Systematic Domain Swaps of Iterative, Non-reducing Polyketide Synthases Provide a Mechanistic Understanding and Rationale For Catalytic Reprogramming

Adam G. Newman, Anna L. Vagstad, Philip A. Storm, and Craig A. Townsend*

Department of Chemistry, The Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218

Contact Information: * E-mail: ctownsend@jhu.edu

SUPPLEMENTARY INFORMATION

Cloning Details.

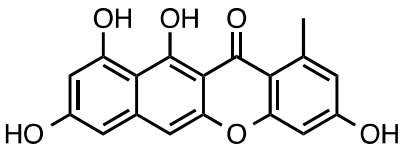
A *C*-terminal 6×His tagged Pks4 SAT-KS-MAT construct was cloned by overlap extension PCR of exon fragments from genomic DNA. The resulting spliced fragment was used as template for Pks4-SAT-s1 Pks4-NKA-3 primers. The resulting PCR product was digested with NdeI and NotI and ligated into pET24a(+) to yield pEPks4-NKA-1. This plasmid was cut with NdeI and EcoRI and inserted into plasmid pEPks4-SATn-1[1] to yield pEPks4-NKAn. The resulting plasmid contains the *N*-terminal codon optimized sequence of pEPks4-SATn-1.

A full-length *C*-terminal 6×His tagged CTB1 construct was cloned from pCR-CTB1-ex #3 (a pCR blunt plasmid containing CTB1 exon 3) template with primers CTB1-S3+1 and CTB1-ex6-3 and pCR-CTB1-Ex7-9 #3 (a pCR blunt plasmid containing CTB1 exons 7-9) template with primers CTB1-ex7-5 and CTB1-3. The resulting PCR products were spliced together using overlap extension PCR with outside primers CTB1-S3+1 and CTB1-3. The resulting overlap extension PCR product was digested with NheI-HF and NotI-HF (New England Biolabs, Ipswich, MA) and inserted into pECTB1-NKA6 to give pECTB1.

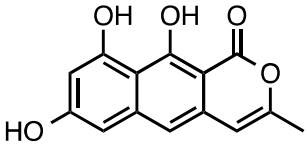
A *C*-terminal 6×His tagged CTB1 SAT-KS-MAT/Pks1 PT-ACP_2-TE chimeric full-length PKS was cloned using Gibson assembly cloning to give plasmid pEM4P6-1.[2] The crossover sites (CTB1 S1293 and Pks1 P1290) were selected to be five amino acids upstream of a conserved threonine at the *N*-terminal end of the PT domain. The CTB1 SAT-KS-MAT fragment was PCR amplified from pECTB1 template with T7 and M4P6-3 primers. The Pks1 PT-ACP_2-TE fragment was PCR amplified from pEPks1alt template with M4P6-5 and T7 term primers. The resulting fragments where incubated with Nde1 and Not1-HF digested pET24a(+) vector in equimolar concentrations (0.25 pmol each) with 0.08 U T5 exonuclease, 0.5 U Phusion DNA Polymerase, and 80 U *Taq* DNA ligase in 1× isothermal reaction buffer (5% PEG-8000, 100 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM each of the four dNTPs, and 1 mM NAD) to a final volume of 20 μL. Assembly reactions were incubated for 1 hour at 50 °C. Following incubation, the reactions were diluted with 80 μL ddH_2O and directly electorporated into *E. coli* BL21(DE3) cells. Cells were plated on LB agar plates with 50 μg/mL kanamycin and incubated overnight at 37 °C. Colonies were selected, screened, and sequenced to ensure proper chimeric insertion.

Chemical Characterization.

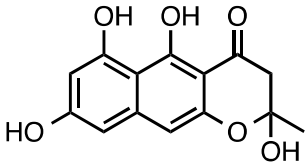
All mass data unless otherwise stated was collected on a Shimadzu LC-IT-TOF (Shimadzu Corporation, Kyoto, Japan) in positive ion mode fitted with a Luna C18(2) column (2.0 × 150 mm, 3 μ; Phenomenex, Torrence, CA) using a linear gradient of 5% to 85% solvent B over 30 min at 0.2 mL/min. HPLC retention times are reported for a Prodigy 5u ODS3 column (4.6 × 250 mm, 5μ; Phenomenex, Torrence, CA) using a linear gradient of 5% to 85% solvent B over 30 min at 1 mL/min. Solvent A was water + 0.1% formic acid and solvent B was acetonitrile + 0.1% formic acid in both cases. The λ_{max} for each compound was recorded for the HPLC peak maximum at 280 nm.



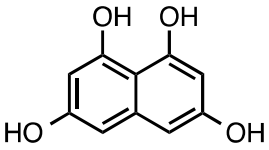
3,8,10,11-tetrahydroxy-1-methyl-12*H*-benzo[*b*]xanthen-12-one (pre-bikaverin, 2).
λ_{max} (nm): 226, 254, 276, 302, 332, 348, 428. HPLC retention time: 33.0 min. HRMS (*m/z*): calculated exact mass for $C_{18}H_{13}O_6$ [MH^+], 325.0712; found [MH^+], 325.0703. These data are in complete agreement with literature values for **2**.[3]



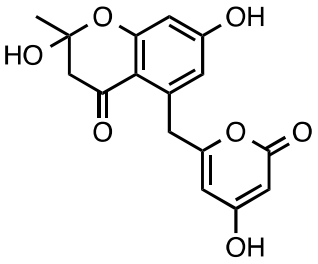
7,9,10-trihydroxy-3-methyl-1*H*-benzo[*g*]isochromen-1-one (*nor*-toralactone, 4).
λ_{max} (nm): 268, 278, 394. HPLC retention time: 28.7 min. HRMS (*m/z*): calculated exact mass for $C_{14}H_{11}O_5$ [MH^+], 259.0607; found [MH^+], 259.0606. These data are in complete agreement with literature values for **4**.[4]



