

Supplementary

Table S1: Antimycobacterial activity against *M. aurum* A+ from agar overlay assays of selected filamentous actinobacteria grown in six different growth media.

+ = zone of growth inhibition observed - = no zone of inhibition observed

Test Strain	Middlebrook	Czapek	DSMZ #553	Modified Bennett's	ISP-2	JCM #61
<i>Actinomadura napierensis</i> B60 ^T	+	-	-	-	+	-
<i>Actinomadura rudentiformis</i> HMC1 ^T	-	-	-	-	-	-
<i>Actinomadura</i> strain M2	-	+	-	-	-	
<i>Actinomadura</i> strain M27	-	-	+	-	-	-
<i>Amycolatopsis circi</i> S1.3 ^T	-	-	+	+	+	-
<i>Amycolatopsis</i> strain CP1B7Th	+	+	+	+	+	+
<i>Amycolatopsis</i> strain 3B4Tu	+	+	+	+	+	+
<i>Kribbella speibonae</i> SK5	-	-	-	+	+	-
<i>Kribbella</i> strain 1B8Tu	-	-	+	+	+	-
<i>Microbispora</i> strain SMA-HA3	-	-	-	-	-	-
<i>Micromonospora equina</i> Y22 ^T	-	-	-	-	-	-
<i>Micromonospora</i> strain Muiz4Y	-	-	+	-	-	-
<i>Micromonospora</i> strain M27	-	-	+	-	+	-
<i>Micromonospora tulbaghia</i> TVU1 ^T	-	-	-	-	-	-
<i>Nocardia</i> strain 2017S B10 Fri	-	-	+	-	-	-
<i>Nocardia</i> strain 2017S B3Th	-	-	-	-	-	-
<i>Nocardia</i> strain N12	-	-	+	-	-	-
<i>Nocardia</i> strain N15	-	-	-	-	-	-
<i>Nocardia rhamnosiphila</i> GM202 ^T	-	-	-	-	-	-

<i>Nocardia</i> strain N1	-	-	-	-	-	-
<i>Nonomuraea</i> strain QMC26	+	+	-	-	-	-
<i>Nonomuraea</i> strain SMA-BO4	-	-	-	-	-	-
<i>Streptomyces africanus</i> CPJVP-H ^T	-	-	-	-	+	-
<i>Streptomyces</i> strain 268	-	+	-	-	-	-
<i>Streptomyces</i> strain JBGM7	-	-	-	+	-	-
<i>Streptomyces fractus</i> MV32 ^T	-	-	+	-	-	-
<i>Streptomyces</i> strain #15	-	-	+	+	-	-
<i>Streptomyces</i> strain 1J1	-	-	+	-	-	-
<i>Streptomyces</i> strain B1	+	+	+	+	+	-
<i>Streptomyces</i> strain B2	+	+	+	+	+	-
<i>Streptomyces</i> strain C23	-	-	+	-	+	-
<i>Streptomyces</i> strain C3	-	-	-	-	+	-
<i>Streptomyces</i> strain CW2	-	-	+	+	+	+
<i>Streptomyces</i> strain CW5	-	-	-			+
<i>Streptomyces</i> strain HMC5	-	-	-	-	-	+
<i>Streptomyces</i> strain M8	-	-	-	-	-	-
<i>Streptomyces</i> strain PR10	-	+	+	+	+	-
<i>Streptomyces</i> strain PR3	+	+	+	+	+	+
<i>Streptomyces</i> strain PR36	-	-	+	+	-	-
<i>Streptomyces</i> strain PR4	-	-	+	+	+	+
<i>Streptomyces</i> strain Y10	-	-	+	-	+	+
<i>Streptomyces</i> strain Y12	-	-	+	+	-	+
<i>Streptomyces</i> strain Y30	-	-	+	+	-	+

Table S2. Accurate masses of identified antibiotics in the zones of inhibition and liquid culture crude extracts of active strains with theoretical monoisotopic masses, accurate mass differences and mass error.

Liquid: Small scale liquid cultures (25 mL).

Agar: agar-plate cultivation

* The cosine score is generated by GNPS molecular networking and is a measure of spectral similarity, with 1 being an identical match between the query spectrum and the GNPS library spectrum.

