Supporting Information

Natalamycin A, an Ansamycin from a Termite-Associated *Streptomyces* sp.

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General Experimental Procedures

Optical rotations were obtained using a Perkin-Elmer 241 polarimeter, IR spectra were measured on a Bruker Alpha-P FT-IR spectrometer, and UV spectra were acquired on an Amersham Biosciences Ultrospec 5300 Pro Spectrophotometer. All NMR experiments were carried out on a Varian INOVA 600 MHz NMR spectrometer equipped with an indirect detection probe. HPLC purifications were carried out on an Agilent 1100 or 1200 Series HPLC system (Agilent Technologies) equipped with a photo diode array detector. LC-MS analysis was performed on an Agilent 1200 Series HPLC system equipped with a diode array detector and a 6130 Series ESI mass spectrometer using an analytical Phenomenex Luna C18 column (5 μm, 4.6 × 100 mm). High resolution mass spectrometry (HR-MS) analysis was performed on a Waters Micromass Q-ToF Ultima ESI-TOF mass spectrometer at the University of Illinois Urbana-Champaign School of Chemical Sciences Mass Spectrometry Laboratory. The following growth media, prepared from BD Difco™ dehydrated culture media, for bacteria and fungi were used throughout the course of this study:

Broth (per 1 L)

ISP-2: 0.4% yeast extract, 1.0% malt extract, 0.4% glucose, pH 7.3

LB-Miller: 1.0% tryptone, 5.0% yeast extract, 1.0% NaCl, pH 7.0

PD: 0.4% potato starch infusion, 2.0% dextrose, pH 5.1

YM: 0.5% peptic digest of animal tissue, 0.3% yeast extract, 0.3% malt extract, pH 6.2

YP: 1.0% yeast, 2.0% peptone, 1.0% glucose, pH 7.0

Agar (per 1 L)

ISP-2: 0.4% yeast extract, 1.0% malt extract, 0.4% glucose, 1.5% agar, pH 7.2

LB-Miller: 1.0% tryptone, 5.0% yeast extract, 1.0% NaCl, 1.5% agar, pH 7.0

Low nutrient media[1]: chitin (4 g), K_2HPO_4 (0.7 g), KH_2PO_4 (0.3 g), $MgSO_4 \cdot 5H_2O$ (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), $ZnSO_4$ (0.001 g), $MnCl_2$ (0.001 g), agar (20 g), pH 8

PD: 0.4% potato starch infusion, 2.0% dextrose, 1.5% agar, pH 5.6

YM: 0.5% peptic digest of animal tissue, 0.3% yeast extract, 0.3% malt extract, 1.0% dextrose, 2.0% agar, pH 6.2

Isolation of *Streptomyces* spp.

Fungal comb material was collected from a *Macrotermes natalensis* Mn802 nest in South Africa, placed into clean plastic bags, stored at 5 °C, and processed within 1 day from collection. All samples, which were processed separately, were thoroughly fragmented and mixed in 700 µL of ddH_2O. Bacteria were isolated by plating 350 µL of these suspensions on low nutrient media. Isolates with Actinobacteria-like morphology were transferred to YM agar and sub-cultured until pure isolates were obtained. This resulted in a total of 360 Actinobacteria isolates, including *Streptomyces* sp. M56.

Streptomyces sp. M56 was identified and prioritized for analysis on the basis of its antifungal activity (*vide infra*). A region of the 16S rDNA gene was amplified for phylogenetic analysis with general primers [8F and 1540R or 27F and 1492R] using standard DNA extraction and PCR protocols. A nucleotide BLAST search of the partial 16S rRNA sequence for *Streptomyces* sp. M56 revealed that it had a 100% identity match with the partial 16S rRNA sequence for *Streptomyces malaysiensis* strain 1160 GenBank accession number HQ607429.1.

Liquid cultures of *Streptomyces* sp. M56 were prepared by excising YM agar slices (approx. 2 cm × 1 cm) covered with bacterial growth from a plate culture less than 14 d old and adding them to 50 mL ISP-2 liquid medium. The inoculated broth was then incubated at 30 ºC while shaken at 250 rpm. Turbidity was reached within 4 days.

Isolation of *Pseudoxylaria* sp. X802 and *Termitomyces* sp. T112 fungi

Pseudoxylaria sp. X802 was isolated from hyphal cords or stroma appearing on fungal comb material (approx. 15 g) that had been incubated for 7–14 days in the absence of termites in sealed styrofoam cups with tissue paper soaked in ddH_2O to maintain humidity. Material from fresh fruiting structures of *Pseudoxylaria* sp. X802 was placed directly onto YM agar plates for further propagation. *Termitomyces* sp. T112 was obtained by placing nodules from fresh fungal comb material directly onto YMA plates. Both fungi were subcultured on YM agar plates until pure isolates were obtained. All incubations were performed in the dark at 25 °C.

Liquid fungal cultures were prepared by excising YM agar slices (approx. 2 cm × 1 cm) covered with fungal growth from a plate culture 14–21 d old and adding them to 50 mL PD liquid medium. This was incubated at 30 ºC and shaken at 250 rpm for approximately 14–21 d to obtain sufficient biomass.

Streptomyces sp. M56 16S rRNA Sequence

5′–3′ direction

“CGGCCGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTG
GGACAAGCCCTGGAAACGGGGTCTAATACCGGATACGACGCGTTCCCGCATGGGATACGT
GTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGGGGTGAT
GGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACT
GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA
AGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAG
CAGGGAAGAAGCGTGAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAG
CAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCG
TAGGCGGCTTGTCGCGTCGGATGTGAAAGCCCGGGGCTTAACTCCGGGTCTGCATTCGAT
ACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGC
AGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAG
GAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTT
GGGAACTAGGTGTGGGCGACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTTCC
CCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACA
AGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACAT
ACACCGGAAACATCCAGAGATGGGTGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGC
TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCC
TGTGTTGCCAGCGGGTTATGCCGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGA
AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAAT
GGCCGGTACAATGAGCTGCGAAGCCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCA
GTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATC
AGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAA
GTCGGTAACACCCGAAGCCGG”

GenBank accession number: KJ511242

Screening of *Streptomyces* sp. M56 against Fungi

For antifungal screening, 20 µL of a turbid bacterial/spore solution of *Streptomyces* sp. M56 grown in ISP-2 broth was placed in the center of a PD agar plate. The plates were incubated for 7 d at 30 °C, in which time dense bacterial growth with a radius of approx. 0.3–0.5 cm was observed. Two methods for subsequent inoculation with fungi were used:

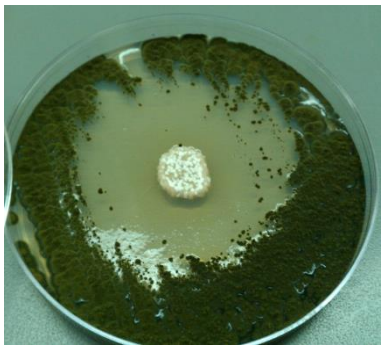
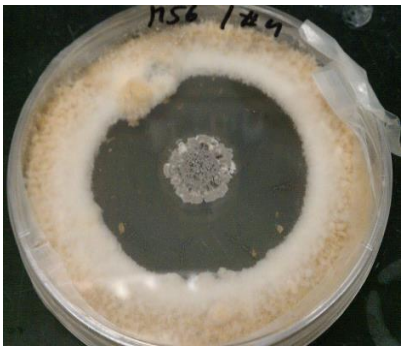
Method A: Plates were inoculated with 100 µL of a turbid mycelia/spore suspension of the respective fungi in PD broth. The fungal suspension was carefully spread on the plate with a sterile cotton swab without touching *Streptomyces* sp. M56.

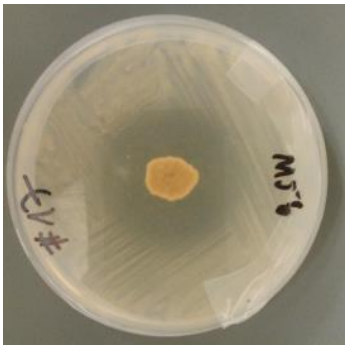
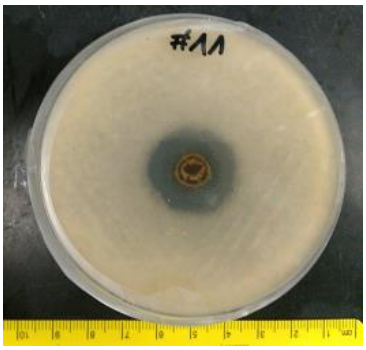
Method B: Agar slices with fungal growth were excised and placed at the edge of the assay plate.

All assay plates were incubated at ambient temperature in the dark. The effects of Actinobacteria inhibition on fungal growth were evaluated 3, 5, 7, 10, 14, and 21 days after fungal inoculation. Measurements were done using the edge of the bacterial growth as the point of reference. The measured zone of inhibition (ZOI) is equivalent to the average distance between the bacteria and the region of normal fungal growth. As a control, a mycelia/spore suspension of the respective fungus was spread onto a sterile PD agar plate and monitored over time to check its viability.

Table S1. Antifungal activity of *Streptomyces* sp. M56 against various fungi

Fungus	Assay pictures	
Alternaria alternate		
Method and time	Method B, 7 d	Method B, 14 d
ZOI (triplicate)	16 mm	14 mm

Cladosporium perangustum		
Method and time	Method A, 14 d	Method B, 7 d
ZOI (triplicate)	15 mm	16 mm
Pleosporales sp. LH222		
Method and time	Method A, 21 d	Method B, 7 d
ZOI (triplicate)	14 mm	12 mm
Pseudoxylaria sp. X802		
Method and time	Method B, 21 d	
ZOI (triplicate)	17 mm (full inhibition)	

Termitomyces sp. T112		
Method and time	Method B, 21 d	
ZOI (triplicate)	16 mm (full inhibition)	
Trichoderma sp. ATT151		
Method and time	Method A, 14 d	
ZOI (triplicate)	7 mm	
Umbelopsis isabellina		
Method and time	Method A, 7 d	Method B, 7 d
ZOI (triplicate)	4 mm	5 mm

Metabolomic Profile Time Course Studies of *Streptomyces* sp. M56

For metabolomic profiling, 14 ISP-2 agar plates were each inoculated with a 50 µL aliquot of a turbid bacterial/spore suspension of *Streptomyces* sp. M56 in ISP-2 broth. The suspension was evenly distributed over the agar surface, and the plates were incubated at 30 °C in the dark. For each time point (3, 5, 7, 9, 10, 12, and 14 days after inoculation) two agar plates were cut into squares, consolidated, and soaked in *i*PrOH overnight. The solvent was filtered, removed under reduced pressure, and the crude extract was dissolved in a 50% MeOH/H_2O solution (1 mL). Of this mixture, a 100 µL aliquot was used for reverse-phase LC-MS analysis, which was performed using an analytical Phenomenex Luna C18 column (5 µm, 4.6 × 100 mm) (flow rate: 0.7 mL/min, program (30 min total): 0-20 min linear gradient 10% MeCN/H_2O (+ 0.1% formic acid (FA)) to 100% MeCN + 0.1% FA, then 3 min 100% MeCN + 0.1% FA isocratic elution, then 1 min ramp down to and 6 min isocratic elution with 10% MeCN/H_2O (+ 0.1% FA) (Figure S1 and S2)).

