Bioinspired nucleolipid as a low molecular weight oleogelator for oil-in-water nanoemulsions

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Preparation of the Oleogels and Characterization

Complementary data concerning gel Formation

Critical gelation concentration determination assays for NL 4 and NL 5

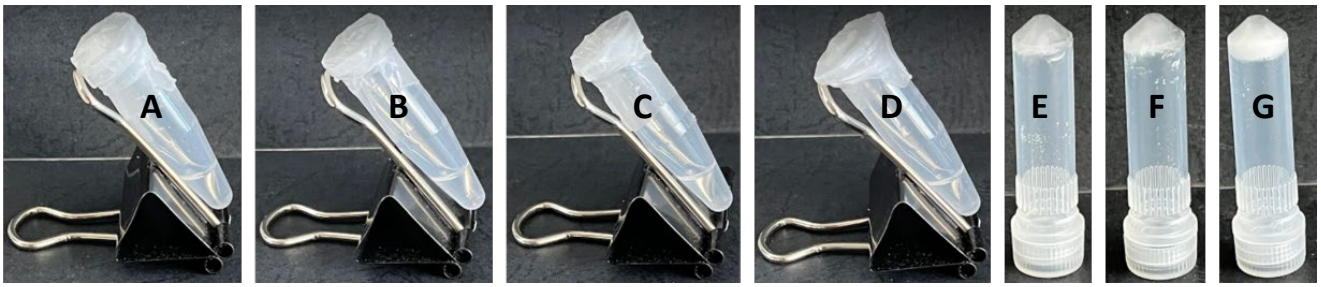


Figure S1: NL **4**-based gelation assays in MCT oil (Miglyol® 812N) : A: 6% w/w, B: 8% w/w, C: 10 % w/w, D: 12%w/w, E: 14% w/w, F: 15% w/w, G: 16% w/w.

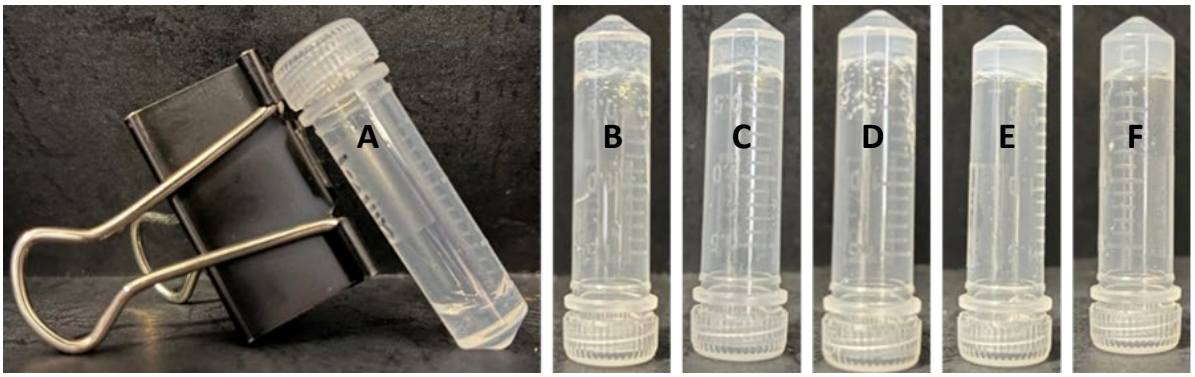


Figure S2: NL **5**-based gelation assays in MCT oil (Miglyol® 812N) : A: 1% w/w, B: 1.5% w/w, C: 2 % w/w, D: 3%w/w, E: 4% w/w, F: 5% w/w.

Rheological study

Rheological measurements were performed on a Kinexus® Pro+ rheometer (Malvern Instruments Ltd., Orsay, France) with steel plate-plate geometry (diameter: 20 mm). The lower plate has a Peltier temperature control system and all gel samples were studied at ambient temperature (25 ± 0.01°C) unless specified. The gel was placed on the bottom geometry using a plastic spatula and the oleogels rested for a 30 min equilibration before the experiments started. All measurements were carried out within the linear viscoelastic regime (LVR) involving no disruption of the gel structure. LVR was determined through an amplitude strain sweep experiment (shear strain: 0.001 – 100 %, frequency: 12.57 rad.s^{-1} (2 Hz). Elastic G' and viscous G" moduli were evaluated by performing a frequency sweep experiment from 0.1257 to 12.57 rad.s^{-1} at a shear strain of 0.1 % (independence of moduli with the applied strain). At classical angular frequency of 1 Hz, the harmonic distortion values indicate a poor quality of the signal and therefore of the measurement (HD > 2%), which makes it unusable. Previous results of the literature reported higher angular frequency (10 rad.s^{-1}) for suitable characterization of organogel.[1] Thixotropic behavior was measured by a step-strain measurement composed of three steps repeated three times: 1) shear strain of 0.1 % for 30 minutes (within the LVR), 2) shear strain of 10 % for 2 minutes (outside of the LVR), 3) shear strain of 0.1 % until stabilization (within the LVR).

The determination of gel-sol transition temperature was determined by rheology. The oleogel (5 % w/w NL **5**) was gradually heated from 25 to 85 °C at 0.1 % shear strain (heating rate 5 °C/min).The sol-gel transition temperature was recorded at 79.5 °C as soon as the gel became liquid.

At least three replicates were measured for each sample.

At 2% w/w, the gel displayed a modification of its rheological properties with 75,86 °C as a Tgel/sol, G'=29.4 kPa and G''=5.83 kPa revealing that the mechanical properties depend on the concentration of oleogelator. As the oleogel at 5% w/w displayed both higher strength and faster gelation time, it was selected for nanoemulsion formulation (1% w/w final concentration in the nanoemulsion).

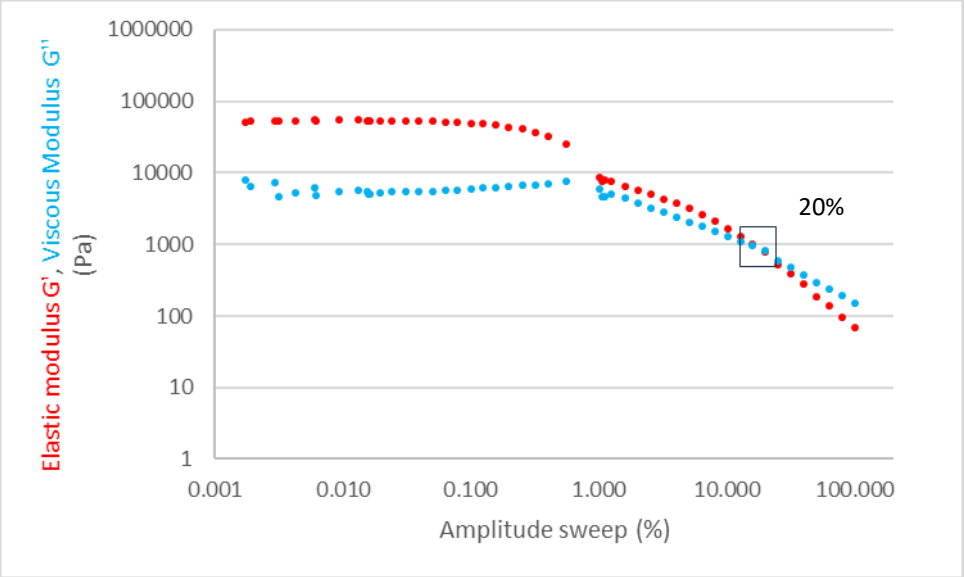


Figure S3: Amplitude sweep experiment of NL **5** based oleogel 5 % w/w in Miglyol® 812N

Additional rheological data concerning NL 5 gelation at 2% w/w.

