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Cloning of hybrid PKS modules

The cloning, expression, and purification of Pik Mod5[1], Pik Mod5-PikTE[2], Pik Mod6[1], Pik TE[3], DEBS Mod5[4], DEBS Mod5-DEBS TE[4], DEBS Mod6[4], DEBS TE[5], Juv Mod6[6], and Juv Mod7[6] have been previously reported. Pik Mod6, DEBS Mod6, Juv Mod6, and Juv Mod7 hybrids were generated by inserting a restriction site at the 3' end of the alignment consensus for each antiSMASH[7] annotated ACP domain to enable insertion of the respective non-native TE domains (Figure S1).

The hybrid TE proteins utilized in this study were generated by the following procedures:

The Juv TE domain region was determined by sequence alignment to Pik and DEBS and PCR amplified from pET21-Juv Mod7 using primers 5'-ccaaccgaattcaccggcgcggcgggcgggccacc-3' and 5'-ccaaccctcgagtcatgcggccgcaagcttcggaacgcg-3' and subsequently inserted into pET28b using *Eco*RI and *Xho*I. This construct was then used as the template for two rounds of mutagenesis following the QuikChange site-directed mutagenesis (Stratagene) protocol to remove an internal *Bam*HI site using primers 5'- ggggcctggccggacccccggcaggactg-3' and 5'-cagtcctgccgggggtccggccaggcccc-3', and *Hind*III site using primers 5'-gtgcggccgcaagtttcggaacgcgag-3' and 5'-cgcctggcggtaccggatcgcctggc-3'. This modified construct was then used for generating the Juv TE hybrid PKS modules as described below.

Pik Mod5-TE hybrids:
pET24b Pik Mod5-Pik TE[2] was digested with *Hind*III and *Xho*I to allow for fusion with the DEBS TE using primers 5'-ccaaccaagcttagcgggactcccgcccgggaagcg-3' and 5'-ccaaccctcgagtgaattccctccgcccagccaggc-3', and Juv TE using primers 5'-ccaaccaagcttaccggcgcggcgggcgggccacc-3' and 5'-ccaaccctcgagtgcggccgcaagcttcggaacgcg-3'.

Pik Mod6–TE hybrids:
The Pik Mod6-TE hybrids were constructed from pET24b Pik Mod6[1] in two steps. First, Pik Mod6 was truncated at the 3' end to its corresponding ACP domain by digestion with *Eco*RI and *Hind*III followed by ligation with a similarly digested PCR amplification product generated using primers 5'-gacagctcacccgaattc-3' and 5'-ccaaccaagcttcagctcgtcgctgatgcgctcggc-3'. Next this intermediate was digested with *Hind*III and *Xho*I to allow for fusion with the DEBS TE using primers 5'-ccaaccaagcttagcgggactcccgcccgggaagcg-3' and 5'-ccaaccctcgagtgaattccctccgcccagccaggc-3', and the Juv TE using primers 5'-ccaaccaagcttaccggcgcggcgggcgggccacc-3' and 5'-ccaaccctcgagtgcggccgcaagcttcggaacgcg-3'.

DEBS Mod5-TE hybrids:
pET28b DEBS Mod5-DEBS TE[4] was digested with *Bam*HI and *Xho*I to allow for fusion with Pik TE using primers 5'-ccaaccggatcctccggggccgacaccggc-3' and 5'-ccttccctcgagtcagcccgccccctcgatgcc-3', DEBS TE using primers 5'-ccaaccggatccagcgggactcccgcccgggaagcg-3' and 5'-ccaaccctcgagtcatgaattccctccgcccagccaggc-3', and Juv TE using primers 5'-ccaaccggatccaccggcgcggcgggcgggccacc-3' and 5'-ccaaccctcgagtcatgcggccgcaagcttcggaacgcg-3'.

DEBS Mod6-TE hybrids:
The DEBS Mod6-TE hybrids were constructed from pET24b DEBS Mod6[4] in two steps. First, DEBS Mod6 was truncated at the 3' end to its corresponding ACP domain by digestion with *Xba*I and *Eco*RI followed by ligation with a similarly digested PCR amplification product generated using primers 5'-cccctctagaaataattttgtttaactttaagaagg-3' and 5'-ccaaccgaattcgagctgctgtcctatgtggtcg-3'. Next this intermediate was digested with *Eco*RI and HindIII to allow for fusion with the Pik TE using primers 5'-ccaaccgaattctccggggccgacaccggc-3'

and 5’-ccaaccaagcttgcccgccccctcgatgcc-3’, and the Juv TE using primers 5’-ccaaccgaattcaccggcgcggcgggcgggccacc-3’ and 5’-ccaaccaagctttgcggccgcaagcttcggaacgcg-3’.

Juv Mod6-TE hybrids:
The Juv Mod6-TE hybrids were constructed from pET28b Juv Mod6[6] in two steps. First, Juv Mod6 was truncated at the 3’ end to its corresponding ACP domain by digestion with *Nde*I and *Hind*III followed by ligation with a similarly digested PCR amplification product generated using primers 5’- ccaacccatatgtcgaacgagcagaagctccgc-3’ and 5’-ccaaccaagcttgagcagcccggccaggtgctcggc-3’. Next this intermediate was digested with *Hind*III and *Xho*I to allow for fusion with the Pik TE using primers 5’- ccaaccaagctttccggggccgacaccggc-3’ and 5’- ccaaccctcgagtcacttgcccgcccctcga-3’, the DEBS TE using primers 5’-ccaaccaagcttagcgggactcccgcccgggaagcg-3’ and 5’- ccaaccctcgagtcatgaattccctccgcccagccaggc-3’, and the Juv TE using primers 5’- ccaaccaagcttaccggcgcggcgggcgggccaacc-3’ and 5’-ccaaccctcgagtcatgcggccgcaagcttcggaacgcg-3’.

Juv Mod7-TE hybrids:
The Juv Mod7-TE hybrids were constructed from pET21b Juv Mod7[6] in two steps. First, Juv Mod7 was truncated at the 3’ end to its corresponding ACP domain by digestion with *Kpn*I and *Hind*III followed by ligation with a similarly digested PCR amplification product generated using primers 5’- cgcctggcggtaccggatcgcctggc-3’ and 5’- ccaaccaagcttgagcaggccgtgcaggtgcgcggc-3’. Next this intermediate was digested with *Hind*III and *Xho*I to allow for fusion with the Pik TE using primers 5’- ccaaccaagctttccggggccgacaccggc-3’ and 5’- ccaaccctcgagcttgcccgcccctcga-3’, and the DEBS TE using primers 5’- ccaaccaagcttagcgggactcccgcccgggaagcg-3’ and 5’-ccaaccctcgagtgaattccctccgcccagccaggc-3’.

Juv Mod6 PikTE$_{S148A}$:
The JuvMod6 PikTE$_{S148A}$ was constructed from pET28b JuvMod6 PikTE and pET28 PikTE$_{S148A}$ in two steps. First, PikTE$_{S148A}$ was amplified to contain an N terminal *HindIII* site 5’-CCAACCAAGCTTTCCGGGGCCGACACCGGC – 3’ and an *XhoI* site 5’-CCAACCCTCGAGTCACTTGCCCGCCCCCTCGA – 3’. Both the amplicon and the pET28b JuvMod6 PikTE were digested with *HindIII* and *XhoI* with subsequent ligation yielding the desired inactive TE fusion.

Figure S1. Determination of the post-ACP restriction site (black arrow) for incorporating non-native TE domains. The location was chosen from alignment of the antiSMASH[7] annotated ACP domain regions (orange highlight) for each PKS module. Sequences were aligned using T-coffee[8] and rendered with ESPript[9].

PKS protein biochemistry

E. coli cell culture, protein purification, and enzymatic reactions were performed using water obtained from a Millipore Milli-Q system with Millipore Q-Gard 2/Quantum Ex Ultrapure organex cartridges. *E. coli* culture growth was performed in 15 mL sterile tubes for the seed cultures and 2.8 L Corning Fernbach flasks with deep baffles (3x) for protein expression cultures. Reagents were obtained from the following sources: LB broth (Miller) and glycerol was obtained from EMD. Isopropyl-β-D-thiogalactopyranoside (IPTG), Kanamycin sulfate (Kan), and Ampicillin (Amp) were obtained from Gold Biotechnology. NaCl, $CaCl_2$ and imidazole were obtained from Fisher Scientific. Lysozyme was purchased from RPI, PD-10 colums were purchased from GE scientific, and Ni-NTA agarose resin was purchased from Qiagen. The pH of all solutions was monitored via a Symphony SB70P pH meter calibrated according to the manufacturer's specifications. Optical density (OD_{600}) was determined using an Eppendorf Biophotometer and *E. coli* cell lysis was accomplished using a 550 Sonic Dismembrator (Fisher Scientific). All solutions were autoclaved or sterile filtered (0.2 μm) prior to use.

Buffers:
lysis: HEPES (50 mM), NaCl (300 mM), imidazole (10 mM), glycerol (10% v/v), pH 8.0.
wash: HEPES (50 mM), NaCl (300 mM), imidazole (30 mM), glycerol (10% v/v), pH 8.0.
elution HEPES (50 mM), NaCl (300 mM), imidazole (300 mM), glycerol (10% v/v), pH 8.0.
storage: HEPES (50 mM), NaCl (150 mM), EDTA (1 mM), glycerol (20% v/v), pH 7.2.
Stock solutions:
hexaketide substrates (50 mM in DMSO), 2-vinylpyridine (500 mM in DMSO), ascorbic acid (500 mM in H_2O), sodium metabisulfite (100 mM in H_2O).

Protein production

A starter culture was generated by inoculating 5 mL of LB broth containing Kan or Amp (50 mg/L) with fresh transformants of *E. coli* (BAP1)[8] cells containing the corresponding plasmids for expression of the respective PKS proteins and grown overnight at 37 ˚C. Following overnight growth, the entire starter culture was subsequently used to inoculate an expression culture of 1 L of TB containing Kan or Amp (50 mg/L) and grown at 37 ˚C to an OD_{600} of 0.3-0.4. The expression cultures were then cooled to 18 ˚C and growth was maintained until an OD_{600} of 0.7-0.8 was reached, at which point protein expression was induced via addition of IPTG (350 μM) and the cultures were incubated at 200 RPM at 18 ˚C for 20 hours.