To compare growth on solid media with liquid fermentation, 25 mL ISP-2 liquid broth was inoculated with 50 µL of a turbid bacterial/spore solution in ISP-2 broth and incubated for 10 d at 30 °C with shaking at 250 rpm. The culture was extracted using EtOAc (75 mL) and the solvent was removed under reduced pressure. The resulting crude extract was dissolved in a 50% MeOH/H_2O solution (1 mL) and a 100 µL aliquot was used for LC-MS studies (Figure S3).

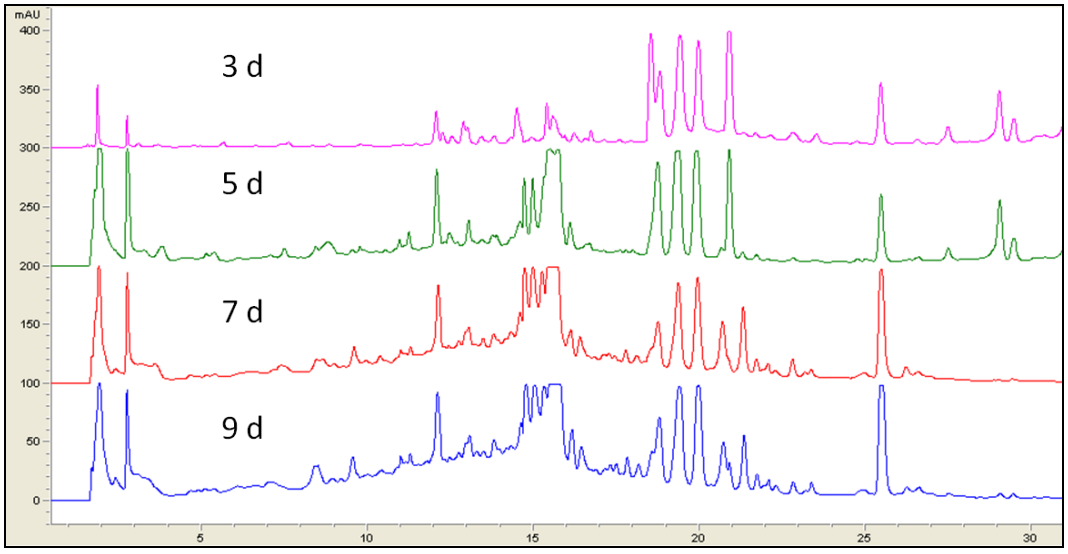


Figure S1. LC-MS diagram (254 nm) of metabolite extracts from *Streptomyces* sp. M56 grown on ISP-2 agar plates after 3 d (pink), 5 d (green), 7 d (red) and 9 d (blue)

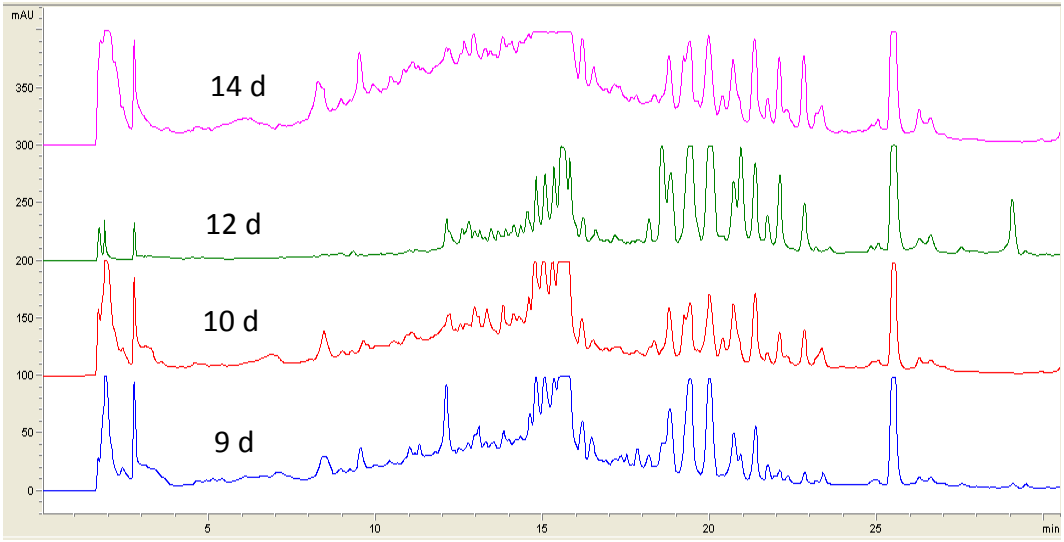


Figure S2. LC-MS diagram (254 nm) of metabolite extracts from *Streptomyces* sp. M56 grown on ISP-2 agar plates after 9 d (blue), 10 d (red), 12 d (green) and 14 d (pink)

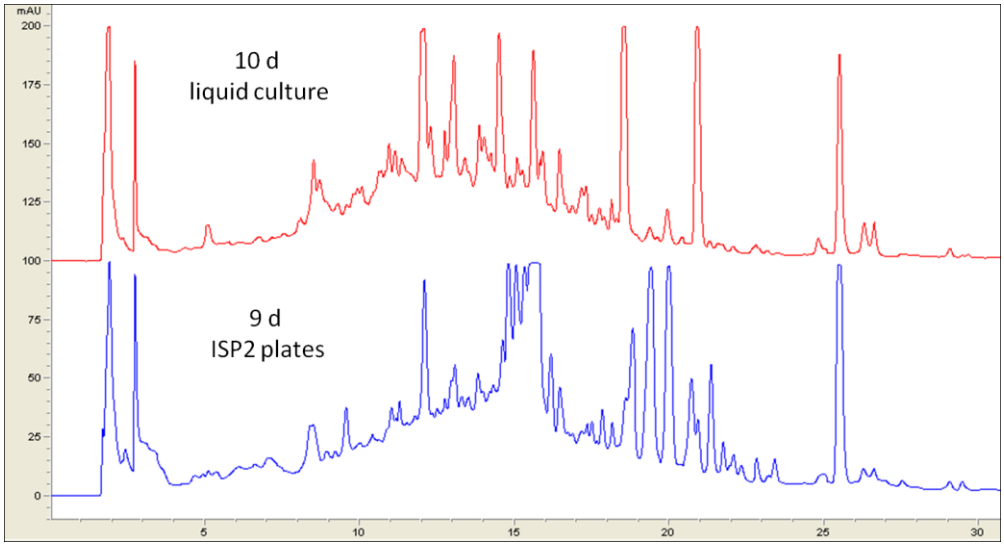


Figure S3. LC-MS trace (254 nm) of M56 metabolite extracts from an ISP-2 liquid culture after 10 d (red) and from ISP-2 agar plates after 9 d (blue)

Preparative-Scale Purification of Ansamycins

Streptomyces sp. M56 was grown on 50 ISP-2 agar plates (14 cm diameter) for 10 days at 30 °C. The agar was then cut into squares, consolidated, and soaked overnight in *i*PrOH. The *i*PrOH phase was filtered, the solvent was removed under reduced pressure, and the crude extract dissolved in a 5:1 MeOH:H_2O mixture (200 mL). The extract was loaded onto an activated pre-packed C_{18} Sep-Pak cartridge (10 g, Waters) which was equilibrated with 20% MeOH/H_2O. The charged column was then washed with two column volumes of 20% MeOH/H_2O to remove very polar compounds followed by step gradient elution with two column volumes of each of the following solvent mixtures: 40% MeOH/H_2O, 60% MeOH/H_2O, 80% MeOH/H_2O, 100% MeOH, and 100% acetone.

Each fraction was tested for antifungal and antimicrobial activity in triplicate against standardized bacterial and yeast strains from the American Type Culture Collection (*B. subtilis* (ATCC 6633), *E. coli* (ATCC 25922) and *S. cerevisiae* (ATCC 9763)) and co-isolated fungi *Pseudoxylaria* sp. X802 and *Termitomyces* sp. T112. MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) values for *B. subtilis*, *E. coli*, and *S. cerevisiae* were determined using broth-dilution techniques.[2] *B. subtilis*, and *E. coli* were grown in LB-Miller broth (5 mL), and *S. cerevisiae* was grown in YP broth (5 mL) at 30 °C overnight to an $OD_{600nm} > 0.9$. The cultures were diluted 1:100 with LB-Miller broth to an approximate OD_{600nm} of 0.05, and 100 µL aliquots of diluted culture were added to a series of wells in a 96-well plate. A 0.1 mg/100 µL DMSO stock solution of the crude material from each fraction was used for testing (titration from 1 µL up to 12 µL of substrate solution, in 1 µL steps), and streptomycin sulfate and amphotericin B were used as positive controls. After incubation at 30 °C for 12 h the OD_{600nm} was measured using a SpectraMax M5 Multi-Mode Microplate Reader. Cultures in wells showing no growth were plated on agar plates (LB for *E. coli* and *B. subtilis* assays, and YP for *S. cerevisiae* assays) and incubated at 30 °C overnight to verify loss of viability. MIC_{50} values were calculated using XLfit 4.2 software.[3]

For antifungal assays against *Pseudoxylaria* sp. X802 and *Termitomyces* sp. T112, PD agar plates were inoculated with 100 µL of a turbid mycelia/spore suspension in PD broth, and the surface was allowed to dry using the laminar air flow of the biosafety cabinet. Sterile paper discs soaked with a DMSO solution (0.1 mg/100 µL) of the crude material from each fraction were placed onto the inoculated plates. All plates were stored at room temperature and were monitored for 21 d.

Fractions eluted with 80% and 100% MeOH exhibited a clear zone of inhibition in disc diffusion assays against *Pseudoxylaria* sp. X802 and *Termitomyces* sp. T112 (ZOI 0.5–1.0 cm), a MIC of 35 ± 5 µg/mL for *B. subtilis*, and an MFC of 45 ± 5 µg/mL for *S. cerevisiae*. These fractions were subsequently analyzed by LC-MS, which indicated a complex mixture of secondary metabolites.

Fractions eluted with 80% and 100% MeOH were consolidated and subsequently purified by preparative reverse-phase HPLC (Agilent 1100 Series HPLC system, C_{18} column, Phenomenex Luna, 250 × 21.2 mm, 5 µm) with a flow rate of 10 mL/min using a linear gradient from 30% MeOH/H_2O to 100% MeOH for 30 min, then 100% MeOH for the next 10 min, then down to 30% MeOH/H_2O within one minute and further isocratic elution for 9 min. Fractions were collected for every minute starting at 6 min and ending at 40 min to afford 34 fractions. These fractions were assayed against *B. subtilis*, *S. cerevisiae*, *Pseudoxylaria* sp. X802, and *Termitomyces* sp. T112. Fractions 17–26 exhibited modest to moderate activity against *Pseudoxylaria* sp. X802 and *Termitomyces* sp. T112 (Figure S4), and against *B. subtilis* and *S. cerevisiae*.

