

SUPPLEMENTARY INFORMATION

Artemannuols A–C, three novel sesquiterpenoid-flavonol hybrids with antihepatoma activity from *Artemisia annua*

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List of Supplementary Information

General Experimental Instruments and Procedures	4
ECD Calculations	4
Antihepatoma assay	4
Figure S1. Key chemical shifts difference of 1-bisabolen-3-ols according to the references (<i>J. Nat. Prod.</i> , 2014, 77, 1708–1717; <i>J. Chem. Eco.</i> , 2014, 40, 1260–1268)	5
S1. ¹ H NMR (600 MHz, acetone-d ₆) of compound 1	6
S2. ¹³ C NMR (DEPT) (150 MHz, acetone-d ₆) of compound 1	7
S3. ¹ H- ¹ H COSY (600 MHz, acetone-d ₆) of compound 1	8
S4. HSQC (600 MHz, acetone-d ₆) of compound 1	8
S5. HMBC (600 MHz, acetone-d ₆) of compound 1	9
S6. ROESY (600 MHz, acetone-d ₆) of compound 1	9
S7. [α] _D spectrum of compound 1 in MeOH	10
S8. IR of compound 1	10
S9. ECD and UV of compound 1	11
S10. HRESIMS of compound 1	12
S11. ¹ H NMR (600 MHz, acetone-d ₆) of compound 2	13
S12. ¹³ C NMR (DEPT) (150 MHz, acetone-d ₆) of compound 2	14
S13. ¹ H- ¹ H COSY (600 MHz, acetone-d ₆) of compound 2	15
S14. HSQC (600 MHz, acetone-d ₆) of compound 2	15
S15. HMBC (600 MHz, acetone-d ₆) of compound 2	16
S16. ROESY (600 MHz, acetone-d ₆) of compound 2	16
S17. [α] _D spectrum of compound 2 in MeOH	17
S18. IR of compound 2	17
S19. ECD and UV of compound 2	18
S20. HRESIMS of compound 2	19
S22. ¹³ C NMR (DEPT) (150 MHz, CD ₃ OD) of compound 3	21
S23. ¹ H- ¹ H COSY (600 MHz, CD ₃ OD) of compound 3	21
S24. HSQC (600 MHz, CD ₃ OD) of compound 3	22
S25. HMBC (600 MHz, CD ₃ OD) of compound 3	22
S26. ROESY (600 MHz, CD ₃ OD) of compound 3	23
S27. [α] _D spectrum of compound 3 in MeOH	23

S28. IR of compound 3	24
S29. ECD and UV of compound 3	25
S30. HRESIMS of compound 3	26

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-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). 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 GF₂₅₄ plates (Yantai Jiangyou Silicon Development Company, Yantai, China), and spots were monitored under UV light or by heating after sprayed with 10% H₂SO₄ 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. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-CBM-20 system (Shimadzu, Kyoto, Japan) with Agilent XDB-C₁₈ (5 μ m, 9.4 \times 250 mm) columns.

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 $\sigma = \sim 0.3$ eV.

Antihepatoma assay

The antihepatoma assay of the compounds **1–3** 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% CO₂ for 24 h. The culture medium was replaced with fresh medium containing different concentrations of **compounds 1–3**, and cells were incubated for additional 48 h. After removal of the medium, 100 μ L of MTT reagent (1mg/mL) was added into each well, and the plates were kept in incubator for 4 h. After that, 100 μ L 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 $[(A_{490} \text{ control} - A_{490} \text{ treated})/A_{490} \text{ control}] \times 100\%$. The antihepatoma assay of compounds was expressed as IC₅₀ values calculated by GraphPad Prism 5 (GraphPad Software, California, USA).

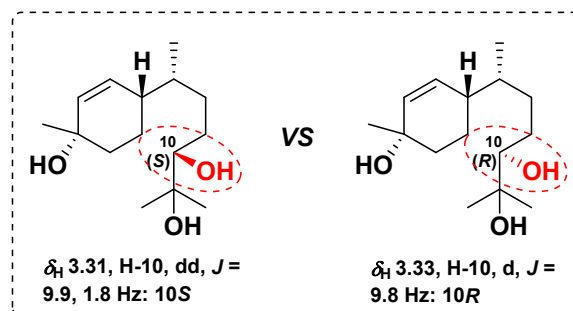
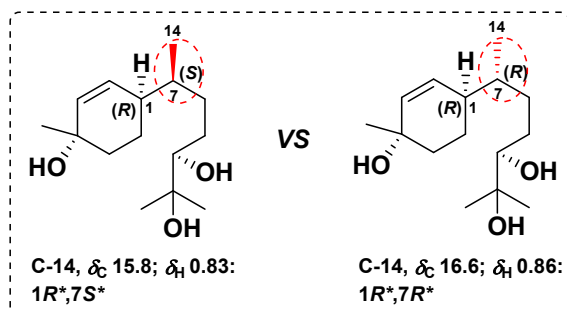
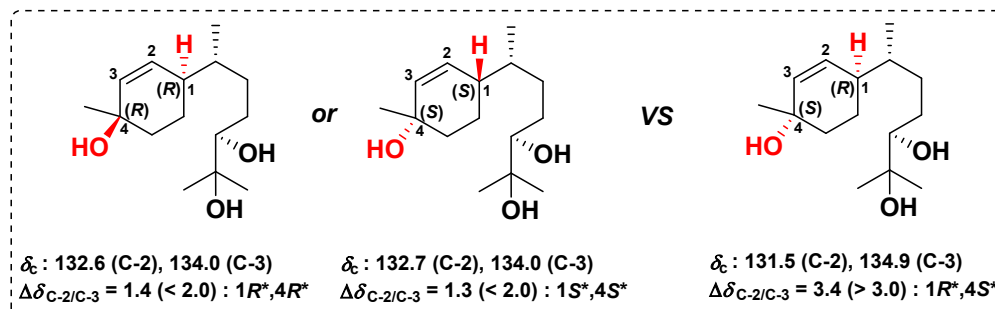
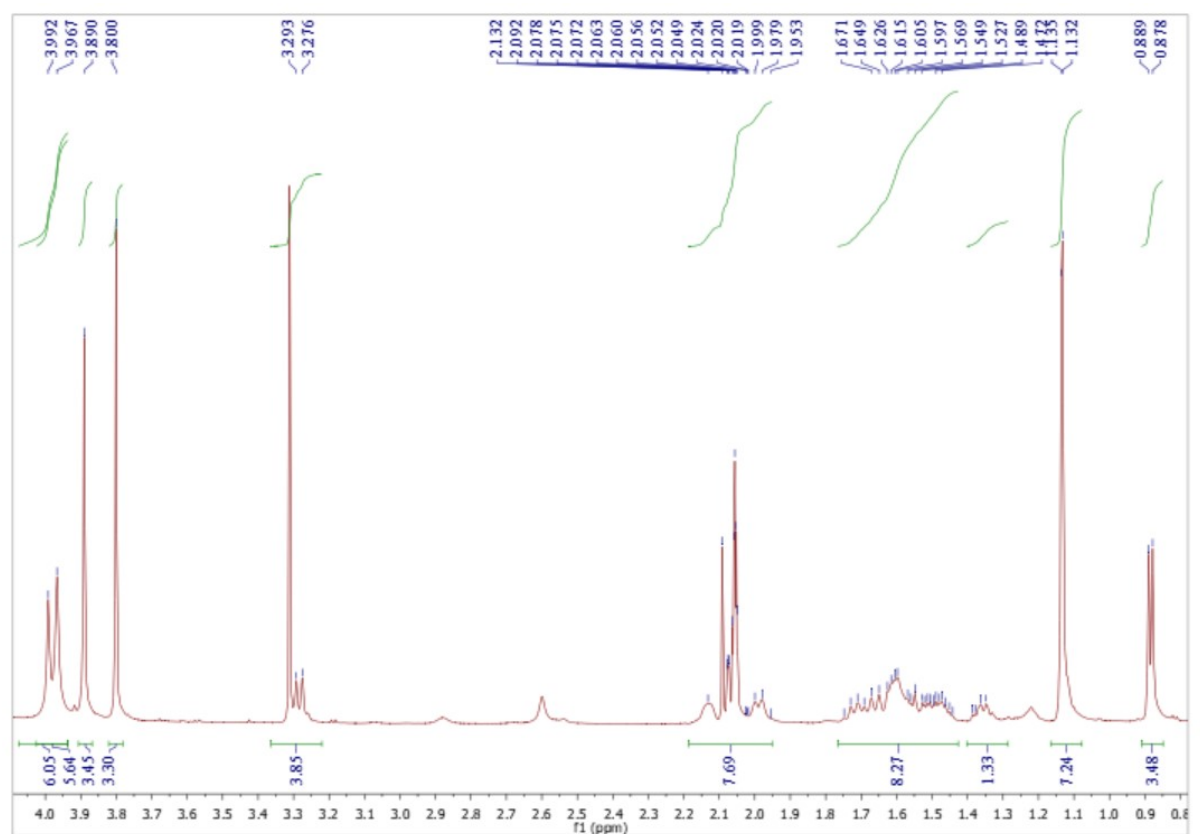
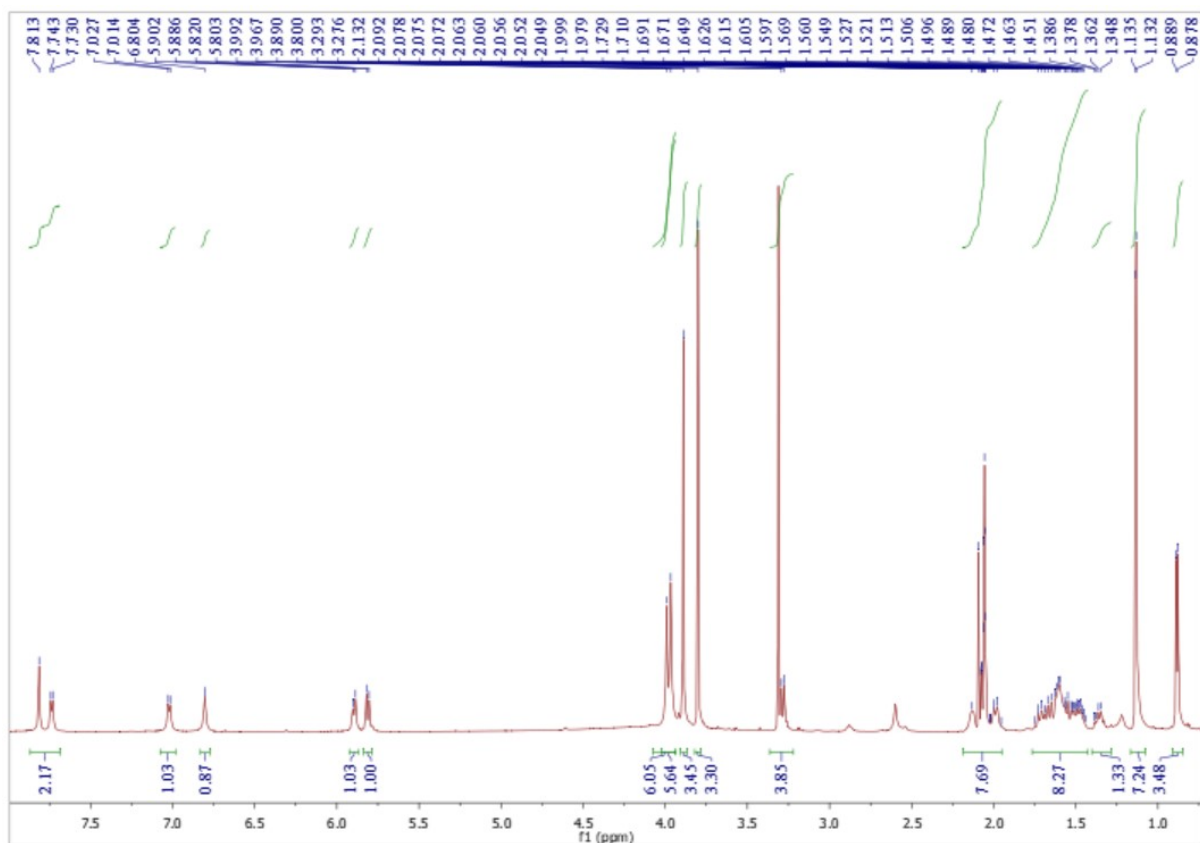
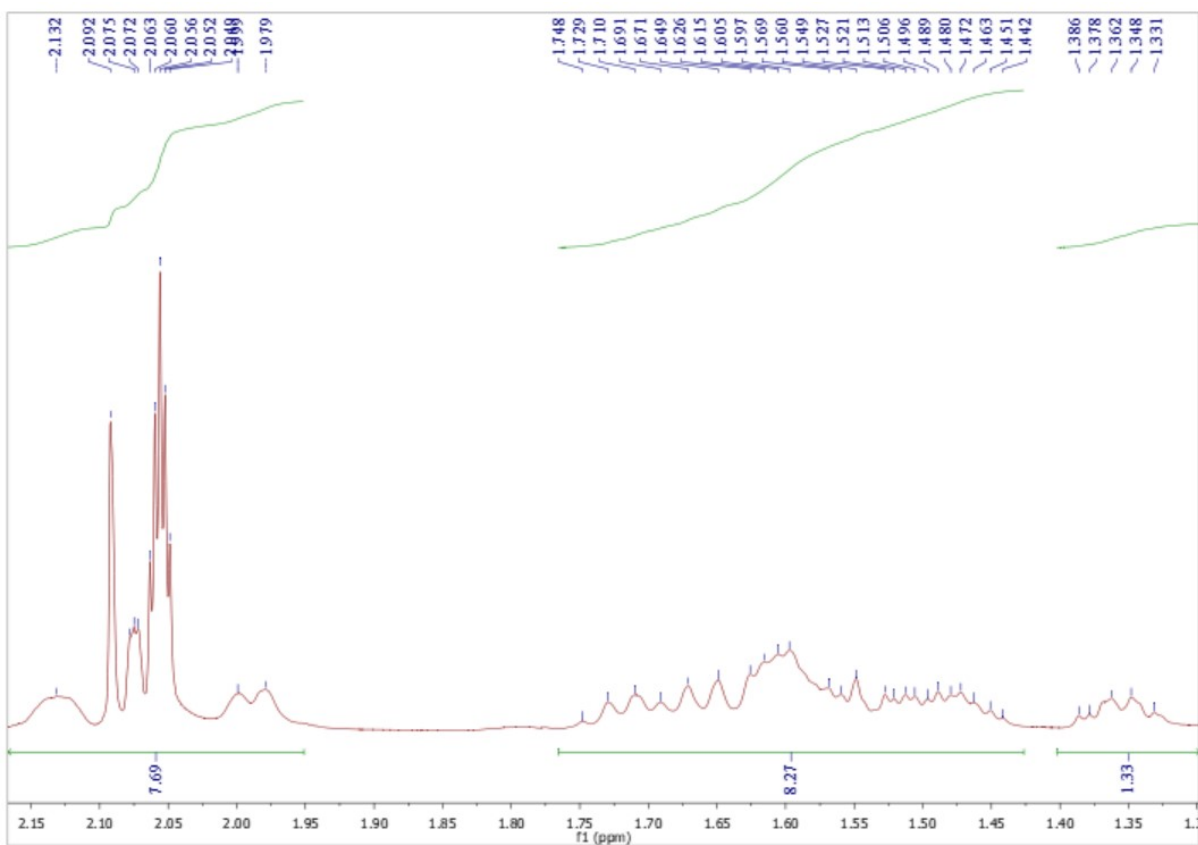


