Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

A biologically relevant fluorescent probe to assess the binding of ceramide to the CERT transfer protein

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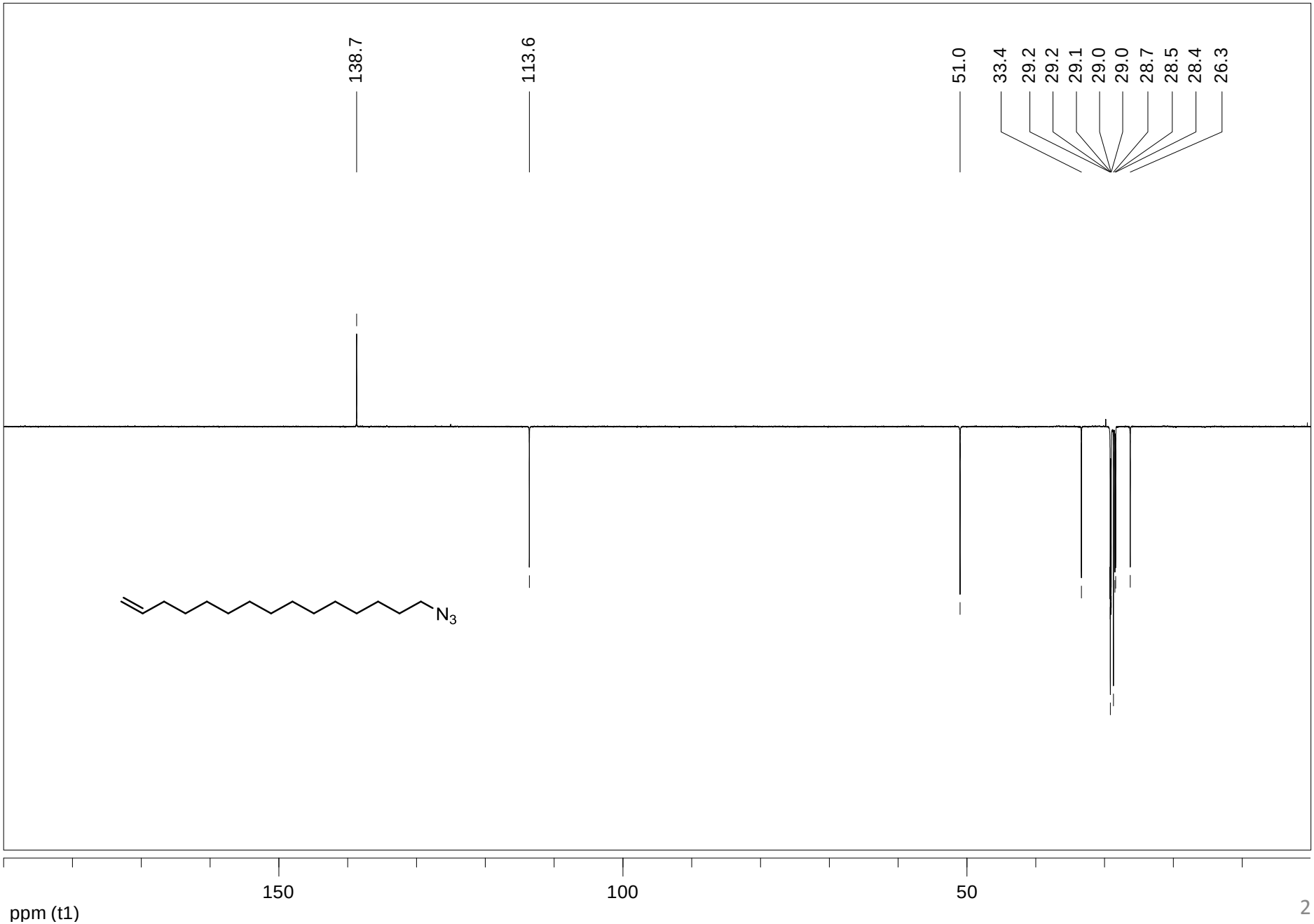
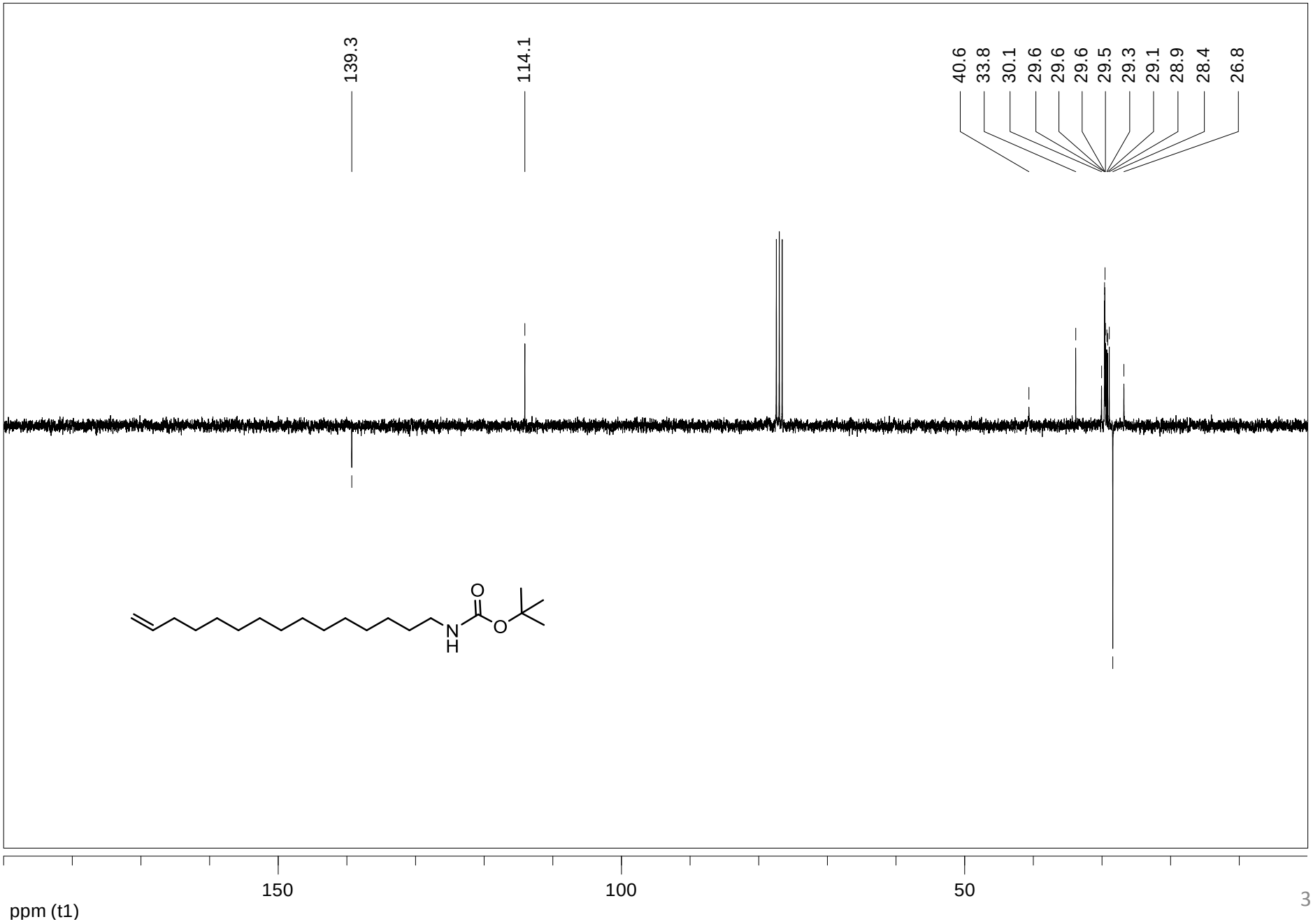
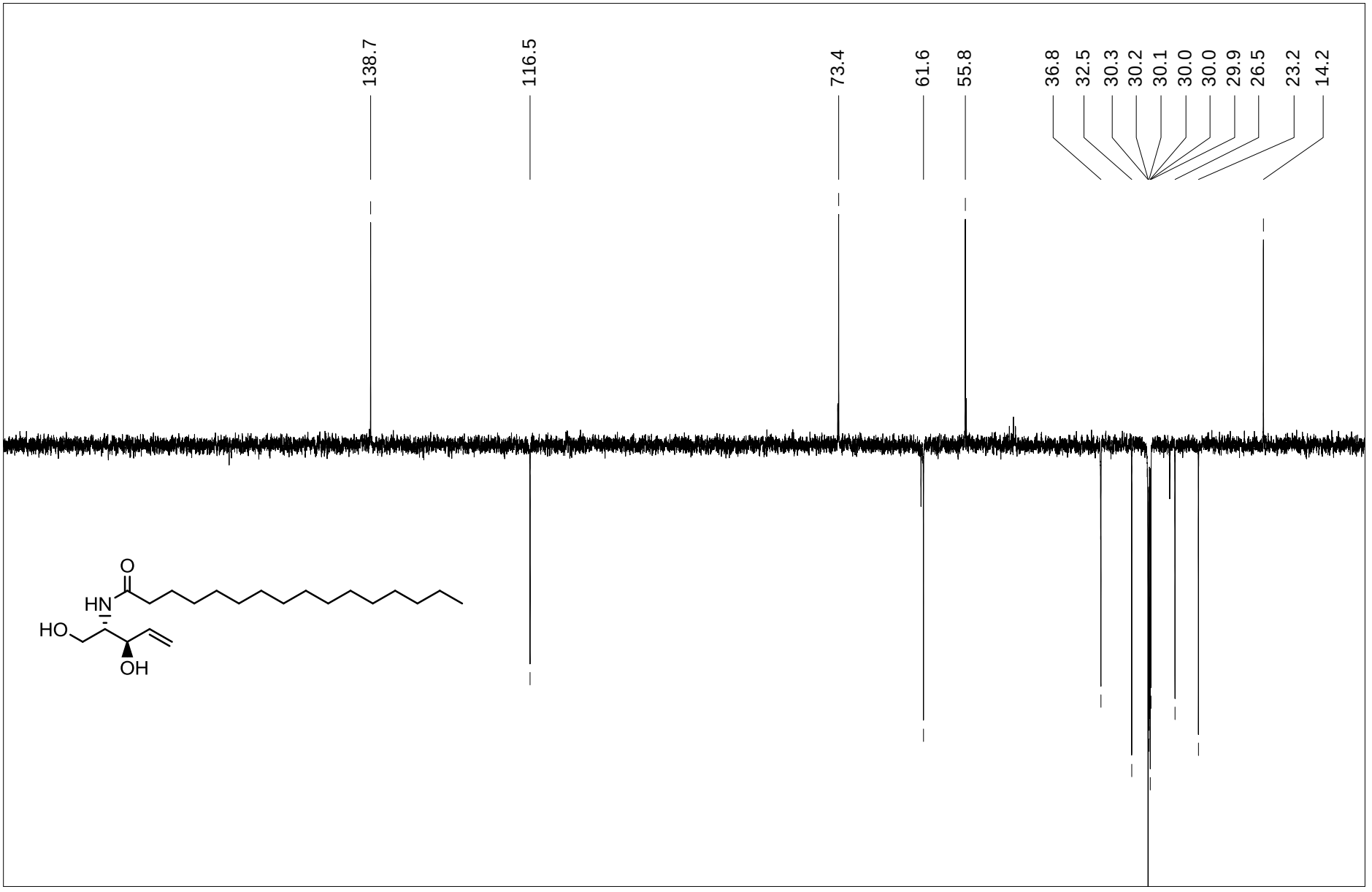
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **8**