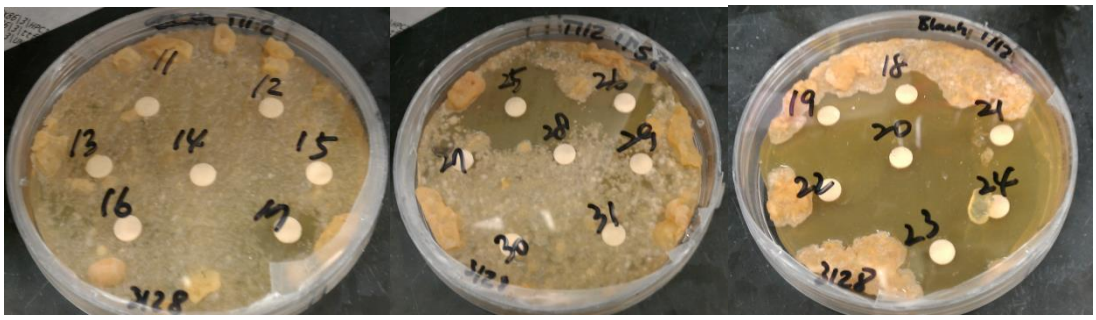


Figure S4. Disc assay against *Termitomyces* sp. T112 with material fractionated by preparative reverse-phase HPLC

Fraction 17 was further purified by semi-preparative reverse-phase HPLC (Agilent 1200 Series HPLC system, Phenyl-hexyl column, Phenomenex Luna, 250 × 10.0 mm, 5 µm, flow rate: 2 mL/min) using isocratic elution with 33% MeCN/H_2O (+ 0.1% FA) over 35 min to afford compounds **1** (0.7 mg, t_R 27.7 min), **3** (t_R 2.5 mg, 16.7 min), **8** (t_R 1.8 mg, 24.2 min), and **9** (t_R 0.5 mg, 31.3 min) (Figure S5 and S6).

Fraction 18 was purified by semi-preparative reverse-phase HPLC (Phenyl-hexyl column, Phenomenex Luna, 250 × 10.0 mm, 5 µm, flow rate: 2 mL/min) using isocratic elution with 35% MeCN/H_2O (+ 0.1% FA) over 35 min to furnish compound **5** (1.1 mg, t_R 36.9 min).

Fractions 20 and 21 were consolidated and separated by semi-preparative reverse-phase HPLC (Phenyl-hexyl column, Phenomenex Luna, 250 × 10.0 mm, 5 µm, flow rate: 2 mL/min) using isocratic elution with 43% MeCN/H_2O (+ 0.1% FA) over 30 min to yield compound **7** (1.6 mg, t_R 18.9 min).

Fraction 22 was purified by semi-preparative reverse-phase HPLC (Phenyl-hexyl column, Phenomenex Luna, 250 × 10.0 mm, 5 µm, flow rate: 2 mL/min) using isocratic elution with 45% MeCN/H_2O (+ 0.1% FA) over 30 min to afford compound **6** (1.2 mg, t_R 20.0 min).

Fraction 25 was purified by semi-preparative reverse-phase HPLC (Phenyl-hexyl column, Phenomenex Luna, 250 × 10.0 mm, 5 µm, flow rate: 2 mL/min) using isocratic elution with 53% MeCN/H_2O (+ 0.1% FA) over 30 min to furnish compounds **2** (1.5 mg, t_R 13.0 min) and **4** (1.0 mg, t_R 19.1 min).

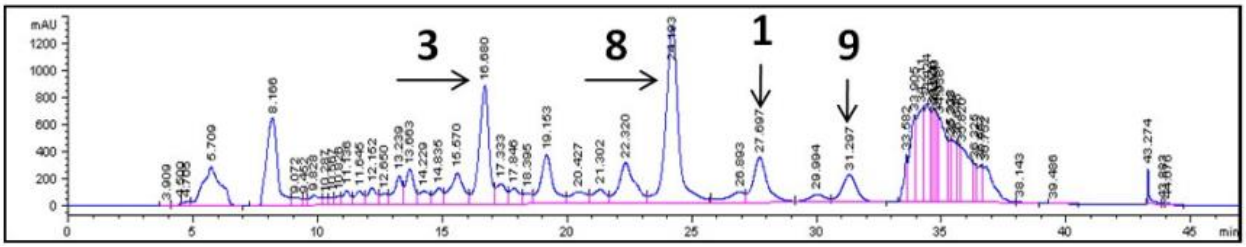


Figure S5. Trace from semi-preparative HPLC purification of fraction 17 (254 nm, flow rate: 2 mL/min, 33% MeCN/H_2O + 0.1% FA for 35 min)

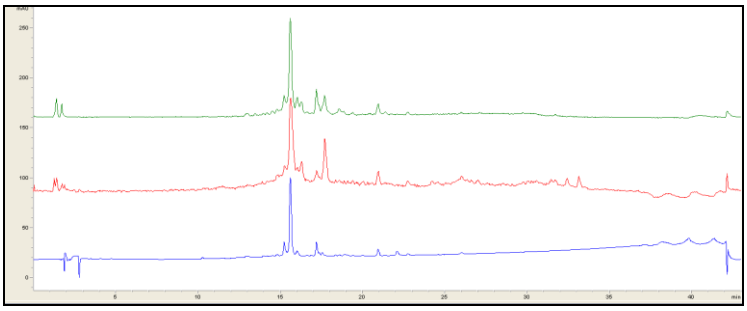


Figure S6. LC-MS trace for **1** (green: negative ion scan, red: positive ion scan, blue: 254 nm, flow rate: 0.7 mL/min, LC-MS program: linear gradient 0–30 min from 10% MeCN/H_2O (+ 0.1% FA) to 100% MeCN + 0.1% FA, then 5 min isocratic elution with 100% MeCN + 0.1% FA, then ramp down over 1 min to and 6 min isocratic elution with 10% MeCN/H_2O (+ 0.1% FA))

Spectroscopic Data for New Compounds

1: amorphous powder; 0.7 mg (50 × 14 cm diameter agar plates); $[\alpha]_D^{25}$ -16.5 (*c* 0.04, MeOH); IR (KBr) ν_{max} 3475, 3375, 3285, 2920, 1727, 1700, 1655, 1439, 1354, 1283, 1187, 1026 cm^{-1}; UV (MeOH) λ_{max} (log ε) 220 (4.2), 254 (1.8), 292 (1.0) nm; HR-ESIMS *m/z* 697.3310 $[M + Na]^+$ (calcd for $C_{35}H_{50}N_2O_{11}Na$: 697.3312).

Antifungal activity:

S. cerevisiae: MFC_{50} = 55 ± 2 µg/mL; MFC = 100 µg/mL

Antimicrobial activity:

B. subtilis: MIC_{50} = 85 ± 2 µg/mL; MIC = 150 µg/mL

8: amorphous powder; 1.8 mg (50 × 14 cm diameter agar plates); $[\alpha]_D^{25}$ +36.4 (*c* 0.03, MeOH); IR (KBr) ν_{max} 3454, 3330, 2930, 2835, 1710, 1658, 1595, 1450, 1390, 1150 cm^{-1}; UV (MeOH) λ_{max} (log ε) 213 (3.6), 264 (4.1), 336 (3.2) nm; HR-ESIMS *m/z* 600.2880 $[M + Na]^+$ (calcd for $C_{29}H_{43}N_3O_9Na$: 600.2897).

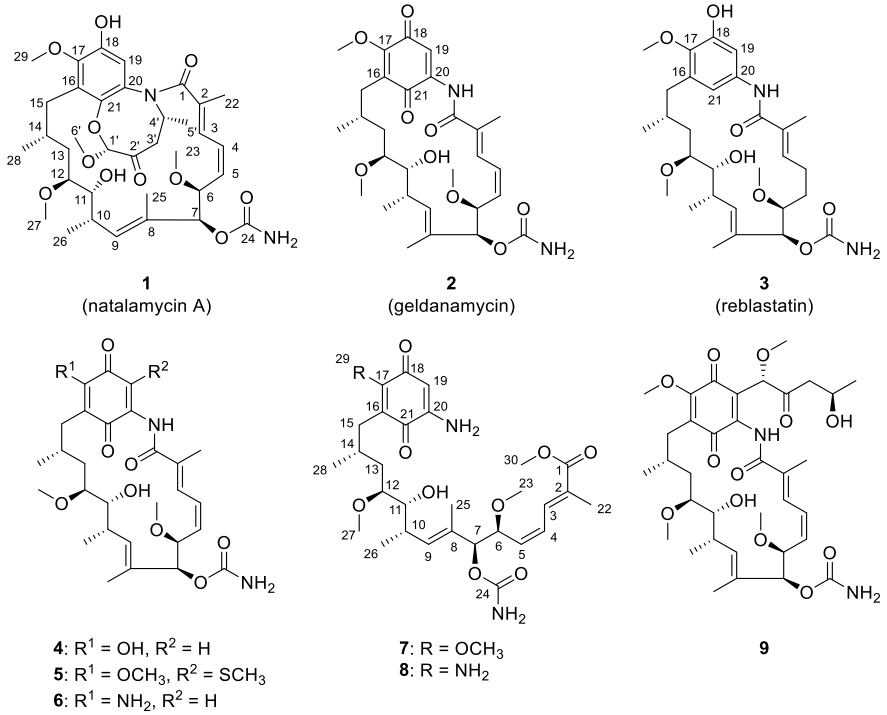


Figure S7. Chemical structures of isolated geldanamycin analogs. Note that the labels for **1** *do not* correspond to the atom labels used for quantum-chemical NMR calculations.

Table S2. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** in $CDCl_3$

position	δ_C ($CDCl_3$), type[a]	δ_H (J in Hz)	COSY	HMBC
1	173.2, C			
2	137.6, C			
3	123.7, CH	5.75, br d (12.0)	H-4	C-1, C-5, C-22
4	130.1, CH	6.22, dd (12.0, 11.0)	H-3, H-5	C-2, C-6
5	128.2, CH	4.96, dd (11.0, 10.0)	H-4, H-6	C-3, C-7
6	75.4, CH	3.81, overlap	H-5, H-7	C-4, C-8
7	82.9, CH	4.91, d (8.5)	H-6	C-5, C-9, C-24, C-25
8	130.6, C			
9	134.1, CH	5.11, d (10.0)	H-10	C-7, C-25, C-26
10	35.2, CH	2.32, m	H-9, H-11, H-26	C-8
11	73.4, CH	3.59, m	H-10, H-12	C-26
12	80.6, CH	2.78, m	H-11, H-13	C-27
13	31.5, CH_2	1.64, m; 0.55, m	H-12, H-14	C-28
14	30.8, CH	2.22, m	H-13, H-15, H-28	C-13, C-15
15	34.4, CH_2	2.97, m; 2.65, m	H-14	C-13, C-14, C-16, C-17, C-21, C-28
16	130.3, C			
17	147.1, C			
18	–			
19	115.1, CH	6.66, s		C-17, C-20, C-21
20	129.5, C			
21	144.2, C			
22	16.9, CH_3	1.93, s		C-1, C-2, C-3
23	57.0, CH_3	3.09, s		C-6
24	156.9, C			
25	13.0, CH_3	1.30, s		C-7, C-8, C-9
26	19.4, CH_3	1.04, d (6.0)	H-10	C-9, C-10, C-11
27	57.4, CH_3	3.31, s		C-12
28	16.9, CH_3	0.60, d (6.5)	H-14	C-13, C-14, C-15
29	62.4, CH_3	3.81, s		C-17
1′	107.0, CH	4.94, s		C-21, C-3′, C-6′
2′	202.0, C			
3′	42.4, CH_2	2.44, dd (11.0, 4.0)	H-4′	C-2′, C-4′
		2.39, dd (11.0, 10.5)		
4′	49.4, CH	5.44, m	H-3′, H-5′	
5′	20.8, CH_3	1.34, d (7.0)	H-4′	C-3′, C-4′
6′	59.7, CH_3	3.57, s		C-1′
24-NH_2		4.54, br s		

[a] The assignments were based on ^{1}H-^{1}H COSY, gHSQC, TOCSY, and gHMBC experiments. C–H multiplicities were assigned through a gHSQCad experiment, and CH versus CH_3 was distinguished through ^{1}H integration.

