Shornephine A: Structure, chemical stability and P-glycoprotein inhibitory properties of a rare diketomorpholine from an Australian marine-derived *Aspergillus* sp.

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1 DNA Taxonomic Analysis of CMB-M081F

1.1 ITS Gene Sequence of CMB-M081F

CAGGGATCATTGTTGAGTTTTACTGATTGCAAAGAATCACACTCAGACTGCAAGCTTTCAGAACAG
GGTTCATGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGCGAGTCGCCCCCCGGCGGCCAGCAACG
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BLAST search (closest match):

Aspergillus terreus strain CO1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: gb|KC582297.1| Length: 583 Number of Matches: 1

Related Information

Range 1: 2 to 239 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
407 bits(220)	2e-110	233/238(98%)	5/238(2%)	Plus/Minus

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Aspergillus terreus strain CO1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

GenBank: KC582297.1

FASTA Graphics

Go to:

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DEFINITION  Aspergillus terreus strain CO1 18S ribosomal RNA gene, partial
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            ribosomal RNA gene, partial sequence.
ACCESSION   KC582297
VERSION     KC582297.1  GI:499109366
KEYWORDS    .
SOURCE      Aspergillus terreus
  ORGANISM  Aspergillus terreus
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Eurotiomycetes; Eurotiomycetidae; Eurotiales; Trichocomaceae;
            mitosporic Trichocomaceae; Aspergillus.
REFERENCE   1  (bases 1 to 583)
  AUTHORS   Suja,M., Vasuki,S. and Sajitha,N.
  TITLE     Isolation of endophytic fungi from Seaweeds
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 583)
  AUTHORS   Suja,M., Vasuki,S. and Sajitha,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2013) CAS in Marine Biology, Faculty of Marine
            Sciences, Annamalai University, Parangipettai, Cuddalore, Tamilnadu
            608502, India
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2 Cytotoxicity (MTT) Assays

The MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was modified from that previously described[1] using adherent cell lines SW620 and KB-3-1, and their respective P-gp over-expressing daughter cell lines SW620 Ad300 and KB-V1. Briefly, cells were harvested with trypsin and dispensed into 96-well microtiter assay plates at 2,000 cells/well for SW620, SW620 Ad300 and KB-3-1, and 5,000 cells/well for KB-V1, then incubated for 18 h at 37 °C with 5% CO_2 (to allow cells to attach). Test compounds were dissolved in 5% DMSO in PBS (v/v) and aliquots (20 μL) tested over a series of final concentrations ranging from 10 nM to 30 μM. Control wells were treated with 5% aqueous DMSO. After 68 h incubation at 37 °C with 5% CO_2, an aliquot (20 μL) of MTT in PBS (4 mg/mL) was added to each well (final concentration of 0.4 mg/mL), and the microtiter plates incubated for a further 4 h at 37 °C with 5% CO_2. After this final incubation the medium was aspirated and precipitated formazan crystals dissolved in DMSO (100 μL/well). The absorbance of each well was measured at 580 nm and IC_{50} values were calculated as the concentration of analyte required for 50% inhibition of cancer cell growth (compared to negative controls). All experiments were performed in duplicate.

3 Chromatograms

3.1 HPLC-DAD chromatogram of CMB-M081F M1 agar plate extract

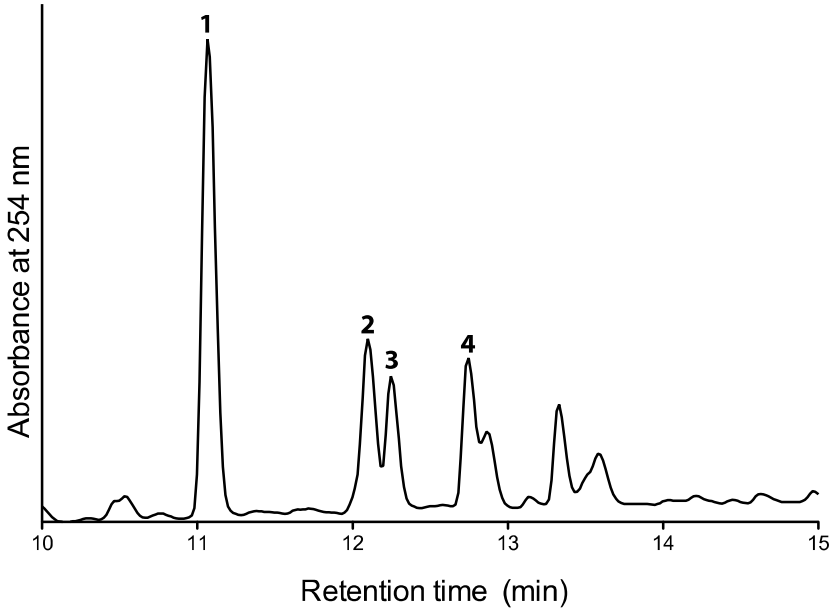


Figure S1. Expansion of HPLC-DAD (254 nm) chromatogram of the crude extract CMB-M81F highlighting the DKM **1** and DKPs **2**–**4**.

3.2 HPLC-DAD-ESIMS chromatograms of Mosher esters

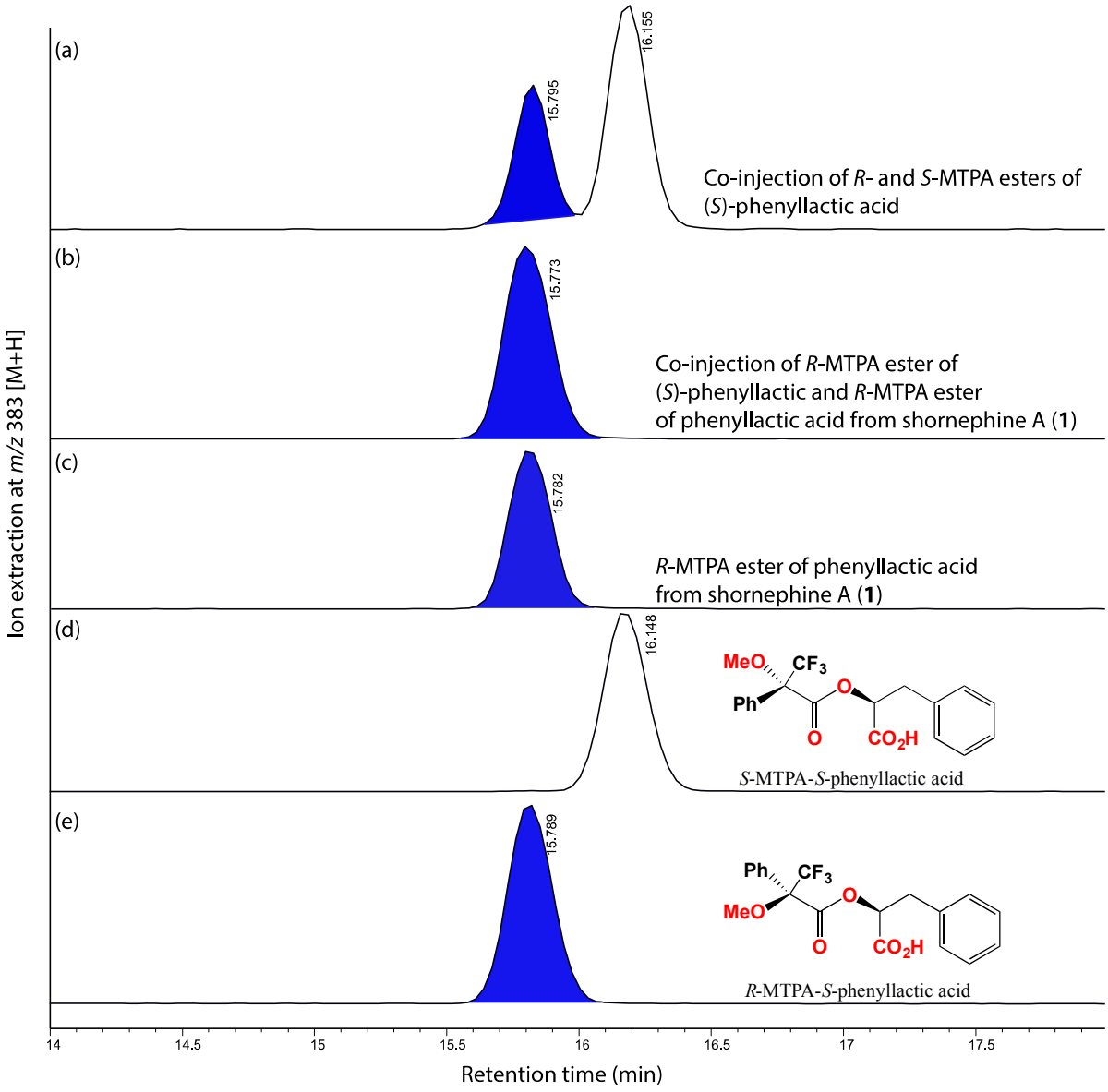


Figure S2. Analytical HPLC (Zorbax SB-C_8 5 µm 150 × 4.6 mm column, 1.0 mL/min, gradient elution from 90% H_2O:MeCN to 100% MeCN over 15 min followed by a 5 min hold at 100% MeCN, with isocratic 0.05% HCO_2H in MeCN modifier), monitoring single ion extractions at *m/z* 383 $[M+H]^+$ for the Mosher ester of phenyllactic acid). (a) Co-injection of *R*- and *S*-MTPA esters of authentic (*S*)-phenyllactic acid, (b) Co-injection of *R*-MTPA ester of authentic (*S*)-phenyllactic acid with the *R*-MTPA ester of phenyllactic acid recovered from hydrolysis of shornephine A (**1**), (c) *R*-MTPA ester of phenyllactic acid recovered from hydrolysis of shornephine A (**1**) (d) *S*-MTPA ester of authentic (*S*)-phenyllactic acid and (e) *R*-MTPA ester of authentic (*S*)-phenyllactic acid.

3.3 Methanolysis of shornephine A (1)

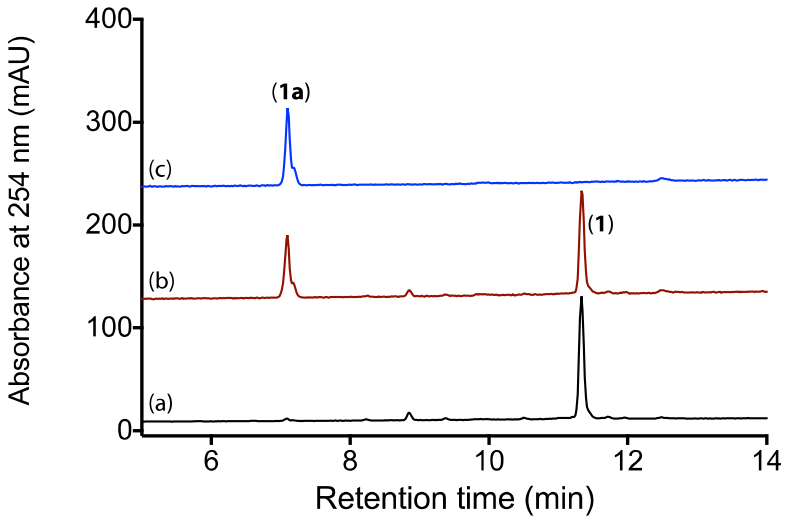


Figure S3. HPLC-DAD (254 nm) chromatogram of shornephine A (**1**) after incubation in MeOH for (a) 30 min, (b) 8 h and (c) 24 h (100% conversion to **1a**)

3.4 Methanolysis of *cyclo*-(L-phenylalanine-L-mandelic acid) (17)

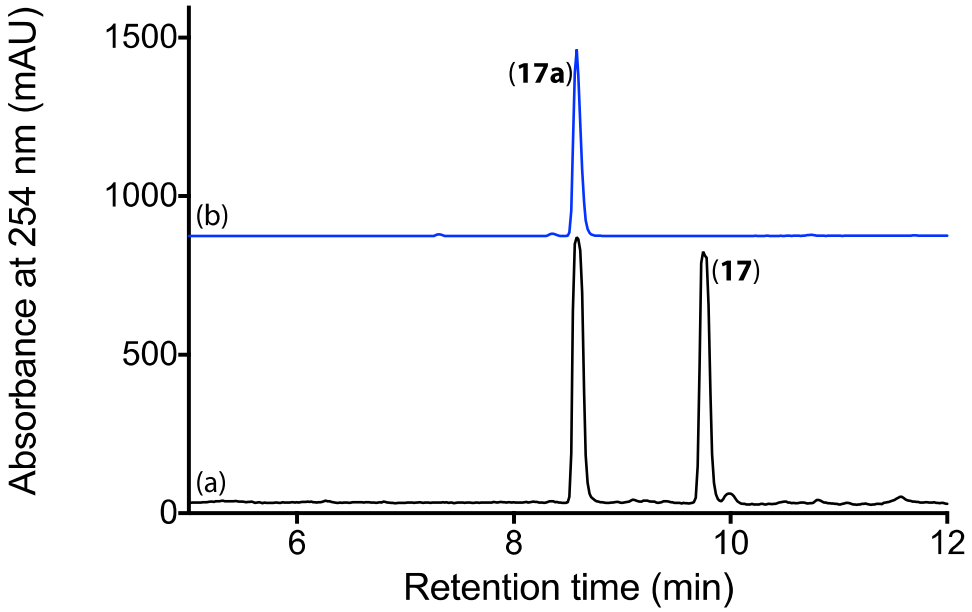


Figure S4. HPLC-DAD (254 nm) chromatogram of *cyclo*-(L-phenylalanine-L-mandelic acid) (**17**) after incubation in MeOH for (a) 30 min and (b) 1 h (100% conversion to **17a**)

3.5 Methanolysis of *cyclo*-(*N*-methyl-L-tyrosine-L-phenyllactic acid) (26)

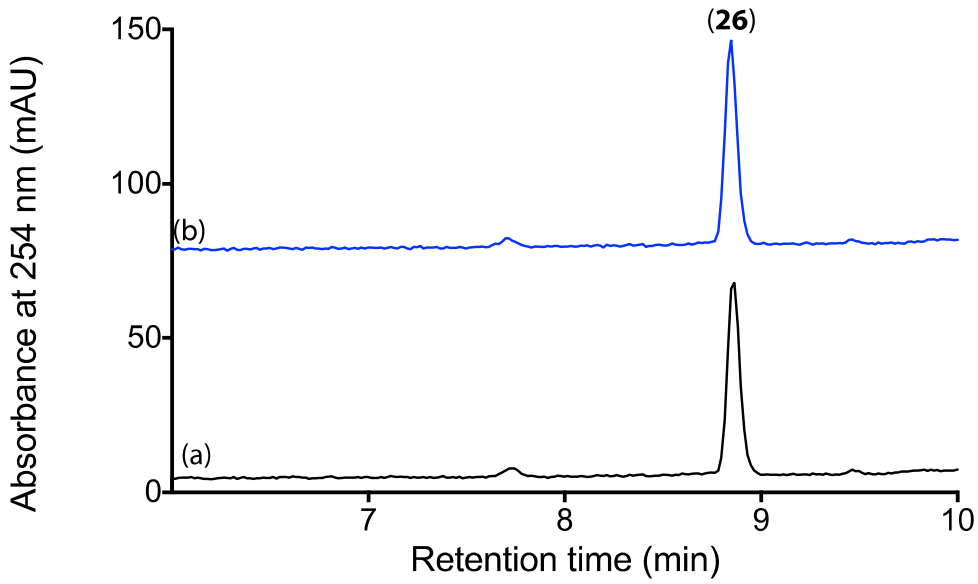


Figure S5. HPLC-DAD (254 nm) chromatogram of *cyclo*-(*N*-methyl-L-tyrosine-L-phenyllactic acid) (**26**) after incubation in MeOH for (a) 3 h and (b) 24 h.

