Supporting information

for

Characterization of Three Tailoring Enzymes in Dutomycin Biosynthesis and Generation of a Potent Antibacterial Analogue

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Table S1. Plasmids used in this study.

Plasmid	Description	Reference
pLS51	*ks1* (part of *dutA*) in pJET1.2	this work
pLS52	*ks2* in pJET1.2	this work
pLS53	*ks3* in pJET1.2	this work
pLS54	*ks1* (part of *dutA*) in pKC1139	this work
pLS55	*ks2* in pKC1139	this work
pLS56	*ks3* in pKC1139	this work
pSW70	*dutGT2* fragment in pJET1.2	this work
pSW75	*dutGT2* fragment in pKC1139	this work
pSW82	*dutMT1* fragment in pJET1.2	this work
pSW91	*dutMT1* fragment in pKC1139	this work
pSW138	*dutGT1* fragment in pJET1.2	this work
pSW140	*dutGT1* fragment in pKC1139	this work
pSUN151	*dutMT1* in pJET1.2	this work
pSUN168	*dutMT1* in pET28a	this work

Table S2. Primers used in this study.*

Primer	Sequence
KSα-F	5'-TSGCSTGCTTCGAYGCSATC-3'
KSα-R	5'-TGGAANCCGCCGAABCCGCT-3'
DutMT1-F1	5'-AA**AAGCTT**CCTGCGCGACATGGTCCTGT-3'
DutMT1-R1	5'-AA**TCTAGA**CGACCAGGTTCTCGATCACG-3'
DutMT1-F2	5'-AA**CATATG**ACAGCTCCCGCTCT-3'
DutMT1-R2	5'-AA**CTCGAG**TCAGTTACCCGTGTCCCGGCGTT-3'
DutGT2-F	5'-AA**AAGCTT**ACGGCATCCTGGACGAGCAT-3'
DutGT2-R	5'-AA**TCTAGA**TTCGGCTGGGCGACGATCAA-3'
DutGT1-F	5'- AA**AAGCTT**GATCCGCGCGCTGTA-3'
DutGT1-R	5'- AA**TCTAGA**ATCTCTGTGCAGATCGGTGA -3'
DutA-Check1	5'-GTCTCCACGGGCTGTACCTC-3'
DutA-Check2	5'-ATCAGCCGGCTGGGCAGATG-3'
DutMT1-Check1	5'- ATGACAGCTCCCGCTCTCGAA -3'
DutMT1-Check2	5'- ATTTATTGGCGTCATCAGGTTGT -3'
DutGT2-Check1	5'-AGATCAAGCACAGTCCGGA-3'
DutGT2-Check2	5'-AAGCGACTCTGGAAGAGG-3'
DutGT1-Check1	5'-ATGGTCGACGAGTACGT-3'
DutGT1-Check2	5'- TGACGATGTCGATGGCCAA-3'
M13-47	5'-CGCCAGGGTTTTCCCAGTCACGAC-3'
RM-V	5'-GAGCGGATAACAATTTCACACAGG-3'

* Restriction sites are shown in bold.

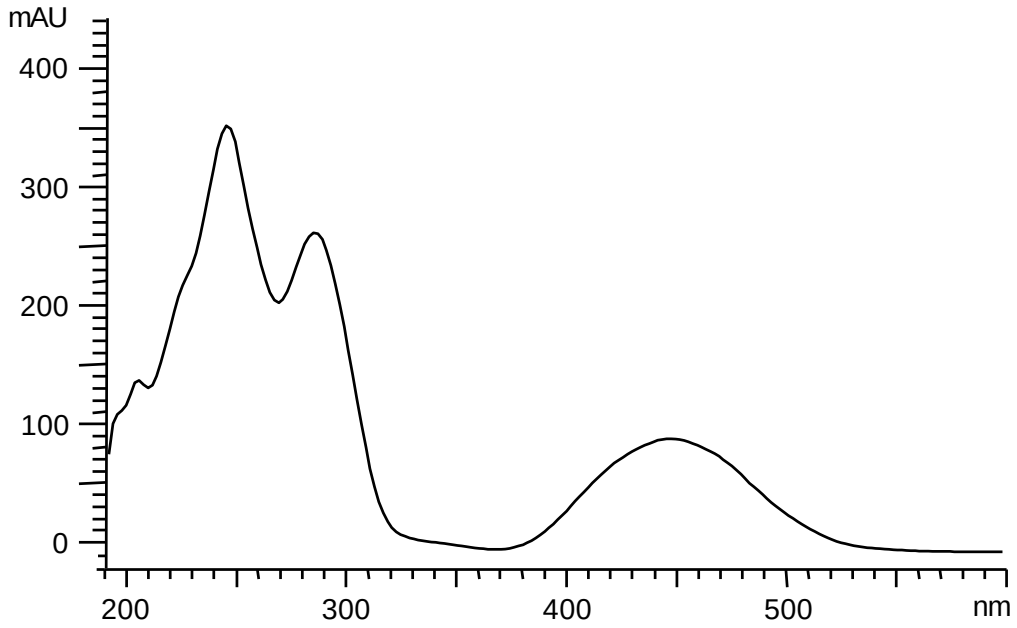


Figure S1. UV spectrum of **1** and **2**.

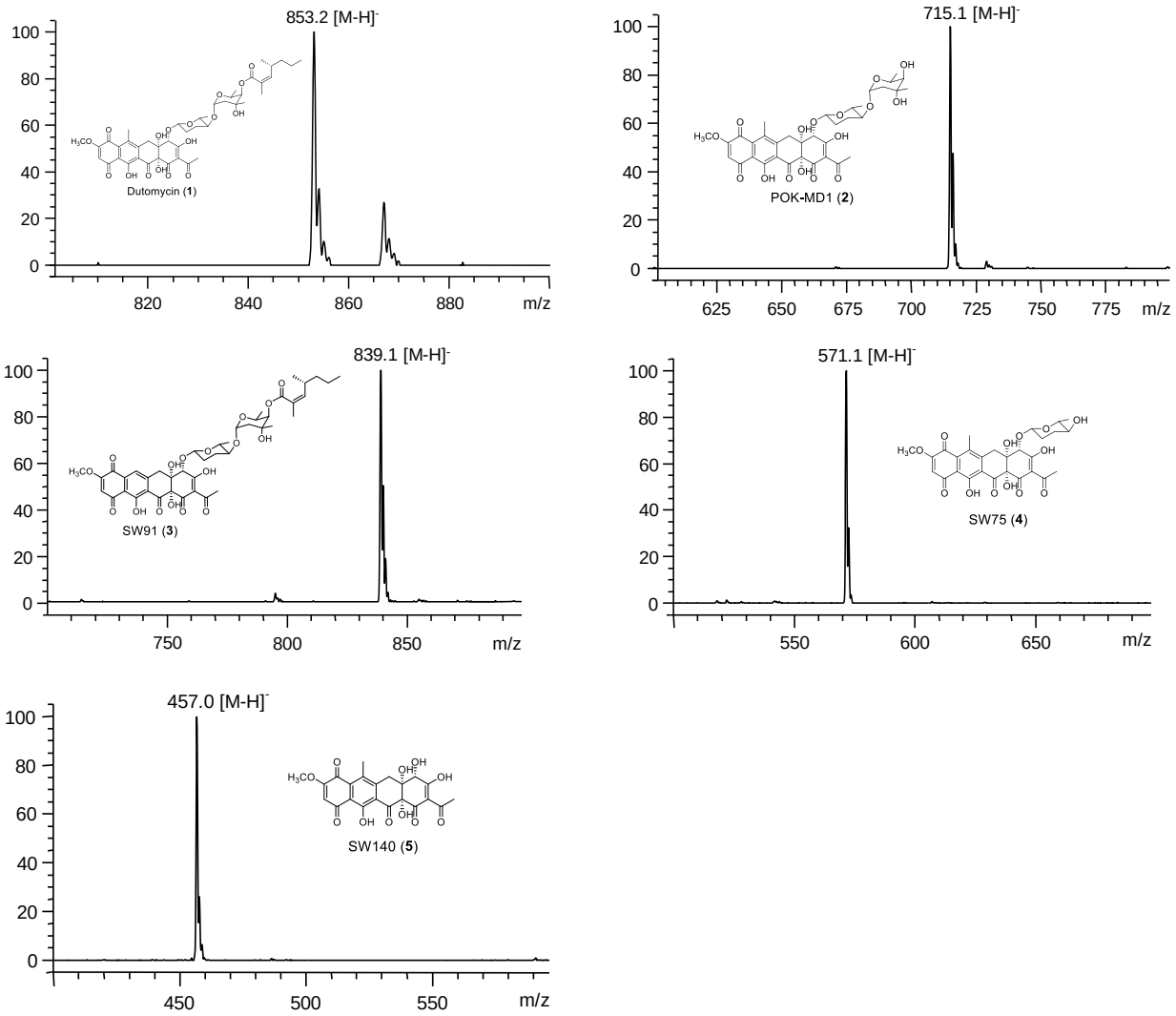
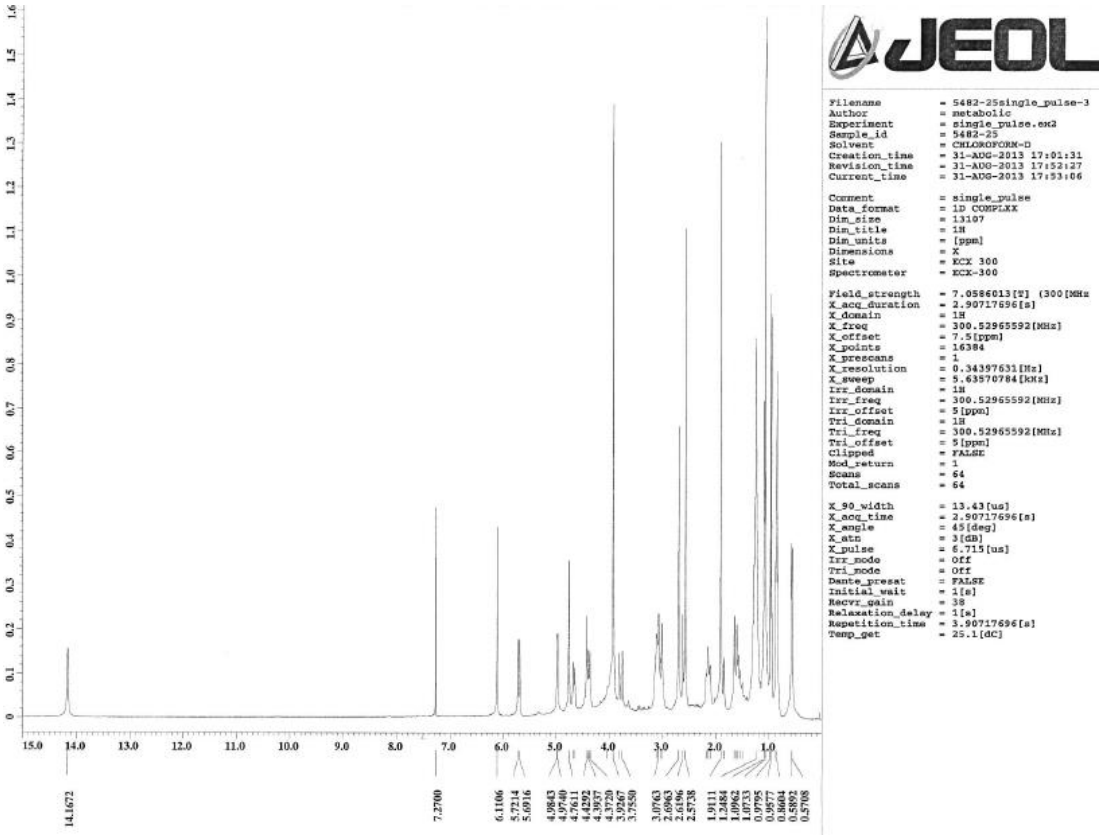


