Supporting Information

Discovery of Druggability-Improved Analogues by Investigation of LL-D49194α1 Biosynthetic Pathway

Lei Dong,† ‡ Yi Shen,† Xian-Feng Hou,‡ Wen-Jun Li,*,† § and Gong-Li Tang*,‡

†State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275, China.
‡State Key Laboratory of Bio-organic and Natural Products Chemistry, Center for Excellence in Molecular Synthesis, Shanghai Institute of Organic Chemistry, University of Chinese Academy of Sciences (CAS), CAS, Shanghai, 2000032, China
§Southern Laboratory of Ocean Science and Engineering (Guangdong, Zhuhai), Zhuhai, 519000, China

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Supplementary Materials and Methods

General

Reagents and chemicals were purchased from TCI (shanghai) or Sigma-Aldrich, unless noted otherwise. Oligonucleotide primers synthesis was performed at Suzhou Genewiz Biotechnology Co., Ltd., and are listed in Table S2. PCR amplification was performed using either KOD Plus (TOYOBO) DNA polymerase or Taq DNA polymerase. DNA sequencing was performed at the Biosune (Shanghai) Biotechnology Co., Ltd. Analytical HPLC was performed on an Agilent 1200 series system. Preparative HPLC was performed on a Shimadzu LC-20-AT system. Silica gel column chromatography was carried out using SiliaFlash P60. NMR spectra were obtained on Agilent DD2 600Mhz system and chemical shifts were calibrated with reference solvent signals (\(^1\)H NMR: CDCl\(_3\) 7.26 ppm, \(^{13}\)C NMR: CDCl\(_3\) 77.16 ppm). Data for \(^1\)H NMR are listed as follows: chemical shift (\(\delta\), ppm ), multiplicity (s = singlet, brs = broad singlet, d = doublet, brd = broad doublet, t = triplet, m = multiplet or unresolved). Coupling constants (\(J\)) are in terms of Hertz. LC-MS were obtained on a ThermoFisher UHPLC Ultimate3000 system with a compact mass spectrometer (LTQ XL). HR-ESI-MS were recorded on an Agilent 1260 Infinity system with a time-of-light (TOF) mass analyzer. All biological assays were conducted in three independent replicates.

Strains and media

A LLD producer, *Streptomyces vinaceusdrappus* NRRL 15735 was purchased from the American Agricultural Research Service (ARS). *S. vinaceusdrappus* NRRL 15735 was cultivated at 30 °C, in MS medium (2% soybean powder, 2% mannitol, 2% agar, pH = 7.3±0.2) for 5 days, and used as the source for routine inoculation. For genome extraction, *S. vinaceusdrappus* NRRL 15735 was cultivated in shaking flask in modified YEME medium (0.3% yeast extract, 0.5% peptone, 0.3% malt extract, 1% glucose, 10% sucrose, pH=7.3±0.2).

Standard DNA engineering experiments were carried out using *Escherichia coli* DH5α competent cells (Invitrogen). *E. coli* cells bearing manipulated plasmids were cultivated in Luria-Betani medium and were selected with apramycin.
Whole genome sequencing and analysis

Whole genome sequencing of \textit{S. vinaceusdrappus} NRRL 15735 was performed on an Illumina Hiseq2000 system, and the resulting reads were assembled using SOAP denovo2,\(^1\) yielding the genome size was 8, 094, 394 bp, comprised of 7543 genes, 39 scaffolds were annotated. The open reading frames (ORFs) were predicted using online tool 2ndFind (http://biosyn.nih.go.jp/2ndfind), and manually adjusted according to the homologous domains matched in the NCBI database.

Construction of Gene Deletion and Complementation Mutants

The gene deletion mutants of \textit{lldA1, lldM1, lldB3, lldB4, lldM2, lldB5, lldO2, lldM3, lldM4, lldO6, lldO7, lldO10} were constructed using a homologous sequence replacement strategy as previously published.\(^2\) The PCR template is the genome DNA, amplification was performed with the following primers respectively: lldA1-L/RH-F/R, lldM1-L/RH-F/R, lldB3-L/RH-F/R, lldB4-L/RH-F/R, lldM2-L/RH-F/R, lldB5-L/RH-F/R, lldO2-L/RH-F/R, lldM3-L/RH-F/R, lldM4-L/RH-F/R, lldO6-L/RH-F/R, lldO7-L/RH-F/R, lldO10-L/RH-F/R (Table S2), Mutant plasmids pTG2027, pTG5028, pTG5029, pTG5030, pTG5031, pTG5032, pTG5033, pTG5034, pTG5035, pTG5036, pTG5037, pTG5038 were constructed for gene replacement (Table S1), and then introduced into \textit{S. vinaceusdrappus} NRRL 15735 through intergeneric conjugation from \textit{E. coli} S17-1. The double crossover mutants (from TG5019 to TG5030, Table S1), were selected with apramycin-sensitive colonies and whose genotype were tested by PCR (from t-A1-F/R to t-O10-F/R, Table S2).

Production and Analysis of LL-D49194 (LLD)

\textit{S. vinaceusdrappus} NRRL 15735 WT and recombinant strains were cultivated in a seed culture of modified TSB (tryptic soy broth 3%; complemented with FeSO\(_4\).7H\(_2\)O 0.0001%, MnCl\(_2\).4H\(_2\)O 0.0001%, ZnSO\(_4\).7H\(_2\)O 0.0001%) at 30 °C for 36 h, then 6mL of seed solution was transferred into a 0.5 L flask containing 0.1 L fermentation broth (soluble starch 6%, yeast extract 1%, glucose 1%, NaCl 0.3%, K\(_2\)HPO\(_4\) 0.1%, MgSO\(_4\).7H\(_2\)O 0.1%, DIAION HP20, 3%, Trace sault 0.1 mL (stored in 1000× stock solution: CuSO\(_4\).5H\(_2\)O 7%, FeSO\(_4\).7H\(_2\)O 1%, MnCl\(_2\).4H\(_2\)O 0.8%, ZnSO\(_4\).7H\(_2\)O 0.2%, CoCl\(_2\).7H\(_2\)O 0.006%), pH =7.3±0.2) and incubated at
29 °C for 6 days. The incubation broth was collected and then centrifugalized (5000 rpm for 20 minutes), the supernatant was removed, and all the mycelia and macro resin HP20 were collected. Two volume of acetone was added and stirred for 2 h, and then ultrasonic extraction for 0.5 h. After evaporated the organic layer, the water layer was partitioned with triple volume ethyl acetate to afford EtOAc extract.

HPLC analysis was performed on a ThermoFisher ODS-2 HYPERSIL column (5 μm, 250×4.6 mm) equilibrated with a 50% solvent A (water and 0.1% formic acid) and solvent B (acetonitrile and 0.1% formic acid) program, and then analyzed with the following gradient program: 0-5 min, 10% B; 5-24 min, a binary linear gradient from 10%-90% B; 24-26 min constant 90% B; 26-27 min, gradient from 90%-10% B; 27-31 min constant 10% B. The program was performed at a flow rate of 1.0 mL/min and a column temperature of 20 °C, UV detection at 400 nm using an Agilent 1200 series system. The MS data of related compounds were obtained by LC-MS analysis on a ThermoFisher LTQ XL under the same conditions.

**Purification conditions for 7 and 8**
The EtOAc extract from a 7 L liquid fermentation flask of mutant strain *S. vinaceusdrappus* TG5022 was dropwise added to silica-gel column chromatography, and then eluted stepwise with a dichloromethane:methanol gradient (100:0 to 90:10). Components that contained 7 and 8 were further purified by semi-HPLC using a Thermo ODS-2 HYPERSIL 5 μm column, gradient flow at a rate of 2 mL/min from 63%-67% acetonitrile in water over 15 minutes to yield a yellow solid 7 (0.84 mg) and of a yellow solid 8 (1.59 mg), respectively.

**Purification conditions for 9**
The EtOAc extract from a 7 L liquid fermentation flask of mutant strain *S. vinaceusdrappus* TG5024 was dropwise added to silica-gel column chromatography, and then eluted stepwise with a dichloromethane:methanol gradient (100:0 to 90:10). Components that contained 9 were further purified by semi-HPLC using a Thermo ODS-2 HYPERSIL 5 μm column, gradient flow at a rate of 2 mL/min from 60%-65% acetonitrile in water over 15 minutes to yield an orange solid 9 (8.41 mg).
Purification conditions for 5
The EtOAc extract from a 20 L liquid fermentation flask of mutant strain *S. vinaceusdrappus* TG5021 was dropwise added to silica-gel column chromatography, and then eluted stepwise with a dichloromethane:methanol gradient (100:0 to 85:15). Components that contained 5 were further purified by semi-HPLC using a Thermo ODS-2 HYPERSIL 5 μm column, gradient flow at a rate of 2 mL/min from 10%-58% acetonitrile in water over 15 minutes to yield a yellow solid 5 (12.63 mg).

