Supporting Information

Rational Domain Swaps Reveal Insights About Chain Length Control by Ketosynthase Domains in Fungal Non-Reducing Polyketide Synthases

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Table of Contents

Supplemental Methods

Fungal strains and molecular genetic manipulations

A. nidulans strains used in this study are listed in **Table S1**. All primers used in this study are listed in **Table S2**.

Gene deletions were carried out in LO4852 for AN3386 and in LO2026 for the other 8 PKS genes by replacing the target gene with the selection marker, *Aspergillus fumigatus pyroA* (*AfpyroA*) gene (**Figure S1A)**. Chimeric PKS genes were constructed after gene deletion. For SAT domain swaps as shown in **Figure S1B**, the *afoE* SAT plus a 120bp upstream promoter region was replaced with a DNA cassette containing 1) a selection marker [*Aspergillus fumigatus pyrG* (*AfpyrG*) or *riboB* (*AfriboB*) *gene*] followed by 2) a 401bp *A. nidulans alcA* promoter [*alcA*(p)] followed by 3) the SAT portion of a donor gene starting from the start codon and ending at the selected junction site, so that the chimeric gene was under the control of the *alcA* promoter. This strategy was also applied to the extended domain swap between AN3386 and *afoE* except that the donating portion of AN3386 and receiving region of *afoE* were extended.

Two ~1000bp-fragments upstream and downstream of each targeted DNA region were amplified from *A. nidulans* genomic DNA by PCR and fused together with the replacement cassette by fusion PCR.[1] Protoplast production and transformation were carried out as described.[1] Three to five transformants for each genotype were analyzed by diagnostic PCR with three primer sets. When the external primers in the first round of PCR were used, the difference in the sizes of targeted DNA region before and after replacement allowed the determination of correct gene replacement. When one of the external primers and the primer located inside the cassette were used, the correct mutant gave the PCR product of the expected size, otherwise no product was present.

Fermentation and LC-MS analysis

For fermentation, $2.5x10^7$ spores of each *A. nidulans* strain were grown in 25 mL liquid LMM medium (15 g/L lactose, 6 g/L $NaNO_3$, 0.52 g/L KCl, 0.52 g/L $MgSO_4 \cdot 7H_2O$, 1.52 g/L KH_2PO_4, and 1 ml/L trace elements)[2] in 125 mL flasks at 37°C with shaking at 180 rpm and supplemented with uracil (1 g/L), uridine (10 mM), riboflavin (2.5 mg/L), or pyridoxine (0.5 mg/L) when necessary. For *alcA*(p)-inducing conditions, cyclopentanone at a final concentration of 10 mM was added to the medium after 18 h of incubation. Culture medium was collected 48 h after cyclopentanone induction by filtration and extracted once with the same volume of EtOAc. The mycelium collected was subsequently soaked in 25 mL of MeOH and 25mL of MeOH/DCM (1:1) and respectively sonicated for 1 h. After removal of the cell debris by filtration, MeOH and MeOH/DCM (1:1) were combined,

concentrated, resuspended in 25 mL of ddH_2O, and extracted with the same volume of EtOAc once. The EtOAc layer was evaporated in *vacuo*, redissolved in 0.2 mL of 20% DMSO/MeOH, and 10 μL was injected for HPLC-DAD-MS analysis.

The solvent gradient for HPLC was 95% MeCN/H_2O (solvent B) in 5% MeCN/H_2O (solvent A), both containing 0.05% formic acid: 0% B from 0 to 5 min, 0 to 100% B from 5 to 35 min, maintained at 100% B from 35 to 40 min, 100 to 0% B from 40 to 45 min, and re-equilibration with 0% B from 45 to 50 min. Conditions for MS included a capillary voltage 5.0 kV, a sheath gas flow rate at 60 arbitrary units, an auxiliary gas flow rate at 10 arbitrary units, and the ion transfer capillary temperature at 350°C. HPLC-DAD-MS analysis was carried out using a ThermoFinnigan LCQ Advantage ion trap mass spectrometer with a reverse phase C_{18} column (Alltech Prevail C_{18}; particle size, 3 μm; column, 2.1 by 100 mm) at a flow rate of 125 μL/min.

Isolation and identification of secondary metabolites

For structure elucidation of compound **5** which was mainly produced in mycelium, $4.0x10^8$ spores of CW1335 were cultivated in 400 mL liquid LMM medium in 2000 mL flasks (total 5 flasks) at 37°C with shaking at 180 rpm and induced with cyclopentanone at 18 h. The culture mycelium was collected through filtration 48 h after induction, and soaked in 200 mL of MeOH twice followed by 1 h sonication. After removal of the cell debris by filtration, MeOH was combined, concentrated, resuspended in 50 mL of ddH_2O, and extracted with the same volume of EtOAc three times. The crude extract evaporated from EtOAc layer (40.45 mg) was then purified by reverse-phase HPLC with a Phenomenex Luna C_{18} column (5-μm particle size; 250 by 21.2 mm) at a flow rate of 5.0 mL/min and measured by a UV detector at 254 nm. The gradient system was 100% MeCN (solvent B) in 5% MeCN/H_2O (solvent A), both containing 0.05% trifluoroacetic acid, as follows: equilibration with 0% solvent B from 0 to 5 min, 0% to 100% solvent B from 5 to 35 min, 100% solvent B from 35 to 40 min, 100% to 0% solvent B from 40 to 43 min, and reequilibration with 0% solvent B from 43 to 48 min. Compound **5** (8.57 mg) was eluted at 35.0 min.

The NMR data for compound **5** were highly similar to that of established compound **2**. It has a molecular formula of $C_{17}H_{26}O_3$ on the basis of its HRESIMS, ^{13}C NMR, and DEPT spectroscopic data, representing five indices of hydrogen deficiency (IHD). The ^1H and ^{13}C NMR spectra of **5** exhibited signals for a C_9H_{19} alkyl side chain [δ_H 0.87 (3H, t, J = 7.0 Hz), 1.13 – 1.28 (12H), 1.49 (2H, m), and 2.72 (2H, t, J = 7.8 Hz); δ_C 13.4 ~ 32.8], a downfield methyl group [δ_H 1.88 (3H, s); δ_C 6.6 (q)] and an aldehyde group [δ_H 9.97 (1H, s, H-1) and δ_C 193.2 (d, C-1)] attached to an aromatic ring[δ_H 6.29 (1H, s); δ_C 108.6 ~ 164.0], which highly resembles data of the penta-substitute aromatic moiety and aliphatic side chain of the known compound **2**. The 2D NMR (gHMQC, gHMBC, and

gCOSY) analysis also assisted in assigning the structure of compound **5**.

