SUPPORTING INFORMATION

Cytochalasins from an Australian marine sediment-derived *Phomopsis* sp. (CMB-M0042F): Acid-mediated intra-molecular cycloadditions enhance chemical diversity

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1 Taxonomy of CMB-M0042F

Genomic DNA of CMB-M0042F was extracted from the mycelia using the DNeasy Plant Mini Kit (Qiagen) as per the manufacturers protocol. The rRNA genes were amplified by PCR using the universal primers ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) purchased from Sigma-Aldrich. The PCR mixture (50 μL) contained 1 μL of genomic DNA (20–40 ng), 200 μM of each deoxynucleoside triphosphate (dNTP), 1.5 mM $MgCl_2$, 0.3 μM of each primer, 1 U of *Taq* DNA polymerase (Fisher Biotec) and 5 μl of PCR buffer. PCR was performed using the following conditions: initial denaturation at 95 °C for 3 min, 30 cycles in series of 94 °C for 30 s (denaturation), 55 °C for 60 s (annealing) and 72 °C for 60 s (extension), followed by one cycle at 72 °C for 6 min. The PCR products were purified with PCR purification kit (Qiagen) and sequenced. The BLAST search showed the amplified ITS sequence (GenBank accession no. KU641387) has 99% homology with other members of the genus *Phomopsis* sp.

ITS gene sequence of CMB-M0042F

GGCGCACCCAGAAACCCTTTGTGAACTTATACCTTACTGTTGCCTCGGCGTACGC
TGGCCCCTAGGGGTCCCTCTTGTCTCAAGAGGAGCAGGCGCGCCGGCGGCCAAG
TTAACTCTTGTTTTTACACTGAAACTCTGAGAAAAAAAACAAAATGAATCAAAA
CTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGC
GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCTCCGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTC
AAGCCTGGCTTGGTGTTGGGGCACTGCCTGTAAAAGGGCAGGCCCTCAAATCTA
GTGGCGAGCTCGCCAGGACCCCGAGCGTAGTAGTTAAACCCTCGCTTTGGAAGG
CCCTGGCGGTGCCCTGCCGTTAAACCCCCAACTTTTGAAAATTTGACCTCGGATC
AGGTAGGAATACCCGCTGAACTTAAGCATA (519 bp)

BLAST search of CMB-M0042F (closest match)

Phomopsis sp. EXH1_17 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: gb|KF227860.1| Length: 560 Number of Matches: 1

Related Information

Range 1: 75 to 560 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
863 bits(467)	0.0	480/486(99%)	1/486(0%)	Plus/Plus

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            ||||||
Sbjct  555  ACCTCG  560
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Phomopsis sp. EXH1_17 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: KF227860.1

FASTA Graphics

Go to:

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VERSION     KF227860.1  GI:632125214
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SOURCE      Phomopsis sp. EXH1_17
  ORGANISM  Phomopsis sp. EXH1_17
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            Sordariomycetes; Sordariomycetidae; Diaporthales; Valsaceae;
            Phomopsis.
REFERENCE   1  (bases 1 to 560)
  AUTHORS   Rachel,M.R. and John,D.D.W.
  TITLE     Biodiversity of fungal endophytes in Semi evergreen vine thickets
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 560)
  AUTHORS   Rachel,M.R. and John,D.D.W.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-JUN-2013) Biological and Physical Sciences,
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2 Cultivation of CMB-M0042F

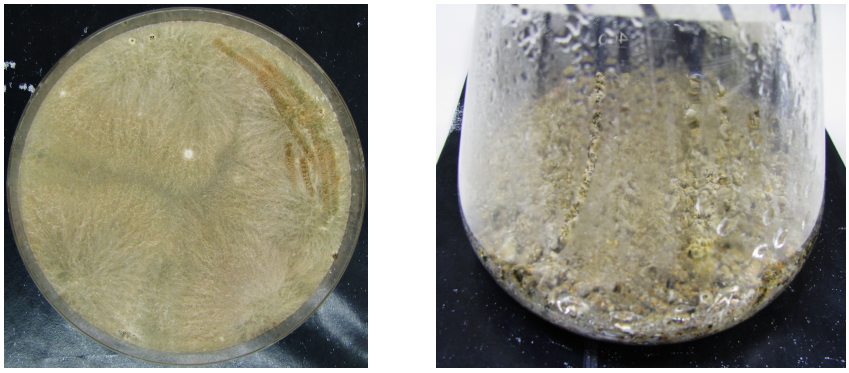

Figure S1. Colony photos of fungus *Phomopsis* sp. CMB-M0042F on saline M1 agar plates (left) and saline rice solid media (right) after 25 days' cultivation at 26.5 °C

3 HPLC chromatograms

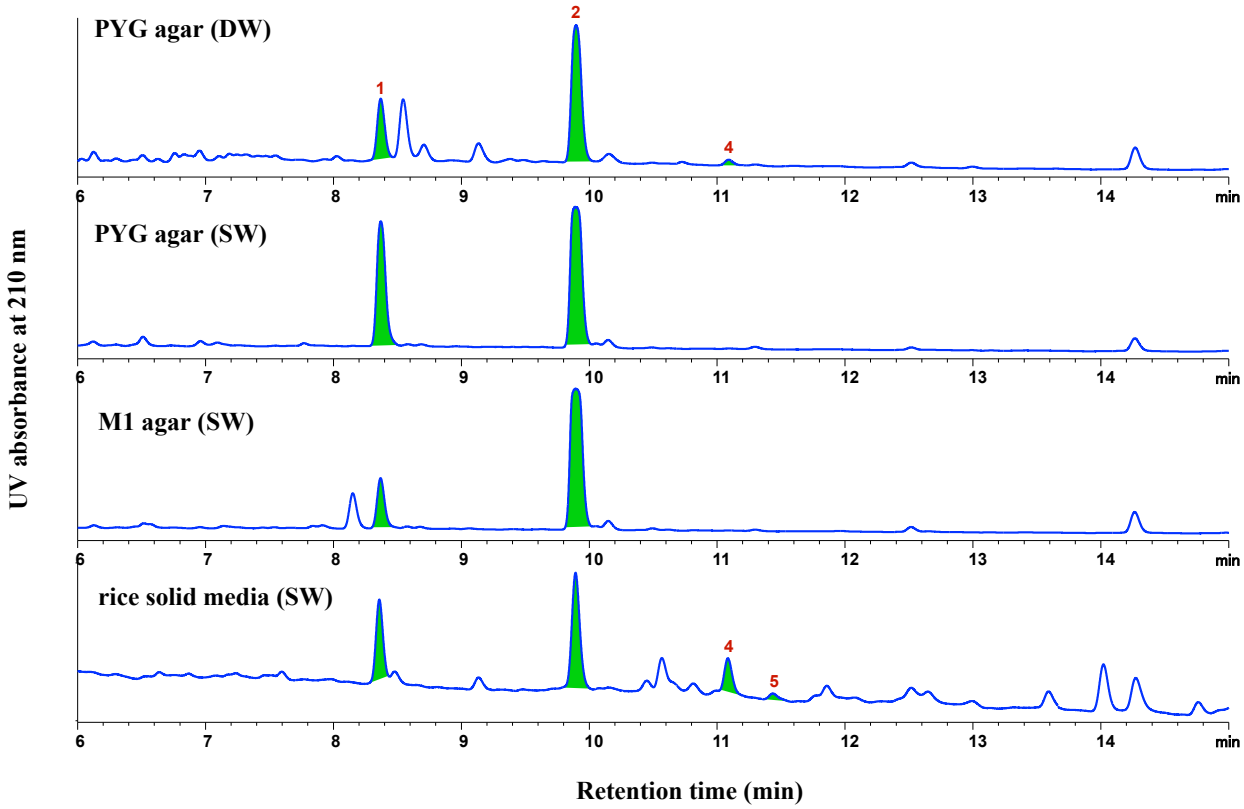

Figure S2. HPLC-DAD profiling (210 nm) of the EtOAc crude extract of fungus *Phomopsis* sp. (CMB-M0042F) on different culture media

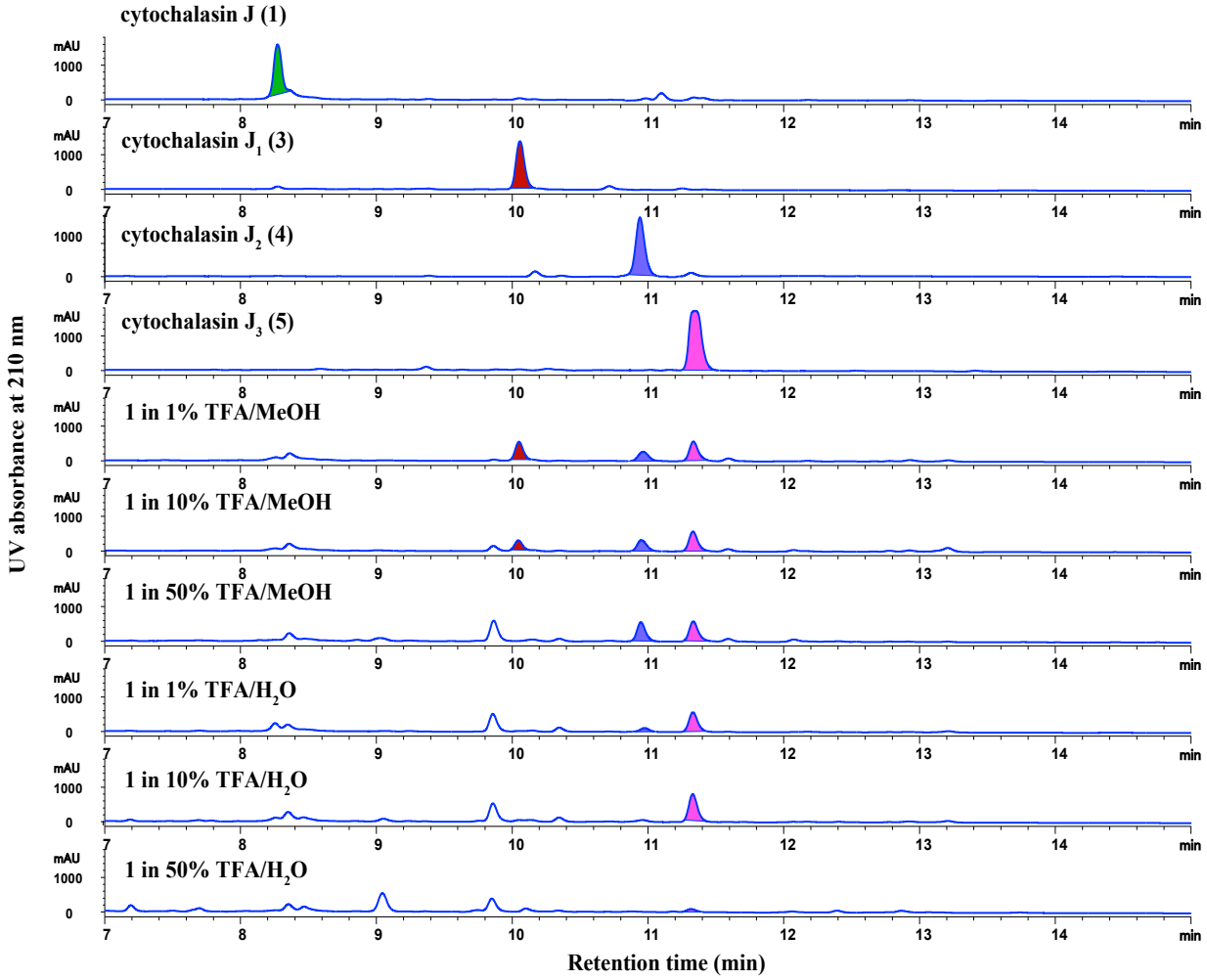

Figure S3. HPLC-DAD profiles for the TFA treatments of cytochalasin J (**1**)

