Supporting Information

Exploring the divalent effect in fucosidase inhibition with stereoisomeric pyrrolidine dimers

Audrey Hottin,[a] Daniel W. Wright,[b] Elena Moreno-Clavijo,[c] Antonio J. Moreno-Vargas,[c] Gideon J. Davies[b] and Jean-Bernard Behr*[a]

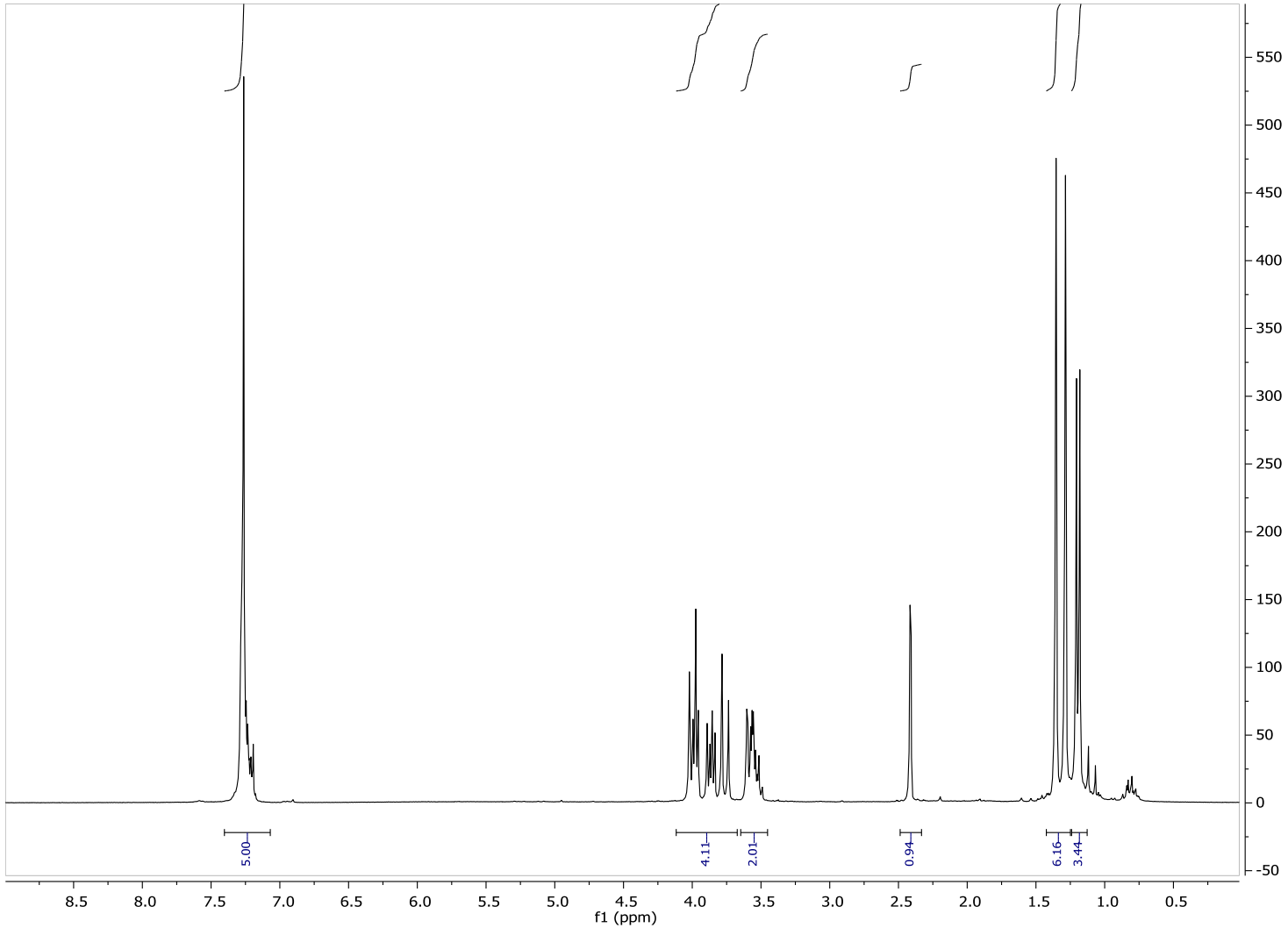
Supporting Information

E-mail: jb.behr@univ-reims.fr

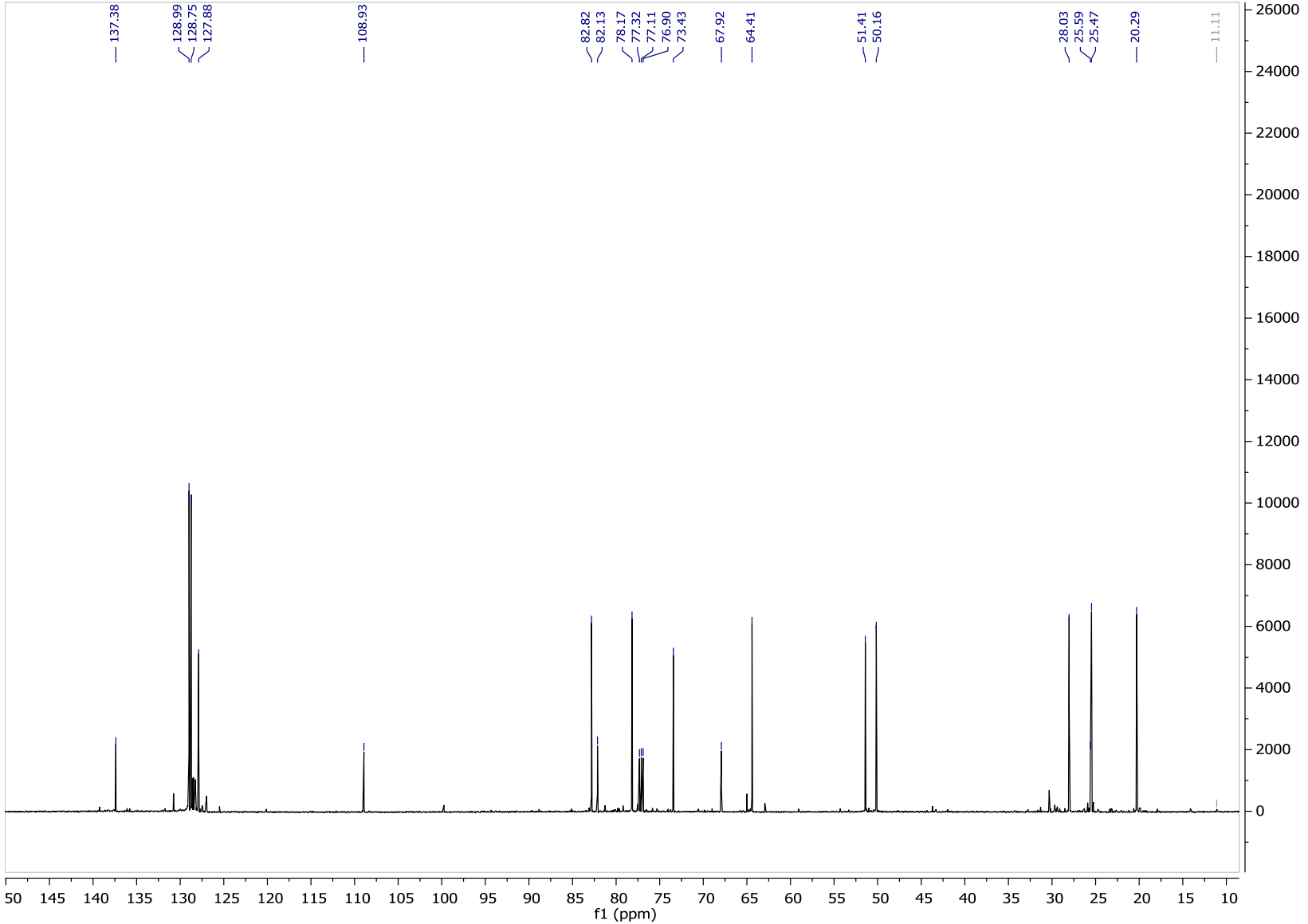
Chemistry :

***General considerations*:** All reagents and solvents were commercially available in high purity and used as received. Silica gel F_{254} (0.2 mm) was used for TLC plates, detection being carried out by spraying with an alcoholic solution of phosphomolybdic acid, *p*-anisaldehyde or an aqueous solution of $KMnO_4$ (2%) / Na_2CO_3 (4%), followed by heating. Flash column chromatography was performed over silica gel M 9385 (40-63 μm) Kieselgel 60. NMR spectra were recorded on Bruker AC 250 (250 MHz for ^1H, 62.5 MHz for ^{13}C) or 600 (600 MHz for ^1H, 150 MHz for ^{13}C) spectrometers. Chemical shifts are expressed in parts per million (ppm) and were calibrated to the residual solvent peak. Coupling constants are in Hz and splitting pattern abbreviations are: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; qt, quintuplet; m, multiplet. IR spectra were recorded with an IR^{TM} plus MIDAC spectrophotometer and are expressed in cm^{-1}. Optical rotations were determined at 20 °C with a Perkin-Elmer Model 241 polarimeter in the specified solvents. High Resolution Mass Spectra (HRMS) were performed on Q-TOF Micro micromass positive ESI (CV = 30 V).

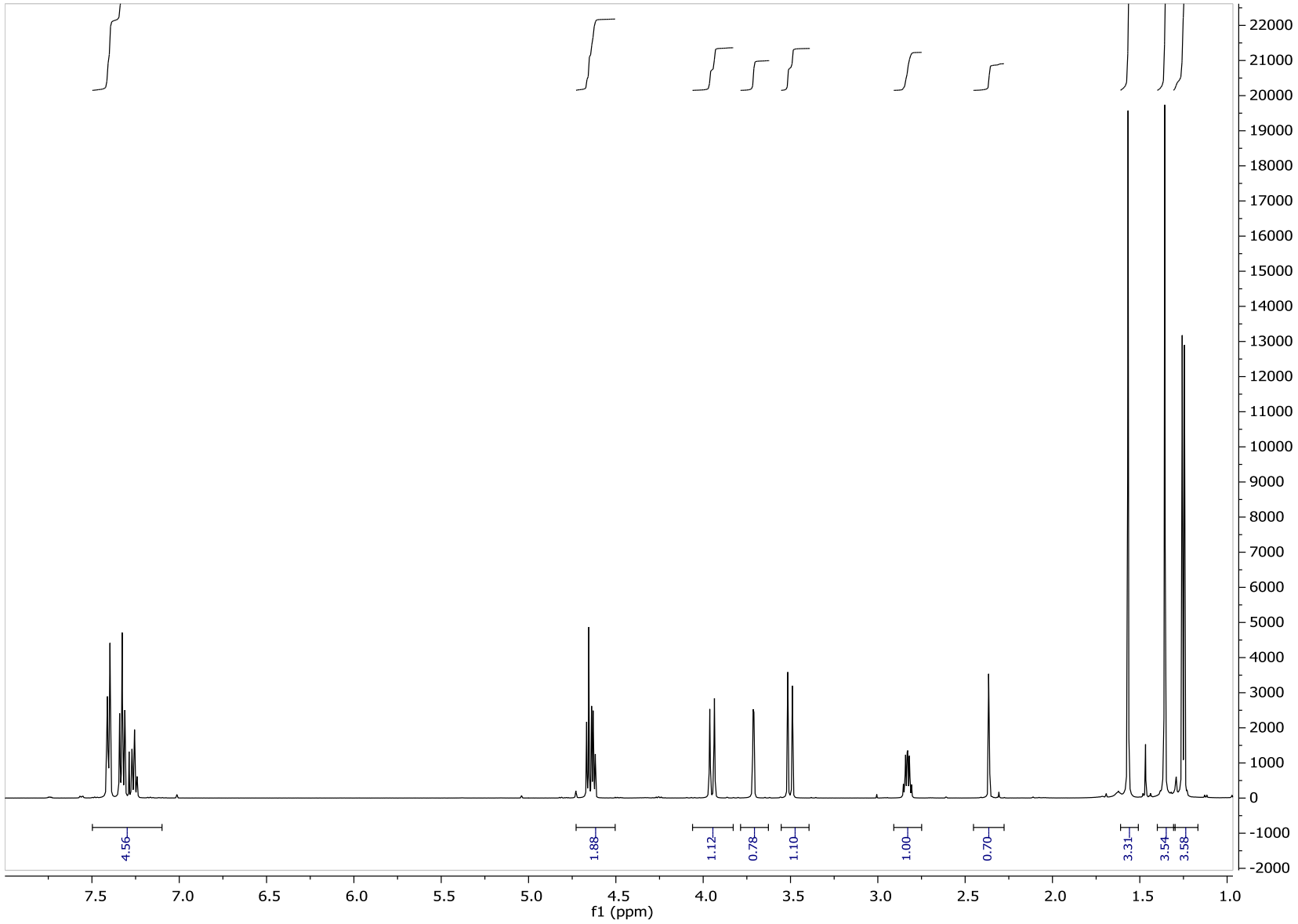
^{1}H-NMR spectrum of compound 7 ($CDCl_3$, 600 MHz)



