Supporting information

Cytotoxicity-Guided Isolation of Elatostemanosides I-VI from *Elatostema tenuicaudatum* W.T.Wang and Their Cytotoxicity Activities

Quoc-Dung Tran Huynh,[ab#] Thuy-Tien Thi Phan,[bc#] Ta-Wei Liu,[d] Truc-Ly Thi Duong,[e] Su-Jung Hsu,[d] Ching-Chuan Kuo,[f] Man-Hsiu Chu,[d] Yun-Han Wang,[a] Thanh-Vu Nguyen,[g] Yao-An Shen,[hij] Ju-Jui Fan,[kl] Dang-Khoa Nguyen,[m] Thanh-Hoa Vo[no] and Ching-Kuo Lee*[adqr]

[a] Ph.D. Program in Clinical Drug Development of Herbal Medicine, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan.
[b] Institute of Pharmaceutical Education and Research, Binh Duong University, Thu Dau Mot 820000, Binh Duong, Vietnam.
[c] Graduate Institute of Biomedical Materials and Tissue Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, 11031, Taiwan
[d] School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei 11042, Taiwan
[e] Faculty of Traditional medicine, Can Tho University of Medicine and Pharmacy, Can Tho 900000, Vietnam
[f] Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 35053, Taiwan
[g] Biotechnology Center Of Ho Chi Minh City, Ho Chi Minh City 700000, Vietnam
[h] Department of Pathology, School of Medicine, College of Medicine, Taipei Medical University, Taipei 110301, Taiwan
[i] Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei 110301, Taiwan
[j] International Master/Ph.D. Program in Medicine, College of Medicine, Taipei Medical University, Taipei 110301, Taiwan
[k] School of Biomedical Engineering, Taipei Medical University, Taipei 11031, Taiwan
[l] International PhD Program for Biomedical Engineering, Taipei Medical University, Taipei 11031, Taiwan
[m] Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh 700000, Viet Nam
[n] University of Health Sciences, Vietnam National University Ho Chi Minh City, Ho Chi Minh 700000, Vietnam
[o] Center for Discovery and Development of Healthcare Product, Vietnam National University Ho Chi Minh City, Ho Chi Minh 700000, Vietnam
[p] University of Health Sciences, Vietnam National University Ho Chi Minh City, Ho Chi Minh 700000, Vietnam
[q] Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei 11042, Taiwan
[r] Department of Chemistry, Chung Yuan Christian University, Zhongli District, Taoyuan 32023, Taiwan
# These authors contributed equally

Figure S1. ^{1}H-NMR spectrum of compound 1

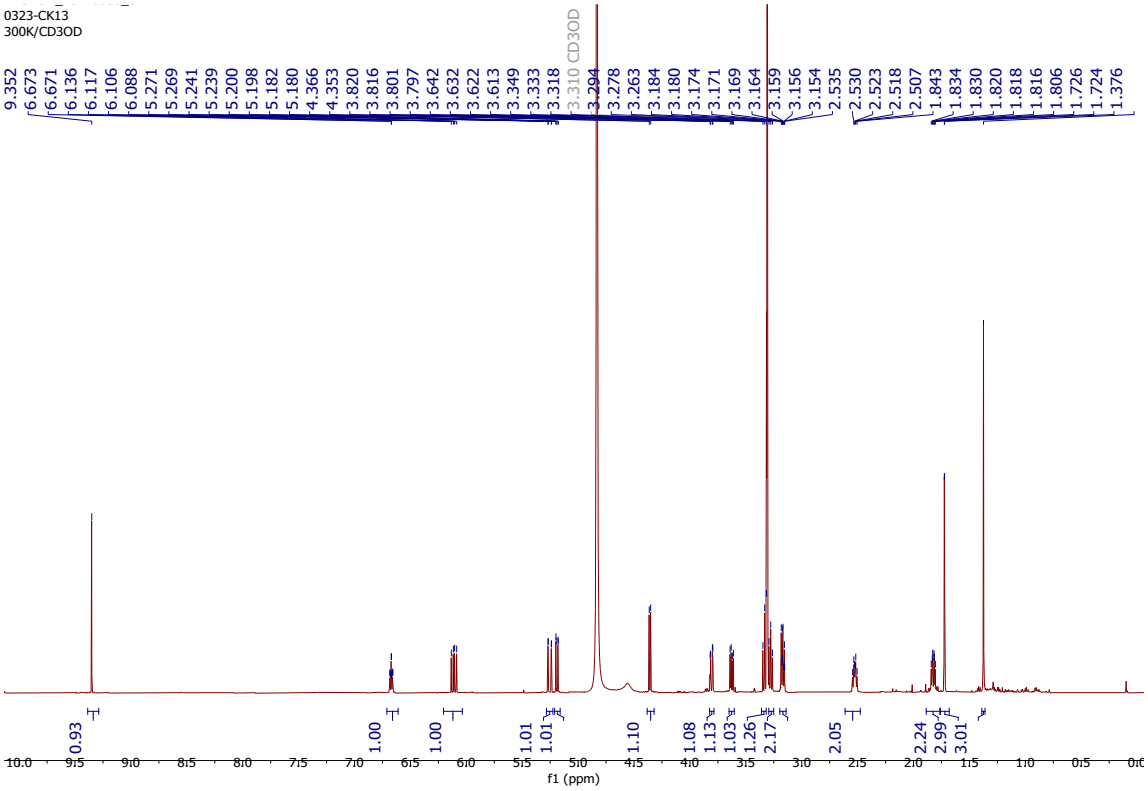


Figure S2. ^{13}C-NMR spectrum of compound 1

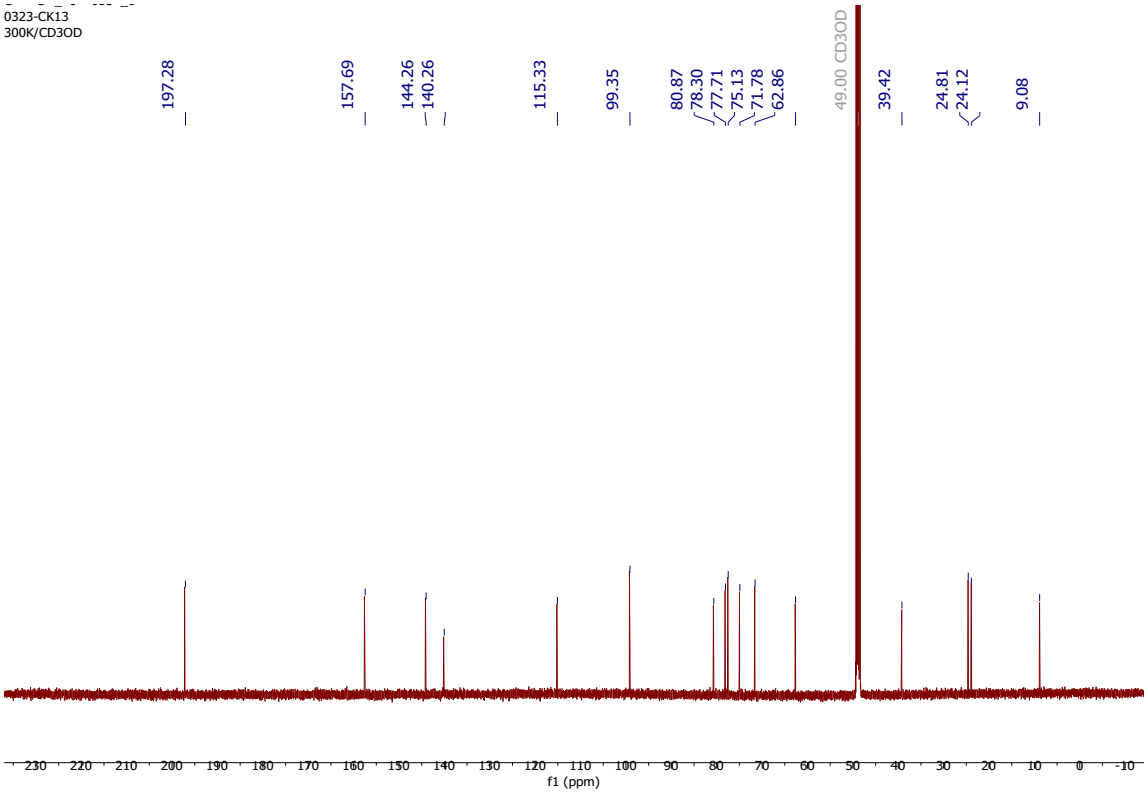


Figure S3. DEPT NMR spectrum of compound 1

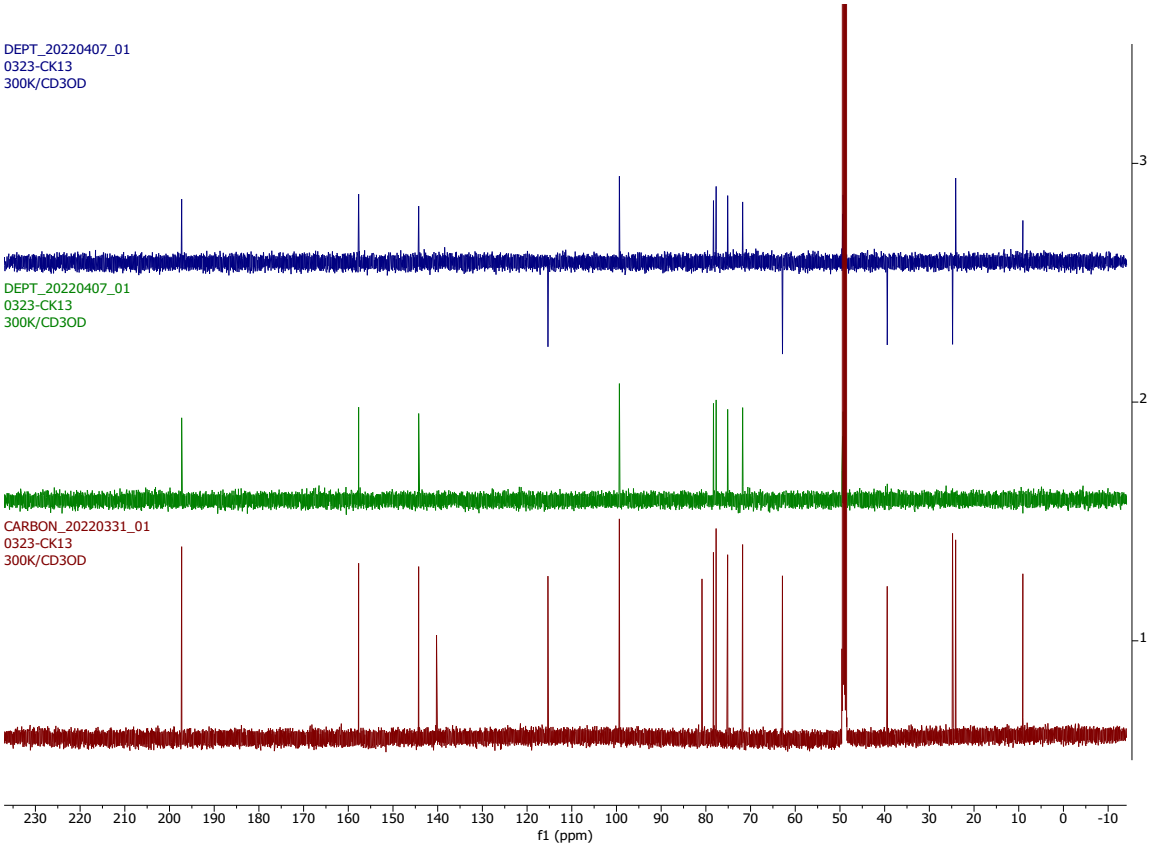


Figure S4. HSQC spectrum of compound 1

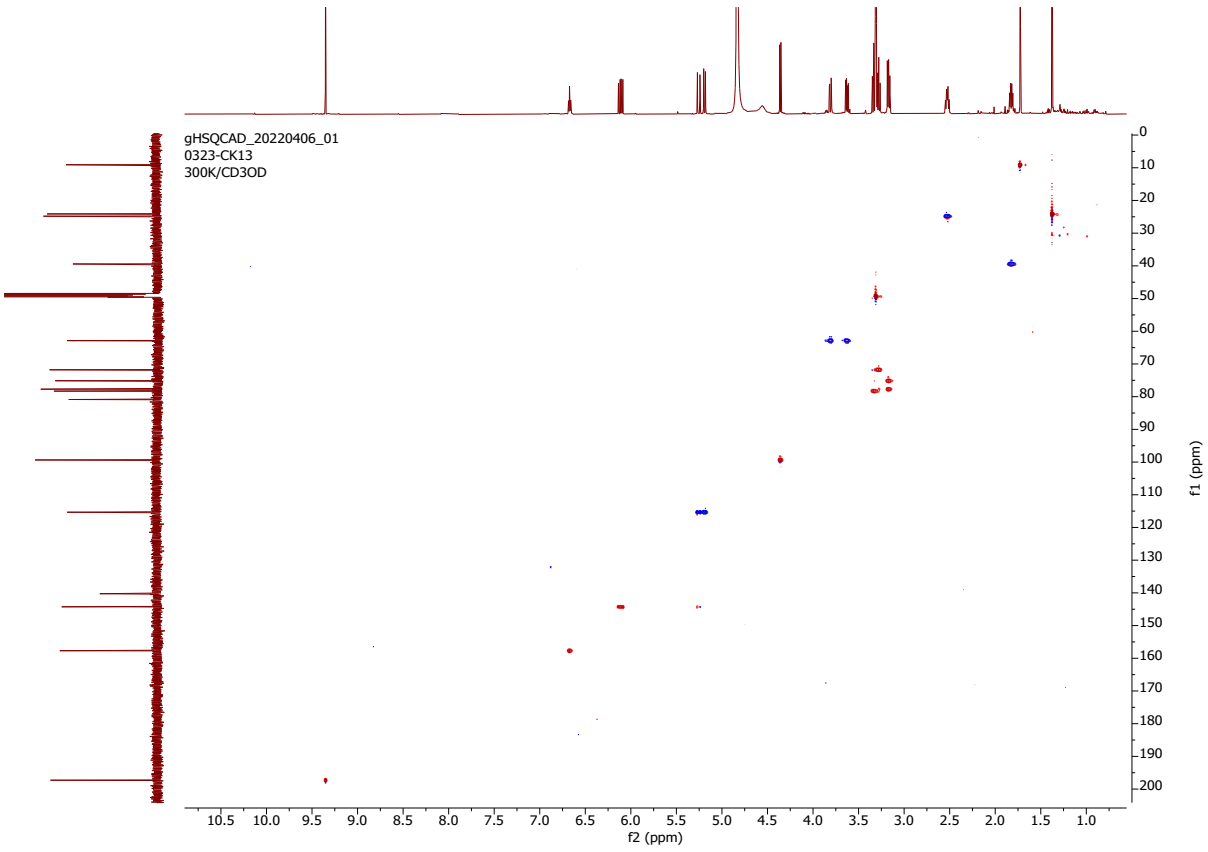


Figure S5. HMBC NMR spectrum of compound 1

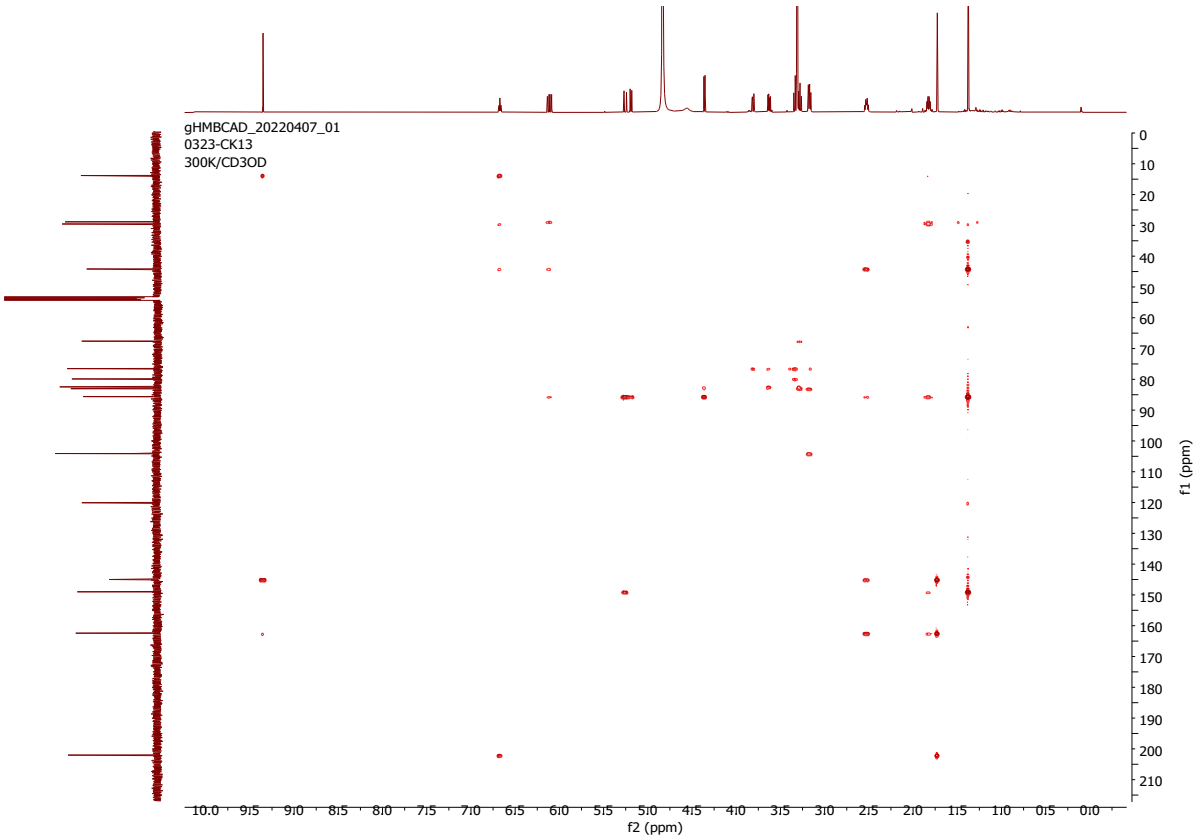


Figure S6. COSY NMR spectrum of compound 1

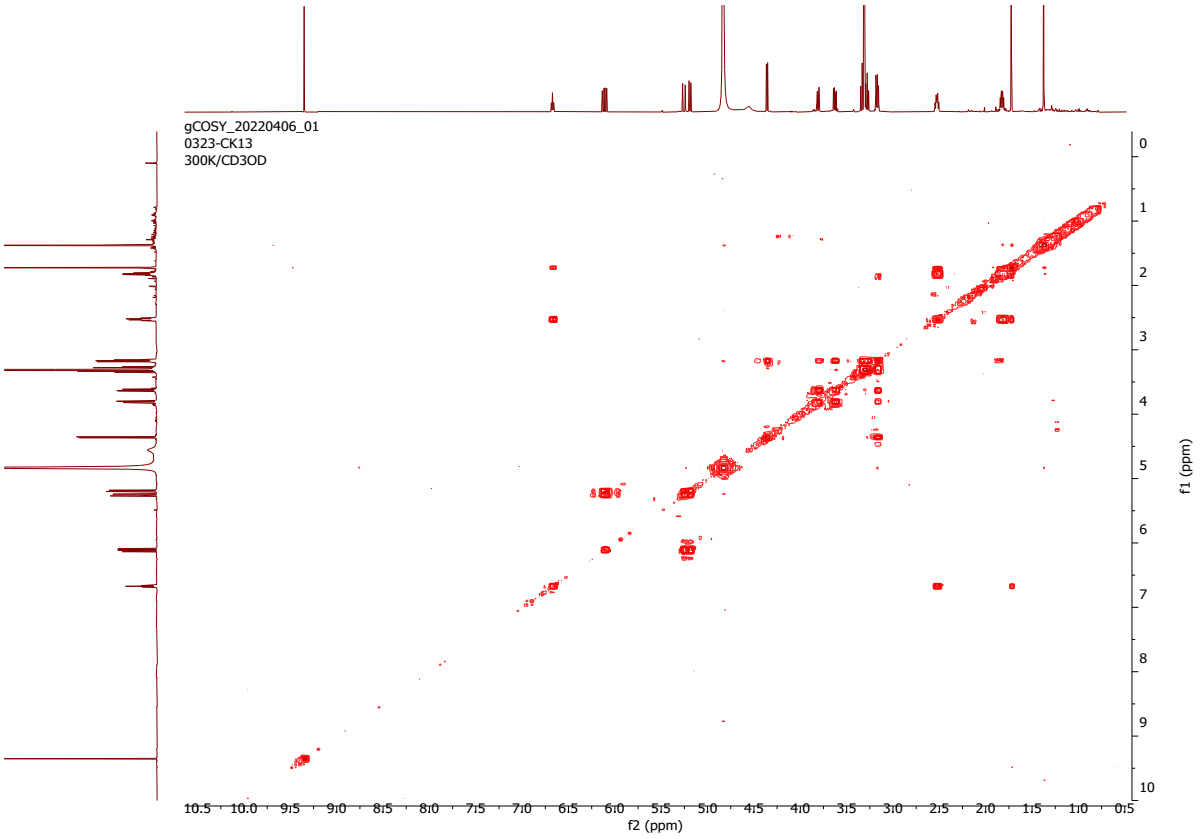


Figure S7. NOESY NMR spectrum of compound 1

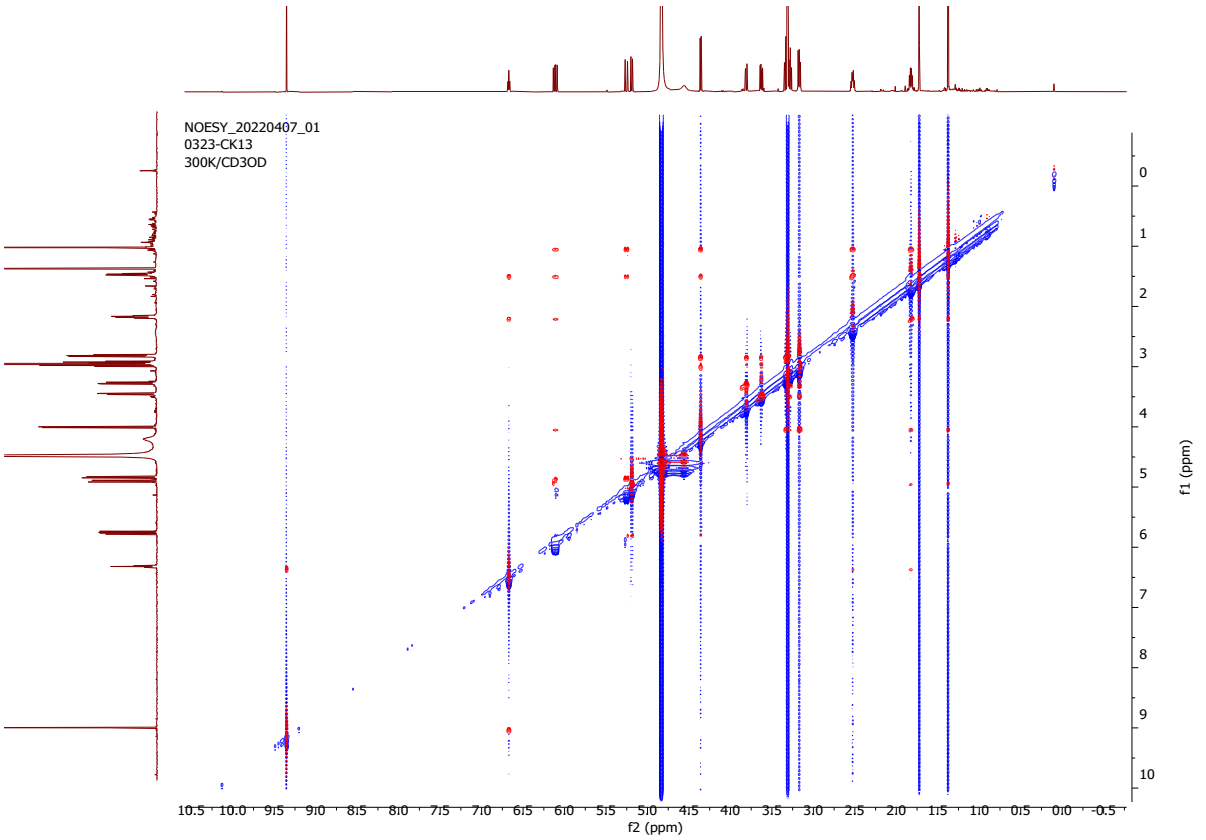
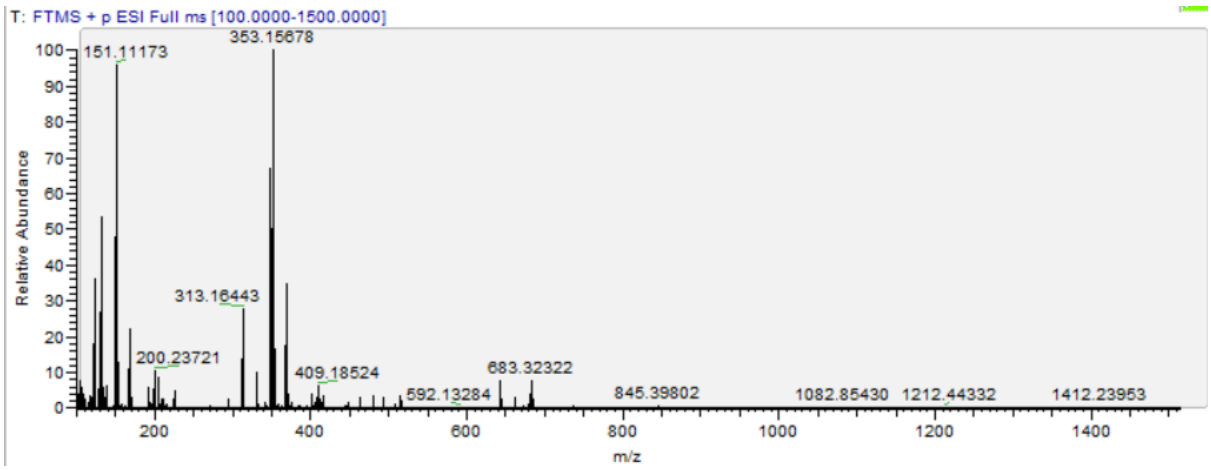