For structure elucidation of compound **4**, CW1339 was grown in 30 mL of liquid LMM (total 80 flasks) and harvested as described in "Fermentation and LC-MS analysis" section. The culture medium was collected through filtration, extracted twice with an equal volume of EtOAc, and evaporated as described above. The crude extract (241.2 mg) was applied to a Merck Si gel column (230 to 400 mesh; ASTM) and eluted with $CHCl_3$/MeOH mixtures of increasing polarity (fraction A, 1:0; fraction B, 49:1; fraction C, 19:1; fraction D, 9:1; and fraction E, 7:3). Fraction E (27.81 mg) was further purified by reverse-phase HPLC with a Phenomenex Luna C_{18} column (5-µm particle size; 250 by 21.2 mm) at a flow rate of 10.0 mL/min and measured by a UV detector at 254 nm. The gradient system was MeCN (solvent B) in 5% MeCN/H_2O (solvent A), both containing 0.05% trifluoroacetic acid, as follows: equilibration with 0% solvent B from 0 to 5 min, 0% to 100% solvent B from 5 to 35 min, 100% solvent B from 35 to 40 min, 100% to 0% solvent B from 40 to 43 min, and reequilibration with 0% solvent B from 43 to 48 min. Compound **4** (1.67 mg) eluted at 35.0 min.

The NMR data for compound **4** were also highly similar to that of established compound **2**. The mass difference and the ^{13}C NMR resonance at 176.6 were indicative of a carboxylic acid functionality. 2D NMR experiments established the C18 position of the carboxylic acid and fully corroborated our proposed structure.

Compound Identification

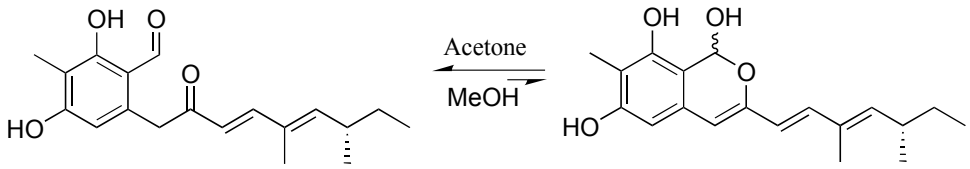
Nuclear magnetic resonance (NMR) spectra of **1** and **4** were respectively collected on a Varian Mercury Plus 400 spectrometer and a Varian 400-MR 2-Channel NMR Spectrometer.

Compound **1**: brown solid; For UV and ESI-MS data, see **Figure S2**; For ^1H and ^{13}C NMR data, see **Table S3**.

Compound **4**: yellow solid; For UV and ESI-MS data, see **Figure S2**; For ^1H and ^{13}C NMR data, see **Table S3**.

Supplemental Discussion on 1 and products in equilibrium

Compound **1** in the current text was the product of a genetic knock out in an earlier project.[12] The minor products as marked with an asterisk in the current text were isolated and characterized by NMR, using acetone-d_6. We had found that their spectra matched that of the major product. Given this result and the chemical structure of the major product, we reasoned that the minor products were cyclic hemiacetals in equilibrium with the major product. The identity of the solvent may affect the equilibrium. We have now performed HRMS analysis of compound **1** and the minor products. In positive mode the measured masses for **1a**, and **1b** were 299.1639, and 299.1658, respectively, corresponding to metabolites with a molecular formula of $C_{19}H_{23}O_3$ $[M+H-H_2O]^+$. For **1** the parent ion of 317.1753 is observed ($C_{19}H_{25}O_4$ $[M+H]^+$), but the spectrum also displays a minor peak of 299.1642 ($C_{19}H_{23}O_3$ $[M+H-H_2O]^+$).



1 **1a** and **1b**

As Figure 1 in the main text shows, there is no clear sign of the hemiacetal forms of **2** and **4**. The equilibrium may favor the hemiacetals less than with **1**, but it cannot be discounted that equilibrium was not reached before the samples underwent the LC/MS experiment.

Table S1. Products from NR-PKSs used in this study.

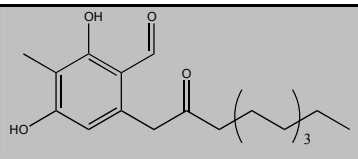
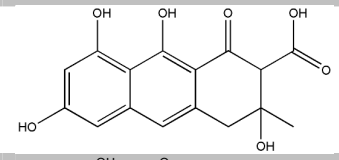
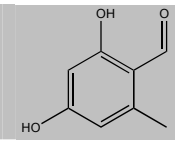
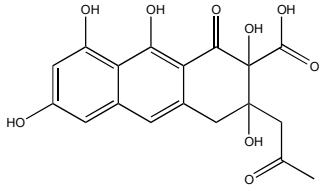
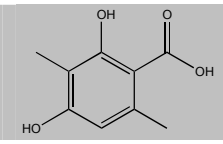
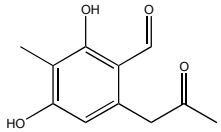
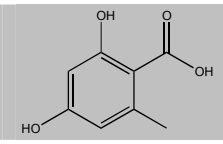
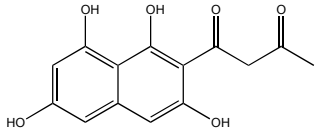
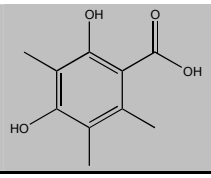
Gene	Starter unit[3]	Product released from NR-PKS	Protein homology with AfoE[a]	Supplemental references
AN3386			39%	3
AN0150			28%	4
AN3230			43%	3
AN6000			28%	5,6
AN6448			32%	3,7
AN7903			57%	3
AN7909			25%	8,9
AN8209			34%	10
AN8383			30%	3,11,12

