C NMR** (300 MHz, *CDCl$_3$*) δ 174.81, 174.47, 174.35, 52.31, 38.41, 36.83, 36.33, 31.90, 30.59, 29.66, 29.63, 29.63, 29.61, 29.53, 29.51, 29.38, 29.36, 29.35, 29.35, 29.34, 29.34, 29.34, 29.31, 29.16, 25.78, 25.67, 22.68, 21.77, 14.11, 14.11. **ESI-MS** *m/z* calcd for $C_{30}H_{58}N_2O_4$ [M +H]$^+$: 511.45. Found: 511.58.

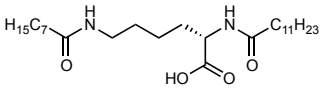
N^{α}-Dodecanoyl-N^{ε}-butanoyl-L-lysine (33)



The procedure above was used with N^{α}-dodecanoyl-L-lysine (**38**) (0.010 g, 0.030 mmol) and butryl chloride (5 µL, 0.046 mmol) to yield **33** (0.011 g, 0.027 mmol, 87 %).

^{1}H NMR (500 MHz, *CDCl$_3$*) δ 6.96 (bs, 1H), 6.37 (bs, 1H), 4.41 (bs, 1H), 3.20 (bs, 2H), 2.22 (m, 2H), 2.17 (t, *J* = 7.3 Hz, 2H), 1.83 (m, 1H), 1.75-1.42 (m, 6H), 1.42-1.16 (m, 19H), 0.92 (t, *J* = 7.4 Hz, 3H), 0.88 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR** (400 MHz, *CDCl$_3$*) δ 174.11, 174.02, 174.02, 57.93, 38.83, 38.51, 36.43, 31.90, 29.68, 29.64, 29.60, 29.59, 29.45, 29.43, 29.35, 29.31, 28.94, 25.80, 22.67, 19.22, 14.09, 13.75. **ESI-MS** *m/z* calcd for $C_{22}H_{42}N_2O_4$ [M +H]$^+$: 399.32. Found: 399.42.

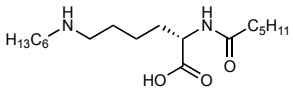
N^{α}-Dodecanoyl-N^{ε}-octanoyl-L-lysine (34)



The procedure above was used with N^{α}-dodecanoyl-L-lysine (**38**) (0.011 g, 0.033 mmol) and octanoyl chloride (9 µL, 0.049 mmol) to yield **34** (0.009 g, 0.019 mmol, 57 %).

^{1}H NMR (500 MHz, *CDCl$_3$*) δ 6.85 (d, *J* = 6.9 Hz, 1H), 5.79 (t, *J* = 5.4 Hz, 1H), 4.49 (m, 1H), 3.38 (m, 1H), 3.19 (m, 1H), 2.27 (td, *J* = 7.3, 3.3, 2H), 2.19 (t, *J* = 7.7 Hz, 2H), 1.90 (m, 1H), 1.82 (s, 1H), 1.68-1.57 (m, 4H), 1.57-1.48 (m, 2H), 1.45-1.15 (m, 26H), 0.87 (t, *J* = 6.8 Hz, 6H). **^{13}C NMR** (400 MHz, *CDCl$_3$*) δ 174.74, 174.58, 173.96, 52.26, 38.54, 36.76, 36.35, 31.90, 31.67, 30.73, 29.64, 29.62, 29.53, 29.37, 29.34, 29.31, 29.23, 29.11, 28.98, 25.78, 25.68, 22.68, 22.59, 21.82, 14.11, 14.05. **ESI-MS** *m/z* calcd for $C_{26}H_{50}N_2O_4$ [M +H]$^+$: 455.39. Found: 455.52.

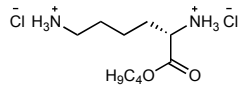
N^{α}-Hexanoyl-N^{ε}-hexyl-L-lysine (35)



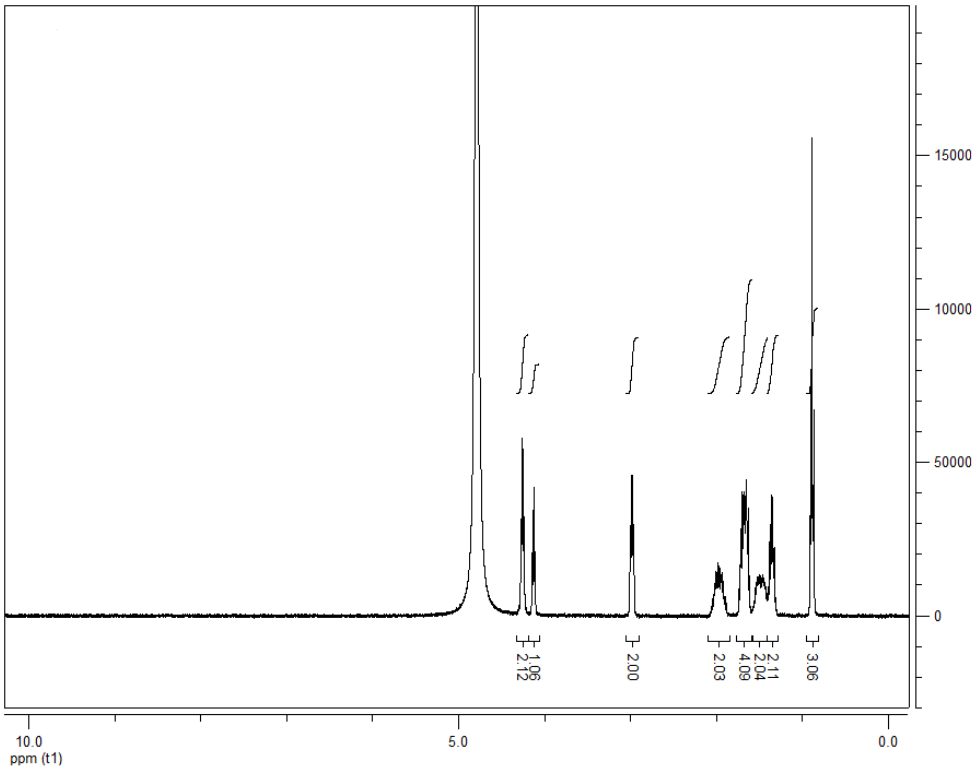
22 (0.03 g, 0.079 mmol) was dissolved in MeOH and the following were added: hexanal (14 µL, 0.12 mmol), acetic acid (9 µL, 0.16 mmol), $Pd(OH)_2$ on carbon (3 mg). The mixture was subjected to 40 bar of H_2 in a Parr Bomb while stirring at 50 °C for 18 hrs. The mixture was cooled to room temperature for 1 hr before opening Parr Bomb to atmosphere. The $Pd(OH)_2$ on carbon was filtered and the filtrate was concentrated under reduced pressure. The residue was purified via silica column chromatography (5:1:1:1 EtOAc:MeOH:H_2O:CH_3CN, Rf = 0.3) to yield **35** as a yellowish oil (8.6 mg, 0.026 mmol, 33 %).

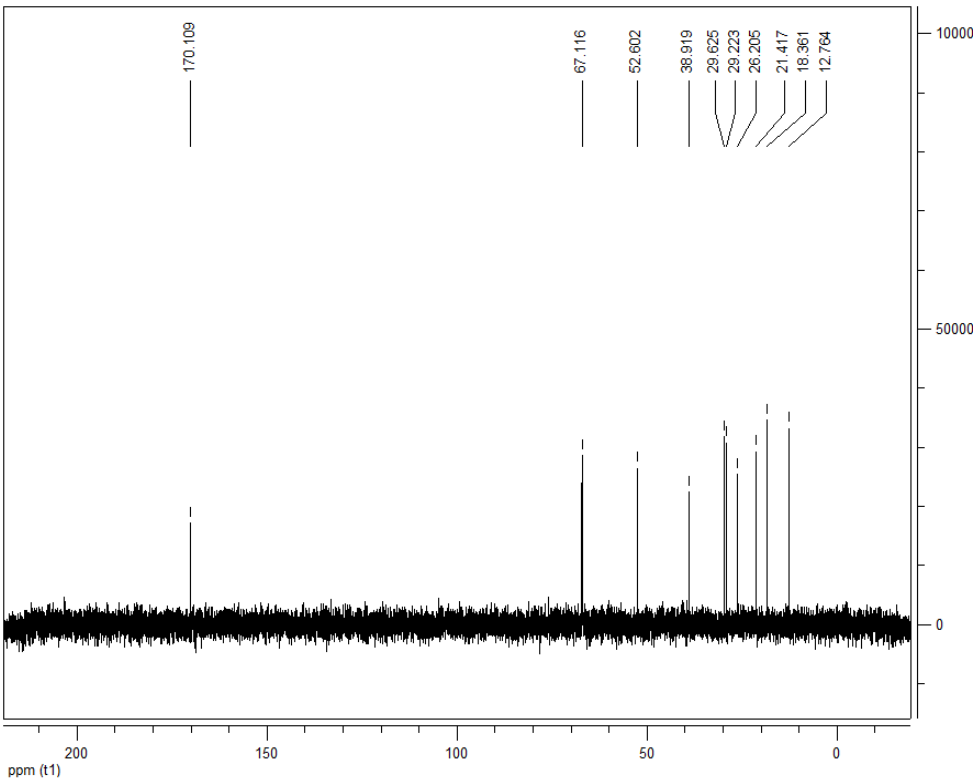
^{1}H NMR (300 MHz, *CDCl$_3$*) δ 6.77 (bs, 1H), 4.20 (td, *J* = 5.2, 5.8 Hz, 1H), 2.87 (m, 3H), 2.18 (bt, *J* = 7.6 Hz, 2H), 1.87 (m, 1H), 1.80-1.53 (m, 7H), 1.52-1.12 (m, 13H), 0.89 (t, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (500 MHz, *CDCl$_3$*) δ 176.34, 173.25, 53.62, 47.90, 47.48, 36.56, 31.95, 31.47, 31.22, 29.68, 26.40, 25.93, 25.75, 25.45, 22.44 , 22.41, 13.97, 13.91. **ESI-MS** *m/z* calcd for $C_{18}H_{36}N_2O_3$ [M +H]$^+$: 329.28. Found: 329.29.

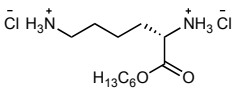
v) Spectroscopic Data



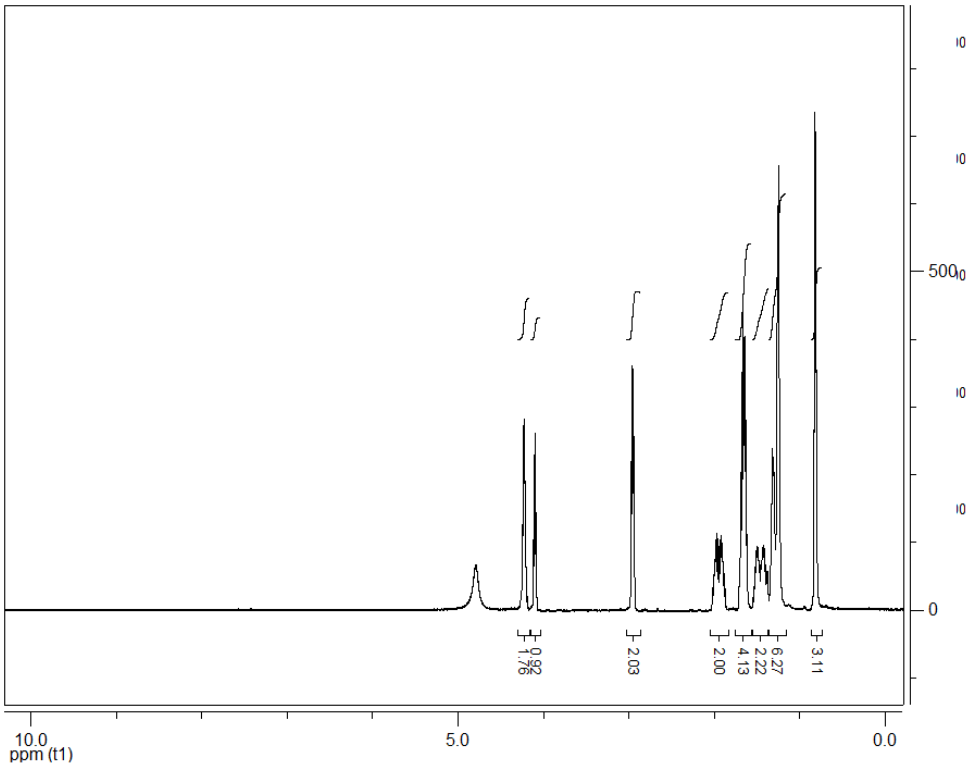
4






Cl H3N
NH3 Cl
H13C6O
O

5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0.26
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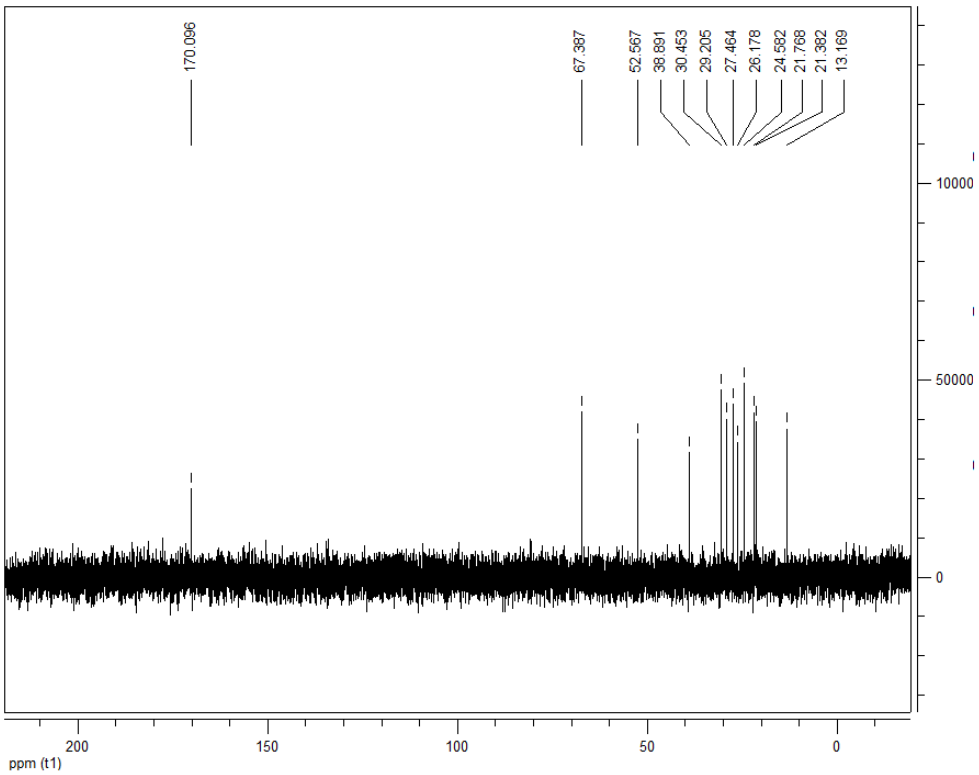
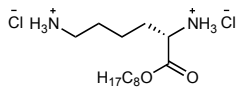
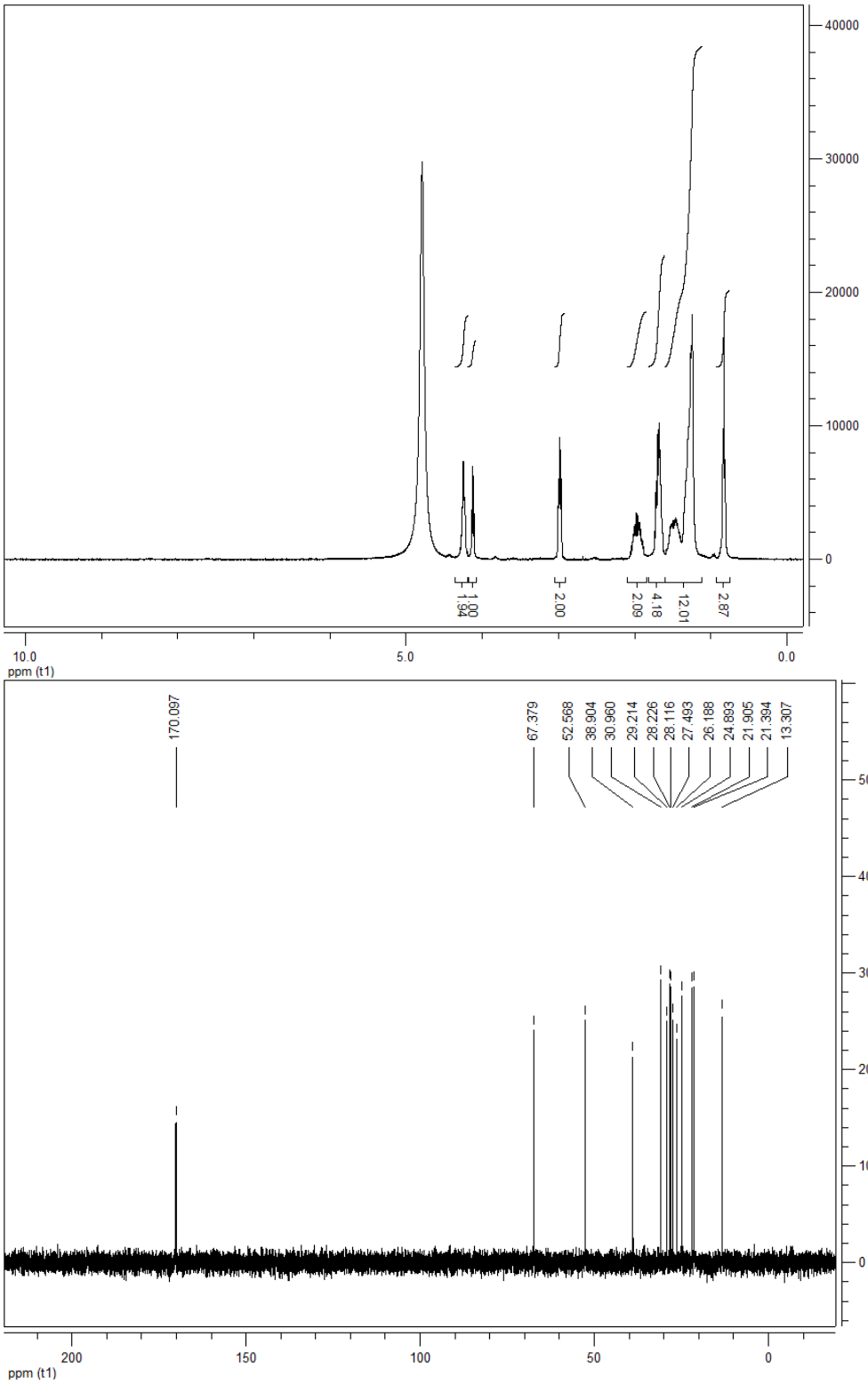
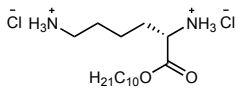
2.00
4.13
2.22
6.27
3.11
10.0
ppm (t1)
5.0
0.0