Figure S8. HR-ESI-MS spectra of compound 1



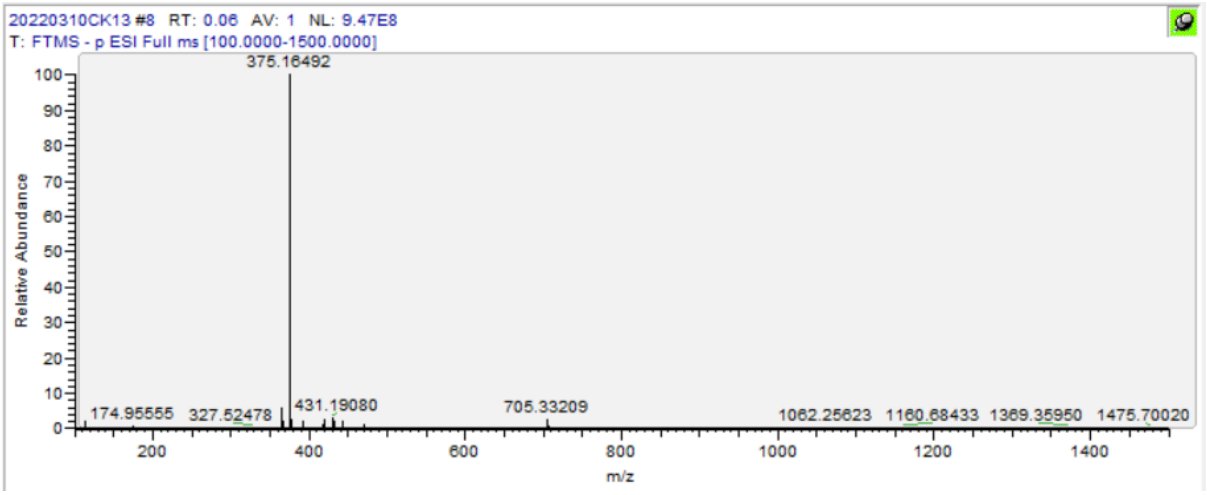
Elemental composition

Single mass

Mass: 353.15678

Max. results 10 Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{16}H_{26}O_7Na$	3.5	-0.833



Elemental composition

Single mass

Mass: 375.16492

Max. results 10 Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{17}H_{27}O_9$	4.5	-0.103

Figure S9. UV spectrum of compound 1

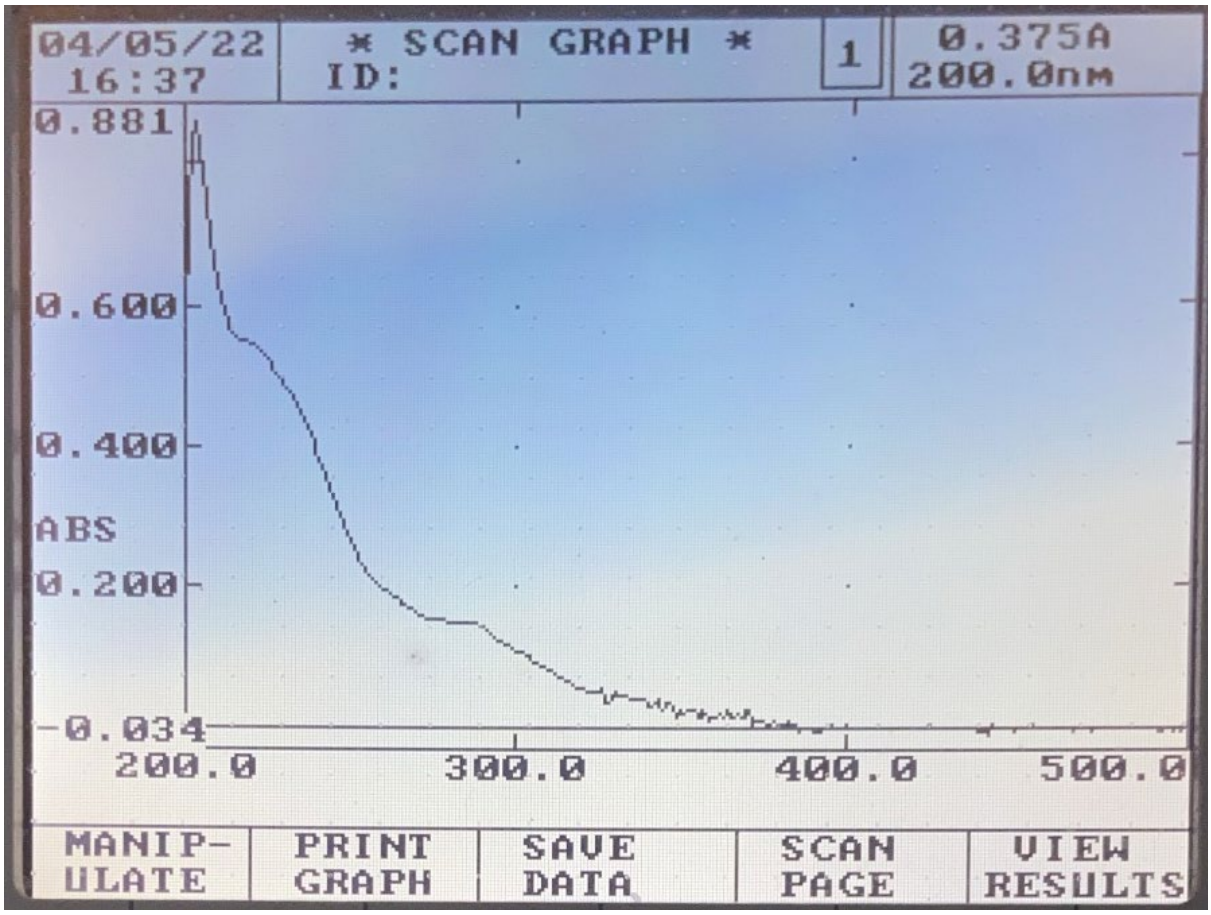
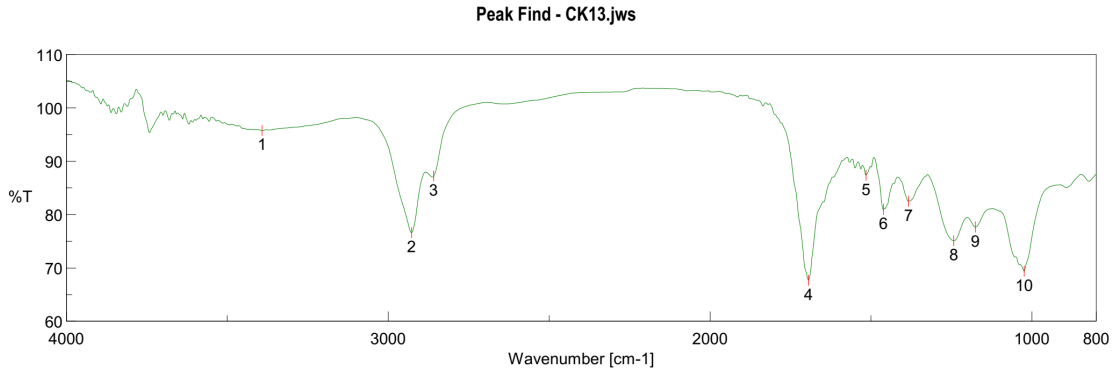


Figure S10. IR spectrum of compound 1



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3392.17	95.7439	2	2927.41	76.6019	3	2858.95	87.188
4	1694.16	67.6572	5	1515.78	87.3484	6	1461.78	80.945
7	1383.68	82.462	8	1242.9	75.0577	9	1175.4	77.6305
10	1024.02	69.3625						

