

Skeletal editing via multi-step engineering of a modular polyketide synthase

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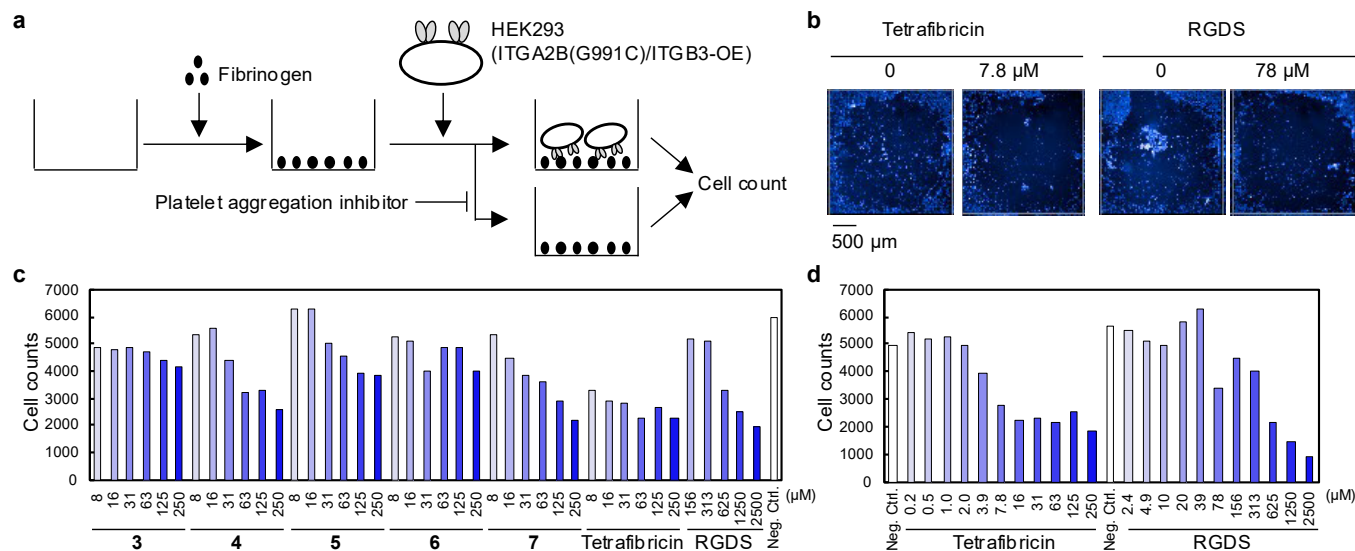
Supplementary Discussion

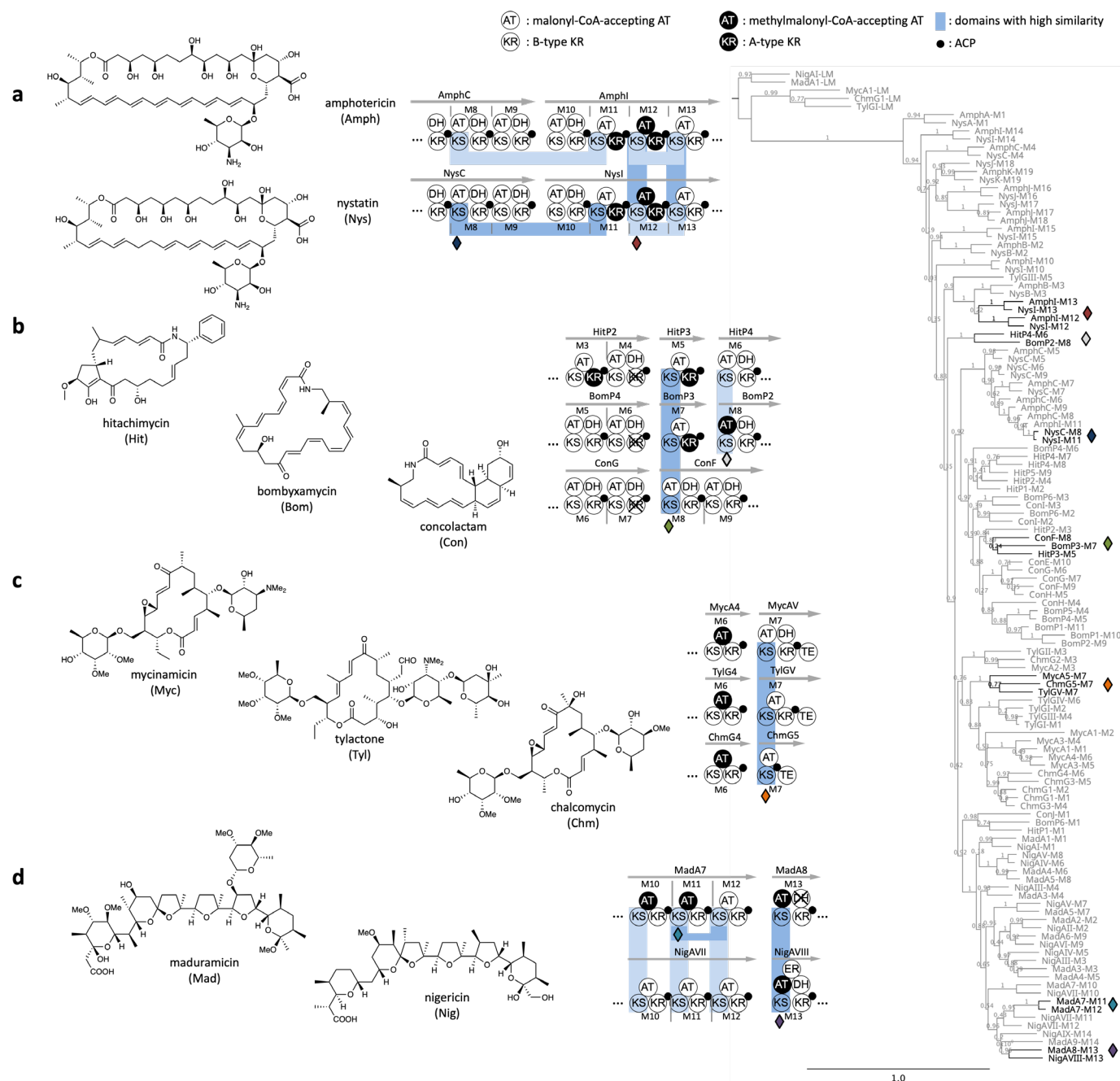
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Supplementary Discussion

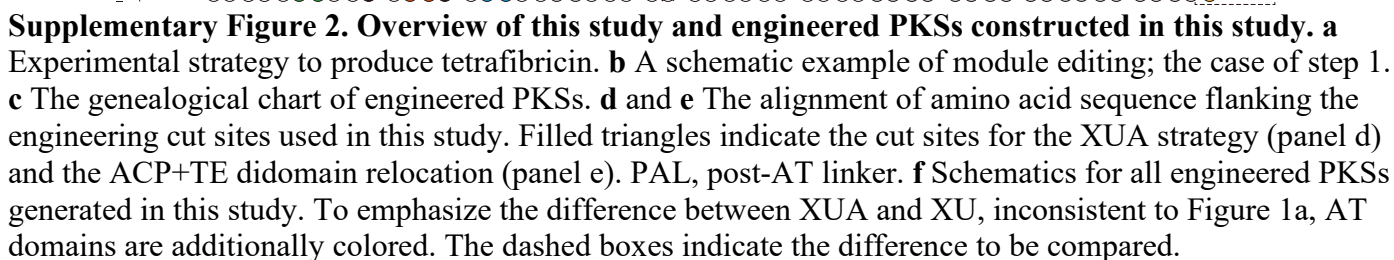
To evaluate the biological activity of tetra fibrin accessed here, we constructed an *in vitro* platelet aggregation model since we could not use human blood samples. In this model, we used a fibrinogen-coated microtiter plate to which the HEK293 cells overexpressing ITGA2B(G991C) (an active form variant of ITGA2B) and ITGB3 were bound (panel a). An inhibitory effect by the tested compound was measured as the number of Hoechst33342 stained cells remaining on the plate after washing (panel b). The RGDS peptide was used as a positive control. Among the tested compounds, 3, 4, 5, 6, 7, and tetra fibrin, tetra fibrin showed the strongest inhibitory activity, while 4 and 7 slightly inhibited (panel c). Further analysis showed that the engineered tetra fibrin exhibited an IC₅₀ approximately 10-fold lower than that of RGDS (panel d), which was consistent with the literature (ref. 34).

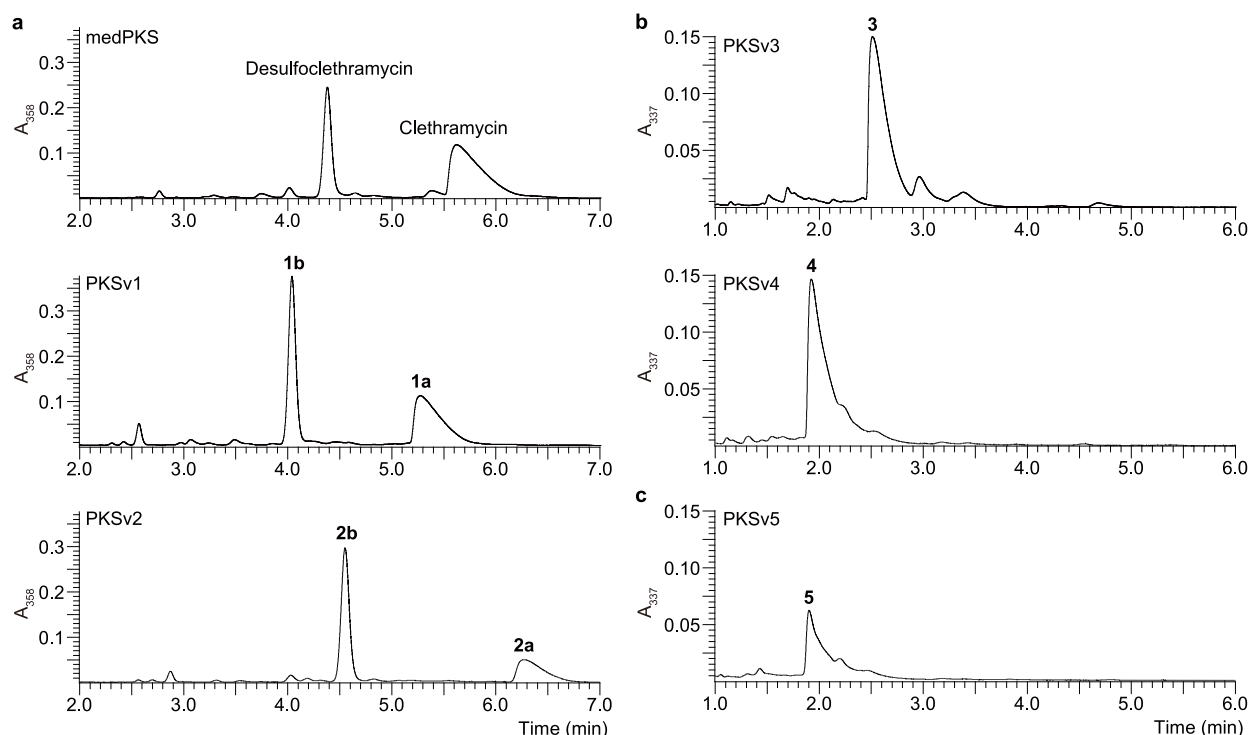




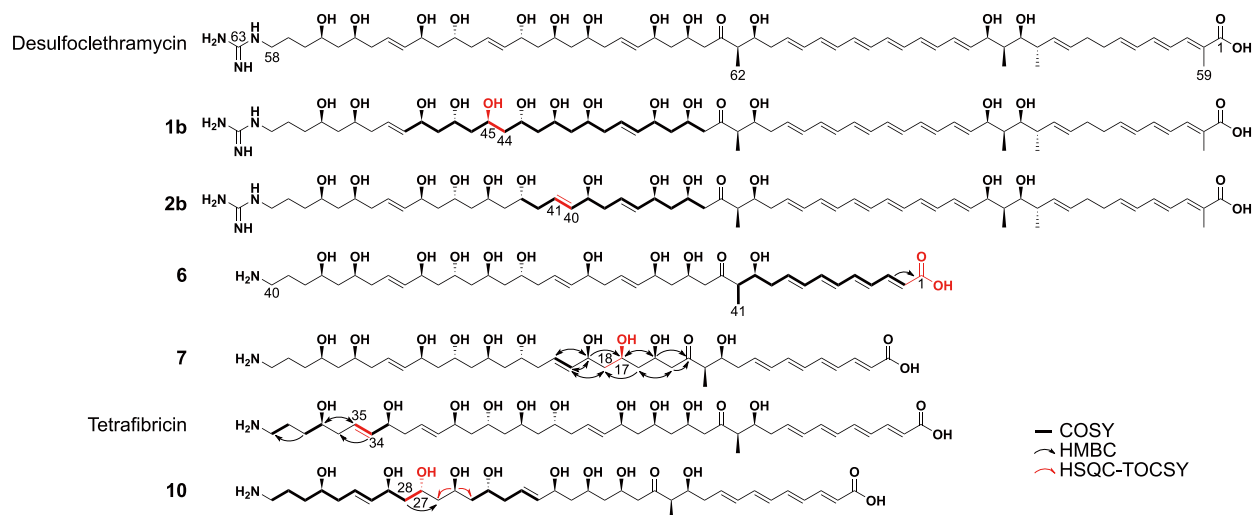
Supplementary Figure 1. Phylogenetic analysis of KS domains from various *cis*-AT PKS pathways.

Phylogenetic analysis of KS domains from various *cis*-AT PKS pathways. Overall, the analysis indicates that KSs with high sequence identity have identical composition of upstream processing domains, but their downstream processing domains can be variable. **a** Comparison of polyene macrolides amphotericin and nystatin PKSs. **b** Comparison of polyene macrolactams hitachimycin, bombyxamycin, and concolactam PKSs. **c** Comparison of macrolides mycinamicin, tylactone, and chalconicmycin PKSs. **d** Comparison of polyethers maduramicin and nigericin PKSs.

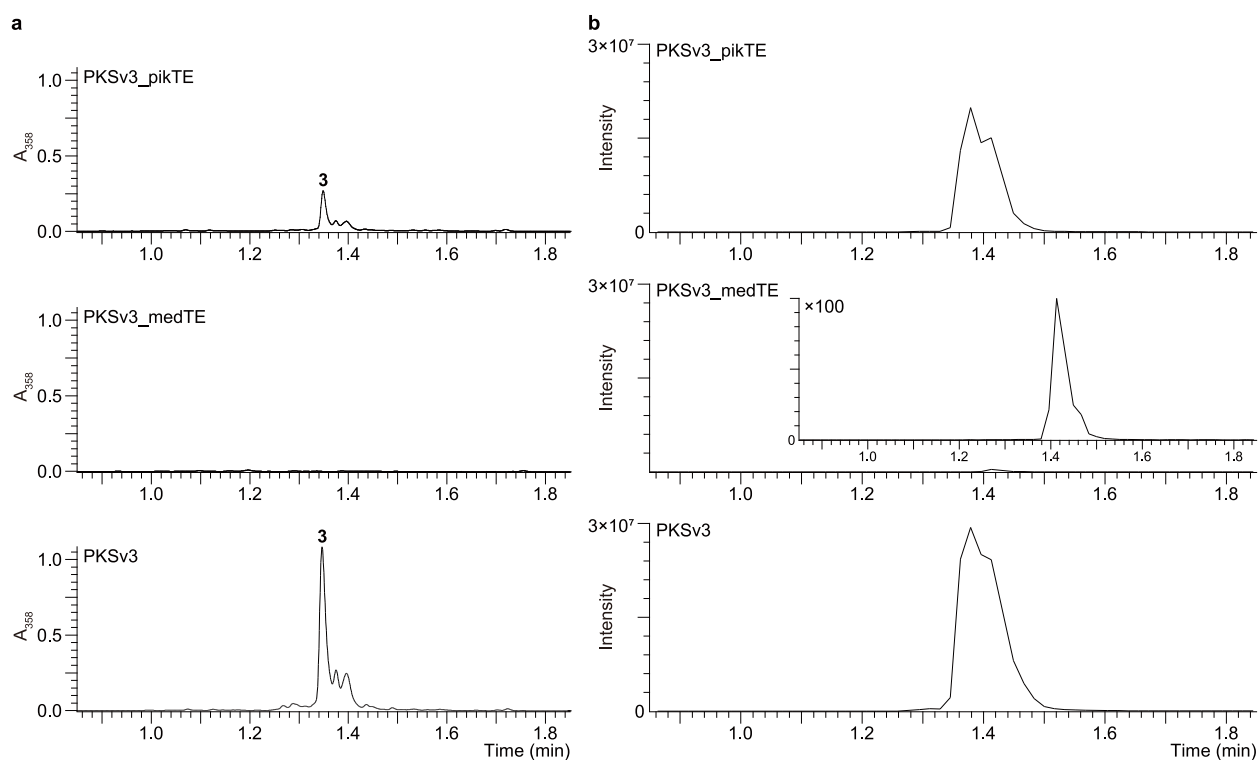




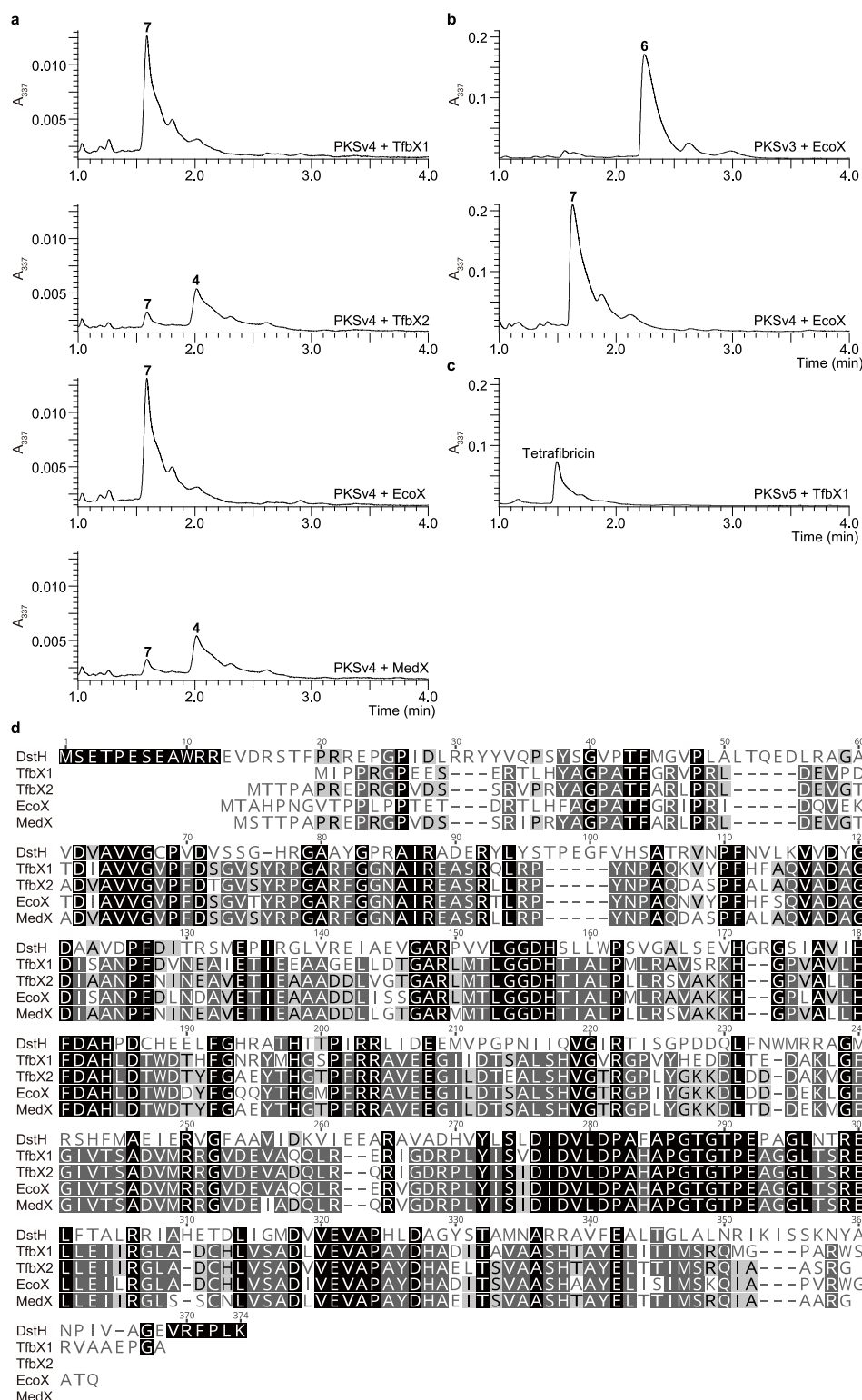
Supplementary Figure 3. Analytical UHPLC of medPKS and PKSv1–v5 products. The culture extracts of *S. lividans* TK23 $\Delta redD-X$ carrying medPKS, PKSv1, PKSv2, PKSv3, PKSv4, or PKSv5 gene were analyzed by UHPLC (isocratic; see Method section for detail). **a** Production of clethramycin+desulfoclethramycin, **1a+1b** and **2a+2b**. UV chromatograms at 358 nm are indicated. **b** and **c** Production of **3**, **4** (panel b), and **5** (panel c). UV chromatograms at 337 nm are indicated. Each chromatogram is representative of three biological replicates.



