

Supporting Information

Design, synthesis, and biological evaluation of new arjunolic acid derivatives as anticancer agents

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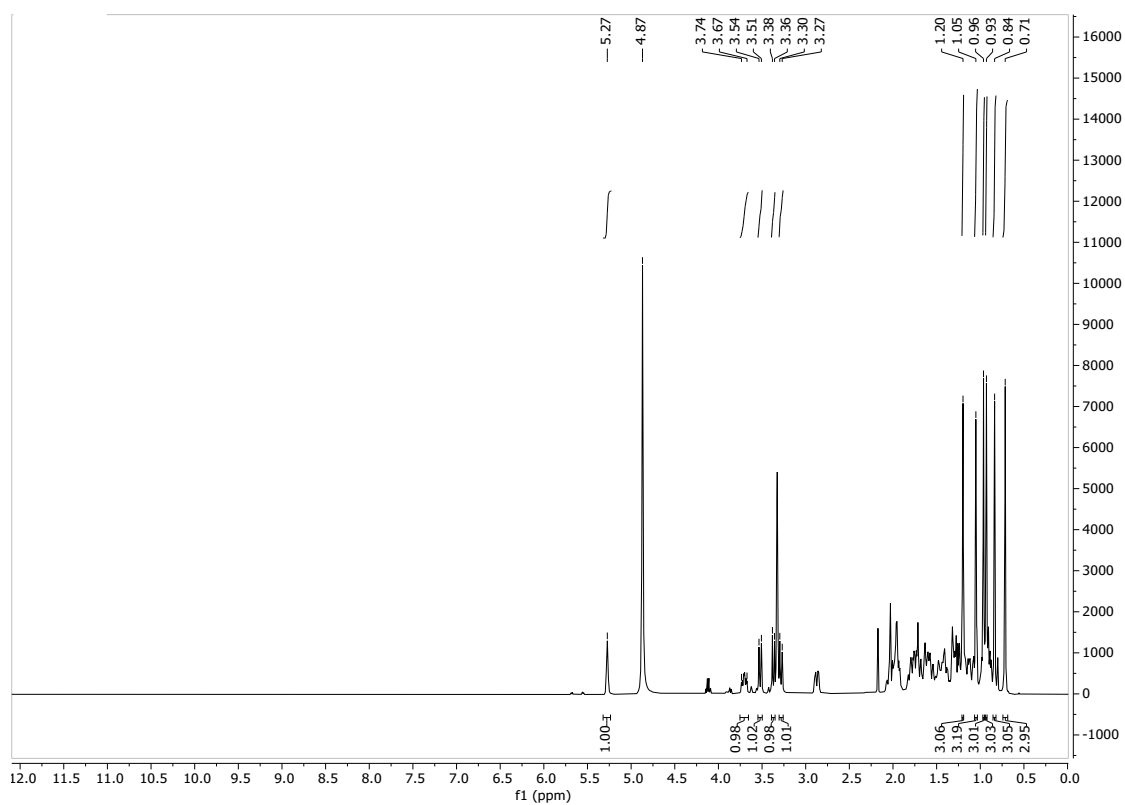
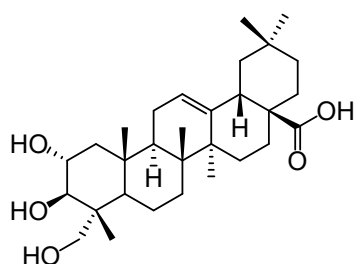
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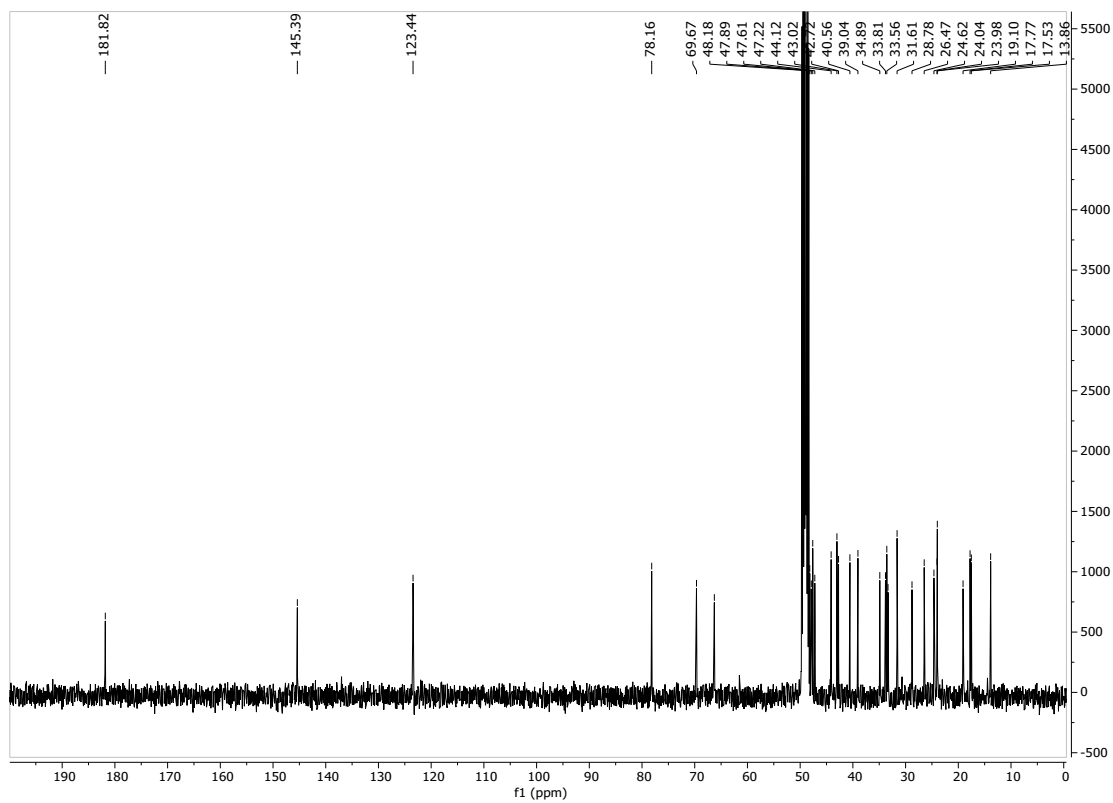
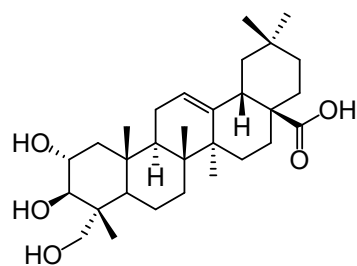
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1.1 $^1\text{H-NMR}$ spectrum for Arjunolic acid 1 recorded in CH_3OD

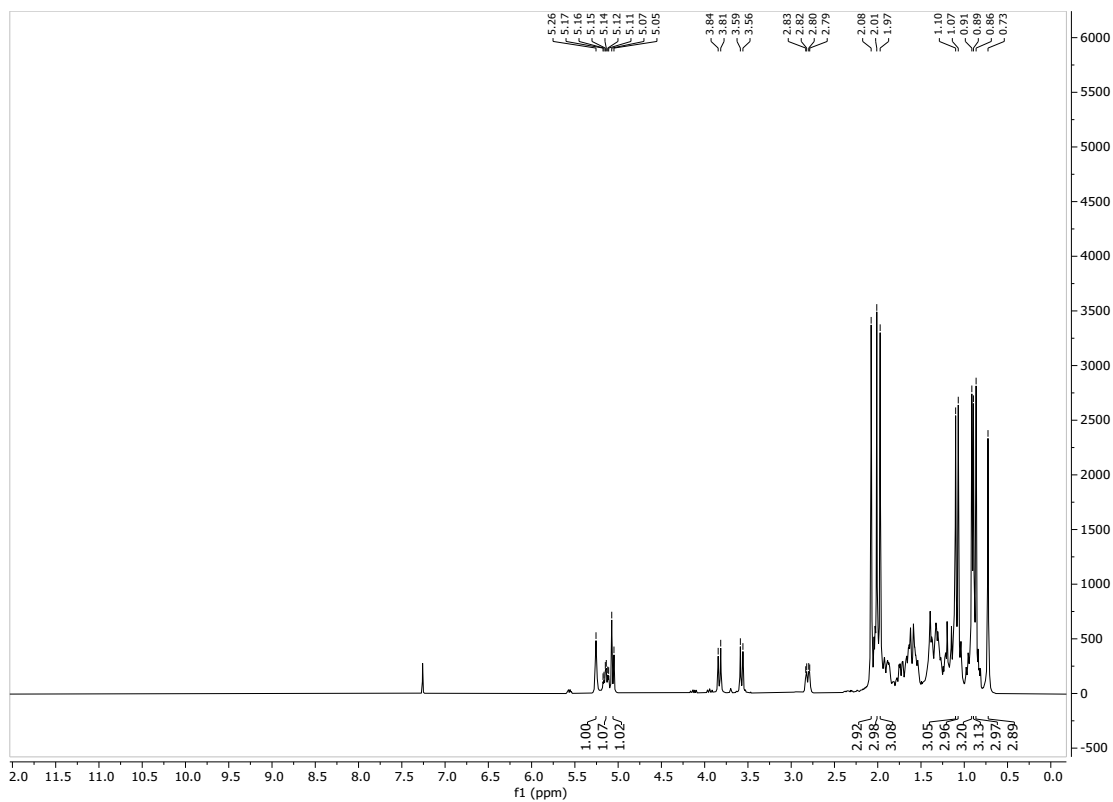
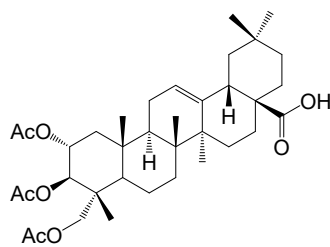


1.2 $^{13}\text{C-NMR}$ spectrum for Arjunolic acid 1 recorded in CH_3OD

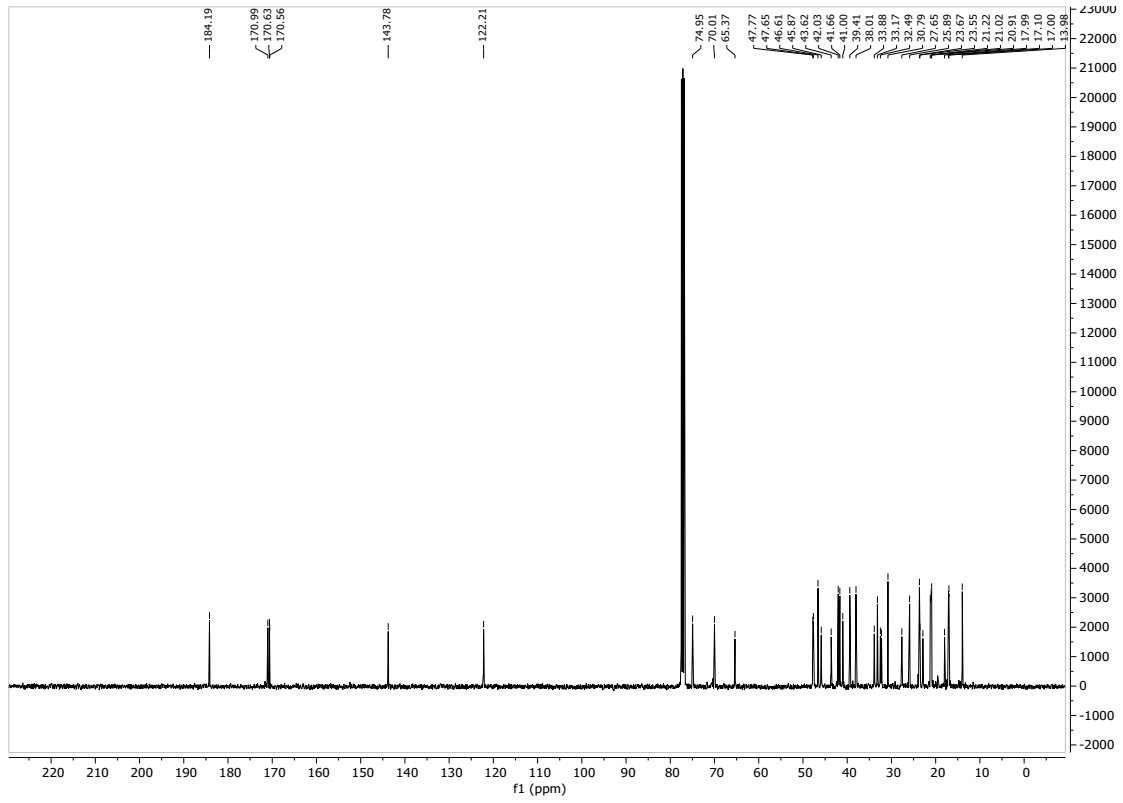
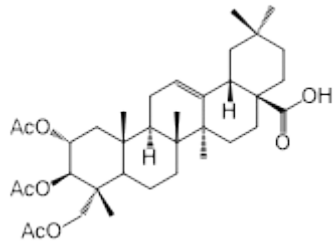


2. NMR data for compound 2

2.1 $^1\text{H-NMR}$ spectrum for compound 2 recorded in CDCl_3

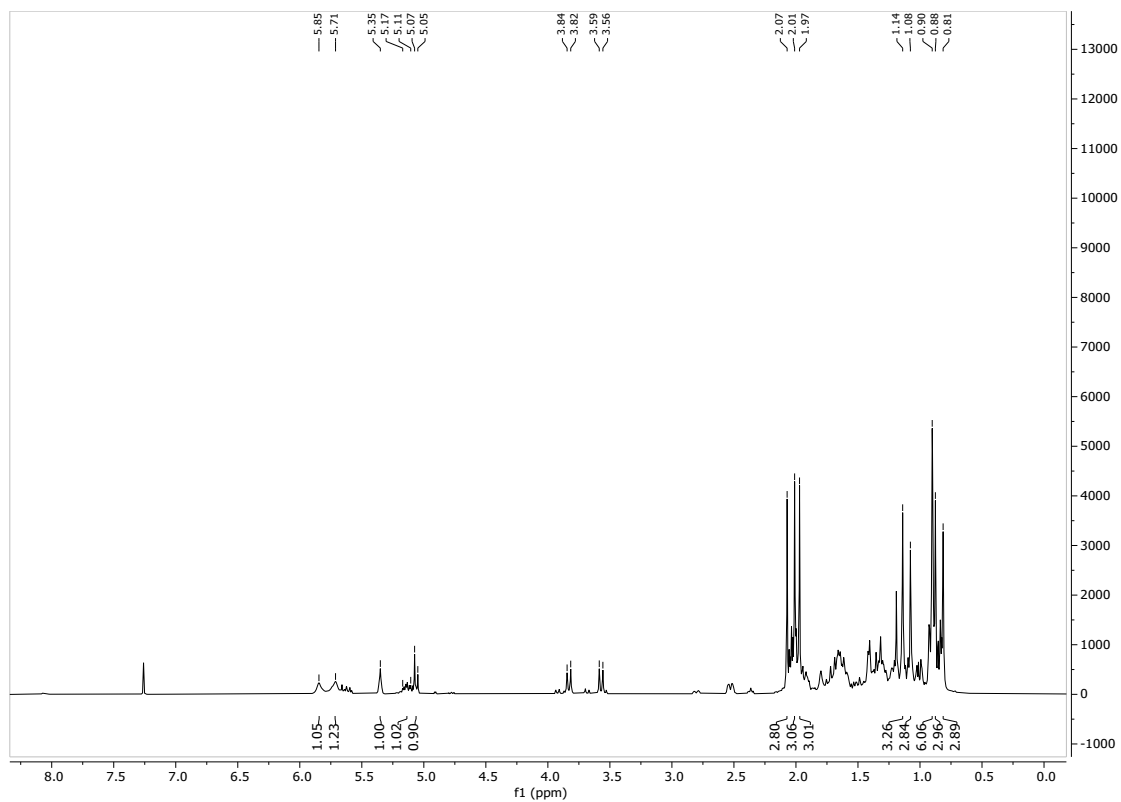
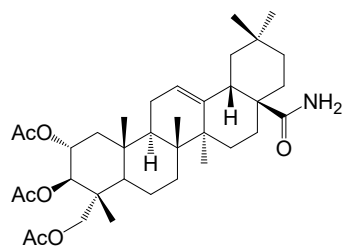


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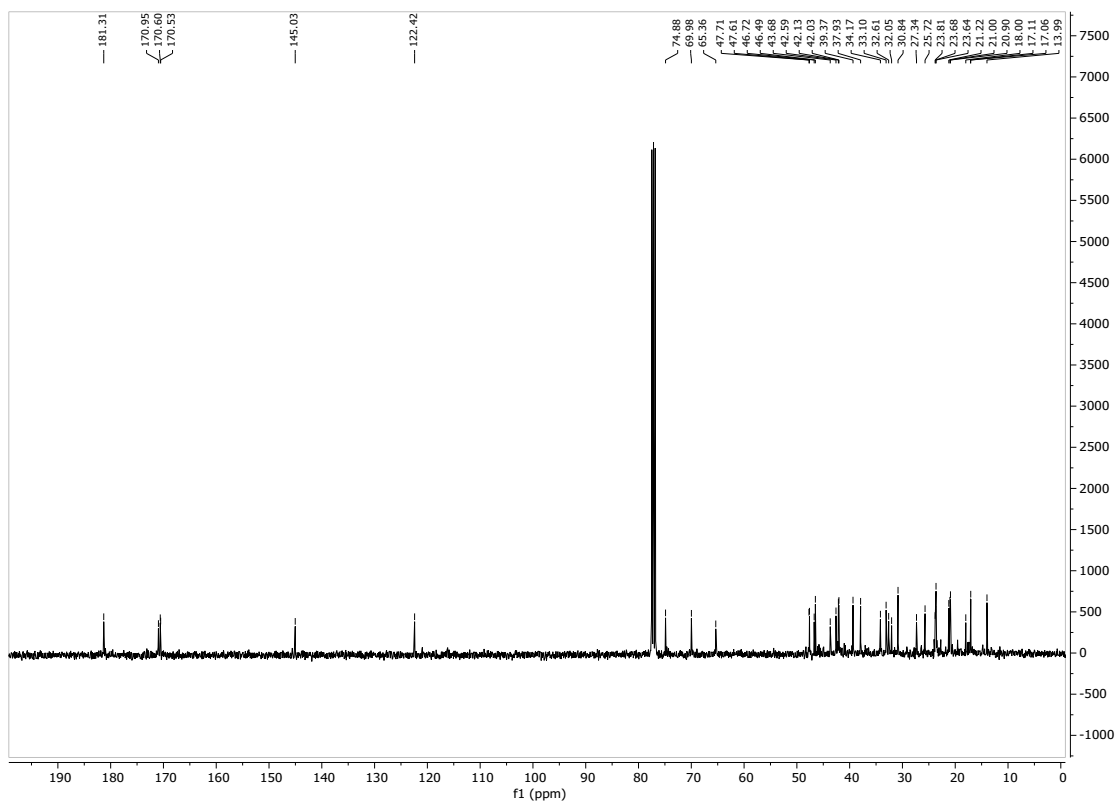
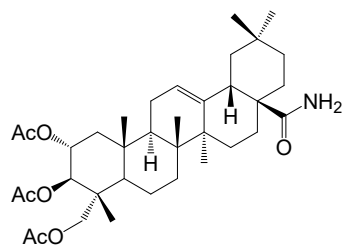


3. NMR data for compound 3

3.1 ^1H -NMR spectrum for compound 3 recorded in CDCl_3

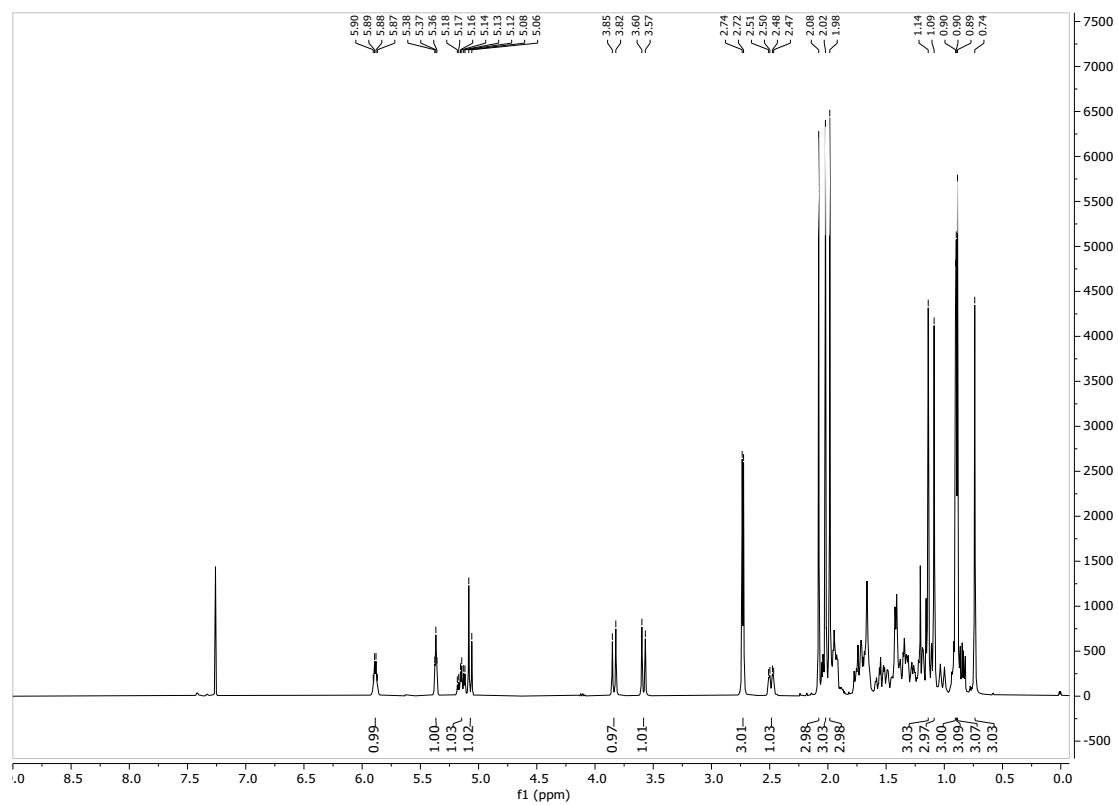
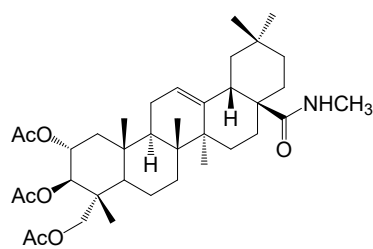


3.2 ^{13}C -NMR spectrum for compound 3 recorded in CDCl_3

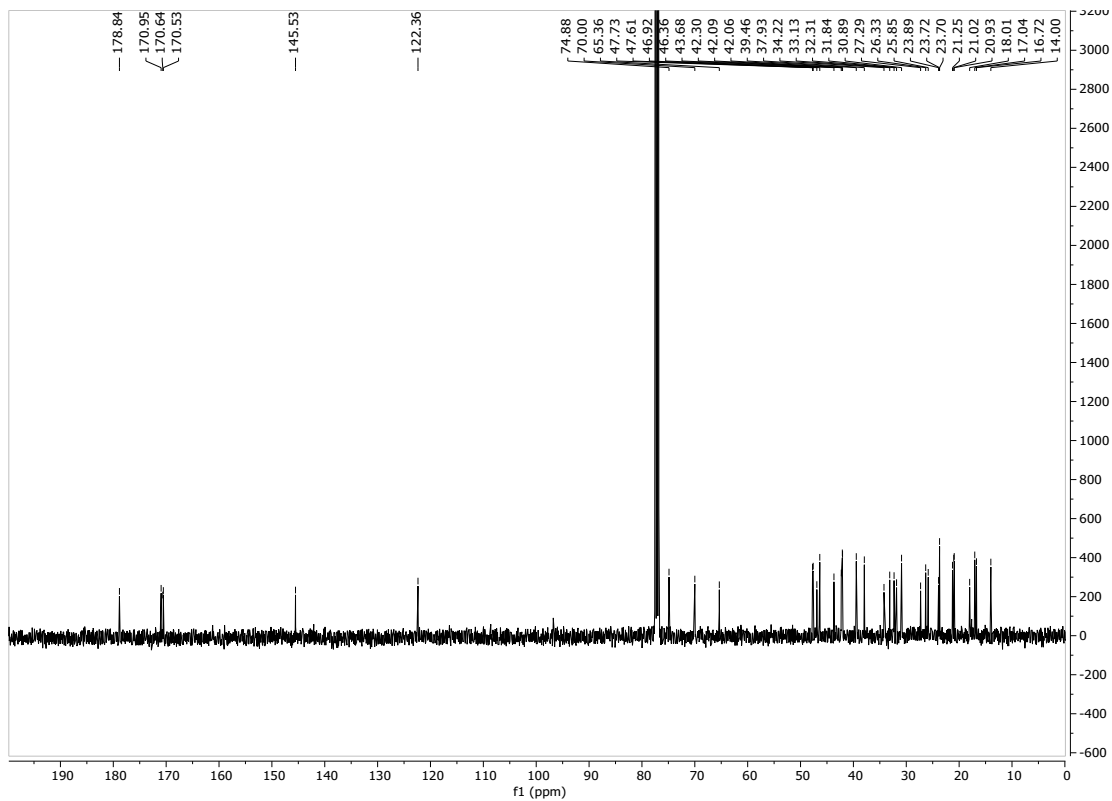
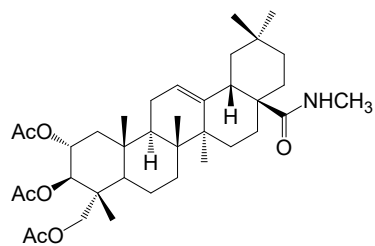


