



Supporting Information

for

Nanangenines: drimane sesquiterpenoids as the dominant metabolite cohort of a novel Australian fungus, *Aspergillus nanangensis*

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Details of cultivation media, fractionation schemes, NMR spectra and tabulated 2D NMR data for all compounds, detailed X-ray crystallographic details and CCDC deposition numbers, bioassay procedures and genomic data

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Supplementary methods

Single crystal X-ray diffraction crystal structure determinations

In each case, a suitable specimen was selected and attached to a fibre or nylon loop with Exxon Paratone N, then quenched in a cold nitrogen gas stream from an Oxford Cryosystems Cryostream. Data were collected with a four-circle kappa goniometer and CCD detector equipped SuperNova X-ray diffractometer, using mirror monochromated Cu K α radiation generated from a microsource. Data processing was undertaken with CrysAlisPro [1] software and included a multi-scan absorption correction. Subsequent computations were carried out with the assistance of ShelXle [1], WinGX [2,3], PLATON [4,5] and XNPP [6]. The structures were obtained using ShelXT [7] and extended and refined with ShelXL [8]. In general non-hydrogen atoms in the asymmetric unit were modelled with anisotropic displacement parameters and a riding atom model was used for the hydrogen atoms. Images with displacement ellipsoid representations were generated with Olex2 [9]. Crystallographic data are summarised in Table S2.

General procedure for synthesis of 4-bromobenzoate derivatives: A solution of nanangenine to be derivatised (0.03 mmol), 4-bromobenzoyl chloride (6.6 mg, 0.03 mmol) and triethylamine (50 μ L) in MeCN (950 μ L) was heated at 70 $^{\circ}$ C for 4 h. The solution was washed with sodium bicarbonate solution (3 M, 50 mL) and extracted into ethyl acetate before being concentrated in vacuo. The residue was purified by preparative HPLC (Hypersil C₁₈, isocratic 100% MeCN, 20 mL min⁻¹). Crystals suitable for X-ray diffraction were grown by slow evaporation of methanolic solutions.

Nanangenine A (1): A colourless blade-like crystal of **1** was used for the data collection and the structure was obtained and modelled in the space group $P2_1$ (#4). The asymmetric unit was found to contain four crystallographically-independent molecules. The hydroxy hydrogens were located in final difference maps and in general modelled with isotropic displacement parameters. The displacement parameter for one of the model hydroxy hydrogen atoms (H3O_4) was tied to that of the associated oxygen atom. In addition to an intramolecular hydrogen bond, intermolecular hydrogen bonds link molecules in discrete layers on the (002) planes of the unit cell. A depiction of one the molecules with 50% displacement ellipsoids is provided in Figure S3. The Parsons intensity quotients based Flack parameter determined by ShelXL was 0.06(3). The PLATON calculated Bayesian probability estimate that the assigned absolute structure is correct is 1.0. The probability estimate that the assignment is incorrect is 0.0, while that for an inversion twin is 8.0×10^{-51} . Accordingly, the assigned absolute structure is confirmed. CCDC reference: 1940618.

9-O-(4-Bromobenzoyl)-nanangenine B (2b): A colourless columnar crystal was used for the data collection and the structure was obtained in the space group $P2_1$ (#4). The asymmetric unit contains two crystallographically-independent molecules. The diffraction data are pseudo-orthorhombic and a structure can be obtained and modelled in $P2_12_12_1$ (#19), however the pendant alkyl residue is poorly resolved. Relatively large displacement envelopes on the alkyl residue mask disorder. The hydroxy hydrogen sites were located in final difference maps and modelled with isotropic displacement parameters. An Olex2 depiction of one of the molecules, with 50% displacement ellipsoids, is provided in Figure S4. The Parsons intensity quotients based Flack parameter determined by ShelXL was -0.003(7) and accordingly the assigned absolute structure is confirmed. CCDC reference: 1940620.

1-O-(4-Bromobenzoyl)-isonanangenine B (3b): A pale orange prismatic fragment cut from a larger crystal was used for the data collection and the structure was obtained in the space group $P2_1$ (#4). The asymmetric unit contains two crystallographically independent molecules. The asymmetric unit contains two crystallographically-independent molecules, one of which exhibits minor site disorder of

the bromobenzoate residue. Two orientations were modelled for the disordered residue, with respective occupancies refined and then fixed at 0.93 and 0.07. A rigid body was applied in modelling the minor occupancy bromobenzoate sites. Isotropic displacement parameters were used for the minor occupancy sites. The hydroxy hydrogen position was located in final difference maps and refined with isotropic displacement parameters. The two independent molecules are linked by a hydrogen bond between the hydroxy residue of one and the furanone carbonyl moiety of the second. An Olex2 depiction of one of the molecules, with 75% displacement ellipsoids, is provided in Figure S5. The Parsons intensity quotients based Flack parameter determined by ShelXL refined to $-0.017(5)$. The PLATON calculated Bayesian probability estimate that the assigned absolute structure is correct is 1.0. The probability estimate that the assignment is incorrect or is an inversion twin is 0.0. CCDC reference: 1941156.

Nanangenine C (4): A colourless prismatic crystal of **4** was used for the data collection and the structure was obtained in the space group $P2_12_12_1$ (#19). The asymmetric unit contains **4** linked by a hydrogen bond to a water molecule; the water hydrogen positions were inferred from final difference maps. A depiction of the molecule with 50% displacement ellipsoids is provided in Figure S6. The Parsons intensity quotients based Flack parameter determined by ShelXL was 0.10(9) and that of XNPP refined to 0.12(7). The PLATON calculated Bayesian probability estimate that the assigned absolute structure is correct is 1.0. The probability estimate that the assignment is incorrect is 1.0×10^{-37} , while that for an inversion twin is 1.0×10^{-6} . Accordingly, though not definitively determined, the assigned absolute structure appears likely to be correct. CCDC reference: 1940619.

Nanangenine G (9): A colourless plate like crystal of **9** was used for the data collection and the structure was obtained in the space group $P2_12_12_1$ (#19). The pedant alkyl residue of **9** is disordered about two orientations. Site occupancies were refined and then fixed at 0.6 and 0.4; isotropic displacement parameters were used for the minor occupancy sites. The hydroxy hydrogen positions for all but that of O3 were located in final difference maps and refined with isotropic displacement parameters. Adjacent molecules are linked by hydrogen bonds. A depiction of the molecule with 50% displacement ellipsoids is provided in Figure S7. The ShelXL and XNPP calculated Flack parameter refined to 0.10(10) and 0.08(9) respectively. The PLATON calculated Bayesian probability estimate that the assigned absolute structure is correct is 1.0. The probability estimate that the assignment is incorrect is 4.0×10^{-24} , while that for an inversion twin is 4.0×10^{-5} . Accordingly, though not definitively determined, the assigned absolute structure is likely correct. CCDC reference: 1940621.

Bioassay of compounds

Purified metabolites were dissolved in DMSO to provide stock solutions of 10,000 µg/mL. An aliquot of each stock solution was transferred to the first lane of Rows B to G in a 96-well microtitre plate and two-fold serially diluted with DMSO across the 12 lanes of the plate to provide a 2,048-fold concentration gradient. Bioassay medium was added to an aliquot of each test solution to provide a 100-fold dilution into the final bioassay, thus yielding a test range of 100 to 0.05 µg/mL in 1% DMSO. Row A contained no test compound (as a reference for no inhibition) and row H was uninoculated (as a reference for complete inhibition).

CyTOX is an indicative bioassay platform for discovery of antitumour actives. NS-1 (ATCC TIB-18) mouse myeloma, DU-145 (ATCC HTB-81) prostate cancer, MCF-7 (ATCC HTB-22) breast cancer, and NFF (ATCC PCS-201) human neonatal foreskin fibroblast cells were each inoculated in 96-well microtitre plates (190 µL) at 50,000 cells/mL in DMEM (Dulbecco's Modified Eagle Medium + 10% fetal bovine serum (FBS) + 1% penicillin/streptomycin (10,000 U mL⁻¹ / 10,000 µg mL⁻¹, Life Technologies Cat. No. 15140122), together with resazurin (250 µg mL⁻¹; 10 µL) and incubated in 37 °C (5% CO₂) incubator. The plates were incubated for 96 h during which time the positive control wells change colour from a blue to pink colour. The absorbance of each well was measured at 605 nm using a Spectromax plate reader (Molecular Devices).

ProTOX is a generic bioassay platform for antibiotic discovery. In the present study *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922) were used as indicative species for Gram-positive and Gram-negative antibacterial activity. A bacterial suspension (50 mL in 250 mL flask) was prepared in nutrient media by cultivation for 24 h at 250 rpm, 28 °C. The suspension was diluted to an absorbance of 0.01 absorbance units per mL, and 10 µL aliquots were added to the wells of a 96-well microtitre plate, which contained the test compounds dispersed in nutrient broth (Amyl) with resazurin (12.5 µg mL⁻¹). The plates were incubated at 28 °C for 24 h during which time the positive control wells change colour from a blue to light pink colour. MIC end points were determined visually. The absorbance was measured using Spectromax plate reader (Molecular Devices) at 605 nm and the IC₅₀ values determined graphically.

EuTOX is a generic bioassay platform for antifungal discovery. In the present study, the yeast *Candida albicans* (ATCC 10231) was used as indicative species for antifungal activity. A yeast suspension (50 mL in 250 mL flask) was prepared in 1% malt extract broth by cultivation for 24 h at 250 rpm, 28 °C. The suspension was diluted to an absorbance of 0.005 absorbance units per mL for *C. albicans*. Aliquots (20 µL and 30 µL) of *C. albicans* were applied to the wells of a 96-well microtitre plate, which contained the test compounds dispersed in malt extract agar containing bromocresol green (50 µg mL⁻¹). The plates were incubated at 28 °C for 24 h during which time the positive control wells change colour from a blue to yellow colour. MIC end points were determined visually. The absorbance was measured using Spectromax plate reader (Molecular Devices) at 620 nm and the IC₅₀ determined graphically.

Phytox is a generic bioassay platform for herbicidal discovery. In the present study, *Eragrostis tef* (teff) seed was used as indicative species for herbicidal discovery. Teff seeds (10 to 15) were dispensed using a LabTIE seed dispenser into the wells of a 96-well microtitre plate, which contained the test compounds dispersed in 200 µL of agar (1% w/v) per well. The plates were placed in a tray wrapped with a semi-opaque bag, exposed to 1600 lux (within the tray) using Power-GLO (20 W) and Sun-GLO (20 W) tubes, and incubated for 72 h at 24 °C.

Genomic DNA extraction

A. nanangensis was grown in potato dextrose broth (BD Bioscience) at room temperature. Mycelium was collected from one week-old liquid culture and ground in liquid nitrogen. The frozen ground mycelium was extracted using a buffer with 10mM Tris, 10 mM EDTA, 1% SDS and incubated at 70 °C for 20 min. This is followed by precipitation of the SDS and cell debris with equal volume of 2.8 M potassium acetate and clean-up with chloroform/isoamyl alcohol (24:1) extraction. Finally, the genomic DNA was precipitated from the supernatant using 0.7 volume isopropanol and the DNA pellet was dissolved in TE buffer. The quality of the genomic DNA was checked by gel electrophoresis.

Bioinformatics analyses

Alignment of *A. nanangensis* MST-FP2251 ITS to an undescribed sequence: *A. nanangensis* ITS sequence was retrieved from genome sequence and is deposited on GenBank (MK979278). ITS sequences of *A. janus* and an uncultured fungus were obtained from NCBI at accessions EU021598.1 and GQ921753.1, respectively. Sequences were trimmed based on length of the shortest sequence, and the multiple sequence alignment was constructed using Clustal Omega v1.2.4 [10].

Alignment of *A. nanangensis* MST-FP2251 with other *Aspergilli*: ITS sequences of *Aspergillus* species were obtained from NCBI (Table S13) and were aligned using MAFFT with the L-INS-i option. ML tree was constructed using RAXML-NG v0.6.0 [11] from 1000 bootstrap replicates. BI tree was constructed using MrBayes v3.2.6 [12] from 200000 generations. Both trees were constructed under the General Time Reversible (GTR) substitution model.

Alignment of AstC and homologs listed in Shinohara et al: Though Shinohara et al. list the revised annotation of AstC as AORIB40_05408, its amino acid sequence of was retrieved from AspGD [13] under the locus tag AO090026000582. AORIB40_NS.05916 was obtained from AspGD at coordinates Chr3_A_oryzae_RIB40:3512882-3513472(-). All other sequences were retrieved from NCBI via their accession codes. A multiple sequence alignment was constructed using ClustalOmega v1.2.4 [10].

Alignment of AstC to tBLASTn hits: Local BLAST databases were constructed inside Geneious 10.2.6 and AstC homologs were identified using tBLASTn. A multiple sequence alignment of the AstC homologs was constructed using MUSCLE v3.8.31 [14] and a phylogenetic tree was constructed under the JTT model using FastTree 2.1.5 [15].

Custom Python scripts: Homologous gene clusters were identified using a custom Python script, named clusterblaster (<https://github.com/gamcil/clusterblaster>). Briefly, the script takes as input a set of query sequences in FASTA format (presumably clustered), searches a pre-constructed DIAMOND [16] database consisting of protein sequences obtained from NCBI, then leverages the 'identical protein group' functionality of NCBI Entrez to ascertain the original genomic context of hit proteins (i.e. start and end on an assembly scaffold). Hits are then grouped by scaffold and ordered by location; those that meet user-defined clustering thresholds (maximum intergenic distance, minimum number of proteins conserved) are reported.

Gene cluster homology was visualised using crosslinker (<https://github.com/gamcil/crosslinker>). Briefly, crosslinker takes as input GenBank files of gene clusters, performs pairwise alignments between all sets of protein sequences, then draws a to-scale SVG figure depicting gene cluster homology with shaded boxes linking homologous proteins, the darkness of which indicates the degree of amino acid identity.

Supplementary tables

Table S1. Recipes for microbiological media.

Glycerol Casein Agar (AC)	
<i>Ingredient</i>	<i>Quantity</i>
Glycerol (Chem-Supply)	30 g
Casein peptone (Amyl)	2 g
K ₂ HPO ₄ (Chem-Supply)	1 g
NaCl (Chem-Supply)	1 g
MgSO ₄ ·7H ₂ O (AnalaR)	0.5 g
Trace element solution (mL)*	5 mL
Distilled H ₂ O	1000 mL
Bacteriological agar (Amyl)	20 g
Autoclave	
*Trace element solution	
CaCl ₂ ·2H ₂ O	3 g
FeC ₆ O ₇ H ₅	1 g
MnSO ₄	0.2 g
ZnCl ₂	0.1 g
CuSO ₄ ·5H ₂ O	0.025 g
Na ₂ B ₄ O ₇ ·10H ₂ O	0.02 g
CoCl ₂	0.004 g
Na ₂ MoO ₄ ·2H ₂ O	0.01 g
Deionised water	1000 mL
Filter sterilise	
Czapeks Agar (CZ)	
<i>Ingredient</i>	<i>Quantity</i>
Czapeks Dox Media (Oxoid)	99.88 g
Distilled water	2200 mL
Malt Extract Agar (MA)	
<i>Ingredient</i>	<i>Quantity</i>
Bacteriological peptone (Difco)	3 g
Malt extract (Amyl)	60 g
Bacteriological glucose (Amyl)	60 g
Distilled water	1000 mL
Adjust pH to 5.5	
Bacteriological agar (Amyl)	20 g
Autoclave	
Yeast Extract Sucrose Agar (YS)	
<i>Ingredient</i>	<i>Quantity</i>
Yeast Extract (Difco)	20 g
Sucrose (Amyl)	150 g
Bacteriological Agar (Amyl)	20 g

Distilled water	1000 mL
Autoclave	

Cracked Wheat (BL)

<i>Ingredient</i>	<i>Quantity</i>
Cracked wheat	46 g
Distilled water	30 mL
Autoclave	

Pearl Barley (PB)

<i>Ingredient</i>	<i>Quantity</i>
Pearl barley	48 g
Distilled water	35 mL
Autoclave	

Jasmine Rice (JR)

<i>Ingredient</i>	<i>Quantity</i>
Jasmine rice	50 g
Distilled water	25 mL
Autoclave	

Basmati rice (BS)

<i>Ingredient</i>	<i>Quantity</i>
Basmati rice	44 g
Distilled water	30 mL
Autoclave	

Table S2. Crystallographic data for **1**, **2b**, **3b**, **4** and **9**.

	Nanangenine A (1)	9-O-(4-Bromobenzoyl)-nanangenine B (2b)	1-O-(4-Bromobenzoyl)-isonanangenine B (3b)	Nanangenine C (4)	Nanangenine G (9)
Formula	C ₁₅ H ₂₂ O ₅	C ₂₈ H ₃₅ BrO ₇	C ₂₈ H ₃₅ BrO ₇	C ₂₁ H ₃₄ O ₆	C ₂₁ H ₃₆ O ₆
Molecular Weight	282.32	563.47	563.47	382.48	384.50
Crystal System	monoclinic	monoclinic	monoclinic	orthorhombic	orthorhombic
Space Group	<i>P</i> 2 ₁ (#4)	<i>P</i> 2 ₁ (#4)	<i>P</i> 2 ₁ (#4)	<i>P</i> 2 ₁ 2 ₁ 2 ₁ (#19)	<i>P</i> 2 ₁ 2 ₁ 2 ₁ (#19)
<i>a</i> (Å)	15.7957(2)	7.7558(2)	14.88851(10) Å	7.6270(4)	6.4497(2)
<i>b</i> (Å)	10.30070(10)	14.0642(2)	6.10835(4) Å	9.5926(3)	7.9709(4)
<i>c</i> (Å)	19.6228(2)	24.5340(3)	29.4427(3) Å	28.6181(8)	40.3829(19)
β	113.6310(10)°	90.460(2)°	101.3693(7)°	-	-
<i>V</i> (Å ³)	2925.04(6)	2676.06(9)	2625.10(4)	2093.76(13)	2076.08(16)
<i>D</i> _c (g cm ⁻³)	1.282	1.399	1.426 g	1.213	1.230
<i>Z</i>	8	4	4	4	4
Crystal Size (mm)	0.295×0.080×0.039	0.168×0.040×0.033	0.134×0.120×0.044	0.382×0.229×0.059	0.188×0.187×0.016
Crystal Colour	colourless	colourless	colourless	colourless	colourless
Crystal Habit	blade	columnar	prism	prism	late
Temperature (Kelvin)	150.0(5)	100.0(5)	100.0(1)	150.0(5)	150.0(5)
λ (Cu K α , Å)	1.5418	1.54184	1.5418	1.54184	1.5418
μ (Cu K α , mm ⁻¹)	0.788	2.462	2.509	0.713	0.720
<i>T</i> _{max,max} (multi-scan)	0.884, 1.00	0.934, 1.00	0.874, 1.00	0.696, 1.00	0.737, 1.00
2 θ _{max}	152.59°	153.23°	152.64°	148.88°	171.94°
<i>hkl</i> range	−19 18, −12 12, −24 24	−8 7, −17 17, −30 30	−18 18, −7 7, −36 34	−8 9, −11 11, −35 35	−8 8, −9 9, −50 50
<i>N</i>	65936	52872	74047	36004	47388
<i>N</i> _{ind}	11984 (<i>R</i> _{merge} 0.0338)	10615 (<i>R</i> _{merge} 0.0506)	10685 (<i>R</i> _{merge} 0.0448)	4173 (<i>R</i> _{merge} 0.1085)	4335 (<i>R</i> _{merge} 0.0772)
<i>N</i> _{iobs}	11783(<i>I</i> > 2 σ (<i>I</i>))	9834(<i>I</i> > 2 σ (<i>I</i>))	10457(<i>I</i> > 2 σ (<i>I</i>))	3841(<i>I</i> > 2 σ (<i>I</i>))	3937(<i>I</i> > 2 σ (<i>I</i>))
<i>N</i> _{var}	781	665	672	252	281
Residuals <i>R</i> 1(<i>F</i>), <i>wR</i> 2(<i>F</i> ²)	0.0403, 0.1112	0.0522, 0.1327	0.0337, 0.0844	0.0474, 0.1342	0.0555, 0.1350
GoF(all)	1.301	1.220	1.159	1.361	1.267
Residual Extrema (e ⁻ Å ⁻³)	−0.260, 0.203	−0.573, 1.496	−0.672, 1.215	−0.298, 0.296	−0.322, 0.335

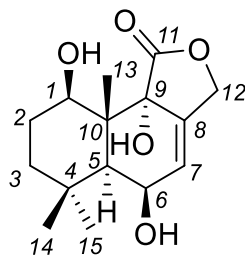


Table S3. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for nanangenine A (**1**) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (<i>J</i> in Hz)	HMBC	COSY	NOESY
1	69.0	4.12, dd (8.4, 7.8)	2, 3, 9, 13	1-OH, 2	1-OH, 2, 5, 9-OH
2	26.2	1.54, m	1, 4, 5, 14	1, 3	1, 3, 13
3	41.5	1.22, m	4, 14	2	1, 2, 6-OH, 13, 15
4	33.6				
5	45.0	1.52, d (5.0)	3, 4, 6, 9, 12, 13	6	1, 3, 6, 9-OH, 14, 15
6	63.3	4.30, m		5, 6-OH	5, 6-OH, 7, 15
7	128.2	5.92, m	5, 9	6, 12a/b	6, 6-OH, 12b
8	131.1				
9	76.1				
10	42.4				
11	179.3				
12a	70.6	5.00, ddd (12.2, 2.5, 2.4)	7, 8, 9, 11	7, 12b	6, 7
12b		4.91, ddd (12.2, 1.3, 1.2)		7, 12a	7
13	12.6	0.92, s	1, 5, 9, 10,		2, 3, 1-OH
14	24.3	1.23, s			1, 2, 15
15	31.9	1.02, s	3, 4, 5, 15		5, 6, 14
1-OH		4.76, s	1	1	1, 2, 13
6-OH		4.81, br d (5.8)	6	6	6, 7, 9-OH, 14
9-OH		6.50, s	3, 8, 9, 11		1, 5, 6-OH,

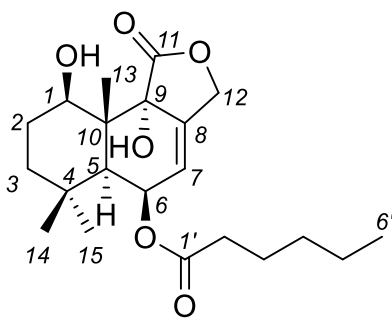


Table S4. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for nanangenine B (2) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (<i>J</i> in Hz)	HMBC
1	68.8	4.19, m	2, 9, 13
2	26.0	1.57, m ^b	
3	41.4	1.29, m ^c	1, 2, 4, 5,
4	33.1		1, 4, 6, 9, 10, 13,
5	43.9	1.87, d (4.94)	14, 15
6	66.1	5.47, m	5, 7, 8, 1'
7	122.0	5.88, m	5, 6, 9, 12
8	135.6		
9	75.7		
10	42.5		
11	178.5		
12a	70.4	5.03, m	6, 7, 8, 9, 11
12b		4.94, m	6, 7, 8, 9, 11
13	12.3	0.95, s	1, 5, 9, 10
14	24.1	1.07, s	3, 4, 5, 15
15	30.6	0.91, s	3, 4, 5, 14
1'	172.5		
2'a	34.1	2.32, m	1', 3', 4'
2'b		2.27, m	1', 3', 4'
3'	24.0	1.55, m ^b	1', 4', 5'
4'	31.6	1.23, m ^c	3', 5'
5'	21.8	1.27, m ^c	4', 6'
6'	13.8	0.84, t (7.12)	4', 5'
1-OH		4.73, br s	1, 2, 10
9-OH		6.78, br s	8, 9, 10, 11

^{b, c} indicates overlapping resonances.

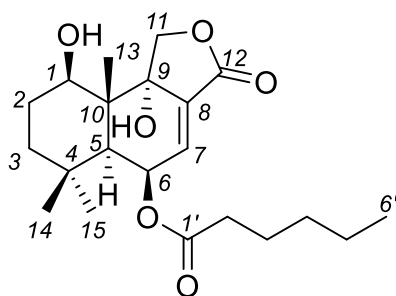


Table S5. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for isonanangenine B (**3**) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (J in Hz)	HMBC
1	69.2	3.96, ddd (11.4, 4.9, 4.9)	9, 10, 13
2a	27.7	1.58, m ^b	
2b		1.53, m	
3a	42.2	1.31, m ^c	1, 2, 4, 5, 14
3b		1.24, m	1, 4, 5, 14
4	32.4		
5	44.6	1.99, d (5.07)	4, 10, 13, 14, 15
6	66.6	5.62, dd (4.5, 4.4)	5, 7, 8, 9, 1'
7	132.1	6.46, d (3.88)	4, 5, 6, 8, 9, 12
8	133.6		
9	75.6		
10	43.7		
11a	76.9	4.43, d (10.4)	9, 10, 12
11b		4.20, d (10.4)	8, 9, 12, 13
12	169.1		
13	12.7	0.96, s	1, 5, 9, 10
14	24.6	1.09, s	3, 4, 5, 15
15	33.3	0.93, s	4, 5, 14
1'	172.7		
2'a	34.3	2.36, m	1', 3', 4'
2'b		2.29, m	1', 3', 4'
3'	24.3	1.55, m ^b	4', 5'
4'	31.0	1.25, m ^c	3', 5'
5'	22.2	1.26, m ^c	4', 6'
6'	14.2	0.84, t (6.8)	4', 5'
1-OH		4.65, br s	1, 10
9-OH		5.64, br s	9, 11

^{b, c} indicates overlapping resonances.

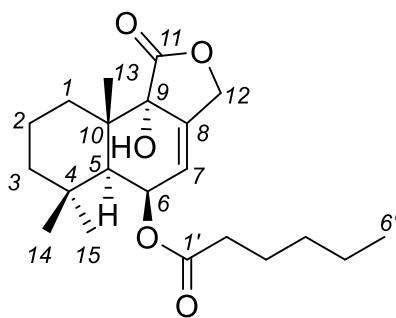


Table S6. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for nanangenine C (**4**) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (<i>J</i> in Hz)	HMBC
1	29.9	1.95, dd (13.9, 4.3)	2, 9, 13
2a	17.8	1.58, m	
2b		1.46, m	
3	44.8	1.33, m	1, 2, 4, 5,
4	33.7		
5	44.5	1.98, d (4.9)	4, 6, 9, 10, 13, 14, 15
6	66.5	5.48, m	5, 7, 8, 1'
7	121.8	5.76, m	5, 6, 9, 12
8	136.9		
9	73.6		
10	37.6		
11	174.8		
12	68.7	4.80, m	7, 8, 9, 11
13	18.3	1.01, s	1, 5, 9, 10
14	24.6	1.09, s	3, 4, 5, 15
15	32.5	0.92, s	3, 4, 5, 14
1'	172.7		
2'	34.5	2.28, m	1', 3', 4'
3'	24.3	1.53, m	1', 4', 5'
4'	31.0	1.26, m ^b	3', 5'
5'	22.2	1.26, m ^b	4', 6'
6'	14.1	0.84, t (7.1)	4', 5'
9-OH		6.30, br s	8, 9, 10, 11

^b indicates overlapping resonances.

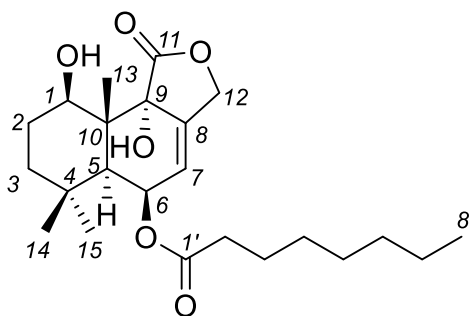


Table S7. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for nanangenine D (5) in $\text{DMSO}-d_6$.

