

## **Supplementary material**

# **Genome-Guided discovery of pretilactam from *Actinosynnema pretiosum* ATCC 31565**

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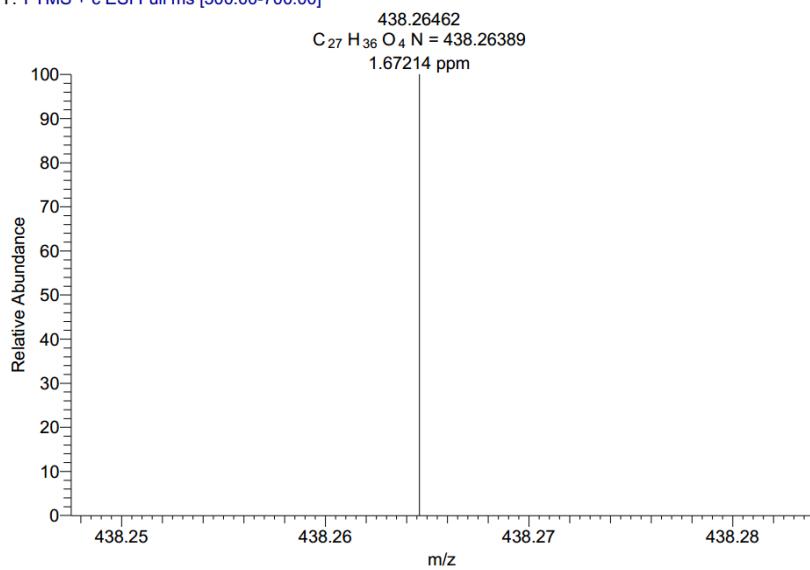
## Contents

<b>Figure S1</b>	AntiSMASH of the genomic DNA of ATCC 31565.....	S3
<b>Figure S2</b>	HR(+)ESIMS of pretilactam ( <b>1</b> ).....	S4
<b>Figure S3</b>	$^1\text{H}$ NMR spectrum (800 MHz) of pretilactam ( <b>1</b> ) in DMSO- <i>d</i> <sub>6</sub> .....	S4
<b>Figure S4</b>	$^{13}\text{C}$ NMR spectrum (200 MHz) of pretilactam ( <b>1</b> ) in DMSO- <i>d</i> <sub>6</sub> .....	S5
<b>Figure S5</b>	$^1\text{H}$ - $^1\text{H}$ COSY spectrum (800 MHz) of pretilactam ( <b>1</b> ) in DMSO- <i>d</i> <sub>6</sub> .....	S5
<b>Figure S6</b>	HSQC spectrum (200 MHz) of pretilactam ( <b>1</b> ) in DMSO- <i>d</i> <sub>6</sub> .....	S6
<b>Figure S7</b>	HMBC spectrum (200 MHz) of pretilactam ( <b>1</b> ) in DMSO- <i>d</i> <sub>6</sub> .....	S6
<b>Figure S8</b>	TOCSY spectrum (800 MHz) of pretilactam ( <b>1</b> ) in DMSO- <i>d</i> <sub>6</sub> .....	S7
<b>Figure S9</b>	NOESY spectrum (800 MHz) of pretilactam ( <b>1</b> ) in DMSO- <i>d</i> <sub>6</sub> .....	S7
<b>Figure S10</b>	UV spectrum of pretilactam ( <b>1</b> ).....	S8
<b>Figure S11</b>	IR spectrum of pretilactam ( <b>1</b> ).....	S8

