SUPPLEMENTARY INFORMATION

Artemeriopolides A–D, two types of sesquiterpenoid dimers with rare carbon skeletons from *Artemisia eriopoda* and their antihepatoma cytotoxicity

Xiao-Feng He,[‡a] Qi-Hao Li,[‡a] Tian-Ze Li,[a] Yun-Bao Ma,[a] Wei Dong,[a] Ke-Xin Yang,[a,b] Chang-An Geng,[a] Hao-Wei Zhang,[a,b] Yuan Wang,[a,b] and Ji-Jun Chen*[a,b]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[‡]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

*Corresponding author. Prof. Dr. Ji-Jun Chen, State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, 132[#] Lanhei Road, Kunming 650201, Yunnan, *People's Republic of China*.

Tel.: +86 871 65223265, Fax: +86 871 65227197, *E-mail address*: chenjj@mail.kib.ac.cn (Ji-Jun Chen).

List of Supplementary Information

General Experimental Instruments and Procedures

A Shimadzu LC/MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan) was used to gain the HRESIMS. UV spectra were conducted on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan), and IR (KBr) spectra were obtained on a Bio-Rad FTS-135 spectrometer (Hercules, California, USA). 1D and 2D NMR spectra were conducted on Advance III-400 or III-600 spectrometers (Bruker, Bremerhaven, Germany) with TMS as the internal standard. Optical rotations were determined on a JASCO P-1020 digital polarimeter (Horiba, Tokyo, Japan). X-ray crystallographic analyses using Cu Kα radiation were performed on a Bruker D8 QUEST instrument (Bruker, Karlsruher, Germany). Electronic circular dichroism (ECD) spectra were measured on an Applied Photophysics Circular Dichromatograph (Applied Photophysics, Britain). Thin-layer chromatography (TLC) analyses were performed on silica gel GF_{254} plates (Yantai Jiangyou Silicon Development Company, Yantai, China), and spots were monitored under UV light or by heating after sprayed with 10% H_2SO_4 in EtOH (*v/v*). Silica gel (200~300 mesh, Linyi Haixiang, Linyi, China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography. Medium pressure liquid chromatography (MPLC) separation was conducted on a Dr-Flash II apparatus using an MCI gel CHP 20P column (75~150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-CBM-20 system (Shimadzu, Kyoto, Japan) with Agilent XDB-C_{18} (5 μm, 9.4 × 250 mm) columns.

Cell cycle and apoptosis assays were performed on a flow cytometer (FACSCalibur, Becton Dickinson Co., Ltd., New Jersey, USA). Western blot assay was conducted on vertical electrophoresis apparatus (DYCZ-24DN, Liuyi Biotechnology Co., Ltd., Beijing, China) and a Semi-Dry Blotter (YRDIMES, wealtec Co., Ltd., Nevada, USA.), exposured by MultiSpectral imaging system (BioSpectrum, Analytik Jena Co., Ltd., Jena, Germany).

ECD Calculations

The conformation search was performed by Spartan '14 software using molecular mechanics MMFF94x. The appropriate low-energy conformers were selected and optimized in the gas phase by semi-empirical method in Gaussian 09 program package, and were further optimized and analyzed for frequency using the density functional theory (DFT) at the B3LYP/6-31G(d,p) level, resulting in no imaginary frequencies. Solvent effects were taken into consideration by using the conductor polarizable continuum model (CPCM). All the conformers were used for calculating electronic circular dichroism (ECD) by the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d,p) level with the CPCM model in MeOH.1 The overall calculated ECD curves were generated by Boltzmann weighting of the selected low-energy conformers using SpecDis 1.62 with σ = ~0.3 eV.

Cytotoxicity assays

The cytotoxicity of the compounds **1**–**4** was tested by the MTT assay. Briefly, cells in a density of 3×10^4 cells/well were seeded into 96-well plates and incubated at 37 °C with 5% CO_2 for 24 h. The culture medium was replaced with fresh medium containing different concentrations of guaianolide dimers, and cells were incubated for additional 48 h. After removal of the medium, 100 μL of MTT reagent (1mg/mL) was added into each well, and the plates were kept in incubator for 4 h. After that, 100 μL of dimethyl sulfoxide (DMSO) was added into each well, and the plates were measured at 490 nm using microplate reader (BIO-RAD, USA). The inhibitory ratio was calculated as [(A_{490} control – A_{490} treated)/A_{490} control] ×100%. The cytotoxicity of compounds was expressed as IC_{50} values calculated by GraphPad Prism 5

(GraphPad Software, California, USA).

Cell migration and invasion assays

Cell migration and invasion of HepG2 cells were evaluated by Transwell assays (Corning, USA). For cell migration assay, HepG2 cells (10×10^5/mL) were seeded on the upper chambers. After adherence, the cells were maintained in serum-free DMEM with various concentrations of compound **1** for 48 h. Then, cells in the upper chambers were wiped, and the migrated cells were fixed in 70% ethanol and stained with crystal violet solution (0.1%) for 30 min. After that, images were taken by imaging system (Olympus IX73). For cell invasion assay, matrigel (BD Biosciences) was diluted to 1:50 in pre-cool DMEM medium and added to the upper chamber 6 h prior to seeding the cells. The subsequent procedures were the same as above.

Flow cytometry assays

We assessed the effect of compound **1** on cell cycle and apoptosis by flow cytometry. HepG2 cells were seeded into 6-well plates at a density of 3×10^5 cells per well, cultured overnight. Then, cells were treated with various concentrations (0.0, 16.8, 33.6, and 67.2 μM) of **1** for 12 h (cell cycle) or 48h (apoptosis). In cell cycle assay, cells were collected and fixed in 70% ethanol at -20 °C overnight. Thereafter, HepG2 cells were resuspended in PBS containing in staining buffer of 100 μg/mL PI and 200 μg/mL RNase A. In apoptosis assay, cells were harvested and suspended in binding buffer, and stained with fluorochrome Annexin V/PI for 15 min. Finally, the cells were analyzed by flow cytometry. Cell cycle and apoptosis assays were analyzed by using a BD AccuriC6 flow cytometer (BD Biosciences, San Jose, CA, USA).

Western blot

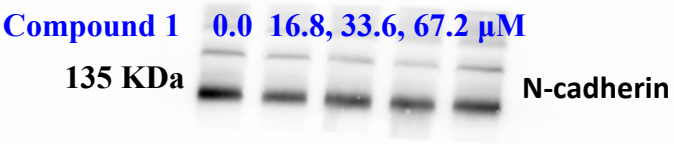
The expression of apoptosis-related proteins was determined by Western blot. Briefly, HepG2 cells were seeded into 6-well plates and treated with compound **1** for 48 h. The cells were collected and lysed in RIPA buffer to extract total protein, and the protein concentration was determined by BCA method. Samples were then fractionated using SDS-PAGE and transferred to PVDF membranes. Then, the membranes were incubated with specific primary antibodies at 4 °C overnight. The secondary antibody was incubated for 2 h at room temperature. Proteins were detected by ECL solution (Advansta, USA) and photographed by the multispectral imaging system (UVP, USA).

Table S1 Crystal data and structure refinement for compound **1**

Identification code	global	
Empirical formula	C29 H40 O5	
Formula weight	468.61	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	P1	
Unit cell dimensions	a = 9.7122(3) Å	α= 94.3130(10)°.
	b = 10.0265(3) Å	β= 101.0300(10)°.
	c = 19.5820(6) Å	γ = 90.1650(10)°.
Volume	1866.07(10) $Å^3$	
Z	3	
Density (calculated)	1.251 Mg/m^3	
Absorption coefficient	0.668 mm^{-1}	
F(000)	762	
Crystal size	0.280 x 0.240 x 0.120 mm^3	
Theta range for data collection	2.31 to 72.34°.	
Index ranges	-10<=h<=11, -12<=k<=12, -24<=l<=24	
Reflections collected	58997	
Independent reflections	14179 [R(int) = 0.0424]	
Completeness to theta = 72.34°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.92 and 0.80	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	14179 / 3 / 934	
Goodness-of-fit on F^2	1.066	
Final R indices [I>2sigma(I)]	R1 = 0.0414, wR2 = 0.1065	
R indices (all data)	R1 = 0.0419, wR2 = 0.1068	
Absolute structure parameter	0.00(5)	
Largest diff. peak and hole	0.710 and -0.306 $e.Å^{-3}$	

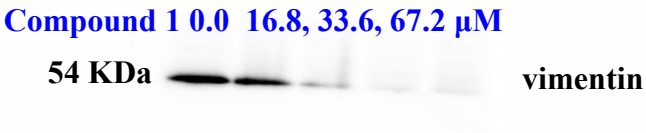
Table S2 Crystal data and structure refinement for compound **3**

Identification code	global
Empirical formula	C30 H38 O5
Formula weight	478.60
Temperature	100(2) K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	P 1 21 1
Unit cell dimensions	a = 9.979(2) Å = 90°. b = 10.231(3) Å □= 94.762(13)°. c = 25.066(6) Å □ = 90°.
Volume	2550.3(10) $Å^3$
Z	4
Density (calculated)	1.246 Mg/m^3
Absorption coefficient	0.665 mm^{-1}
F(000)	1032
Crystal size	0.340 x 0.150 x 0.020 mm^3
Theta range for data collection	1.77 to 79.72°.
Index ranges	-10<=h<=12, -12<=k<=12, -30<=l<=30
Reflections collected	30700
Independent reflections	9060 [R(int) = 0.1034]
Completeness to theta = 79.72°	84.5 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.99 and 0.60
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9060 / 1 / 639
Goodness-of-fit on F^2	1.076
Final R indices [I>2sigma(I)]	R1 = 0.0832, wR2 = 0.1991
R indices (all data)	R1 = 0.1242, wR2 = 0.2199
Absolute structure parameter	0.22(13)
Largest diff. peak and hole	0.365 and -0.438 e.$Å^{-3}$


Compound 1
0.0
16.8, 33.6, 67.2 µM
135 KDa
N-cadherin

Compound 1 0.0 16.8, 33.6, 67.2 µM

125-135 KDa  **E-cadherin**


Compound 1 0.0 16.8, 33.6, 67.2 µM
54 KDa
vimentin

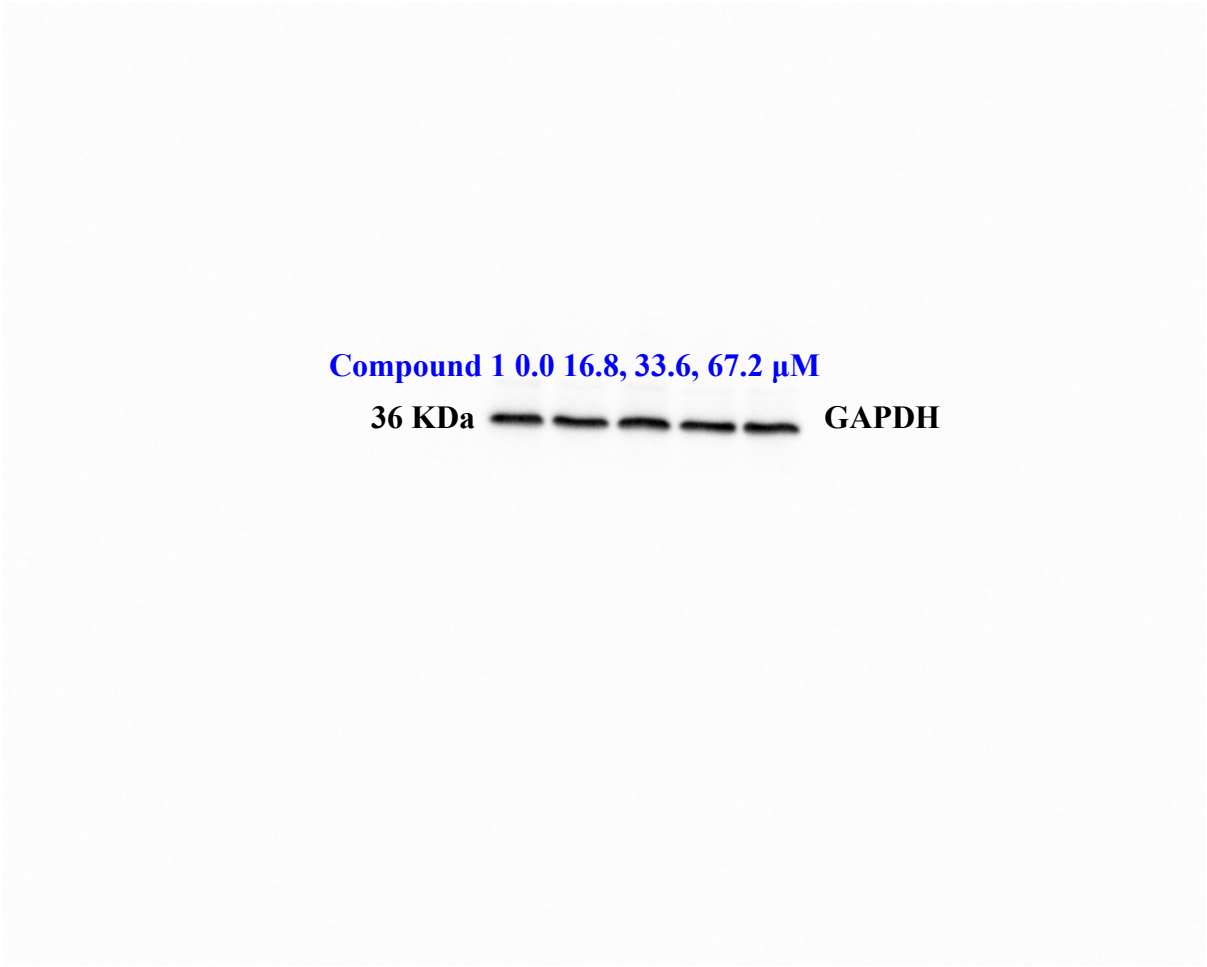


Figure S1 All raw images of Western blot of the key-related proteins with cell migration and invasion (N-cadherin, E-cadherin, and vimentin).

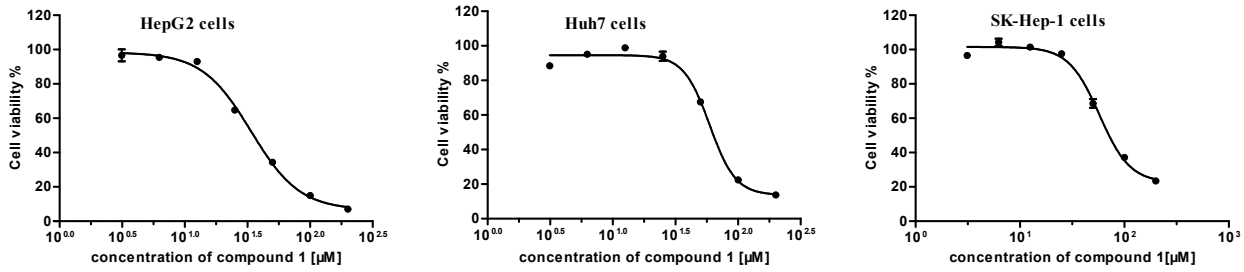
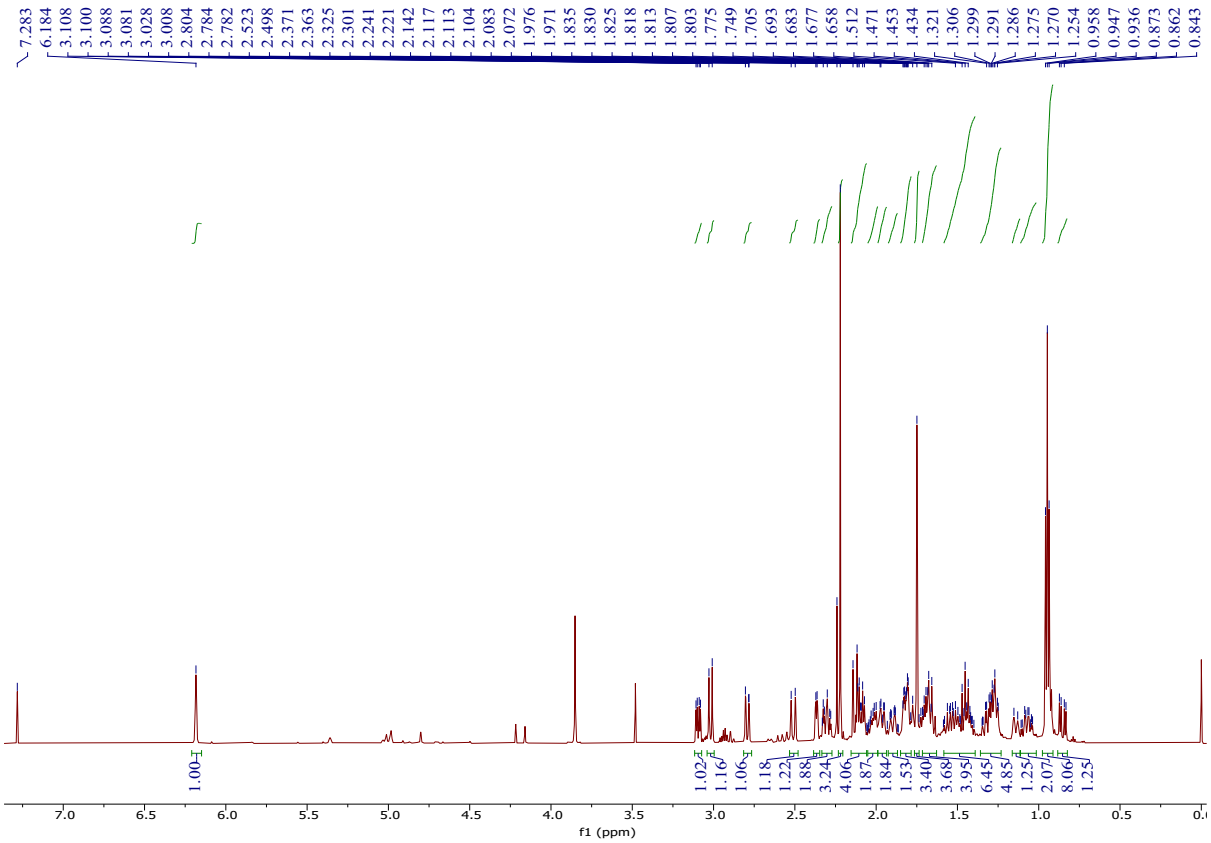
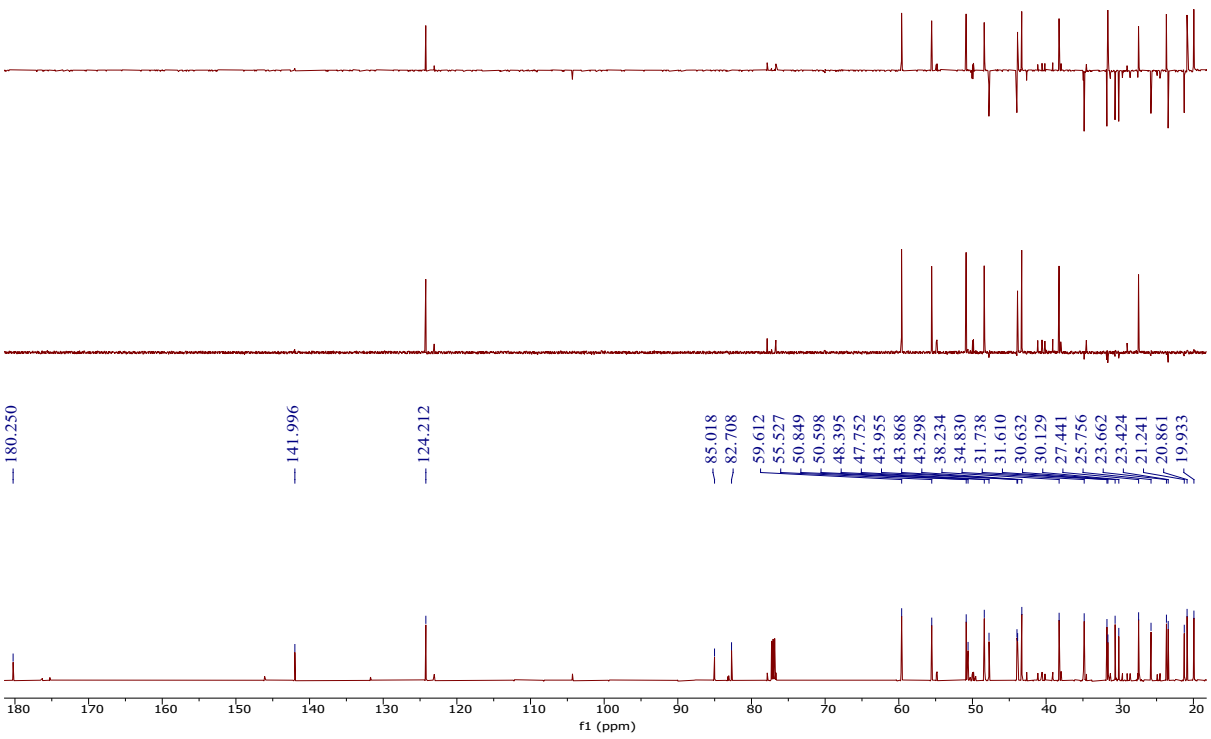


Figure S2. Cell viability of compound **1** in HCC cells was evaluated by MTT assay.

