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Supporting Information

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Supporting Information

for

Combinatorial Mutasynthesis of Scrambled Beauvericins, Cyclooligomer Depsipeptide Cell Migration Inhibitors from *Beauveria bassiana*

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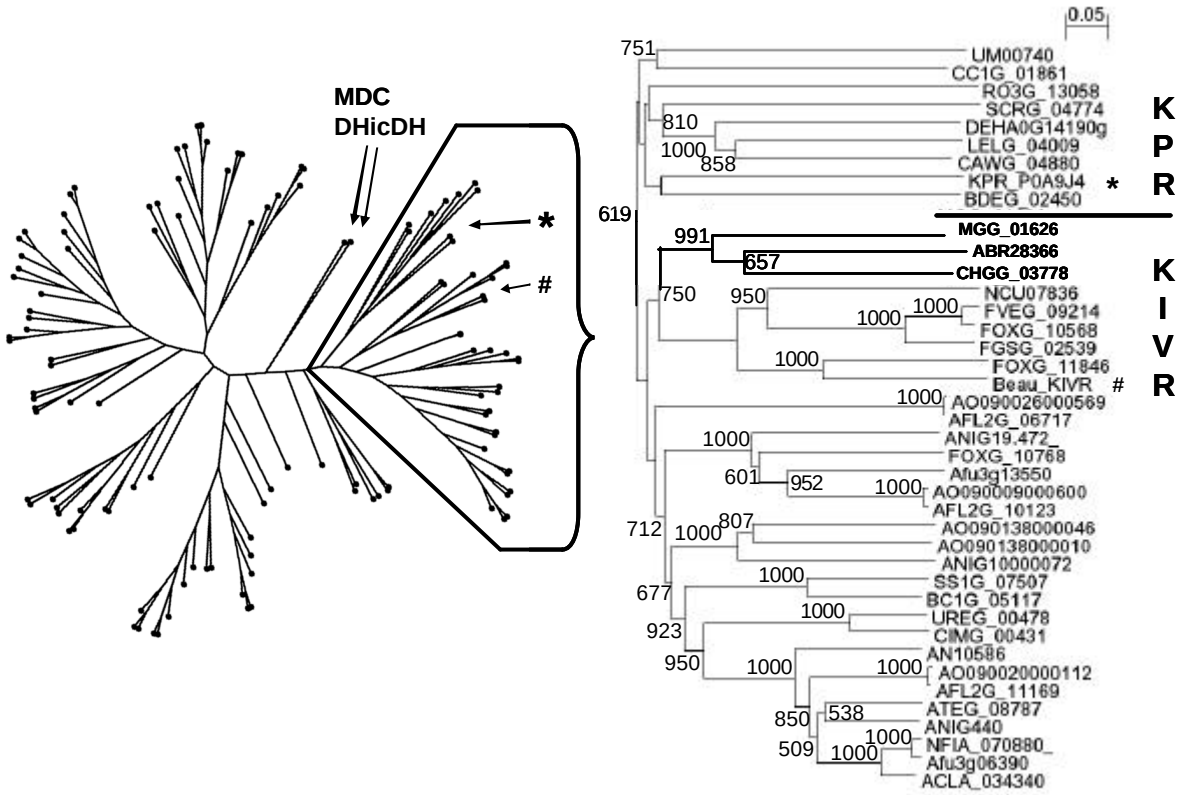


Figure S1. Phylogenomic Analysis of Fungal COG1893 Proteins. 118 deduced proteins annotated as "ketopantoate reductase" (COG1893) were collected from the currently (February 2008) available 30 fungal genome sequences at the Fungal Genome Initiative (FGI) website (http://www.broad.mit.edu/annotation/fungi/fgi/). Multiple sequence alignments of these sequences with the *B. bassiana* KIVR (Beau_KIVR, emphasized by a #) and the *E. coli* PanE ketopantoate reductase (KPR_P0A9J4, emphasized by a *) were generated in VectorNTI. Bootstrapped trees were calculated in CLUSTALX with the neighbor-joining method using 1000 repeats, and rooted with the *Lactobacillus delbrueckii* D-hydroxyisocaproate dehydrogenase (DHicDH, CAA46324) and the *Streptococcus bovis* malic enzyme (MDC, AAB07709) as outgroups. The radial tree of the full alignment (*left*) was drawn with Phylodraw 0.8 (http://pearl.cs.pusan.ac.kr/phylodraw/). The phylogram (*right*) of the indicated section of the tree was plotted with NJPlot (http://pbil.univ-lyon1.fr/software/njplot.html), with significant (>500) bootstrap values indicated near the forks. The scale shows the numbers of substitutions per site. Proposed clades for ketopantoate reductases and ketoisovalerate reductases are shown on the *right*. Sequences are labeled with the locus name, consisting of a letter code for the organism and a numerical identifier. Organism codes: ACLA, *Aspergillus clavatus*; AFL, *A. flavus*; Afu, *A. fumigatus*; AN, *A. nidulans*; ANIG, *A. niger*; AO, *A. oryzae*; ATEG, *A. terreus*; BC1G, *Botrytis cinerea*; BDEG, *Batrachochytrium dendrobatidis*; CAWG, *Candida albicans*; CC1G, *Coprinus cinereus*; CHGG, *Chaetomium globosum*; CIMG, *Coccidioides immitis*; DEHA, *Debaromyces hansenii*; FGSG, *Fusarium graminearum*; FOXG, *F. oxyspo-*

