Supporting Information

Two separate gene clusters encode the biosynthetic pathway for the meroterpenoids austinol and dehydroaustinol in *Aspergillus nidulans*

Hsien-Chun Lo, Ruth Entwistle, Chun-Jun Guo, Manmeet Ahuja, Edyta Szewczyk, Jui-Hsiang Hung, Yi-Ming Chiang, Berl R. Oakley,* and Clay C. C. Wang*

*Correspondence should be addressed to B.O. (boakley@ku.edu) and C.W. (clayw@usc.edu).

Table of Contents

Detailed structural characterization

Compound **4** was isolated as slight yellowish gum. The molecular formula was found to be $C_{26}H_{38}O_5$ by its ^{13}C NMR and DEPT spectral data, suggesting eight indices of hydrogen deficiency (IHD). The ^1H, ^{13}C, gHMQC, and gHMBC NMR spectroscopic data of compound **4** (Table S3) including the 3β-hydroxy group (δ_H 3.11 and δ_C 78.3), the CH-5 methine group (δ_H 0.56 and δ_C 54.4), and three methyl groups (δ_H 0.78 and δ_C 15.9, CH_3-13; δ_H 0.73 and δ_C 15.5, CH_3-14; and δ_H 0.94 and δ_C 27.8, CH_3-15) exhibit typical 3β-hydroxy-4,4,8,10-tetramethyldecalin partial structure that were observed in many triterpenoids.[1] This suggested that compound **4** is an intermediate biosynthesized before the spiro ring formation in the austinol biosynthetic pathway. The NMR data also showed signals for one downfield methine group [δ_H 3.91 (1H, q, *J* = 6.4 Hz, H-5′) and δ_C 62.5 (C-5′)], one terminal double bond [δ_H 4.82 and 5.29 (each 1H, br s, H_2-1′), and δ_C 112.0 (C-1′) and 146.8 (C-2′)], one carboxymethyl ester [δ_H 3.67 (3H, s, OMe), and δ_C 52.0 (OMe) and 169.2 (C-8′)], and two ketone groups [δ_C 204.1 (C-6′) and 209.4 (C-4′)]. These data indicated that compound **4** have similar partial structure of C and D rings with preaustinoid A, an early intermediate in the austinol biosynthetic pathway.[2,3] Comparison of the ^1H and ^{13}C NMR spectra between compound **4** and preaustinoid A revealed that the only difference in C and D rings is that compound **4** do not contain hydroxyl group on C-5′. This resulted in the quartet splitting of H-5′ (δ_H 3.91, 1H, *J* = 6.4 Hz) and the doublet splitting of H_3-10′ (δ_H 1.19, 3H, *J* = 6.4 Hz) in its ^1H NMR. gHMBC also confirmed the assigned structure. Therefore, the structure of this new meroterpene, named protoaustinoid A, was assigned as **4**. However, protoaustinoid A is not stable and became a mixture of more than two compounds in the NMR tube during acquiring its 2D spectra in $CDCl_3$. The ^1H NMR spectra of the mixture showed the disappearance of H-5′ methine proton at δ_H 3.91 and ^{13}C NMR spectra of that showed the appearance of a hydroperoxy carbon at δ_c 95.3 (Figure S9 and S10). These evidences indicated that protoaustinoid A autooxidized to its 5′-hydroperoxide derivatives **4′** in $CDCl_3$ due to the susceptible H-5′ in the β-diketone ring D. However, the stereochemistry of C-5′ of protoaustinoid A **4** remains to be determined.

Compound **6** was isolated as colorless needles. The molecular formula was found to be $C_{26}H_{32}O_7$ by its ^{13}C NMR, DEPT and HRESIMS spectral data, suggesting eleven IHD. The IR spectrum showed hydroxyl (3383 cm^{-1}) and ester or ketone functionalities (1759 and 1715 cm^{-1}). The ^1H NMR spectrum of compound **6** (Table S4) exhibited signals for five methyl groups [δ_H 1.24, 1.27, 1.35, 1.39, and 1.55 (each 3H)], one acetyl group [δ_H 2.02 (3H, s)], one methoxyl group [δ_H 3.70 (3H, s)], two terminal olefinic protons [δ_H 5.34 and 5.90 (each 1H, br s)], and one pair of deshielded *cis* 1,2-disubstituted olefinic protons [δ_H 6.01 and 6.73 (each 1H, d, *J* = 10.0 Hz)]. The gHMBC correlations of the deshielded 1, 2-disubstituted olefinic protons with a carbonylic carbon [δ_C 164.2, C-3] suggested the presence of an α,β-unsaturated δ-lactone moiety which has been observed in many meroterpenes.[2,4] Comparison the ^1H and ^{13}C NMR of compound **6** with those of preaustinoid A3 (**5**) suggested that compound **6** has similar partial structure of A, B and C rings with preaustinoid A3.[2] In gHMBC spectrum, the key long-range ^1H–^{13}C correlations between methyl H_3-9′ (δ_H 1.27) and ketone C-4′ (δ_C 211.1); as well as between acetyl

H_3-10′ (δ_H 2.02) and ketone C-5′ (δ_C 201.3) and tertiary hydroxyl C-6′ (δ_C 92.9) established the partial structure of D ring. NOESY correlation between H_3-9′ and H_3-10′ suggested that the acetyl group should locate on the β face. Thus, the structure of this meroterpene, named preaustinoid A4, was assigned as shown in **6**.

Compound **8**, colorless needles, analyzed to have molecular formula $C_{25}H_{30}O_7$ by its ^{13}C NMR, DEPT and HRESIMS spectral data. The ^{1}H and ^{13}C NMR spectral data of compound **8** (Table S5) were similar to those of isoaustinone **7**,[2] except for the existence of a secondary hydroxyl group at C-11 in compound **8**. This caused the downfield shift of H_α-11 from δ_H 2.88 in **7** to δ_H 4.25 in **8**. The C-11 hydroxyl group was assigned to locate at β face due to the NOESY correlation with H_3-12. 2D NMR spectra (Table S5) also confirmed the assigned structure. Therefore, compound **8** was established to be 11β-hydroxyisoaustinone.

Compound **10** was isolated as colorless amorphous solid. This compound has molecular formula $C_{26}H_{32}O_7$ and is a constitutional isomer of preaustinoid A4 **6**. These two compounds have similar ^{13}C NMR spectra especially for those signals located on the A, B, and C ring (Table S6 and S4). The major difference of gHMBC spectrum is that methyl H_3-9′ (δ_H 1.06) connected to the tertiary hydroxyl C-4′ (δ_C 92.9) in compound **10**, unlike compound **6** of which H_3-9′ is connected to ketone C-4′. Moreover, the D_2O exchangeable hydroxyl proton (δ_H 4.56) displays long-range ^{1}H-^{13}C correlations with C-3′ (δ_C 50.1), C-4′ (δ_C 92.2) and C-5′ (δ_C 202.6). These data suggested that the acetyl and hydroxyl groups are attached to C-4′ in compound **10**. The acetyl group is located at β face due to its NOESY correlation with H_3-9′. Thus, the structure of this meroterpene, named preaustinoid A5, was assigned as shown in **10**.

Compound **12** was isolated as colorless plates. Its ^{1}H and ^{13}C NMR spectra were nearly identical with those of isoaustinone **7** suggested that **12** is a stereoisomer of **7**. The major spectral difference of compound **12** and **7** were those signals from CH-5′ (δ_H 4.46 and δ_C 84.5 in **12**; δ_H 4.28 and δ_C 76.3 in **7**) and CH_3-10′ (δ_H 1.15 and δ_C 18.1 in **12**; δ_H 1.28 and δ_C 12.6 in **7**) (Table S7 and S9). This suggested that compound **12** is a 5′*R* isomer of **7**. However, no obvious NOESY correlation of H_3-10′ with other protons led us to perform the single-crystal X-ray diffraction study (Figure S5). This confirmed the assigned structure of compound **12**, (5′*R*)-isoaustinone.

The molecular formula of Compound **13** was deduced to be $C_{25}H_{30}O_7$ from its ^{13}C NMR, DEPT and HRESIMS spectral data, representing eleven IHD. Its ^{1}H, ^{13}C, and gHMQC spectra exhibit signals for three double bonds (including one 1,2-disubstituted and two tetrasubstituted olefins) and three carbonyl groups, suggesting that **13** is a pentacyclic compound. Moreover, the ^{1}H, ^{13}C, and gHMQC NMR data of **13** also revealed signals for five singlet methyl groups [δ_H 1.40 (6H, H_3-14 and H_3-15), 1.62 (3H, H_3-12), 1.79 (3H, H_3-9′), and 1.83 (3H, H_3-13)], one doublet methyl group [δ_H 1.47 (3H, J = 6.4 Hz, H_3-10′)], three methylene groups [δ_H 1.63 and 1.86 (each 1H, H_2-6), 1.72 and 2.81 (each 1H, H_2-7), and 3.02 and 3.23 (each 1H, H_2-1′)], two oxygenated methine protons [δ_H 4.70 (1H, H-5′) and 5.16 (1H, H-11)], and one *cis* 1,2-disubsitituted olefin [δ_H 5.95 (1H, H-2) and 6.42 (1H, H-1)] (Table S8). The ^{1}H and ^{13}C NMR signals in A, B, and E rings of **13** are similar to those of isoaustinone **7**, 11β-hydroxyisoaustinone **8**, and (5′*R*)-isoaustinone **12** and can be assigned unambiguously *via* the ^{1}H–^{13}C gHMBC correlations (Table S8). Similar to 11β-hydroxyisoaustinone **8**, there is a hydroxyl group located on C-11 in **13** due to the gHMBC correlations of H-11 (δ_H 5.16) to C-9 (δ_C 142.0) and C-10 (δ_C 134.5). However, unlike H-11 in 11β-hydroxyisoaustinone **8** which flanked by two quaternary carbons, H-11 in **13** has ^{1}H–^{1}H COSY correlations with the methylene (H_2-1′). The planar structure of C and D rings were then established from gHMBC correlations (Figure S6a). The H-5′ is located at α face due to its NOESY correlation with H_2-7 (Figure S6b). Thus, the structure of this novel meroterpene, named neoaustinone, was assigned as shown in **13**.

Five known compounds were identified as 3,5-dimethyloresellinic acid **3**,[5] preaustinoid A3 **5**,[2] isoaustinone **7**,[2] austinolide **9**,[2] and austinoneol A **11**[4] by comparing their NMR (Table S9 and S10) and MS data as well as optical rotations with those reported in the literature.[4]

Supplemental methods

Isolation and identification of secondary metabolites

For structure elucidation, each *A. nidulans* deletant was cultivated at 37 °C on ~20 solid YAG plates at 2.25 × 10^7 spores per 15-cm plates (~55 ml of medium per plate). After 3 days, agar was chopped into small pieces and then soaked in 800 ml of 1:1 CH_2Cl_2/MeOH for 24 hr twice. After filtration, the combined extract was evaporated *in vacuo* to yield a residue, which was suspended in water (500 ml) (Water layer was further acidified to pH 2 for AN9259.4 deletant) and then partitioned with ethyl acetate (500 ml) three times. The combined ethyl acetate layer was evaporated *in vacuo* to afford a crude extract (the weight for each deletant is list below). The crude extract was applied to a Si gel column (Merck, 230 to 400 mesh, ASTM, 20 × 80 mm) and eluted with 250 ml CH_2Cl_2/MeOH mixtures of increasing polarity (fraction A, 1:0; fraction B, 19:1; fraction C, 9:1; fraction D, 7:3). All the meroterpenoids were eluted in fraction B and the weight of each fraction B per liter of medium was list below.

Deletion	Crude weight (mg)	Fraction B weight (mg)	Silica gel used (g)	Crude weight (mg/L)	Fraction B weight (mg/L)
AN8379.4	790	184.0	51.4	658	153.3
AN9247.4	340	219.9	22.1	378	244.4
AN9248.4	370	248.8	24.1	296	199.1
AN9249.4	190	104.0	12.4	200	109.5
AN9253.4	680	189.8	44.2	591	165.0
AN9259.4	600	50.5	39.0	600	50.5
AN11205.4	400	210.0	26.0	421	221.1
AN11214.4	430	187.0	28.0	344	149.6

Fraction B was further purified by reverse phase HPLC [Phenomenex Luna 5µm C18 (2), 250 × 10 mm] with a flow rate of 5.0 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 % TFA. The HPLC gradient condition of each deletant is listed below.

Gradient condition for AN8379.4 deletant:
50 to 70 % B from 0 to 5 min, 70 to 75 % B from 5 to 15 min, 75 to 85 % B from 15 to 20 min, 85 to 100 % B from 20 to 25, maintained at 100 % B from 25 to 30 min, 100 to 50 % B from 30 to 32 min, and re-equilibration with 50 % B from 33 to 36 min. Compound **4** (6.4 mg/L of medium) was eluted at 8.3 min.

Gradient condition for AN9247.4 deletant:
50 to 65 % B from 0 to 15 min, 65 to 100 % B from 15 to 20 min, maintained at 100 % B from 20 to 22 min, 100 to 55 % B from 22 to 24 min, and re-equilibration with 55 % B from 24 to 26 min. Compound **13** (9.7 mg/L of medium), **1** (7.5 mg/L of medium), **2** (7.1 mg/L of medium), **5** (15.9 mg/L of medium), **11** (19.1 mg/L of medium), and **12** (9.0 mg/L of medium) were eluted at 4.7, 5.5, 5.8, 7.3, 7.6, and 7.9 min, respectively.

Gradient condition for AN9248.4 deletant:
50 to 68 % B from 0 to 5 min, 68 to 68 % B from 5 to 20 min, 68 to 100 % B from 20 to 23 min ,maintained at 100 % B from 23 to 25 min, 100 to 50 % B from 25 to 27 min, and re-equilibration with 50 % B from 27 to 29 min. Compound **9** (28.8 mg/L of medium), **12** (1.9 mg/L of medium), and **7** (3.7 mg/L of medium) were eluted at 7.2, 7.9, and 8.0 min, respectively.

Gradient condition for AN9249.4 deletant:
50 to 68 % B from 0 to 3 min, 68 to 70 % B from 3 to 20 min, 70 to 100 % B from 20 to 23 min, maintained at 100 % B from 23 to 25 min, 100 to 50 % B from 25 to 27 min, and re-equilibration with 50 % B from 27 to 29 min. Compound **12** (17.1 mg/L of medium) were eluted at 7.6 min.

Gradient condition for AN9253.4 deletant:
55 to 57 % B from 0 to 2 min, 57 to 62 % B from 2 to 12 min, 62 to 100 % B from 12 to 17 min, maintained at 100 % B from 17 to 19 min, 100 to 55% B from 19 to 21 min, and re-equilibration with 55 % B from 21 to 23 min. Compound **8** (21.7 mg/L of medium) and **7** (32.3 mg/L of medium) were eluted at 5.1 and 9.3 min, respectively.

Gradient condition for AN9259.4 deletant:
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. Compound **3** (5.1 mg/L of medium) was eluted at 5.1 min.

Gradient condition for AN11205.4 deletant:
The gradient system was same with that applied in AN9253.4 deletant. Compound **11** (11.9 mg/L of medium), **6** (21.3 mg/L of medium), and **10** (7.1 mg/L of medium) were eluted at 7.6, 8.0, and 8.4 min, respectively.

Gradient condition for AN11214.4 deletant:

50 % B from 0 to 5 min, 55 % B from 5 to 7 min, 55 to 100 % B from 7 to 10 min, maintained at 100 % B from 10 to 12 min, 100 to 50% B from 12 to 14 min, and re-equilibration with 50% B from 14 to 16 min. Compound **5** (26.7 mg/L of medium) was eluted at 7.3 min.

Supplemental references

(1) Chiang, Y. M.; Kuo, Y. H. *J. Org. Chem.* **2002**, 7656-7661.

(2) Fill, T.P.; Pereira, G.K.; Geris dos Santos, R.M.; Barisson, A.; Rodrigues-Fo, E. *Z. Naturforsch.* **2007**, *62b*, 1035-1044.

(3) Geris, R. M.; Rodrigues-fo, E. *Phytochemistry* **2002**, *61*, 907-912.

(4) Santos, R. M. G. D.; Rodrigues-Filho, E. *J. Brazil. Chem. Soc.* **2003**, *14*, 722-727.

(5) Hirota, A.; Morimitsu, Y. ; H. H. ; *Biosci. Biotech. Biochem.* **1997**, *61*, 647-650.

Table S1. *A. nidulans* strains used in this study.

