BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Supporting Information

for

Bacterial terpene biosynthesis: challenges and opportunities for pathway engineering

Eric J. N. Helfrich, Geng-Min Lin, Christopher A. Voigt and Jon Clardy

Beilstein J. Org. Chem. **2019,** *15,* 2889–2906. doi:10.3762/bjoc.15.283

Additional figures and tables

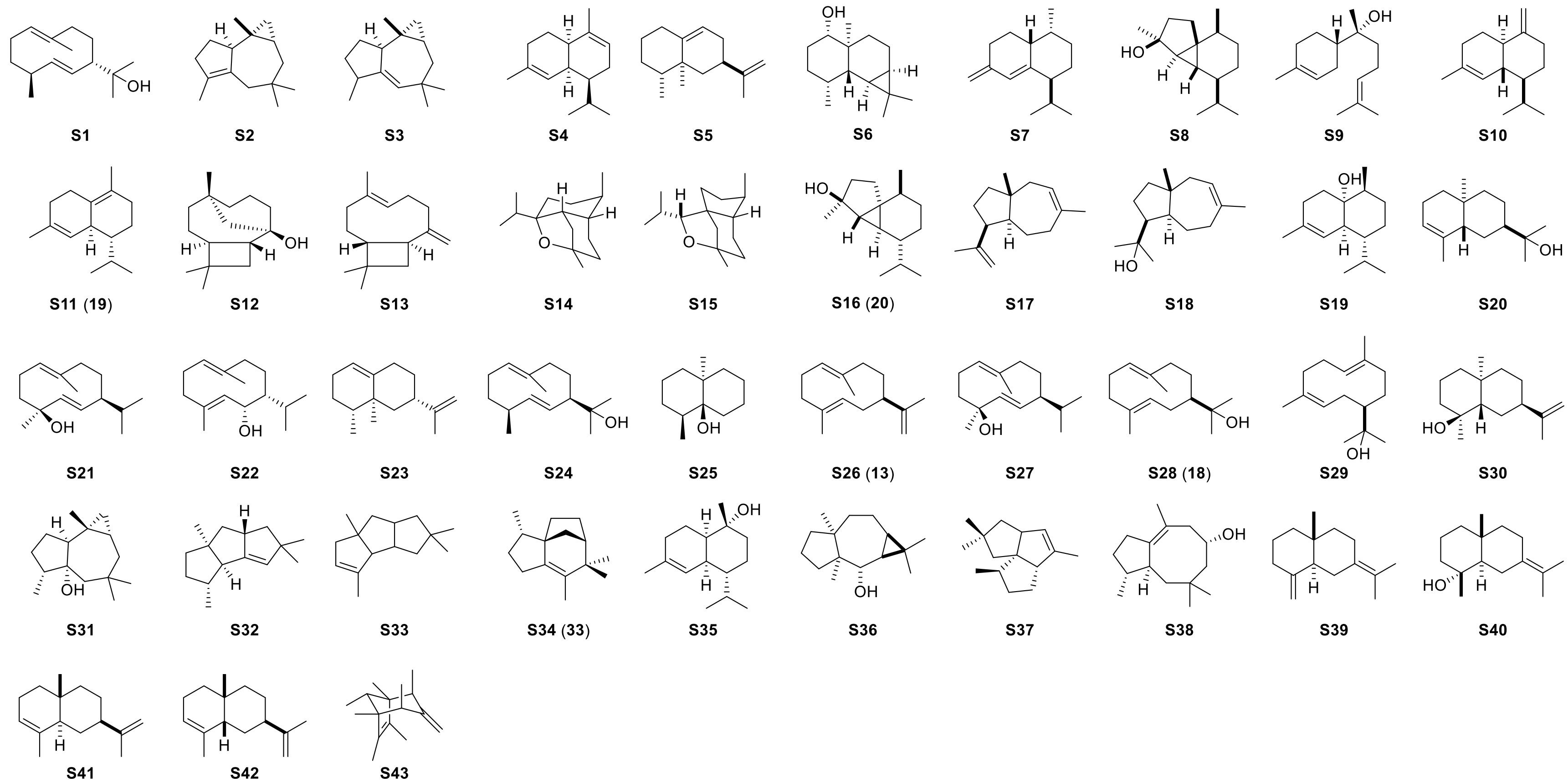
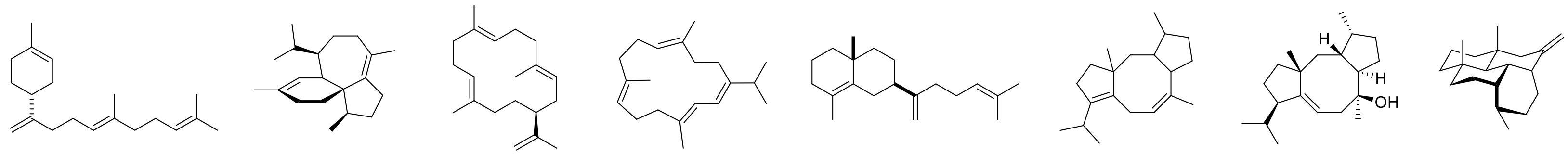
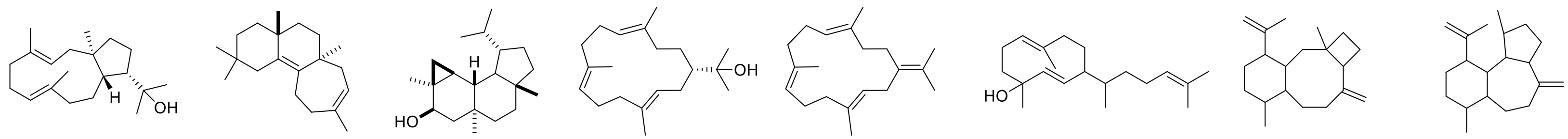


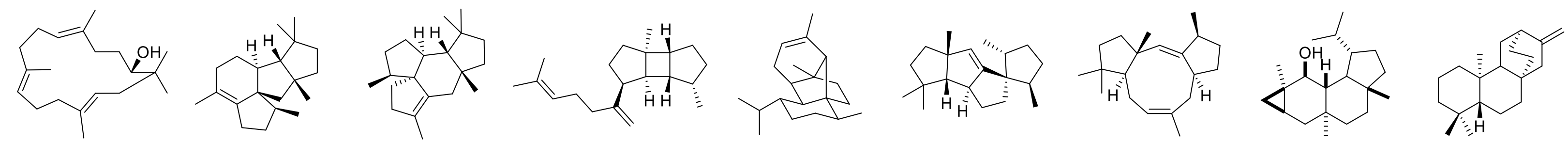
Figure S1: Characterized bacterial sesquiterpenes.



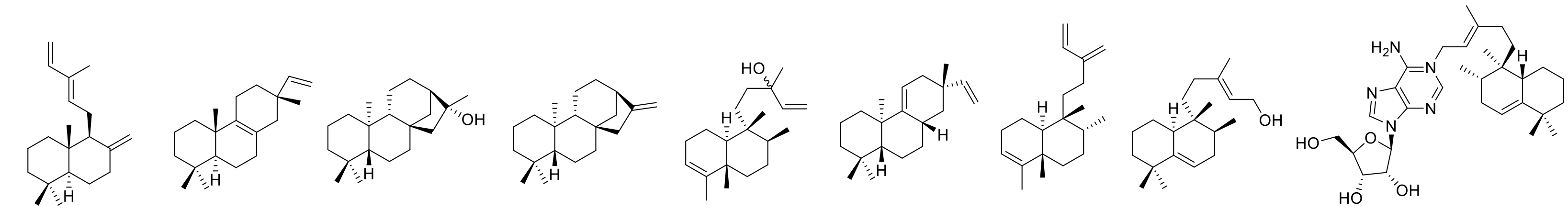
S44 S45 S46 S47 S48 S49 S50 (23) S51



S52 S53 S54 S55 S56 S57 S58 S59



S60 S61 S62 S63 (39) S64 S65 S66 S67 S68



S69 S70 S71 S72 (16) S73 S74 S75 S76 S77

Figure S2: Characterized bacterial diterpenes.

Table S1: Characterized bacterial terpene cyclases.