rum; FVEG, *F. verticilloides*; LELG, *Lodderomyces elongisporus*; MGG, *Magnaporthe grisea*; NCU, *Neurospora crassa*; NFIA, *Neosartorya fischeri*; RO3G, *Rhizopus oryzae*; SCRG, *Saccharomyces cerevisiae*; SS1G, *Sclerotinia sclerotiorum*; UM, *Ustillago maydis*; UREG, *Uncinocarpus reesii*.

2. SUPPLEMENTAL EXPERIMENTAL PROCEDURES

2.1. Construction of the *kivr* disruption vector: The *kivr* disruption cassette included two PCR-amplified DNA fragments covering the 5' and the 3' region of the *kivr* gene cloned in between the left and right borders of the T-DNA region of the pAg1-H3 vector [32] at the *Sma*I - *Xho*I and *Sph*I - *Hin*dIII sites, respectively. The 5' region of the *kivr* gene (EU886196, bp 9,233-11,194) was amplified as a *Stu*I - *Sal*I fragment, while the 3' region (bp 6,903-9,031) was amplified as a *Sph*I - *Hin*dIII fragment. Primers KIVR1 and 2 were used for the amplification of the 5' region of the *kivr* gene, and primers KIVR3 and 4 were used for the amplification of the 3' region of the *kivr* gene.

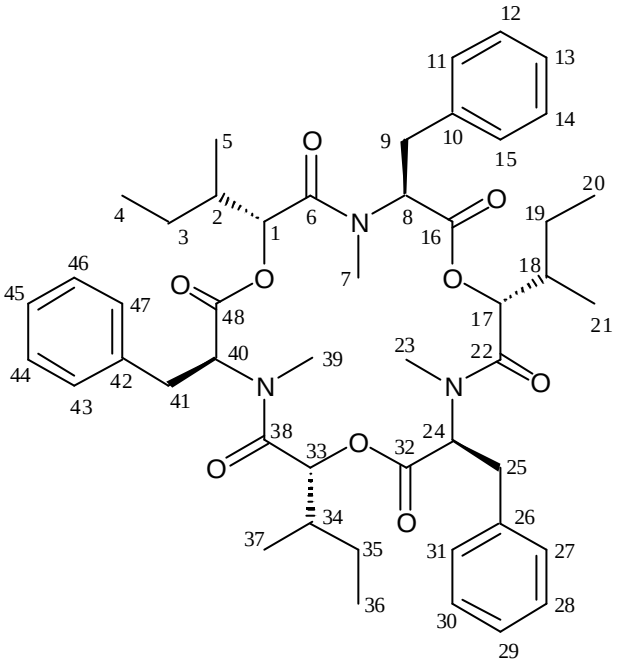
KIVR1: 5'-act**aggCCT**CGCCAAAAGAAC-3' *Stu*I
KIVR2: 5'-AGC**GTCGAC**AACCAGCTTG-3' *Sal*I
KIVR3: 5'-gct**gcaTGC**GAGCTACATGTC-3' *Sph*I
KIVR4: 5'-GTCTACACC**AAGCTT**AACGAGCC-3' *Hin*dIII

(*Small case*: sequences introduced by the primers. *Capital letters*: natural sequence from the *kivr* locus. *Bold*: restriction sites used for plasmid construction). The resulting construct was digested with *Bam*HI, the ends were filled in, and the 0.94-kb *Sma*I restriction fragment from pCB1524, containing the *bar* selectable marker gene, was inserted in between the fragments for the 5' and 3' *kivr* regions.