Strain name	Growth medium	Source	Compound	Cosine score*	Theoretical monoisotopic mass (Da)	Experimental Mass (Da)	Accurate mass difference (Da)	Mass Error (ppm)
<i>Kribbella</i> strain 1B8Tu	DSMZ #553	Agar	Actinomycin D	0.78	1254.6284	1254.6314	-0.0030	-2.391
		Liquid		0.78	1254.6284	1254.6310	-0.0026	-2.072
<i>Streptomyces</i> strain C23	ISP-2	Agar	Puromycin	0.96	471.2230	471.2236	-0.0006	-1.273
		Liquid		0.98	471.2230	471.2231	-0.0001	-0.212
<i>Streptomyces</i> strain CW2	ISP-2	Agar	Actinomycin D	0.84	1254.6284	1254.6284	0.0000	0.000
		Liquid		0.8	1254.6284	1254.6310	-0.0026	-2.072
	JCM #61	Agar		0.84	1254.6284	1254.6298	-0.0014	-1.116
		Liquid		0.79	1254.6284	1254.6294	-0.0010	-0.797
<i>Streptomyces</i> strain PR3	ISP-2	Agar	Valinomycin	0.9	1110.6311	1110.6348	-0.0037	-3.331
		Liquid		0.95	1110.6311	1110.6331	-0.0020	-1.801
	DSMZ #553	Agar		0.94	1110.6311	1110.6315	-0.0004	-0.360
		Liquid		0.88	1110.6311	1110.6300	0.0011	0.990
	Modified Bennett's	Agar		0.79	1110.6311	1110.6302	0.0009	0.810
		Liquid		0.92	1110.6311	1110.6346	-0.0035	-3.151
	Czapek	Agar		0.92	1110.6311	1110.6348	-0.0037	-3.331
		Liquid		0.88	1110.6311	1110.6348	-0.0037	-3.331
	JCM #61	Agar		0.8	1110.6311	1110.6331	-0.0020	-1.801
		Liquid		0.72	1110.6311	1110.6315	-0.0004	-0.360
	Middlebrook	Agar		0.82	1110.6311	1110.6300	0.0011	0.990
		Liquid		0.81	1110.6311	1110.6302	0.0009	0.810
<i>Streptomyces</i> strain PR10	ISP-2	Agar	Actinomycin D	0.77	1254.6284	1254.6262	0.0022	1.754
		Liquid		0.78	1254.6284	1254.6294	-0.0010	-0.797
	DSMZ #553	Agar		0.86	1254.6284	1254.626	0.0024	1.913
		Liquid		0.78	1254.6284	1254.6289	-0.0005	-0.399
	Modified Bennett's	Agar		0.87	1254.6284	1254.6283	0.0001	0.080
		Liquid		0.8	1254.6284	1254.6298	-0.0014	-1.116
	Czapek	Agar		0.84	1254.6284	1254.6300	-0.0016	-1.275
		Liquid		0.72	1254.6284	1254.6301	-0.0017	-1.355
<i>Streptomyces</i> strain PR36	DSMZ #553	Agar	Valinomycin	0.8	1110.6311	1110.6346	-0.0035	-3.151
		Liquid		0.85	1110.6311	1110.629	0.0021	1.891

Table S3: *In vitro* antitubercular activity (nM) against *M. tuberculosis* H37Rv^T of selected South African actinobacterial strains. Two technical repeats, one biological repeat.

Strain Name	Growth Medium	7H9_ADC_GLU_ TX	7H9_CAS_GLU_ TX	7H9_ADC_GLU_ TW
<i>Actinomadura napierensis</i> B60 ^T	Middlebrook	> 62.5	26.1	> 62.5
<i>Actinomadura napierensis</i> B60 ^T	ISP-2	2.3	1.3	1.6
<i>Actinomadura</i> strain M2	Czapek	> 62.5	6.9	25.7
<i>Actinomadura</i> strain M27	DSMZ #553	> 62.5	21.7	> 62.5
<i>Amycolatopsis circi</i> S1.3 ^T	Modified Bennett's	> 62.5	> 62.5	5.2
<i>Amycolatopsis circi</i> S1.3 ^T	DSMZ #553	> 62.5	30.4	30.9
<i>Amycolatopsis circi</i> S1.3 ^T	ISP-2	> 62.5	6.8	50.2
<i>Amycolatopsis</i> strain CP1B7Th	ISP-2	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain CP1B7Th	DSMZ #553	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain CP1B7Th	Modified Bennett's	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain CP1B7Th	Czapek	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain CP1B7Th	JCM #61	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain CP1B7Th	Middlebrook	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain 3B4Tu	ISP-2	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain 3B4Tu	DSMZ #553	> 62.5	> 62.5	23.7
<i>Amycolatopsis</i> strain 3B4Tu	Modified Bennett's	54.4	29.9	0.79