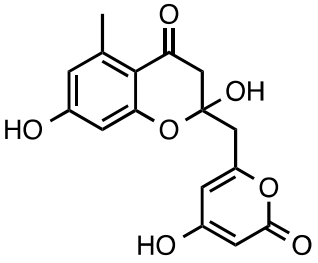
2,5,6,8-tetrahydroxy-2-methyl-2,3-dihydro-4*H*-benzo[*g*]chromen-4-one (YWA1, 5).
λ_{max} (nm): 228, 280, 324, 334, 408. HPLC retention time: 21.6 min. HRMS (*m/z*): calculated exact mass for $C_{14}H_{13}O_6$ [MH^+], 277.0712; found [MH^+], 277.0670. These data are in complete agreement with literature values for **5**.[5]



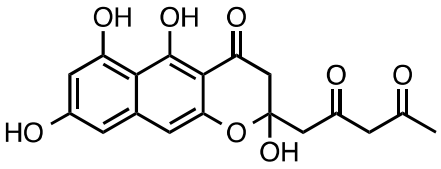
naphthalene-1,3,6,8-tetraol (THN, 6).
λ_{max} (nm): 266, 300. HPLC retention time: 14.2 min. HRMS (*m/z*): calculated exact mass for $C_{10}H_9O_4$ [MH^+], 193.0501; found [MH^+], 193.0494. MS data was collected on a Waters Acquity/Xeno-G2 UPLC-MS in positive ion mode. These data are in complete agreement with literature values for **6**.[6]



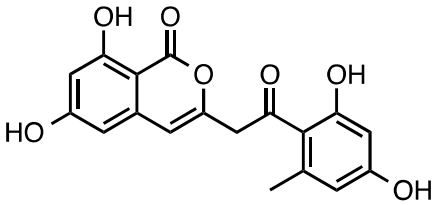
6-((3,6-dihydroxy-6-methyl-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)methyl)-4-hydroxy-2*H*-pyran-2-one (SEK4, 7).
λ_{max} (nm): 280. HPLC retention time: 15.9 min. HRMS (*m/z*): calculated exact mass for $C_{16}H_{15}O_7$ [MH^+], 319.0818; found [MH^+], 319.0806. SEK4 coelutes with its isomer SEK4b, **8**. These data are in complete agreement with literature values for **7**.[7-9]



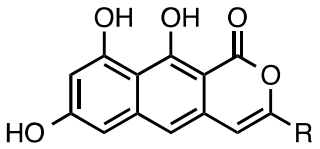
2,5,7-trihydroxy-2-((4-hydroxy-2-oxo-2*H*-pyran-6-yl)methyl)chroman-4-one (SEK4b, 8).
λ_{max} (nm): 280. HPLC retention time: 15.9 min. HRMS (*m/z*): calculated exact mass for $C_{16}H_{15}O_7$ [MH^+], 319.0818; found [MH^+], 319.0806. SEK4b coelutes with its isomer SEK, **7**. These data are in complete agreement with literature values for **8**.[9,10]



1-(2,5,6,8-tetrahydroxy-4-oxo-3,4-dihydro-2*H*-benzo[*g*]chromen-2-yl)pentane-2,4-dione (11).
λ_{max} (nm): 230, 284, 326, 338, 416. HPLC retention time: 17.6 min. HRMS (*m/z*): calculated exact mass for $C_{18}H_{17}O_8$ [MH^+], 361.0923; found [MH^+], 361.0913; [MH^+-H_2O], 343.0808. MS data was collected on a Waters Acquity/Xeno-G2 UPLC-MS in positive ion mode. The λ_{max} and shape of the UV-vis spectrum for this molecule are similar to those for YWA1 (**5**) with which it likely shares the same core architecture (See Figures 3, S8).[5] This peak converts to pre-bikaverin (**2**) with time. Taken together, these data support the proposed structural assignment. This structure is consistent with polyketide cyclization logic.

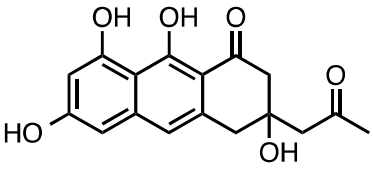


3-(2-(2,4-dihydroxy-6-methylphenyl)-2-oxoethyl)-6,8-dihydroxy-1*H*-isochromen-1-one (SMA93, 12).
λ_{max} (nm): 244, 278, 328. HPLC retention time: 20.0 min. HRMS (*m/z*): calculated exact mass for $C_{12}H_{11}O_5$ [MH^+], 235.0607; found [MH^+], 235.0603. These data are in complete agreement with literature values for **12**.[11]

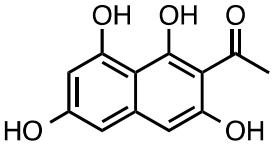


R = $CH_2COCH_2COCH_3$, **13**
R = CH_2COCH_3, **14**

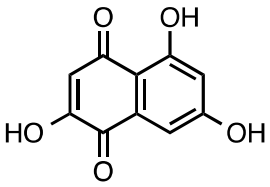
1-(7,9,10-trihydroxy-1-oxo-1*H*-benzo[*g*]isochromen-3-yl)pentane-2,4-dione (13) or 7,9,10-trihydroxy-3-(2-oxopropyl)-1*H*-benzo[*g*]isochromen-1-one (14).
λ_{max} (nm): 270, 280, 392. HPLC retention time: 24.8 min. HRMS (*m/z*): calculated exact mass for $C_{18}H_{15}O_7$ (**13**) [MH^+], 345.0818; mass not found. Calculated exact mass for $C_{16}H_{13}O_6$ (**14**) [MH^+], 301.0712; mass not found. The λ_{max} and shape of the UV-vis spectrum for this molecule are similar to those for *nor*-toralactone (**4**) and norpyrone (**19**), suggesting a shared core architecture (See Figures 4, S8).[4,12] Because a mass for this peak could not be found, we propose structures **13** and **14** as the most reasonable assignments for this product. Both are consistent with polyketide cyclization logic.



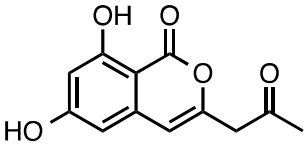
3,6,8,9-tetrahydroxy-3-(2-oxopropyl)-3,4-dihydroanthracen-1(2*H*)-one (15).
λ_{max} (nm): 226, 272, 398. HPLC retention time: 20.9 min. HRMS (*m/z*): calculated exact mass for $C_{17}H_{17}O_6$ [MH^+], 317.1025; found [MH^+], 317.1015. The λ_{max} and shape of the UV-vis spectrum for this molecule are similar to those for atrochrysone, suggesting a shared core architecture (See Figures 5, S8).[13,14] Taken together, these data support the proposed structural assignment. This assignment is in agreement with polyketide cyclization logic.



