Supporting Information

Jejucarbosides B-E, Chlorinated Cycloaromatized Enediynes, from a Marine *Streptomyces* sp.

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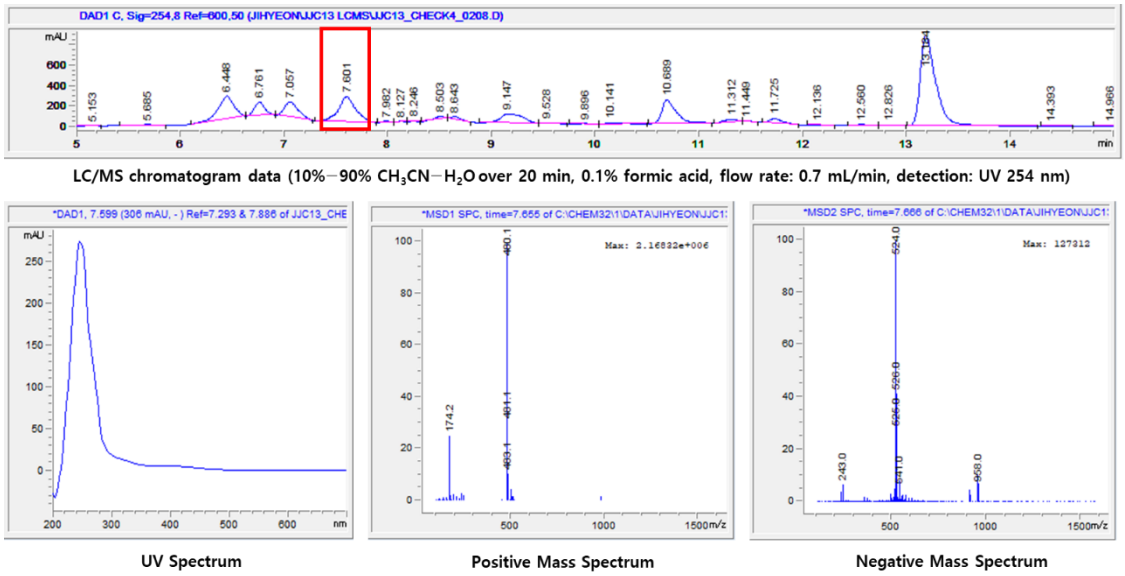
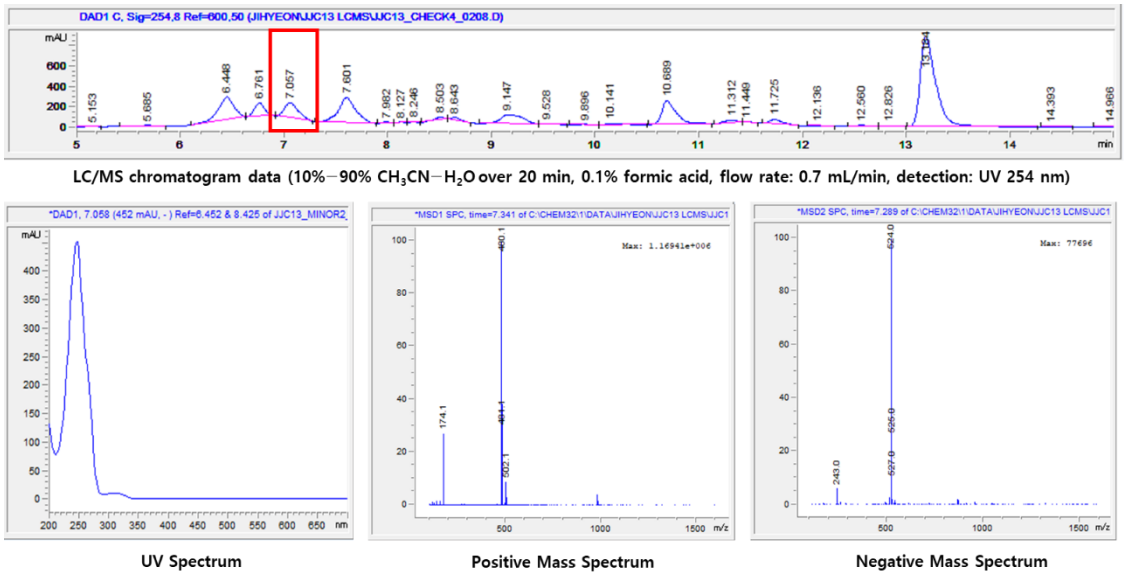
Figure S1. LC/MS profile of the extract of *Streptomyces* sp. JJC13 showing the characteristic mono-chlorinated pattern ($[M+H]^+$:$[M+2+H]^+$ = 3:1) of jejucarbosides A-E.

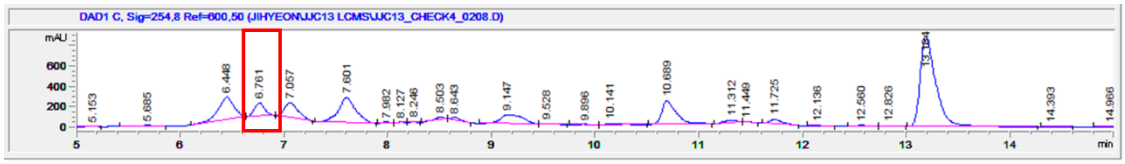
a. Jejucarboside A



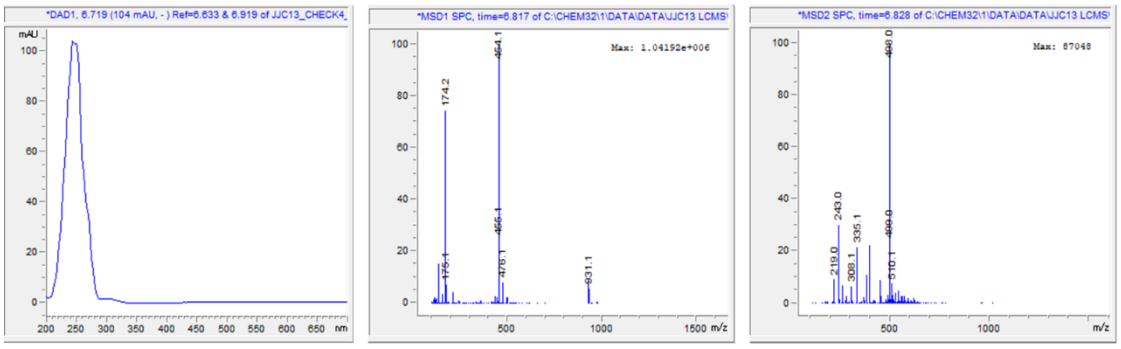
b. Jejucarboside B (**1**)



c. Jejucarboside C (**2**)

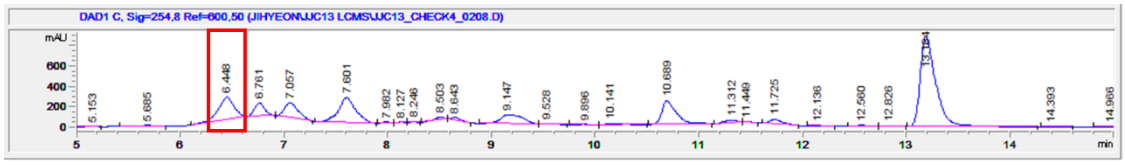


LC/MS chromatogram data (10%−90% CH_3CN-H_2O over 20 min, 0.1% formic acid, flow rate: 0.7 mL/min, detection: UV 254 nm)

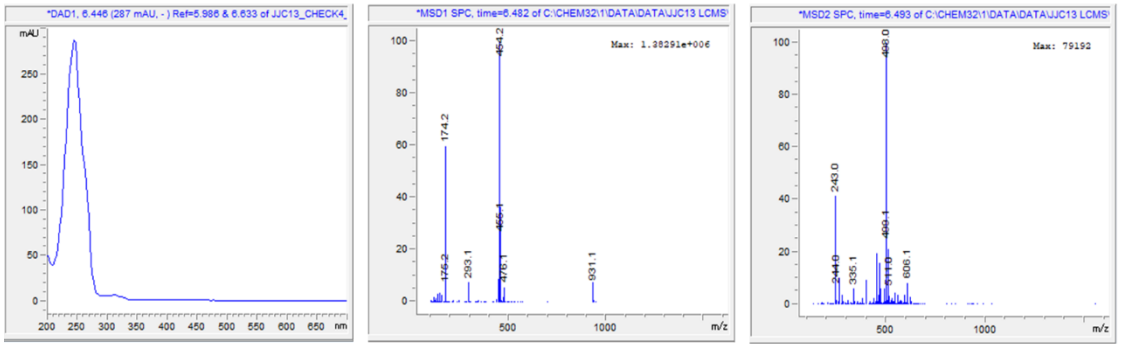


UV Spectrum | Positive Mass Spectrum | Negative Mass Spectrum

d. Jejucarboside D (**3**)

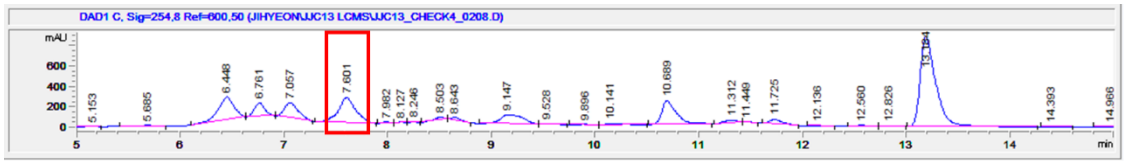


LC/MS chromatogram data (10%−90% CH_3CN-H_2O over 20 min, 0.1% formic acid, flow rate: 0.7 mL/min, detection: UV 254 nm)

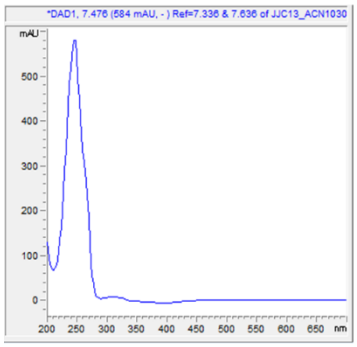


UV Spectrum | Positive Mass Spectrum | Negative Mass Spectrum

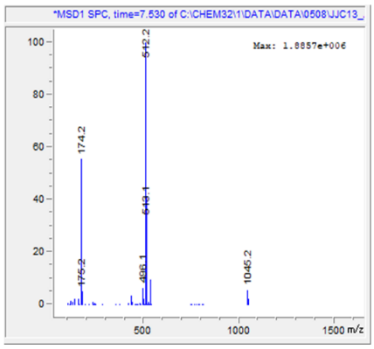
e. Jejucarboside E (**4**)



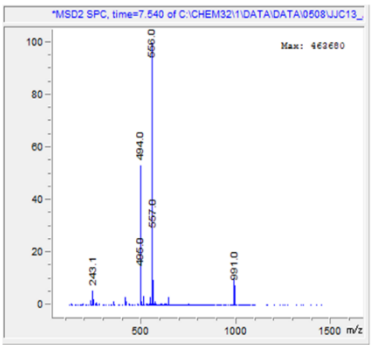
LC/MS chromatogram data (10%−90% CH_3CN-H_2O over 20 min, 0.1% formic acid, flow rate: 0.7 mL/min, detection: UV 254 nm)



UV Spectrum



Positive Mass Spectrum



Negative Mass Spectrum

Figure S2. ^{1}H NMR spectrum of jejucarboside B (**1**) at 850 MHz in CD_3CN.