Figure S2. ESI-MS spectra of **1-5**.

a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X_freq = 300.52965592[MHz]
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X_prescans = 1
X_resolution = 0.34397631[Hz]
X_sweep = 5.63570784[kHz]
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Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Tri_domain = 1H
Tri_freq = 300.52965592[MHz]
Tri_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
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Total_scans = 64
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X_acq_time = 2.90717696[s]
X_angle = 45[deg]
X_atn = 3[dB]
X_pulse = 6.715[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 38
Relaxation_delay = 1[s]
Repetition_time = 3.90717696[s]
Temp_get = 25.1[dC]
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7.2700
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5.7214
5.6916
4.0843
4.0740
3.9761
4.4291
4.3937
4.3720
3.9267
3.7550
3.0763
2.6963
2.6196
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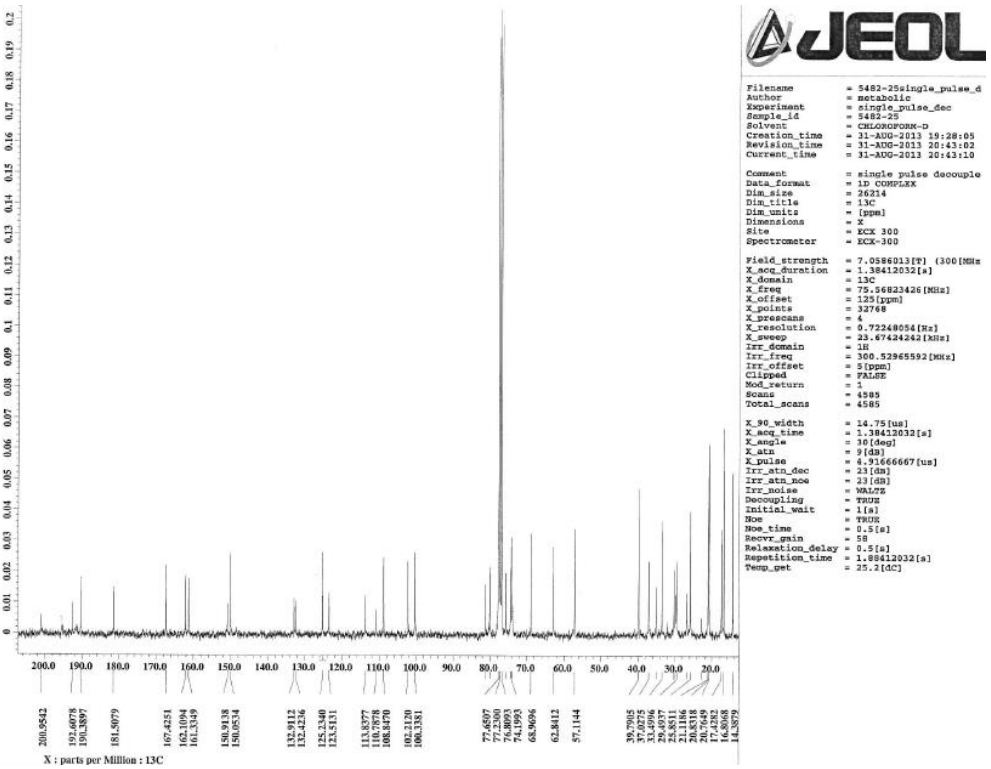
0.9597
0.9681
0.5892
0.5708

b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X_sweep = 23.67424242[kHz]
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Irr_freq = 300.52965592[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
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Total_scans = 4585
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Irr_atn_noe = 23[dB]
Irr_noise = WALTZ
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162.1094
161.3349
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132.9312
132.4236
125.2340
123.5131
113.8377
110.7878
108.8470
102.2120
100.3381
77.4597
77.2480
76.8093
74.1993
68.9696
62.8412
57.1144
39.7905
37.0275
31.1996
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X : parts per Million : 13C