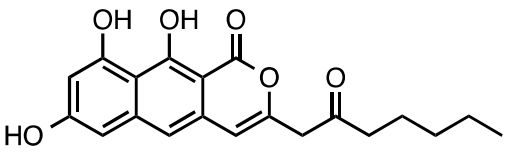
1-(1,3,6,8-tetrahydroxynaphthalen-2-yl)ethan-1-one (ATHN, 16).
λ_{max} (nm): 230, 280, 320, 330, 404. HPLC retention time: 24.9 min. HRMS (*m/z*): calculated exact mass for $C_{12}H_{11}O_5$ [MH^+], 235.0607; found [MH^+], 235.1326. These data are in complete agreement with literature values for **16**.[6]



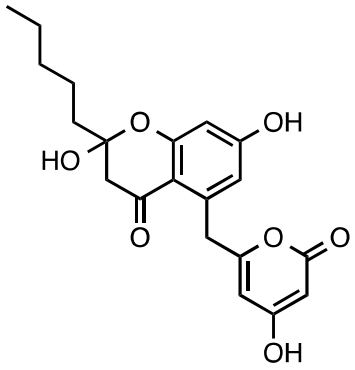
2,5,7-trihydroxynaphthalene-1,4-dione (flaviolin, 17).
λ_{max} (nm): 216, 262, 306, 446. HPLC retention time: 18.5 min. HRMS (*m/z*): calculated exact mass for $C_{10}H_7O_5$ [MH^+], 207.0294; found [MH^+], 207.0287. MS data was collected on a Waters Acquity/Xeno-G2 UPLC-MS in positive ion mode. These data are in complete agreement with literature values for **17**.[6]



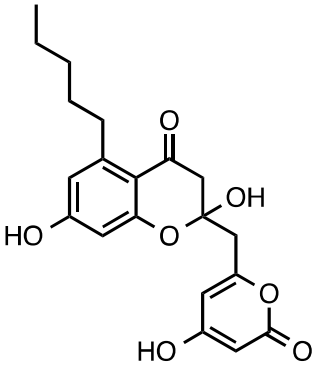
6,8-dihydroxy-3-(2-oxopropyl)-1*H*-isochromen-1-one (18).
λ_{max} (nm): 244, 278, 328. HPLC retention time: 20.0 min. HRMS (*m/z*): calculated exact mass for $C_{12}H_{11}O_5$ [MH^+], 235.0607; found [MH^+], 235.0603. These data are in complete agreement with literature values for **18**.[6]



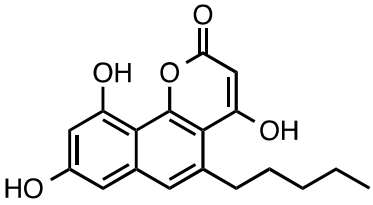
7,9,10-trihydroxy-3-(2-oxoheptyl)-1*H*-benzo[*g*]isochromen-1-one (norpyrone, 19).
λ_{max} (nm): 272, 280, 390. HPLC retention time: 35.1 min. HRMS (*m/z*): calculated exact mass for $C_{20}H_{21}O_6$ [MH^+], 357.1338; found [MH^+], 357.1340. These data are in complete agreement with literature values for **19**.[12]



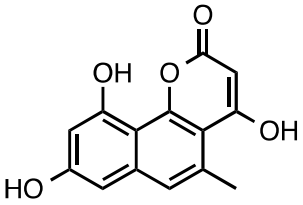
2,7-dihydroxy-5-((4-hydroxy-2-oxo-2*H*-pyran-6-yl)methyl)-2-pentylchroman-4-one (Hex-SEK4, 20).
λ_{max} (nm): 232, 278. HPLC retention time: 24.7 min. HRMS (*m/z*): calculated exact mass for $C_{20}H_{23}O_7$ [MH^+], 375.1444; found [MH^+], 375.1441. Hex-SEK4 coelutes with its isomer Hex-SEK4b, **21**. These data are in complete agreement with literature values for **20**.[15]



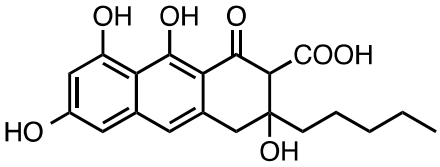
2,7-dihydroxy-2-((4-hydroxy-2-oxo-2*H*-pyran-6-yl)methyl)-5-pentylchroman-4-one (Hex-SEK4b, 21).
λ_{max} (nm): 232, 278. HPLC retention time: 24.7 min. HRMS (*m/z*): calculated exact mass for $C_{20}H_{23}O_7$ [MH^+], 375.1444; found [MH^+], 375.1441. Hex-SEK4b coelutes with its isomer Hex-SEK4, **20**. These data are in complete agreement with literature values for **21**.[16]



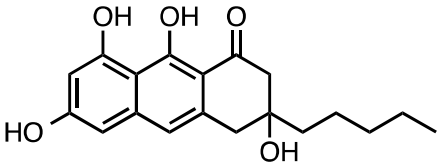
4,8,10-trihydroxy-5-pentyl-2*H*-benzo[*h*]chromen-2-one (Hex-pannorin, 22).
λ_{max} (nm): 232, 278, 288, 322, 368. HPLC retention time: 28.5 min. HRMS (*m/z*): calculated exact mass for $C_{18}H_{19}O_5$ [MH^+], 315.1233; found [MH^+], 315.1220. The λ_{max} and shape of the UV-vis spectrum for this molecule are similar to those for pannorin (**23**), suggesting a shared core architecture (See Figures 8, S10).[17] Taken together, these data support the proposed structural assignment. This assignment is in agreement with polyketide cyclization logic.