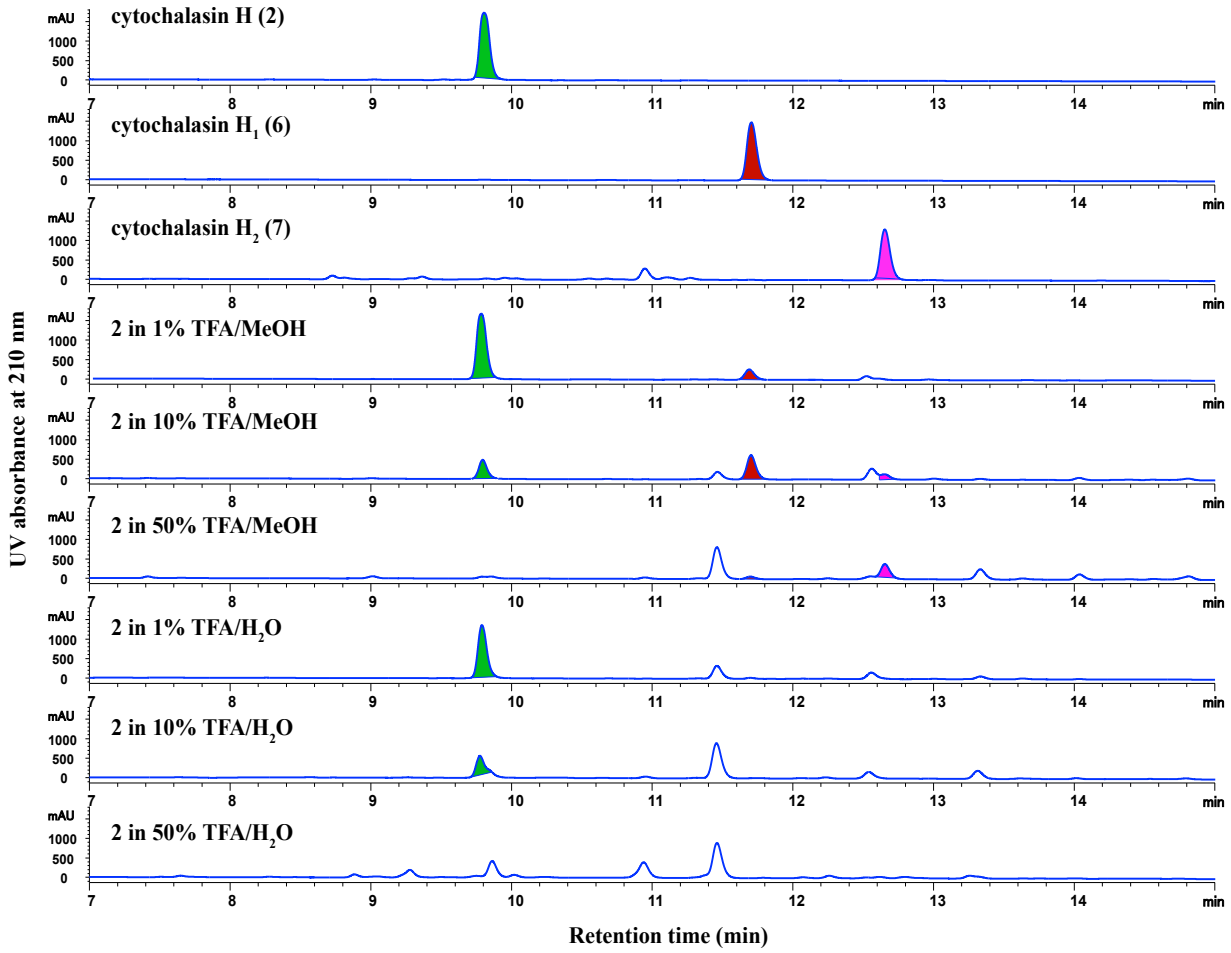

Figure S4. HPLC-DAD profiles for the TFA treatments of cytochalasin H (**2**)

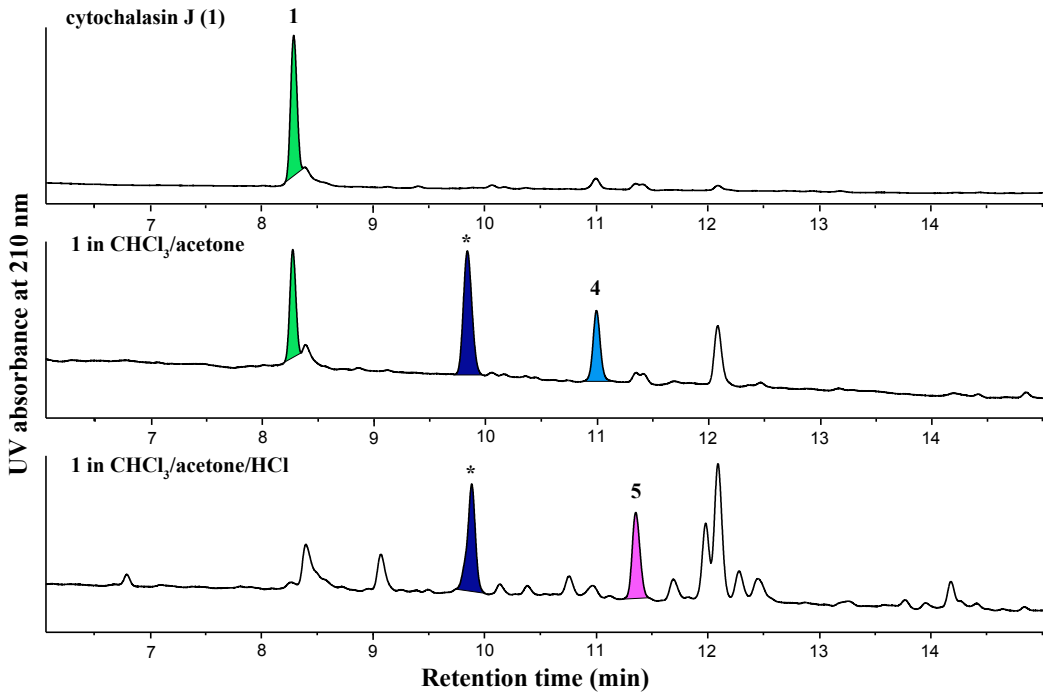

Figure S5. HPLC-DAD profiles for the the $CHCl_3$/acetone/acid treatment of cytochalasin J (**1**)

Peak * shows *m/z* = 450 $[M+H]^+$

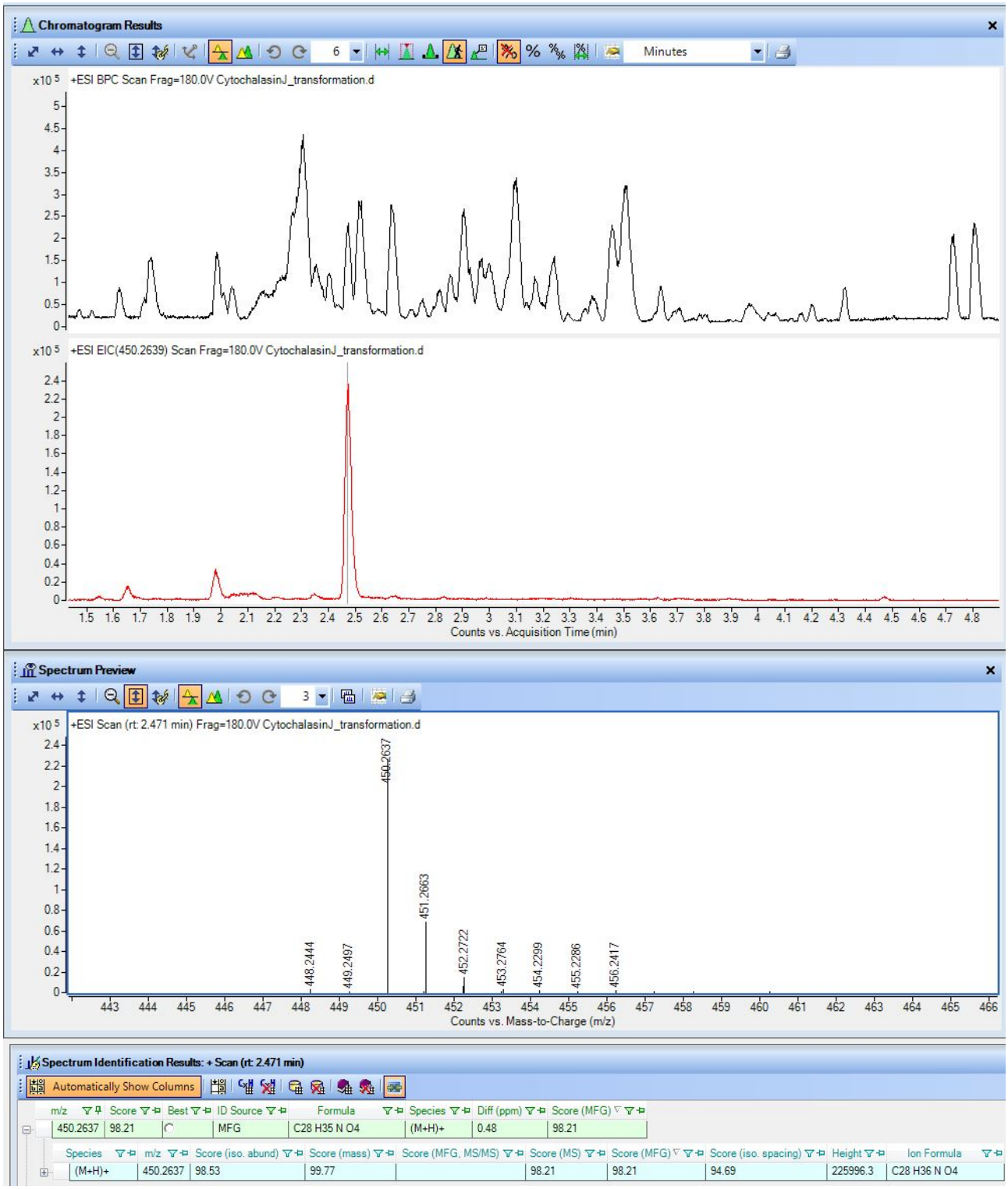

Figure S6. HPLC-QTOF profiles for the $CHCl_3$/acetone/acid treatment of cytochalasin J (**1**)

*Extracted ion chromatogram at 450.2637 gives MF of $C_{28}H_{35}NO_4$ (matched that of phomopchalasin B)

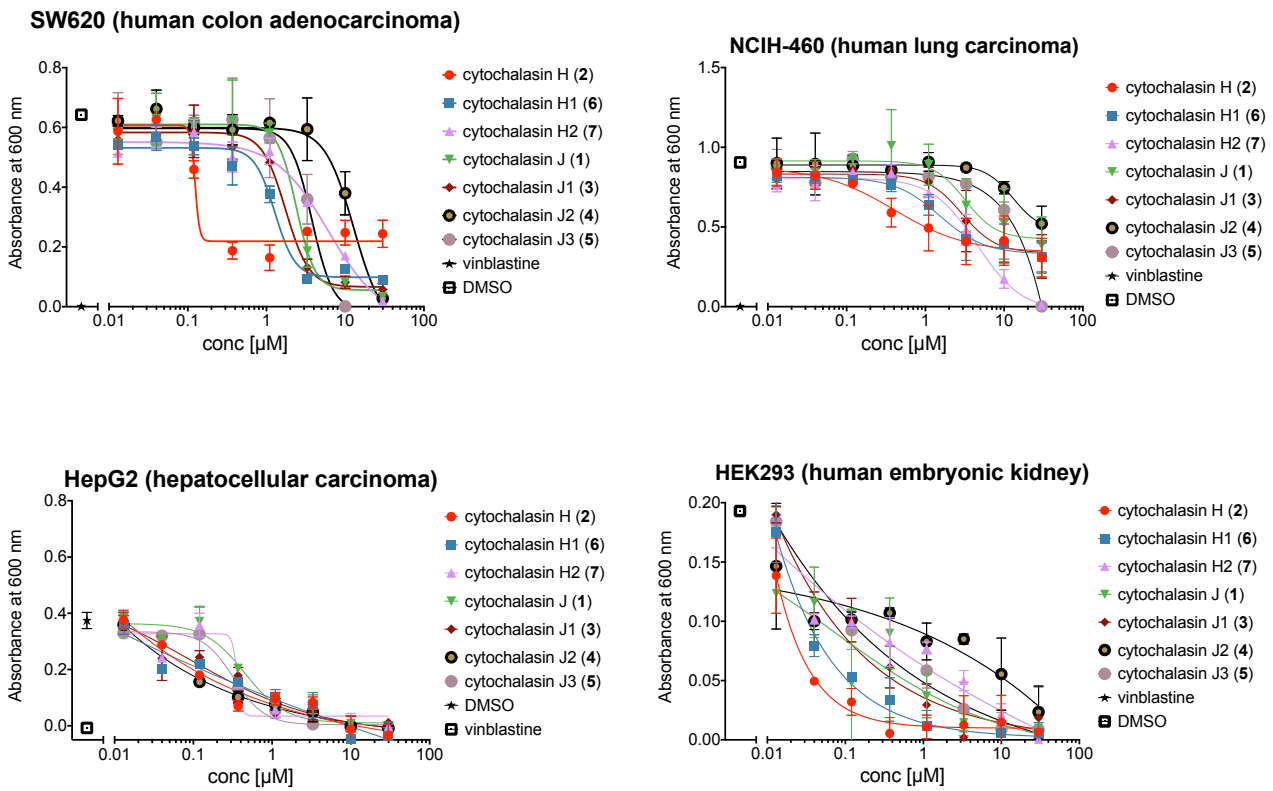

Figure S7. Cytotoxicity assay of the cytochalasins (**1**–**7**) against SW620 (human colon cancer cell line), NCIH-460 (human lung carcinoma), HepG2 (hepatocellular carcinoma) and HEK293 (human embryonic kidney) cell lines