Table S3. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** in $CDCl_3$ at 25 ºC and 55 ºC

position	25 ºC		55 ºC	
	δ_C[a], type	δ_H (J in Hz)[a]	δ_C[a], type	δ_H (J in Hz)[a]
1	173.2, C		173.2, C	
2	137.6, C		137.7, C	
3	123.7, CH	5.75, br d (12.0)	123.8, CH	5.73, br d (11.0)
4	130.1, CH	6.22, dd (12.0, 11.0)	130.0, CH	6.23, dd (11.0, 11.0)
5	128.2, CH	4.96, dd (11.0, 10.0)	128.7, CH	5.01, dd (11.0, 10.0)
6	75.4, CH	3.81, overlap	75.8, CH	3.84, overlap
7	82.9, CH	4.91, d (8.5)	83.1, CH	4.94, d (9.0)
8	130.6, C		130.7, C	
9	134.1, CH	5.11, d (10.0)	134.5, CH	5.13, d (11.0)
10	35.2, CH	2.32, m	35.4, CH	2.37, m
11	73.4, CH	3.59, m	74.0, CH	3.60, dd (10.0, 2.5)
12	80.6, CH	2.78, m	81.3, CH	2.85, m
13	31.5, CH_2	1.64, m; 0.55, m	31.8, CH_2	1.60, m; 0.62, m
14	30.8, CH	2.22, m	31.1, CH	2.26, m
15	34.4, CH_2	2.97, m; 2.65, m	32.9, CH_2	3.01, m; 2.68, m
16	130.3, C		130.7, C	
17	147.1, C		147.2, C	
18	–		–	
19	115.1, CH	6.66, s	115.5, CH	6.67, s
20	129.5, C		129.6, C	
21	144.2, C		144.4, C	
22	16.9, CH_3	1.93, s	17.1, CH_3	1.95, s
23	57.0, CH_3	3.09, s	57.1, CH_3	3.13, s
24	156.9, C		157.3, C	
25	13.0, CH_3	1.30, s	13.2, CH_3	1.34, s
26	19.4, CH_3	1.04, d (6.0)	19.5, CH_3	1.06, d (6.5)
27	57.4, CH_3	3.31, s	57.5, CH_3	3.34, s
28	16.9, CH_3	0.60, d (6.5)	17.6, CH_3	0.66, d (6.5)
29	62.4, CH_3	3.81, s	62.5, CH_3	3.84, s
1′	107.0, CH	4.94, s	107.2, CH	4.95, s
2′	202.0, C		201.8, C	
3′	42.4, CH_2	2.44, dd (11.0, 4.0)	42.6, CH_2	2.41, m
		2.39, dd (11.0, 10.5)		2.39, m
4′	49.4, CH	5.44, m	49.7, CH	5.46, m
5′	20.8, CH_3	1.34, d (7.0)	20.9, CH_3	1.36, d (6.5)
6′	59.7, CH_3	3.57, s	59.7, CH_3	3.58, s
24-NH_2		4.54, br s		4.51, br s

[a] The assignments were based on ^{1}H-^{1}H COSY, gHSQCad, TOCSY, and gHMBC experiments. C–H multiplicities were assigned through gHSQCad, and CH versus CH_3 was distinguished through ^{1}H integration.

Table S4. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** in CD_3OD

position	δ_C (CD_3OD), type[a]	δ_H (*J* in Hz)	COSY	HMBC
1	174.3, C			
2	137.2, C			
3	123.9, CH	5.86, br d (12.0)	H-4	C-1, C-5, C-22
4	129.7, CH	6.28, dd (12.0, 11.0)	H-3, H-5	C-2, C-6
5	128.7, CH	5.02, dd (11.0, 10.0)	H-4, H-6	C-3, C-7
6	75.9, CH	3.91, dd (10.0, 10.0)	H-5, H-7	C-4, C-8
7	82.9, CH	4.85, overlap	H-6	C-5, C-9, C-24, C-25
8	131.1, C			
9	134.7, CH	5.10, d (11.0)	H-10	C-7, C-25, C-26
10	35.6, CH	2.43, m	H-9, H-11, H-26	C-8
11	73.6, CH	3.55, dd (10.0, 2.5)	H-10, H-12	C-26
12	81.2, CH	2.84, m	H-11, H-13	C-27
13	32.3, CH_2	1.68, m; 0.67, m	H-12, H-14	C-28
14	31.1, CH	2.21, m	H-13, H-15, H-28	C-13, C-15
15	32.2, CH_2	2.99, m; 2.75, m	H-14	C-13, C-14, C-16, C-17, C-21, C-28
16	131.1, C			
17	148.3, C			
18	_			
19	116.0, CH	6.63, s		C-17, C-20, C-21
20	127.9, C			
21	143.1, C			
22	15.9, CH_3	1.90, s		C-1, C-2, C-3
23	56.1, CH_3	3.10, s		C-6
24	159.9, C			
25	12.7, CH_3	1.39, s		C-7, C-8, C-9
26	18.7, CH_3	0.99, d (6.5)	H-10	C-9, C-10, C-11
27	56.3, CH_3	3.35, s		C-12
28	16.8, CH_3	0.64, d (6.5)	H-14	C-13, C-14, C-15
29	60.7, CH_3	3.76, s		C-17
1′	107.1, CH	4.99, s		C-21, C-3′, C-6′
2′	203.0, C			
3′	41.7, CH_2	2.41, m	H-4′	C-2′, C-4′
		2.37, m		
4′	50.2, CH	5.32, m	H-3′, H-5′	
5′	19.8, CH_3	1.33, d (6.5)	H-4′	C-3′, C-4′
6′	58.9, CH_3	3.59, s		C-1′

[a] The assignments were based on ^{1}H-^{1}H COSY, gHSQCad, TOCSY, and gHMBC experiments. C–H multiplicities were assigned through gHSQCad, and CH versus CH_3 was distinguished through ^{1}H integration.

Table S5. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 HMz) data of **8** in CD_3OD

Position	**8**			
	δ_C (CD_3OD)[a]	δ_H (*J* in Hz)	COSY	HMBC
1	169.8, C			
2	130.9, C			
3	133.0, CH	7.51, br d (12.0)	H-4	C-1, C-5, C-22
4	128.9, CH	6.64, dd (12.0, 11.0)	H-3, H-5	C-2, C-6
5	134.8, CH	5.61, dd (11.0, 10.0)	H-4, H-6	C-3, C-7
6	78.6, CH	4.34, dd (10.0, 6.5)	H-5, H-7	C-4, C-8
7	81.1, CH	4.92, d (6.5)	H-6	C-5, C-9, C-24, C-25
8	131.8, C			
9	133.5, CH	5.34, d (9.0)	H-10	C-7, C-25, C-26
10	35.8, CH	2.38, m	H-9, H-11, H-26	C-8
11	75.2, CH	3.52, dd (9.5, 2.0)	H-10, H-12	C-26
12	82.3, CH	3.18, m	H-11, H-13	C-27
13	36.0, CH_2	1.58 m; 1.09, m	H-12, H-14	C-28
14	29.8, CH	1.81, m	H-13, H-15, H-28	C-13, C-15
15	32.0, CH_2	2.27, dd (13.0, 8.5)	H-14	C-13, C-14, C-16, C-17, C-21, C-28
		2.23, dd (13.0, 6.0)		
16	107.6, C			
17	151.4, C			
18	_			
19	95.3, CH	5.40, s		C-17, C-21
20	_			
21	179.6, C			
22	12.5, CH_3	1.93, s		C-1, C-2, C-3
23	56.9, CH_3	3.27, s		C-6
24	158.7, C			
25	14.7, CH_3	1.63, s		C-7, C-8, C-9
26	17.7, CH_3	0.96, d (6.0)	H-10	C-9, C-10, C-11
27	56.9, CH_3	3.33, s		C-12
28	20.3, CH_3	0.78, d (6.5)	H-14	C-13, C-14, C-15
29				C-17
30	52.4, CH_3	3.75, s		C-1

[a] The assignments were based on ^{1}H-^{1}H COSY, gHSQCad, TOCSY, and gHMBC experiments. C–H multiplicities were assigned through gHSQCad, and CH versus CH_3 was distinguished through ^{1}H integration.

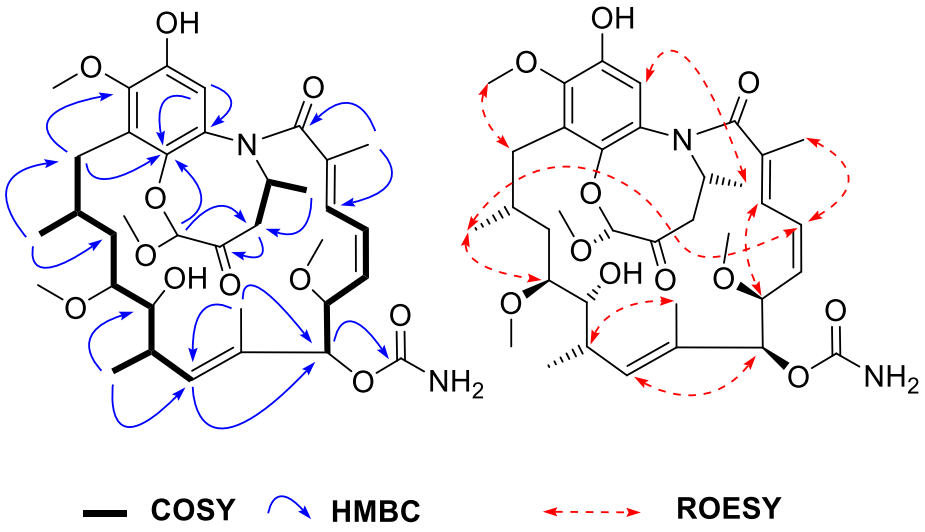


Figure S8. Key HMBC, COSY and ROESY correlation of natalamycin A.

