

SUPPLEMENTARY MATERIALS

Synthesis, Structure and In Vitro Biological Evaluation of Semi-synthetic Derivatives of Betulin

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Experimental

A. Synthesis - Materials and methods

The chemical reagents were purchased from the Sigma-Aldrich. All melting points (mp) were determined using an Electrothermal IA 9300 apparatus, and are uncorrected. ^1H and ^{13}C NMR spectra were performed on a Bruker Avance III 600 (Bruker Corporation, Billerica, MA, USA) using CDCl_3 as solvent. Chemical shifts (δ) were referenced to the signal of the CDCl_3 and are given in parts per million (ppm). Infrared spectra were recorded on an IRAffinity-1 spectrometer (Shimadzu, Kyoto, Japan). HRMS spectra were recorded on a Bruker Impact II (Bruker Corporation, Billerica, MA, USA). The course of the reaction was monitored by thin-layer chromatography using Silica gel 60 254F plates (Merck, Darmstadt, Germany), and mixture dichloromethane/ethanol (60:1, v/v) as an eluent. The spots of the chromatograms were visualized by spraying with an 10% ethanolic solution of H_2SO_4 and heating to 100°C . Purification was conducted by column chromatography using silica gel 60 (0.063-0.200 mm, Merck, Darmstadt, Germany) as a solid phase, and mixture dichloromethane/ethanol (60:1, v/v) as an eluent.

