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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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*. 
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 Kα 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 μL) in MeCN (950 μL) was heated at 70 °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 P2₁(#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 (H3O₄) 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 P2₁(#4). The asymmetric unit contains two crystallographically-independent molecules. The diffraction data are pseudo-orthorhombic and a structure can be obtained and modelled in P2₁2₁2₁ (#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 P2₁(#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 Flack parameter is 0.10(10) and that of XNPP refined to 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 $4.0 \times 10^{-37}$, while that for an inversion twin is $1.0 \times 10^{-6}$. 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 $4.0 \times 10^{-24}$, while that for an inversion twin is $4.0 \times 10^{-5}$. 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 contains 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)** | **Quantity** |
|------------------------------|--------------|
| 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         |

*Trace element solution*

| Ingredient | Quantity |
|------------|----------|
| 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)** | **Quantity** |
|-----------------------|--------------|
| Czapeks Dox Media (Oxoid) | 99.88 g |
| Distilled water       | 2200 mL     |

| **Malt Extract Agar (MA)** | **Quantity** |
|---------------------------|--------------|
| 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)** | **Quantity** |
|------------------------------------|--------------|
| Yeast Extract (Difco)              | 20 g         |
| Sucrose (Amyl)                     | 150 g        |
| Bacteriological Agar (Amyl)        | 20 g         |
Distilled water 1000 mL
Autoclave

| Ingredient         | Quantity |
|--------------------|----------|
| Cracked wheat      | 46 g     |
| Distilled water    | 30 mL    |

| Ingredient         | Quantity |
|--------------------|----------|
| Pearl barley       | 48 g     |
| Distilled water    | 35 mL    |

| Ingredient         | Quantity |
|--------------------|----------|
| Jasmine rice       | 50 g     |
| Distilled water    | 25 mL    |

| Ingredient         | Quantity |
|--------------------|----------|
| Basmati rice       | 44 g     |
| Distilled water    | 30 mL    |
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        | C₁₅H₂₂O₅          | C₂₈H₃₅BrO₇                              | C₂₈H₃₅BrO₇                                 | C₂₁H₃₄O₆         | C₂₁H₳₃O₆         |
| Molecular Weight | 282.32            | 563.47                                  | 563.47                                     | 382.48           | 384.50           |
| Crystal System | monoclinic        | monoclinic                              | monoclinic                                 | orthorhombic      | orthorhombic      |
| Space Group    | P₂₁(#4)           | P₂₁(#4)                                 | P₂₁(#4)                                    | P₂₁;P₂₁;P₂₁(#19) | P₂₁;P₂₁;P₂₁(#19) |
| a (Å)          | 15.7957(2)        | 7.7558(2)                               | 14.88881(10) Å                            | 7.6270(4)        | 6.4497(2)        |
| b (Å)          | 10.3007(10)       | 14.0642(2)                              | 6.10835(4) Å                              | 9.5926(3)        | 7.9709(4)        |
| c (Å)          | 19.6228(2)        | 24.5340(3)                              | 29.4427(3) Å                              | 28.6181(8)       | 40.3829(19)      |
| β (º)          | 113.6310(10)      | 90.460(2)                               | 101.3693(7) º                            | -                | -                |
| V (Å³)         | 2925.04(6)        | 2676.06(9)                              | 2625.10(4) Å                              | 2093.76(13)      | 2076.08(16)      |
| Dc (g cm⁻³)    | 1.282             | 1.399                                   | 1.426 g                                    | 1.213            | 1.230            |
| Z              | 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 Kα, Å)   | 1.5418            | 1.5418                                  | 1.5418                                    | 1.5418           | 1.5418           |
| μ(Cu Kα, mm⁻¹) | 0.788             | 2.462                                   | 2.509                                     | 0.713            | 0.720            |
| Tmin, max (multi-scan) | 0.884, 1.00 | 0.934, 1.00                           | 0.874, 1.00                               | 0.696, 1.00      | 0.737, 1.00      |
| 2θmax         | 152.59º           | 153.23º                                 | 152.64º                                   | 148.88º          | 171.94º          |
| hkl 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 9, –9 9, –50 50 |
| N*            | 65936             | 52872                                   | 74047                                     | 36004            | 47388            |
| N⊥            | 11984 (Rmerge 0.0338) | 10615 (Rmerge 0.0506) | 10685 (Rmerge 0.0448) | 4173 (Rmerge 0.1085) | 4335 (Rmerge 0.0772) |
| Nhobs         | 11783(I > 2σ(I))  | 9834(I > 2σ(I))                        | 10457(I > 2σ(I))                          | 3841(I > 2σ(I))  | 3937(I > 2σ(I))  |
| Nvar          | 781               | 665                                     | 672                                        | 252              | 281              |
| Residuals     | 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. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for nanangenine A (1) in DMSO-$d_6$.