Figure S11. ^{1}H-NMR spectrum of compound 2

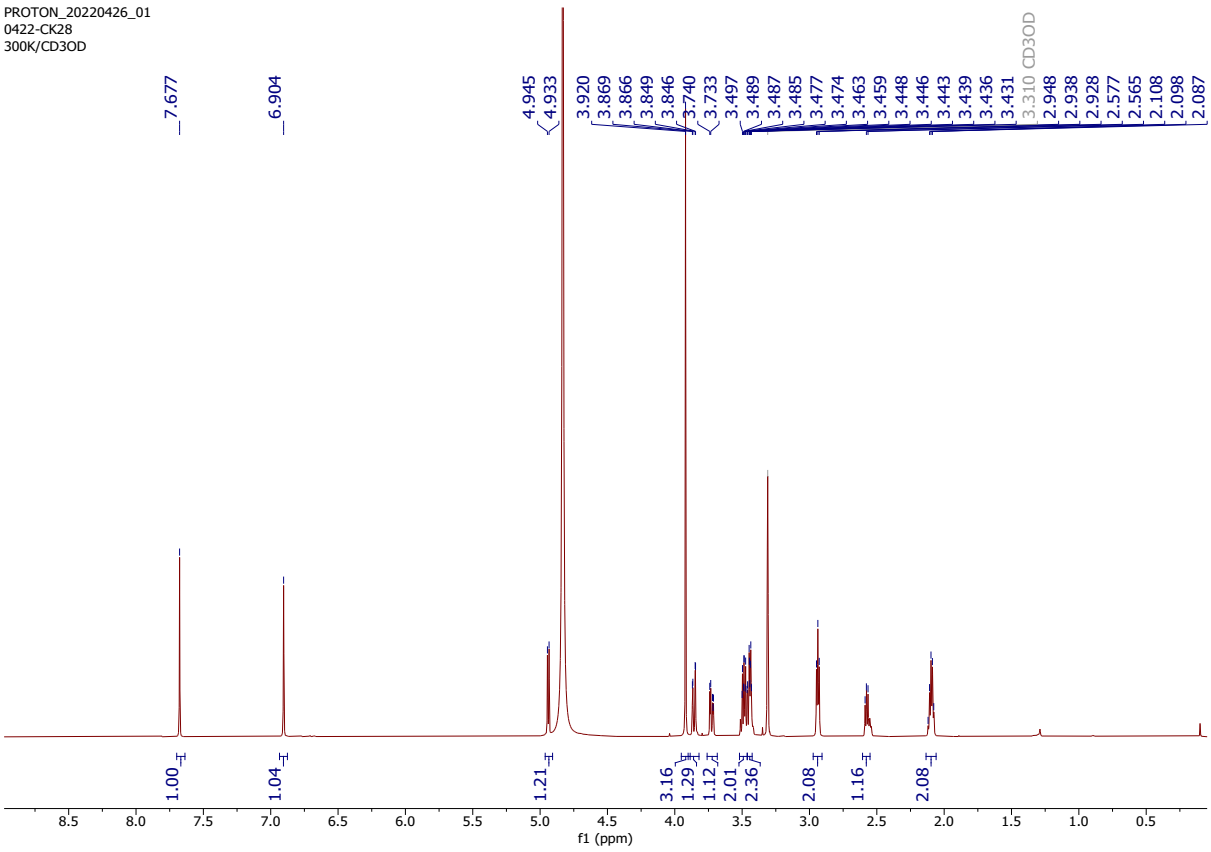


Figure S12. ^{13}C-NMR spectrum of compound 2

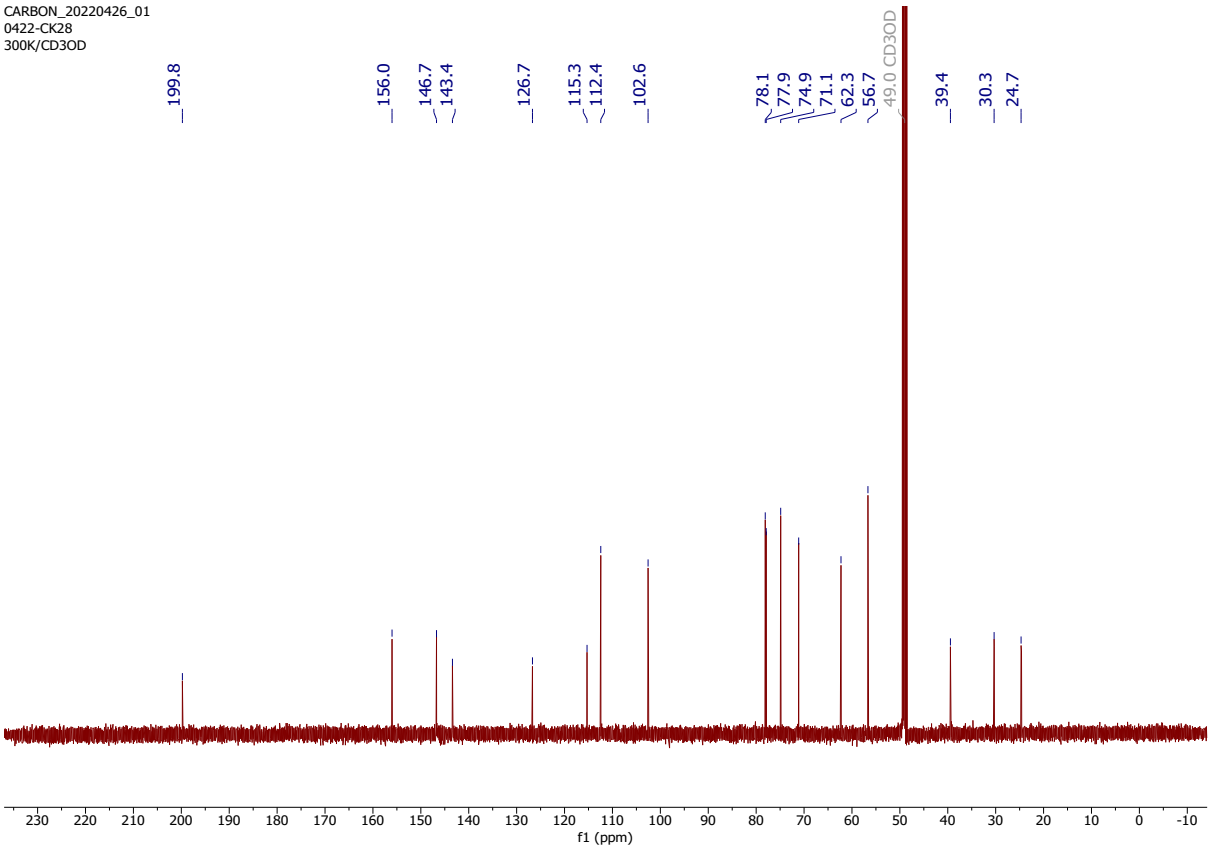


Figure S13. DEPT NMR spectrum of compound 2

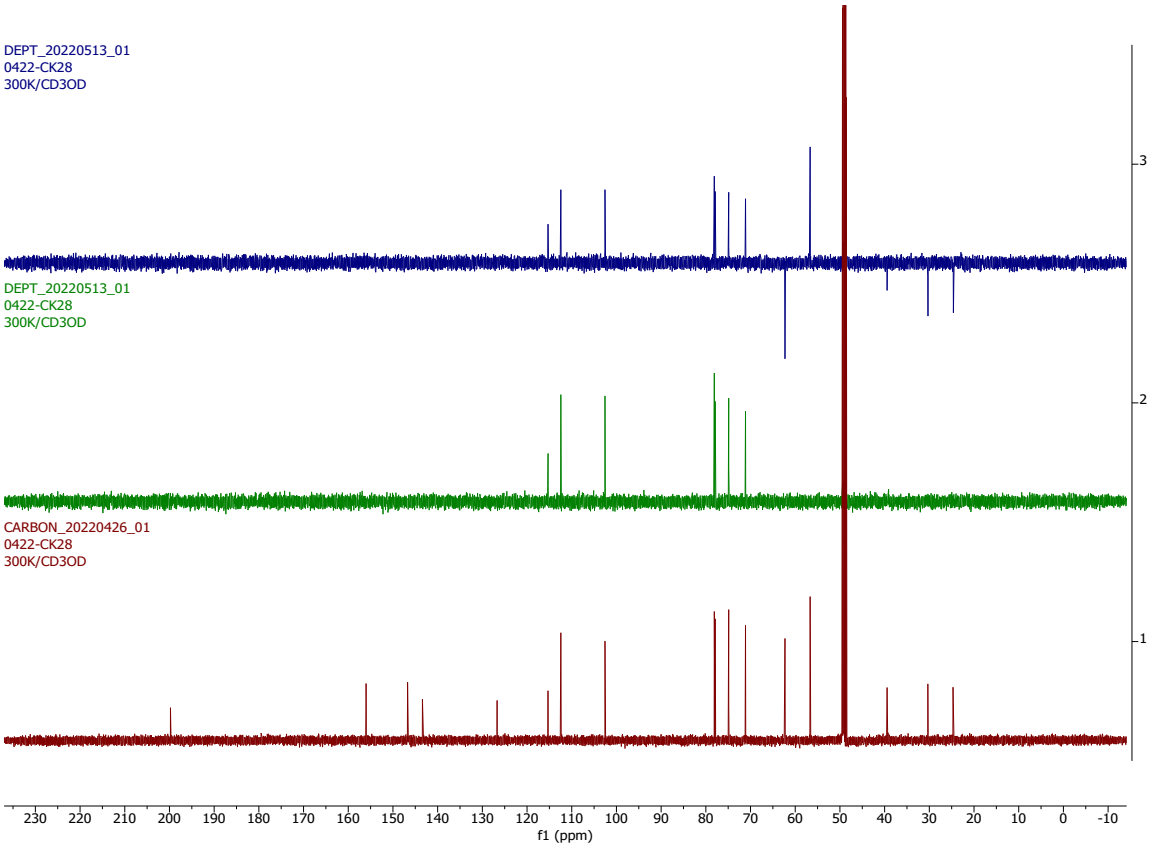


Figure S14. HSQC spectrum of compound 2

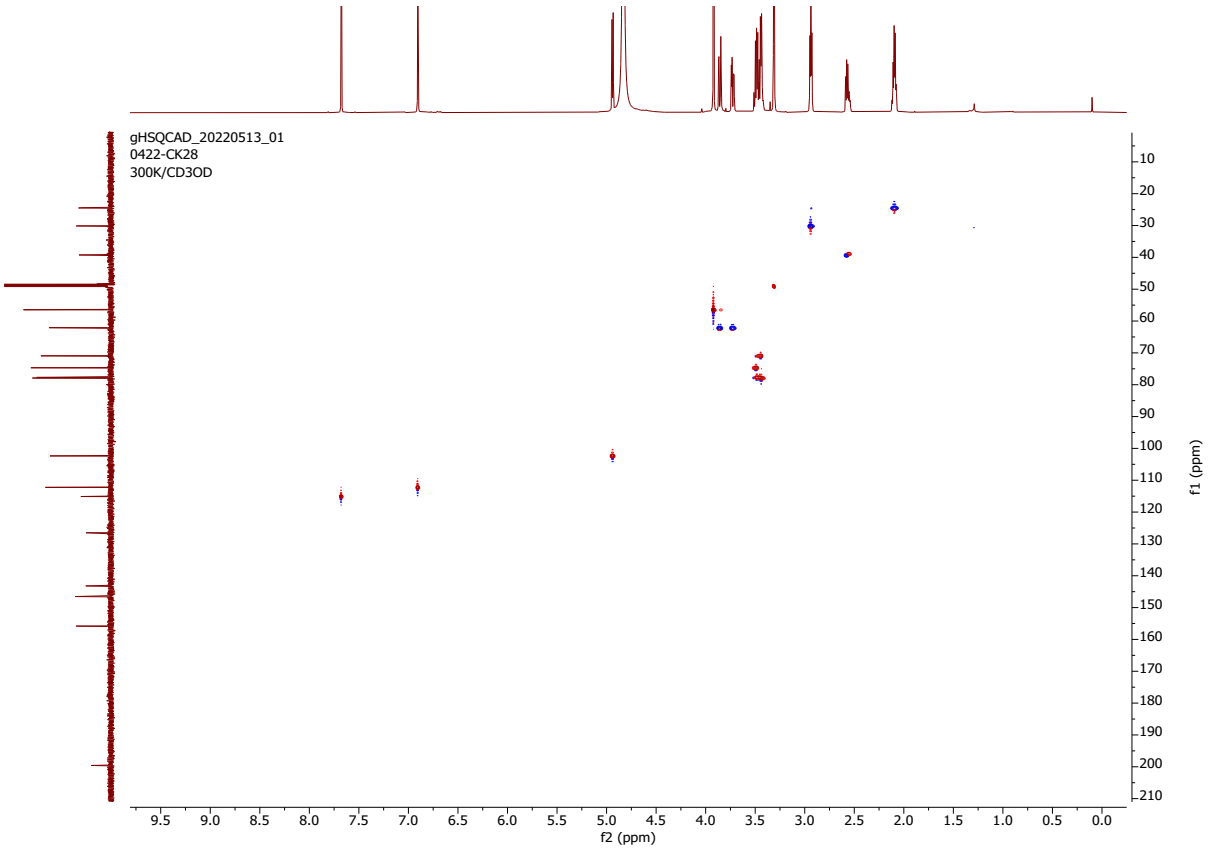


Figure S15. HMBC spectrum of compound 2

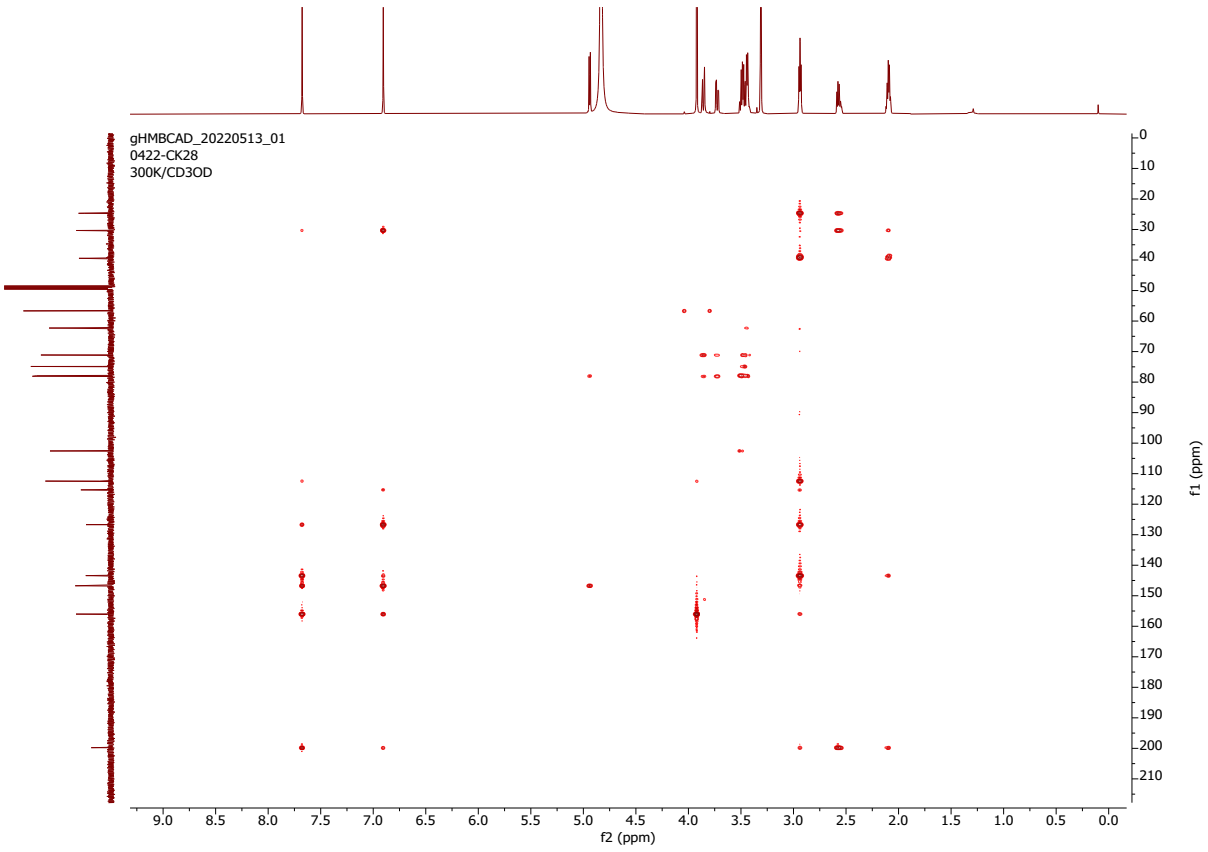


Figure S16. COSY spectrum of compound 2

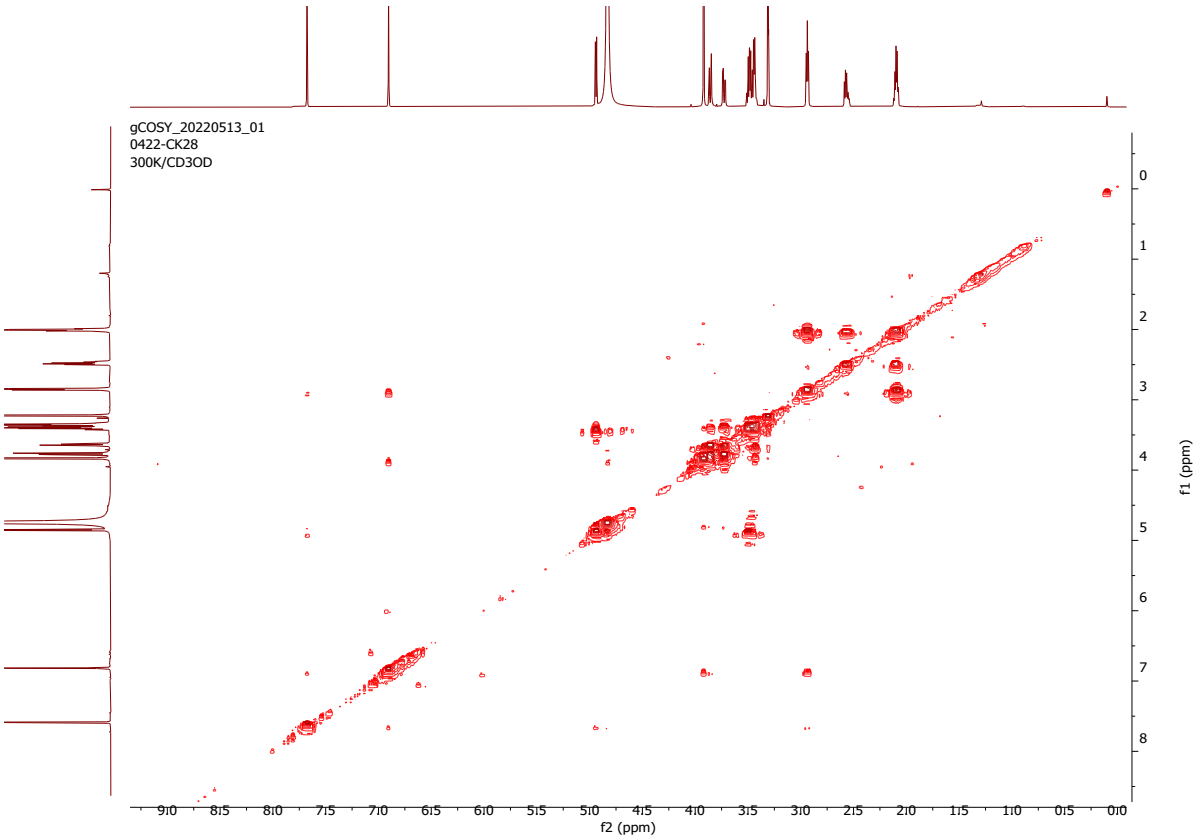


Figure S17. NOESY spectrum of compound 2

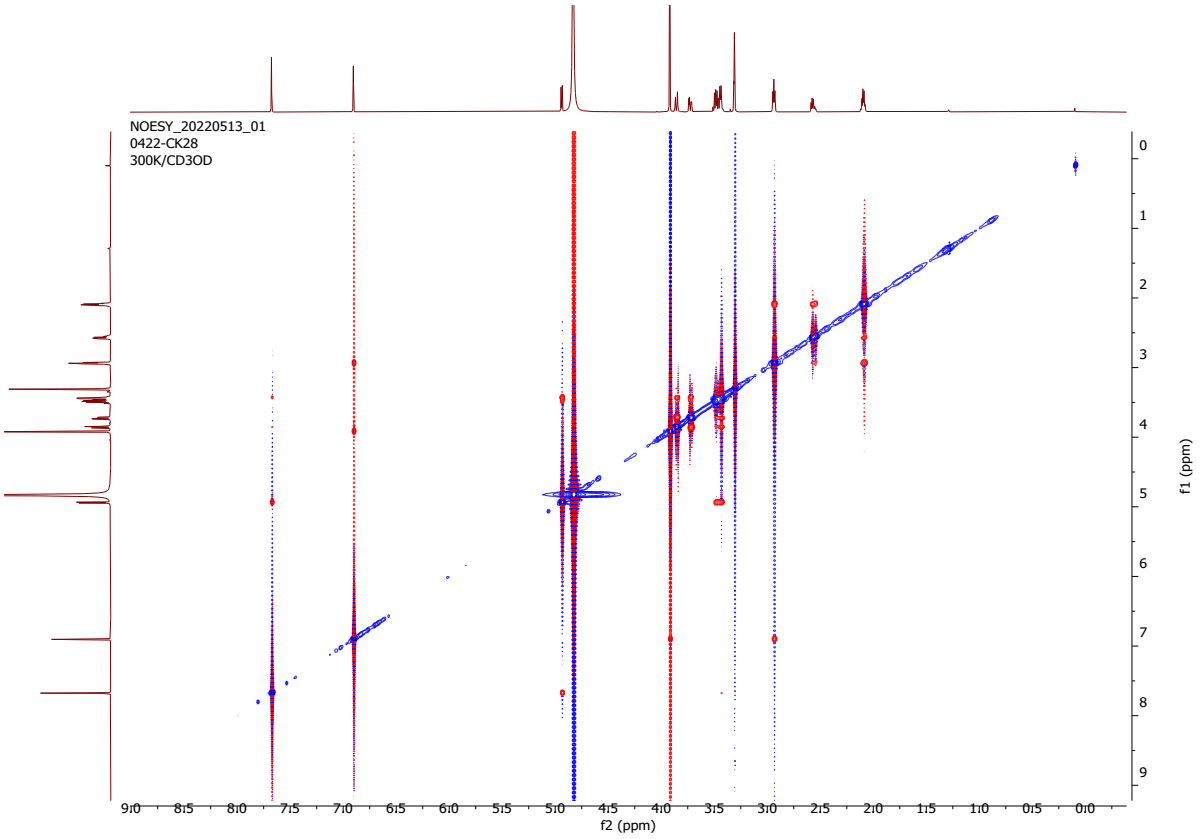
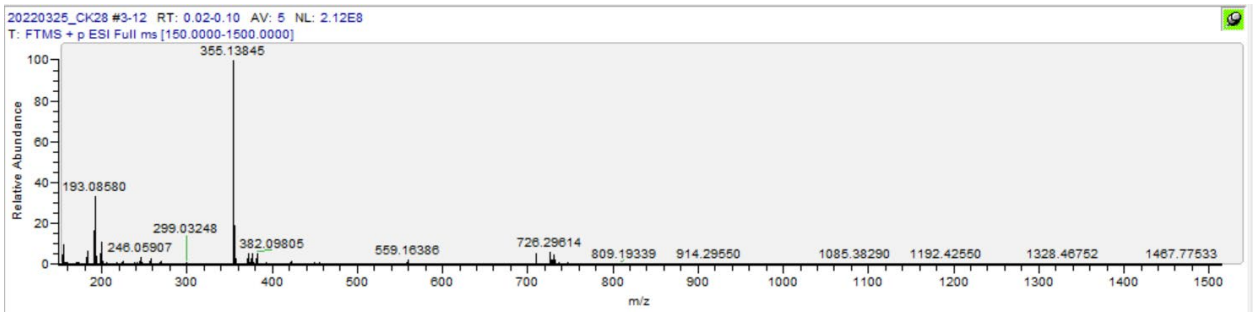


Figure S18. HR-ESI-MS spectra of compound 2



Elemental composition

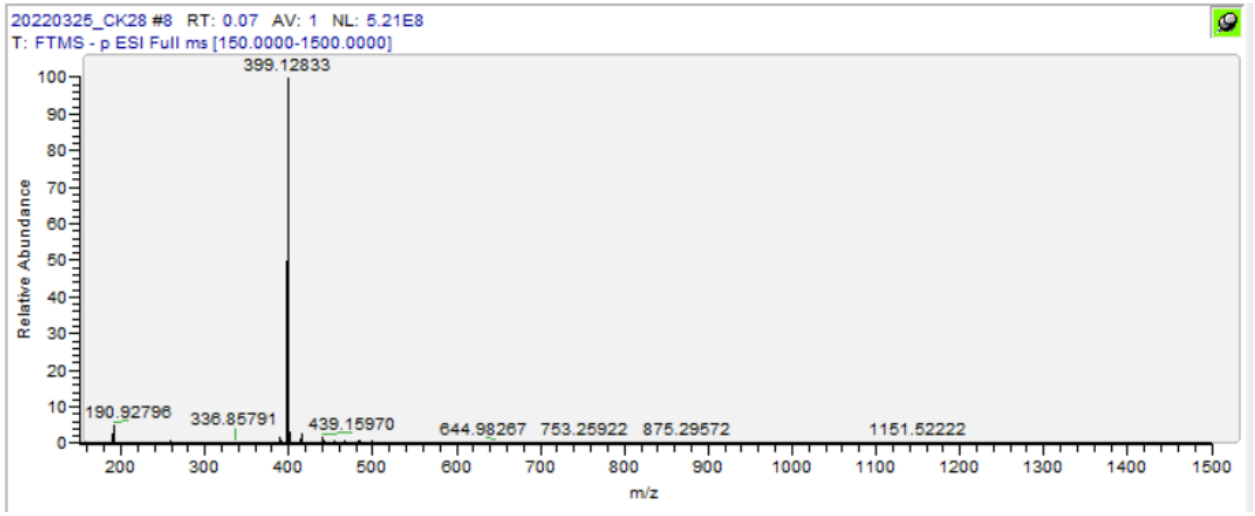
Single mass

Mass: 355.13845

Max. results 10

Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{17}H_{23}O_8$	6.5	-0.828



Elemental composition

Single mass

Mass: 399.12833

Max. results 10

Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{18}H_{23}O_{10}$	7.5	-0.610

Figure S19. UV spectrum of compound 2

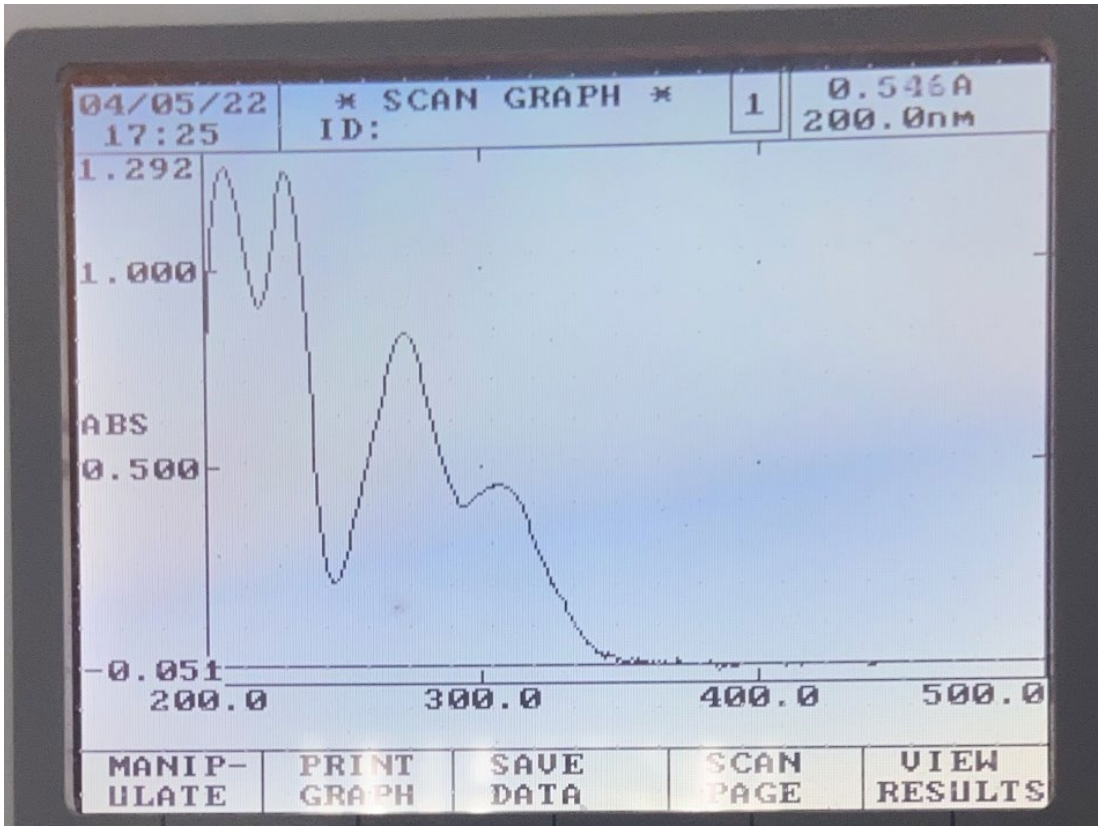
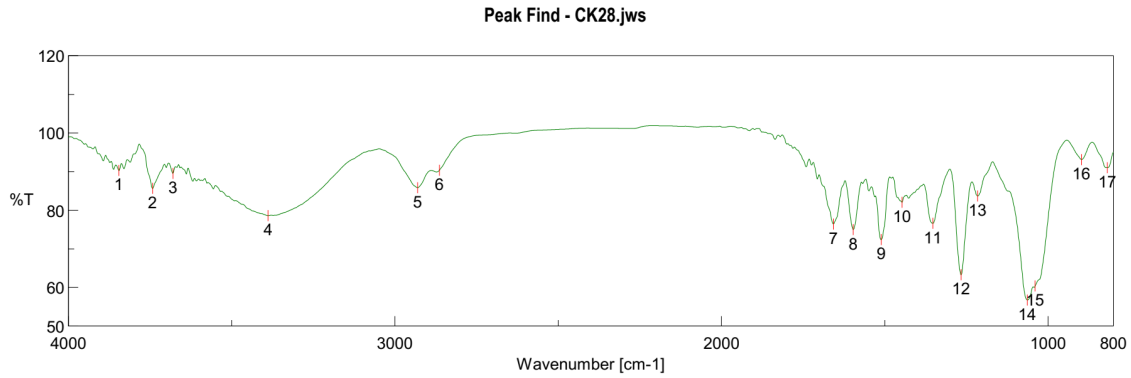


Figure S20. IR spectrum of compound 2



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3845.36	90.2716	2	3742.19	85.6259	3	3679.51	89.4822
4	3388.32	78.5835	5	2930.31	85.8124	6	2863.77	90.3292
7	1657.52	76.3876	8	1596.77	74.979	9	1510.95	72.3557
10	1447.31	82.0136	11	1352.82	76.5968	12	1266.04	63.2542
13	1215.9	83.6578	14	1063.55	56.7262	15	1039.44	60.3581
16	897.701	93.0801	17	818.634	90.9478	18	676.892	86.1439
19	592.039	85.5411	20	412.692	104.924			

