Supplementary Information

Synthesis and cytotoxic activity of novel A-ring cleaved ursolic acid derivatives in human non-small cell lung cancer cells

Vanessa I. S. Mendes[a,b], Geoffrey A. Bartholomeusz[c], Mary Ayres[c], Varsha Gandhi[c,*], Jorge A. R. Salvador[a,b,*]

[a]*Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, 3000-548, Coimbra, Portugal. E-mail: salvador@ci.uc.pt; Fax: +351 239 488 503; Tel: +351 239 488 400*

[b]*Center for Neuroscience and Cell Biology, Coimbra, Portugal*

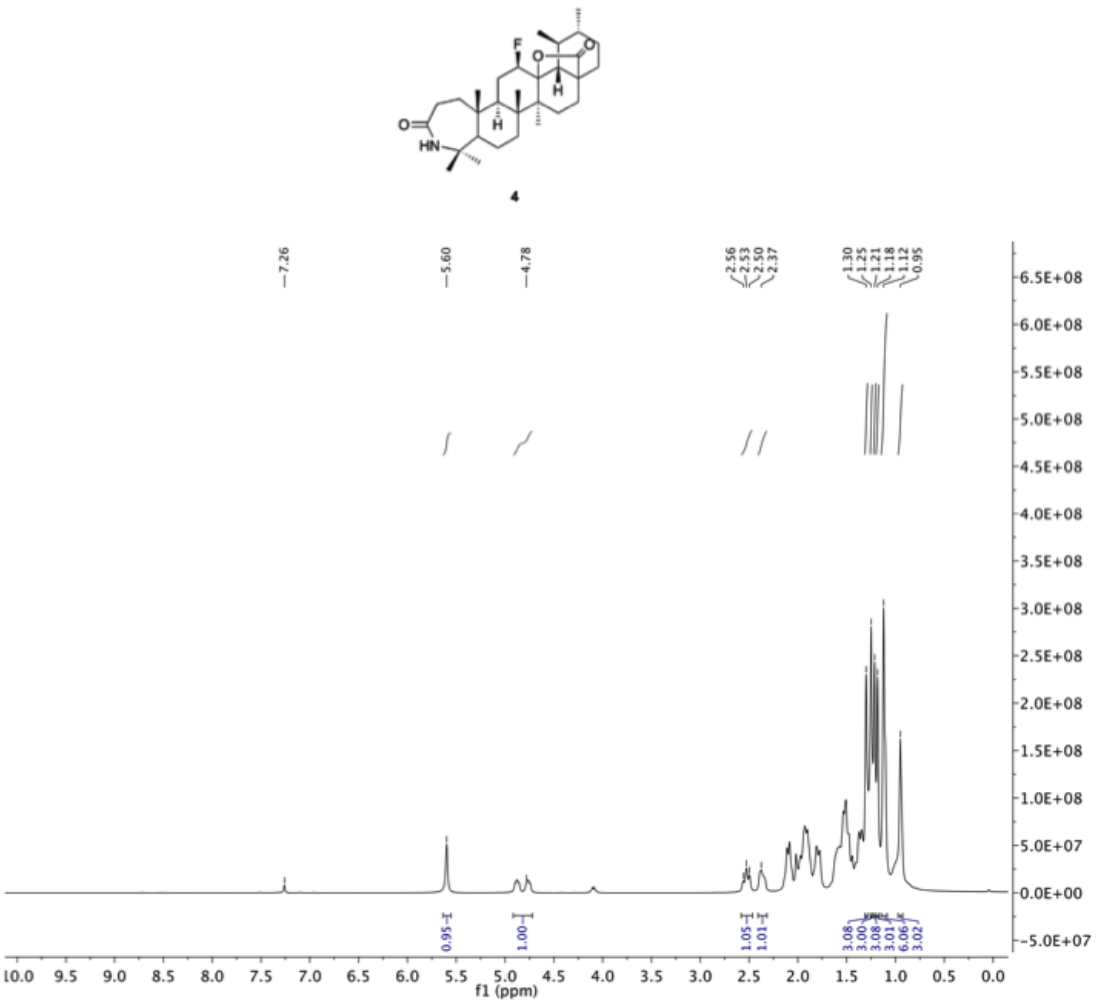
[c]*Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. E-mail: vgandhi@mdanderson.org; Fax: +1 713-794-4316; Tel: +1 713-792-2989*

*Equal contributions and corresponding authors.

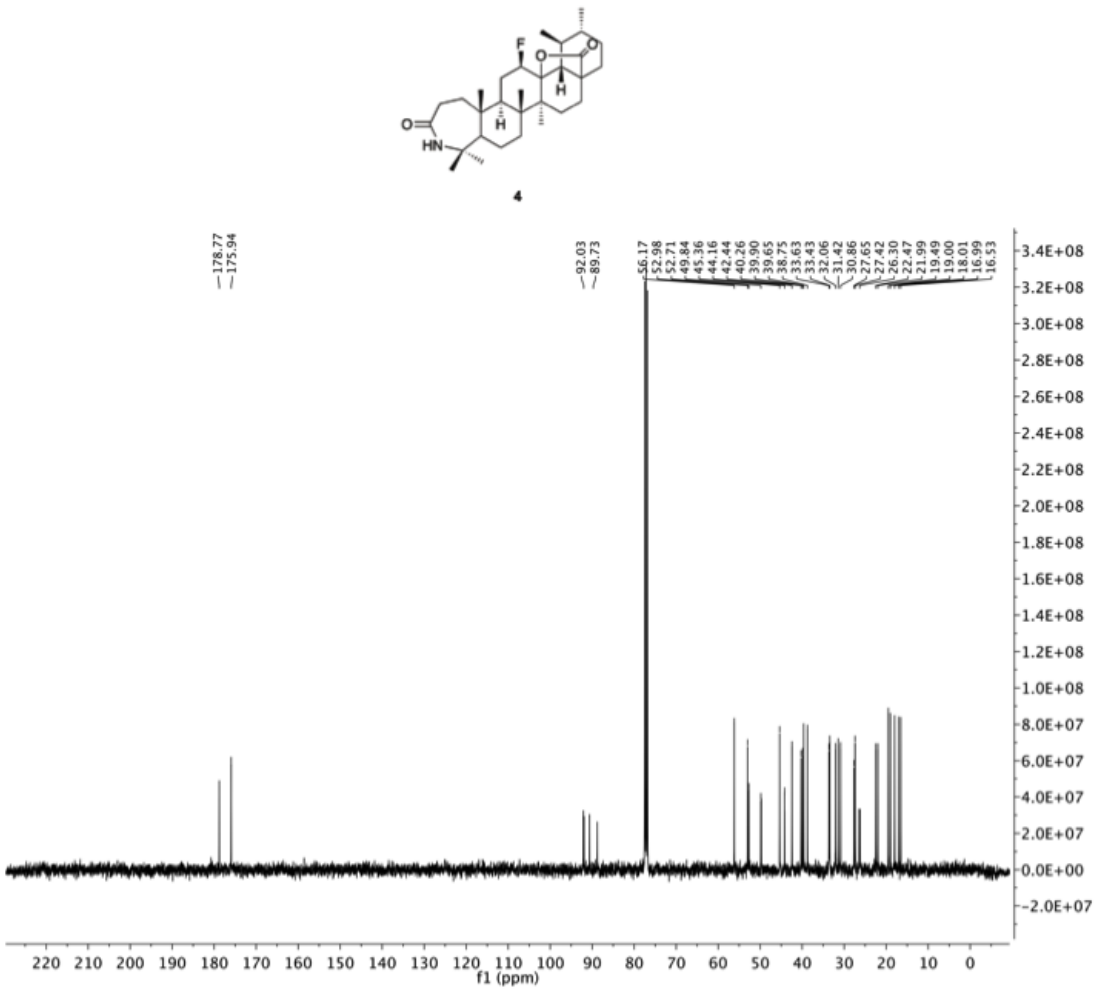
Table of Contents

1. NMR data for compound 4:

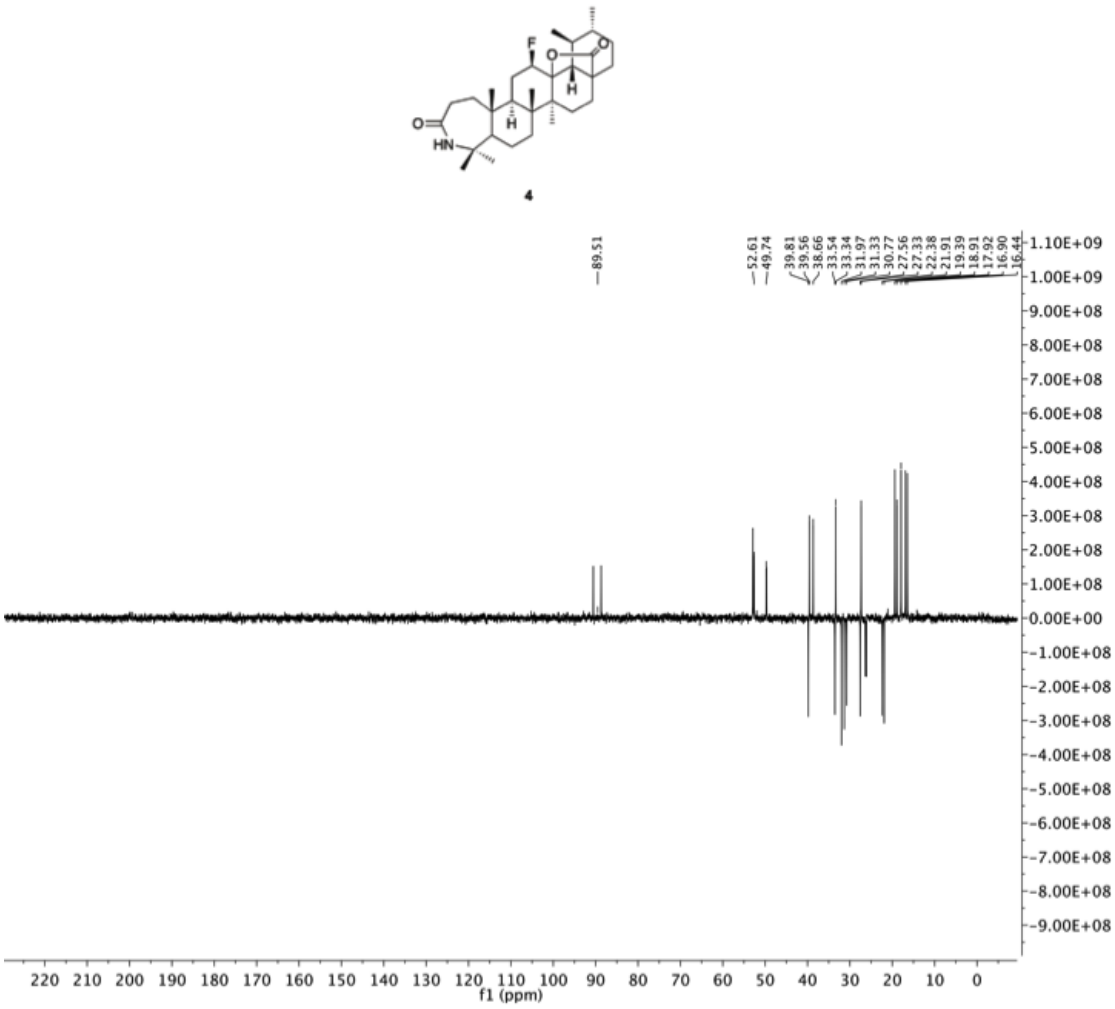
1.1. ^{1}H NMR spectrum for compound 4 recorded in $CDCl_3$