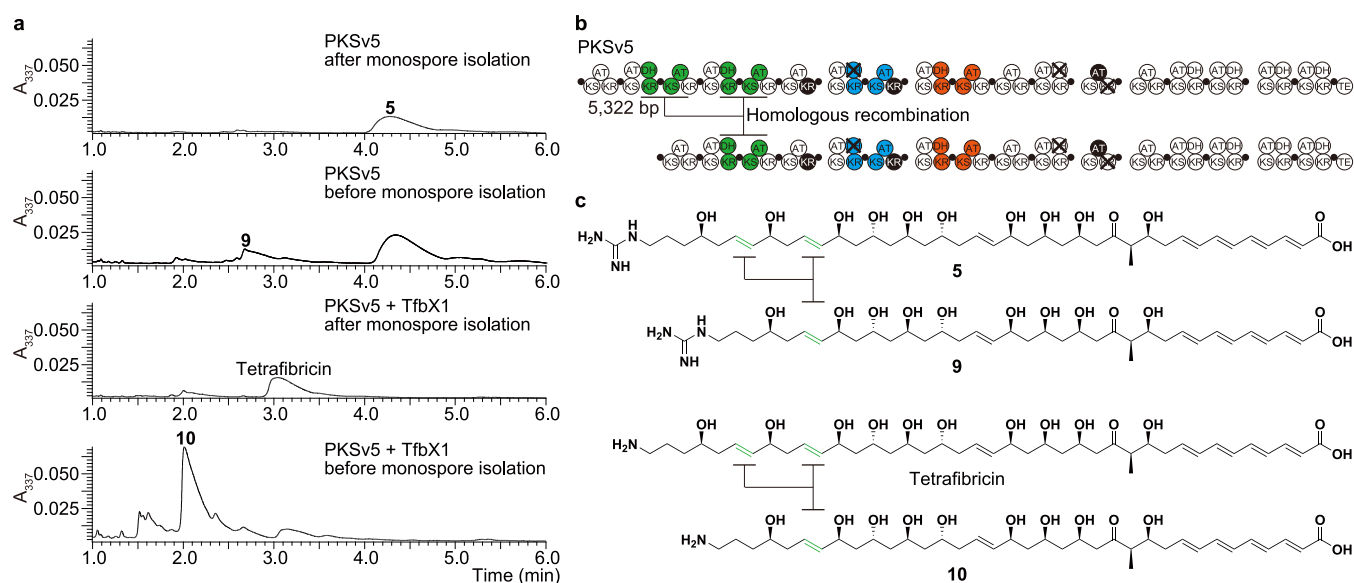
Supplementary Figure 4. Key 2D NMR correlations used for structural determination. The intended substructures in each step are colored red. Bold bonds, COSY; black full arrows, HMBC; red half arrows, HSQC-TOCSY.



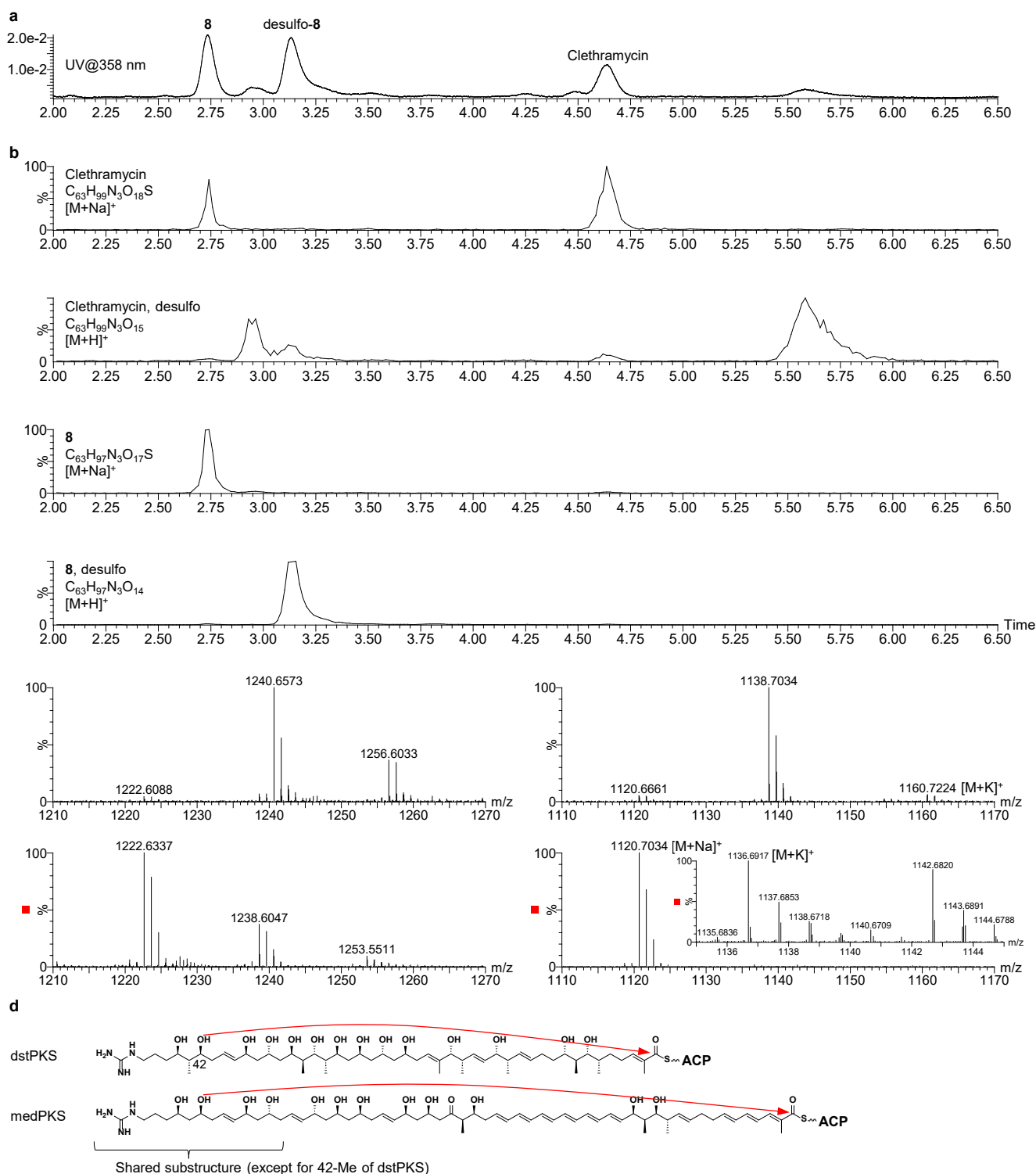
Supplementary Figure 5. Analytical UHPLC of PKSv3 TE variants products. The culture extracts of *S. lividans* TK23 $\Delta redD-X$ carrying PKSv3, PKSv3_medTE, or PKSv3_pikTE gene were analyzed by UHPLC (gradient; see Method section for detail). **a** UV chromatograms at 337 nm. **b** Mass chromatograms at m/z 824.4903. Each chromatogram is representative of three biological replicates.



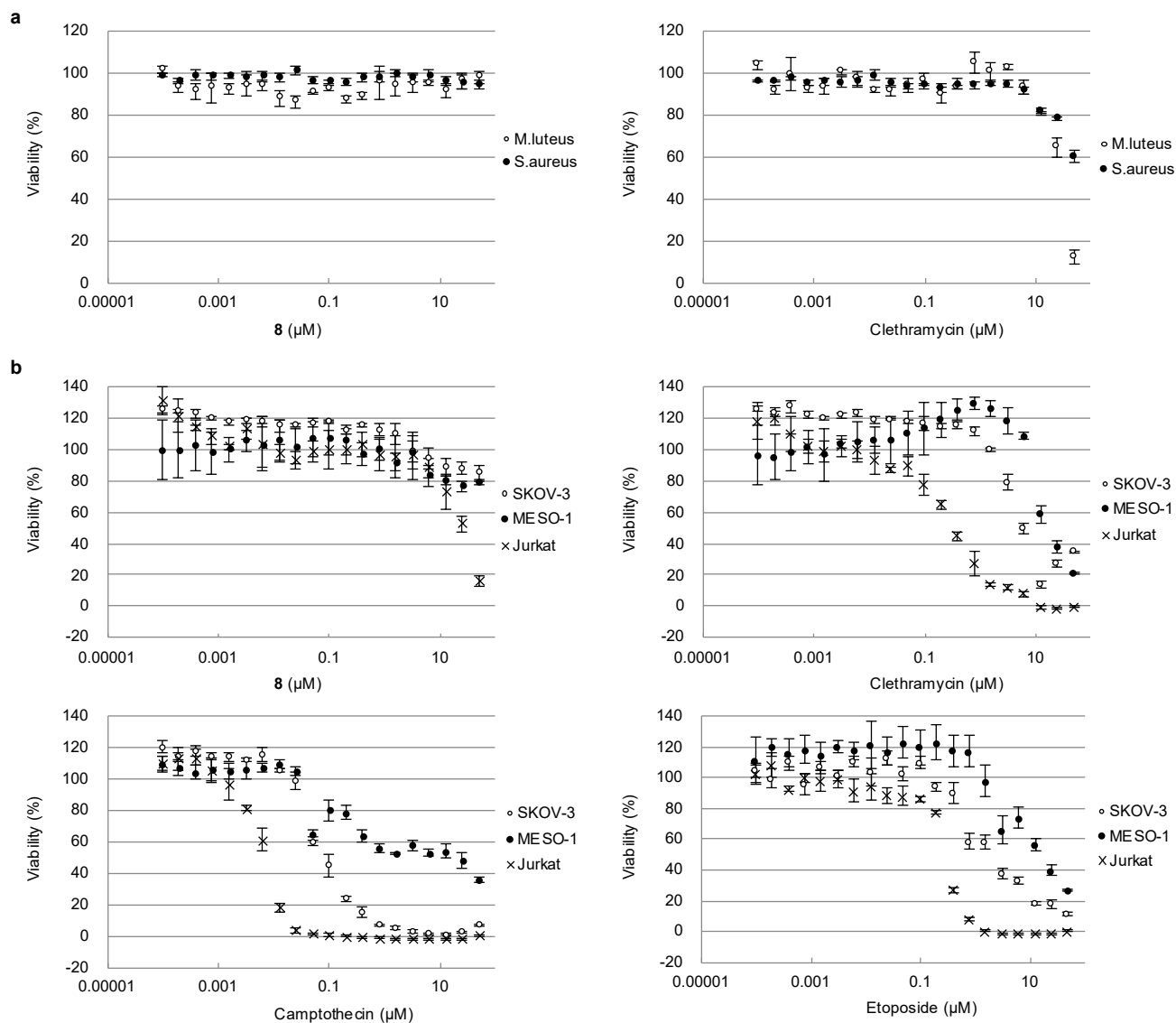
Supplementary Figure 6. De-guanidinylation by amidinohydrolases. **a** Co-expression of *tfbX1*, *tfbX2*, *ecoX*, or *medX* with PKSv4. The culture extracts of transformants were analyzed by UHPLC (isocratic 15% B; see Method section for detail). **b** Production of 6 and 7. The culture extracts of transformants were analyzed by UHPLC (isocratic 17% B; see Method section for detail). **c** Production of tetrafibricin. The culture extracts of transformants were analyzed by UHPLC (isocratic 15% B; see Method section for detail). Each chromatogram is representative of three biological replicates. **d** Sequence alignment of selected amidinohydrolases. Conserved residues are highlighted in black (among all five sequences), dark grey (among 4 out of 5), and light grey (among 3 out of 5).



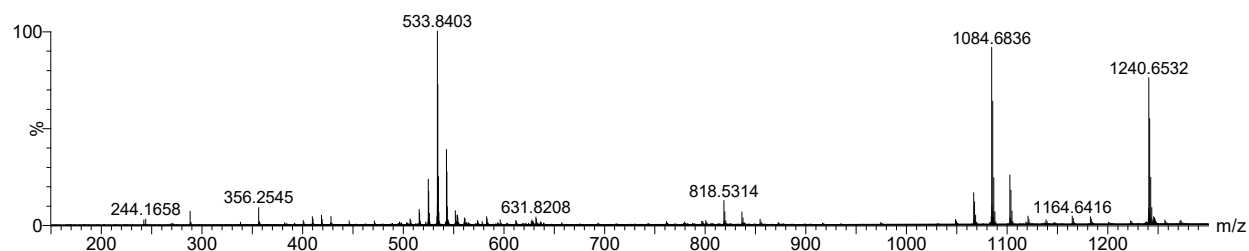
Supplementary Figure 7. Module-skipped by-products. By-products **9** and **10** were observed in the culture broth of transformants significantly before monospore isolation. **a** The culture extracts of *S. lividans* TK23 $\Delta redD-X$ carrying PKSv5 or PKSv5+*tfbX1* before and after monospore isolation (see ‘Transformation of *Streptomyces lividans* TK23 $\Delta redD-X$ ’ in Method), respectively. UV chromatograms at 337 nm are indicated. Each chromatogram is representative of three biological replicates. **b** The possible homologous recombination site is schematically illustrated.



Supplementary Figure 8. Production of 8. **a** UV chromatogram at 358 nm. The culture extract of *S. lividans* TK23 $\Delta redD-X$ carrying PKSv6 was analyzed by UHPLC (isocratic). **b** The extracted ion chromatograms for clethramycin $[M+Na]^+$, desulfoclethramycin $[M+H]^+$, **8** $[M+Na]^+$ and desulfo-**8** $[M+H]^+$, from top to bottom. **c** The mass spectra of clethramycin and **8**, and desulfoclethramycin and desulfo-**8** show that the isotopic peaks of $[8+K]^+$ and $[desulfo-8+K]^+$ overlap with the $[clethramycin+Na]^+$ and $[desulfoclethramycin+Na]^+$ peaks, respectively. Each chromatogram is representative of three biological replicates. **d** The final module intermediates of DstPKS and MedPKS. The arrow indicates the dstTE-catalyzed reaction to form the macrocyclic polyketides.

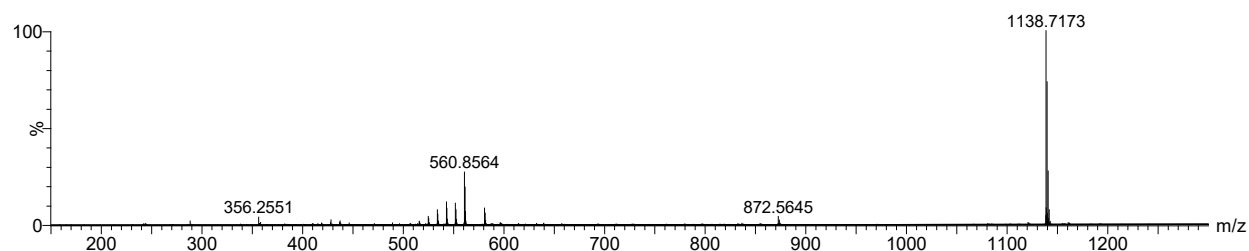


Supplementary Figure 9. Biological activities of 8. a Antibacterial assay of **8** and clethramycin. **b** Cytotoxicity of **8** and clethramycin, as well as camptothecin and etoposide as positive controls.



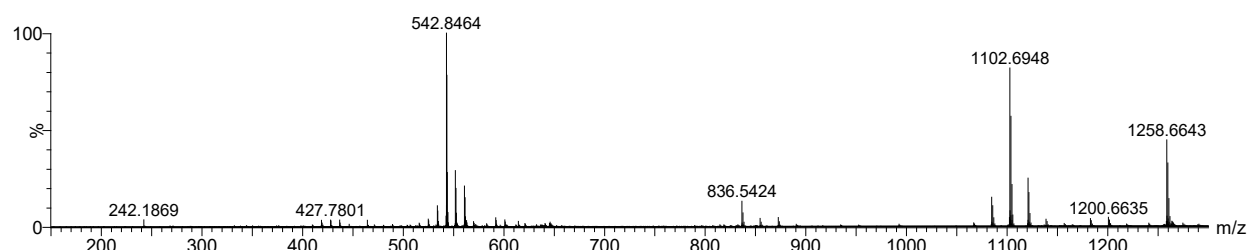
Supplementary Figure 10.

High resolution mass spectrum for clethramycin.



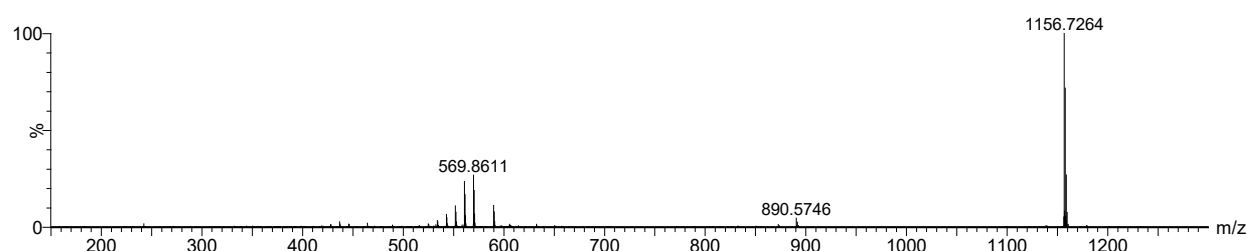
Supplementary Figure 11.

High resolution mass spectrum for desulfoclethramycin.



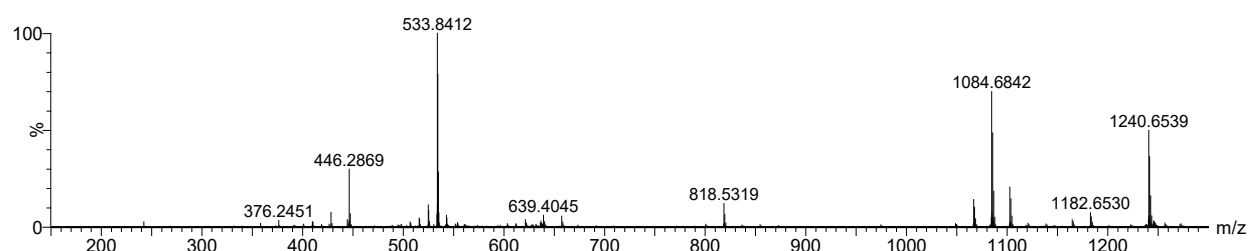
Supplementary Figure 12.

High resolution mass spectrum for 1a.



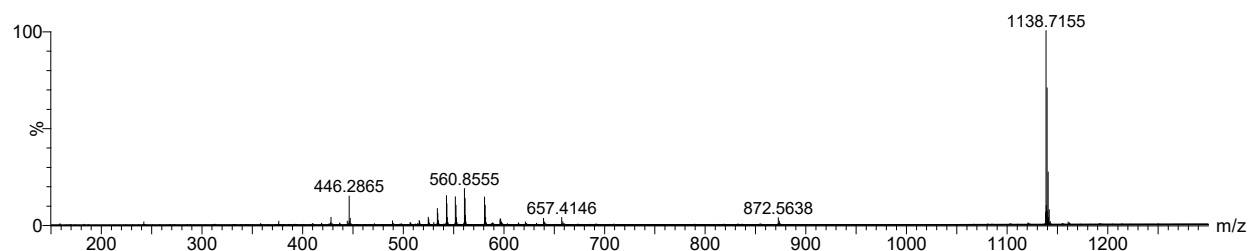
Supplementary Figure 13.

High resolution mass spectrum for 1b.

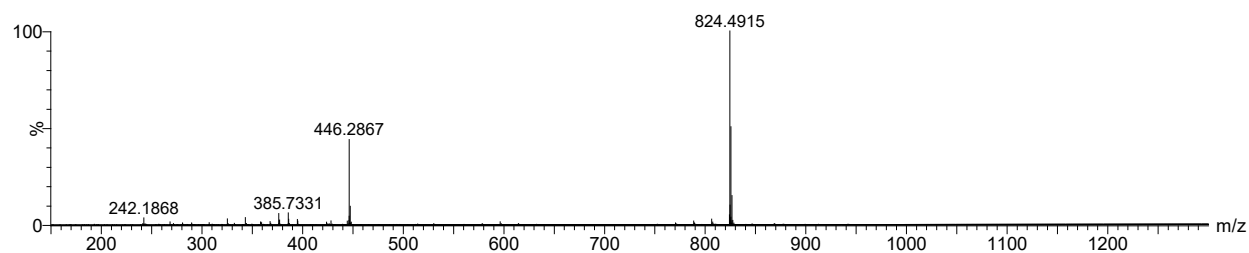


Supplementary Figure 14.

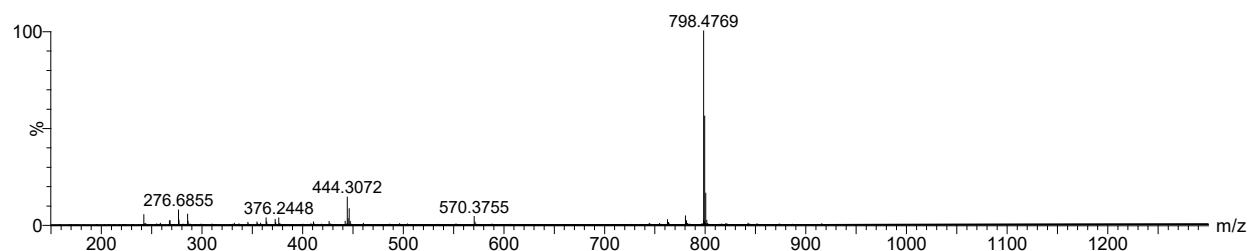
High resolution mass spectrum for 2a.