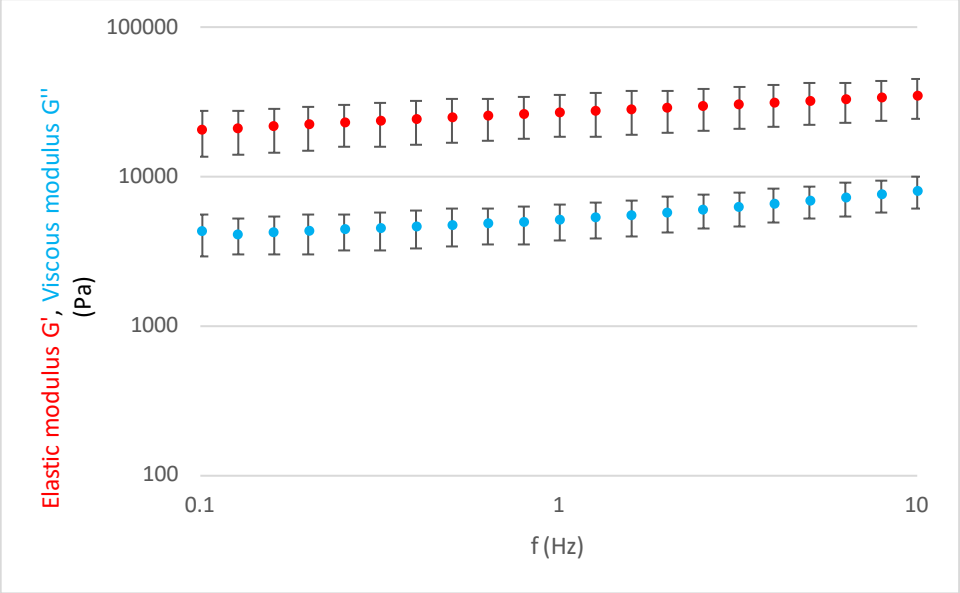


Figure S4: Frequency sweep experiment of NL **5** based oleogel 2 % w/w in Miglyol® 812N

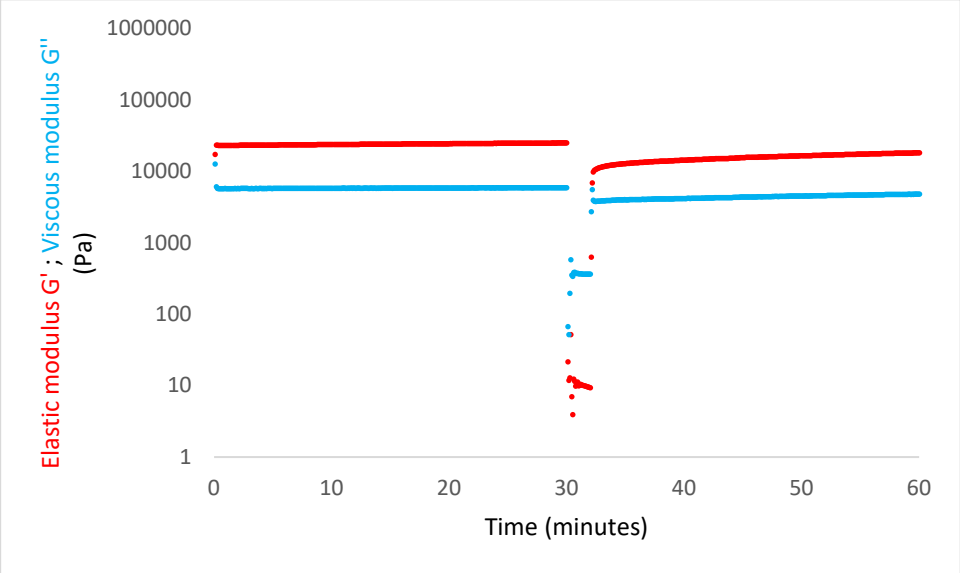


Figure S5: Step-strain experiment of NL **5** based oleogel 2 % w/w in Miglyol® 812N at a fixed shear strain of 0.1%

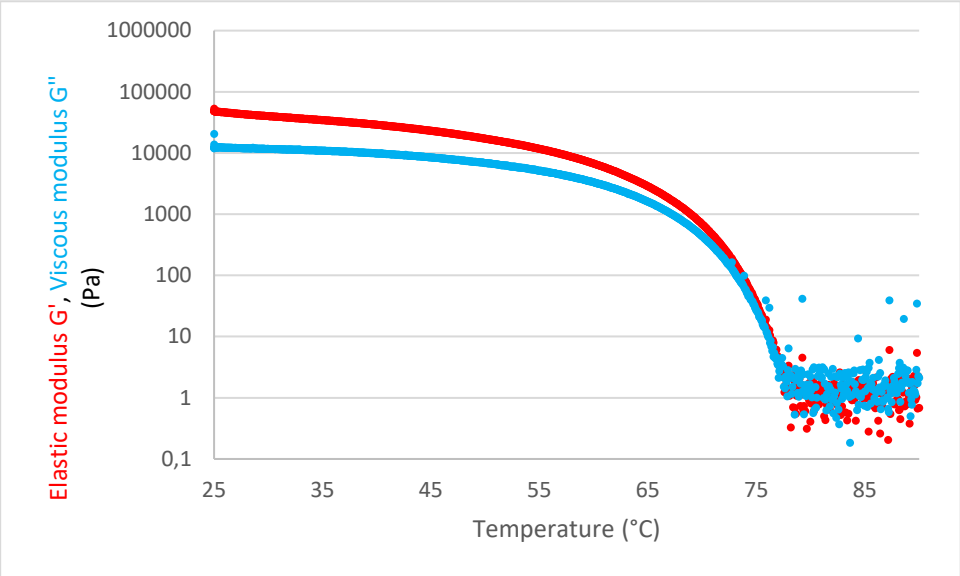


Figure S6: Sol-Gel transition of NL **5** based oleogel 2 % w/w in Miglyol® 812N

Supplementary data for gelled Nanoemulsion

Table 1: Composition of Nanoemulsion

	Composition	Quantity (% w/w)
Oil phase	Miglyol® 812N	20
	Lipoid E80®	1,2
	NL **5**	1
Aqueous phase	Tween® 80	2,5
	Milli-Q water	Qsp 100