Terpene[a]	Uniprot #	Name	Producing Strain	Host[b]	Study[c]	Isoprenoid Flux	Ref
Sesquiterpene							
(+)-allohedycaryol (**S1**)	B2HGU3	mmar_3220	*Mycobacterium marinum*	SUKA22	B	*fps*	1
african-2-ene (**S2**)* / african-1-ene (**S3**)*	D5SKM9	sclav_p0985	*Streptomyces clavuligerus*	SUKA22	B	*fps*	1
(-)-α-amorphene (**S4**)	D9XDR8		*Streptomyces viridochromogenes*	BL21(DE3)	A/B	endogenous	2-4
aristolochene (**S5**)*	A0A117E6J5		*Streptomyces acidiscabies*	WT			5
avermitilol (**S6**)	Q82RR7	sav76	*Streptomyces avermitilis*	BL21(DE3)	A	N/A	5
				SUKA17	B	*fps*	5
bicyclosesquiphellandrene (**S7**)*	A9GK58	sce6369	*Sorangium cellulosum*	SUKA22	B	*fps*	1
10-*epi*-cubebol (**S8**)*[,e]				C43(DE3)	B	MEV pathway	6
(-)-*epi*-α-bisabolol (**S9**)	G5EKN0	SC2	*Streptomyces citricolor*	BL21(DE3)	A	N/A	7
				TK21	B	endogenous	7
(-)-γ-cadinene (**S10**)	C7PLV2		*Chitinophaga pinensis*	BL21(DE3)	A/B	endogenous	2, 3
(-)-δ-cadinene (**S11**, **19**)	B5GS26	sscg_02150/ sclav_p0328	*Streptomyces clavuligerus*	BL21(DE3)	A	N/A	8
				JM109(DE3)	B	MEV pathway	8
				SUKA22	B	*fps*	1
(+)-caryolan-1-ol (**S12**)	B1W019	gcoA	*Streptomyces griseus*	BL21(DE3)	A	N/A	9
				TK21	B	endogenous	9
				SUKA22	B	*fps*	1
(-)-(*E*)-β-caryophyllene (**S13**)	K0K750		*Saccharothrix espanaensis*	BL21(DE3)	A/B	endogenous	3, 10
corvol ether A (**S14**)* / corvol ether B (**S15**)*	E4N7E5		*Kitasatospora setae*	BL21(DE3)	A	N/A	4, 11, 12
epi-cubebol (**S16**, **20**)*	D2B747		*Streptosporangium roseum*	BL21(DE3)	A/B	endogenous	3, 4, 10, 12
(+)-dauca-8,11-diene (**S17**)* / (+)-isodauc-8-en-11-ol (**S18**)*	F2R8C1	sven_0552	*Streptomyces venezuelae*	SUKA22	B	*fps*	1
				BL21(DE3)	A	N/A	12, 13
(+)-epicubenol (**S19**)	B1W477	gecA	*Streptomyces griseus*	BL21(DE3)	A	N/A	14
				TK21	B	endogenous	14
				SUKA22	B	*fps*	1
(+)-eremophilene (**S20**)	A9FZ87	geoA/ sce8852	*Sorangium cellulosum*	BL21(DE3)	A	N/A	15

				C43(DE3)	B	endogenous	15
				SUKA22	B	*fps*	1
7-*epi*-α-eudesmol (**S21**)*	D9XD61		*Streptomyces viridochromogenes*	BL21(DE3)	A/B	endogenous	2, 3
(-)-germacradien-4-ol (**S22**)	G5EKM9	SC1	*Streptomyces citricolor*	BL21(DE3)	A	N/A	7
				TK21	B	endogenous	7
	A0A097ZQD8	slt18_1246	*Streptomyces lactacystinaeus*	SUKA22	B	*fps*	1
germacradien-6-ol (**S23**)	E8W6C7		*Streptomyces pratensis*	BL21(DE3)	A	N/A	4, 16
1(10),5-germacradien-11-ol (**S24**)/ geosmin (**S25**)	Q9X839	sco6073/ ScGS	*Streptomyces coelicolor*	pLysS	A	N/A	17-21
	Q82L49	sav2163/ geoA	*Streptomyces avermitilis*	BL21(DE3)	A	N/A	22
	B2IUI7	NP2	*Nostoc punctiforme*	JM109	B	endogenous	23
				pLysS	A	N/A	24
	B0FLN6	spterp13	*Streptomyces peucetius*	BL21(DE3)	A	N/A	25
1(10),5-germacradien-11-ol (**S24**)*[d]	A9F845	sce1440	*Sorangium cellulosum*	C43(DE3)	B	endogenous	6
germacrene A (**S26**, **13**)*	Q8YN85	NS1/ alr4685	*Nostoc sp.* PCC 7120	JM109	B	endogenous	23
				BL21(DE3)	A	N/A	23
				SUKA22	B	*fps*	1
	Q3MBN2	ava_1982	*Anabaena variabilis*	SUKA22	B	*fps*	1
(4*S*,7*S*)-germacrene D-4-ol (**S27**)	A0A127Q4J7		*Collimonas pratensis*	BL21(DE3)	A	N/A	4
hedycaryol (**S28**, **18**)*	A0A2N3Y098		*Saccharopolyspora spinosa*	BL21(DE3)	A/B		10
(2Z,6E)-hedycaryol (**S29**)*	E4MYY0	HcS	*Kitasatospora setae*	BL21(DE3)	B	endogenous	26
(+)-intermedeol (**S30**)	B5GTQ6	sclav_p0635	*Streptomyces clavuligerus*	SUKA22	B	*fps*	1
				BL21(DE3)	A	N/A	13
isoafricanol (**S31**)	G2P5T1		*Streptomyces violaceusniger*	WT			27
	A0A291SJC7		*Streptomyces malaysiensis*	BL21(DE3)	A	N/A	28
isohirsut-1-ene (cucumene, **S32**)	B5GLM7		*Streptomyces clavuligerus*	SUKA22	B	*fggps*	1, 29
				BL21(DE3)	A	N/A	30
isohirsut-4-ene (**S33**)*	A0A097ZQA4	slt18_1880	*Streptomyces lactacystinaeus*	SUKA22	B	*ggps*	1, 29
(+)-*epi*-isozizaene (**S34**, **33**)	Q9K499	sco5222	*Streptomyces coelicolor*	BL21(DE3)	A	N/A	31-34
	Q82IV1	sav3032	*Streptomyces avermitilis*	BL21(DE3)	A	N/A	35
(+)-T-muurolol (**S35**)	B5GW45	sscg_03688/ sclav_p0068	*Streptomyces clavuligerus*	BL21(DE3)	A	N/A	8
				JM109(DE3)	B	MEV pathway	8
				SUKA22	B	*fps*	1
	A7NH01		*Roseiflexus castenholzii*	BL21(DE3)	A/B	endogenous	2-4, 36

	A5UZ14	roseRS_3509	*Roseiflexus sp.* RS-1	SUKA22	B	*fps*	8
neomeranol B (**S36**)*	C9Z4F7		*Streptomyces scabiei*	BL21(DE3)	A	N/A	13
pentalenene (**S37**)	Q55012	PenA	*Streptomyces exfoliatus*	BL21(DE3)	A	N/A	37, 38
				XD	B	endogenous	39
	Q82IY4	sav2998/ PtlA	*S. avermitilis*	BL21(DE3)	A	N/A	40
pristinol (**S38**)	B5H7H3		*Streptomyces pristinaespiralis*	BL21(DE3)	A	N/A	41
selina-4(15),7(11)-diene (**S39**)*	L8ERG6		*Streptomyces rimosus*	WT			42
	B5HDJ6		*Streptomyces pristinaespiralis*	BL21(DE3)	A/B	endogenous	2
selina-7(11)-ene-4-ol (**S40**)*[,d]	A0A097ZQE4	sspSK_3051	*Streptomyces* sp. SK 1894	SUKA22	B	*fps*	1
α-selinene (**S41**)*	A9B3Q4	haur_2988	*Herpetosiphon aurantiacus*	SUKA22	B	*fps*	1
5-*epi*-α-selinene (**S42**)*	B2J4A4	NP1/ npun_R3832	*Nostoc punctiforme*	JM109	B	endogenous	23
				BL21(DE3)	A	N/A	23
				SUKA22	B	*fps*	1
sodorifen (**S43**)[e]	A0A318P116/ A0A318P5Q0	sod_c20750/ sod_c20760	*Serratia plymuthica* 4Rx13	BL21(DE3)	A/B	N/A	43, 44
Type I Diterpene							
axinyssene (**S44**)*[#]	A0A084SQV5	CysTC2	*Archangium violaceum*	BW25113	B	MEV pathway	45
bonnadiene (**S45**)	A0A1G9UQQ0	BdS	*Allokutzneria albata*	BL21(DE3)	A	N/A	46
(*S*)-cembrene A (**S46**)	A0A1G9S4L4	CAS	*Allokutzneria albata*	BL21(DE3)	A	N/A	47
cembrene C (**S47**)	Q1AYR1	rxyl_0493	*Rubrobacter xylanophilus*	SUKA22	B	*ggps*	1
clavulatriene A (**S48**)*[,d]	B5H135	sclav_p1169	*Streptomyces clavuligerus*	SUKA22	B	*ggps*	1, 29
cyclooctat-7(8),10(14)-diene (**S49**)*	A0A097ZQD0	slt18_1078	*Streptomyces lactacystinaeus*	SUKA22	B	*ggps*	1, 29
cyclooctat-9-en-7-ol (**S50**, **23**)	C9K1X5	CotB2	*Streptomyces melanosporofaciens*	BL21(DE3)	A	N/A	48, 49
				S. albus	B	*ggps*	48
				BL21(DE3)	A/B	*dxs/dxr/idi/ggps*	49-51
				Rosetta2	A	N/A	52
hydropyrene (**S51**)	D5SK09	sclav_p0765/ HpS	*Streptomyces clavuligerus*	SUKA22	B	*ggps*	1, 29
				BL21(DE3)	A	N/A	53
18-hydroxydolabella-3,7-diene (**S52**)	C7PA56	HdS	*Chitinophaga pinensis*	BL21(DE3)	A	N/A	54
				NB	B	endogenous	54
lydicene (**S53**)*[#]	A0A0D4DXU3	StlTC	*Streptomyces lydicus*	BW25113	B	MEV pathway	45
neoverrucosan-5β-ol (**S54**)*[#]	H6KZW8	SapTC1	*Saprospira grandis*	BW25113	B	MEV pathway	45