<i>Amycolatopsis</i> strain 3B4Tu	Czapek	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain 3B4Tu	JCM #61	> 62.5	> 62.5	> 62.5
<i>Amycolatopsis</i> strain 3B4Tu	Middlebrook	> 62.5	> 62.5	> 62.5
<i>Kribbella speibonae</i> SK5	Modified Bennett's	> 62.5	26.6	> 62.5
<i>Kribbella speibonae</i> SK5	ISP-2	> 62.5	9.8	> 62.5
<i>Kribbella</i> strain 1B8Tu	ISP-2	> 62.5	> 62.5	> 62.5
<i>Kribbella</i> strain 1B8Tu	Modified Bennett's	> 62.5	> 62.5	15.6
<i>Micromonospora</i> strain Muiz4Y	DSMZ #553	> 62.5	> 62.5	> 62.5
<i>Micromonospora</i> strain 1J1	DSMZ #553	> 62.5	5.9	> 62.5
<i>Micromonospora</i> strain M27	DSMZ #553	> 62.5	> 62.5	> 62.5
<i>Micromonospora</i> strain M27	ISP-2	> 62.5	> 62.5	> 62.5
<i>Nocardia</i> strain B10S Fri	DSMZ #553	61.1	40.5	52.9
<i>Nocardia</i> strain N12	DSMZ #553	> 62.5	> 62.5	> 62.5
<i>Nonomuraea</i> strain QMC26	Middlebrook	> 62.5	31.1	> 62.5
<i>Nonomuraea</i> strain QMC26	Czapek	> 62.5	31.5	> 62.5
<i>Streptomyces africanus</i> CPJVR-H ^T	ISP-2	3.4	> 62.5	5.2
<i>Streptomyces</i> strain #15	DSMZ #553	> 62.5	24.0	> 62.5
<i>Streptomyces</i> strain #15	Modified Bennett's	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain 268	Czapek	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain B1	Czapek	> 62.5	31.0	> 62.5

<i>Streptomyces</i> strain B1	Modified Bennett's	> 62.5	6.0	27.8
<i>Streptomyces</i> strain B1	ISP-2	> 62.5	9.6	43.1
<i>Streptomyces</i> strain B1	Middlebrook	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain B1	DSMZ #553	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain B2	Middlebrook	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain B2	DSMZ #553	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain B2	Modified Bennett's	> 62.5	29.7	23.2
<i>Streptomyces</i> strain B2	Czapek	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain B2	ISP-2	> 62.5	29.5	> 62.5
<i>Streptomyces</i> strain C3	ISP-2	> 62.5	29.5	> 62.5
<i>Streptomyces</i> strain CW5	JCM #61	0.6	0.2	< 0.12
<i>Streptomyces</i> strain HMC5	JCM #61	25.6	2.8	7.9
<i>Streptomyces</i> strain JBGM7	Modified Bennett's	> 62.5	26.5	> 62.5
<i>Streptomyces</i> strain M8	DSMZ #553	> 62.5	6.1	6.5
<i>Streptomyces fractus</i> MV32 ^T	DSMZ #553	0.8	< 0.12	< 0.12
<i>Streptomyces</i> strain PR4	DSMZ #553	> 62.5	14.9	> 62.5
<i>Streptomyces</i> strain PR4	Modified Bennett's	2.1	2.4	0.397
<i>Streptomyces</i> strain PR4	ISP-2	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain PR4	JCM #61	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain Y10	DSMZ #553	< 0.12	< 0.12	< 0.12
<i>Streptomyces</i> strain Y10	JCM #61	0.5	< 0.12	< 0.12
<i>Streptomyces</i> strain Y10	ISP-2	> 62.5	> 62.5	> 62.5
<i>Streptomyces</i> strain Y12	Modified Bennett's	> 62.5	2.3	> 62.5
<i>Streptomyces</i> strain Y12	JCM #61	> 62.5	16.2	> 62.5
<i>Streptomyces</i> strain Y12	DSMZ #553	> 62.5	> 62.5	> 62.5

<i>Streptomyces</i> strain Y30	Modified Bennett's	> 62.5	16.5	> 62.5
<i>Streptomyces</i> strain Y30	DSMZ #553	> 62.5	> 62.5	> 62.5