Figure S7: Macroscopical aspect of gelled NE-NL **5** 24 hours after formation

Table 2: Comparative physico-chemical characteristics of NEs

	Non-gelled NE (without NL **5** oleogelator)	NE-NL **5** based *
Mean droplet size (nm)	164.0 ± 3.8	141.5 ± 3.7
PdI	0.106 ± 0.007	0.096 ±0.021
Zeta Potential (mV)	−27.4 ± −1.7	−28.0 ± −2.1

**measured before aqueous phase gelation*

Nanoemulsion rheologic characterization

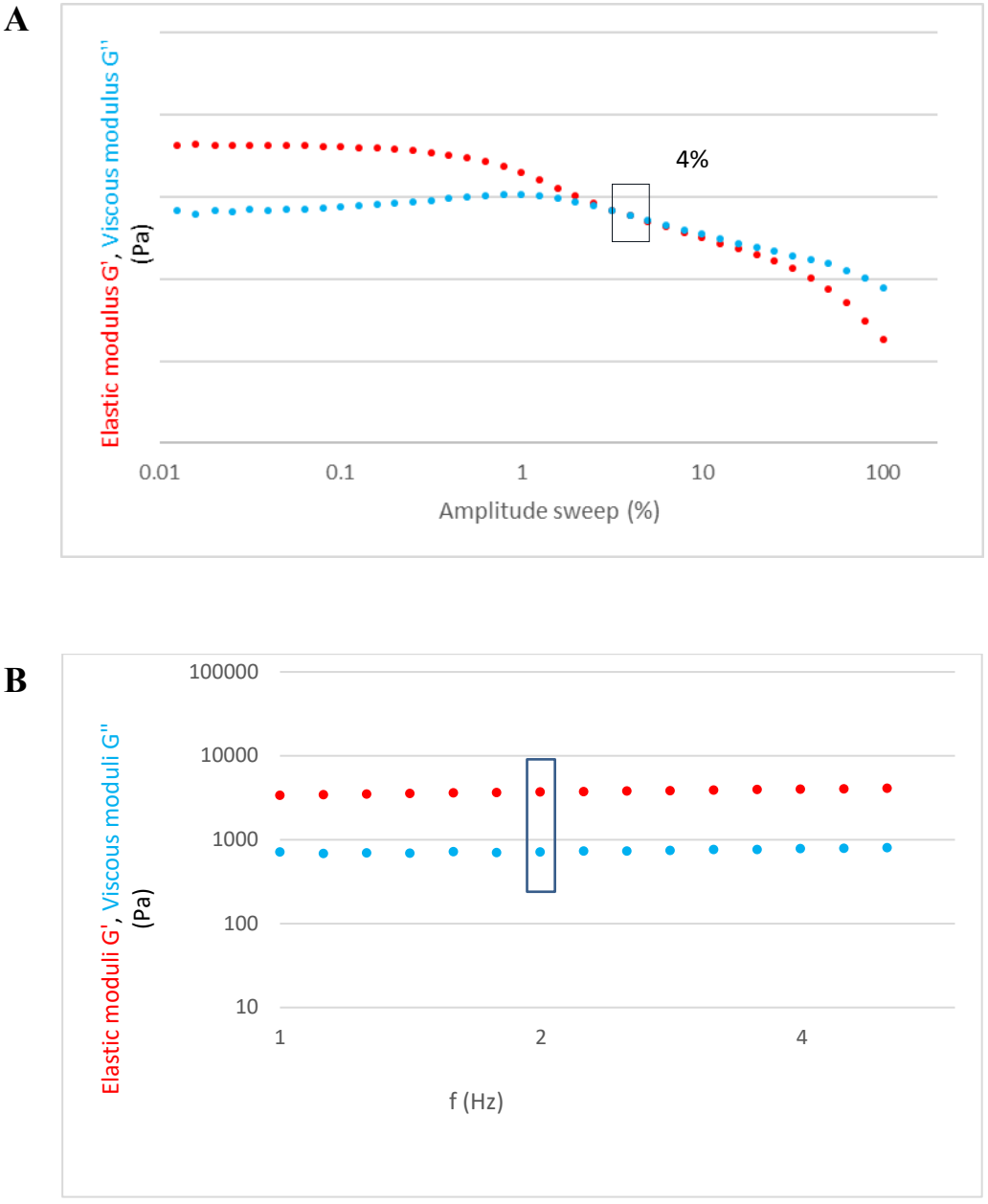


Figure S8: A-Amplitude sweep experiment of gelled NE-NL 5 based
B-Frequency sweep experiment of gelled NE-NL **5** based

DRX study

X-ray diffraction data for NL **3** and NL **4** based oleogels were collected at the IECB X-ray facility (CNRS UMS 3033 – INSERM US001, University of Bordeaux) with a Rigaku FRX rotating anode (2.9 kW) diffractometer using CuKα wavelength with a partial chi goniometer (AFC11). The X-ray source is equipped with high flux Osmic Varimax mirrors and a Pixel-Hybride HyPix6000 detector. Data were processed with the Rigaku Oxford Diffraction CrysalisPro software (version1.171.40.69a)[2]. The crystal structures were solved with Shelxt[3] and refined by full-matrix least-squares method on F^2 with Shelxl-2014 within Olex2[4]. Non-H atoms were refined with anisotropic displacement parameters. H-atoms were refined in the riding-model approximation, with Uiso(H)=1.2Ueq (CH, CH_2, NH). RIGU restraints were apply to model geometry of the molecules and thermal motion parameters. The cif files have been deposited to the CCDC with numbers **2386437** and **2386438** and contain all data statistics.

The wide-angle diffraction pattern from NL **5** based oleogel was measured at 290K on the same rotating anode. The oleogel sample was mounted in MicroLoop from MiteGen. The diffraction pattern corresponds to a 360° rotation along the phi axis (perpendicular to the direct beam with omega and chi axes at the 0 position) with an exposure time of 720 sec. Data were integrated with CrysalisPro with median filter and a baseline correction. Indexation was performed using Crysfire 2020 (version 1.0.8) and Dicvol and gives a monoclinic crystal lattice with a= 36.81 Å, b= 3.81 Å, c= 25.03 Å and ß= 99.55 °(Figure S10) . The X-ray diffraction patterns of the NL 5-based oleogel are in agreement with data described for similar molecular structures[5].