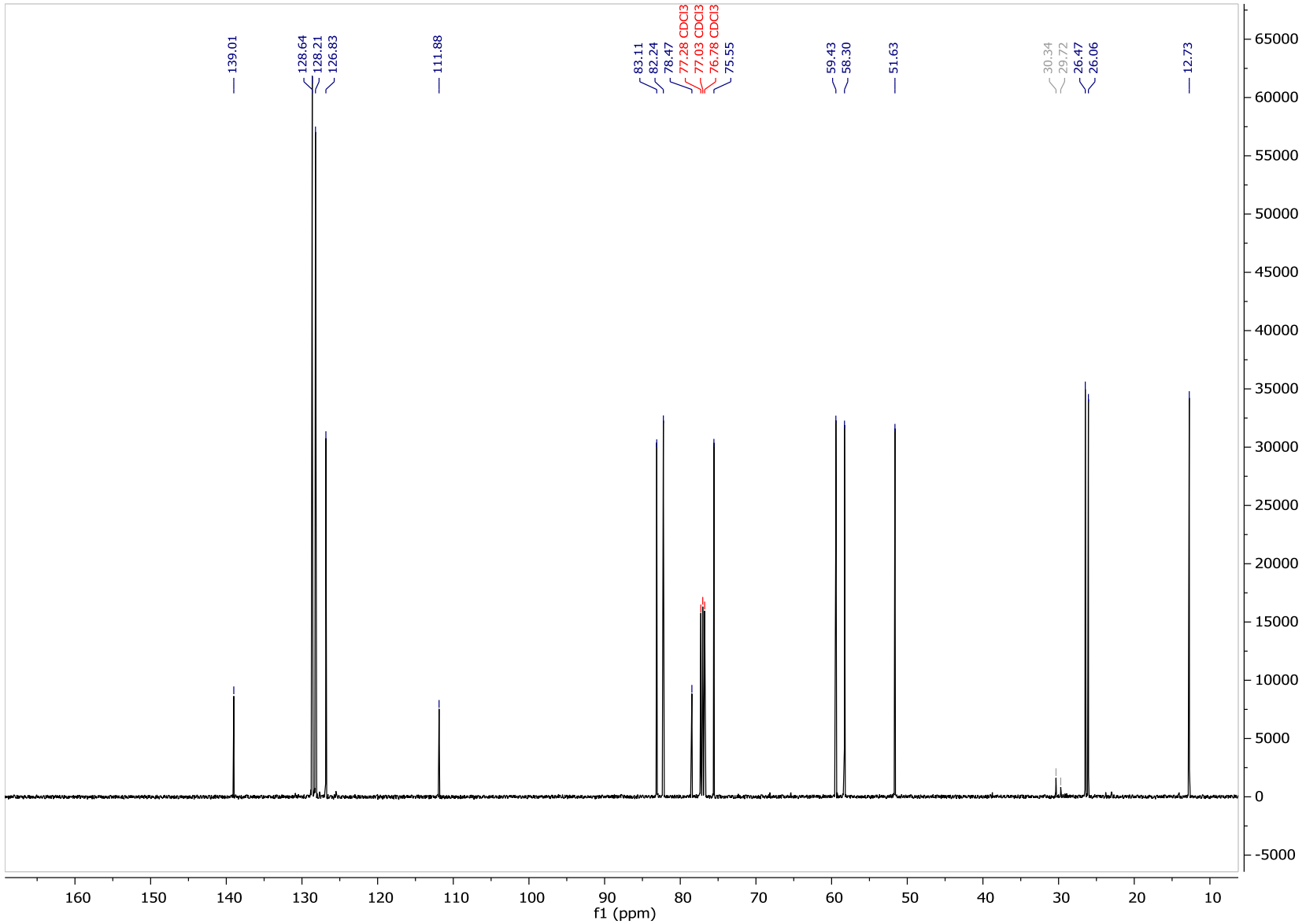
^{13}C-NMR spectrum of compound 7 ($CDCl_3$, 151 MHz)



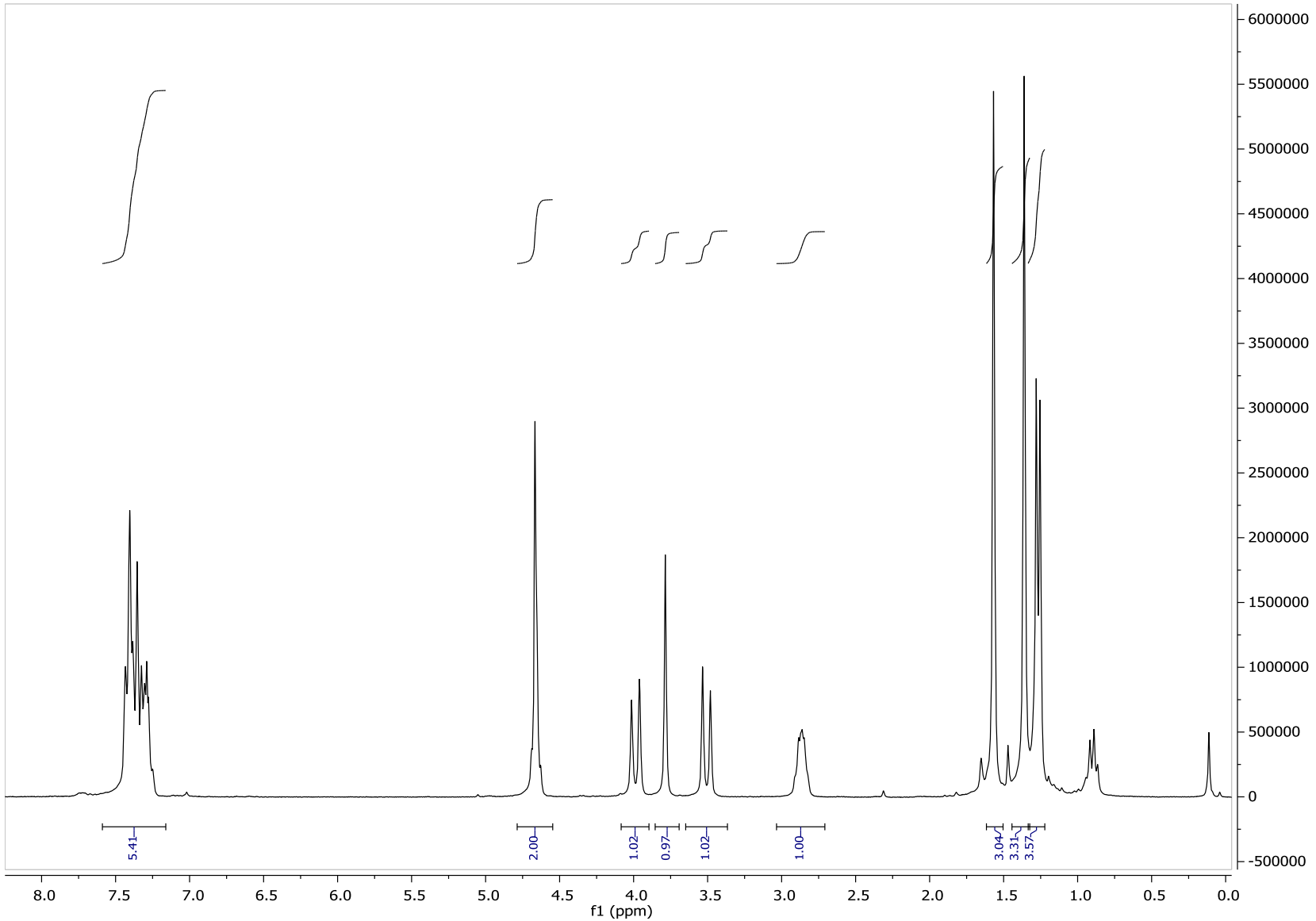
^{1}H-NMR spectrum of compound 8 ($CDCl_3$, 500 MHz)