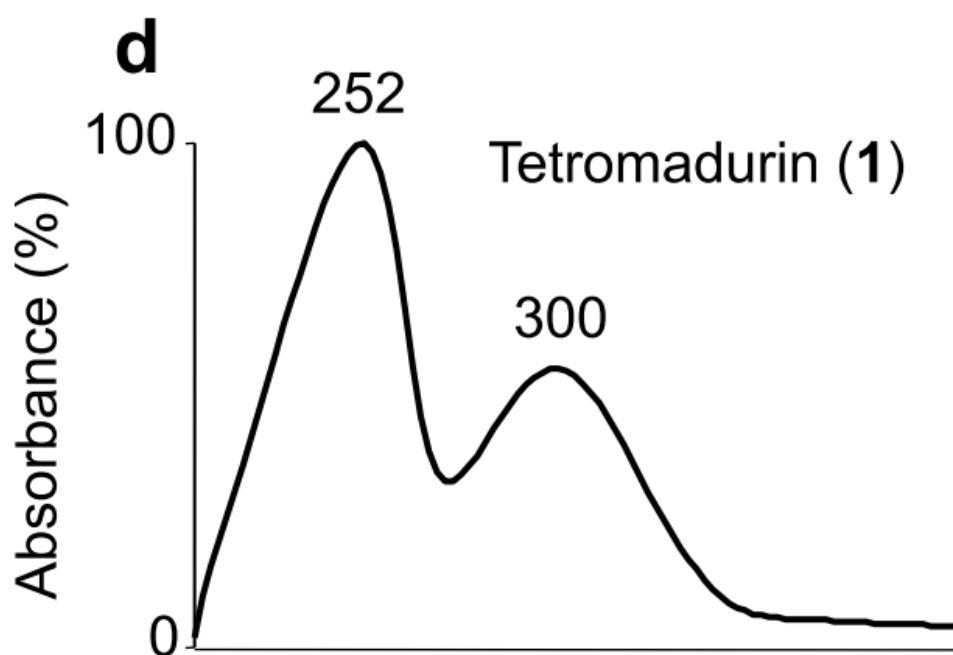
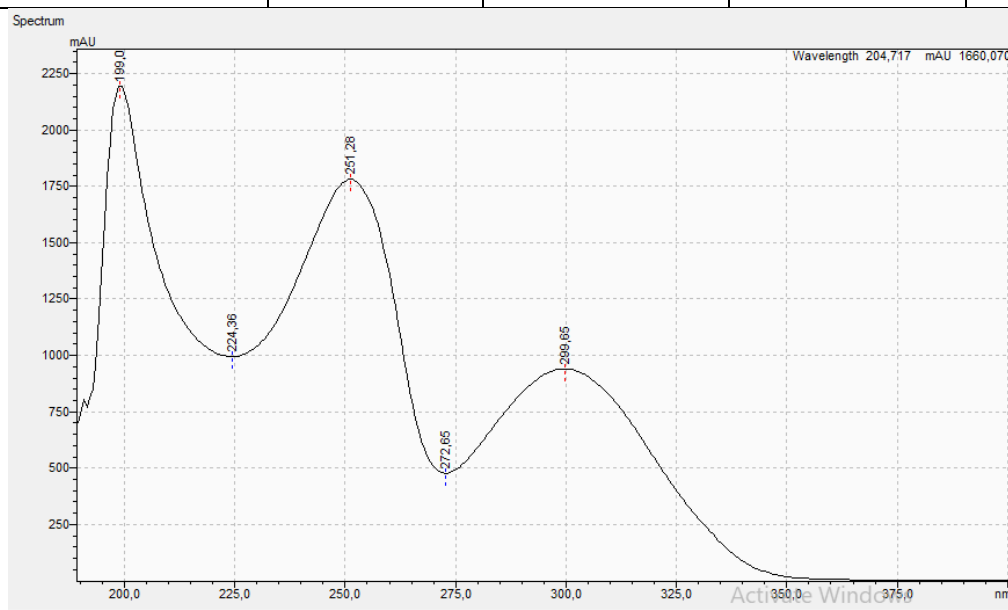


Figure S1: Comparison of experimentally obtained UV spectrum of identified tetromadurin (top) vs published spectrum of tetromadurin (bottom) showing a strong match between the two [28].

Table S4: Carbon and hydrogen NMR spectroscopic data of tetromadurin presented in original isolation paper by [29] compared to the experimental data measured from tetromadurin obtained in this study.