Figure S21. ^{1}H-NMR spectrum of compound 3

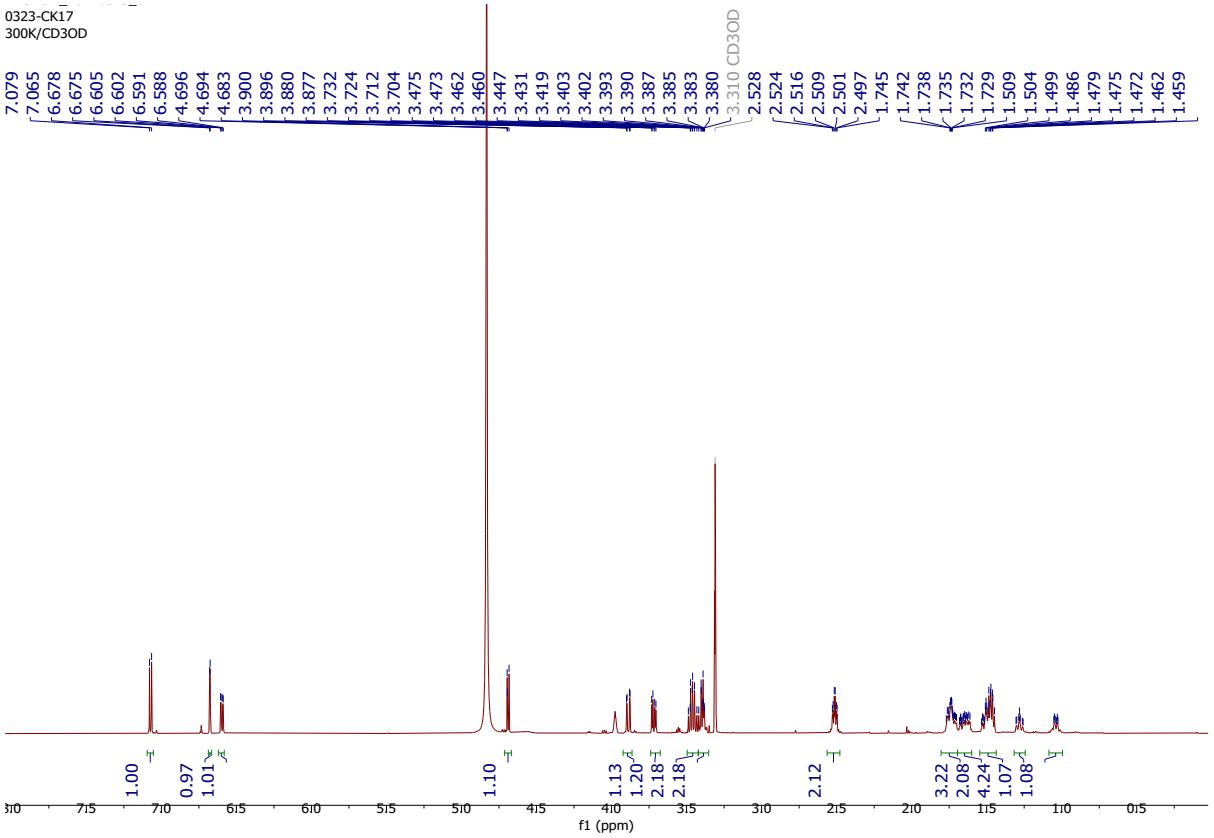


Figure S22. ^{13}C-NMR spectrum of compound 3

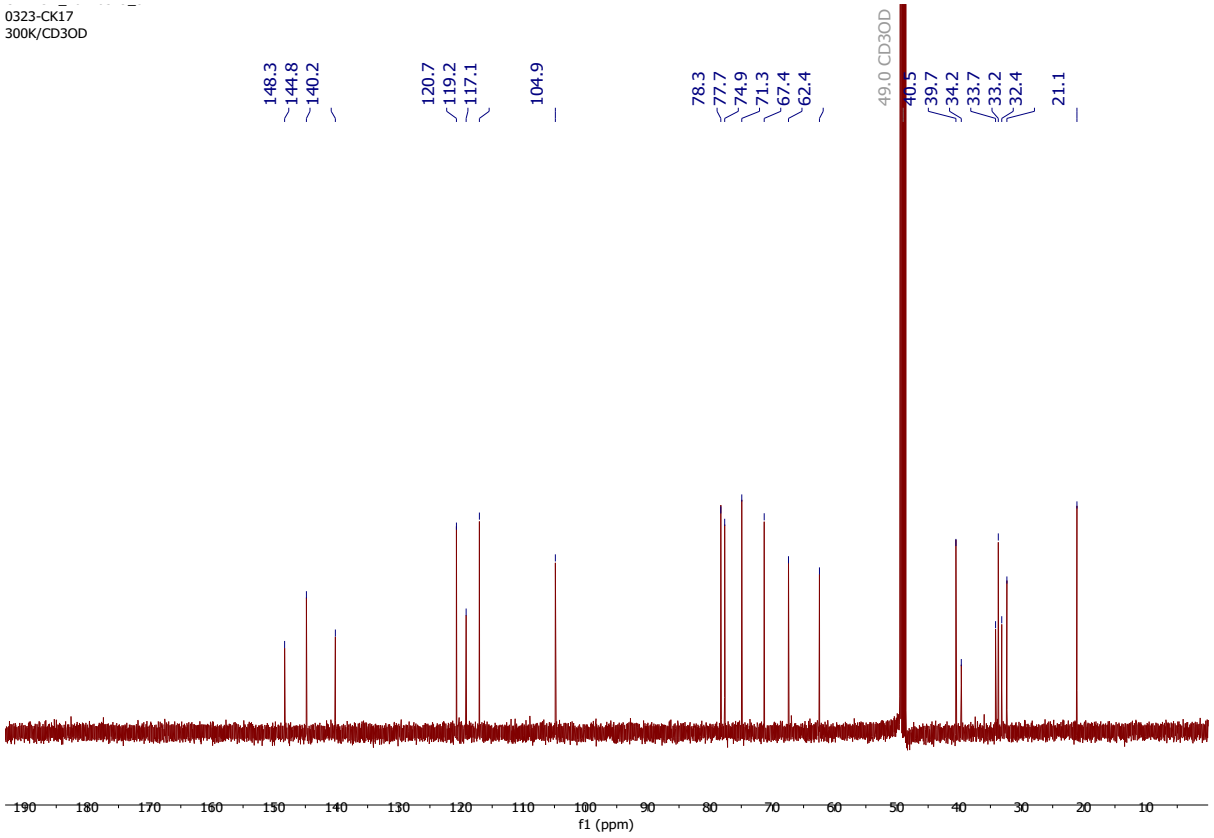


Figure S23. DEPT NMR spectrum of compound 3

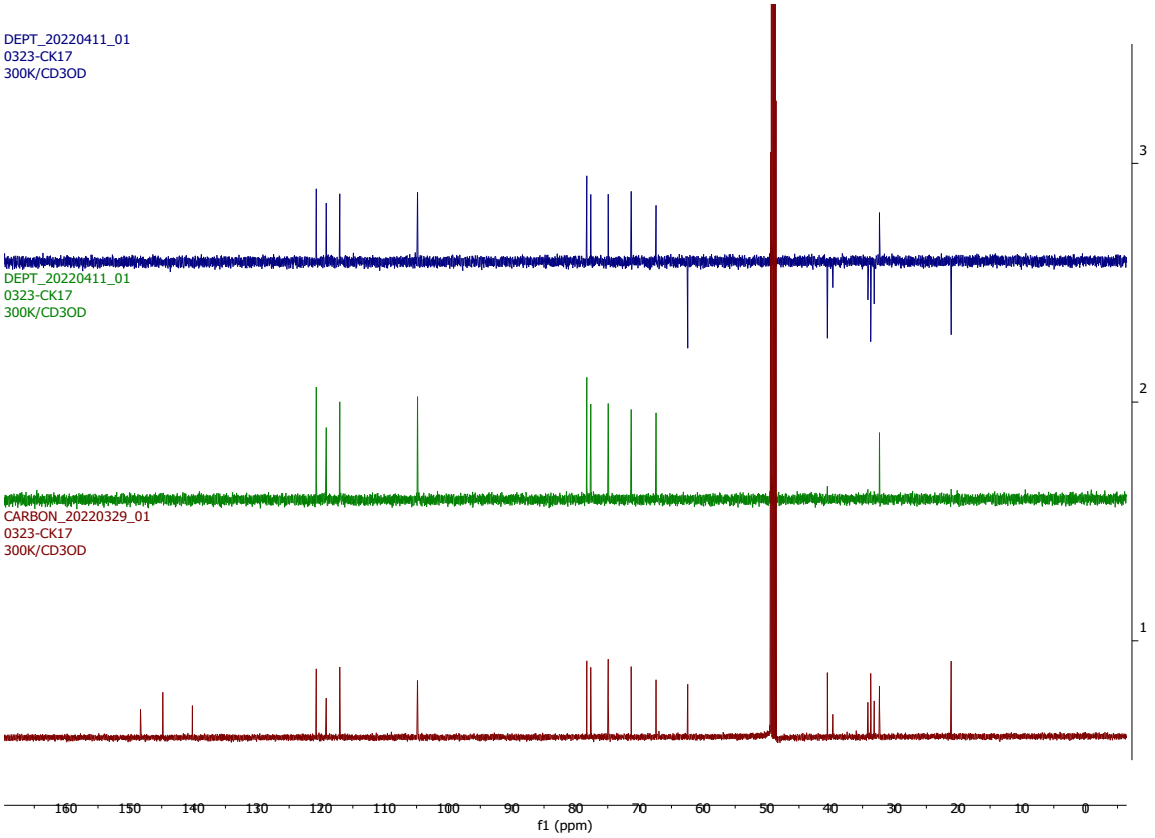


Figure S24. HSQC NMR spectrum of compound 3

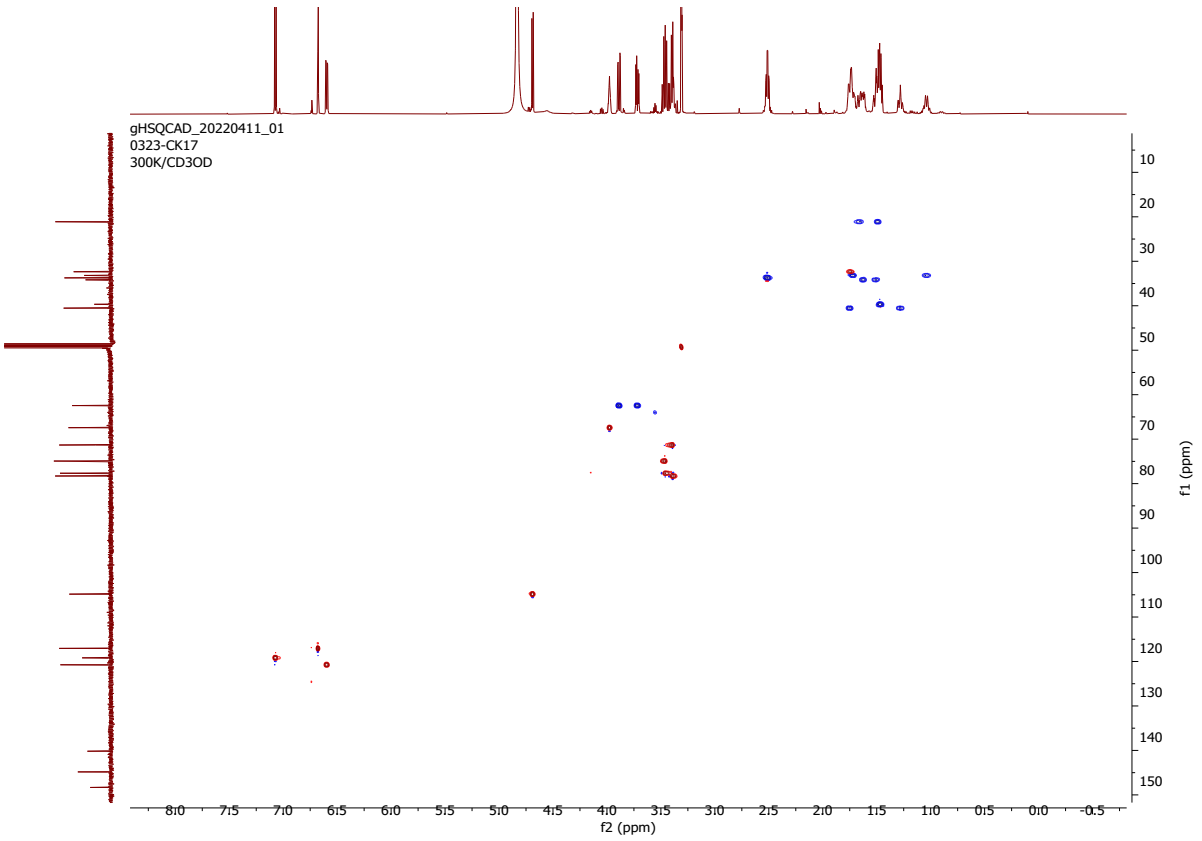


Figure S25. HMBC NMR spectrum of compound 3

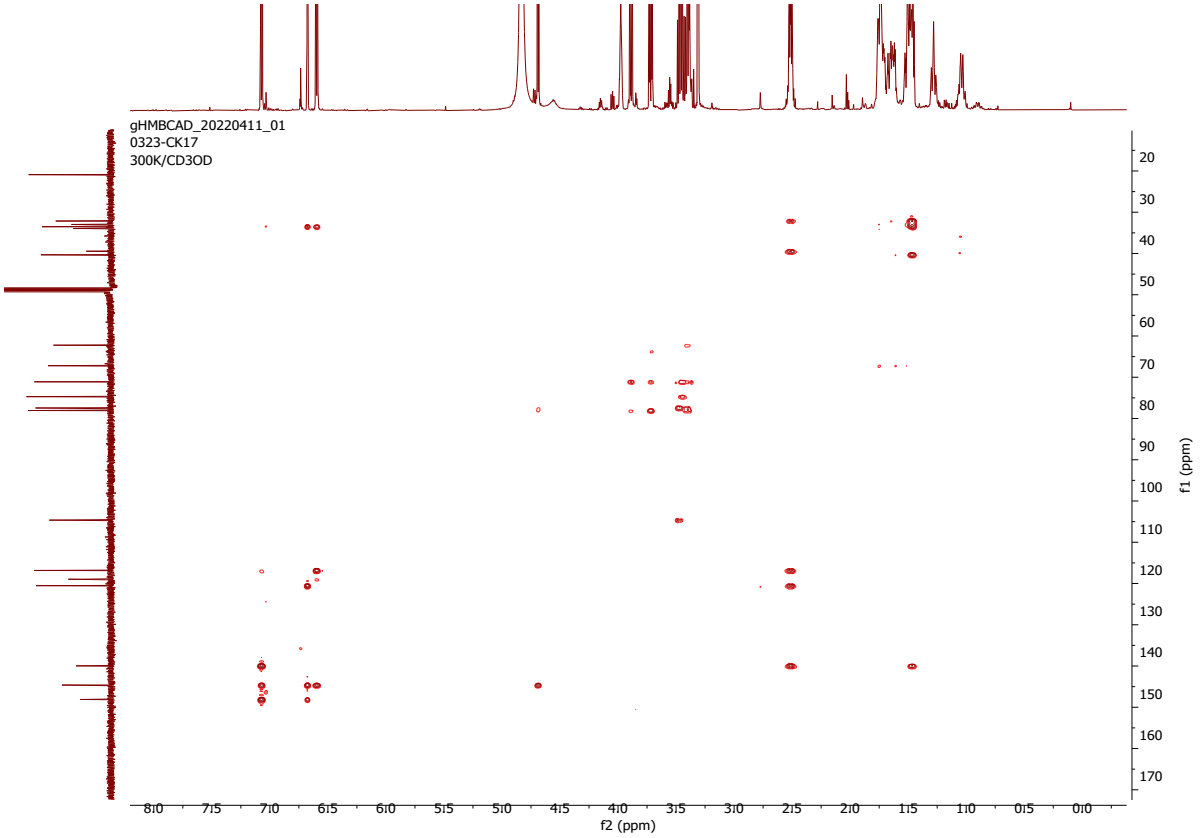


Figure S26. COSY NMR spectrum of compound 3

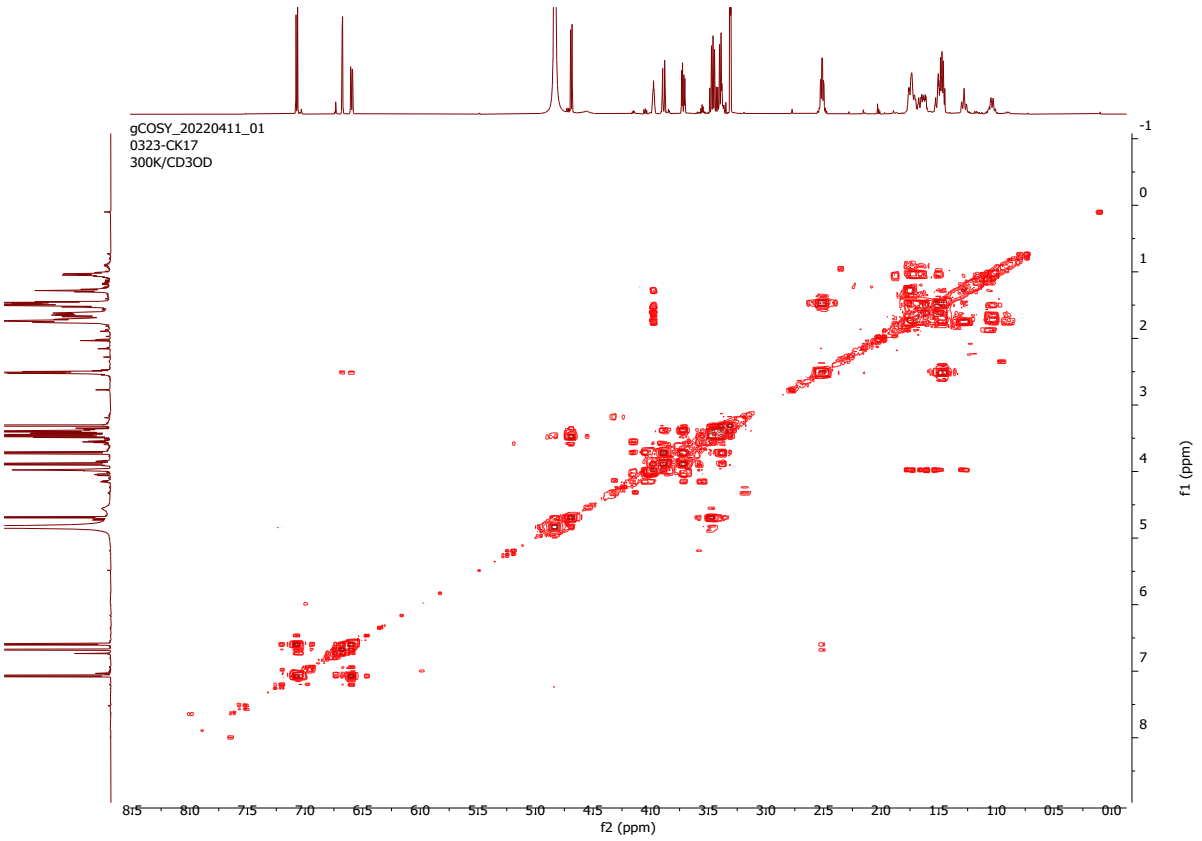


Figure S27. NOESY NMR spectrum of compound 3

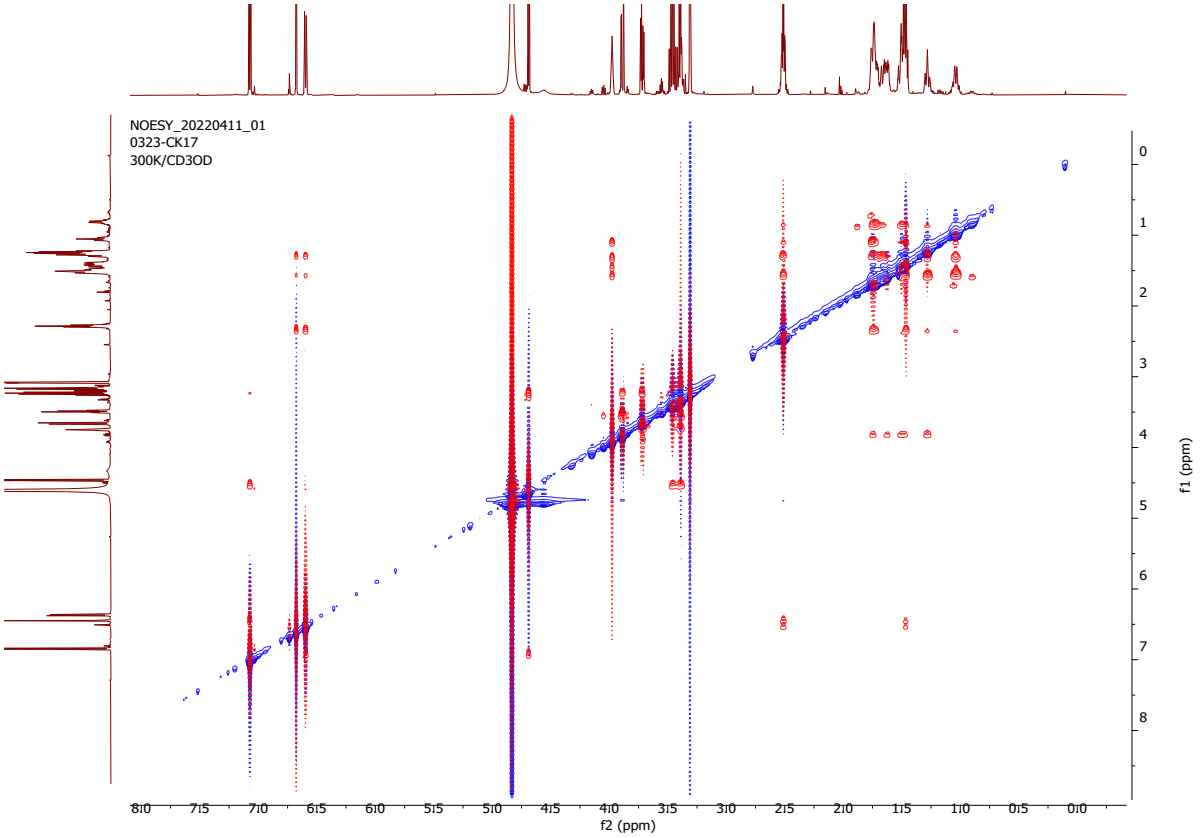
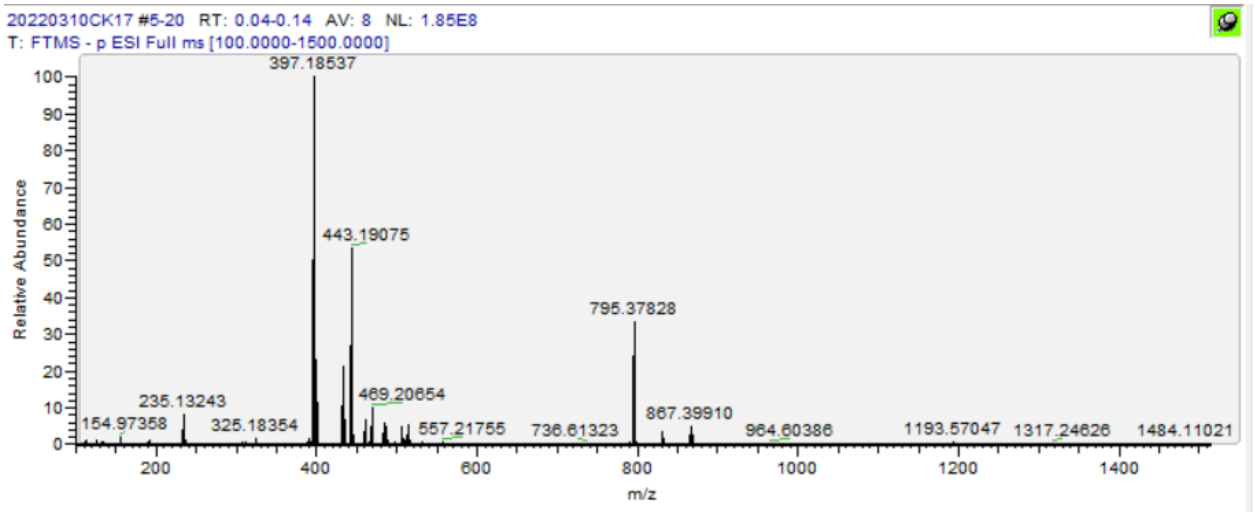


Figure S28. HR-ESI-MS spectra of compound 3



Elemental composition

Single mass

Mass: 397.18536

Max. results 10

Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{20}H_{29}O_8$	6.5	-0.842

Elemental composition

Single mass

Mass: 795.37828

Max. results 10

Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{40}H_{59}O_{16}$	11.5	-1.863

Figure S29. UV spectrum of compound 3

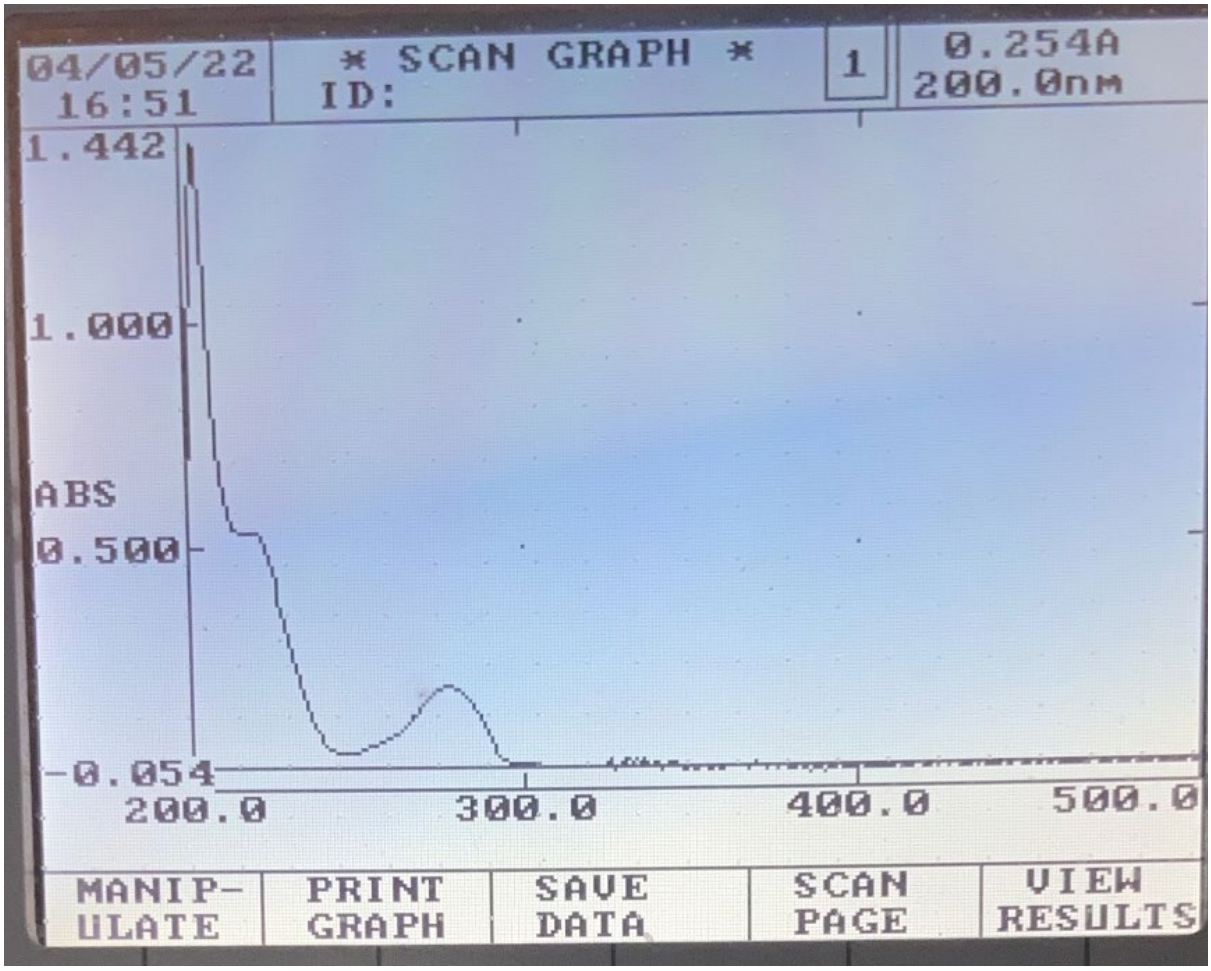
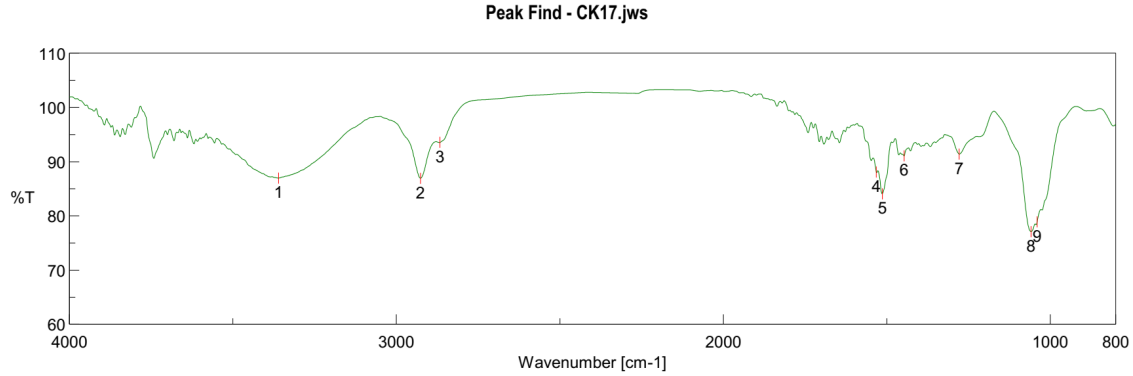


Figure S30. IR spectrum of compound 3



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3360.35	86.9582	2	2926.45	86.9222	3	2866.67	93.5405
4	1532.17	88.0805	5	1513.85	84.053	6	1447.31	91.0375
7	1278.57	91.3784	8	1058.73	77.0789	9	1040.41	78.8758