Fungal strain or Transformant(s)	Gene mutation(s)	Genotype
LO2026[a]		*pyrG*89*; pyroA*4, *nkuA::argB*, *riboB*2, *stcJ::riboB*
LO3270, LO3272, LO3273	AN8376.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2,*stcJ::riboB;* AN8376.4*::AfpyrG*
LO3284, LO3287	AN8377.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2,*stcJ::riboB;* AN8377.4*::AfpyrG*
LO3239, LO3240, LO3241	AN8378.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8378.4*::AfpyrG*
LO3244, LO3245, LO3246	AN8379.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8379.4*::AfpyrG*
LO3250, LO3251	AN8380.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8380.4*::AfpyrG*
LO3307, LO3308, LO3309	AN8381.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8381.4*::AfpyrG*
LO3254, LO3255, LO3256	AN8382.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8382.4*::AfpyrG*
LO3448, LO3449, LO3450	AN8383.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8383.4*::AfpyrG*
LO3259, LO3260,LO3261	AN8384.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB*; AN8384.4*::AfpyrG*
LO3264, LO3266	AN8385.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8385.4*::AfpyrG*
LO3311, LO3314	AN11077.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN11077.4*::AfpyrG*
LO3275, LO3277	AN11085.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN11085.4*::AfpyrG*
LO3279, LO3280, LO3281	AN8387.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8387.4*::AfpyrG*
LO4011, LO4012	AN9244.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9244.4*::AfpyrG*
LO4016, LO4017, LO4018	AN9245.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9245.4*::AfpyrG*
LO3829, LO3830, LO3831	AN9246.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9246.4*::AfpyrG*
LO3824, LO3825, LO3826	AN9247.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9247.4*::AfpyrG*
LO3819, LO3821	AN9248.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9248.4*::AfpyrG*
LO3814, LO3815, LO3816	AN9249.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9249.4*::AfpyrG*
LO3809, LO3810, LO3811	AN9250.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9250.4*::AfpyrG*
LO3804, LO3805, LO3806	AN9251.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9251.4*::AfpyrG*
LO3799, LO3800, LO3801	AN9252.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9252.4*::AfpyrG*
LO3794, LO3795	AN9253.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9253.4*::AfpyrG*
LO3790, LO3791	AN9254.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9254.4*::AfpyrG*
LO3784, LO3785, LO3786	AN11214.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN11214.4*::AfpyrG*
LO3540, LO3541	AN11205.4Δ	*pyrG*89*; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;*

		AN11205.4::*AfpyrG*
LO3603, LO3604, LO3605	AN9256.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9256.4::*AfpyrG*
LO6199, LO6200, LO6201	AN11647.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN11647.4::*AfpyrG*
LO3434, LO3435	AN9257.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9257.4::*AfpyrG*
LO3458, LO3459, LO3460	AN11217.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN11217.4::*AfpyrG*
LO3453, LO3454, LO3455	AN11206.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN11206.4::*AfpyrG*
LO3289, LO3290, LO3291	AN9259.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9259.4::*AfpyrG*
LO3463, LO3464, LO3465	AN11648.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN11648.4::*AfpyrG*
LO3438, LO3439, LO3440	AN9260.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9260.4::*AfpyrG*
LO3443,LO3444, LO3445	AN9261.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN9261.4::*AfpyrG*
LO3303, LO3304, LO3305	AN8142.4Δ	*pyrG89; pyroA*4, *nkuA::argB; riboB*2, *stcJ::riboB;* AN8142.4::*AfpyrG*

[a]: LO2026 which carries *stcJ*Δ and *nkuA*Δ was used as the parental strain for generating deletants in this study.

Table S2. Primers used in this study

primer	Sequence (5′→3′)
AN8376.4P1	GTG GCT GAT CGG TAT GAG G
AN8376.4P2	AGT TGT TAT GCA GTG CAG TCG
AN8376.4P3	CGA AGA GGG TGA AGA GCA TTG TTC TTT TGT AGC GGG TTT CG
AN8376.4P4	GCA TCA GTG CCT CCT CTC AGA CAG GAT GCC ATA TGA CGA TTG ACC
AN8376.4P5	GAG TGC ACA AGG CTC TAC AGG
AN8376.4P6	GGA TCC CTT TTT CAT GAC ACC
AN8377.4P1	GTT TCT TGG GCT GAT TTT GC
AN8377.4P2	TGC ATC CAT TTT CTC ACT GC
AN8377.4P3	CGA AGA GGG TGA AGA GCA TTG CGC ACC CTA GAA GAC AAA GC
AN8377.4P4	GCA TCA GTG CCT CCT CTC AGA CAG TCC CCA GAT AAG CTC AGT GC
AN8377.4P5	ACG GAT ACG GCA ACA CTA CC
AN8377.4P6	GAT GGG CCA GCA GAT ATG AC
AN8378.4P1	AAG CAG CTC CCA GAA AGA AAG
AN8378.4P2	AAT GAG GAT ATG GGC TCT GC
AN8378.4P3	CGA AGA GGG TGA AGA GCA TTG AGC TCC ATG CTT GAA AAT GC
AN8378.4P4	GCA TCA GTG CCT CCT CTC AGA CAG GGG CCA TGT ATA CCC TAA TGC
AN8378.4P5	CCT GGA ACC AGA TCA AGA GC
AN8378.4P6	CAT TGG CAG ACT CAT GAT ACG
AN8379.4P1	GCA GGA CCC TTC ATT CTG C
AN8379.4P2	GCC ATG TAA CAG AAT CCA TCC
AN8379.4P3	CGA AGA GGG TGA AGA GCA TTG CAA GCT GAT TTG TCC CAT CC
AN8379.4P4	GCA TCA GTG CCT CCT CTC AGA CAG TCT CTG TAT GCC CGA GTT CC
AN8379.4P5	GGC AGG TCA TCT AGC ATT GG
AN8379.4P6	CGG TAA CAG TTG GGT ACA TGG
AN8380.4P1	AGA TGT CCG GCA TTA GCA AC
AN8380.4P2	TTC AGG AGC CTT GAG CGT AG
AN8380.4P3	CGA AGA GGG TGA AGA GCA TTG CGT AGT TGA TGC GTC GAA AG
AN8380.4P4	GCA TCA GTG CCT CCT CTC AGA CAG CAG GTG TGC AAA CAA AAA GG
AN8380.4P5	GGC CAT GAA GGA GAA ATG G
AN8380.4P6	CCA GGT TCT AGG CTC AGA GG
AN8381.4P1	GAA GCT CTC AGA TGC TAC TG
AN8381.4P2	CCA GTG ATA GGA CAC GTT AAG
AN8381.4P3	CGA AGA GGG TGA AGA GCA TTG CTA TGA CCG ACG GTT CTA AC
AN8381.4P4	GCA TCA GTG CCT CCT CTC AGA CAG GAA CCT TGT GAC TGA ATT ATG C
AN8381.4P5	CTA ACT TTG CTT GTA GTG CTG
AN8381.4P6	GAC TTT CGA TCG TGG CTT TTG
AN8382.4P1	TGG GCA GGA TTA TGA AGT CG
AN8382.4P2	GAA GTC GTC CTA CCC GAA CC
AN8382.4P3	CGA AGA GGG TGA AGA GCA TTG GGA ATG CCC TAT GAT TTT GC
AN8382.4P4	GCA TCA GTG CCT CCT CTC AGA CAG AAA TGT GAG GGA GTG ATG TGC
AN8382.4P5	ACA ATT CGA AGC TGA AAC GC
AN8382.4P6	TTT GAA GCA AAA AGG GAA CC
AN8383.4P1	CGG TTT TTA GTT GGG TTT CG
AN8383.4P2	ATC TTG AGA ACG GCT TGT GC
AN8383.4P3	CGA AGA GGG TGA AGA GCA TTG GCC TGT ATA GGG CAA AGT GC
AN8383.4P4	GCA TCA GTG CCT CCT CTC AGA CAG AGT ATT TAG TGC CTA TCC TCG
AN8383.4P5	TAA CAA CGA TGC TGG TTT GG
AN8383.4P6	CAT ACT AAG CAG CGC AAT GG
AN8384.4P1	AAG CTC AAG AGG GGT TAC TGC
AN8384.4P2	CAA GAA TCC CCA ACC TAT TCC
AN8384.4P3	CGA AGA GGG TGA AGA GCA TTG GCA CTC ATC ACG GAG AGA CC

AN8384.4P4 GCA TCA GTG CCT CCT CTC AGA CAG TTC GGT CCG AGC TGT TTA TC
AN8384.4P5 CAT ATA GAG GCC GCA AGA CC
AN8384.4P6 CAG AAA GGC CGA GAA CAA AG

AN8385.4P1 GGG GCC TCA ATG TAG ATT CC
AN8385.4P2 GTT GAA TTC GGC GAT GAG AC
AN8385.4P3 CGA AGA GGG TGA AGA GCA TTG GGG GAA CGA TCT CAG AAG TG
AN8385.4P4 GCA TCA GTG CCT CCT CTC AGA CAG ATT GAT ACC ACG CAC CTT CG
AN8385.4P5 GAG ACC CCG GTT CTA AGC TC
AN8385.4P6 AGG ATG GCG TCA CAA GCT AC

AN11077.4P1 AGA AGG CGC TGT AGG TAT CG
AN11077.4P2 CGC ACT CAG GAA ACT CAA GG
AN11077.4P3 CGA AGA GGG TGA AGA GCA TTG TCG GTT CGA GCT TCT GTA GG
AN11077.4P4 GCA TCA GTG CCT CCT CTC AGA CAG AGA GCC TTG CAT TTG TAT TCG
AN11077.4P5 GGA ATG AAA AGG CCT GAA CC
AN11077.4P6 GAC CGA GAA GAC AAC CTT GG

AN11085.4P1 GAG CCT TGC ATT TGT ATT CG
AN11085.4P2 GTC GTT CAA GCC GTA GTT AGG
AN11085.4P3 CGA AGA GGG TGA AGA GCA TTG GAC CGA GAA GAC AAC CTT GG
AN11085.4P4 GCA TCA GTG CCT CCT CTC AGA CAG GCG CGT ATA GAC CTG AGA CAC
AN11085.4P5 CCT TGT ACC AGG AAA TGC AAC
AN11085.4P6 GGG ATG TGT CGC ATT CTA GTC

AN8387.4P1 GCT GGA ACG GTT ACA TCT GC
AN8387.4P2 AGC GGC TCA GAC TAC AAT GG
AN8387.4P3 CGA AGA GGG TGA AGA GCA TTG TGA TGT CTT TCG ATG CTT GC
AN8387.4P4 GCA TCA GTG CCT CCT CTC AGA CAG GGG AGG GGT ATT GTC TCA GG
AN8387.4P5 TAC TAA CCT CTG CCG GCT TG
AN8387.4P6 GCA ACG TCA TCT CCA AAT CC

AN9244.4P1 GTT GCG GAT GAA GGA AAC C
AN9244.4P2 GCT CCA CCA GCT TTA GCC G
AN9244.4P3 CGA AGA GGG TGA AGA GCA TTG CGA TGC TAA CGT ACG GAC C
AN9244.4P4 GCA TCA GTG CCT CCT CTC AGA CAG GCT TGA TAG AGA TCA TTG
AN9244.4P5 CCT GCA TTC CCC AGA ATG C
AN9244.4P6 CCT GGT AGT TTG GCA CGC

AN9245.4P1 CCA AGT GGG CTG GCA ATG TG
AN9245.4P2 GTCCAGATCGAATACTGTGTG
AN9245.4P3 CGA AGA GGG TGA AGA GCA TTG CAA GTG AGC GTG AAT CAT CTT
AN9245.4P4 GCA TCA GTG CCT CCT CTC AGA CAG CGG GAA CTC GGC TAA AGC TGC
AN9245.4P5 TTC AGT AAG CTC GTC ACC AC
AN9245.4P6 TTA CAA ACG ACG GCA ACA GC

AN9246.4P1 GGT GCA CCC TGT TAT TTT GC
AN9246.4P2 GAA GTC ATC CTG CCA AAT GC
AN9246.4P3 CGA AGA GGG TGA AGA GCA TTG GGT AAC AGC CCC TAC CTT CC
AN9246.4P4 GCA TCA GTG CCT CCT CTC AGA CAG ACA CAT GTC CTC GAT CAT GC
AN9246.4P5 CTC GAC GTT GAG GTT TCA GC
AN9246.4P6 ACT TGA GTA TCC CGG AAT CG

AN9247.4P1 CAA TGC TGG GTT GAA CTG C
AN9247.4P2 AAC TCT GTA GCA ATT TGC ATC G
AN9247.4P3 CGA AGA GGG TGA AGA GCA TTG GAA GGT AGG GGC TGT TAC CC
AN9247.4P4 GCA TCA GTG CCT CCT CTC AGA CAG GGC AGA TGG TCA GAT TAG GG
AN9247.4P5 AGT CGG GGA TCT TCT TAG CC
AN9247.4P6 GCC CTG GAA GAT TCT TTG C

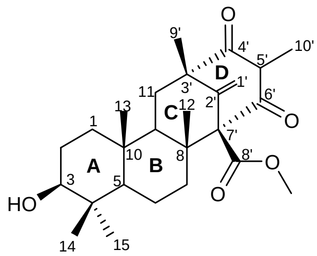
AN9248.4P1 CGG GCA AAC AGA CAA ATA TCC
AN9248.4P2 AAA TTT TGT CAG ACG ACC TTC C

AN92484P3	CGA AGA GGG TGA AGA GCA TTG TCT CAT GGC ATT GAT TCT TCC
AN9248.4P4	GCA TCA GTG CCT CCT CTC AGA CAG GCT GTC TTC TGT CCG CTA TTG
AN9248.4P5	AAT ACC GGT TAG AAG CGA AGC
AN9248.4P6	GGC TGA AGG ATC TCC AAT ACC
AN9249.4P1	CGA GGA ACA GAA GGC TGT G
AN9249.4P2	TTG CGC TAT CGT CTC AAG C
AN9249.4P3	CGA AGA GGG TGA AGA GCA TTG GGA AAT CCC ATG CAC TAC G
AN9249.4P4	GCA TCA GTG CCT CCT CTC AGA CAG GGC TCA CAG ATG ATG GAA CC
AN9249.4P5	GGG GTT CAA GTC AGG ATG C
AN9249.4P6	TGG GAA TTG AAC TCG TCT CC
AN9250.4P1	GCA AGA AGG CAG GAA ATA CG
AN9250.4P2	AGA GAC AAG CGA GGC TAT GG
AN9250.4P3	CGA AGA GGG TGA AGA GCA TTG CCA ATG AGT TGC AAA ACA CG
AN9250.4P4	GCA TCA GTG CCT CCT CTC AGA CAG ACC GGC GAG GTG TAT GTA TG
AN9250.4P5	CAA GGA GGA AGT TCG GGA AG
AN9250.4P6	AGA GGT CAT CCC AAG ATT GC
AN9251.4P1	CAG CTG TTG GAG TTT TTC AGC
AN9251.4P2	TCA GCG CAA AAG ACA AAG C
AN9251.4P3	CGA AGA GGG TGA AGA GCA TTG TCC TCA ACT TTC GTG TTT TGC
AN9251.4P4	GCA TCA GTG CCT CCT CTC AGA CAG ACA CCC TTC CCT CAT AGC AG
AN9251.4P5	TGT GAC TCG TCC GAC TTG AC
AN9251.4P6	TGA ATA CTT CGC CGT GTG AC
AN9252.4P1	CAC ATG AAC CAG GCA CTA GC
AN9252.4P2	GCG GTC GTT TTA TCA AGT GC
AN9252.4P3	CGA AGA GGG TGA AGA GCA TTG AAG CCA GCG AAA CTG TTG AC
AN9252.4P4	GCA TCA GTG CCT CCT CTC AGA CAG GCA CGC CTT TCC AAA ATA AG
AN9252.4P5	CGT ATT TCC TGC CTT CTT GC
AN9252.4P6	ATC TTT GCA AGT GGC CAG AG
AN9253.4P1	AAC GGT TTG CTT CTG ACA CC
AN9253.4P2	CGG CAG TAC TCA CAA CAT GC
AN9253.4P3	CGA AGA GGG TGA AGA GCA TTG CGG TCA GGG ATA TTC ATA GGC
AN9253.4P4	GCA TCA GTG CCT CCT CTC AGA CAG TGG CCT TTG AGA ATG AGA CC
AN9253.4P5	TTG GTC GTT GGT GAA AAT AGG
AN9253.4P6	GGA CTG CAG AAG CAG TTG G
AN9254.4P1	GTG GTA GCA CTC GAC TGA CC
AN9254.4P2	TTC AGC ATT ACG GCT CTG C
AN9254.4P3	CGA AGA GGG TGA AGA GCA TTG CTA GCG AGA CGG CTA CAA GG
AN9254.4P4	GCA TCA GTG CCT CCT CTC AGA CAG CGG TTA CTC TGG AAC CAT GC
AN9254.4P5	CAG GAG ACG CAA ACA ATT CC
AN9254.4P6	GTC CGC ATA TTC AGG AGA CG
AN11214.4P1	TGA AGG AAC TGG GTT TGT CC
AN11214.4P2	CTC AGC AGA GGA GCA AGA GC
AN11214.4P3	CGA AGA GGG TGA AGA GCA TTG CCC TTT GGC TCC TAA TAT CG
AN11214.4P4	GCA TCA GTG CCT CCT CTC AGA CAG TCA CTA TAC CCG CCT TGA CC
AN11214.4P5	TGT AAC TGA TTC AAT CCC ATG C
AN11214.4P6	AGA CAT ATG GGG CCT TTG C
AN11205.4P1	AAC TTT CTG CGC GCC TAC C
AN11205.4P2	CTC GAC TTG ATA TCT CCG TG
AN11205.4P3	CGT CAG ACA CAG AAT AAC TC TGT ACC TGT CAT AGC ATT AAG
AN11205.4P4	GCA TCA GTG CCT CCT CTC AGA CAG TCA GCC AGG AGG AGA TCG AG
AN11205.4P5	TCG CTG TCA CCT TCA CTA TG
AN11205.4P6	TAG GGT ACT GTT GGT GCA AG
AN9256.4P1	TCG AGT CCA TTT TGG CCA AG