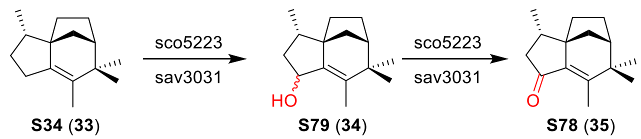
(*R*)-nephthenol (**S55**) / cembrene C isomer (**S56**)	M1V9Q0	DtcycA	*Streptomyces* sp. SANK 60404	BL21(DE3)	A	N/A	55
obscuronatin (**S57**)*	A9B3Q3	haur_2987	*Herpetosiphon aurantiacus*	SUKA22	B	*ggps*	1
odyverdiene A (**S58**)* / odyverdiene B (**S59**)*	A0A097ZQD1	nd90_0354	*Streptomyces sp.* ND90	SUKA22	B	*ggps*	1, 29
S60[d]	M1VDX3	DtcycB	*Streptomyces sp.* SANK 60404	BL21(DE3)	A	N/A	55
phomopsene (**S61**)/ allokutznerene (**S62**)	A0A1G9VRW7	PmS	*Allokutzneria albata*	BL21(DE3)	A	N/A	46
spata-13,17-diene (**S63**, **39**)	UPI000BAF81BA	SpS	*Streptomyces xinghaiensis*	BL21(DE3)	A	N/A	36, 56
spiroalbatene (**S64**)	A0A1H0BVV7	SaS	*Allokutzneria albata*	BL21(DE3)	A	N/A	36, 46
(-)-spiroviolene (**S65**)	P0DPK6	SvS	*Streptomyces violens*	BL21(DE3)	A	N/A	57
tsukubadiene (**S66**)	I2N045	stsu_20912	*Streptomyces tsukubensis*	SUKA22	B	*ggps*	1, 29
				BL21(DE3)	A	N/A	36, 57
verrucosane-2β-ol (**S67**)*[#]	A9WGY1	ChlTC2	*Chloroflexus aurantiacus*	BW25113	B	MEV pathway	45
	A0A178MLU0	ChlTC5	*Chloroflexus islandicus*	BW25113	B	MEV pathway	45
TypeII/I Diterpene[f]							
ent-atiserene (**S68**)	DBL2U6/ DBL2U4	PtmT2/ PtmT1	*Streptomyces platensis*	BL21(DE3)	A/B	*dxs/dxr/idi/ggps*	58, 59
biformene (**S69**)	A0A0H5BB10/ A0A0H5BN57	CldB/ CldD	*Streptomyces cyslabdanicus*	JM109(DE3)	A	NA	60, 61
				SUKA22	B	*ggps*	
isopimara-8,15-diene (**S70**)	A8M709/ A8M708	sare_1288/ sare_1287	*Salinispora arenicola*	C41(DE3)	B	*ggps*	62
(16*R*)-*ent*-kauran-16-ol (**S71**)	DBL2U6/ DBL2U9	PtmT2/ PtmT3	*Streptomyces platensis*	BL21(DE3)	A/B	*dxs/dxr/idi/ggps*	58, 59
ent-kaur-16-ene (**S72**, **16**)	Q45221/ Q45222	blr2149/ bl42150	*Bradyrhizobium japonicum*	C41(DE3)	B	endogenous	63
	Q989L7/ Q989L6		*Mesorhizobium japonicum*	C41(DE3)	B	*dxs/dxr/idi/ggps*	64
	P55538/ P55537		*Sinorhizobium fredii*	C41(DE3)	B	*dxs/dxr/idi/ggps*	64
	Q8KLD5/ Q8KLD4		*Rhizobium etli*	C41(DE3)	B	*dxs/dxr/idi/ggps*	64
(+)-kolavelool (**S73**)	A9AWD5/ A9AWD6	haur_2145/ haur_2146	*Herpetosiphon aurantiacus*	BL21(DE3)	A	N/A	65

ent-pimara-9(11),15-diene (**S74**)	Q5KSN5/ Q5KSN4		*Streptomyces sp.* KO-3988	M15/pREP4	A	N/A	66, 67
terpentetriene (**S75**)	Q9AJE4/ Q9AJE3		*Kitasatospora griseola*	M15/pREP4	A	N/A	68, 69
				TK23	B	endogenous	68
tuberculosinol (**S76**)/ 1-tuberculosinyladenosinec (**S77**)	O50406/ P9WJ61	Rv3377c/ Rv3378c	*Mycobacterium tuberculosis*	BL21(DE3)	A	N/A	70-73

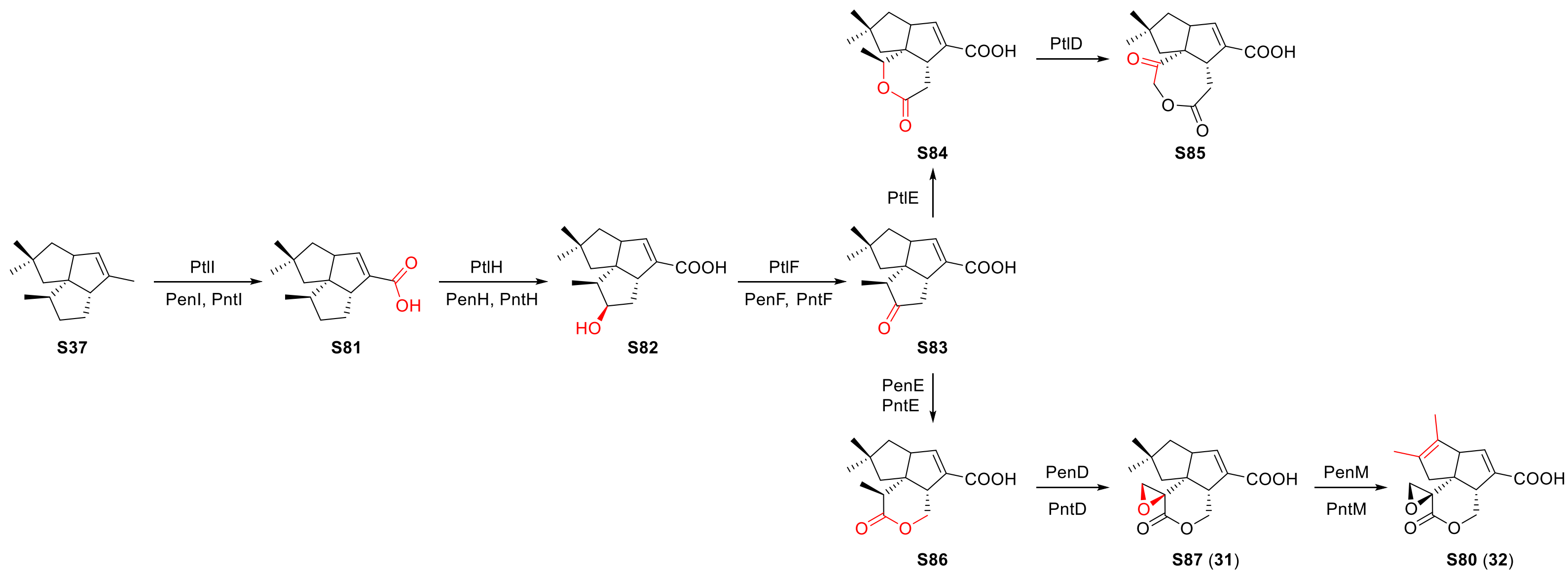
[a]terpenes labeled with asterisk have unclear absolute stereochemistry, and those labeled with pound signs are the product of UbiA-type terpene cyclases; [b]BL21(DE3), BW25113, C41(DE3), C43(DE3), JM109, JM109(DE3), M15/pREP4, pLysS [BL21(DE3)pLysS], and Rossetta2(DE3) are *Escherichia coli* strains. SUKA16, SUKA17, and SUKA22 are *Streptomyces avermitilis* strains. TK21 and TK23 are *Streptomyces lividans* strains. NB: *Nicotiana benthaniana*. XD: *Xanthophyllomyces dendrorhous*. WT: terpenes are detected in the headspace of wild-type strains, and the assignment of terpene cyclase was based on analysis of genomic sequence and exclusion of other common terpene cyclases. [c]A: *in vitro*, B: *in vivo* studies; [d]several products were produced; [e]requires two enzymes: methyltransferase / cyclase; [f]accession numbers and protein names in the order of type II diterpene synthase / type I diterpene synthase.

Table S2: Functional characterization of bacterial tailoring enzymes for terpene modification.

