Supporting information for:

Red Emitting Neutral Fluorescent Glycoconjugates for Membrane Optical Imaging

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Materials and methods for Langmuir experiments

Chemicals

1-Palmitoyl-2-OleoylPhosphatidylCholine (POPC) of analytical grade was purchased from Sigma-Aldrich (Saint Quentin-Fallavier, France) and used without further purification. It was dissolved in a chloroform/methanol (9:1, v/v) mixture at a final concentration of 1×10^{-3}M. Glycolipidic probes were dissolved in dimethylsulfoxide at a precise concentration of 8.5×10^{-4} M. These mixtures were used as spreading solutions. Ultrapure water (resistivity = 18.2 MΩ.cm) obtained from PURELAB Option-Q 7 water purification system (Veolia Water STI, France), was used as subphase or to prepare a 150 mM Phosphate-Buffered Saline (PBS) solution, pH 7.4.

Langmuir monolayer formation and surface pressure measurements

Langmuir monolayer experiments were performed on rectangular computer-controlled Langmuir-Blodgett trough (KSV Instrument Ltd., Finland): the KSV 2000 model (three multi-compartment systems, V = 85 mL ± 1 mL, S = 119.25 cm^2). This trough working in a symmetrical compression mode was made of Teflon. It was enclosed in a filtered air flow cabinet to avoid dust deposition. The surface pressure π defined as γ_0 - γ, where γ_0 is the surface tension of the pure aqueous subphase and γ the surface tension exerted by the lipids at the subphase surface, was measured using a platinum plate with an accuracy of ± 0.05 mN/m. The POPC monolayers were formed on a 150 mM PBS solution (pH 7.4) used as subphase. PBS has been chosen because it is isotonic and non-toxic to cells. It was commonly used in biological research, and especially to prepare the culture cell which has been used elsewhere for *in cellulo* membrane imaging experiments. Experiments were carried out at a constant temperature (22°C ± 0.5°C) with a water circulating bath (Lauda E100, Lauda France).

POPC monolayer was formed by deposition of an aliquot of 14 µL of the spreading solutions at a clean interface by means of a Hamilton micro-syringe. The experimental error on the spread volume gave a standard deviation of ± 0.8 $Å^2$/molecule for the molecular area (A). After complete evaporation of the solvent (~15 min), the spread lipids were symmetrically compressed by two mobile barriers made in Delrin at a constant rate of 3.75 $cm^2\ min^{-1}$, giving a compression rate of 4.15 $Å^2.molecule^{-1}.min^{-1}$. The isotherm diagram of the monolayer representing the surface pressure π as a function of mean molecular area occupied by one molecule (A expressed in $Å^2$ per molecule) was recorded. After compression, the monolayer could possibly be decompressed to record the compression/decompression isotherms.

Carbohydrate-based fluorescent probe penetration investigations

A POPC monolayer was formed on a 150 mM PBS solution (pH 7.4) as explained above and after recording of a first compression/decompression (control) isotherm, the monolayer was compressed again up to a defined lateral pressure (initial surface pressure π_i). A 15 min lag time was necessary for the monolayer relaxation and for checking the monolayer stability at

fixed constant surface pressure before dye injection. To investigate the penetration properties, the compounds dissolved in DMSO were injected under the compressed POPC monolayer, into the PBS subphase gently stirred with a magnetic bar at a final concentration of 10^{-6} M. The injection was performed with a Hamilton micro-syringe at a constant area. The kinetics of surface pressure variation due to subsequent probe interaction with the monolayer was recorded. The maximal surface pressure increase ($\Delta\pi$) was determined from the kinetics curves. Each injection was repeated twice and performed independently in duplicate with a fresh film and subphase. Injections of a DMSO alone or probes at the same final concentration were performed as controls.

After penetration, the monolayer was decompressed to zero, and an overall compression/decompression cycle was recorded again.

Brewster Angle Microscopy experiments

The morphology of the monolayers at the air/water interface, before and after probe interaction, was observed by Brewster Angle Microscopy. This technique uses the zero reflectance of an air/water surface for parallel polarized light at the Brewster angle of incidence (53° for the air/water interface). The different phases of a monolayer lead to a measurable change in reflectivity, thus allowing the visualization of monolayer morphology. The Brewster Angle Microscope (EP3-SW, Nanofilm, Germany) mounted on the KSV 2000 Langmuir trough was equipped with a laser (532 nm, 50 mW), a polarizer, an analyzer and a CCD camera with a x10 magnification lens. The Brewster Angle Microscopy (BAM) images coded in gray level were recorded with CCD scanning camera, using proprietary motor control circuitry with completely hands-off computer-controlled system. The spatial lateral resolution of the microscope was about 2 µm and the image size was 493 x 383 µm.

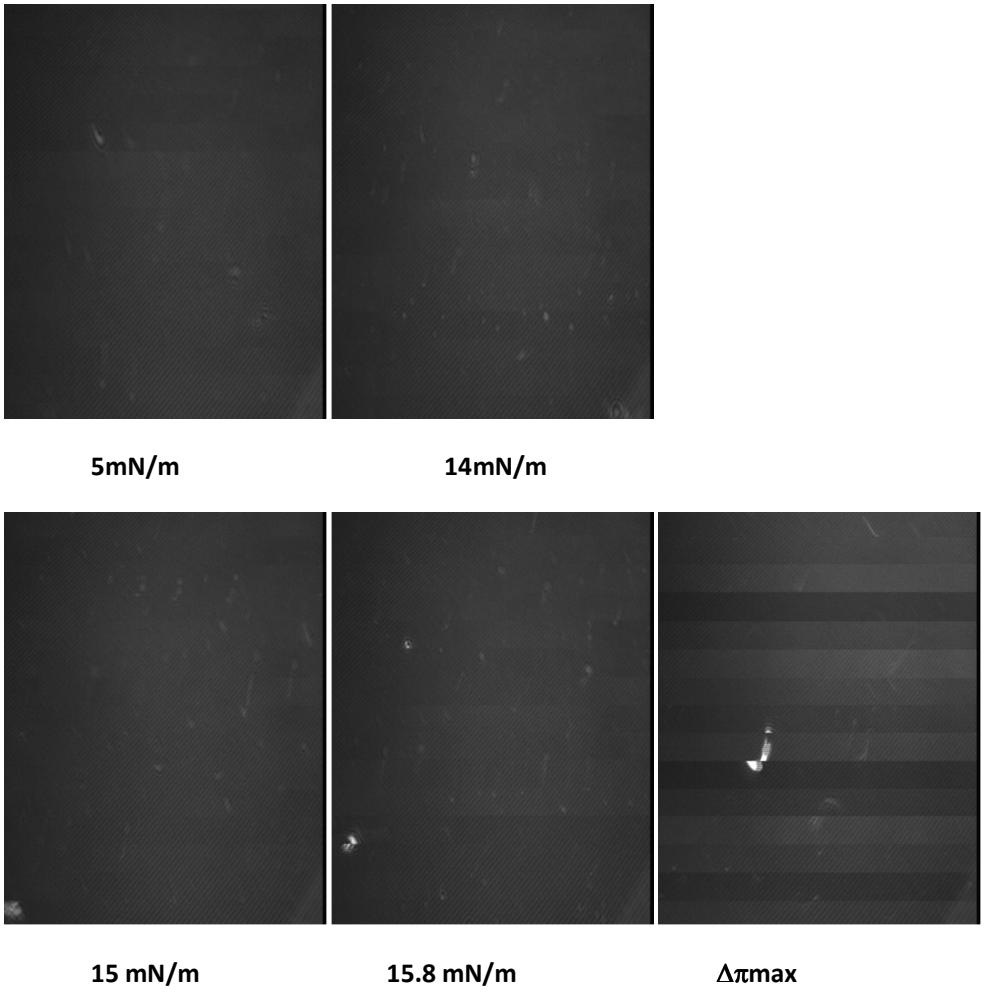

Figure S1. Monolayer morphology visualized by Brewster Angle Microscopy before and during the insertion of probe **14** into a POPC monolayer initially compressed at an initial surface pressure (π_i) of 5 mN/m. The phospholipid organization was unchanged (Image size: 483 μm x 383 μm).

5 mN /m **DPPC alone compressed at 17 mN/m** **π_{max}=17 mN/m**

Figure S2. Monolayer morphology visualized by Brewster Angle Microscopy before and once the maximal surface pressure (πmax) was attained after insertion of probe **14** into a DPPC monolayer initially compressed at an initial surface pressure (π_i) of 5 mN/m (Image size: 483 µm x 383 µm).

Imaging figures

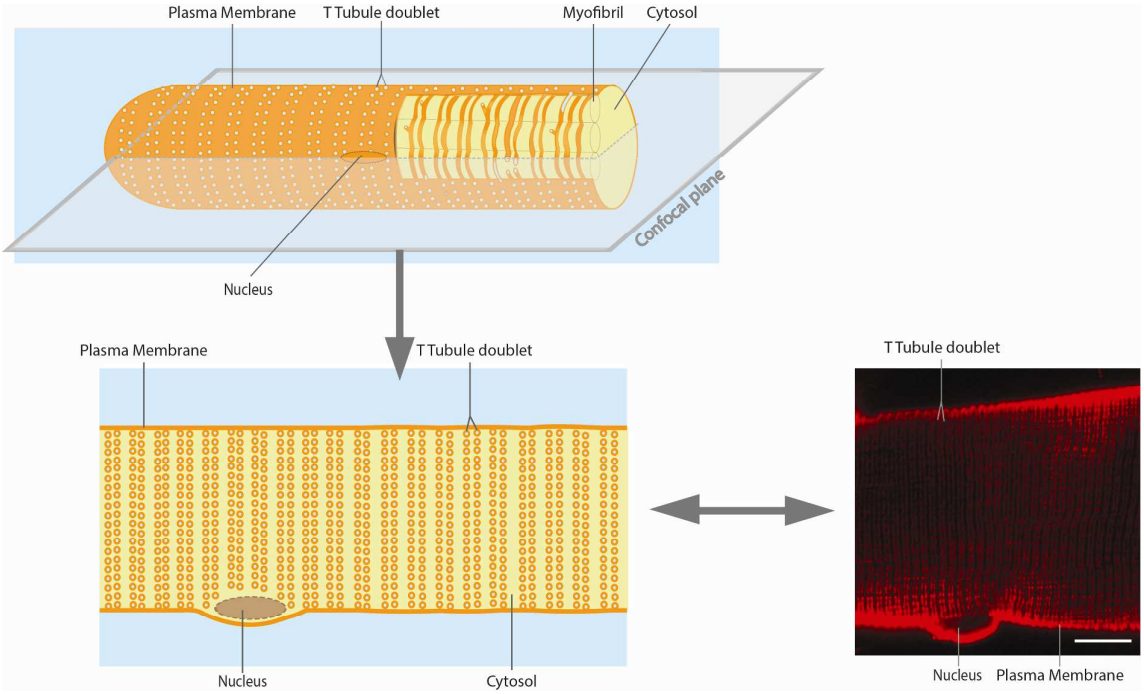

Figure S3. Complexity of the plasma membrane system in a skeletal muscle fiber.

A: 3D drawing of a muscle fiber. The plasma membrane system, in orange, is composed of the surface membrane and its invaginations, the T-tubules. Cytoplasm is indicated in yellow. B: Drawing of a confocal slice of a skeletal muscle fiber, following the confocal plane indicated in A. The t-tubules are organized in transversal doublets. C: Confocal image (xy) of fluorescence of a skeletal muscle fiber stained with Di-8-ANEPPS. T-tubules appear as paired transversal lines in this image. White bar= 15 µm.