Figure S31. ^{1}H-NMR spectrum of compound 4

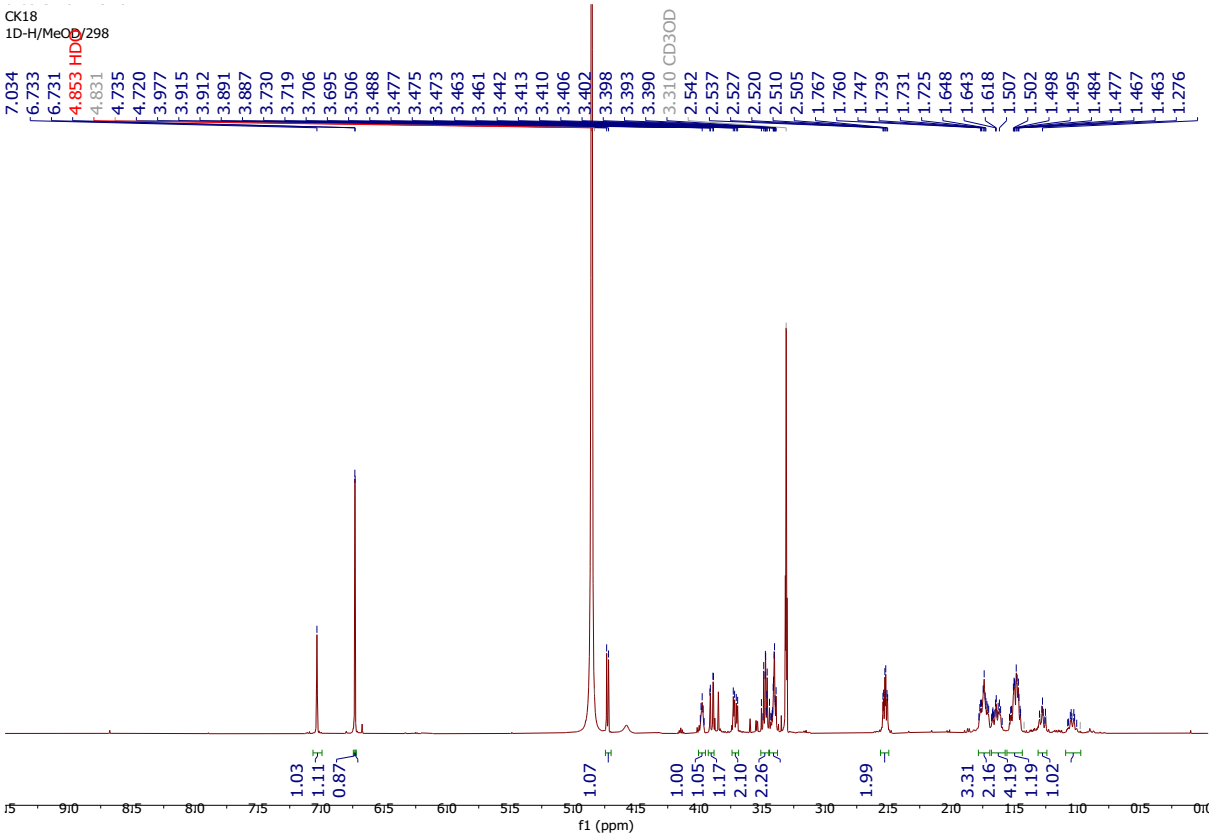


Figure S32. ^{13}C-NMR spectrum of compound 4

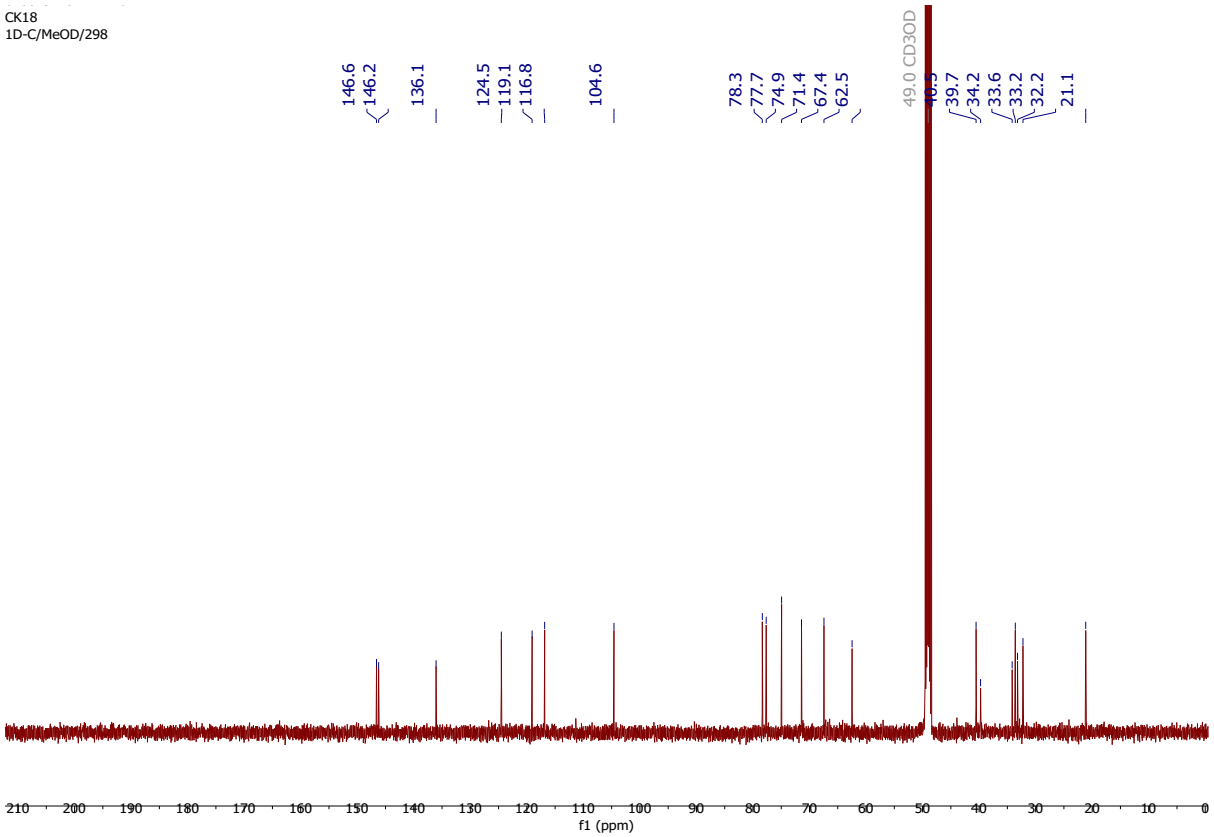


Figure S33. HSQC NMR spectrum of compund 4

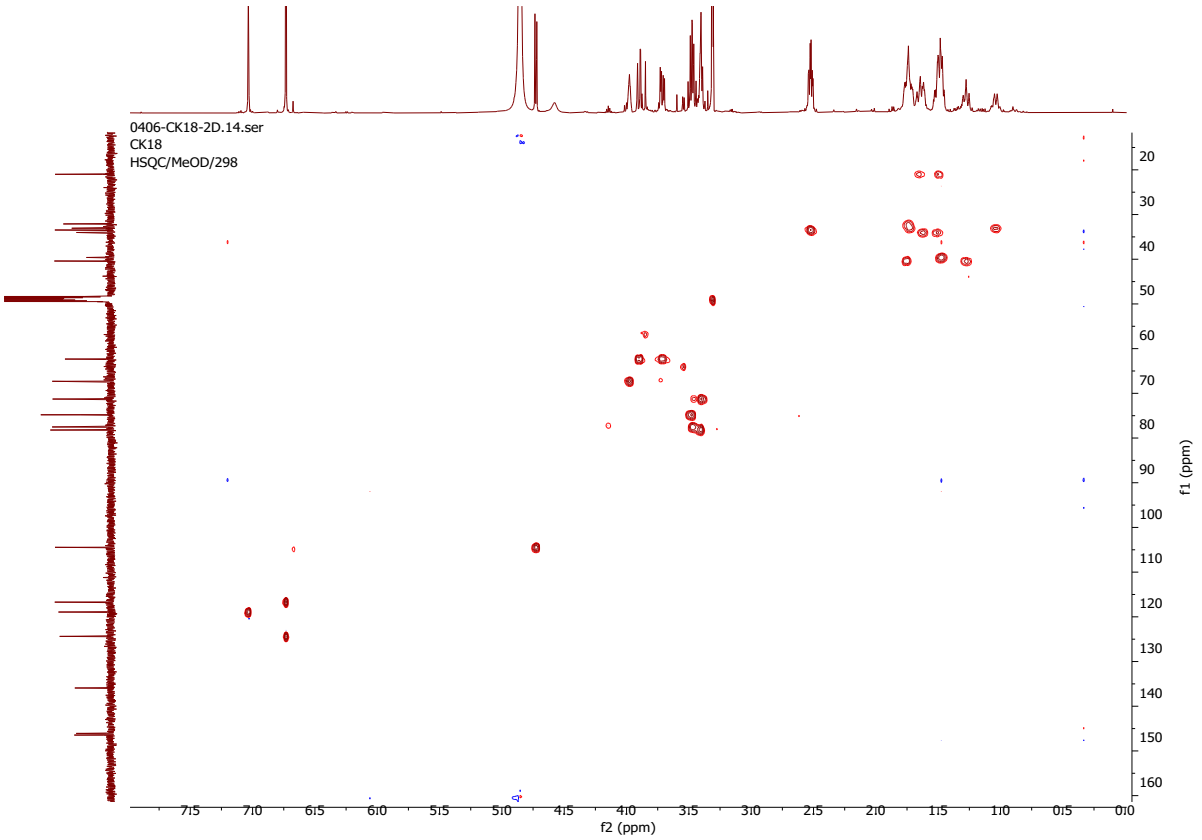


Figure S34. HMBC NMR spectrum of compound 4

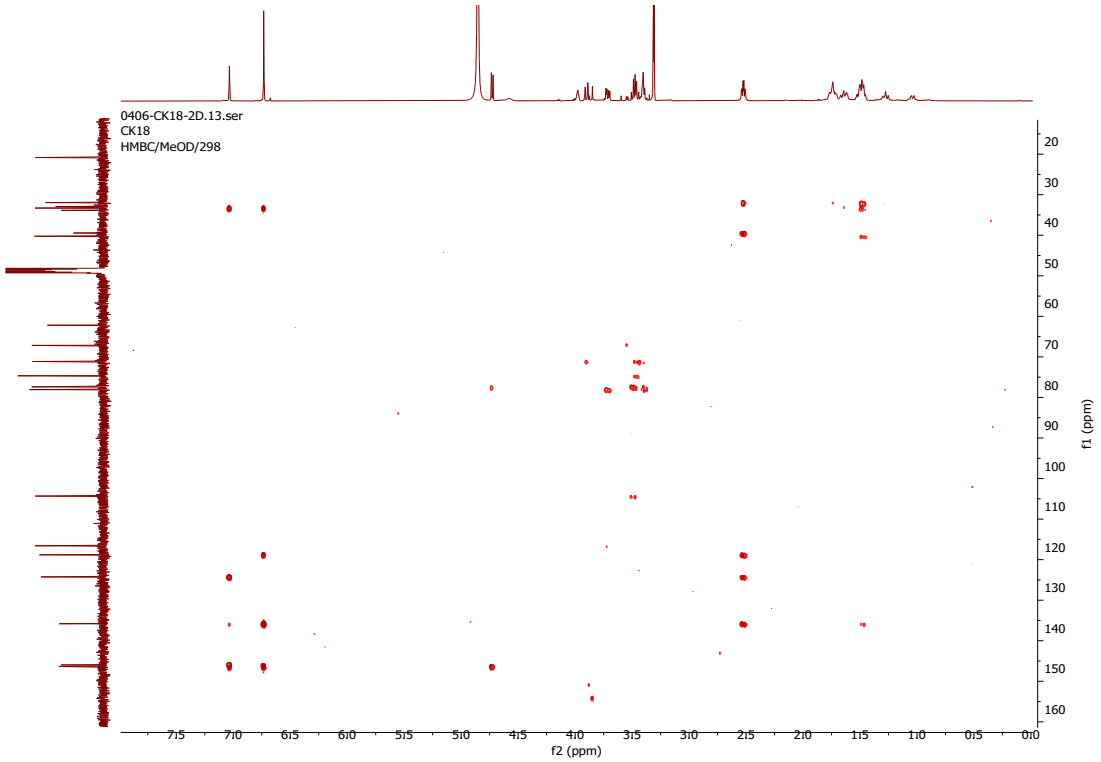


Figure S35. COSY NMR spectrum of compound 4

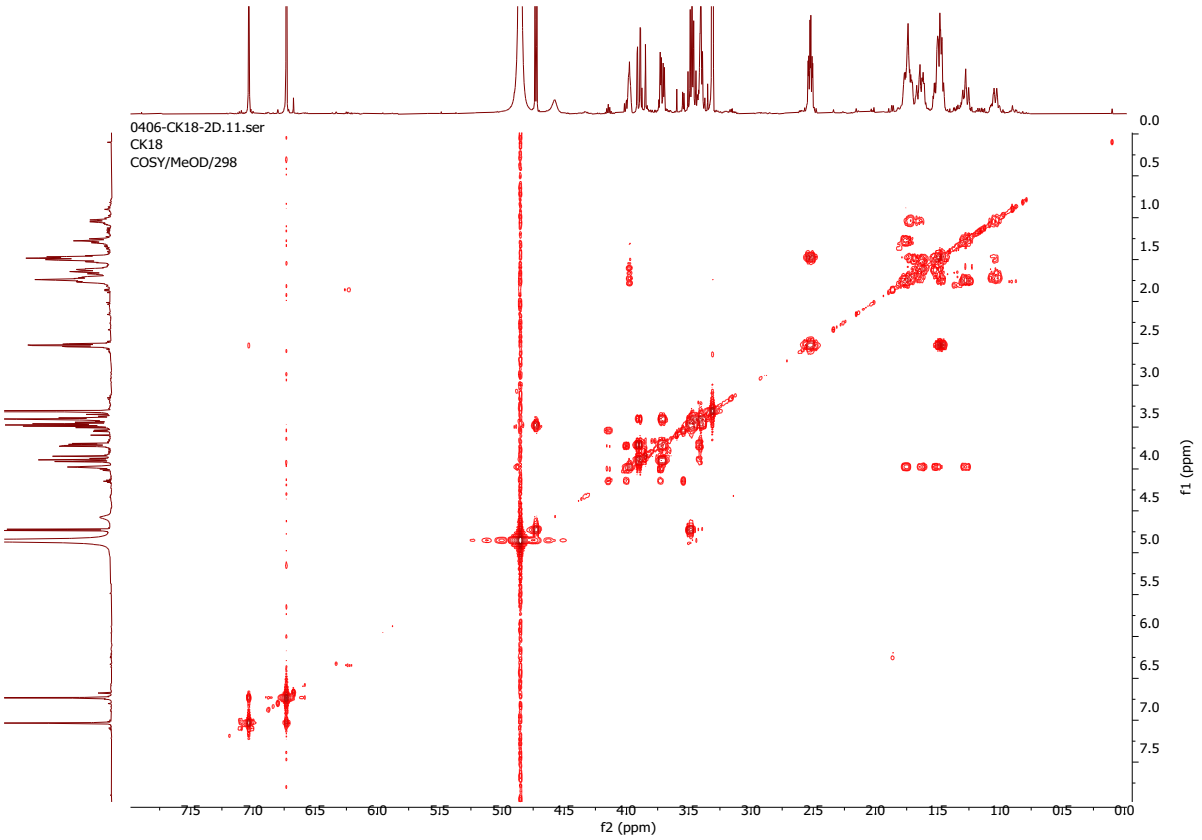


Figure S36. NOESY NMR spectrum of compound 4

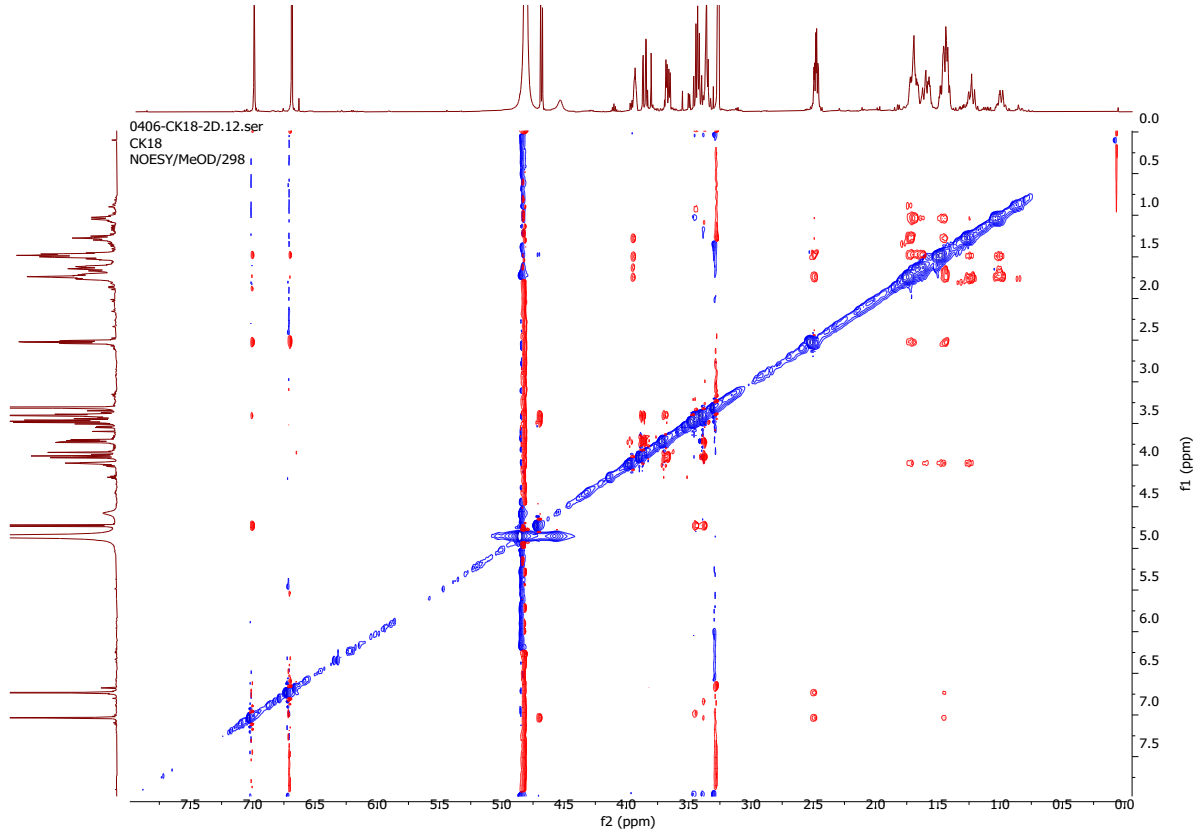
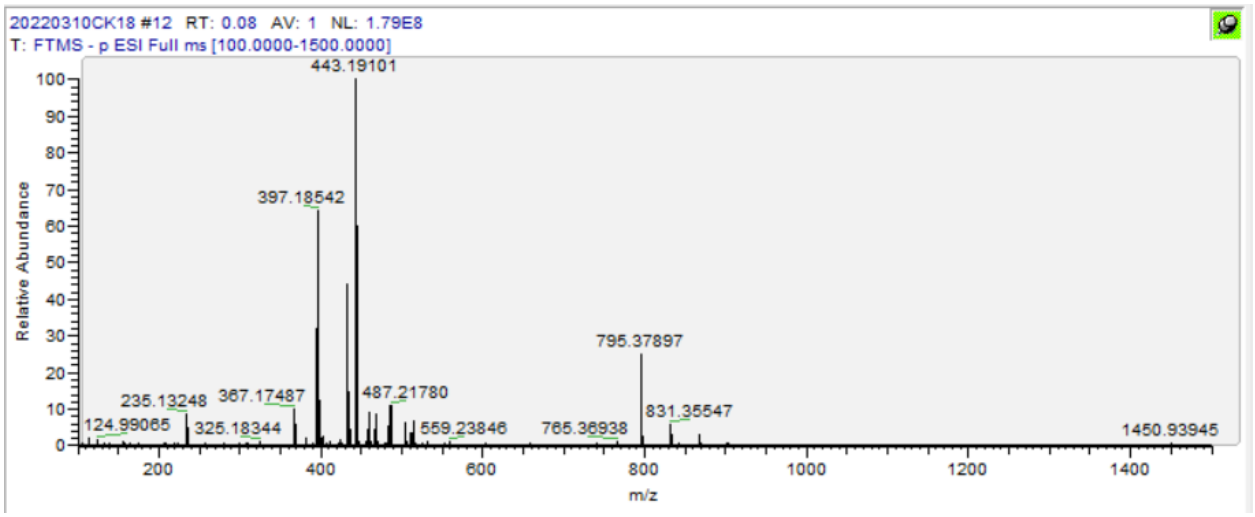
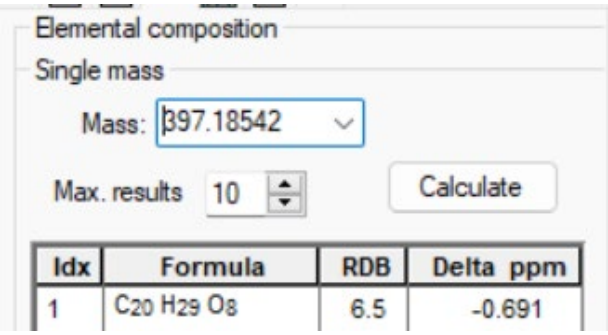
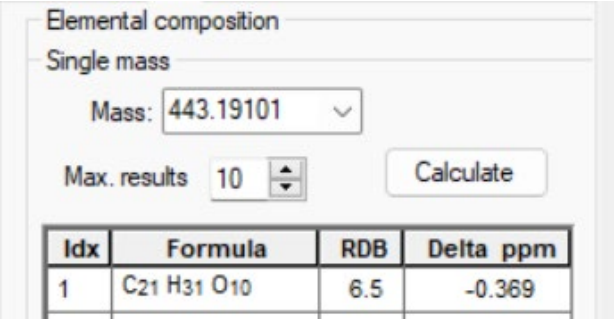


Figure S37. HR-ESI-MS spectra of compound 4







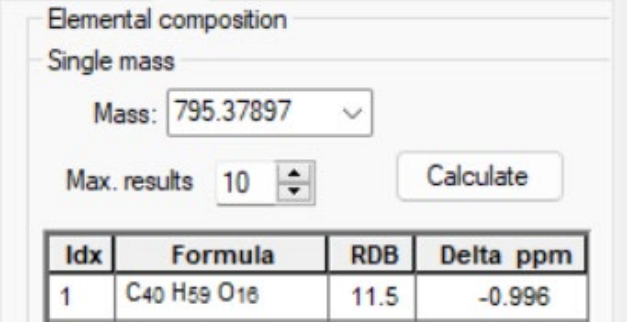


Figure S38. UV spectrum of compound 4

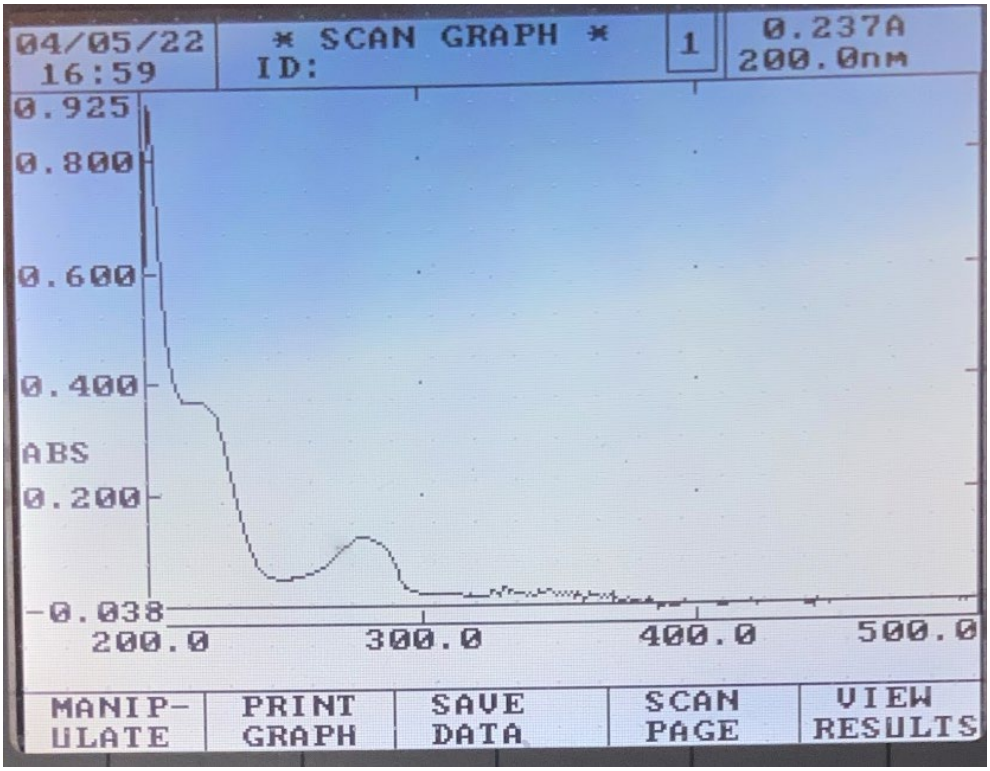
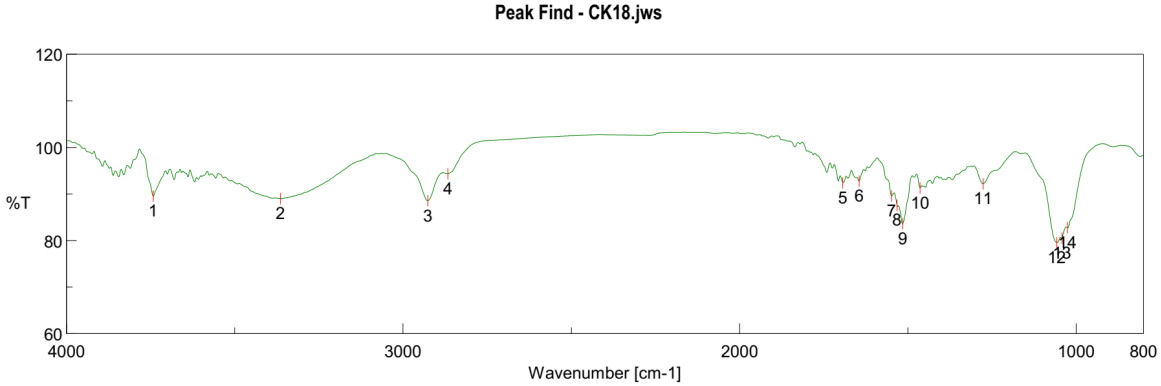


Figure S39. IR spectrum of compound 4



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3742.19	89.5361	2	3364.21	88.9585	3	2926.45	88.5
4	2866.67	94.3321	5	1693.19	92.3753	6	1644.98	92.7417
7	1548.56	89.5438	8	1532.17	87.5184	9	1515.78	83.5985
10	1463.71	91.2965	11	1276.65	92.1671	12	1057.76	79.5008
13	1042.34	80.5251	14	1025.94	82.7239			