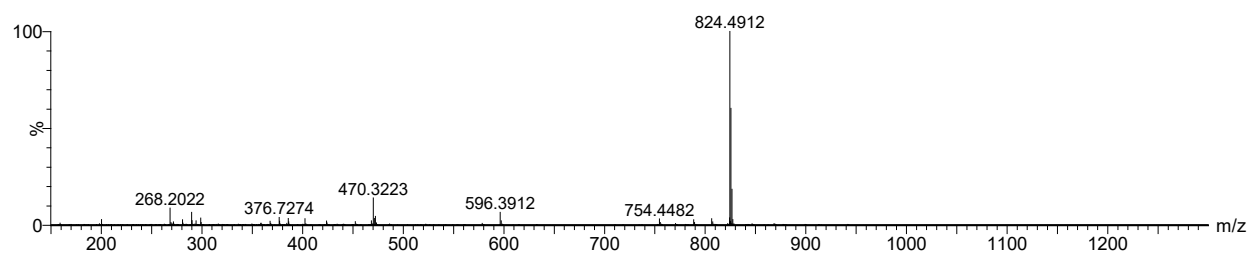
Supplementary Figure 15.
High resolution mass spectrum for **2b**.



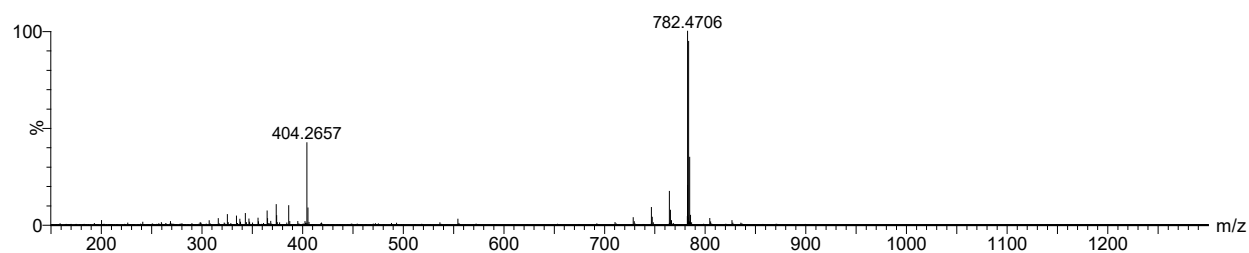
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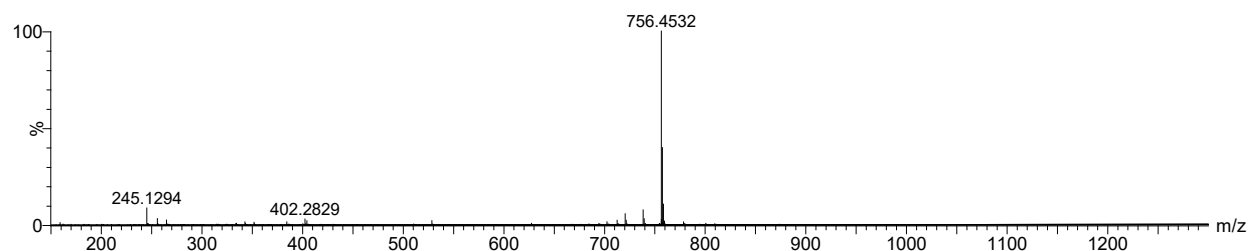
Supplementary Figure 17.
High resolution mass spectrum for **4**.



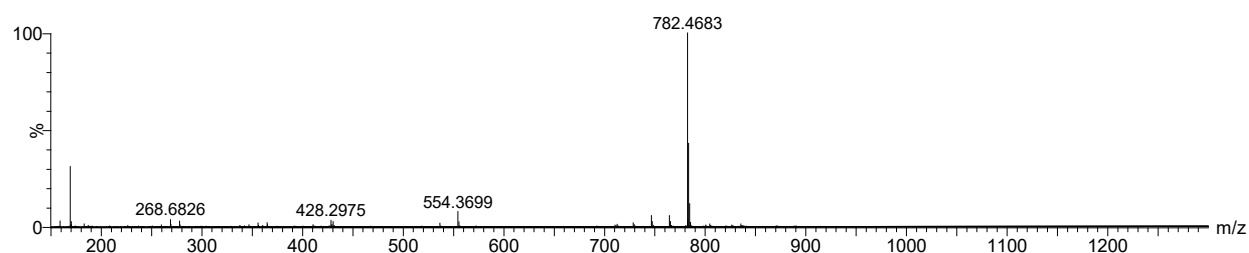
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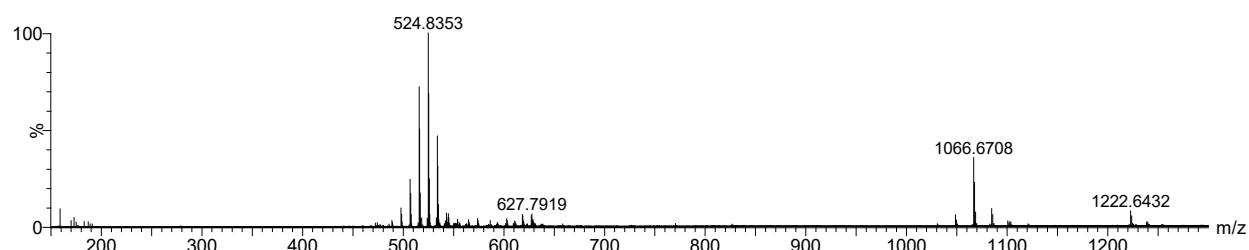
Supplementary Figure 19.
High resolution mass spectrum for **6**.



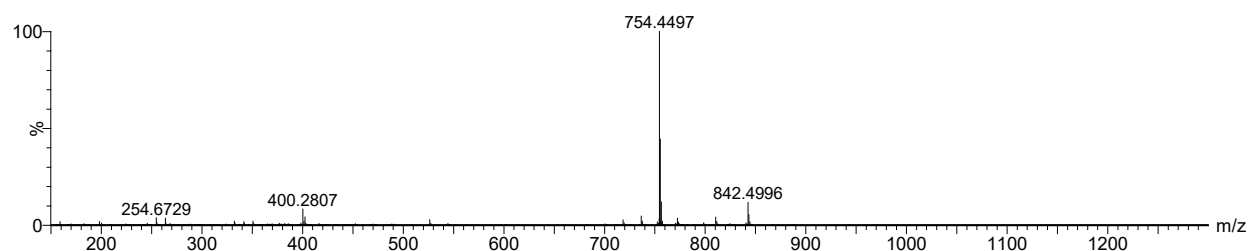
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High resolution mass spectrum for **7**.



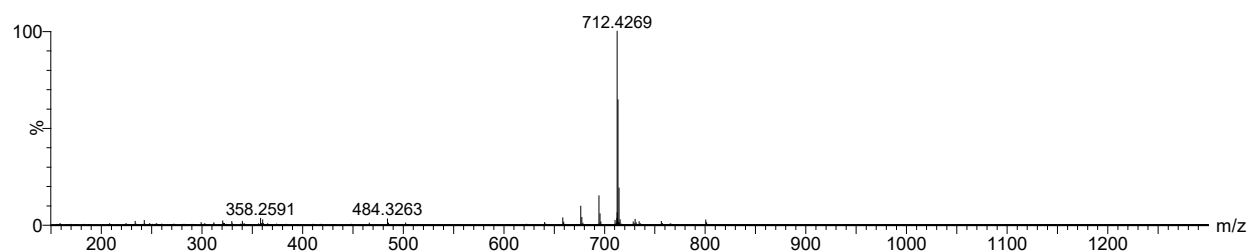
Supplementary Figure 21.
High resolution mass spectrum for tetrafibricin.



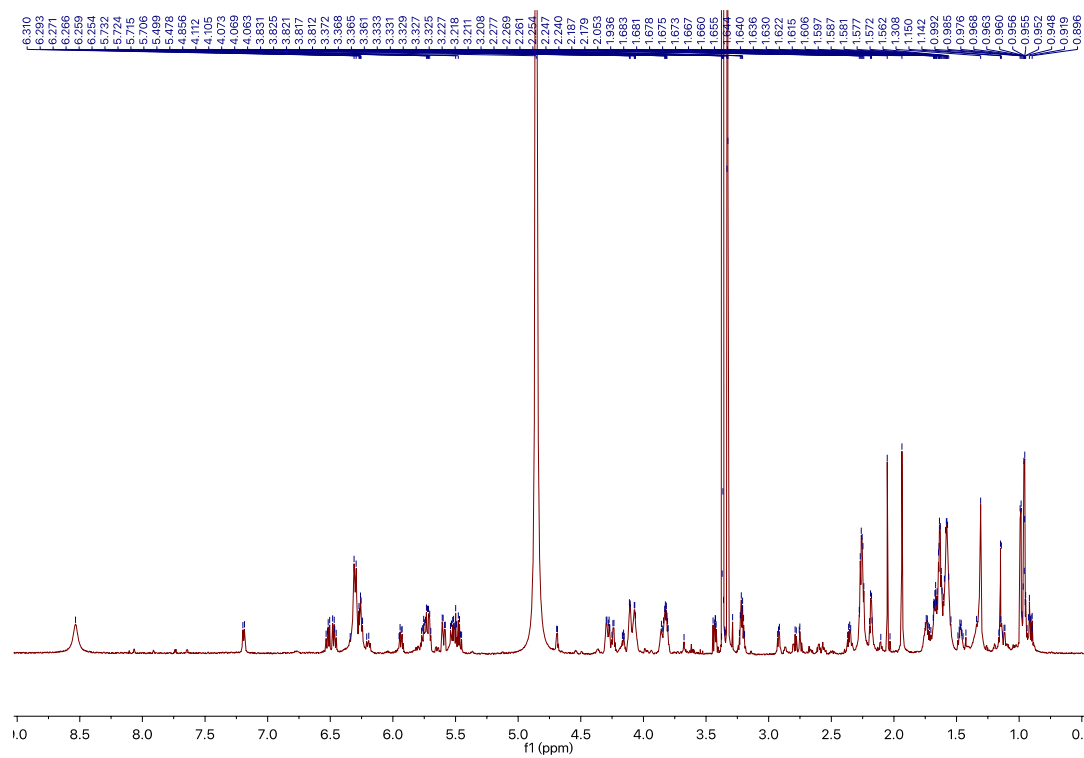
Supplementary Figure 22.
High resolution mass spectrum for **8**.



Supplementary Figure 23.
High resolution mass spectrum for **9**.

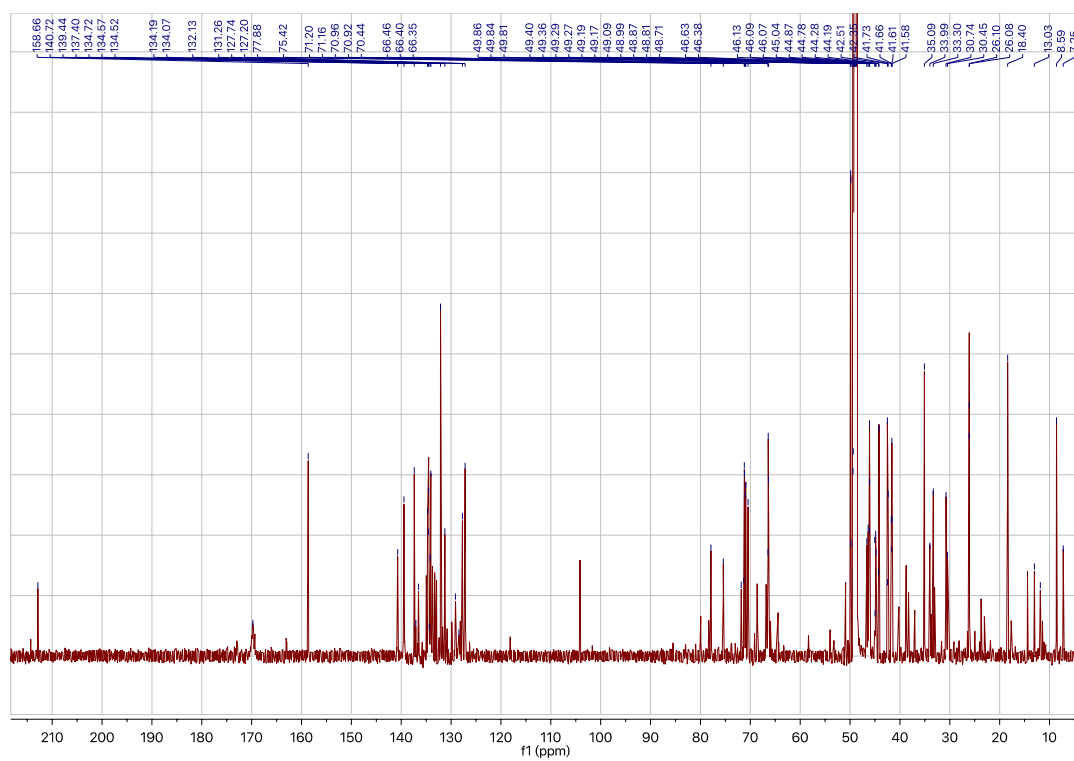


Supplementary Figure 24.
High resolution mass spectrum for **10**.

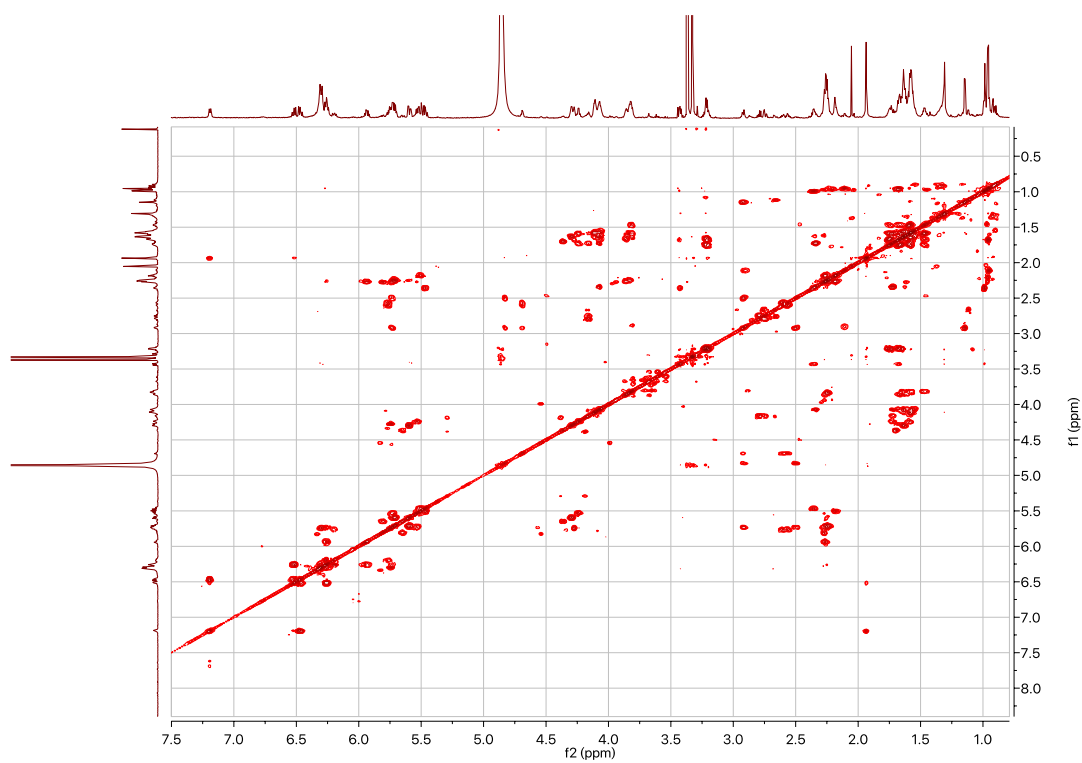


Supplementary Figure 25.

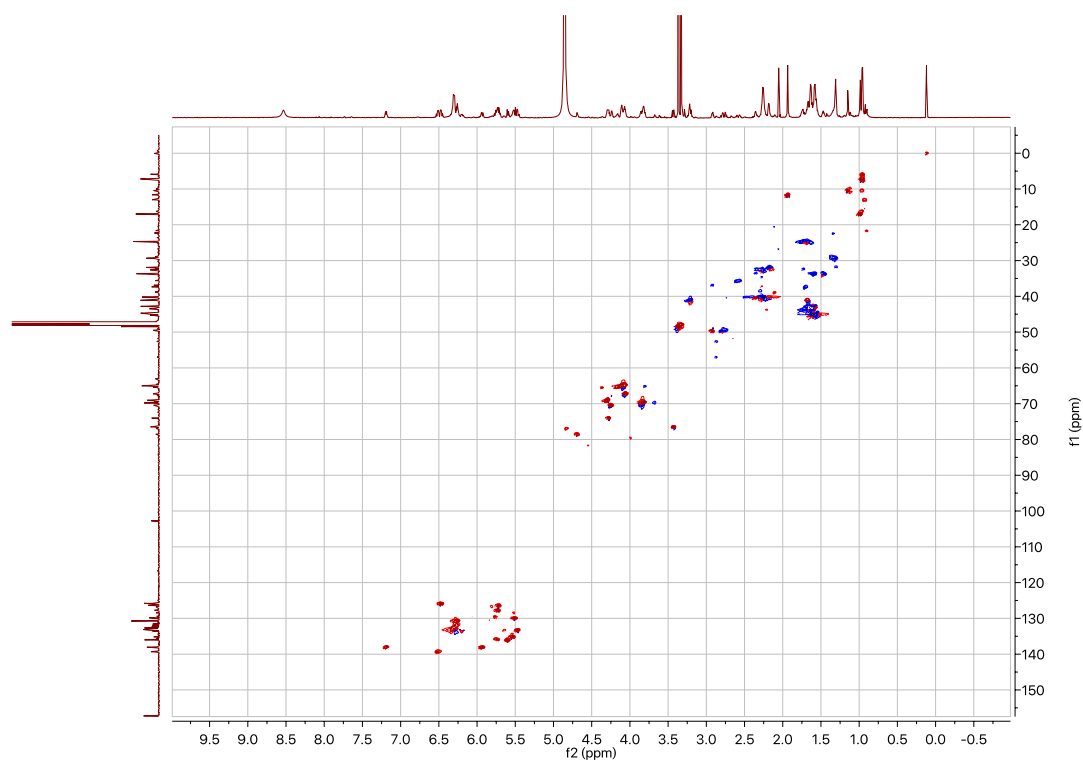
^1H NMR spectrum (methanol- d_4 , 900 MHz) for **1b**



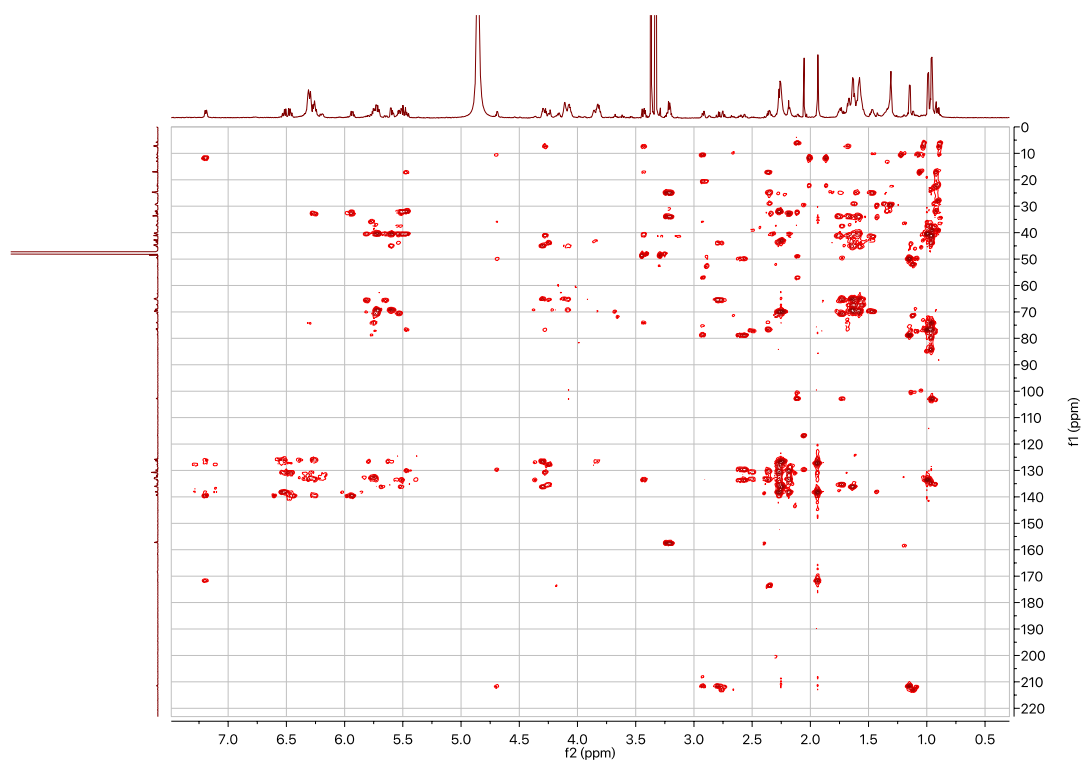
Supplementary Figure 26.
 ^{13}C NMR spectrum (methanol- d_4 , 225 MHz) for **1b**



