Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Heronamides with unreported skeletons from a deep-sea *Streptomyces*: discovery and biosynthesis

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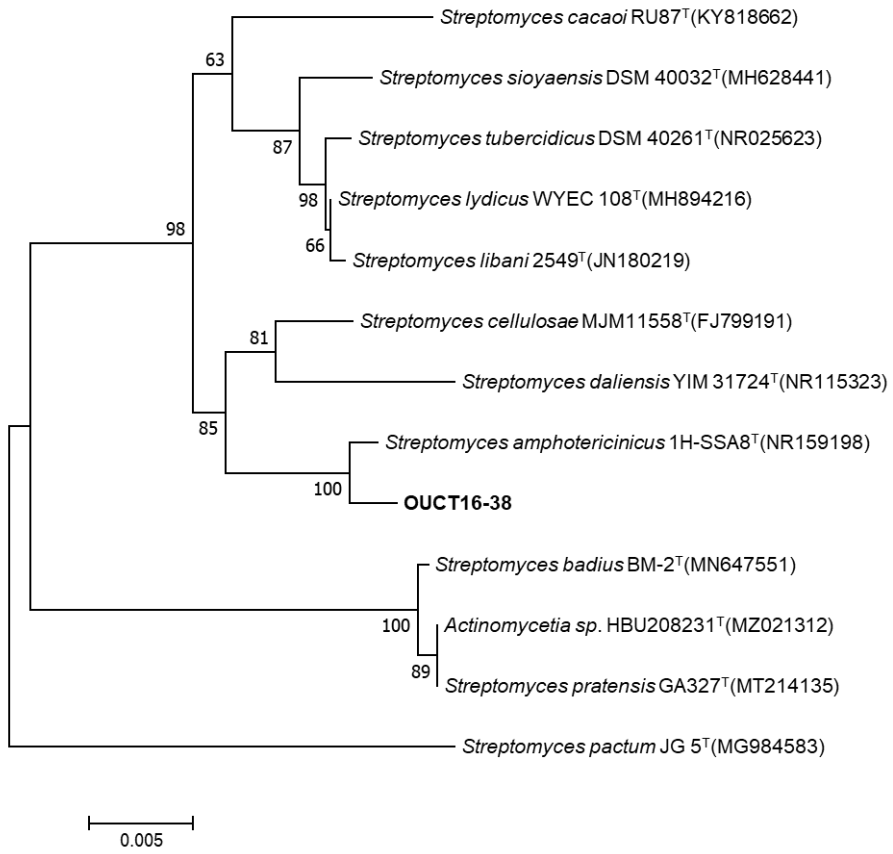

Figure S1. Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequence of the OUCT16-38 strain

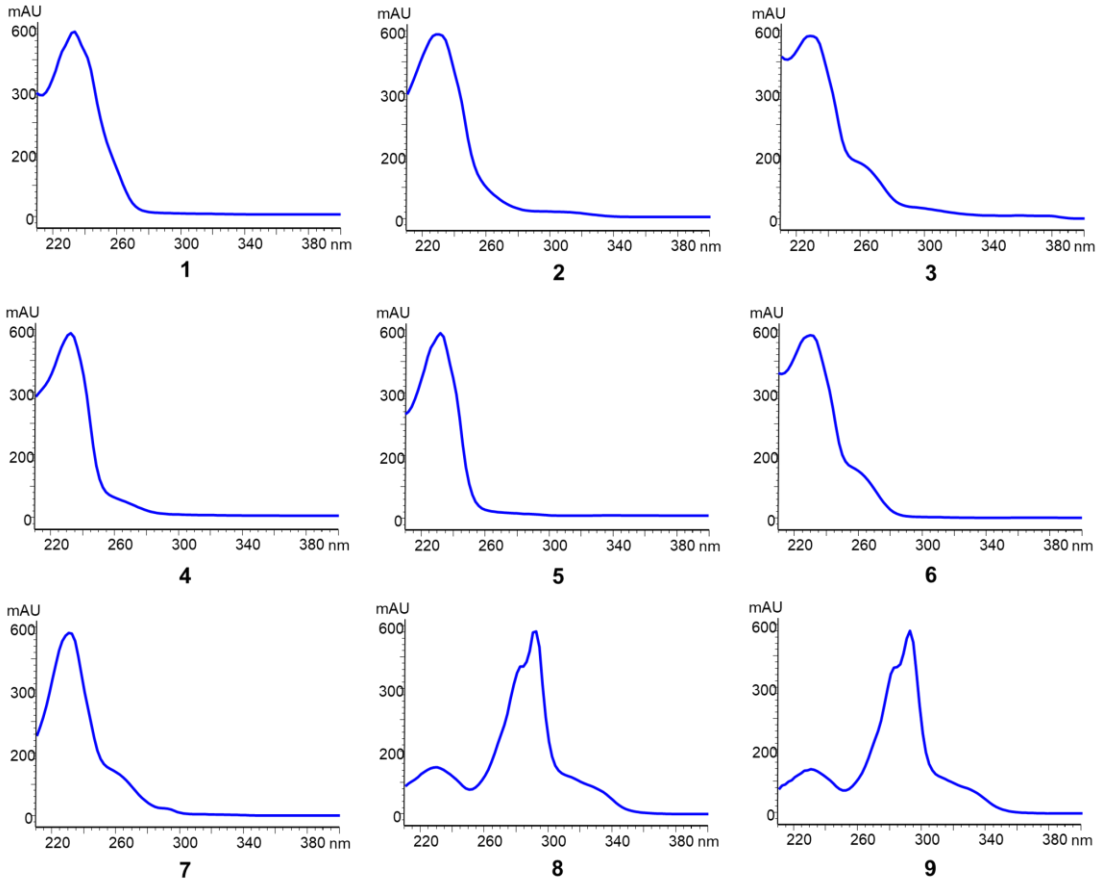

Figure S2. UV spectra of **1**−**9**

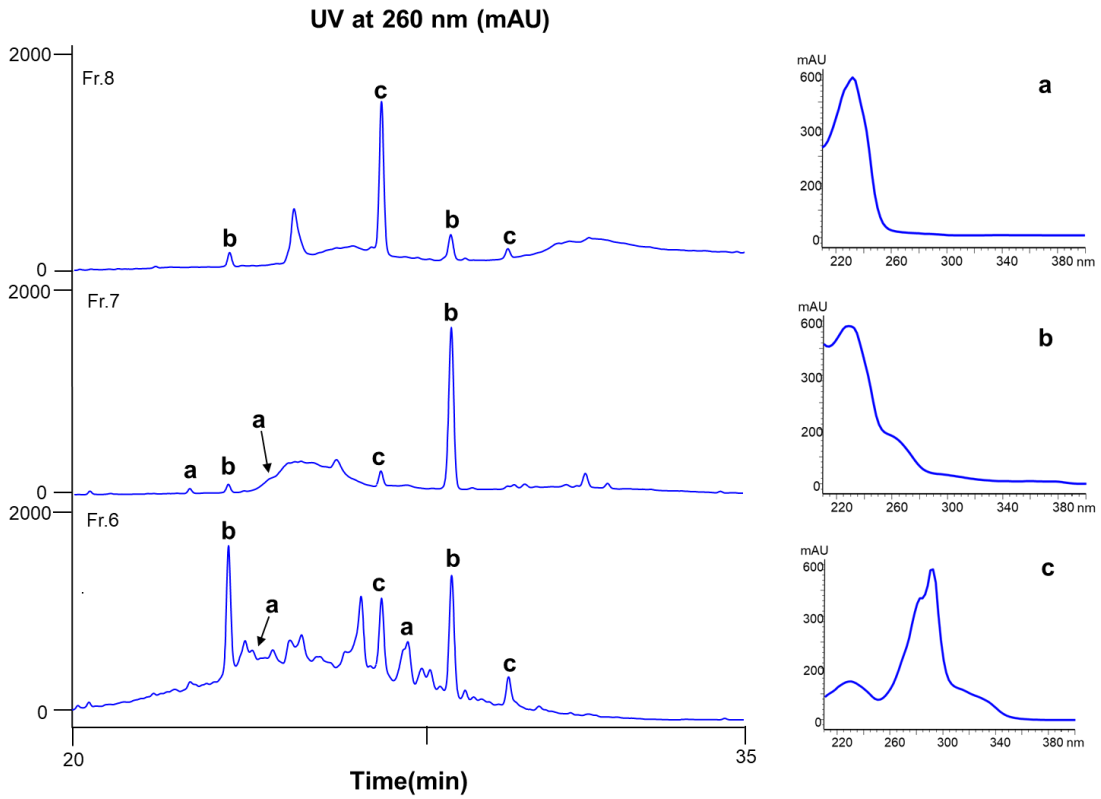

Figure S3. HPLC analysis of Fr.6~Fr.8. Peaks displaying heronamide-type UV spectra were labeled.

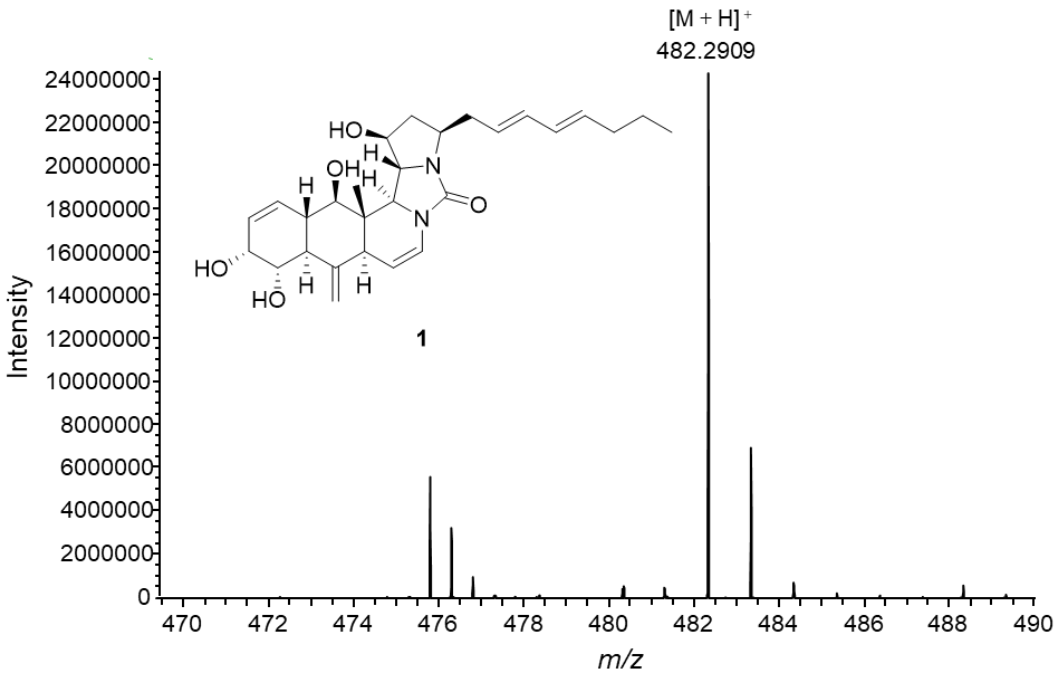

Figure S4. HRESIMS spectrum of **1**

Figure S5. IR spectrum of **1**

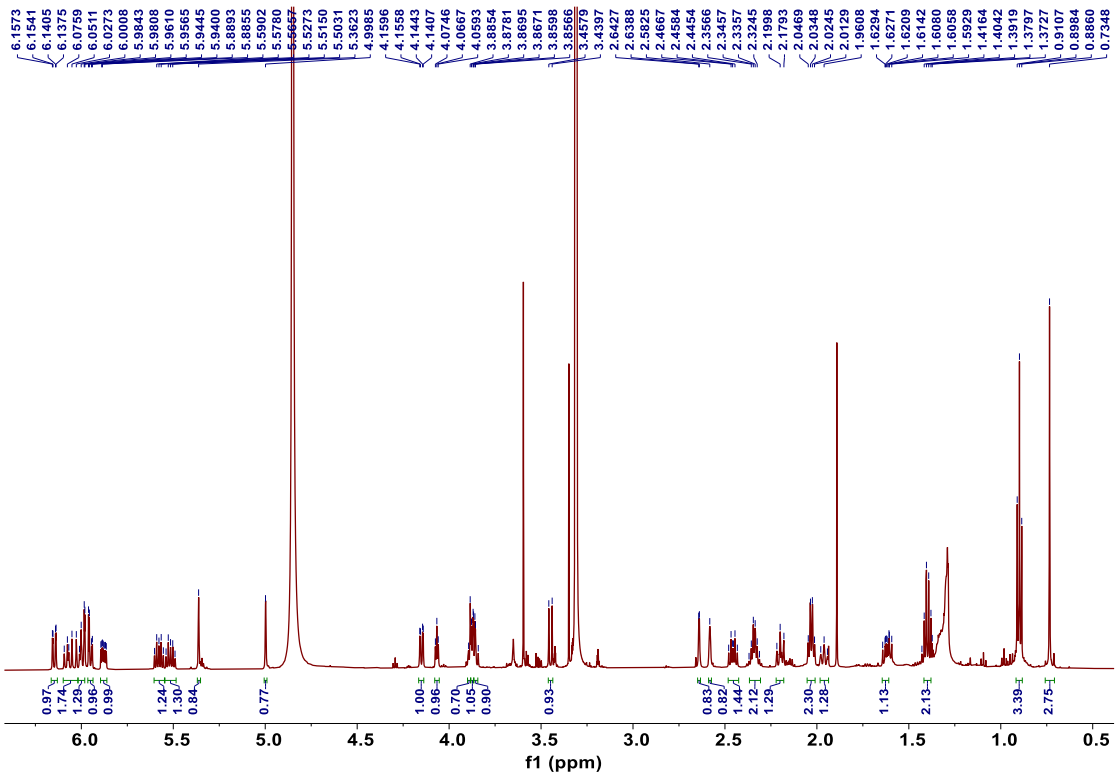

Figure S6. ^{1}H NMR spectrum of **1** in CD_3OD (600 MHz)

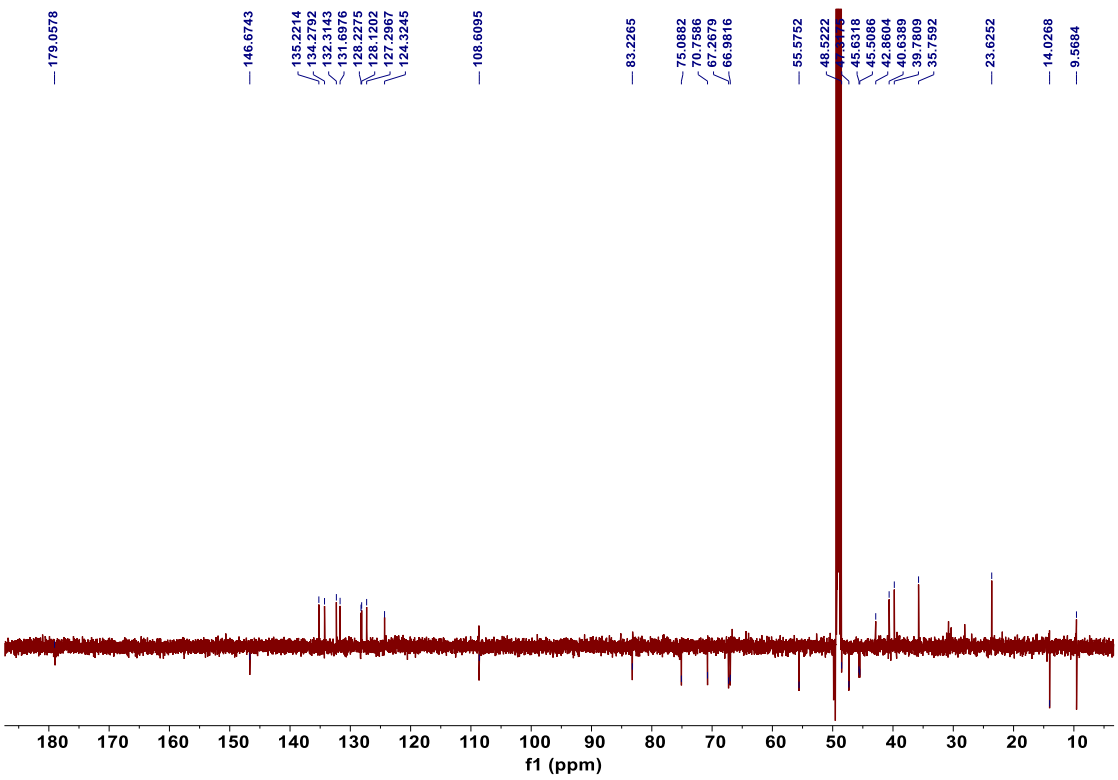

Figure S7. DEPTQ spectrum of **1** in CD_3OD (150 MHz)

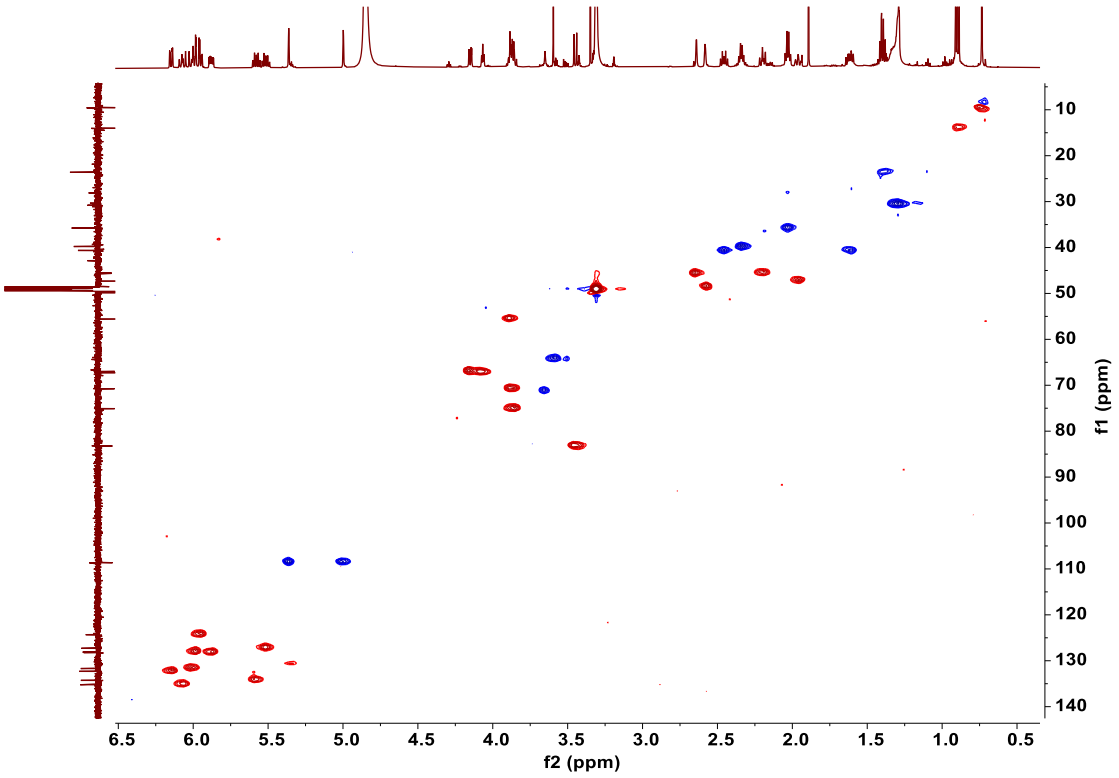

Figure S8. HSQC spectrum of **1** in CD_3OD (600 MHz)