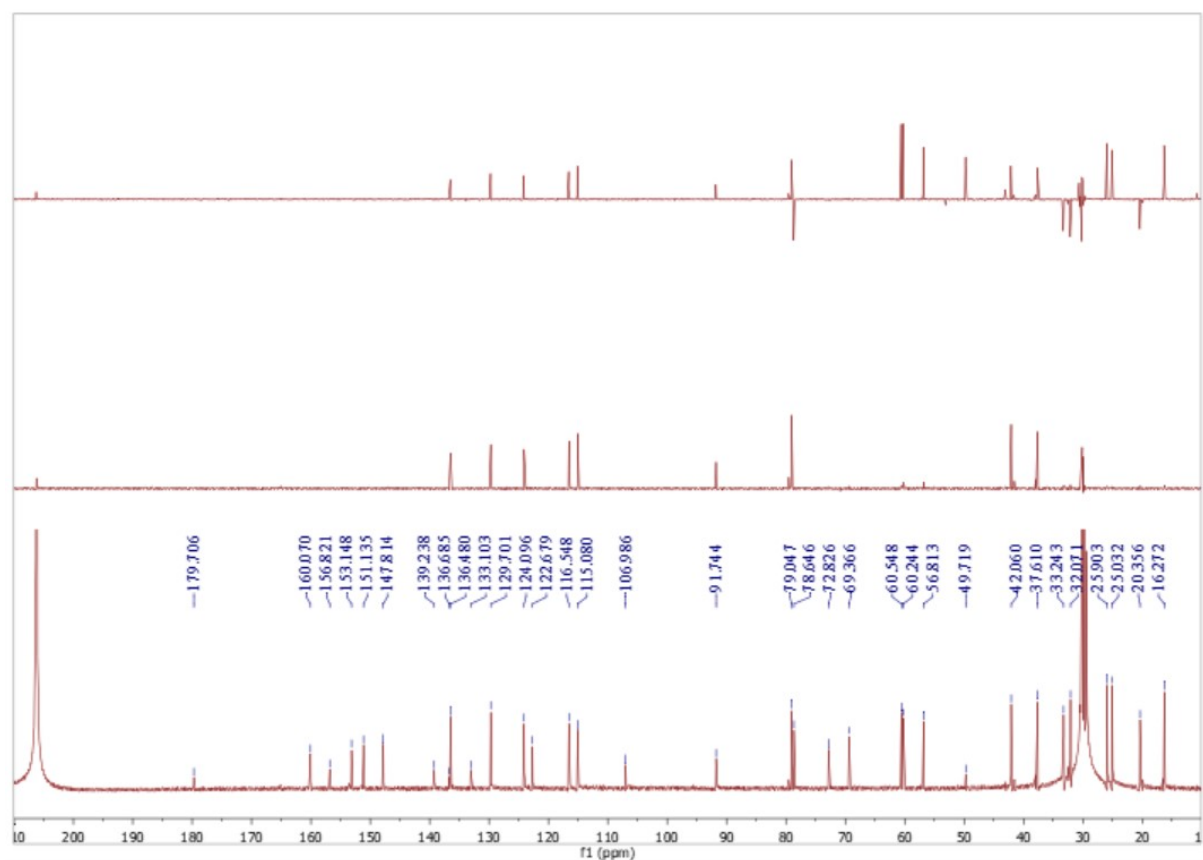
Figure S1. Key chemical shifts differences of 1-bisabolene-3-ols according to the references (*J. Nat. Prod.*, 2014, 77, 1708–1717; *J. Chem. Eco.*, 2014, 40, 1260–1268)

S1. ¹H NMR (600 MHz, acetone-d₆) of compound 1

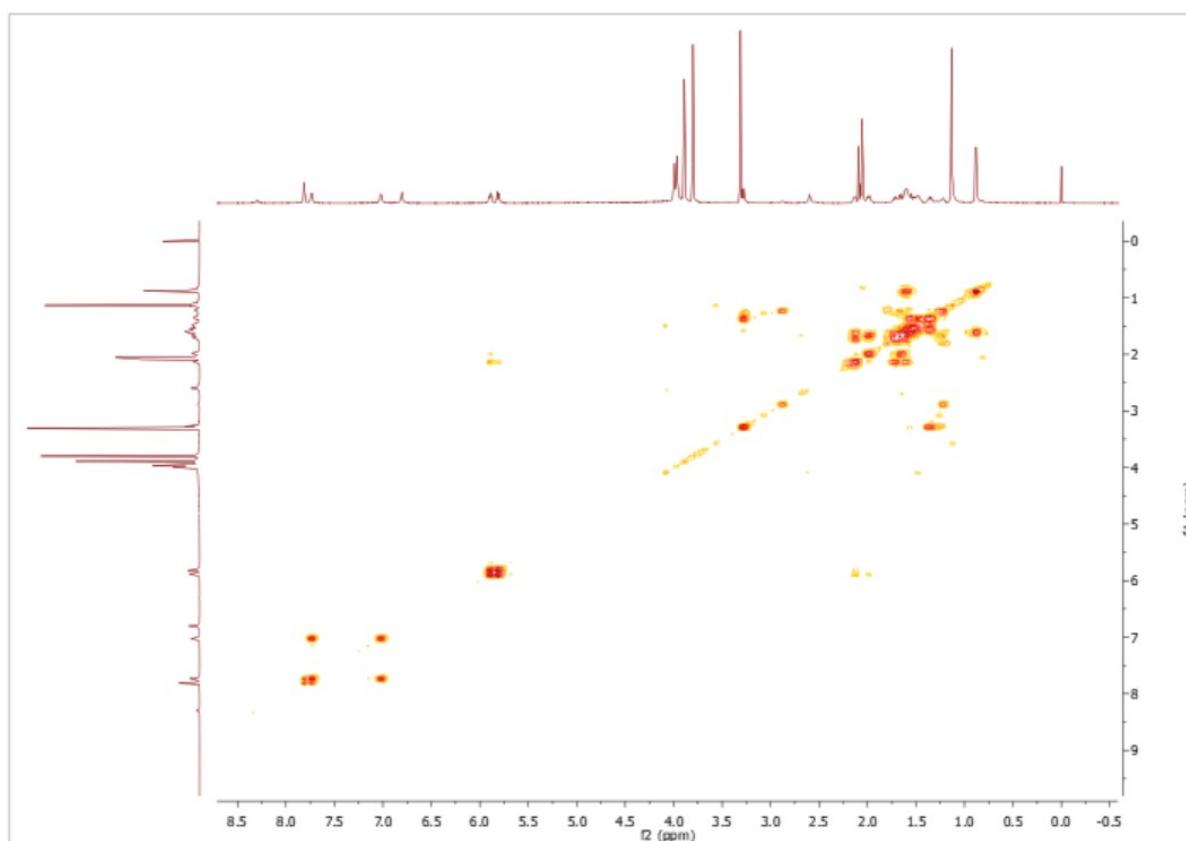




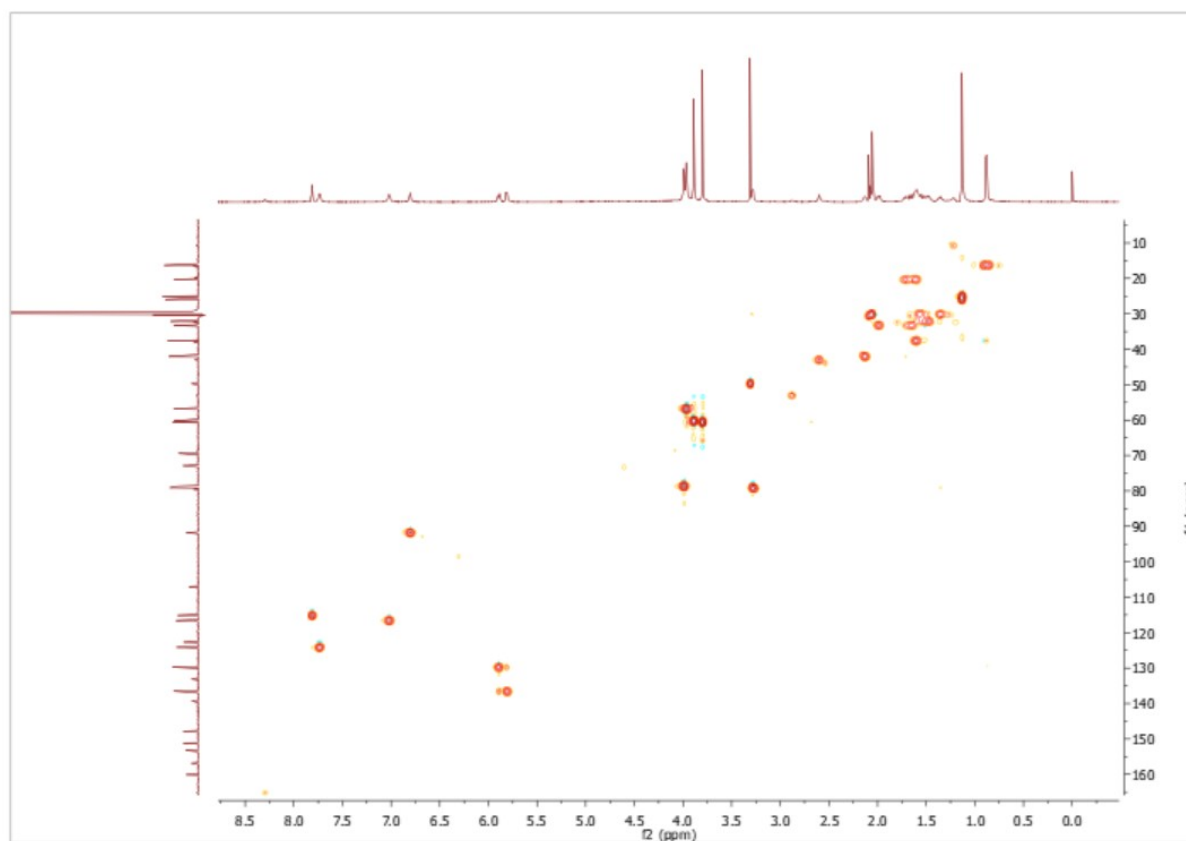
S2. ^{13}C NMR (DEPT) (150 MHz, acetone- d_6) of compound **1**



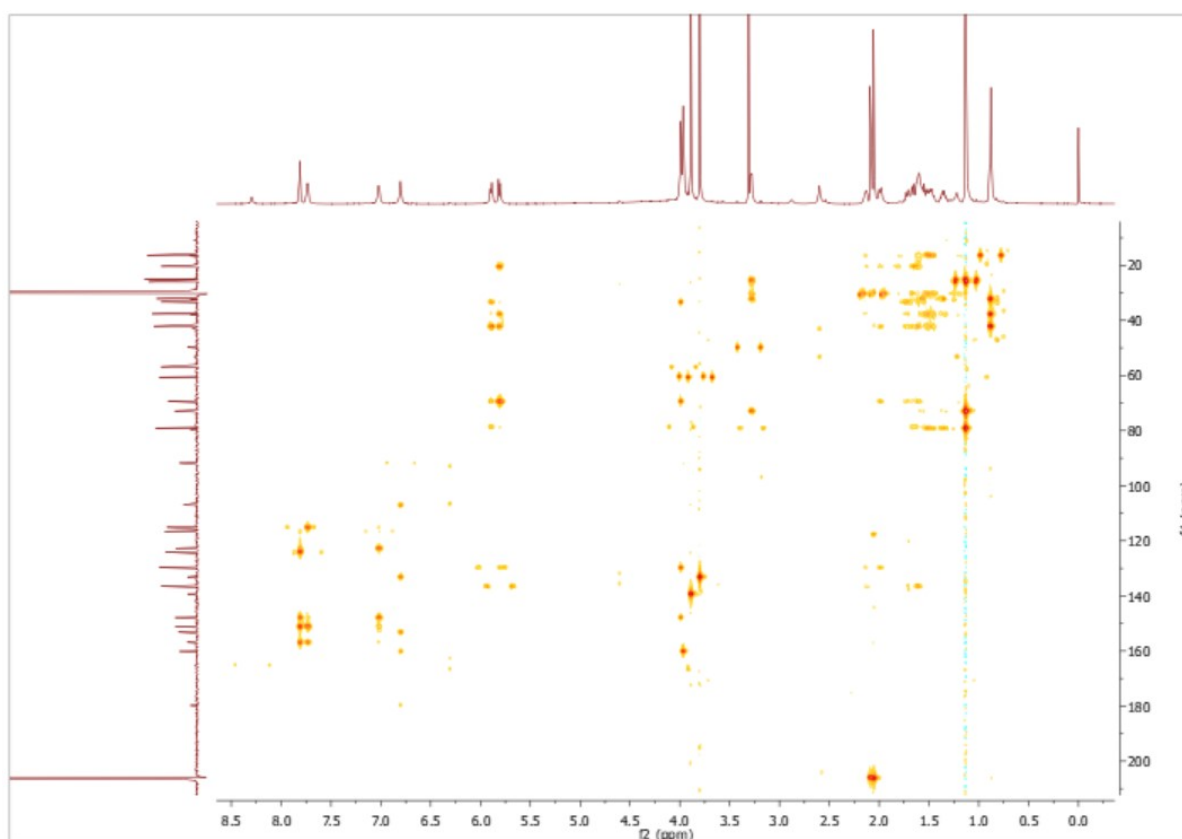
S3. ^1H - ^1H COSY (600 MHz, acetone- d_6) of compound **1**



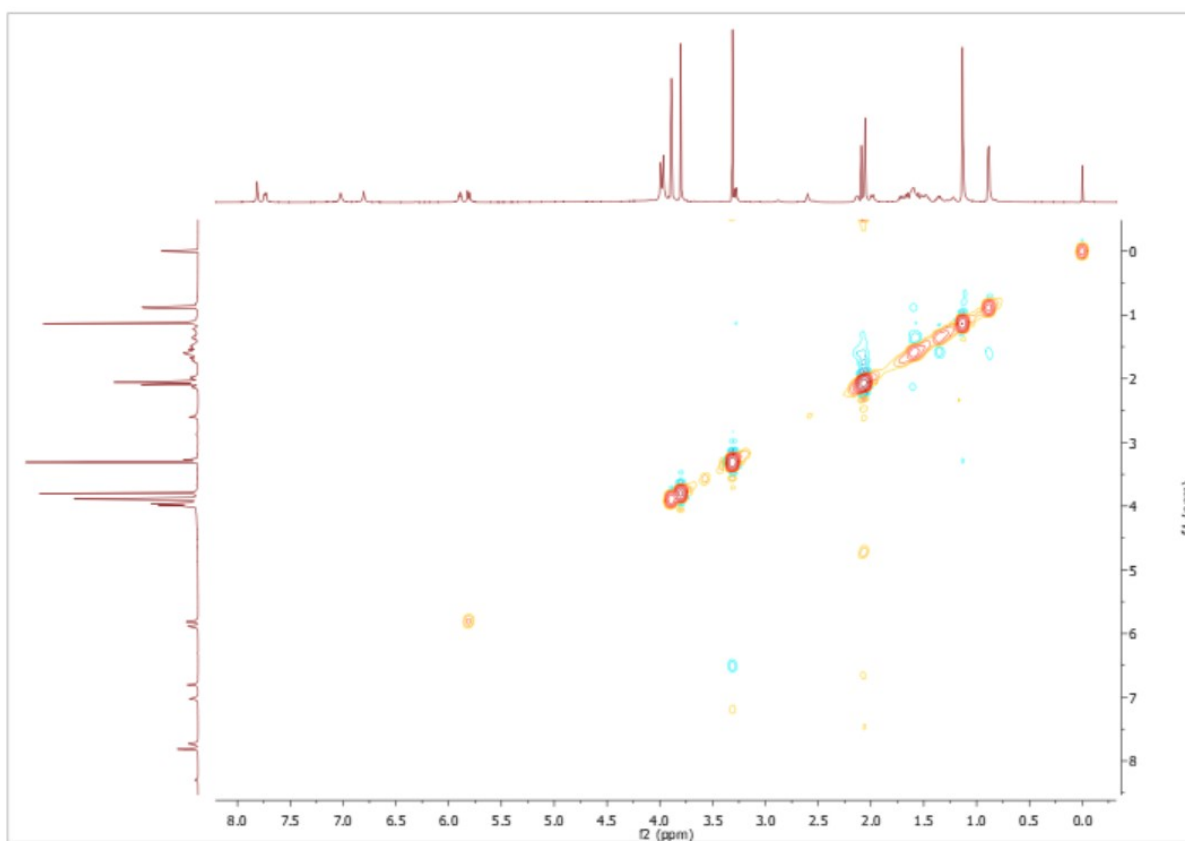
S4. HSQC (600 MHz, acetone- d_6) of compound **1**