Figure S1. ^1H NMR, compound 6

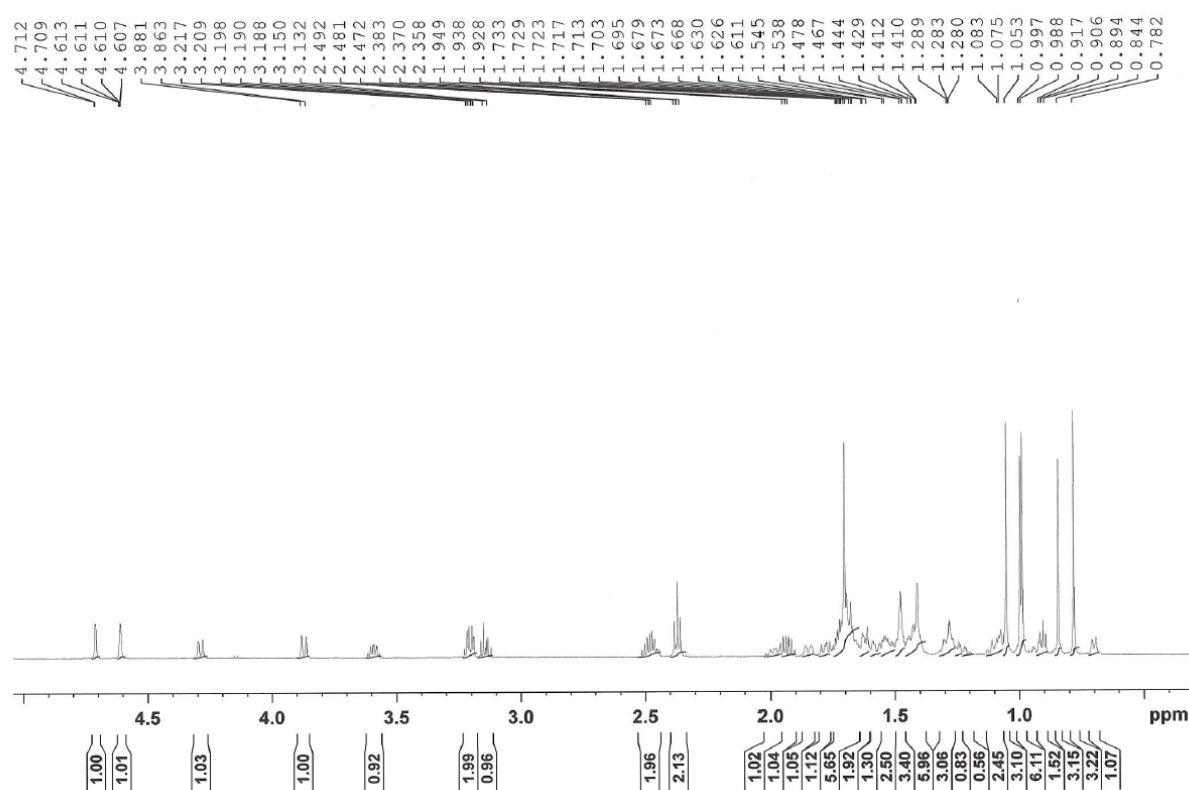


Figure S2. ^{13}C NMR, compound 6

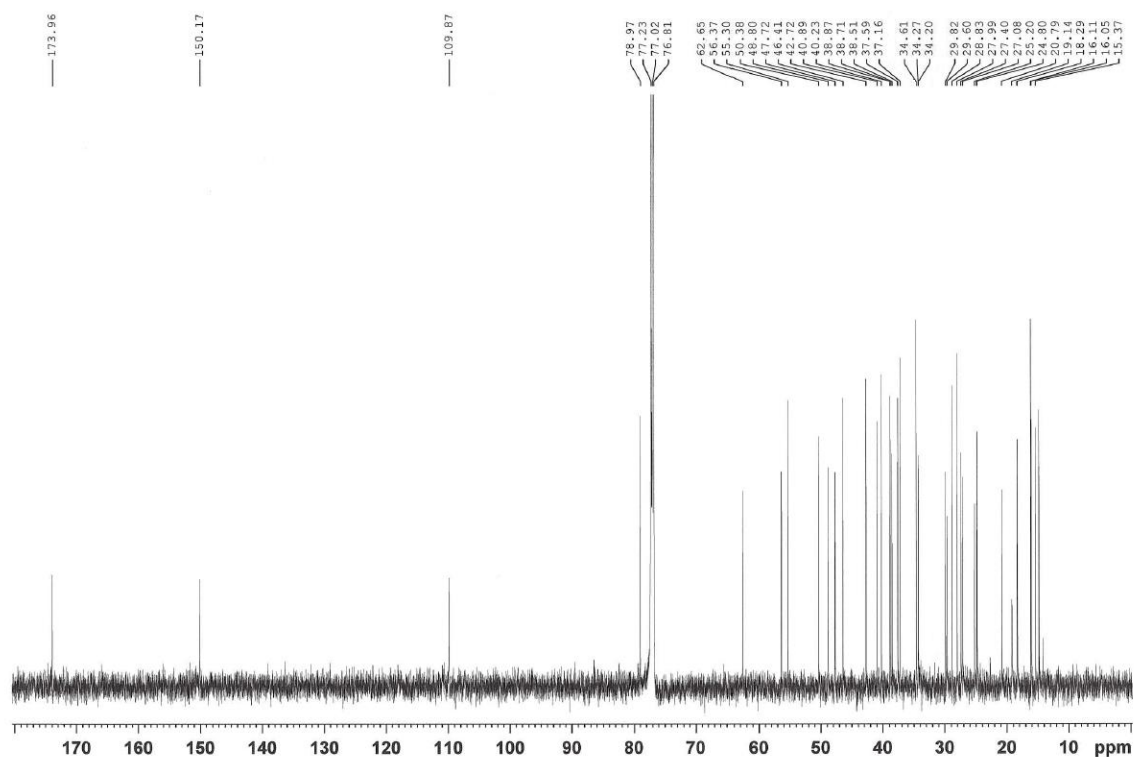


Figure S3. IR, compound 6

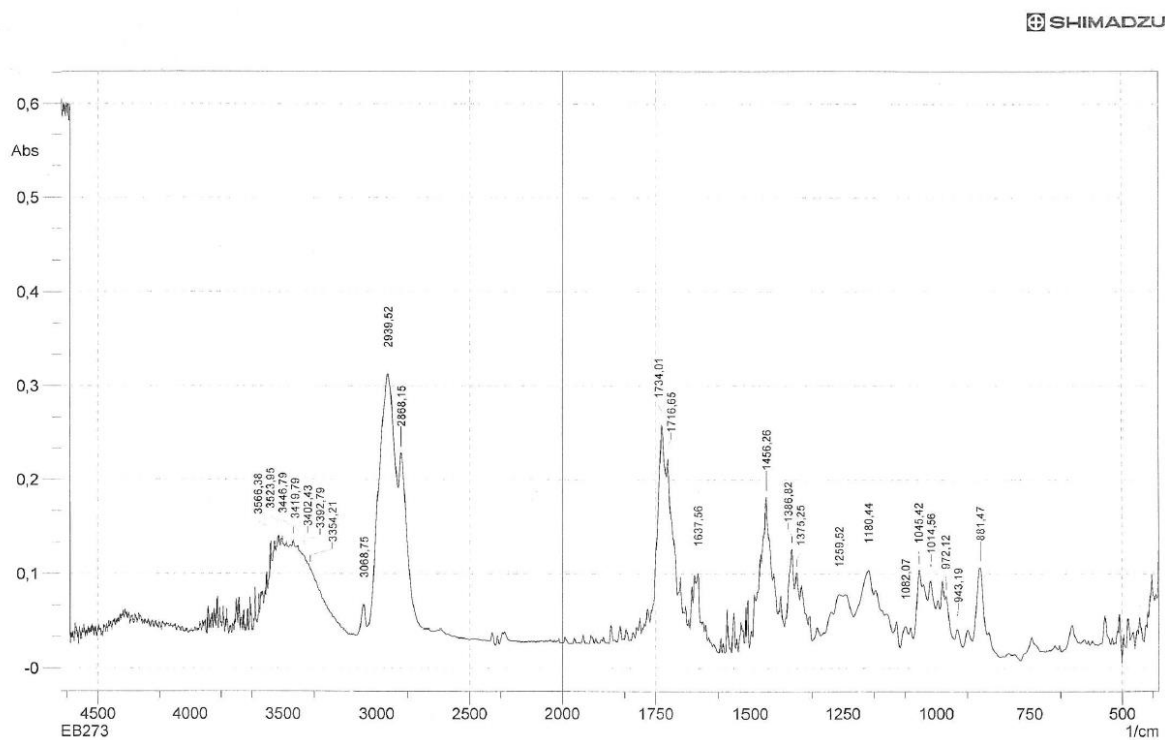


Figure S4. HRMS, compound 6

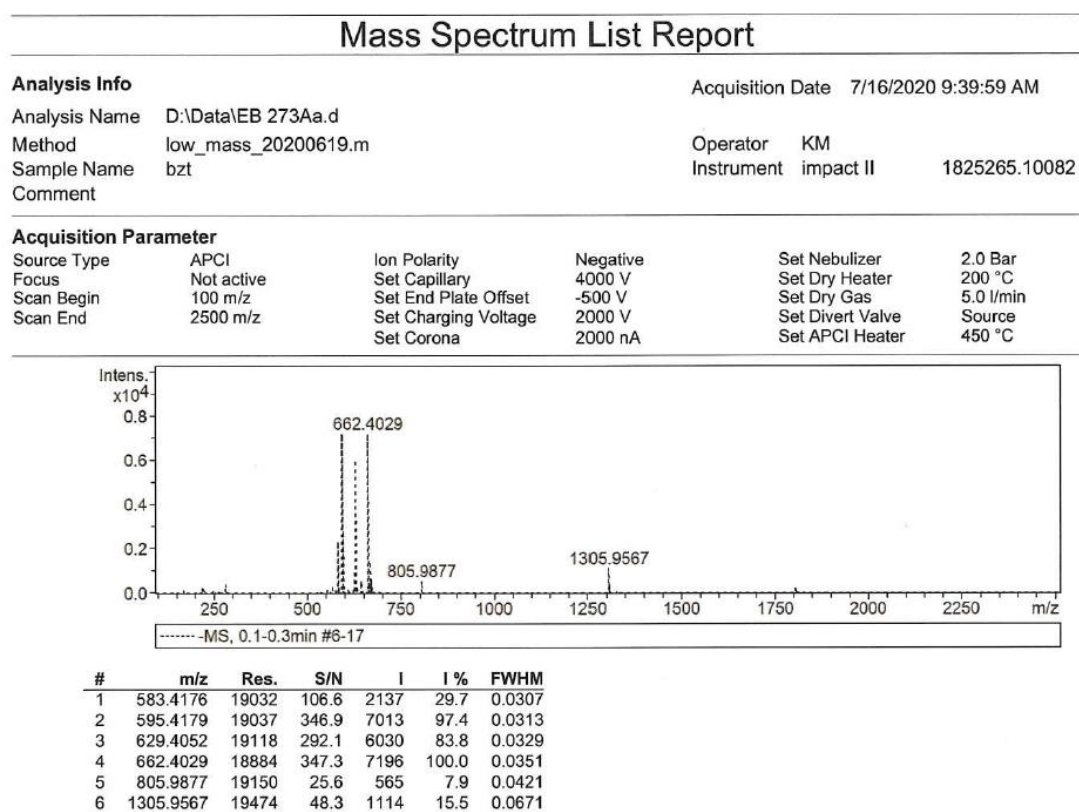


Figure S5. ¹H NMR, compound 7

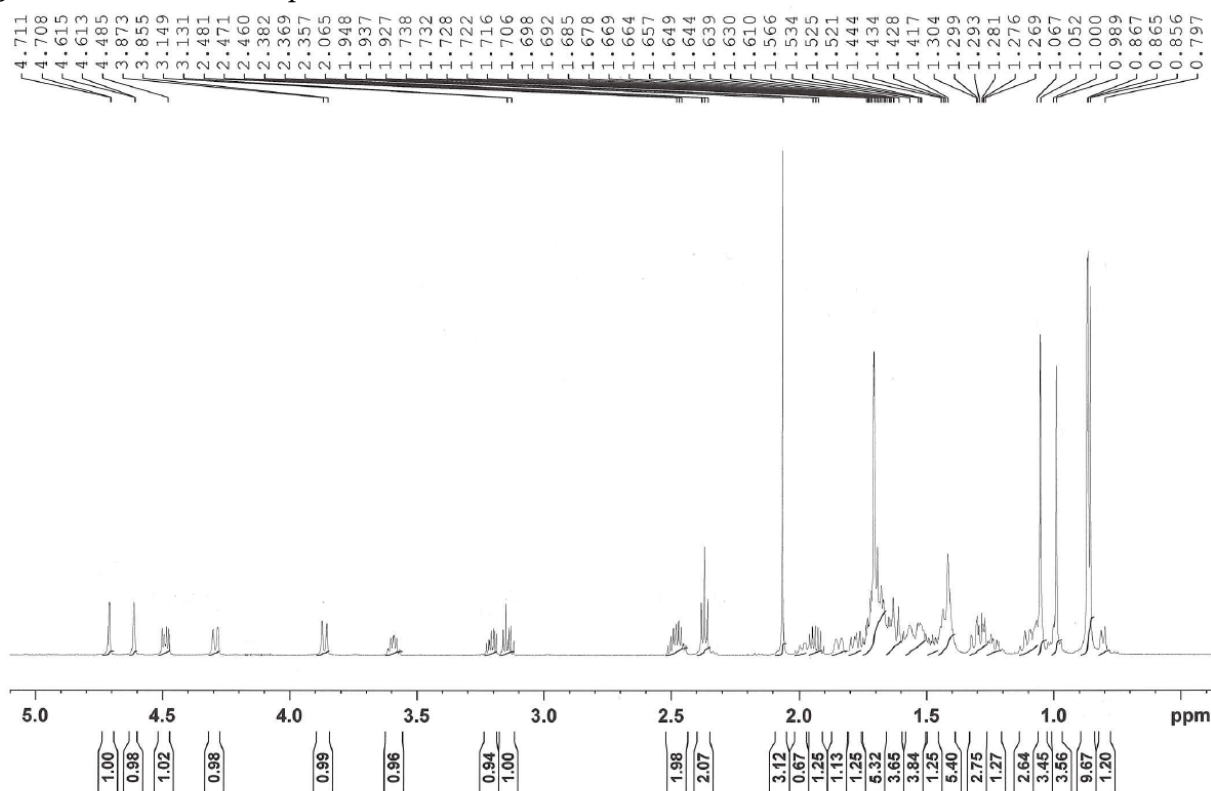


Figure S6. ^{13}C NMR, compound 7

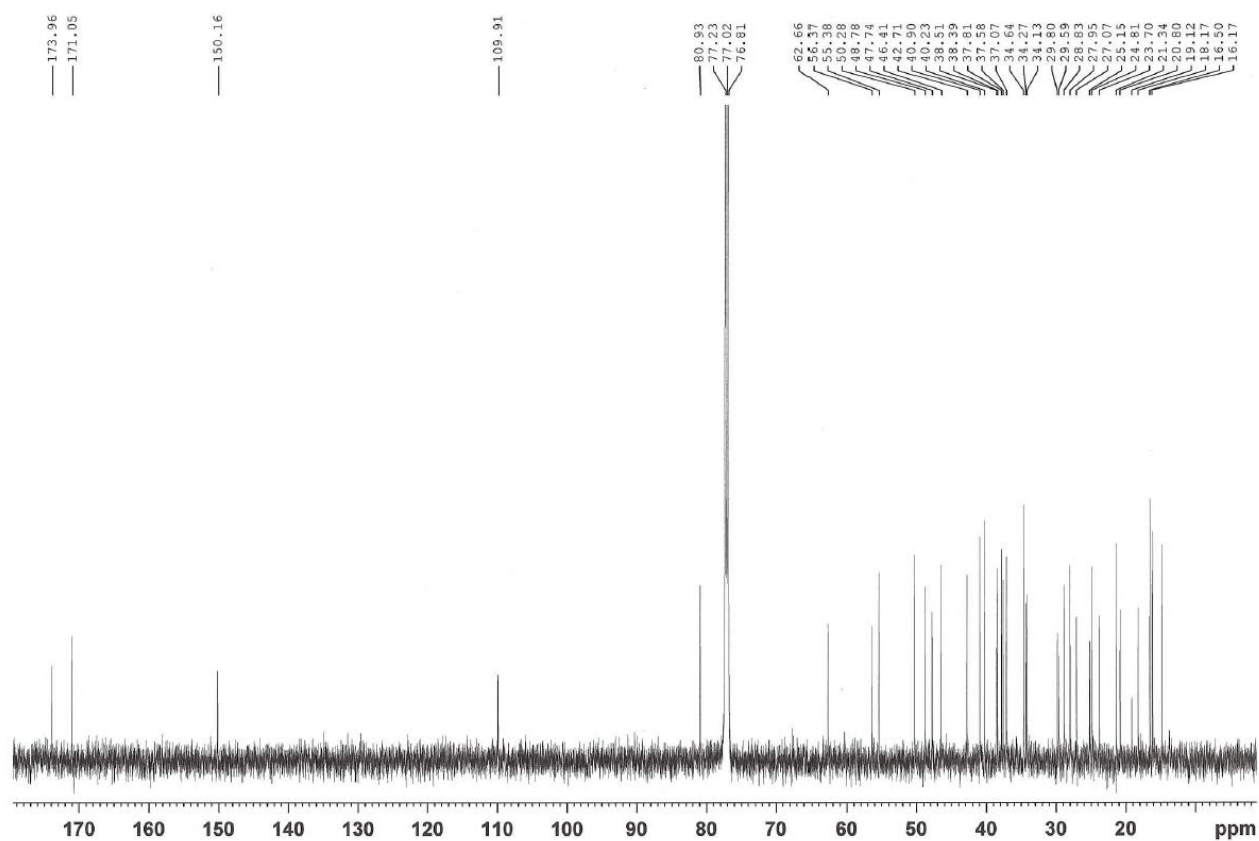


Figure S7. IR, compound 7