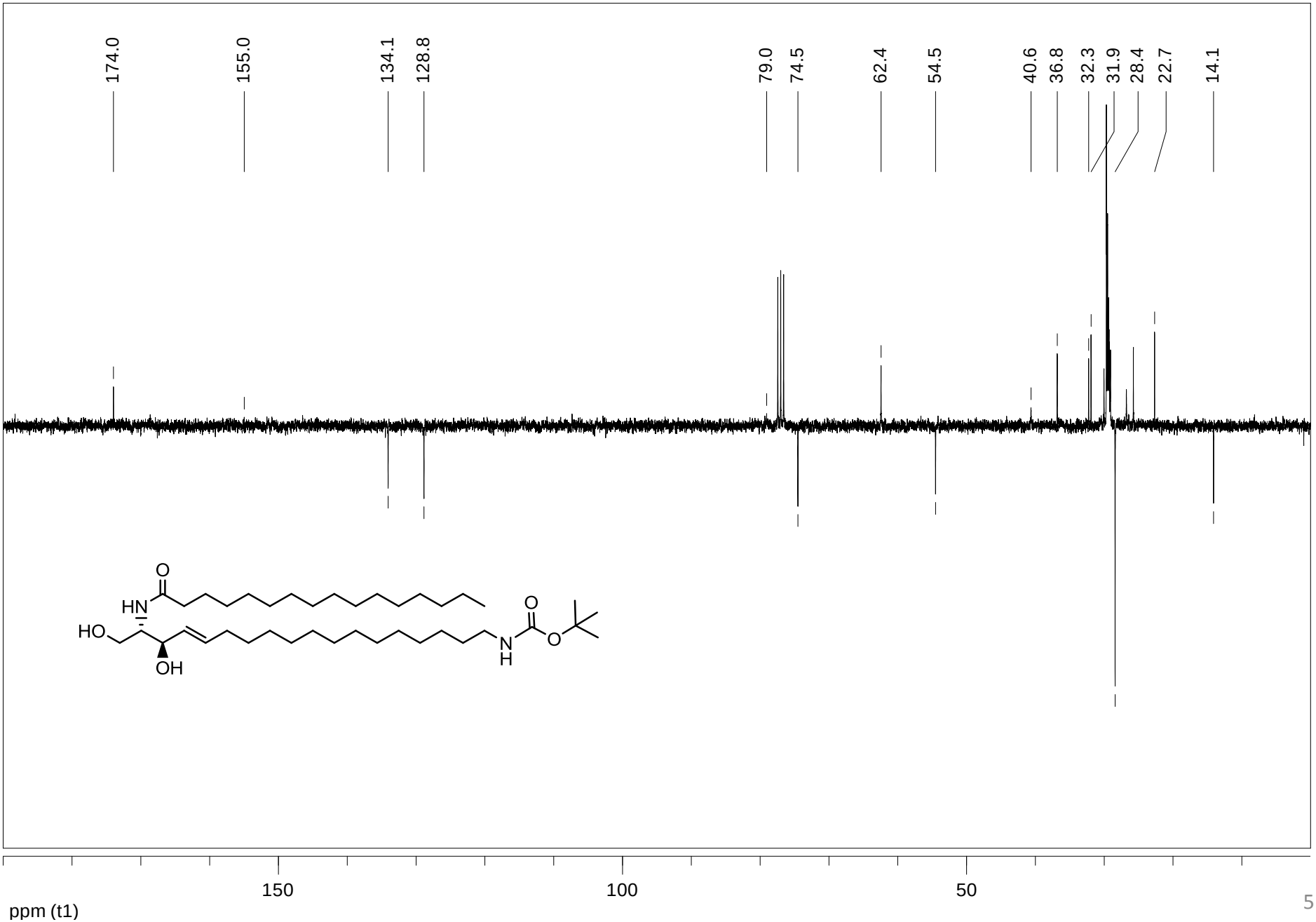
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **9**



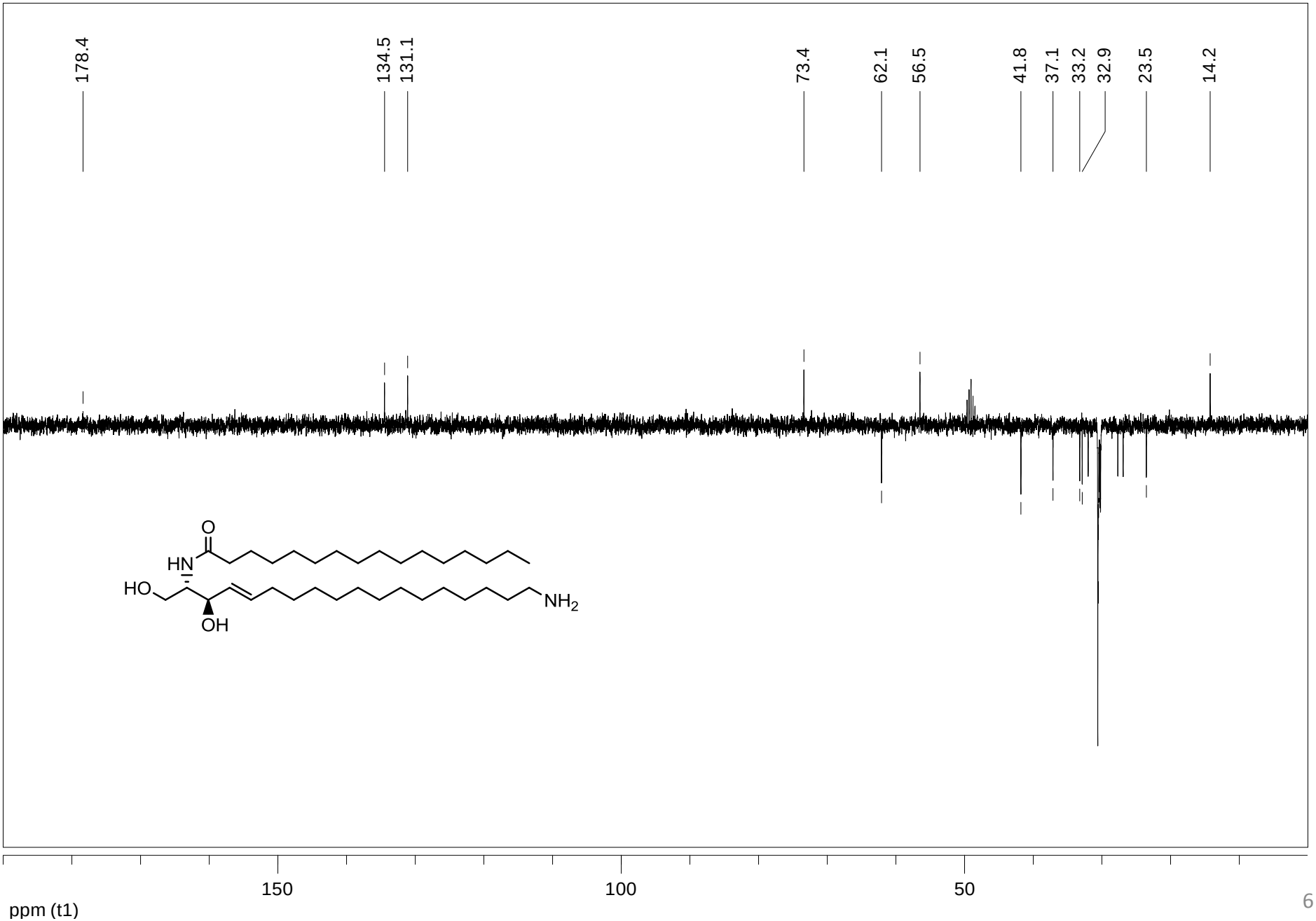
^{13}C NMR spectrum (CD_3OD, 75 MHz) of compound **3**