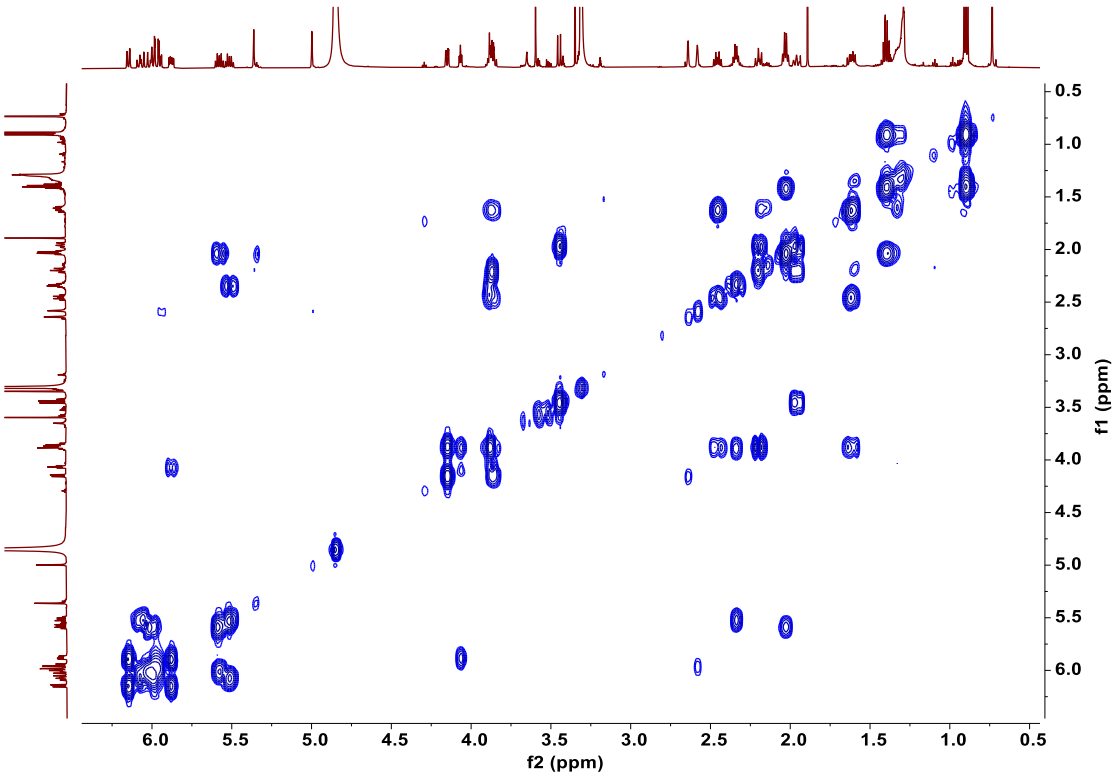

Figure S9. COSY spectrum of **1** in CD_3OD (600 MHz)

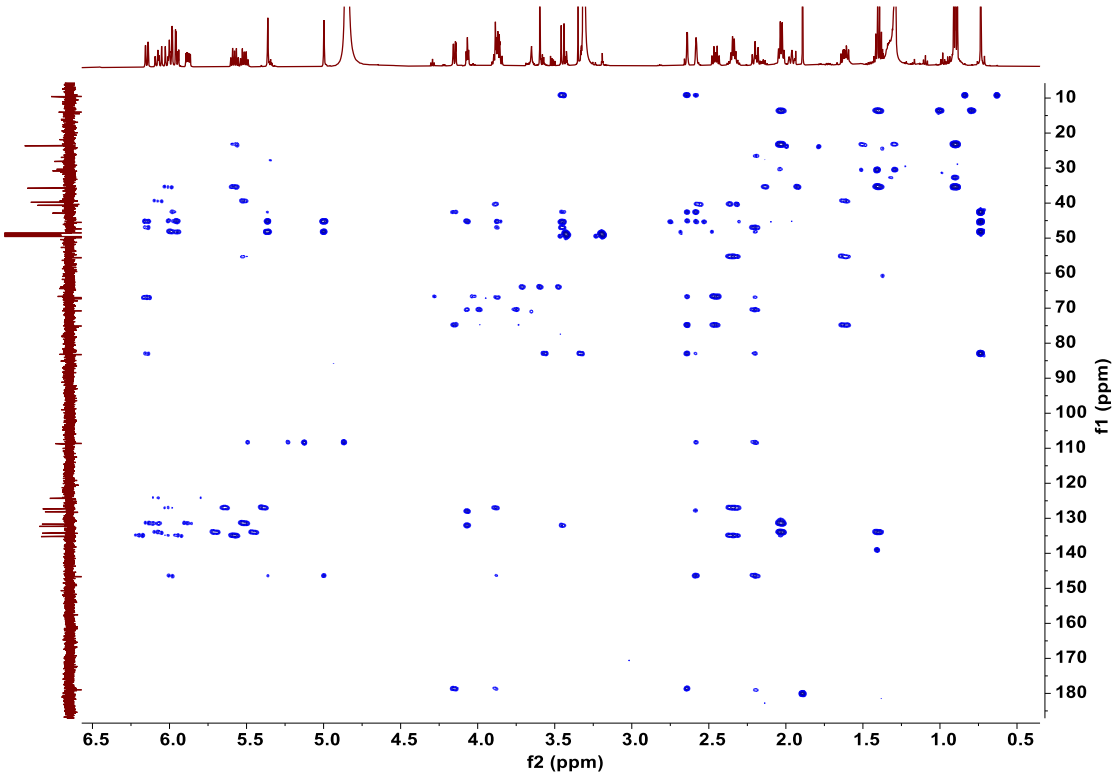

Figure S10. HMBC spectrum of **1** in CD_3OD (600 MHz)

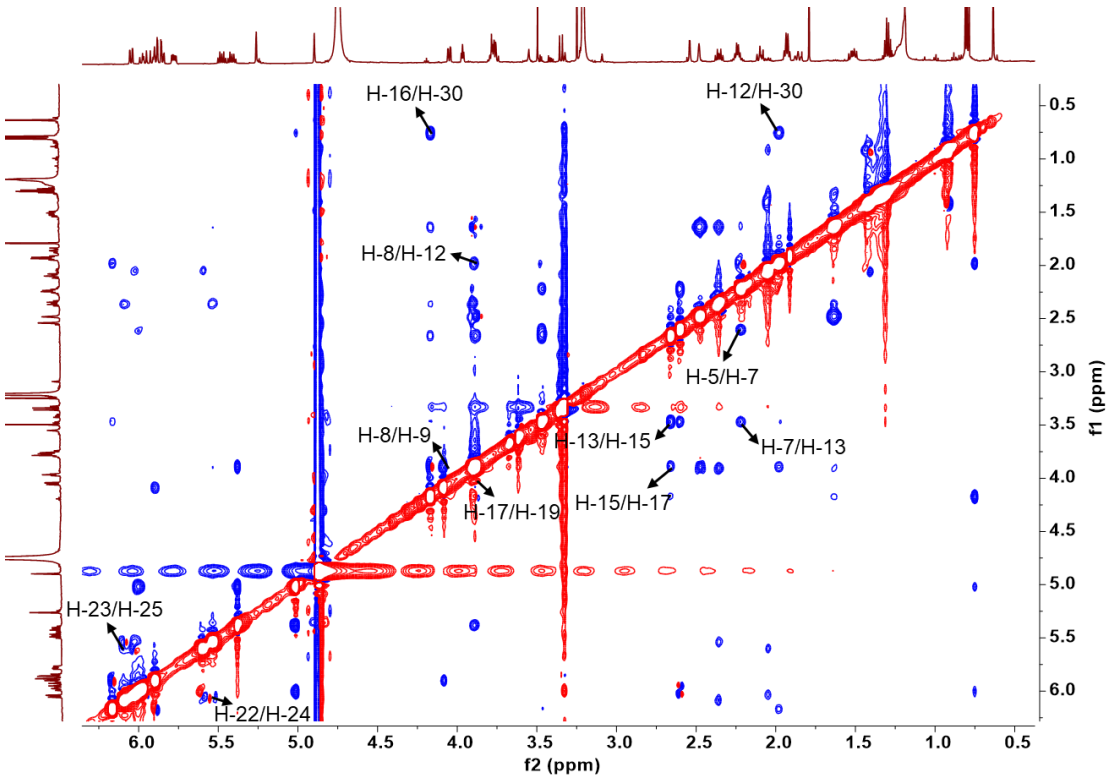

Figure S11. NOESY spectrum of **1** in CD_3OD (600 MHz)

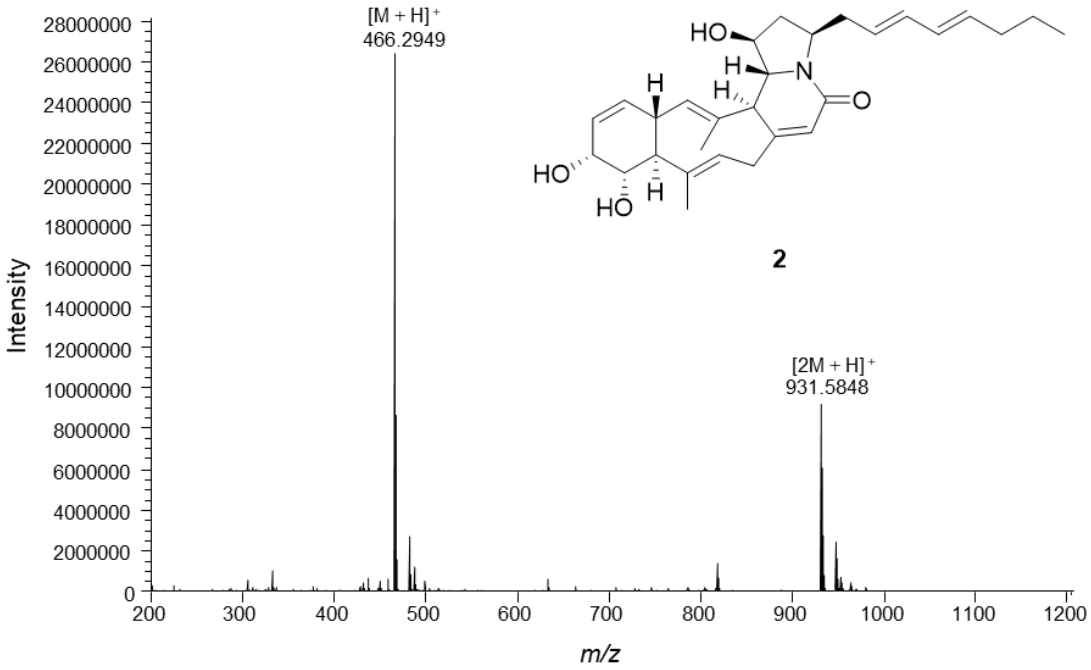

Figure S12. HRESIMS spectrum of **2**

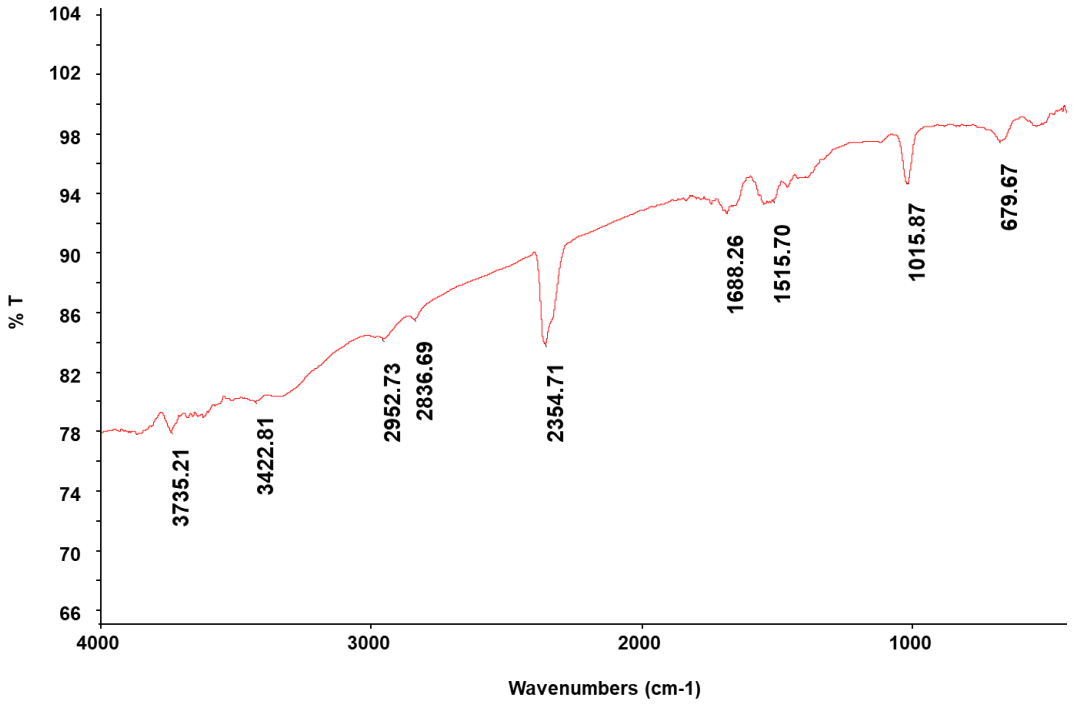

Figure S13. IR spectrum of **2**

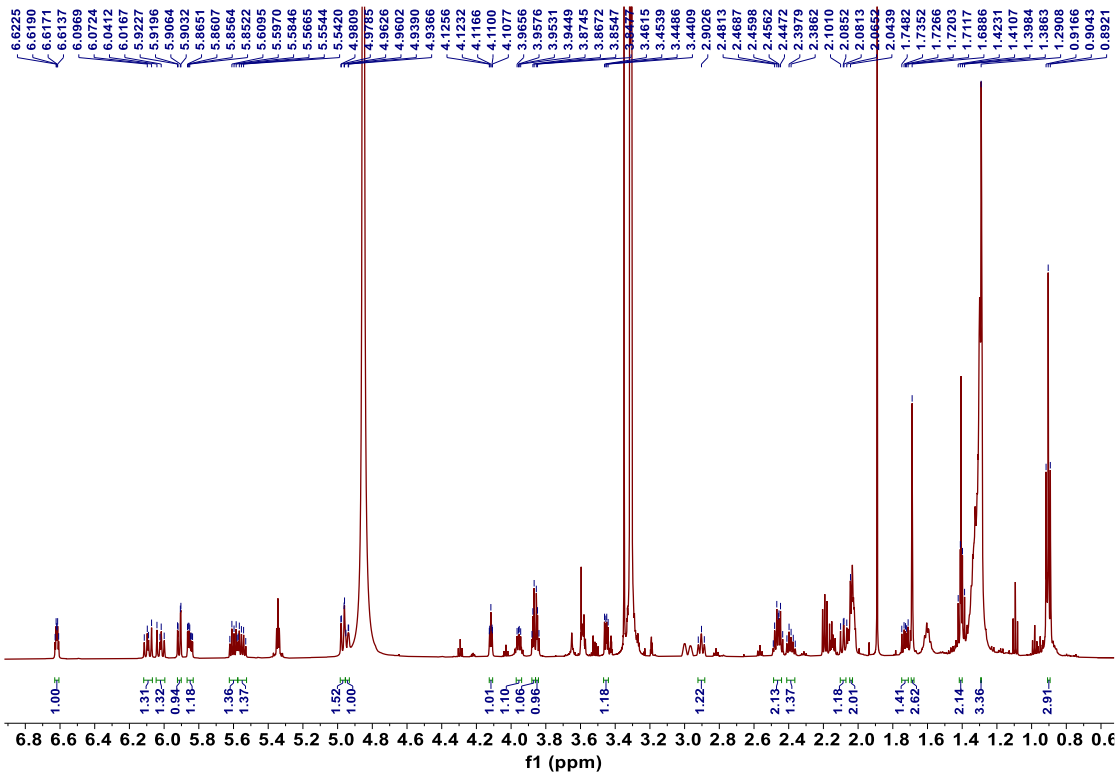

Figure S14. ^{1}H NMR spectrum of **2** in CD_3OD (600 MHz)

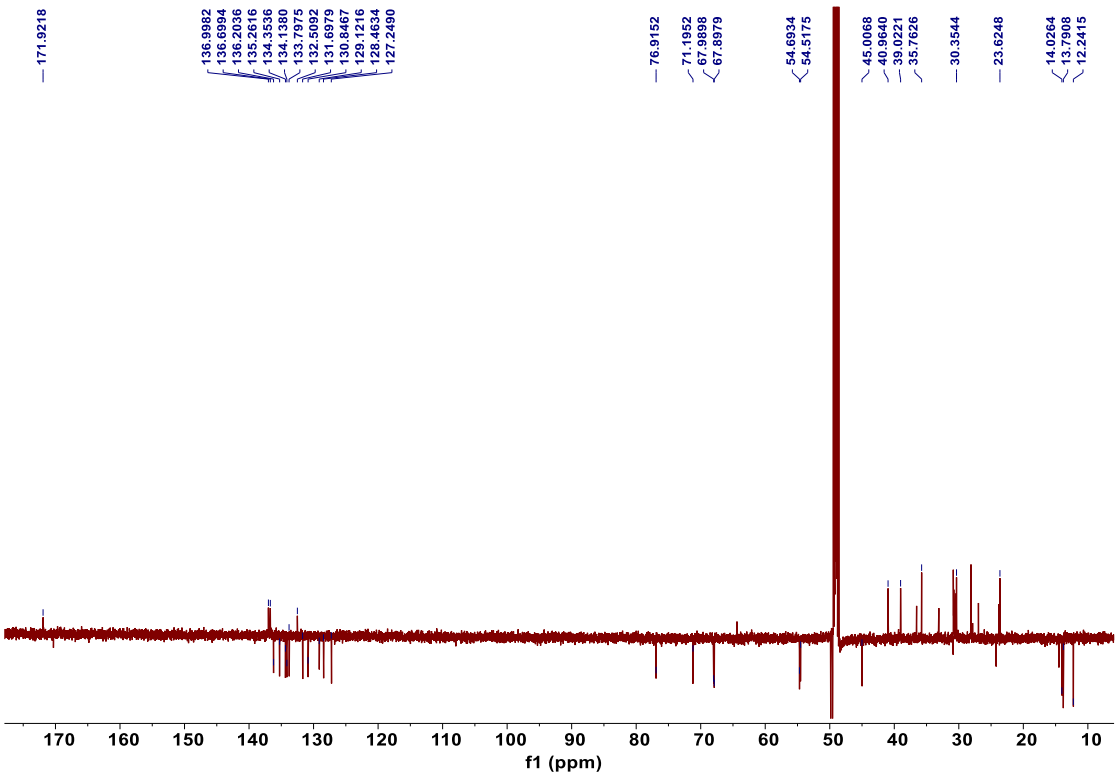

Figure S15. DEPTQ spectrum of **2** in CD_3OD (150 MHz)