Pos.	δ_{C}	δ_{H} , mult. (J in Hz)	HMBC	COSY	ROESY
1	68.7	4.17, ddd (10.9, 5.9, 0.9)	2, 3, 9, 10, 13	2, 1-OH	1-OH, 2, 3, 5, 9-OH
2	25.9	1.57, m	1, 3, 4	1, 3	1, 3, 13, 14
3	41.3	1.28, m	1, 5, 14	2	1, 2, 5, 14, 15
4	33.1				
5	43.8	1.87, d (4.9)	4, 6, 9, 10, 13, 14, 15	5, 6	1, 3, 6, 9-OH, 15
6	66.0	5.46, m	7, 8, 10, 1'	5, 7, 12a/b	5, 7, 15
7	121.9	5.88, m	5, 6, 8, 9, 11	6, 12a/b	6, 12b
8	135.5				
9	75.6				
10	42.4				
11	178.4				
12a	70.3	5.03, ddd (12.8, 4.8, 2.5)	6, 7, 8, 11	6, 7, 12b	12b
12b		4.94, ddd (12.8, 1.7, 1.2)	6, 7, 8, 9, 11	6, 7, 12a	7, 12a
13	12.2	0.94, s	1, 5, 9, 10		1-OH, 2
14	24.0	1.06, s	3, 5, 15		2, 3, 15
15	31.5	0.90, s	3, 4, 5, 14		3, 5, 6, 14
1'	172.2				
2'a	34.0	2.33, m	1', 3', 4'	2'b, 3'	2'b
2'b		2.27, m	1', 3', 4'	2'a, 3'	2'a
3'	24.2	1.53, m	2', 4', 5'	4'	4'
4'	28.2	1.24, m	3', 5', 6'	3'	3'
5'	28.3	1.24, m	3', 6'	6'	
6'	31.0	1.22, m	7', 8'	5', 7', 8'	
7'	21.9	1.24, m	6'	6', 8'	8'
8'	13.9	0.84, t (7.9)	6', 7'	7'	7'
1-OH		4.71, d (0.9)	1, 2, 10	1	1, 13
9-OH		6.77 s	8, 9, 10, 11		1, 5

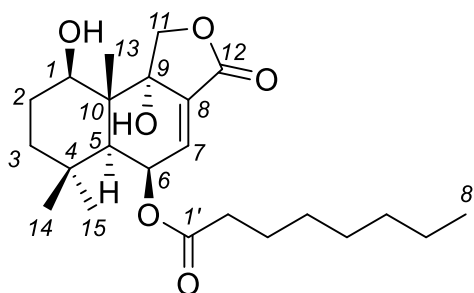


Table S8. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for isonanangenine D (**6**) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (J in Hz)	HMBC	COSY	ROESY
1	68.6	3.95, ddd (12.0, 5.9, 3.9)	3, 9, 10, 13	2, 1-OH	1-OH, 2, 3b, 5, 9-OH
2	27.2	1.53, m	1, 3	1, 3	1, 1-OH, 14
3a	41.7	1.31, ddd (13.3, 3.5, 3.5)	1	2	3b, 14
3b		1.23, m			2, 3a, 5
4	32.9				
5	44.1	1.99, d (5.2)	3, 6, 9, 10, 13, 14, 15	6, 13 (wk)	1, 3b, 6, 9-OH, 15
6	66.0	5.59, dd (5.1, 3.9)	7, 8, 10, 1'	5, 7, 11a	5, 7, 15
7	131.6	6.45, d (3.9)	5, 8, 9, 12	6	6
8	133.2				
9	75.0				
10	43.2				
11a	76.4	4.41, dd (10.2, 0.5)	9, 10	11b	11b, 13
11b		4.19, br d (10.2)	8, 9, 12	11a	11a
12	168.6				
13	12.3	0.94, s	1, 9, 10	5 (wk)	1-OH, 11a, 14
14	24.2	1.08, s	3, 4, 5, 15	14 (wk)	2, 3a, 13
15	31.9	0.91, s	3, 4, 5, 14	15 (wk)	6
1'	172.2				
2'a	33.9	2.36, m	1', 3', 4'	2'b, 3'	2'b
2'b		2.27, m	1', 3', 4'	2'a, 3'	2'a
3'	24.2	1.53, m	1', 4', 5'	2'a, 2'b, 4'	4'
4'	28.2	1.23, m	2', 3'	3'	3'
5'	28.2	1.23, m	6'	6'	
6'	31.0	1.21, m		5', 7', 8'	
7'	21.9	1.23, m	5', 6', 8'	8'	8'
8'	13.9	0.83, t (7.2)	6', 7'	7'	7'
1-OH		4.62, d (5.1)	1, 2, 10	1	1, 2, 13
9-OH		5.61 br s	8, 9, 11		1, 5

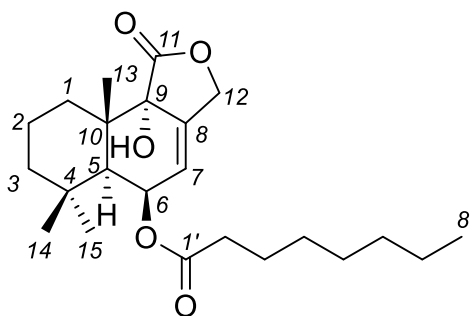


Table S9. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for nanangenine E (7) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (J in Hz)	HMBC	COSY	ROESY
1a	29.4	1.95, ddd (15.0, 13.5, 4.3)	3, 10, 13	1b, 2a/2b	1b
1b		1.81, dm (13.5)	3, 10, 13	1a, 2a/2b	1a, 13
2a	17.4	1.59, m		1a/b, 3a/b	2b, 13, 14
2b		1.47, m		1a/b, 3a/b	2a
3a	44.3	1.33, dm (13.0)		2a/b, 3b	3b, 14
3b		1.19, m		2a/b, 3a	3a, 5
4	33.3				
5	44.0	1.97, d (4.9)	4, 9, 10, 13, 14	6	3b, 6, 9-OH, 15
6	66.0	5.47, m	7, 10	5, 7, 12a,	5, 7, 15
7	121.3	5.77, m	3, 5, 9, 12	6, 12a	6, 12b
8	136.5				
9	73.1				
10	37.2				
11	174.3				
12a	68.2	4.87, ddd (12.8, 2.5, 2.5)	7, 8, 11	6, 7, 12b	12b
12b		4.74, ddd (12.8, 1.2, 1.2)	7, 8, 9, 11	12a	7, 12a, 13
13	18.1	0.99, s	1, 5, 9, 10		1b, 2a, 12b, 14
14	24.2	1.08, s	3, 4, 5, 15	14	2a, 3a, 13, 15
15	32.1	0.91, s	3, 4, 5, 14	15	5, 6, 14
1'	172.2				
2'a	34.0	2.32, m	1', 3', 4'	2'b	2'b, 4'
2'b		2.25, m	1', 3', 4'	2'a	2'a, 4'
3'	24.2	1.52, m	1', 2', 4', 5'	4'	2'a, 2'b, 4'
4'	28.3	1.24, m	5', 6'	3'	3'
5'	28.2	1.24, m	4', 6'	6'	
6'	31.0	1.21, m	4', 5'		
7'	21.9	1.24, m	5', 6'	8'	8'
8'	13.9	0.84, t (7.2)	6', 7'	7'	7'
9-OH		6.24 s	8, 9, 10, 11		5

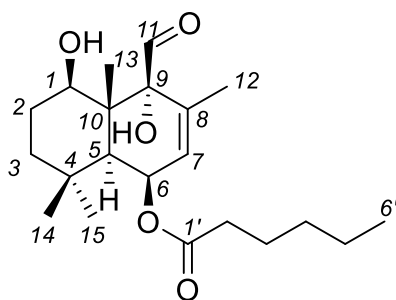


Table S10. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for nanangenine F (**8**) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult (J in Hz)	HMBC	COSY	ROESY
1	68.5	3.88, ddd (11.9, 5.8, 5.8)	9, 10, 13	2a, 1-OH	1-OH, 2b, 3a, 5, 9-OH
2a	27.3	1.51, m	1, 3	1	3b, 9-OH, 13, 14
2b		1.47, m	1, 3		1-OH, 3a
3a	41.6	1.26, m	1	3b	1, 2b, 14
3b		1.21, m		3a	2a, 5
4	32.8				
5	43.5	1.84, d (4.7)	4, 9, 10, 13, 14	6	1, 3b, 6, 9-OH, 15
6	66.5	5.37, m	7, 8, 10, 1'	5, 7, 12	5, 7, 15
7	124.0	5.53, dq (5.1, 1.5)	5, 6, 9, 12	6, 12	6, 12
8	137.2				
9	80.1				
10	47.0				
11	203.3	9.49, br s	7 (wk), 8 (wk), 9	9-OH	1-OH, 9-OH, 12, 13
12	19.5	1.51, dd, (1.3, 1.3)	7, 8, 9	6, 7	7, 11
13	11.5	1.06, s	1, 9, 10		2a, 11
14	24.3	1.05, s	3, 4, 5, 15		15, 2'a, 2'b,
15	31.8	0.89, s	3, 4, 5, 14		3a, 5, 6, 14
1'	172.3				
2'a	34.1	2.30, m	1', 3', 4'	3'	14, 3', 4'
2'b		2.23, m	1', 3', 4'	3'	14, 3', 4'
3'	23.9	1.53, m	1', 2', 4', 5'	2'a/b, 4'	2'a, 2'b
4'	30.6	1.25, m	5'	3', 6'	2'a, 2'b
5'	21.7	1.26, m	4'	3', 6'	6'
6'	13.7	0.84, t (7.0)	4', 5'	5'	5'
1-OH		4.74, d, (5.7)	1, 2, 10	1	1, 11
9-OH		5.46, s	8, 9, 10, 11	11	1, 2a, 5, 11

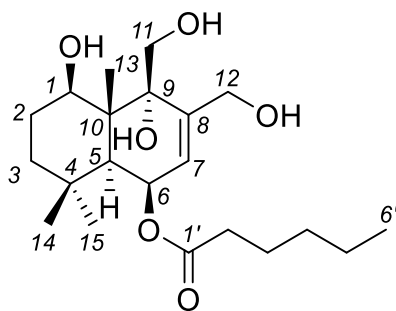


Table S11. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for nanangenine G (**9**) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (J in Hz)	HMBC	COSY	NOESY
1	69.1	3.93 dd (11.2, 4.3)		1-OH, 2	2, 3, 9-OH
2	28.3	1.56, m ^b	15	1, 3	1, 3
3	41.6	1.23, m ^c	1, 2, 4, 5,	2	1, 2, 5, 14
4	33.5				
5	44.9	1.83, d (4.4)	4, 10, 13, 15	6	1, 3, 6, 9-OH, 15
6	67.0	5.41, m	7, 8, 10, 1'	7	5, 7
7	121.4	5.78, br d (5.3)	5, 6, 8, 9, 11	6, 12a	6, 12a, 12-OH
8	144.1				
9	75.6				
10	45.9				
11a	62.2	3.73, m	8, 9, 10	11b, 11-OH	1-OH, 9-OH, 11b, 11-OH, 12a
11b		3.67, m	8, 9, 10	11a, 11-OH	9-OH, 11a, 11-OH, 12a
12a	61.0	4.05, m	7, 8, 12-OH	7, 12b, 12-OH	7, 11b, 12-OH
12b		4.02, m	7, 8, 12-OH	12a, 12-OH	7, 11b, 12-OH
13	12.5	1.06, s	1, 5, 9		2, 11a
14	24.4	1.03, s	3, 4, 5		2, 15, 2'a
15	32.4	0.87, br s	3, 5, 15		3, 5, 6, 14
1'	172.7				
2'a	34.5	2.24, m	1', 3', 4'	3'	3', 5'
2'b		2.21, m	1', 3', 4'	3'	3'
3'	24.2	1.53, m ^b	4', 5'	2'a, 2'b, 4'	2'a
4'	30.9	1.25, m ^c	3', 5', 6'	3', 5'	2'a, 6'
5'	22.0	1.26, m ^c	3', 6'	4', 6'	6'
6'	13.9	0.83, t (7.1)	4', 5'	5'	3', 5'
1-OH		5.07, br s	1, 2, 10	1	9-OH, 11-OH, 12-OH
9-OH		4.41, br s	8, 9, 10, 12		1, 1-OH, 5, 11a, 11-OH, 12-OH
11-OH		5.27, br s	8, 11	11a, 11b	1-OH, 9-OH, 12-OH
12-OH		4.88, br s		12a, 12b	1-OH, 9-OH, 11-OH, 12b

^{b, c} indicates overlapping resonances.

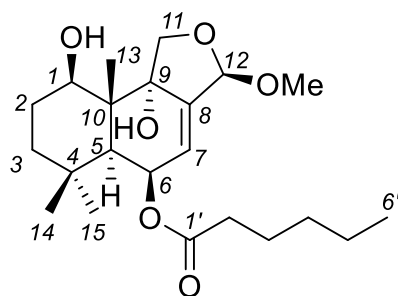


Table S12. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data for nanangenine H (**10**) in $\text{DMSO-}d_6$.

Pos.	δ_{C}	δ_{H} , mult. (J in Hz)	HMBC	COSY	ROESY
1	68.4	3.93, ddd (11.8, 5.4, 5.0)	9, 10, 13	2a, 1-OH	1-OH, 2a, 2b, 3b, 5, 9-OH
2a	27.5	1.53, m	1, 3, 4	1, 2b	3b
2b		1.48, m		2a, 3a	1, 1-OH, 3a
3a	41.9	1.27, m	1	2b	2b, 14
3b		1.22, m			2a
4	32.8				
5	44.3	1.95, d (4.0)	4, 9, 10, 14, 15	6, 13	1, 3b, 6, 9-OH, 15
6	67.1	5.46, ddd (5.0, 3.9, 1.4)	7, 8, 10, 12, 1'	5, 7	5, 7, 15
7	120.3	5.57, dd (3.9, 1.4)	5, 9, 12	6, 12	6
8	145.0				
9	78.3				
10	43.5				
11a	74.5	3.84, d (9.7)	7, 9	11b	11b, 13
11b		3.76, d (9.7)	7, 8, 9, 12	11a	1-OH, 11a
12	101.9	5.31, t (1.4)	7, 9, 11, 12-OMe	6, 7	9-OH, 12-OMe
13	11.7	0.92, s	1, 5, 9, 10	5	1-OH, 11a, 15, 3'
14	24.3	1.08, s	3, 4, 5, 15	14	3a, 13, 15, 3'
15	32.1	0.89, s	3, 4, 5, 14	15	5, 6, 14
1'	172.3				
2'a	34.1	2.32, m	1', 3', 4'	3'	
2'b		2.23, m	1', 3', 4'	3'	
3'	24.0	1.52, m	1', 2', 4', 5', 6'	2'a/b, 4'	13, 14
4'	30.5	1.24, m	5',	3'	
5'	21.7	1.25, m	4'	6'	6'
6'	13.9	0.84, t (7.1)	4', 5'	5'	5'
12-OMe	54.1	3.26, s	12		12
1-OH		4.37, d (5.4)	1, 2, 10	1	1, 2b, 11b, 13
9-OH		4.93, s	8, 9, 10, 11		1, 5, 12

Table S13. NCBI accessions of internal transcribed spacer (ITS) region sequences used in Figure S48.

Organism	NCBI accession
<i>Aspergillus flavus</i> CBS 569.65	AF027863
<i>Aspergillus tubingensis</i> NRRL 4875	EF661193
<i>Aspergillus niger</i> CBS 554.65	EF661186
<i>Aspergillus terreus</i> CBS 601.65	EF669586
<i>Aspergillus carneus</i> CBS 494.65	EF669611
<i>Aspergillus brevijanensis</i> CBS 111.46	EF669582
<i>Aspergillus janus</i> CBS 118.45	EF669578
<i>Aspergillus nanangensis</i> MST-FP2251	MK979278

Table S14. Predicted functions and best BLAST hit loci for genes in the putative nanangenine biosynthetic gene cluster (BGC).

Scaffold_3: 476387-509910	Size (nt / aa)	Predicted function	Best BLAST loci (identity/coverage %)
FE257_006540	11080/3467	Oxidoreductase	<i>A. heteromorphus</i> BO70DRAFT_410469 (63.92/90)
FE257_006541	7788/2384	HR-PKS	<i>A. ustus</i> 00536 (66.74/99)
FE257_006542	1773/543	Haloacid dehydrogenase-like	<i>A. ustus</i> 00537 (66.30/99)
FE257_006543	1778/519	FAD-binding oxidoreductase	<i>A. ustus</i> 00538 (60.58/98)
FE257_006544	1580/526	Cytochrome P450	<i>A. ustus</i> 00539 (75.25/96)
FE257_006545	1003/312	Hydrolase	<i>A. calidoustus</i> ASPCAL02981 (72.46/97)
FE257_006546	923/307	Short-chain dehydrogenase	<i>A. lentulus</i> ALT_1008 (83.55/98)
FE257_006547	2023/460	Cytochrome P450	<i>T. stipitatus</i> TSTA_007840 (67.43/100)

Table S15. Pairwise amino acid alignment identity (%) and similarity (%) scores between putative nanangenines BGC in *A. nanangensis* MST-FP2251 and homologs in *A. ustus*, *A. calidoustus*, *A. insuetus* and *A. pseudodeflectus*.

<i>A. nanangensis</i> MST-FP2251 (Locus tag: FE257_)									BGC similarity (%) / BGC conservation (%)
Locus tag	006540	006541	006542	006543	006544	006545	006546	006547	
Protein length	3467	2384	543	519	526	312	307	460	
<i>A. ustus</i> CBS 3.3904 (Locus tag: HK57_)									
Gene no.		00536	00537	00538	00539	00540	00541		64.59/75
Identity (%)		66.26	64.53	59.2	65.48	61.43	62.58		
Similarity (%)		77.2	74.15	73.62	74.83	70.25	74.54		
<i>A. calidoustus</i> CBS 12160 (Locus tag: ASPCAL)									
Gene no.		02977	02978	02979	02980	02981	02982		65.24/75
Identity (%)		66.4	61.66	56.17	69.55	70.83	64.87		
Similarity (%)		76.92	73.42	73.24	79.7	83.65	76.58		
<i>A. insuetus</i> CBS 107.25 (JGI Portal: Aspins1)									
Protein ID		305363	294198	315989	315991	270963	270964		64.72/75
Identity (%)		65.89	60.77	54.68	69.29	71.15	65.19		
Similarity (%)		76.94	70.35	70.55	79.4	84.29	76.58		
<i>A. pseudodeflectus</i> CBS 756.74 (JGI Portal: Asppdef1)									
Protein ID		276134	267293	276137	276138				55.74/50
Identity (%)		66.19	61.66	54.2	69.36				
Similarity (%)		76.63	73.24	70.23	79.51				

Total BGC similarity (%) and conservation (%) were calculated as follows: similarity = sum(sequence length × identity) / maximum score, where maximum score = sum(sequence length × 100); and conservation = number of conserved genes / number of total genes.

Supplementary figures

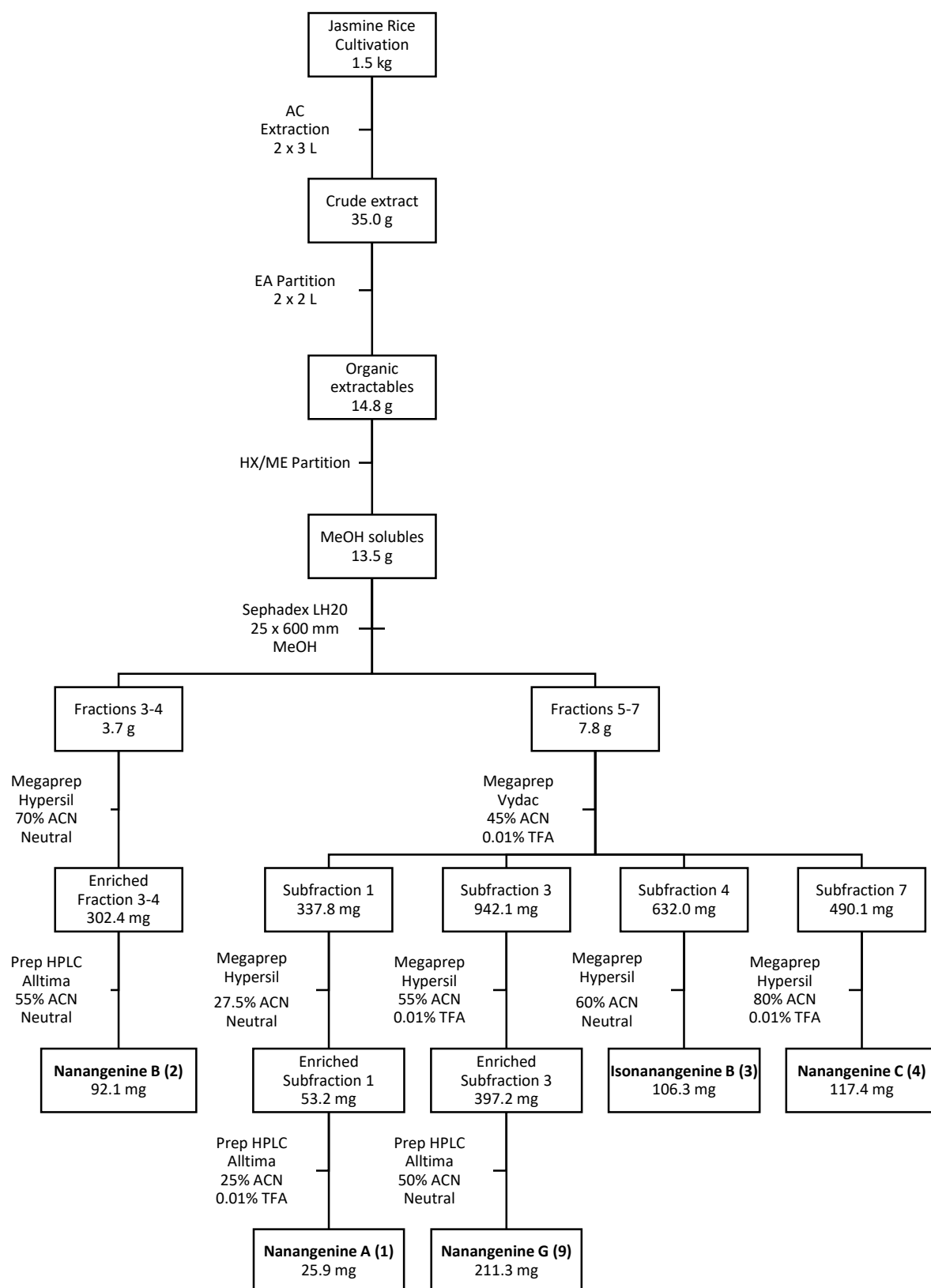


Figure S1. Fractionation scheme for culture A (jasmine rice)

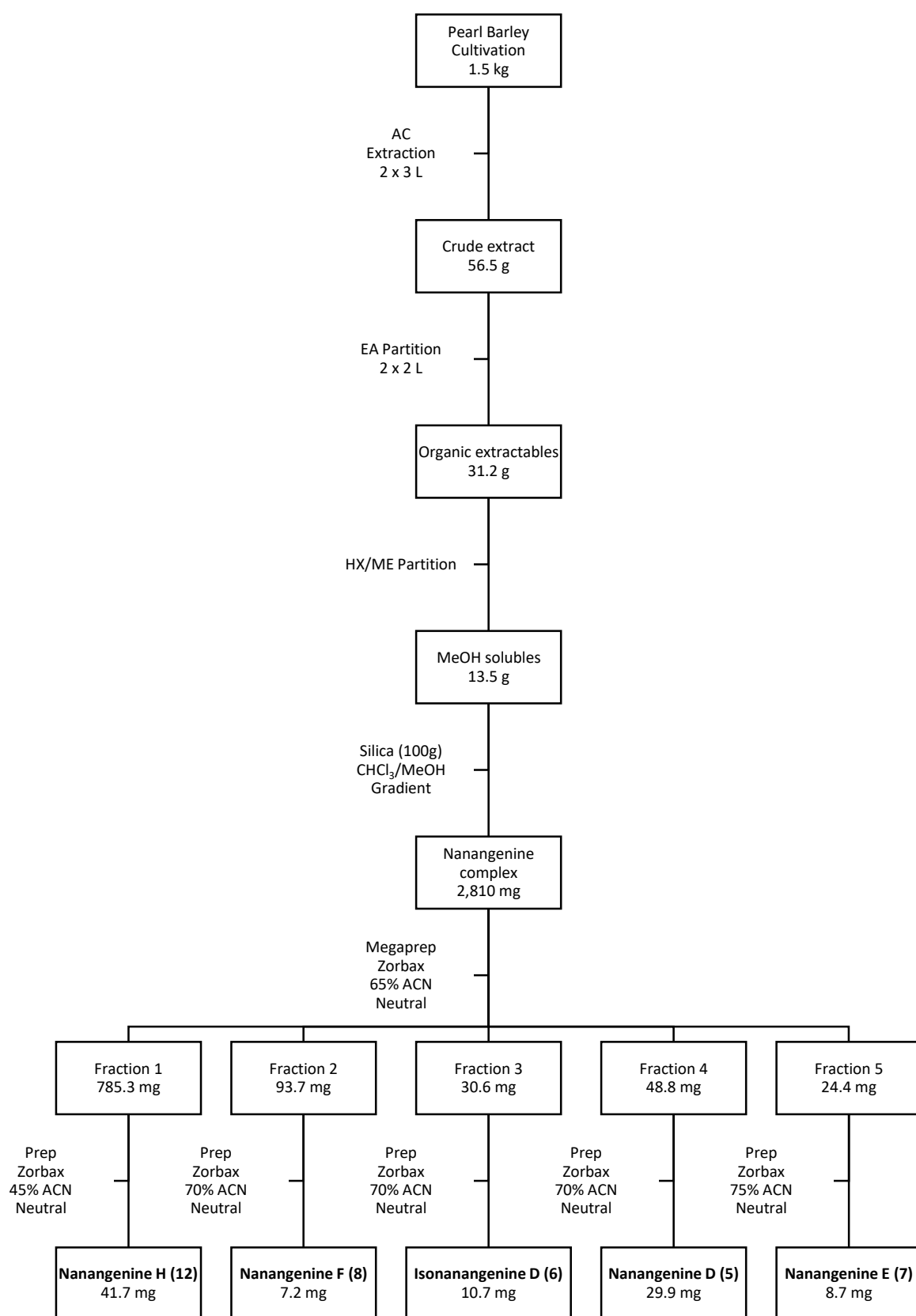


Figure S2. Fractionation scheme for culture B (pearl barley)

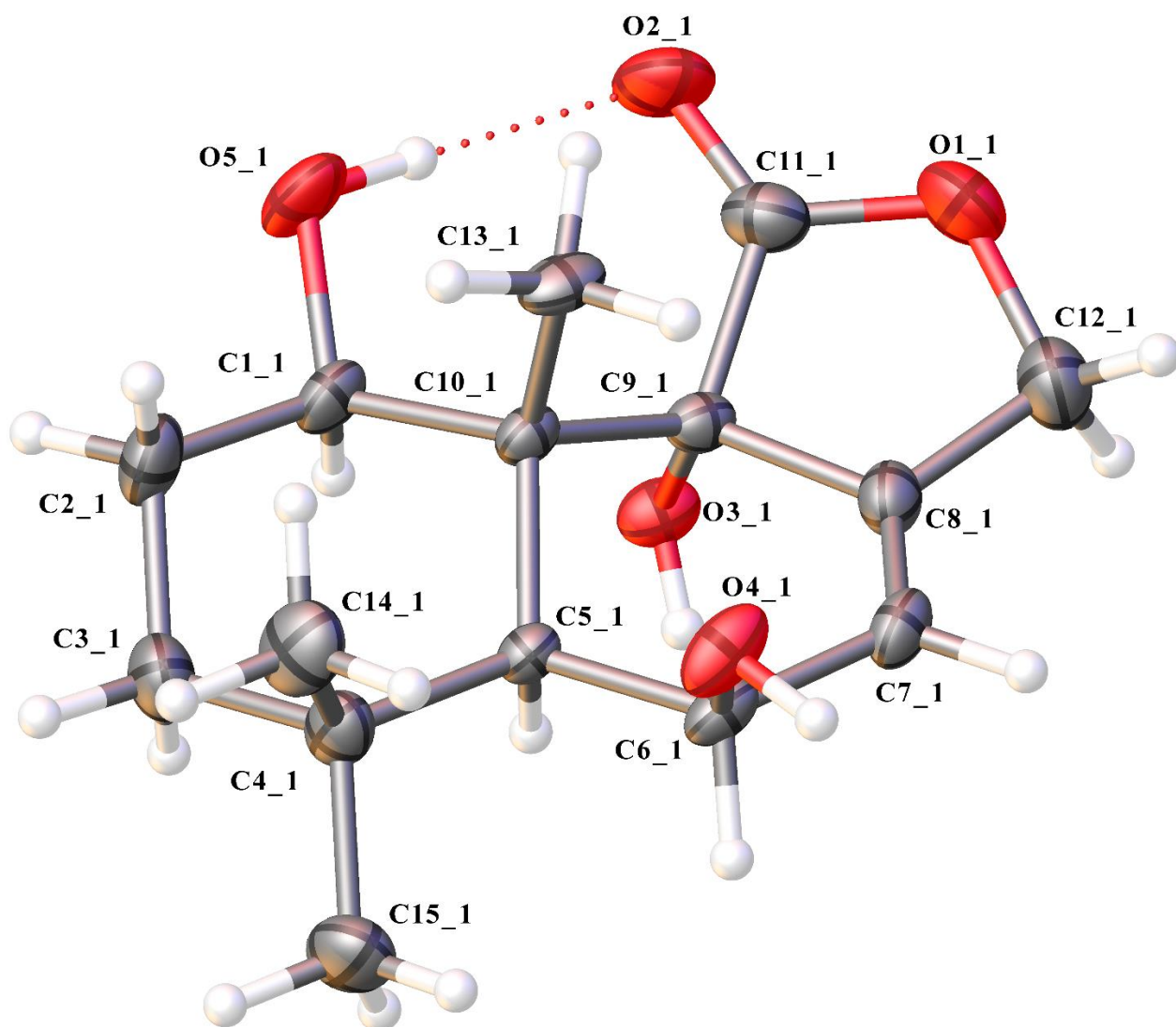


Figure S3. Olex2 depiction of one of the four crystallographically-independent molecules of nanangenine A (**1**), with 50% displacement ellipsoids and showing the intramolecular hydrogen bond.

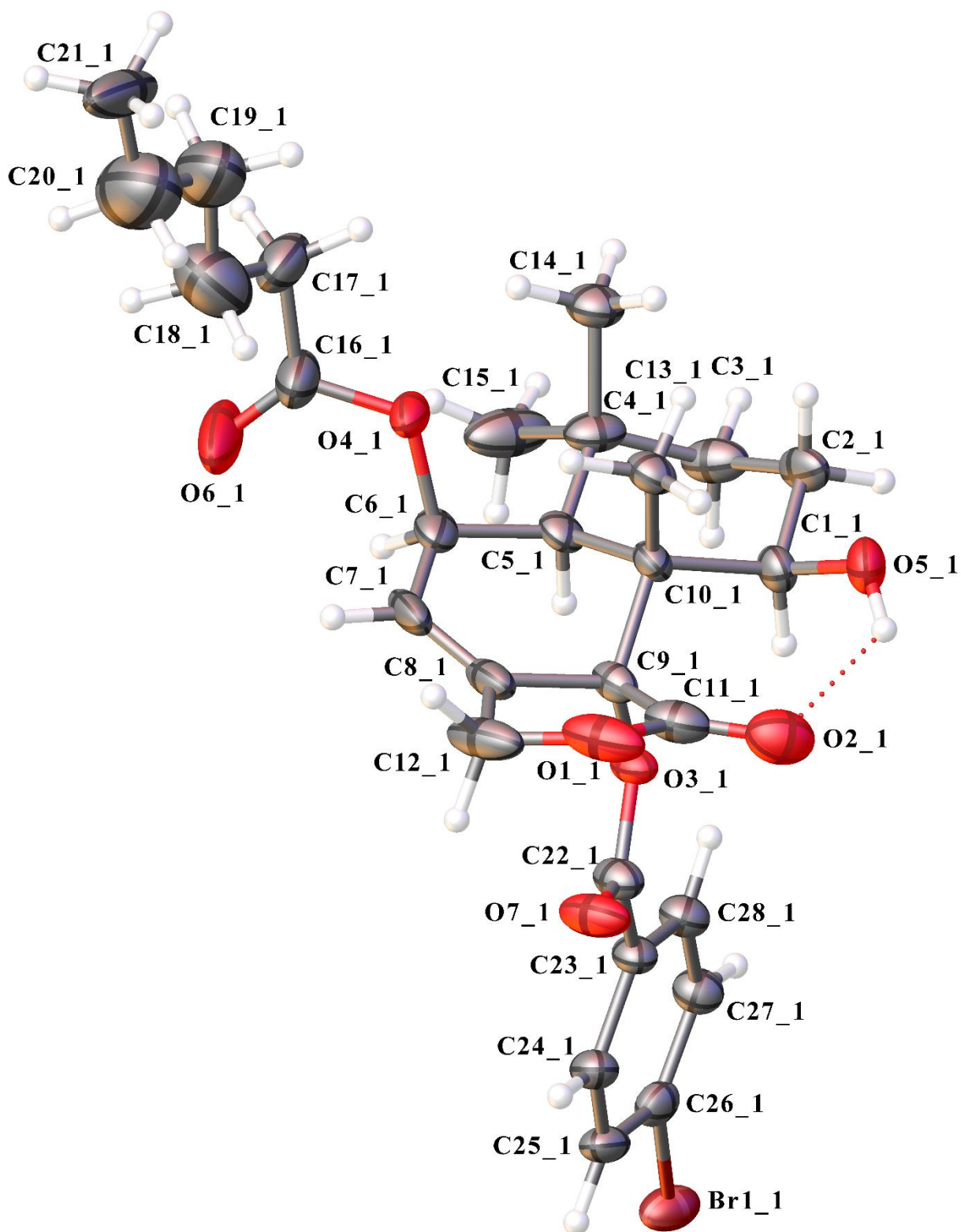


Figure S4. Olex2 depiction of one of the two crystallographically-independent molecules of 9-O-(4-bromobenzoyl)-nanangenine B (**2b**) with 50% displacement ellipsoids.