S5. HMBC (600 MHz, acetone-d₆) of compound **1**



S6. ROESY (600 MHz, acetone-d₆) 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, 13-DEC-2022

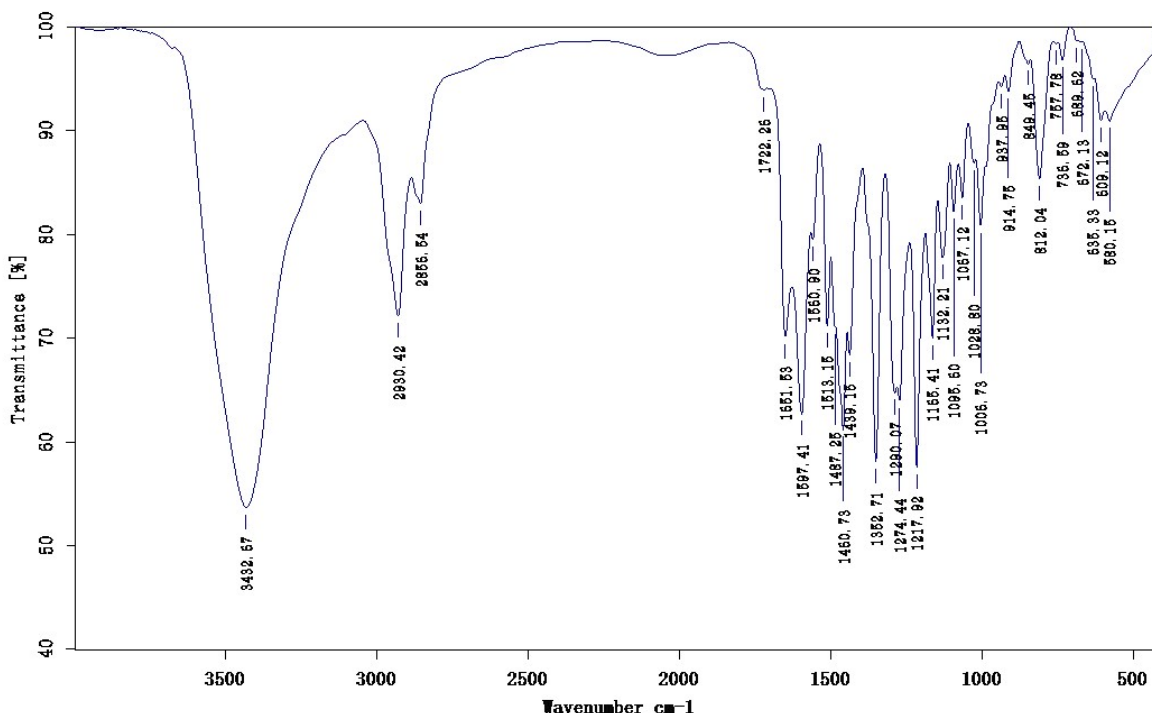
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Time Delay : Disabled

Delay between Measurement : Disabled

<u>n</u>	<u>Average</u>	<u>Std.Dev.</u>	<u>% RSD</u>	<u>Maximum</u>	<u>Minimum</u>					
5	59.59	1.16	1.94	61.22	58.16					
<u>S.No</u>	<u>Sample ID</u>	<u>Time</u>	<u>Result</u>	<u>Scale</u>	<u>OR °Arc</u>	<u>WLG.nm</u>	<u>Lg.mm</u>	<u>Conc.g/100ml</u>	<u>Temp.</u>	
1	janh-7a	03:19:44 PM	61.22	SR	0.060	589	100.00	0.098	20.2	
2	janh-7a	03:19:51 PM	60.20	SR	0.059	589	100.00	0.098	20.2	
3	janh-7a	03:19:58 PM	59.18	SR	0.058	589	100.00	0.098	20.2	
4	janh-7a	03:20:04 PM	59.18	SR	0.058	589	100.00	0.098	20.1	
5	janh-7a	03:20:11 PM	58.16	SR	0.057	589	100.00	0.098	20.1	

S8. IR of compound **1**

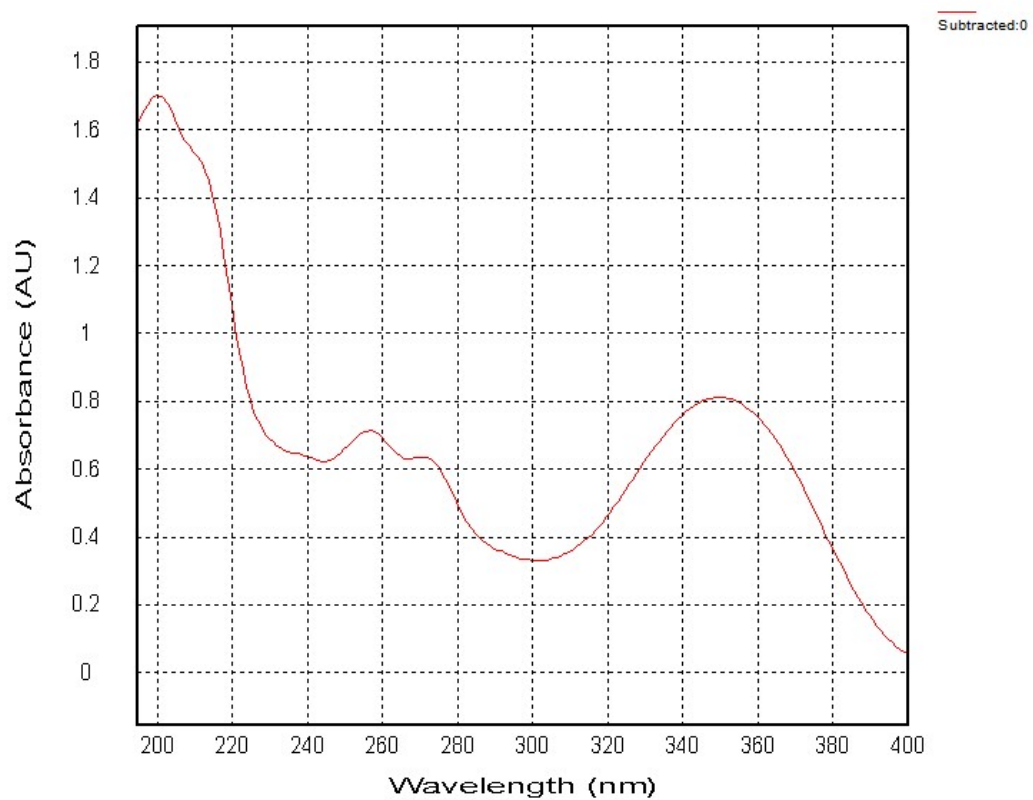
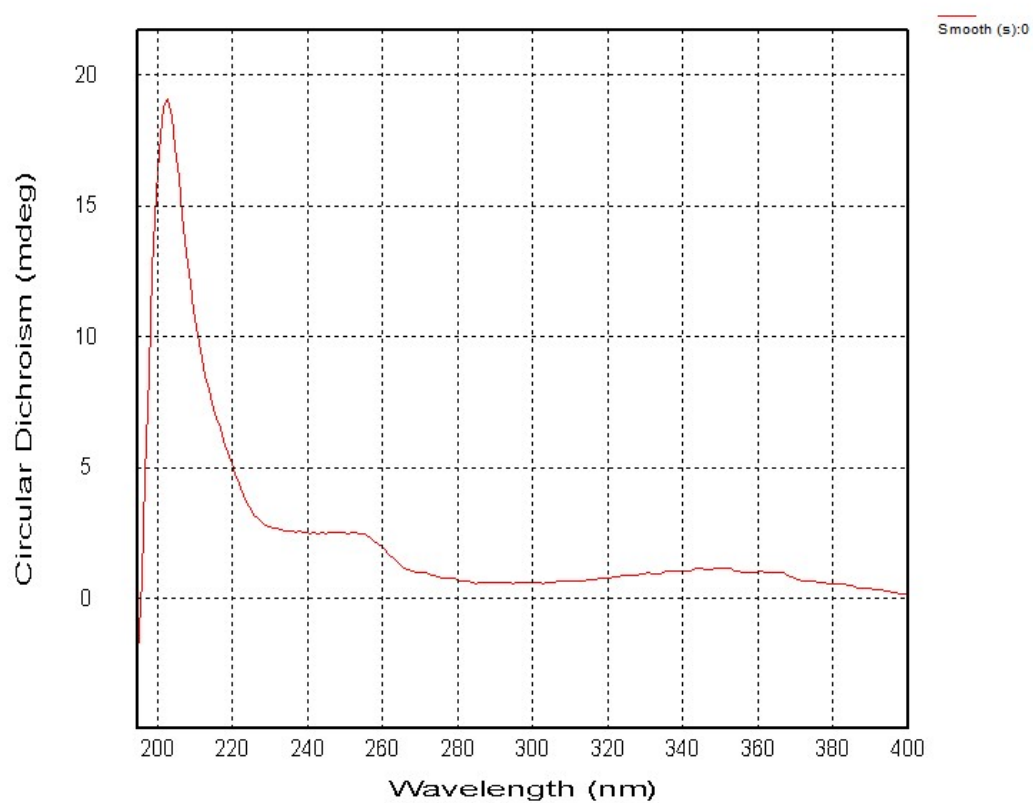


Sample Name: Janh-7a
 Sample Form: KBr
 Path of File: E:\data
 Date of Measurement: 2023/1/14

Resolution: 4
 Aperture Setting: 6 mm
 Number of Background Scans: 16
 Number of Sample Scans: 16

Beamsplitter Setting: KBr
 Source Setting: MIR
 Instrument Type: BRUKER VERTEX 70
 Soft Version: OPUS 8.1

S9. ECD and UV of compound 1



S10. HRESIMS of compound 1

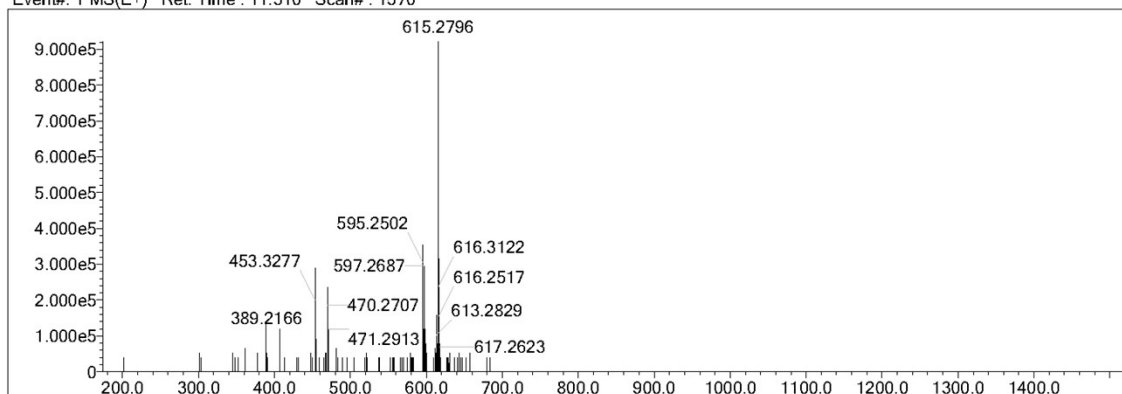
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	300	O	2	0	12	H
C	4	0	150	15N	3	0	0	Na
N	3	0	1					

Error Margin (ppm): 50
 HC Ratio: unlimited
 Max Isotopes: all
 MSn Iso RI (%): 75.00

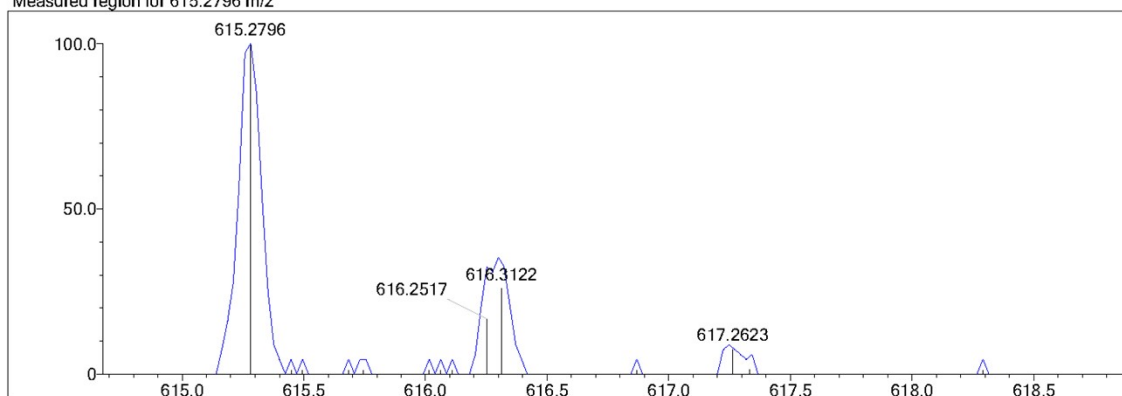
DBE Range: -2.0 - 1000.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

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

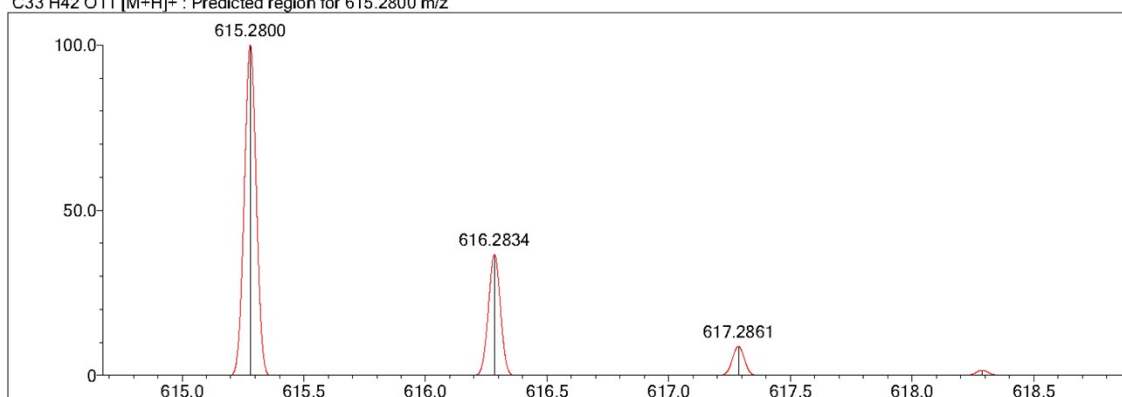
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Measured region for 615.2796 m/z