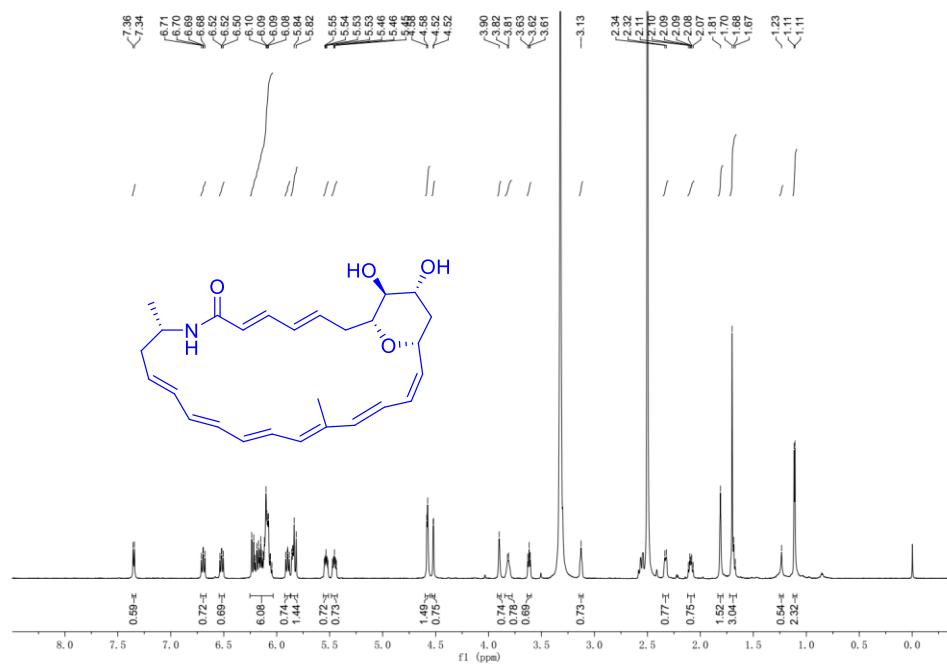
Identified secondary metabolite regions																											
Region	Type	From	To	Most similar known cluster	Similarity	MIBiG	BGC-ID																				
The following regions are from record NODE_1:																											
1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Region 1	Terpene	46280	66796	SF2575	polyketide	6%	BGC0000269																				
Region 2	Lanthipeptide	230028	253096	Tetronasin	polyketide	9%	BGC0000163																				
Region 3	Terpene	3209146	3229317	Geosmin	other	100%	BGC0001181																				
Region 4	Bacteriocin	3837575	3848375																								
Region 5	Indole	4468806	4489927	Frankiamycin	polyketide	14%	BGC0001197																				
Region 6	Nrpsfragment-Otherks-T1pk	4936116	4980643																								
Region 7	Nrps-T1pk	5169664	5327702	Salinilactam	polyketide	88%	BGC0000142																				
Region 8	Nucleoside	5384654	5405622	Tunicamycin	other	64%	BGC0000880																				
Region 9	Nrps-T1pk	5652921	5732628	Tirandamycin	hybrid	40%	BGC0001052																				
Region 10	Otherks	5814814	5854503	Calcimycin	polyketide	13%	BGC0000032																				
Region 11	Nrpsfragment-T1pk	5878730	5975542	Ansamitocin	polyketide	97%	BGC0000020																				
Region 12	Lassopeptide	6034265	6056728	SF2575	polyketide	4%	BGC0000269																				
Region 13	Nrps-Phosphonate-T1pk	6066797	6154441	Himastatin	NRPS	8%	BGC0001117																				
Region 14	Nrps-Otherks	6328654	6476784	Naphthyridinomycin	NRPS	60%	BGC0000394																				
Region 15	Terpene	6599385	6618894	Isorenieratene	terpene	85%	BGC0000664																				
Region 16	Thiopeptide	6643965	6673418																								
Region 17	T1pk	6679293	6719988	Coelimycin	polyketide	8%	BGC0000038																				
Region 18	T1pk	6828788	6884394																								
Region 19	T1pk	6917680	6962500	Kedarcidin	polyketide	6%	BGC0000081																				
Region 20	T1pk	7248874	7310026	Spinosad	polyketide	8%	BGC0000148																				
Region 21	Terpene	7319928	7341610	Brasilicardin A	terpene	45%	BGC0000632																				
Region 22	Nrps	7369240	7462182	Marformycins	NRPS	12%	BGC0001214																				
Region 23	Nrps	7499669	7542655																								
Region 24	Nrpsfragment-Other-T1pk	7542698	7611214	Lasalocid	polyketide	16%	BGC0000087																				
Region 25	T1pk	7645186	7703975	Coelimycin	polyketide	25%	BGC0000038																				
Region 26	Oligosaccharide	7915377	7958960																								
Region 27	Nrps	8017917	8071251	Erythrochelin	NRPS	85%	BGC0000349																				