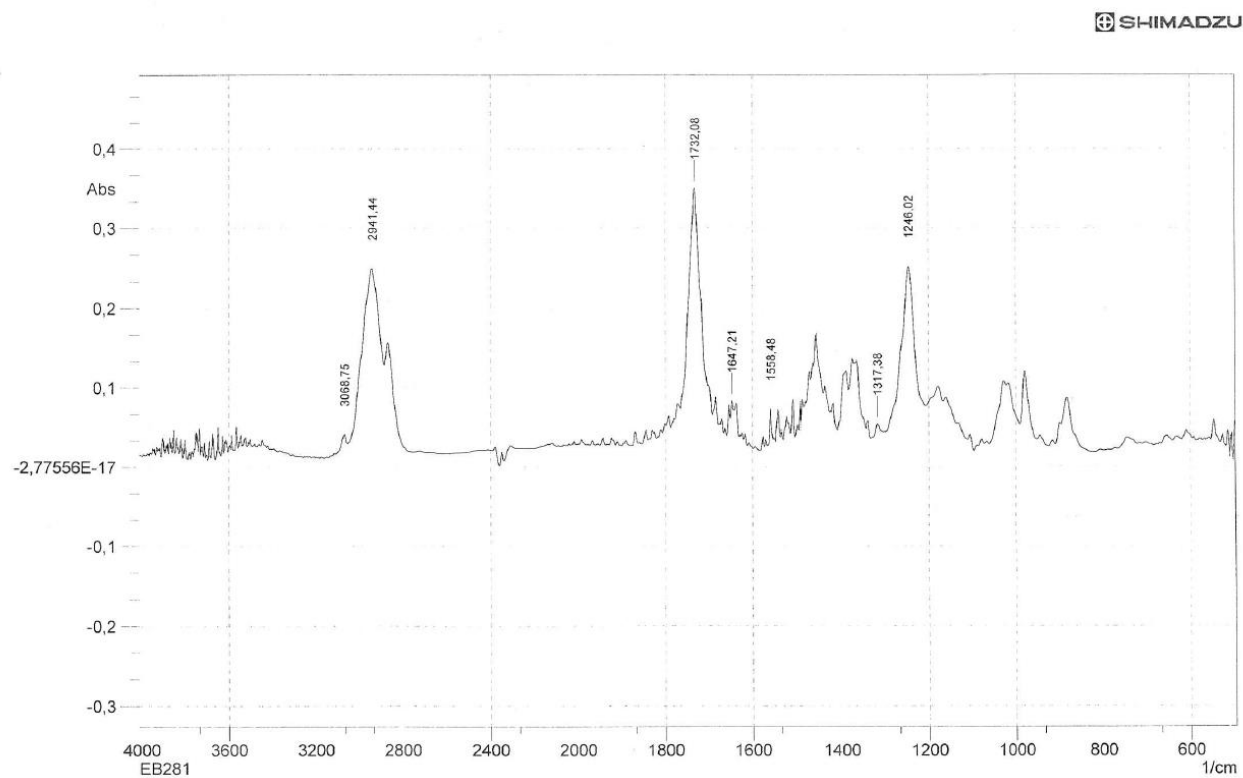


Figure S8. HRMS, compound 7

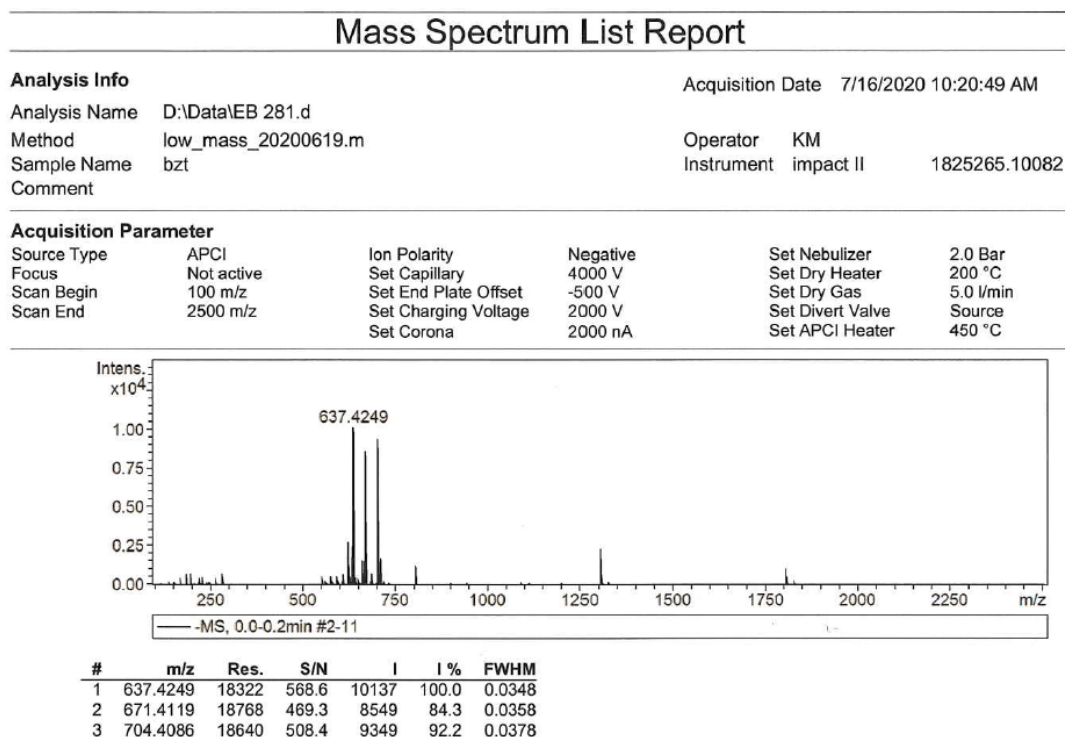


Figure S9. ¹H NMR, compound 8

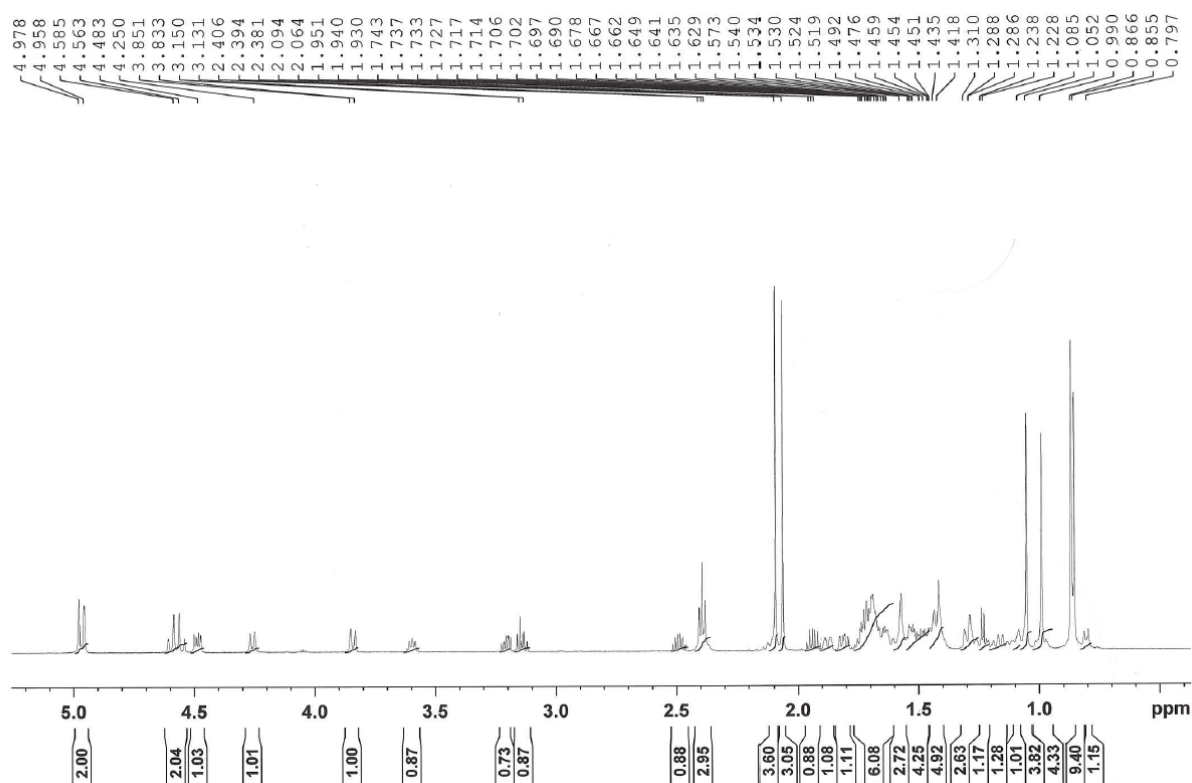


Figure S10. ^{13}C NMR, compound 8

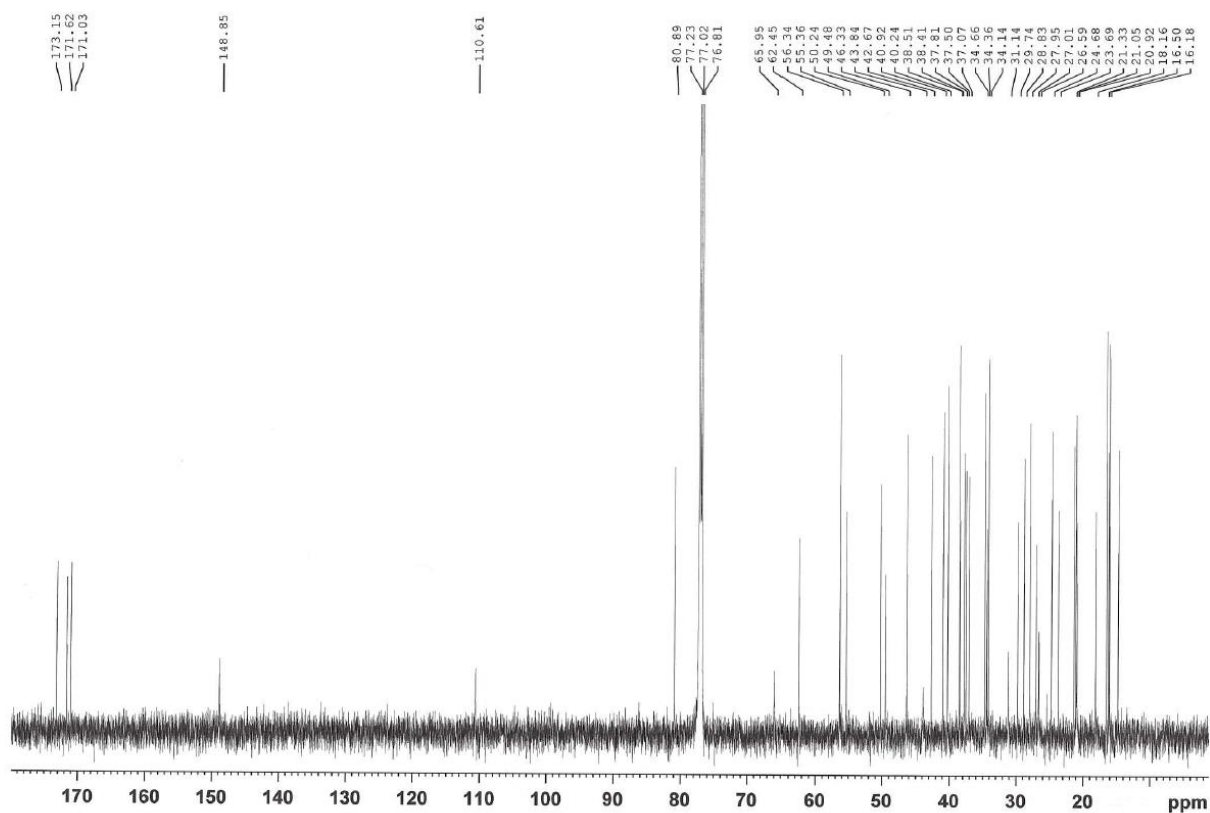


Figure S11. IR, compound 8

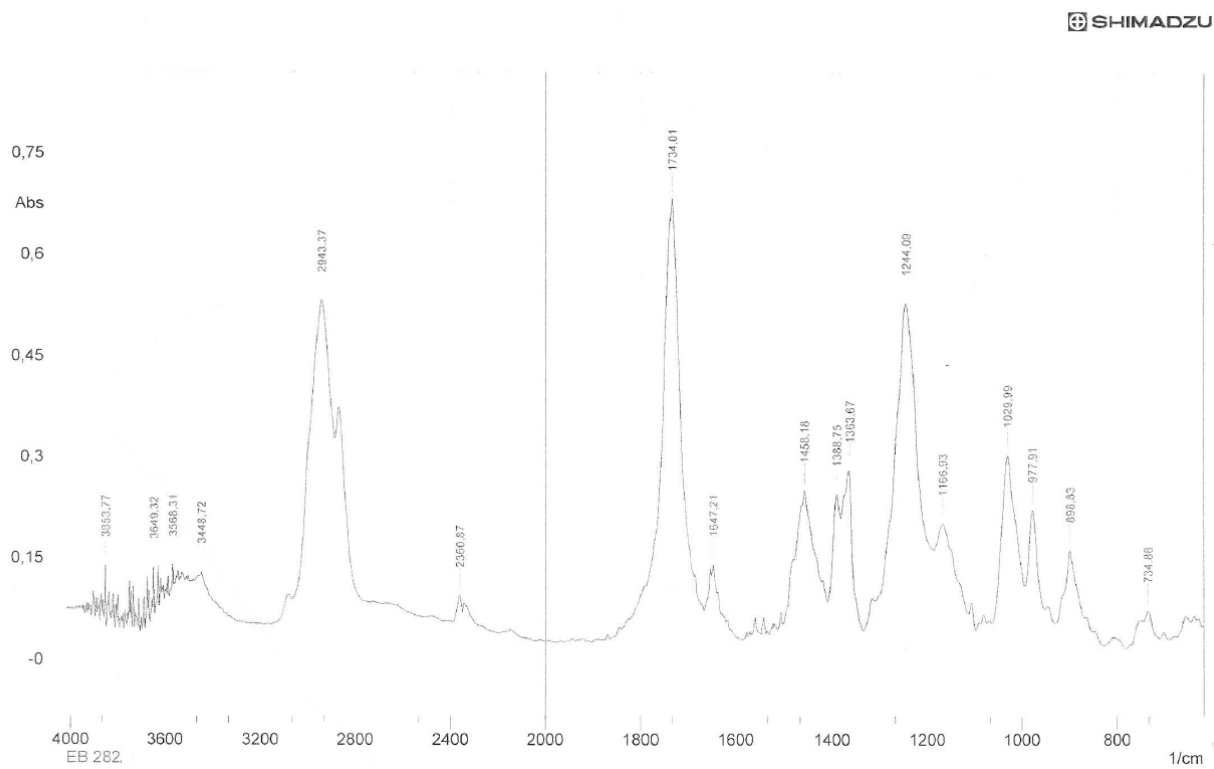


Figure S12. HRMS, compound 8

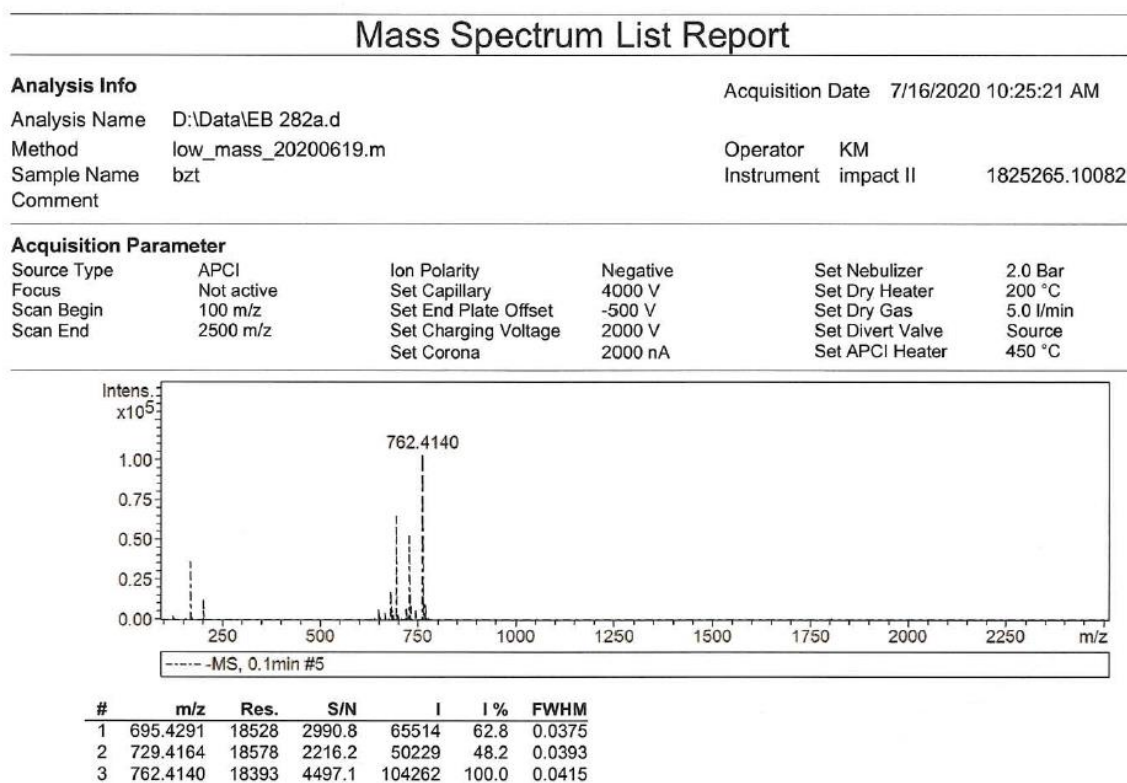


Figure S13. ¹H NMR, compound 9

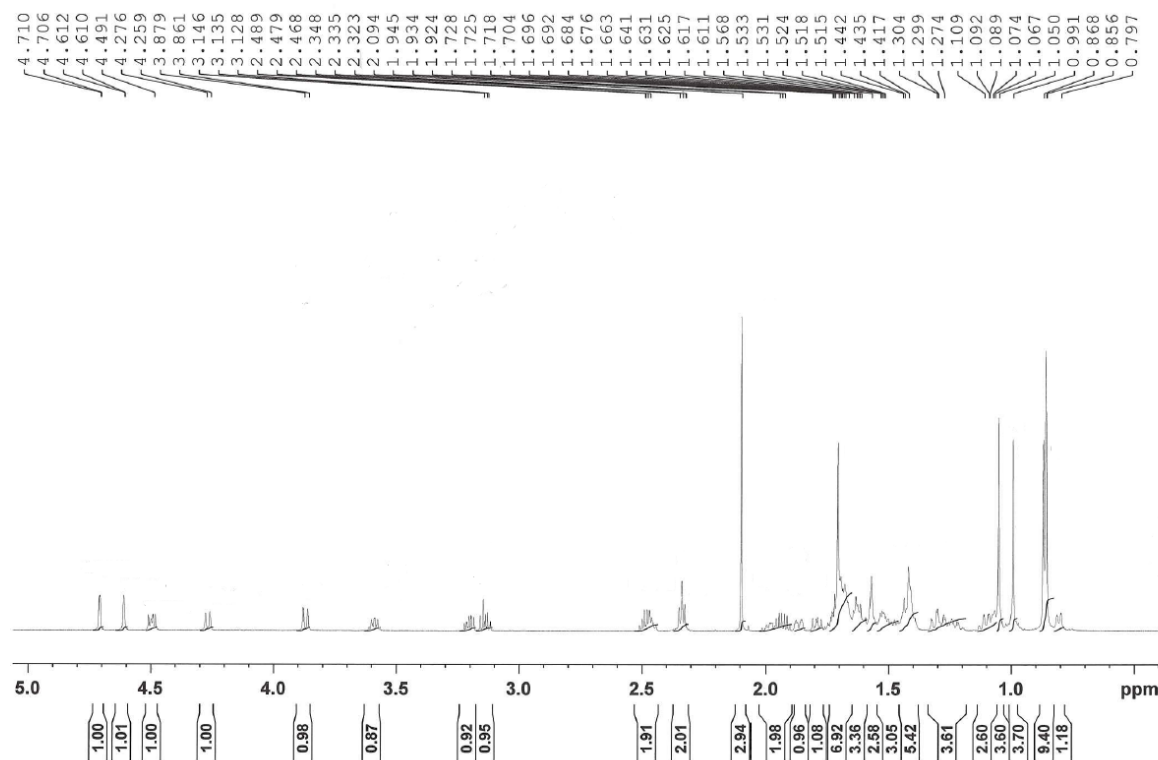


Figure S14. ^{13}C NMR, compound **9**

