Supplementary material for: "Two distinct conformers of the cyclic heptapeptide phakellistatin 2 isolated from the Fijian marine sponge *Stylotella aurantium*"

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JCC/Stang

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Structure numbering used for restraints

Calculation of structures

ROEs were obtained using a T-ROESY pulse sequence with a 300 ms mixing time. ROE's were classified into weak (1.8 - 5.0 Å), medium (1.8 - 3.5 Å) and strong (1.8 - 2.5 Å) categories by contour counting. Molecular dynamics calculation were carried out using XPLOR 3.851¹, modified for swapping of prochiral groups. Throughout the calculations a forcefield with purely repulsive non bonded interaction terms (REPEL) was used, with no electrostatic potential being applied. Circular template structures with good geometry were defined, annealed at 300 K, minimised, and checked for the correct chirality and the geometry of the residues¹. These template structures were then used in the structure calculations.

Structure calculation used an *ab initio* simulated annealing strategy (YASAP 3.0)^{2,3}. Sum averaging⁴ was used for all methyl, methylene, and aromatic ring atom pairs. Assignment of prochiral groups was achieved by floating assignment and swapping of prochiral groups^{5,6}. A reduced set of non-bonded interactions and a reduced representation of the amino acid side chains was used during the conformation search phase⁷. During all stages of the simulated annealing calculation the temperature was maintained by coupling to a heat bath⁸ with a coupling frequency of 10 ps⁻¹. 100 initial starting structures were calculated by randomisation of the backbone ϕ and ψ angles using an elastic bond between 7 Pro C and 1 Phe N to relieve strain. The simulated annealing protocol was divided into four stages:

1. 60 ms of annealing at a temperature of 2000 K with a timestep of 2 fs during which the force constants for the distance restraints were increased to their final value in 5 steps. This stage of the calculation used the reduced sidechain representation consisting of a 2.25 Å radius C α atom.
2. cooling from 2000 to 1000 K over 40 ps during which force constants for non bonded interactions were increased to their final value.
3. cooling from 1000K to 100 K over 20 ps with a time step of 1 fs.
4. 200 steps of minimisation.

In all cases the lengths of restraints for methyl residues was extended by 0.5 Å to allow for the differential relaxation of methyls. A soft potential was used in the *ab initio* simulated annealing protocol for the distance restraints.

During the refinement stage the lowest energy structures from the ensemble were refined by a single round of simulated annealing with slow cooling. The high temperature step consisted of 600 ps of cooling from 1500 K to 100 K with a timestep of 3 fs followed 4000 rounds of minimisation. A square well potential was used for the distance restraint function. Clusters of low energy structures with a similar backbone geometry were selected to represent the final structures.

Calculations were carried out on a SGI O2 computer. The overlay and display of structures was achieved using Molmol.⁹

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Phakellistatin 2 (1a, more polar conformer) in CD₃OD

Table S1 ¹H (δ/ppm, proton count, multiplicity, *J* (Hz)), 400 MHz, ¹³C (δ/ppm, multiplicity), 100 MHz and 2D NMR data in CD₃OD for phakellistatin 2 (1a, more polar conformer).