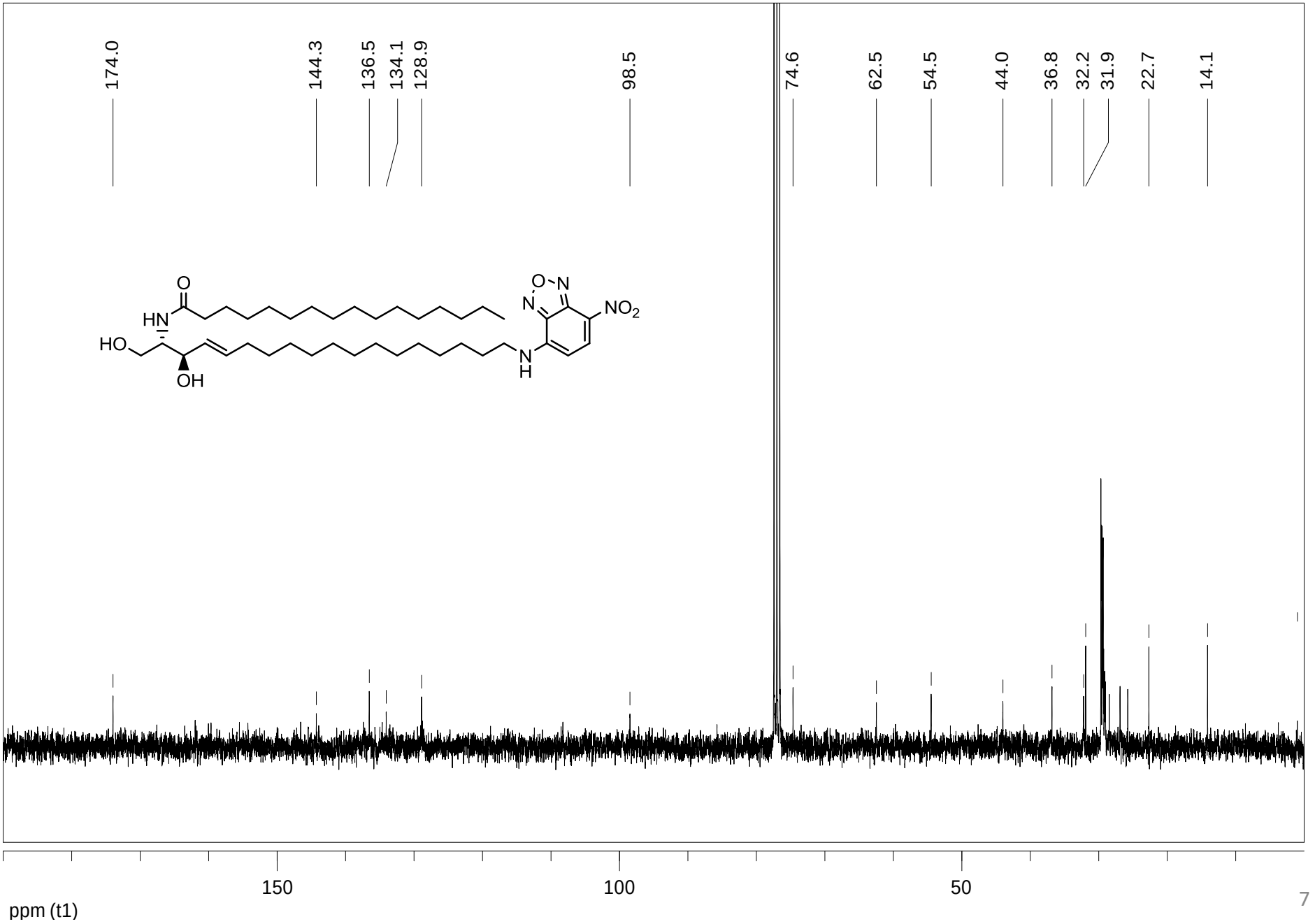
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **10**



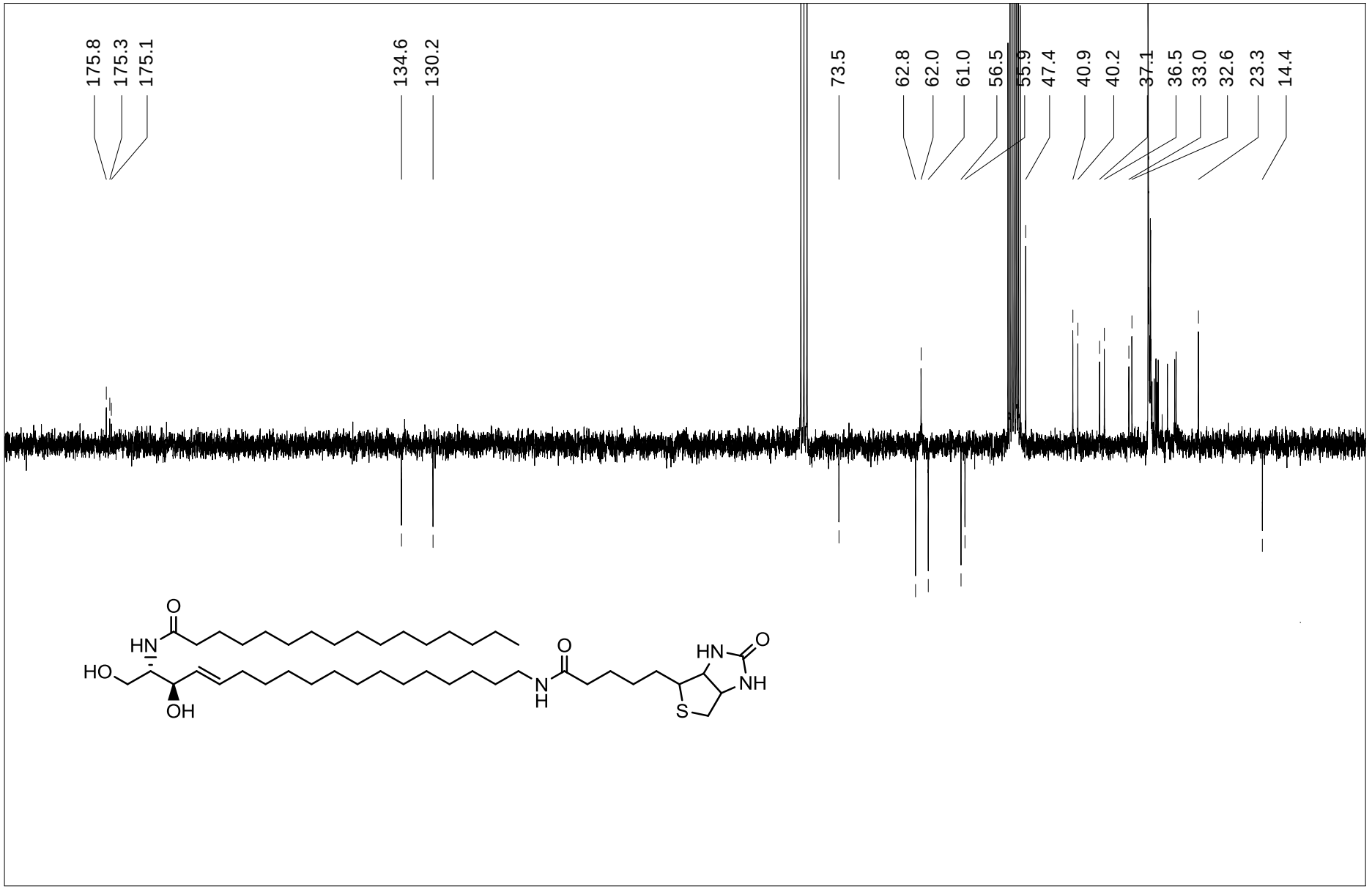
^{13}C NMR spectrum (CD_3OD, 75 MHz) of compound **13**



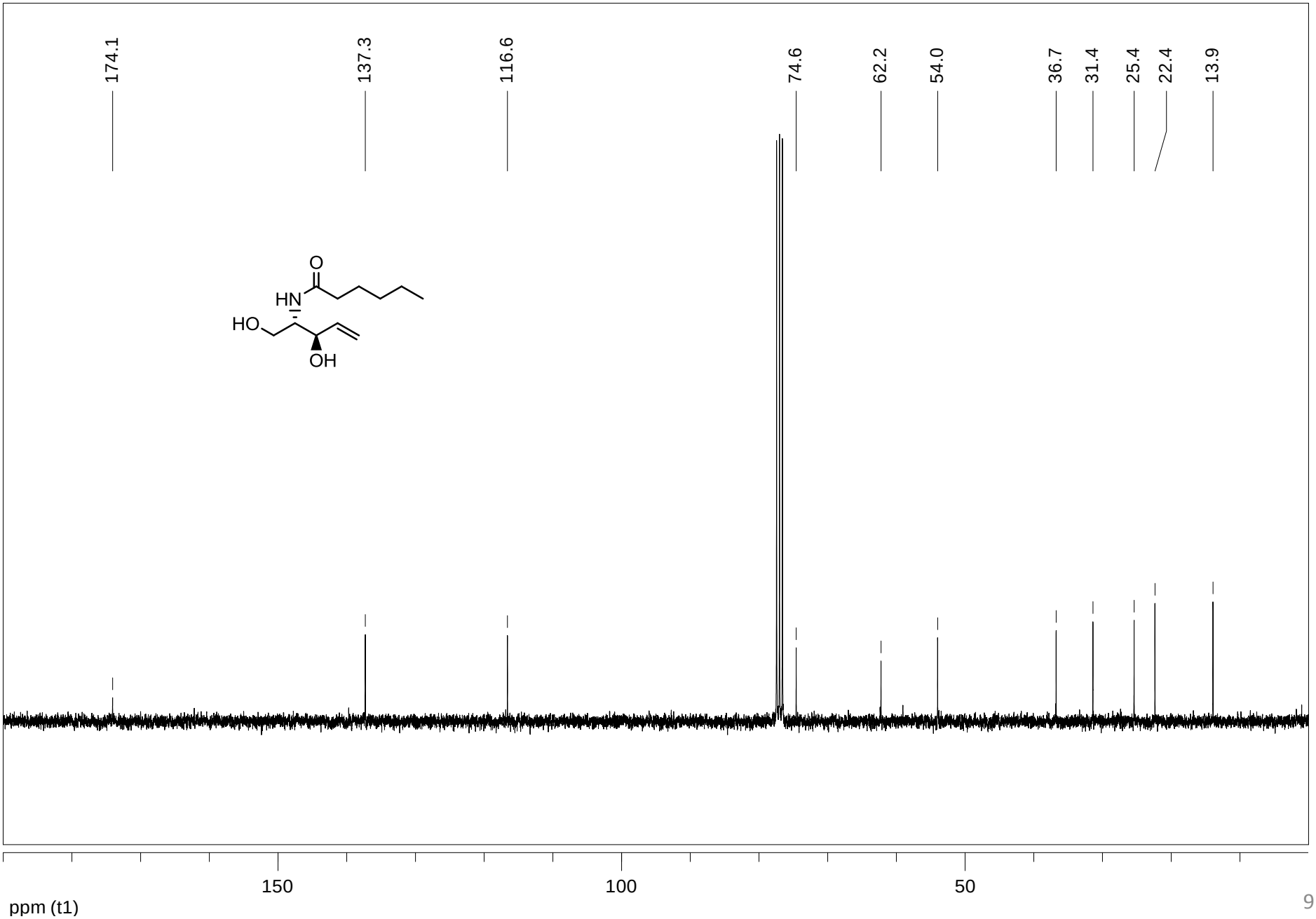
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **16**