Uniprot #	Name	Class[a]	Strain	Host[b]	Study[c]	Substrate[d]	Reaction	Ref
Albaflavenone (S78, 35)								
Q9K498	CYP107A1 sco5223	CYP	*Streptomyces coelicolor*	BL21(DE3)	A	*epi*-isozizaene (**S34, 33**)	hydroxylation-oxidation	74, 75
Q81IV2	CYP107A2 sav3031	CYP	*Streptomyces avermitilis*	SUKA16	B	*epi*-isozizaene (**S34, 33**)	hydroxylation-oxidation[e]	35

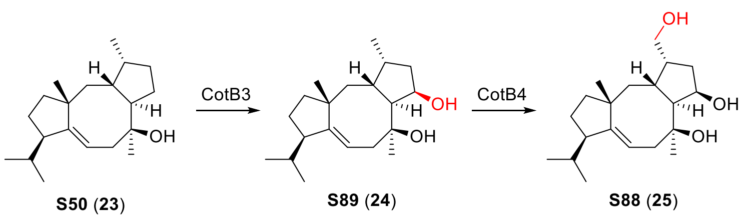


Uniprot #	Name	Class[a]	Strain	Host[b]	Study[c]	Substrate[d]	Reaction	Ref
Pentalenolactone (**S80**, **32**)								
Q82IY3/ E3VWK7/ E3VWJ1	PtlI/ PenI/ PntI[f]	CYP	*Streptomyces avermitilis/ Streptomyces exfoliatus/ Streptomyces arenae*	BL21(DE3)	A	pentalenene (**S37**)	4e-oxidation[g]	76, 77
Q82IZ1/ E3VWK0/ E3VWI4	PtlH/ PenH/ PntH[f]	AKG	as above	BL21(DE3)	A	1-deoxypentaleniate (**S81**)	hydroxylation	77-79
Q82IY9 E3VWK2/ E3VWI6	PtlF/ PenF/ PntF[f]	DH	as above	BL21(DE3)	A	**S82**	2e-oxidation	77, 80
Q82IY8	PtlE	FMO	*Streptomyces avermitilis*	BL21(DE3)	A	**S83**	Baeye-Villiger oxidation	81
Q82IY7	PtlD	AKG	*Streptomyces avermitilis*	BL21(DE3)	A	neopentalenolactone D (**S84**)	dehydrogenation	77
E3VWK3/ E3VWI7	PenE/ PntE	FMO	*Streptomyces exfoliatus/ Streptomyces arenae*	BL21(DE3)	A	**S83**	Baeye-Villiger oxidation	77
E3VWK4/ E3VWI8	PenD/ PntD	AKG	as above	BL21(DE3)	A	pentalenolactone D (**S86**)	dehydrogenation-epoxidation	77
E3VWJ9/ E3VWI3	PenM/ PntM	CYP	as above	BL21(DE3)	A	pentalenolactone F (**S87**, **31**)	rearrangement	82



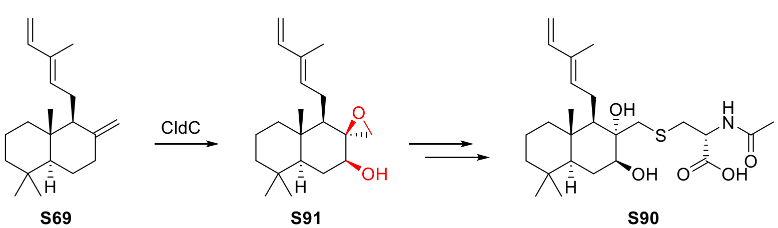
Cyclooctatin (S88, 25)

C9K1X6	CotB3	CYP	*Streptomyces melanosporofaciens*	*S. albus*	B	cyclooctat-9-en-7-ol (**S50**, **23**)	hydroxylation	48
C9K1X7	CotB4	CYP	*Streptomyces melanosporofaciens*	*S. albus*	B	**S89** (**24**)	hydroxylation	48



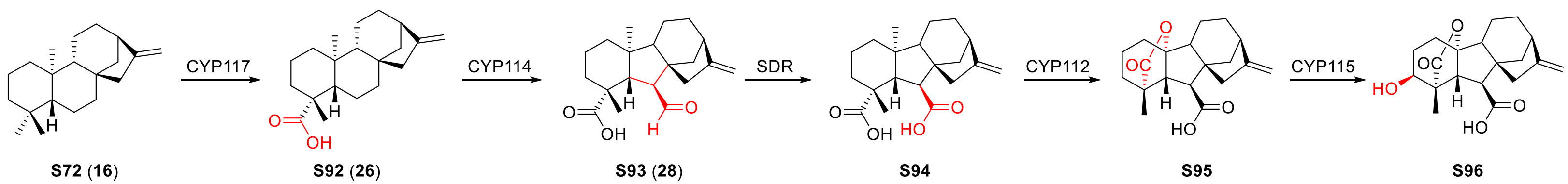
Cyslabdan (S90)

A0A0H5B5M5	CldC	CYP	*Streptomyces cyslabdanicus*	SUKA22	C	biformene (**S69**)	hydroxylation-epoxidation	60



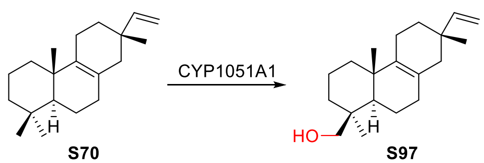
Gibberellinsh

P55540/ Q59205	CYP117A2	CYP	*Sinorhizobium fredii/ Bradyrhizobium diazoefficiens*	SM	C	*ent*-kaurene (**S72**, **16**)	6e-oxidation	83
P55543/ Q59204	CYP114	CYP	as above	SM	C	*ent*-kaurenoic acid (**S92**, **26**)	hydroxylation/ ring contraction	83
P55541/ Q45219	SDR	DH	as above	SM	C	**S93** (**28**)	2e-oxidation	83
P55544/ Q59203	CYP112A2	CYP	as above	SM	C	gibberellin A12 (**S94**)	demethylation-lactonization	83
UPI00040E8C18/ Q98A71	SMCYP115/ MlCYP115	CYP	*Sinorhizobium meliloti / Mesorhizobium japonicum*	SM	C	gibberellin A9 (**S95**)	hydroxylation	84



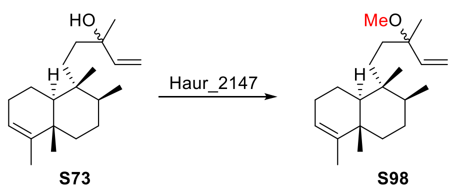
Isopimara-8,15-dien-19-ol (**S97**)

A8M707	CYP1051A1 sare_1286	CYP	*Salinispora arenicola*	C43(DE3)	B	isopimara-8,15-diene (**S70**)	hydroxylation	62



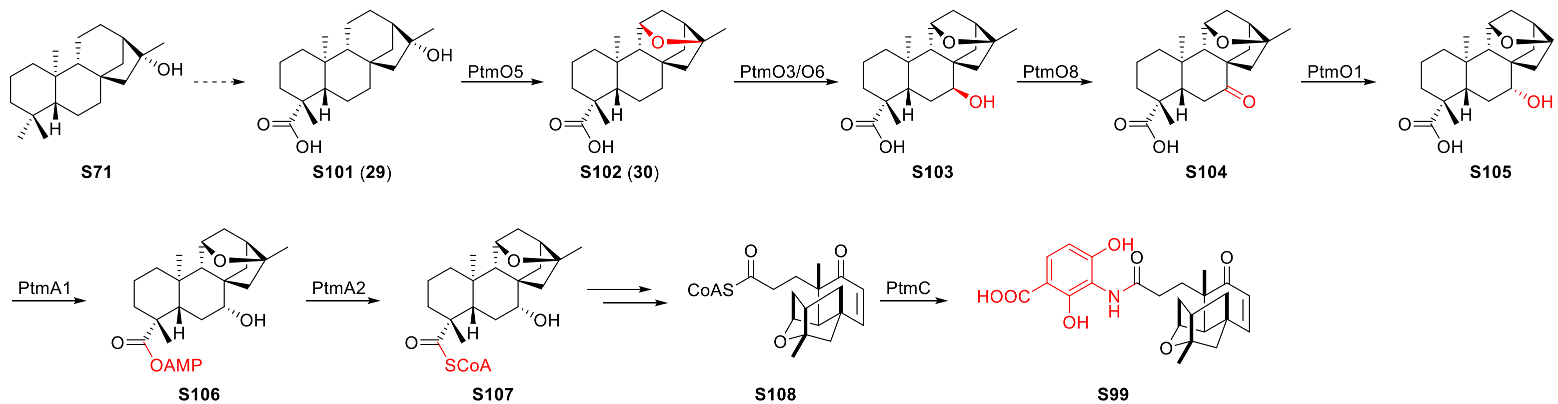
(+)-*O*-methylkolavelool (**S98**)