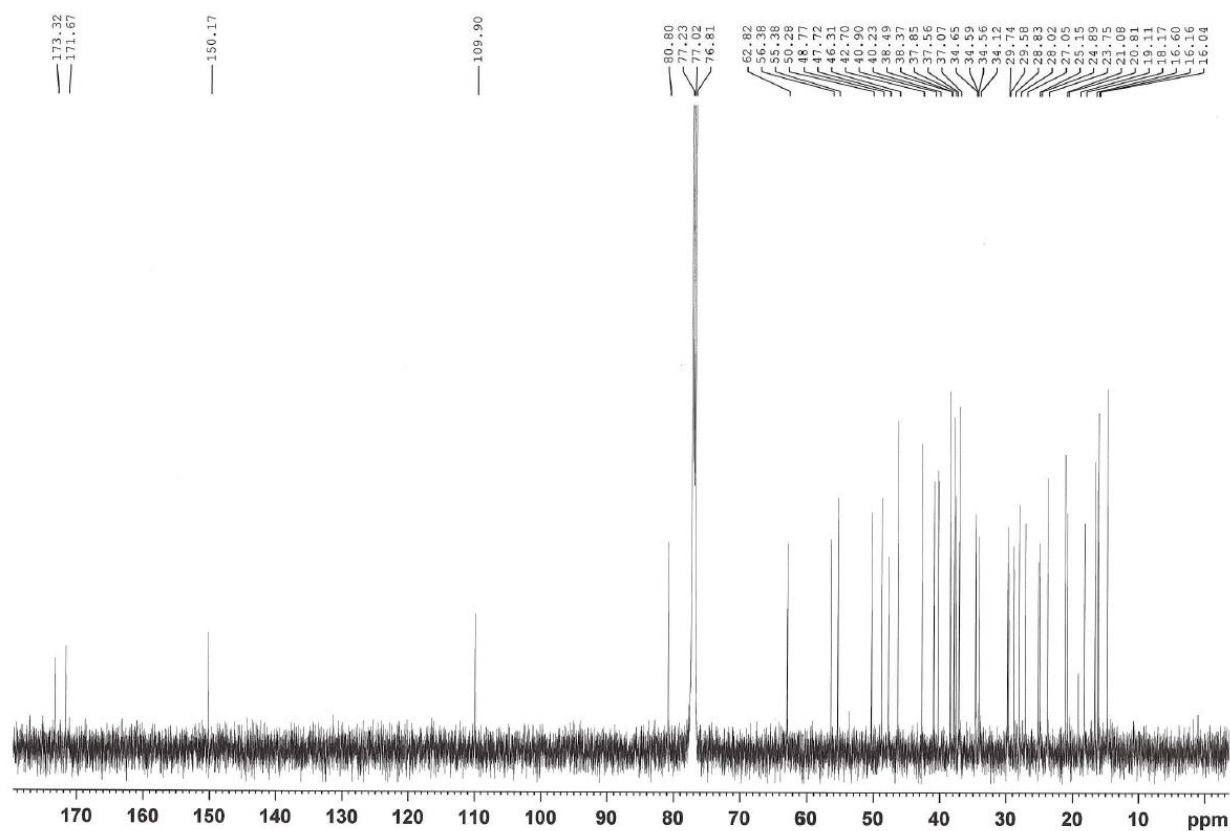


Figure S15. IR, compound **9**

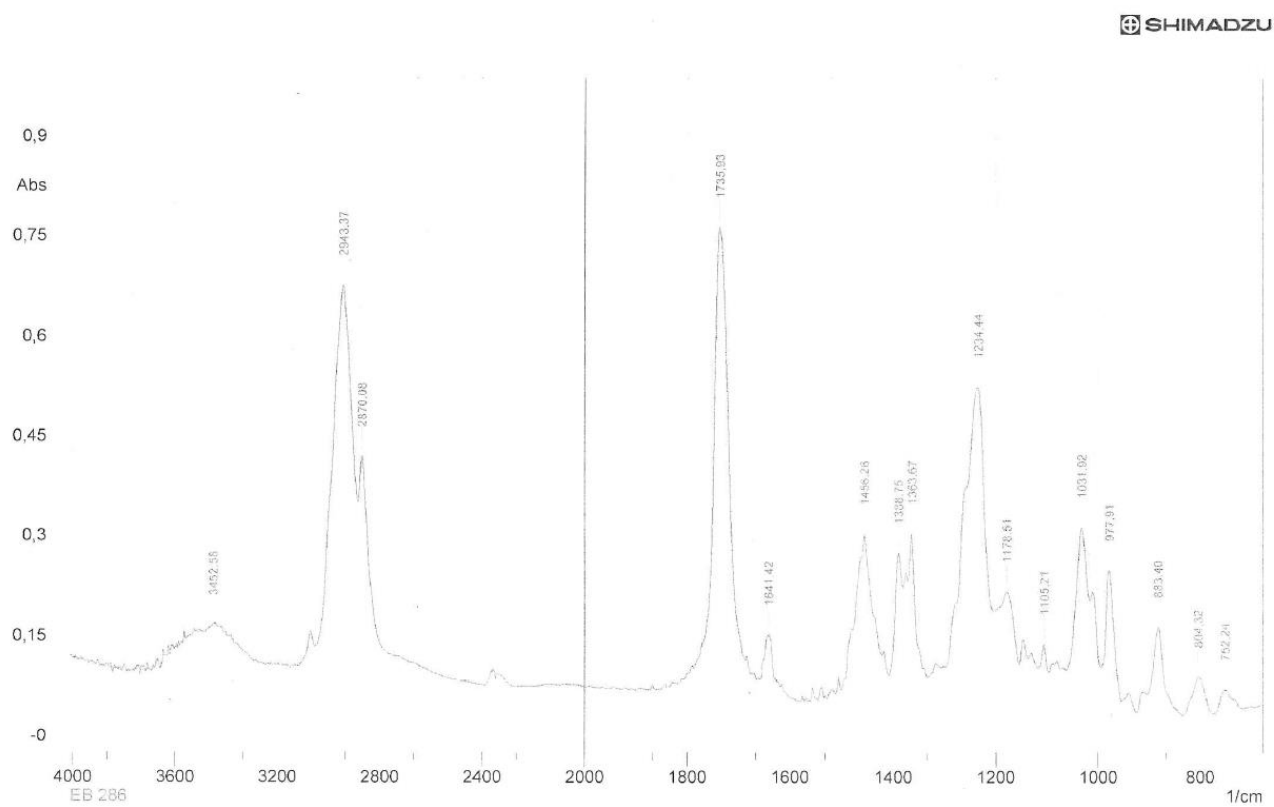


Figure S16. HRMS, compound 9

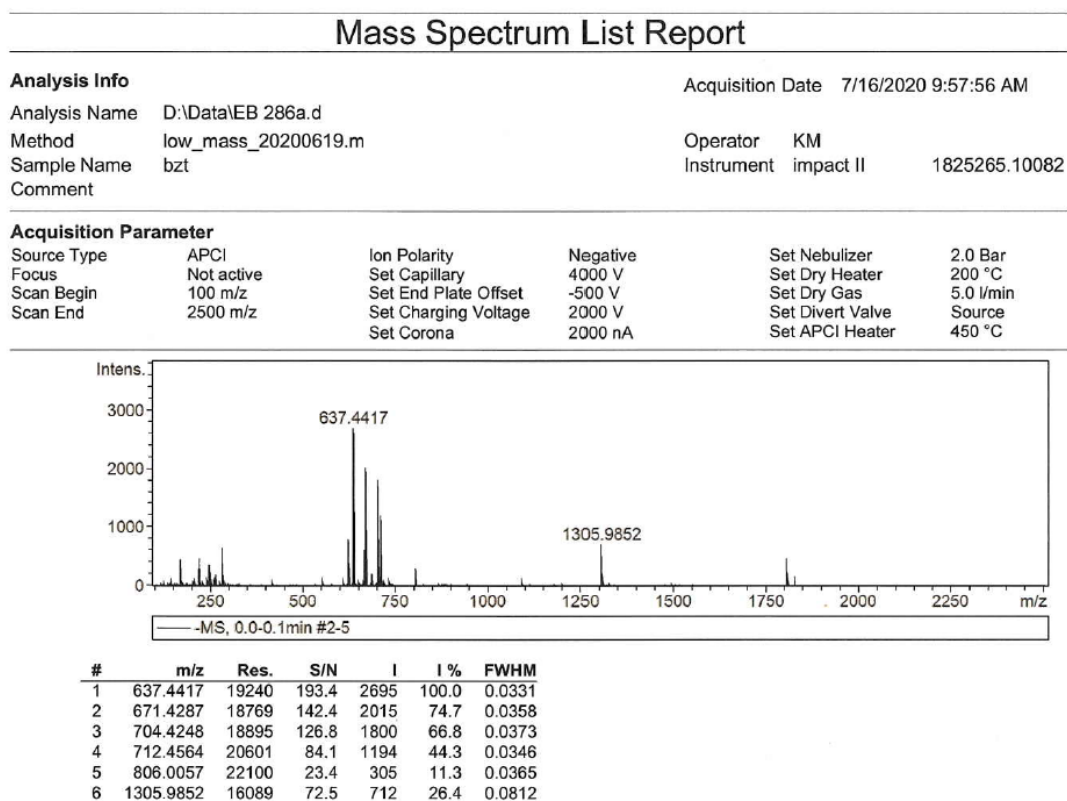


Figure S17. ¹H NMR, compound 10

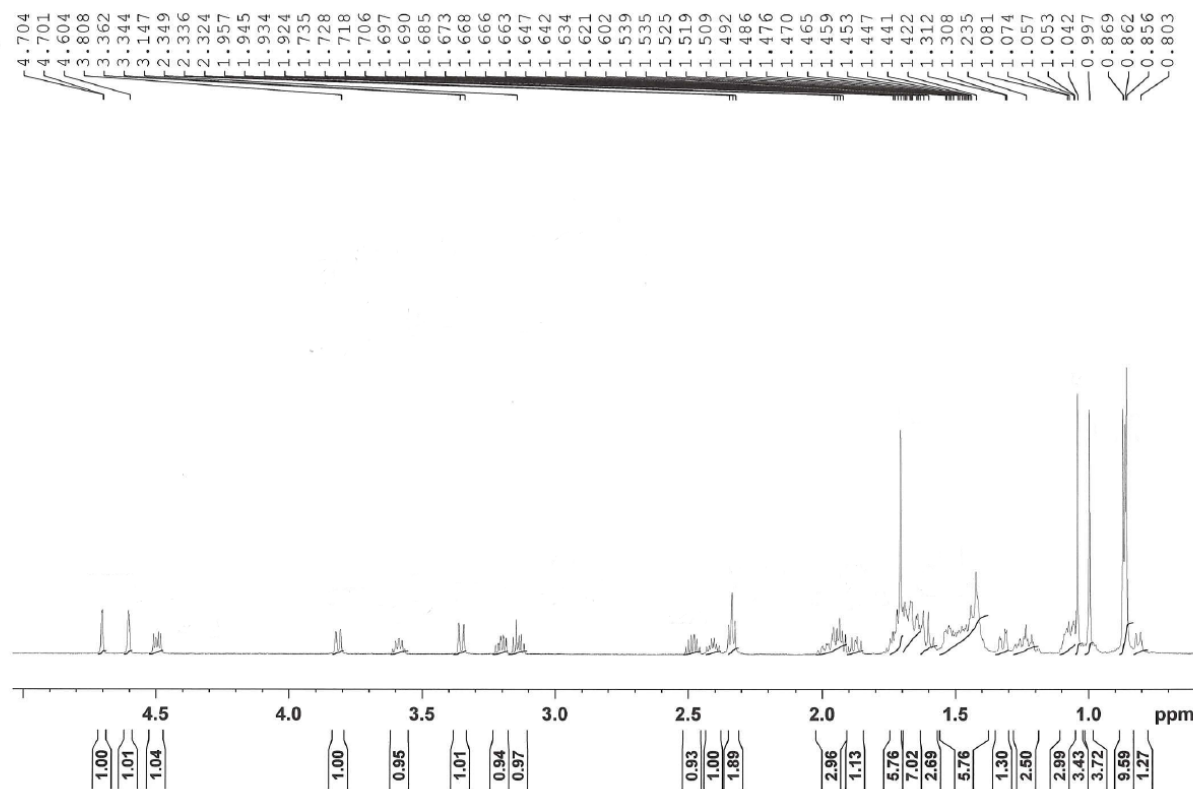


Figure S18. ^{13}C NMR, compound 10

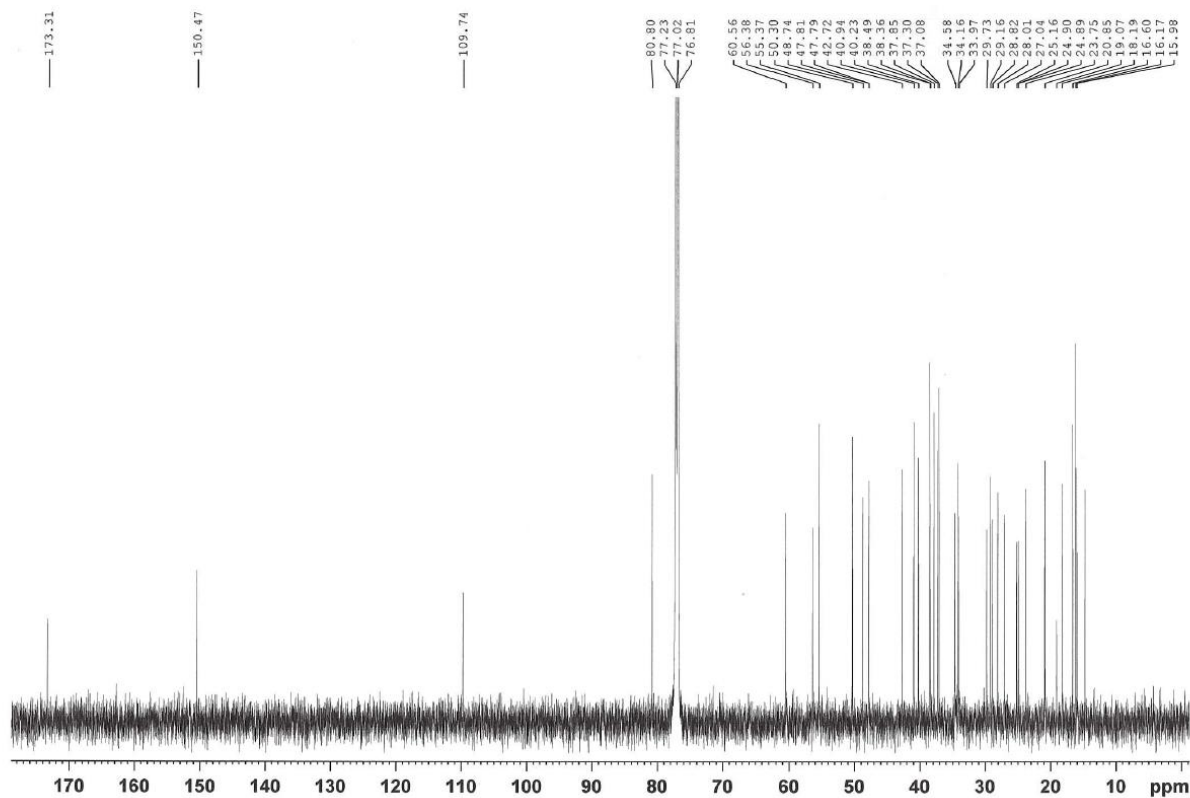


Figure S19. IR, compound 10

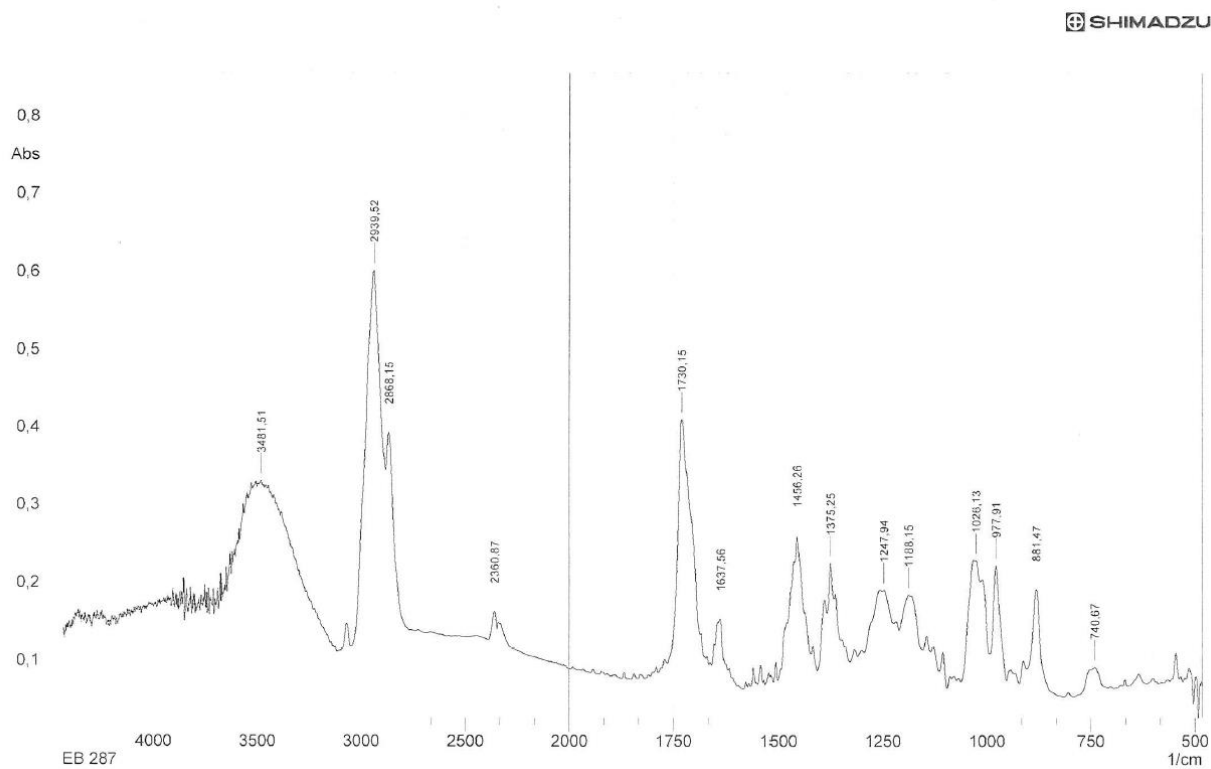
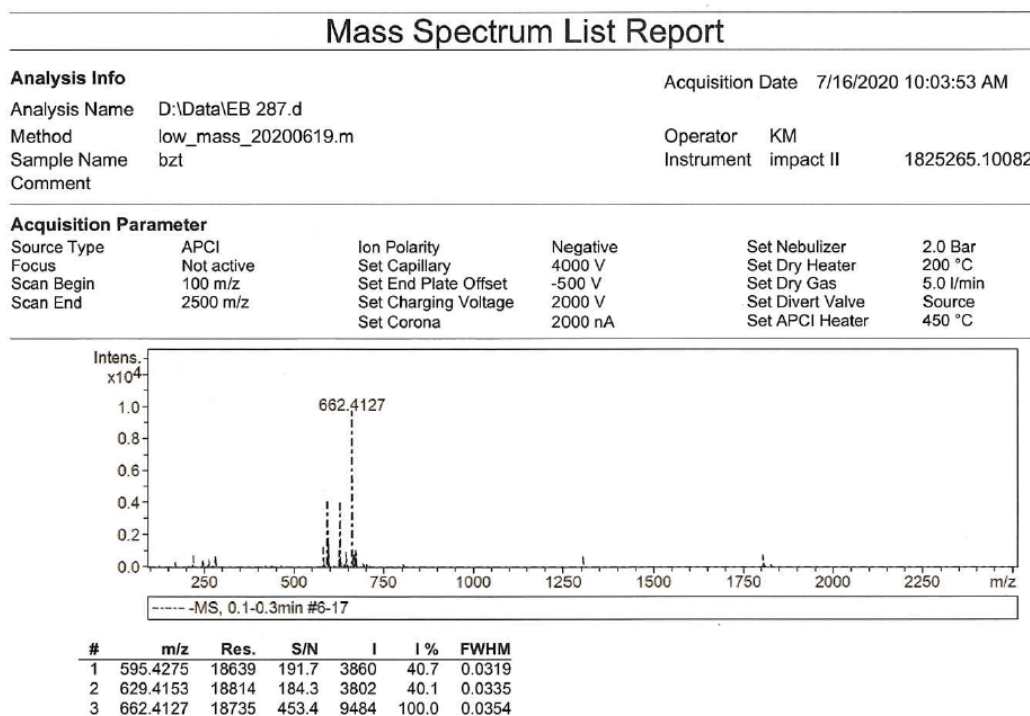


Figure S20. HRMS, compound 10