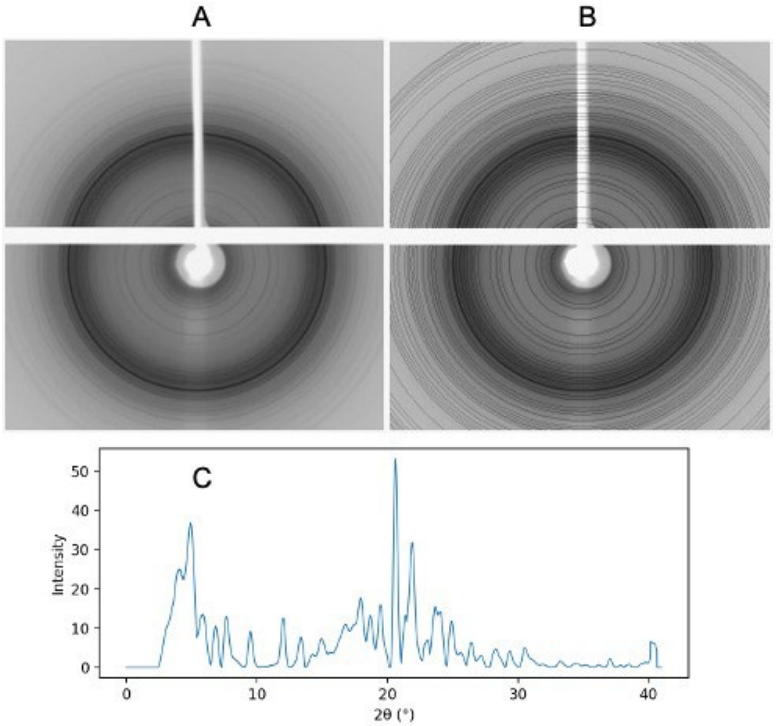


Figure S9: Diffraction pattern from NL **5** based oleogel (A). Superimposition of the indexation rings from the monoclinic cell obtained to the diffraction pattern showing the good fit of the data (B). 2D plot of the diffraction data (C).

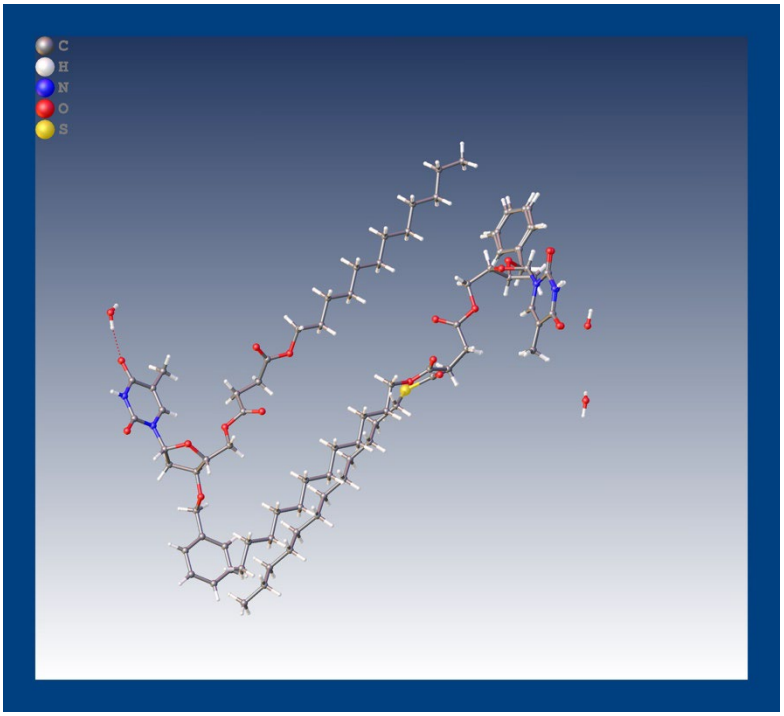


Figure S10: Superimposition of two molecule of compounds **3** and **4** showing the different positions of the fatty acid chains.

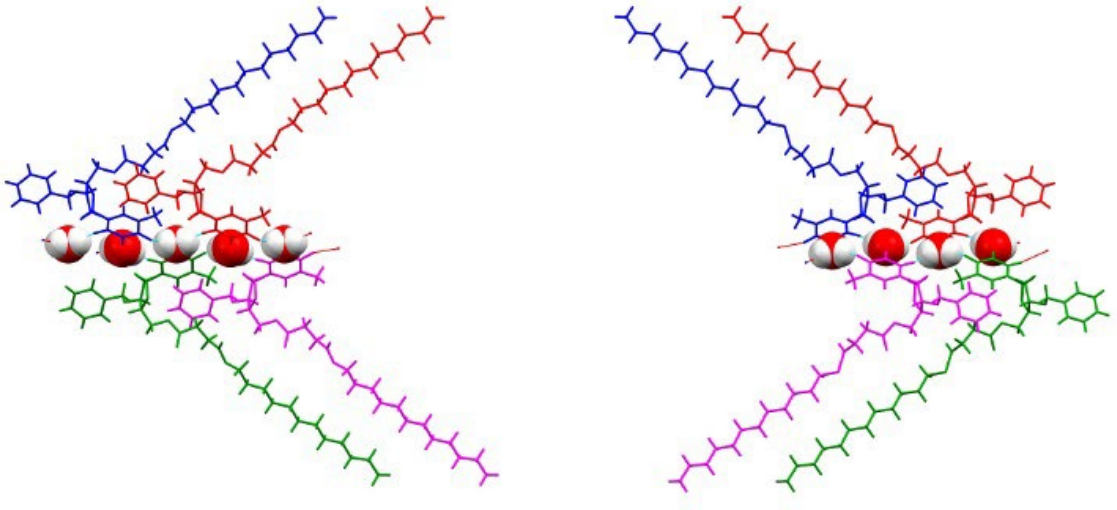


Figure S11: View of the crystal packings along the a axis of compound **4** (left) and **3** (right) showing the same positions of the water molecules involved in hydrogen bonding with the nucleobase moieties (in CPK).

Solid NMR study

Experiments were performed at a MAS frequency of 11 kHz on a triple resonance 4 mm MAS probe using 600 MHz ^{1}H Larmor frequency spectrometers (Bruker Biospin). Radiofrequency (RF) field strengths were ~90-100 kHz for ^{1}H and 50-60 kHz for ^{13}C. Chemical shifts were calibrated using DSS as an internal reference. A cross-polarization (CP) contact time of 1 ms was used for a decoupling strength of 90 kHz using SPINAL-64. A total of 20,480 scans and 512 scans were used for CP and INEPT spectra respectively.

In vitro cytotoxicity assay

A MTS tretrazolium assay was performed on immortalized keratinocyte cell line. Cells were plated at 2000 cells by well in 96 well plates in Keratinocyte Growth Medium-2 (KGM™-2, Promocell). Due to the lipophilic chemical characteristic of the oleogelator, the NL **5** was prior contact with cells formulated in oil-in-water (o/w) NE at 2% and diluted in culture medium at 1/1000e (v/v). The cell cultures were kept at 37°C, 5% CO_2 for 24 hours prior to NE exposure, to allow the cells to attach to the microplate. Prior to incubation, the cells were washed with 200 µL phosphate-buffered saline (PBS); then, 100 µL of dilution of emulsion was distributed in the wells, and plates were incubated for 4 hours, 24 and 48 hours at 37°C, 5% CO_2. After incubation times, NE was removed and the cells were rinsed with 200 µL of PBS. MTS were performed 4h, 24h and 48h after treatment. MTS is based on the conversion of a tetrazolium salt into a colored formazan product by mitochondrial activity of viable cells at 37°C. The amount of formazan which is directly proportional to the number of living cells in culture was measured at 492 nm by microplate reader. Optical Density (OD) results were compared with the control cells not exposed to NE-NL **5**.