Carbon #	δC (100 MHz, $CDCl_3$) [29]	Type	δH (400 MHz, $CDCl_3$) [29]	δC (100 MHz, $CDCl_3$) (Experimental)	δH (400 MHz, $CDCl_3$) (Experimental)
C-1	171.2	C	-	-	-
C-2	96.2	C	-	-	-
C-3	196.0	C	-	-	-
C-4	83.3	CH	4.67 br s	83.3	4.68
C-5	37.2	CH	2.45, t, 9.8	36.96	2.46
C-6	43.5	CH	1.64	43.3	1.66
C-7	76.3	CH	4.02, br s	76.4	4.04
C-8	35.9	CH	1.52, ??	35.7	1.53
C-9	34.9	CH ₂	0.98 1.36	34.79	0.99 1.36
C-10	36.1	CH	2.72, dq, 3.6, 9.8	35.79	2.74
C-11	137.6	CH	5.24, d, 10.4	137.4	5.23
C-12	135.9	C	-	-	-
C-13	85.0	CH	3.62-2.72	84.8	3.69
C-14	32.3	CH ₂	1.18 1.80	32.18	1.22 1.80
C-15	31.3	CH ₂	1.20 1.34	31.1	1.24 1.42
C-16	31.2	CH ₂	1.58	31.0	1.5
C-17	92.1	CH	3.42, d, 10.8	92.0	3.44
C-18	134.5	C	2.26, m		
C-19	131.1	CH	5.84, br dd, 6.0, 8.0	131.2	5.85
C-20	32.2	CH ₂	2.26, m	32.1	2.27
C-21	83.3	CH	3.62-3.72	83.3	3.66
C-22	40.2	CH	1.80	40.4	1.84
C-23	44.5	CH ₂	1.30 1.96, dd, 9.4, 12.4		
C-24	82.4	C	-	-	-
C-25	82.9	CH	3.62 - 3.72	-	-
C-26	25.2	CH ₂	1.85 - 1.95	25.96	1.81
C-27	22.8	CH ₂	1.66 1.80	22.49	1.66 1.83
C-28	81.3	CH	3.62-3.72	81.2	3.73
C-29	70.5	CH	3.97, dt, 2.6, 7.2		
C-30	28.2	CH ₂	1.23 1.35	27.9	1.42
C-31	10.5	CH ₃	0.93, t, 7.2	10.2	0.95
C-32	25.6	CH ₃	1.06, s	25.37	1.08
C-33	16.2	CH ₃	1.00, d, 6.4	16.07	1.02
C-34	10.4	CH ₃	1.72, s	10.2	1.74
C-35	17.3	CH ₃	0.66, d, 7.6	17.1	0.66
C-36	55.9	CH ₂	3.94, 4.25, d, 11.2	55.6	3.94 4.27
C-37	17.8	CH ₃	0.91, d, 7.2	17.56	0.93
C-38	65.6	CH ₂	3.88 4.36, dd, 2.6, 13.0	65.4	3.88 4.38