Purification conditions for 11 and 12
The EtOAc extract from a 8 L liquid fermentation flask of mutant strain *S. vinaceusdrappus* TG5020 was dropwise added to silica-gel column chromatography, and then eluted stepwise with a dichloromethane:methanol gradient (100:0 to 85:15). Components that contained 11 and 12 were further purified by semi-HPLC using a Thermo ODS-2 HYPERSIL 5 μm column, gradient flow at a rate of 2 mL/min from 20%-50% acetonitrile in water over 15 minutes to yield an orange solid 11 (3.82 mg) and an orange solid 12 (5.49 mg), respectively.

Purification conditions for 10
The EtOAc extract from a 14 L liquid fermentation flask of mutant strain *S. vinaceusdrappus* TG5025 was dropwise added to silica-gel column chromatography, and then eluted stepwise with a dichloromethane:methanol gradient (100:0 to 90:10). Components that contained 10 were further purified by semi-HPLC using a Thermo ODS-2 HYPERSIL 5 μm column, gradient flow at a rate of 2 mL/min from 20%-65% acetonitrile in water over 15 minutes to yield a yellow solid 10 (6.65 mg).

Purification conditions for 4
The EtOAc extract from a 14 L liquid fermentation flask of mutant strain *S. vinaceusdrappus* TG5030 was dropwise added to silica-gel column chromatography, and then eluted stepwise with a dichloromethane:methanol gradient (100:0 to 90:10). Components that contained 4 were further purified by semi-HPLC using a Thermo ODS-2 HYPERSIL 5 μm column, gradient flow at a rate of 2 mL/min from 30%-60% acetonitrile in water over 15 minutes to yield an orange
solid 4 (5.74 mg).

**Cancer cell viability assay of LL-D49194α1 and Analogues**

All cell-culture work was carried out in a biological safety cabinet. HL60 and B16F10 cells were maintained in a 5% CO₂, 37 °C, humidified atmosphere in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin/penicillin. LL-D49194α1 and analogues were dissolved in DMSO as 20 mM stock solution. Cells were collected and determined using a hemacytometer, with the final concentration of cell suspension was approximately 16,000 cells/50 μL. According to a preliminary experiment, compound stock solution was added and diluted with RPMI-1640 media (supplementary with FBS and antibiotics) to achieve final concentrations ranged from 1 nM to 4000 nM (0.1% v/v final concentration of DMSO). After incubated at 5% CO₂ and 37 °C for 48 h, cell viability was assessed using CCK-8 assay. 0.1% DMSO-treated cells were served as negative control. IC₅₀ value was calculated and fitted using GraphPad Prism7, curves are in Figure S50.

**Reactions of LL-D49194α1, 5 and 4 with Duplex DNA**

Alkylation buffer (10 mM potassium phosphate buffer, pH 7.0) was prepared as previously published.³ Duplex DNA oligomers d(AATTACGTAATT) (0.5 mM stock solution in alkylation buffer) were added in 10.0 μL (5 nmol) to four separate solutions designated as vials 1-4, respectively, LL-D49194α1 (10 mM stock solution in DMSO, 1.0 μL, 10 nmol, vial 2), 5 (10 mM stock solution in DMSO, 1.0μL, 10 nmol, vial 3) and 4 (10 mM stock solution in DMSO, 1.0 μL, 10 nmol, vial 4) were added, all 4 vials were then complemented to 100 μL with alkylation buffer. All samples were rotated in a speed of 350 rpm at room temperature. After 12 hours, 5 μL aliquot were removed from each of the 4 vials and diluted with MeCN in 10-fold. Then the samples were injected and analyzed on an Agilent 1260 Infinity system (gradient elution with 1 mM triethylammonium acetate buffer and acetonitrile) with a ESI-TOF spectrometry in negative ionization mode, capillary exit voltage was -80V.

**Solubility assay of LL-D49194α1, 5 and 4**

All related compounds were calibrated by diluting the 5 mM stock solution to 200, 150, 100,
75, 50, 25 μM, UV absorbance was measured by HPLC using a Thermo ODS-2 HYPERSIL 5μm column, gradient flow at a rate of 1mL/min from 5%-90% MeCN in water over 15 minutes. The aqueous solubility of LL-D49194α1 and analogues was measured with the shake-flask method as previously published.¹ 1.0 mg of compound was diluted to 50 mM, after stirred at room temperature for over 24 h. The solutions were filtered and diluted in 100-fold, then compared the UV absorbance of each compound to calibration curve to measure its aqueous solubility (Figure S51). Internal standard = Trioxacarcin A.²

**Computation of Octanol-Water partition coefficient**

The logP (logarithm of the n-octanol and water partition-coefficient) for all LL-D49194 analogues were calculated using a local software XLOGP3.³ Software were downloaded via website (http://www.sioc-ccbg.ac.cn/?p=42&software=xlogp3). Numbers of hydrogen bond donors, hydrogen bond acceptors and rotatable bonds were included and listed in Table S12.

**Human liver microsome assay of LL-D49194α1, 5 and 4**

Metabolic ratio assay *in vitro* was determined using a microsome method as previously published.⁴ A mixture of PBS (50mM, pH 7.4) and NADPH regenerating system (Sigma-Aldrich) were incubated in a water bath for 5 minutes at 37 °C, and then cooled down in ice box. Next, compound in 20 mM stock solution was added and vortexed (final concentration 0.2 mM, 1% DMSO), 10 μL mixture was withdrawn immediately as to sample, then acetonitrile within 50 μM internal standard was added (4:1, v/v) to quench the reaction. Ice-cold human live microsomes (Bioivt, 10-donor) were added (final concentration of 1 mg/mL) and then incubated in water bath for 2 h at 37 °C, another mixture was withdrawn as t1 sample and quenched as before. Metabolic ratio was analyzed on a HPLC system using a Thermo ODS-2 HYPERSIL 5 μm column, gradient flow at a rate of 1mL/min from 5%-82% MeCN in water over 13 minutes. Internal standard = Trioxacarcin A.

**Analytical data**

LL-D49194α1 (1): yellow solid; for NMR data see Figures S1 and S2; HR-ESI-MS (positive ion) found m/z 1015.3767 (calcd [M + Na]^+ for formula C₄₈H₆₄O₂₂Na m/z = 1015.3781).
**LL-D49194β2 (2):** yellow solid; for NMR data see Figures S3 and S4; HR-ESI-MS (positive ion) found \( m/z \) 973.3651 (calcd \([M + Na]^+\) for formula \( \text{C}_{46}\text{H}_{62}\text{O}_{21}\text{Na}\) \(m/z = 973.3676\)).

4: orange solid; for NMR data see Table S7 and Figures S10 to S15; HR-ESI-MS (positive ion) found \( m/z \) 457.1129 (calcd \([M + H]^+\) for formula \( \text{C}_{23}\text{H}_{21}\text{O}_{10}\) \(m/z = 457.1129\)).

5: yellow solid; for NMR data see Table S7 and Figures S16 to S21; HR-ESI-MS (positive ion) found \( m/z \) 487.1235 (calcd \([M + H]^+\) for formula \( \text{C}_{24}\text{H}_{33}\text{O}_{11}\) \(m/z = 487.1235\)).

7: yellow solid; for NMR data see Table S11 and Figures S34 to S39; HR-ESI-MS (positive ion) found \( m/z \) 685.2103 (calcd \([M + Na]^+\) for formula \( \text{C}_{32}\text{H}_{38}\text{O}_{15}\text{Na}\) \(m/z = 685.2105\)).

8: yellow solid; for NMR data see Table S6 and Figures S8 and S9; HR-ESI-MS (positive ion) found \( m/z \) 829.2887 (calcd \([M + Na]^+\) for formula \( \text{C}_{39}\text{H}_{50}\text{O}_{18}\text{Na}\) \(m/z = 829.2889\)).

9: orange solid; for NMR data see Table S12 and Figures S40 to S45; HR-ESI-MS (positive ion) found \( m/z \) 829.2892 (calcd \([M + Na]^+\) for formula \( \text{C}_{39}\text{H}_{50}\text{O}_{18}\text{Na}\) \(m/z = 829.2889\)).

10: yellow solid; for NMR data see Table S13 and Figures S46 to S51; HR-ESI-MS (positive ion) found \( m/z \) 811.2786 (calcd \([M + Na]^+\) for formula \( \text{C}_{39}\text{H}_{48}\text{O}_{17}\text{Na}\) \(m/z = 811.2784\)).

11: orange solid; for NMR data see Table S9 and Figures S22 to S27; HR-ESI-MS (positive ion) found \( m/z \) 666.2387 (calcd \([M + \text{NH}_4]^+\) for formula \( \text{C}_{31}\text{H}_{46}\text{O}_{15}\text{N}\) \(m/z = 666.2392\)).