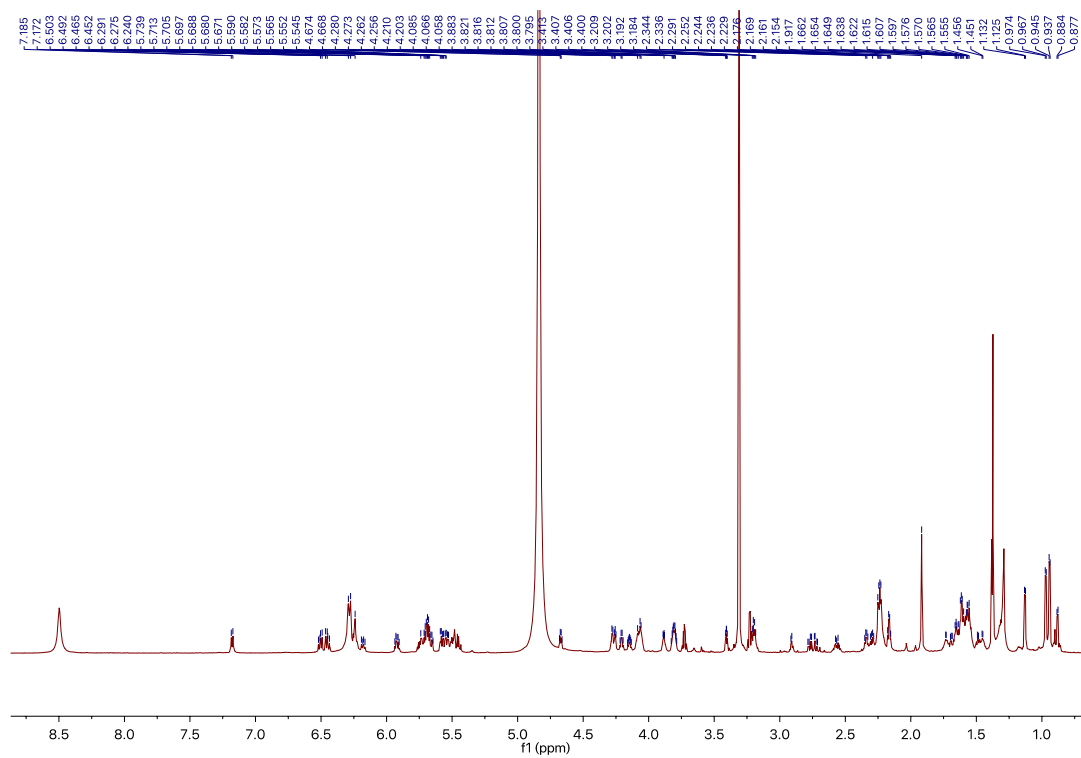
Supplementary Figure 27.
COSY spectrum (methanol- d_4 , 900 MHz) for **1b**



Supplementary Figure 28.
HSQC NMR spectrum (methanol- d_4 , 225 MHz) for **1b**

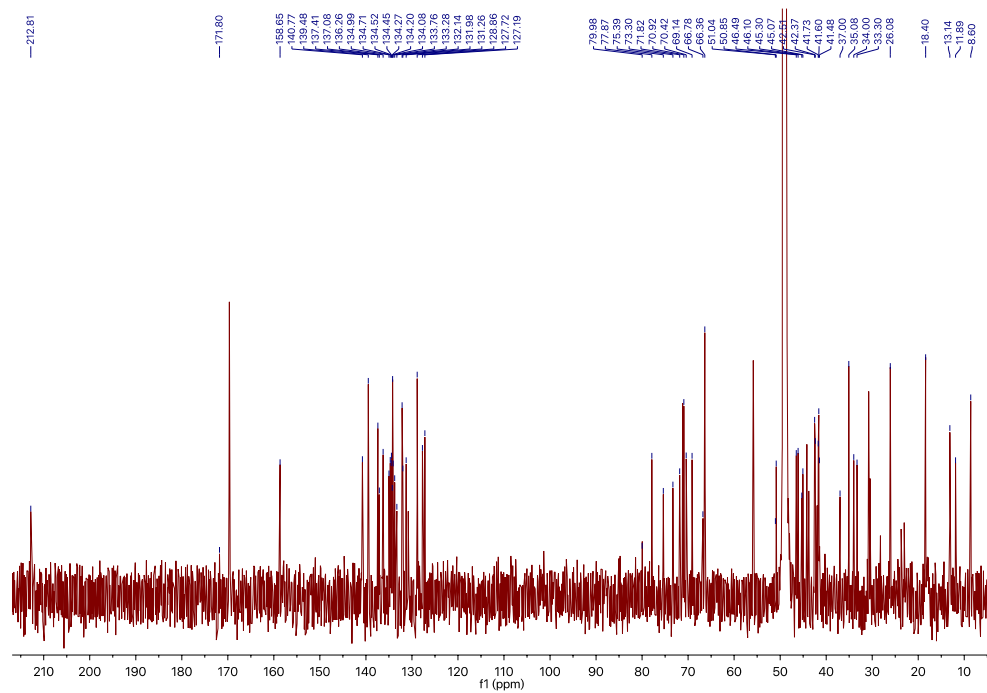


Supplementary Figure 29.
HMBC NMR spectrum (methanol-*d*₄, 225 MHz) for **1b**



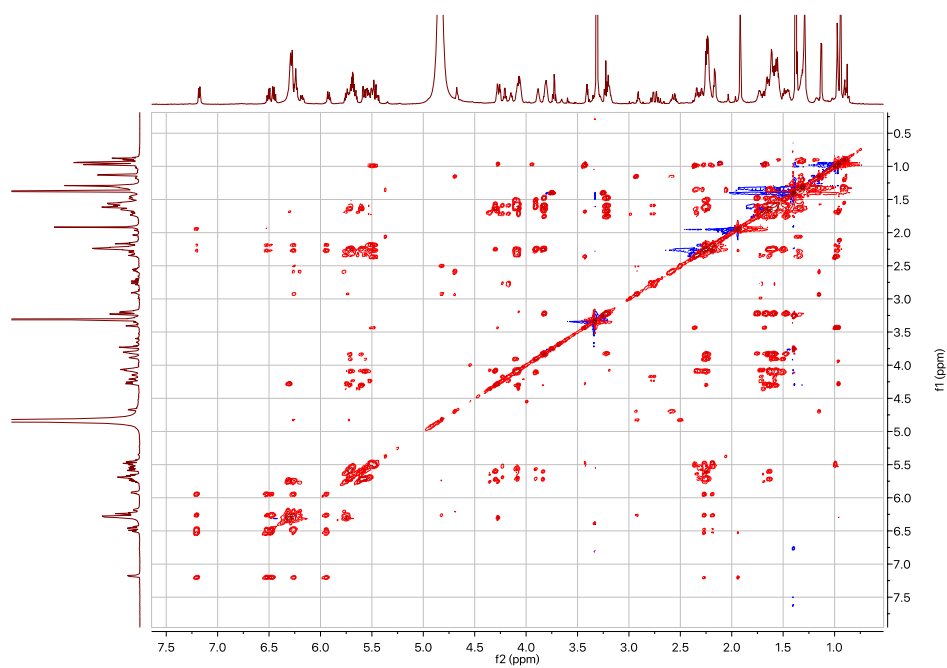
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^1H NMR spectrum ($\text{methanol-}d_4$, 900 MHz) for **2b**

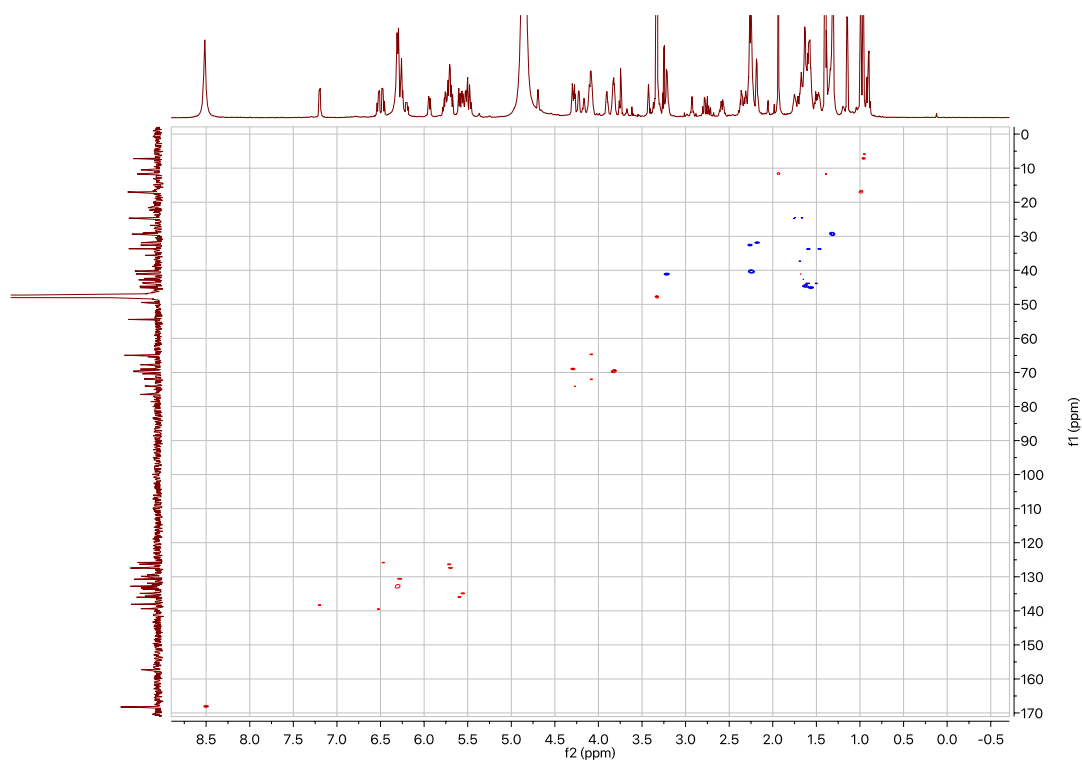


Supplementary Figure 31.

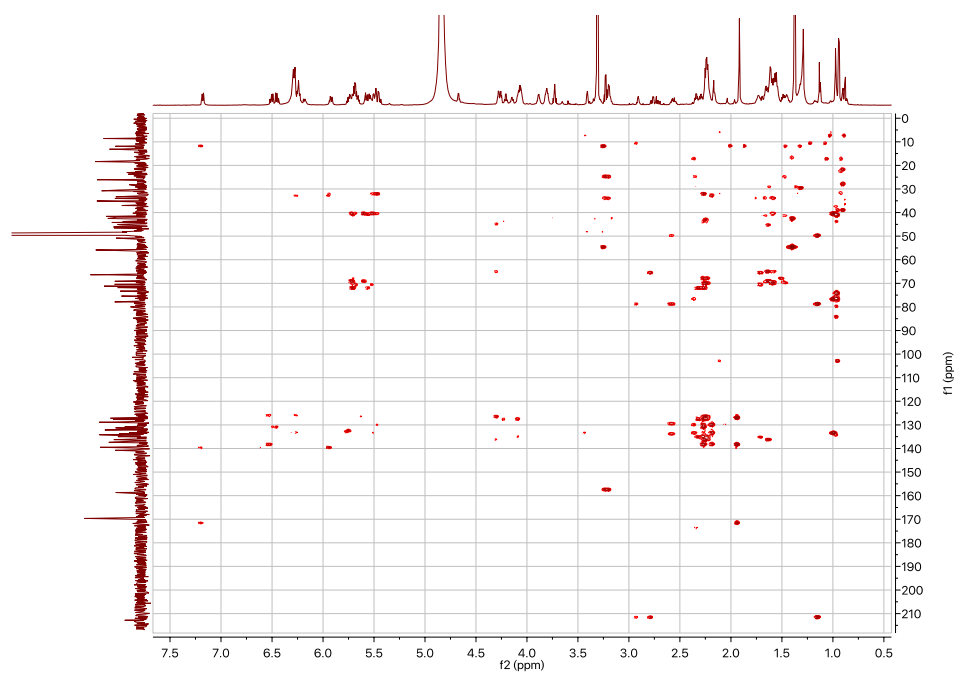
^{13}C NMR spectrum (methanol- d_4 , 225 MHz) for **2b**



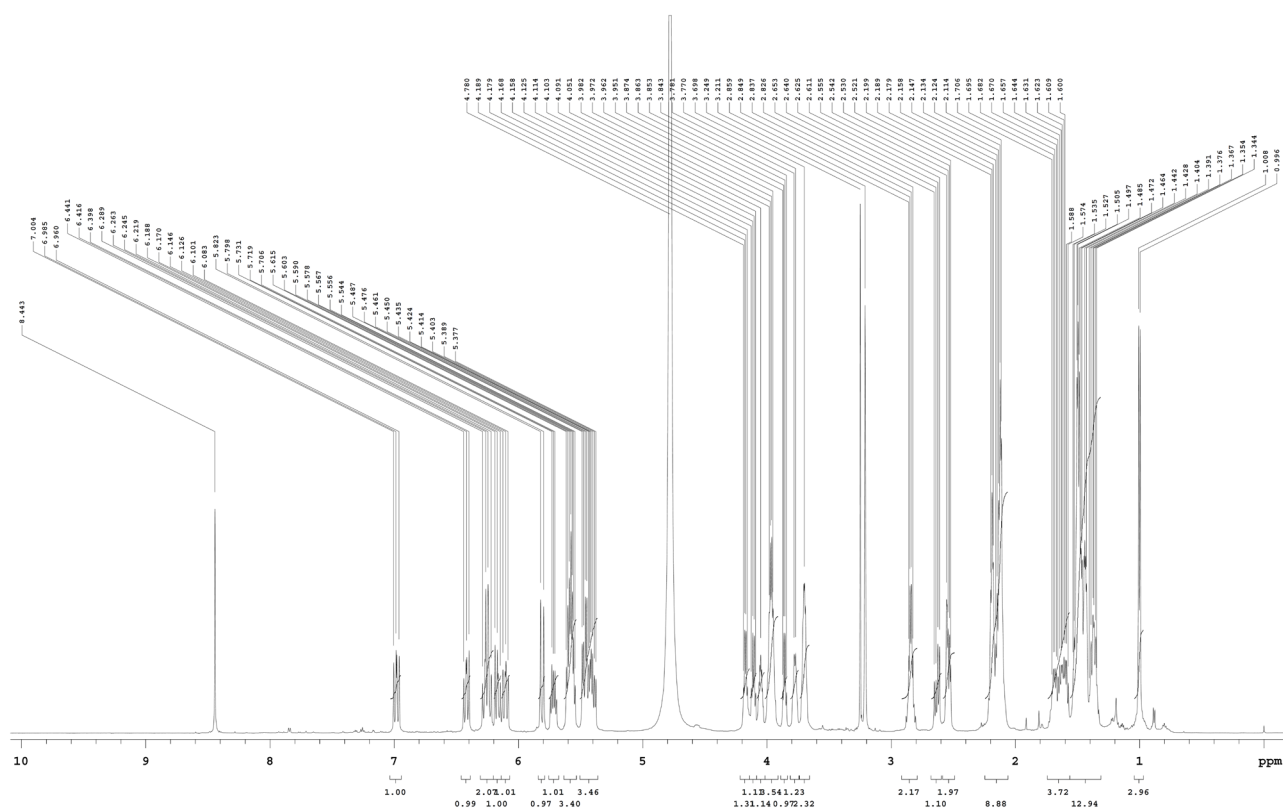
Supplementary Figure 32.
COSY spectrum (methanol-*d*₄, 900 MHz) for **2b**



Supplementary Figure 33.
HSQC spectrum (methanol- d_4 , 225 MHz) for **2b**

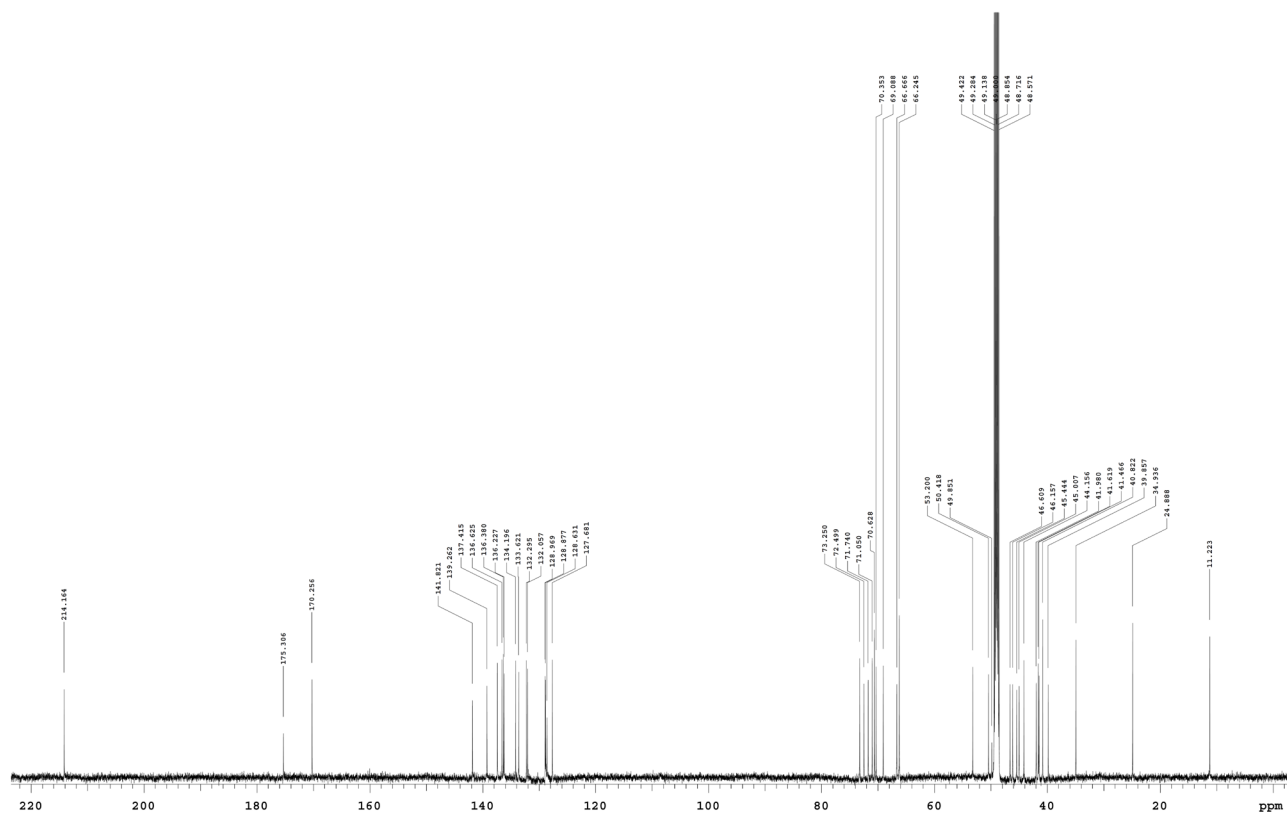


Supplementary Figure 34.
HMBC spectrum (methanol-*d*₄, 225 MHz) for **2b**

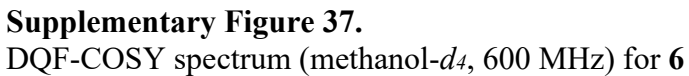


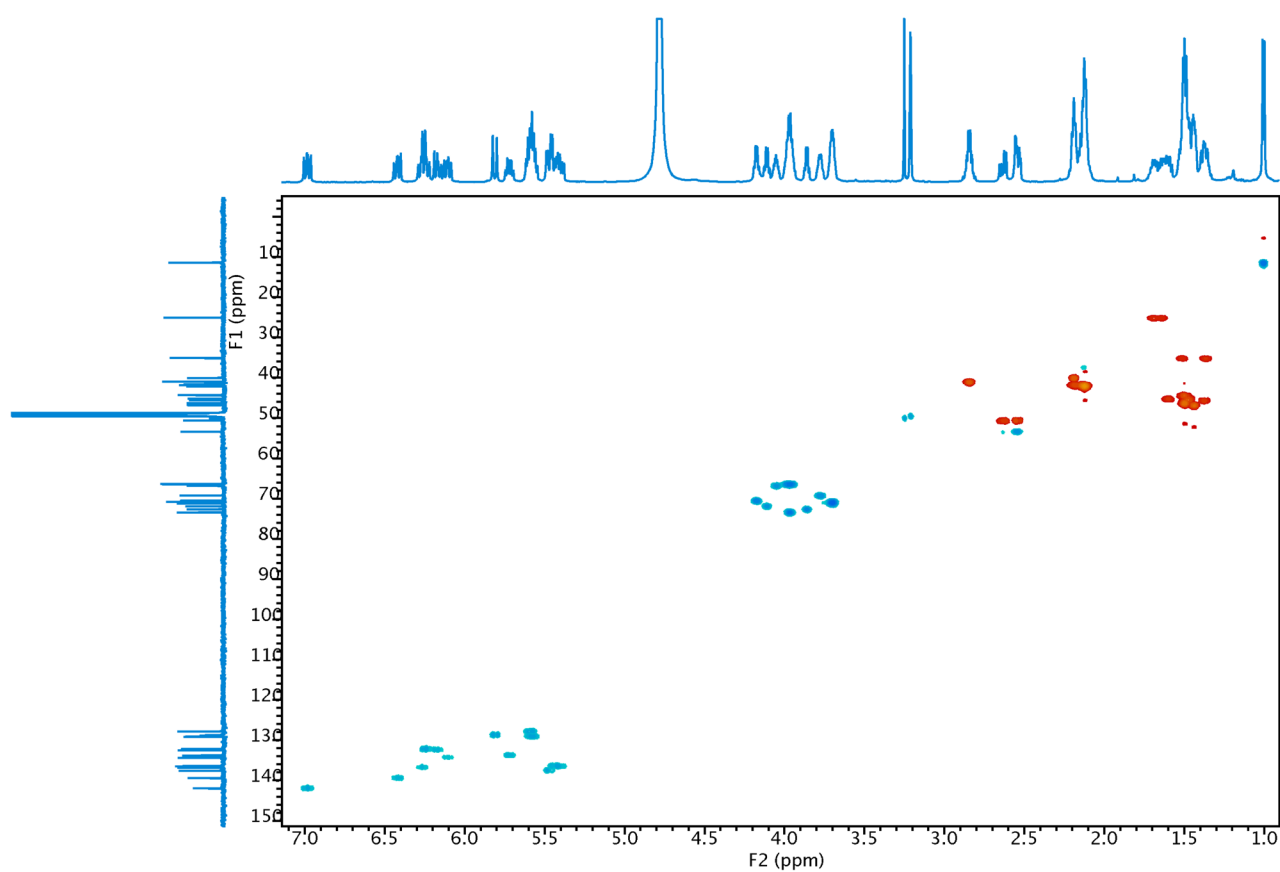
Supplementary Figure 35.