B. Lipophilicity

Table S1. Lipophilicity parameters of standard compounds; determined experimentally (R_{M0} ; mobile phase acetone:buffer Tris, pH 7.4) and literature values ($\log P_{lit}$)

Reference compound	R_{M0}	$\log P_{lit}$	$\log P_{TLC}$	b	r
acetanilide	0.53	1.21	1.21	-0.013	0.957
prednisone	0.62	1.62	1.31	-0.015	0.930
4-bromoacetophenone	1.87	2.43	2.76	-0.025	0.993
benzophenone	2.30	3.18	3.26	-0.031	0.994
anthracene	3.47	4.45	4.61	-0.041	0.994
dibenzyl	3.62	4.79	4.78	-0.043	0.996
DDT	4.80	6.38	6.15	-0.056	0.996

DDT- dichlorodiphenyltrichloroethane

b is the slope and r is the correlation coefficient for the linear relationship $R_M = R_{M0} + bC$

The below calibration curve equation was used to determine the experimental lipophilicity values ($\log P_{TLC}$) of the tested compounds:

$$\log P_{TLC} = 1.1572 R_{M0} + 0.5937 \quad (r = 0.993, SD=0.203)$$

Table S2. The correlation matrix for theoretically and experimentally obtained lipophilicity parameters of the tested betulin derivatives

