Electronic Supplementary Information

Triterpenes from the fruits of *Rosa laevigata* with acetylcholinesterase and Aβ-aggregation inhibitory activities

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S.1. Materials and methods

S.1.1. General methods

Mass spectra were determined on a HRESI-MS: MicroTOF spectrometer (Bruker Daltonics, CA). NMR spectra were recorded on a Bruker ARX-500 spectrometer with TMS as internal standard in MeOD or dimethyl sulfoxide-d_6 (DMSO-d_6). Silica gel (100-200 mesh, Qingdao Marine Chemical Co., China); Polyamide (200-400 mesh, Qingdao Marine Chemical Co., China); Reversed-phase C_{18} silica gel (ODS 70–80 μm, Merck, Germany) were used for column chromatography and silica gel GF254 (Qingdao Marine Chemical Co., China) for TLC. 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), Huperzine A, Acetylthiocholine (ATCI), acetylcholinesterase (AChE), trolox, amyloid-β ($A\beta_{1\text{-}40}$), and thioflavin T (ThT) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Solvents were of industrial purity and distilled prior to use.

S.1.2. Plant material

Fruits of *R. laevigata* were collected from Nanning, Guangxi province, P. R. China, in August 2011. The voucher specimens (No. RLSY1108) are lodged in the Herbarium of Shenyang University of Chemical Technology, Shenyang 110142, P.R. China. The plant material was dried at 50 ^{o}C for 48 h in a hot air oven, and then reduced to powder using a grinder, and the powder was passed through a sieve (No. 60).

S.1.3. AChE inhibitory assay

The substrate acetylthiocholine was hydrolyzed by acetylcholinesterase to produce thiocholine, and then the thiocholine interacted with DTNB to give a yellow color. Using an Ellman assay, the half maximal inhibitory concentration (IC_{50}) was calculated for extracts and compounds **1-6** with a range of concentrations (2, 10, 30, 60, 100, 150, 200 μg mL^{-1}) [1,2] The each of the wells of 96-microtiter well plates was added 140 μL phosphate buffer (0.1M pH 7.4), 20 μL of different concentrations of sample, 15 μL acetylcholinesterase (0.4 U mL^{-1}), followed by incubating for 20 min at 4℃. Then, 10 μL ATCI and 20μL DTNB were added, followed by incubating for 30 min at

37℃. The absorbance of each sample (A_s) was measured at 405nm using a microplate reader. A blank positive control was set up by adding 20 μL Galanthamine instead of 20 μL sample solution. Blank groups contained all components except sample (A_0), the background group (A_b) contain all components except acetylcholinesterase, complete inhibition group was 100 μg mL^{-1} Huperzine-A (A_c) was used as the substitute sample. The inhibition rate (%) was calculated by the following equation:

$$\text{inhibition rate}\,(\%) = \frac{(A_0 - A_c) - (A_s - A_b)}{(A_0 - A_c)} \times 100\,\%$$

S.1.4. Self-mediated $A\beta_{1\text{-}40}$ aggregation assay

The thioflavin T (ThT) based fluorometric assay was established for measuring the self-mediated $A\beta_{1\text{-}40}$ aggregation.[3] Lyophilized $A\beta_{1\text{-}40}$ was dissolved in DMSO to obtain a 4 mM solution and then diluted into 250 μM solution with 0.1 M sodium phosphate buffer (pH 7.4). Aliquots (10 μL) of $A\beta_{1\text{-}40}$ were dissolved in 0.1 M sodium phosphate buffer (pH 7.4), to give a final concentration of 50 μM of $A\beta_{1\text{-}40}$. For co-incubation experiments,10 μL of the test compounds in 0.1 M sodium phosphate buffer pH 8.0 solution (final concentration 100 μM) were added into aliquots (10 μL) of $A\beta_{1\text{-}40}$ (final concentration 50μM). The reaction mixtures were incubated at room temperature for 24 h and 50 μL of ThT (100 μM) in 0.1 M sodium phosphate buffer (pH 7.4) was added. Fluorescence was monitored at 450 nm and emission at 485 nm using a a BIO-RAD microplate reader (Model 680, Thermo Fisher, Fermont, CA, USA). Each assay was run in triplicates along with trolox as reference agents. The fluorescence intensities in the presence and absence of inhibitors were compared using appropriate controls containing 1% DMSO and the percentage of inhibition was calculated using the equation: $100 - (IF_i/IF_o \times 100)$ where IF_i and IF_o are the fluorescence intensities obtained for $A\beta_{1\text{-}40}$ in the presence and absence of inhibitor, respectively.

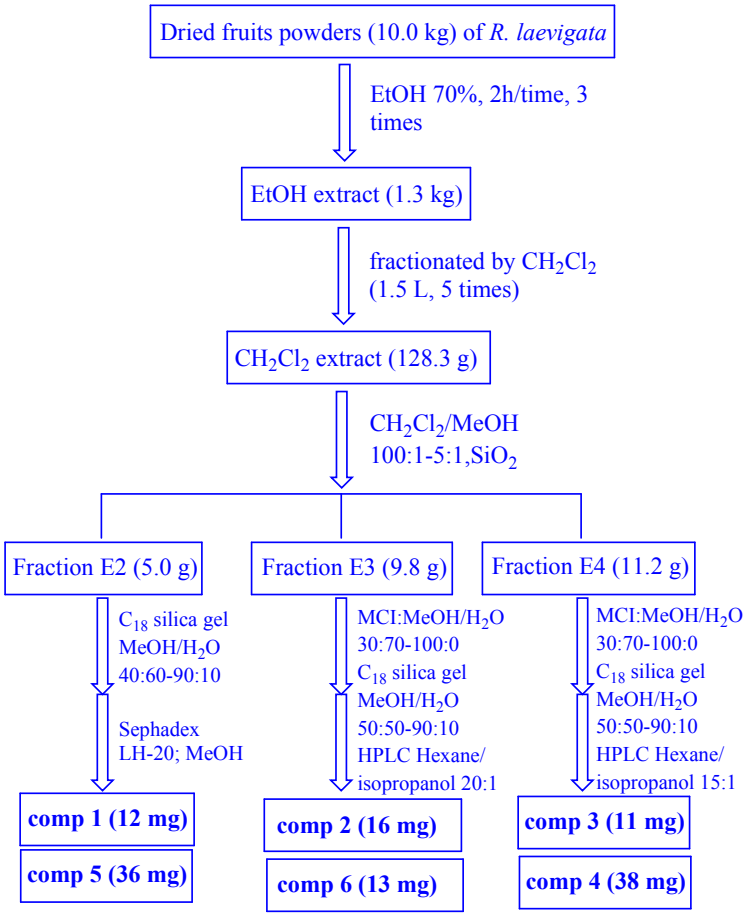
S.1.5. Molecular modeling (docking) studies

Docking of compounds **1** and **4** into the acetylcholinesterase was performed with Molegro virtual docker (Molegro).[4] The X-ray crystal structure of the enzyme AChE (PDB code 1W6R)[5]

was obtained from the RCSB Protein Data Bank. The co-crystal ligand fasciculin was extracted to define the binding site in docking. The structures of compounds were constructed and energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The docking experiment on AChE was carried out by superimposing the minimized ligand into the receptor. Ten runs were performed and five poses returned. All the other docking parameters were set as default. The pose for which the biotin entity of **1** and **4** binds to AChE in a similar way as observed in the original ligand-enzyme complex was exported and examined with Discovery Studio Visualizer 4.0 (Accelrys).

S.1.6. Extraction and isolation

Dried fruits powders (10.0 kg) of *R. laevigata*. were extracted with 70% ethanol (60 L) under reflux (80-85 ^{o}C) condition three times. The ethanol extract was concentrated *in vacuo* to yield a reddish-brown crude extract (1308 g, 13.1 %), which was dissolved in 1.5 L of water and then fractionated by dichloromethane (CH_2Cl_2) (1.5 L × 5), and CH_2Cl_2 extract (128.3 g, 1.28 %) fractions were obtained. Then the CH_2Cl_2-soluble extract (98.5 g) was subjected to purification by silica gel column using CH_2Cl_2–MeOH (100:1→ 5:1) solvent to gain fractions E1–E7, among which Fr. E2 (5.0 g) was purified by reversed-phase C_{18} silica gel using MeOH/H_2O with gradient solvent system of (60:40→ 90:10) to gain E2-1–10, sequentially by column chromatography (Sephadex LH-20; MeOH) to yield compounds **1** (12 mg) from E2-3 and **5** (36 mg) from E2-5. Frs. E3 (9.8 g) and E4 (11.2 g) were applied to a column of MCI gel (methanol/water 30:70→100:0) respectively to collect major fractions. the major fractions of Frs. E3 were then combined on the basis of TLC analysis, and finally performed by reversed-phase C_{18} silica gel using MeOH/H_2O with gradient solvent system of (50:50→ 90:10) and HPLC (Hexane/isopropanol 20:1) to obtain compounds **2** (16 mg), **6** (13 mg). In the same way, compounds **3** (11 mg), **4** (38 mg) were isolated from Frs. E4.



S.1.7. Characteristic data of compounds

Laevigaterpene A (**1**): colorless needles; HRESIMS *m/z*: $[M+Na]^+$ 495.3478 (calcd For $C_{30}H_{48}O_4Na^+$, 495.3445), see Table 1 for ^1H NMR, ^{13}C NMR.

Laevigaterpene B (**2**): colorless needles; HRESIMS *m/z*: 599.3719 $[M + Na]^+$ (calcd $C_{37}H_{52}O_5Na^+$,599.3707), see Table 1 for ^1H NMR, ^{13}C NMR.

3-*O*-*trans*-*p*-coumaroyl alphitolic acid (3), an amorphous white powder (MeOH), mp 201~203 °C. ESI-MS (m/z): 641 $[M+Na]^+$. ^1H-NMR (500M Hz, MeOD): δ_H 7.62 (1H, d, *J*=16 Hz, H-3′), 7.46 (2H, d, *J*=8.5 Hz, H-3″, H-5″), 6.80 (2H, d, *J*=8.5 Hz, H-2″, H-6”), 6.38 (1H, d, *J*=16 Hz, H-2′), 4.70 (1H, d, *J*=1.8 Hz H_2-30), 4.58 (1H, d, *J*=1.8 Hz, H_2-30), 4.77 (1H, m, H-2), 3.05 (1H, m, H-19), 3.96 (1H, d *J*=9.6 Hz, H-3), 1.70 (3H, s, H-29), 1.03 (3H, s, H-24), 0.98 (3H, s, H-27), 0.97 (3H, s, H-25), 0.92 (3H, s, H-23), 0.87 (3H, s, H-26). ^{13}C-NMR (125 Hz, MeOD): δ_C 49.4 (C-1), 67.8 (C-2), 85.5 (C-3), 39.5 (C-4), 55.4 (C-5), 18.1 (C-6), 35.4 (C-7), 41.9 (C-8), 50.4 (C-9), 39.4 (C-10), 22.2

(C-11), 26.7 (C-12), 40.6 (C-13), 43.6 (C-14), 30.7 (C-15), 33.3 (C-16), 56.5 (C-17), 51.8 (C-18), 48.6 (C-19), 151.9 (C-20), 31.7 (C-21), 39.6 (C-22), 29.0 (C-23), 17.9 (C-24), 19.4 (C-25), 16.6 (C-26), 15.0 (C-27), 180.6 (C-28), 110.1 (C-29), 19.5 (C-30), 169.6 (C-1′), 115.7 (C-2′), 146.2 (C-3′), 127.3 (C-1″), 131.1 (C-2″), 116.8 (C-3″), 161.1 (C-4″), 116.8 (C-5″), 131.1 (C-6″).