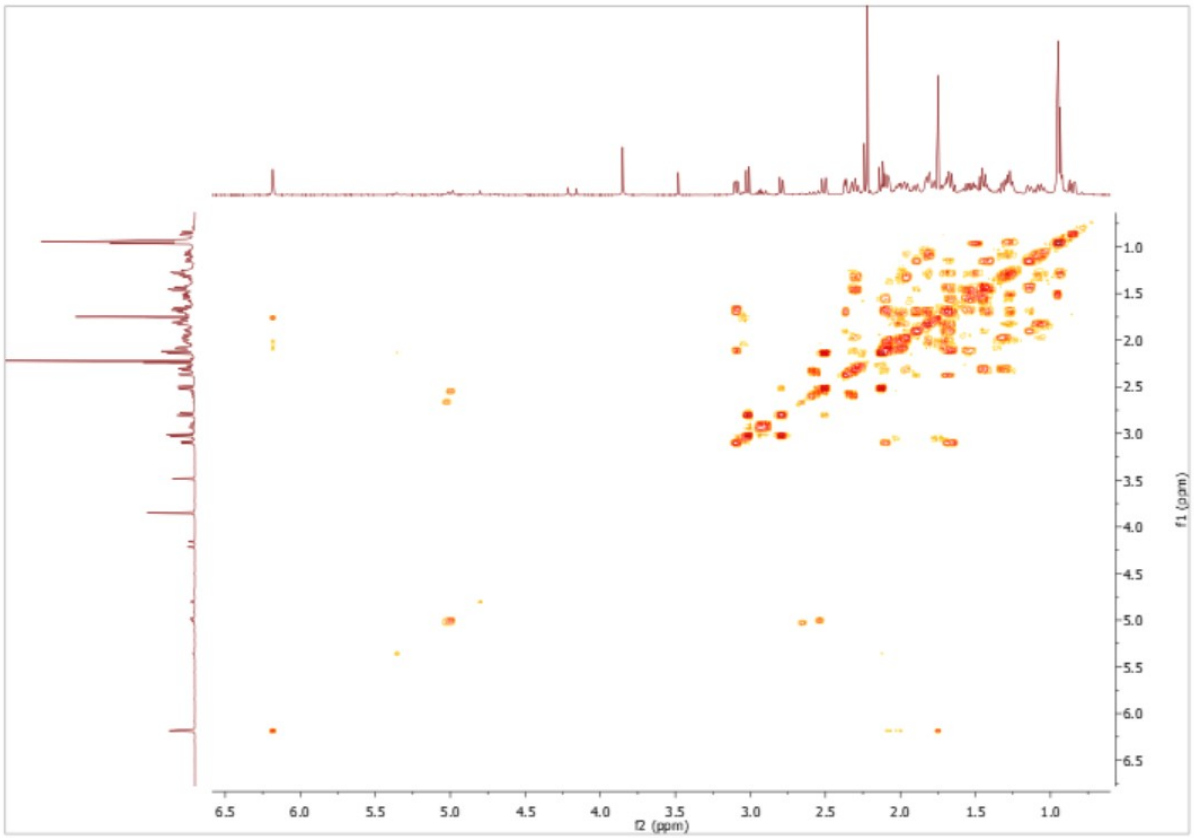
S1. ^{1}H NMR (600 MHz, $CDCl_3$) of compound **1**



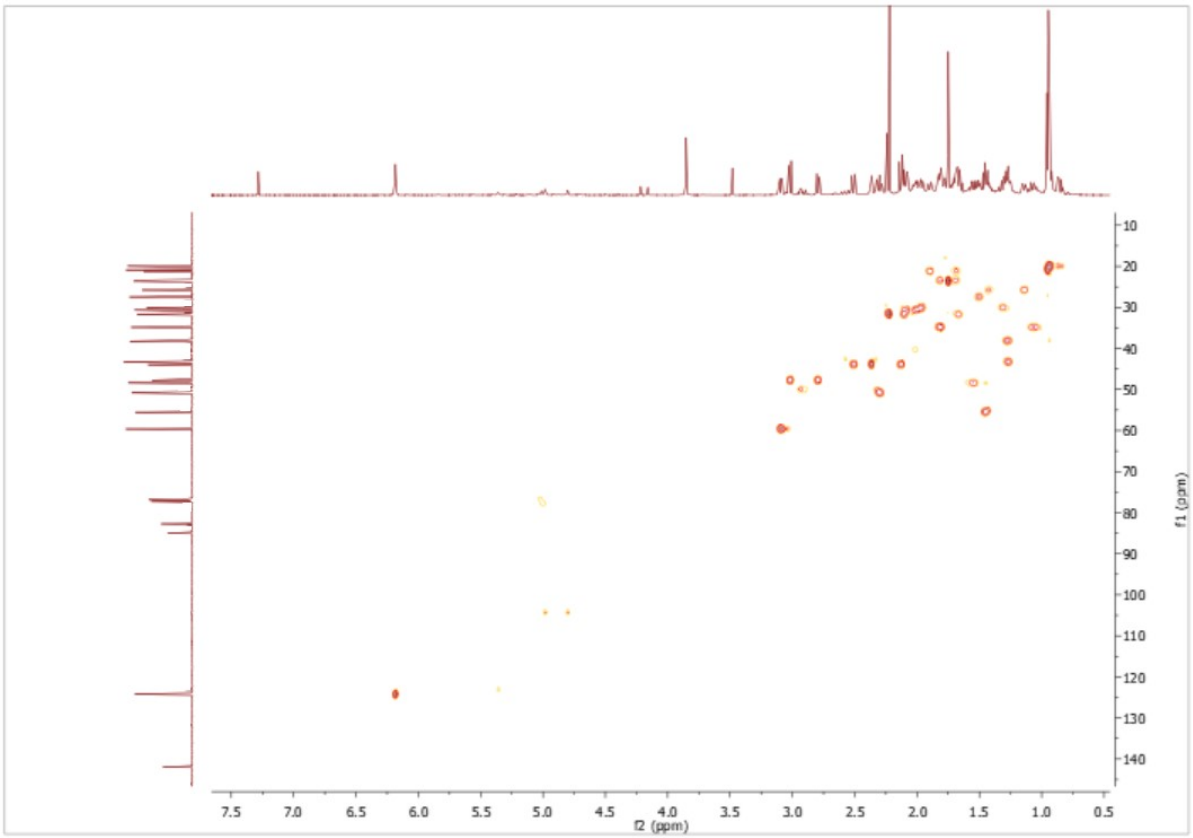
S2. ^{13}C NMR (DEPT) (150 MHz, $CDCl_3$) of compound **1**



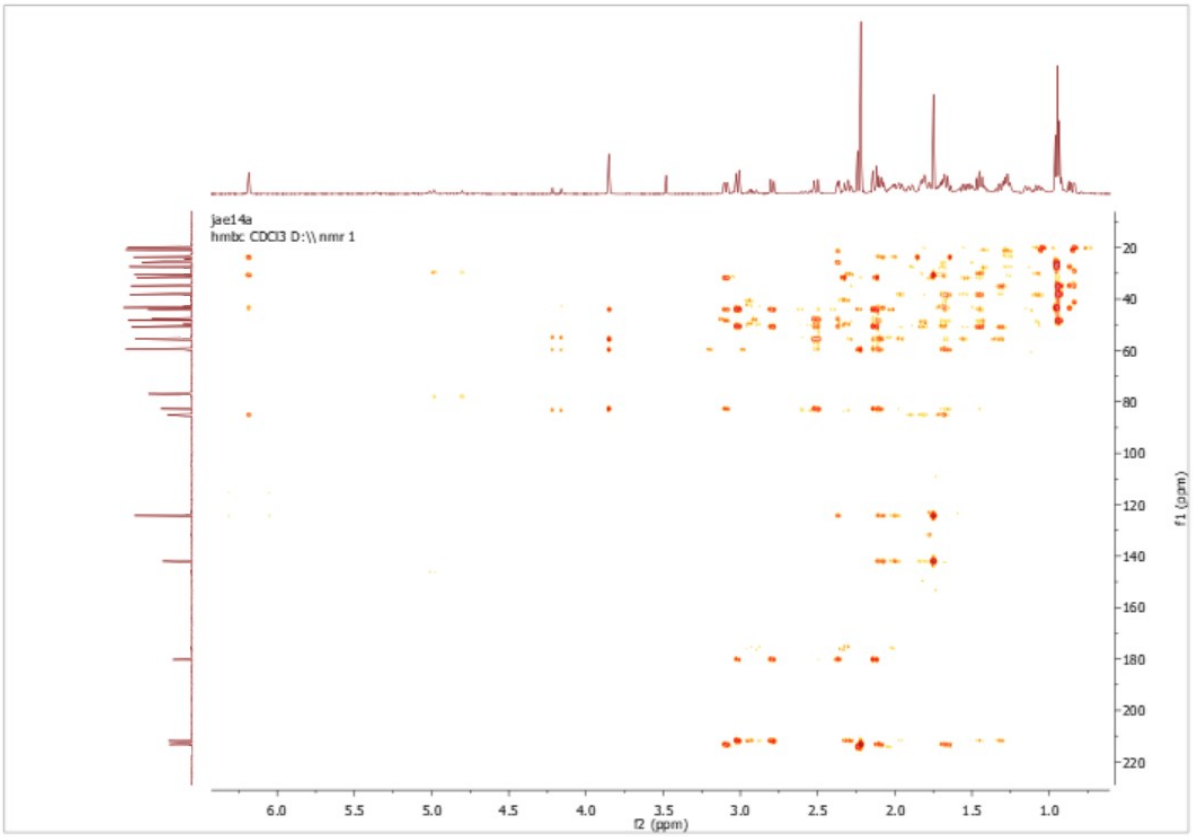
S3. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) of compound **1**



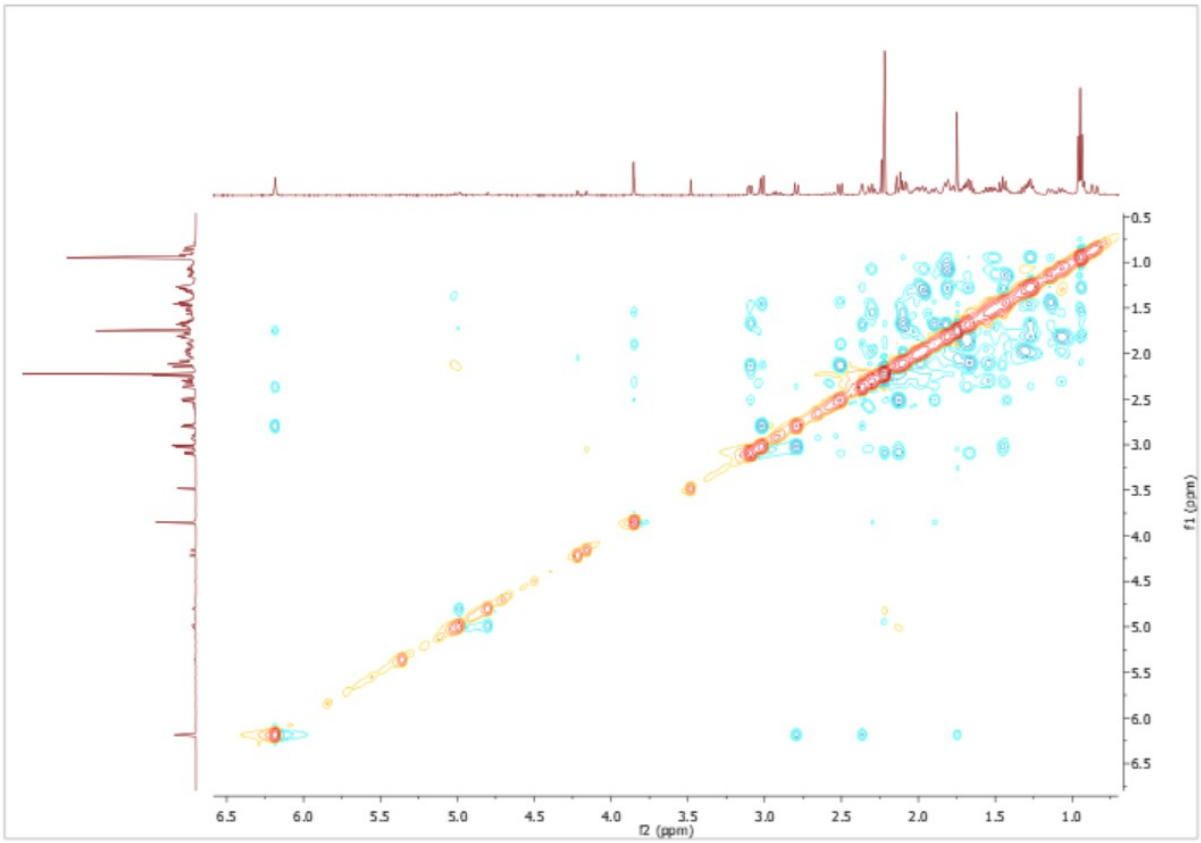
S4. HSQC (600 MHz, $CDCl_3$) of compound **1**



S5. HMBC (600 MHz, $CDCl_3$) of compound **1**



S6. ROESY (600 MHz, $CDCl_3$) of compound **1**



S7. $[\alpha]_D$ spectrum of compound **1** in MeOH

Rudolph Research Analytical

This sample was measured on an Autopol VI, Serial #91058
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : Tuesday, 08-JUN-2021

Set Temperature : OFF

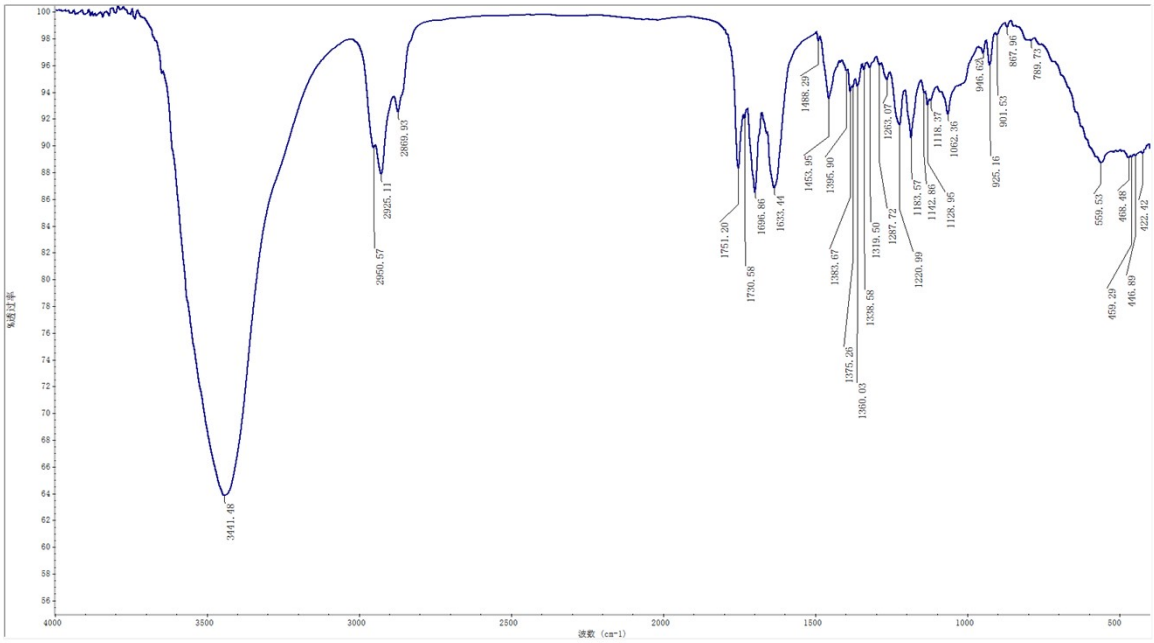
Time Delay : Disabled

Delay between Measurement : Disabled

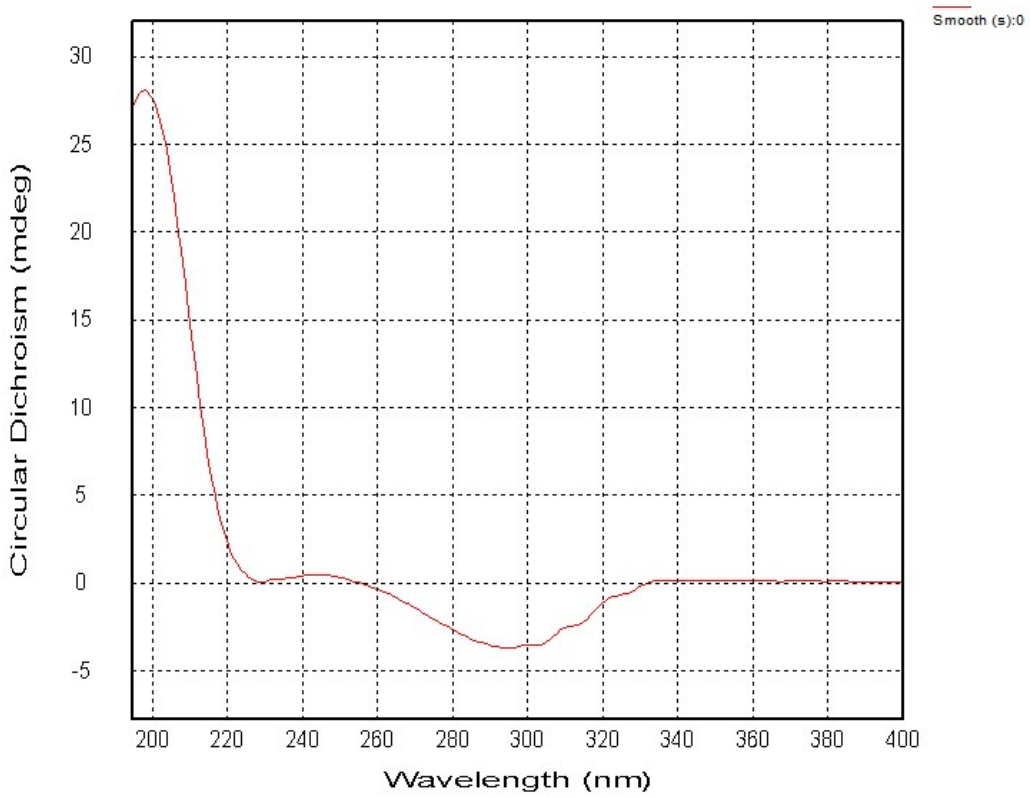
n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	-12.69	0.41	-3.23	-12.22	-13.15

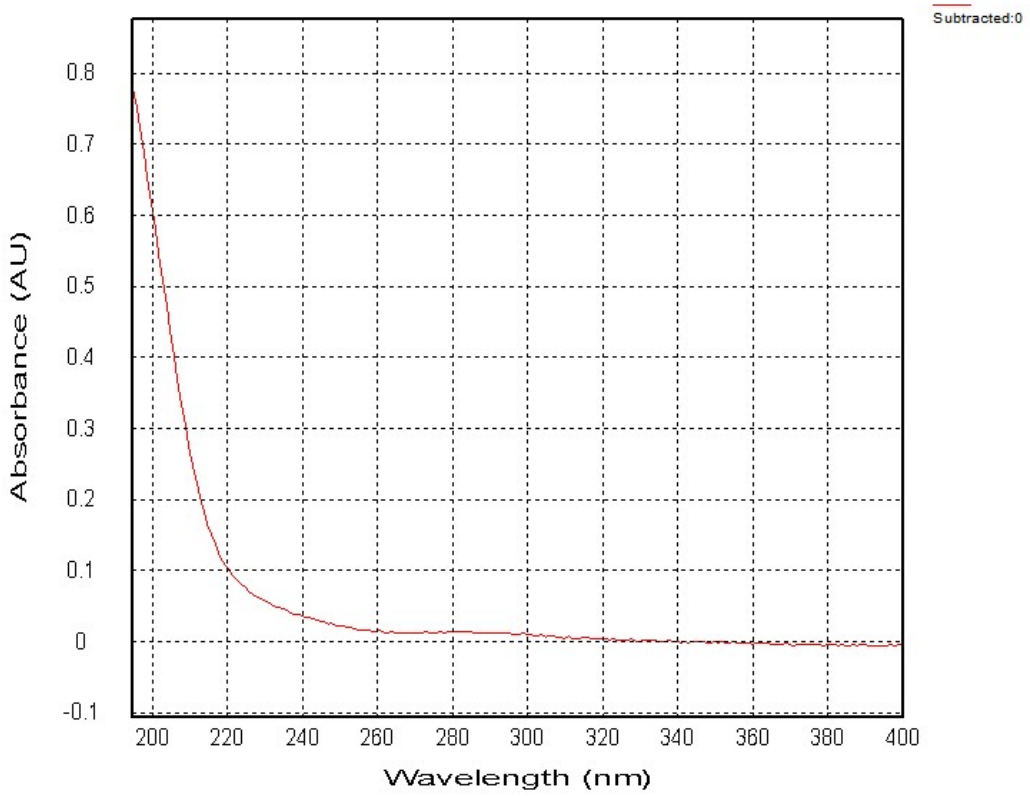
S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	jae-14a	10:47:10 AM	-12.22	SR	-0.0132	589	100.00	0.108	24.4
2	jae-14a	10:47:19 AM	-12.41	SR	-0.0134	589	100.00	0.108	24.4
3	jae-14a	10:47:27 AM	-12.59	SR	-0.0136	589	100.00	0.108	24.4
4	jae-14a	10:47:35 AM	-13.15	SR	-0.0142	589	100.00	0.108	24.4
5	jae-14a	10:47:43 AM	-13.06	SR	-0.0141	589	100.00	0.108	24.4