4 Tabulated NMR data and NMR spectra

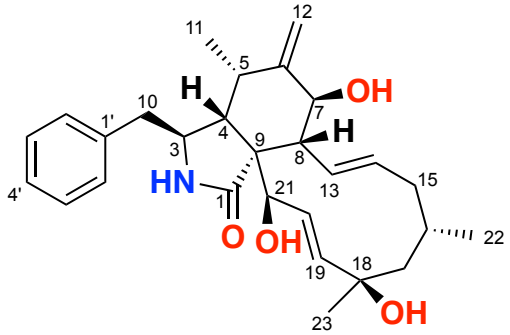

Table S1. NMR (600 MHz, $CDCl_3$) data for cytochalasin J (**1**)

Pos.	δ_H (mult., J (Hz))	δ_C	COSY	HMBC (^{1}H to ^{13}C)
1	–	176.1	–	–
2-NH	5.73 (br s)	–	–	4, 9
3	3.31 (m)	54.1	4, 10a, 10b	5, 1'
4	2.61 (m)[d]	50.3	3, 5	1, 3, 5, 6, 9, 10, 21
5	2.92 (m)[b]	33.1	4, 11	6, 7, 9, 11
6	–	148.5	–	–
7	3.83 (d, 10.7)	70.0	8, 12a, 12b	5, 6, 12, 13
8	2.88 (m)[b]	46.0	7, 13	1, 7, 9, 13, 14, 21
9	–	53.1	–	–
10	a 2.92 (m)[b]	45.7	3, 10b	3, 4, 1', 2'/6'
	b 2.59 (dd, 13.6, 8.2)[d]		3, 10a	3, 1', 2'/6'
11	1.11 (d, 6.7)	14.2	5	4, 5, 6
12	a 5.34 (s)[a]	114.1	7	5, 6, 7
	b 5.12 (s)		7	5, 6, 7
13	5.73 (dd, 15.5, 9.8)[c]	127.9	8, 14	8, 9, 15
14	5.33 (m)[a]	138.1	13, 15a, 15b	8, 15
15	a 2.02 (m)	42.8	14, 15b, 16	13, 14, 16, 17, 22
	b 1.81 (m)[e]		14, 15a, 16	13, 14, 16, 17
16	1.80 (m)[e]	28.6	15a, 15b, 17, 22	–
17	a 1.90 (dd, 14.4, 3.4)	53.9	16, 17b	15, 16
	b 1.57 (dd, 14.4, 3.3)		16, 17a	16, 18, 19, 22, 23
18	–	74.7	–	–
19	5.75 (dd, 16.8, 2.2)[c]	137.2	20	21, 23
20	6.03 (dd, 16.8, 2.6)	131.2	19, 21	18, 21
21	4.08 (dd, 2.6, 2.2)	76.8	20	4, 8, 9, 19, 20
22	1.04 (d, 6.6)	26.6	16	15, 16, 17
23	1.36 (s)	31.3	–	17, 18, 19
1'	–	137.6	–	–
2'/6'	7.15 (dd, 8.0, 1.3)	129.3	3'/5'	10, 2'/6', 4'
3'/5'	7.32 (dd, 8.0, 7.1)	129.0	2'/6', 4'	1', 3'/5'
4'	7.24 (dd, 7.1, 1.3)	127.3	3'/5'	2'/6'

[a,b,c,d,e] Overlapping signals and assignments were supported by HSQC and HMBC.

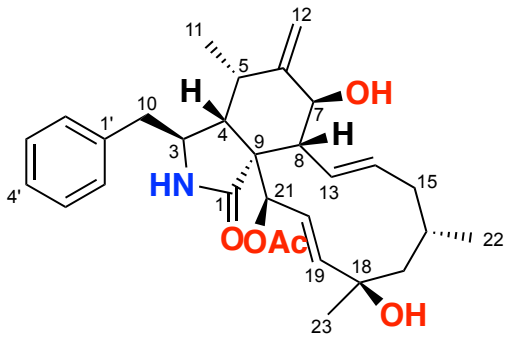

Table S2. NMR (600 MHz, $CDCl_3$) data for cytochalasin H (**2**)

Pos.	δ_H (mult., J (Hz))	δ_C	COSY	HMBC (^{1}H to ^{13}C)
1	–	174.4	–	–
2-NH	5.60 (br s)	–	3	1, 3, 4, 9
3	3.25 (m)	53.8	4, 10a, 10b, 2-NH	1, 4, 5, 1'
4	2.12 (dd, 4.0, 4.0)	50.6	3, 5	1, 3, 5, 6, 9, 10, 21
5	2.78 (qd, 6.6, 4.0)	33.0	4, 11	3, 4, 6, 11, 12
6	–	148.0	–	–
7	3.83 (d, 10.7)	69.9	8, 12a, 12b	5, 6, 8, 9, 12, 13
8	2.94 (dd, 10.7, 9.8)	47.3	7, 13	1, 6, 7, 9, 13, 14, 21
9	–	51.9	–	–
10	a 2.86 (dd, 13.6, 4.2)	45.7	3, 10b	3, 4, 1', 2'/6'
	b 2.65 (dd, 13.6, 9.9)		3, 10a	3, 4, 1', 2'/6'
11	0.99 (d, 6.6)	14.2	5	4, 5, 6
12	a 5.35 (s)	114.3	7	5, 6, 7
	b 5.11 (s)		7	5, 6, 7
13	5.73 (dd, 15.2, 9.8)	127.2[c]	8, 14	7, 8, 15
14	5.40 (m)	138.9	13, 15a, 15b	8, 15, 16
15	a 2.04 (m)	42.9	14, 15b, 16	13, 14, 16, 17, 22
	b 1.79 (m)[a]		14, 15a, 16	13, 14, 16, 17
16	1.81 (m)[a]	28.6	15a, 15b, 17, 22	–
17	a 1.87 (dd, 14.4, 2.4)	53.9	16, 17b	15, 16
	b 1.56 (dd, 14.4, 2.0)		16, 17a	18, 19, 22, 23
18	–	74.5	–	–
19	5.53 (m)[b]	138.3	20	20, 21, 23
20	5.86 (dd, 16.4, 2.4)	126.1	19, 21	18, 19, 21
21	5.55 (m)[b]	77.6	20	4, 8, 9, 19, 20, 21-O<u>CO</u>CH_3
22	1.04 (d, 6.1)	26.6	16	15, 16, 17
23	1.34 (s)	31.3	–	17, 18, 19
1'	–	137.5	–	–
2'/6'	7.14 (d, 7.2)	129.1	3'/5'	10, 2'/6', 3'/5'
3'/5'	7.32 (dd, 7.2, 7.2)	129.2	2'/6', 4'	1', 3'/5'
4'	7.24 (d, 7.2)	127.2[c]	3'/5'	2'/6'
21-O<u>CO</u>CH_3	–	170.3	–	–
21-OCO<u>CH_3</u>	2.24 (s)	21.0	–	21-O<u>CO</u>CH_3

[a,b,c] Overlapping signals and assignments were supported by HSQC and HMBC.

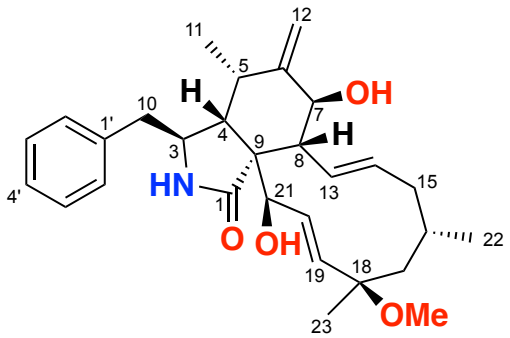

Table S3. NMR (600 MHz, $CDCl_3$) data for cytochalasin J_1 (**3**)

Pos.	δ_H (mult., J (Hz))	δ_C	COSY	HMBC (^{1}H to ^{13}C)
1	–	175.6	–	–
2-NH	5.41 (br s)	–	–	9, 10
3	3.29 (m)	53.9	4, 10a, 10b	4, 5, 1'
4	2.60 (m)[a]	50.6	3, 5	3, 5, 6, 8, 9, 10, 11, 21
5	2.92 (m)[b]	33.1	4, 11	6, 7, 9, 12
6	–	148.5	–	–
7	3.83 (d, 10.7)	70.1	8, 12a, 12b	6, 12, 13
8	2.91 (m)[b]	45.9	7, 13	1, 7, 9, 13, 14, 21
9	–	53.0	–	–
10	a 2.92 (m)[b]	46.0	3, 10b	3, 4, 1', 2'/6'
	b 2.59 (m)[a]		3, 10a	3, 4, 1', 2'/6'
11	1.11 (d, 6.7)	14.1	5	4, 5, 6
12	a 5.33 (s)	114.0	7	5, 6, 7
	b 5.12 (s)		7	5, 6, 7
13	5.72 (m)[c]	128.1	8, 14	7, 8, 15
14	5.36 (m)	137.8	13, 15a, 15b	8, 15
15	a 1.98 (m)	43.0	14, 15b, 16	13, 14, 16, 17, 22
	b 1.76 (m)[d]		14, 15a, 16	13, 14, 16
16	1.76 (m)[d]	28.0	15a, 15b, 17, 22	14
17	1.72 (br s)	51.7	16	15, 16, 18, 19, 22, 23
18	–	79.0	–	–
19	5.74 (dd, 16.8, 1.8)[c]	137.0	20	21, 23
20	6.00 (dd, 16.8, 2.7)	131.5	19, 21	18, 21
21	4.16 (dd, 2.7, 1.8)	77.2	20	8, 9, 19, 20
22	1.02 (d, 5.5)	26.2	16	15, 16, 17
23	1.29 (s)	24.5	–	17, 18, 19
1'	–	137.7	–	–
2'/6'	7.15 (d, 7.4)	129.3	3'/5'	10, 2'/6', 3'/5', 4'
3'/5'	7.32 (dd, 7.3, 7.4)	129.1	2'/6', 4'	1', 2'/6', 3'/5'
4'	7.24 (d, 7.3)	127.2	3'/5'	2'/6'
18-OMe	3.22 (s)	50.8	–	18
7-OH	2.00 (s)	–	7	6, 7, 8
21-OH	1.81 (d, 5.7)	–	21	9, 21

[a,b,c,d] Overlapping signals and assignments were supported by HSQC and HMBC.

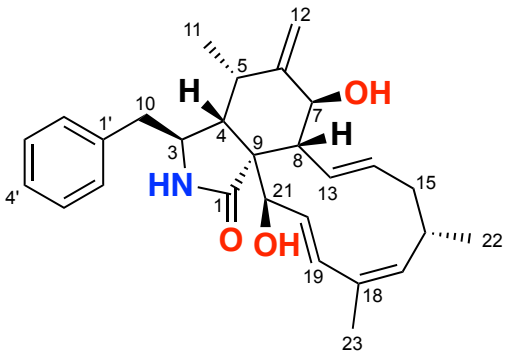

Table S4. NMR (600 MHz, $CDCl_3$) data for cytochalasin J_2 (**4**)

Pos.	δ_H (mult., J (Hz))	δ_C	COSY	HMBC (^{1}H to ^{13}C)
1	–	175.7	–	–
2-NH	5.50 (br s)	–	–	3, 4
3	3.28 (m)	53.9	4, 10a, 10b	5
4	2.57 (dd, 5.2, 3.5)	50.7	3, 5	6, 10, 21
5	2.92 (qd, 6.7, 5.2)	32.9	4, 11	3, 4, 6, 11, 12
6	–	148.0	–	–
7	3.70 (dd, 10.7, 1.3)	69.6	8, 12a, 12b	5, 6, 12, 13
8	3.06 (dd, 10.7, 9.6)	46.4	7, 13	1, 6, 7, 9, 13, 14
9	–	49.4	–	–
10	a 2.90 (dd, 13.5, 4.1)	45.7	3, 10b	3, 1', 2'/6'
	b 2.62 (dd, 13.5, 9.5)		3, 10a	3, 4, 1', 2'/6'
11	1.09 (d, 6.7)	13.7	5	4, 5, 6
12	a 5.29 (s)	114.3	7	5, 6, 7
	b 5.10 (s)		7	5, 6, 7
13	5.97 (ddd, 15.8, 9.6, 1.4)	130.9	8, 14	8, 15
14	5.68 (m)	137.1	13, 15a, 15b	8, 15
15	a 2.22 (dd, 13.0, 10.4)	43.0	14, 15b, 16	13, 14, 16, 17, 22
	b 1.98 (dd, 13.0, 4.8)		14, 15a, 16	13, 14, 16, 17, 22
16	2.75 (m)	31.9	15a, 15b, 17, 22	–
17	5.26 (d, 7.5)	136.1	16, 23	19, 22, 23
18	–	132.6	–	–
19	6.85 (dd, 16.6, 1.3)	135.4	20	17, 18, 20, 21, 23
20	5.72 (dd, 16.6, 3.5)	125.3	19, 21	18, 21
21	4.20 (dd, 3.5, 1.3)	77.3	20	4, 8, 9, 19, 20
22	1.04 (d, 6.9)	24.2	16	15, 16, 17
23	1.83 (s)	21.1	17	17, 18, 19
1'	–	137.7	–	–
2'/6'	7.15 (dd, 8.0, 1.3)	129.3	3'/5'	10, 2'/6', 4'
3'/5'	7.32 (dd, 8.0, 7.1)	129.0	2'/6', 4'	1', 3'/5'
4'	7.25 (d, 7.1)	127.2	3'/5'	2'/6'