3-*O*-*cis*-*p*-coumaroyl alphitolic acid (4), an amorphous white powder (MeOH), mp 208~210 °C. ESI-MS (m/z): 641 $[M+Na]^+$. ^{1}H-NMR (500M Hz, MeOD): δ_H 7.54 (2H, d, *J*=8.5 Hz, H-3″, H-5″), 6.77 (1H, d, *J*=12.5 Hz, H-3′), 6.63 (2H, d, *J*=8.5 Hz, H-2″, H-6″), 5.74 (1H, d, *J*=12.5 Hz, H-2′), 4.70 (1H, d, *J*=1.8 Hz H_2-30), 4.58 (1H, d, *J*=1.8 Hz, H_2-30), 4.77 (1H, m, H-2), 3.05 (1H, m, H-19), 3.96 (1H, d *J*=9.6 Hz, H-3), 1.60 (3H, s, H-29), 1.09 (3H, s, H-24), 0.93 (3H, s, H-27), 0.87 (3H, s, H-25), 0.84 (3H, s, H-23), 0.75 (3H, s, H-26). ^{13}C-NMR (125 Hz, MeOD): δ_C 49.2 (C-1), 67.8 (C-2), 85.2 (C-3), 40.5 (C-4), 56.6 (C-5), 19.4 (C-6), 35.3 (C-7), 41.9 (C-8), 50.4 (C-9), 39.4 (C-10), 22.2 (C-11), 26.8 (C-12), 39.6 (C-13), 43.6 (C-14), 30.7 (C-15), 33.3 (C-16), 57.5 (C-17), 51.8 (C-18), 49.5 (C-19), 151.9 (C-20), 31.6 (C-21), 38.1 (C-22), 29.1 (C-23), 17.8 (C-24), 17.9 (C-25), 16.6 (C-26), 15.1 (C-27), 180.0 (C-28), 110.2 (C-29), 19.6 (C-30), 168.5 (C-1′), 117.4 (C-2′), 144.6 (C-3′), 127.8 (C-1″), 133.6 (C-2″), 115.7 (C-3″), 159.9 (C-4″), 115.7 (C-5″), 133.6 (C-6″).

3-*O*-*trans*-*p*-coumaroyl maslinic acid (5), an amorphous white powder (MeOH), mp 278~281 °C. ESI-MS (m/z): 618 $[M]^+$. ^{1}H-NMR (500M Hz, MeOD): δ_H 7.65 (1H, d, *J*=16 Hz, H-3′), 7.48 (2H, d, *J*=8.5 Hz, H-3″, H-5″), 6.83 (2H, d, *J*=8.5 Hz, H-2″, H-6″), 6.41 (1H, d, *J*=16 Hz, H-2′), 5.28 (1H, t, *J*=3.5 Hz), 3.65 (1H, td, *J*=9.5 Hz), 2.90 (1H, d, *J*=9.5 Hz), 1.21 (3H, s), 1.08 (3H, s), 0.97 (3H, s), 0.96 (3H, s), 0.93 (3H, s), 0.92 (3H, s), 0.86 (3H, s). ^{13}C-NMR (125 Hz, MeOD): δ_C 49.5 (C-1), 67.6 (C-2), 85.5 (C-3), 42.7(C-4), 56.5 (C-5), 19.4 (C-6), 33.8 (C-7), 40.6 (C-8), 48.5 (C-9), 39.2 (C-10), 24.1 (C-11), 123.3 (C-12), 145.4 (C-13), 42.9 (C-14), 28.8 (C-15), 24.6 (C-16), 47.6 (C-17), 42.9 (C-18), 47.4 (C-19), 31.6 (C-20), 34.9 (C-21), 33.8 (C-22), 29.2 (C-23), 18.3 (C-24), 17.1 (C-25), 17.7 (C-26), 26.4 (C-27), 180.1 (C-28), 33.5 (C-29), 24.0 (C-30), 169.6 (C-1′), 115.8 (C-2′), 146.2 (C-3′), 127.3 (C-1″), 131.1 (C-2″), 116.9 (C-3″), 161.2 (C-4″), 116.9 (C-5″), 131.1 (C-6″)。

3-*O*-*cis*-*p*-coumaroyl maslinic acid (6), an amorphous white powder (MeOH), mp 283~285 °C.

ESI-MS (m/z): 618 $[M]^+$. ^{1}H-NMR (500M Hz, MeOD): δ_H δ7.56 (2H, d, *J*=8.5 Hz, H-3″, H-5″), 6.79 (1H, d, *J*=12.5 Hz, H-3′), 6.65 (2H, d, *J*=8.5 Hz, H-2″, H-6″), 5.76 (1H, d, *J*=12.5 Hz, H-2′), 5.24 (1H, t, *J*=3.5 Hz), 3.61 (1H, td, *J*=9.5 Hz), 2.90 (1H, d, *J*=9.5 Hz), 1.19 (3H, s), 1.06 (3H, s), 0.96 (3H, s), 0.95 (3H, s), 0.93 (3H, s), 0.91 (3H, s), 0.85 (3H, s). ^{13}C-NMR (125 Hz, MeOD): δ_C 49.5 (C-1), 67.6 (C-2), 85.2 (C-3), 42.7(C-4), 56.5 (C-5), 19.5 (C-6), 33.8 (C-7), 40.6 (C-8), 48.5 (C-9), 39.2 (C-10), 24.0 (C-11), 123.3 (C-12), 145.5 (C-13), 42.9 (C-14), 28.9 (C-15), 24.6 (C-16), 47.6 (C-17), 42.9 (C-18), 47.3 (C-19), 31.6 (C-20), 34.9 (C-21), 33.8 (C-22), 29.2 (C-23), 18.2 (C-24), 17.1 (C-25), 17.7 (C-26), 26.4 (C-27), 180.1 (C-28), 33.6 (C-29), 24.0 (C-30), 168.6 (C-1′), 117.4 (C-2′), 144.7 (C-3′), 127.8 (C-1″), 133.6 (C-2″), 115.7 (C-3″), 159.9 (C-4″), 115.7 (C-5″), 133.6 (C-6″)。

*S.1.8. Synthesis of benzoyl ester (**1a**) of Laevigaterpene A (**1**).*

A sample of laevigaterpene A (**1**, 3.0 mg) was mixed with 200 μL of benzoyl chloride and 300 μL of CH_2Cl_2. The mixture was stirred at room temperature for 12 h. Four milliliters of H_2O was added, and the resulting solution was extracted with CH_2Cl_2 (3 × 2 mL). The combined organic phase was then washed successively with 25% aqueous Na_2CO_3 (3 × 1 mL) and H_2O (3 × 1 mL) and dried over Na_2SO_4. The residue obtained upon evaporation was subjected to HPLC purification with CH_3OH-H_2O (3:1) elution at 2 mL/min and UV detection at 220 nm to afford the 2-*O*-benzoyl derivative of **1**. The products were identified by MS and ^{1}H NMR analysis.

HRESIMS *m/z*: 599.3735 $[M + Na]^+$ (calcd $C_{37}H_{52}O_5Na^+$,599.3707).

S.1.9. Quantum chemical ECD calculation.

Initial structures were constructed based on information of NOESY correlations, and conformational analysis was performed by using the MMFF molecular mechanics force field. Further optimization of the structures in methanol was performed using the Gaussian 09 program at B3LYP/6-31 G (d, p). The optimized structures were shown in table S1 and S2. Optimization was confirmed by computation of frequency. Conformational distribution of the optimized structures was investigated at PM3 level and suggested the major conformers (> 95%). ECD spectra of

different conformers were simulated using a Gaussian function. The overall theoretical ECD spectra were obtained according to the Boltzmann weighting of each conformers. After hypsochromic-shifting 20 nm by UV correction, in the 200-400 nm region, the theoretically calculated ECD spectra of **1b** and **2a** were in good agreement with the experimental ECD spectra of **1** and **2** (Figure 4).

S.1.10. SH-SY5Y neuroblastoma cell toxicity studies

The SH-SY5Y neuroblastoma cells were plated at a density of 1×10^4 cells/well in 200 μL of complete media consisting of DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C with 5% CO_2. The cells were incubated overnight before treatment with 100 μL of test sample solutions and select controls (galanthamine, Sigma–Aldrich Chemical Co. St. Louis, MO, USA) at various concentrations (0-200 μM/mL) in 2% DMSO. Cell viability was determined colorimetrically using MTT assay.[6] Then 20 μL of the MTT solution was added into each well. After incubation for 4 h at 37 °C, the cells were finally lysed with 150 μL of DMSO. The absorbance was measured at 490 nm with a microplate reader (ELX 800, Bio-tek, USA). The value for cell viability was expressed as the percentage of the control value. The results were expressed as a percent reduction of MTT relative to untreated controls that included 2% DMSO and the average absorbance value for each treatment was subtracted with the absorbance reading of wells containing only media, MTT reagent, and detergent reagent.