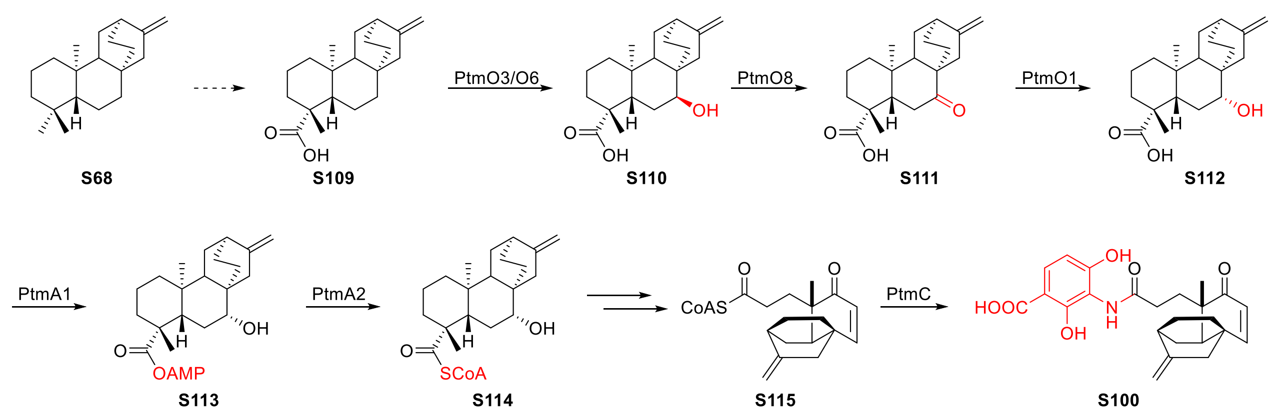
A9AWD7	Haur_2147	MT	*Herpetosiphon aurantiacus*	BL21(DE3)	A	(+)-kolavelool (**S73**)	methylation	65



Platensimycin (**S99**) / **Platencin** (**S100**)

DBL2V0	PtmO5	CYP	*Streptomyces platensis*	BL21(DE3)	A	**S101** (**29**)	hydroxylation/ ether formation[i]	59, 85
DBL2U7	PtmO3	AKG	as above	BL21(DE3)	A/B	**S102** (**30**), **S109**	hydroxylation	86
DBL2V2	PtmO6	AKG	as above	BL21(DE3)	A/B	**S103**, **S110**	hydroxylation	86
DBL2W5	PtmO8	DH	as above	BL21(DE3)	A/B	**S104**, **S111**	oxidation	86
A0A0A0UXJ2	PtmO1	DH	as above	BL21(DE3)	A/B	**S105**, **S112**	reduction	86
DBL2T7	PtmA1	CoAS	as above	mutant	B	**S106**, **S113**	adenylation	87
DBL2U1	PtmA2	CoAS	as above	BL21(DE3)	A/B	**S107**, **S114**	ligation	87
DBL2W0	PtmC	NAT	as above	mutant	B	**S108**, **S115**	ligation	88

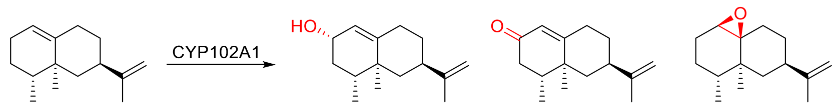




Others

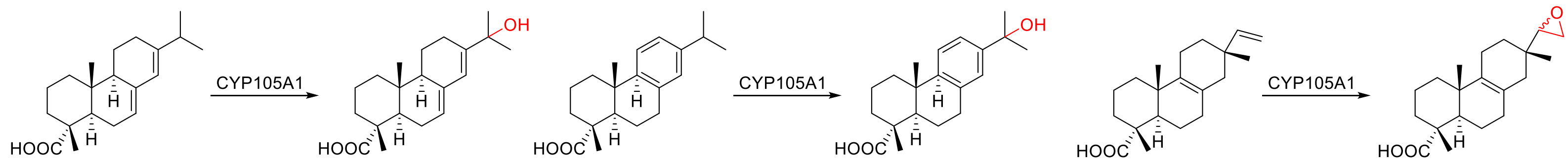
P14779	P450$_{BM3}$	CYP	*Bacillus megaterium*	DH5α	A/C	*valencene (**S116**)	oxidation	89

	CYP102A1							



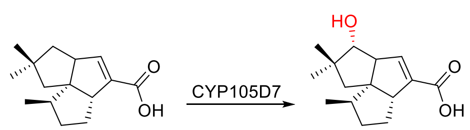
S116 S117 S118 S119

P18326	CYP105A1	CYP	*Streptomyces griseolus*	JM109	A/C	*abietic acid (**S120**)	hydroxylation	90, 91
				BM	C			90
				JM109	A/C	*dehydroabietic acid (**S121**)	hydroxylation	90
				BM	C			90
				JM109	A/C	*isopimaric acid (**S122**)	epoxidation	90
				BM	C			90



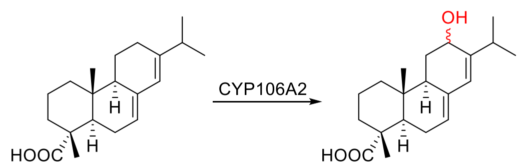
S120 S123 S121 S124 S122 S125

Q825I8	CYP105D7 sav7469	CYP	*S. avermitilis*	SUKA13	B	*1-deoxypentalenate (**S81**)	hydroxylation	92
				BL21(DE3)	A			92



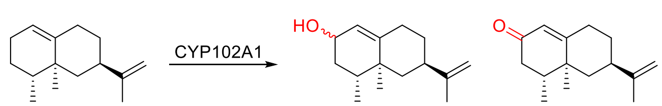
S81 S126

Q06069	CYP106A2	CYP	*Bacillus megaterium*	BL21(DE3)	A	*abietic acid (**S120**)	hydroxylation	93
				BM	C			



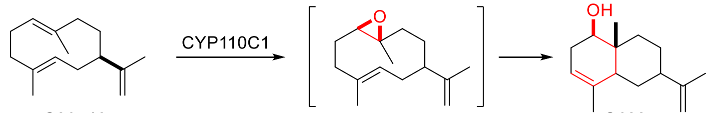
S120 S127

U5U1Z3	CYP109B1	CYP	*Bacillus subtilis*	BL21(DE3)	A/C	*valencene (**S116**)	hydroxylation/ oxidation	94



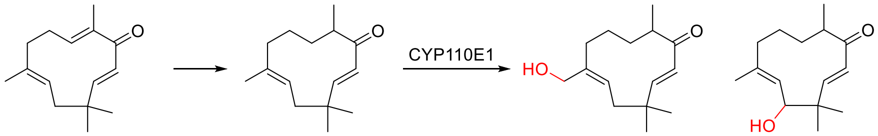
S116 *rac*-S117 S118

Q8YN84	CYP110C1	CYP	*Nostoc punctiforme*	JM109 / BL21(DE3)	B	germacrene A (**S26**, **13**)	epoxidation-cyclization	23



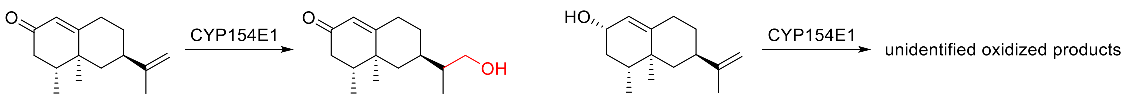
S26 (**13**) **S128**

Q8YMU4	CYP110E1	CYP	*Nostoc sp.* PCC 7120	BL21(DE3)	C	*2,3-dihydrozerumbone (**S130**)[j]	hydroxylation	15



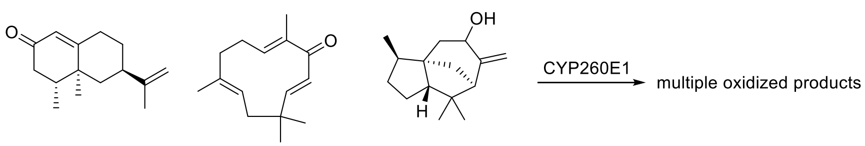
S129 **S130** **S131** **S132**

Q47KL3	CYP154E1	CYP	*Thermobifida fusca*	BL21(DE3)	A	*(+)-nootkatone (**S118**)	hydroxylation	95
						*(+)-nootkatol (**S117**)	oxidation	95



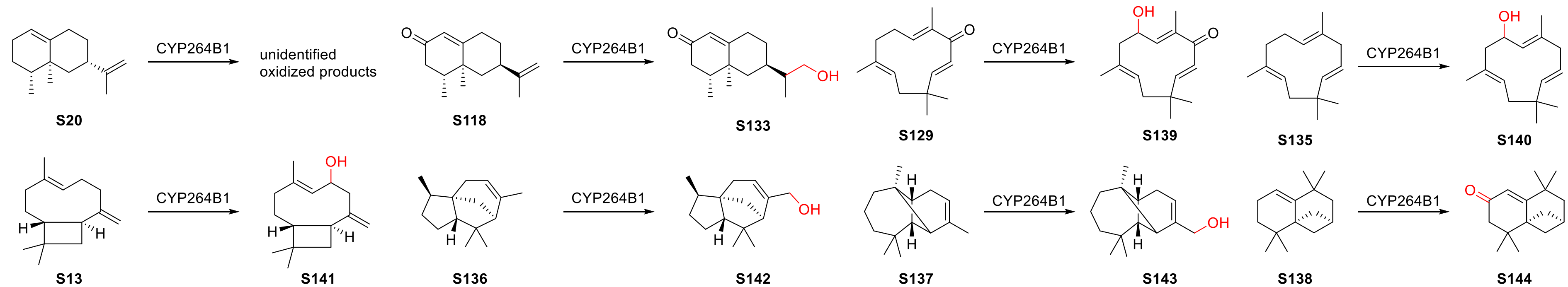
S118 **S133** **S117**

A9FDB7	CYP260A1 sce1588	CYP	*Sorangium cellulosum* So ce56	BL21(DE3)	A/C	*(+)-nootkatone (**S118**)	oxidation	96, 97
					C	*zerumbone (**S129**)	oxidation	97
					C	*(+)-3(15)-cedren-4-ol (**S134**)	oxidation	97