12: orange solid; for NMR data see Table S10 and Figures S28 to S33; HR-ESI-MS (positive ion) found \( m/z \) 796.3023 (calcd \([M + \text{NH}_4]^+\) for formula \( \text{C}_{37}\text{H}_{50}\text{O}_{18}\text{N}\) \(m/z = 796.3022\)).
**Table S1.** Strains and plasmids used in this study

| Strain/Plasmid | Characteristics | Reference |
|----------------|-----------------|-----------|
| **Strains**    |                 |           |
| *E. coli* DH5α | Host for general cloning | Invitrogen |
| *E. coli* S17-1 | Donor strain for intergeneric conjugation between *E. coli* and *S. vinaceusdrappus* NRRL 15735 | 2 |
| *S. vinaceusdrappus* NRRL15735 | Wild type strain, LLD producing | NRRL |
| *S. vinaceusdrappus* TG5019 | *lldA1* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5020 | *lldM1* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5021 | *lldB3* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5022 | *lldB4* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5023 | *lldM2* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5024 | *lldB5* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5025 | *lldO2* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5026 | *lldM3* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5027 | *lldM4* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5028 | *lldO6* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5029 | *lldO7* gene replacement mutant | This work |
| *S. vinaceusdrappus* TG5030 | *lld10* gene replacement mutant | This work |
| **Plasmids**  |                 |           |
| pKC1139 | Apramycin resistance and thermo-sensitive plasmid | 2 |
| pTG5027 | pKC1139 vector derivative for gene replacement of *lldA1* | This work |
| pTG5028 | pKC1139 vector derivative for gene replacement of *lldM1* | This work |
| pTG5029 | pKC1139 vector derivative for gene replacement of *lldB3* | This work |
| pTG5030 | pKC1139 vector derivative for gene replacement of *lldB4* | This work |
| pTG5031 | pKC1139 vector derivative for gene replacement of *lldM2* | This work |
| pTG5032 | pKC1139 vector derivative for gene replacement of *lldB5* | This work |
| pTG5033 | pKC1139 vector derivative for gene replacement of *lldO2* | This work |
| pTG5034 | pKC1139 vector derivative for gene replacement of *lldM3* | This work |
| pTG5035 | pKC1139 vector derivative for gene replacement of *lldM4* | This work |
| pTG5036 | pKC1139 vector derivative for gene replacement of *lldO6* | This work |
| pTG5037 | pKC1139 vector derivative for gene replacement of *lldO7* | This work |
| pTG5038 | pKC1139 vector derivative for gene replacement of *lldO10* | This work |
### Table S2. Primers used in this study

| Primer     | Sequence (5' to 3')                                |
|------------|----------------------------------------------------|
| lldA1-LH-F | ATAGGATCCAGGGGAGCACAGATGGAA                       |
| lldA1-LH-R | ATATCTAGAGGTGATGGCGACTCTGCG                       |
| lldA1-RH-F | ATATCTAGACGCCGGAGGACCCGGCTCA                     |
| lldA1-RH-R | ATAAAGCTTCTCGAGGCGCTCTCAGAGA                     |
| lldM1-LH-F | ATAGGATCCGACACGACGCCGACACGTAC                    |
| lldM1-LH-R | ATATCTAGAGGTGATGGCGACTCTGCG                       |
| lldM1-RH-F | ATATCTAGAGGTGACAGTTCGAGTTGGTTCG                 |
| lldM1-RH-R | ATAAAGCTTCTCGAGGCGACCCGAAGCAGC                   |
| lldB3-LH-F | ATAGAATTCCCTGTCCAGCTCACCAGCTGCC                 |
| lldB3-LH-R | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldB3-RH-F | ATATCTAGAGGTGACCTCCTGAGTGAGG                   |
| lldB3-RH-R | ATAAAGCTTCTCGAGGCGACCCGAAGCAGC                   |
| lldB4-LH-F | ATAGAATTCCGAGGGGATGTGAGCACTCC                  |
| lldB4-LH-R | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldB5-LH-F | ATAGAATTCCACTGACCGACCGACGACG                  |
| lldB5-LH-R | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldB5-RH-F | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldB5-RH-R | ATAAAGCTTCTCGAGGCGACCCGAAGCAGC                   |
| lldO2-LH-F | ATAGAATTCCGAGGGGATGTGAGCACTCC                  |
| lldO2-LH-R | ATATCTAGAGGTGAGCTGACACGC                         |
| lldO2-RH-F | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldO2-RH-R | ATAAAGCTTCTCGAGGCGACCCGAAGCAGC                   |
| lldM3-LH-F | ATAGAATTCCGAGGGGATGTGAGCACTCC                  |
| lldM3-LH-R | ATATCTAGAGGTGAGCTGACACGC                         |
| lldM3-RH-F | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldM3-RH-R | ATAAAGCTTCTCGAGGCGACCCGAAGCAGC                   |
| lldM4-LH-F | ATAGAATTCCGAGGGGATGTGAGCACTCC                  |
| lldM4-LH-R | ATATCTAGAGGTGAGCTGACACGC                         |
| lldM4-RH-F | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldM4-RH-R | ATAAAGCTTCTCGAGGCGACCCGAAGCAGC                   |
| lldO6-LH-F | ATAGAATTCCGAGGGGATGTGAGCACTCC                  |
| lldO6-LH-R | ATATCTAGAGGTGAGCTGACACGC                         |
| lldO6-RH-F | ATATCTAGACATCGTGGGTCCTCCGAGTCCTG                |
| lldO6-RH-R | ATAAAGCTTCTCGAGGCGACCCGAAGCAGC                   |
| lldO7-LH-F   | ATAGAATTCAGCGCCTGCTGCTG | lldO7-LH-R   | ATATCTAGAGGGTTCCGAGTTCCGAGG | lldO7-RH-F   | ATATCTAGACTGCGCTGCTTCTTCTC | lldO7-RH-R   | ATAAAGCTTGGTGCTGCTCAACGCCCAGA |
|------------|-------------------------|------------|-----------------------------|------------|-----------------------------|------------|-------------------------------|
| lldO10-LH-F | ATAGAATTCAGAGGGTTCCGAGG | lldO10-LH-R | ATATCTAGAATCGGCGACCAGGGCTC | lldO10-RH-F | ATATCTAGACAACGCTGGCTCCGGTTC | lldO10-RH-R | ATAAAGCTTAACTGCTGCTGCTGCCAGA |
| t-A1-F     | GGGCCCAGAAGAAGCTGATCG  | t-A1-R     | ACCAGGCGAGAAGCTGGTAG        | t-M1-F     | GAAAATACGCGCAGCGAATG        | t-M1-R     | GCTGTCGAGAAGACCCCTTCT       |
| t-B3-F     | ACCAGGCGAGAAGCTGGTAG   | t-B3-R     | GGGCGAGACCAGCTGGTAG         | t-B4-F     | CCGGTCAGCGACTCCGCGAT       | t-B4-R     | CCAAGATAATGCTGCCCAGTCT    |
| t-B5-F     | CGTGTCAACGCTGGCTGGTC   | t-B5-R     | TTCACGCGAGCAAGCTGGTAG       | t-O2-F     | CTCATCGAGGAGACGTCTTC       | t-O2-R     | CGTCAGGCAGCCAGCATG         |
| t-M3-F     | GATGTTGTCGCGCGGTGTG   | t-M3-R     | TCCTCAAGCTGGCTCCTC          | t-M4-F     | TACGGACCGAGTGCGGATGG       | t-M4-R     | CTCGTCCTGCGTCGCAGATC       |
| t-M6-F     | AGGTGCTGCTACTACGGACGGA | t-M6-R     | CTCGTCCTGCGTCGACGCA        | t-O7-F     | TGGCCTGATGCGCTGAGAAGA     | t-O7-R     | TGGCCTGACGCTCGTGAGCAGCA    |
| t-O10-F    | ACAACGCGGTGTGGCGTGAAG  | t-O10-R    | TGGCCTGACGCTCGTGAGAAGA     |
Table S3. Comparative analysis of biosynthetic gene clusters of LL-D49194 in *S. vinaceusdrappus* NRRL 15735 and TXNs in *S. bottropensis* DO-45 (NRRL 12051)

