

Supporting Information

Agelasine Diterpenoids and Cbl-b Inhibitory Agelifेरins from the Coralline Demosponge *Astrosclera willeyana*

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Supporting Information

Figure S1. ^1H NMR spectrum (600 MHz) of Agelasine W (**1**) in CD_3OD .

Figure S2. ^{13}C NMR spectrum (150 MHz) of Agelasine W (**1**) in CD_3OD .

Figure S3. HSQC spectrum of Agelasine W (**1**) in CD_3OD .

Figure S4. HMBC spectrum of Agelasine W (**1**) in CD_3OD .

Figure S5. COSY spectrum of Agelasine W (**1**) in CD_3OD .

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Figure S27. ^1H NMR spectrum (600 MHz) of *N*(1)-methylisoageliferin (**4**) in CD_3OD .

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Figure S29. HSQC spectrum of *N*(1)-methylisoageliferin (**4**) in CD_3OD .

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Figure S31. COSY spectrum of *N*(1)-methylisoageliferin (**4**) in CD_3OD .

Figure S32. ECD spectrum of *N*(1)-methylisoageliferin (**4**).

Figure S33. HRESIMS of *N*(1)-methylisoageliferin (**4**).

Figure S34. IR spectrum of *N*(1)-methylisoageliferin (**4**).

Figure S35. UV spectrum of *N*(1)-methylisoageliferin (**4**).

Table S1. ^1H NMR (600 MHz) data for compounds **5-10** in CD_3OD .

Table S2. ^{13}C NMR (150 MHz) data for compound **5-10** in CD_3OD .

Table S3. ^{13}C NMR (150 MHz) data for compound **3** and closely related clerodane diastereomers in CDCl_3 .

179B034_46.1.fid

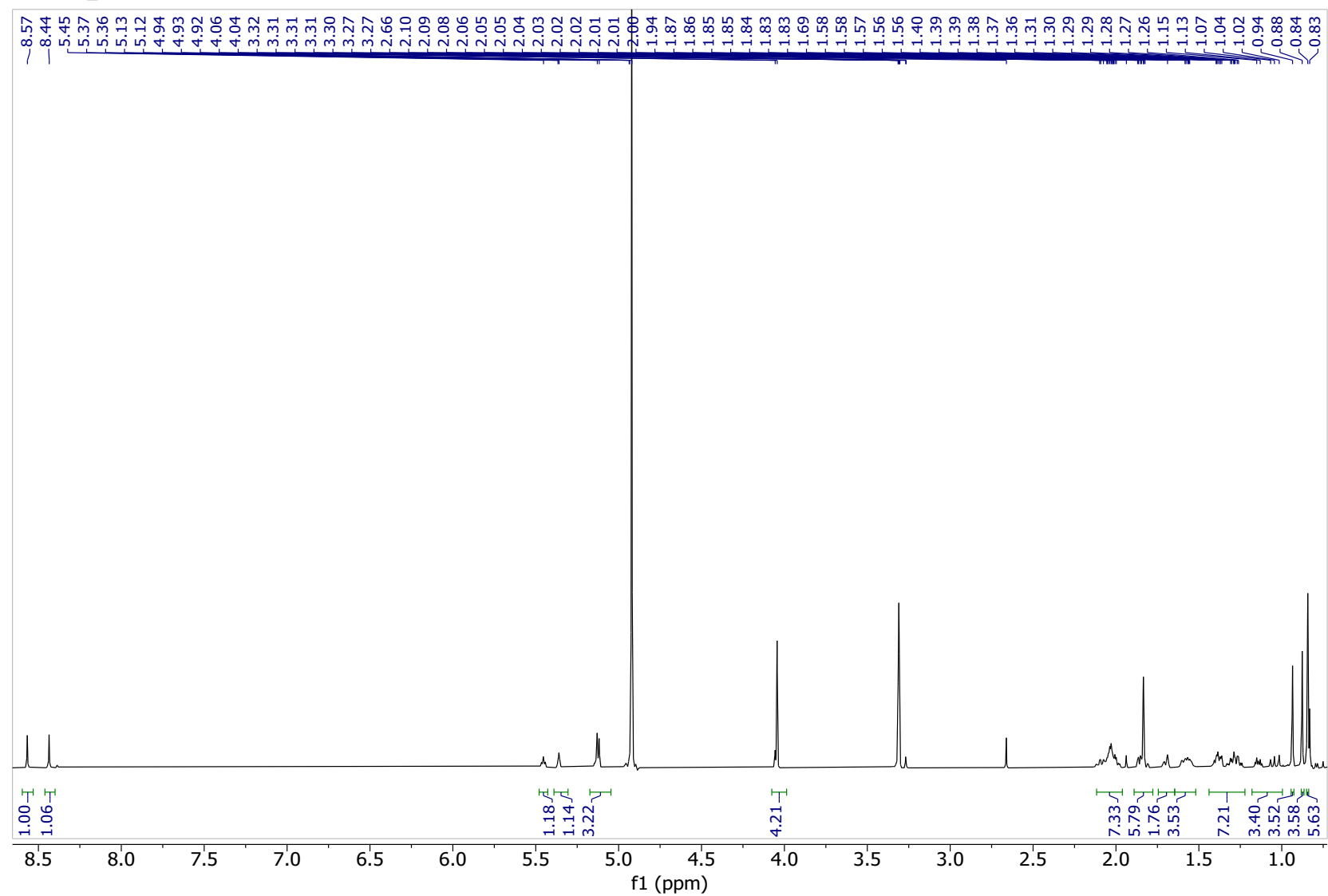


Figure S1. ¹H NMR spectrum (600 MHz) of Agelasine W (1) in CD₃OD.

179B034_46.8.fid

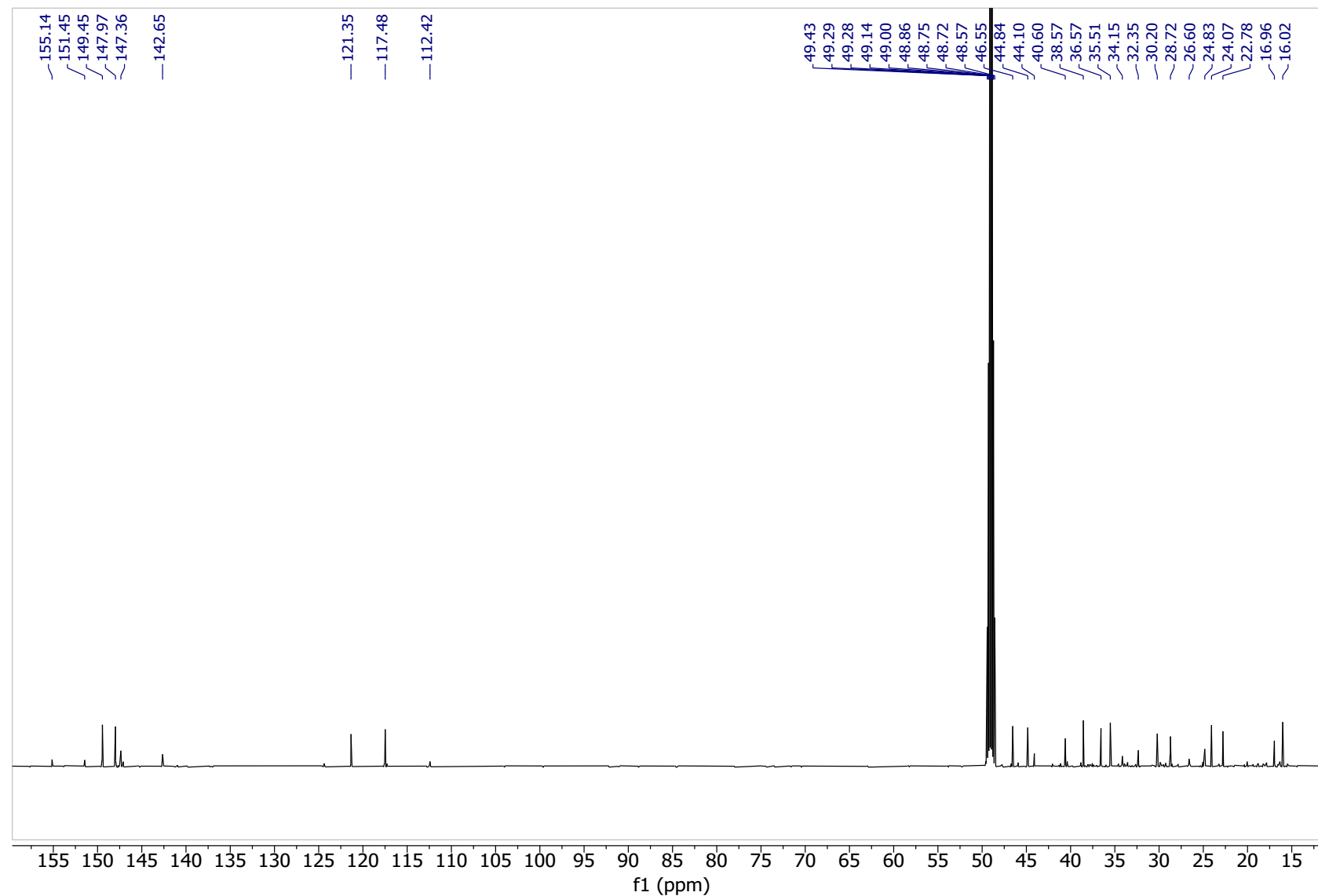


Figure S2. ^{13}C NMR spectrum (150 MHz) of Agelasine W (1) in CD_3OD .

179B034_46.5.ser

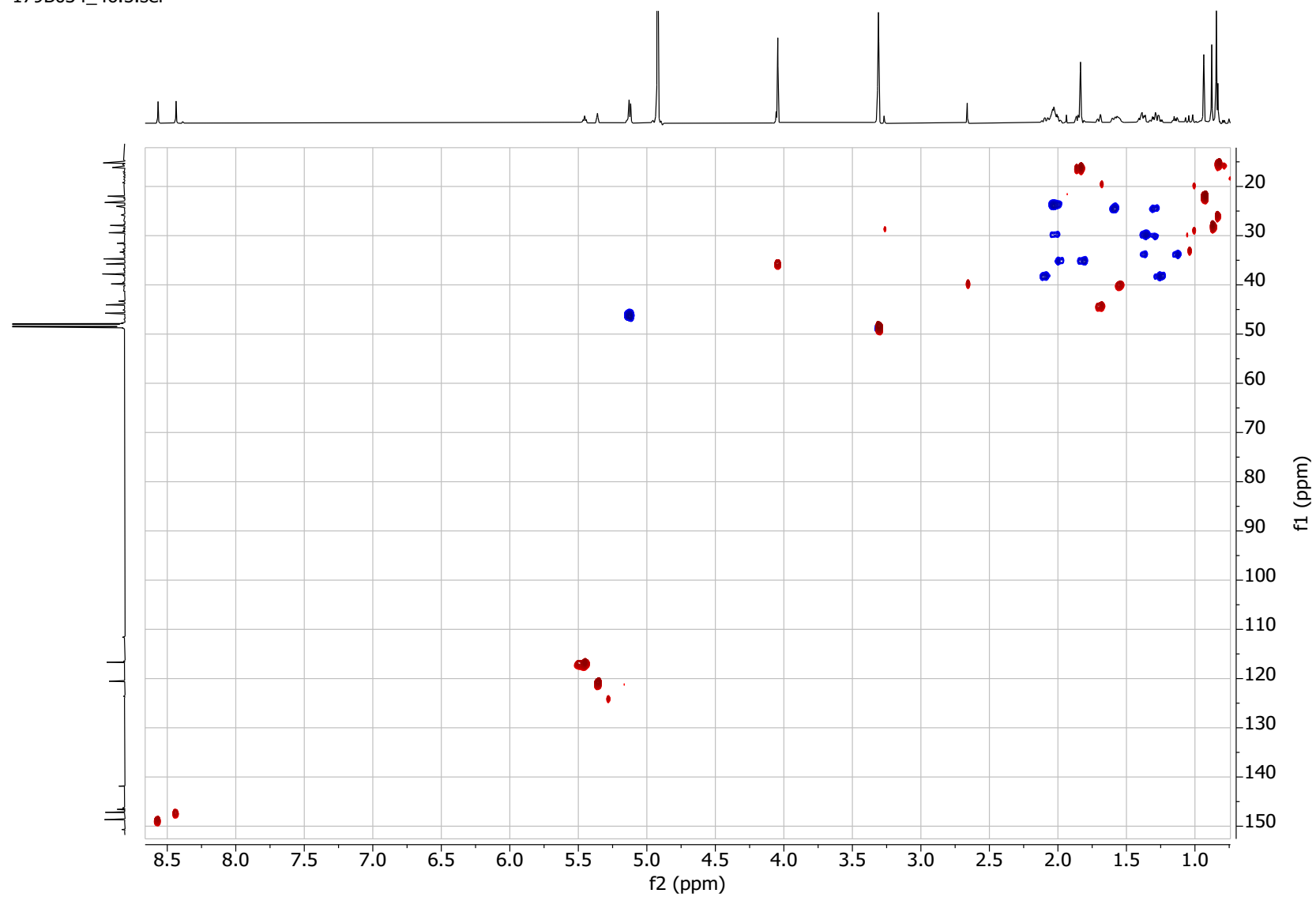


Figure S3. HSQC spectrum of Agelasine W (1) in CD_3OD .

179B034_46.7.ser

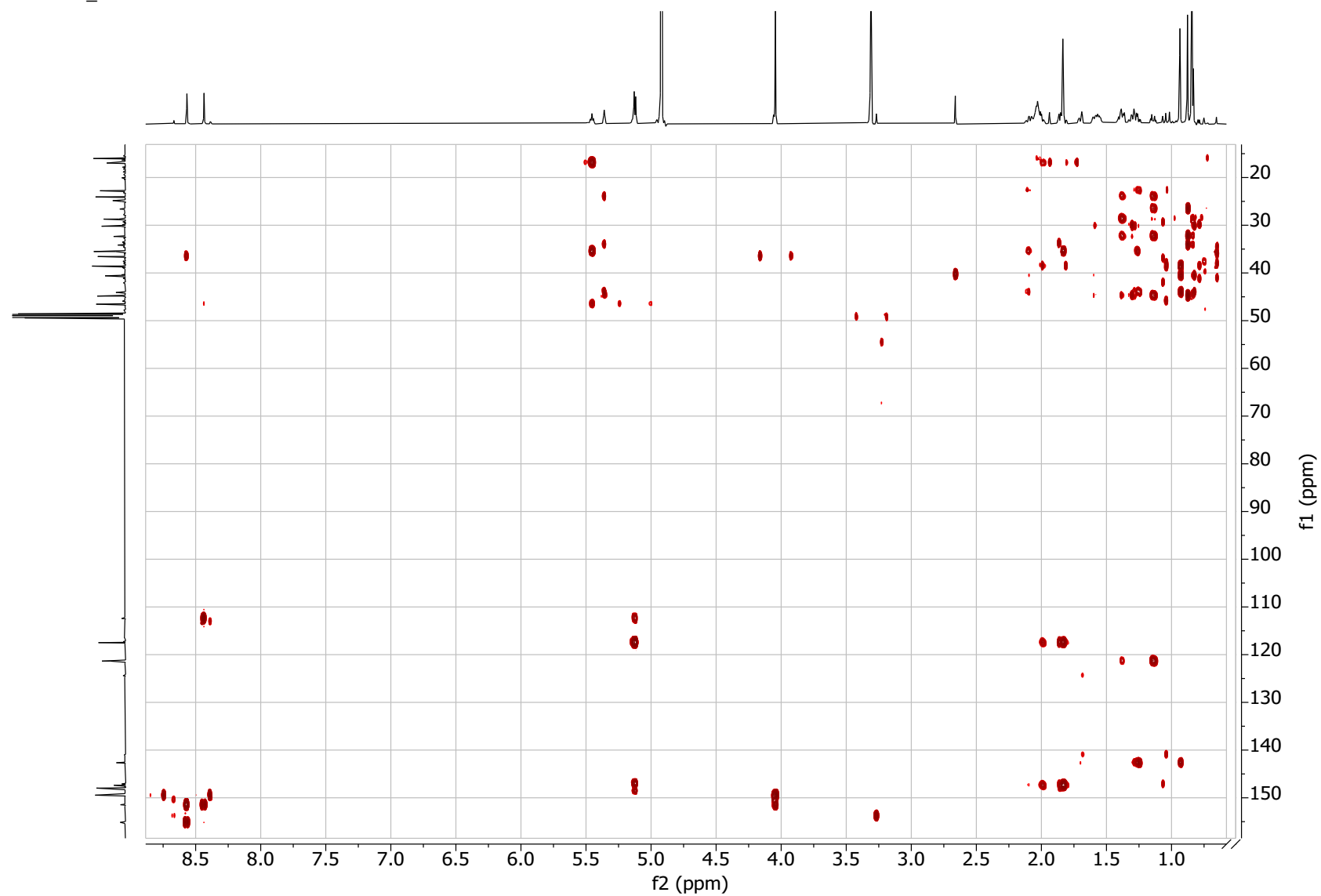


Figure S4. HMBC spectrum of Agelasine W (**1**) in CD_3OD .