Table S2. *A. nidulans* strains used in this study

strain	related mutation(s)	genotype
LO2026	*stcJ*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2;stcJ*::*AfriboB*
CW1062	*stcJ*Δ; *mdpG*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; mdpG*::*AfpyroA*
CW1093	*stcJ*Δ; *mdpG*Δ; SAT^{afoE}::*alcA(p)*-SAT^{mdpG}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; mdpG*::*AfpyroA;*SAT^{afoE}::*AfpyrG-alcA(p)*-SAT^{mdpG}
CW1068	*stcJ*Δ; *pkfA*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; pkfA*::*AfpyroA*
CW1097	*stcJ*Δ; *pkfA*Δ; SAT^{afoE}::*alcA(p)*-SAT^{pkfA}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; pkfA*::*AfpyroA;*SAT^{afoE}:: *AfpyrG-alcA(p)*-SAT^{pkfA}
CW1071	*stcJ*Δ; *aptA*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; aptA*::*AfpyroA*
CW1102	*stcJ*Δ; *aptA*Δ; SAT^{afoE}::*alcA(p)*-SAT^{aptA}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; aptA*::*AfpyroA;*SAT^{afoE}:: *AfpyrG-alcA(p)*- SAT^{aptA}
CW1074	*stcJ*Δ; *pkbA*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; pkbA*::*AfpyroA*
CW1107	*stcJ*Δ; *pkbA*Δ; SAT^{afoE}:: *alcA(p)*- SAT^{pkbA}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; pkbA*::*AfpyroA*; SAT^{afoE}:: *AfpyrG-alcA(p)*- SAT^{pkbA}
CW1080	*stcJ*Δ; *pkeA*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; pkeA*::*AfpyroA*
CW1114	*stcJ*Δ; *pkeA*Δ; SAT^{afoE}::*alcA(p)*-SAT^{pkeA}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; pkeA*::*AfpyroA*; SAT^{afoE}:: *AfpyrG-alcA(p)*- SAT^{pkeA}
CW1084	*stcJ*Δ; *orsA*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; orsA*::*AfpyroA*
CW1117	*stcJ*Δ; *orsA*Δ; SAT^{afoE}::*alcA(p)*-SAT^{orsA}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; orsA*::*AfpyroA*; SAT^{afoE}:: *AfpyrG-alcA(p)*- SAT^{orsA}
CW1086	*stcJ*Δ; *wA*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; wA*::*AfpyroA*
CW1122	*stcJ*Δ; *wA*Δ; SAT^{afoE}::*alcA(p)*-SAT^{wA}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; wA*::*AfpyroA*; SAT^{afoE}:: *AfpyrG-alcA(p)*- SAT^{wA}
CW1092	*stcJ*Δ; *ausA*Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; ausA*::*AfpyroA*
CW1127	*stcJ*Δ; *ausA*Δ; SAT^{afoE}::*alcA(p)*-SAT^{ausA}	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2; stcJ*::*AfriboB; ausA*::*AfpyroA*; SAT^{afoE}:: *AfpyrG-alcA(p)*- SAT^{ausA}
LO4852	*ST*Δ; *alcA*(p)-AN3380.1; *alcA*(p)-AN3381.1	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2;stcA-W*Δ*;* ANID_03381.1-ANID_03380.1:: *AfpyrG-alcA*(p)-ANID_03381.1-*alcA*(p)-ANID_03380.1
LO4925	*ST*Δ; *alcA*(p)-AN3380.1; *alcA*(p)-AN3381.1; *alcA*(p)-AN3386.1	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2;stcA-W*Δ*;* ANID_03381.1-ANID_03380.1:: *AfpyrG-alcA*(p)-ANID_03381.1-*alcA*(p)-ANID_03380.1; ANID_03386.1:: *AfriboB-alcA*(p)-ANID_03386.1
CW1331	*ST*Δ; *alcA*(p)-AN3380.1; *alcA*(p)-AN3381.1; AN3386.1Δ	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2;stcA-W*Δ*;* ANID_03381.1-ANID_03380.1:: *AfpyrG-alcA*(p)-ANID_03381.1-*alcA*(p)-ANID_03380.1; ANID_03386.1::*AfpyroA*
CW1335	*ST*Δ; *alcA*(p)-AN3380.1; *alcA*(p)-AN3381.1; AN3386.1Δ; SAT^{afoE}::*alcA(p)*-SAT$^{AN3386.1}$	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2;stcA-W*Δ*;* ANID_03381.1-ANID_03380.1:: *AfpyrG-alcA*(p)-ANID_03381.1-*alcA*(p)-ANID_03380.1; ANID_03386.1::*AfpyroA*; SAT^{afoE}::*AfriboB-alcA(p)*-SAT$^{ANID_03386.1}$
CW1339	*ST*Δ; *alcA*(p)-AN3381.1; *alcA*(p)-AN3380.1;	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2;stcA-W*Δ*;* ANID_03381.1-ANID_03380.1:: *AfpyrG-alcA*(p)-

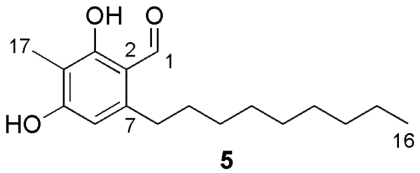
	AN3386.1Δ; (SAT-KS-AT)^{afoE}::*alcA(p)*-(SAT-KS-AT)$^{AN3386.1}$	ANID_03381.1-*alcA*(p)-ANID_03380.1; ANID_03386.1::*AfpyroA*; (SAT-KS-AT)^{afoE}::*AfriboB-alcA(p)*-(SAT-KS-AT)$^{ANID\_03386.1}$
CW1344	*ST*Δ; *alcA*(p)-AN3380.1; *alcA*(p)-AN3381.1; AN3386.1Δ; (SAT-KS-AT-PT)^{afoE}::*alcA(p)*-(SAT-KS-AT-PT)$^{AN3386.1}$	*pyrG89*; *pyroA4*; *nkuA*::*argB*; *riboB2;stcA-W*Δ*;* ANID_03381.1-ANID_03380.1:: *AfpyrG-alcA*(p)-ANID_03381.1-*alcA*(p)-ANID_03380.1; ANID_03386.1::*AfpyroA*; (SAT-KS-AT-PT)^{afoE}::*AfriboB-alcA(p)*-(SAT-KS-AT-PT)$^{ANID\_03386.1}$

Table S3. Primers used in this study.