| Gene      | Amino acid | Protein homolog (No.), [origin]; S/I (%) | TXN homolog, S/I (%) | Proposed function                     |
|-----------|------------|------------------------------------------|----------------------|---------------------------------------|
| orf(-1)   | 288        | Transposase (KES02929), [S. toyocaensis]; (87/85) | /                    | Transposase                           |
| lldRg1    | 267        | SLA_3077 (BAU8399), [S. laurentii ATCC 31255]; (83/70) | TnxRg2 (81/68)       | SARP-family regulator                 |
| lldB1     | 310        | ADK74_10340 (KOG47576), [S. decoyicus]; (74/66) | TnxB5 (70/60)        | dNDP-hexose-4-ketoreductase           |
| lldB2     | 194        | PokS7 (ACN64855), [S. diastatochromogenes]; (86/74) | TnxB6 (80/72)        | dTDP-4-dehydrorhamnose 3,5-epimerase  |
| lldM1     | 410        | GonCM (CUW01197), [S. caniferus]; (82/72) | TnxM1 (81/71)        | dNDP-hexose-3-C-methyltrasferase      |
| lldB3     | 420        | TnxB10 (AKT74299), [S. bottropensis DO-45]; (68/56) | TnxB10 (68/56)       | GTB-type glycosyltransferase          |
| lldB4     | 430        | TnxB10 (AKT74299), [S. bottropensis DO-45]; (68/58) | ?                    | GTB-type glycosyltransferase          |
| lldRr1    | 505        | CK936_34450 (PAU44521), [S. albireticulat]; (75/60) | ?                    | MFS transporter                       |
| lldM2     | 339        | TnxM4 (AKT74307), [S. bottropensis DO-45]; (81/69) | TnxM4 (81/69)        | O-Methyltrasferase                    |
| lldB5     | 423        | TnxB12 (AKT74304), [S. bottropensis DO-45]; (75/65) | TnxB12 (75/65)       | GTB-type glycosyltransferase          |
| lldB6     | 391        | TnxB11 (AKT74303), [S. bottropensis DO-45]; (74/63) | TnxB11 (74/63)       | O-Acyltrasferase                     |
| lldO1     | 168        | ACZ90_22755 (KUJ68064), [S. albicat]; (76/69) | ?                    | VOC-family, extradiol dioxygenase     |
| lldU1     | 390        | PA111_01900 (EHN12885), *Patulibacter medicamentivorans*; (89/81) | TnxU4 (82/71)        | HTH-42 superfamily, unknown          |
| lldU2     | 126        | Hypothetical protein (WP_030583115), [S. sclerotialus]; (74/57) | TnxU3 (67/51)        | Unknown                               |
| lldO2     | 400        | TnxO12 (AKT74306), [S. bottropensis DO-45]; (81/72) | TnxO12 (81/72)       | Cytochrome P450                       |
| lldO3     | 240        | TnxC4 (AKT74300), [S. bottropensis DO-45]; (77/69) | TnxC4 (77/69)        | Ketoreductase                         |
| lldM3     | 357        | MetLA2 (AAT45283), [S. tuberkidicus]; (81/71) | TnxM2 (79/70)        | O-Methyltrasferase                    |
| lldM4     | 348        | MetLA1 (AAT45282), [S. tuberkidicus]; (85/79) | ?                    | O-Methyltrasferase                    |
| lldU3     | 143        | TnxO11 (AKT74297), [S. bottropensis DO-45]; (86/78) | TnxO11 (86/78)       | NTF2-like superfamily, unknown       |
| lldC1  | 306 | TnxC3 (AKT74295), [S. bottropensis DO-45]; (87/84) | TnxC3 (87/84) | 2,3-Cyclase |
| lldO4  | 187 | TnxO10 (AKT74294), [S. bottropensis DO-45]; (83/77) | TnxO10 (83/77) | Flavin reductase |
| lldC2  | 261 | TnxC2 (AKT74293), [S. bottropensis DO-45]; (93/89) | TnxC2 (93/89) | Ketoreductase |
| lldC3  | 154 | TnxO9 (AKT74292), [S. bottropensis DO-45]; (91/88) | TnxO9 (91/88) | Pyrone synthase |
| lldO5  | 373 | TnxO8 (AKT74291), [S. bottropensis DO-45]; (86/81) | TnxO8 (86/81) | Hydroxylase |
| lldU4  | 175 | TnxO7 (AKT74290), [S. bottropensis DO-45]; (95/90) | TnxO7 (95/90) | NTF2-like superfamily, unknown |
| lldO6  | 421 | TnxO6 (AKT74289), [S. bottropensis DO-45]; (95/92) | TnxO6 (95/92) | Cytochrome P450 |
| lldRg2 | 341 | TnxRg5 (AKT74288), [S. bottropensis DO-45]; (88/84) | TnxRg5 (88/84) | YaY-family regulator |
| lldRr2 | 471 | TnxRr2 (AKT74287), [S. bottropensis DO-45]; (95/89) | TnxRr2 (95/89) | MFS transporter |
| lldB7  | 322 | TnxB8 (AKT74286), [S. bottropensis DO-45]; (88/83) | TnxB8 (88/83) | dTDP-hexose 3-ketoreductase |
| lldB8  | 485 | TnxB7 (AKT74285), [S. bottropensis DO-45]; (88/83) | TnxB7 (88/83) | dTDP-hexose 2,3-dehydratase |
| lldH1  | 373 | TnxH2 (AKT74281), [S. bottropensis DO-45]; (91/86) | TnxH2 (91/86) | Epoxide hydrolase |
| lldO7  | 409 | TnxO5 (AKT74280), [S. bottropensis DO-45]; (97/90) | TnxO5 (97/90) | Cytochrome P450 |
| lldH2  | 494 | TnxH1 (AKT74279), [S. bottropensis DO-45]; (90/86) | TnxH1 (90/86) | α/β-Hydrolase |
| lldO8  | 107 | TnxO4 (AKT74278), [S. bottropensis DO-45]; (98/95) | TnxO4 (98/95) | Ferredoxin |
| lldO9  | 101 | TnxO3 (AKT74277), [S. bottropensis DO-45]; (98/95) | TnxO4-C_terminal | Ferredoxin reductase (only 1/4) |
| lldU5  | 370 | TnxU2 (AKT74276), [S. bottropensis DO-45]; (90/83) | TnxU2 (90/83) | HTH-42 superfamily, unknown |
| lldO10 | 408 | TnxO2 (AKT74275), [S. bottropensis DO-45]; (94/91) | TnxO2 (94/91) | Cytochrome P450 |
| lldU6  | 122 | TnxU1 (AKT74274), [S. bottropensis DO-45]; (85/82) | TnxU1 (85/82) | Unknown |
| lldO11 | 345 | TnxO1 (AKT74273), [S. bottropensis DO-45]; (91/87) | TnxO1 (91/87) | NADP+-dependent oxidoreductase (ER) |
| lldRr3 | 457 | TnxRr1 (AKT74272), [S. bottropensis DO-45]; (76/68) | TnxRr1 (76/68) | MFS transporter |
| lldO12 | 260 | TnxP4 (AKT74271), [S. bottropensis DO-45]; (92/86) | TnxP4 (92/86) | NAD(P)+-dependent oxidoreductase(SDR) |
| lldA4  | 411 | TxnP3 (AKT74270), [S. bottropensis DO-45]; (85/80) | TtxnP3 (85/80) | CoA transferase/carnitine dehydratase |
| lldA5  | 543 | TxnP2 (AKT74269), [S. bottropensis DO-45]; (88/84) | TtxnP2 (88/84) | 2-Isopropylmalate/Citramalate synthase |
| lldA6  | 346 | TxnA5 (AKT74268), [S. bottropensis DO-45]; (93/90) | TtxnA5 (93/90) | KS-III (S-H-H) |
| lldC4  | 318 | TxnC1 (AKT74267), [S. bottropensis DO-45]; (90/83) | TtxnC1 (90/83) | 1-Cyclase/aromatase |
| lldA7  | 590 | TxnP1 (AKT74266), [S. bottropensis DO-45]; (84/75) | TtxnP1 (84/75) | ATP-dependent CoA synthase |
| lldRg3 | 203 | TxnRg4 (AKT74265), [S. bottropensis DO-45]; (97/91) | TtxnRg4 (97/91) | 2-Component regulator (MerR-family) |
| lldRg4 | 393 | TxnRg3 (AKT74264), [S. bottropensis DO-45]; (80/71) | TtxnRg3 (80/71) | 2-Component kinase |
| lldRg5 | 292 | TxnRg2 (AKT74263), [S. bottropensis DO-45]; (94/91) | TtxnRg2 (94/91) | SARP-family regulator |
| lldA1  | 420 | TxnA1 (AKT74262), [S. bottropensis DO-45]; (96/92) | TtxnA1 (96/92) | KSα |
| lldA2  | 406 | TxnA2 (AKT74261), [S. bottropensis DO-45]; (92/87) | TtxnA2 (92/87) | KSβ |
| lldA3  | 89  | TxnA3 (AKT74260), [S. bottropensis DO-45]; (94/90) | TtxnA3 (94/90) | ACP |
| lldA8  | 564 | TxnA4 (AKT74259), [S. bottropensis DO-45]; (82/77) | TtxnA4 (82/77) | MAT |
| lldB9  | 115 | TxnB4-C(231-345) (AKT74258), [S. bottropensis DO-45]; (97/96) | TtxnB4-C-terminal | Pyruvate dehydrogenase-β (only 1/3) |
| lldB10 | 110 | TxnB3-N(1-104) (AKT74257), [S. bottropensis DO-45]; (87/80) | TtxnB3-N-terminal | Pyruvate dehydrogenase-α (only 1/3) |
| lldB11 | 292 | TxnB2 (AKT74256), [S. bottropensis DO-45]; (94/90) | TtxnB2 (94/90) | Glucose-1-P thymidylyltransferase |
| lldB12 | 331 | TxnB1 (AKT74255), [S. bottropensis DO-45]; (94/90) | TtxnB1 (94/90) | dTDP-glucose 4,6-dehydratase |
| orf(+1)| 334 | IQ63_37240 (KND26343), [S. acidiscabies]; (90/81) | / | Hydroxymethylbilane synthase |