| Pos. | $\delta_C$ | $\delta_H$, mult. ($J$ 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  | 70.6       | 4.91, ddd (12.2, 1.3, 1.2)    | 7, 8, 9, 11 | 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. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for nanangenine B (2) in DMSO-$d_6$.

| Pos. | $\delta_c$ | $\delta_H$, mult. ($J$ in Hz) | HMBC |
|------|------------|-----------------------------|------|
| 1    | 68.8       | 4.19, m                      | 2, 9, 13 |
| 2    | 26.0       | 1.57, m<sup>b</sup>         |      |
| 3    | 41.4       | 1.29, m<sup>c</sup>         | 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<sup>b</sup>         | 1', 4', 5' |
| 4'   | 31.6       | 1.23, m<sup>c</sup>         | 3', 5' |
| 5'   | 21.8       | 1.27, m<sup>c</sup>         | 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 |

<sup>b, c</sup> indicates overlapping resonances.
Table S5. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for isonanagenine B (3) in DMSO-$d_6$.

| Pos. | $\delta_{C}$ | $\delta_{H}$, 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. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for nanangenine C (4) in DMSO-$d_6$.

| Pos. | $\delta$C | $\delta$H, mult. ($J$ 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<sup>b</sup>           | 3', 5' |
| 5'   | 22.2      | 1.26, m<sup>b</sup>           | 4', 6' |
| 6'   | 14.1      | 0.84, t (7.1)                 | 4', 5' |
| 9-OH |           | 6.30, br s                    | 8, 9, 10, 11 |

<sup>b</sup>indicates overlapping resonances.

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

| Pos. | $\delta_C$ | $\delta_H$, 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′ | 2b, 3′ | 2b |
| 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. $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data for isonanangenine D (6) in DMSO-$d_6$.

| Pos. | $\delta_C$ | $\delta_H$, 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   | 3, 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. $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data for nanangenine E (7) in DMSO-$d_6$.

| Pos. | $\delta_C$ | $\delta_H$, 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   | 17.4       | 1.81, dm (13.5)             | 3, 10, 13 | 1a, 2a/2b | 1a, 13 |
| 2a   | 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, 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      |                            |      |       |       |
| 2a   | 34.0       | 2.32, m                     | 1', 3', 4' | 2'b | 2'b, 4' |
| 2b   | 2.25, m    | 1', 3', 4'                  | 2a | 2'a, 4' |
| 3'   | 4.87       | 2.15, m                     | 1', 2', 4', 5' | 4' | 2'a, 2'b, 4' |
| 4'   | 2.24       | 1.24, m                     | 5', 6' | 3' | 3' |
| 5'   | 2.28       | 1.24, m                     | 4', 6' | 6' |
| 6'   | 31.0       | 1.21, m                     | 4', 5' |      |       |
| 7'   | 31.0       | 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. $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data for nanangenine F (8) in DMSO-$d_6$.

| Pos. | $\delta_C$ | $\delta_H$, 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  | 22.3       | 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. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for nanangenine G (9) in DMSO-$d_6$.