179B034_46.6.ser



Figure S5. COSY spectrum of Agelasine W (**1**) in CD₃OD.

1/9B034_46.9.ser

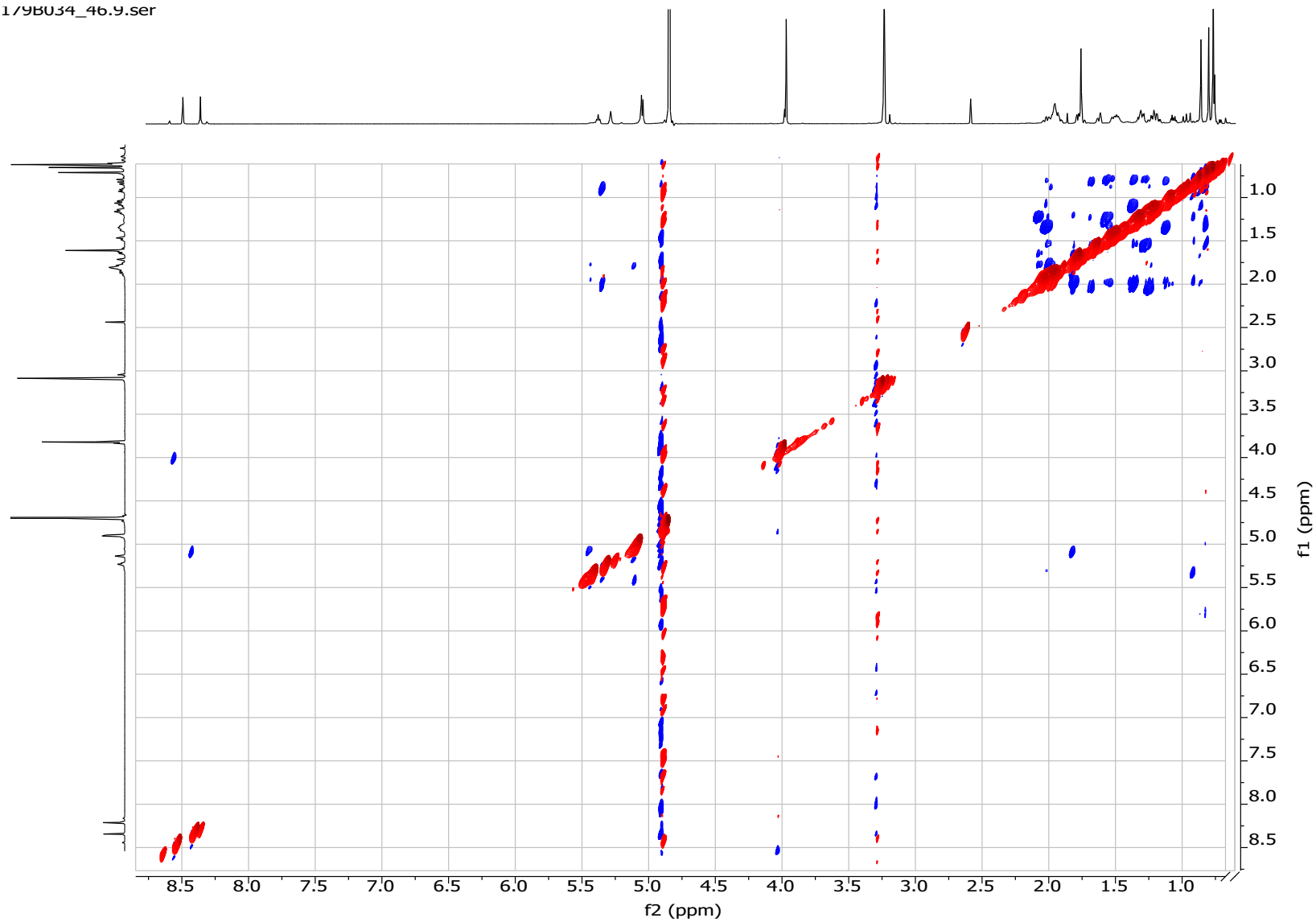


Figure S6. NOESY spectrum of Agelaine W (**1**) in CD₃OD.

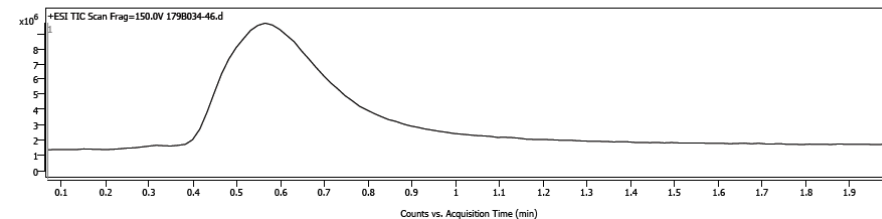
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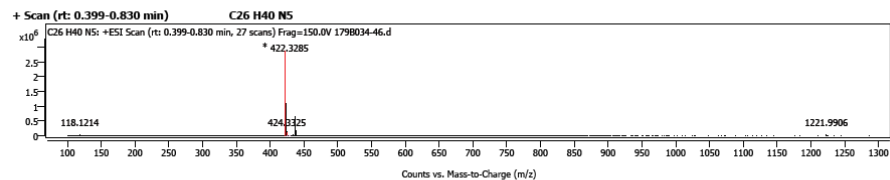
Sample Information

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Sample ID		Acq. Time (Local)	10/29/2019 8:47:47 AM (UTC-05:00)
Instrument	Instrument 1	Method Path (Acq)	D:\MassHunter\Methods\FJA_SM_LowFlow.m
MS Type	QTOF	Version (Acq SW)	6200 series TOF/6500 series Q-TOF B.09.00 (B9044.1 SP1)
Inj. Vol. (ul)	1	IRM Status	Some ions missed
Position	Vial 16	Method Path (DA)	
Plate Pos.		Target Source Path	
Operator		Result Summary	

Sample Chromatograms



Sample Spectra



Spectrum Identification Table

Best ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes MFG		C26 H40 N5	M+	422.3285	0.31		84.22			84.22	

MassHunter Qual 10.0
(End of Report)

Figure S7. HRESIMS spectrum of Agelasine W (1).

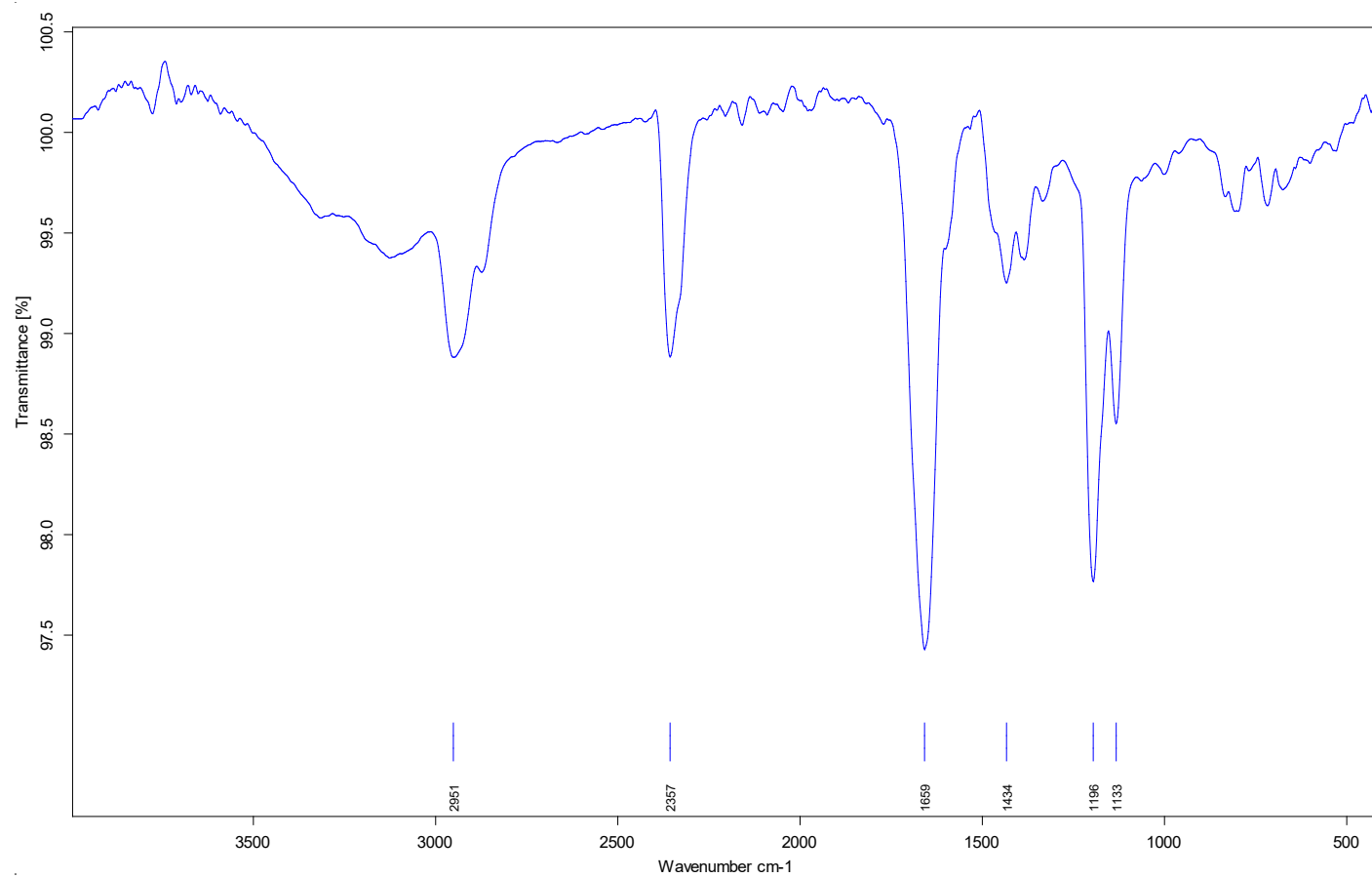


Figure S8. IR spectrum (neat) of Agelasine W (1).

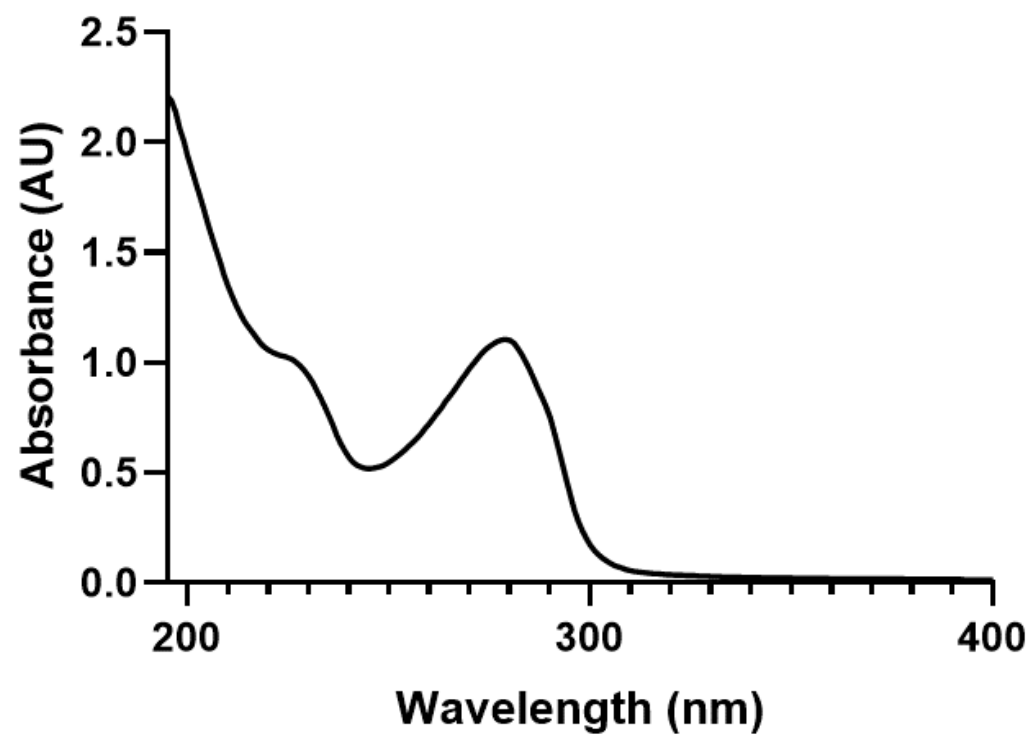


Figure S9. UV spectrum of Agelasine W (1).