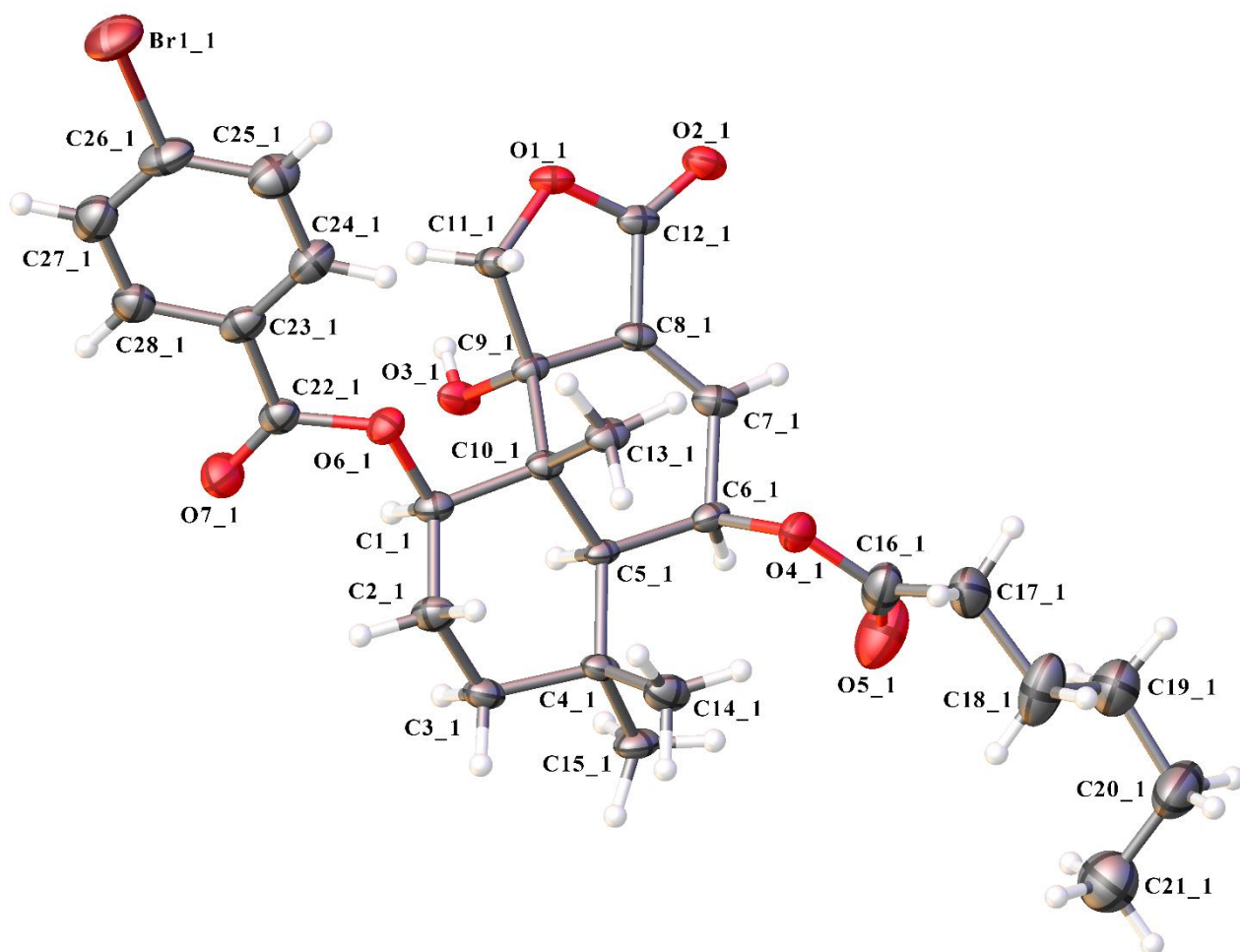


Figure S5. Olex2 depiction of one of the two crystallographically-independent molecules of 1-O-(4-bromobenzoyl)-isonanangenine B (**3b**) with 75% displacement ellipsoids.

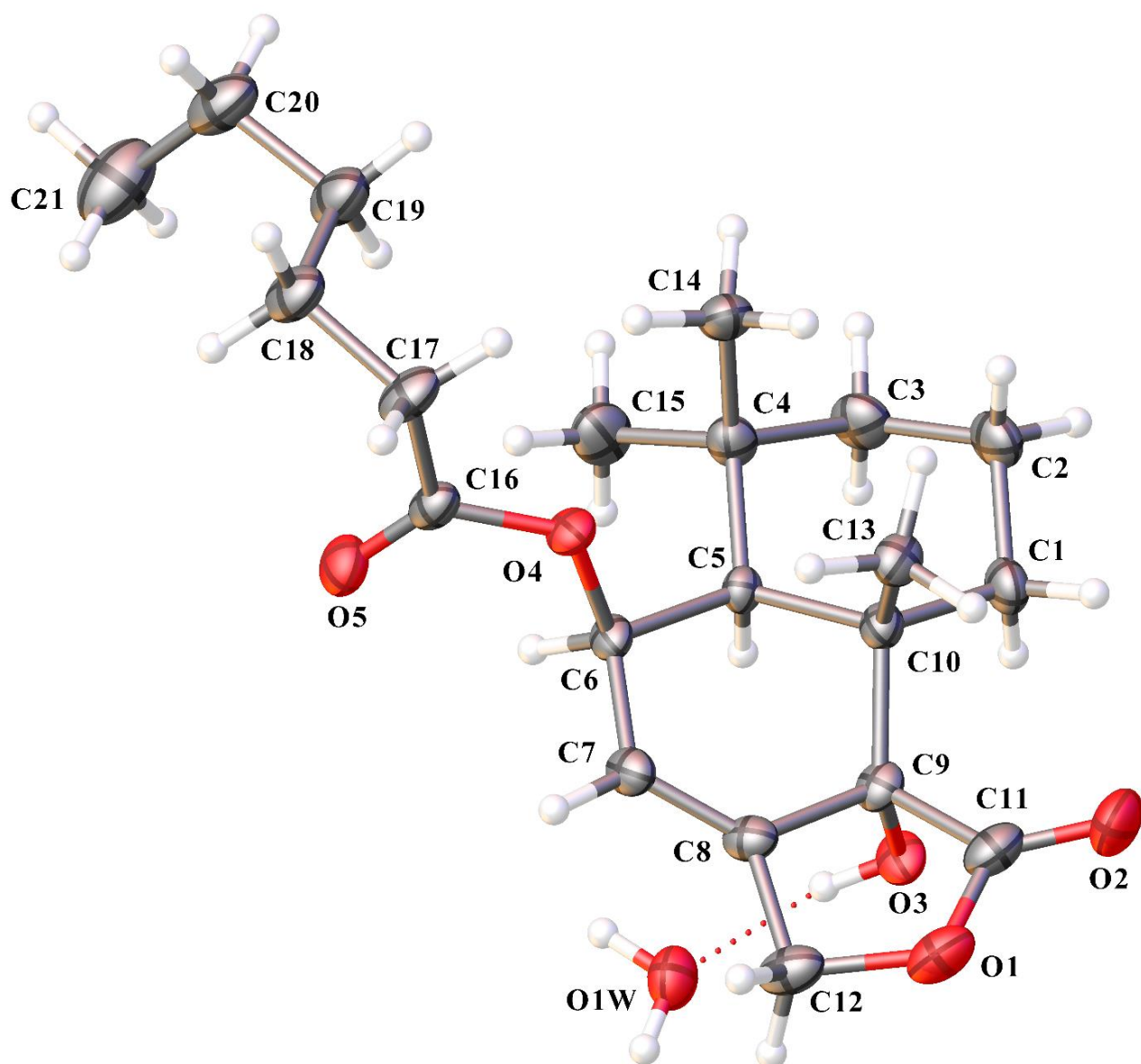


Figure S6. Olex2 depiction of nanangenine C (4) and a hydrogen bond-linked water molecule, with 50% displacement ellipsoids.

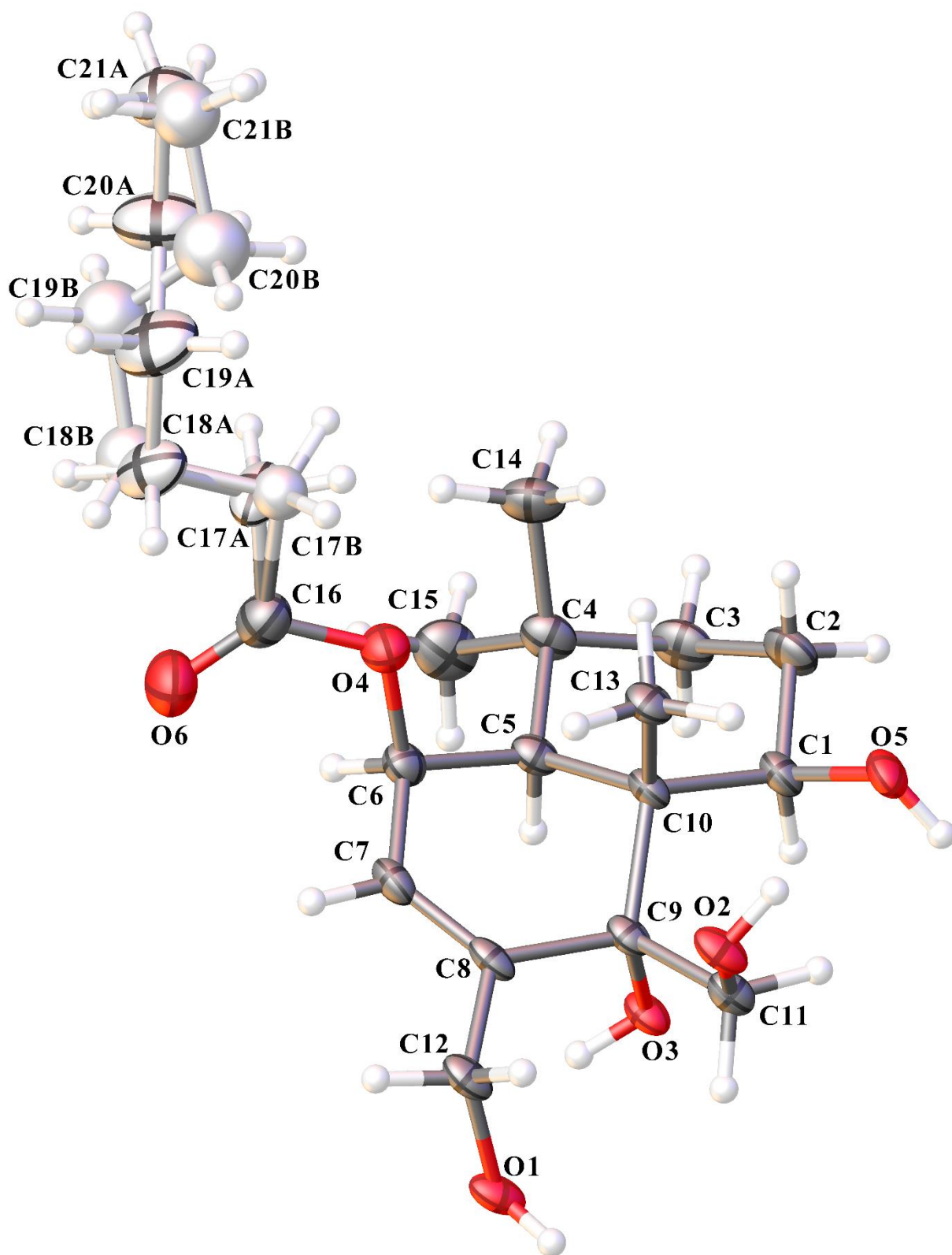


Figure S7. Olex2 depiction of nanangenine G (9) with 50% displacement ellipsoids and disordered sites shown with 'faded' colour.

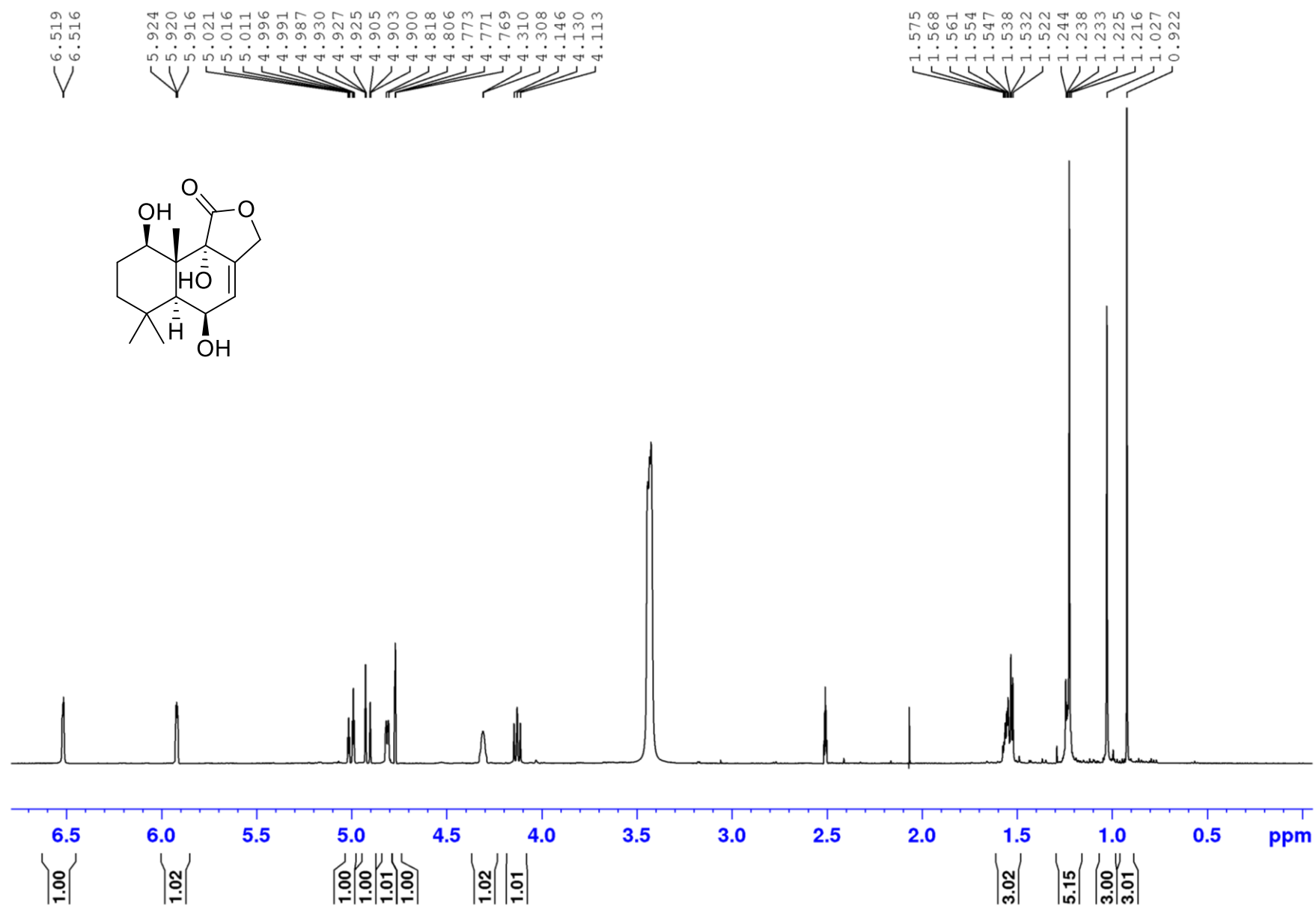


Figure S8. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of nanangenine A (1).

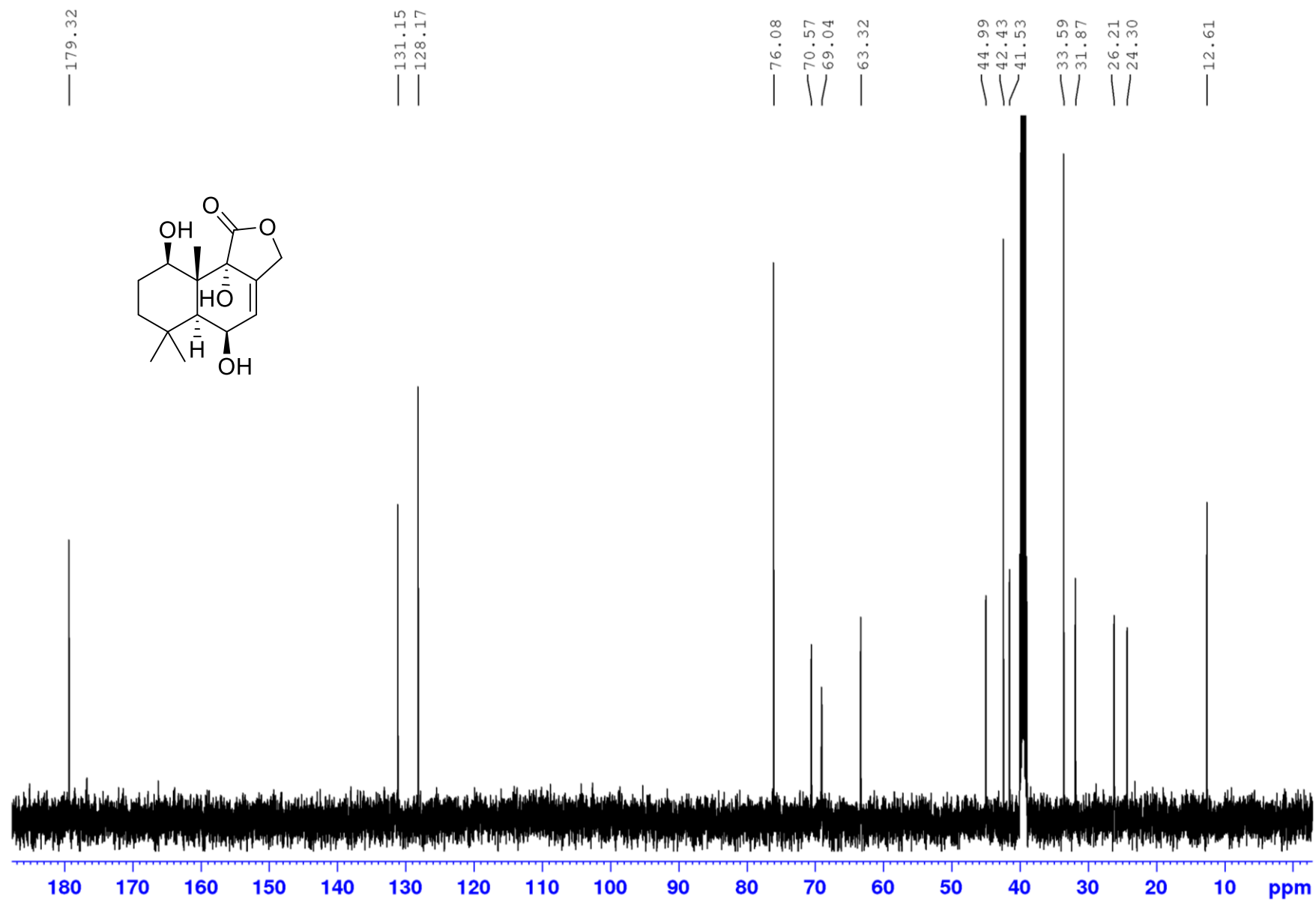


Figure S9. ¹³C NMR spectrum (150 MHz, DMSO-*d*₆) of nanangenine A (1).

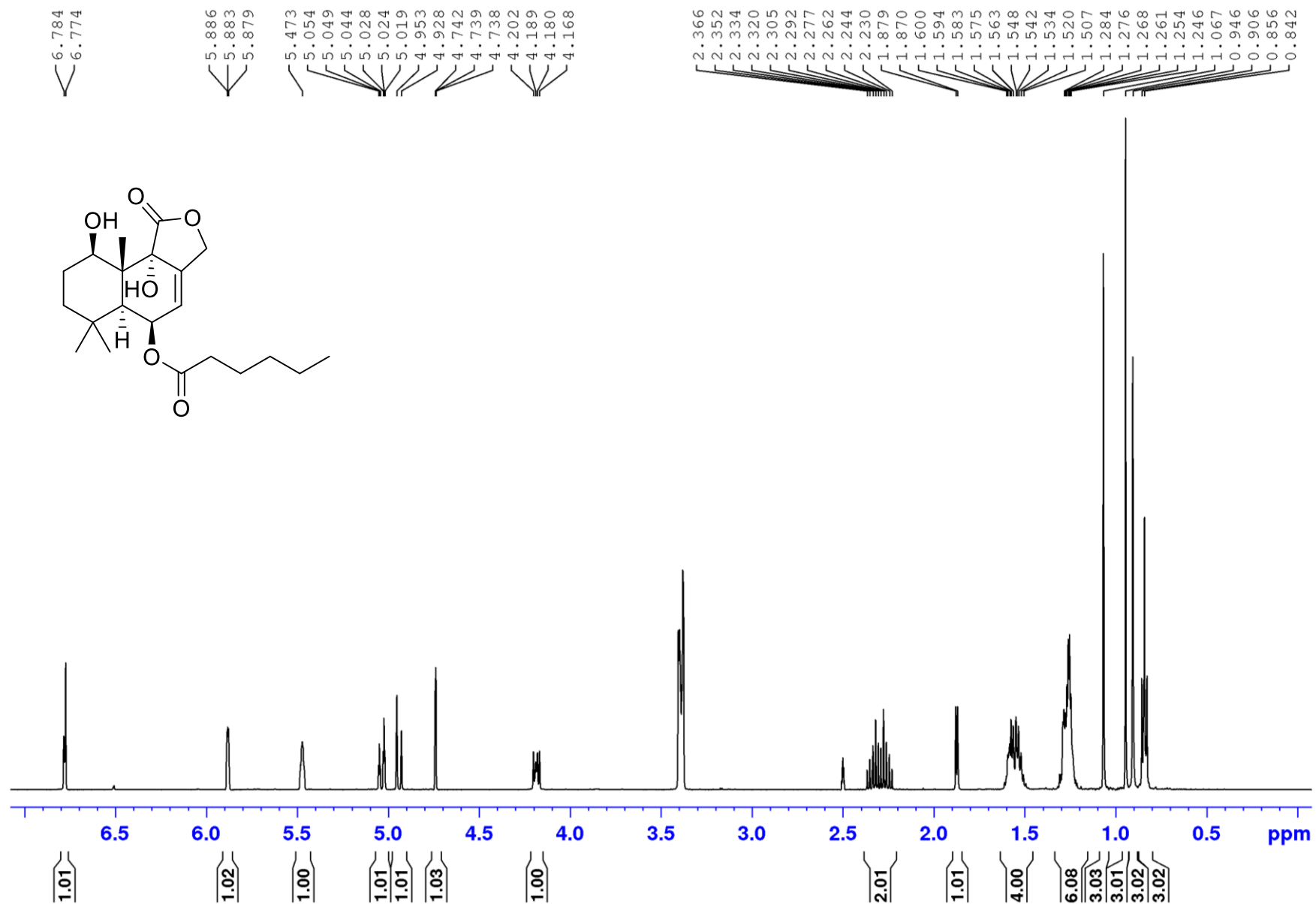


Figure S10. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of nanangenine B (2).

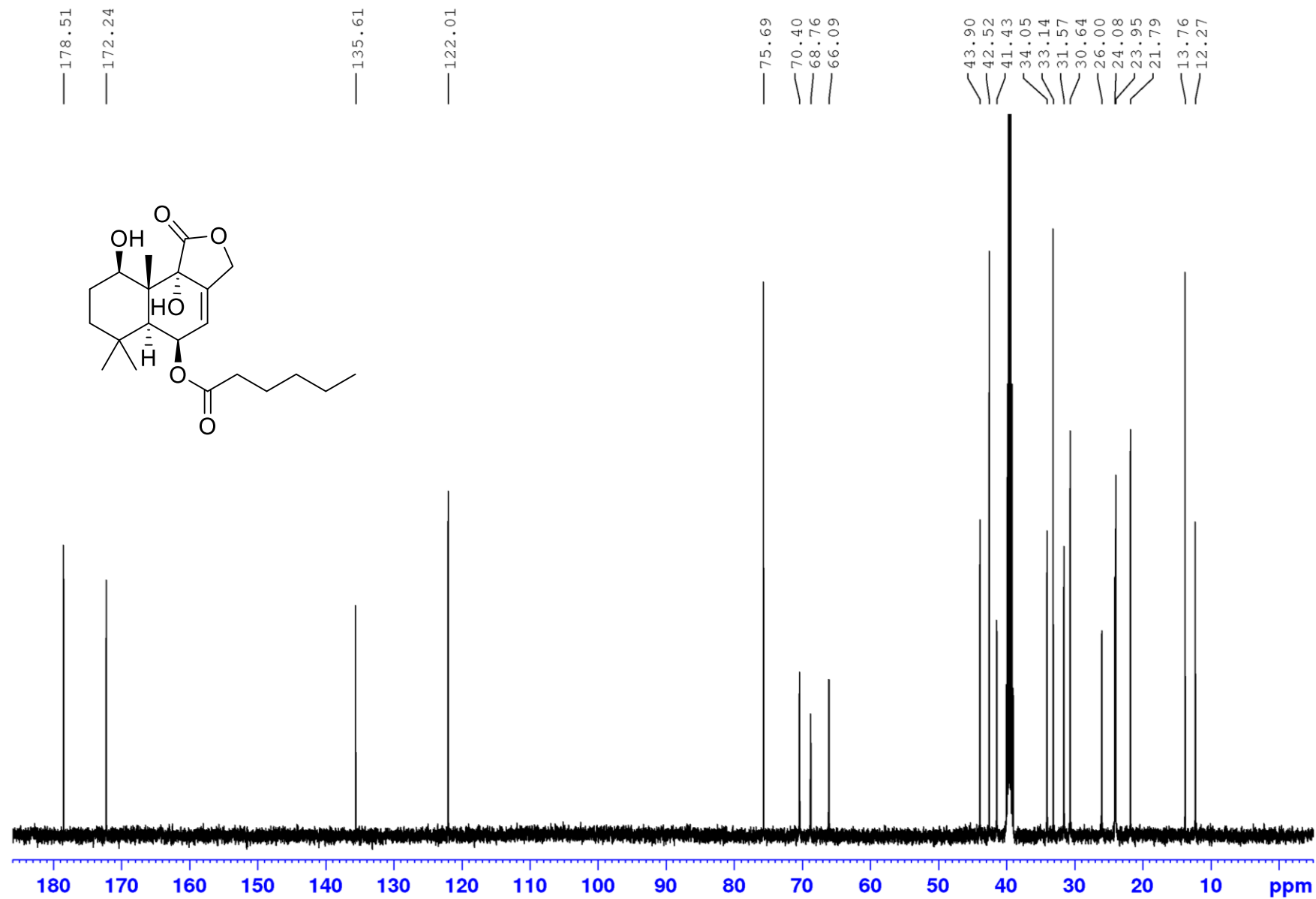


Figure S11. ¹³C NMR spectrum (125 MHz, DMSO-*d*₆) of nanangene B (2).

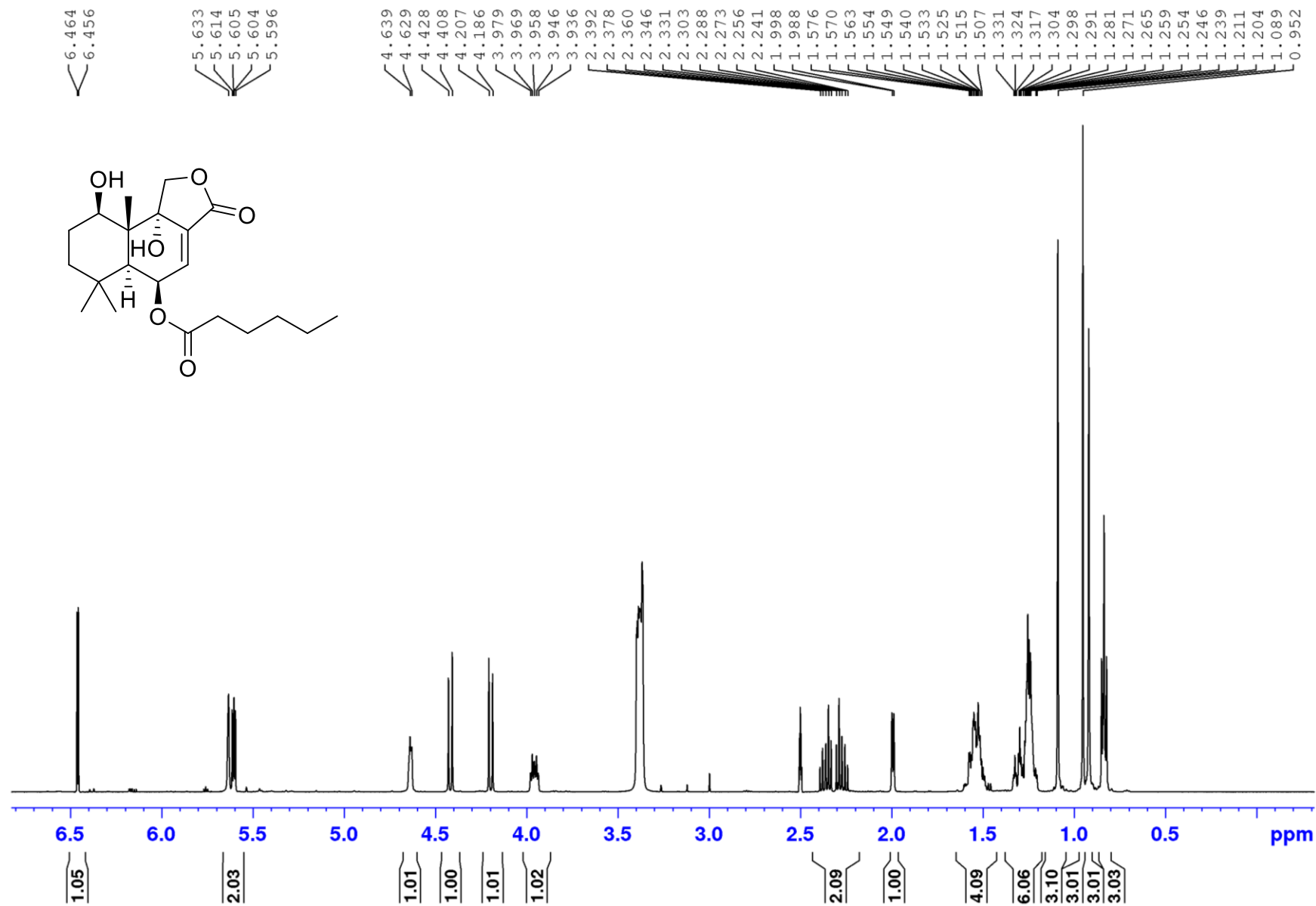


Figure S12. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of isonanangienine B (3).