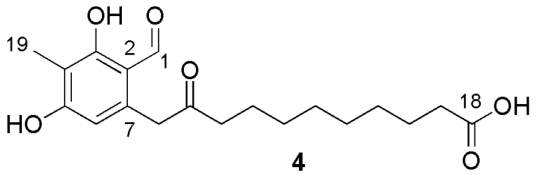
primer	sequence (5'→3')
AN0150.1F1	TGACTGAACCCTGCTAGGC
AN0150.1F2	GAACCCCGGACTGGAAGTAAC
AN0150.1R3	CGAAGAGGGTGAAGAGCATTG GCTGTTGCTTTCGGTTGTTAG
AN0150.1F4	GCATCAGTGCCTCCTCTCAGACAG CAGACTCGGCCTGAGATAATA
AN0150.1R5	GGGGTAGCCAACCGCACAATG
AN0150.1R6	CATTTTCTGCGCTAGTTCACC
SWAP.F1	GCAAGTACGCCATCTTCGAT
SWAP.F2	GCTTGCCCTGAAGACTTACA
SWAP.R3	CGAAGAGGGTGAAGAGCATTG AGCGAGCGGGTCAAGAG
AN0150swap_F4	ATCCTATCACCTCGCCTCAAA ATGCCCGTATATACTCCTC
AN0150swap_R5	TGACGTTTCGAATCCTTGC
AN0150swap_F6	GCAAGGATTCGAAACGTCA CAGCTTCAACCGGTCCTGC
SWAP.R7	CGTCGTTAGCAGTGACCTTG
SWAP.R8	CGCCCTCTGTATGCCCAATA
AN3230.1F1	GTTCCATCTCCTGCGCTAATC
AN3230.1F2	AGTTGAACAGGGCGTAGACAC
AN3230.1R3	CGAAGAGGGTGAAGAGCATTG GCCTTGCGCAGCGATAAC
AN3230.1F4	GCATCAGTGCCTCCTCTCAGACAG ATGTTGTGTGGAGGGTAGAA
AN3230.1R5	ATAATCATACGTGGCGTTGC
AN3230.1R6	TACCGTCCAGCTCATAGTATC
AN3230swap_F4	ATCCTATCACCTCGCCTCAAA ATGCAGGGTTCCAGTCACAC
AN3230swap_R5	TTGGTCGAGGCTGGCAAAG
AN3230swap_F6	CTTTGCCAGCCTCGACCAA CAGCTTCAACCGGTCCTGC
AN6000.1F1	TGCACGCGGAACTCTCTGT
AN6000.1F2	TCCGGAAGGTCATCGATCAC
AN6000.1R3	CGAAGAGGGTGAAGAGCATTG TCAAGCGCTCATTACGTTCAA
AN6000.1F4	GCATCAGTGCCTCCTCTCAGACAG ACGCTTCAGCTACCAATTTAG
AN6000.1R5	TCTGCTCCTCGGGTGTAGACT
AN6000.1R6	CGAAGAGGATGCAGTCCAAT
AN6000swap_F4	ATCCTATCACCTCGCCTCAAA ATGAAAGACAATACGCATAGC
AN6000swap_R5	CAAGTCGGTTATCTTGATCTC
AN6000swap_F6	GAGATCAAGATAACCGACTTG CAGCTTCAACCGGTCCTGC
AN6448.1F1	ATTTGGCGAAGTAGTTAGT
AN6448.1F2	GTAAATGCTGACGACGTGAAC
AN6448.1R3	CGAAGAGGGTGAAGAGCATTG CTAGTCATCTCGCTTAGAGGA
AN6448.1F4	GCATCAGTGCCTCCTCTCAGACAG GTTAGAAAGGACTGAACGACC
AN6448.1R5	AGACAACGCAGGATACGAG
AN6448.1R6	GGGAAGGAGGATATAAGTGC
AN6448swap_F4	ATCCTATCACCTCGCCTCAAA ATGGCTCCGGCACCATCC
AN6448swap_R5	GTTCAATGGAGCAGGCACAG
AN6448swap_F6	CTGTGCCTGCTCCATTGAAC CAGCTTCAACCGGTCCTGC
AN7903.1F1	GATGTTTATTTAGCCGTTTC
AN7903.1F2	CCGTACGACAGACATCAG
AN7903.1R3	CGAAGAGGGTGAAGAGCATTG GTGCGAGTAGCGATTATGCAA
AN7903.1F4	GCATCAGTGCCTCCTCTCAGACAG TCTTAACCCTTGAATAATCTC
AN7903.1R5	GCAAGTAGTCAGGAGACTAGA
AN7903.1R6	TGCTGCTTATGTATTGTGG
AN7903swap_F4	ATCCTATCACCTCGCCTCAAA ATGCTTGGTCATCGGGACTT
AN7903swap_R5	GGAGTAGGAGTACTGGTATC
AN7903swap_F6	GATACCAGTACTCCTACTCC CAGCTTCAACCGGTCCTGC
AN7909.1F1	GACAGGATAATCTCGGACTC
AN7909.1F2	CATACTGGCGCGATAGATG
AN7909.1R3	CGAAGAGGGTGAAGAGCATTG CAGGTATAATGTGCACGTCTC
AN7909.1F4	GCATCAGTGCCTCCTCTCAGACAG TGCTGTTCTATTGCCGTGAAG