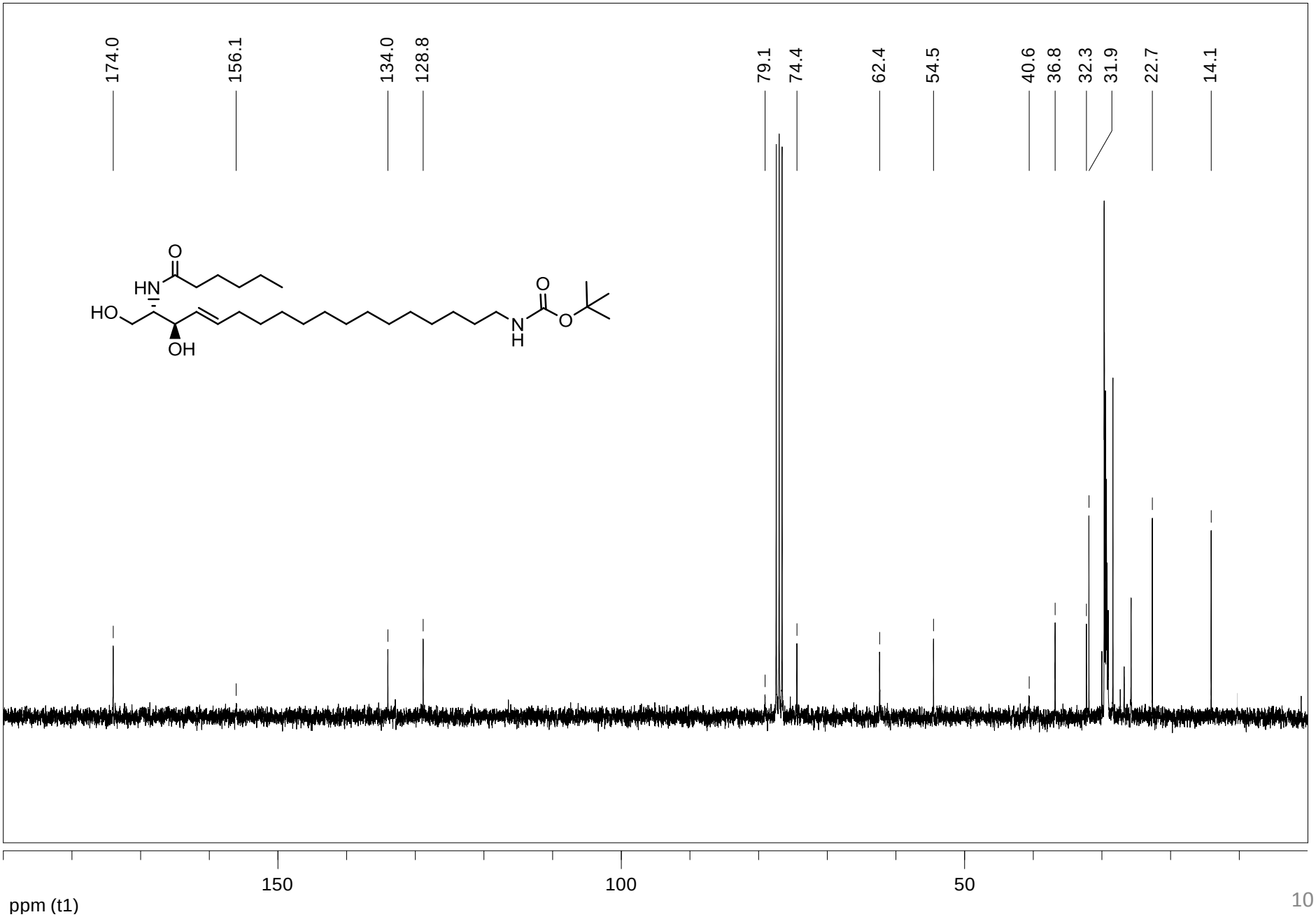
^{13}C NMR spectrum ($CDCl_3$+CD_3OD, 75 MHz) of compound **19**



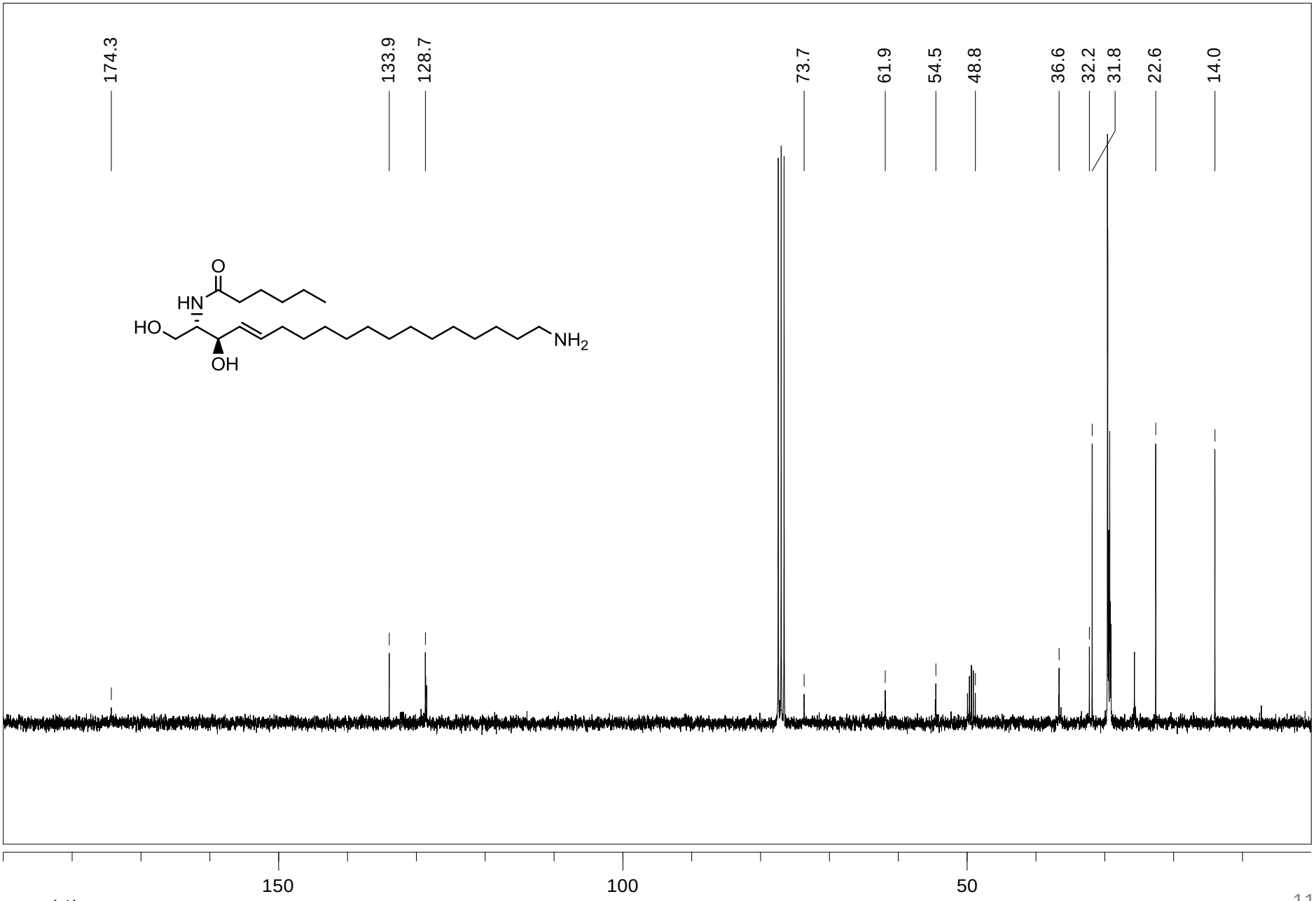
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **4**



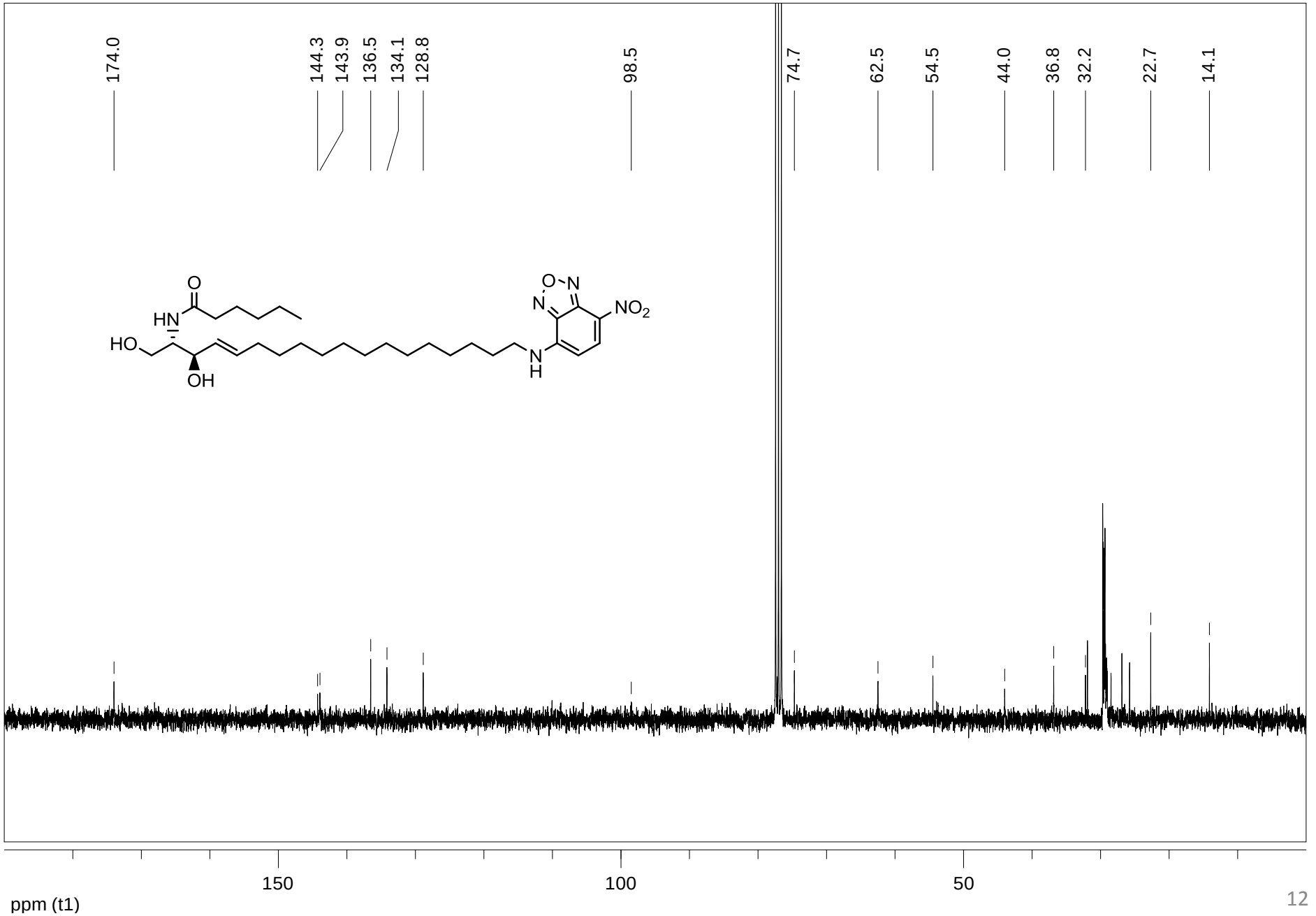
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **11**