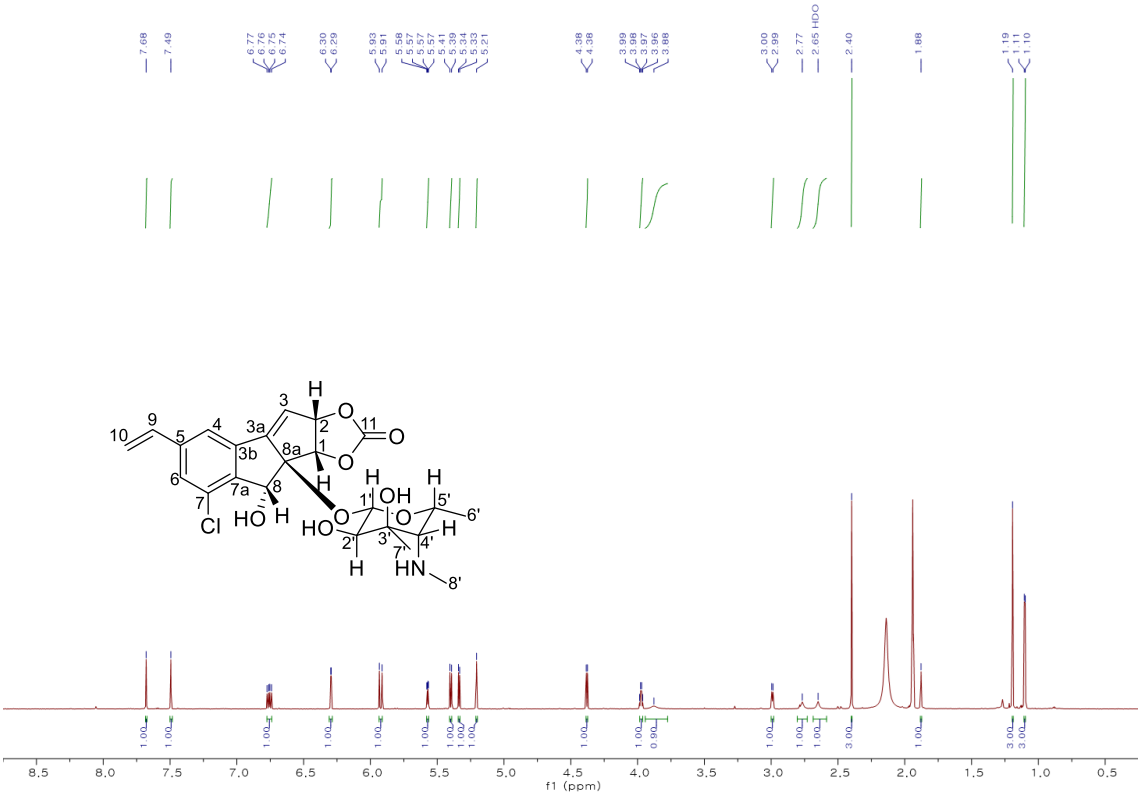


Figure S3. ^{13}C NMR spectrum of jejucarboside B (**1**) at 850 MHz in CD_3CN.

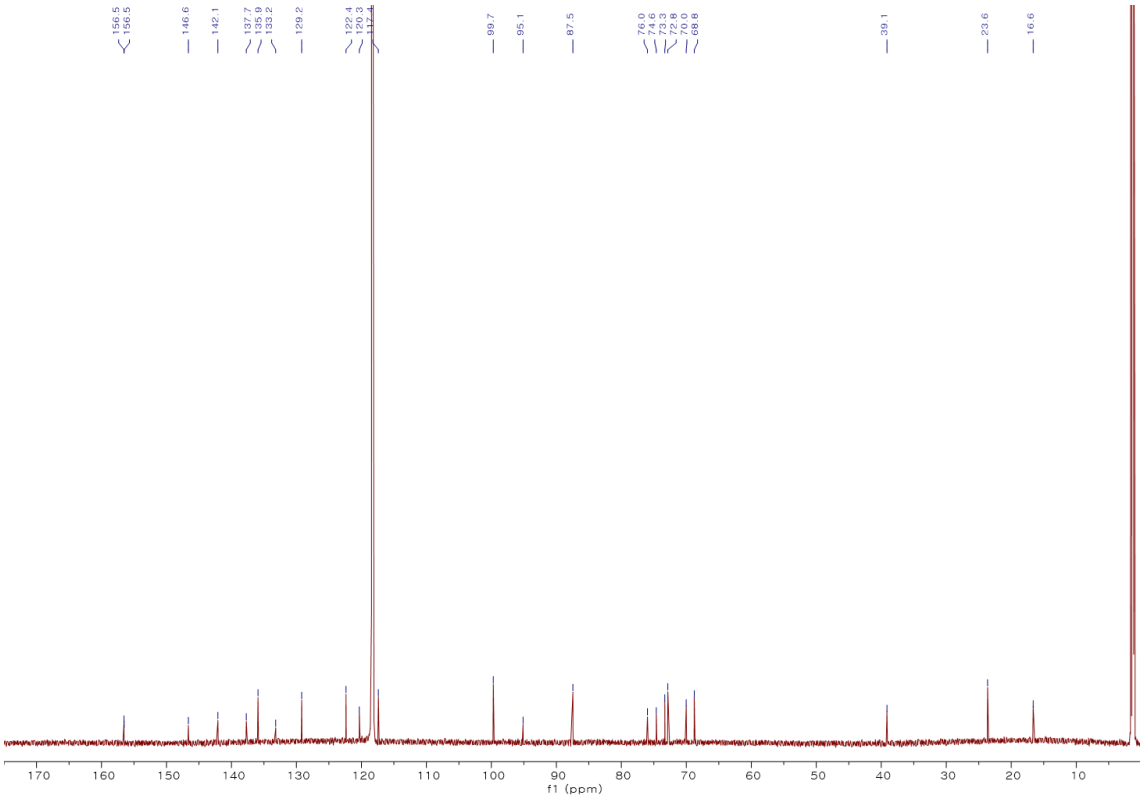


Figure S4. COSY NMR spectrum of jejucarboside B (**1**) at 850 MHz in CD_3CN.

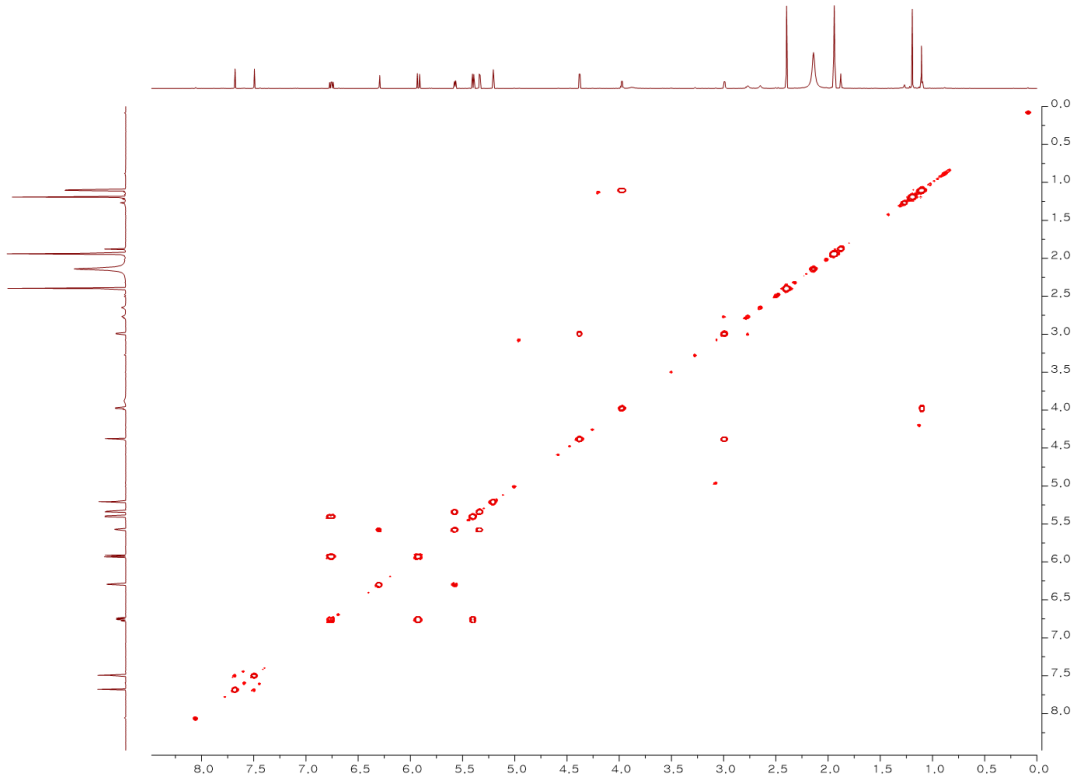


Figure S5. HSQC NMR spectrum of jejucarboside B (**1**) at 850 MHz in CD_3CN.

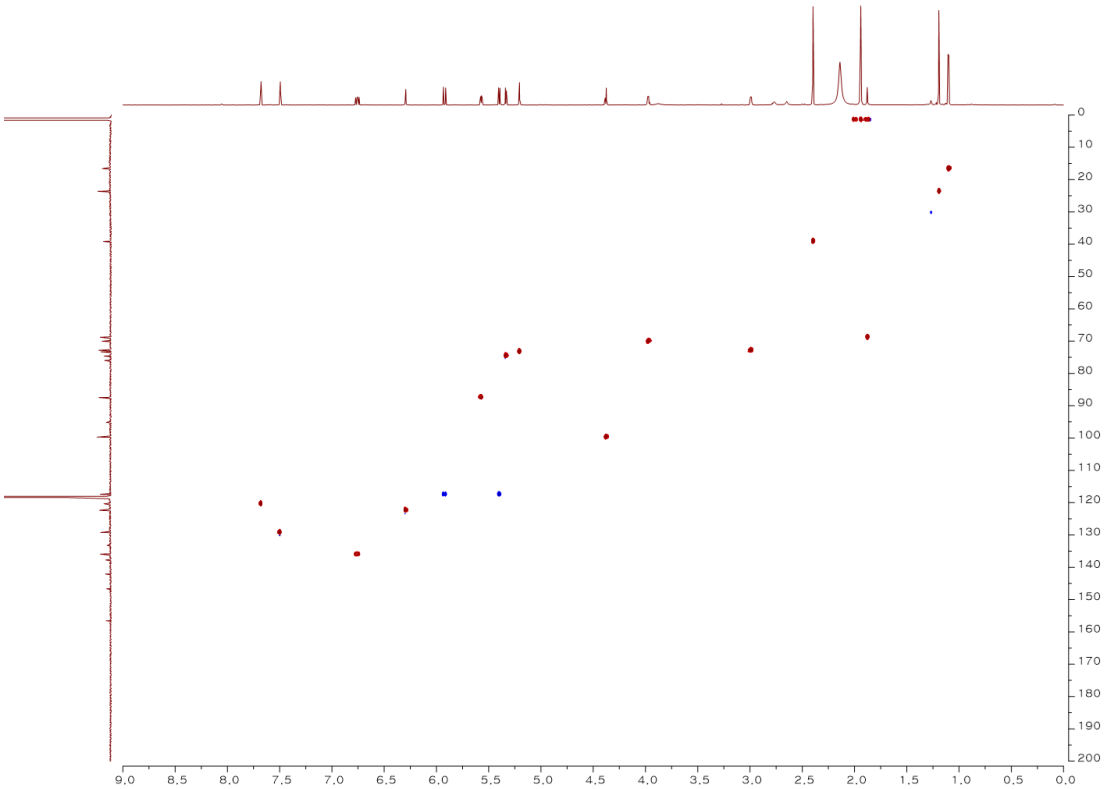


Figure S6. HMBC NMR spectrum of jejucarboside B (**1**) at 850 MHz in CD_3CN.