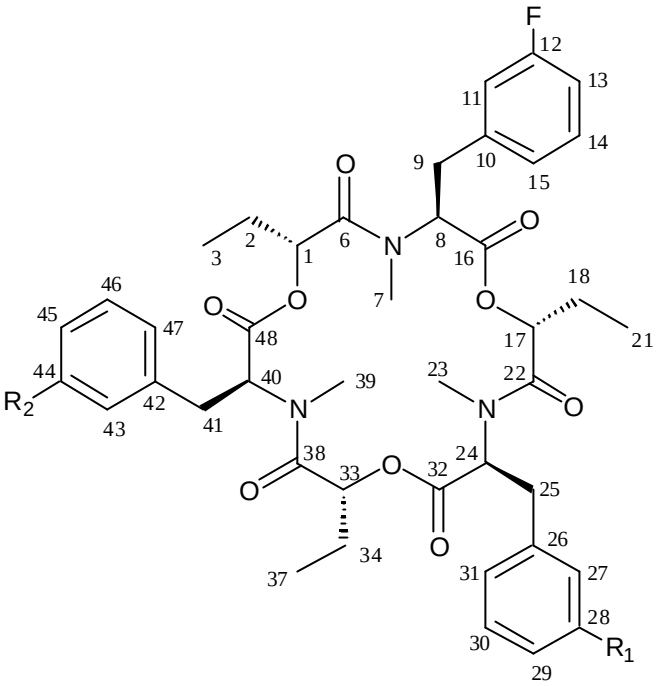
2.2. Isolation of genomic DNA from *B. bassiana*: Total DNA was isolated from *B. bassiana* by grinding 0.1 g of freeze-dried mycelia in a mixture (1:1, 3 mL) of Buffer HSE (Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] 10 mM, sucrose 0.5 mM, EDTA 20 mM, pH 6.9) and Tris-EDTA buffer (Tris [2-amino-2-(hydroxymethyl)-propane-1,3-diol] 50 mM, EDTA [Ethylene diamine tetracetic acid] 20 mM, pH 8.0) and incubating (64 °C, 30 min) after addition of SDS (10% sodium dodecyl sulfate, 150 µL) and proteinase K (20 mg mL^{-1}, 32 µL). After centrifugation (13,000 g, 10 min), the cleared supernatant was extracted with phenol:chloroform:isoamyl alcohol (25:24:1) followed by extraction with chloroform. The DNA was precipitated with isopropanol,

spooled onto a glass rod, washed with ethanol (80%), and resuspended in Tris (500 µL, 10 mM, pH 8.0). The solution was incubated (37 ^{o}C, 30 min) after addition of RNase (3 µL, 5 mg mL^{-1}), precipitated with isopropanol, washed in 80 % ethanol, and resuspended in Tris (200 µL, 10 mM, pH 8.0).

2.3. Chemical characterization of beauvericin analogues: Optical rotations were measured with a Jasco DIP-370 digital polarimeter using MeOH as the solvent, and ^{1}H NMR spectra were recorded in [D_6]acetone on a Bruker DRX-500 instrument at 500 MHz for the isolated compounds **4**, **8-13**, **17** and **18**. High-resolution FABMS were obtained with a JEOL HX110A mass spectrometer. The chemical shift values (δ) are given in parts per million (ppm) and the coupling constants are given in hertz.



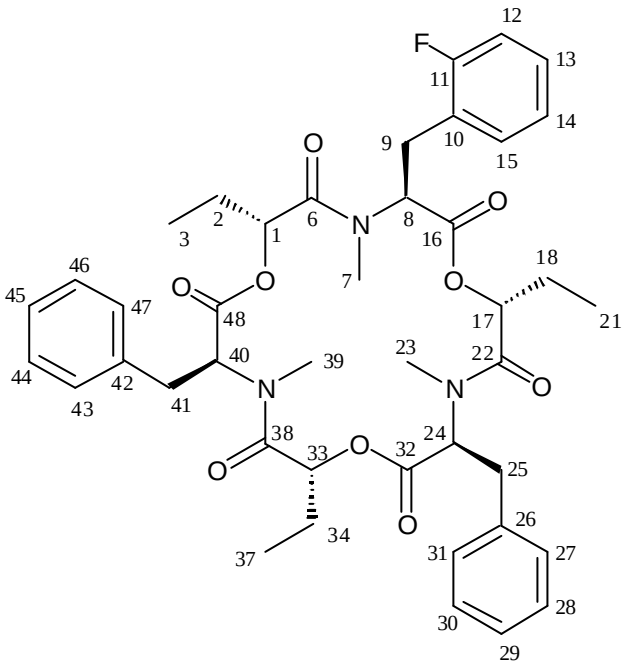
Beauvericin C (4): white powder; $[\alpha]^{25}_{D}$ +40.5 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.31- 7.18 (15H, m, H-11- H-15, H-27- H-31 and H-43- H-47), 5.44 (3H, dd, *J* = 11.4, 5.1 Hz, H-8, H-24 and H-40), 5.11 (3H, d, *J* = 7.8 Hz, H-1, H-17 and H-33), 3.28 (3H, dd, *J* = 14.4, 5.1 Hz, H-9a, H-25a and H-41a), 3.07 (3H, dd, *J* = 14.4, 11.5 Hz, H-9b, H-25b and H-41b), 3.04 (9H, s, CH_3-7, CH_3-23 and CH_3-39), 1.77-1.69 (3H, m, H-2, H-18 and H-34), 0.82- 0.75 (6H, m, CH_2-3, CH_2-19 and CH_2-35), 0.75 (9H, d, *J* = 6.7 Hz, CH_3-5, CH_3-21 and CH_3-37), 0.67 (9H, t, *J* = 7.0 Hz, CH_3-4, CH_3-20 and CH_3-36); HRFABMS *m/z* 826.4656 [*M* +1]$^{+}$ (calcd for $C_{48}H_{64}N_3O_9$ 826.4643).