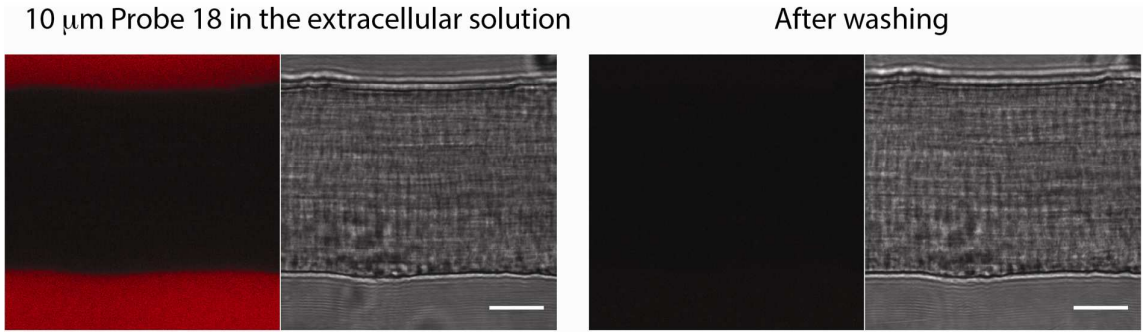

Figure S4. Probe **18** staining experiment.

Confocal images (xy) of fluorescence and transmitted images of a skeletal muscle fiber during incubation with the probe **18**, and after washing. No staining is detected. White bar = 10 μm.

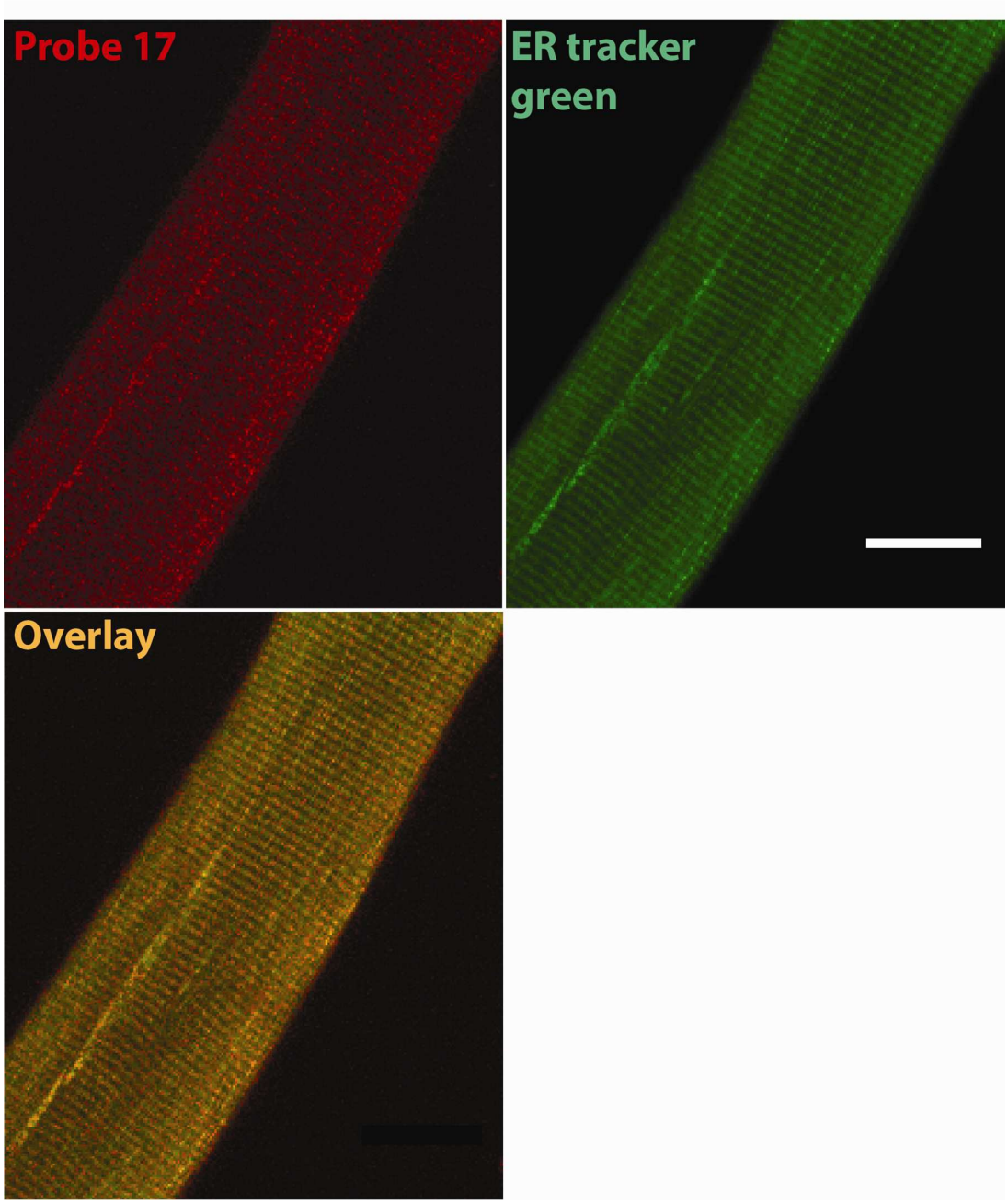

Figure S5. Colocalization of probe **17** and ER tracker Green.

Confocal images (xy) of fluorescence of a skeletal muscle fiber loaded with the probe 17 (upper left) and ER tracker Green (upper right). The overlay (lower left) shows a perfect colocalization of the two probes. White bar = 20 μm. Experiment were repeated on 4 cells, with similar results.

General Information for synthesis:

Solvents were of HPLC or reagent quality and purchased commercially. Starting materials were purchased commercially and used without further purification. Compounds were characterised by using 1H and 13C NMR spectroscopy. The spectra were recorded on a Bruker AC 200 operating at 200.13 MHz and 50.32 for respectively and on a Bruker Advance operating at 500.10 for 1H and 125.75 MHz for 13C. Chemical shifts are reported as δ values (ppm) with reference to the residual solvent peaks.
For proton, data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, b=broad), coupling constants in Hz. UV/vis absorption measurements were recorded on a JASCO V550 spectrometer. Fluorescence spectra were measured using a Horiba-Jobin Yvon Fluorolog-3 spectrofluorimeter, equipped with a red-sensitive Hamamatsu R928 photomuliplier tube. Spectra were reference corrected for both the excitation source light intensity variation (lamp and grating) and the emission spectral response (detector and grating). All solvents were of spectrophotometric grade. Coumarin 153 laser grade was purchased from Acros. All air- or moisture-sensitive reactions were carried out in flame-dried glassware under Ar atmosphere. DMF was freshly distilled over CaH_2. Thin-layer chromatography (tlc) was performed with Merck 60F254 precoated silica gel plates. Column chromatography was carried out using Merck silica gel 60 (70-230 mesh).

^{1}H NMR and ^{13}C NMR spectra

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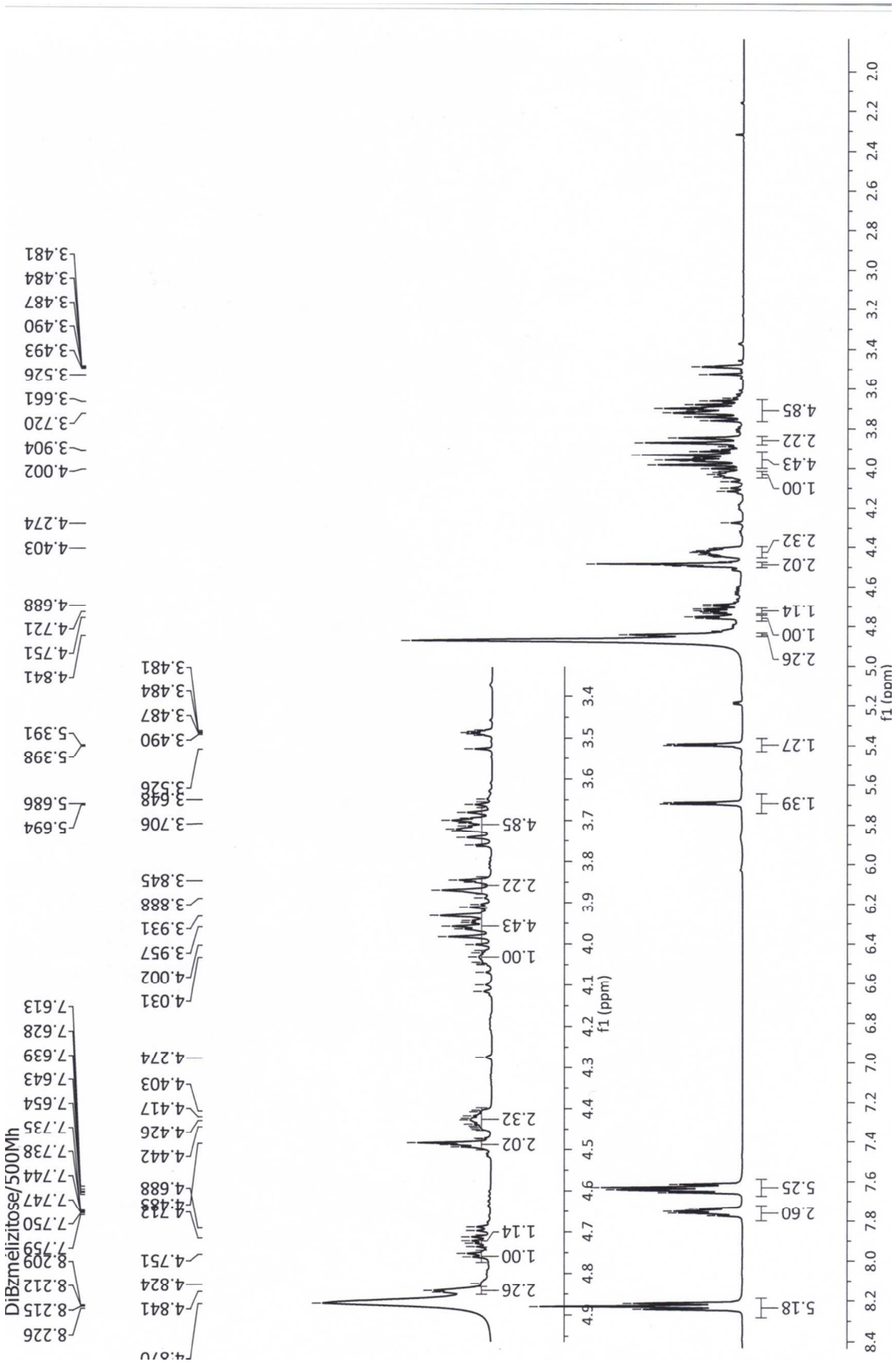