No	Residue	Atom	¹³ C	¹ H	COSY ¹ H- ¹ H	HMBC C→H
1	Phe	α	55.3 (d)	4.37 (1H, dd 4.0)	H1β _A , H1β _B , NH1	H1β _A , H1β _B
		β	38.0 (t)	A. 3.14 (1H, m) B. 2.89 (1H, m)	H1α H1α, H1α [*]	H1(3/5), H1α
		1	136.5 (s)			H1(3/5), H1β _A , H1β _B
		2/6	129.7 (d)	7.20 (2H, dd, 4.4, 8.4)	H1(3/5), H1(4)	H1(2/6), H1β _A , H1β _B
		3/5	128.3 (d)	7.28 (2H, dd, 2.2, 9.6)	H1(2/6), H1(4)	H1(3/5)
		4	130.2 (d)	7.30 (1H, dd, 4.4, 8.4)	H1(2/6), H1(3/5)	H1(3/5)
		CO	172.4 (s)			H1α, H1β _A , H1β _B
		NH		8.77 (1H, bs)	H1α	
2	Pro	α	61.9 (d)	3.18 (1H, dd, 8.0)	H2β _B	
		β	31.3 (t)	A. 1.88 (1H, m) B. 0.95 (1H, m)		H2α
		γ	22.5 (t)	A. 1.60 (1H, m) B. 1.35 (1H, m)	H2γ _B H2γ _A	H2α
		δ	47.5 (t)	A. 3.42 (1H, m) B. 3.30 (1H, m)	H2δ _B H2δ _A	H2α
		CO	172.6 (s)			H2α, H2β _B
		NH		9.05 (1H, d, 8.0)	H3α	
3	Ile	α	59.4 (d)	3.72 (1H, dd, 8.4)	H3β, NH3	3γCH ₃
		β	35.3 (t)	1.83 (1H, bm)	H3α, H3δCH ₃	3γCH ₃ , H3α
		γ	26.7 (t)	A. 1.32 (1H, m) B. 1.07 (1H, m)	H3γ _B H3γ _A	3γCH ₃ , 3δCH ₃ , H3α
		γCH ₃	15.9 (q)	0.69 (3H, d, 6.4)	H3β	
		δCH ₃	10.3 (q)	0.76 (3H, d, 6.8)		
		CO	172.6 (s)			H5α
4	Ile	NH		9.05 (1H, d, 8.0)	H3α	
		α	55.4 (d)	4.30 (1H, dd, 9.2)	H4β, NH4	4γCH ₃ , 4δCH ₃
		β	37.3 (d)	1.73 (1H, m)	H4α	H4α, 4γCH ₃ , 4δCH ₃
		γ	25.7 (t)	A. 1.62 (1H, m) B. 1.14 (1H, m)	H4γ _B H4γ _A	H4α, 4γCH ₃ , 4δCH ₃
		γCH ₃	14.8 (q)	0.85 (3H, d, 6.8)		
		δCH ₃	10.6 (q)	0.85 (3H, d, 7.2)		
5	Pro	CO	174.1 (s)			H5α
		NH		7.95 (1H, d, 8.8)	H4α	
		α	62.8 (d)	4.51 (1H, d, 8.0)	H5β _B	
		β	32.4 (t)	A. 2.22 (1H, m) B. 2.10 (1H, m)		H5α
		γ	22.6 (t)	A. 1.86 (1H, m) B. 1.60 (1H, m)	H5α H5γ _B	H5α
		δ	47.3 (t)	A. 3.54 (1H, m) B. 3.42 (1H, m)	H5δ _B H5δ _A	H5α
6	Tyr	CO	172.8 (s)			
		α	53.9 (d)	4.62 (1H, bm)	H6β, NH6	
		β	37.8 (t)	3.07 (2H, m)	H6α	H6(3/5)
		1	127.0 (s)			H6α, H6(2/6), H6β
		2/6	115.8 (d)	6.66 (2H, d, 8.8)	H6(3/5)	
		3/5	131.6 (d)	6.85 (2H, d, 8.4)	H6(2/6)	H6β
		4	157.4 (s)			H6(2/6), H6(3/5)
		CO	170.1 (s)			H6α, H6β
7	Pro	NH		6.76 (1H, d, 4.8)	H6α	
		α	59.5 (d)	4.40 (1H, dd, 8.0)	H7β _B , H7δ _A	
		β	31.3 (t)	A. 2.05 (1H, m) B. 1.80 (1H, m)		H7α
		γ	22.2 (t)	2.05 (1H, m) 1.80 (1H, m)	H7α	
		δ	48.0 (t)	A. 3.54 (1H, m) B. 3.35 (1H, m)	H7δ _B H7δ _A	H7α, H74β, H7α
		CO	172.4 (s)			

Table S2 Restraints used for nOe restrained structure calculation of phakellistatin 2 (**1a**, more polar conformer) in CD₃OD.