AN9256.4P2	CAC GCC ATG ATC ATC AGA G
AN9256.4P3	CGT CAG ACA CAG AAT AAC TC CAG CAT GCC GAC TCC AAG G
AN9256.4P4	GCA TCA GTG CCT CCT CTC AGA CAG CAG TAA AGC CAG CCA TCA ATG
AN9256.4P5	CAG TAC CAC GTC CCT TAG AA
AN9256.4P6	GAG GTT TTA ACG GAG GTT TTG
AN11647.4P1	AGT CGT CCT CGG CTTTCAC
AN11647.4P2	GTG TGC AGG AAA AAC CAC AC
AN11647.4P3	CGA AGA GGG TGA AGA GCA TTG TCT CCG TGT TGC ACA TCA AC
AN11647.4P4	GCA TCA GTG CCT CCT CTC AGA CAG CCA GAT CAT CAA CCA CCT G
AN11647.4P5	CCA ATG AGC AAG GCA TCG TG
AN11647.4P6	CTC CAG CAC TAG CCA GAA G
AN9257.4P1	TGG TTG ATG ATC TGG ACA GC
AN9257.4P2	ACG AGG ACT TGC AAA AGA GC
AN9257.4P3	CGA AGA GGG TGA AGA GCA TTG GGA ATG TTT GCG CAA CTA GC
AN9257.4P4	GCA TCA GTG CCT CCT CTC AGA CAG GGG TGG GAA ATC AGG AAA AG
AN9257.4P5	ACA GAC TGC TCT CCC TAC CG
AN9257.4P6	GAC TGC TCA CGA CAC TGC AC
AN11217.4P1	AAG ACG TCG GTC TCC ATG AC
AN11217.4P2	TGC TGG AGA CCT GGA GAT TC
AN11217.4P3	CGA AGA GGG TGA AGA GCA TTG TCA TCG CAT GTA ACC ACA GG
AN11217.4P4	GCA TCA GTG CCT CCT CTC AGA CAG CTT GTC TCG TGG CTT TTT CG
AN11217.4P5	CGA CTG GTG CTG TTG TAT GC
AN11217.4P6	GTG GTT TTT CCT GCA CAC G
AN11206.4P1	GGT CGA TCA TGA CAA TCA CG
AN11206.4P2	CAT ACC TAG GCT GTG CAT GG
AN11206.4P3	CGA AGA GGG TGA AGA GCA TTG CCT TGC TAT GTG CAT CAA CG
AN11206.4P4	GCA TCA GTG CCT CCT CTC AGA CAG TCA GAA CAG GCT GGA ATG TG
AN11206.4P5	GGC TGA AGC TGC TTA TCG TC
AN11206.4P6	CTA GTG AGC CGG CTT CAA AC
AN9259.4P1	GGA GAT ATT GCA AAG CAC ACC
AN9259.4P2	ACG CAC TGC ACT GTG TAT CC
AN9259.4P3	CGA AGA GGG TGA AGA GCA TTG CGG CAA TCA TGC ATC TAG G
AN9259.4P4	GCA TCA GTG CCT CCT CTC AGA CAG CTA GAT CGC TGG GAA GGT TG
AN9259.4P5	ACG TCT GGA CTT GGG ATG AG
AN9259.4P6	CTT GTG TCT TGG AAG CAT CG
AN11648.4P1	GCC CAC CAT AAT GTC AAA GC
AN11648.4P2	GCA GGT TCT GCG GTT ATA CG
AN11648.4P3	CGA AGA GGG TGA AGA GCA TTG TTT CGA TCC GTT CAT CTT CG
AN11648.4P4	GCA TCA GTG CCT CCT CTC AGA CAG CCC GAT TGC TTC TTG TAT GC
AN11648.4P5	GGC GCT GAT TAG TGT TTC G
AN11648.4P6	ATT TAC AGA GGC CCT GTT GG
AN9260.4P1	TCA GGA GAA GCT TTG GAA GG
AN9260.4P2	GAT TTG CCA GCA ACC ATC C
AN9260.4P3	CGA AGA GGG TGA AGA GCA TTG TTG GCA CAA TAG GCA ATC G
AN9260.4P4	GCA TCA GTG CCT CCT CTC AGA CAG ATA TTT ACG CCC AAA TCA TGC
AN9260.4P5	ACT TGA TCC TCG GGT TTT CC
AN9260.4P6	TCA GCA TGA TCG CCT AAC C
AN9261.4P1	TGG AAT CGG TTT GTC ATG G
AN9261.4P2	GGC GTA CGA TGT AGA TCA GG
AN9261.4P3	CGA AGA GGG TGA AGA GCA TTG CTT CTG TAA CCG CGT TGT CC
AN9261.4P4	GCA TCA GTG CCT CCT CTC AGA CAGTCA ATT AAC ACC CTG CGA TTC
AN9261.4P5	AGG GCA AAG GAC ATA TCA CG
AN9261.4P6	TTC CTG ACC AAG GAT TCA GAC

AN8142.4P1	ACC AGT ATG CCA AAA ACA AGG
AN8142.4P2	CCC GAT TGT CCA TAG TAT TCG
AN8142.4P3	CGA AGA GGG TGA AGA GCA TTG TAC CAC CTC GCA ATG TCT AGC
AN8142.4P4	GCA TCA GTG CCT CCT CTC AGA CAG TGT ACG TTT GTG GCA TTT GG
AN8142.4P5	GCT CGT AGG ACC ACA CAA GG
AN8142.4P6	GGA AGA CCG TTT GTT GAA CG
Internal primer	Used for diagnostic PCR
PyrGR	CGG GAG CAG CGT AGA TGC C
PyrGF	GAG TTA TTC TGT GTC TGA CG

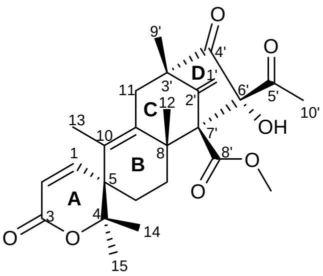
Blue and red sequences are tails that anneal to the *A. fumigatus pyrG* fragment (*AfpyrG*) during fusion PCR.



protoaustinoid A (**4**)

Table S3. NMR data for compound **4** (400 and 100 MHz in $CDCl_3$)

Position	δ H (*J* in Hz)	δ C
1	H_α: 0.66, td (13.2, 4.0) H_β: 1.50, m	38.3, CH_2
2	1.50, m	26.9, CH_2
3	3.11, dd (11.2, 4.4)	78.3, CH
4	—	38.3, C
5	0.56, dd (6.0, 2.0)	54.4, CH
6	1.55, m	18.2, CH_2
7	H_α: 2.24, td (14.0, 4.0) H_β: 2.13, dt (14.0, 3.2)	33.1, CH_2
8	—	47.0, C
9	0.56, dd (12.4, 3.2)	51.9, CH
10	—	37.5, C
11	H_α: 1.60, m H_β: 1.92, dd (12.4, 3.2)	37.7, CH_2
12	1.21, s	17.6, CH_3
13	0.78, s	15.9, CH_3
14	0.73, s	15.5, CH_3
15	0.94, s	27.8, CH_3
1'	H_a: 4.82, brs H_b: 5.29, brs	112.0, CH_2
2'	—	146.8, C
3'	—	52.2, C
4'	—	209.4, C
5'	3.91, q (6.4)	62.5, CH
6'	—	204.1, C
7'	—	73.0, C
8'	—	169.2, C
9'	1.37, s	21.4, CH_3
10'	1.19, s	6.5, CH_3
-OCH_3	3.67, s	52.0, CH_3

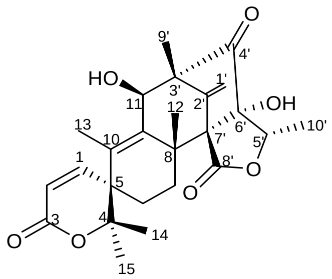


preaustinoid A4 (**6**)

Table S4. NMR data for compound **6** (400 and 100 MHz in $CDCl_3$)

Position	δ H (*J* in Hz)	δ C	HMBC[a]	COSY	NOESY
1	6.73, d (10.0)	147.2, CH	3, 4, 5, 6	H-2	H-2, H_α-7
2	6.01, d (10.0)	118.8, CH	3, 5	H-1	H-1
3	—	164.2, C			
4	—	85.7, C			
5	—	45.5, C			
6	1.60, m	27.4, CH_2	1, 5, 7	H_2-7	H_2-7, H_3-12, H_3-15
7	H_α: 3.11, dt (13.6, 4.4)	27.3, CH_2	5, 6, 8, 12	H_2-6, H_β-7	H-1, H_2-6, H_β-7
	H_β:1.04, dt (13.6, 3.6)		8, 12	H_2-6, H_α-7	H_2-6, H_α-7, H_3-12
8	—	45.6, C			
9	—	135.3, C			
10	—	129.5, C			
11	H_α:2.86, d (14.4)	43.0, CH_2	9, 10, 3', 4'	H_β-11	H_β-11, H_3-13, H_3-9'
	H_β:2.28, dd (14.4, 1.6)		8, 9, 10, 2', 3', 4', 9'	H_α-11, H_3-13	H_α-11, H_3-12, H_3-9'
12	1.39 s	24.0, CH_3	7, 8, 9, 10, 7'		H_2-6, H_β-7, H_β-11
13	1.55, d (1.6)	15.2, CH_3	5, 9, 10	H_β-11	H_α-11, H_3-14
14	1.24, s	23.0, CH_3	4, 5		H_3-13
15	1.35, s	25.7, CH_3	4, 5		H_2-6
1'	H_a: 5.90, br s	109.6, CH_2	2', 3', 7'		H_b-1'
	H_b: 5.34, br s		2', 3', 7'		H_a-1', H_3-9'
2'	—	146.5, C			
3'	—	54.9, C			
4'	—	211.1, C			
5'	—	201.3, C			
6'	—	92.9, C			
7'	—	65.8, C			
8'	—	169.5, C			
9'	1.27, s	16.3, CH_3	11, 2', 3', 4'		H_2-11, H_b-1', H_3-10’
10'	2.02, s	26.7, CH_3	5', 6'		H_3-9'
-OCH_3	3.70, s	54.9, CH_3	8'		
-OH	4.68, bs				

[a]: HMBC correlations are from proton(s) to the indicated carbon.

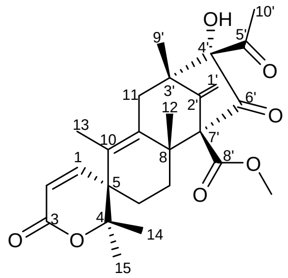


11β-hydroxyisoaustinone (**8**)

Table S5. NMR data for compound **8** (400 and 100 MHz in DMSO-d_6)

Position	δ H (*J* in Hz)	δ C	HMBC[a]	COSY	NOESY
1	6.55, d (10.0)	148.1, CH	3, 4, 5, 14	H-2	H-2
2	5.97, d (10.0)	117.9, CH	3, 5	H-1	H-1
3	—	163.4, C			
4	—	85.6, C			
5	—	45.2, C			
6	$H_α$: 1.50, m	26.2, CH_2	5	$H_β$-6, H_2-7	
	$H_β$: 1.74, td (14.0, 3.6)		1, 4, 5, 9, 10	$H_α$-6, H_2-7	H_3-12
7	$H_α$: 1.45, m	26.2, CH_2	5, 6	H_2-6, $H_β$-7	
	$H_β$: 2.73, td (14.0, 3.6)			H_2-6, $H_α$-7	H_3-12
8	—	40.3, C			
9	—	139.7, C			
10	—	131.3, C			
11	4.25, d (4.8)	74.1, CH	8, 9, 10, 13, 2', 3', 4'	OH-11	H_3-13, OH-11
12	1.46, s	23.4, CH_3	7, 8, 9, 7'		$H_β$-6, $H_β$-7, OH-11
13	1.54, s	13.8, CH_3	5, 9, 10		H-11, H_3-14
14	1.26, s	22.8, CH_3	4, 5, 15		H_3-13
15	1.30, s	25.7, CH_3	4, 5, 14		
1'	H_a: 5.03, br s	108.9, CH_2	2', 3', 4', 7'		H_b-1'
	H_b: 5.15, br s		2', 3', 4’, 7'		H_a-1', H_3-9'
2'	—	144.3, C			
3'	—	61.8, C			
4'	—	210.3, C			
5'	4.37, q (6.4)	77.0, CH	4', 6', 10’	H_3-10'	H_3-10'
6'	—	90.8, C			
7'	—	65.8, C			
8'	—	172.4, C			
9'	1.18, s	13.2, CH_3	11, 2', 3', 4'		H_b-1'
10'	1.13, d (6.4)	13.0, CH	5', 6'	H-5'	H_3-5', OH-6'
6'-OH	6.68, bs	—	4', 6', 7'		H_3-10'
11-OH	5.66, d (4.8)	—	9, 11, 3’	H-11	H-11, H_3-12,

[a]: HMBC correlations are from proton(s) to the indicated carbon.

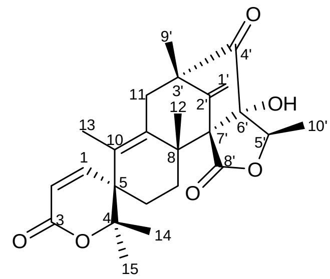


preaustinoid A5 (**10**)

Table S6. NMR data for compound **10** (400 and 100 MHz in $CDCl_3$)

Position	δ H (*J* in Hz)	δ C	HMBC[a]	COSY	NOESY
1	6.34, d (10.0)	146.5, CH	3, 4	H-2	H-2
2	6.02, d (10.0)	119.2, CH	3, 4, 5	H-1	H-1
3	—	164.2, C			
4	—	85.7, C			
5	—	45.5, C			
6	1.59, m	26.6, CH_2	5, 7, 8	H_2-7	H_2-7
7	H_α: 2.00, td (14.0, 1.6)	24.5, CH_2	6, 8	H_2-6, H_β-7	H_2-6, H_β-7
	H_β: 1.79, dt (14.0, 3.6)		6, 8, 12	H_2-6, H_α-7	H_2-6, H_α-7, H_3-12
8	—	47.2, C			
9	—	136.1, C			
10	—	131.0, C			
11	H_α: 2.00, dq (14.4, 1.2)	40.6, CH_2	9, 10, 3', 4'	H_β-11,H_3-13	H_β-11, H_3-9'
	H_β: 3.05, d (14.4)		8, 9, 10, 2', 3', 9'	H_α-11	H_α-11, H_3-13, H_3-9'
12	1.48, s	21.7, CH_3	7,8,9,7'		H_β-7
13	1.69, d (1.2)	15.2, CH_3	5, 9, 10	H_α-11	H_β-11, H_3-14
14	1.25, s	23.1, CH_3	4, 5		H_3-13
15	1.36, s	25.8, CH_3	4, 5		
1'	H_a: 5.15, s	108.1, CH_2	2', 3', 7'		H_b-1',
	H_b:5.36, s		3', 7'		H_a-1',H_3-9'
2'	—	149.6, C			
3'	—	50.1, C			
4'	—	92.2, C			
5'	—	202.6, C			
6'	—	207.4, C			
7'	—	71.5, C			
8'	—	167.7, C			
9'	1.06, s	16.3, CH_3	11, 2', 3', 4'		H_β-11, H_b-1', H_3-10'
10'	2.21, s	28.2, CH_3	4',5'		H_3-9'
OCH_3	3.76, s	52.4, CH_3	8'		
OH	4.56, bs		3',4',5'		

[a]: HMBC correlations are from proton(s) to the indicated carbon.

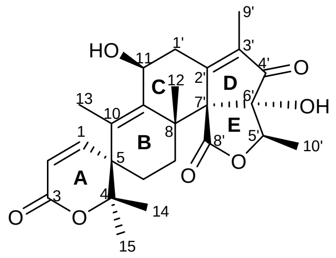


(5'*R*)-isoaustinone (**12**)

Table S7. NMR data for compound **12** (400 and 100 MHz in $CDCl_3$)

Position	δ H (*J* in Hz)	δ C	HMBC[a]	COSY	NOESY
1	6.53, d (10.0)	146.7, CH	3, 4, 5, 6	H-2	H-2
2	6.02, d (10.0)	119.4, CH	3, 5	H-1	H-1
3	—	164.0, C			
4	—	85.9, C			
5	—	45.6, C			
6	1.62, m	27.1, CH_2	1, 2, 5, 7, 9, 10	H_2-7	H_2-7
7	H_α: 2.60, td (13.6, 4.4)	26.2, CH_2	6, 8, 12, 7'	H_2-6, H_β-7	H_2-6, H_β-7
	H_β: 1.80, dt (13.6, 3.6)			H_2-6, H_α-7	H_2-6, H_α-7
8	—	41.5, C			
9	—	136.5, C			
10	—	129.5, C			
11	H_α: 2.89, d (14.4)	42.9, CH_2	3, 8, 9, 10, 2', 3', 4',9'	H_β-11	H_3-9', H_β-11, H_3-13
	H_β: 2.28, dd (14.4, 2.0)		9, 10, 2', 3', 4'	H_α-11, H_3-13	H_3-12, H_α-11
12	1.43, d (0.8)	22.2, CH_3	8, 9, 7'		H_β-11
13	1.57, d (1.6)	15.2, CH_3	5, 9, 10	H_β-11	H_α-11
14	1.24, s	23.1, CH_3	4, 5		
15	1.37, s	25.7, CH_3	4, 5		
1'	H_a: 5.27, d (0.8)	108.9, CH_2	11, 2', 3', 4',7'	H_b-1'	
	H_b: 5.30, d (0.8)		11, 2', 3', 4',7'	H_a-1'	H_3-9'
2'	—	147.3, C			
3'	—	55.6, C			
4'	—	214.0, C			
5'	4.46, q (6.8)	84.5, CH	6', 8'	H_3-10'	H_3-10'
6'	—	90.7, C			
7'	—	64.5, C			
8'	—	172.1, C			
9'	1.26, s	14.8, CH_3	2', 3', 4', 11		H_b-1'
10'	1.15, d (6.8)	18.1, CH_3	5', 6'	H-5'	H-5'
OH	2.96, bs	—			

[a]: HMBC correlations are from proton(s) to the indicated carbon.

