Supporting Information

A gene cluster containing two fungal polyketide synthases encode the biosynthetic pathway of a polyketide, asperfuranone, in *Aspergillus nidulans*

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Complete Refs. 1, 2, 4, & 5

(1) Askenazi, M.; Driggers, E. M.; Holtzman, D. A.; Norman, T. C.; Iverson, S.; Zimmer, D. P.; Boers, M. E.; Blomquist, P. R.; Martinez, E. J.; Monreal, A. W.; Feibelman, T. P.; Mayorga, M. E.; Maxon, M. E.; Sykes, K.; Tobin, J. V.; Cordero, E.; Salama, S. R.; Trueheart, J.; Royer, J. C.; Madden, K. T. *Nat Biotechnol* **2003**, *21*, 150-6.

(2) Galagan, J. E.; Calvo, S. E.; Cuomo, C.; Ma, L. J.; Wortman, J. R.; Batzoglou, S.; Lee, S. I.; Basturkmen, M.; Spevak, C. C.; Clutterbuck, J.; Kapitonov, V.; Jurka, J.; Scazzocchio, C.; Farman, M.; Butler, J.; Purcell, S.; Harris, S.; Braus, G. H.; Draht, O.; Busch, S.; D'Enfert, C.; Bouchier, C.; Goldman, G. H.; Bell-Pedersen, D.; Griffiths-Jones, S.; Doonan, J. H.; Yu, J.; Vienken, K.; Pain, A.; Freitag, M.; Selker, E. U.; Archer, D. B.; Penalva, M. A.; Oakley, B. R.; Momany, M.; Tanaka, T.; Kumagai, T.; Asai, K.; Machida, M.; Nierman, W. C.; Denning, D. W.; Caddick, M.; Hynes, M.; Paoletti, M.; Fischer, R.; Miller, B.; Dyer, P.; Sachs, M. S.; Osmani, S. A.; Birren, B. W. *Nature* **2005**, *438*, 1105-15.

(4) Machida, M.; Asai, K.; Sano, M.; Tanaka, T.; Kumagai, T.; Terai, G.; Kusumoto, K.; Arima, T.; Akita, O.; Kashiwagi, Y.; Abe, K.; Gomi, K.; Horiuchi, H.; Kitamoto, K.; Kobayashi, T.; Takeuchi, M.; Denning, D. W.; Galagan, J. E.; Nierman, W. C.; Yu, J.; Archer, D. B.; Bennett, J. W.; Bhatnagar, D.; Cleveland, T. E.; Fedorova, N. D.; Gotoh, O.; Horikawa, H.; Hosoyama, A.; Ichinomiya, M.; Igarashi, R.; Iwashita, K.; Juvvadi, P. R.; Kato, M.; Kato, Y.; Kin, T.; Kokubun, A.; Maeda, H.; Maeyama, N.; Maruyama, J.; Nagasaki, H.; Nakajima, T.; Oda, K.; Okada, K.; Paulsen, I.; Sakamoto, K.; Sawano, T.; Takahashi, M.; Takase, K.; Terabayashi, Y.; Wortman, J. R.; Yamada, O.; Yamagata, Y.; Anazawa, H.; Hata, Y.; Koide, Y.; Komori, T.; Koyama, Y.; Minetoki, T.; Suharnan, S.; Tanaka, A.; Isono, K.; Kuhara, S.; Ogasawara, N.; Kikuchi, H. *Nature* **2005**, *438*, 1157-61.

(5) Nierman, W. C.; Pain, A.; Anderson, M. J.; Wortman, J. R.; Kim, H. S.; Arroyo, J.; Berriman, M.; Abe, K.; Archer, D. B.; Bermejo, C.; Bennett, J.; Bowyer, P.; Chen, D.; Collins, M.; Coulsen, R.; Davies, R.; Dyer, P. S.; Farman, M.; Fedorova, N.; Feldblyum, T. V.; Fischer, R.; Fosker, N.; Fraser, A.; Garcia, J. L.; Garcia, M. J.; Goble, A.; Goldman, G. H.; Gomi, K.; Griffith-Jones, S.; Gwilliam, R.; Haas, B.; Haas, H.; Harris, D.; Horiuchi, H.; Huang, J.; Humphray, S.; Jimenez, J.; Keller, N.; Khouri, H.; Kitamoto, K.; Kobayashi, T.; Konzack, S.; Kulkarni, R.; Kumagai, T.; Lafon, A.; Latge, J. P.; Li, W.; Lord, A.; Lu, C.; Majoros, W. H.; May, G. S.; Miller, B. L.; Mohamoud, Y.; Molina, M.; Monod, M.; Mouyna, I.; Mulligan, S.; Murphy, L.; O'Neil, S.; Paulsen, I.; Penalva, M. A.; Pertea, M.; Price, C.; Pritchard, B. L.; Quail, M. A.; Rabbinowitsch, E.; Rawlins, N.; Rajandream, M. A.; Reichard, U.; Renauld, H.; Robson, G. D.; Rodriguez de Cordoba, S.; Rodriguez-Pena, J. M.; Ronning, C. M.; Rutter, S.; Salzberg, S. L.; Sanchez, M.; Sanchez-Ferrero, J. C.; Saunders, D.; Seeger, K.; Squares, R.; Squares, S.; Takeuchi, M.; Tekaia, F.; Turner, G.; Vazquez de Aldana, C. R.; Weidman, J.; White, O.; Woodward, J.; Yu, J. H.; Fraser, C.; Galagan, J. E.; Asai, K.; Machida, M.; Hall, N.; Barrell, B.; Denning, D. W. *Nature* **2005**, *438*, 1151-6.

Detailed Structural Characterization

Asperfuanone (**1**) was isolated as light yellow gum. The molecular formula was found to be $C_{19}H_{24}O_5$ by its ^{13}C NMR, DEPT, and HRESIMS spectral data, representing eight indices of hydrogen deficiency (IHD). The IR spectrum showed absorptions for hydroxyl (3385 cm^{-1}) and conjugated carbonyl (1682 and 1659 cm^{-1}) functionalities. The ^1H NMR spectrum in acetone-d_6 (Table S3) exhibited signals for four methyl groups [δ_H 0.86 (t, J = 7.6 Hz), 1.01 (d, J = 6.4 Hz), 1.34 (s), and 1.90 (br s)], two methylenes [δ_H 1.35 and 1.45 (each 1H, m); 2.91 (dd, J = 18.0, 8.4 Hz) and 3.51 (dd, J = 18.0, 5.2 Hz)], two methine protons [δ_H 2.55 (m) and 4.13 (dd, J = 8.4, 5.2 Hz)], three olefinic protons [δ_H 5.88 (br d, J = 10.0 Hz), 6.84 (d, J = 15.2 Hz), and 7.41 (d, J = 15.2 Hz)], and one unusual down field aromatic proton [δ_H 8.82 (s)]. The ^1H, ^{13}C NMR and COSY data revealed the presence of a 4,6-dimethyl-2,4-octadienone moiety. The remainder of the structure was established by HMBC and COSY correlations. HMBC long-range ^{13}C–^1H correlations (Figure S2a) including C-10/H_2-11, H-12, H-16; C-11/H-12; C-12/H_2-11, H_3-19; C-13/H_2-11, H-12, H_3-19; C-14/H-12, H-16, H_3-19; and C-15/H_2-11, H-16 established 5,6-dihydroxy-5-methyl-6,7-dihydro-5*H*-isobenzofuran-4-one moiety. The 4,6-dimethyl-2,4-octadienone side chain was thus attached to the C-9 of the furan ring. The unusual down field aromatic proton was attributed to the two carbonyl groups conjugated with the furan ring. ROESY correlations (Figure S2b) for H_α-11/H-12 and H_β-11/H_3-19 suggested that CH_3-19 should be on the same face as H_β-11. The stereochemistry of C-3 was assigned as 3*S* since asperfuraone (**1**) is biosynthesized from compound **2** that the C-3 stereochemistry has been determined previously.[1] The absolute configuration of C-12 was determined by a modified Mosher's method.[2] Treatment of **1** with 1.5 equivalent of (*R*)- and (*S*)-2-methyoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) afforded the (*S*)- and (*R*)-MTPA esters (**1a** and **1b**, respectively). $\Delta\delta$ values ($\delta_S - \delta_R$) of H_α-11 and H_β-11 showed positive values, while H_3-19 was negative (Figure S3), thus indicating a 12*R*-configuration. Therefore, the stereochemistry of asperfuranone (**1**) was 3*S*, 12*R*, and 13*S*.