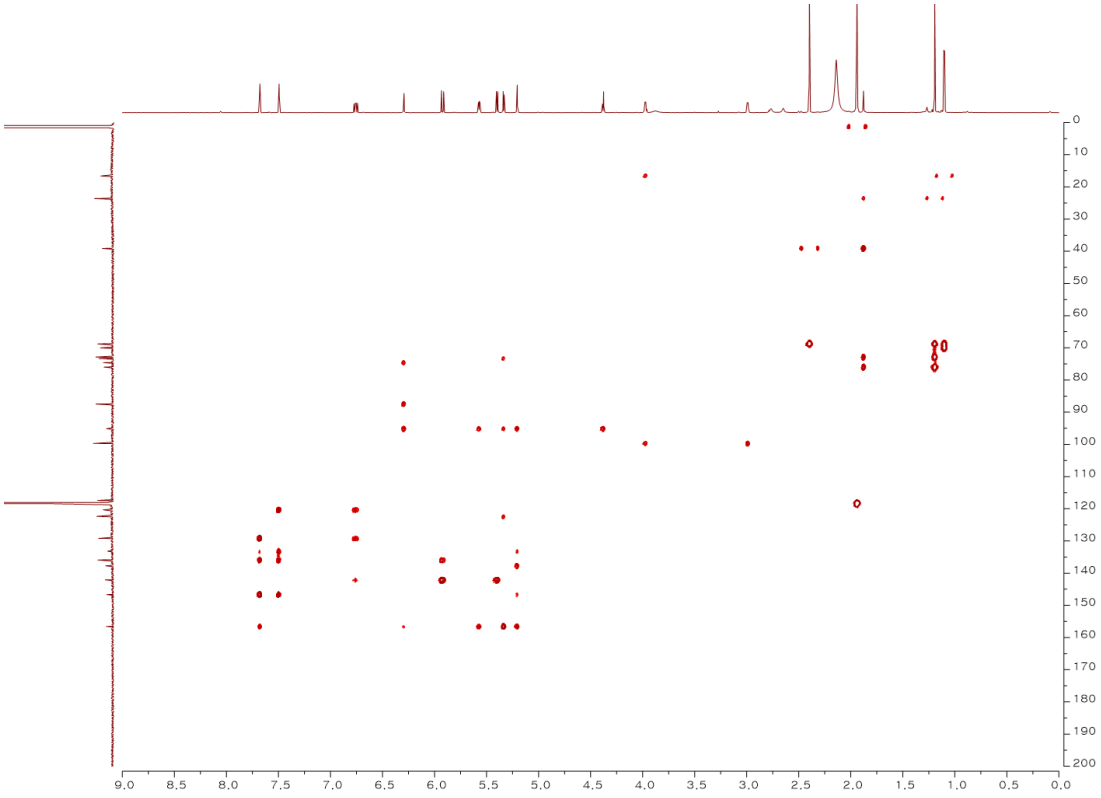


Figure S7. ROESY NMR spectrum of jejucarboside B (**1**) at 850 MHz in CD_3CN.

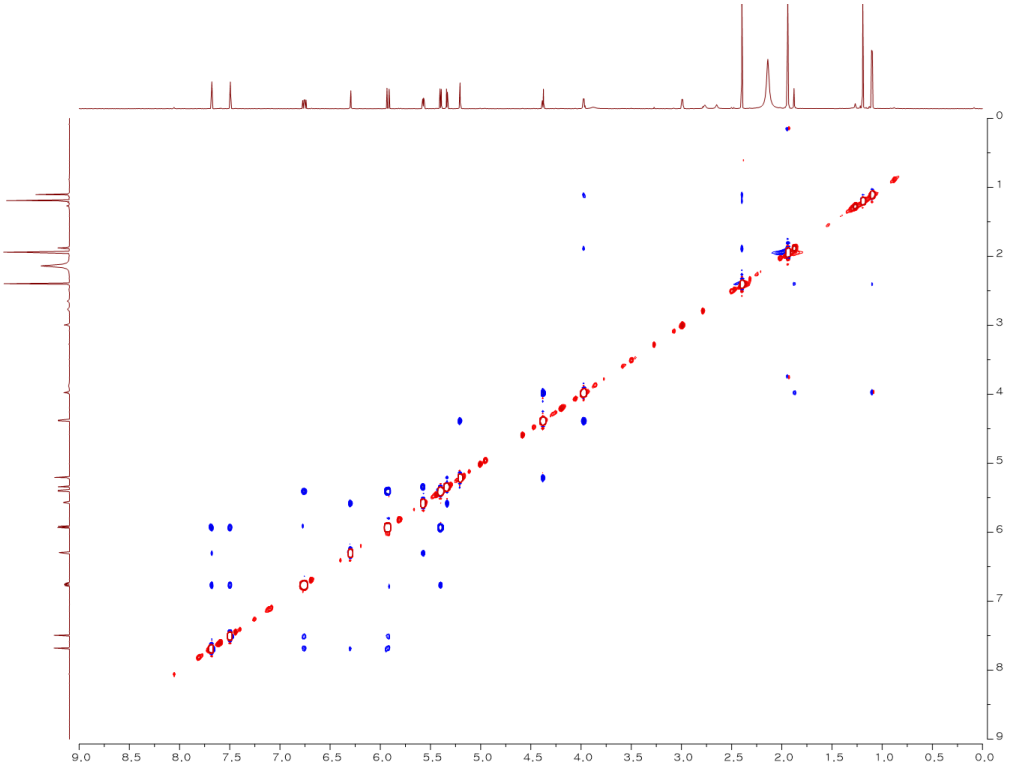


Figure S8. ^{1}H NMR spectrum of jejucarboside C (**2**) at 850 MHz in CD_3CN.

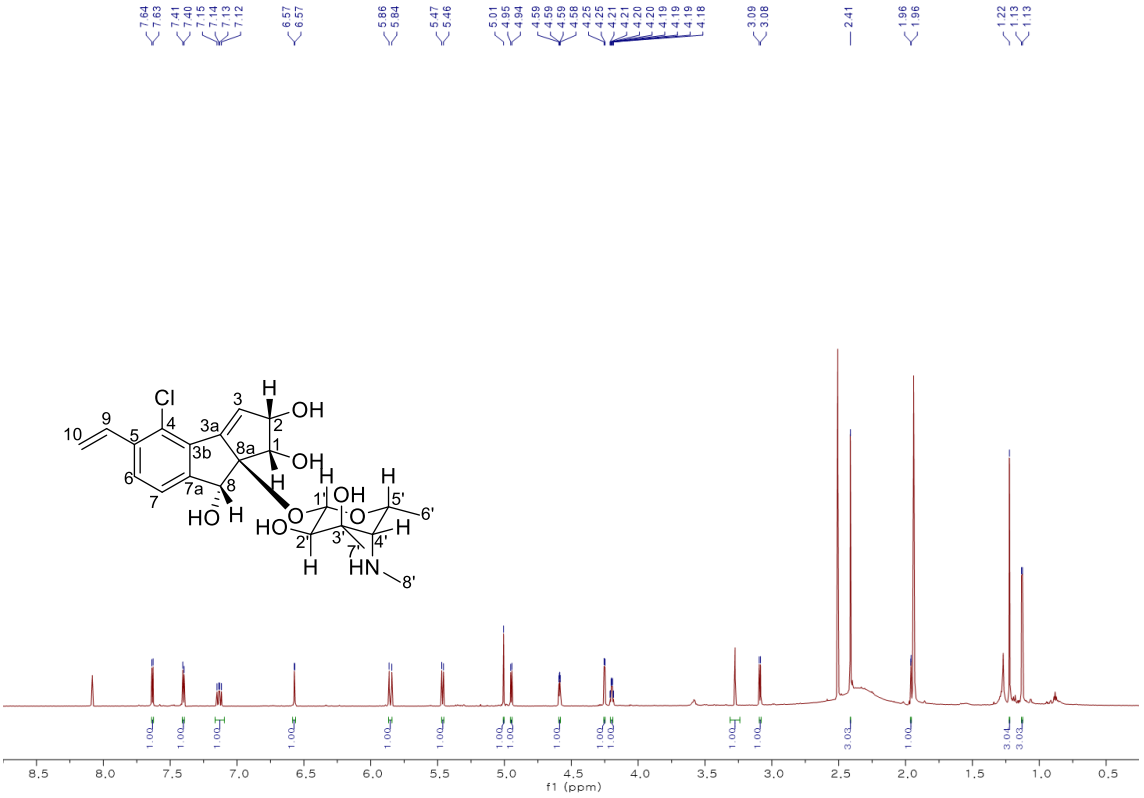


Figure S9. ^{13}C NMR spectrum of jejucarboside C (**2**) at 850 MHz in CD_3CN.

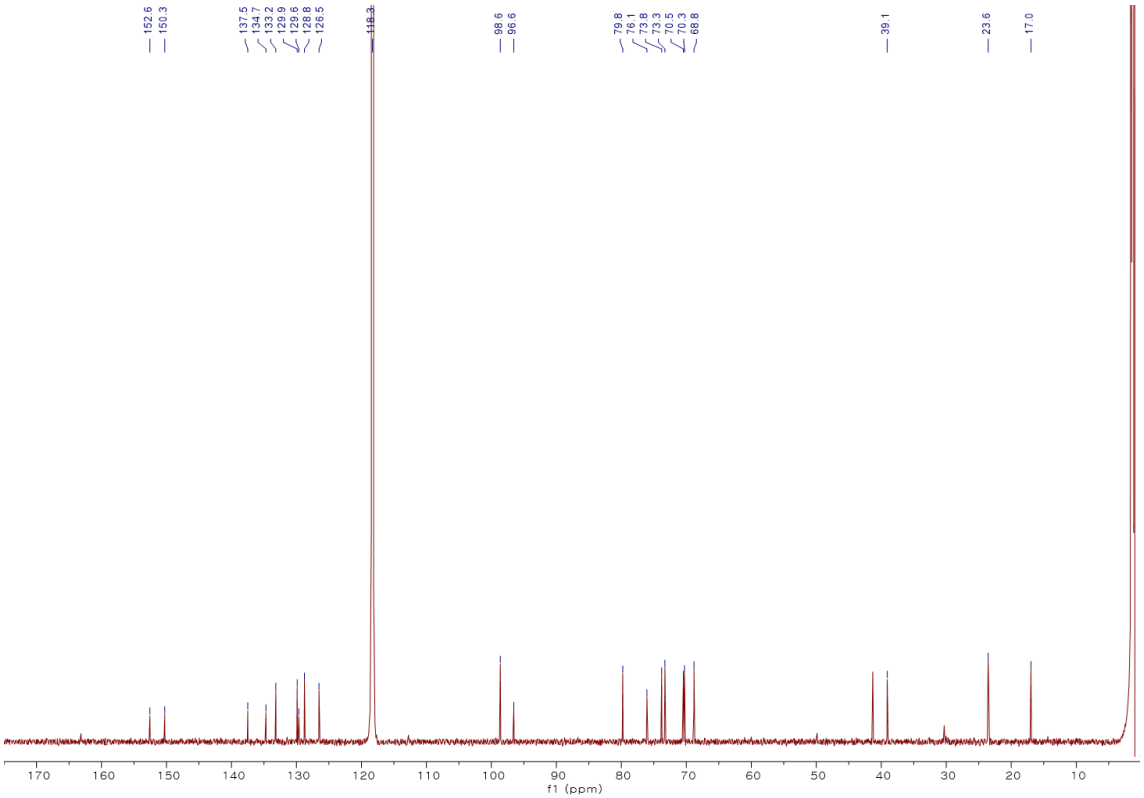


Figure S10. COSY NMR spectrum of jejucarboside C (**2**) at 850 MHz in CD_3CN.