1/9B034_49.1.tid

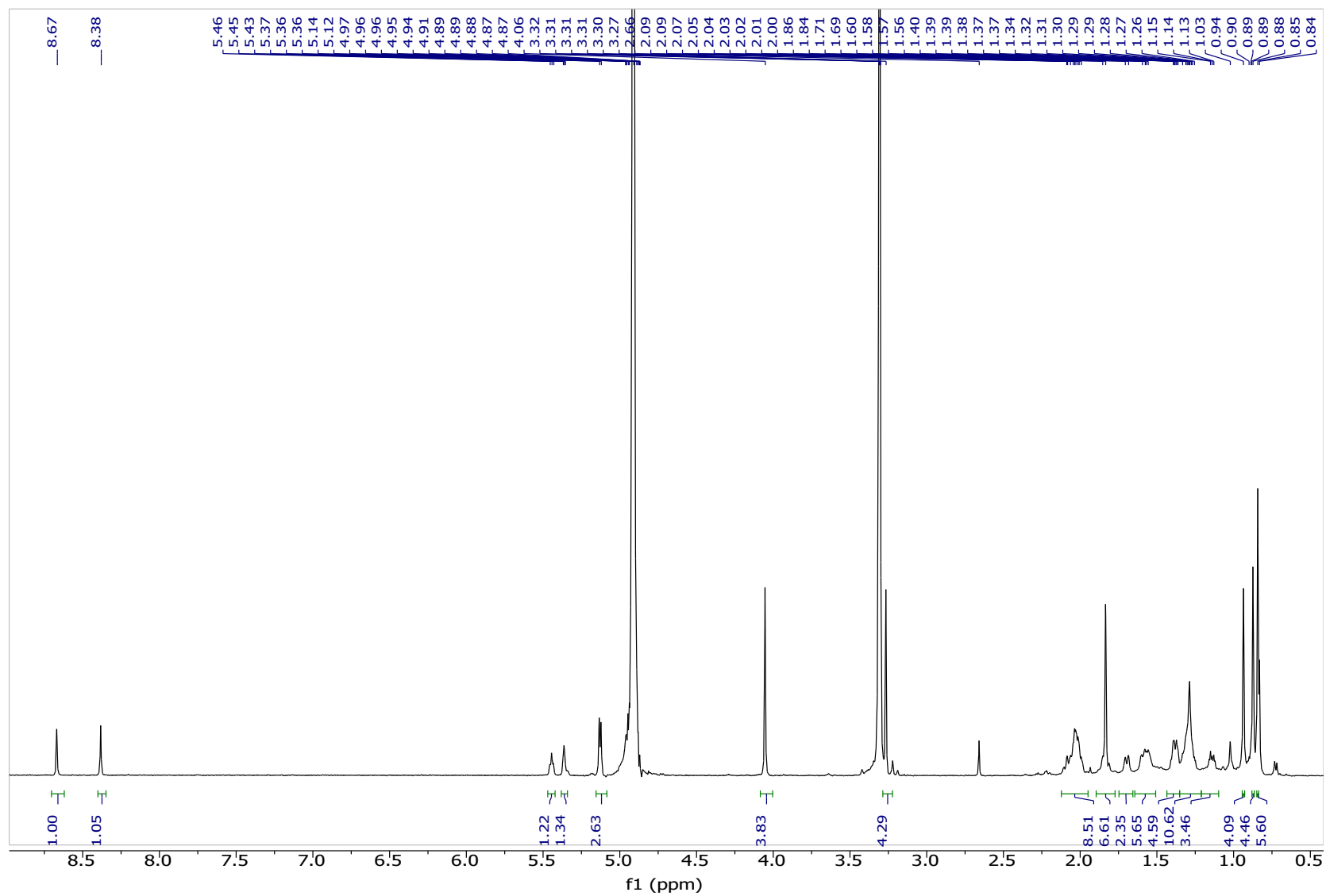


Figure S10. ^1H NMR spectrum (600 MHz) of Agelasine X (**2**) in CD_3OD .

179B034_49.5.tif

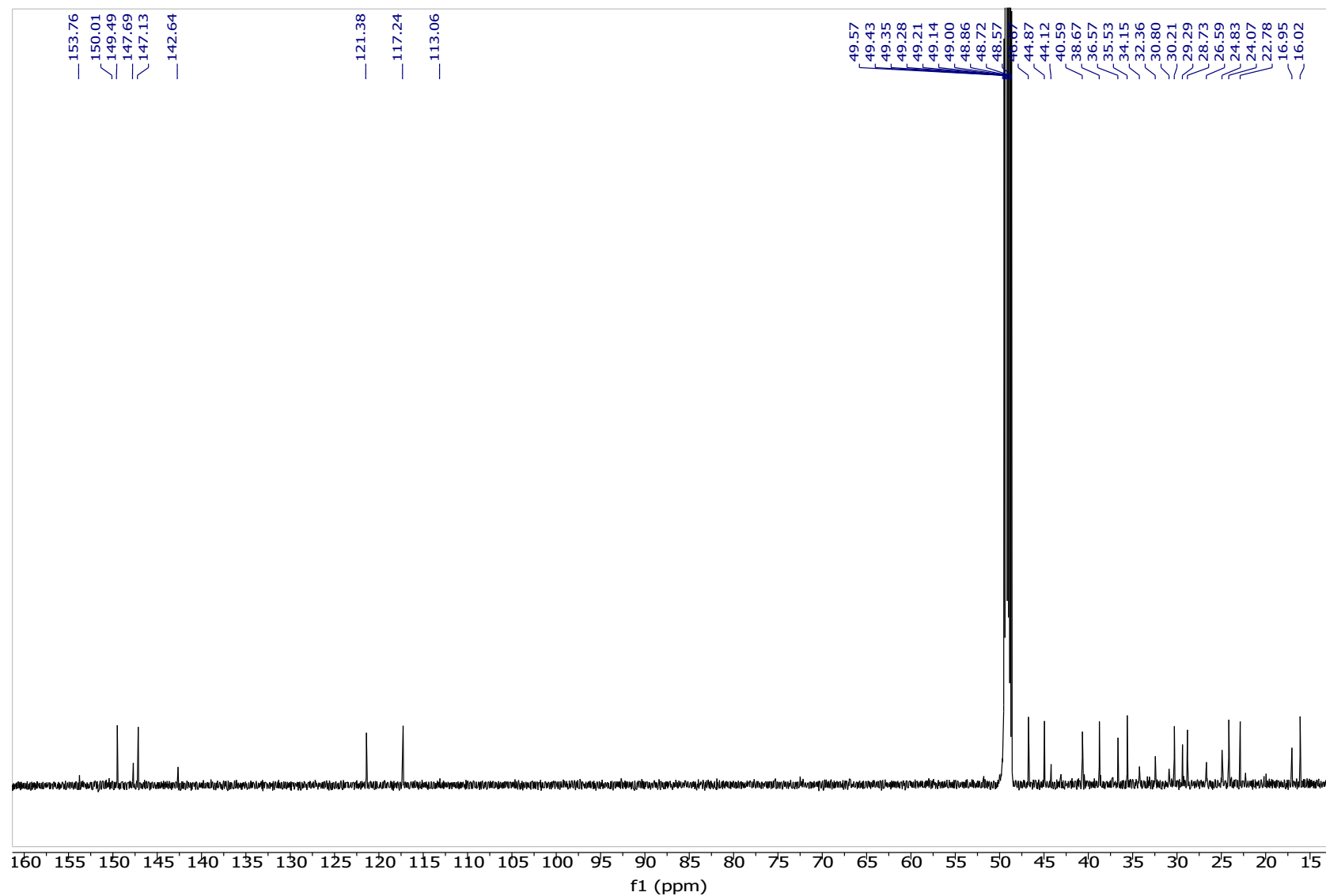


Figure S11. ¹³C NMR spectrum (150 MHz) of Agelasine X (**2**) in CD₃OD.

179B034_49.2.ser

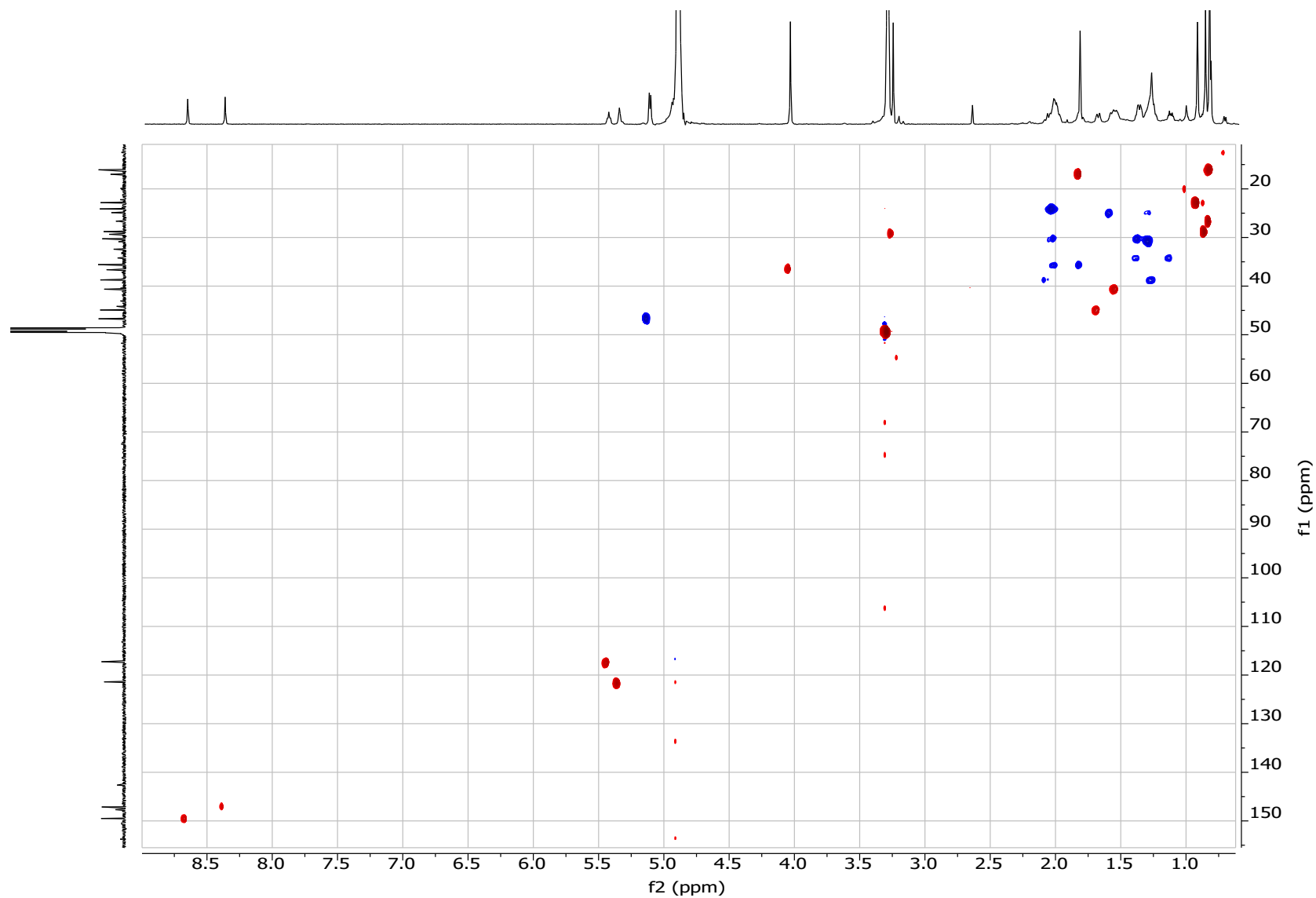


Figure S12. HSQC spectrum of Agelasine X (**2**) in CD_3OD .

1/9B034_49.4.ser

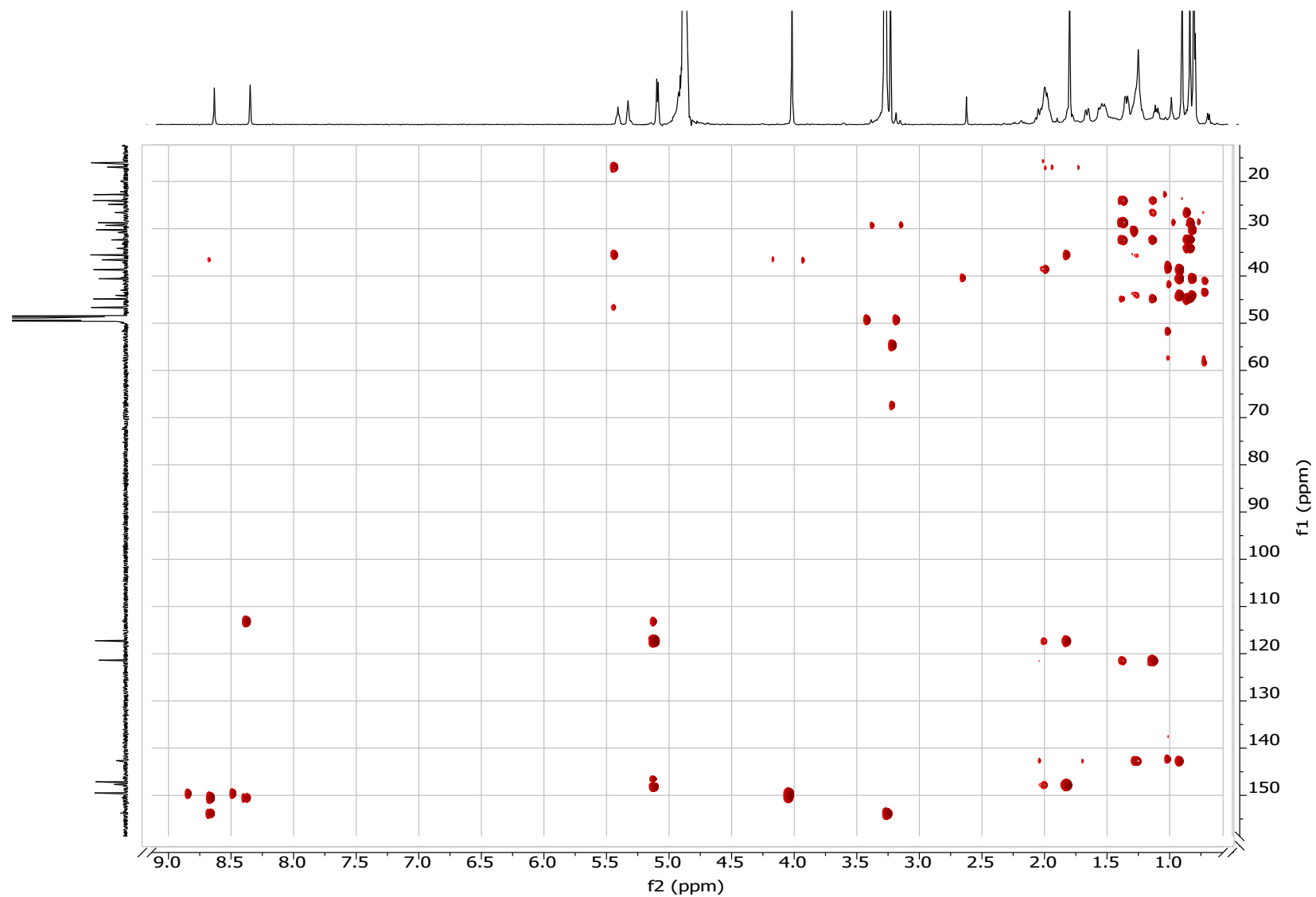


Figure S13. HMBC spectrum of Agelasine X (**2**) in CD₃OD.

Figure S14. COSY spectrum of Agelasine X (**2**) in CD₃OD.