Compound **2**, a colorless solid, has similar ^1H and ^{13}C NMR data with asperfuranone (**1**) on the 4,6-dimethyl-2,4-octadienone side chain. The 4,6-dihydroxy-5-methyl-benzaldehyde moiety was established by HMBC correlations. Therefore, the structure of

compound **2** was identified as 2,4-dihydroxy-6-(5,7-dimethyl-2-oxo-*trans*-3-*trans*-5-nonadienyl)-3-methylbenzaldehyde. This structure was then confirmed by comparing its NMR data as well as optical rotations with those reported in the literature.[1]

Supplemental Methods

Isolation and identification of secondary metabolites

For structural elucidation, asperfuranone (**1**) was purified from 3 L of LMM medium inoculated with *alcA*(p)-AN1029.3 *A. nidulans* in the induction condition and extracted with EtOAc as described above. The mycelium which also containing asperfuraone (**1**) was extracted separately with 500 mL MeOH twice, with each sonicated for 1 h. Both extract were pooled to get 164.3 mg of crude extract. This extract was then applied to a SiO_2 gel column (Merck 230-400 mesh, ASTM, 20 × 60 mm) and eluted with $CHCl_3$-MeOH mixtures of increasing polarity (fraction A, 1:0, 200 mL; fraction B, 19:1, 200 mL; fraction C, 9:1, 200 mL; fraction D, 7:3, 200 mL). Fraction C (67.3 mg), which contained asperfuraonone (**1**), 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: 42 to 45 % B from 0 to 20 min, 45 to 100 % B from 20 to 26 min, maintained at 100 % B from 26 to 31 min, 100 to 42 % B from 31 to 32 min, and re-equilibration with 42 % B from 32 to 37 min. Asperfuranone (**1**, 32.8 mg) was eluted at 15.0 min. 2,4-Dihydroxy-6-(5,7-dimethyl-2-oxo-*trans*-3-*trans*-5-nonadienyl)-3-methylbenzaldehyde (**2**) was purified from 3 L of LMM medium inoculated with *alcA*(p)-AN1029.3, AN1033.3Δ *A. nidulans* as described above. Crude extract was applied to a SiO_2 gel column (Merck 230-400 mesh, ASTM, 40 × 190 mm) and eluted with $CHCl_3$-MeOH mixtures of increasing polarity (fraction A, 1:0, 1000 mL; fraction B, 19:1, 1000 mL; fraction C, 19:1, 1000 mL; fraction D, 7:3, 1000 mL). Fraction B (326.4 mg) 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: 60 % B from 0 to 5 min, 60 to 100 % B from 5 to 25 min, maintained at 100 % B from 25 to 30 min, 100 to 60 % B from 30 to 31 min, and re-equilibration with 60 % B

from 31 to 35 min. Compound **2** (71.6 mg), **3** (51.6 mg), and **4** (50.3 mg) were elute at 12.6, 22.4, and 24.4 min, respectively.

Preparation of (*S*)- and (*R*)-MTPA esters of asperfuranone (1)

To a CH_2Cl_2 solution (0.5 mL) of asperfuranone (**1**, 2.0 mg) were added 4-(dimethylamino)-pyridine (4.0 eq) and (*R*)- or (*S*)-MTPACl (1.5 eq) at room temperature, and stirring for overnight. After evaporation of solvent, the (*S*)- and (*R*)-MTPA esters (**1a** and **1b**, respectively) were 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: 40 to 100 % B from 0 to 20 min, maintained at 100 % B from 20 to 24 min, 100 to 40 % B from 24 to 26 min, and re-equilibration with 40 % B from 26 to 30 min. Compound **1a** (1.2 mg), and **1b** (1.5 mg) were elute at 19.1 and 19.4 min, respectively.

(*S*)-MTPA ester of asperfuraonone (1a): colorless gum; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.277 (s, H-16), 7.65 - 7.55 (m, 2 H), 7.49 - 7.39 (m, 3 H), 7.449 (d, *J* = 15.6 Hz, H-6), 6.465 (d, *J* = 15.6 Hz, H-7), 5.868 (d, *J* = 10.0 Hz, H-4), 5.645 (dd, *J* = 10.0, 6.0 Hz, H-12), 3.770 (dd, *J* = 17.6, 6.0 Hz, H_a-11), 3.598 (s, 3 H, OMe), 3.087 (dd, *J* = 17.6, 10.0 Hz, H_b-11), 2.501 (m, H-3), 1.868 (s, H_3-18), 1.52 - 1.21 (m, H_2-2), 1.318 (s, H_3-19), 1.019 (d, *J* = 6.8 Hz, H_3-17), 0.864 (t, *J* = 7.6 Hz, H_3-1); ESIMS *m*/*z* (rel. int.): 549.0 $[M+H]^+$ (100), 315.1 (30).

(*R*)-MTPA ester of asperfuraonone (1b): colorless gum; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.275 (s, H-16), 7.69 - 7.62 (m, 2 H), 7.49 - 7.39 (m, 3 H), 7.443 (d, *J* = 15.6 Hz, H-6), 6.455 (d, *J* = 15.2 Hz, H-7), 5.866 (d, *J* = 10.0 Hz, H-4), 5.591 (dd, *J* = 10.0, 5.6 Hz, H-12), 3.748 (dd, *J* = 17.6, 5.6 Hz, H_a-11), 3.634 (s, 3 H, OMe), 2.942 (dd, *J* = 17.6, 10.0 Hz, H_b-11), 2.499 (m, H-3), 1.864 (s, H_3-18), 1.51 - 1.23 (m, H_2-2), 1.396 (s, H_3-19), 1.018 (d, *J* = 6.4 Hz, H_3-17), 0.863 (t, *J* = 7.6 Hz, H_3-1); ESIMS *m*/*z* (rel. int.): 549.0 $[M+H]^+$ (100), 315.1 (30).

Supplemental References

(1) Matsuzaki, K.; Tahara, H.; Inokoshi, J.; Tanaka, H.; Masuma, R.; Omura, S. *J Antibiot (Tokyo)* **1998**, *51*, 1004-11.

(2) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J Am Chem Soc* **1991**, *113*, 4092-4096.