**Figure S1** AntiSMASH of the genomic DNA of ATCC 31565

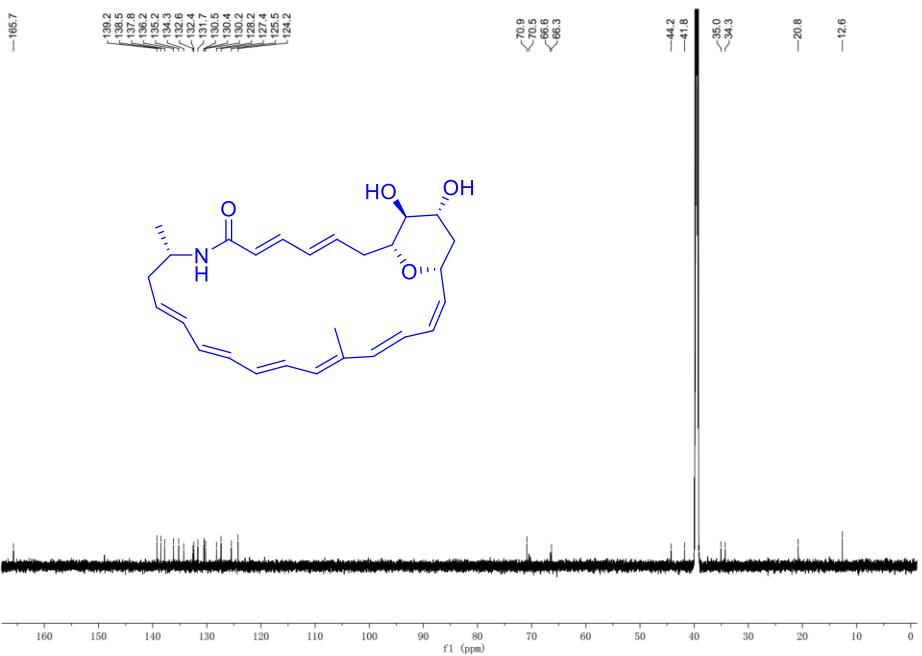
M-2 + #88 RT: 1.33 AV: 1 NL: 1.14E5  
T: FTMS + c ESI Full ms [300.00-700.00]



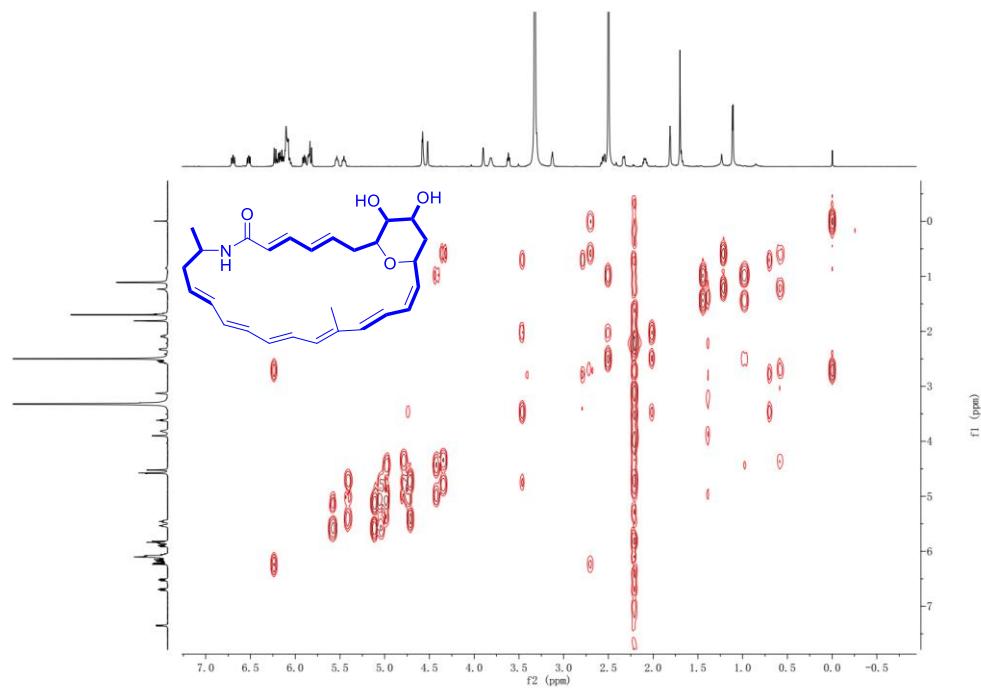
**Figure S2** HR(+)ESIMS of pretilactam (**1**)