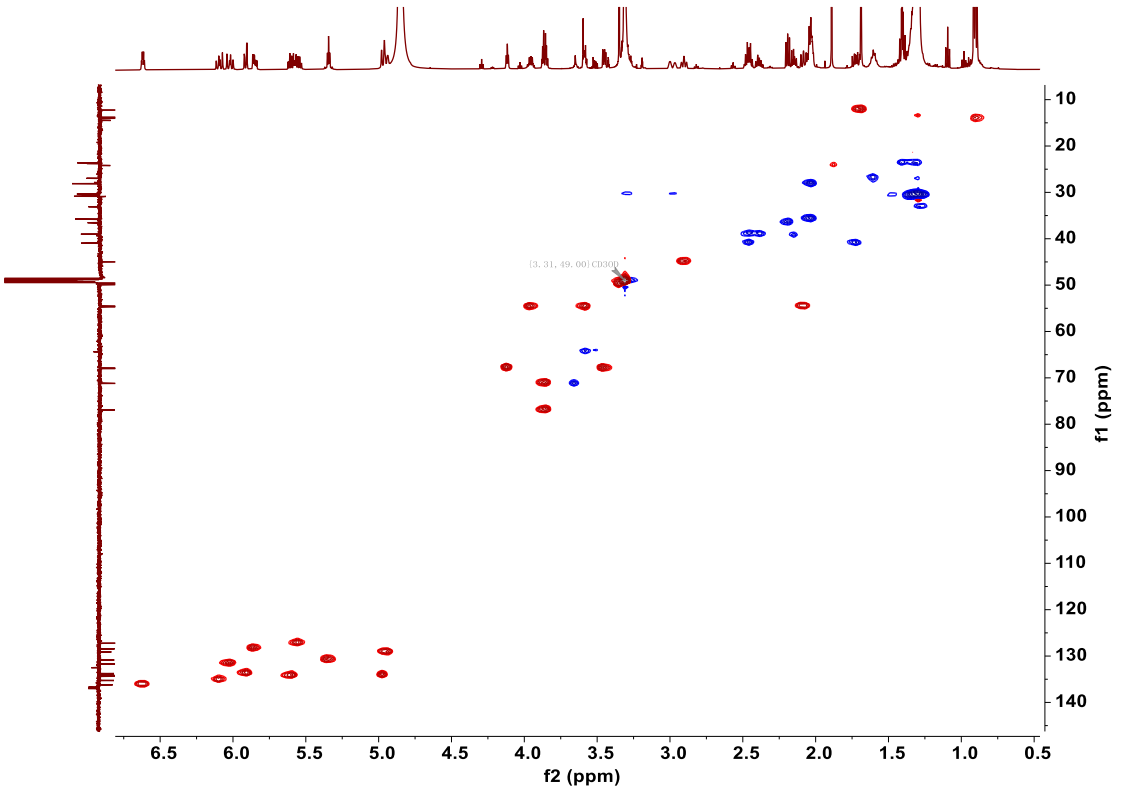

Figure S16. HSQC spectrum of **2** in CD_3OD (600 MHz)

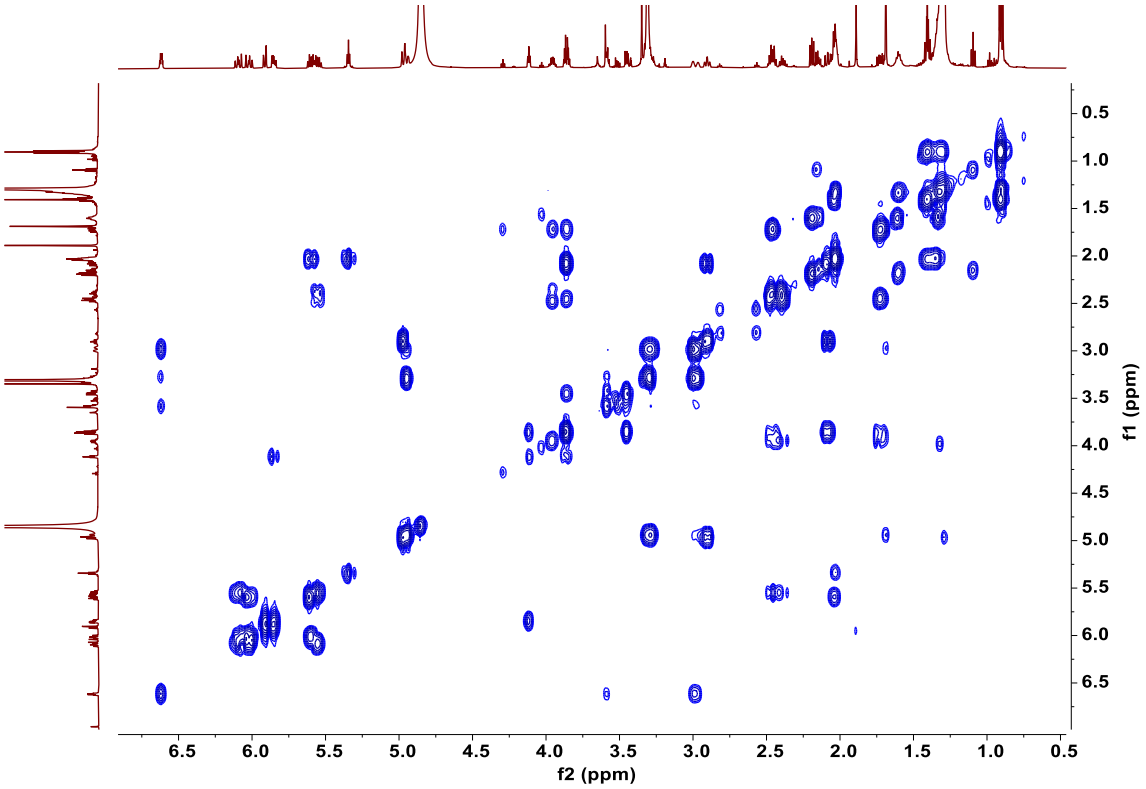

Figure S17. COSY spectrum of **2** in CD_3OD (600 MHz)

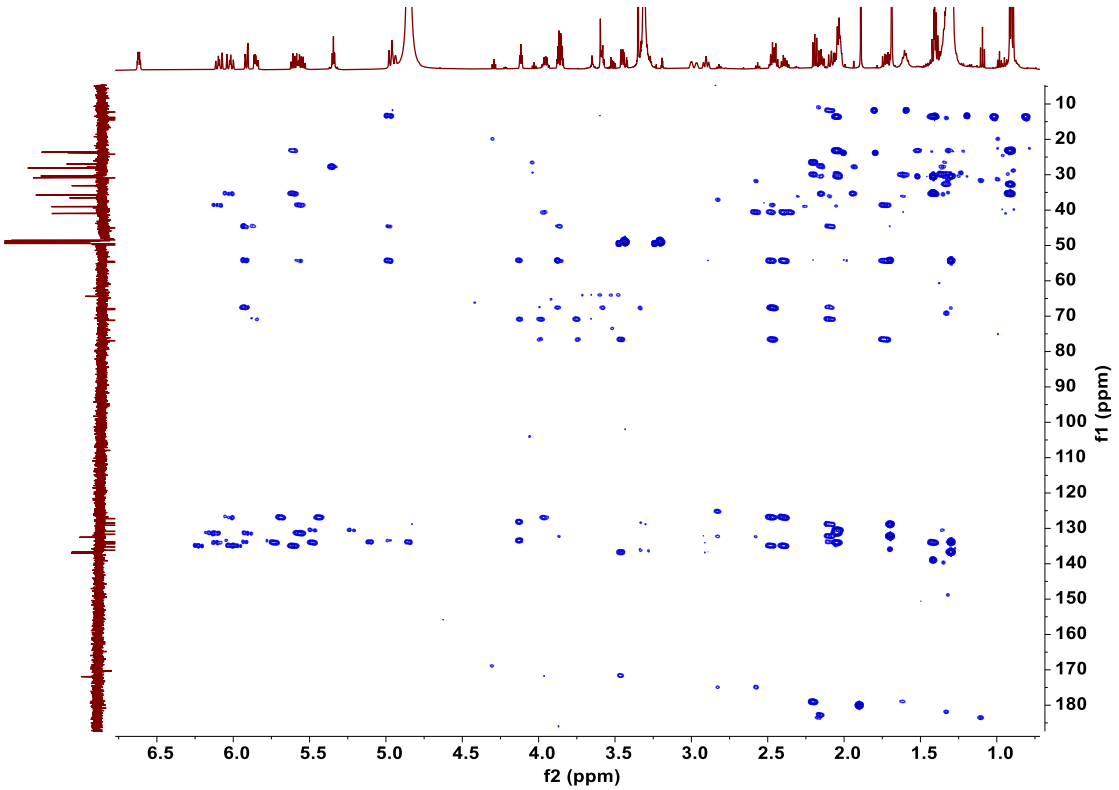

Figure S18. HMBC spectrum of **2** in CD_3OD (600 MHz)

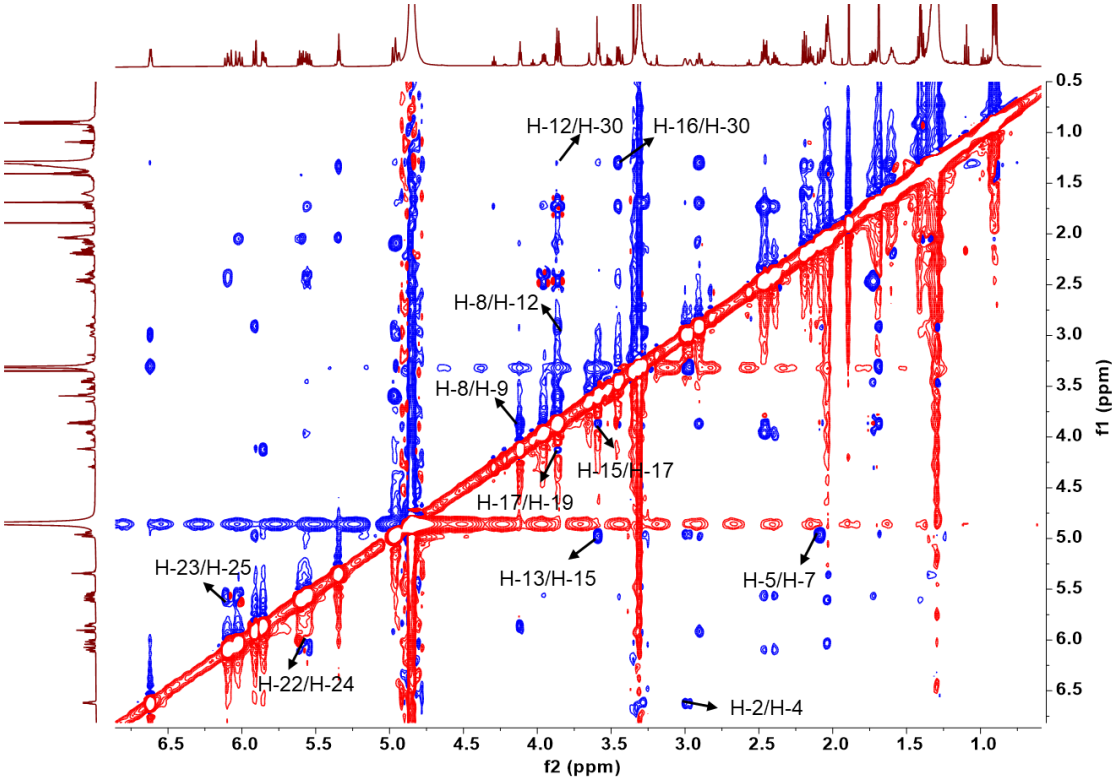

Figure S19. NOESY spectrum of **2** in CD_3OD (600 MHz)

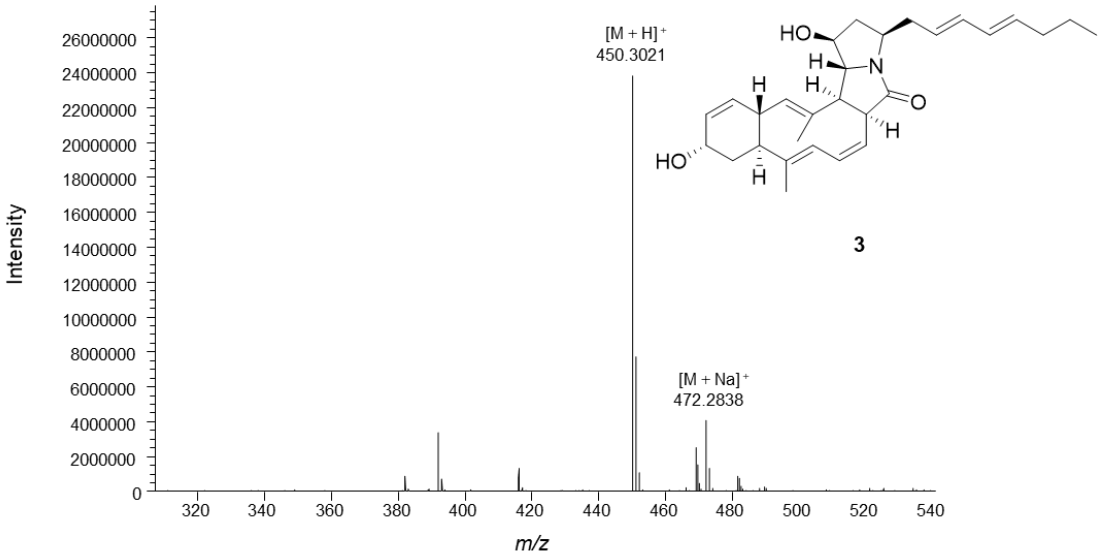

Figure S20. HRESIMS spectrum of **3**

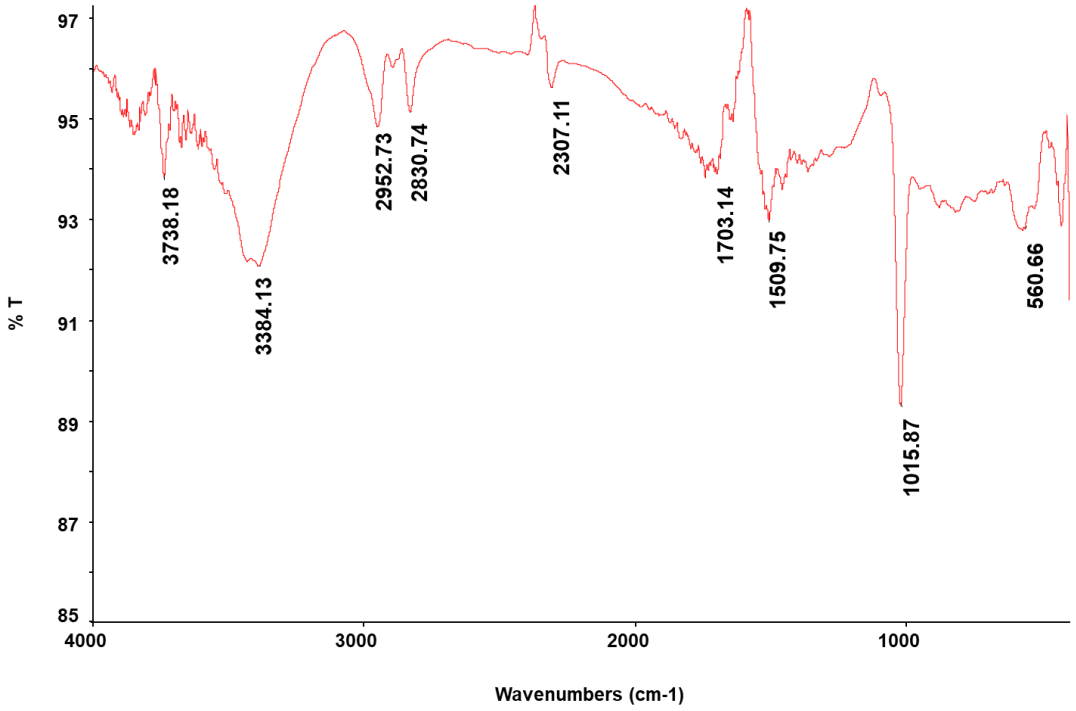

Figure S21. IR spectrum of **3**

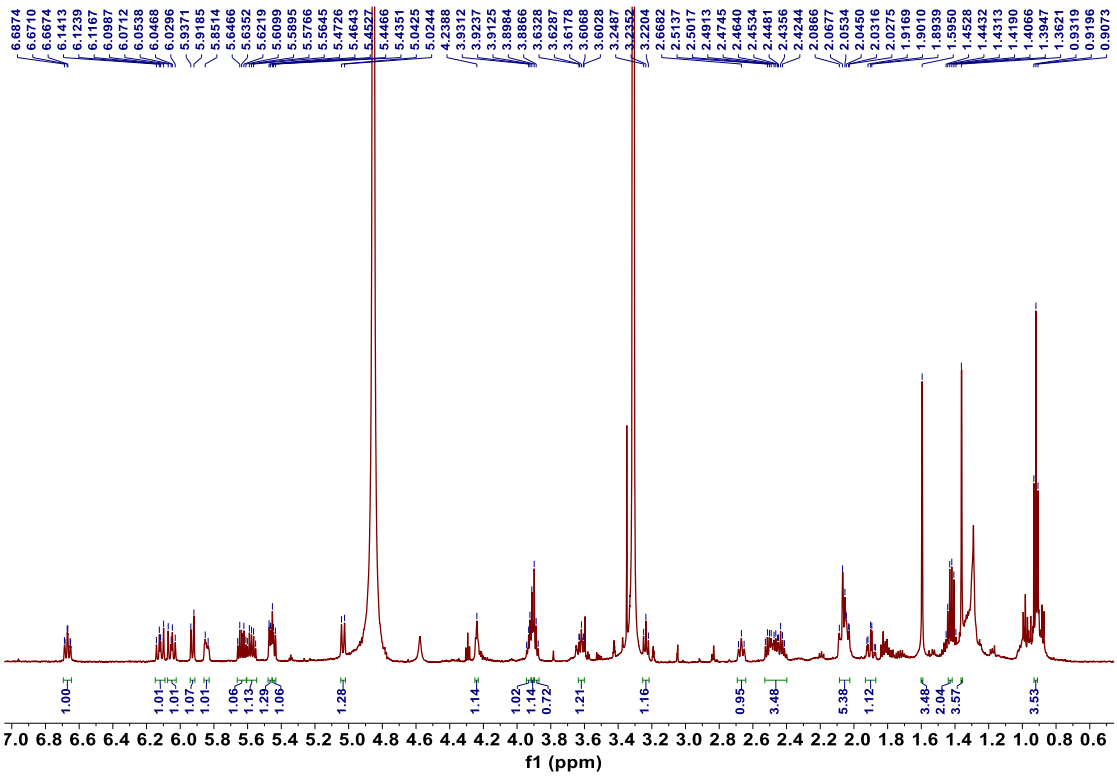

Figure S22. ^{1}H NMR spectrum of **3** in CD_3OD (600 MHz)

