Supporting Information

Total Synthesis of Thermoactinoamide A and Its Analogue

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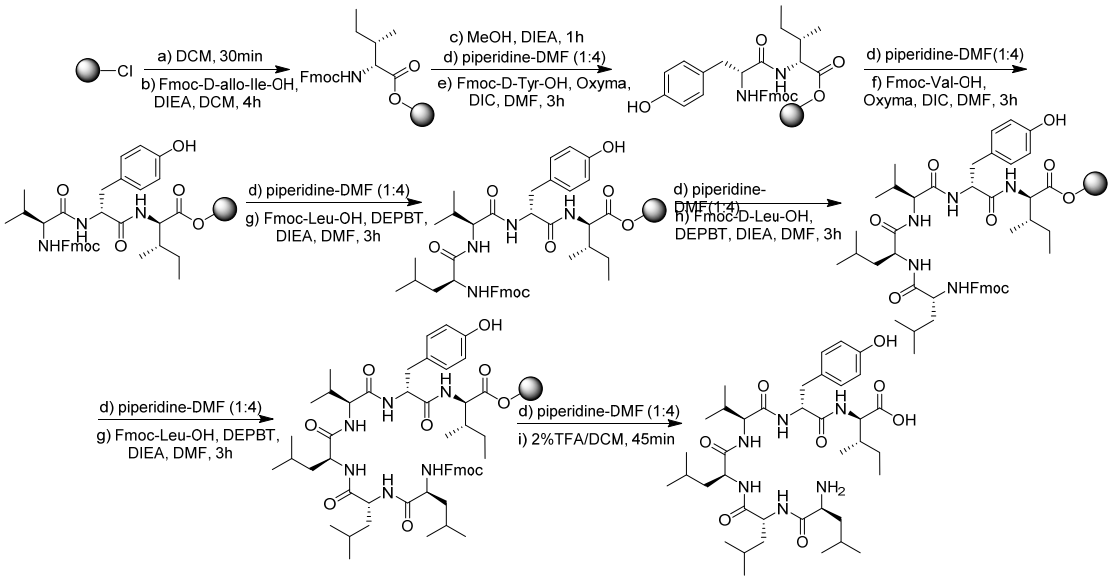
Synthesis of anti-thermoactinoamideA (1b)



Scheme S1. Synthesis of anti-thermoactinoamideA**(1b)**linker for SPPS.

Table S1. Evaluation bioactivity of thermoactinoamides**.**

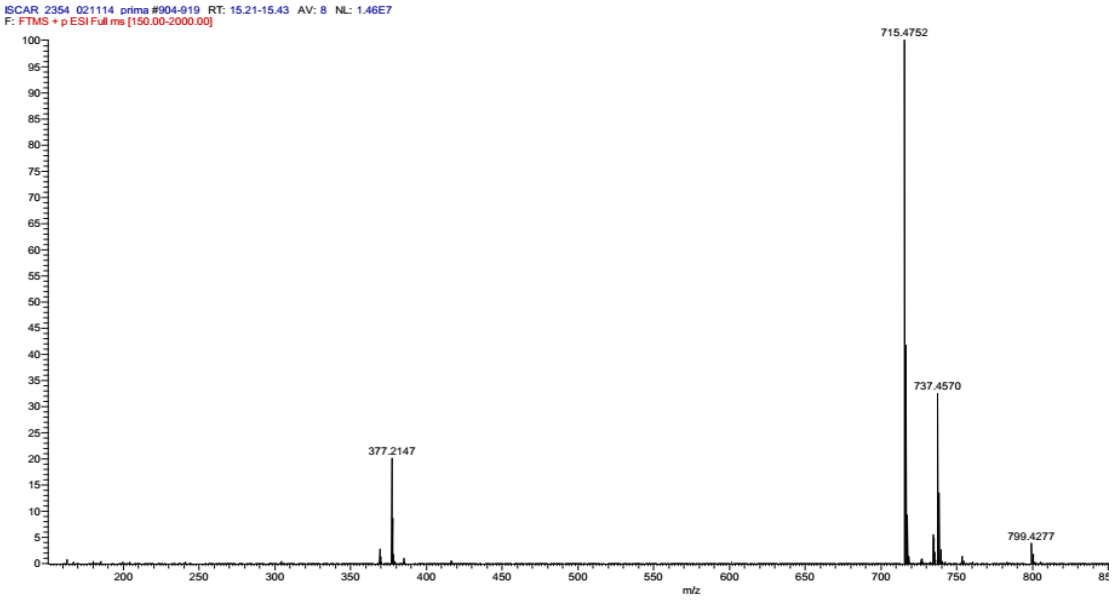
Cmpd	antitumor						antiviral		antituberculous	anti-inflammatory	
	K562	A549	Hela	HT29	SK-BR-3	Huh7	H3N2	EV71	TB	COX-1	COX-2
	observed IC_{50} (uM)[a]										
1a	NA	NA	NA	NA	NA	>30	NA	NA	NA	NA	NA
2a	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1b	NA	NA	NA	NA	NA	>30	NA	NA	NA	NA	NA
2b	NA	NA	NA	NA	NA	>30	NA	NA	NA	NA	NA

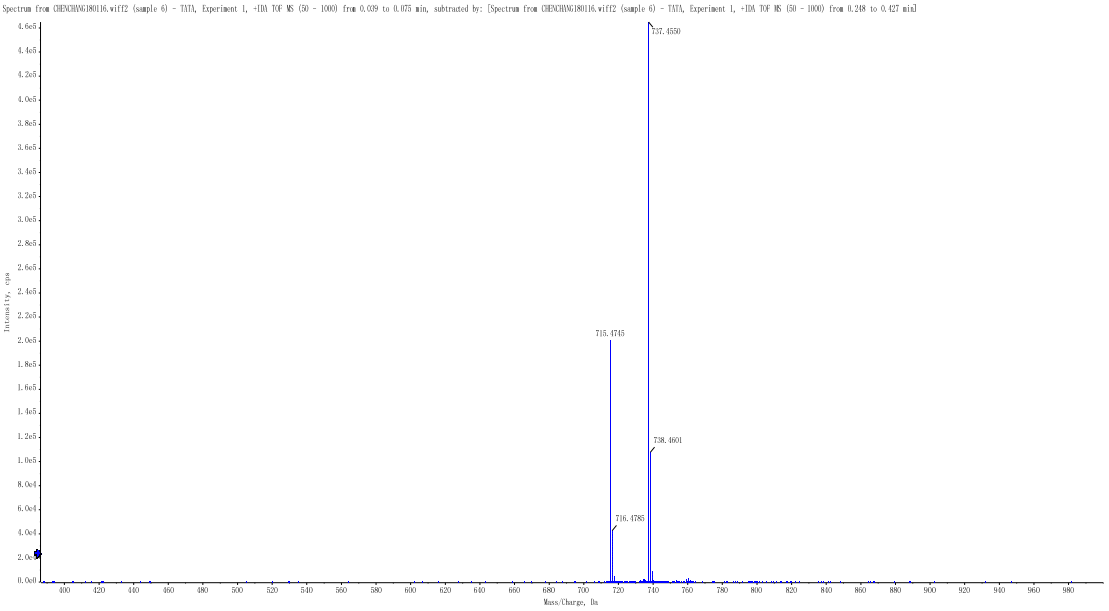
[a]The concentration of tested compound was 30 μM. If the survival cancer cells were below 70%, IC_{50} was calculated by using Graphpad Prism 5.0; If the survival cancer cells were 70%-85%, IC_{50} was marked as > 30 μM; Otherwise, IC_{50} marked as NA (no inhibition)**.**