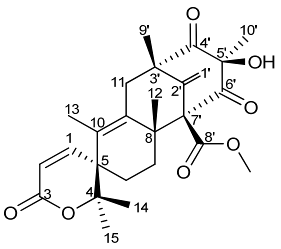


neoaustinone (**13**)

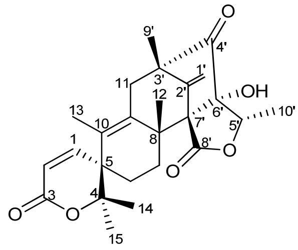
Table S8. NMR data for compound **13** (400 and 100 MHz in $CDCl_3$)

Position	δ H (*J* in Hz)	δ C	HMBC[a]	COSY	NOESY
1	6.42, d (9.6)	147.4, CH	3, 4, 5, 6	H-2	H-2, H_α-7
2	5.95, d (9.6)	118.6, CH	3, 5	H-1	H-1
3	—	164.8, C			
4	—	86.1, C			
5	—	45.5, C			
6	H_α: 1.63, m	25.9, CH_2	5	H_β-6, H_2-7	H-1, H_β-6, H_β-7, H-5'
	H_β:1.86, td (12.8, 3.6)		1, 4, 5	H_α-6, H_2-7	H_α-6, H-11
7	H_α: 2.81,td (12.8, 3.6)	28.3, CH_2		H_2-6, H_β-7	H-1, H_β-7, H-5'
	H_β:1.72, dt (12.8, 3.6)		5, 8	H_2-6, H_α-7	H_α-6, H_α-7
8	—	40.3, C			
9	—	142.0, C			
10	—	134.5, C			
11	5.16, dt (6.8, 4.4)	67.6, CH	9, 10, 2'	H_2-1'	H_3-13, H_2-1'
12	1.62, s	30.4, CH_3	7, 8, 9, 7'		H_β-1'
13	1.83, s	15.5, CH_3	5, 9, 10		H-11
14*	1.40, s	23.2, CH_3	4, 5, 15		
15*	1.40, s	25.6, CH_3	4, 5, 14		
1'	H_α: 3.02, dd (14.0, 4.4)	34.7, CH_2	9, 11, 2', 7'	H-11, H_β-1'	H-11, H_β-1'
	H_β: 3.23 dd (14.0, 4.4)		11, 2', 3'	H-11, H_α-1'	H-11, H_α-1'
2'	—	169.9, C			
3'	—	137.2, C			
4'	—	203.4, C			
5'	4.70, q (6.4)	79.4, CH	4', 6', 10'	H_3-10'	H_2-7, H_3-10'
6'	—	85.4, C			
7'	—	61.6, C			
8'	—	173.0, C			
9'	1.79, brs	8.4, CH_3	2', 3', 4'	H_β-1'	
10'	1.47, d (6.4)	15.2, CH_3	5', 6'	H-5'	H-5'
6'-OH	3.61, s				
11-OH	1.92 d (6.8)				

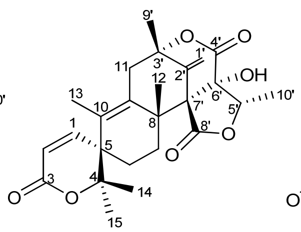
[a]: HMBC correlations are from proton(s) to the indicated carbon.



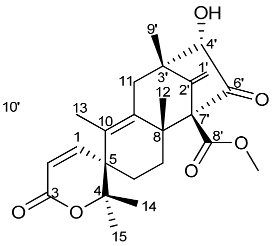
preaustinoid A3 (**5**)



isoaustinone (**7**)



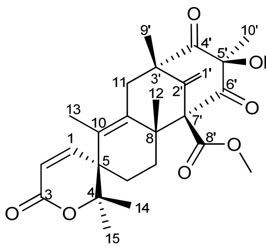
austinolide (**9**)



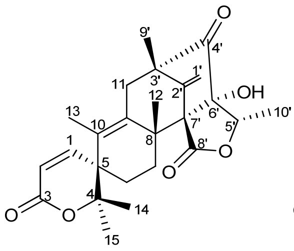
austinoneol A (**11**)

Table S9. ^{1}H NMR data for compound **5, 7, 9**, **11** (400 MHz in $CDCl_3$)

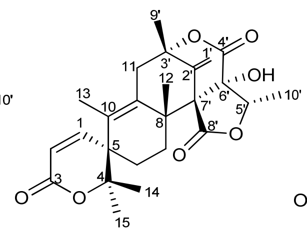
	δ H (*J* in Hz)			
Position	**5**	**7**	**9**	**11**
1	6. 36, d (9.6)	6.57, d (10.0)	6.70, d (10.0)	6.41, d (10.0)
2	6.03, d (9.6)	6.02, d (10.0)	6.06, d (10.0)	6.04, d (10.0)
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	H_α:1.53 m H_β: 1.61 m	1.62, m	H_α: 1.62, m H_β: 1.52, dd (14.0, 3.6)	1.58, m
7	H_α: 2.04, dt (14.8, 3.6) H_β: 2.66, td (17.6, 3.6)	H_α: 2.576, td (13.2, 4.8) H_β: 1.81, dt (13.2, 3.6)	H_α: 3.03, td (14.0, 3.6) H_β: 1.72, dt (14.0, 3.6)	H_α: 2.36, m H_β: 1.75 dt (14.0, 4.0)
8	—	—	—	—
9	—	—	—	—
10	—	—	—	—
11	H_α: 2.21, dd (14.4, 1.2) H_β: 2.95, d (15.6)	H_α: 2.88, d (14.4) H_β: 2.27, dq (14.4, 1.6)	H_α: 3.20, d (16.4), H_β: 2.40, dq (16.4, 1.2)	H_α: 2.95, d (14.8) H_β: 1.97,dq (14.8, 1.6)
12	1.48, s	1.42, s	1.37, s	1.40, s
13	1.49, s	1.56, d (1.6)	1.67, d (1.2)	1.65, d (1.6)
14	1.22, s	1.24, s	1.25, s	1.36, s
15	1.36, s	1.37, s	1.38, s	1.23, s
1'	H_a: 4.99, d (1.0); H_b: 5.49, d (1.0)	H_a: 5.25, d (1.0); H_b: 5.23, d (1.0)	H_a: 5.33, d (1.4); H_b: 5.60, d (1.4)	H_a: 5.00, br s; H_b: 5.24, br s
2'	—	—	—	—
3'	—	—	—	—
4'	—	—	—	3.68, s
5'	—	4.28, q (6.4)	4.40, q (6.4)	—
6'	—	—	—	—
7'	—	—	—	—
8'	—	—	—	—
9'	1.50, s	1.30, s	1.66, s	1.33, s
10'	1.20, s	1.28, d (6.4)	1.28, d (6.4)	3.70, s
OCH_3	3.76, s	—	—	—
OH	2.90, br s	2.72, br s	3.62, br s	



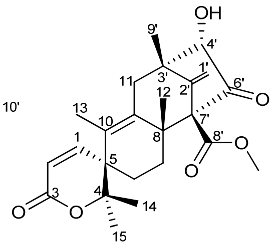
preaustinoid A3 (**5**)



isoaustinone (**7**)



austinolide (**9**)



austinoneol A (**11**)

Table S10. ^{13}C NMR data for compound **5, 7, 9**, **11** (100 MHz in $CDCl_3$)

	δ C			
Position	**5**	**7**	**9**	**11**
1	145.9, CH	147.0, CH	147.1, CH	146.6, CH
2	119.7, CH	119.2, CH	119.5, CH	119.2, CH
3	163.9, C	164.1, C	164.2, C	164.2, C
4	85.7, C	85.9, C	86.0, C	85.7, C
5	45.4, C	45.6, C	46.0, C	45.5, C
6	26.2, CH_2	27.0, CH_2	27.0, CH_2	26.4, CH_2
7	24.6, CH_2	26.0, CH_2	25.6, CH_2	24.4, CH_2
8	47.0, C	40.9, C	41.7, C	46.0, C
9	136.2, C	136.0, C	134.2, C	136.9, C
10	131.9, C	129.7, C	140.0, C	130.7, C
11	41.1, CH_2	42.5, CH_2	41.9, CH_2	36.1, CH_2
12	25.9, CH_3	22.2, CH_3	22.9, CH_3	20.9, CH_3
13	15.5, CH_3	15.1, CH_3	15.4, CH_3	15.3, CH_3
14	22.9, CH_3	23.1, CH_3	22.9, CH_3	25.8, CH_3
15	25.9, CH_3	25.7, CH_3	25.9, CH_3	23.1, CH_3
1'	113.9, CH_2	108.1, CH_2	115.0, CH_2	107.4, CH_2
2'	143.8, C	146.2, C	134.4, C	148.4, C
3'	51.1, C	55.2, C	83.8, C	46.5, C
4'	206.1, C	212.4, C	171.5, C	82.6, C
5'	78.1, C	76.3, CH	79.1, CH	—
6'	203.6, C	90.6, C	80.8, C	210.4, C
7'	71.8, C	66.1, C	63.1, C	69.1, C
8'	168.8, C	172.1, C	171.0, C	167.9, C
9'	22.8, CH_3	15.1, CH_3	23.7, CH_3	19.8, CH_3
10'	16.6, CH_3	12.6, CH_3	11.5, CH_3	—
OCH_3	52.6, CH_3	—	—	52.2, CH_3

ORFs		Homologs	
Gene	Predicted size (aa)	Match from BLAST search at NCBI (accession no.)	Identity/similarity (%)
AN8380.4	151	Terpene cyclase (AusN)[*Aspergillus nidulans*] (in this study)	46/68
AN8382.4	241	Hypothetical protein	
AN9250.4	516	TqaD (O-acetyltransferase) [*Penicillium aethiopicum*] (ADY16688)	29/45
AN9251.4	535	GA14-synthase (P450 monooxygenase) [*Gibberella intermedia*](CAF31353)	36/58
AN9252.4	140	Hypothetical protein	
AN9256.4	143	Hypothetical protein	
AN11647.4	94	cyclopentadecanone 1,2-monooxygenase [*Pseudomonas sp.* HI-70](BAE93346)	42/61
AN11217.4	620	Bcmfs1(Major facilitator superfamily) [*Botryotinia fuckeliana*] (AAF64435)	45/63

Table S11. Annotation of genes internal to the cluster A and B not required for austinol/dehydroaustinol biosynthesis.

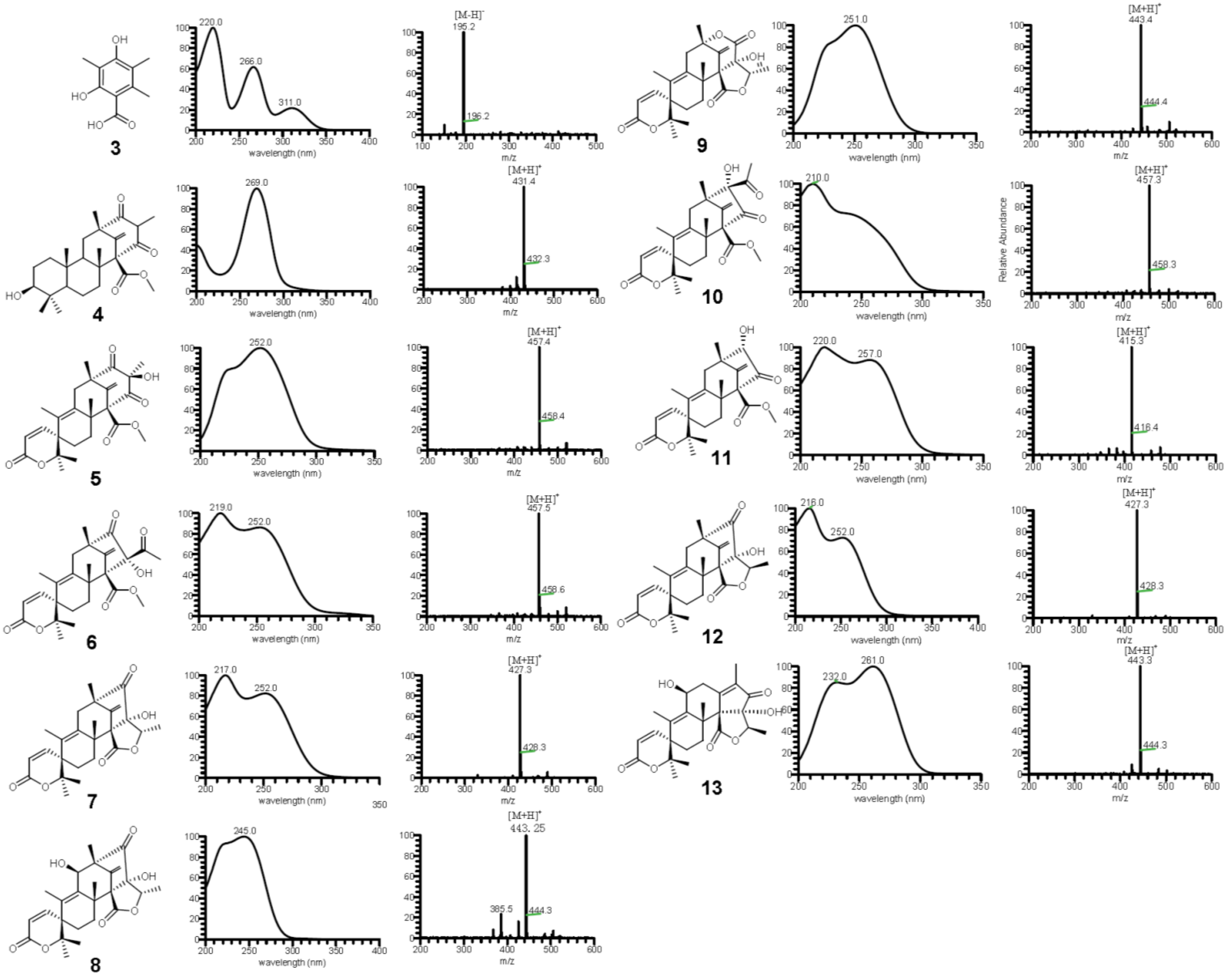


Figure S1. UV-Vis and ESIMS spectra of compounds isolated from *A. niduans*.

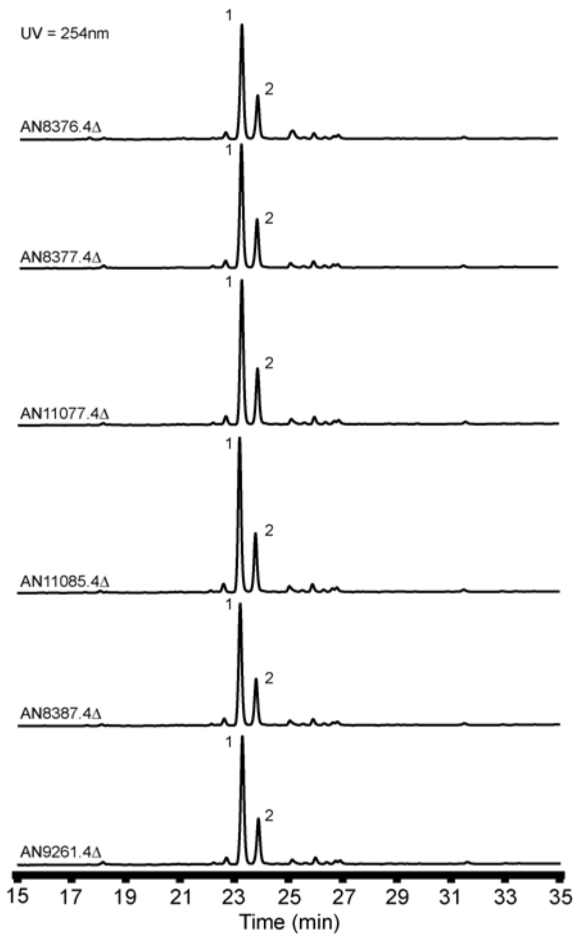


Figure S2. HPLC profiles of extracts from the remaining knockout strains not shown in figure 4 and 5 as detected by UV absorption at 254 nm.