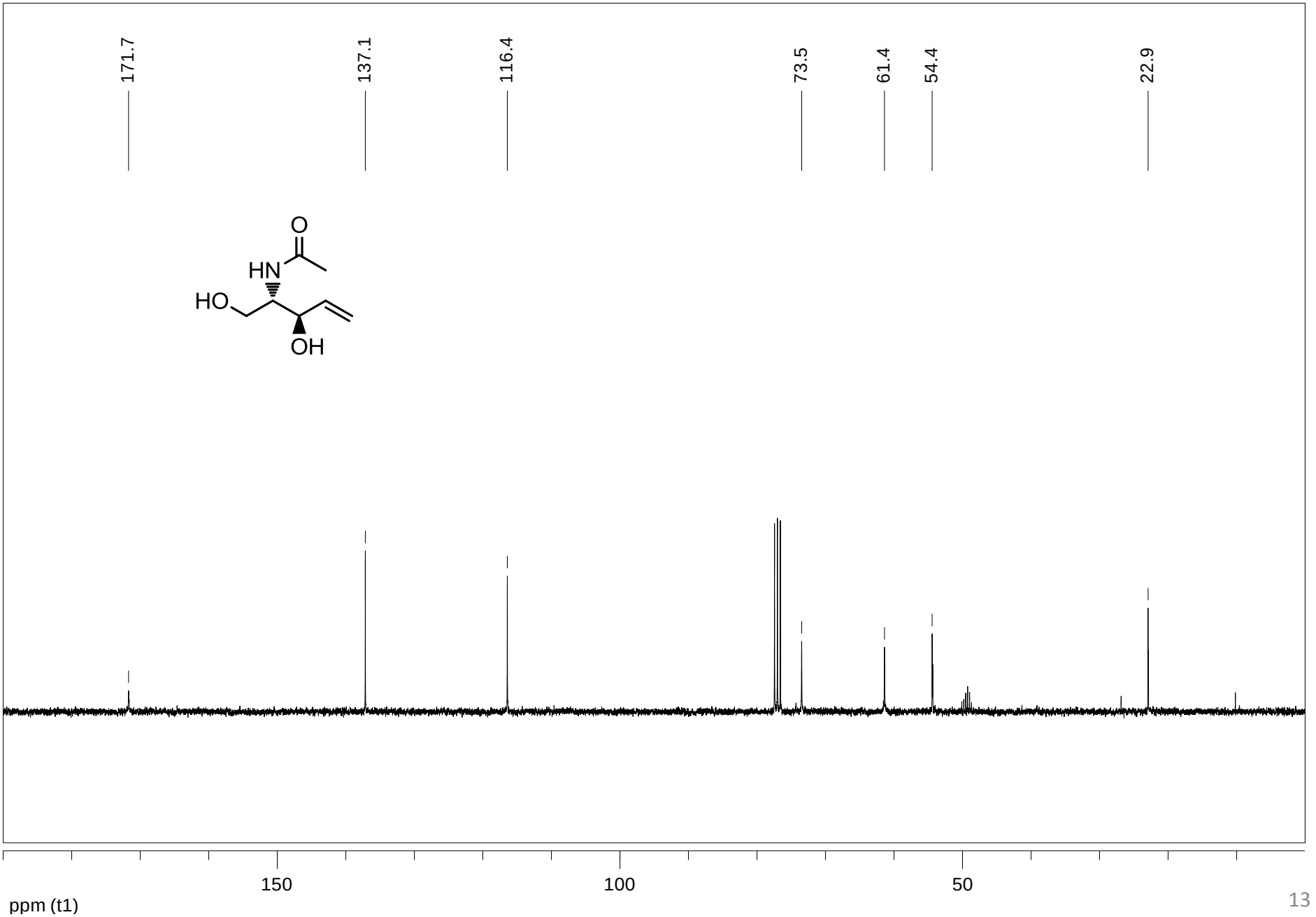
^{13}C NMR spectrum ($CDCl_3$+CD_3OD, 75 MHz) of compound **14**



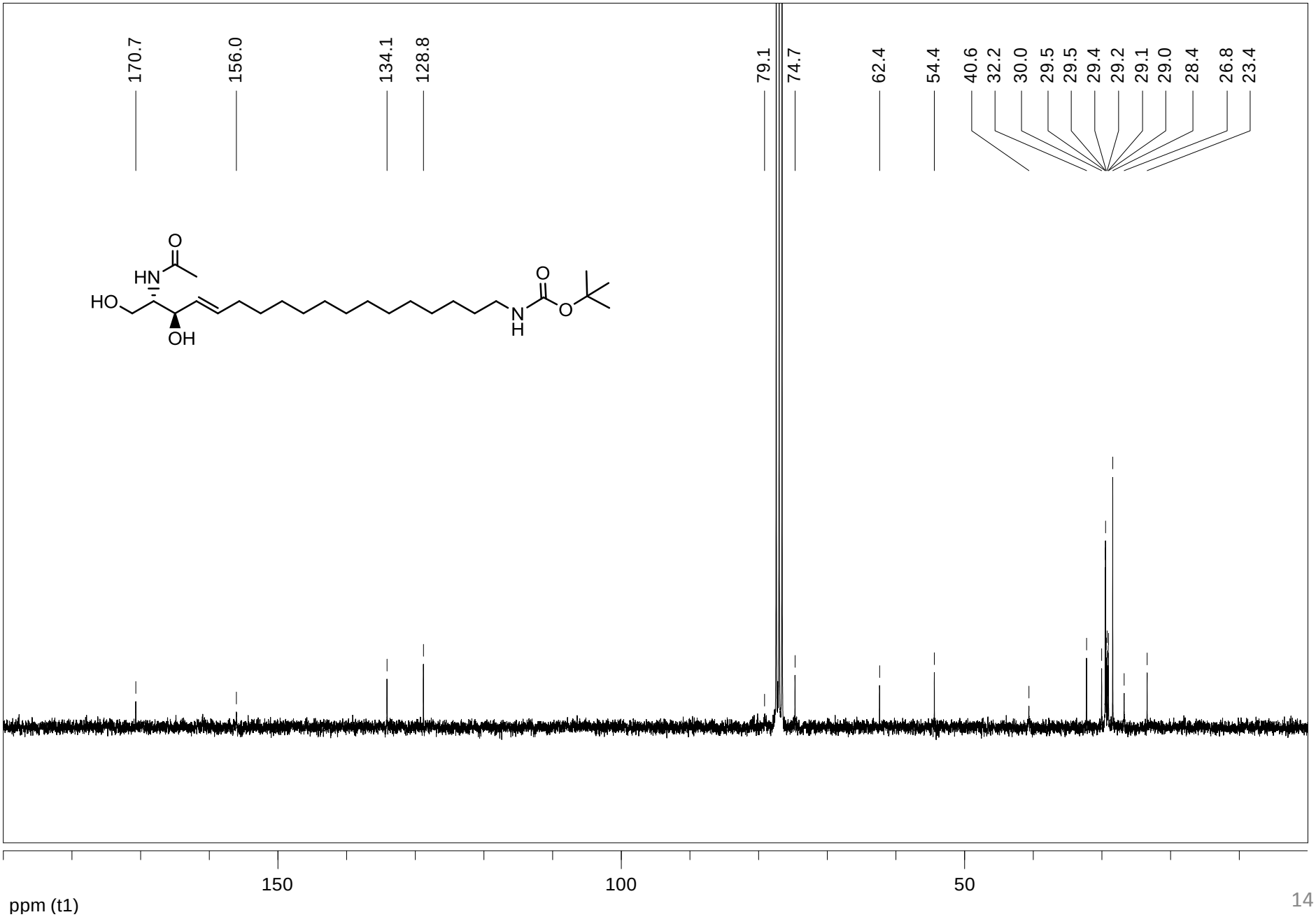
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **17**