amino acid	pos.	δ_C, type		
		Natural(ppm) 175MHz-CD_3SOCD_3	Synthetic (ppm) 75MHz-CD_3OD	Anti-Thermoactinoamide A 75MHz-CD_3OD
D-Tyr	1	171.2, -C=O	172.9, -C=O	172.6, -C=O
	2	54.8, -NH-CH-	56.1, -NH-CH-	56.1, -NH-CH-
	3	37.6, CH_2	37.5, CH_2	36.1, CH_2
	4	126.6, C	129.5, C	129.7, C
	5/9	129.9, -C=C-	130.0, -C=C-	127.2, -C=C-
	6/8	114.7, -C=C-	115.0, -C=C-	115.1, -C=C-
	7	155.8, C	156.1,C	156.2, C
L-Val	1	170.5, -C=O	171.1,-C=O	171.2, -C=O
	2	60.0, -NH-CH-	58.7, -NH-CH-	58.4, -NH-CH-
	3	29.0, CH	29.4, CH	31.1, CH
	4	17.4, CH_3	15.4, CH_3	17.4, CH_3
	5	18.6, CH_3	18.01, CH_3	17.8, CH_3
L-Leu(I)	1	170.7, -C=O	172.1,-C=O	172.4, -C=O
	2	50.6, -NH-CH-	51.9, -NH-CH-	52.5, -NH-CH-
	3	39.2, CH_2	39.0, CH_2	39.4, CH_2
	4	24.0, CH	24.8, CH	24.6, CH
	5	22.8, CH_3	22.2, CH_3	22.23, CH_3
	6	20.9, CH_3	19.8, CH_3	19.8, CH_3
D-Leu(II)	1	171.4, -C=O	173.1,-C=O	173.3, -C=O
	2	50.5, -NH-CH-	51.2, -NH-CH-	51.2, -NH-CH-
	3	40.8, CH_2	40.7, CH_2	40.6, CH_2
	4	24.2, CH	26.7, CH	24.7, CH
	5	22.5, CH_3	21.9, CH_3	22.21, CH_3
	6	21.8, CH_3	20.7, CH_3	21.2, CH_3
L-Leu(III)	1	171.7, -C=O	173.4,-C=O	173.4, -C=O
	2	51.1, -NH-CH-	52.7, -NH-CH-	52.6, -NH-CH-
	3	39.3, CH_2	40.1, CH_2	40.1, CH_2
	4	24.0, CH	24.5, CH	24.5, CH
	5	22.1, CH_3	21.5, CH_3	21.7, CH_3
	6	22.0, CH_3	21.2, CH_3	21.5, CH_3
D-allo-Ile	1	170.8, -C=O	172.5,-C=O	172.5, -C=O
	2	55.8, -NH-CH-	56.5, -NH-CH-	57.5, -NH-CH-
	3	35.9, CH	31.7, CH	36.0, CH
	4	14.3, CH_3	14.0, CH_3	13.7, CH_3
	5	25.5, CH_2	28.9, CH_2	25.9, CH_2
	6	11.2, CH_3	10.0, CH_3	10.7, CH_3

Comparison of Thermoactinoamide A (Naturalvs Synthetic) ^{13}C NMR and ^{13}C NMR Spectrum of Anti-Thermoactinoamide A

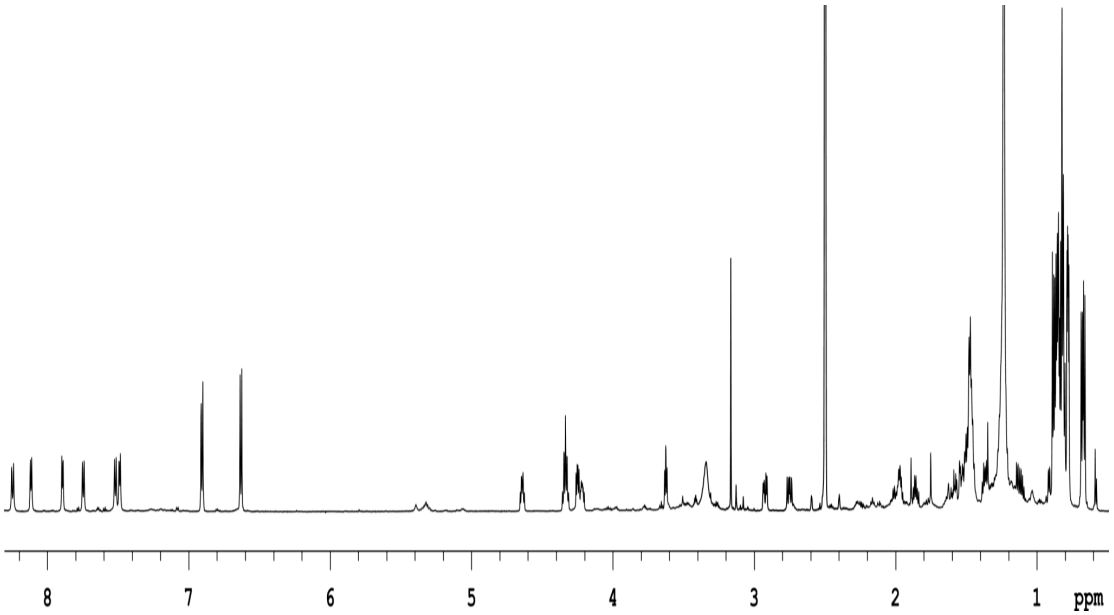
Figure S1. Comparative High-resolution ESI mass spectrum of syntheticthermoactinoamideA (lower) and natural thermoactinoamide A(upper)





Hit	Formula	m/z	RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Found
1	$C_{38}H_{62}N_6O_7$	715.4753	11.0	-1.1	1			NA/NA

Figure S2. Comparative [1]HNMR spectra for synthetic thermoactinoamideA(lower, 300MHz, CD_3OD) and natural thermoactinoamideA(upper,700MHz, CD_3SOCD_3)