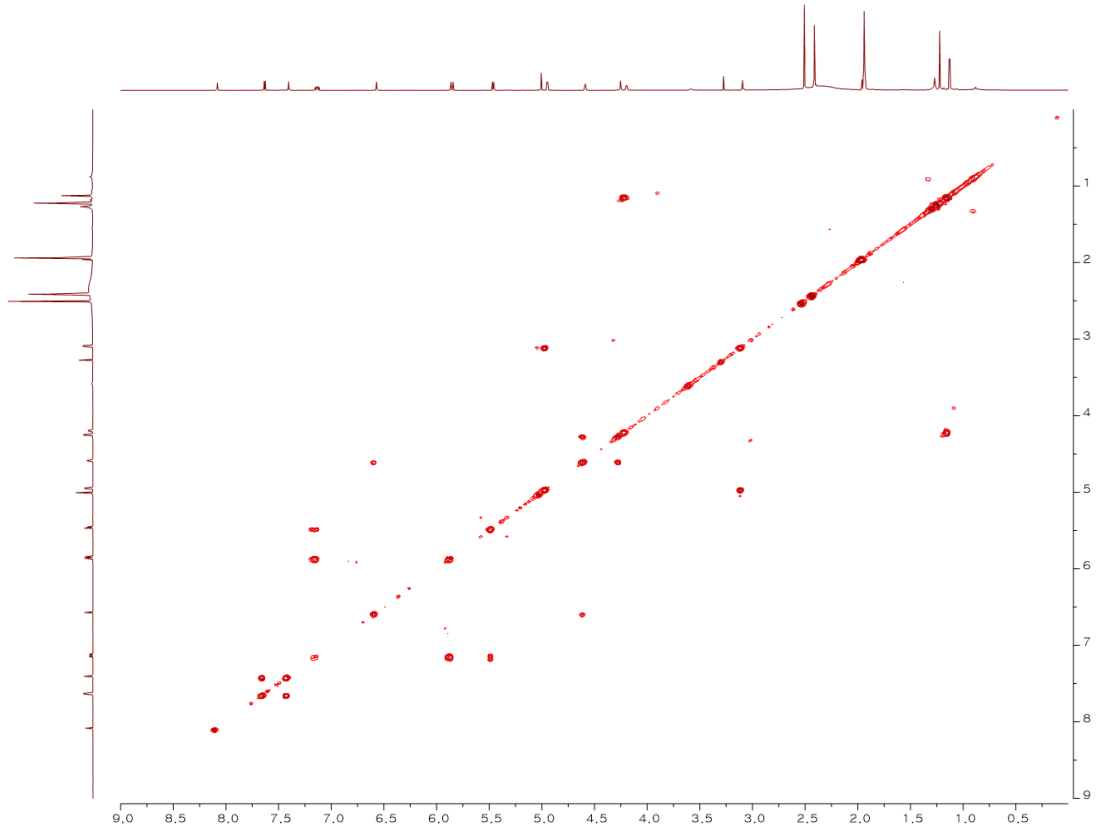


Figure S11. HSQC NMR spectrum of jejucarboside C (**2**) at 850 MHz in CD_3CN.

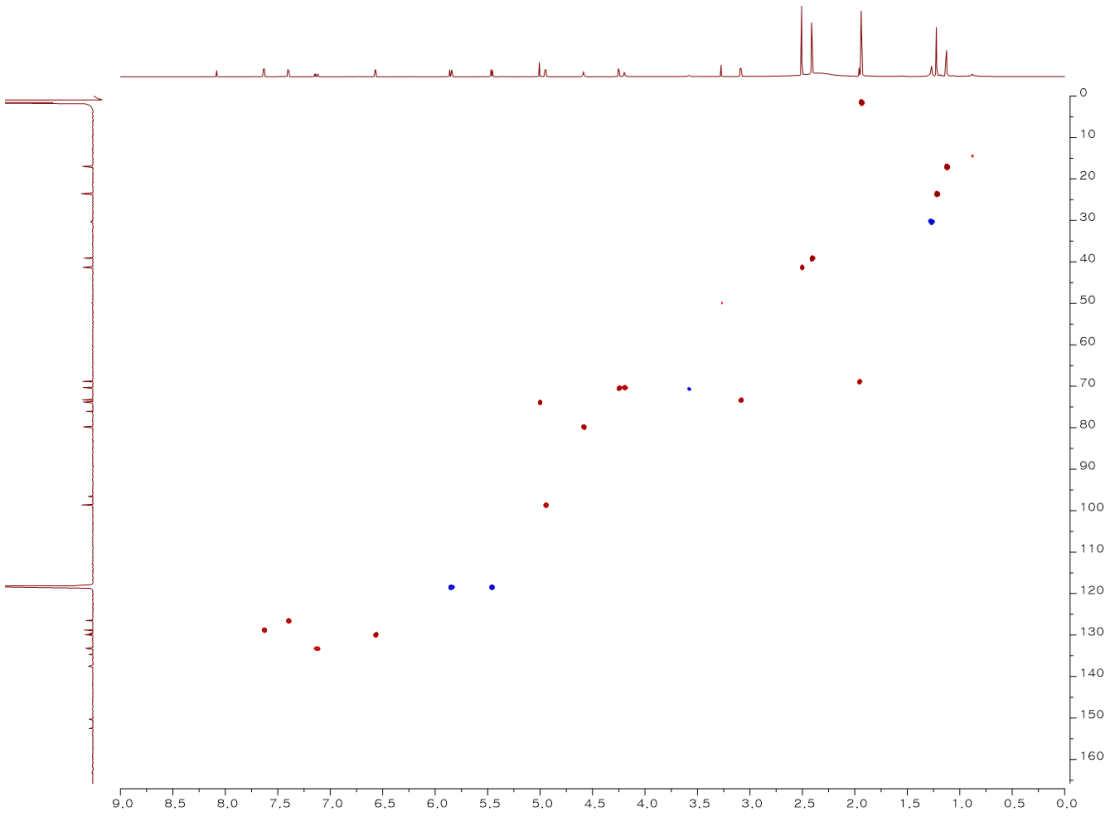


Figure S12. HMBC NMR spectrum of jejucarboside C (**2**) at 850 MHz in CD_3CN.

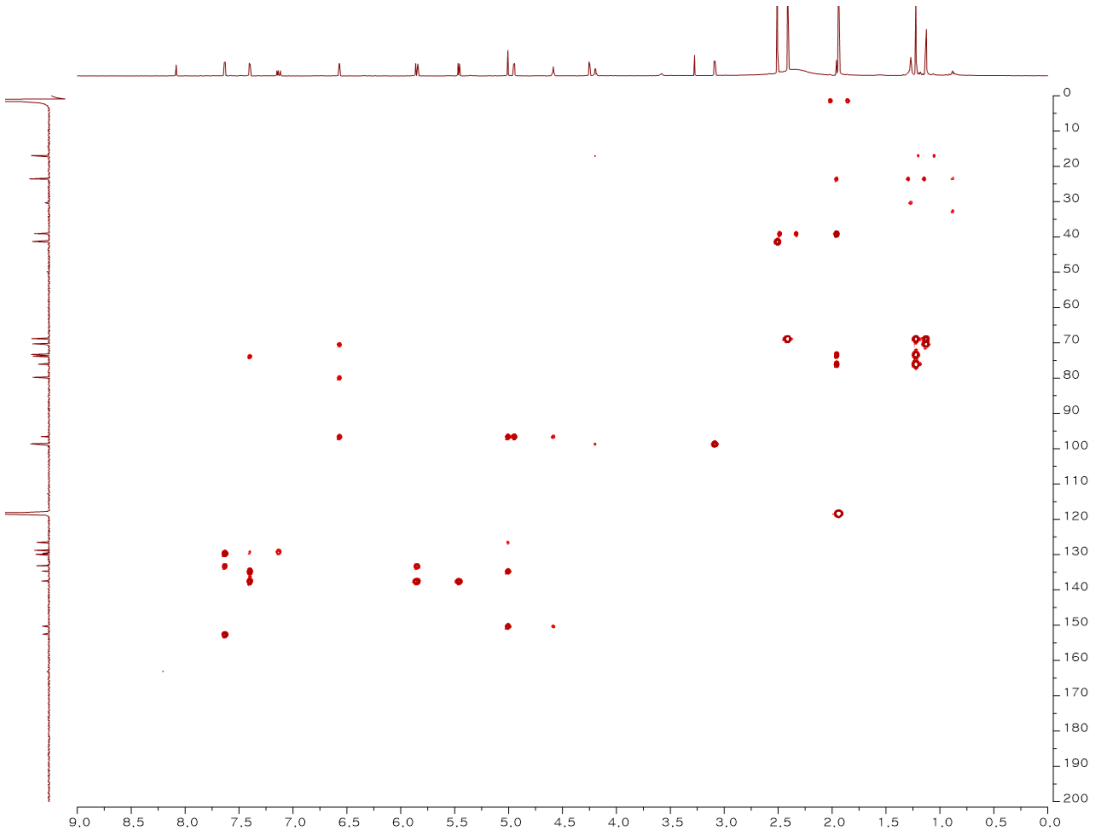


Figure S13. ROESY NMR spectrum of jejucarboside C (**2**) at 850 MHz in CD_3CN.

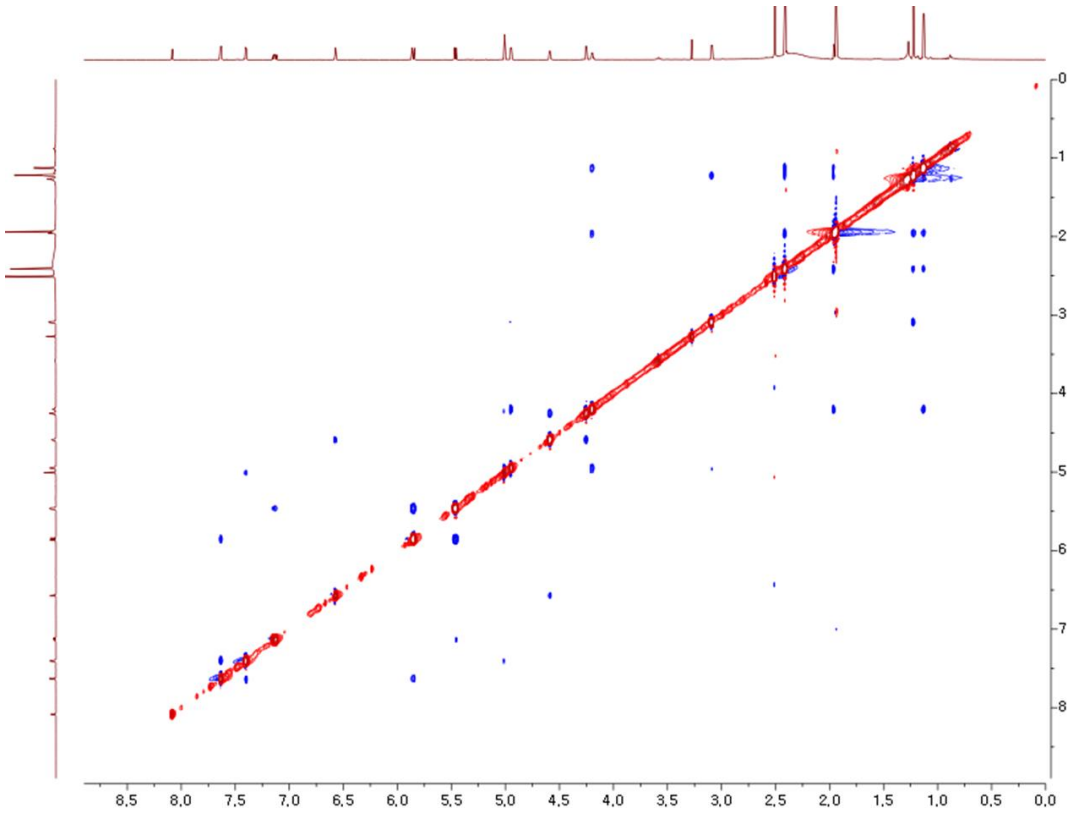


Figure S14. ^{1}H NMR spectrum of jejucarboside D (**3**) at 850 MHz in CD_3CN.