Single Crystal X-ray Diffraction Experimental Procedure

Colorless block crystals of **1** were obtained through slow evaporation from $CHCl_3$ with trace MeOH in a glass vial. A crystal was mounted in a 15 K helium cold stream on a Bruker D8 three-circle fixed chi goniometer with an Apex II CCD detector at the Advanced Photon Source synchrotron in Argonne National Laboratories (ChemMatCARS sector 15 beamline, experimental station ID-B), and a sphere of data to $2\theta = 31.6°$ was collected. Integration was performed using SAINT,[4] and a multi-scan absorption correction was applied to the data using SADABS.[5] A total of 33421 reflections were collected, with 10598 unique reflections and 10533 reflections observed [$I>2\sigma(I)$]. The structure was solved through intrinsic phasing (SHELXT-2013) and refined by full-matrix least-squares on F^2 (SHELXL-2013) using 553 parameters and 6 restraints (1 ISOR restraint required) (Figure S8).[6] Hydrogens were initially added using the riding model and methyl group hydrogens were refined as rigid idealized groups. Hydrogens on heteroatoms, initially placed through the riding model or assigned on the basis of residual electron density, were allowed to refine free with isotropic thermal parameters by removal of SHELXL AFIX directives, which were re-introduced / added after free refinement. Anomalous dispersion and absorption coefficients (f' and f'') for C, Cl, N, O were calculated for the synchrotron radiation wavelength used (0.41328 Å). The absolute stereochemistry was confirmed using Bayesian statistics on 4731 select Bijvoet pairs with a Hooft y parameter of 0.00(4).[7]

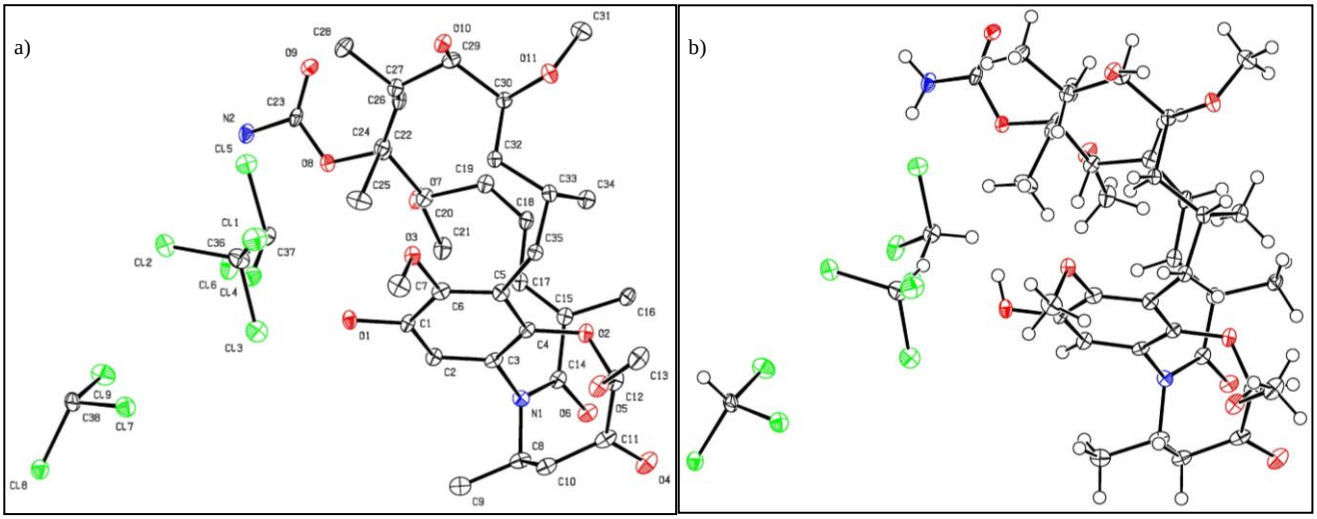


Figure S9. ORTEP plot of **1** a) with labels and no hydrogens displayed for clarity, and b) with hydrogens displayed. Thermal ellipsoids are shown at 50% probability. The X-ray atom labels *do not* correspond to the atom labels used for quantum-chemical NMR calculations.

Table S6. Crystal and structure refinement data for **1**

Identification code	kt29-076res_a	
Empirical formula	$C_{38}H_{53}Cl_9N_2O_{11}$	
Formula weight	1032.87	
Temperature	15.0 K	
Radiation	Synchrotron	
Wavelength	0.41328 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 12.9360(18) Å	α= 90°.
	b = 17.448(2) Å	β= 90°.
	c = 21.111(3) Å	γ = 90°.
Volume	4764.9(11) Å^3	
Z	4	
Density (calculated)	1.440 Mg/m^3	
Absorption coefficient	0.303 mm^{-1}	
F(000)	2144	
Color	Colorless	
Description	Block	
Crystal size	0.65 x 0.6 x 0.5 mm^3	
θ range for data collection	0.880 to 15.787°.	
Index ranges	$-9 \le h \le 16$, $-22 \le k \le 22$, $-27 \le l \le 27$	
Reflections collected	33421	
Independent reflections	10598 [R_{int} = 0.0390]	
Observed reflections [$I>2\sigma(I)$]	10533	
Completeness to θ = 14.357°	94.0%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.8503 and 0.6576	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	10598 / 6 / 553	
Goodness-of-fit on F^2	1.002	
Final R indices [$I>2\sigma(I)$]	R_1 = 0.0635, wR_2 = 0.1840	
R indices (all data)	R_1 = 0.0649, wR_2 = 0.1913	
Absolute structure parameter (Hooft y)	0.00(4)	
Largest diff. peak and hole	0.834 and -0.594 e.Å^{-3}	

Computational Methods

All DFT electronic structure and NMR calculations were performed in *Gaussian 09 Revision A.02*,[8] and conformational searches were performed in *Schrödinger Release 2013-2 MacroModel 10.1*.[9] Initial searches to locate conformer candidates of **1** were performed in *MacroModel 10.1* using the advanced conformational search tool and macrocycle conformational sampling tool. C-clamp conformer candidates were located by first minimizing the X-ray crystal structure of **1** using molecular mechanics with the Merck Molecular Force Field (MMFF)[10] and then using the advanced conformational search tool with the torsional sampling / Monte-Carlo Multiple Minimum (MCMM)[11] algorithm and MMFF minimization. The torsion between the macrocycle amide and diene was restricted by restricting the dihedral angle of the carbonyl and vinylic methyl group to -58.1º ± 5º. Extended conformer candidates were located through using the macrocycle conformational sampling tool with the OPLS_2005 force field[12] and with 4r distance dependent electrostatic treatment.

The computational procedure for the NMR calculations developed by Tantillo and co-workers was used,[13] and all reported empirically scaled NMR chemical shift calculations were performed at the SCRF-mPW1PW91/6-311+G(2d,p)//B3LYP/6-31+G(d,p) level of theory.[14,15] Therefore the conformer candidates (all within a 12.5 kJ/mol window)[16] were first subjected to optimization and frequency (opt+freq) calculations in *Gaussian 09* using B3LYP/6-31+G(d,p). Occasionally for difficult optimization cases (i.e., where the molecular geometry appears to "oscillate" and does not converge) it was necessary to stop and resume the job with calculating the analytic Hessian once using the opt=calcfc directive before proceeding with optimization using the default GEDIIS (Geometry optimization method using an Energy-represented Direct Inversion in the Iterative Subspace)[17] algorithm. Frequency lists were checked to ensure that no imaginary frequencies were present (i.e., structure does not represent a saddle point on the potential energy hypersurface). Conformer candidates with absolute *G* values that were 2.5 kcal/mol above the global minimum at 298.15 K were excluded from further calculations. Through this procedure, two candidate conformers – one C-clamp (**1a**) and one extended (**1b**) – were located, and **1a** was found to be the global minimum. NMR calculations were performed using the GIAO (gauge-independent atomic orbitals)[18] method using the SCRF-mPW1PW91/6-311+G(2d,p) level of theory, where SCRF (Self-Consistent Reaction Field) refers to inclusion of solvent effects arising from chloroform using the SMD implicit solvation model[19] with default radii. The calculated NMR isotropic shifts were empirically scaled according to the formula:

$\delta = (b - \sigma) / -m$, where δ is the calculated chemical shift referenced to TMS, σ is the calculated isotropic chemical shielding value, b is the y-intercept, and m is the slope. ^{1}H shifts used the following scaling factors: m = -1.0936, b = 31.8018, and ^{13}C shifts used the following scaling factors: m = -1.0533, b = 186.5242, as obtained from the CHESHIRE CCAT website.[13d] Methyl group proton and the carbamate nitrogen proton chemical shifts were averaged to account for chemical shift averaging on the experimental NMR timescale. The chemical shifts from both conformers were Boltzmann averaged at 298.15 K to yield the final calculated isotropic chemical shifts.

Gaussian 09 calculations were performed using the Odyssey cluster maintained by Research Computing (RC) of the Faculty of Arts and Science (FAS) at Harvard University. Comprised of multiple nodes, each node contains 4 AMD Opteron 6376 "Abu Dhabi" processors (where each processor has 8 floating point units (FPU), 16 integer cores, and 16 MB cache), 256 GB RAM, 250 GB local scratch space, and all nodes are linked with FDR Infiniband (IB) fabric. Each *Gaussian 09* calculation (either opt+freq or NMR) was executed under the CentOS 6.4 environment using a maximum of 64 integer cores and 64 GB RAM in shared memory parallel (SMP) fashion through the %nprocshared=64 and %mem=64GB directives, respectively, with non-exclusive node assignment. Opt+freq calculations would typically take 2–4 days, and NMR calculations would typically take 6 – 9 hours.