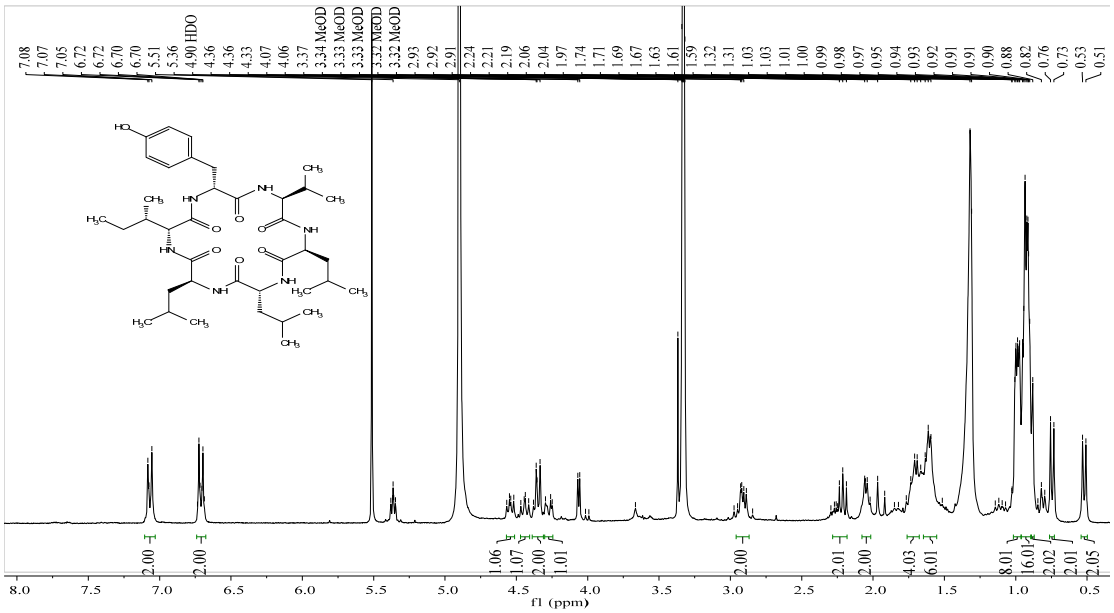


Figure S3.[13]C NMR spectra for synthetic thermoactinoamideA (75 MHz, CD_3OD)

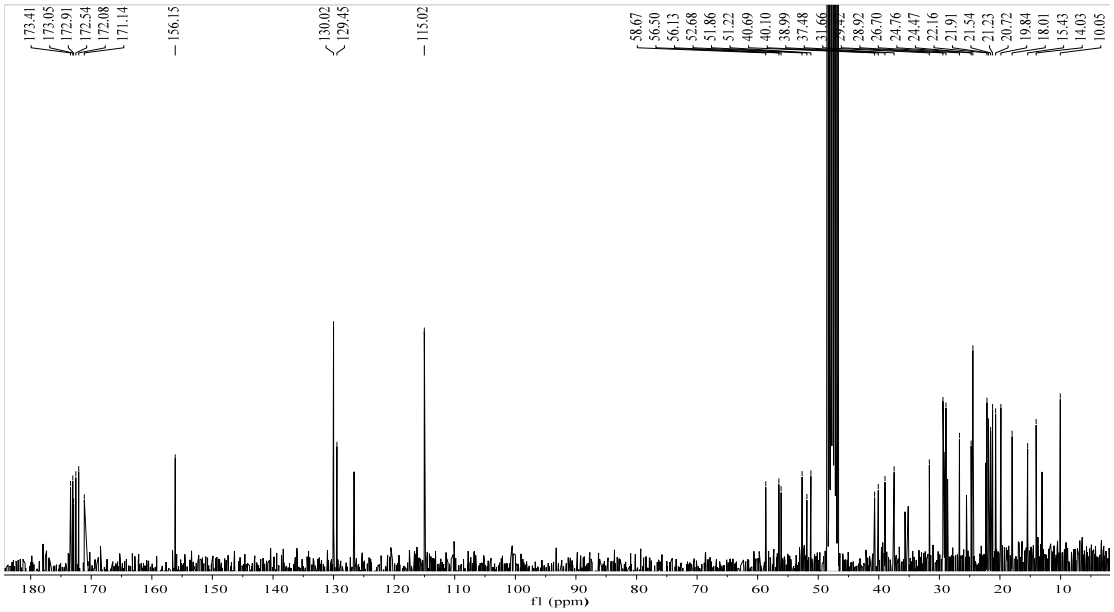


Figure S4.DEPT spectra for synthetic thermoactinoamideA (75 MHz, CD_3OD)

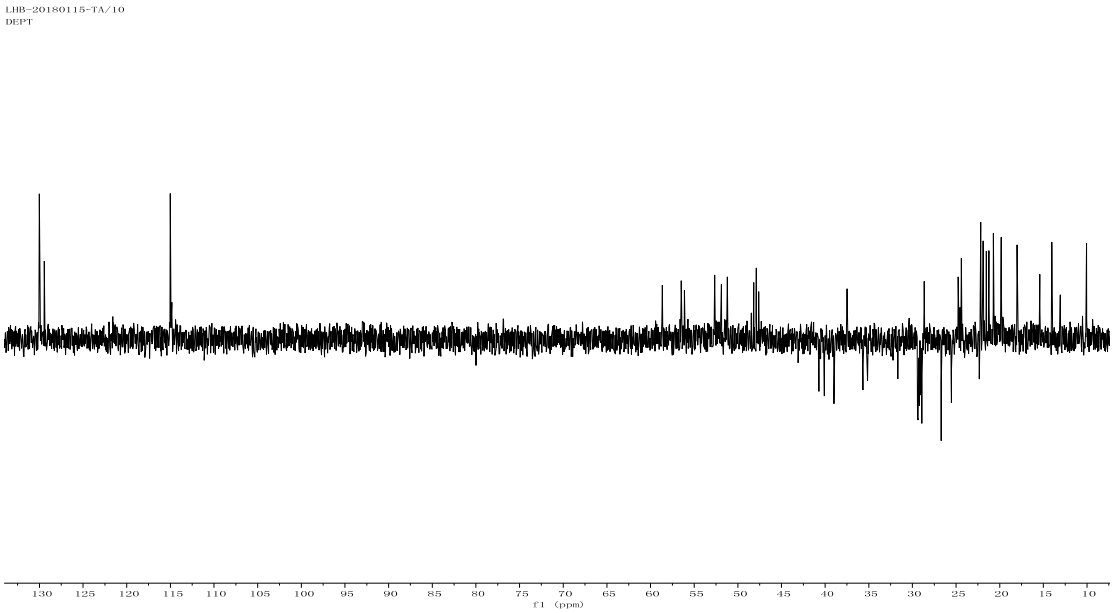


Figure S5. Comparative H-H COSYspectrum for synthetic thermoactinoamideA(lower, 300 MHz, CD_3OD) and natural thermoactinoamide A(upper,700MHz, CD_3SOCD_3).