Protein purification

A single pass purification scheme was employed to minimize the length of time each protein was processed since previous work has shown the most reproducible in vitro activity is achieved with short purification times.[9] Purification by Ni-NTA affinity chromatography provided each protein in yields (Table S1) and purities (Figure S2) sufficient for enzymatic analysis. Although the Juv Mod6 hybrids contained significant contaminating species within the purified protein fractions (lanes 13-16, Figure S2), our previous study[6] had shown Juv Mod6 retains high catalytic efficiency as a cell-free lysate, thus alleviating our concern over the purity of these proteins.

Protein expression cultures were cooled to 4 ˚C and harvested by centrifugation (6,500 x g, 10 min, 4 ˚C). The pelleted cells were then suspended in 5 mL of lysis buffer per gram of cells via vortex. Cell lysis was accomplished by the addition of 0.4 mg/mL lysozyme and the solution was then sonicated on ice (100 x 3s with 10s rest periods). The resulting cellular lysate was then pelleted by centrifugation (60,000 x g, 30 min, 4 ˚C) and the supernatant was applied to 6 mL of pre-equilibrated Ni-NTA resin. After binding, the column was washed with 15 column volumes of wash buffer and the target protein was subsequently eluted with 4 column volumes of elution buffer. Elution fractions were determined by their absorption at 280 nm, pooled, and buffer exchanged into storage buffer using a pre-equilibrated PD-10 column. After buffer exchange, the elution fractions were once again monitored via their absorption at 280 nm, pooled, flash frozen in liquid N_2, and stored at -80 ˚C.

Module	TE	Lane number	MW (kDa)	Purification yield (mg)
Pik Mod5	DEBS	1	186	41
	Juv	2	186	40
	Pik	3	187	37
Pik Mod6	DEBS	4	138	39
	Juv	5	139	34
	Pik	6	143	26
DEBS Mod5	DEBS	7	186	41
	Juv	8	187	27
	Pik	9	188	32
DEBS Mod6	DEBS	10	178	24
	Juv	11	178	22
	Pik	12	179	22
Juv Mod6	---	13	165	35
	DEBS	14	187	26
	Juv	14	188	31
	Pik	16	189	30
Juv Mod7	DEBS	17	186	26
	Juv	18	187	20
	Pik	19	188	21

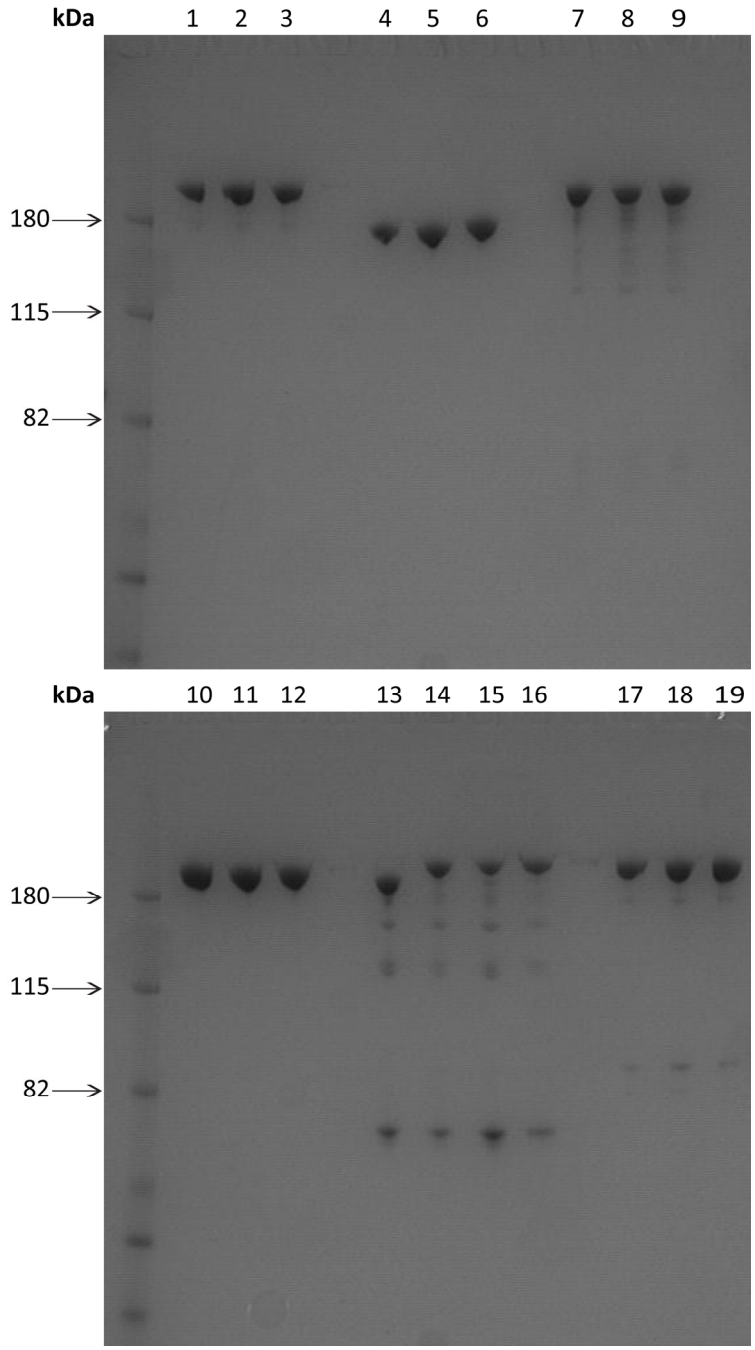


Figure S2 NuPAGE® Bis-Tris Mini gel of purified PKS modules in MOPS running buffer with BenchMark™ Pre-stained Protein Ladder. Lanes: 1. Pik Mod5-DEBS TE (186 kDa), 2. Pik Mod5-Juv TE (186 kDa), 3. Pik Mod5-Pik TE (187 kDa), 4. Pik Mod6-DEBS TE (138 kDa), 5. Pik Mod6-Juv TE (139 kDa), 6. Pik Mod6-Pik TE (143 kDa), 7. DEBS Mod5-DEBS TE (186 kDa), 8. DEBS Mod5-Juv TE (187 kDa), 9. DEBS Mod5-Pik TE (188 kDa), 10. DEBS Mod6-DEBS TE (178 kDa), 11. DEBS Mod6-Juv TE (178 kDa), 12. DEBS Mod6-Pik TE (179 kDa), 13. Juv Mod6 (165 kDa), 14. Juv Mod6-DEBS TE (187 kDa), 15. Juv Mod6-Juv TE (188 kDa), 16. Juv Mod6-Pik TE (189 kDa), 17. Juv Mod7-DEBS TE (186 kDa), 18. Juv Mod7-Juv TE(187 kDa), 19. Juv Mod7-PikTE (188 kDa).

Enzymatic reactions

All analytical scale reactions were performed in triplicate at a volume of 50 µL and quenched with 3 volumes of MeOH (150 µL), clarified by centrifugation (17,000 x g, 30 min, 4 ˚C) and analyzed for product formation by HPLC. 2-vinylpyridine (Sigma) was employed as a thiol scavenger.

Reactions containing NBOM protected Pik hexaketide[10] were performed in two steps. First, a solution containing ascorbic acid (25mM final concentration), sodium metabisulfite (1mM final concentration), NBOM protected substrate (1mM final concentration), and H_2O (requisite dead volume) was irradiated under a consumer facial tanning lamp at a height of 14 cm (Verseo #AH129c) for 20 min to furnish the deprotected Pik hexaketide **2**. After photolysis, the solution was diluted with reaction buffer and catalysis was initiated via the addition of enzyme and incubated for 4 hours.

HPLC analysis

Macrolactone production was monitored via analytical high-performance liquid chromatography (HPLC) using a Shimadzu LC-20AD instrument.

Product formation in Table 1 was quantified using Phenomenex Luna 5µ C18 250 x 4.6 mm column (serial 466013-1) monitoring at a wavelength of 236 nm. Separation was accomplished by the following method: 1.5 mL/min, solvent A: H_2O + 0.1% formic acid, solvent B: MeCN + 0.1% formic acid, 5% B 0-1 min, 5-100% B linear gradient 1-12 min, 100% B 12-15 min, 5% B 15-17.5 min.

Product formation in Table 2 and 3 was quantified using a Zorbax SB-Phenyl 3.5 µM 4.6 x 150 mm column (part number 863953-912) monitoring at a wavelength of 236 nm. Separation was accomplished by the following method: 3.0 mL/min, solvent A: H_2O + 0.1% formic acid, solvent B: MeCN + 0.1% formic acid, 5% B 0-1 min, 5-70% B linear gradient 1-13 min, 100% B 13-15 min, 5% B 15-17 min.

LC-HRMS analysis

Analytical liquid chromatography-mass spectrometry (LC-MS) was performed on an Agilent LC system (1290 series) coupled to an Agilent QTOF mass spectrometer (6500 series) using a Phenomenex Synergi 4µ Hydro RP 100 x 2 mm column (serial 48836-5) heated to 50°C. Method: 0.4 mL/min, solvent A: H_2O + 0.1% formic acid, solvent B: MeCN + 0.1% formic acid, 0% B 0-2 min,0-100% B linear gradient 2-10 min, 100% 10-11 min, 0% B 11-12 min, 0-1 min were diverted to waste.