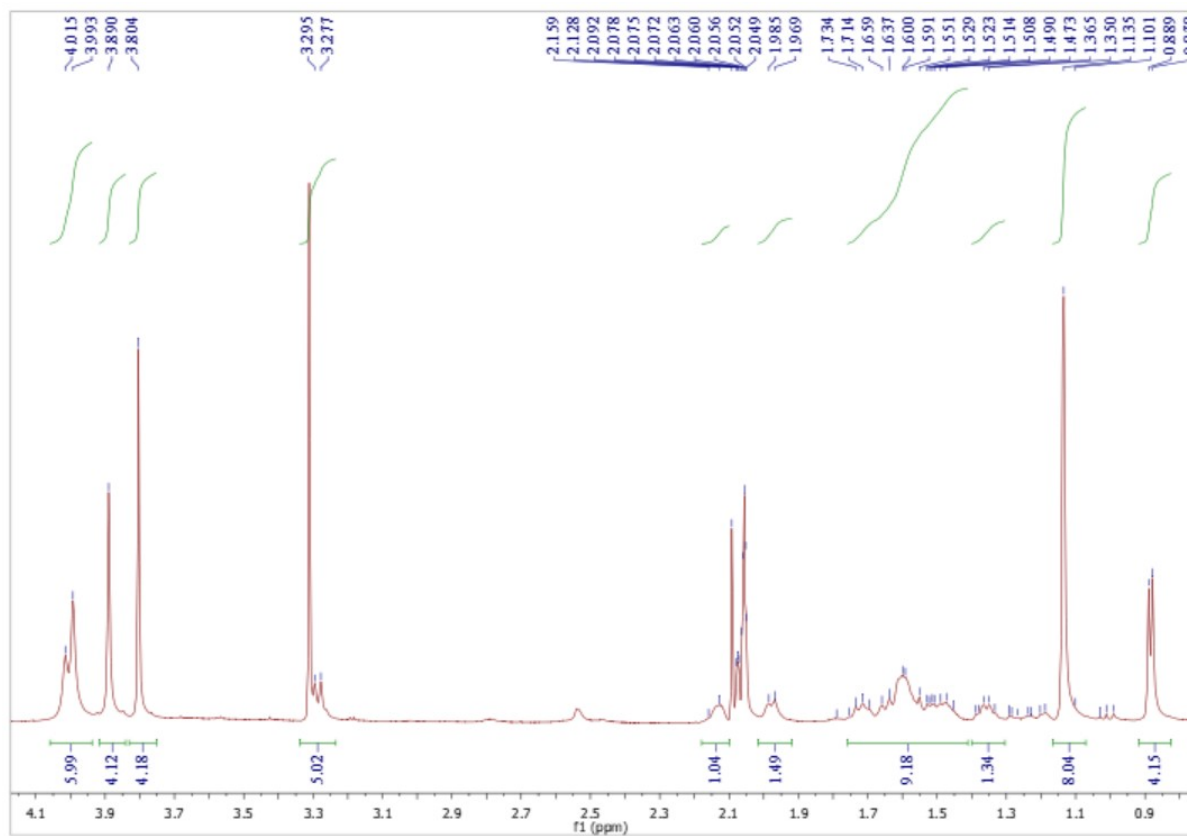
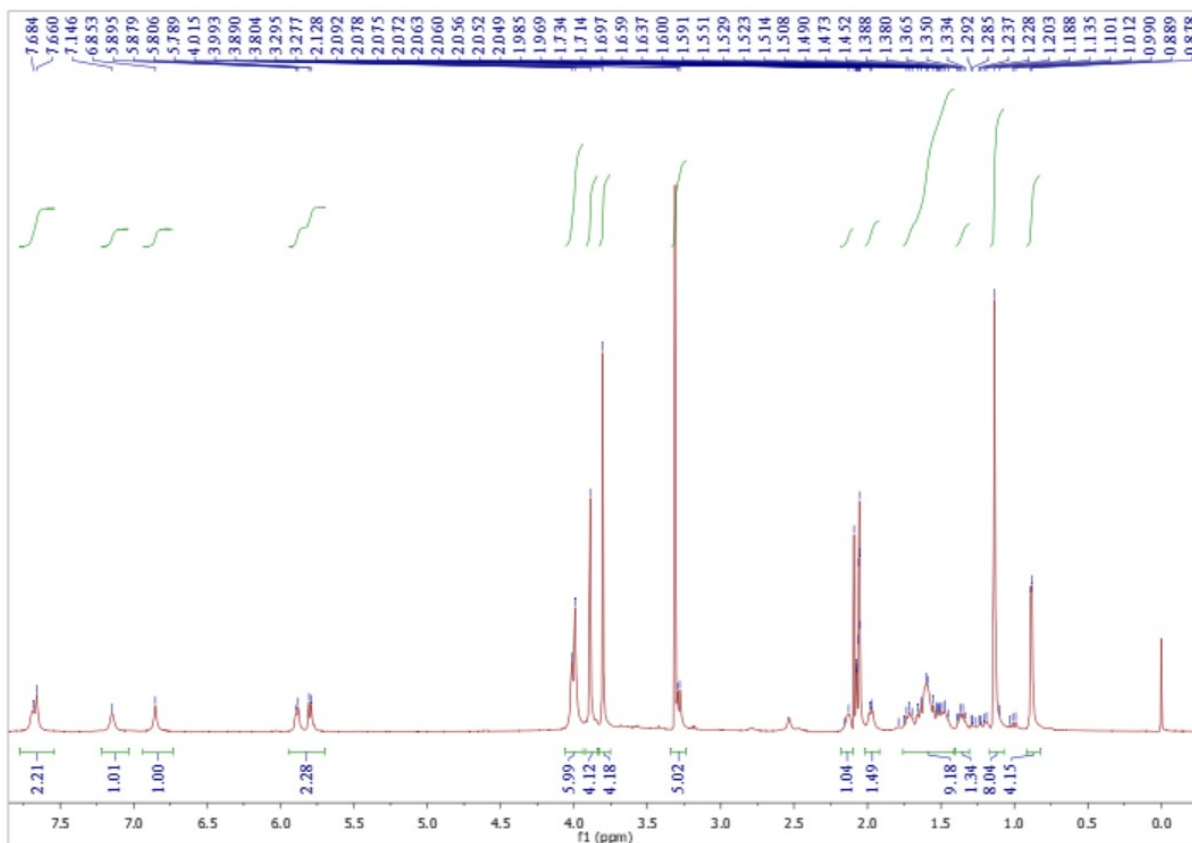


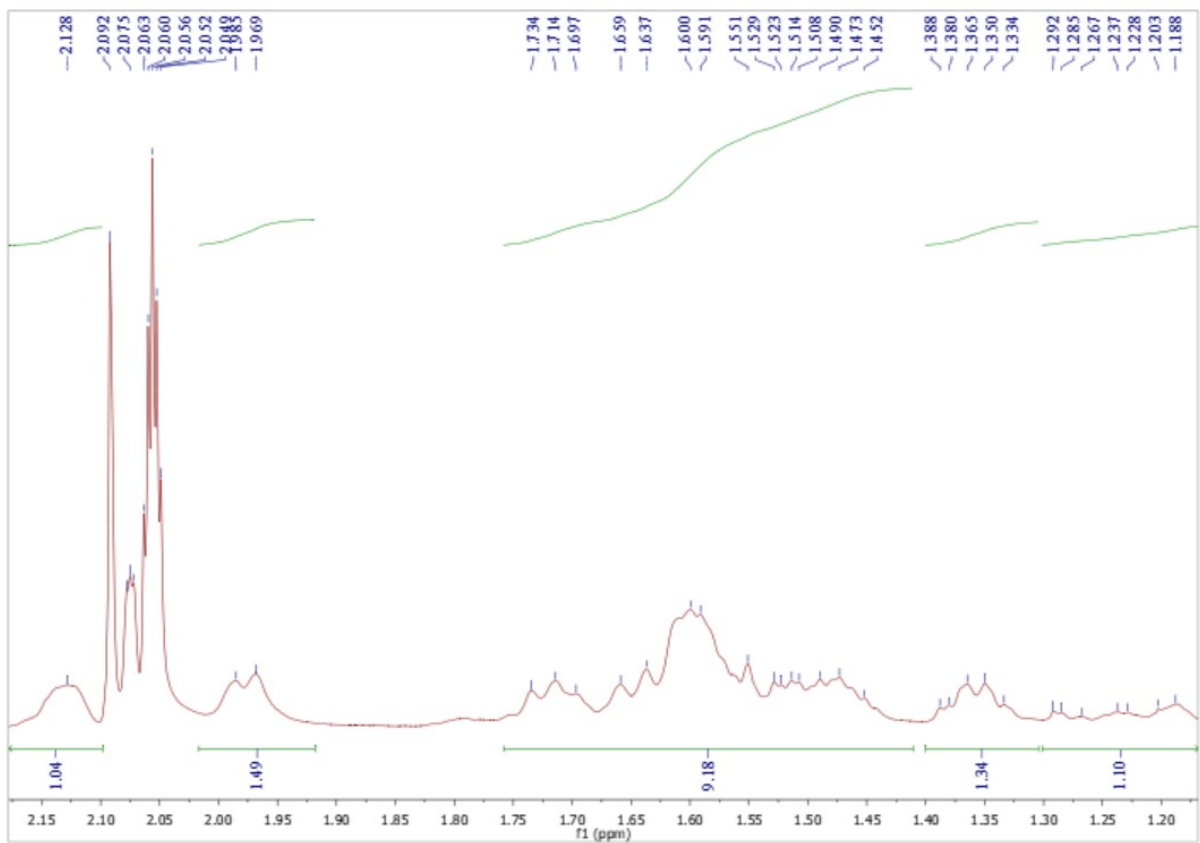
C33 H42 O11 [M+H]⁺ : Predicted region for 615.2800 m/z



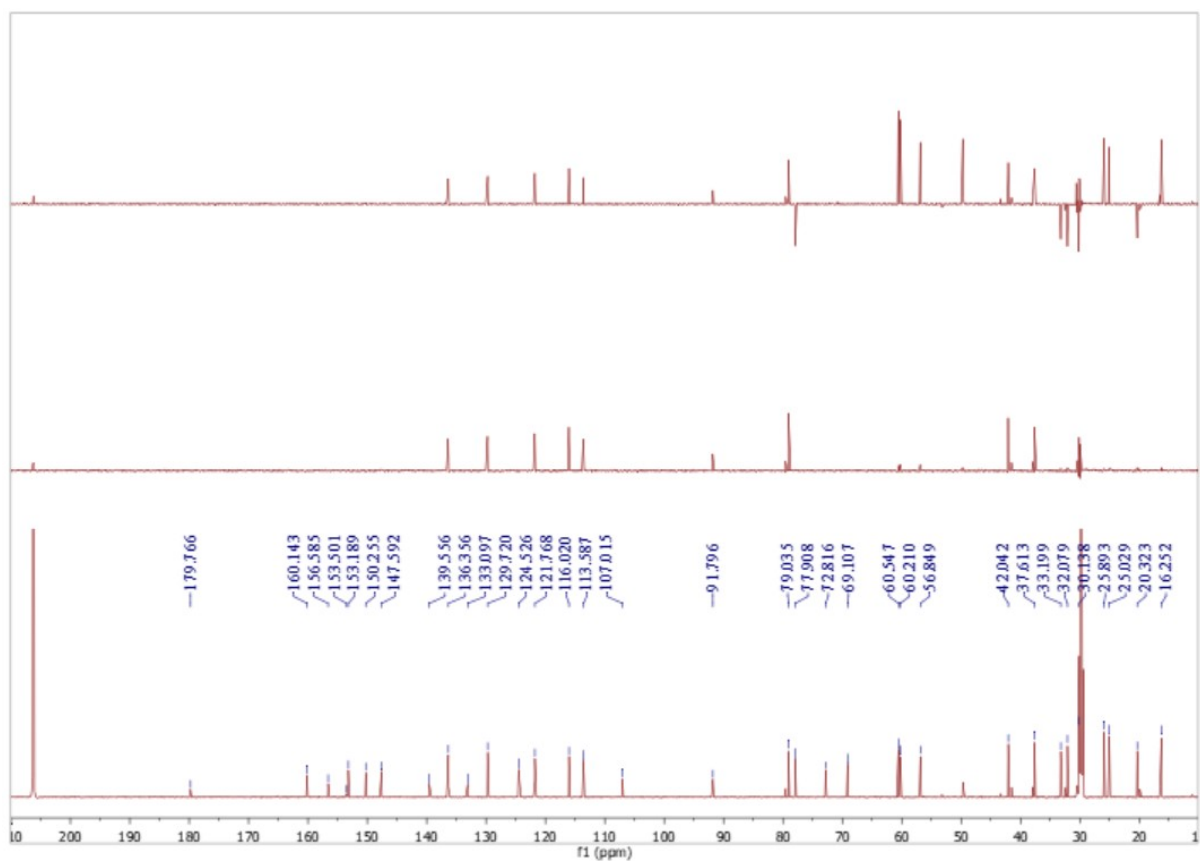
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	39.32	C33 H42 O11	[M+H] ⁺	615.2796	615.2800	-0.4	-0.65	39.32	13.0