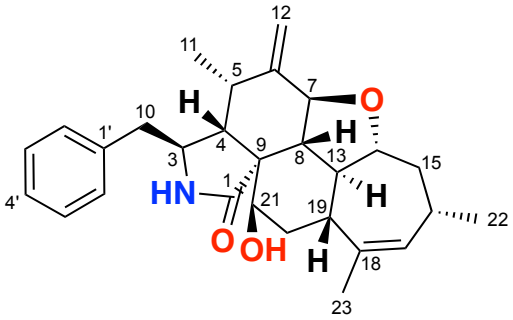

Table S5. NMR (600 MHz, $CDCl_3$) data for cytochalasin J_3 (**5**)

Pos.	δ_H (mult., J (Hz))	δ_C	COSY	HMBC (^{1}H to ^{13}C)
1	–	175.8	–	–
2-NH	5.49 (br s)	–	3	3, 4, 9
3	3.34 (m)	53.6	4, 10a, 10b, 2-NH	1, 4, 5, 1'
4	2.51 (m)[a]	47.5	3, 5	3, 5, 9, 10, 11
5	3.01 (m)	36.6	4, 11, 12a, 12b	3, 4, 11
6	–	149.3	–	–
7	4.08 (dd, 13.0, 1.8)	77.0	8, 12a, 12b	8, 12
8	2.47 (dd, 13.0, 12.8)	44.0	7, 13	1, 6, 7, 9, 13, 14, 19, 21
9	–	48.6	–	–
10	a 2.97 (dd, 13.6, 3.0)	45.7	3, 10b, 2'/6'	3, 4, 1', 2'/6'
	b 2.52 (dd, 13.6, 9.2)[a]		3, 10a	3, 1', 2'/6'
11	1.21 (d, 6.7)	15.1	5	4, 5, 6
12	a 5.38 (s)	114.0	5, 7	5, 6, 7
	b 5.21 (s)		5, 7	5, 6, 7
13	1.83 (ddd, 12.8, 10.4, 9.0)	45.2	8, 14, 19	8, 14, 15, 18, 19
14	3.69 (ddd, 11.6, 9.0, 2.7)	88.2	13, 15a, 15b	16, 19
15	a 1.99 (ddd, 11.6, 3.7, 2.7)	40.1	14, 15b, 16	13, 14, 16, 17, 22
	b 1.39 (ddd, 11.6, 11.6, 11.6)		14, 15a, 16	13, 14, 16, 17, 22
16	2.13 (m)	30.5	15a, 15b, 17, 22	–
17	5.24 (br s)	133.6	16, 19, 23	15, 16, 19, 23
18	–	138.4	–	–
19	2.34 (ddd, 13.0, 10.4, 3.9)	35.5	13, 17, 20a, 20b, 23	–
20	a 2.63 (ddd, 14.5, 13.0, 2.7)	34.7	19, 20b, 21	13, 18, 19, 21
	b 1.95 (ddd, 14.5, 3.9, 2.9)		19, 20a, 21	9, 13, 19, 21
21	3.78 (br dd, 2.9, 2.7)	71.2	20a, 20b	8, 9, 19
22	1.10 (d, 7.2)	24.8	16	15, 16, 17
23	1.74 (s)	23.8	17, 19	17, 18, 19
1'	–	137.3	–	–
2'/6'	7.15 (d, 7.0)	129.5	10a, 3'/5'	10, 2'/6', 3'/5', 4'
3'/5'	7.32 (dd, 7.4, 7.0)	129.0	2'/6', 4'	1', 3'/5'
4'	7.26[b]	127.3	3'/5'	2'/6', 3'/5'

[a] Overlapping signals and assignments supported by HSQC and HMBC;
[b] Overlapped with residual $CHCl_3$ signal.

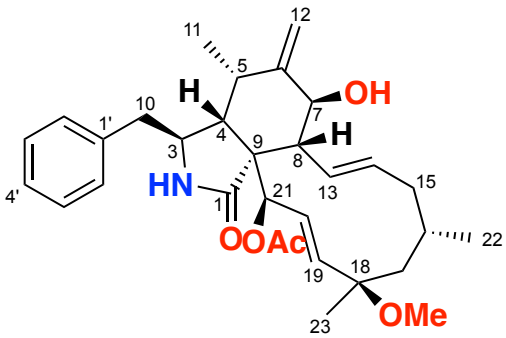

Table S6. NMR (600 MHz, $CDCl_3$) data for cytochalasin H_1 (**6**)

Pos.	δ_H (mult., *J* (Hz))	δ_C	COSY	HMBC (^{1}H to ^{13}C)
1	–	174.3	–	–
2-NH	5.52 (br s)	–	3	–
3	3.26 (ddd, 9.8, 4.5, 4.4)	53.9	2, 4, 10a, 10b	4
4	2.14 (dd, 4.4, 4.4)	50.7	3, 5	–
5	2.80 (qd, 6.7, 4.4)	33.0	4, 11	3, 4, 6, 11, 12
6	–	147.9	–	–
7	3.83 (d, 10.8)	69.9	8	6, 12, 13
8	2.97 (dd, 10.8, 9.8)	47.3	7, 13	1, 7, 9, 13, 14
9	–	51.7	–	–
10	a 2.87 (dd, 13.5, 4.5)	45.8	3, 10b	3, 1', 2'/6'
	b 2.65 (dd, 13.5, 9.8)		3, 10a	3, 4, 1', 2'/6'
11	1.01 (d, 6.7)[a]	14.1	5	4, 5, 6
12	a 5.34 (s)	114.4	–	5, 6, 7
	b 5.12 (s)		–	5, 6, 7
13	5.73 (dd, 15.4, 9.8)	127.4	8, 14	8, 15
14	5.43 (m)	138.6	13, 15a, 15b	–
15	a 2.01 (m)	43.2	14, 15b, 16	–
	b 1.78 (m)[b]		14, 15a, 16	–
16	1.79 (m)[b]	27.9	15a, 15b, 17a, 17b, 22	–
17	a 1.74 (d, 15.0)	52.0	16, 17b	15, 16, 18, 19, 22, 23
	b 1.71 (d, 15.0)		16, 17a	15, 16, 18, 19, 22, 23
18	–	78.9	–	–
19	5.53 (dd, 15.3, 2.1)[c]	138.4	20	20, 21, 23
20	5.80 (dd, 15.3, 3.5)	126.2	19, 21	21
21	5.52 (m)[c]	78.1	20	8
22	1.02 (d, 6.1)[a]	26.3	16	15, 16
23	1.27 (s)	24.3	–	17, 18, 19
1'	–	137.5	–	–
2'/6'	7.15 (d, 7.2)	129.2	3'/5'	10, 3'/5', 4'
3'/5'	7.33 (dd, 7.7, 7.2)	129.1	2'/6', 4'	1', 2'/6'
4'	7.26[d]	127.3	3'/5'	2'/6'
18-OMe	3.15 (s)	50.9	–	18
21-O<u>CO</u>CH_3	–	170.2	–	–
21-OCO<u>CH_3</u>	2.25 (s)	21.0	–	21-O<u>CO</u>CH_3

[a,b,c] Overlapping signals and assignments supported by HSQC and HMBC; [d] Overlapped with residual $CHCl_3$ signal.

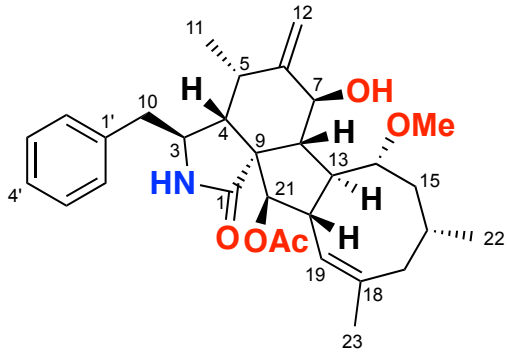

Table S7. NMR (600 MHz, $CDCl_3$) data for cytochalasin H_2 (**7**)

Pos.	δ_H (mult., J (Hz))	δ_C	COSY	HMBC (^{1}H to ^{13}C)
1	–	176.8	–	–
2-NH	5.27 (br s)	–	3	3, 4, 9
3	3.32 (m)[a]	53.0	2, 4, 10	1, 4, 5
4	2.22 (dd, 6.0, 1.3)	45.2	3, 5	1, 5, 6, 9, 10, 21
5	2.75 (qd, 6.7, 6.0)	32.3	4, 11	3, 4, 6, 11, 12
6	–	149.1	–	–
7	4.15 (d, 9.4)	71.2	8	5, 6, 12, 13
8	2.25 (dd, 10.0, 9.4)	52.3	7, 13	1, 7, 9, 13, 14
9	–	56.6	–	–
10	2.61 (m)	43.9	3	3, 1', 2'/6'
11	0.94 (d, 6.7)	13.4	5	4, 5, 6
12	a 5.21 (s)	114.1	–	5, 6, 7
	b 5.01 (s)		–	5, 6, 7
13	3.00 (ddd, 10.0, 9.8, 9.8)	50.7	8, 14, 20	8, 14, 19
14	3.07 (dd, 9.8, 7.6)	87.2	13, 15a	13, 16, 20, 14-OMe
15	a 1.76 (m)	39.3	14, 15b, 16	13, 14
	b 1.67 (m)		15a	14
16	1.97 (m)	31.8	15a, 17a, 22	–
17	a 2.56 (dd, 14.5, 6.2)	38.0	16, 17b	16, 18, 19, 22, 23
	b 1.81 (dd, 14.5, 3.5)		17a	
18	–	133.7	–	–
19	5.64 (d, 8.5)	127.4	20	17, 20, 21, 23
20	2.65 (ddd, 9.8, 8.5, 2.7)	52.2	13, 19, 21	13, 14, 21
21	5.08 (d, 2.7)	82.6	20	1, 8, 13, 19
22	1.03 (d, 6.9)	24.7	16	15, 16, 17
23	1.75 (s)	27.0	–	17, 18, 19
1'	–	137.7	–	–
2'/6'	7.12 (dd, 8.1, 1.1)	129.3	3'/5'	10, 4'
3'/5'	7.31 (ddd, 8.1, 7.4)	128.9	2'/6', 4'	1'
4'	7.23 (dd, 7.4, 1.1)	127.0	3'/5'	2'/6'
14-OMe	3.34 (s)[a]	54.5	–	14
21-O<u>CO</u>CH_3	–	170.0	–	–
21-OCO<u>CH_3</u>	2.16 (s)	21.6	–	21-O<u>CO</u>CH_3
7-OH	5.73 (s)	–	–	6, 7

[a] Overlapping signals and assignments supported by HSQC and HMBC.