Beauvericin G_3H_1 (5), R_1=R_2=H: HRFABMS *m/z* 760.3605 $[M+1]^+$ (calcd for $C_{42}H_{51}FN_3O_9$ 760.3609).

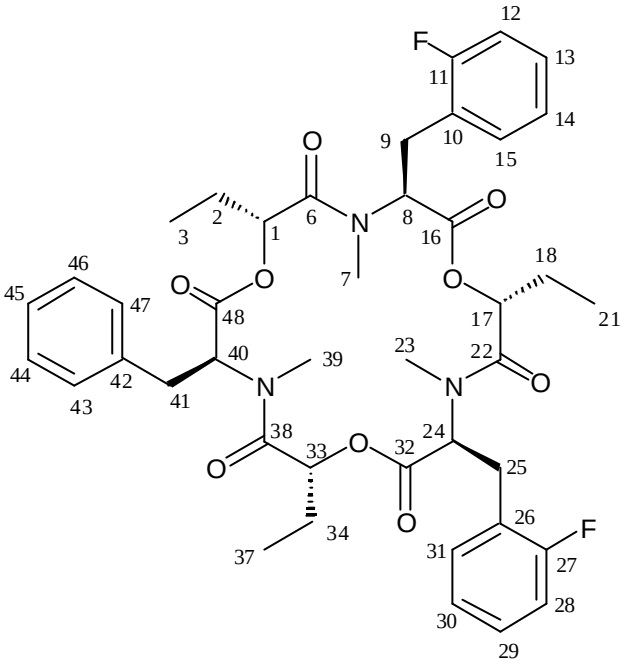
Beauvericin G_3H_2 (6), R_1=F, R_2=H: HRFABMS *m/z* 778.3491 $[M+1]^+$ (calcd for $C_{42}H_{50}F_2N_3O_9$ 778.3515).

Beauvericin G_3H_3 (7), R_1=R_2=F: HRFABMS *m/z* 796.3383 $[M+1]^+$ (calcd for $C_{42}H_{49}F_3N_3O_9$ 796.3421).

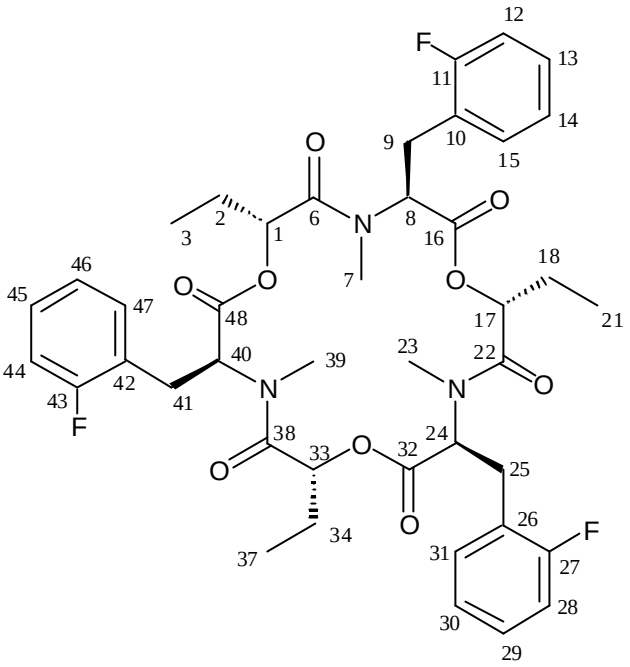


Beauvericin G_3I_1 (8): white powder; $[\alpha]^{25}_D$ –10.4 (*c* 0.3, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.34 (1H, t, *J* = 7.4 Hz, H-14), 7.07 (1H, dd, *J* = 9.9, 7.4 Hz, H-12),