C-39	180.2	C	-	-	-
C-40	153.5	C	-	-	-
C-41	90.1	CH ₂	4.78, 5.18, d, 2.8	90.1	4.80 5.20
C-42	58.9	CH ₃	3.34	58.5	3.35

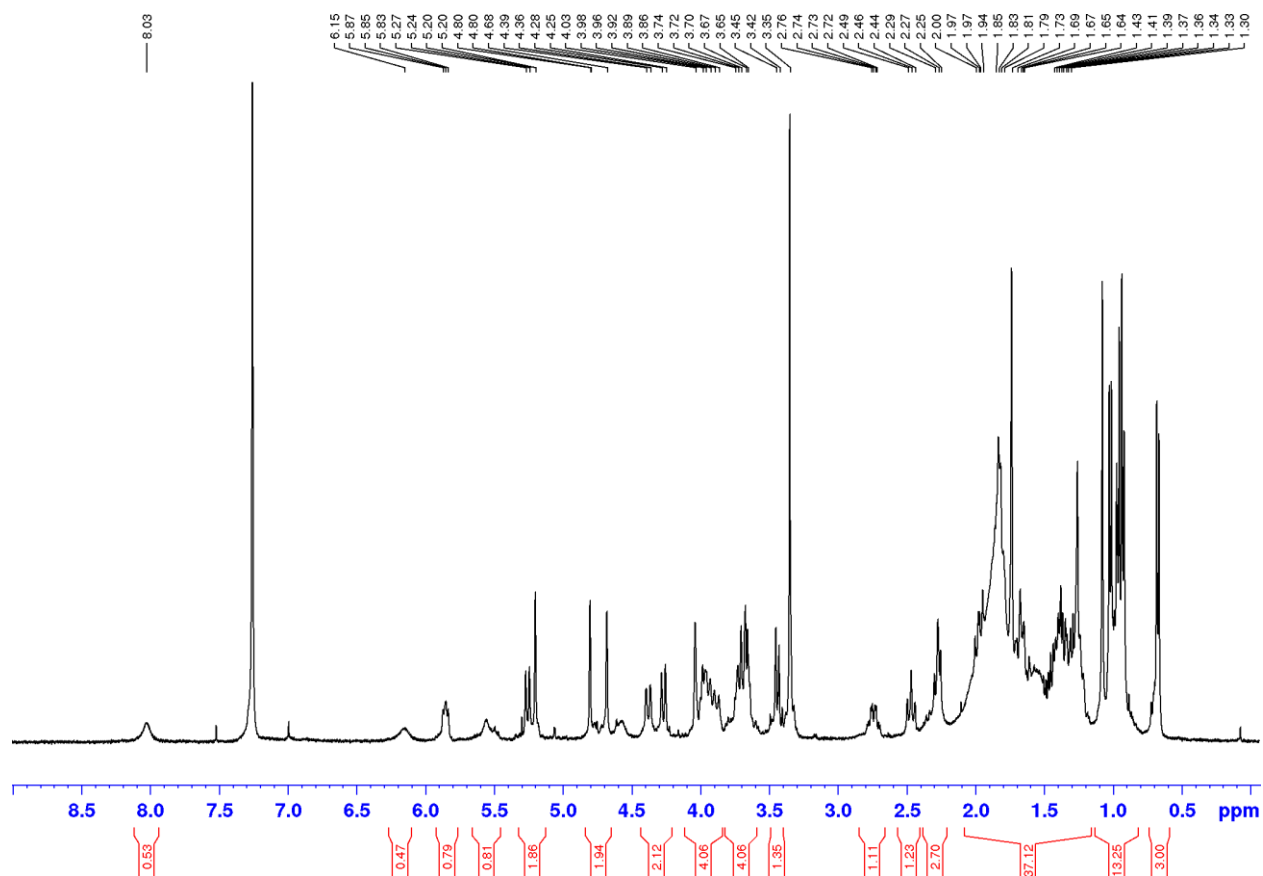


Figure S2. ¹H NMR spectrum of tetromadurin (400 MHz, CDCl₃)