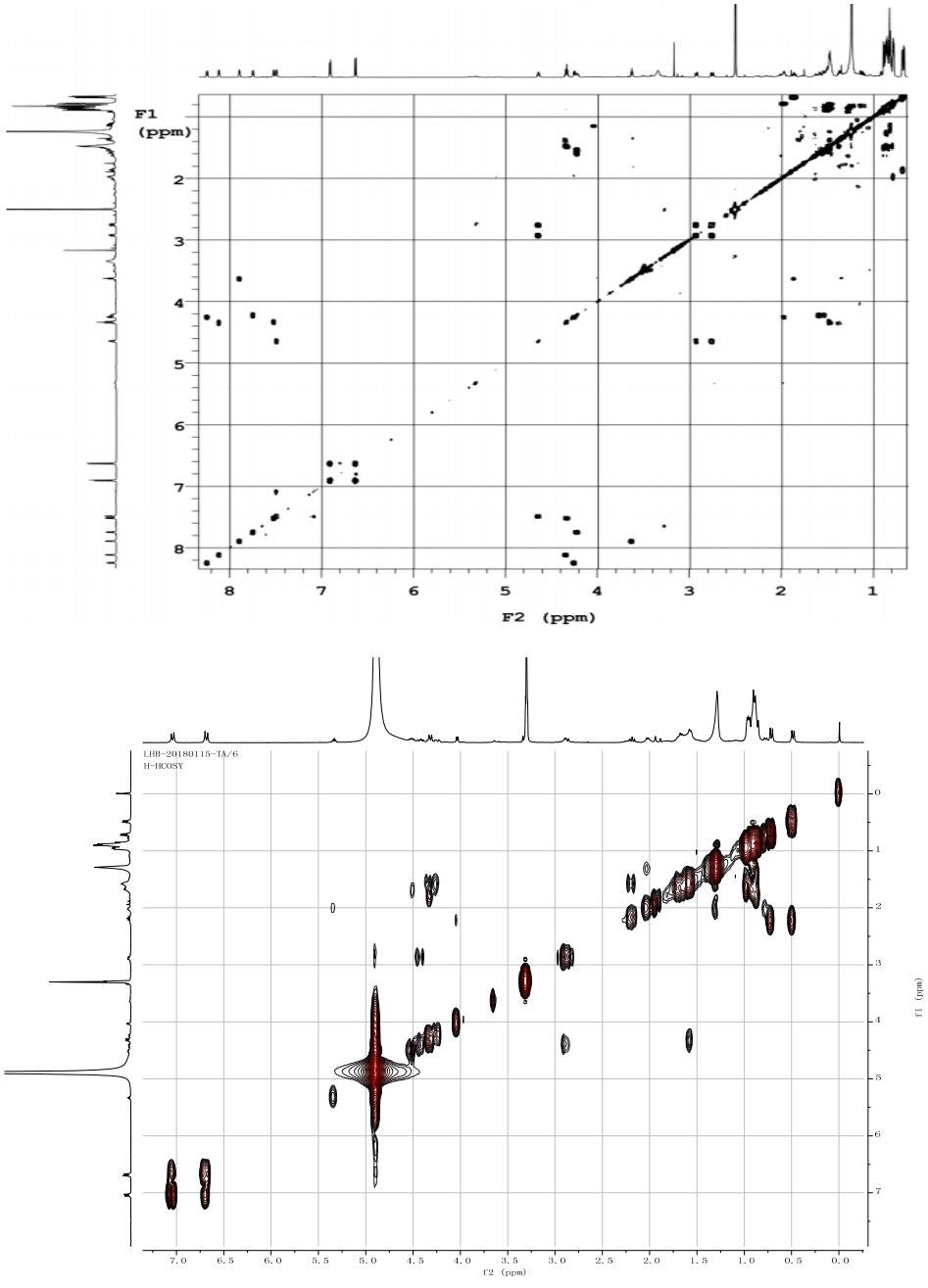


Figure S6. Comparative HSQC spectrum for synthetic thermoactinoamideA(lower, 300 MHz, CD_3OD) and natural thermoactinoamide A(upper,700 MHz, CD_3SOCD_3).

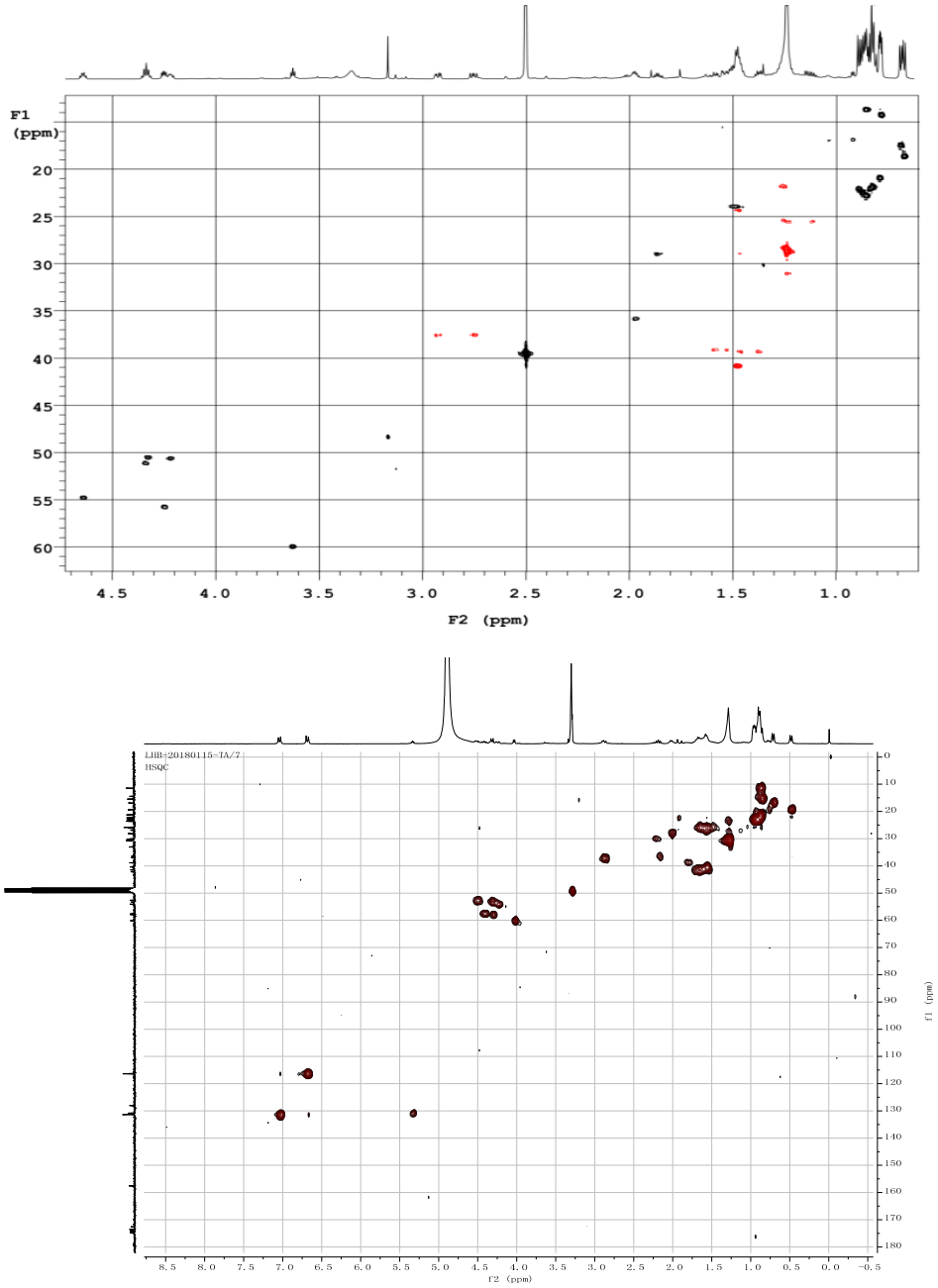


Figure S7. Comparative HMBC spectrum for synthetic thermoactinoamideA(lower, 300 MHz, CD_3OD) and natural thermoactinoamide A(upper,700 MHz, CD_3SOCD_3)