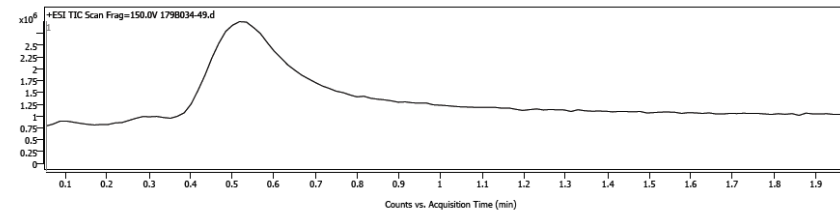
Analysis Report



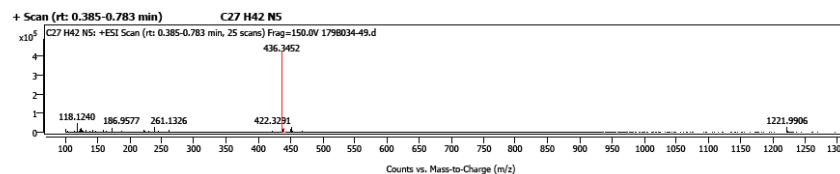
Sample Information

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Sample ID		Acq. Time (Local)	10/29/2019 8:58:41 AM (UTC-05:00)
Instrument	Instrument 1	Method Path (Acq)	D:\MassHunter\Methods\FIA_SM_LowFlow.m
MS Type	QTOF	Version (Acq SW)	6200 series TOF/6500 series Q-TOF B.09.00 (B9044.1 SP1)
Inj. Vol. (ul)	1	IRM Status	Some ions missed
Position	Vial 18	Method Path (DA)	C:\Users\bokesch\ Desktop\QTOF-DATAANALYSIS-ADMIN by HEIDIB\We\102919\1798034-49.d\Results\Qual\Version4\IRB.m
Plate Pos.		Target Source Path	
Operator		Result Summary	

Sample Chromatograms



Sample Spectra



Spectrum Identification Table

Best ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MPG)	Lib/DB
Yes: MPG	C27 H42 N5		M+	436.3452	3.76		92.33			92.33	

MassHunter Qual 10.0
(End of Report)

Figure S15. HRESIMS spectrum of Agelasine X (2).

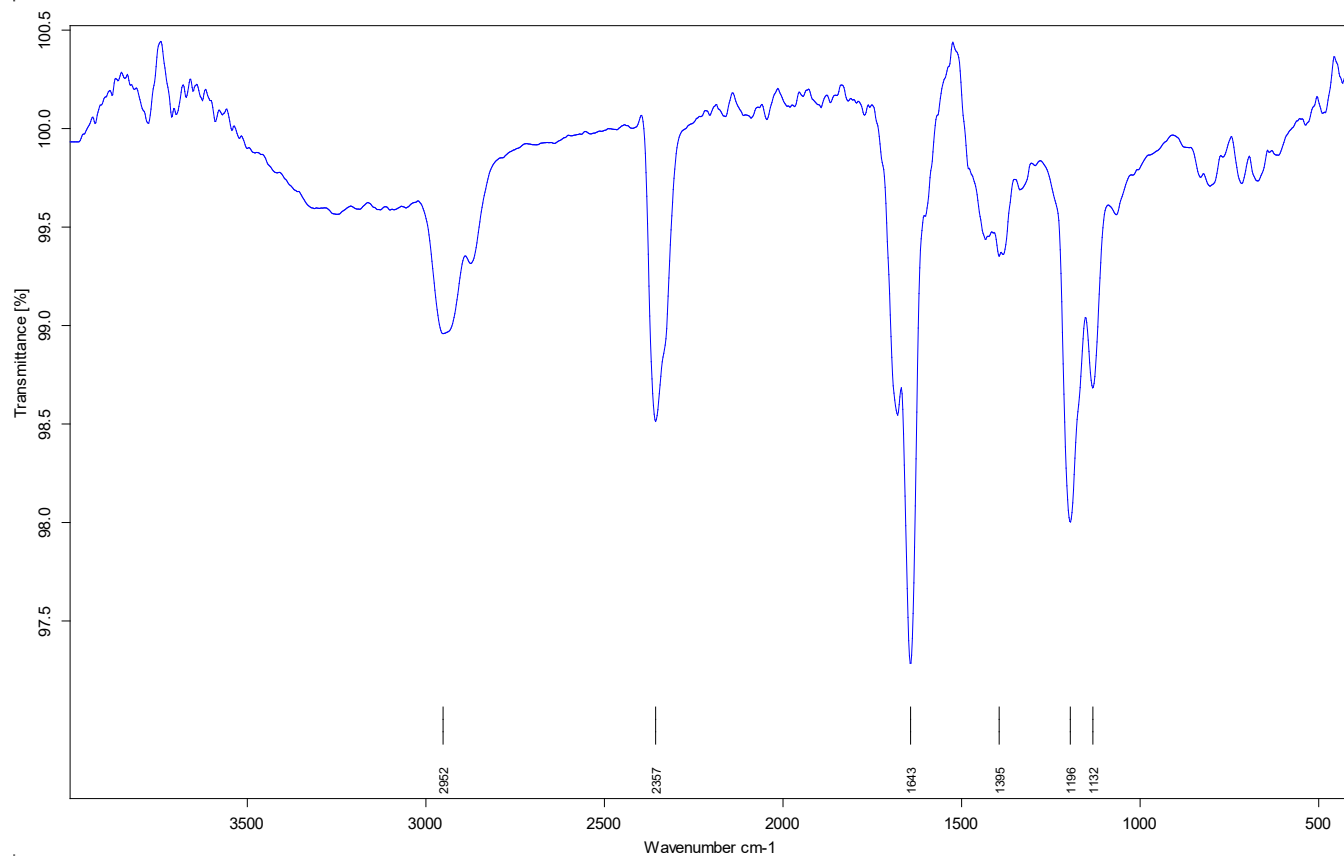


Figure S16. IR spectrum (neat) of Agelazine X (**2**).

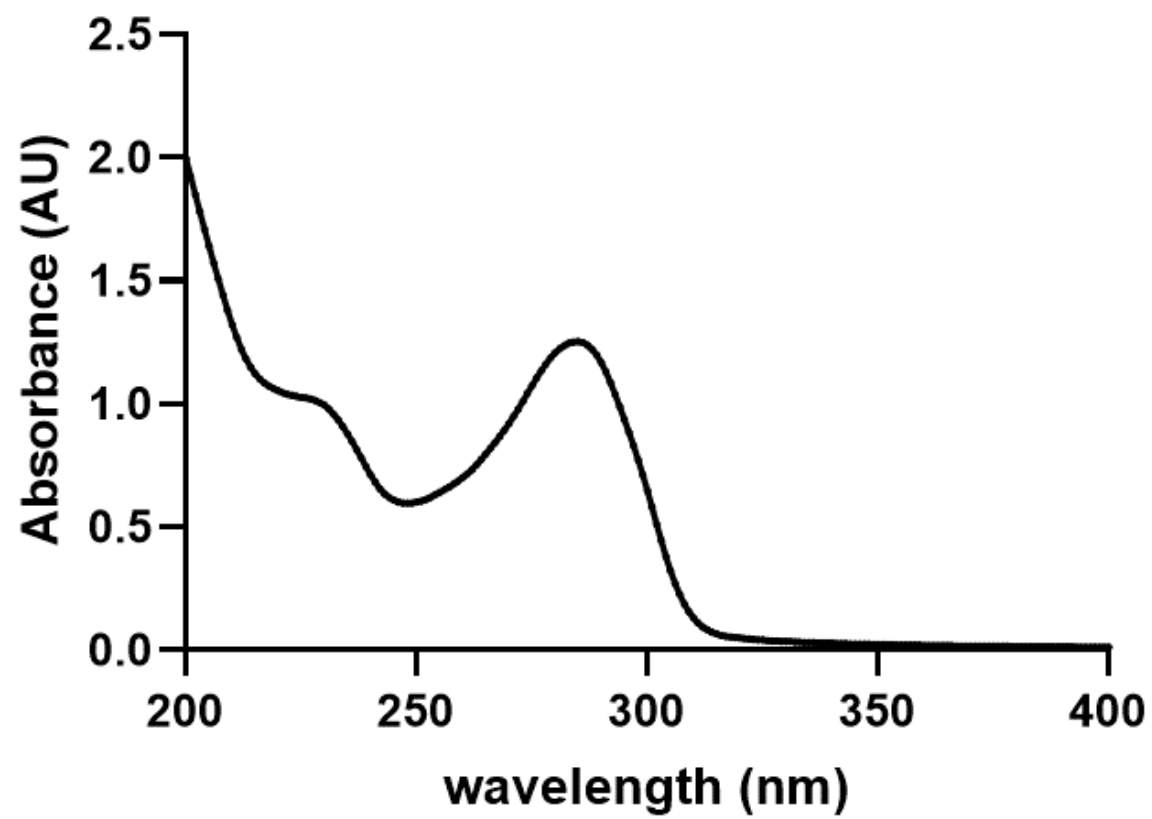


Figure S17. UV spectrum of Agelasine X (2).

1/9B034_4/.1.tif

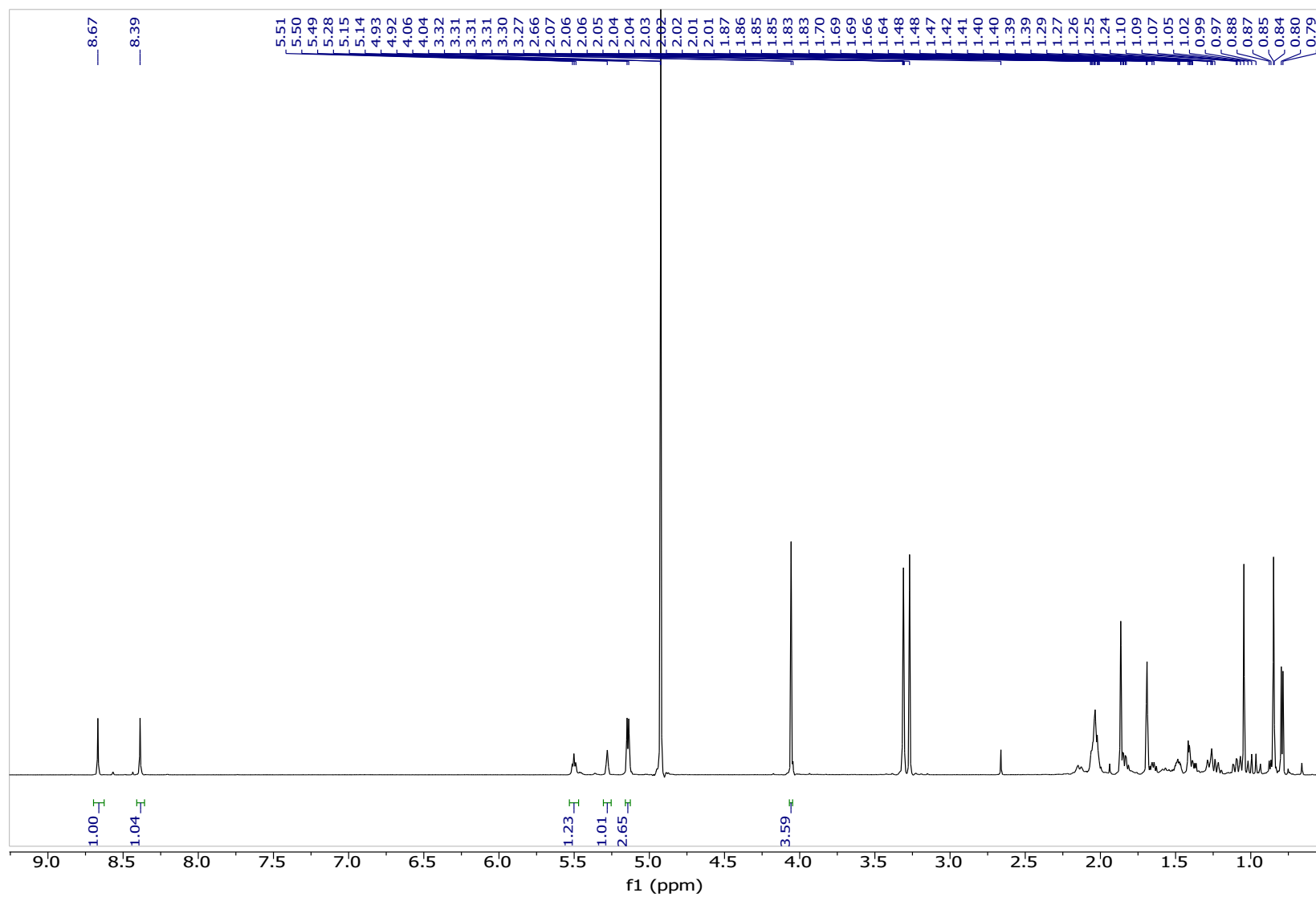


Figure S18. ¹H NMR spectrum (600 MHz) of Agelasine Y (3) in CD₃OD.

1/9B034_47.8.tif

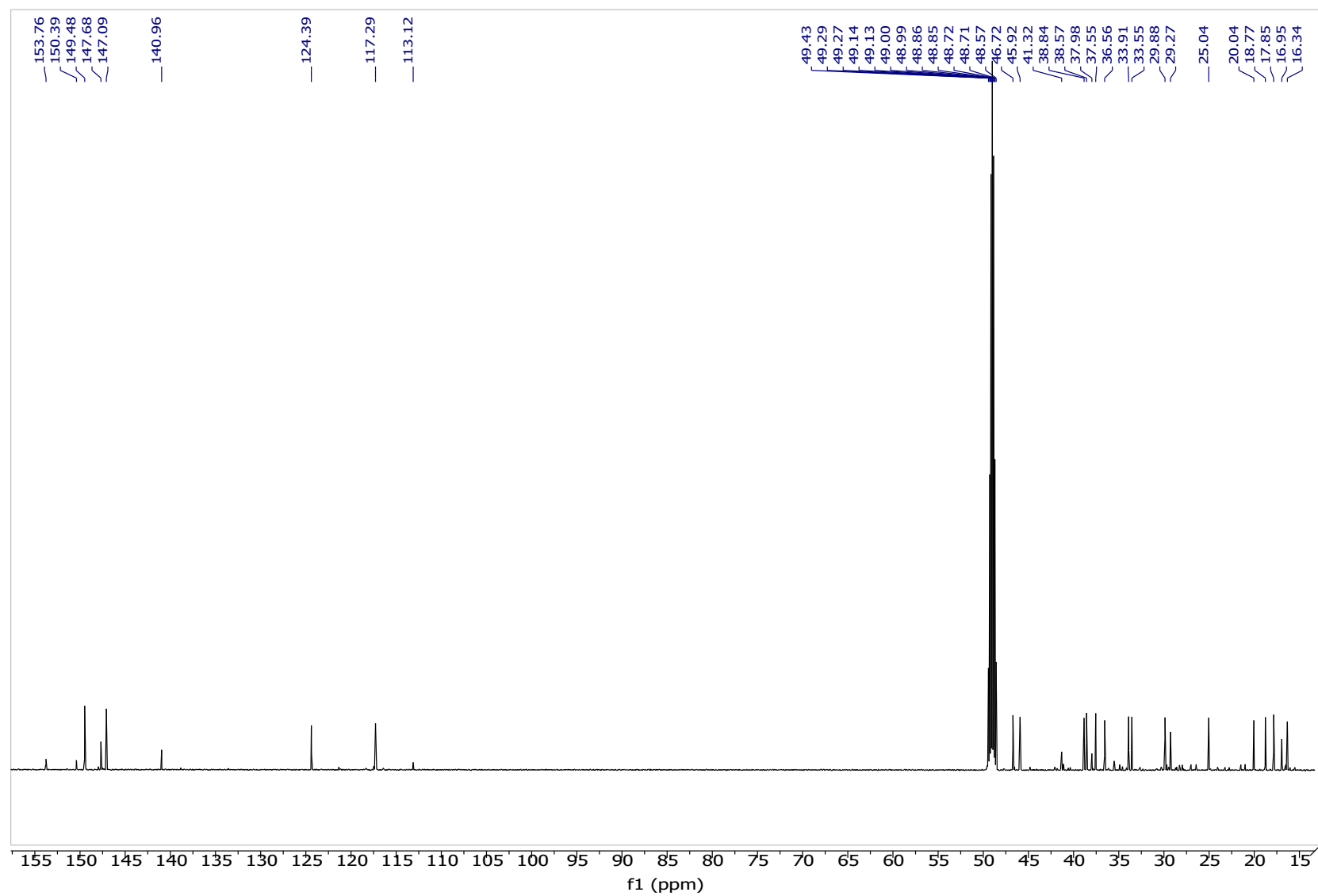


Figure S19. ¹³C NMR spectrum (150 MHz) of Agelasine Y (**3**) in CD₃OD.