4. NMR data for compound 4

4.1 $^1\text{H-NMR}$ spectrum for compound 4 recorded in CDCl_3

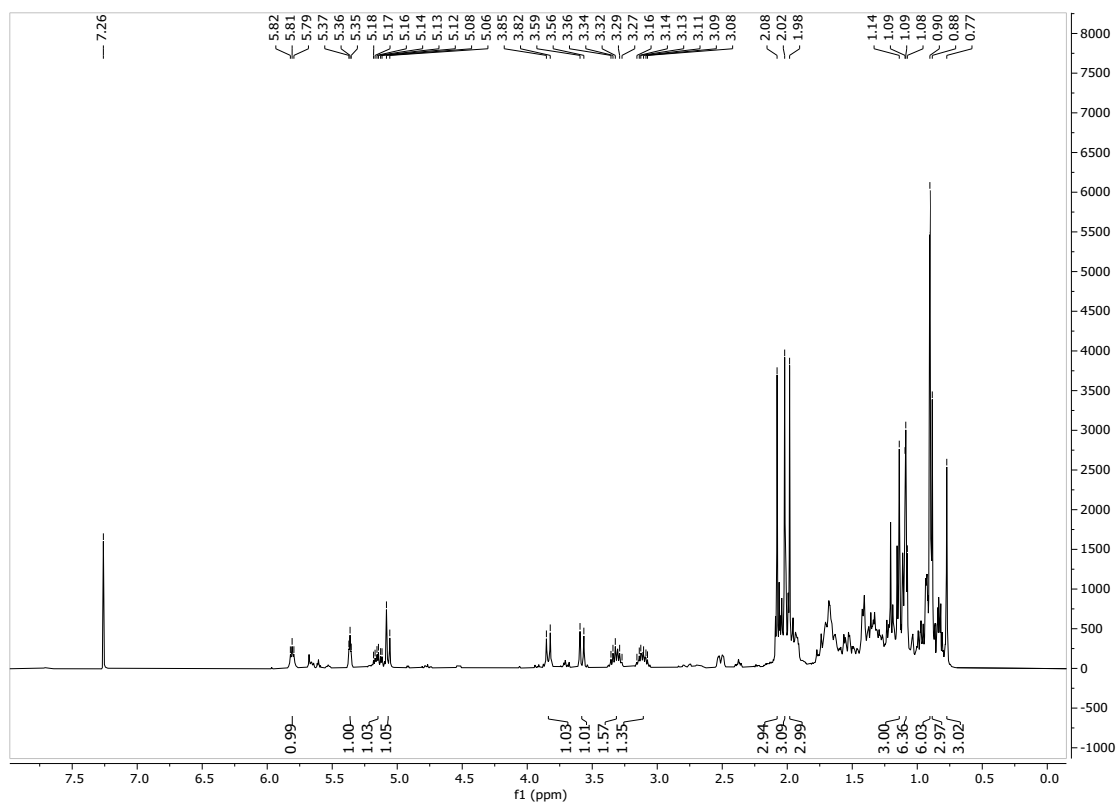
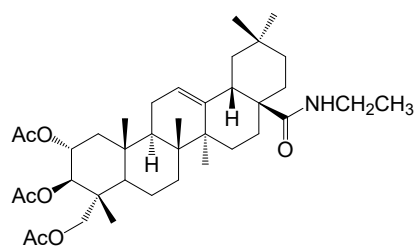


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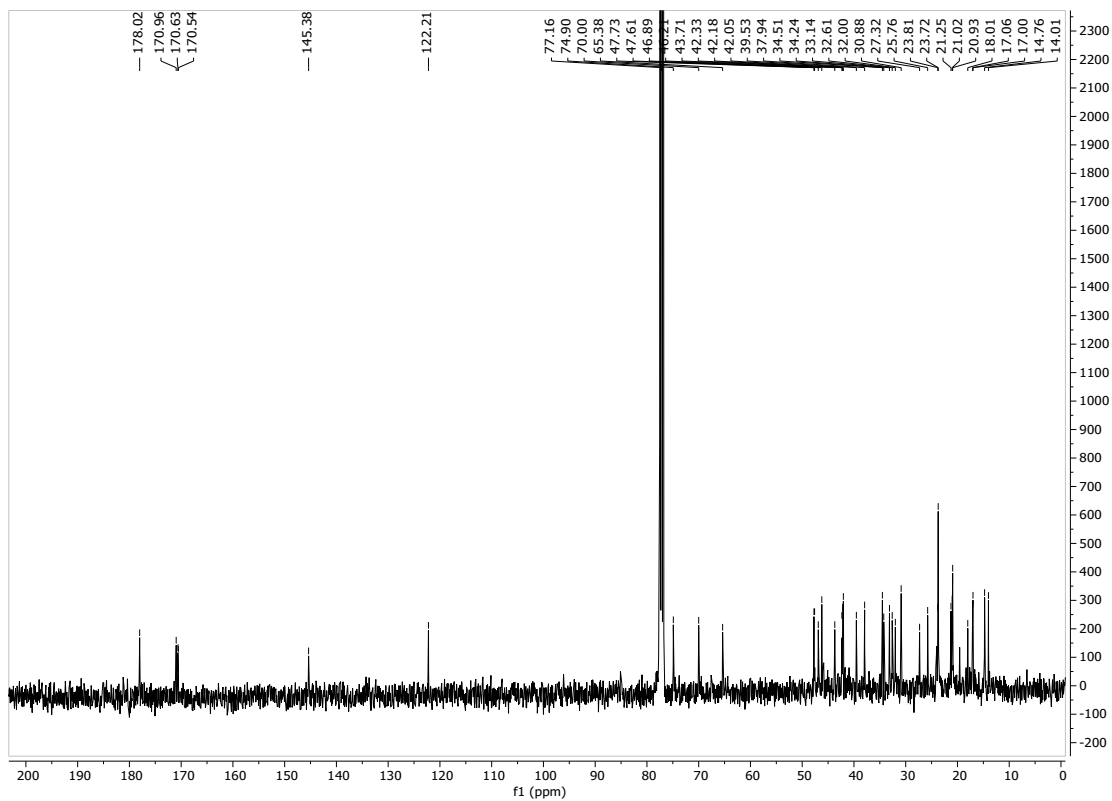
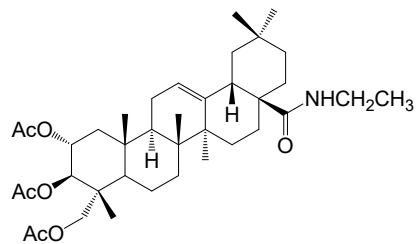


5. NMR data for compound 5

5.1 $^1\text{H-NMR}$ spectrum for compound 5 recorded in CDCl_3

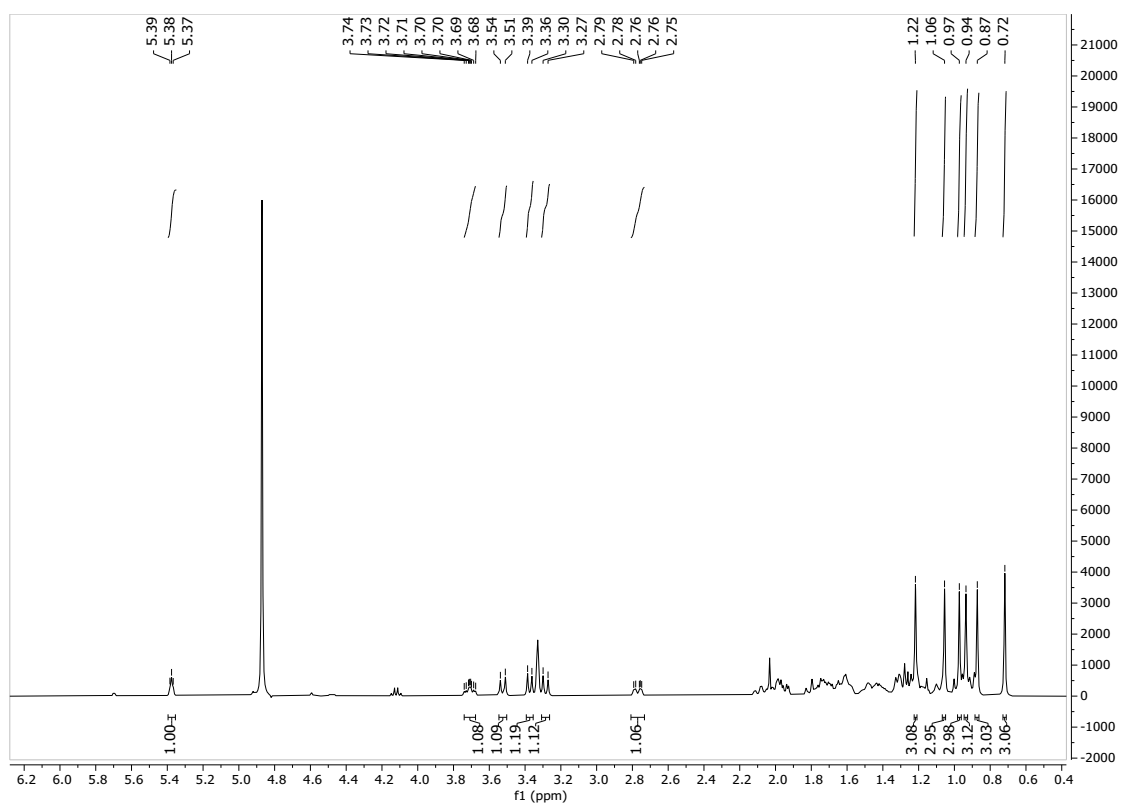
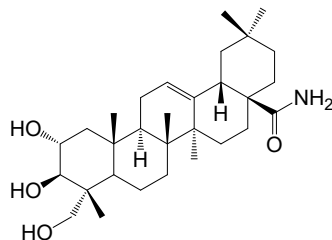


5.2 $^{13}\text{C-NMR}$ spectrum for compound 5 recorded in CDCl_3

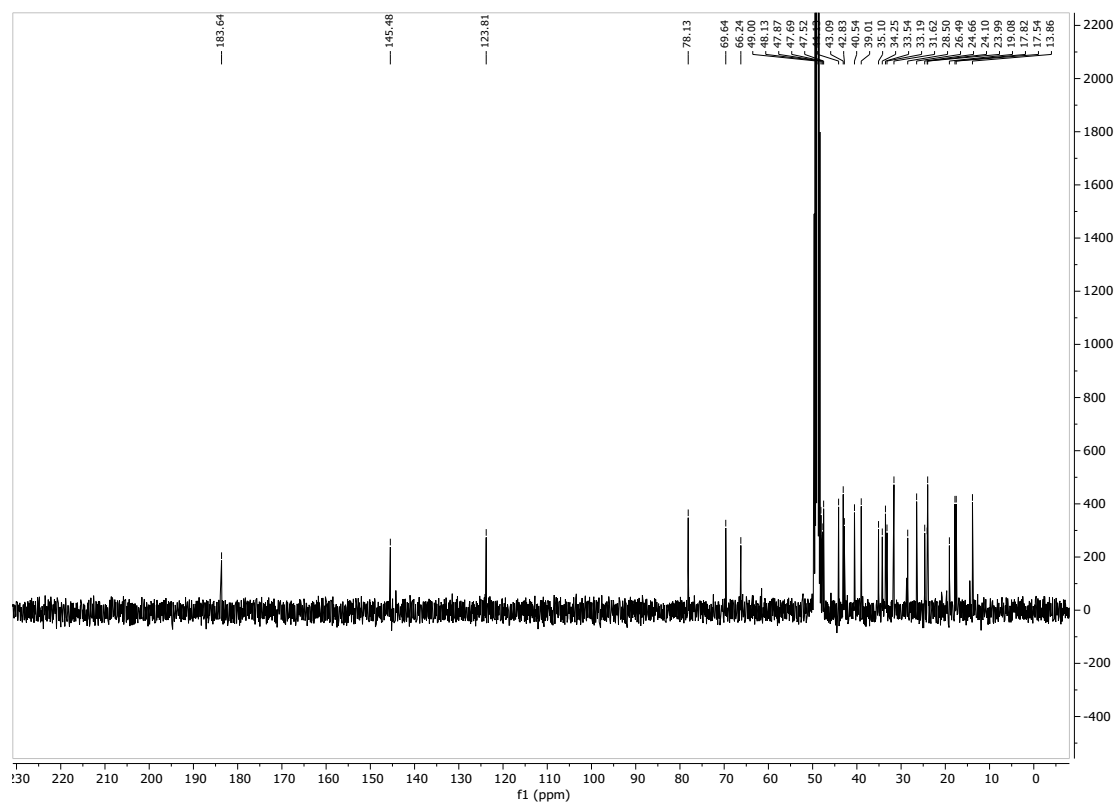
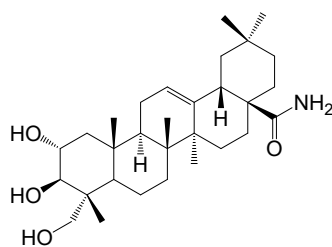


6. NMR data for compound 6

6.1 $^1\text{H-NMR}$ spectrum for compound 6 recorded in CH_3OD

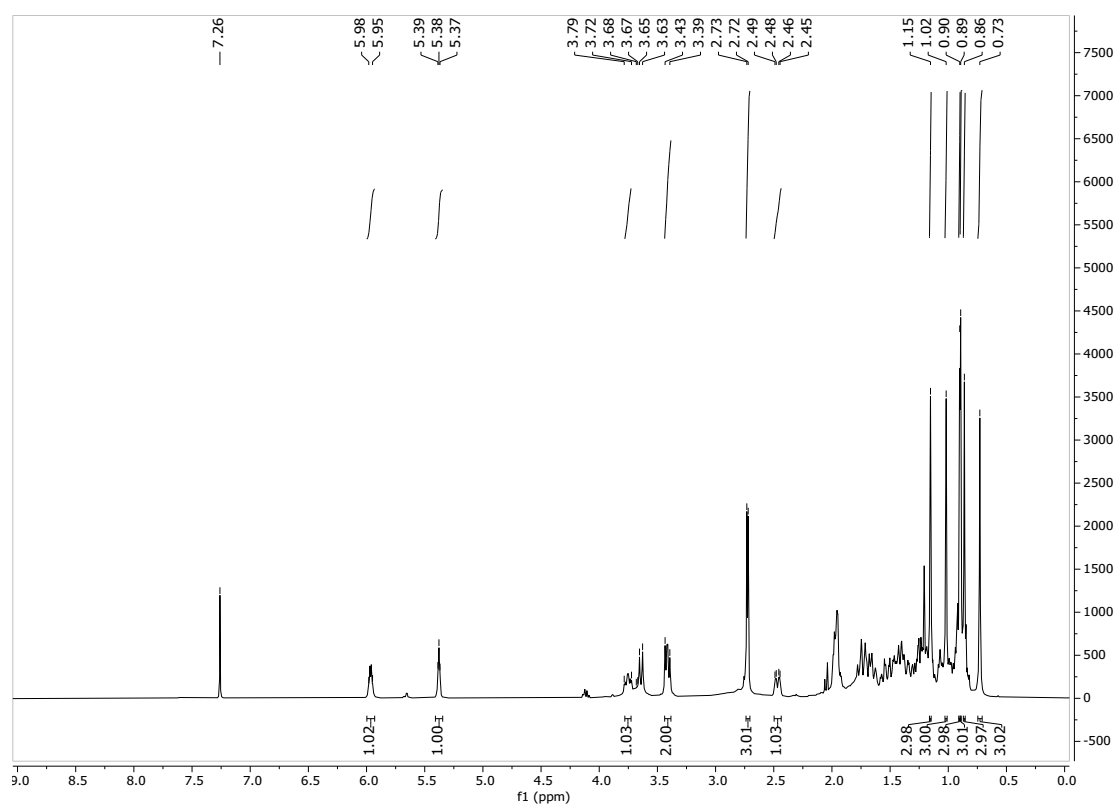
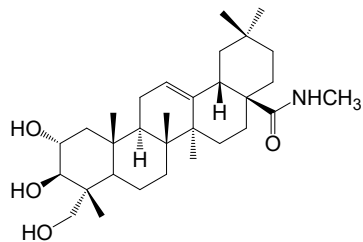


6.2 ^{13}C -NMR spectrum for compound 6 recorded in CH_3OD

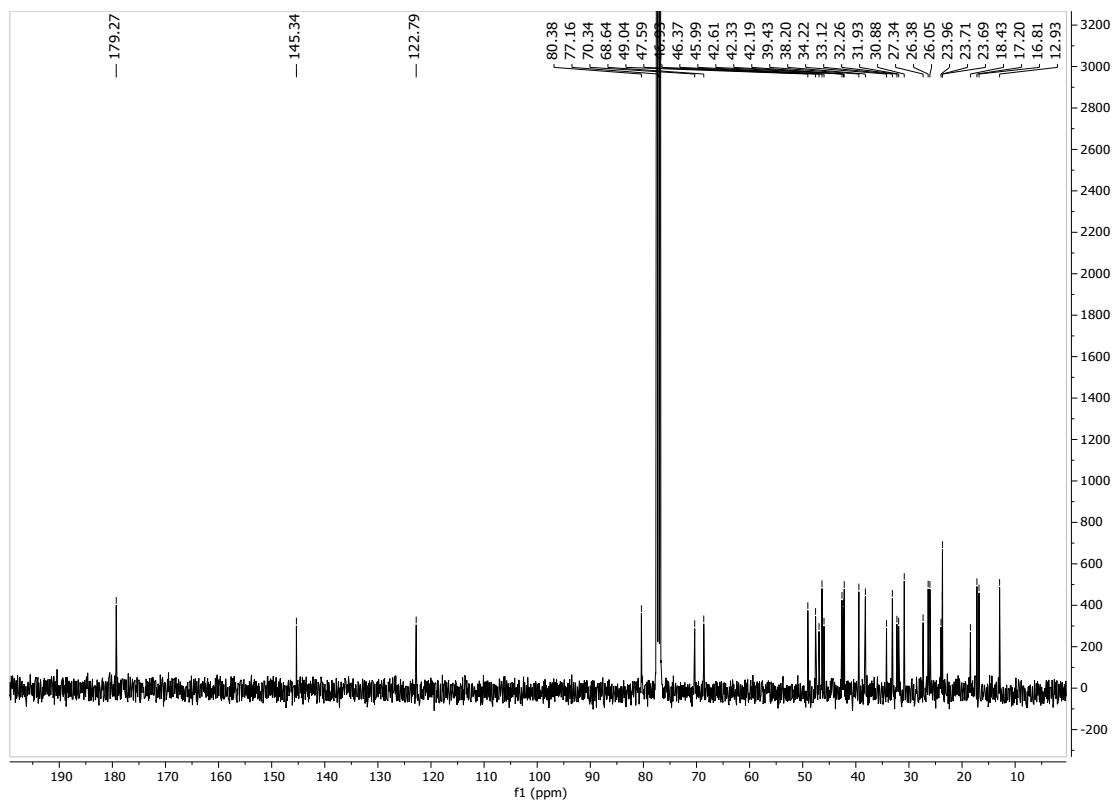
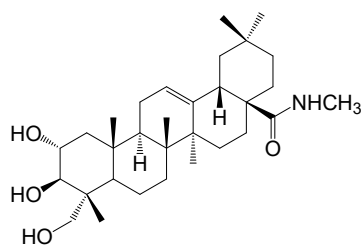


7. NMR data for compound 7

7.1 $^1\text{H-NMR}$ spectrum for compound 7 recorded in CDCl_3

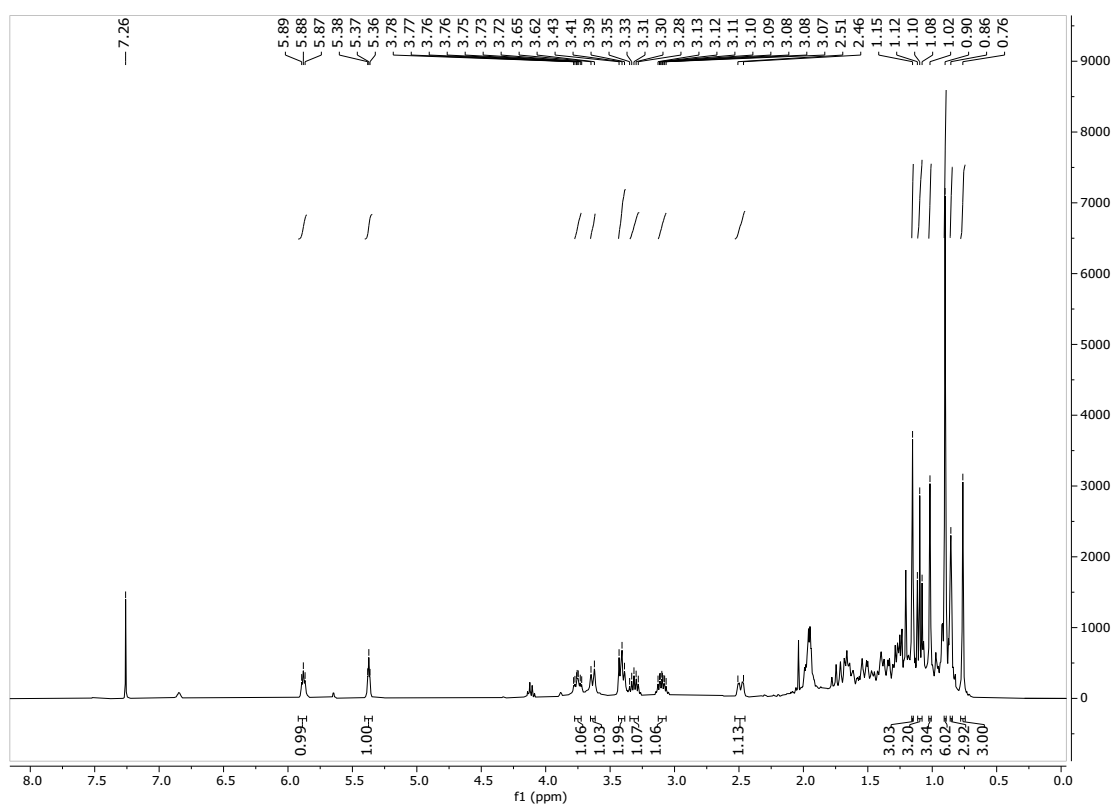
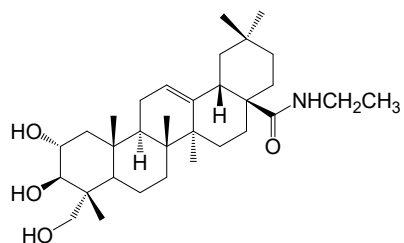


7.2 ^{13}C -NMR spectrum for compound 7 recorded in CDCl_3

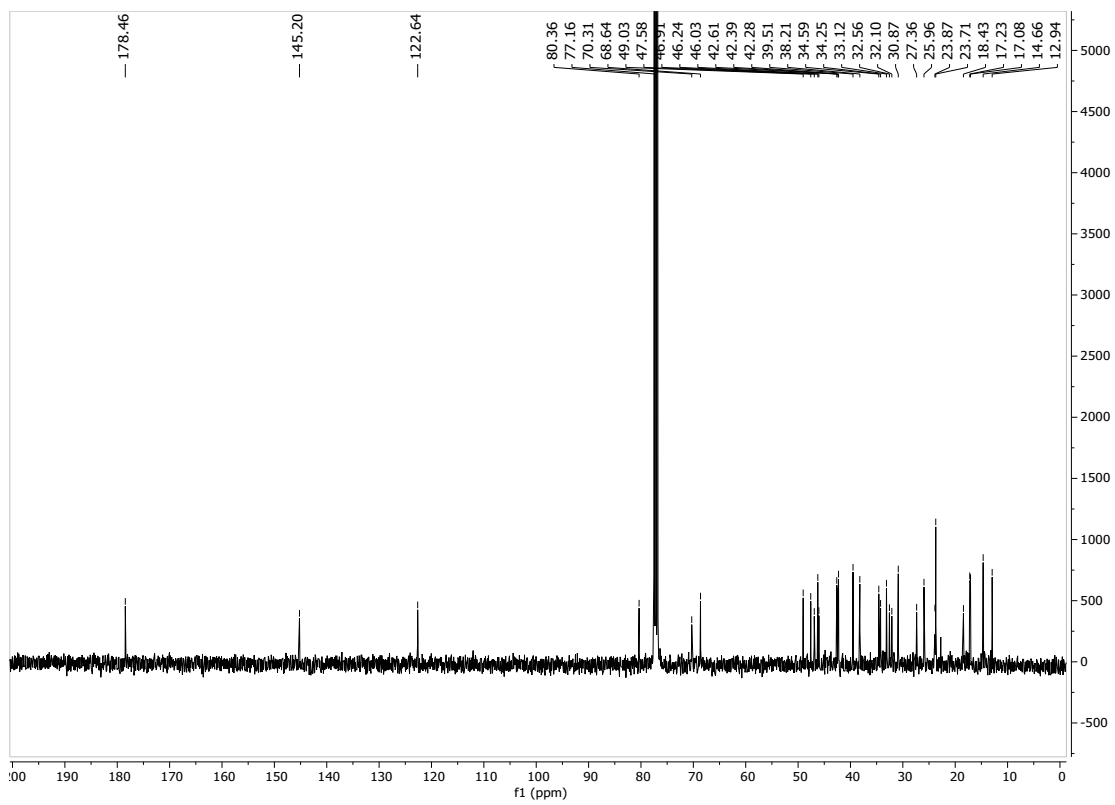
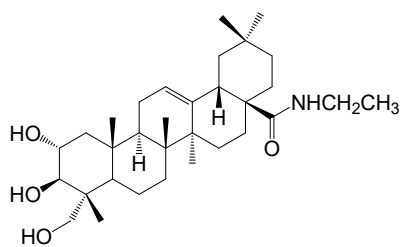