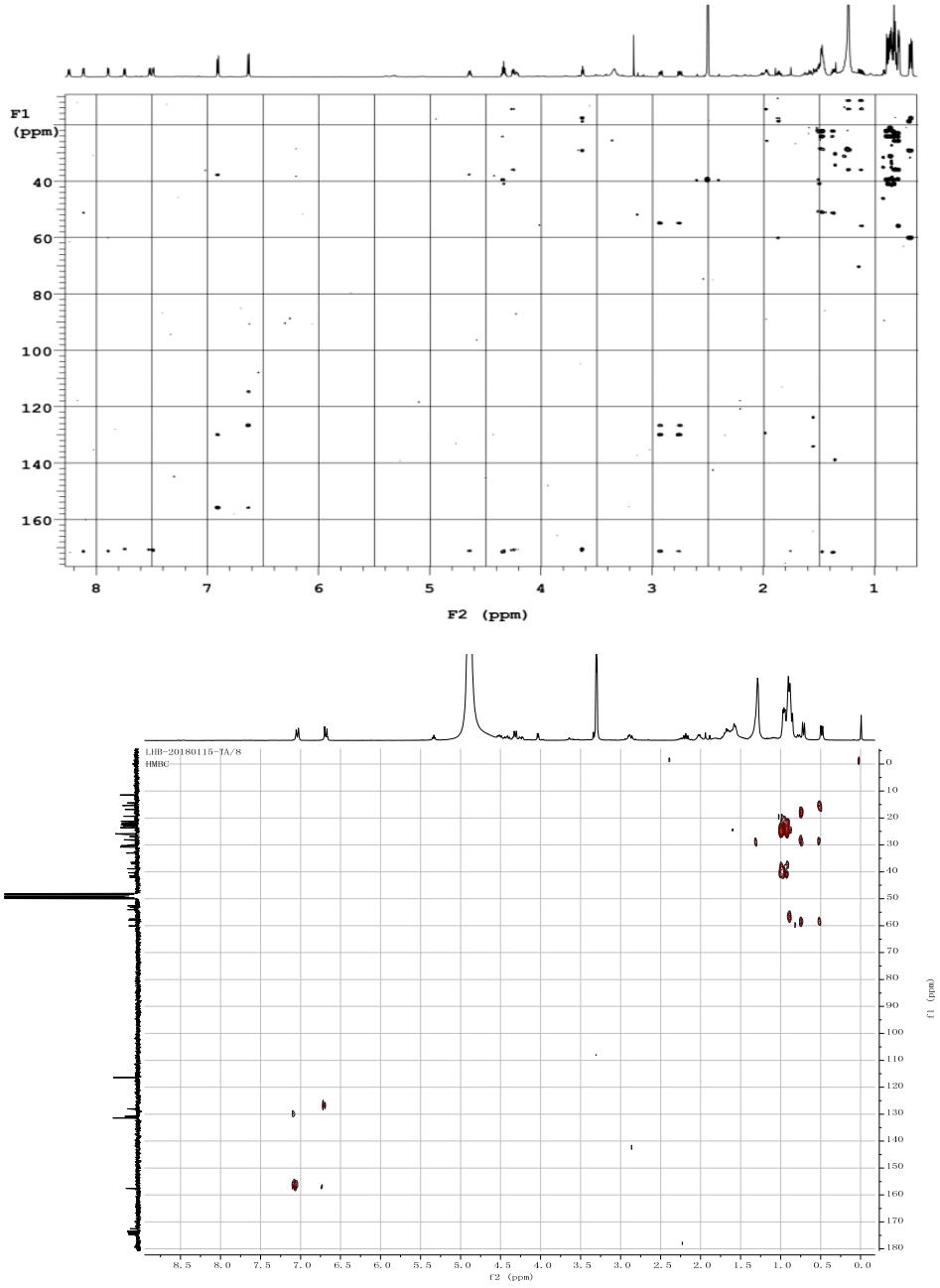


Figure S8. Comparative NOESY spectrum for synthetic thermoactinoamideA(lower, 300 MHz, CD_3OD) and natural thermoactinoamide A (upper,700 MHz, CD_3SOCD_3)

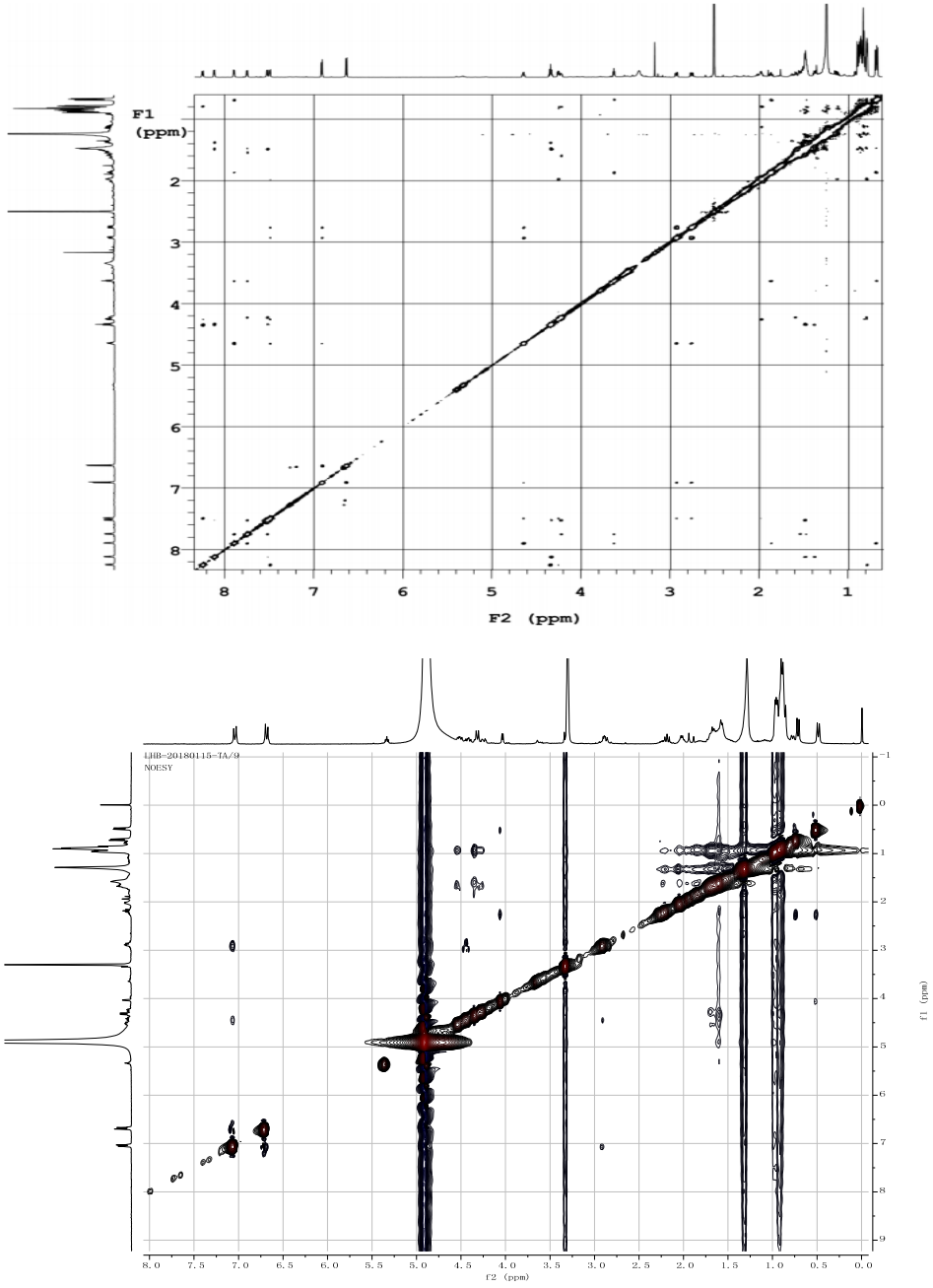


Figure S9.UV trace (210nm) from HPLC analysis of a crude reaction mixture of thermoactinoamide A: isocratic elution 75% MeOH/H_2O over 30 min at a flow rate of 1mL/min. Elution time = 17.617 min