1.2. ^{13}C NMR spectrum for compound 4 recorded in $CDCl_3$

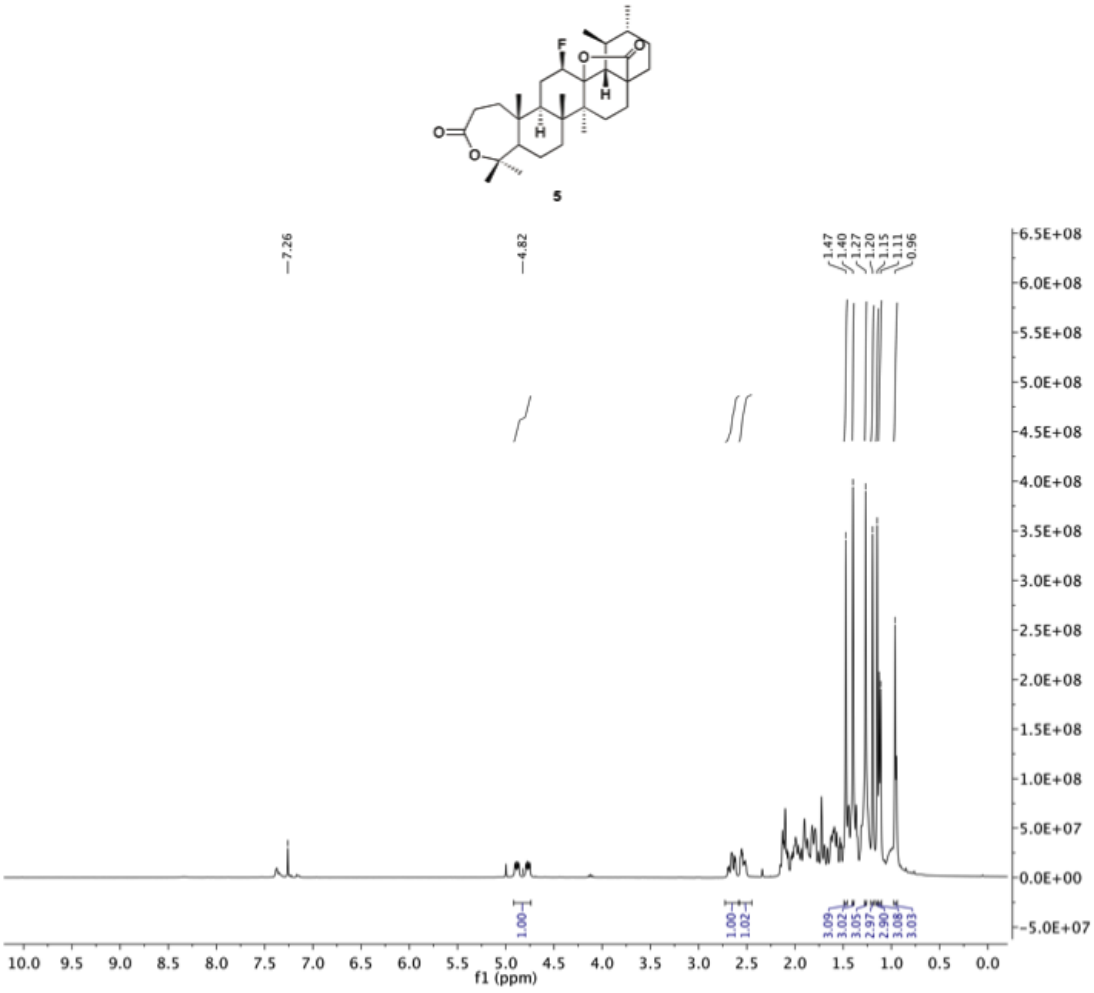


1.3. DEPT-135 NMR spectrum for compound 4 recorded in $CDCl_3$

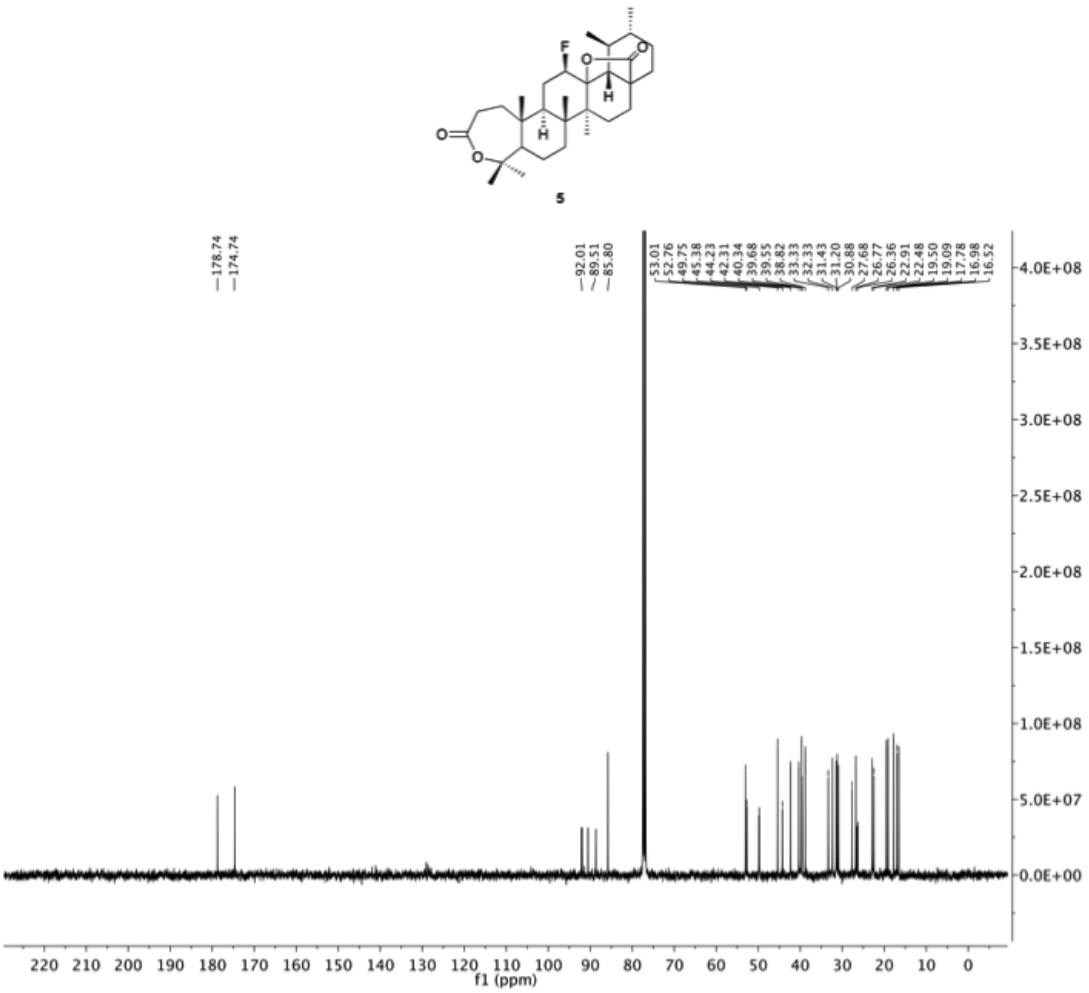


2. NMR data for compound 5:

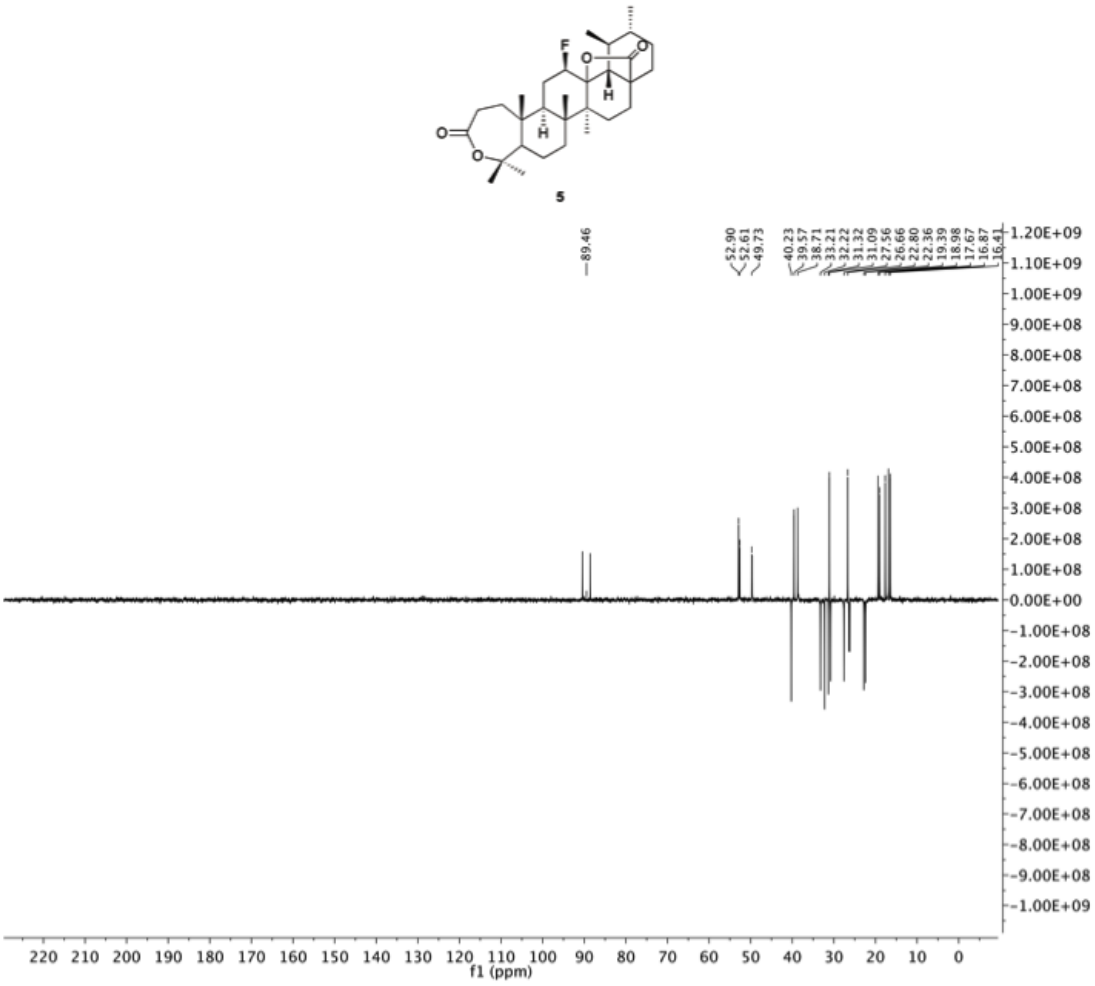
2.1. ^{1}H NMR spectrum for compound 5 recorded in $CDCl_3$



2.2. ^{13}C NMR spectrum for compound 5 recorded in $CDCl_3$

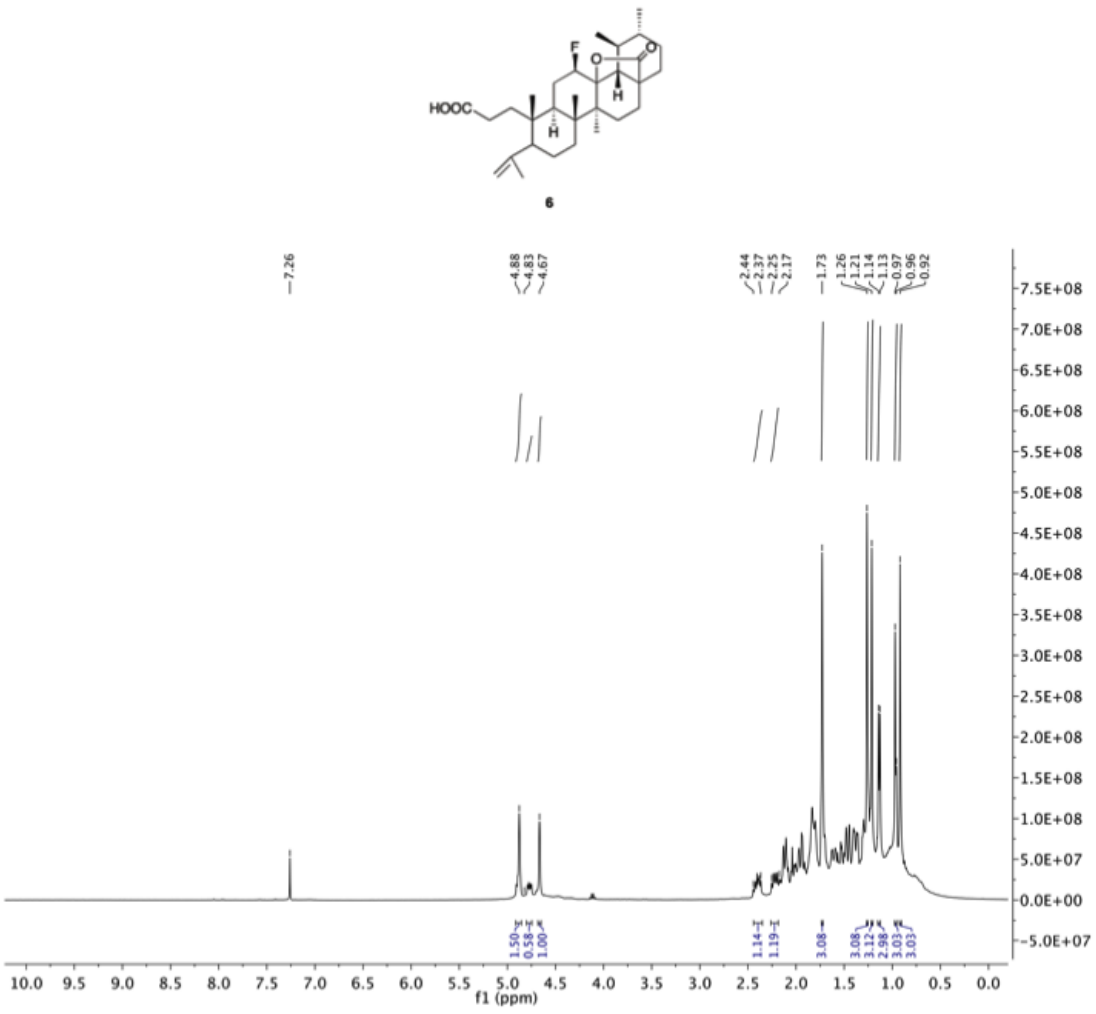


2.3. DEPT-135 NMR spectrum for compound 5 recorded in $CDCl_3$

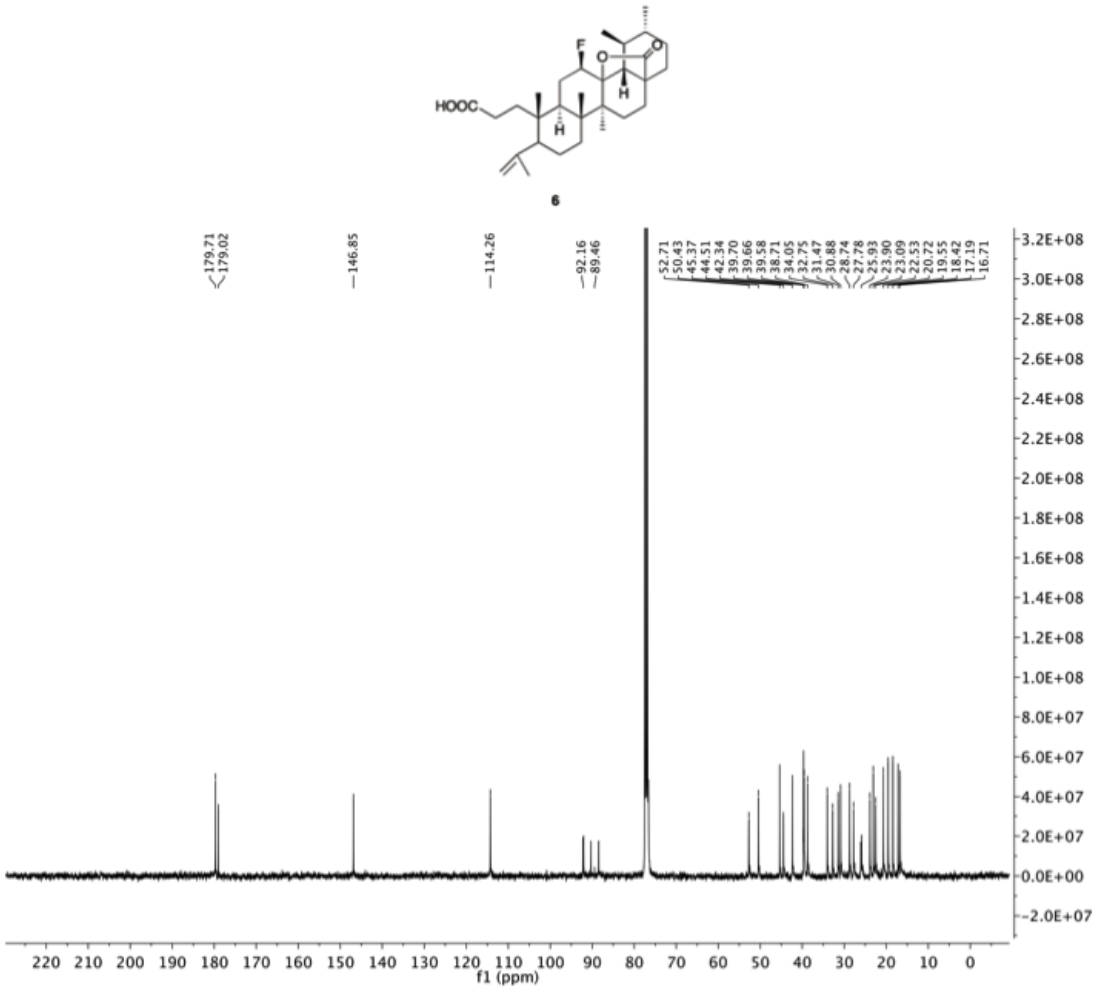


3. NMR data for compound 6:

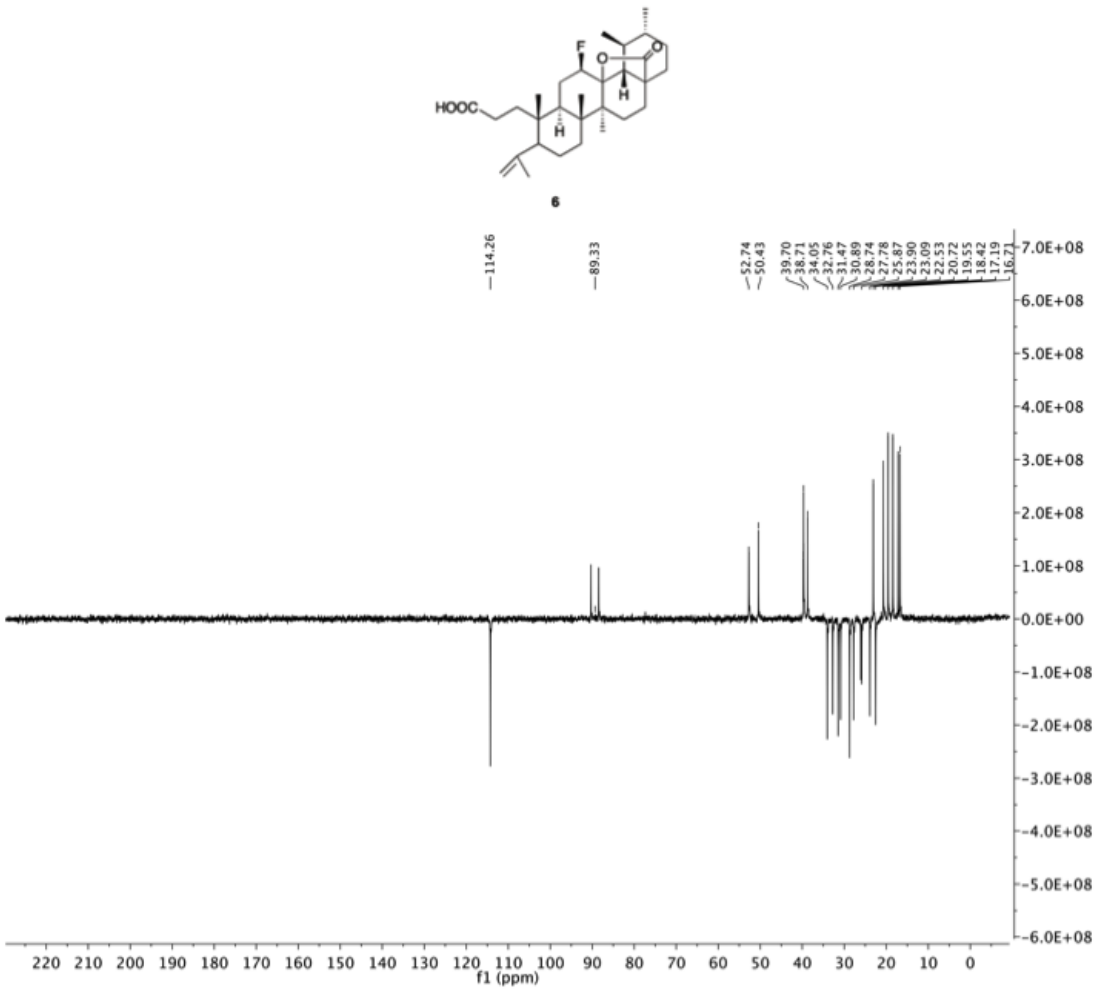
3.1. ^{1}H NMR spectrum for compound 6 recorded in $CDCl_3$