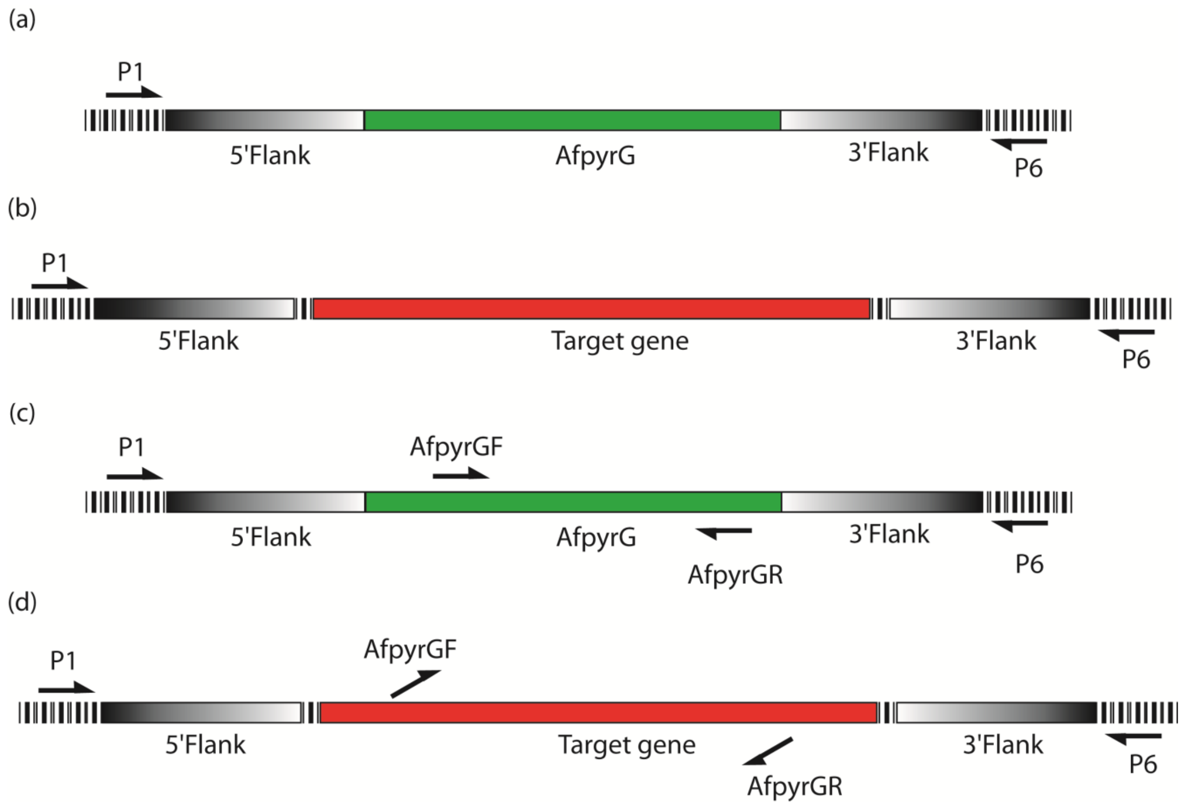
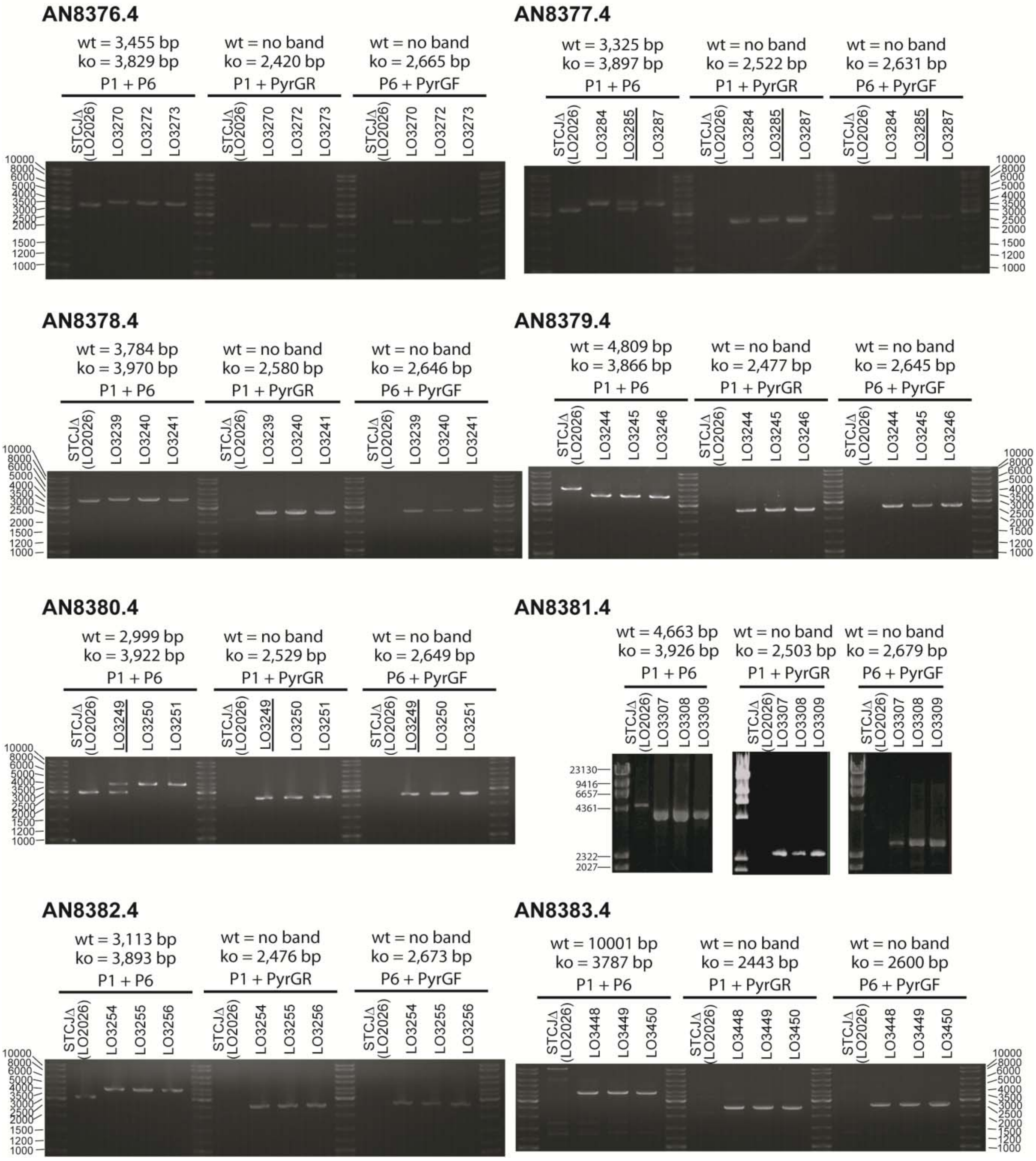
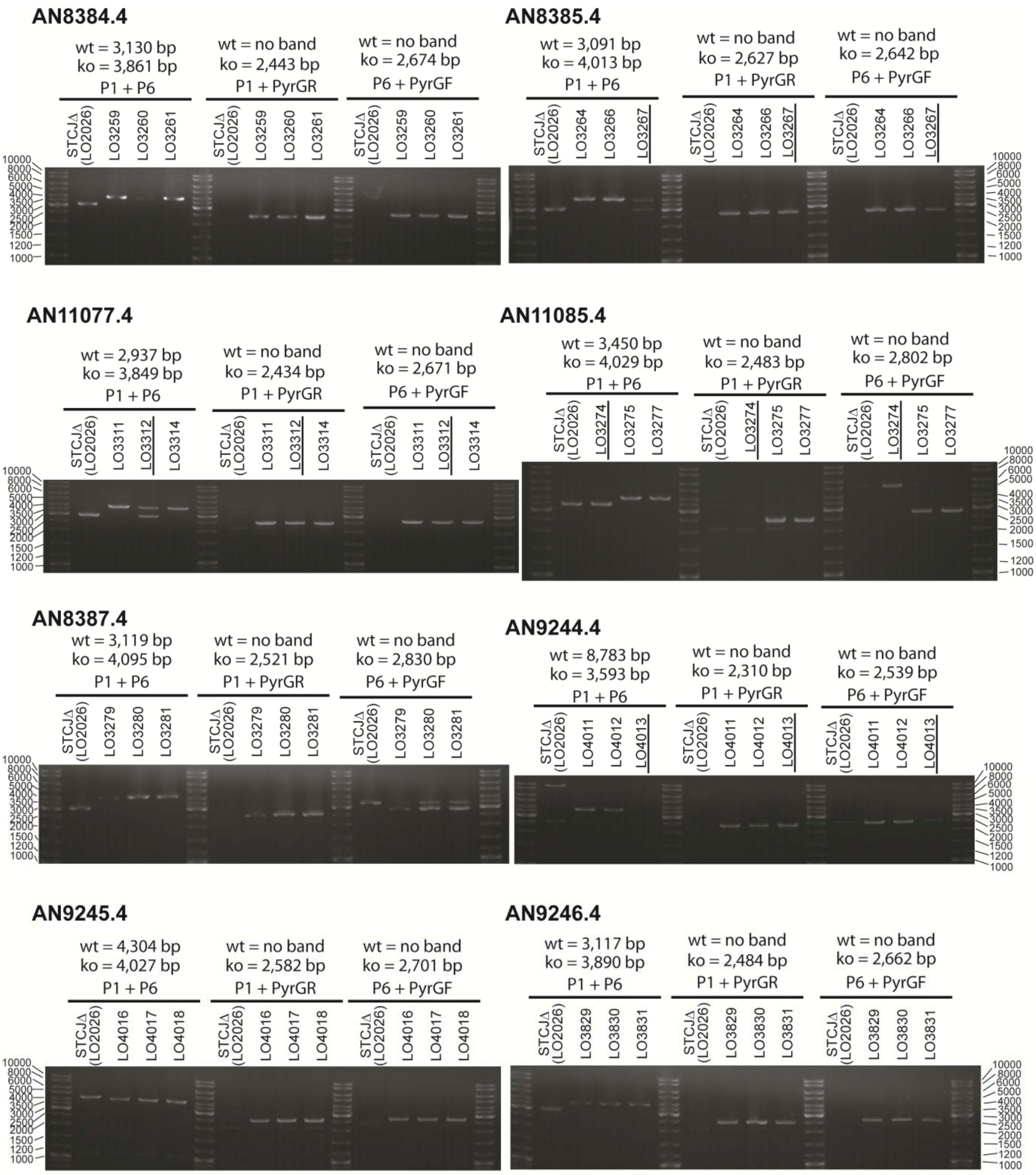
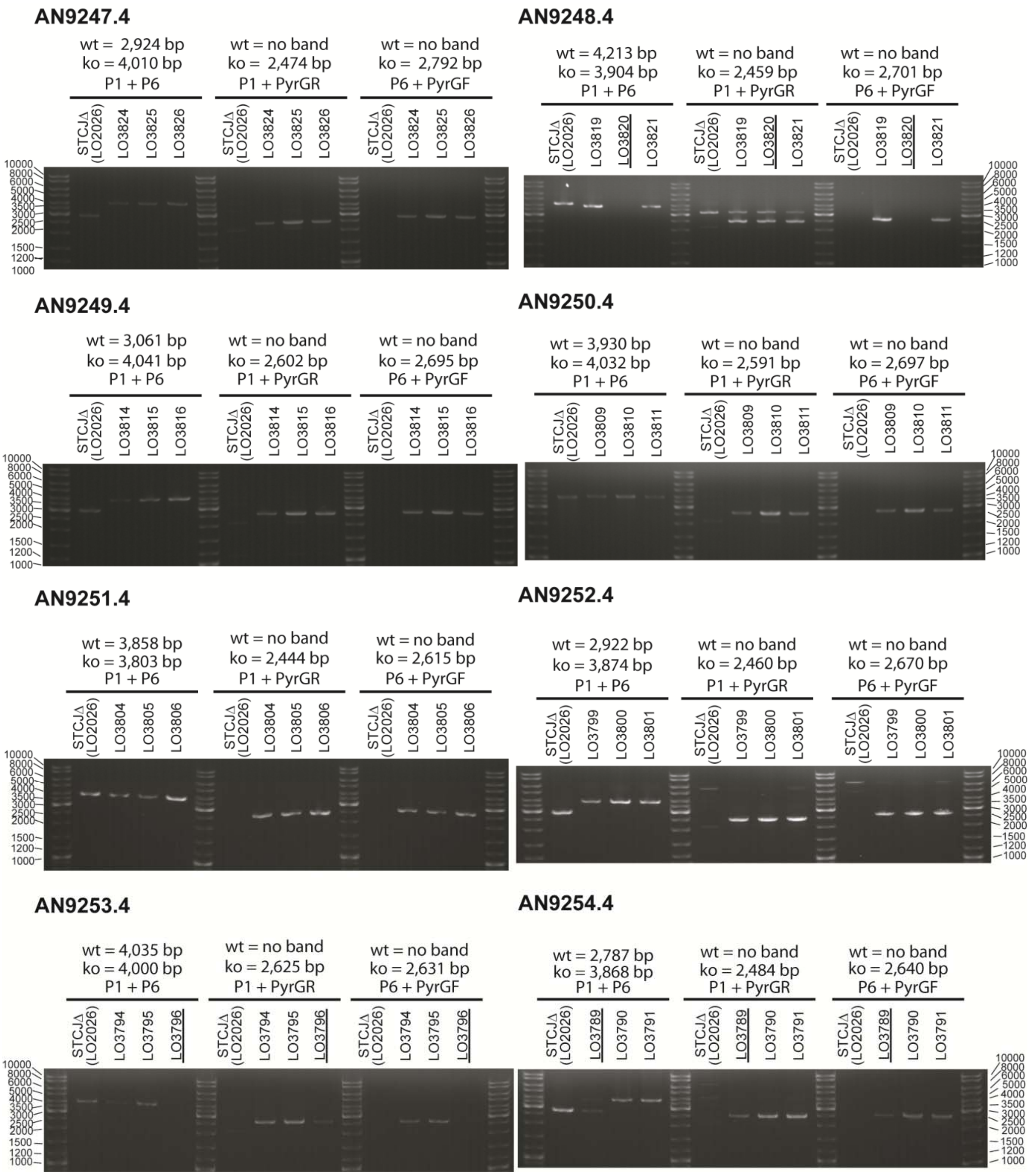


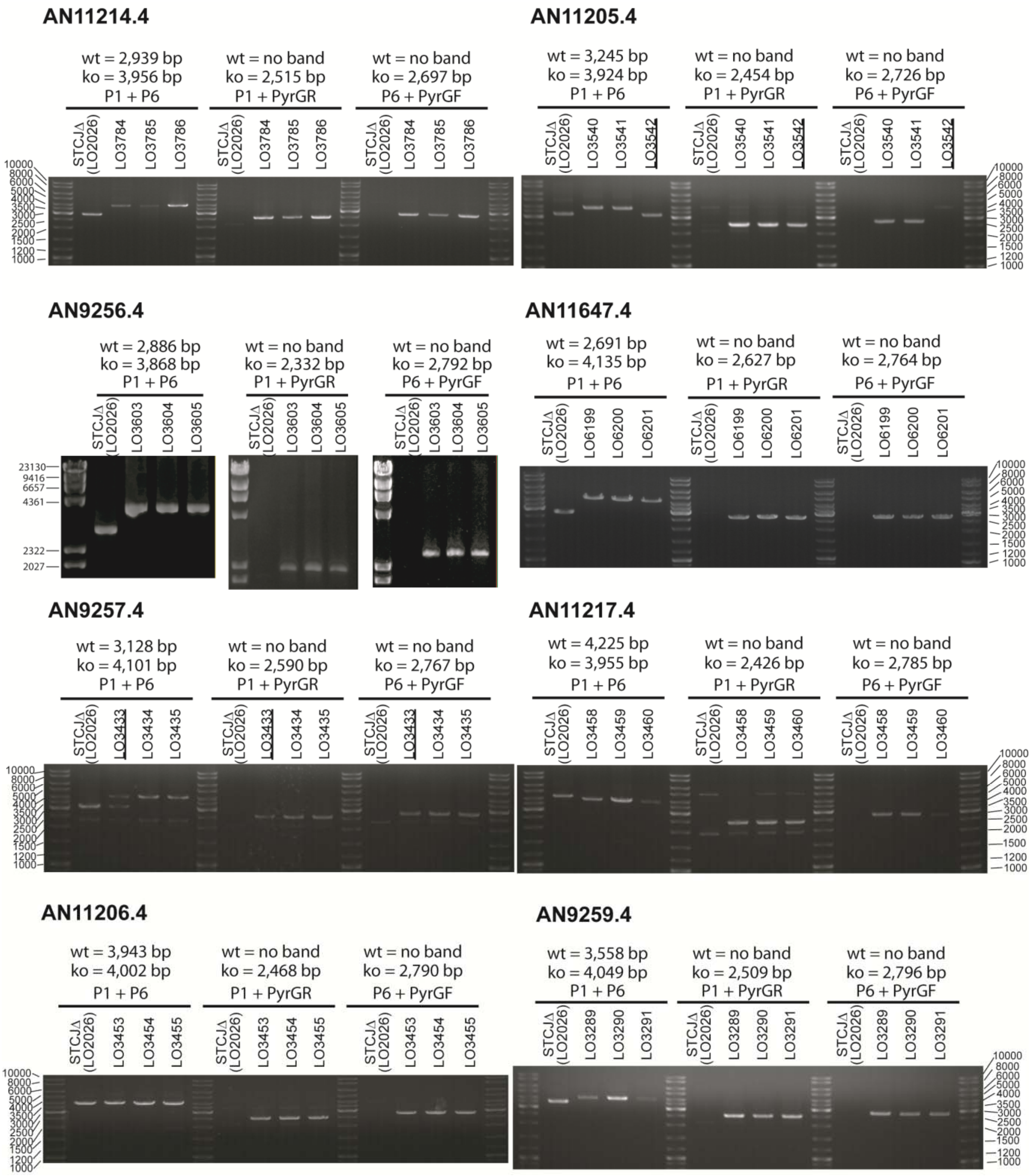
Figure S3. Schematic of the Diagnostic PCR strategy. We used two redundant strategies to determine if the target gene had been deleted by replacement with *AfpyrG*. In one strategy, DNA from transformants is amplified with two primers, P1 from the chromosomal region just outside of the 5' flank of the transforming DNA fragment and P6 from just outside of the 3' flank. If the target gene is different in size from the *AfpyrG* gene, which was used as a selectable marker for transformation, the PCR fragment amplified from a correct transformant (a) will be different in size from the fragment amplified if the target gene is intact (b). In some instances the target gene and the *AfpyrG* cassette will be of comparable size and a second strategy is applied. In the second strategy, P1 or P6 are used with internal primers specific to the *AfpyrG* cassette. For example, if the target gene has been replaced by the *AfpyrG* gene (c), P1 and AfpyrGR will amplify a fragment of a predictable size. If the target gene has not been replaced (d), the AfpyrGR primer will not anneal and there will be no specific amplification. Likewise AfpyrGF and P6 are used in combination and amplification will only occur if the target gene has been replaced by *AfpyrG*.