S11. ¹H NMR (600 MHz, acetone-d₆) of compound 2

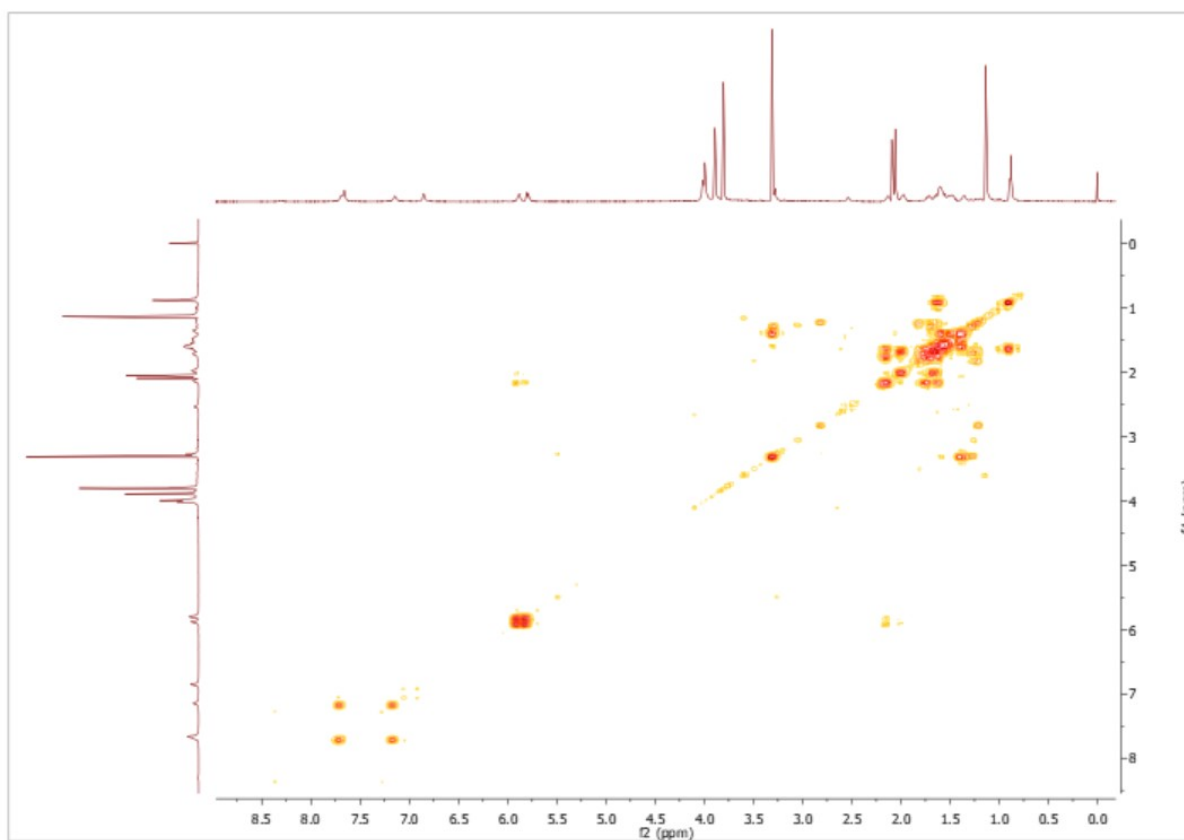




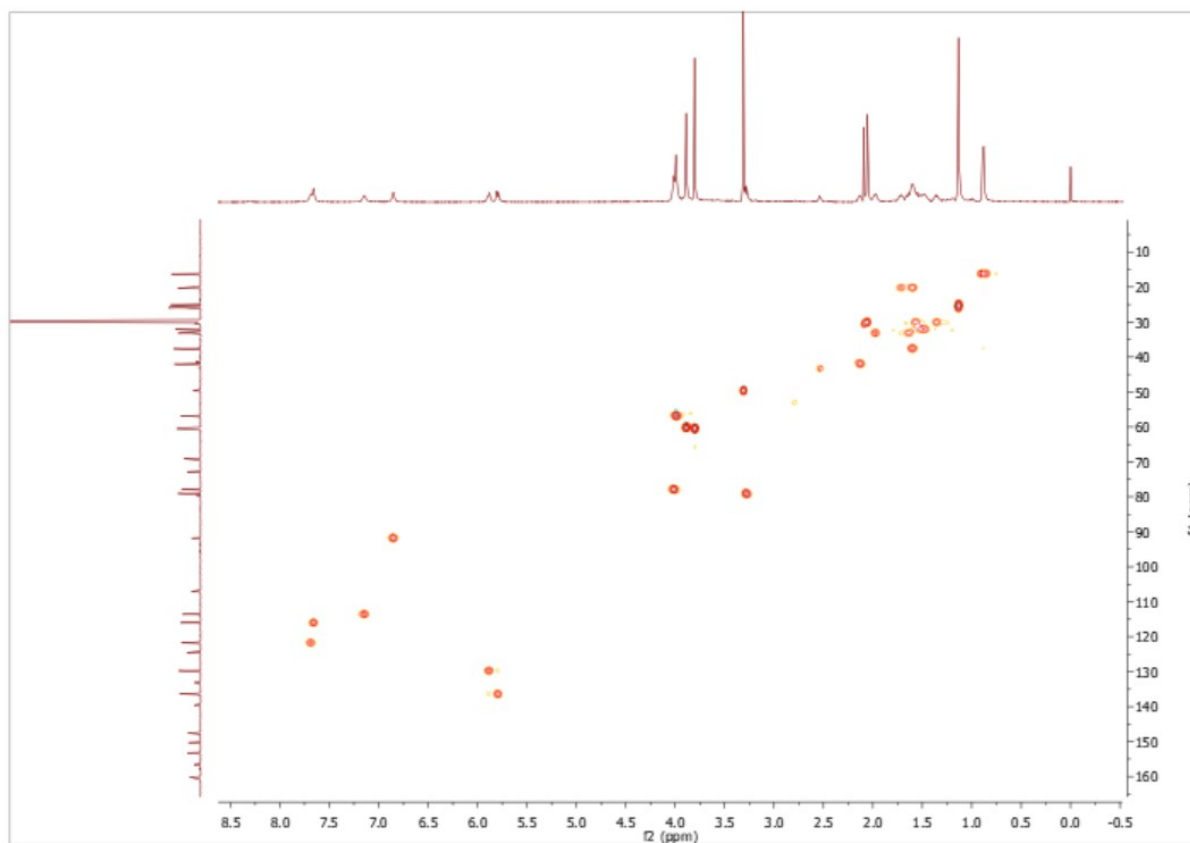
S12. ^{13}C NMR (DEPT) (150 MHz, acetone- d_6) of compound **2**



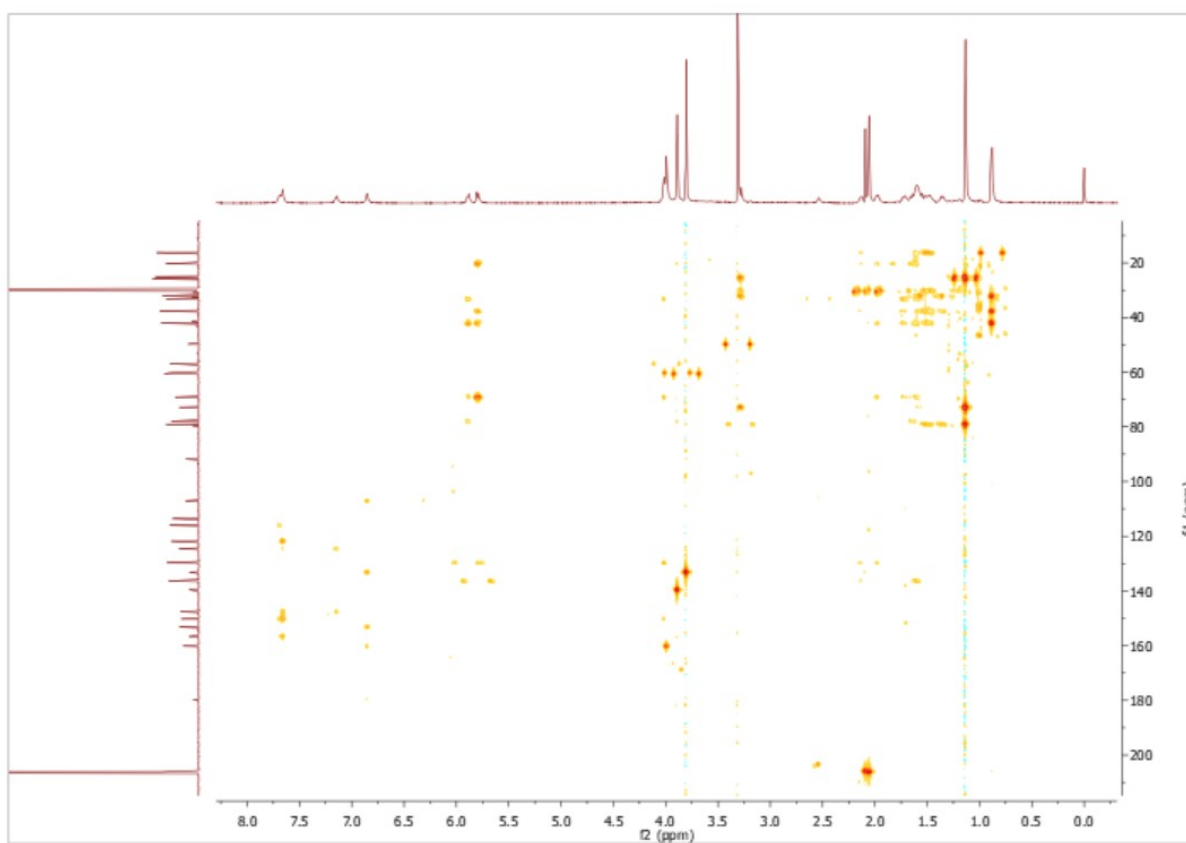
S13. ^1H - ^1H COSY (600 MHz, acetone- d_6) of compound **2**



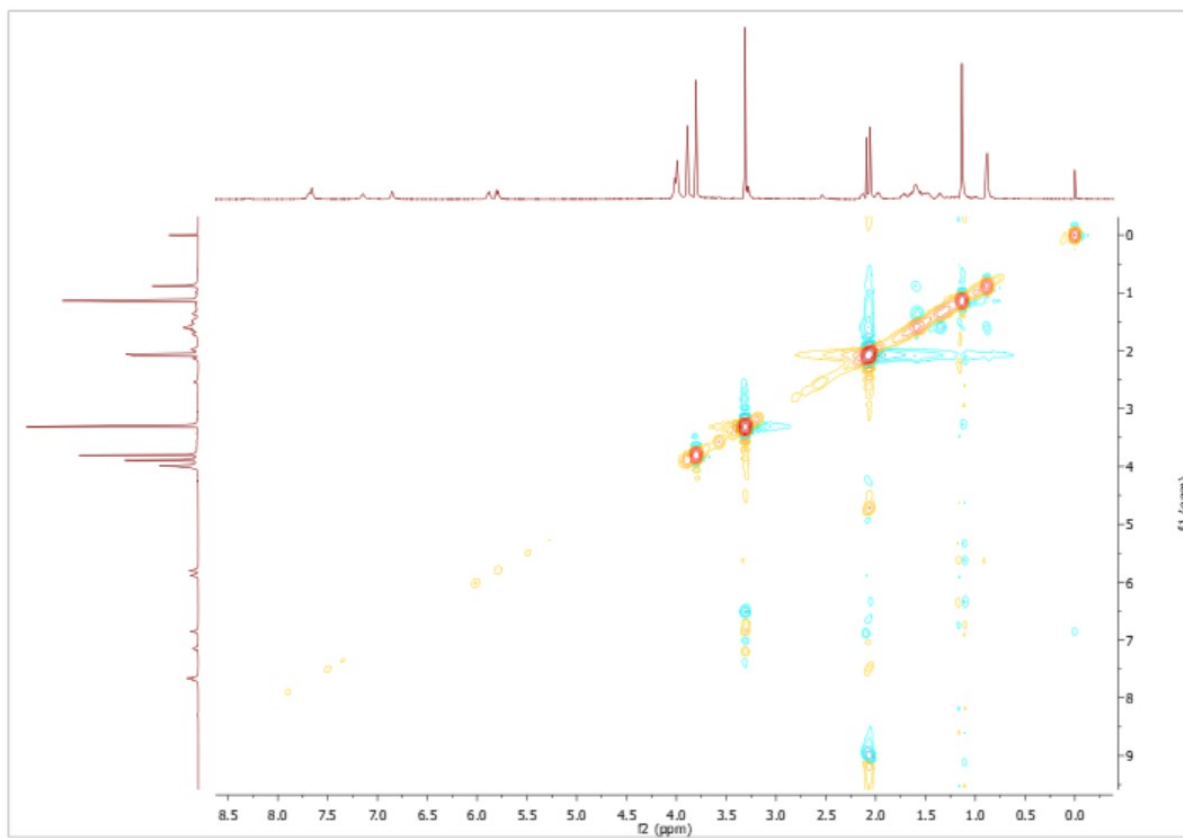
S14. HSQC (600 MHz, acetone- d_6) of compound **2**



S15. HMBC (600 MHz, acetone-d₆) of compound 2



S16. ROESY (600 MHz, acetone-d₆) 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, 13-DEC-2022

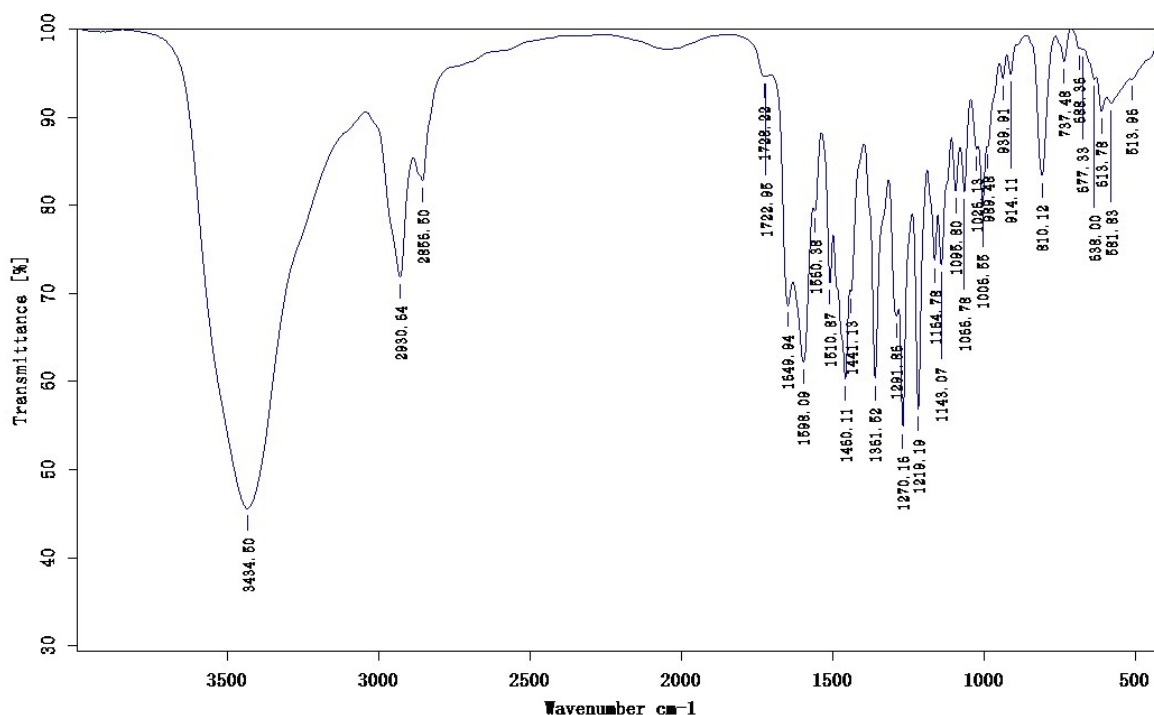
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Delay between Measurement : Disabled