S8. IR of compound **1**



S9. ECD and UV of compound **1**





S10. HRESIMS of compound **1**

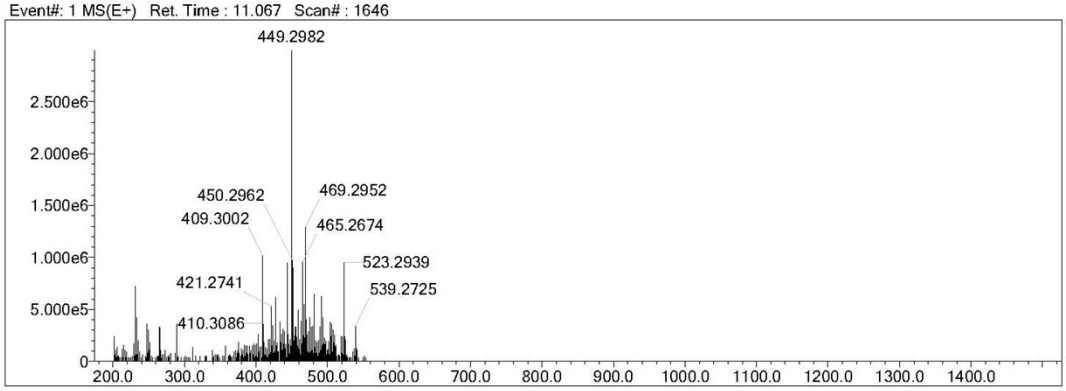
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	300	O	2	0	12	H
C	4	0	150	15N	3	0	0	Na
N	3	0	1					

Error Margin (ppm): 30
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

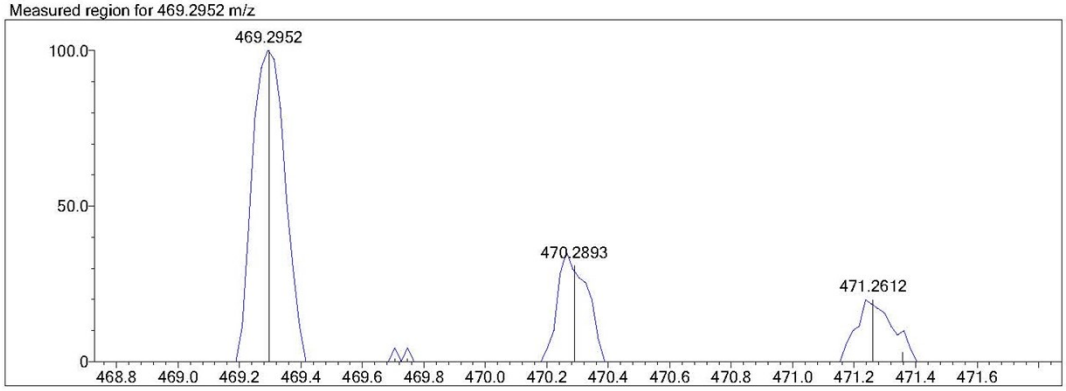
DBE Range: -2.0 - 1000.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 500

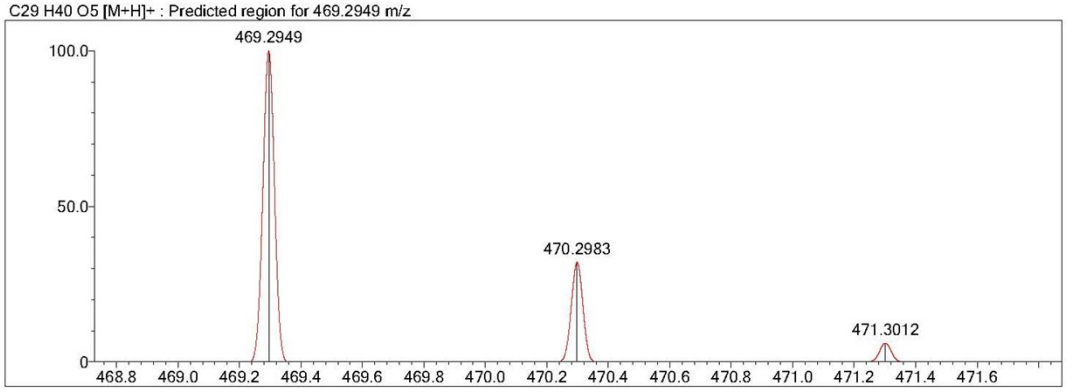
Event#: 1 MS(E+) Ret. Time : 11.067 Scan# : 1646



Measured region for 469.2952 m/z

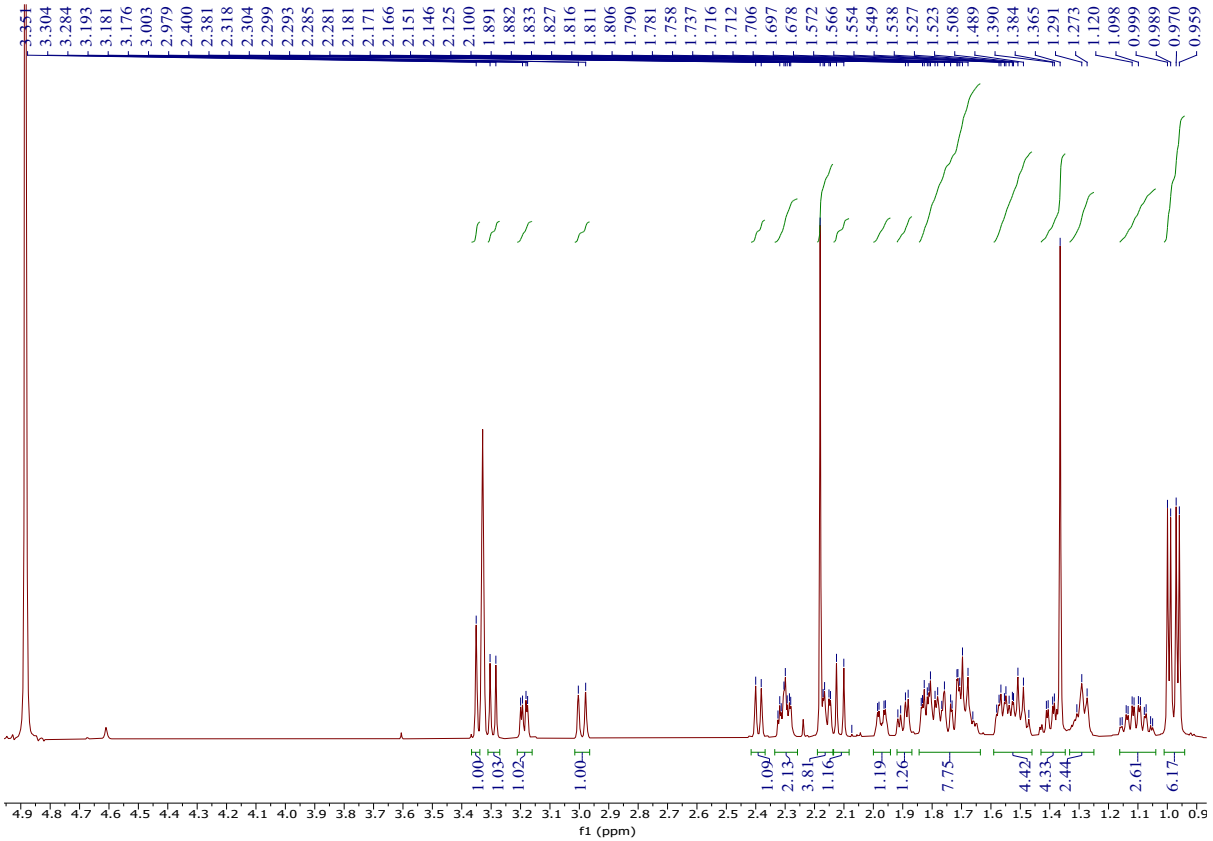


C29 H40 O5 [M+H]+ : Predicted region for 469.2949 m/z

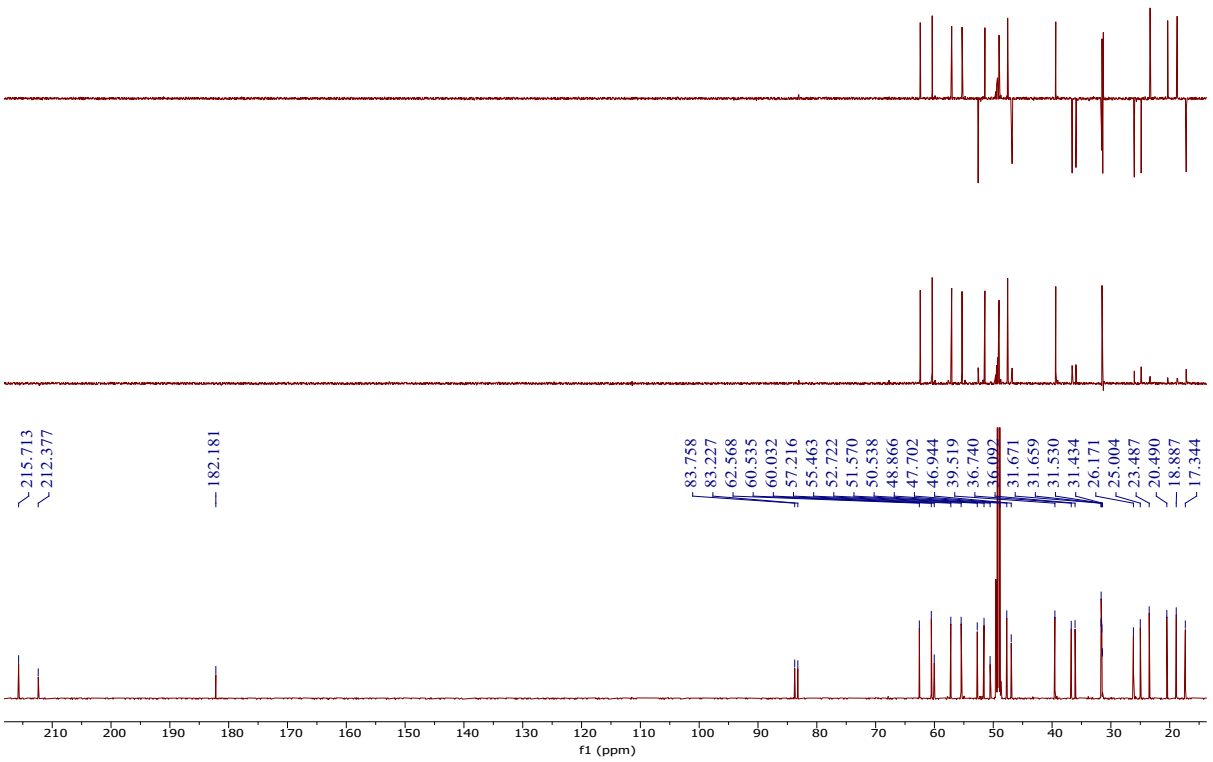


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	25.04	C29 H40 O5	[M+H]+	469.2952	469.2949	0.3	0.64	25.04	10.0

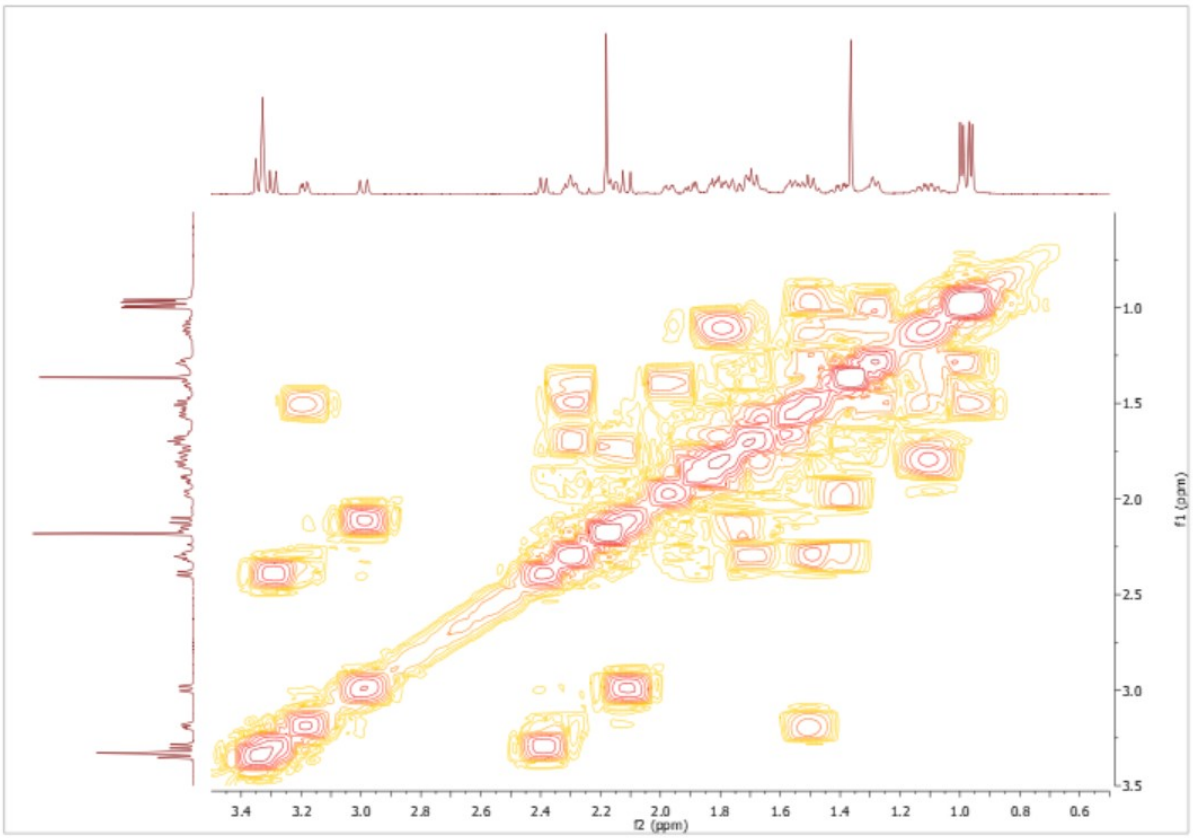
S11. ^{1}H NMR (600 MHz, CD_3OD) of compound **2**



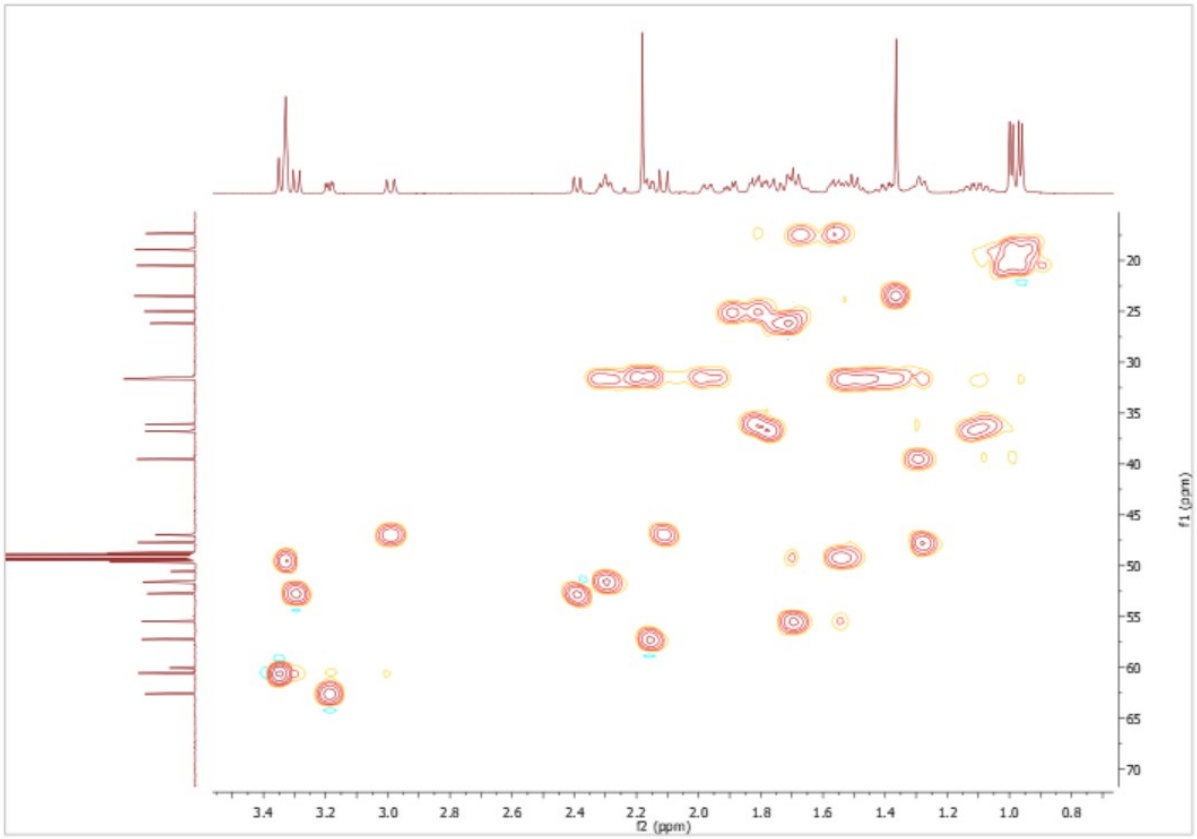
S12. ^{13}C NMR (DEPT) (150 MHz, CD_3OD) of compound **2**



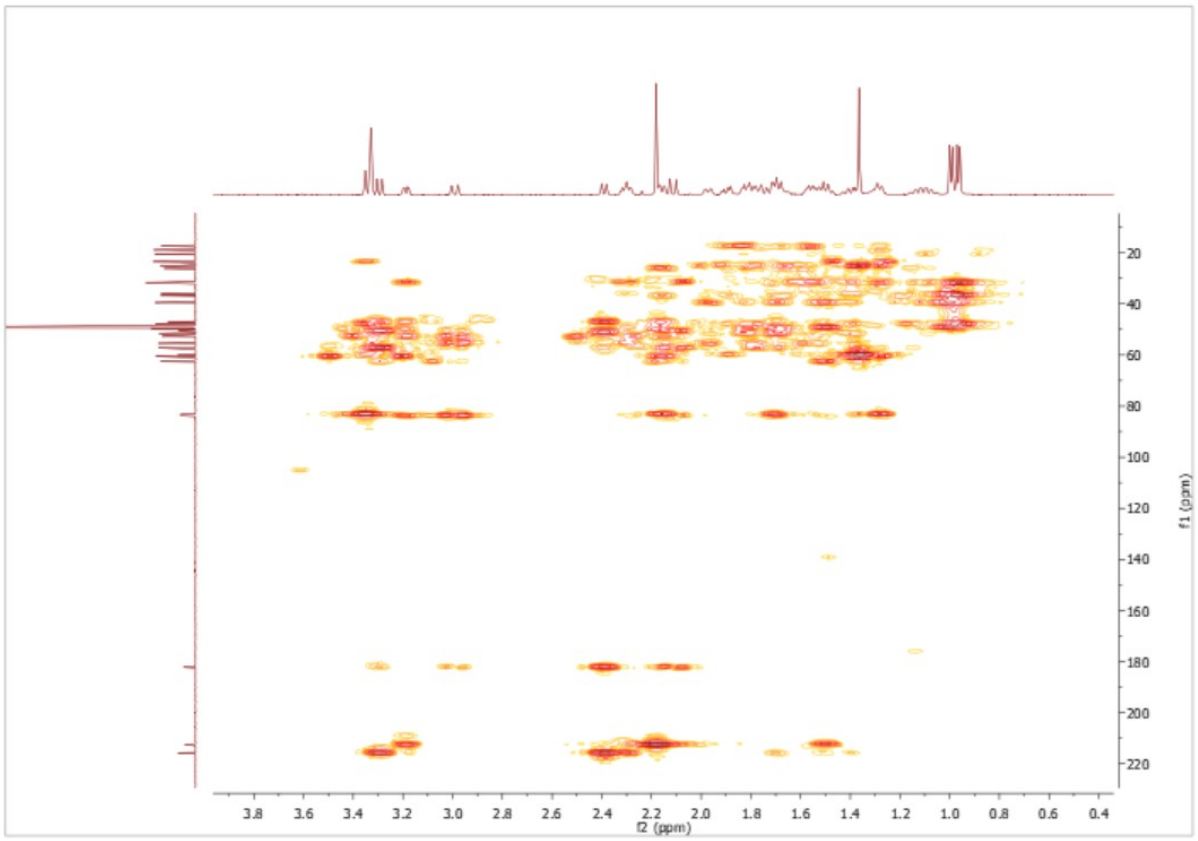
S13. ^{1}H-^{1}H COSY (600 MHz, CD_3OD) of compound **2**