AN7909.1R5	TTGGACGTCGACCGGATCAAA
AN7909.1R6	GAGAAGCCGCATATACGAG
AN7909swap_F4	ATCCTATCACCTCGCCTCAAA ATGGCTCCAAATCACGTTC
AN7909swap_R5	CGTAGGACTAAGCTGCCCA
AN7909swap_F6	TGGGCAGCTTAGTCCTACG CAGCTTCAACCGGTCCTGC
AN8209.1F1	AGCGAAAAGCCGACGTTGC
AN8209.1F2	CGCACTCTGGAAACGAACTC
AN8209.1R3	CGAAGAGGGTGAAGAGCATTG GCTTTGGTTTTGGCTTAGGTT
AN8209.1F4	GCATCAGTGCCTCCTCTCAGACAG GGGCCACTTCCTATACCTG
AN8209.1R5	TATTGCGACTTACGACAACCT
AN8209.1R6	TCCATAGTTACGCGATAGAAA
AN8209swap_F4	ATCCTATCACCTCGCCTCAAA ATGGAGGACCCATACCGTG
AN8209swap_R5	GTCGCTAATTCCTCCATCC
AN8209swap_F6	GGATGGAGGAATTAGCGAC CAGCTTCAACCGGTCCTGC
AN8383.1F1	TGTGAGACCCGTCGAAGC
AN8383.1F2	TAGAAACCAGAATTGCCGTAT
AN8383.1R3	CGAAGAGGGTGAAGAGCATTG ATCCTCGTTGTGGTAATGGAA
AN8383.1F4	GCATCAGTGCCTCCTCTCAGACAG AATGAGACACAGTCGCTTAAC
AN8383.1R5	GCTTGCGCCAGAGTATCATA
AN8383.1R6	AGATAGATGGCCTAGTATGG
AN8383swap_F4	ATCCTATCACCTCGCCTCAAA ATGGGGTCCCTTGATGATA
AN8383swap_R5	TCTGCCATTTATGTGCTCG
AN8383swap_F6	CGAGCACATAAATGGCAGA CAGCTTCAACCGGTCCTGC
AN3386.4F1	GATGTTCGCTCGTATCGT
AN3386.4F2	TTGGAAGACCGGCGATCATC
AN3386.4R3	CGAAGAGGGTGAAGAGCATTG TTGTCGGCTACTCTATACTC
AN3386.4F4	GCATCAGTGCCTCCTCTCAGACAG TGGAGACGAGCAATTATATC
AN3386.4R5	CCGCCCTTGCTTCTACTCCC
AN3386.4R6	GCCTGTGTGATTGGACTG
pyrGF2	CAATGCTCTTCACCCTCTTCG
AN3386SATswap.R5	GCTTATGGTGGCCGGAAGC
AN3386SATswap.F6	GCTTCCGGCCACCATAAGC CGCGTCGAGCCAGTCATG
AN3386ATswap.R5	CTGGTTATATTTCAAGGGAAGGG
AN3386ATswap.F6	CCCTTCCCTTGAAATATAACCAG CTCGCTGGGGCAGGGCTG
AN3386ATswap.R7	ATCGCTTTGTCGCCTTGTC
AN3386ATswap.R8	TGGCCGCTTACCGCATCAA
AN3386PTswap.R5	ATTTTCCTGGTTAGCAGTATCT
AN3386PTswap.F6	AGATACTGCTAACCAGGAAAAT ACGAAGGCCAAATCCAAGTCC
AN3386PTswap.R7	GGATCACCTGGCTGGTCTTG
AN3386PTswap.R8	CATCTGTGCGTAACTCATGC

Blue and red sequences are tails that anneal to the selection marker (*AfpyroA* or *AfriboB*) fragment during fusion PCR. Green sequence is a tail that anneals to the *alcA* promoter fragment during fusion PCR.



5



4

Table S4. NMR data for compounds **5** and **4** (400 and 100 MHz)[a].

	5 (acetone-d_6)		**4** (CD_3OD)	
position	δ_C	δ_H	δ_C	δ_H
1	193.2 (CH)	9.97 (s)	193.7 (CH)	9.80 (s)
2	111.6 (C)	—	112.7 (C)	—
3	163.7 (C)	—	164.3 (C)	—
4	108.6 (C)	—	110.2 (C)	—
5	163.0 (C)	—	163.4 (C)	—
6	109.1 (CH)	6.29 (s)	110.5 (CH)	6.26 (s)
7	146.7 (C)	—	138.1 (C)	—
8	31.2 (CH_2)	2.72 (t, 7.8)	45.4 (CH_2)	4.01 (s)
9	32.8 (CH_2)	1.49 (m)	209.0 (C)	—
10	29.2[b] (CH_2)	1.13-1.28	41.8 (CH_2)	2.58 (t, 7.4)
11	29.2[b] (CH_2)	1.13-1.28	23.5 (CH_2)	1.57 (m)
12	29.2[b] (CH_2)	1.13-1.28	29.1[b] (CH_2)	1.23-1.38
13	29.1[b] (CH_2)	1.13-1.28	29.0[b] (CH_2)	1.23-1.38
14	31.7 (CH_2)	1.13-1.28	29.0[b] (CH_2)	1.23-1.38
15	22.4 (CH_2)	1.13-1.28	28.9[b] (CH_2)	1.23-1.38
16	13.4 (CH_3)	0.74 (t, 7.0)	24.9 (CH_2)	1.57 (m)
17	6.3 (CH_3)	1.88 (s)	33.8 (CH_2)	2.27 (t, 7.4)
18			176.6 (C)	—
19			6.1 (CH_3)	2.01 (s)

[a]Figures in parentheses are multiplicities and coupling constants (*J*) in Hz.
[b]Values in the same column may be interchanged.

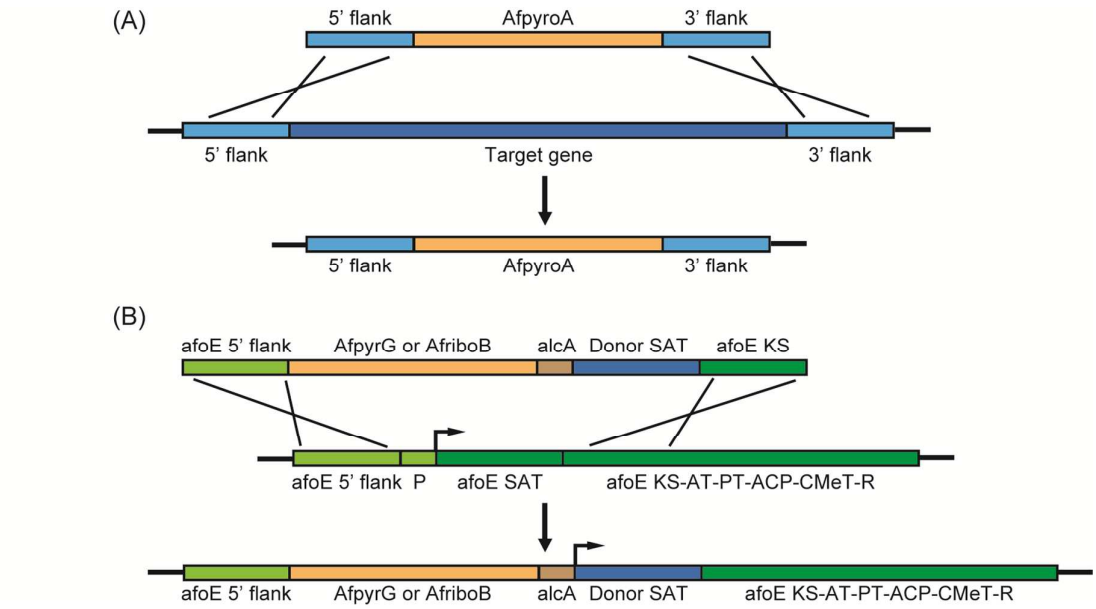


Figure S1. Gene deletion (A) and domain swap (B) strategies in this study. Promoter is abbreviated as P.