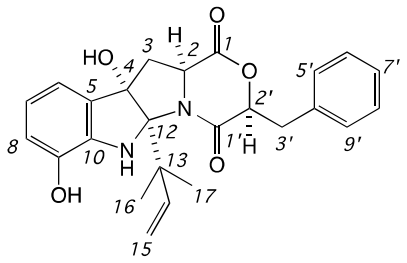


Table S1. 1D and 2D NMR (600 MHz, $CDCl_3$) data of shornephine A (**1**)

pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	ROESY	$^1H - ^{13}C$ HMBC
1		167.7			
2	4.32, d *(11.1)*	57.3	3a/b	2′, 16/17	1, 3, 4
3	a 3.26, d *(13.7)*	36.9	2, 3b		1, 2, 4, 5, 12
	b 2.81, dd *(13.7, 11.1)*		2, 3a		1, 2, 4, 5
4		88.5			
4-OH	2.07, br s			3b	3, 4
5		131.5			
6	6.91, d *(7.7)*	117.1	7	3a	4, 7, 10
7	6.69[b], m	117.3[e]	8		5, 9
8	6.67[b], m	121.2[e]	6, 7		7, 10
6	6.91, d *(7.7)*	117.1	7	3a	4, 7, 10
9		141.4			
9-OH	[d]				
10		135.9			
11-NH	6.34, s			16, 17	4, 5
12		94.9			
13		44.9			
14	6.39, dd *(17.3, 10.6)*	144.1	15a/b	3b, 16/17	13, 16/17
15	a 5.18, d *(17.3)*	113.1	14, 15b	16/17	13, 14
	b 5.11, d *(10.6)*		14, 15a		13, 14
16	1.38, s	22.9		2, 14	12, 13, 14
17	1.38, s	25.8		2, 14	12, 13, 14
1′		165.9			
2′	4.76, dd *(8.8, 1.6)*	78.5	3′a/b	2, 3′a	3′, 4′
3′	a 3.32, d *(15.1)*	34.4	2′, 3′b	2′	4′, 5′/9′
	b 2.92, dd *(15.1, 8.8)*		2′, 3′a		1′, 2′, 4′, 5′/9′
4′		136.2			
5′/9′	7.20[b]	126.7[f]			
6′/8′	7.20[b]	129.3[f]			
7′	7.20[b]	128.5			

[a] ^{13}C NMR assignments supported by gHSQC and gHMBC data.
[b,c] overlapping signals.
[d] not observed.
[e,f] assignments are interchangeable

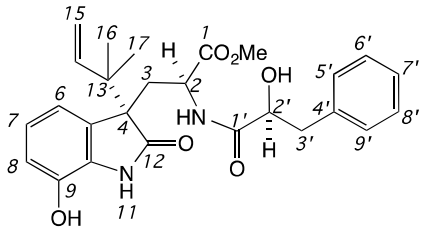


Table S2. 1D and 2D NMR (600 MHz, DMSO-d_6) data of *seco*-shornephine A methyl ester (**1a**)

pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	ROESY	$^1H - ^{13}C$ HMBC
1		172.5			
1-OMe	3.38, s	52.1			1
2	3.79, ddd, (*9.7, 8.0, 5.4*)	49.8	3a/b, 2-NH	2-NH, 6	1, 1′, 3
2-NH	7.35, d, (*8.0*)		2	2, 2′, 3b	1′, 2
3	a 2.32, dd, (*14.2*, *5.4)*	32.8	3b, 2	6	1, 2, 4, 5, 12, 13
	b 2.24, dd, (*14.2, 9.7*)		3a, 2	2-NH	1, 2, 4, 5, 12, 13
4		55.8			
5		130.0			
6	6.56, d, (*7.4*)	117.0	7	2, 3a, 14, 16, 17	4, 8, 10
7	6.78, dd, (*8.0*, *7.4*)	121.4	6, 8		5, 9
8	6.70, d, (*8.0*)	115.6	7		6, 9, 10
9		141.5			
9-OH	9.50, br s				
10		131.0			
11-NH	10.20, br s				
12		179.7			
13		42.3			
14	6.04, dd, (*17.4*, *10.8*)	143.5	15a/b	6, 16, 17	13, 17
15	a 5.07, dd, (*10.8*, *0.6*)	113.8	14		13
	b 4.99, dd, (*17.4*, *0.6*)		14		13, 14
16	1.01, s	22.1		6, 14	4, 13, 14, 17
17	0.93, s	21.8		6, 14	4, 13, 14, 16
1′		173.5			
2′	3.90, dd, (*9.6*, *3.3*)	72.5	3′a/b	2-NH	1′
2′-OH	5.53, br s		2′		
3′	a 2.79, dd, (*13.8*, *3.3*)	40.4	2′, 3′b		2′, 4′, 5′/9′
	b 2.61, dd, (*13.8*, *9.6*)		2′, 3′a		2′, 4′, 5′/9′
4′		138.8			
5′/9′	7.22, d, (*7.2*)	129.7	6′/8′		5′/9′, 7′
6′/8′	7.26, ddd, (*7.2*, *7.2, 0.6*)	128.2	5′/9′, 7′		6′/8′, 4′
7′	7.18, td, (*7.2*, *0.6*)	126.3	6′/8′		5′/9′

[a] ^{13}C NMR assignments supported by gHSQC and gHMBC data.

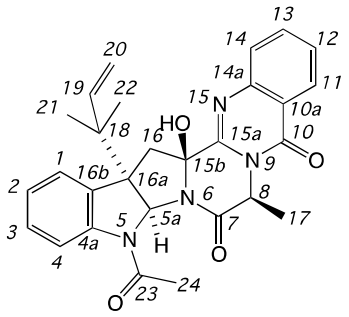


Table S3. 1D and 2D NMR (600 MHz, $CDCl_3$) data of 15b-β-hydroxyl-5-*N*-acetyladreemin (**2**)[2]

pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	ROESY	$^1H - ^{13}C$ HMBC
1	7.42, d *(7.5)*	124.4	2		3, 16b
2	7.21, dd *(7.5, 7.5)*	124.9	1, 3		1, 3, 4, 4a, 16b
3	7.45, dd *(7.5, 7.5)*	130.2	2, 4		1, 16b
4	8.07, br s	[b]	3		
4a		142.5			
5a	6.04, br s	80.2		21, 22, 24	
7		168.4			
8	5.34, q *(7.1)*	53.5	17		7, 10, 15a, 17
10		160.1			
10a		120.7			
11	8.27, d *(7.5)*	127.1	12		10, 12, 14a
12	7.77, dd *(7.5, 7.5)*	128.3	11, 13		10a, 11
13	7.51, dd *(8.1, 7.5)*	134.9	12, 14		11, 14a
14	7.72, d *(8.1)*	127.8	13		10, 10a, 13
15a		150.6			
15b		89.2			
16	α 3.15, d *(14.8)*	43.8		21, 22	15a, 15b, 18
	β 3.06, d *(14.8)*			21, 22	5a, 15b
16a		59.4			
16b		133.3			
17	1.64, d *(7.1)*	18.3	8		7, 8
18		40.5			
19	5.78, dd *(17.0, 10.9)*	143.4	20α,β	16α, 21, 22	18, 22
20	α 5.13, d *(10.9)*	115.9	19	21, 22	18, 19
	β 5.11, d *(17.0)*		19	21, 22	18, 19
21	0.98, s	22.6	22	5a, 19	18, 19, 22
22	1.17, s	22.8	21	5a, 19	18, 19, 21
23		170.8			
24	2.65, br s	23.6		21, 22	23
15b-OH	2.21, br s				15a

[a] ^{13}C NMR assignments supported by gHSQC and gHMBC data
[b] not observed

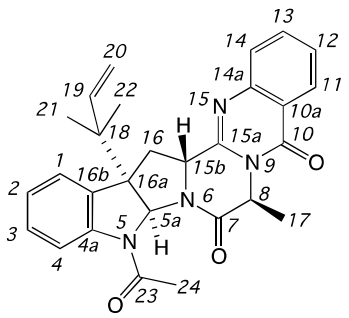


Table S4. 1D and 2D NMR (600 MHz, $CDCl_3$) data of 5-*N*-acetyladreemin (**3**)[2]

pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	ROESY	$^1H - ^{13}C$ HMBC
1	7.39, d (*7.8*)	124.7	2		4, 16
2	7.18, dd (*7.8, 7.8*)	124.5	1, 3		4
3	7.36, dd (*7.8*, *7.2*)	130.1	2, 4		1, 4a
4	8.03, br s	121.2	3		2
4a		142.2			
5a	6.04, br s	81.2		16β, 21, 22	16, 18
7		169.1			
8	5.41, q (*7.2*)	53.6	17		7
10		160.1			
10a		121.2			
11	8.27, dd (*7.8, 1.2*)	127.8	12		
12	7.50, dd (*7.8*, *7.2*)	127.5	11, 13		10a
13	7.72, ddd (*8.4*, *7.2*, *1.2*)	135.2	12, 14		14a
14	7.66, d (*8.4*)	126.3	13		
14a		149.4			
15a		151.1			
15b	4.41, dd (*10.3*, *5.5*)	90.2	16α,β	16α,β, 17	15a
16a		60.1			
16b		132.3			
16	α 3.02, dd (*12.8, 5.5*)	59.2	15b, 16β		18
	β 2.69[b]		15b, 16α		18
17	1.44, d (*7.2*)	19.1	8	15b	
18		41.2			
19	5.85, dd (*17.4*, *11.0*)	145.3	20α, β	16β, 21, 22	
20	α 5.12 d (*17.4*)	115.1	19, 20β		21, 22
	β 5.11, d (*11.0*)		19, 20α	21, 22	21, 22
21	1.21, s	22.1	22		4a, 18, 20
22	1.03, s	22.3	21		4a, 18, 20
23		171.4			
24	2.67, s	23.6			

[a] ^{13}C NMR assignments supported by gHSQC and gHMBC data.
[b] Overlapping signal

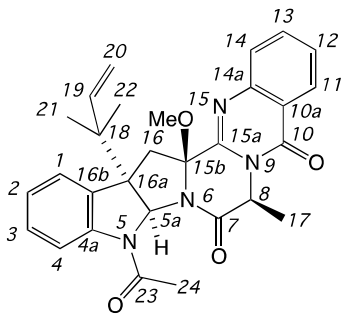


Table S5. 1D and 2D NMR (600 MHz, $CDCl_3$) of 15b-β-methoxy-5-*N*-acetylardeemin (**4**)

pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	ROESY	$^1H - ^{13}C$ HMBC
1	7.35, d (*7.8*)	123.8	2	16β	3, 4a
2	7.13, dd (*7.8*, *0.6*)	123.9	1, 3		4
3	7.31, dd (*7.8*, *0.6*)	128.5	2, 4		1, 4a
4	7.98, br d (*7.8*)	119.5	3		
4a		143.0			
5a	6.10, br s	80.5		24	
7		168.0			
8	5.29, q, (*6.6*)	54.9	17		7, 15a, 17
10		160.0			
10a		120.8			
11	8.29, d (*7.8*)	126.9	12		10, 13, 14a
12	7.54, dd (*7.8*, *1.8*)	127.9	11, 13		10a, 14
13	7.80, dd (*7.8*, *1.8*)	134.8	12, 14		11, 14a
14	7.75, d (*7.8*)	127.9	13		10a, 12
14a		146.3			
15a		148.1			
15b		92.7			
15b-OMe	2.70, s	52.1		16α, 17	15b
16	α 3.15, d (*14.4*)	37.4	16β	1, 21, 22	5a, 15b
	β 2.79, d (*14.4*)		16α	19	15a, 18
16a		59.1			
16b		134.2			
17	1.64, d (*6.6*)	18.9	8	15b-OMe	7, 8
18		40.9			
19	5.85, dd (*17.5*, *10.8*)	143.3	20α, β	16b	
20	α 5.12, d (*17.5*)	114.9	19		18
	β 5.11, d (*10.8*)		19		18
21	1.20, s	22.6		16α	18, 19, 22
22	1.01, s	23.3		16α	18, 19, 21
23					
24	2.66, br s	23.9		5a	