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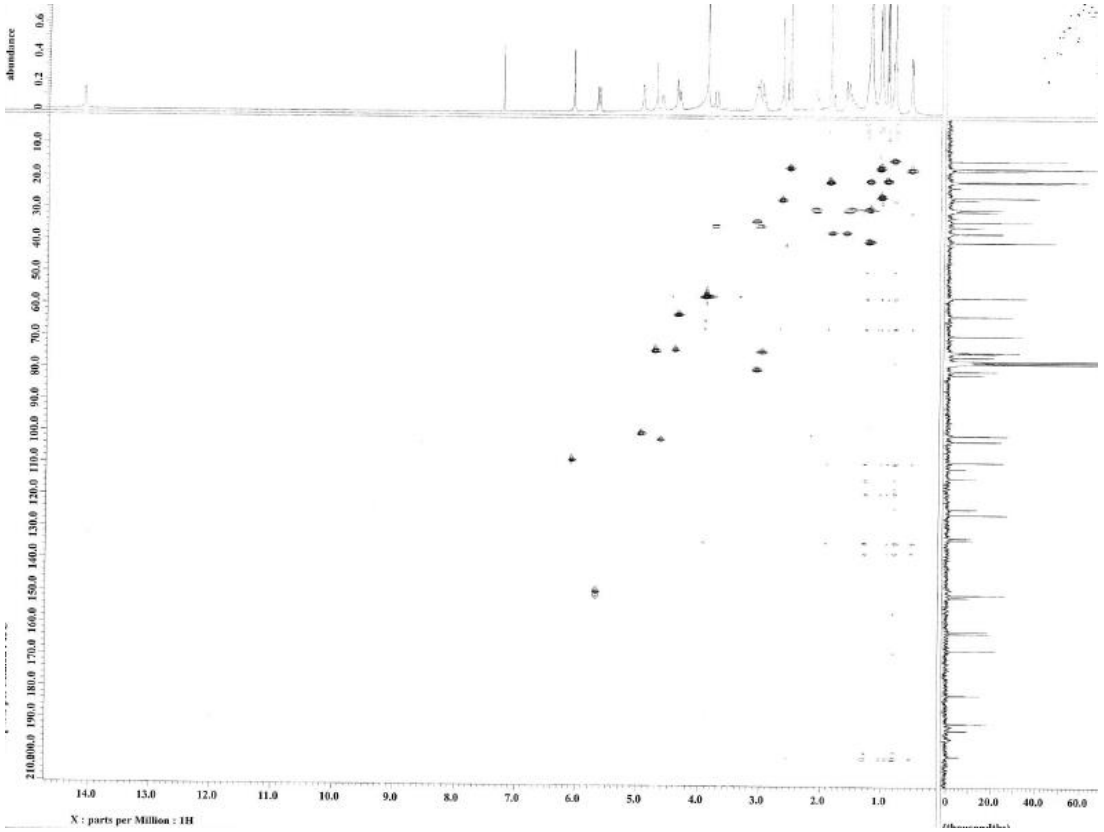
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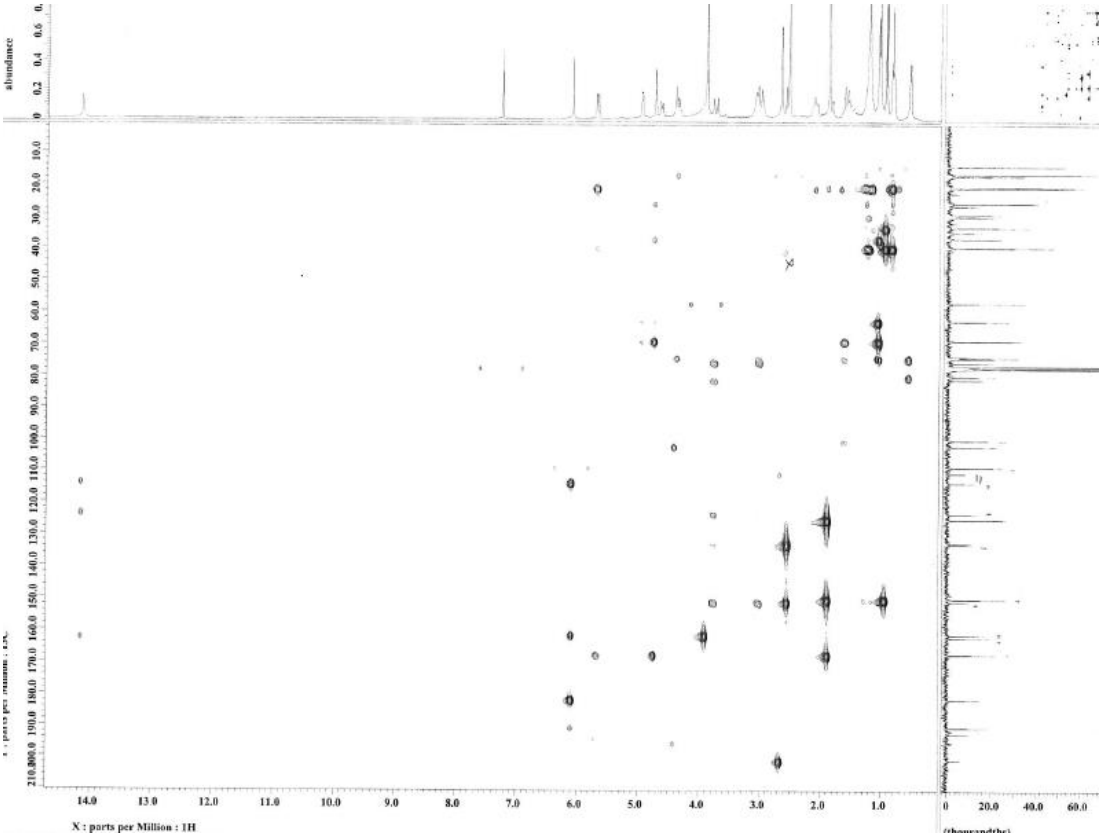
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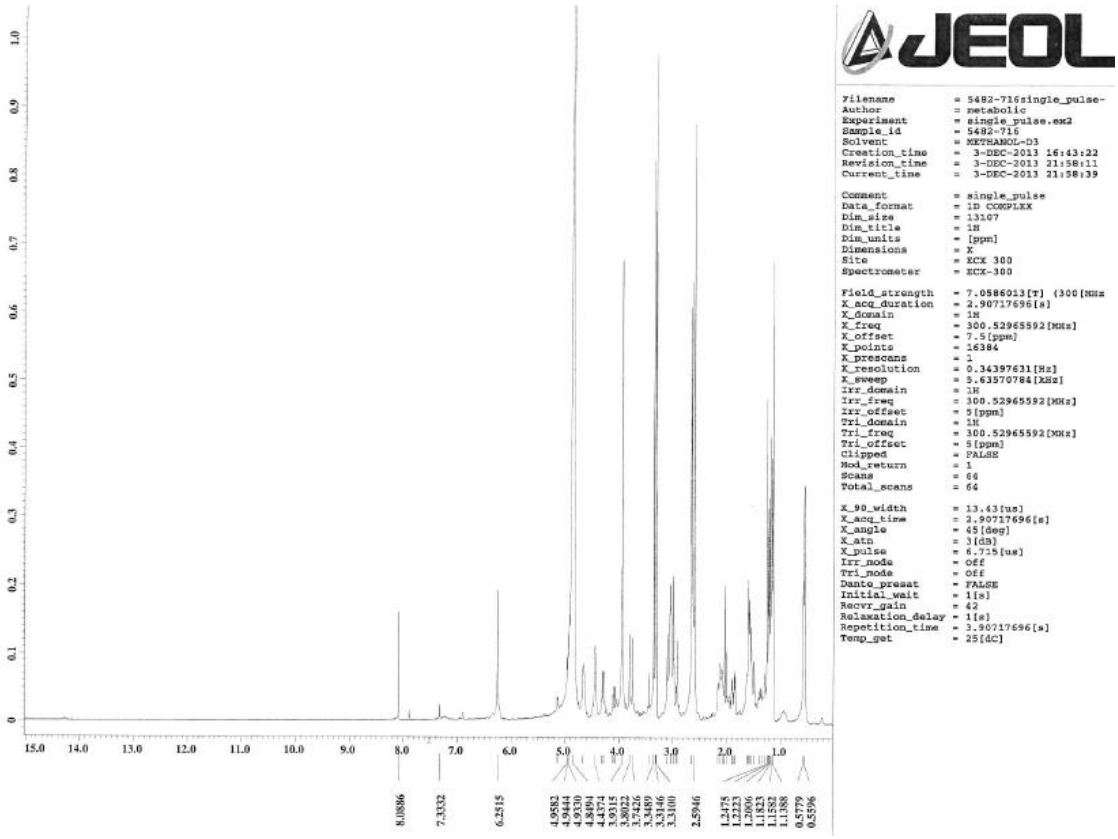

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Dim_units       = [ppm]
Dimensions      = X
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Spectrometer    = ECX-300

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X_acq_duration  = 2.90717696[s]
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X_offset        = 7.5[ppm]
X_points        = 16384
X_prescans      = 1
X_resolution    = 0.34397631[Hz]
X_sweep         = 5.63570784[kHz]
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Irr_freq        = 300.52965592[MHz]
Irr_offset      = 5[ppm]
Tri_domain      = 1H
Tri_freq        = 300.52965592[MHz]
Tri_offset      = 5[ppm]
Clipped         = FALSE
Mod_return      = 1
Scans           = 64
Total_scans     = 64

X_90_width      = 13.43[us]
X_acq_time      = 2.90717696[s]
X_angle         = 45[deg]
X_atn           = 3[dB]
X_pulse         = 6.715[us]
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Tri_mode        = Off
Dante_presat    = FALSE
Initial_wait    = 1[s]
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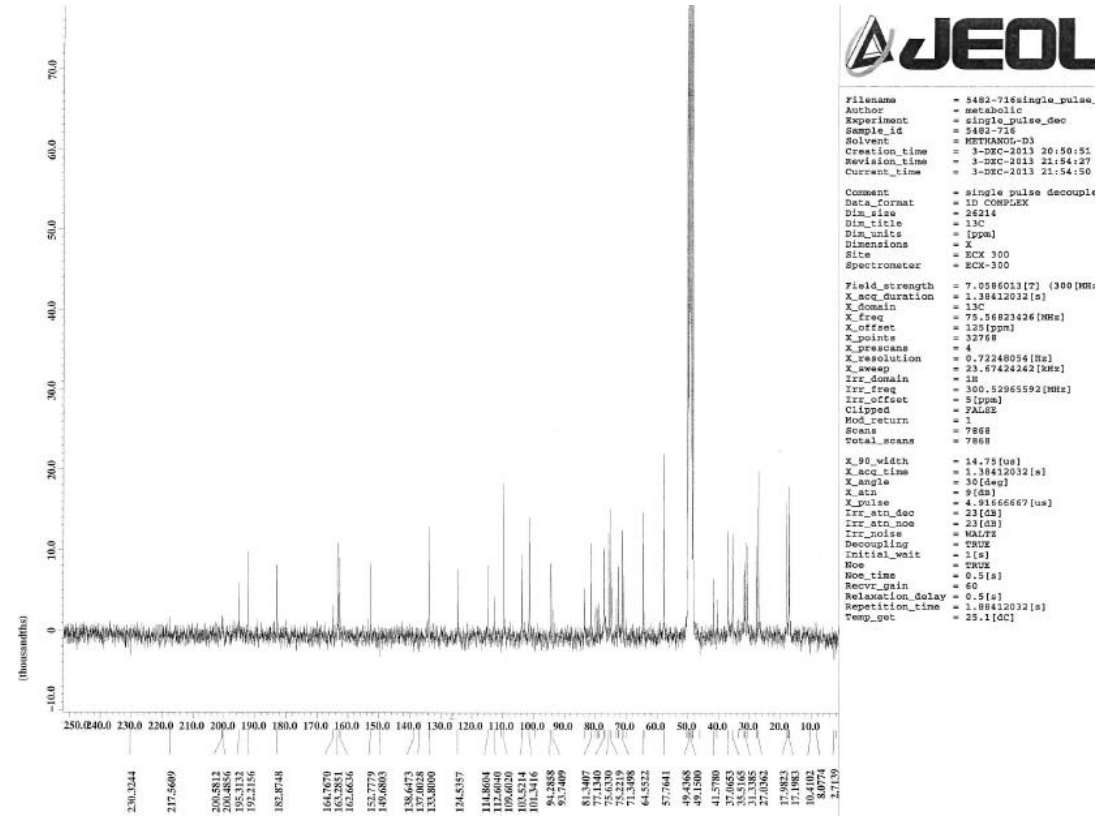