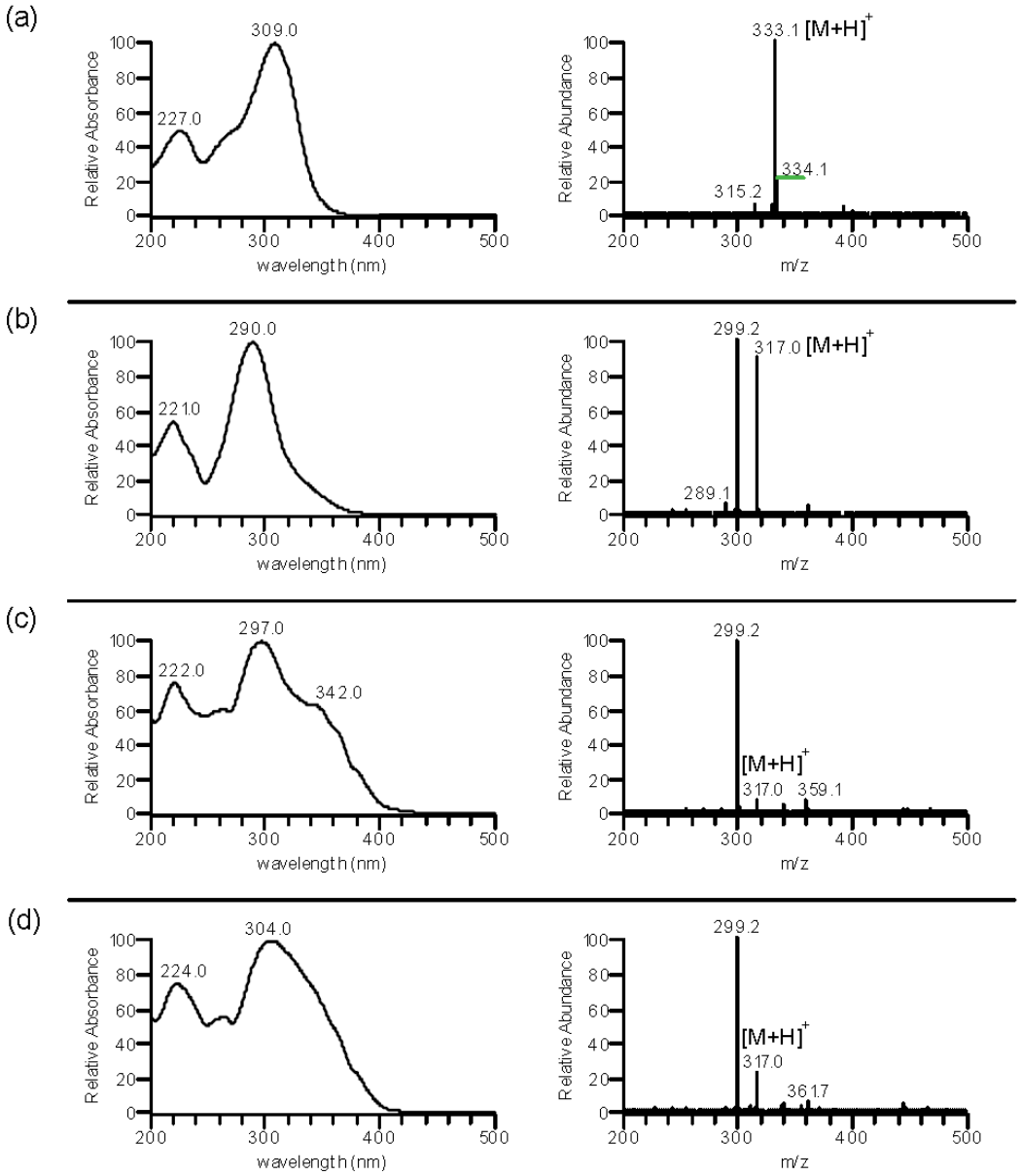


Figure S1. UV-Vis and ESIMS spectra (positive mode) of (a) asperfuranone (**1**), (b) 2,4-dihydroxy-6-(5,7-dimethyl-2-oxo-*trans*-3-*trans*-5-nonadienyl)-3-methylbenzaldehyde (**2**), (c) compound **3**, and (d) compound **4**.

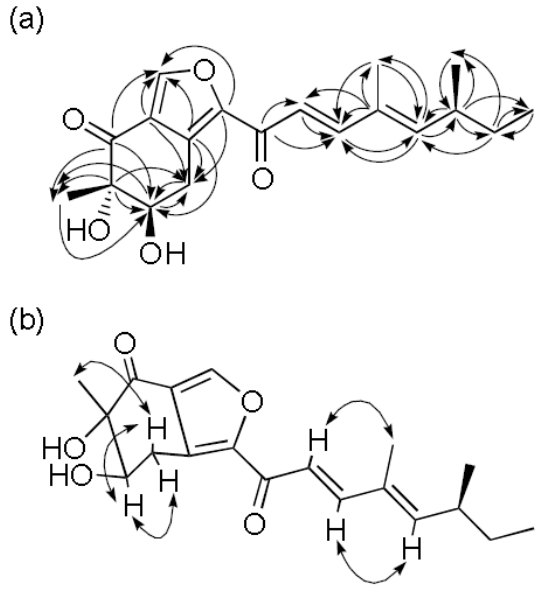


Figure S2. (a) Key HMBC and (b) key ROESY correlations of asperfuranone (**1**).

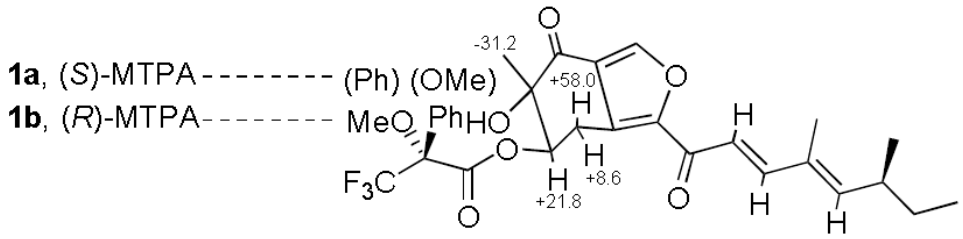


Figure S3. Δδ values [Δδ (in Hz) = $\delta_S - \delta_R$] obtained from the (*S*)- and (*R*)-MTPA esters (**1a** and **1b**, respectively).

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

strain	genotype
alcA(p)-AN1029.3	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3
alcA(p)-AN1029.3, AN1027.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1027.3::*AfpyroA*
alcA(p)-AN1029.3, AN1028.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1028.3::*AfpyroA*
alcA(p)-AN1029.3, AN1030.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1030.3::*AfpyroA*
alcA(p)-AN1029.3, AN1031.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1031.3::*AfpyroA*
alcA(p)-AN1029.3, AN1032.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1032.3::*AfpyroA*
alcA(p)-AN1029.3, AN1033.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1033.3::*AfpyroA*
alcA(p)-AN1029.3, AN1034.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1034.3::*AfpyroA*
alcA(p)-AN1029.3, AN1035.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1035.3::*AfpyroA*
alcA(p)-AN1029.3, AN11287.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN11287.3::*AfpyroA*
alcA(p)-AN1029.3, AN1036.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1036.3::*AfpyroA*
alcA(p)-AN1029.3, AN1037.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN1037.3::*AfpyroA*
alcA(p)-AN1029.3, AN11288.3Δ	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; AN1029.3::*AfpyrG-alcA*(p)-AN1029.3; AN11288.3::*AfpyroA*

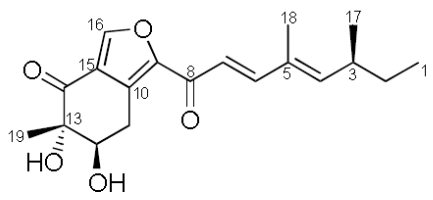
AN1029.3::*AfpyrG-alcA*(p)-AN1029.3 is a replacement of the endogenous promoter of AN1029.3 with the *alcA* promoter and the *A. fumigatus pyrG* gene (*AfpyrG*). *AfpyroA* is the *A. fumigatus pyroA* gene and *AfriboB* is the *A. fumigatus riboB* gene.