Figure S40. 1H-NMR spectrum of compound 5

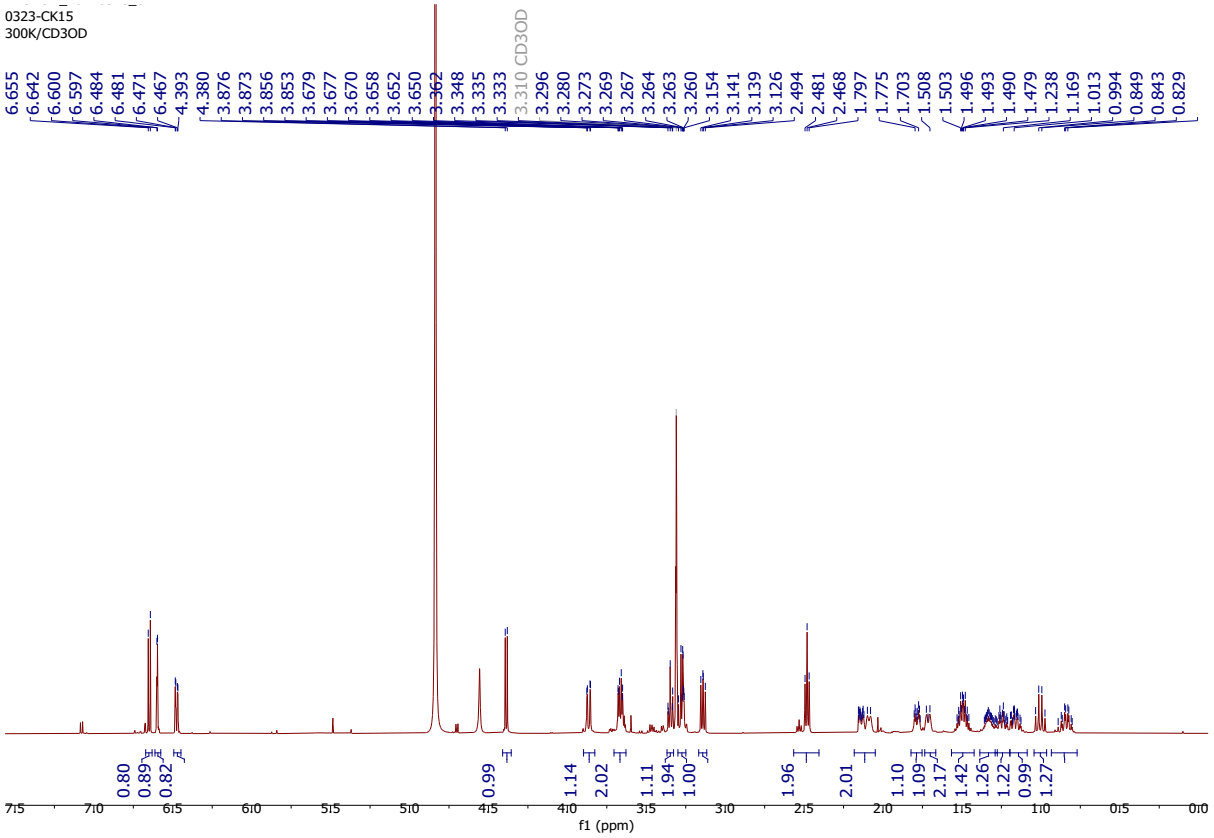


Figure S41. 13C-NMR spectrum of compound 5

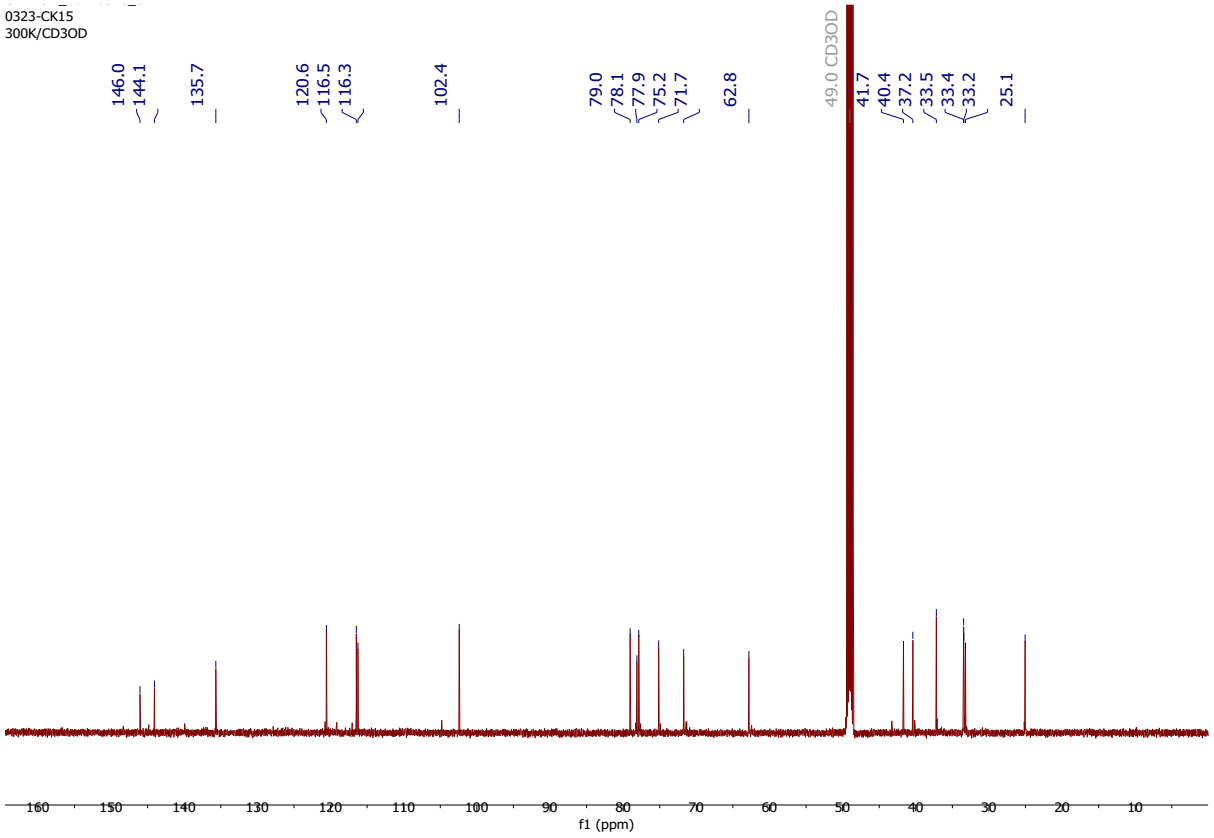


Figure S42. DEPT NMR spectrum of compound 5

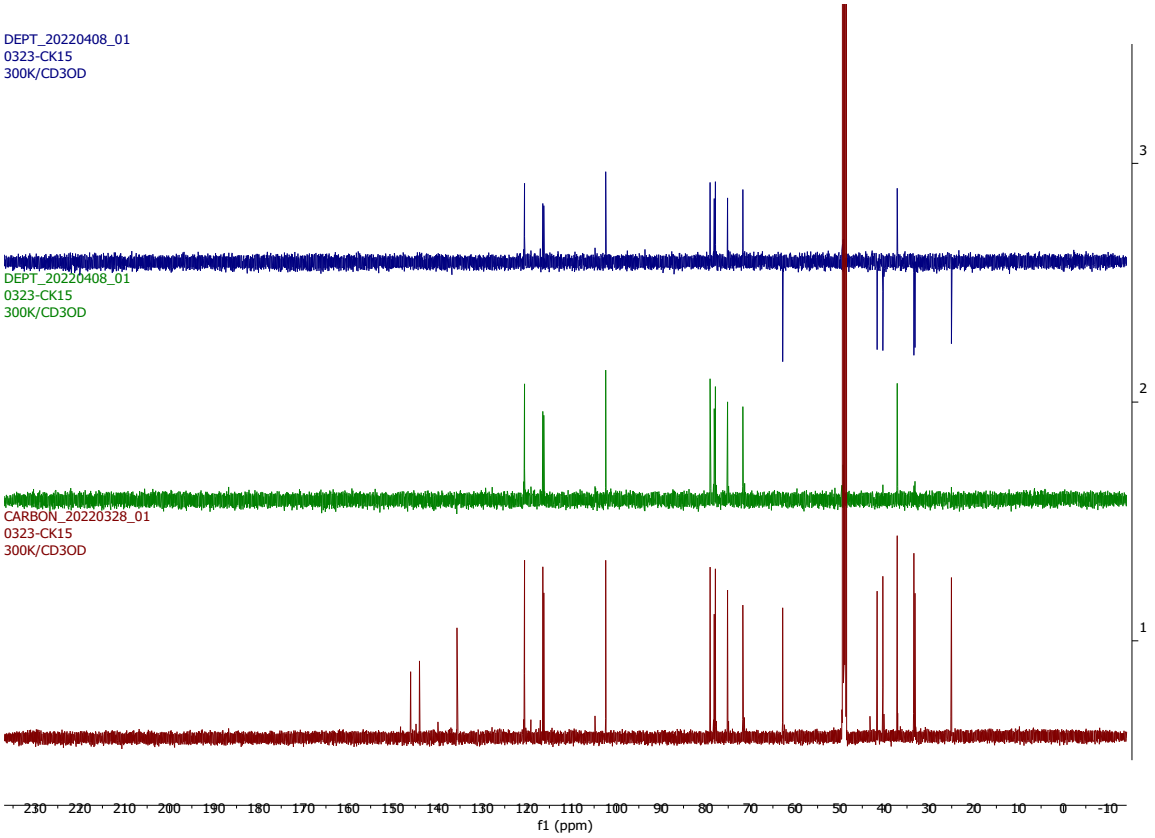


Figure S43. HSQC NMR spectrum of compound 5

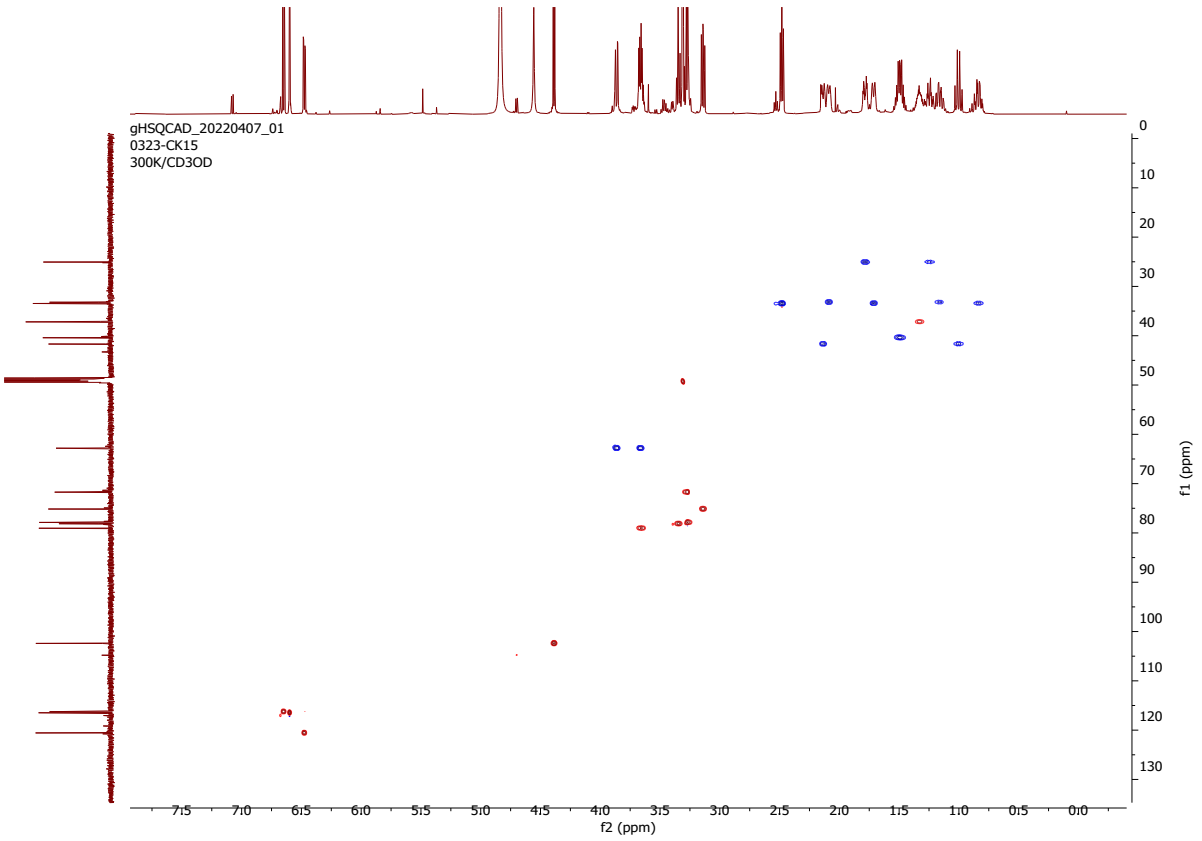


Figure S44. HMBC NMR spectrum of compound 5

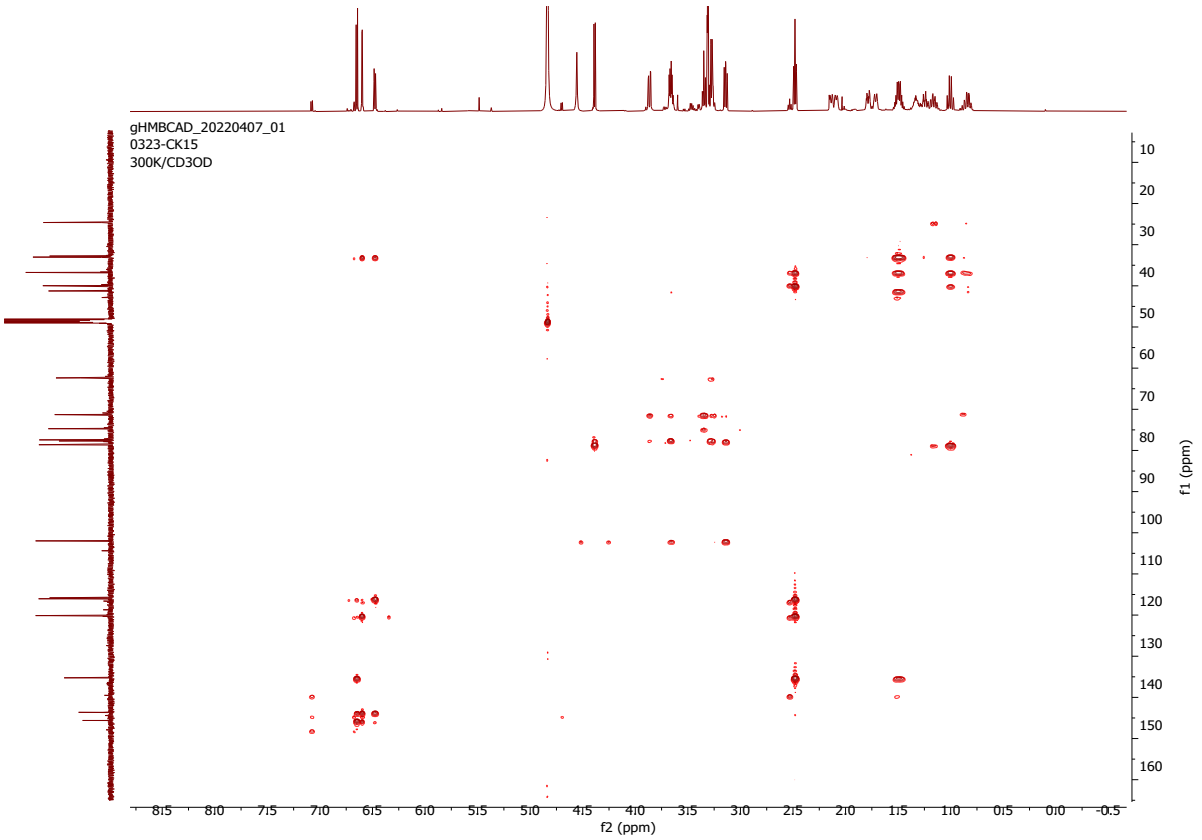


Figure S45. COSY NMR spectrum of compound 5

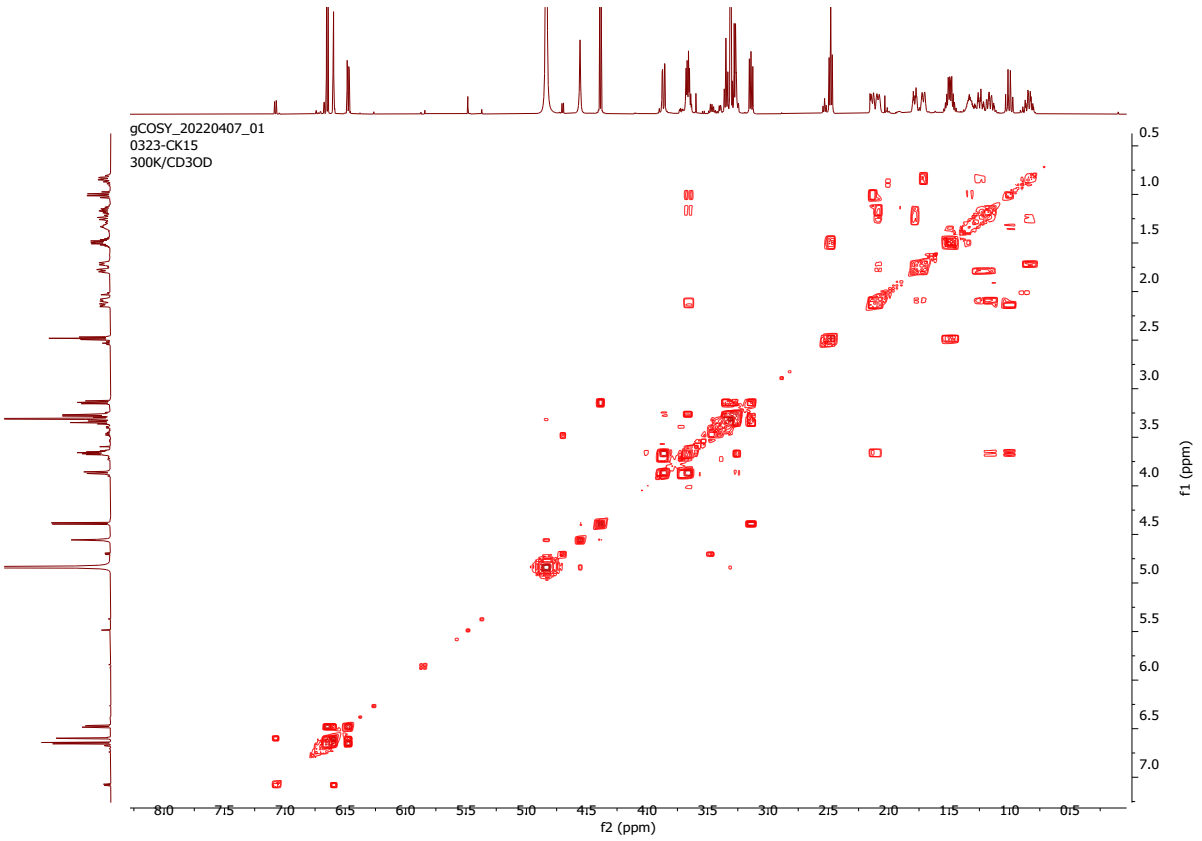


Figure S46. NOESY NMR of compound 5

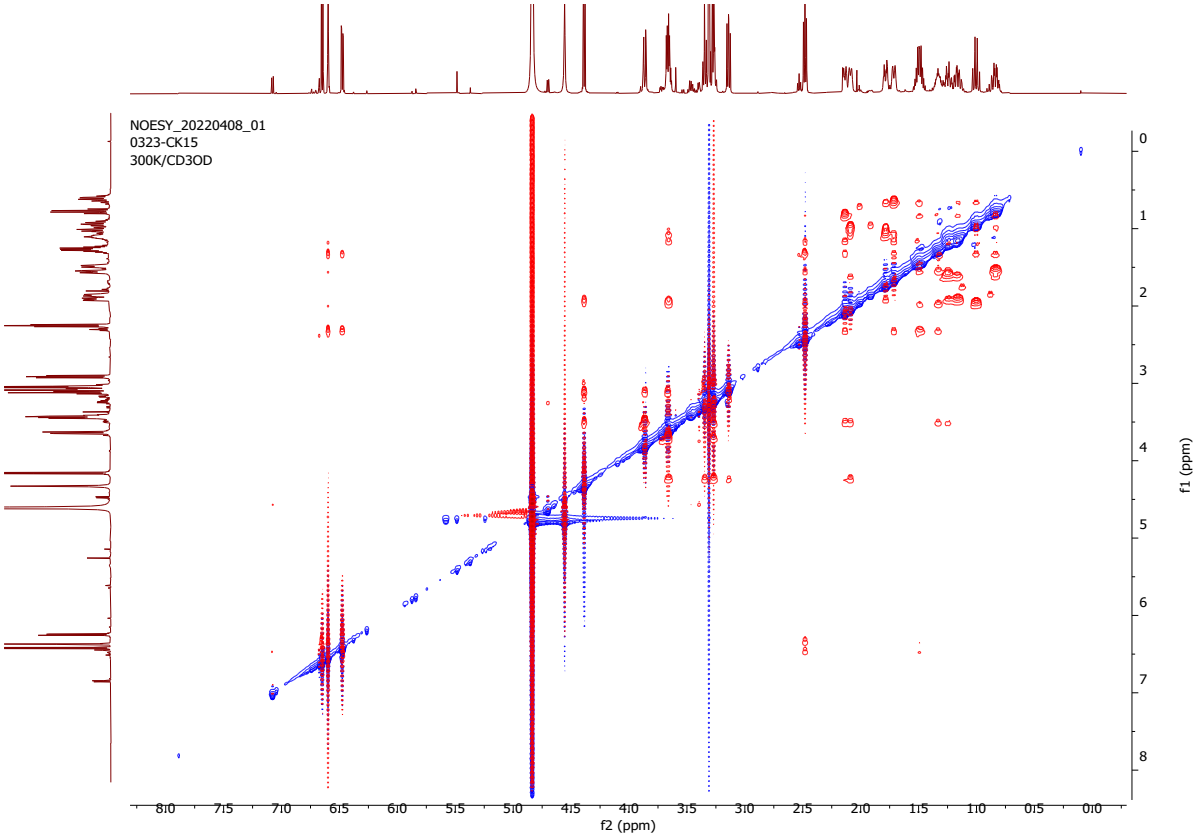
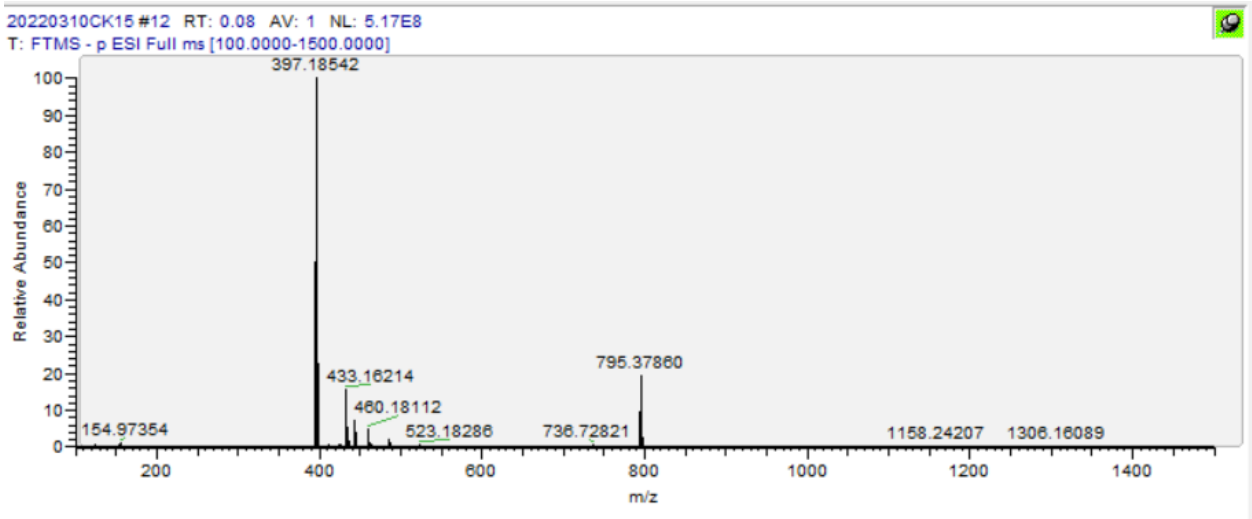


Figure S47. HR-ESI-MS spectra of compound 5



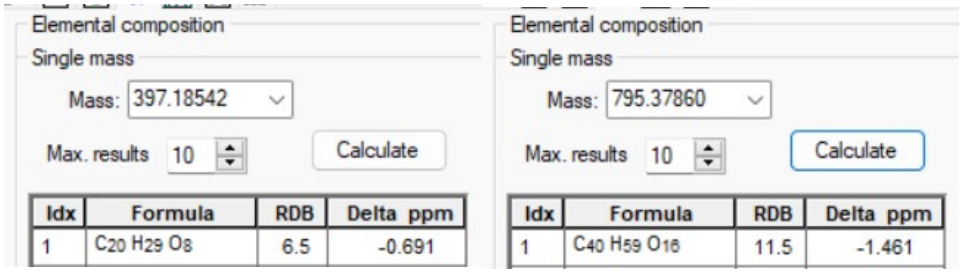


Figure S48. UV spectrum of compound 5

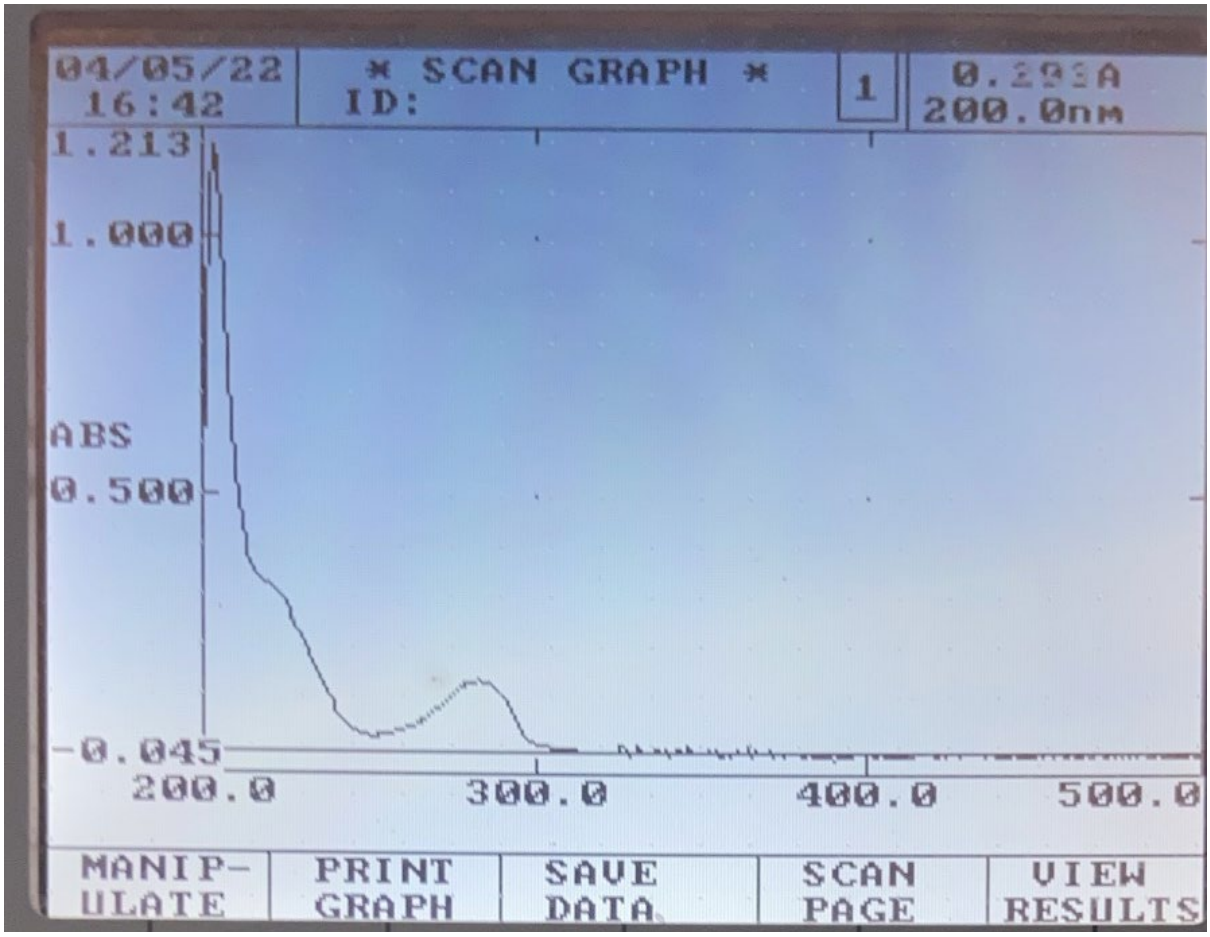
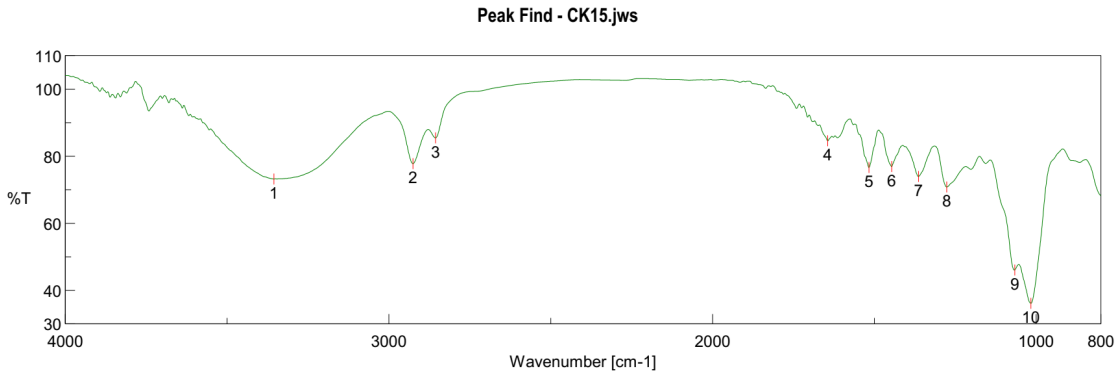


Figure S49. IR spectrum of compound 5



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3355.53	73.1777	2	2925.48	77.7508	3	2856.06	85.4187
4	1644.02	84.5493	5	1516.74	76.6176	6	1446.35	76.8802
7	1363.43	73.893	8	1276.65	70.7743	9	1066.44	46.0631
10	1016.3	36.1115						