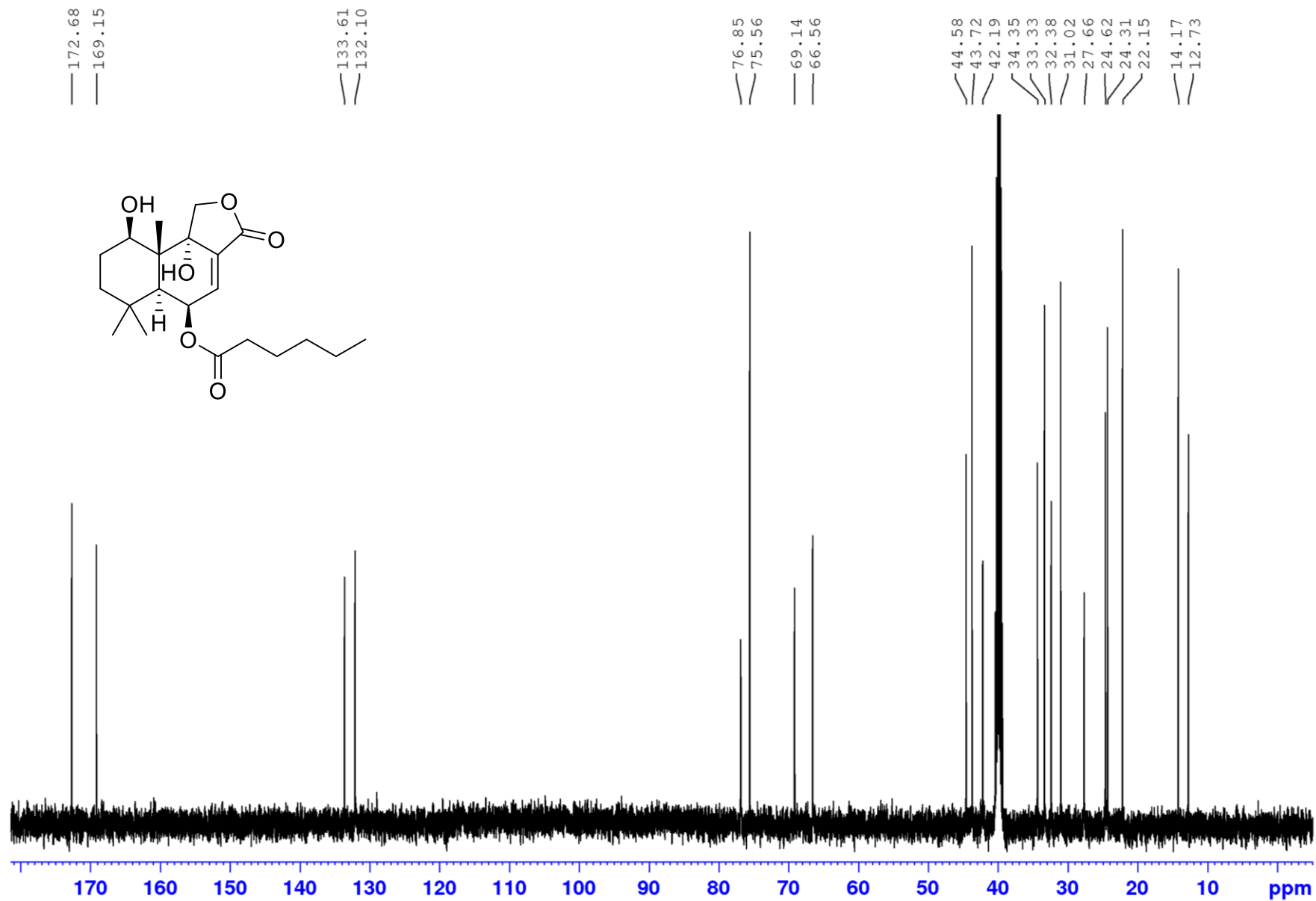


Figure S13. ¹³C NMR spectrum (125 MHz, DMSO-*d*₆) of isonanangienine B (3).

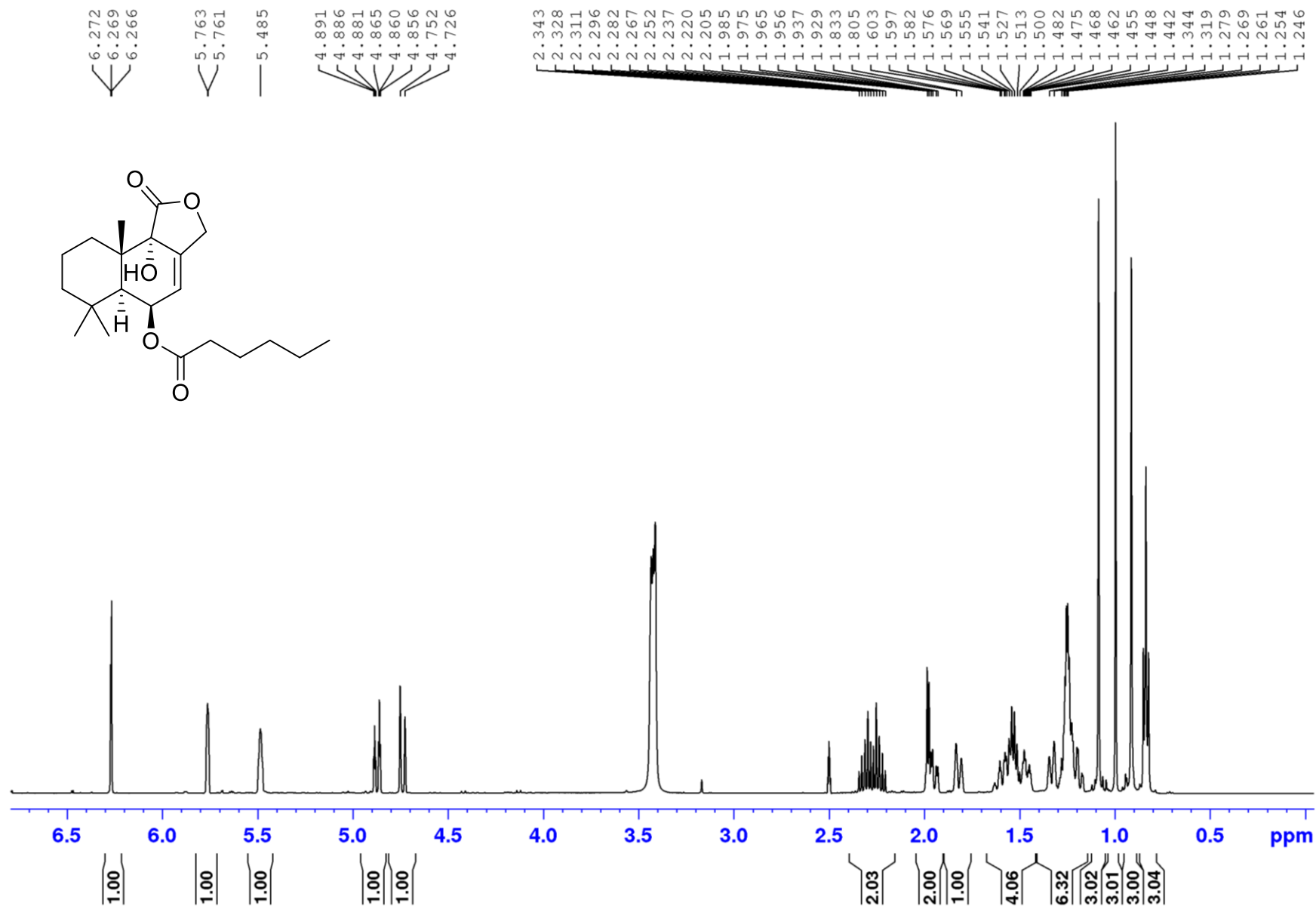


Figure S14. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of nanangene C (4).

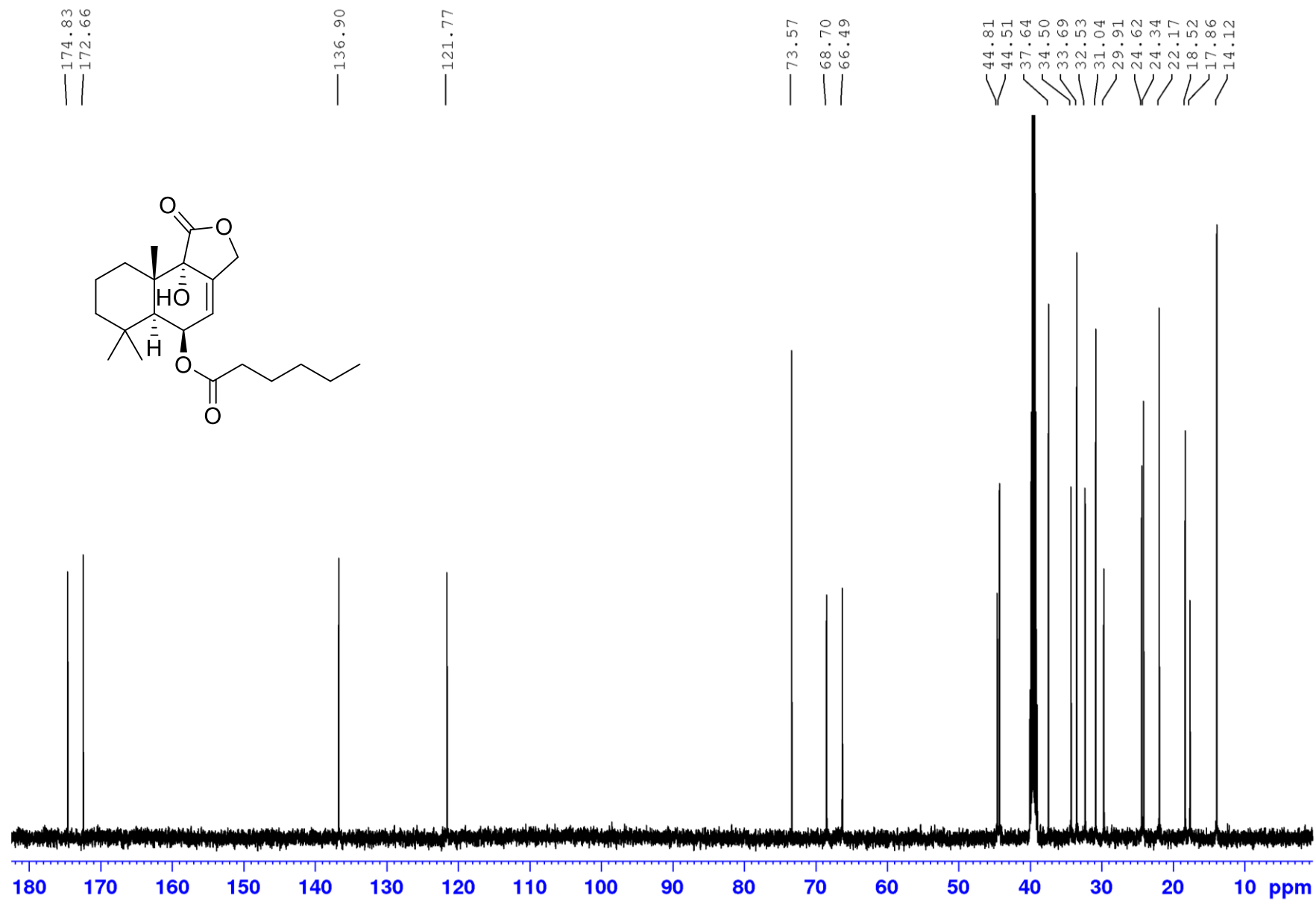


Figure S15. ^{13}C NMR spectrum (125 MHz, $\text{DMSO}-d_6$) of nanangenine C (4).

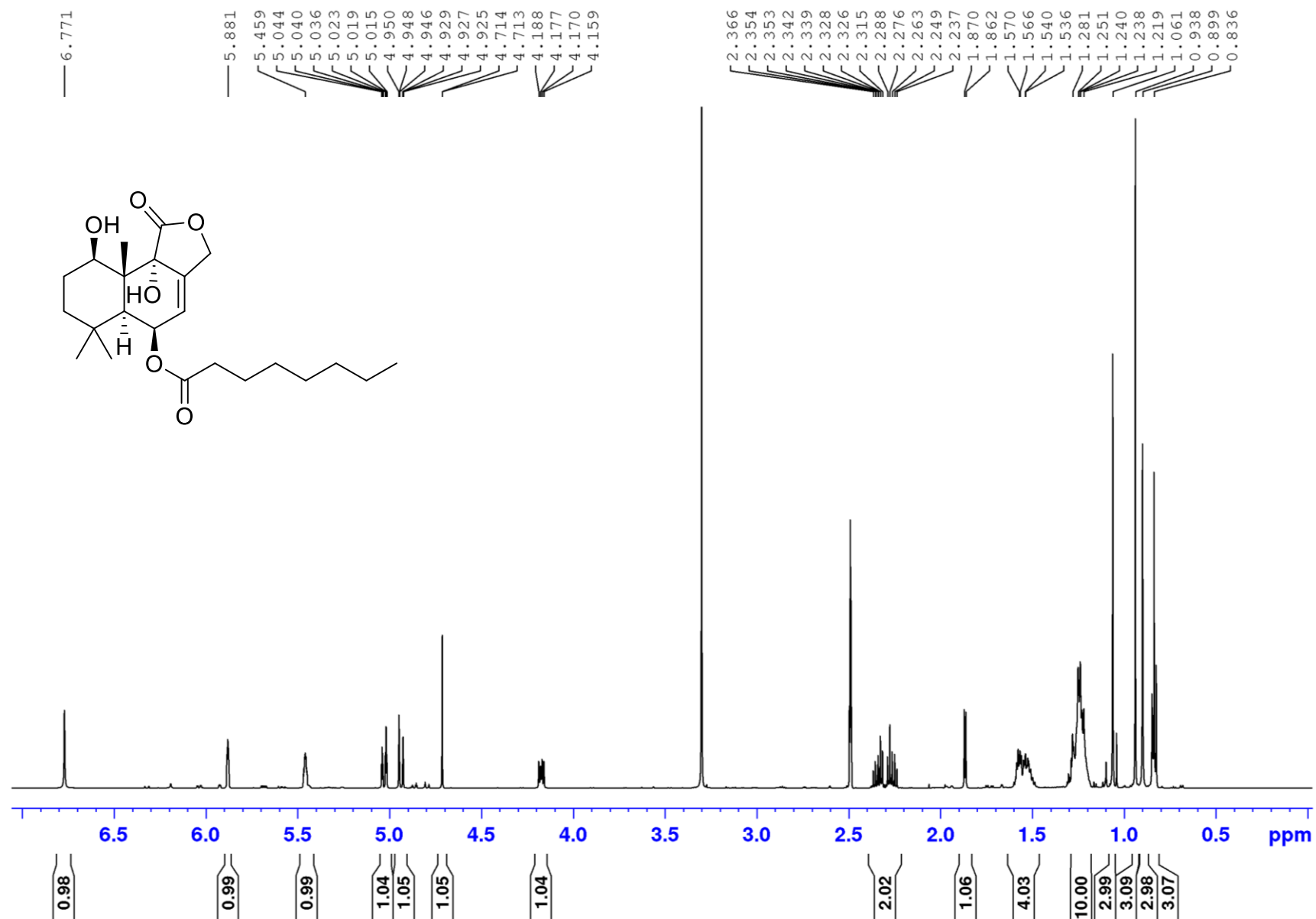


Figure S16. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of nanangene D (5).

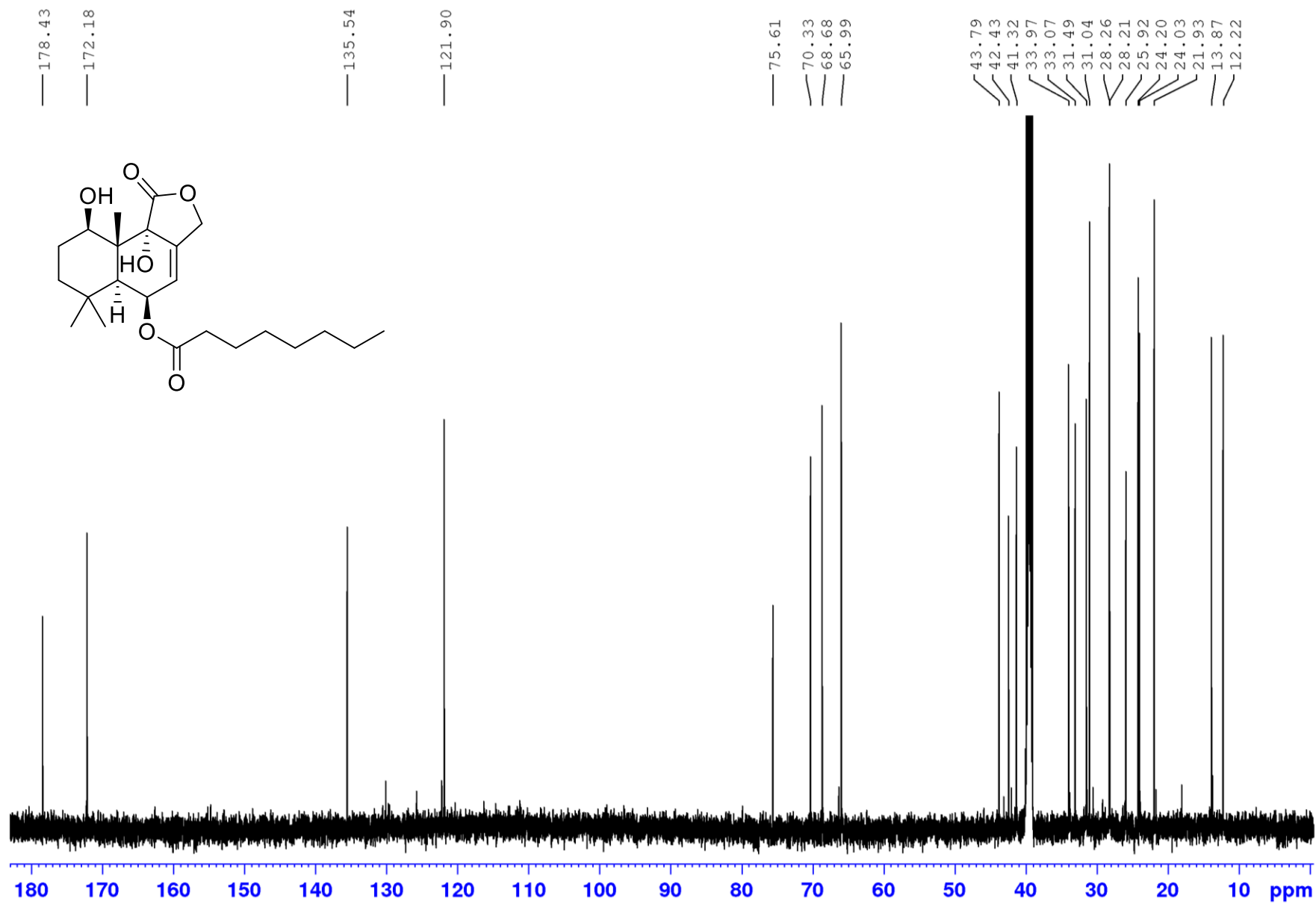


Figure S17. ^{13}C NMR spectrum (150 MHz, $\text{DMSO}-d_6$) of nanangenine D (5).

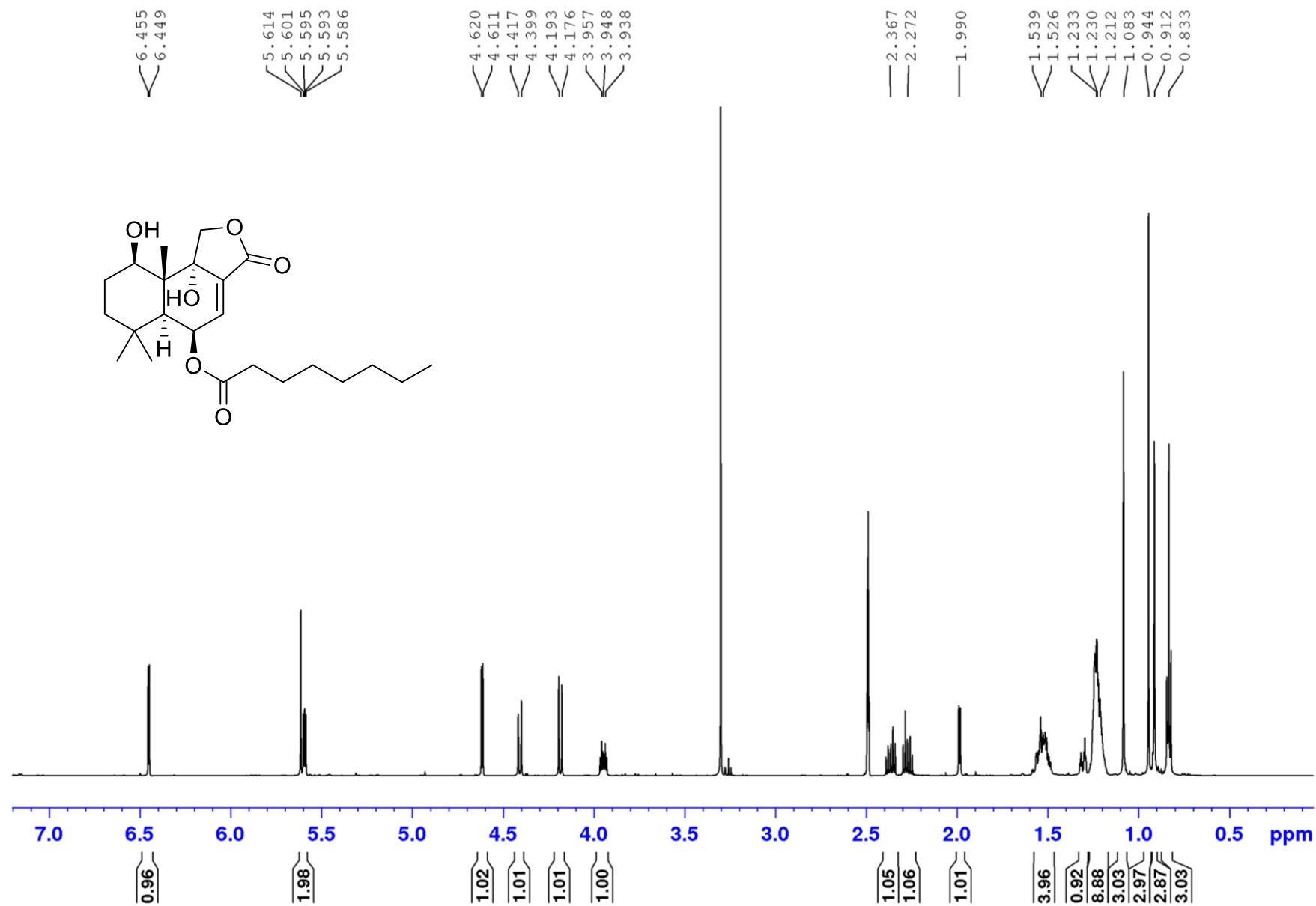


Figure S18. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of isonanangienine D (6).

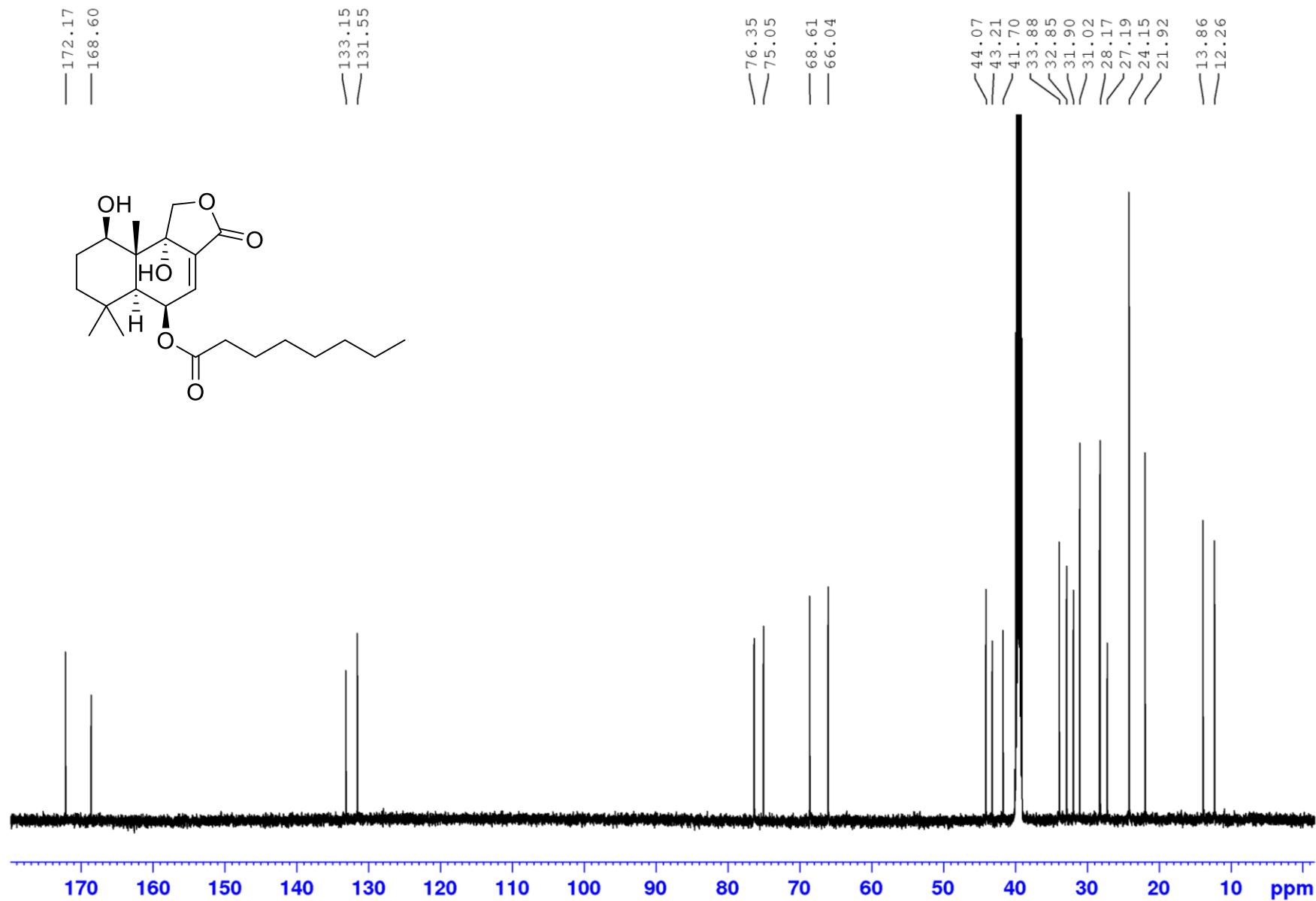


Figure S19. ¹³C NMR spectrum (150 MHz, DMSO-*d*₆) of isonanangienine D (6).

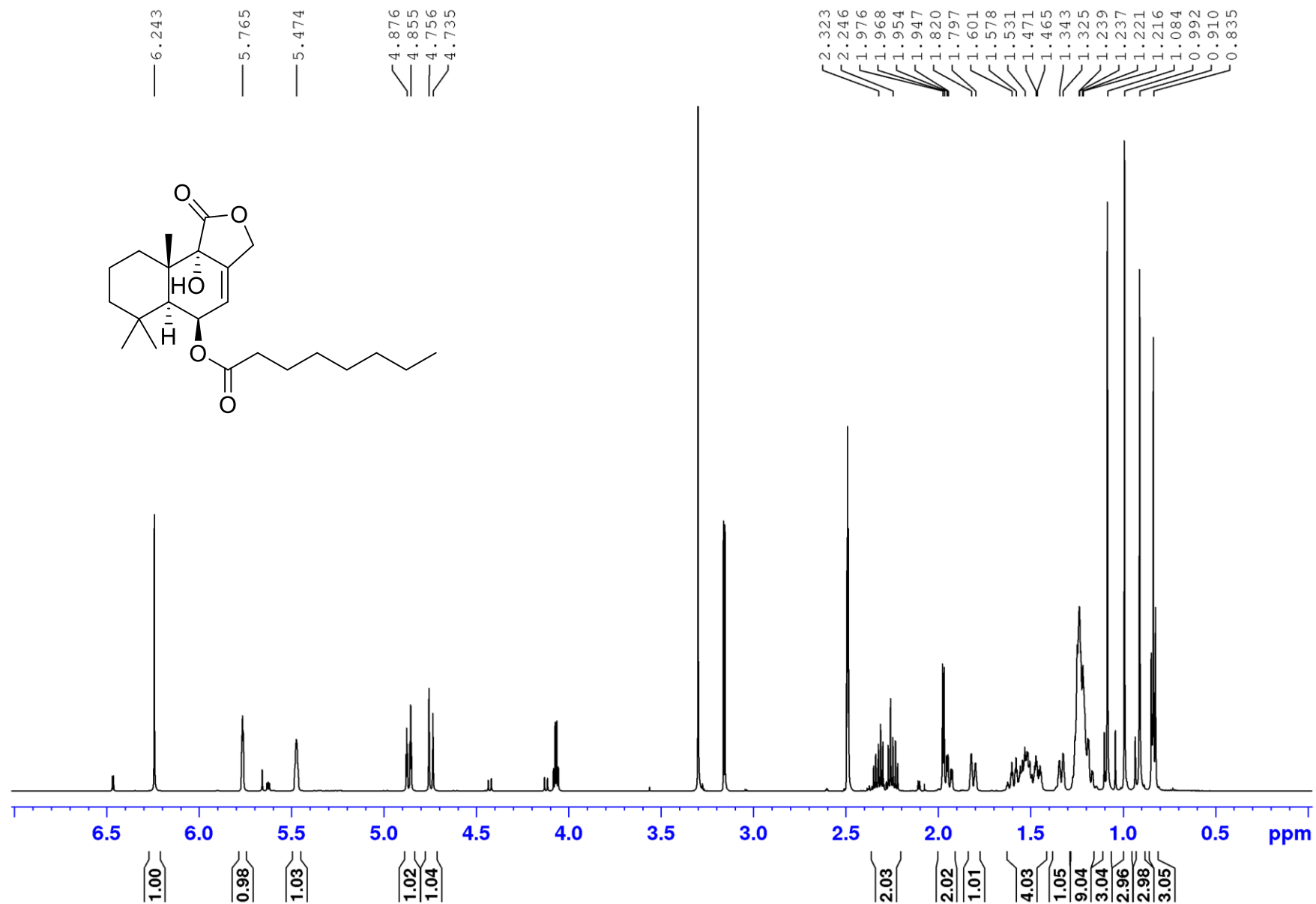


Figure S20. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of nanangenine E (7).

