Supporting Information

Biochemical Characterization of a Multiple Prenyltransferase from *Tolypocladium inflatum*

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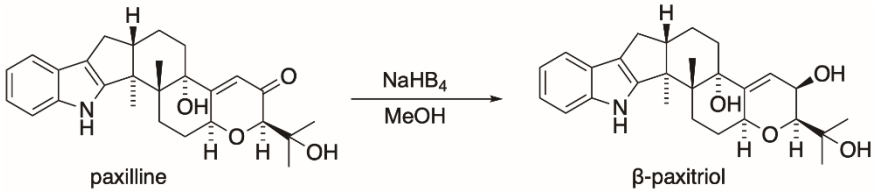
Contents

Supplementary experimental section

Compound identification based on GNPS analysis

Solid fermentation of *T. inflatum* was carried out in a 500 mL Fernbach flasks containing 80 g of rice and incubated at 25°C for 14 days. The fermented rice substrate was extracted with ethyl acetate, and the organic solvent was evaporated to dryness under vacuum to afford the crude extract. Crude extracts were subjected to UPLC-ESI-HRMS measurements (AB SCEIX Triple TOF 6600) with IDA acquisition modes. MS data preprocessing for feature-based molecular networking was performed using MZmine 2.53. MS data were analyzed via classical or feature-based molecular networking workflows, both of which are available in the GNPS web platform (https://gnps.ucsd.edu). The network file based on positive ion mode MS data can be found and accessed at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=5af9d8f55b1449c08dda185ea9c581c9. Cytoscape V3.10.1 was used for visualization of relationships among compounds with yfileds circle layout.

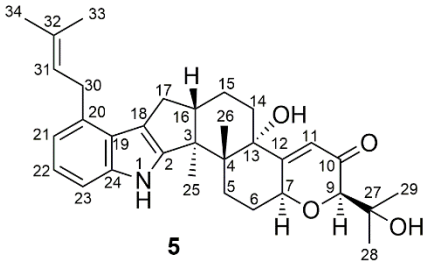
Synthesis of β-paxitriol



paxilline β-paxitriol

To a solution of paxilline (100.0 mg, 9.2 mmol) in MeOH (25 mL) was added $NaBH_4$ (52 mg, 55.0 mmol) and the mixture was stirred for 30 min at ambient temperature. The mixture was quenched by the addition of 1 mol NH_4Cl solution 12 ml .The mixture was added 1 mol HCl solution 25 ml and stirred for 30 min at ambient temperature. The aqueous layer was extracted with ethyl acetate , and the combined organic layers were washed with $NaHCO_3$, brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification of the residue by preparative liquid chromatograph to gave β-paxitriol (10.0 mg, 914.8 μmol, 10%). The ^1H NMR data (Fig. S21) are in good agreement with the reported data (Miles et al. 1992).

Table S1: ^{1}H and ^{13}C NMR data of the compounds 20-prenylpaxilline (**5**) and 22-prenylpaxilline (**10**).



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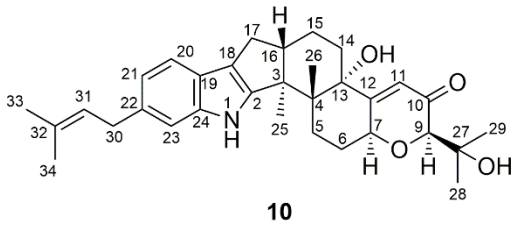

10

	5		**10**	
position	H-1	C-13	H-1	C-13
1	7.73 (1H, s)	-NH	7.64 (1H, s)	-NH
2	/	151.0	/	151.2
3	/	50.6	/	50.9
4	/	43.3	/	43.3
5	2.80 (1H, td, *J* = 13.6, 5.0 Hz) 1.47 (1H, dd, *J* = 13.2, 4.0 Hz)	28.2	2.82 (1H, m) 1.43 (1H, m)	28.1
6	2.33 (1H, m) 1.80 (1H, m)	28.6	2.33 (1H, m) 1.90 (1H, m)	28.6
7	4.86 (1H, m)	72.7	4.85 (1H, t, *J* = 9.0 Hz)	72.7
8	/	/	/	/
9	3.73 (1H, d, *J* = 2.0 Hz)	83.4	3.71 (1H, d, *J* = 1.8 Hz)	83.3
10	/	199.4	/	199.4
11	5.89 (1H, d, *J* = 7.9 Hz)	119.8	5.87 (1H, d, *J* = 1.6 Hz)	119.6
12	/	168.3	/	168.4
13	/	77.7	/	77.6
14	2.05 (1H, m) 1.66 (1H, m)	34.5	2.05 (1H, m) 1.67 (1H, m)	34.4
15	2.05 (1H, m) 1.82 (1H, m)	21.0	2.05 (1H, m) 1.82 (1H, m)	21.0
16	2.89 (1H, m)	49.6	2.82 (1H, m)	49.5
17	2.89 (1H, m) 2.61 (1H, m)	29.2	2.74 (1H, m) 2.42 (1H, m)	27.4
18	/	117.1	/	117.4
19	/	124.5	/	123.3
20	/	133.2	7.35(1H, d, *J* = 8.0 Hz)	118.6
21	6.86 (1H, d, *J* = 7.1 Hz)	119.2	6.92(1H, d, *J* = 8.0 Hz)	120.9
22	7.02 (1H, t, *J* = 7.1Hz)	121.2	/	134.7
23	7.15 (1H, d, *J* = 7.1 Hz)	109.4	7.11(1H, s)	110.9
24	/	139.8	/	140.4
25	1.54 (3H, s)	16.3	1.62 (3H, s)	16.3
26	1.05 (3H, s)	19.8	1.02 (3H, s)	19.8
27	4.10 (1H, s)	72.6	4.10 (1H, s)	72.7
28	1.31 (3H, s)	24.3	1.31 (3H, s)	24.3
29	1.31 (3H, s)	26.7	1.31 (3H, s)	26.7
30	3.61 (2H, d, *J* = 7.0 Hz)	32.1	3.41 (2H, d, *J* = 7.2 Hz)	34.6
31	5.41 (1H, t, *J* = 7.0 Hz)	123.7	5.36 (1H, t, *J* = 7.2 Hz)	124.3
32	/	132.0	/	131.9
33	1.77 (3H, s)	18.1	1.74 (3H, s)	17.8
34	1.76 (3H, s)	25.9	1.74 (3H, s)	25.7

Table S2: DNA and protein sequences of *tolF*. Highlighted in yellow is the intron region.

Gene	DNA sequence	Protein sequence
TorF	ATGACTTTTGACAAGGAGGCGGTGACAGCTCCAGCACGCG ATAGCAAACATGCTGCAGACCTGGAATACTGGACGCAACAT GTTGTCCCTATTATCAGCTCCCTCCTAAAGTCTGCCGGATCC TACTCGCCTGACGACCAGGACGCGCACATACGTACCCTATC AGAACATGTCTTCCCGAACCTTGGTCCGCGGCCATCCATGG CTCATACCAGGTCCTTTTTGACCCAGACCGGCTCCCCTCTCC AGCCCAGCATCAACTTCAGCTCCGGAAAGCCCCAAGTACGT TACTGCTGGGAGCTGCTGGGAGCTCAAGGCGGCAGCGATG GCGACCCGCTTGCGGTGGAGGCAGCGCGAGAGATACTGTC TTATCTTTCCACGGCCTTTGGCTTCAGCACACGATGGAGCG ACGCCTGGCTGTCCGCATTTGCTCCAACACTAGAAGAAGCG AAATCCGTCCAGGTCAAGCTCCCGAAGTGGCTGGCGAGCTT CACGTCGGCGGAGGAAGAAGTGCCCGCGTTGAAGCGACTT CCCTTTGCCTTTGTTGCCTTTGACTTGAGTGGCCCCAAGAC GTCTATGAAGGCGTACTTTAATCCCAAGGGCAAGGAAATCG CAACTGGGAAGCCAGCAGCGGATGCGACTTGGAGTACTCTT CGCAGTCTGAAACCGTCTCTGAACACGGCGTCAATCGACAT CCTTGAGCAGTGAGTCCGAGCTCGGCCTTGGCCATTTGATG TTTCTTCAATGTTCAGACATTTGCTTACTTCGTCTGGAACAG ATTCCTTGCCGAACGCGCAGTACCTTCCACGGTCGAGCTTG TGGGAATCGACTGTGTCGACGAAGCCAGTCTGTCGGATGCA AGGGTCAAGCTCTACGTCTATACCTTGAGCAACTCCTTCGA AACGGTTCGCGACTACGTGACCCTGGGCGGCCGTCTCCAGG ATGAGACTACCTTGAAGGGCTTGGACATCTTGCACGACATT TGGCACCTCCTGCTCCAAGAGCCCGAAGGCGTCAACGACG ACTACAACAAGCCCGTCAACGACGGTTCCATCCTCTGCCAG AAGCTGTACTTTAGCTTCGAGATGAGGCCCGGCAGGGAGCT TCCCGAAGTCAAGTCCTACGTACCGACTTGGAACTATGTAC GGAGCGATGCGGAAACCGTTCAAAACTACGAGGAGGTCTT CCGAAGATGCGGGTACGAGTGGGGAGAGGATGGGAGGTAC AAGAAACTGTTCGAGAGTGCTTTGTGTGTTTCCCTCCCTAC CATGATGTAAGCATTCACTAACCGTTCATTCTCAGCGGGCCG GTGGAACACAACCGCCCGAAGCCGGTCCATTGCGACGCAT CGTTCCTATACTCCGAAAAGAAGGGGACATATCAGACACTG TACTACAGCCCACCGCTCGGAGAAGAAGAATACAAGTAG	MTFDKEAVTAPAR DSKHAADLEYWT QHVVPIISSLLKSA GSYSPDDQDAHIR TLSEHVFPNLGPRP SMAHTRSFLTQTG SPLQPSINFSSGKP QVRYCWELLGAQ GGSDGDPLAVEAA REILSYLSTAFGFS TRWSDAWLSAFAP TLEEAKSVQVKLP KWLASFTSAEEEV PALKRLPFAFVAFD LSGPKTSMKAYFN PKGKEIATGKPAA DATSTLRSLKPSLN TASIDILEQFLAER AVPSTVELVGIDCV DEASLSDARVKLY VYTLSNSFETVRD YVTLGGRLQDETT LKGLDILHDIWHL LLQEPEGVNDDYN KPVNDGSILCQKL YFSFEMRPGRELP EVKSYVPTWNYV RSDAETVQNYEEV FRRCGYEWGEDG RYKKLFESAFGPV EHNRPKPVHCDAS FLYSEKKGTYQTL YYSPPLGEEEYK

Table S3: Plasmids used in this study.