Figure S50. 1H-NMR spectrum of compound 6

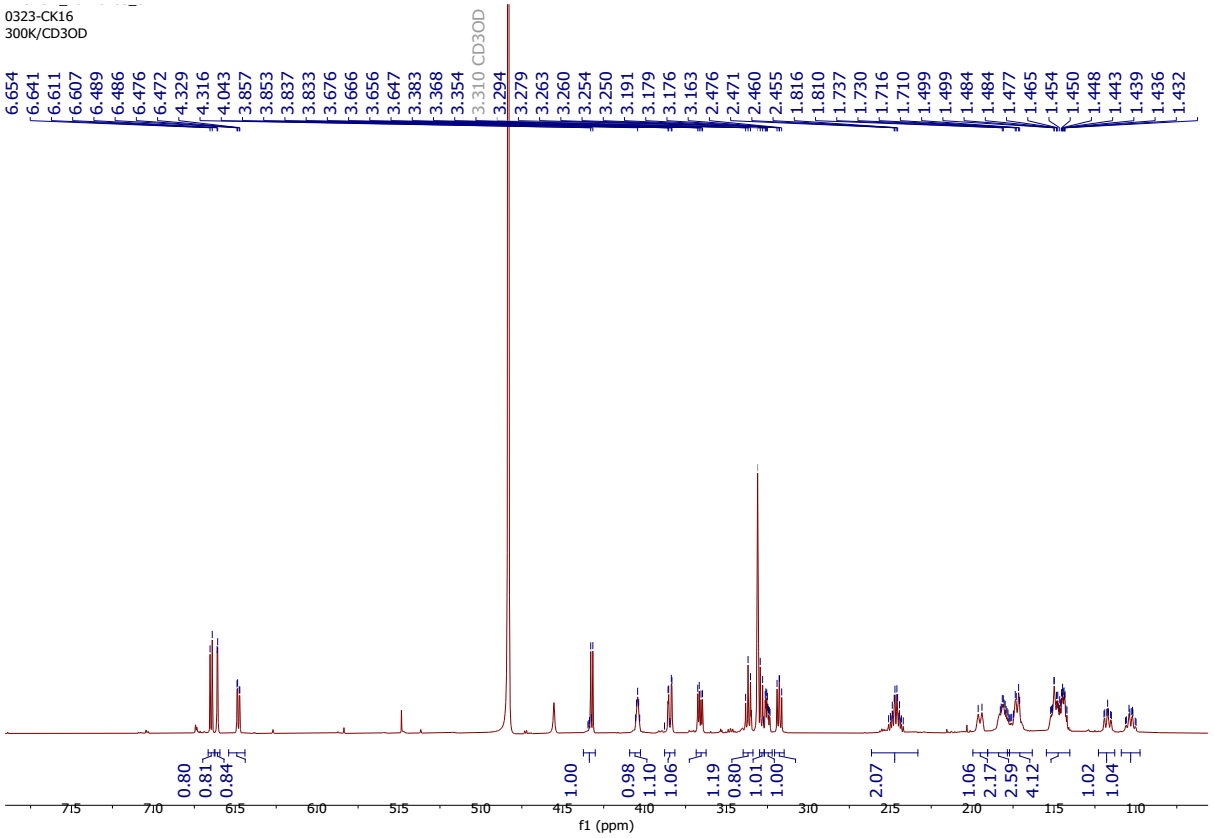


Figure S51. 13C-NMR spectrum of compound 6

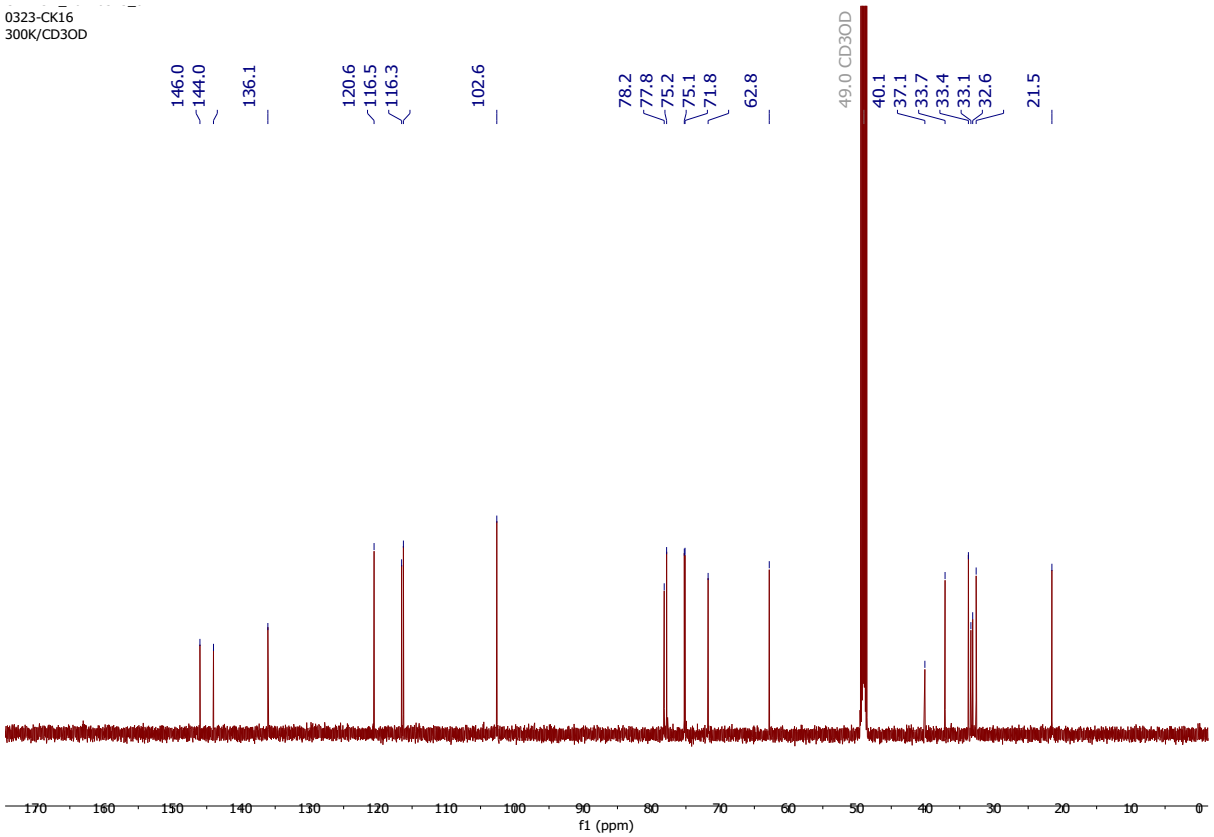


Figure S52. DEPT NMR spectrum of compound 6

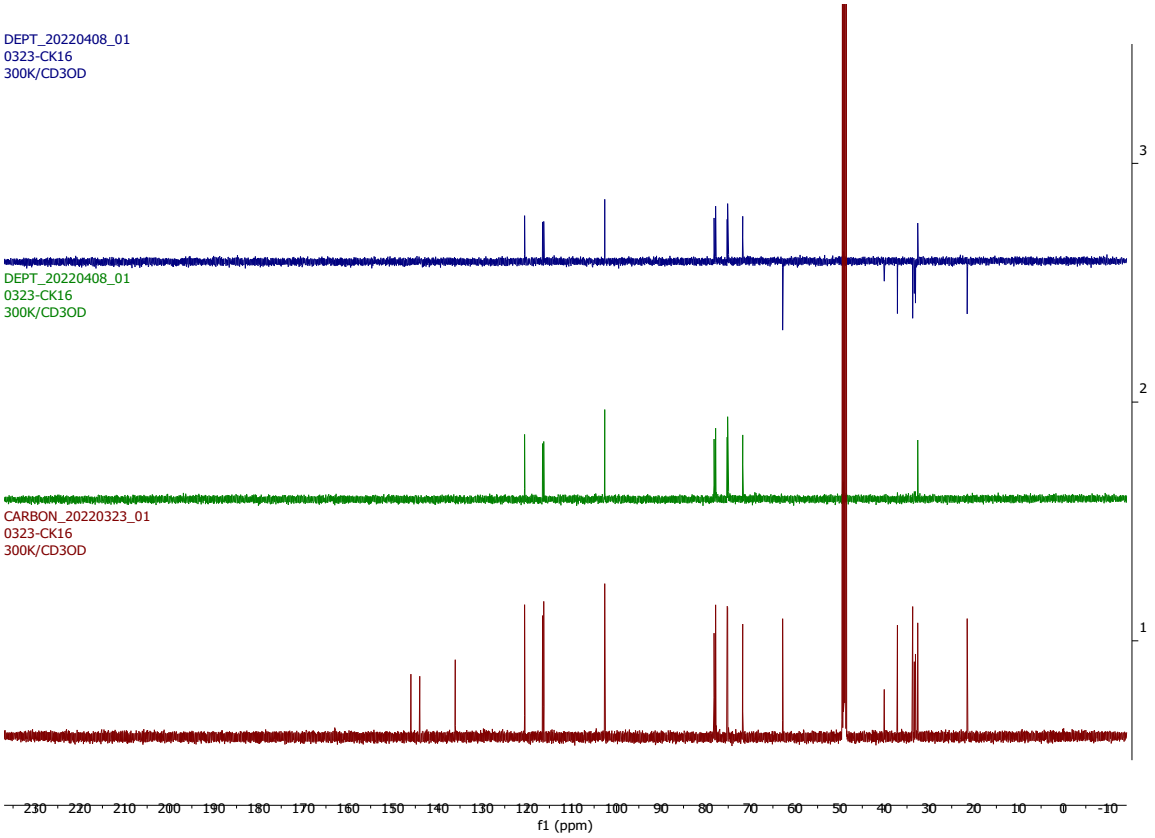


Figure S53. HSQC NMR of compound 6

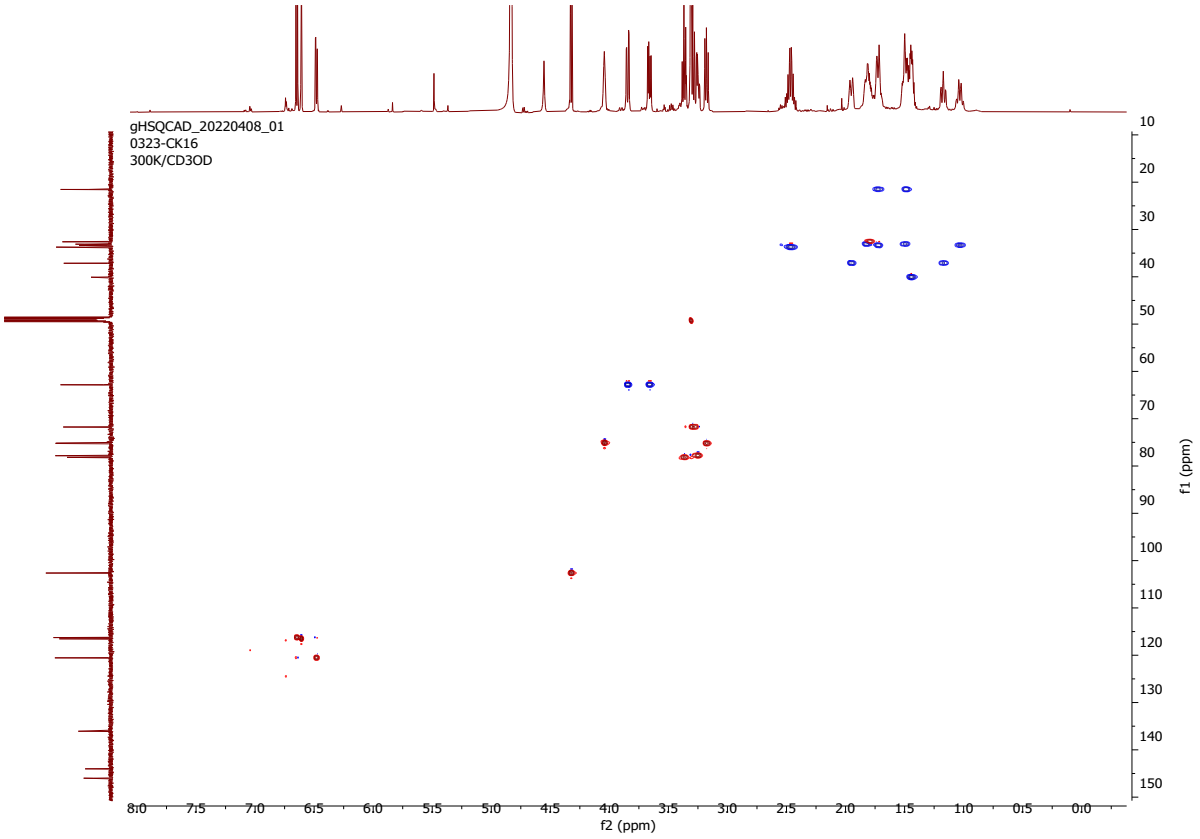


Figure S54. HMBC NMR of compound 6

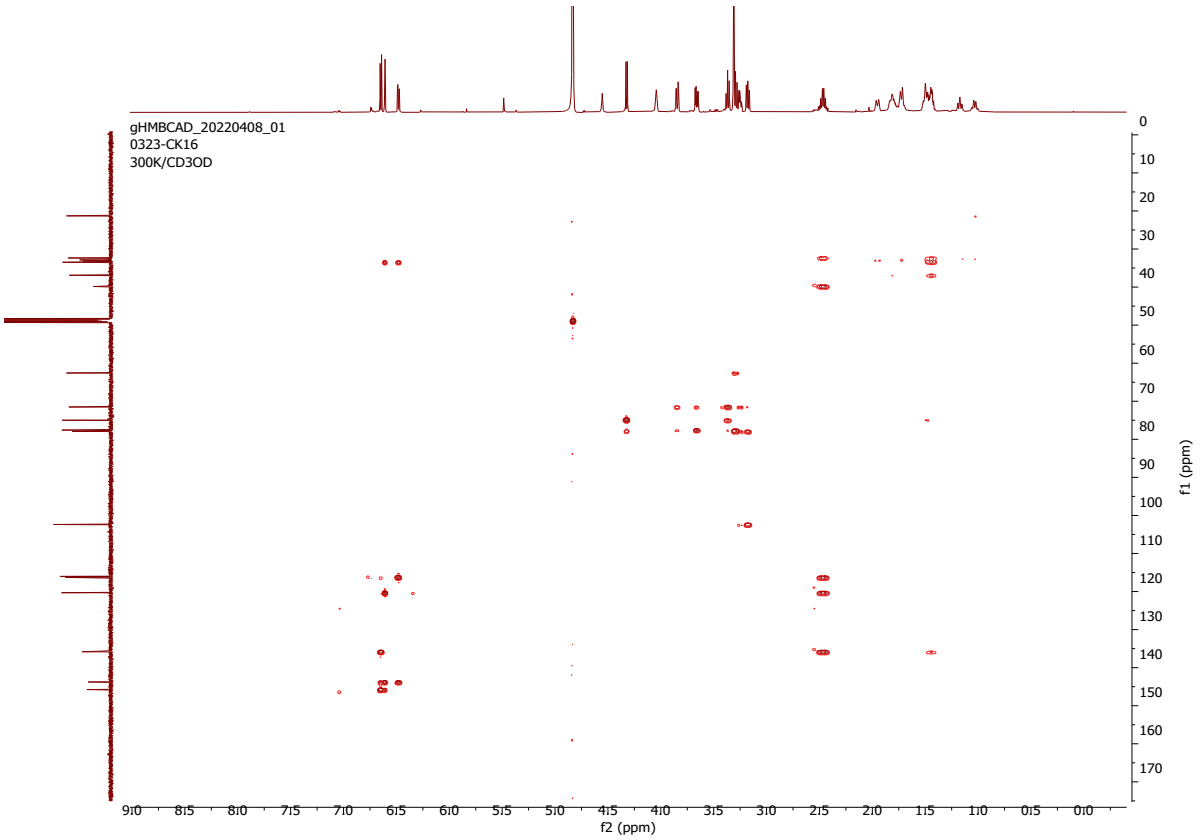


Figure S55. COSY NMR of compound 6

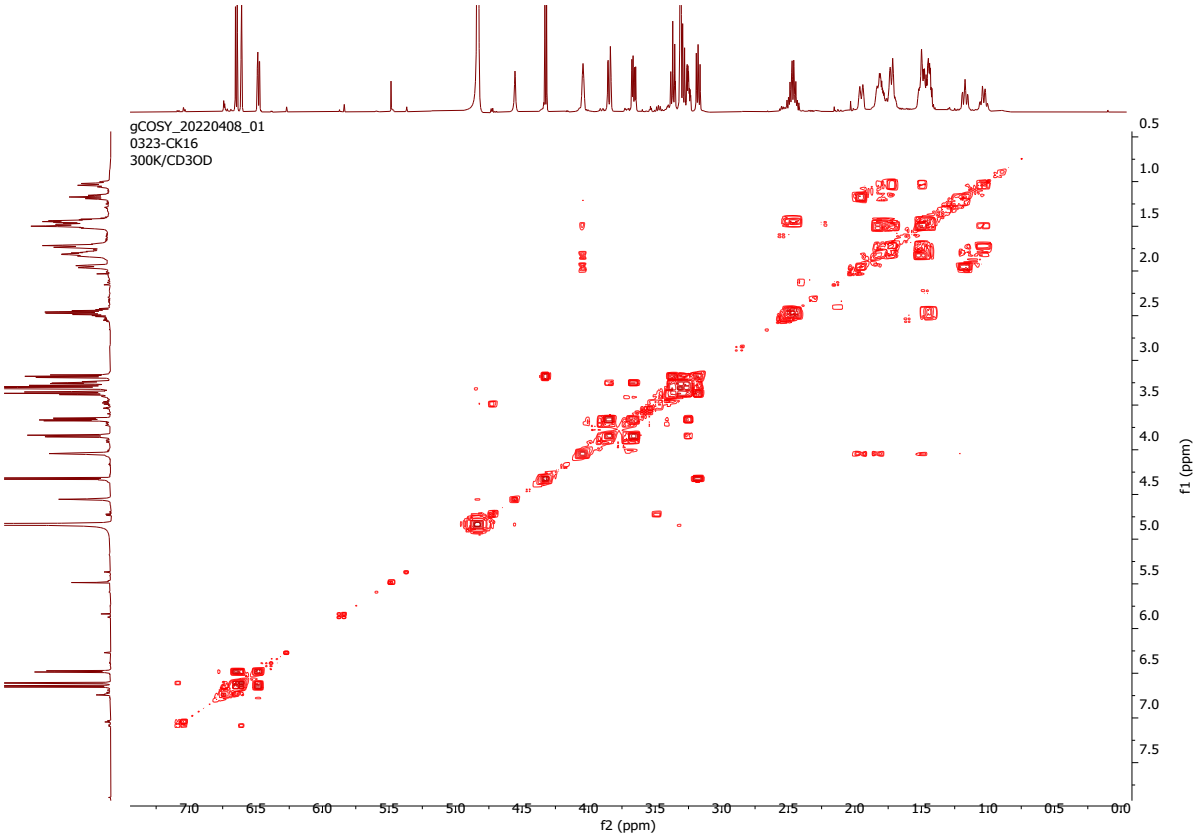


Figure S56. NOESY NMR of compound 6

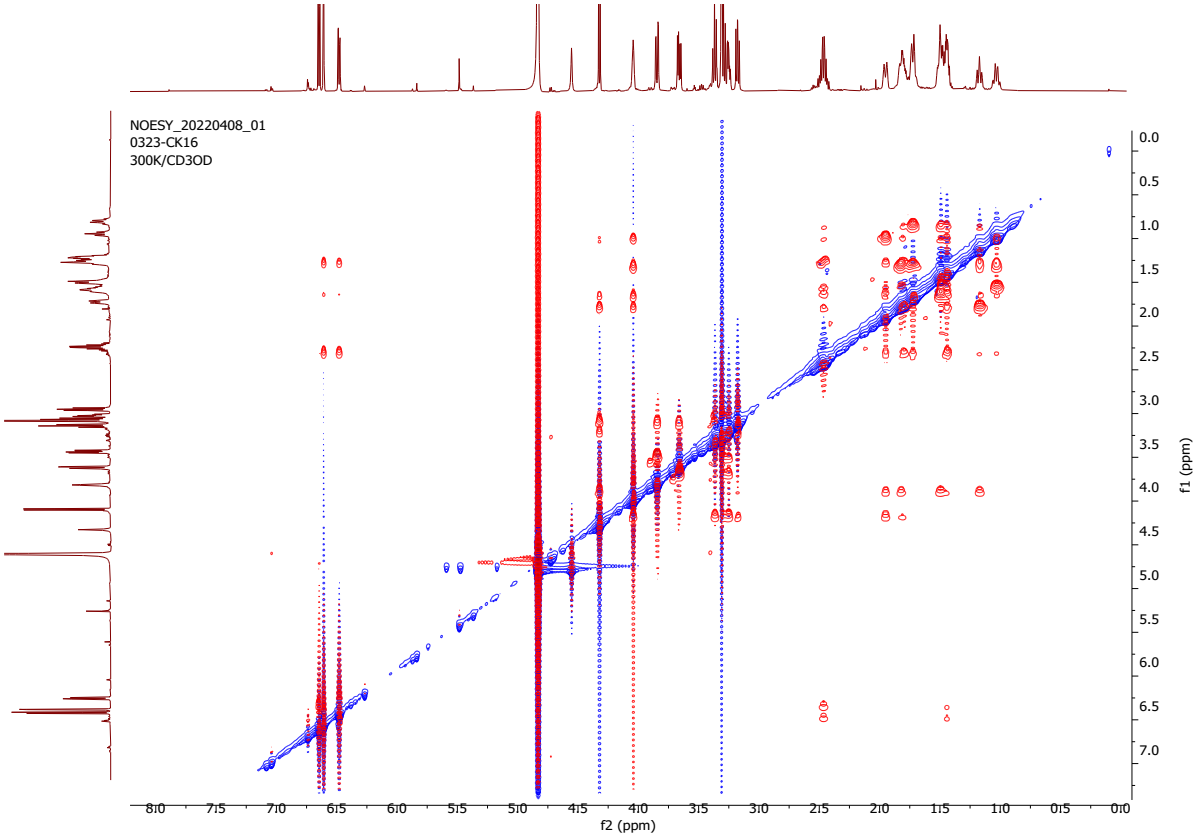
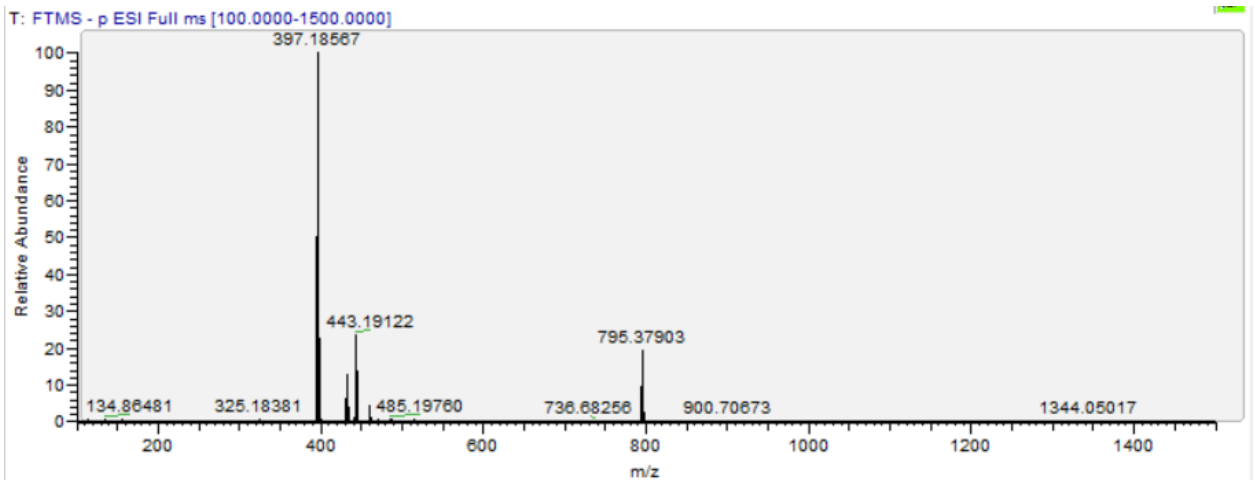


Figure S57. HR-ESI-MS spectra of compound 6



Elemental composition

Single mass

Mass: 397.18567

Max. results 10

Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{20}H_{29}O_8$	6.5	-0.061

Elemental composition

Single mass

Mass: 795.37903

Max. results 10

Calculate

Idx	Formula	RDB	Delta ppm
1	$C_{40}H_{59}O_{16}$	11.5	-0.920

Figure S58. UV spectrum of compound 6

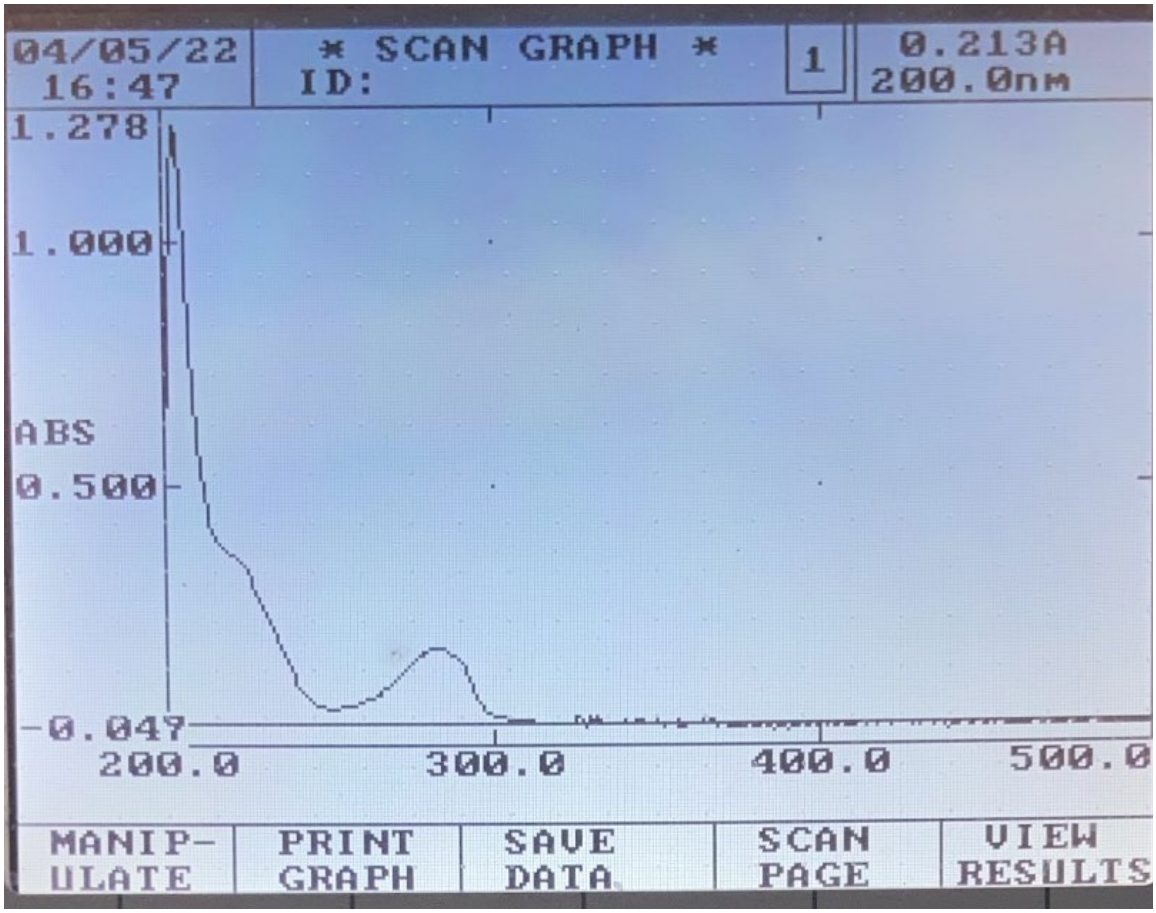
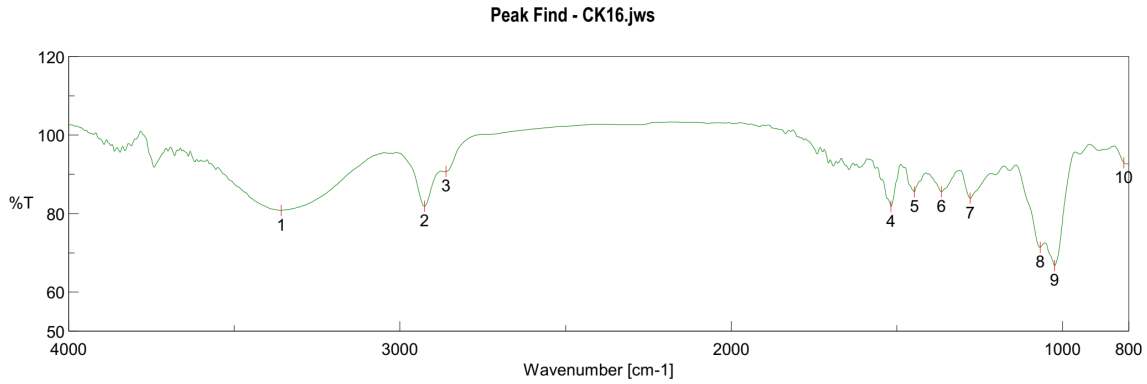