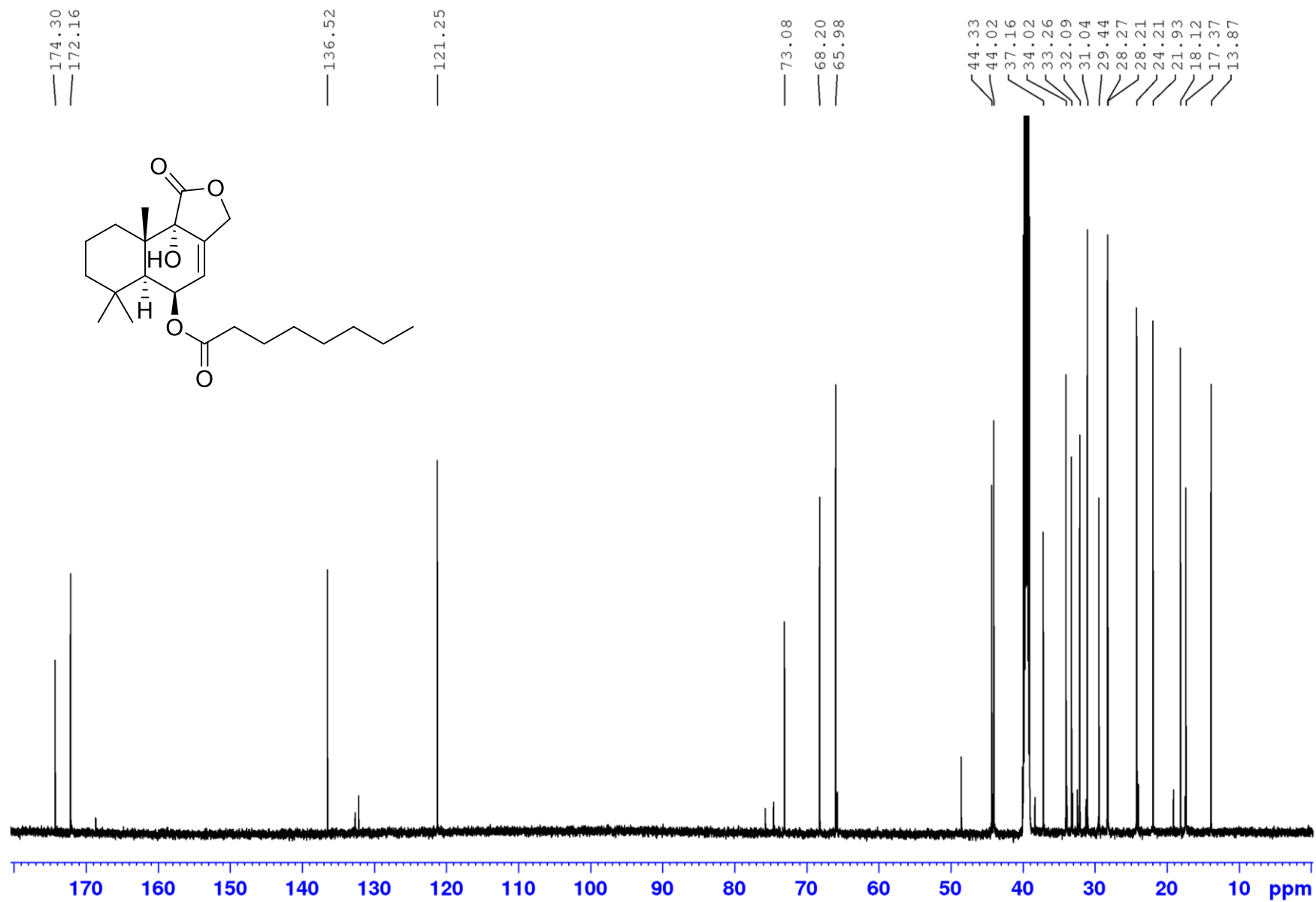


Figure S21. ^{13}C NMR spectrum (150 MHz, $\text{DMSO}-d_6$) of nanangene E (7).

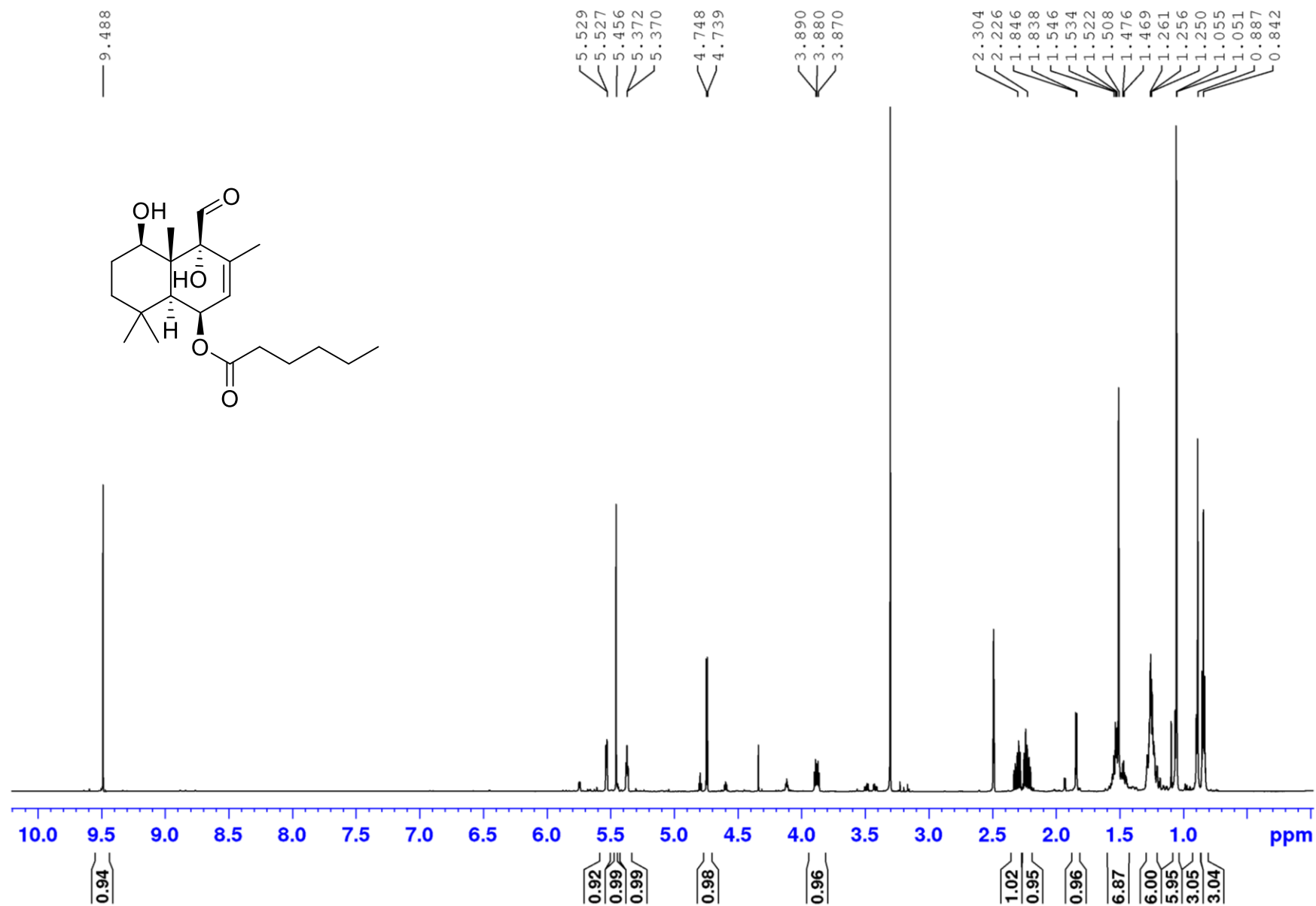


Figure S22. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of nanangene F (8).

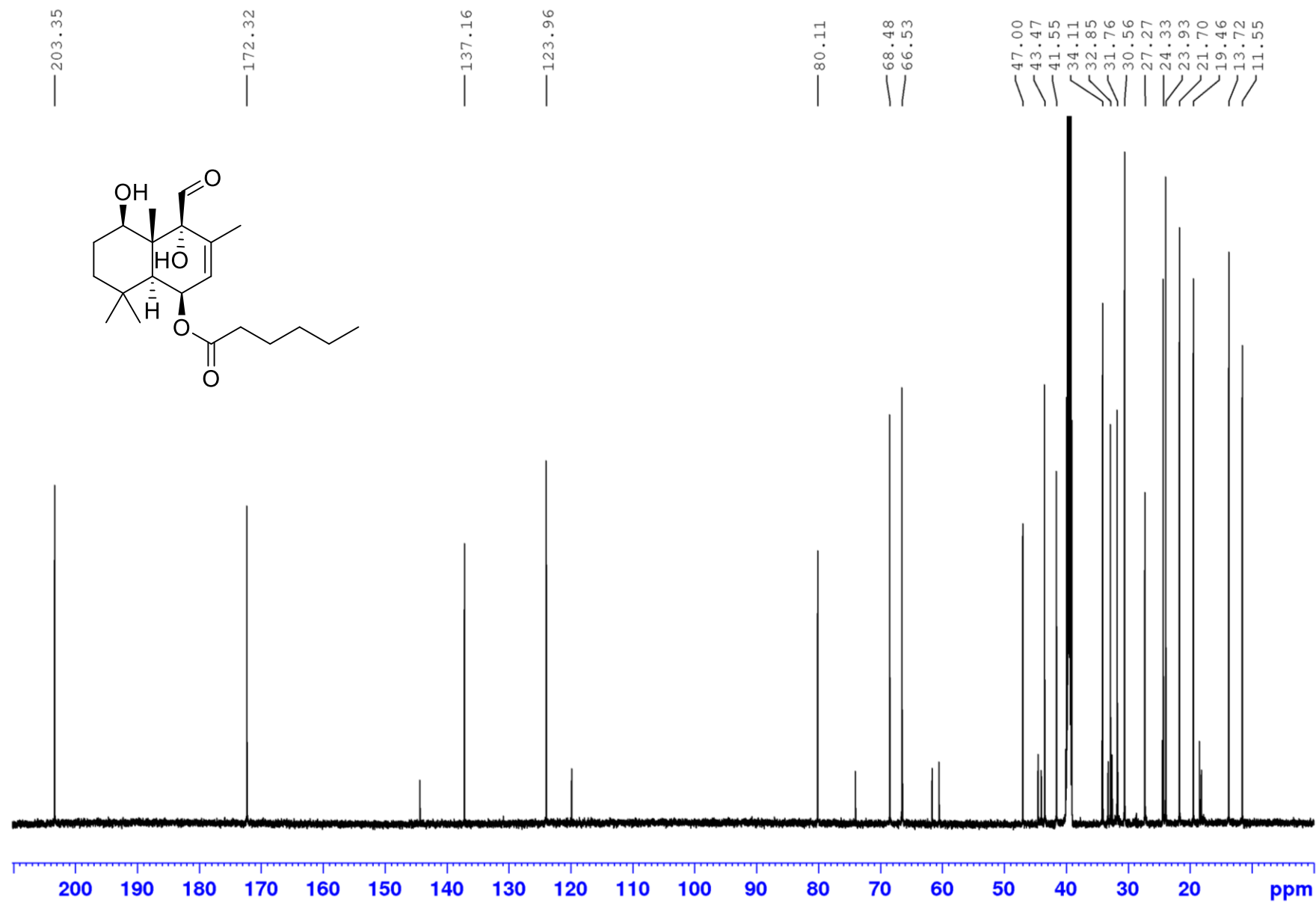


Figure S23. ^{13}C NMR spectrum (150 MHz, $\text{DMSO}-d_6$) of nanangenine F (8).

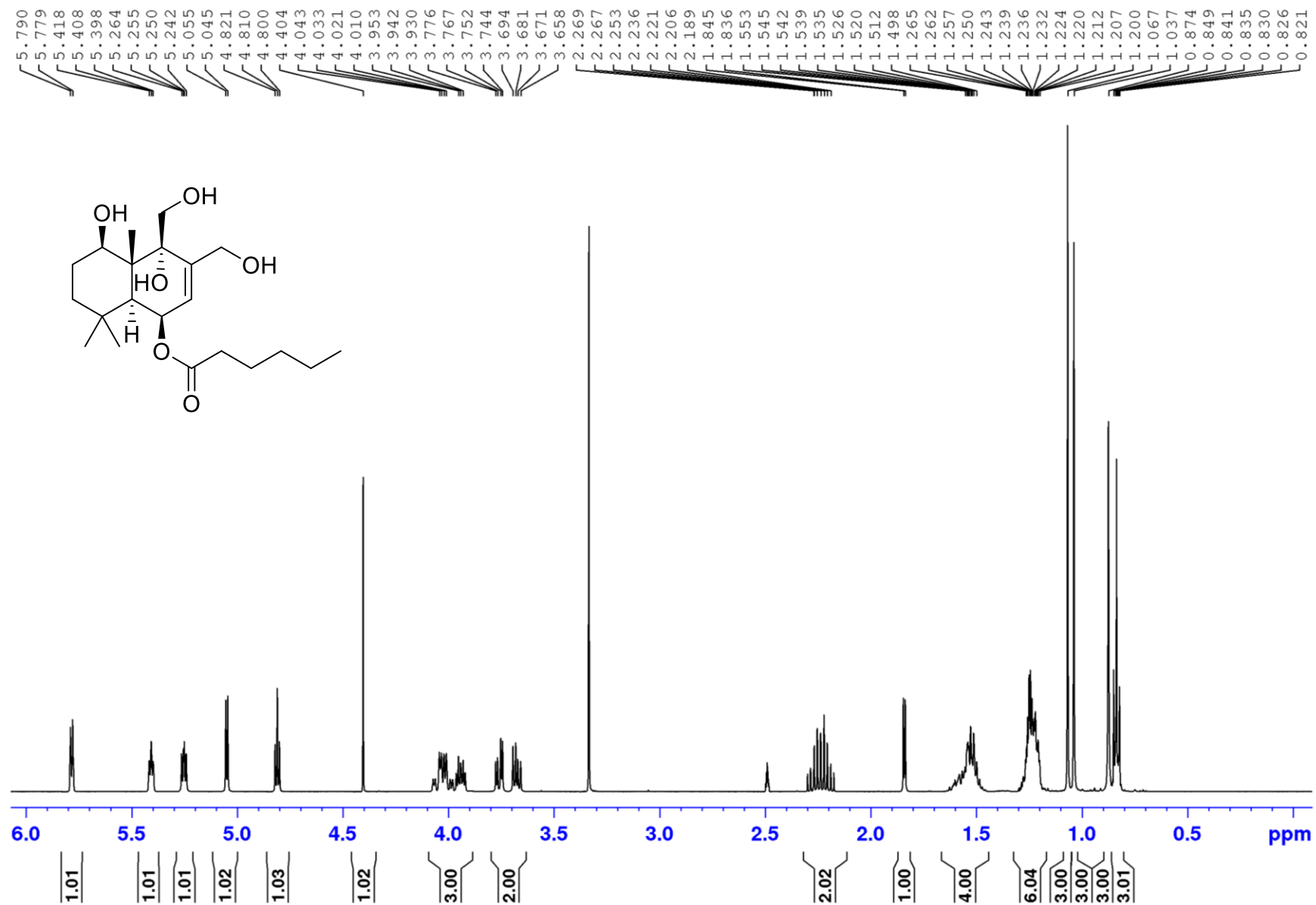


Figure S24. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of nanangenine G (9).

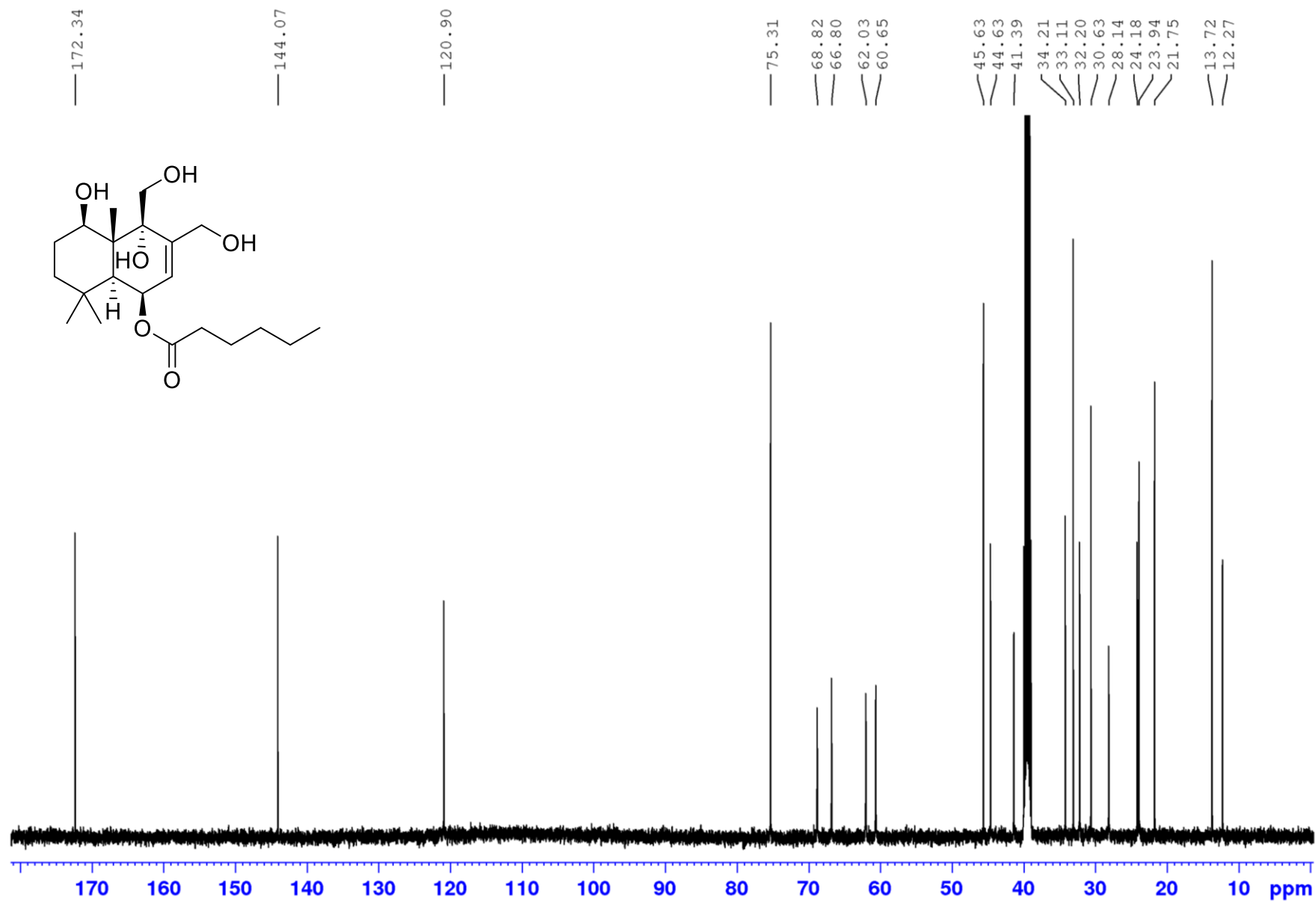


Figure S25. ^{13}C NMR spectrum (125 MHz, $\text{DMSO}-d_6$) of nanangenine G (9).

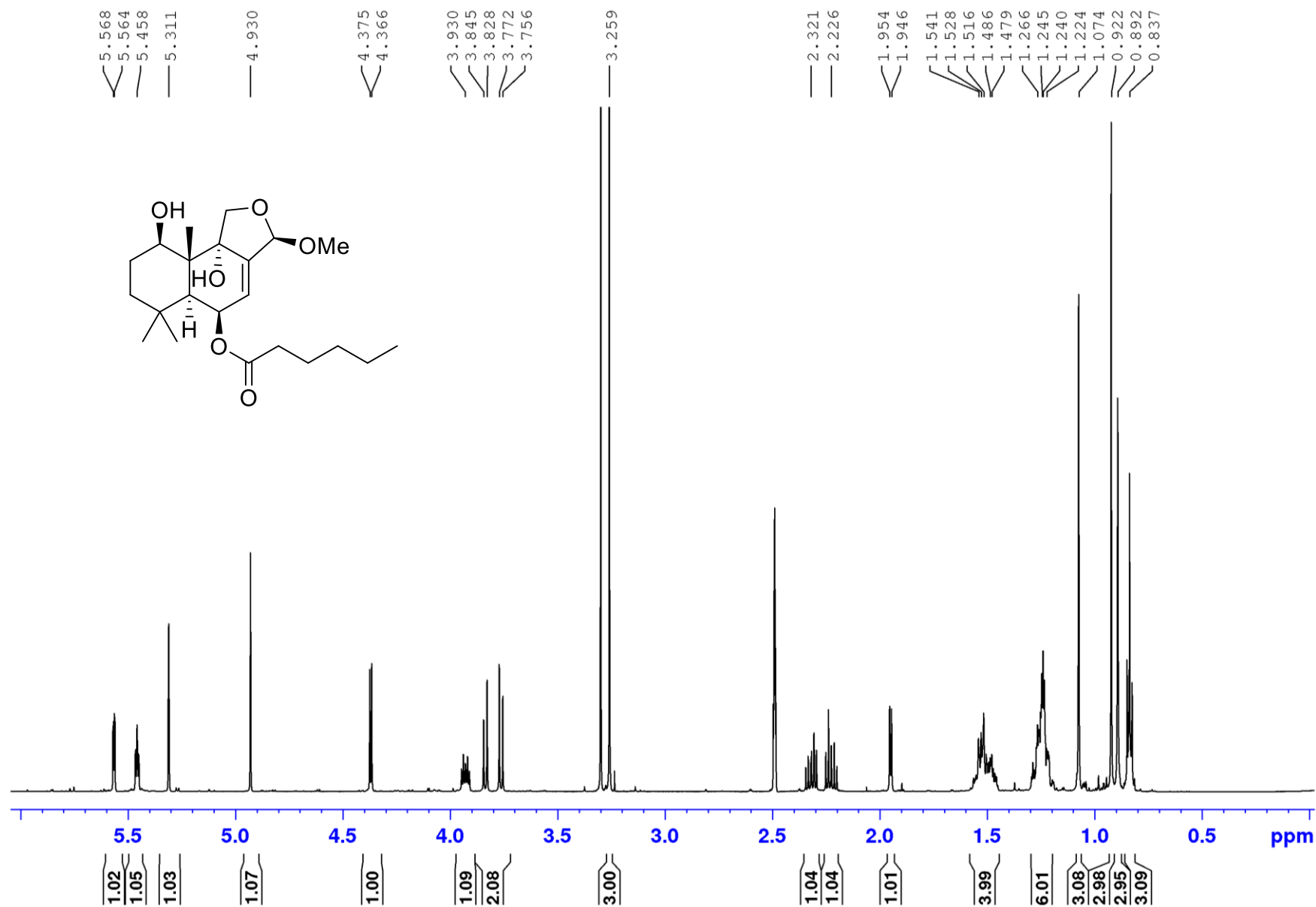


Figure S26. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of nanangenine H (10).

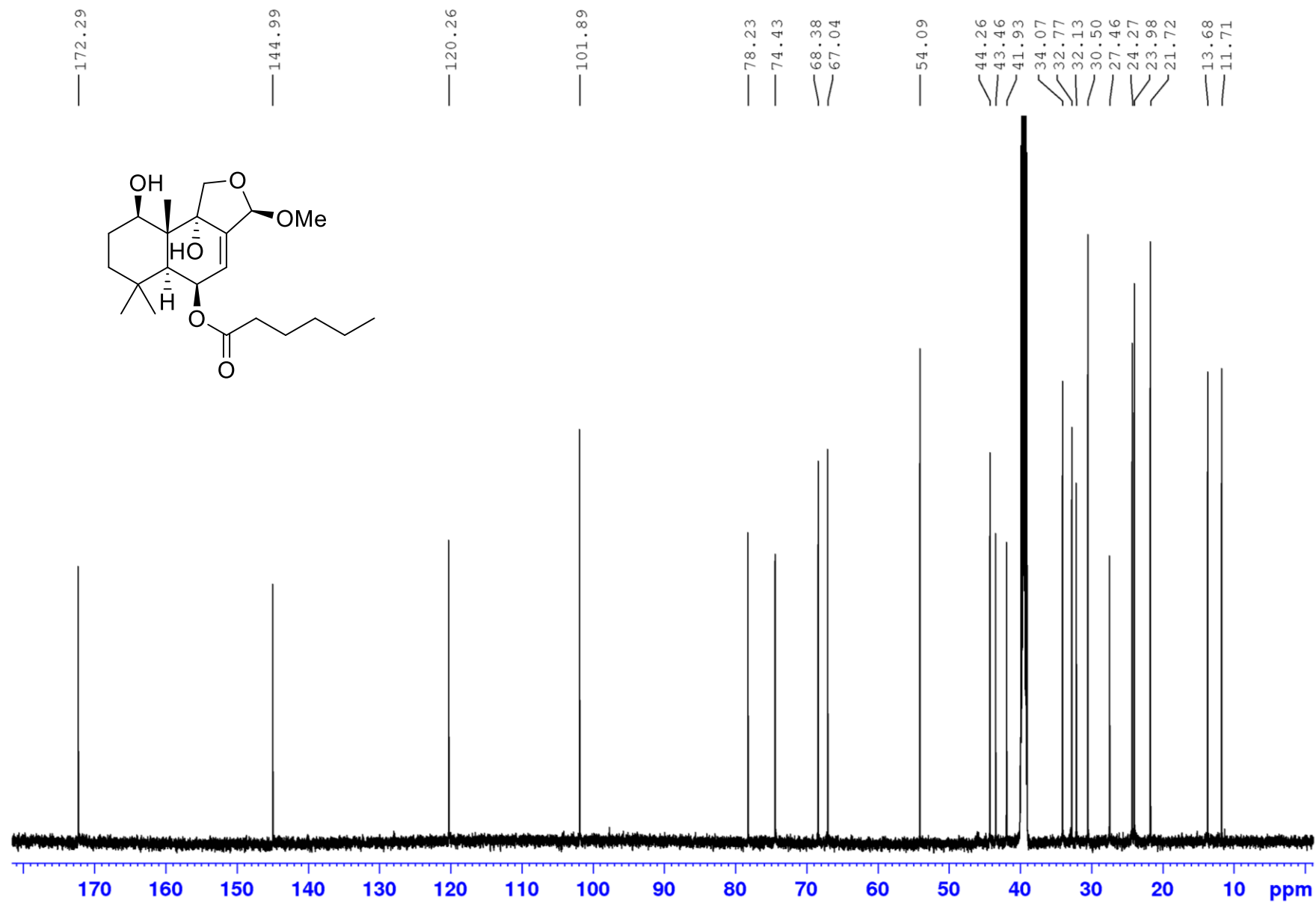


Figure S27. ¹³C NMR spectrum (150 MHz, DMSO-*d*₆) of nanangenine H (10).