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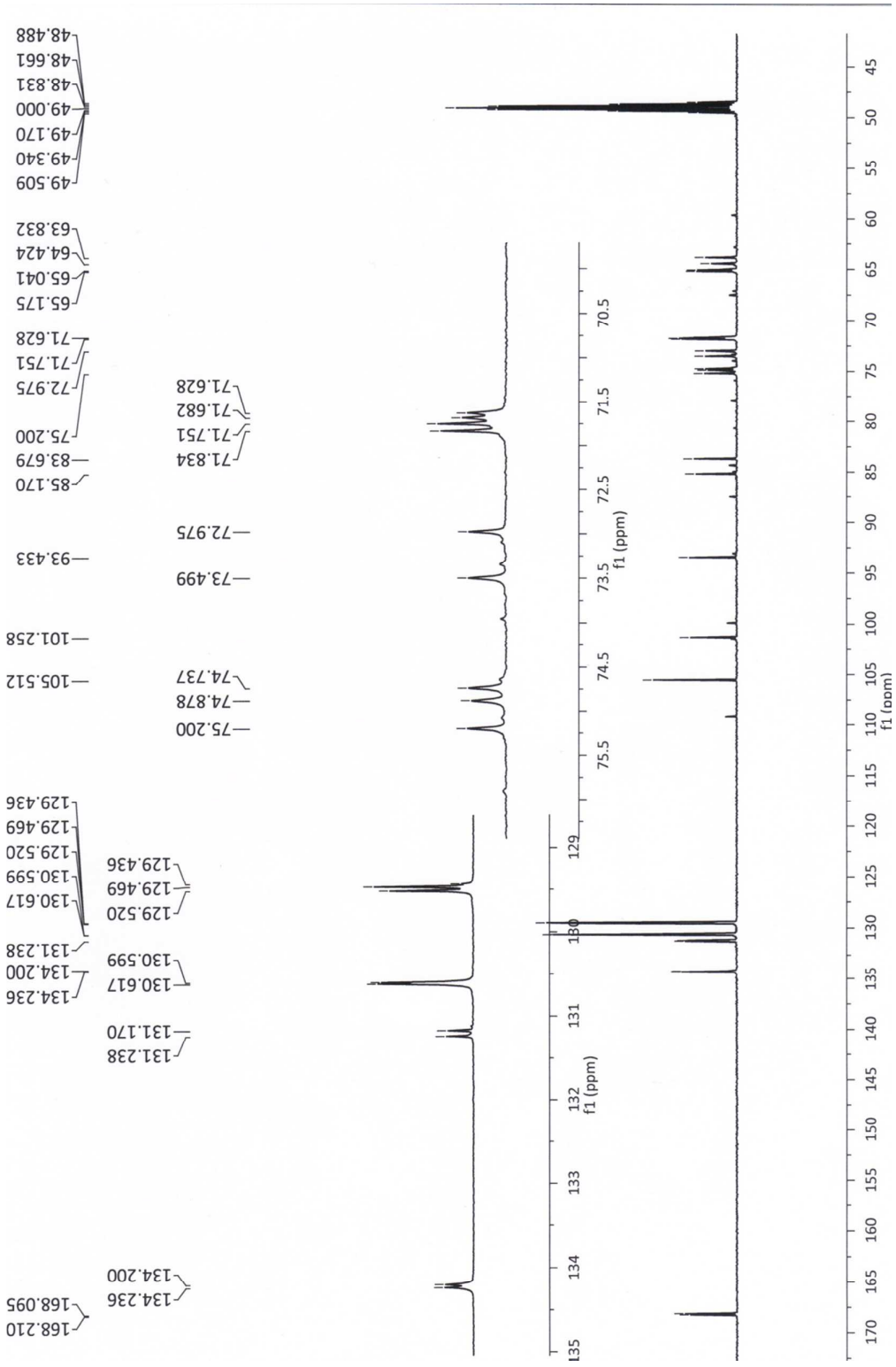

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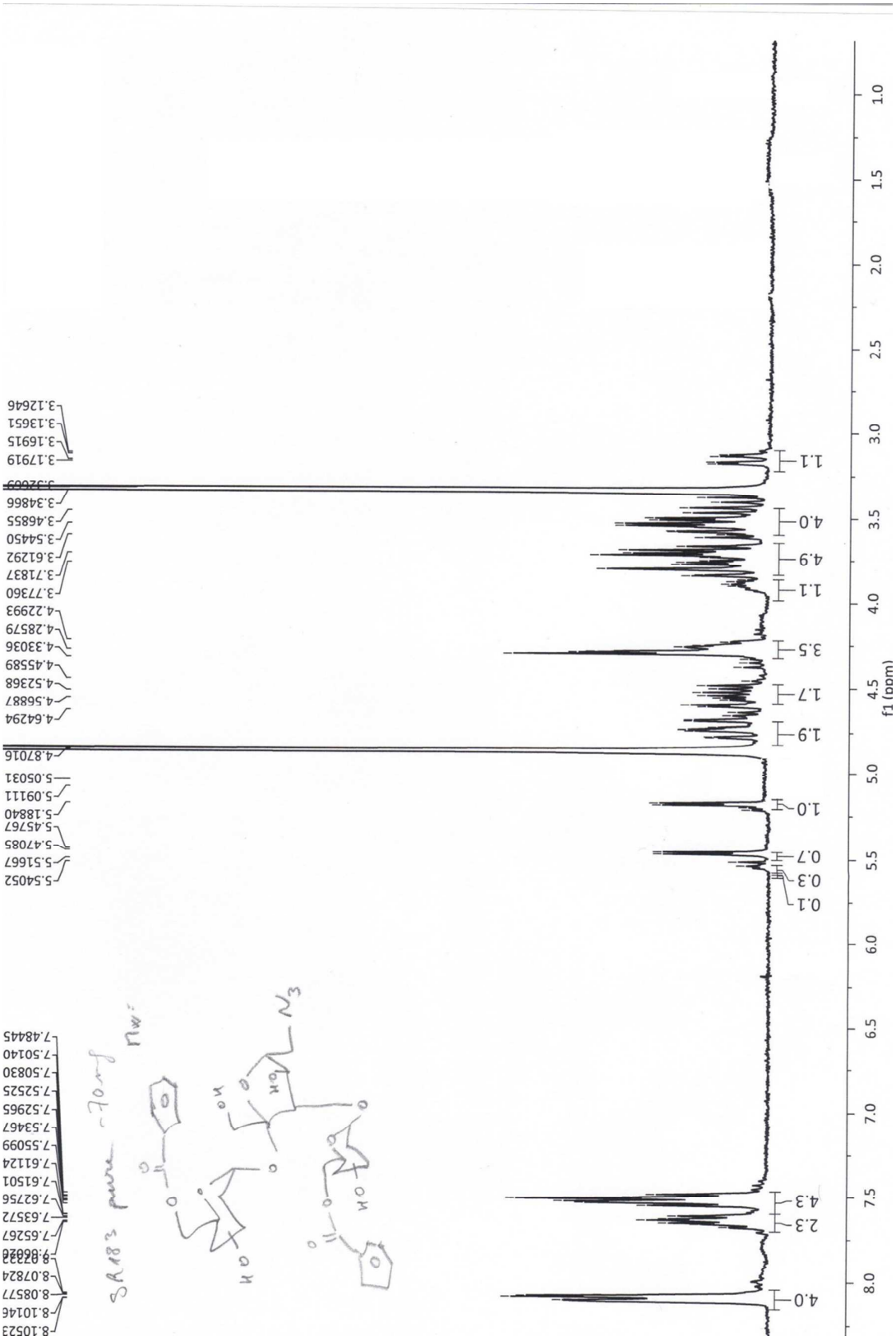

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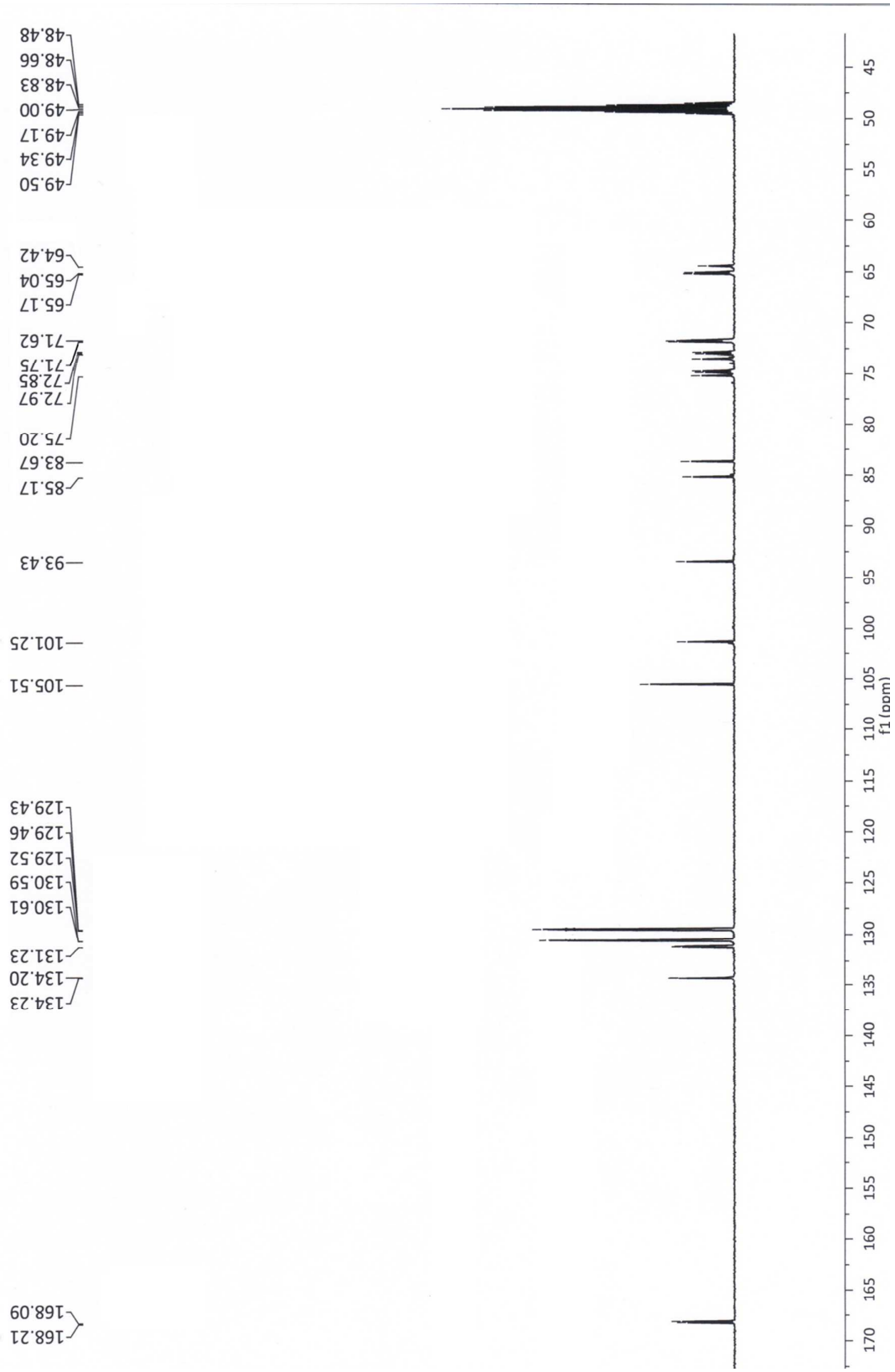

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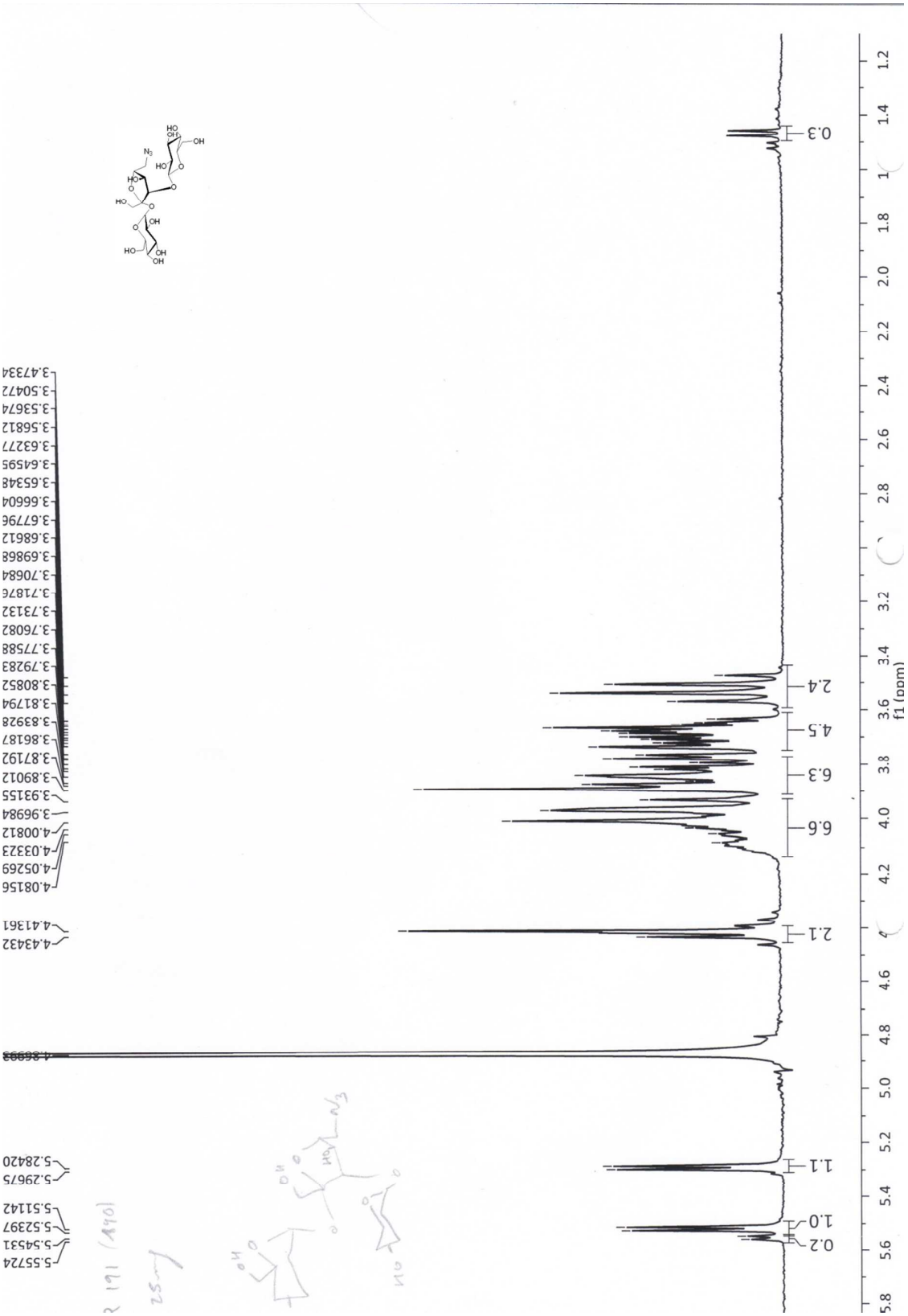