1/9B034_47.5.ser

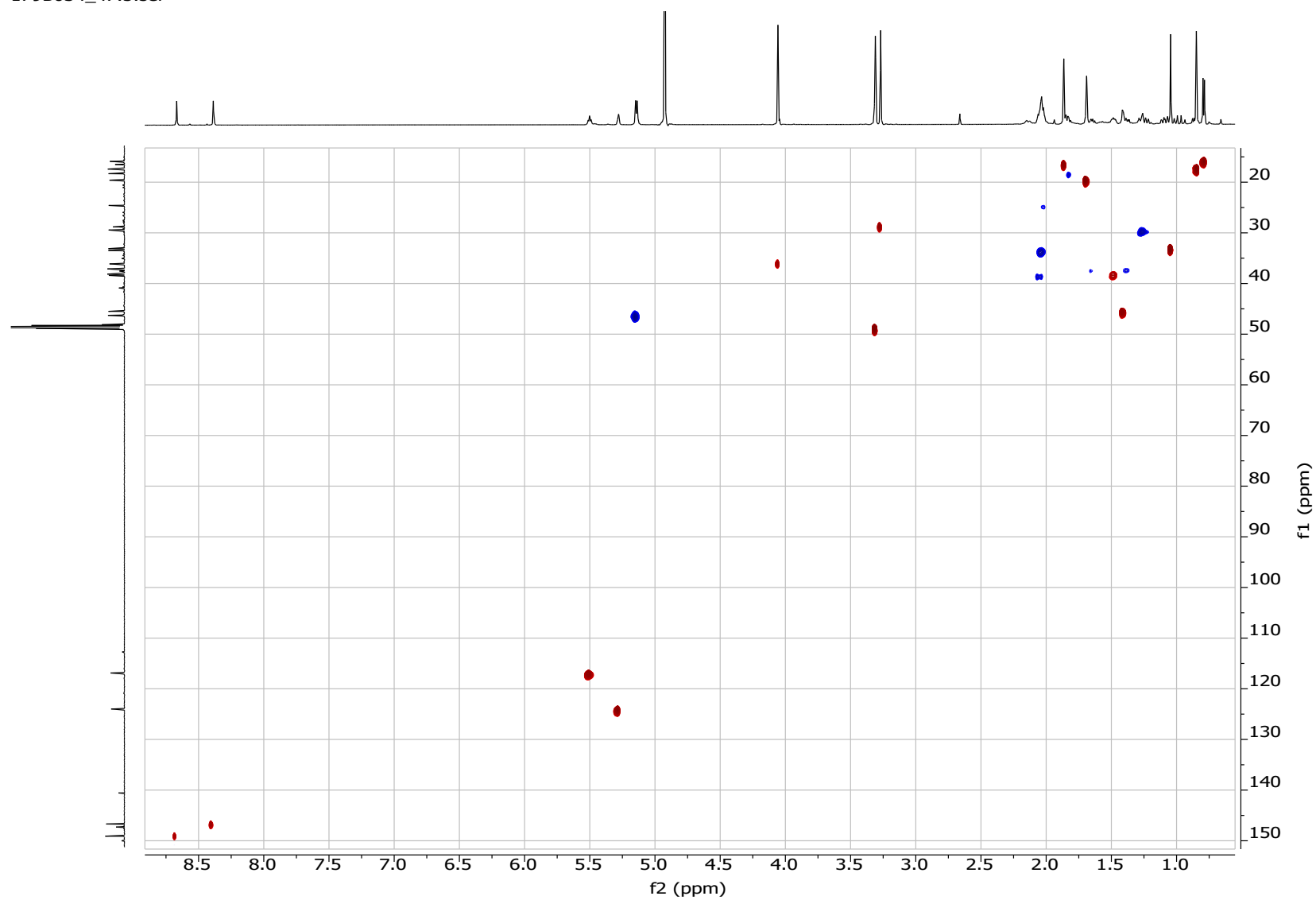


Figure S20. HSQC spectrum of Agelaine Y (**3**) in CD_3OD .

1/9B034_47/.ser

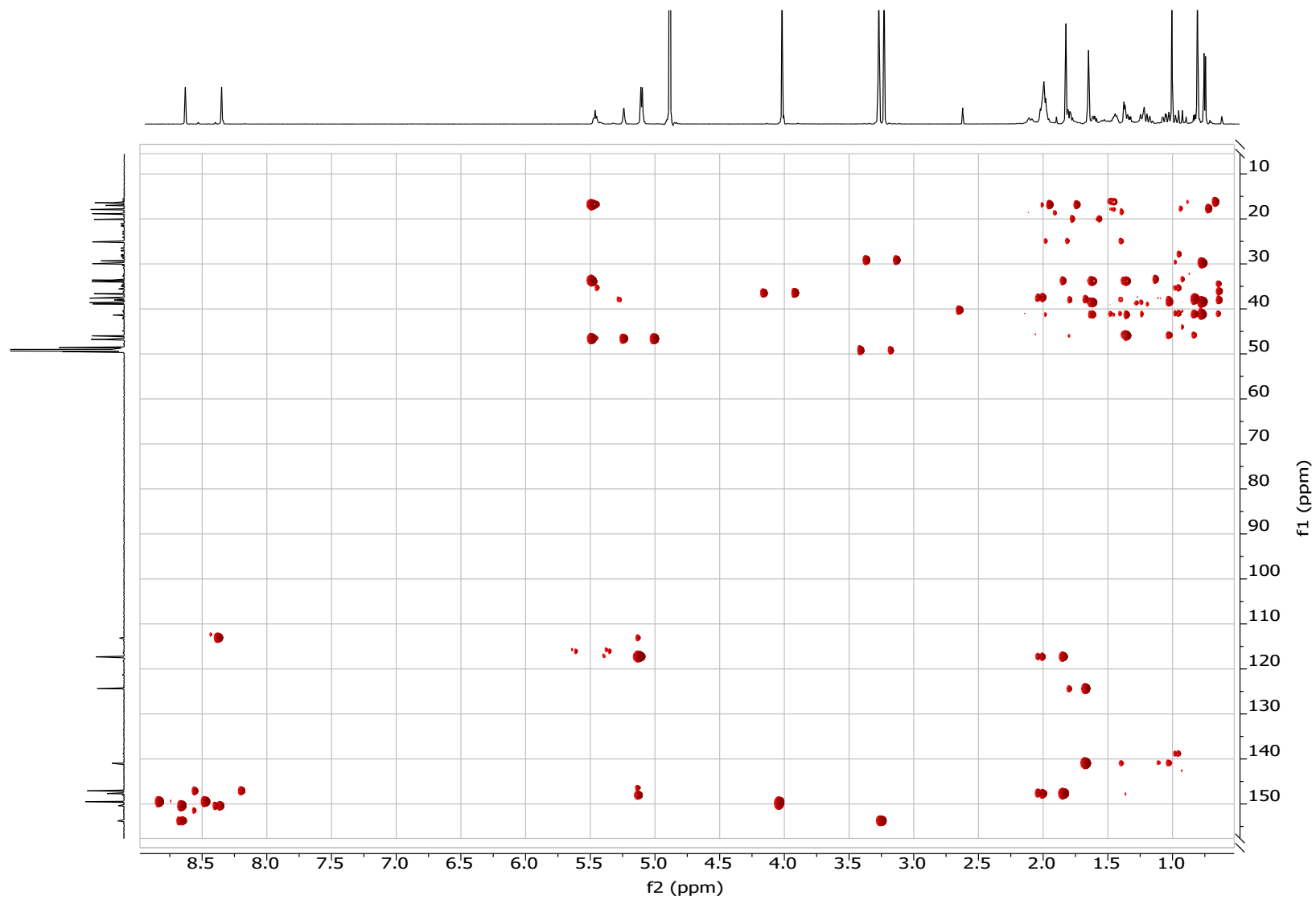


Figure S21. HMBC spectrum of Agelastine Y (**3**) in CD_3OD .

1/9B034_47.b.ser

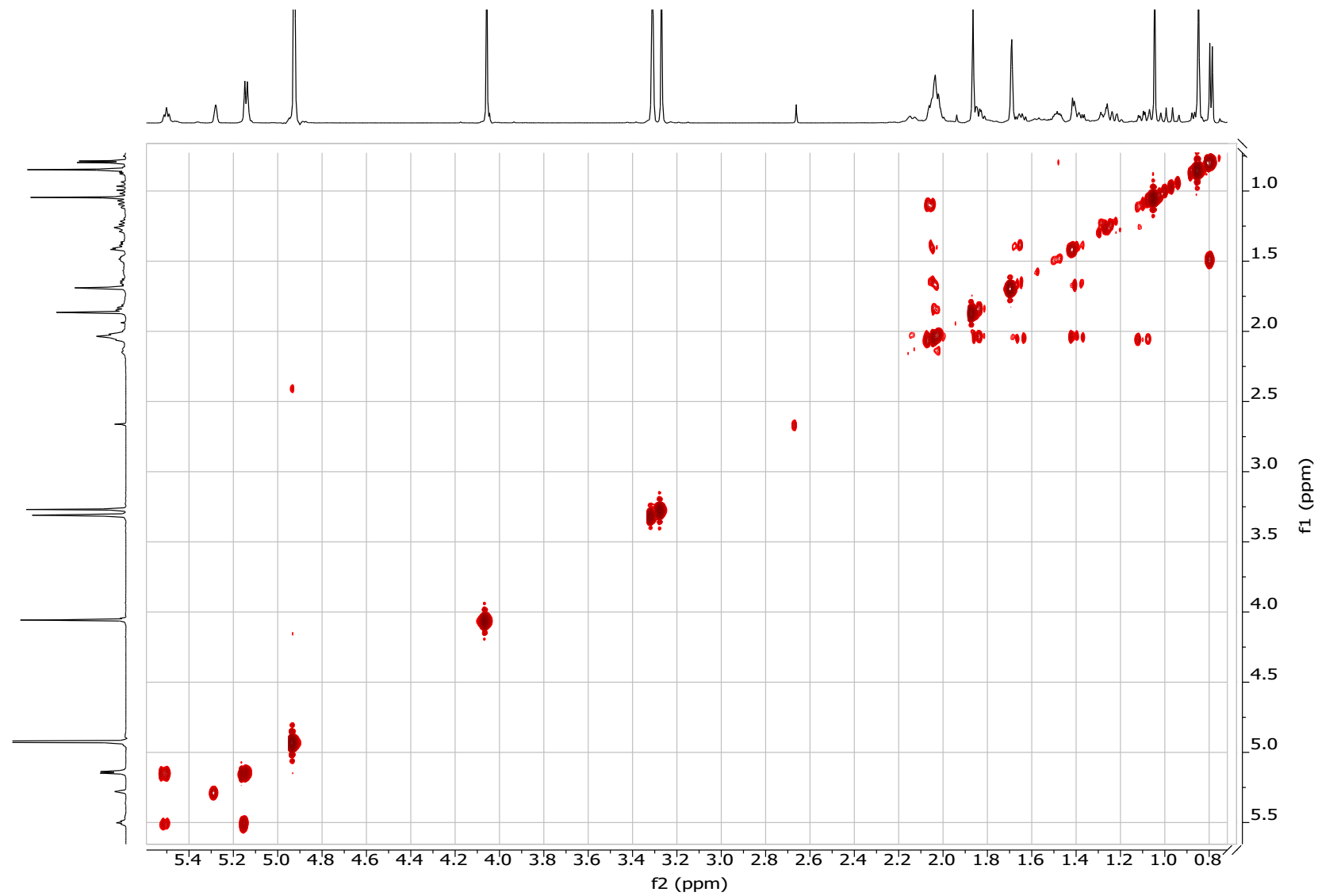


Figure S22. COSY spectrum of Agelasine Y (3) in CD₃OD.

1/9B034_4/.10.ser

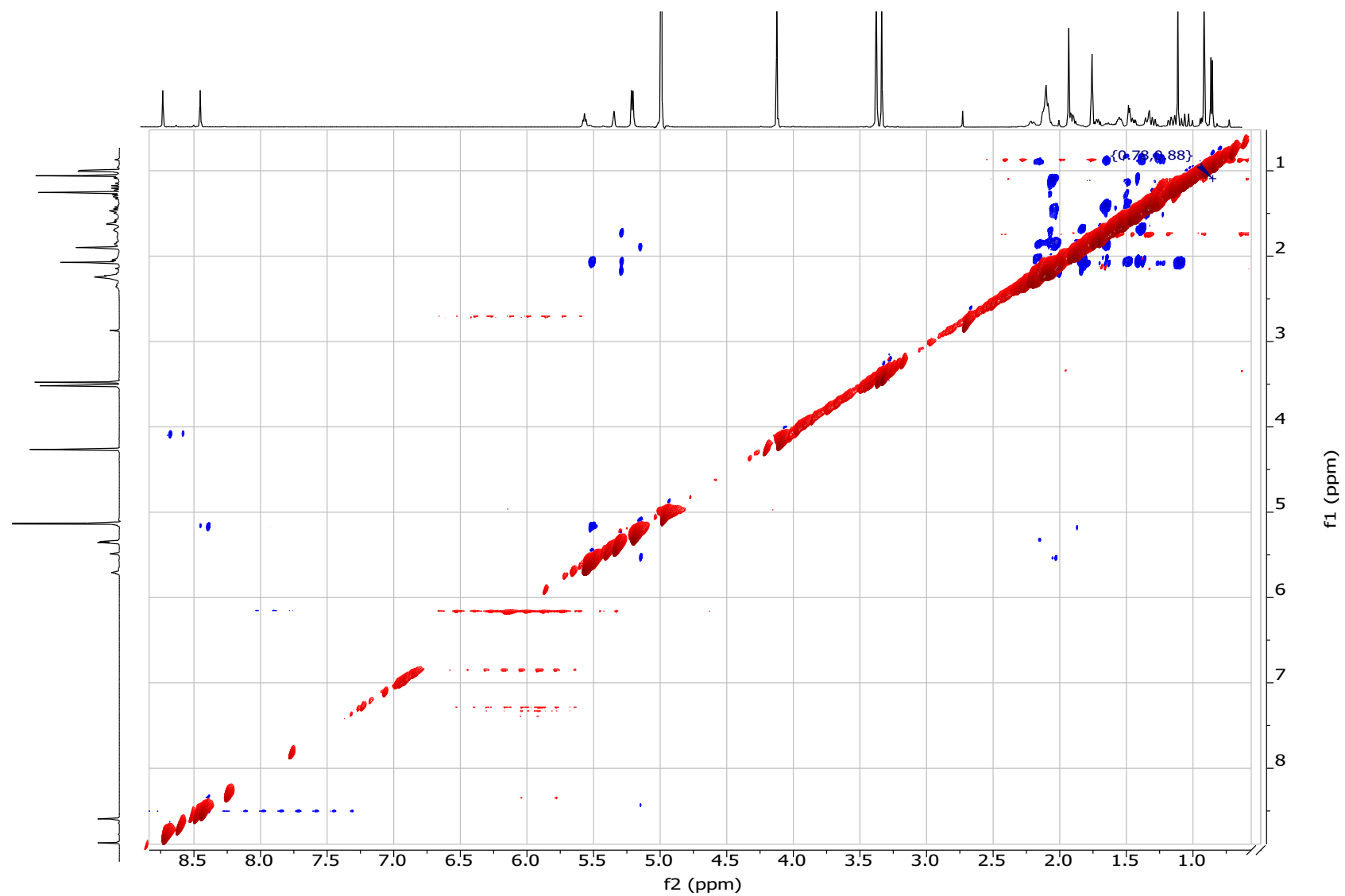


Figure S23. NOESY spectrum of Agelasine Y (**3**) in CD₃OD.