170.096
67.387
52.567
38.891
30.453
29.205
27.464
26.178
24.582
21.768
21.382
13.169
10000
50000
0
200
150
100
50
0
ppm (t1)

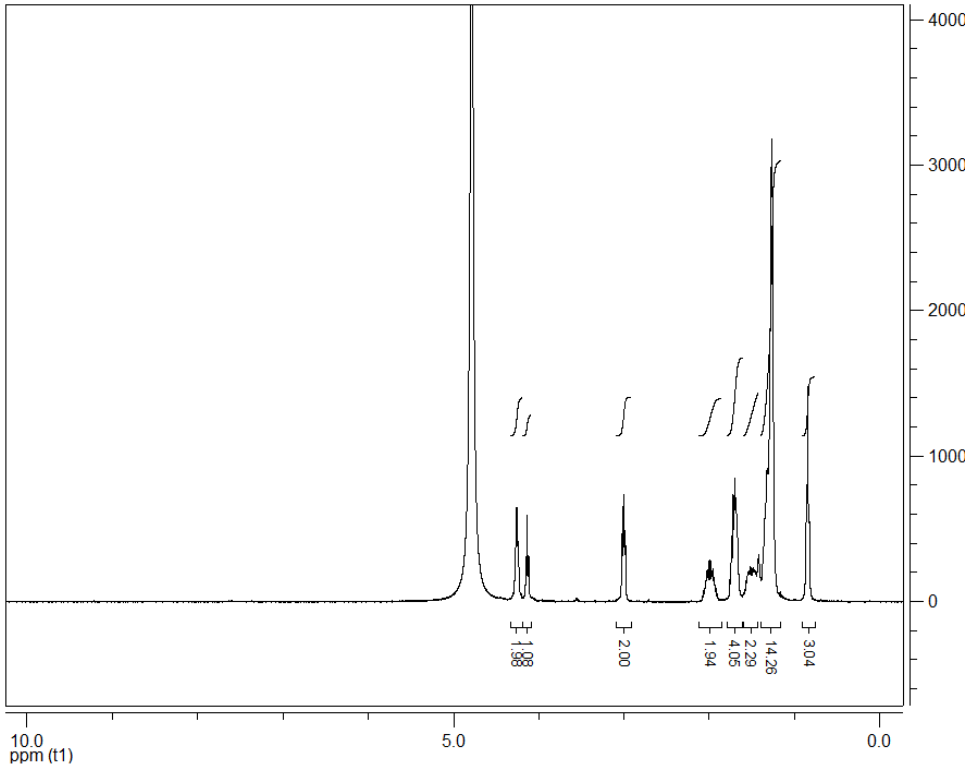


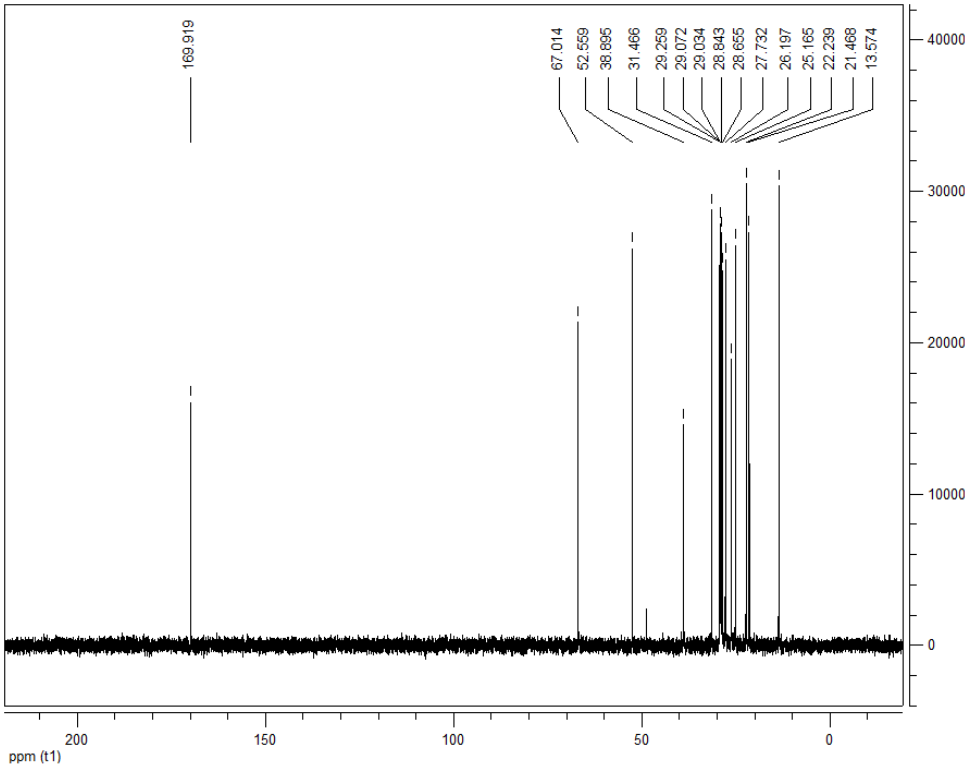
6

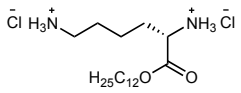

40000
30000
20000
10000
0
1.00
1.04
2.00
2.69
4.18
12.01
2.87
10.0
5.0
0.0
ppm (t1)
170.097
67.379
52.568
38.904
30.960
29.214
28.226
28.116
27.493
26.188
24.893
21.905
21.394
13.307
50000
40000
30000
20000
10000
0
200
150
100
50
0
ppm (t1)



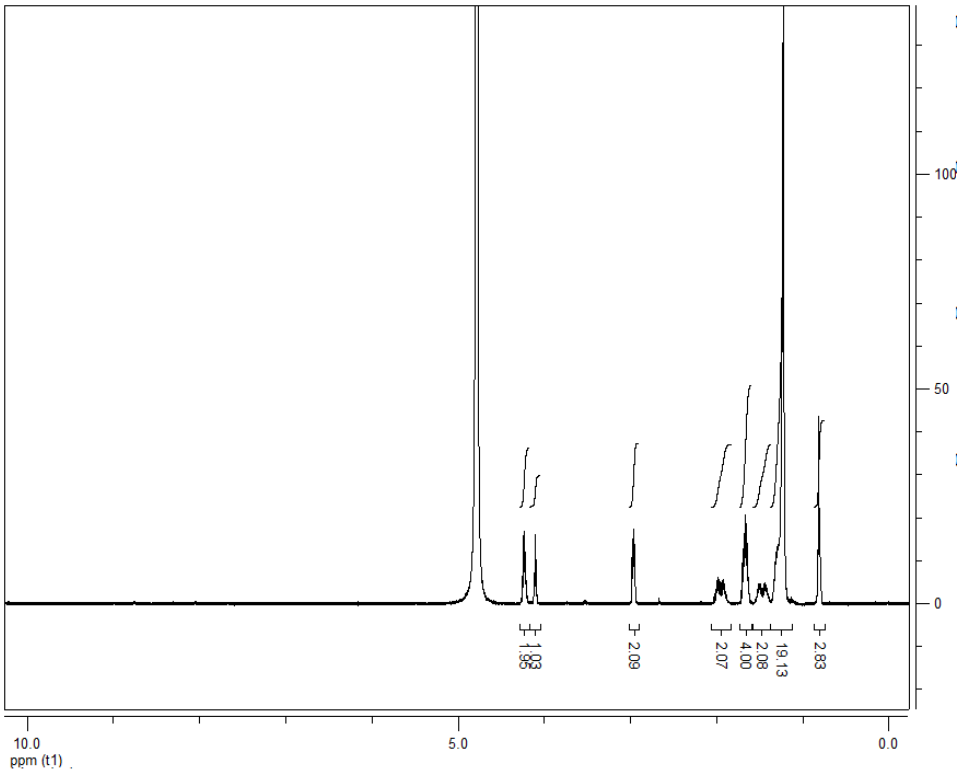
7

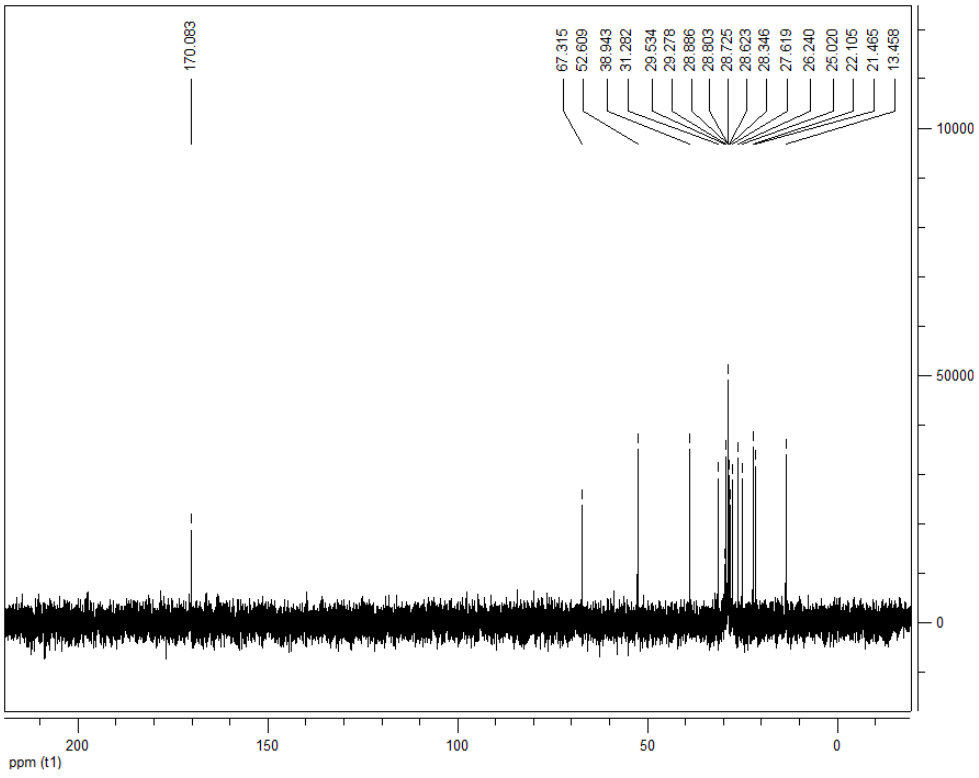


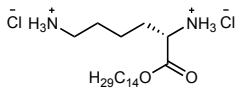



Cl H3N
NH3 Cl
H25C12O
O

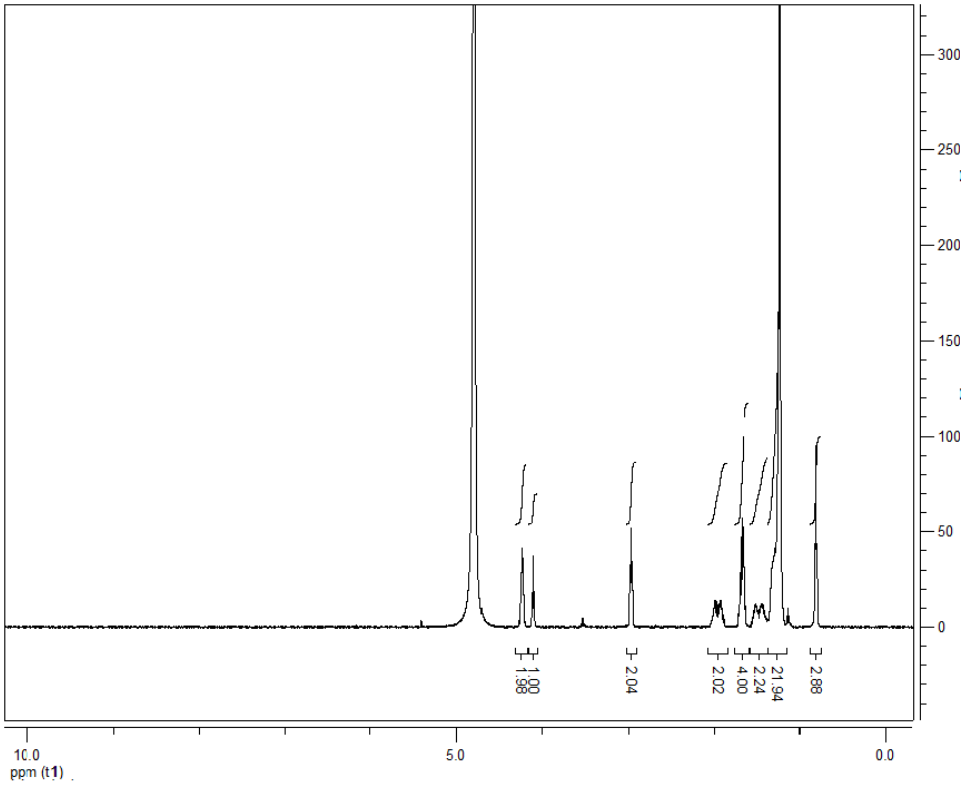
8

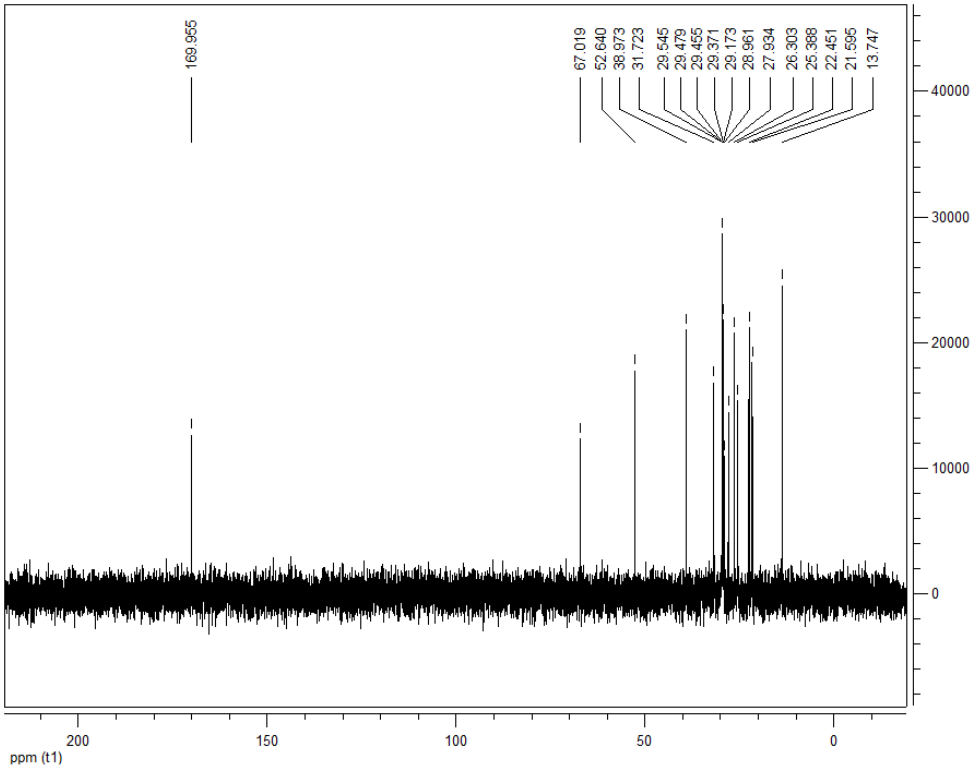

100
50
0
1.03
1.96
2.09
2.07
4.00
2.08
19.13
2.83
10.0
5.0
0.0
ppm (t1)

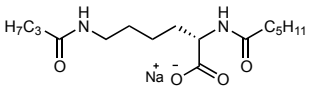

170.083
67.315
52.609
38.943
31.282
29.534
29.278
28.886
28.803
28.725
28.623
28.346
27.619
26.240
25.020
22.105
21.465
13.458
10000
50000
0
200
150
100
50
0
ppm (t1)


Cl H3N
NH3 Cl
H29C14O
O

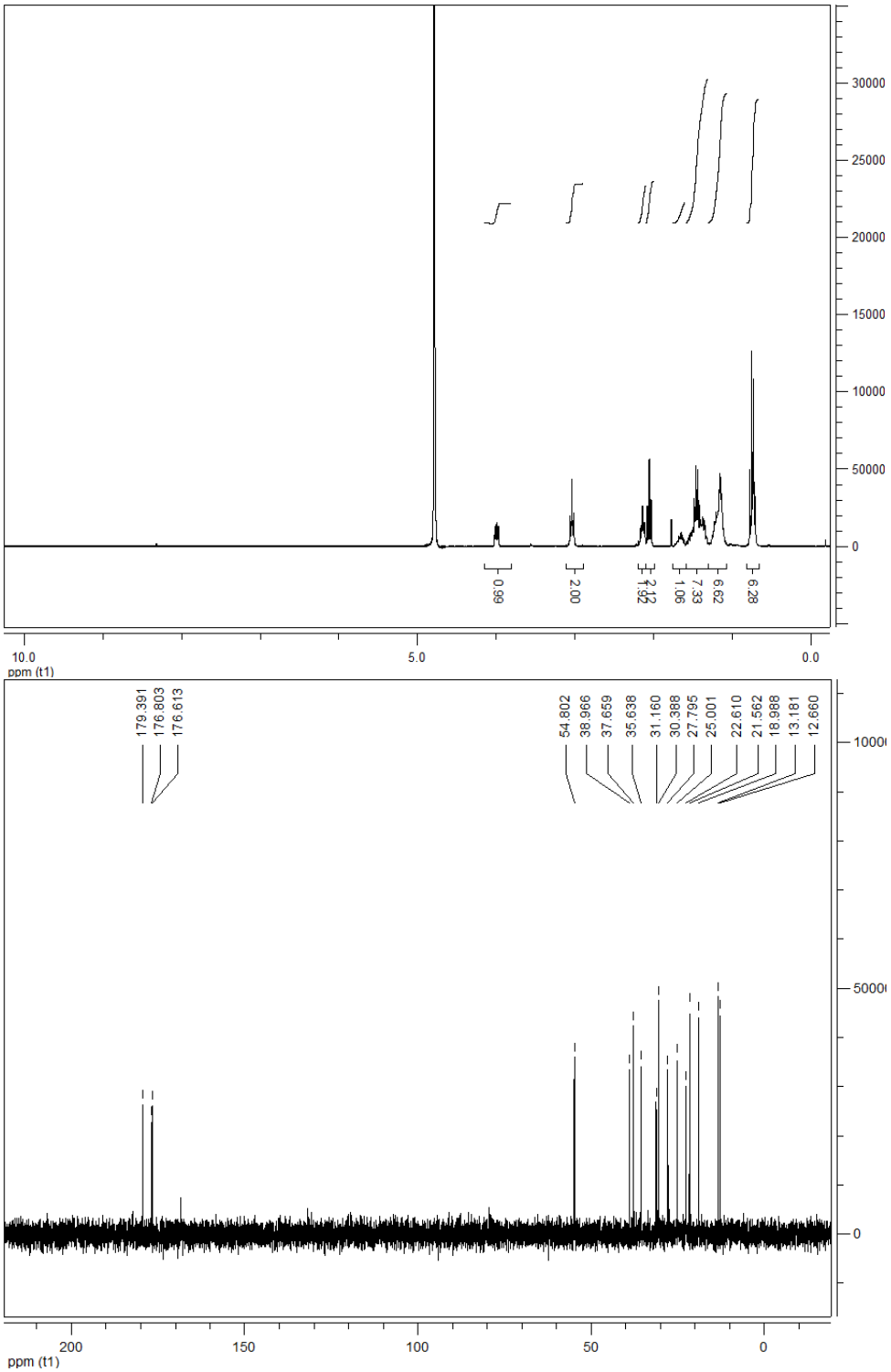
9

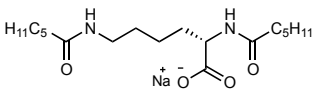

1.00
1.98
2.04
2.02
4.00
2.24
21.64
2.88
10.0
5.0
0.0
ppm (t1)