3.2. ^{13}C NMR spectrum for compound 6 recorded in $CDCl_3$

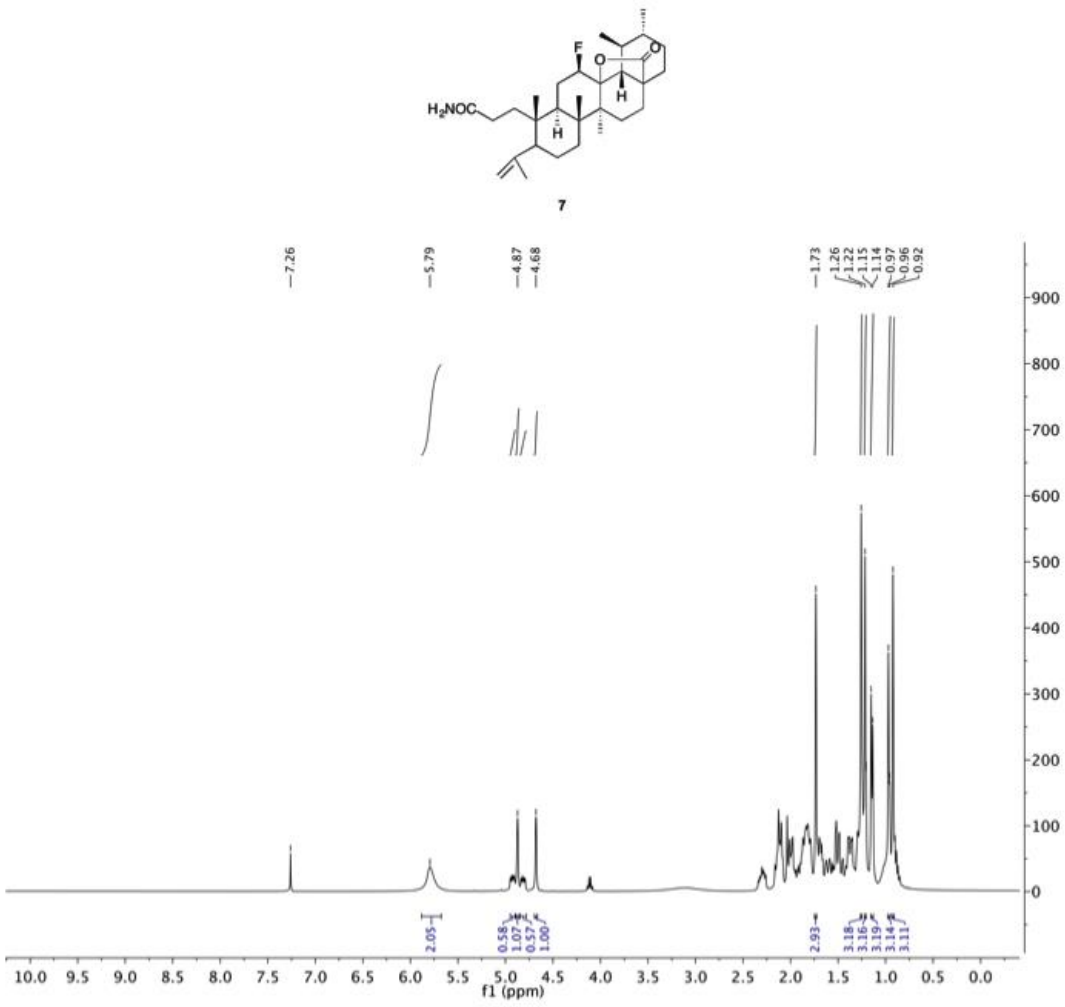


3.3. DEPT-135 NMR spectrum for compound 6 recorded in $CDCl_3$

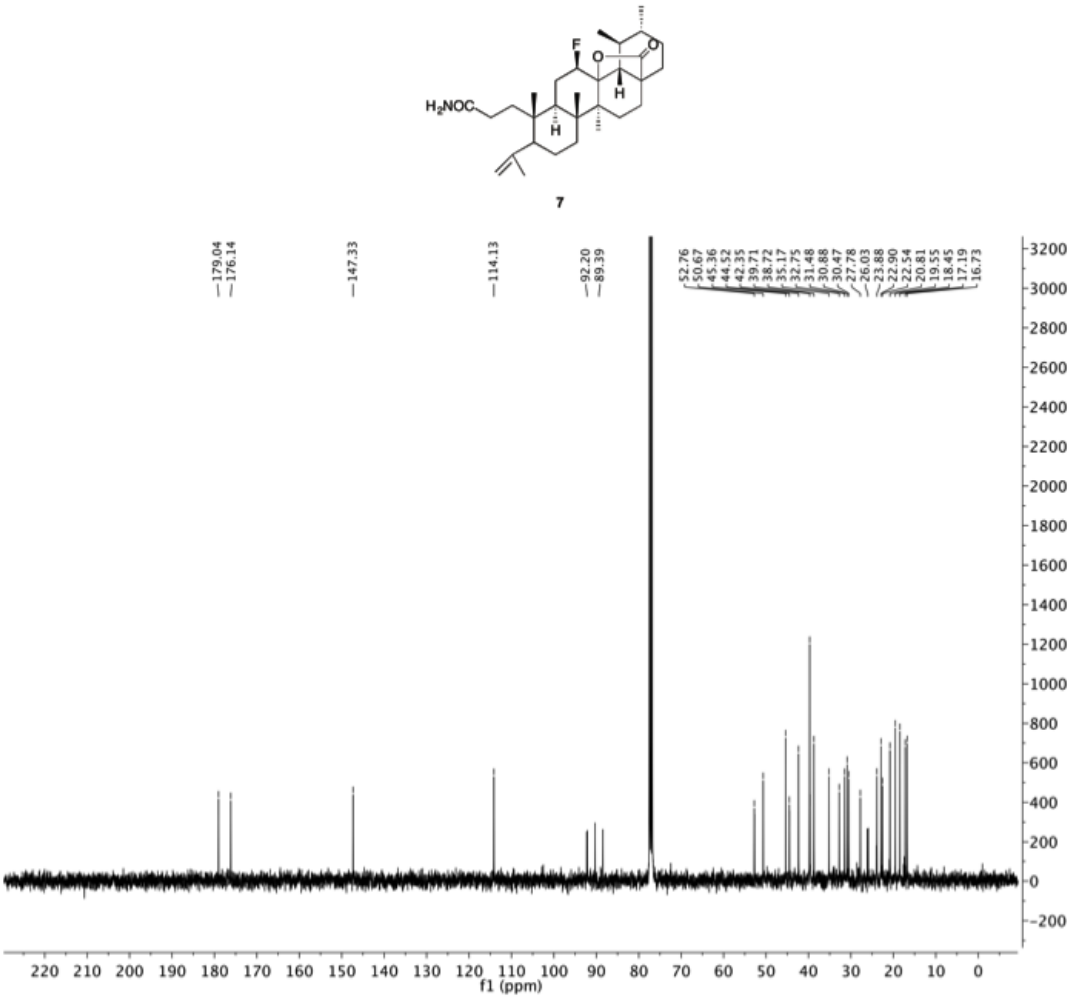


4. NMR data for compound 7:

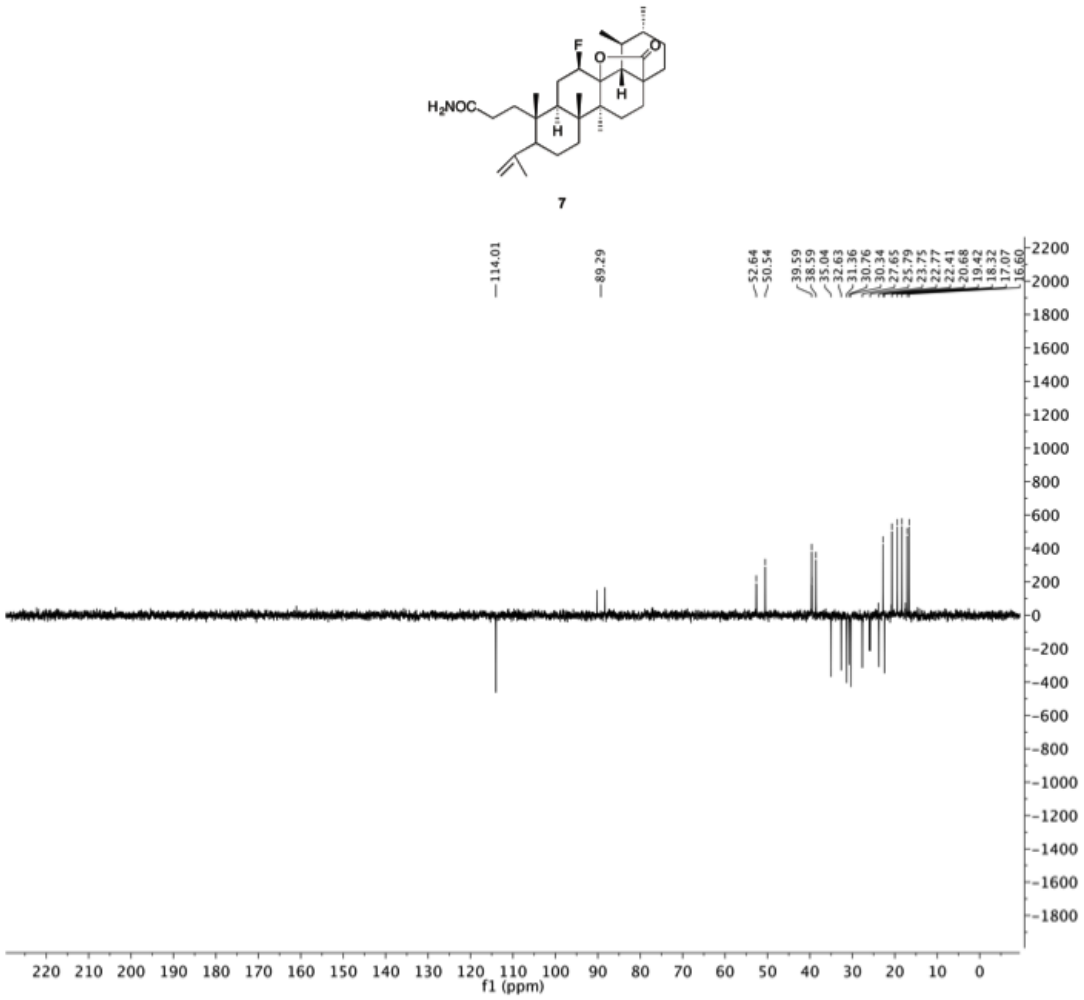
4.1. ^{1}H NMR spectrum for compound 7 recorded in $CDCl_3$



4.2. ^{13}C NMR spectrum for compound 7 recorded in $CDCl_3$

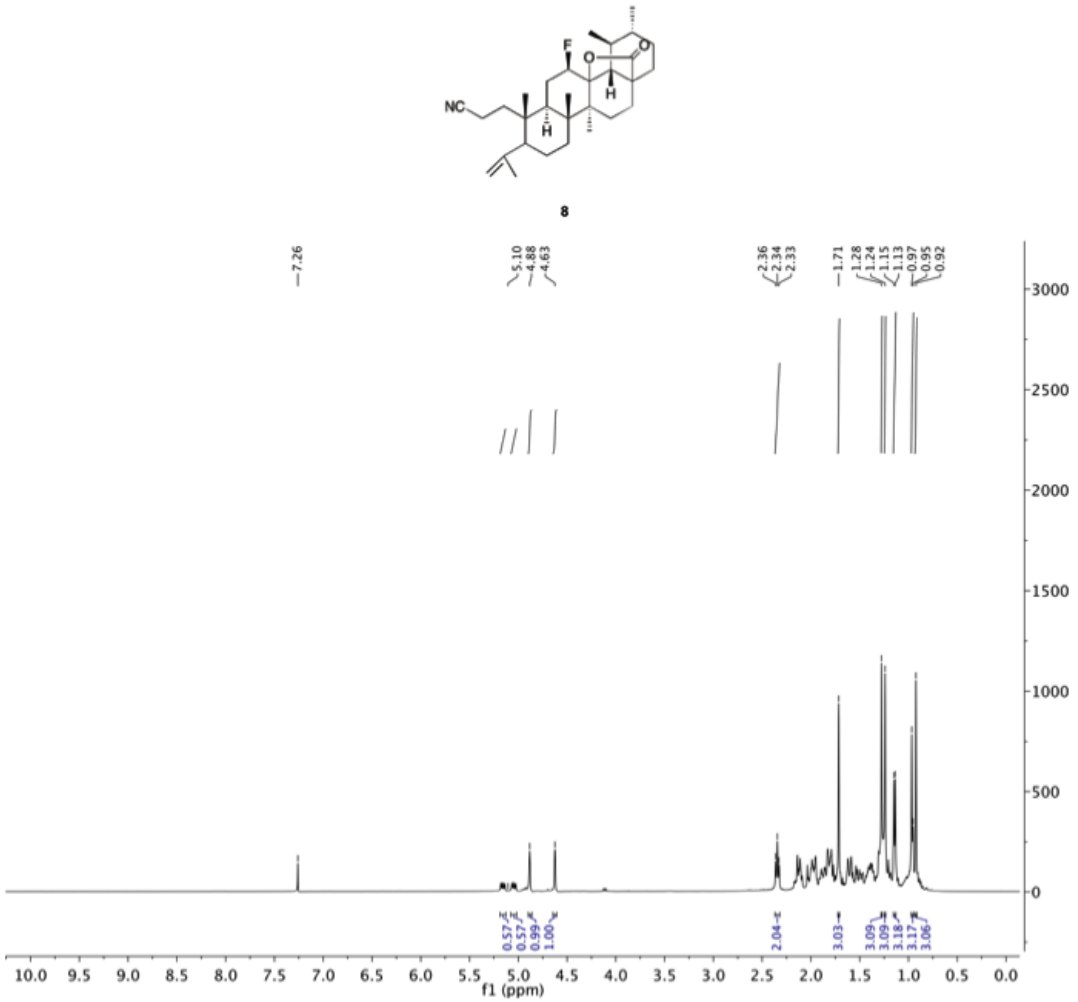


4.3. DEPT-135 NMR spectrum for compound 7 recorded in $CDCl_3$

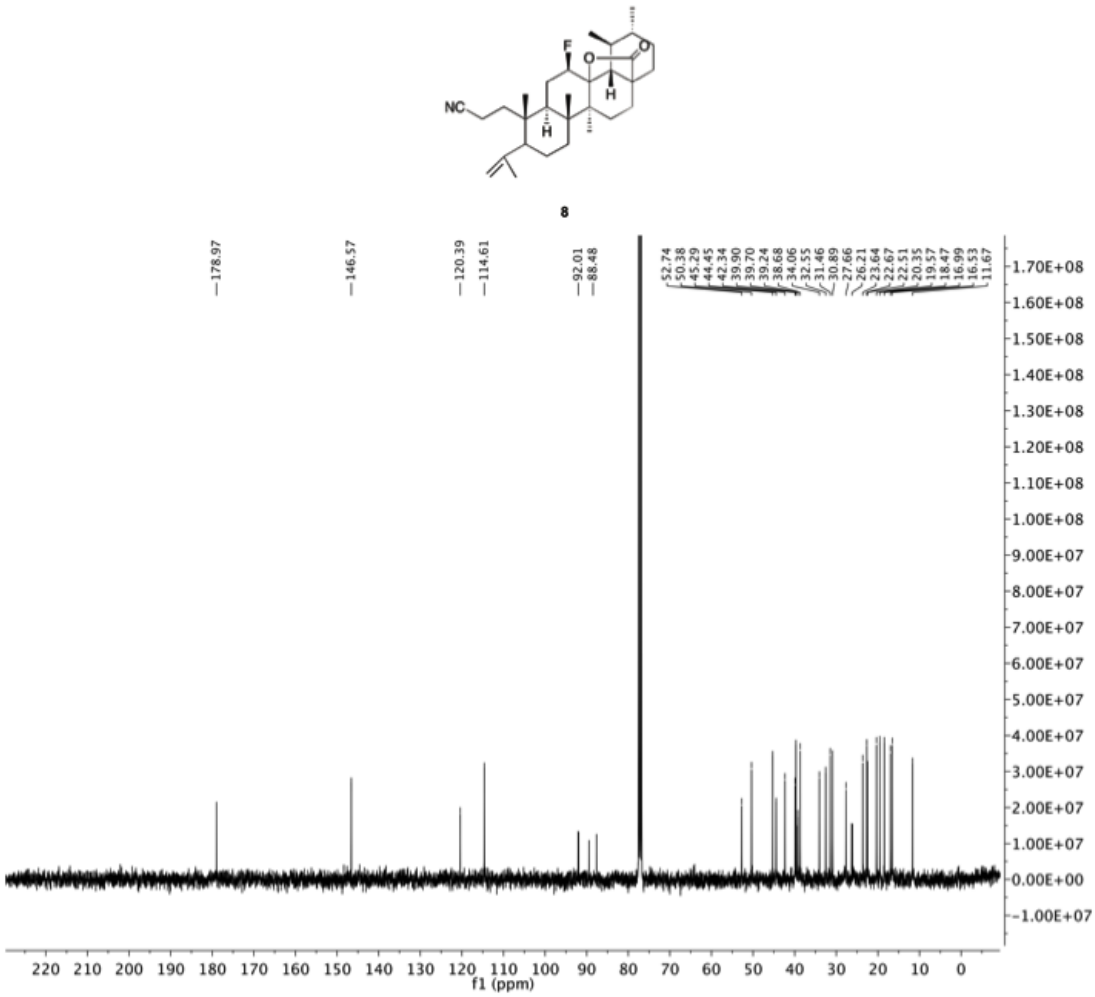


5. NMR data for compound 8:

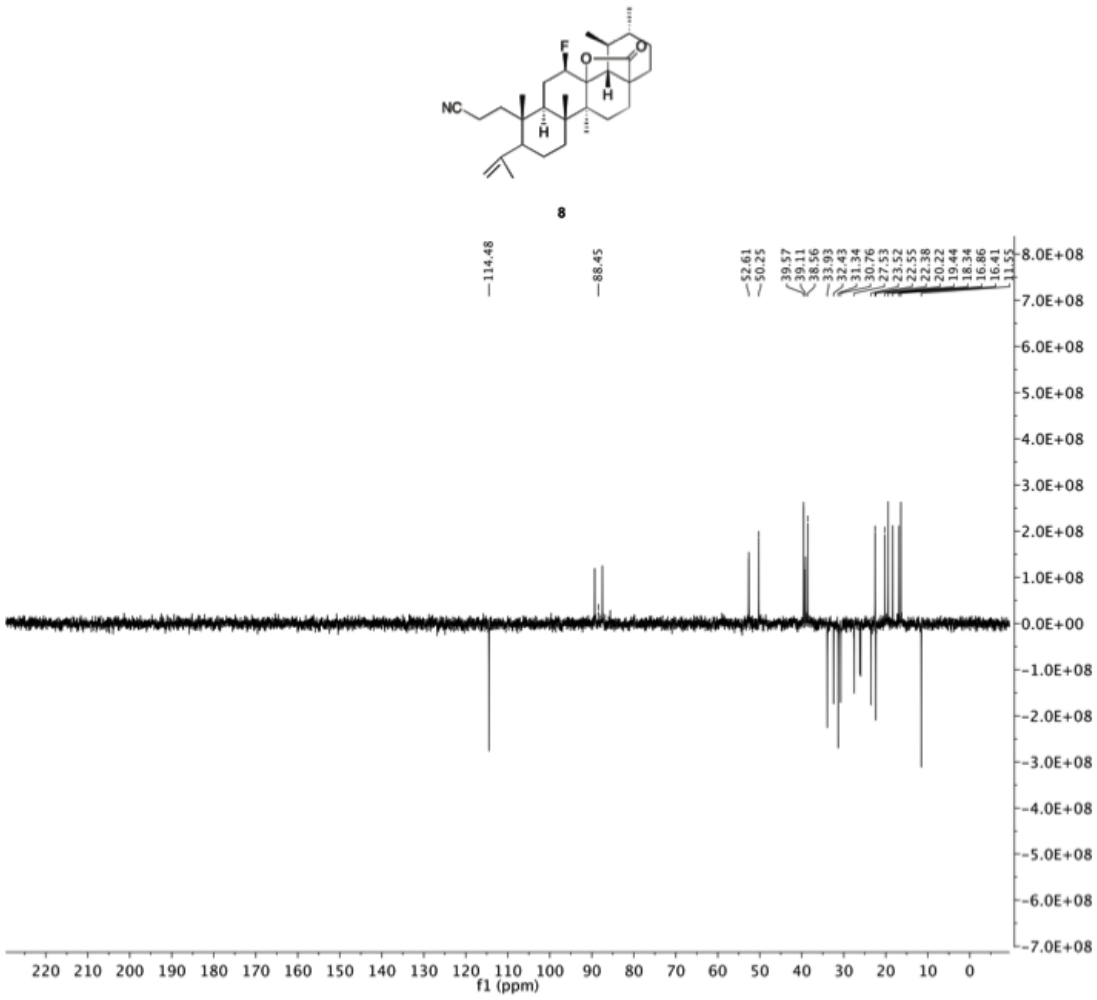
5.1. ^{1}H NMR spectrum for compound 8 recorded in $CDCl_3$