8. NMR data for compound 8

8.1 ¹H-NMR spectrum for compound 8 recorded in CDCl₃

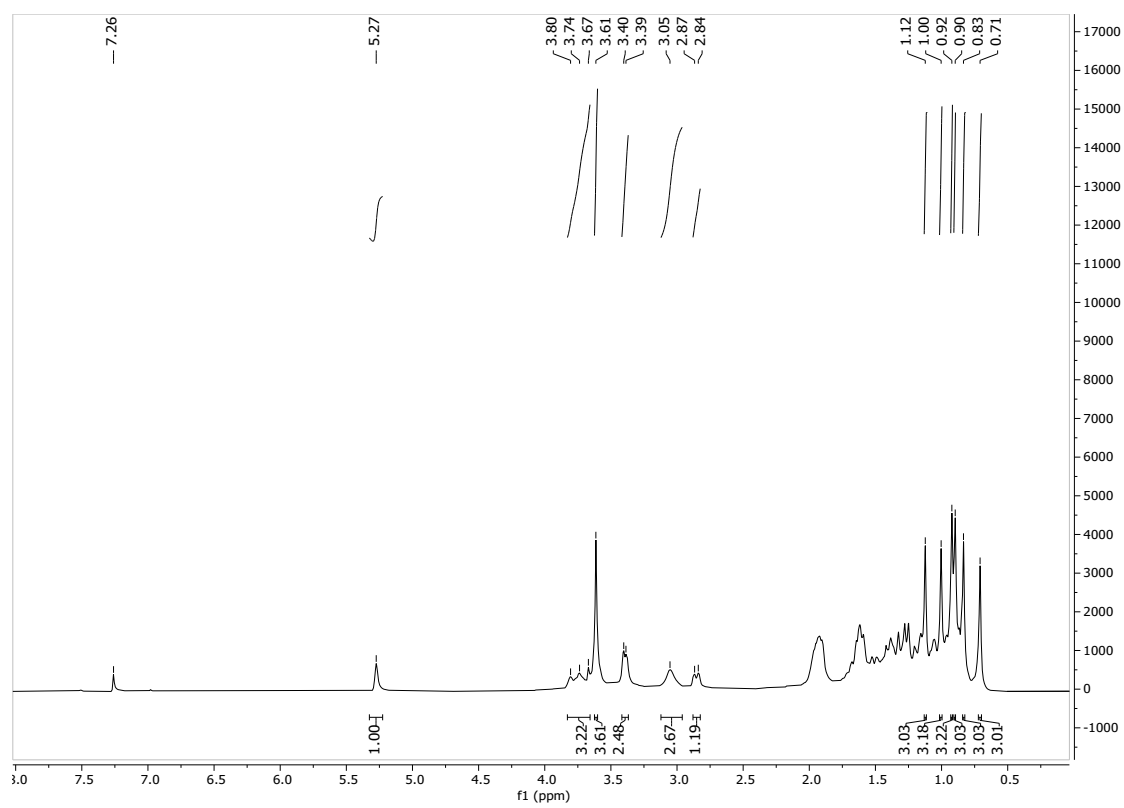
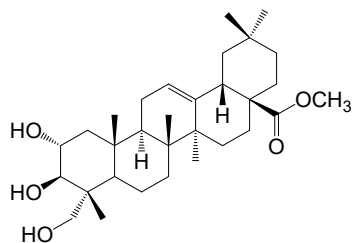


8.2 ^{13}C -NMR spectrum for compound 8 recorded in CDCl_3

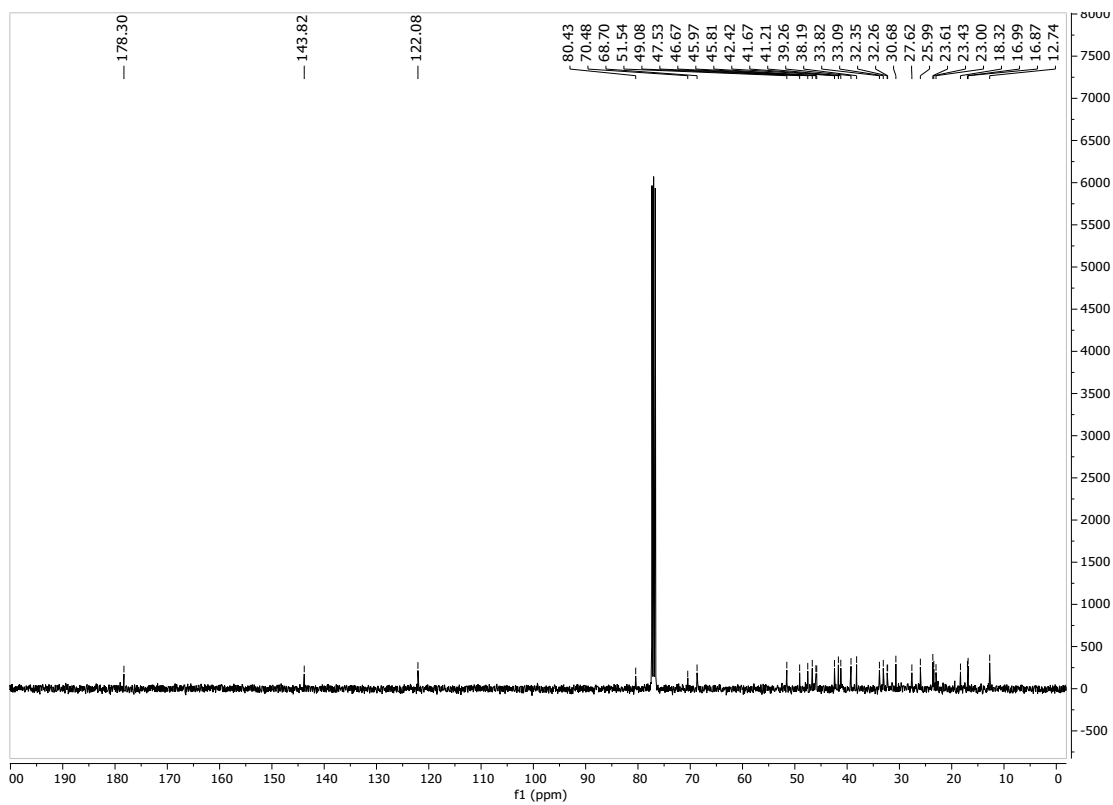
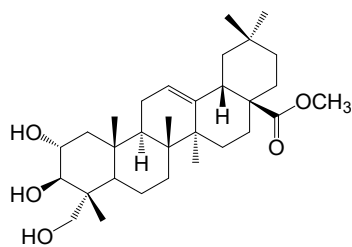


9. NMR data for compound 9

9.1 $^1\text{H-NMR}$ spectrum for compound 9 recorded in CDCl_3

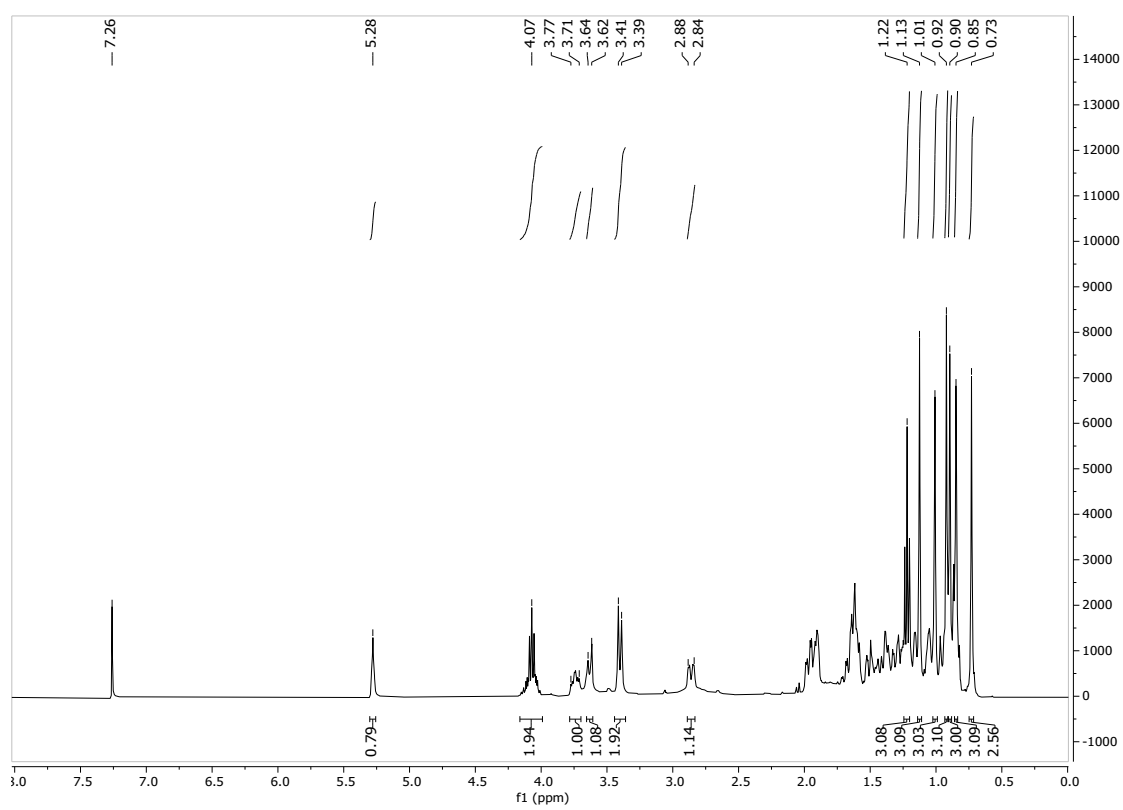
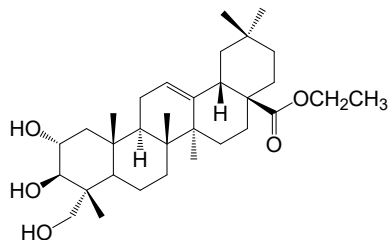


9.2 ^{13}C -NMR spectrum for compound 9 recorded in CDCl_3

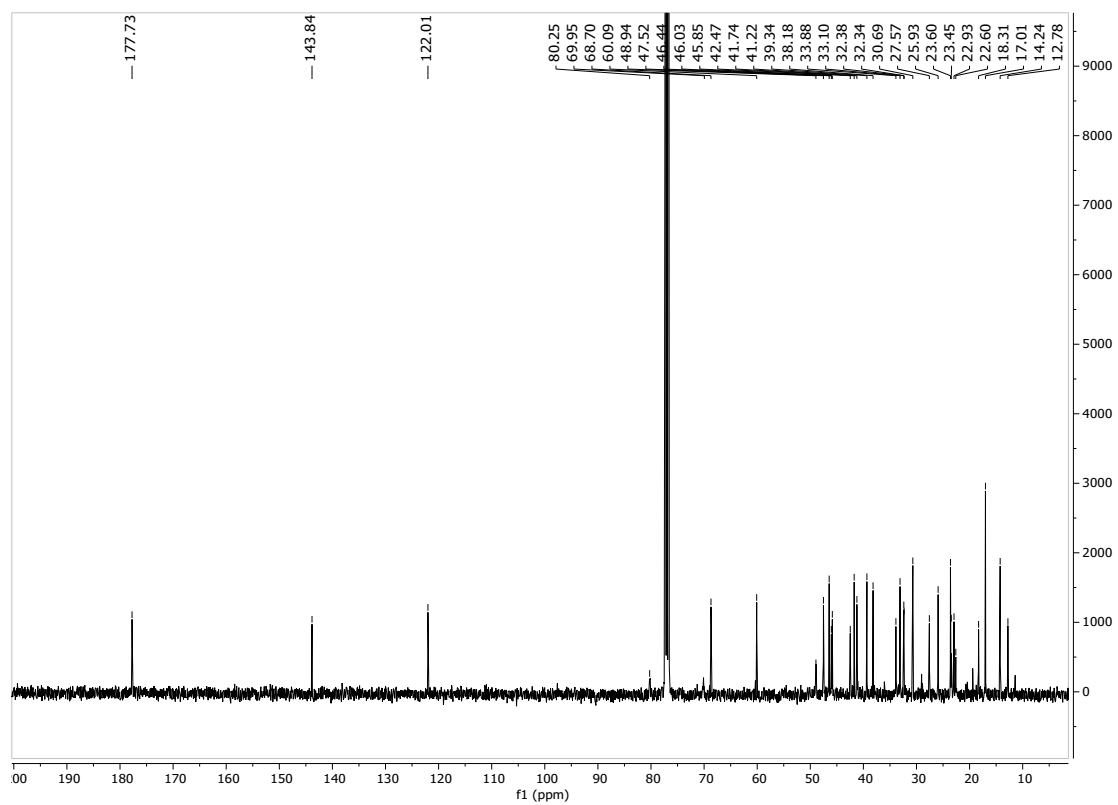
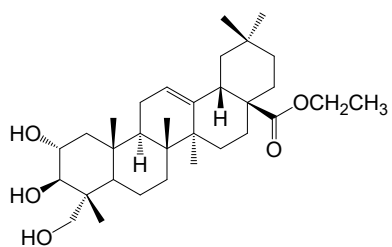


10. NMR data for compound 10

10.1 ¹H-NMR spectrum for compound 10 recorded in CDCl₃

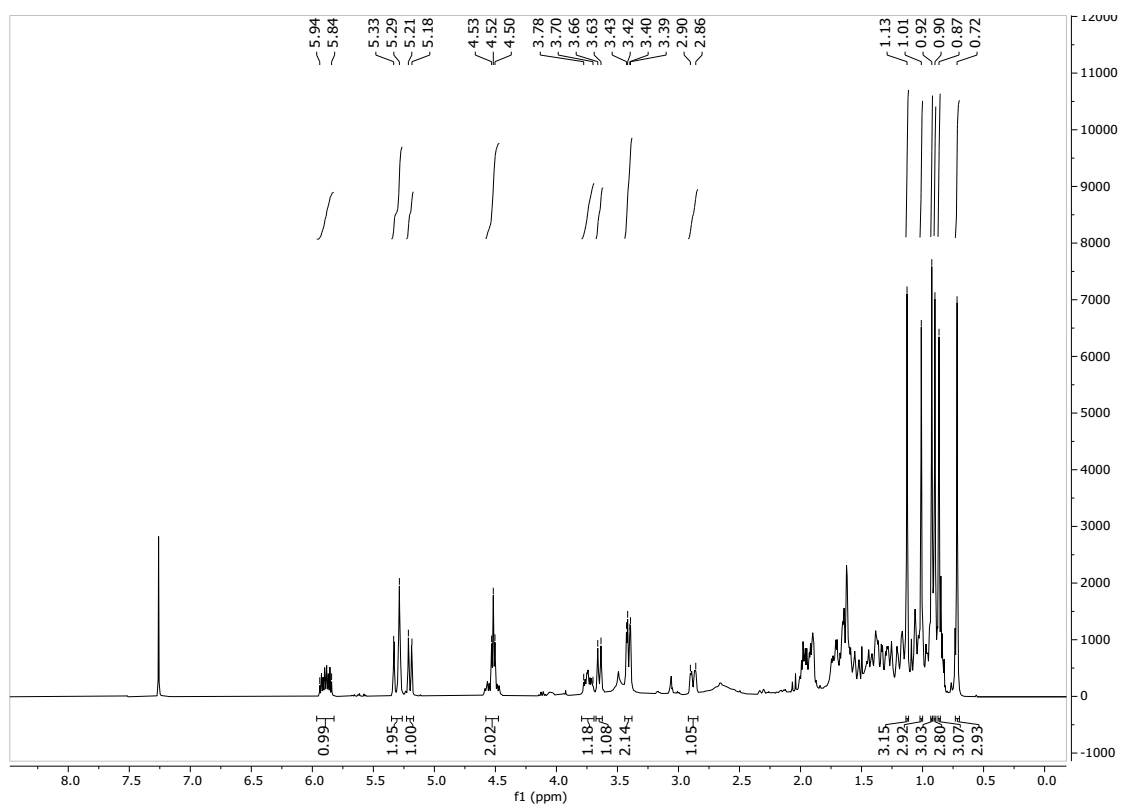
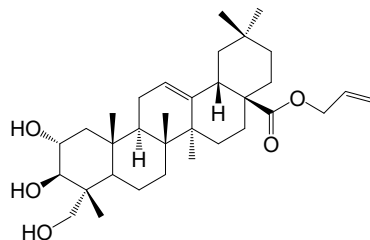


10.2 ^{13}C -NMR spectrum for compound 10 recorded in CDCl_3

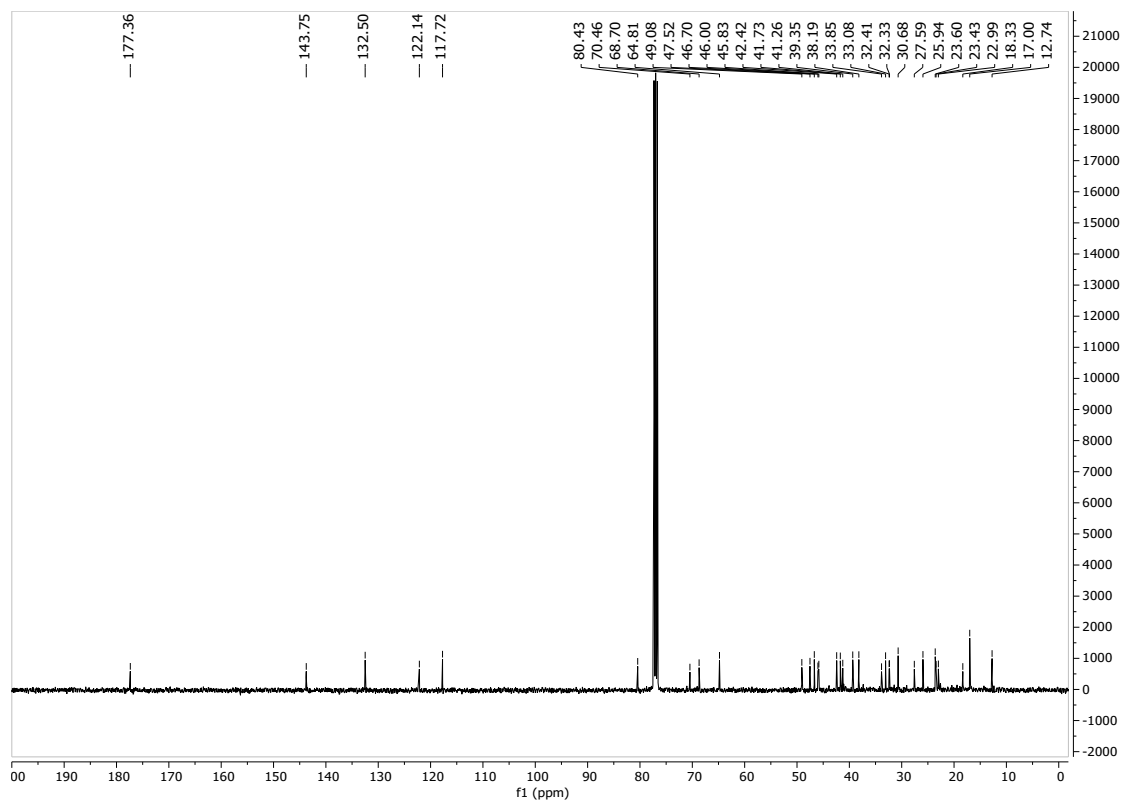
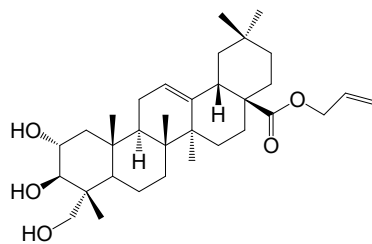


11. NMR data for compound 12

11.1 ¹H-NMR spectrum for compound 12 recorded in CDCl₃

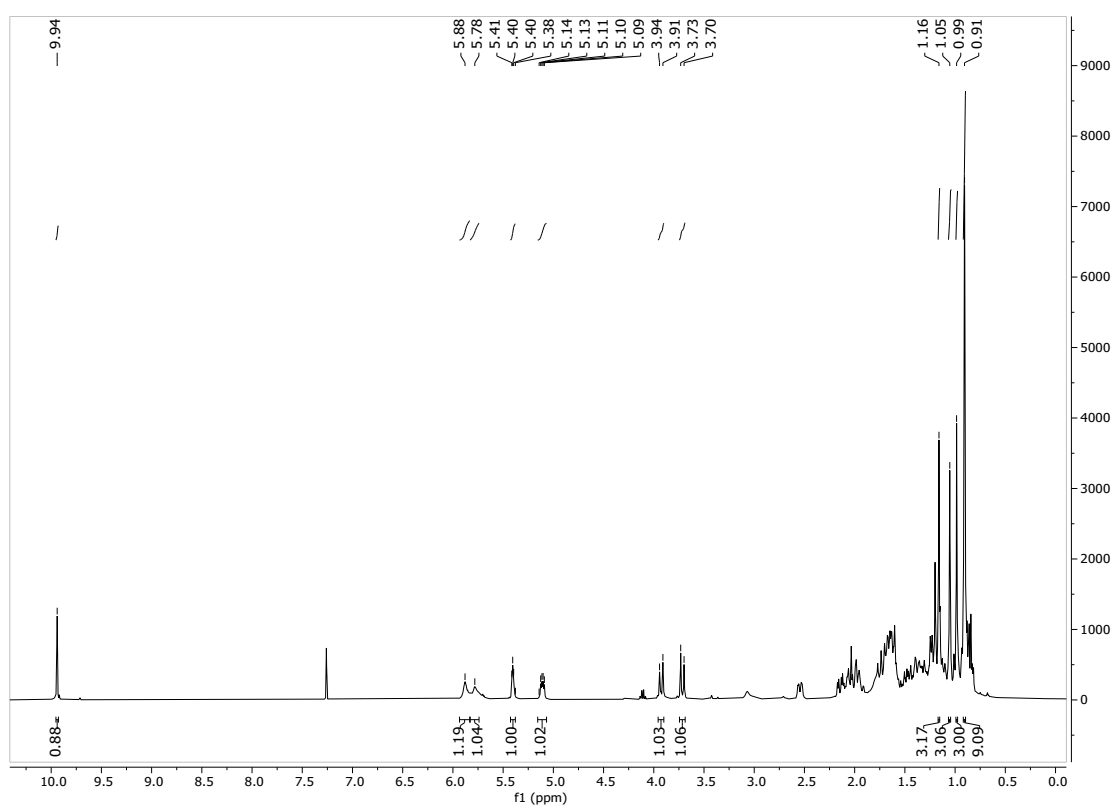
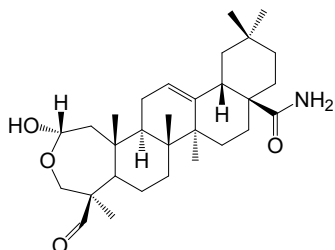