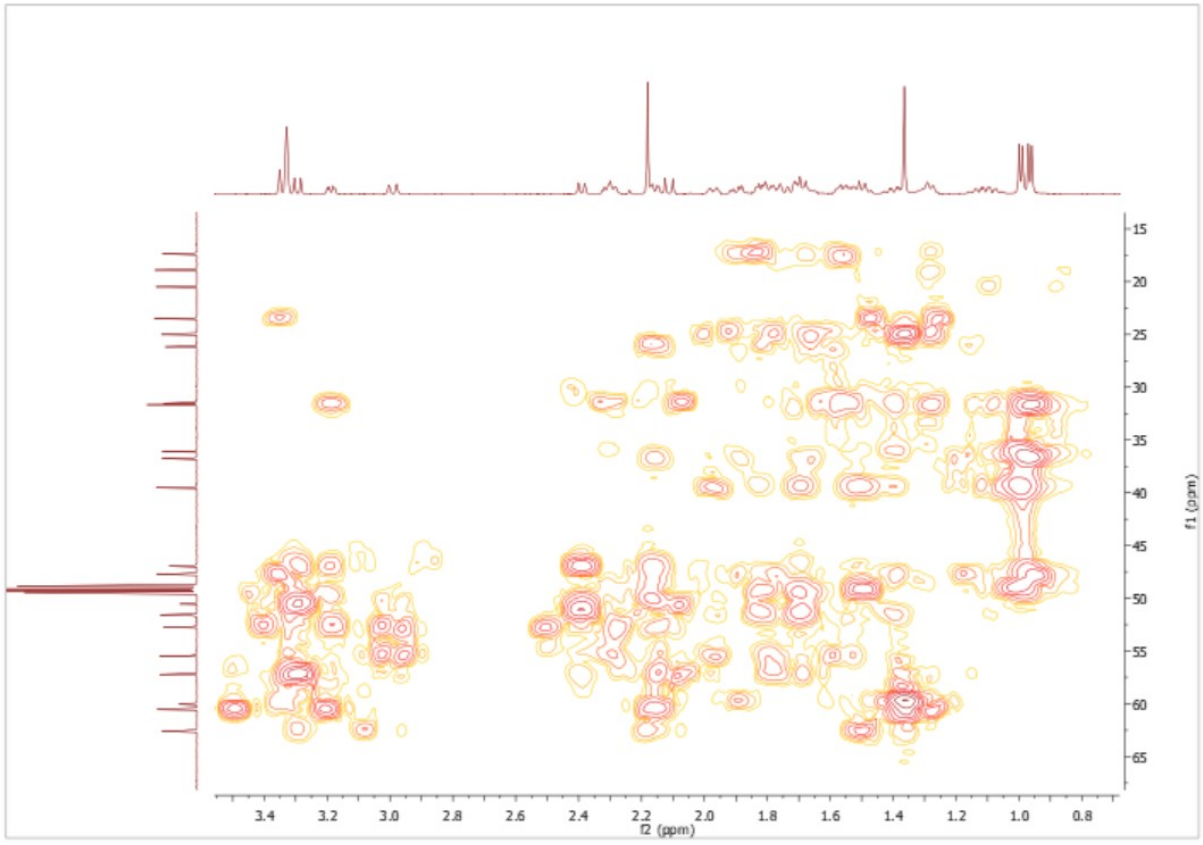


S14. HSQC (600 MHz, CD_3OD) of compound **2**

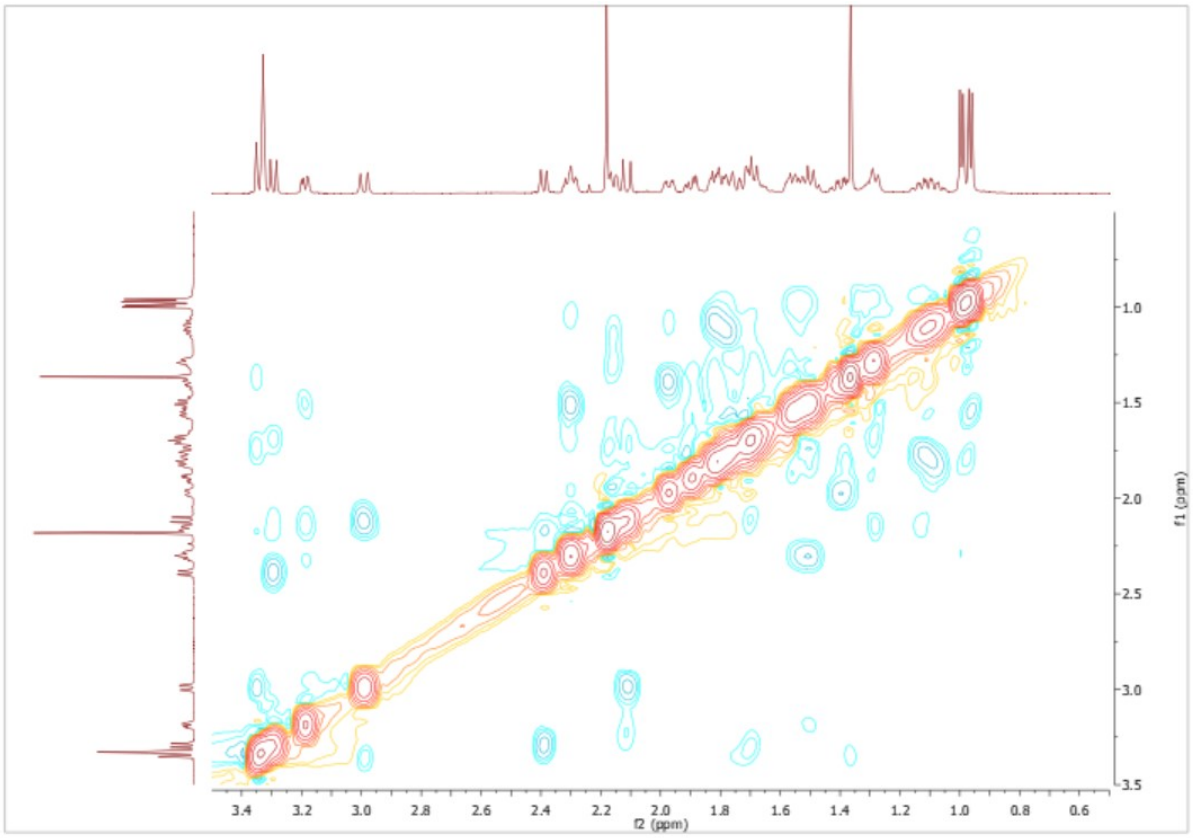


S15. HMBC (600 MHz, CD_3OD) of compound **2**





S16. ROESY (600 MHz, CD_3OD) of compound **2**



S17. $[\alpha]_D$ spectrum of compound **2** in MeOH

Rudolph Research Analytical

This sample was measured on an Autopol VI, Serial #91058
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : Tuesday, 10-AUG-2021

Set Temperature : OFF

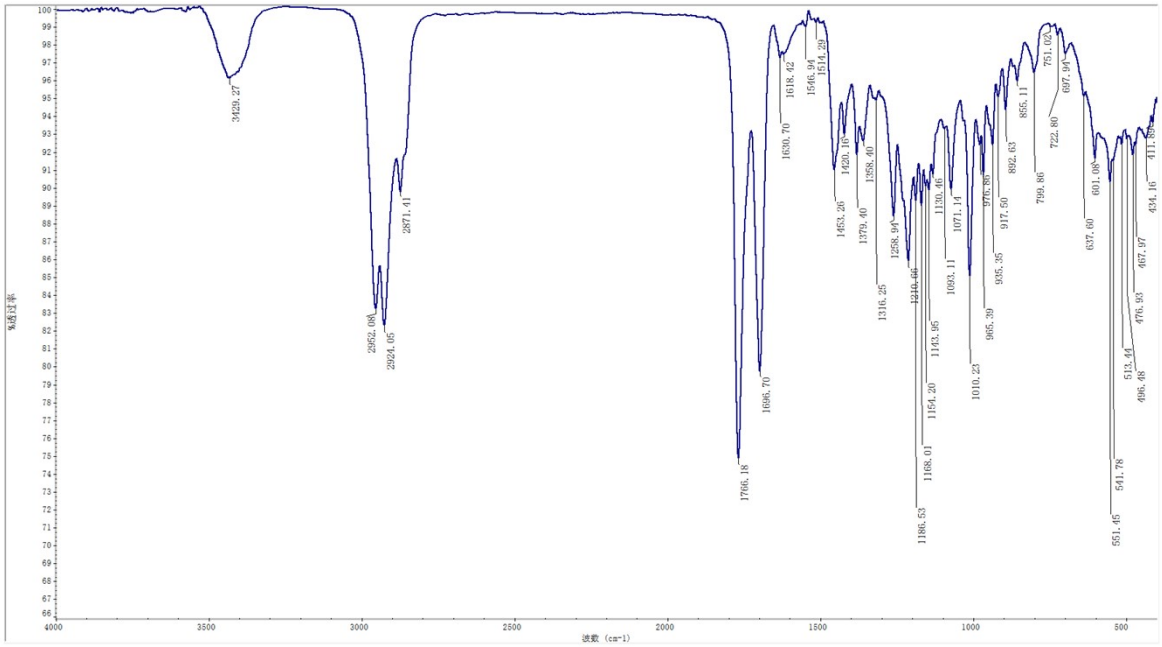
Time Delay : Disabled

Delay between Measurement : Disabled

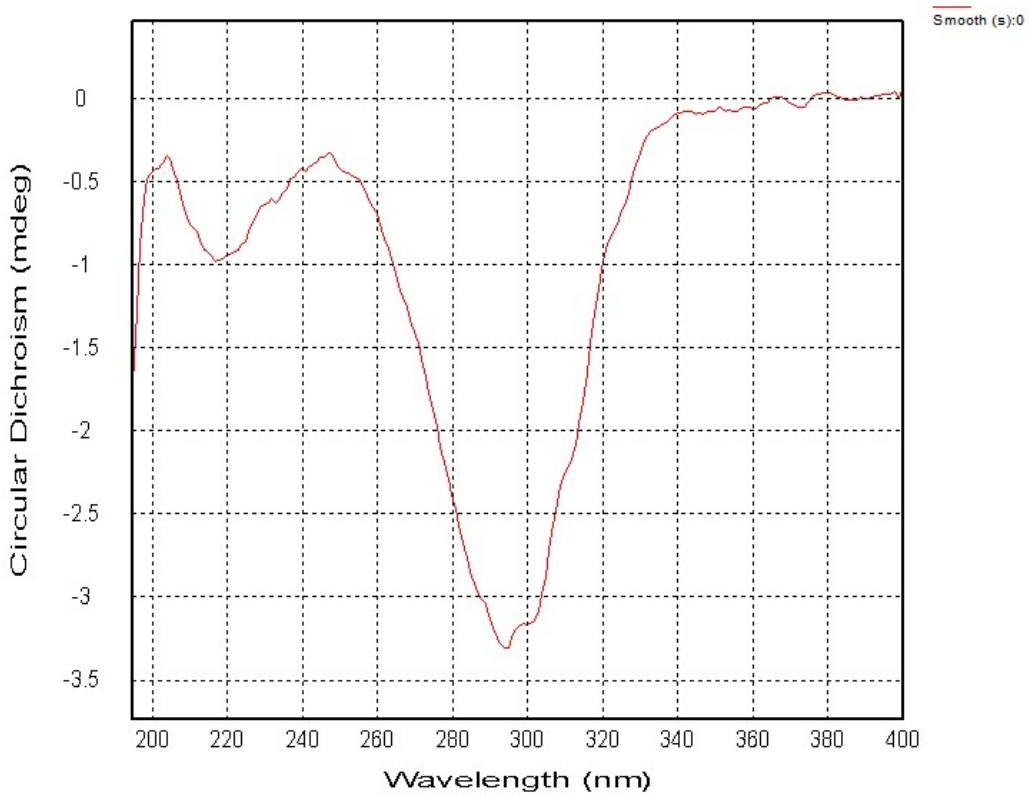
n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	-81.06	0.22	-0.27	-80.78	-81.35

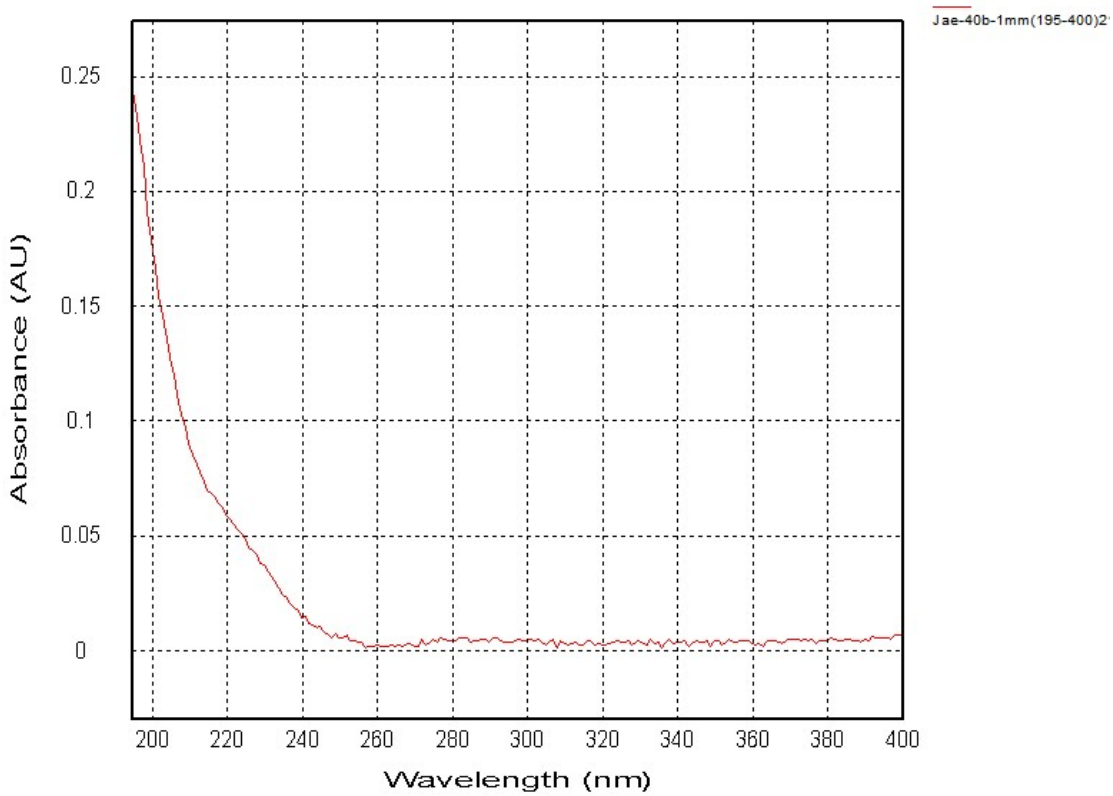
S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	jae-40b	10:01:57 AM	-80.99	SR	-0.1142	589	100.00	0.141	26.1
2	jae-40b	10:02:06 AM	-80.99	SR	-0.1142	589	100.00	0.141	26.1
3	jae-40b	10:02:14 AM	-80.78	SR	-0.1139	589	100.00	0.141	26.1
4	jae-40b	10:02:23 AM	-81.35	SR	-0.1147	589	100.00	0.141	26.1
5	jae-40b	10:02:30 AM	-81.21	SR	-0.1145	589	100.00	0.141	26.1

S18. IR of compound **2**



S19. ECD and UV of compound **2**



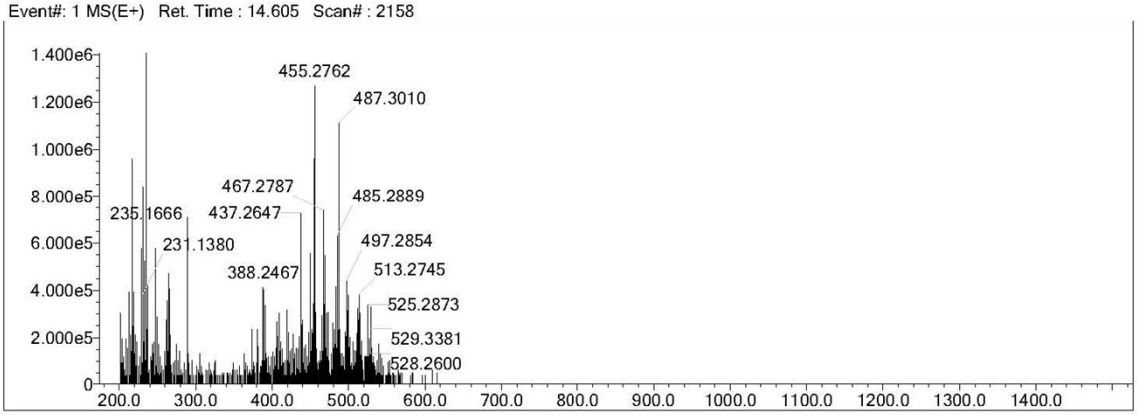


S20. HRESIMS of compound **2**

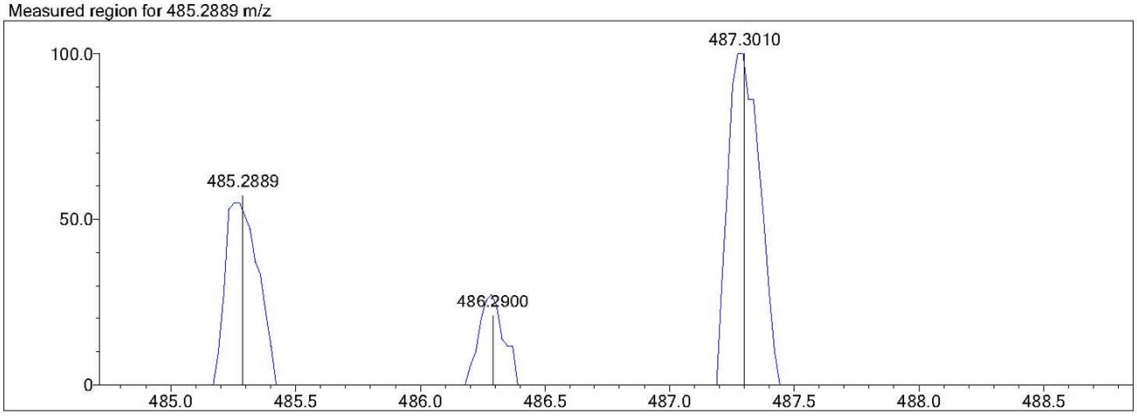
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	300	O	2	0	12	H
C	4	0	150	15N	3	0	0	Na
N	3	0	1					

Error Margin (ppm):	30	DBE Range:	-2.0 - 1000.0	Electron Ions:	both
HC Ratio:	unlimited	Apply N Rule:	yes	Use MSn Info:	no
Max Isotopes:	all	Isotope RI (%):	1.00	Isotope Res:	10000
MSn Iso RI (%):	75.00	MSn Logic Mode:	AND	Max Results:	500

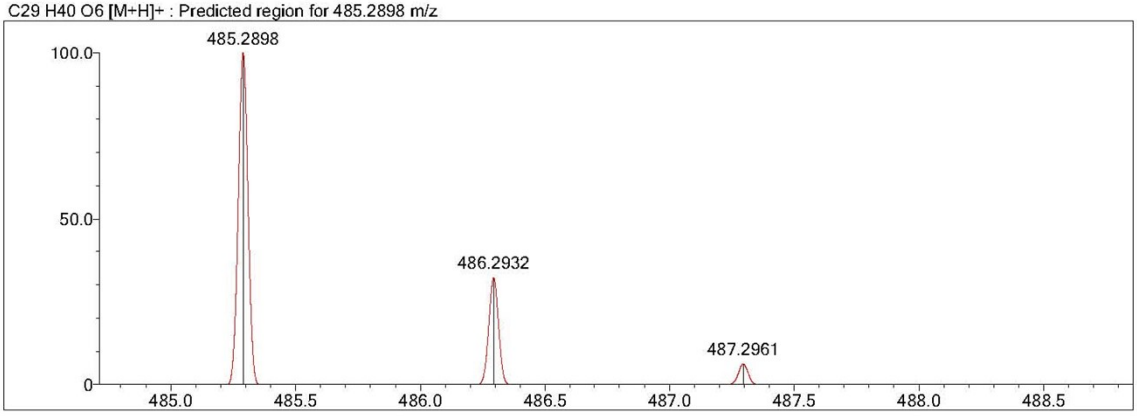
Event#: 1 MS(E+) Ret. Time : 14.605 Scan# : 2158