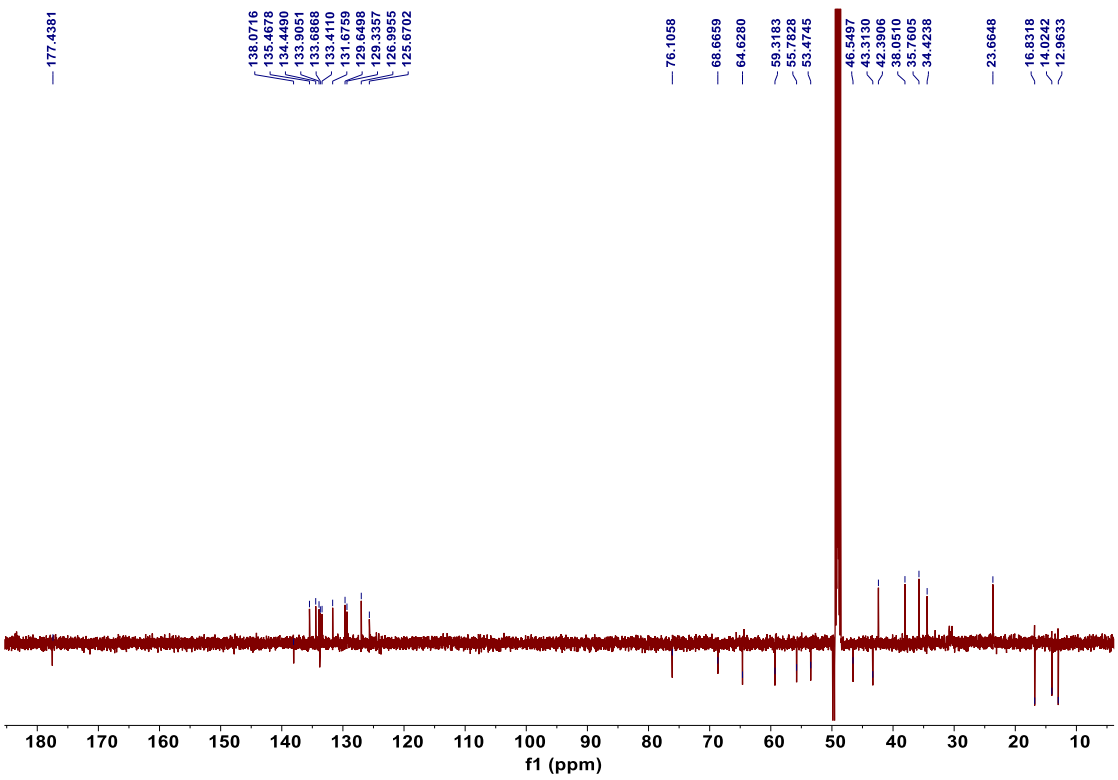

Figure S23. DEPTQ spectrum of **3** in CD_3OD (150 MHz)

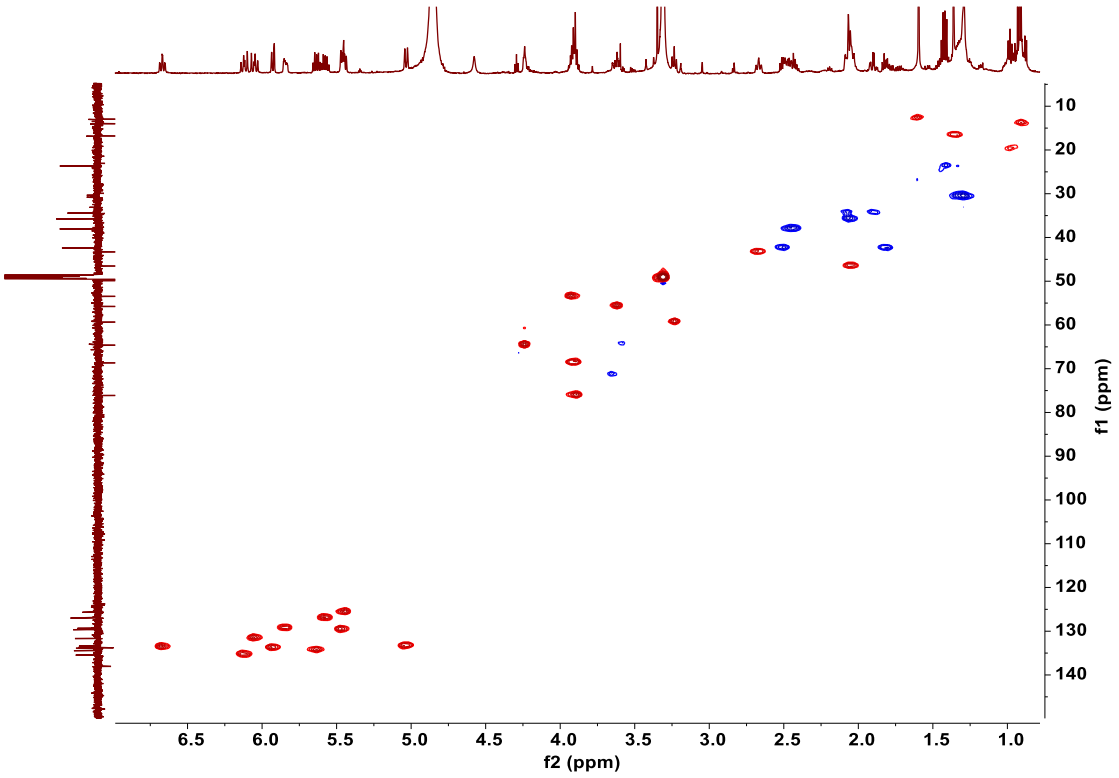

Figure S24. HSQC spectrum of **3** in CD_3OD (600 MHz)

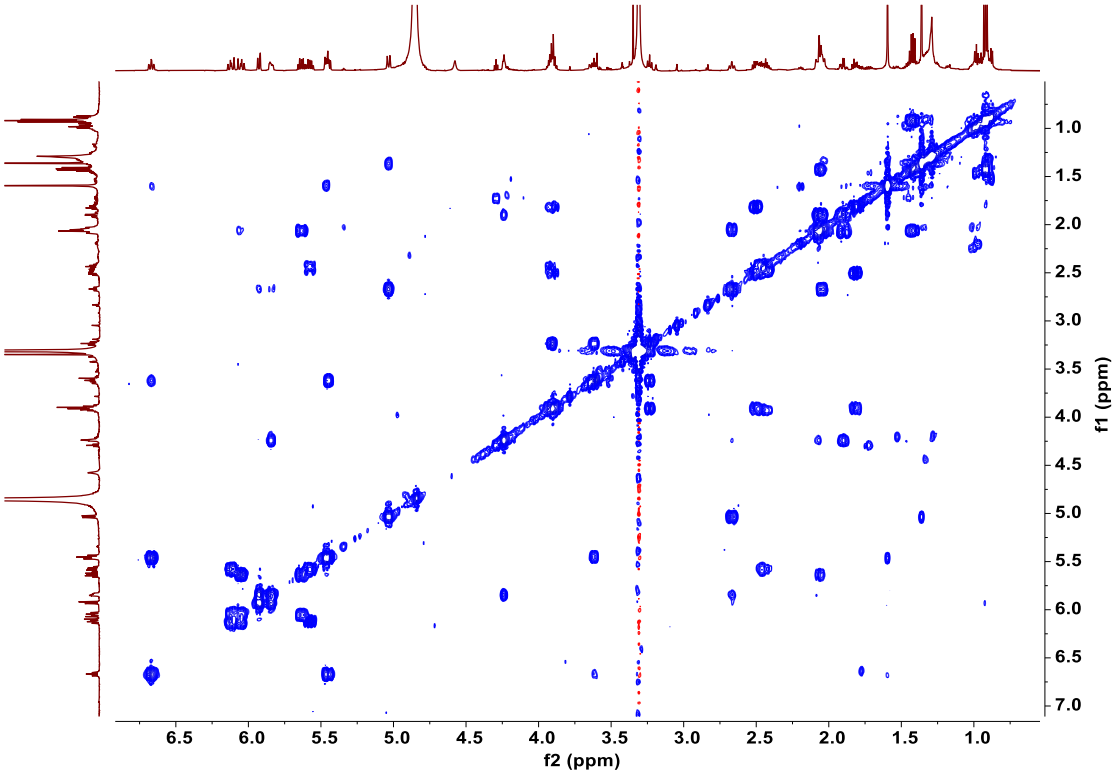

Figure S25. COSY spectrum of **3** in CD_3OD (600 MHz)

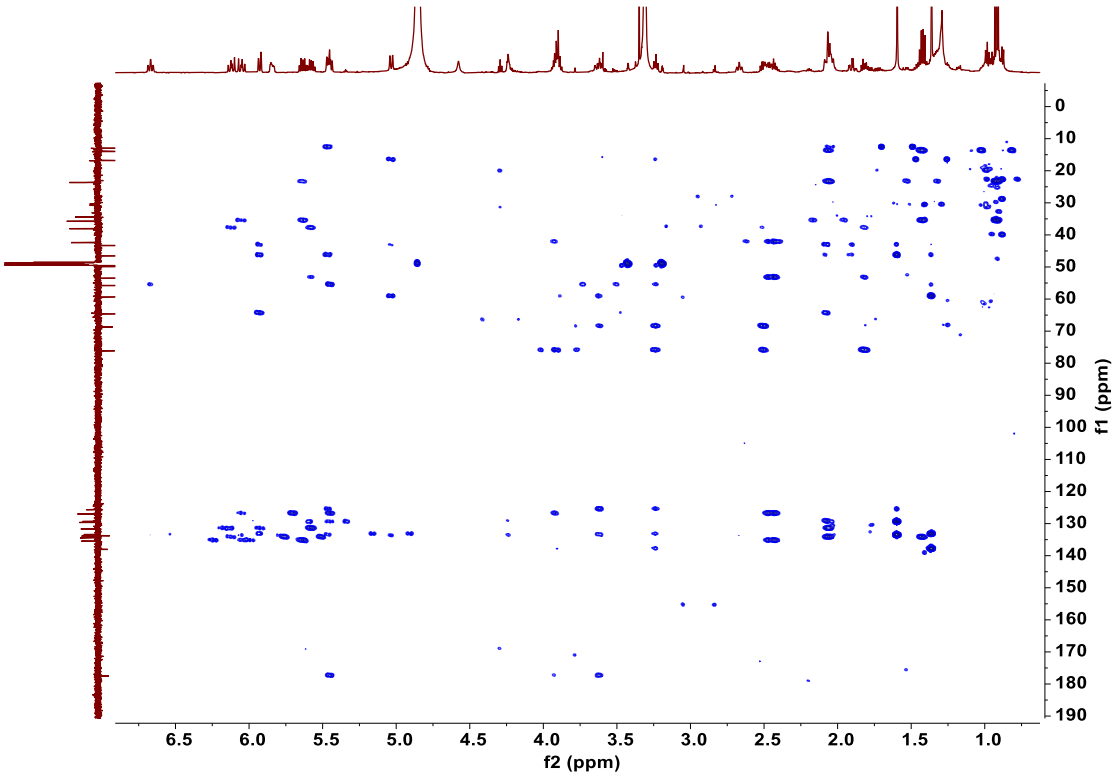

Figure S26. HMBC spectrum of **3** in CD_3OD (600 MHz)

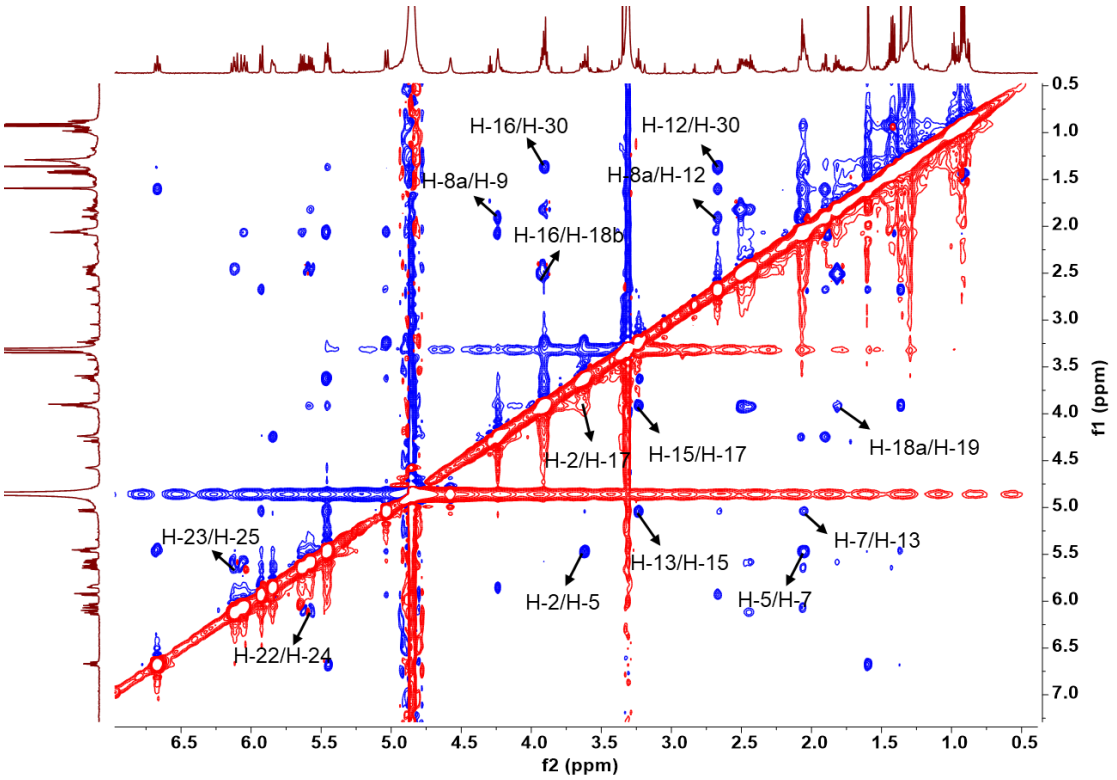

Figure S27. NOESY spectrum of **3** in CD_3OD (600 MHz)

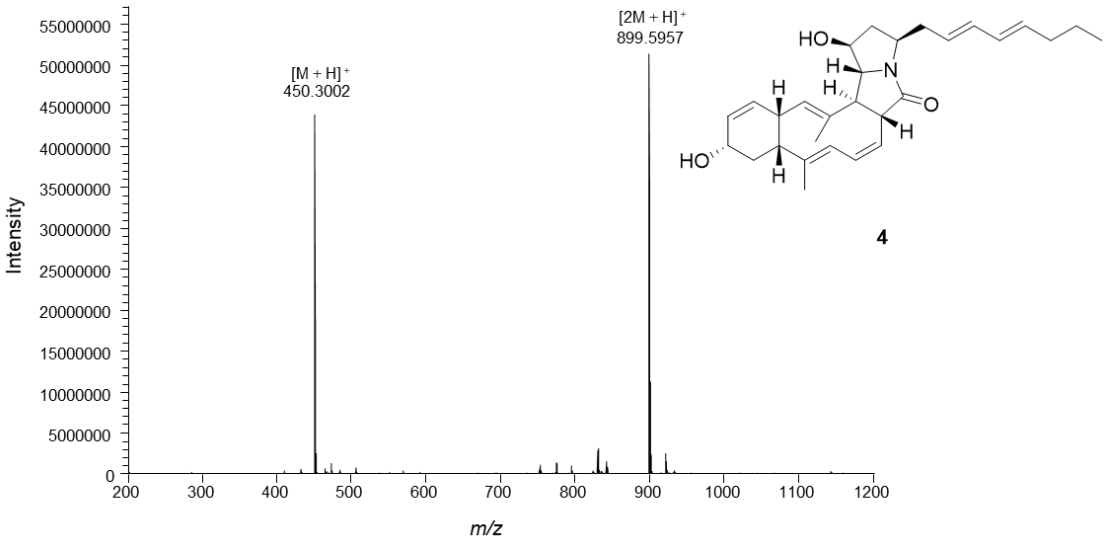

Figure S28. HRESIMS spectrum of **4**

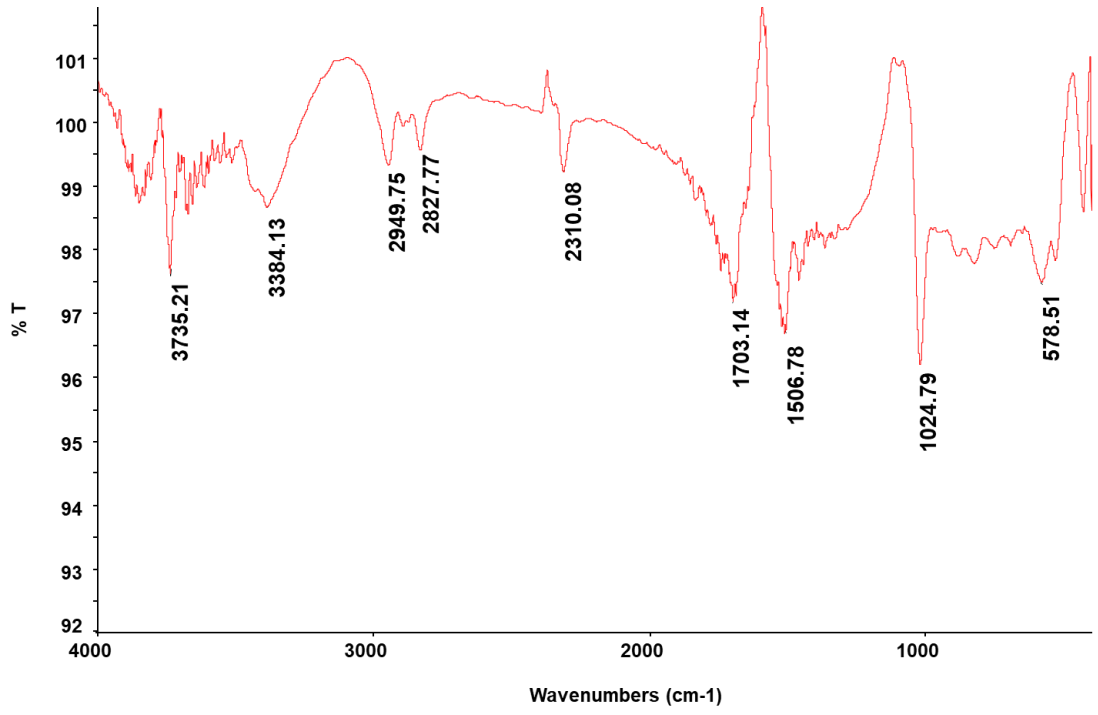

Figure S29. IR spectrum of **4**

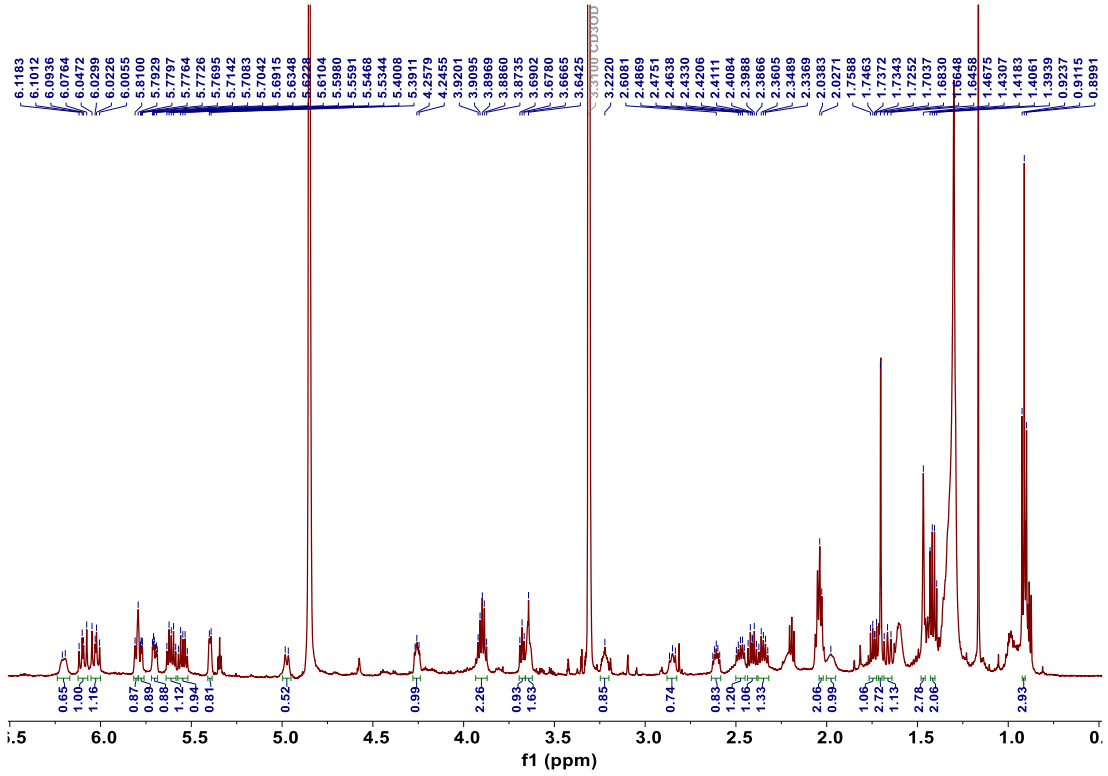

Figure S30. ^{1}H NMR spectrum of **4** in CD_3OD (600 MHz)