11.2 ^{13}C -NMR spectrum for compound 12 recorded in CDCl_3

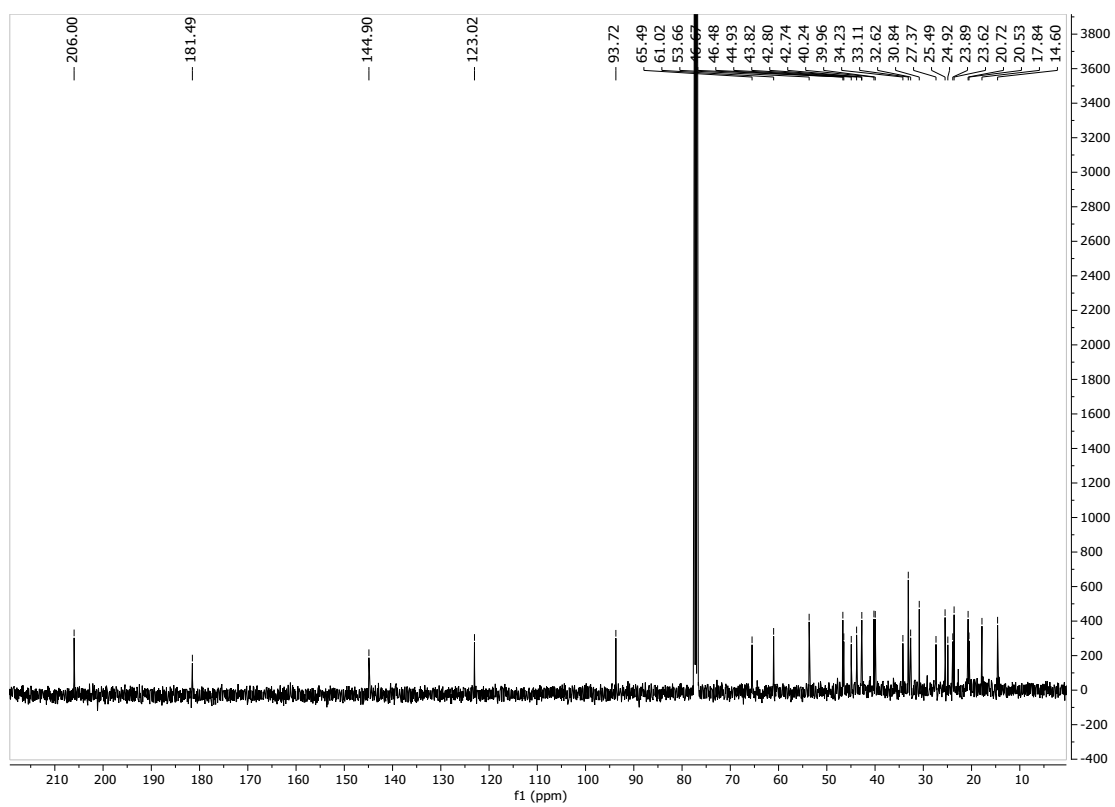
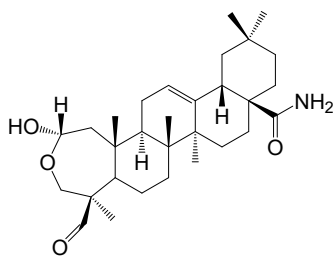


12. NMR data for compound 14

12.1 ¹H-NMR spectrum for compound 14 recorded in CDCl₃

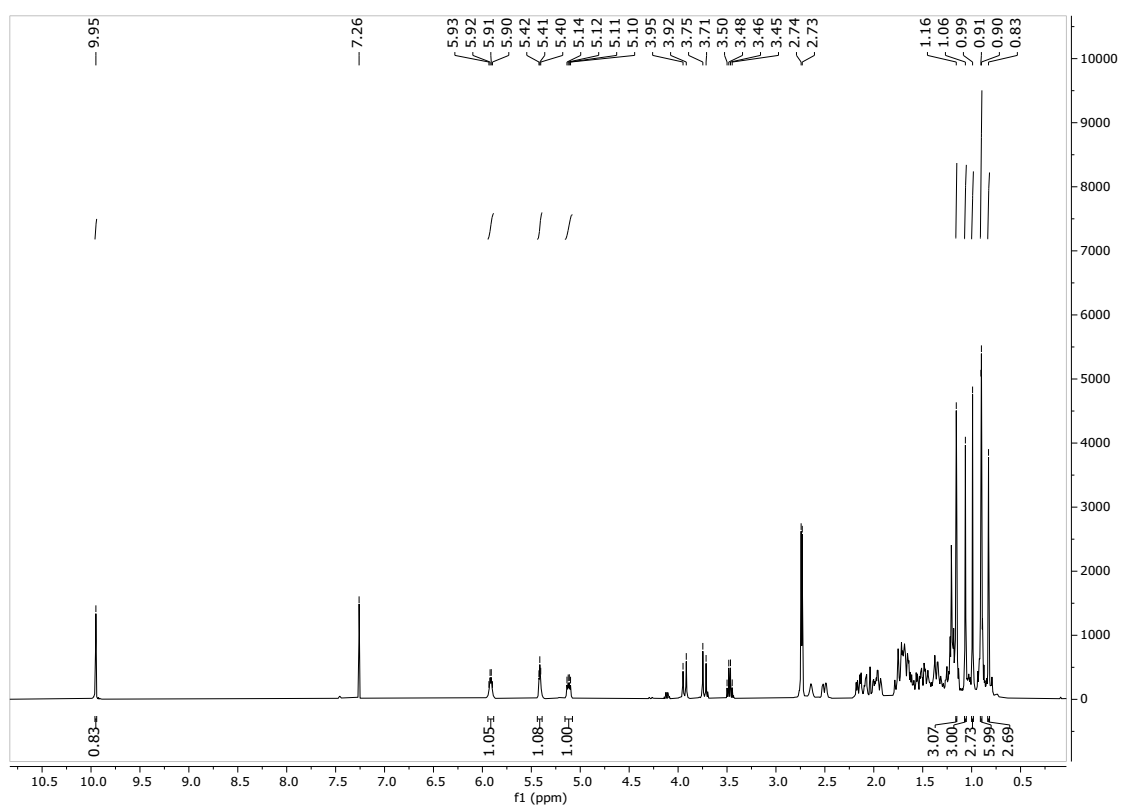
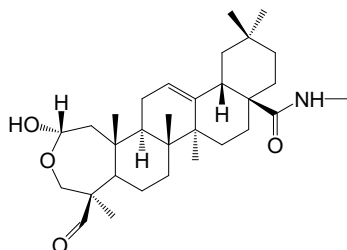


12.2 ^{13}C -NMR spectrum for compound 14 recorded in CDCl_3

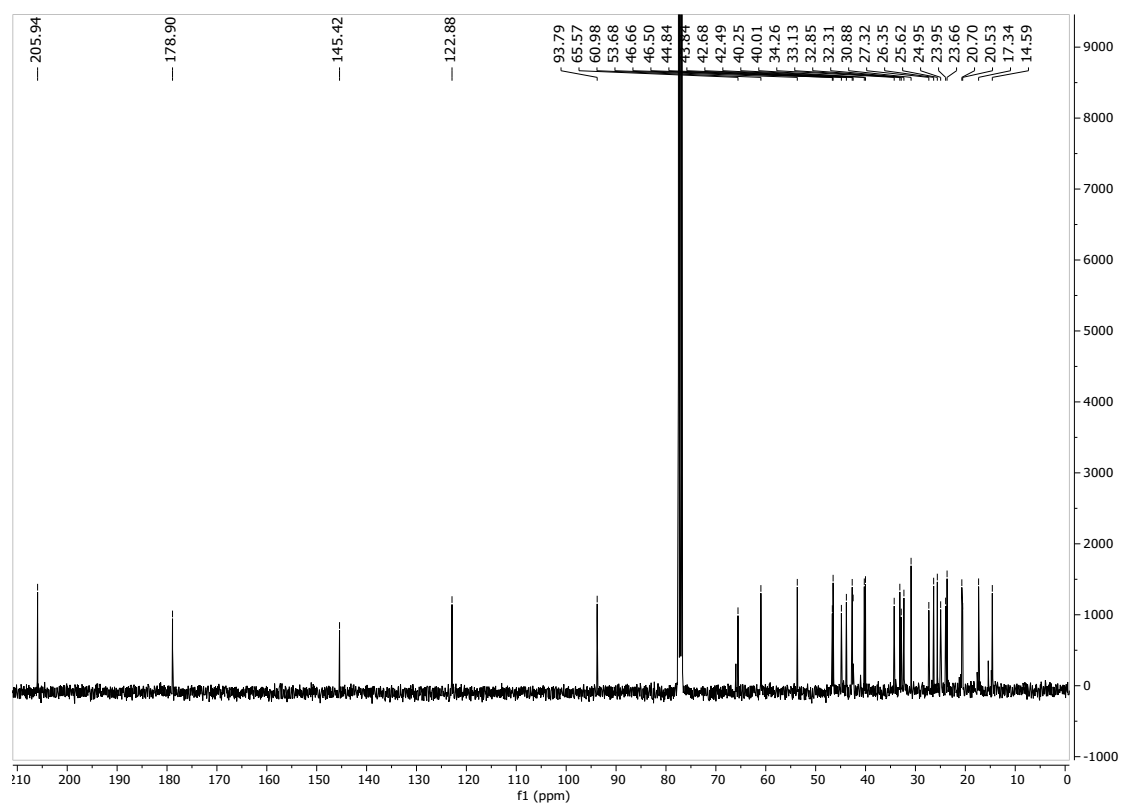
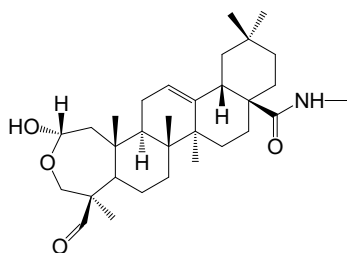


13. NMR data for compound 15

13.1 ^1H -NMR spectrum for compound 15 recorded in CDCl_3

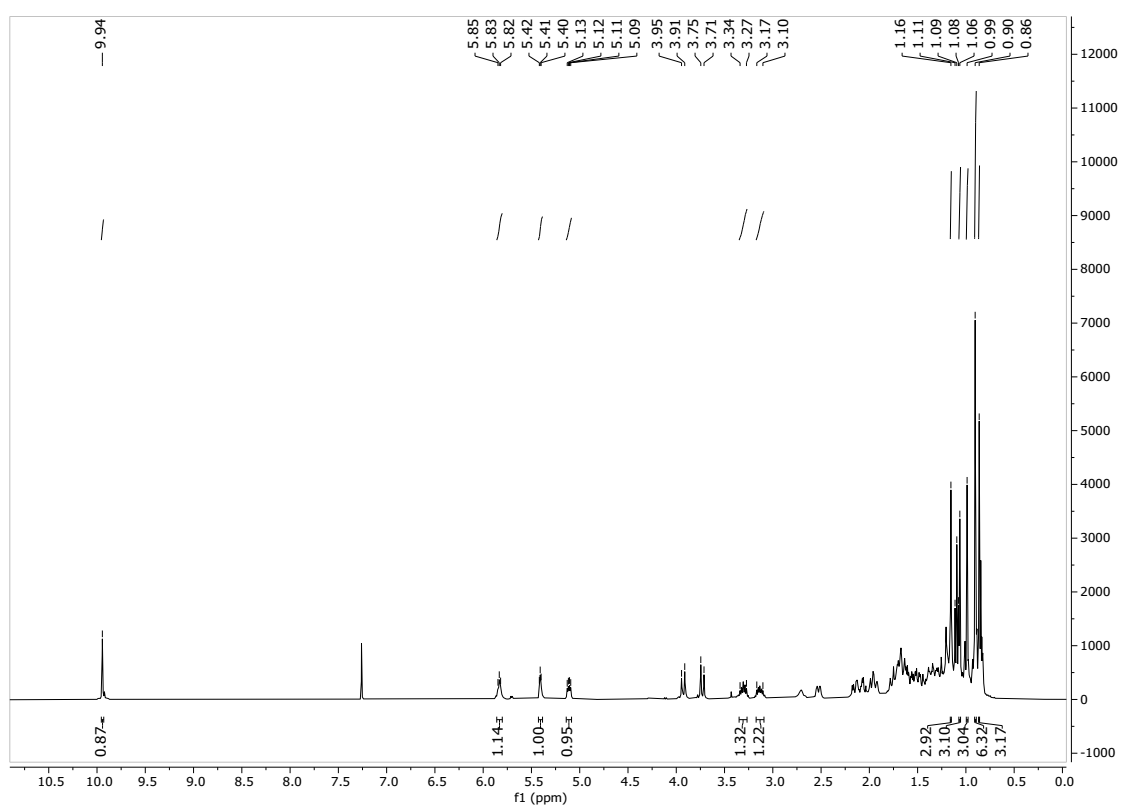
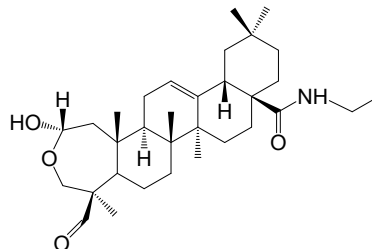


13.2 ^{13}C -NMR spectrum for compound 15 recorded in CDCl_3

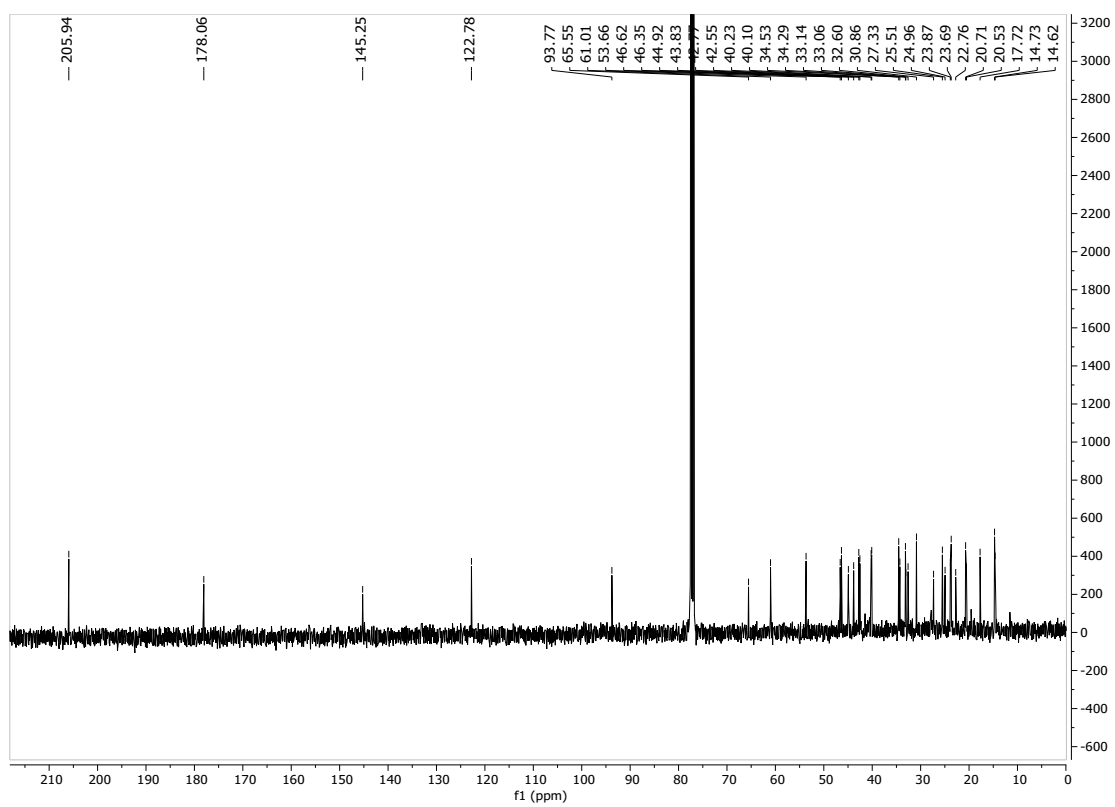
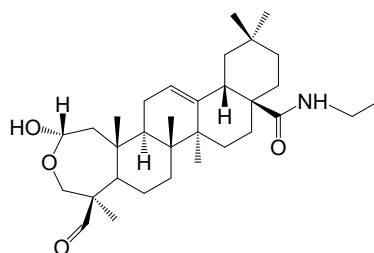


14. NMR data for compound 16

14.1 ¹H-NMR spectrum for compound 16 recorded in CDCl₃

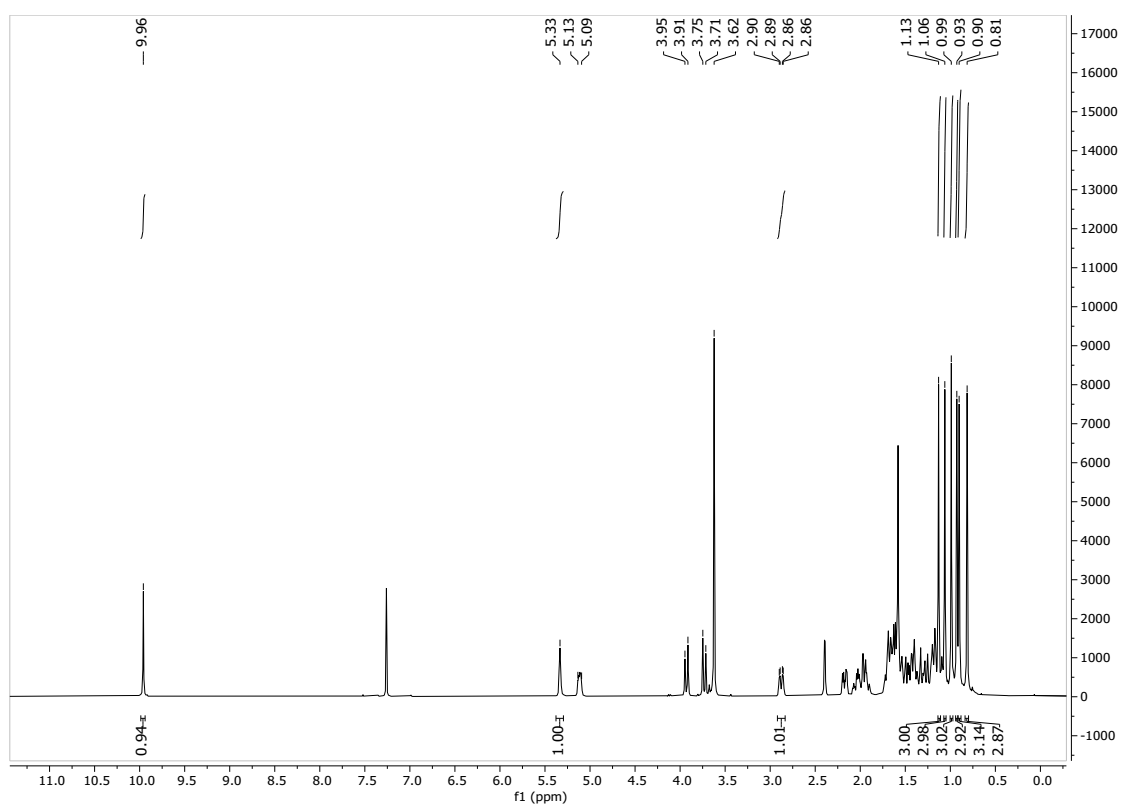
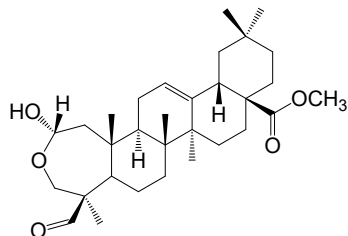


14.2 ^{13}C -NMR spectrum for compound 16 recorded in CDCl_3

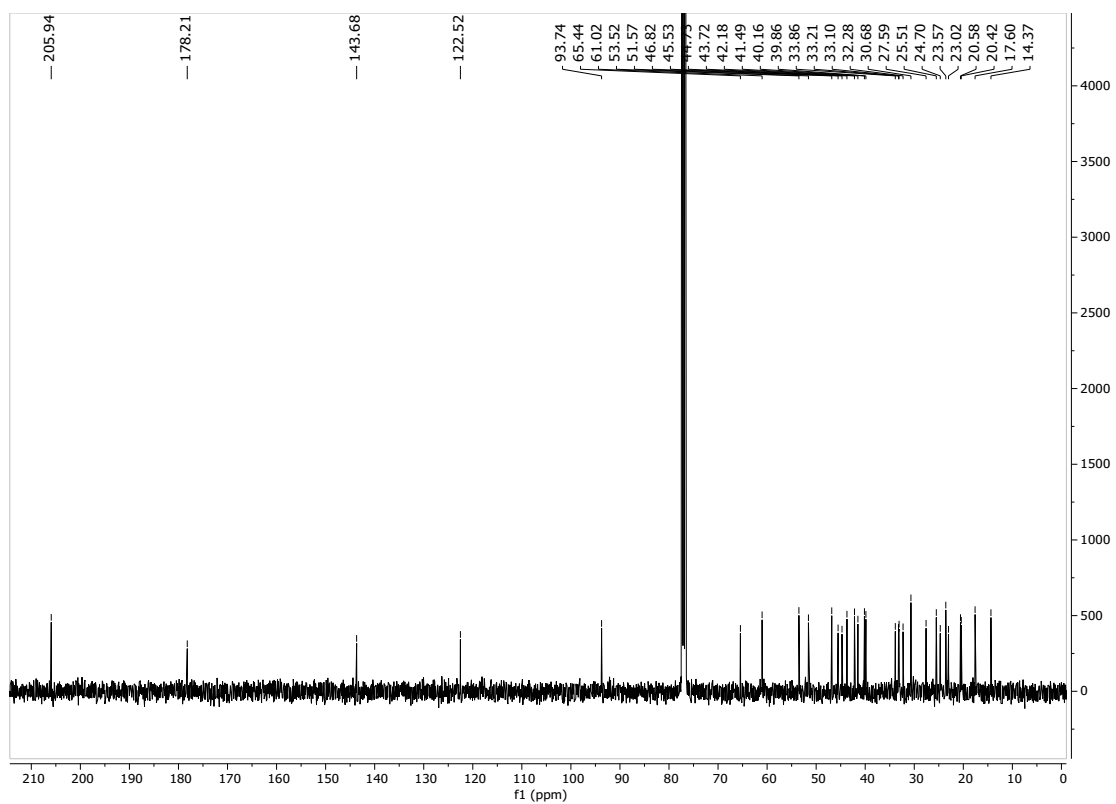
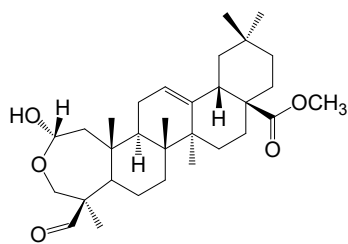


15. NMR data for compound 17

15.1 ^1H -NMR spectrum for compound 17 recorded in CDCl_3

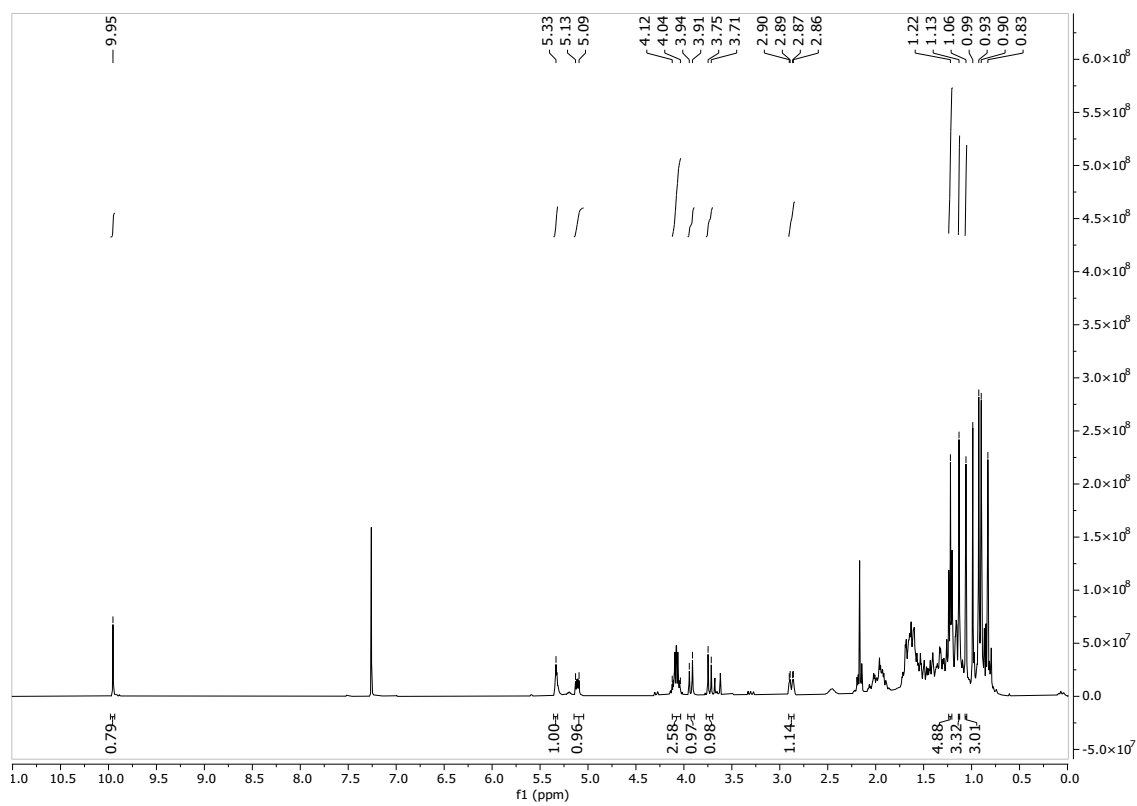
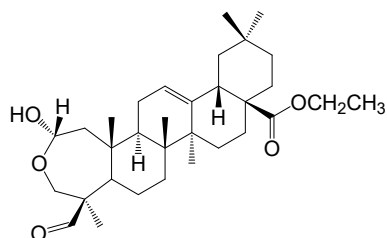