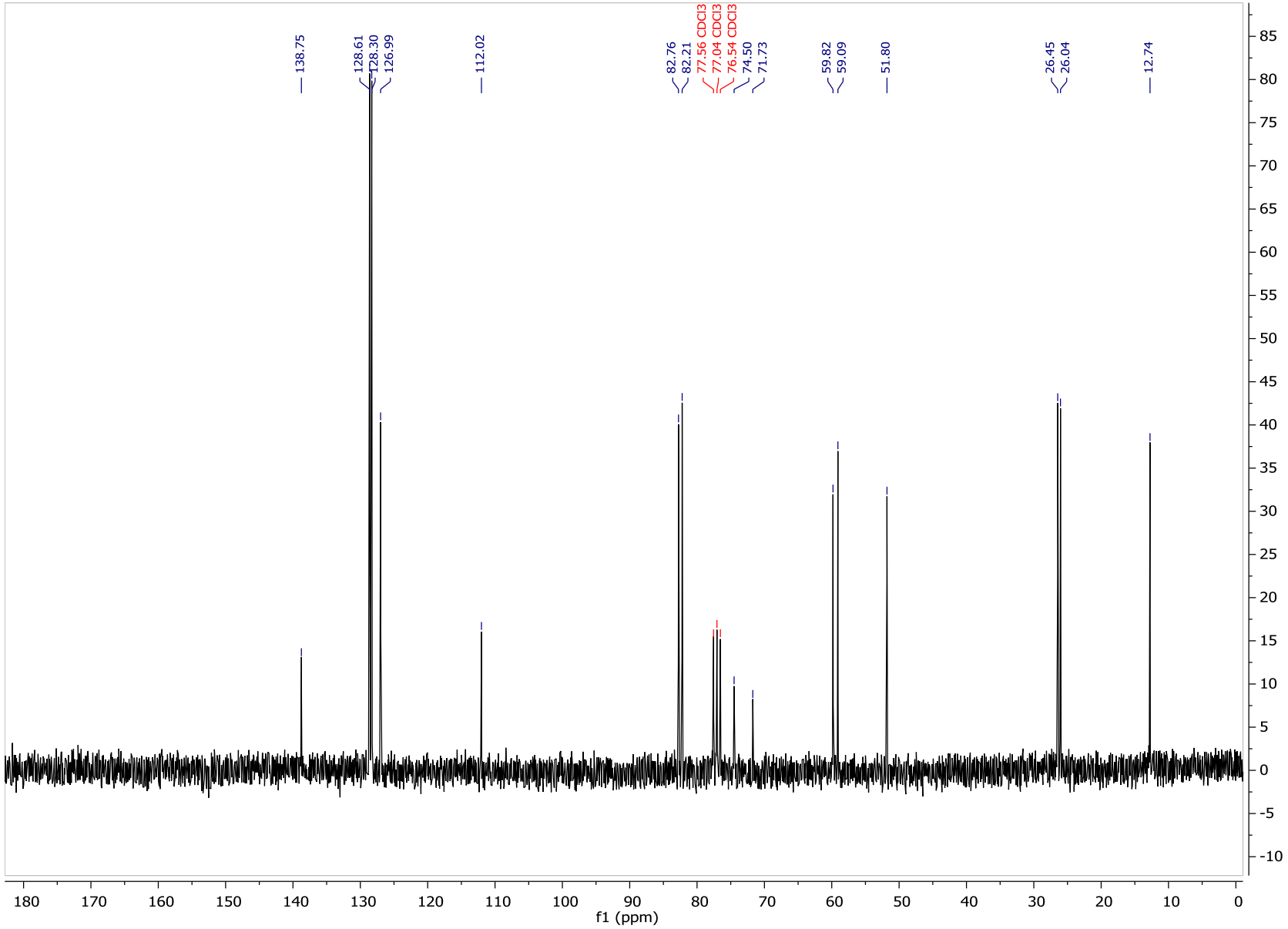
^{13}C-NMR spectrum of compound 8 ($CDCl_3$, 126 MHz)



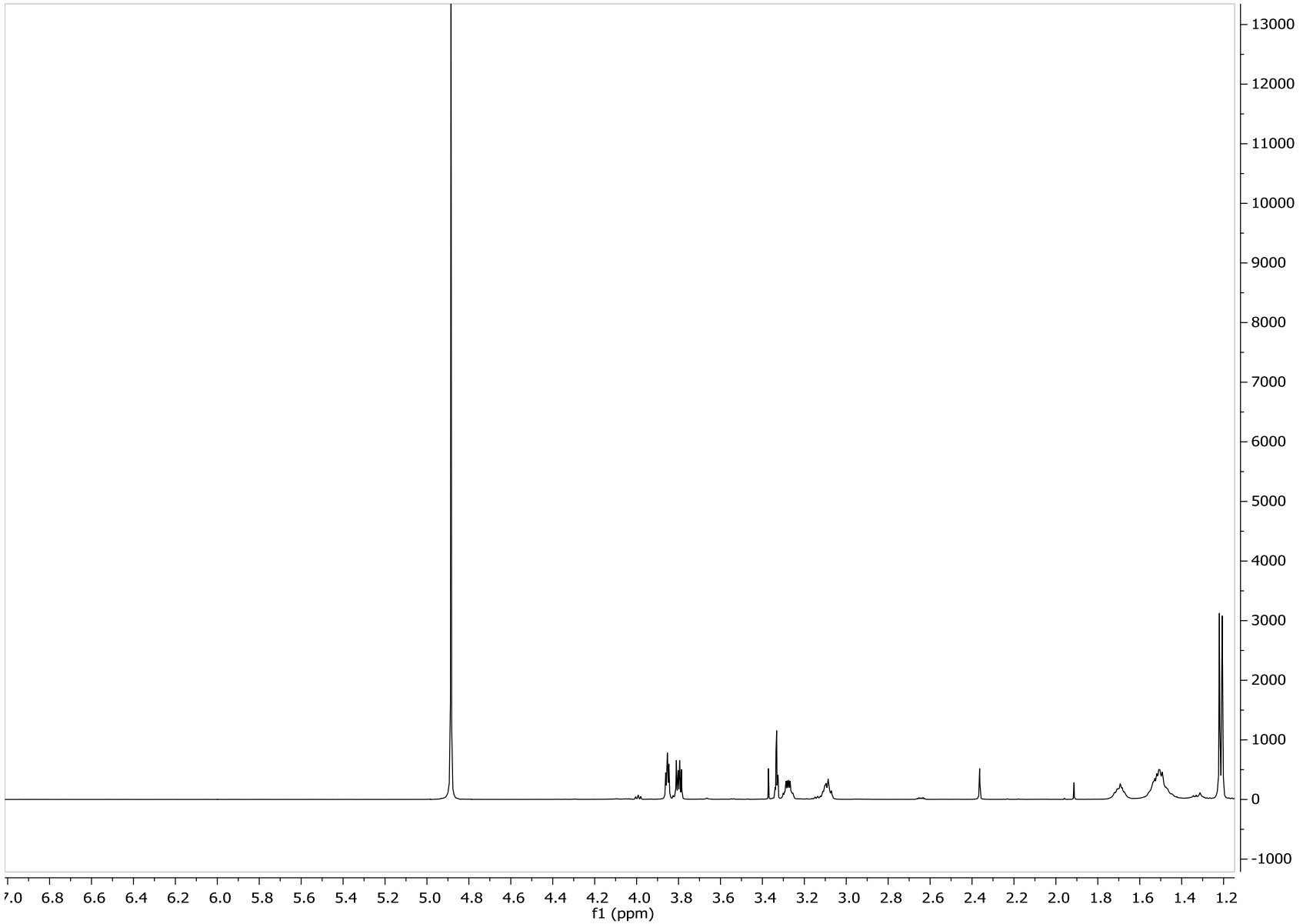
^{1}H-NMR spectrum of compound 9 ($CDCl_3$, 250 MHz)



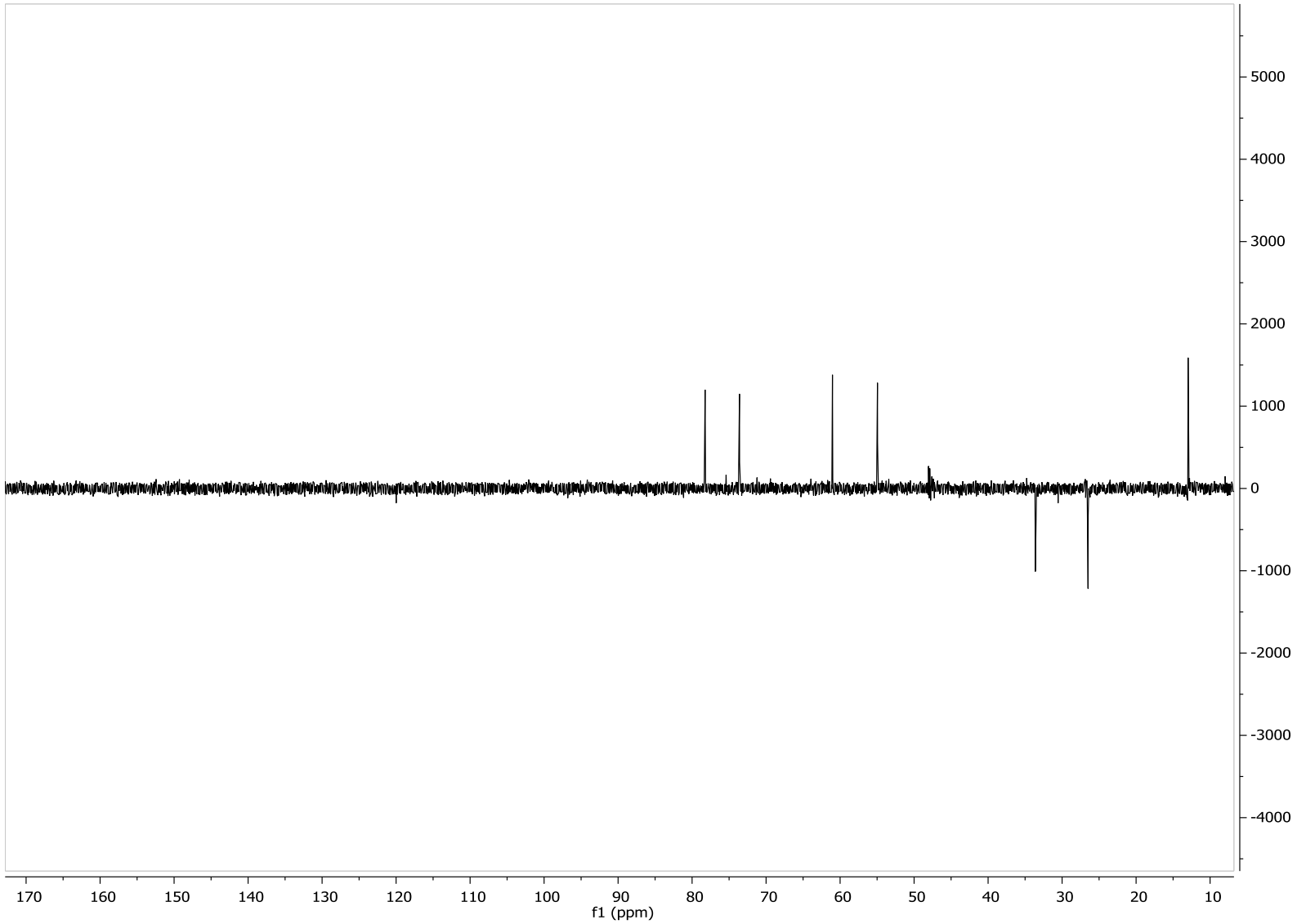
^{13}C-NMR spectrum of compound 9 ($CDCl_3$, 63 MHz)