AN8376.4
wt = 3,455 bp
ko = 3,829 bp
P1 + P6
wt = no band
ko = 2,420 bp
P1 + PyrGR
wt = no band
ko = 2,665 bp
P6 + PyrGF
STCJΔ (LO2026) LO3270 LO3272 LO3273
AN8377.4
wt = 3,325 bp
ko = 3,897 bp
P1 + P6
wt = no band
ko = 2,522 bp
P1 + PyrGR
wt = no band
ko = 2,631 bp
P6 + PyrGF
STCJΔ (LO2026) LO3284 LO3285 LO3287
AN8378.4
wt = 3,784 bp
ko = 3,970 bp
P1 + P6
wt = no band
ko = 2,580 bp
P1 + PyrGR
wt = no band
ko = 2,646 bp
P6 + PyrGF
STCJΔ (LO2026) LO3239 LO3240 LO3241
AN8379.4
wt = 4,809 bp
ko = 3,866 bp
P1 + P6
wt = no band
ko = 2,477 bp
P1 + PyrGR
wt = no band
ko = 2,645 bp
P6 + PyrGF
STCJΔ (LO2026) LO3244 LO3245 LO3246
AN8380.4
wt = 2,999 bp
ko = 3,922 bp
P1 + P6
wt = no band
ko = 2,529 bp
P1 + PyrGR
wt = no band
ko = 2,649 bp
P6 + PyrGF
STCJΔ (LO2026) LO3249 LO3250 LO3251
AN8381.4
wt = 4,663 bp
ko = 3,926 bp
P1 + P6
wt = no band
ko = 2,503 bp
P1 + PyrGR
wt = no band
ko = 2,679 bp
P6 + PyrGF
STCJΔ (LO2026) LO3307 LO3308 LO3309
AN8382.4
wt = 3,113 bp
ko = 3,893 bp
P1 + P6
wt = no band
ko = 2,476 bp
P1 + PyrGR
wt = no band
ko = 2,673 bp
P6 + PyrGF
STCJΔ (LO2026) LO3254 LO3255 LO3256
AN8383.4
wt = 10001 bp
ko = 3787 bp
P1 + P6
wt = no band
ko = 2443 bp
P1 + PyrGR
wt = no band
ko = 2600 bp
P6 + PyrGF
STCJΔ (LO2026) LO3448 LO3449 LO3450


AN8384.4
wt = 3,130 bp
ko = 3,861 bp
P1 + P6
wt = no band
ko = 2,443 bp
P1 + PyrGR
wt = no band
ko = 2,674 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3259
LO3260
LO3261
AN8385.4
wt = 3,091 bp
ko = 4,013 bp
P1 + P6
wt = no band
ko = 2,627 bp
P1 + PyrGR
wt = no band
ko = 2,642 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3264
LO3266
LO3267
AN11077.4
wt = 2,937 bp
ko = 3,849 bp
P1 + P6
wt = no band
ko = 2,434 bp
P1 + PyrGR
wt = no band
ko = 2,671 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3311
LO3312
LO3314
AN11085.4
wt = 3,450 bp
ko = 4,029 bp
P1 + P6
wt = no band
ko = 2,483 bp
P1 + PyrGR
wt = no band
ko = 2,802 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3274
LO3275
LO3277
AN8387.4
wt = 3,119 bp
ko = 4,095 bp
P1 + P6
wt = no band
ko = 2,521 bp
P1 + PyrGR
wt = no band
ko = 2,830 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3279
LO3280
LO3281
AN9244.4
wt = 8,783 bp
ko = 3,593 bp
P1 + P6
wt = no band
ko = 2,310 bp
P1 + PyrGR
wt = no band
ko = 2,539 bp
P6 + PyrGF
STCJΔ (LO2026)
LO4011
LO4012
LO4013
AN9245.4
wt = 4,304 bp
ko = 4,027 bp
P1 + P6
wt = no band
ko = 2,582 bp
P1 + PyrGR
wt = no band
ko = 2,701 bp
P6 + PyrGF
STCJΔ (LO2026)
LO4016
LO4017
LO4018
AN9246.4
wt = 3,117 bp
ko = 3,890 bp
P1 + P6
wt = no band
ko = 2,484 bp
P1 + PyrGR
wt = no band
ko = 2,662 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3829
LO3830
LO3831
10000
8000
6000
5000
4000
3500
3000
2500
2000
1500
1200
1000


AN9247.4
wt = 2,924 bp
ko = 4,010 bp
P1 + P6
wt = no band
ko = 2,474 bp
P1 + PyrGR
wt = no band
ko = 2,792 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3824
LO3825
LO3826
AN9248.4
wt = 4,213 bp
ko = 3,904 bp
P1 + P6
wt = no band
ko = 2,459 bp
P1 + PyrGR
wt = no band
ko = 2,701 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3819
LO3820
LO3821
AN9249.4
wt = 3,061 bp
ko = 4,041 bp
P1 + P6
wt = no band
ko = 2,602 bp
P1 + PyrGR
wt = no band
ko = 2,695 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3814
LO3815
LO3816
AN9250.4
wt = 3,930 bp
ko = 4,032 bp
P1 + P6
wt = no band
ko = 2,591 bp
P1 + PyrGR
wt = no band
ko = 2,697 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3809
LO3810
LO3811
AN9251.4
wt = 3,858 bp
ko = 3,803 bp
P1 + P6
wt = no band
ko = 2,444 bp
P1 + PyrGR
wt = no band
ko = 2,615 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3804
LO3805
LO3806
AN9252.4
wt = 2,922 bp
ko = 3,874 bp
P1 + P6
wt = no band
ko = 2,460 bp
P1 + PyrGR
wt = no band
ko = 2,670 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3799
LO3800
LO3801
AN9253.4
wt = 4,035 bp
ko = 4,000 bp
P1 + P6
wt = no band
ko = 2,625 bp
P1 + PyrGR
wt = no band
ko = 2,631 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3794
LO3795
LO3796
AN9254.4
wt = 2,787 bp
ko = 3,868 bp
P1 + P6
wt = no band
ko = 2,484 bp
P1 + PyrGR
wt = no band
ko = 2,640 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3789
LO3790
LO3791
10000
8000
6000
5000
4000
3500
3000
2500
2000
1500
1200
1000


AN11214.4
wt = 2,939 bp
ko = 3,956 bp
P1 + P6
wt = no band
ko = 2,515 bp
P1 + PyrGR
wt = no band
ko = 2,697 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3784
LO3785
LO3786
AN11205.4
wt = 3,245 bp
ko = 3,924 bp
P1 + P6
wt = no band
ko = 2,454 bp
P1 + PyrGR
wt = no band
ko = 2,726 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3540
LO3541
LO3542
AN9256.4
wt = 2,886 bp
ko = 3,868 bp
P1 + P6
wt = no band
ko = 2,332 bp
P1 + PyrGR
wt = no band
ko = 2,792 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3603
LO3604
LO3605
23130
9416
6657
4361
2322
2027
AN11647.4
wt = 2,691 bp
ko = 4,135 bp
P1 + P6
wt = no band
ko = 2,627 bp
P1 + PyrGR
wt = no band
ko = 2,764 bp
P6 + PyrGF
STCJΔ (LO2026)
LO6199
LO6200
LO6201
AN9257.4
wt = 3,128 bp
ko = 4,101 bp
P1 + P6
wt = no band
ko = 2,590 bp
P1 + PyrGR
wt = no band
ko = 2,767 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3433
LO3434
LO3435
AN11217.4
wt = 4,225 bp
ko = 3,955 bp
P1 + P6
wt = no band
ko = 2,426 bp
P1 + PyrGR
wt = no band
ko = 2,785 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3458
LO3459
LO3460
AN11206.4
wt = 3,943 bp
ko = 4,002 bp
P1 + P6
wt = no band
ko = 2,468 bp
P1 + PyrGR
wt = no band
ko = 2,790 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3453
LO3454
LO3455
AN9259.4
wt = 3,558 bp
ko = 4,049 bp
P1 + P6
wt = no band
ko = 2,509 bp
P1 + PyrGR
wt = no band
ko = 2,796 bp
P6 + PyrGF
STCJΔ (LO2026)
LO3289
LO3290
LO3291
10000
8000
6000
5000
4000
3500
3000
2500
2000
1500
1200
1000

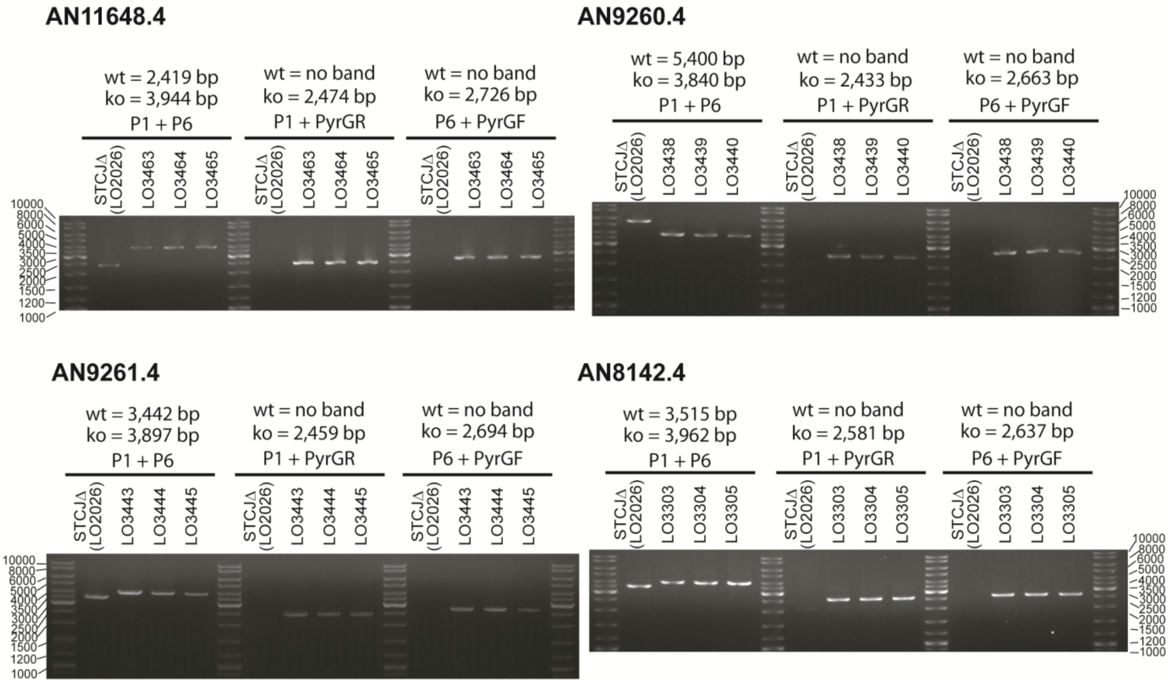


Figure S4. Results of diagnostic PCR for all the gene deletion strains. Strain numbers underlined are incorrect transformants that were not used for metabolite analysis.

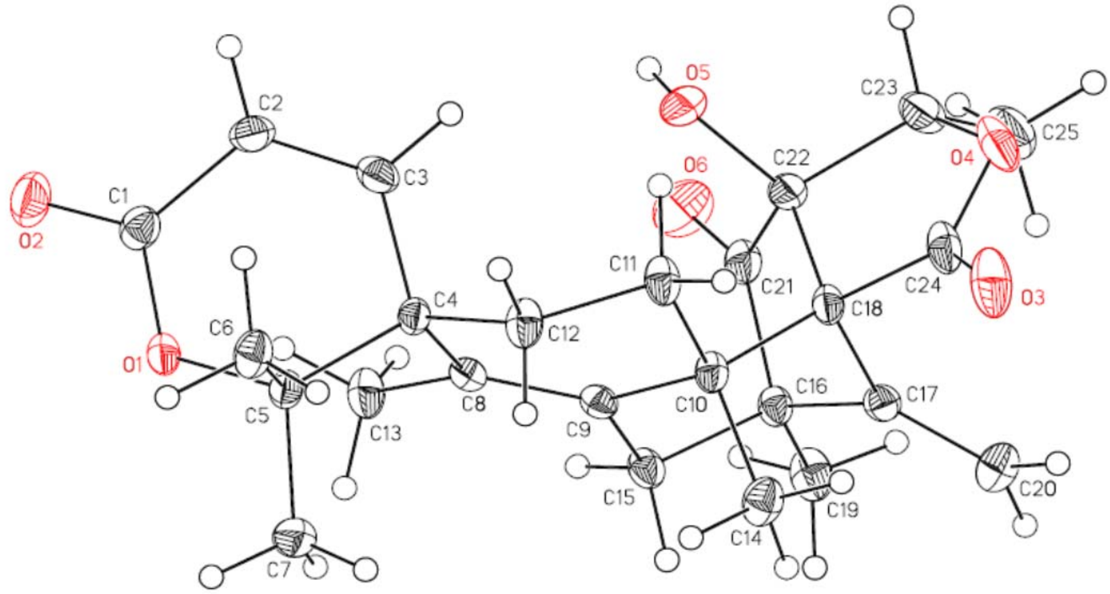


Figure S5. ORTEP drawing of (5'*R*)-isoaustinone **12**.

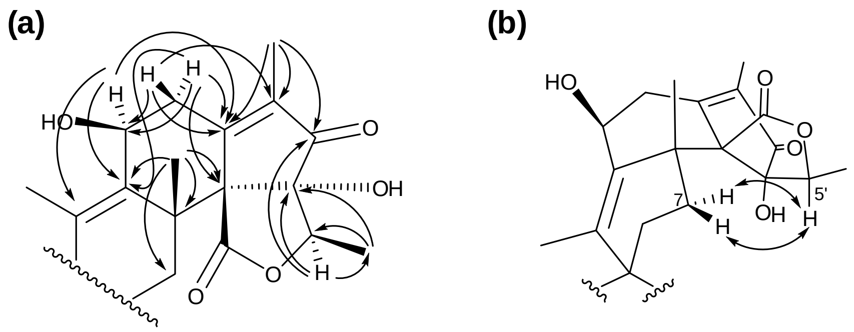


Figure S6. (a) Key gHMBC and (b) NOESY correlations in C, D, and E rings of neoaustinone **13**.