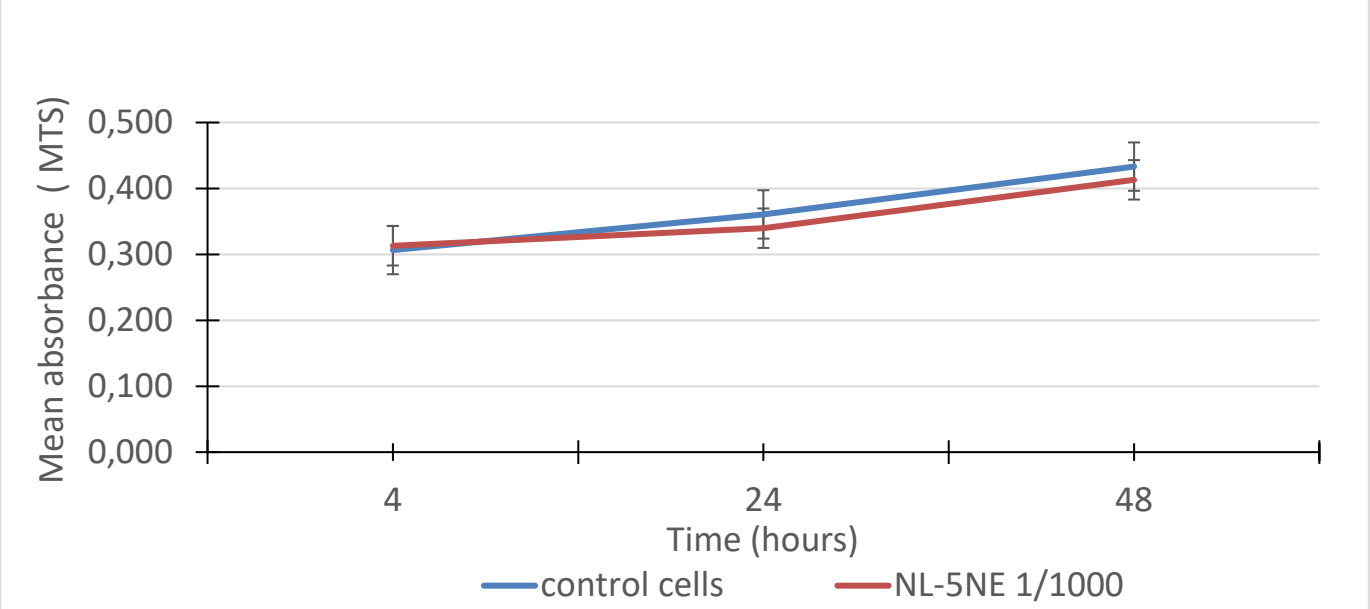


Figure S12: Cytotoxicity evaluation of NE-NL **5**. MTS tretrazolium assay performed on cells exposed to medium alone or medium with NE-NL **5**.

[1] C. A. Palla, M. E. Carrín and D. B. Genovese. Conference: International Conference on Food Innovation 2014 - FoodInnova 2014.

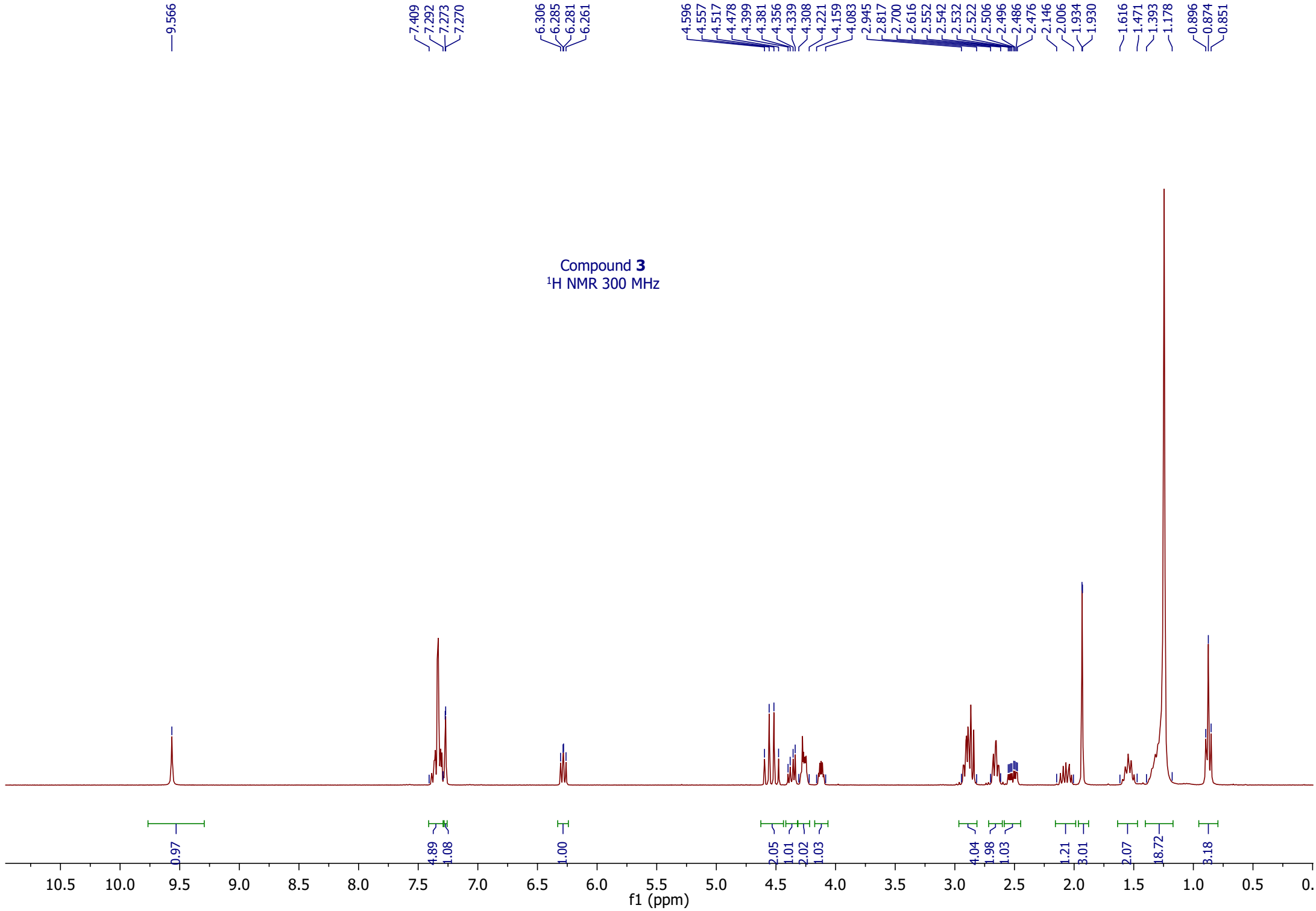
[2] CrysalisPro (Rigaku Oxford Diffraction, 2020).