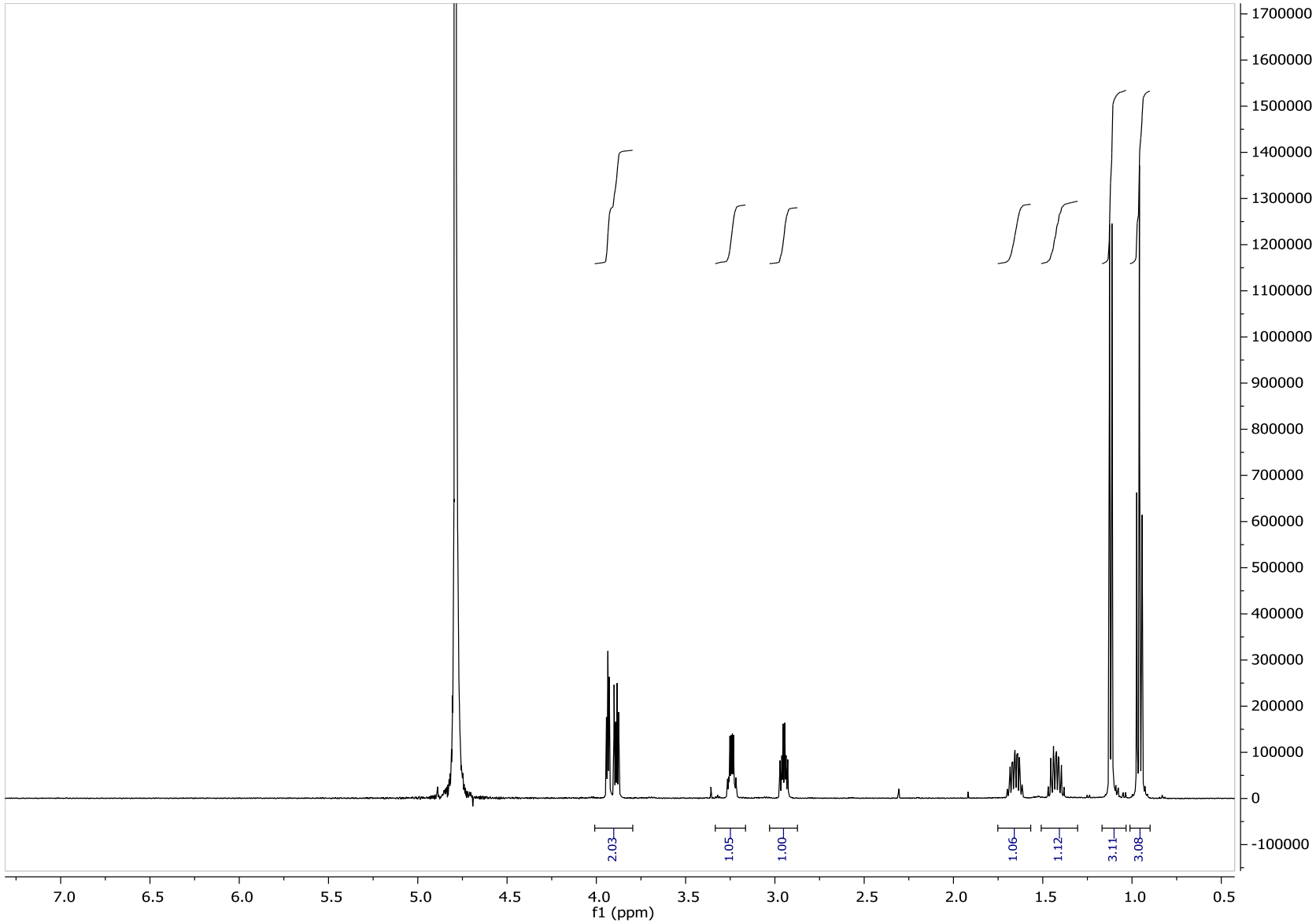
^{1}H-NMR spectrum of compound 1 (CD_3OD, 500 MHz)



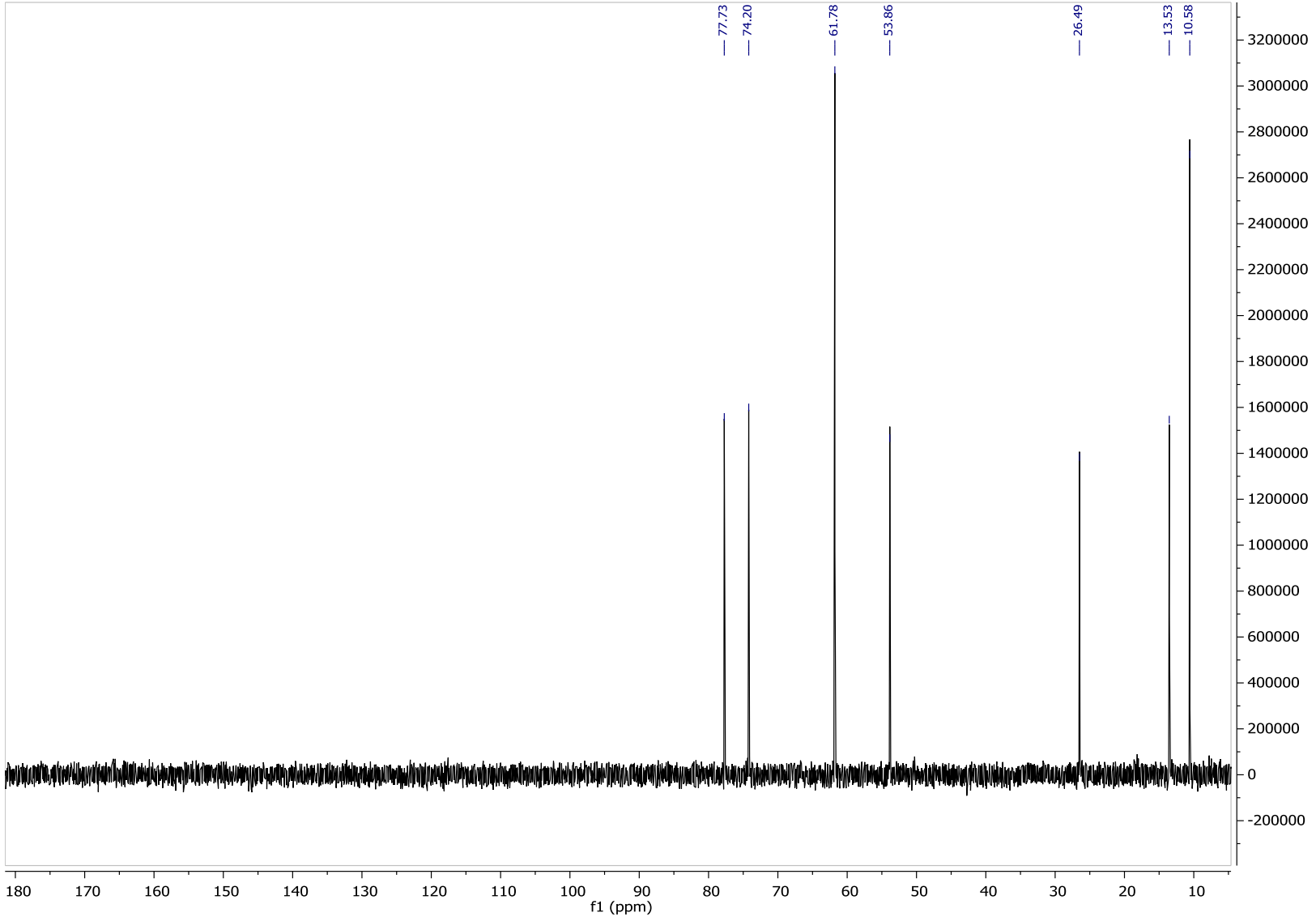
^{13}C-NMR (DEPT) spectrum of compound 1 (CD_3OD, 126 MHz)



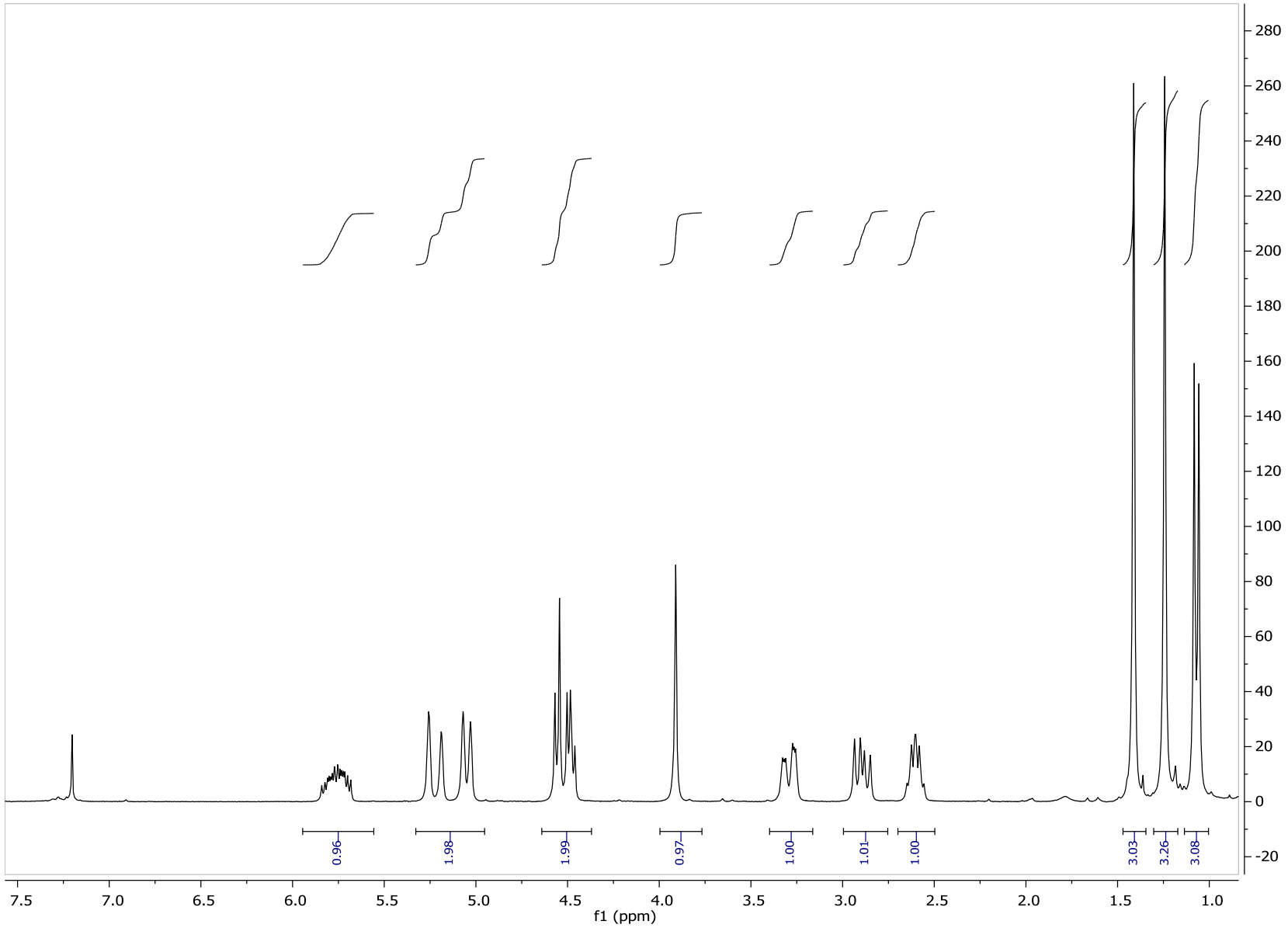
^{1}H-NMR spectrum of compound 3 (D_2O, 500 MHz)


1700000
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
2.03
1.05
1.00
1.06
1.12
3.11
3.08
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