Primer	Sequence 5'-3'	Restriction site	Vector
TolF-F1	ccggaattcgagctcggtaccATGACTTTTGACAAGGAGGCGGTG	*KpnI*	pUARA2
TolF-R1	tactacagatccccgggtaccCTACTTGTATTCTTCTTCTCCGAG		
TolF-F2	ctcggtaccctcgagggatccATGACTTTTGACAAGGAGGCGGTG	*BamHI*	pCold
TolF-R2	gacaagcttgaattcggatccCTACTTGTATTCTTCTTCTCCGAG		
TolF-F3	caccatcaccatcacggatccATGACTTTTGACAAGGAGGCGGTG	*BamHI*	pQE30
TolF-R3	accgagctcgcatgcggatccCTACTTGTATTCTTCTTCTCCGAG		
TolF-F4	cagcaaatgggtcgcggatccATGACTTTTGACAAGGAGGCGGTG	*BamHI*	pET28a
TolF-R4	acggagctcgaattcggatccCTACTTGTATTCTTCTTCTCCGAG		
TolF-F5	cgcgatatcgtcgacggatccATGACTTTTGACAAGGAGGCGGTG	*BamHI*	pMal-c5x
TolF-R5	acctgcagggaattcggatccCTACTTGTATTCTTCTTCTCCGAG		
TerF-F1	ccggaattcgagctcggtaccATGACTATCGACAAGAAGCCGGTG	*KpnI*	pUARA2
TerF-R1	tactacagatccccgggtaccCTATTCGTACTCTTCGCCATCTTTC		
TerF-F2	cgcgatatcgtcgacggatccATGACTATCGACAAGAAGCCGGTG	*BamHI*	pMal-c5x
TerF-R2	acctgcagggaattcggatccCTATTCGTACTCTTCGCCATCTTTC		

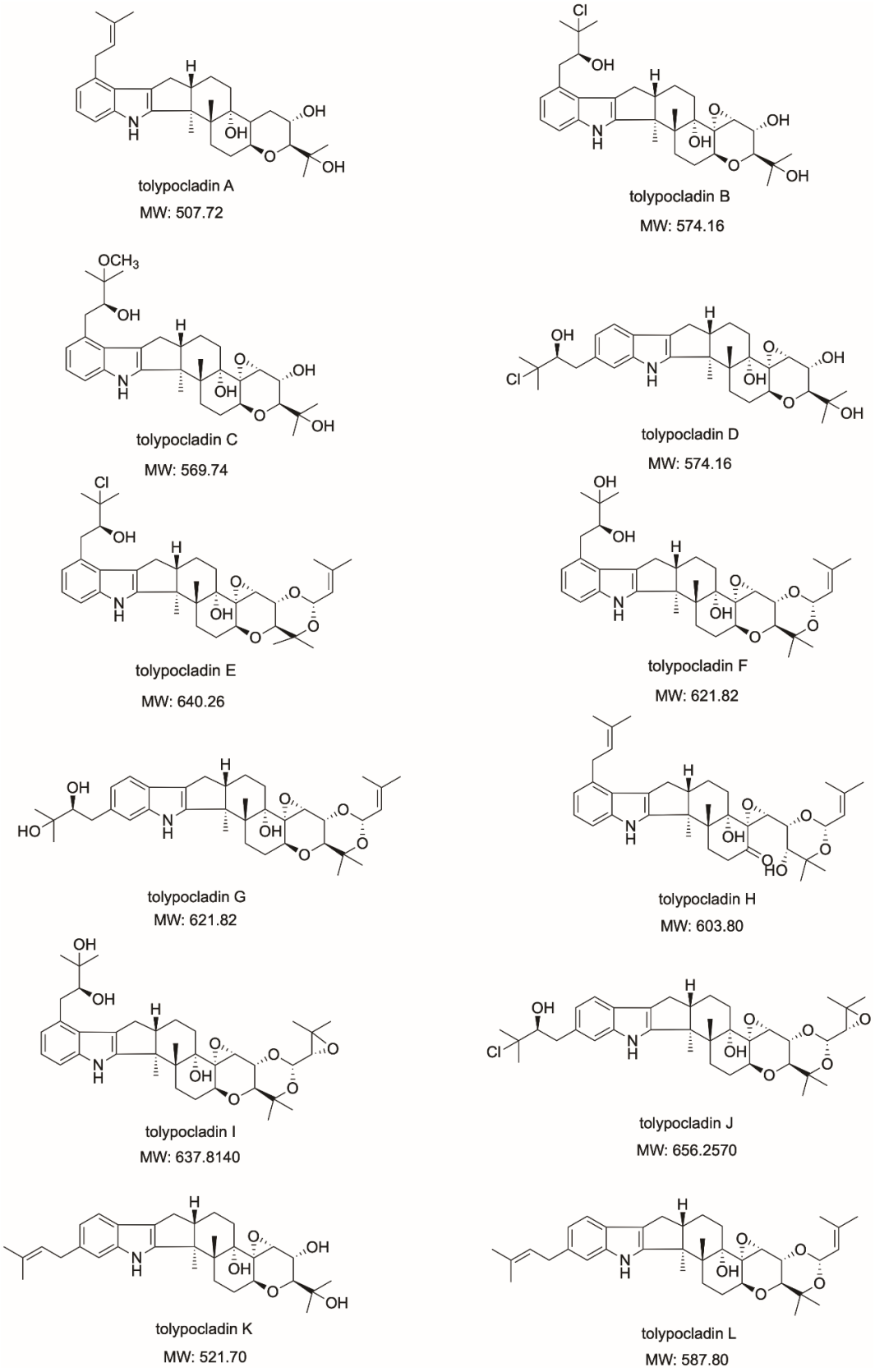

Fig. S1 Chemical structure of tolypocladin A-L (Xu et al. 2019a; Xu et al. 2019b).

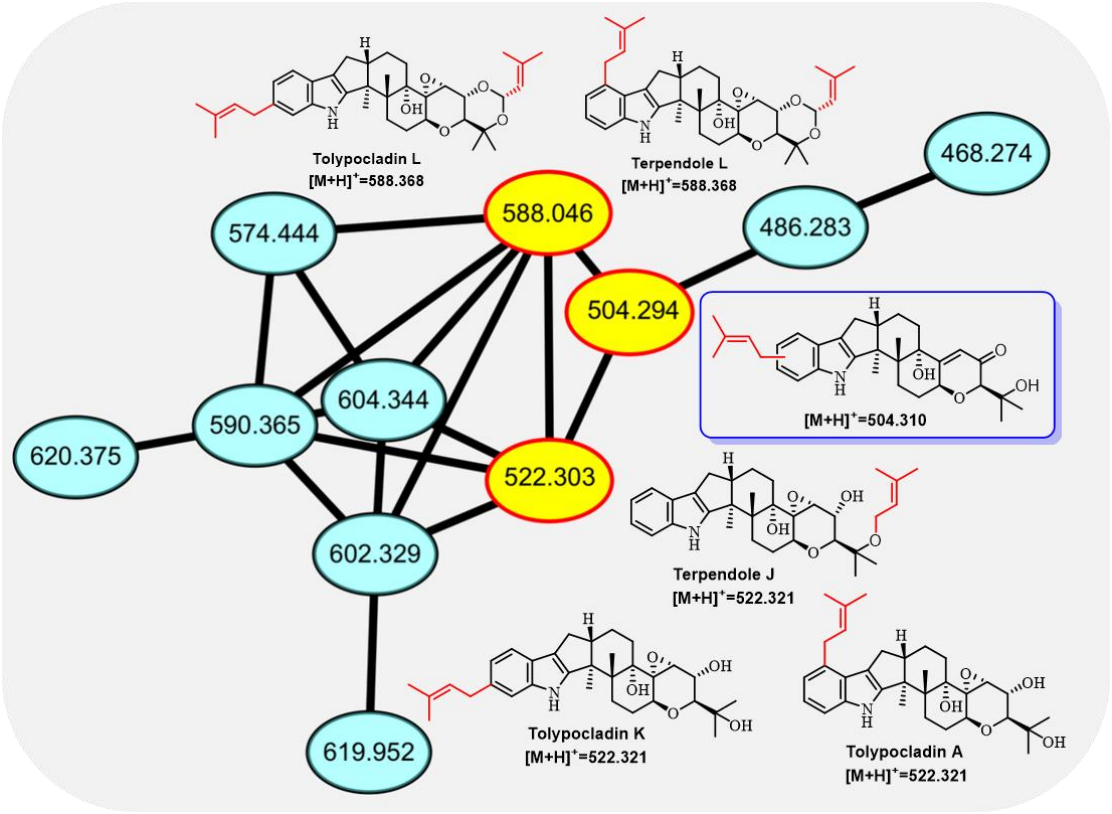

Fig. S2 Molecular network of the metabolic products from *T. inflatum*.

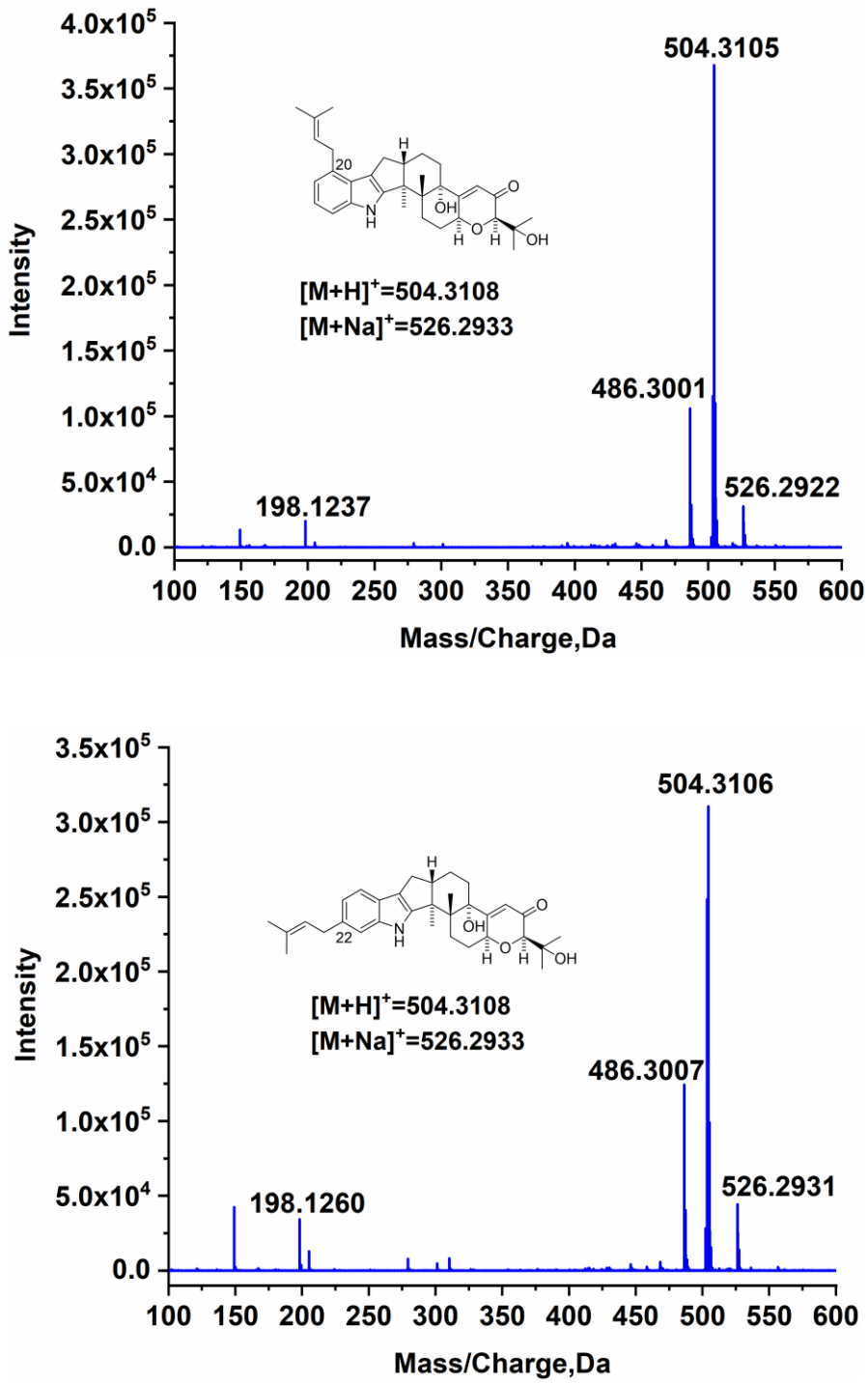

Fig. S3 LC-MS analysis of 20-prenylpaxilline and 22-prenylpaxilline.