Table S2. Primers used in this study

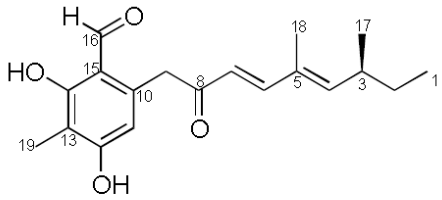
primer	Sequence (5′→3′)
alcA_AN1029.3P1	GGA GCG ACA GAA CCA AAG TC
alcA_AN1029.3P2	TGG GCC ATG GGC TAT CTT CC
alcA_AN1029.3P3	CGA AGA GGG TGA AGA GCA TTG TTA CGA GCG AGT TAC GAA CG
alcA_AN1029.3P4	ATC CTA TCA CCT CGC CTC AAA ATG GCG TGT CCC ACC AGA CG
alcA_AN1029.3P5	TGC TGG GGT ATG GCT ATC TC
alcA_AN1029.3P6	ATG GCA GTG AGC AGA CAT TG
AN1027.3P1	ACG CGG TAA GTT TTG TCT CC
AN1027.3P2	AGC ACT TCG TTG GAA ACA GG
AN1027.3P3	CGA AGA GGG TGA AGA GCA TTG CTC TCA CGT GGC TTC TAG ACC
AN1027.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GTC TTT GGG CTT TTG AGT CG
AN1027.3P5	CTG CGC CTT GTC TTC TCT CC
AN1027.3P6	GGG AGC ACA AAT TCT TAG CC
AN1028.3P1	GTT CCT ACC CGA TAC GTT GG
AN1028.3P2	GCA TCT GCT ACG AGT GTT AAG C
AN1028.3P3	CGA AGA GGG TGA AGA GCA TTG TCC TTC TGT CAG AGA CAC TGG
AN1028.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GGA TTT CAT GTC CAG TGT CG
AN1028.3P5	TGT CAA GGA GCG AAG ATT GG
AN1028.3P6	CAG TGG AAC AAC CGT AGA CC
AN1030.3P1	GCC TAC AGC ATT CCA TTT CG
AN1030.3P2	GCC ATC TCC GTG GTA TTT GC
AN1030.3P3	CGA AGA GGG TGA AGA GCA TTG ACG GTA ACG TGG TTC AGT GC
AN1030.3P4	GCA TCA GTG CCT CCT CTC AGA CAG AAT ACT GCG CTT GGT GAT GG
AN1030.3P5	GTA GAA TGC CGC TGA CAA GG
AN1030.3P6	TTT TAC ATA CCC CCG GAA CC
AN1031.3P1	GGA TGT GAC GTC TGT CTA TGG
AN1031.3P2	TAG GAG CTG GGT TCC TGT TCC
AN1031.3P3	CGA AGA GGG TGA AGA GCA TTG TCT GGG ACT CTA CCT CCT TCC
AN1031.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GAA GAT TGG CCT TGG TAC GG
AN1031.3P5	GAT CTC TTA TCC GCC CTC AAG G
AN1031.3P6	AGC CAC GTC TTT GAC TAC CG
AN1032.3P1	ACC TCA GCA CCA CTT TCT GG
AN1032.3P2	CAA ACC TCA CAA CCC TCT CG
AN1032.3P3	CGA AGA GGG TGA AGA GCA TTG CAA TGG CAG ATC GTC TAT GC
AN1032.3P4	GCA TCA GTG CCT CCT CTC AGA CAG AAG GAT CAG GAT GCA ACT CC
AN1032.3P5	TCT GCA TGG TCG CCT AGT AGG
AN1032.3P6	AGC TGG TGC ATC TTC TCT GC
AN1033.3P1	TAA AGA GCC AGA CCC AAA GC
AN1033.3P2	GGC AAA TAA GAC AGC GAA GC
AN1033.3P3	CGA AGA GGG TGA AGA GCA TTG AGG ATG CCG GTA CCA GTA GG
AN1033.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TGT CTA AAG GCG TCC ACT GC
AN1033.3P5	ATA TCG CGG GCT ATA TCA GG
AN1033.3P6	CAT CTG TCC AGT TCA GAC AAG G
AN1034.3P1	GCT TGT TCT CGC TGA GTT GC
AN1034.3P2	CGC TGA GTT GCT TGA GAA GG
AN1034.3P3	CGA AGA GGG TGA AGA GCA TTG CTG TCC CTT GGG GAA GTA GG
AN1034.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TCA CCT CAA CTC CGC TAT CC
AN1034.3P5	ACG GCA TGC TTA AGA AGG AGA AGG
AN1034.3P6	AAG AGC CCT GTT TCG TAC CC
AN1035.3P1	CTG GCG TAT TCT GGT GTT GG
AN1035.3P2	GCC ATG CCC TGT AAC TTG AGT AG
AN1035.3P3	CGA AGA GGG TGA AGA GCA TTG TGG GTG TGC AAG ATA GG
AN1035.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TTT CCG ACT TAT GCC TGA GC
AN1035.3P5	GAG TCT GGA CCC TGT TTT CG

AN1035.3P6	CGC TTG GAC AGA AAC TCA CC
AN11287.3P1	CAT CTT CAT GGT CCC TGT GG
AN11287.3P2	CGA TAT CGA TCT GAC CAA GC
AN11287.3P3	CGA AGA GGG TGA AGA GCA TTG GAG ATC TTG GAG GGG AAT CG
AN11287.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TAT CTT GCC AGG ACC AAA CC
AN11287.3P5	TCA ACG AGA CCC TCT TGA CC
AN11287.3P6	CGA TAA TGT CCT CCC TCC TGA AC
AN1036.3P1	ATG GAG TTG GAG CCT GTA CC
AN1036.3P2	GCC TGT ACC TTT GTC GAT AAG C
AN1036.3P3	CGA AGA GGG TGA AGA GCA TTG GAA TCT GTG CCA GTT GAT GG
AN1036.3P4	GCA TCA GTG CCT CCT CTC AGA CAG AGT TTG CTC TGG GTG TCT GG
AN1036.3P5	CTC TTA CCT GCG AGG ACT GG
AN1036.3P6	ATG TCC CCT CCG CTT TTA GC
AN1037.3P1	GCG AAT TGG TGG TAT CAT CG
AN1037.3P2	GTT GGG AGG AAT TTG AGT GG
AN1037.3P3	CGA AGA GGG TGA AGA GCA TTG TGG AAC TGG TTA GGC ACA GC
AN1037.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CTT GGC TAG GCG TAT GAT GG
AN1037.3P5	GAA ATC TCC GGA CCA TGT GC
AN1037.3P6	TGA CCT GAC TCC AGA AAT CTC C
AN11288.3P1	TCC ATA ACC ACC ATC CAT CG
AN11288.3P2	GGC TGG AAT CAA CAA AGC TCC
AN11288.3P3	CGA AGA GGG TGA AGA GCA TTG ACG GAA GCC CAG ATA TTT CC
AN11288.3P4	GCA TCA GTG CCT CCT CTC AGA CAG ATC GCC ATC CTA CTC AAT CC
AN11288.3P5	GCT CCG ACT GAC AAA TCT CC
AN11288.3P6	AAA TCC AAG TCG TCC TCA CG
AN1038.3P1	AAC CTT GTT CCG CAT AGA CC
AN1038.3P2	CTG ACC GGT AAT TCC TCA CC
AN1038.3P3	CGA AGA GGG TGA AGA GCA TTG GGC AGT CGG GTT ACA ATA GC
AN1038.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TTA CGA AAC TCG ACC CCT GAT G
AN1038.3P5	AGA CCA GCC AAT CAC TCA CC
AN1038.3P6	ATG GAC CTC GAT CCT TGC CAT AC