Table S8. Diagnostic ROESY (600 MHz, $CDCl_3$) data for cytochalasins J_3 (**5**) and H_2 (**7**)

Pos.	cytochalasin J_3 (**5**)		cytochalasin H_2 (**7**)	
	δ_H (mult., *J* (Hz))	ROESY	δ_H (mult., *J* (Hz))	ROESY
3	3.34 (m)	7, 10a, 11	3.32 (m)[a]	10, 11
4	2.51 (m)[a]	5	2.22 (dd, 6.0, 1.3)	5, 14, 7-OH
5	3.01 (m)	4, 8	2.75 (qd, 6.7, 6.0)	4, 8, 7-OH
7	4.08 (dd, 13.0, 1.8)	3, 12a, 13, 21	4.15 (d, 9.4)	12a, 13
8	2.47 (dd, 13.0, 12.8)	5, 14	2.25 (dd, 10.0, 9.4)	5, 14, 7-OH
10	a 2.97 (dd, 13.6, 3.0)	3, 11	2.61 (m)	3
	b 2.52 (dd, 13.6, 9.2)[a]	–	–	–
11	1.21 (d, 6.7)	3, 10a, 12b	0.94 (d, 6.7)	3, 12b
12	a 5.38 (s)	7	a 5.21 (s)	7
	b 5.21 (s)	11	b 5.01 (s)	11
13	1.83 (ddd, 12.8, 10.4, 9.0)	7, 15b, 20a	3.00 (ddd, 10.0, 9.8, 9.8)	7, 17b, 21
14	3.69 (ddd, 11.6, 9.0, 2.7)	8, 15a, 16, 19, 20b	3.07 (dd, 9.8, 7.6)	4, 8, 16, 15b, 20, 7-OH
15	a 1.99 (ddd, 11.6, 3.7, 2.7)	14, 16	a 1.76 (m)	–
	b 1.39 (ddd, 11.6, 11.6, 11.6)	13	b 1.67 (m)	14
16	2.13 (m)	14, 15a	1.97 (m)	14, 20
17	5.24 (br s)	22, 23	a 2.56 (dd, 14.5, 6.2)	–
	–	–	b 1.81 (dd, 14.5, 3.5)	13
19	2.34 (ddd, 13.0, 10.4, 3.9)	14, 20b	5.64 (d, 8.5)	21, 23
20	a 2.63 (ddd, 14.5, 13.0, 2.7)	13, 21, 23	2.65 (ddd, 9.8, 8.5, 2.7)	14, 16
	b 1.95 (ddd, 14.5, 3.9, 2.9)	14, 19, 23	–	–
21	3.78 (br dd, 2.9, 2.7)	7, 20a	5.08 (d, 2.7)	13, 19
22	1.10 (d, 7.2)	17	1.03 (d, 6.9)	–
23	1.74 (s)	17, 20a, 20b	1.75 (s)	19
7-OH	–	–	5.73 (s)	4, 5, 8, 14

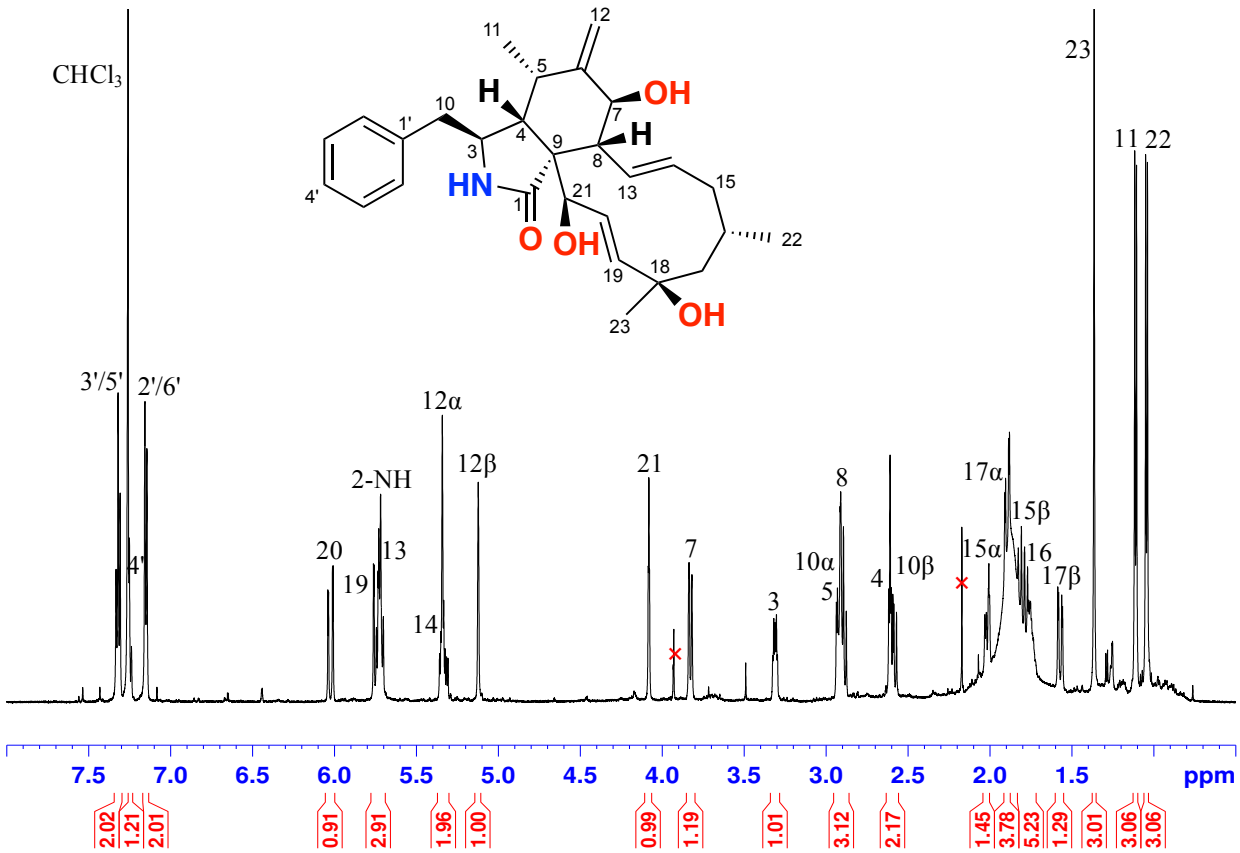

Figure S8. ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum of cytochalasin J (**1**)

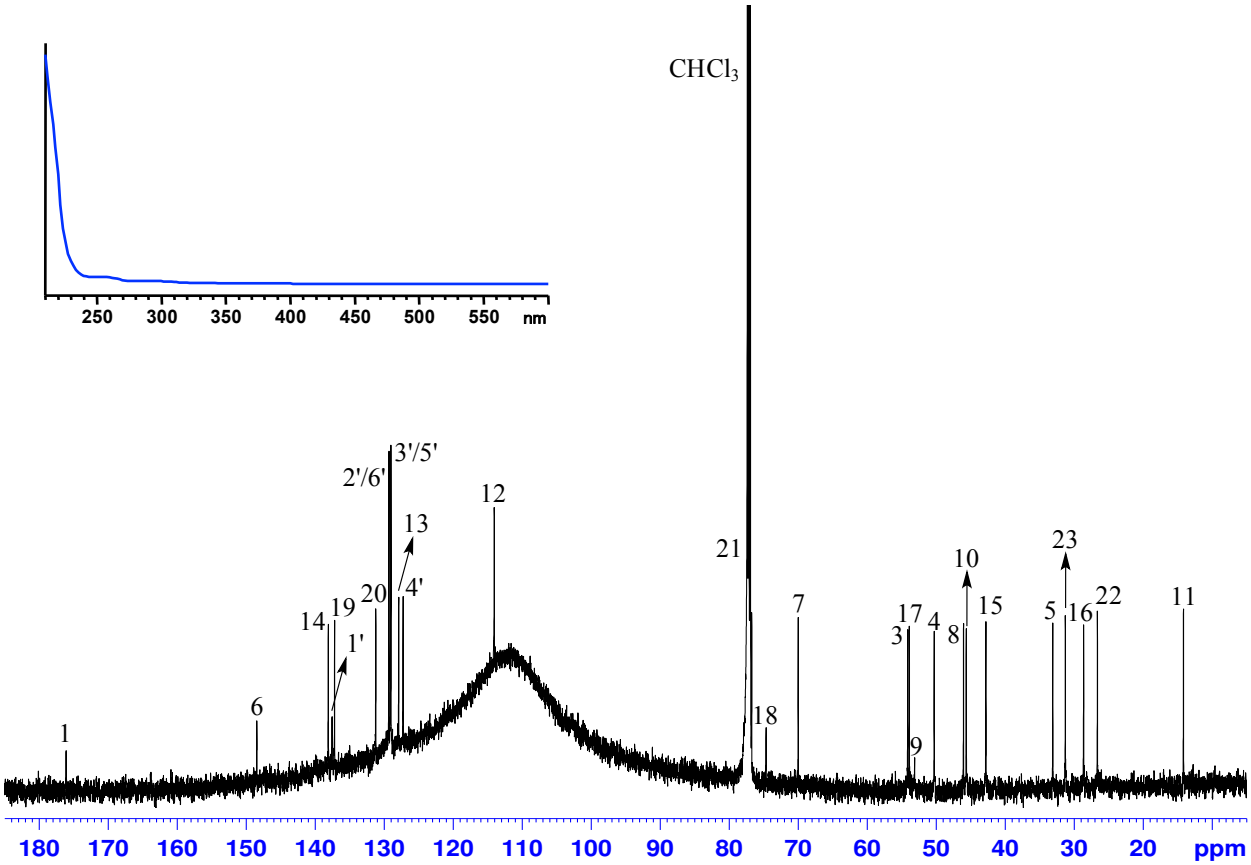

Figure S9. ^{13}C-NMR (150 MHz, $CDCl_3$) and UV spectra of cytochalasin J (**1**)

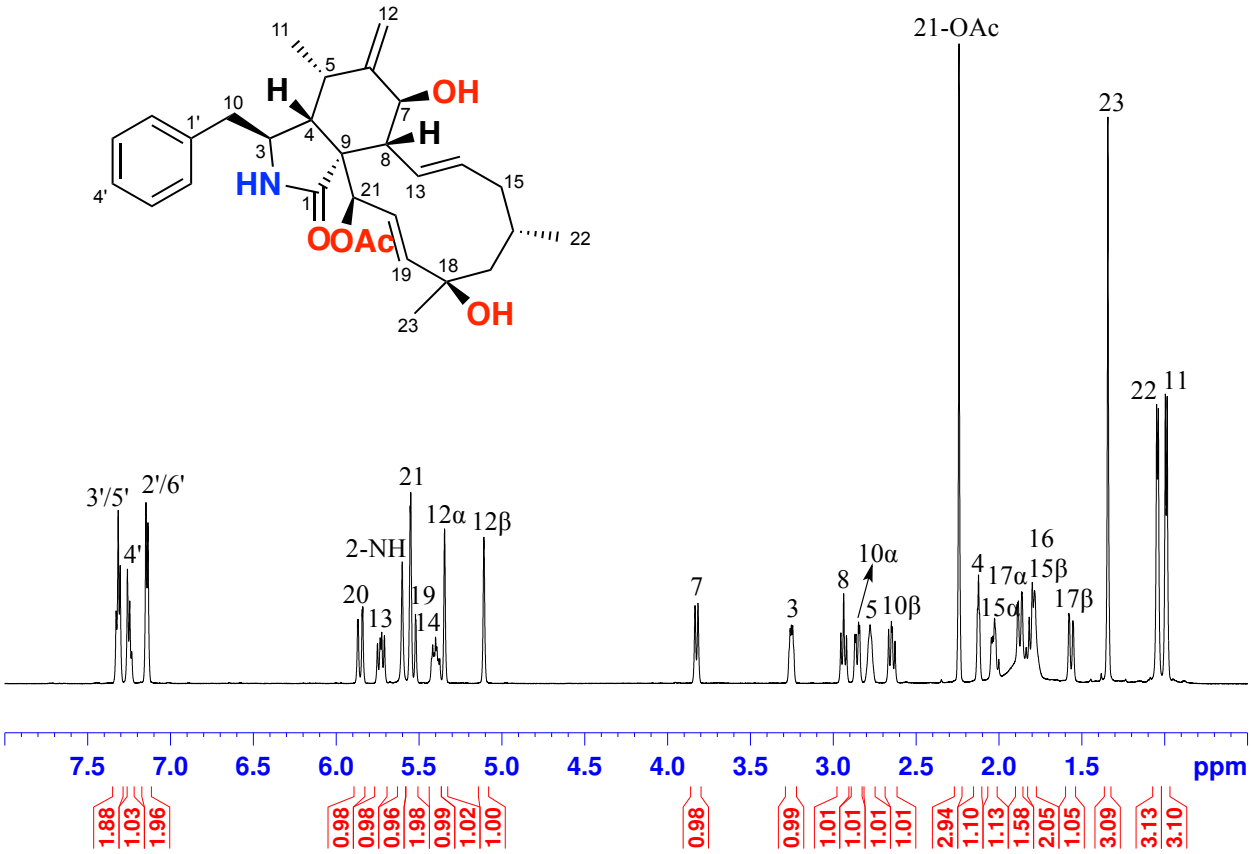

Figure S10. ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum of cytochalasin H (**2**)