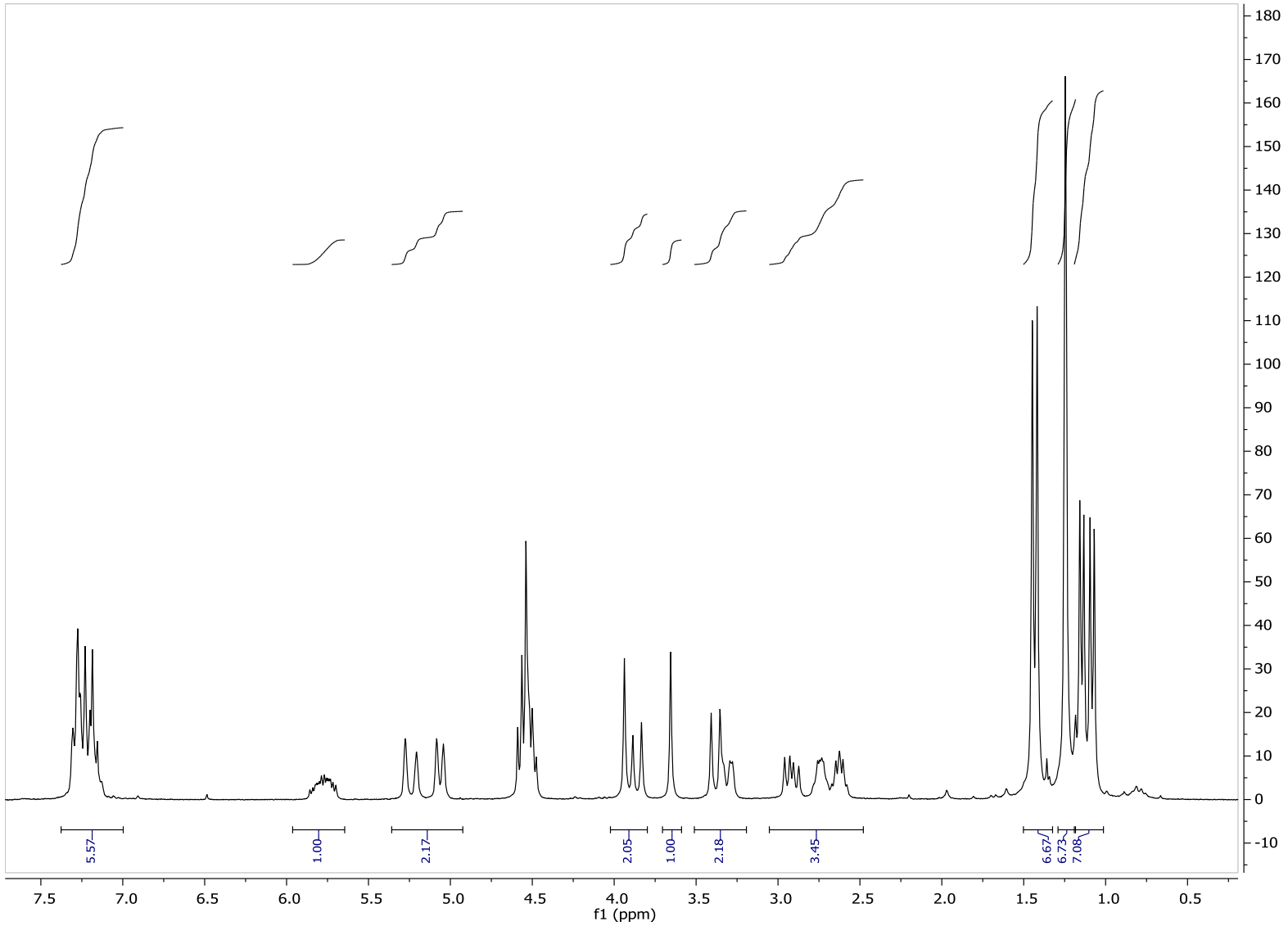
^{13}C-NMR spectrum of compound 3 (D_2O, 126 MHz)



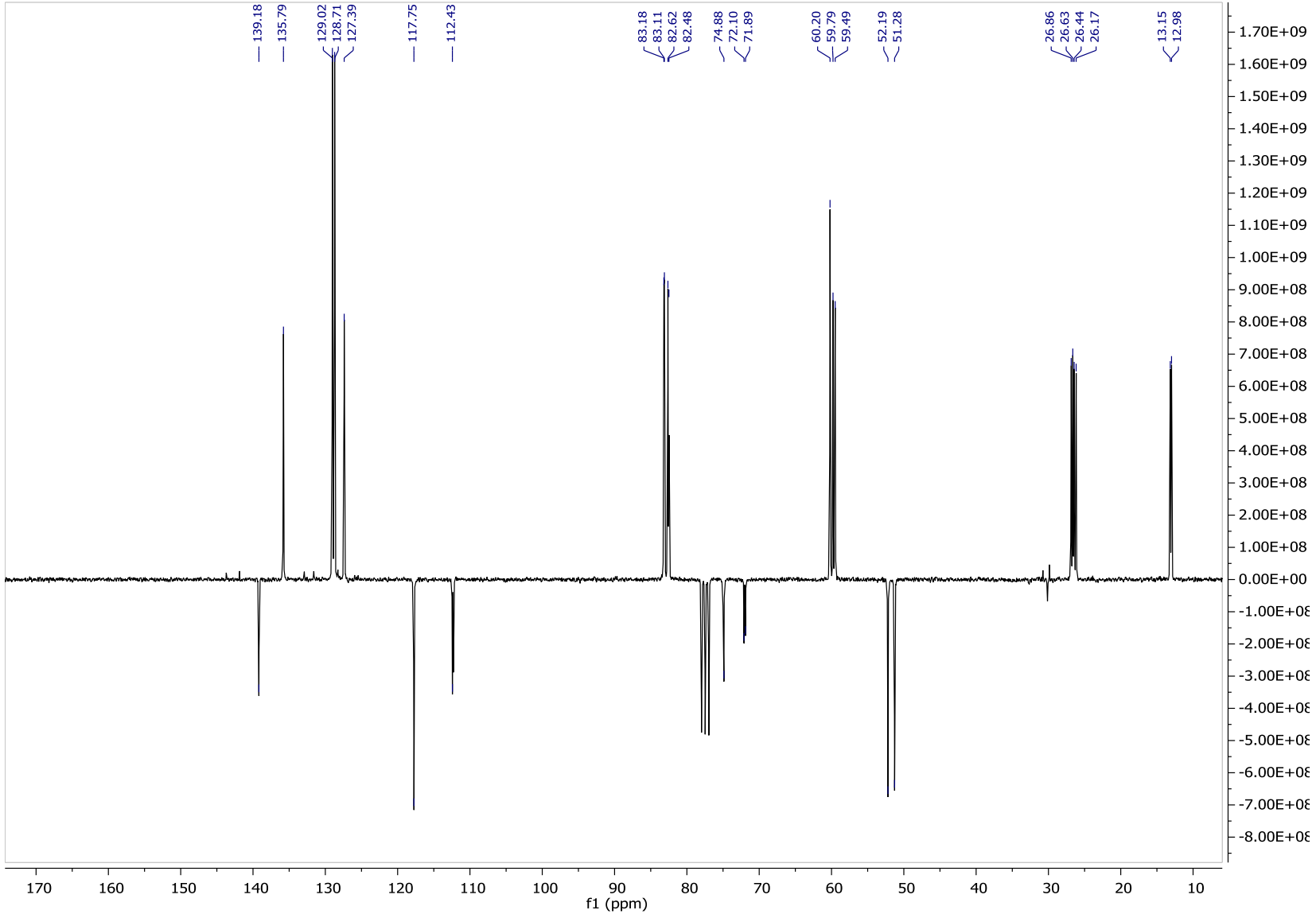
^{1}H-NMR spectrum of compound 12 ($CDCl_3$, 500 MHz)



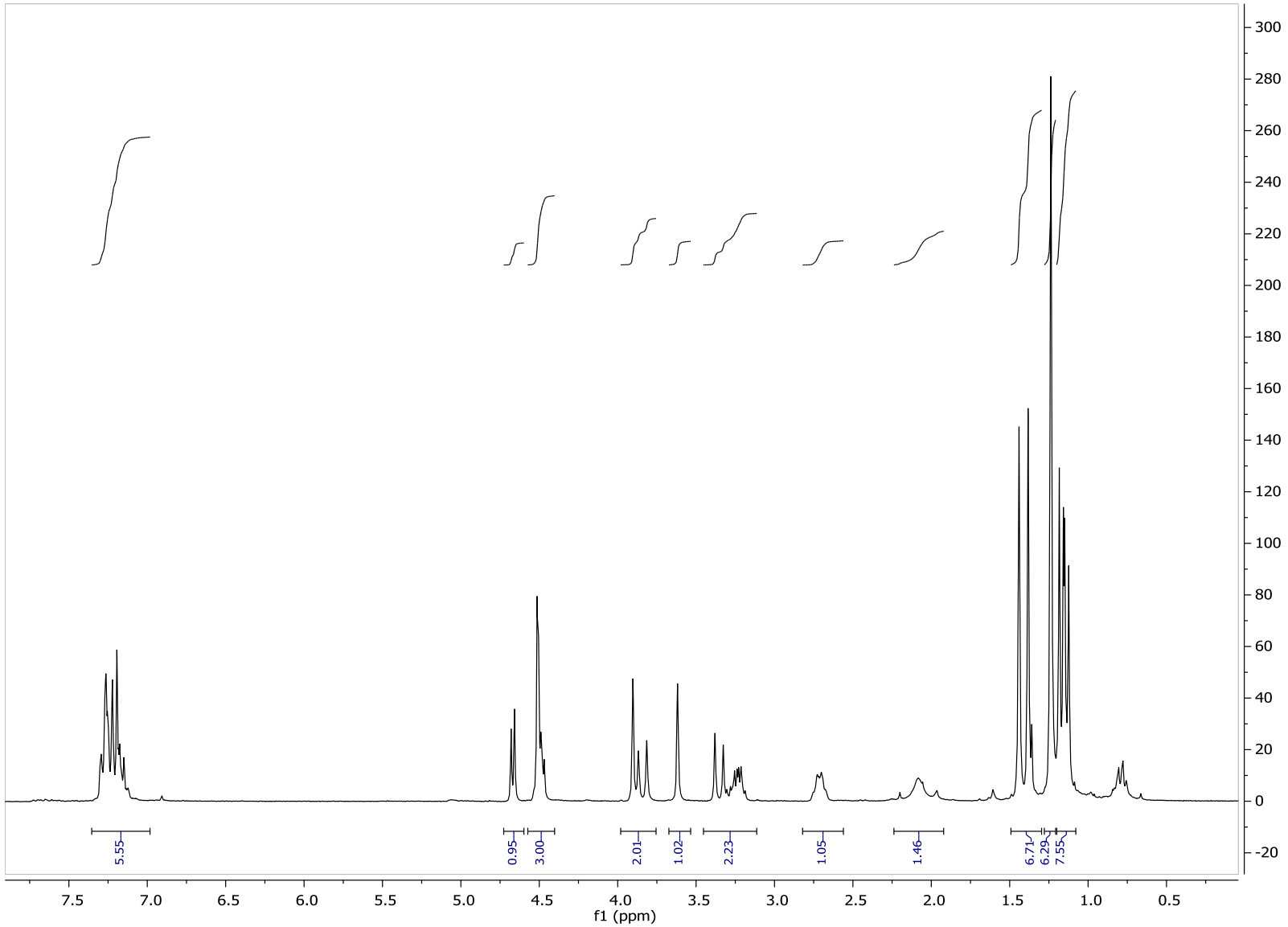
^{1}H-NMR spectrum of compound 13 ($CDCl_3$, 250 MHz)