Measured region for 485.2889 m/z

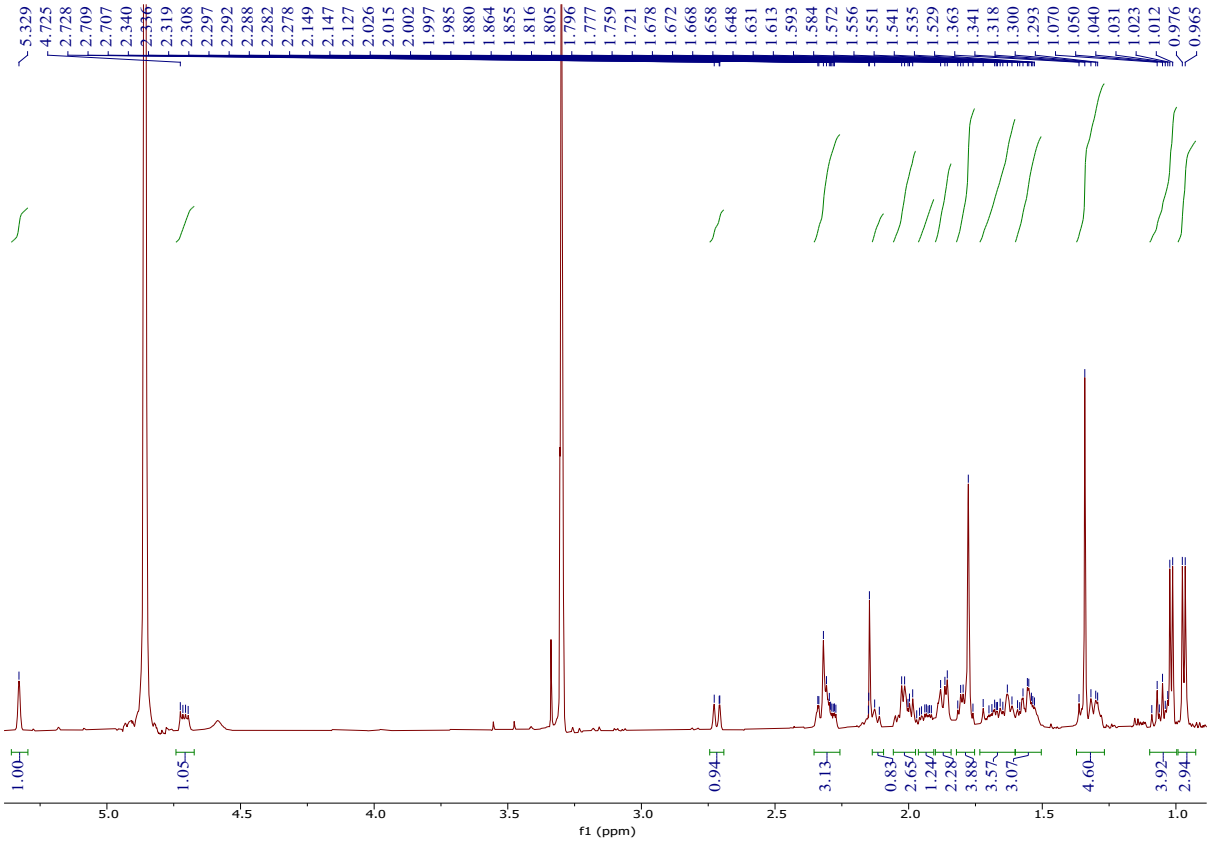


C29 H40 O6 [M+H]+ : Predicted region for 485.2898 m/z

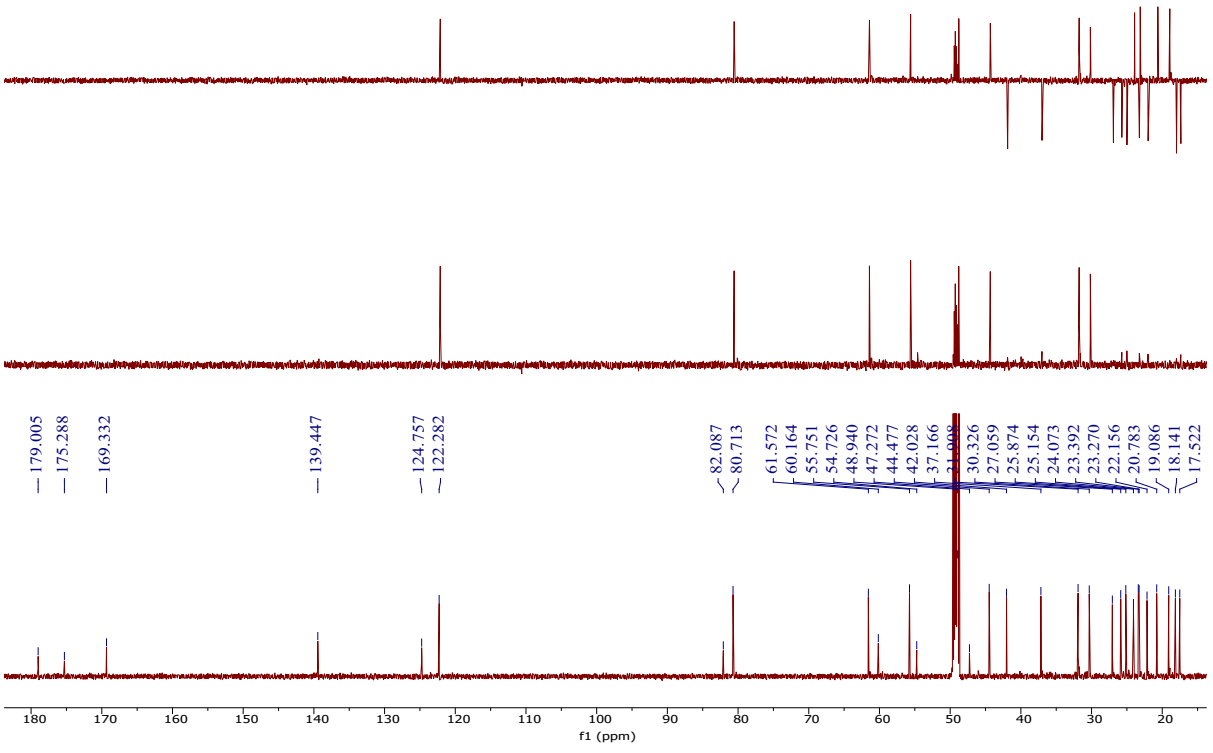


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	17.27	C29 H40 O6	[M+H]+	485.2889	485.2898	-0.9	-1.85	17.64	10.0

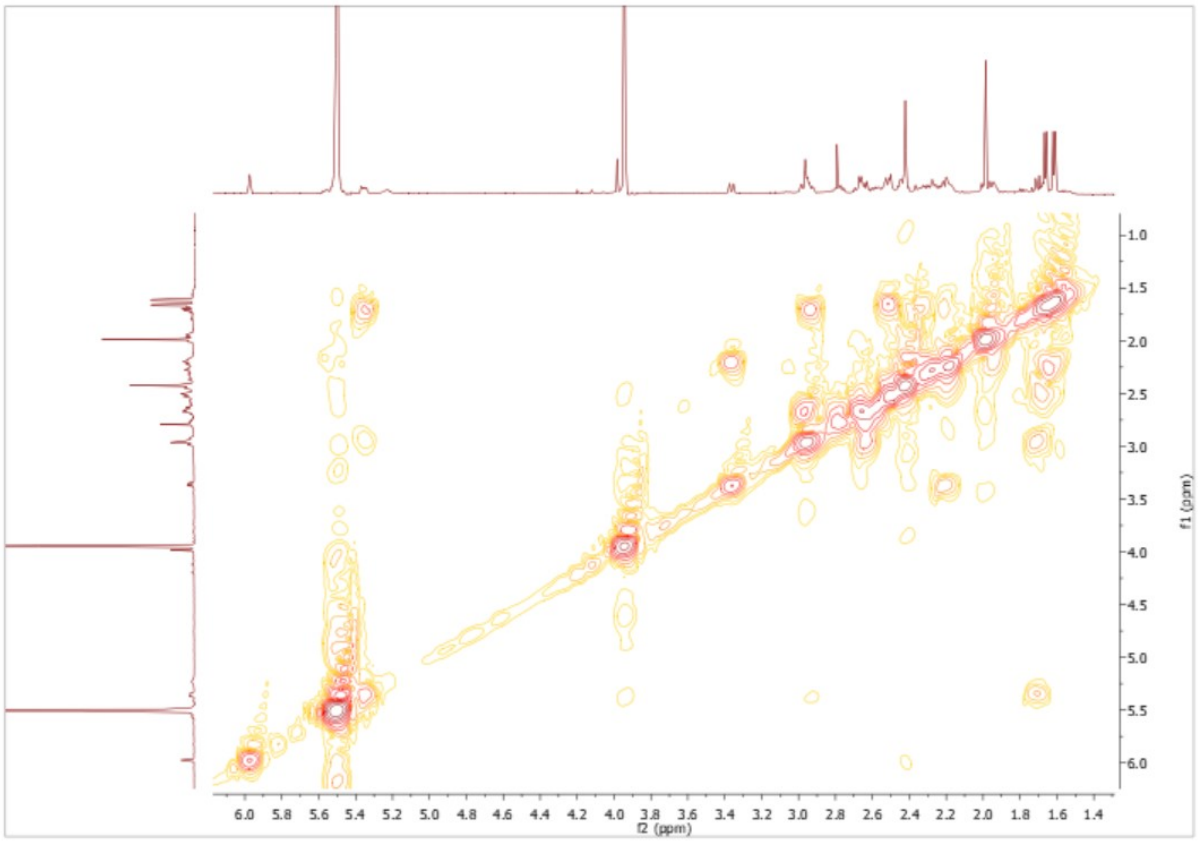
S21. ^{1}H NMR (600 MHz, CD_3OD) of compound **3**



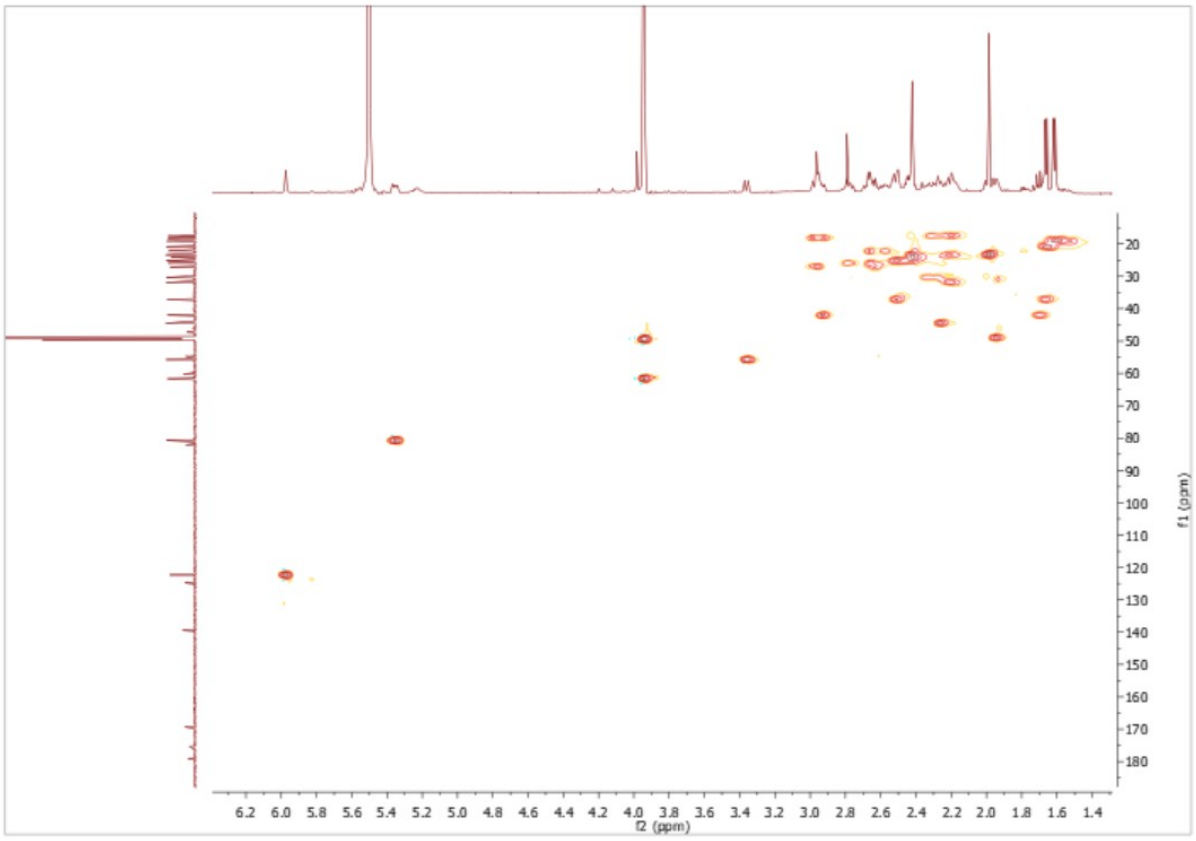
S22. ^{13}C NMR (DEPT) (150 MHz, CD_3OD) of compound **3**

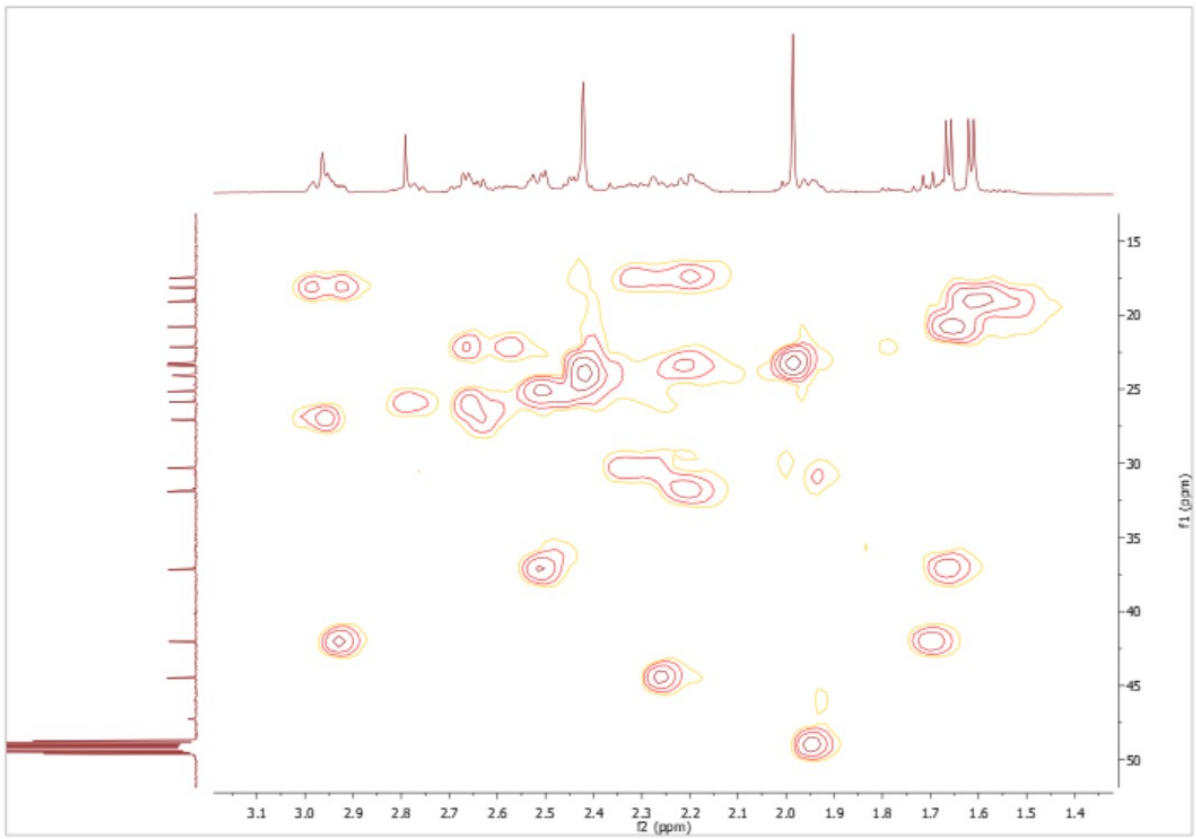


S23. ^{1}H-^{1}H COSY (600 MHz, CD_3OD) of compound **3**

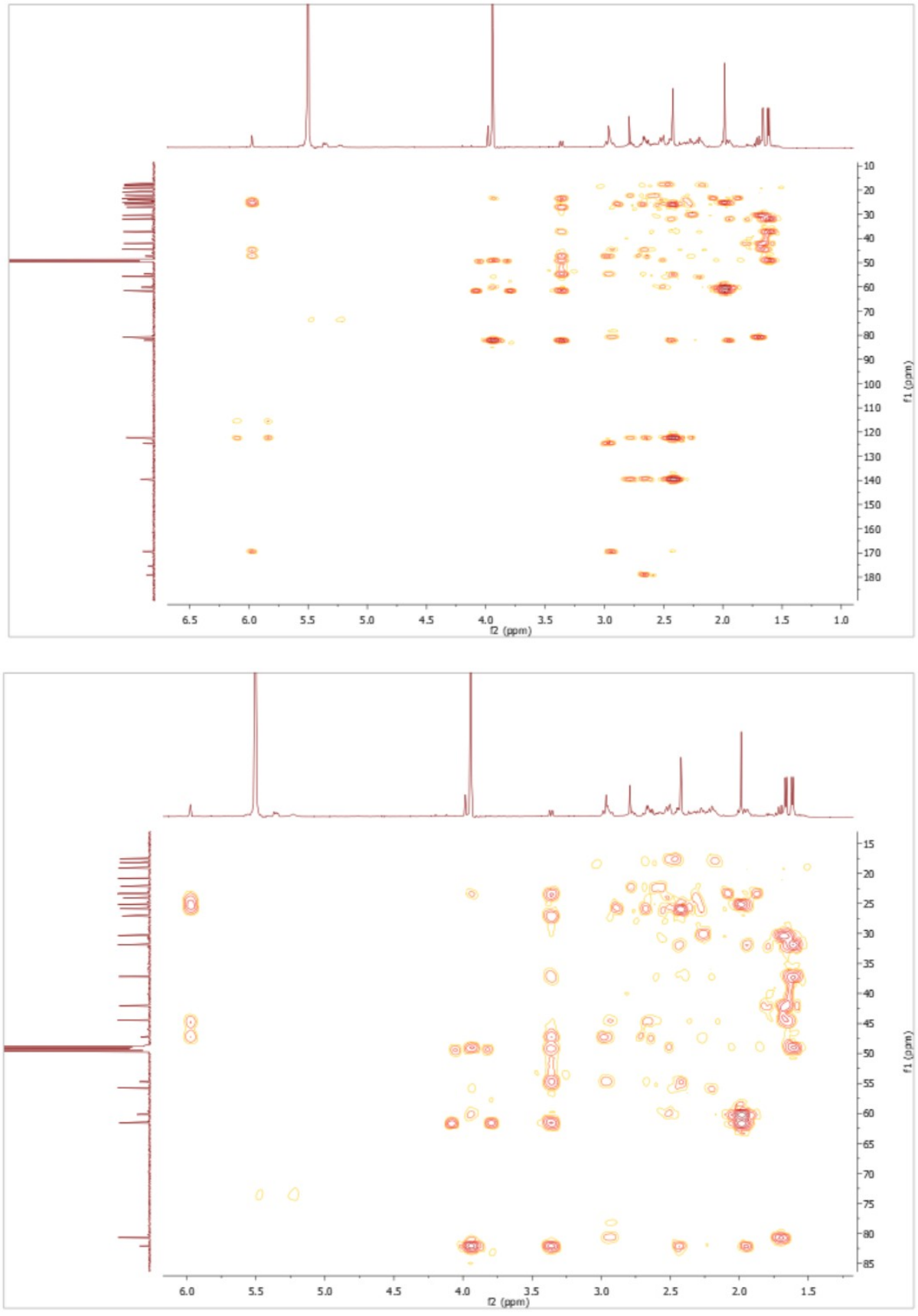


S24. HSQC (600 MHz, CD_3OD) of compound **3**

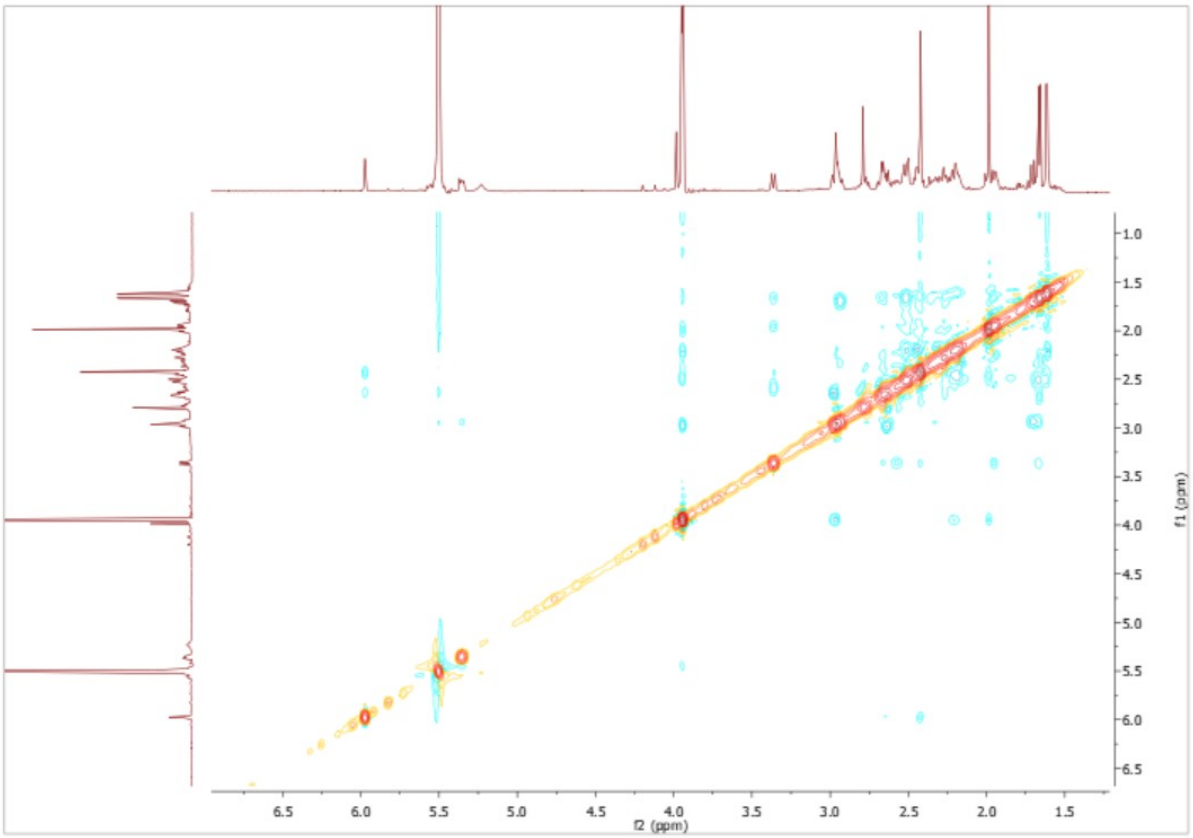




S25. HMBC (600 MHz, CD_3OD) of compound **3**



S26. ROESY (600 MHz, CD_3OD) of compound **3**



S27. $[\alpha]_D$ spectrum of compound **3** in MeOH

Rudolph Research Analytical

This sample was measured on an Autopol VI, Serial #91058
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : Tuesday, 10-AUG-2021