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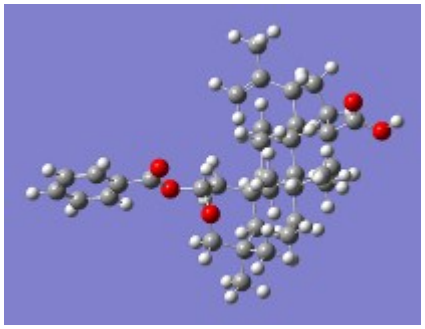
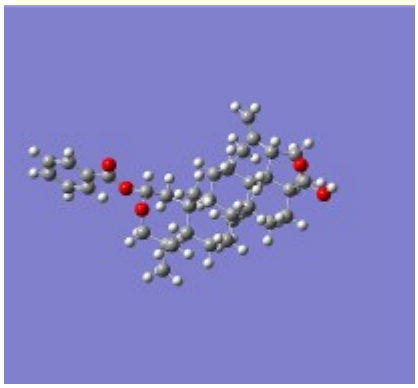
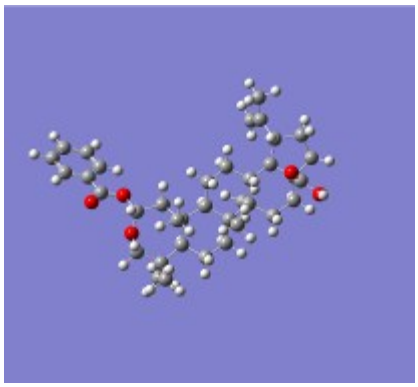
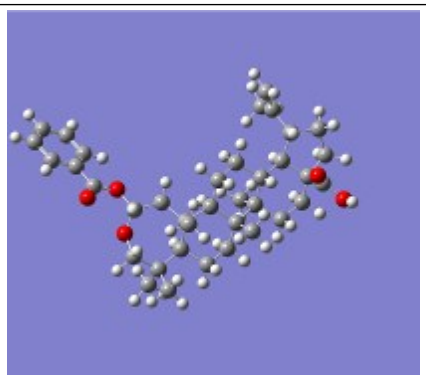
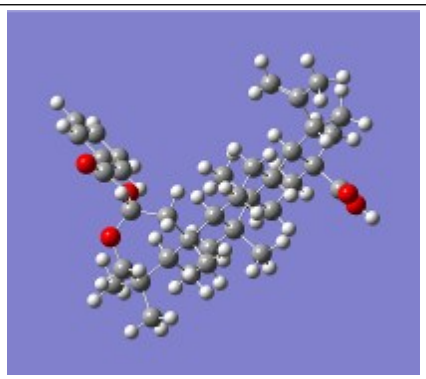
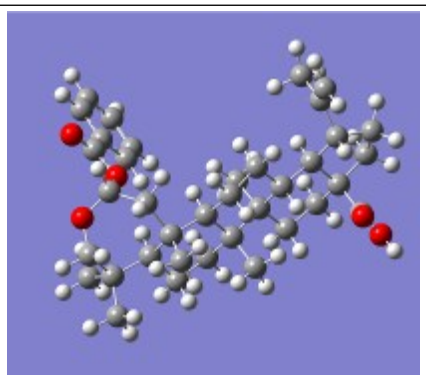
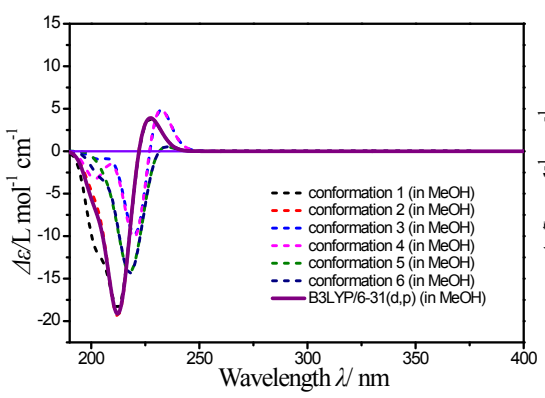
Table S1. Conformations of **1b** were Obtained after the Optimization.

no.	conformer	population (%)
1b-1		17.94
1b-2		81.58
1b-3		0.09
1b-4		0.38

1b-5		0.00
1b-6		0.01



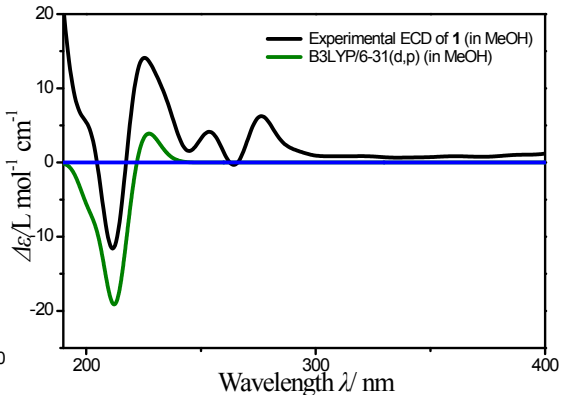


Table S2. Conformations of **2a** were Obtained after the Optimization.

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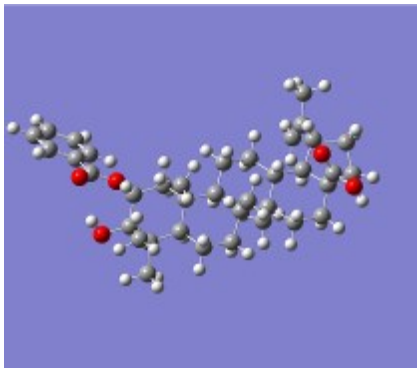
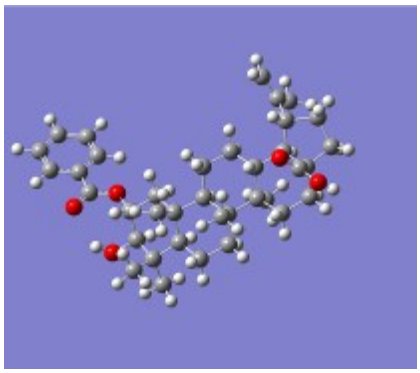
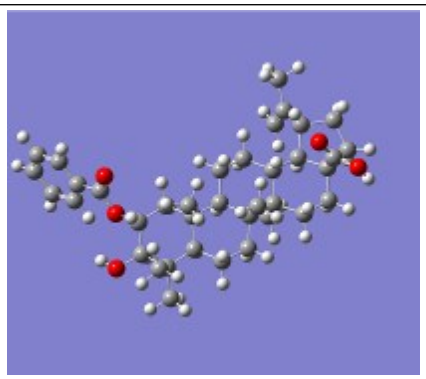
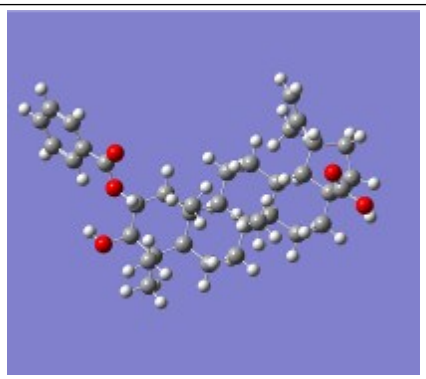
2a-1		11.73
2a-2		56.62
2a-3		5.52
2a-4		26.13

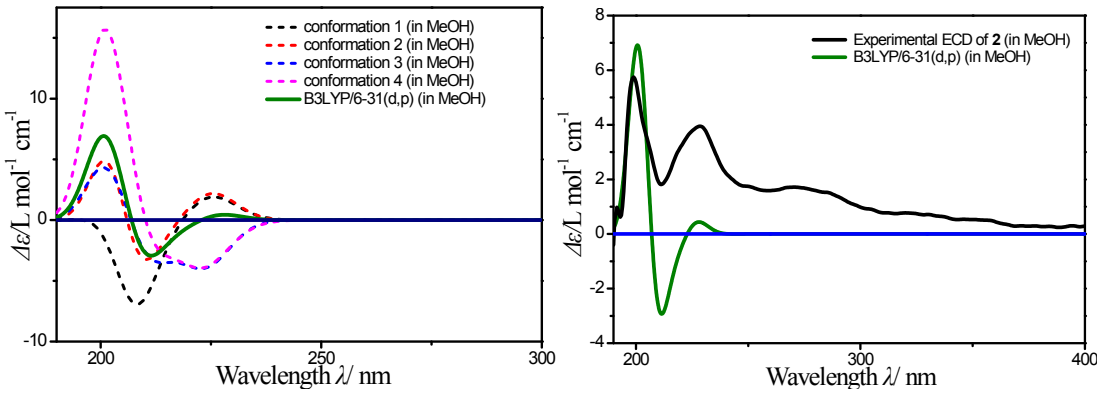

conformation 1 (in MeOH)
conformation 2 (in MeOH)
conformation 3 (in MeOH)
conformation 4 (in MeOH)
B3LYP/6-31(d,p) (in MeOH)
Δε/L mol-1 cm-1
Wavelength λ/ nm
Experimental ECD of 2 (in MeOH)
B3LYP/6-31(d,p) (in MeOH)
Δε/L mol-1 cm-1
Wavelength λ/ nm

Table S3. AChE inhibition activity of compounds **1-6**, positive control and different fractions.

Experiment	**1**	**2**	**3**	**4**	**5**	**6**	**CH_2Cl_2 fraction**	**EtOH fraction**	**Galant-hamine**
AChE (IC_{50}, μg/mL)	14.4	27.5	36.1	15.2	28.5	25.2	26.4	36.3	3.6 μM (reference 6)
the maximal inhibition ratio %	90.4	94.1	89.4	91.9	85.5	91.5	87.5	78.3	--

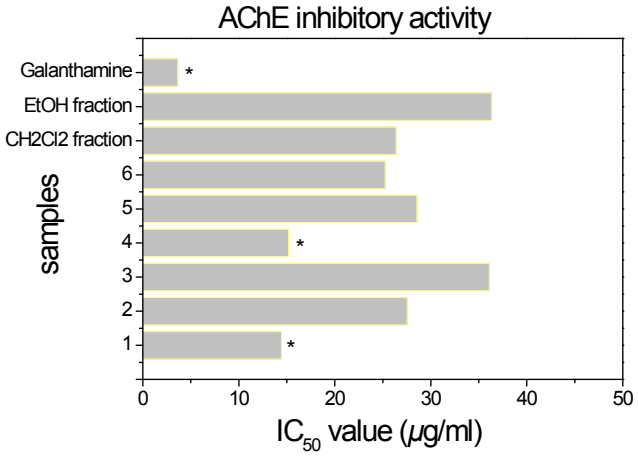


Table S4. Inhibition of self-mediated aggregation of $A\beta_{1-40}$ by compounds **1-6**

Experiment	**1**	**2**	**3**	**4**	**5**	**6**	**Trolox**
Inhibition at 100 μM (%)	--	65.2 ± 10	--	--	--	--	50.5 ± 5.9

Table S5. MTT reduction cytotoxicity assay in SH-SY5Y neuroblastoma cells of compounds **1-6**.