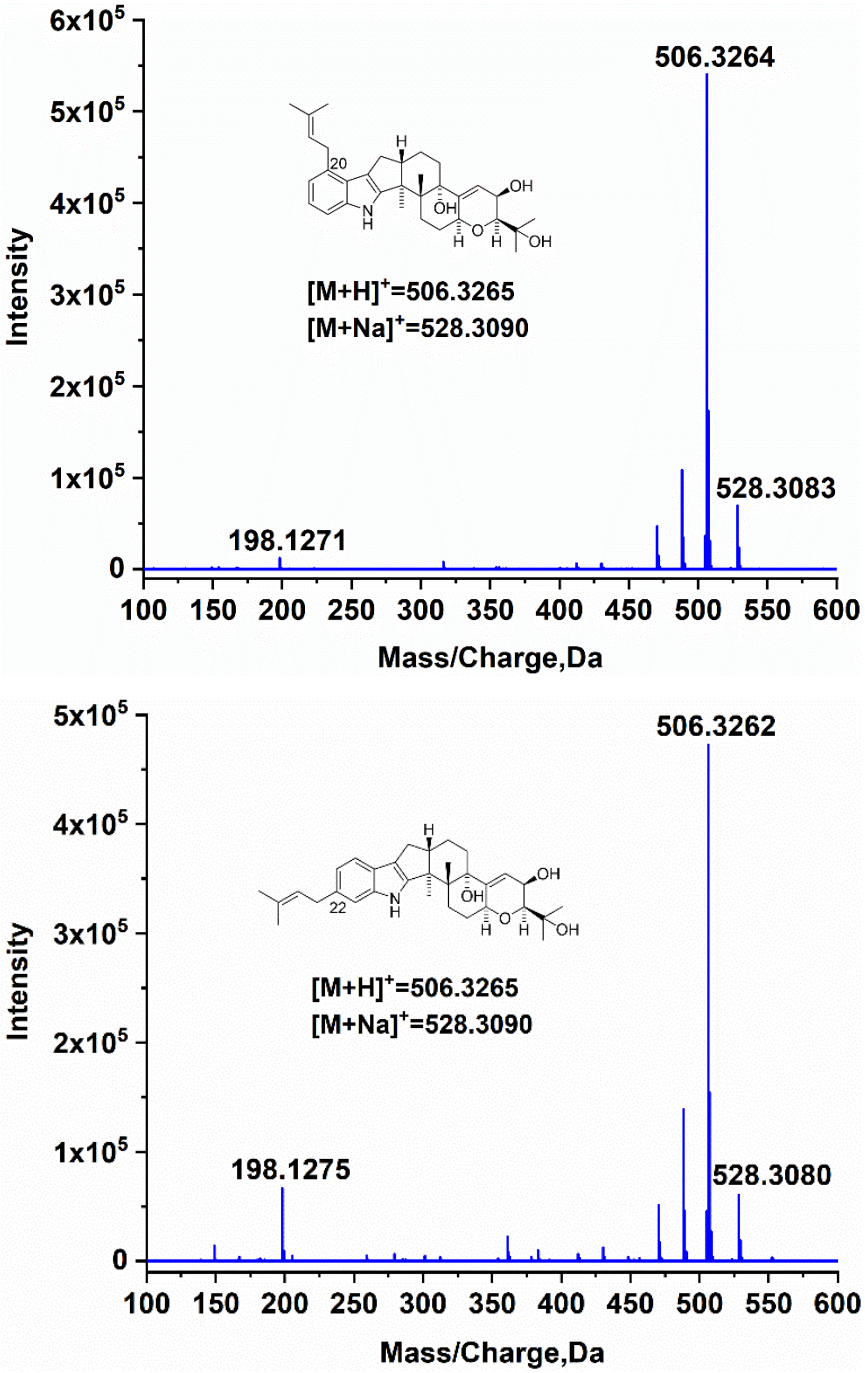

Fig. S4 LC-MS analysis of 20-prenylpaxitriol and 22-prenylpaxitriol.

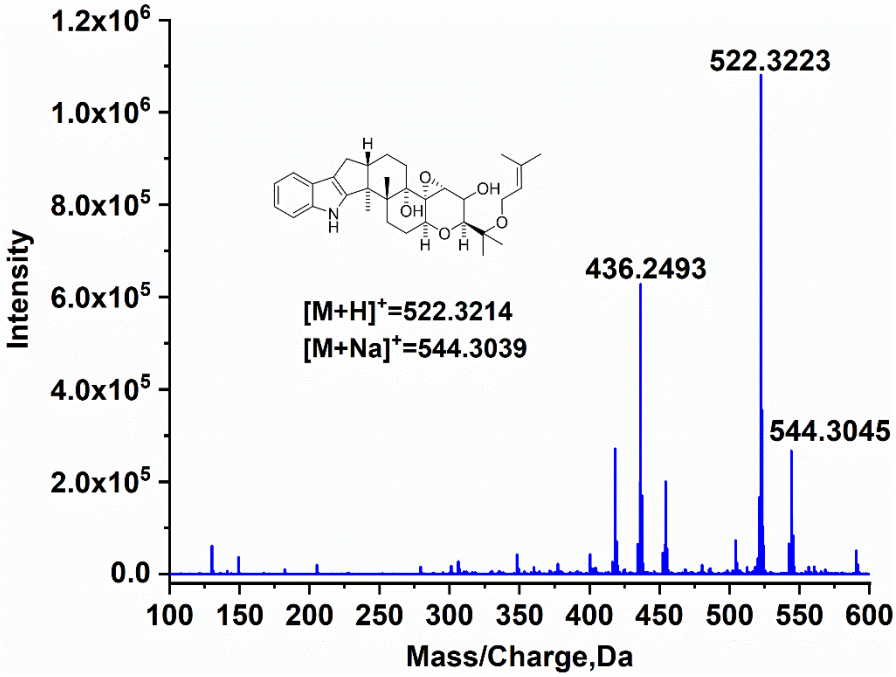

Fig. S5 LC-MS analysis of prenylated terpendole I formed by *in vivo* assay.

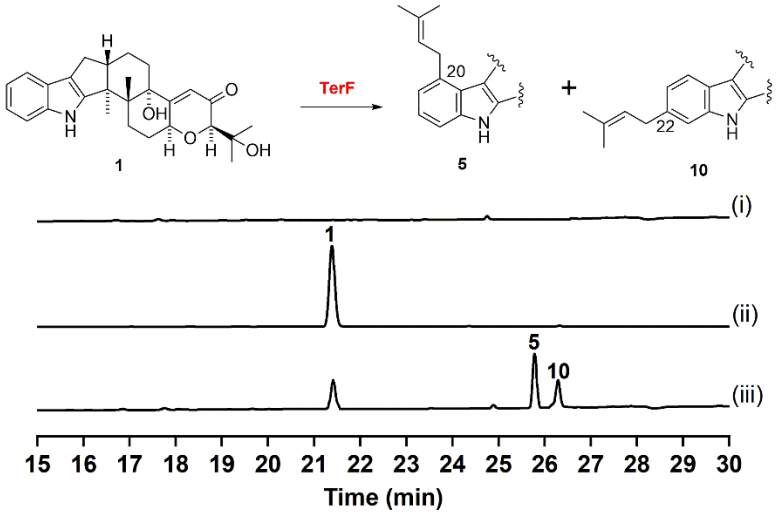

Fig. S6 HPLC profiles of feeding experiments to *AO-terF* with paxilline (**1**). (i) *AO-WT*; (ii) *AO-WT* + **1**; (iii) *AO-terF* + **1**.

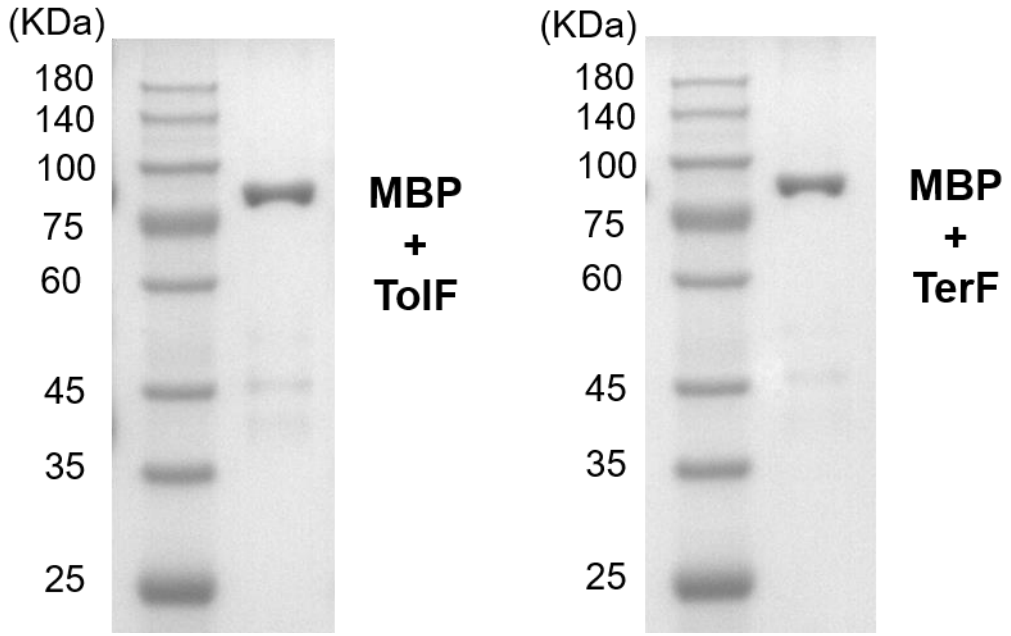

Fig. S7 SDS-PAGE of the heterogeneously expressed proteins. TolF ≈ 47 KDa + MBP tag = 42.5 KDa (~89.5 KDa). TerF≈ 46.2 KDa + MBP tag = 42.5 KDa (~88.8 KDa).

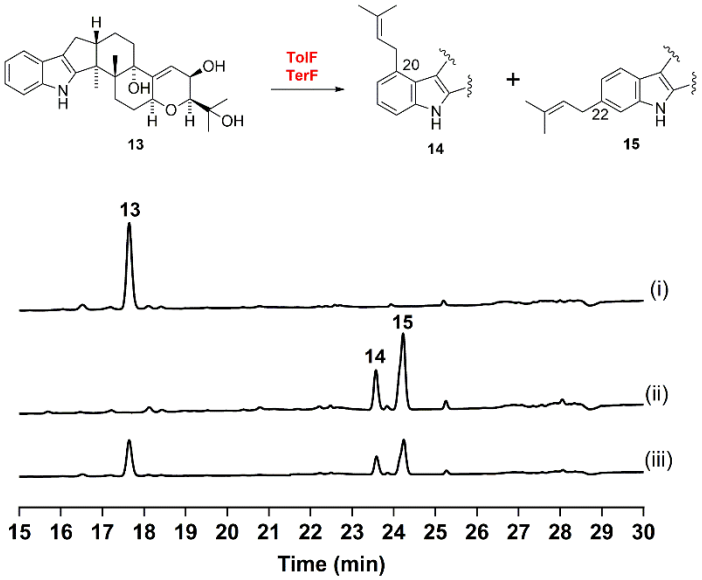

Fig. S8 HPLC profiles of the prenylated β-paxitriol formed by in vitro assay. (i) boiled enzyme with **13** and DMAPP; (ii) TolF with **13** and DMAPP; (iii) TerF with **13** and DMAPP.