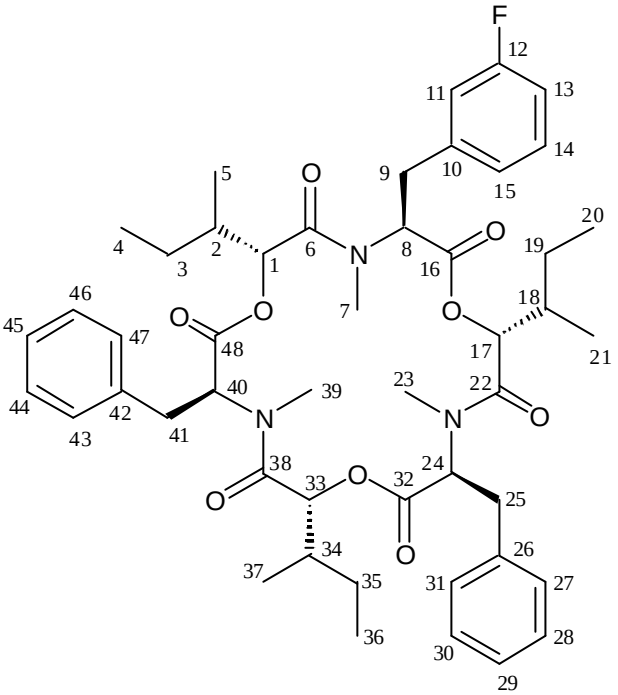
7.31- 7.16 (11H, m, H-15, H-27- H-31 and H-43- H-47), 7.01 (1H, t, *J* = 7.4 Hz, H-13), 5.39- 5.32 (3H, m, H-8, H-24 and H-40), 5.23- 5.18 (2H, m, H-1 and H-17), 5.08 (1H, dd, *J* = 10.0, 5.4 Hz, H-33), 3.28 (1H, dd, *J* = 14.2, 5.0 Hz, H-9a), 3.25- 3.20 (2H, m, H-25a and H-41a), 3.12 (1H, dd, *J* = 14.2, 10.6 Hz, H-9b), 3.09- 3.02 (2H, m, H-25b and H-41b), 2.99, 2.97 and 2.93 (3H each, s, CH_3-7, CH_3-23 and CH_3-39), 1.65- 1.47 (6H, m, CH_2-2, CH_2-18 and CH_2-34), 0.67 (3H, t, *J* = 7.4 Hz, CH_3-37), 0.63 (3H, t, *J* = 7.4 Hz, CH_3-3/ CH_3-21), 0.62 (3H, t, *J* = 7.4 Hz, CH_3-3/ CH_3-21); HRFABMS *m/z* 760.3603 $[M+1]^+$ (calcd for $C_{42}H_{51}FN_3O_9$ 760.3604).



Beauvericin G_3I_2 (9): white powder; $[\alpha]^{25}_D$ –8.1 (*c* 0.2, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.36- 7.33 (2H, m, H-14 and H-30), 7.30- 7.18 (7H, m, H-15, H-31 and H-43- H-47), 7.12- 7.04 (4H, m, H-12, H-13, H-28 and H-29), 5.39- 5.32 (3H, m, H-8, H-24 and H-40), 5.26 (1H, dd, *J* = 10.5, 5.5 Hz, H-17), 5.13 (2H, dd, *J* = 9.5, 5.0 Hz, H-1 and H-33), 3.30- 3.21 (3H, m, H-9a, H-25a and H-41a), 3.15- 3.05 (3H, m, H-9b, H-25b and H-41b), 3.01, 2.98 and 2.96 (3H each, s, CH_3-7, CH_3-23 and CH_3-39), 1.63- 1.46 (6H, m, CH_2-2, CH_2-18 and CH_2-34), 0.66 (6H, t, *J* = 7.0 Hz, CH_3-3 and CH_3-37), 0.62 (3H, t, *J* = 7.5 Hz, CH_3-21); HRFABMS *m/z* 778.3501 $[M+1]^+$ (calcd for $C_{42}H_{50}F_2N_3O_9$ 778.3510).

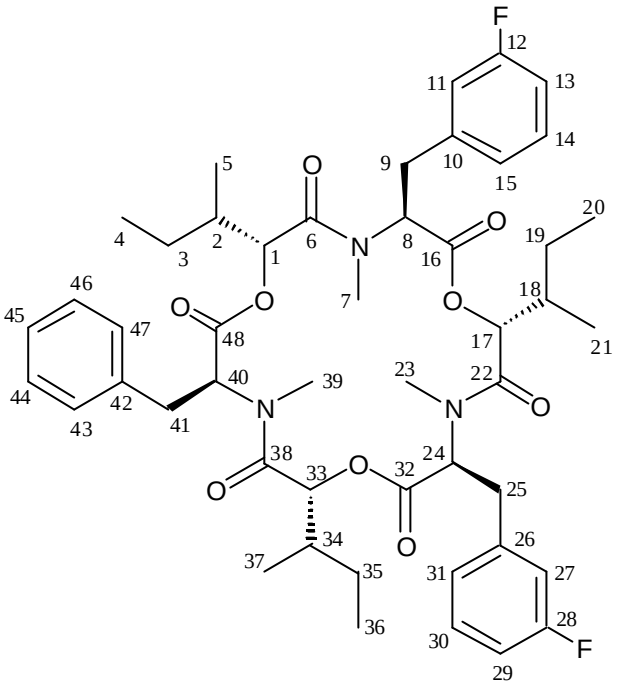


Beauvericin G_3I_3 (10): HRFABMS *m/z* 796.3405 $[M+1]^+$ (calcd for $C_{42}H_{49}F_3N_3O_9$ 796.3415).