	XLOGP2	MLOGP	ALOGPs	AC LogP	ALOGP	XLOGP3	LogP_{TLC}
XLOGP2	1.000	0.990	0.988	0.994	0.993	0.999	0.861
MLOGP	0.990	1.000	0.964	0.984	0.996	0.994	0.828
ALOGPs	0.988	0.964	1.000	0.993	0.980	0.984	0.833
AC LogP	0.994	0.984	0.993	1.000	0.995	0.994	0.814
ALOGP	0.993	0.996	0.980	0.995	1.000	0.996	0.811
XLOGP3	0.999	0.994	0.984	0.994	0.996	1.000	0.854
LogP_{TLC}	0.861	0.828	0.833	0.814	0.811	0.854	1.000

Table S3. Molecular descriptors for betulin and its derivatives **2-3** and **5-10**

Compound	MW	HA	HD	RB	TPSA	MR
betulin	442.72	2	2	2	40.46	136.30
2	484.75	3	1	4	46.53	146.04
3	542.79	5	1	7	72.83	156.94
5	484.75	3	1	4	46.53	146.04
6	631.03	3	1	9	97.13	187.95
7	673.06	4	0	11	103.20	197.69
8	731.10	6	0	14	129.50	208.59
9	673.06	4	0	11	103.20	197.69
10	631.03	3	1	9	97.13	187.95

MW-molecular weight (g/mol); HA-hydrogen bond acceptors; HD-hydrogen bond donors; RB-rotational bonds; TPSA- topological polar surface (Å²); MR-molar refractivity