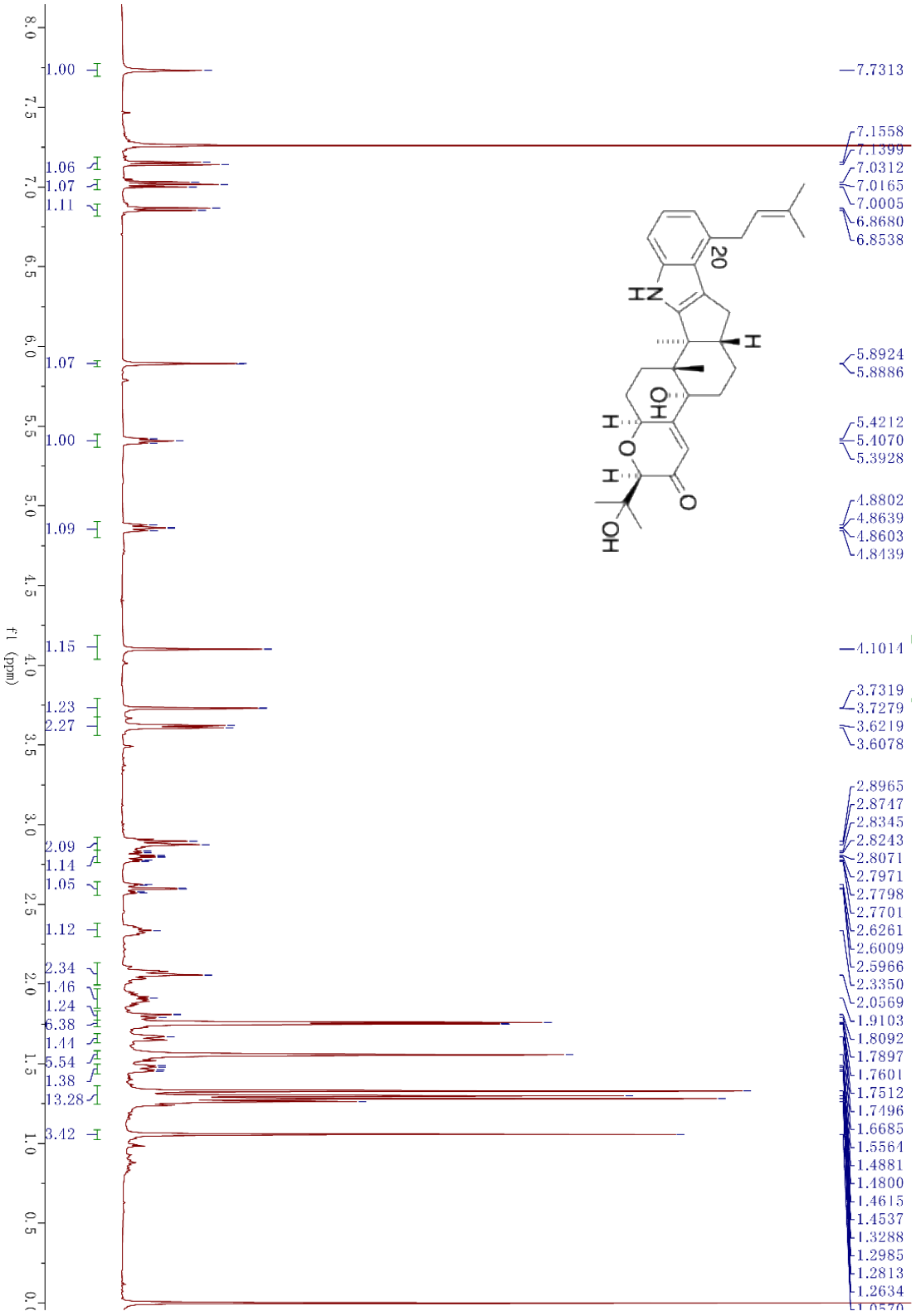

Fig. S9 ^{1}H-NMR of 20-prenylpaxilline.

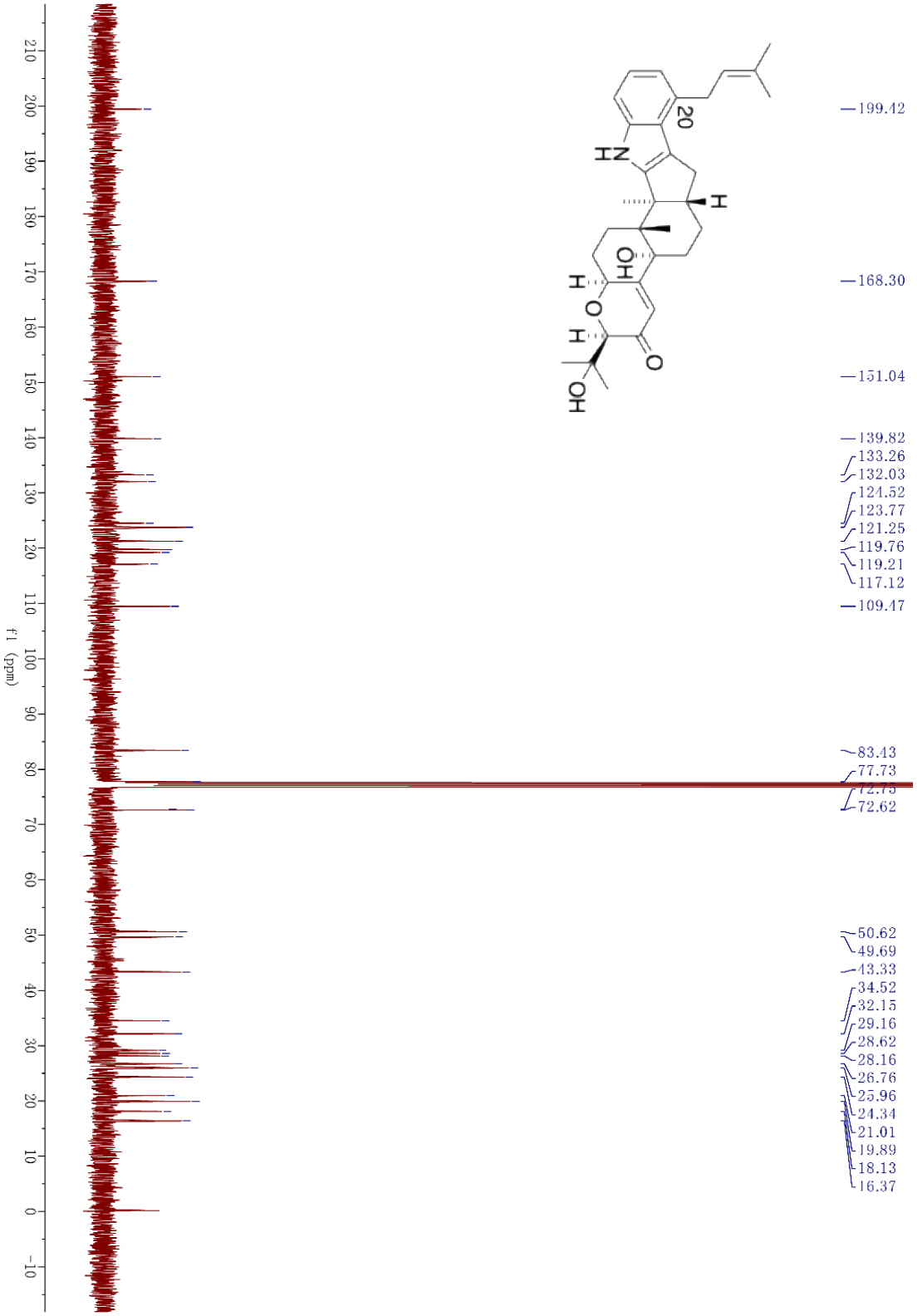

Fig. S10 ^{13}C-NMR of 20-prenylpaxilline.

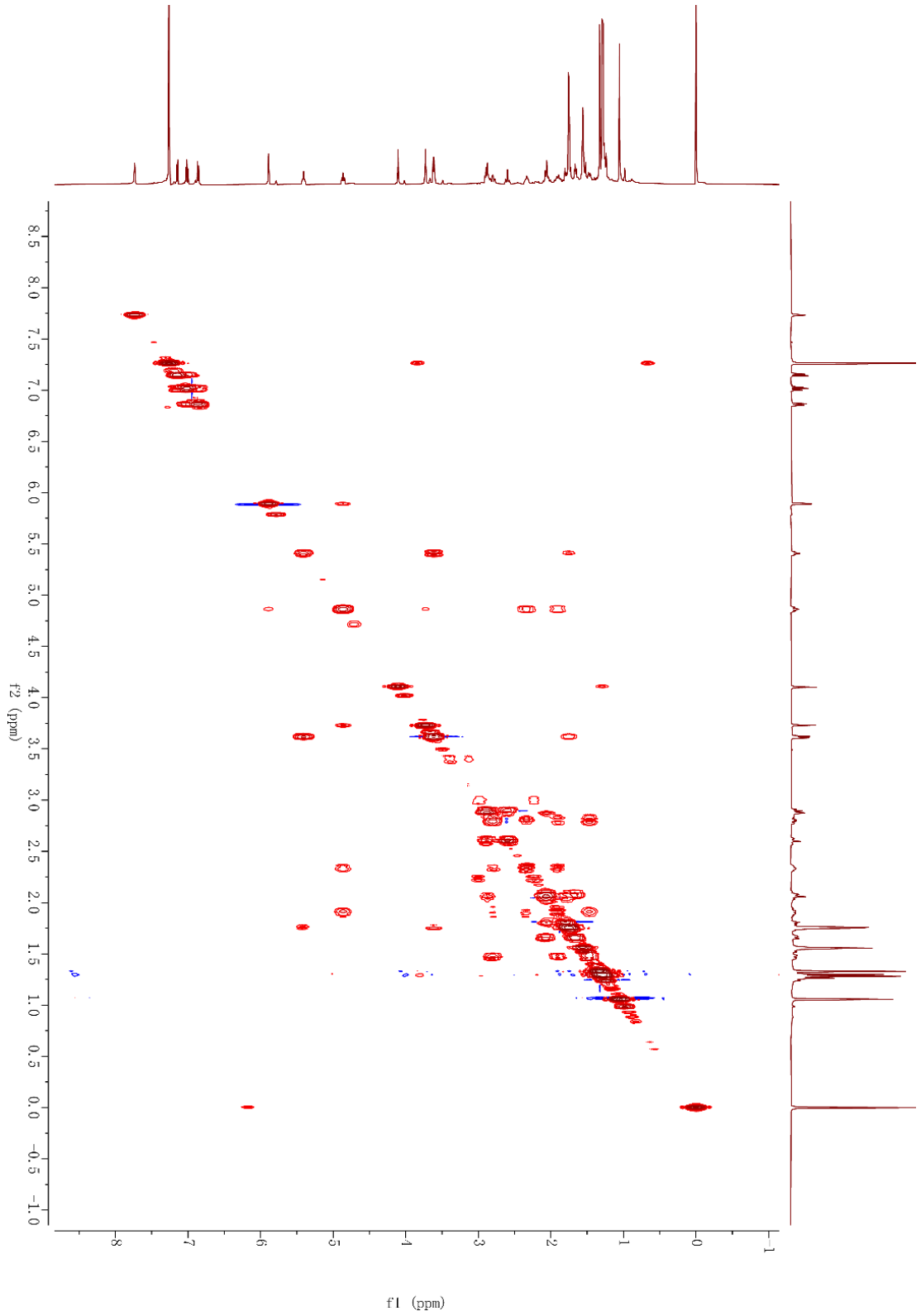

Fig. S11 H-H COSY of 20-prenylpaxilline.