The sequence of LL-D49194 biosynthetic gene cluster has been deposited into GenBank under accession no. MK501817.
Table S4. NMR data of 7

| Position | $^1$C δ(ppm) | $^1$H δ(ppm) | Intensity | Multiplicity | HMBC Correlation | COSY Correlation | NOESY Correlation |
|----------|---------------|---------------|-----------|--------------|------------------|------------------|-------------------|
| 1        | 203.4         | 4.94          | 1H        | d (12.8, 5.5 Hz) | 1, 3             | H-3α             | H-3α              |
| 2        | 67.8          | 2.74(m)       | 1H        | m            | 1, 2, 4, 4a       | H-2, H-4         | H-2               |
| 3        | 37.1          | 2.18(β)       | 1H        | m            | 1, 2             | H-4              |                   |
| 4        | 62.0          | 5.47          | 1H        | brs          | 2, 9a            | H-3α, H-3β       | H-3β, H-10-OMe    |
| 4a       | 129.6         | 7.47          | 1H        | s            | 6-Me, 8a, 10      | H-6-Me, H-10-OMe |                   |
| 5        | 116.5         | 7.47          | 1H        | s            |                   |                   |                   |
| 6        | 114.8         | 7.47          | 1H        | s            |                   |                   |                   |
| 7        | 142.9         | 7.47          | 1H        | s            |                   |                   |                   |
| 8        | 151.7         | 7.47          | 1H        | s            |                   |                   |                   |
| 8a       | 114.8         | 7.47          | 1H        | s            |                   |                   |                   |
| 9        | 162.6         | 7.47          | 1H        | s            |                   |                   |                   |
| 9a       | 107.3         | 7.47          | 1H        | s            |                   |                   |                   |
| 10       | 144.5         | 7.47          | 1H        | s            |                   |                   |                   |
| 10a      | 135.9         | 7.47          | 1H        | s            |                   |                   |                   |
| 11       | 69.3          | 5.17          | 1H        | d (4.1 Hz)   | 7, 8, 12, 13      | H-12             | H-6-Me, H-12      |
| 12       | 71.4          | 4.84          | 1H        | d (4.1 Hz)   | 14, 15            | H-11             | H-11              |
| 13       | 102.5         | 4.84          | 1H        | d (4.1 Hz)   | 14, 15            | H-11             |                   |
| 14       | 69.2          | 4.84          | 1H        | d (4.1 Hz)   | 14, 15            | H-11             |                   |
| 15       | 104.4         | 4.84          | 1H        | d (4.1 Hz)   | 14, 15            | H-11             |                   |
| 16       | 94.5          | 5.14          | 1H        | s            | 16-OMe, 1'        |                   |                   |
| 17       | 48.0          | 2.86          | 2H        | d (13.9, 5.5 Hz) | 13, 14          |                   |                   |
| 1'       | 95.1          | 5.38          | 1H        | d (3.5 Hz)   | 16, 2', 5'        | H-2''            | H-16-OMe, H-2'', H-2β |
| 2'       | 34.7          | 2.02(α)       | 1H        | d (14.8, 3.8 Hz) | 1', 4'          | H-1'             |                   |
| 3'       | 70.0          | 2.02(α)       | 1H        | d (14.8 Hz)  | 1', 4'           | H-1'             |                   |
| 4'       | 74.6          | 3.22          | 1H        | d (6.8 Hz)   | 2', 3', 5', 6', 7' | H-2β             | H-7              |
| 5'       | 63.7          | 4.58          | 1H        | d (12.6, 6.1 Hz) | 1', 4', 6'      | H-6'             |                   |
| 6'       | 16.6          | 1.32          | 3H        | d (6.6 Hz)   | 4', 5'           | H-5'             |                   |
| 7        | 26.2          | 1.30          | 3H        | s            | 2', 4'           | H-4'             |                   |
| 6-Me     | 20.5          | 2.61          | 3H        | s            | 5, 6, 7         | H-5, H-11        |                   |
| 10-OMe   | 63.0          | 3.93          | 3H        | s            | 10              | H-4, H-5         |                   |
| 13-OMe   | 53.0          | 3.74          | 3H        | s            | 13              |                   |                   |
| 16-OMe   | 59.0          | 3.52          | 3H        | s            | 16              | H-1'             |                   |
| 9-OH     | 13.94         | 1H            | s         | 8a, 9, 9a    |                   |                   |                   |

$^1$H NMR: 600 MHz, $^1$C NMR: 150 MHz (in CDCl$_3$)
### Table S5. NMR data of 8

| position | $^{13}$C | $^1$H | multiplicity   |
|----------|---------|------|--------------|
|          | $\delta$(ppm) | $\delta$(ppm) | intensity |
| 1        | 203.0   |       |              |
| 2        | 68.1    | 4.78 | 1H dd (12.7, 5.6 Hz) |
| 3        | 36.8    | 2.82(α) | 1H m |
|          |         | 2.21(β) | 1H m |
| 4        | 67.6    | 5.39 | 1H t |
| 4a       | 126.7   |       |              |
| 5        | 116.8   | 7.50 | 1H s |
| 6        | 115.1   |       |              |
| 7        | 143.1   |       |              |
| 8        | 151.8   |       |              |
| 8a       | 115.0   |       |              |
| 9        | 163.4   |       |              |
| 9a       | 107.5   |       |              |
| 10       | 145.0   |       |              |
| 10a      | 135.7   |       |              |
| 11       | 69.3    | 5.17 | 1H d (4.1 Hz) |
| 12       | 71.3    | 4.85 | 1H d (4.0 Hz) |
| 13       | 102.5   |       |              |
| 14       | 69.1    |       |              |
| 15       | 104.5   |       |              |
| 16       | 94.5    | 5.14 | 1H s |
| 17       | 48.0    | 2.87 | 2H dd (13.1, 5.5 Hz) |
| 1'       | 95.1    | 5.37 | 1H d (3.4 Hz) |
| 2'       | 34.7    | 2.02(α) | 1H dd (14.7, 3.8 Hz) |
|          |         | 1.85(β) | 1H d (14.7 Hz) |
| 3'       | 70.0    |       |              |
| 4'       | 74.6    | 3.21 | 1H m |
| 5'       | 63.7    | 4.54 | 1H dd (12.7, 6.0 Hz) |
| 6'       | 16.9    | 1.31 | 3H d (6.5 Hz) |
| 7'       | 26.2    | 1.30 | 3H s |
| 1''      | 98.2    | 5.32 | 1H d (3.9 Hz) |
| 2''      | 36.0    | 1.95(α) | 1H dd (14.9, 4.3 Hz) |
|          |         | 1.56(β) | 1H s |
| 3''      | 70.2    |       |              |
| 4''      | 74.6    | 3.21 | 1H m |
| 5''      | 63.7    | 4.57 | 1H dd (13.2, 6.6 Hz) |
| 6''      | 17.0    | 1.38 | 3H d (6.6 Hz) |
| 7''      | 26.2    | 1.21 | 3H s |
| 6-Me     | 20.5    | 2.60 | 3H s |
| 10-OMe   | 62.9    | 3.85 | 3H s |
| 13-OMe   | 53.0    | 3.74 | 3H s |
| 16-OMe   | 58.9    | 3.52 | 3H s |
| 9-OH     | 14.16   | 1H s |