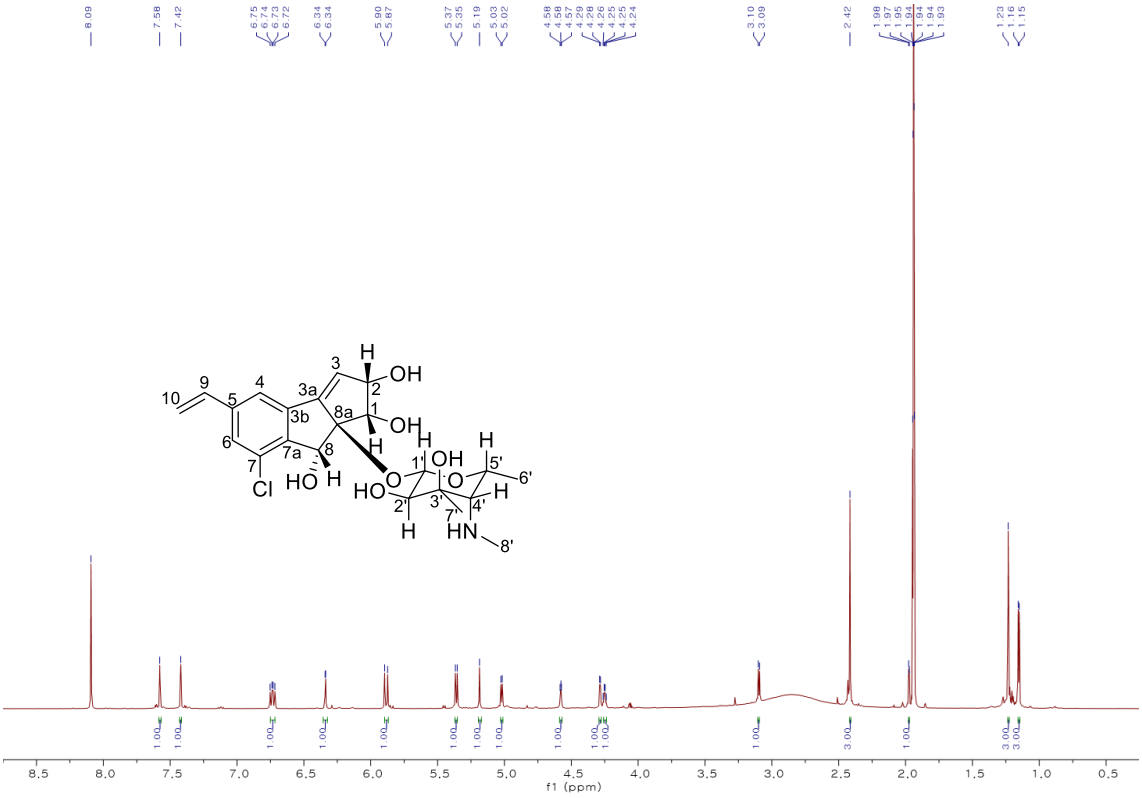


Figure S15. ^{13}C NMR spectrum of jejucarboside D (**3**) at 850 MHz in CD_3CN.

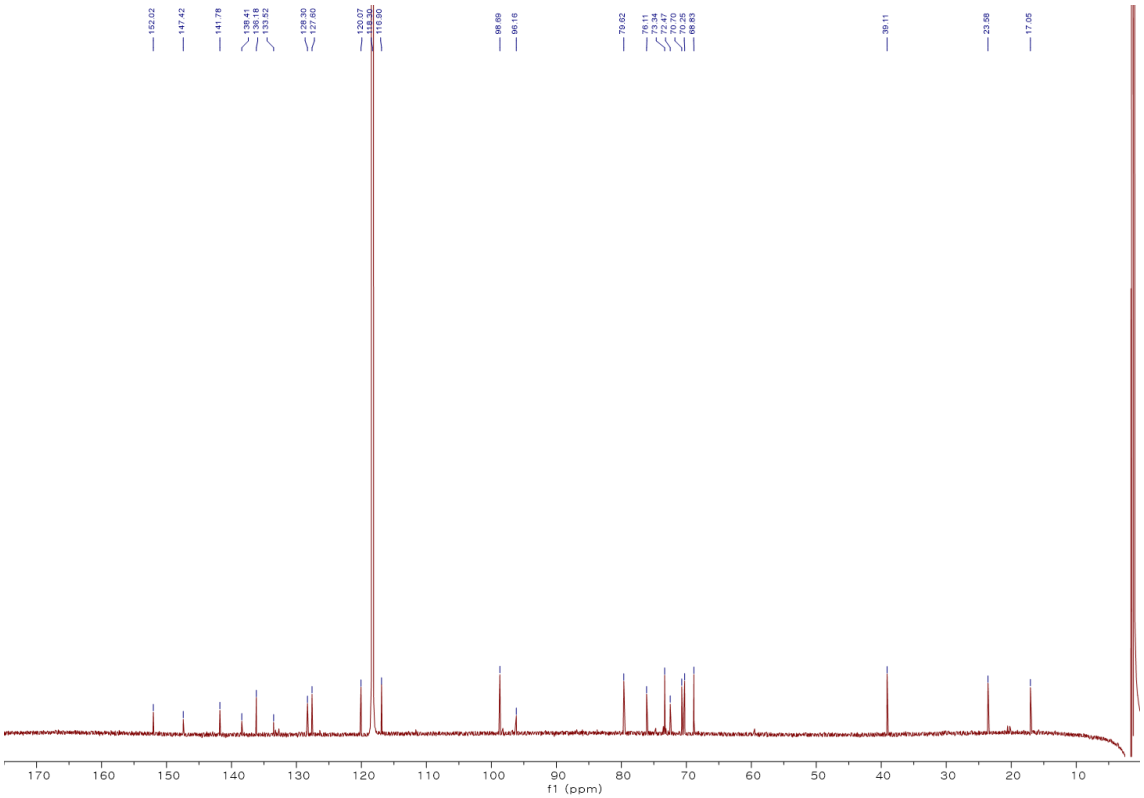


Figure S16. COSY NMR spectrum of jejucarboside D (**3**) at 850 MHz in CD_3CN.

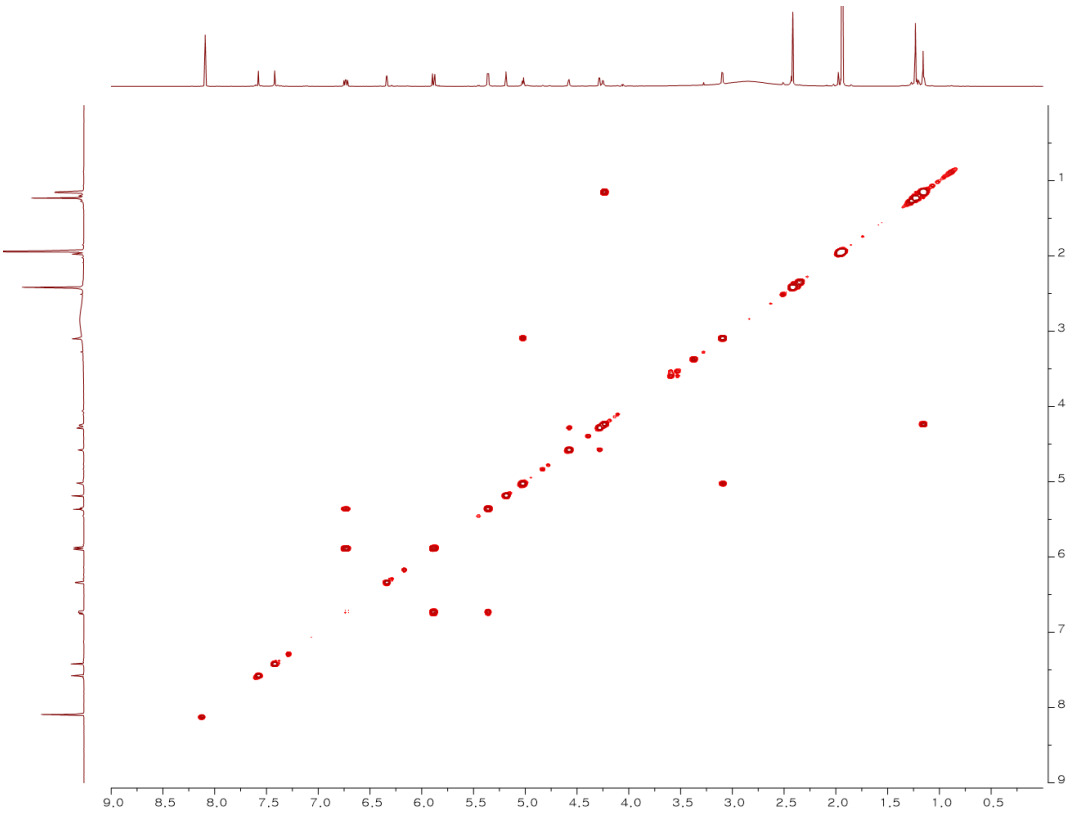


Figure S17. HSQC NMR spectrum of jejucarboside D (**3**) at 850 MHz in CD_3CN.

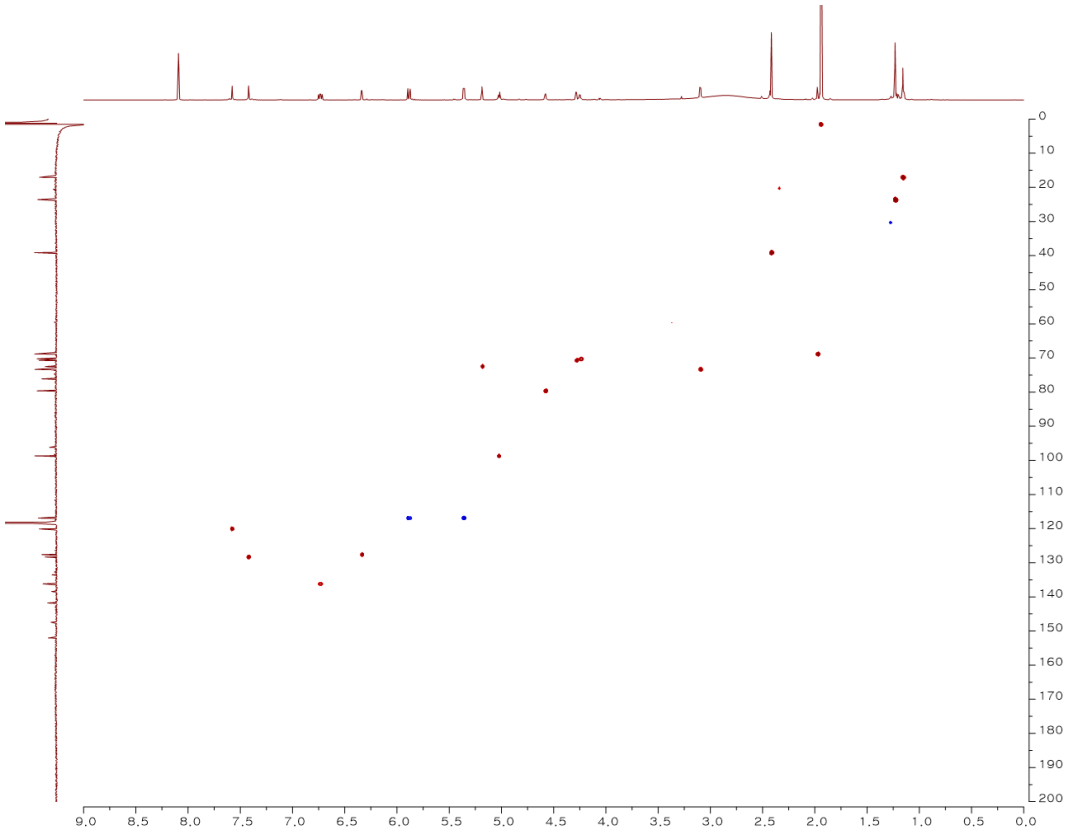


Figure S18. HMBC NMR spectrum of jejucarboside D (**3**) at 850 MHz in CD_3CN.