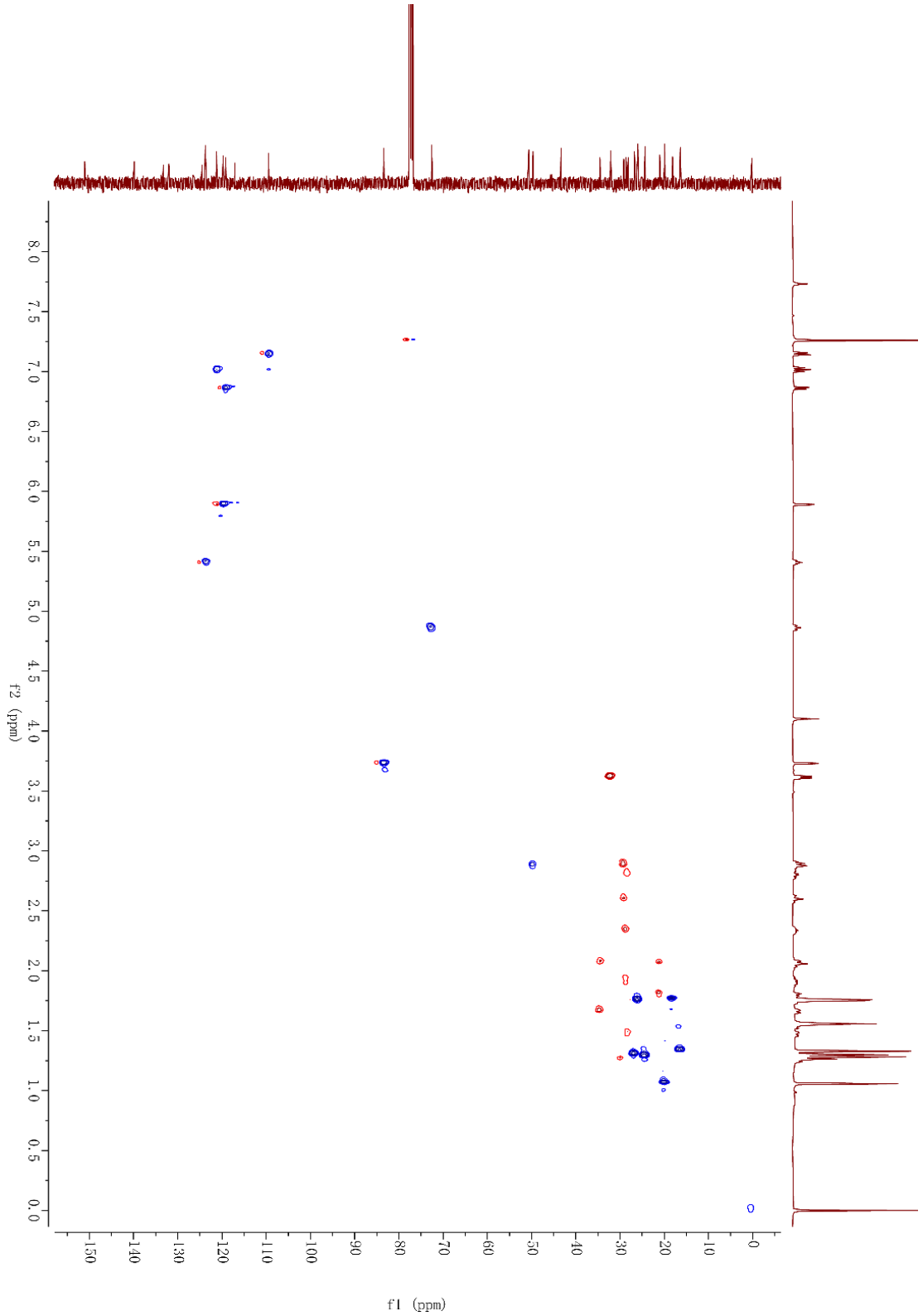

Fig. S12 HSQC of 20-prenylpaxilline.

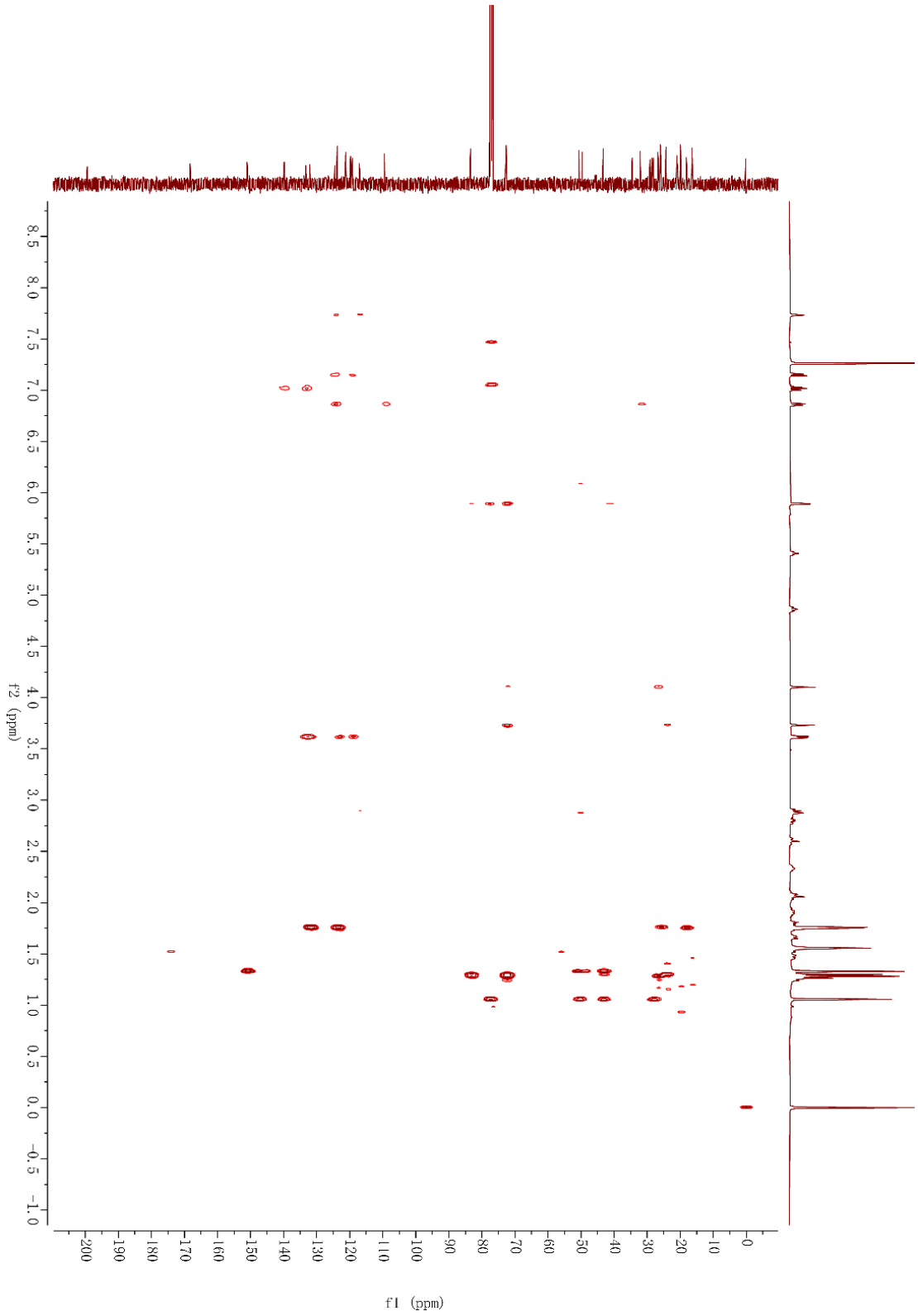

Fig. S13 HMBC of 20-prenylpaxilline.

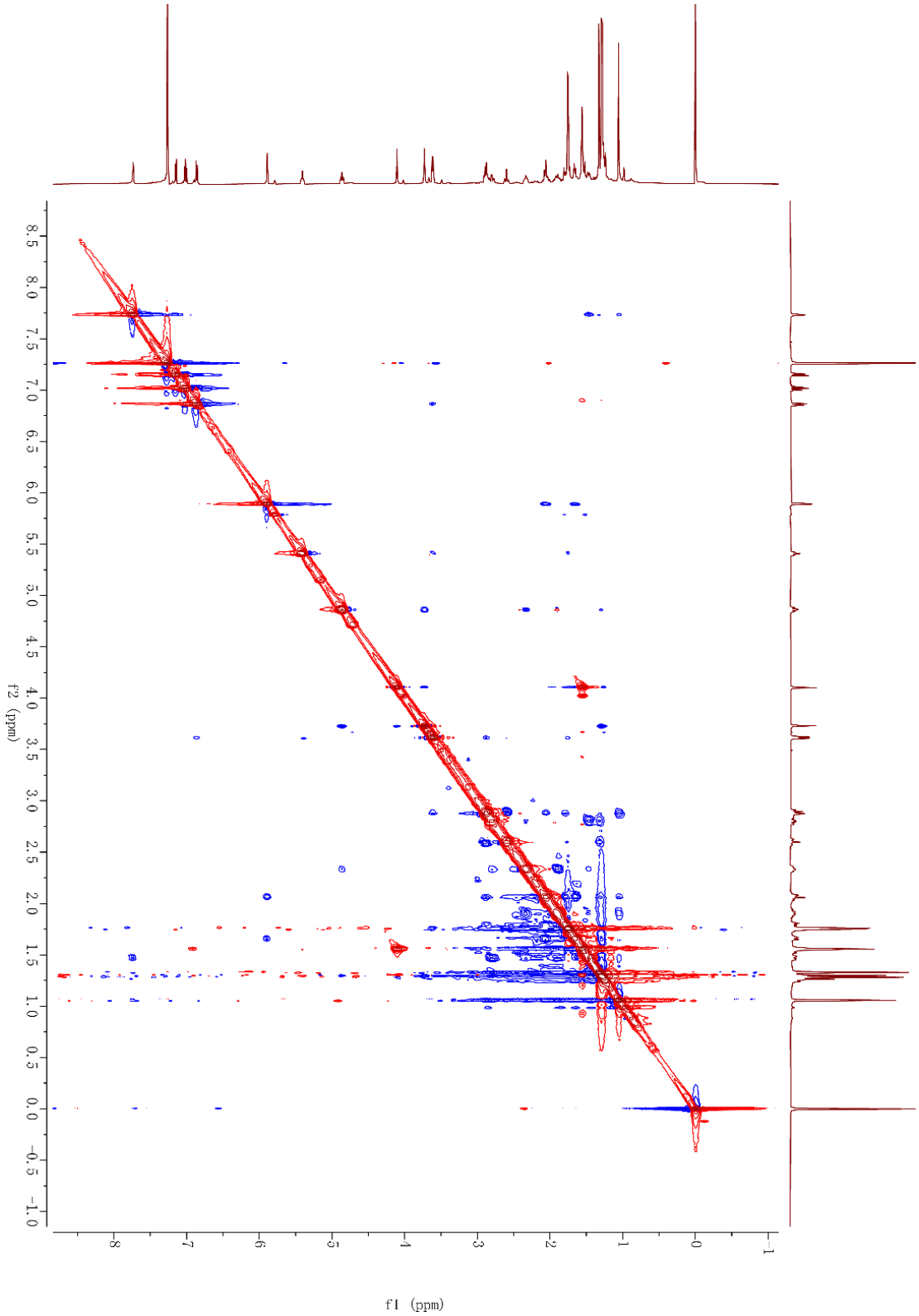

Fig. S14 NOESY of 20-prenylpaxilline.