Note: 1 hartree = 1 a.u. ≈ 627.5095 kcal/mol

Computational Data

Figure S10. General atom labeling for C-clamp (**1a**) and extended (**1b**) conformer

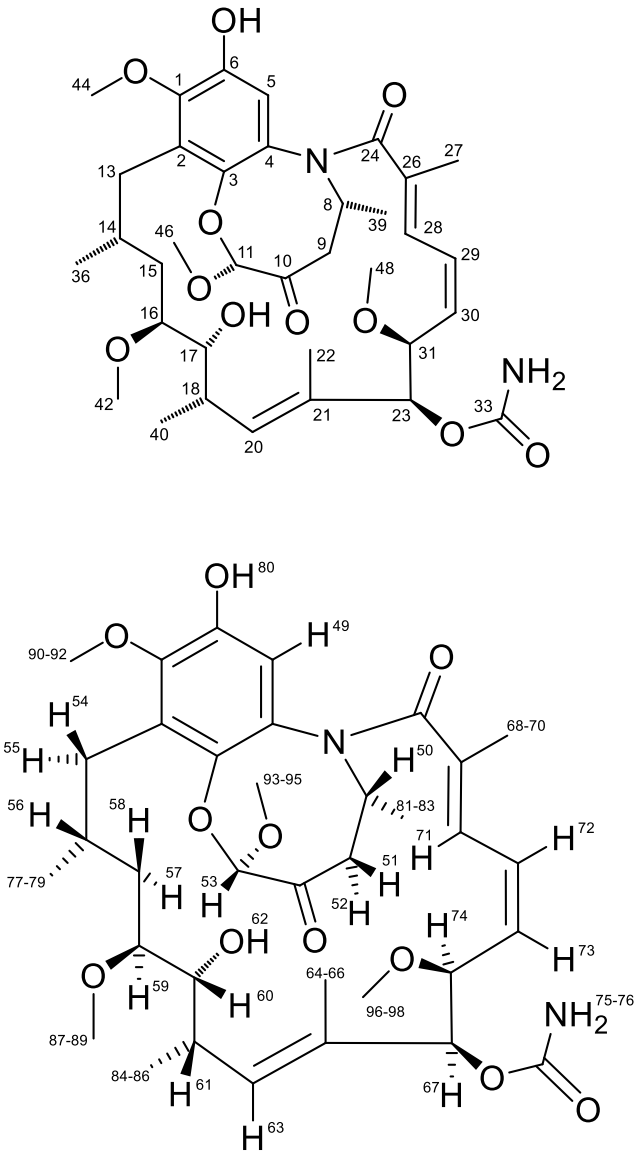


Figure S11. Geometry of C-clamp conformer **1a**

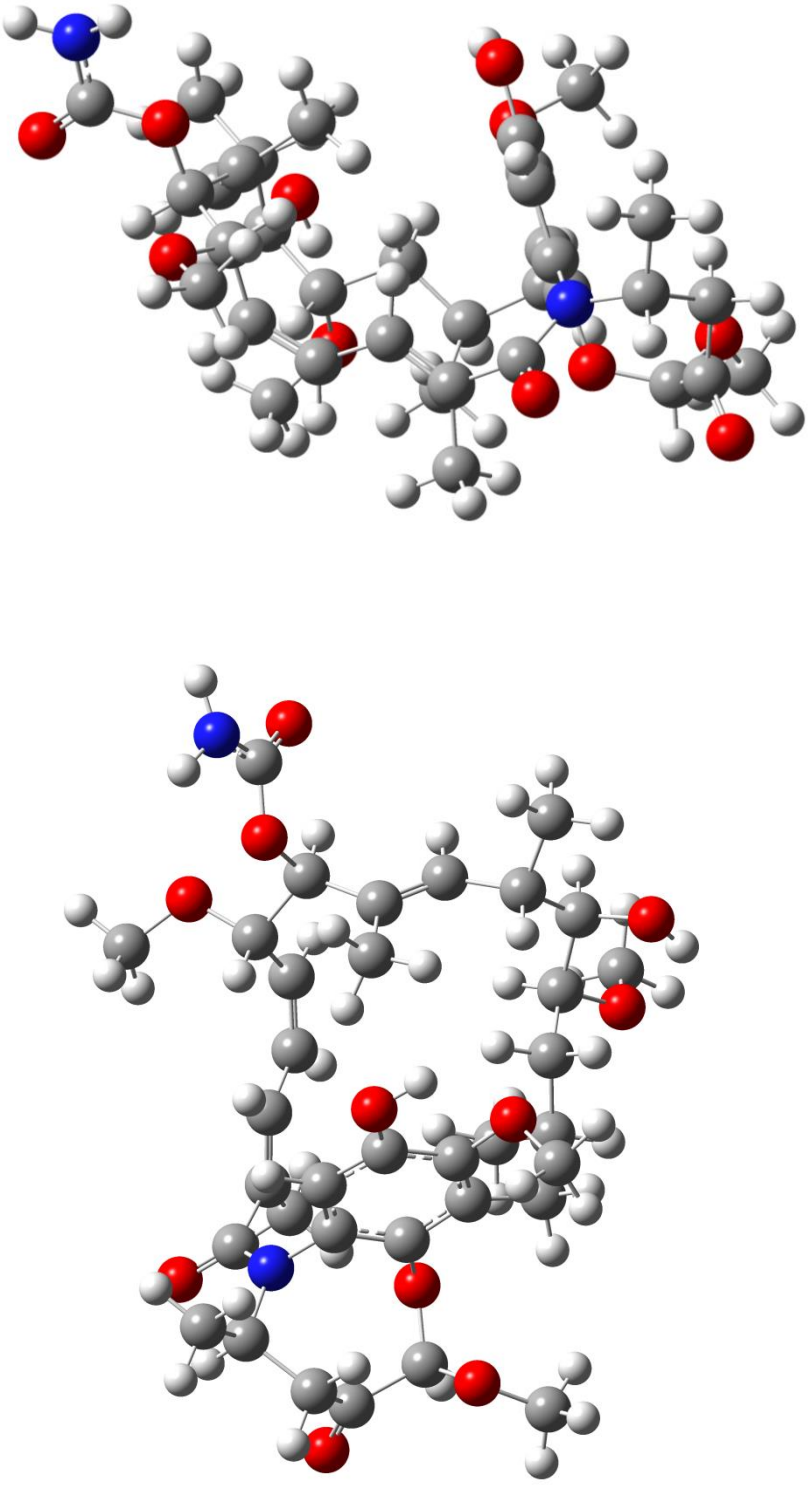


Table S7. Coordinates of C-clamp conformer **1a** (gas-phase B3LYP/6-31+G(d,p))

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	1.110497	1.195873	1.978158
2	6	0	1.822987	1.436402	0.793758
3	6	0	2.568595	0.361364	0.263100
4	6	0	2.436627	-0.937822	0.782515
5	6	0	1.652796	-1.157778	1.919648
6	6	0	1.020601	-0.086995	2.540092
7	7	0	3.138874	-2.043857	0.202258
8	6	0	4.422165	-2.460513	0.828814
9	6	0	5.331348	-1.224362	1.006400
10	6	0	5.552892	-0.540615	-0.331266
11	6	0	4.782615	0.750180	-0.682935
12	8	0	3.368049	0.579165	-0.854629
13	6	0	1.734138	2.777670	0.092484
14	6	0	0.588698	2.913349	-0.962839
15	6	0	-0.804165	2.850638	-0.303026
16	6	0	-1.994913	2.994948	-1.258714
17	6	0	-3.338092	3.274316	-0.539179
18	6	0	-3.741191	2.266890	0.556906
19	8	0	-3.288189	4.572778	0.054989
20	6	0	-3.936177	0.873651	0.004213
21	6	0	-3.437671	-0.283892	0.473027
22	6	0	-2.492212	-0.411861	1.641931
23	6	0	-3.903910	-1.592051	-0.141442
24	6	0	2.755053	-2.681557	-0.966489
25	8	0	3.369100	-3.659742	-1.391312
26	6	0	1.622808	-2.123869	-1.797013
27	6	0	2.099822	-1.715911	-3.170916
28	6	0	0.337300	-2.144040	-1.375207
29	6	0	-0.801146	-1.710715	-2.176280
30	6	0	-2.116213	-1.803748	-1.888993
31	6	0	-2.788884	-2.482216	-0.724028
32	8	0	-4.555680	-2.371003	0.898501
33	6	0	-5.906476	-2.501598	0.821833
34	8	0	-6.621351	-1.981161	-0.015738
35	7	0	-6.358282	-3.273940	1.857951
36	6	0	0.745741	1.925922	-2.127322
37	8	0	0.290887	-0.298921	3.672868
38	8	0	6.353677	-0.965271	-1.144161
39	6	0	4.243469	-3.236541	2.136840
40	6	0	-5.053584	2.722027	1.236164
41	8	0	-1.729763	4.112259	-2.134829
42	6	0	-2.403523	4.075891	-3.386045
43	8	0	0.392167	2.192800	2.627046
44	6	0	1.166318	2.989705	3.543275
45	8	0	5.105387	1.690203	0.305981
46	6	0	5.533797	2.968537	-0.172966
47	8	0	-3.442935	-3.672311	-1.181905
48	6	0	-2.569772	-4.764231	-1.428898
49	1	0	1.531788	-2.157651	2.318694
50	1	0	4.880168	-3.123066	0.092183
51	1	0	6.306927	-1.563190	1.368396
52	1	0	4.912449	-0.526697	1.737795
53	1	0	5.125234	1.063500	-1.673391
54	1	0	1.598454	3.570308	0.833727
55	1	0	2.677915	2.972733	-0.419206
56	1	0	0.704267	3.923734	-1.373651
57	1	0	-0.869886	3.626791	0.466758
58	1	0	-0.921855	1.891107	0.211436
59	1	0	-2.100221	2.085606	-1.866732
60	1	0	-4.133245	3.262043	-1.304810

61	1	0	-2.951610	2.276445	1.313531
62	1	0	-2.819393	5.141168	-0.575988
63	1	0	-4.647059	0.812251	-0.822166
64	1	0	-2.897721	-1.104286	2.387201
65	1	0	-1.521849	-0.813639	1.328751
66	1	0	-2.308694	0.544969	2.132273
67	1	0	-4.640429	-1.402983	-0.925309
68	1	0	1.284715	-1.451830	-3.844516
69	1	0	2.777454	-0.857952	-3.096048
70	1	0	2.664642	-2.541730	-3.614810
71	1	0	0.132146	-2.509998	-0.372143
72	1	0	-0.560699	-1.244359	-3.127651
73	1	0	-2.816820	-1.422282	-2.630716
74	1	0	-2.074021	-2.747608	0.067332
75	1	0	-7.314390	-3.586648	1.795642
76	1	0	-5.699817	-3.848545	2.361235
77	1	0	0.566016	0.893664	-1.811405
78	1	0	0.040555	2.161225	-2.929761
79	1	0	1.754801	1.971358	-2.547290
80	1	0	-0.206790	0.516005	3.851040
81	1	0	3.828344	-2.612536	2.934321
82	1	0	3.584281	-4.095703	1.980309
83	1	0	5.214382	-3.613374	2.475598
84	1	0	-5.882569	2.727292	0.518289
85	1	0	-5.318849	2.034236	2.044819
86	1	0	-4.947134	3.730113	1.642959
87	1	0	-2.170752	3.151105	-3.933314
88	1	0	-3.493764	4.156563	-3.279303
89	1	0	-2.042971	4.931132	-3.962428
90	1	0	0.472118	3.708019	3.982979
91	1	0	1.606332	2.365961	4.330458
92	1	0	1.966407	3.523594	3.019821
93	1	0	4.777916	3.428771	-0.819876
94	1	0	5.683438	3.593998	0.708600
95	1	0	6.480221	2.881458	-0.719916
96	1	0	-3.198058	-5.595356	-1.756342
97	1	0	-1.836159	-4.536391	-2.213231
98	1	0	-2.033190	-5.057423	-0.513736

Rotational constants (GHZ): 0.0838621 0.0490005 0.0399060

T = 298.15 K

Zero-point correction= 0.823965 (Hartree/Particle)
Thermal correction to Energy= 0.876091
Thermal correction to Enthalpy= 0.877036
Thermal correction to Gibbs Free Energy= 0.736540
Sum of electronic and zero-point Energies= -2300.036666
Sum of electronic and thermal Energies= -2299.984540
Sum of electronic and thermal Enthalpies= -2299.983596
Sum of electronic and thermal Free Energies= -2300.124091

Table S8. NMR isotropic and chemical shifts for **1a** (SCRF-mPW1PW91/6-311+G(2d,p)//B3LYP/6-31+G(d,p), SCRF = SMD, solvent = chloroform)