Set Temperature : OFF

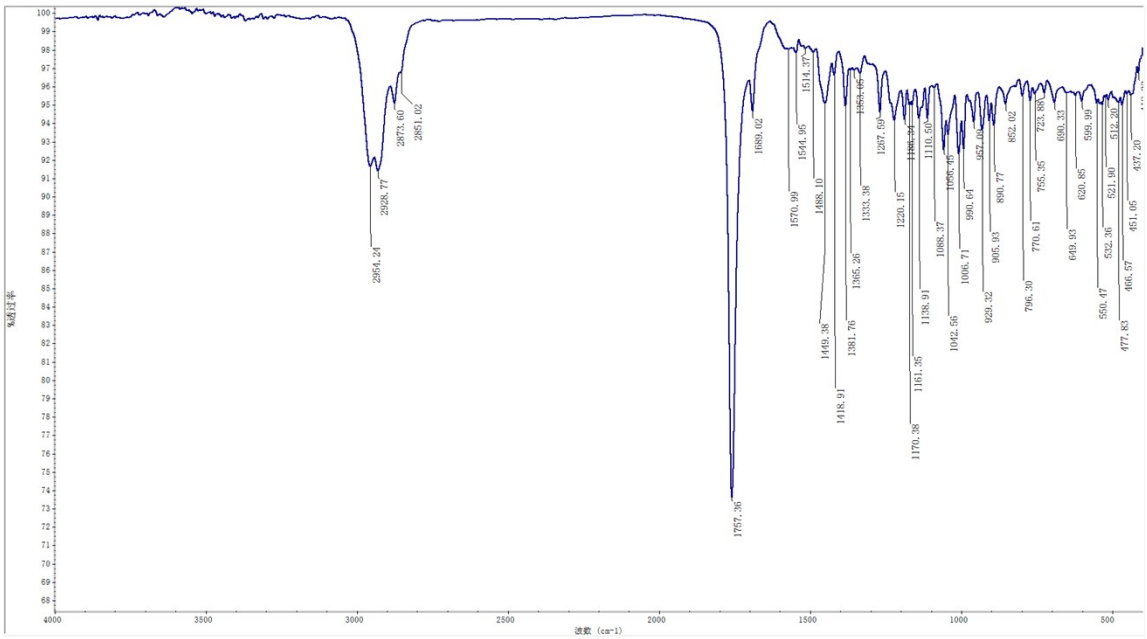
Time Delay : Disabled

Delay between Measurement : Disabled

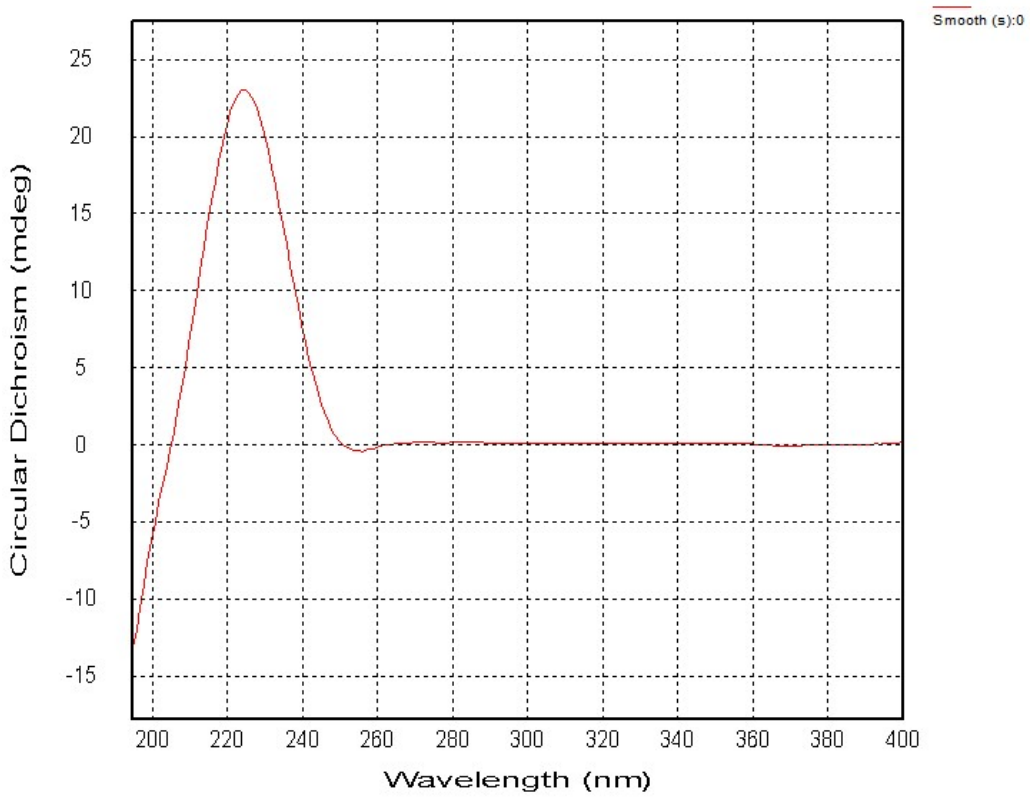
n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	38.59	0.57	1.47	39.57	38.19

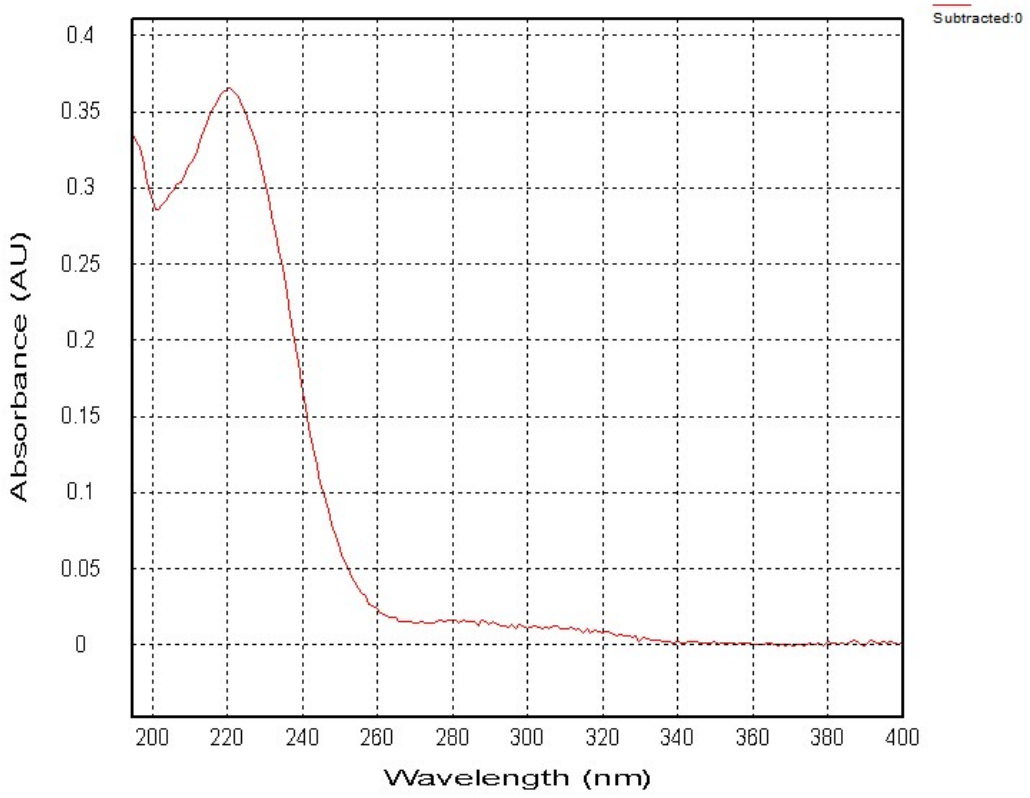
S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	jae-42b	10:40:22 AM	39.57	SR	0.0459	589	100.00	0.116	26.6
2	jae-42b	10:40:30 AM	38.45	SR	0.0446	589	100.00	0.116	26.6
3	jae-42b	10:40:38 AM	38.53	SR	0.0447	589	100.00	0.116	26.6
4	jae-42b	10:40:47 AM	38.19	SR	0.0443	589	100.00	0.116	26.7
5	jae-42b	10:40:55 AM	38.19	SR	0.0443	589	100.00	0.116	26.7

S28. IR of compound **3**



S29. ECD and UV of compound **3**





S30. HRESIMS of compound **3**

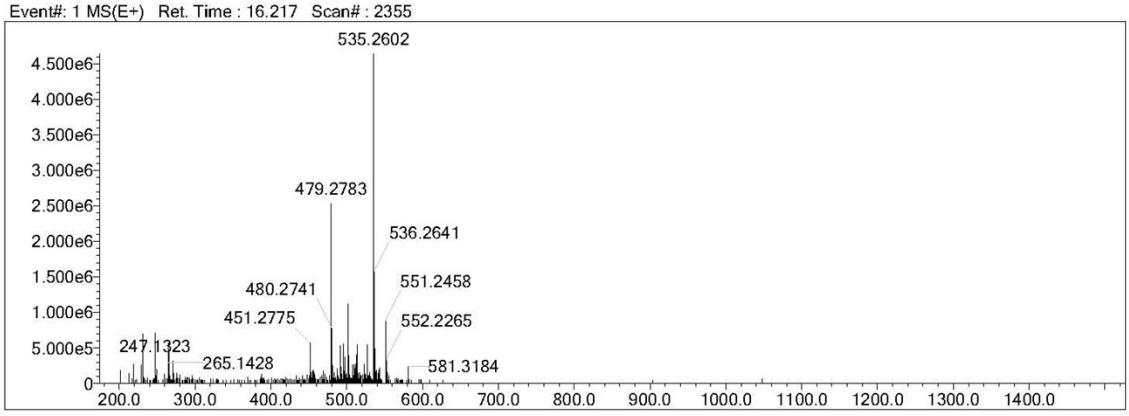
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	300	O	2	0	12	H
C	4	0	150	15N	3	0	0	Na
N	3	0	1					

Error Margin (ppm): 30
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

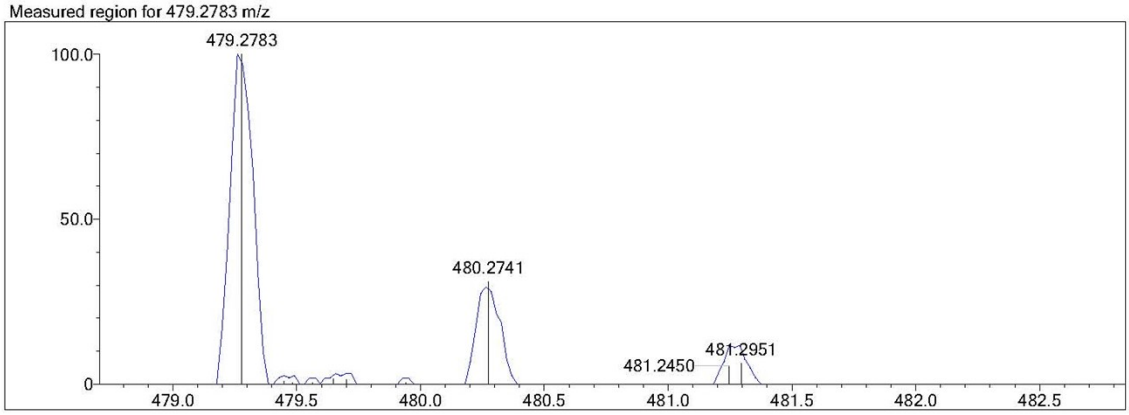
DBE Range: -2.0 - 1000.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 500

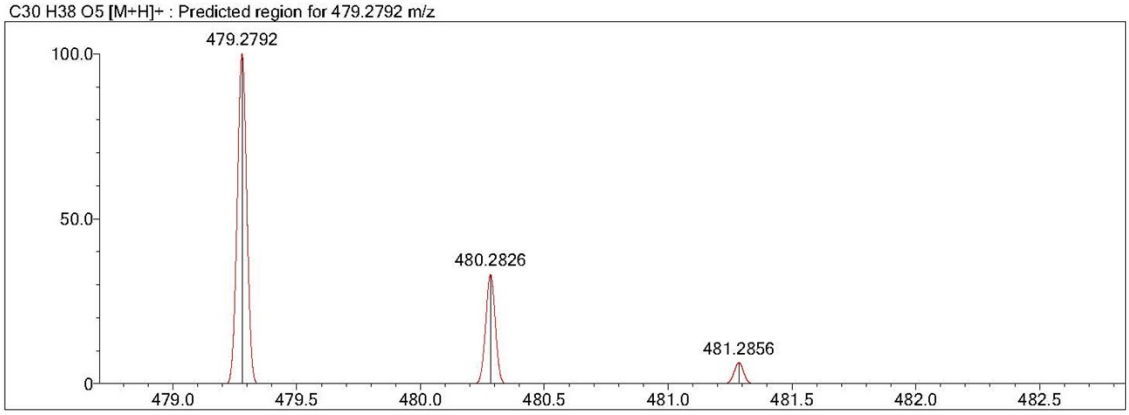
Event#: 1 MS(E+) Ret. Time : 16.217 Scan# : 2355



Measured region for 479.2783 m/z

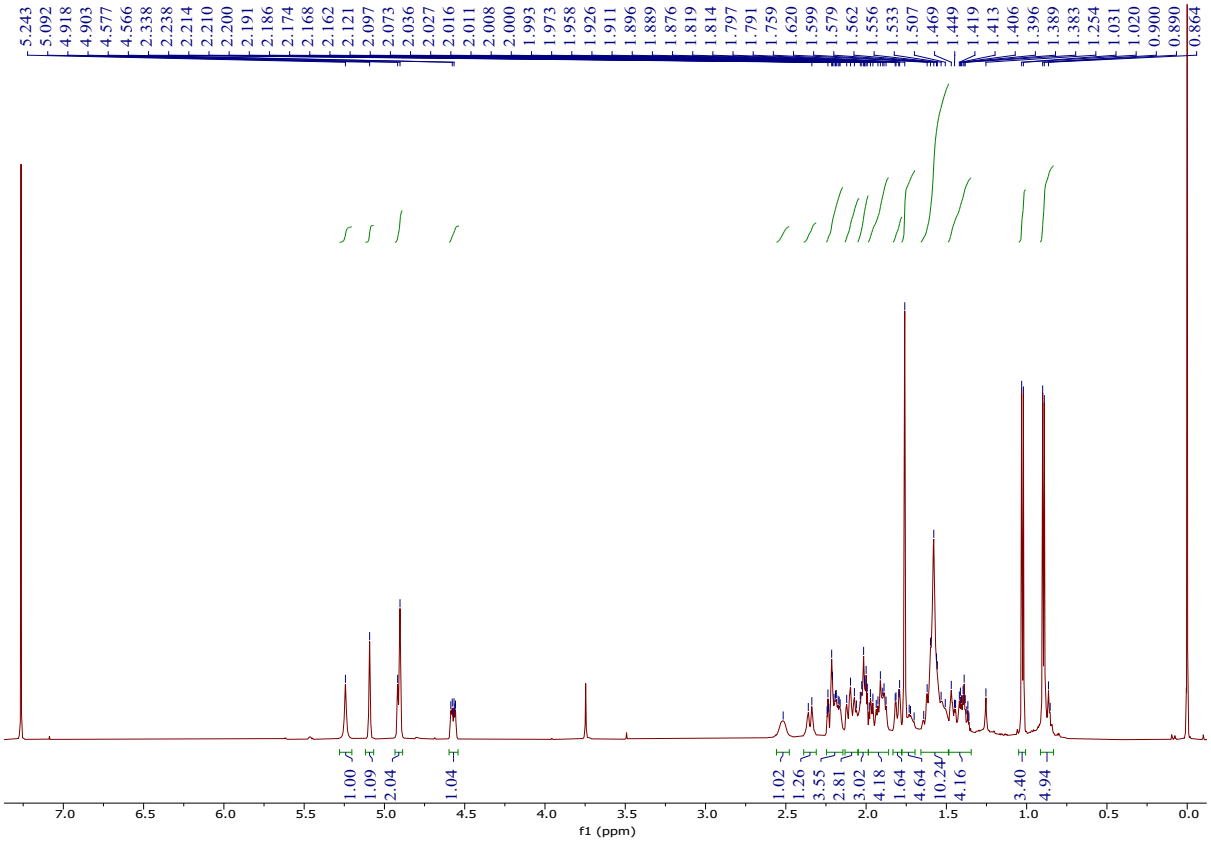


C30 H38 O5 [M+H]+ : Predicted region for 479.2792 m/z

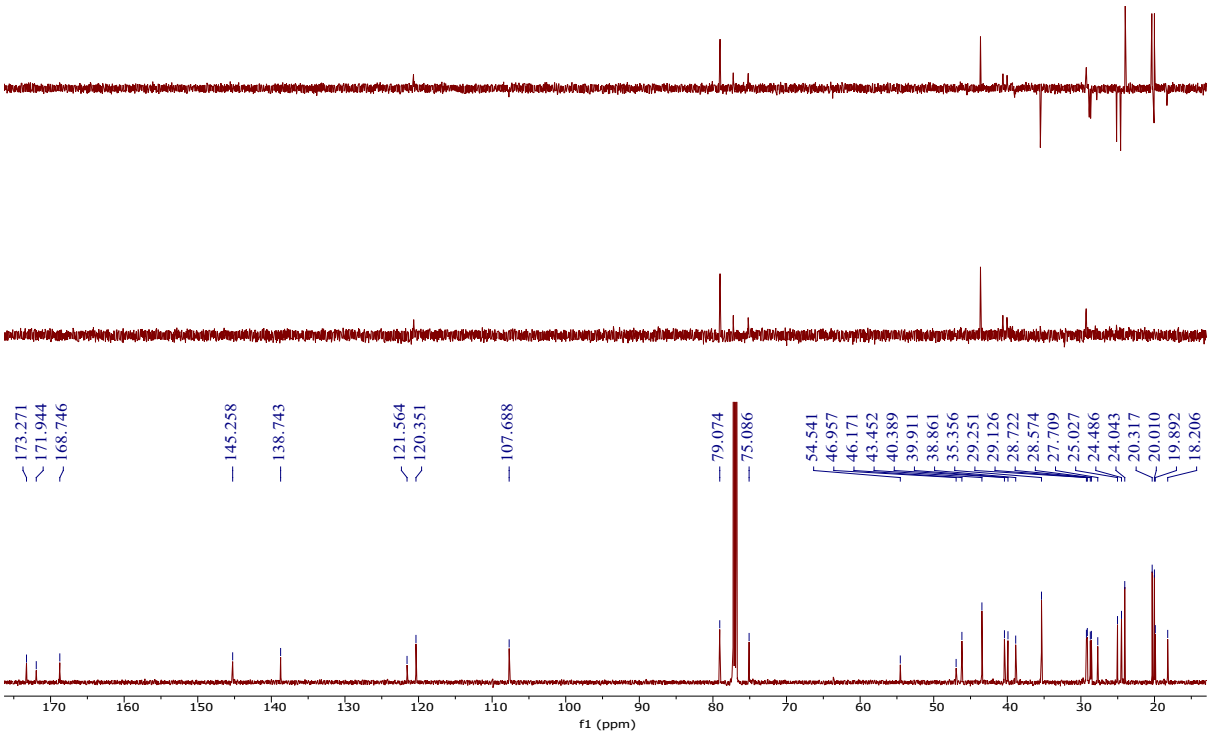


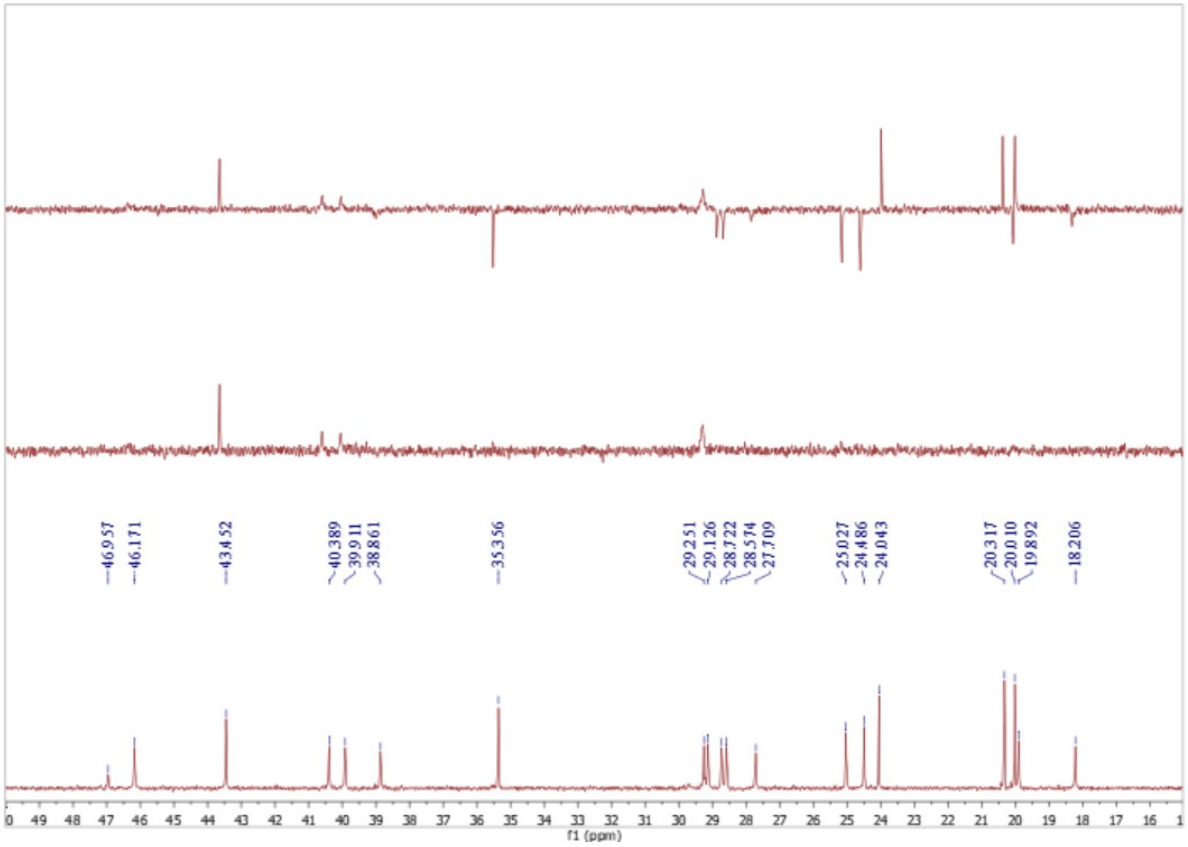
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	59.26	C30 H38 O5	[M+H]+	479.2783	479.2792	-0.9	-1.88	60.60	12.0