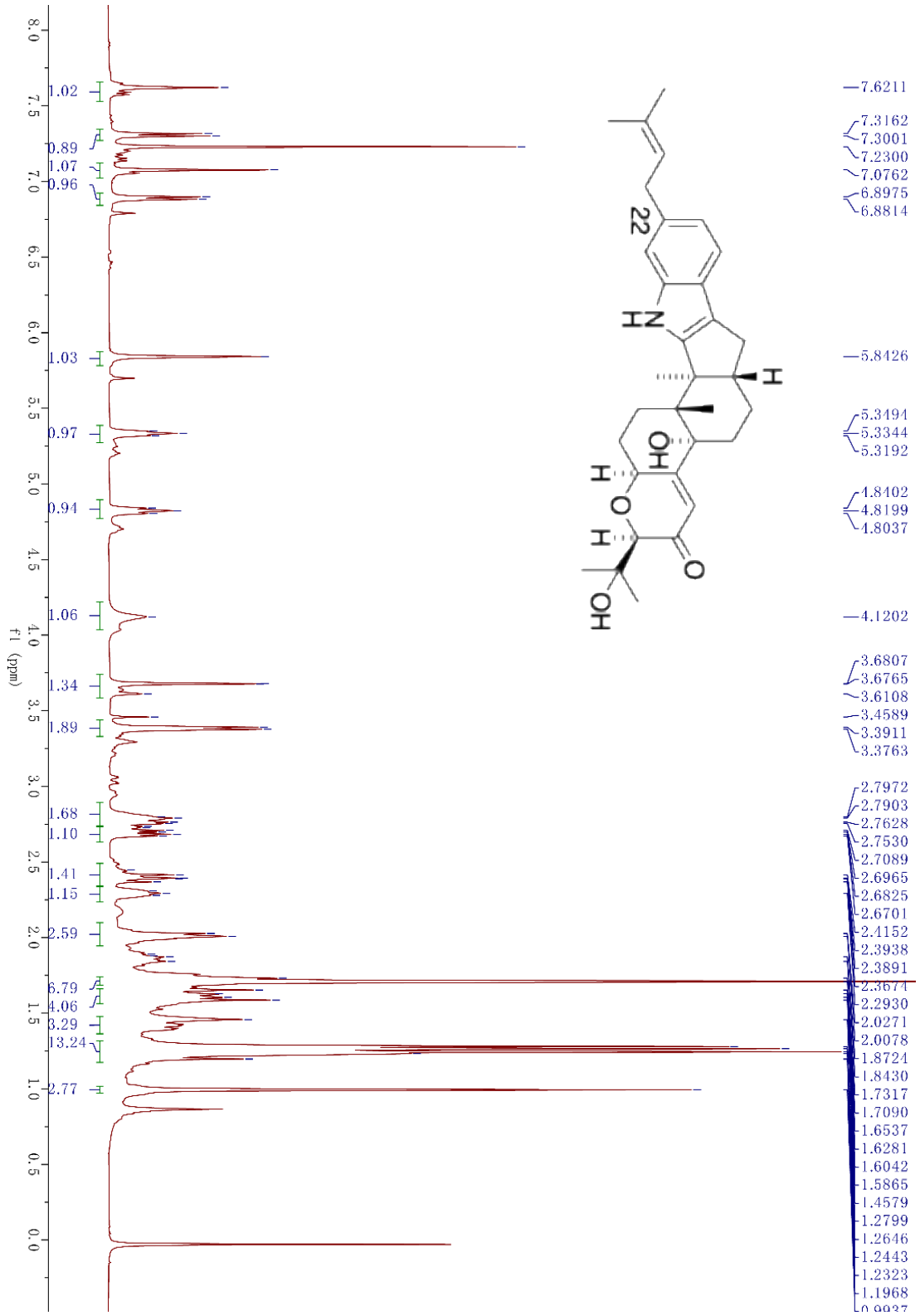

Fig. S15 ^{1}H-NMR of 22-prenylpaxilline.

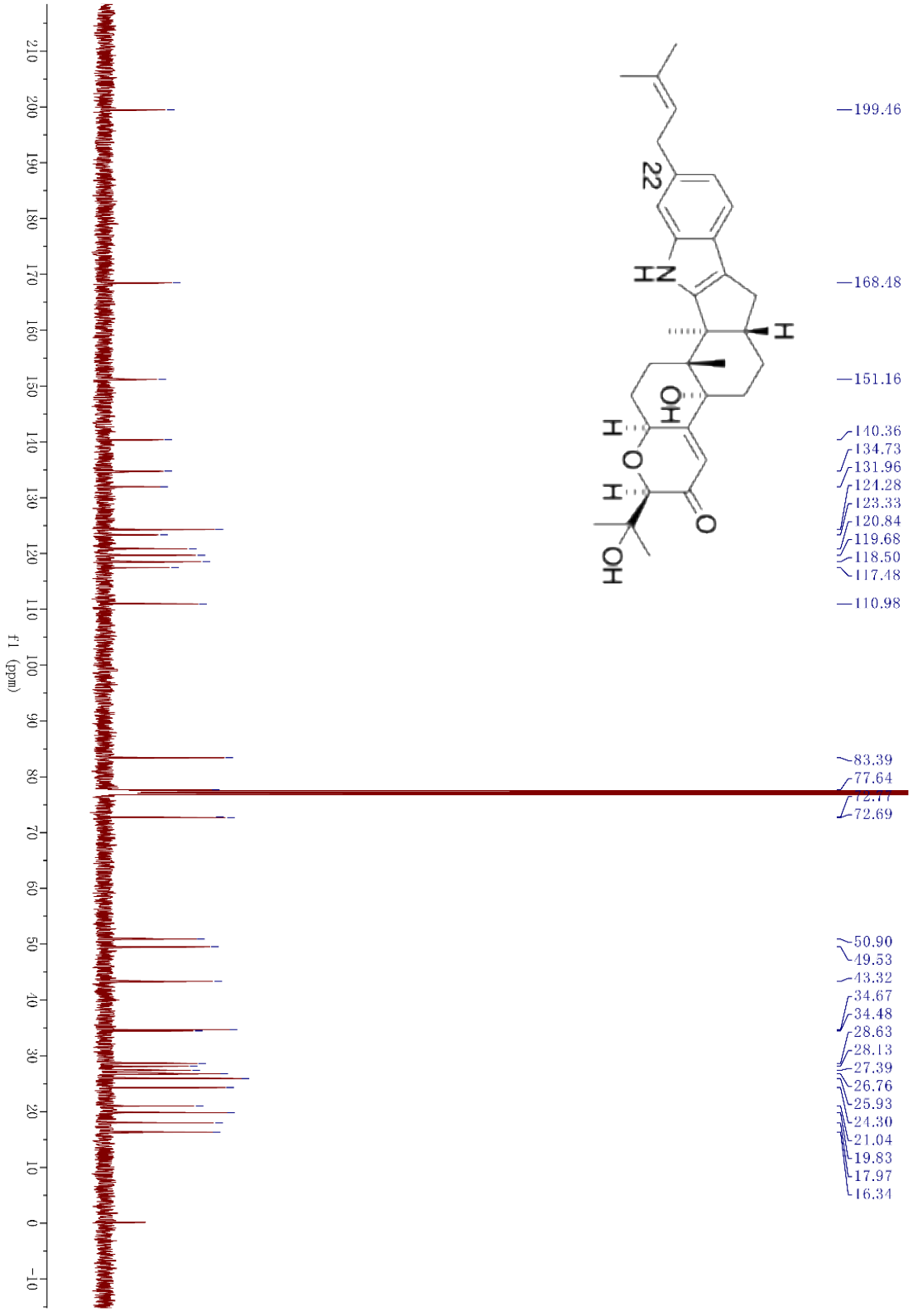

Fig. S16 ^{13}C-NMR of 22-prenylpaxilline.

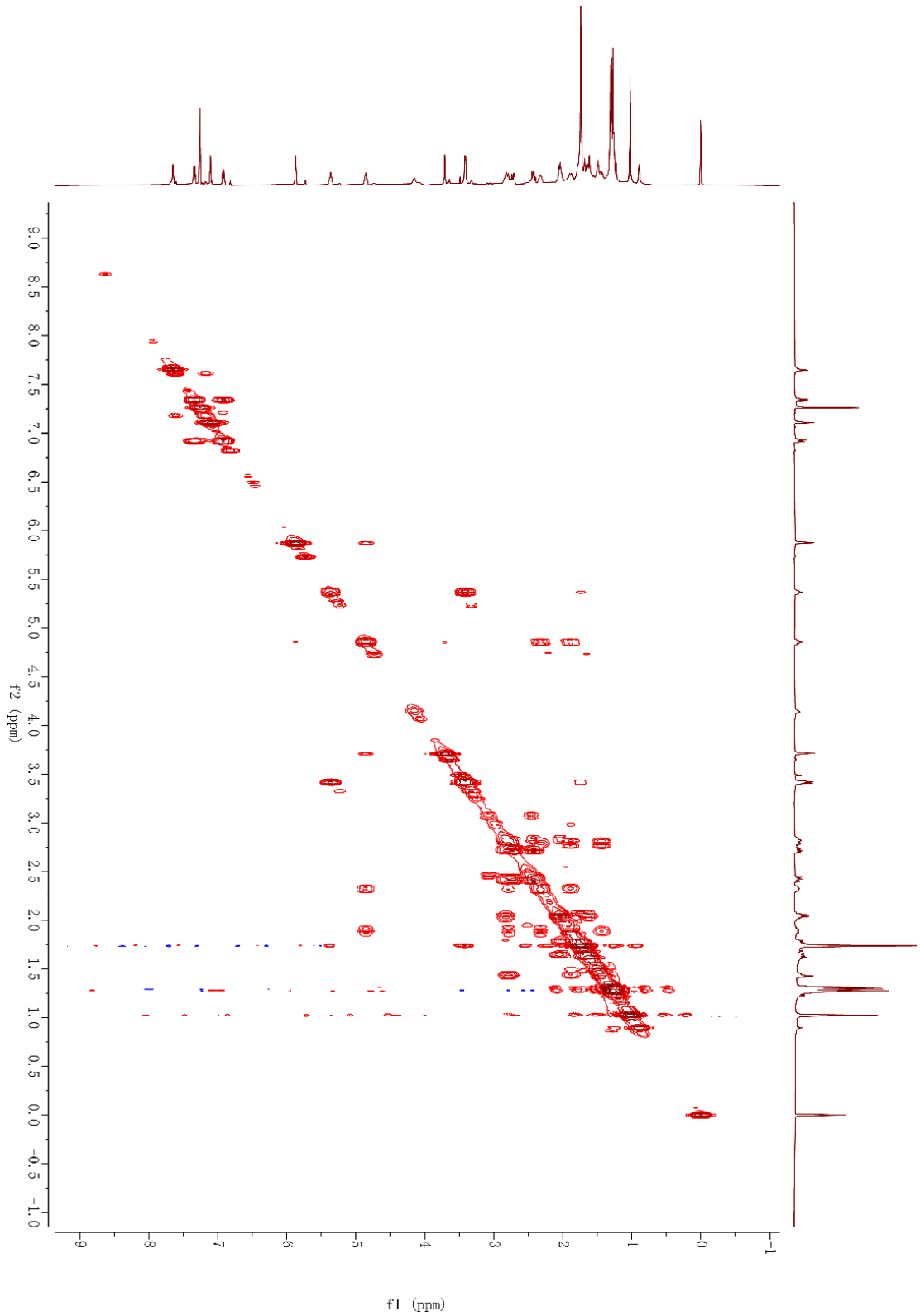

Fig. S17 H-H COSY of 22-prenylpaxilline.