[a] ^{13}C NMR assignments supported by gHSQC and gHMBC data

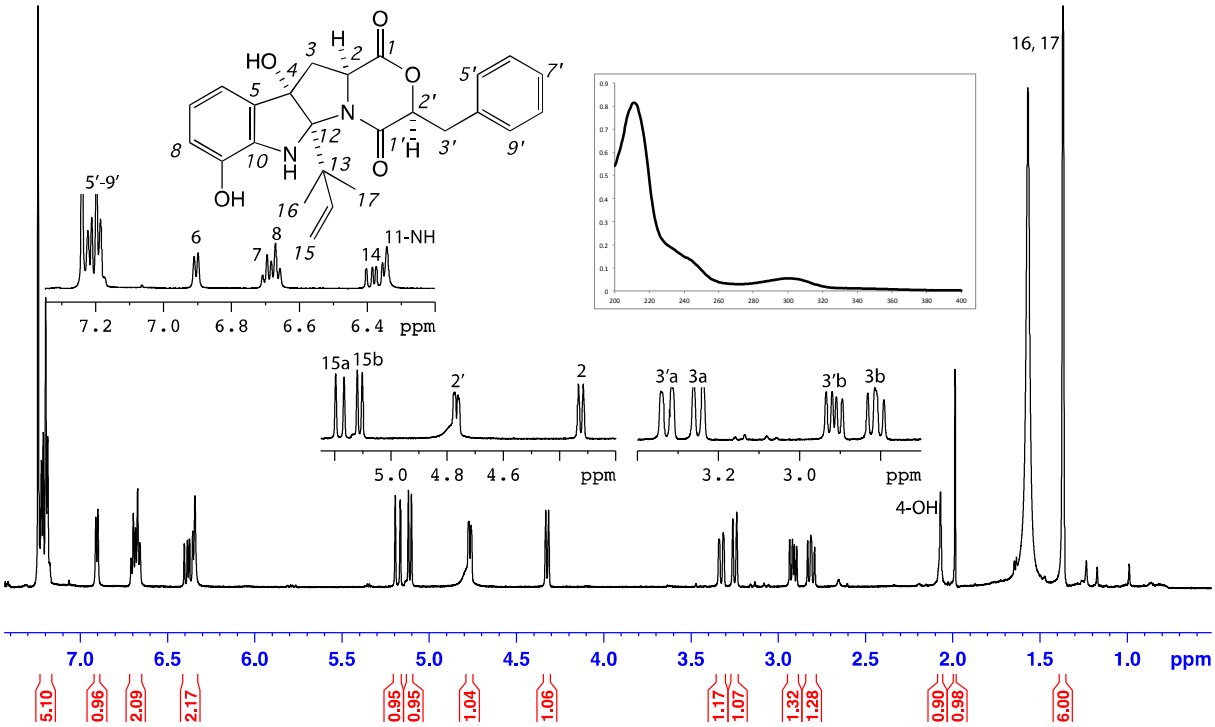


Figure S6. ^{1}H NMR (600 MHz, $CDCl_3$) and UV-vis spectra of shornephine A (**1**)

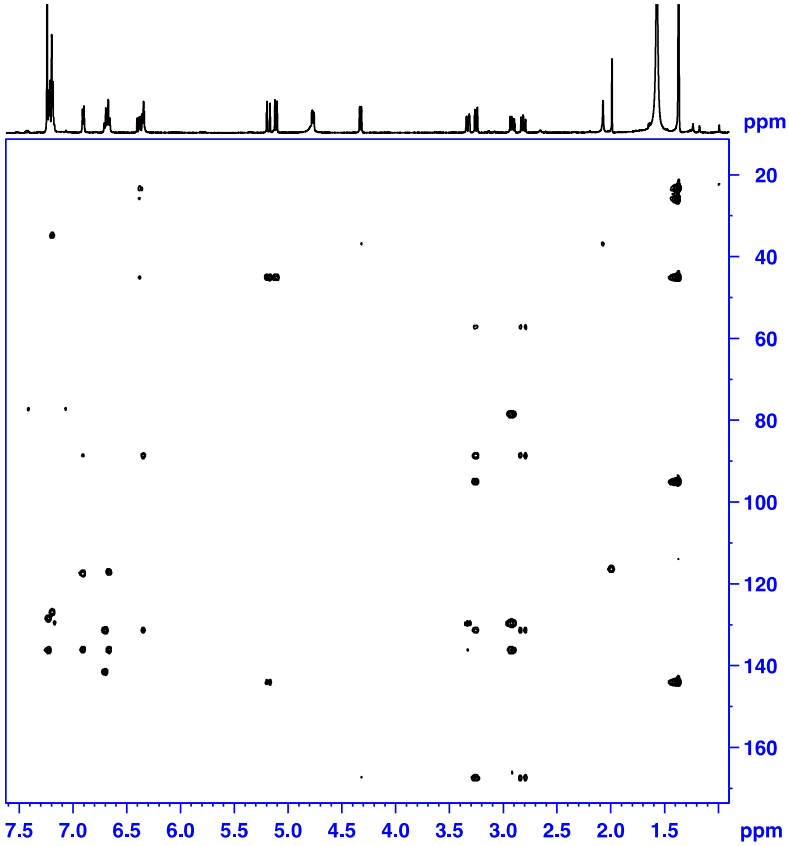


Figure S7. HMBC NMR (600 MHz, $CDCl_3$) spectrum of shornephine A (**1**)

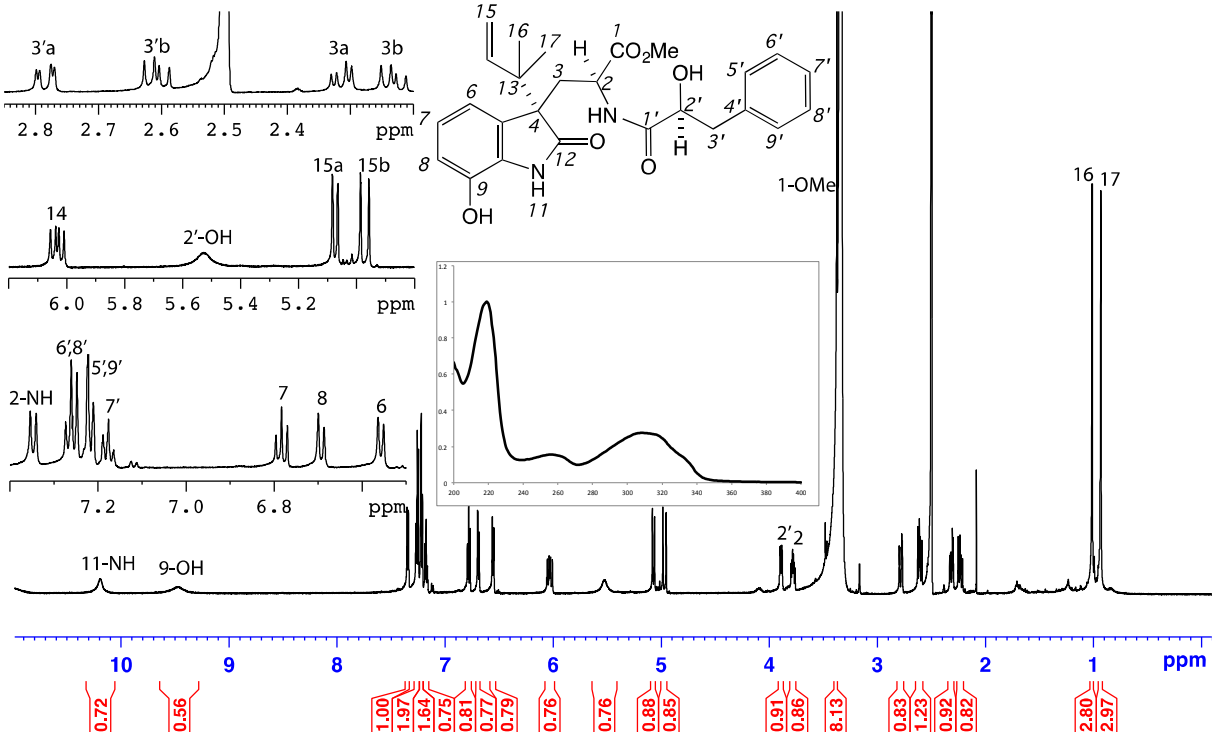


Figure S8. ^{1}H NMR (600 MHz, DMSO-d_6) and UV-vis spectra of *seco*-shornephine A methyl ester (**1a**)

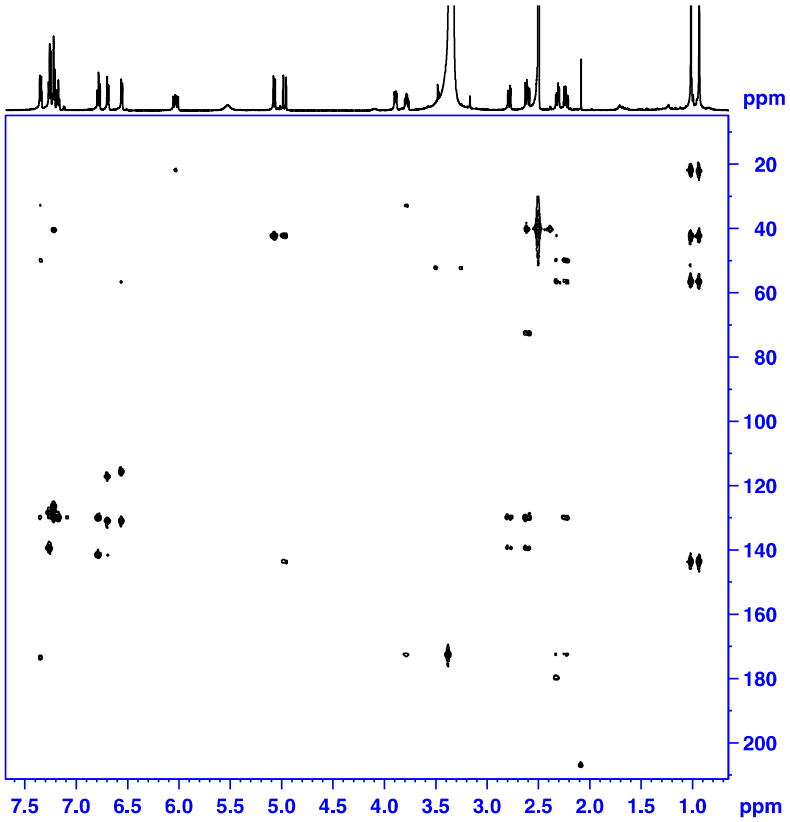


Figure S9. HMBC NMR (600 MHz, DMSO-d_6) spectrum of *seco*-shornephine A methyl ester (**1a**)

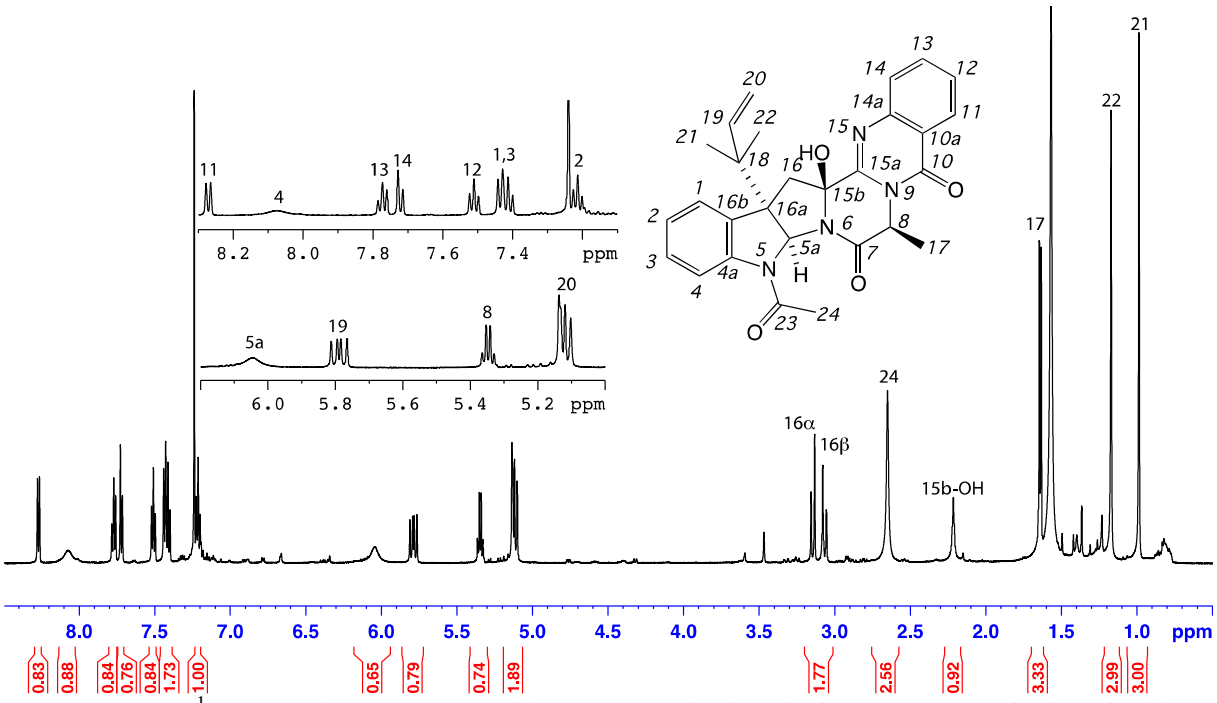


Figure S10. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of 15b-β-hydroxy-5-*N*-acetylardeemin (**2**)

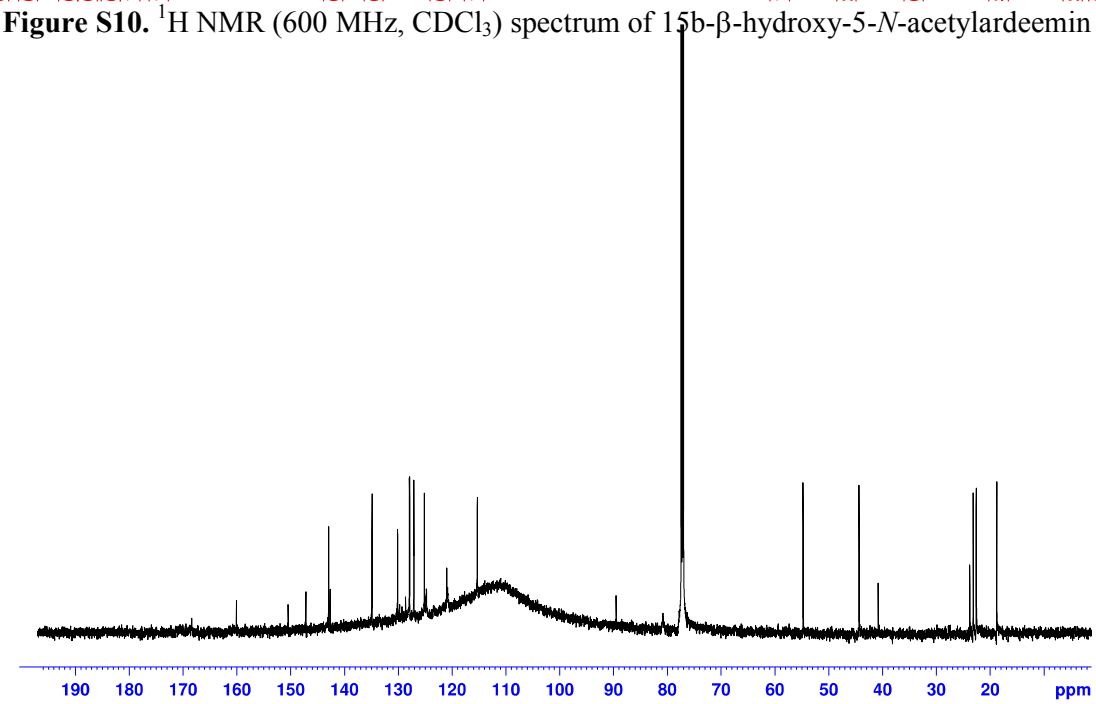


Figure S11. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of 15b-β-hydroxy-5-*N*-acetylardeemin (**2**)