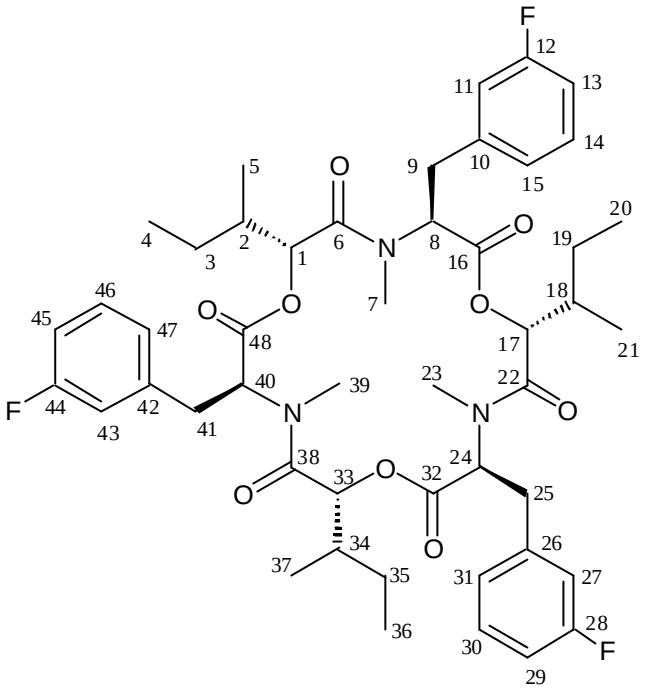


Beauvericin CH_1 (11): white powder; $[\alpha]^{25}_D$ +28.9 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.31 (1H, dd, *J* = 14.0, 7.8 Hz, H-13), 7.30- 7.18 (10H, m, H-27- H-31 and H-43- H-47), 7.15 (1H, d, *J* = 7.7 Hz, H-15), 7.10 (1H, d, *J* = 9.9 Hz, H-11), 6.98 (1H, td, *J* = 7.7, 1.9 Hz, H-14), 5.48- 5.39 (3H, m, H-8, H-24 and H-40), 5.16- 5.11 (3H, m, H-1, H-17 and H-33), 3.31 (1H, dd, *J* = 15.6, 5.0 Hz, H-9a), 3.28 (2H, dd, *J* = 14.7, 4.9 Hz, H-25a and H-41a), 3.13 (1H, dd, *J* = 15.6, 11.7 Hz, H-9b), 3.10 (2H, dd,

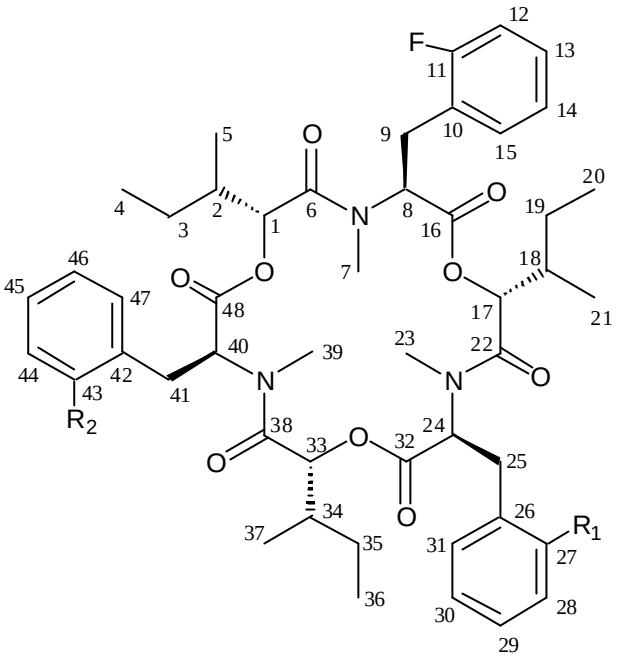
J = 14.7, 12.4 Hz, H-25b and H-41b), 3.06, 3.05 and 3.03 (3H each, s, CH_3-7, CH_3-23 and CH_3-39), 1.79- 1.68 (3H, m, H-2, H-18 and H-34), 0.91- 0.78 (6H, m, CH_2-3, CH_2-19 and CH_2-35), 0.78- 0.73 (9H, m, CH_3-5, CH_3-21 and CH_3-37), 0.73- 0.65 (9H, m, CH_3-4, CH_3-20 and CH_3-36); HRFABMS *m/z* 844.4568 [*M* +1] (calcd for $C_{48}H_{63}FN_3O_9$ 844.4548).