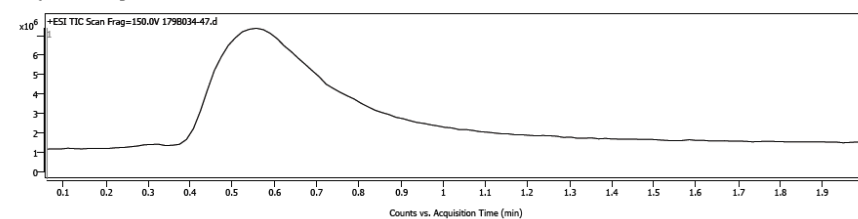
Analysis Report



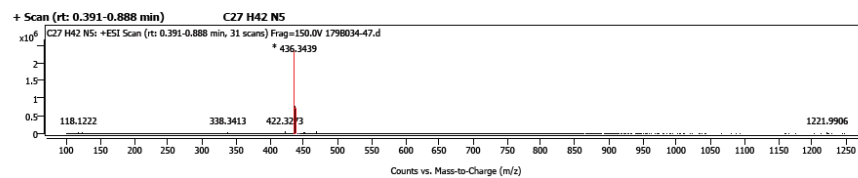
Sample Information

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Sample ID		Acq. Time (Local)	10/29/2019 8:53:13 AM (UTC-05:00)
Instrument	Instrument 1	Method Path (Acq)	D:\MassHunter\Methods\FIA_SM_LowFlow.m
MS Type	QTOF	Version (Acq SW)	6200 series TOF/6500 series Q-TOF B.09.00 (B9044.1 SP1)
Inj. Vol. (ul)	1	IRM Status	Some ions missed
Position	Vial 17	Method Path (DA)	
Plate Pos.		Target Source Path	
Operator		Result Summary	

Sample Chromatograms



Sample Spectra



Spectrum Identification Table

Best ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes_MFG		C27 H42 N5	M+	436.3439	0.51		97.80			97.80	

MassHunter Qual 10.0
(End of Report)

Figure S24. HRESIMS spectrum of Agelasine Y (3).

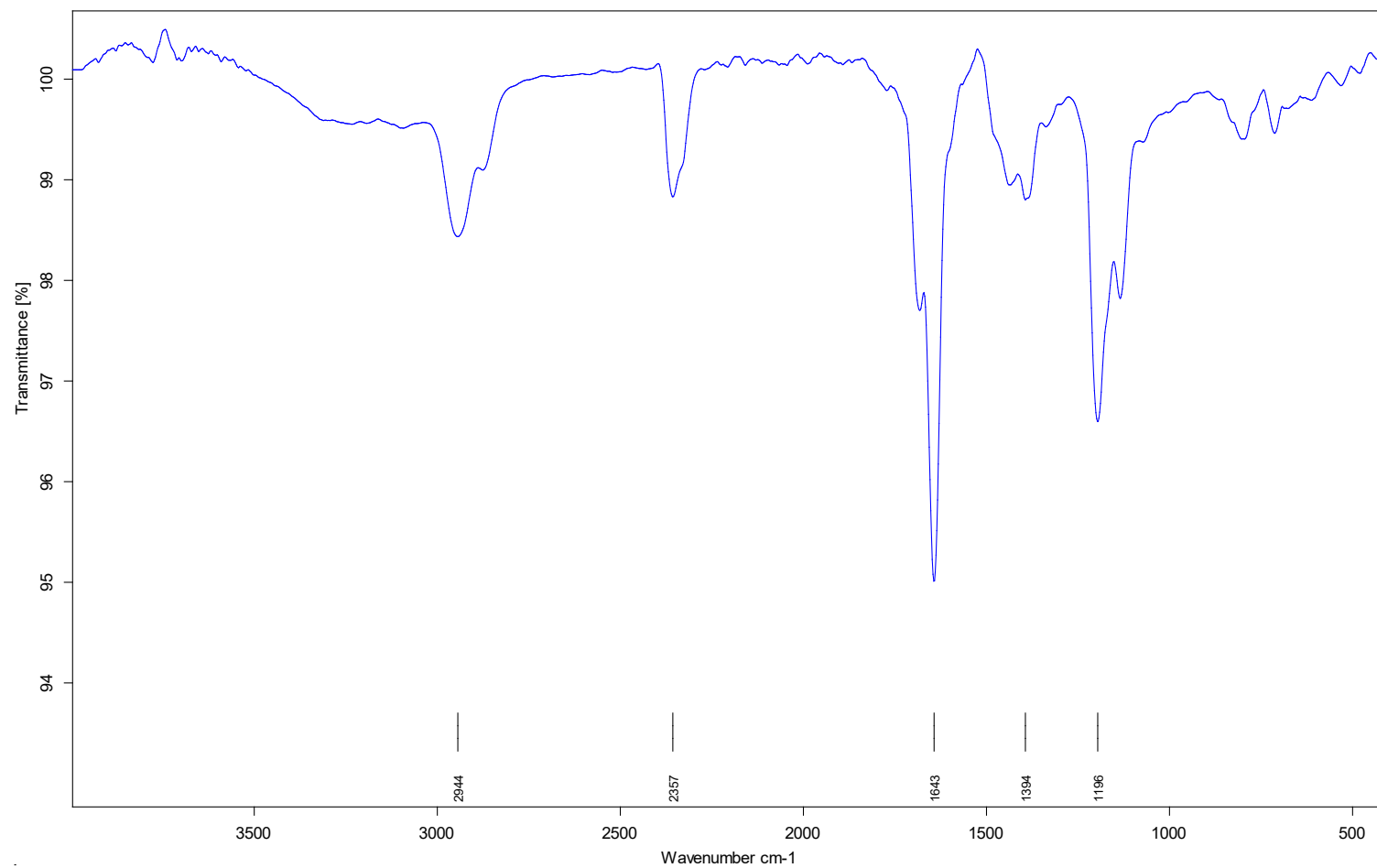


Figure S25. IR spectrum (neat) of Agelasine Y (**3**).

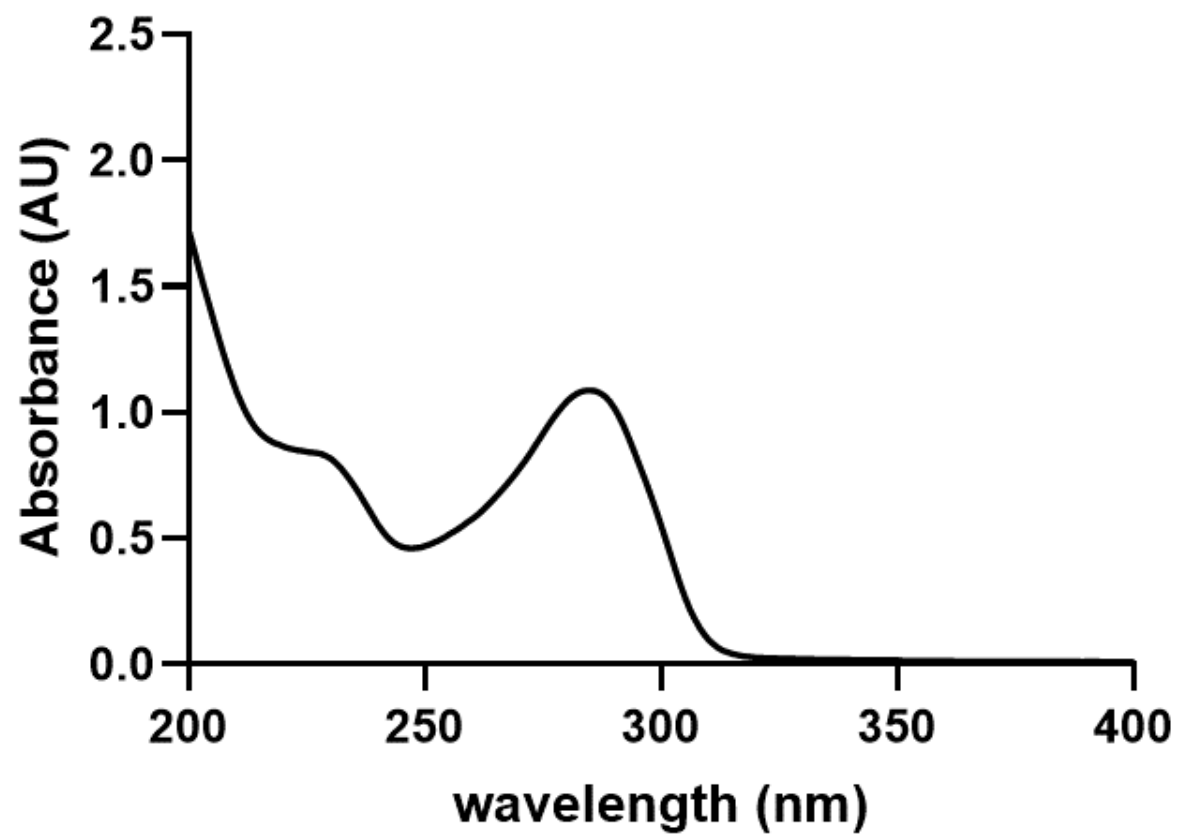


Figure S26. UV spectrum of Agelasine Y (3).

Figure S27. ^1H NMR spectrum (600 MHz) of *N*(1)-methylisoageliferin (**4**) in CD_3OD .

179B053_31.5.fid

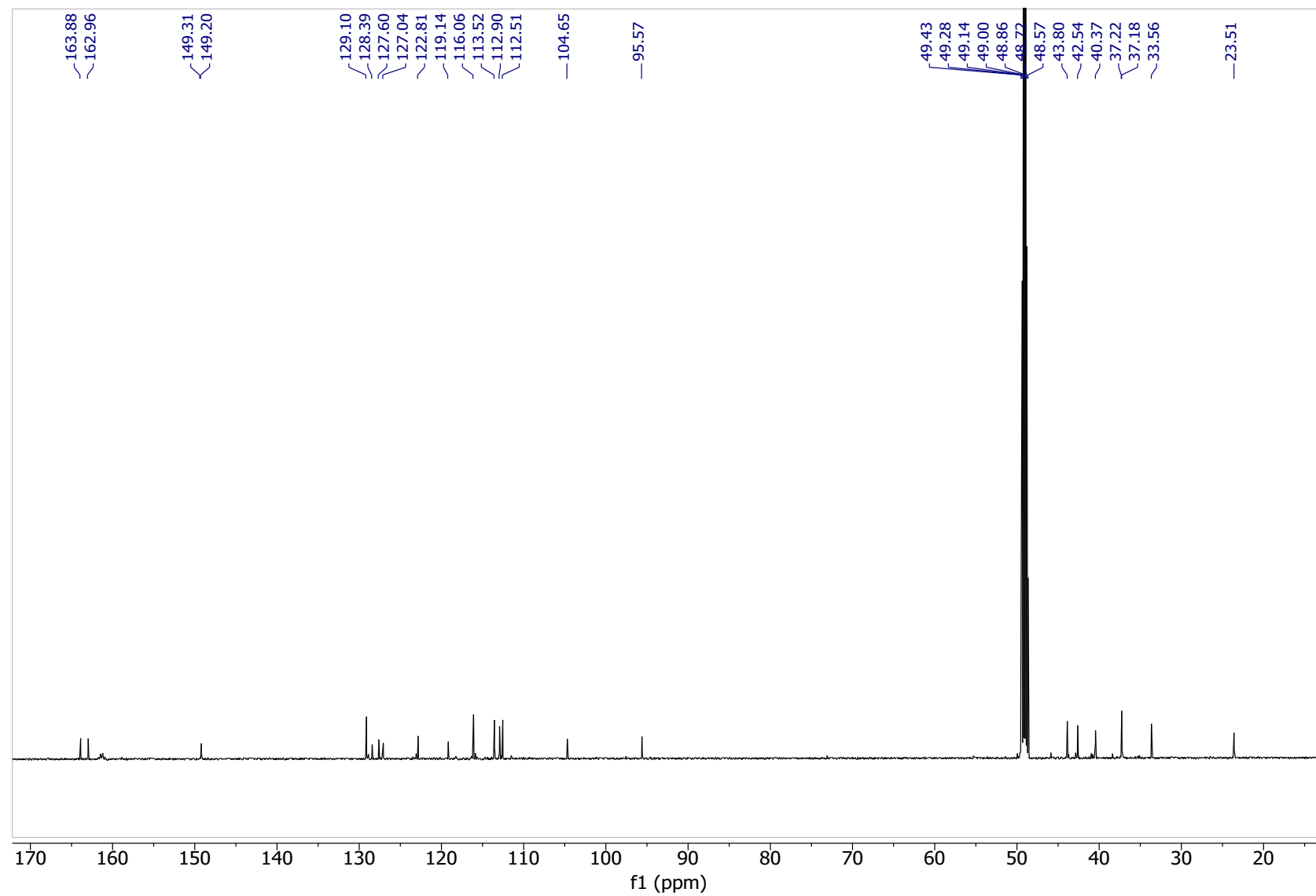


Figure S28. ^{13}C NMR spectrum (150 MHz) of *N*(1)-methylisoageliferin (**4**) in CD_3OD .