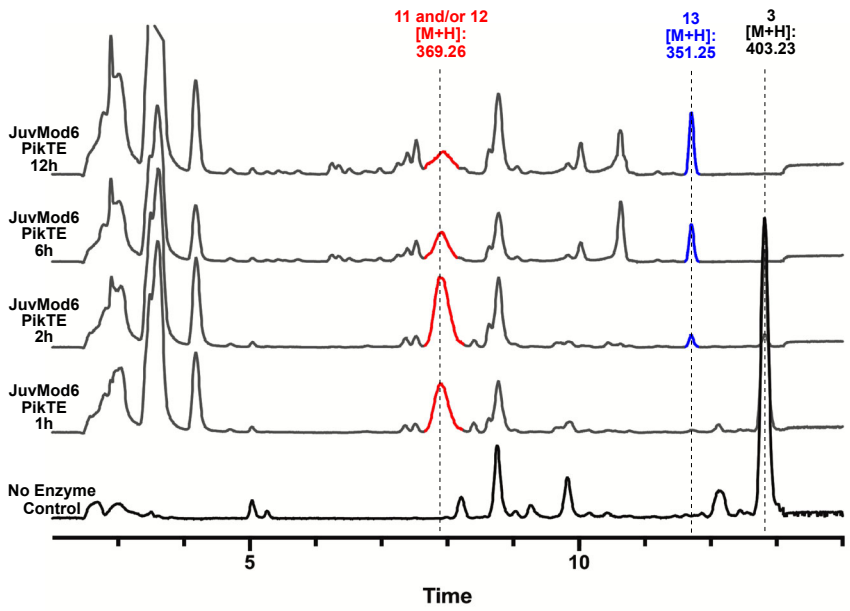


Figure S3: TIC LC-HRMS analysis of JuvMod6-PikTE time course showing disappearance of starting material and appearance of a substance corresponding to the linear acid or hemiketal species (compounds **11** and/or **12**). In a time-dependent manner, the level of **11**/**12** (red line) is reduced while the dehydrated pyran **13** (blue line) becomes the major product.

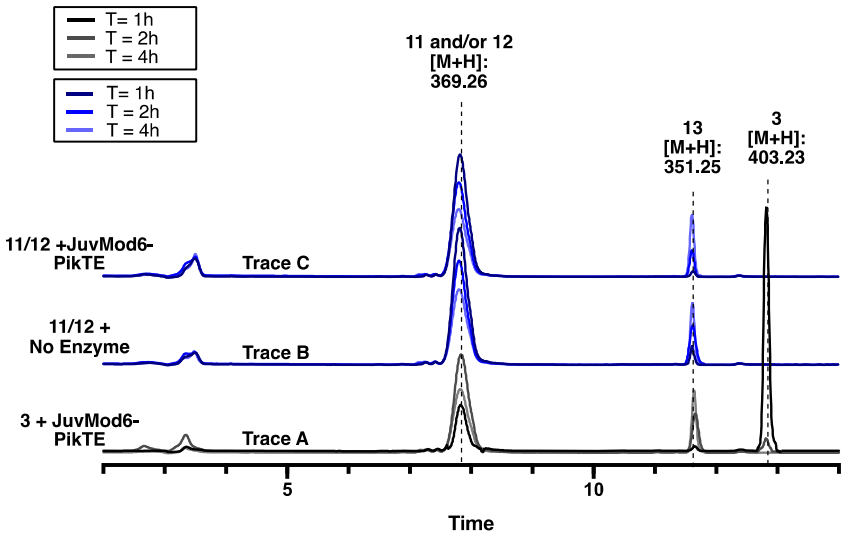
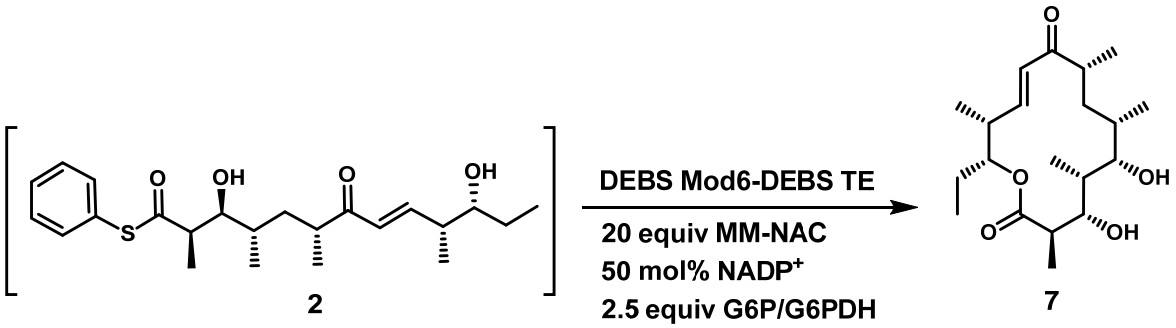


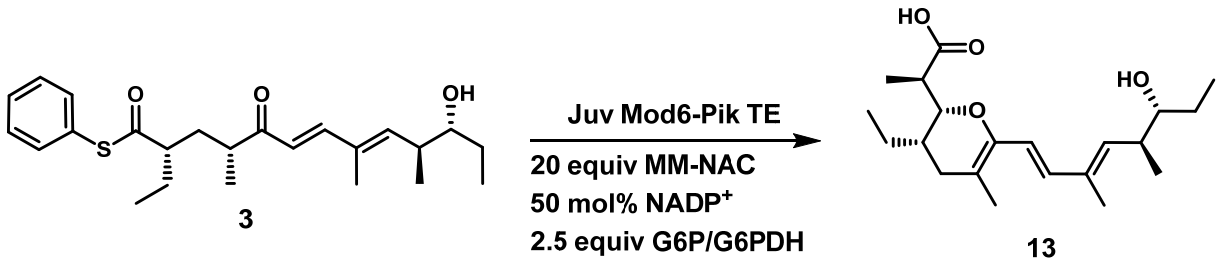
Figure S4: LC-HRMS analysis (UV extracted at 285 nm) of isolated **11/12** mixture under enzymatic reaction buffered conditions (see below) over time with and without enzyme in comparison to original reaction of **3** to **13**. Analysis indicates that the original reaction consumes starting material in two hours with clear formation of **13** after 4 hours (Trace A). Compounds **11/12** display turnover to the dehydrated pyran **13** in a similar time-dependent manner with (Trace C) and without (Trace B) Juv Mod6-Pik TE. Continued time points not displayed for the original reaction (**3** +JuvMod6-PikTE) shows an increase in **13** over the 12-hour period (Figure S3). However, isolated **11**/**12** shows continued depletion that does not correspond to an increase in **13**.



Reaction conditions: sodium phosphate buffer (400 mM, 20% v/v glycerol, 92 mL total, pH = 7.2), Pik hexaketide **2** (51 mg, 0.09 mmol, 1 mM), MM-SNAC (20 equiv, 20 mM), $NADP^+$ (0.5 equiv, 0.5 mM), glucose-6-phosphate (2.5 equiv, 2.5 mM), glucose-6-phosphate dehydrogenase (2 units/mL), 2-vinylpyridine (8 mM), ascorbic acid (25 mM), sodium metabisulfite (1 mM), DEBS Mod6-DEBS TE (1 μM, 0.1 mol%), 20 hours, stationary, RT.

Workup and purification: Quenched with acetone (2x volume, 184 mL), placed in a -20 °C freezer for 1 h and filtered through a celite plug. Remaining insoluble material was suspended in acetone and this solution was used to rinse the celite plug. Acetone was removed through rotary evaporation and the aqueous layer was saturated with NaCl and extracted 3x EtOAc. Combined organic layers were concentrated. **7** was purified directly by preparatory HPLC using a Phenomenex Luna 5u C18 250 x 21.2mm column (serial 444304-4) monitoring at 250nM. Method 9mL/min, A: H_2O + 0.1% formic acid, B: MeCN + 0.1% formic acid, 5% B 0-5 min, 5-100% B linear gradient 5-45 min, 100% B 45-65 min, 5% 65-75 min.

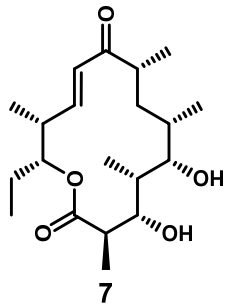
7 from Pik hexaketide **2** (4.1 mg, 0.089 mmol, 12.6% yield).
^{1}H NMR (700 MHz, CD_3OD) δ 7.11 (dd, *J* = 15.9, 4.5 Hz, 1H), 6.27 (dd, *J* = 15.9, 2.0 Hz, 1H), 5.10 (ddd, *J* = 9.4, 4.5, 1.8 Hz, 1H), 3.44 (dd, *J* = 9.0, 1.5 Hz, 1H), 3.41 (dd, *J* = 7.2, 1.3 Hz, 1H), 2.69 (ddd, *J* = 8.7, 4.3, 2.0 Hz, 1H), 2.63 (ddd, *J* = 10.8, 7.0, 4.0 Hz, 1H), 2.55 (m, 1H), 1.78 (m, 1H), 1.76 (ddd, *J* = 14.1, 7.0, 4.9 Hz, 1H), 1.63 (ddd, *J* = 14.0, 7.4, 4.6 Hz, 1H), 1.41 – 1.35 (m, 1H), 1.31 – 1.26 (m, 1H), 1.25 (s, 1H), 1.22 (d, *J* = 4.0 Hz, 1H), 1.20 (t, *J* = 4.4 Hz, 1H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.15 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.95 (m, 3H), 0.93 (d, *J* = 7.4 Hz, 2H).
^{13}C NMR (700 MHz; CD_3OD) δ 206.07, 177.72, 152.84, 124.72, 78.40, 78.10, 75.00, 45.90, 45.30, 42.93, 40.80, 37.45, 35.71, 26.52, 19.41, 18.03, 15.61, 10.92, 10.34, 8.92.
HRMS: Calculated $[M+Na]^+$ 377.2298, found 377.2307



Reaction conditions: sodium phosphate buffer (400 mM, 20% v/v glycerol, 204 mL total, pH = 7.2), Tyl hexaketide **3** (41 mg, 0.10 mmol, 0.5 mM), MM-SNAC (20 equiv, 10 mM), $NADP^+$ (0.5 equiv, 0.25 mM), glucose-6-phosphate (2.5 equiv, 1.25 mM), glucose-6-phosphate dehydrogenase (2 units/mL), Juv Mod6 (4 μM, 0.8 mol%), 20 hours, stationary, RT.

Workup and purification: Quenched with acetone (2x volume, 400 mL), placed in a -20 °C freezer for 1 h and filtered through a celite plug. Remaining insoluble material was suspended in acetone and this solution was used to rinse the celite plug. Acetone was removed through rotary evaporation and the aqueous layer was saturated with NaCl and extracted 3x EtOAc. Combined organic layers were concentrated. **13** was purified directly by preparatory HPLC using a Phenomenex Luna 5u C18 250 x 21.2mm column (serial 444304-4) monitoring at 250nM. Method 9mL/min, A: H_2O + 0.1% formic acid, B: MeCN + 0.1% formic acid, 5% B 0-5 min, 5-100% B linear gradient 5-45 min, 100% B 45-65 min, 5% 65-75 min.