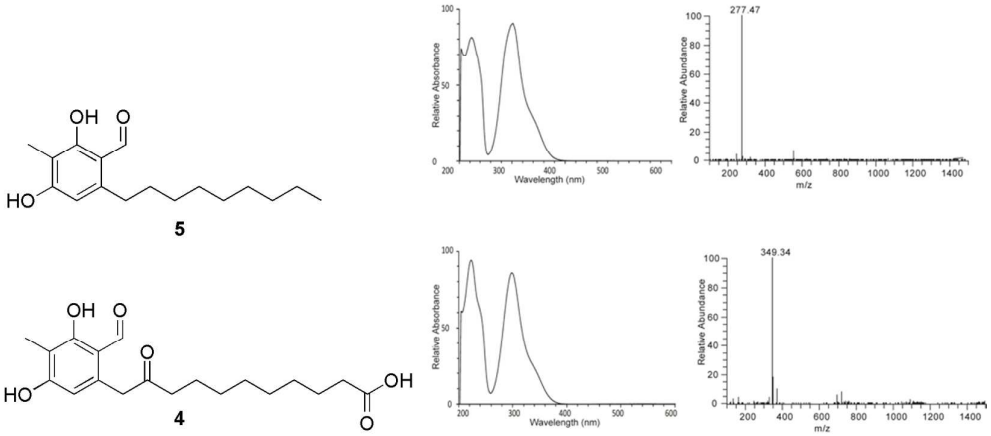


Figure S2. UV-Vis and ESIMS spectra (negative mode) of compounds **5** and **4**.

5 **4**

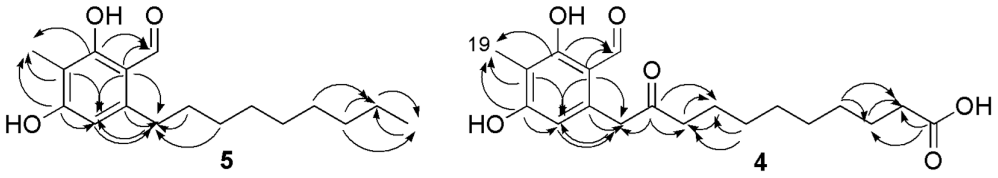


Figure S3. HMBC correlations of compounds **5** and **4**.

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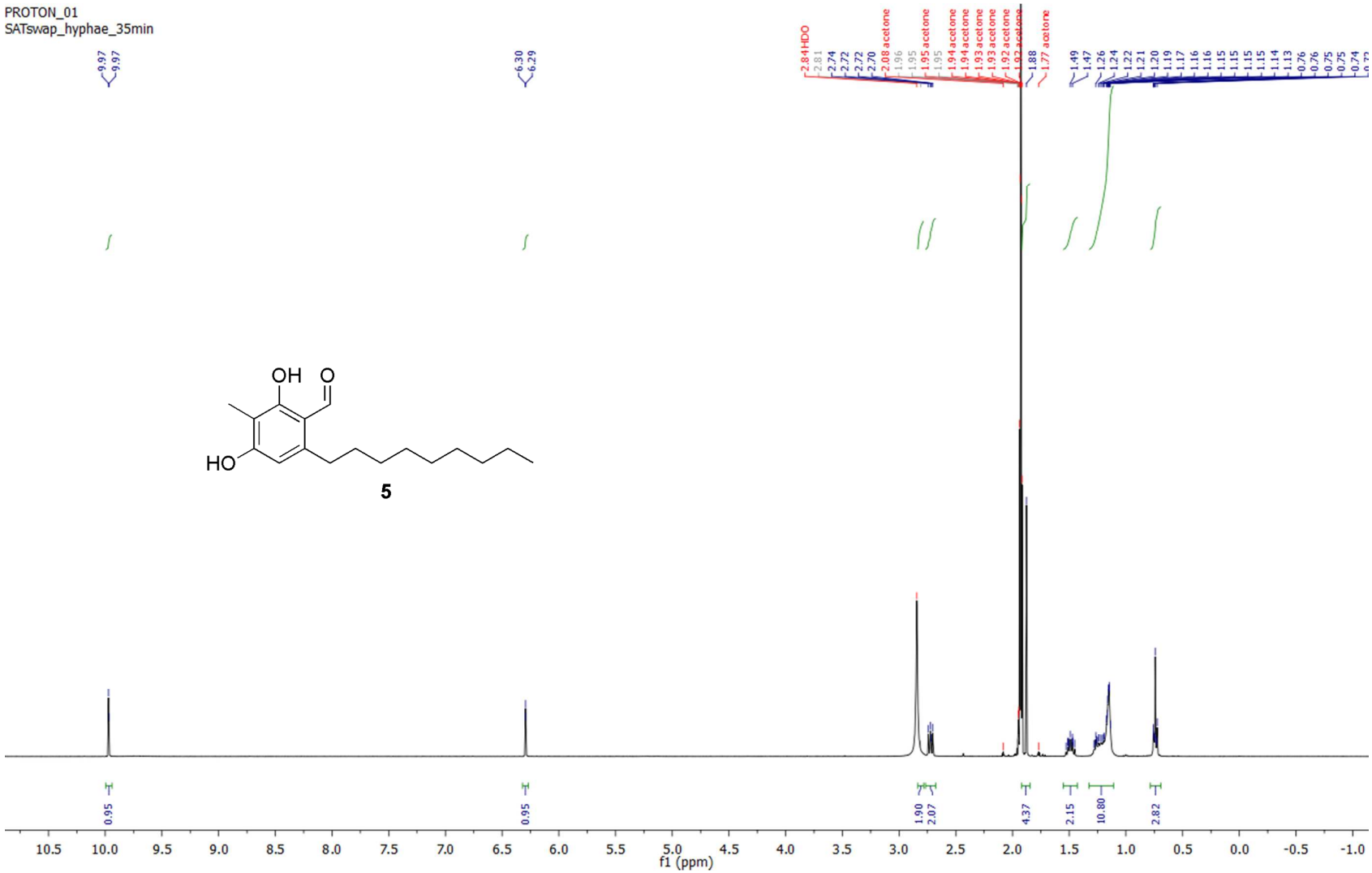