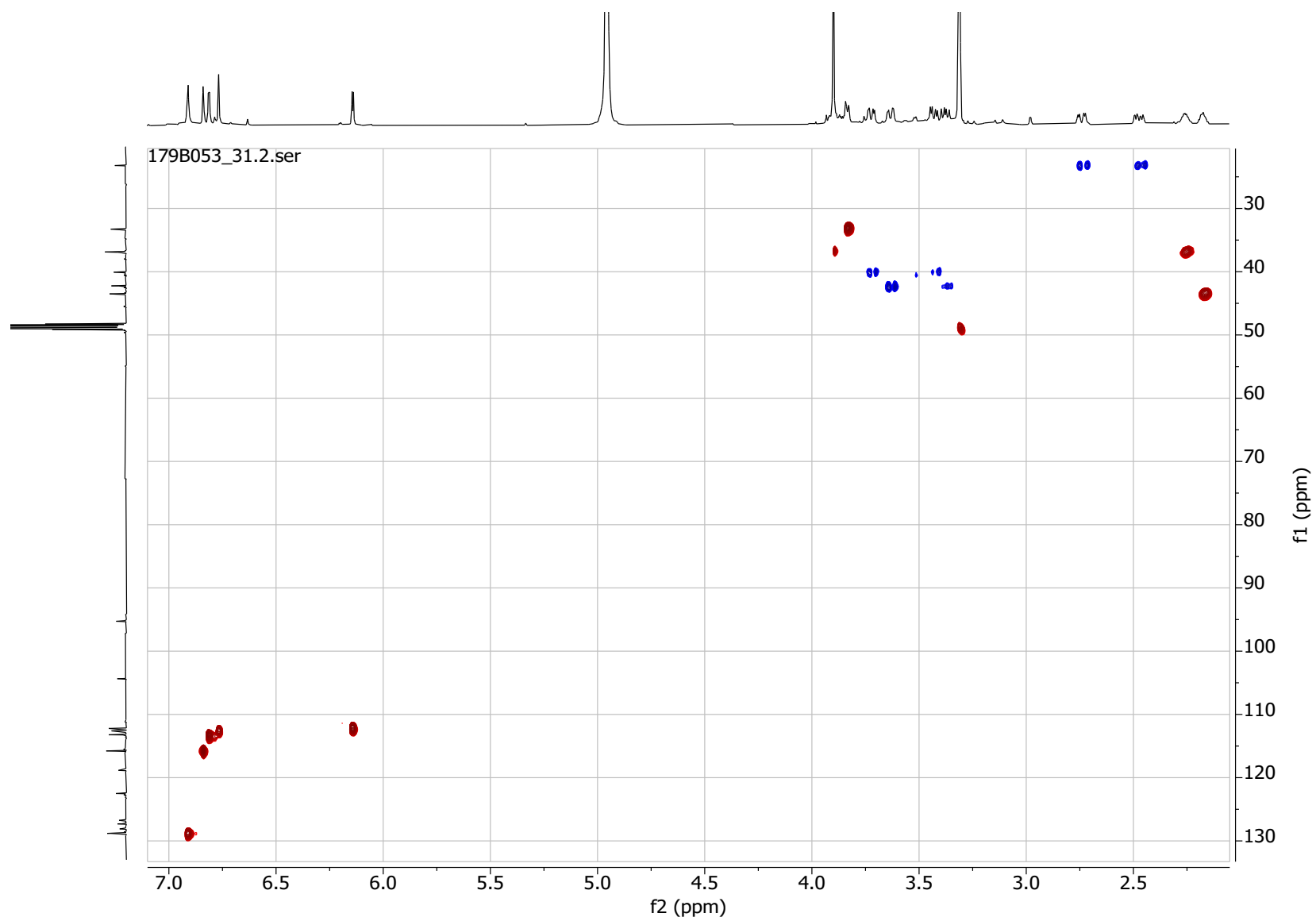


Figure S29. HSQC spectrum of *N*(1)-methylisoageliferin (**4**) in CD_3OD .

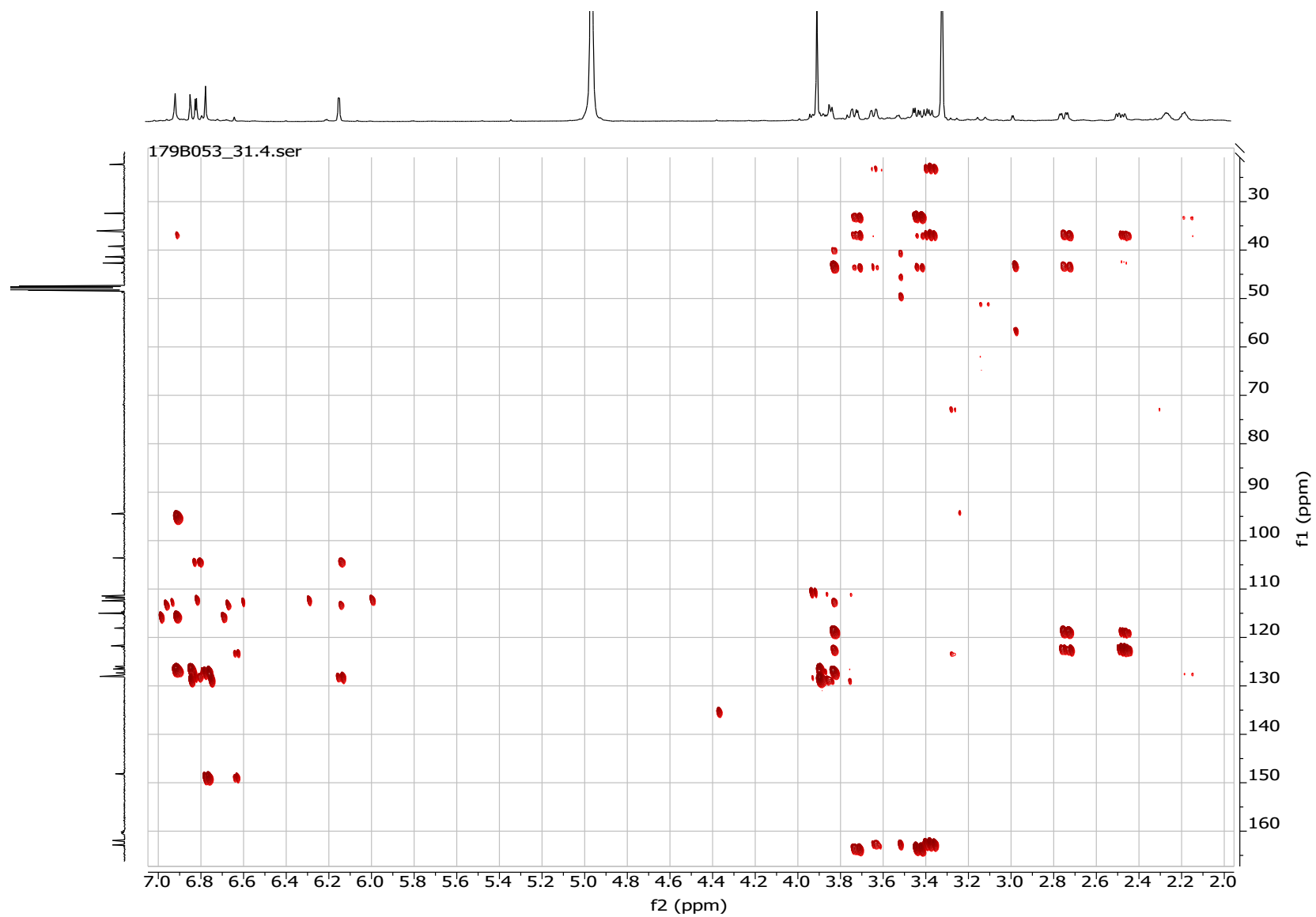


Figure S30. HMBC spectrum of *N*(1)-methyloageliferin (**4**) in CD_3OD .

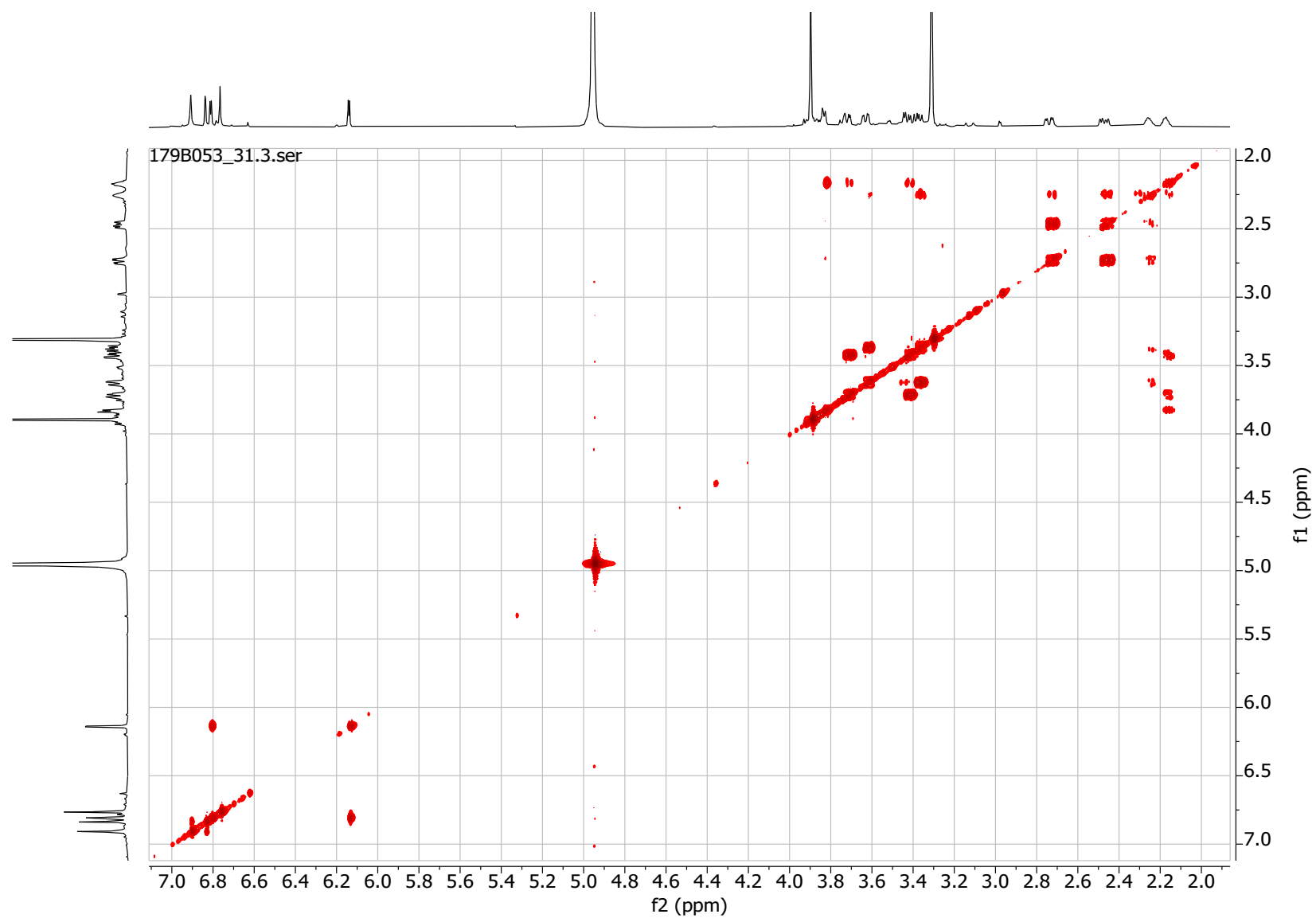


Figure S31. COSY spectrum of *N*(1)-methylisoageliferin (**4**) in CD₃OD.

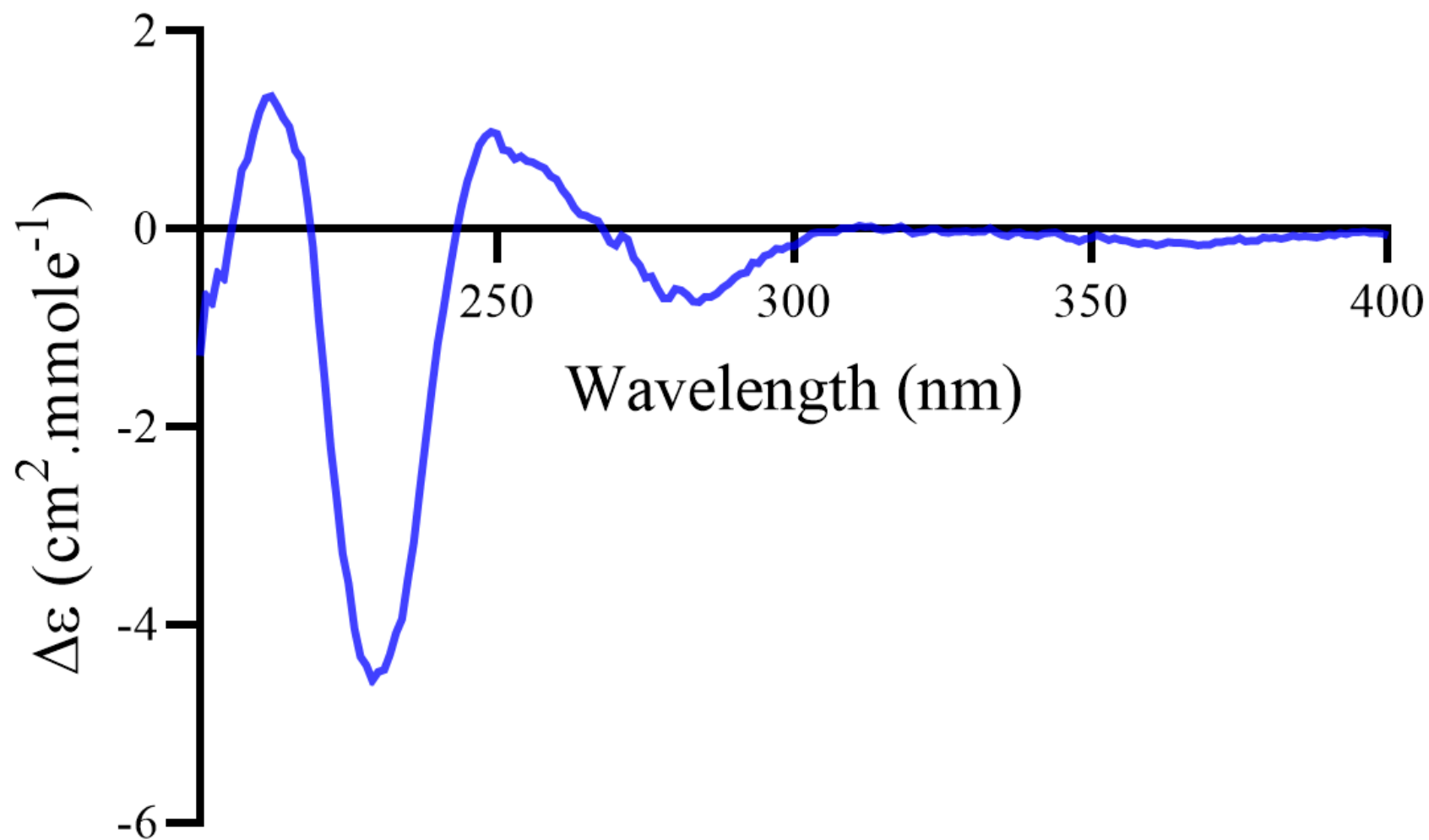


Figure S32. ECD spectrum of *N*(1)-methylisoageliferin (**4**).