[3] G.M. Sheldrick, Crystal structure refinement with SHELXL, *Acta Cryst.*, 2015, **71**, 3. https://doi.org/10.1107/S2053229614024218

[4] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program, *J. Appl. Cryst.,* 2009, **42**, 339. https://doi.org/10.1107/S0021889808042726

[5] N. Campins, P. Dieudonné, M. W. Grinstaff and P. Barthélémy, Nanostructured assemblies from nucleotide-based amphiphiles. *New J. Chem.*, 2007, **31**, 1928. https://doi.org/10.1039/B704884J.

Compounds 3-5 NMR spectra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1.471
1.393
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1.98
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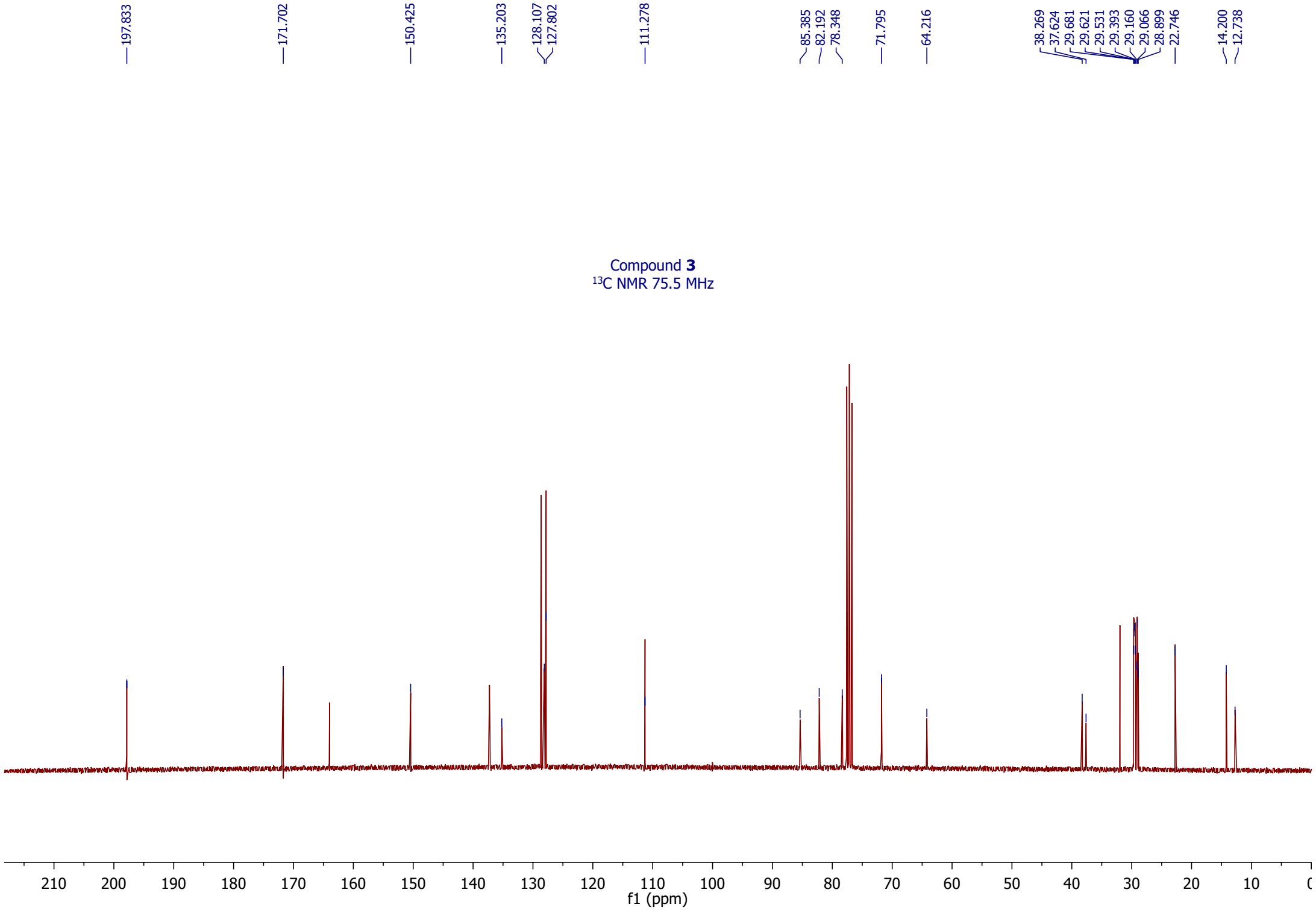
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Compound 3
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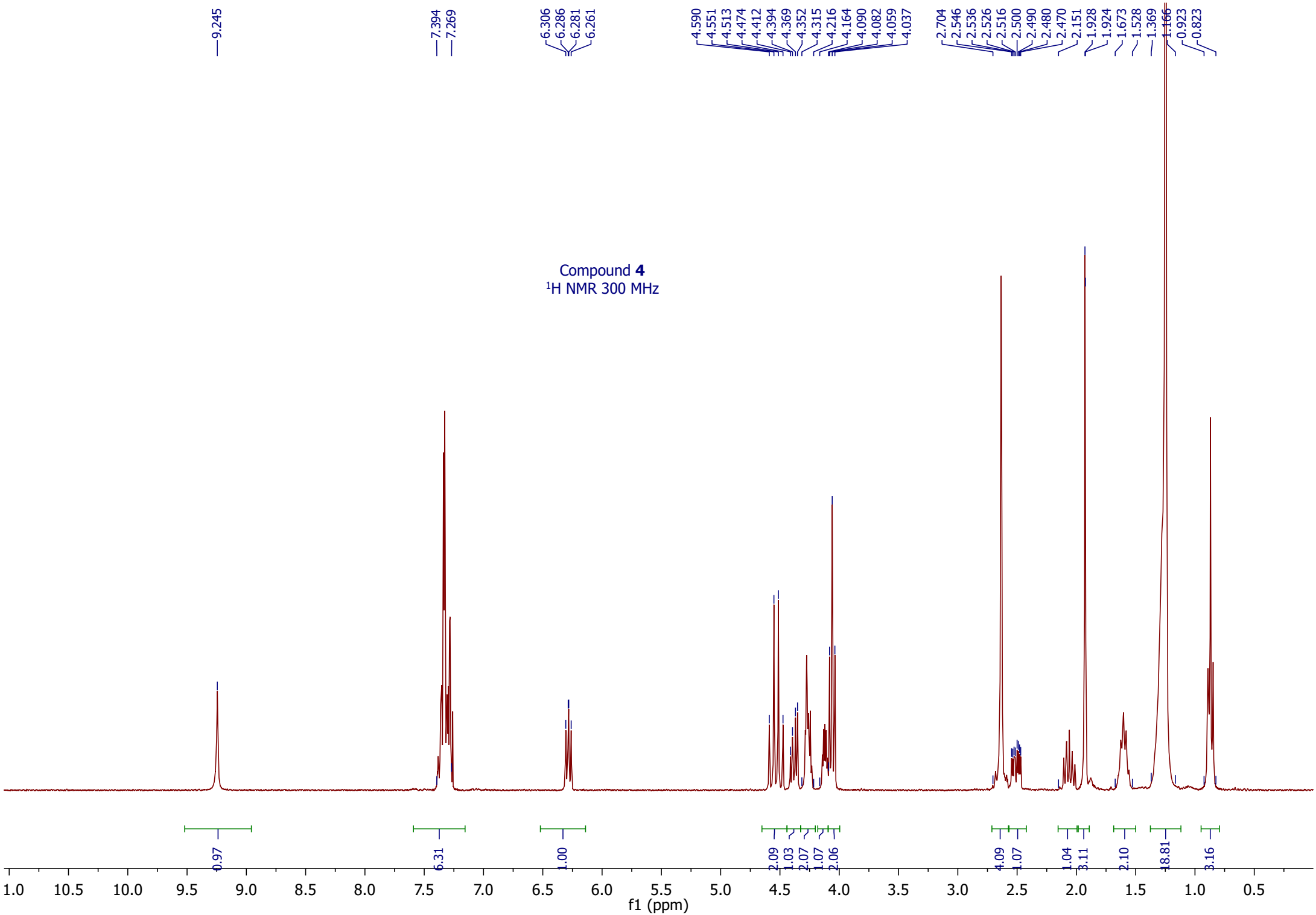