$^1$H NMR: 600 MHz, $^{13}$C NMR: 150 MHz (in CDCl$_3$)
Table S6. NMR data of 9

| position | $^{13}$C δ(ppm) | $^1$H δ(ppm) | intensity | multiplicity | HMBC correlation | COSY correlation | NOESY correlation |
|----------|------------------|--------------|-----------|--------------|------------------|------------------|------------------|
| 1        | 203.5            | 5.06         | 1 H       | dd (12.2 Hz) | 1, 3             | H-3a, H-3β       |                  |
| 2        | 67.8             | 5.74         | 1 H       | dd (11.3 Hz) | 1, 2, 3, 4a      | H-2, H-4         |                  |
| 3        | 33.7             | 4.83         | 1 H       | dd (3.9 Hz)  | 14, 15           | H-11             | H-13-OMe         |
| 4        | 129.6            | 7.45         | 1 H       | s            | 6-Me, 8a, 10     | H-13-OMe         |                  |
| 5        | 116.4            | 5.15         | 1 H       | d (4.0 Hz)   | 7, 8, 12, 13     | H-12             |                  |
| 6        | 144.8            | 114.8        |           |              |                  |                  |                  |
| 7        | 142.8            | 114.8        |           |              |                  |                  |                  |
| 8        | 151.7            | 114.8        |           |              |                  |                  |                  |
| 8a       | 162.6            | 107.3        |           |              |                  |                  |                  |
| 9        | 144.5            | 144.5        |           |              |                  |                  |                  |
| 10       | 104.4            | 69.1         |           |              |                  |                  |                  |
| 10a      | 135.9            | 94.3         | 1 H       | s            | 16-OMe, 1         | H-16-OMe         |                  |
| 11       | 94.3             | 5.11         | 1 H       | s            | 16-OMe, 1         | H-16-OMe         |                  |
| 12       | 69.3             | 4.83         | 1 H       | d (3.9 Hz)   | 14, 15           | H-11             |                  |
| 13       | 102.4            | 69.1         |           |              |                  |                  |                  |
| 14       | 104.4            | 69.1         |           |              |                  |                  |                  |
| 15       |                  |              |           |              |                  |                  |                  |
| 16       | 94.3             | 5.11         | 1 H       | s            | 16-OMe, 1         | H-16-OMe         |                  |
| 17       | 48.0             | 2.85         | 2 H       | dd (14.4 Hz) | 13, 14           |                  |                  |
| 1'       | 150.7            | 5.36         | 1 H       | dd (11.3 Hz) | 1, 2, 3, 4a      | H-2, H-4         |                  |
| 2'       | 35.7             | 2.01         | 1 H       | d (4.0 Hz)   | 1, 4             |                  |                  |
| 3'       | 69.3             |              |           |              |                  |                  |                  |
| 4'       | 84.1             | 3.30         | 1 H       | s            | 2', 3', 5', 6', 7', 1' | H-6'             |                  |
| 5'       | 64.0             | 4.50         | 1 H       | dd (12.8 Hz) | 1', 4', 6'       | H-6'             | H-9-OH, H-6'     |
| 6'       | 17.8             | 1.28         | 3 H       | d (6.9 Hz)   | 4', 5'           |                  | H-4', H-5'       |
| 7'       | 26.8             | 1.32         | 3 H       | s            | 2', 4'           |                  |                  |
| 1''      | 101.2            | 4.99         | 1 H       | d (3.7 Hz)   | 4', 2', 5'       | H-2''            |                  |
| 2''      | 35.7             | 1.99         | 1 H       | d (3.9 Hz)   | 1', 3'           | H-1''            |                  |
| 3''      | 10.2             | 70.2         |           |              |                  |                  |                  |
| 4''      | 74.4             | 3.17         | 1 H       | s            | 2', 3', 5', 6', 7', 1' | H-6'             |                  |
| 5''      | 64.0             | 4.54         | 1 H       | dd (12.8 Hz) | 1', 4', 6'       | H-6'             |                  |
| 6''      | 16.6             | 1.22         | 3 H       | d (6.5 Hz)   | 4', 5'           |                  |                  |
| 7''      | 26.3             | 1.27         | 3 H       | s            | 2', 4'           |                  |                  |
| 6-Me     | 20.5             | 2.59         | 3 H       | s            | 5, 6, 7         |                  |                  |
| 10-OMe   | 63.0             | 3.92         | 3 H       | s            | 10             |                  | H-4             |
| 13-OMe   | 53.0             | 3.73         | 3 H       | s            | 13             | H-13-OMe         | H-12, H-9-OH    |
| 16-OMe   | 59.1             | 3.50         | 3 H       | s            | 16             |                  | H-16           |

$^1$H NMR: 600 MHz, $^{13}$C NMR: 150 MHz (in CDCl₃)
Table S7. NMR data of 5

| position | $^1$H δ(ppm) | $^{13}$C δ(ppm) | intensity | multiplicity | HMBC correlation | COSY correlation | NOESY correlation |
|----------|--------------|-----------------|-----------|--------------|------------------|------------------|-------------------|
| 1        | 202.0        |                 |           |              |                  |                 |                   |
| 2        | 36.8         | 2H t (7.1 Hz)   | 1, 4, 9a  | H-3n, H-3β   |                  |                 |                   |
| 3        | 39.5         | 2H t (7.0 Hz)   | 1, 4, 4a  | H-2n, H-2β   |                  |                 |                   |
| 4        | 195.0        |                 |           |              |                  |                 |                   |
| 5        | 118.5        | 7.76 H         | s         | 6-Me, 8a, 10 | H-6-Me, H-10-Ome|                 |                   |
| 6        | 116.7        |                 |           |              |                  |                 |                   |
| 7        | 142.9        |                 |           |              |                  |                 |                   |
| 8        | 151.5        |                 |           |              |                  |                 |                   |
| 8a       | 117.0        |                 |           |              |                  |                 |                   |
| 9        | 162.7        |                 |           |              |                  |                 |                   |
| 9a       | 110.3        |                 |           |              |                  |                 |                   |
| 10       | 147.5        |                 |           |              |                  |                 |                   |
| 10a      | 136.5        |                 |           |              |                  |                 |                   |
| 11       | 69.3         | 5.24 H         | d (4.0 Hz)| 7, 8, 12, 13 | H-12             | H-6-Me          |                   |
| 12       | 71.7         | 4.90 H         | d (4.0 Hz)| 14, 15       | H-11             | H-13-Ome        |                   |
| 13       | 102.4        |                 |           |              |                  |                 |                   |
| 14       | 69.2         |                 |           |              |                  |                 |                   |
| 15       | 103.8        |                 |           |              |                  |                 |                   |
| 16       | 88.7         | 5.38 H         | brs       | 14           | H-16-OH(1), H-16-OH(2)|                 |                   |
| 17       | 49.0         | 3.18(α) H      | d (5.3 Hz)| 13, 14       |                  | H-5, H-11       |                   |
|          | 2.96(β)      | 1H d (5.3 Hz)  |           | 13, 14       |                  |                 |                   |
| 6-Me     | 20.4         | 2.64 H         | s         | 5, 6, 7      | H-5, H-11        |                  |                   |
| 10-Ome   | 63.5         | 3.95 H         | s         | 10           |                  | H-5             |                   |
| 13-Ome   | 53.1         | 3.75 H         | s         | 13           |                  | H-12            |                   |
| 9-OH     | 15.02        | 1H s           |           | 8a, 9, 9a    |                  |                 |                   |
| 16-OH(1) | 3.83         | 1H brd         |           | 15, 16       | H-16             | H-16-OH(2)      |                   |
| 16-OH(2) | 3.50         | 1H brs         |           | 16           | H-16             | H-16-OH(1)      |                   |

$^1$H NMR: 600 MHz, $^{13}$C NMR: 150 MHz (in CDCl$_3$)
Table S8. NMR data of 11

| Position | $^{13}$C (δ(ppm)) | $^1$H (δ(ppm)) | Intensity | Multiplicity | HMBC Correlation | COSY Correlation | NOESY Correlation |
|----------|------------------|----------------|-----------|--------------|-----------------|-----------------|------------------|
| 1        | 203.4            | dd (12.8, 5.5 Hz) |           | 1, 3         | H-3α, H-3β      | H-3α            |                   |
| 2        | 67.8             | dd (2.8 Hz)     | 4.94      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 3        | 2.76(α)          | m              | 1, 2, 4, 4a| 1H           | H-2, H-4        | H-2, H-4        |                   |
| 4        | 62.0             | dd (12.8, 5.5 Hz) |           | 1, 3         | H-3α, H-3β      | H-3α            |                   |
| 4a       | 129.7            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 5        | 116.5            | dd (12.8, 5.5 Hz) |           | 1, 3         | H-3α, H-3β      | H-3α            |                   |
| 6        | 114.8            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 7        | 151.7            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 8        | 142.8            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 9        | 145.5            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 9a       | 135.9            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 10        | 139.3            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 10a      | 139.3            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 11       | 69.3             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 12       | 71.4             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 13       | 102.4            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 14       | 69.2             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 15       | 104.4            | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 16       | 94.6             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 17       | 48.0             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 1'       | 95.1             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 2'       | 29.3             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 3'       | 67.7             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 4'       | 70.8             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 5'       | 62.4             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 6'       | 16.6             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 6-Me     | 20.5             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 10-Me    | 63.0             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 13-Me    | 53.0             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |
| 16-Me    | 58.9             | dd (2.8 Hz)     | 2.18      | 1H           | H-2, H-4        | H-2, H-4        |                   |