S31. ^{1}H NMR (600 MHz, $CDCl_3$) of compound **4**

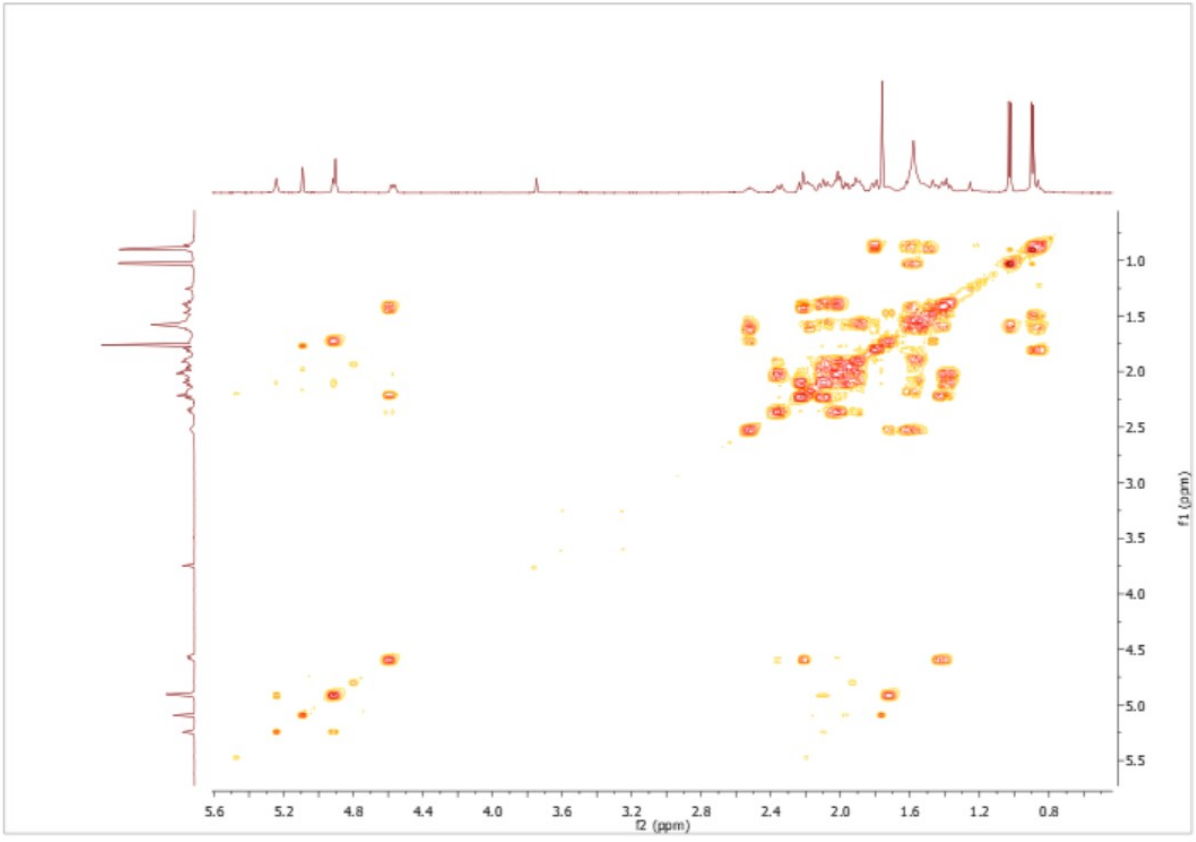


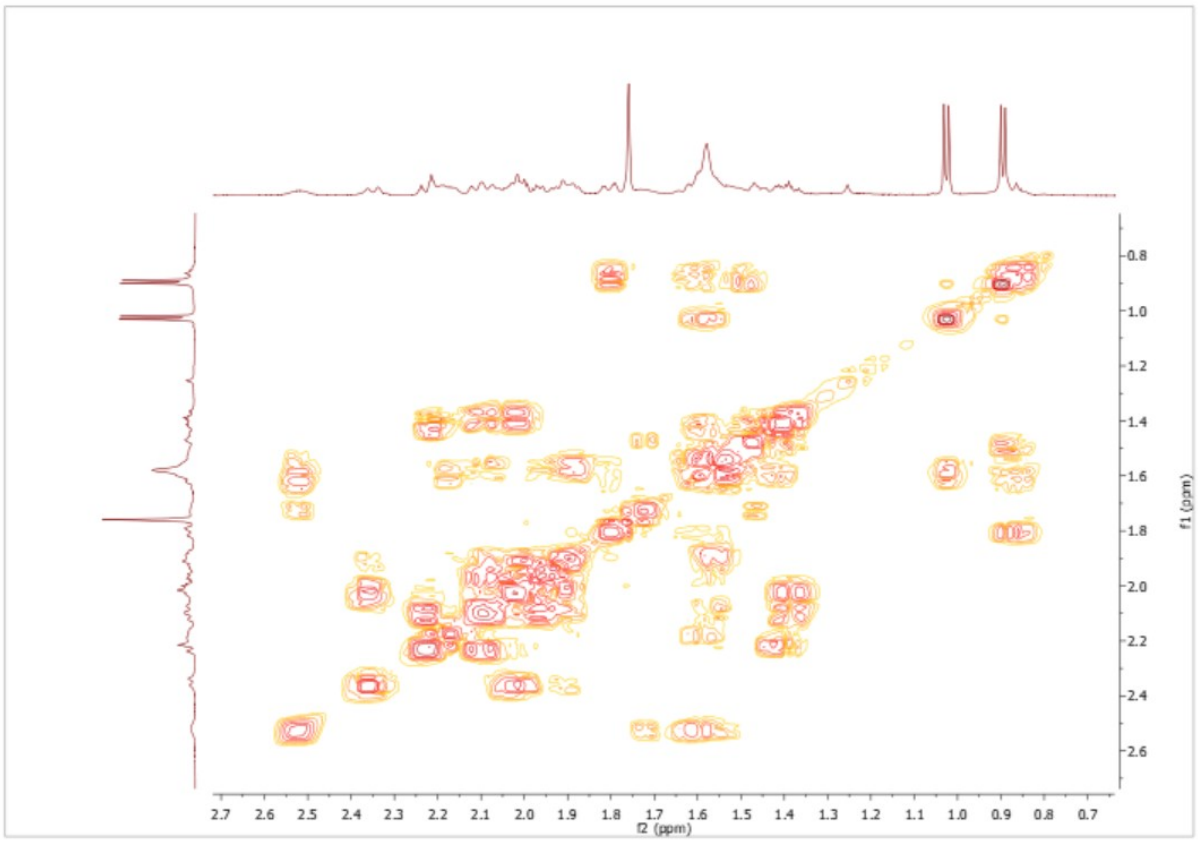
S32. ^{13}C NMR (DEPT) (150 MHz, $CDCl_3$) of compound **4**



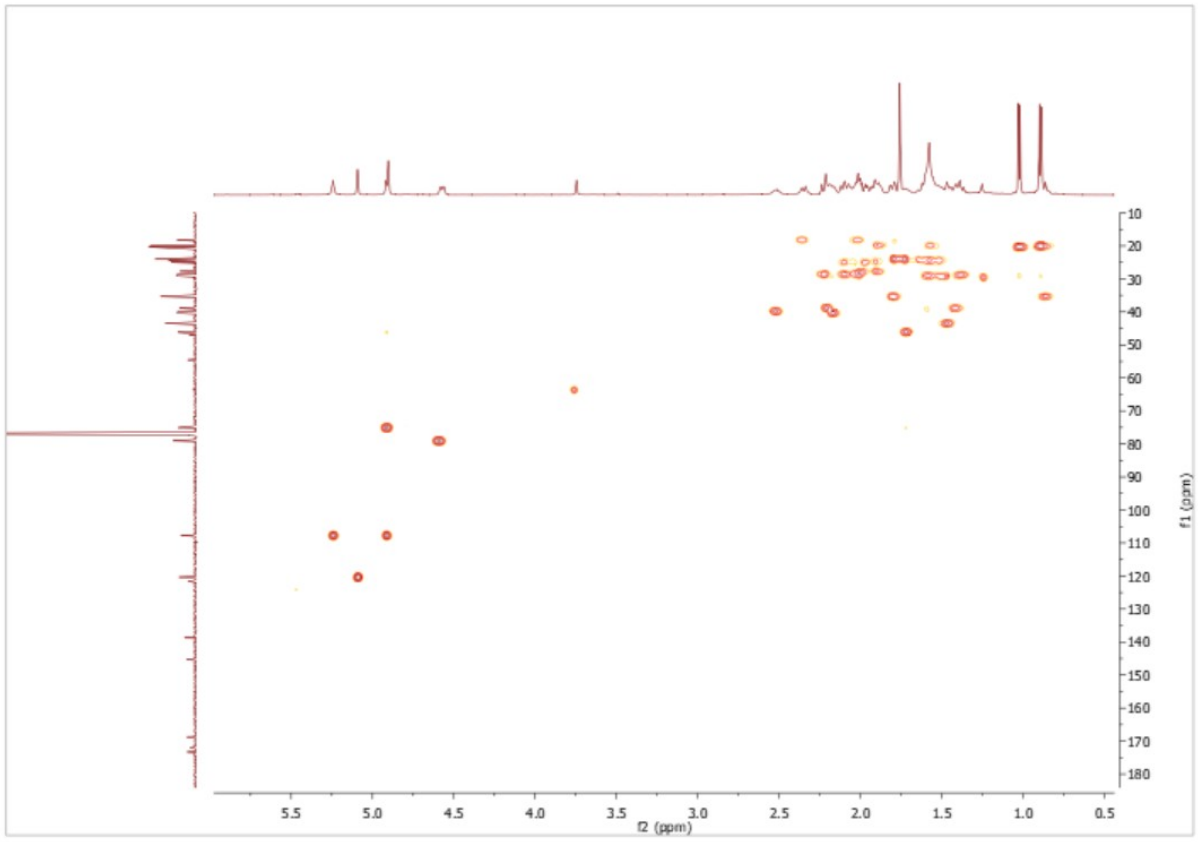


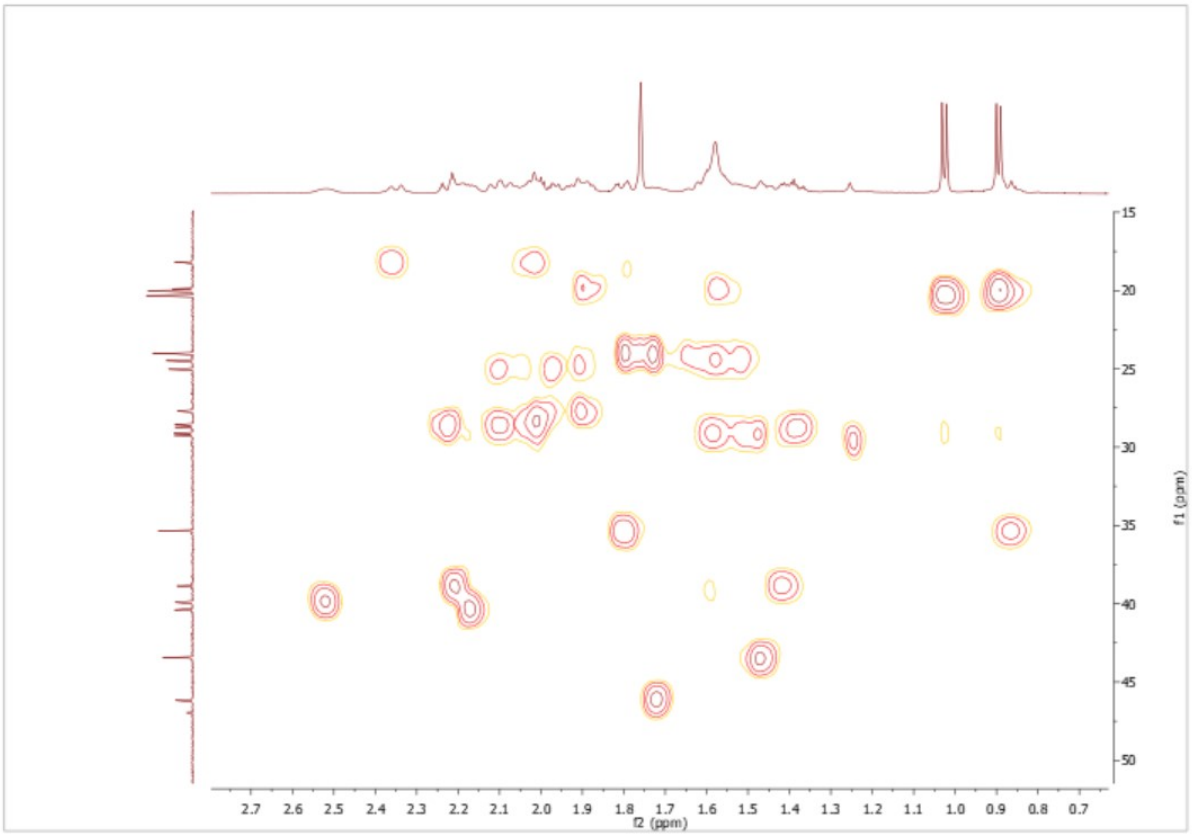
S33. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) of compound **4**



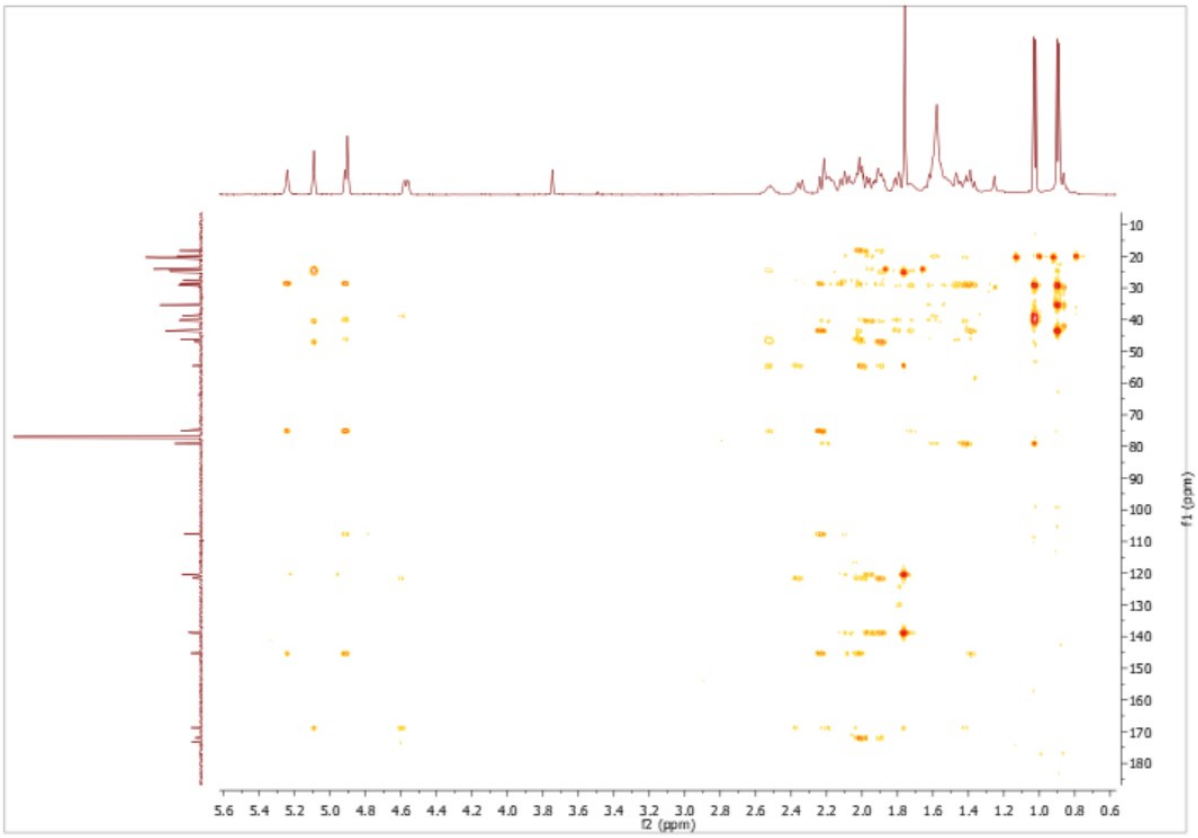


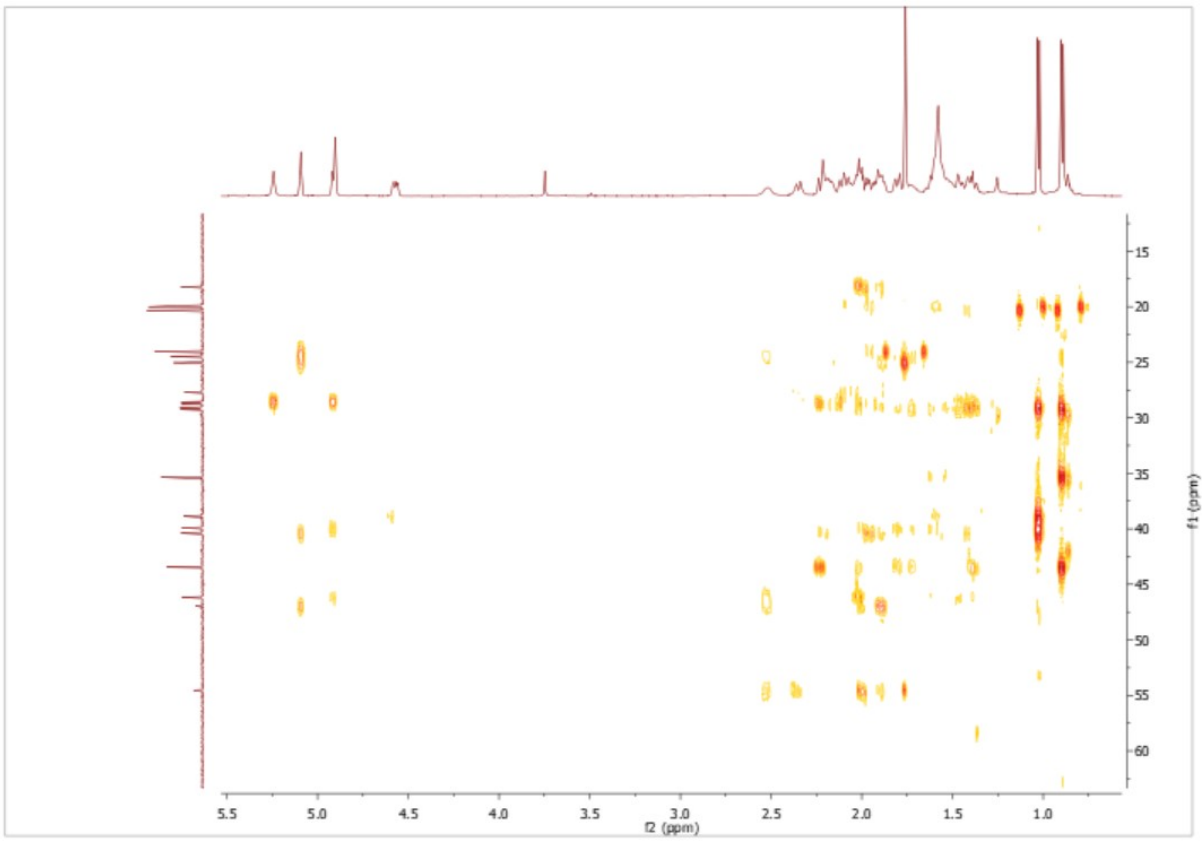
S34. HSQC (600 MHz, $CDCl_3$) of compound **4**