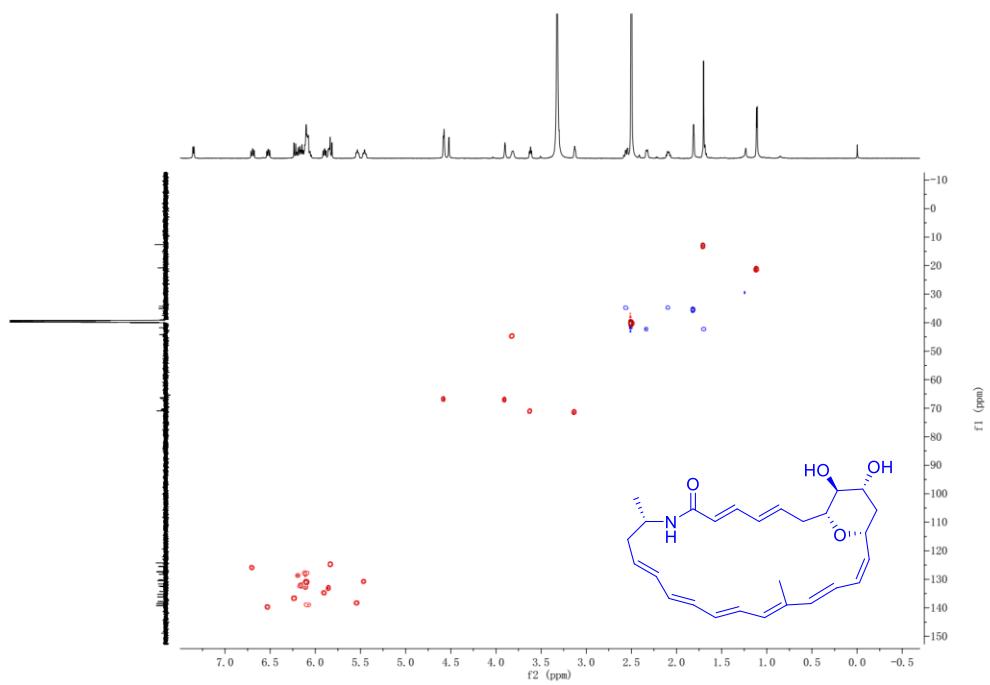
**Figure S3**  $^1H$  NMR spectrum (800 MHz) of pretilactam (**1**) in  $DMSO-d_6$



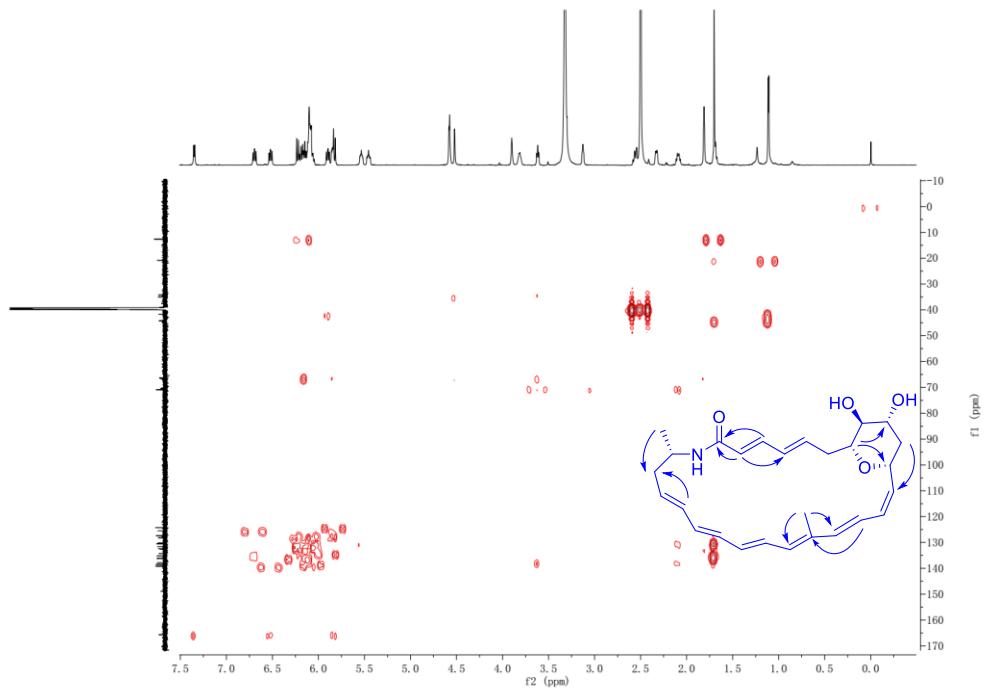
**Figure S4**  $^{13}\text{C}$  NMR spectrum (200 MHz) of pretilactam (**1**) in  $\text{DMSO}-d_6$