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Spectrometer    = ECX-300

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X_acq_duration  = 1.38412032[s]
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X_freq          = 75.56823426[MHz]
X_offset        = 125[ppm]
X_points        = 32768
X_prescans      = 4
X_resolution    = 0.72248056[Hz]
X_sweep         = 23.67424242[kHz]
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Irr_freq        = 300.52965592[MHz]
Irr_offset      = 5[ppm]
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X_pulse         = 4.91666667[us]
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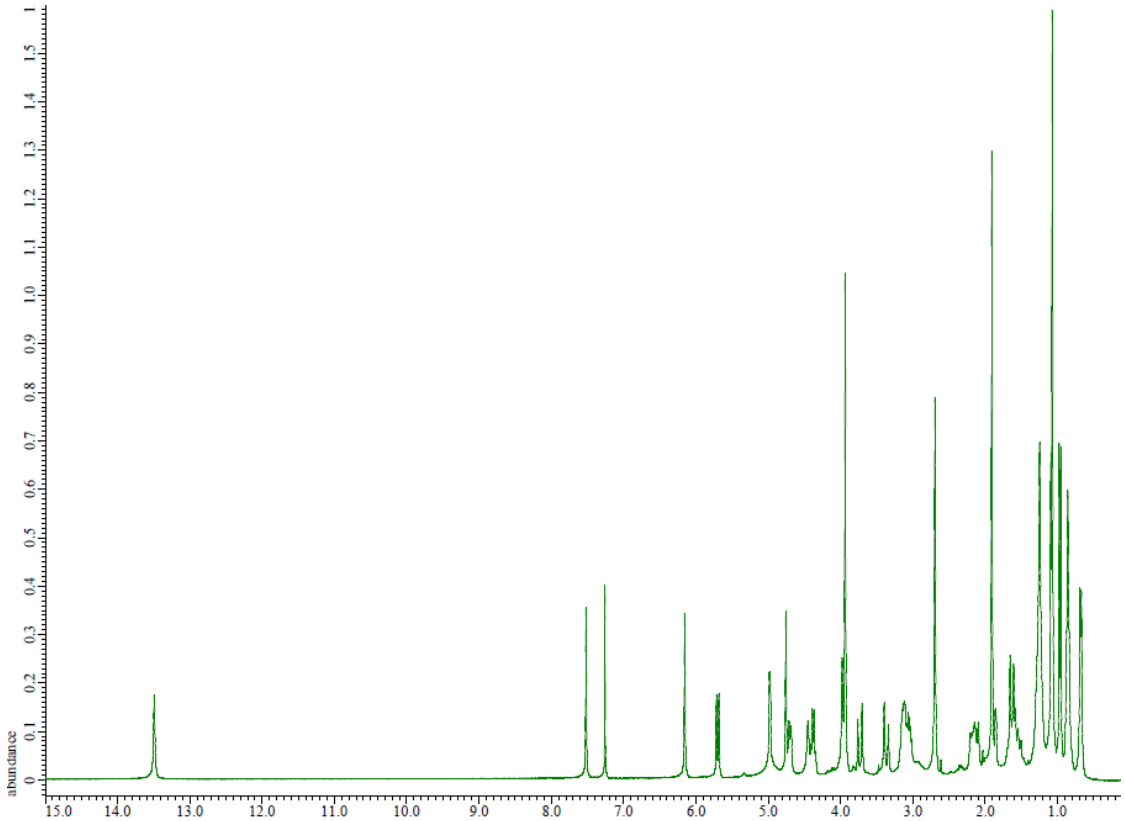
g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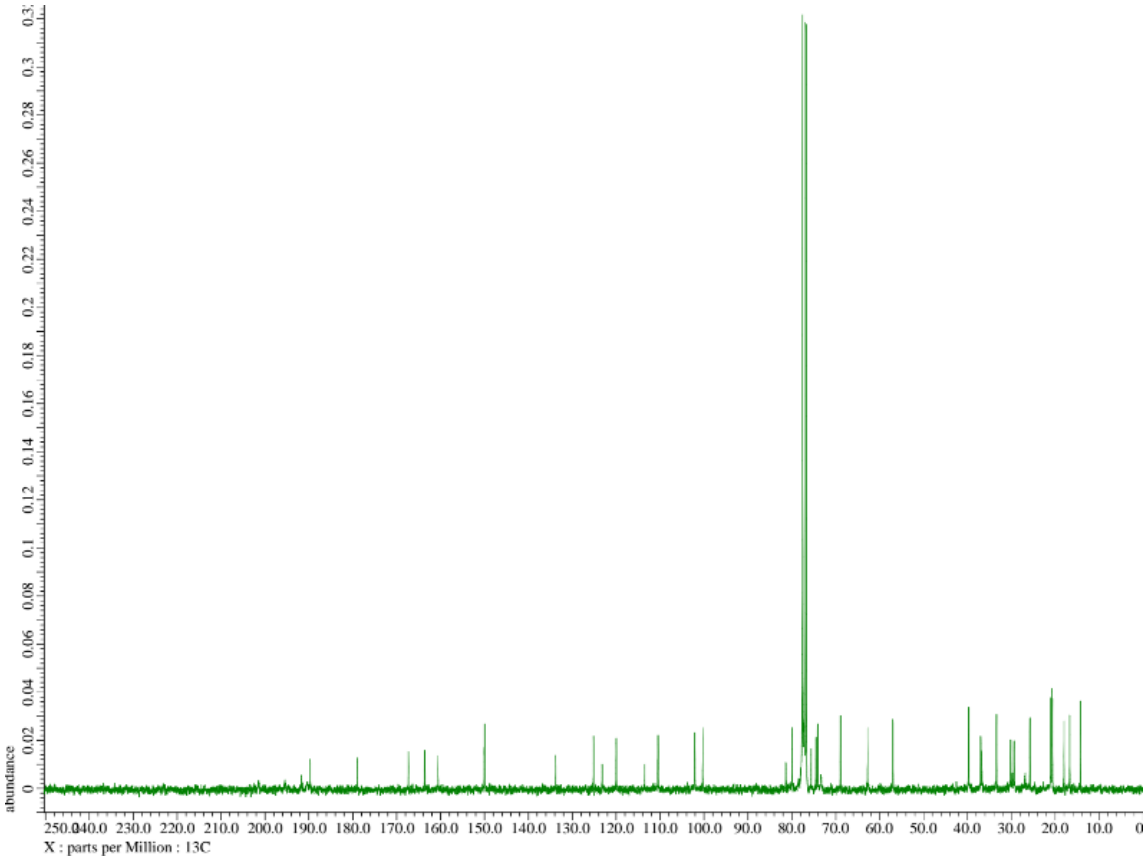
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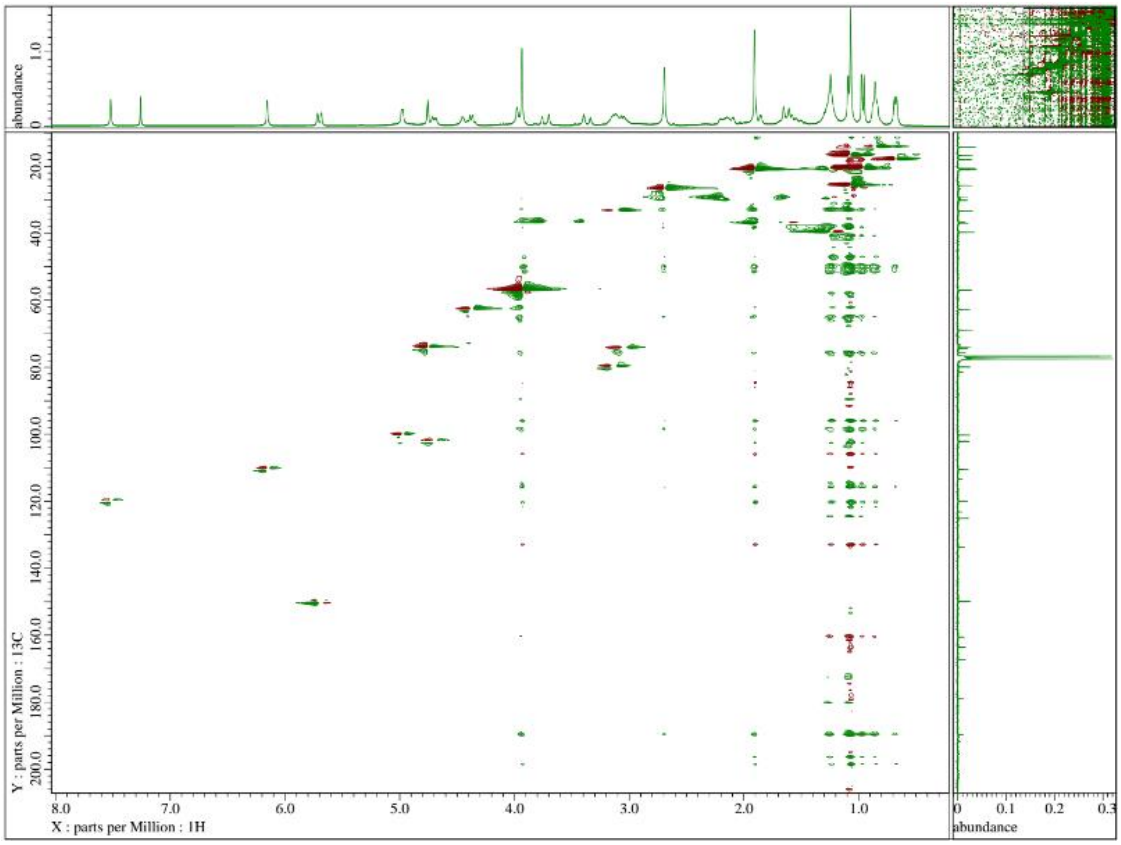
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X : parts per Million : 1H
abundance