| Pos. | $\delta_{C}$ | $\delta_{H}$, 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, 6 | 2 | 1, 2, 5, 14 |
| 4    | 33.5         | 4.02, m                        |      |      |       |
| 5    | 44.9         | 1.83, d (4.4)                  | 4, 10, 13, 15 | 6 | 1, 3, 6, 9-OH, 15 |
| 6    | 121.4        | 5.78, br d (5.3)               | 5, 6, 8, 9, 11 | 6, 12a | 6, 12a, 12-OH |
| 7    | 144.1        |                               |      |      |       |
| 8    | 75.6         |                               |      |      |       |
| 9    | 45.9         |                               |      |      |       |
| 10   | 62.2         | 3.73, m                        | 8, 9, 10 | 11b, 11-OH | 1-OH, 9-OH, 11b, 11-OH, 12a |
| 11a  | 3.67, m       | 8, 9, 10 | 11a, 11-OH | 9-OH, 11a, 11-OH, 12a |
| 11b  | 61.0         | 4.05, m                        | 7, 8, 12-OH | 12b, 12-OH | 7, 11b, 12-OH |
| 12a  | 4.02, m       | 7, 8, 12-OH | 12b, 12-OH | 7, 11b, 12-OH |
| 12b  | 12.5         | 1.06, s                        | 1, 5, 9 | | 2, 11a |
| 13   | 24.4         | 1.03, s                        | 3, 4, 5 | | 2, 15, 2a |
| 14   | 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, 2b, 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. $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data for nanangenine H (10) in DMSO-$d_6$.

| Pos. | $\delta_C$ | $\delta_H$, 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  | 37.6, 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'   | 13.9       | 0.84, t (7.1)               | 4', 5' | 5'  |       |
| 12-OMe | 54.1      | 3.26, s                     | 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   | 1, 5, 12 |
Table S13. NCBI accessions of internal transcribed spacer (ITS) region sequences used in Figure S48.

| Organism                  | NCBI accession |
|---------------------------|----------------|
| Aspergillus flavus CBS 569.65 | AF027863       |
| Aspergillus tubingensis NRRL 4875 | EF661193       |
| Aspergillus niger CBS 554.65 | EF661186       |
| Aspergillus terreus CBS 601.65 | EF669586       |
| Aspergillus carneus CBS 494.65 | EF669611       |
| Aspergillus brevijanus CBS 111.46 | EF669582     |
| Aspergillus janus CBS 118.45 | EF669578       |
| Aspergillus nanangensis 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               | A. heteromorphus BO70DRAFT_410469 (63.92/90) |
| FE257_006541               | 7788/2384     | HR-PKS                       | A. ustus 00536 (66.74/99)             |
| FE257_006542               | 1773/543      | Haloacid dehydrogenase-like  | A. ustus 00537 (66.30/99)             |
| FE257_006543               | 1778/519      | FAD-binding oxidoreductase   | A. ustus 00538 (60.58/98)             |
| FE257_006544               | 1580/526      | Cytochrome P450               | A. ustus 00539 (75.25/96)             |
| FE257_006545               | 1003/312      | Hydrolase                     | A. calidoustus ASPCAL02981 (72.46/97) |
| FE257_006546               | 923/307       | Short-chain dehydrogenase    | A. lentulus ALT_1008 (83.55/98)       |
| FE257_006547               | 2023/460      | Cytochrome P450               | T. stipitatus 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*.

| **A. nanangensis** 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 |

| **A. ustus** CBS 3.3904 (Locus tag: HK57_) | |
|-----------------------------------------------|---------------------------------------------|
| Gene no. | 00536 | 00537 | 00538 | 00539 | 00540 | 00541 | 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 |

| **A. calidoustus** CBS 12160 (Locus tag: ASPCAL) | |
|-----------------------------------------------|---------------------------------------------|
| Gene no. | 02977 | 02978 | 02979 | 02980 | 02981 | 02982 | 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 |