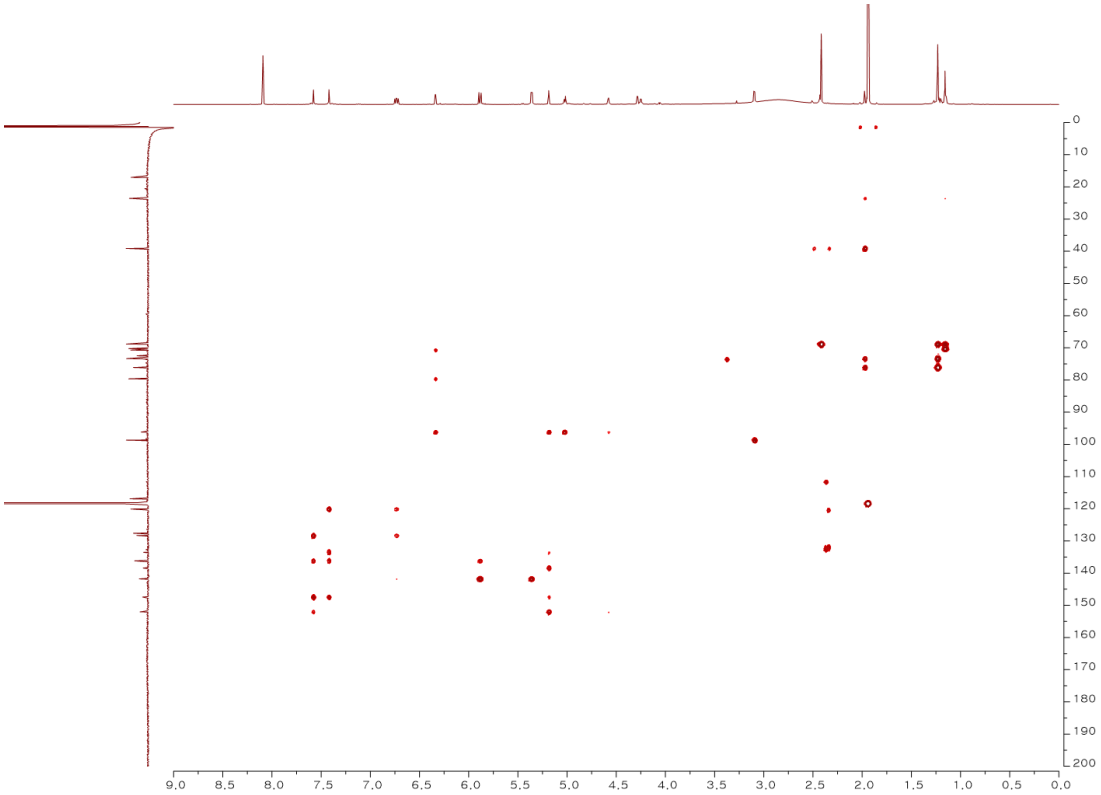


Figure S19. ROESY NMR spectrum of jejucarboside D (**3**) at 850 MHz in CD_3CN.

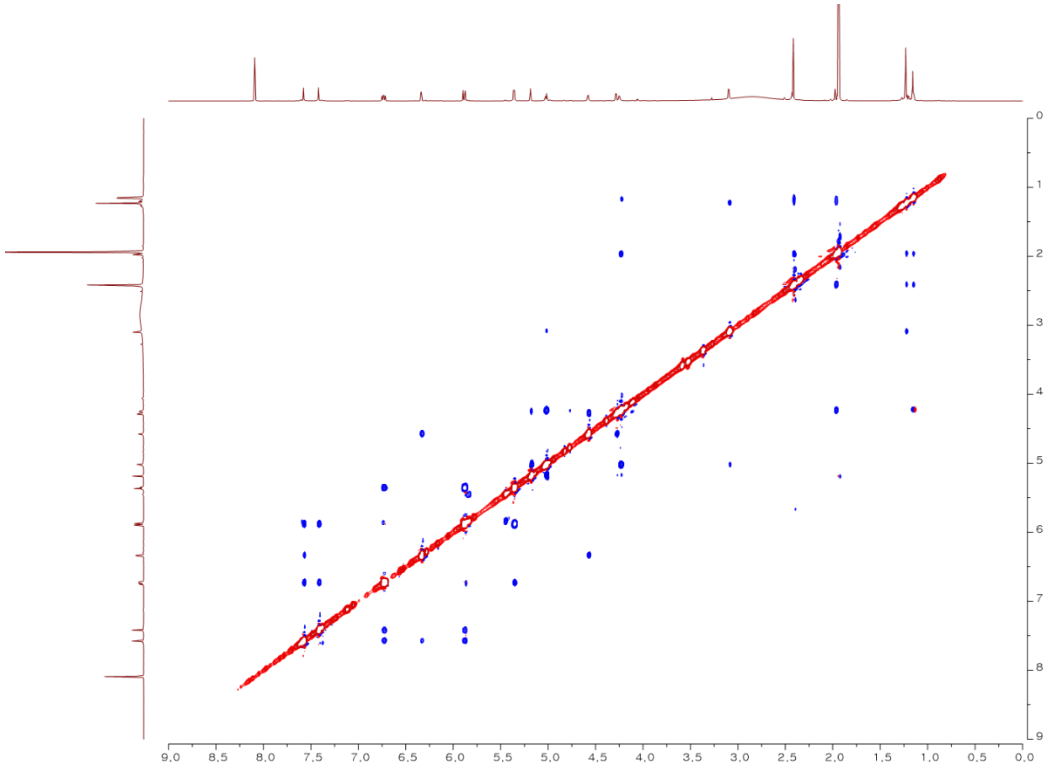


Figure S20. ^{1}H NMR spectrum of jejucarboside E (**4**) at 850 MHz in CD_3CN.

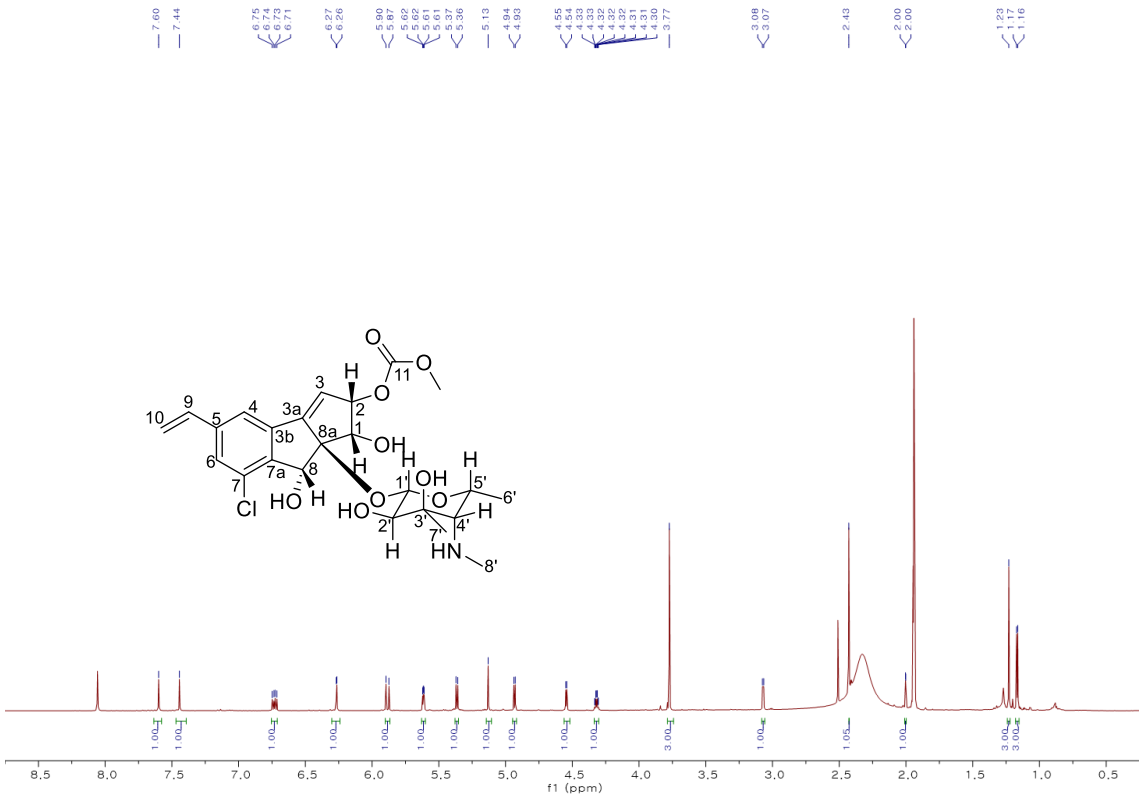


Figure S21. ^{13}C NMR spectrum of jejucarboside E (**4**) at 850 MHz in CD_3CN.

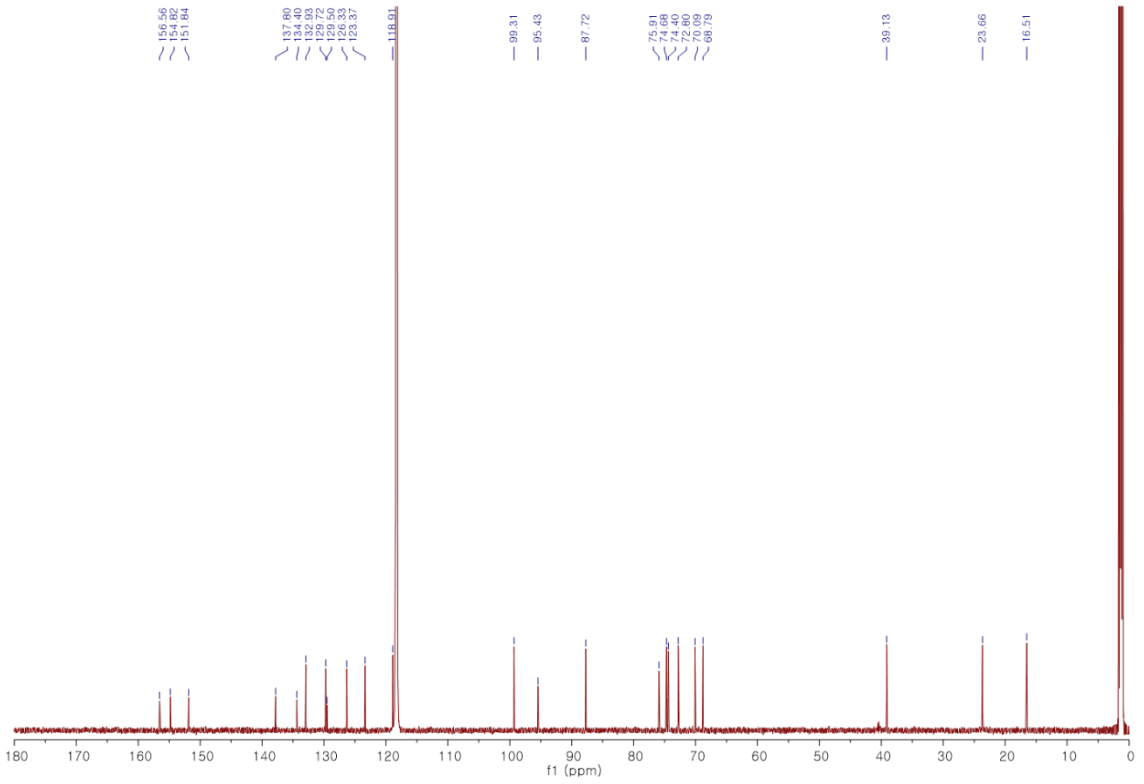


Figure S22. COSY NMR spectrum of jejucarboside E (**4**) at 850 MHz in CD_3CN.