5.2. ^{13}C NMR spectrum for compound 8 recorded in $CDCl_3$

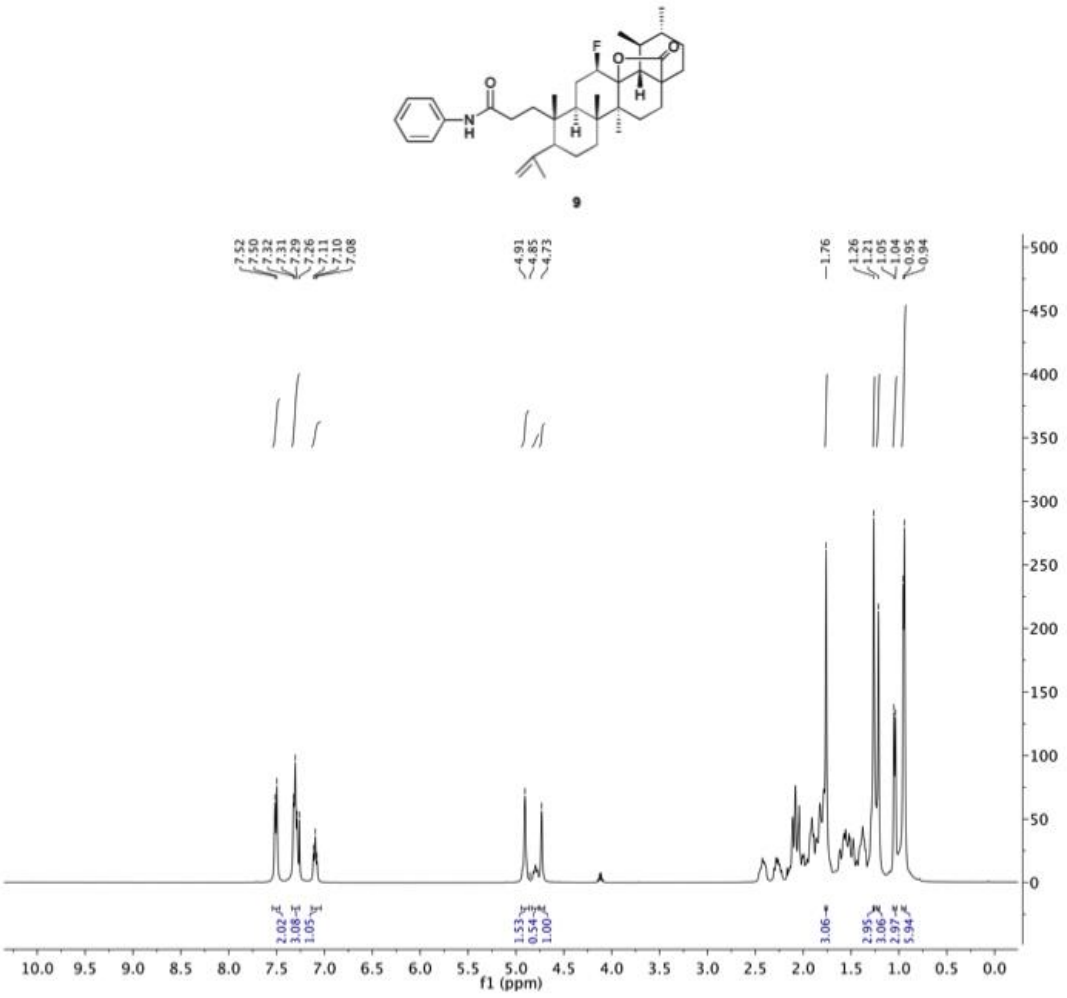


5.3. DEPT-135 NMR spectrum for compound 8 recorded in $CDCl_3$

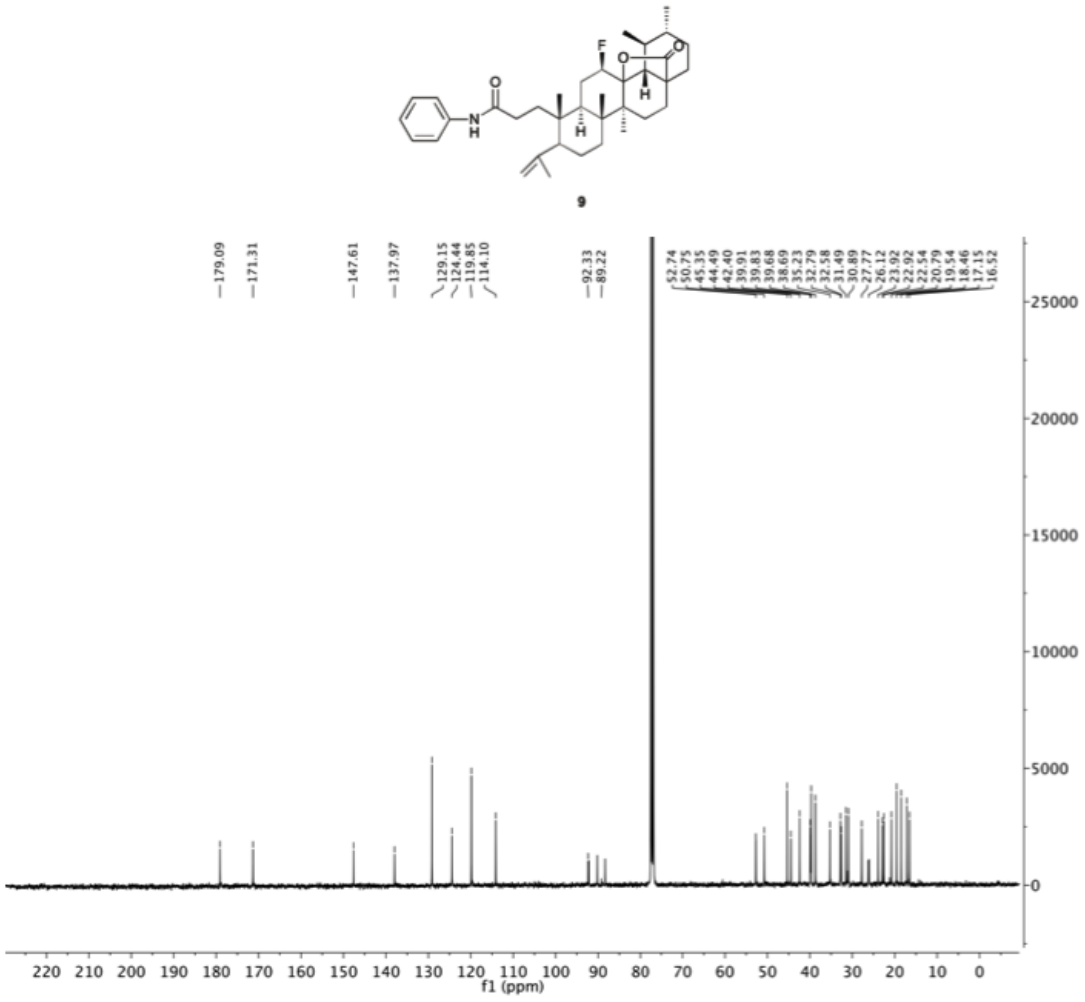


6. NMR data for compound 9:

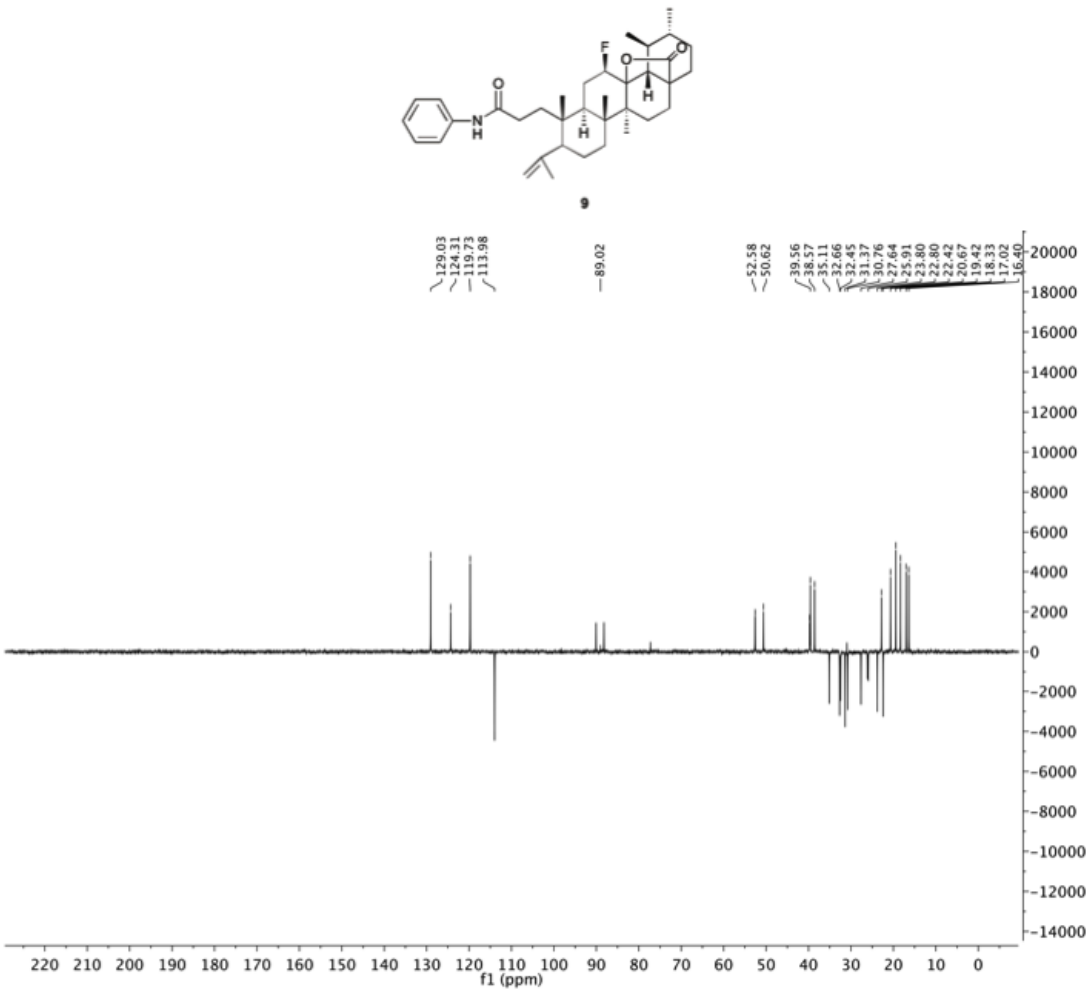
6.1. ^{1}H NMR spectrum for compound 9 recorded in $CDCl_3$



6.2. ^{13}C NMR spectrum for compound 9 recorded in $CDCl_3$

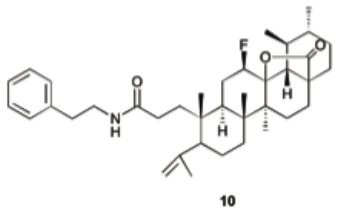


6.3. DEPT-135 NMR spectrum for compound 9 recorded in $CDCl_3$

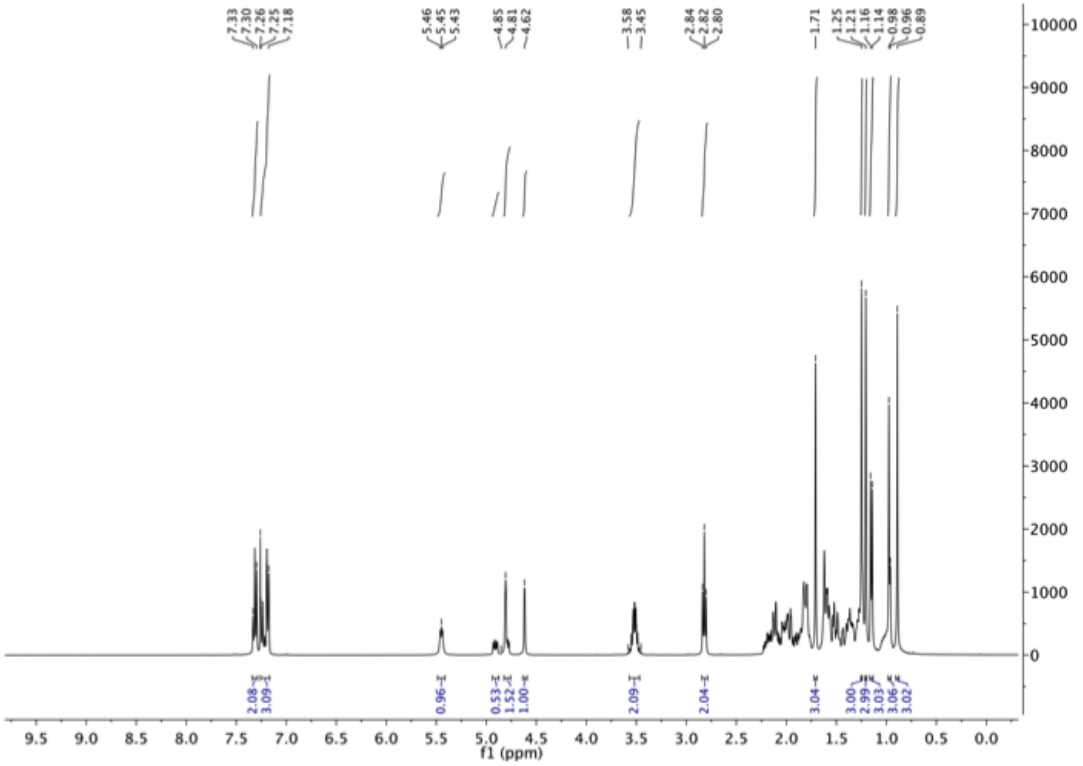


7. NMR data for compound 10:

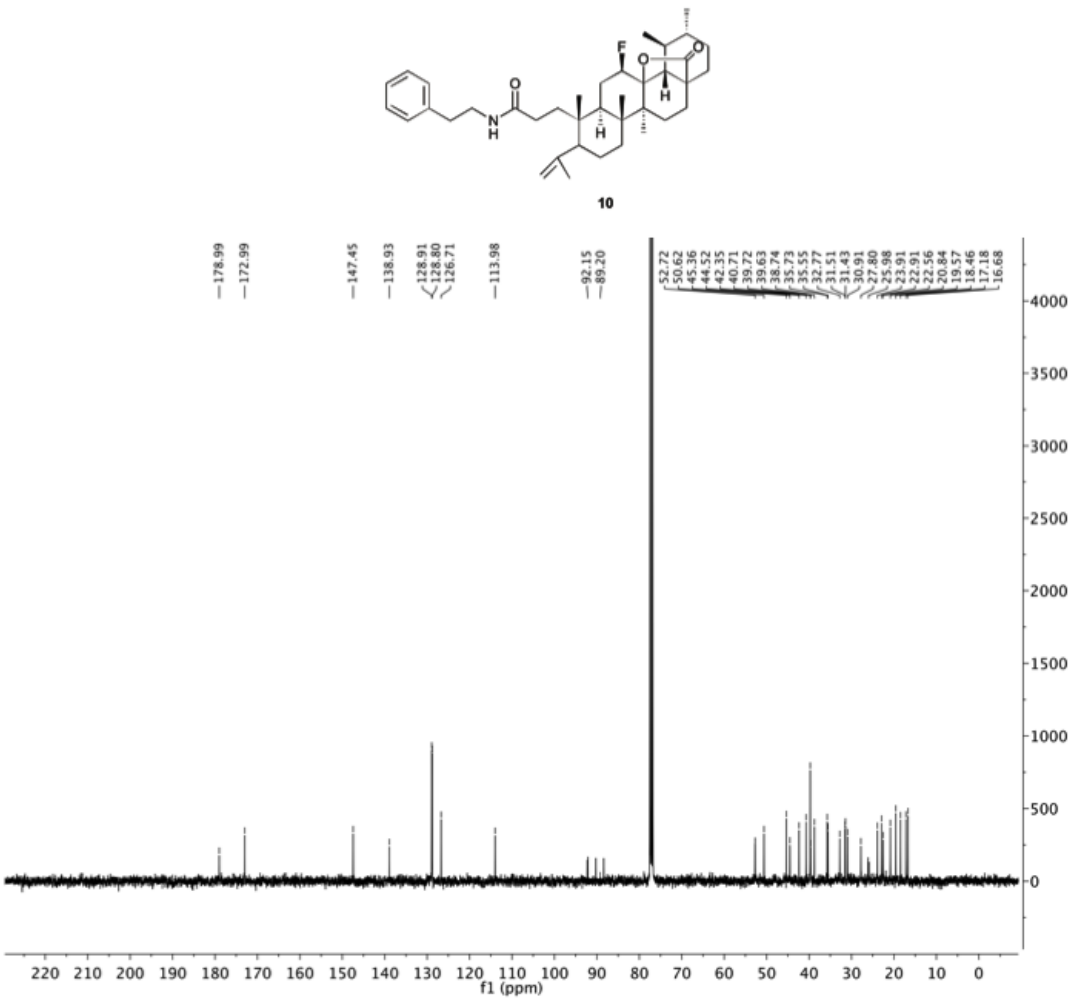
7.1. ^{1}H NMR spectrum for compound 10 recorded in $CDCl_3$