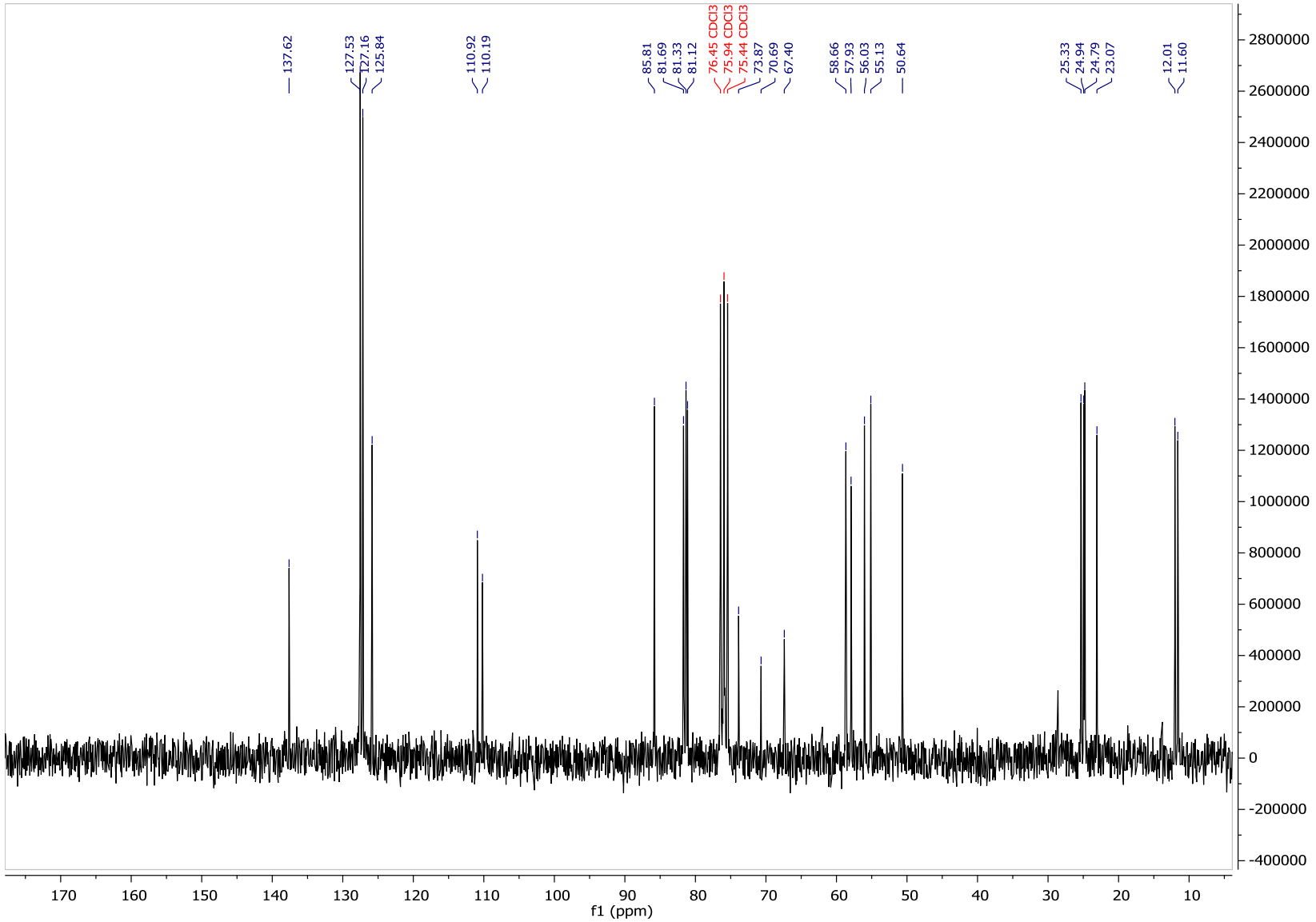
^{13}C-NMR (JMOD) spectrum of compound 13 ($CDCl_3$, 63 MHz)



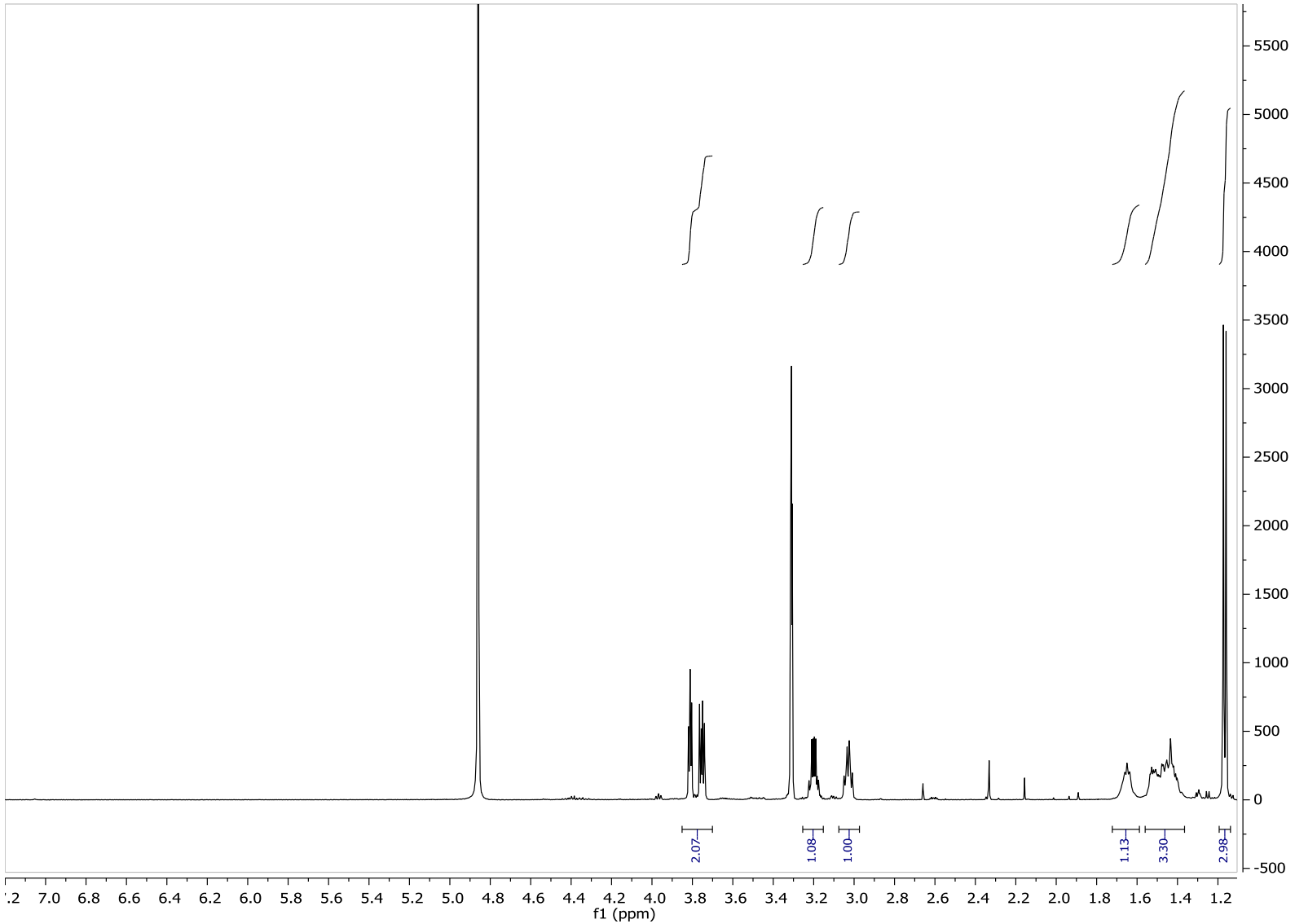
^{1}H-NMR spectrum of compound 14 ($CDCl_3$, 250 MHz)



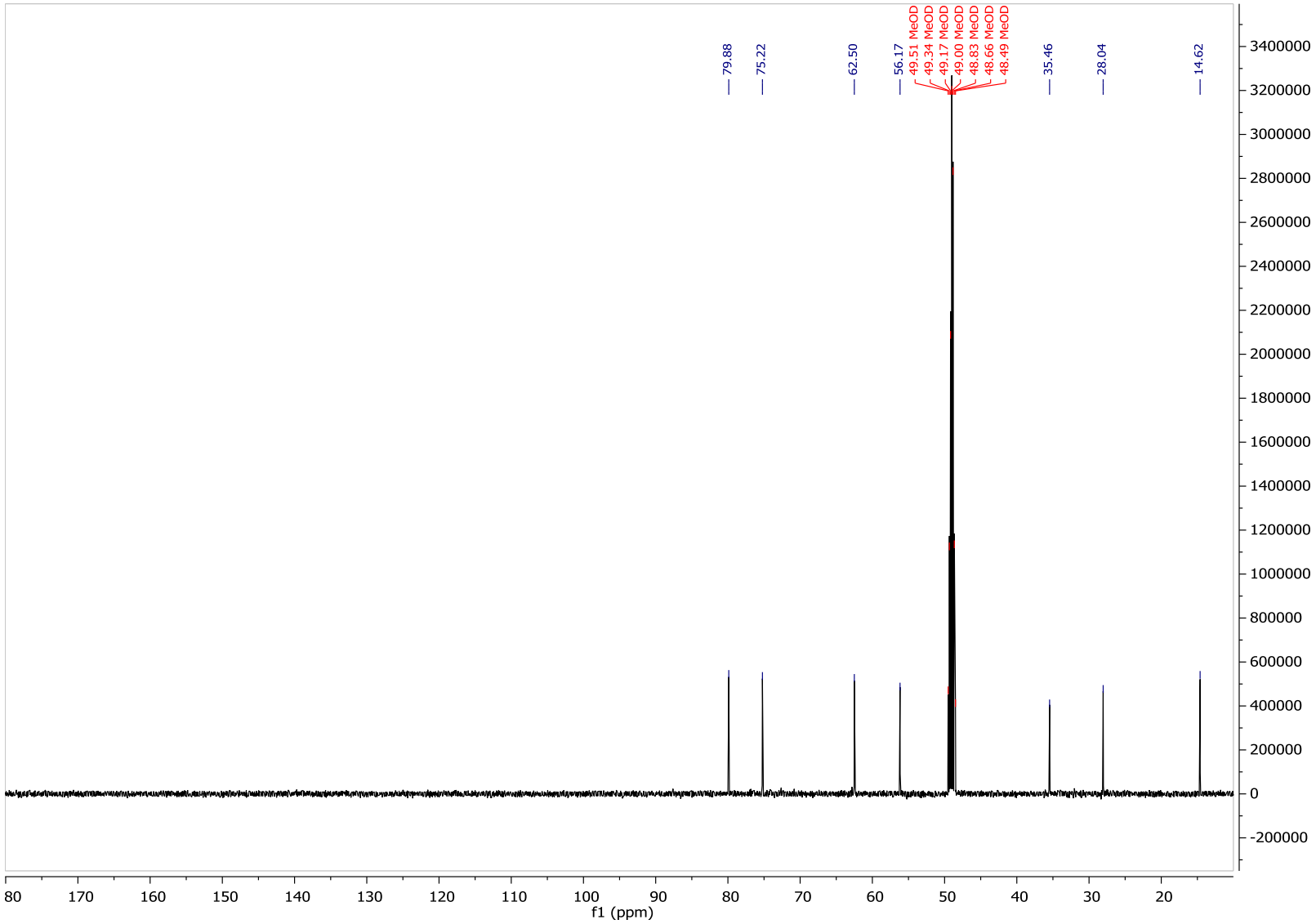
^{13}C-NMR spectrum of compound 14 ($CDCl_3$, 63 MHz)