$^1$H NMR: 600 MHz, $^{13}$C NMR: 150 MHz (in CDCl$_3$)
Table S9. NMR data of 12

| position | \(^{13}C\) δ (ppm) | \(^{1}H\) δ (ppm) | intensity | multiplicity | HMBC correlation | COSY correlation | NOESY correlation |
|----------|--------------------|------------------|------------|---------------|-----------------|-----------------|------------------|
| 1        | 203.4              |                  |            |               |                 |                 |                  |
| 2        | 67.8               | 4.94             | 1H         | dd (12.8, 5.5 Hz) | 1, 3            | H-3a, H-3β       | H-3a             |
| 3        | 37.1               | 2.76(a)          | 1H         | m             | 1, 2, 4, 4a     | H-2, H-4        | H-2, H-4         |
| 4        | 2.18(b)            | 5.46             | 1H         | t             | 2, 9a           | H-3a, H-3β      | H-3a, H-2a, H-2'a, H-3' |
| 4a       |                    |                  |            |               |                 |                 |                  |
| 5        | 116.5              | 7.47             | 1H         | s             | 6-Me, 8a, 10    | H-6-Me, H-10-OMe |                  |
| 6        | 114.8              |                  |            |               |                 |                 |                  |
| 7        | 142.8              |                  |            |               |                 |                 |                  |
| 8        | 151.7              |                  |            |               |                 |                 |                  |
| 8a       | 114.8              |                  |            |               |                 |                 |                  |
| 9        | 162.6              |                  |            |               |                 |                 |                  |
| 9a       | 107.3              |                  |            |               |                 |                 |                  |
| 10       | 144.5              |                  |            |               |                 |                 |                  |
| 10a      | 135.9              |                  |            |               |                 |                 |                  |
| 11       | 69.3               | 5.17             | 1H         | d (4.1 Hz)    | 7, 8, 12, 13    | H-12            | H-6-Me, H-12     |
| 12       | 71.5               | 4.85             | 1H         | d (4.1 Hz)    | 14, 15          | H-11            | H-11, H-13-OMe   |
| 13       | 102.4              |                  |            |               |                 |                 |                  |
| 14       | 69.2               |                  |            |               |                 |                 |                  |
| 15       | 104.4              |                  |            |               |                 |                 |                  |
| 16       | 94.6               | 5.11             | 1H         | s             | 16-OMe, 1'      | H-16-OMe, H-5'  |                  |
| 17       | 48.0               | 2.86             | 2H         | dd (13.4, 5.5 Hz) | 13, 14        |                 |                  |
| 1'       | 94.8               | 5.38             | 1H         | brd (2.2 Hz)  | 16, 5', 2', 3' | H-2'a, H-2'β   | H-2'a, H-16-OMe, H-2'a |
| 2'       | 30.1               | 2.09(a)          | 1H         | m             | 1', 4'          | H-1', H-3'      | H-4, H-1'        |
| 3'       | 66.4               | 4.04             | 1H         | brs           | 1'             | H-2'a, H-2'β, H-4' |                  |
| 4'       | 79.8               | 3.47             | 1H         | brs           | 2', 5', 6', 1' | H-3', H-5'     | H-1''            |
| 5'       | 62.1               | 4.48             | 1H         | dd (12.7, 6.1 Hz) | 1', 3', 4', 6' | H-4', H-6'     | H-16, H-6'       |
| 6'       | 16.8               | 1.27             | 3H         | d (6.5 Hz)    | 4', 5'         | H-5''          | H-1''            |
| 1''      | 101.0              | 4.99             | 1H         | brd (3.5 Hz)  | 4', 2', 3'     | H-2'a, H-2'β    | H-6', H-2''β, H-4' |
| 2''      | 30.4               | 2.20(a)          | 1H         | m             | 1', 4'          | H-1', H-3'      | H-4', H-1'       |
| 3''      | 67.7               | 3.94             | 1H         | brd (3.5 Hz)  | 1', 5'         | H-2'a, H-2'β    |                   |
| 4''      | 70.7               | 3.50             | 1H         | brd (3.3 Hz)  | 2', 5', 6''    | H-3', H-5'     |                   |
| 5''      | 62.5               | 4.30             | 1H         | dd (13.2, 6.5 Hz) | 1', 3', 4', 6'' | H-4', H-6'     |                   |
| 6''      | 16.4               | 1.25             | 3H         | d (6.7 Hz)    | 4', 5''        | H-5'''         | H-5'''           |
| 6-Me     | 20.5               | 2.61             | 3H         | s             | 5, 6, 7        | H-5, H-11      |                  |
| 10-OMe   | 63.0               | 3.93             | 3H         | s             | 10            | H-5           |                  |
| 13-OMe   | 53.0               | 3.75             | 3H         | s             | 13            | H-12          |                  |
| 16-OMe   | 59.1               | 3.51             | 3H         | s             | 16            | H-1', H-16     |                  |
| 9-OH     | 13.94              | 1.14             | 1H         | s             | 8a, 9, 9a      |                |                  |

\(^1H\) NMR: 600 MHz, \(^{13}C\) NMR: 150 MHz (in CDCl\(_3\))
Table S10. NMR data of 10

| position | $^{13}$C δ(ppm) | $^1$H δ(ppm) | intensity | multiplicity | HMBC correlation | COSY correlation | NOESY correlation |
|----------|-----------------|--------------|-----------|--------------|------------------|------------------|--------------------|
| 1        | 202.0           |              |           | m            | 1, 4, 9a         |                  | H-3                |
| 2        | 36.8            | 3.11         | 2H        | m            | 1, 4, 9a         |                  | H-2                |
| 3        | 39.5            | 3.01         | 2H        | m            | 1, 4, 9a         |                  | H-3                |
| 4        | 195.0           |              |           |              |                  |                  |                    |
| 4a       | 121.7           |              |           |              |                  |                  |                    |
| 5        | 118.4           | 7.74         | 1H        | s            | 6-Me, 8a, 10     | H-10-OMe         |                    |
| 6        | 116.9           |              |           |              |                  |                  |                    |
| 7        | 143.1           |              |           |              |                  |                  |                    |
| 8        | 151.5           |              |           |              |                  |                  |                    |
| 8a       | 116.9           |              |           |              |                  |                  |                    |
| 9        | 162.7           |              |           |              |                  |                  |                    |
| 9a       | 110.2           |              |           |              |                  |                  |                    |
| 10       | 147.5           |              |           |              |                  |                  |                    |
| 10a      | 136.5           |              |           |              |                  |                  |                    |
| 11       | 69.2            | 5.17         | 1H        | d (4.1 Hz)   | 7, 8, 12, 13    | H-12             | H-6-Me, H-12       |
| 12       | 71.4            | 4.84         | 1H        | d (4.1 Hz)   | 14, 15           | H-11             | H-11, H-13-OMe     |
| 13       | 102.5           |              |           |              |                  |                  |                    |
| 14       | 69.1            |              |           |              |                  |                  |                    |
| 15       | 104.4           |              |           |              |                  |                  |                    |
| 16       | 94.4            | 5.11         | 1H        | s            | 16-OMe, 1'      | H-16-OMe         |                    |
| 17       | 48.0            | 2.88(a)      | 1H        | d (5.9 Hz)   | 14               |                  |                    |
| 17’      | 95.0            | 5.36         | 1H        | m            | 16, 3', 5'      | H-2'α, H-2'β     | H-16-OMe, H-2'β   |
| 2’       | 35.7            | 2.02(a)      | 1H        | dd (14.6, 4.2 Hz) | 1', 3'         | H-1'             |                    |
| 3’       | 69.3            |              |           |              |                  |                  |                    |
| 4’       | 84.1            | 3.31         | 1H        | s            | 3', 5', 6', 7', 1’ | H-5', H-1”      |                    |
| 5’       | 64.0            | 4.52         | 1H        | dd (13.4, 6.7Hz) | 1', 4', 6'    | H-6’            | H-4’, H-6’         |
| 6’       | 17.8            | 1.29         | 3H        | d (6.1 Hz)   | 4', 5'          | H-5’            | H-5’               |
| 7’       | 26.8            | 1.33         | 3H        | s            | 2', 3', 4'      | H-1”            |                    |
| 1’*      | 101.2           | 5.00         | 1H        | d (3.8 Hz)   | 4', 3', 5'      | H-2”α, H-2”β    | H-4’, H-7’, H-2”α |
| 2’*      | 35.7            | 1.91(a)      | 1H        | brs          | 1’, 3’, 4’      | H-1”            | H-1”               |
| 3’*      | 70.2            |              |           |              |                  |                  |                    |
| 4’*      | 74.5            | 3.18         | 1H        | s            |                  |                  |                    |
| 5’*      | 64.0            | 4.55         | 1H        | dd (13.1, 6.5 Hz) | 1’, 4’, 6’    | H-6”            | H-6”               |
| 6’*      | 16.6            | 1.23         | 3H        | d (6.6 Hz)   | 4’, 5’          | H-5”            | H-5”               |
| 7’*      | 26.3            | 1.28         | 3H        | s            | 2’, 3’, 4’      | H-1”            |                    |
| 6-Me     | 20.5            | 2.62         | 3H        | s            | 5, 6, 7         | H-11            |                    |
| 10-OMe   | 63.5            | 3.95         | 3H        | s            | 10              | H-5             |                    |
| 13-OMe   | 53.0            | 3.73         | 3H        | s            | 13              | H-12            |                    |
| 16-OMe   | 59.1            | 3.50         | 3H        | s            | 16              | H-16, H-1’      |                    |
| 9-OH     | 15.03           | 1H           | s         | 8a, 9, 9a     |                  |                  |                    |