15.2 ^{13}C -NMR spectrum for compound 17 recorded in CDCl_3

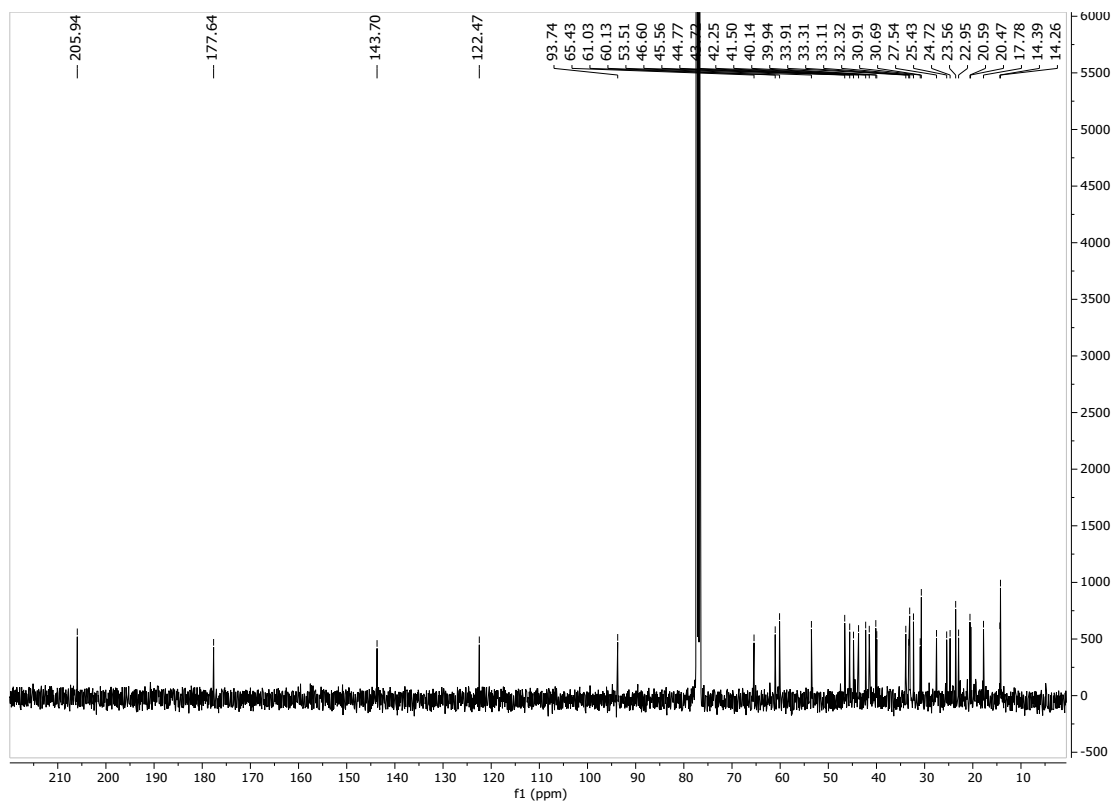
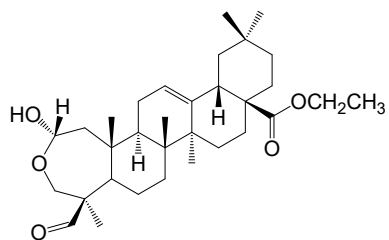


16. NMR data for compound 18

16.1 ^1H -NMR spectrum for compound 18 recorded in CDCl_3

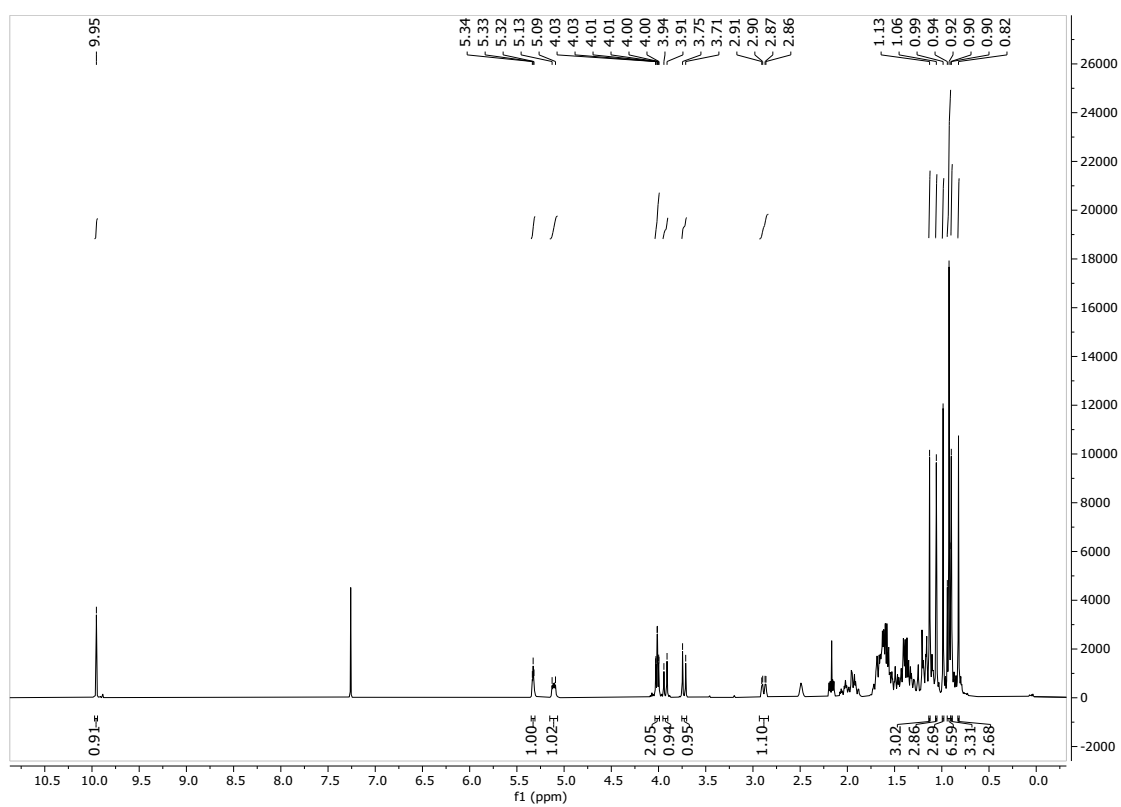
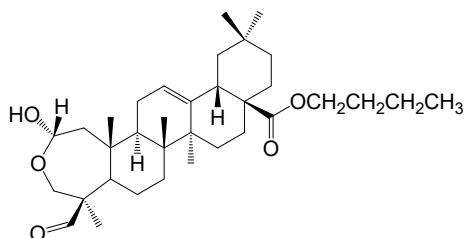


16.2 ^{13}C -NMR spectrum for compound 18 recorded in CDCl_3

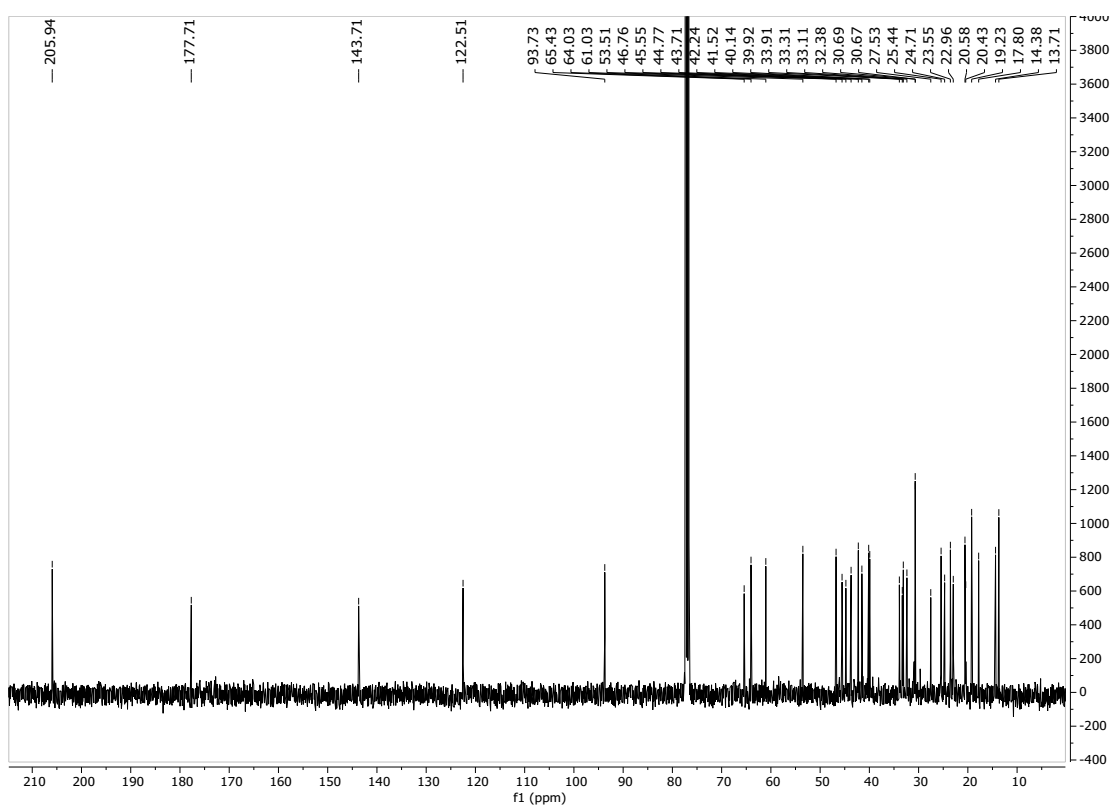
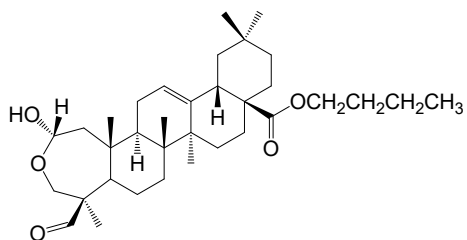


17. NMR data for compound 19

17.1 ¹H-NMR spectrum for compound 19 recorded in CDCl₃

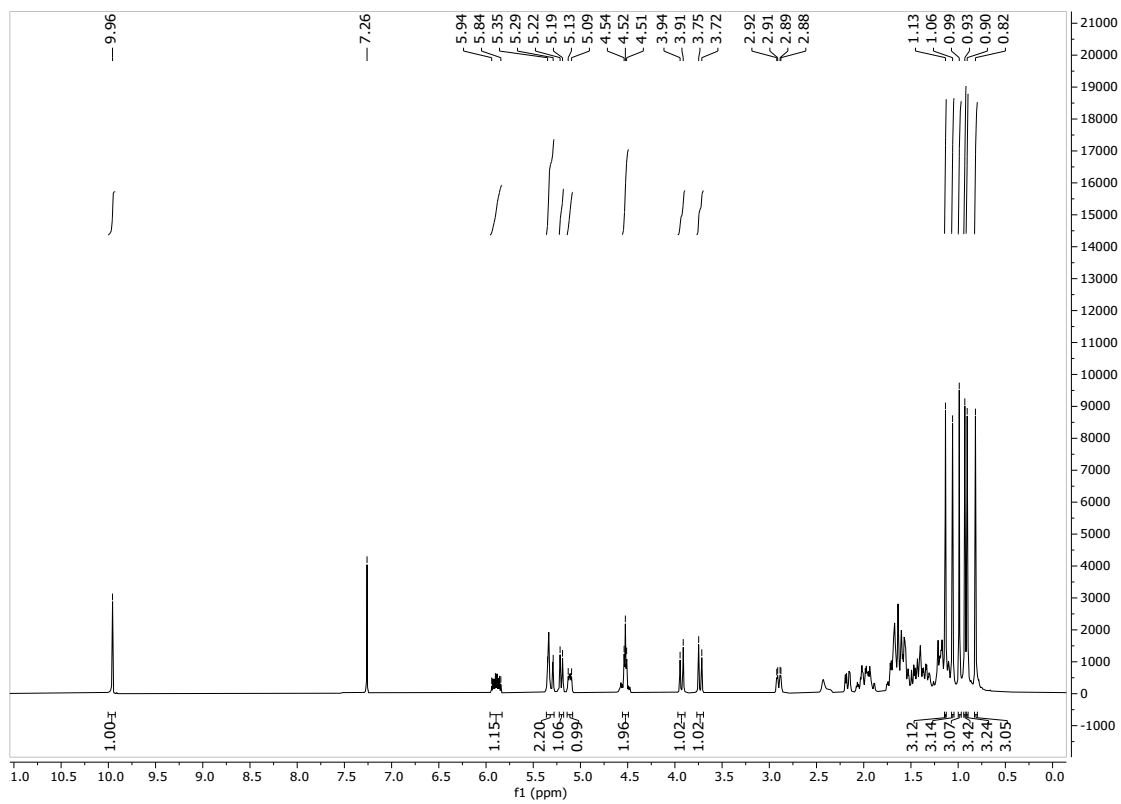
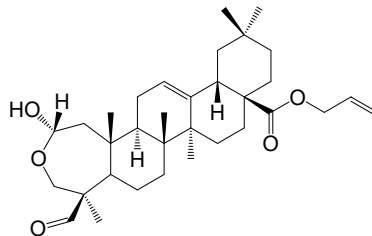


17.2 ^{13}C -NMR spectrum for compound 19 recorded in CDCl_3

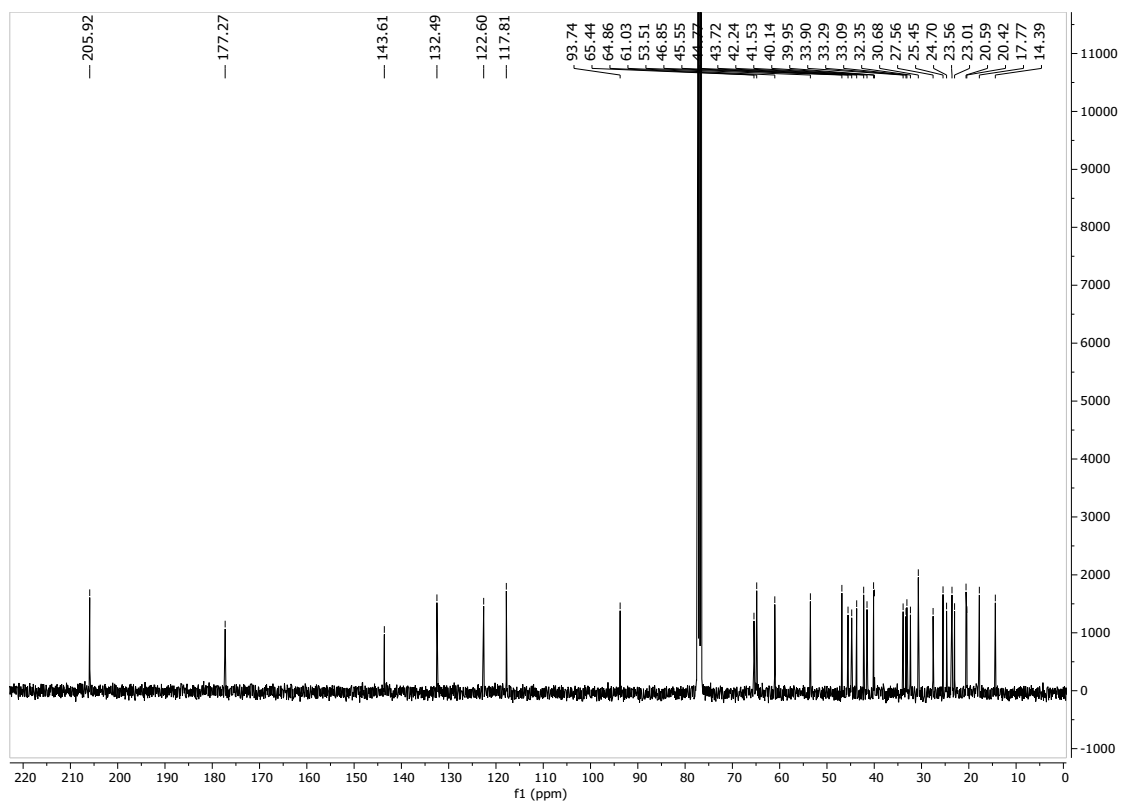
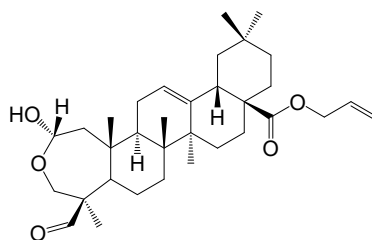


18. NMR data for compound 20

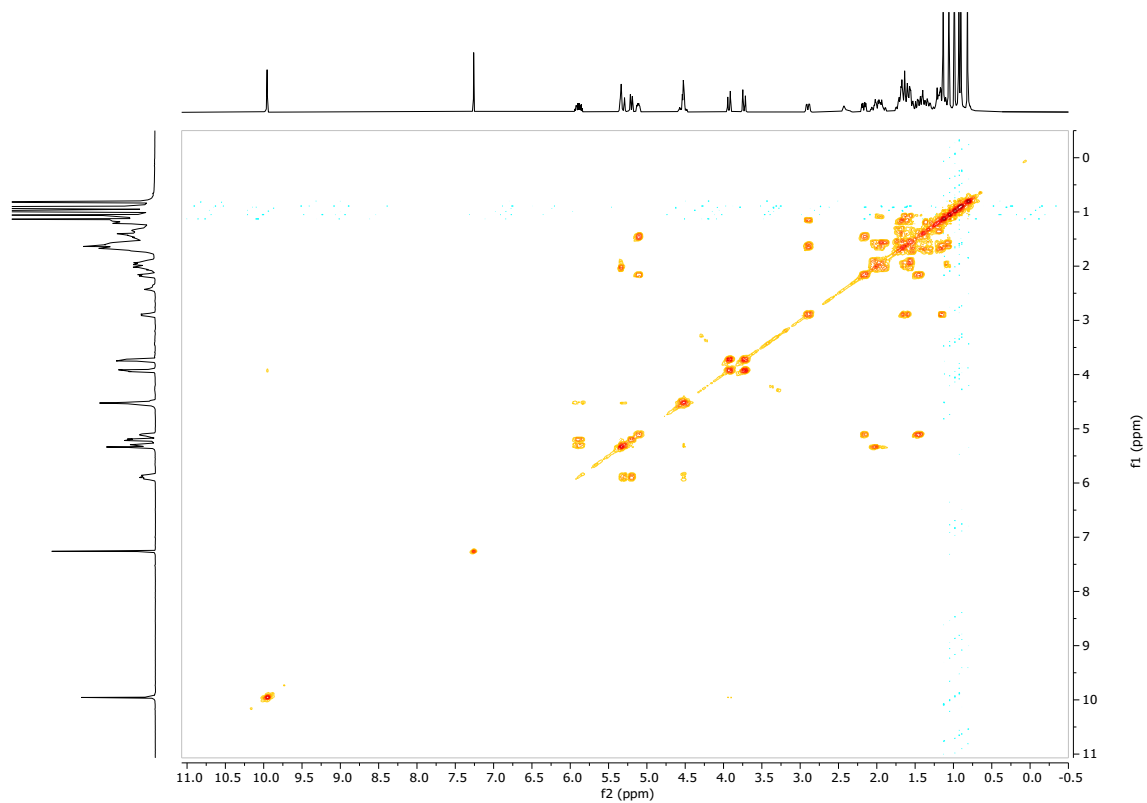
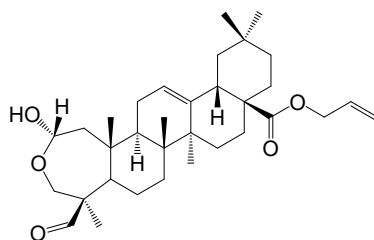
18.1 ¹H-NMR spectrum for compound 20 recorded in CDCl₃



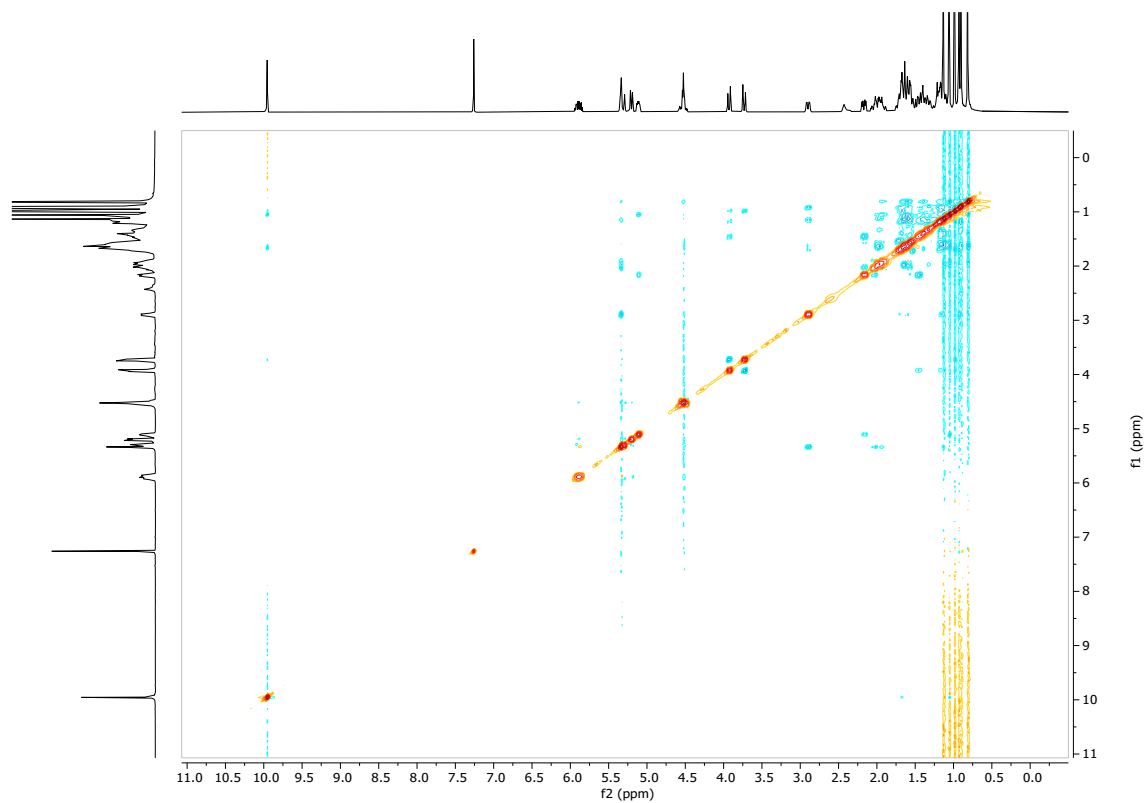
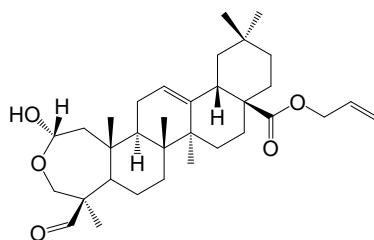
18.2 ^{13}C -NMR spectrum for compound 20 recorded in CDCl_3



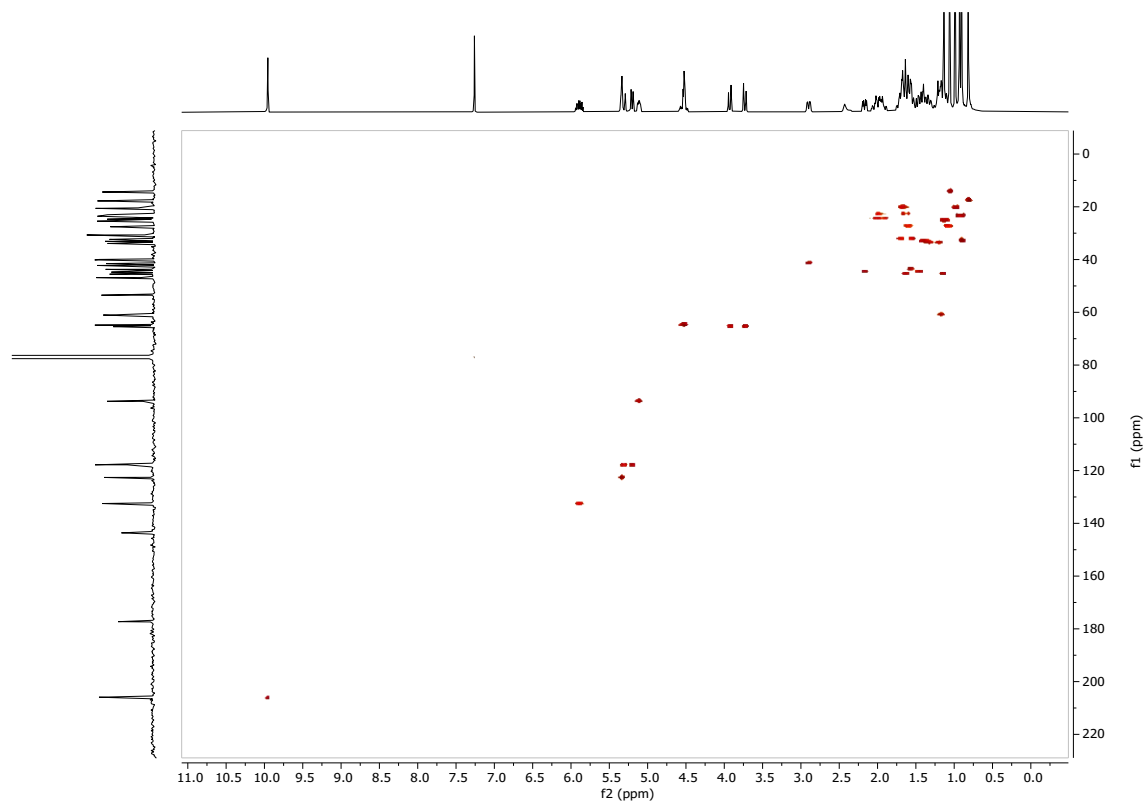
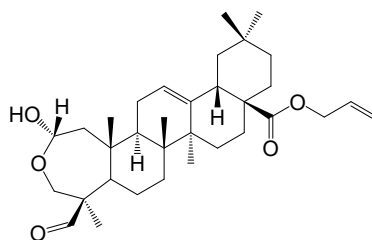
18.3 COSY spectrum for compound 20 recorded in CDCl₃