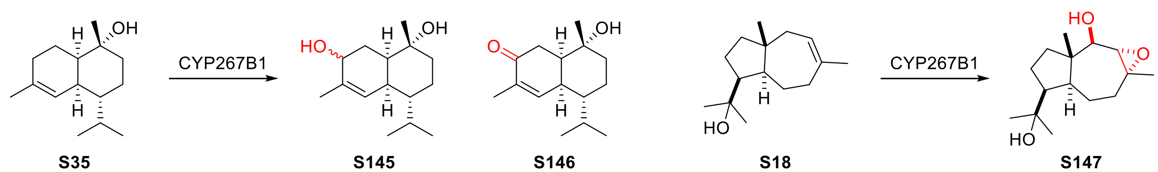


S118 **S129** **S134**

A9FZ85	CYP264B1 sce8851	CYP	*Sorangium cellulosum* So ce56	C43(DE3)	A/B/C	(+)-eremophilene (**S20**)	hydroxylation	15, 97
				C43(DE3) / BL21(DE3)	C	*(+)-nootkatone (**S118**)	hydroxylation	97
					C	*zerumbone (**S129**)	hydroxylation	97
					A/C	*α-humulene (**S135**)	hydroxylation	97, 98
					C	*β-caryophyllene (**S13**)	hydroxylation	97
					C	*α-cedrene (**S136**)	hydroxylation	97
					A/C	*(+)-α-longipinene (**S137**)	hydroxylation	98
					A/C	*(-)-isolongifolene (**S138**)	4e-oxidation	98



A9ERX9	CYP267B1	CYP	*Sorangium cellulosum* So ce56	C43(DE3)	A	*(+)-T-muurolol (**S35**)	hydroxylation-oxidation	99
					A	*(+)-isodauc-8-en-11-ol (**S18**)	epoxidation-hydroxylation	99



[a]AKG: α-ketoglutarate-dependent non-heme iron enzyme, CoAS: acyl-CoA synthetase, CYP: cytochrome P450, DH: NAD(P)$^+$-dependent dehydrogenase, FMO: Flavin-dependent monooxygenase, MT: methyltransferase, NAT: *N*-acetyl transferase; [b]BL21(DE3), C43(DE3), DH5α, JM109: *E. coli* strains. SUKA13, SUKA16, SUKA22: *S. avermitilis* strains. BM: *Bacillus megaterium* MS941. SM: *Sinorhizobium meliloti* 1021. [c]A: in vitro, B: in vivo: the heterologous host expresses both cyclase and tailoring enzymes, or gene deletion / complementation experiments in the producing strains, C: growing cells or resting cells expressing tailoring enzymes are fed with substrate directly; [d]substrates marked with asterisk are not produced by any enzyme(s) encoded nearby the tailoring enzymes; [e]epoxidation took place when expressed in *S. avermitilis*, which might come from the action of endogenous P450 of the heterologous host. Also see Figure 8 in the main text; [f]only Ptl enzymes were experimentally verifies. Pen and Pnt enzymes' function were implied by sequence homology. [g]6e-oxidaion was expected, but *in vitro* reaction only catalyzed 4e-oxidation; [h]*Sinorhizobium fredii* enzymes were studied by feeding substrates to *Sinorhizobium meliloti* 1021 expressing enzymes of interest. Substrates were also fed to *S. fredii / Bradyrhizobium diazoefficiens* deletion strains to map out the biosynthetic pathway; [i]in the in vitro studies, HCl was required to trigger the ether ring formation; [j]it was claimed to come from the reduction of zerumbone (the fed substrate, **S128**) by endogenous enzymes of *E. coli*.

References

1. Yamada, Y.; Kuzuyama, T.; Komatsu, M.; Shin-ya, K.; Omura, S.; Cane, D. E.; Ikeda, H., Terpene synthases are widely distributed in bacteria. *Proceedings of the National Academy of Sciences* **2015,** *112*, 857-862.

2. Rabe, P.; Dickschat, J. S., Rapid chemical characterization of bacterial terpene synthases. *Angewandte Chemie - International Edition* **2013,** *52*, 1810-1812.

3. Rabe, P.; Schmitz, T.; Dickschat, J. S., Mechanistic investigations on six bacterial terpene cyclases. *Beilstein Journal of Organic Chemistry* **2016,** *12*, 1839-1850.

4. Rinkel, J.; Rabe, P.; Garbeva, P.; Dickschat, J. S., Lessons from 1,3-Hydride Shifts in Sesquiterpene Cyclizations. *Angewandte Chemie International Edition* **2016,** *55*, 13593-13596.

5. Chou, W. K. W.; Fanizza, I.; Uchiyama, T.; Komatsu, M.; Ikeda, H.; Cane, D. E., Genome Mining in Streptomyces avermitilis : Cloning and Characterization of SAV_76, the Synthase for a New Sesquiterpene, Avermitilol. *Journal of the American Chemical Society* **2010,** *132*, 8850-8851.

6. Schifrin, A.; Khatri, Y.; Kirsch, P.; Thiel, V.; Schulz, S.; Bernhardt, R., A single terpene synthase is responsible for a wide variety of sesquiterpenes in Sorangium cellulosum Soce56. *Organic & Biomolecular Chemistry* **2016,** *14*, 3385-3393.

7. Nakano, C.; Kudo, F.; Eguchi, T.; Ohnishi, Y., Genome Mining Reveals Two Novel Bacterial Sesquiterpene Cyclases: (−)-Germacradien-4-ol and (−)-epi-α-Bisabolol Synthases from Streptomyces citricolor. *ChemBioChem* **2011,** *12*, 2271-2275.

8. Hu, Y.; Chou, W. K. W.; Hopson, R.; Cane, D. E., Genome Mining in Streptomyces clavuligerus: Expression and Biochemical Characterization of Two New Cryptic Sesquiterpene Synthases. *Chemistry & Biology* **2011,** *18*, 32-37.

9. Nakano, C.; Horinouchi, S.; Ohnishi, Y., Characterization of a Novel Sesquiterpene Cyclase Involved in (+)-Caryolan-1-ol Biosynthesis in Streptomyces griseus. *Journal of Biological Chemistry* **2011,** *286*, 27980-27987.

10. Dickschat, J. S.; Pahirulzaman, K. A. K.; Rabe, P.; Klapschinski, T. A., An Improved technique for the rapid chemical characterisation of bacterial terpene cyclases. *ChemBioChem* **2014,** *15*, 810-814.

11. Rabe, P.; Pahirulzaman, K. A. K.; Dickschat, J. S., Structures and biosynthesis of corvol ethers - Sesquiterpenes from the actinomycete kitasatospora setae. *Angewandte Chemie - International Edition* **2015,** *54*, 6041-6045.

12. Rabe, P.; Dickschat, J. S., The EIMS fragmentation mechanisms of the sesquiterpenes corvol ethers A and B, epi -cubebol and isodauc-8-en-11-ol. *Beilstein Journal of Organic Chemistry* **2016,** *12*, 1380-1394.

13. Rabe, P.; Rinkel, J.; Klapschinski, T. A.; Barra, L.; Dickschat, J. S., A method for investigating the stereochemical course of terpene cyclisations. *Organic & Biomolecular Chemistry* **2016,** *14*, 158-164.

14. Nakano, C.; Tezuka, T.; Horinouchi, S.; Ohnishi, Y., Identification of the SGR6065 gene product as a sesquiterpene cyclase involved in (+)-epicubenol biosynthesis in Streptomyces griseus. *The Journal of Antibiotics* **2012,** *65*, 551-558.

15. Schifrin, A.; Ly, T. T. B.; Günnewich, N.; Zapp, J.; Thiel, V.; Schulz, S.; Hannemann, F.; Khatri, Y.; Bernhardt, R., Characterization of the Gene Cluster CYP264B1- geo A from Sorangium cellulosum So ce56: Biosynthesis of (+)-Eremophilene and Its Hydroxylation. *ChemBioChem* **2015,** *16*, 337-344.

16. Rabe, P.; Barra, L.; Rinkel, J.; Riclea, R.; Citron, C. A.; Klapschinski, T. A.; Janusko, A.; Dickschat, J. S., Conformational Analysis, Thermal Rearrangement, and EI-MS Fragmentation Mechanism of (1(10)E,4E,6S,7R)-Germacradien-6-ol by13C-Labeling Experiments. *Angewandte Chemie - International Edition* **2015,** *54*, 13448-13451.