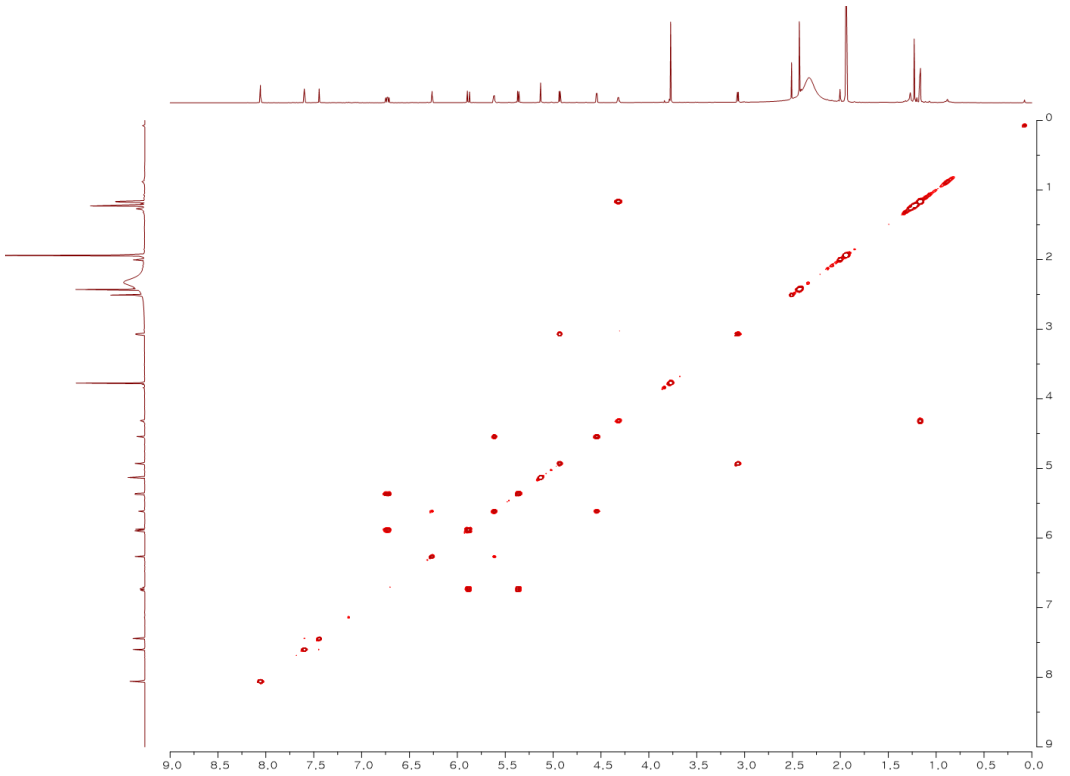


Figure S23. HSQC NMR spectrum of jejucarboside E (**4**) at 850 MHz in CD_3CN.

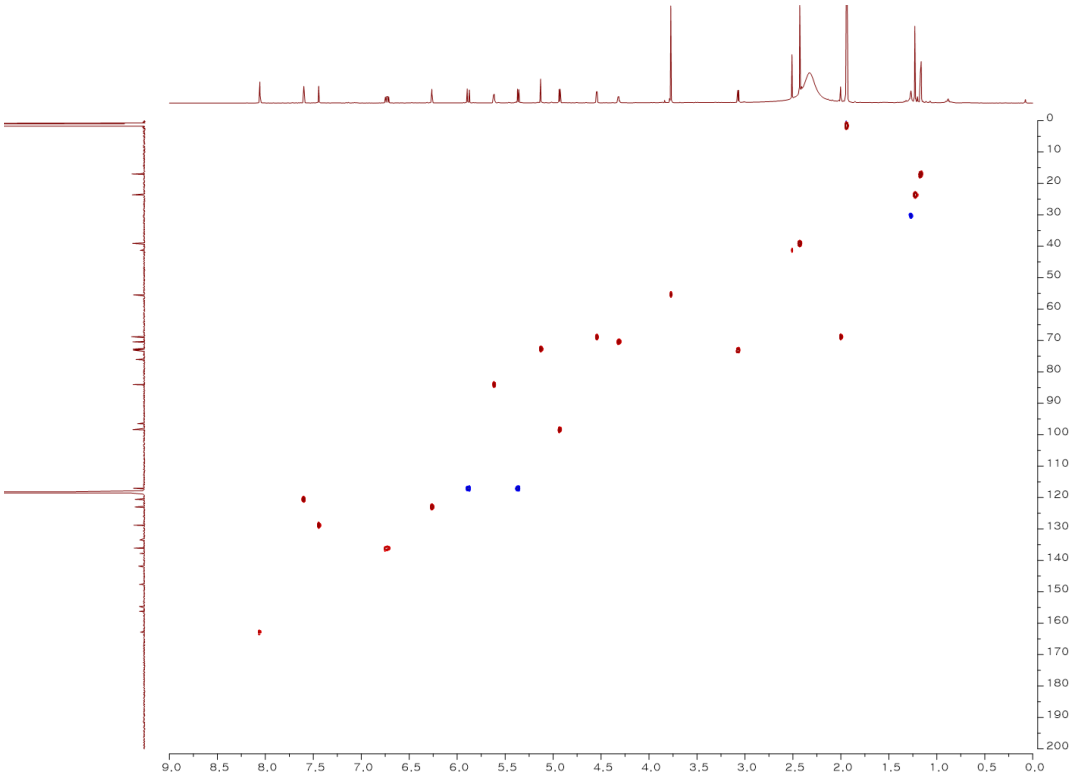


Figure S24. HMBC NMR spectrum of jejucarboside E (**4**) at 850 MHz in CD_3CN.

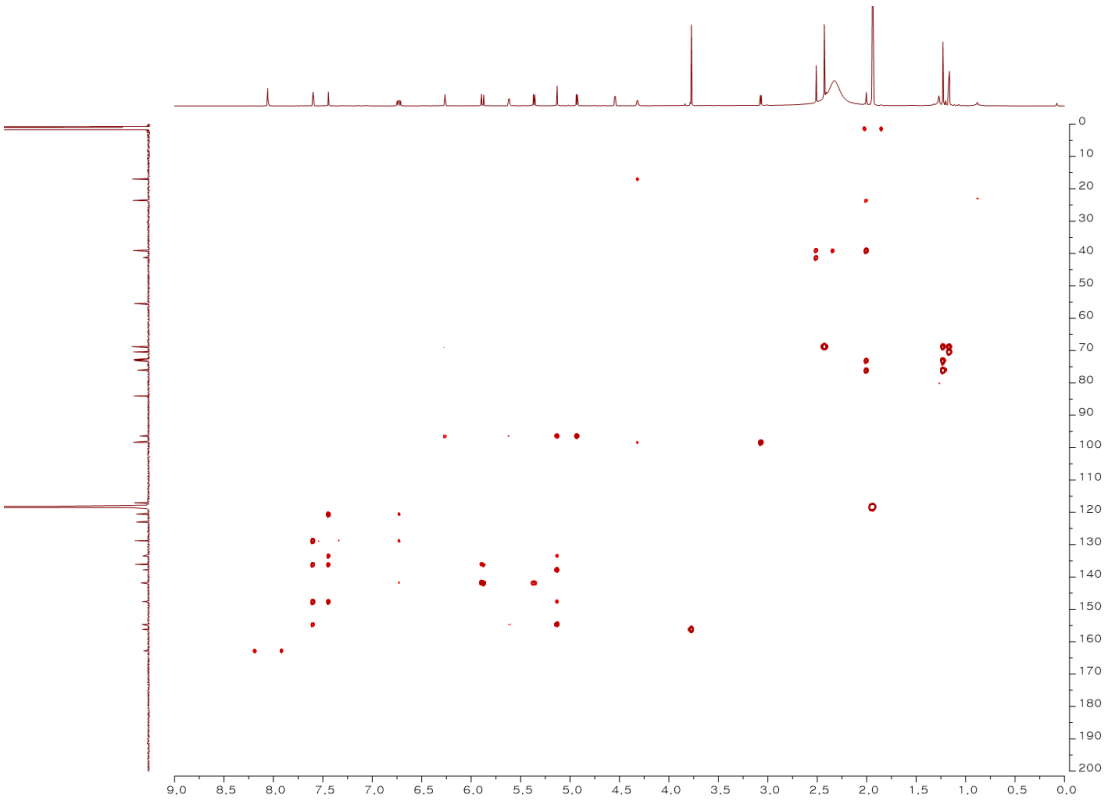


Figure S25. ROESY NMR spectrum of jejucarboside E (**4**) at 850 MHz in CD_3CN.

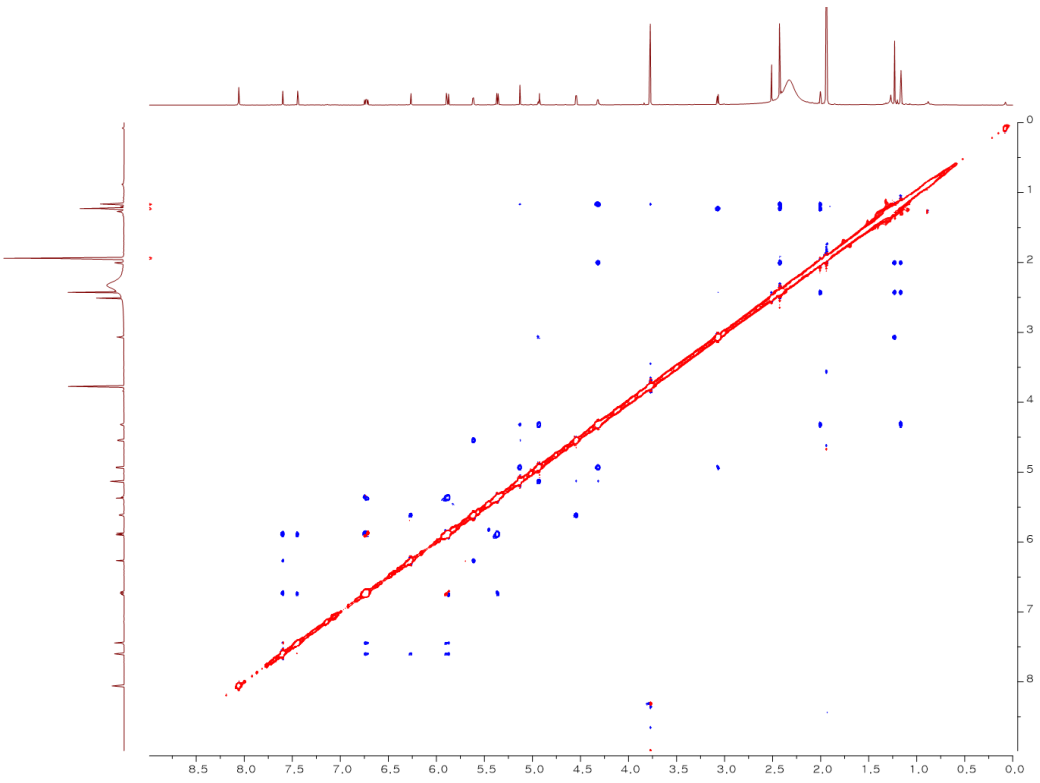


Table S1. NMR spectroscopic data of jejucarboside A (800 MHz, CD_3CN) and jejucarbosides B-E (**1-4**) (850 MHz, CD_3CN).