13 from Tyl hexaketide **3** (4.2 mg, 0.01 mmol, 11.7% yield).
^{1}H NMR (700 MHz, CD_3OD) δ 6.49 (d, *J* = 15.5 Hz, 1H), 6.21 (d, *J* = 15.5 Hz, 1H), 5.46 (d, *J* = 9.8 Hz, 1H), 3.95 – 3.91 (m, 1H), 3.37 – 3.34 (m, 1H), 2.79 – 2.73 (m, 1H), 2.64 – 2.59 (m, 1H), 2.23 (dd, *J* = 18.0, 6.2 Hz, 1H), 1.86 – 1.79 (m, 2H), 1.78 (s, 2H), 1.73 (s, 2H), 1.69 – 1.64 (m, 2H), 1.57 – 1.51 (m, 2H), 1.47 – 1.41 (m, 2H), 1.39 – 1.33 (m, 2H), 1.17 (d, *J* = 6.9 Hz, 2H), 1.00 (d, *J* = 6.9 Hz, 2H), 0.96 (t, *J* = 7.5 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H).
^{13}C NMR (700 MHz; CD_3OD) δ 178.84, 145.04, 136.20, 135.13, 133.34, 118.42, 108.00, 79.91, 78.19, 42.17, 39.55, 36.59, 33.35, 28.38, 25.59, 17.60, 17.40, 12.83, 11.43, 11.26, 10.90.
HRMS: Calculated $[M+H]^+$ 351.2530, found 351.2533


O
OH
OH
O
O

7

8.0
7.5
7.0
6.5
6.0
5.5
5.0
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2.0
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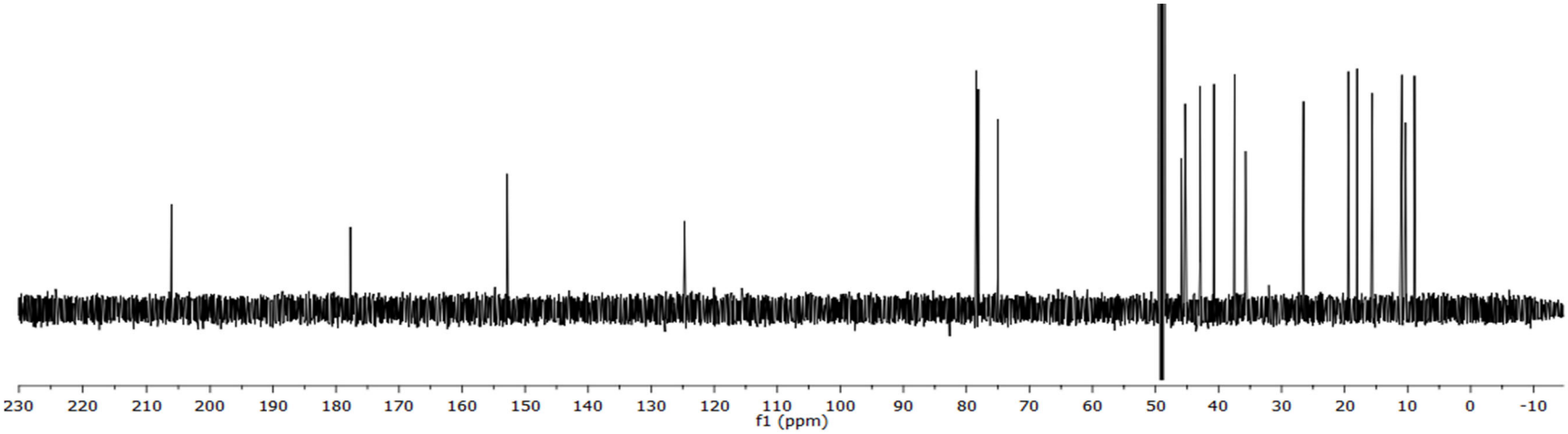
f1 (ppm)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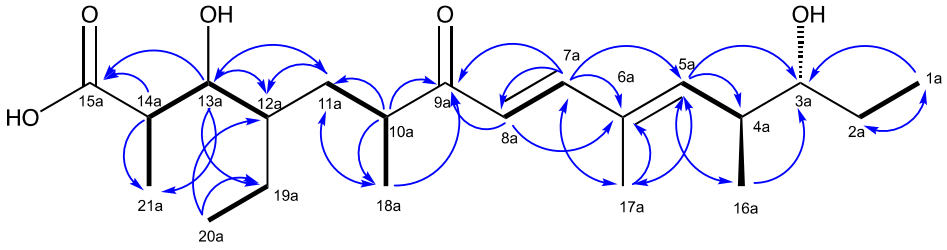
Cosy Correlation

HMBC Correlation



11a

COSY and HMBC correlations seen from easily discernable, single peak hydrogens. Overlapping signals as well as clear correlations from obscured hydrogens are denoted in the table. NMRs taken in D-6 Acetone.

Position	δ ^{1}H (mult, J Hz)	δ ^{13}C	COSY	HMBC
1a	0.96 (t, J = 7.4)	10.8$^{\alpha}$	2a', 2a''	2a, 3a
2a'	1.56 - 1.48 (m)	28.9$^{\alpha}$	1a, 2a'', 3a	1a
2a''	1.41 - 1.28 (obs)$^{\beta,\delta}$			
3a	3.38 - 3.34 (m)$^{\beta}$	77.1$^{\alpha}$	2a', 2a'', 4a	
3a-OH	4.43 (d, J = 7.3)		3a	
4a	2.66 - 2.61 (m)	40.6$^{\alpha}$	3a	3a, 5a, 6a, 16a
5a	5.96 (d, J = 8.7)	146.4	4a	3a, 4a, 16a, 17a
6a		133.6		
7a	7.12 (d, J = 16.1)	148.0$^{\alpha}$	8a	5a, 6a, 8a, 9a, 17a
8a	5.96 (d, J = 16.3)	125.8	7a	6a, 7a, 9a
9a		206.4		
10a	3.76 - 3.71 (m)	40.4	11a', 11a'', 18a	9a, 11a, 18a
11a'	1.65 (ddd, J = 13.9, 7.5, 4.1)	36.4	10a, 11a''	12a, 13a, 18a
11a''	1.41 - 1.28 (obs)$^{\delta}$			
12a	1.41 - 1.28 (obs)$^{\beta,\delta}$	38.4		
13a	3.53 (dd, J = 9.9, 2.6)	73.8	12a, 14a	11a, 12a, 15a, 21a
14a	2.27 (qd, J = 7.3, 2.5)	41.0	13a, 21a	15a, 21a
15a		178.4		
16a	1.01 (d, J = 6.2)	17.9$^{\alpha}$	4a	3a, 5a
17a	1.80 (s)	12.8$^{\alpha}$		5a, 6a, 7a, 16a
18a	1.00 (d, J = 5.5)	18.4	10a	9a, 11a
19a'	1.25 - 1.18 (m)	23.7	19a''	13a
19a''	0.93 - 0.89 (obs.)$^{\delta}$			
20a	0.81 (t, J = 7.4)	11.1	19a''	12a, 19a
21a	1.08 (d, J = 6.7)	11.7	14a	13a, 14a, 15a

$^{\alpha}$ Peaks assigned based on relative height

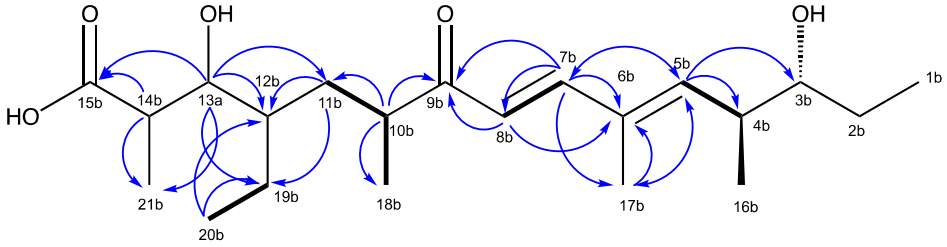
$^{\beta}$ May be interchanged between isomers

$^{\delta}$ Signals cannot be unambiguously assigned due to heavy overlap

Obs. Indicates the peak is obscured but is visibly present due to 2D correlations in the designated range

Cosy Correlation

HMBC Correlation



11b

COSY and HMBC correlations seen from easily discernable, single peak hydrogens. Overlapping signals as well as clear correlations from obscured hydrogens are denoted in the table. NMRs taken in D-6 Acetone

Position	δ ^{1}H (mult, J Hz)	δ ^{13}C	COSY	HMBC
1b	0.94 - 0.90 (m)$^{\beta}$	10.8$^{\alpha}$		
2b'	1.54 - 1.33 (m)$^{\beta,\delta}$	28.8$^{\alpha}$		
2b''	1.54 - 1.33 (m)$^{\beta,\delta}$			
3b	3.45 - 3.40 (m)$^{\beta}$	76.9$^{\alpha}$		
3b-OH	3.69 (d, J = 5.7)		3b	
4b	2.71 - 2.66 (m)	39.80	3b	
5b	6.12 (d, J = 9.9)	146.6	4b	3b, 4b, 7b, 17b
6b		133.9$^{\alpha}$		
7b	7.33 (d, J = 15.8)	148.8	8b	5b, 6b, 8b, 9b, 17b
8b	6.26 (d, J = 15.8)	124.6	7b	6b, 9b
9b		206.1		
10b	3.32 - 3.27 (m)	42.3	11b, 18b	9b, 11b, 18b
11b'	2.16 (ddd, J = 13.5, 9.4, 3.6)	33.2$^{\alpha}$	10b, 11b''	12b, 19b
11b''	1.35 - 1.28 (m)			
12b	1.41 - 1.28 (obs)$^{\beta,\delta}$	39.6$^{\alpha}$		
13b	3.78 (dd, J = 9.6, 2.7)	71.6		11b, 12b, 15b, 19b, 21b
14b	2.36 (qd, J = 7.3, 2.7)	40.6$^{\alpha}$		15b, 21b
15b		176.3		
16b	1.05 (d, J = 6.9)	17.5$^{\alpha}$	4b	3b, 5b
17b	1.85 (s)	12.8$^{\alpha}$		5b, 6b
18b	1.07 (d, J = 7.05)	19.2	10b	9b, 10b, 11b
19b'	1.41 - 1.28 (m)	22.4		
19b''	1.15 - 1.12 (m)			20a
20b	0.86 (t, J = 7.4)	10.6	19b', 19b''	12b, 19b
21b	1.09 (d, J = 6.0)	11.4$^{\alpha}$	14b	13b, 15b