^1H NMR spectrum ($\text{methanol-}d_4$, 600 MHz) for **6**

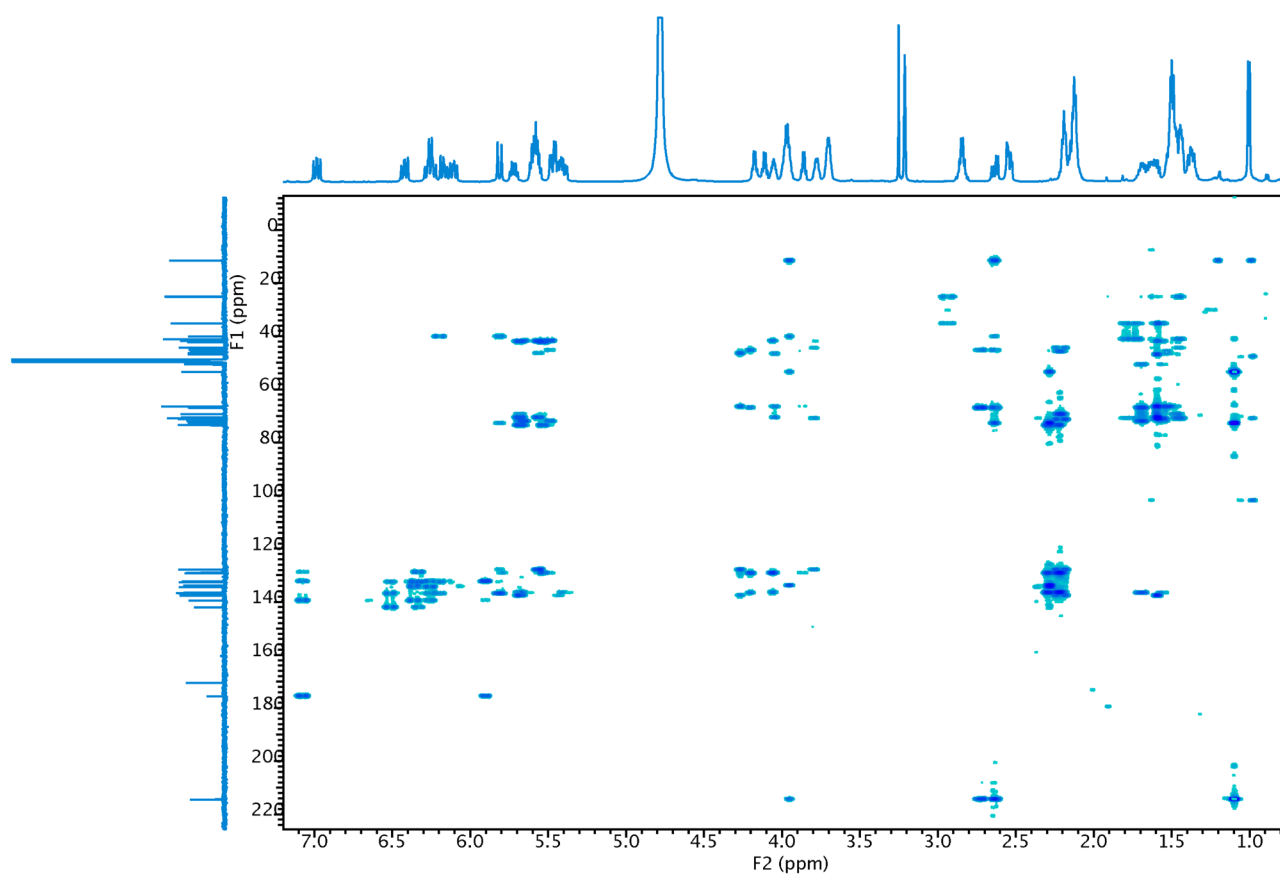


Supplementary Figure 36.
 ^{13}C NMR spectrum (methanol- d_4 , 150 MHz) for **6**

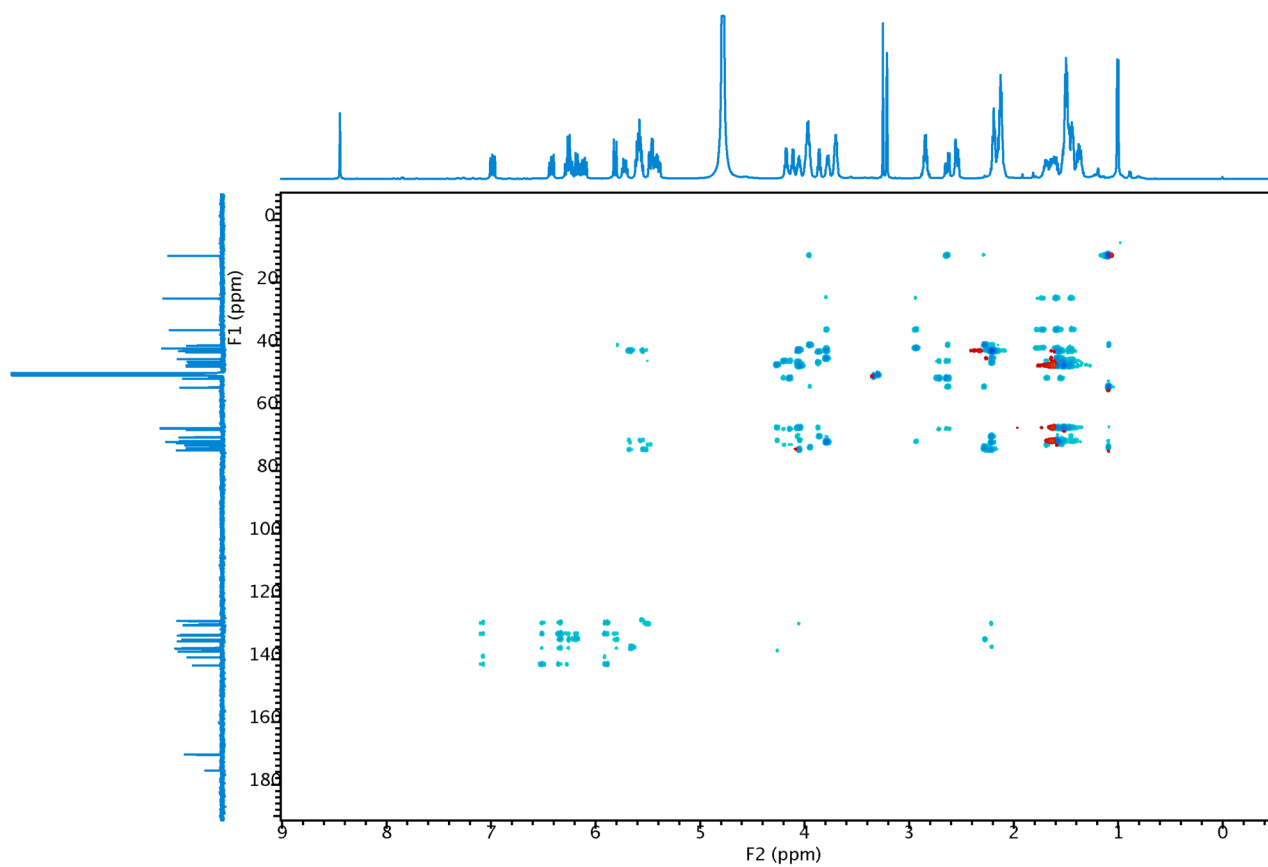




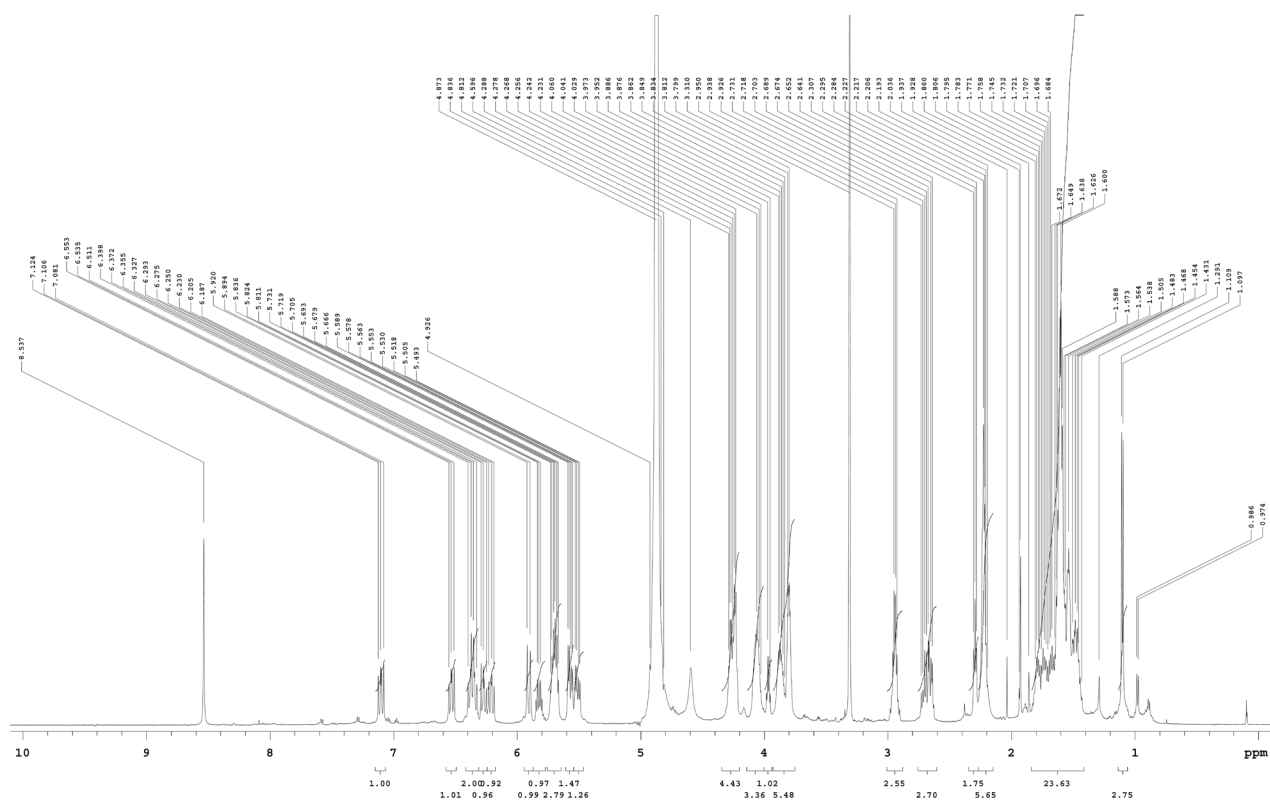
Supplementary Figure 38.
HSQCAD spectrum (methanol- d_4 , 600 MHz) for **6**



Supplementary Figure 39.
HMBCAD spectrum (methanol-*d*₄, 600 MHz) for **6**

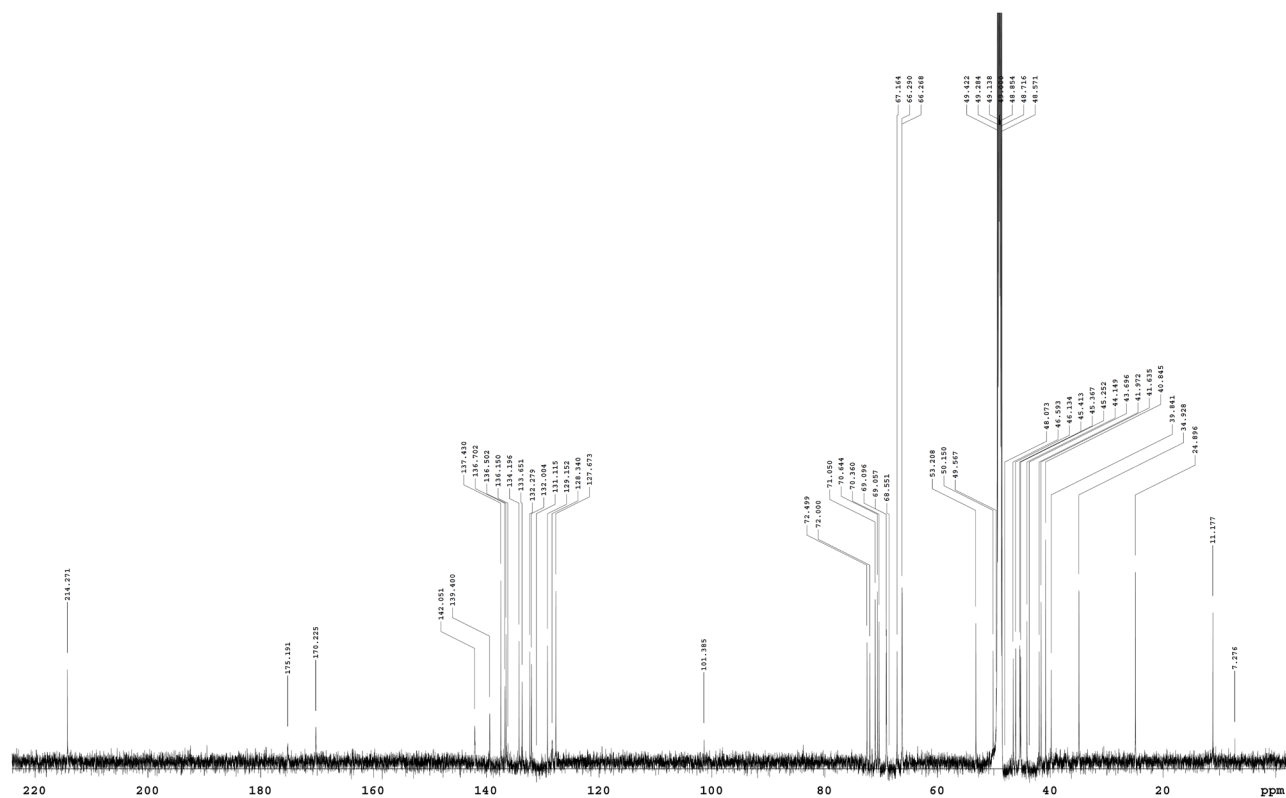


Supplementary Figure 40.
HSQC-TOCSY spectrum (methanol- d_4 , 600 MHz) for **6**

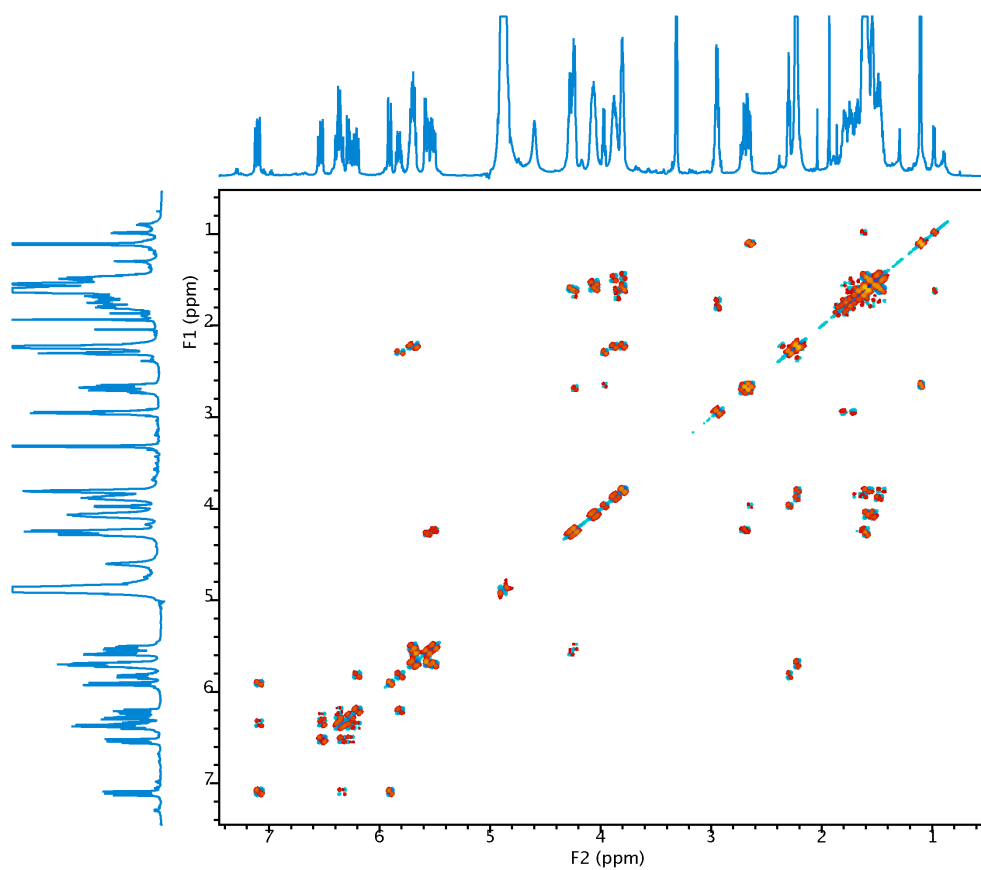


Supplementary Figure 41.

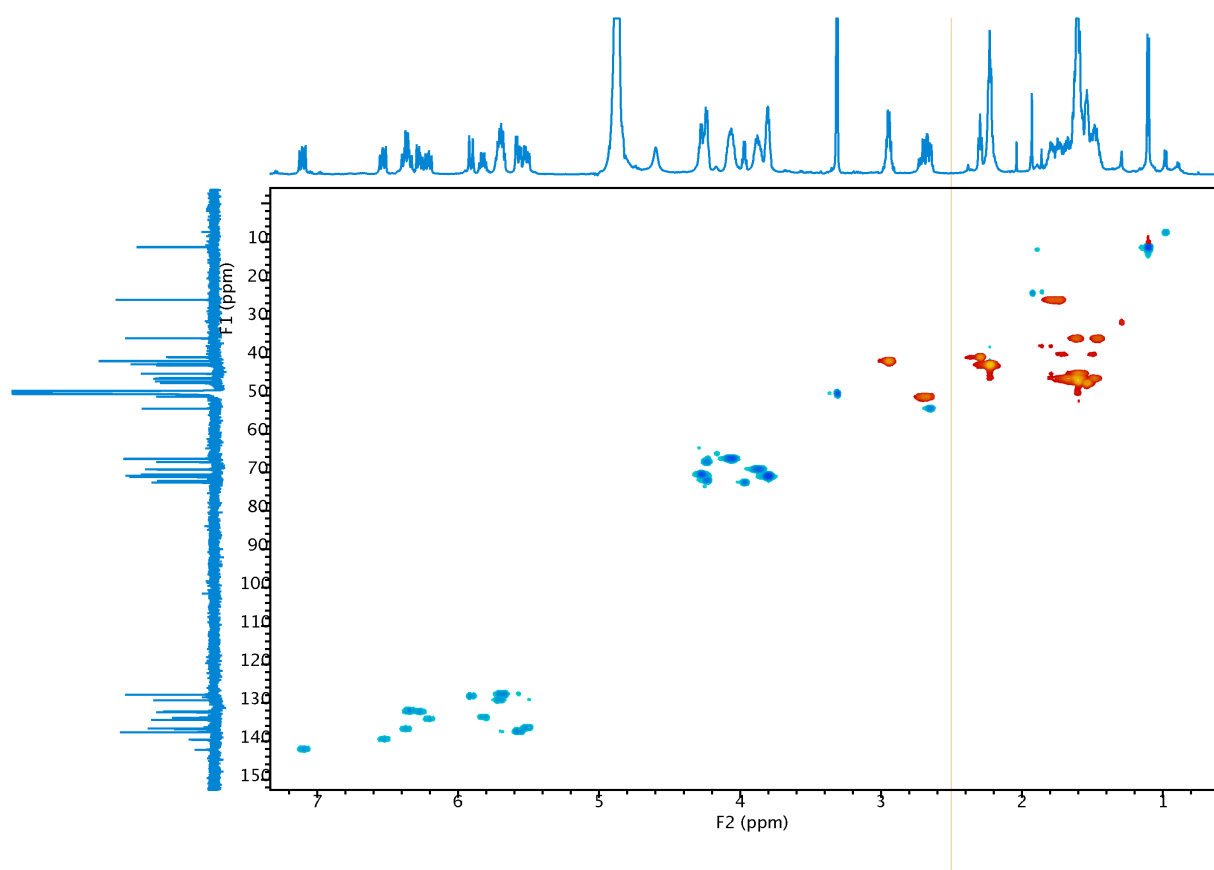
^1H NMR spectrum ($\text{methanol-}d_4$, 600 MHz) for **7**