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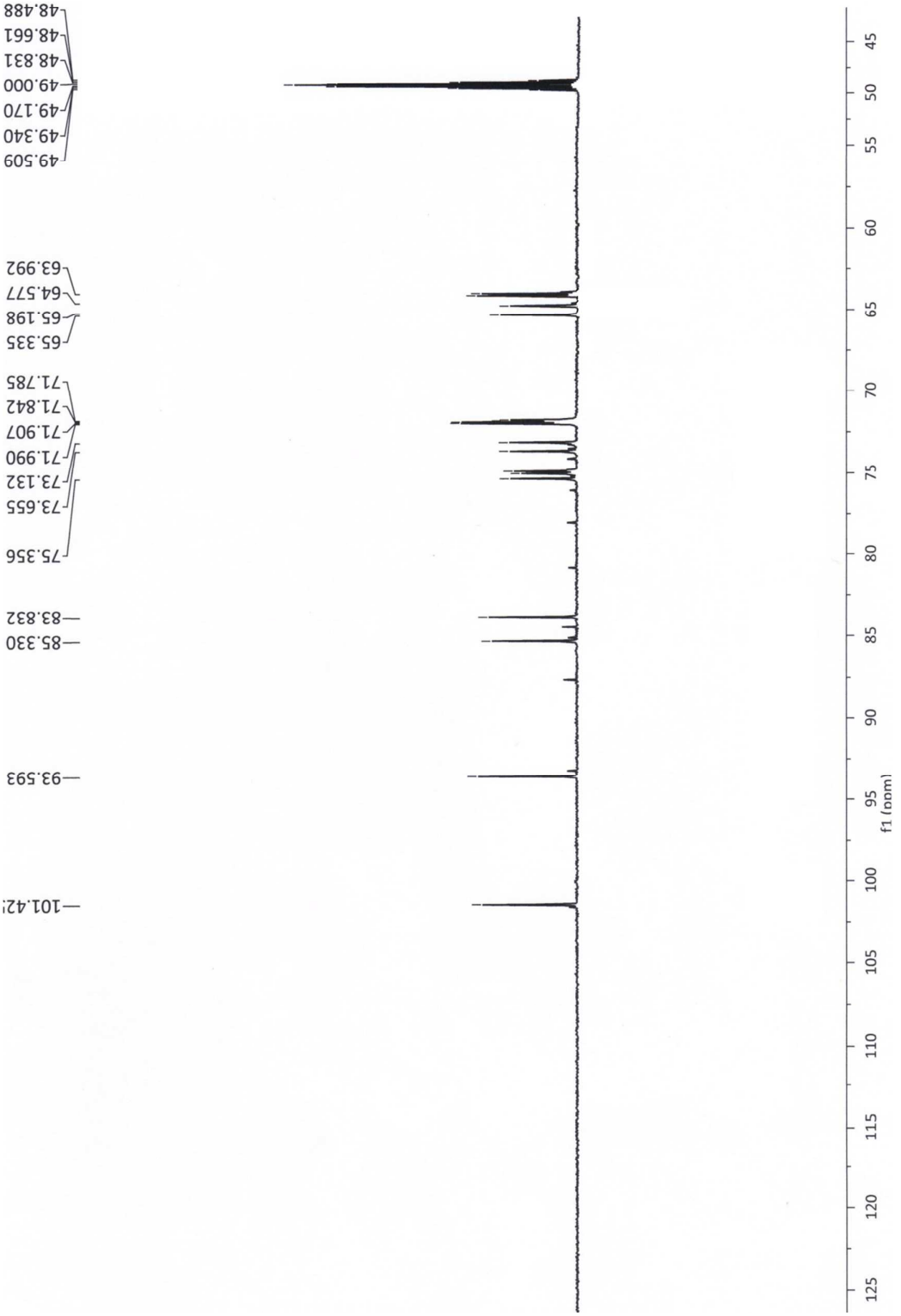
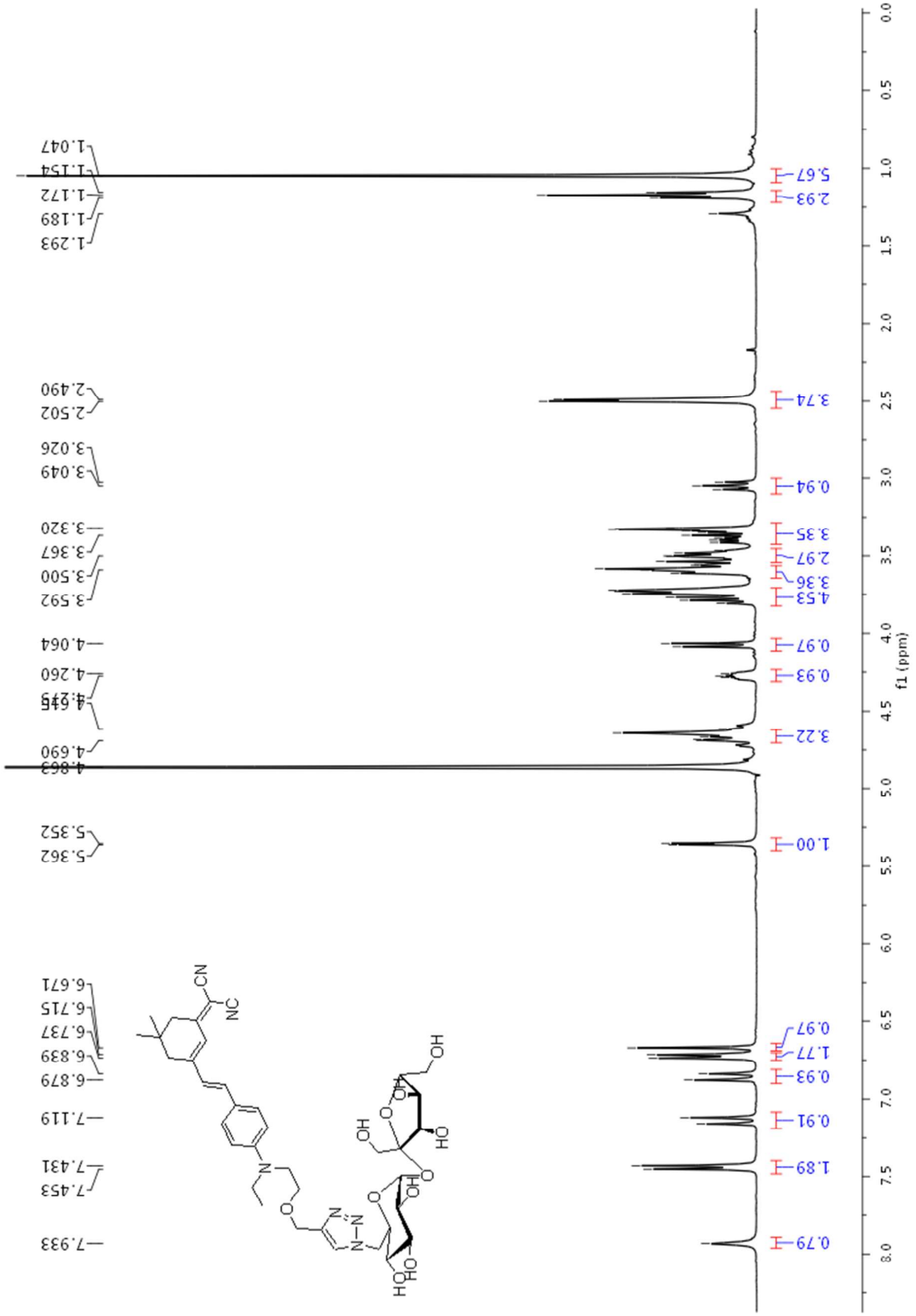


Compound 13



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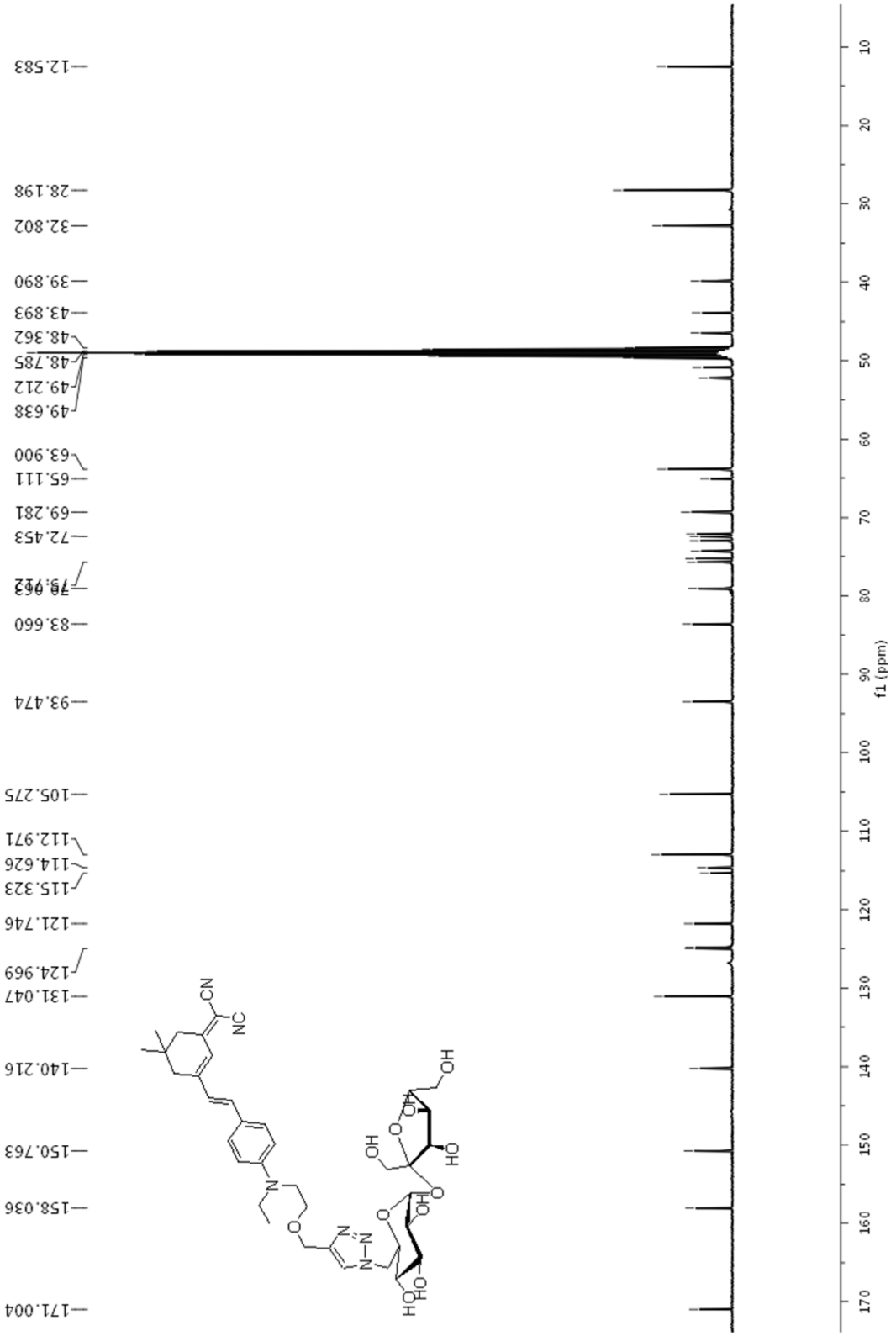
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105.275
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115.323
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N
f1 (ppm)