$^{\alpha}$ Peaks assigned based on relative height

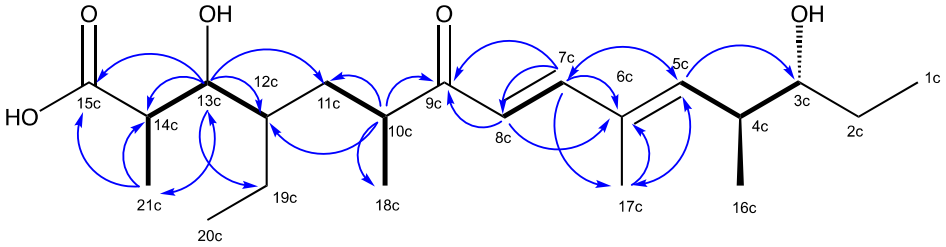
$^{\beta}$ May be interchanged between isomers

$^{\delta}$ Signals cannot be unambiguously assigned due to heavy overlap

Obs. Indicates the peak is obscured but is visibly present due to 2D correlations in the designated range

Cosy Correlation

HMBC Correlation



11c

COSY and HMBC correlations seen from easily discernable, single peak hydrogens. Overlapping signals as well as clear correlations from obscured hydrogens are denoted in the table. NMRs taken in D-6 Acetone

Position	δ ^{1}H (mult, J Hz)	δ ^{13}C	COSY	HMBC
1c	0.94 - 0.90 (m)$^{\beta}$	10.8		
2c'	1.41 - 1.28 (obs)$^{\beta,\delta}$	28.7$^{\alpha}$		
2c''	1.41 - 1.28 (obs)$^{\beta,\delta}$			
3c	3.45 - 3.40 (m)$^{\beta}$	76.9$^{\alpha}$	4b	
3c-OH				
4c	2.71 - 2.66 (m)	39.80	5b	5b, 6b
5c	6.01 (d, J = 10.1)	146.0	4b	3b, 7b, 17b
6c		133.9$^{\alpha}$		
7c	7.26 (d, J = 15.8)	148.1$^{\alpha}$	8b	5b, 6b, 8b, 9b, 17b
8c	6.26 (d, J = 15.7)	123.9	7b	6b, 9b
9c		204.0		
10c	3.02 (h, J = 6.9)	43.2$^{\alpha}$	11b', 18b	9b, 11b, 12b, 18b
11c'	1.99 (ddd, J = 14.7, 8.9, 4.3)	33.7	10b, 11b''	
11c''	1.27 - 1.23 (m)		11b'	19b
12c	1.41 - 1.28 (obs)$^{\beta,\delta}$	41.6		
13c	3.83 (dd, J = 6.8, 4.9)	73.8	12b, 14b	11b, 12b, 14b, 15b, 19b, 21b
14c	2.66 - 2.59 (m)	43.3	21b	12b, 13b, 15b, 21b
15c		177.0		
16c	1.04 (d, J = 6.6)	17.4	4b	3b, 5b
17c	1.84 (s)	12.7$^{\alpha}$		5b, 6b
18c	1.09 - 1.06 (obs.)$^{\delta}$	18.2		
19c	1.49 - 1.43 (obs.)$^{\delta}$	24.4$^{\alpha}$		
19c	1.41 - 1.33 (obs.)$^{\delta}$			
20c	0.94 - 0.90 (obs.)	11.3		
21c	1.17 (d, J = 7.0)	12.9$^{\alpha}$	14b	13b, 14b, 15b

$^{\alpha}$ Peaks assigned based on relative height

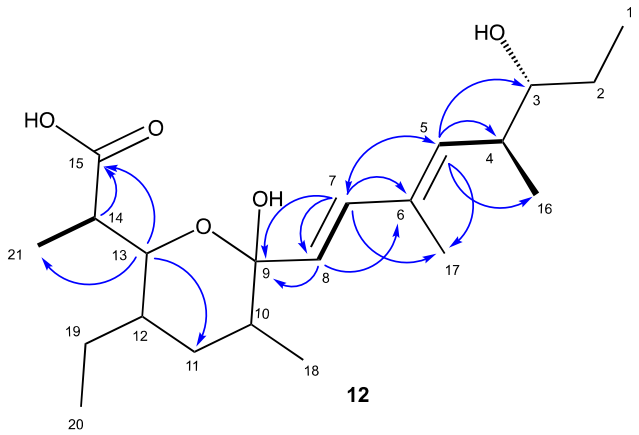
$^{\beta}$ May be interchanged between isomers

$^{\delta}$ Signals cannot be unambiguously assigned due to heavy overlap

Obs. Indicates the peak is obscured but is visibly present due to 2D correlations in the designated range

Cosy Correlation

HMBC Correlation



12

COSY and HMBC correlations seen from easily discernable, single peak hydrogens. Overlapping signals as well as clear correlations from obscured hydrogens are denoted in the table. NMRs taken in D-6 Acetone

Position	δ ^{1}H (mult, J Hz)	δ ^{13}C	COSY	HMBC
1	obs.$^{\delta}$	10.8$^{\alpha,\beta}$		
2'	1.41 - 1.28 (obs)$^{\beta,\delta}$	28.6$^{\alpha}$		
2''	1.41 - 1.28 (obs)$^{\beta,\delta}$			
3	3.38 - 3.34 (m)$^{\beta}$	77.0$^{\alpha}$		
3' OH				
4	2.65 - 2.60 (obs.)$^{\delta}$	39.1		
5	5.51 (d, J = 9.6)	136.4	4	3, 4, 7, 16
6		133.4		
7	6.38 (d, J = 15.8)	135.4	8	5, 8, 9, 17
8	5.61 (d, J = 15.9)	130.6	7	6, 9
9		97.8		
10	2.16 - 2.12 (m)	32.0$^{\alpha}$		
11'	1.41 - 1.28 (obs)$^{\beta,\delta}$	33.5		
11''	1.28 - 1.23 (obs.)$^{\delta}$			
12	1.49 - 1.45 (obs.)$^{\delta}$	39.3	13	11
13	4.29 (dd, J = 10.3, 2.7)	74.4$^{\alpha}$	12	11, 14, 15, 21
14	2.75 (qd, J = 7.0, 2.5)	40.7$^{\alpha}$	21	
15		175.9		
16	1.06 - 1.02 (obs.)$^{\delta}$	17.7$^{\alpha}$		4, 5
17	1.73 (s)	12.9		5, 6
18	0.89 - 0.85 (obs.)	16.9		9, 11
19'	1.49 - 1.43 (obs.)$^{\delta}$	24.5$^{\alpha}$		
19''	1.41 - 1.33 (obs.)$^{\delta}$			
20	obs.$^{\delta}$	obs.$^{\delta}$		
21	1.10 (d, J = 7.1)	9.3	14	13, 14, 15

$^{\alpha}$ Peaks assigned based on relative height

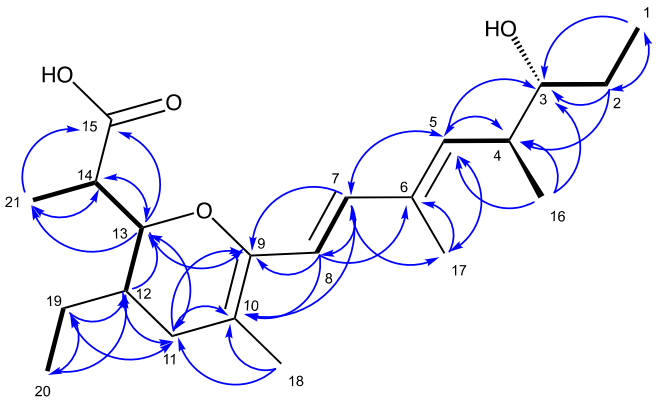
$^{\beta}$ Protons cannot be unambiguously assigned, may be interchanged between isomers

$^{\delta}$ Signals cannot be unambiguously assigned due to heavy overlap

Cosy Correlation

HMBC Correlation

Obs. Indicates the peak is obscured but is visibly present due to 2D correlations in