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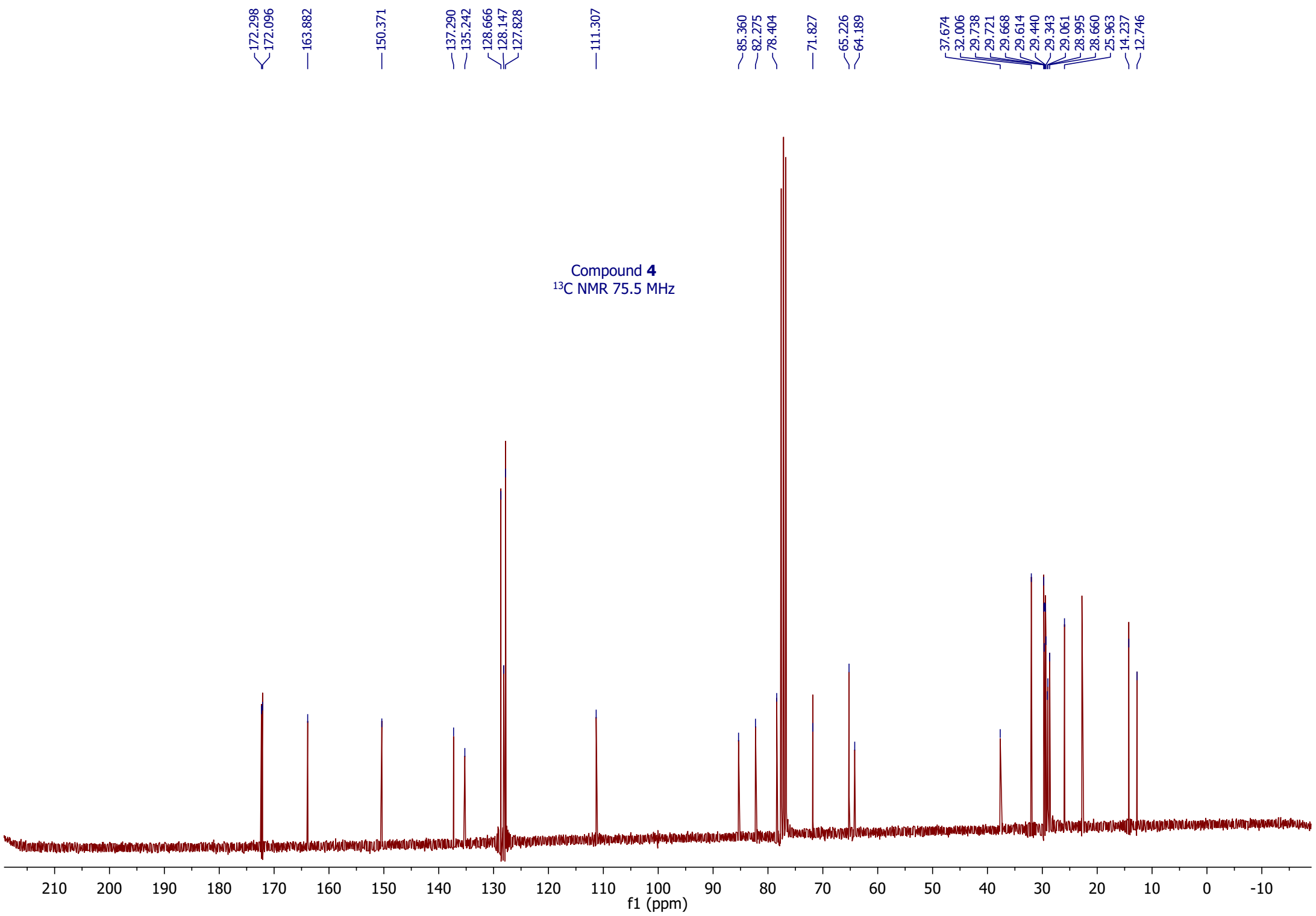
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Compound 4
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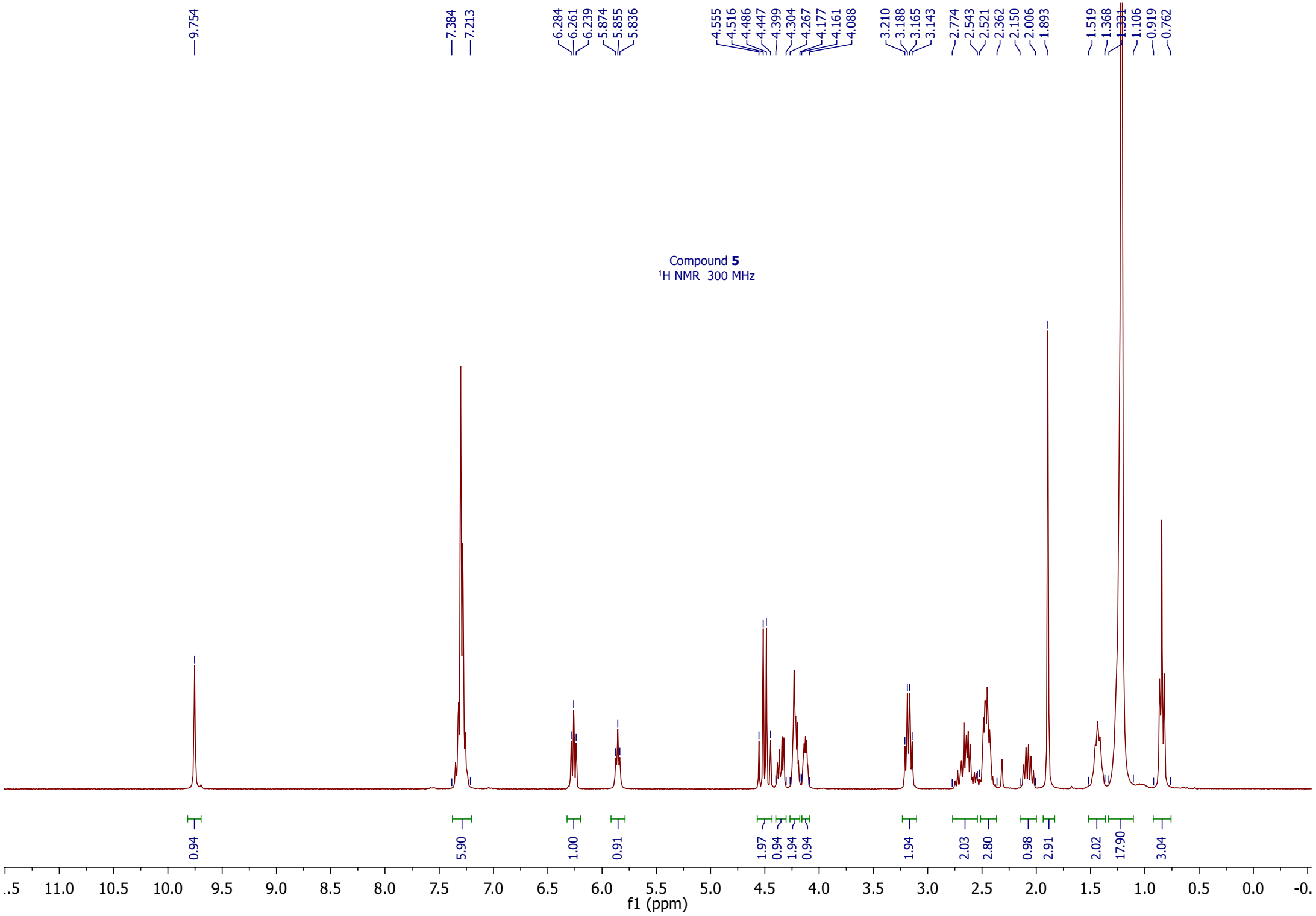
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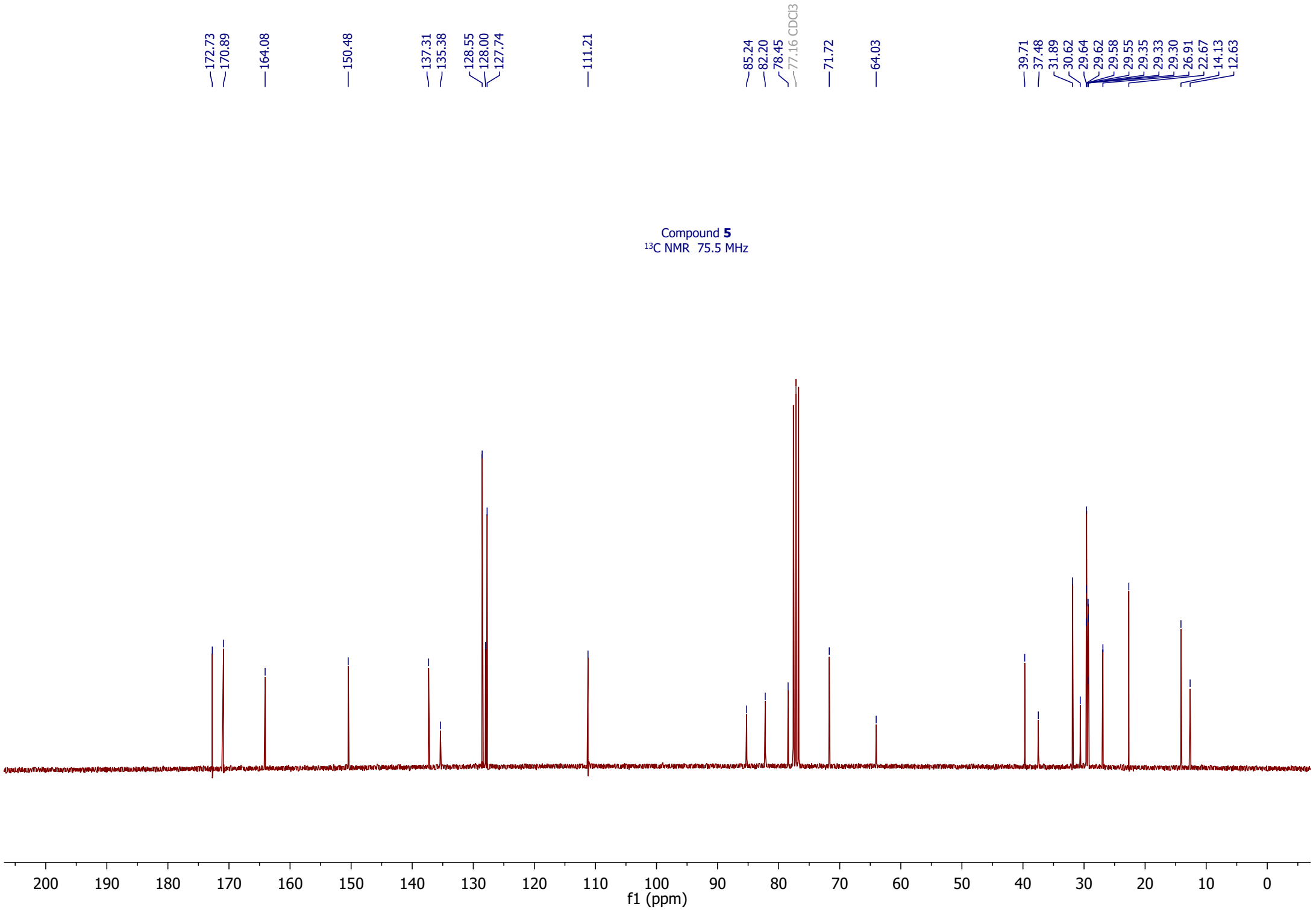
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Compound 5
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f1 (ppm)