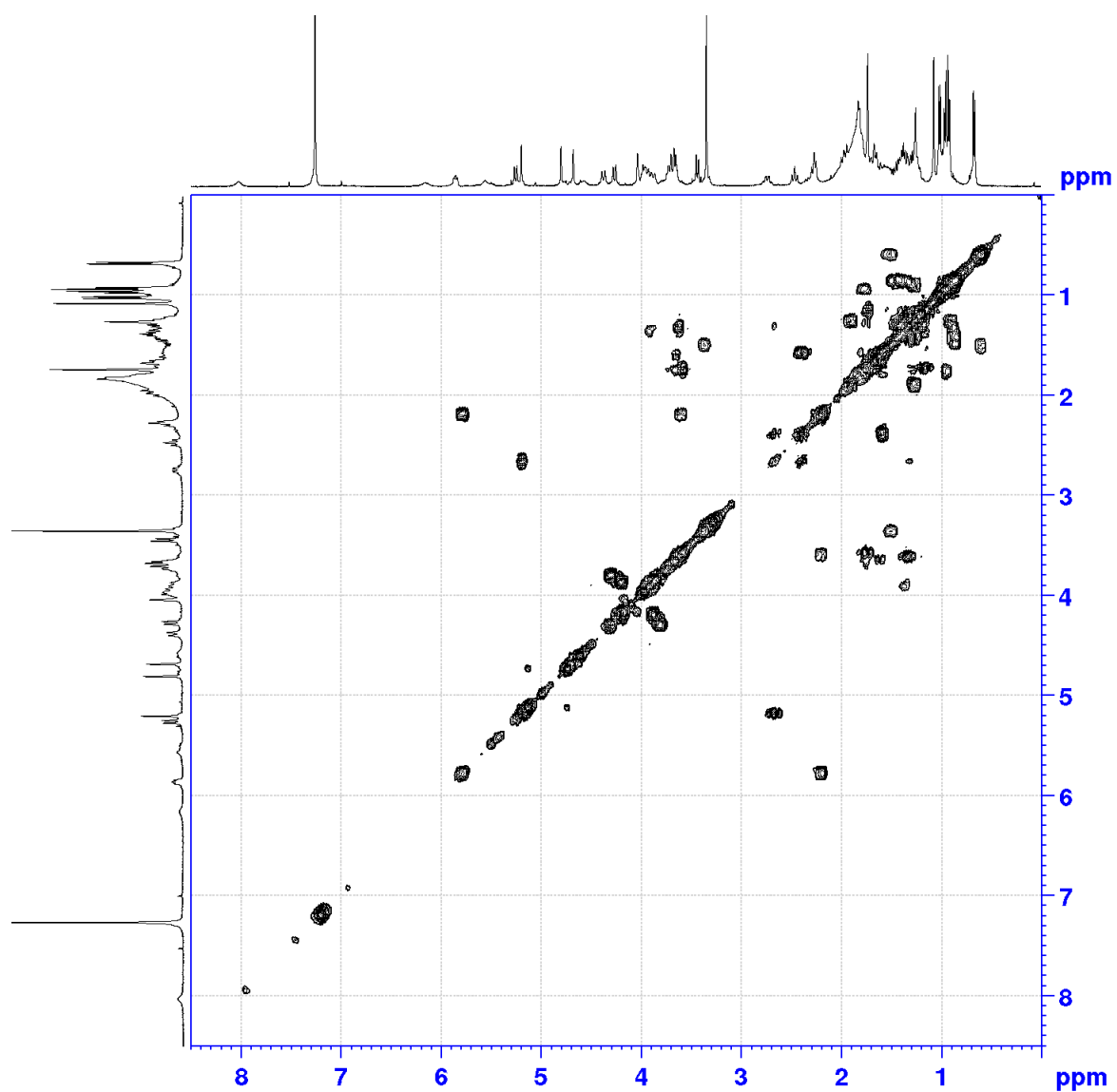


Figure S3. ^1H - ^1H COSY spectrum of tetromadurin

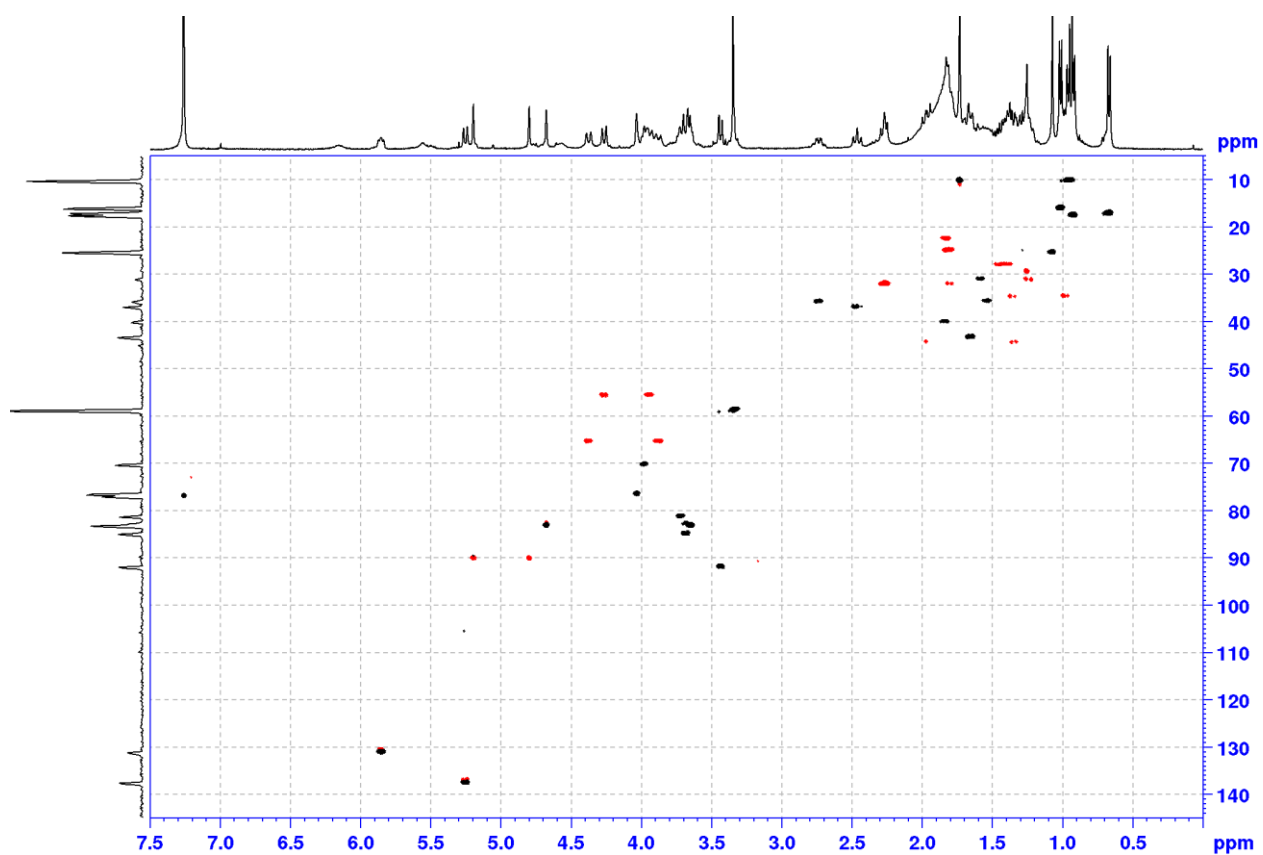