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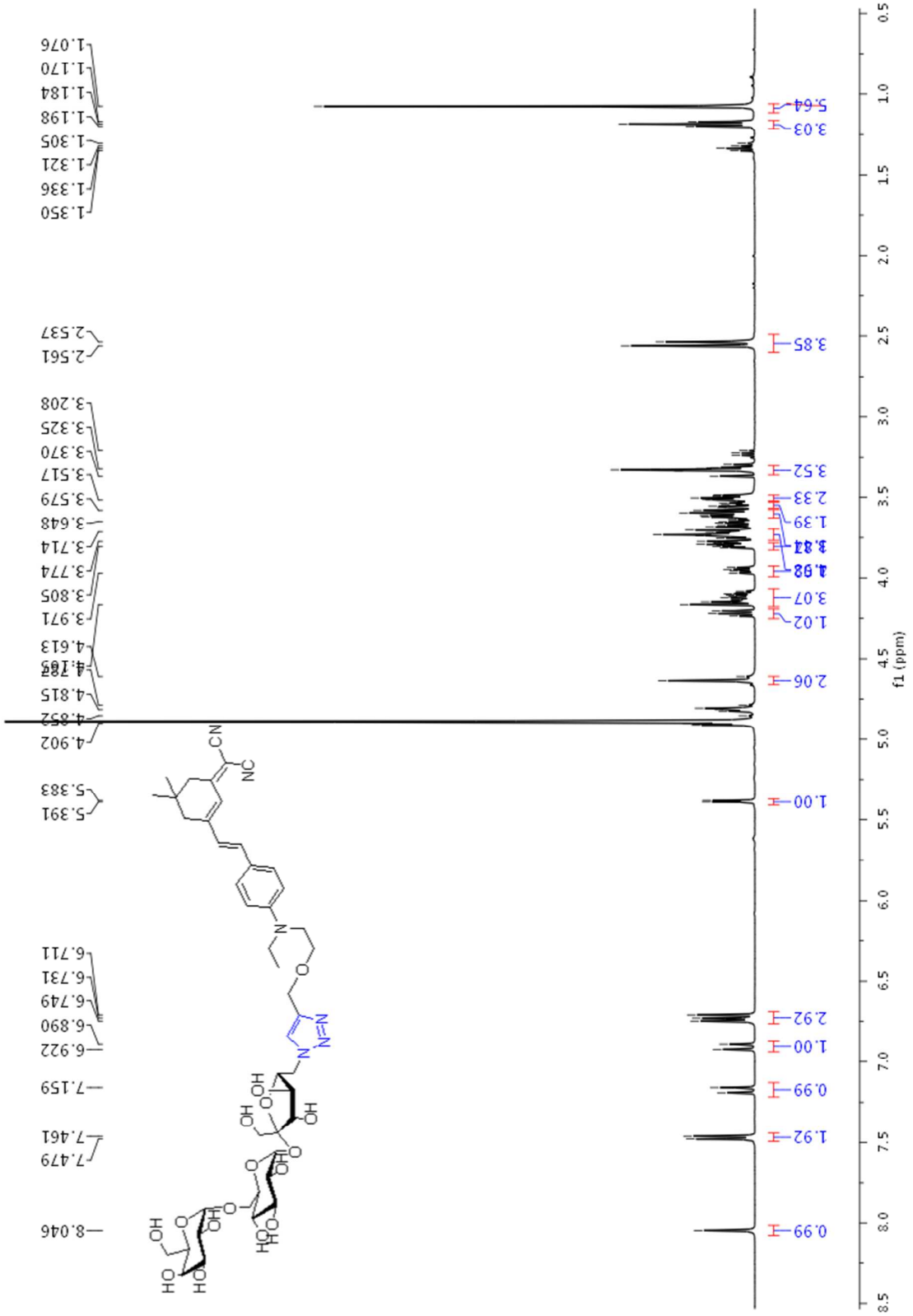
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3.714
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4.785
4.815
4.852
4.902
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3.03
3.85
3.52
2.33
1.39
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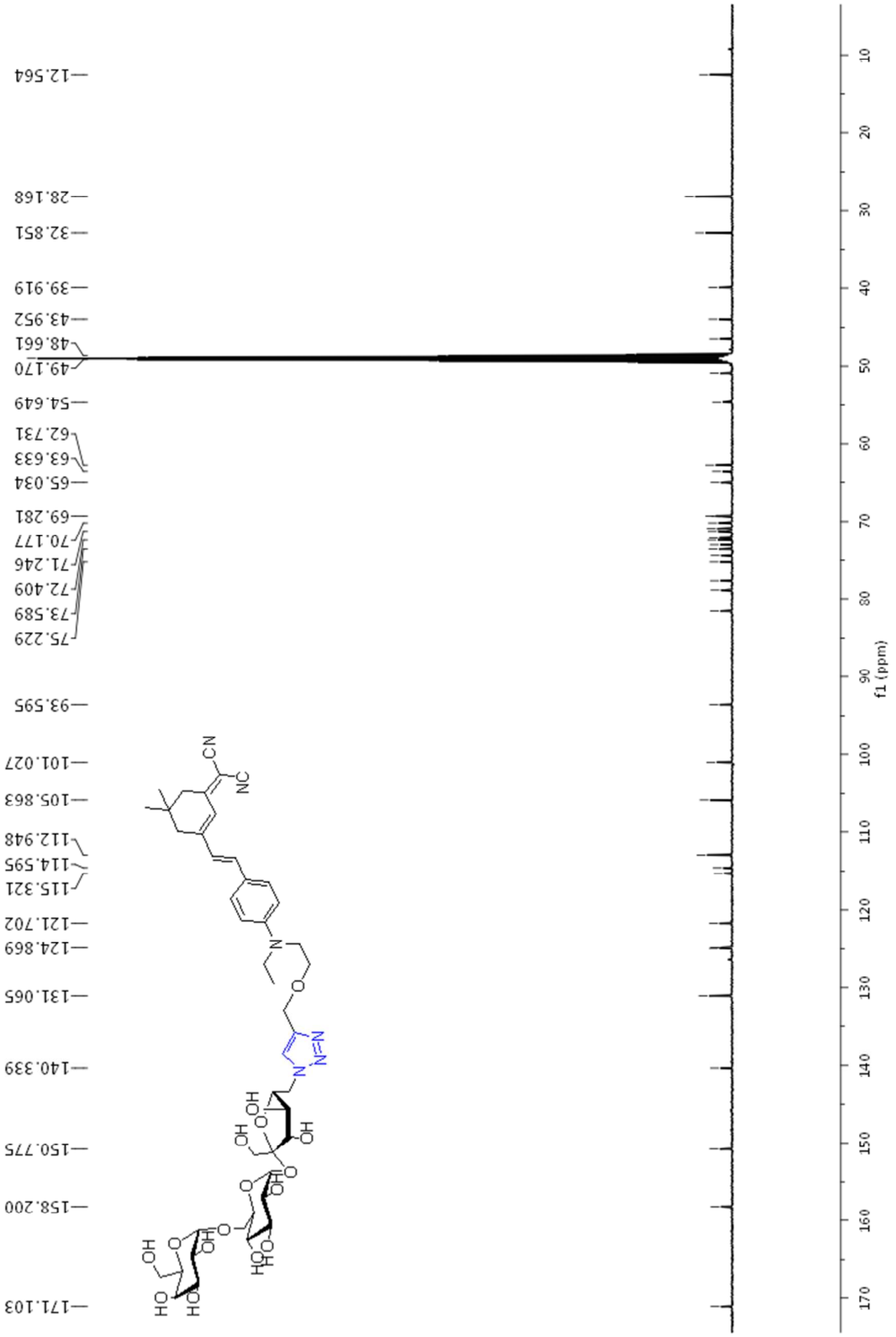
f1 (ppm)

Compound 14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70.177
71.246
72.409
73.589
75.229
93.595
101.027
105.863
112.948
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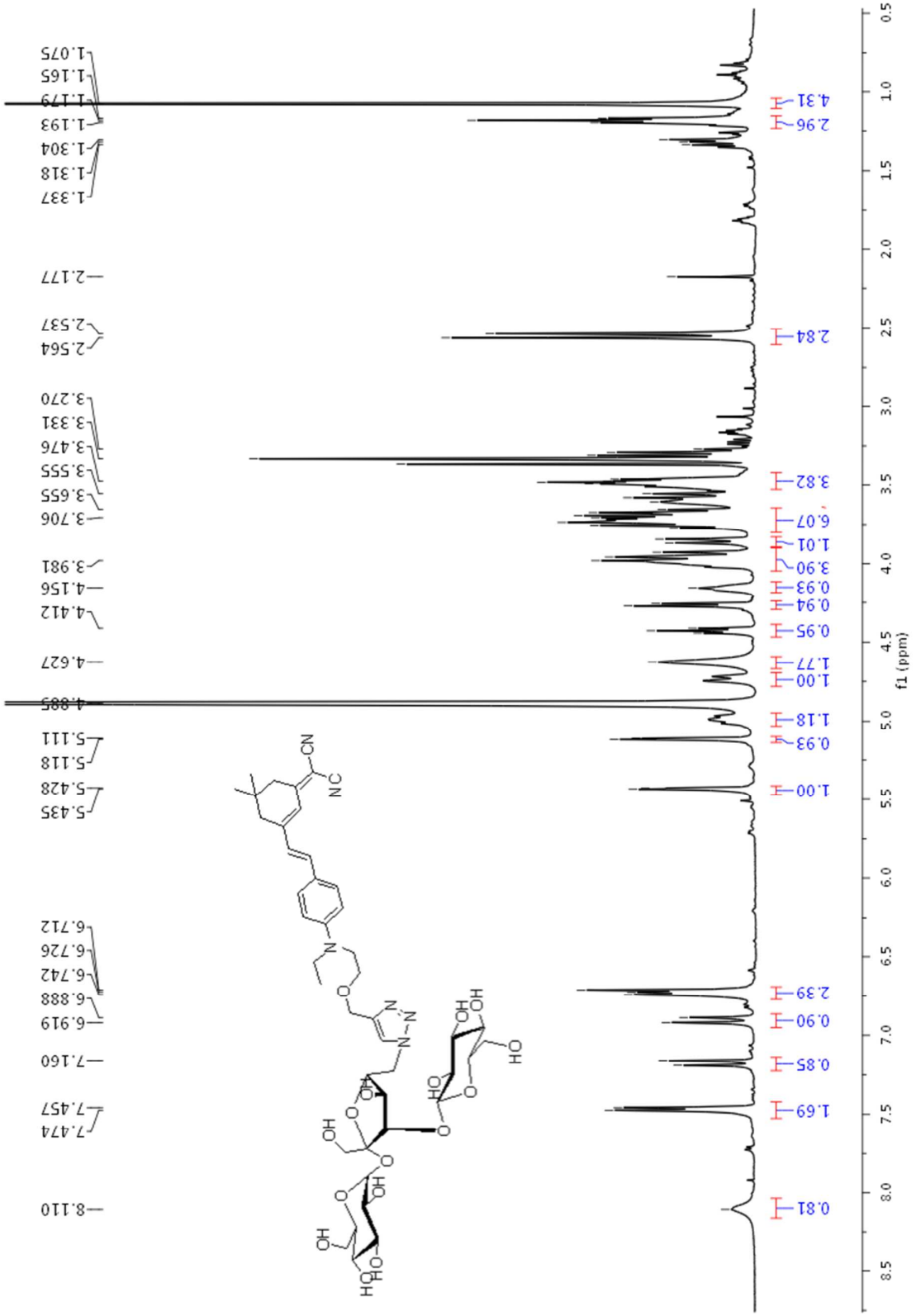
f1 (ppm)