| **A. insuetus** CBS 107.25 (JGI Portal: Aspins1) | |
|-----------------------------------------------|---------------------------------------------|
| Protein ID | 305363 | 294198 | 315989 | 315991 | 270963 | 270964 | 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 |

| **A. pseudodeflectus** CBS 756.74 (JGI Portal: Asppdef1) | |
|-----------------------------------------------|---------------------------------------------|
| Protein ID | 276134 | 267293 | 276137 | 276138 | 276138 | 276138 | 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-\textit{O}-(4-bromobenzoyl)-nanangenine B (\textit{2b}) with 50\% displacement ellipsoids.
Figure S5. Olex2 depiction of one of the two crystallographically-independent molecules of 1-O-(4-bromobenzoyl)-isonanangene 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. $^1$H NMR spectrum (500 MHz, DMSO-$d_6$) of nanangenine A (1).
Figure S9. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of nanangenine A (1).
Figure S10. $^1$H NMR spectrum (500 MHz, DMSO-$d_6$) of nanangenine B (2).
Figure S11. $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$) of nanangenine B (2).
Figure S12. $^1$H NMR spectrum (500 MHz, DMSO-$d_6$) of isonanangenine B (3).
Figure S13. $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$) of isonanangenine B (3).
Figure S14. $^1$H NMR spectrum (500 MHz, DMSO-$d_6$) of nanangenine C (4).
Figure S15. $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$) of nanangenine C (4).
Figure S16. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of nanangenine D (5).
Figure S17. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of nanangenine D (5).
Figure S18. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of isonanangenine D (6).
Figure S19. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of isonanagenine D (6).
Figure S20. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of nanangenine E (7).
Figure S21. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of nanangenine E (7).
Figure S22. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of nanangenine F (8).
Figure S23. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of nanangenine F (8).
Figure S24. $^1$H NMR spectrum (500 MHz, DMSO-$d_6$) of nanangenine G (9).
Figure S25. $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$) of nanangenine G (9).
Figure S26. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of nanangenine H (10).
Figure S27. $^{13}$C 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 isonanangenine B (3).

Figure S31. HRMS–ESI(−) spectrum of nanangenine 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 isonanangene B (3).

Figure S41. UV–vis spectrum (MeCN) of nanangenine C (4)
**Figure S42.** UV–vis spectrum (MeCN) of nanangene 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)
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.
| Protein            | Sequence                                                                 | Identity (%) |
|--------------------|---------------------------------------------------------------------------|--------------|
| *A. nanangensis*   | **AstC**<sup>1</sup> FE257_006542                                       | 50.00        |
| *L. bicolor*       | XP_001877382                                                              | 40.65        |
| *P. roqueforti*    | CDM29845                                                                  | 36.46        |
| *A. bisporus*      | XP_006461126                                                              | 35.74        |
| *A. niger*         | XP_001391086                                                              | 34.50        |
| *A. oryzae*        | AO090026000580                                                            | 34.17        |
| *T. atroviride*    | XP_013943363                                                              | 32.93        |
| *A. terreus*       | XP_001217376                                                              | 32.63        |
| *A. oryzae*        | AO090026000576                                                            | 32.40        |

<sup>1</sup> **AstC** denotes the AstC protein domain.

**Figure S50.** Multiple sequence alignment of FE257_006542, AstC and orthologous proteins. Bold red text highlights the DDxxD motif, while bold blue text highlights the conserved cysteine residues. Asterisk (*) indicates the stop codon in the multiple sequence alignment matrix.

**Table S62.** Pairwise identity (%) matrix showing the identity between various proteins. The table is structured with proteins listed in the first column and their pairwise identities displayed in the corresponding rows.

**Note:** The alignment and figures indicate the conservation and identity patterns among the proteins, with highlighted regions indicating key motifs or conserved areas.
**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.

| Pairwise identity (%) matrix | A_nanangensis_MST-FP2251 | A_janus_NRRL_1787 | Uncultured_fungus |
|-----------------------------|--------------------------|-------------------|-------------------|
| 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 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.
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