Blue and red sequences are tails that anneal to the *A. fumigatus pyroA* (*AfpyroA*) or alcA promoter fragment during fusion PCR



1

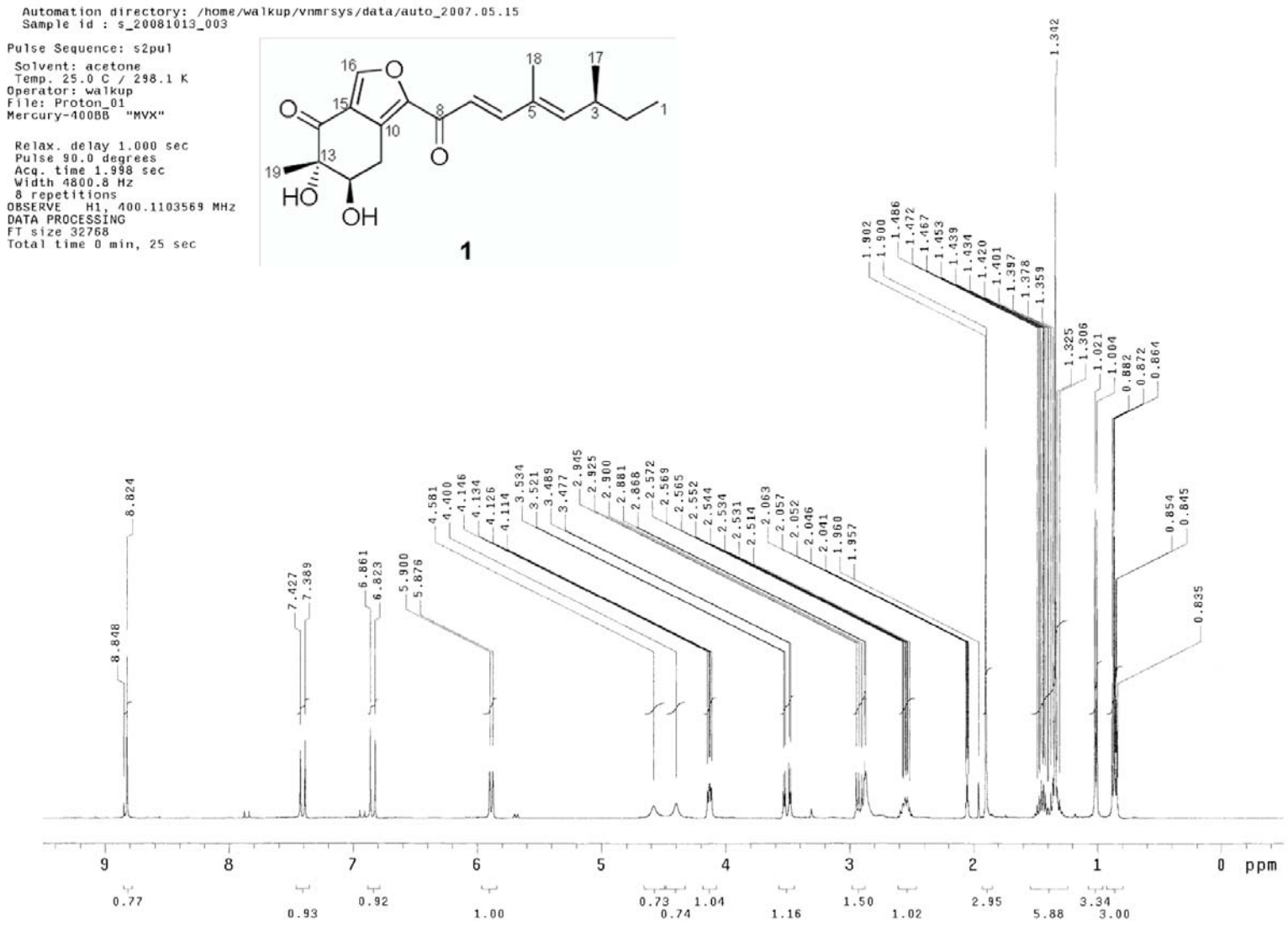


2

Table S3. ^{1}H and ^{13}C NMR data for compounds **1** and **2** (400 and 100 MHz in acetone-d_6)

	1		**2**	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1	12.3, CH_3	0.86, t (7.6)	12.3, CH_3	0.85, t (7.2)
2	30.7, CH_2	1.35, 1.45, m	30.7, CH_2	1.32, 1.43, m
3	35.9, CH	2.55, m	35.8, CH	2.53, m
4	150.9, CH	5.88, br d (10.0)	150.6, CH	5.84, br d (9.6)
5	133.3, qC	—	133.1, qC	—
6	149.6 ,CH	7.41, d (15.2)	149.3 ,CH	7.36, d (15.6)
7	122.1, CH	6.84, d (15.2)	124.4, CH	6.27, d (15.6)
8	184.9, qC	—	197.0, qC	—
9	128.3, qC	—	44.2, CH_2	4.25, s
10	135.9, qC	—	139.8, qC	—
11	29.3, CH_2	2.91, dd (H_β, 18.0, 8.4) 3.51, dd (H_α, 18.0, 5.2)	111.7, CH	6.47, s
12	75.1, CH	4.13, dd (8.4, 5.2)	163.7, qC	—
13	78.8, qC	—	110.7, qC	—
14	188.6, qC	—	164.9, qC	—
15	147.9, qC	—	113.7, qC	—
16	154.2, CH	8.82, s	195.1, CH	9.90, s
17	20.4, CH_3	1.01, d (6.4)	20.4, CH_3	0.99, d (6.8)
18	12.8, CH_3	1.90, br s	12.6, CH_3	1.82, br s
19	18.7, CH_3	1.34, s	7.4, CH_3	2.03, s
12-OH	—	4.40[a], br s	—	9.68, br s
13-OH	—	4.58[a], br s	—	—
14-OH	—	—	—	12.78, s

[a] Values bearing the same superscript may be interchanged.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Sample id : s_20081013_003
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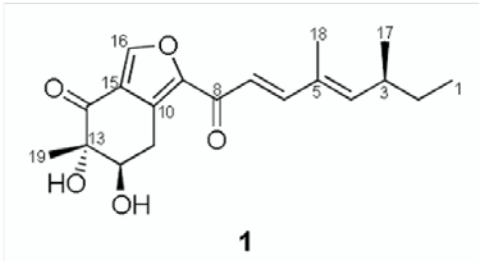
Solvent: acetone
Temp. 25.0 C / 298.1 K
Operator: walkup
File: Proton_01
Mercury-400BB "MVX"
Relax. delay 1.000 sec
Pulse 90.0 degrees
Acq. time 1.998 sec
Width 4800.8 Hz
8 repetitions
OBSERVE H1, 400.1103569 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 25 sec
HO
OH
1
8.848
8.824
7.427
7.389
6.861
6.823
5.900
5.878
4.581
4.400
4.146
4.134
4.126
4.114
3.534
3.521
3.489
3.477
2.945
2.925
2.900
2.881
2.868
2.572
2.569
2.565
2.552
2.544
2.534
2.531
2.514
2.063
2.057
2.052
2.046
2.041
1.960
1.957
1.902
1.900
1.486
1.472
1.467
1.453
1.439
1.434
1.420
1.401
1.397
1.378
1.359
1.342
1.325
1.306
1.021
1.004
0.882
0.872
0.864
0.854
0.845
0.835
9
8
7
6
5
4
3
2
1
0 ppm
0.77
0.93
0.92
1.00
0.73
0.74
1.04
1.16
1.50
1.02
2.95
5.88
3.34
3.00