169.955
67.019
52.640
38.973
31.723
29.545
29.479
29.455
29.371
29.173
28.961
27.934
26.303
25.388
22.451
21.595
13.747
200
150
100
50
0
ppm (t1)

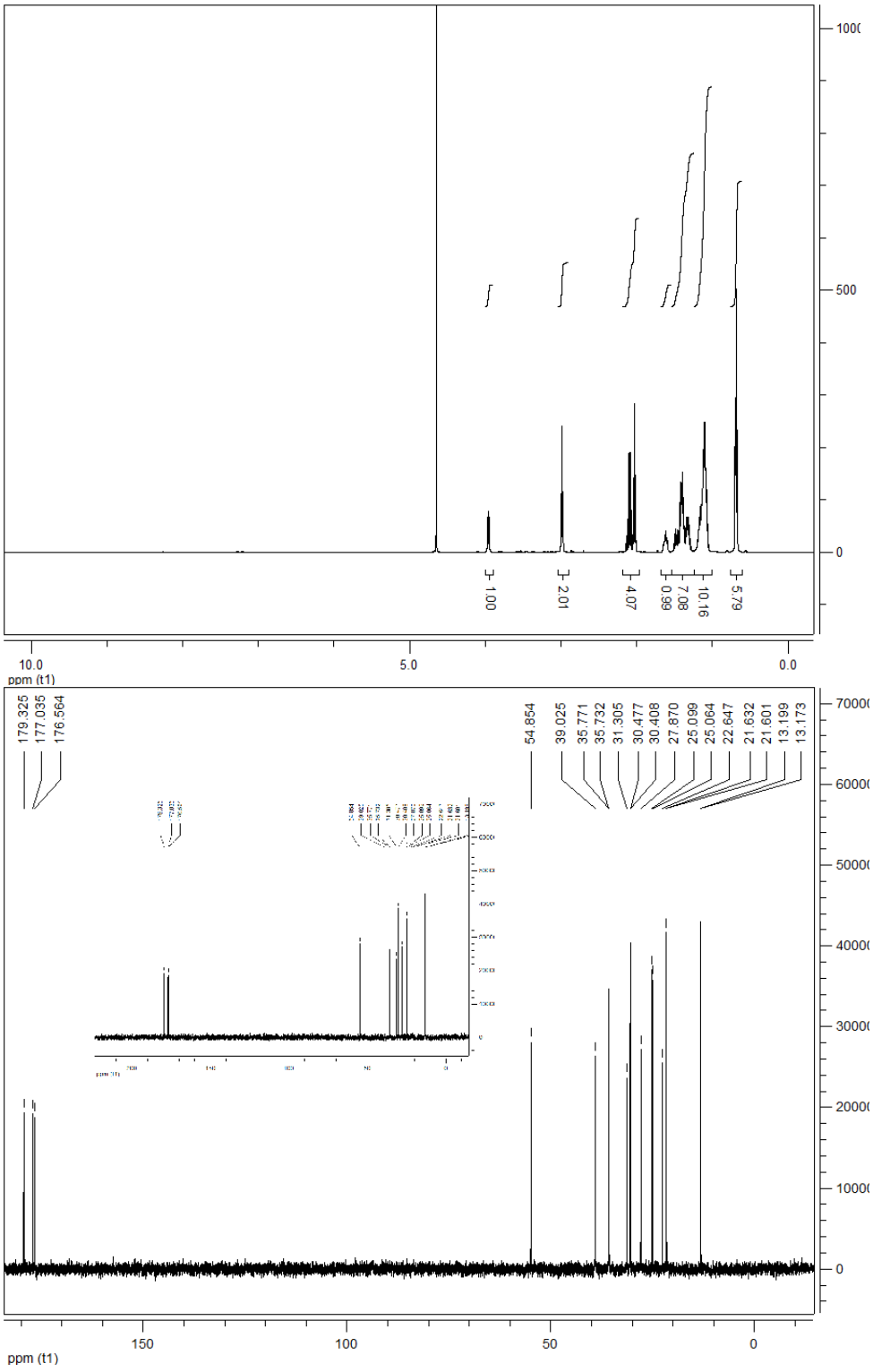

H7C3
H
N
O
H
N
C5H11
O
Na
+
-
O
O

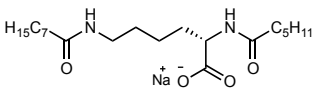
10


30000
25000
20000
15000
10000
50000
0
0.99
2.00
2.12
1.92
1.06
7.33
6.62
9.28
10.0
5.0
0.0
ppm (t1)
179.391
176.803
176.613
54.802
38.966
37.659
35.638
31.160
30.388
27.795
25.001
22.610
21.562
18.988
13.181
12.660
10000
50000
0
200
150
100
50
0
ppm (t1)

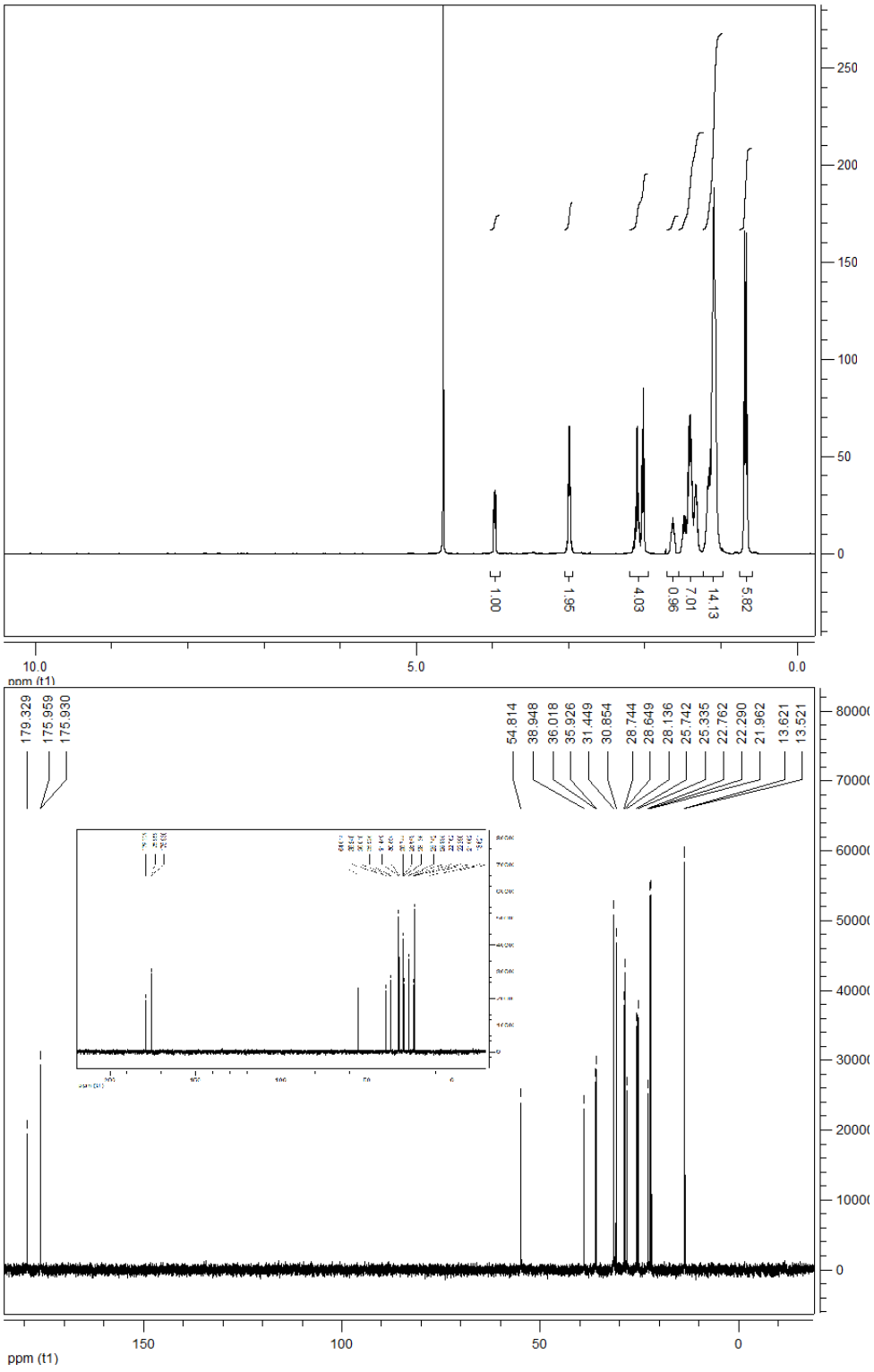


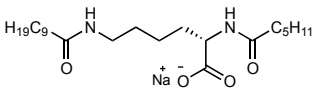
11


1.00
2.01
4.07
0.99
7.08
10.16
5.79
10.0
5.0
0.0
ppm (t1)
179.325
177.035
176.564
54.854
39.025
35.771
35.732
31.305
30.477
30.408
27.870
25.099
25.064
22.647
21.632
21.601
13.199
13.173
150
100
50
0
ppm (t1)

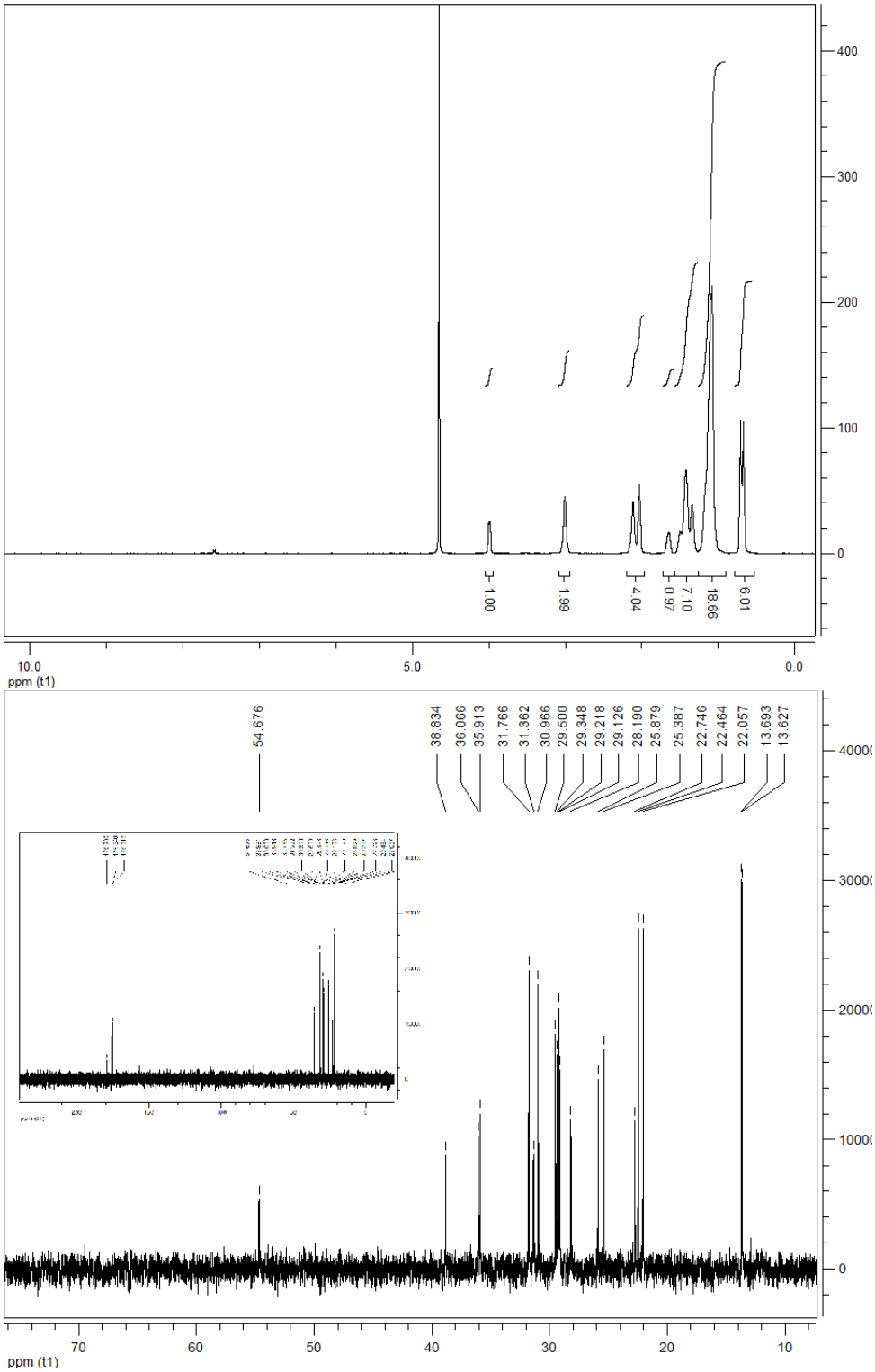


12




H19C9
H
N
O
H
N
C5H11
O
+
Na
-
O
O

13


1.00
1.99
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0.97
7.10
18.66
6.01
10.0
5.0
0.0
ppm (t1)
54.676
38.834
36.066
35.913
31.766
31.362
30.966
29.500
29.348
29.218
29.126
28.190
25.879
25.387
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22.464
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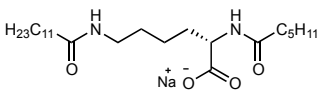
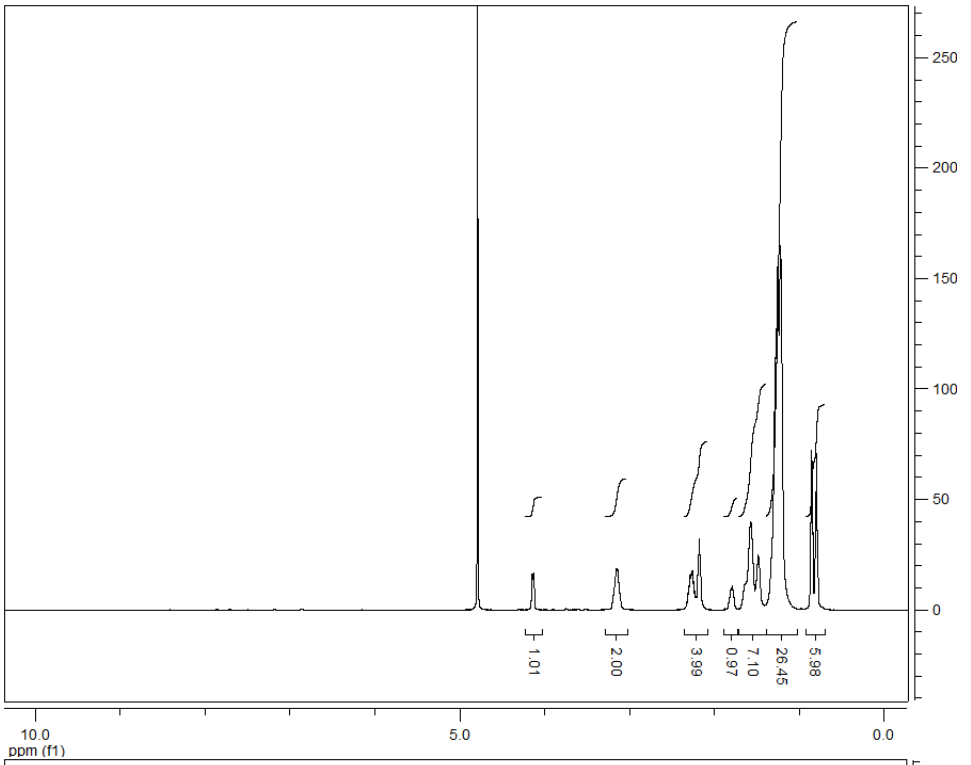
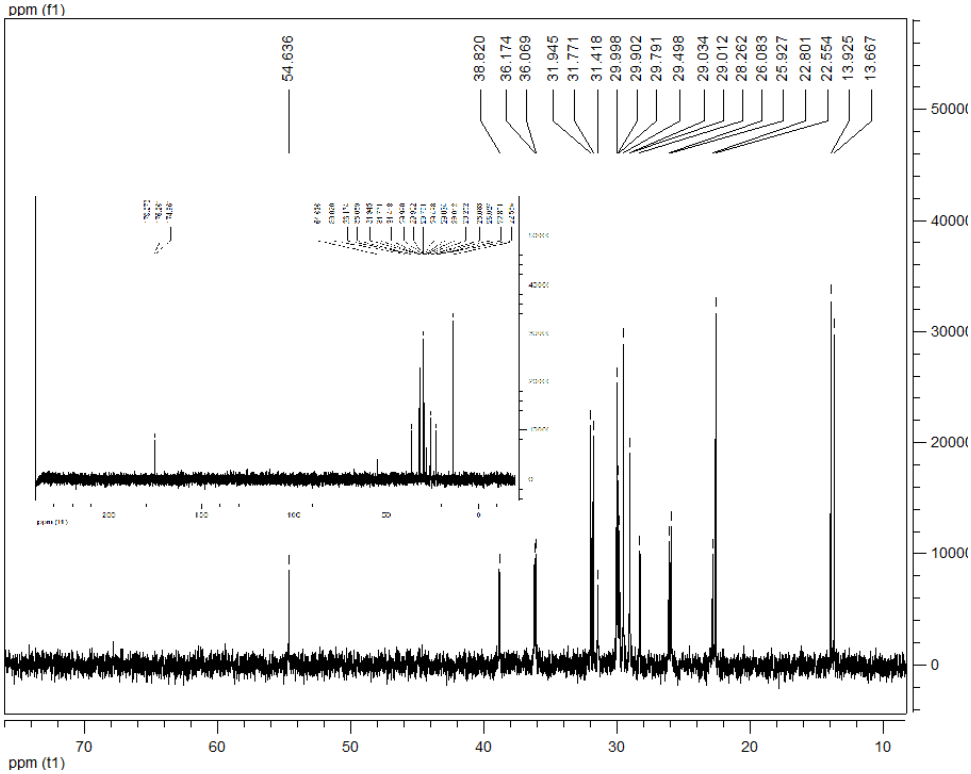
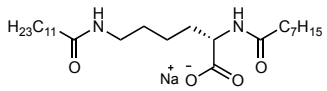
13.693
13.627
70
60
50
40
30
20
10
ppm (t1)