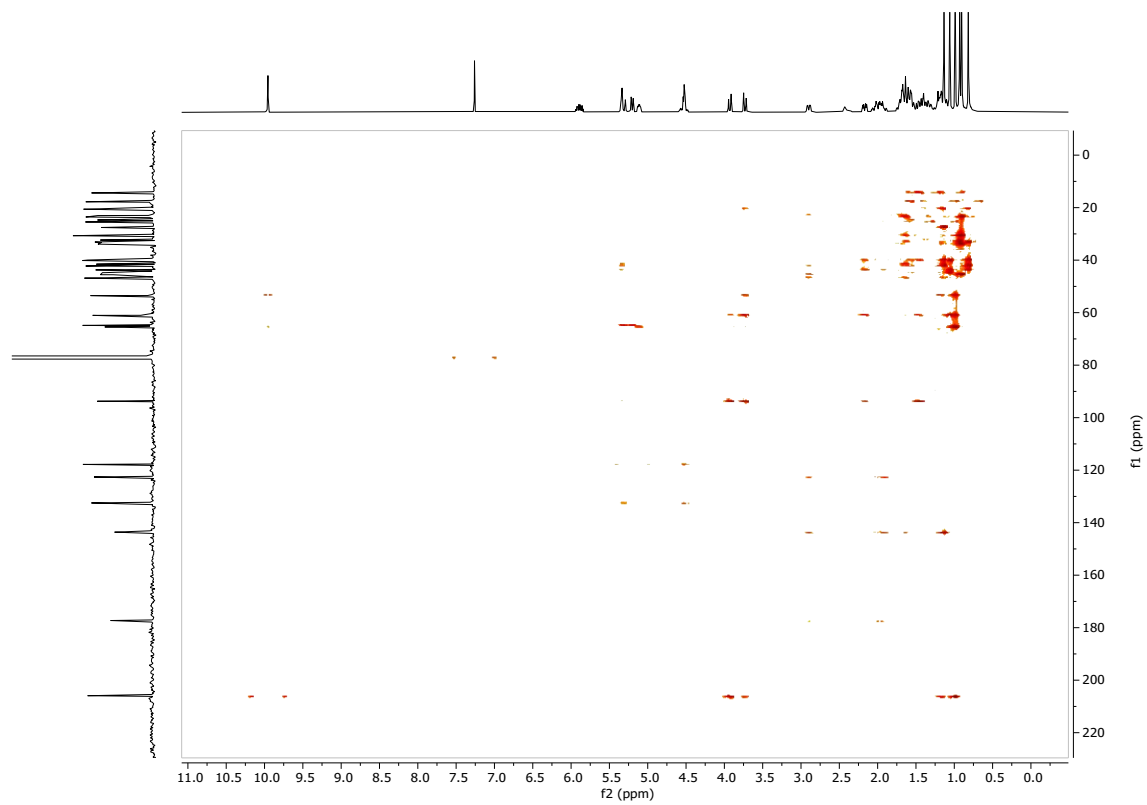
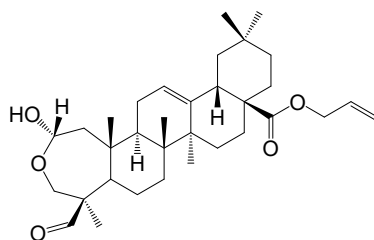
18.4 NOESY spectrum for compound 20 recorded in CDCl₃



18.4 HSQC spectrum for compound 20 recorded in CDCl₃

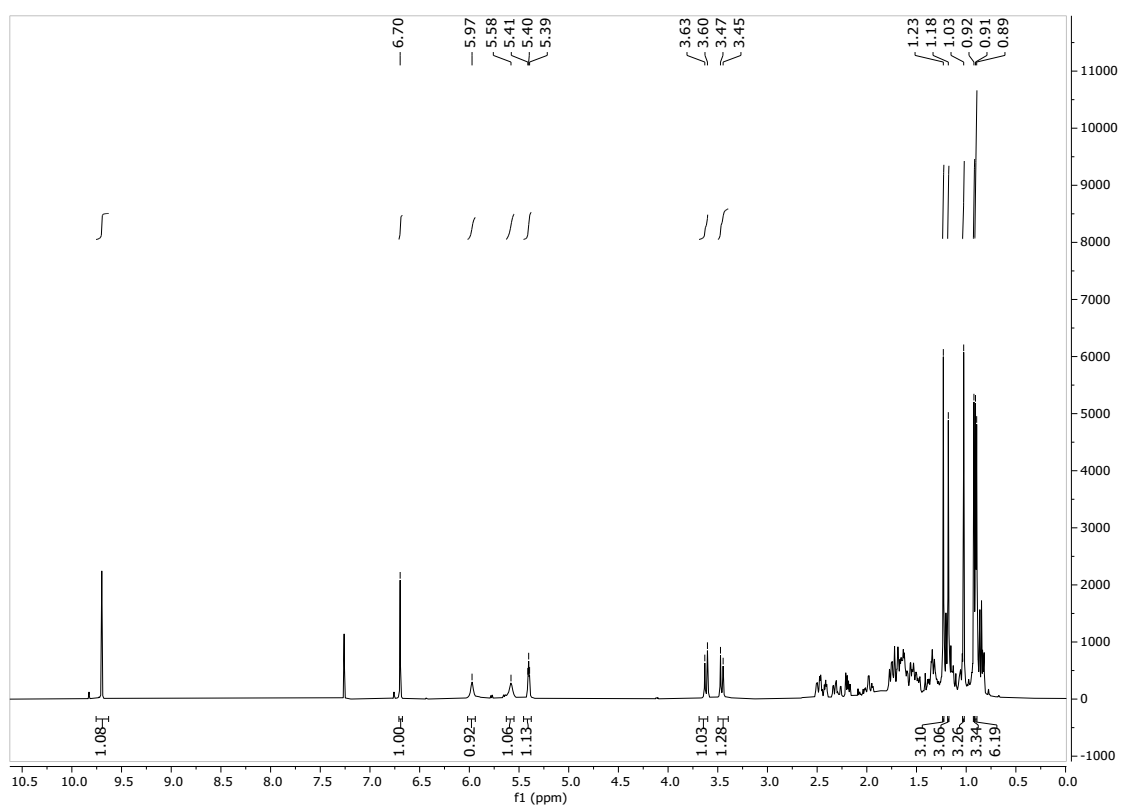
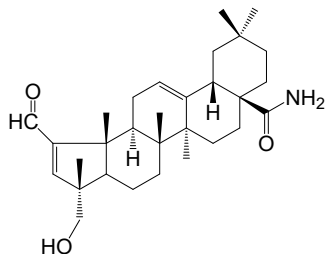


18.4 HMBC spectrum for compound 20 recorded in CDCl₃

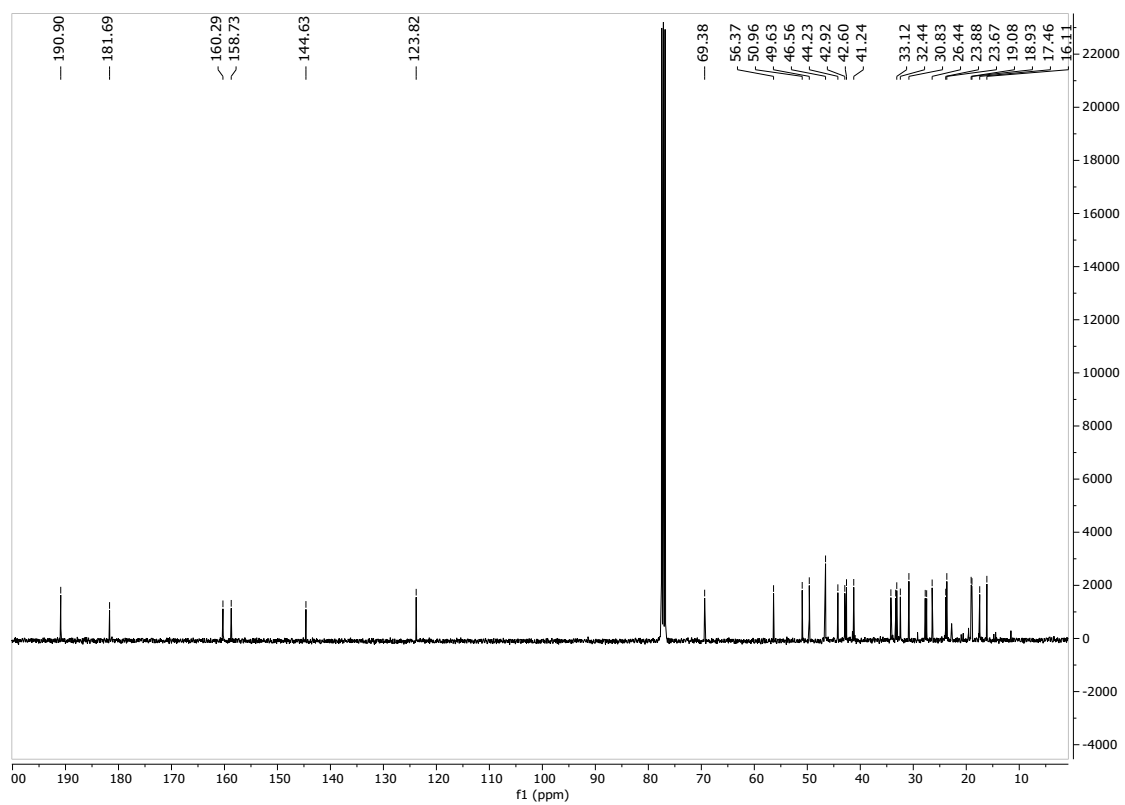
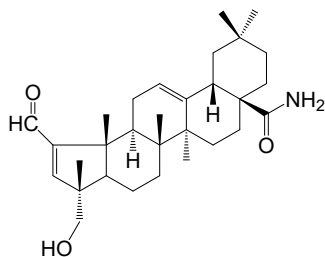


19. NMR data for compound 22

19.1 ^1H -NMR spectrum for compound 22 recorded in CDCl_3

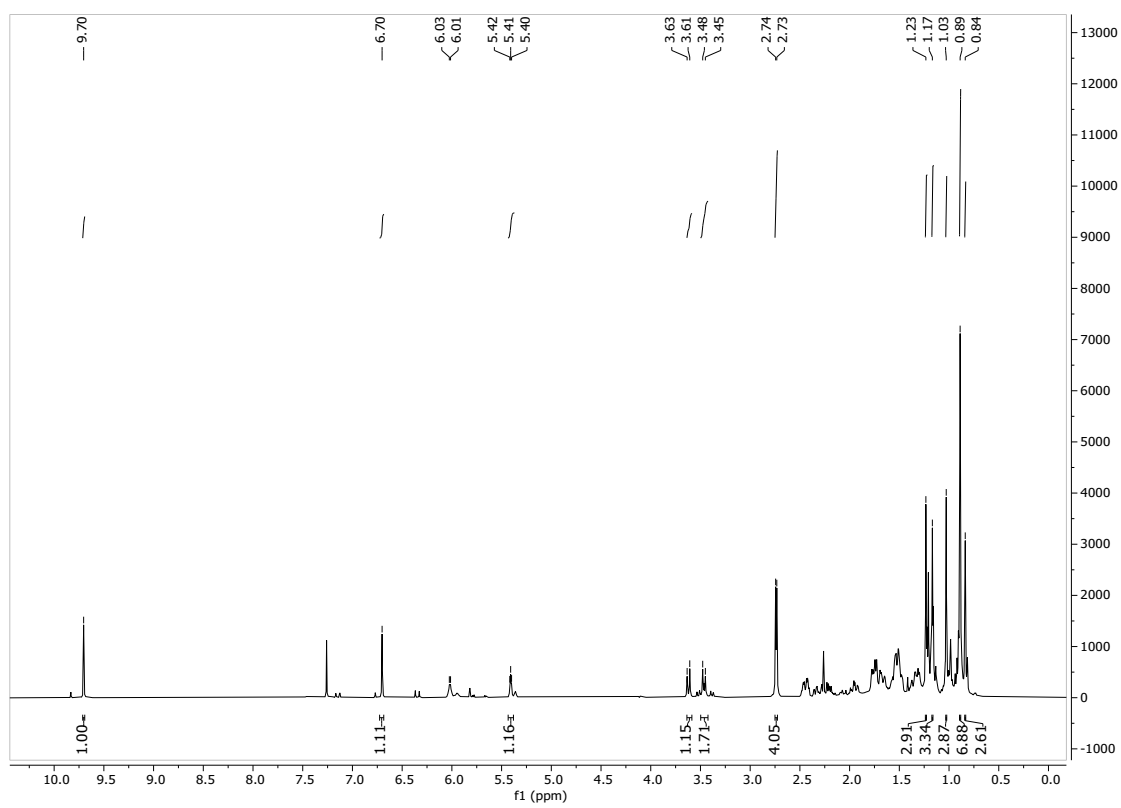
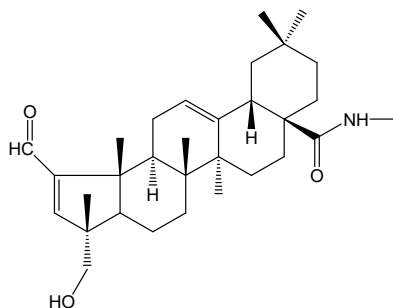


19.2 ^{13}C -NMR spectrum for compound 22 recorded in CDCl_3

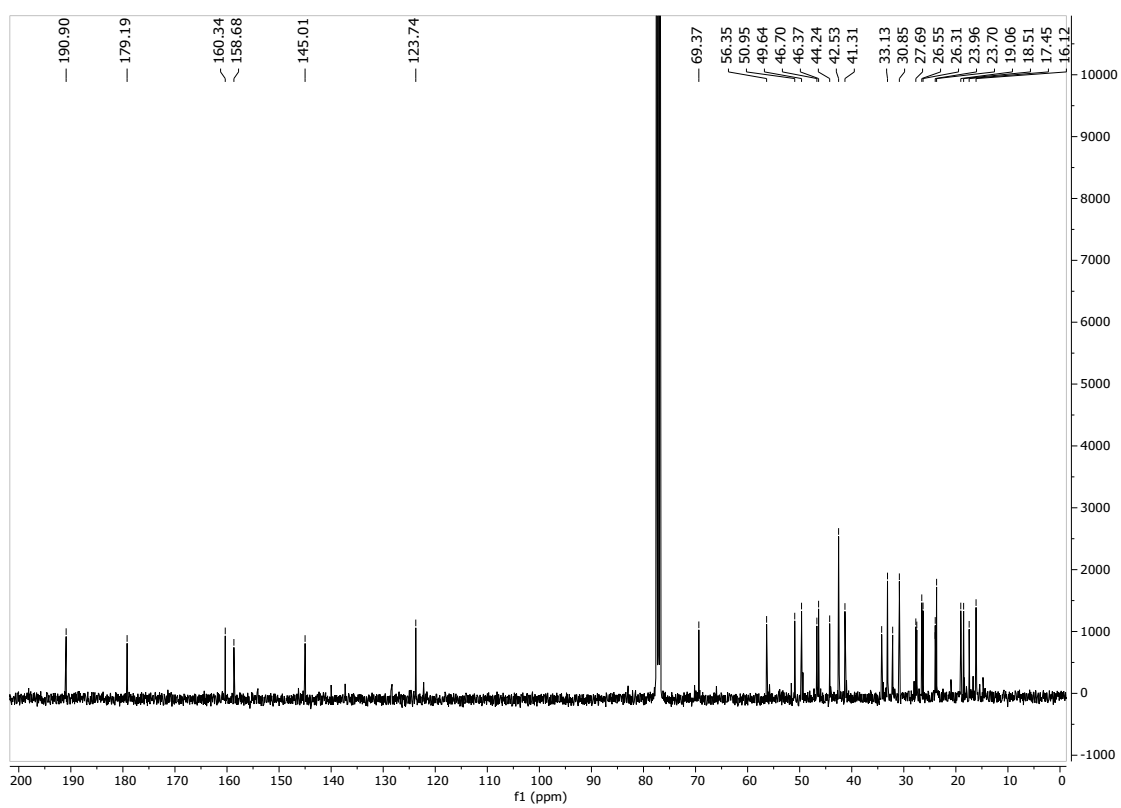
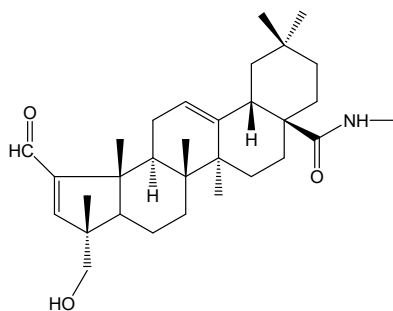


20. NMR data for compound 23

20.1 ¹H-NMR spectrum for compound 23 recorded in CDCl₃

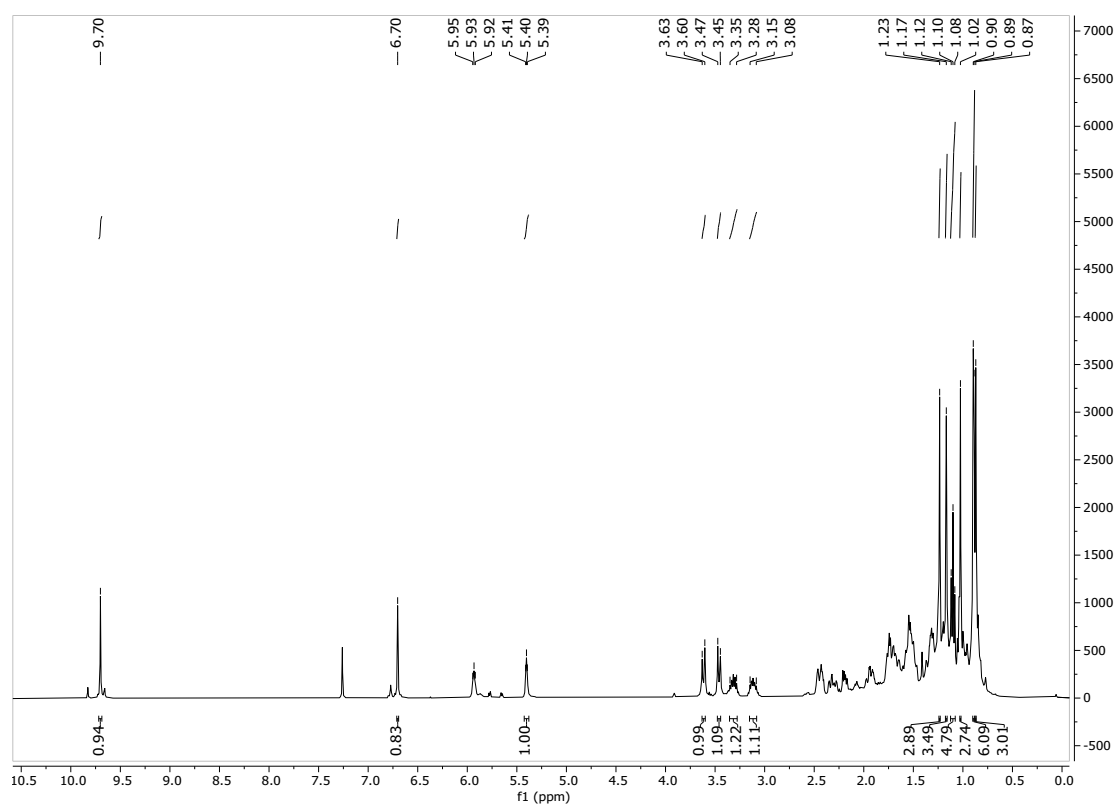
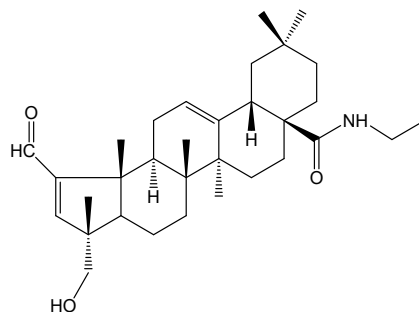


20.2 ^{13}C -NMR spectrum for compound 23 recorded in CDCl_3

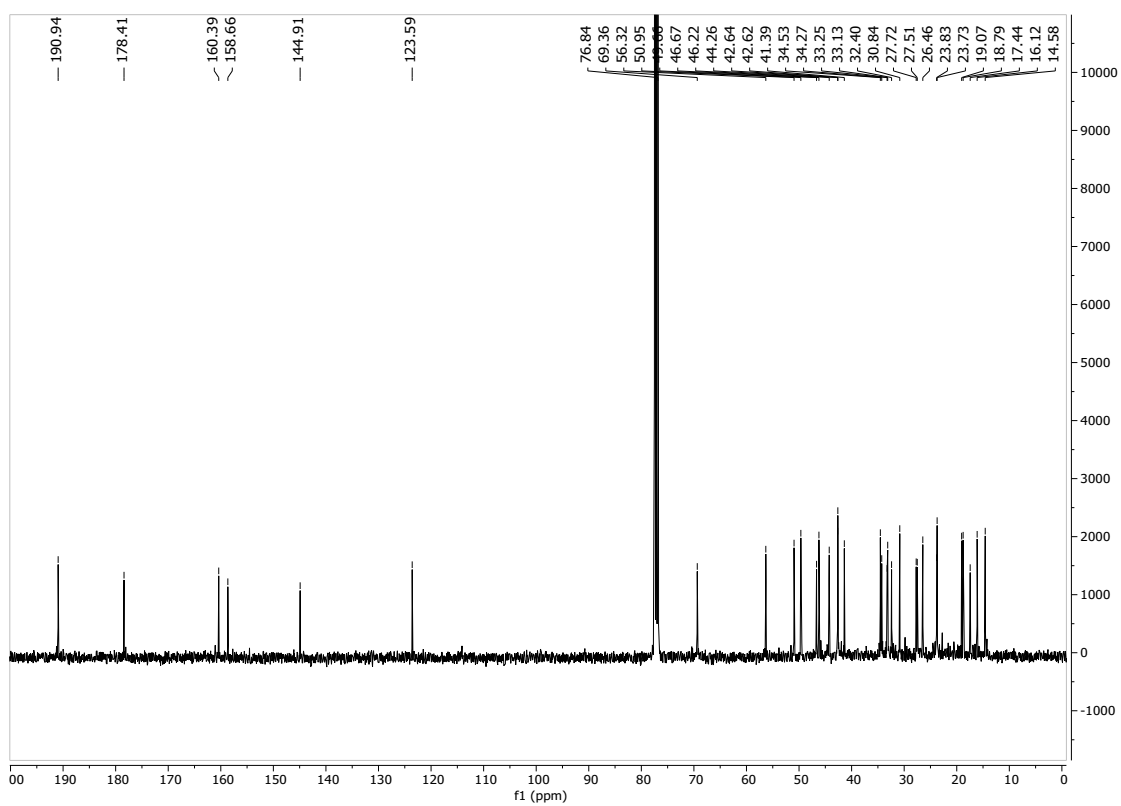
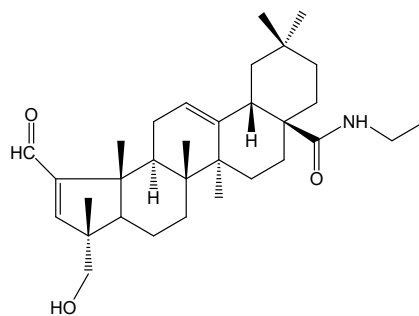