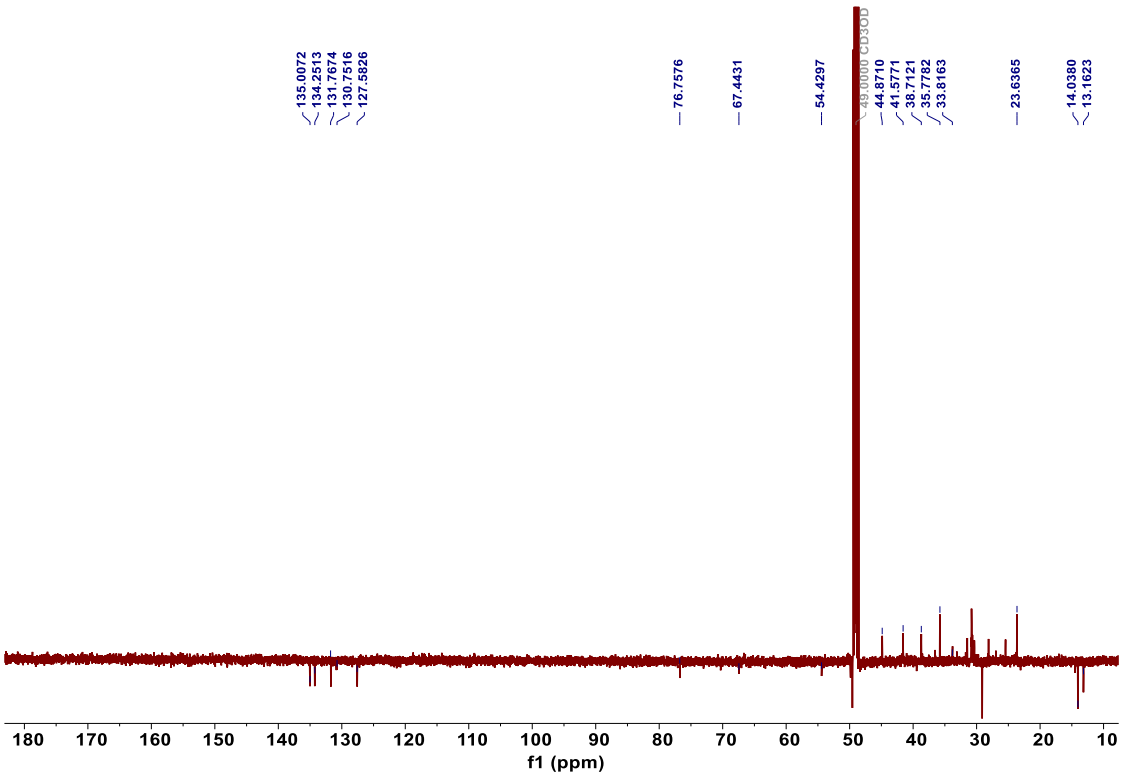

Figure S31. DEPTQ spectrum of **4** in CD_3OD (150 MHz)

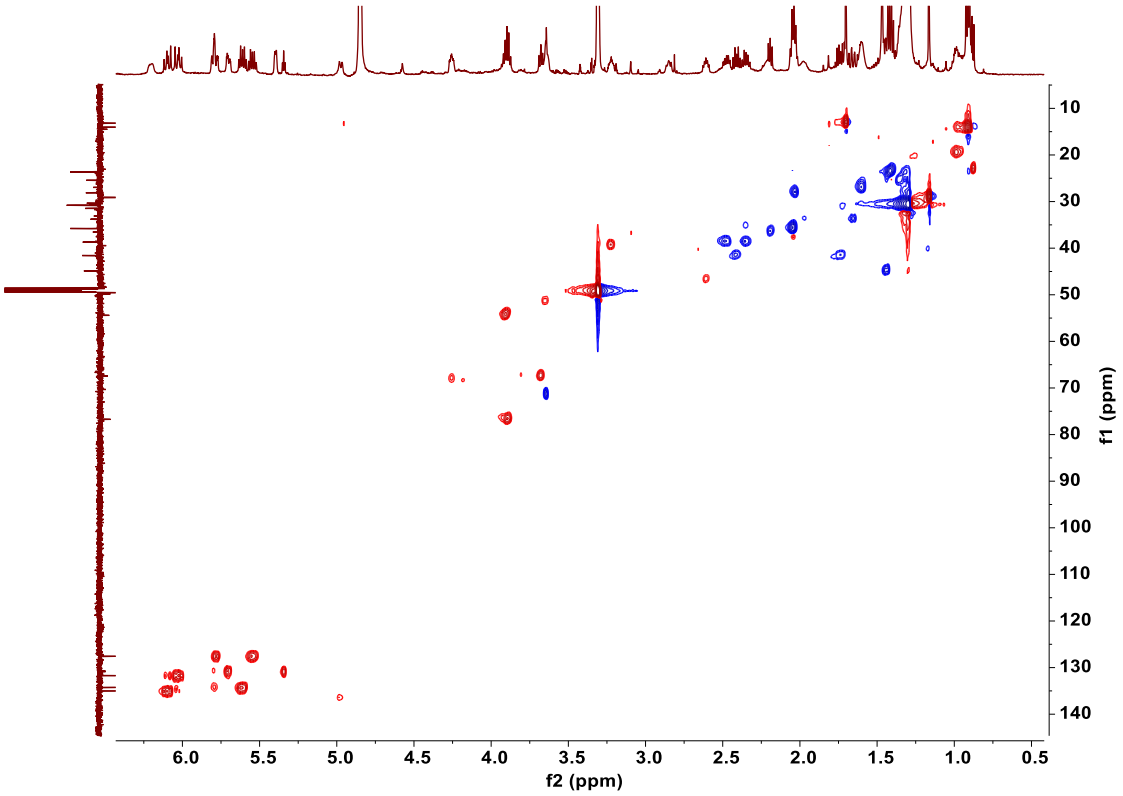

Figure S32. HSQC spectrum of **4** in CD_3OD (600 MHz)

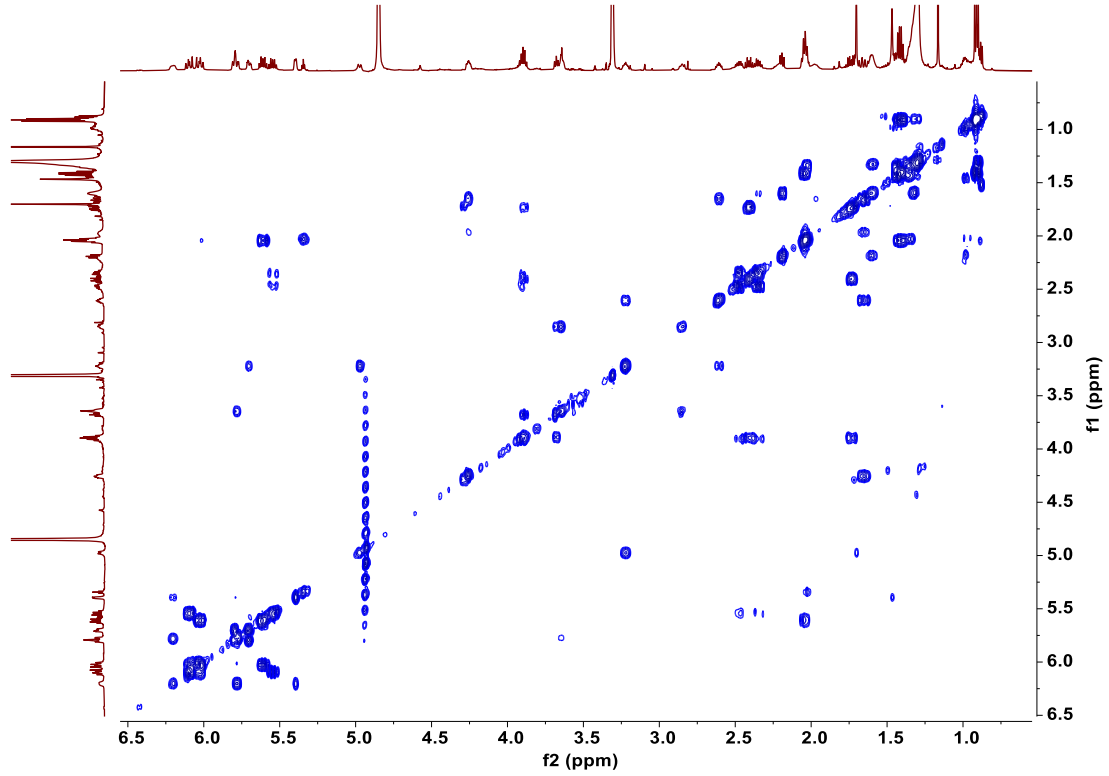

Figure S33. COSY spectrum of **4** in CD_3OD (600 MHz)

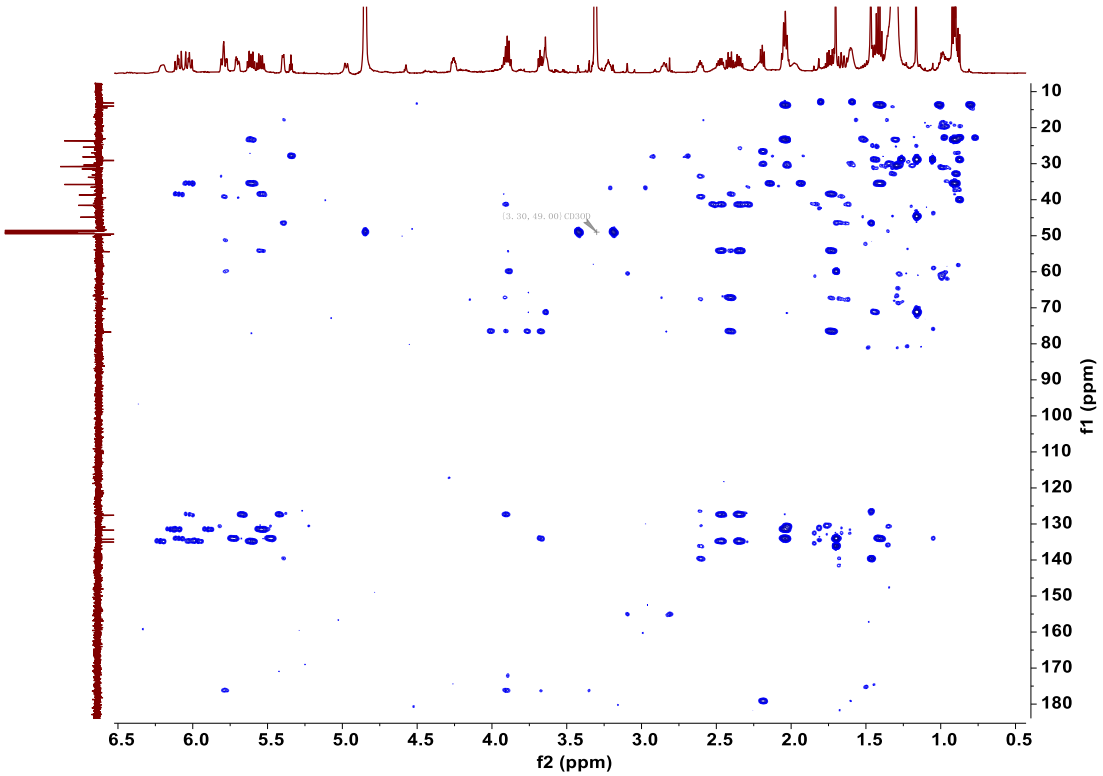

Figure S34. HMBC spectrum of **4** in CD_3OD (600 MHz)

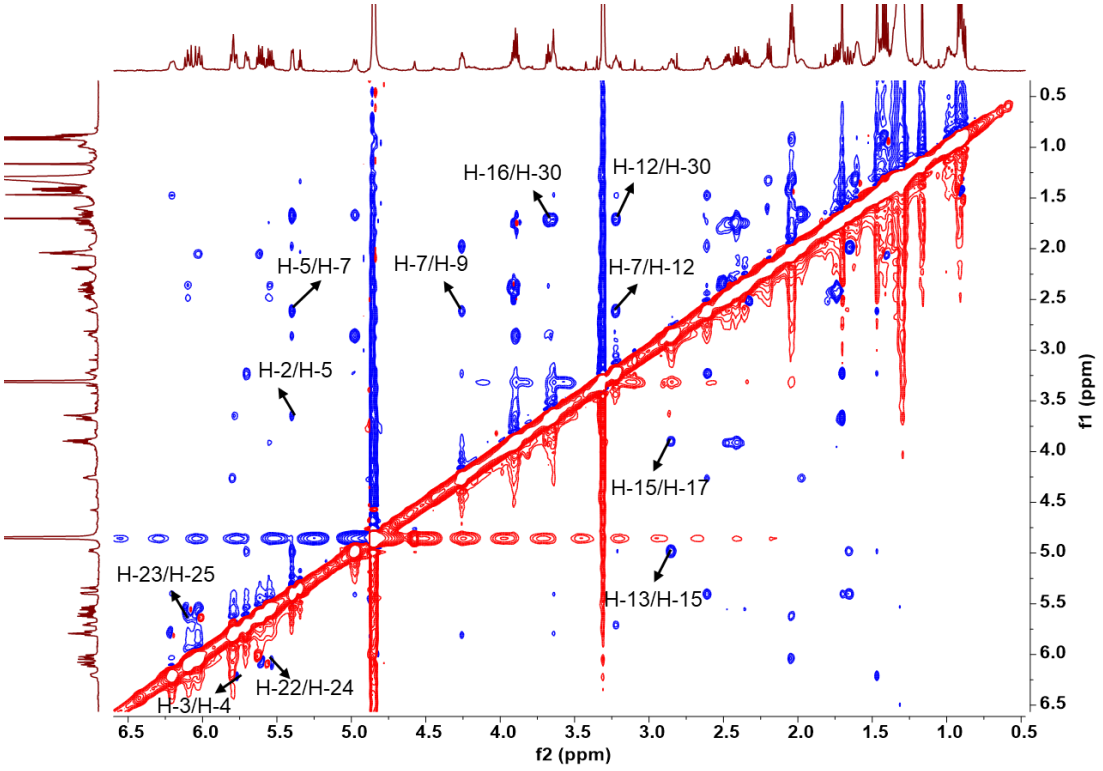

Figure S35. NOESY spectrum of **4** in CD_3OD (600 MHz)