H23C11
H
N
O
H
N
C5H11
O
Na
O
O

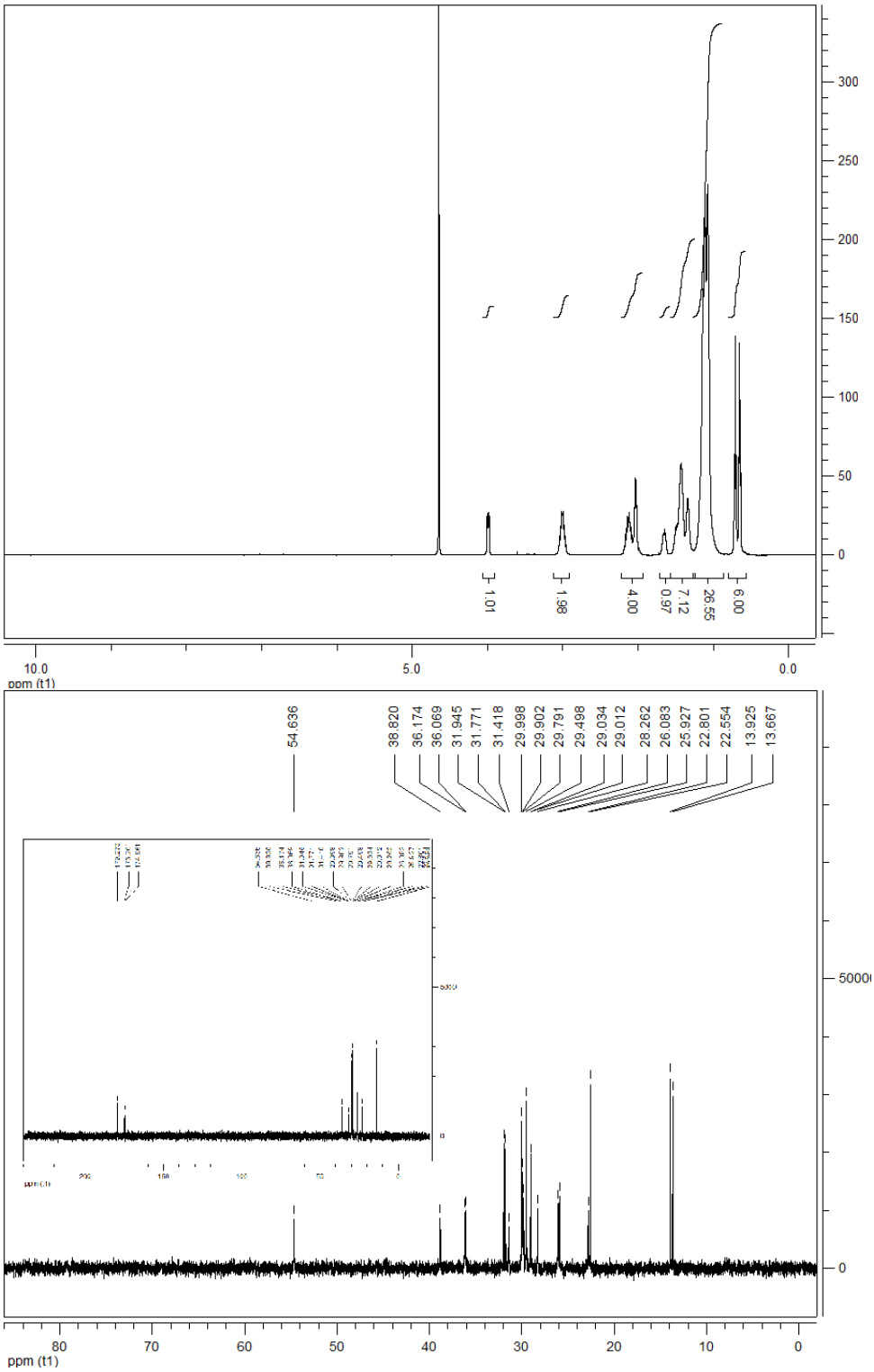
14

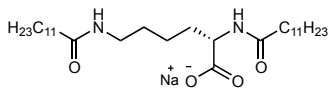

250
200
150
100
50
0
1.01
2.00
3.99
0.97
7.10
29.45
5.98
10.0
5.0
0.0
ppm (f1)


54.636
38.820
36.174
36.069
31.945
31.771
31.418
29.998
29.902
29.791
29.498
29.034
29.012
28.262
26.083
25.927
22.801
22.554
13.925
13.667
50000
40000
30000
20000
10000
0
70
60
50
40
30
20
10
ppm (t1)

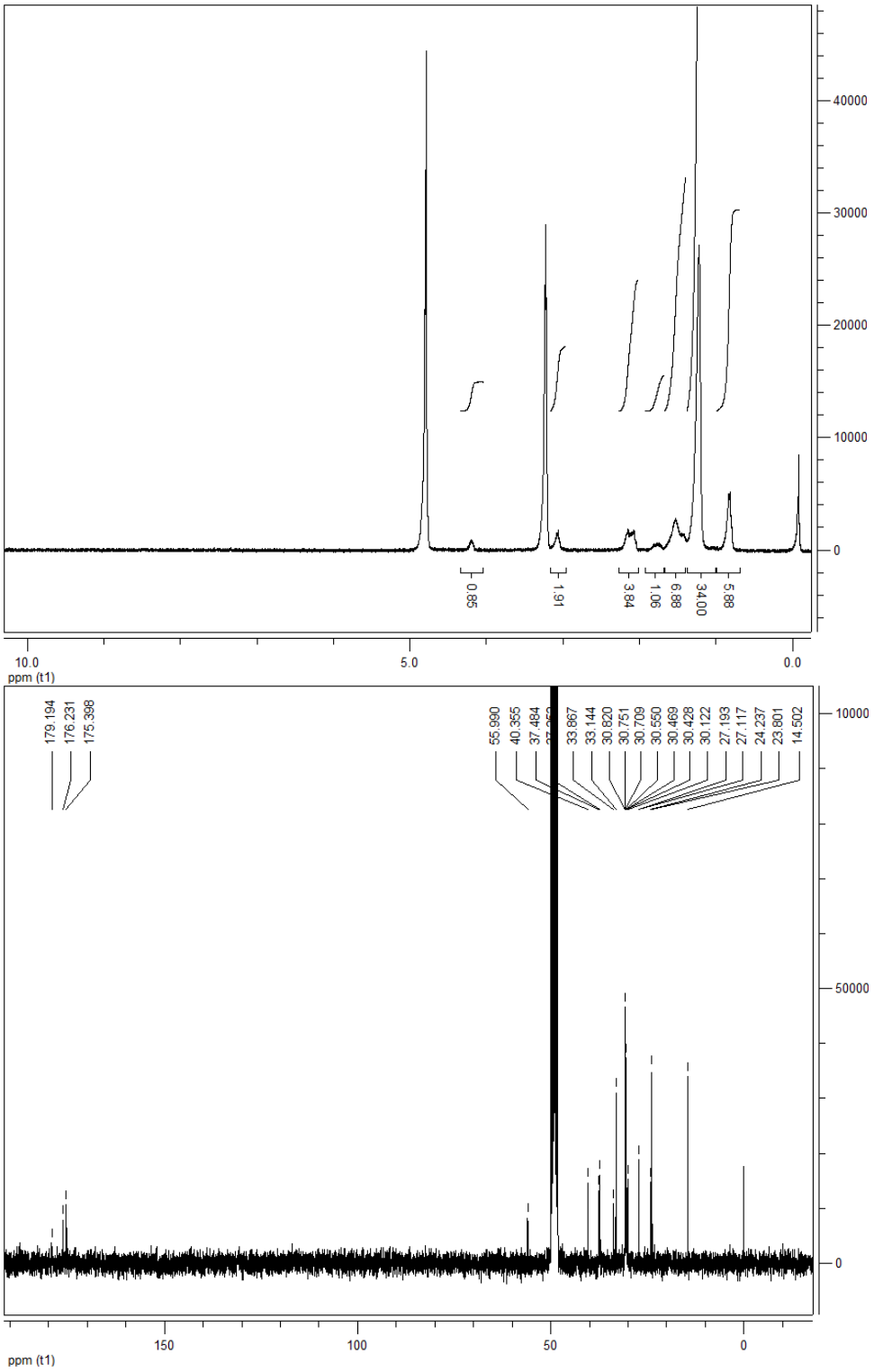

H23C11
H
N
H
N
C7H15
O
O
O
Na
+
-
O
O

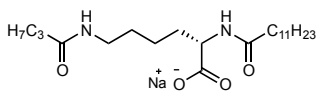
16


300
250
200
150
100
50
0
1.01
1.98
4.00
0.97
7.12
26.55
9.00
10.0
5.0
0.0
ppm (t1)
54.636
38.820
36.174
36.069
31.945
31.771
31.418
29.998
29.902
29.791
29.498
29.034
29.012
28.262
26.083
25.927
22.801
22.554
13.925
13.667
50000
0
80
70
60
50
40
30
20
10
0
ppm (t1)

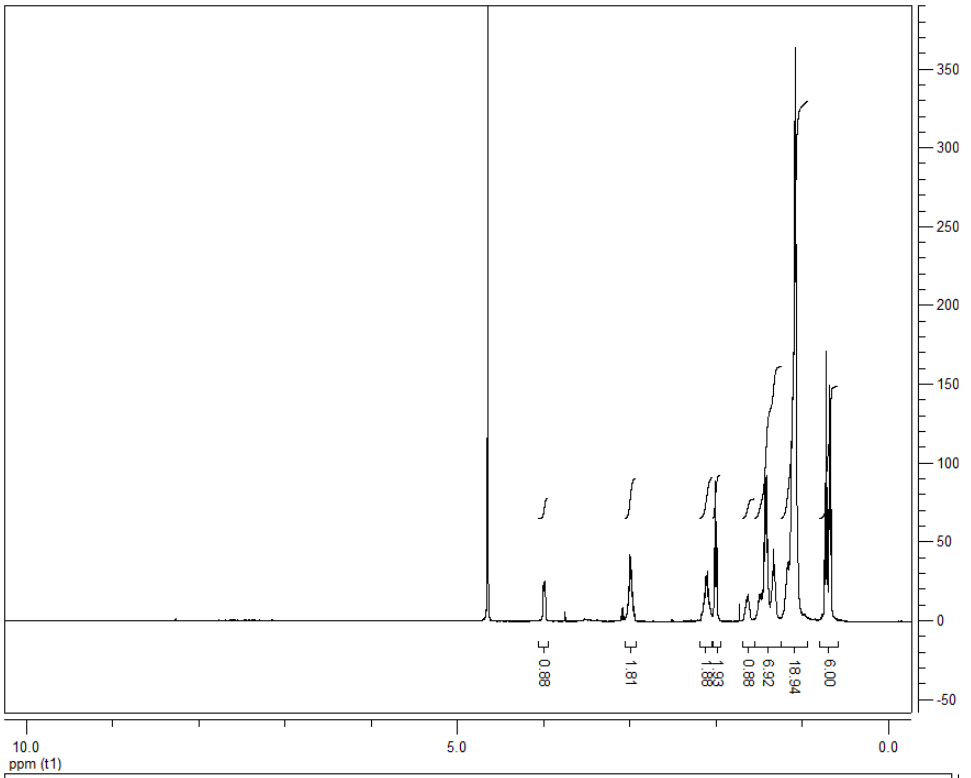

H23C11
H
N
O
H
N
C11H23
O
Na
+
-
O
O

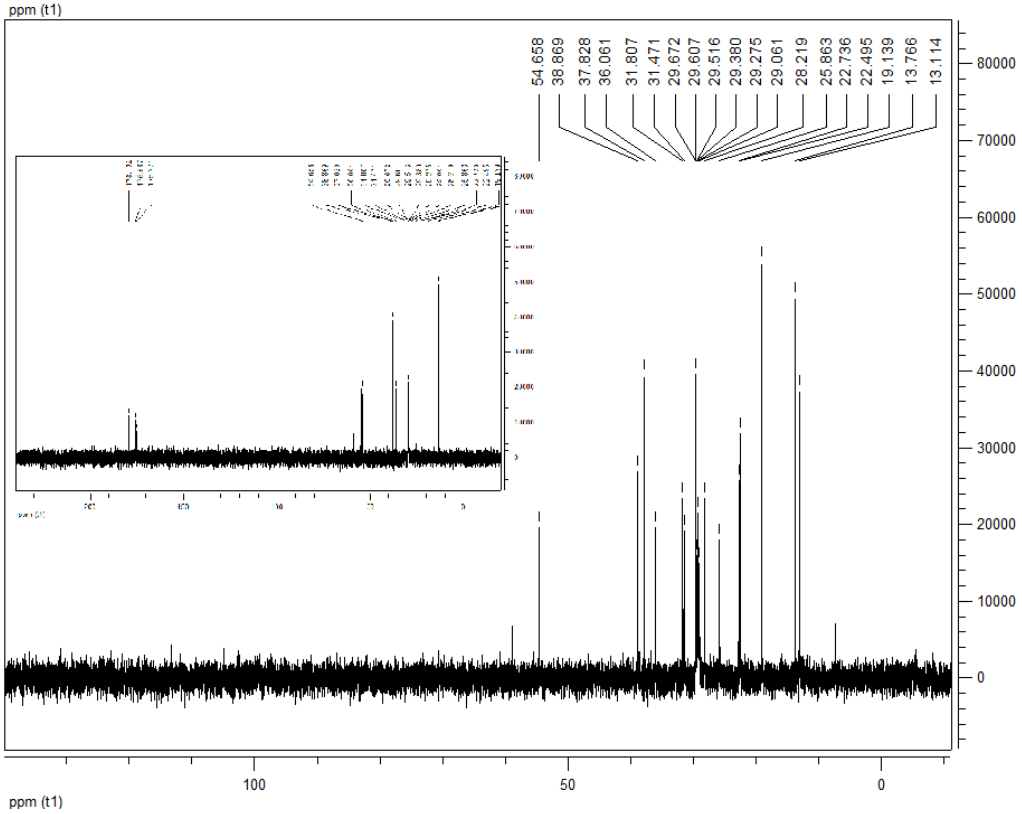
17

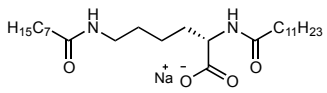

40000
30000
20000
10000
0
0.85
1.91
3.84
1.06
6.88
34.00
5.88
10.0
5.0
0.0
ppm (t1)
179.194
176.231
175.398
55.990
40.365
37.484
33.867
33.144
30.820
30.751
30.709
30.550
30.469
30.428
30.122
27.193
27.117
24.237
23.801
14.502
10000
50000
0
150
100
50
0
ppm (t1)


H7C3
H
N
O
H
N
C11H23
O
Na
+
-
O
O

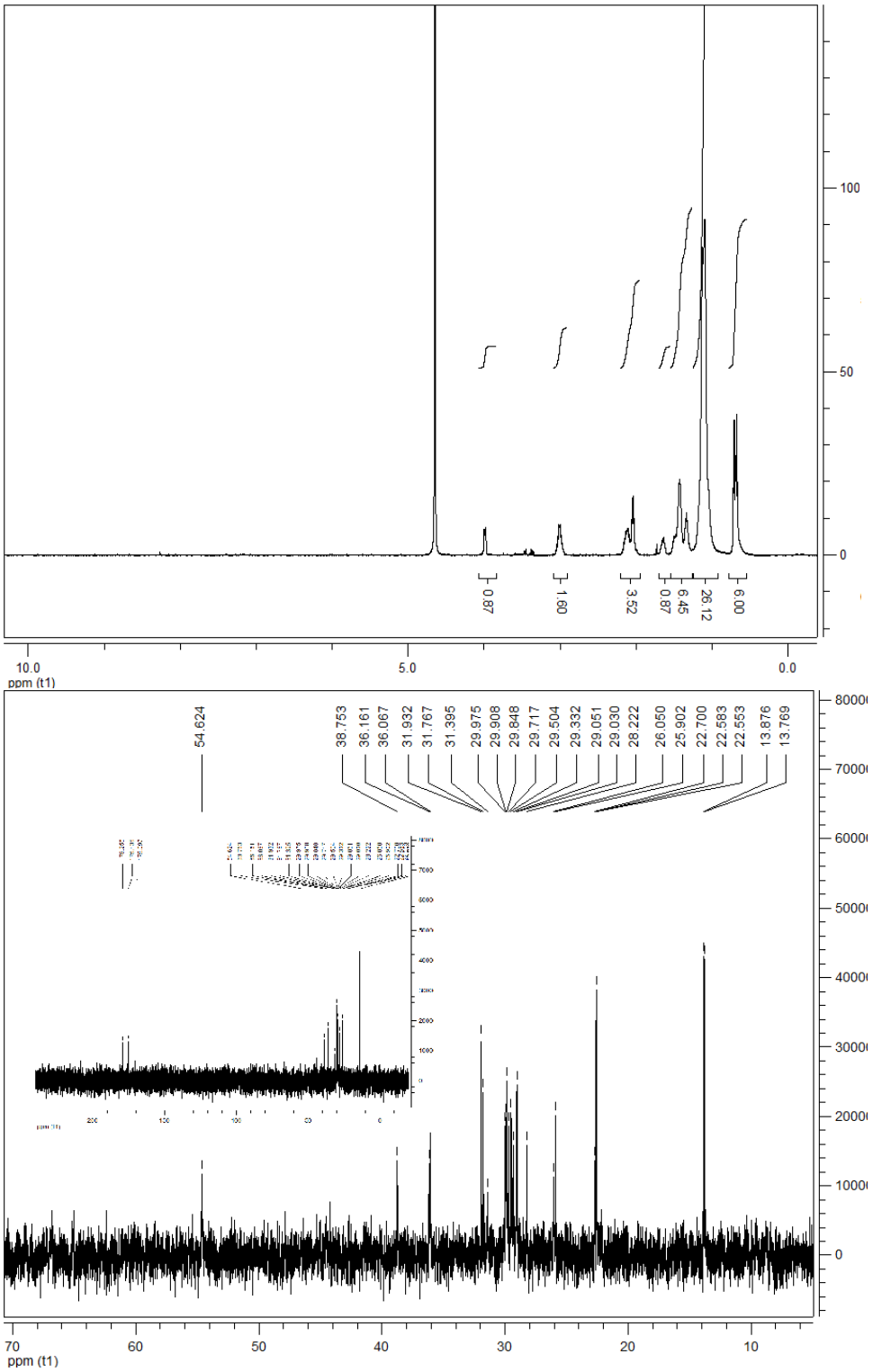
18

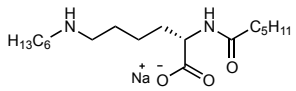

0.88
1.81
1.93
1.88
0.88
6.92
18.94
6.00
10.0
5.0
0.0
ppm (t1)