4,8,10-trihydroxy-5-methyl-2*H*-benzo[*h*]chromen-2-one (pannorin, 23).
λ_{max} (nm): 230, 276, 288, 320, 364. HPLC retention time: 20.1 min. HRMS (*m/z*): calculated exact mass for $C_{14}H_{11}O_5$ [MH^+], 259.0607; found [MH^+], 259.0606. These data are in complete agreement with literature values for **23**.[4,17]

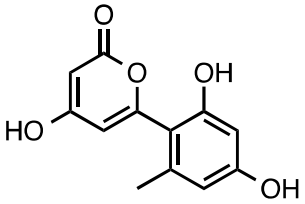


3,6,8,9-tetrahydroxy-1-oxo-3-pentyl-1,2,3,4-tetrahydroanthracene-2-carboxylic acid (Hex-atrochrysone carboxylic acid, 24).
λ_{max} (nm): 224, 268, 318, 378. HPLC retention time: 29.0 min. HRMS (*m/z*): calculated exact mass for $C_{20}H_{23}O_7$ [MH^+], 375.1444; found [MH^+], 375.1440. The λ_{max} and shape of the UV-vis spectrum for this molecule are similar to those for atrochrysone, suggesting a shared core architecture (See Figures 5, S10).[13,14] Taken together, these data support the proposed structural assignment. This assignment is in agreement with polyketide cyclization logic.

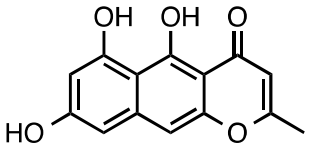


3,6,8,9-tetrahydroxy-3-pentyl-3,4-dihydroanthracen-1(2*H*)-one (Hex-atrochrysone, 25).
λ_{max} (nm): 228, 272, 320, 294. HPLC retention time: 32.6 min. HRMS (*m/z*): calculated exact mass for $C_{19}H_{23}O_5$ [MH^+], 331.1546; found [MH^+], 331.1535. The λ_{max} and shape of the UV-vis

spectrum for this molecule are similar to those for atrochrysone, suggesting a shared core architecture (See Figures 5, S10).[13,14] Taken together, these data support the proposed structural assignment. This assignment is in agreement with polyketide cyclization logic.



6-(2,4-dihydroxy-6-methylphenyl)-4-hydroxy-2*H*-pyran-2-one (31).
λ_{max} (nm): 304. HPLC retention time: 14.3 min. HRMS (*m/z*): calculated exact mass for $C_{12}H_{11}O_5$ [MH^+], 235.0607; found [MH^+], 235.0604. MS data was collected on a Waters Acquity/Xeno-G2 UPLC-MS in positive ion mode. A synthetic standard of this material was provided by Dr. Ikuro Abe. HPLC chromatogram for this product is presented in Figure S12. These data are in complete agreement with the standard.[18,19]



5,6,8-trihydroxy-2-methyl-4*H*-benzo[*g*]chromen-4-one (*nor*-rubrofusarin, 32).
λ_{max} (nm): 224, 278, 328, 412. HPLC retention time: 27.0 min. HRMS (*m/z*): calculated exact mass for $C_{14}H_{11}O_5$ [MH^+], 259.0607; found [MH^+], 259.0599. MS data was collected on a Waters Acquity/Xeno-G2 UPLC-MS in positive ion mode. These data are in complete agreement with literature values for **32**.[20]

Supplementary Tables.

Table S1. Percent identity/similarity/gaps for global pairwise alignment of NR-PKSs. Alignment conducted using EMBOSS Needle with default settings (http://www.ebi.ac.uk/Tools/psa/).

	PksA	**ACAS**	**Pks4**	**CTB1**	**wA**	**Pks1**
PksA						
ACAS	30.3/45.9/23.1					
Pks4	36.9/55.6/8.9	32.8/48.6/20.3				
CTB1	36.8/53.8/11.5	29.4/42.9/25.5	36.0/52.4/11.9			
wA	38.5/54.8/12.2	32.3/56.7/26.0	43.0/60.1/10.5	37.5/54.9/8.8		
Pks1	35.8/52.2/11.8	33.7/47.1/25.3	38.2/53.6/11.2	35.3/53.3/7.9	43.3/60.8/6.2	

Table S2. Protein constructs used in this study. Experimental cloning details provided in given references. Asterisks indicate revised sequence numbering described in given references.

Protein	Encodes	Plasmid	Vector	Tag	MW (kDa)	Ref.
PksA SAT-KS-MAT	M1-S1294	pENKA4	pET24a	*C*-His	143.7	[12]
PksA PT	E1305-I1677	pEPT2	pET24a	*C*-His	42.4	[12]
PksA ACP	I1677-S1839	pEACP41	pET28a	*N*-His	20.6	[21]
PksA TE	D1812-A2109	pETE2	pET28a	*N*-His	36.4	[12]
ACAS PT	S1318-A1669	pEACAS-PT2	pET24a	*C*-His	40.0	[16]
ACAS ACP	A1668-S1771	pEACAS-ACP2	pET28a	*N*-His	13.3	[16]
ACTE	Full protein	pEACTE	pET28a	*N*-His	37.5	[16]
Pks4 SAT-KS-MAT	M1-A1283*[22]	pEPks4-NKAn	pET24a	*C*-His	140.8	This study
Pks4 PT	R1284-P1631*[22]	pEPks4-PT	pET24a	*C*-His	39.6	[16]
Pks4 ACP	P1631-K1764*[22]	pEPks4-ACP	pET28a	*N*-His	16.2	[16]
Pks4 TE	P1765-N2036*[22]	pEPks4-TE	pET28a	*N*-His	32.2	[16]
CTB1 SAT-KS-MAT	M1-S1293	pECTB1-NKA6	pET24a	*C*-His	140.1	[4]
CTB1 PT	S1293-I1654	pECTB1-PT	pET24a	*C*-His	41.2	[4]
CTB1 ACP_2	S1637-K1909	pECTB1-ACP	pET28a	*N*-His	31.7	[4]
CTB1 TE	R1910-S2196	pECTB1-TE	pET28a	*N*-His	33.6	[4]
CTB1 full-length	Full protein	pECTB1	pET24a	*C*-His	238.0	This study
wA PT	T1287-P1619	pEwA-PT	pET24a	*C*-His	38.5	[16]
wA ACP_2	P1619-H1879	pEwA-ACP	pET28a	*N*-His	30.4	[16]
wA TE	L1859-V2157	pEwA-TE2	pET28a	*N*-His	34.9	[16]
Pks1 SAT-KS-MAT	M1-K1291*[6]	pEPks1-NKA	pET24a	*C*-His	141.3	[22]
Pks1 PT	K1291-P1640*[6]	pEPks1-PT	pET24a	*C*-His	39.4	[16]
Pks1 ACP_2	P1640-K1908*[6]	pEPks1-ACP	pET28a	*N*-His	30.9	[16]
Pks1 TE	P1881-E2183*[6]	pEPks1-TEalt	pET28a	*N*-His	35.7	[6]
Pks1 full-length	Full protein	pEPks1alt	pET24a	*C*-His	238.1	[6]
CTB1 SAT-KS-MAT/Pks1 PT-ACP_2-TE Chimera	CTB1: M1-S1293 Pks1: P1290-E2183*[6]	pEM4P6	pET24a	*C*-His	237.1	This study