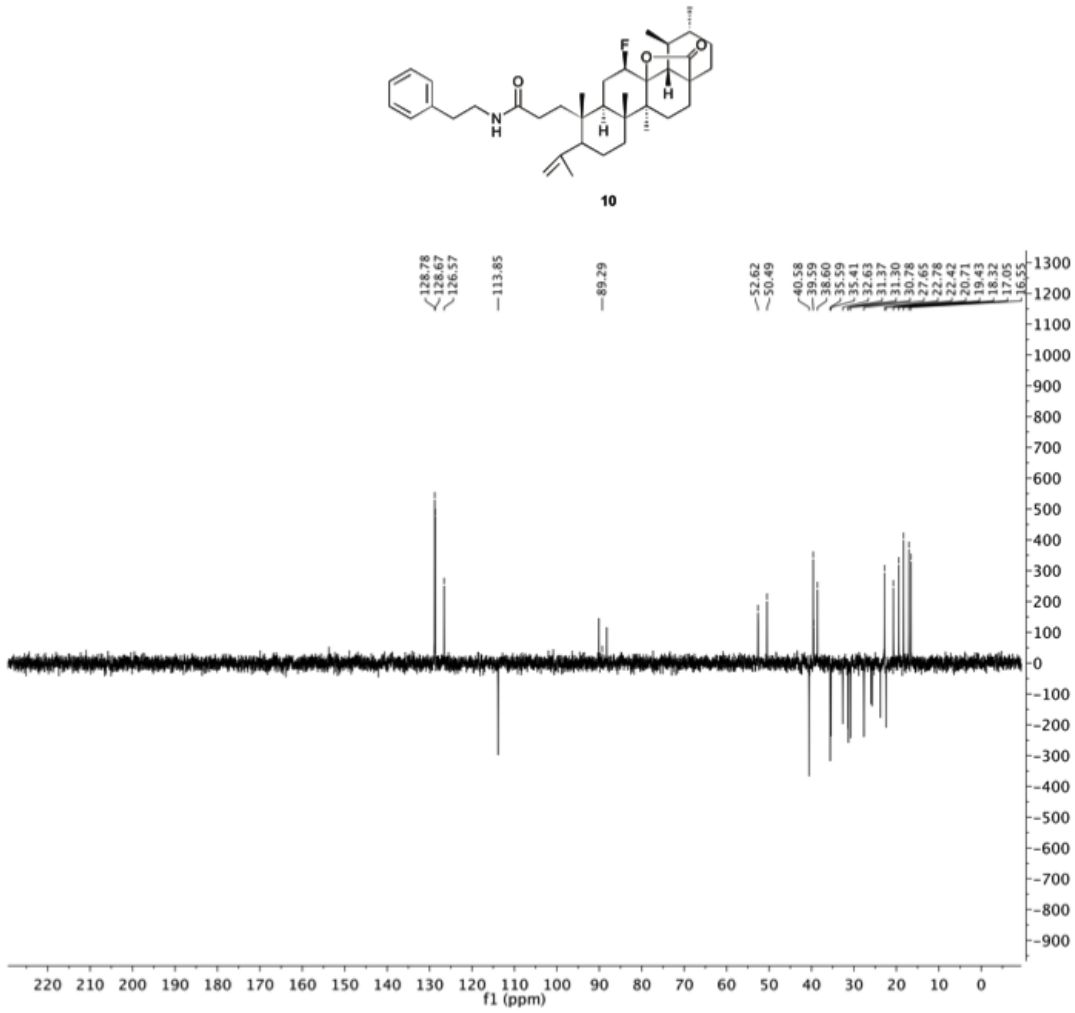
10



7.2. ^{13}C NMR spectrum for compound 10 recorded in $CDCl_3$

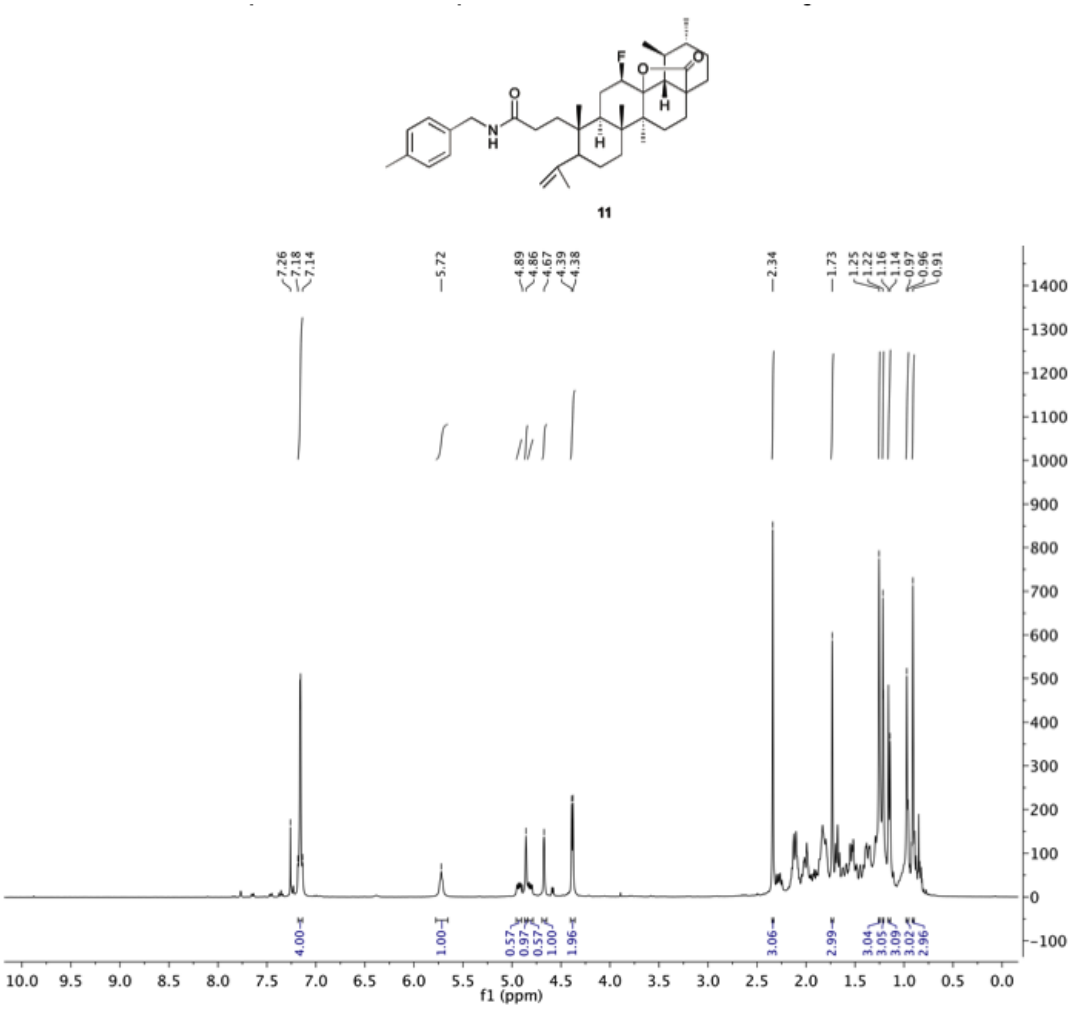


7.3. DEPT-135 NMR spectrum for compound 10 recorded in $CDCl_3$

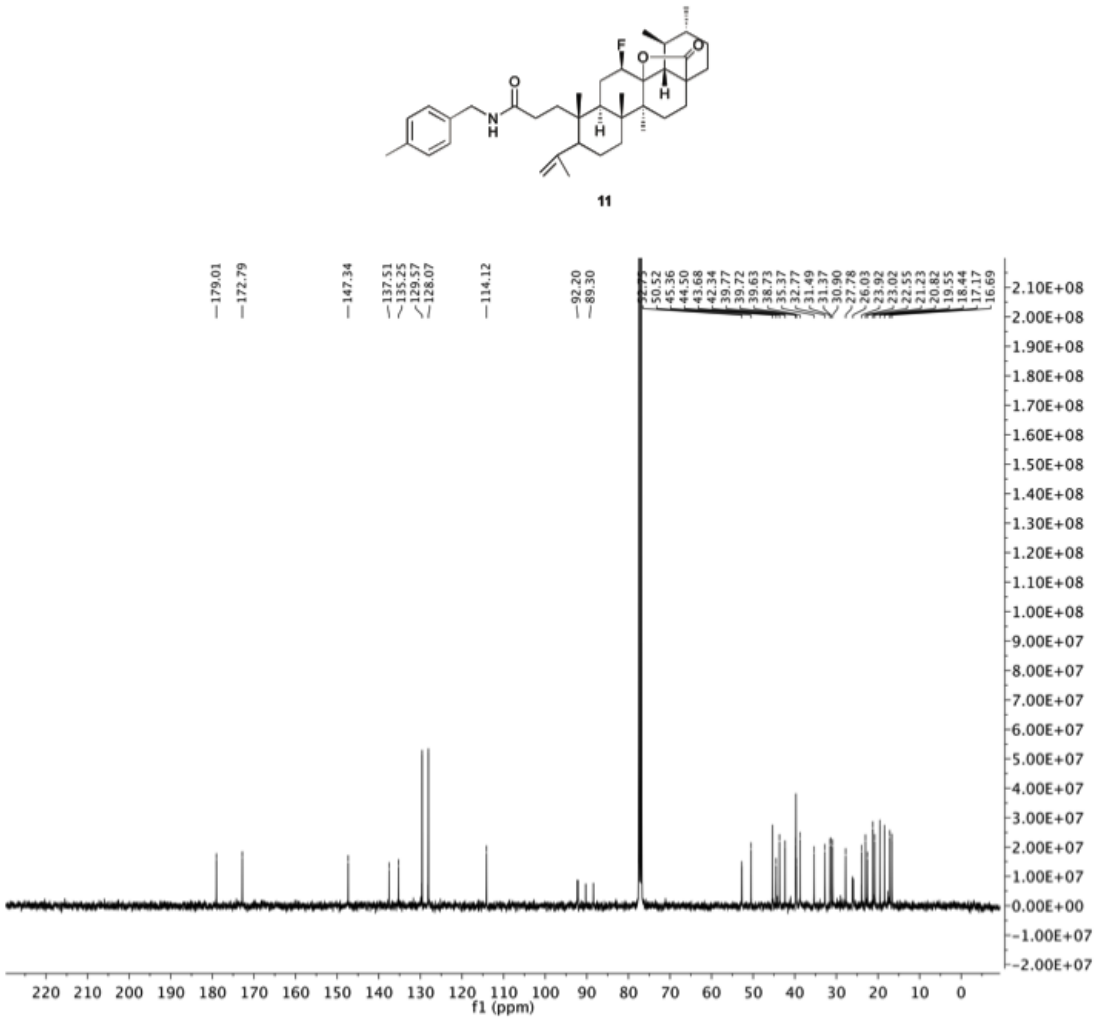


8. NMR data for compound 11:

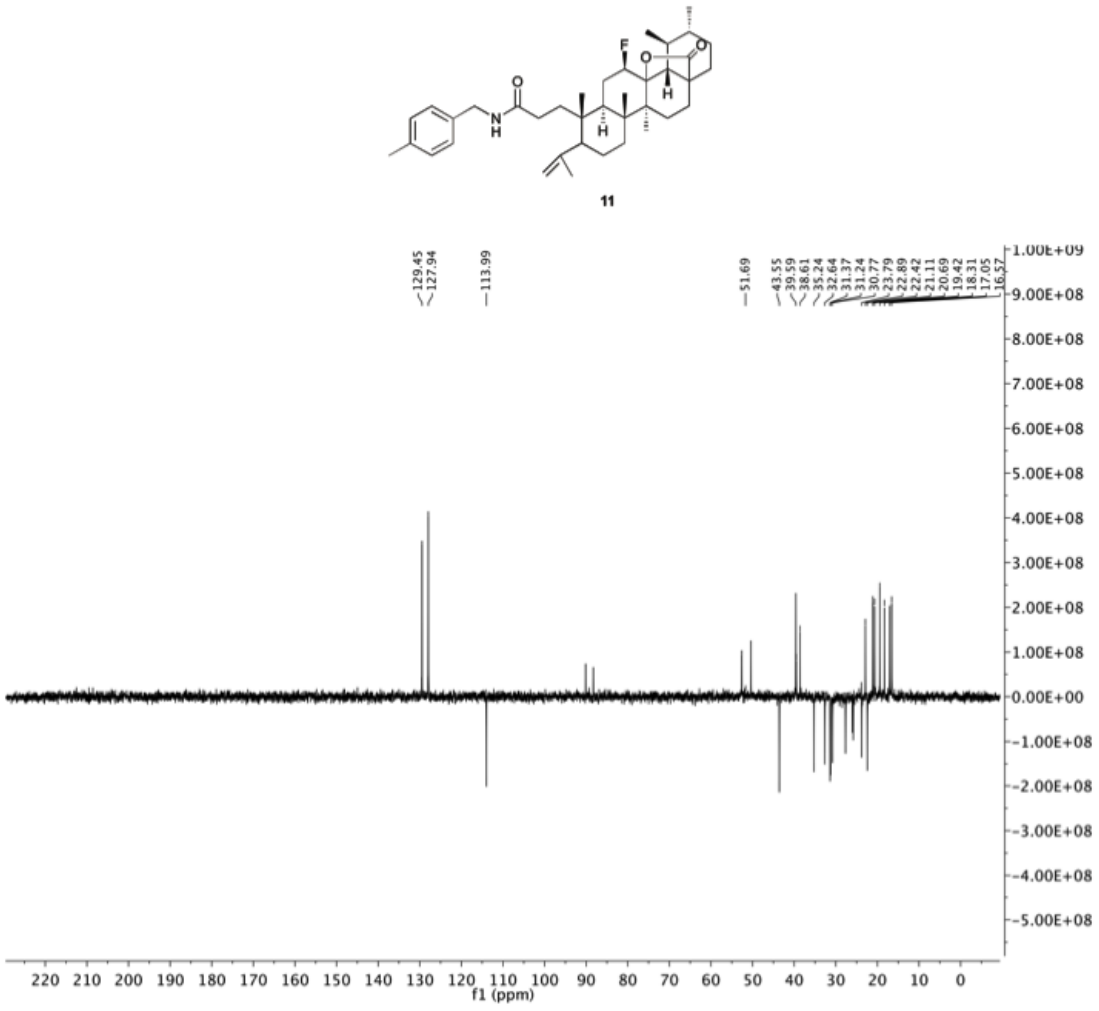
8.1. ^{1}H NMR spectrum for compound 11 recorded in $CDCl_3$



8.2. ^{13}C NMR spectrum for compound 11 recorded in $CDCl_3$

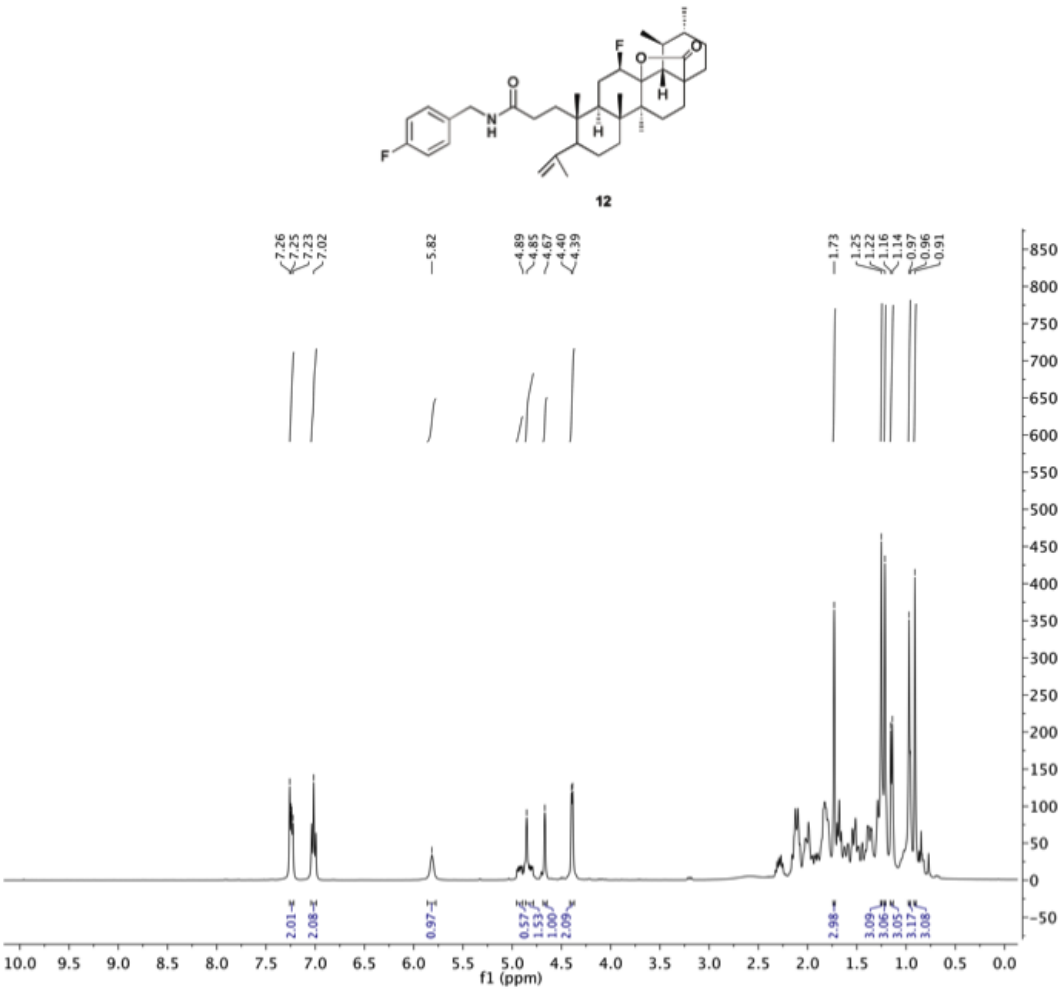


8.3. DEPT-135 NMR spectrum for compound 11 recorded in $CDCl_3$

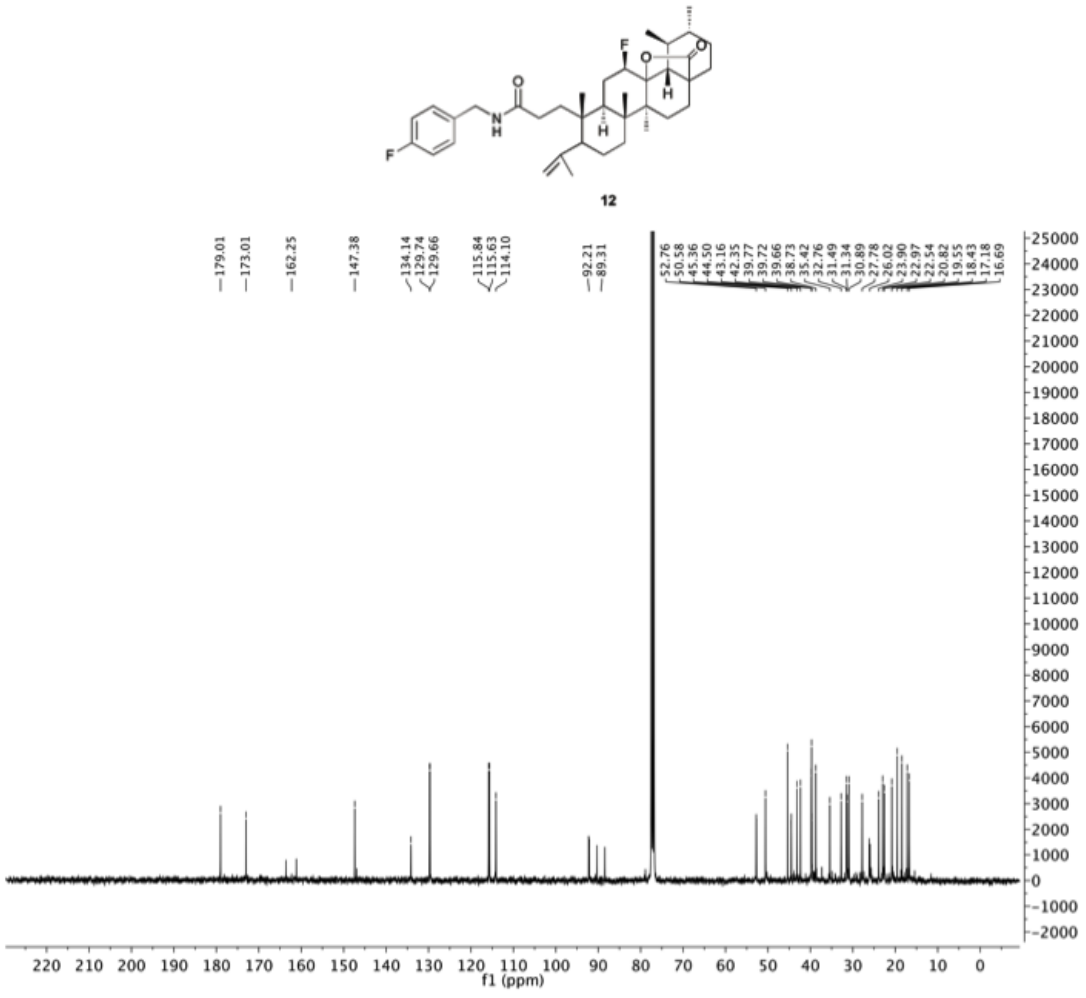


9. NMR data for compound 12:

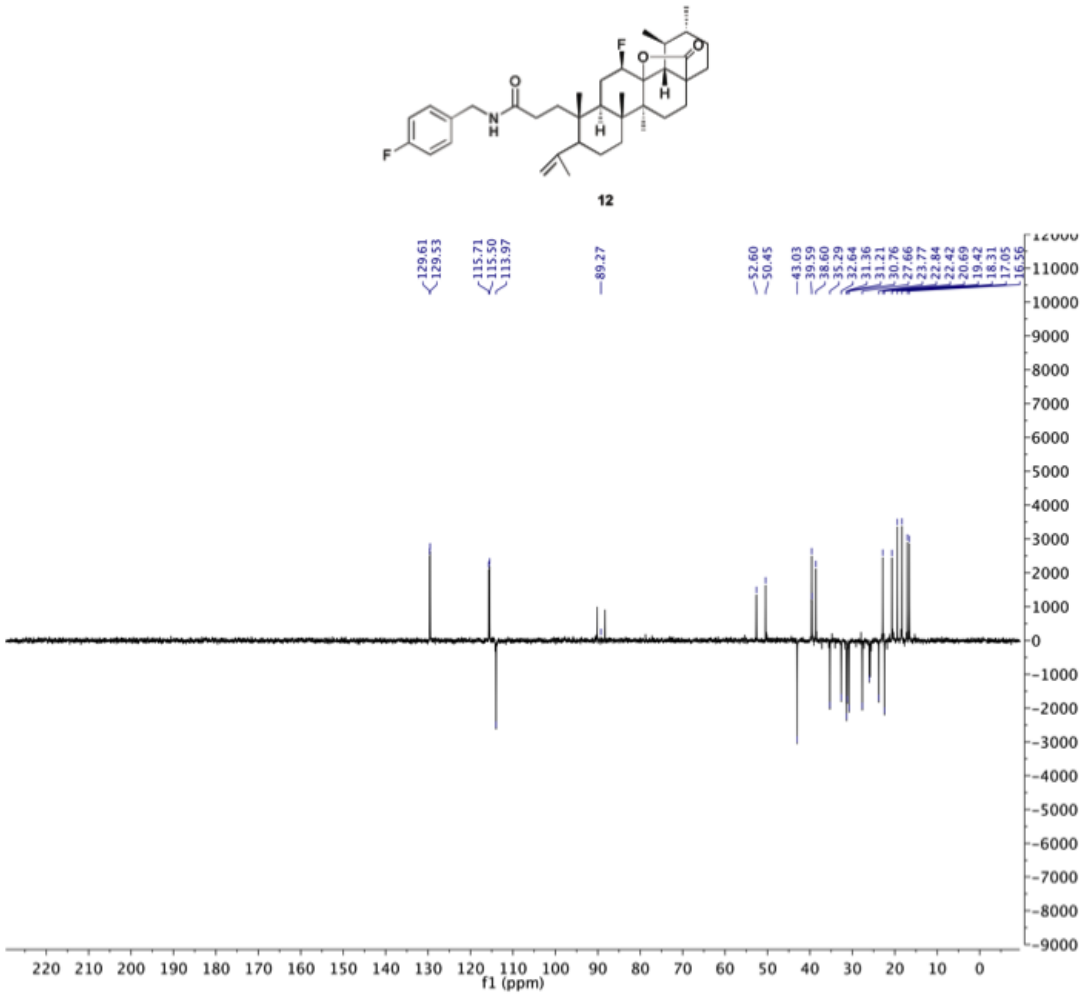
9.1. ^{1}H NMR spectrum for compound 12 recorded in $CDCl_3$