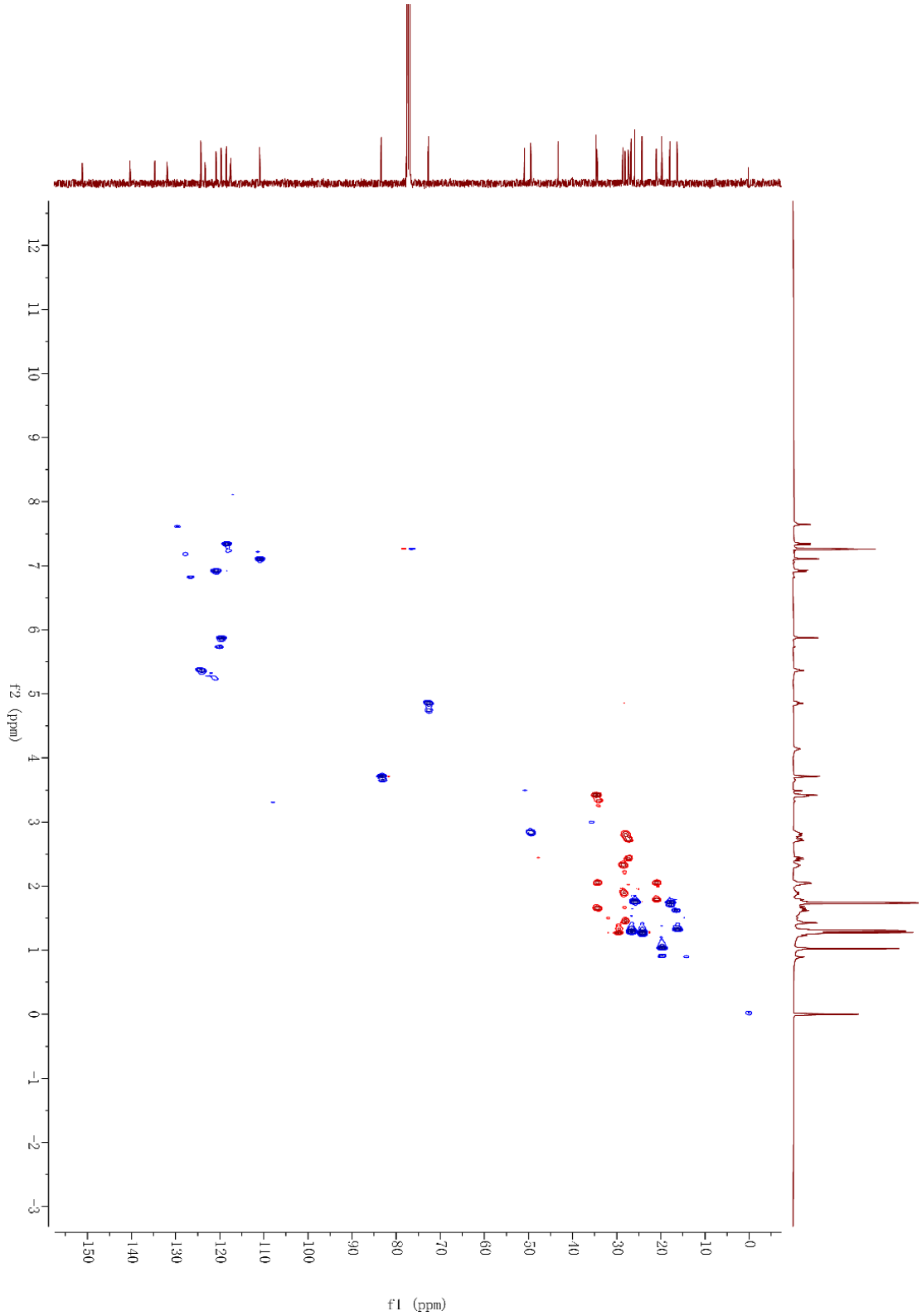

Fig. S18 HSQC of 22-prenylpaxilline.

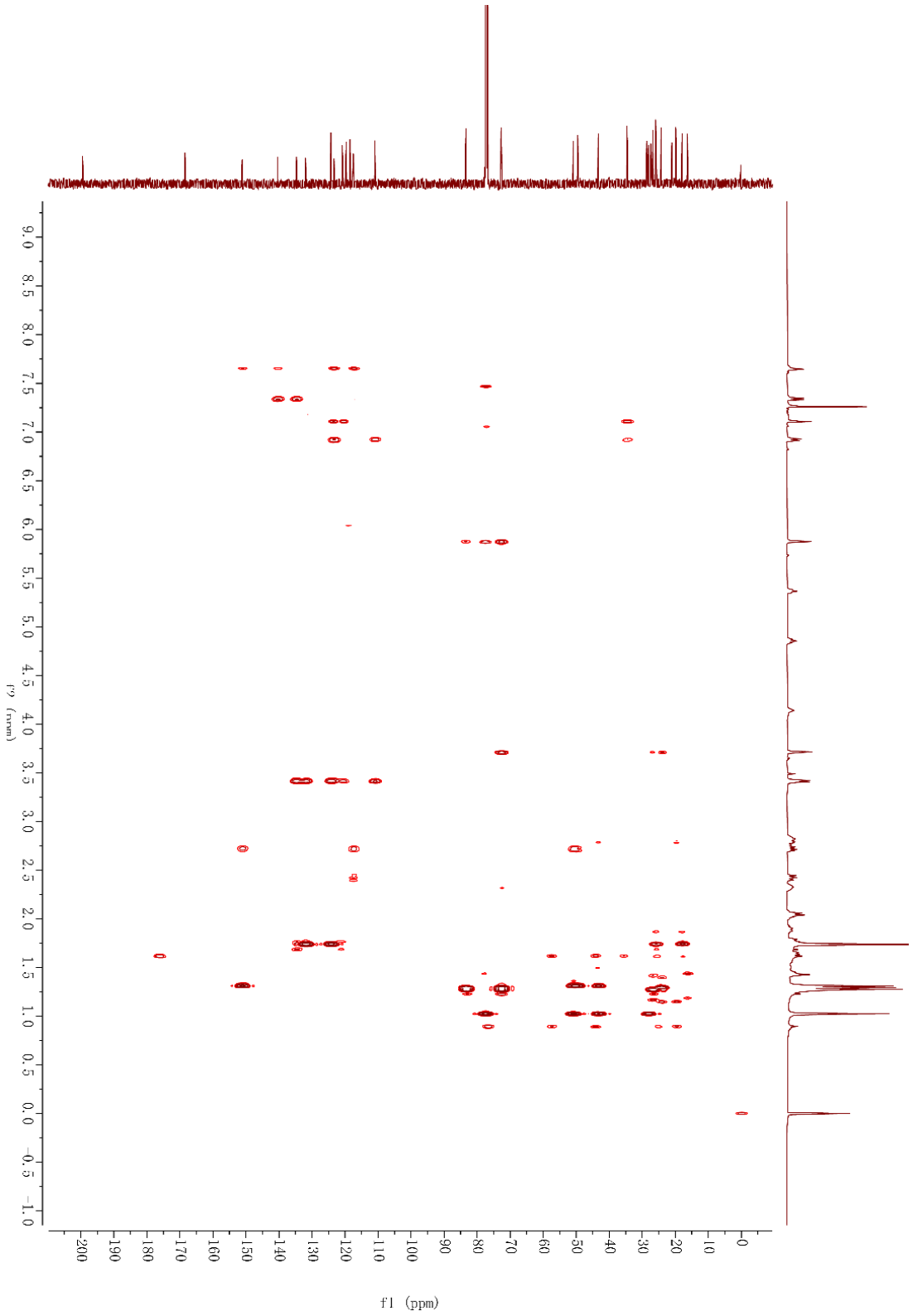

Fig. S19 HMBC of 22-prenylpaxilline.

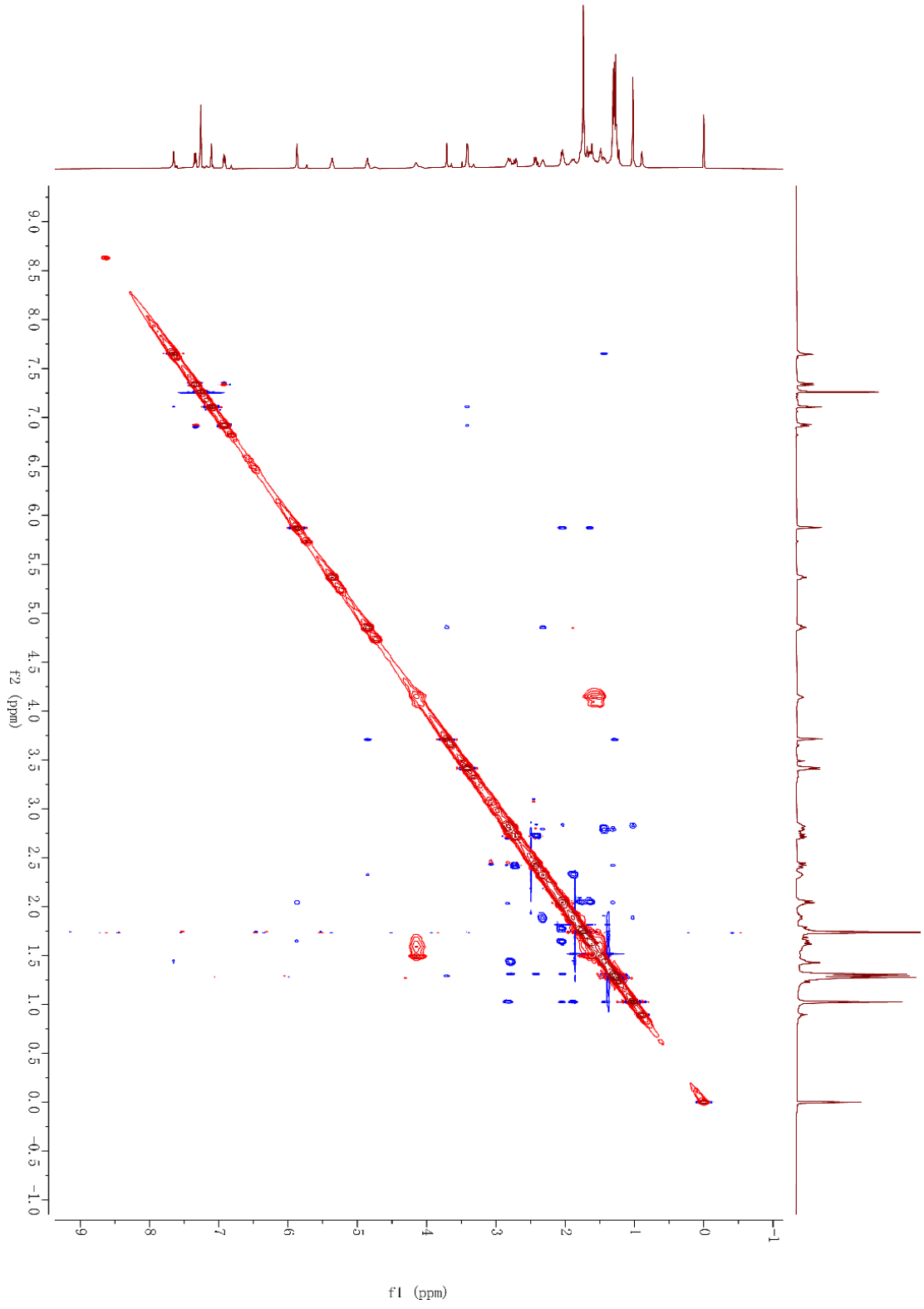

Fig. S20 NOESY of 22-prenylpaxilline.