54.658
38.869
37.828
36.061
31.807
31.471
29.672
29.607
29.516
29.380
29.275
29.061
28.219
25.863
22.736
22.495
19.139
13.766
13.114
100
50
0
ppm (t1)

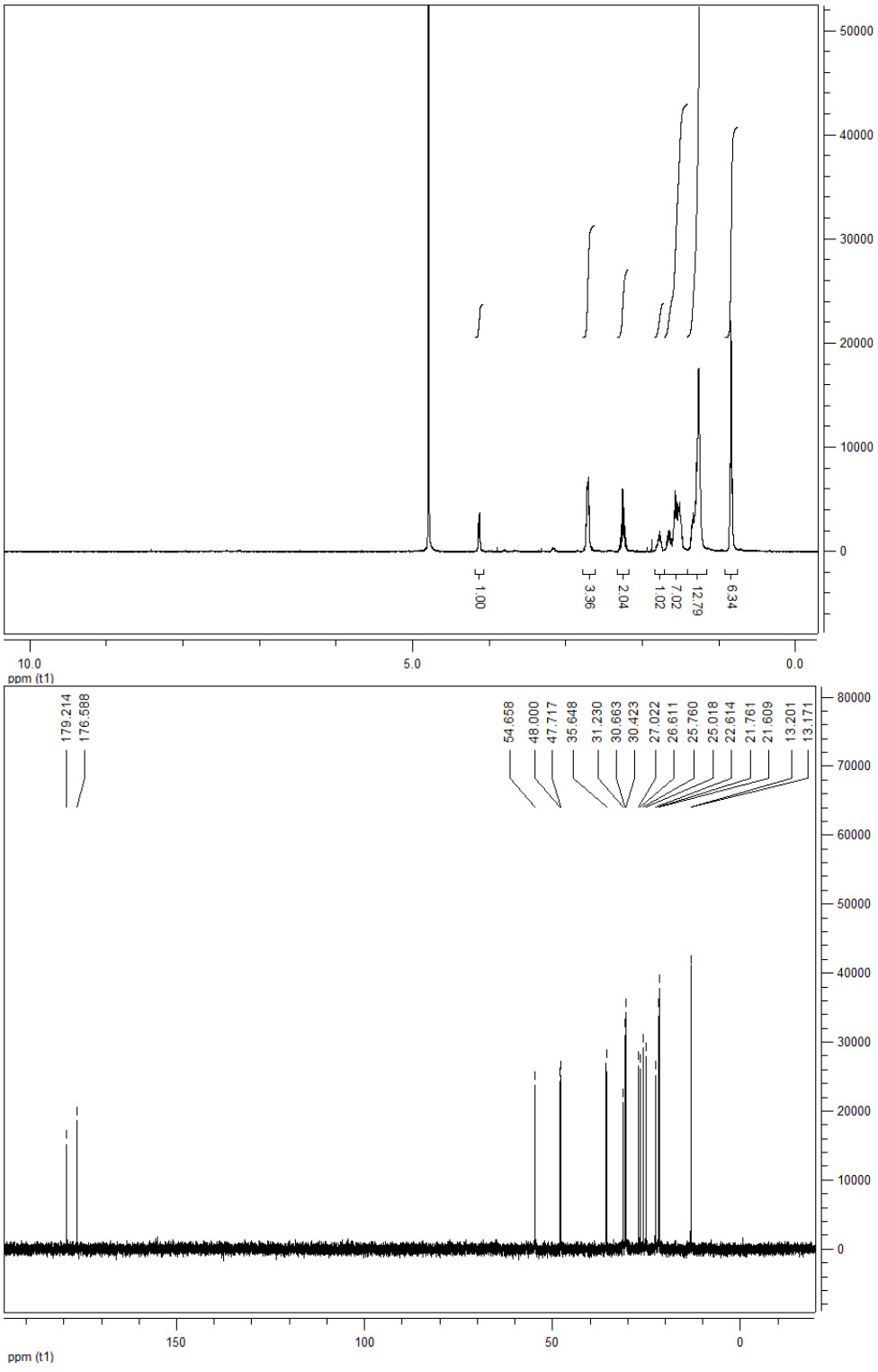

H15C7
H
N
O
H
N
C11H23
O
Na
+
-
O
O

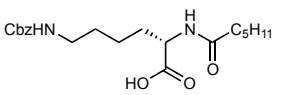
19


0.87
1.60
3.52
0.87
6.45
26.12
9.00
10.0
5.0
0.0
ppm (t1)
54.624
38.753
36.161
36.067
31.932
31.767
31.395
29.975
29.908
29.848
29.717
29.504
29.332
29.051
29.030
28.222
26.050
25.902
22.700
22.583
22.553
13.876
13.769
70
60
50
40
30
20
10
ppm (t1)

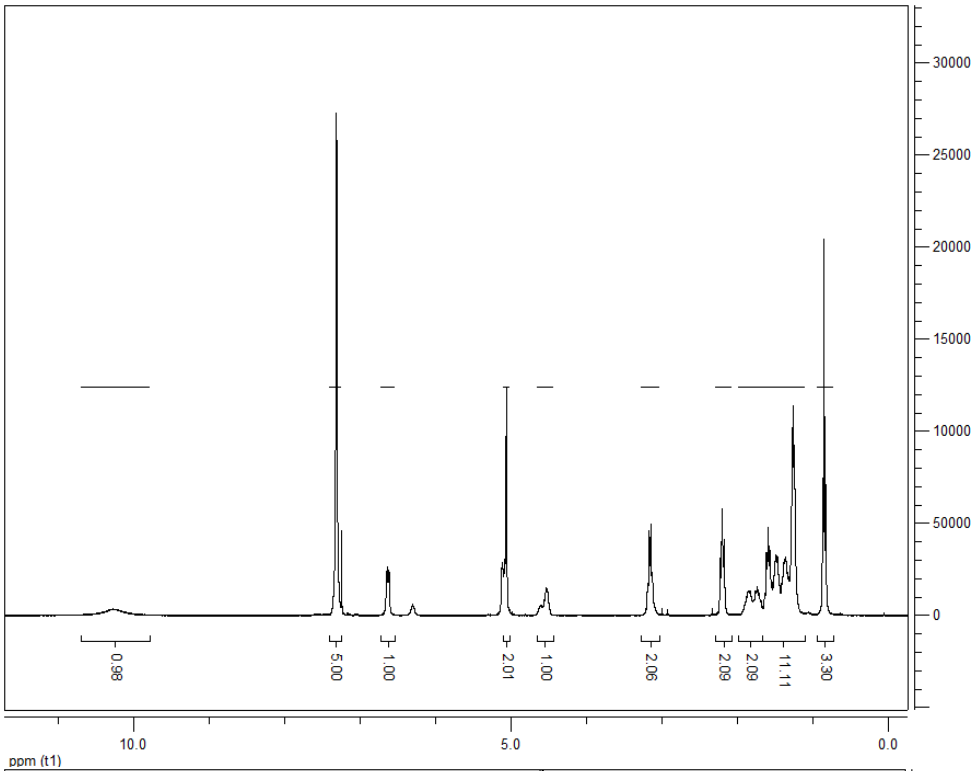

H13C6
N
H
N
H
C5H11
O
Na
O
O

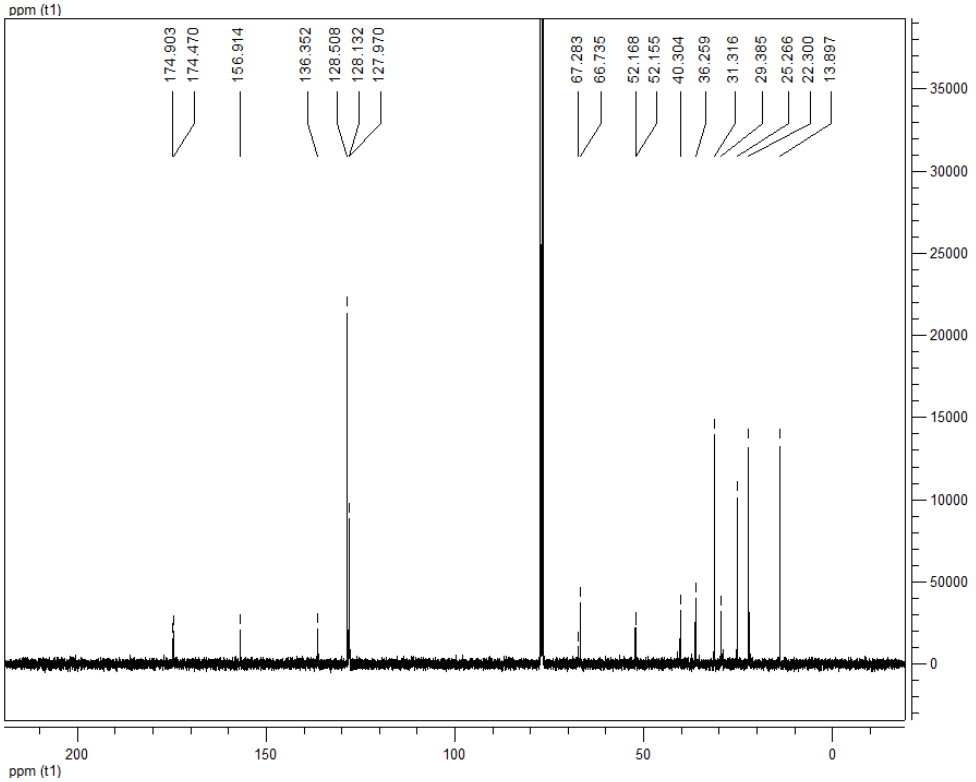
21

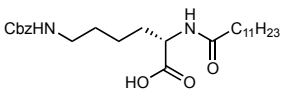

50000
40000
30000
20000
10000
0
1.00
3.36
2.04
1.02
7.02
12.79
6.34
10.0
5.0
0.0
ppm (t1)
179.214
176.588
54.658
48.000
47.717
35.648
31.230
30.663
30.423
27.022
26.611
25.760
25.018
22.614
21.761
21.609
13.201
13.171
80000
70000
60000
50000
40000
30000
20000
10000
0
150
100
50
0
ppm (t1)