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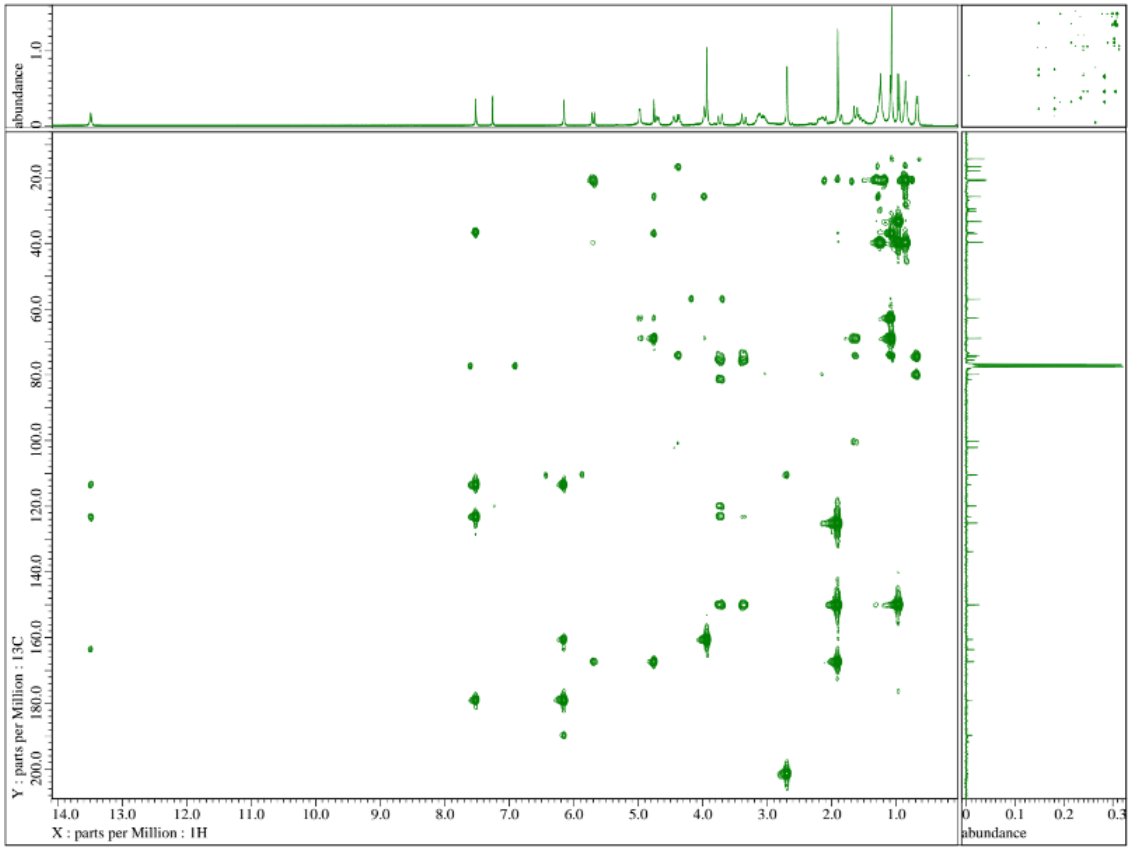
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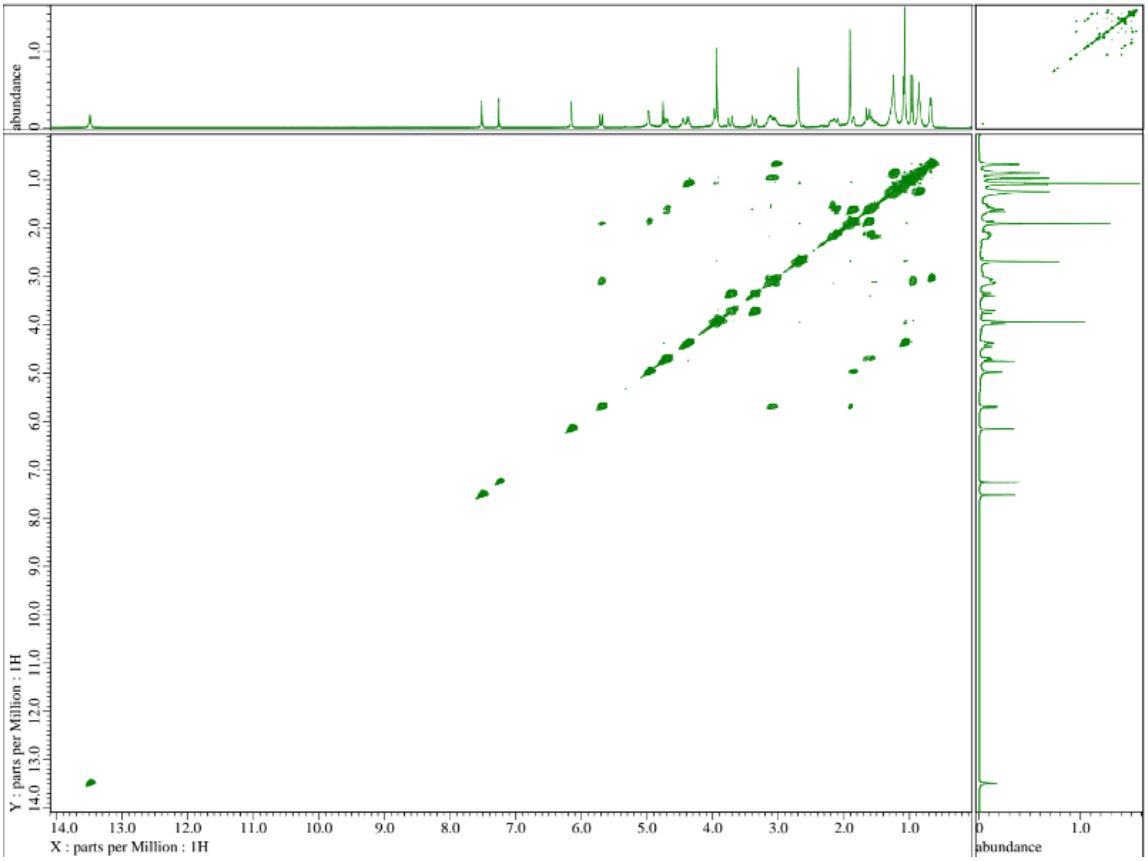
abundance