Compound 15



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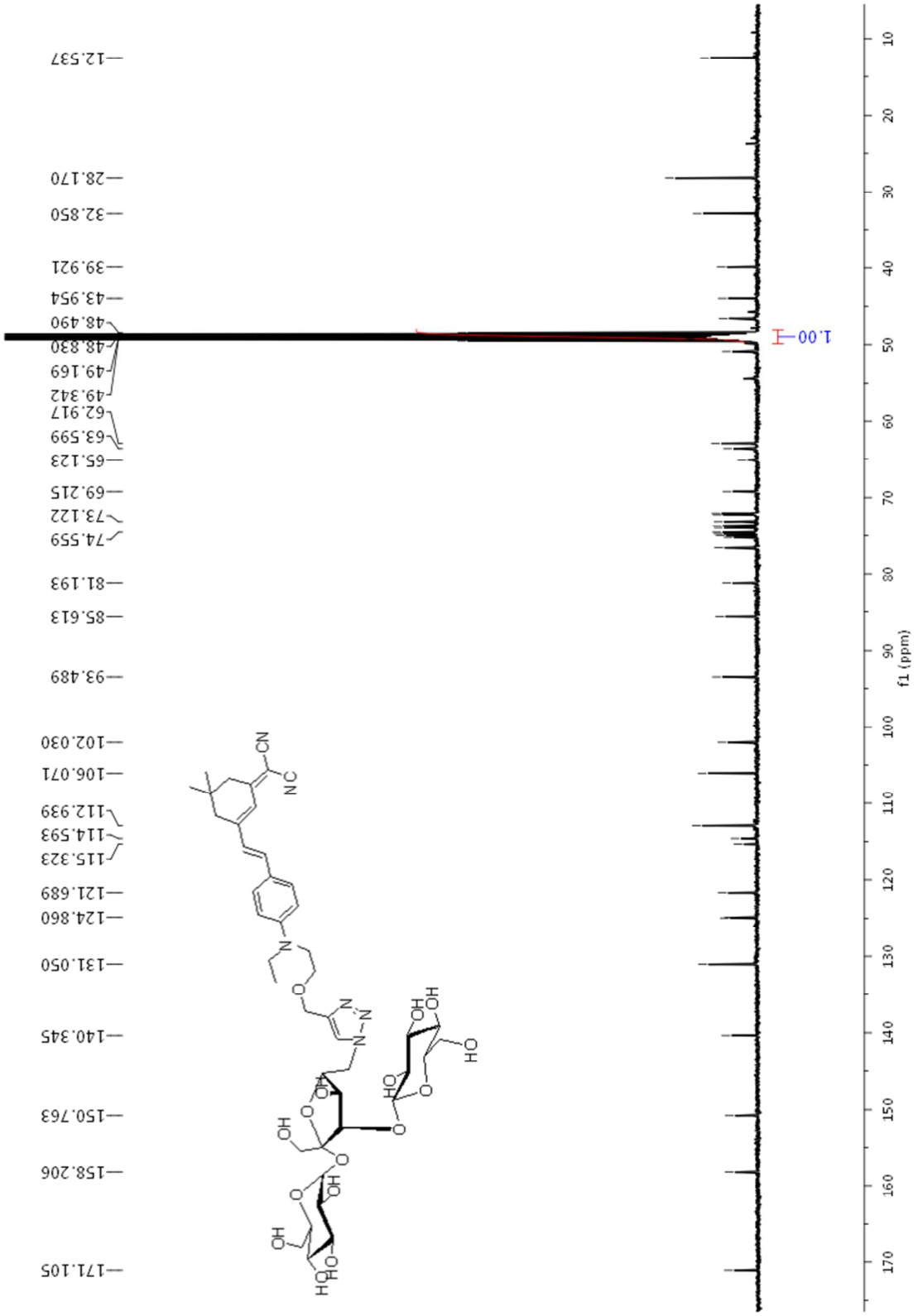
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69.215
73.122
74.559
81.193
85.613
93.489
102.030
106.071
112.939
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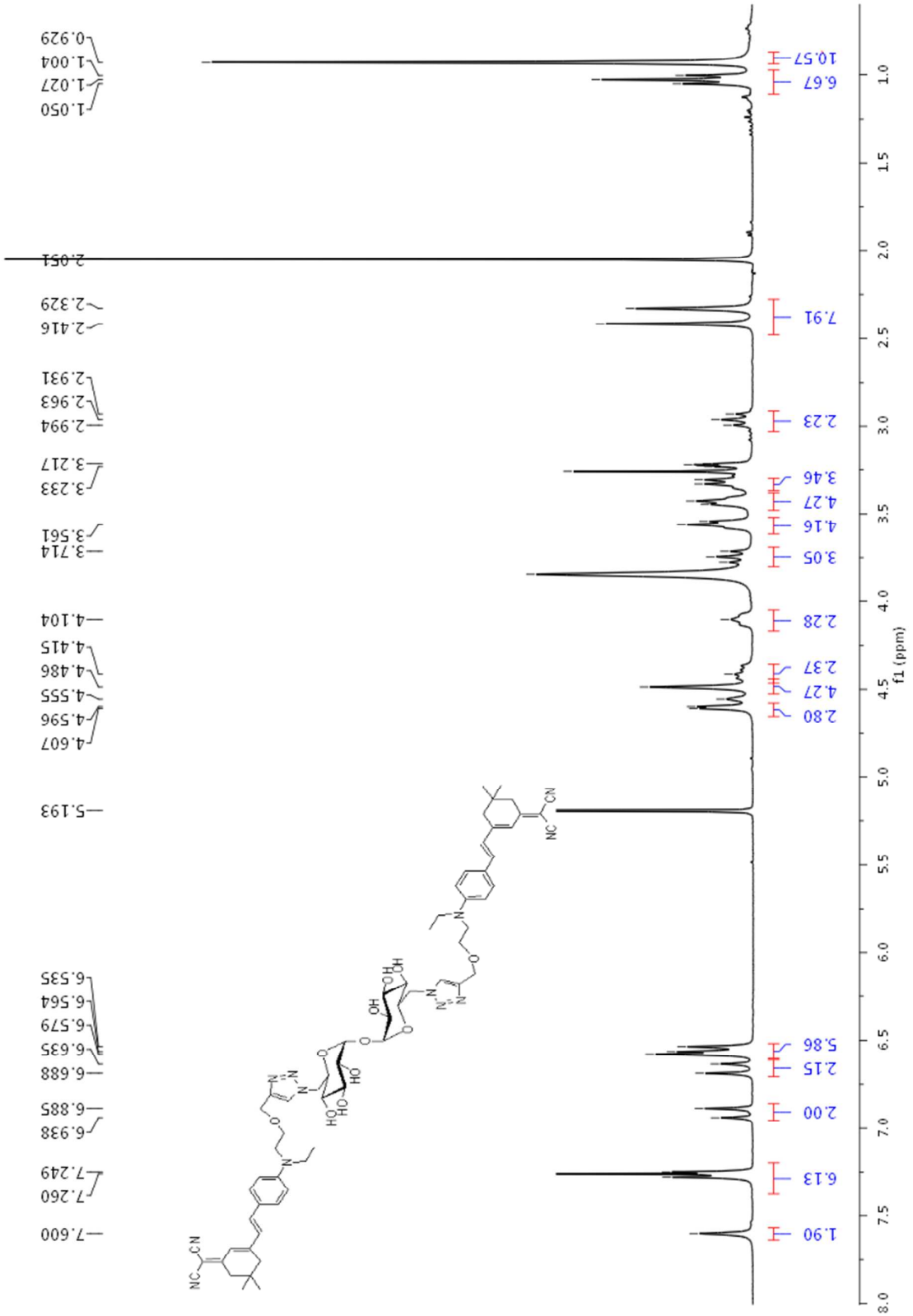
f1 (ppm)