Experiment	**1**	**2**	**3**	**4**	**5**	**6**	**Galant-hamine**
Cell Viability at 40μM $\pm$ SEM (%)	75 %	98 %	80 %	85 %	96 %	97 %	96 %
Cytotoxicity (IC_{50}, μg/mL)	>100	>100	55.9	75.0	>100	>100	>100

Figure.1. ^{1}H NMR spectrum (500MHz, MeOD) of compound **1**

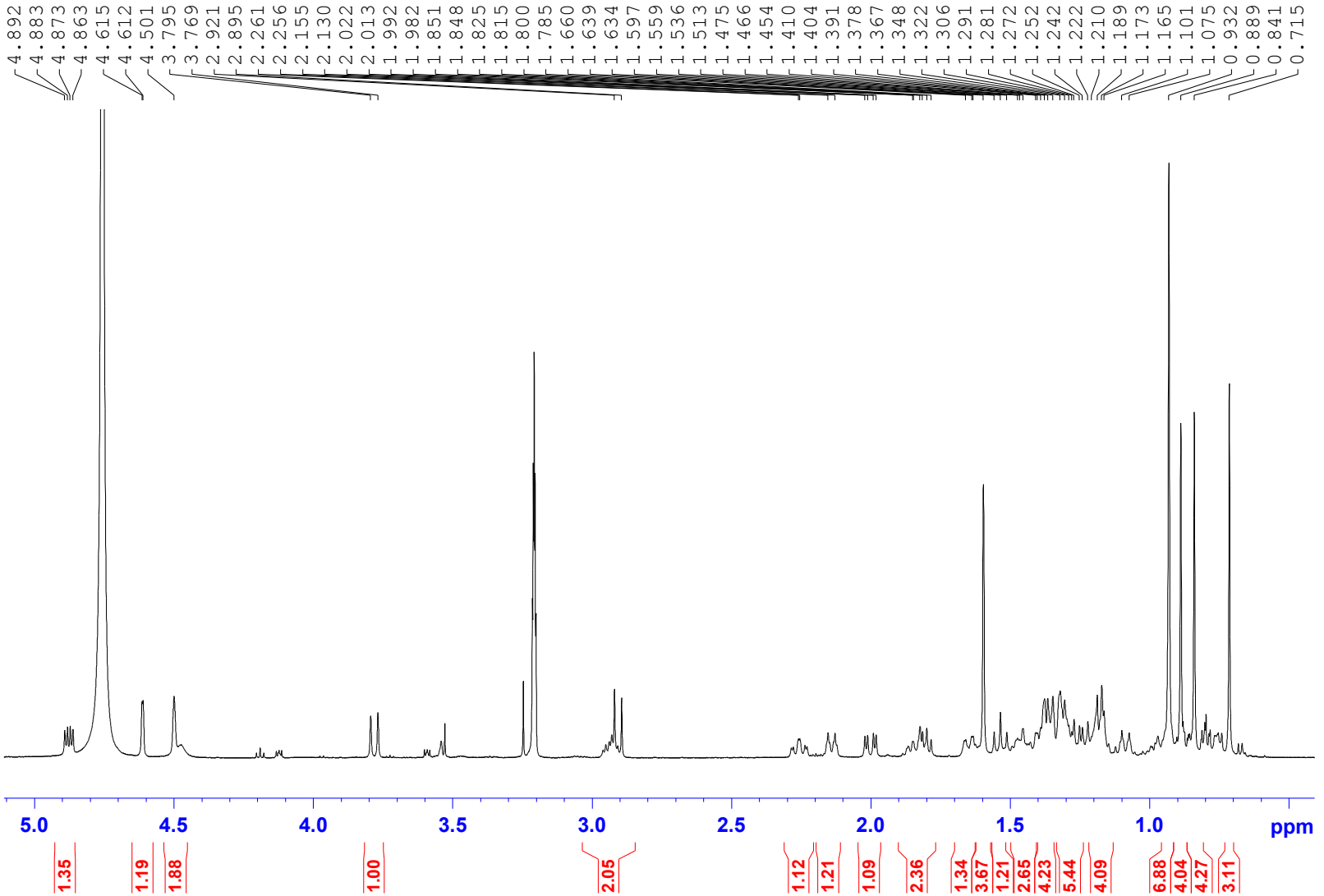


Figure.2. ^{13}C NMR spectrum (125MHz, MeOD) of compound **1**

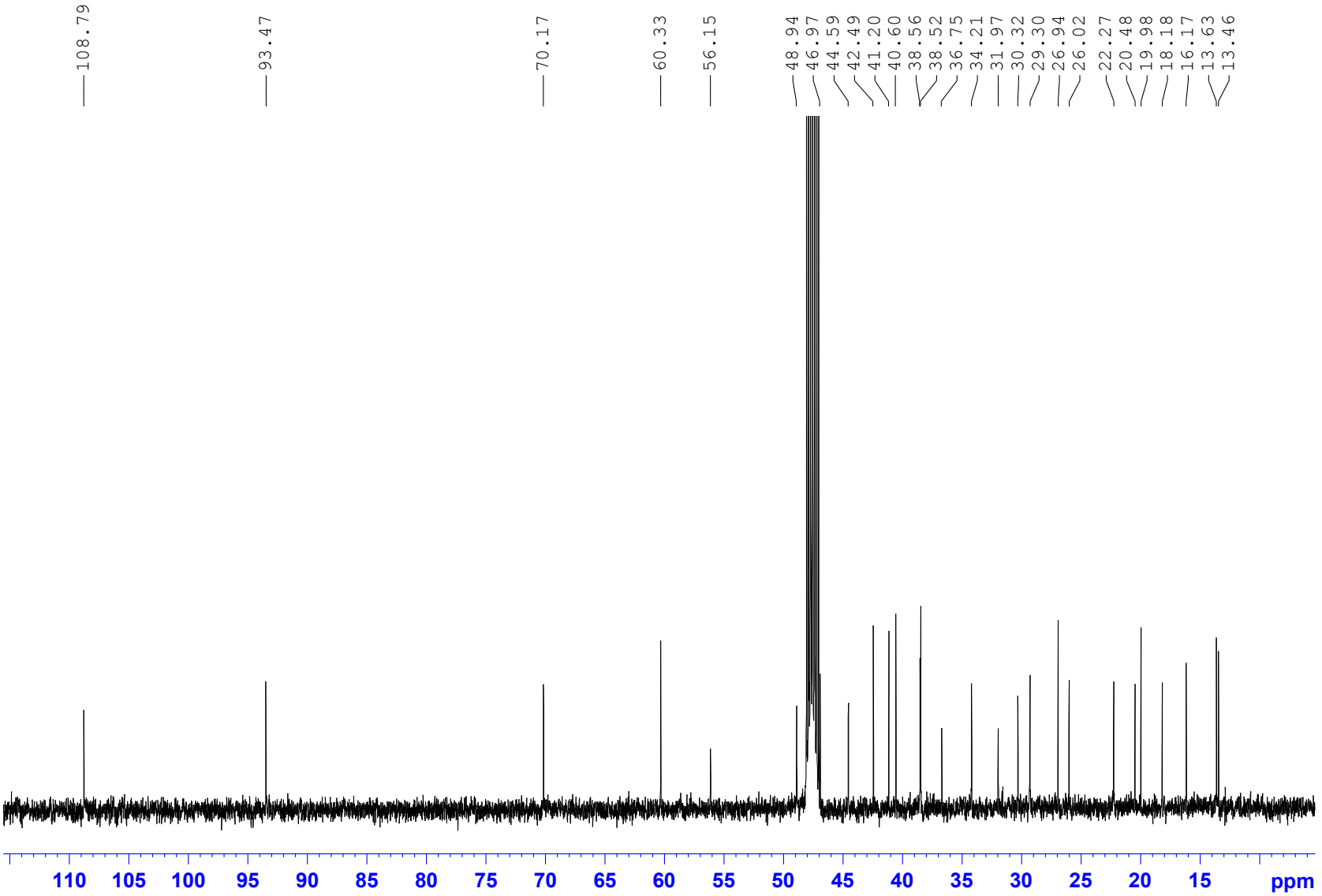


Figure.3. HSQC spectrum (500MHz, MeOD) of compound **1**

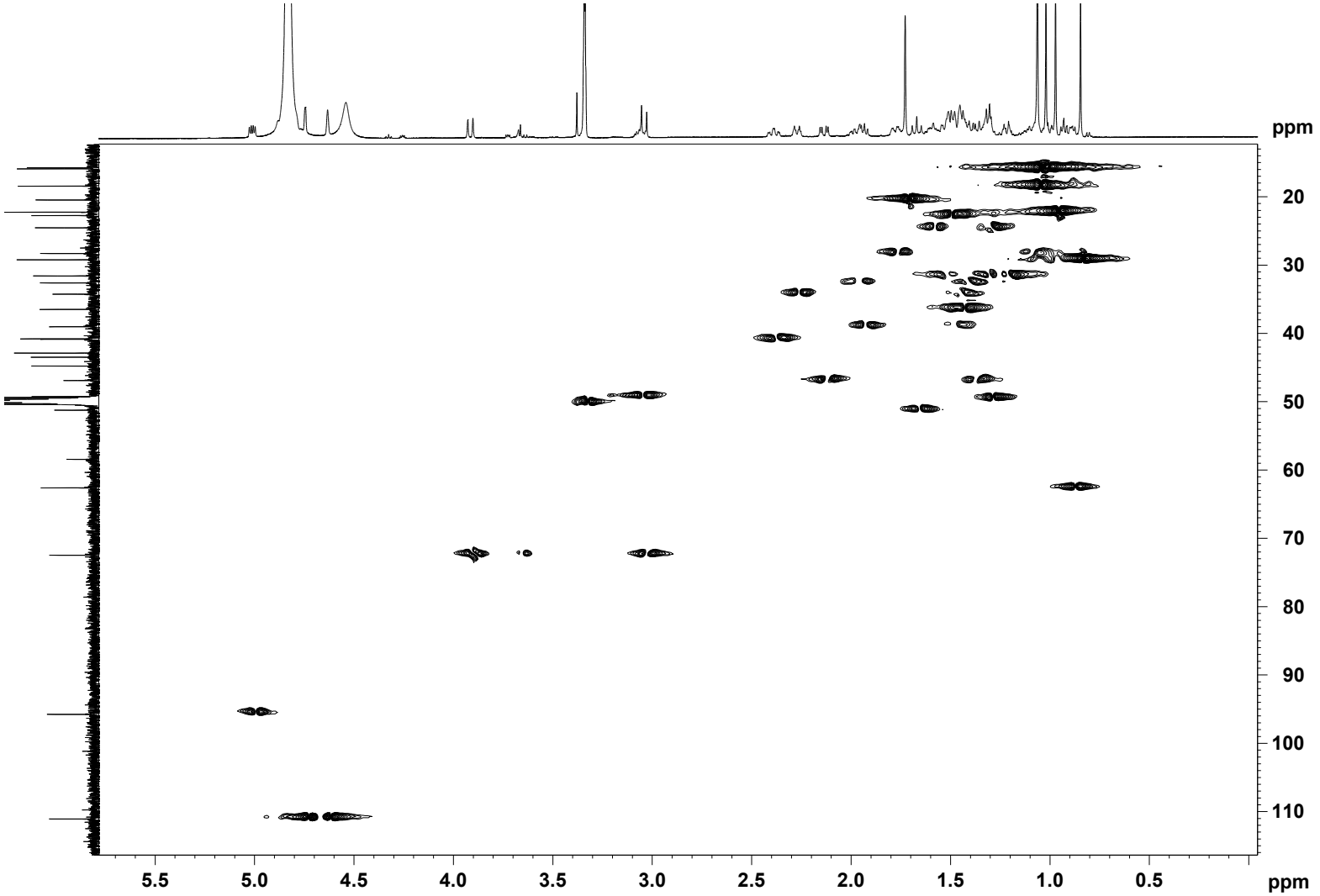


Figure.4. HMBC spectrum (500MHz, MeOD) of compound **1**