Figure S4. ^{1}H NMR of compound **5**

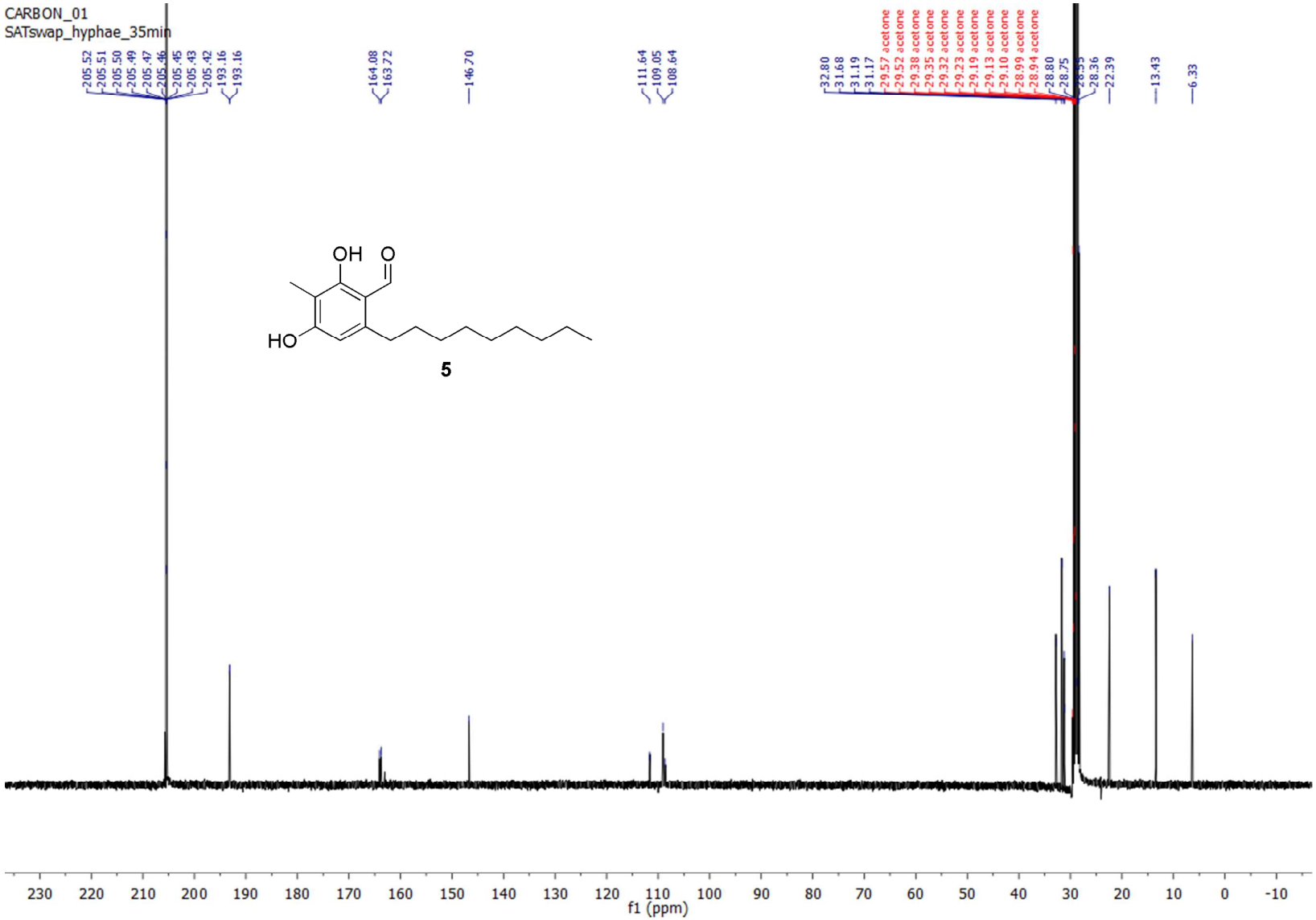

Figure S5. ^{13}C NMR of compound **5**