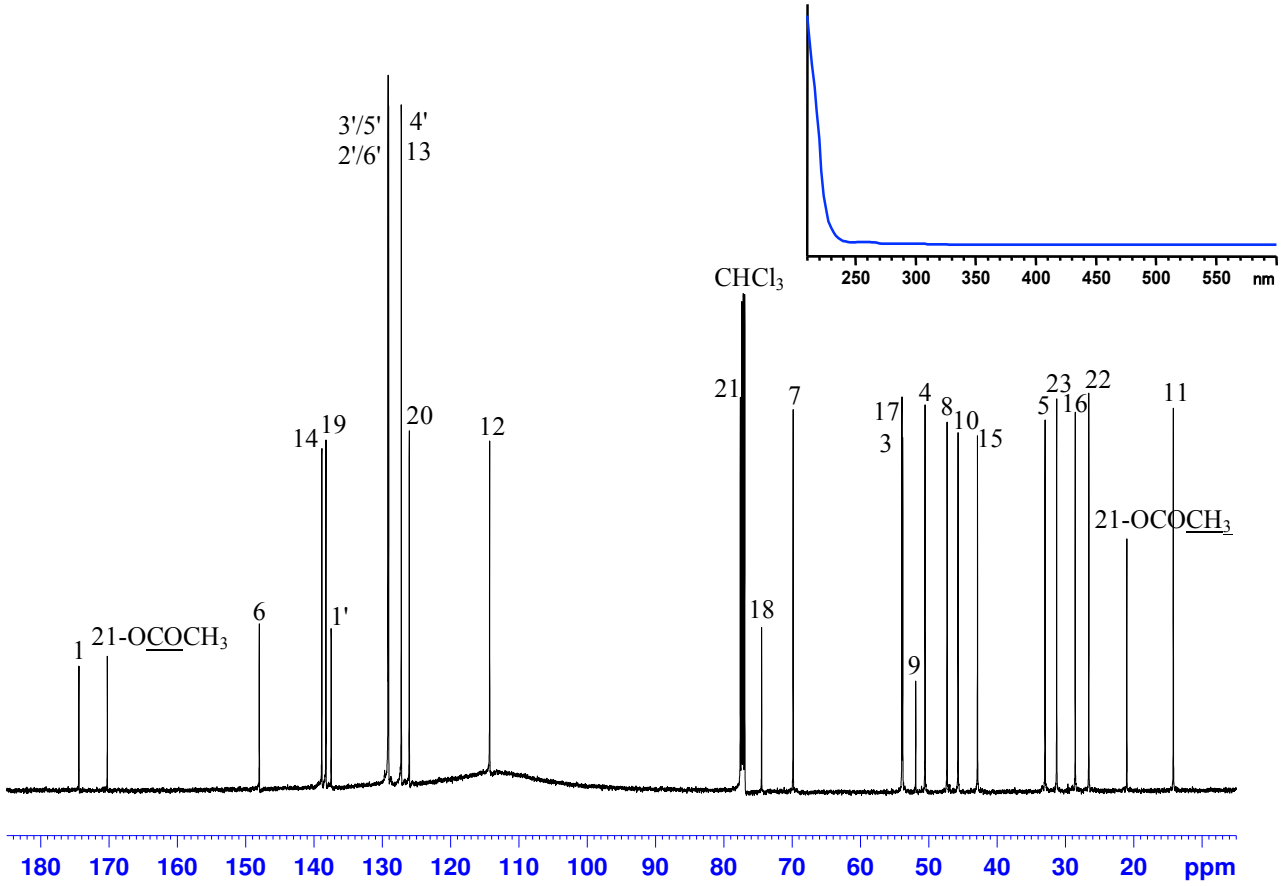

Figure S11. ^{13}C-NMR (150 MHz, $CDCl_3$) and UV spectra of cytochalasin H (**2**)

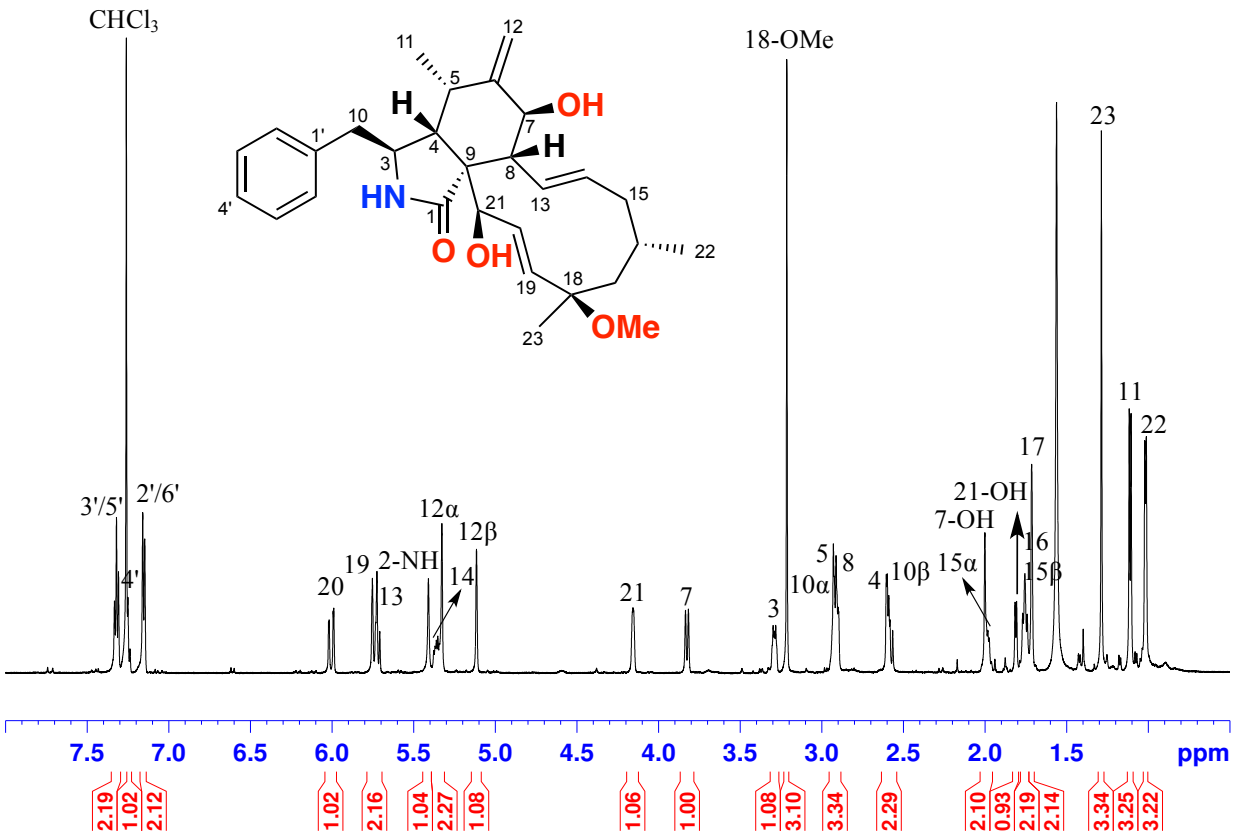

Figure S12. ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum of cytochalasin J_1 (**3**)

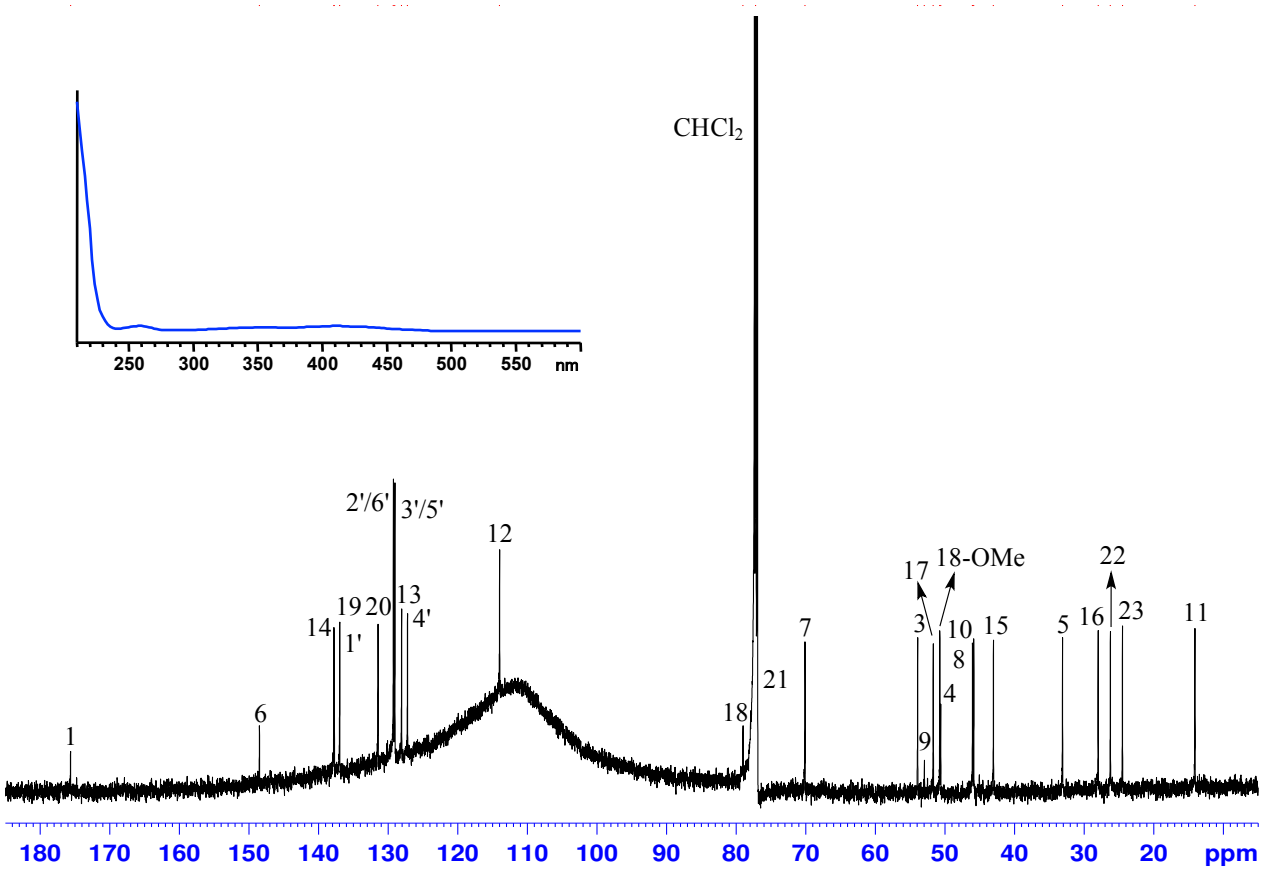

Figure S13. ^{13}C-NMR (150 MHz, $CDCl_3$) and UV spectra of cytochalasin J_1 (**3**)

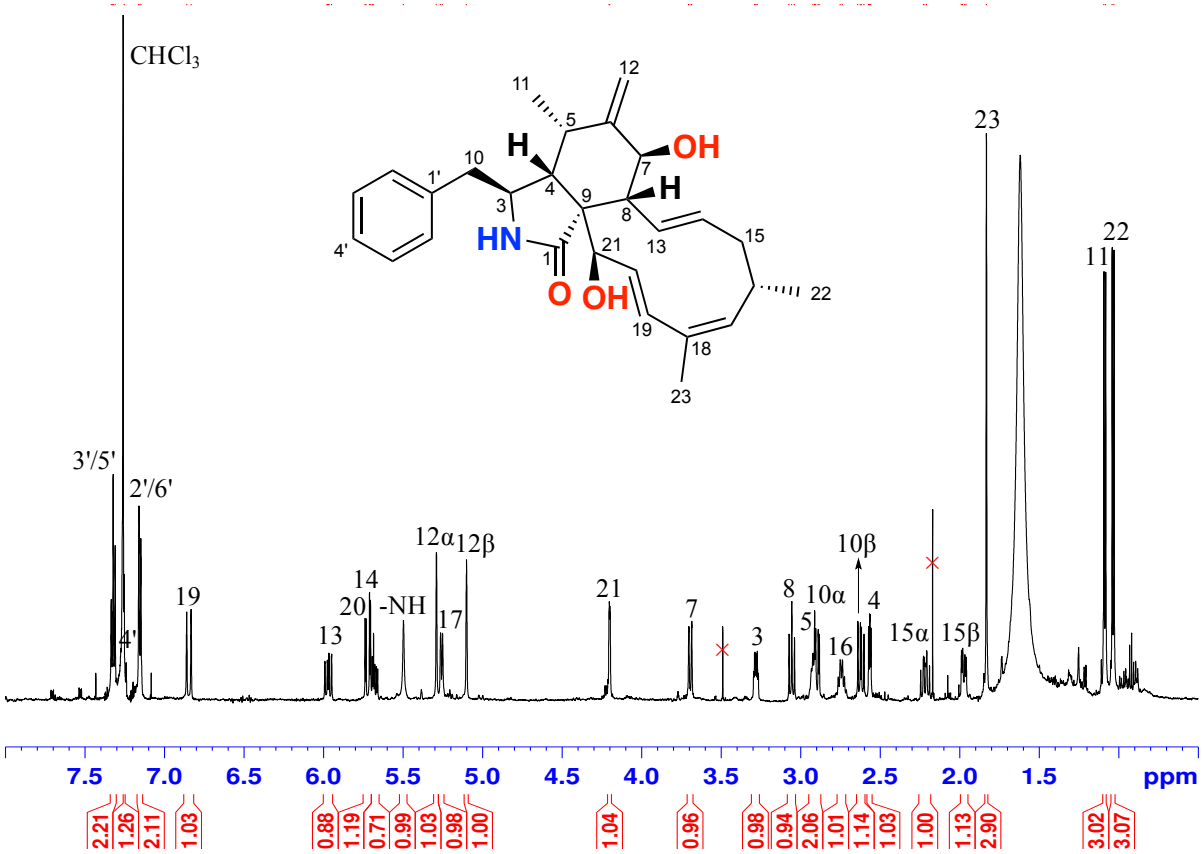

Figure S14. ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum of cytochalasin J_2 (**4**)