k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abundance

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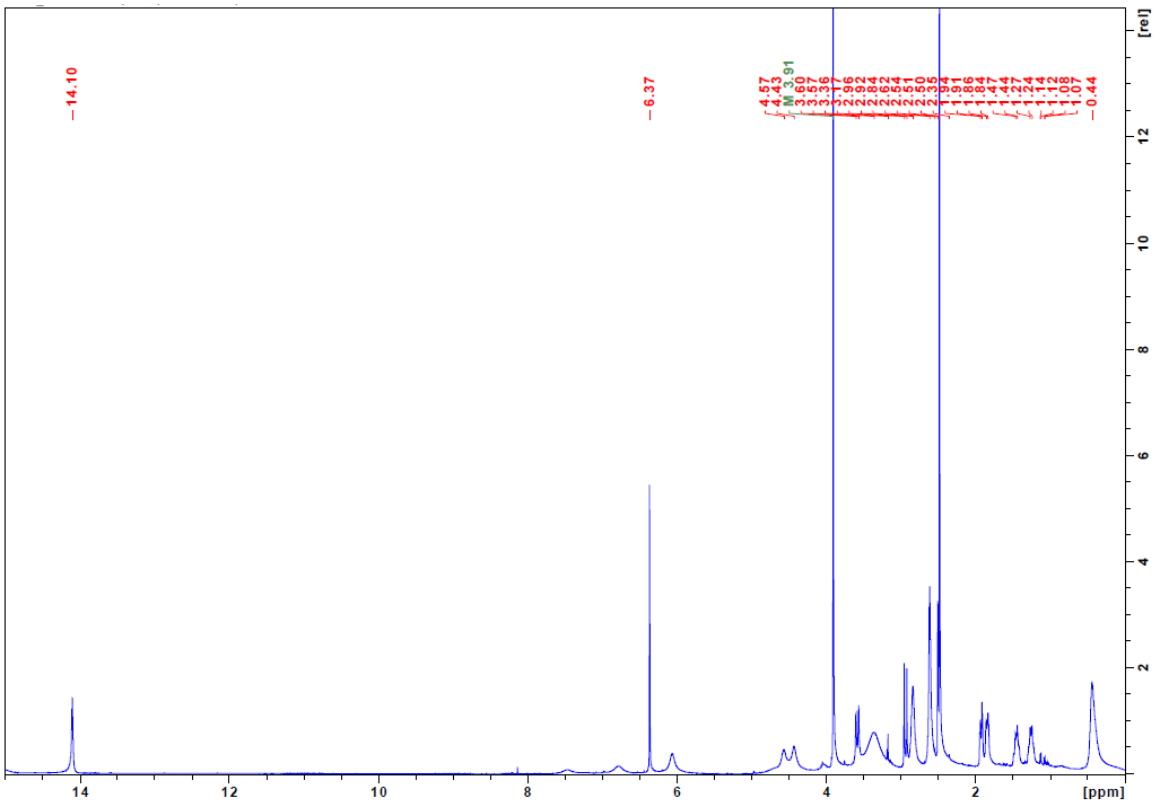
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1.91
1.86
1.84
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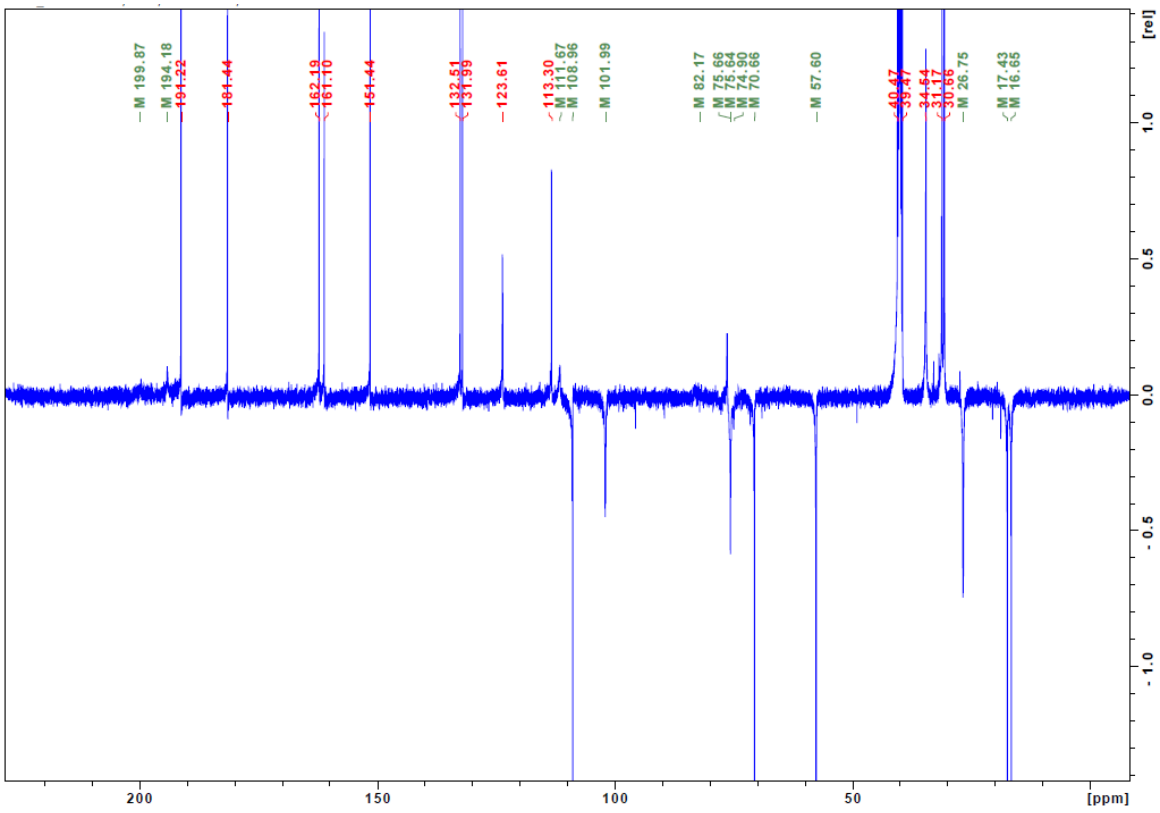
1.27
1.24
1.14
1.12
1.08
1.07
0.44
[rel]
[ppm]

m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M 194.18
191.22
181.44
162.19
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151.44
132.51
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123.61
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111.67
108.96
M 101.99
M 82.17
M 75.66
M 75.64
M 75.60
M 70.86
M 57.60
40.47
39.47
34.54
31.17
30.66
M 26.75
M 17.43
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0.0
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- 1.0
200
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50
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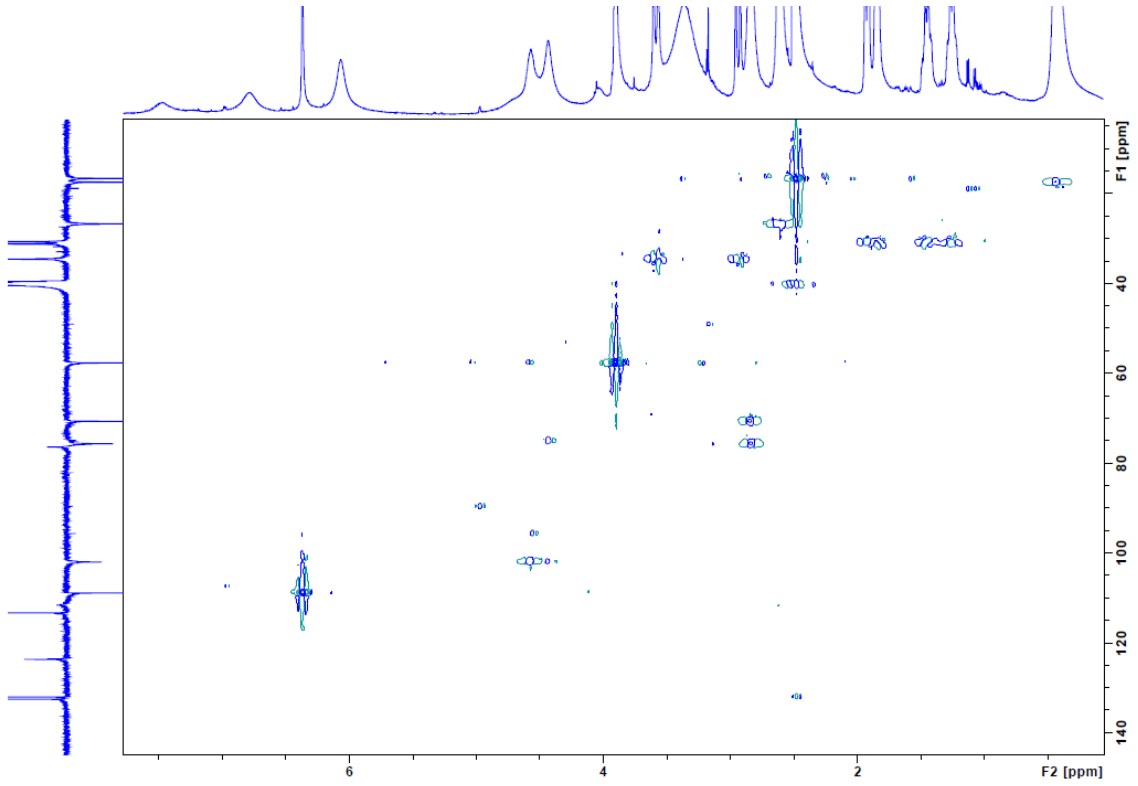
n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80
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120
140
6
4
2
F2 [ppm]