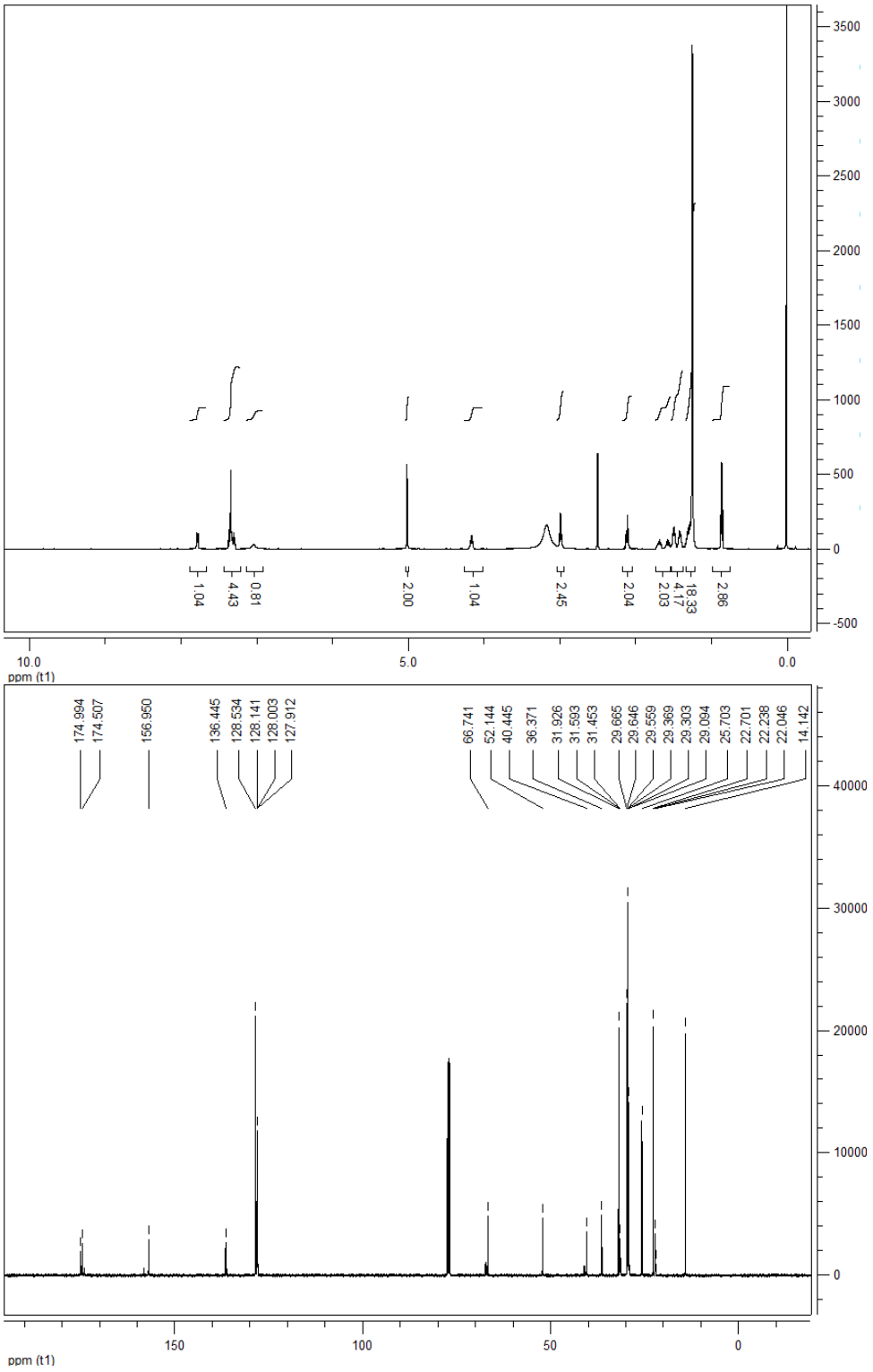
22

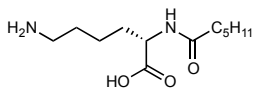




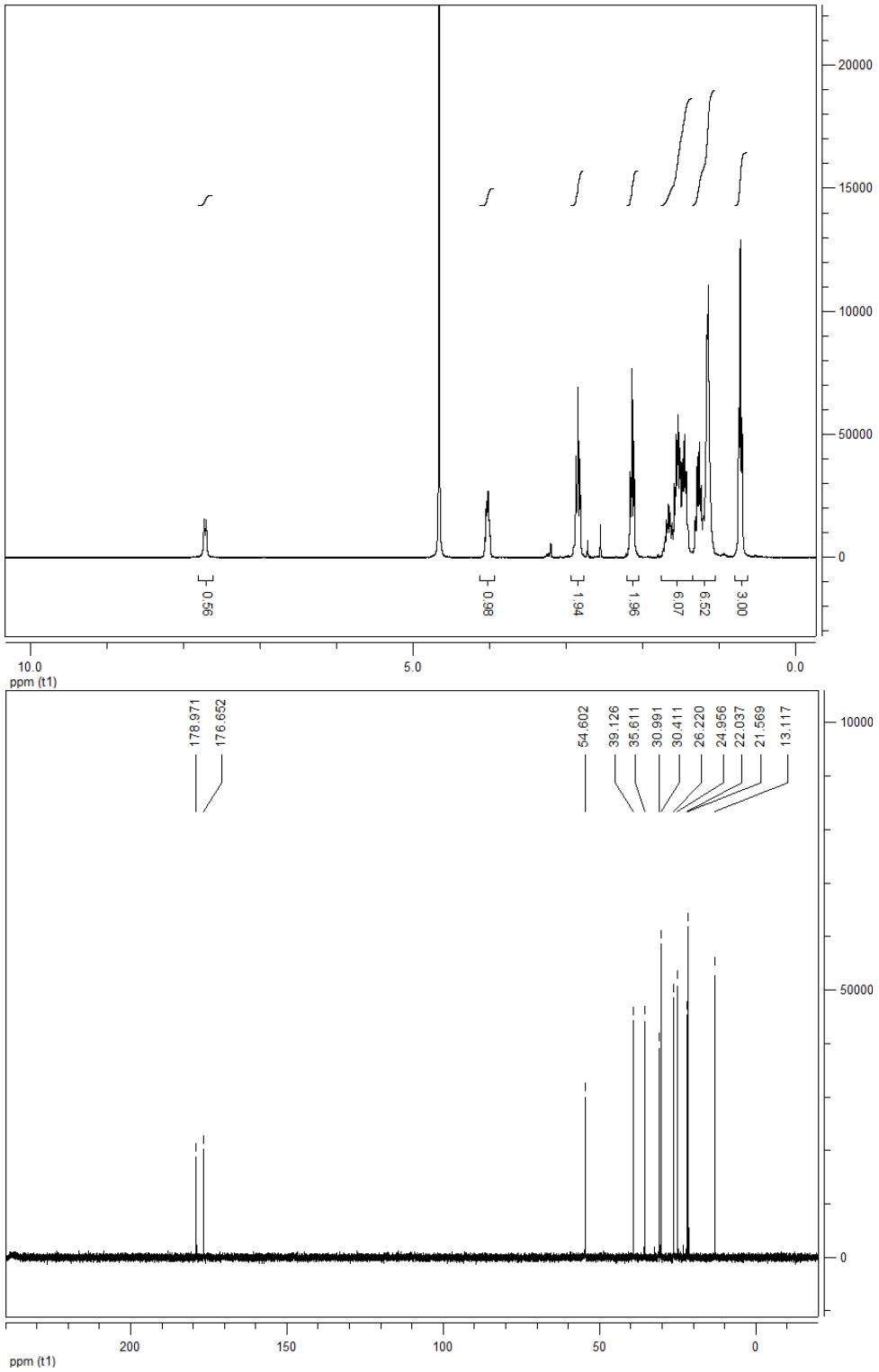


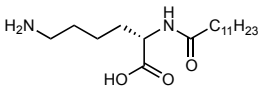
23



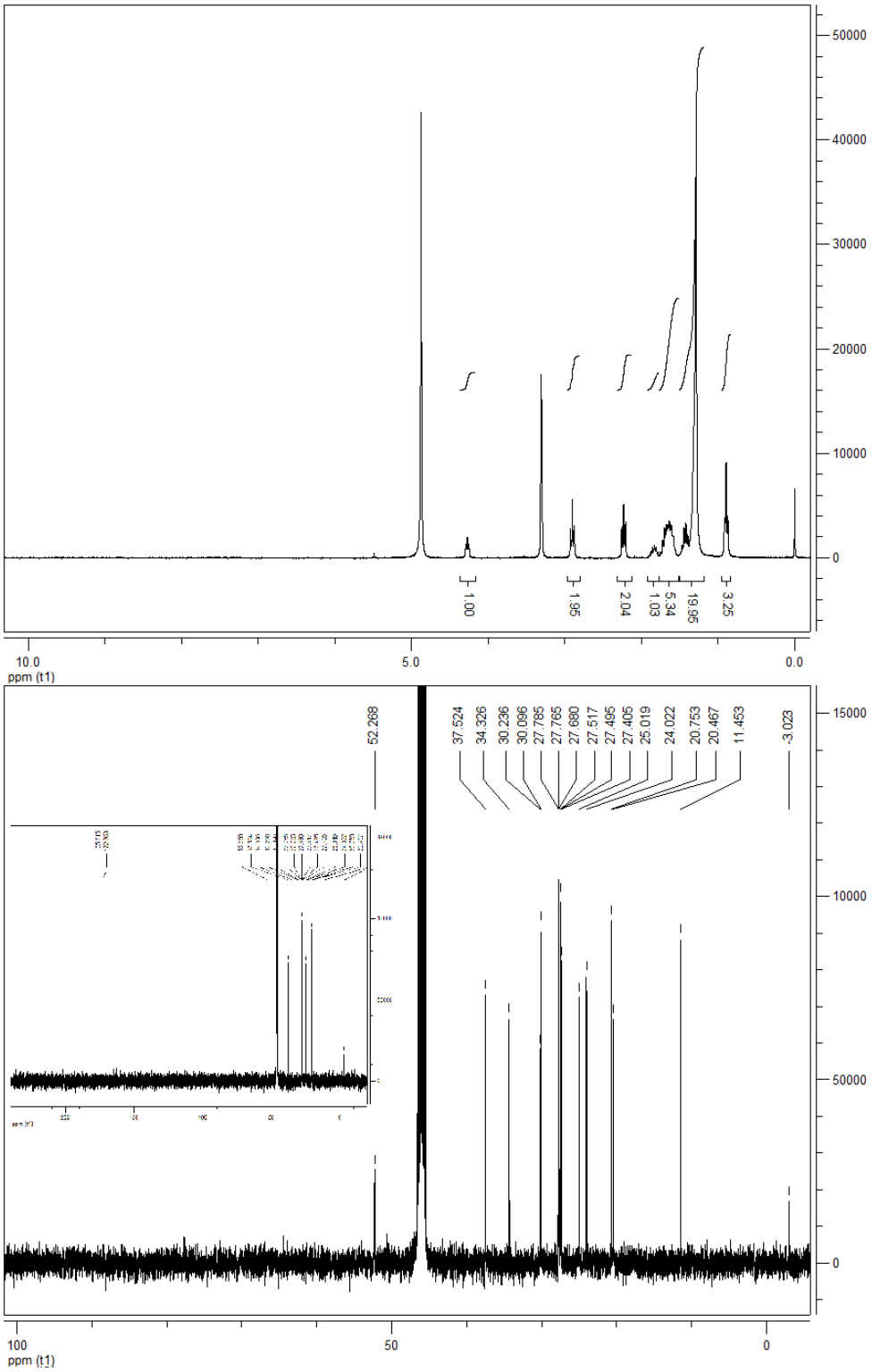


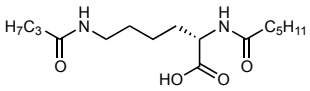
24



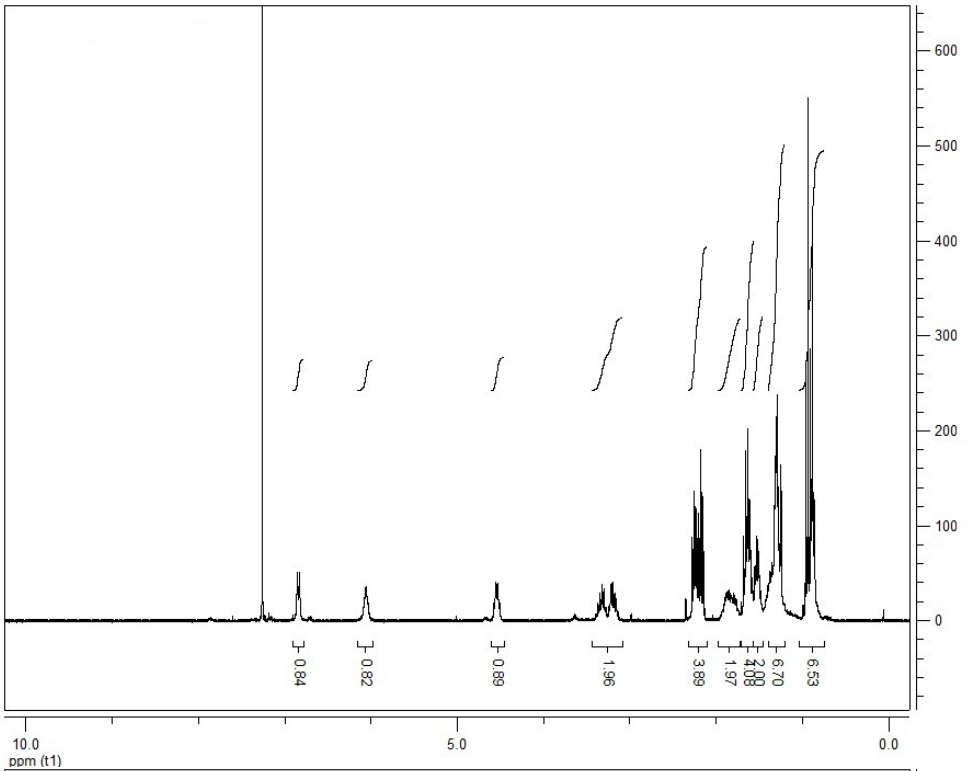

H2N
H
N
C11H23
O
HO
O

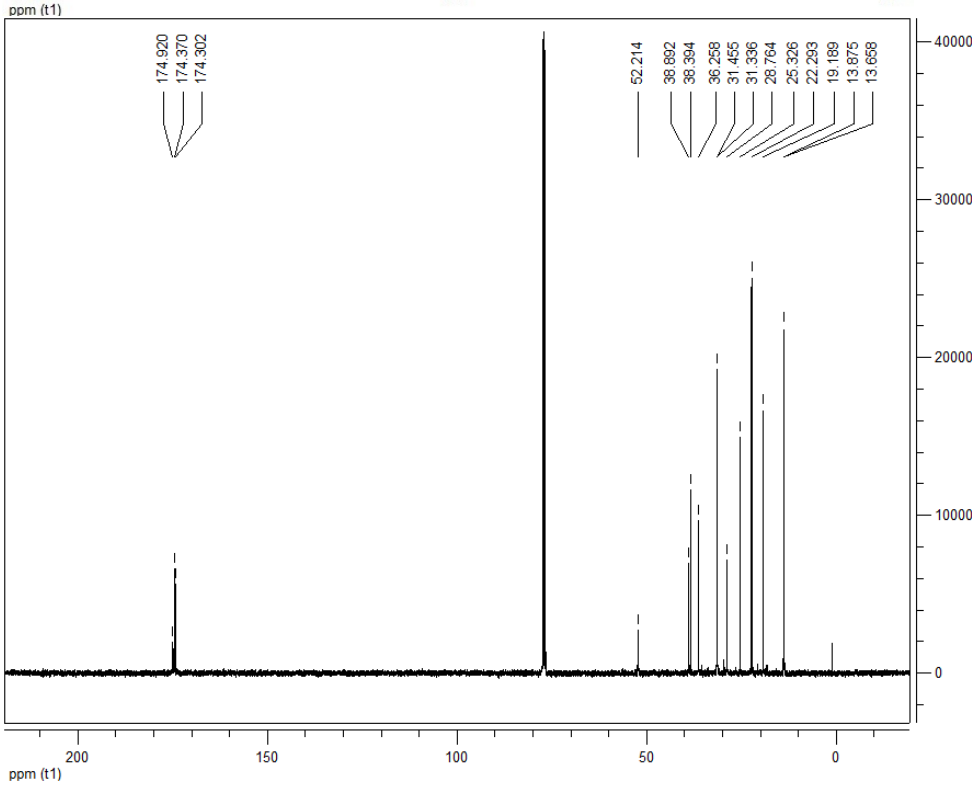
25

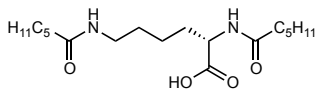

50000
40000
30000
20000
10000
0
1.00
1.95
2.04
1.03
5.34
19.95
3.25
10.0
ppm (t1)
5.0
0.0
52.268
37.524
34.326
30.236
30.096
27.785
27.765
27.680
27.517
27.495
27.405
25.019
24.022
20.753
20.467
11.453
-3.023
15000
10000
50000
0
100
ppm (t1)
50
0


H7C3
H
N
O
H
N
C5H11
O
HO
O

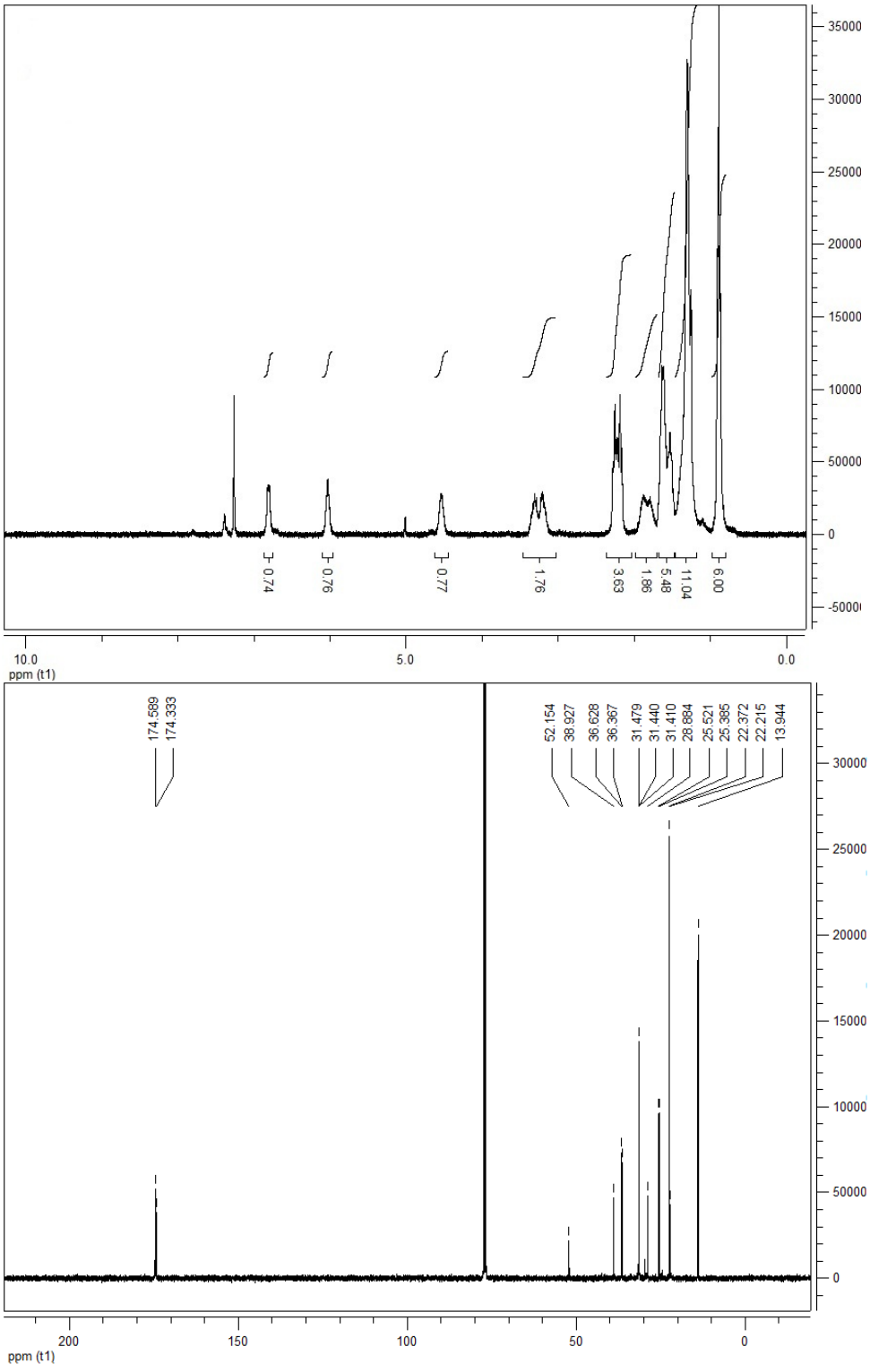
26

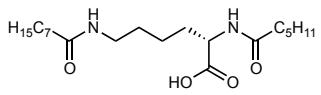

600
500
400
300
200
100
0
0.84
0.82
0.89
1.96
3.89
1.97
4.08
2.00
9.70
9.53
10.0
5.0
0.0
ppm (t1)


174.920
174.370
174.302
52.214
38.892
38.394
36.258
31.455
31.336
28.764
25.326
22.293
19.189
13.875
13.658
40000
30000
20000
10000
0
200
150
100
50
0
ppm (t1)

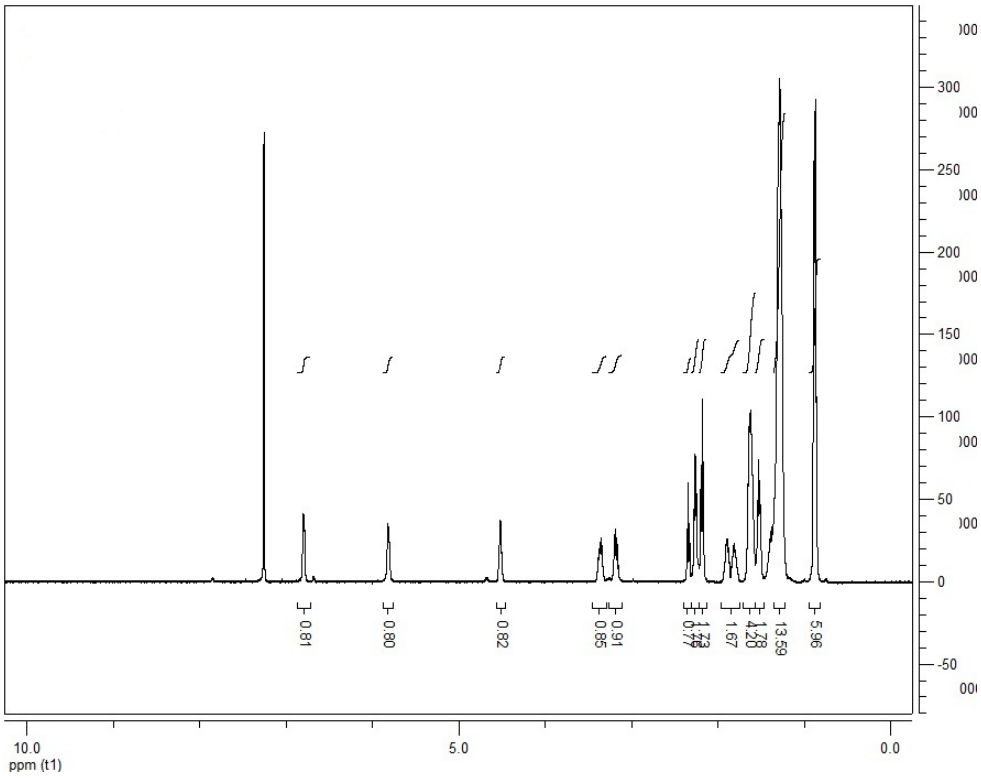


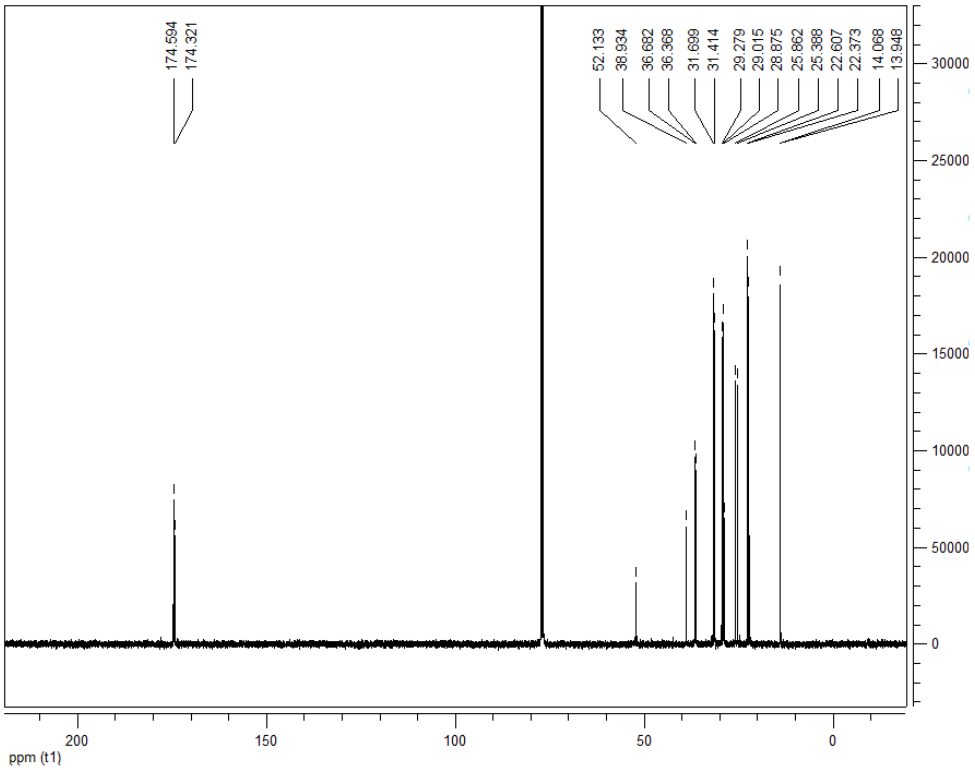
27

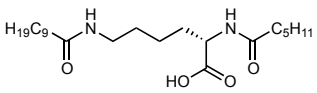

0.74
0.76
0.77
1.76
3.63
1.86
5.48
11.04
6.00
10.0
ppm (t1)
5.0
0.0
174.589
174.333
52.154
38.927
36.628
36.367
31.479
31.440
31.410
28.884
25.521
25.385
22.372
22.215
13.944
200
150
100
50
0
ppm (t1)