Atom	Isotropic	Scaled Shift	Experiment	|Δ|	Atom	Isotropic	Scaled Shift	Experiment	|Δ|
C1	33.6681	145.12	147.1	2.0	H61	29.156	2.42	2.32	0.10
C2	46.5614	132.88	130.3	2.6	H62	29.6793	1.94	n.d.	
C3	36.0143	142.89	144.2	1.3	H63	26.0777	5.23	5.11	0.12
C4	51.9767	127.74	129.5	1.8	H64	30.0107	1.64		
C5	66.3336	114.11	115.1	1.0	H65	30.8268	0.89		
C6	32.5814	146.15	n.d.	n.d.	H66	29.8489	1.79		
C8	131.5418	52.20	49.4	2.8	H67	26.4093	4.93	4.91	0.02
C9	142.8047	41.51	42.4	0.9	H68	29.5187	2.09		
C10	-27.455	203.15	202.0	1.2	H69	29.9204	1.72		
C11	75.3853	105.51	107.0	1.5	H70	29.5657	2.04		
C13	152.7922	32.03	34.4	2.4	H71	25.7183	5.56	5.75	0.19
C14	150.2484	34.44	30.8	3.6	H72	24.7874	6.41	6.22	0.19
C15	154.5783	30.33	31.5	1.2	H73	26.4414	4.90	4.96	0.06
C16	103.5401	78.78	80.6	1.8	H74	27.6591	3.79	3.81	0.02
C17	112.6345	70.15	73.4	3.2	H75	27.6937	3.76		
C18	147.1834	37.35	35.2	2.2	H76	27.2843	4.13		
C20	45.8206	133.58	134.1	0.5	H77	31.6741	0.12		
C21	45.3573	134.02	130.6	3.4	H78	30.9843	0.75		
C22	173.2706	12.58	13.0	0.4	H79	30.8349	0.88		
C23	101.8967	80.35	82.9	2.6	H80	26.0926	5.22	n.d.	
C24	7.2138	170.24	173.2	3.0	H81	30.4341	1.25		
C26	36.5841	142.35	137.6	4.8	H82	30.3024	1.37		
C27	169.7885	15.89	16.9	1.0	H83	30.4595	1.23		
C28	58.6	121.45	123.7	2.2	H84	31.0768	0.66		
C29	50.2923	129.34	130.1	0.8	H85	31.0901	0.65		
C30	53.1127	126.66	128.2	1.5	H86	30.0545	1.60		
C31	109.5591	73.07	75.4	2.3	H87	28.7537	2.79		
C33	23.4038	154.87	156.9	2.0	H88	28.1098	3.38		
C36	171.3527	14.40	16.9	2.5	H89	28.2697	3.23		
C39	166.3821	19.12	20.8	1.7	H90	27.701	3.75		
C40	167.7334	17.84	19.4	1.6	H91	28.0659	3.42		
C42	130.275	53.40	57.0	3.6	H92	27.8561	3.61		
C44	124.77	58.63	62.4	3.8	H93	27.6555	3.79		
C46	125.7968	57.65	59.7	2.0	H94	28.1152	3.37		
C48	130.6322	53.06	57.4	4.3	H95	28.0725	3.41		
H49	24.5342	6.65	6.66	0.01	H96	28.4509	3.06		
H50	26.1537	5.16	5.44	0.28	H97	28.5388	2.98		
H51	29.6112	2.00	2.39	0.39	H98	28.9191	2.64		
H52	29.2426	2.34	2.44	0.10	avg(H64–66)		1.44	1.30	0.14
H53	26.6902	4.67	4.94	0.27	avg(H68–70)		1.95	1.93	0.02
H54	28.9171	2.64	2.65	0.01	avg(H75–76)		3.94	4.54	0.60
H55	28.6106	2.92	2.97	0.05	avg(H77–79)		0.58	0.60	0.02
H56	29.438	2.16	2.22	0.06	avg(H81–83)		1.28	1.34	0.06
H57	30.0116	1.64	1.64	0.00	avg(H84–86)		0.97	1.04	0.07
H58	31.243	0.51	0.55	0.04	avg(H87–89)		3.13	3.31	0.18
H59	28.7399	2.80	2.78	0.02	avg(H90–92)		3.59	3.81	0.22
H60	27.8101	3.65	3.59	0.06	avg(H93–95)		3.52	3.57	0.05
					avg(H96–98)		2.89	3.09	0.20

Statistics with experimental data for **1a** only: ^{1}H CMAD: 0.12 ppm, Max. Deviation: 0.60 ppm (H75)
^{13}C CMAD: 2.2 ppm, Max. Deviation: 4.8 ppm (C26)

Figure S12. Geometry of extended conformer **1b**

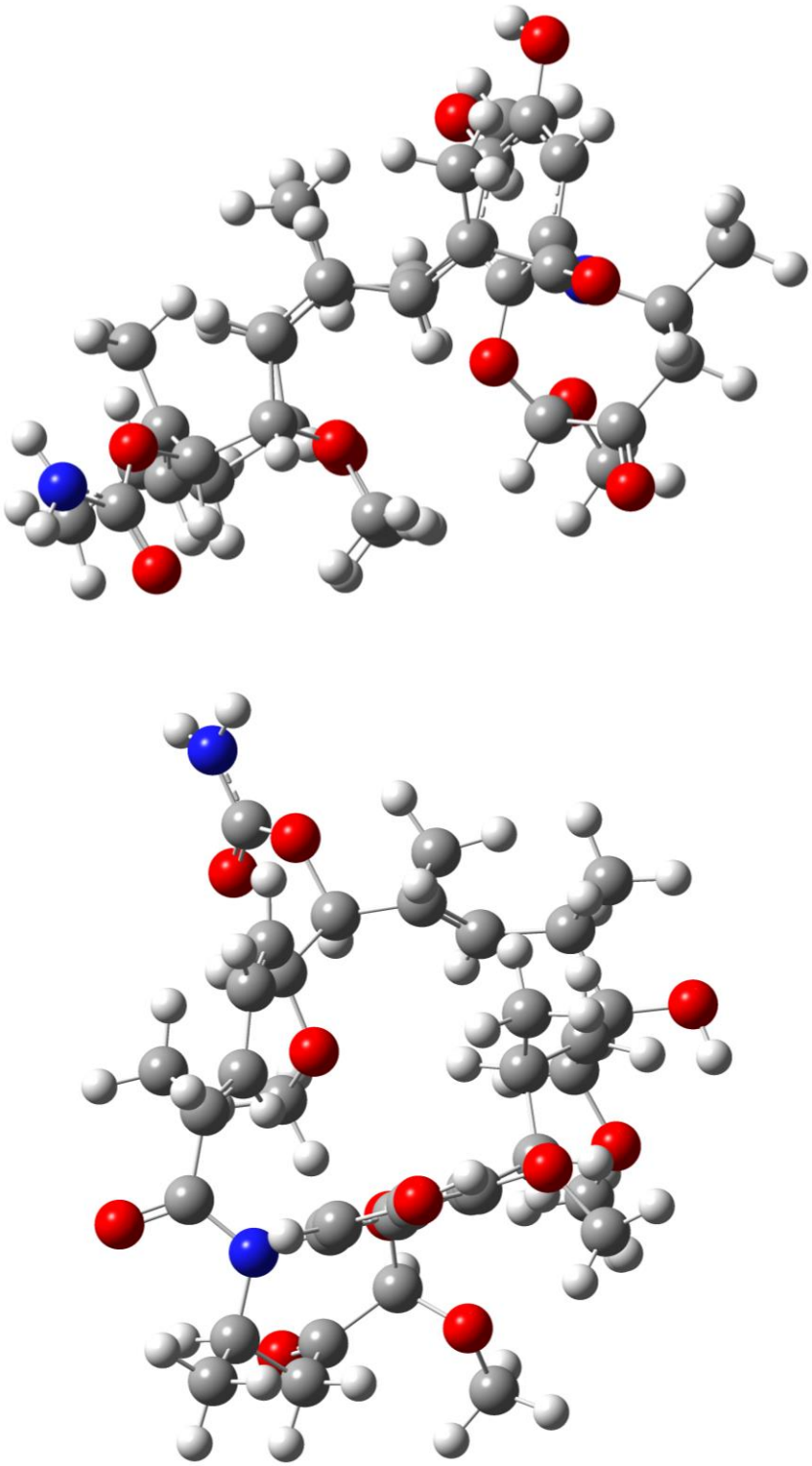


Table S9. Coordinates of extended conformer **1b** (gas-phase B3LYP/6-31+G(d,p))

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-3.152220	1.544583	-1.959123
2	6	0	-2.255974	1.452209	-0.885813
3	6	0	-2.355266	0.314090	-0.060484
4	6	0	-3.213896	-0.749375	-0.380314
5	6	0	-4.048112	-0.653082	-1.500584
6	6	0	-4.026481	0.495702	-2.283515
7	7	0	-3.265872	-1.932951	0.431317
8	6	0	-4.261538	-1.999281	1.533524
9	6	0	-4.185529	-0.726287	2.399139
10	6	0	-2.789733	-0.522544	2.955343
11	6	0	-1.873633	0.544534	2.309572
12	8	0	-1.464921	0.194028	1.004852
13	6	0	-1.145831	2.464279	-0.693460
14	6	0	0.192221	1.997416	-1.337593
15	6	0	1.367058	2.937028	-0.985146
16	6	0	1.905418	2.840944	0.448096
17	6	0	3.388796	3.248560	0.597620
18	6	0	4.403995	2.392416	-0.189708
19	8	0	3.560407	4.610718	0.202930
20	6	0	4.280262	0.932514	0.175674
21	6	0	4.059504	-0.122110	-0.629814
22	6	0	3.886367	-0.035230	-2.127919
23	6	0	4.003322	-1.494801	0.011289
24	6	0	-2.754444	-3.139088	-0.021819
25	8	0	-3.141823	-4.212427	0.441063
26	6	0	-1.681004	-3.129955	-1.081359
27	6	0	-2.032453	-3.932659	-2.308617
28	6	0	-0.471486	-2.601529	-0.780404
29	6	0	0.718800	-2.708243	-1.617592
30	6	0	2.011554	-2.521958	-1.273310
31	6	0	2.622324	-2.219234	0.071964
32	8	0	4.928794	-2.369141	-0.702454
33	6	0	5.693415	-3.203923	0.054371
34	8	0	5.656399	-3.276974	1.271010
35	7	0	6.489443	-3.968297	-0.747052
36	6	0	0.080929	1.878173	-2.866743
37	8	0	-4.840845	0.588467	-3.374614
38	8	0	-2.368276	-1.158410	3.905238
39	6	0	-5.689760	-2.252675	1.035447
40	6	0	5.840977	2.884160	0.088602
41	8	0	1.112057	3.736785	1.262181
42	6	0	1.104934	3.451459	2.652493
43	8	0	-3.168312	2.634617	-2.821696
44	6	0	-3.932930	3.757478	-2.347462
45	8	0	-2.506040	1.803787	2.288681
46	6	0	-2.643474	2.414586	3.571274
47	8	0	1.744429	-1.476723	0.907797
48	6	0	1.776398	-1.857326	2.280787
49	1	0	-4.707185	-1.471454	-1.766666
50	1	0	-3.949670	-2.850362	2.142210
51	1	0	-4.863746	-0.859708	3.247998
52	1	0	-4.506206	0.152504	1.831736
53	1	0	-0.948475	0.572645	2.900298
54	1	0	-1.424279	3.420178	-1.147434
55	1	0	-0.993226	2.645893	0.369715
56	1	0	0.424512	1.003206	-0.934440
57	1	0	1.091043	3.979860	-1.185604
58	1	0	2.185359	2.708529	-1.673870
59	1	0	1.782958	1.815326	0.824304