o

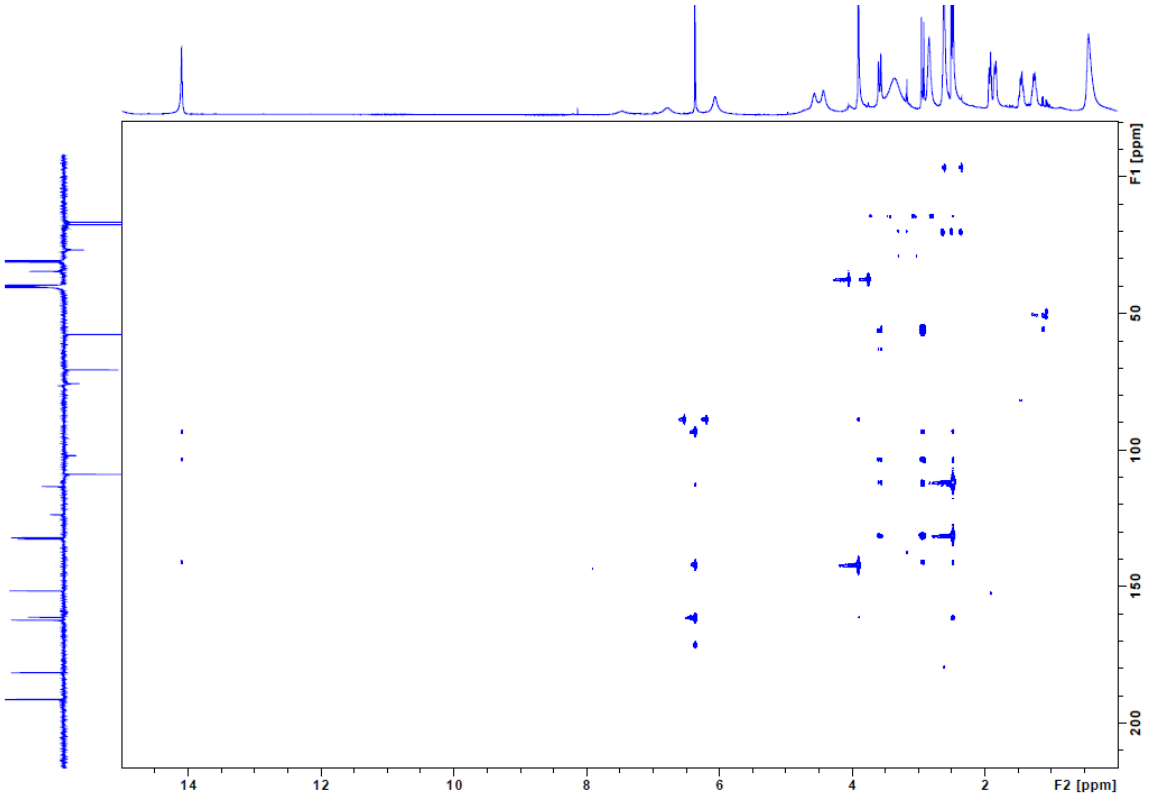

Figure S3. NMR spectra of **1**-**4**. a) ^{1}H NMR spectrum of **1**; b) ^{13}C NMR spectrum of **1**; c) HSQC spectrum of **1**; d) HMBC spectrum of **1**; e) ^{1}H NMR spectrum of **2**; f) ^{13}C NMR spectrum of **2**; g) ^{1}H NMR spectrum of **3**; h) ^{13}C NMR spectrum of **3**; i) HSQC spectrum of **3**; j) HMBC spectrum of **3**; k) ^{1}H-^{1}H COSY spectrum of **3**; l) ^{1}H NMR spectrum of **4**; m) ^{13}C NMR spectrum of **4**; n) HSQC spectrum of **4**; o) HMBC spectrum of **4**.

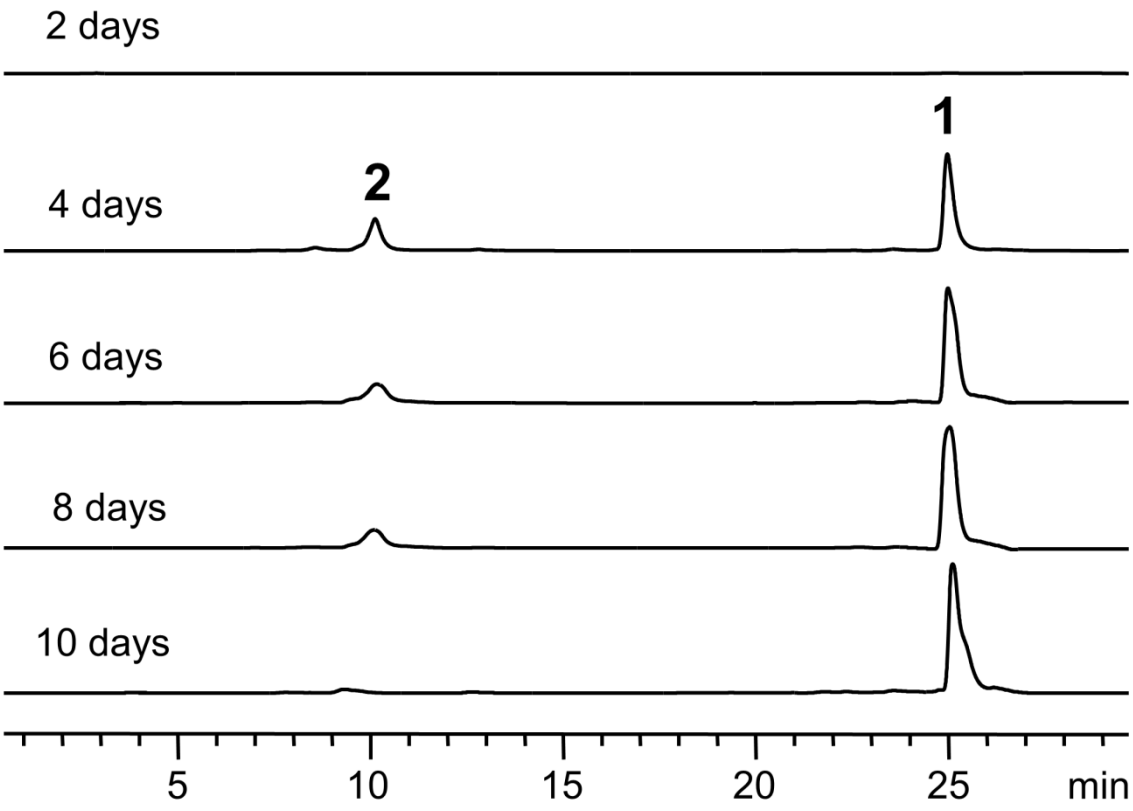

Figure S4. Time course analysis of the production of **1** and **2** by *S. minoensis* NRRL B-5482**.**

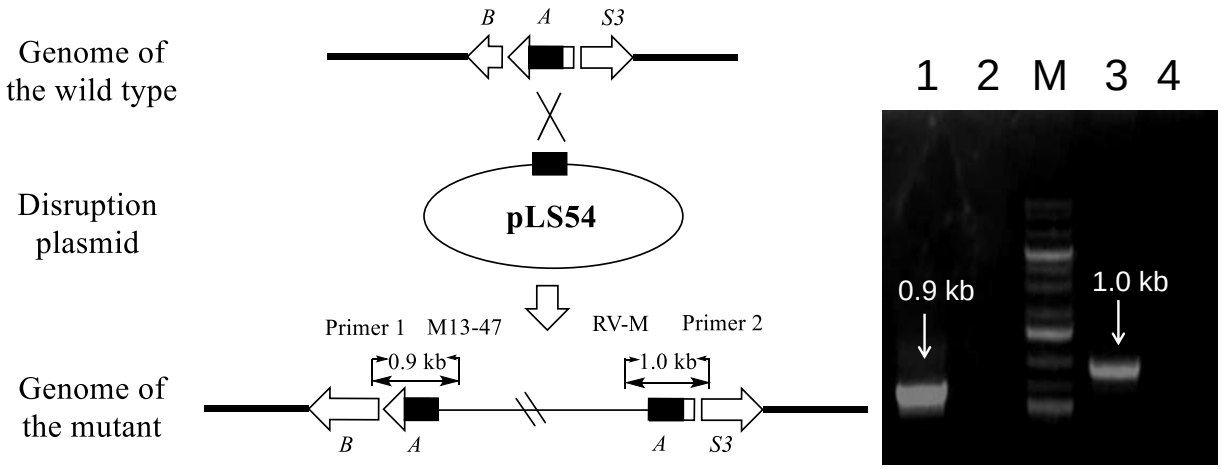

Figure S5. PCR verification of the *Streptomyces minoensis* NRRL B-5482-ΔDutA. M: 1-kb Plus DNA ladder; 1: 1.0-kb PCR product from the mutant with primers M13-47 and DutA-Check1; 2: PCR product from the wild type with primers M13-47 and DutA-Check1; 3: PCR product from the wild type with primers RM-V and DutA-Check2; 4: 1.0-kb PCR product from the mutant with primers RM-V and DutA-Check2.