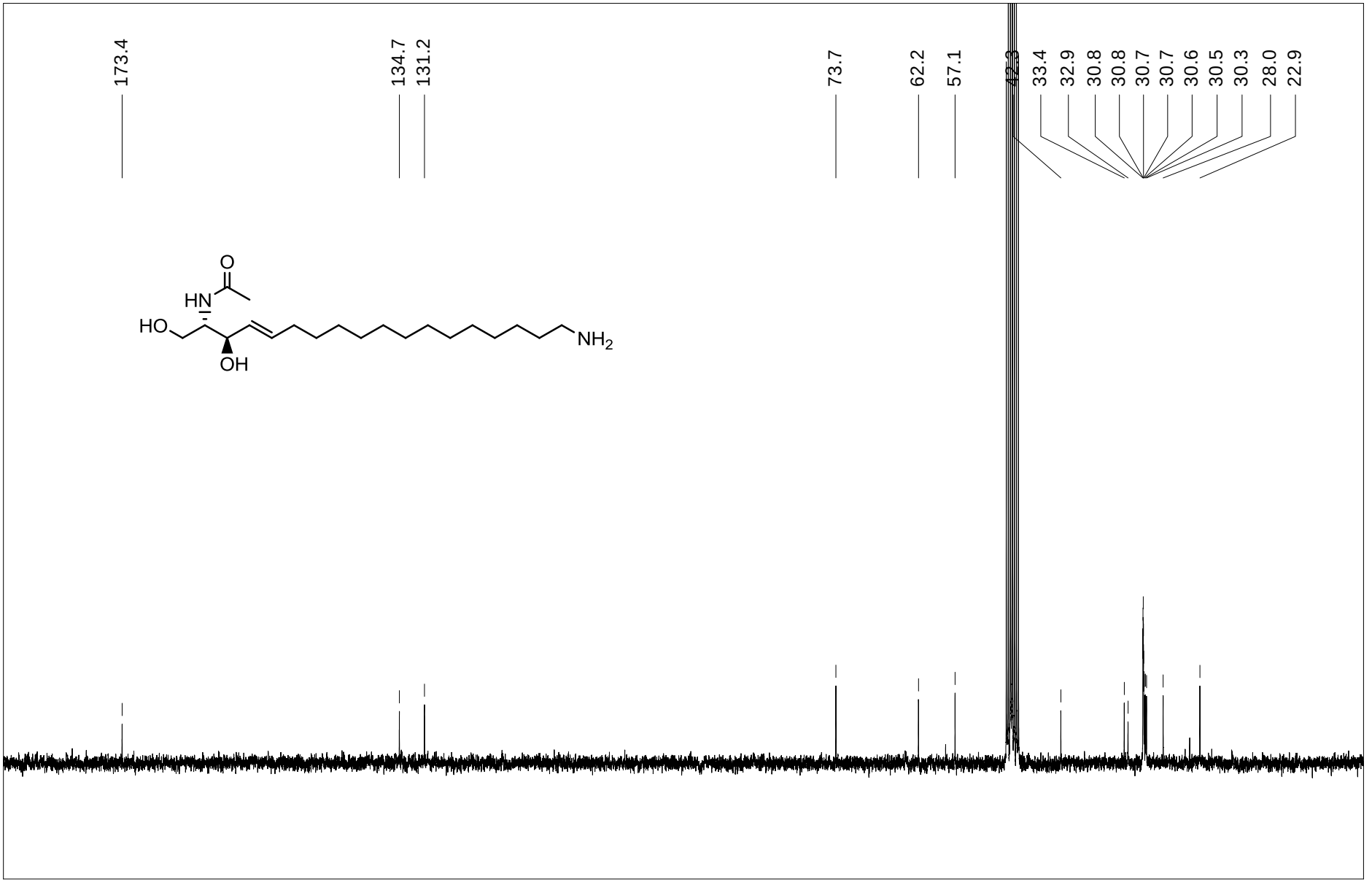
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **5**



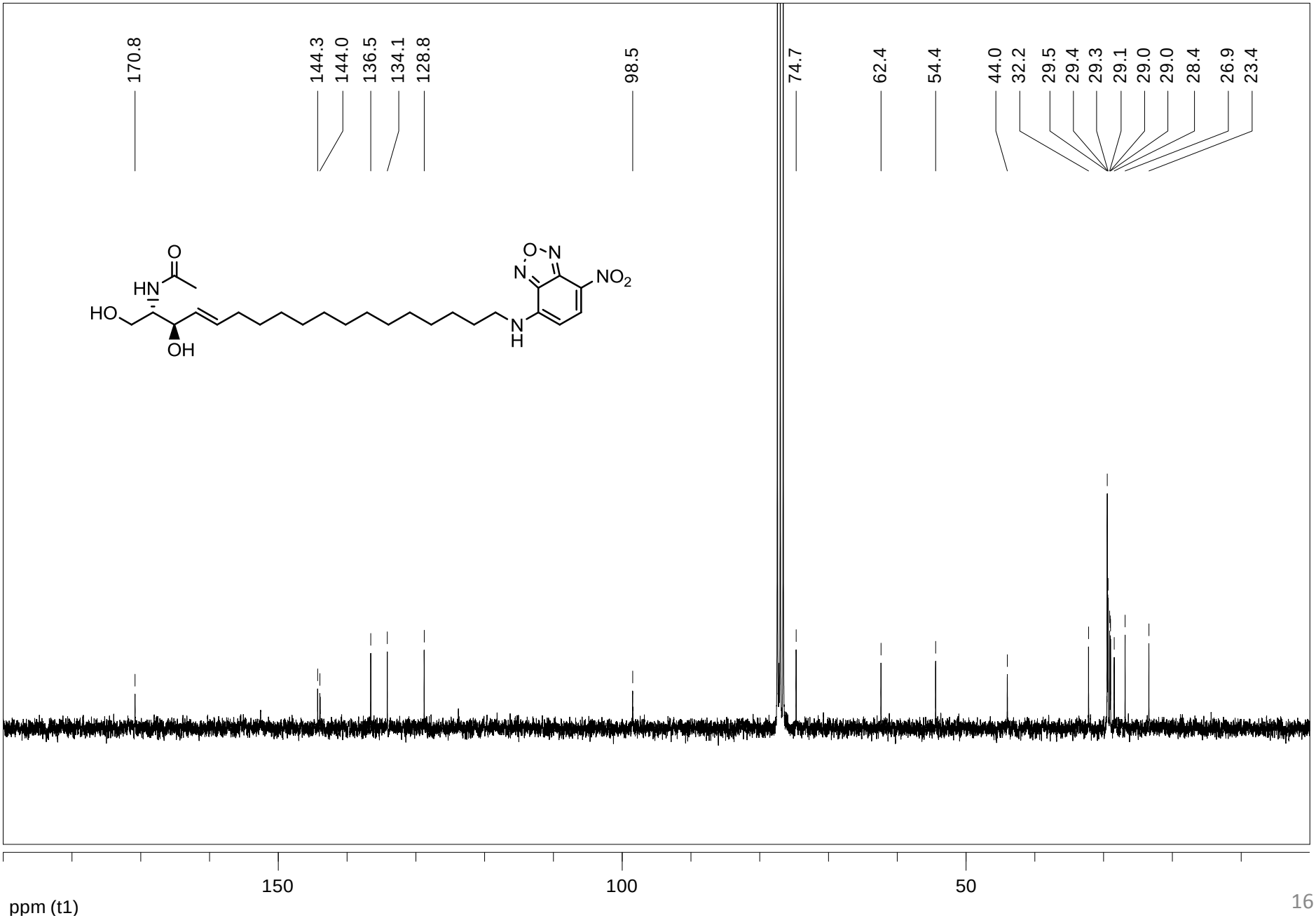
^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **12**