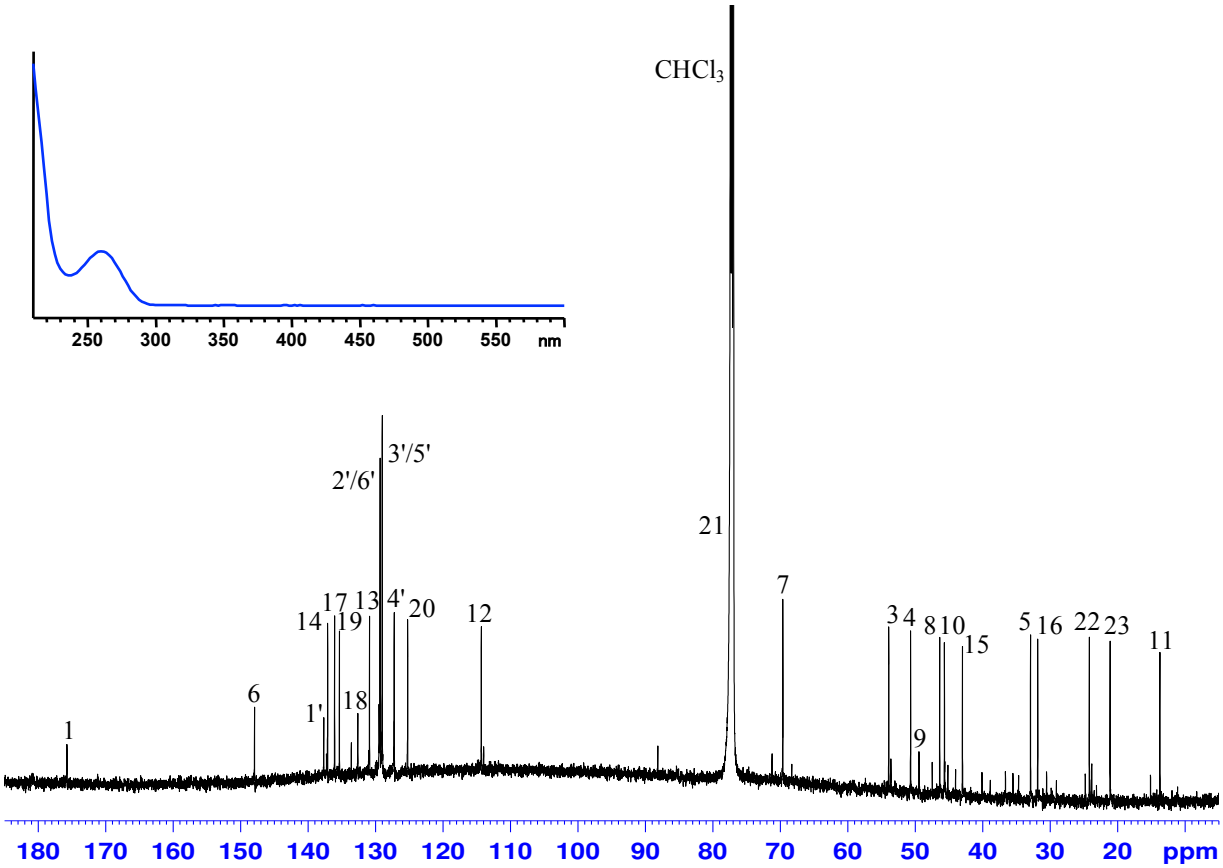

Figure S15. ^{13}C-NMR (150 MHz, $CDCl_3$) and UV spectra of cytochalasin J_2 (**4**)

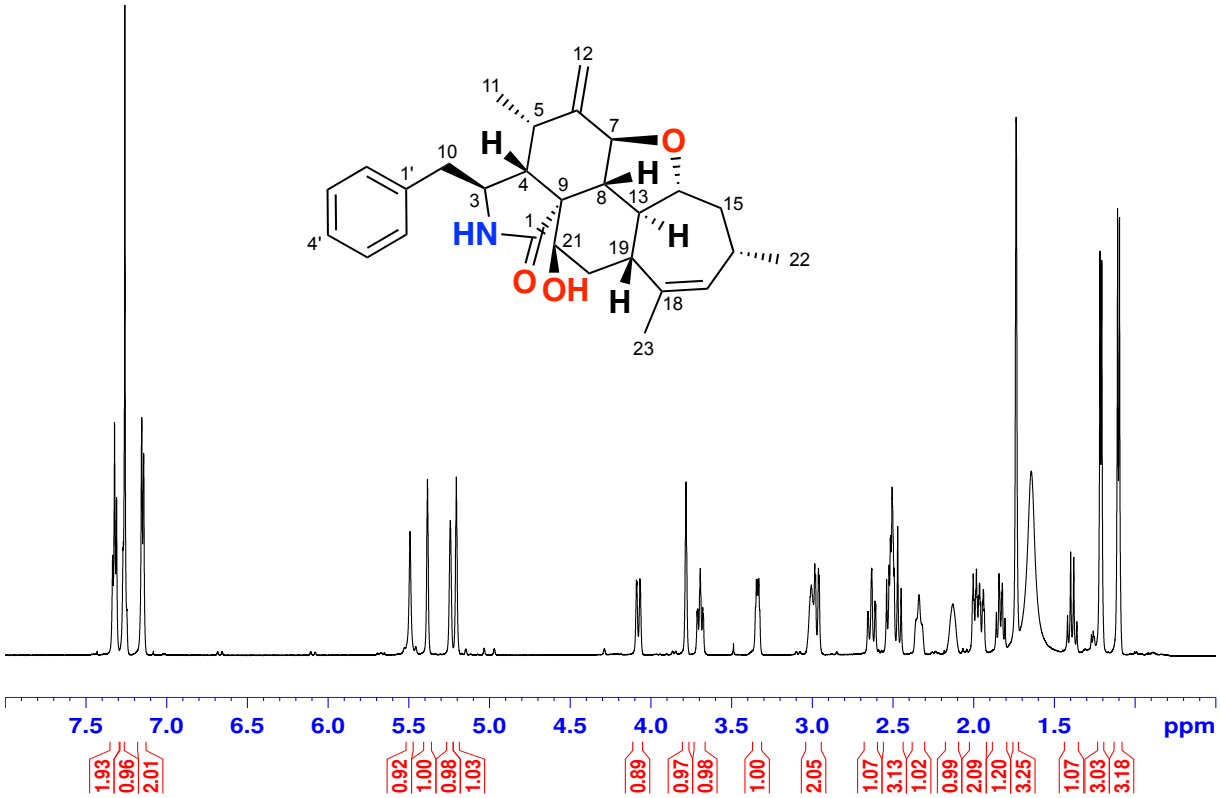

Figure S16. ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum of cytochalasin J_3 (**5**)

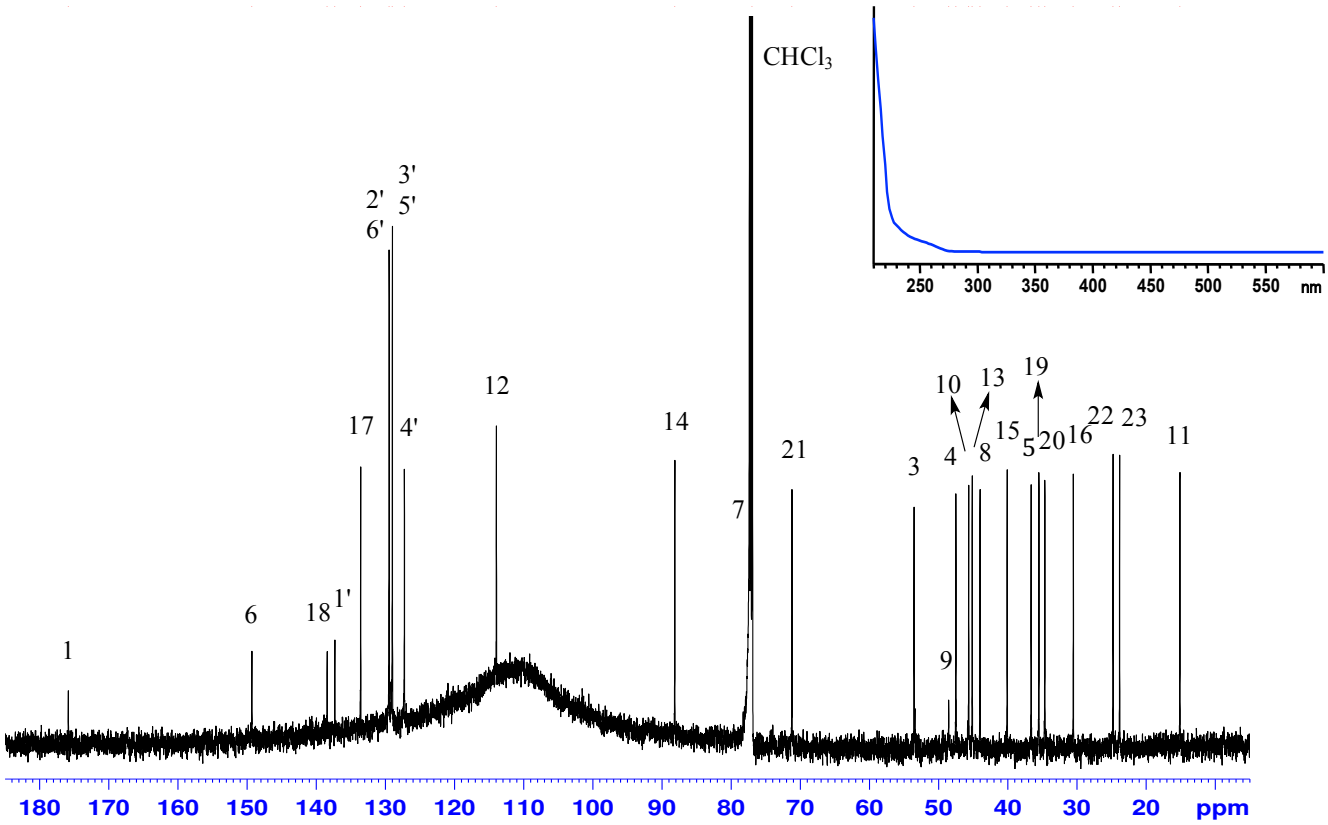

Figure S17. ^{13}C-NMR (150 MHz, $CDCl_3$) and UV spectra of cytochalasin J_3 (**5**)