H15C7
H
N
O
H
N
C5H11
O
HO
O

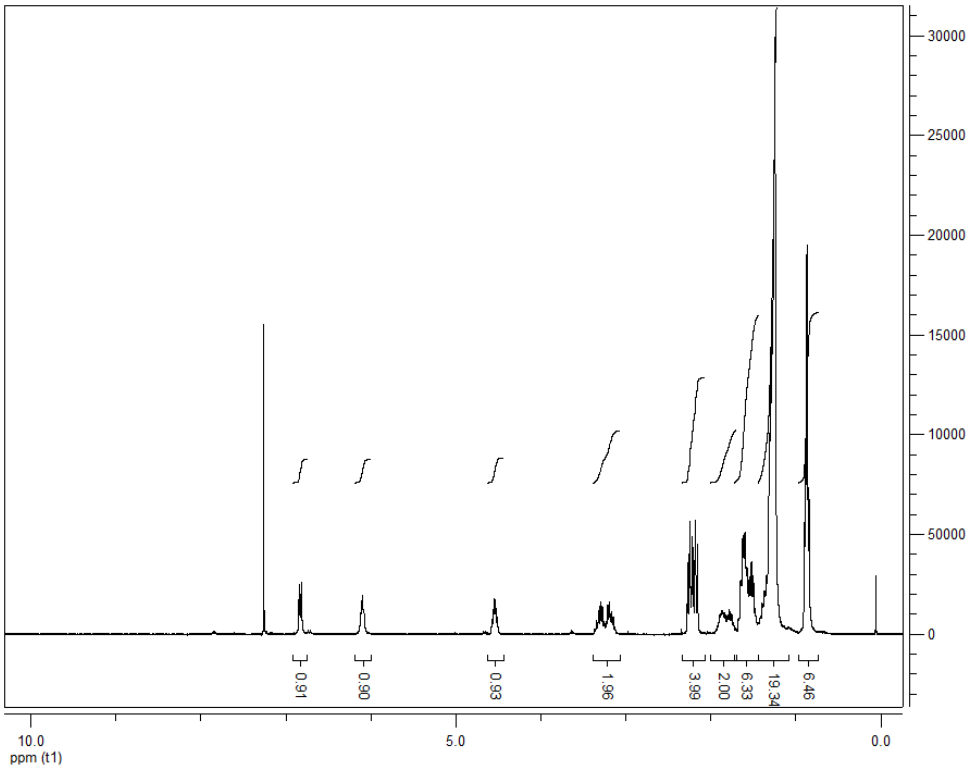
28

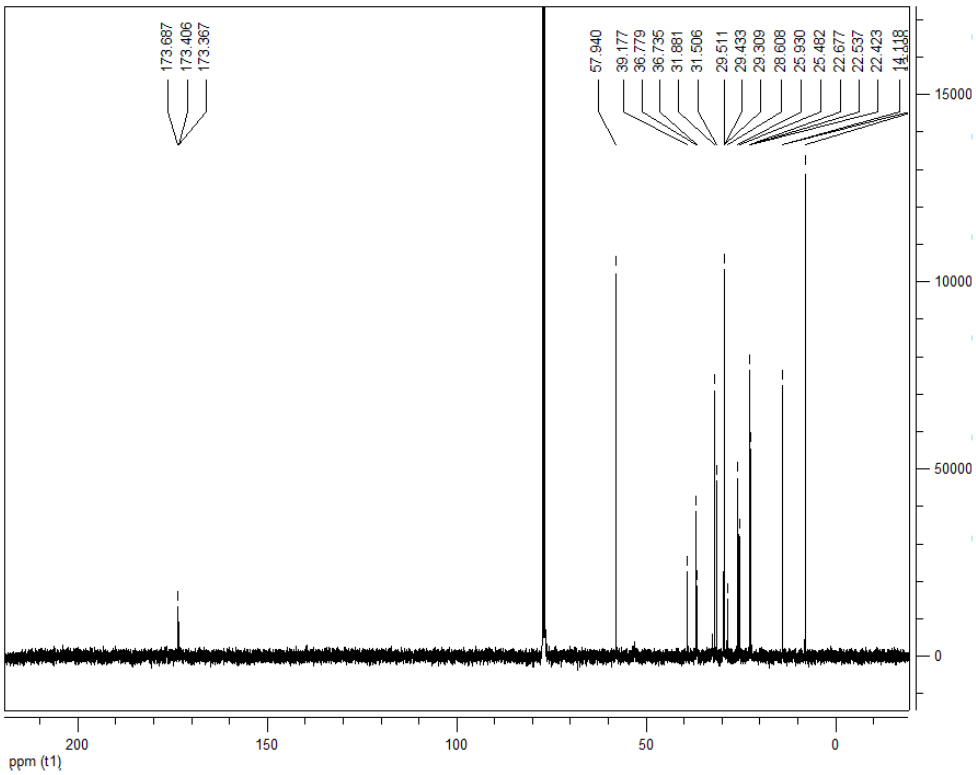

0.81
0.80
0.82
0.85
0.91
1.97
4.78
13.59
5.96
10.0
ppm (t1)
5.0
0.0

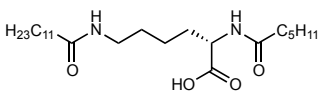

174.594
174.321
52.133
38.934
36.682
36.368
31.699
31.414
29.279
29.015
28.875
25.862
25.388
22.607
22.373
14.068
13.948
200
150
100
50
0
ppm (t1)


H19C9
H
N
O
H
N
C5H11
O
HO
O

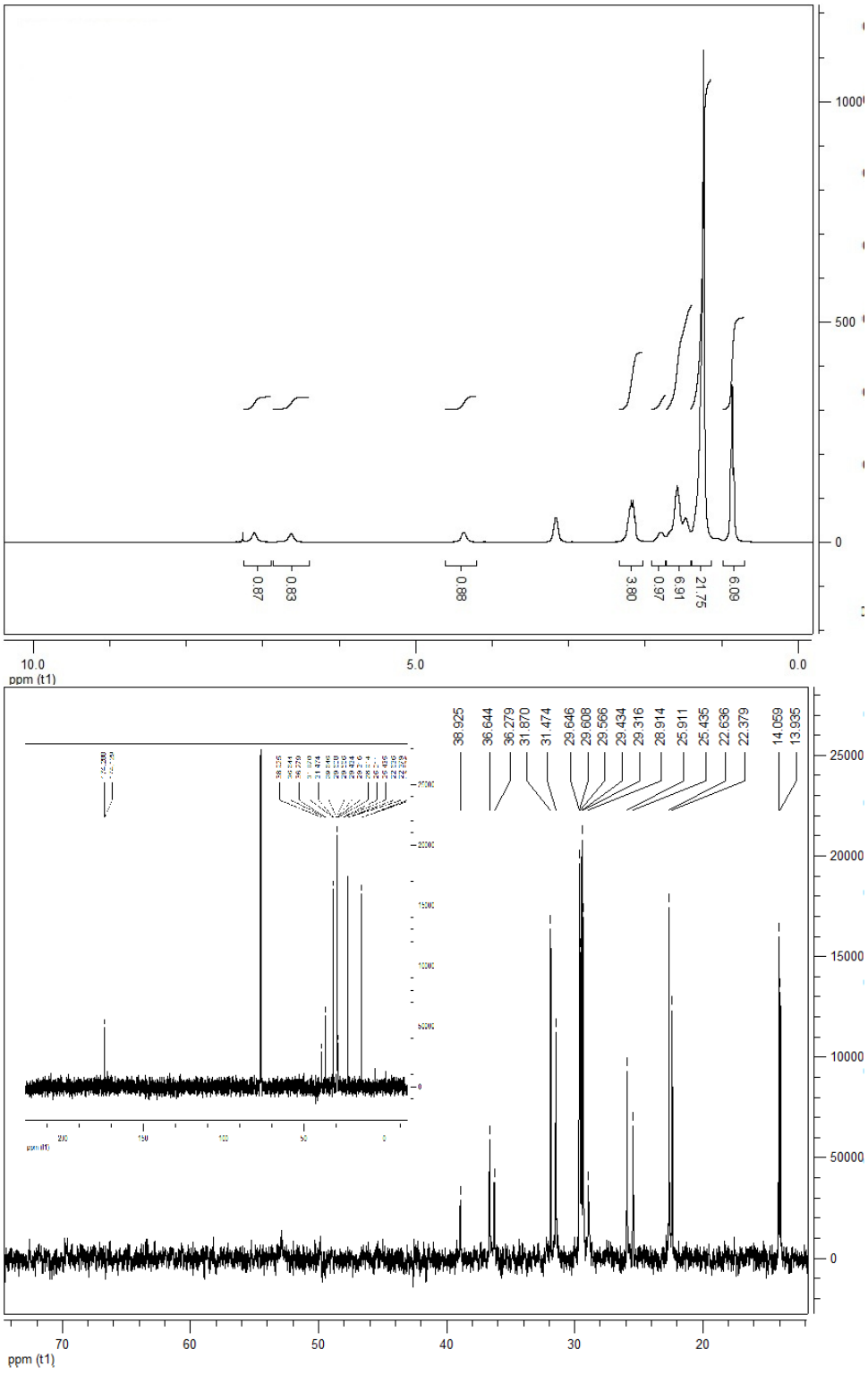
29

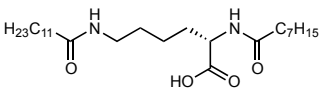

30000
25000
20000
15000
10000
50000
0
0.91
0.90
0.93
1.96
3.99
2.00
6.33
19.34
6.46
10.0
ppm (t1)
5.0
0.0


173.687
173.406
173.367
57.940
39.177
36.779
36.735
31.881
31.506
29.511
29.433
29.309
28.608
25.930
25.482
22.677
22.537
22.423
14.118
15000
10000
50000
0
200
150
100
50
0
ppm (t1)

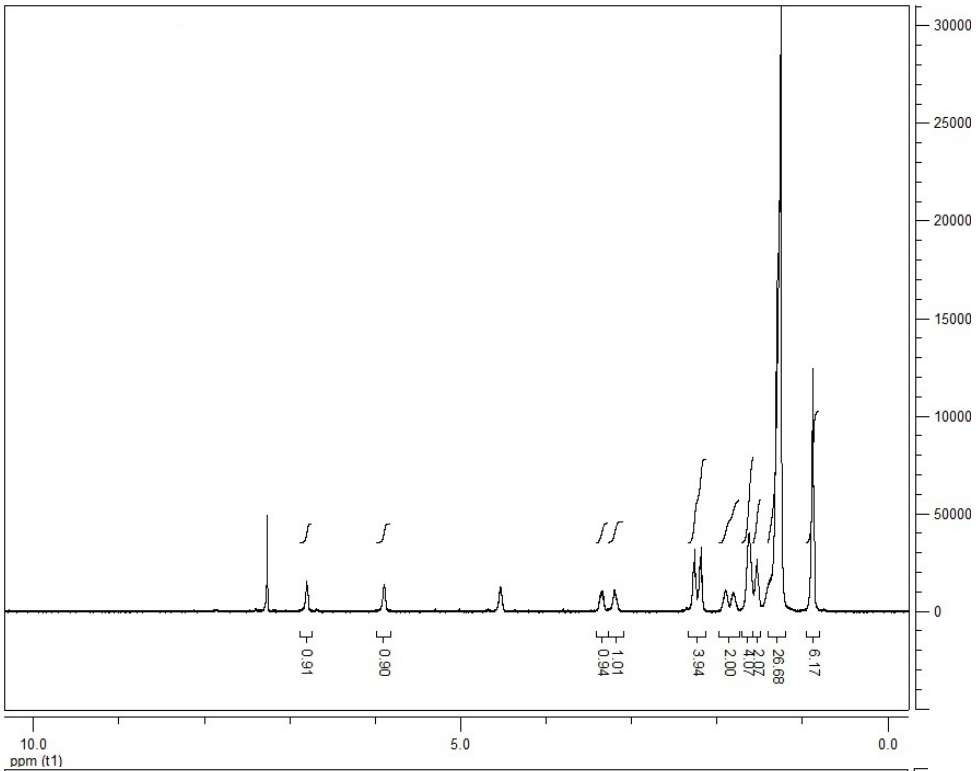

H23C11
H
N
O
H
N
C5H11
O
HO
O

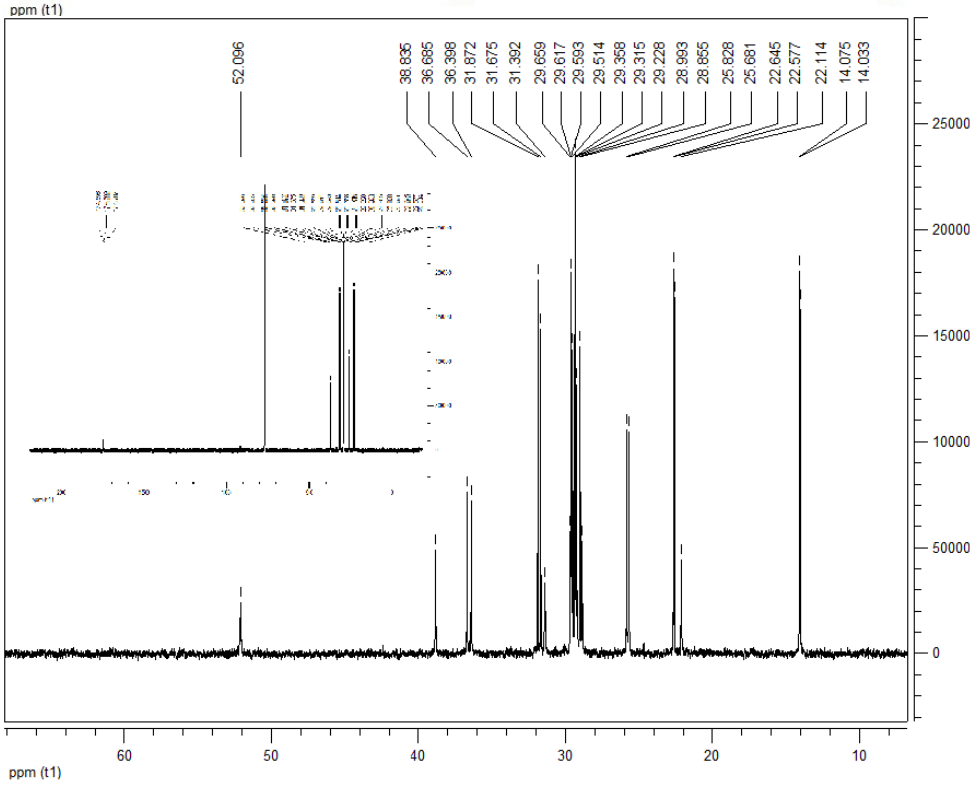
30

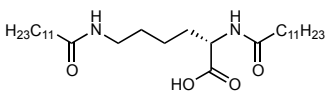

0.87
0.83
0.88
3.80
0.97
6.91
21.75
6.09
10.0
5.0
0.0
ppm (t1)
38.925
36.644
36.279
31.870
31.474
29.646
29.608
29.566
29.434
29.316
28.914
25.911
25.435
22.636
22.379
14.059
13.935
70
60
50
40
30
20
ppm (t1)