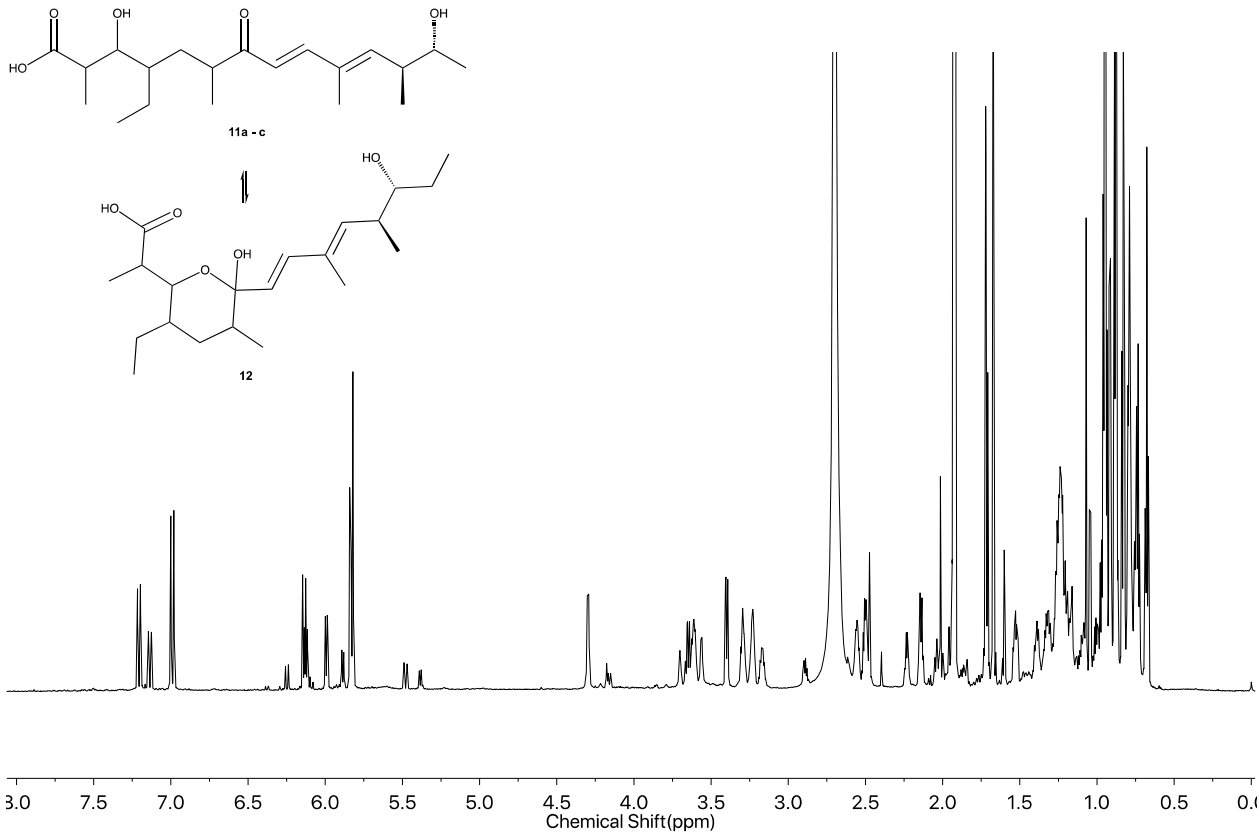


13

the designated rang

NMRs taken in D-6 Acetone

Position	δ ^{1}H (mult, J Hz)	δ ^{13}C	COSY	HMBC
1	0.92 (t, J = 7.4)	10.9	2', 2''	2, 3
2'	1.39 - 1.32 (m)	28.7	1, 3	1, 3, 4
2''	1.46 - 1.40 (m)		1	1
3	3.38 (dt, J = 8.5, 4.2)	77.3	2'	
3' OH				
4	2.64 - 2.57 (m)	39.3	5, 16	5
5	5.52 (d, J = 9.9)	136.6	4	3, 4, 7, 17
6		134.5		
7	6.51 (d, J = 15.4)	133.2	8	5, 8, 9, 10, 17
8	6.23 (d, J = 15.4)	118.3	7	6, 9, 10
9		144.7		
10		107.6		
11'	1.82 (dd, J = 18.7, 8.0)	33.1	11''	10, 12, 19
11''	2.24 (dd, J = 18.1, 6.2)		11'	9, 10, 12, 13, 19
12	1.72 - 1.66 (m)	36	13	11, 13
13	3.98 (dd, J = 7.3, 5.4)	79.6	12, 14	9, 11, 14, 15, 21
14	2.81 - 2.72 (m)	41.5	13, 21	13, 15
15		176.5		
16	1.01 (d, J = 6.9)	17.9	4	3, 4, 5
17	1.78 (s)	12.8		5, 6, 7
18	1.74 (s)	17.5		10, 11
19'	1.26 - 1.19 (m)	25.1	20	11, 12, 20
19''	1.60 - 1.52 (m)		20	11, 12, 20
20	0.95 (t, J = 7.6)	11.3	19', 19''	12, 19
21	1.16 (d, J = 6.9)	11.2	14	14, 15