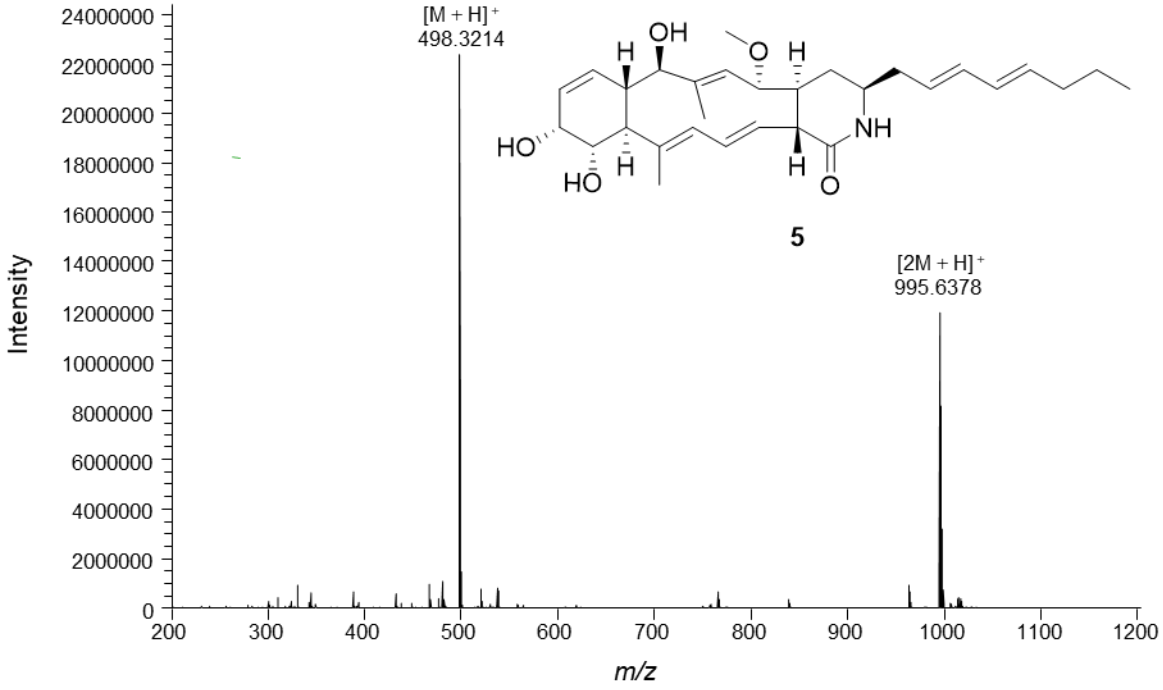

Figure S36. HRESIMS spectrum of **5**

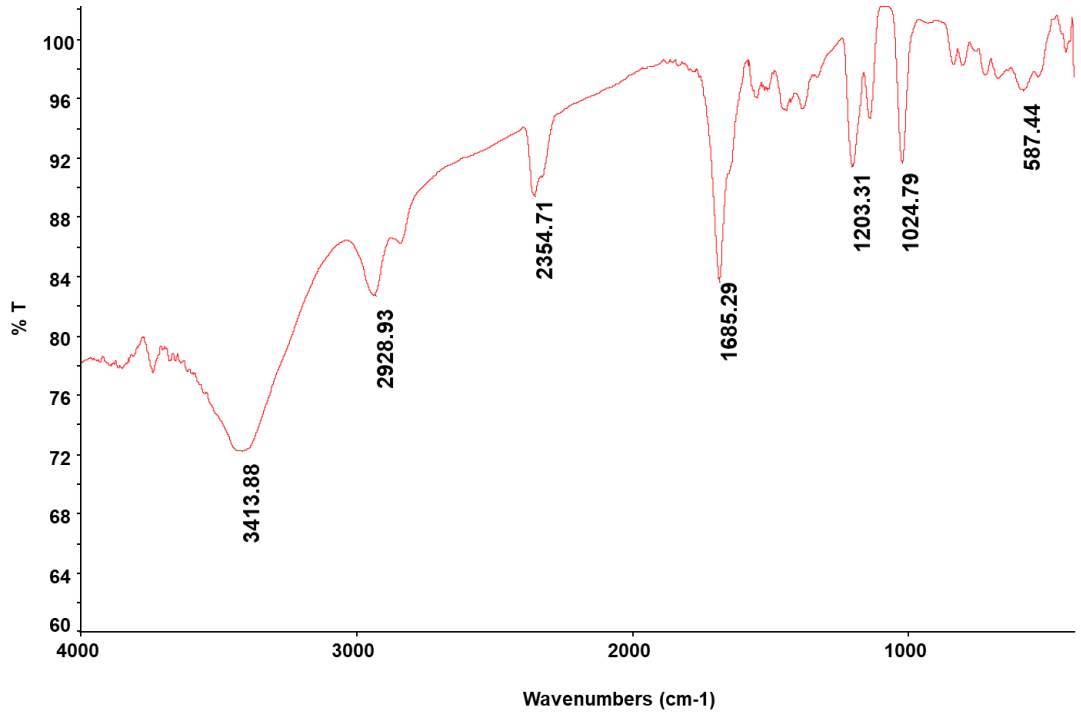

Figure S37. IR spectrum of **5**

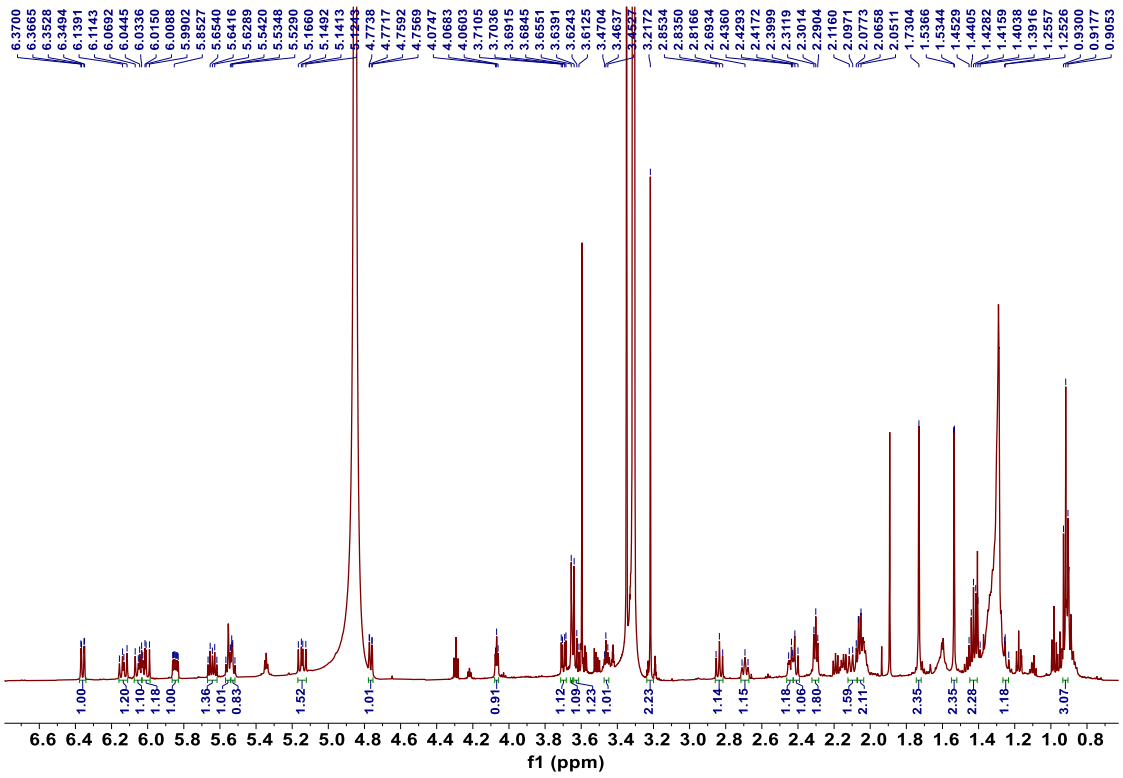

Figure S38. ^{1}H NMR spectrum of **5** in CD_3OD (600 MHz)

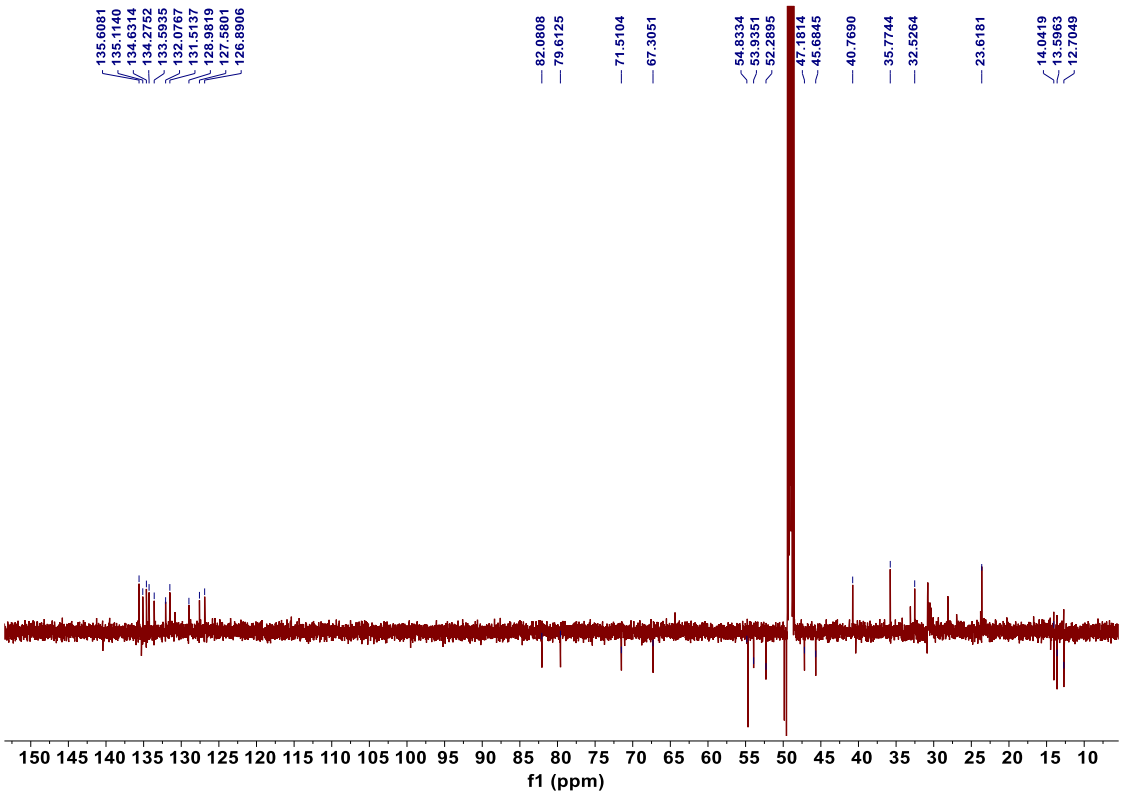

Figure S39. DEPTQ spectrum of **5** in CD_3OD (150 MHz)

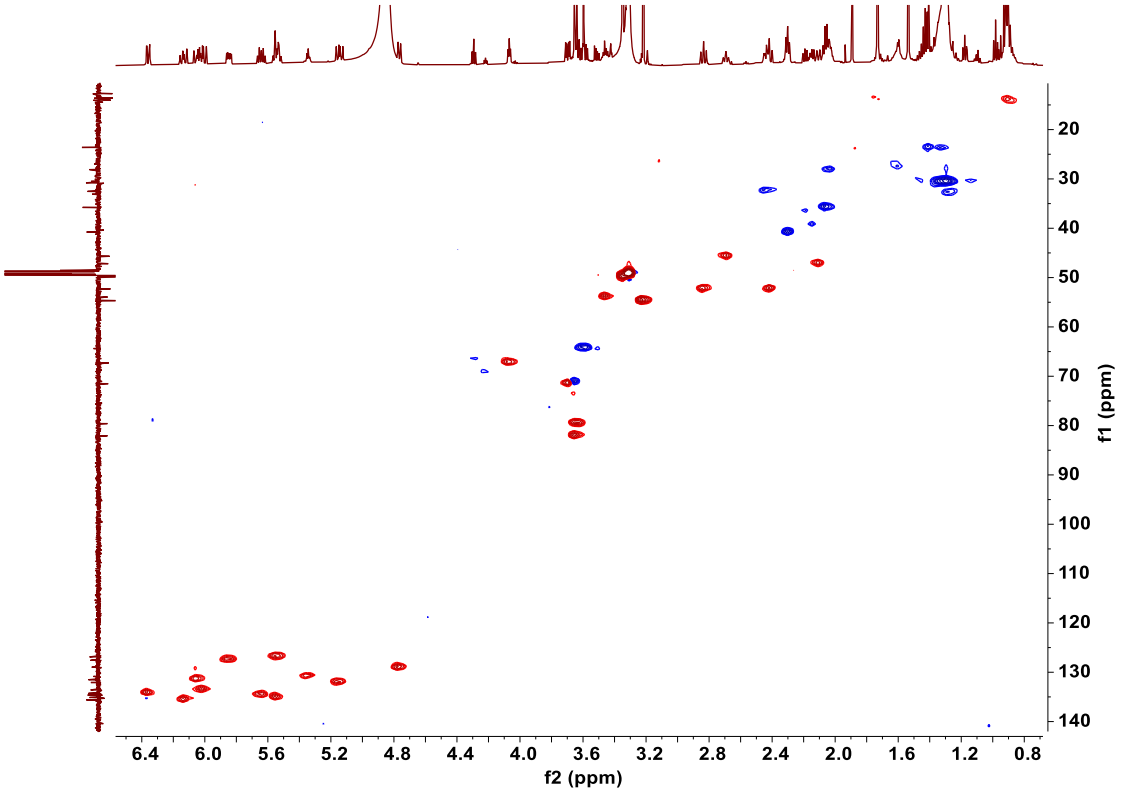

Figure S40. HSQC spectrum of **5** in CD_3OD (600 MHz)

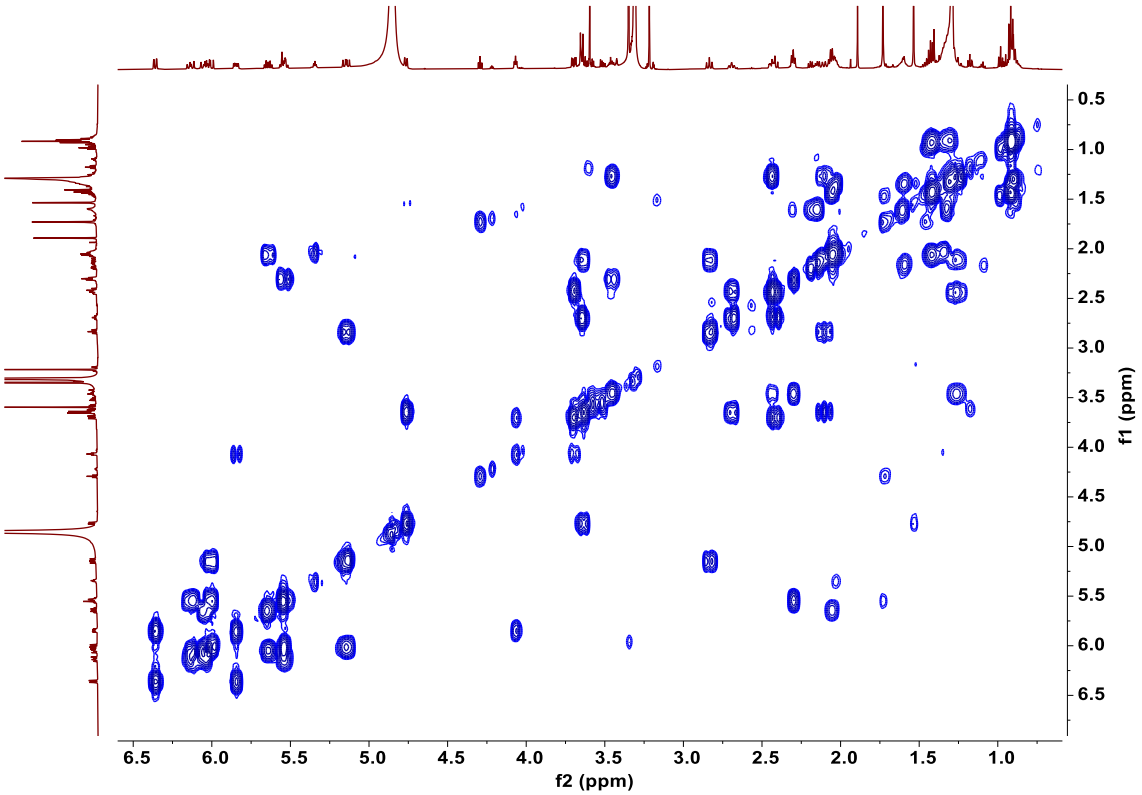

Figure S41. COSY spectrum of **5** in CD_3OD (600 MHz)

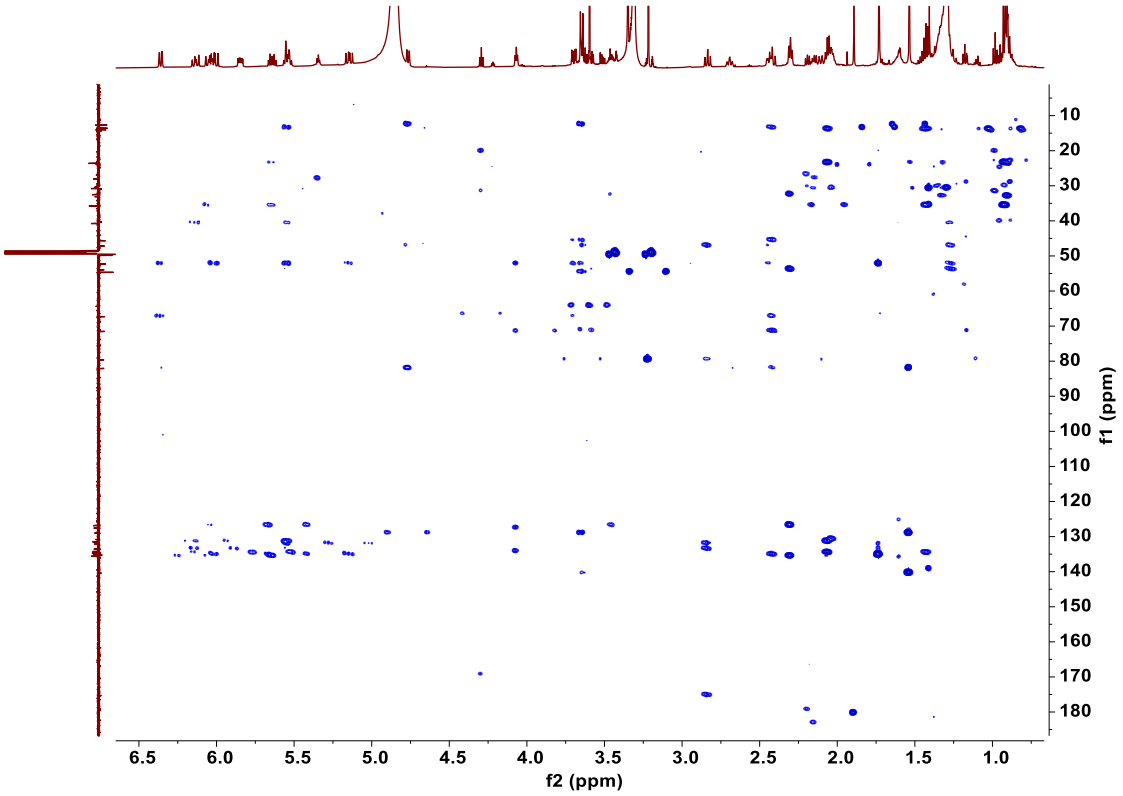

Figure S42. HMBC spectrum of **5** in CD_3OD (600 MHz)

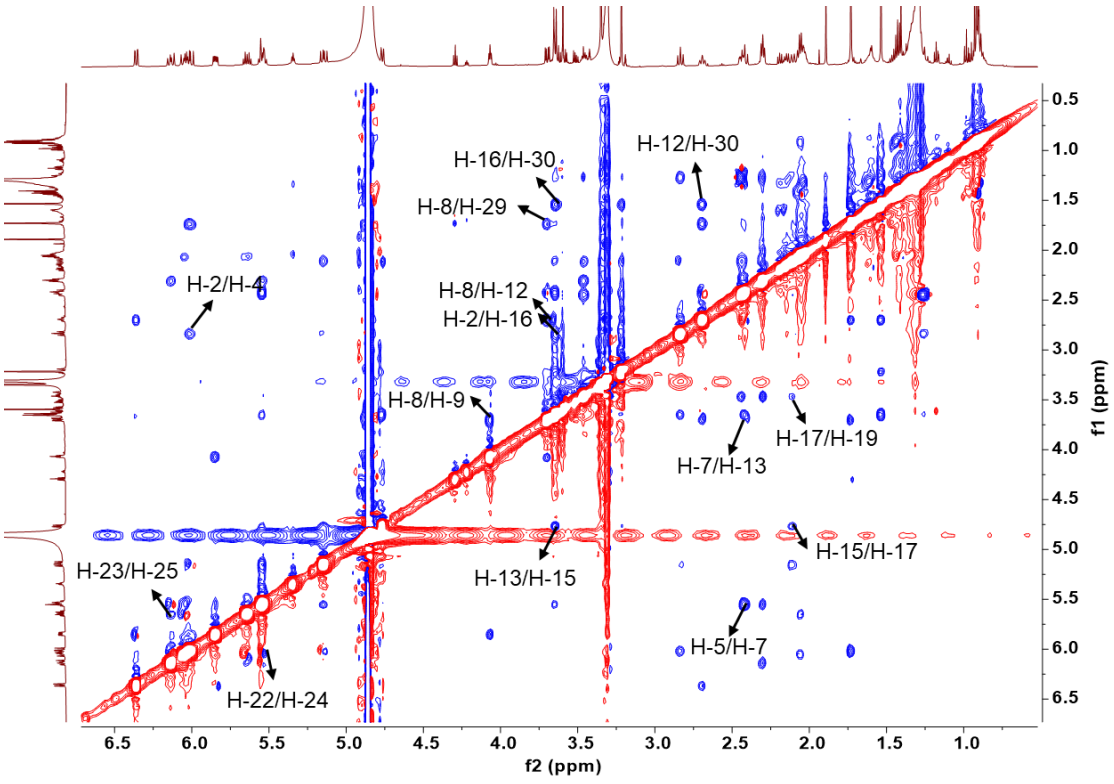

Figure S43. NOESY spectrum of **5** in CD_3OD (600 MHz)