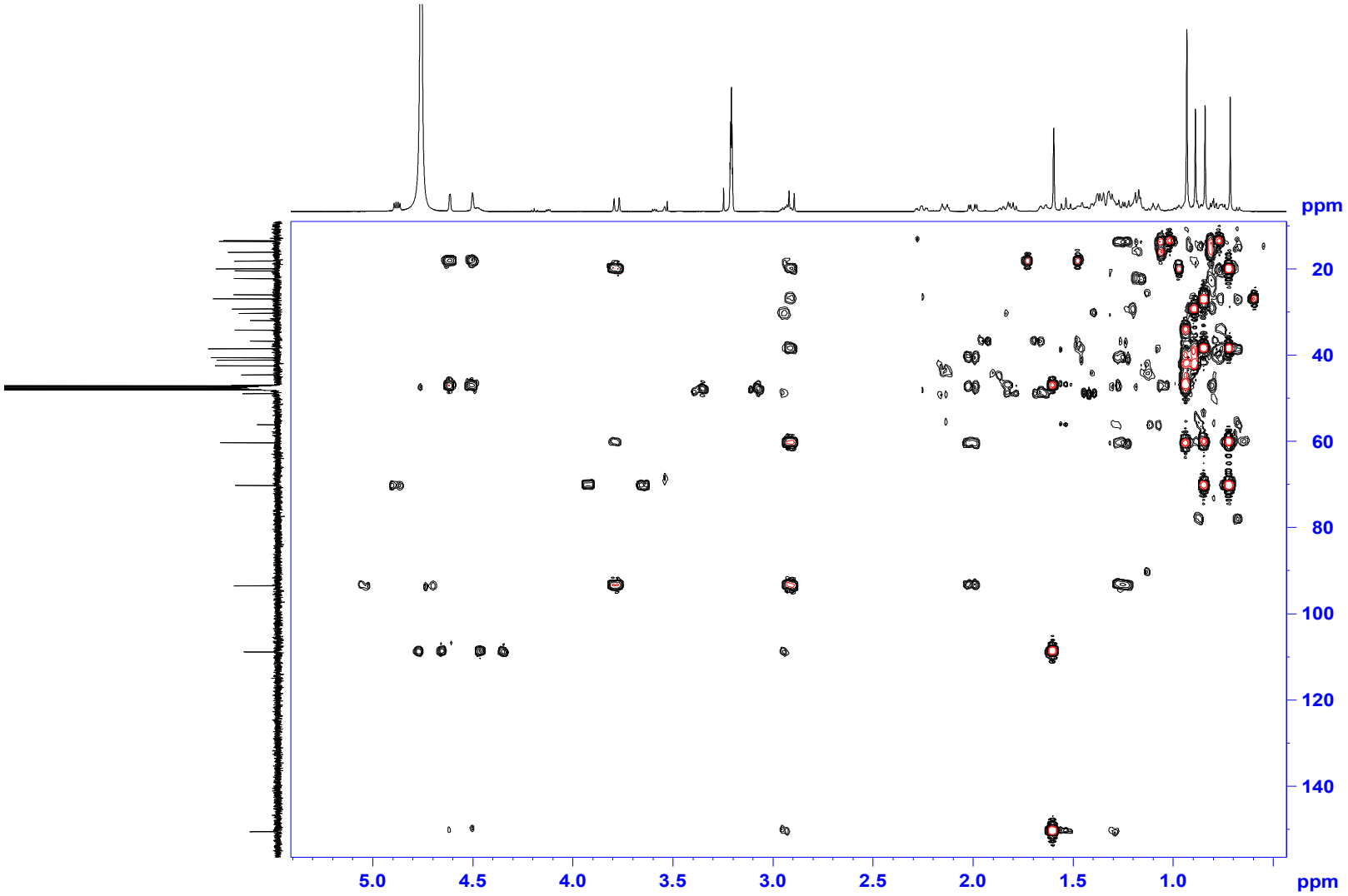


Figure.5. NOESY spectrum (500MHz, MeOD) of compound **1**

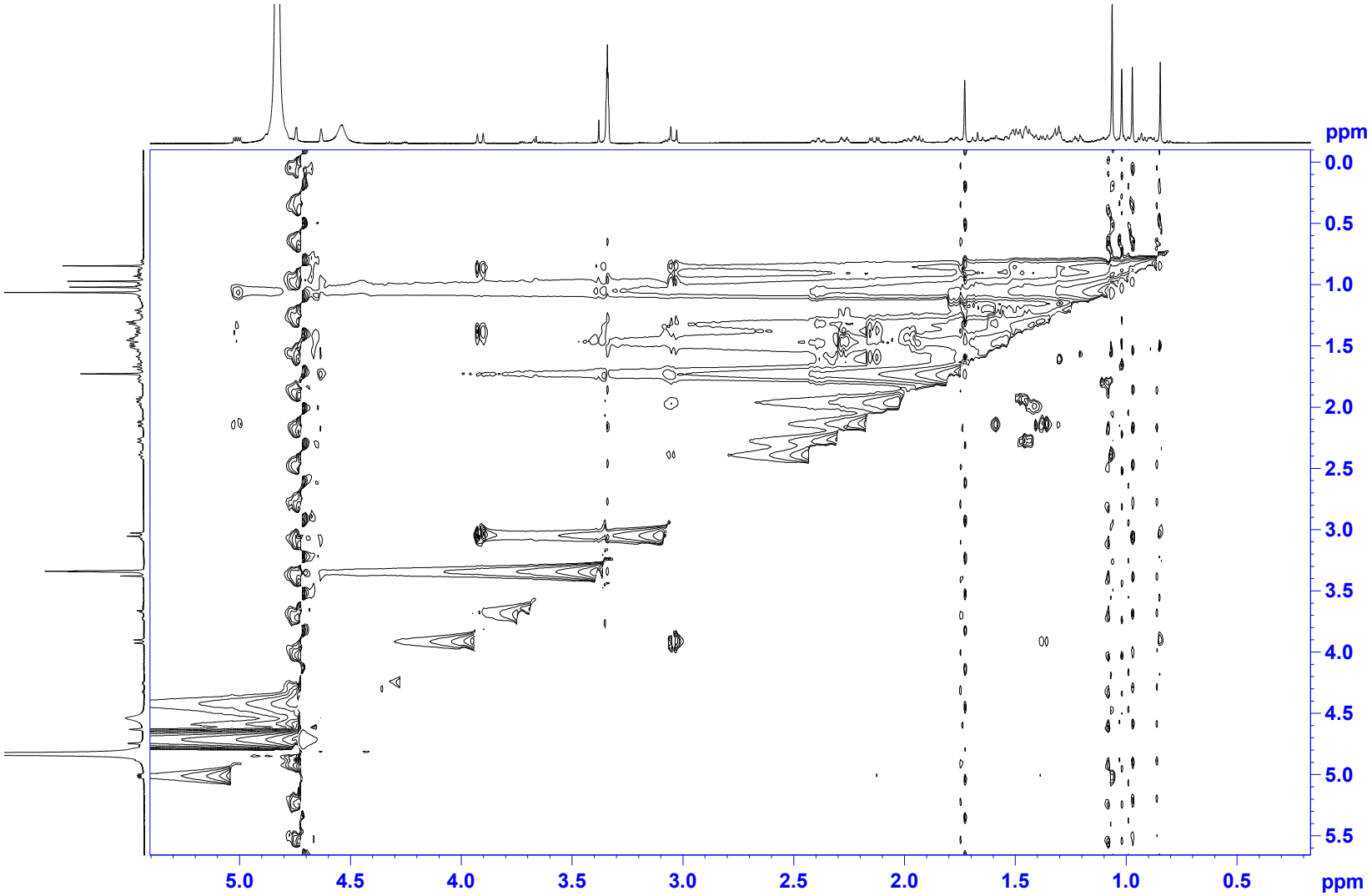


Figure.6. The HREIMS spectrum of compound **1**

Mass Spectrum Molecular Formula Report

Analysis Info D:\Data\20141217CEYANG\JZ-11_1-d,4_01_4408.d

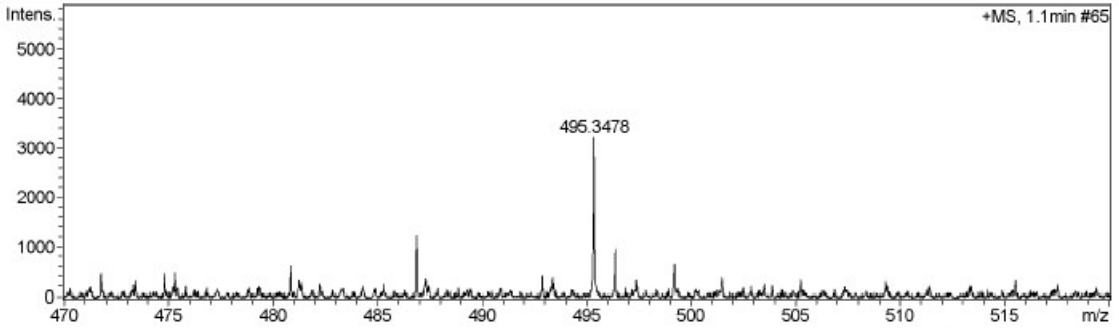
Analysis Name		Acquisition Date	12/17/2014 6:42:33 PM
Method	20131026_ceyang.m	Operator	micrOTOF-Q
Sample Name	JZ-11	Instrument / Ser#	Bruker Customer 125
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.2 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source