Figure S4. HSQC NMR spectrum of tetromadurin

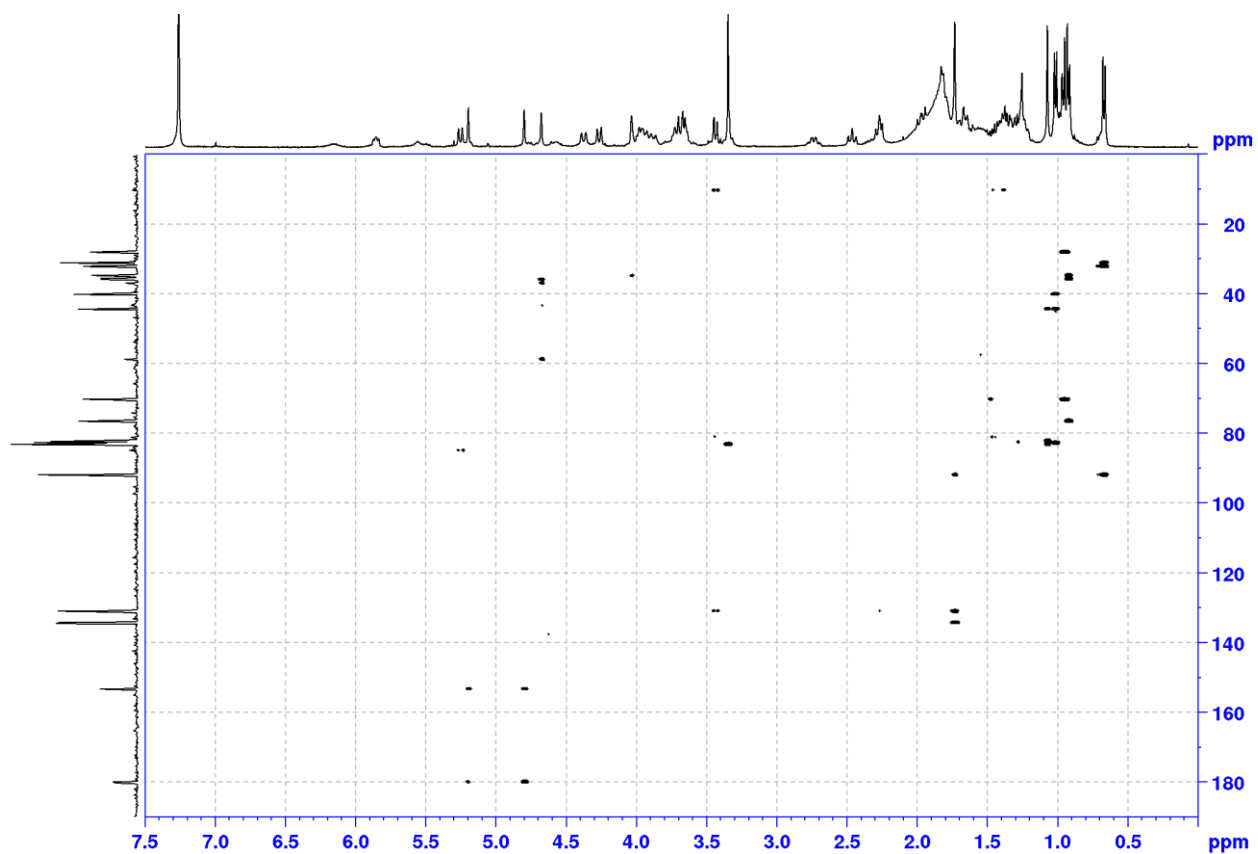


Figure S5. HMBC NMR spectrum of tetromadurin