Table S3. Primers used in this study for cloning the Pks4 SAT-KS-MAT tridomain fragment. The revised sequence numbering for Pks4 was used.[22]

Primer	Sequence
Pks4-SAT-s1	GTAACATATGGCGAGCAGCGCCGACGTGTATGTGTTC
Pks4-NKA-3	GTAAGCGGCCGCCGCTTGTACAACTGCACCGACC
CTB1-S3+1	AAGCGCTAGCTAAGTTCTATCTTGCAGGTGCCAGTGTCGA
CTB1-ex6-3	ATTAGCATGCTGCTCGACGATACTAGAGAACGTGCATGA
CTB1-ex7-5	CACGTTCTCTAGTATCGTCGAGCAGCATGCTAAT
CTB1-3	GTAAGCGGCCGCTGATGAAACAATCCCCAAACC
M4P6-5	CCGTGGTTGTTGCCGCGTCACCCAAACTGGCGACCACCTC
M4P6-3	GAGGTGGTCGCCAGTTTGGGTGACGCGGCAACAACCACGG
T7	TAATACGACTCACTATAGGG
T7 Term	GCTAGTTATTGCTCAGCGG

Supplementary Figures.

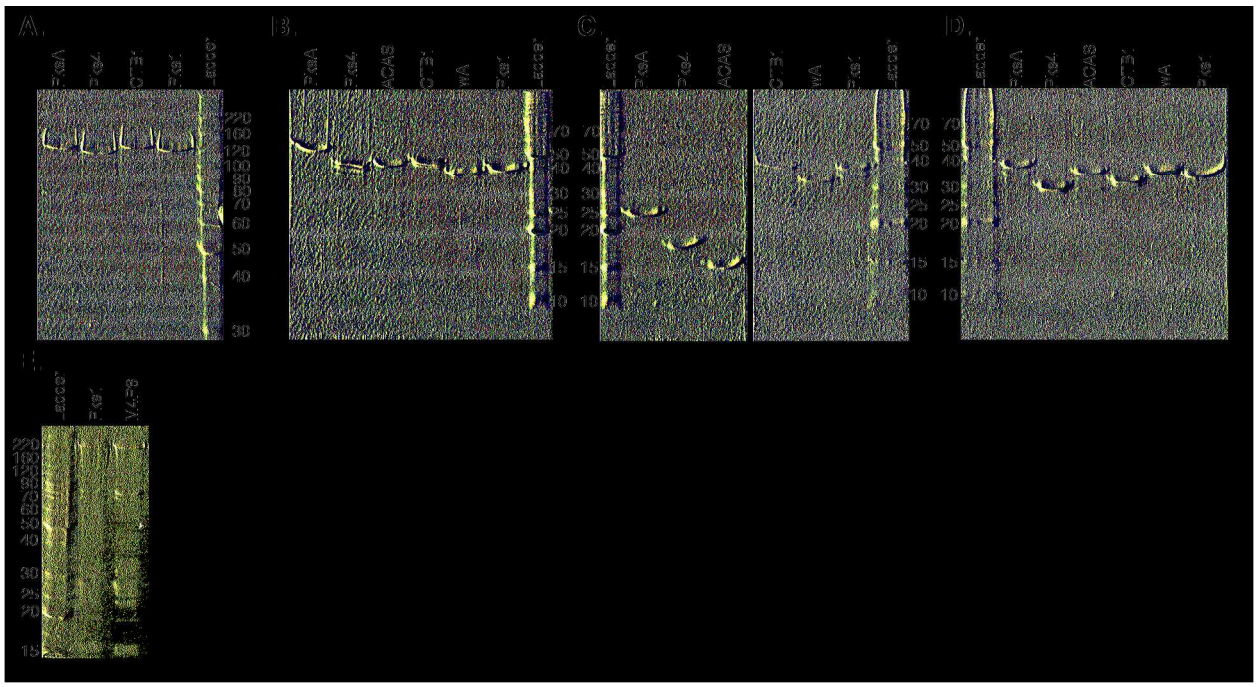


Figure S1. Purified proteins used in this study separated by SDS-PAGE and stained with Coomassie Blue. BenchMark Protein Ladder (Invitrogen, Grand Island, NY) was used as a molecular weight standard (indicated in kDa). A) 10% SDS-PAGE of SAT-KS-MAT proteins, B) 15% SDS-PAGE of PT proteins, C) 15% SDS-PAGE of ACP_n proteins, D) 15% SDS-PAGE of TE proteins, and E) 12% SDS-PAGE of full-length PKS.

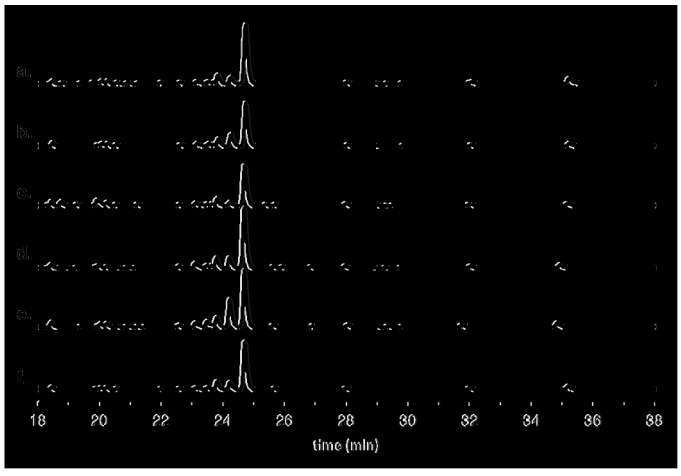


Figure S2. Product profile for combinatorial minimal PksA. Reactions with PksA SAT-KS-MAT and a) PksA ACP; b) Pks4 ACP; c) ACAS ACP; d) CTB1 ACP_2; e) wA ACP_2; f) Pks1 ACP_2. This reaction profile has been previously characterized.[15]