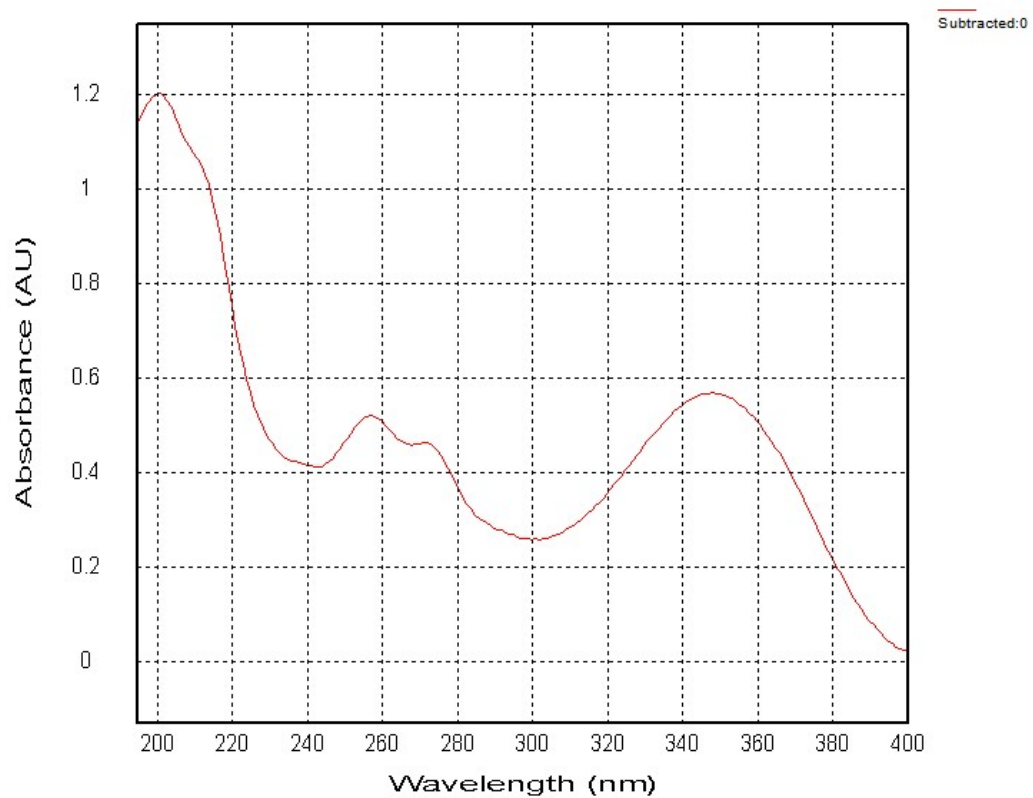
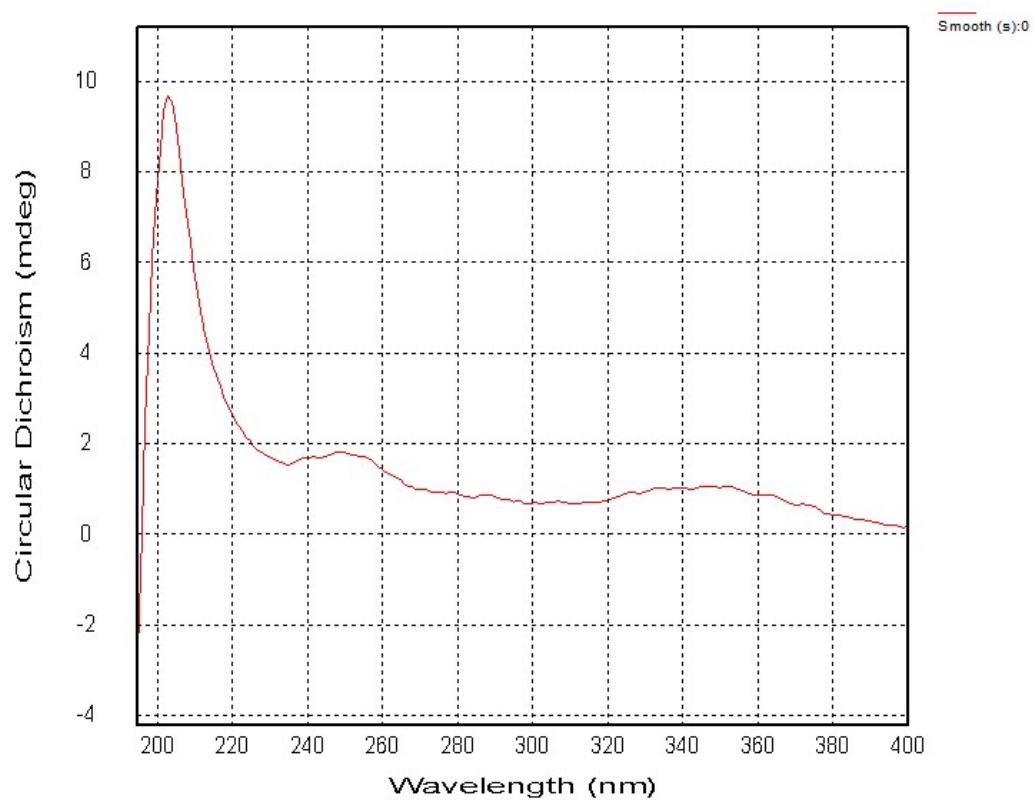
<u>n</u>	<u>Average</u>	<u>Std.Dev.</u>	<u>% RSD</u>	<u>Maximum</u>	<u>Minimum</u>					
5	52.09	1.23	2.36	52.99	50.75					
<u>S.No</u>	<u>Sample ID</u>	<u>Time</u>	<u>Result</u>	<u>Scale</u>	<u>OR °Arc</u>	<u>WLG.nm</u>	<u>Lg.mm</u>	<u>Conc.g/100ml</u>	<u>Temp.</u>	
1	janh-7b	03:25:24 PM	52.99	SR	0.071	589	100.00	0.134	20.2	
2	janh-7b	03:25:31 PM	52.99	SR	0.071	589	100.00	0.134	20.1	
3	janh-7b	03:25:37 PM	52.99	SR	0.071	589	100.00	0.134	20.1	
4	janh-7b	03:25:44 PM	50.75	SR	0.068	589	100.00	0.134	20.1	
5	janh-7b	03:25:51 PM	50.75	SR	0.068	589	100.00	0.134	20.0	

S18. IR of compound 2



Sample Name: Janh-7b	Resolution: 4	Beamsplitter Setting: KBr
Sample Form: KBr	Aperture Setting: 6 mm	Source Setting: MIR
Path of File: E:\data	Number of Background Scans: 16	Instrument Type: BRUKER VERTEX 70
Date of Measurement: 2023/1/15	Number of Sample Scans: 16	Soft Version: QPUS.1

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): 50

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

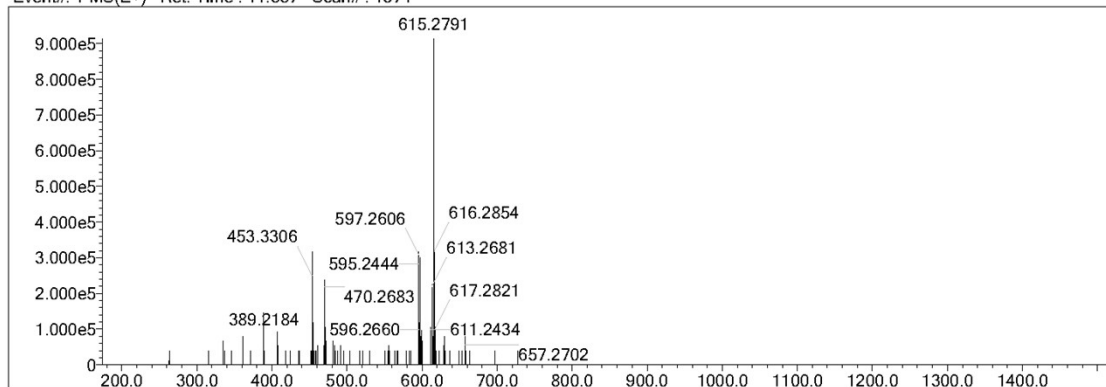
Electron Ions: both

Use MSn Info: no

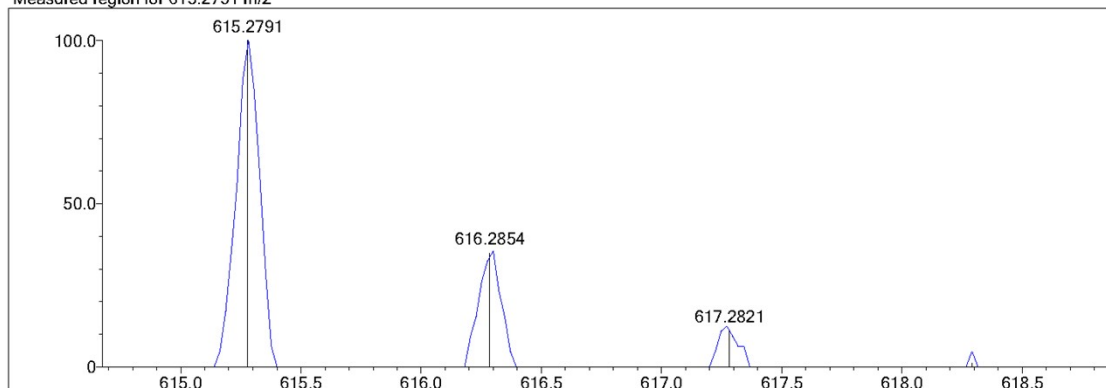
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Max Results: 500

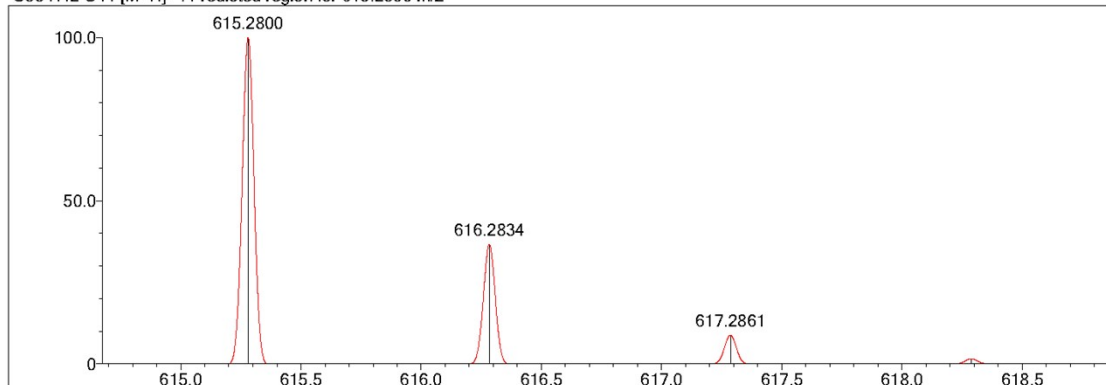
Event#: 1 MS(E+) Ret. Time : 11.337 Scan# : 1574



Measured region for 615.2791 m/z

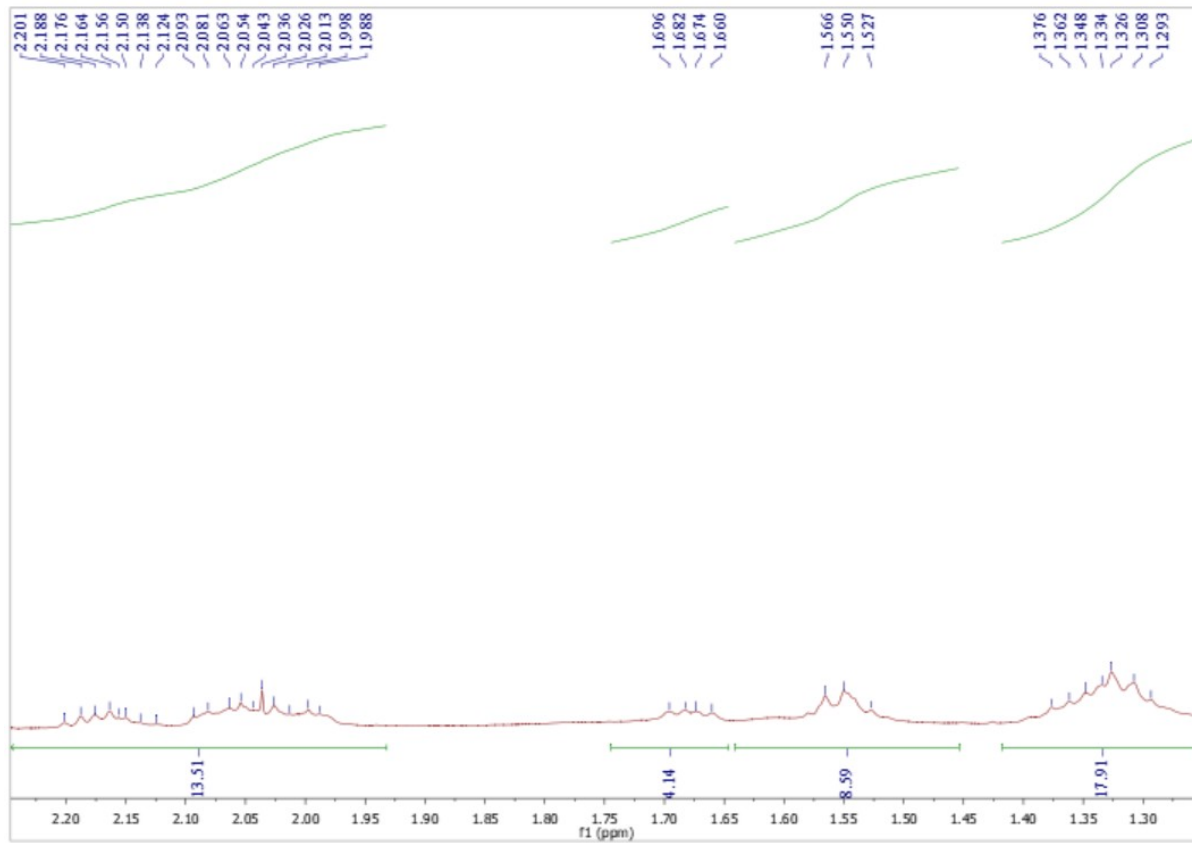
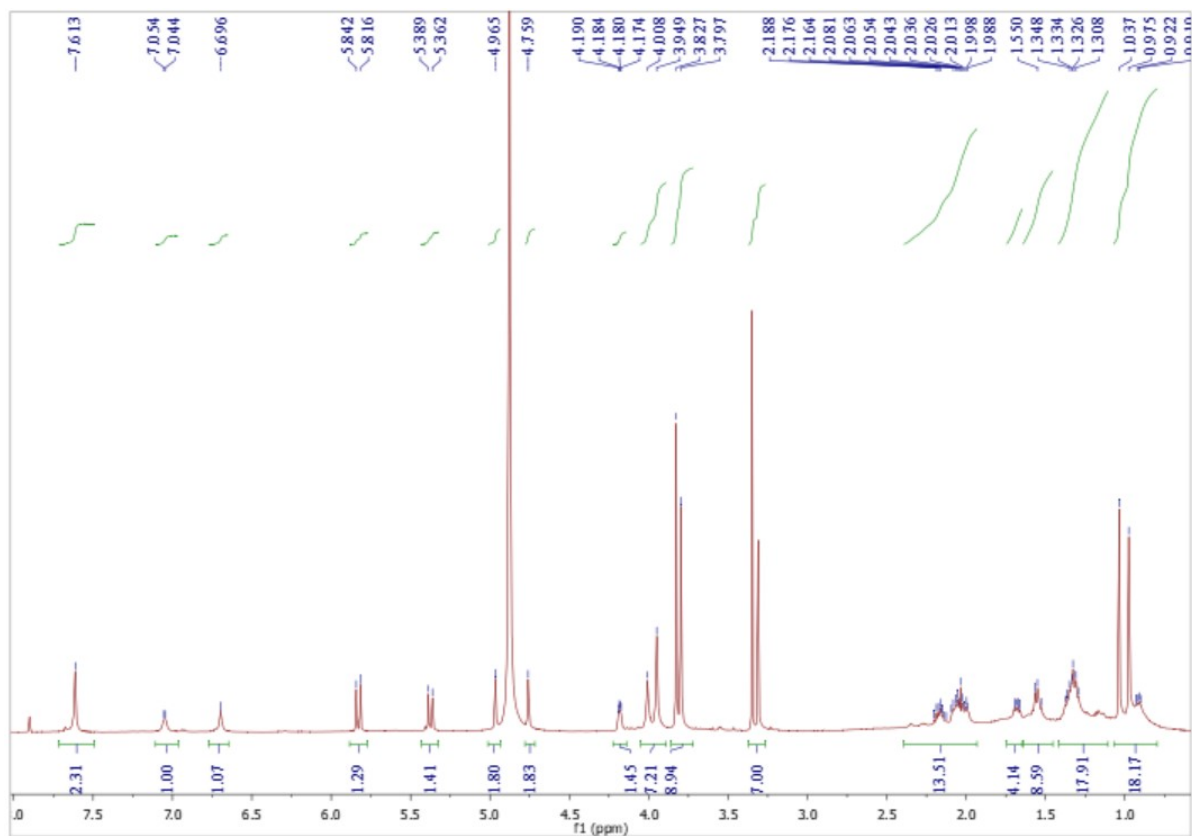