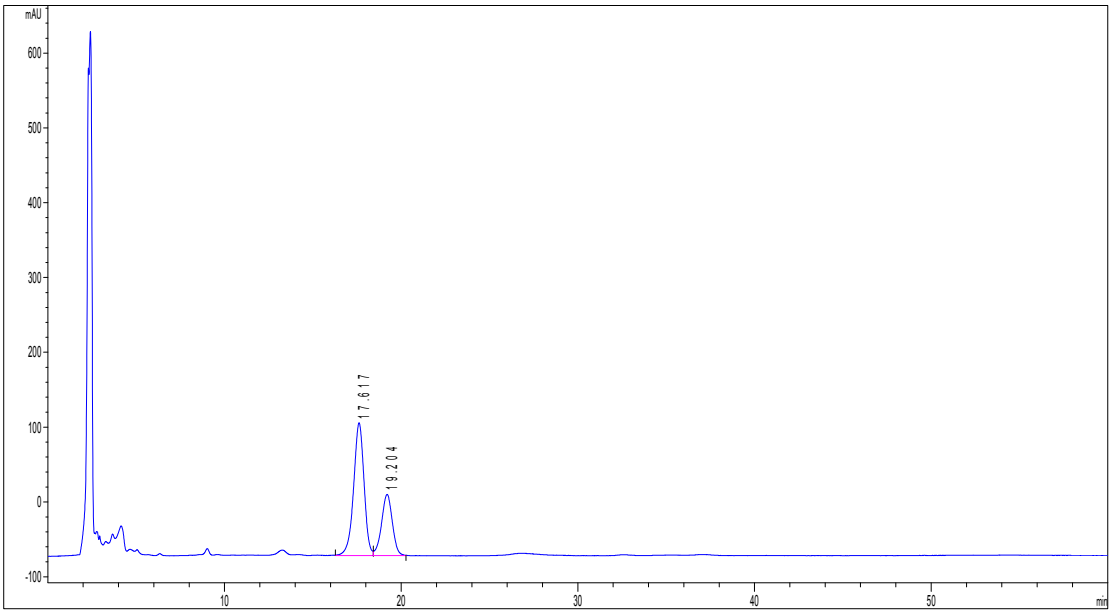


Figure S10.IR spectra of thermoactinoamide A

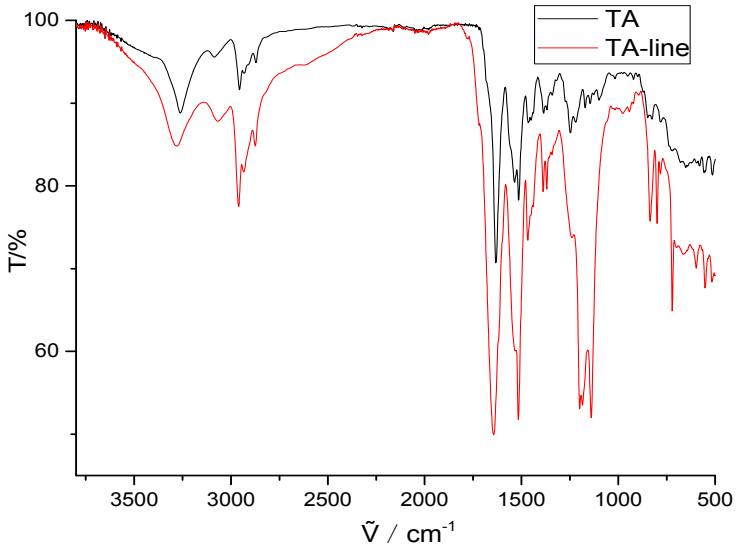
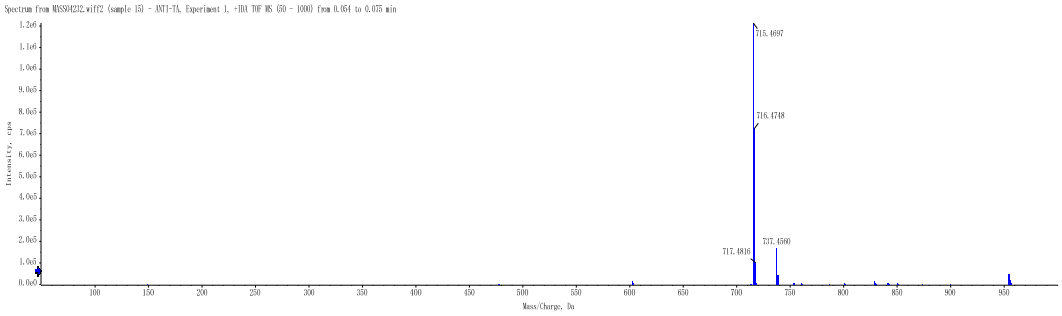
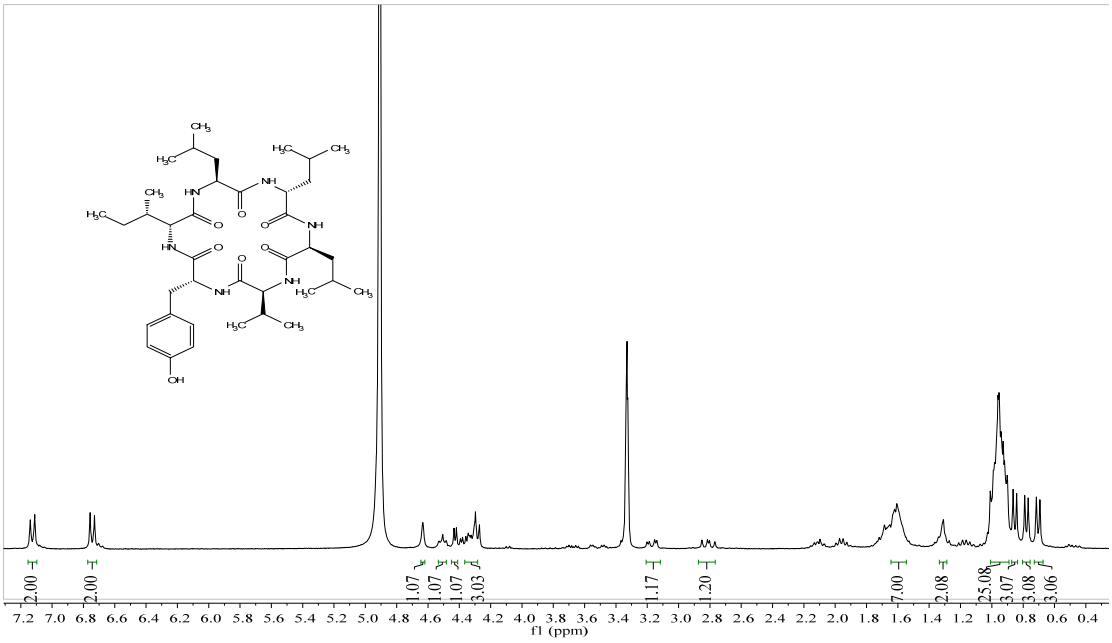


Figure S11. High-resolution ESI mass spectrum of anti-thermoactinoamide A



Hit	Formula	m/z	RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Found
1	$C_{38}H_{62}N_6O_7$	715.4753	11.0	-3.8	1			NA/NA

Figure S12. ^{1}H and ^{13}C spectra ofanti-thermoactinoamideA .