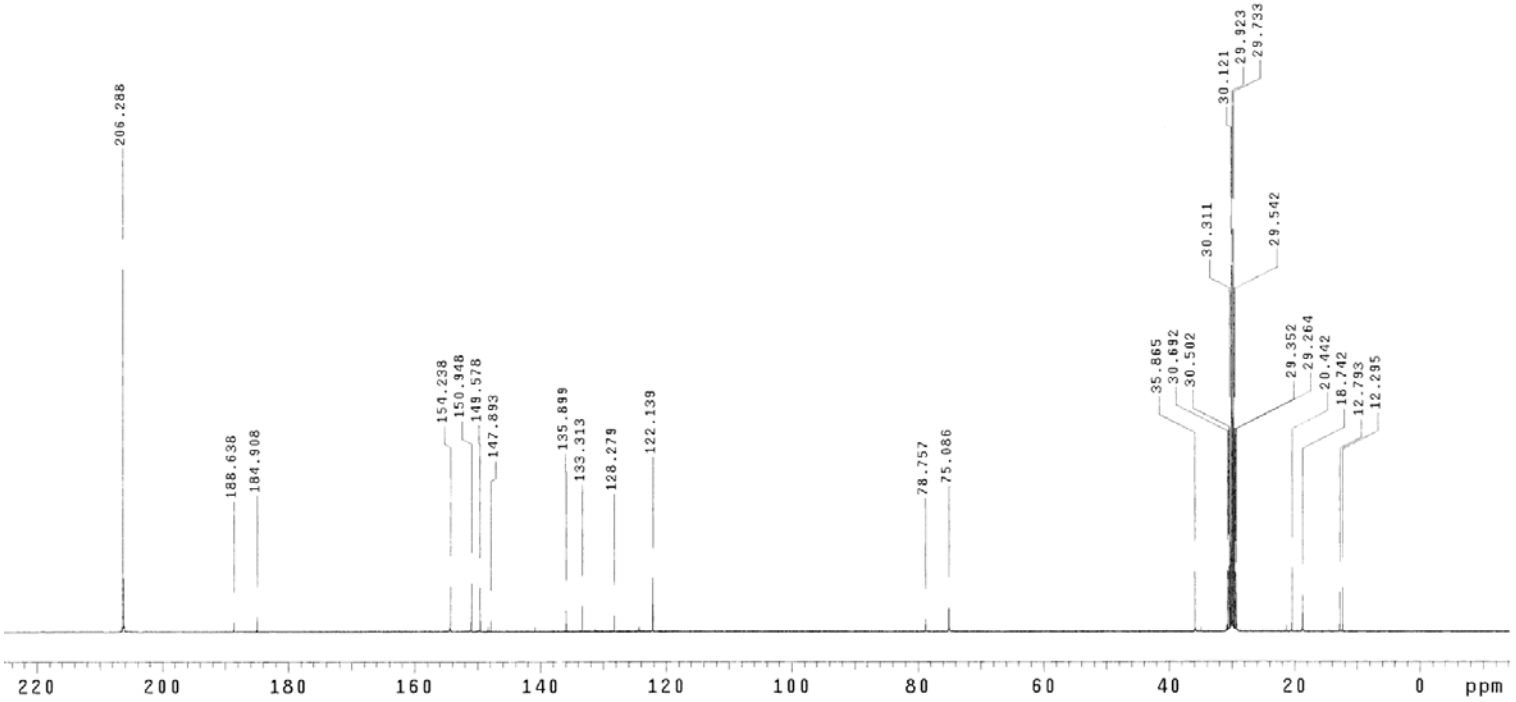
Automation directory: /home/walkup/vnmrsys/data/auto_2007.05.15
Sample id : s_20081010_001

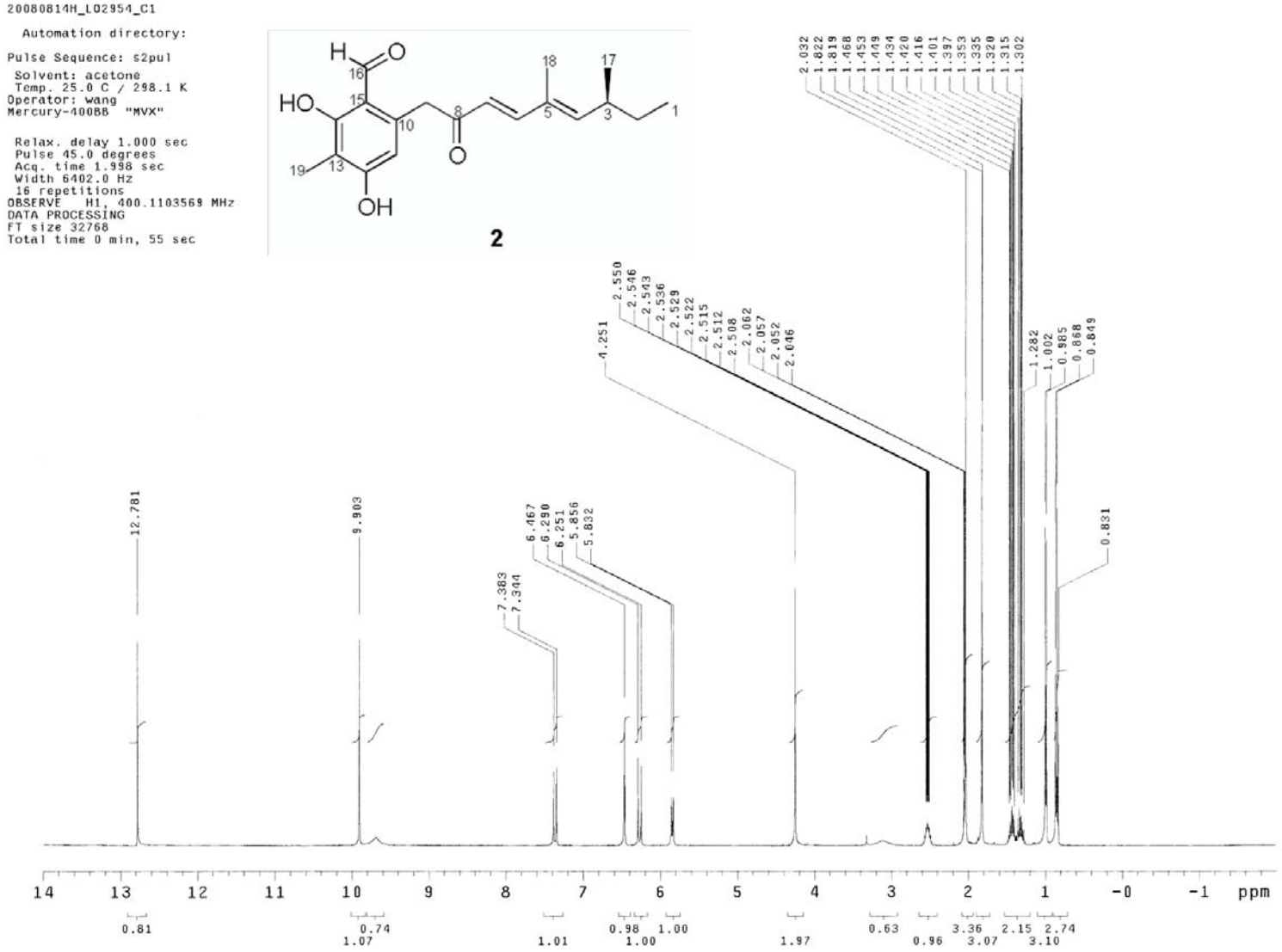
Pulse Sequence: s2pul
Solvent: acetone
Temp. 25.0 C / 298.1 K
Operator: walkup
File: Carbon_01
Mercury-400BB "MVX"