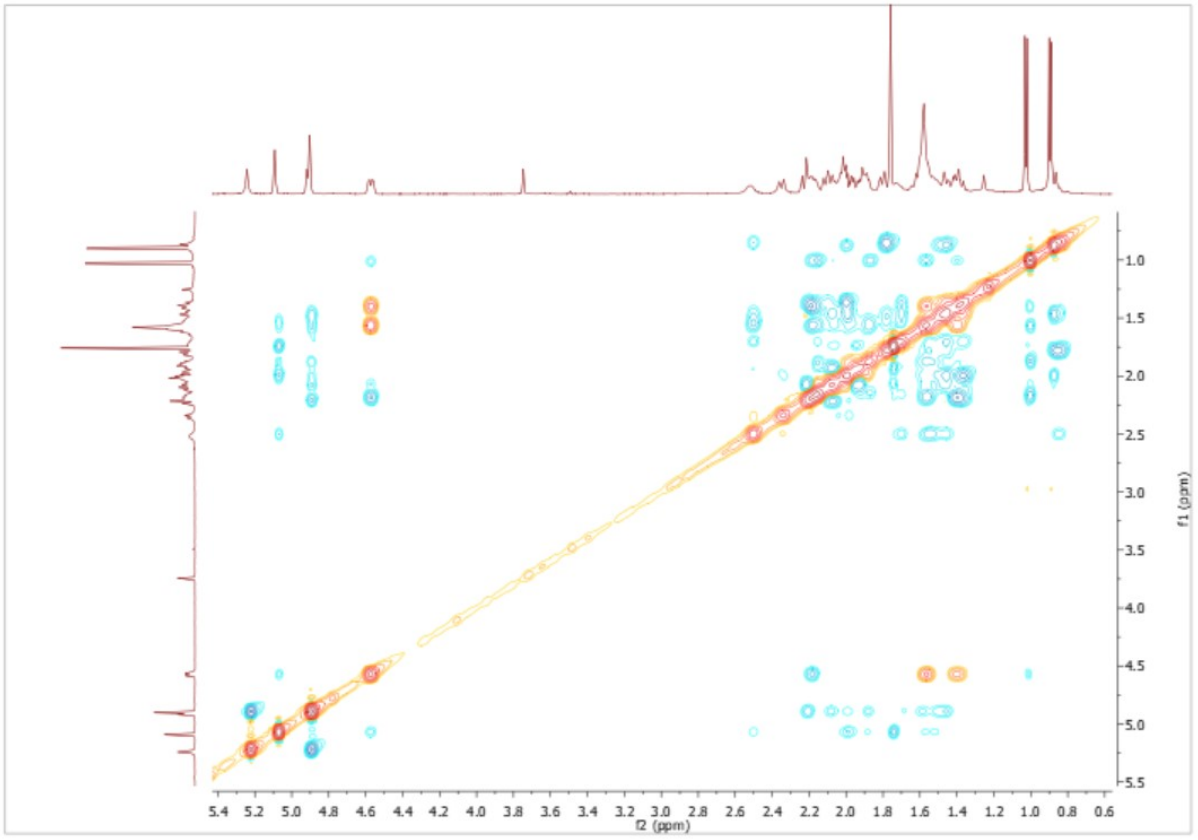


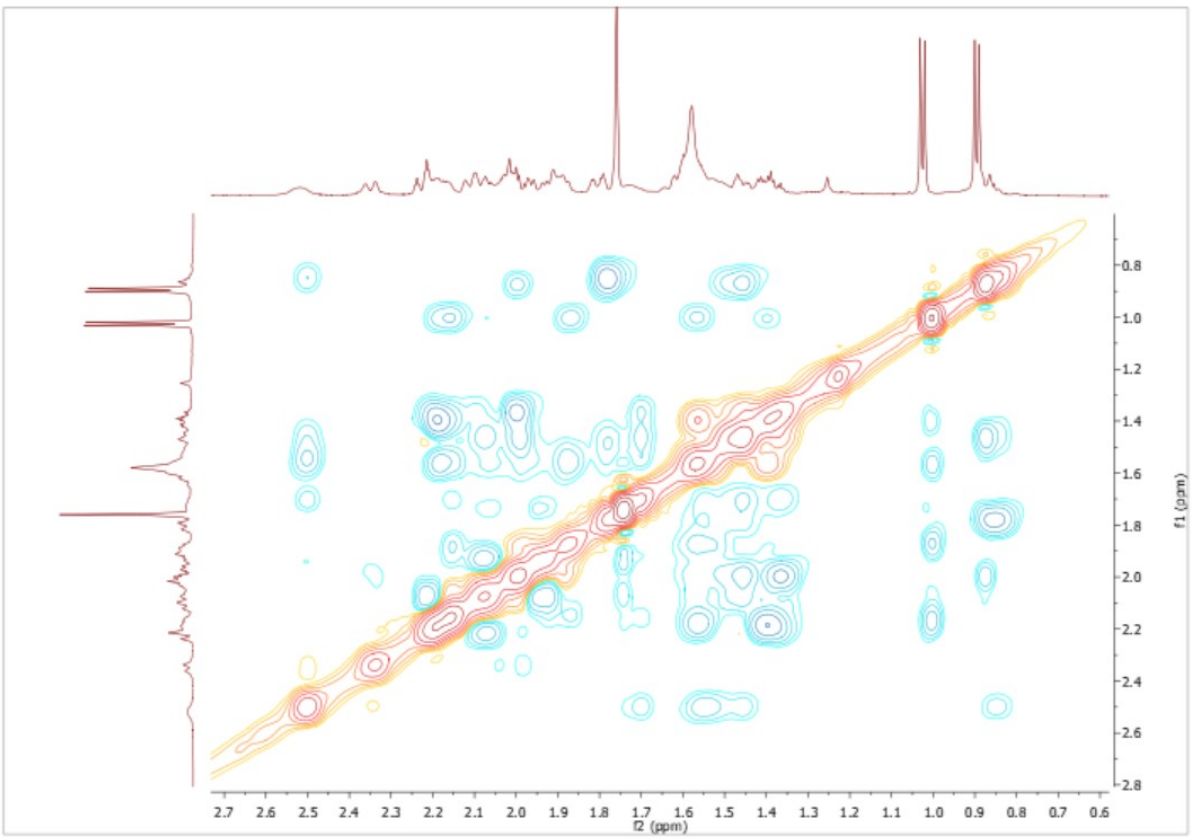
S35. HMBC (600 MHz, $CDCl_3$) of compound **4**





S36. ROESY (600 MHz, $CDCl_3$) of compound **4**





S37. $[\alpha]_D$ spectrum of compound **4** in MeOH

Rudolph Research Analytical

This sample was measured on an Autopol VI, Serial #91058
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : Tuesday, 08-JUN-2021

Set Temperature : OFF

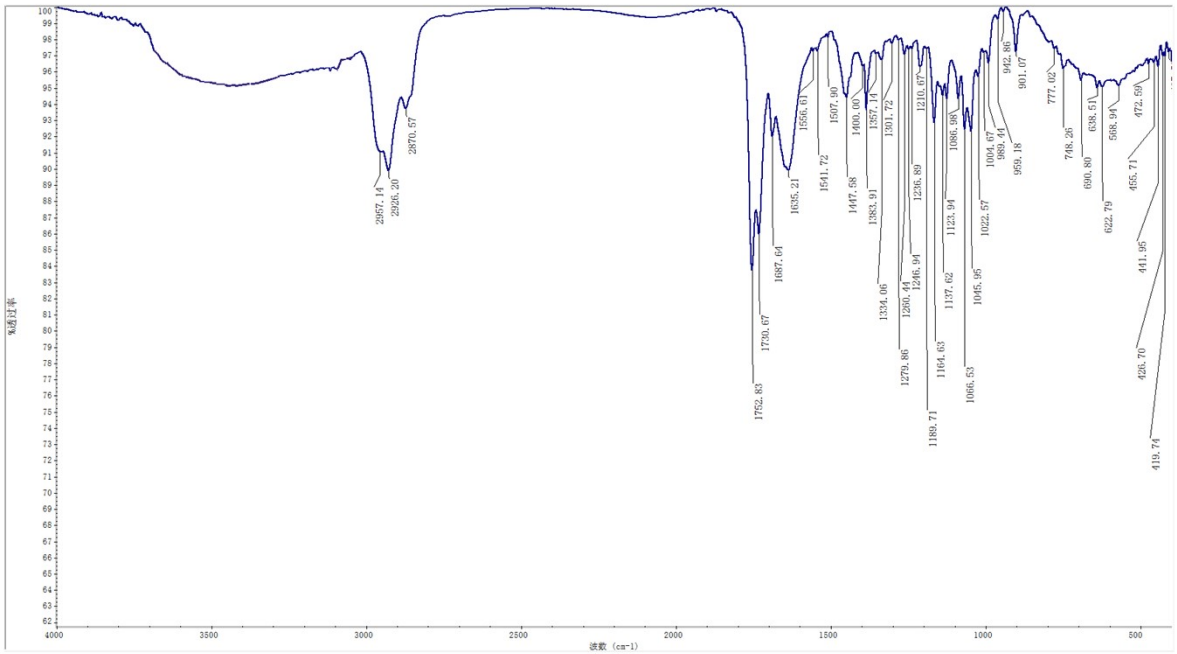
Time Delay : Disabled

Delay between Measurement : Disabled

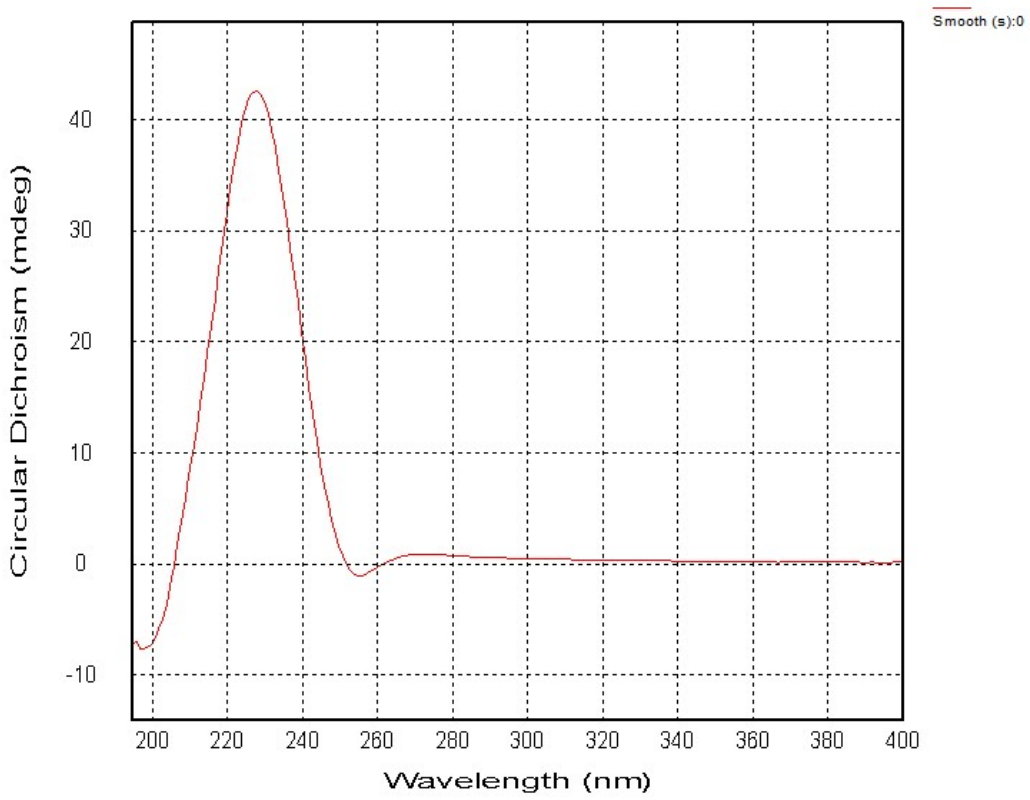
n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	114.85	0.50	0.43	115.55	114.35

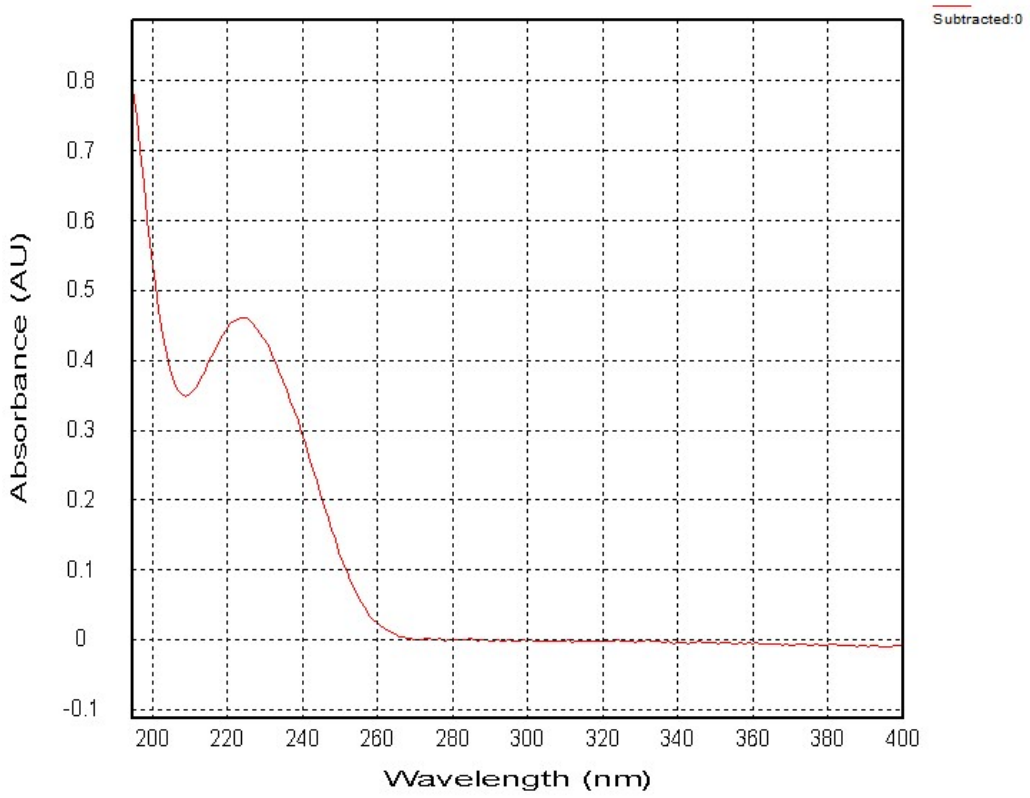
S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	jae-10a-4	01:09:24 PM	114.55	SR	0.2291	589	100.00	0.200	25.3
2	jae-10a-4	01:09:33 PM	114.35	SR	0.2287	589	100.00	0.200	25.3
3	jae-10a-4	01:09:41 PM	115.55	SR	0.2311	589	100.00	0.200	25.3
4	jae-10a-4	01:09:49 PM	115.20	SR	0.2304	589	100.00	0.200	25.3
5	jae-10a-4	01:09:57 PM	114.60	SR	0.2292	589	100.00	0.200	25.3

S38. IR of compound **4**



S39. ECD and UV of compound **4**





S40. HRESIMS of compound **4**

Elmt	Val.	Min	Max
H	1	0	300
C	4	0	150
N	3	0	0

Elmt	Val.	Min	Max
O	2	0	12
15N	3	0	0

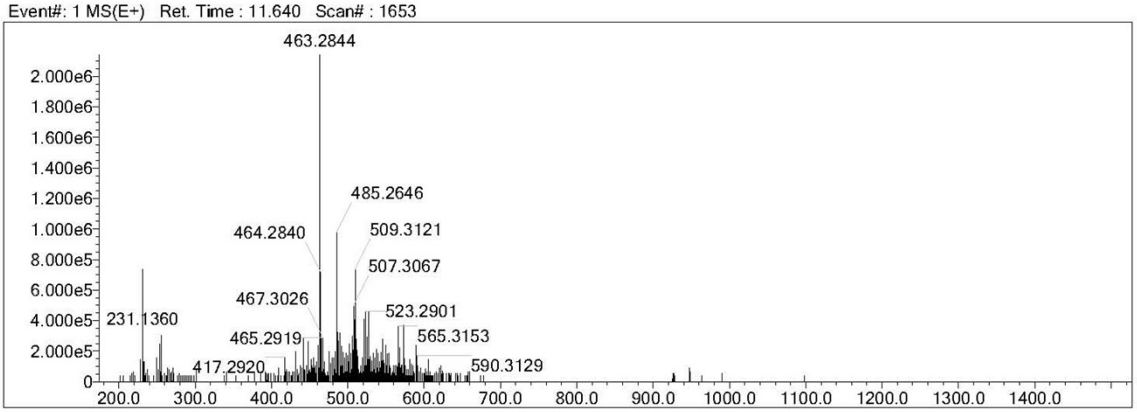
Use Adduct
H
Na

Error Margin (ppm): 30
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

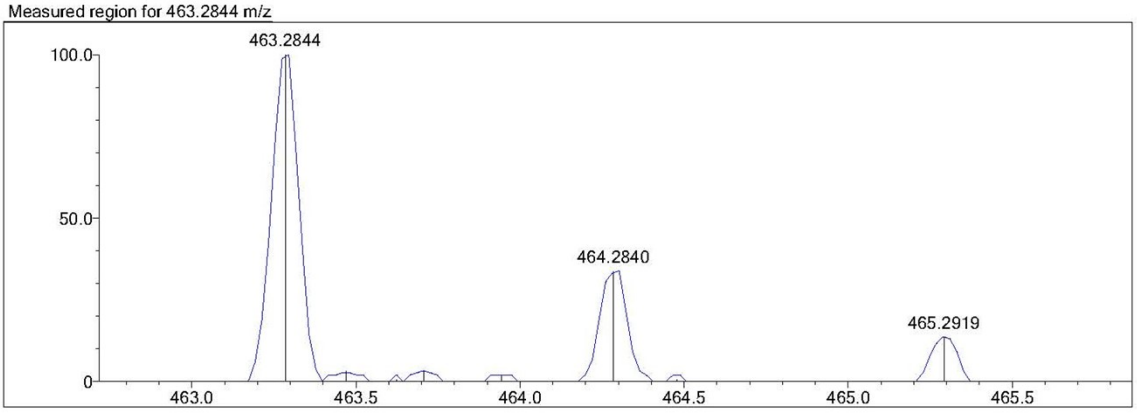
DBE Range: -2.0 - 1000.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 500

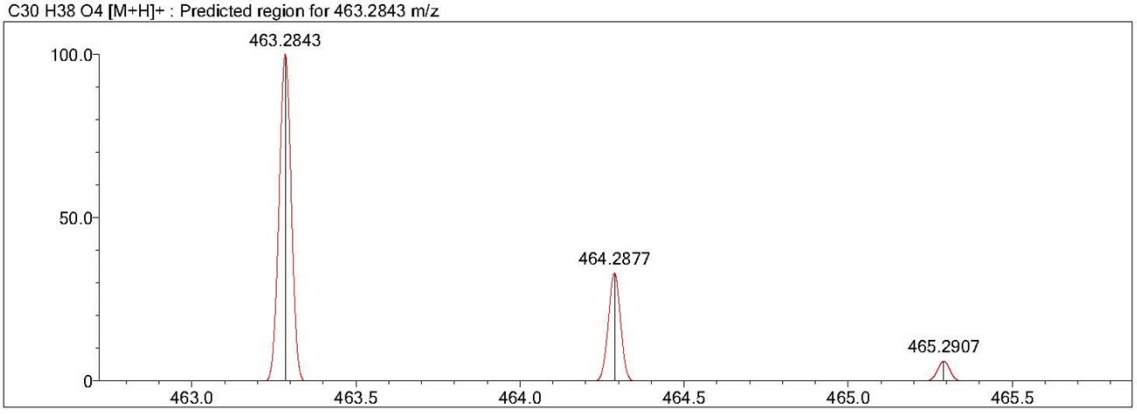
Event#: 1 MS(E+) Ret. Time : 11.640 Scan# : 1653



Measured region for 463.2844 m/z



C30 H38 O4 [M+H]+ : Predicted region for 463.2843 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	54.26	C30 H38 O4	[M+H]+	463.2844	463.2843	0.1	0.22	54.26	12.0