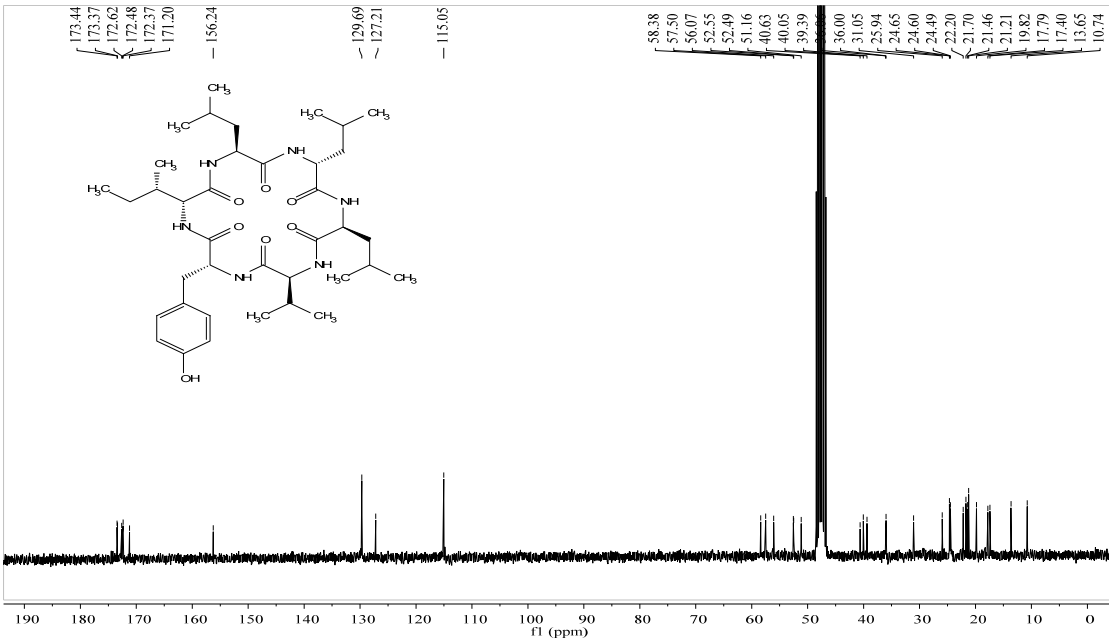


Figure S13. ^{13}C and DEPT spectra of anti-thermoactinoamideA

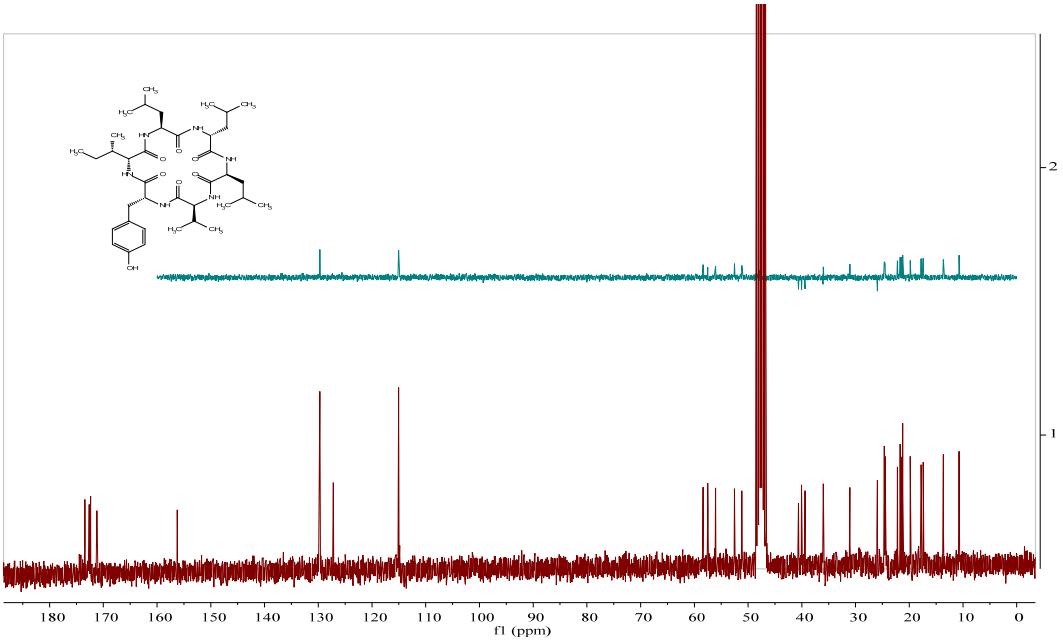


Figure S14. H-H COSYspectrum for synthetic anti-thermoactinoamide A

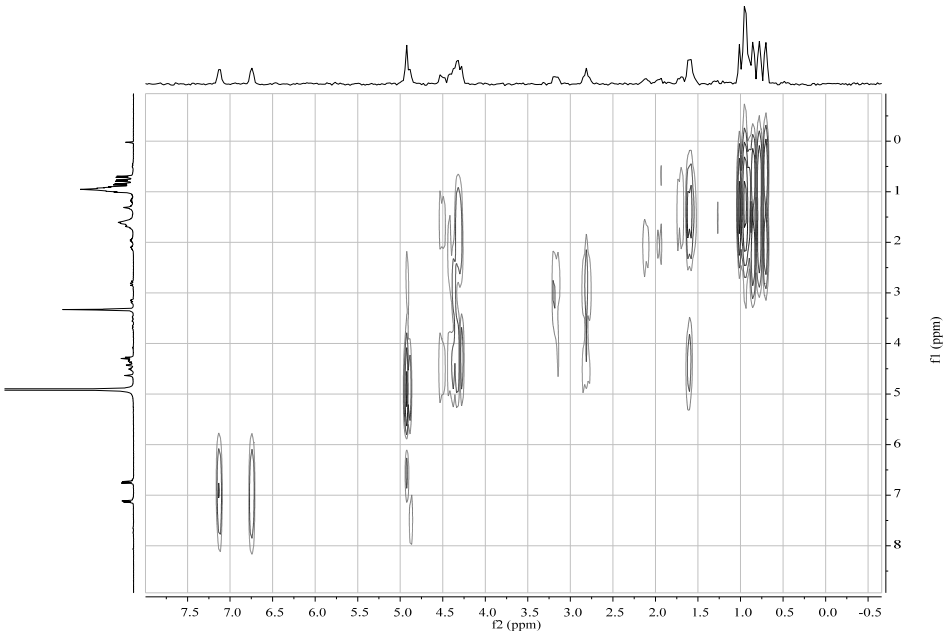


Figure S15. HSQC spectrum for synthetic anti-thermoactinoamide A

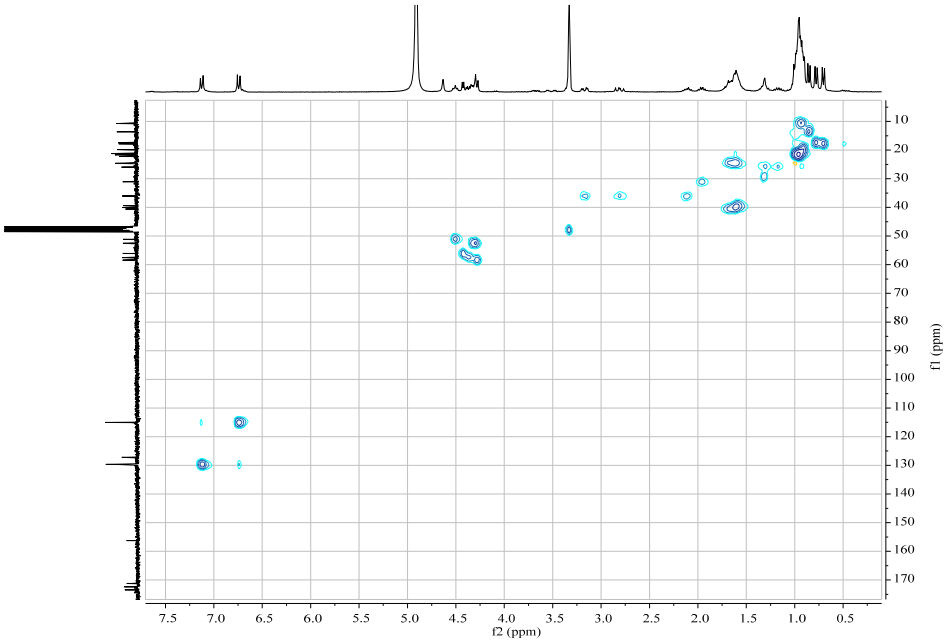


Figure S16. HMBC spectrum for synthetic anti-thermoactinoamide A