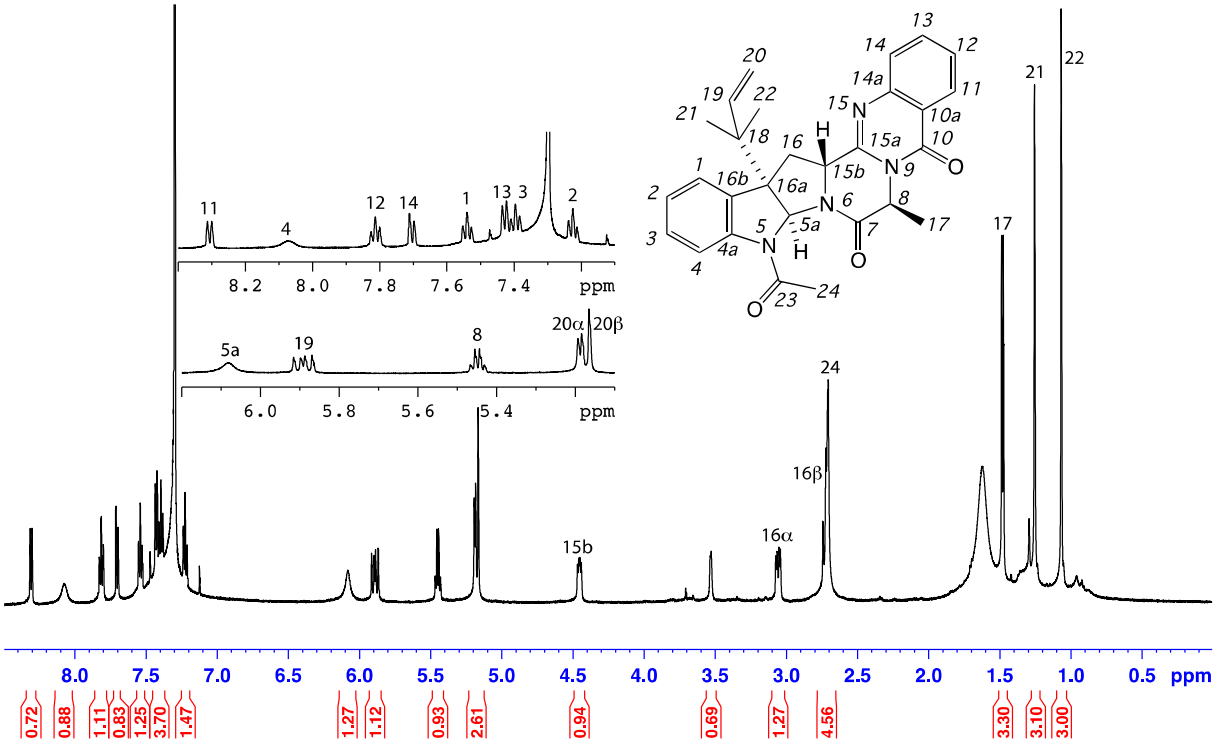


Figure S12. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of 5-*N*-acetylardeemin (**3**)

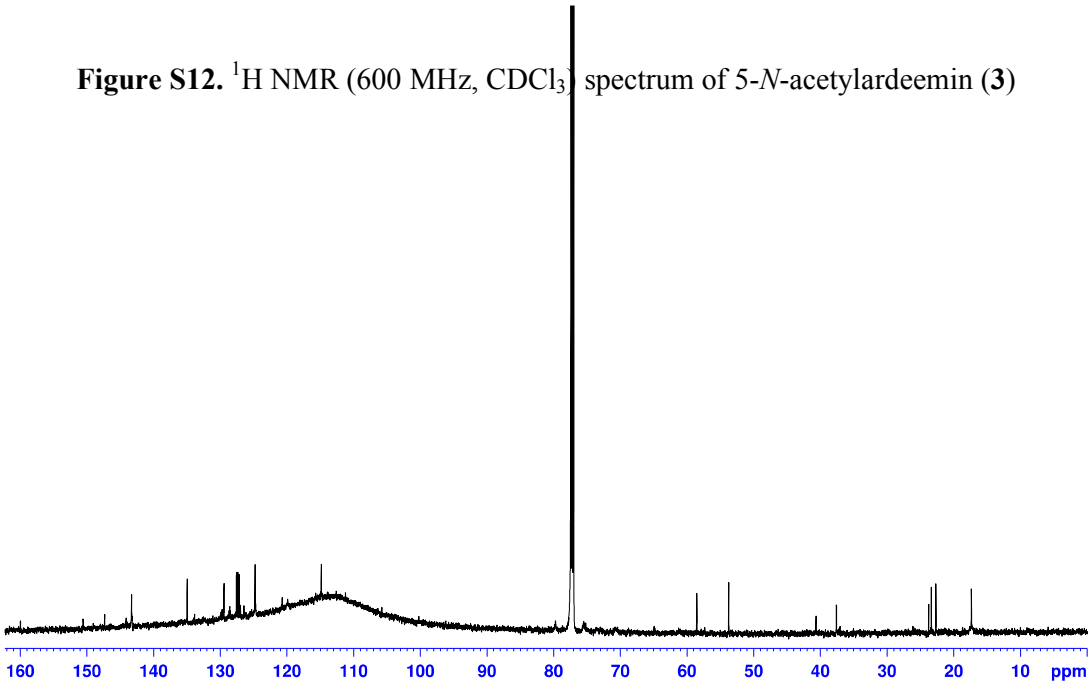


Figure S13. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of 5-*N*-acetylardeemin (**3**)

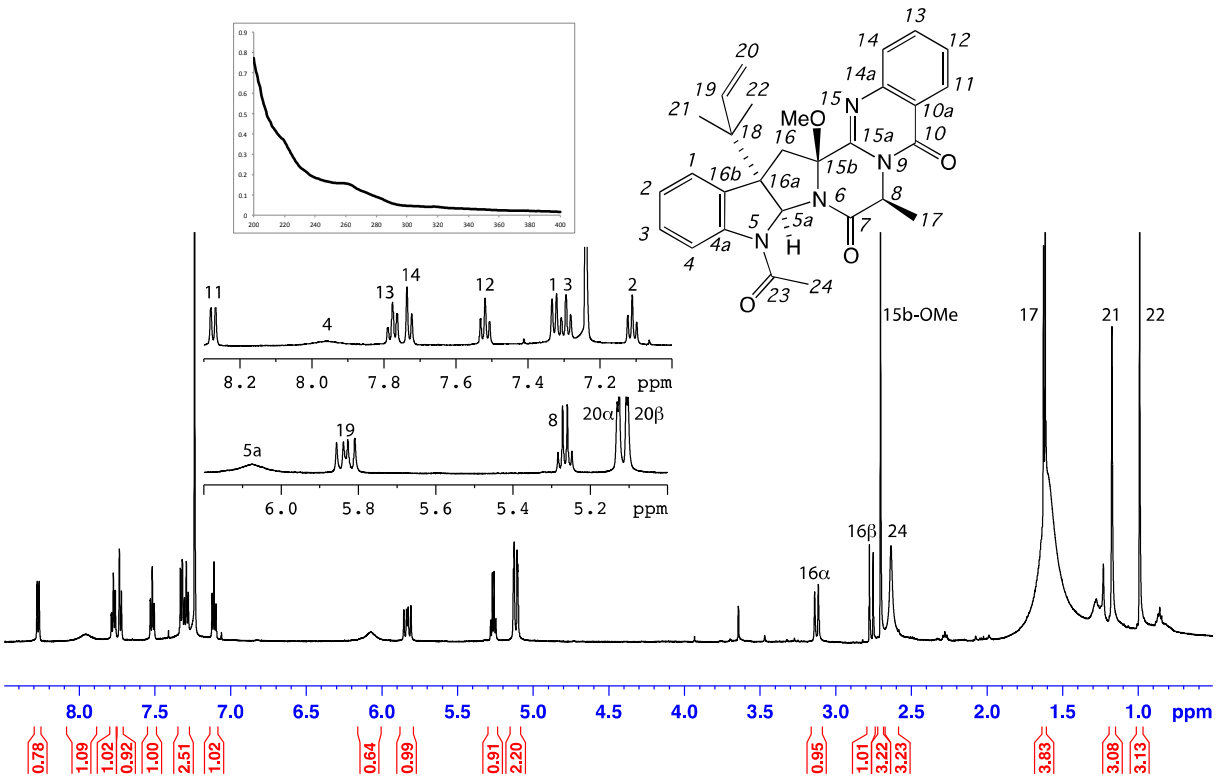


Figure S14. ^{1}H NMR (600 MHz, $CDCl_3$) and UV-vis spectra of 15b-β-methoxy-5-*N*-acetylardeemin (**4**)

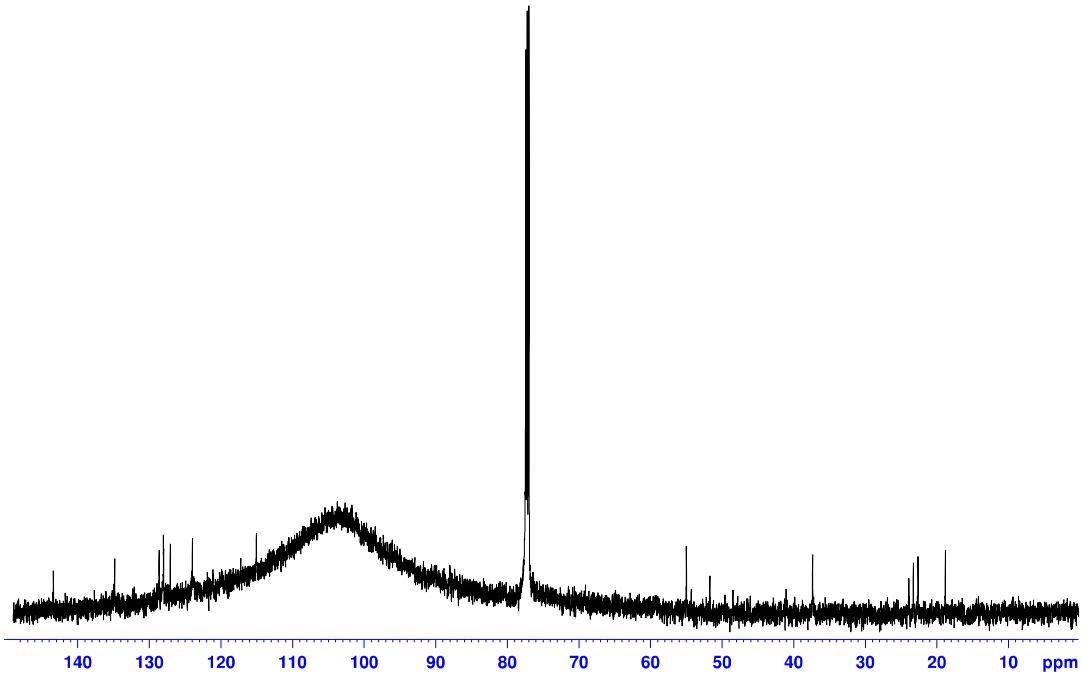


Figure S15. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of 15b-β-methoxy-5-*N*-acetylardeemin (**4**)

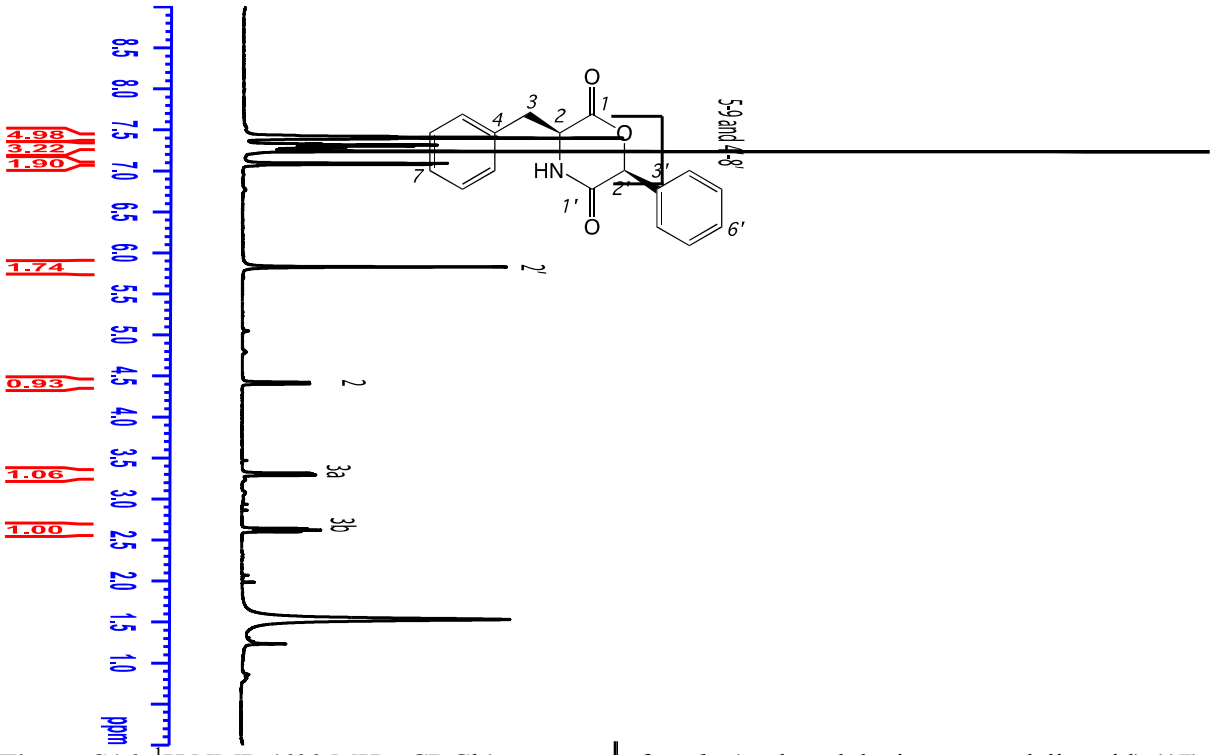


Figure S16. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-phenylalanine-L-mandelic acid) (**17**)