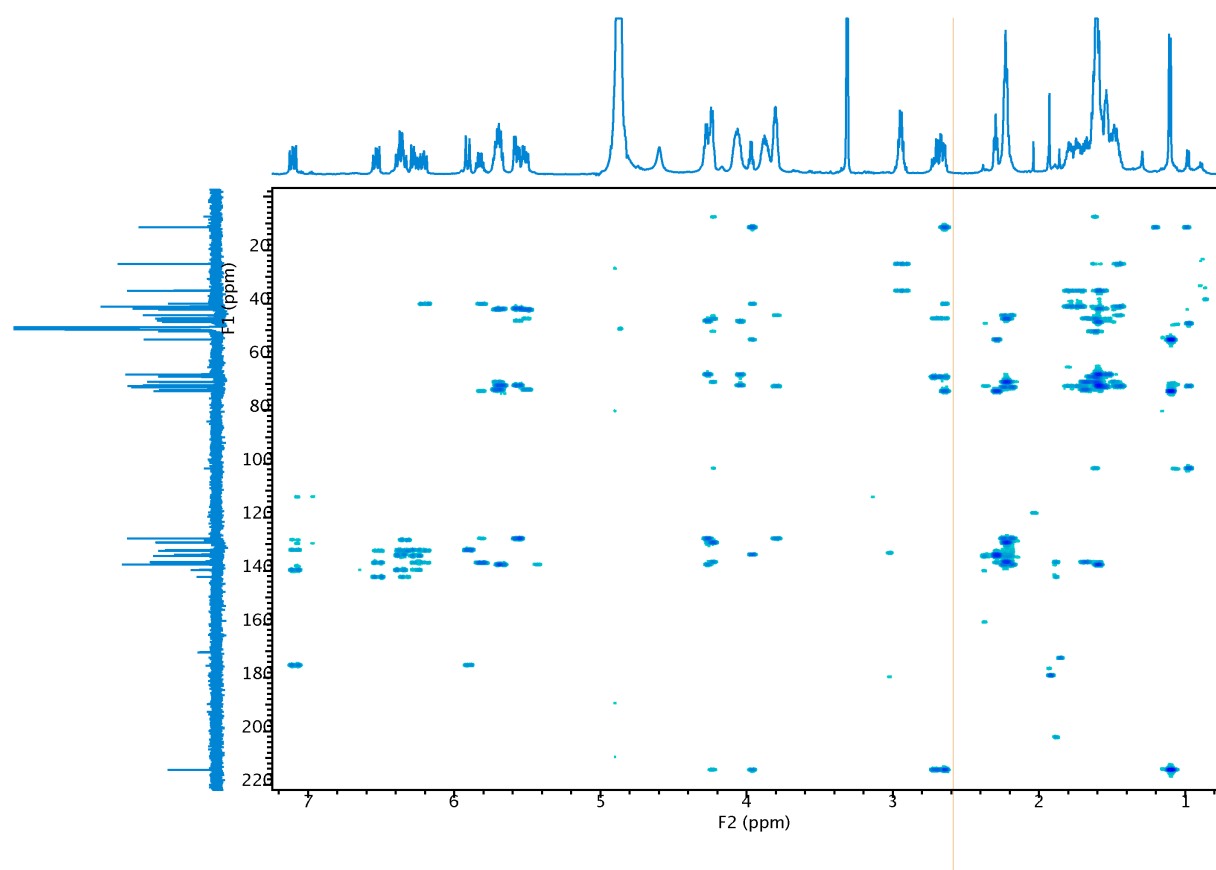
Supplementary Figure 42.
 ^{13}C NMR spectrum (methanol- d_4 , 150 MHz) for **7**



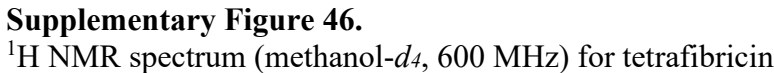
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DQF-COSY spectrum (methanol- d_4 , 600 MHz) for **7**

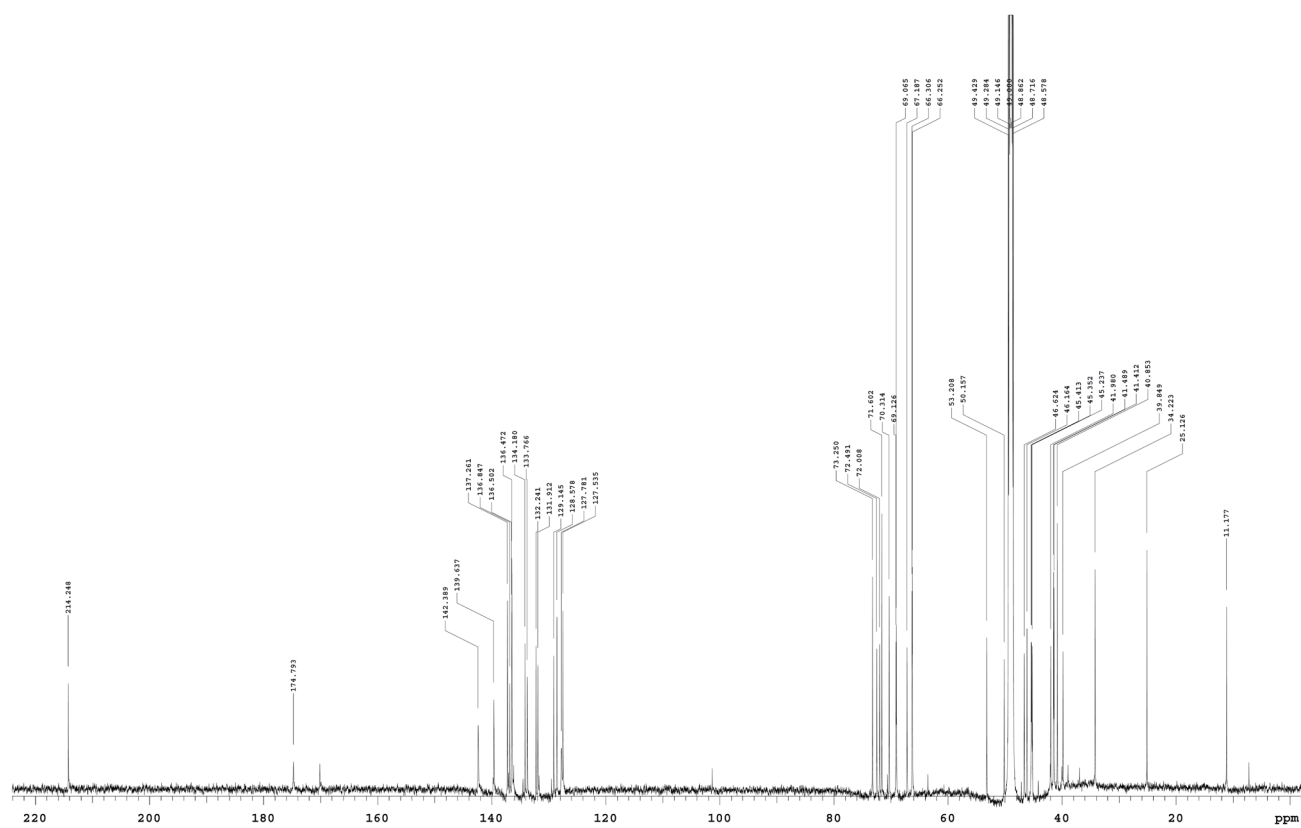


Supplementary Figure 44.
HSQCAD spectrum (methanol- d_4 , 600 MHz) for **7**



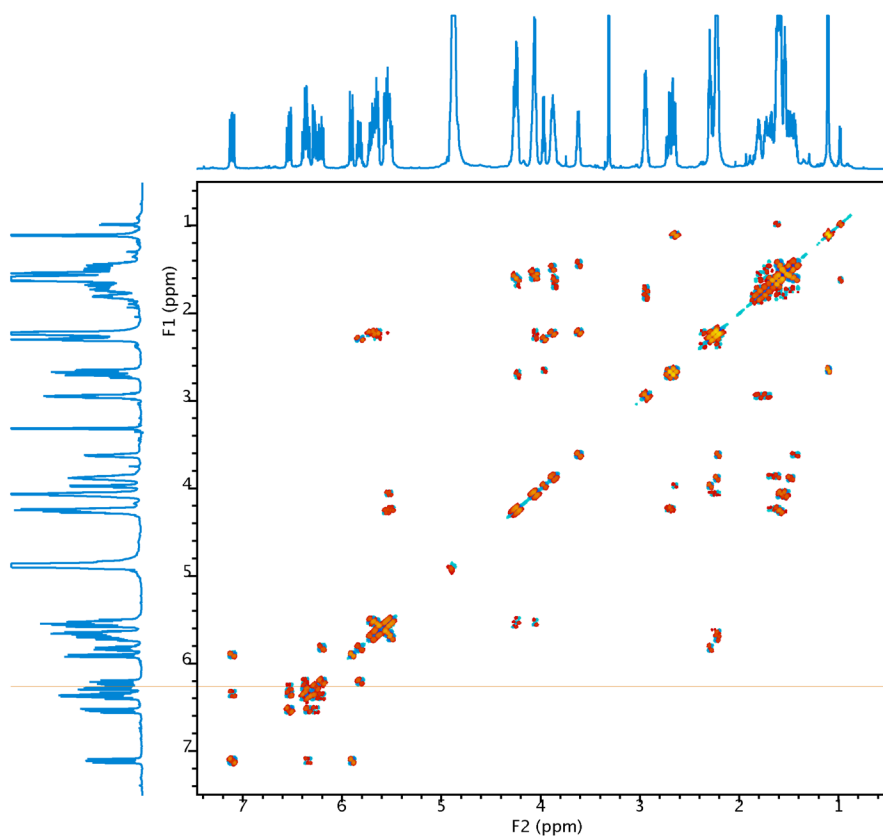
Supplementary Figure 45.
HMBCAD spectrum (methanol-*d*₄, 600 MHz) for **7**



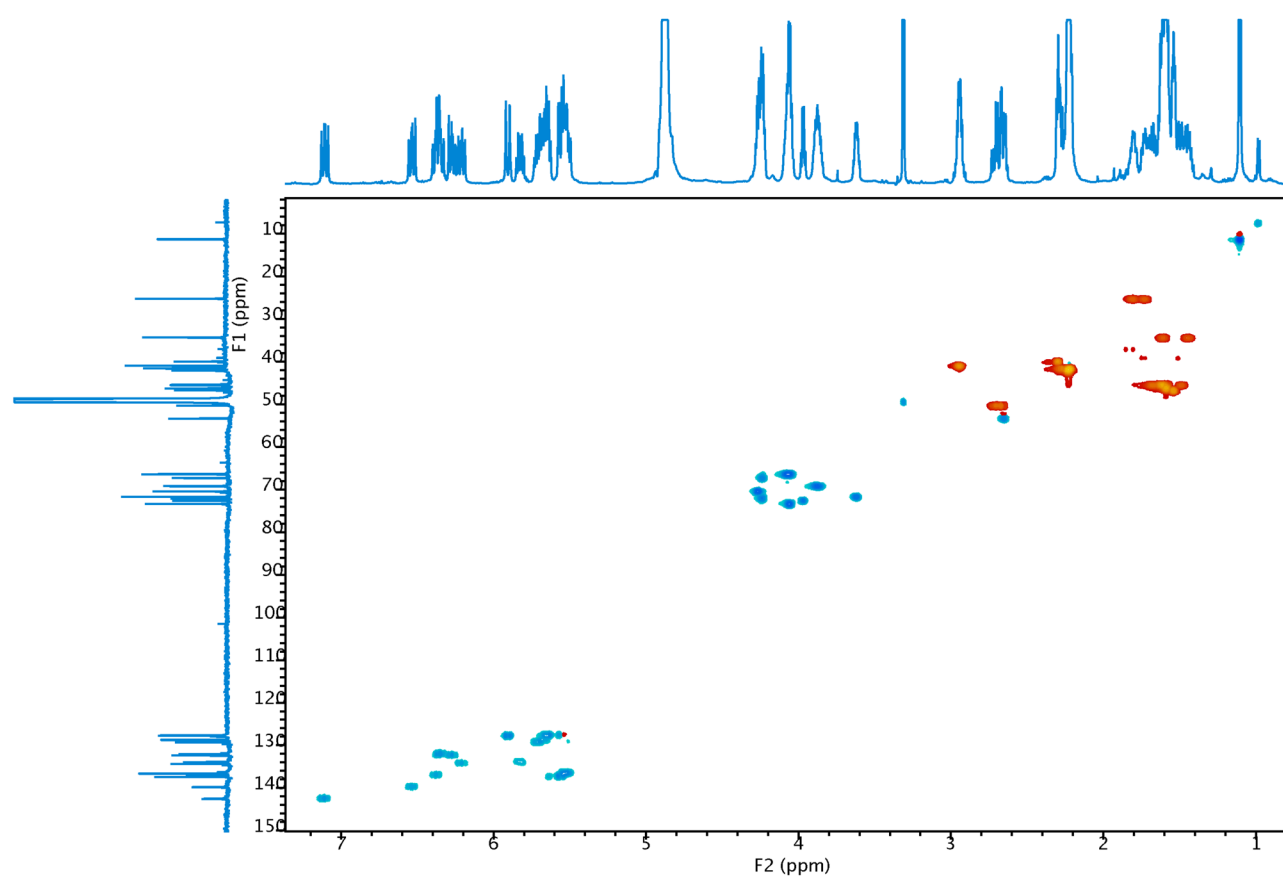


Supplementary Figure 47.

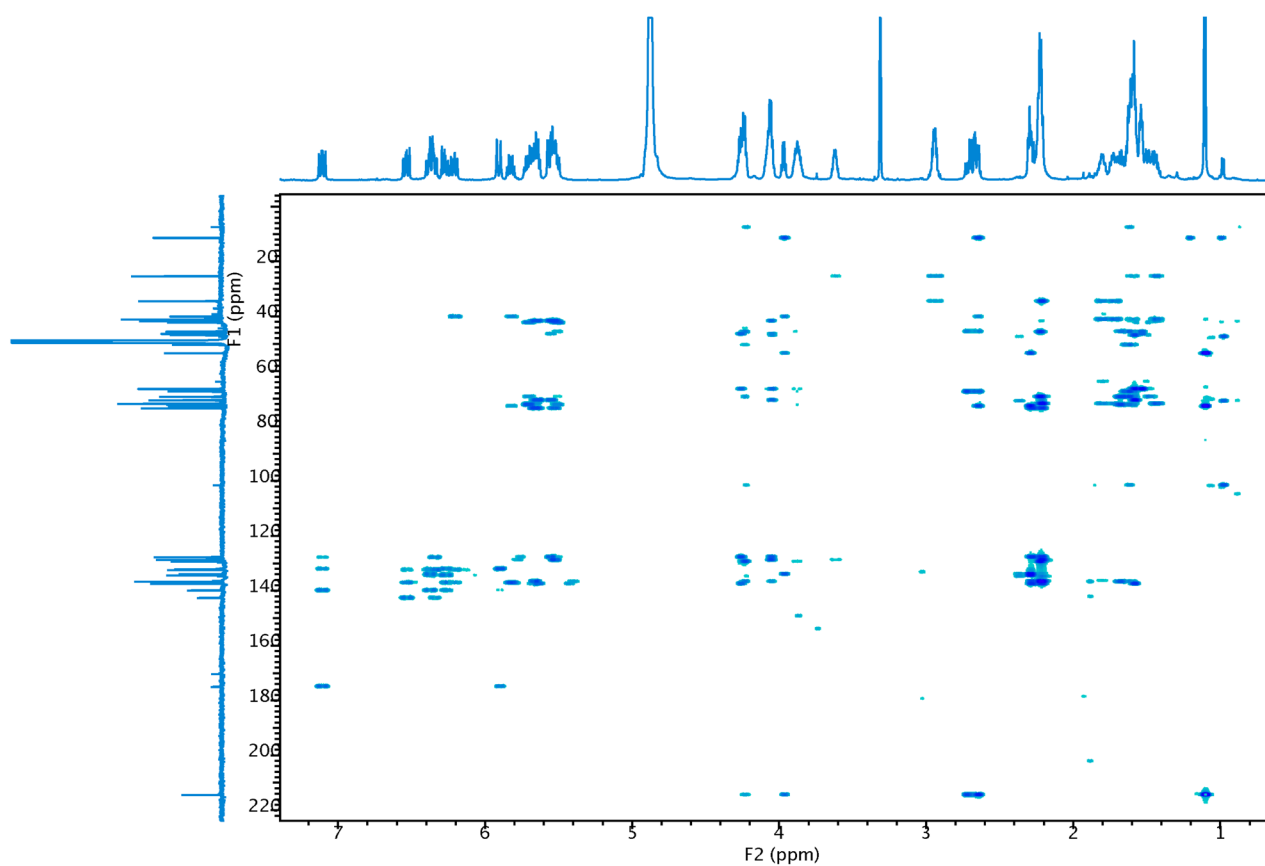
¹³C NMR spectrum (methanol-*d*₄, 150 MHz) for tetrafibricin



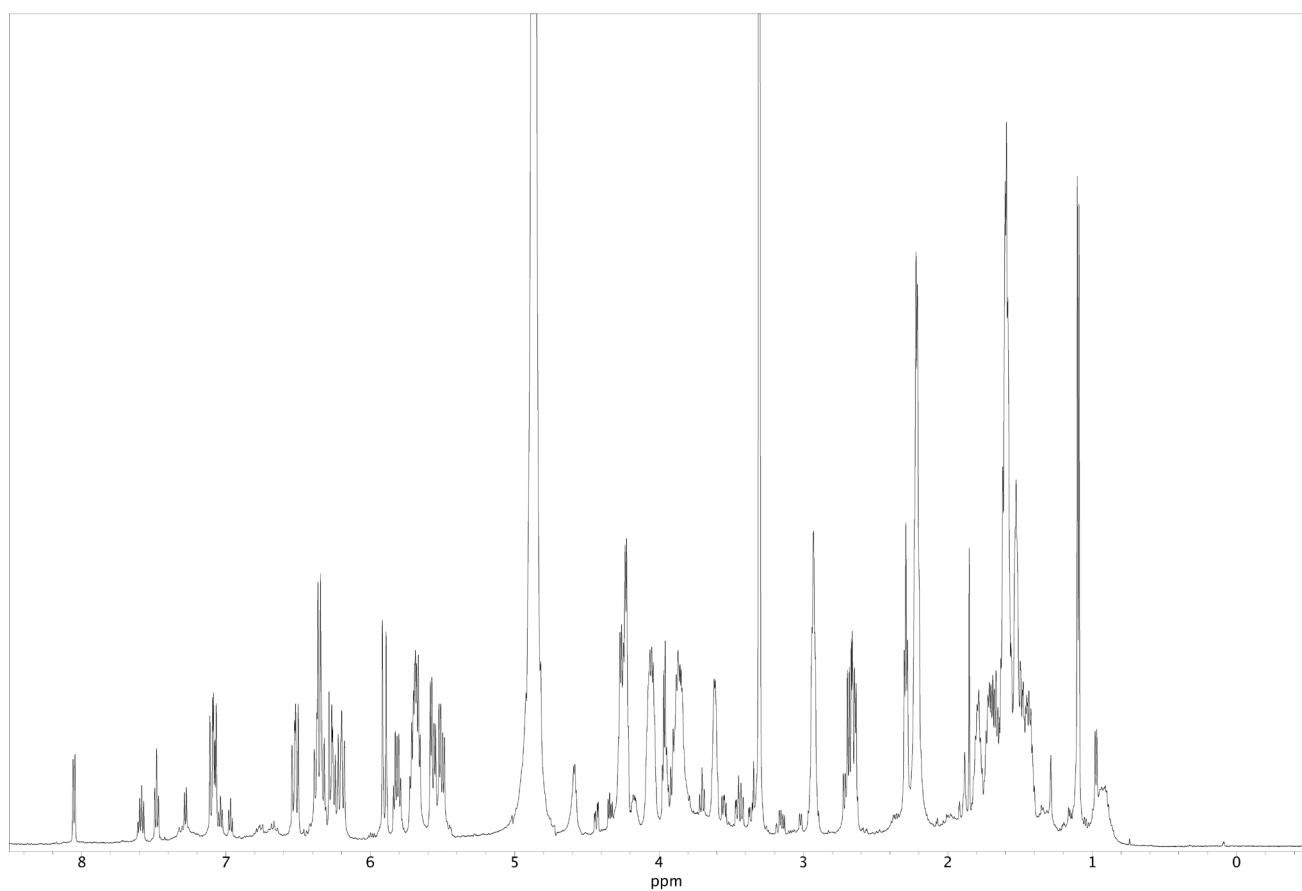
Supplementary Figure 48.
DQF-COSY spectrum (methanol-*d*₄, 600 MHz) for tetrafibricin



Supplementary Figure 49.
HSQCAD spectrum (methanol- d_4 , 600 MHz) for tetrafibricin

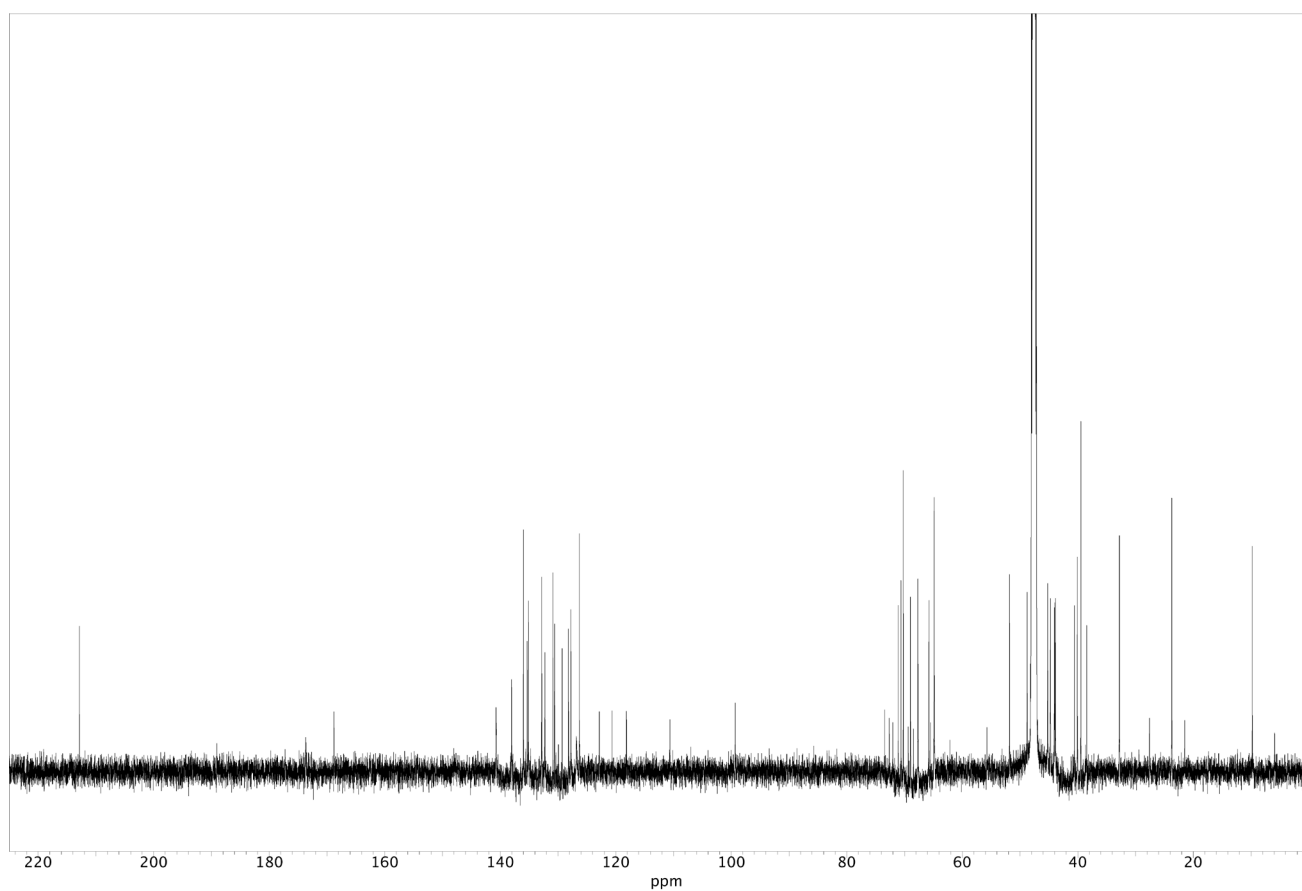


Supplementary Figure 50.
HMBCAD spectrum (methanol-*d*₄, 600 MHz) for tetrafibricin