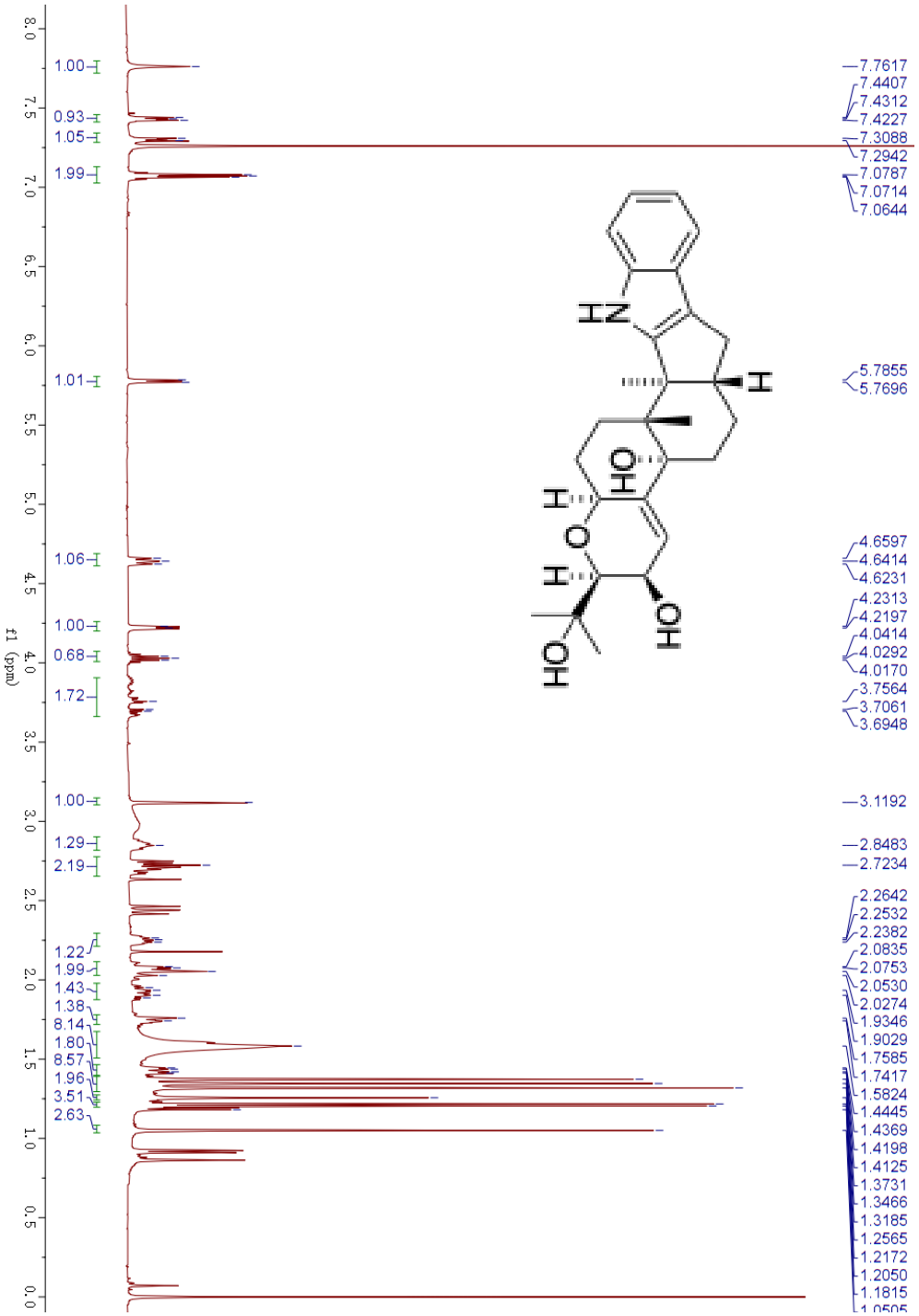

Fig. S21 ^{1}H-NMR of β-paxitriol.

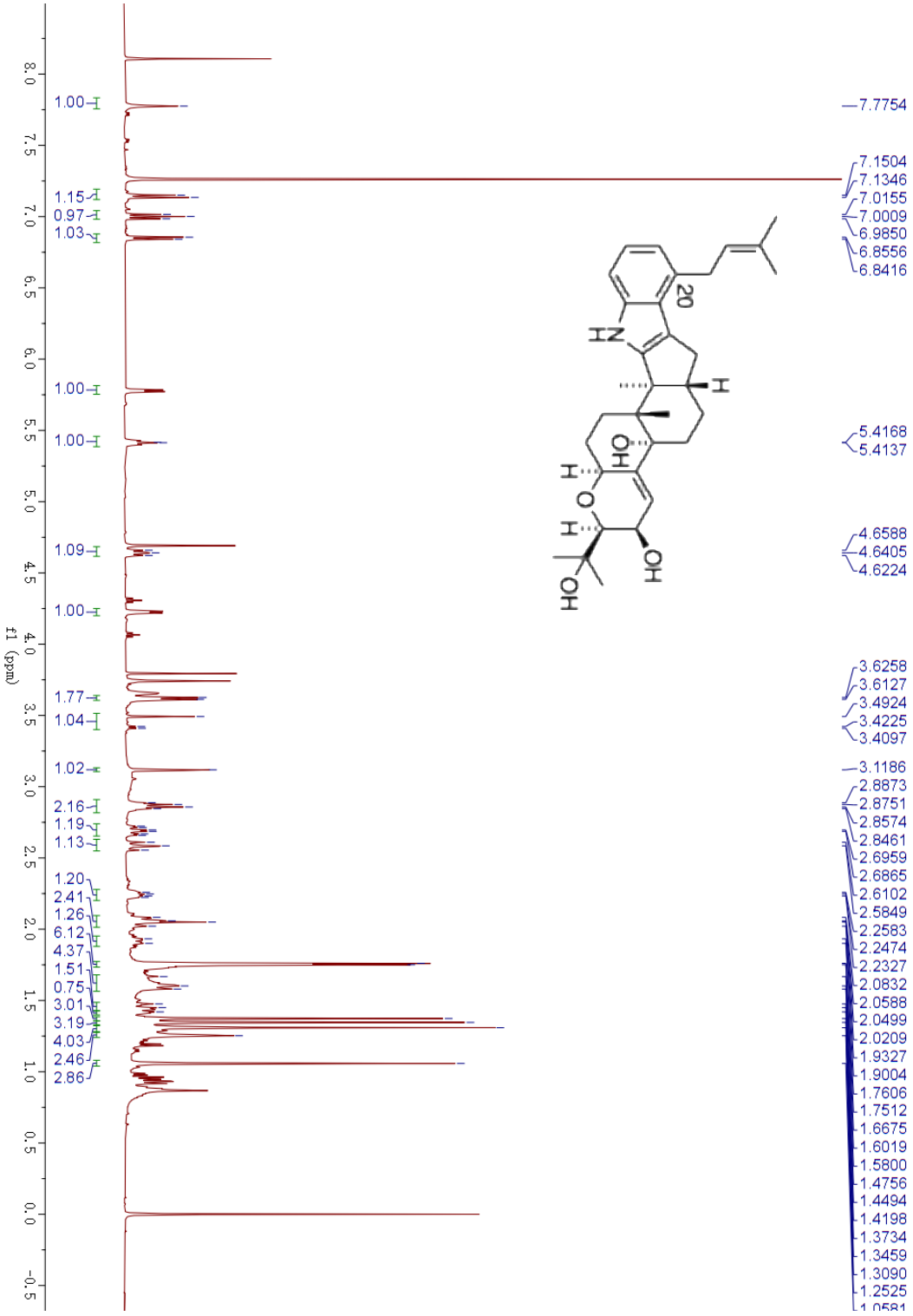

Fig. S22 ^{1}H-NMR of 20-prenylpaxitriol.

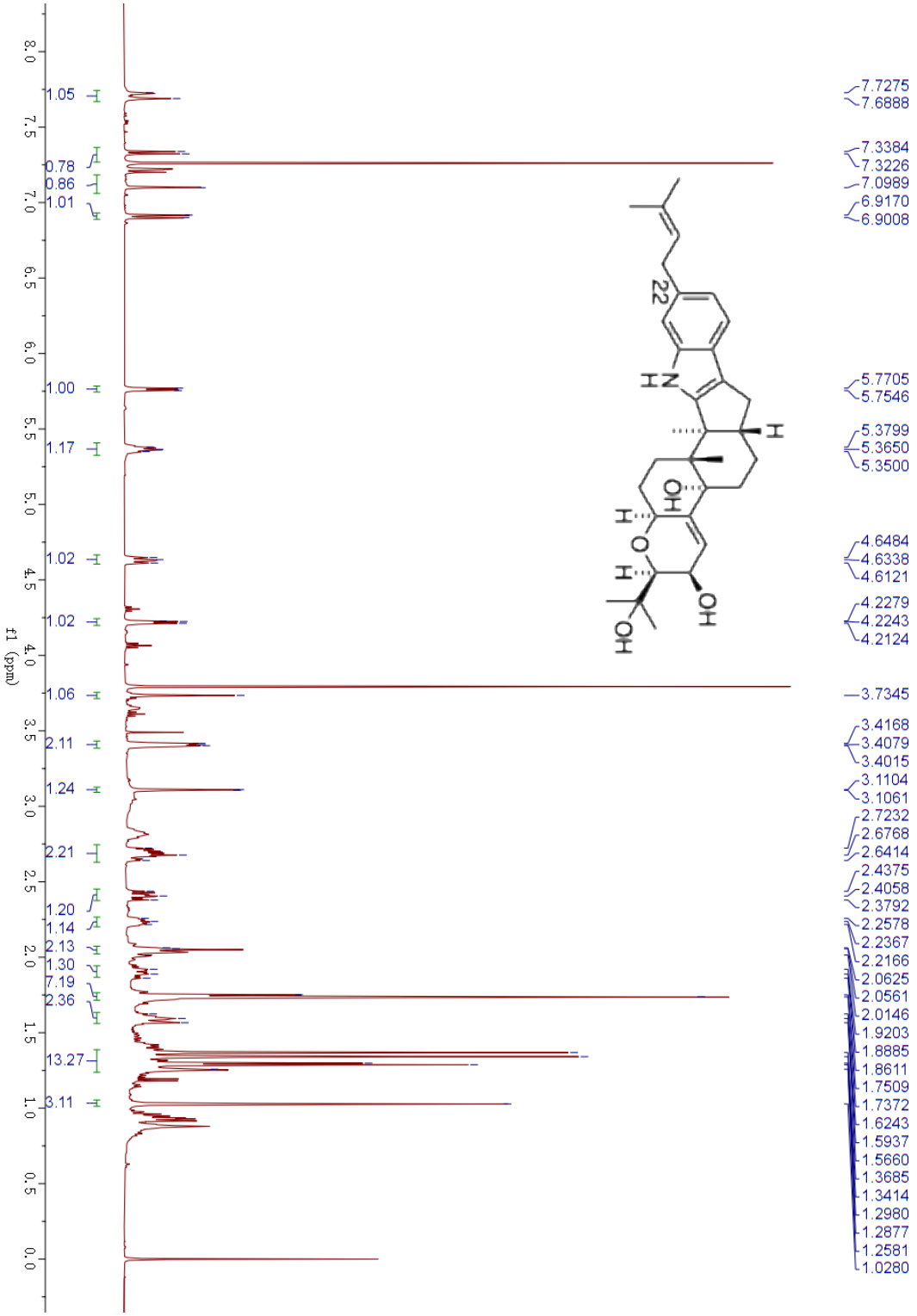

Fig. S23 ^{1}H-NMR of 22-prenylpaxitriol.

References:

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Xu LL, Pang X-J, Shi Q, Xian P-J, Tao Y-D, Yang X-L (2019b) Two new prenylated indole diterpenoids from *Tolypocladium* sp. and their antimicrobial activities. Chem Biodivers 16(6):e1900116 https://doi.org/10.1002/cbdv.201900116