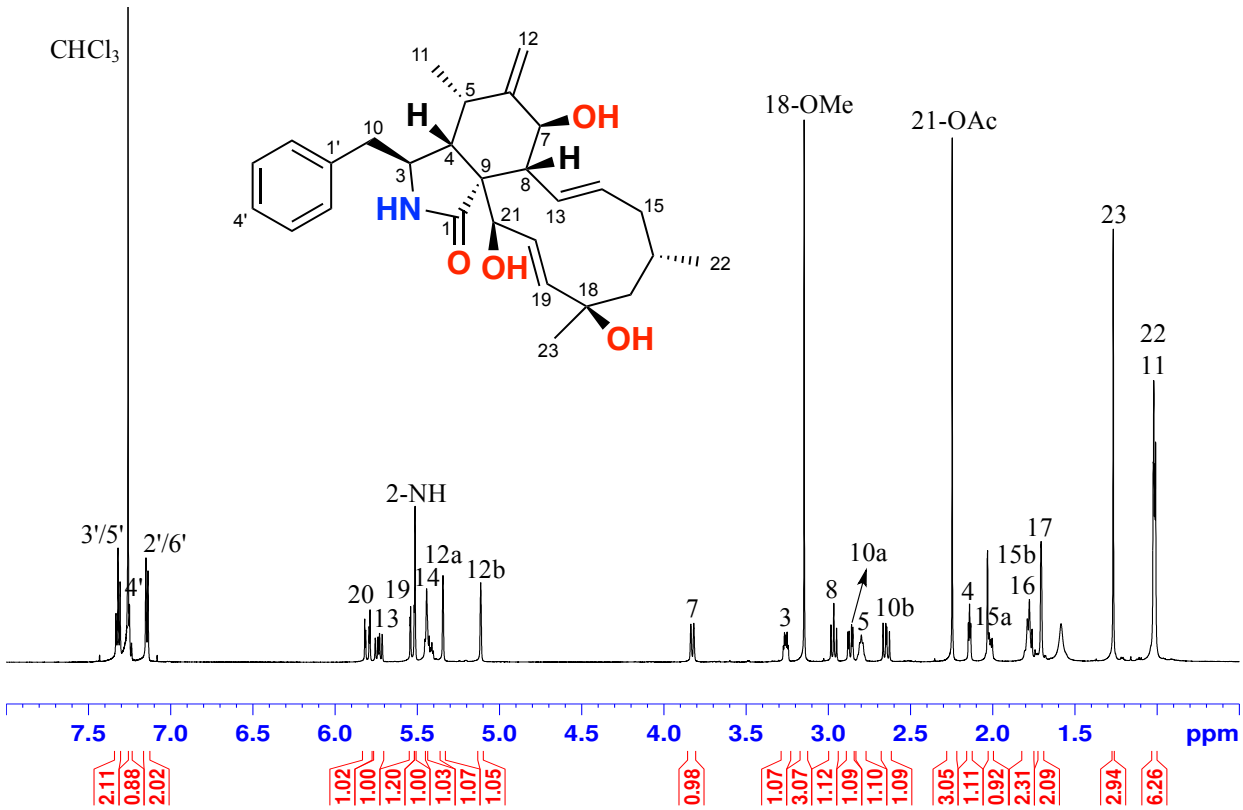

Figure S18. ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum of cytochalasin H_1 (**6**)

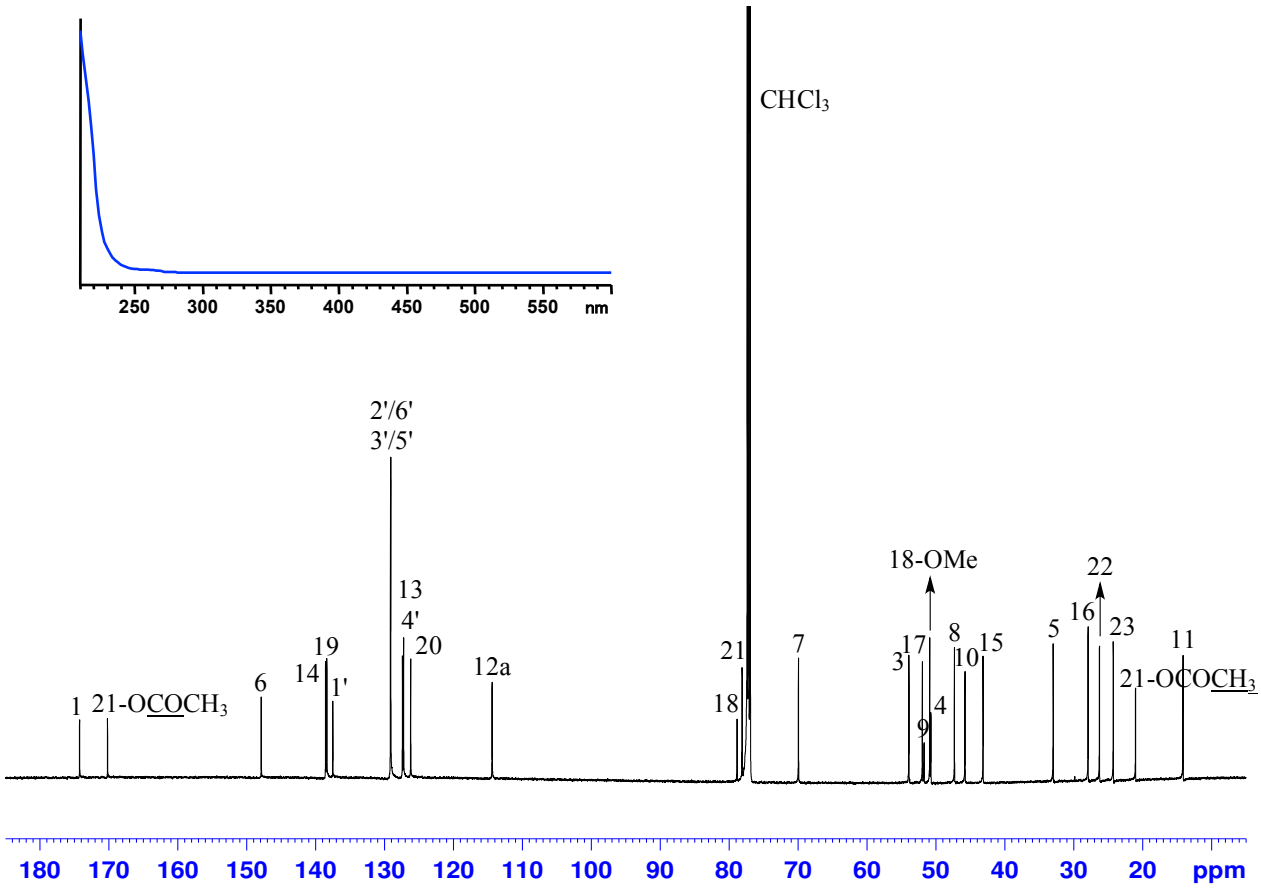

Figure S19. ^{13}C-NMR (150 MHz, $CDCl_3$) and UV spectra of cytochalasin H_1 (**6**)

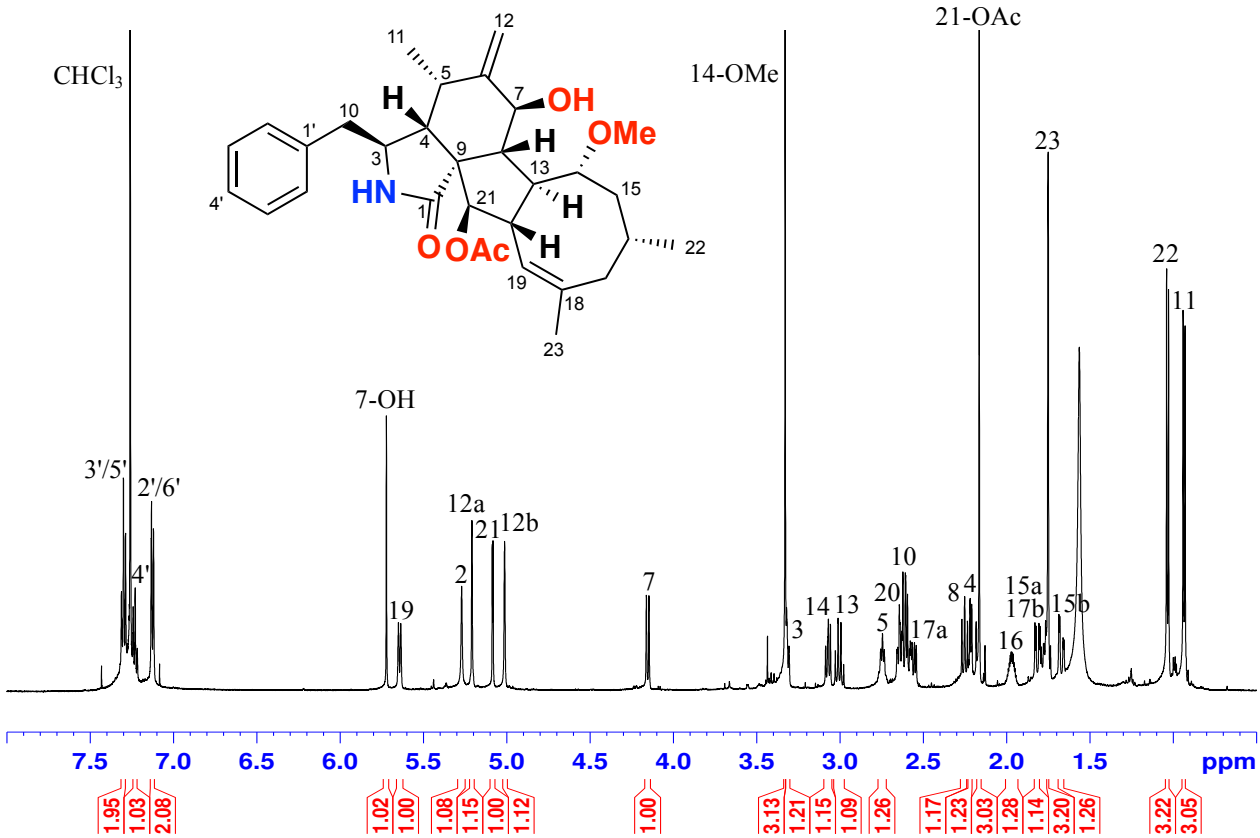

Figure S20. ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum of cytochalasin H_2 (**7**)

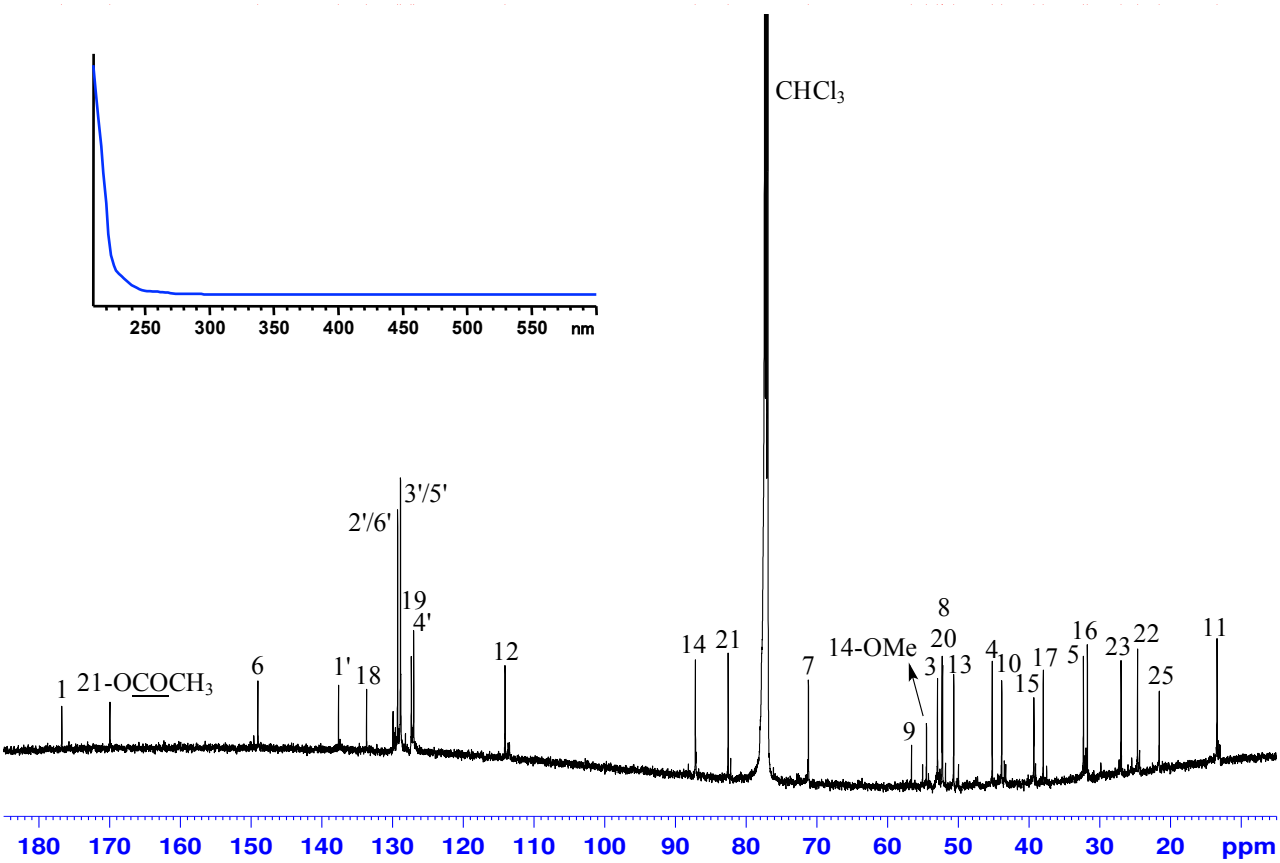

Figure S21. ^{13}C-NMR (150 MHz, $CDCl_3$) and UV spectra of cytochalasin H_2 (**7**)