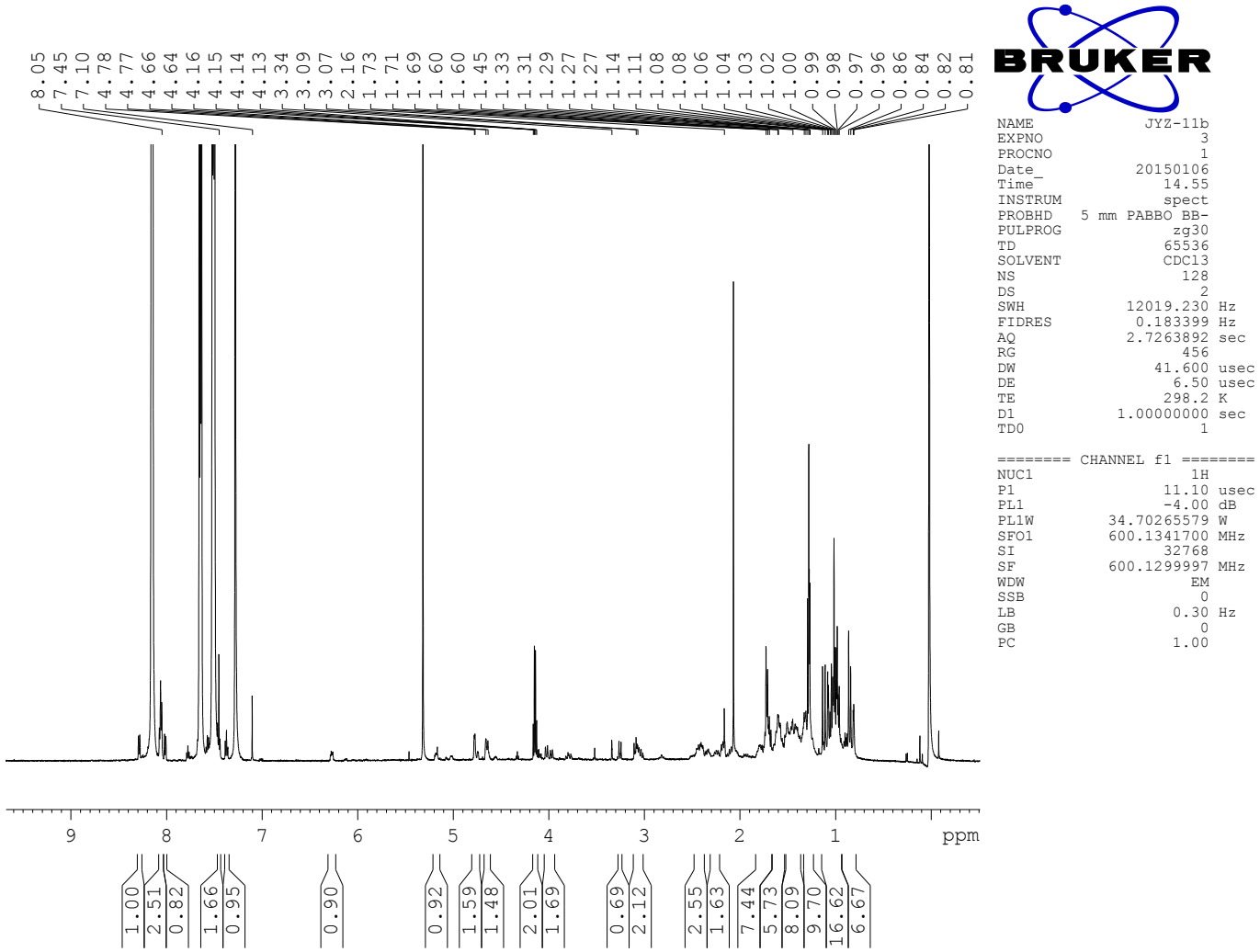
Generate Molecular Formula Parameter

Formula, min.	C30H48O4Na						
Formula, max.							
Measured m/z	495.348	Tolerance	7	ppm	Charge	1	
Check Valence	no	Minimum	0		Maximum	0	
Nirogen Rule	no	Electron Configuration	both				
Filter H/C Ratio	no	Minimum	0		Maximum	3	
Estimate Carbon	yes						



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 30 H 48 Na 1 O 4	0.029	495.3445	-6.67	-6.17	-3.30	6.50	ok	even

Figure.7. [1]H NMR spectrum (500MHz, MeOD) of compound **1a**



BRUKER

NAME	JYZ-11b	
EXPNO	3	
PROCNO	1	
Date_	20150106	
Time	14.55	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	128	
DS	2	
SWH	12019.230	Hz
FIDRES	0.183399	Hz
AQ	2.7263892	sec
RG	456	
DW	41.600	usec
DE	6.50	usec
TE	298.2	K
D1	1.00000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	1H	
P1	11.10	usec
PL1	-4.00	dB
PL1W	34.70265579	W
SFO1	600.1341700	MHz
SI	32768	
SF	600.1299997	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

Figure.8. The HREIMS spectrum of compound **1a**

Mass Spectrum Molecular Formula Report

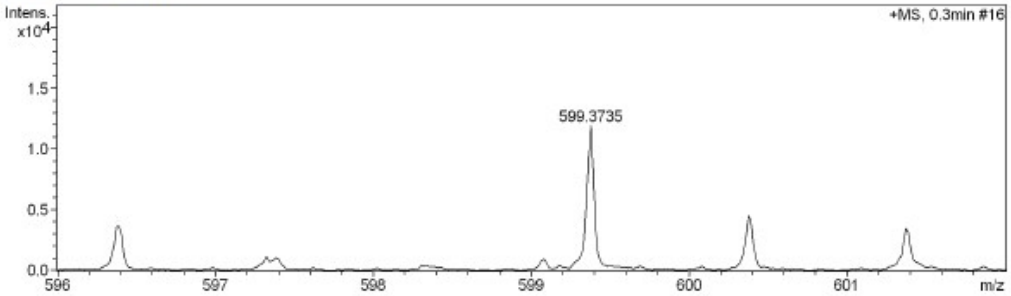
Analysis Info D:\Data\20150107ceyang\JZ-11A.d
Analysis Name
Method 20131026ceyang.m
Sample Name JZ-11A
Comment
Acquisition Date 1/8/2015 12:21:06 PM
Operator micrOTOF-Q
Instrument / Ser# Bruker Customer 125

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C37H52O5Na						
Formula, max.							
Measured m/z	599.374	Tolerance	5	ppm	Charge	1	
Check Valence	no	Minimum	0		Maximum	0	
Nirogen Rule	no	Electron Configuration	both				
Filter H/C Ratio	no	Minimum	0		Maximum	3	
Estimate Carbon	yes						



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 37 H 52 Na 1 O 5	0.113	599.3707	-4.72	-2.62	-2.83	11.50	ok	even

Bruker Daltonics DataAnalysis 3.4 printed: 1/12/2015 3:03:09 PM Page 1 of 1

Figure.9. ECD spectrum (MeOD) of compound **1a**

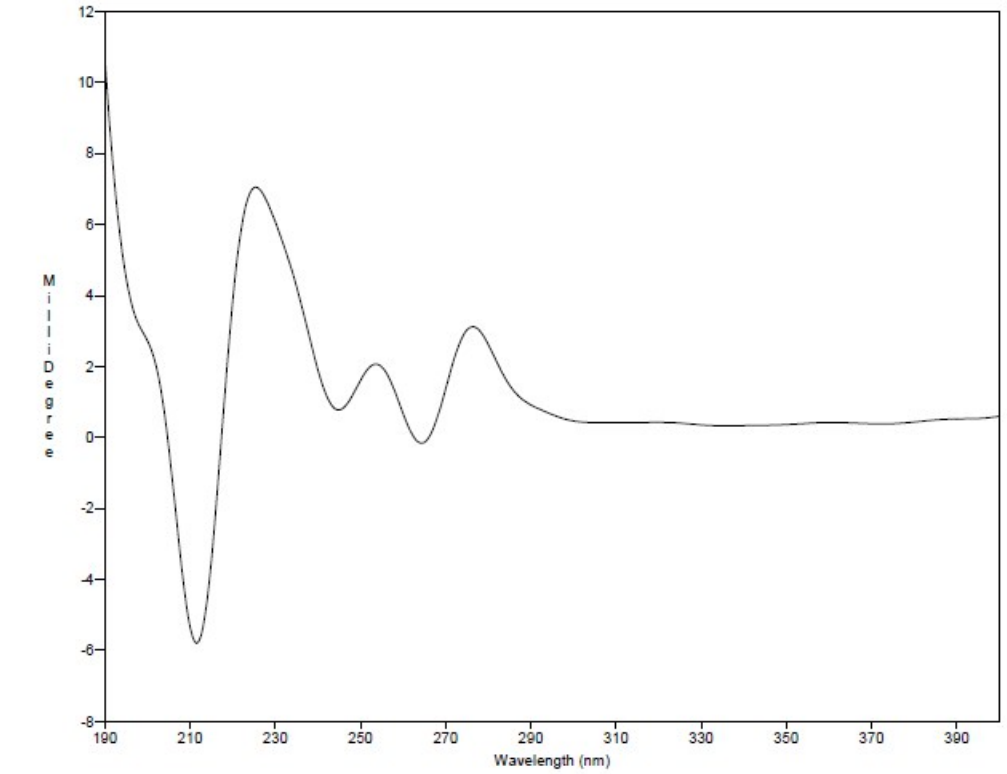
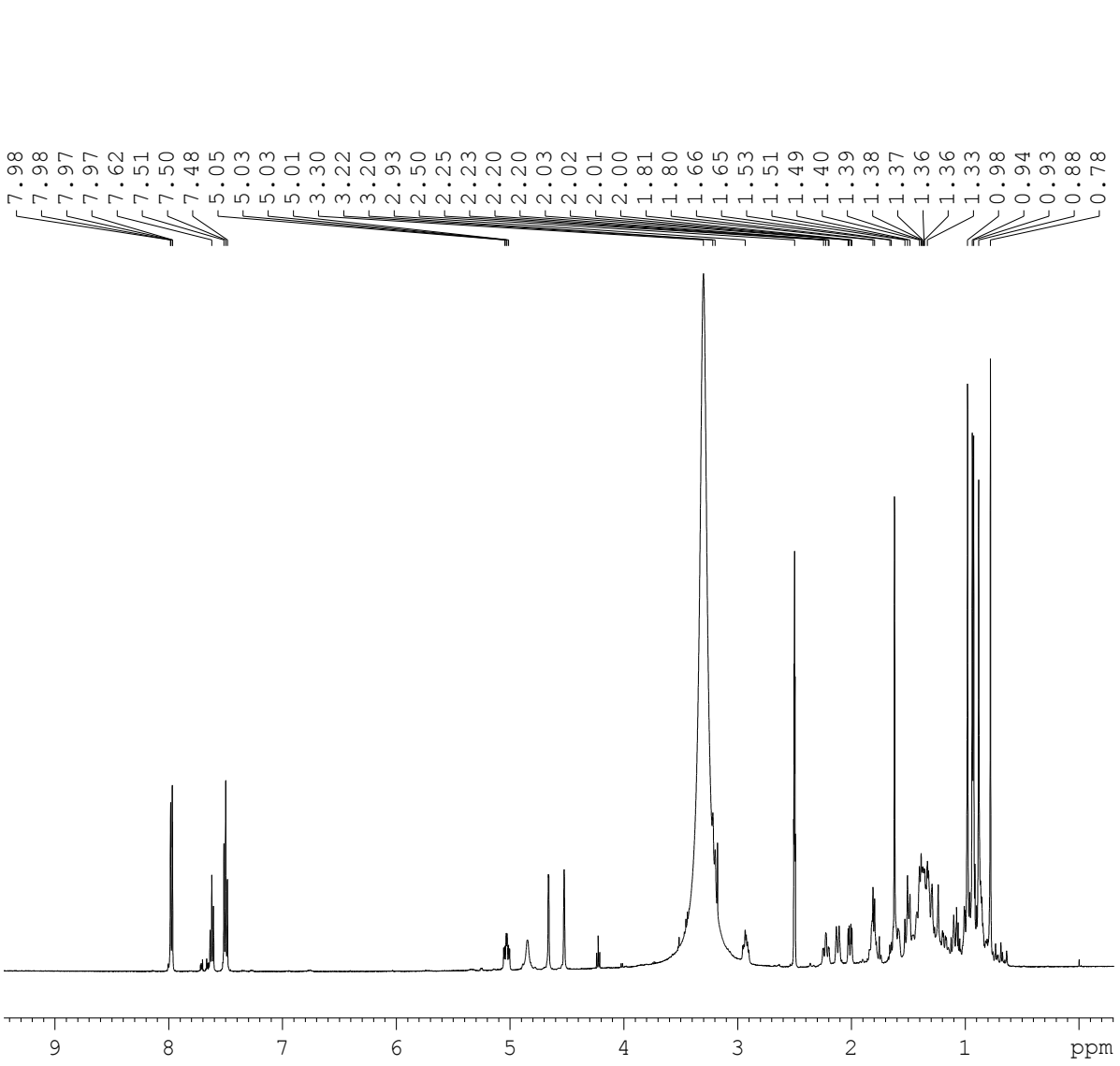