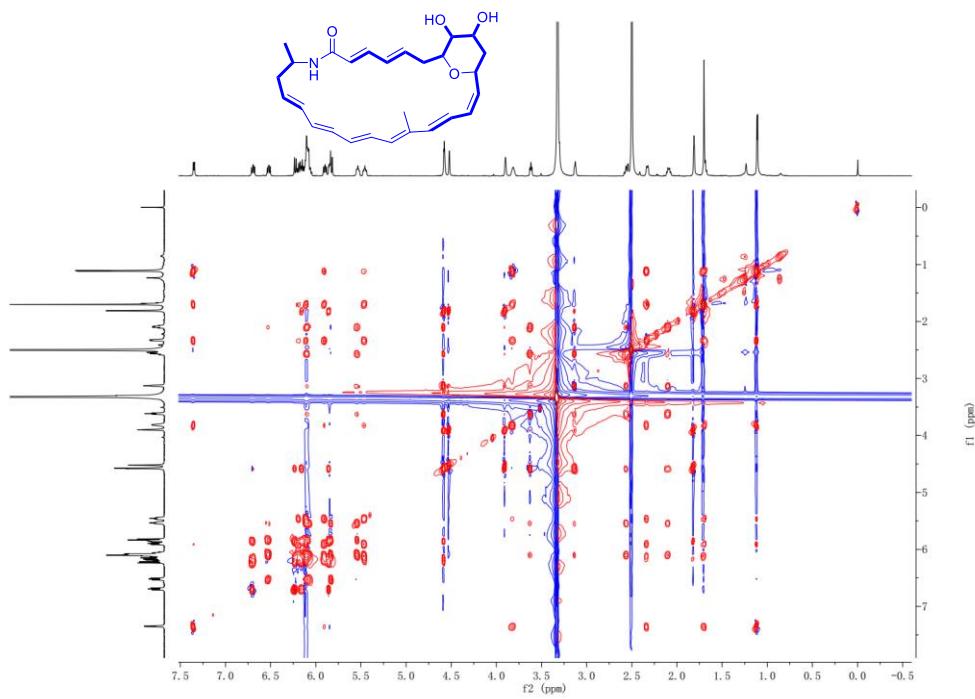
**Figure S5**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (800 MHz) of pretilactam (**1**) in  $\text{DMSO}-d_6$



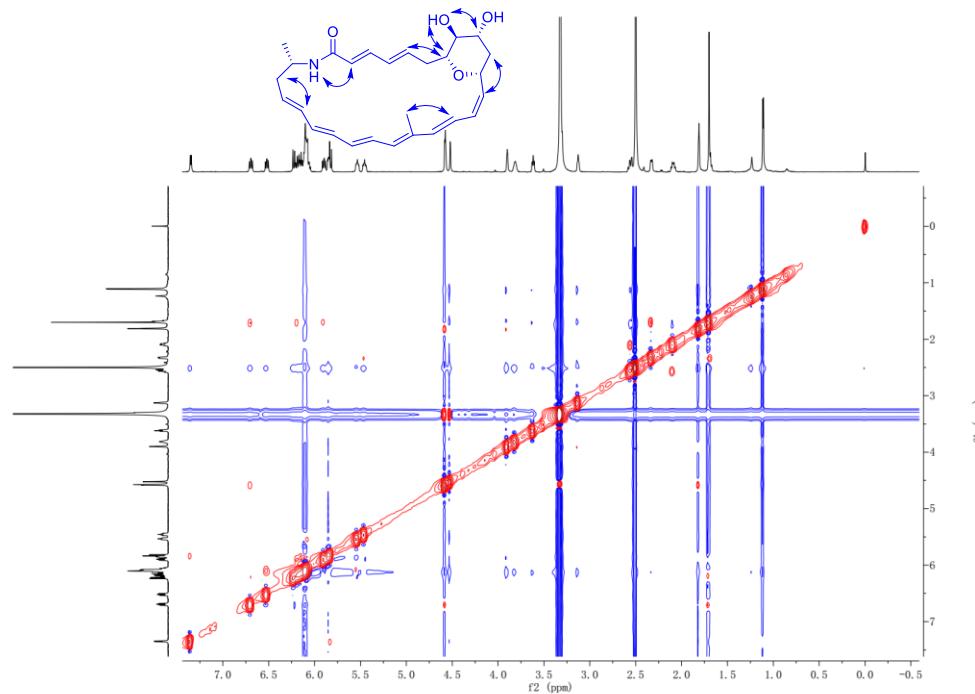
**Figure S6** HSQC spectrum (200 MHz) of pretilactam (**1**) in  $\text{DMSO}-d_6$