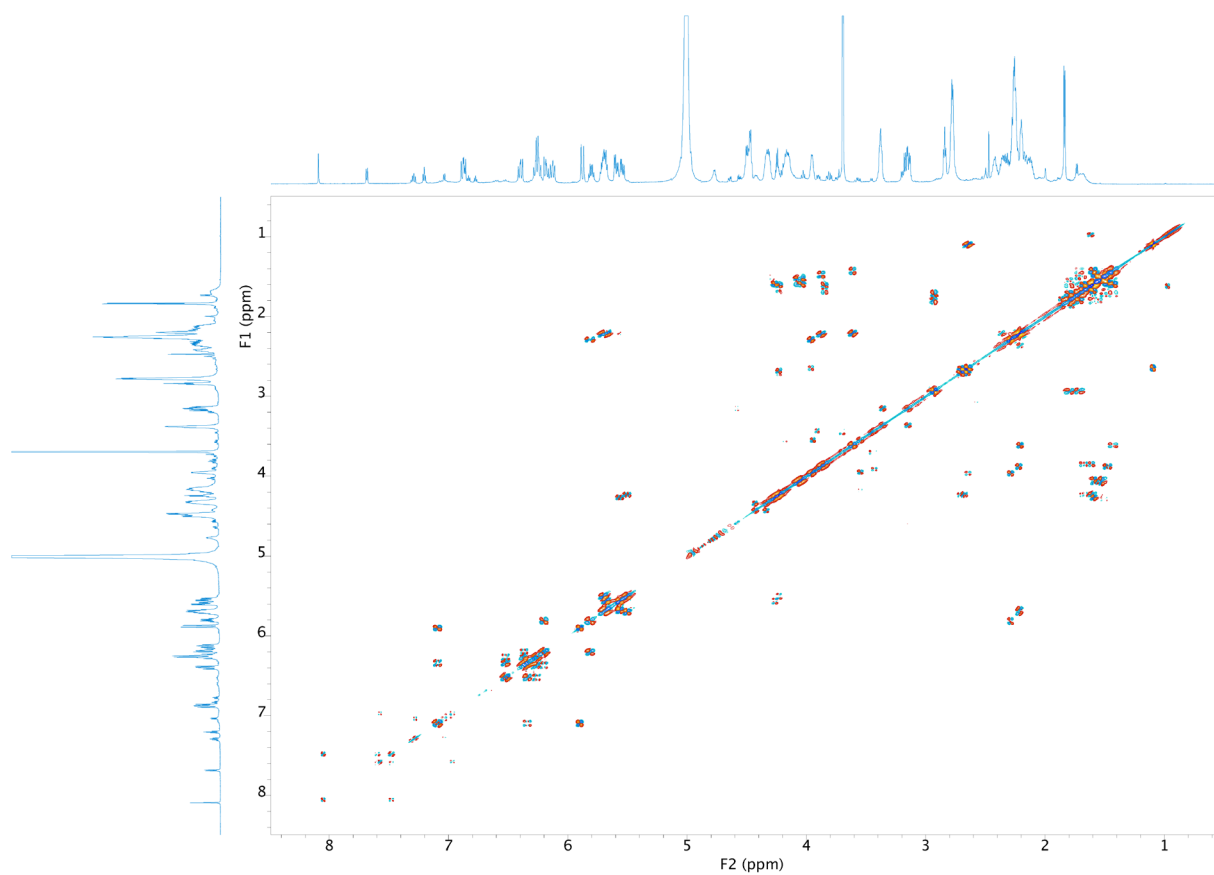
Supplementary Figure 51.

^1H NMR spectrum (methanol- d_4 , 600 MHz) for **10**

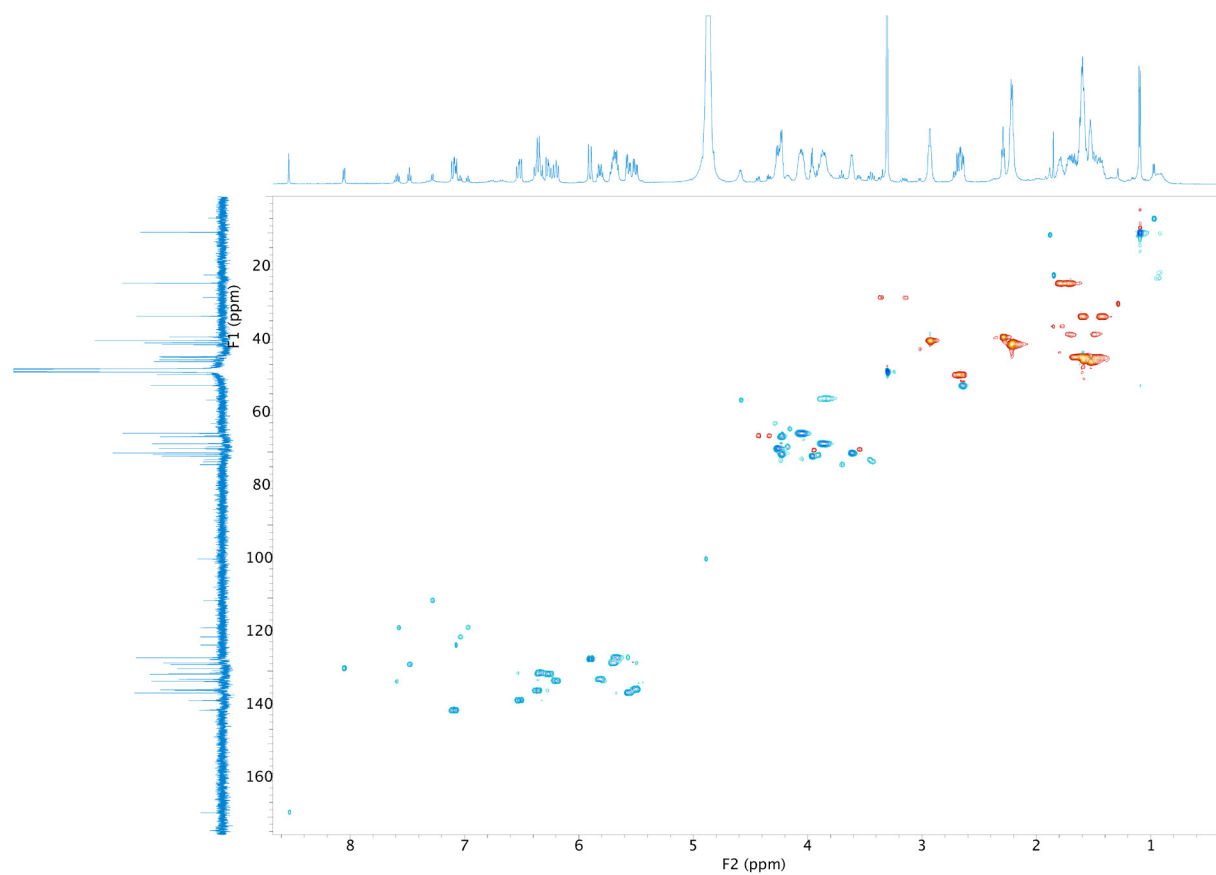


Supplementary Figure 52.

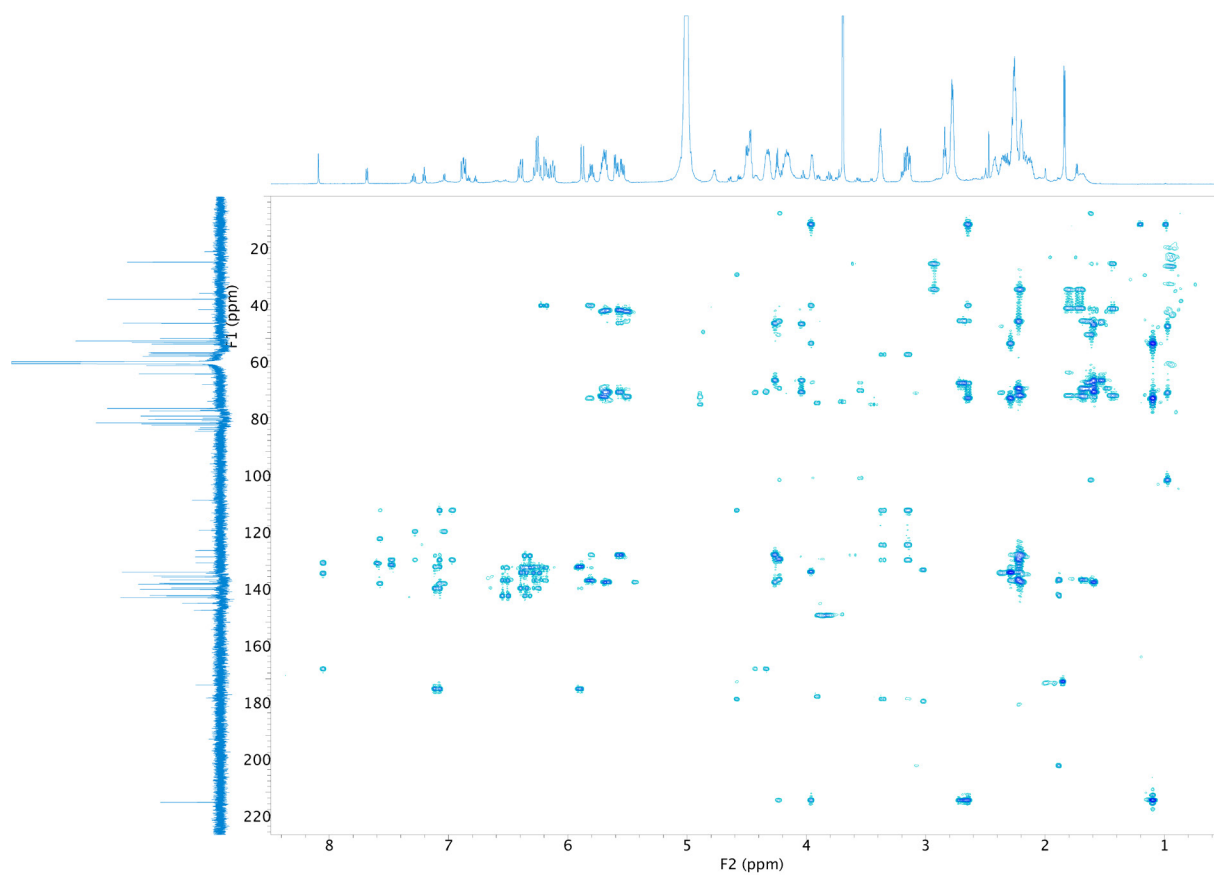
^{13}C NMR spectrum (methanol- d_4 , 150 MHz) for **10**



Supplementary Figure 53.
DQF-COSY spectrum (methanol- d_4 , 600 MHz) for **10**



Supplementary Figure 54.
HSQCAD spectrum (methanol- d_4 , 600 MHz) for **10**



Supplementary Figure 55.
HMBCAD spectrum (methanol- d_4 , 600 MHz) for **10**

Supplementary Table 1.

HR-MS analyses of clethramycin derivatives.

Compound name	formula	adduct	calculated	observed	error (ppm)
Clethramycin	C ₆₃ H ₉₉ N ₃ O ₁₈ S	Na	1240.6537	1240.6532	-0.4
desulfo-clethramycin	C ₆₃ H ₉₉ N ₃ O ₁₅	H	1138.7149	1138.7173	2.1
1a	C ₆₃ H ₁₀₁ N ₃ O ₁₉ S	Na	1258.6642	1258.6643	0.1
1b	C ₆₃ H ₁₀₁ N ₃ O ₁₆	H	1156.7255	1156.7264	0.8
2a	C ₆₃ H ₉₉ N ₃ O ₁₈ S	Na	1240.6537	1240.6539	0.2
2b	C ₆₃ H ₉₉ N ₃ O ₁₅	H	1138.7149	1138.7155	0.5
3	C ₄₂ H ₆₉ N ₃ O ₁₃	H	824.4903	824.4915	1.5
4	C ₄₀ H ₆₇ N ₃ O ₁₃	H	798.4747	798.4769	2.8
5	C ₄₂ H ₆₉ N ₃ O ₁₃	H	824.4903	824.4912	1.1
6	C ₄₁ H ₆₇ NO ₁₃	H	782.4685	782.4706	2.7
7	C ₃₉ H ₆₅ NO ₁₃	H	756.4529	756.4532	0.4
Tetrafibricin	C ₄₁ H ₆₇ NO ₁₃	H	782.4685	782.4683	-0.3
8	C ₆₃ H ₉₇ N ₃ O ₁₇ S	Na	1222.6431	1222.6432	0.1
9	C ₃₈ H ₆₃ N ₃ O ₁₂	H	754.4485	754.4497	1.6
10	C ₃₇ H ₆₁ NO ₁₂	H	712.4267	712.4269	0.3

Supplementary Table 2.NMR data for **1b** in methanol-*d*₄ at 900 MHz for ¹H and 225 MHz for ¹³C

Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)	Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)
1	171.7	-	33	66.8	4.16 (m)
2	128.6	-	34	26.4	1.73 (m), ovl 1.59 (m), ovl
3	140.7	7.17 (d, 11.7)	35	71.8	4.24 (dd, 14.4, 7.2)
4	127.3	6.47 (dd, 14.9, 11.7)	36	136.5	5.53 (m), ovl
5	140.9	6.52 (dd, 14.9, 10.7)	37	129.1	5.73 (m), ovl
6	132.1	6.26 (m), ovl	38	42.5	2.25 (m)
7	139.4	5.93 (dt, 14.6, 7.0)	39	70.4	3.83 (m)
8	34.1	2.27 (m)	40	46.6	1.58 (m), ovl
9	33.3	2.18 (m)	41	68.5	4.07 (m), ovl
10	131.2	5.50 (dt, 15.1, 8.0)	42	46.6	1.58 (m), ovl
11	134.7	5.45 (dt, 8.2, 15.3)	43	68.5	4.07 (m), ovl
12	41.8	2.33 (m)	44	46.6	1.58 (m), ovl
13	78.1	3.41 (dd, 6.4, 5.0)	45	68.5	4.07 (m), ovl
14	42.5	1.68 (m), ovl	46	46.6	1.58 (m), ovl
15	75.4	4.27 (m)	47	68.5	4.07 (m), ovl
16	137.1	5.74 (m), ovl	48	46.4	1.64 (m), ovl
17	132.0	6.28 (m), ovl	49	70.6	4.30 (m), ovl
18	134.1	6.29 (m), ovl	50	137.6	5.58 (dd, 15.5, 6.6)
19	134.3	6.28 (m), ovl	51	127.2	5.72 (m), ovl
20	133.8	6.27 (m), ovl	52	41.7	2.27 (m), ovl
21	134.2	6.27 (m), ovl	53	72.0	3.83 (m)
22	134.5	6.27 (m), ovl	54	41.8	2.25 (m)
23	134.5	6.27 (m), ovl	55	71.0	3.82 (m)
24	134.6	6.27 (m), ovl	56	35.0	1.59 (m), ovl 1.47 (m)
25	133.3	6.27 (m), ovl	57	26.3	1.67 (m) 1.76 (m)
26	132.7	6.27 (m), ovl	58	42.6	3.21 (m)
27	130.9	5.76 (m), ovl	59	13.5	1.93 (s)
28	37.0	2.54 (m) 2.59 (m)	60	18.4	0.97 (d, 6.8)
29	80.0	4.67 (dd, 12.6, 5.4)	61	8.5	0.96 (d, 7.2)
30	51.1	2.92 (dq, 6.8, 6.8)	62	11.8	1.13 (d, 7.2)
31	213.0	-	63	158.7	-
32	51.0	2.78 (dd, 17.1, 9.0) 2.73 (dd, 17.1, 4.5)			

ovl: overlapped

Supplementary Table 3.NMR data for **2b** in methanol-*d*₄ at 900 MHz for ¹H and 225 MHz for ¹³C

Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)	Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)
1	171.8	-	33	66.8	4.15 (dq, 4.5)
2	127.7	-	34	45.0	1.71 (m), 1.58 (m)
3	139.5	7.18 (d, 11.7)	35	71.8	4.20 (dd, 13.5, 7.2)
4	127.2	6.45 (dd, 14.4, 11.7)	36	131.3	5.50 (m)
5	140.8	6.51 (dd, 15.3, 10.8)	37	127.7	5.69 (m)
6	132.1	6.24 (m), ovl	38	41.6	2.25 (m)
7	139.5	5.92 (dt, 15.3, 7.2)	39	73.3	4.08 (m)
8	34.0	2.27 (m)	40	136.2	5.54 (dd, 15.3, 6.3)
9	33.3	2.17 (m)	41	128.9	5.68 (m)
10	131.3	5.50 (m)	42	41.6	2.25 (m)
11	134.7	5.46 (d, 16.2, 9.0)	43	69.1	3.90 (m)
12	41.7	2.35 (m)	44	45.3	1.49 (m) 1.59 (m)
13	77.8	3.41 (dd, 6.3, 5.4)	45	66.4	4.08 (m), ovl
14	42.4	1.68 (m)	46	46.5	1.61 (m), ovl
15	75.4	4.26 (m), ovl	47	66.4	4.08 (m), ovl
16	137.1	5.74 (m), ovl	48	46.1	1.61 (m), ovl
17	132.0	6.27 (m), ovl	49	70.4	4.28 (m)
18	134.1	6.29 (m)	50	137.4	5.60 (dd, 15.3, 7.2)
19	134.3	6.27 (m), ovl	51	127.7	5.71 (m)
20	133.8	6.27 (m), ovl	52	41.6	2.25 (m)
21	134.2	6.27 (m), ovl	53	70.9	3.80 (m), ovl
22	134.5	6.27 (m), ovl	54	41.6	2.25 (m)
23	134.5	6.27 (m), ovl	55	70.9	3.80 (m), ovl
24	135	6.27 (m), ovl	56	35.1	1.59 (m) 1.47 (m)
25	133.3	6.27 (m), ovl	57	26.1	1.67 (m) 1.75 (m)
26	132.1	6.24 (m)	58	42.5	3.19 (m)
27	140.7	6.18 (m)	59	13.1	1.94 (s)
28	37.0	2.57 (m)	60	18.4	0.97 (d, 6.3)
29	80.0	4.67 (dd, 11.7, 6.3)	61	8.6	0.94 (d, 7.2)
30	51.0	2.91 (dq, 6.3)	62	11.9	1.13 (d, 6.3)
31	212.8	-	63	158.7	-
32	50.9	2.79 (dd, 17.1, 8.1) 2.74 (dd, 17.1, 4.5)			

ovl: overlapped

Supplementary Table 4.NMR data for **6** in methanol-*d*₄ at 600 MHz for ¹H and 150 MHz for ¹³C

Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)	Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)
1	175.3	-	22	136.2	5.44 (dd, 15.9, 7.1)
2	128.6	5.81 (d, 15.6)	23	128.9	5.60 (m), ovl
3	141.8	6.98 (dd, 14.8, 11.0)	24	42.0	2.12 (m), ovl
4	132.1	6.24 (dd, 15.7, 11.6)	25	69.1	3.78 (m)
5	139.3	6.42 (dd, 14.7, 11.1)	26	45.4	1.48 (m), ovl 1.37 (m), ovl
6	132.3	6.17 (dd, 14.7, 11.1)	27	*66.2	3.97 (m), ovl
7	136.6	6.27 (dd, 15.2, 11.3)	28	46.6	1.43 (m), ovl
8	134.2	6.10 (dd, 15.2, 11.4)	29	*66.2	3.97 (m), ovl
9	133.6	5.72 (m)	30	46.2	1.50 (m), ovl
10	39.9	2.19 (m), ovl	31	70.4	4.17 (q, 6.7)
11	72.5	3.86 (q, 6.1)	32	137.4	5.47 (dd, 16.2, 6.5)
12	53.2	2.54 (m), ovl	33	127.7	5.60 (m), ovl
13	214.2	-	34	41.6	2.15 (m), ovl 2.12 (m), ovl
14	50.4	2.63 (dd, 16.8, 8.1) 2.54 (dd, 12.7, 5.3)	35	71.1	3.70 (m), ovl
15	66.7	4.05 (m)	36	44.2	1.50 (m), ovl
16	45.0	1.60 (m) 1.47 (m)	37	70.6	3.70 (m), ovl
17	71.7	4.11 (q, 6.7)	38	34.9	1.51 (m), ovl 1.36 (m), ovl
18	136.4	5.40 (dd, 15.3, 7.4)	39	24.9	1.69 (m)
19	129.0	5.58 (m), ovl	40	40.8	2.84 (m)
20	41.5	2.19 (m), ovl 2.13 (m), ovl	41	11.2	1.00 (d, 7.0)
21	73.3	3.96 (m), ovl			

ovl: overlapped

*exchangeable

Supplementary Table 5.NMR data for **7** in methanol-*d*₄ at 600 MHz for ¹H and 150 MHz for ¹³C