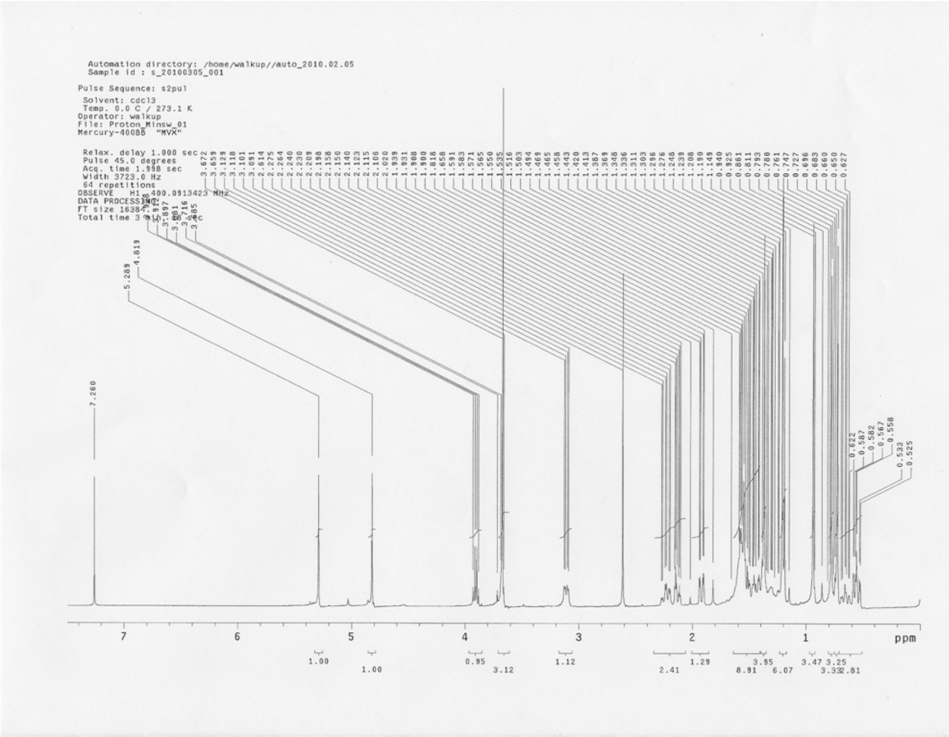


Figure S7.[1]H NMR spectrum of compound **4**

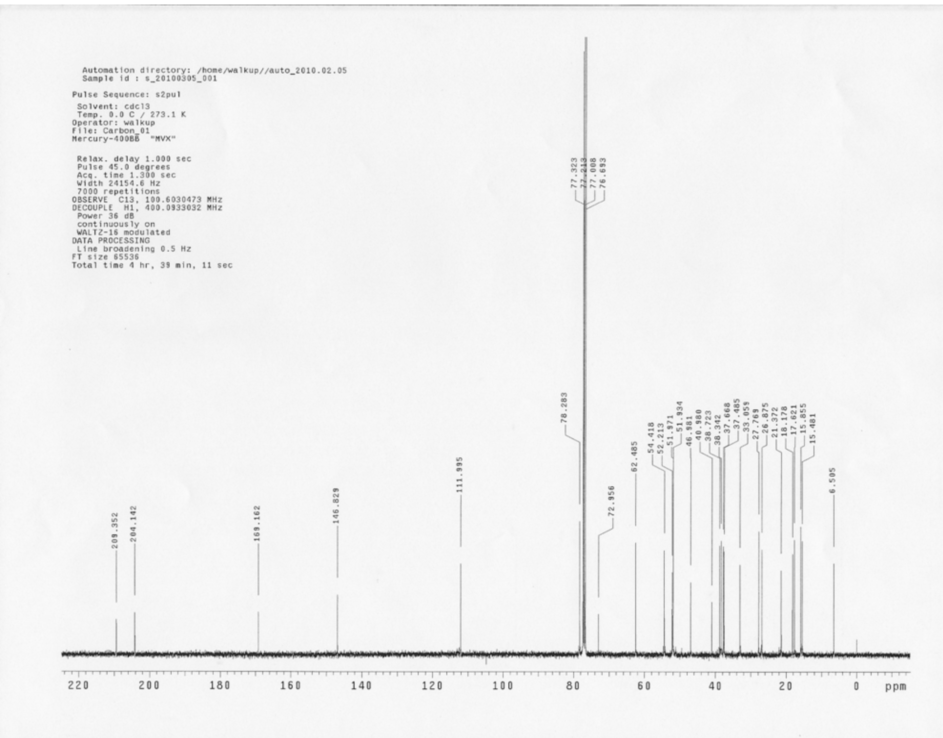


Figure S8.[13]C NMR spectrum of compound **4**

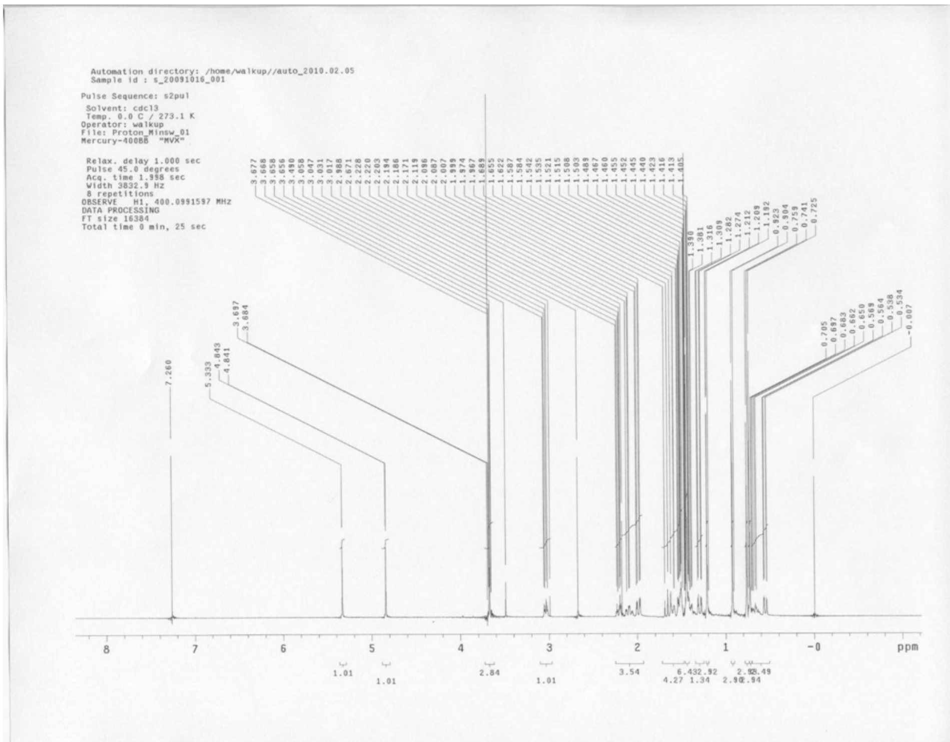


Figure S9. ^{1}H NMR spectrum of compound **4** after autooxidation

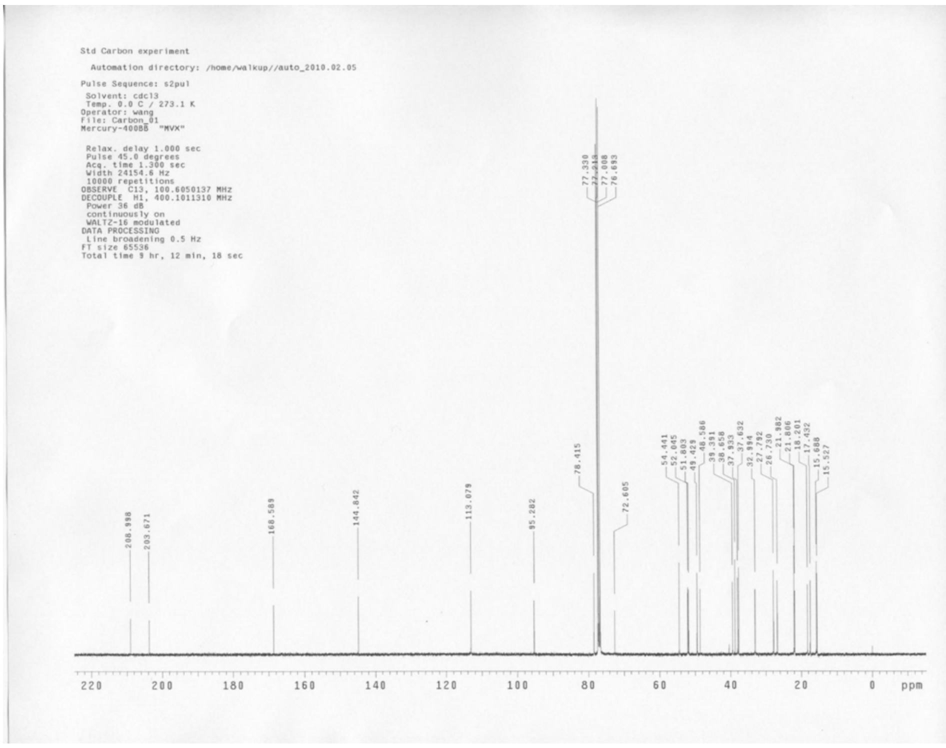


Figure S10. ^{13}C NMR spectrum of compound **4** after autooxidation

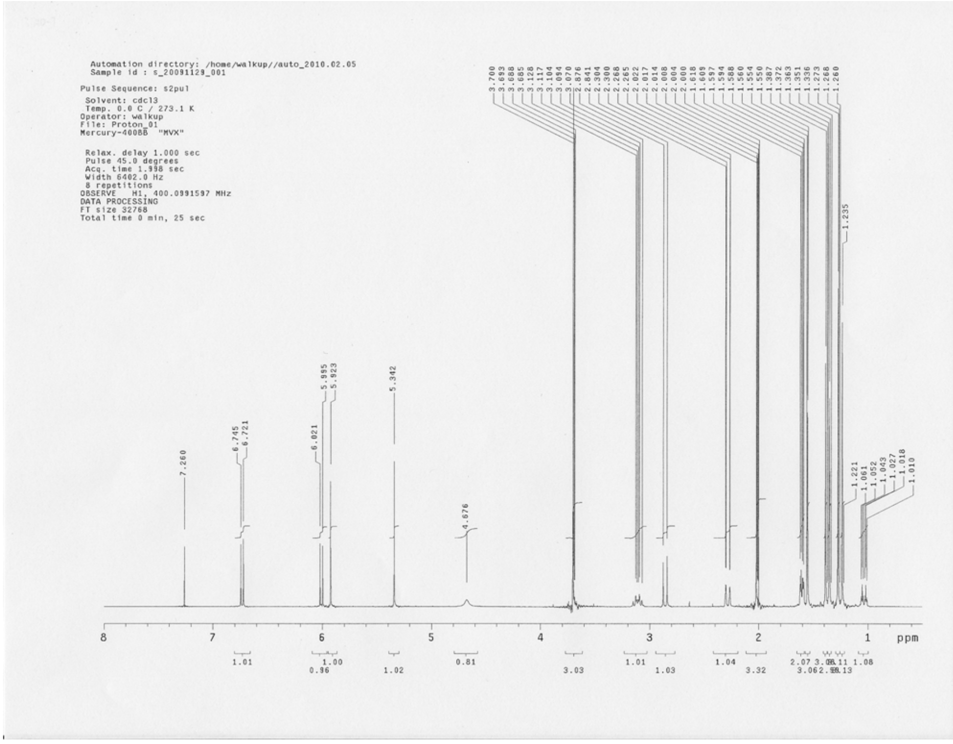


Figure S11.[1]H NMR spectrum of compound **6**

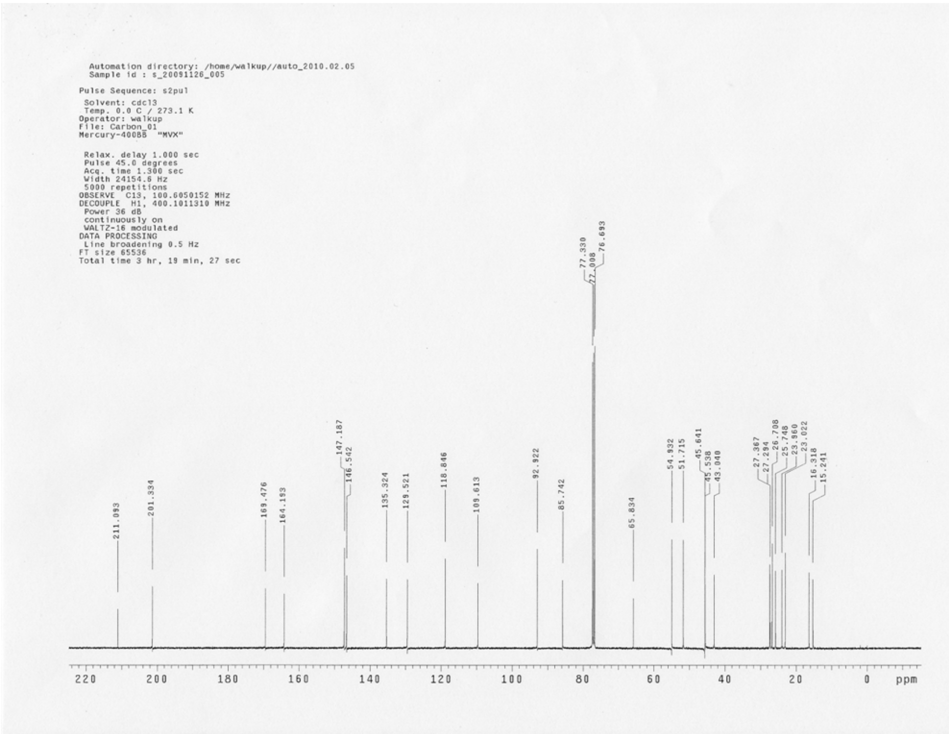


Figure S12.[13]C NMR spectrum of compound **6**

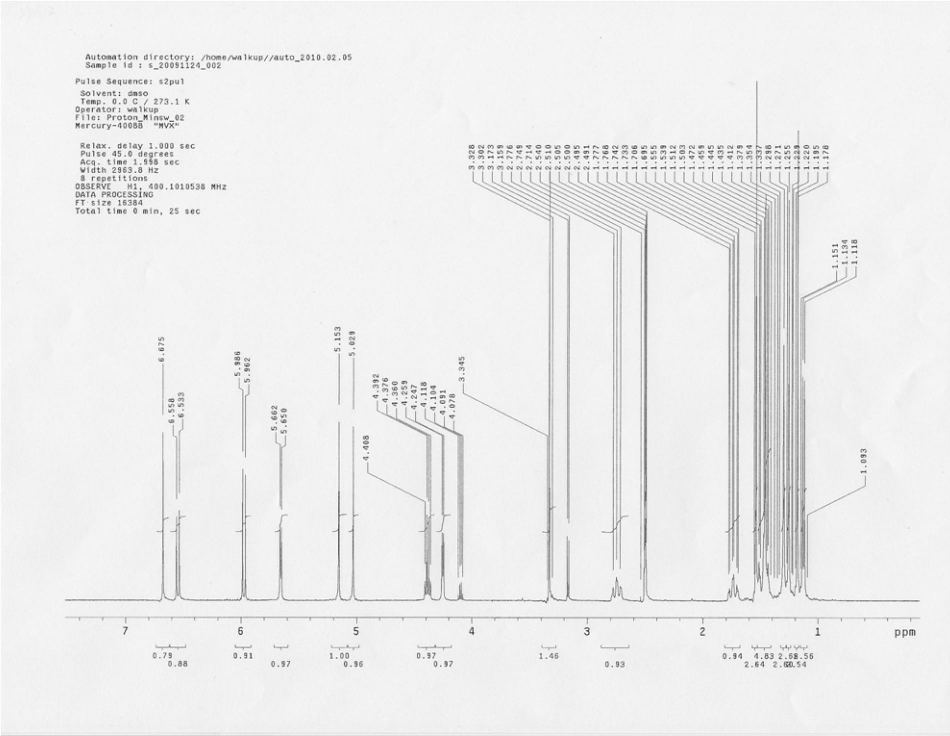


Figure S13. ^{1}H NMR spectrum of compound **8**

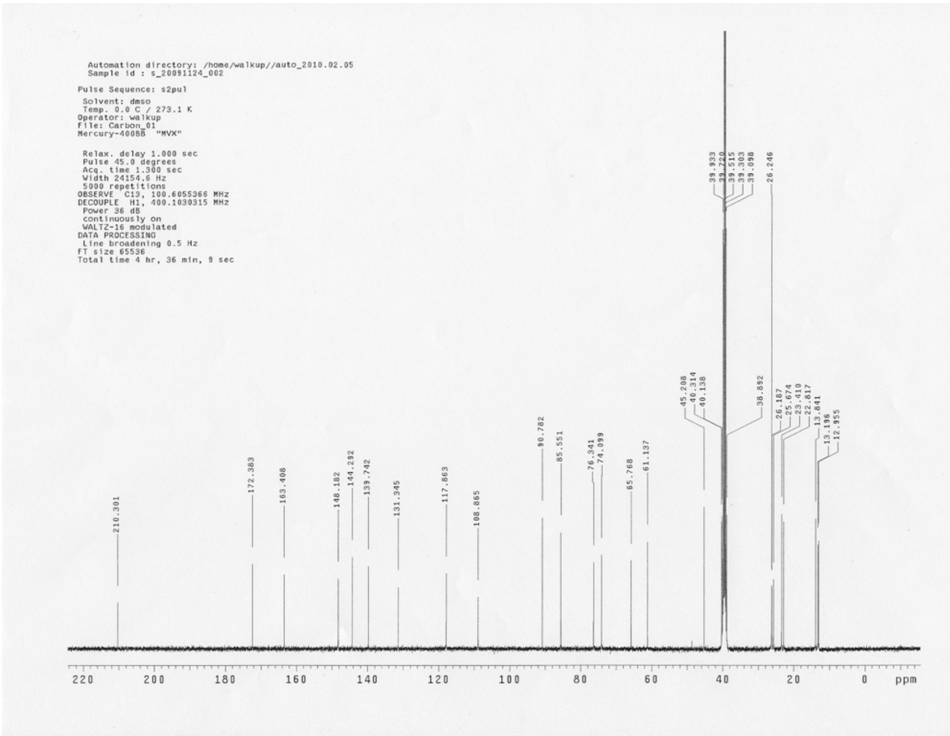


Figure S14. ^{13}C NMR spectrum of compound **8**

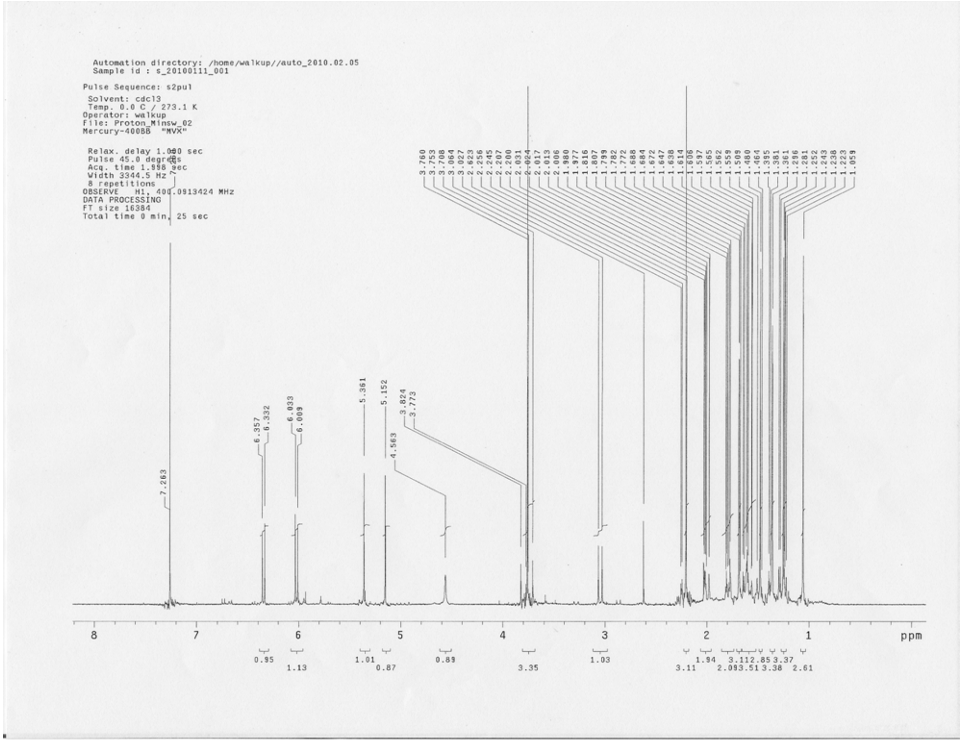