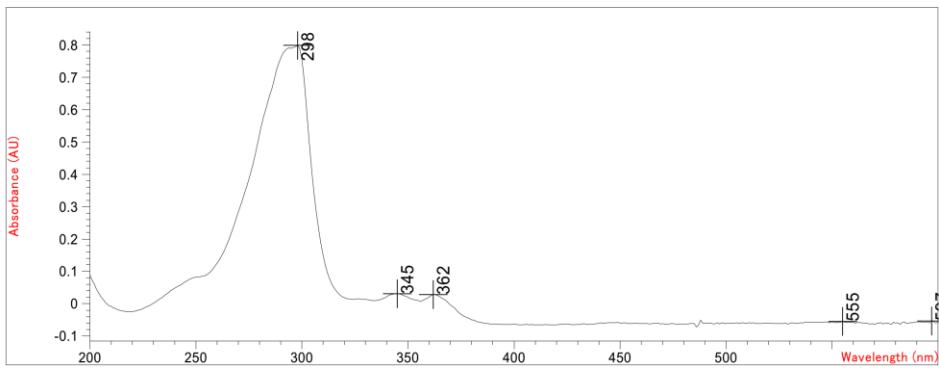
**Figure S7** HMBC spectrum (200 MHz) of pretilactam (**1**) in  $\text{DMSO}-d_6$



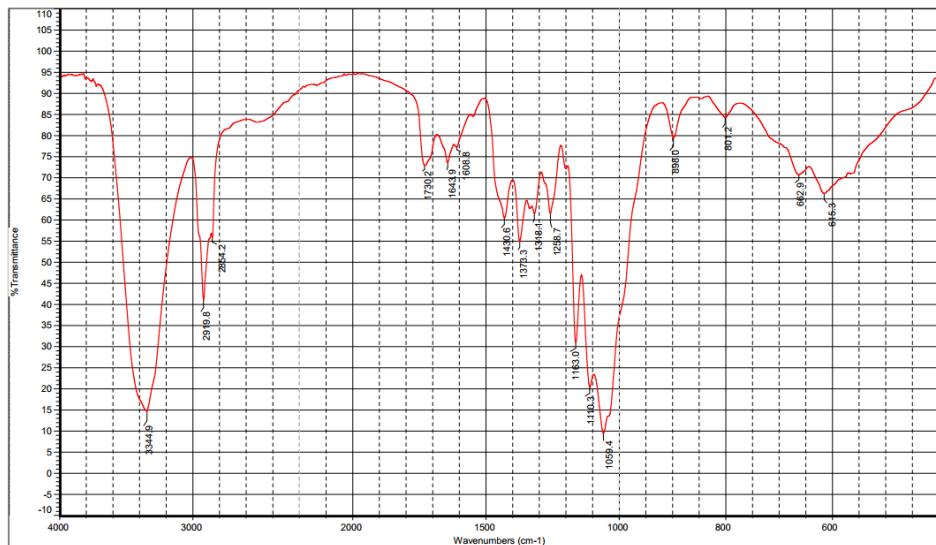
**Figure S8** TOCSY spectrum (800 MHz) of pretilactam (**1**) in  $\text{DMSO}-d_6$



**Figure S9** NOESY spectrum (800 MHz) of pretilactam (**1**) in  $\text{DMSO}-d_6$



**Figure S10** UV spectrum of pretilactam (**1**)



**Figure S11** IR spectrum of pretilactam (**1**)