60	1	0	3.650277	3.148961	1.665496
61	1	0	4.201493	2.540065	-1.254059
62	1	0	2.806296	5.100915	0.563866
63	1	0	4.420883	0.727928	1.239083
64	1	0	4.497289	-0.793265	-2.628392
65	1	0	2.845970	-0.217425	-2.419341
66	1	0	4.180123	0.940932	-2.517673
67	1	0	4.356413	-1.421250	1.040581
68	1	0	-1.197702	-4.026179	-3.004231
69	1	0	-2.350382	-4.937979	-2.011812
70	1	0	-2.872399	-3.474273	-2.846509
71	1	0	-0.348740	-2.092195	0.168671
72	1	0	0.554150	-3.008775	-2.650103
73	1	0	2.753312	-2.712680	-2.045089
74	1	0	2.841178	-3.189397	0.550946
75	1	0	7.193133	-4.531848	-0.297912
76	1	0	6.581562	-3.750404	-1.726566
77	1	0	-0.133035	2.852146	-3.322976
78	1	0	1.019137	1.505977	-3.292773
79	1	0	-0.713583	1.190233	-3.167758
80	1	0	-4.570141	1.383181	-3.863727
81	1	0	-6.060106	-1.419265	0.429524
82	1	0	-5.721643	-3.169809	0.441652
83	1	0	-6.365108	-2.378133	1.888718
84	1	0	6.103311	2.746036	1.144831
85	1	0	6.560176	2.317485	-0.511021
86	1	0	5.937354	3.945818	-0.150847
87	1	0	0.798082	2.412805	2.841827
88	1	0	2.082670	3.617266	3.125105
89	1	0	0.379720	4.129846	3.108533
90	1	0	-3.866682	4.521653	-3.124199
91	1	0	-4.981591	3.478205	-2.188734
92	1	0	-3.519252	4.147037	-1.411399
93	1	0	-1.669828	2.510058	4.069726
94	1	0	-3.060060	3.407294	3.394440
95	1	0	-3.322461	1.851904	4.225122
96	1	0	2.770709	-1.719131	2.725705
97	1	0	1.059240	-1.218569	2.799817
98	1	0	1.474148	-2.905843	2.406978

Rotational constants (GHZ): 0.0801004 0.0502993 0.0418240

T = 298.15 K

Zero-point correction= 0.823321 (Hartree/Particle)
Thermal correction to Energy= 0.875688
Thermal correction to Enthalpy= 0.876632
Thermal correction to Gibbs Free Energy= 0.735523
Sum of electronic and zero-point Energies= -2300.034578
Sum of electronic and thermal Energies= -2299.982211
Sum of electronic and thermal Enthalpies= -2299.981267
Sum of electronic and thermal Free Energies= -2300.122376

Table S10. NMR isotropic and chemical shifts for **1b** (SCRF-mPW1PW91/6-311+G(2d,p)//B3LYP/6-31+G(d,p), SCRF = SMD, solvent = chloroform)

Atom	Isotropic	Scaled Shift	Experiment	|Δ|	Atom	Isotropic	Scaled Shift	Experiment	|Δ|
C1	34.2704	144.55	147.1	2.6	H61	29.0163	2.55	2.32	0.23
C2	43.9255	135.38	130.3	5.1	H62	29.8517	1.78	n.d.	n.d.
C3	37.0159	141.94	144.2	2.3	H63	25.9468	5.35	5.11	0.24
C4	50.3624	129.27	129.5	0.2	H64	30.1056	1.55		
C5	67.6551	112.85	115.1	2.2	H65	30.2583	1.41		
C6	34.2792	144.54	n.d.	n.d.	H66	29.6681	1.95		
C8	130.347	53.33	49.4	3.9	H67	26.0887	5.22	4.91	0.31
C9	144.0587	40.32	42.4	2.1	H68	29.6691	1.95		
C10	-35.4354	210.73	202.0	8.7	H69	29.7132	1.91		
C11	77.8843	103.14	107.0	3.9	H70	30.4415	1.24		
C13	152.3038	32.49	34.4	1.9	H71	24.2402	6.91	5.75	1.16
C14	146.1362	38.34	30.8	7.5	H72	25.1993	6.04	6.22	0.18
C15	149.5956	35.06	31.5	3.6	H73	25.7355	5.55	4.96	0.59
C16	97.4207	84.59	80.6	4.0	H74	27.3909	4.03	3.81	0.22
C17	111.441	71.28	73.4	2.1	H75	27.476	3.96		
C18	146.5901	37.91	35.2	2.7	H76	27.1008	4.30		
C20	44.2278	135.10	134.1	1.0	H77	31.1906	0.56		
C21	46.0994	133.32	130.6	2.7	H78	31.3	0.46		
C22	172.3616	13.45	13.0	0.4	H79	31.2964	0.46		
C23	105.3481	77.07	82.9	5.8	H80	26.0067	5.30	n.d.	n.d.
C24	5.0296	172.31	173.2	0.9	H81	30.621	1.08		
C26	42.6289	136.61	137.6	1.0	H82	30.0181	1.63		
C27	172.0248	13.77	16.9	3.1	H83	30.6611	1.04		
C28	56.534	123.41	123.7	0.3	H84	30.967	0.76		
C29	55.8195	124.09	130.1	6.0	H85	30.9455	0.78		
C30	49.0377	130.53	128.2	2.3	H86	30.017	1.63		
C31	101.6999	80.53	75.4	5.1	H87	28.4171	3.10		
C33	24.9336	153.41	156.9	3.5	H88	27.9304	3.54		
C36	166.3652	19.14	16.9	2.2	H89	28.079	3.40		
C39	168.1706	17.42	20.8	3.4	H90	27.7944	3.66		
C40	168.1099	17.48	19.4	1.9	H91	28.0595	3.42		
C42	130.1738	53.50	57.0	3.5	H92	27.8924	3.57		
C44	124.2557	59.12	62.4	3.3	H93	28.3589	3.15		
C46	131.5936	52.15	59.7	7.5	H94	28.0822	3.40		
C48	128.4689	55.12	57.4	2.3	H95	28.7646	2.78		
H49	24.7323	6.46	6.66	0.20	H96	27.9692	3.50		
H50	26.8086	4.57	5.44	0.87	H97	28.1783	3.31		
H51	29.7403	1.89	2.39	0.50	H98	28.3623	3.15		
H52	28.9562	2.60	2.44	0.16	avg(H64–66)		1.64	1.30	0.34
H53	26.664	4.70	4.94	0.24	avg(H68–70)		1.70	1.93	0.23
H54	29.5633	2.05	2.65	0.60	avg(H75-76)		4.13	4.54	0.41
H55	28.1077	3.38	2.97	0.41	avg(H77–79)		0.49	0.60	0.11
H56	30.2469	1.42	2.22	0.80	avg(H81–83)		1.25	1.34	0.09
H57	30.6226	1.08	1.64	0.56	avg(H84–86)		1.06	1.04	0.02
H58	30.2054	1.46	0.55	0.91	avg(H87–89)		3.35	3.31	0.04
H59	28.1585	3.33	2.78	0.55	avg(H90–92)		3.55	3.81	0.26
H60	27.7423	3.71	3.59	0.12	avg(H93–95)		3.11	3.57	0.46
					avg(H96–98)		3.32	3.09	0.23

Statistics with experimental data for **1b** only: ^{1}H CMAD: 0.38 ppm, Max. Deviation: 1.16 ppm (H71)
^{13}C CMAD: 3.2 ppm, Max. Deviation: 8.7 ppm (C10)

Table S11. Final Boltzmann-averaged NMR shifts for **1** (SCRF-mPW1PW91/6-311+G(2d,p)//B3LYP/6-31+G(d,p), SCRF = SMD, solvent = chloroform)

Atom	Boltzmann-averaged shift	Experiment	|Δ|	Atom	Boltzmann-averaged shift	Experiment	|Δ|
C1	145.04	147.1	2.1	C46	56.88	59.7	2.8
C2	133.23	130.3	2.9	C48	53.35	57.4	4.0
C3	142.76	144.2	1.4	H49	6.62	6.66	0.04
C4	127.95	129.5	1.5	H50	5.08	5.44	0.36
C5	113.93	115.1	1.2	H51	1.99	2.39	0.40
C6	145.93	n.d.	n.d.	H52	2.38	2.44	0.06
C8	52.36	49.4	3.0	H53	4.68	4.94	0.26
C9	41.34	42.4	1.1	H54	2.56	2.65	0.09
C10	204.21	202.0	2.2	H55	2.98	2.97	0.01
C11	105.18	107.0	1.8	H56	2.06	2.22	0.16
C13	32.09	34.4	2.3	H57	1.56	1.64	0.08
C14	34.99	30.8	4.2	H58	0.64	0.55	0.09
C15	30.99	31.5	0.5	H59	2.87	2.78	0.09
C16	79.60	80.6	1.0	H60	3.66	3.59	0.07
C17	70.31	73.4	3.1	H61	2.44	2.32	0.12
C18	37.43	35.2	2.2	H62	1.92	n.d.	n.d.
C20	133.80	134.1	0.3	H63	5.25	5.11	0.14
C21	133.92	130.6	3.3	avg (H64-66)	1.47	1.30	0.17
C22	12.70	13.0	0.3	H67	4.97	4.91	0.06
C23	79.89	82.9	3.0	avg(H68-70)	1.92	1.93	0.01
C24	170.53	173.2	2.7	H71	5.75	5.75	0.00
C26	141.55	137.6	4.0	H72	6.36	6.22	0.14
C27	15.59	16.9	1.3	H73	4.99	4.96	0.03
C28	121.73	123.7	2.0	H74	3.82	3.81	0.01
C29	128.60	130.1	1.5	avg(H75-76)	3.97	4.54	0.57
C30	127.20	128.2	1.0	avg(H77-79)	0.57	0.60	0.03
C31	74.11	75.4	1.3	H80	5.23	n.d.	n.d.
C33	154.66	156.9	2.2	avg (H81-83)	1.28	1.34	0.06
C36	15.07	16.9	1.8	avg (H84-86)	0.98	1.04	0.06
C39	18.89	20.8	1.9	avg (H87-89)	3.16	3.31	0.15
C40	17.79	19.4	1.6	avg (H90-92)	3.59	3.81	0.22
C42	53.42	57.0	3.6	avg (H93-95)	3.47	3.57	0.01
C44	58.70	62.4	3.7	avg (H96-98)	2.95	3.09	0.14

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