^{13}C NMR spectrum (CD_3OD, 75 MHz) of compound **15**

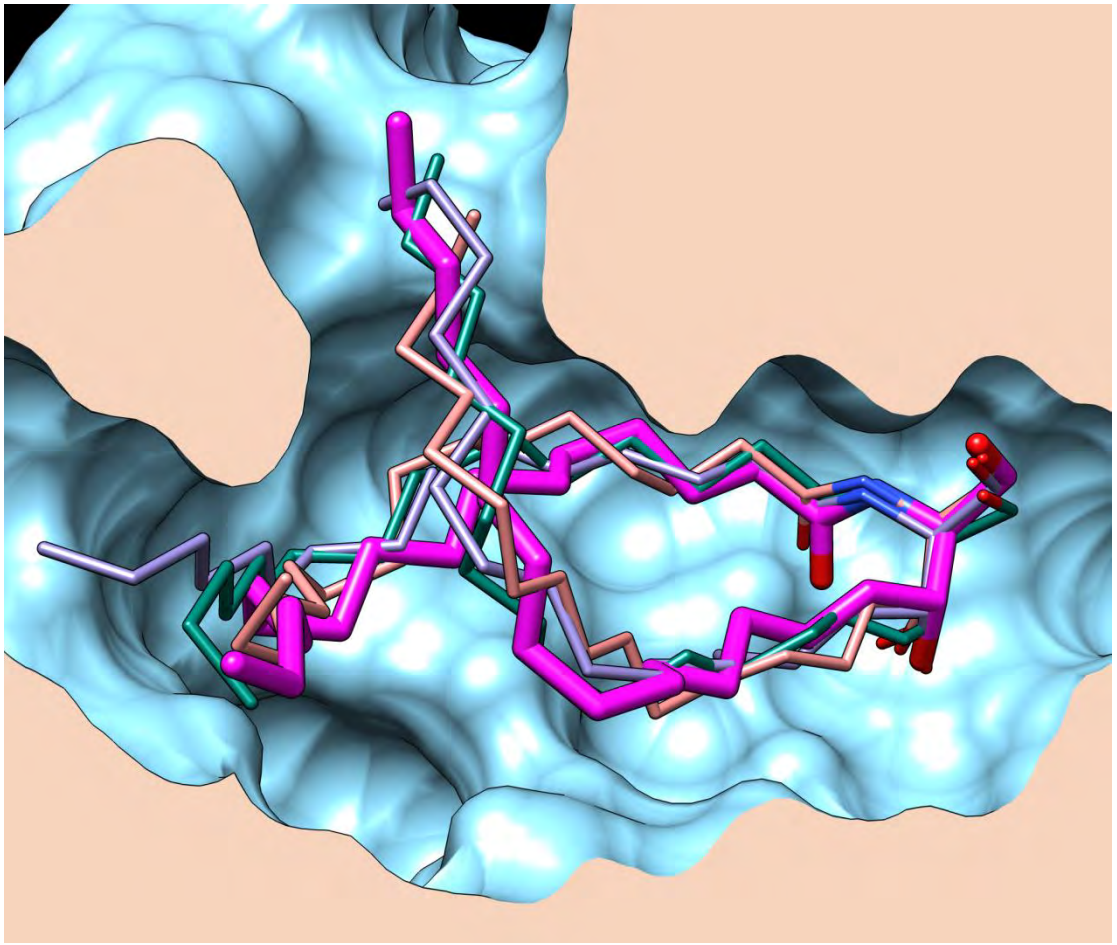


^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of compound **18**

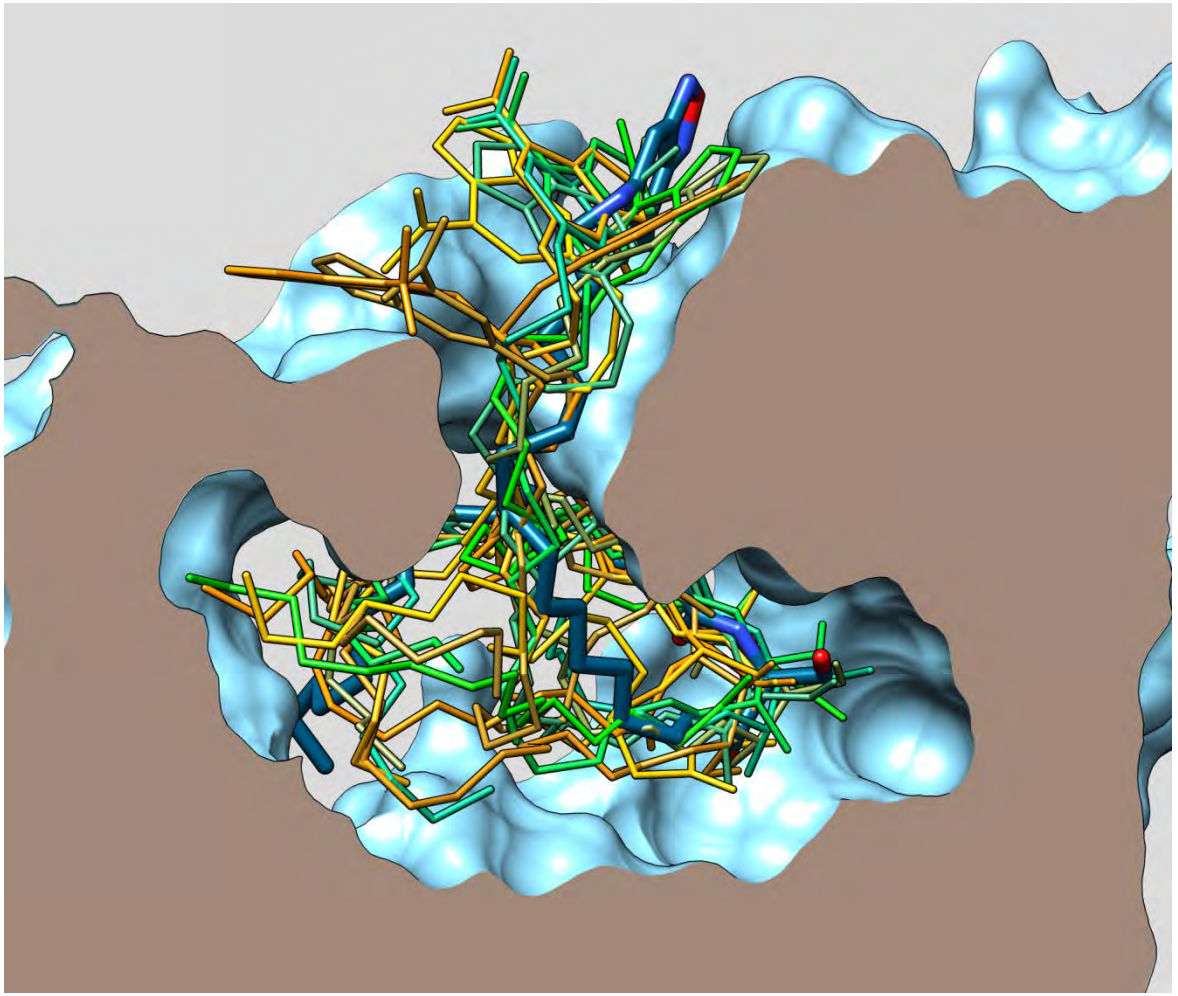


Superposition of cristallographic pose (2E3O, magenta) with docked poses (light purple, cyan, salmon) of C16-ceramide .

These results show a good agreement in reproducing conformation and hydrogen bond network at polar head level. Calculation was done in 2E3N cavity (light blue) after structural alignments and gives RMSD of 1.19, 1.05, 1.13 Angstroms respectively. Calculation was done using Molegro templates (H donor, H acceptor, steric) relative to cristallographic ceramide and flexible residues (45 residues around cavity).



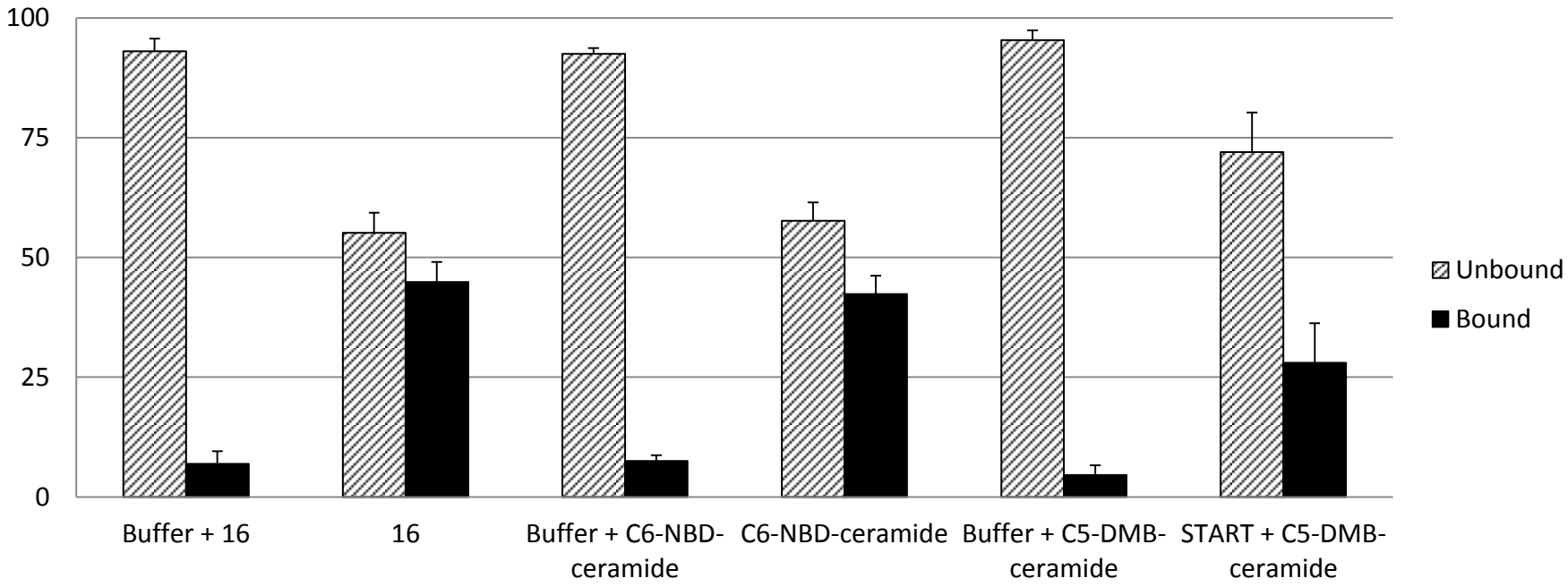
Superposition of eleven docked poses of probe **16** in clipped 2E3N cavity (light blue) corresponding to putative conformations with fluorophore found outside cavity.
The pose shown in Fig. 3B is highlighted (blue sticks).



Details of H-bonding calculated using Molegro Virtual Docker

ID	Donor	Energy	Length	Additional Info
0	(TYR 553) OH – O1 (Ligand)	-2.5	2.6562	Donor rotatable;;[AA-A-D: 138.106 (100%)]
1	(Ligand) N1 – OE1 (GLU 446)	-2.5	2.76944	;[AA-A-D: 158.639 (100%)];[D-H-A: 152.709 (100%)] [AA-A-H: 151.152 (100%)]
2	(Ligand) O2 – OE1 (GLU 446)	-2.5	2.8515	Donor rotatable;;[AA-A-D: 127.954 (100%)]
3	(GLN 467) NE2 – O2 (Ligand)	-2.5	2.80439	;[AA-A-D: 126.524 (100%)];[D-H-A: 43.4826 (0%)] [AA-A-H: 130.659 (100%)];[D-H-A: 151.047 (100%)] [AA-A-H: 116.454 (100%)]
4	(Ligand) O3 – OD1 (ASN 504)	-2.5	3.01814	Donor rotatable;;[AA-A-D: 122.144 (100%)]
5	(THR 447) OG1 – N3 (Ligand)	-1.66026	2.49923	Donor rotatable;