C33 H42 O11 [M+H]⁺ : Predicted region for 615.2800 m/z

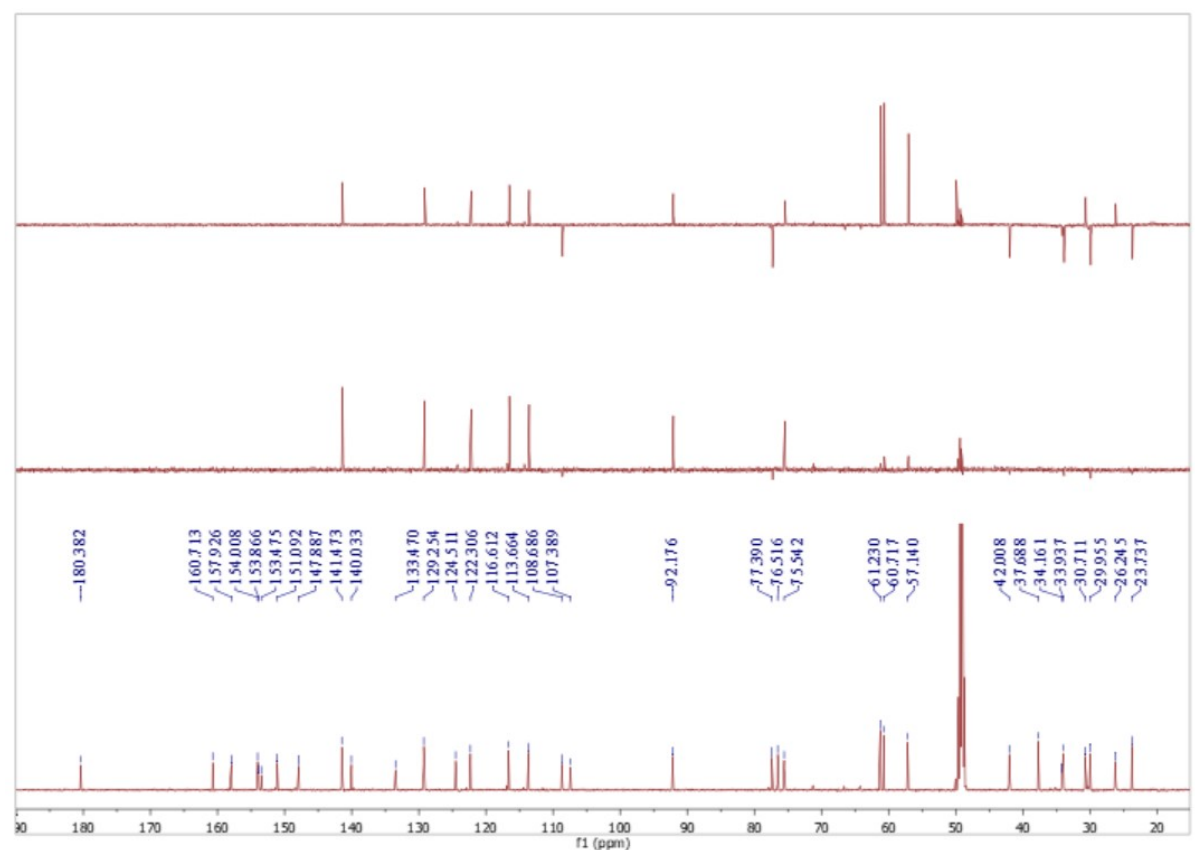


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	79.98	C33 H42 O11	[M+H] ⁺	615.2791	615.2800	-0.9	-1.46	80.91	13.0

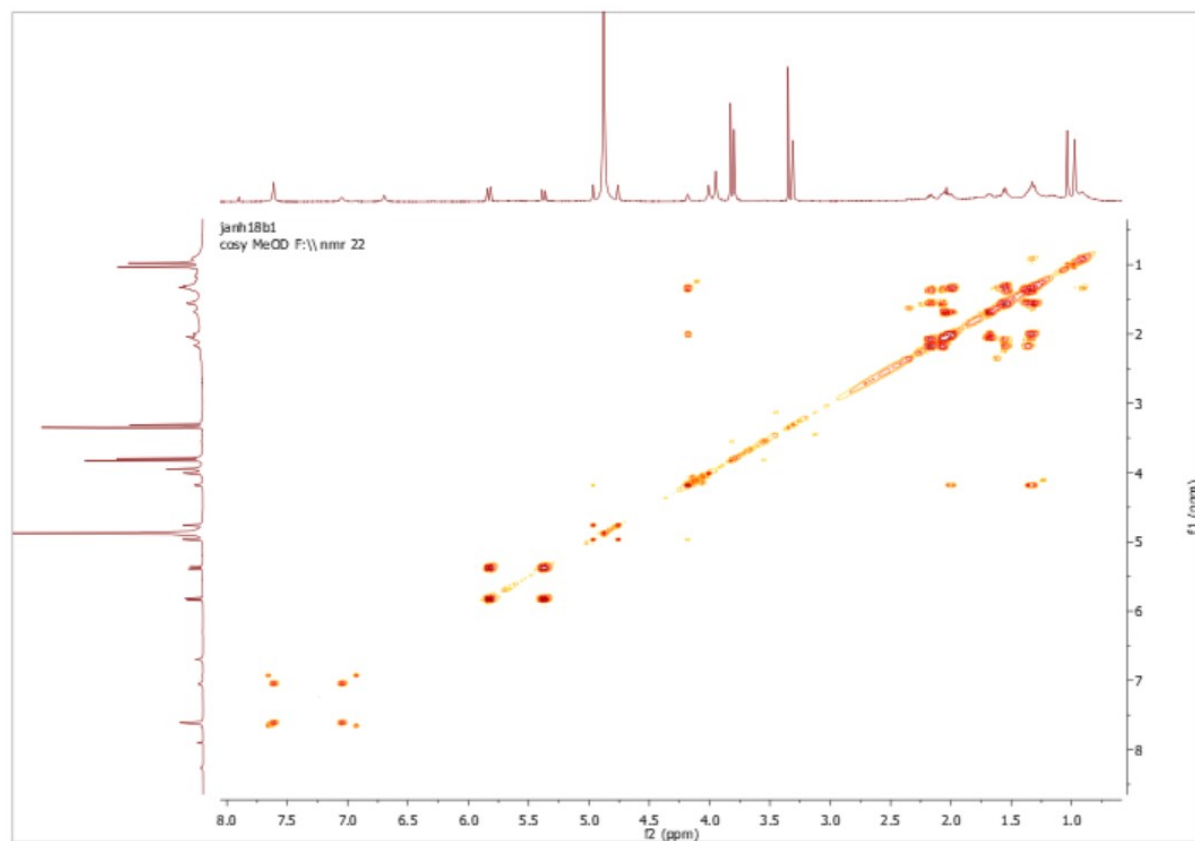
S21. ¹H NMR (600 MHz, CD₃OD) of compound 3



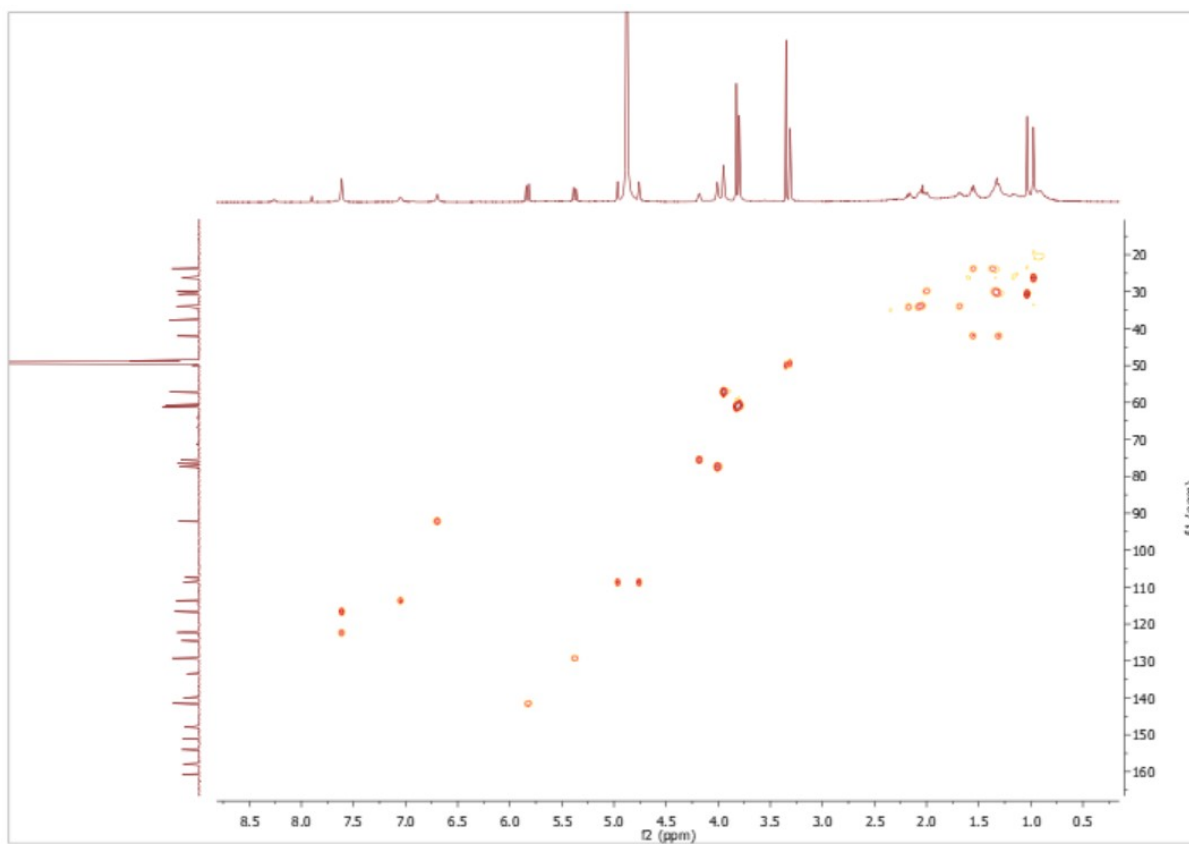
S22. ^{13}C NMR (DEPT) (150 MHz, CD_3OD) of compound **3**



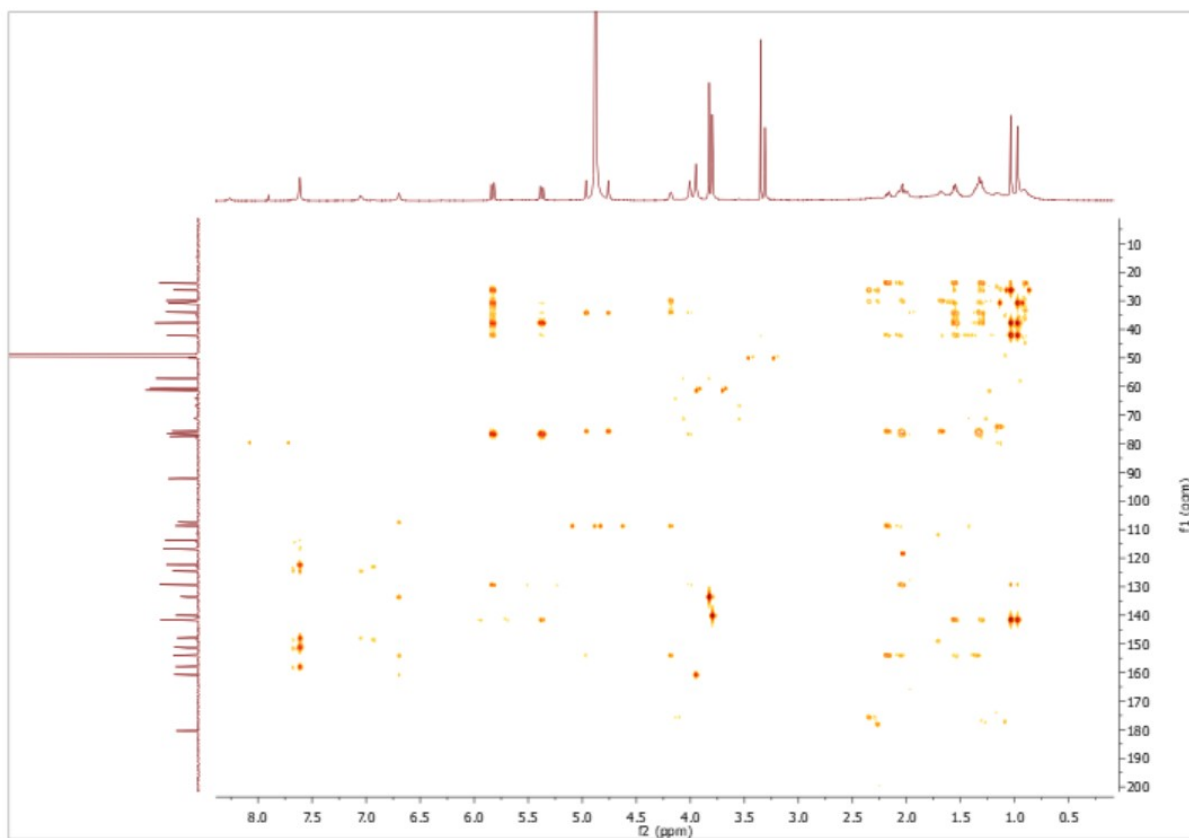
S23. ^1H - ^1H COSY (600 MHz, CD_3OD) of compound **3**



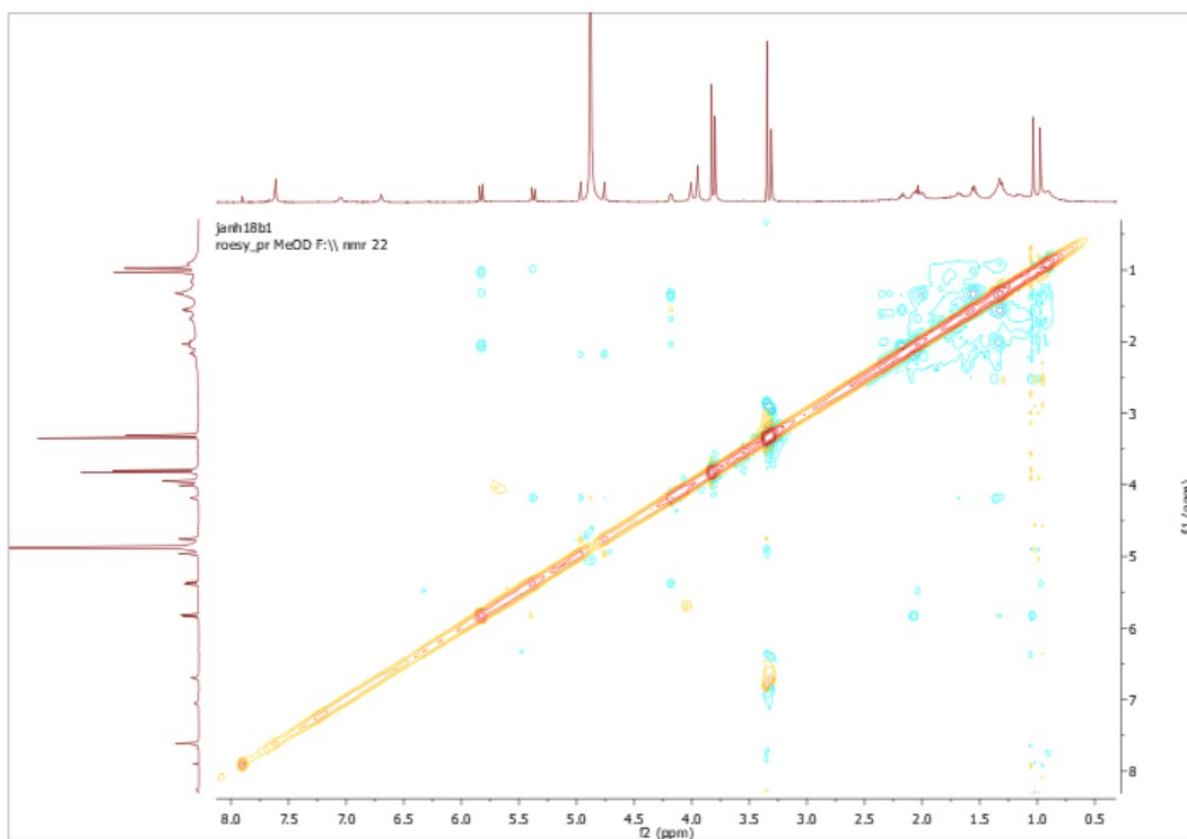
S24. HSQC (600 MHz, CD₃OD) of compound **3**