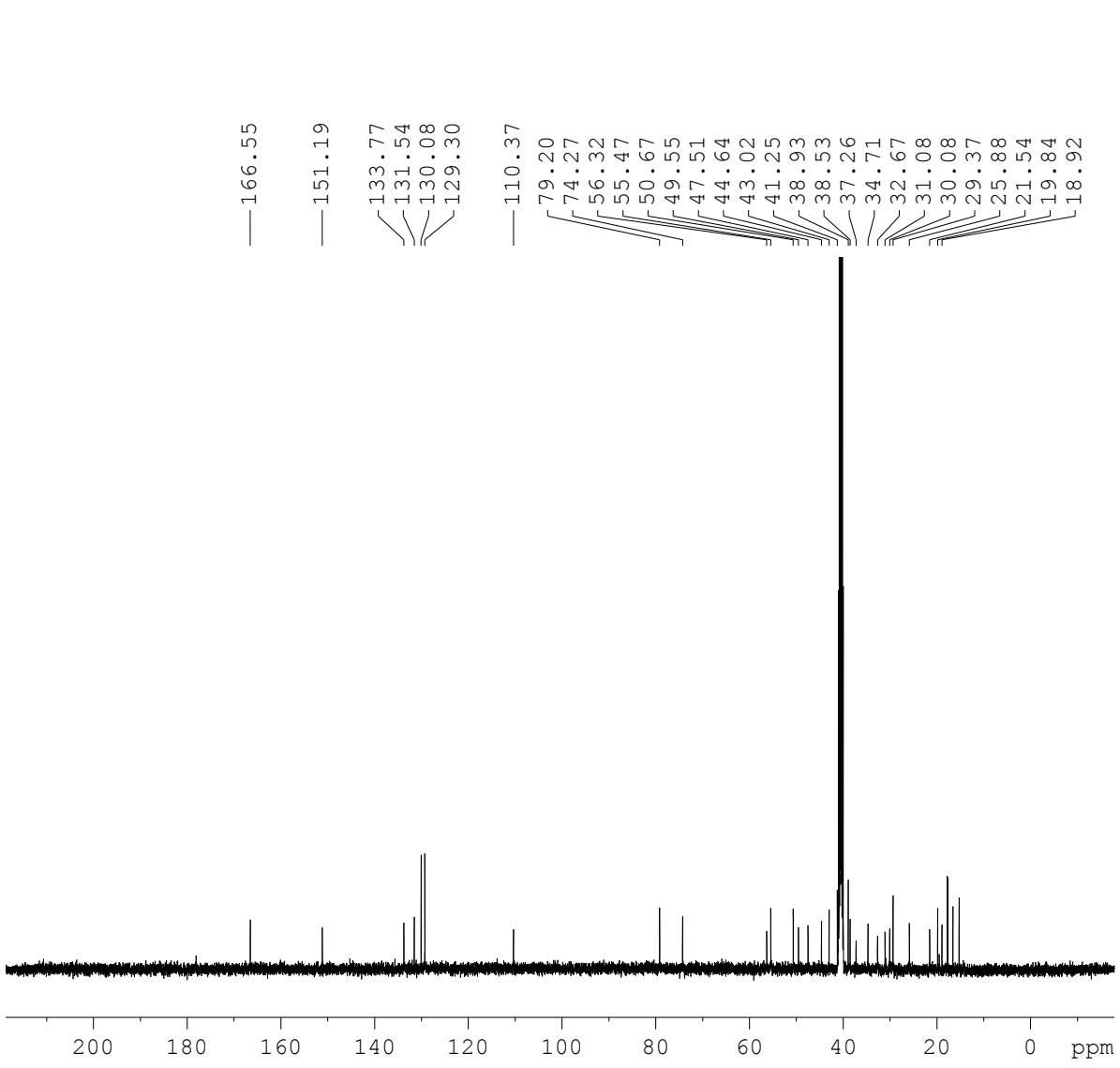
Figure.10. ^{1}H NMR spectrum (500MHz, MeOD) of compound **2**



BRUKER

NAME	jyz-13
EXPNO	2
PROCNO	1
Date_	20140922
Time	10.05
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	0
SWH	7500.000 Hz
FIDRES	0.114441 Hz
AQ	4.3691168 sec
RG	124.23
DW	66.667 usec
DE	10.19 usec
TE	316.1 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	12.00 usec
ND0	2
TD	128
SFO1	125.7703 MHz
FIDRES	218.226349 Hz
SW	222.095 ppm
FnMODE	QF
SI	32768
SF	500.1300062 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00
SI	1024
MC2	QF
SF	125.7577890 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

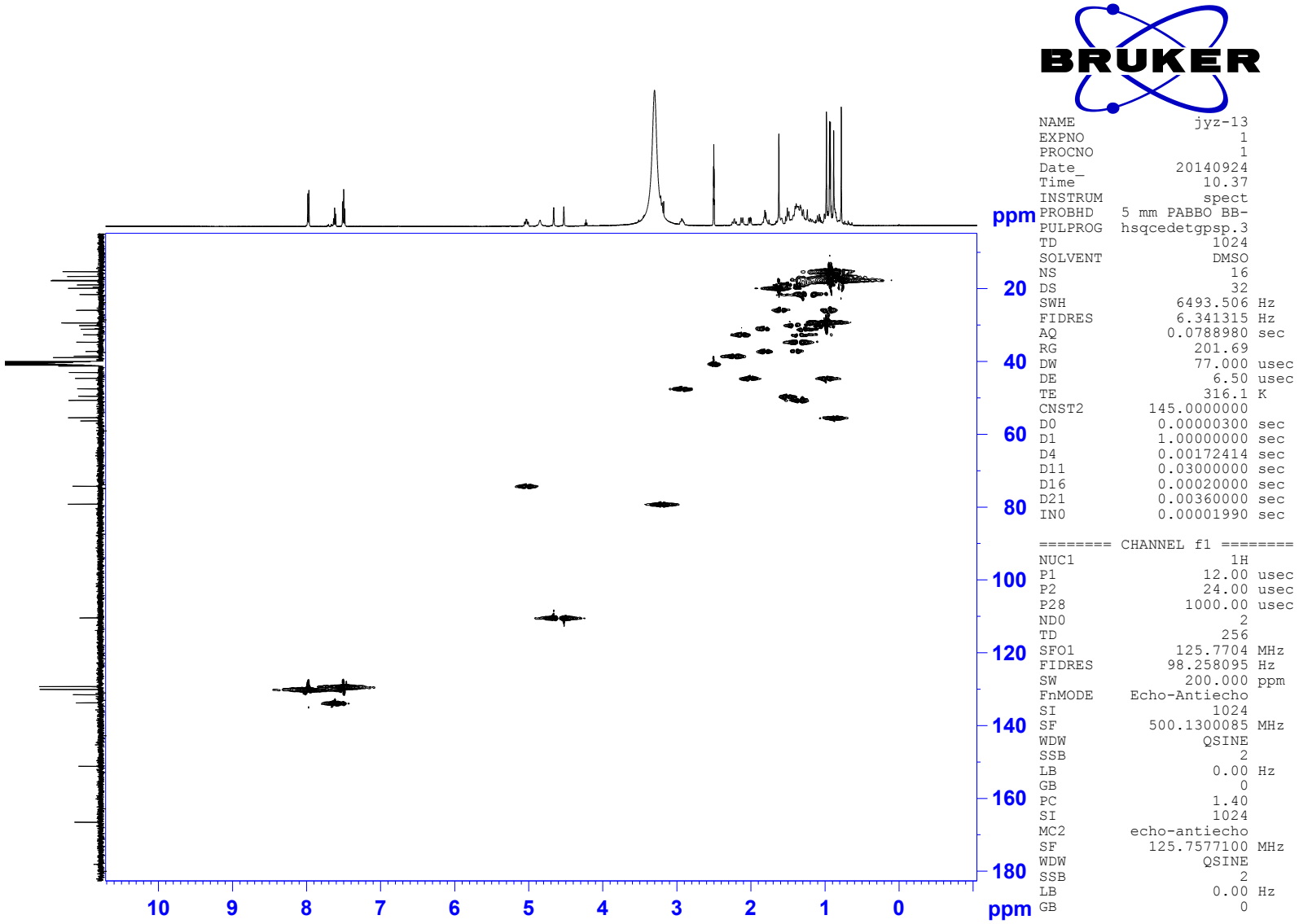
Figure.11. ^{13}C NMR spectrum (125MHz, MeOD) of compound **2**



BRUKER

NAME	jyz-13
EXPNO	3
PROCNO	1
Date_	20140922
Time	13.49
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	1024
DS	4
SWH	29761.904 Hz
FIDRES	0.454131 Hz
AQ	1.1010548 sec
RG	201.69
DW	16.800 usec
DE	6.50 usec
TE	316.1 K
D1	2.00000000 sec
D11	0.03000000 sec
======== CHANNEL f1 ========	
NUC1	13C
P1	8.90 usec
SI	32768
SF	125.7577362 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