	Jejucarbosde A		**Jejucarboside B (1)**		**Jejucarboside C (2)**		**Jejucarboside D (3)**		**Jejucarboside E (4)**	
Position	δ_C, Type	δ_H, Mult (*J* in Hz)	δ_C, Type	δ_H, Mult (*J* in Hz)	δ_C, Type	δ_H, Mult (*J* in Hz)	δ_C, Type	δ_H, Mult (*J* in Hz)	δ_C, Type	δ_H, Mult (*J* in Hz)
1	74.4, CH	5.32, d (7.0)	74.6, CH	5.34, d (7.0)	70.5, CH	4.25, d (5.5)	70.7, CH	4.28, d (5.0)	68.9, CH	4.54, d (6.0)
2	87.7, CH	5.59, dd (7.0, 3.0)	87.5, CH	5.57, dd (7.0, 3.0)	79.8, CH	4.59, dd (5.5, 3.0)	79.6, CH	4.58, dd (5.0, 3.0)	84.0, CH	5.61, dd (6.0, 3.0)
3	123.4, CH	6.53, d (3.0)	122.4, CH	6.30, d (3.0)	129.9, CH	6.57. d (3.0)	127.6, CH	6.34, d (3.0)	123.0, CH	6.27, d (3.0)
3a	154.8, C		156.5, C		150.3, C		152.0, C		154.6, C	
3b	134.4, C		137.7, C		134.7, C		138.4, C		137.8, C	
4	129.5, C		120.3, CH	7.68, s	129.6, C		120.1, CH	7.58, s	120.5, CH	7.60, s
5	137.8, C		142.1, C		137.5, C		141.8, C		141.8, C	
6	129.7, CH	7.73, d (8.0)	129.2, CH	7.49, s	128.8, CH	7.63, d (8.0)	128.3, CH	7.42, s	128.8, CH	7.44, s
7	126.3, CH	7.46, d (8.0)	133.2, C		126.5, CH	7.40, d (8.0)	133.5, C		133.5, C	
7a	151.8, C		146.6, C		152.6, C		147.4, C		147.6, C	
8	74.7, CH	5.01, s	73.3, CH	5.21, s	73.8, CH	5.01, s	72.5, CH	5.19, s	72.7, CH	5.13, s
8a	95.4, C		95.1, C		96.6, C		96.2, C		96.4, C	
9	132.9, CH	7.16, dd (18.0, 11.0)	135.9, CH	6.76, dd (18.0, 11.0)	133.2, CH	7.13, dd (17.5, 11.0)	136.2, CH	6.75, dd (18.0, 11.0)	136.1, CH	6.73, dd (17.5, 11.0)
10	118.9, CH_2	5.52, dd (11.0, 1.0)	117.4, CH_2	5.40, d (11.0)	118.3, CH_2	5.46, d (11.0)	116.9, CH_2	5.36, d (11.0)	117.1, CH_2	5.36, d (11.0)
		5.91, dd (18.0, 1.0)		5.92, d (18.0)		5.85, d (17.5)		5.88, d (18.0)		5.88, d (17.5)
11	156.6, C		156.5, C						156.2, C	
11-OMe									55.5, CH_3	3.77, s
1'	99.3, CH	4.27, d (7.5)	99.7, CH	4.38, d (7.5)	98.6, CH	4.95, d (8.0)	99.3, CH	5.02, d (8.0)	98.3, CH	4.93, d (8.0)
2'	72.8, CH	3.01, d (7.5)	72.8, CH	2.99, d (7.5)	73.3, CH	3.08, d (8.0)	72.8, CH	3.09, d (8.0)	73.1, CH	3.07, d (8.0)
3'	75.9, C		76.0, C		76.1, C		75.9, C		76.1, C	
4'	68.8, CH	1.88, m	68.8, CH	1.88, s	68.8, CH	1.95, d (1.5)	68.8, CH	1.97, d (1.5)	68.8, CH	2.00, d (1.5)
5'	70.1, CH	3.86, m	70.0, CH	3.97, q (6.5)	70.3, CH	4.20, qd (6.5, 1.5)	70.1, CH	4.24, qd (6.5, 1.5)	70.4, CH	4.32, qd (6.5, 1.5)
6'	16.5, CH_3	1.08, d (6.5)	16.6, CH_3	1.10, d (6.5)	17.0, CH_3	1.13, d (6.5)	16.5, CH_3	1.15, d (6.5)	17.0, CH_3	1.17, d (6.5)
7'	23.7, CH_3	1.20, s	23.6, CH_3	1.19, s	23.6, CH_3	1.22, s	23.7, CH_3	1.23, s	23.6, CH_3	1.23, s
8'	39.1, CH_3	2.42, s	39.1, CH_3	2.40, s	39.1, CH_3	2.41, s	39.1, CH_3	2.41, s	39.1, CH_3	2.43, s

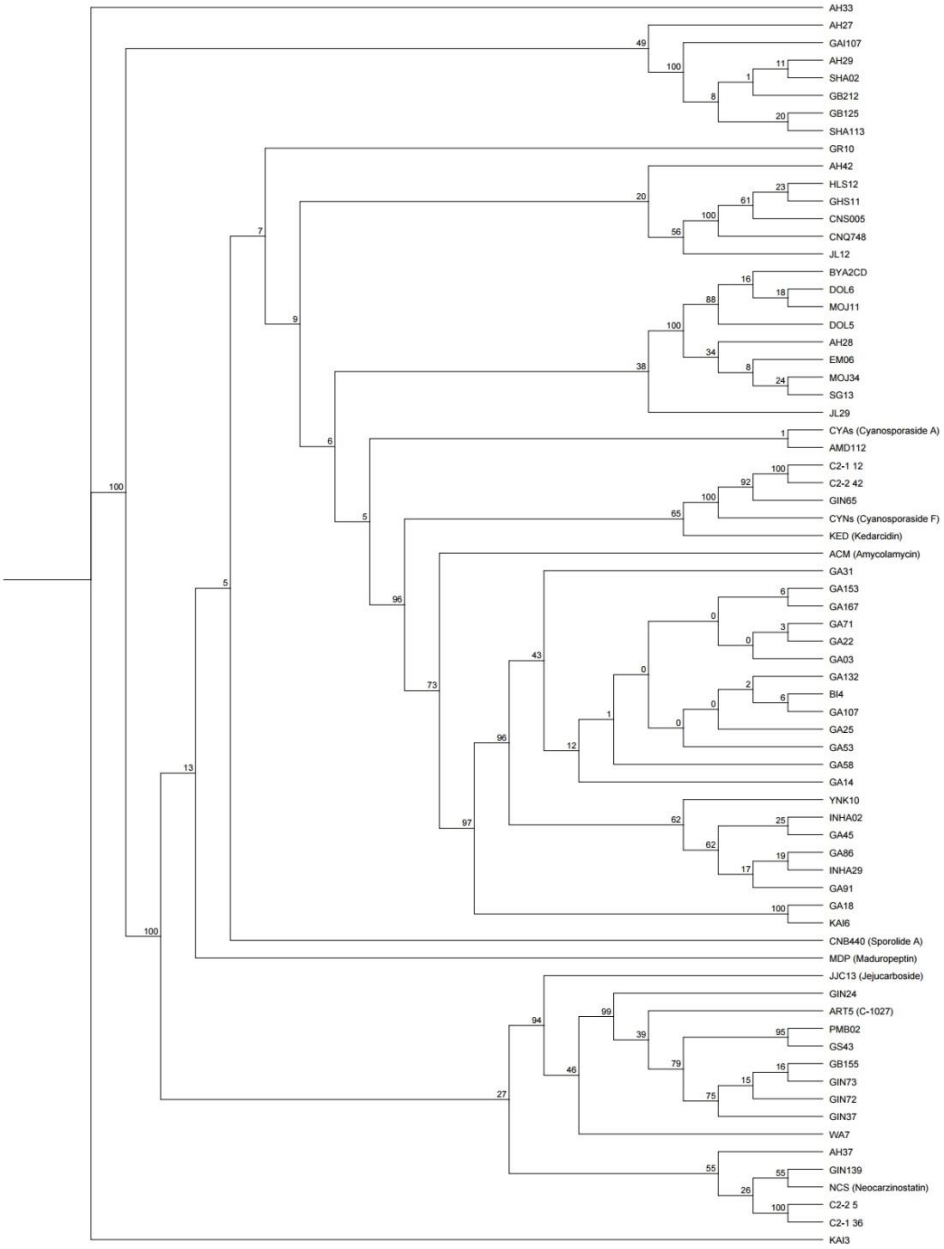
Table S2. Composition of media used for culture optimization.

YEME	**A1C**
Yeast 4g/L Glucose 4g/L Malt 10g/L Sea salt 14g/L	Starch 10g/L Yeast 4g/L Phytone peptone 2g/L Calcium Carbonate 1g/L Sea salt 14g/L
YEME+Humic acid	**YEME+L-ornithine**
Yeast 4g/L Glucose 4g/L Malt 10g/L Humic acid 1g/L Sea salt 14g/L	Yeast 4g/L Glucose 4g/L Malt 10g/L L-Ornithine 2g/L Sea salt 14g/L
YEME+Humic acid+ L-ornithine	**A1**
Yeast 4g/L Glucose 4g/L Malt 10g/L Humic acid 1g/L L-Ornithine 2g/L Sea salt 14g/L	Starch 10g/L Yeast 4g/L Peptone 2g/L Sea salt 14g/L
DSY	**GLY**
Dextrin 10g/L Soytone (soybean meal) 4g/L Yeast 1g/L NaCl 0.5g/L Sea salt 14g/L	Glycerol 20g/L Lactose 10g/L Malt 5g/L Yeast 5g/L Calcium Carbonate 1g/L Sea salt 14g/L
modified K	**YPM**
Yeast 3g/L Glucose 2g/L Mannitol 2g/L Malt 5g/L Starch 5g/L Soytone 5g/L Calcium Carbonate 1g/L Sea salt 14g/L	Yeast 2g/L Mannitol 4g/L Peptone 2g/L Sea salt 14g/L

Phylogenetic analysis of Enediyne Core Gene Amplicons

Translated amino acid sequences of the amplicons and references were aligned using MUSCLE (v5.1) [1], and gap-free consensus regions were extracted for their phylogenetic analysis. Phylogenetic analysis of the alignments was proceeded using RAxML (v8.2.11) [2] plugin installed in Geneious Prime software (v2023.0.4) with GAMMA DAYHOFF protein model and 100 bootstrap replicating number. The final bootstrap support percentage values were calculated by best-scoring maximum likelihood tree searching option in the plugin.

Figure S26. Phylogenetic tree of the amplicons.



References

(1) Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1792-1797. DOI: 10.1093/nar/gkh340.

(2) Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312-1313. DOI: 10.1093/bioinformatics/btu033.