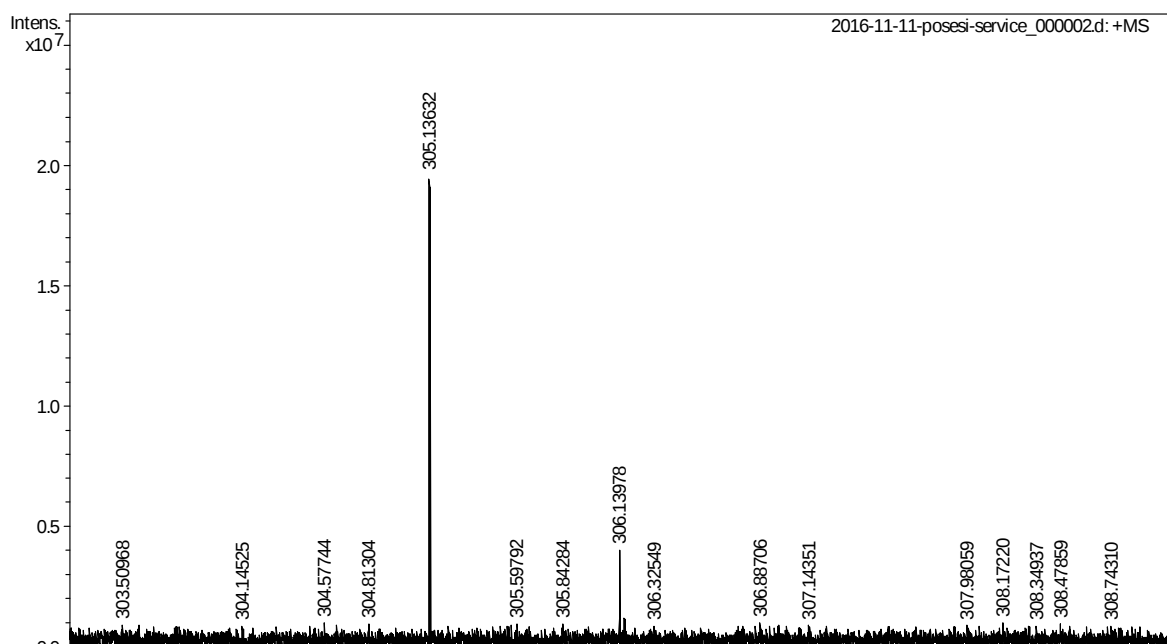


Figure S28. HRMS–ESI(+) spectrum of nanangenine A (**1**)

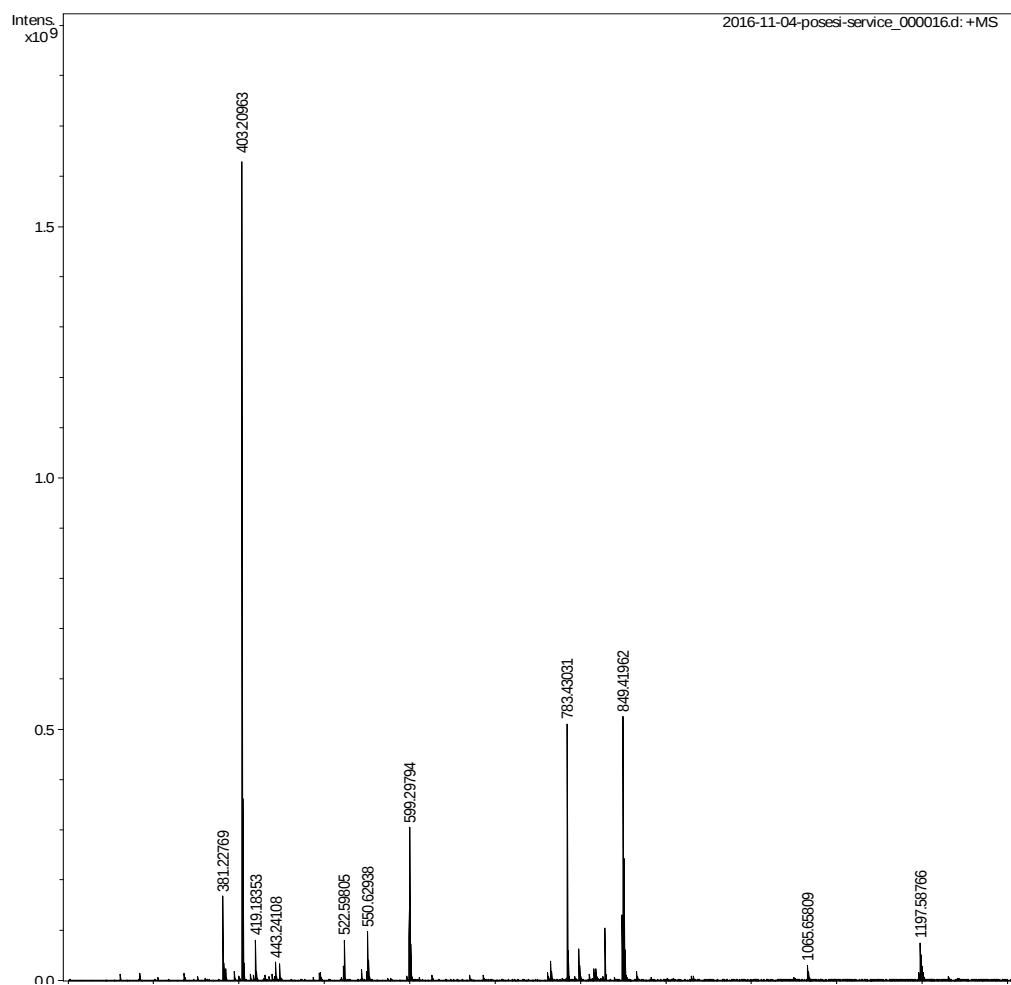


Figure S29. HRMS–ESI(+) spectrum of nanangenine B (**2**).

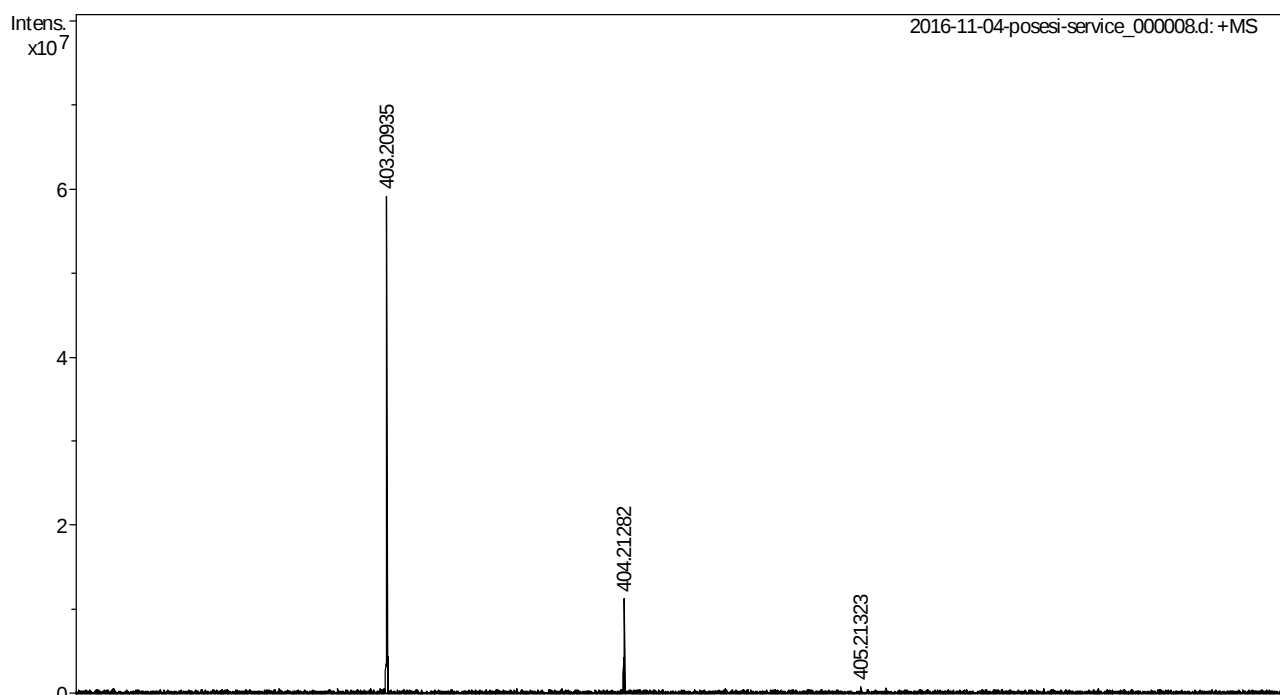


Figure S30. HRMS–ESI(–) spectrum of isonanangene B (**3**).

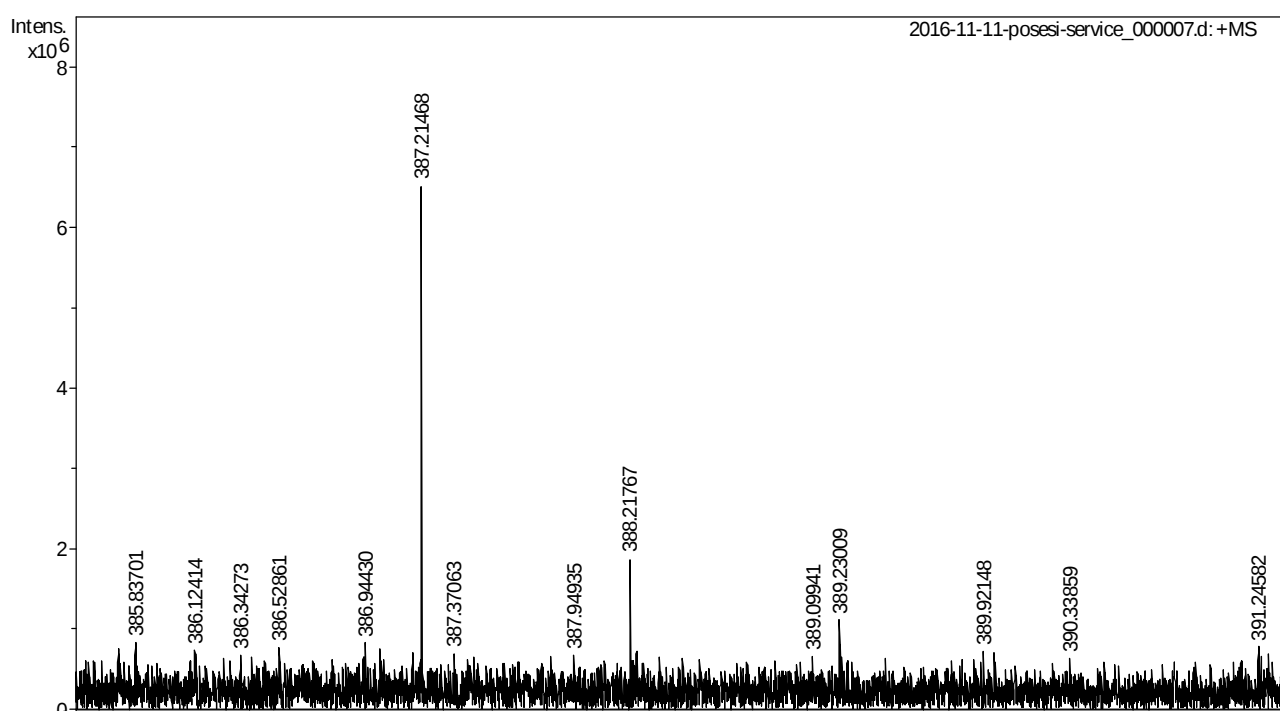


Figure S31. HRMS–ESI(–) spectrum of nanangene C (**4**).

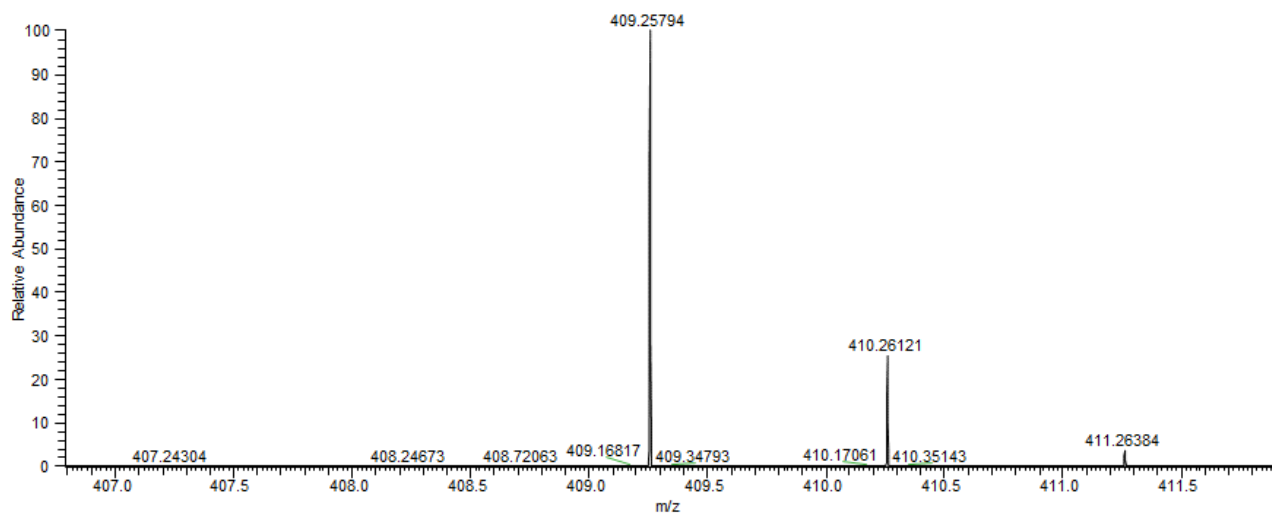


Figure S32. HRMS–ESI(+) spectrum of nanangenine D (5).

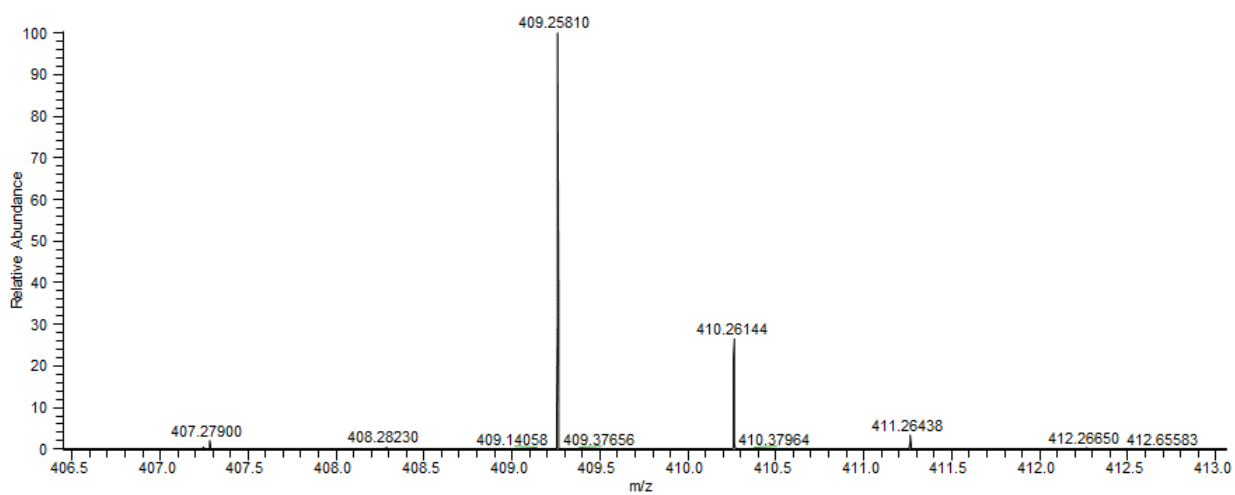


Figure S33. HRMS–ESI(+) spectrum of isonanangenine D (6).

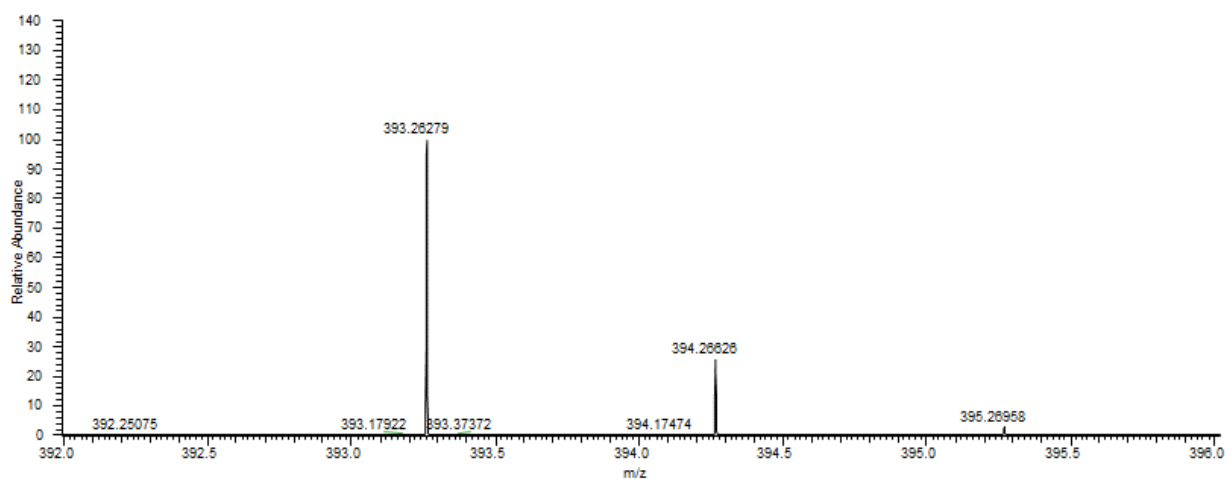


Figure S34. HRMS–ESI(+) spectrum of nanangenine E (7).

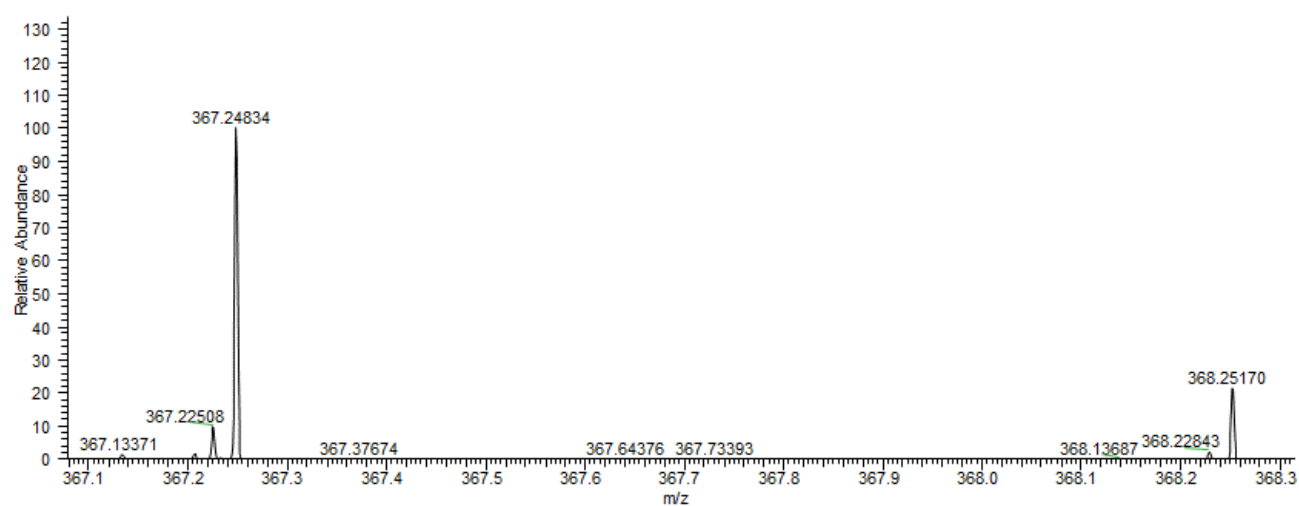


Figure S35. HRMS–ESI(+) spectrum of nanangenine F (8).

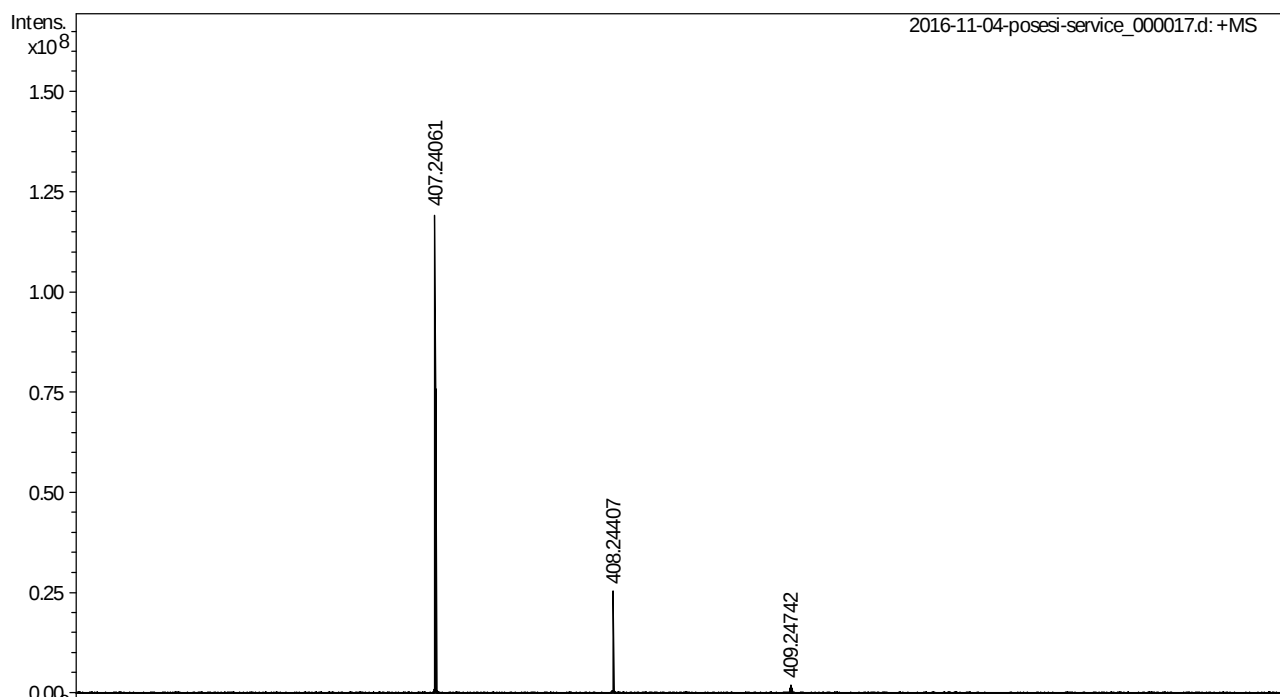


Figure S36. HRMS–ESI(+) spectrum of nanangenine G (**9**).

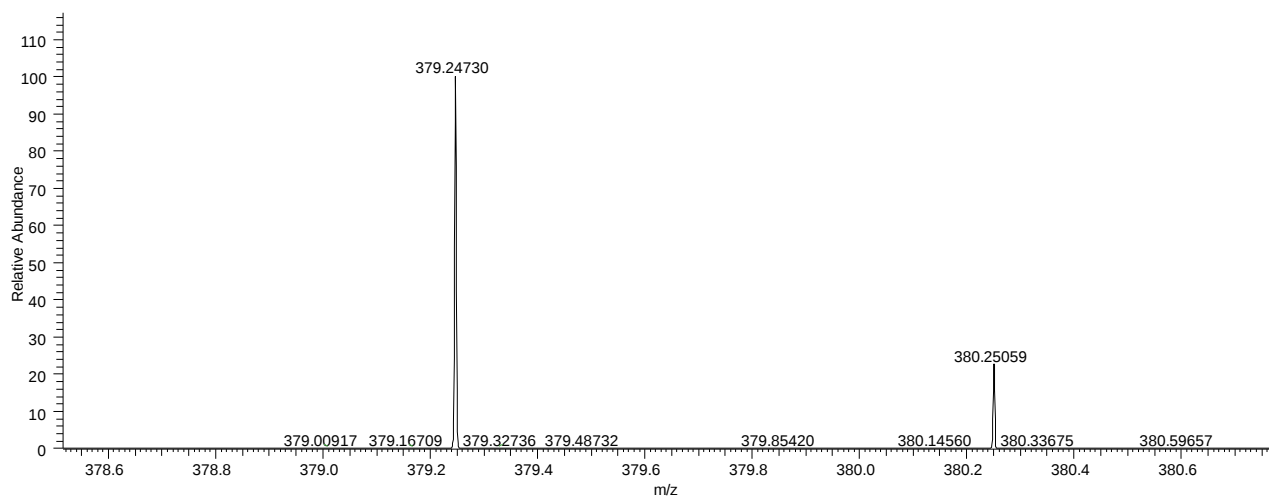


Figure S37. HRMS–ESI(+) spectrum of nanangenine H (**10**).

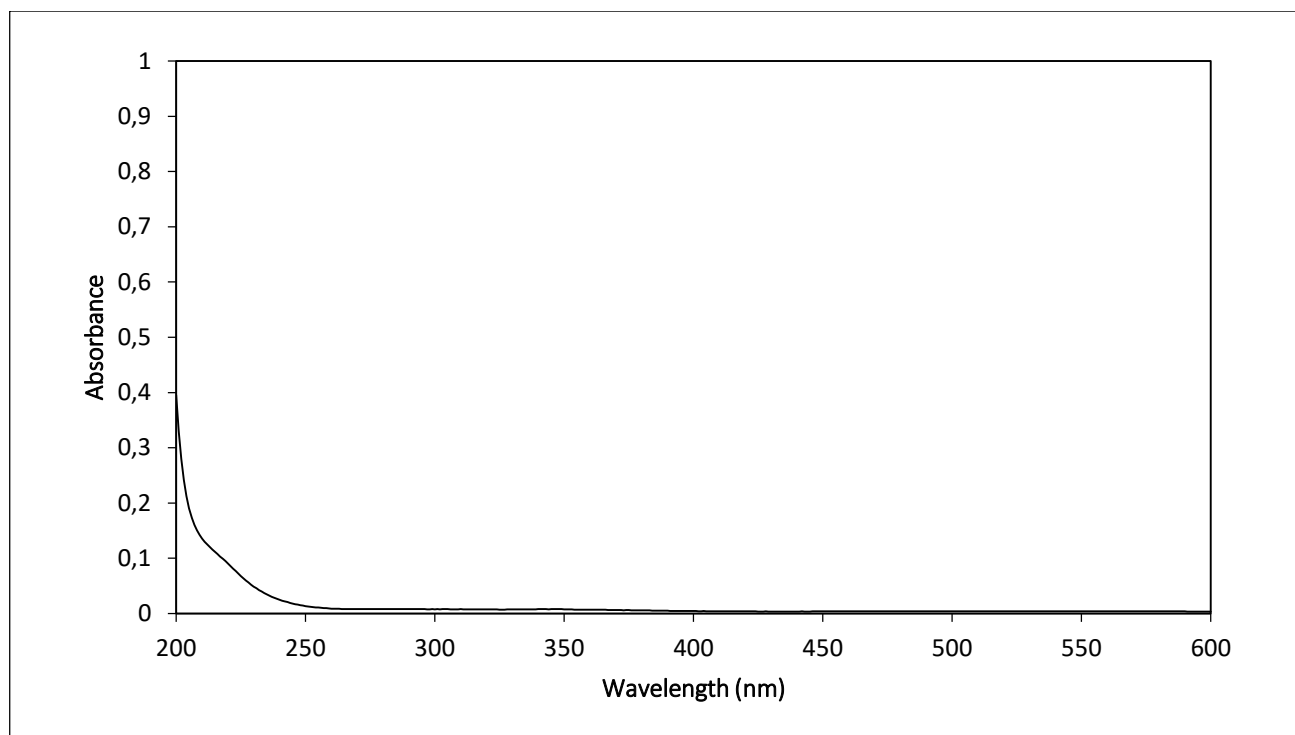


Figure S38. UV-vis spectrum (MeCN) of nanangenine A (1).

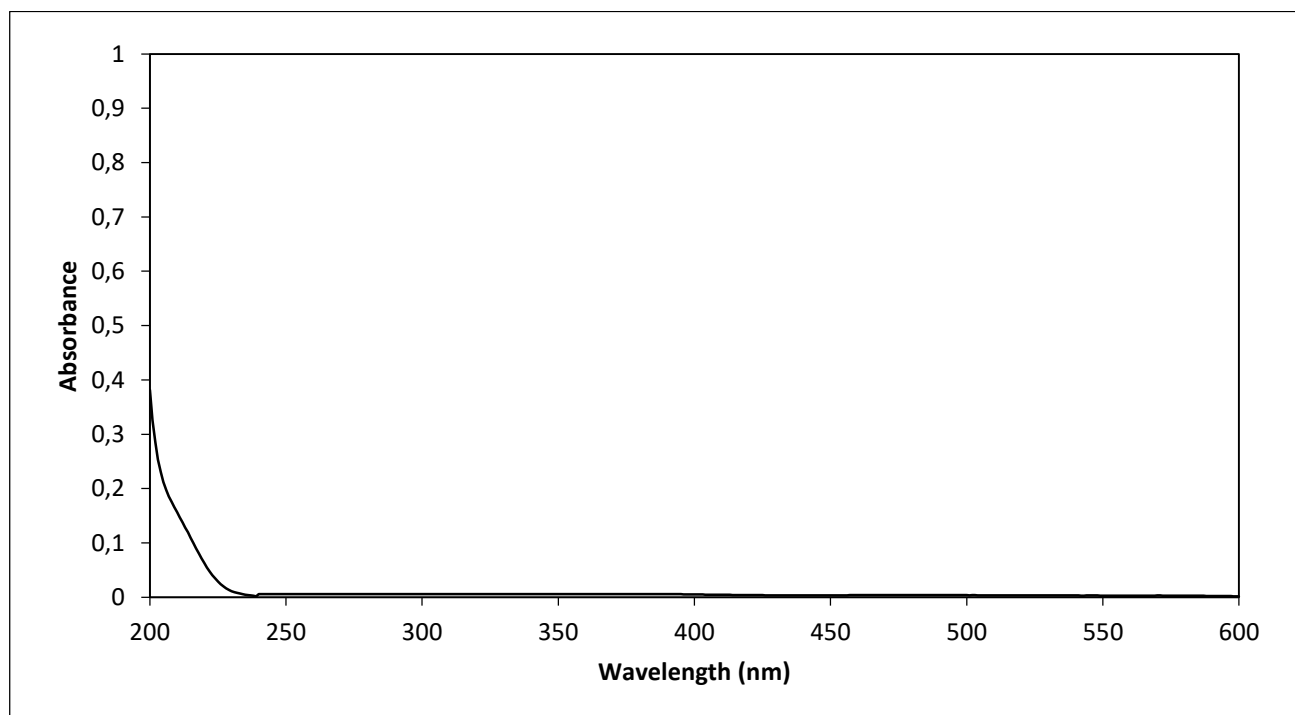


Figure S39. UV-vis spectrum (MeCN) of nanangenine B (2).