Binding assay of **16**, C6-NBD-Cer and C5-DMB-Cer to START domain

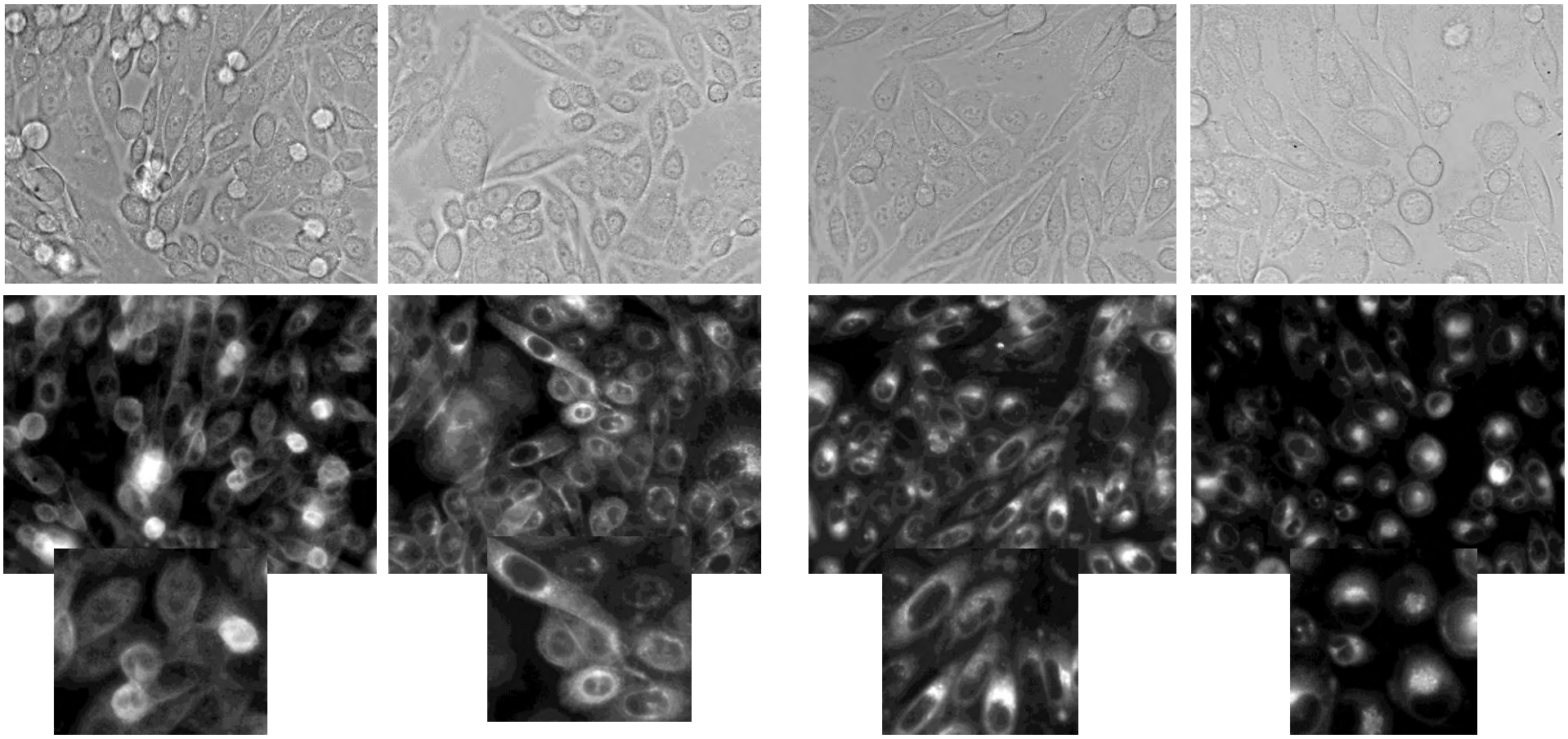


Intracellular distribution of the C5-DMB-Cer

C5-DMB-Cer T0

C5-DMB-Cer T30 min

CHO LYA | CHO LYA hCERT | CHO LYA | CHO LYA hCERT



Filtre GFP Obj 40x

Intracellular distribution of compound **16**

Probe **16**
T0

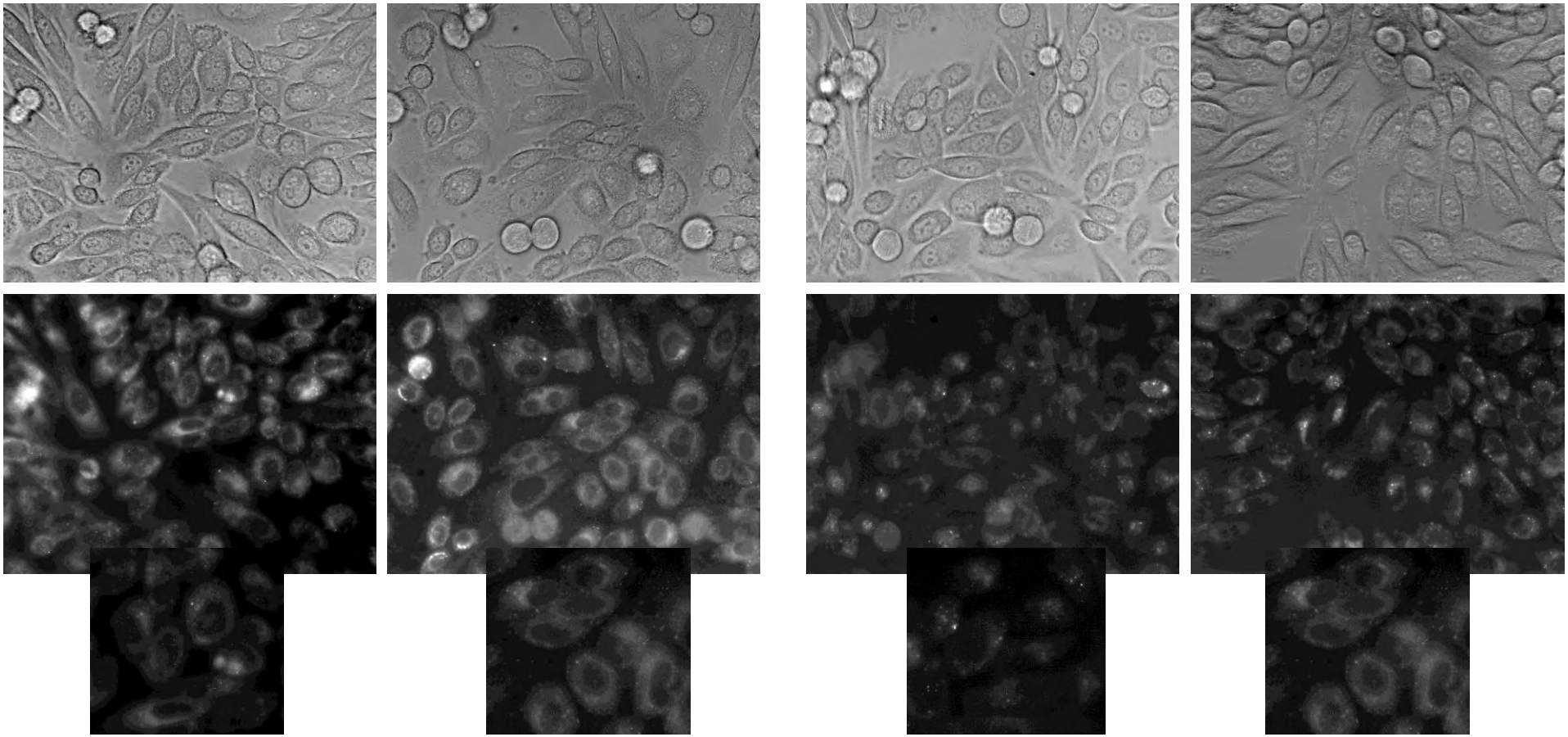
Probe **16**
T30 min

CHO LYA

CHO LYA hCERT

CHO LYA

CHO LYA hCERT



Filtre GFP Obj 40x

Intracellular distribution of the C6-NBD-Cer

C6-NBD-Cer T0

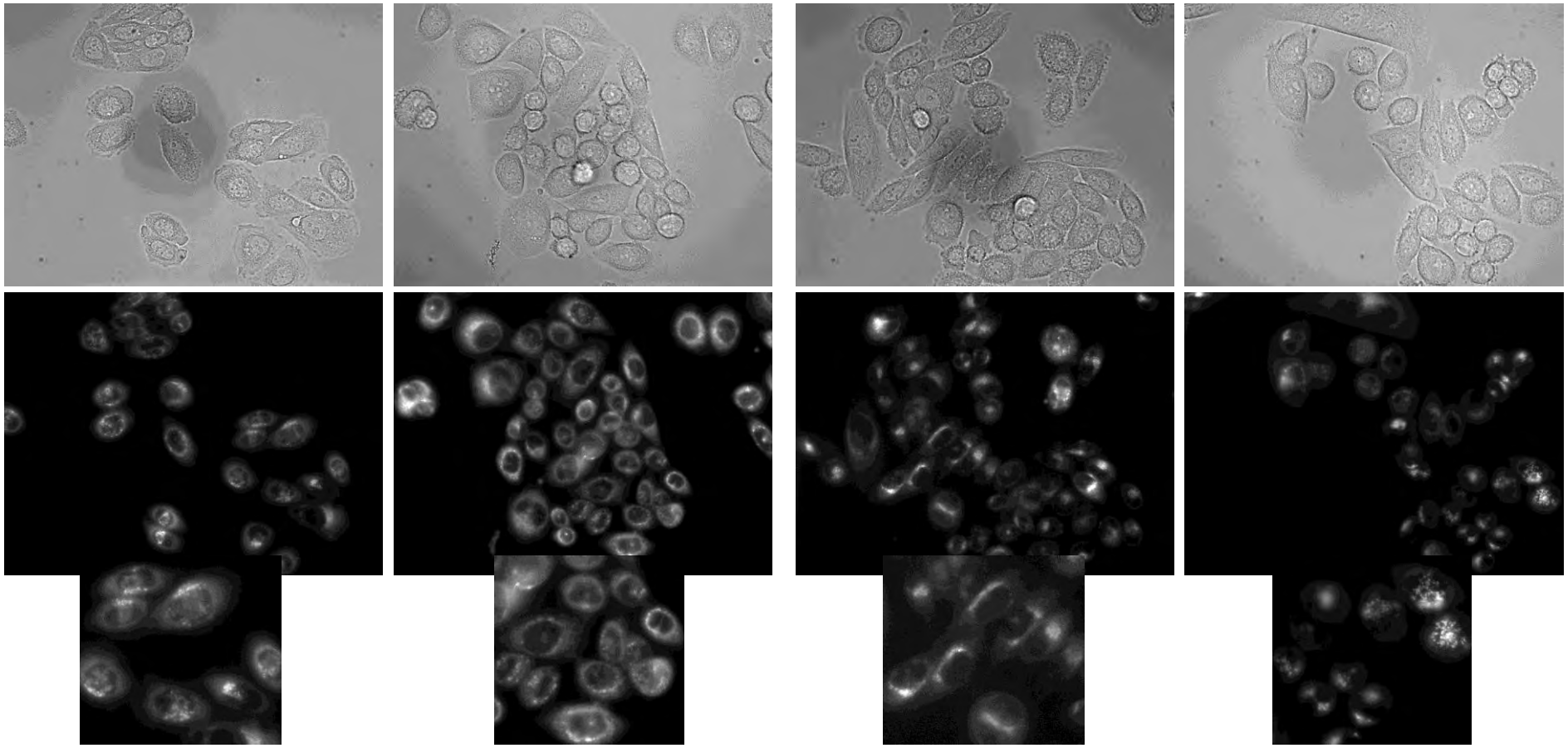
C6-NBD-Cer T30 min

CHO LYA

CHO LYA hCERT

CHO LYA

CHO LYA hCERT



Filtre GFP Obj 40x