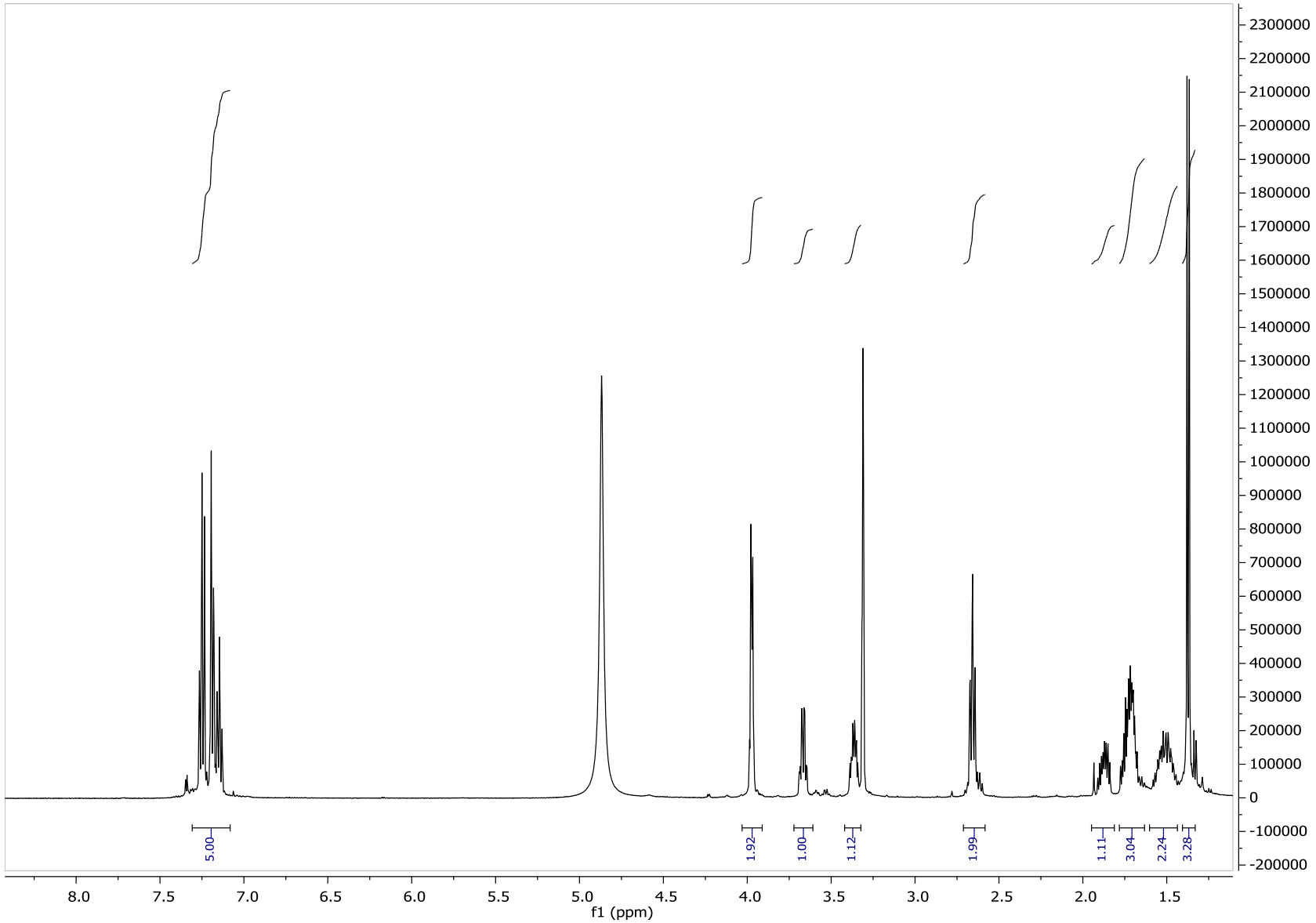
^{1}H-NMR spectrum of compound *meso*-1 (CD_3OD, 500 MHz)



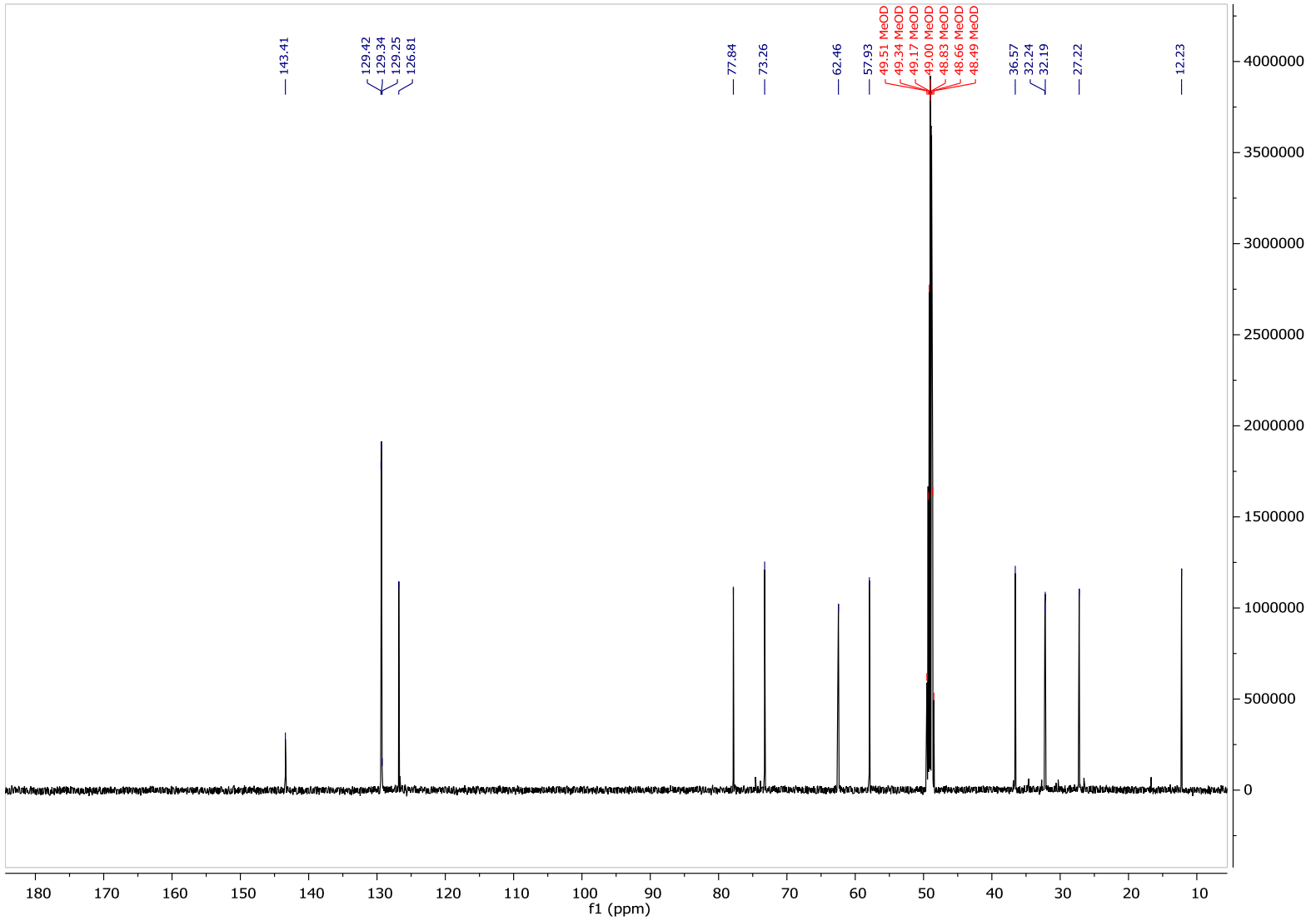
^{13}C-NMR spectrum of compound *meso*-1 (CD_3OD, 126 MHz)



^{1}H-NMR spectrum of compound 4 (CD_3OD, 500 MHz)



^{13}C-NMR spectrum of compound 4 (CD_3OD, 126 MHz)



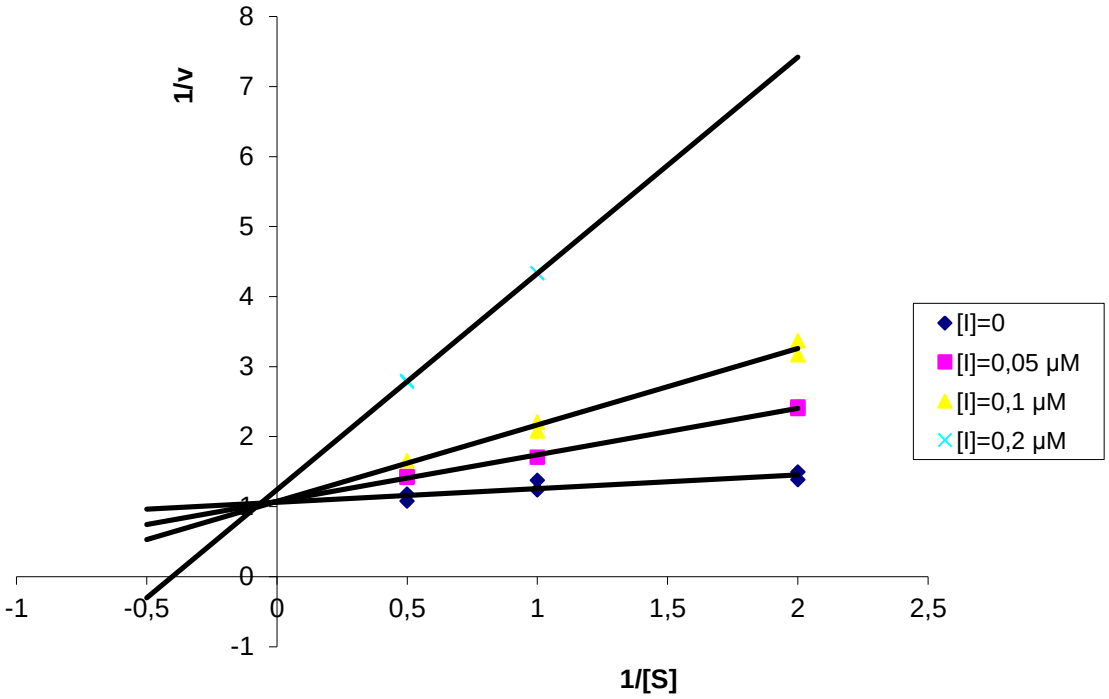
Crystal structure determination :

	*Bt*Fuc2970-**1**
Data collection	
Beamline/Date	DLS I03 02.02.2014
Wavelength (Å)	0.97625
Cell dimensions	
a, *b*, *c* (Å)	56.1,187.7,97.6
α, β, γ (°)	90,94.2,90
Resolution (Å)	67.6-1.83
R_{merge}	0.09(0.79)
$I / \sigma I$	6.8(1.5)
Completeness (%)	99.8(99.8)
Redundancy	3.8(3.9)
Wilson B value	34.7
Refinement	
Resolution (Å)	67.6-2.10
No. reflections	110778
R_{work} / R_{free}	0.19/0.23
No. atoms	
Protein	14332
Ligand/ion	140
Water	773
B-factors	
Protein	44.0
Ligand/ion	60.7
Water	42.9
R.m.s. deviations	
Bond lengths (Å)	0.014
Bond angles (°)	1.5
Ramachandran Statistics (%)	
Preferred	96.1
Allowed	2.9
Outliers	1
PDB codes	5I5R