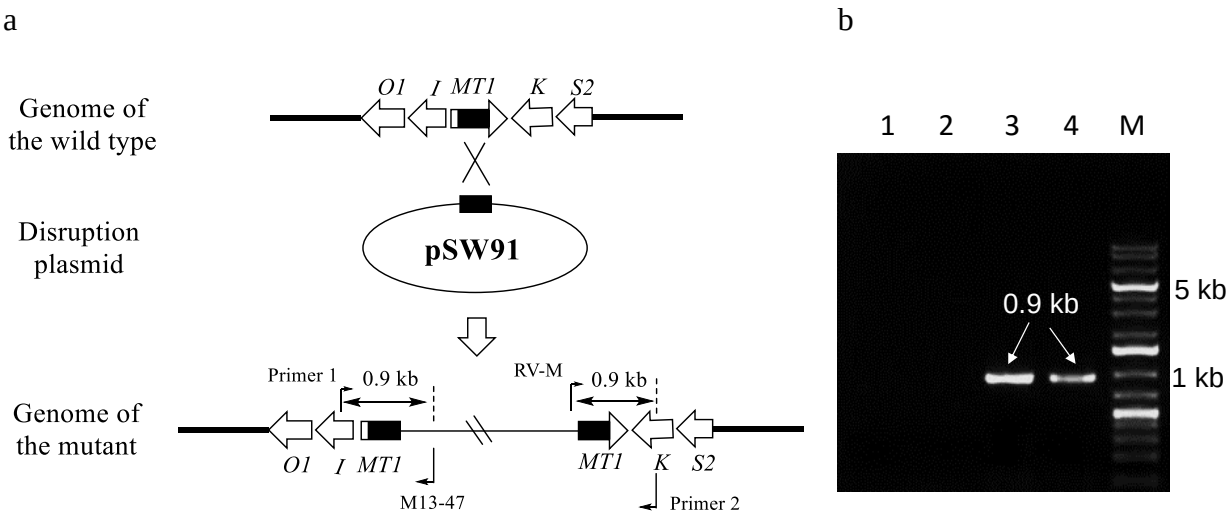

Figure S6. Synthesis of 12-desmethyl-dutomycin (SW91, **3**) by disrupting *dutMT1*. a) Single crossover strategy to disrupt *dutMT1*, b) PCR verification of the *S. minoensis* NRRL B-5482-ΔDutMT1 mutant. M: 1-kb Plus DNA ladder; 1: PCR product from the mutant with primers M13-47 and DutMT1-Check1; 2: PCR product from the wild type with primers M13-47 and DutMT1-Check1; 3: PCR product from the mutant with primers RM-V and DutMT1-Check2; 4: PCR product from the wild type with primers RM-V and DutMT1-Check2.

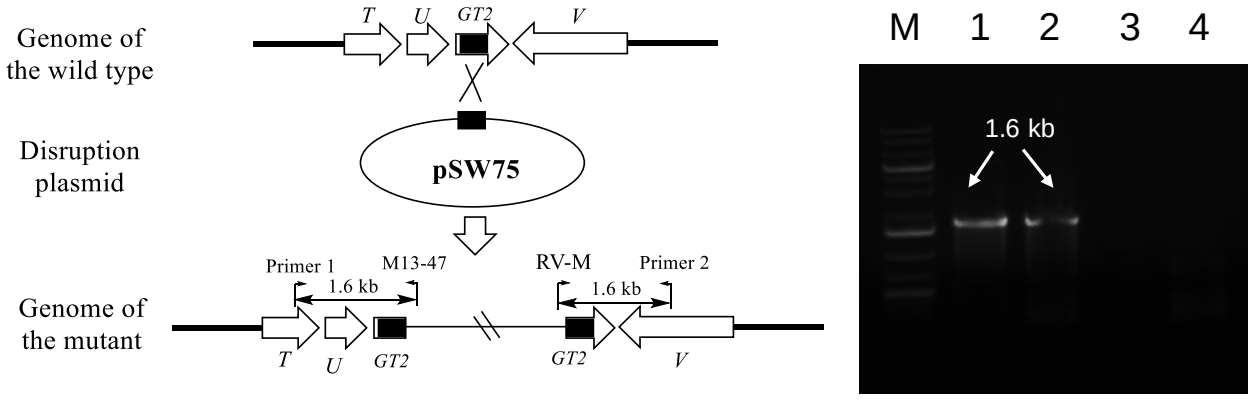

Figure S7. PCR verification of the *S. minoensis* NRRL B-5482-ΔDutGT2 mutant. M: 1-kb Plus DNA ladder; 1: 1.6-kb PCR product from the mutant with primers M13-47 and DutGT2-Check1; 2: 1.6-kb PCR product from the mutant with primers RM-V and DutGT2-Check2; 3: PCR product from the wild type with primers M13-47 and DutGT2-Check1; 4: PCR product from the wild type with primers RM-V and DutGT2-Check2.

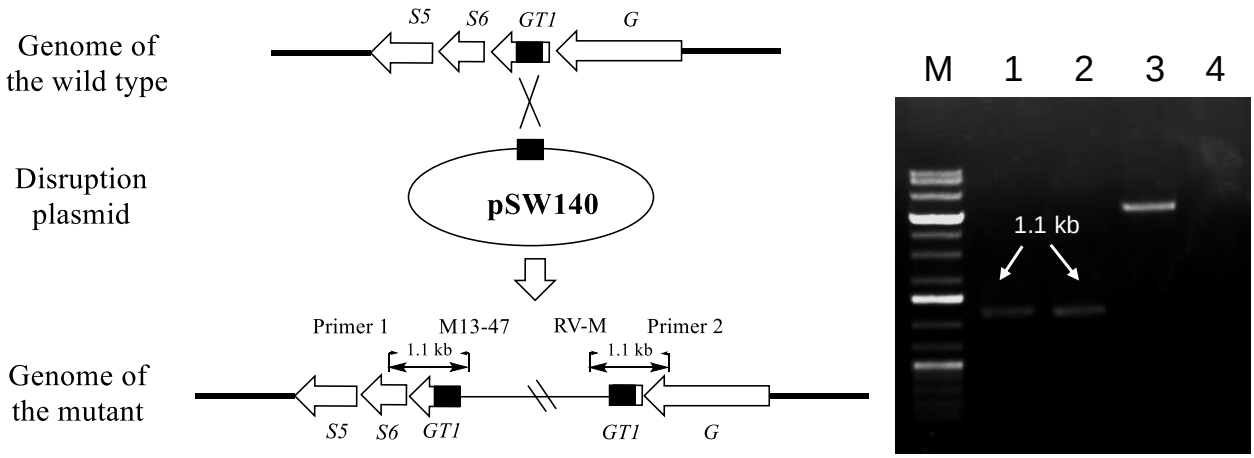

Figure S8. PCR verification of the *S. minoensis* NRRL B-5482-ΔDutGT1 mutant. M: 1-kb Plus DNA ladder; 1: 1.1-kb PCR product from the mutant with primers M13-47 and DutGT1-Check1; 2: 1.1-kb PCR product from the mutant with primers RM-V and DutGT1-Check2; 3: PCR product from the wild type with primers M13-47 and DutGT1-Check1; 4: PCR product from the wild type with primers RM-V and DutGT1-Check2.

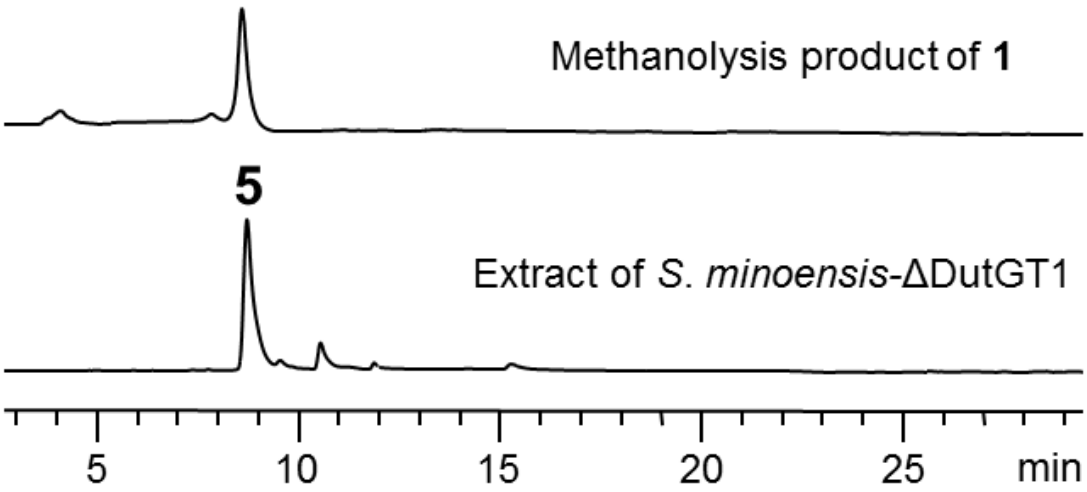

Figure S9. Comparison of the major product (**5**) of *S. minoensis*-ΔDutGT1 with the methanolysis product of **1** on HPLC at 460 nm.