H23C11
H
N
O
H
N
C7H15
O
HO
O

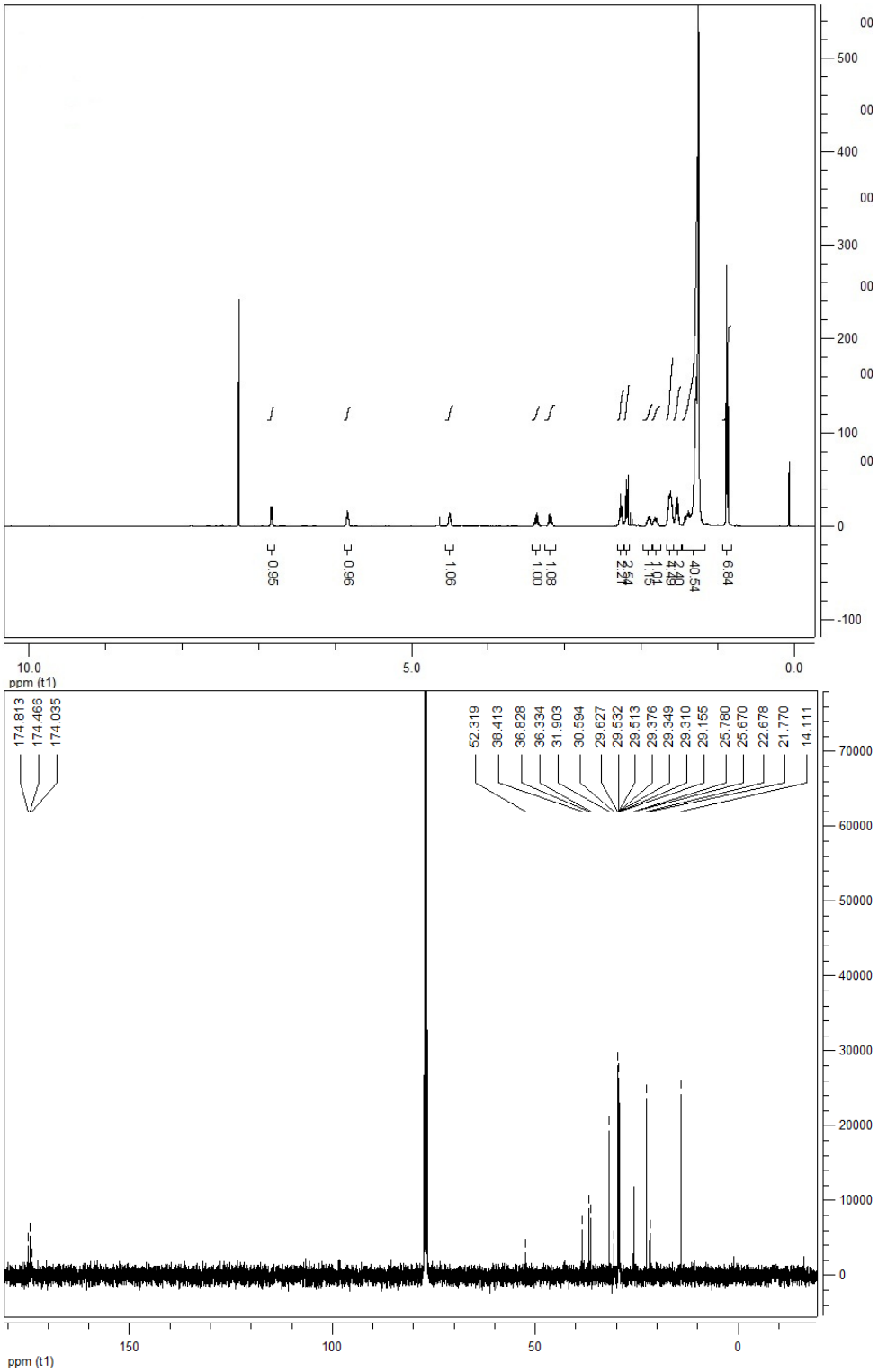
31

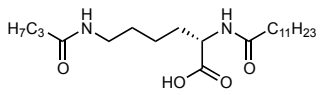

30000
25000
20000
15000
10000
50000
0
0.91
0.90
0.94
1.01
3.94
2.00
4.07
26.98
6.17
10.0
5.0
0.0
ppm (t1)


52.096
38.836
36.685
36.398
31.872
31.675
31.392
29.659
29.617
29.593
29.514
29.358
29.315
29.228
28.993
28.855
25.828
25.681
22.645
22.577
22.114
14.075
14.033
25000
20000
15000
10000
50000
0
60
50
40
30
20
10
ppm (t1)

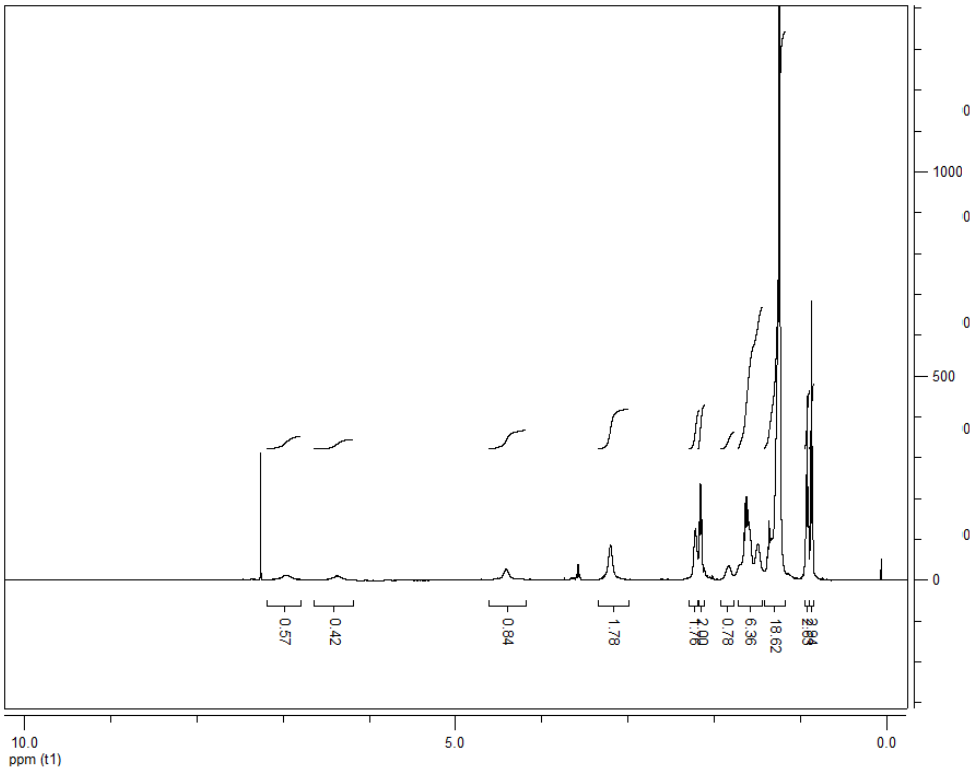

H23C11
H
N
O
H
N
C11H23
O
HO
O

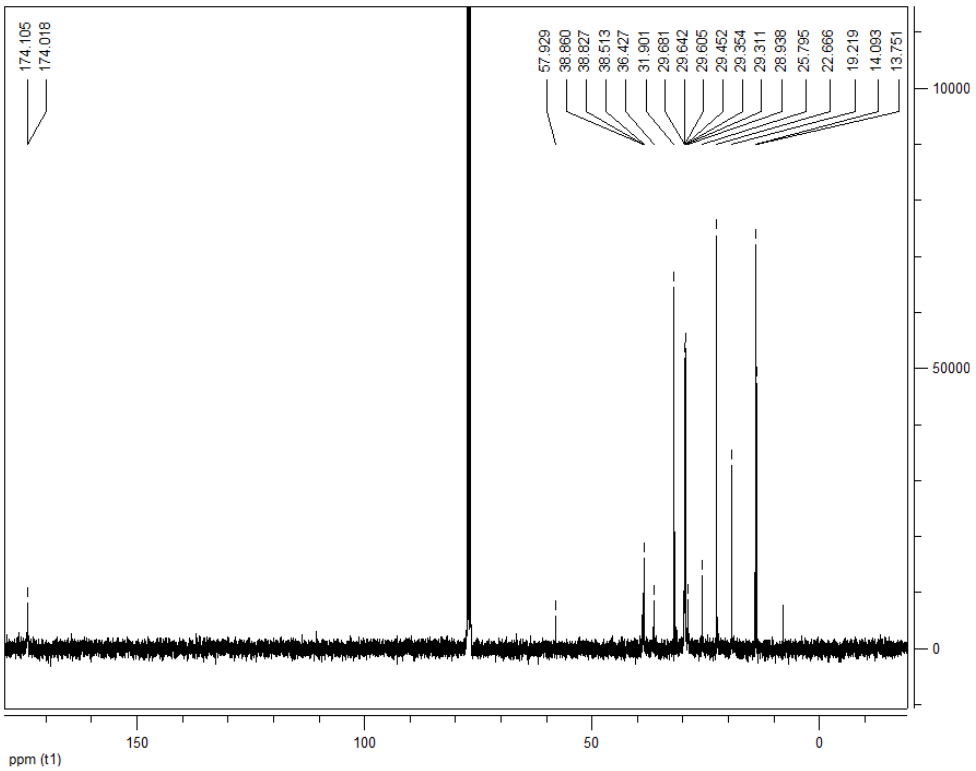
32

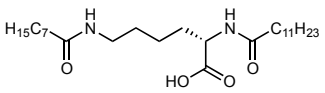

0.96
0.96
1.06
1.08
1.00
2.24
1.15
2.48
2.07
6.84
10.0
5.0
0.0
ppm (t1)
174.813
174.466
174.035
52.319
38.413
36.828
36.334
31.903
30.594
29.627
29.532
29.513
29.376
29.349
29.310
29.155
25.780
25.670
22.678
21.770
14.111
150
100
50
0
ppm (t1)


H7C3
H
N
O
H
N
C11H23
O
HO
O

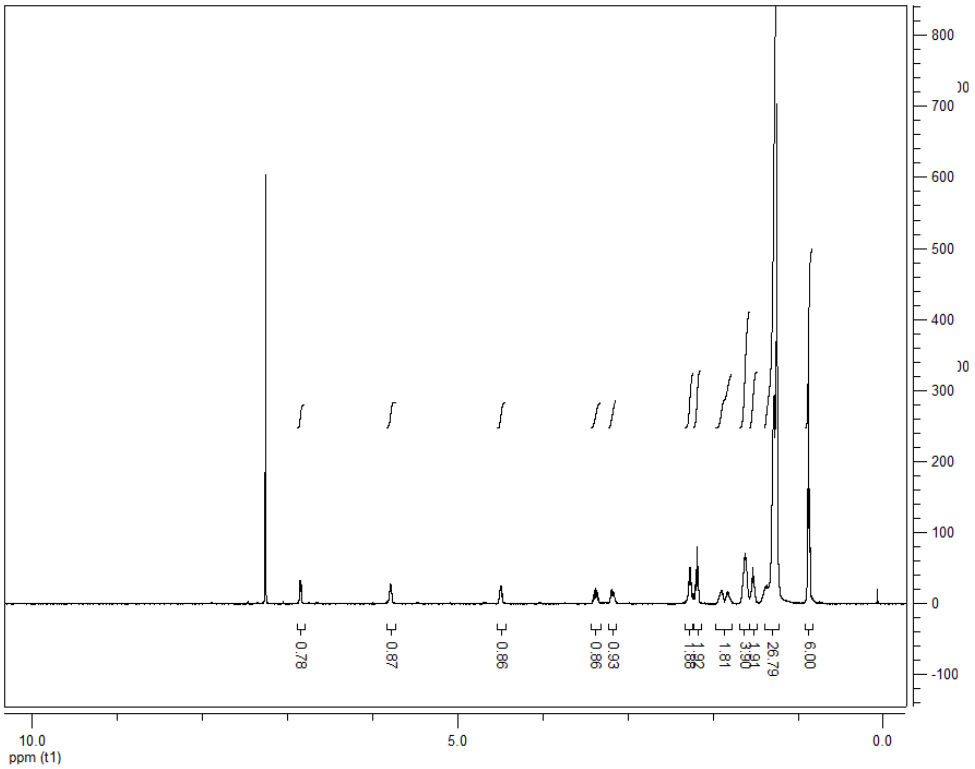
33

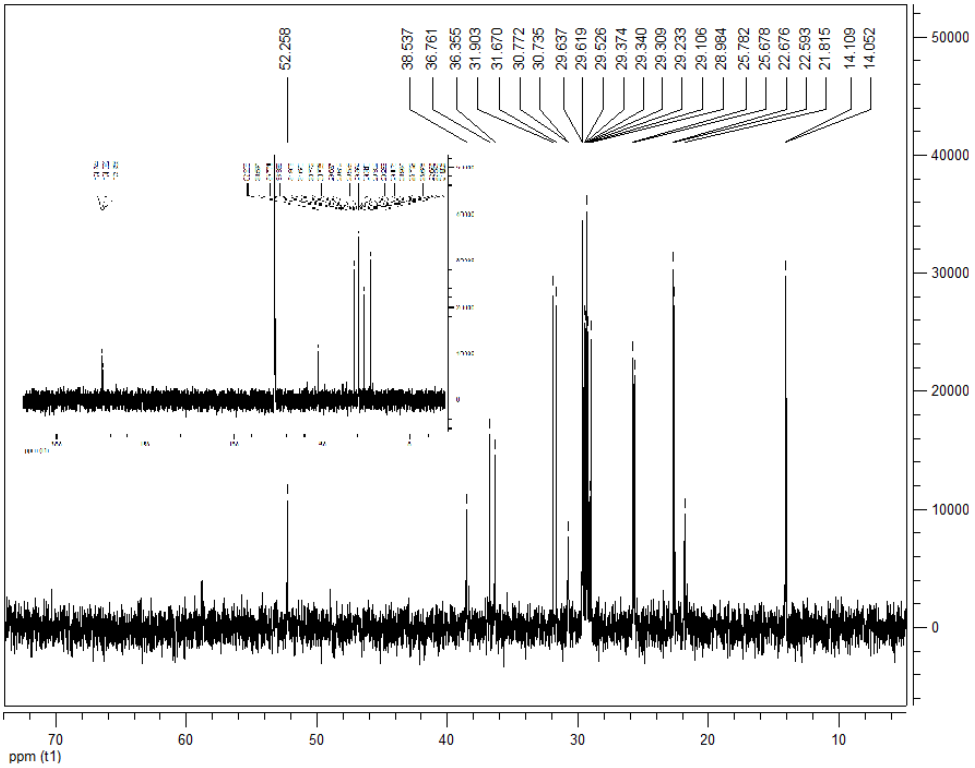

0.57
0.42
0.84
1.78
2.00
0.78
9.36
18.62
10.0
ppm (t1)
5.0
0.0

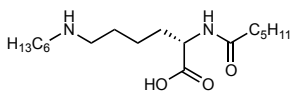

174.105
174.018
57.929
38.860
38.827
38.513
36.427
31.901
29.681
29.642
29.605
29.452
29.354
29.311
28.938
25.795
22.666
19.219
14.093
13.751
150
ppm (t1)
100
50
0



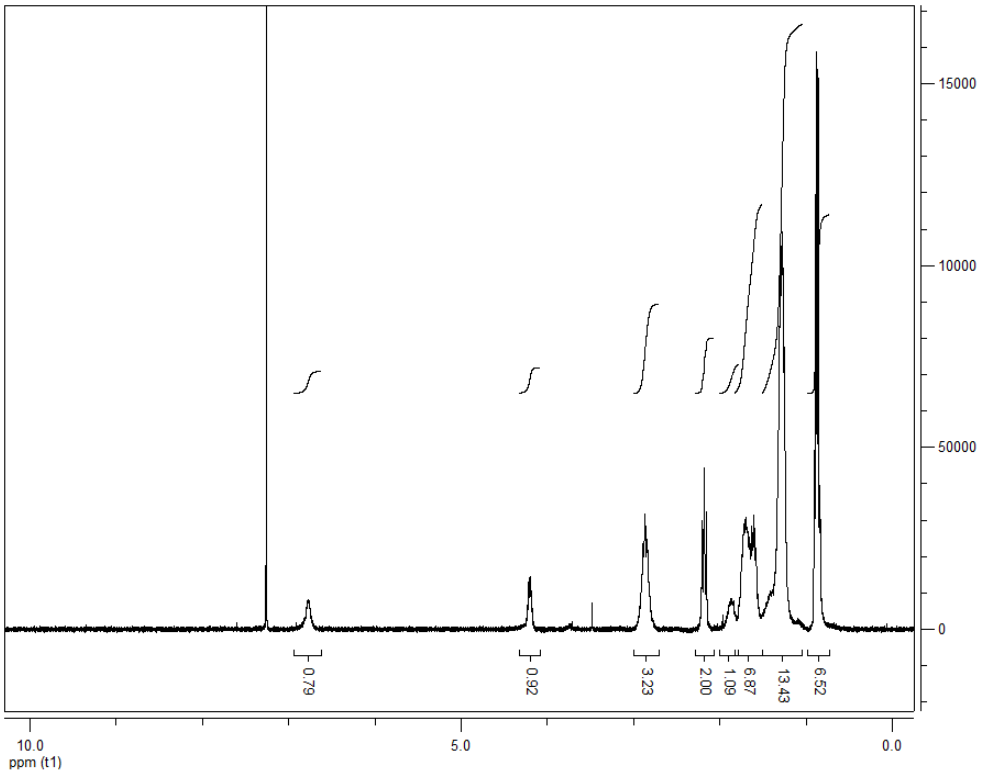
34

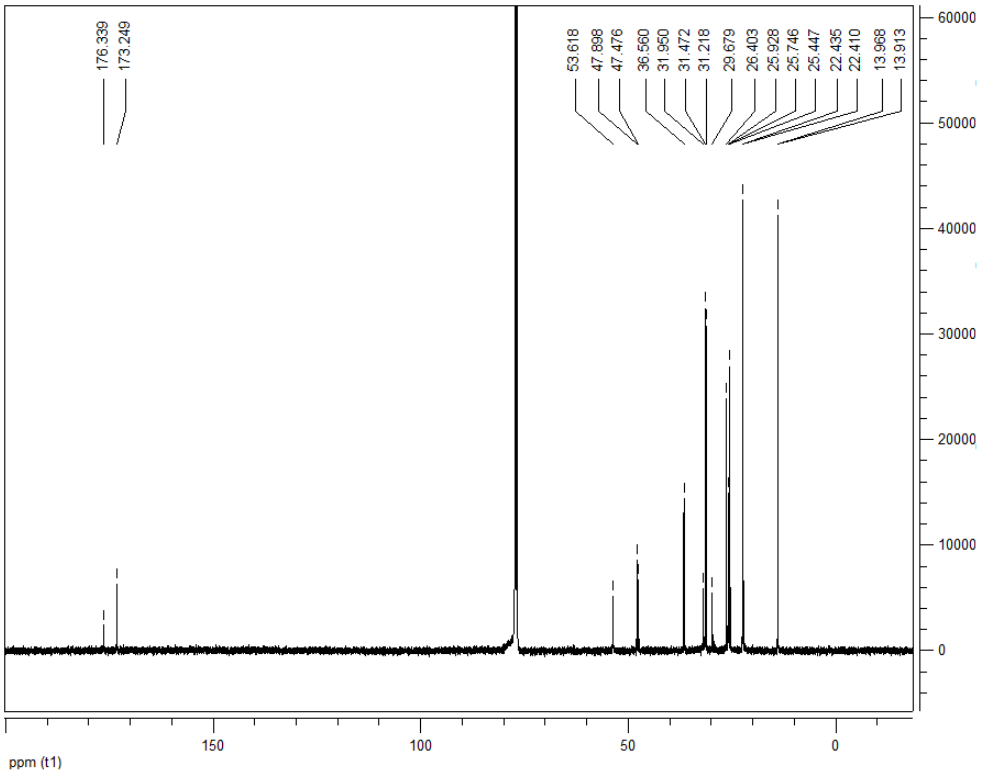






35





vi) References

1. C. A. Knight, J. Hallett and A. L. DeVries, *Cryobiology* 1988, **25**, 55.
2. J. Jackman, M. Noestheden, D. Moffat, J. P. Pezacki, S. Findlay and R. N. Ben, *Biochem. Biophys. Res. Commun.* 2007, **354**, 340.
3. A. Chakrabartty, C. L. Hew, *Eur. J. Biochem.* 1991, **202**, 1057.
4. M. Suzuki, T. Abe and K. Hanabusa, *Colloid Interface Sci.* 2010, **341**, 69.