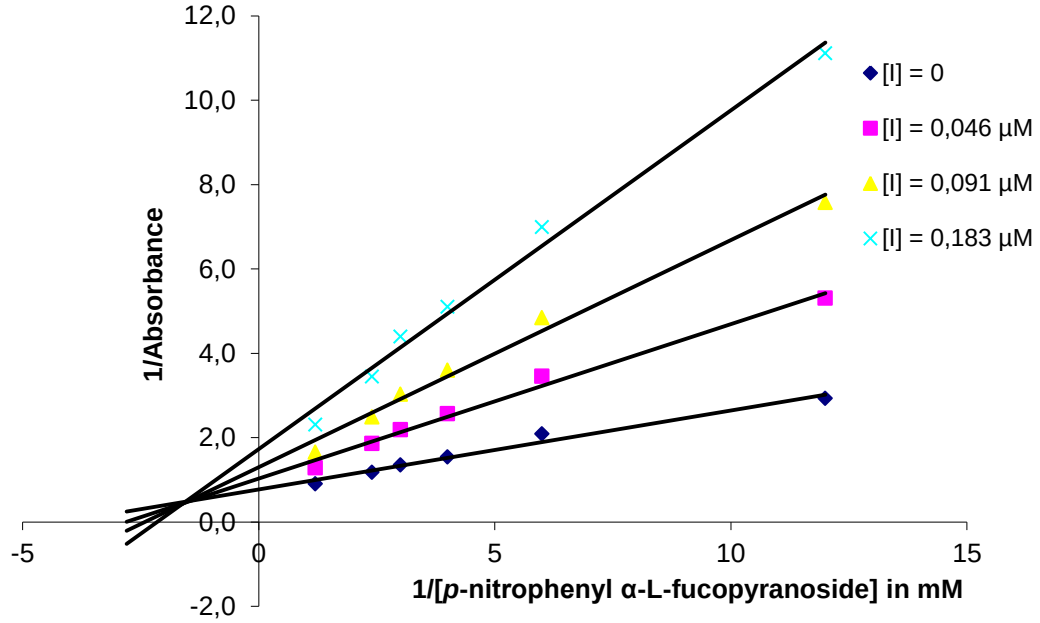
Table S1. MacromolecularX-ray data collection and refinement statistics.

Lineweaver-Burk plots for all tested compounds (bovine kidney fucosidase)

Compound **1**
K_i = 0,023 µM (competitive)

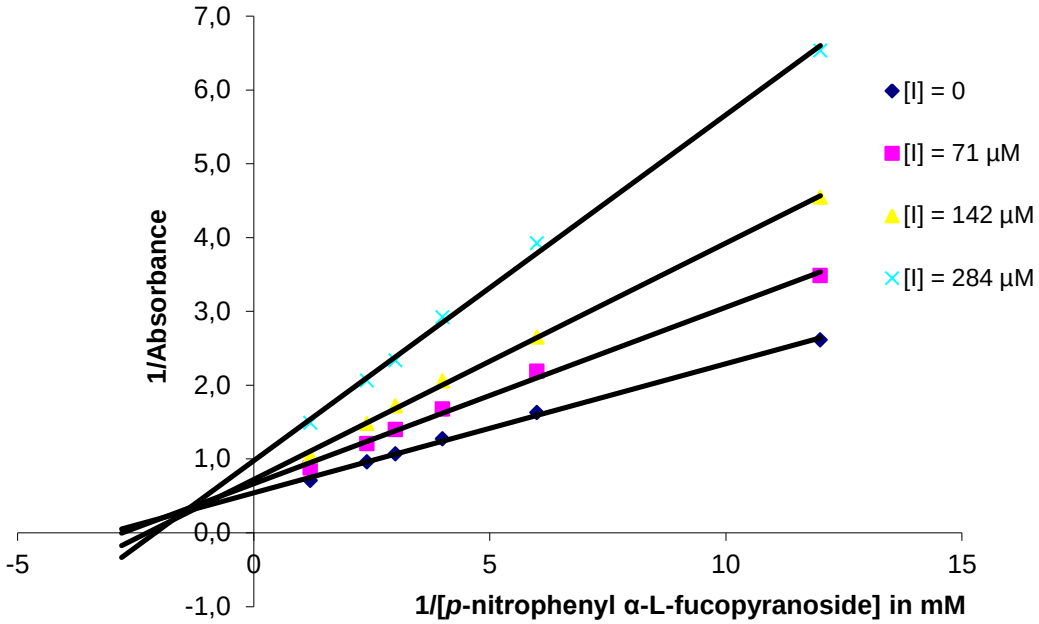


Compound ***meso*-1**
K_i = 0,051 µM (mixed type of inhibition, K' = 0,16 µM)



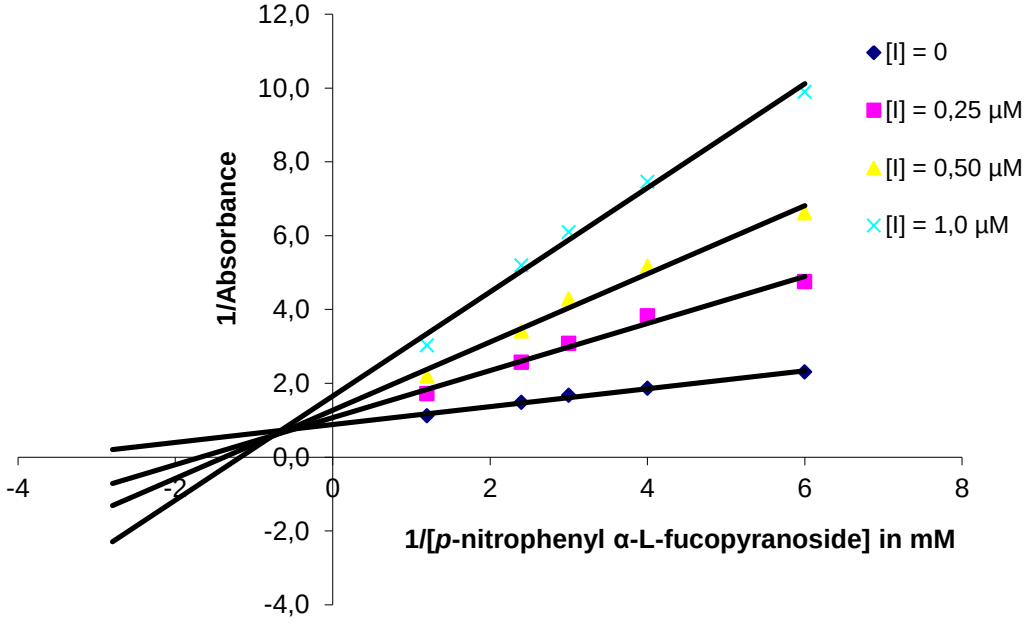
Compound ***ent*-1**

K_i = 178 µM (mixed type of inhibition, K' = 335 µM)



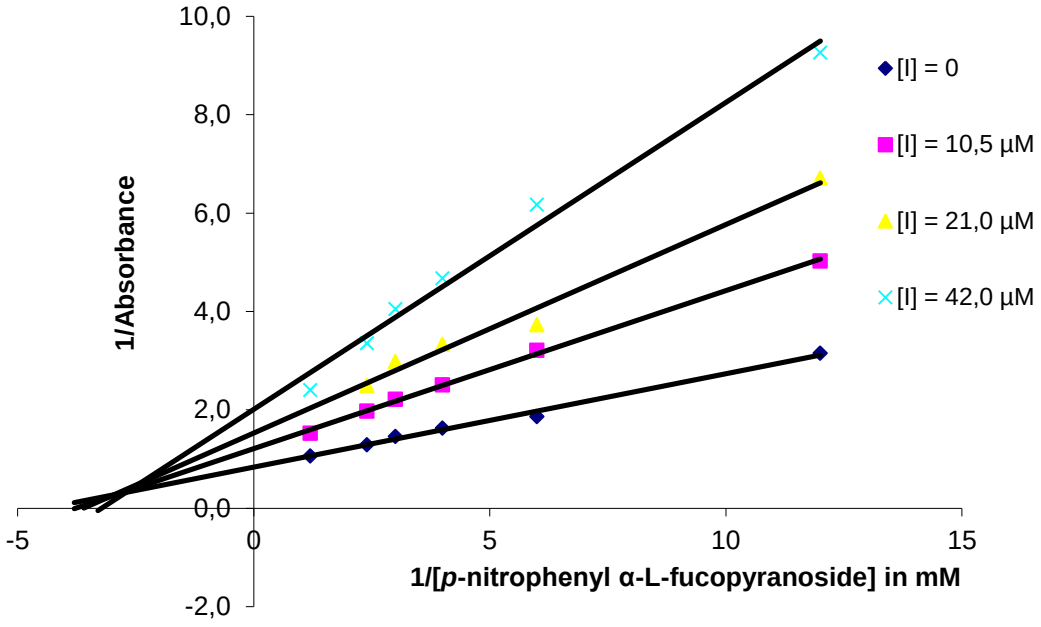
Compound **3**

K_i = 0,18 µM (mixed type of inhibition, K' = 1,12 µM)



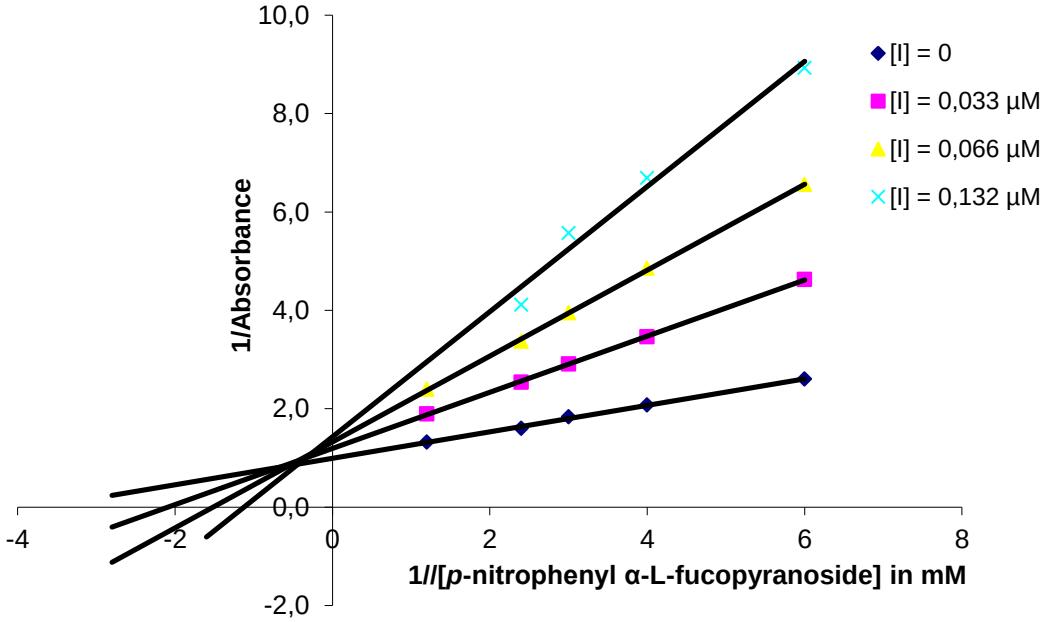
Compound ***ent*-3**

K_i = 12 µM (mixed type of inhibition, K' = 20 µM)



Compound **4**

K_i = 0,031 µM (mixed type of inhibition, K' = 0,108 µM)



***Bt*Fuc2970 inhibition :** Inhibition of *Bt*Fuc2970 fucosidase by compound **1**, yielding a Ki of 1.1μM on this bacterial system. V_0 and V_i are rates in the absence and presence of inhibitor, respectively.