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 24154.6 Hz
5000 repetitions
OBSERVE C13, 100.6077314 MHz
DECOUPLE H1, 400.1124023 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 4 hr, 42 min, 47 sec



1



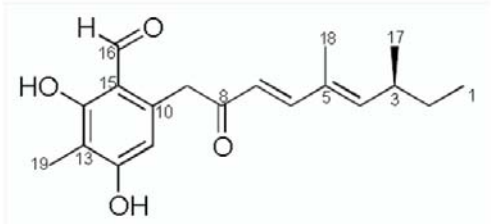

20080814H_LO2954_C1
Automation directory:
Pulse Sequence: s2pul
Solvent: acetone
Temp. 25.0 C / 298.1 K
Operator: wang
Mercury-400BB "MVX"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
16 repetitions
OBSERVE H1, 400.1103568 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 55 sec
2
12.781
9.903
7.383
7.344
6.467
6.290
6.251
5.856
5.832
4.251
2.550
2.546
2.543
2.536
2.529
2.522
2.515
2.512
2.508
2.062
2.057
2.052
2.046
2.032
1.822
1.819
1.468
1.453
1.449
1.434
1.420
1.416
1.401
1.397
1.353
1.335
1.320
1.315
1.302
1.282
1.002
0.985
0.868
0.849
0.831
14 13 12 11 10 9 8 7 6 5 4 3 2 1 -0 -1 ppm
0.81
0.74
1.07
1.01
0.98
1.00
1.00
1.97
0.63
0.96
3.36
3.07
2.15
2.74
3.10

20080814C_LO2954_C1

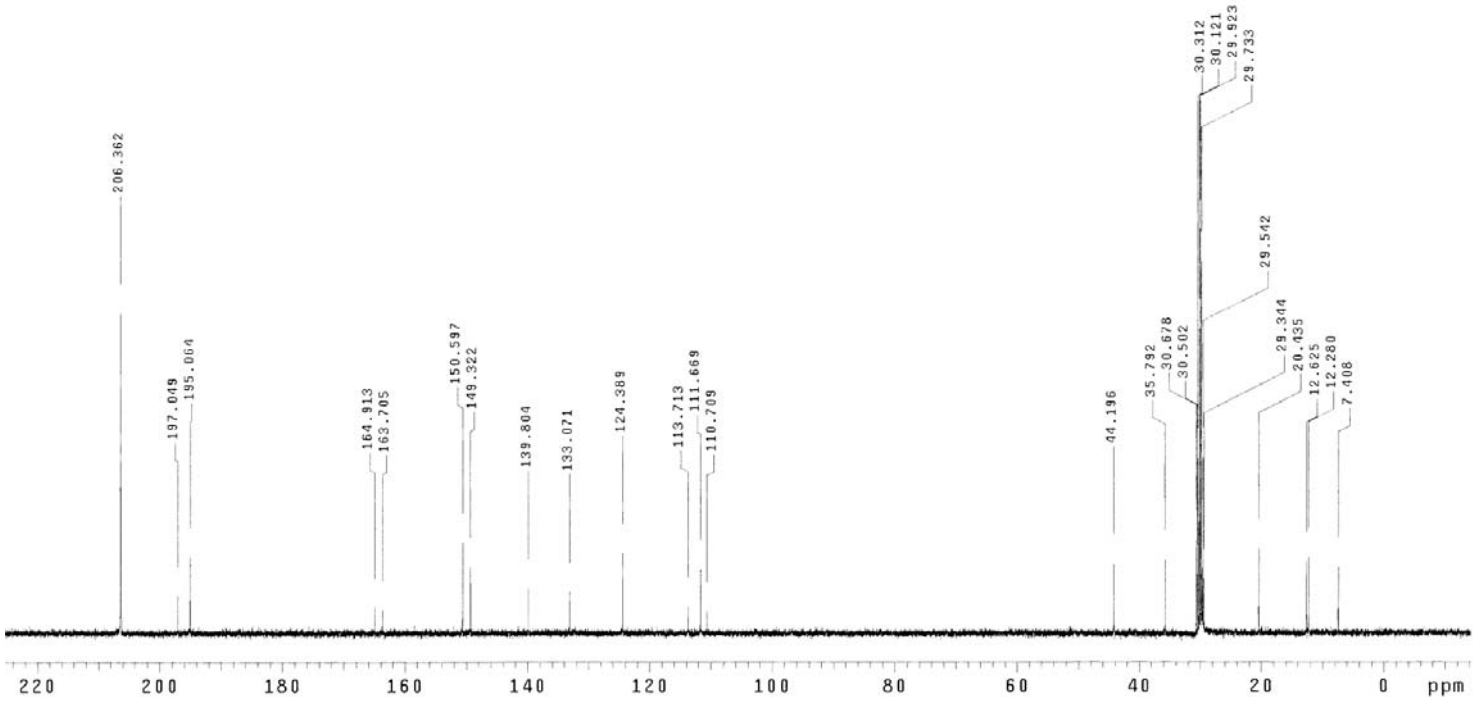
Automation directory:

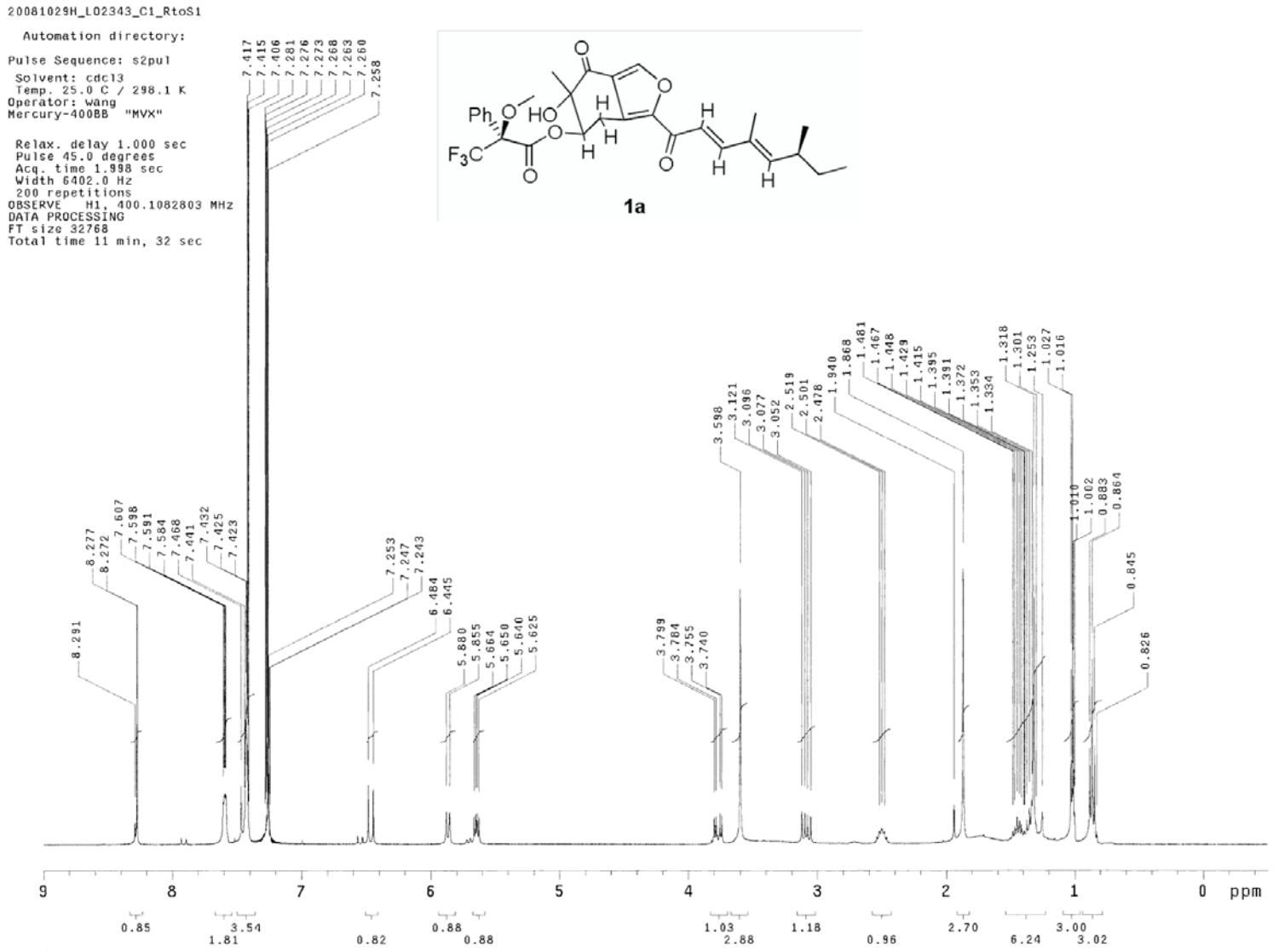
Pulse Sequence: s2pul
Solvent: acetone
Temp. 25.0 C / 298.1 K
Operator: wang
Mercury-400BB "MVX"

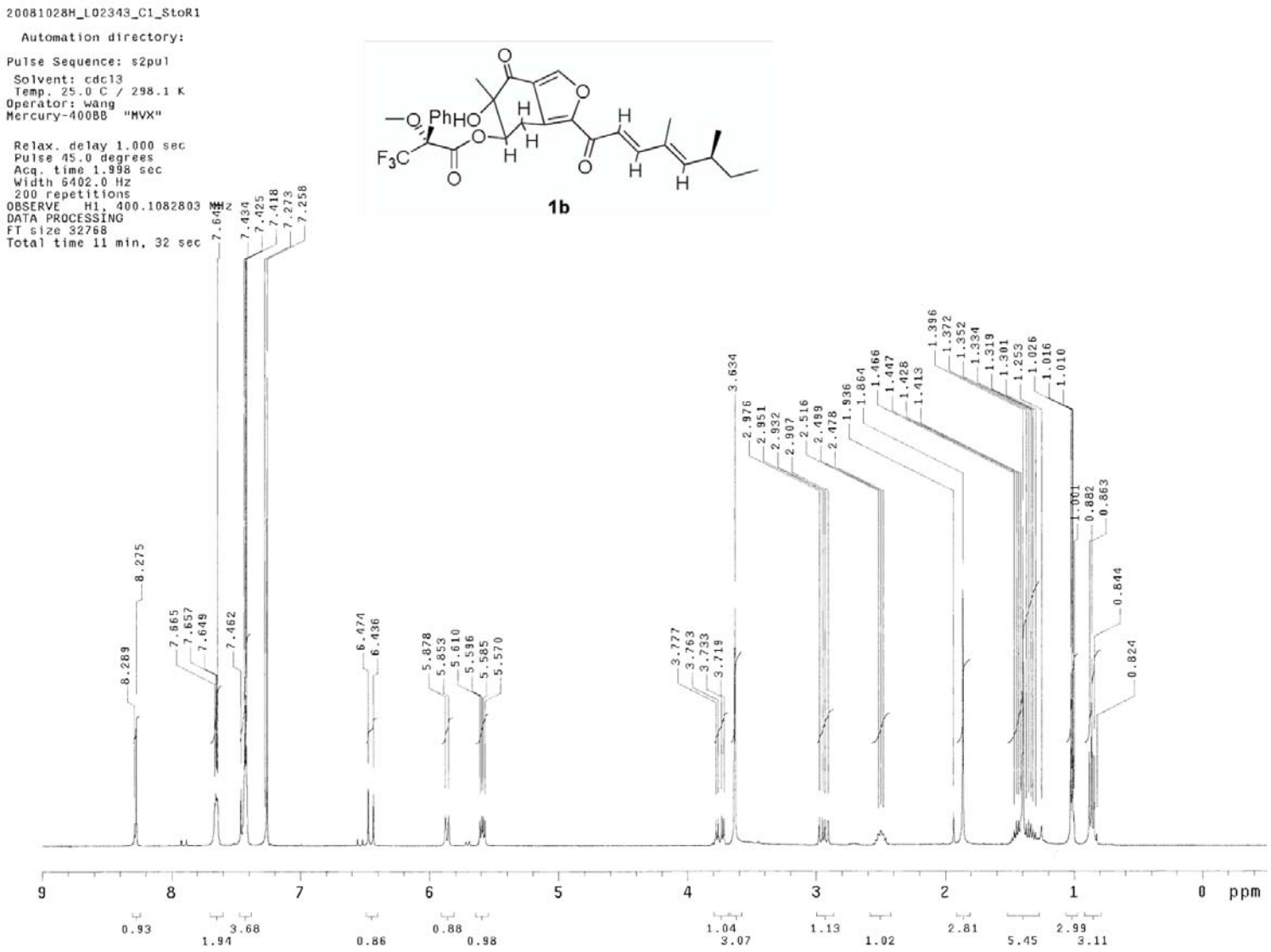
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 24154.6 Hz
60 repetitions
OBSERVE C13, 100.6077328 MHz
DECOUPLE H1, 400.1124023 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 7 min, 11 sec



2




20081029H_LO2343_C1_RtoS1
Automation directory:
Pulse Sequence: s2pul
Solvent: cdcl3
Temp. 25.0 C / 298.1 K
Operator: wang
Mercury-400BB "MVX"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
200 repetitions
OBSERVE H1, 400.1082803 MHz
DATA PROCESSING
FT size 32768
Total time 11 min, 32 sec
1a
ppm


20081028H_LO2343_C1_StoR1
Automation directory:
Pulse Sequence: s2pul
Solvent: cdcl3
Temp. 25.0 C / 298.1 K
Operator: wang
Mercury-400BB "MVX"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
200 repetitions
OBSERVE H1, 400.1082803 MHz
DATA PROCESSING
FT size 32768
Total time 11 min, 32 sec
1b
ppm