$^1$H NMR: 600 MHz, $^{13}$C NMR: 150 MHz (in CDCl$_3$)
Table S11. NMR data of 4

| position | $^{13}$C δ(ppm) | $^{1}$H δ(ppm) | intensity | multiplicity | HMBC correlation | COSY correlation |
|----------|-----------------|----------------|-----------|--------------|------------------|------------------|
| 1        | 201.5           |                |           |              |                  |                  |
| 2        | 35.9            | 3.06           | 2H        | s            | 3, 4, 9a         | H-3α, H-3β       |
| 3        | 36.2            | 3.06           | 2H        | s            | 1, 2, 4a         | H-2α, H-2β       |
| 4        | 200.5           |                |           |              |                  |                  |
| 4a       | 108.8           |                |           |              |                  |                  |
| 5        | 119.0           | 7.98           | 1H        | s            | 8a, 10, 6-Me     |                  |
| 6        | 119.0           |                |           |              |                  |                  |
| 7        | 142.5           |                |           |              |                  |                  |
| 8        | 151.2           |                |           |              |                  |                  |
| 8a       | 117.7           |                |           |              |                  |                  |
| 9        | 158.7           |                |           |              |                  |                  |
| 9a       | 107.6           |                |           |              |                  |                  |
| 10       | 153.5           |                |           |              |                  |                  |
| 10a      | 132.4           |                |           |              |                  |                  |
| 11       | 69.1            | 5.22           | 1H        | d (4.2 Hz)   | 8, 13            | H-12             |
| 12       | 71.9            | 4.89           | 1H        | d (4.2 Hz)   | 14, 15           | H-11             |
| 13       | 102.4           |                |           |              |                  |                  |
| 14       | 69.1            |                |           |              |                  |                  |
| 15       | 105.4           |                |           |              |                  |                  |
| 16       | 68.2            | 3.96(α)        | 1H        | d (12.6 Hz)  | 15               |                  |
|          |                 | 3.83(β)        | 1H        | d (12.6 Hz)  |                  |                  |
| 17       | 48.0            | 2.93(α)        | 1H        | d (4.2 Hz)   | 14               |                  |
|          |                 | 2.77(β)        | 1H        | d (4.2 Hz)   | 14               |                  |
| 6-Me     | 20.1            | 2.63           | 3H        | s            | 5, 7             |                  |
| 13-O Me  | 53.1            | 3.76           | 3H        | s            | 13               |                  |
| 9-OH     | 14.46           | 1H             | s         |              | 9, 8a, 9a        |                  |
| 10-OH    | 13.47           | 1H             | s         | 10, 4a, 10a  |                  |                  |

$^{1}$H NMR: 600MHz, $^{13}$C NMR: 150 MHz (in CDCl$_3$)
| Compound | 1  | 2  | 4  | 5  | 7  | 8  | 9  | 10 | 11 | 12 |
|----------|----|----|----|----|----|----|----|----|----|----|
| Molecular Weight: | 993 | 951 | 456.4 | 486.4 | 662.6 | 806.8 | 806.8 | 788.8 | 648.6 | 778.8 |
| No. of Hydrogen Bond Donors: | 6  | 7  | 3  | 3  | 5  | 6  | 6  | 4  | 5  | 6  |
| No. of Hydrogen Bond Acceptors: | 8  | 8  | 5  | 5  | 6  | 7  | 7  | 6  | 6  | 7  |
| No. of Rotatable Bonds: | 18 | 17 | 5  | 6  | 11 | 14 | 14 | 12 | 11 | 14 |
| No. of Nitrogen and Oxygen Atoms: | 22 | 21 | 10 | 11 | 15 | 18 | 18 | 17 | 15 | 18 |
| No. of Rings: | 10 | 10 | 7  | 7  | 8  | 9  | 9  | 8  | 8  | 9  |
| ClogP | 0.46 | -0.11 | 0.66 | -0.09 | -0.24 | -0.17 | -0.17 | 0.58 | -0.42 | -0.54 |
Figure S1. Construction and verification of S. vinaceusdrappus mutants.

(a) Schematic diagram of gene replacement

(b) All of S. vinaceusdrappus mutants

(c) PCR verification of S. vinaceusdrappus mutants

Genomic DNA from both the S. vinaceusdrappus wild type and mutant strains S. vinaceusdrappus TG5019 (ΔlldA1), TG5020 (ΔlldM1), TG5021 (ΔlldB3), TG5022 (ΔlldB4), TG5023 (ΔlldM2), TG5024 (ΔlldB5), TG5025 (ΔlldO2), TG5026 (ΔlldM3), TG5027 (ΔlldM4), TG5028 (ΔlldO6), TG5029 (ΔlldO7) and TG5030 (ΔlldO10) were extracted and tested by PCR analysis, respectively. A larger gene fragment (larger than 1.5 kb) can be amplified from wild type strain with all PCR testing primers, respectively. Meanwhile a smaller gene fragment (approximately 0.6 -1.0 kb) can be amplified from mutant strains with PCR testing primers, respectively. Lane 1, DNA marker; Lane 2, Mutant strain; Lane 3, WT strain.
Figure S2. $^1$H NMR spectrum of LL-D49194α1 (1)

Figure S3. $^{13}$C NMR spectrum of LL-D49194α1 (1)
Figure S4. $^1$H NMR spectrum of LL-D4919β2 (2)

Figure S5. $^{13}$C NMR spectrum of LL-D4919β2 (2)
Figure S6. $^1$H NMR spectrum of 7

Figure S7. $^{13}$C NMR spectrum of 7
Figure S8. $^1$H-$^1$H COSY spectrum of 7

Figure S9. HMQC spectrum of 7
Figure S10. HMBC spectrum of 7

Figure S11. NOESY spectrum of 7
Figure S12. $^1$H NMR spectrum of 8

Figure S13. $^{13}$C NMR spectrum of 8
Figure S14. $^1$H NMR spectrum of 9

Figure S15. $^{13}$C NMR spectrum of 9
Figure S16. \(^1\)H-\(^1\)H COSY spectrum of 9

Figure S17. HMQC spectrum of 9
Figure S18. HMBC spectrum of 9

Figure S19. NOESY spectrum of 9
Figure S20. $^1$H NMR spectrum of 5

Figure S21. $^{13}$C NMR spectrum of 5
Figure S22. $^1$H-$^1$H COSY spectrum of 5

Figure S23. HMQC spectrum of 5
Figure S24. HMBC spectrum of 5

Figure S25. NOESY spectrum of 5
Figure S26. $^1$H NMR spectrum of 11

Figure S27. $^{13}$C NMR spectrum of 11
Figure S28. $^1$H–$^1$H COSY spectrum of 11

Figure S29. HMQC spectrum of 11
Figure S30. HMBC spectrum of 11

Figure S31. NOESY spectrum of 11
Figure S32. $^1$H NMR spectrum of 12

Figure S33. $^{13}$C NMR spectrum of 12
Figure S34. $^1$H-$^1$H COSY spectrum of 12

Figure S35. HMQC spectrum of 12
Figure S36. HMBC spectrum of 12

Figure S37. NOESY spectrum of 12
Figure S38. $^1$H NMR spectrum of 10

Figure S39. $^{13}$C NMR spectrum of 10
Figure S40. $^1$H-$^1$H COSY spectrum of 10

Figure S41. HMQC spectrum of 10
Figure S42. HMBC spectrum of 10

Figure S43. NOESY spectrum of 10
Figure S44. $^1$H NMR spectrum of 4

Figure S45. $^{13}$C NMR spectrum of 4
Figure S46. $^1$H-$^1$H COSY spectrum of 4

Figure S47. HMQC spectrum of 4
**Figure S48.** HMBC spectrum of 4

**Figure S49.** NOESY spectrum of 4
Figure S50. Cell viability assays of LL-D4919α1 (1) and analogues

4-1 = compound 11; 4-2 = compound 12; 6-2 = compound 8; 9-1 = compound 7;
5-1 = compound 5; 14-1 = compound 10; 38-1 = compound 4; 9-2 = compound 9;
α1 = compound 1, LLD; β2 = compound 2.
Figure S51. Aqueous solubility assay of LL-D49194α1 (1), 4 and 5

Calibration curve for 1 (271 nm)
y = 1.0139x -0.0018, R²=0.9996
aq. Solubility = 0.45 ± 0.02 μmol/mL

Calibration curve for 5 (271 nm)
y = 1.5692x -0.0049, R²=0.9986
aq. Solubility = 2.86 ± 0.02 μmol/mL

Calibration curve for 4 (271 nm)
y = 1.3955x – 0.0002, R²=1
aq. Solubility = 0.38 ± 0.004 μmol/mL
Supplementary References

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