Beauvericin CH_2 (12): white powder; $[\alpha]^{25}_D$ +28.4 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.32 (2H,dd, *J* = 14.0, 7.8 Hz, H-13 and H-29), 7.29- 7.17 (5H, m, H-43- H-47), 7.14 (2H, d, *J* = 7.8 Hz, H-15 and H-31), 7.10 (2H, d, *J* = 10.2 Hz, H-11 and H-27), 6.98 (2H, td, *J* = 7.8, 2.3 Hz, H-14 and H-30), 5.46 (1H, dd, *J* = 11.2, 4.5 Hz, H-40), 5.42 (2H, dd, *J* = 11.7, 4.8 Hz, H-8 and H-24), 5.16- 5.13 (3H, m, H-1, H-17 and H-33), 3.31 (2H, dd, *J* = 14.7, 4.8 Hz, H-9a and H-25a), 3.28 (1H, dd, *J* = 15.8, 5.0 Hz, H-41a), 3.13 (2H, dd, *J* = 14.7, 11.7 Hz, H-9b and H-25b), 3.07 (1H, dd, *J* = 15.8, 11.2 Hz, H-41b), 3.06, 3.04 and 3.03 (3H each, s, CH_3-7, CH_3-23 and CH_3-39), 1.81- 1.70 (3H, m, H-2, H-18 and H-34), 0.92- 0.84 (6H, m, CH_2-3, CH_2-19 and CH_2-35), 0.77 (9H, t, *J* = 7.7 Hz, CH_3-5, CH_3-21 and CH_3-37), 0.72- 0.67 (9H, m, CH_3-4, CH_3-20 and CH_3-36); HRFABMS *m/z* 862.4489 [*M* +1] (calcd for $C_{48}H_{62}F_2N_3O_9$ 862.4454).



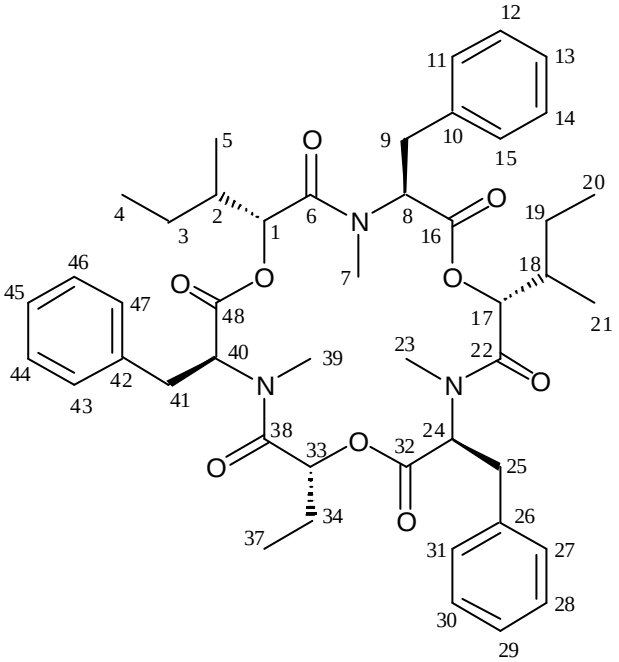
Beauvericin CH_3 (13): white powder; $[\alpha]^{25}_D$ +28.5 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.51 (3H, d, *J* = 7.7 Hz, H-15, H-31 and H-47), 7.32 (3H, dd, *J* = 14.1, 7.7 Hz, H-13, H-29 and H-45), 7.11 (3H, d, *J* = 10.0 Hz, H-11, H-27 and H-43), 6.98 (3H, td, *J* = 7.7, 2.1 Hz, H-14, H-30 and H-46), 5.43 (3H, dd, *J* = 11.5, 4.9 Hz, H-8, H-24 and H-40), 5.16 (3H, d, *J* = 7.6 Hz, H-1, H-17 and H-33), 3.31 (3H, dd, *J* = 14.5, 4.9 Hz, H-9a, H-25a and H-41a), 3.13 (3H, dd, *J* = 14.5, 11.6 Hz, H-9b, H-25b and H-41b), 3.04 (9H, s, CH_3-7, CH_3-23 and CH_3-39), 1.80- 1.74 (3H, m, H-2, H-18 and H-34), 0.91- 0.70 (6H, m, CH_2-3, CH_2-19 and CH_2-35), 0.78 (9H, t, *J* = 7.7 Hz, CH_3-5, CH_3-21 and CH_3-37), 0.71 (9H, t, *J* = 7.2 Hz, CH_3-4, CH_3-20 and CH_3-36); HRFABMS *m/z* 880.4321 [*M* +1] (calcd for $C_{48}H_{61}F_3N_3O_9$ 880.4360).



Beauvericin Cl_1 (14), R_1=R_2=H: HRFABMS *m/z* 844.4568 $[M+1]^+$ (calcd for $C_{48}H_{63}FN_3O_9$ 844.4548).