Compound 16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6.564
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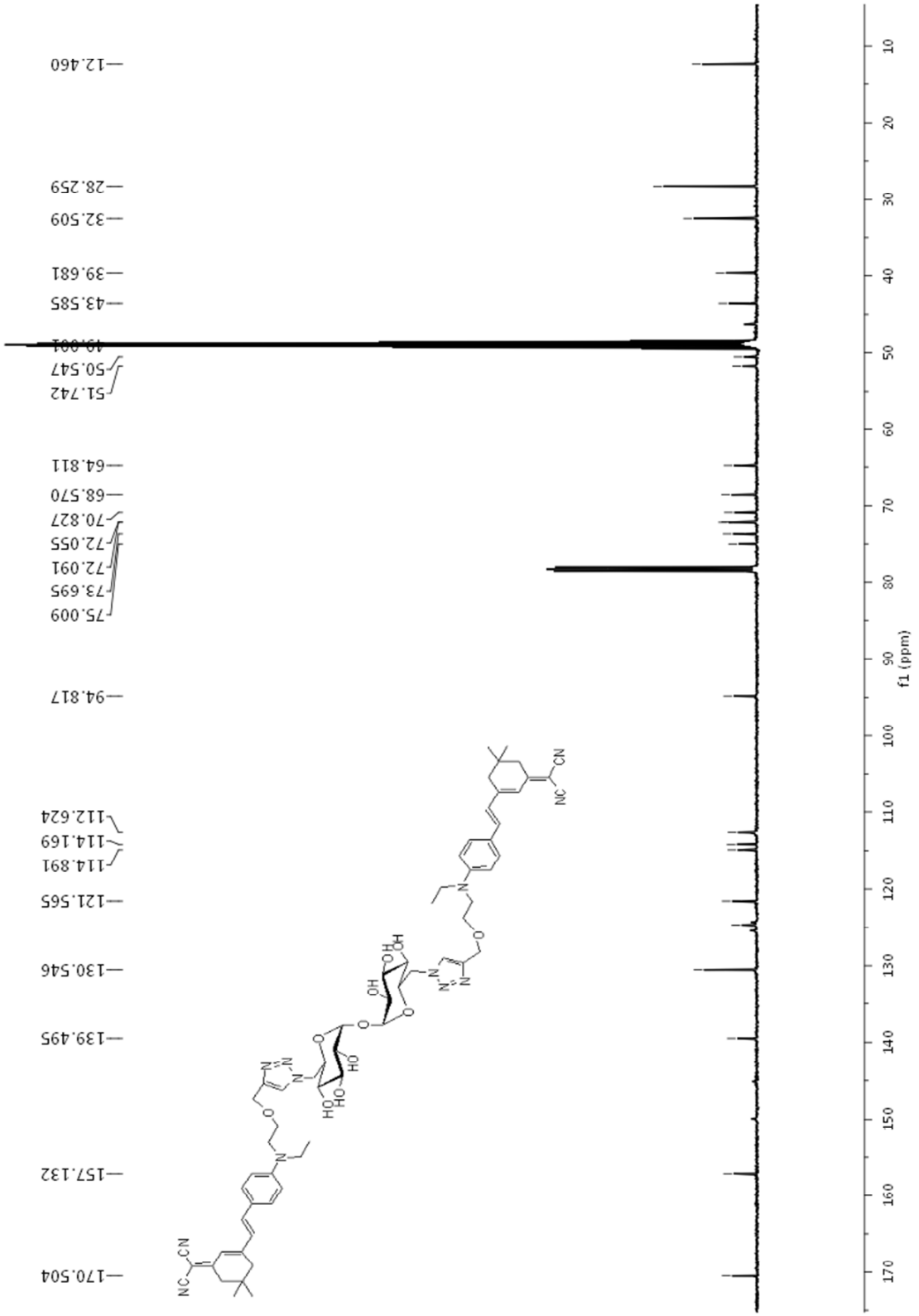
Compound 16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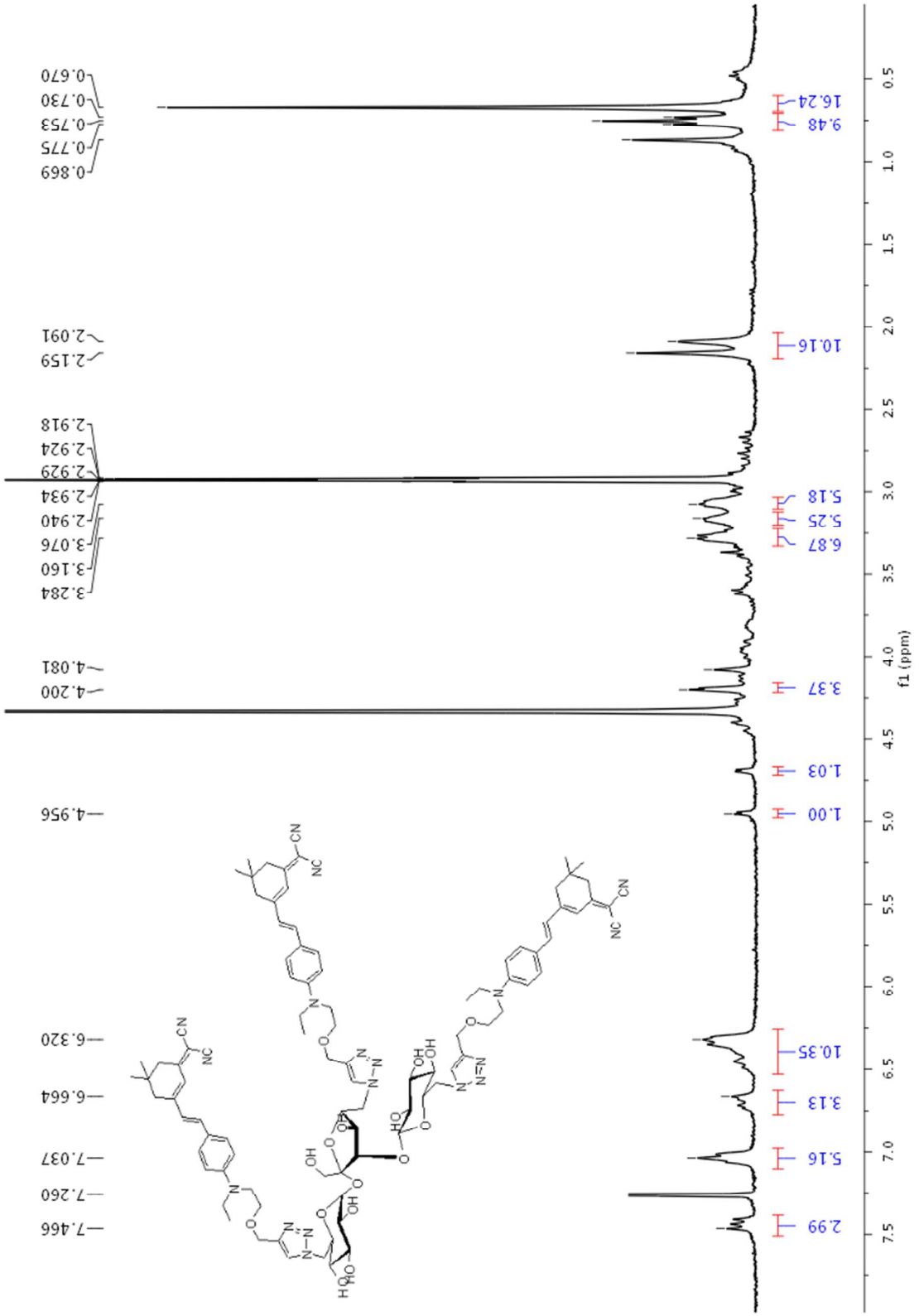
f1 (ppm)

Compound 17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4.956
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6.664
7.037
7.260
7.466
16.24
9.48
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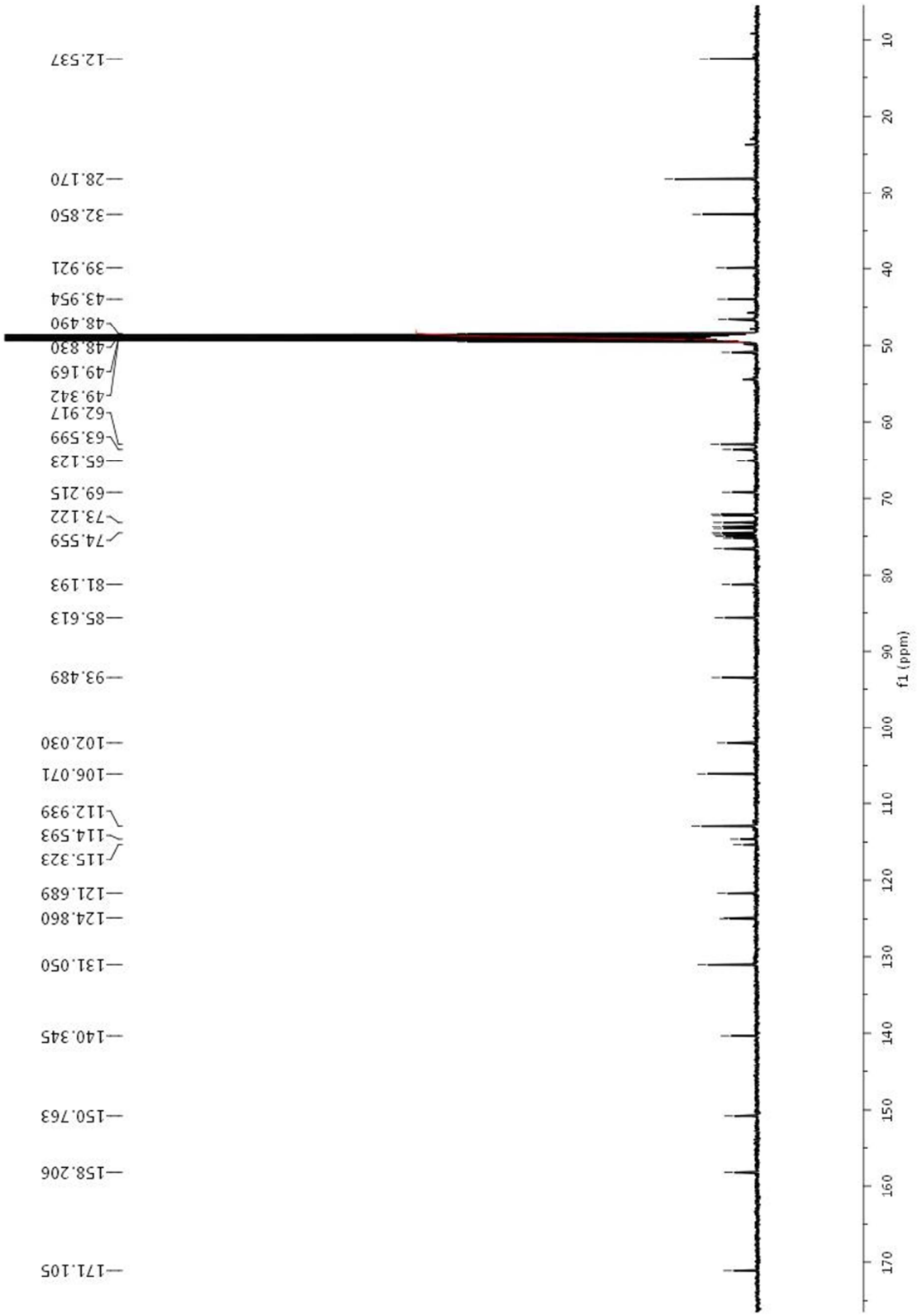
2.99
f1 (ppm)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102.030
106.071
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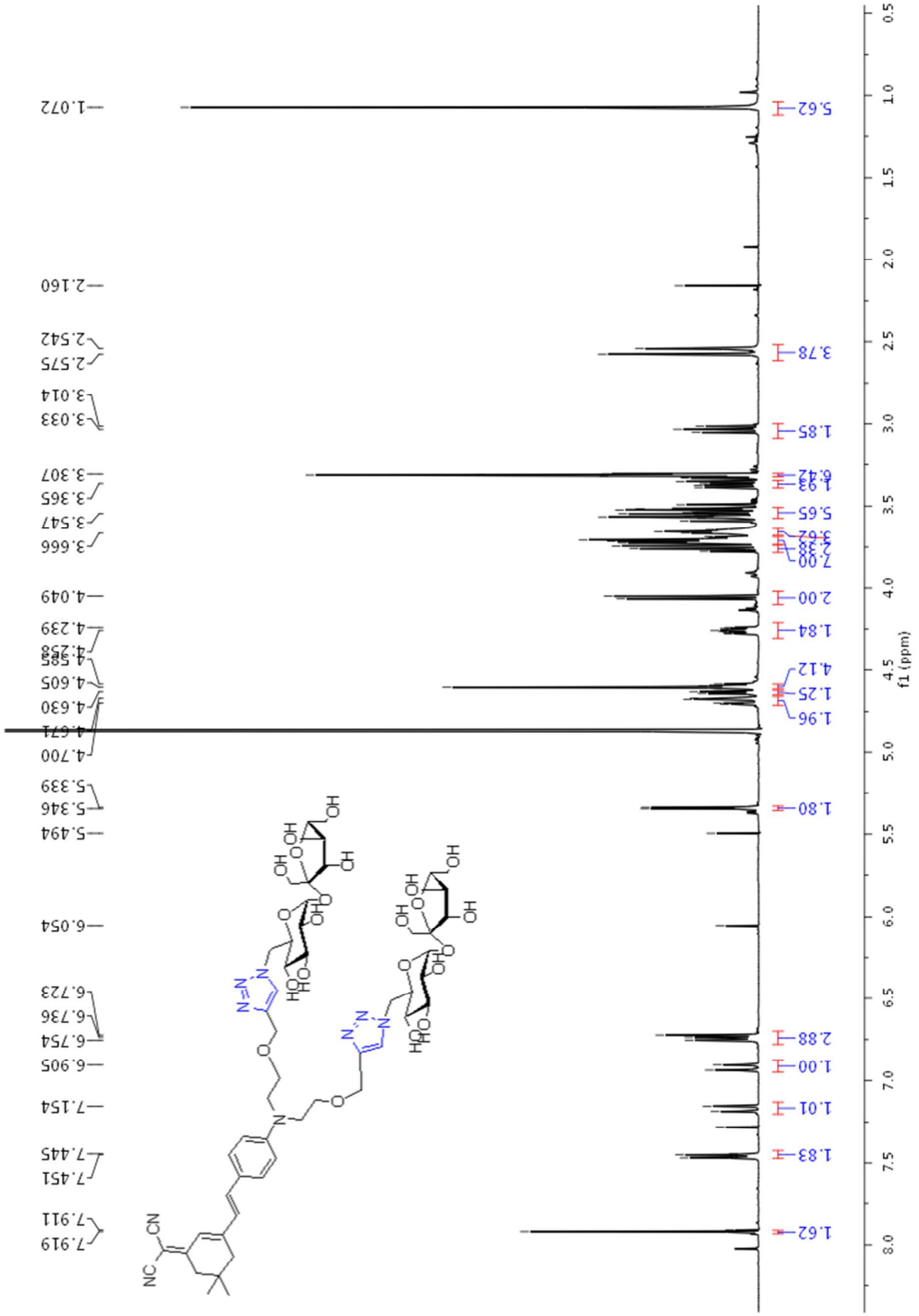
171.105
f1 (ppm)