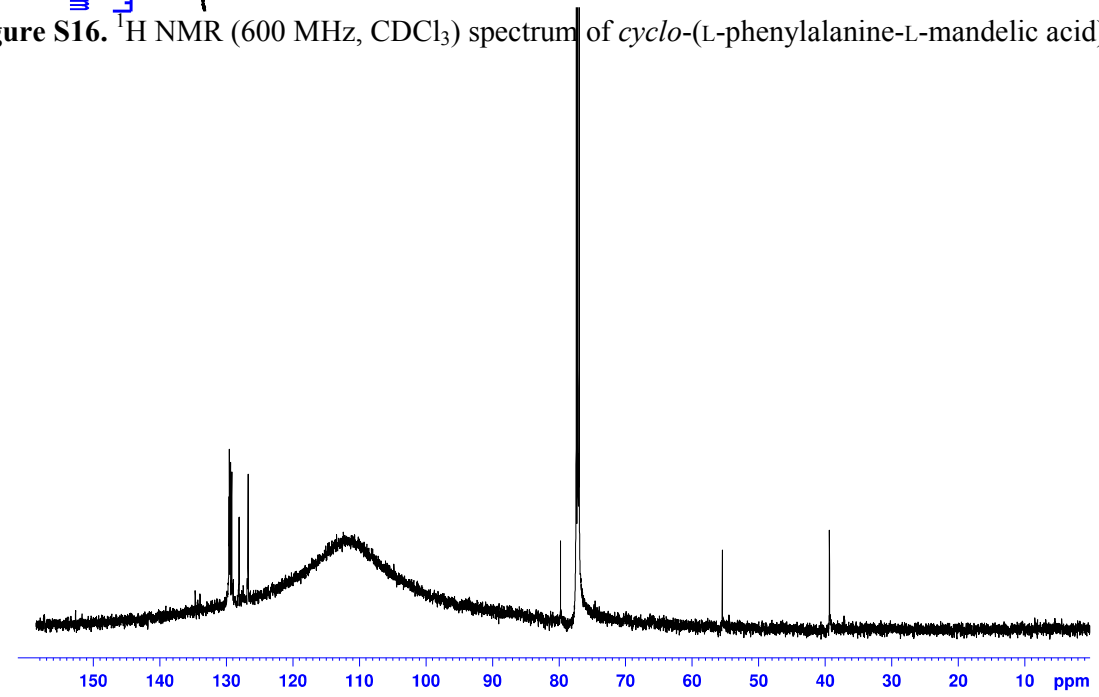


Figure S17. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(L-phenylalanine-L-mandelic acid) (**17**)

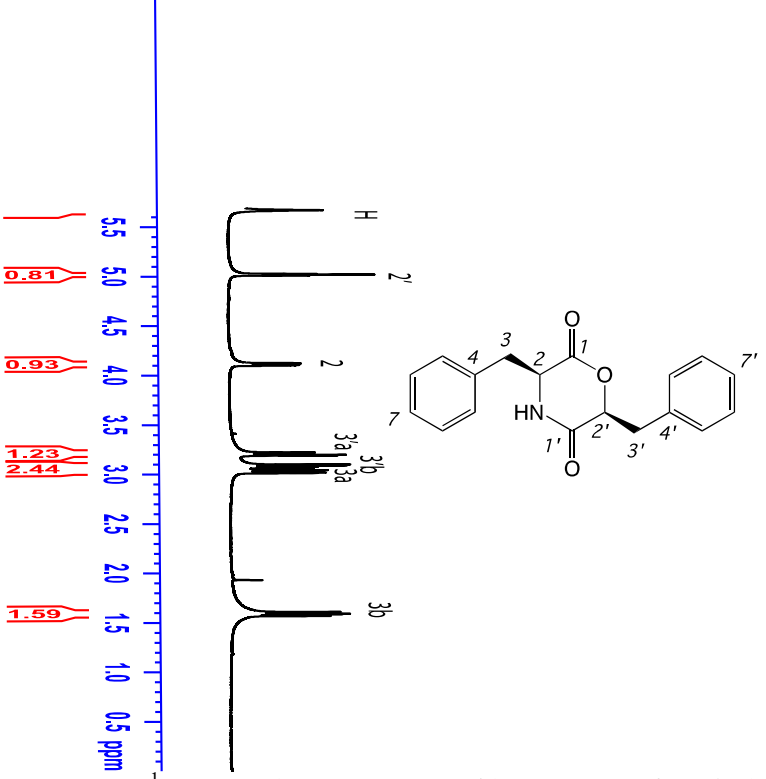


Figure S18. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-phenylalanine-L-phenyllactic acid) (**18**)

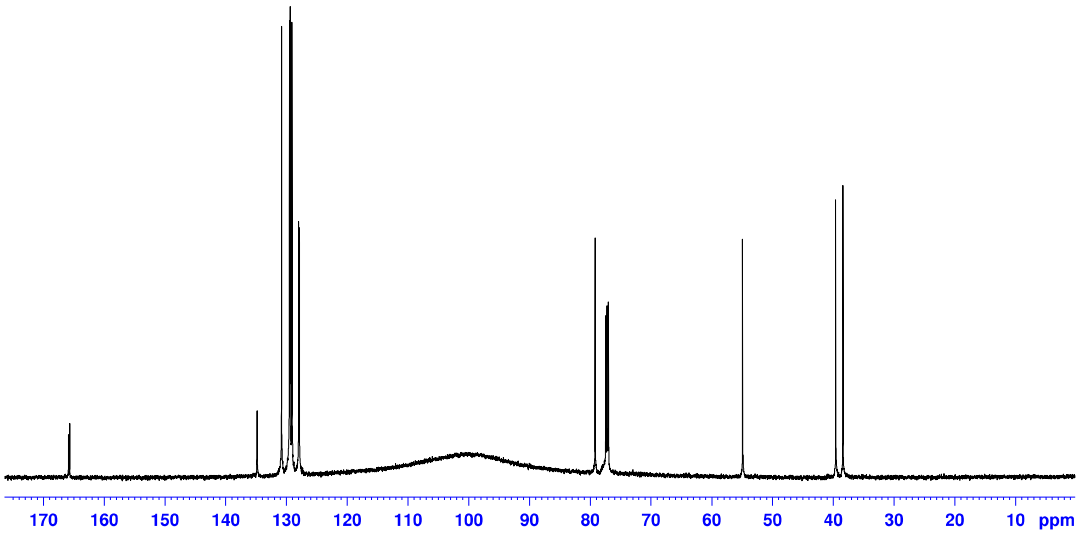


Figure S19. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(L-phenylalanine-L-phenyllactic acid) (**18**)

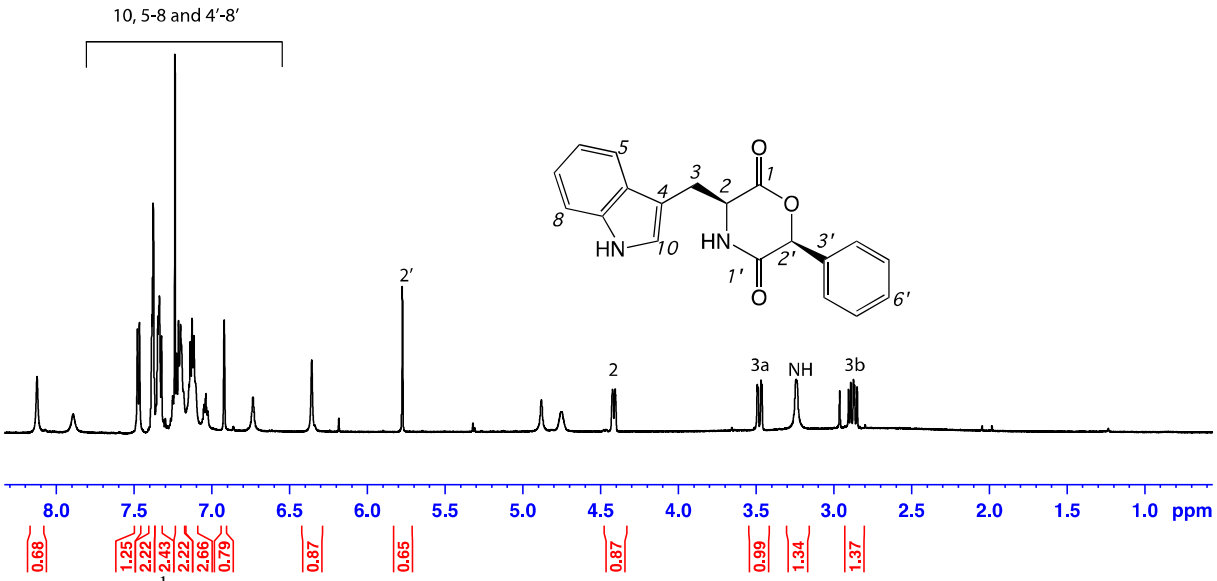


Figure S20. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-tryptophan-L-mandelic acid) (**19**)

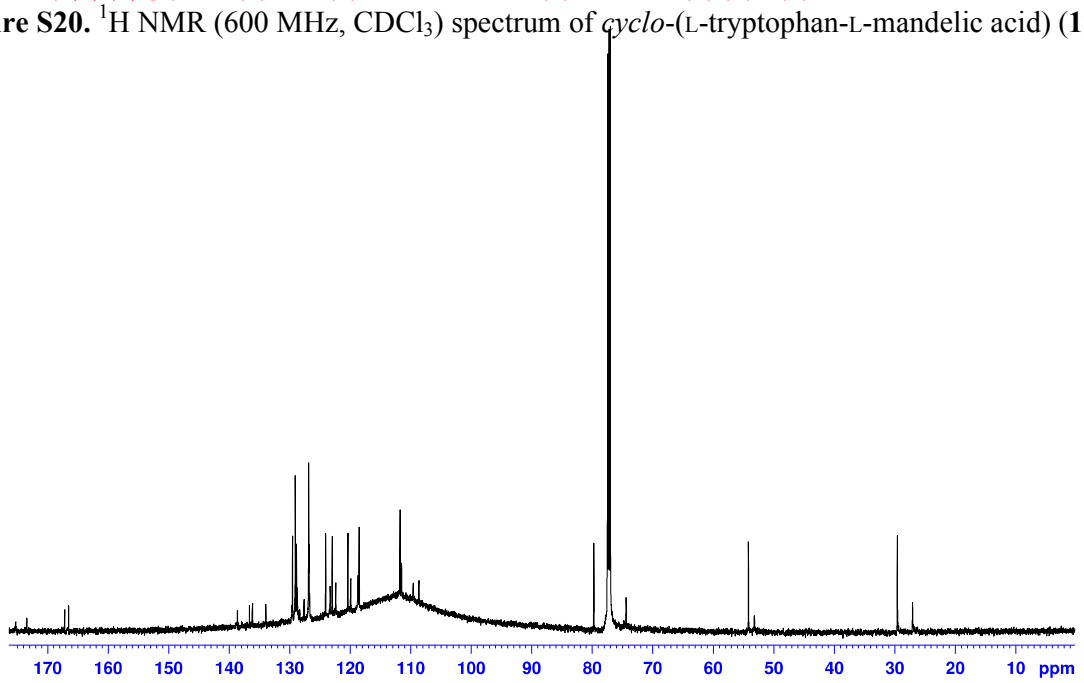


Figure S21. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(L-tryptophan-L-mandelic acid) (**19**)

*impurities present in the spectra due to relative instability of the compounds

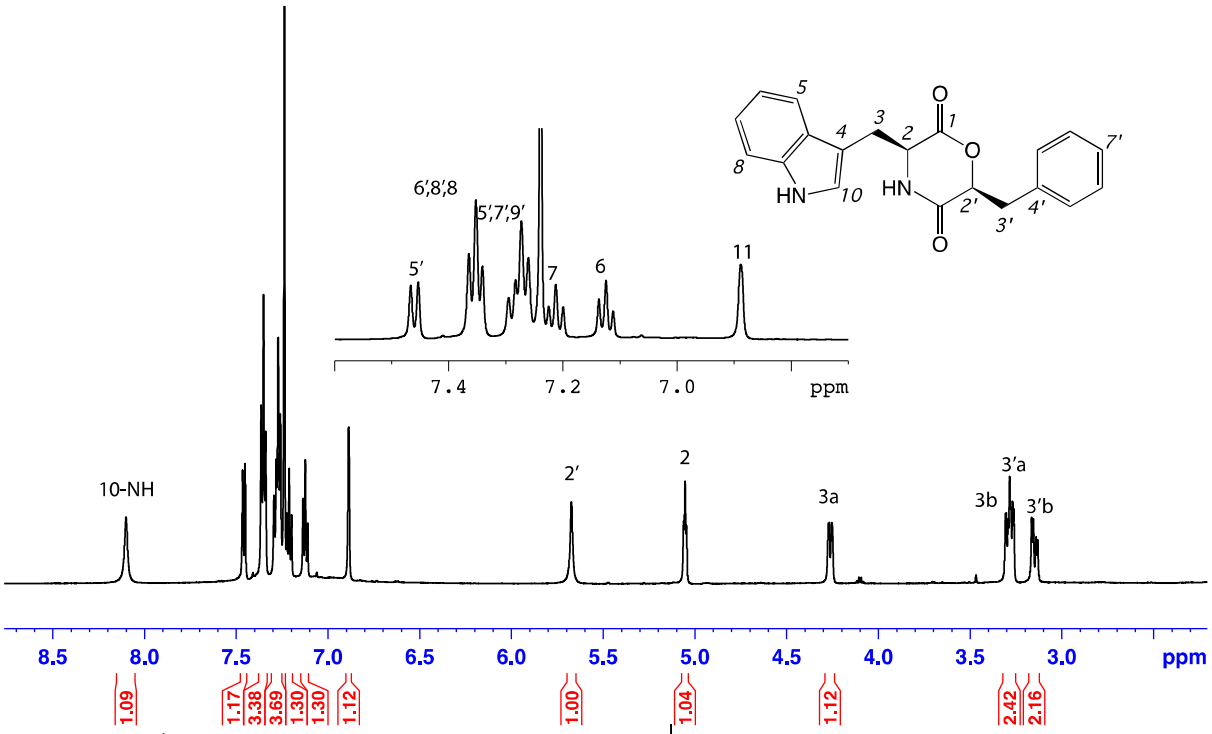


Figure S22. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-tryptophan-L-phenyllactic acid) (**20**)