11a - c
12
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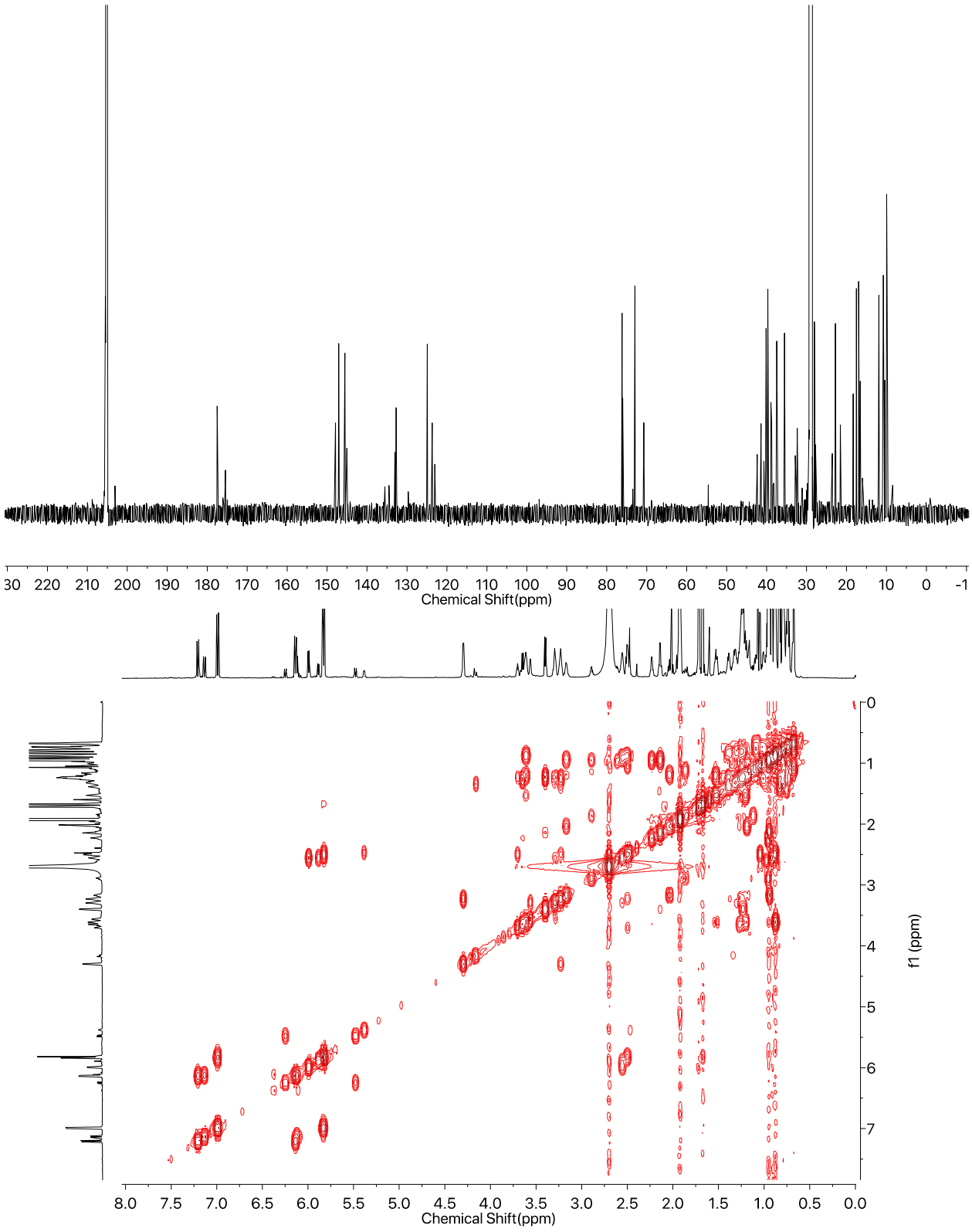
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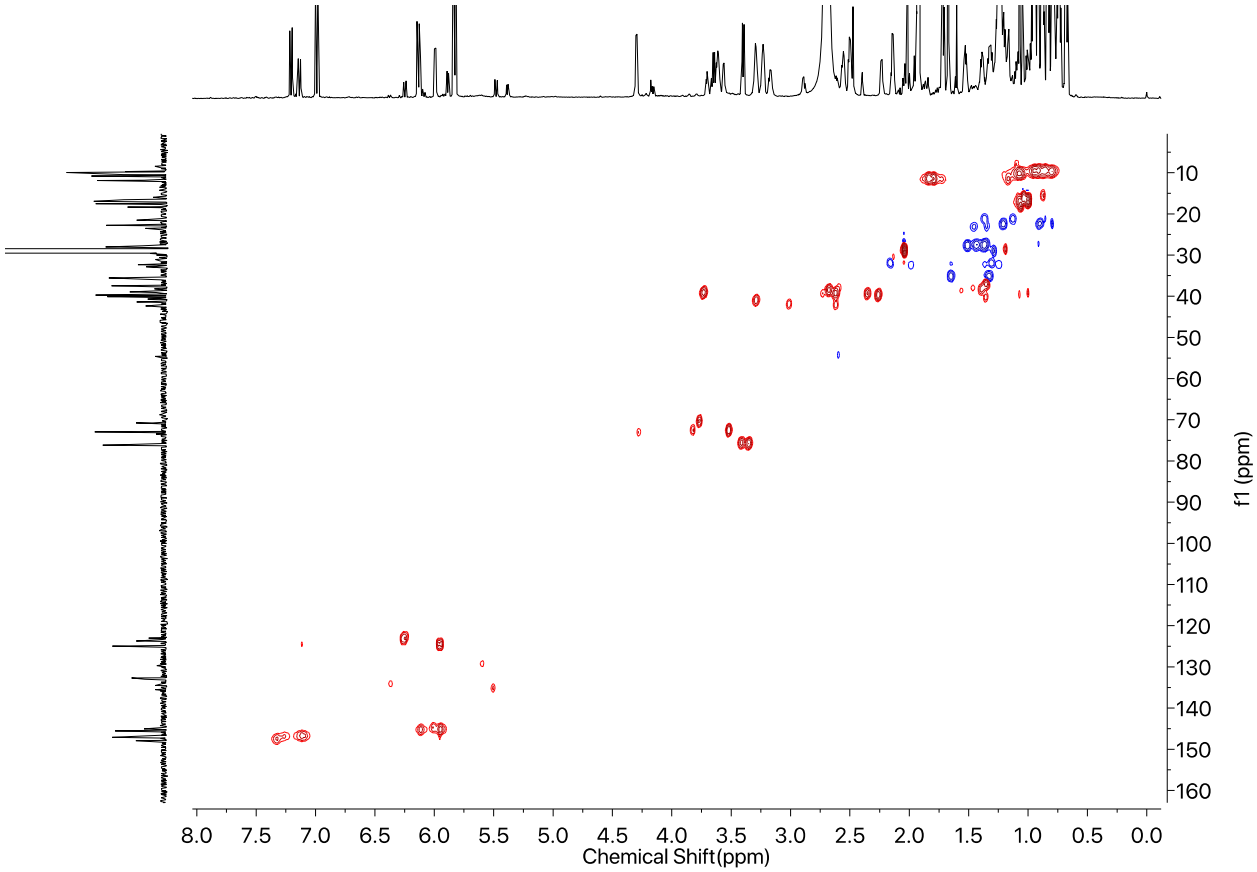
f1 (ppm)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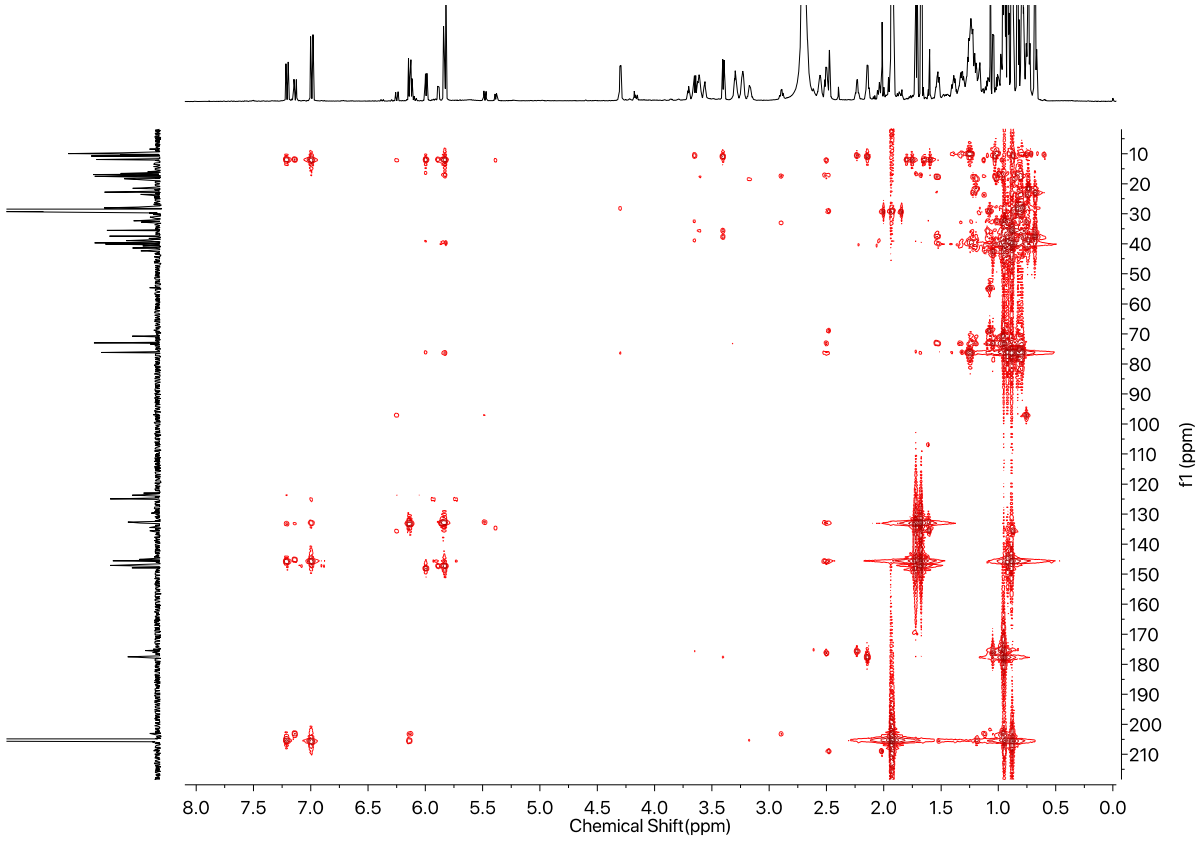
Chemical Shift(ppm)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f1 (ppm)
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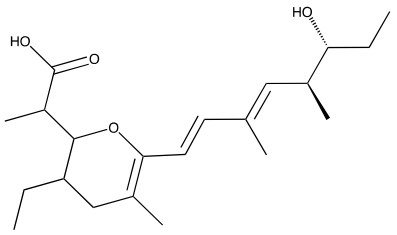
Chemical Shift(ppm)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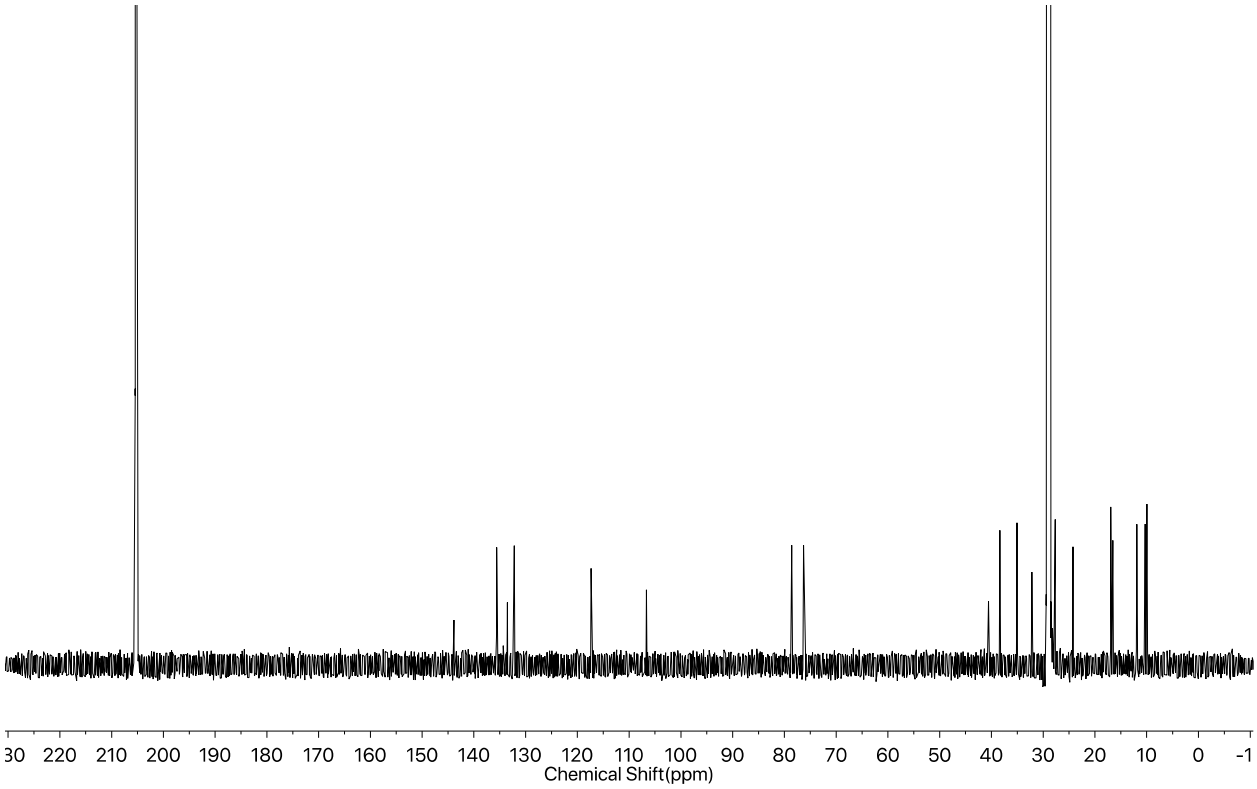
Chemical Shift(ppm)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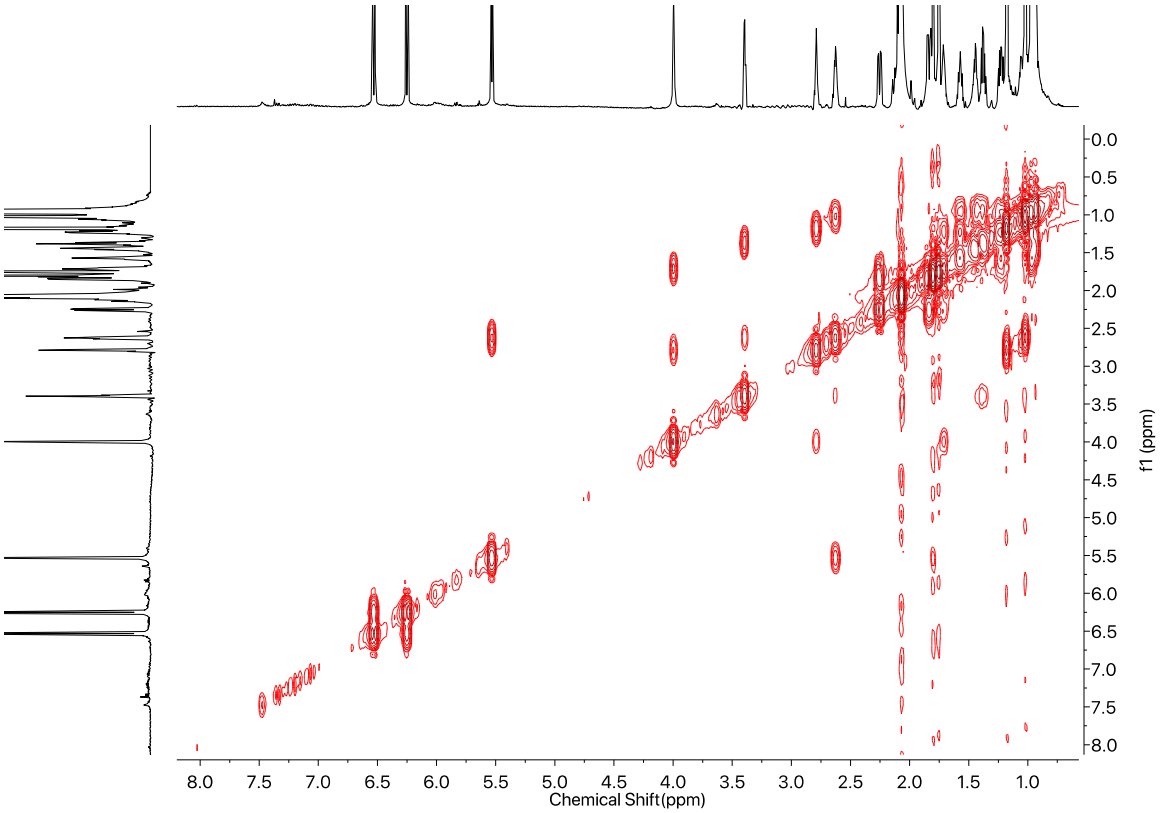
Chemical Shift(ppm)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Chemical Shift(ppm)
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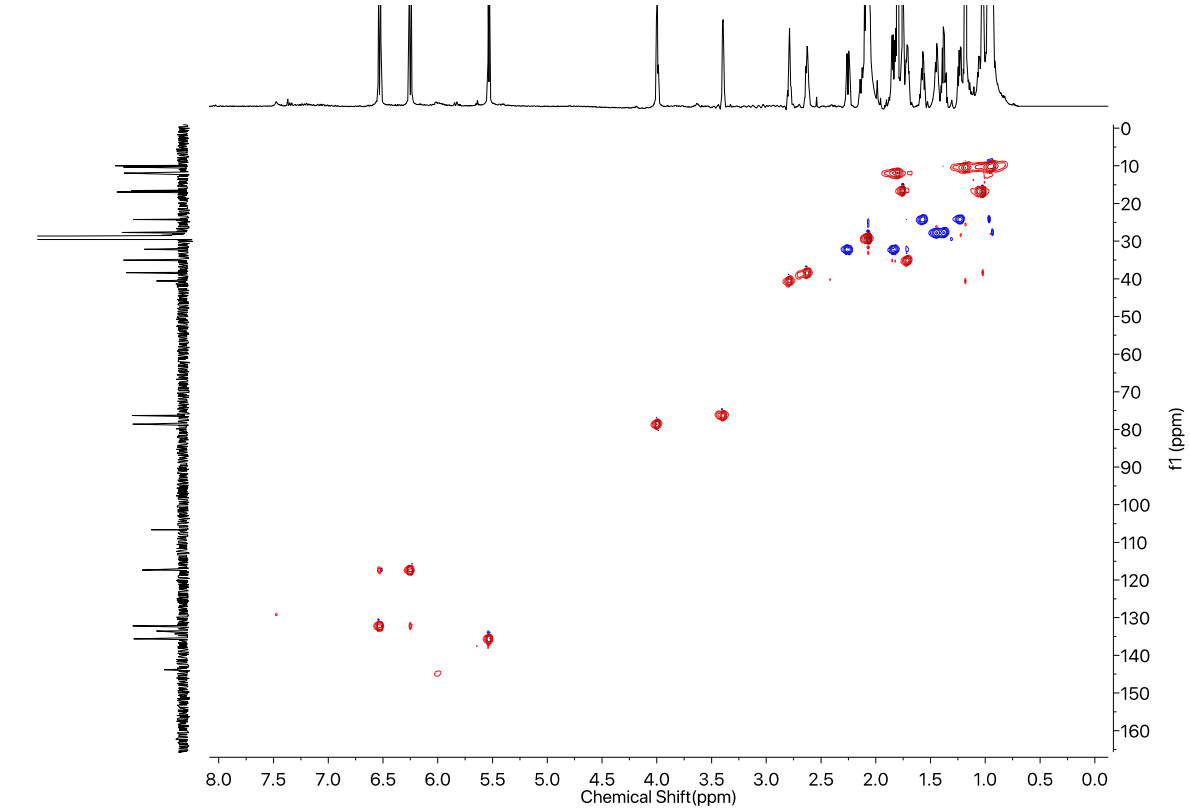
f1 (ppm)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f1 (ppm)
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7.5
7.0
6.5
6.0
5.5
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3.0
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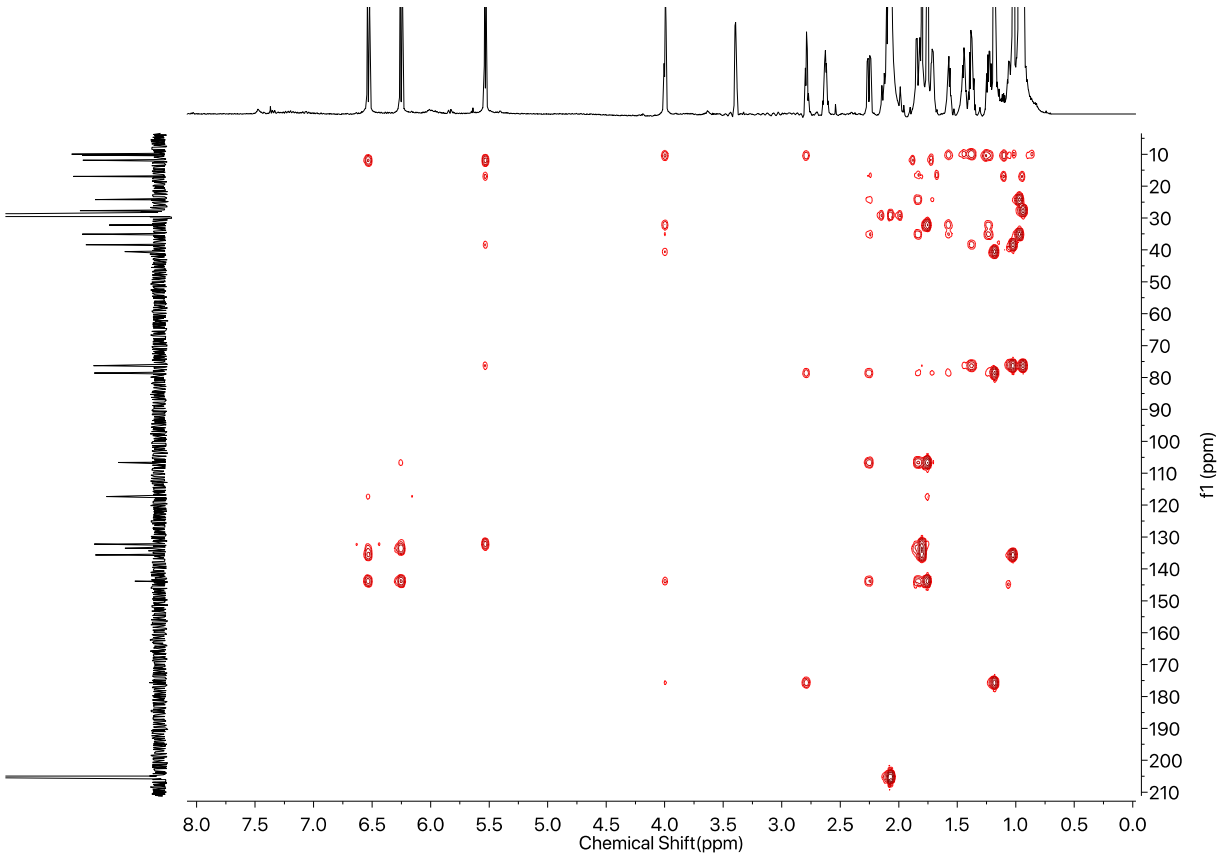
Chemical Shift(ppm)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f1 (ppm)
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6.5
6.0
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3.0
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1.0
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Chemical Shift(ppm)