Figure S15. ^{1}H NMR spectrum of compound **10**

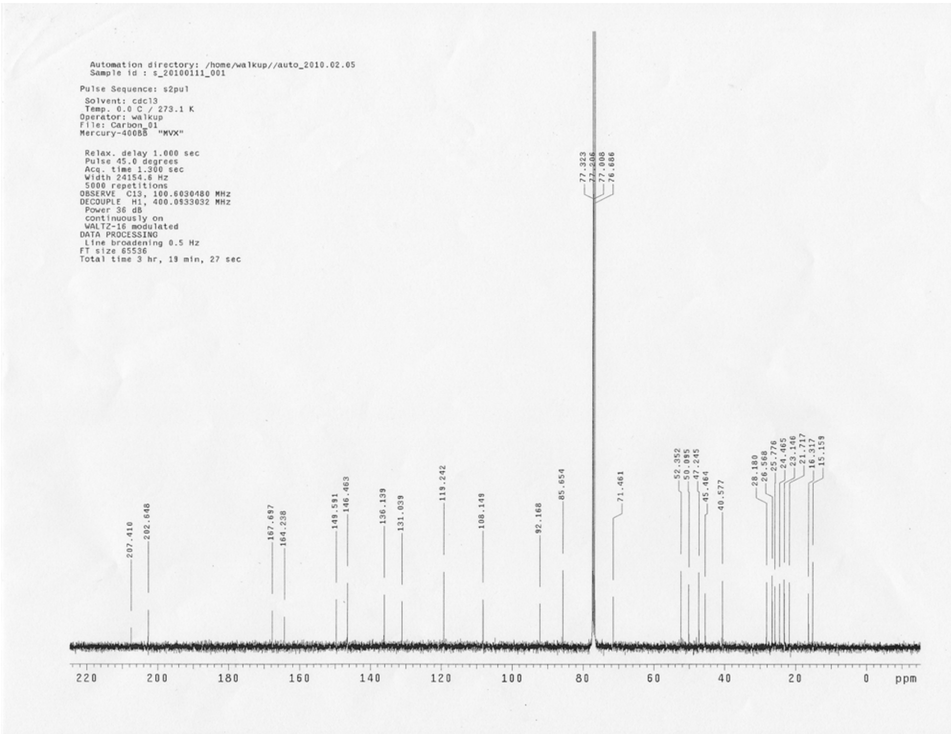


Figure S16. ^{13}C NMR spectrum of compound **10**

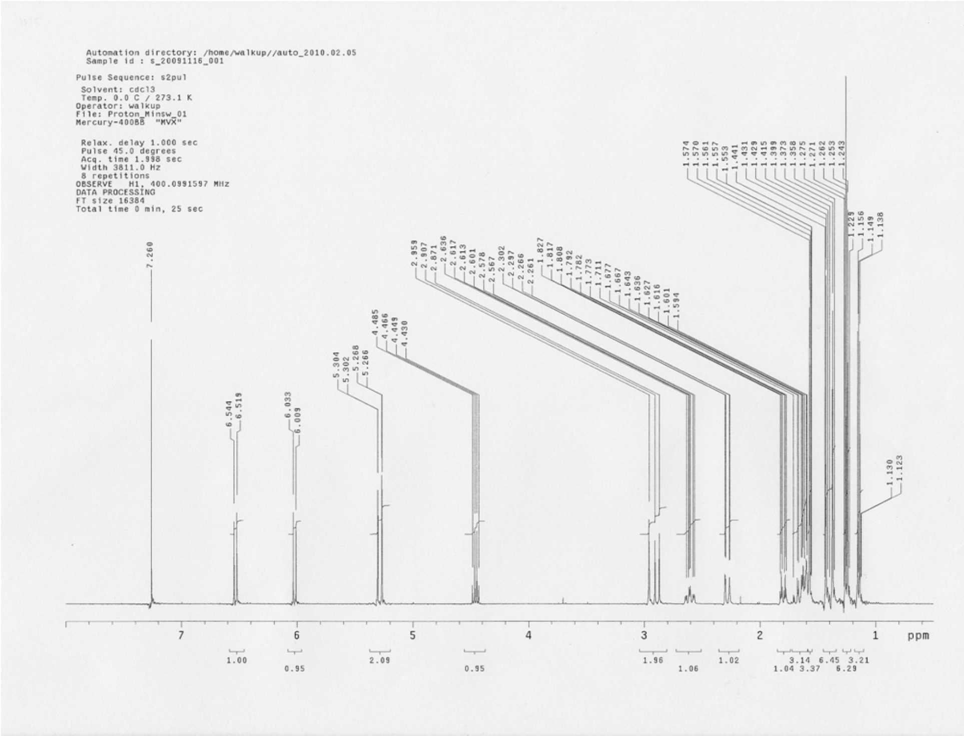


Figure S17. ^{1}H NMR spectrum of compound **12**

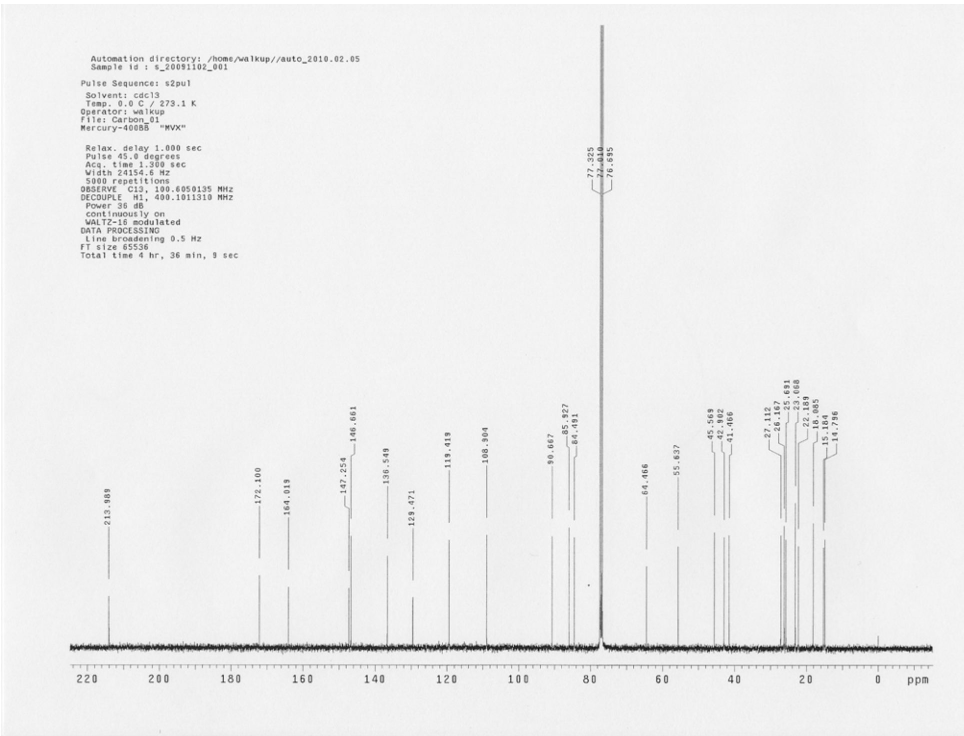


Figure S18. ^{13}C NMR spectrum of compound **12**

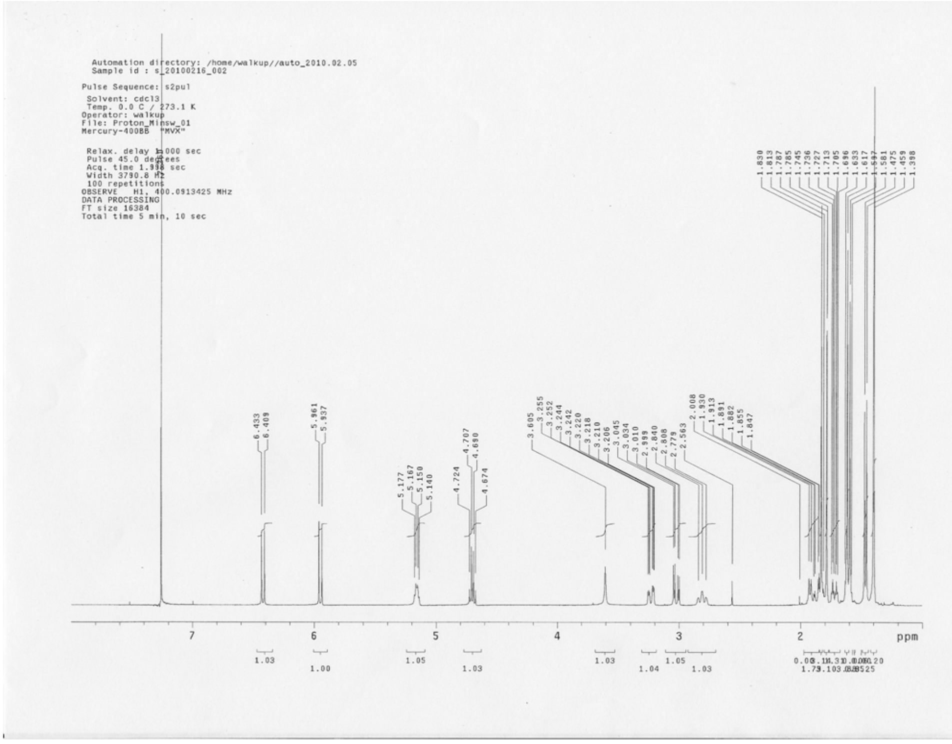


Figure S19.[1]H NMR spectrum of compound **13**

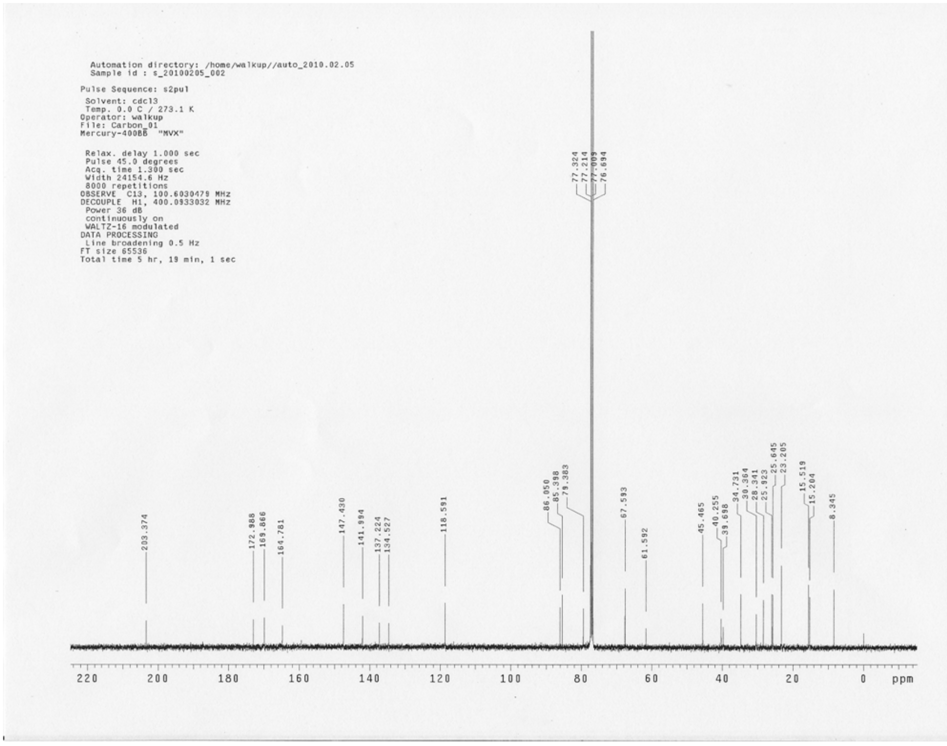


Figure S20.[13]C NMR spectrum of compound **13**

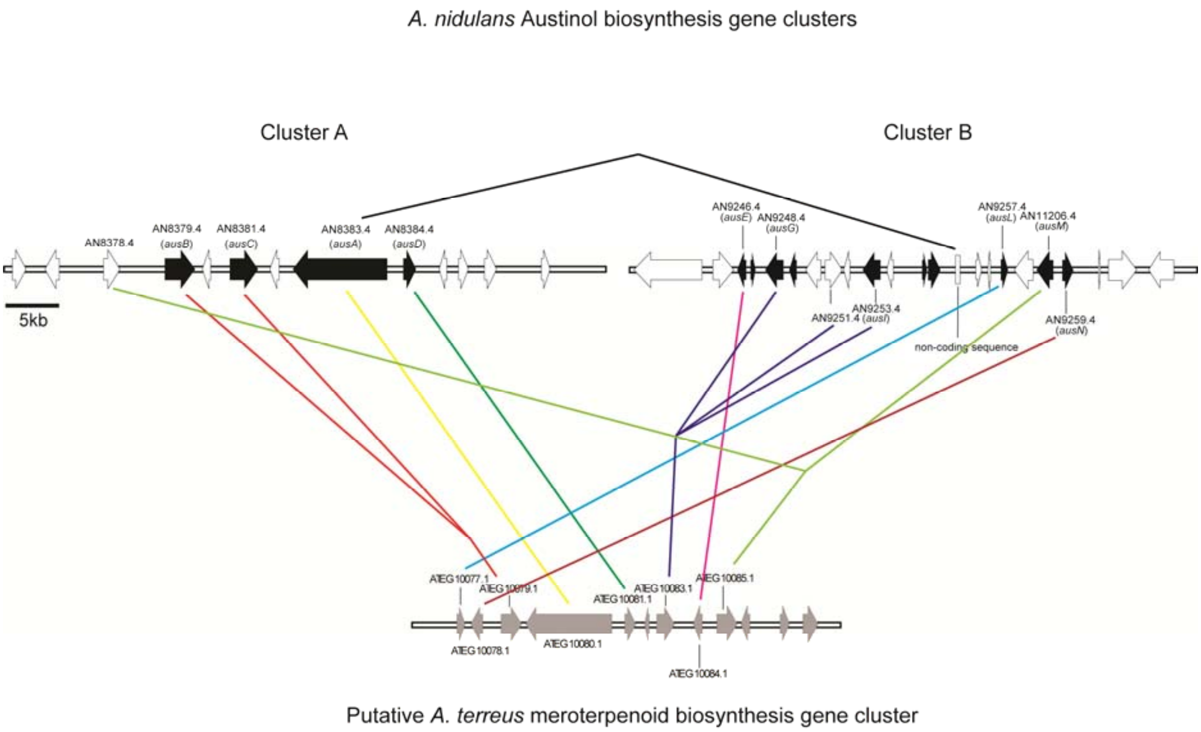


Figure S21. Relationship between homologous genes in the austinol clusters of *A. nidulans* and a putative meroterpenoid cluster of *A. terreus*. Lines connect homologous genes. The homology was determined by BLAST analysis and only homology with E values lower than 1E-26 are shown here.