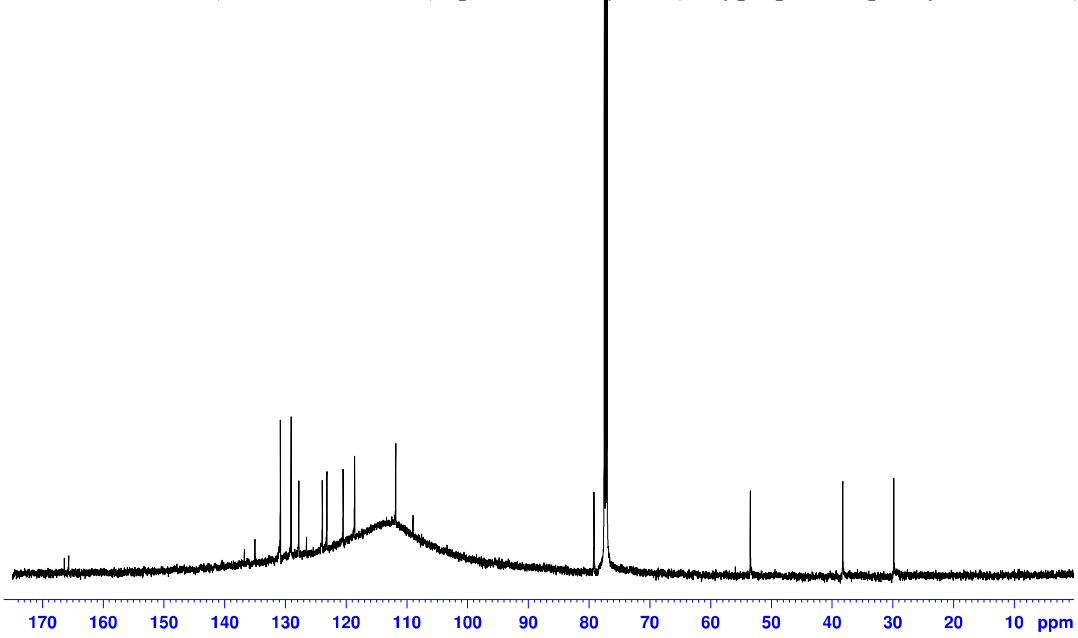


Figure S23. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(L-tryptophan-L-phenyllactic acid) (**20**)

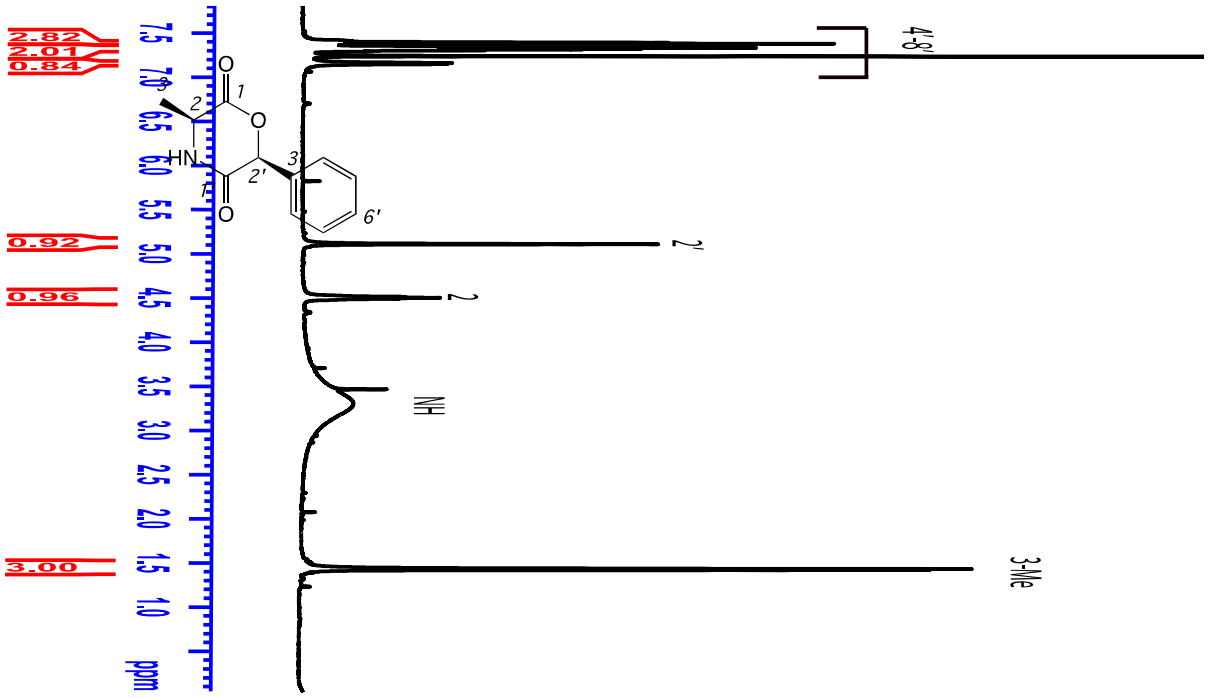


Figure S24. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-alanine-L-mandelic acid) (**21**)

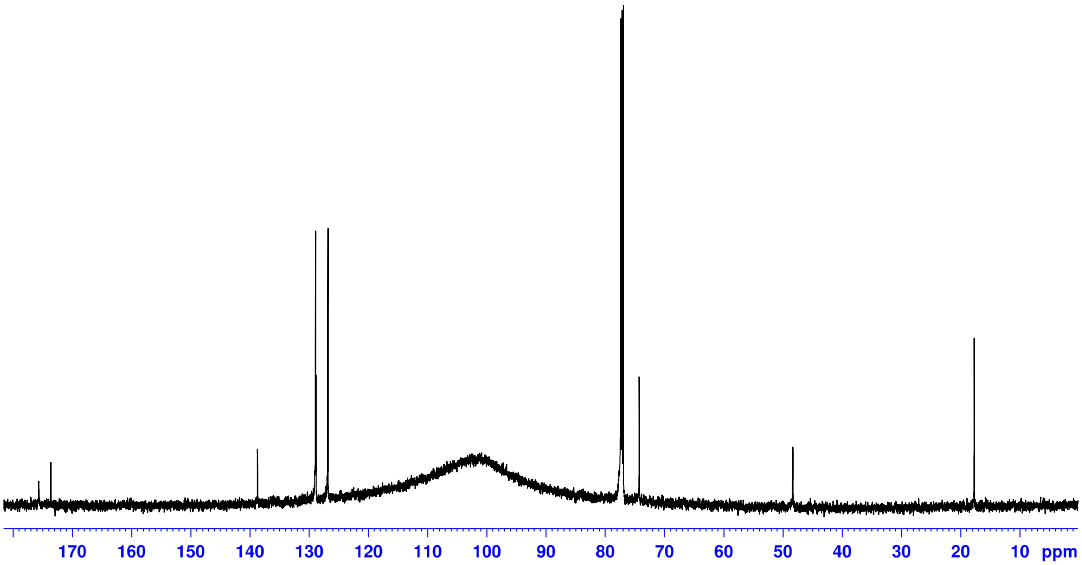


Figure S25. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(L-alanine-L-mandelic acid) (**21**)

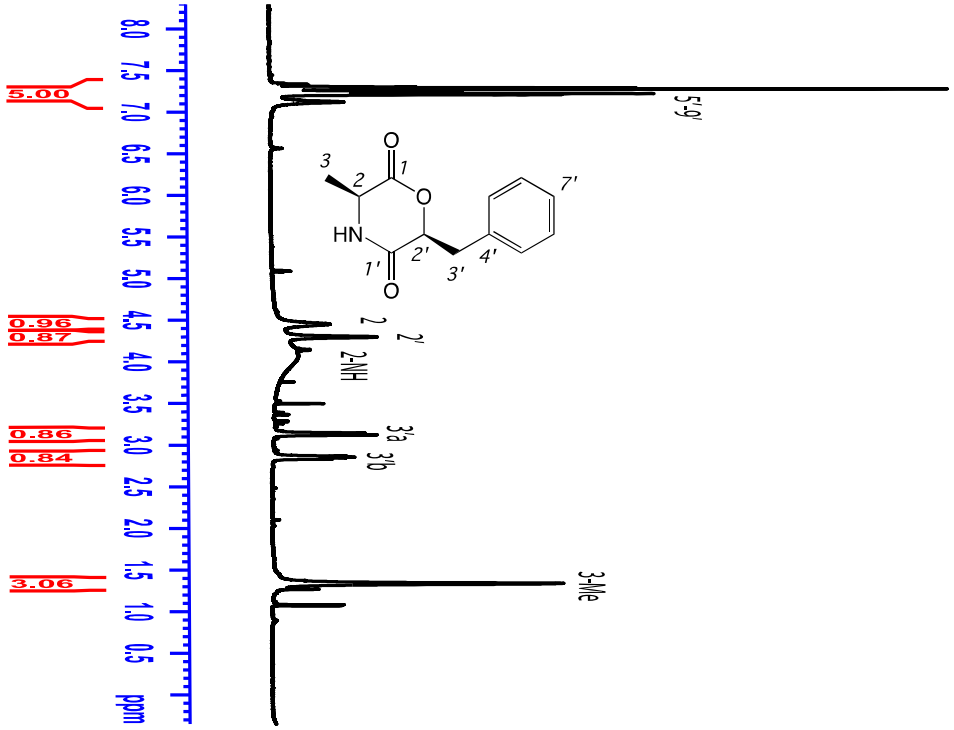


Figure S26. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-alanine-L-phenyllactic acid) (**22**)

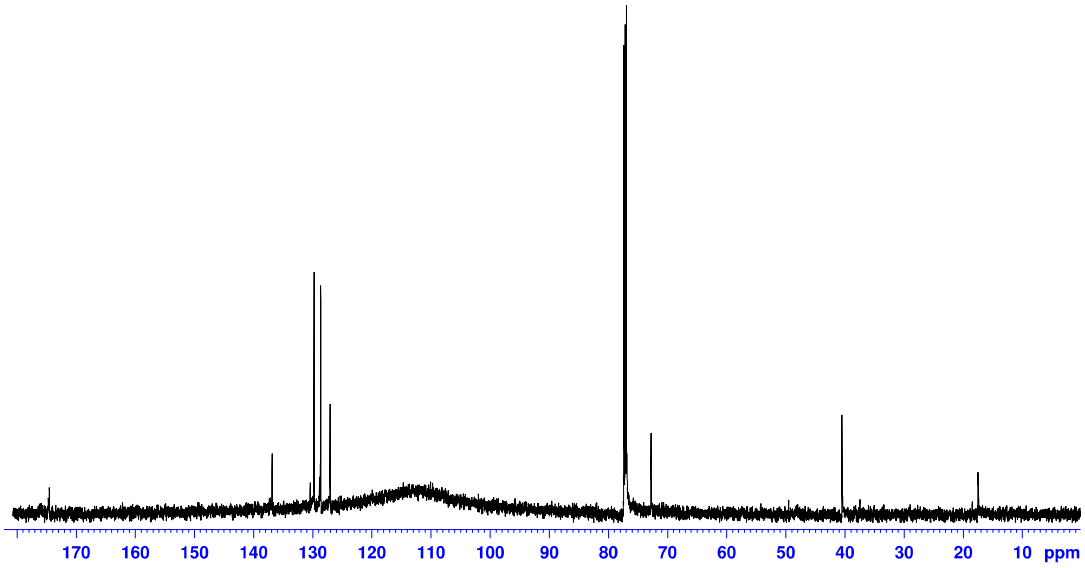


Figure S27. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(L-alanine-L-phenyllactic acid) (**22**)

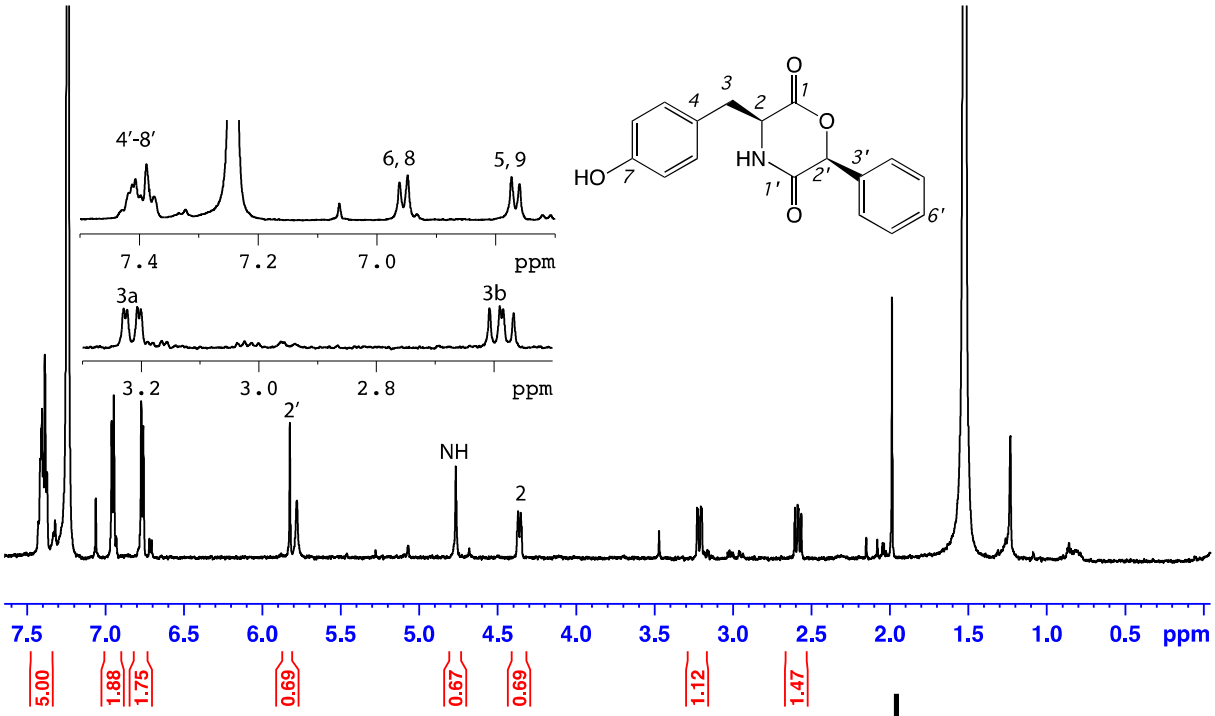


Figure S28. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-tyrosine-L-mandelic acid) (**23**)