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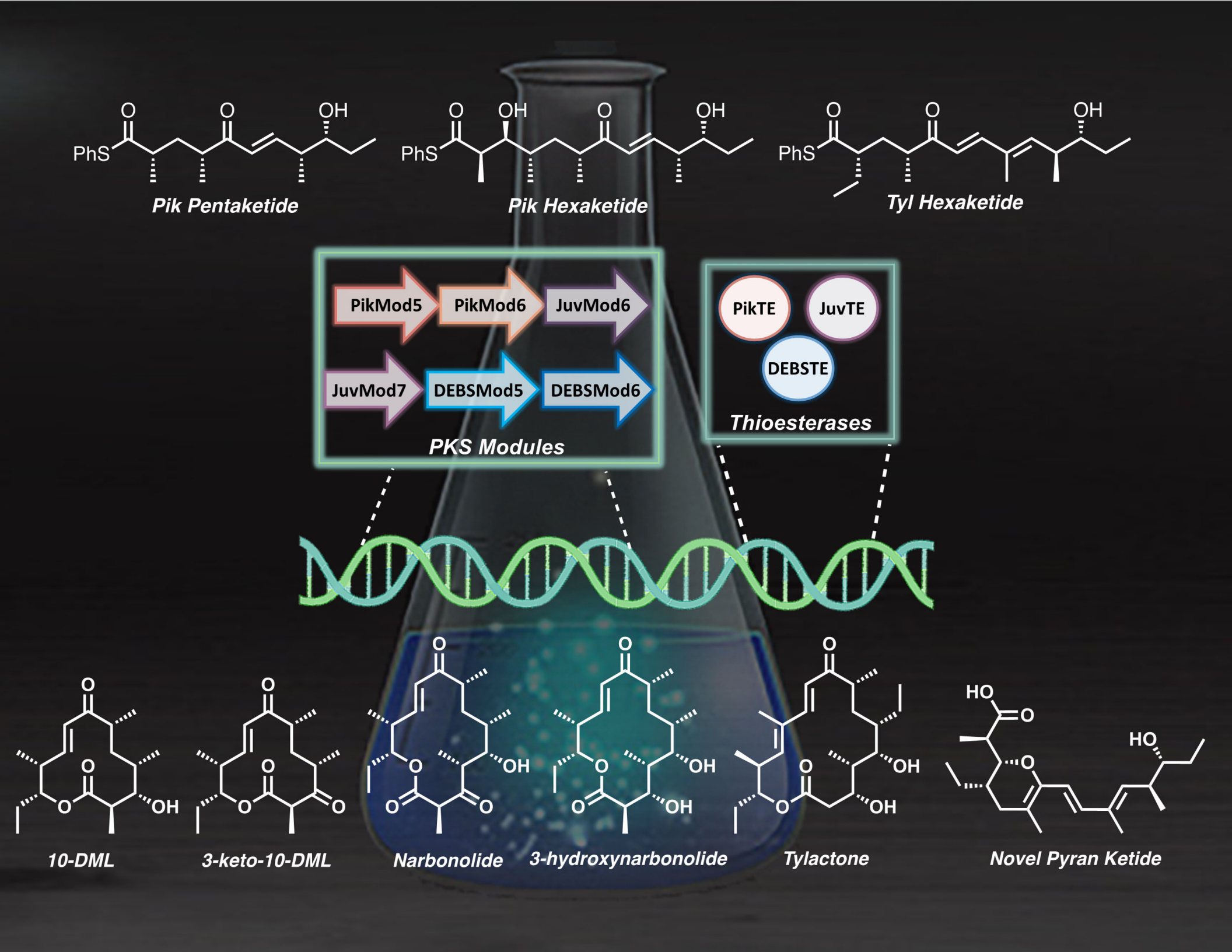
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JuvTE
DEBSTE
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Narbonolide
3-hydroxynarbonolide
Tylactone
Novel Pyran Ketide