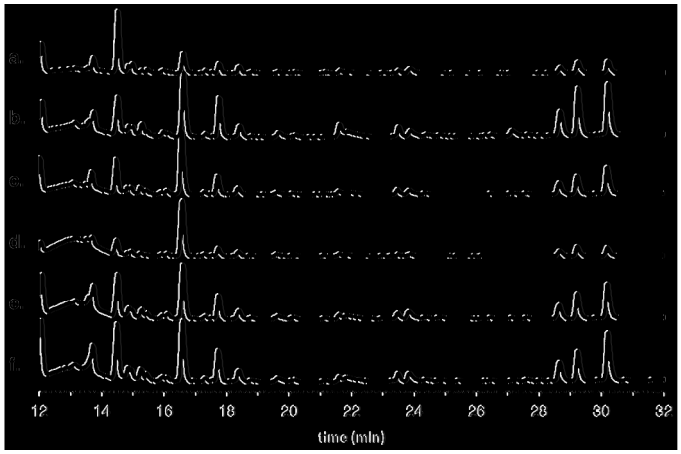


Figure S3. Product profile for combinatorial minimal Pks1. Reactions with Pks1 SAT-KS-MAT and a) Pks1 ACP; b) PksA ACP; c) Pks4 ACP; d) ACAS ACP; e) CTB1 ACP_2; f) wA ACP_2.

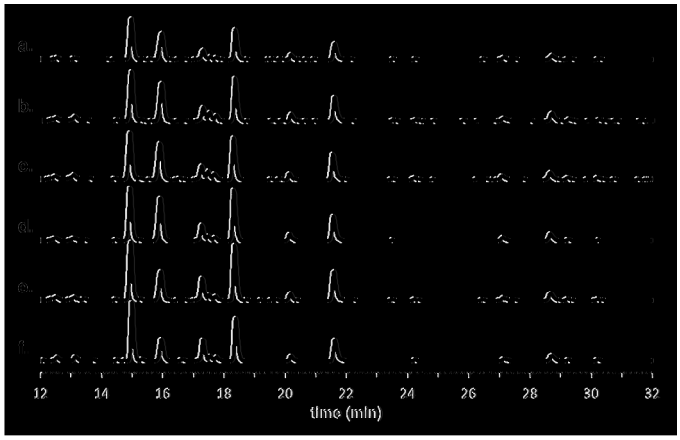


Figure S4. Product profile for combinatorial minimal CTB1. Reactions with CTB1 SAT-KS-MAT and a) CTB1 ACP_2; b) PksA ACP; c) Pks4 ACP; d) ACAS ACP; e) wA ACP_2; f) Pks1 ACP_2. This reaction profile has been previously characterized.[4]

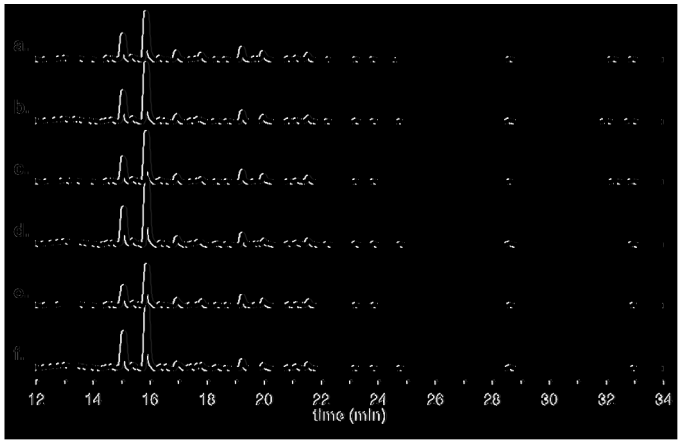


Figure S5. Product profile for combinatorial minimal Pks4. Reactions with Pks4 SAT-KS-MAT and a) Pks4 ACP; b) PksA ACP; c) ACAS ACP; d) CTB1 ACP_2; e) wA ACP_2; f) Pks1 ACP_2. SEK4 (**7**) and SEK4b (**8**) previously characterized.[7,8,10]

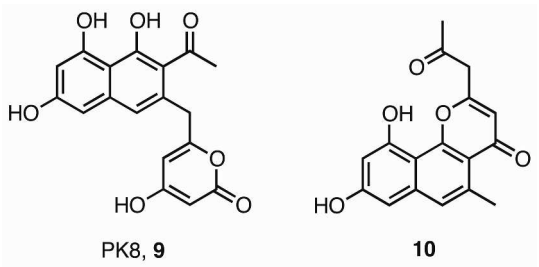


Figure S6. Expected minimal Pks4 products. Products previously characterized.[8]