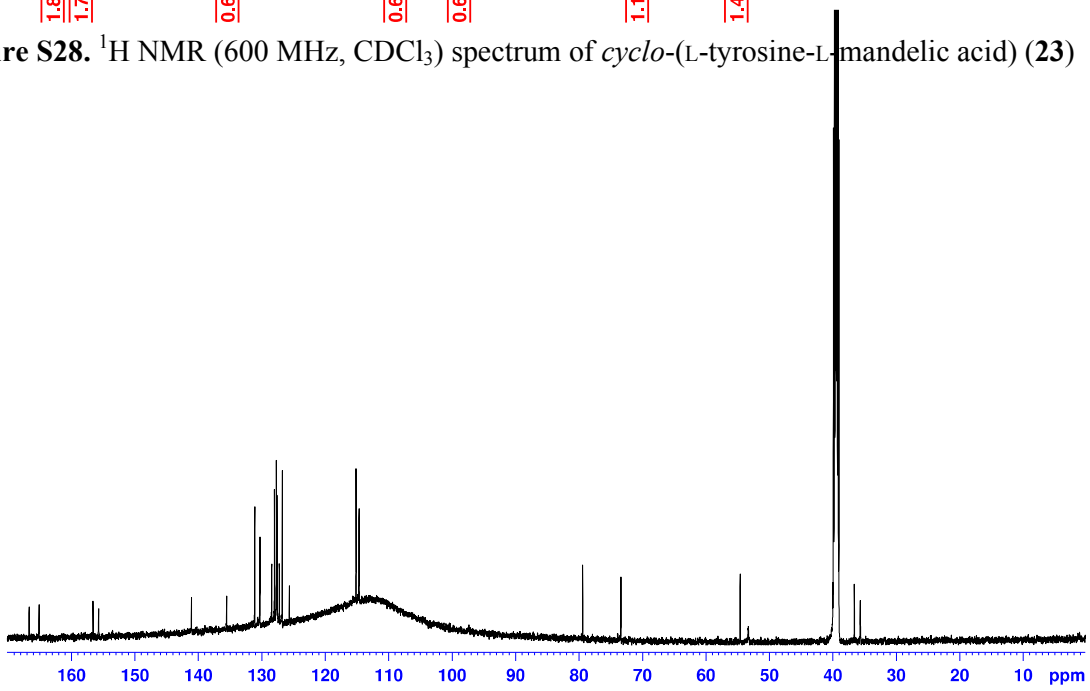


Figure S29. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of *cyclo*-(L-tyrosine-L-mandelic acid) (**23**)

*impurities present in the spectra due to relative instability of the compounds

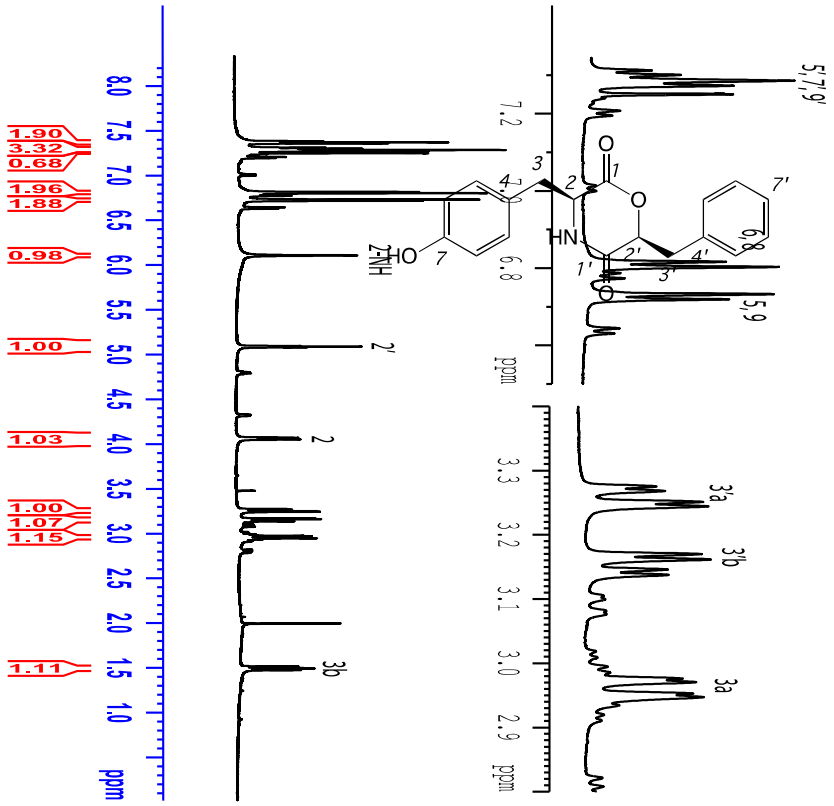


Figure S30. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(L-tyrosine-L-phenyllactic acid) (**24**)

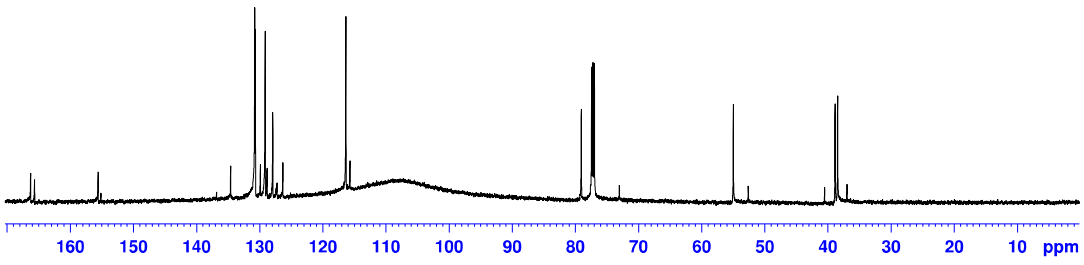


Figure S31. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(L-tyrosine-L-phenyllactic acid) (**24**)

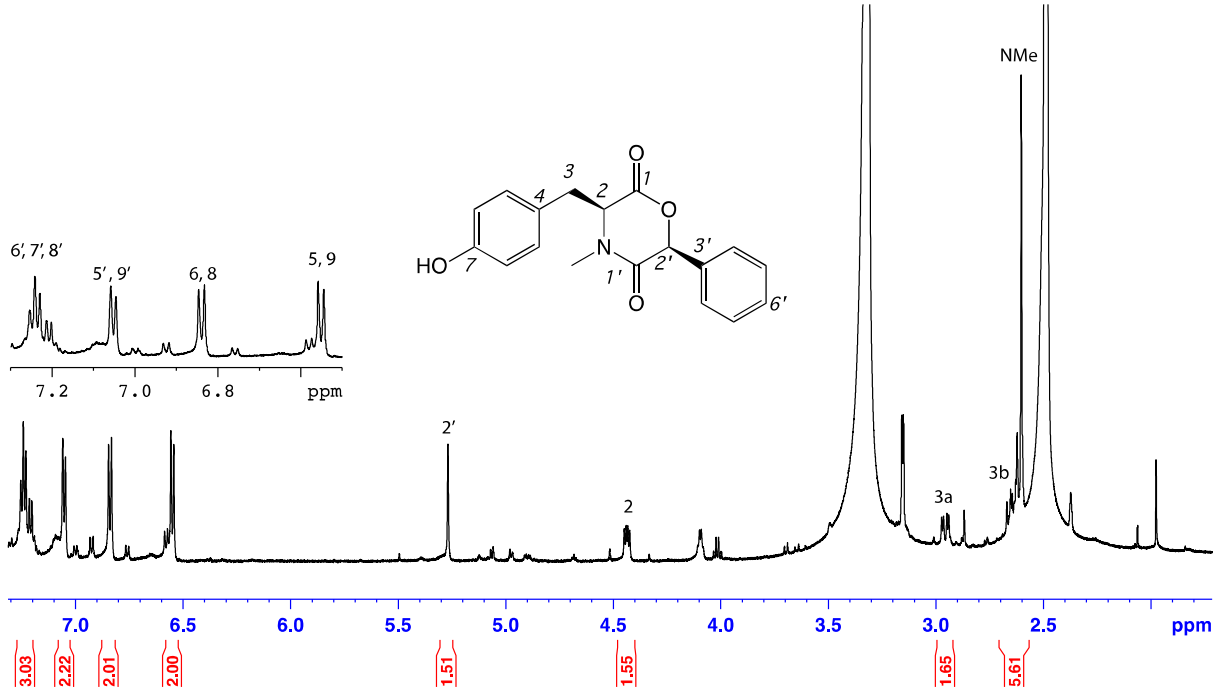


Figure S32. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of *cyclo*-(*N*-methyl-L-tyrosine-L-mandelic acid) (**25**)

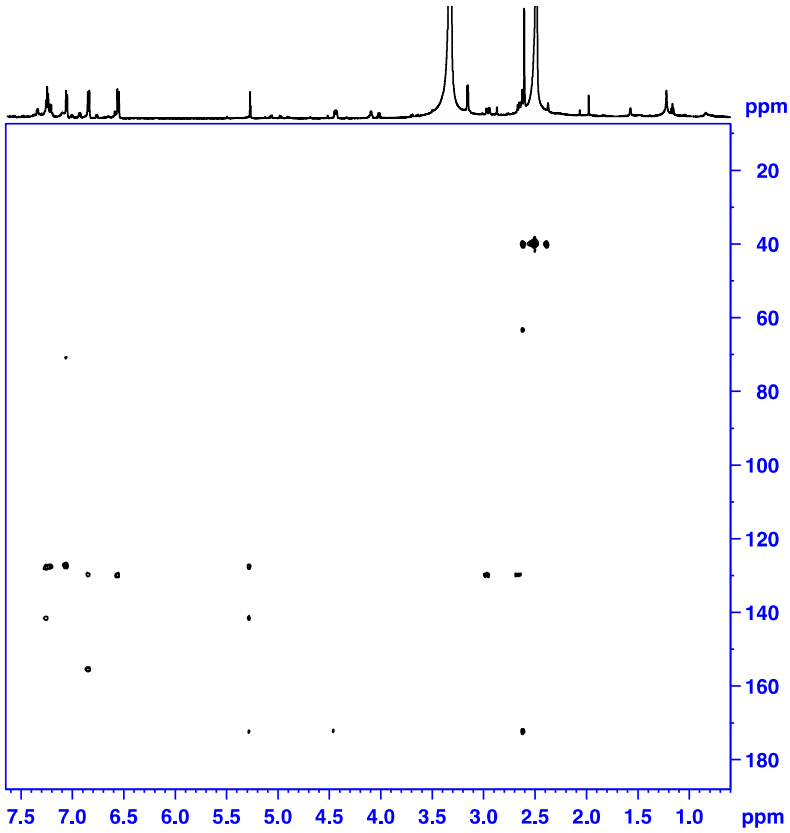


Figure S33. HMBC NMR (600 MHz, DMSO-d_6) NMR spectrum of *cyclo*-(*N*-methyl-L-tyrosine-L-mandelic acid) (**25**)

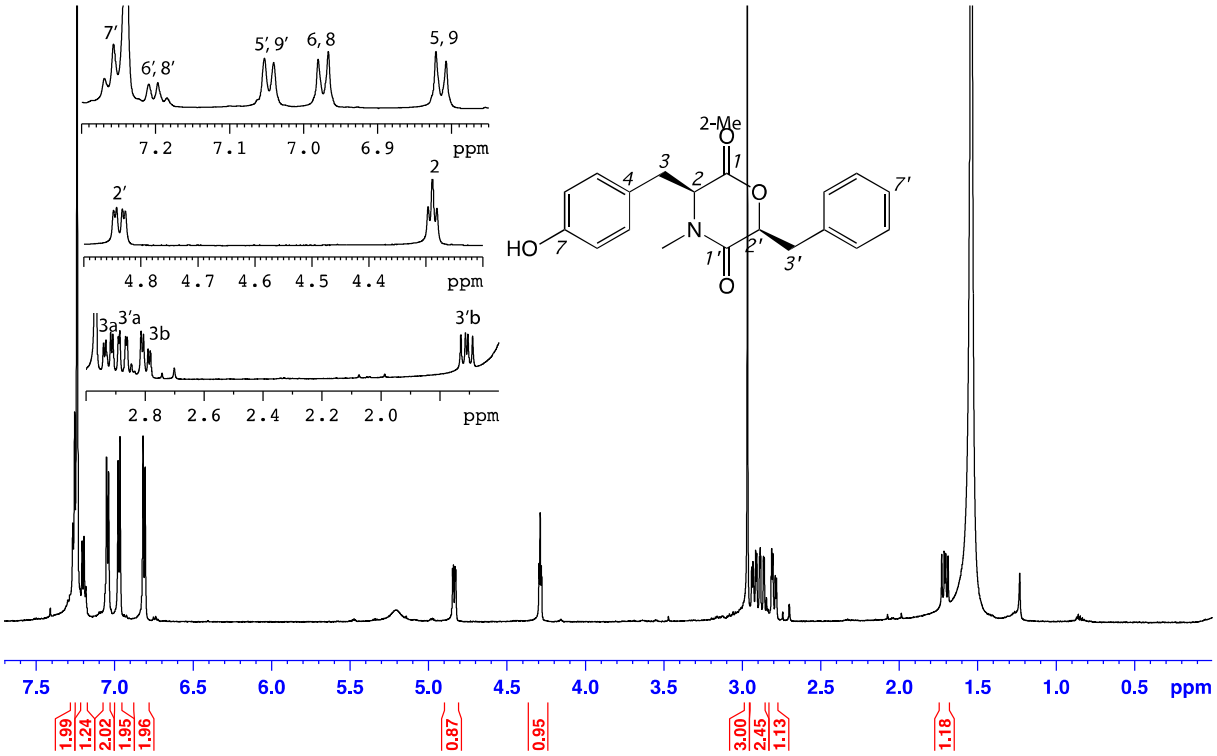


Figure S34. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of *cyclo*-(*N*-methyl-L-tyrosine-L-phenyllactic acid) (**26**)