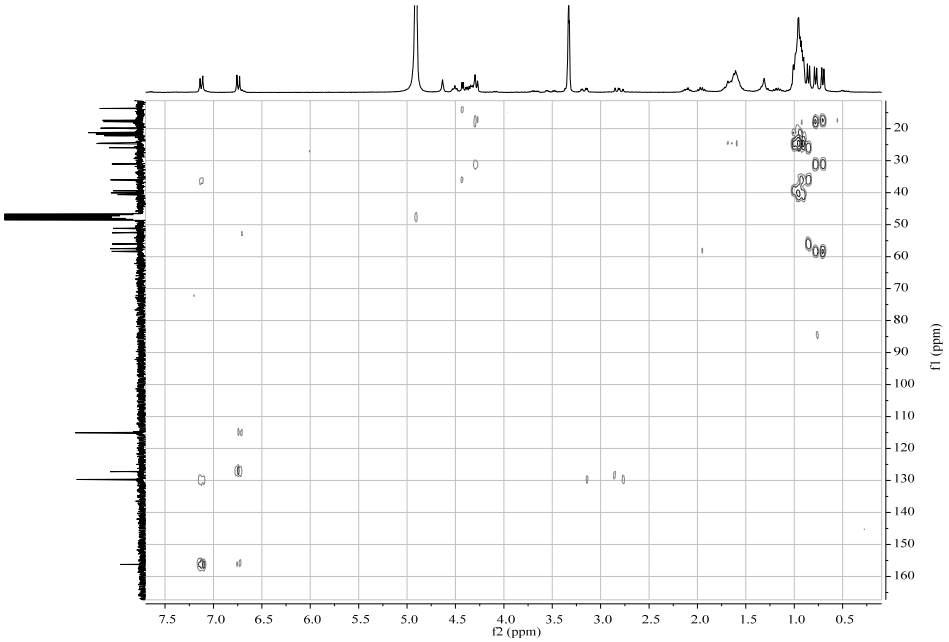


Figure S17. NOESY spectrum for synthetic anti-thermoactinoamide A

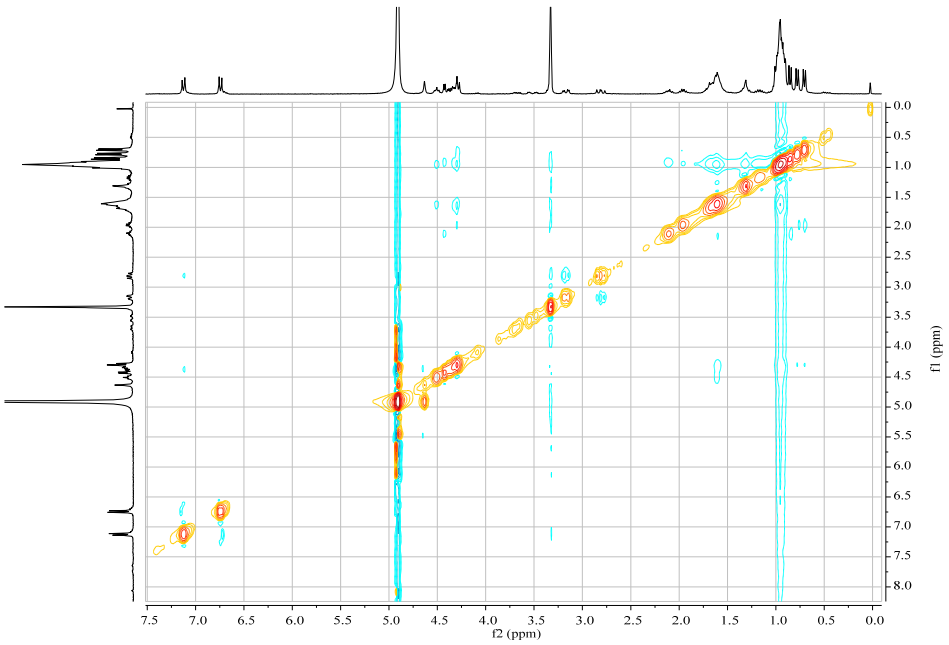


Figure S18. IR spectra of anti-thermoactinoamide A

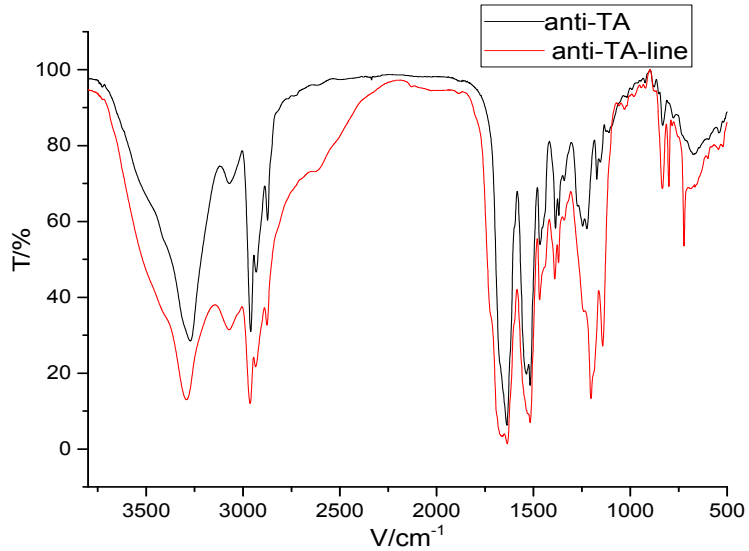


Figure S19. UV trace (210nm) from HPLC analysis of a crude reaction mixture of anti-thermoactinoamide A:isocratic elution 85% MeOH/H_2O over 20 min at aflow rate of 1mL/min. Elution time = 14.818 min