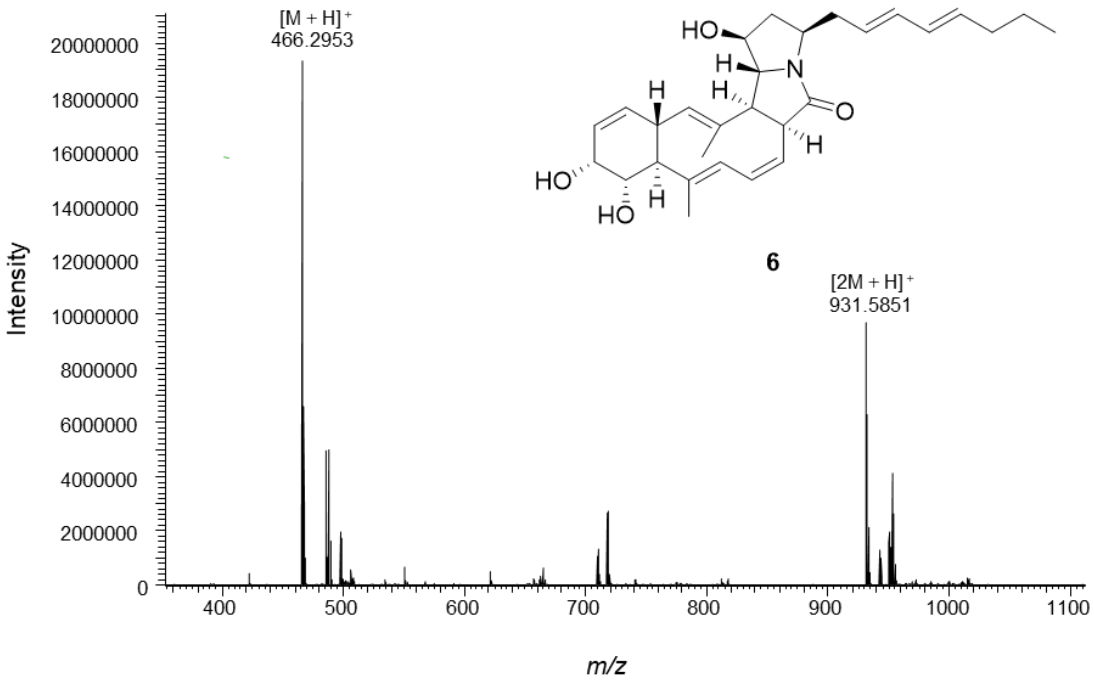

Figure S44. HRESIMS spectrum of **6**

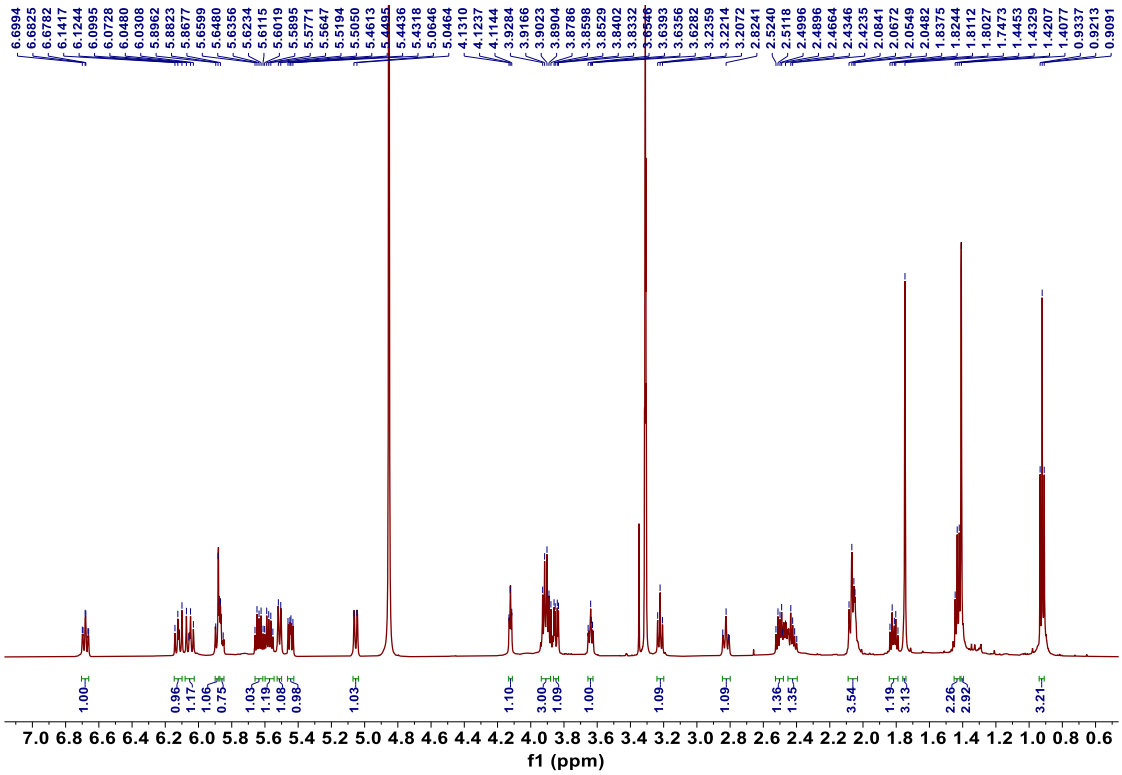

Figure S45. ^{1}H NMR spectrum of **6** in CD_3OD (600 MHz)

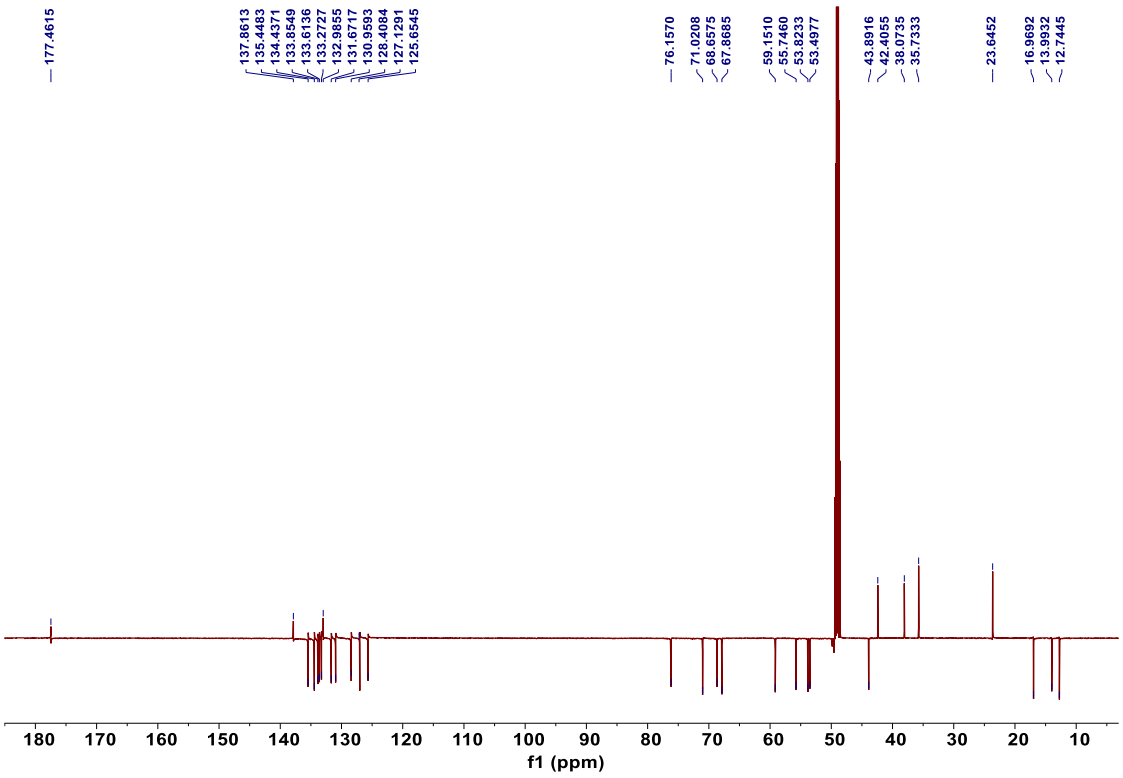

Figure S46. DEPTQ spectrum of **6** in CD_3OD (150 MHz)

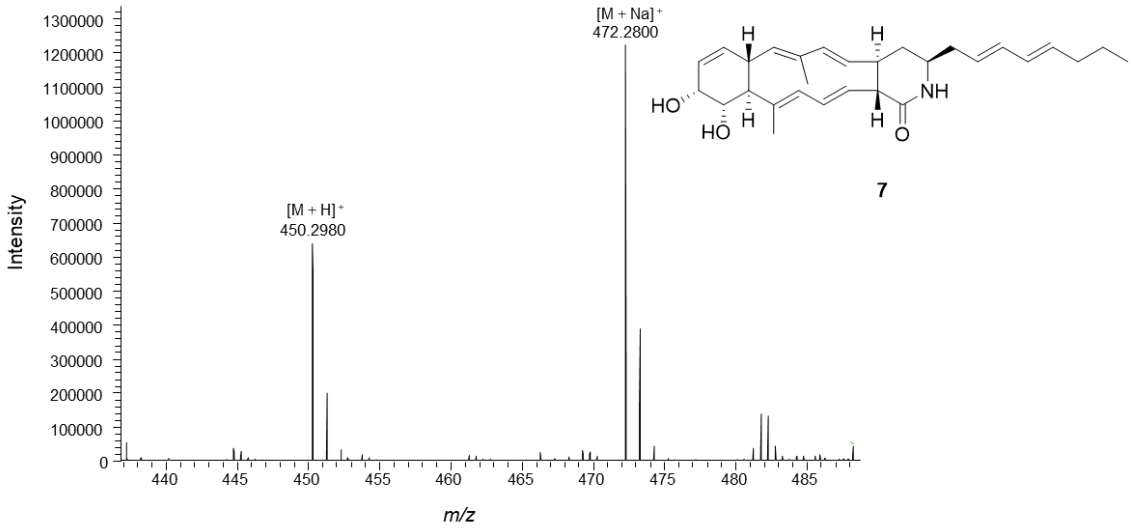

Figure S47. HRESIMS spectrum of **7**

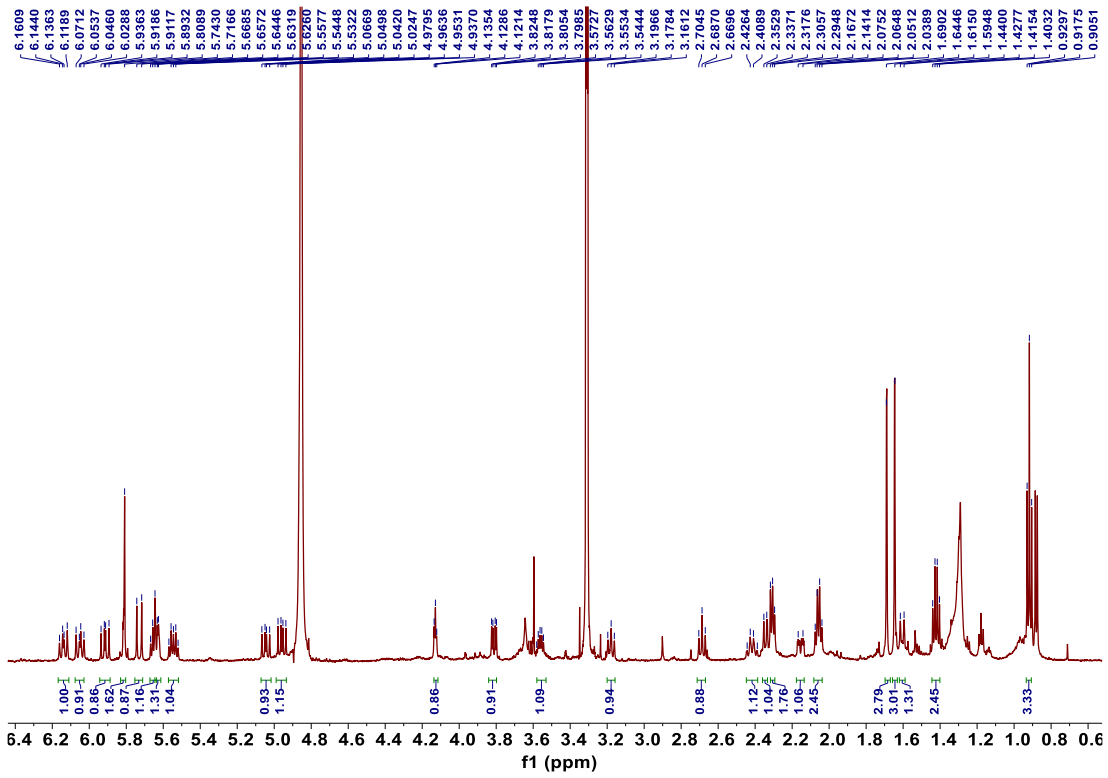

Figure S48. ^{1}H NMR spectrum of **7** in CD_3OD (600 MHz)

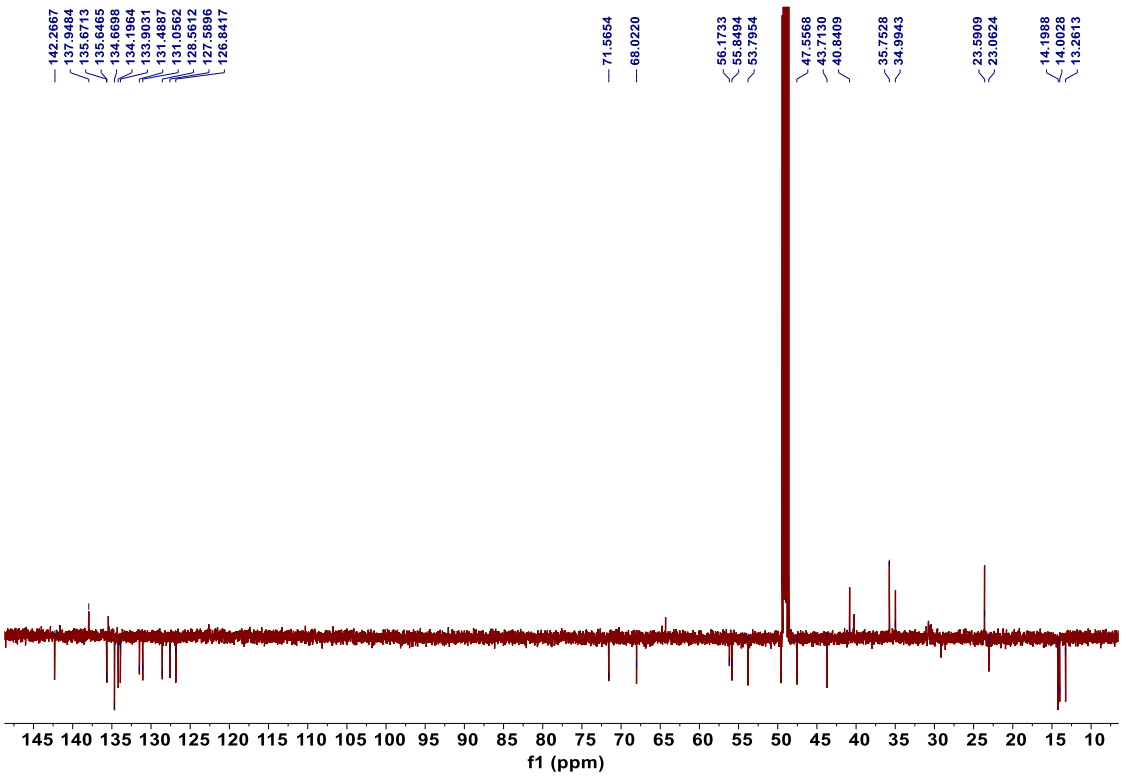

Figure S49. DEPTQ spectrum of **7** in CD_3OD (150 MHz)

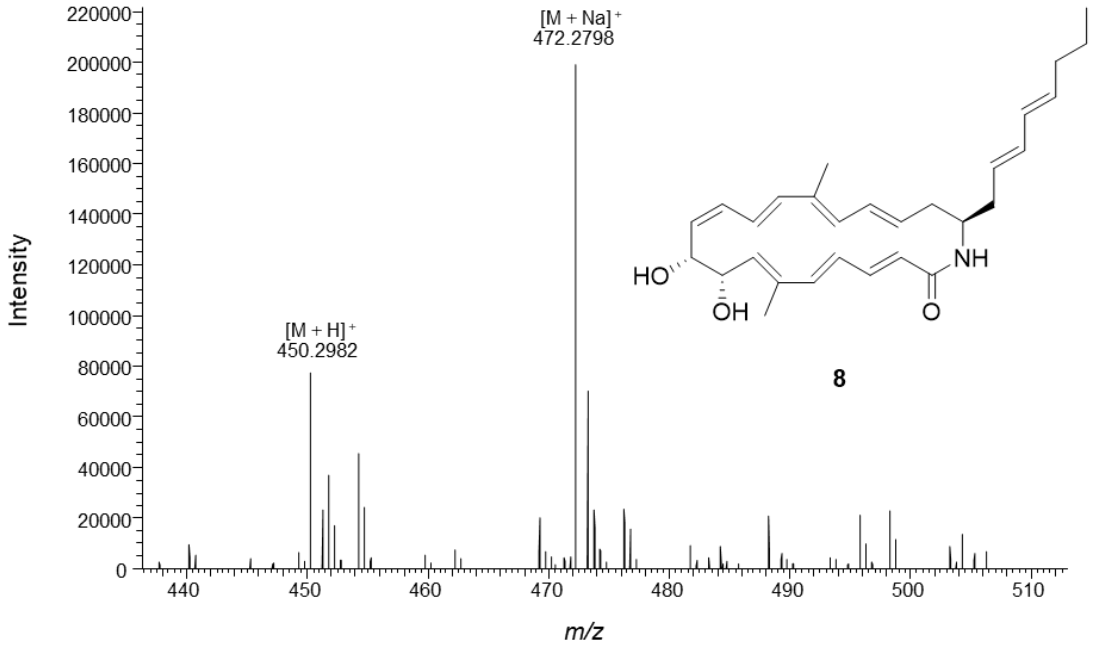

Figure S50. HRESIMS spectrum of **8**

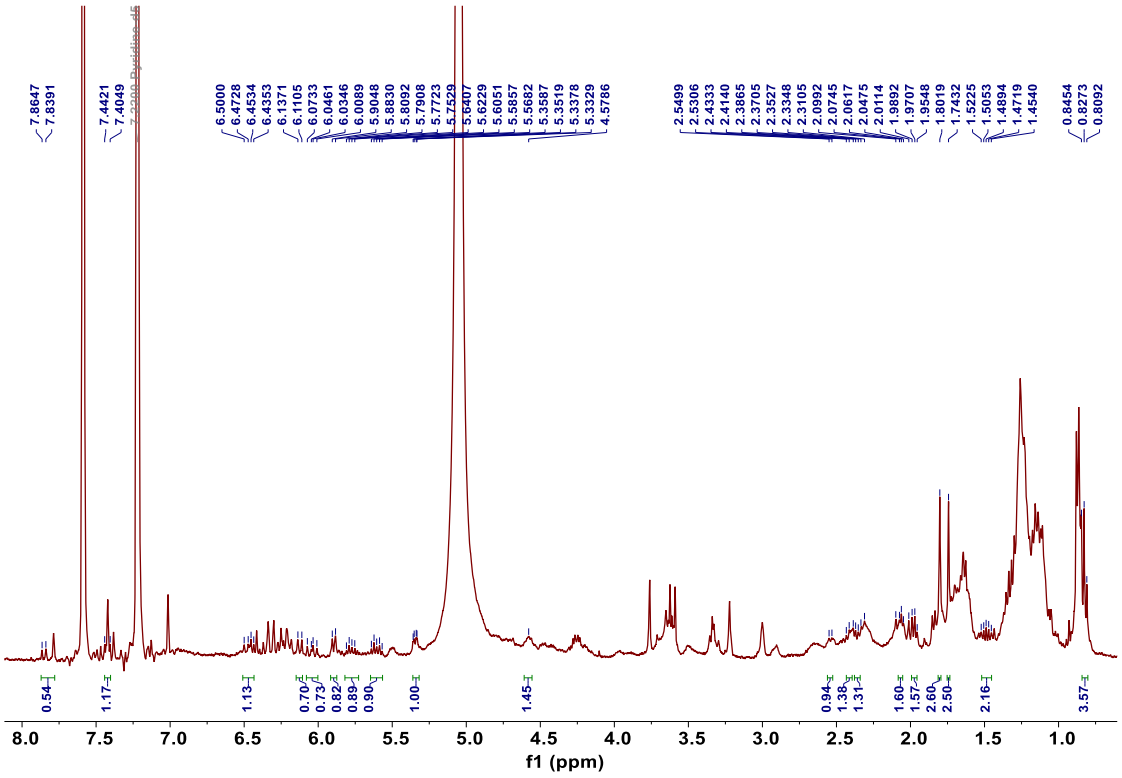

Figure S51. ^{1}H NMR spectrum of **8** in pyridine-d_5 (500 MHz)