Position	δ_C	δ_H (multiplicity, <i>J</i> in Hz)	Position	δ_C	δ_H (multiplicity, <i>J</i> in Hz)
1	172.3	-	21	129.1	5.72 (m)
2	123.8	5.88 (d, 15.1)	22	42.0	2.23 (m), ovl
3	144.9	7.24 (dd, 14.9, 11.4)	23	69.1	3.89 (m), ovl
4	131.2	6.38 (dd, 14.8, 11.4)	24	45.4	1.60 (m) 1.42 (m)
5	141.4	6.62 (dd, 14.7, 11.0)	25	66.28	4.08 (m), ovl
6	131.9	6.30 (dd, 14.8, 11.0)	26	46.6	1.48 (m), ovl
7	137.9	6.44 (dd, 14.9, 10.7)	27	66.25	4.08 (m), ovl
8	134.6	6.23 (dd, 15.1, 10.8)	28	46.1	1.54 (m), ovl
9	134.0	5.84 (dd, 15.1, 8.0)	29	70.3	4.28 (q, 6.2)
10	39.8	2.30 (t, 7.0)	30	137.4	5.58 (dd, 15.5, 6.3)
11	72.5	3.98 (q, 6.2)	31	127.7	5.69 (m)
12	53.3	2.66 (m)	32	41.6	2.23 (m), ovl
13	214.3	-	33	71.0	3.82 (m), ovl
14	50.1	2.73 (dd, 16.1, 8.2) 2.67 (m), ovl	34	44.2	1.54 (m), ovl
15	67.2	4.25 (m), ovl	35	70.6	3.82 (m), ovl
16	45.2	1.56 (m), ovl	36	35.0	1.55 (m), ovl 1.40 (m)
17	69.2	3.89 (m), ovl	37	24.9	1.72 (m) 1.67 (m)
18	45.3	1.56 (m), ovl	38	40.8	2.95 (m)
19	72.0	4.25 (m), ovl	39	11.1	1.11 (d, 6.9)
20	136.5	5.52 (dd, 15.5, 7.1)			

ovl: overlapped

Supplementary Table 6.NMR data for tetrafibricin in methanol-*d*₄ at 600 MHz for ¹H and 150 MHz for ¹³C

Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)	Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)
1	174.8	-	22	42.0	2.22 (m), ovl
2	127.8	5.91 (d, 15.3)	23	69.1	3.88 (m), ovl
3	142.4	7.11 (dd, 15.1, 11.3)	24	45.4	1.57 (m), ovl 1.49 (m), ovl
4	131.9	6.35 (dd, 15.2, 11.6)	25	66.3	4.07 (m), ovl
5	139.6	6.53 (ss, 14.8, 10.9)	26	46.6	1.54 (m), ovl
6	132.2	6.27 (dd, 14.8, 10.7)	27	66.3	4.07 (m), ovl
7	136.8	6.38 (dd, 15.4, 10.7)	28	46.2	1.59 (m), ovl
8	134.2	6.21 (dd, 15.0, 10.9)	29	70.3	4.27 (m), ovl
9	133.8	5.82 (dt, 15.3, 7.4)	30	137.3	5.56 (dd, 14.0, 6.4)
10	39.8	2.30 (m), ovl	31	127.5	5.65 (m), ovl
11	72.5	3.97 (q, 6.2)	32	41.4	2.65 (m), ovl
12	53.2	2.65 (m), ovl	33	73.3	4.06 (m), ovl
13	214.2	-	34	136.5	5.54 (m), ovl
14	50.2	2.71 (dd, 17.0, 8.0), 2.66 (dd, 17.0, 4.3)	35	128.6	5.67 (m), ovl
15	67.2	4.24 (m), ovl	36	41.5	2.28 (m), ovl 2.21 (m), ovl
16	45.2	1.63 (m), ovl 1.44 (m), ovl	37	71.6	3.62 (m)
17	69.1	3.88 (m), ovl	38	34.2	1.61 (m), ovl 1.44 (m), ovl
18	45.4	1.62 (m), ovl	39	25.1	1.81 (m), ovl 1.73 (m), ovl
19	72.0	4.24 (m), ovl	40	40.9	2.94 (m)
20	136.5	5.51 (dd, 15.0, 7.0)	41	11.2	1.10 (d, 7.2)
21	129.1	5.71 (m), ovl			

ovl.: overlapped

Supplementary Table 7.NMR data for **10** in methanol-*d*₄ at 600 MHz for ¹H and 150 MHz for ¹³C

Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)	Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)
1	173.6	-	33	135.1	5.51 (dd, 15.4, 7.2)
2	126.8	5.90 (d, 15.2)	34	127.7	5.7 (m), ovl
3	140.7	7.09 (dd, 15.2, 11.2)	35	40.5	2.22 (m), ovl
4	130.6	6.34 (dd, 15.2, 11.2), ovl	36	67.6	3.88 (m), ovl
5	138.0	6.52 (dd, 14.8, 10.6)	37	44.02	1.47 (m), ovl
6	130.9	6.26 (dd, 15.0, 10.8), ovl	38	*64.84	4.07 (m), ovl
7	135.3	6.37 (dd, 15.2, 10.8)	39	45.2	1.53 (m), ovl
8	132.8	6.2 (dd, 14.8, 10.8)	40	*64.82	4.04 (m), ovl
9	132.2	5.82 (dt, 15.0, 7.3)	41	44.7	1.59 (m), ovl
10	38.4	2.29 (dd, 6.8, 6.8)	42	68.9	4.27 (m), ovl
11	71.1	3.96 (m)	43	136.0	5.56 (dd, 15.4, 6.5)
12	51.8	2.64 (m), ovl	44	126.3	5.68 (m), ovl
13	212.8	-	45	40.0	2.2 (m), ovl
14	48.7	2.69 (m), ovl 2.63 (m), ovl	46	70.2	3.61 (m)
15	65.7	4.23 (m), ovl	47	32.7	1.60 (m), ovl 1.43 (m), ovl
16	43.8	1.62 (m), ovl	48	23.7	1.79 (m), ovl 1.71 (m), ovl
17	67.7	3.85 (m), ovl	49	39.4	2.93 (m)
18	43.95	1.66 (m), ovl	50	9.8	1.1 (d, 6.9)
19	70.6	4.22 (m), ovl	51		

ovl: overlapped

*exchangeable

Supplementary Table 8.NMR data for **8** in methanol-*d*₄ at 600 MHz for ¹H and 150 MHz for ¹³C

Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)	Position	δ _C	δ _H (multiplicity, <i>J</i> in Hz)
1	169.6	-	33	66.5	4.14 (m)
2	127.2	-	34	45.0	1.55 (m), ovl 1.49 (m), ovl
3	140.4	7.21 (d, 10.9)	35	71.9	4.2 (q, 6.8)
4	127.0	6.45 (dd, 15.0, 11.6)	36	136.6	5.51 (m), ovl
5	141.8	6.55 (dd, 14.7, 10.6)	37	129.1	5.7 (m), ovl
6	132.2	6.25 (m), ovl	38	44.9	1.61 (m), ovl
7	140.3	5.94 (dt, 15.1, 6.4)	39	71.3	3.82 (m), ovl
8	34.0	2.28 (m), ovl	40	44.6	1.63 (m), ovl
9	33.0	2.18 (m), ovl	41	68.5	3.99 (m)
10	131.4	5.46 (m), ovl	42	41.9	2.19 (m), ovl
11	134.5	5.46 (m), ovl	43	70.2	4.26 (m), ovl
12	41.3	2.42 (m), ovl	44	137.2	5.56 (m), ovl
13	77.2	3.35 (dd, 6.7, 4.7)	45	128.1	5.67 (m), ovl
14	44.2	1.59 (m), ovl	46	41.7	2.23 (m), ovl
15	73.7	4.25 (m), ovl	47	68.8	3.83 (m), ovl
16	137.7	5.69 (m), ovl	48	45.6	1.65 (m), ovl
17	131.7	6.25 (m), ovl	49	69.8	4.26 (m), ovl
18	134.5	6.25 (m), ovl	50	138.4	5.61 (m), ovl
19	134.3	6.25 (m), ovl	51	125.8	5.63 (m), ovl
20	134.2	6.25 (m), ovl	52	38.0	2.43 (m), ovl 2.38 (m), ovl
21	134.1	6.25 (m), ovl	53	72.9	5.11 (m)
22	133.8	6.25 (m), ovl	54	42.5	1.84 (quin, 6.4) 1.76 (m), ovl
23	133.2	6.25 (m), ovl	55	69.0	3.64 (m)
24	132.2	6.25 (m), ovl	56	34.9	1.59 (m), ovl 1.42 (m), ovl
25	131.7	6.25 (m), ovl	57	26.2	1.73 (m), ovl 1.61 (m), ovl
26	134.9	6.14 (dd, 14.3, 9.9)	58	42.4	3.17 (m)
27	131.2	5.73 (dt, 14.5, 7.7)	59	158.6	-
28	37.2	2.55 (m)	60	13.1	1.93 (s)
29	80.2	4.63 (q, 5.9)	61	18.8	1.00 (d, 6.7)
30	51.1	2.92 (quin, 7)	62	13.8	0.95 (d, 6.7)
31	212.9	-	63	12.8	1.14 (d, 7.1)
32	51.2	2.75 (dd, 17.1, 8.0) 2.69 (dd, 17.5, 4.0)			

ovl: overlapped

Supplementary Table 9.
Plasmids used in this study

Name	Description	Source
(BACs)		
pKU518	a BAC vector	Supplementary ref. 1
pKU518med	the BAC containing the whole BGC of mediomycin; referred as pKU518NBRC12747_P1-P14-G22 in the ref.	Ref. 19
pKU518med_PKSv1	the edited BAC encoding PKSv1	This study
pKU518med_PKSv1_XU	the edited BAC encoding PKSv1_XU	This study
pKU518med_PKSv2	the edited BAC encoding PKSv2	This study
pKU518med_PKSv2_XU	the edited BAC encoding PKSv2_XU	This study
pKU518med_PKSv3	the edited BAC encoding PKSv3	This study
pKU518med_PKSv3_medTE	a modified version of pKU518med_PKSv3; migrated the TE domain only	This study
pKU518med_PKSv3_pikTE	a modified version of pKU518med_PKSv3; transplanted the TE domain of pikromycin PKS	This study
pKU518med_PKSv4	the edited BAC encoding PKSv4	This study
pKU518med_PKSv4_XU	the edited BAC encoding PKSv4_XU	This study
pKU518med_PKSv5	the edited BAC encoding PKSv5	This study
pKU518med_PKSv5_XU	the edited BAC encoding PKSv5_XU	This study
pKU518med_PKSv6	the edited BAC encoding PKSv6	This study
pKU518med_PKSv6(TE)	a modified version of pKU518med_PKSv6; transplanted the TE domain of desertomycin PKS	This study
pKU518med_PKSv6_amphACP-TE	a modified version of pKU518med_PKSv6; transplanted the ACP-TE didomain of amphotericin PKS	This study
(amidinohydrolases)		
pKU565tsr-2cistron	an integration vector	Supplementary ref. 1
pKU565T_ermE*p	an integration vector	This study
pKU565T_ermE*p_medX	the <i>medX</i> expression vector	This study
pKU565T_ermE*p_ecoX	the <i>ecoX</i> expression vector	This study
pKU565T_ermE*p_tfbX1	the <i>tfbX1</i> expression vector	This study
pKU565T_ermE*p_tfbX2	the <i>tfbX2</i> expression vector	This study
(platelet assay)		
pCMV3-C-OFPSpark-ITGA2B	ITGA2B expression plasmid	Sino Biological (HG13067-ACR)
pCMV3-C-OFPSpark-ITGA2B(G991C)	ITGA2B(G991C) expression plasmid	This study
pCMV3-C-GFPSpark-ITGB3	ITGB3 expression plasmid	Sino Biological (HG10787-ACG)

Supplementary Table 10.
Bacterial strains used in this study

Name	Usage / Description	Source
<i>Escherichia coli</i> DH5 α	Routine cloning	Lab stock
<i>E. coli</i> NEB10 β	BAC cloning	NEB
<i>Staphylococcus aureus</i>	Antibacterial activity assay	Lab stock
<i>Micrococcus luteus</i>	Antibacterial activity	Lab stock
<i>Streptomyces lividans</i> TK23 $\Delta redD-X$	Host strain for heterologous expression	
(Transformants of <i>S. lividans</i> TK23 $\Delta redD-X$)	Producing compounds	
/pKU518med	Clethramycin and desulfoclethramycin	Ref. 19
/pKU518med_PKSv1	1a and 1b	This study
/pKU518med_PKSv1_XU	1a and 1b	This study
/pKU518med_PKSv2	2a and 2b	This study
/pKU518med_PKSv2_XU	2a and 2b	This study
/pKU518med_PKSv3	3	This study
/pKU518med_PKSv3_medTE	3	This study
/pKU518med_PKSv3_pikTE	3	This study
/pKU518med_PKSv4	4	This study
/pKU518med_PKSv4_XU	4	This study
/pKU518med_PKSv5	5	This study
/pKU518med_PKSv5_XU	5	This study
/pKU518med_PKSv3/pKU565T_ermE*p_ecoX	6	This study
/pKU518med_PKSv4/pKU565T_ermE*p_tfbX1	7	This study
/pKU518med_PKSv4/pKU565T_ermE*p_tfbX2	4 and 7	This study
/pKU518med_PKSv4/pKU565T_ermE*p_ecoX	7	This study
/pKU518med_PKSv4/pKU565T_ermE*p_medX	4 and 7	This study
/pKU518med_PKSv5/pKU565T_ermE*p_tfbX1	Tetrafibricin	This study
/pKU518med_PKSv6	8	This study
/pKU518med_PKSv6(TE)	8	This study
/pKU518med_PKSv6_amphACP-TE	8	This study

Supplementary Table 11.**Combination of a parent BAC, a pair of target sequences, and donor sequences**

Name	Parent	target 1	target 2	donor
pKU518med_				
PKSv1	pKU518med	target_PKSv1_u	target_PKSv1_d	donor_PKSv1
PKSv1_XU	pKU518med	target_PKSv1_u	target_PKSv1_d	donor_PKSv1_XU
PKSv2	pKU518med_PKSv1	target_PKSv2_u	target_PKSv2_d	donor_PKSv2
PKSv2_XU	pKU518med_PKSv1	target_PKSv2_u	target_PKSv2_d	donor_PKSv2_XU
PKSv3	pKU518med_PKSv2	target_PKSv3_u	target_PKSv3_d	donor_PKSv3
PKSv3_medTE	pKU518med_PKSv2	target_PKSv3_u	target_PKSv3_d	donor_PKSv3_medTE
PKSv3_pikTE	pKU518med_PKSv2	target_PKSv3_u	target_PKSv3_pikTE_d	donor_PKSv3_pikTE
PKSv4	pKU518med_PKSv3	target_PKSv4_u	target_PKSv4_d	donor_PKSv4
PKSv4_XU	pKU518med_PKSv3	target_PKSv4_u	target_PKSv4_d	donor_PKSv4_XU
PKSv5	pKU518med_PKSv4	target_PKSv5_u	target_PKSv5_d	donor_PKSv5
PKSv5_XU	pKU518med_PKSv4	target_PKSv5_XU_u	target_PKSv5_d	donor_PKSv5_XU
PKSv6	pKU518med	target_PKSv6_u	target_PKSv6_d	donor_PKSv6_dstACP-TE
PKSv6(TE)	pKU518med	target_PKSv6(TE)_u	target_PKSv6_d	donor_PKSv6_dstTE
PKSv6_amphACP-TE	pKU518med	target_PKSv6_u	target_PKSv6_d	donor_PKSv6_amphACP-TE

Supplementary Table 12.

Accession codes for sequences behind Supplementary Figure 1.

Name	Accession	Organism	Sequence Length
NysJ	WP_079142143.1	<i>Streptomyces noursei</i>	5438
NysI	WP_067343753.1	<i>Streptomyces noursei</i>	9477
NysK	WP_067343749.1	<i>Streptomyces noursei</i>	2066
NysA	WP_067343733.1	<i>Streptomyces noursei</i>	1366
NysB	WP_067343731.1	<i>Streptomyces noursei</i>	3192
NysC	WP_067343729.1	<i>Streptomyces noursei</i>	11098
AmphJ	WP_052453975.1	<i>Streptomyces nodosus</i>	5639
AmphA	WP_052453973.1	<i>Streptomyces nodosus</i>	1412
AmphB	WP_052453972.1	<i>Streptomyces nodosus</i>	3190
AmphI	WP_043437282.1	<i>Streptomyces nodosus</i>	9510
AmphK	WP_043437281.1	<i>Streptomyces nodosus</i>	2034
AmphC	WP_043437269.1	<i>Streptomyces nodosus</i>	10917
ConJ	WCD56696.1	<i>Streptomyces</i> sp.	1302
ConI	WCD56695.1	<i>Streptomyces</i> sp.	4301
ConH	WCD56694.1	<i>Streptomyces</i> sp.	3560
ConG	WCD56693.1	<i>Streptomyces</i> sp.	3494
ConF	WCD56692.1	<i>Streptomyces</i> sp.	3598
ConE	WCD56691.1	<i>Streptomyces</i> sp.	2046
MadA1	QFU19843.1	<i>Actinomadura</i> sp.	2429
MadA2	QFU19842.1	<i>Actinomadura</i> sp.	2217
MadA3	QFU19841.1	<i>Actinomadura</i> sp.	4043
MadA4	QFU19840.1	<i>Actinomadura</i> sp.	4020
MadA5	QFU19839.1	<i>Actinomadura</i> sp.	4035
MadA6	QFU19838.1	<i>Actinomadura</i> sp.	1669
MadA9	QFU19827.1	<i>Actinomadura</i> sp.	1868
MadA8	QFU19826.1	<i>Actinomadura</i> sp.	2091
MadA7	QFU19825.1	<i>Actinomadura</i> sp.	4770
BomP6	QBL56210.1	<i>Streptomyces</i> sp.	5435
BomP5	QBL56191.1	<i>Streptomyces</i> sp.	1737
BomP4	QBL56184.1	<i>Streptomyces</i> sp.	3533
BomP3	QBL56183.1	<i>Streptomyces</i> sp.	1623
BomP2	QBL56182.1	<i>Streptomyces</i> sp.	3577
BomP1	QBL56181.1	<i>Streptomyces</i> sp.	3738
ChmG1	Q5SFB4	<i>Streptomyces bikiniensis</i>	4441
ChmG2	Q5SFB3	<i>Streptomyces bikiniensis</i>	1974
ChmG3	Q5SFB2	<i>Streptomyces bikiniensis</i>	3788
ChmG4	Q5SFB1	<i>Streptomyces bikiniensis</i>	1612
ChmG5	Q5SFB0	<i>Streptomyces bikiniensis</i>	1350
HitP3	BAR73021.1	<i>Streptomyces scabrisporus</i>	1612
HitP4	BAR73020.1	<i>Streptomyces scabrisporus</i>	5186
HitP5	BAR73019.1	<i>Streptomyces scabrisporus</i>	2004
HitP2	BAR73017.1	<i>Streptomyces scabrisporus</i>	3325
HitP1	BAR73007.1	<i>Streptomyces scabrisporus</i>	3983
MycA5	BAC57032.1	<i>Micromonospora griseorubida</i> A11725	2070
MycA4	BAC57031.1	<i>Micromonospora griseorubida</i> A11725	1582
MycA3	BAC57030.1	<i>Micromonospora griseorubida</i> A11725	3649
MycA2	BAC57029.1	<i>Micromonospora griseorubida</i> A11725	1843
MycA1	BAC57028.1	<i>Micromonospora griseorubida</i> A11725	4307
NigAVII	ABC84471.1	<i>Streptomyces violaceusniger</i>	4787
NigAVIII	ABC84470.1	<i>Streptomyces violaceusniger</i>	2206
NigAIX	ABC84469.1	<i>Streptomyces violaceusniger</i>	1942
NigAX	ABC84465.1	<i>Streptomyces violaceusniger</i>	1299
NigAVI	ABC84461.1	<i>Streptomyces violaceusniger</i>	1701
NigAV	ABC84460.1	<i>Streptomyces violaceusniger</i>	4045
NigAIV	ABC84459.1	<i>Streptomyces violaceusniger</i>	4055
NigAIII	ABC84458.1	<i>Streptomyces violaceusniger</i>	4083
NigAII	ABC84457.1	<i>Streptomyces violaceusniger</i>	2233
NigAI	ABC84456.1	<i>Streptomyces violaceusniger</i>	2415
TylGV	AAB66508.1	<i>Streptomyces fradiae</i>	1841
TylGIV	AAB66507.1	<i>Streptomyces fradiae</i>	1611
TylGIII	AAB66506.1	<i>Streptomyces fradiae</i>	3729
TylGII	AAB66505.1	<i>Streptomyces fradiae</i>	1864
TylGI	AAB66504.1	<i>Streptomyces fradiae</i>	4472

Supplementary References

1. J. H. Kim, M. Komatsu, K. Shin-ya, S. Ōmura, H. Ikeda, Distribution and functional analysis of the phosphopantetheinyl transferase superfamily in Actinomycetales microorganisms. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 6828-6833 (2018).