Residue	Atom 1	Residue	Atom 2	NOE (Å)
1	HD#	1	HA	5.000
1	HD#	1	HB1	3.500
1	HD#	1	HB2	3.500
1	HE#	1	HB1	5.000
1	HN	1	HB1	5.000
1	HA	2	HA	2.500
1	HD#	2	HD2	5.000
1	HD#	2	HB1	5.000
1	HE#	2	HA	5.000
1	HE#	2	HB1	5.000
1	HB2	7	HD1	5.000
1	HN	7	HA	3.500
2	HB1	2	HG2	3.500
2	HD1	2	HG1	3.500
2	HD2	2	HB2	5.000
2	HG1	2	HG2	2.500
2	HG1	2	HB2	3.500
2	HB2	3	HG2#	5.500
3	HD1#	2	HG2	5.500
3	HN	2	HA	5.000
3	HA	3	HG2#	4.000
3	HA	3	HD1#	5.500
3	HA	3	HG12	5.000
3	HA	3	HG11	5.000
3	HB	3	HD1#	4.000
3	HB	3	HG11	3.500
3	HG12	3	HG2#	5.500
3	HG2#	3	HG11	5.500
3	HG2#	3	HD1#	6.000
3	HN	3	HA	5.000
3	HG12	4	HD1#	5.500
3	HG2#	4	HB	5.500
3	HA	6	HA	5.000
4	HA	3	HG12	5.000
4	HA	3	HA	5.000
4	HG11	3	HG2#	5.500
4	HN	3	HA	3.500
4	HA	4	HG2# or 4HD#	4.00
4	HB	4	HG12	3.500
4	HB	4	HG2# or 4HD#	4.000
4	HG12	4	HG2# or 4HD1#	4.000
4	HN	4	HA	5.000
4	HN	4	HB	3.500
4	HB	5	HA	5.000
4	HG2#	5	HB1	5.500
4	HN	5	HD1	5.000
4	HA	6	HA	5.000
4	HD1#	6	HB#	5.500
5	HA	4	HG2#	4.000
5	HA	4	HD1#	4.000
5	HA	4	HA	2.500
5	HG1	4	HG2#	5.500
5	HG2	4	HG2#	3.500

5	HA	5	HB2	3.500
5	HA	5	HB1	3.500
5	HA	5	HG2	5.000
5	HB1	5	HB2	2.500
5	HB1	5	HG1	3.500
5	HB2	5	HG2	5.000
5	HD1	5	HG2	5.000
5	HD1	5	HD2	2.500
5	HD2	5	HG1	3.500
5	HA	6	HA	5.000
5	HA	6	HB#	5.000
6	HN	4	HA	5.000
6	HN	4	HG11	5.000
6	HD#	5	HB1	5.000
6	HN	5	HD1	3.500
6	HN	5	HD2	5.000
6	HD#	6	HA	5.000
6	HD#	6	HB#	3.500
6	HN	6	HA	5.000
6	HN	6	HB#	3.500
6	HA	7	HD2	5.000
6	HD#	7	HD2	5.000
6	HE#	7	HB2	5.000
7	HA	6	HB#	3.500
7	HA	6	HA	2.500
7	HA	7	HB1	3.500
7	HA	7	HB2	3.500
7	HA	7	HD2	5.00
7	HD1	7	HG1	3.500
7	HD1	7	HD2	2.500
7	HD2	7	HG2	3.500
7	HG1	7	HG2	2.500

Table S3 Energies for phakellistatin 2 (1a, more polar conformer) minimum energy conformations in CD₃OD.

E _{total}	E _{bonds}	E _{angle}	E _{impropers}	E _{van der Waals}	E _{nOe}
10.60	1.33	5.47	0.34	2.77	0.67

No NOE violations

Table S4 Statistics for 46 lowest energy structures of phakellistatin 2 (1a, more polar conformer) in CD₃OD.

Average global displacements				Average local RMSDs	Average local displacements		
Res.	BB	Heavy	Heavysc	BB	BB	Heavy	Heavysc
1 Phe	0.04	0.74	0.92	0.00	0.00	0.00	0.00
2 Pro	0.05	0.09	0.09	0.03	0.03	0.07	0.06
3 Ile	0.05	0.36	0.51	0.04	0.03	0.36	0.50
4 Ile	0.04	0.08	0.11	0.02	0.02	0.07	0.09
5 Pro	0.04	0.05	0.06	0.02	0.01	0.02	0.02
6 Tyr	0.04	0.74	0.90	0.02	0.02	0.73	0.89
7 Pro	0.04	0.06	0.07	0.00	0.00	0.00	0.00

Phakellistatin 2 (1b, less polar conformer) in CD₃OD**Table S5** Torsion angle restraints used for structure calculation of phakellistatin 2 (less polar conformer) in CD₃OD.