9.2. ^{13}C NMR spectrum for compound 12 recorded in $CDCl_3$

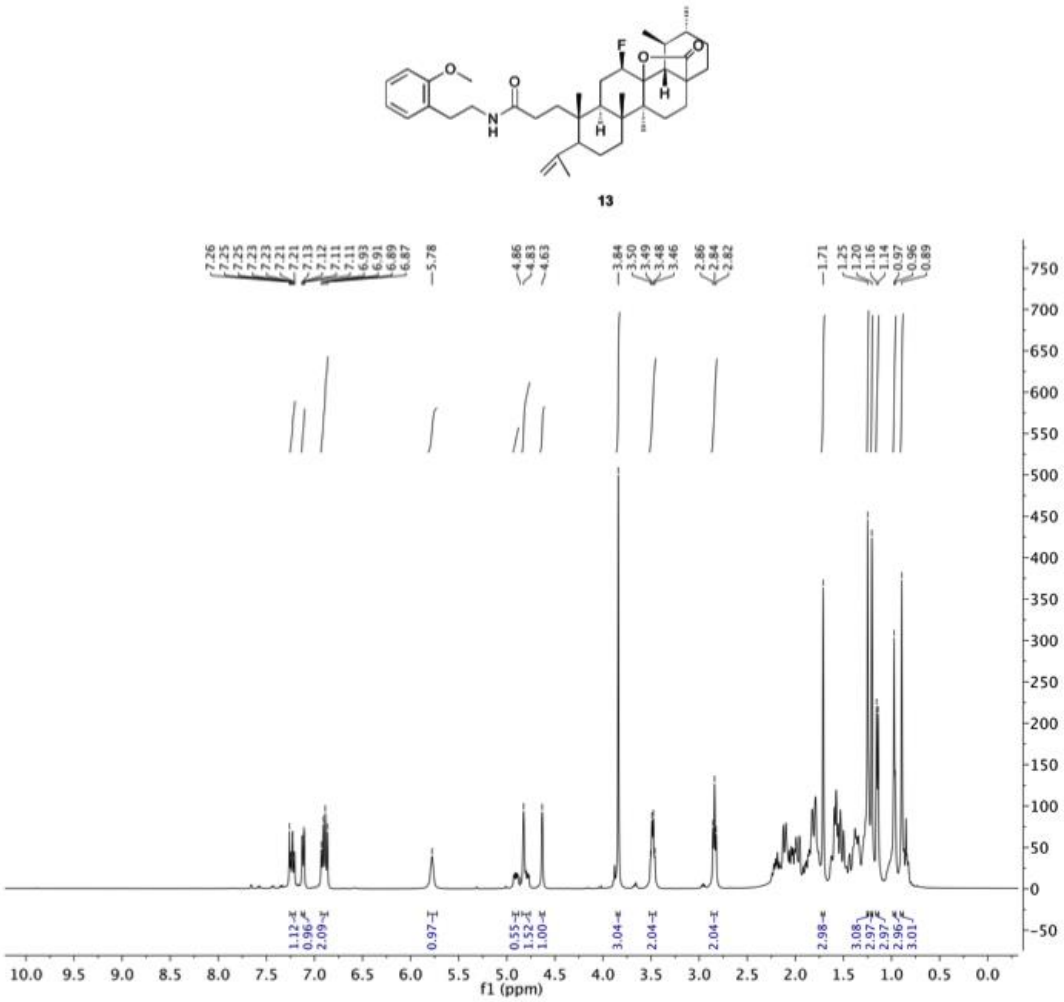


9.3. DEPT-135 NMR spectrum for compound 12 recorded in $CDCl_3$

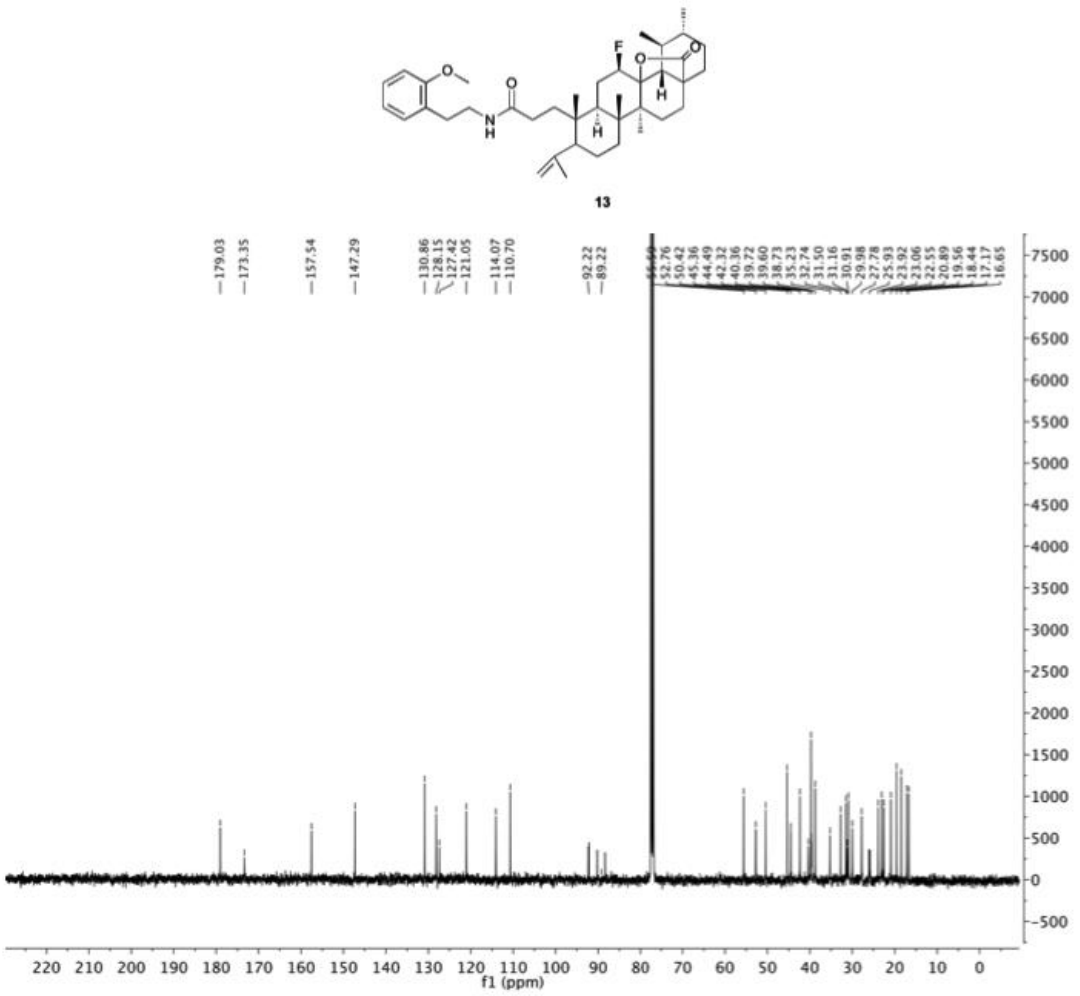


10. NMR data for compound 13:

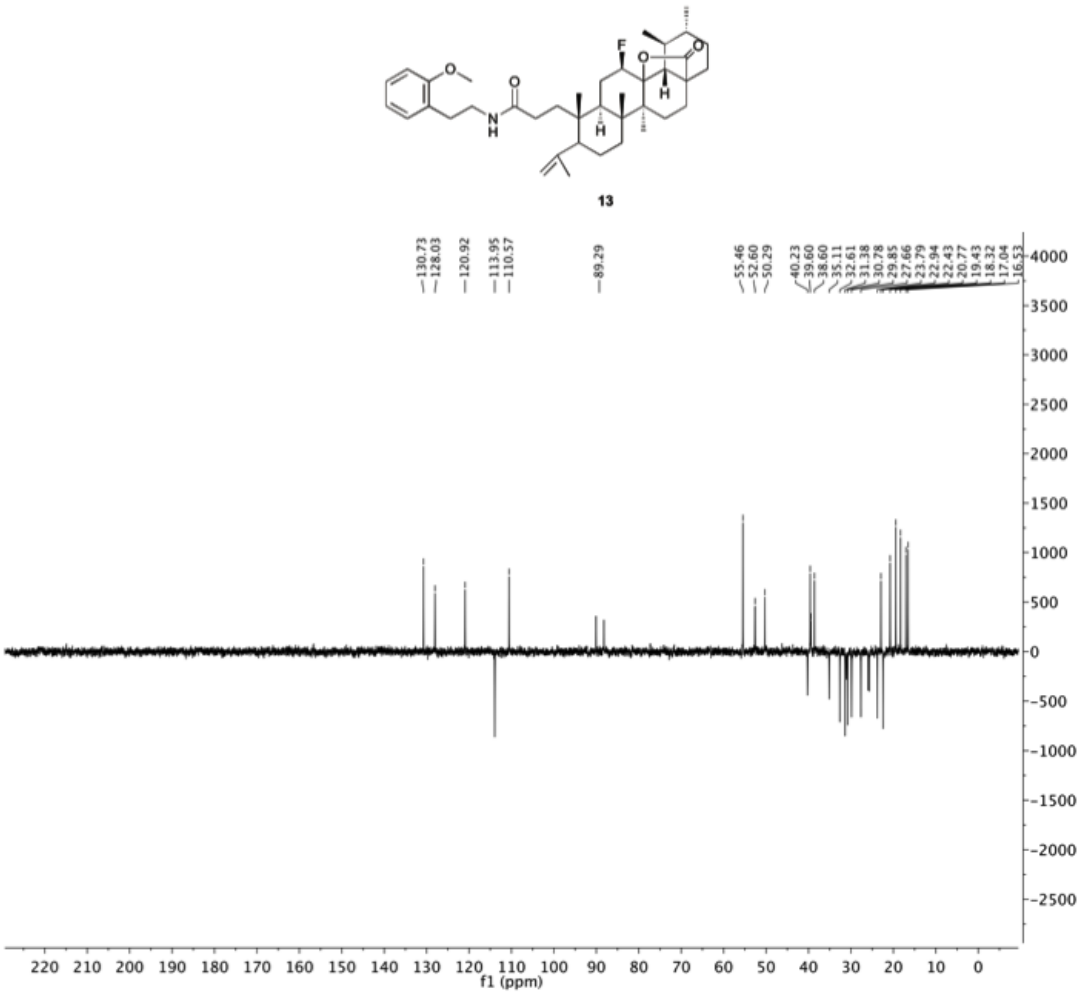
10.1. ^{1}H NMR spectrum for compound 13 recorded in $CDCl_3$



10.2. ^{13}C NMR spectrum for compound 13 recorded in $CDCl_3$

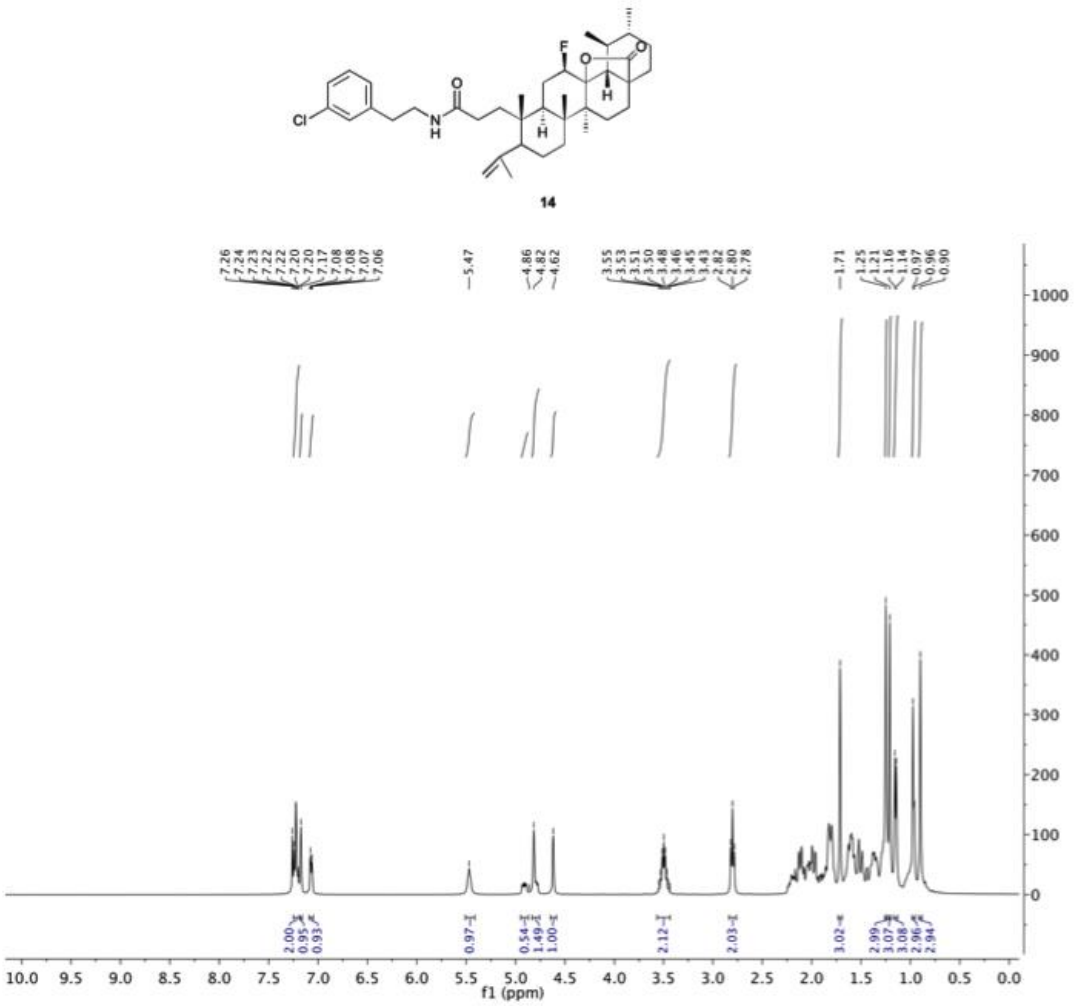


10.3. DEPT-135 NMR spectrum for compound 13 recorded in $CDCl_3$

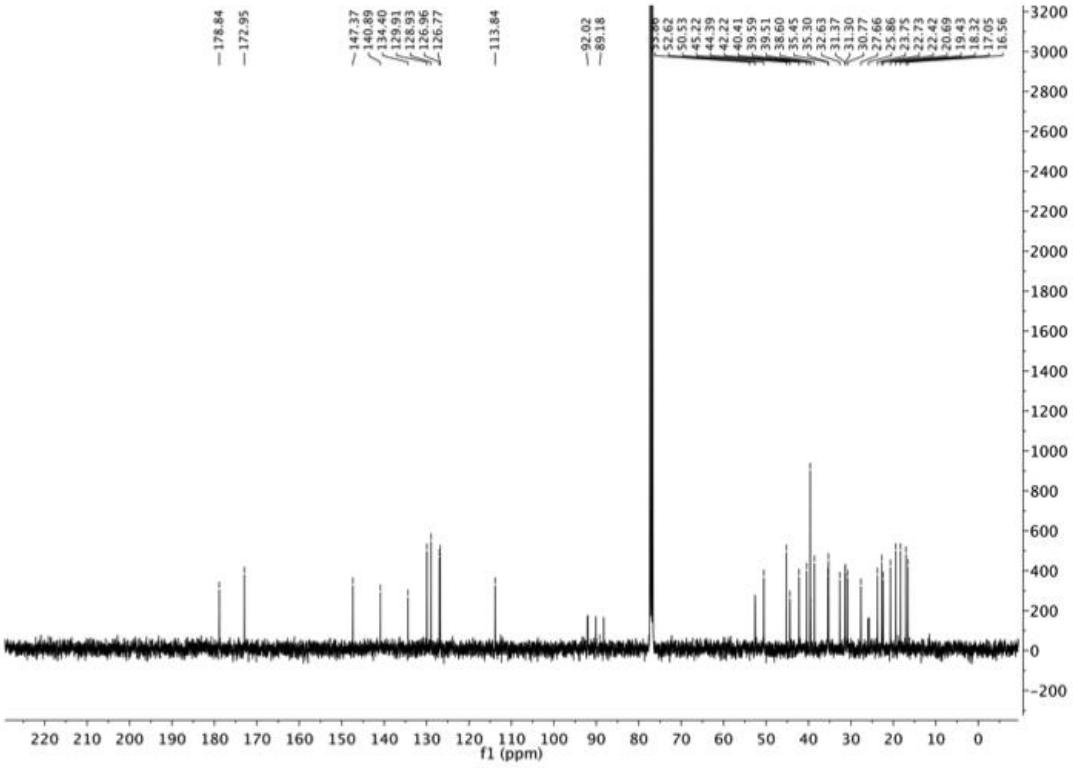


11. NMR data for compound 14:

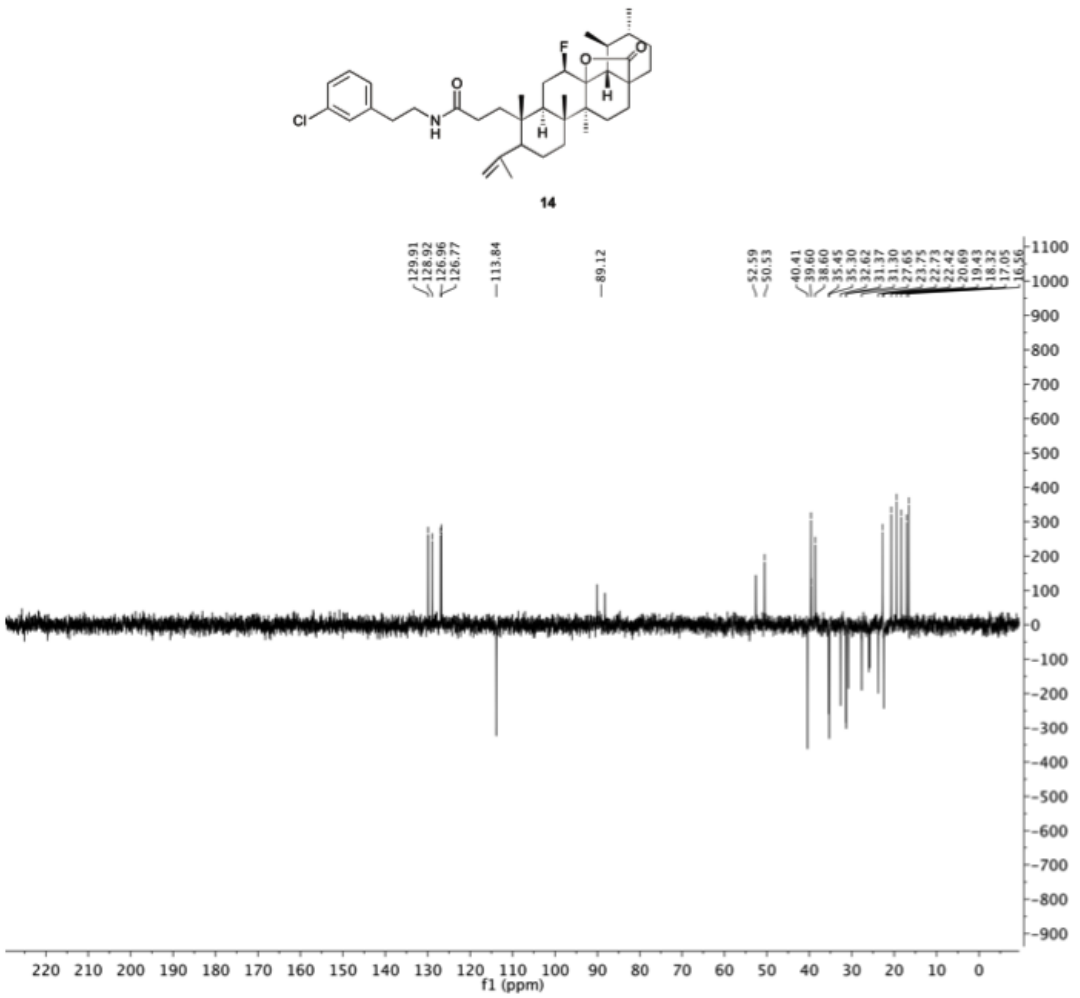
11.1. ^{1}H NMR spectrum for compound 14 recorded in $CDCl_3$