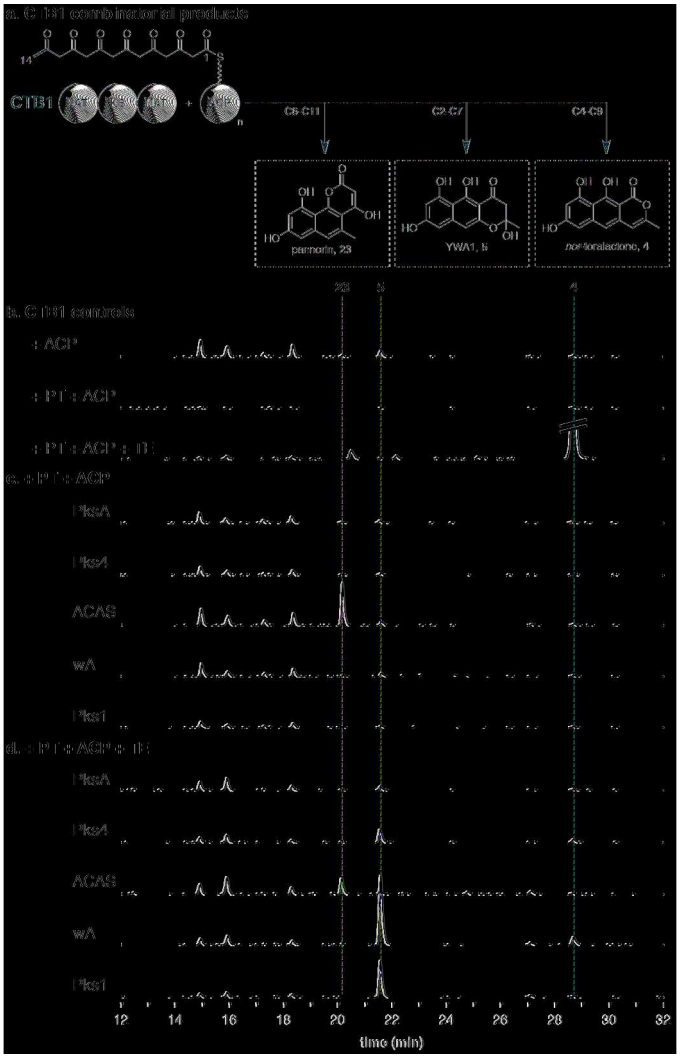


Figure S7. Product analysis of combinatorial reactions with CTB1 SAT-KS-MAT. a) Proposed structures for products of chemical redirection. b) CTB1 control reactions. c) Combinatorial reactions containing PT and ACP_n for the given parent PKS. d) Combinatorial reactions containing PT, ACP_n, and TE for the given parent PKS.

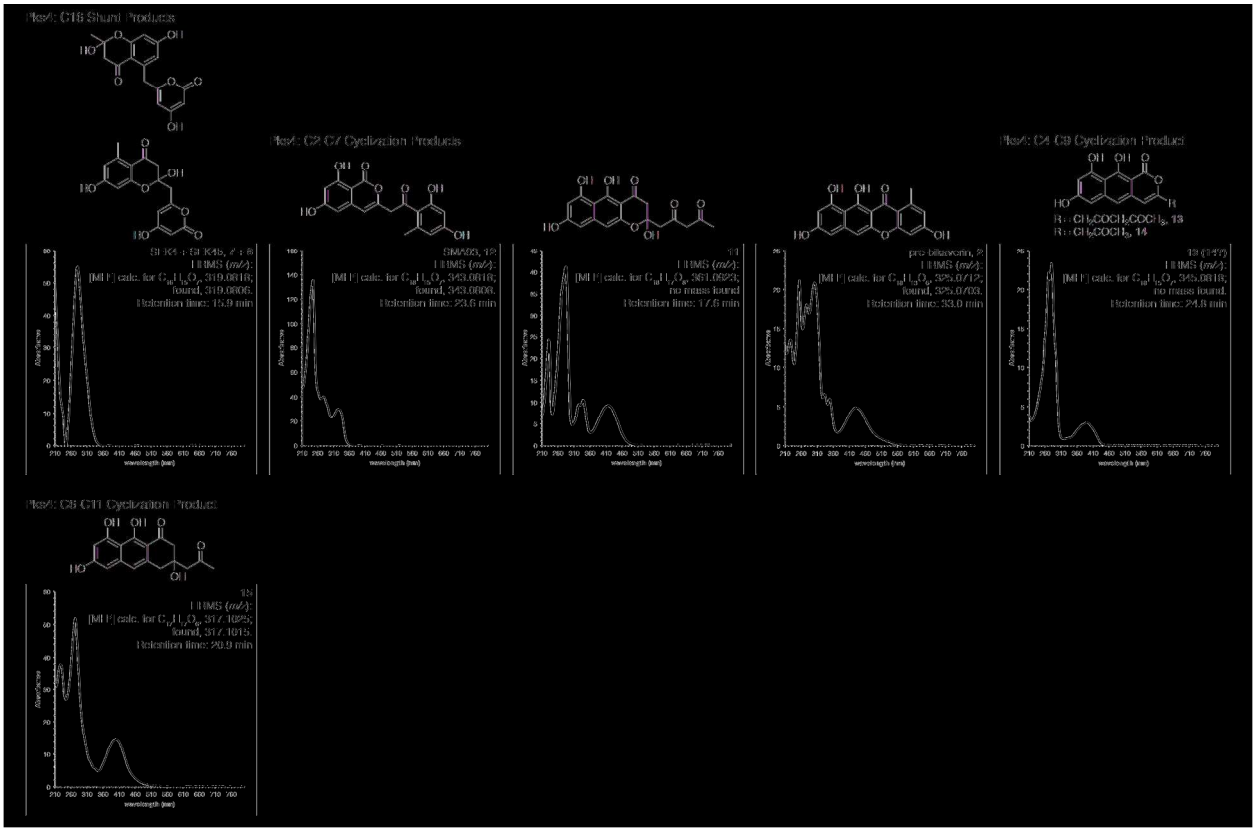


Figure S8. Proposed structures, HRMS data, UV-vis spectra, and retention times for products of combinatorial reactions with Pks4 SAT-KS-MAT. Previously characterized species include SEK4 (**7**),[7] SEK4b (**8**),[10] SMA93 (**12**),[3] pre-bikaverin (**2**).[3]