17. Jiang, J.; He, X.; Cane, D. E., Geosmin Biosynthesis. Streptomyces coelicolor Germacradienol/Germacrene D Synthase Converts Farnesyl Diphosphate to Geosmin. *Journal of the American Chemical Society* **2006,** *128*, 8128-8129.

18. Jiang, J.; Cane, D. E., Geosmin Biosynthesis. Mechanism of the Fragmentation−Rearrangement in the Conversion of Germacradienol to Geosmin. *Journal of the American Chemical Society* **2008,** *130*, 428-429.

19. Cane, D. E.; Watt, R. M., Expression and mechanistic analysis of a germacradienol synthase from Streptomyces coelicolor implicated in geosmin biosynthesis. *Proceedings of the National Academy of Sciences* **2003,** *100*, 1547-1551.

20. Jiang, J.; He, X.; Cane, D. E., Biosynthesis of the earthy odorant geosmin by a bifunctional Streptomyces coelicolor enzyme. *Nature Chemical Biology* **2007,** *3*, 711-715.

21. Harris, G. G.; Lombardi, P. M.; Pemberton, T. A.; Matsui, T.; Weiss, T. M.; Cole, K. E.; Köksal, M.; Murphy, F. V.; Vedula, L. S.; Chou, W. K. W.; Cane, D. E.; Christianson, D. W., Structural Studies of Geosmin Synthase, a Bifunctional Sesquiterpene Synthase with αα Domain Architecture That Catalyzes a Unique Cyclization–Fragmentation Reaction Sequence. *Biochemistry* **2015,** *54*, 7142-7155.

22. Cane, D. E.; He, X.; Kobayashi, S.; Ōmura, S.; Ikeda, H., Geosmin Biosynthesis in Streptomyces avermitilis. Molecular Cloning, Expression and Mechanistic Study of the Germacradienol/Geosmin Synthase. *The Journal of Antibiotics* **2006,** *59*, 471-479.

23. Agger, S. A.; Lopez-Gallego, F.; Hoye, T. R.; Schmidt-Dannert, C., Identification of sesquiterpene synthases from Nostoc punctiforme PCC 73102 and Nostoc sp. strain PCC 7120. *Journal of Bacteriology* **2008,** *190*, 6084-6096.

24. Jiang, J.; Saint, C. P.; Cane, D. E.; Monis, P. T., Isolation and Characterization of the Gene Associated with Geosmin Production in Cyanobacteria. *Environmental Science & Technology* **2008,** *42*, 8027-8032.

25. Ghimire, G. P.; Oh, T. J.; Lee, H. C.; Kim, B. G.; Sohng, J. K., Cloning and functional characterization of the germacradienol synthase (spterp13) from Streptomyces peucetius ATCC 27952. *Journal of Microbiology and Biotechnology* **2008,** *18*, 1216-1220.

26. Baer, P.; Rabe, P.; Citron, C. A.; De Oliveira Mann, C. C.; Kaufmann, N.; Groll, M.; Dickschat, J. S., Hedycaryol synthase in complex with nerolidol reveals terpene cyclase mechanism. *ChemBioChem* **2014,** *15*, 213-216.

27. Riclea, R.; Citron, C. A.; Rinkel, J.; Dickschat, J. S., Identification of isoafricanol and its terpene cyclase in Streptomyces violaceusniger using CLSA-NMR. *Chemical Communications* **2014,** *50*, 4228-4230.

28. Rabe, P.; Samborskyy, M.; Leadlay, P. F.; Dickschat, J. S., Isoafricanol synthase from Streptomyces malaysiensis. *Organic & Biomolecular Chemistry* **2017,** *15*, 2353-2358.

29. Yamada, Y.; Arima, S.; Nagamitsu, T.; Johmoto, K.; Uekusa, H.; Eguchi, T.; Shin-Ya, K.; Cane, D. E.; Ikeda, H., Novel terpenes generated by heterologous expression of bacterial terpene synthase genes in an engineered Streptomyces host. *Journal of Antibiotics* **2015,** *68*, 385-394.

30. Chow, J.-Y.; Tian, B.-X.; Ramamoorthy, G.; Hillerich, B. S.; Seidel, R. D.; Almo, S. C.; Jacobson, M. P.; Poulter, C. D., Computational-guided discovery and characterization of a sesquiterpene synthase from Streptomyces clavuligerus. *Proceedings of the National Academy of Sciences* **2015,** *112*, 5661-5666.

31. Lin, X.; Hopson, R.; Cane, D. E., Genome mining in Streptomyces coelicolor: Molecular cloning and characterization of a new sesquiterpene synthase. *Journal of the American Chemical Society* **2006,** *128*, 6022-6023.

32. Lin, X.; Cane, D. E., Biosynthesis of the Sesquiterpene Antibiotic Albaflavenone in Streptomyces coelicolor . Mechanism and Stereochemistry of the Enzymatic Formation of Epi-isozizaene. *Journal of the American Chemical Society* **2009,** *131*, 6332-6333.

33. Aaron, J. A.; Lin, X.; Cane, D. E.; Christianson, D. W., Structure of Epi-Isozizaene Synthase from Streptomyces coelicolor A3(2), a Platform for New Terpenoid Cyclization Templates. *Biochemistry* **2010,** *49*, 1787-1797.

34. Li, R.; Chou, W. K. W.; Himmelberger, J. A.; Litwin, K. M.; Harris, G. G.; Cane, D. E.; Christianson, D. W., Reprogramming the Chemodiversity of Terpenoid Cyclization by Remolding the Active Site Contour of epi -Isozizaene Synthase. *Biochemistry* **2014,** *53*, 1155-1168.

35. Takamatsu, S.; Lin, X.; Nara, A.; Komatsu, M.; Cane, D. E.; Ikeda, H., Characterization of a silent sesquiterpenoid biosynthetic pathway in Streptomyces avermitilis controlling epi-isozizaene albaflavenone biosynthesis and isolation of a new oxidized epi-isozizaene metabolite. *Microbial Biotechnology* **2011,** *4*, 184-191.

36. Rinkel, J.; Rabe, P.; Dickschat, J. S., The EI-MS Fragmentation Mechanisms of Bacterial Sesquiterpenes and Diterpenes. *European Journal of Organic Chemistry* **2019,** *2019*, 351-359.

37. Cane, D. E.; Sohng, J. K.; Lamberson, C. R.; Rudnicki, S. M.; Wu, Z.; Lloyd, M. D.; Oliver, J. S.; Hubbard, B. R., Pentalenene Synthase. Purification, Molecular Cloning, Sequencing, and High–Level Expression in Escherichia coli of a Terpenoid Cyclase from Streptomyces UC5319. *Biochemistry* **1994,** *33*, 5846-5857.

38. Lesburg, C. A., Crystal Structure of Pentalenene Synthase: Mechanistic Insights on Terpenoid Cyclization Reactions in Biology. *Science* **1997,** *277*, 1820-1824.

39. Melillo, E.; Muntendam, R.; Quax, W. J.; Kayser, O., Heterologous expression of pentalenene synthase (PSS) from Streptomyces UC5319 in Xanthophyllomyces dendrorhous. *Journal of Biotechnology* **2012,** *161*, 302-307.

40. Tetzlaff, C. N.; You, Z.; Cane, D. E.; Takamatsu, S.; Omura, S.; Ikeda, H., A Gene Cluster for Biosynthesis of the Sesquiterpenoid Antibiotic Pentalenolactone in Streptomyces avermitilis †. *Biochemistry* **2006,** *45*, 6179-6186.

41. Klapschinski, T. A.; Rabe, P.; Dickschat, J. S., Pristinol, a Sesquiterpene Alcohol with an Unusual Skeleton from Streptomyces pristinaespiralis. *Angewandte Chemie International Edition* **2016,** *55*, 10141-10144.

42. Rabe, P.; Citron, C. A.; Dickschat, J. S., Volatile Terpenes from Actinomycetes: A Biosynthetic Study Correlating Chemical Analyses to Genome Data. *ChemBioChem* **2013,** *14*, 2345-2354.

43. von Reuss, S.; Domik, D.; Lemfack, M. C.; Magnus, N.; Kai, M.; Weise, T.; Piechulla, B., Sodorifen Biosynthesis in the Rhizobacterium Serratia plymuthica Involves Methylation and Cyclization of MEP-Derived Farnesyl Pyrophosphate by a SAM-Dependent C -Methyltransferase. *Journal of the American Chemical Society* **2018,** *140*, 11855-11862.

44. Duell, E. R.; D'Agostino, P. M.; Shapiro, N.; Woyke, T.; Fuchs, T. M.; Gulder, T. A. M., Direct pathway cloning of the sodorifen biosynthetic gene cluster and recombinant generation of its product in E. coli. *Microbial Cell Factories* **2019,** *18*, 32-32.

45. Yang, Y.-l.; Zhang, S.; Ma, K.; Xu, Y.; Tao, Q.; Chen, Y.; Chen, J.; Guo, S.; Ren, J.; Wang, W.; Tao, Y.; Yin, W.-B.; Liu, H., Discovery and Characterization of a New Family of Diterpene Cyclases in Bacteria and Fungi. *Angewandte Chemie International Edition* **2017,** *56*, 4749-4752.