11.2. ^{13}C NMR spectrum for compound 14 recorded in $CDCl_3$

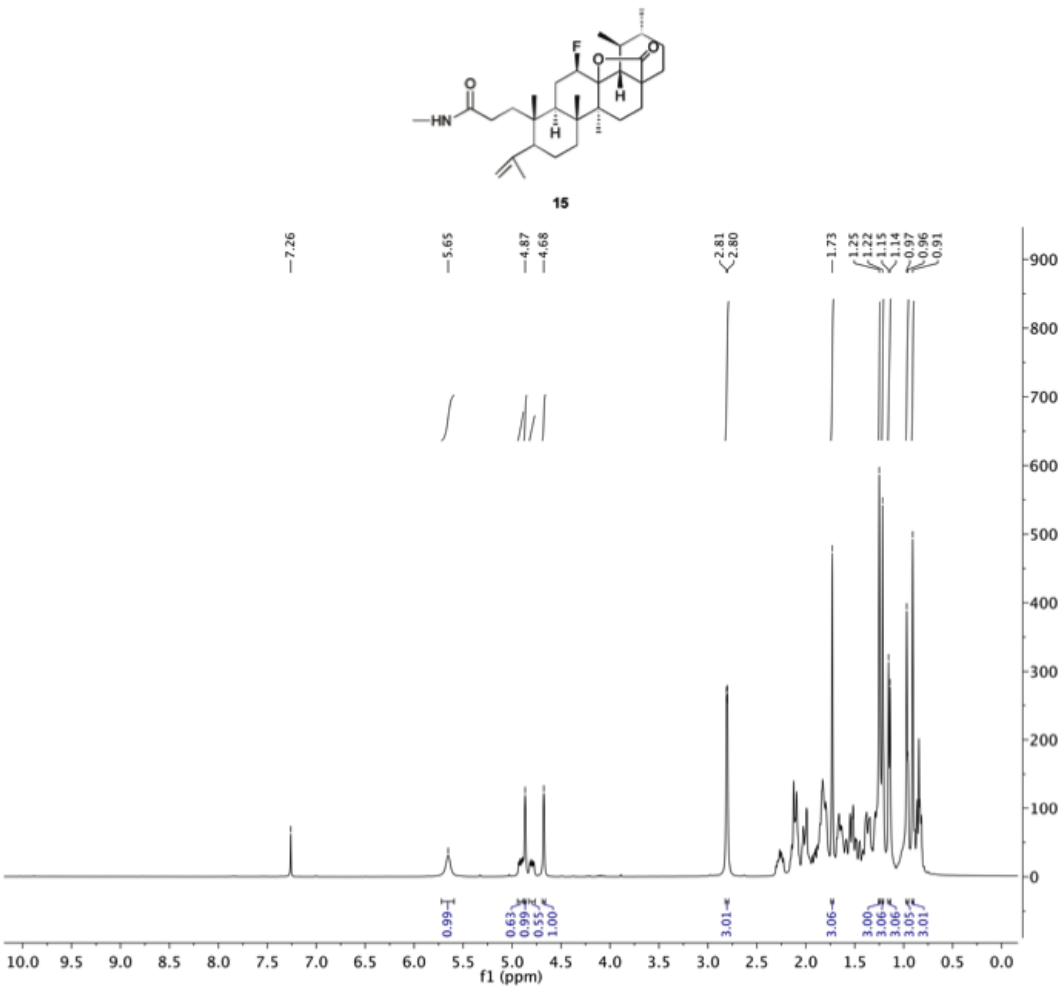


11.3. DEPT-135 NMR spectrum for compound 14 recorded in $CDCl_3$

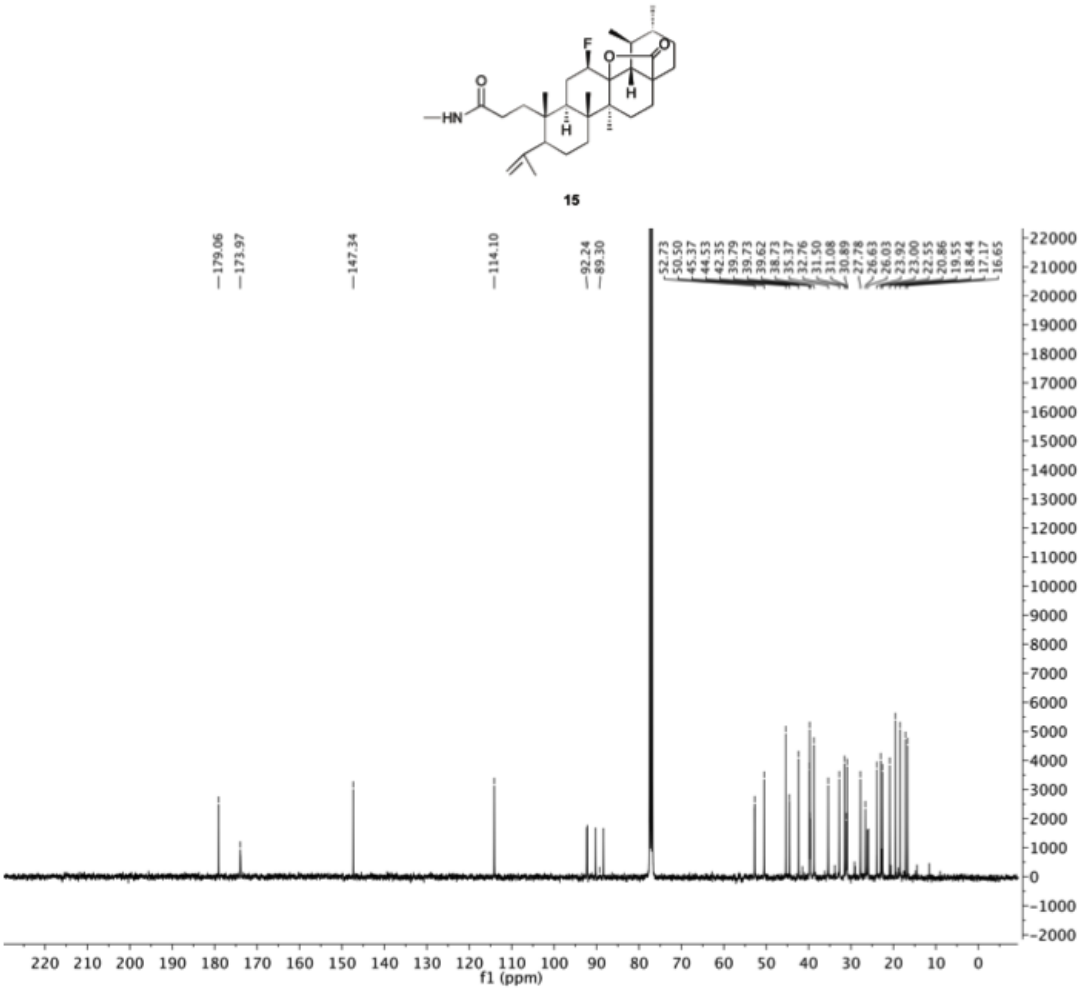


12. NMR data for compound 15:

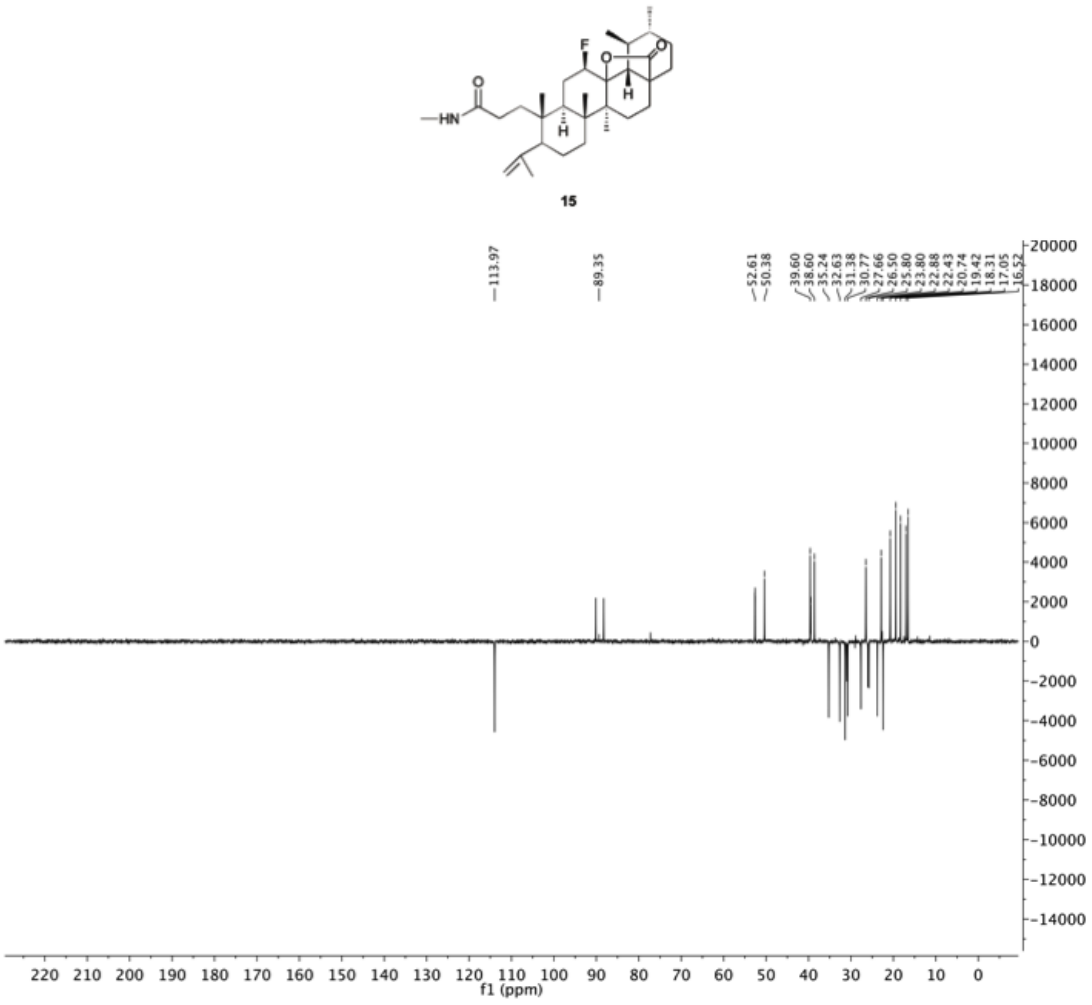
12.1. ^{1}H NMR spectrum for compound 15 recorded in $CDCl_3$