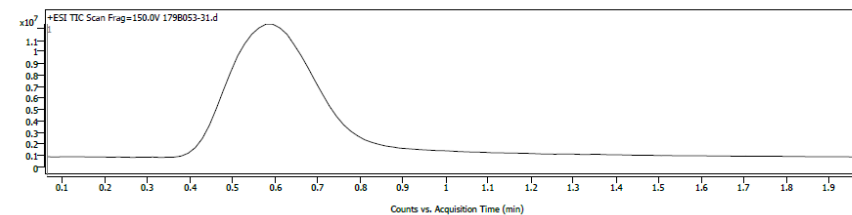
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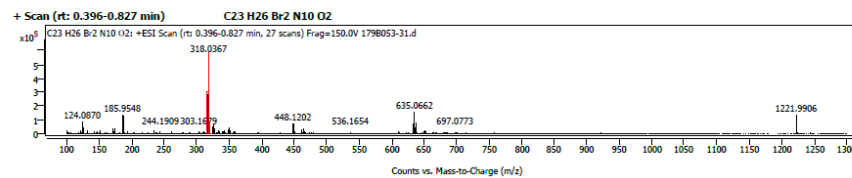
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Instrument	Instrument 1	Method Path (Acq)	C:\Users\admin\Desktop\methods\FIA_SM_LowFlow.m
MS Type	QTOF	Version (Acq SW)	6200 series TOF/6500 series Q-TOF B.09.00 (B9044.1 SP1)
Inj. Vol. (ul)	1	IRM Status	Success
Position	Vial 1	Method Path (DA)	C:\Users\Public\Desktop\QTOF\data\Data\1798053-31.d\Results\Qual\Version4\Default.m
Plate Pos.		Target Source Path	
Operator		Result Summary	

Sample Chromatograms



Sample Spectra



Spectrum Identification Table

Best ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes_MFG		C23 H26 Br2 N10 O2	(M+2H)+2	317.0374	-0.52		99.63			99.63	

MassHunter Qual 10.0
(End of Report)

Figure S33. HRESIMS spectrum of *N*(1)-methylisoageliferin (**4**).

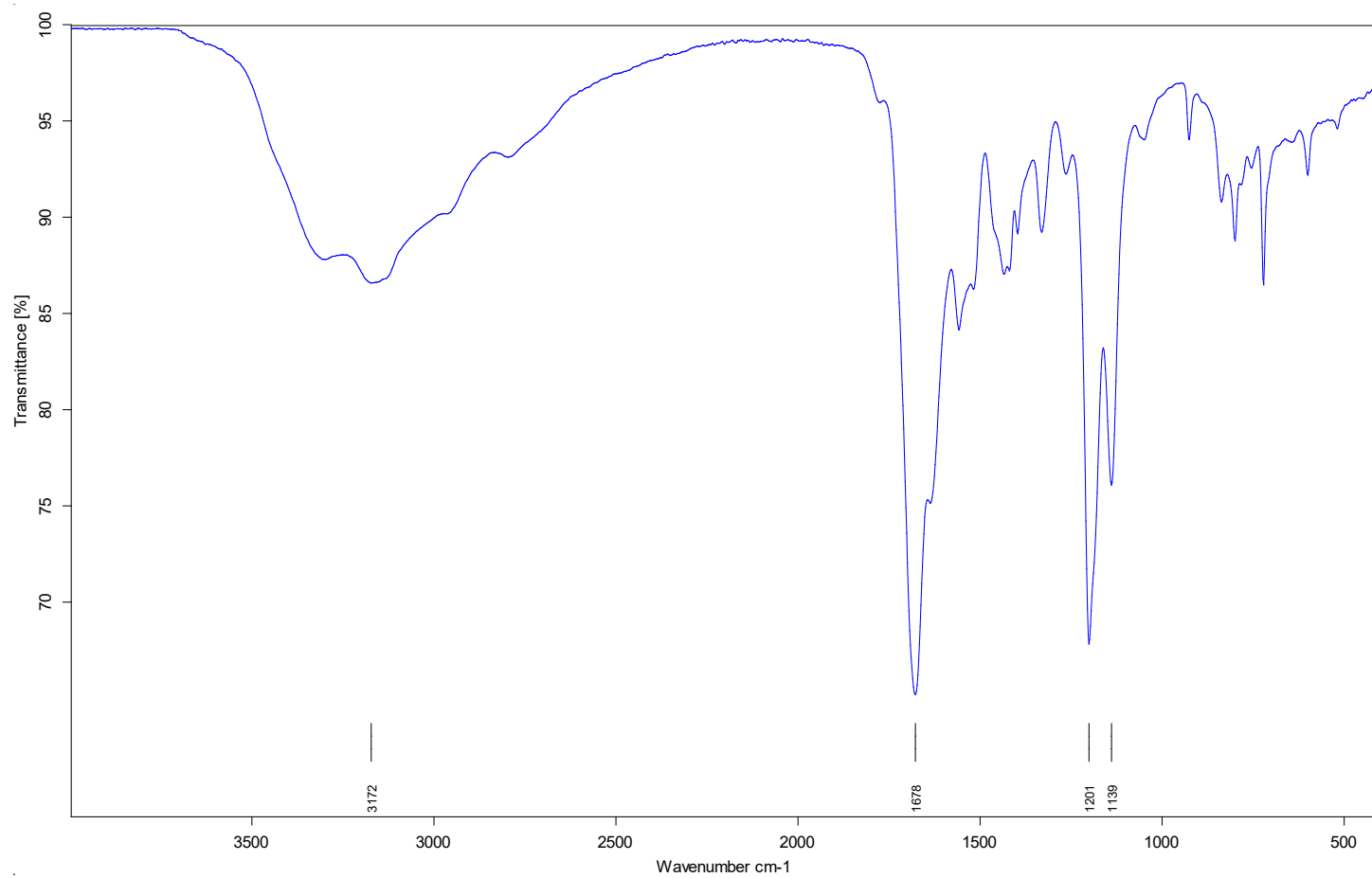


Figure S34. IR spectrum (neat) of *N*(1)-methyloageliferin (**4**).

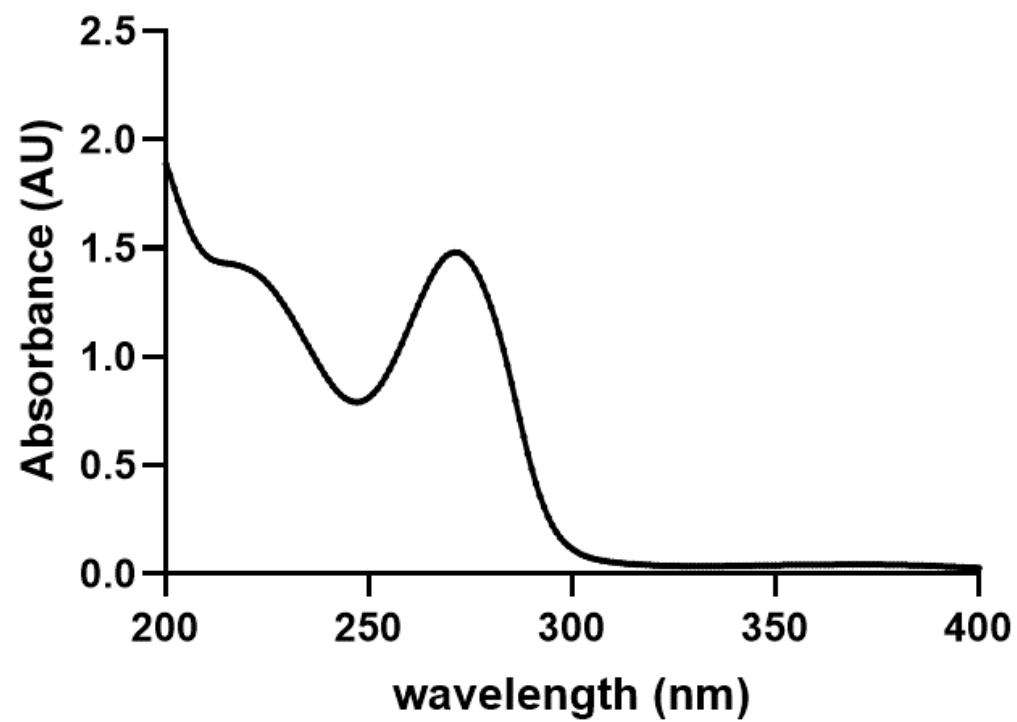


Figure S35. UV spectrum of *N*(1)-methylisoageliferin (**4**).

Table 1 ¹H NMR (600 MHz) data for compounds **5-10** in CD₃OD (*J* in Hz).

position	5	6	7	8	9	10
2	6.97, d (1.5)	6.95, d (1.5)	6.93, d (1.5)	6.92, d (1.5)		6.97, d (1.5)
2'				6.92, d (1.5)	6.91, d (1.5)	
3'	6.22, d (4.0)	6.93, d (1.5)	6.21, d (4.0)			
4	7.25, d (1.5)	7.26, d (1.5)	7.15, d (1.5)	7.12, d (1.5)	7.30, s	7.22, d (1.5)
4'	6.85, d (4.0)	6.81, d (1.5)	6.86, d (4.0)	6.87, d (1.5)	6.81, d (1.5)	6.94, s
8a	3.94, dd (14.8, 3.2)	3.95, dd (14.8, 3.2)	3.93, dd (14.8, 3.2)	3.97, dd (14.8, 3.2)	3.96, dd (14.8, 3.2)	3.95, dd (14.8, 3.2)
8b	3.47, dd (14.8, 4.3)	3.47, dd (14.8, 4.3)	3.43, dd (14.8, 4.3)	3.39, dd (14.8, 4.3)	3.47, dd (14.8, 4.3)	3.48, dd (14.8, 4.3)
8'a	3.87, dd (14.0, 2.7)	3.86, dd (14.0, 2.7)	3.86, dd (14.0, 2.7)	3.89, dd (14.0, 2.7)	3.87, dd (14.0, 2.7)	3.86, dd (14.0, 2.7)
8'b	3.07, dd (14.0, 9.6)	3.02, dd (14.0, 9.6)	3.06, dd (14.0, 9.6)	3.07, dd (14.0, 9.6)	3.02, dd (14.0, 9.6)	3.07, dd (14.0, 9.6)
9	2.03, m	1.99, m	2.04, m	2.03, m	2.00, m	2.02, m
9'	2.19, m	2.18, m	2.19, m	2.17, m	2.18, m	2.20, m
10	3.76, br d (8.5)	3.73, br d (8.5)	3.76, br d (8.5)	3.77, br d (8.5)	3.74, br d (8.5)	3.76, br d (8.5)
10'a	2.85, dd (16.3, 5.3)	2.84, dd (16.3, 5.3)	2.83, dd (16.3, 5.3)	2.82, dd (16.3, 5.3)	2.85, dd (16.3, 5.3)	2.85, dd (16.3, 5.3)
10'b	2.45, ddd (16.3, 9.0, 2.9)	2.42, ddd (16.3, 9.0, 2.9)	2.44, ddd (16.3, 9.0, 2.9)	2.44, ddd (16.3, 9.0, 2.9)	2.43, ddd (16.3, 9.0, 2.9)	2.43, ddd (16.3, 9.0, 2.9)
15	6.85, br s	6.85, br s	6.84, br s	6.85, br s	6.87, br s	6.85, br s
NMe			3.91, s	3.93, s		
N'Me	3.99, s	3.97, s	3.96, s	3.95, s	3.98, s	4.03, s

Table S2. ^{13}C NMR (150 MHz) data for compounds **5-10** in CD_3OD .

position	5	6	7	8	9	10
2	123.2, CH	123.2, CH	129.1, CH	129.0, CH	106.4, C	123.2, CH
2'	111.2, C	129.0, CH	111.2, C	129.0, CH	129.1, CH	112.9, C
3	97.6, C	97.6, C	95.5, C	95.4, C	100.0, C	97.6, C
3'	111.6, CH	95.5, C	111.5, CH	95.4, C	95.5, C	99.1, C
4	114.3, CH	114.3, CH	116.4, CH	116.3, CH	115.2, CH	114.2, CH
4'	114.7, CH	115.8, CH	114.6, CH	115.8, CH	115.8, CH	116.0, CH
5	127.1, C	127.2, C	127.0, C	127.0, C	128.4, C	127.3, C
5'	127.9, C	127.2, C	127.9, C	127.0, C	127.0, C	128.5, C
6	163.3, C	163.3, C	163.9, C	163.5, C	162.5, C	163.3, C
6'	164.0, C	163.6, C	163.9, C	163.8, C	163.6, C	163.0, C
8	38.9, CH_2	38.8, CH_2	38.9, CH_2	39.1, CH_2	38.8, CH_2	39.0, CH_2
8'	43.0, CH_2	43.0, CH_2	42.9, CH_2	42.8, CH_2	43.0, CH_2	43.0, CH_2
9	44.2, CH	44.3, CH	44.2, CH	43.9, CH	44.2, CH	44.2, CH
9'	37.5, CH	37.5, CH	37.5, CH	37.6, CH	37.5, CH	37.4, CH
10	33.4, CH	33.3, CH	33.5, CH	33.7, CH	33.4, CH	33.3, CH
10'	24.9, CH_2	24.9, CH_2	24.9, CH_2	25.0, CH_2	25.0, CH_2	24.8, CH_2
11	127.2, C	127.0, C	127.3, C	127.0, C	127.1, C	127.1, C
11'	123.0, C	123.0, C	123.0, C	122.9, C	123.0, C	123.0, C
13	149.2, C	149.2, C	149.2, C	149.3, C	149.2, C	149.2, C
13'	149.1, C	149.1, C	149.1, C	149.1, C	149.1, C	149.1, C

15	113.3, CH	113.4, CH	113.3, CH	113.3, CH	113.5, CH	113.3, CH
15'	119.4, C	119.5, C	119.5, C	119.6, C	119.5, C	119.4, C
NMe			37.2, CH ₃	37.4, CH ₃		
N'Me	35.3, CH ₃	37.7, CH ₃	35.4, CH ₃	37.7, CH ₃	37.7, CH ₃	36.7, CH ₃

Table S3. ¹³C NMR (150 MHz) data for compounds **3** and closely related clerodane diastereomers¹ in CDCl₃. ^a

position	3	<i>neo-cis-cis-</i> kolavenol	<i>ent-neo-cis-trans-</i> kolavenol	<i>neo-trans-trans-</i> kolavenol	<i>neo-trans-cis-</i> kolavenol
1	17.62	17.74	20.01	17.86	18.25
2	23.92	24.06	25.87	26.89	26.89
3	123.05	123.13	122.41	120.25	120.43
4	139.72	139.89	142.16	144.62	144.55
5	36.78	36.90	38.77	38.34	38.17
6	37.66	37.78	32.32	30.22	36.82
7	28.63	28.80	27.25	25.61	27.49
8	37.27	37.39	37.50	35.15	36.24
9	40.07	40.10	38.59	37.47	38.58
10	44.49	44.66	44.63	45.19	46.40
11	36.28	36.50	36.03	37.86	36.71
12	32.87	32.73	33.42	32.75	32.81
13	148.55	141.11	141.35	141.33	141.02
14	114.27	122.85	122.67	122.70	122.73
15	46.08	59.51	59.48	59.50	59.48
16	16.69	16.53	16.60	16.59	16.55
17	15.83	15.93	15.38	14.86	15.99
18	19.73	19.73	19.35	18.07	18.01
19	33.00	33.08	27.71	20.61	19.94

20	17.16	17.28	26.41	20.42	18.39
2'	146.85				
3' N-Me	36.25				
4'	148.41				
5'	111.97				
6'	152.35				
8'	144.48				
10'N-Me	29.06				

a. Chemical shifts were calibrated against known chloroform signals (δ_C 77.0 ppm)

Reference:

(1) Pelot, K. A.; Hagelthorn, D. M.; Hong, Y. J.; Tantillo, D. J.; Zerbe, P. Diterpene synthase-catalyzed biosynthesis of distinct clerodane stereoisomers. *ChemBioChem* **2019**, 20, 111-117.