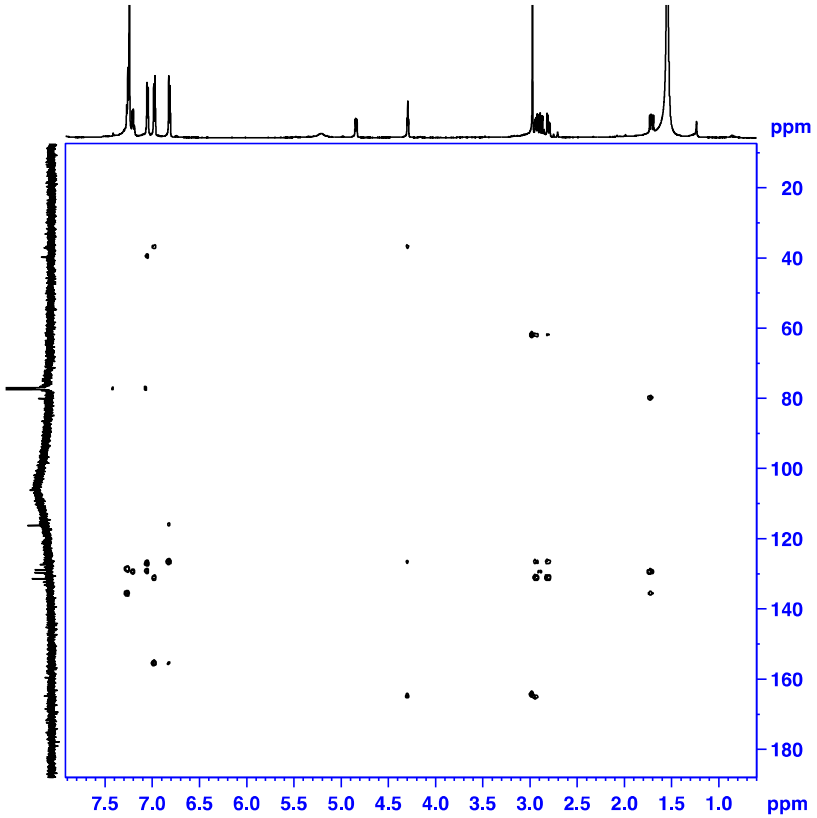


Figure S35. HMBC NMR (150 MHz, $CDCl_3$) spectrum of *cyclo*-(*N*-methyl-L-tyrosine-L-phenyllactic acid) (**26**)

4 Biological Data

4.1 Antibiotic Screening Data for 1−4 and 1a

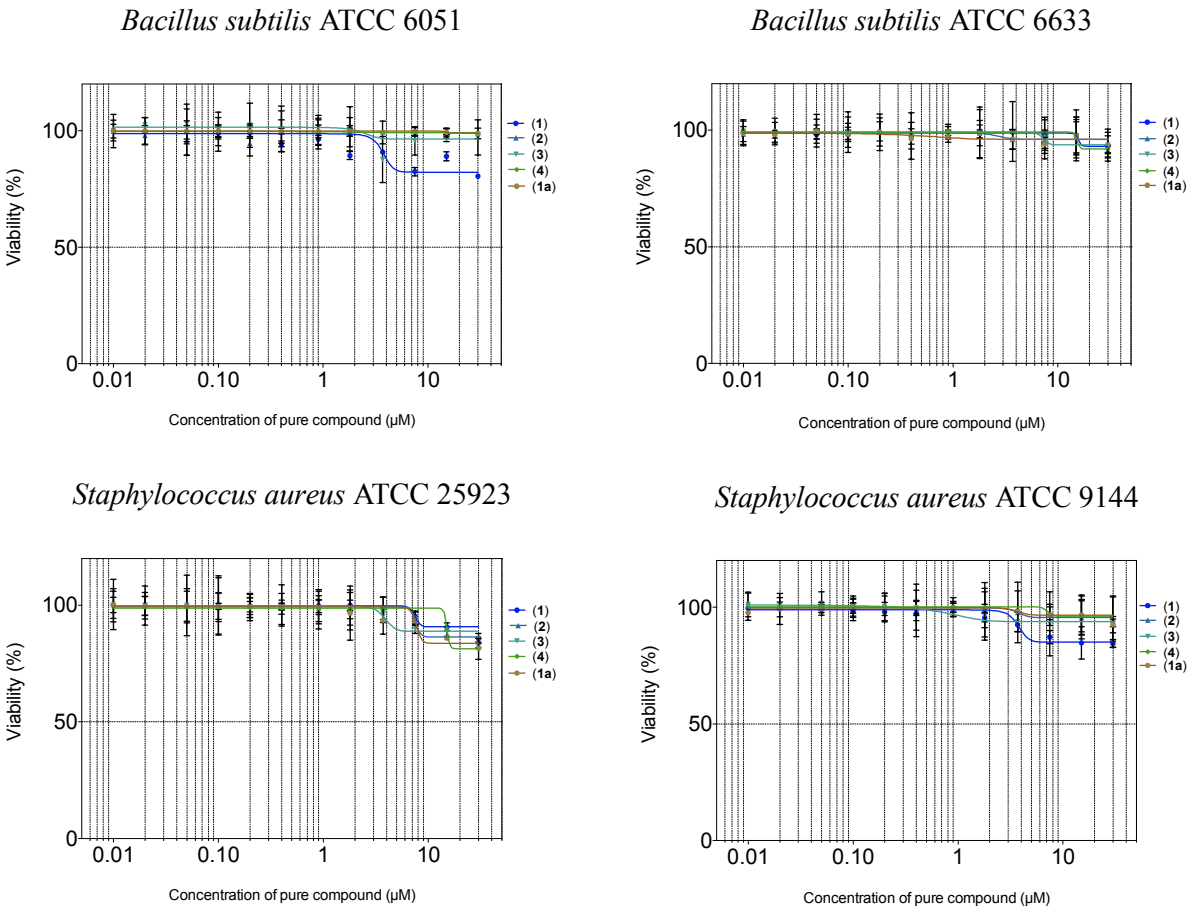
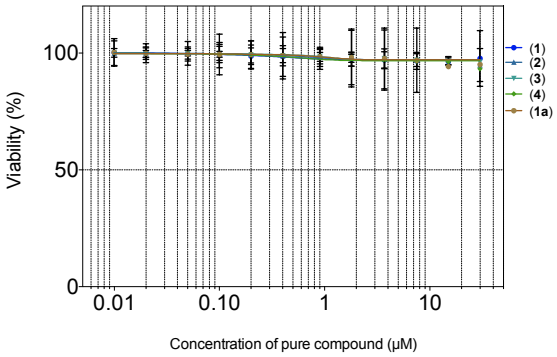
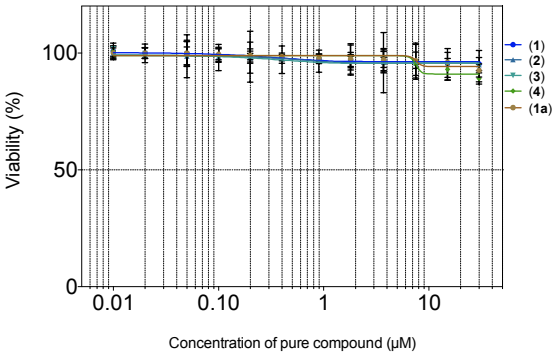


Figure S36. Antibiotic screening of compounds **1**−**4** and **1a** (continued overleaf)

Escherichia coli ATCC 11775



Pseudomonas aeruginosa ATCC 10145



Candida albicans ATCC 90028

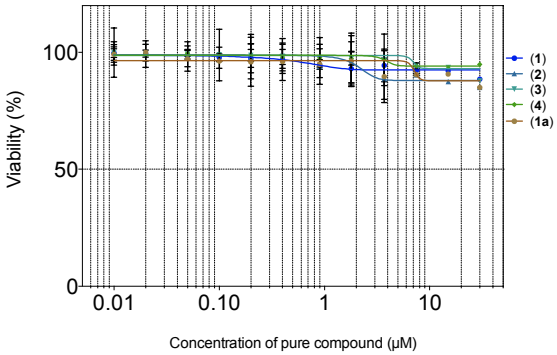


Figure S36. *Continued…* Antibiotic screening of compounds **1**−**4** and **1a**

4.2 Cytotoxicity and Calcein AM Screening Data for 1-4 and synthetic DKMs 17-24 and 19-22

Table S6. Cytotoxicity of compounds and their effects on accumulation of calcein AM in P-gp overexpressing SW620 Ad300 cells using flow cytometry

#	IC_{50} (μM)[a]				FAR[b]
	SW620	SW620 Ad300	KB-3-1	KB-V1	
19	> 30	> 30	> 30	> 30	55.5
24	> 30	> 30	> 30	> 30	42.7
23	> 30	> 30	> 30	> 30	41.3
1	> 30	> 30	> 30	> 30	38.9
2	> 30	> 30	> 30	> 30	30.8
4	> 30	> 30	> 30	> 30	30.5
20					10.4
3					2.4
18					1.8
17					1.3
22					1.2
21					1.0

[a] Cell survival was determined by MTT assay.

[b] FAR (fluorescence arbitrary ratio) = calcein fluorescence intensity (geometric mean) in the presence of compounds **1**–**4** and **17-24**, **19-22** at 20 μM / calcein fluorescence intensity (geometric mean) in the presence of PBS, expressed as a ratio. Positive control is verapamil at 20 μM which FAR = 71.4

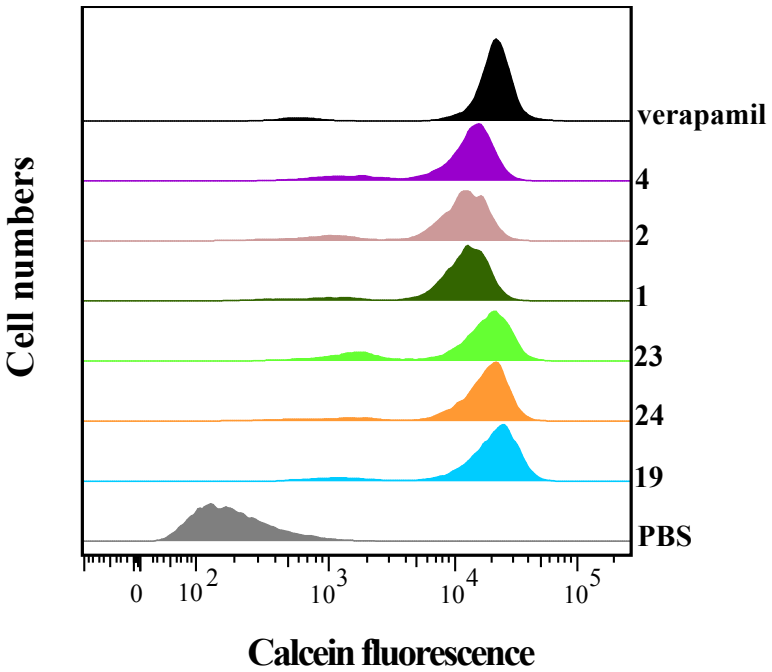


Figure S37. Effect of compounds on accumulation of calcein AM in P-gp-overexpressing SW620 Ad300 cells using flow cytometry

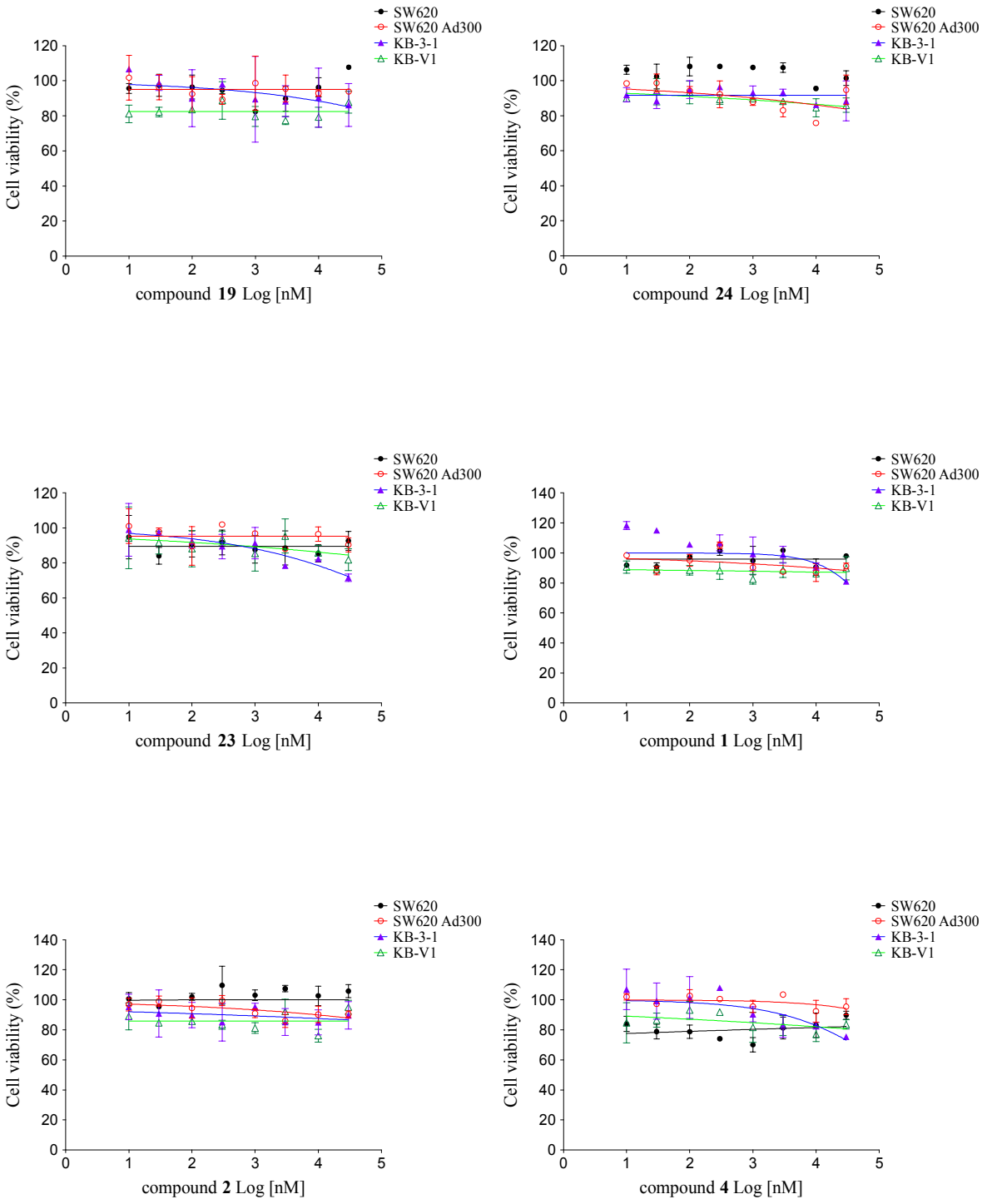


Figure S38. Cytotoxicity of compounds **1**, **2**, **4**, **19**, **23** and **24** against SW620, SW620 Ad300, KB-3-1 and KB-V1

5 References

(1) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Research* **1987**, *47*, 943-946.

(2) Hochlowski, J. E.; Mullally, M. M.; Spanton, S. G.; Whittern, D. N.; Hill, P.; McAlpine, J. B. *J. Antibiot.* **1993**, *46*, 380-386.