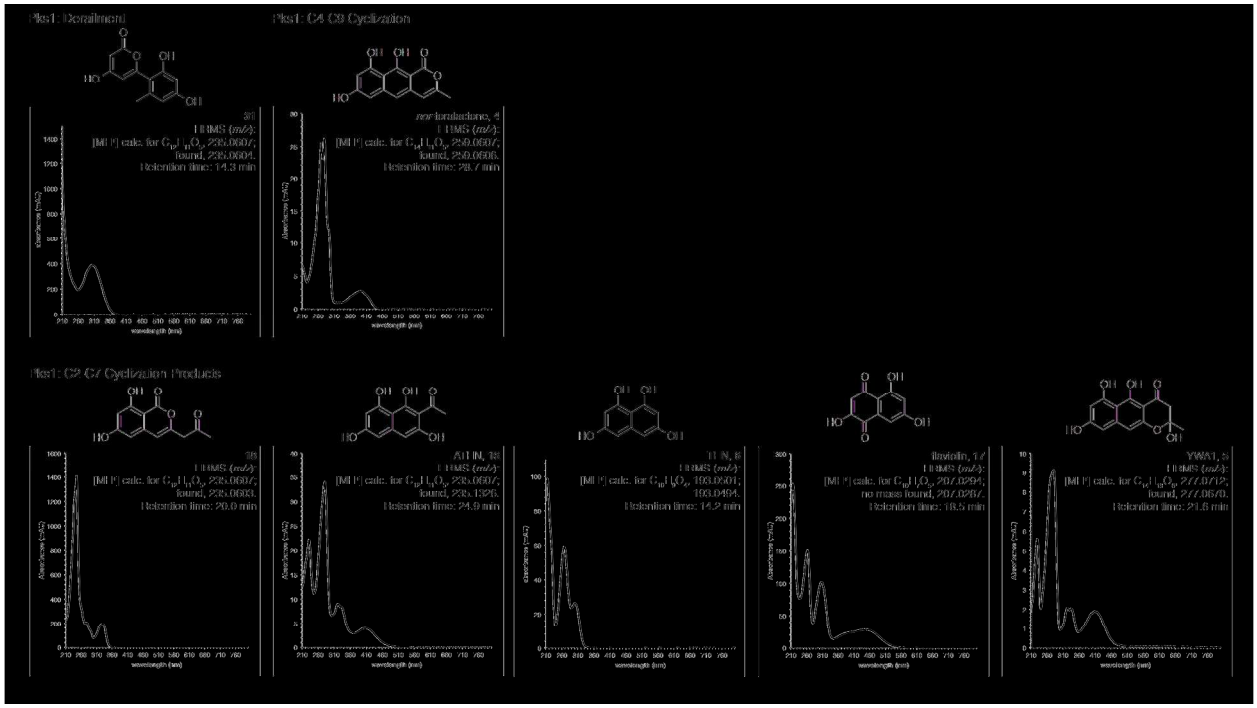


Figure S9. Proposed structures, HRMS data, UV-vis spectra, and retention times for products of combinatorial reactions with Pks1 SAT-KS-MAT. Previously characterized species include 6-(2,4-dihydroxy-6-methylphenyl)-4-hydroxy-2-pyrone (**31**), isocoumarin **18**,[6] ATHN (**16**),[6] THN (**6**), flaviolin (**17**),[6] YWA1 (**5**),[23] and *nor*-toralactone (**4**).[4]

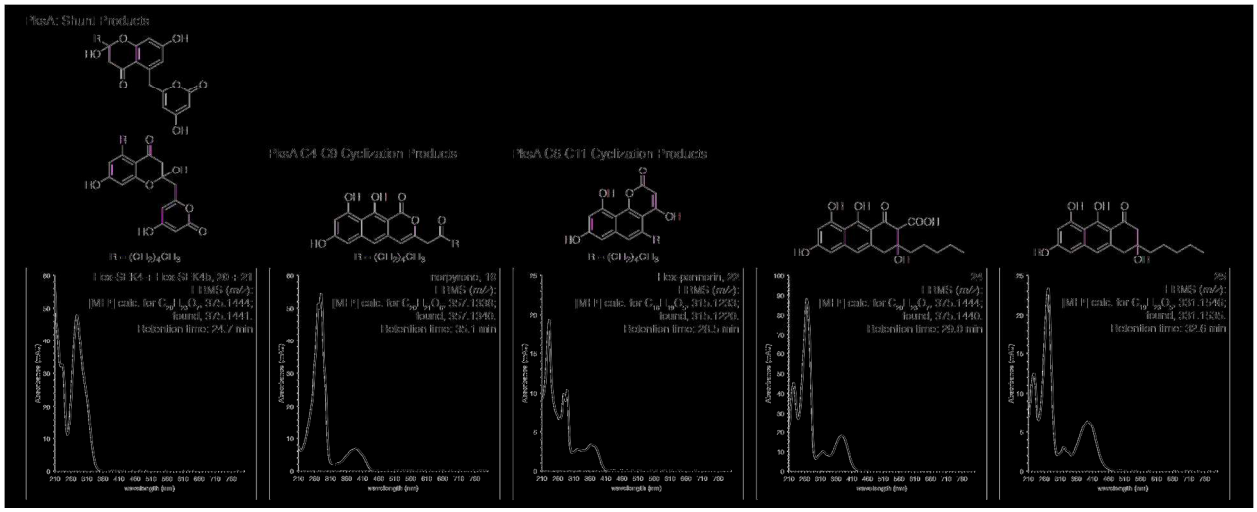


Figure S10. Proposed structures, HRMS data, UV-vis spectra, and retention times for products of combinatorial reactions with PksA SAT-KS-MAT. Previously characterized species include hex-SEK4 (**20**),[15] hex-SEK4b (**21**),[15] and norpyrone (**19**).[12]

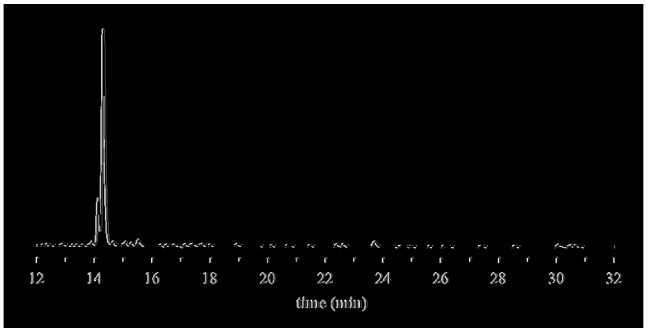


Figure S11. HPLC chromatogram (280 nm) of a synthetic standard of 6-(2,4-dihydroxy-6-methylphenyl)-4-hydroxy-2-pyrone (**31**).

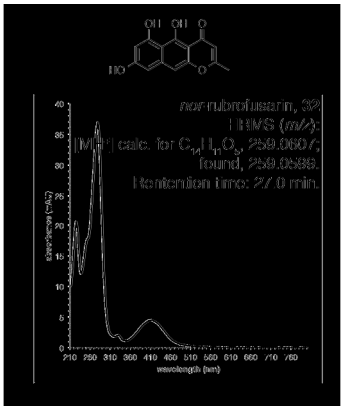


Figure S12. HRMS, UV-vis, and retention time data for *nor*-rubrofusarin (**32**). Data is in accord with literature values.[20]

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