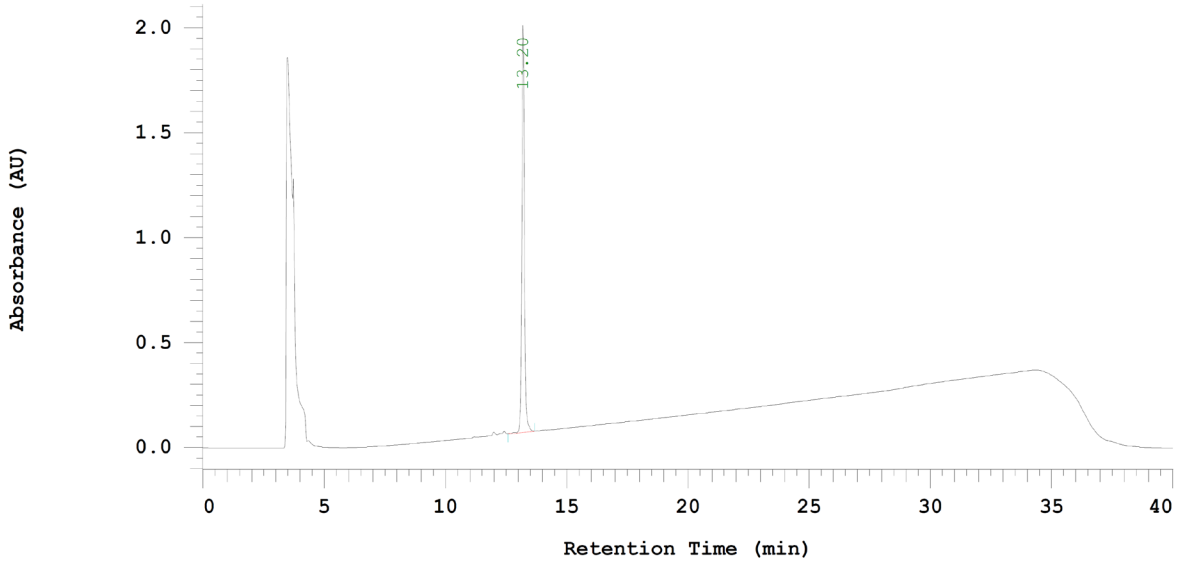
Figure S59. IR spectrum of compound 6



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3358.43	80.7561	2	2926.45	81.8353	3	2860.88	90.6953
4	1517.7	81.7909	5	1447.31	85.4895	6	1365.35	85.518
7	1278.57	83.8522	8	1067.41	71.3434	9	1024.02	66.6787
10	814.777	92.9124	11	414.62	104.834			

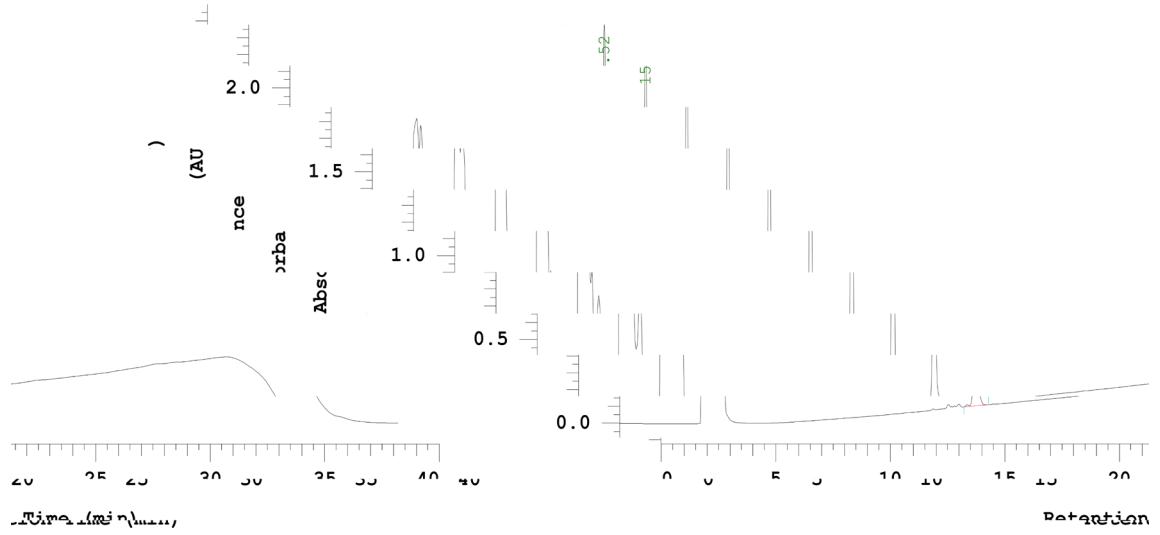
Figure S60. HPLC chromatogram for the purity analysis of compound 1



D-7000 HSM: YONG Series: 0286 Report: modified System: Sys 1

No.	RT	Area	Conc 1	BC
1	13.20	7363169	100.000	BB
		7363169	100.000	

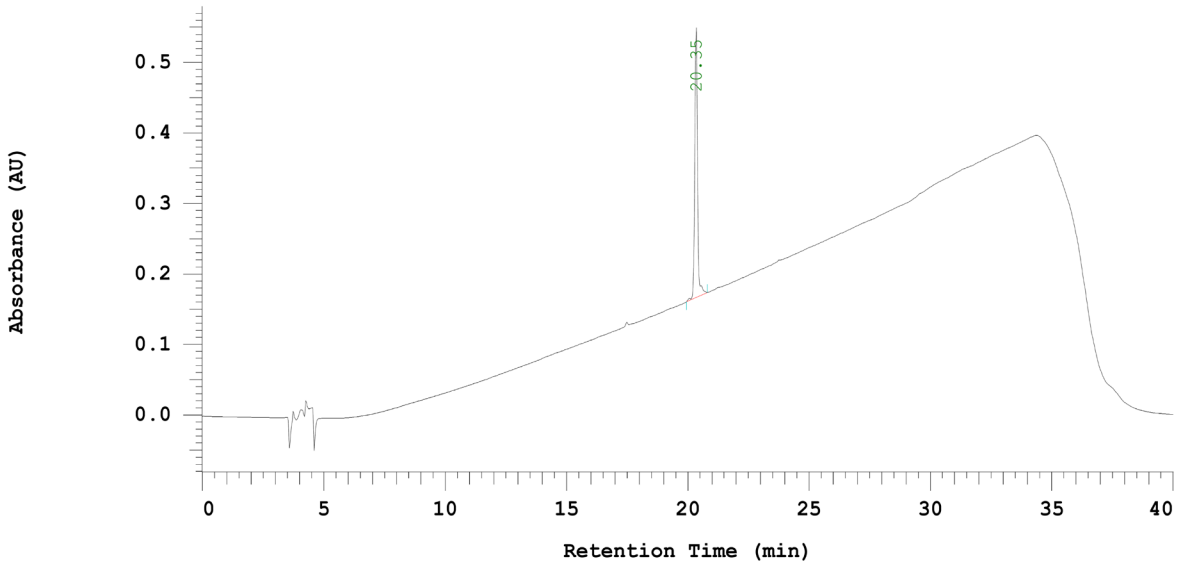
Figure S61. HPLC chromatogram for the purity analysis of compound 2



D-7000 HSM: YONG Series: 0285 Report: modified System: Sys 1

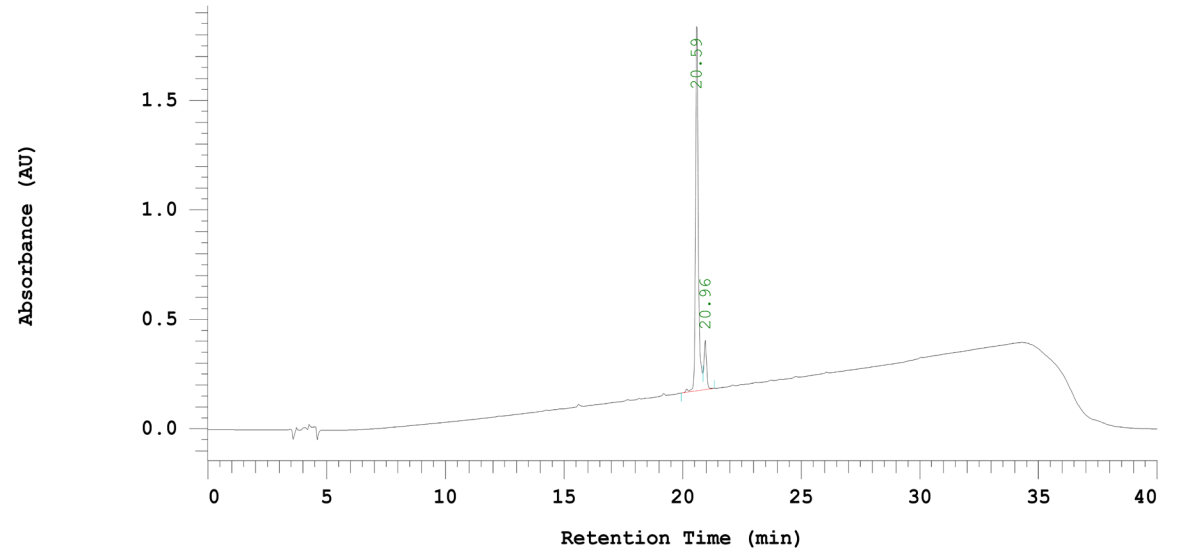
No.	RT	Area	Conc 1	BC
1	15.52	10171891	100.000	BB
		10171891	100.000	

Figure S62. HPLC chromatogram for the purity analysis of compound 3



No.	RT	Area	Conc 1	BC
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		1411617	100.000	

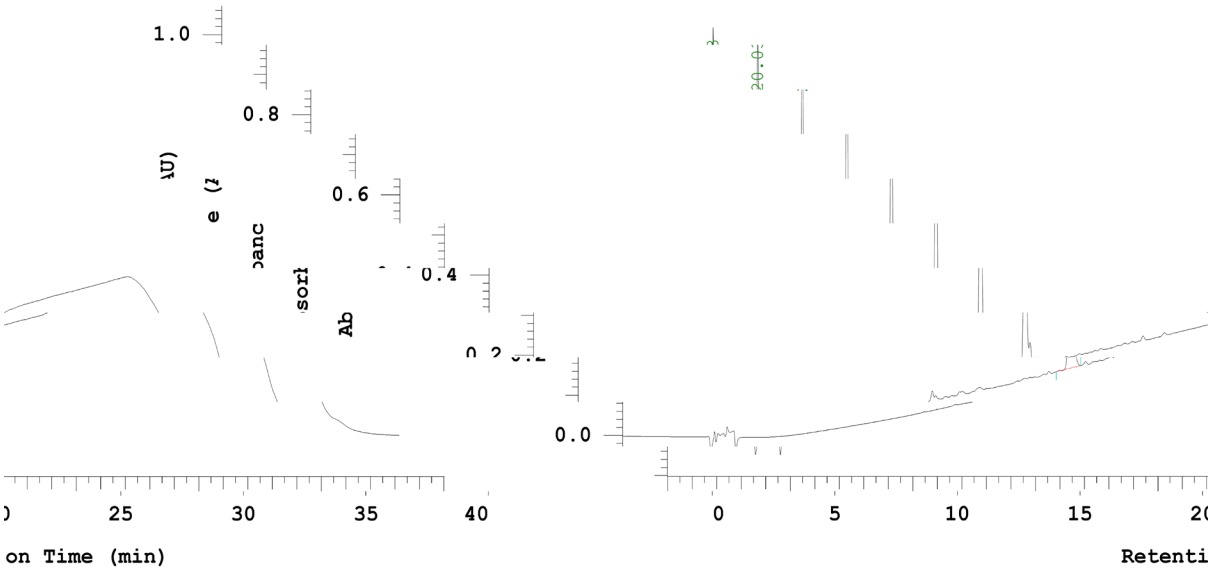
Figure S63. HPLC chromatogram for the purity analysis of compound 4



D-7000 HSM: YONG Series: 0285 Report: modified System: Sys 1

No.	RT	Area	Conc 1	BC
1	20.59	8776182	90.927	BV
2	20.96	875738	9.073	VB
		9651920	100.000	

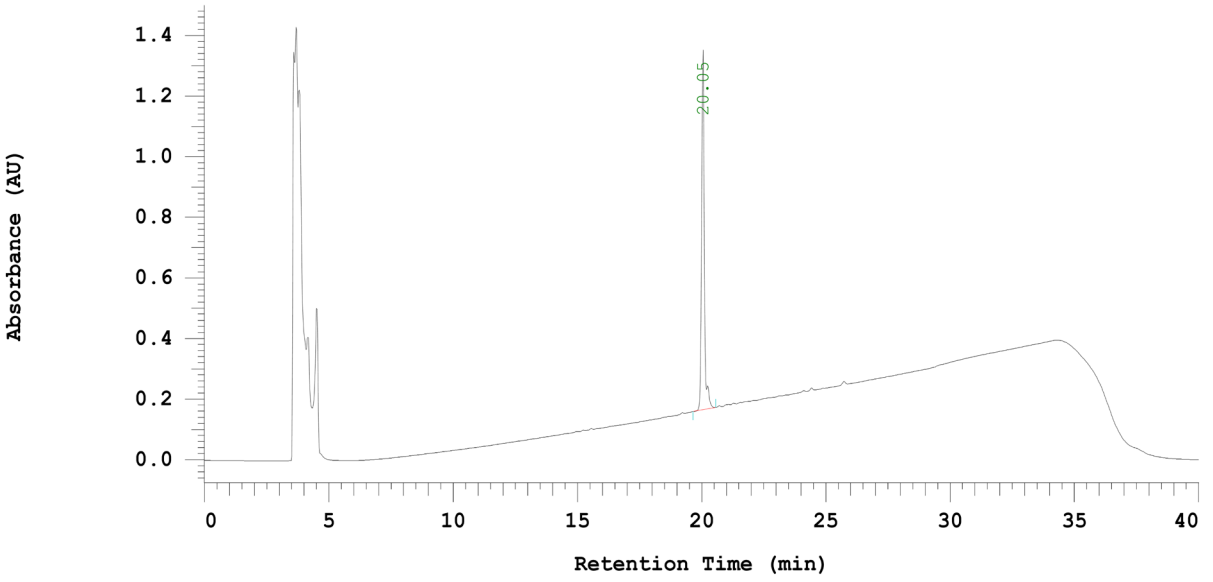
Figure S64. HPLC chromatogram for the purity analysis of compound 5



D-7000 HSM: YONG Series: 0285 Report: modified System: Sys 1

No.	RT	Area	Conc 1	BC
1	20.03	3081597	100.000	BB
		3081597	100.000	

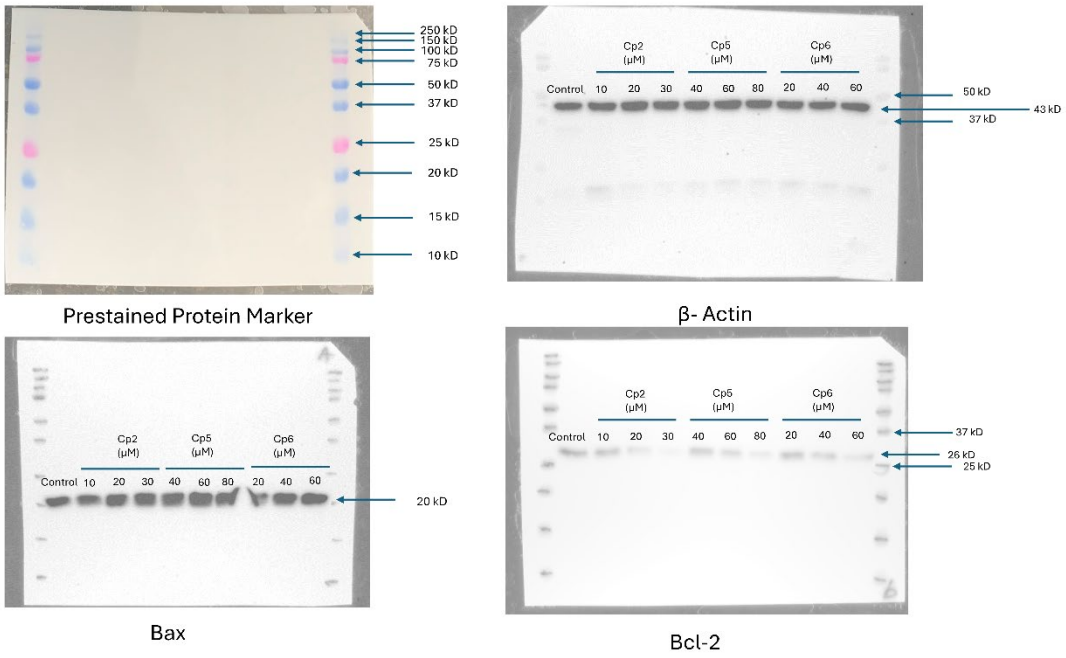
Figure S65. HPLC chromatogram for the purity analysis of compound 6



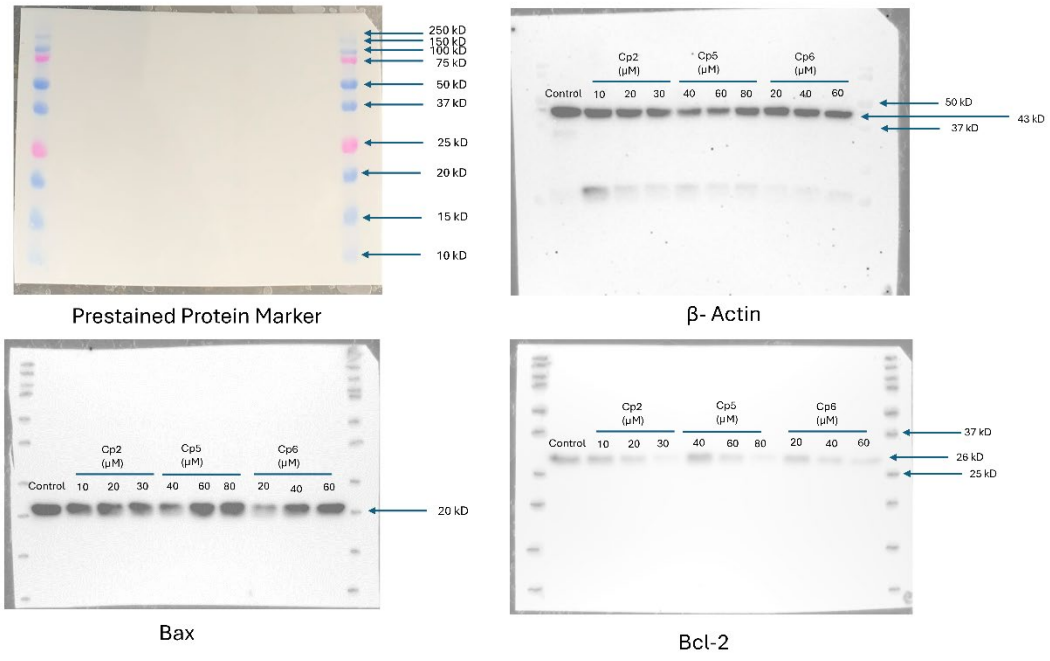
D-7000 HSM: YONG Series: 0285 Report: modified System: Sys 1

No.	RT	Area	Conc 1	BC
1	20.05	4325057	100.000	BB
		4325057	100.000	

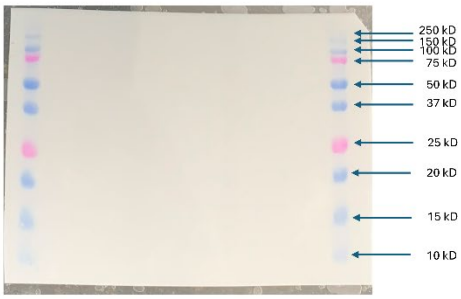
Figure S66. Western-blot analysis



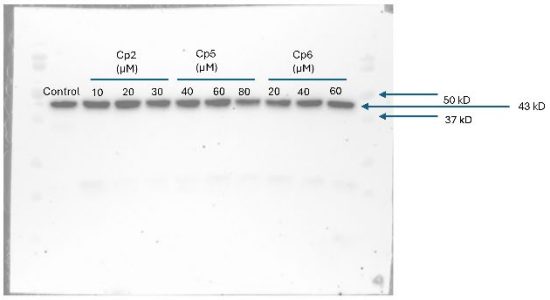
Replicate 1



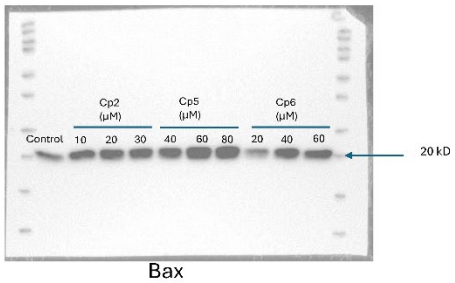
Replicate 2



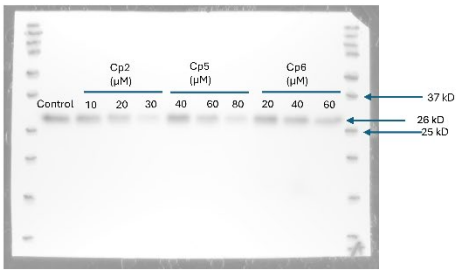
Prestained Protein Marker



β- Actin



Bax



Bcl-2

Replicate 3

Table S1. Energies of calculated compound 1 at RB3LYP/6-31+G(d,p) in methanol

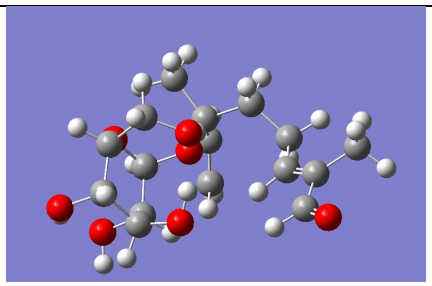
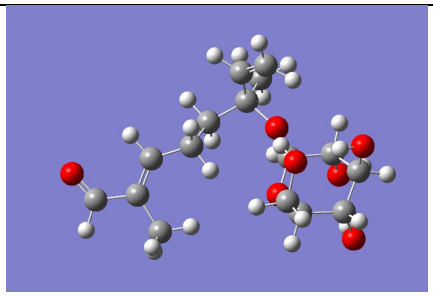
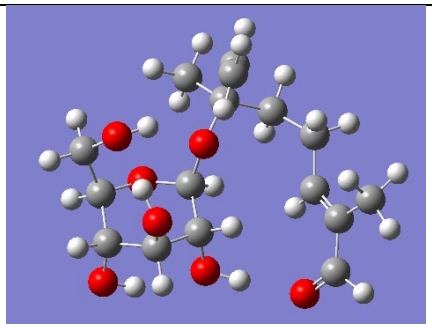
Configuration	Conformer	Structure	E (Hartreee)	Population (%)
7S	1		-1151.939968	96.96
	2		-1151.936701	3.04
7R	1		-1151.949046	100

Table S2. Comparison of the Calculated and Experimental Proton and Carbon Resonances for compound 1

(6S) (6R)

		DP4+	3.86%	96.14%
Nuclei	sp2?	Experimental	Isomer 1	Isomer 2
C	x	197.3	193.2	195.5
C	x	157.7	158.1	152.7
C	x	144.3	135.7	141.6
C	x	140.3	134.6	135.1
C	x	115.3	112.4	110.4
C		99.4	93.4	99.6
C		80.9	85.3	86.6
C		78.3	79.0	83.5
C		77.7	70.6	75.8
C		75.1	70.3	73.4
C		71.8	66.7	71.6
C		62.9	61.79	66.63
C		39.4	38.41	38.06
C		24.8	25.32	30.60
C		24.1	24.58	28.22
C		9.1	7.47	15.19
H	x	9.35	9.00	8.97
H	x	6.67	6.91	6.66
H	x	6.11	5.60	5.38
H	x	5.26	5.40	4.99
H	x	5.19	5.20	4.88
H		4.36	4.69	4.75
H		3.81	3.40	3.37
H		3.63	3.05	3.00
H		3.34	3.21	3.16
H		3.28	3.16	3.08
H		3.17	3.6	2.81
H		3.17	3.57	3.93
H		2.53	2.13	1.94
H		2.53	2.09	1.82
H		1.83	1.23	1.68
H		1.83	1.21	1.14
H		1.72	0.86	0.77
H		1.38	1.13	1.35

Table S3. Assignment of the relative stereochemistry of **1** using DP4+ and representing the DP4+ probabilities for each candidate structure.

(6S) (6R)

	Isomer 1	Isomer 2
sDP4+ (H data)	7.88%	92.12%
sDP4+ (C data)	31.12%	68.88%
sDP4+ (all data)	3.72%	96.28%
uDP4+ (H data)	57.85%	42.15%
uDP4+ (C data)	43.10%	56.90%
uDP4+ (all data)	50.97%	49.03%
DP4+ (H data)	10.50%	89.50%
DP4+ (C data)	25.49%	74.51%
DP4+ (all data)	3.86%	96.14%