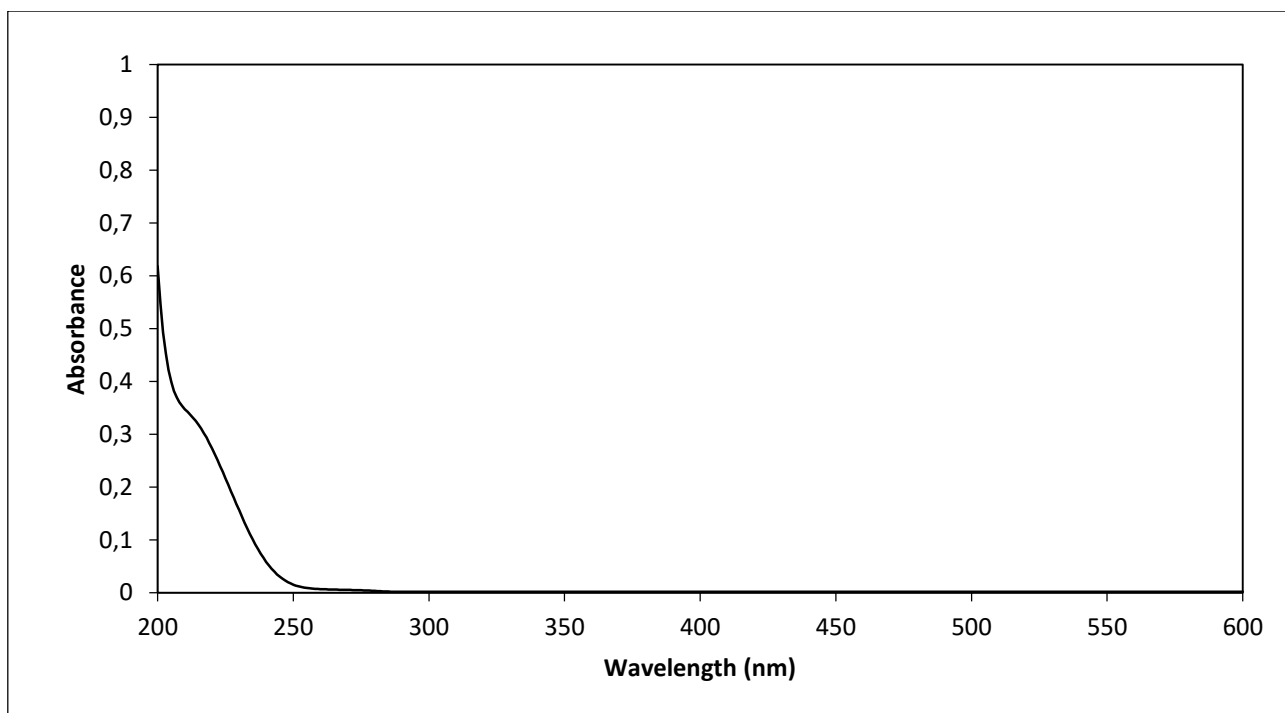


Figure S40. UV-vis spectrum (MeCN) of isonananginenine B (3).

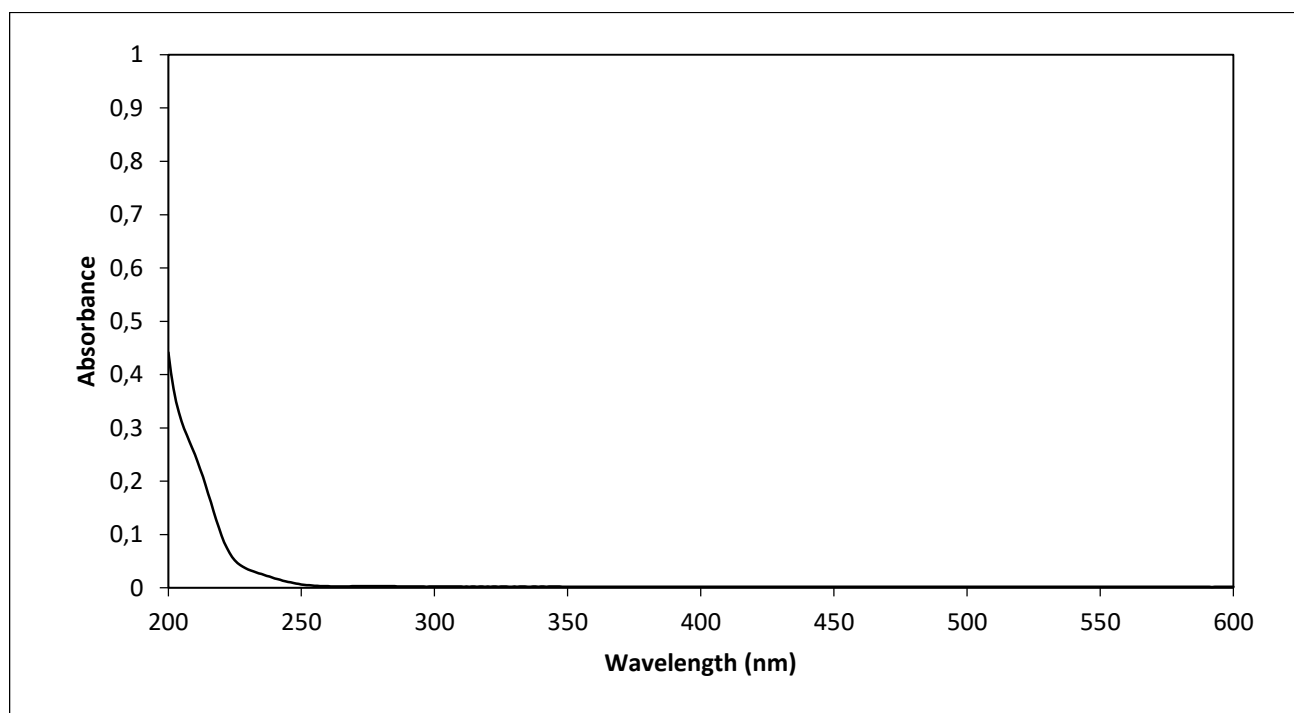


Figure S41. UV-vis spectrum (MeCN) of nananginenine C (4)

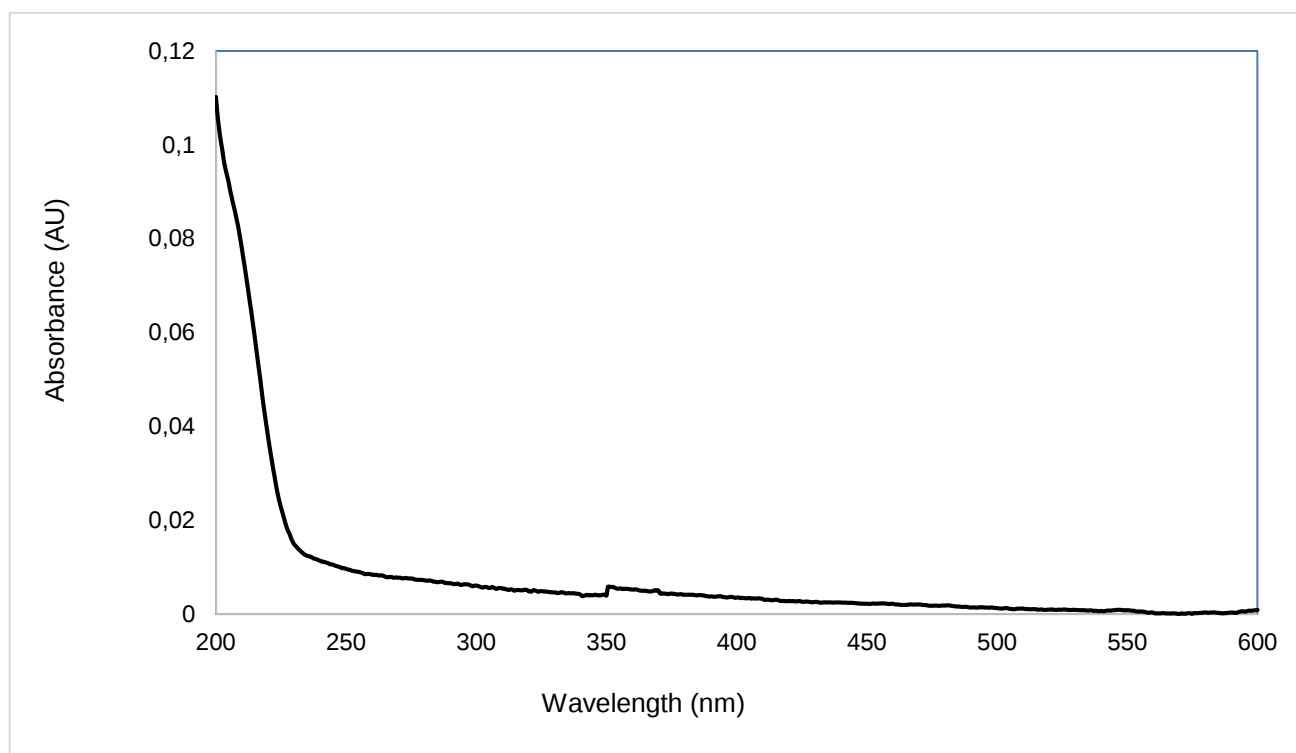


Figure S42. UV-vis spectrum (MeCN) of nanangenine D (5)

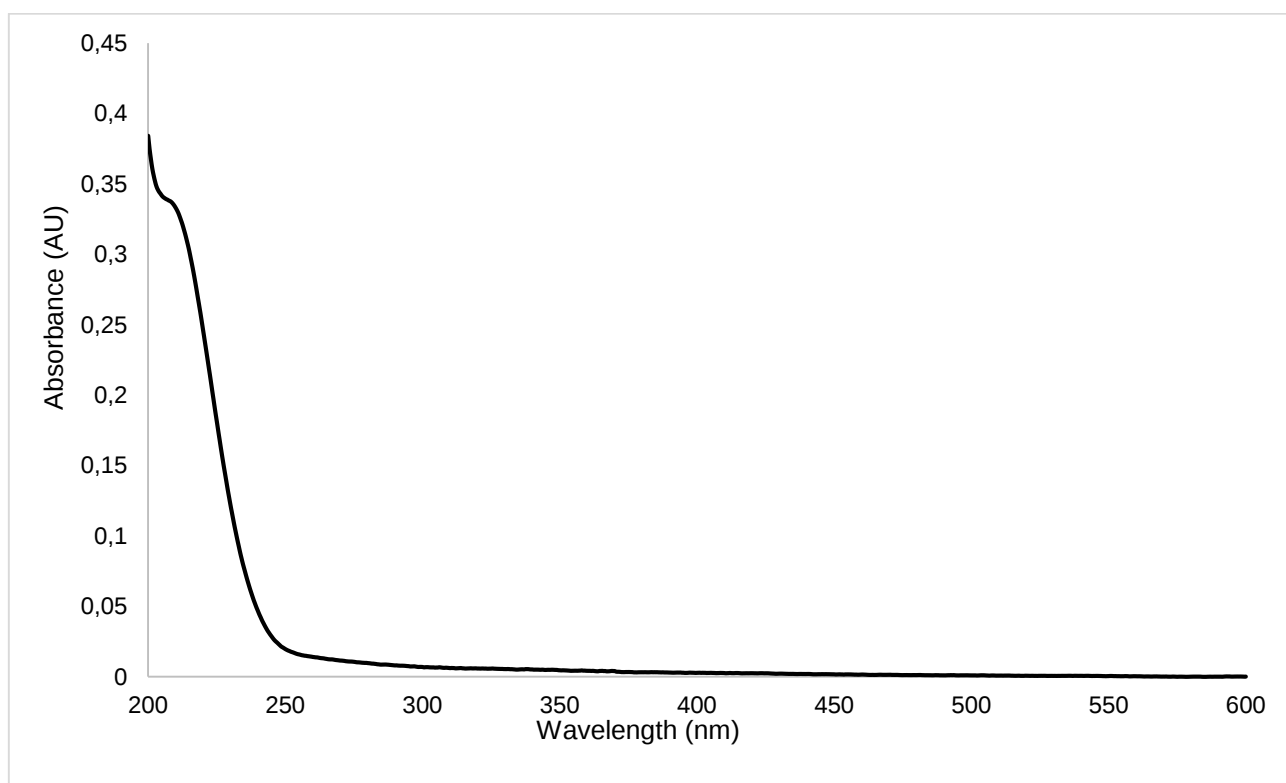


Figure S43. UV-vis spectrum (MeCN) of isonangenine D (6)

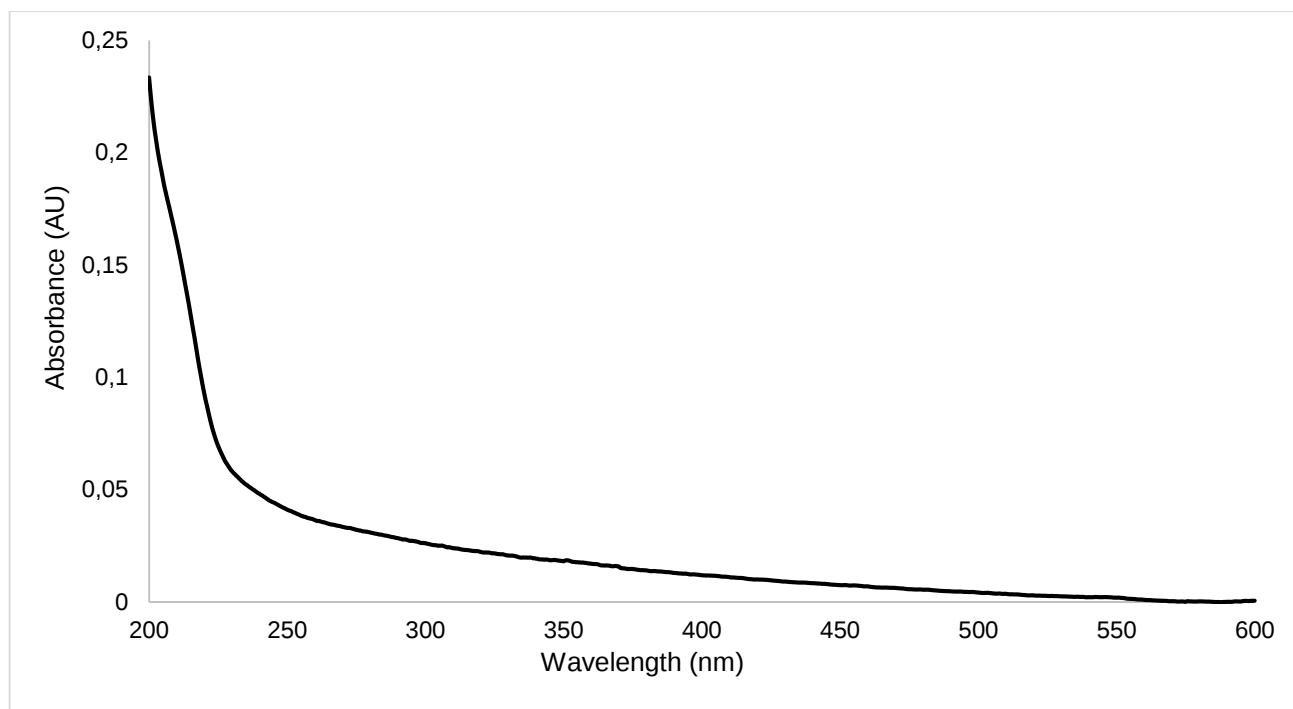


Figure S44. UV-vis spectrum (MeCN) of nanangenine E (7)

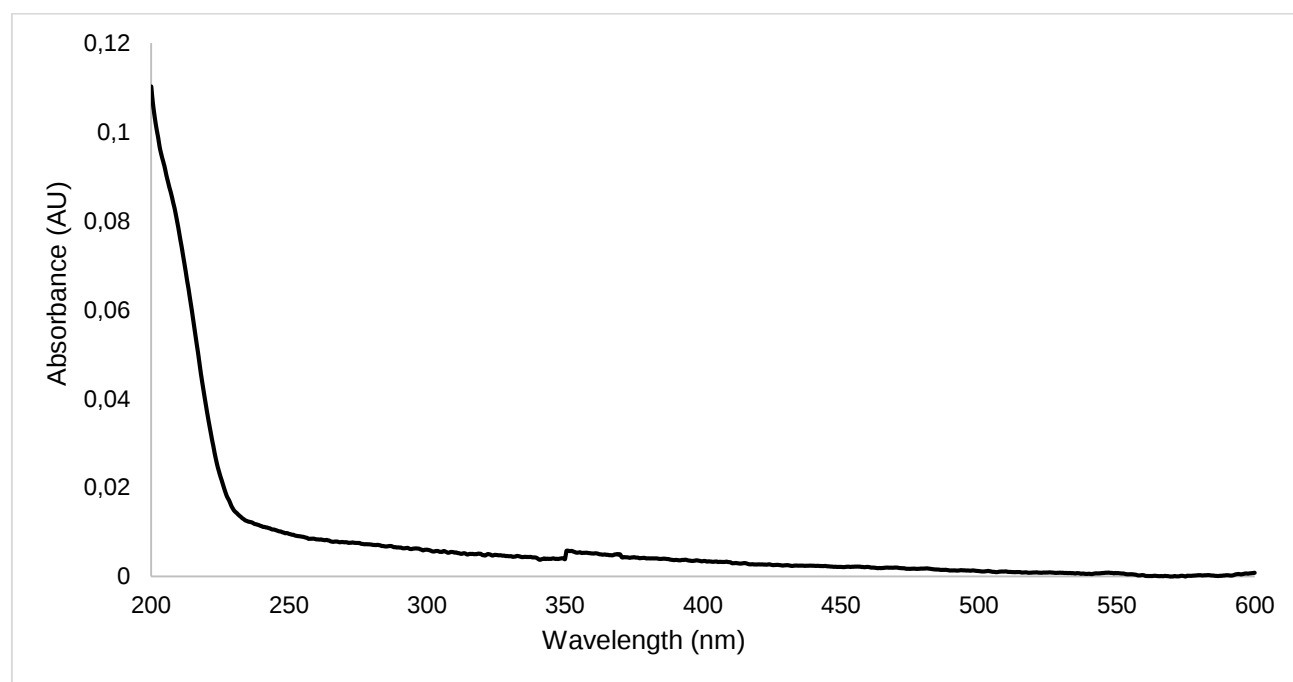


Figure S45. UV-vis spectrum (MeCN) of nanangenine F (8)

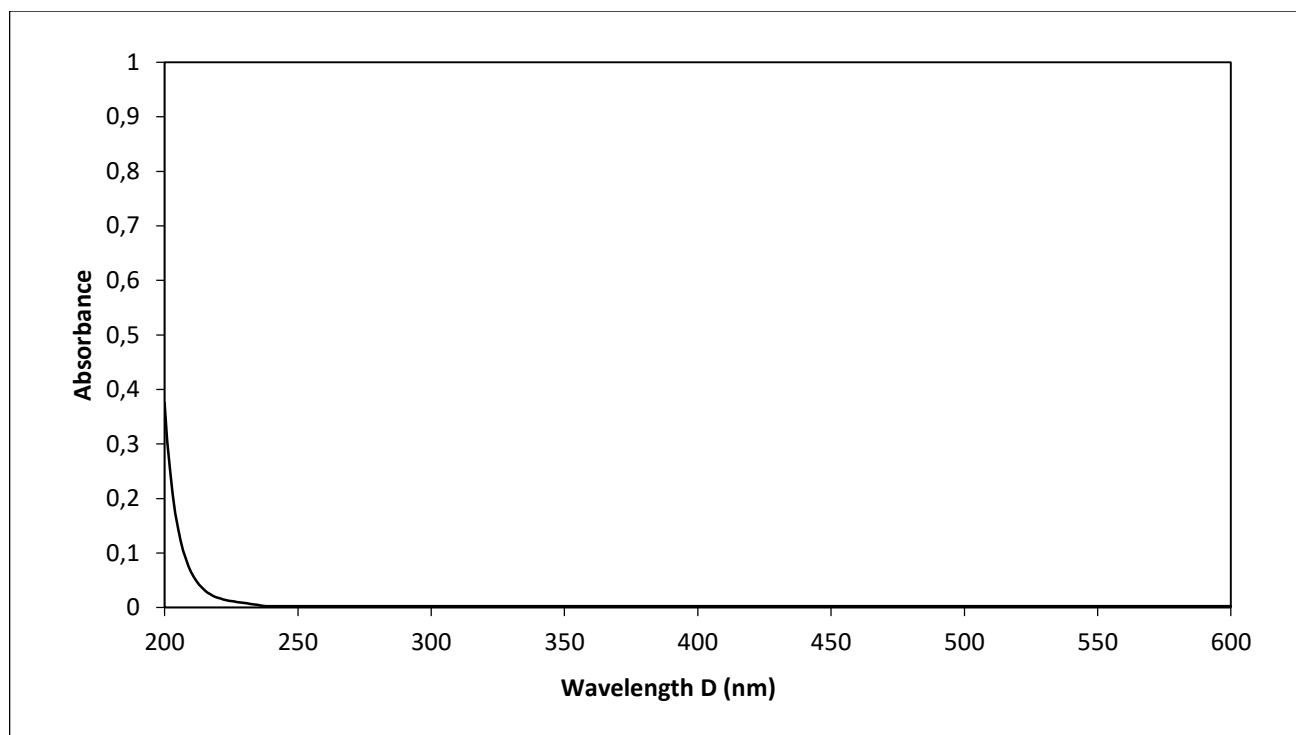


Figure S46. UV-vis spectrum (MeCN) of nanangenine G (**9**)

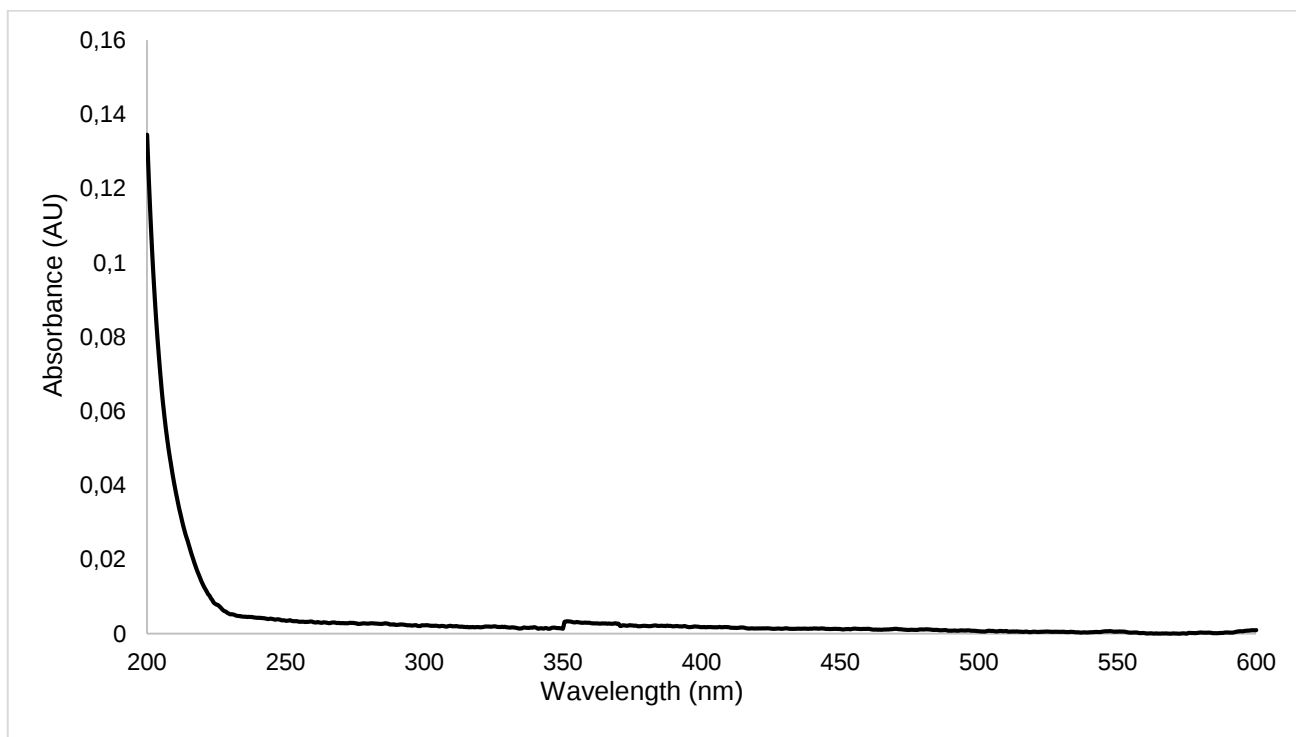
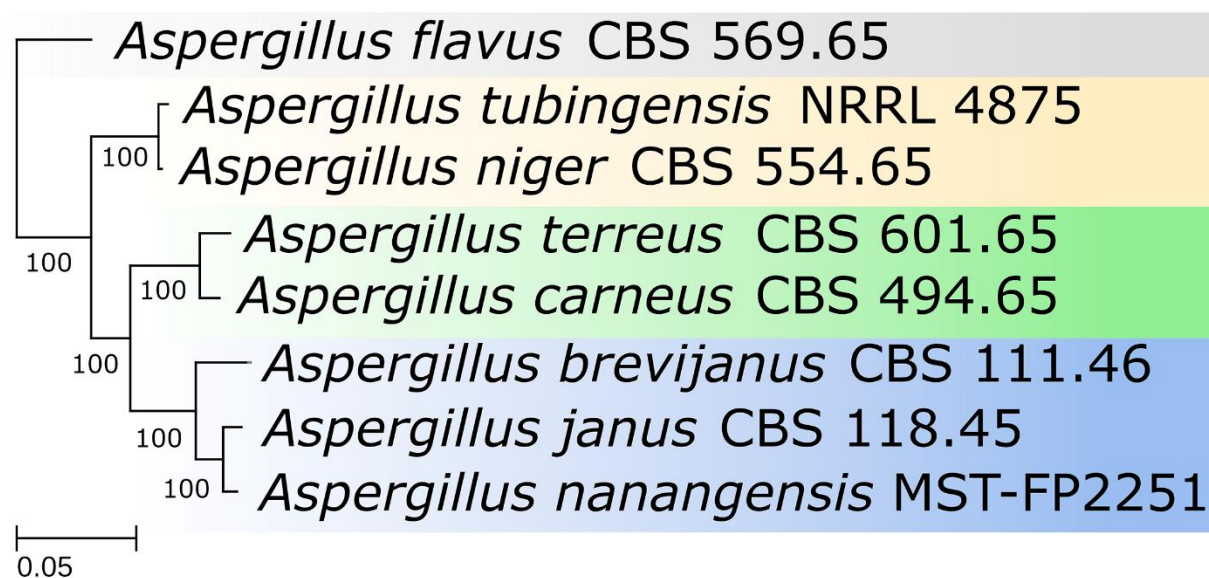
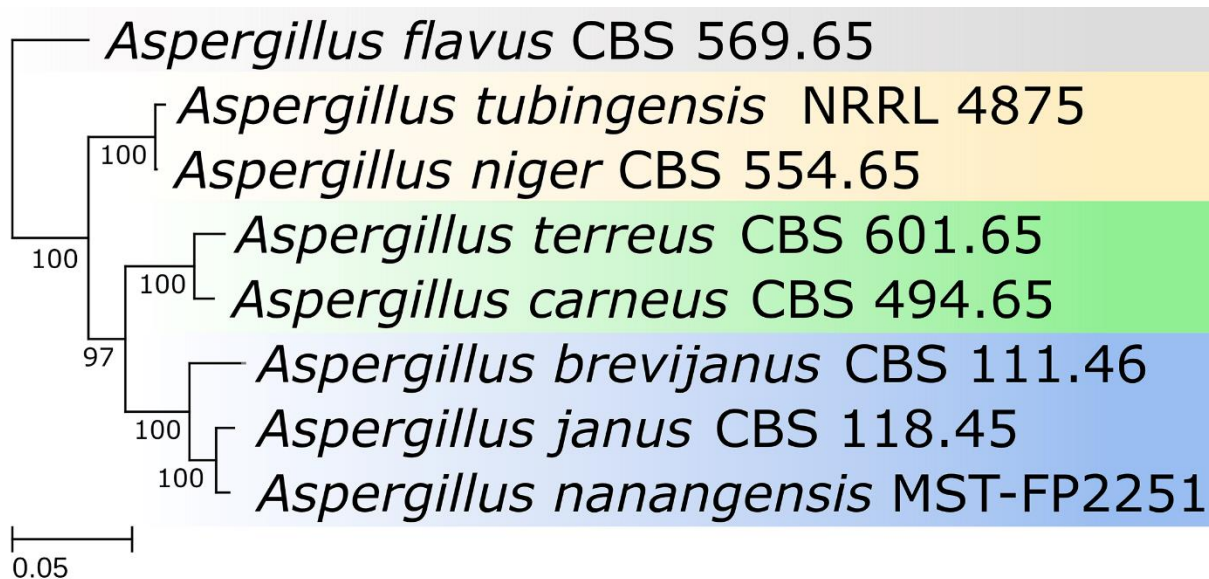


Figure S47. UV-vis spectrum (MeCN) of nanangenine H (**10**)



○ sect. *Flavi* ● sect. *Terrei* ● sect. *Nigri* ● sect. *Jani*

Figure S48. Maximum likelihood (ML; top) and Bayesian Inference (BI; bottom) trees of the internal transcribed spacer (ITS) region of *Aspergillus nanangensis* MST-FP2251 and other *Aspergilli*. *A. nanangensis* clearly clades within section *Jani*.

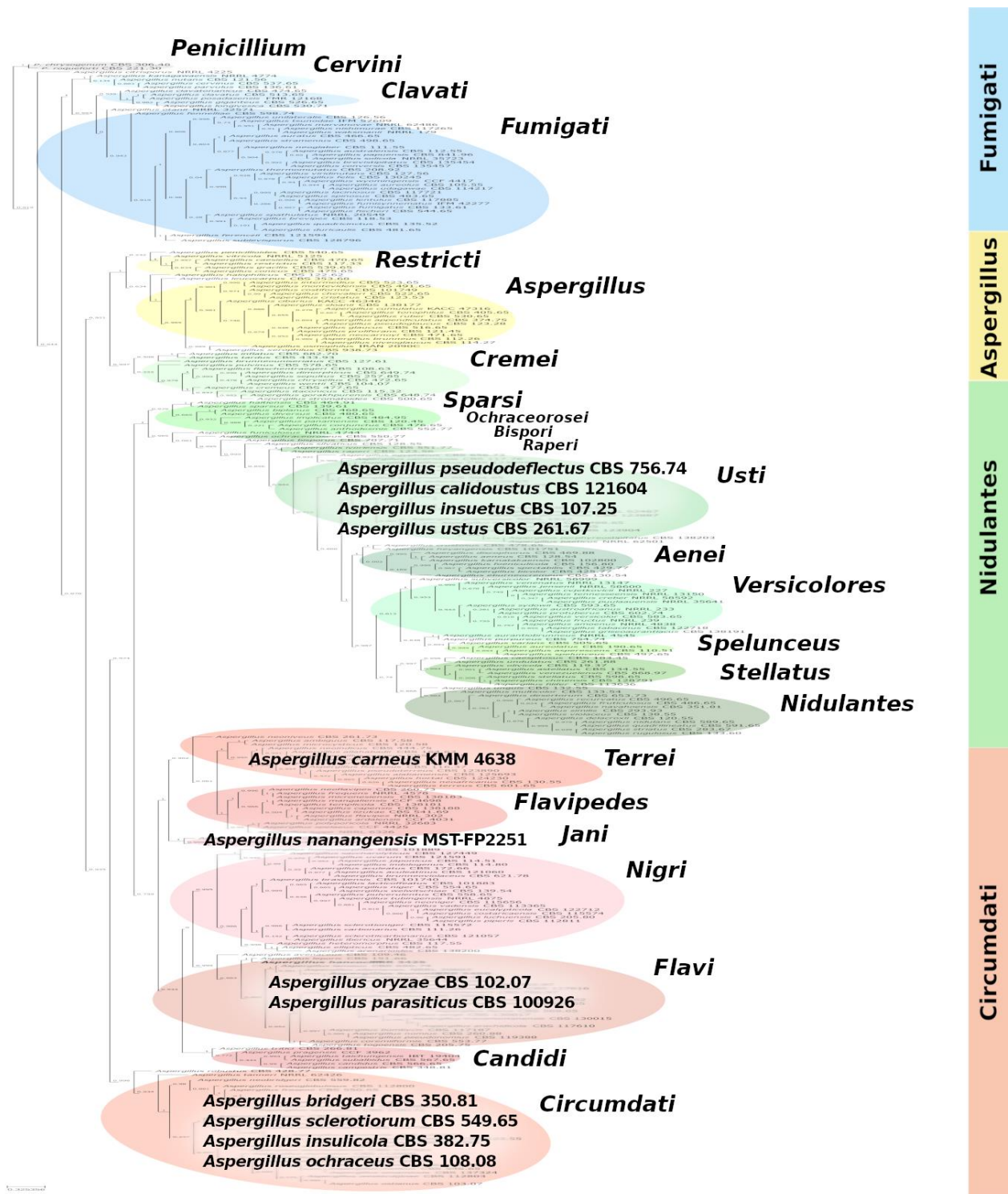


Figure S49. Taxonomic distribution of drimane sesquiterpene production across the *Aspergillus* genus. Underlying phylogenetic tree is built from a concatenated multi-locus alignment consisting of internal transcribed spacer (ITS), beta-tubulin and calmodulin sequences.