21. NMR data for compound 24

21.1 ¹H-NMR spectrum for compound 24 recorded in CDCl₃

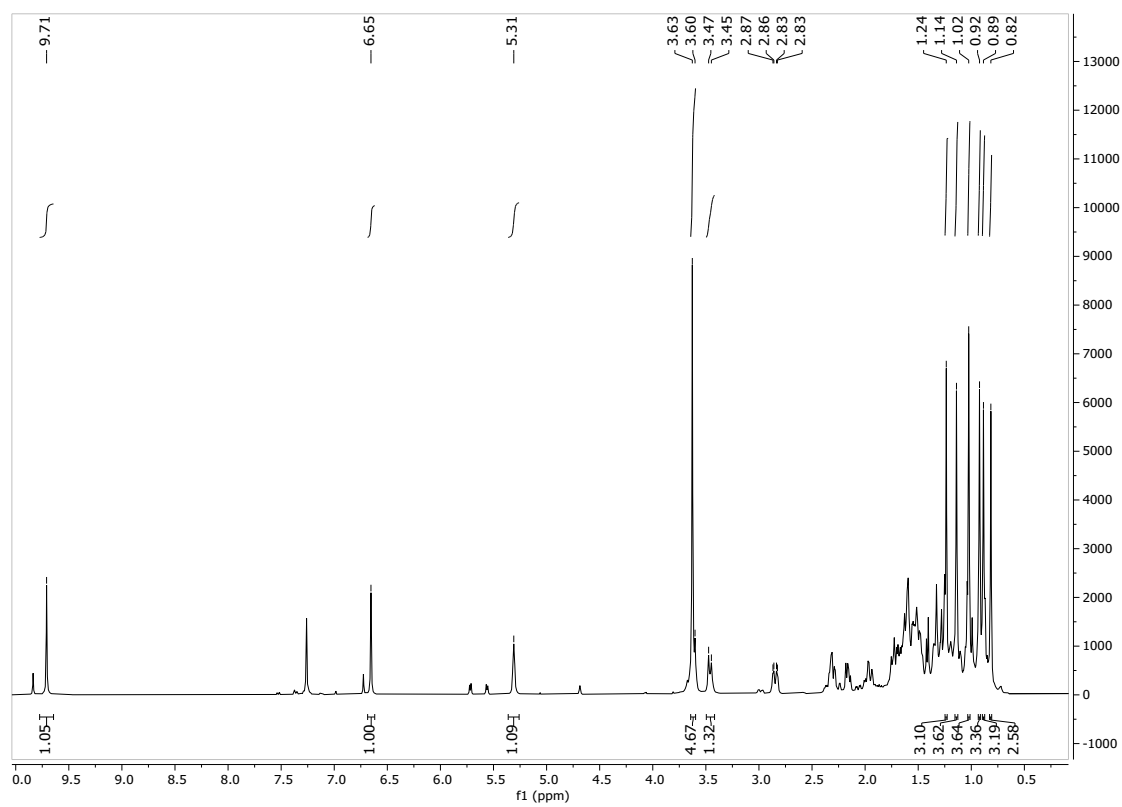
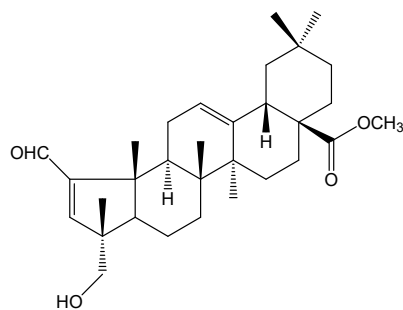


21.2 ^{13}C -NMR spectrum for compound 24 recorded in CDCl_3

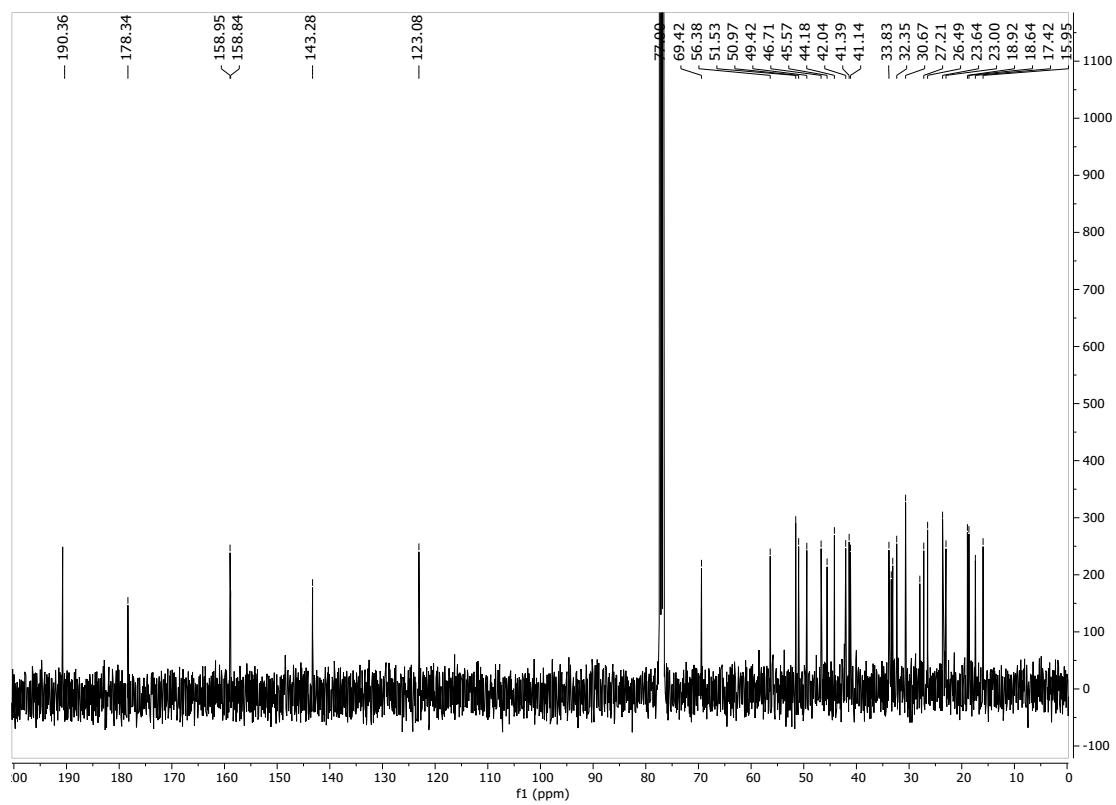
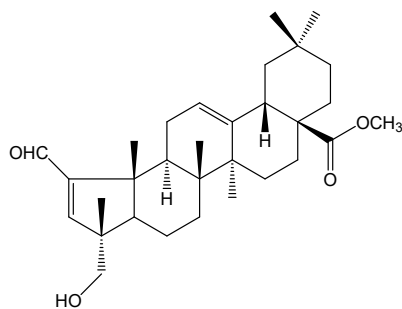


22. NMR data for compound 25

22.1 ¹H-NMR spectrum for compound 25 recorded in CDCl₃

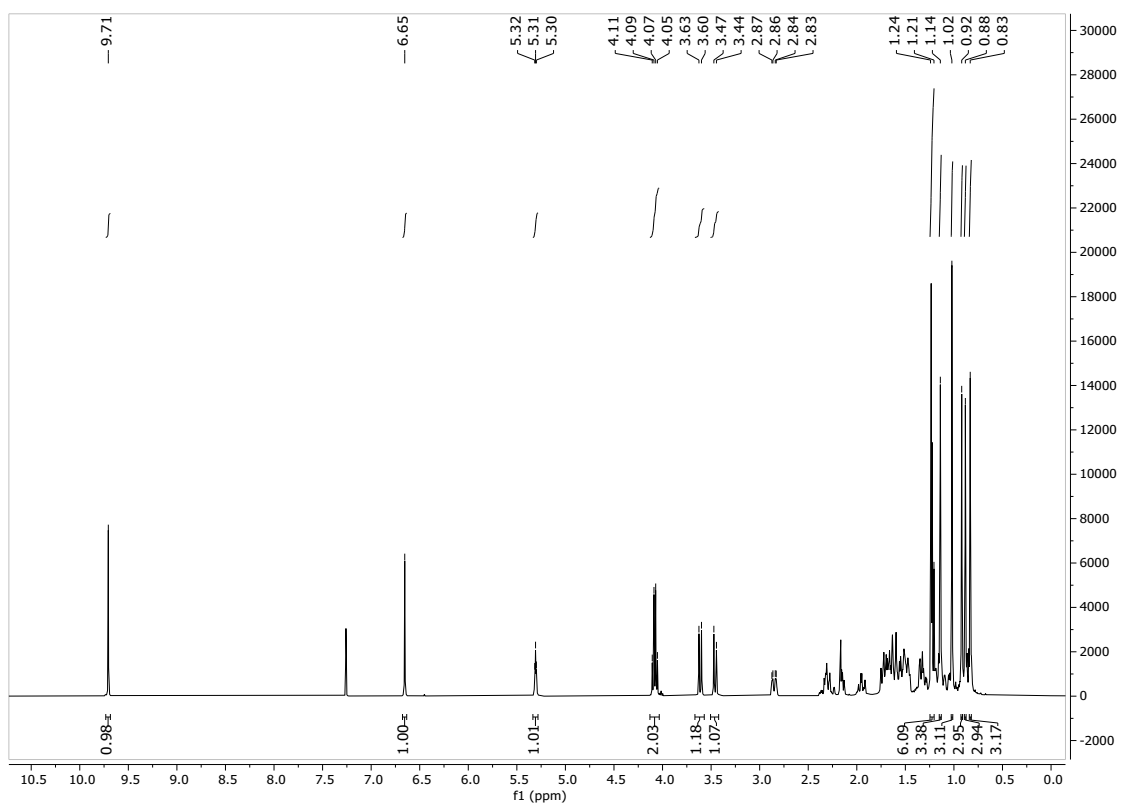
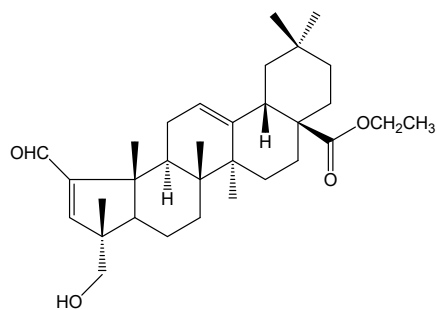


22.2 ^{13}C -NMR spectrum for compound 25 recorded in CDCl_3

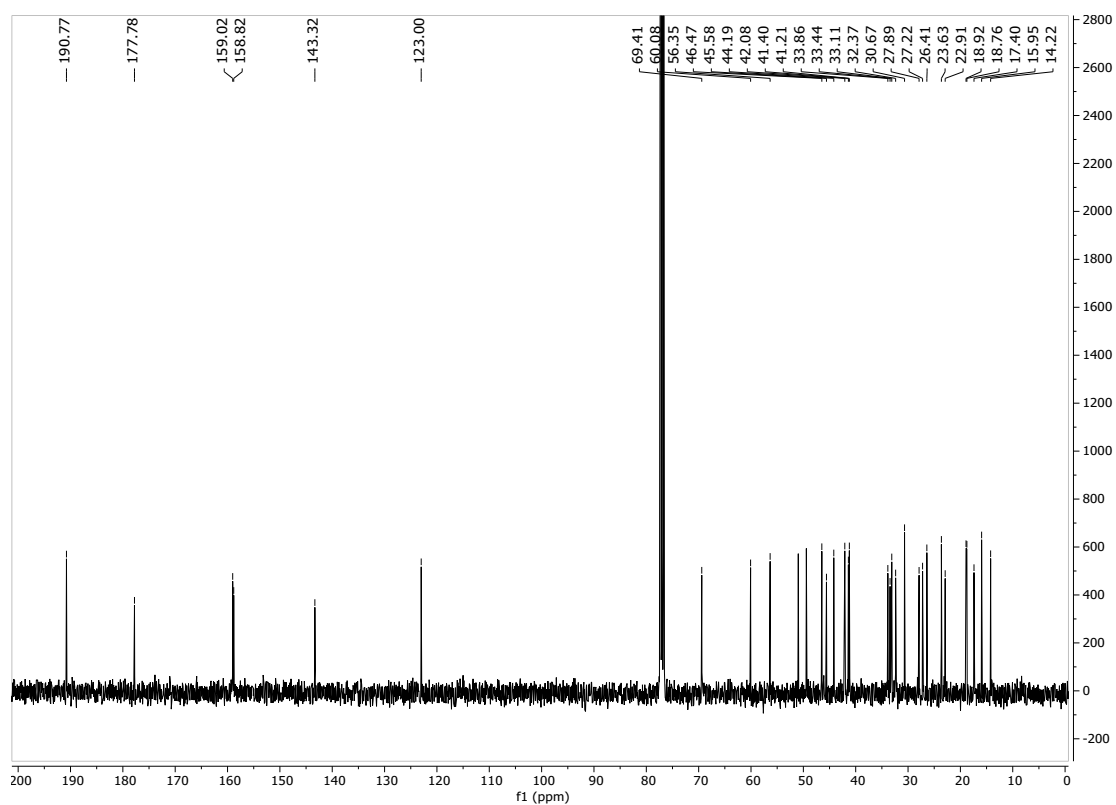
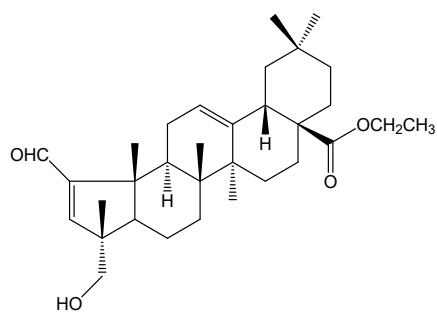


23. NMR data for compound 26

23.1 ^1H -NMR spectrum for compound 26 recorded in CDCl_3

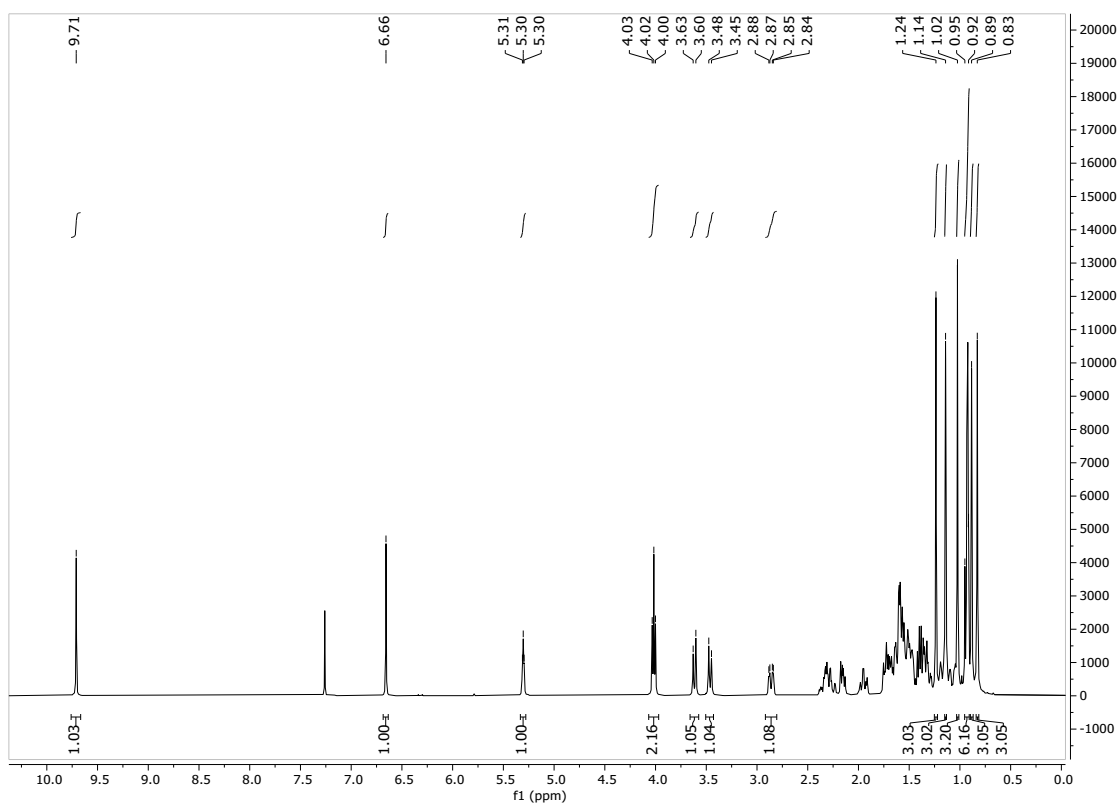
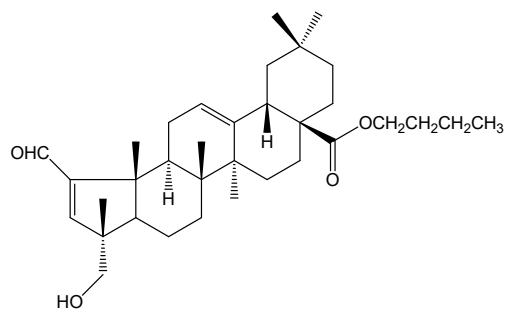


23.2 ^{13}C -NMR spectrum for compound 26 recorded in CDCl_3

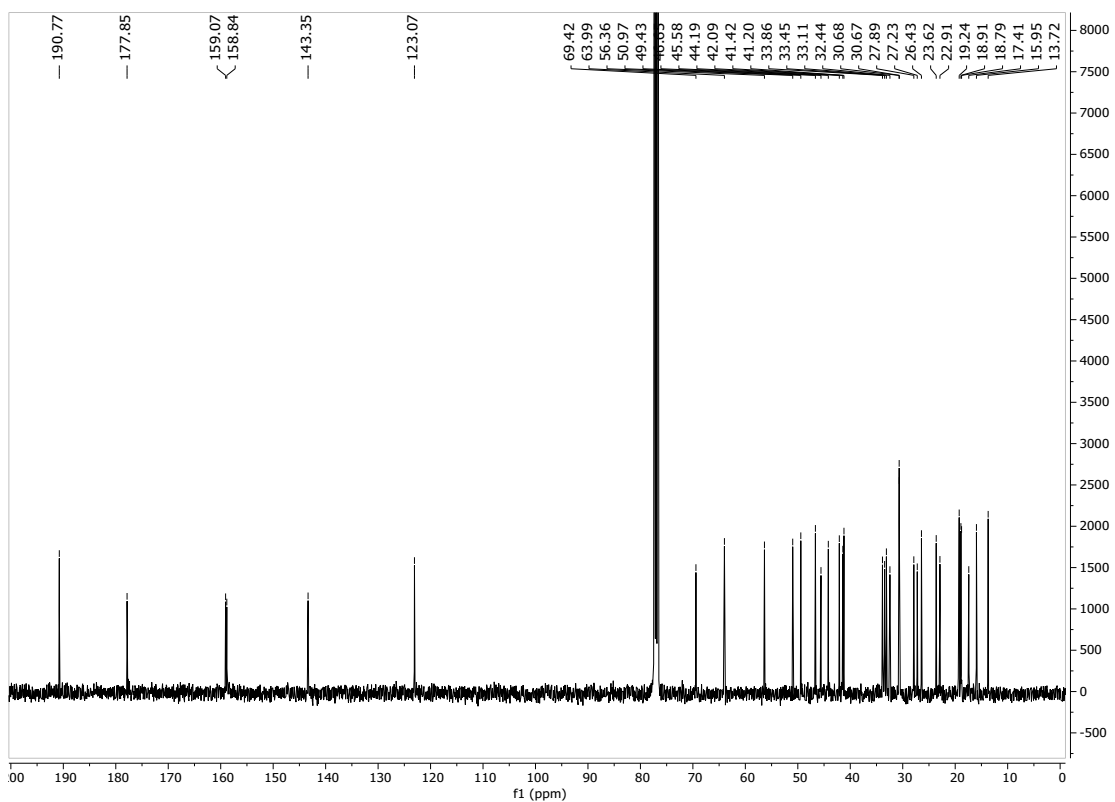
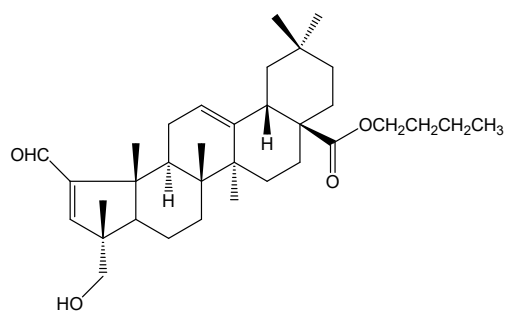


24. NMR data for compound 27

24.1 ¹H-NMR spectrum for compound 27 recorded in CDCl₃

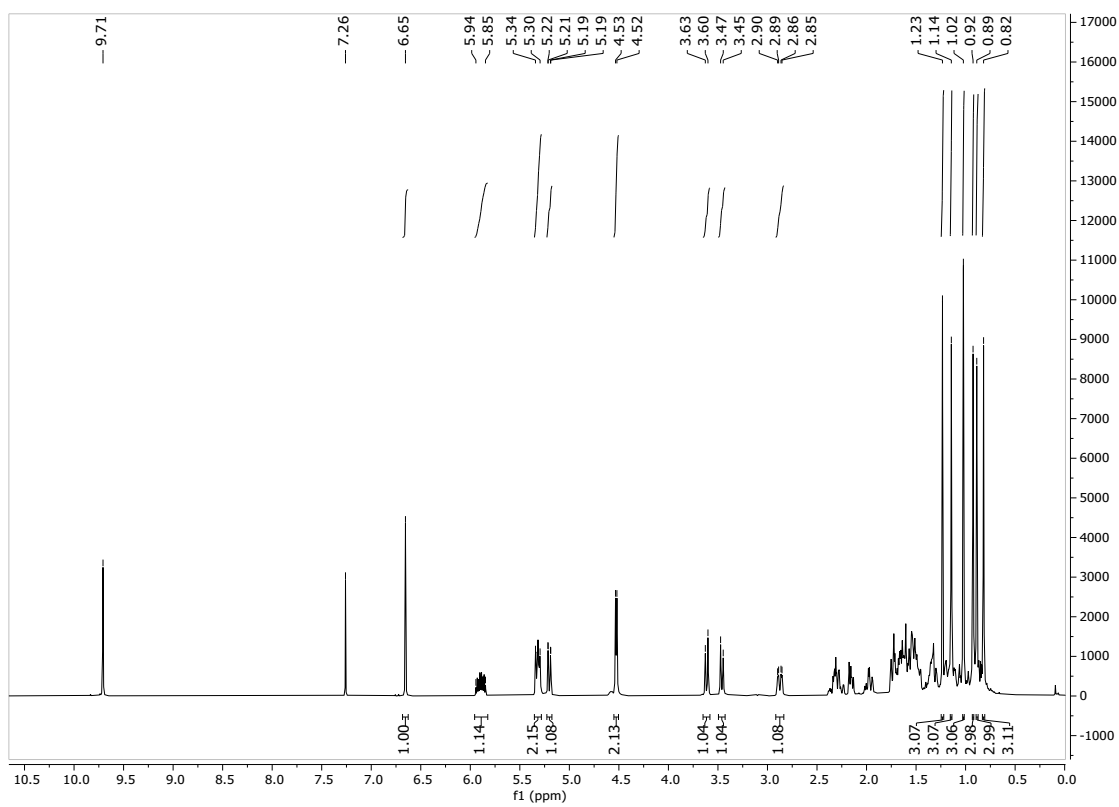
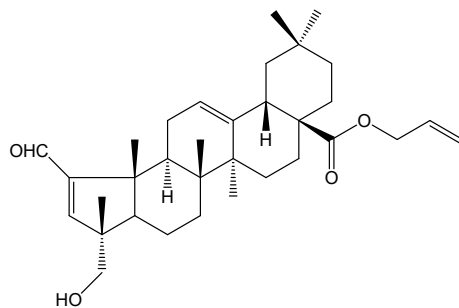


24.2 ^{13}C -NMR spectrum for compound 27 recorded in CDCl_3

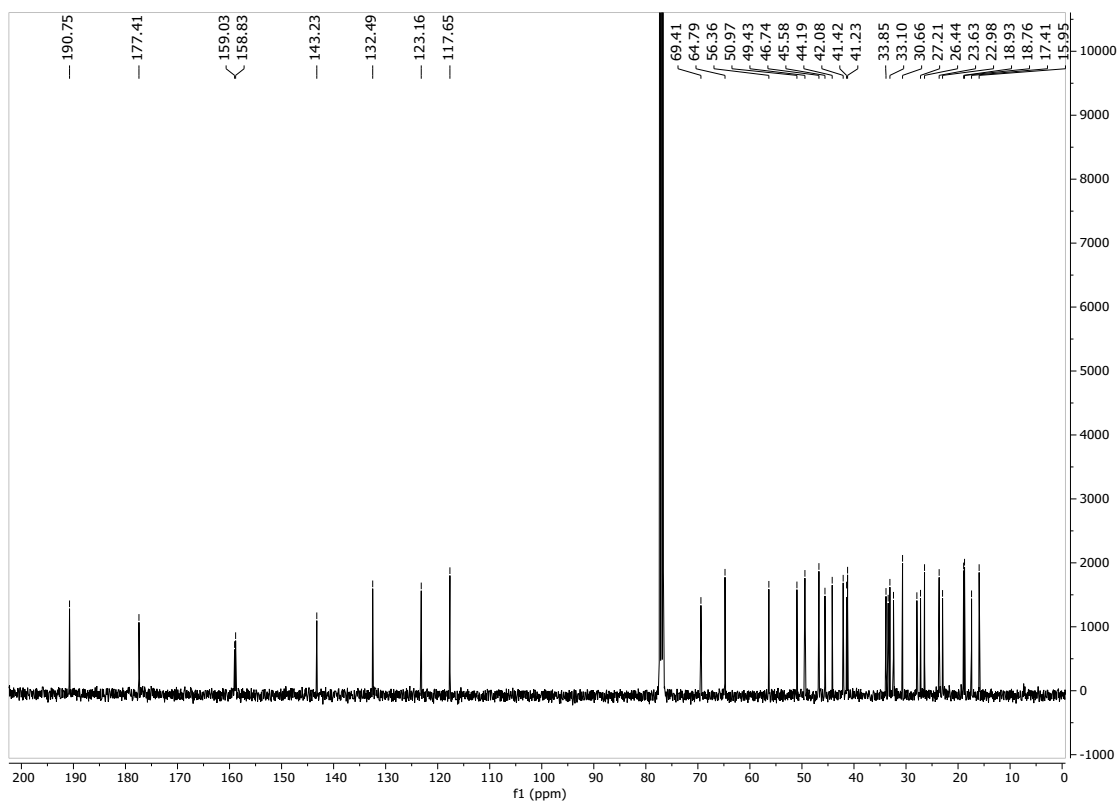
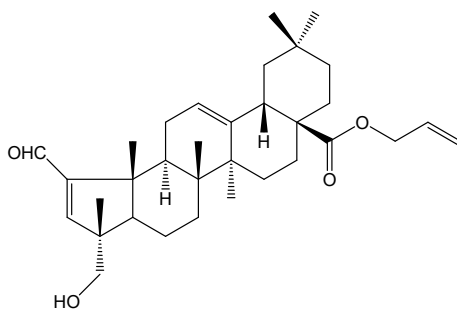