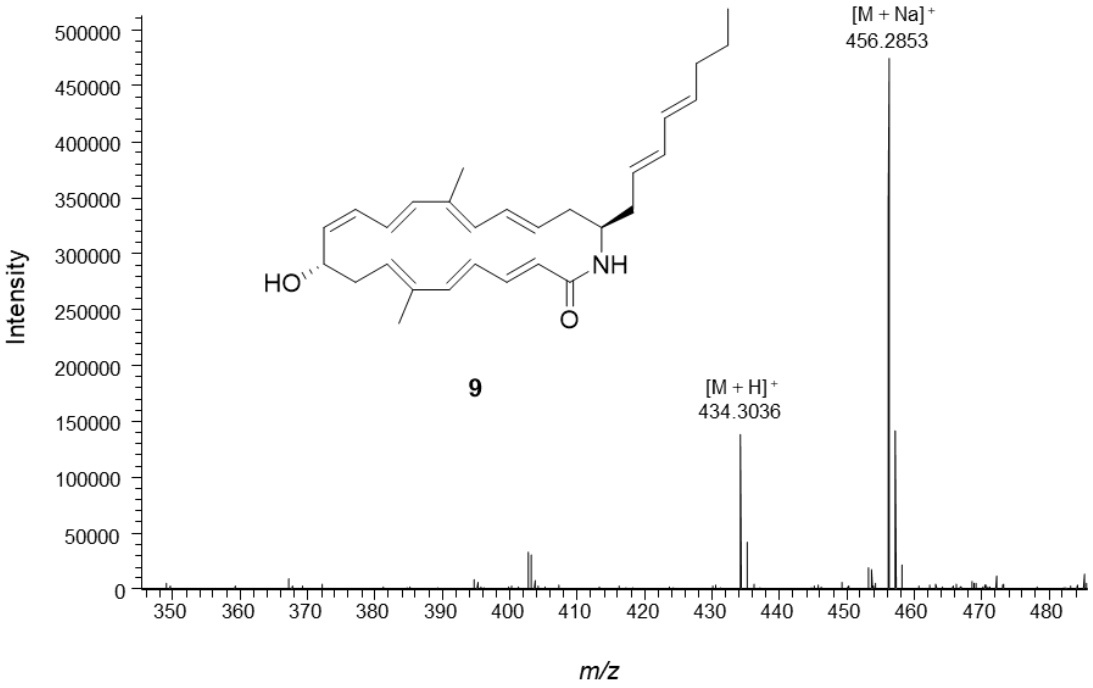

Figure S52. HRESIMS spectrum of **9**

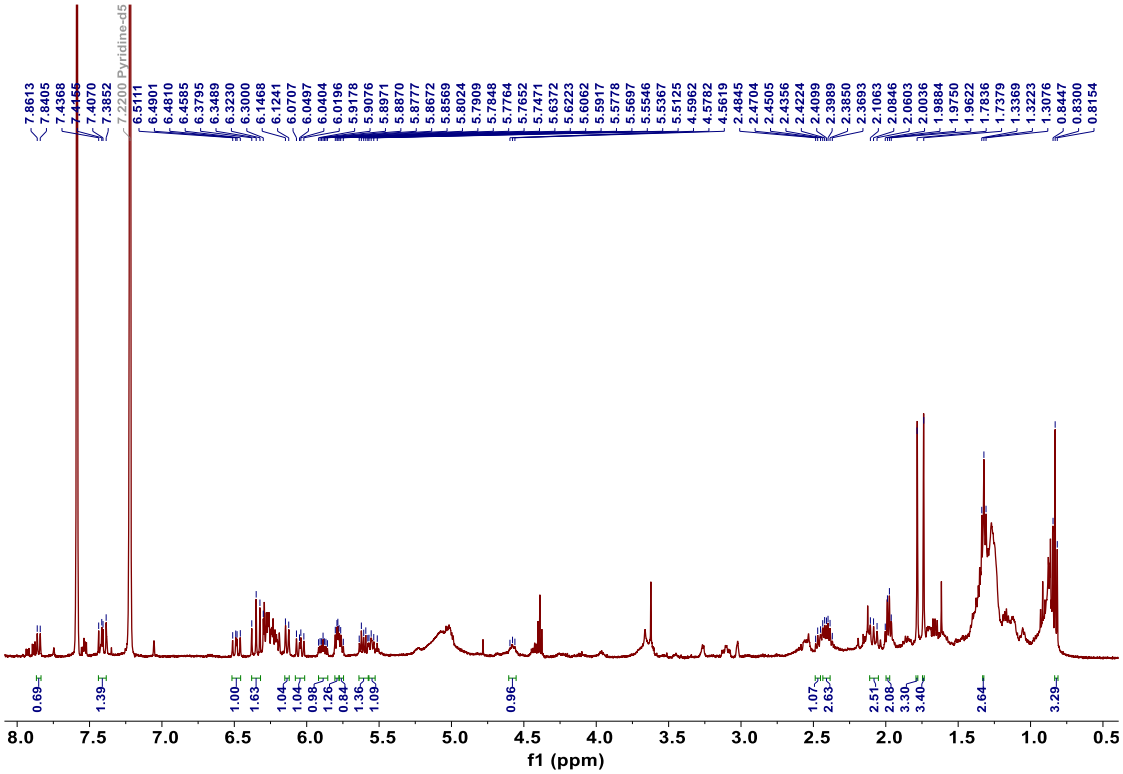

Figure S53. ^{1}H NMR spectrum of **9** in pyridine-d_5 (500 MHz)

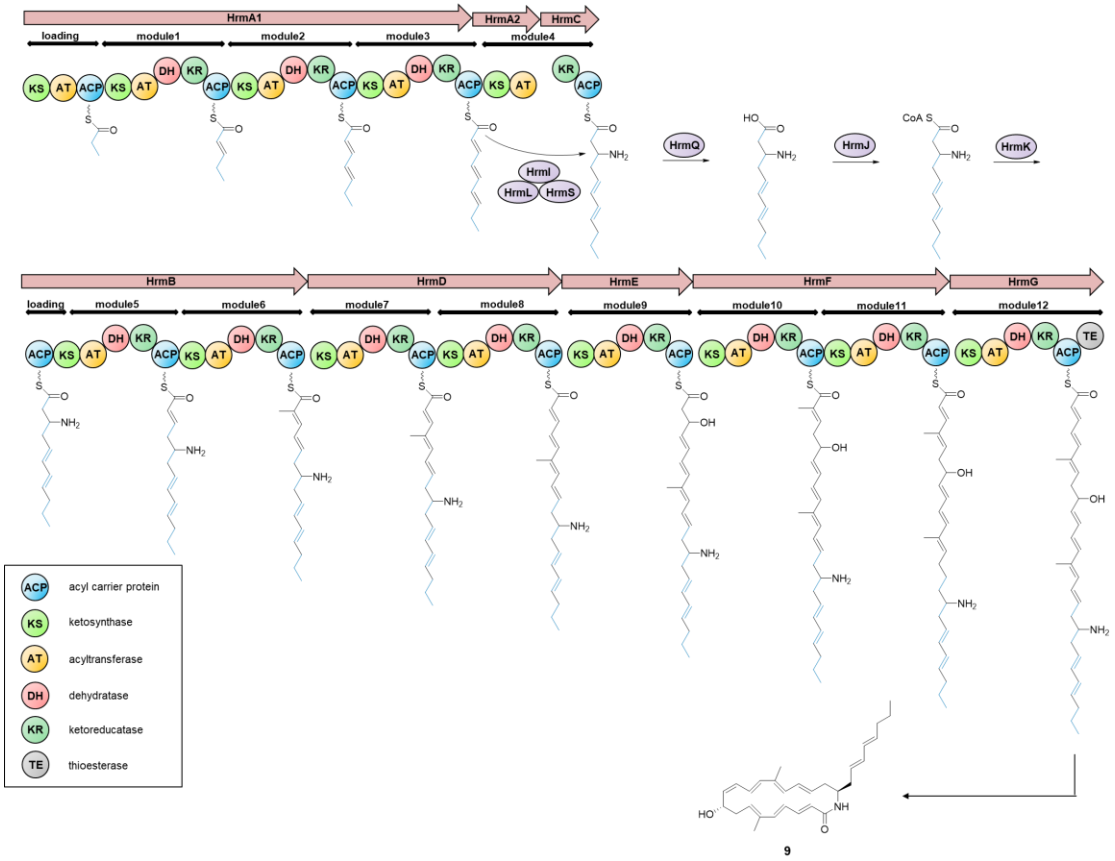

Figure S54. Proposed biosynthetic pathway of compound **9** by *hrm* gene cluster (GenBank Accession No. OR972664) in *Streptomyces* sp. OUCT16-38.

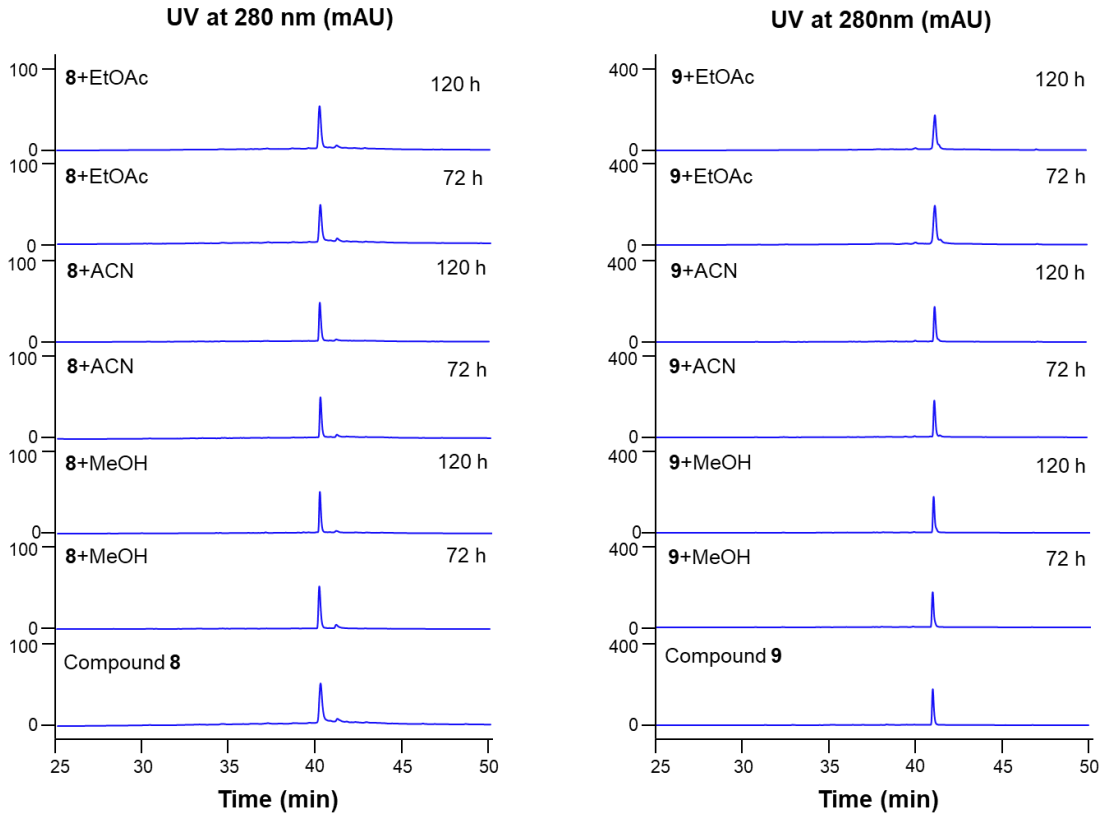

Figure S55. HPLC trace of compounds **8** and **9** retained in MeOH, ACN and EtOAc at room temperature for up to 120 h. The HPLC analysis was performed under gradient conditions (10-100% MeOH) using Agilent Proshell 120 SB-C18 (3 ×150 mm, 2.7 µm) at a flowrate of 0.3 mL/min.

Table S1. Antibacterial activity of the EtOAc extract of the OUCT16-38 strain

Sample	Inhibition zone (mm)		
	S. aureus CCARM 3090	*E. faecium* CCARM 8250	*E. faecalis* CCARM 5172
EtOAc extract	16	18	18
tetracycline	12	32	7

Table S2. Functional prediction of proteins encoded by genes in *hrm* gene cluster and their homologs in known heronamide BGCs (*hrn*, *mla*, *her* and *bec*).

Protein	Size[a]	Proposed function	Homologs	SI/ID[b]	Accession number
HrmA1	6359	Polyketide synthase type I	HrnA1	99/75	SAI82895.1
			MlaA1	100/75	ACO94483.1
			HerA1	100/66	AKD43768.1
			BecA	98/74	ACO94456.1
HrmA2	1036	Polyketide synthase type I	HrnA2	100/81	SAI82896.1
			MlaA2	99/81	ACO94484.1
			HerA2	99/77	AKD43769.1
			BecA	99/74	ACO94456.1
HrmB	3537	Polyketide synthase type I	HrnB	100/83	SAI82900.1
			MlaB	100/83	ACO94488.1
			HerB	100/80	AKD43753.1
			BecB	100/83	ACO94460.1
HrmC	697	Polyketide synthase type I	HrnC	100/77	SAI82898.1
			MlaC	100/77	ACO94486.1
			HerC	100/78	AKD43751.1
			BecC	100/75	ACO94458.1
HrmD	3372	Polyketide synthase type I	HrnD	100/81	SAI82908.1
			MlaD	100/81	ACO94496.1
			HerD	99/78	AKD43761.1
			BecD	99/81	ACO94468.1
HrmE	1642	Polyketide synthase type I	HrnE	100/76	SAI82912.1
			MlaE	100/76	ACO94500.1
			HerE	99/75	AKD43765.1
			BecE	100/77	ACO94472.1
HrmF	3390	Polyketide synthase type I	HrnF	99/79	SAI82911.1
			MlaF	99/80	ACO94499.1
			HerF	99/77	AKD43764.1
			BecF	99/80	ACO94471.1

HrmG	1983	Polyketide synthase type I	HrnG	100/78	SAI82910.1
			MlaG	100/79	ACO94498.1
			HerG	100/76	AKD43763.1
			BecG	100/79	ACO94470.1
HrmH	902	LuxR-type transcriptional regulator	HrnH	99/74	SAI82894.1
			MlaH	99/74	ACO94482.1
			HerH	99/68	AKD43767.1
			BecH	99/74	ACO94455.1
HrmI	365	Glycine oxidase/FAD-dependent oxidoreductase	HrnI	98/77	SAI82897.1
			MlaI	98/78	ACO94485.1
			HerI	98/77	AKD43750.1
			BecI	98/77	ACO94457.1
HrmJ	532	AMP-dependent acyl-CoA synthetase/ligase	HrnJ	98/86	SAI82901.1
			MlaJ	100/86	ACO94489.1
			HerJ	100/81	AKD43754.1
			BecJ	100/84	ACO94461.1
HrmK	313	Acyltransferase	HrnK	100/79	SAI82902.1
			MlaK	100/80	ACO94490.1
			HerK	100/82	AKD43755.1
			BecK	100/77	ACO94462.1
HrmL	505	NRPS adenylation domain	HrnL	97/83	SAI82904.1
			MlaL	97/84	ACO94492.1
			HerL	99/83	AKD43757.1
			BecL	100/81	ACO94464.1
HrmM	198	TetR-type transcriptional regulator	HrnM	100/79	SAI82905.1
			MlaM	100/81	ACO94493.1
			HerM	100/80	AKD43758.1
			BecM	100/86	ACO94465.1
HrmN	526	MFS-type efflux pump	HrnN	98/82	SAI82906.1
			MlaN	99/81	ACO94494.1
			HerN	99/78	AKD43759.1
			BecN	99/79	ACO94466.1
HrmO	412	P450 monooxygenase	HrnO	100/79	AKD43760.1
			MlaO	100/80	ACO94495.1
			HerO	97/82	AKD43760.1
			BecO	100/80	ACO94467.1

HrmP	311	Putative L-amino acid amidase/proline iminopeptidase	HrnP	100/86	SAI82909.1
			MlaP	100/86	ACO94497.1
			HerP	100/88	AKD43762.1
			BecP	99/85	ACO94469.1
HrmQ	195	Thioesterase type II	HrnQ		SAI82914.1
			MlaQ		ACO94502.1
			-		
			BecQ		ACO94474.1
HrmS	78	Peptidyl carrier protein	HrnS	100/82	SAI82903.1
			MlaS	100/79	ACO94491.1
			HerS	100/79	AKD43756.1
			BecS	100/78	ACO94463.1
HrmT	155	SimX2-like protein	HrnT		SAI82913.1
			MlaT		ACO94501.1
			-		
			BecT		ACO94473.1
HrmU	187	Putative NRPS accessory protein	HrnU	98/85	SAI82899.1
			MlaU	98/86	ACO94487.1
			HerU	100/80	AKD43752.1
			BecU	98/84	ACO94459.1

[a]: amino acids; [b]: similarity/identity (%/%)

Table S3. Antibacterial Activity of **1**−**9** (MIC, μg/mL)

Compound	MIC (μg/mL)		
	S. aureus CCARM 3090	*E. faecium* CCARM 5203	*E. faecalis* CCARM 5172
1	>50	>50	>50
2	>50	>50	>50
3	>50	>50	>50
4	>50	>50	>50
5	>50	>50	>50
6	>50	>50	>50
7	>50	50	>50
8	12.5	3.1	3.1
9	>50	3.1	3.1
tetracycline	6.2	1.6	>50

Table S4. Cytotoxicity of **8** and **9** (IC_{50}, μM)

Compound	IC_{50} (μM)				
	A549-Taxol	H1975	H1299	HEL	LX-2
8	4.4±0.4	3.1±0.5	10.1±1.1	4.4±0.6	18.7±1.8
9	3.7±0.2	8.9±0.5	7.6±2.4	5.2±0.1	39.4±7.8
doxorubicin	1.6±0.1	0.1±0.1	1.4±0.1	0.05±0.03	0.4±0.1