A_nanangensis_FE257_006542 (AstC)	-----MVRALVLDLGDVLFNWEAPKSTP----VSRKALGNILSSDIWGDYERGHYT--ESACYEALSARYGF-ETSAIAET	69
A_oryzae_AO090026000582	-----MTKINPYKGLVELKDIVFTS--SSDQIK----LPINTFKSILCCGATAQYQCCGKN--RAQYYSLRLARDFAL--SLADVTAL	73
A_terreus_XP_001217376	----MAITKGPVKALILDFSNVLCWSKPPSNVA----VPPQILKMIMSSDIWHDYECGRYS--REDCYARVADRPHI--SAADMEDT	75
A_niger_XP_001391086	MVVAVPVTSSPDRAILVELNHVIFKS--SFVDVG----IPENTYKSILCCGASTEYQCCGKAG-LDQYYSLRLSDFFDT--PKSQIHDM	78
A_bisporus_XP_006461126	---MAPPQRPFPTAIVFDIGDVLFGWATTKTS----ISPKTLRSILNCPTWFDYERGLA--ENACYYAISQEFNV--NPDEVRA	75
T_atroviride_XP_013943363	MPHSTLTREPKSFHAIVLDLNGVLLSYGGSFPSS----VLKPSQIKNVLDSPTWYDYECCKGISRSQCYQVRVSSEFEM--DVDVFSOT	81
G_trabeum_XP_007864346	---MVAAYRRFSTLICIDIGDLFTWSSSTRTT----ISARTLKAMMSTPTWRLYERGHYS--QQCCYDQLSKDFPPYEPPEIGRA	76
P_roqueforti_CDM29845	--MGKIIKRIYPKGVLIENKNIKAAF--PEIKYVGMNSNISQPDYEFLLKPM--ISSWIGLDGFAQAGVGRKPNASYYITFLQKPEL	76
L_bicolor_XP_001877382	-----IIFDLGDVVFVKWSPTTKTS----ISSRTLRLDLSPTWFNVERGQLA--EEECYQQIGEEFNL--LSGEVRA	65
A_oryzae_AO090026000576 (AstI)	-----MTRQSHYQAIILDLGNVVFWDTSQNPPTAAPNQISLLRTSMKSPVYHSYERGLS--TEECHRLLGKESLHV--DPGQIKEA	78
A_oryzae_AORIB40_NS.05916 (AstK)	-----MCTTFKAAIFDMGGVLFWNPIVDVTQ----VSLKDLGTIINSETWEQFERGKIE--PDCCYHQLGSQIGL--PGSEIAAT	72
A_nanangensis_FE257_006542 (AstC)	FVLARESLKIDTTFKVFHLNHLKAKSNGNLRVYAMSNISQPDYDILLKSKASEDWHLFKDFKVPFSGHVGMRKPDLEFFRHVLRLEIST	154
A_oryzae_AO090026000582	FDTVQATIRPEESFLAFLAELKSRFGEQLKLYAVANMSREDYAMLKSL--PIDWSLFDGVFLSADLGMKRPKELRFRHVLESISM	156
A_terreus_XP_001217376	LKQARKSLQVHHETLLFIQVQVGLDQWGLMVCGMTNTPRPEQDVMSHI--NAEYPVFDRIYISLGMGRKPSICFYQVRVMEIEIGL	158
A_niger_XP_001391086	FSAINKTQVQDHGILACLARKLKAHCRTGTLAIYGCNMSCKDFEKVQGY--SIDWNIIFDGIFISGDMGSKPELRFFSHILDRQL	161
A_bisporus_XP_006461126	FSQARDSLQANHDFISLIRELKAQANGRLRVYAMSNISLPDWEVLRMK--PADWDIFDHVFTSGAVGERKPNLAFYRHVIAATDL	158
T_atroviride_XP_013943363	LEQLTKTVKPHSEFVIAIAKNIKAAF--PEIKYVGMNSNISQPDYEFLLKPM--ISSWIGLDGFAQAGVGRKPNASYYITFLQKPEL	163
G_trabeum_XP_007864346	MDEARDSLTSNDAMAILLRQLKSESNGQLRILAMSNISKPDYMFRLTK--PADWDVFTDVTFTSDAGERKPNLGYRVRVVEKGAI	159
P_roqueforti_CDM29845	ILAVRKSCLVNPVVEALSMKPKGKLEFELYAVNTFSKEDYALVKFL--GFDWSLFRFVRVSSDIGMQKPELRFFYEHVLRQIGL	159
L_bicolor_XP_001877382	FDQARESLSVADEALIDLIRDLTKQSDGRLRIFAMSNISPPDWAVALRTK--PADWSIFDQVFTSGSAGERKPNLGFYEHVLAGTGV	148
A_oryzae_AO090026000576 (AstI)	FDLARQSLRSNPALLDRIRQLKQTR--GAVVYAMNTNIPQAEIYELKES--RAGDMEVDFEVFASGYGSRKRPETEFYRVMGEIGL	160
A_oryzae_AORIB40_NS.05916 (AstK)	FRQTTGCLRDPDARMTSLRLRLKQ--GAVVYAMNTNIPADPFHQLREM--HYEWDLFDGIFVDEFAEGMRKPDLEFYEHLVLRQIDT	152
A_nanangensis_FE257_006542 (AstC)	AAEDVVFV DDKLE NVMSAWSLGMHGIVFQNKEDVQRQLANLFG--SPTERGMEYLSRRKGLQLSMTT-----DIPVQDNFAQLLI	233
A_oryzae_AO090026000582	KPEDTILVDNDTNDILCALSMGLKGILFGST--SVPQALTNLLEYDHISRAEQFLRSKSHLSVHT-----GVTIRENFAQLLI	235
A_terreus_XP_001217376	SGDAIMF DDKLE NVIAAQSVGIRGVLFQSGQDLRRVVLNVLFG--DPVHRGLQFLAANAKMKDSVTNT-----GDTIQDNFAQLLI	237
A_niger_XP_001391086	APSEVIVVDHNTDNVLTAISMGMASVLANSPDDVQSRSLVNIERNPTERGRKFLERNAKNMHSVTHT-----GVLIRENFAQLMI	241
A_bisporus_XP_006461126	PHQHTIFV DDKLE NVLSARSLSGTGIVFDEPSEVKRALRLNIG--DPVQRGGEFLVRNAGKLSITRTTAKHESILPDENFAQLLI	242
T_atroviride_XP_013943363	DSARCVF DDSV ENTVAASALGFKGKLEFELYAVNTFSKEDYALVKFL--SPVDRGMEYMERNAKNMHSVTHT-----GGEQPNFQSQII	242
G_trabeum_XP_007864346	DPHSTIF DDKLE NVLVARSLSGMYGIVFDDQDKVMRALRNLG--DPIQRGWSFMRSHAGHLESVTNT-----GVLVPETFAQLLI	238
P_roqueforti_CDM29845	SSEQVILVDDTSLNLLAAMSMGQGVMSDY--SLYRSILNFVDIDPIGRGTRYLHENAQKHHSFTHT-----GVPVKENFTQLLI	238
L_bicolor_XP_001877382	GPQRTIFV DDKLE NVISARSLSGHGIVFDSPEPVKRALRNLG--DPLARGQAFLENAGNLVSVTENSNDHEAVLLQENFAQLLI	232
A_oryzae_AO090026000576 (AstI)	KAERVVFV DDKE ENVVDARGLGLYGVCFGVVEELRGHLLGI*	201
A_oryzae_AORIB40_NS.05916 (AstK)	SAETIFV DDKLE NVIAAQAVGMVGLHLDLSLATCMELRQLVG-C*	196
A_nanangensis_FE257_006542 (AstC)	LEATNHPELVAME--PGKRTWNYFIGGPNLTDTDFP DDLT SLALSIVPP--APEIISVMDIEVSRNLKDGIVPTYFDDTRPRVD	316
A_oryzae_AO090026000582	LEATGDIDLVELE--YHPTTWNYFIGTPVLTTQTEFF PHDDLT SLATTVLDR--PKDIANEIMDEMCKYRSDDDLMLTFFTFDKNRVD	318
A_terreus_XP_001217376	LELAQDRELVLQ--AGKRTWNYFIGGPKLTATTFP DDMDT SMALSVLPV--AEDVVSVVLEMLKFTVDDGIFMTYFSSRPRVD	320
A_niger_XP_001391086	LEASGDSTLVDIK--PHATTWNYFIGGPKVLTQKRNFP DDLT SLGLTITNA--TPEVANQVLMKILHRYTVDGLIMTFFTFDKNRVD	324
A_bisporus_XP_006461126	LEITGNRALVNLV--EHPQTWNYFDQGGKGLTTEEFF PHDDLT SLGLTILKR--SREIADSVMDKLEMYVDDPDGIIQTYFDHRRPRVD	325
T_atroviride_XP_013943363	LELTQDERLIKLEKRRGPTWNYFPHSNTFNGTTSY DDCDT TSYAMCTLDDIPAHEKEAAMDILNLSPLDNPLCWFNKNRPRIC	327
G_trabeum_XP_007864346	LDDVTQDRNLVALPKDCPRRNWFFSGKPILTTEVF PHDDLT SLGLTIFET--DEAVAQSVIEMDELYVTPDGVVQTYFDHTRPRVD	322
P_roqueforti_CDM29845	LELTGDRSLIDIG--SHRTWNLFIVTPVLTTQADFP DDMDT SLGITILNR--PTHVANLVMDKMLQYRTSDGLMQTFFFTFDKNRVD	321
L_bicolor_XP_001877382	LEVIGDANLVNLV--EHPRTWSFFQGGKGLTTEEFF PHDDLT SLGLTVMKR--DKAVANSVMNEMLEYVDHGTIIQTYFDHRRPRVD	315
A_nanangensis_FE257_006542 (AstC)	PIVCINVSTLFAKHHRSESQSLSTTISWIRDVLYHRAYFAGTRYASPEAFLEFFFTRLTSTLRPG--ALKDDFAGLLTERLRERIGT	399
A_oryzae_AO090026000582	PVVCNVLSLFYKYGRGHEHHTLAWVRQVLIRRAYINGTAFYPMPEAFLYFFFLQHITHLP--QLYDGLKVLKRLQERVRGV	402
A_terreus_XP_001217376	PVVCINVLGVCFRHNRERDVLPTFFHWIRDLINRAYLSGTRYPPSPDLFLFLARLCLAVRNQ--SLREQLVLPVLDRLRERVGA	403
A_niger_XP_001391086	PVVCNVLSLFYQYGRGEEVSDTFDWVQVLVLRARRYIHGTAFYPSPEAFLEFFFSRLRLRLESPTPTYNEQLLRERVAERIGV	409
A_bisporus_XP_006461126	PVVCNVNLSLFYAYGRGELRSTLTFLWEVLLNRAYLDGTRYETAECLFYFMSRLLATSGDP--DLHLLKPLKERVQERIGA	408
T_atroviride_XP_013943363	HGIIANAFRFFALSGQGHKAHTYTLFLCLRLTKAYELGSRYYENIDYMPYILSNLCSRRPTD--PLSALMBRELLKKEIQDRSGC	410
G_trabeum_XP_007864346	PVVCNVNLSLFYSNHRKQELRGLTIWVRVLLNRAYLDGTRYASAECLFYFLSRLLRKAEHDH--ELNDLLNLLKERVQERIGV	405
P_roqueforti_CDM29845	PIVCCNINLIFYQYQGWGNELSETFDWVYQVLQTRTYIHGSAFYPLPEAFFFLSRMLRLKLNHRPCVYIRMRGLLIKRLERLSV	406
L_bicolor_XP_001877382	PVVCVNALTFLYTHGRGSELSRTLQWIKVLLNRAYLDGTRYQTAECLFFLSRLRLASSEDR--ELHALLKPLRLRERIQRERIGV	398
A_nanangensis_FE257_006542 (AstC)	PVDSLALAMRVLSQALGIEC--AADVGR LSEMQCADGG WPCVYIKYGSGLGITNRGVSTAFAVRAIAQFATTSSASA---GMA	480
A_oryzae_AO090026000582	PVDPISLMRLIACNGVGIEH--RMGLNALLS MQNPDG SLDGMTYHYASKRLPIGNQGVSTAMAIKAQCCQANQACGI-----	480
A_terreus_XP_001217376	PGEAVSLAARILACRSFGIDS--ARDMDS LRGKQCEDGG WPEVWYRFASFGLNVNGRLATAFAVRALESPPYGESAVKVM----R	483
A_niger_XP_001391086	PVDAISLAMRLLVCHQVGMRD--TLGLEMLLS MQQPDGG WPLGTIYHYASKQAIAGNRGVSTALAVQAIQVCSQWKKSPNGHPKAT	493
A_bisporus_XP_006461126	DGDSLALAMRILACDFVGIIRD--EVDLRTLTL QCEDGG WEVGMWYKYGSSGISIGNRGLATALAKAVDTMQPQIRFSESPTDT	492
T_atroviride_XP_013943363	DSVDLGAALRTLSSAQMGVYAKRDVQV LLSQQLDGG WNRVWLFYKGKEDIKVGSRGVIAMAVKALRQYVADDE-----	486
G_trabeum_XP_007864346	EGDALALAMRILTCAAVGIRD--DVDMRTL LLPQCEDGG WELGWIKYKYGSSGVNIGNRGLATAFAIKAEIACEDMPASPPTSSTTT	489
P_roqueforti_CDM29845	PVDAASLAMRLIVCHQVGVRH--VSGLK FLSMQEPDGG WEIGTLQYYSKRVLWNGRGTSTALALDAIRALQCPWLAHRF-----	484
L_bicolor_XP_001877382	EGDSIALAMRILVCDVFLGRD--BIDILRS LLPQCEDGG WEIGWIKYKYGSSGLRIGNRGLTALALNAL-----	465
A_nanangensis_FE257_006542 (AstC)	RVDGEGKGEVGRSSPSHGGSRSGTLSAQAKA--LSPKWLQSLLRILRILPALFGVAGVAVVVLVFRV	543
A_oryzae_AO090026000582	-----RIV-----	480
A_terreus_XP_001217376	-----VYTRTERHY---GSP-----	486
A_niger_XP_001391086	-----LVENAI---H-KRRPSFSEKFLGKRPRSGSFRKPLQWILQGSKLRSKVEI-GS-----	505
A_bisporus_XP_006461126	-----PVKHEE---EAPRRVKRSSRIRNHAVQAHF-----	540
T_atroviride_XP_013943363	-----	486
G_trabeum_XP_007864346	-----	517
P_roqueforti_CDM29845	-----	484
L_bicolor_XP_001877382	-----	465

Pairwise Identity (%) Matrix

A_oryzae_AO090026000582	100.00	34.17	51.25	39.58	23.33	37.92	51.04	39.14	36.46
A_terreus_XP_001217376	34.17	100.00	33.54	39.92	27.37	40.53	32.64	40.65	43.00
A_niger_XP_001391086	51.25	33.54	100.00	37.43	22.63	36.44	49.59	39.35	35.64
A_bisporus_XP_006461126	39.58	39.92	37.43	100.00	29.01	56.87	35.74	72.26	42.04
T_atroviride_XP_013943363	23.33	27.37	22.63	29.01	100.00	26.54	22.93	25.38	27.78
G_trabeum_XP_007864346	37.92	40.53	36.44	56.87	26.54	100.00	34.50	60.65	42.17
P_roqueforti_CDM29845	51.04	32.64	49.59	35.74	22.93	34.50	100.00	36.77	33.06
L_bicolor_XP_001877382	39.14	40.65	39.35	72.26	25.38	60.65	36.77	100.00	45.16
A_nanangensis_FE257_006542	36.46	43.00	35.64	42.04	27.78	42.17	33.06	45.16	100.00

Figure S50. Multiple sequence alignment of FE257_006542, AstC and orthologous proteins.

The class I terpene synthase DDxxD motif is highlighted in **bold blue** text; class II DxDTT and QW motifs are highlighted in **bold red** text. Asterisk (*) in bold red text indicates stop codon. Pairwise identity matrix table (bottom): columns correspond to the same sequences in the same order as the rows.

A_nanangensis_MST-FP2251	AGGATCATTACCGAGTGAGGGTCTCGTG6CCCAACCTCCACCCGTGTCTATCTGTACCTGTTGCTTCGGCGGGCCCGCGCT	85
A_janus_NRRL_1787	AGGATCATTACCGAGTGAGGGTCTCGTG6CCCAACCTCCACCCGTGTCTATCTGTACCTGTTGCTTCGGTGGGCCCCGCCGCT	85
Uncultured_fungus	AGGATCATTACCGAGTGAGGGTCTCGTG6CCCAACCTCCACCCGTGTCTATCTGTACCTGTTGCTTCGGCGGGCCCGCGCT	85
A_nanangensis_MST-FP2251	CAGTCGGCCGCCGGGGGGCTTTACAGCCCCAGGCCCGTCCCCGCCGGAGACCCCAACTGAACCTGTTCTGAAAGCTTGCAGT	170
A_janus_NRRL_1787	TGT - CGGCCGCCGGGGGGCTCTCCAGCCCCGGGGCCGTGCC - ACCGAAGACCCCAACTGAACCTGTTCTGAAAGCTTGCAGT	168
Uncultured_fungus	CAGTCGGCCGCCGGGGGGCTTTACAGCCCCAGGCCCGTCCCCGCCGGAGACCCCAACTGAACCTGTTCTGAAAGCTTGCAGT	170
A_nanangensis_MST-FP2251	CTGAGTTGATTATCATTAAATCAGTTAAACTTTCAACAATGGATCTCTTGGTTCGGCATCGATGAAGAACGACGCGAAATGCGA	255
A_janus_NRRL_1787	CTGAGTTGATTATCATTAAATCAGTTAAACTTTCAACAATGGATCTCTTGGTTCGGCATCGATGAAGAACGACGCGAAATGCGA	253
Uncultured_fungus	CTGAGTTGATTATCATTAAATCAGTTAAACTTTCAACAATGGATCTCTTGGTTCGGCATCGATGAAGAACGACGCGAAATGCGA	255
A_nanangensis_MST-FP2251	TAACATAATGTGAATTGCAGAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCGGGGGGCATGCCTG	340
A_janus_NRRL_1787	TAACATAATGTGAATTGCAGAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCGGAGGGCATGCCTG	338
Uncultured_fungus	TAACATAATGTGAATTGCAGAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCGGGGGGCATGCCTG	340
A_nanangensis_MST-FP2251	TCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCTCGTCCCCCTTCCGGGGGACGGGCCGAAAGGCAGCG	425
A_janus_NRRL_1787	TCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCTCGTCCCCCTTCCGGGGGACGGGCCGAAAGGCAGCG	423
Uncultured_fungus	TCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCTCGTCCCCCTTCCGGGGGACGGGCCGAAAGGCAGCG	425
A_nanangensis_MST-FP2251	GCGGCACCGCGTCCGGTCTCGAGCGTATGGGGCTTTGTACCCGCTCAGTAGGCCGGCCGGCGCTGTCGACCCCCAACCTTT	510
A_janus_NRRL_1787	GCGGCACCGCGTCCGGTCTCGAGCGTATGGGGCTTTGTACCCGCTCAGTAGGCCGGCCGGCGCTGTCGACCCCCAACCTTT	508
Uncultured_fungus	GCGGCACCGCGTCCGGTCTCGAGCGTATGGGGCTTTGTACCCGCTCAGTAGGCCGGCCGGCGCTGTCGACCCCCAACCTTT	510
A_nanangensis_MST-FP2251	TATTATTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACCTAA	562
A_janus_NRRL_1787	TATTT - TTTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACCTAA	559
Uncultured_fungus	TATTATTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACCTAA	562

Pairwise identity (%) matrix			
A_nanangensis_MST-FP2251	100.00	94.99	100.00
A_janus_NRRL_1787	94.99	100.00	94.99
Uncultured_fungus	100.00	94.99	100.00

Figure S51. Multiple sequence alignment of internal transcribed spacer (ITS) regions from *A. nanangensis* MST-FP2251, *A. janus* NRRL 1787 and an uncultured fungus previously isolated from soil near Nanango, Queensland. Pairwise identity matrix table (bottom): columns correspond to the same sequences in the same order as the rows.

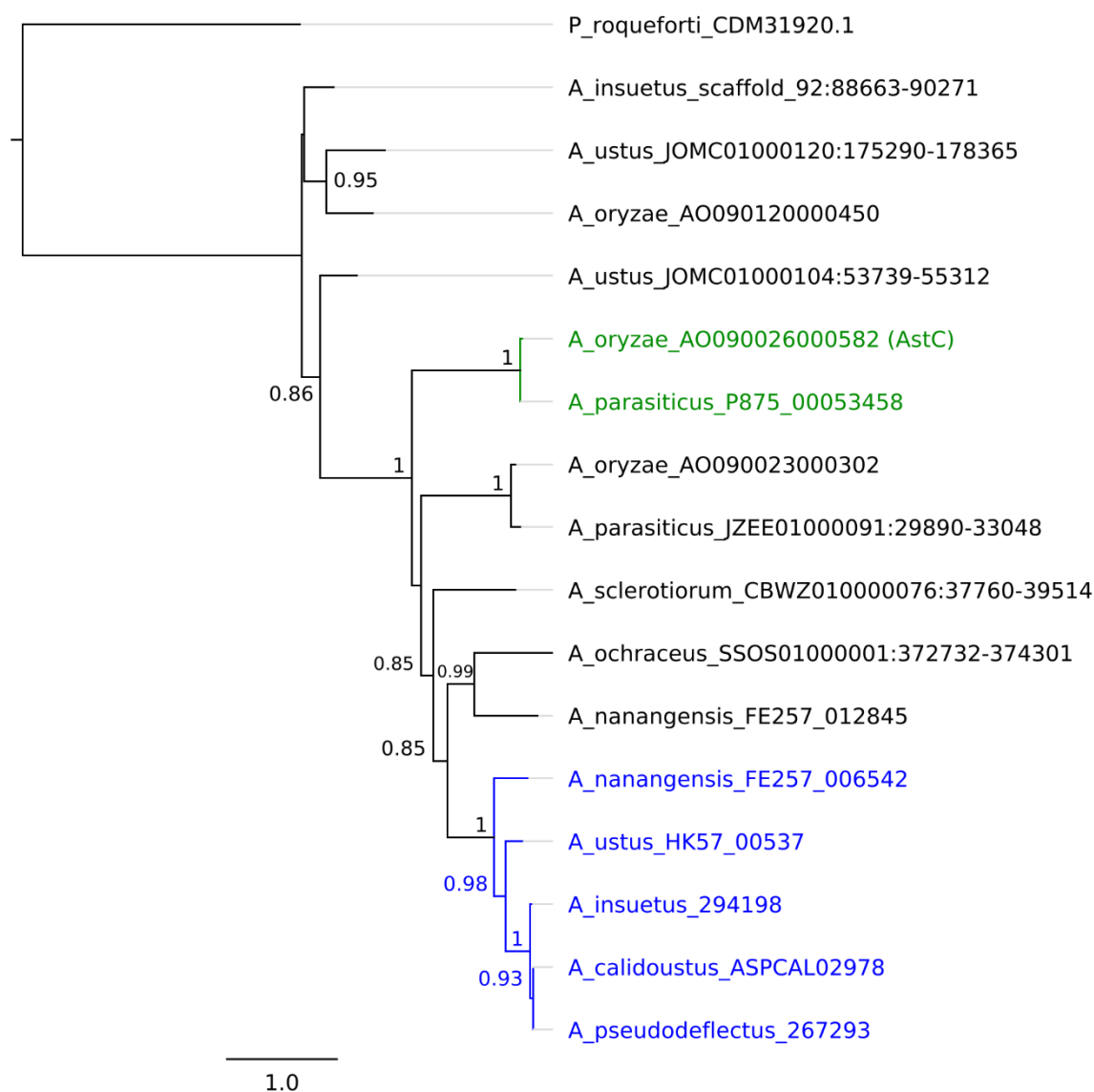


Figure S52. Phylogeny of AstC, the sesquiterpene synthase involved in astellolide C biosynthesis, homologs identified in available genomes of drimane sesquiterpene producing species *A. insuetus*, *A. oryzae*, *A. parasiticus*, *A. ustus*, *A. nanangensis*, *A. calidoustus* and *A. pseudodeflectus*.

Only support values above 0.8 are shown. Green highlighted clade contains AstC. The putative nanangenine cluster terpene synthase and the homologs in the drimane sesquiterpenoid producing-*Aspergilli* from section *Usti* form a distinct clade which is highlighted blue. Sequences were identified via local tBLASTn [17] searches against each genome; names include a locus tag if available, otherwise the coordinates on the assembly scaffold is indicated. Amino acid alignments were built with MUSCLE v3.8.31; the tree was constructed with FastTree 2.1.5 [15] in Geneious 10.2.6 [18] using the JTT model of amino acid substitution.

Supplementary references

1. Hübschle, C. B.; Sheldrick, G. M.; Dittrich, B. “ShelXle: A Qt Graphical User Interface for SHELXL”. *J. Appl. Crystallogr.* **2011**, *44*, 1281–1284. <https://doi.org/10.1107/S0021889811043202>.
2. Farrugia, L. “WinGX Suite for Small-Molecule Single-Crystal Crystallography”. *J. Appl. Crystallogr.* **1999**, *32*, 837–838.
3. Farrugia, L. J. “WinGX and ORTEP for Windows: An Update”. *J. Appl. Crystallogr.* **2012**, *45*, 849–854. <https://doi.org/10.1107/S0021889812029111>.
4. Spek, A. L. “Structure Validation in Chemical Crystallography”. *Acta Crystallogr. D Biol. Crystallogr.* **2009**, *65*, 148–155. <https://doi.org/10.1107/S090744490804362X>.
5. Farrugia, L. J. “PLATON for MS-Windows Taskbar v1.19”; University of Glasgow, **2018**.
6. Parsons, S.; Flack, H. D.; Wagner, T. “Use of Intensity Quotients and Differences in Absolute Structure Refinement”. *Acta Crystallogr. Sect. B Struct. Sci. Cryst. Eng. Mater.* **2013**, *69*, 249–259. <https://doi.org/10.1107/S2052519213010014>.
7. Sheldrick, G. M. “SHELXT - Integrated Space-Group and Crystal-Structure Determination”. *Acta Crystallogr. Sect. Found. Adv.* **2015**, *71* (Pt 1), 3–8. <https://doi.org/10.1107/S2053273314026370>.
8. Sheldrick, G. M. “Crystal Structure Refinement with SHELXL”. *Acta Crystallogr. Sect. C Struct. Chem.* **2015**, *71*, 3–8. <https://doi.org/10.1107/S2053229614024218>.
9. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. “OLEX2: A Complete Structure Solution, Refinement and Analysis Program”. *J. Appl. Crystallogr.* **2009**, *42*, 339–341. <https://doi.org/10.1107/S0021889808042726>.
10. Sievers, F.; Wilm, A.; Dineen, D.; Gibson, T. J.; Karplus, K.; Li, W.; Lopez, R.; McWilliam, H.; Remmert, M.; Soding, J.; Thompson, J. D.; Higgins, D. G. “Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega”. *Mol. Syst. Biol.* **2011**, *7*, 539.
11. Kozlov, A. M.; Darriba, D.; Flouri, T.; Morel, B.; Stamatakis, A. “RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference”. *Bioinformatics* **2019**, 1–3.
12. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D. L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M. A.; Huelsenbeck, J. P., “MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space”. *Syst. Biol.* **2012**, *61*, 539–542.
13. Cerqueira, G. C.; Arnaud, M. B.; Inglis, D. O.; Skrzypek, M. S.; Binkley, G.; Simison, M.; Miyasato, S. R.; Binkley, J.; Orvis, J.; Shah, P.; Wymore, F.; Sherlock, G.; Wortman, J. R. “The Aspergillus Genome Database: multispecies curation and incorporation of RNA-Seq data to improve structural gene annotations”. *Nucleic Acids Res.* **2014**, *42* (Database issue), D705–10.
14. Edgar, R. C. “MUSCLE: multiple sequence alignment with high accuracy and high throughput”. *Nucleic Acids Res.* **2004**, *32*, 1792–1797.
15. Price, M. N.; Dehal, P. S.; Arkin, A. P. “FastTree 2--approximately maximum-likelihood trees for large alignments”. *PLoS One* **2010**, *5*, e9490.
16. Buchfink, B.; Xie, C.; Huson, D. H. “Fast and sensitive protein alignment using DIAMOND”. *Nat. Methods* **2015**, *12*, 59–60.
17. Camacho, C.; Coulouris, G.; Avagyan, V.; Ma, N.; Papadopoulos, J.; Bealer, K.; Madden, T. L. “BLAST+: architecture and applications”. *BMC Bioinformatics* **2009**, *10*, 421.
18. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Meintjes, P.; Drummond, A. “Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data”. *Bioinformatics* **2012**, *28*, 1647–1649.