	<i>J</i> /Hz	Angle (deg.)
1HN-1N-1CA-1HA	1.5	107 ± 30
3HN-3N-3CA-3HA	7.9	148 ± 30
4HN-4N-4CA-4HA	9.3	151 ± 30
6HN-6N-6CA-6HA	4.8	128 ± 30

Table S6 Restraints used for nOe restrained structure calculation of phakellistatin 2 (1b, less polar conformer) in CD₃OD.

Residue	Atom 1	Residue	Atom 2	NOE (Å)
1	HD#	1	HA	3.500
1	HD#	1	HB1	3.500
1	HE#	1	HA	5.000
1	HE#	1	HB1	5.000
1	HA	2	HA	2.500
1	HD#	2	HA	3.500
1	HD#	2	HD2	3.500
1	HE#	2	HA	5.000
1	HE#	2	HB2	5.000
2	HA	2	HB1	5.000
2	HA	2	HG1	5.000
2	HA	2	HB2	3.500
2	HD1	2	HB2	5.000
2	HD1	2	HG1	3.500
2	HD1	2	HG2	5.000
3	HA	3	HG11	5.000
3	HA	3	HG12	3.500
3	HA	3	HD1#	5.500
3	HA	3	HG2#	4.000
3	HB	3	HG2#	4.000
3	HD1#	3	HG11	4.000
3	HD1#	3	HG12	4.000
3	HG11	3	HB	3.500
4	HA	4	HG11	5.000
4	HA	4	HD1#	4.000
4	HD1#	4	HG12	4.000
4	HG2#	5	HB1	5.500
5	HA	4	HA	2.500
5	HA	4	HB	5.000
5	HA	4	HD1#	4.000
5	HG1	4	HG12	5.000
5	HA	5	HB1	3.500
5	HA	5	HB2	3.500
5	HA	5	HG1	5.000
5	HB1	5	HG1	3.500
5	HB1	5	HG2	5.000
5	HD1	5	HG1	3.500
5	HD2	5	HB2	5.000
5	HD2	5	HG1	5.000
5	HD2	5	HG2	3.500
6	HD#	6	HA	5.000
6	HD#	6	HB#	3.500
6	HE#	6	HB#	5.000
6	HD#	7	HA	5.000
6	HD#	7	HD2	5.000

6	HE#	7	HD2	5.000
7	HA	3	HB	5.000
7	HG1	5	HG2	5.000
7	HA	6	HA	3.500
7	HA	6	HB1	3.500
7	HA	7	HB1	3.500
7	HA	7	HG2	5.000
7	HD1	7	HG1	3.500
7	HD2	7	HB2	5.000
7	HD2	7	HG2	3.500

Table S7 Energies for phakellistatin 2 (**1b**, less polar conformer) minimum energy conformations in CD₃OD.

E _{total}	E _{bonds}	E _{angle}	E _{improper}	E _{van der Waals}	E _{NOE}
14.18	1.33	7.75	0.62	2.76	1.72

Table S8 NOE violations for phakellistatin 2 (**1b**, less polar conformer) in CD₃OD.

Restraint	Actual distance (Å)	Restraint distance (Å)	E _{NOE}
1PheHD1/1PheHD2-2ProHD2	3.606	3.50	0.563
7ProHA-6TyrHA	3.622	3.50	0.746

Table S9 Statistics for 35 lowest energy structures of phakellistatin 2 (**1b**, less polar conformer) in CD₃OD.

Average global displacements				Average local RMSDs	Average local displacements		
Res #	BB	Heavy	Heavysc	BB	BB	Heavy	Heavysc
Name							
1 Phe	0.05	0.60	0.75	0.00	0.00	0.00	0.00
2 Pro	0.08	0.15	0.11	0.06	0.05	0.13	0.11
3 Ile	0.10	0.36	0.44	0.08	0.09	0.36	0.45
4 Ile	0.07	0.24	0.33	0.06	0.06	0.24	0.33
5 Pro	0.02	0.03	0.03	0.03	0.01	0.01	0.01
6 Tyr	0.03	0.69	0.84	0.01	0.00	0.69	0.84
7 Pro	0.02	0.02	0.02	0.00	0.00	0.00	0.00

Phakellistatin 2 (1c, less polar conformer) in CDCl₃**Table S10** Restraints used for nOe restrained structure calculation of phakellistatin 2 (less polar conformer) in CDCl₃.