S25. HMBC (600 MHz, CD₃OD) of compound **3**



S26. ROESY (600 MHz, CD₃OD) 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, 13-DEC-2022

Set Temperature : 20.0

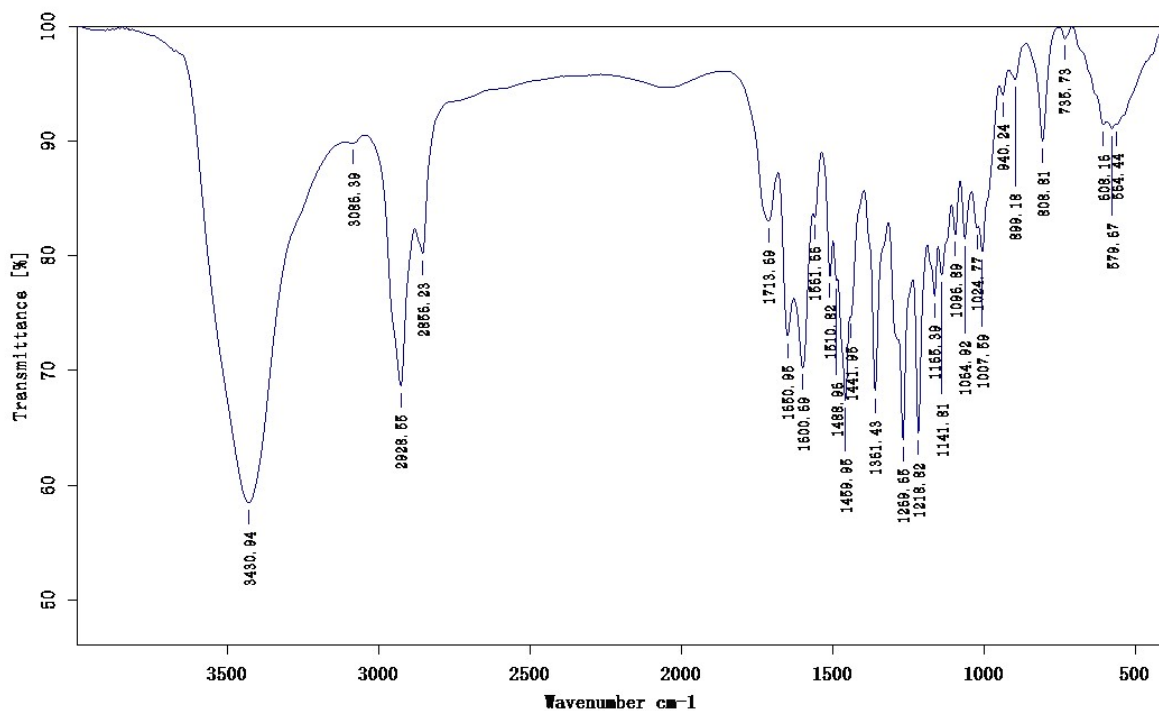
Time Delay : Disabled

Delay between Measurement : Disabled

<u>n</u>	<u>Average</u>	<u>Std.Dev.</u>	<u>% RSD</u>	<u>Maximum</u>	<u>Minimum</u>
5	-9.11	0.97	-10.64	-8.04	-10.71

<u>S.No</u>	<u>Sample ID</u>	<u>Time</u>	<u>Result</u>	<u>Scale</u>	<u>OR °Arc</u>	<u>WLG.nm</u>	<u>Lg.mm</u>	<u>Conc.g/100ml</u>	<u>Temp.</u>
1	janh-18b-1	03:30:30 PM	-8.93	SR	-0.010	589	100.00	0.112	20.2
2	janh-18b-1	03:30:37 PM	-10.71	SR	-0.012	589	100.00	0.112	20.2
3	janh-18b-1	03:30:43 PM	-8.93	SR	-0.010	589	100.00	0.112	20.2
4	janh-18b-1	03:30:50 PM	-8.93	SR	-0.010	589	100.00	0.112	20.1
5	janh-18b-1	03:30:57 PM	-8.04	SR	-0.009	589	100.00	0.112	20.1

S28. IR of compound 3

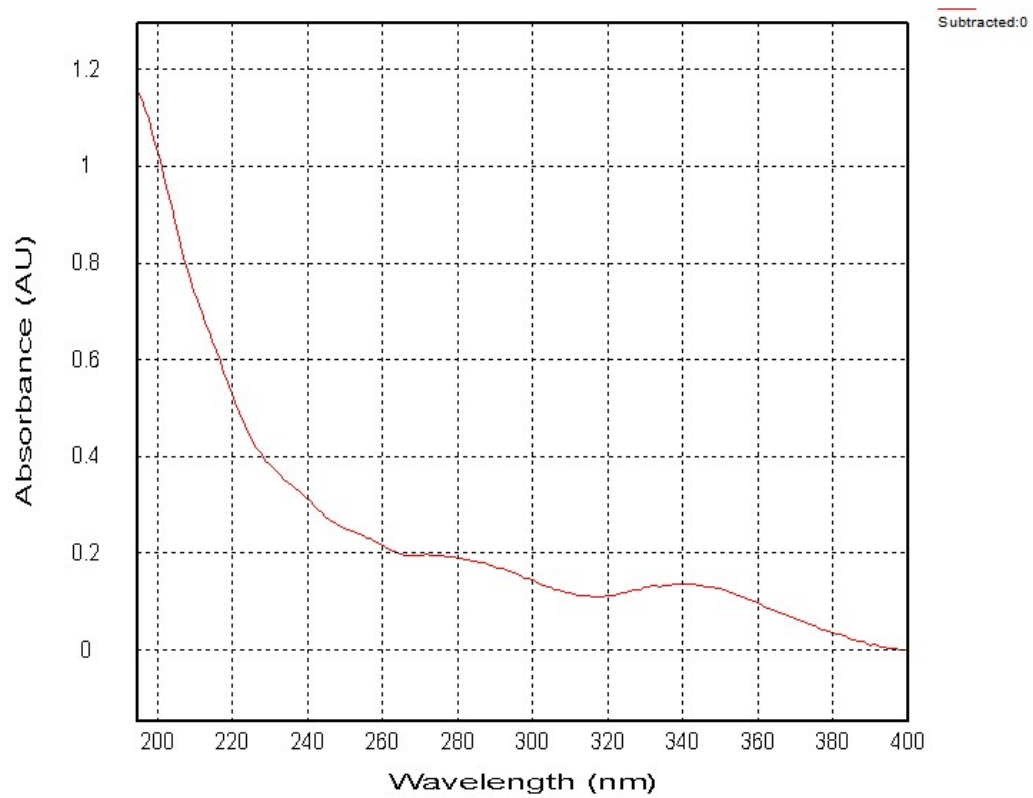
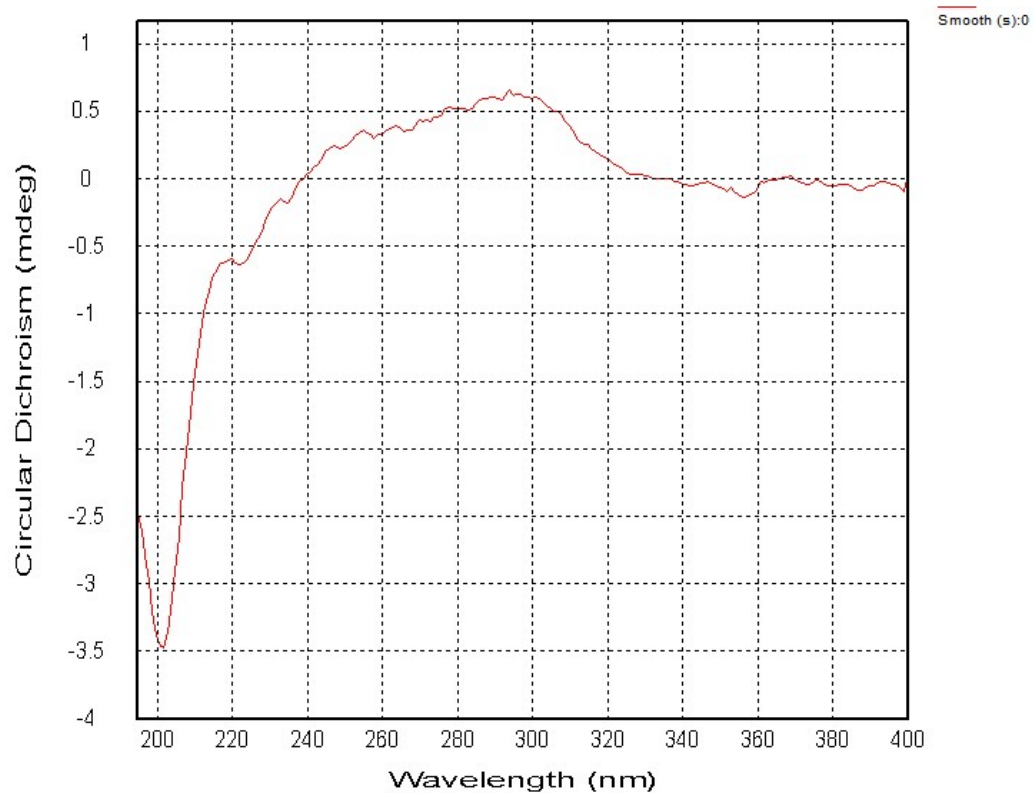


Sample Name: Janh-18b-1
 Sample Form: KBr
 Path of File: E:\data
 Date of Measurement: 2023/1/16

Resolution: 4
 Aperture Setting: 6 mm
 Number of Background Scans: 16
 Number of Sample Scans: 16

Beamsplitter Setting: KBr
 Source Setting: MIR
 Instrument Type: BRUKER VERTEX 70
 Soft Version: OPUS 8.1

S29. ECD and UV of compound 3



S30. HRESIMS of compound 3

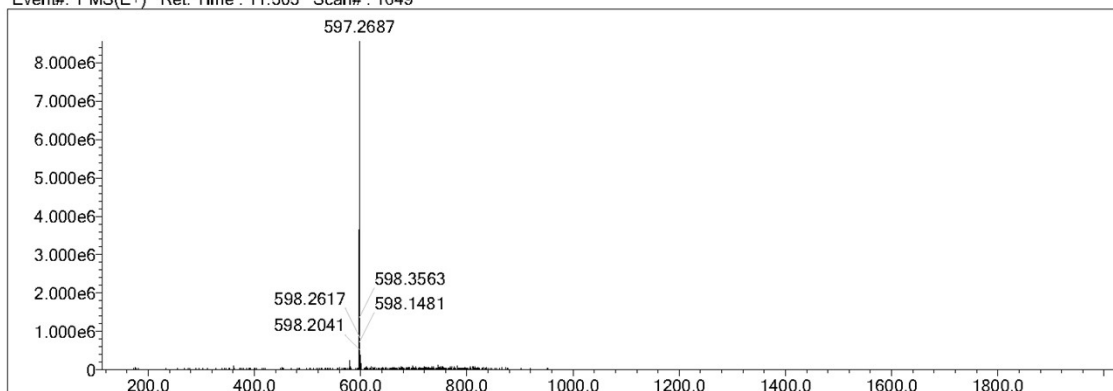
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	300	O	2	0	12	H
C	4	0	150	15N	3	0	0	Na
N	3	0	1					

Error Margin (ppm): 50
 HC Ratio: unlimited
 Max Isotopes: all
 MSn Iso RI (%): 75.00

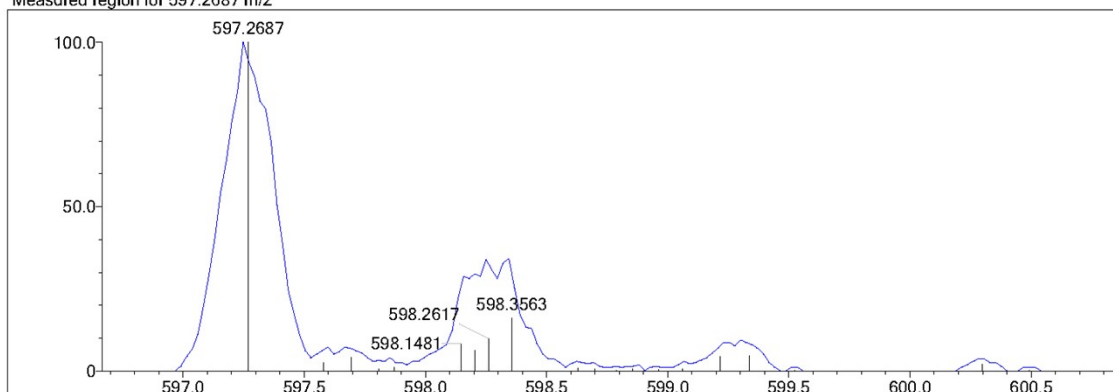
DBE Range: -2.0 - 1000.0
 Apply N Rule: yes
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

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

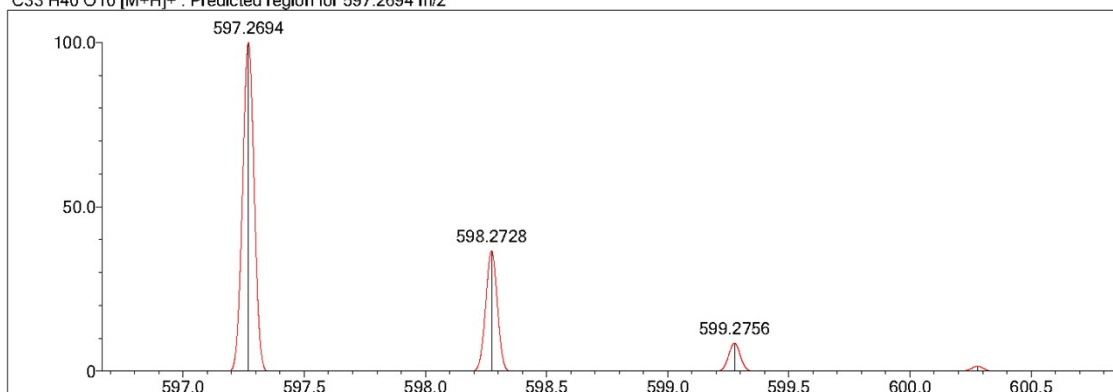
Event#: 1 MS(E+) Ret. Time : 11.505 Scan#: 1649



Measured region for 597.2687 m/z



C33 H40 O10 [M+H]⁺ : Predicted region for 597.2694 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	41.25	C33 H40 O10	[M+H] ⁺	597.2687	597.2694	-0.7	-1.17	41.43	14.0