Figure.12. HSQC spectrum (500MHz, MeOD) of compound **2**

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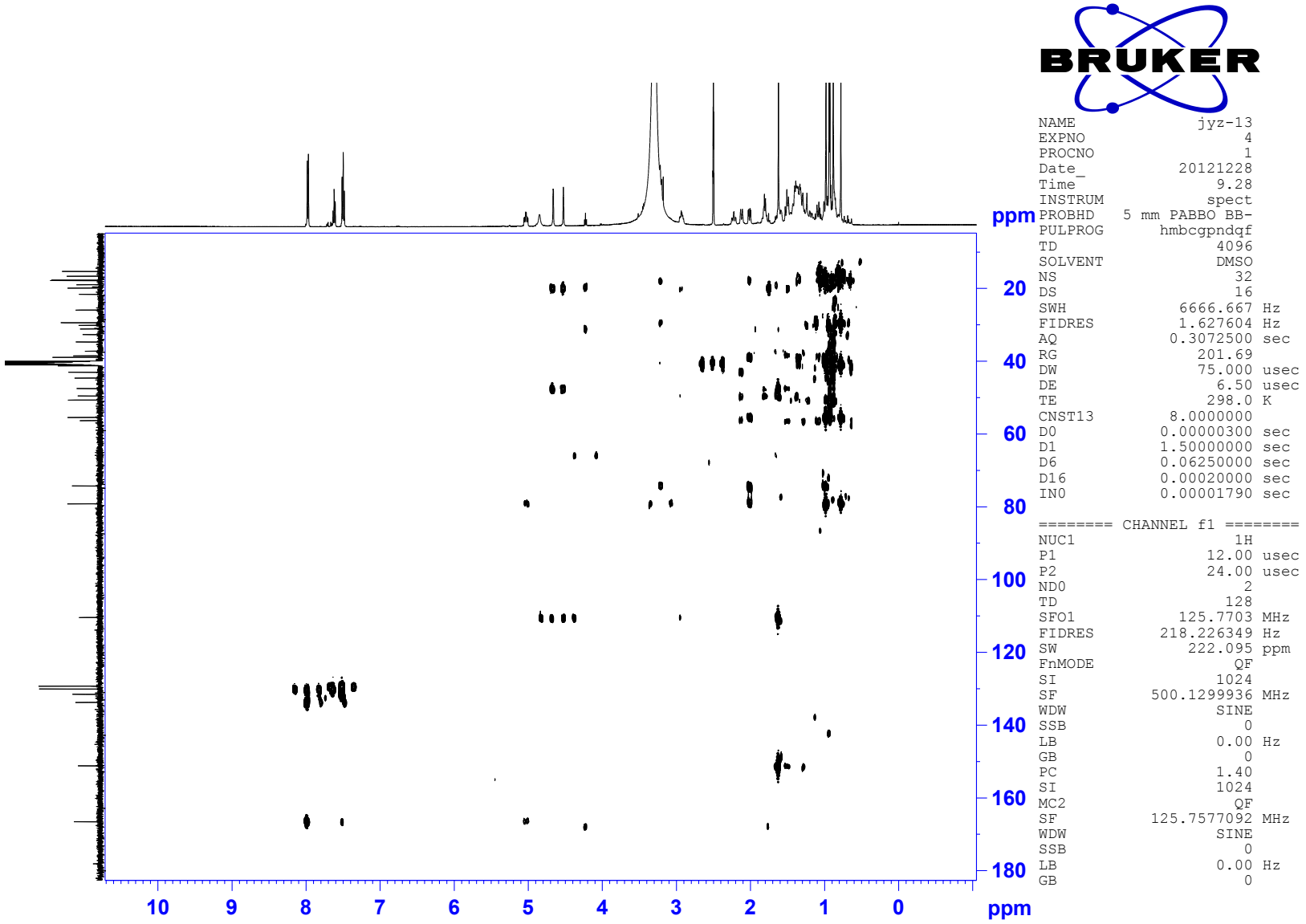
EXPNO                     1
PROCNO                    1
Date_              20140924
Time                  10.37
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG   hsqcedetgpsp.3
TD                     1024
SOLVENT                DMSO
NS                       16
DS                       32
SWH                6493.506 Hz
FIDRES             6.341315 Hz
AQ                 0.0788980 sec
RG                    201.69
DW                    77.000 usec
DE                     6.50 usec
TE                    316.1 K
CNST2            145.0000000
D0                 0.00000300 sec
D1                 1.00000000 sec
D4                 0.00172414 sec
D11                0.03000000 sec
D16                0.00020000 sec
D21                0.00360000 sec
IN0                0.00001990 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     12.00 usec
P2                     24.00 usec
P28                  1000.00 usec
ND0                       2
TD                      256
SFO1               125.7704 MHz
FIDRES              98.258095 Hz
SW                    200.000 ppm
FnMODE     Echo-Antiecho
SI                     1024
SF              500.1300085 MHz
WDW                    QSINE
SSB                       2
LB                     0.00 Hz
GB                        0
PC                     1.40
SI                     1024
MC2            echo-antiecho
SF               125.7577100 MHz
WDW                    QSINE
SSB                       2
LB                     0.00 Hz
GB                        0
```

Figure.13. HMBC spectrum (500MHz, MeOD) of compound **2**



BRUKER

NAME	jyz-13
EXPNO	4
PROCNO	1
Date_	20121228
Time	9.28
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	hmbcgpndqf
TD	4096
SOLVENT	DMSO
NS	32
DS	16
SWH	6666.667 Hz
FIDRES	1.627604 Hz
AQ	0.3072500 sec
RG	201.69
DW	75.000 usec
DE	6.50 usec
TE	298.0 K
CNST13	8.0000000
D0	0.00000300 sec
D1	1.50000000 sec
D6	0.06250000 sec
D16	0.00020000 sec
IN0	0.00001790 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	12.00 usec
P2	24.00 usec
ND0	2
TD	128
SFO1	125.7703 MHz
FIDRES	218.226349 Hz
SW	222.095 ppm
FnMODE	QF
SI	1024
SF	500.1299936 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40
SI	1024
MC2	QF
SF	125.7577092 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

Figure.14. The HREIMS spectrum of compound **2**

Mass Spectrum Molecular Formula Report

Analysis Info D:\Data\20141218\JZ-13.d

Analysis Name
Method tune_wide_pos.m
Sample Name JZ-13
Comment

Acquisition Date 12/18/2014 7:55:30 PM
Operator micrOTOF-Q
Instrument / Ser# Bruker Customer 125

Acquisition Parameter

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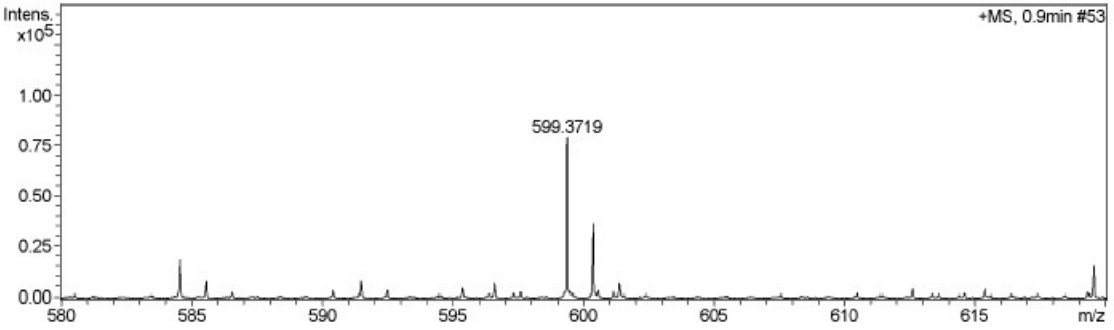
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C37H52O5Na						
Formula, max.							
Measured m/z	599.372	Tolerance	5	ppm	Charge	1	
Check Valence	no	Minimum	0		Maximum	0	
Nirogen Rule	no	Electron Configuration	both				
Filter H/C Ratio	no	Minimum	0		Maximum	3	
Estimate Carbon	yes						



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
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Figure.15. ECD spectrum (MeOD) of compound **2**

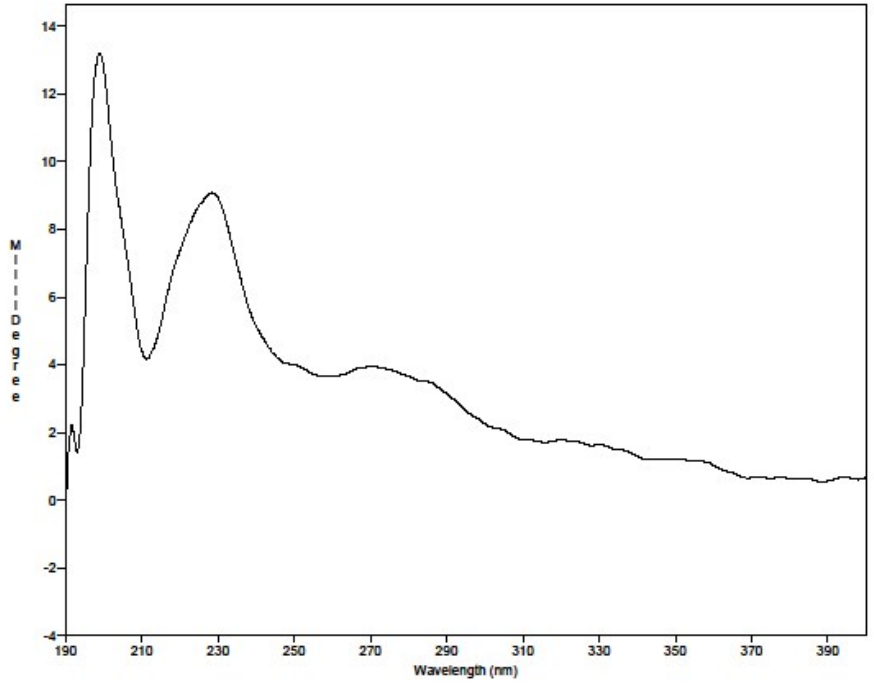

Figure.16. Stereoviews of compounds **1** and **2**

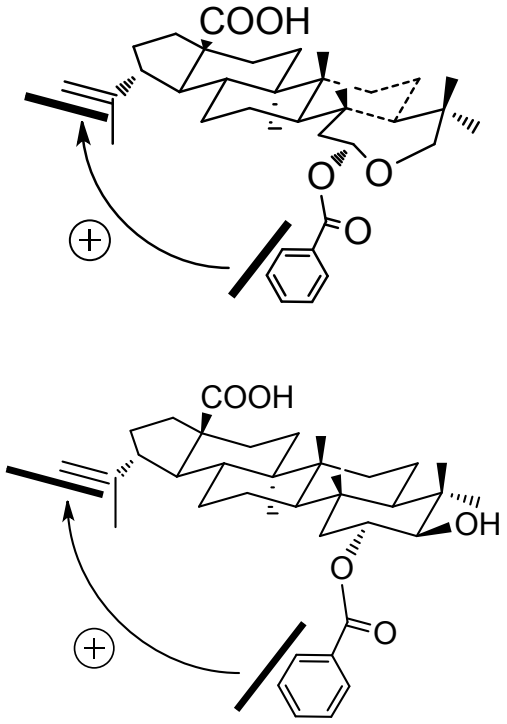

Figure.17. HPLC figures of compounds **1 – 6**