Residue	Atom 1	Residue	Atom 2	NOE (Å)
1	HA	1	HB2	5.000
1	HA	1	HB1	3.500
1	HB1	1	HB2	2.500
1	HN	1	HB2	3.500
1	HN	1	HA	5.000
1	HA	2	HD2	5.000
1	HA	2	HD1	3.500
1	HD#	6	HB#	5.000
1	HD#	6	HA	5.000
1	HE#	6	HB#	5.000
1	HN	6	HA	5.000
1	HN	6	HD#	5.000
1	HD#	7	HA	5.000
1	HD#	7	HD1	5.000
1	HD#	7	HD2	5.000
1	HN	7	HD2	5.000
2	HD2	1	HB1	5.000
2	HA	2	HD1	5.000
2	HA	2	HG1	5.000
2	HA	2	HG2	5.000
2	HB1	2	HA	5.000
2	HB1	2	HD1	5.000
2	HB1	2	HB2	2.500
2	HB2	2	HD2	5.000
2	HD1	2	HD2	2.500
2	HG2	2	HD1	5.000
2	HG2	2	HD2	5.000
3	HD1#	2	HD1	5.500
3	HB	3	HA	3.500
3	HG11	3	HA	5.000
3	HG12	3	HA	5.000
3	HG2#	3	HA	5.500
3	HN	3	HA	5.000
3	HN	4	HA	5.000
3	HD1#	5	HB1	5.500
3	HG2#	5	HB1	5.500
3	HN	5	HD1	5.000
4	HN	1	HB1	5.000
4	HN	2	HD2	5.000
4	HN	2	HA	5.000
4	HN	3	HD1#	5.500
4	HA	4	HG2#	5.500
4	HB	4	HA	5.000
4	HN	4	HG2#	5.500
4	HN	4	HA	5.000
4	HB	5	HD1	5.000
4	HB	5	HA	5.000
4	HD1#	6	HA	5.500
5	HA	4	HA	3.500
5	HA	4	HG2#	5.500
5	HB1	4	HB	5.000
5	HB2	4	HB	5.000
5	HA	5	HD1	5.000
5	HG1	5	HD1	5.000
5	HG1	5	HD2	5.000

5	HG2	5	HD1	5.000
5	HG2	5	HD2	5.000
6	HD#	1	HB2	3.500
6	HD#	1	HB1	5.000
6	HD#	1	HA	5.000
6	HE#	1	HB2	5.000
6	HE#	3	HG2#	5.500
6	HD#	5	HA	5.000
6	HA	6	HB#	5.000
6	HN	6	HB#	5.000
6	HA	7	HD1	5.000
6	HD#	7	HD2	5.000
6	HE#	7	HD2	5.000
7	HD2	1	HB2	5.000
7	HA	6	HA	2.500
7	HB1	7	HA	5.000
7	HB1	7	HD2	5.000
7	HB1	7	HB2	2.500
7	HB2	7	HA	5.00
7	HB2	7	HD2	5.000
7	HD1	7	HD2	2.500
7	HG1	7	HA	5.00
7	HG1	7	HD2	5.000
7	HG2	7	HD2	5.000
7	HG2	7	HA	5.00

Table S11 Energies for phakellistatin 2 (**1c**, less polar conformer) in CDCl₃.

E _{total}	E _{bonds}	E _{angle}	E _{improper}	E _{van der Waals}	E _{nOe}
13.66	1.79	9.26	0.94	0.60	1.07

No NOE violations

Table S12 Statistics for 66 lowest energy structures of phakellistatin 2 (**1c**, less polar conformer) in CDCl₃.

Average global displacements				Average local RMSDs	Average local displacements			
Res	BB	Heavy	Heavysc	BB				
1 Phe	1	0.06	0.73	0.91	0.00	0.00	0.00	0.00
2 Pro	2	0.04	0.05	0.04	0.03	0.01	0.03	0.03
3 Ile	3	0.07	0.96	1.35	0.04	0.04	0.93	1.31
4 Ile	4	0.07	0.44	0.62	0.04	0.03	0.42	0.59
5 Pro	5	0.05	0.08	0.09	0.03	0.03	0.05	0.04
6 Tyr	6	0.06	0.72	0.89	0.03	0.02	0.70	0.86
7 Pro	7	0.04	0.07	0.08	0.00	0.00	0.00	0.00