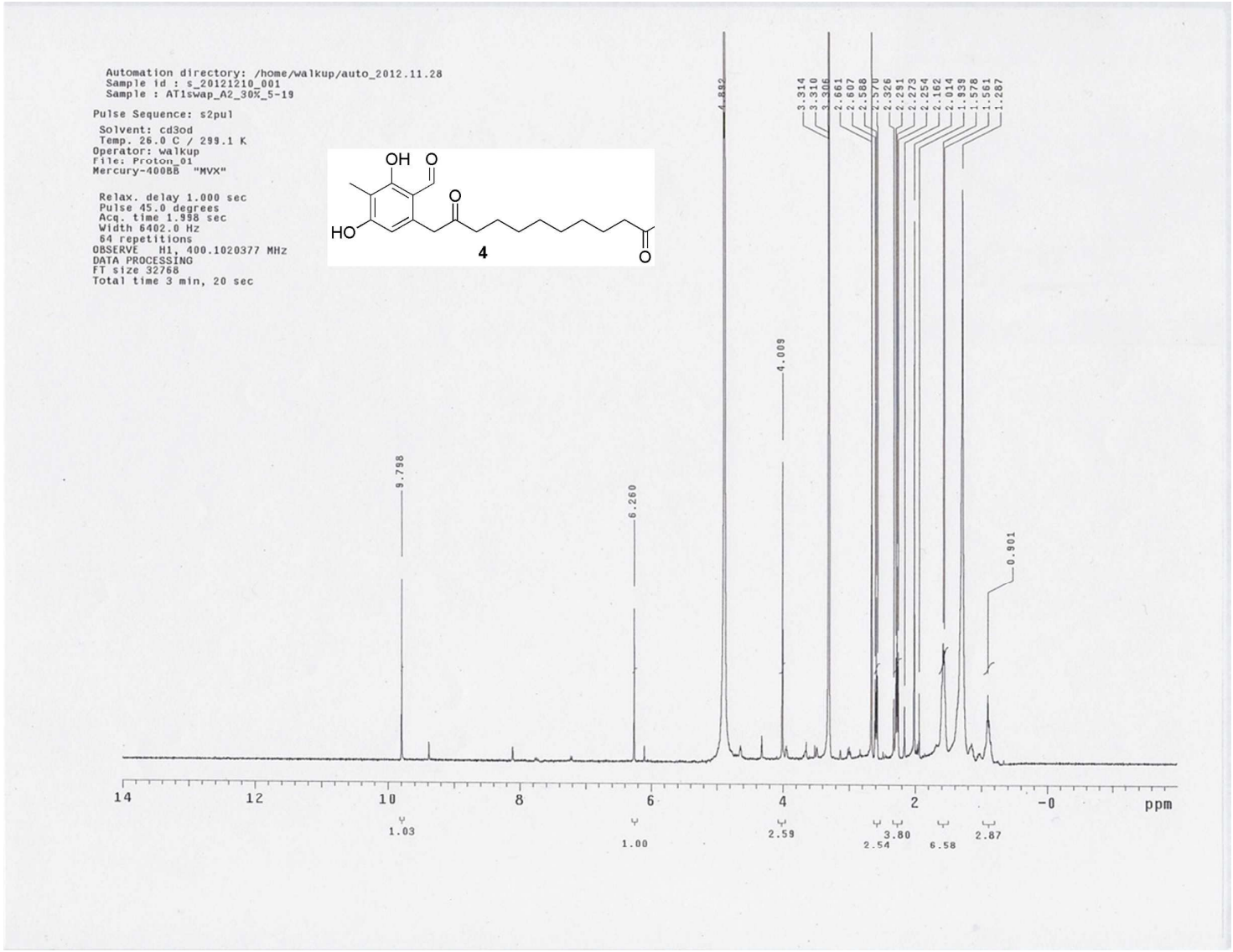

Figure S6. ^{1}H NMR of compound **4**

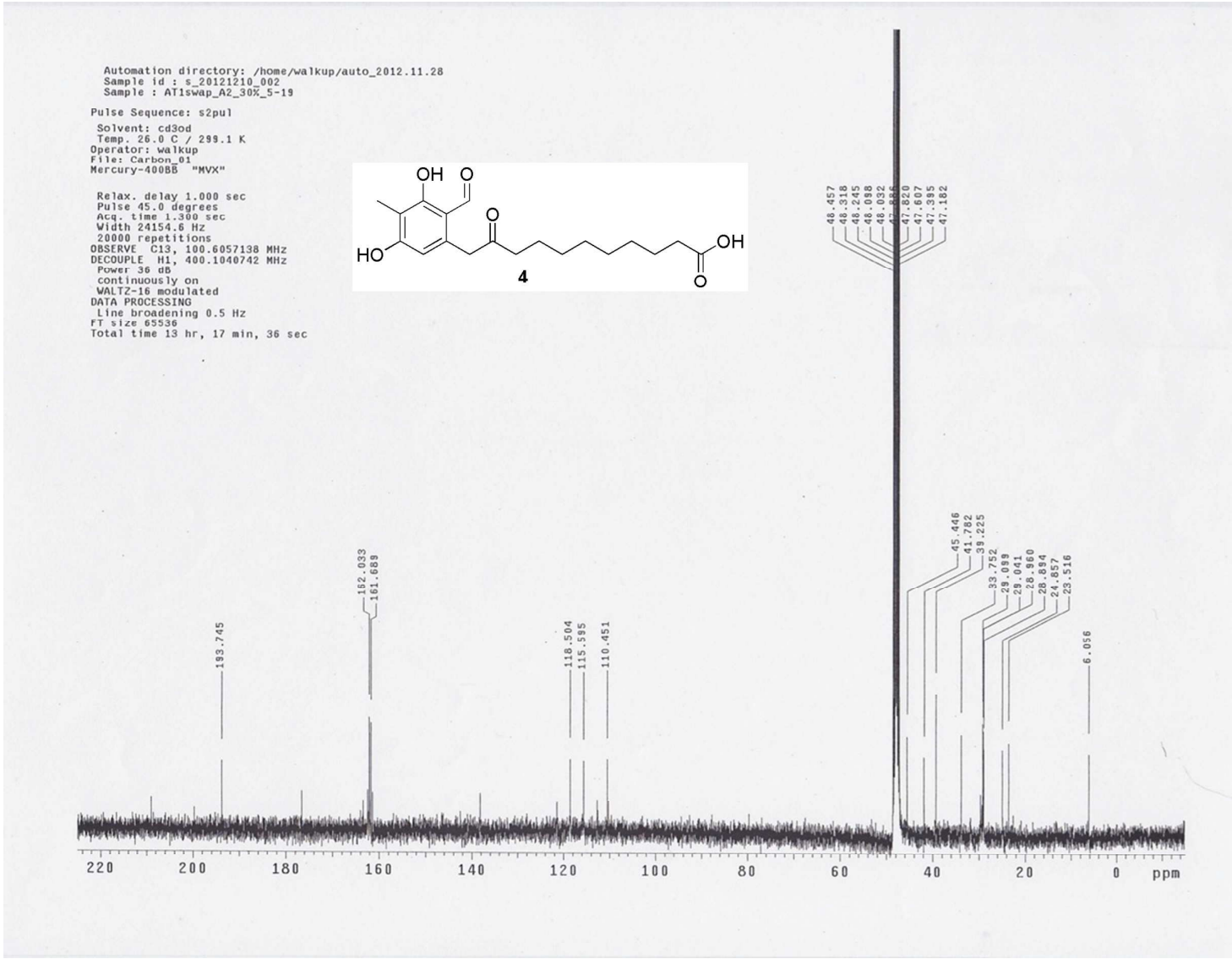

Figure S7. ^{13}C NMR of compound **4**