12.2. ^{13}C NMR spectrum for compound 15 recorded in $CDCl_3$

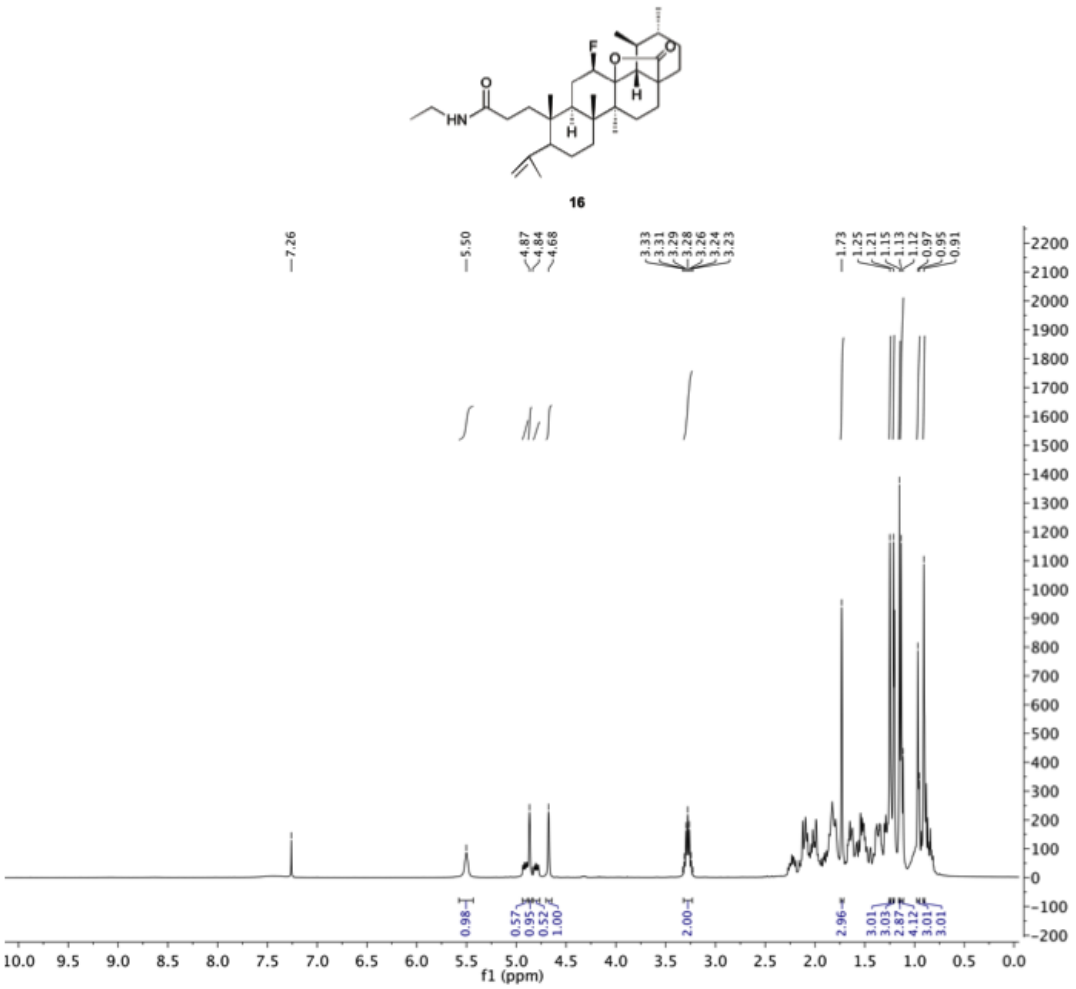


12.3. DEPT-135 NMR spectrum for compound 15 recorded in $CDCl_3$

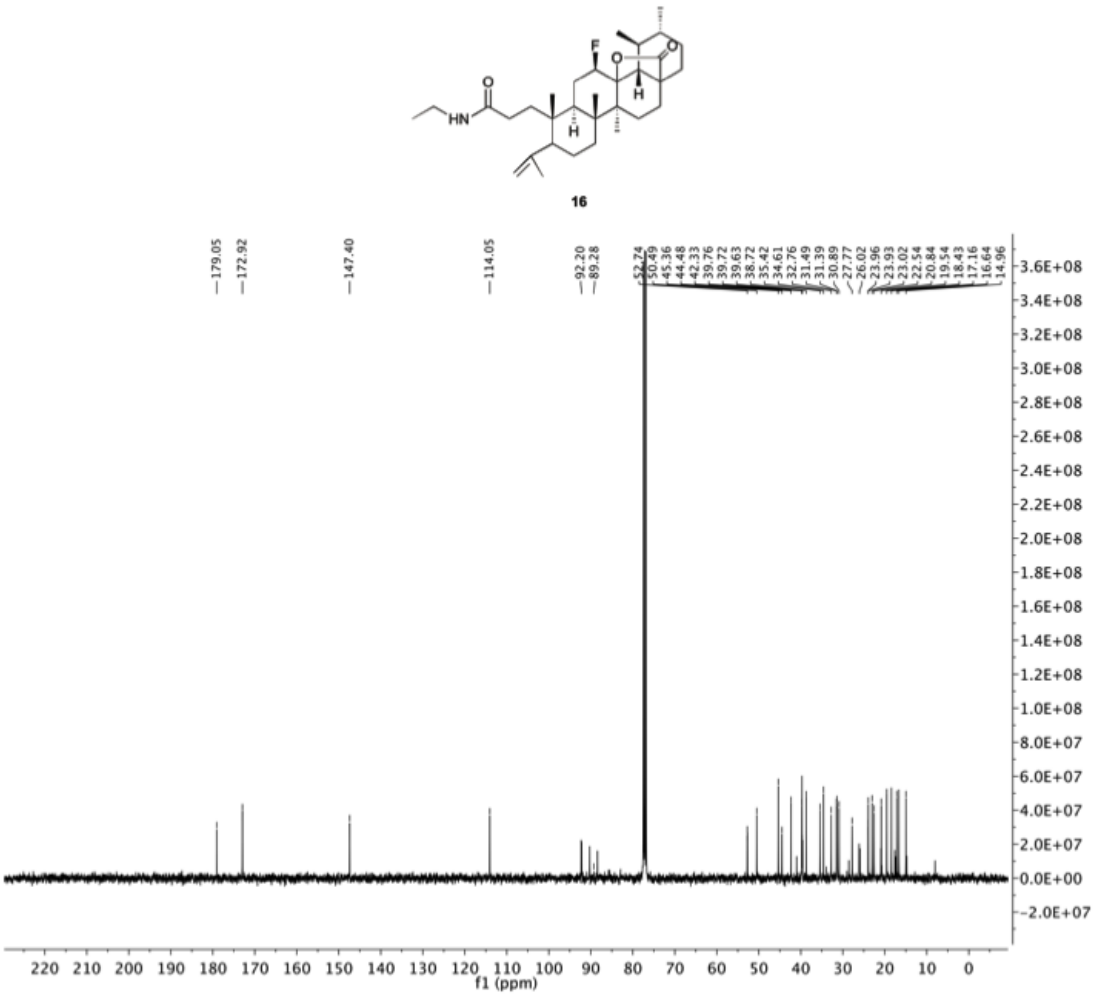


13. NMR data for compound 16:

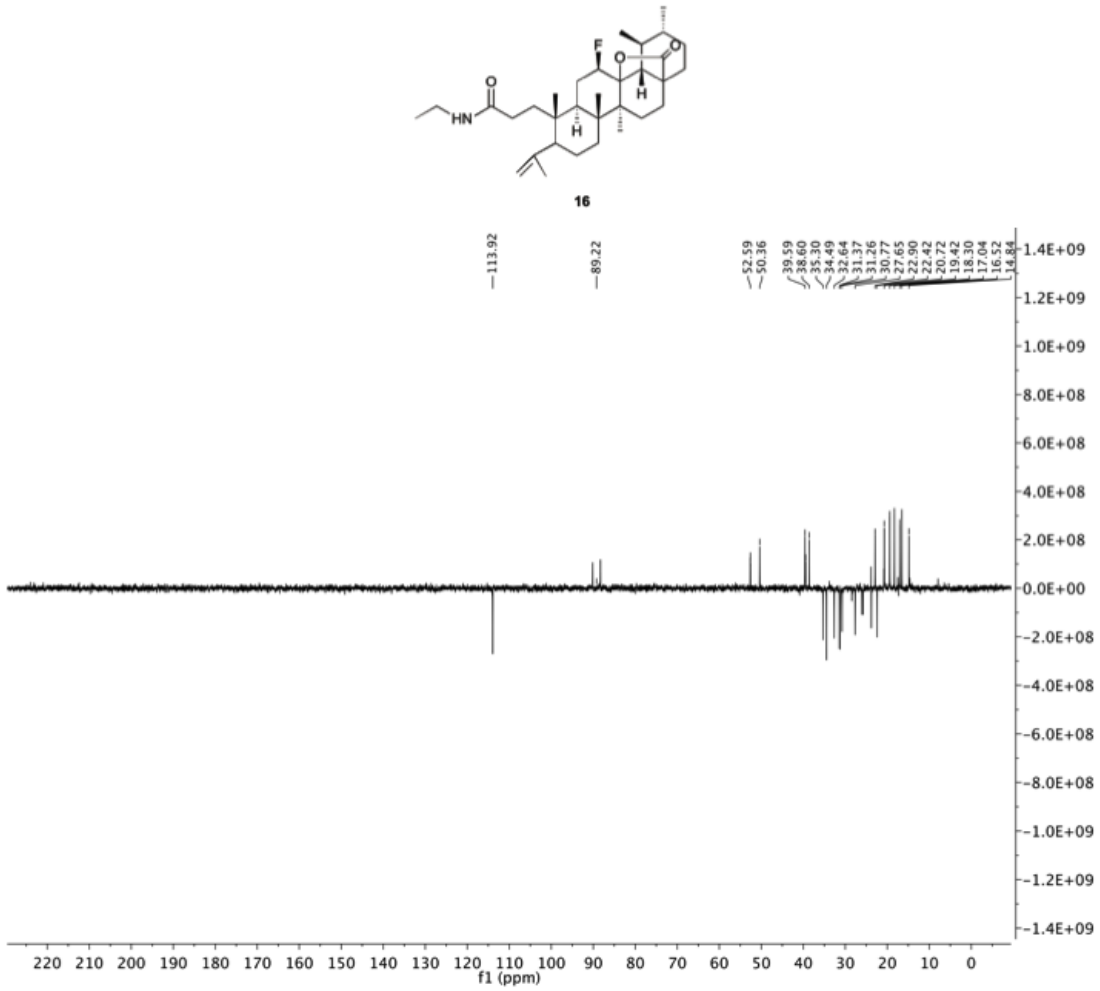
13.1. ^{1}H NMR spectrum for compound 16 recorded in $CDCl_3$



13.2. ^{13}C NMR spectrum for compound 16 recorded in $CDCl_3$

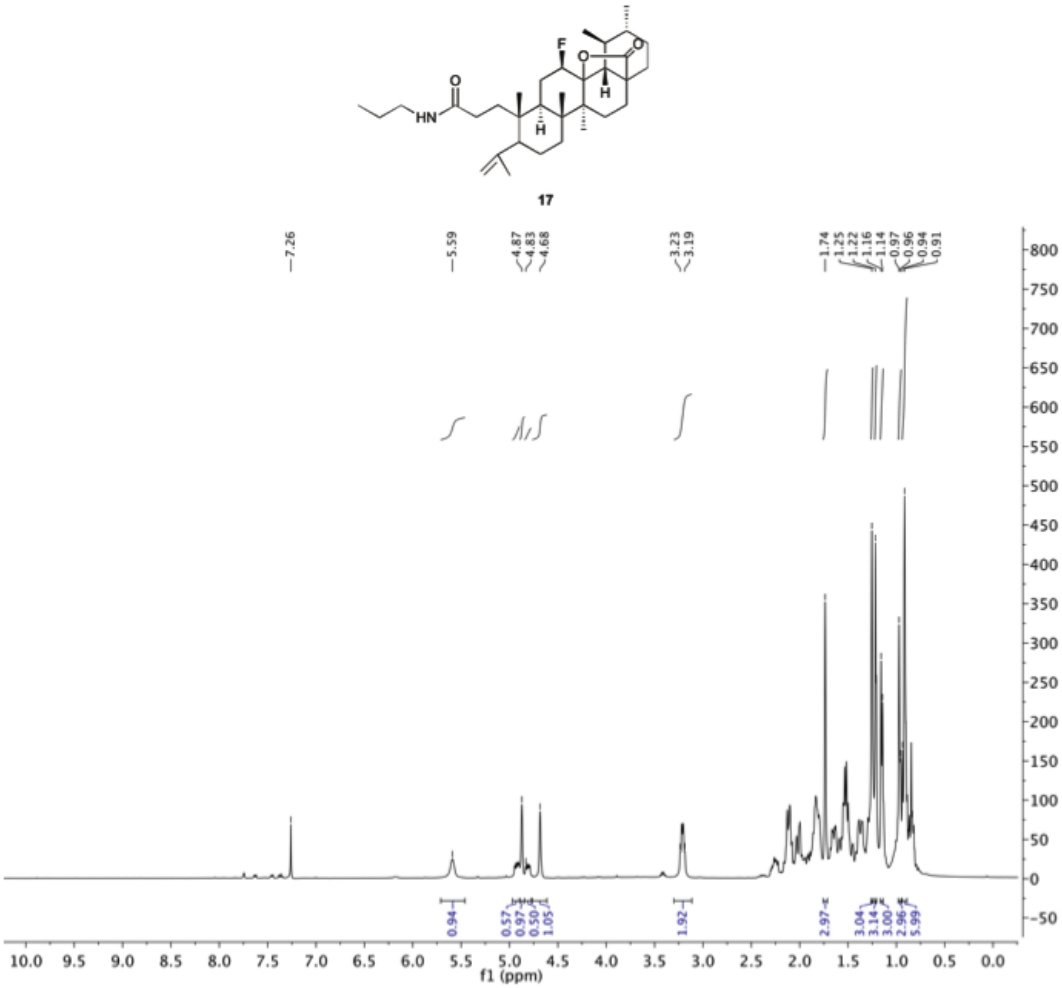


13.3. DEPT-135 NMR spectrum for compound 16 recorded in $CDCl_3$

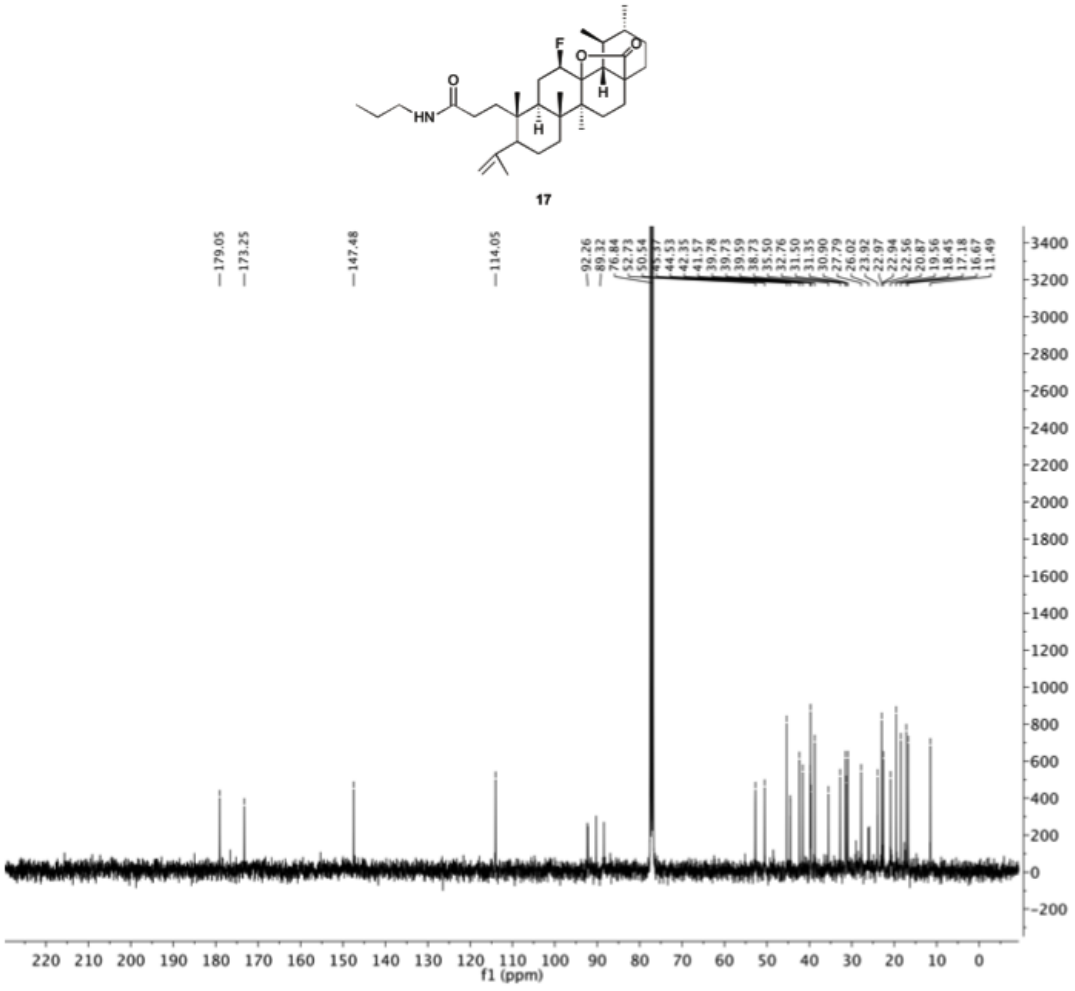


14. NMR data for compound 17:

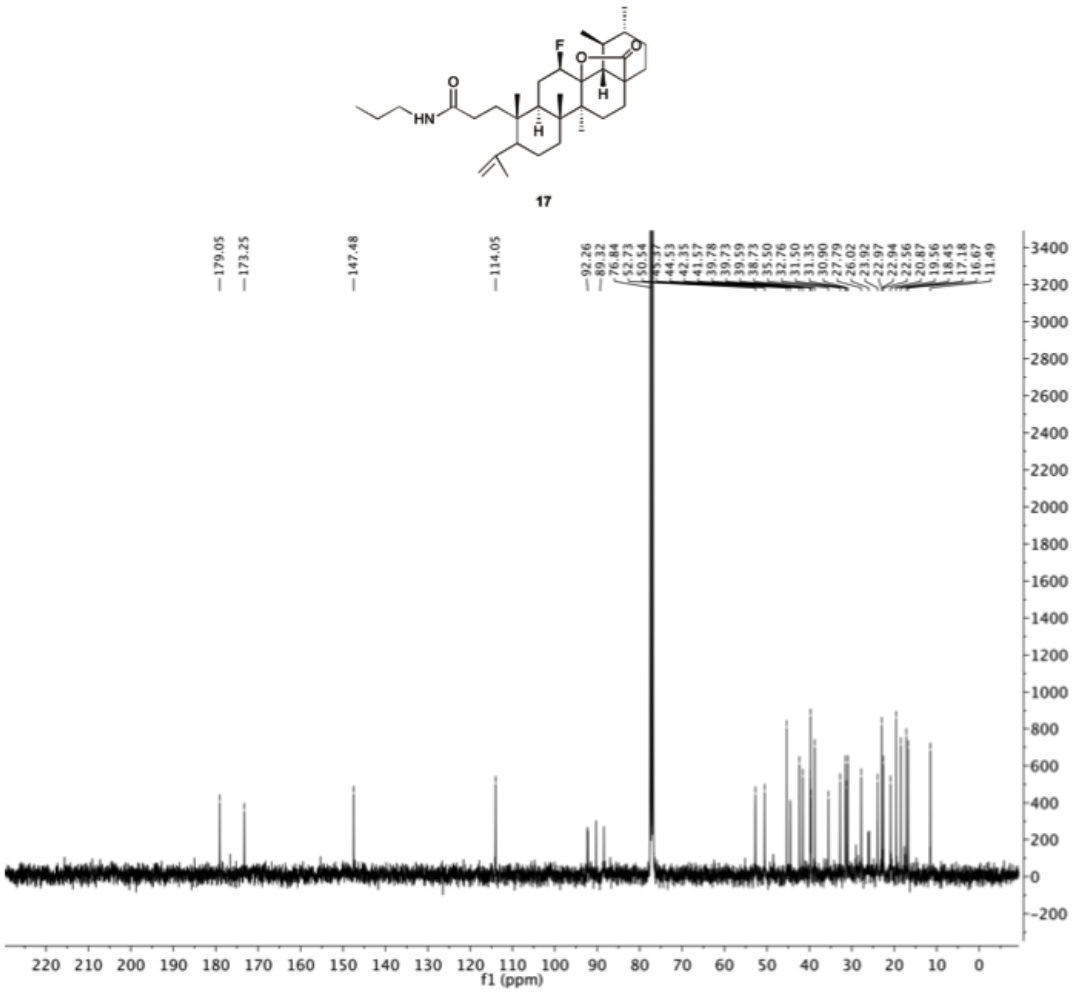
14.1. ^{1}H NMR spectrum for compound 17 recorded in $CDCl_3$



14.2. ^{13}C NMR spectrum for compound 17 recorded in $CDCl_3$



14.3. DEPT-135 NMR spectrum for compound 17 recorded in $CDCl_3$



15. Effect of compound 17 on cell-cycle distribution

The effect of compound **17** on the cell-cycle distribution of H460 cell line was explored. Cells were treated with DMSO (0.15%), compound **17** (3.5 or 4 μM) for 24h and 72h, respectively, and the cell cycle profile was evaluated by FACS analysis, after staining cells with propidium iodide (PI). As it can be observed in Fig 1, there was no change in any population at any concentration or time point, suggesting **C17** treatment in H460 cells does not affect the cell cycle profile.

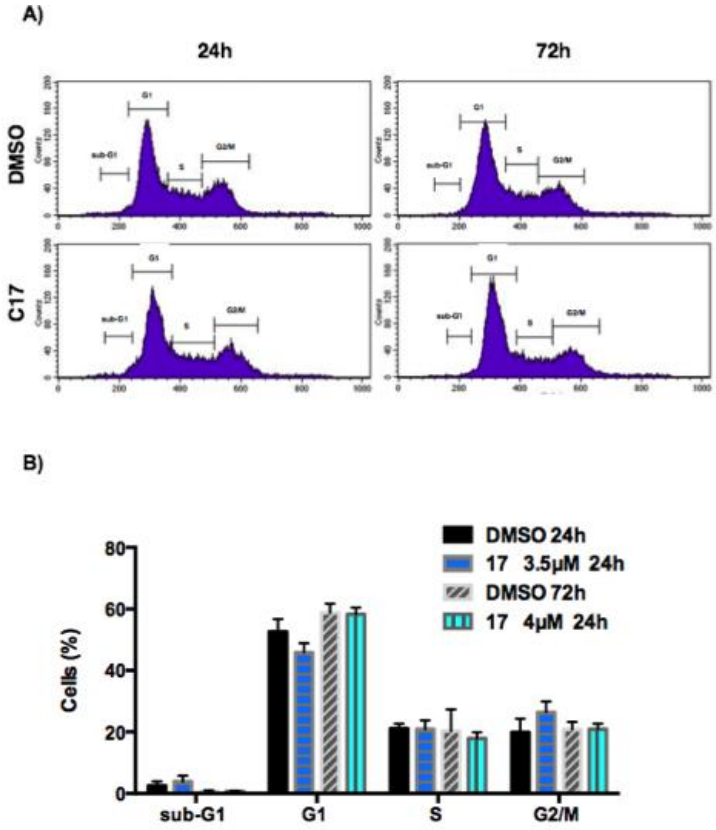


Figure 1. Effect of compound **17** on cell cycle distribution. H460 cells treated with vehicle or compound **17** at the indicated concentrations for 24 and 72h were assessed for cell cycle, using PI staining and flow cytometer analysis. A) Representative histograms of cell cycle analysis are shown. B) The bar graphic depicts the cell population (%) per cell cycle phase. The values represent the mean ± SD of three independent experiments.

15.1. Cell Cycle Assay

Cell cycle was assessed by PI flow cytometry assay. H460 cells were plated in dish 100mmx20mm for 24h and 72h treatment experiment ($3x10^5$ cells/dish and $0.8x10^5$, respectively, H460 CTR5 cell line, 10 ml RPMI-1640-10% FBS) and after 24h, cells were treated with DMSO alone or drugs (in 5ml RPMI-1640-10% FBS, total volume 15ml) for 24 and 72h. DMSO or drug-treated cells ($1x10^6$ cells per experiment) were harvested by tripsinization, collected by centrifuged, washed with PBS and collected as a pellet. Cell were fixed overnight at 4°C by adding 70% ethanol and gently vortexing it. Cells were then removed from ethanol by centrifugation and washed twice with PBS, followed by 15 min incubation, at room temperature, with cold PI solution (PBS, 25 µg/mL PI and 2.5 µg/mL DNAse-free RNAse). Cell cycle analysis was assessed by flow cytometry using a fluorescence activated cell sorter (FACS) (BD Accuri™ C6 flow cytometer (BD Biosciences)).