25. NMR data for compound 28

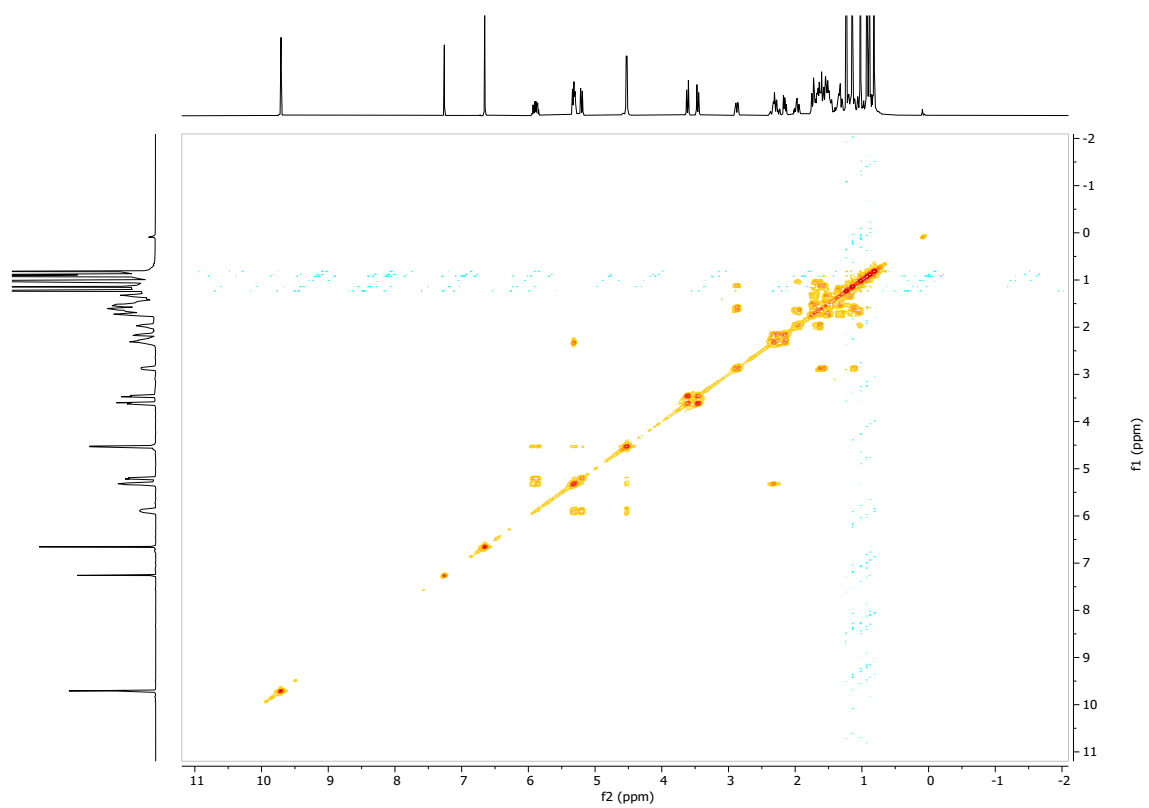
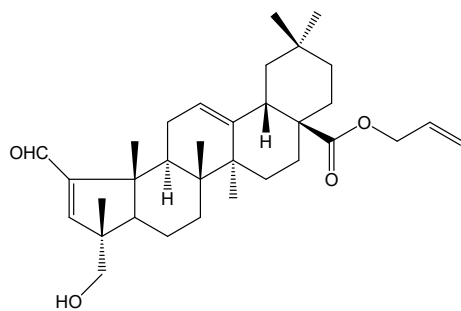
25.1 ¹H-NMR spectrum for compound 28 recorded in CDCl₃



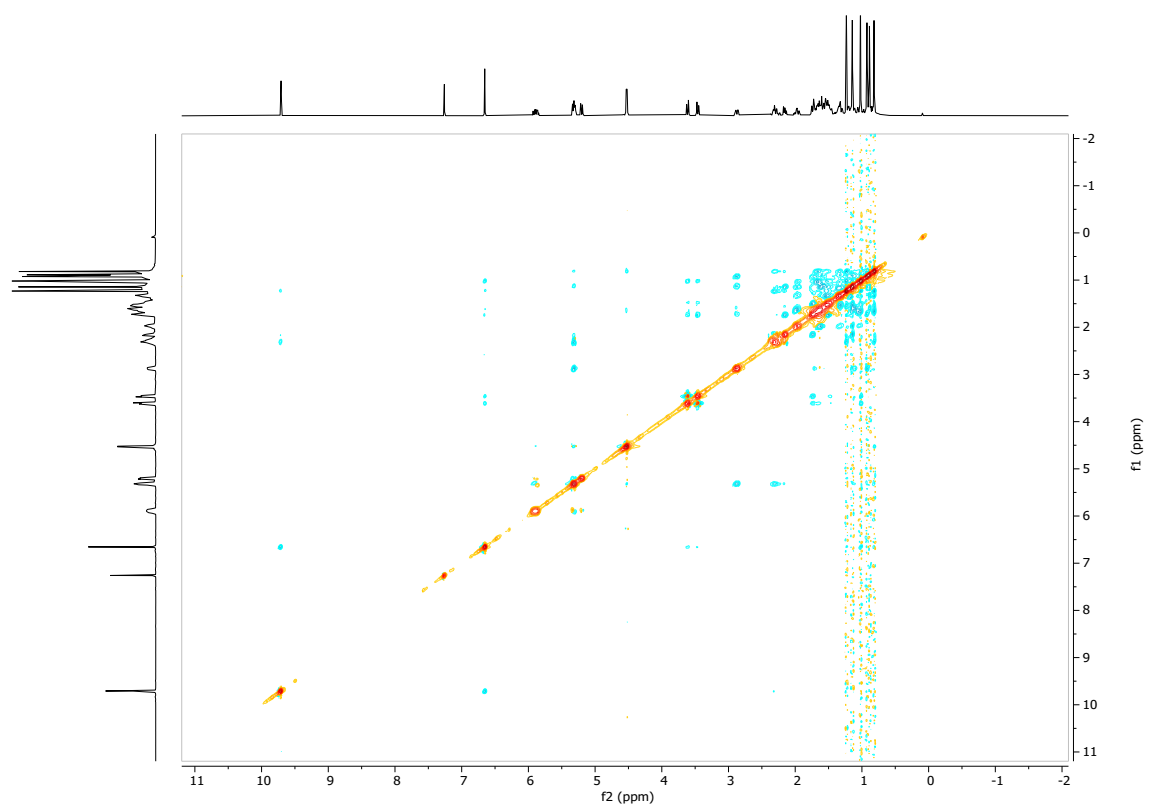
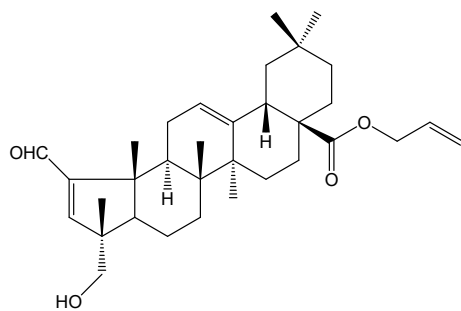
25.2 ^{13}C -NMR spectrum for compound 28 recorded in CDCl_3



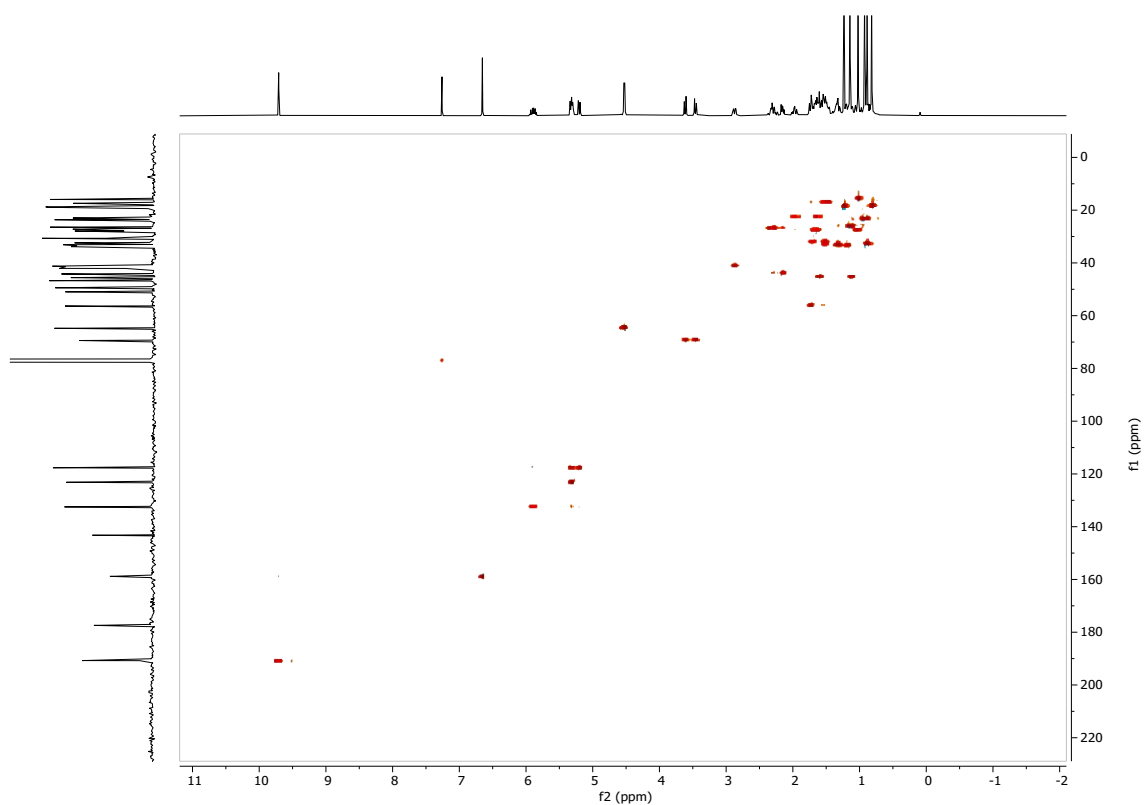
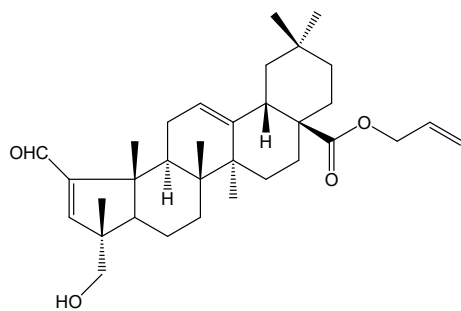
25.3 COSY spectrum for compound 28 recorded in CDCl₃



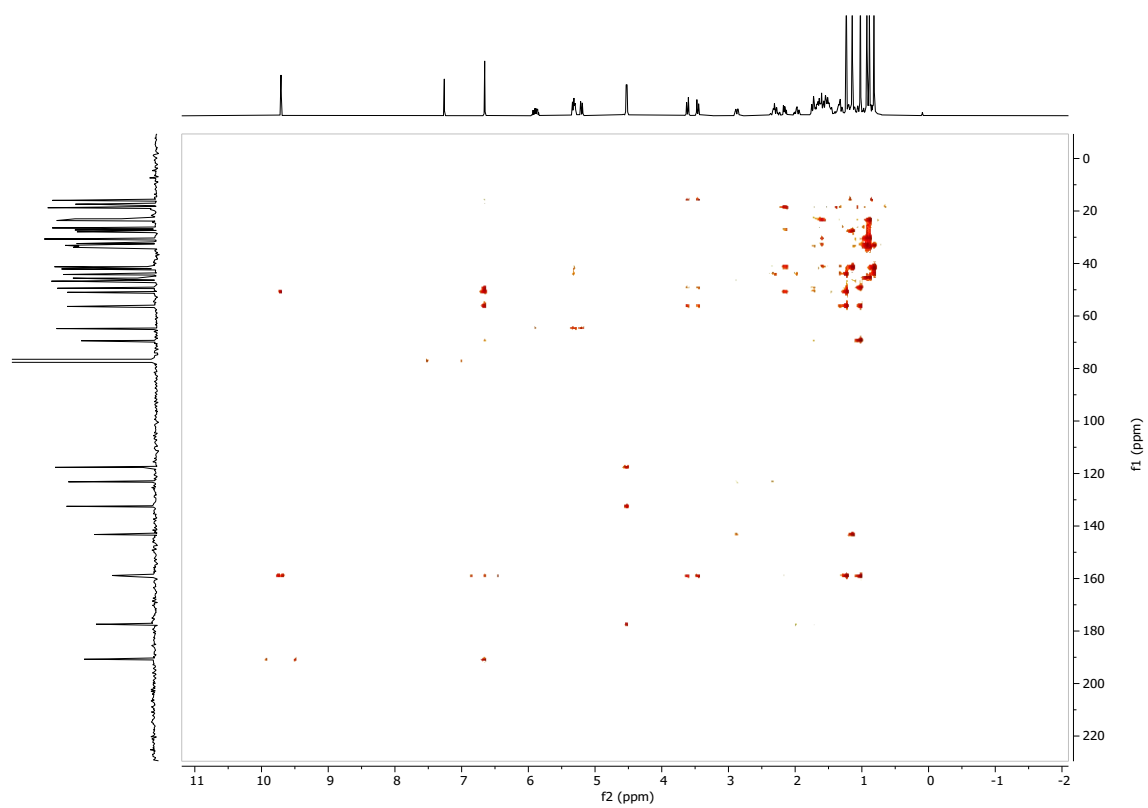
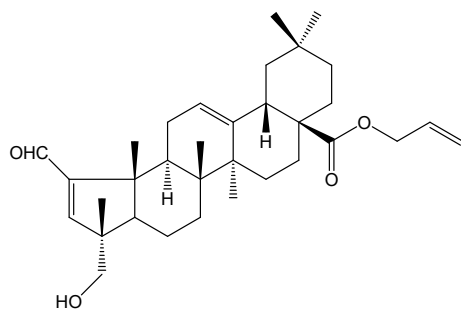
25.4 NOESY spectrum for compound 28 recorded in CDCl₃



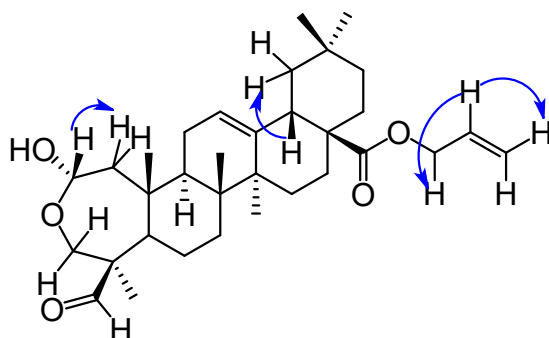
25.5 HSQC spectrum for compound 28 recorded in CDCl₃



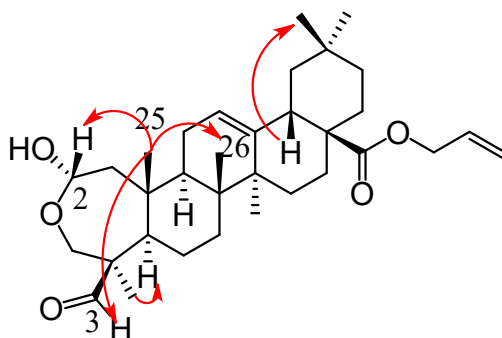
25.6 HMBC spectrum for compound 28 recorded in CDCl₃



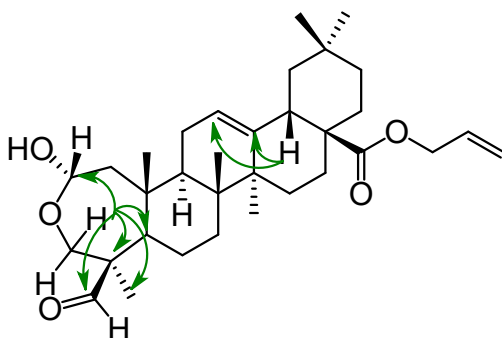
26. Some COSY correlations for compound 20



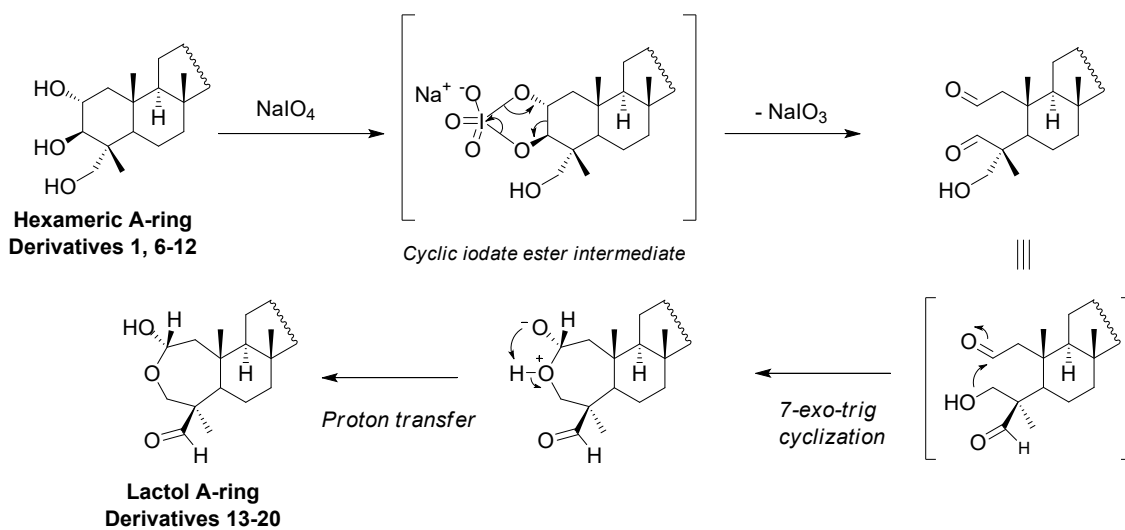
27. Some NOESY correlations for compound 20



28. Some HMBC correlations for compound 20

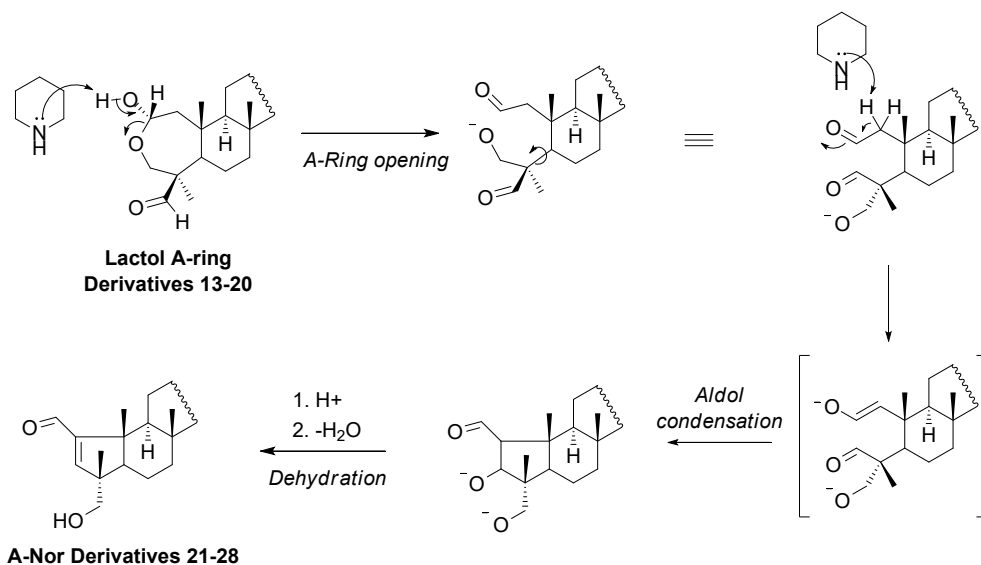


32. Proposed mechanism for the formation of derivatives 13-20.



Conditions: NaIO_4 , $\text{MeOH}/\text{H}_2\text{O}$, r.t.;

33. Proposed mechanism for the formation of derivatives 21-28.



Conditions: one-pot- i - Piperidine, acetic acid, dry benzene, reflux $60\text{ }^\circ\text{C}$, N_2 ; ii - anhydrous MgSO_4 , reflux $60\text{ }^\circ\text{C}$, N_2 .

34. In Silico studies.

After generation of SMILES notation, drug-likeness and absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters were predicted for compound **26** using SwissADME^[1] and pkCSM^[2] online softwares, as previously performed by some of us.^[3]

34.1 In silico molecular properties of compound 26 using the SwissADME predictive database.^a

Descriptor		Value
Molecular weight		496.72 g/mol
iLog P		4.37
MLog P		5.16
nON		4
nOHNH		1
n-rot		5
TPSA		63.60 Å ²
Drug-likeness	Lipinski	Yes; 1 violation (MLogP>4.15)
	Veber	Yes
Medicinal Chemistry	PAINS	0 alert
	Brenk	2 alerts: aldehyde, isolated alkene

^a Octanol-water partition coefficient (LogP; iLogP is *implicit* LogP; MLogP is *Moriguchi* LogP); number of hydrogen bond acceptors (nON); number of hydrogen bond donors (nOHNH); number of rotatable bonds (n-rot); topological polar surface area (TPSA); Lipinski's rule of five: molecular weight < 500 Da; Log P < 5; n-OHNH < 5; n-OHNH < 10. A maximum of 1 violation is permitted. Veber's parameters: n-rot ≤ 10; TPSA ≤ 140 Å² or total of nON and nOHNH ≤ 12.

34.2 *In silico* pharmacokinetic (absorption, distribution, metabolism, and excretion) and toxicity parameters of compound 26 using pkCSM and SwissADME predictive databases.^a

Property	Model Name	Predicted Value
Absorption	Water solubility	-6.102 ^b
	Intestinal absorption (human)	100%
	P-glycoprotein substrate	Yes
	P-glycoprotein I and II inhibitor	Yes
Distribution	BBB permeant	No
	LogBB	-0.572
	LogPS	-1.429
Metabolism	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
	CYP2D6 substrate	No
	CYP3A4 substrate	Yes
Excretion	Log total clearance	0.078 ^c
	Renal OCT2 substrate	No
Toxicity	AMES toxicity	No
	hERG I inhibitor	No
	hERG II inhibitor	No
	Hepatotoxicity	No
	Skin Sensitisation	No

^a Blood-brain barrier (BBB); logarithm of permeability in blood-brain barrier (logBB) < -1 are poorly distributed; blood-brain permeability-surface area product (logPS) > -2 are considered to penetrate the central nervous system and logPS < -3 are considered as unable to penetrate the central nervous system; human ether-a-go-go-related gene (hERG). ^b log(mol/L). ^c log(mL/min/kg).

References:

- 1- Daina, A.; Michielin, O.; Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* 2017, 7, 42717.
- 2- Pires, D.E.V.; Blundell, T.L.; Ascher, D.B. pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J. Med. Chem.* 2015, 58, 4066–4072.
- 3- a) Serrano JL, Lopes D, Reis MJA, Boto REF, Silvestre S, Almeida P. Bis-thiobarbiturates as Promising Xanthine Oxidase Inhibitors: Synthesis and Biological Evaluation. *Biomedicines*. 2021 Oct 11;9(10):1443. b) Cruz-Vicente P, Gonçalves AM, Ferreira O, Queiroz JA, Silvestre S, Passarinha LA, Gallardo E. Discovery of Small Molecules as Membrane-Bound Catechol-O-methyltransferase Inhibitors with Interest in Parkinson's Disease: Pharmacophore Modeling, Molecular Docking and In Vitro Experimental Validation Studies. *Pharmaceuticals (Basel)*. 2021 Dec 31;15(1):51.