Compound 18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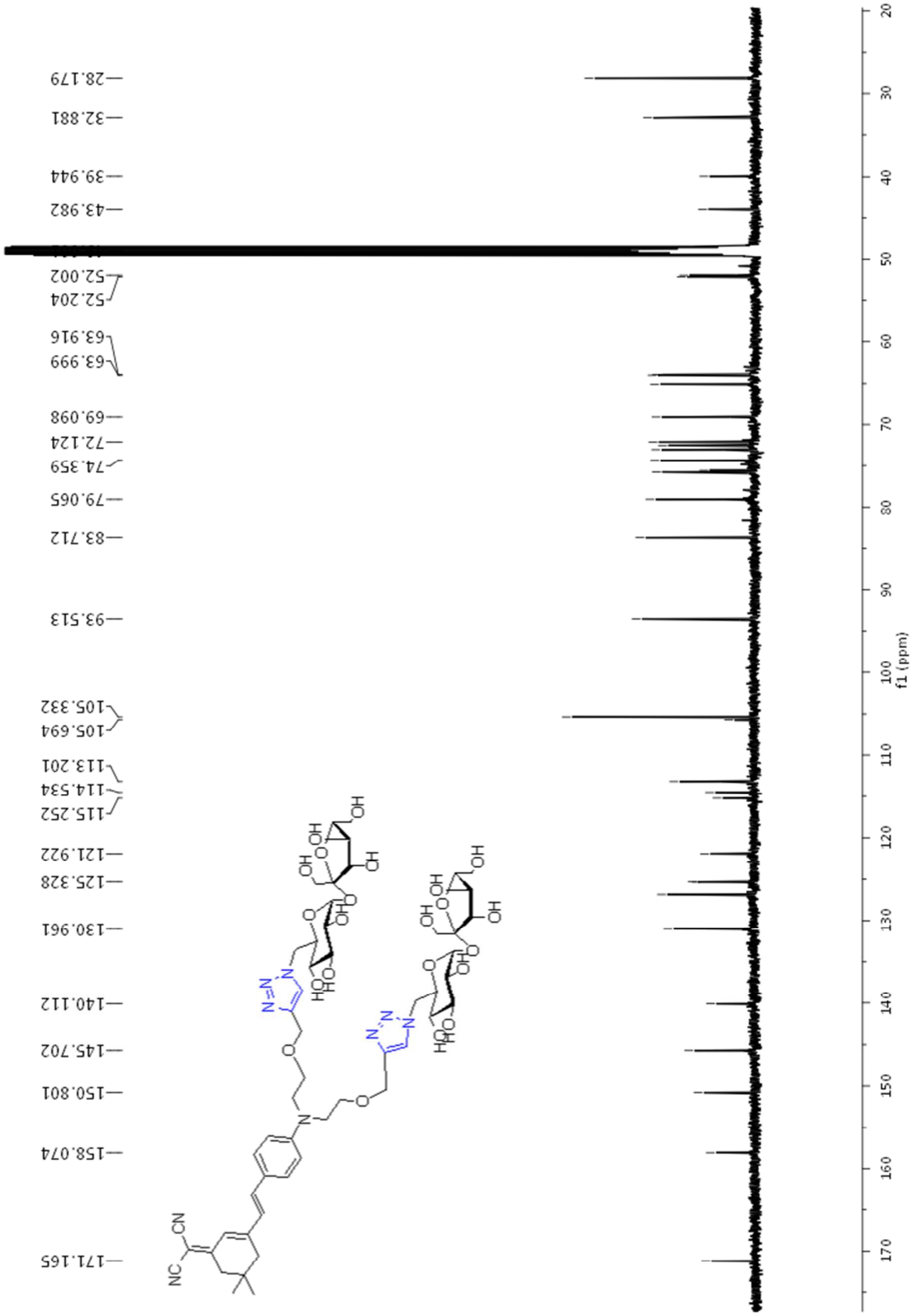
Compound 18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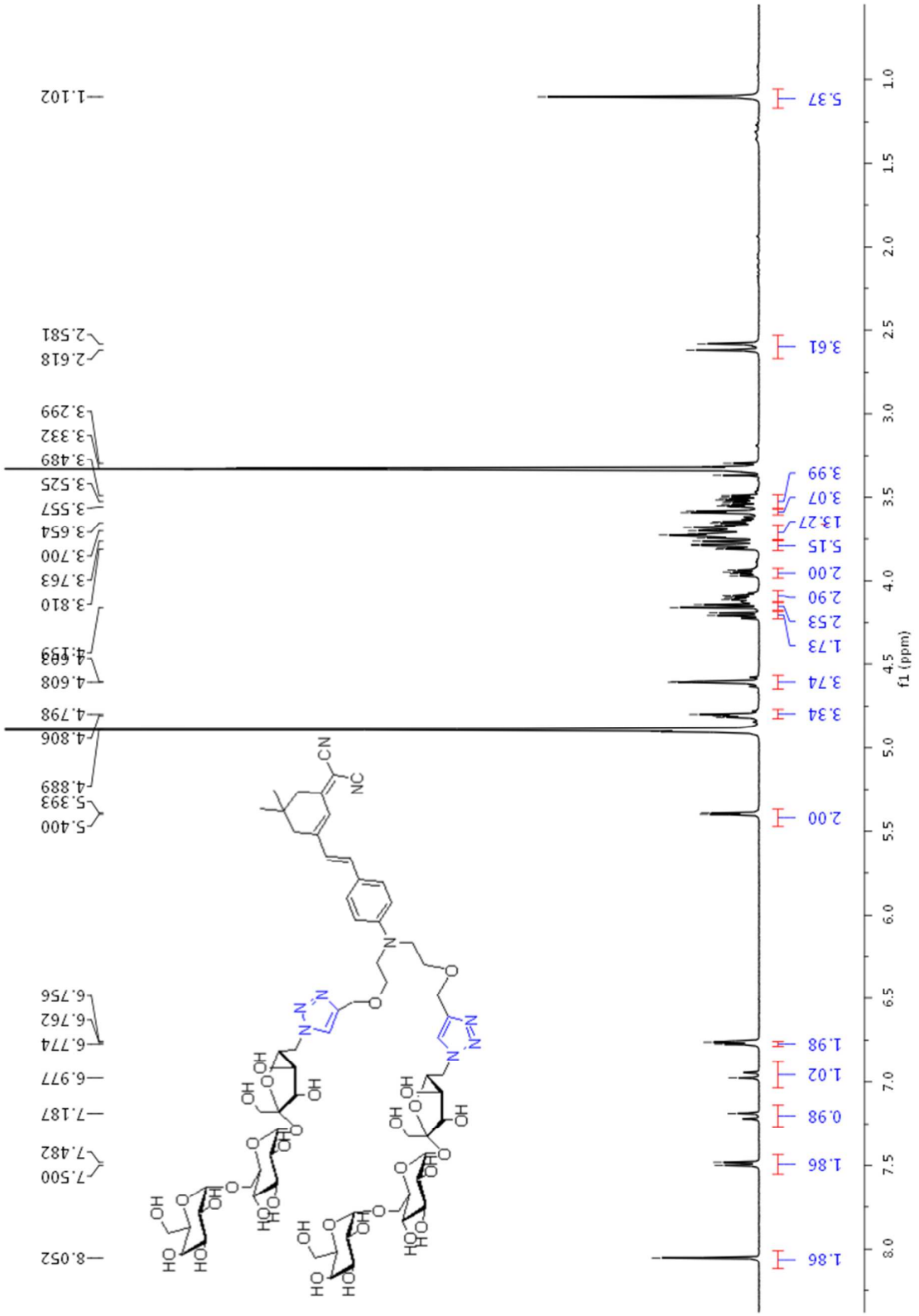
Compound 19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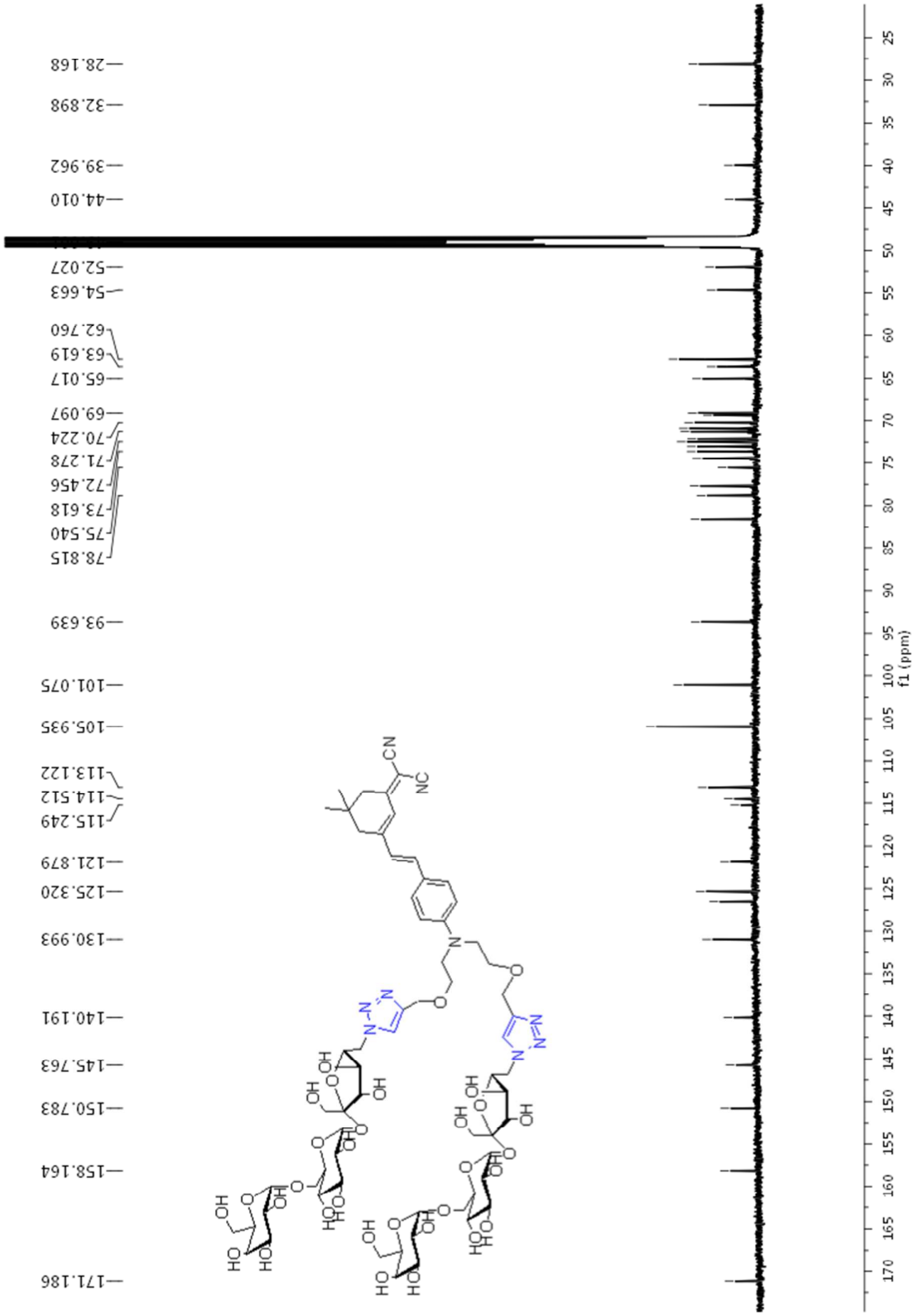
f1 (ppm)

Compound 19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78.815
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140.191
145.763
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158.164
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f1 (ppm)