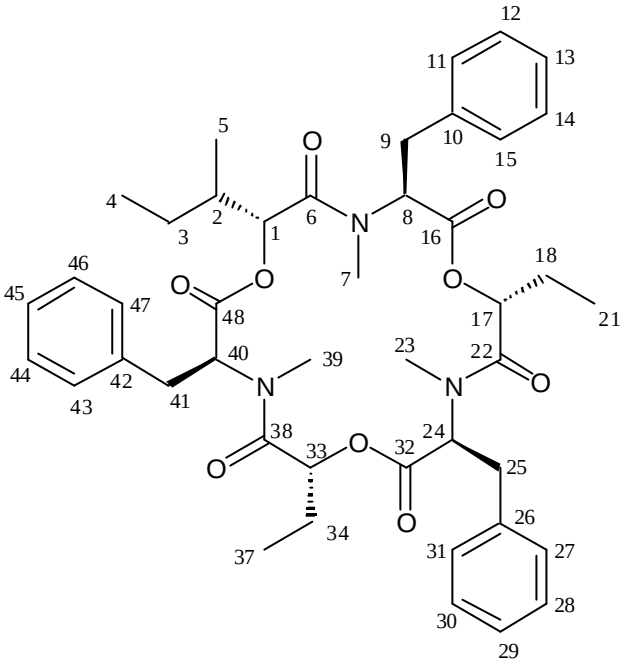
Beauvericin Cl_2 (15), R_1=F, R_2=H: HRFABMS *m/z* 862.4460 $[M+1]^+$ (calcd for $C_{48}H_{62}F_2N_3O_9$ 862.4454).

Beauvericin Cl_3 (16), R_1=R_2=F: HRFABMS *m/z* 880.4346 $[M+1]^+$ (calcd for $C_{48}H_{61}F_3N_3O_9$ 880.4360).



Beauvericin BG_1 (17): white powder; $[\alpha]^{25}_D$ +16.9 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.31- 7.18 (15H, m, H-11- H-15, H-27- H-31 and H-43- H-47), 5.67 (1H,

dd, *J* = 11.2, 5.3 Hz, H-8/H-24/H-40), 5.57 (1H, dd, *J* = 11.9, 4.8 Hz, H-8/H-24/H-40), 5.31 (1H, d, *J* = 9.7 Hz, H-1/H-17), 5.22 (1H, d, *J* = 8.5 Hz, H-1/H-17), 5.11 (1H, dd, *J* = 9.1, 4.4 Hz, H-8/H-24/H-40), 4.58 (1H, m, H-33), 3.30 (9H, s, CH_3-7, CH_3-23 and CH_3-39), 3.27- 3.20 (3H, m, H-9a, H-25a and H-41a), 3.05 (3H, m, H-9b, H-25b and H-41b), 1.80- 1.60 (3H, m, H-2, H-18 and H-34), 0.88- 0.75 (4H, m, CH_2-3, and CH_2-19), 0.79 (3H, t, *J* = 7.4 Hz, CH_3-37), 0.76 (3H, d, *J* = 7.0 Hz, CH_3-5/CH_3-21), 0.70 (3H, d, *J* = 7.0 Hz, CH_3-5/CH_3-21), 0.69- 0.64 (6H, m, CH_3-4 and CH_3-20); HR-FAB-MS *m/z* 798.4338 [*M* +1] (calcd for $C_{46}H_{60}N_3O_9$ 798.4330).



Beauvericin AG_2 (18): white powder; $[\alpha]^{25}_D$ +12.8 (*c* 0.2, MeOH); ^{1}H NMR (500 MHz, [D_6]acetone) 7.29- 7.19 (15H, m, H-11- H-15, H-27- H-31 and H-43- H-47), 5.44 (3H, dd, *J* = 9.8, 4.1 Hz, H-8, H-24 and H-40), 5.14 (1H, d, *J* = 7.3, Hz, H-1), 5.14- 5.12 (2H, m, H-17 and H-33), 3.29 (3H, dd, *J* = 14.4, 4.8 Hz , H-9a, H-25a and H-41a), 3.09 (3H, dd, *J* = 14.4, 11.7 Hz, H-9b, H-25b and H-41b), 3.05 (9H, s, CH_3-7, CH_3-23 and CH_3-39), 1.75- 1.55 (5H, m, H-2, CH_2-18 and CH_2-34), 0.89- 0.86 (2H, m, CH_2-3), 0.75 (3H, d, *J* = 6.7Hz, CH_3-4), 0.76- 0.67 (12H, m, CH_3-4, CH_3-5, CH_3-21 and CH_3-37); HRFABMS *m/z* 770.3985 [*M* +1] (calcd for $C_{44}H_{56}N_3O_9$ 770.4017).