46. Lauterbach, L.; Rinkel, J.; Dickschat, J. S., Two Bacterial Diterpene Synthases from Allokutzneria albata Produce Bonnadiene, Phomopsene, and Allokutznerene. *Angewandte Chemie International Edition* **2018,** *57*, 8280-8283.

47. Rinkel, J.; Lauterbach, L.; Rabe, P.; Dickschat, J. S., Two Diterpene Synthases for Spiroalbatene and Cembrene A from Allokutzneria albata. *Angewandte Chemie International Edition* **2018,** *57*, 3238-3241.

48. Kim, S. Y.; Zhao, P.; Igarashi, M.; Sawa, R.; Tomita, T.; Nishiyama, M.; Kuzuyama, T., Cloning and Heterologous Expression of the Cyclooctatin Biosynthetic Gene Cluster Afford a Diterpene Cyclase and Two P450 Hydroxylases. *Chemistry and Biology* **2009,** *16*, 736-743.

49. Meguro, A.; Motoyoshi, Y.; Teramoto, K.; Ueda, S.; Totsuka, Y.; Ando, Y.; Tomita, T.; Kim, S.-Y.; Kimura, T.; Igarashi, M.; Sawa, R.; Shinada, T.; Nishiyama, M.; Kuzuyama, T., An Unusual Terpene Cyclization Mechanism Involving a Carbon-Carbon Bond Rearrangement. *Angewandte Chemie International Edition* **2015,** *54*, 4353-4356.

50. Görner, C.; Häuslein, I.; Schrepfer, P.; Eisenreich, W.; Brück, T., Targeted Engineering of Cyclooctat-9-en-7-ol Synthase: A Stereospecific Access to Two New Non-natural Fusicoccane-Type Diterpenes. *ChemCatChem* **2013,** *5*, 3289-3298.

51. Tomita, T.; Kim, S.-Y.; Teramoto, K.; Meguro, A.; Ozaki, T.; Yoshida, A.; Motoyoshi, Y.; Mori, N.; Ishigami, K.; Watanabe, H.; Nishiyama, M.; Kuzuyama, T., Structural Insights into the CotB2-Catalyzed Cyclization of Geranylgeranyl Diphosphate to the Diterpene Cyclooctat-9-en-7-ol. *ACS Chemical Biology* **2017,** *12*, 1621-1628.

52. Driller, R.; Janke, S.; Fuchs, M.; Warner, E.; Mhashal, A. R.; Major, D. T.; Christmann, M.; Brück, T.; Loll, B., Towards a comprehensive understanding of the structural dynamics of a bacterial diterpene synthase during catalysis. *Nature Communications* **2018,** *9*, 3971-3971.

53. Rinkel, J.; Rabe, P.; Chen, X.; Köllner, T. G.; Chen, F.; Dickschat, J. S., Mechanisms of the Diterpene Cyclases β-Pinacene Synthase from Dictyostelium discoideum and Hydropyrene Synthase from Streptomyces clavuligerus. *Chemistry - A European Journal* **2017,** *23*, 10501-10505.

54. Dickschat, J. S.; Rinkel, J.; Rabe, P.; Beyraghdar Kashkooli, A.; Bouwmeester, H. J., 18-Hydroxydolabella-3,7-diene synthase – a diterpene synthase from Chitinophaga pinensis. *Beilstein Journal of Organic Chemistry* **2017,** *13*, 1770-1780.

55. Meguro, A.; Tomita, T.; Nishiyama, M.; Kuzuyama, T., Identification and Characterization of Bacterial Diterpene Cyclases that Synthesize the Cembrane Skeleton. *ChemBioChem* **2013,** *14*, 316-321.

56. Rinkel, J.; Lauterbach, L.; Dickschat, J. S., Spata-13,17-diene Synthase-An Enzyme with Sesqui-, Di-, and Sesterterpene Synthase Activity from Streptomyces xinghaiensis. *Angewandte Chemie International Edition* **2017,** *56*, 16385-16389.

57. Rabe, P.; Rinkel, J.; Dolja, E.; Schmitz, T.; Nubbemeyer, B.; Luu, T. H.; Dickschat, J. S., Mechanistic Investigations of Two Bacterial Diterpene Cyclases: Spiroviolene Synthase and Tsukubadiene Synthase. *Angewandte Chemie International Edition* **2017,** *56*, 2776-2779.

58. Rudolf, J. D.; Dong, L.-B.; Cao, H.; Hatzos-Skintges, C.; Osipiuk, J.; Endres, M.; Chang, C.-Y.; Ma, M.; Babnigg, G.; Joachimiak, A.; Phillips, G. N.; Shen, B., Structure of the ent -Copalyl Diphosphate Synthase PtmT2 from Streptomyces platensis CB00739, a Bacterial Type II Diterpene Synthase. *Journal of the American Chemical Society* **2016,** *138*, 10905-10915.

59. Rudolf, J. D.; Dong, L.-B.; Manoogian, K.; Shen, B., Biosynthetic Origin of the Ether Ring in Platensimycin. *Journal of the American Chemical Society* **2016,** *138*, 16711-16721.

60. Ikeda, H.; Shin-ya, K.; Nagamitsu, T.; Tomoda, H., Biosynthesis of mercapturic acid derivative of the labdane-type diterpene, cyslabdan that potentiates imipenem activity against methicillin-resistant Staphylococcus aureus: cyslabdan is generated by mycothiol-mediated xenobiotic detoxification. *Journal of Industrial Microbiology & Biotechnology* **2016,** *43*, 325-342.

61. Yamada, Y.; Komatsu, M.; Ikeda, H., Chemical diversity of labdane-type bicyclic diterpene biosynthesis in Actinomycetales microorganisms. *The Journal of Antibiotics* **2016,** *69*, 515-523.

62. Xu, M.; Hillwig, M. L.; Lane, A. L.; Tiernan, M. S.; Moore, B. S.; Peters, R. J., Characterization of an Orphan Diterpenoid Biosynthetic Operon from Salinispora arenicola. *Journal of Natural Products* **2014,** *77*, 2144-2147.

63. Morrone, D.; Chambers, J.; Lowry, L.; Kim, G.; Anterola, A.; Bender, K.; Peters, R. J., Gibberellin biosynthesis in bacteria: Separate ent-copalyl diphosphate and ent-kaurene synthases in Bradyrhizobium japonicum. *FEBS Letters* **2009,** *583*, 475-480.

64. Hershey, D. M.; Lu, X.; Zi, J.; Peters, R. J., Functional Conservation of the Capacity for ent-Kaurene Biosynthesis and an Associated Operon in Certain Rhizobia. *Journal of Bacteriology* **2014,** *196*, 100-106.

65. Nakano, C.; Oshima, M.; Kurashima, N.; Hoshino, T., Identification of a New Diterpene Biosynthetic Gene Cluster that Produces O -Methylkolavelool in Herpetosiphon aurantiacus. *ChemBioChem* **2015,** *16*, 772-781.

66. Ikeda, C.; Hayashi, Y.; Itoh, N.; Seto, H.; Dairi, T., Functional analysis of eubacterial ent-copalyl diphosphate synthase and pimara-9(11),15-diene synthase with unique primary sequences. *Journal of Biochemistry* **2007,** *141*, 37-45.

67. Kawasaki, T.; Kuzuyama, T.; Kuwamori, Y.; Matsuura, N.; Itoh, N.; Furihata, K.; Seto, H.; Dairi, T., Presence of Copalyl Diphosphate Synthase Gene in an Actinomycete Possessing the Mevalonate Pathway. *The Journal of Antibiotics* **2004,** *57*, 739-747.

68. Dairi, T.; Hamano, Y.; Kuzuyama, T.; Itoh, N.; Furihata, K.; Seto, H., Eubacterial diterpene cyclase genes essential for production of the isoprenoid antibiotic terpentecin. *Journal of Bacteriology* **2001,** *183*, 6085-6094.

69. Hamano, Y.; Kuzuyama, T.; Itoh, N.; Furihata, K.; Seto, H.; Dairi, T., Functional analysis of eubacterial diterpene cyclases responsible for biosynthesis of a diterpene antibiotic, terpentecin. *Journal of Biological Chemistry* **2002,** *277*, 37098-37104.

70. Nakano, C.; Okamura, T.; Sato, T.; Dairi, T.; Hoshino, T., Mycobacterium tuberculosis H37Rv3377c encodes the diterpene cyclase for producing the halimane skeleton. *Chemical Communications* **2005**, 1016-1016.

71. Nakano, C.; Hoshino, T., Characterization of the Rv3377c Gene Product, a Type-B Diterpene Cyclase, from the Mycobacterium tuberculosis H37 Genome. *ChemBioChem* **2009,** *10*, 2060-2071.

72. Nakano, C.; Ootsuka, T.; Takayama, K.; Mitsui, T.; Sato, T.; Hoshino, T., Characterization of the Rv3378c Gene Product, a New Diterpene Synthase for Producing Tuberculosinol and (13 R , S)-Isotuberculosinol (Nosyberkol), from the Mycobacterium tuberculosis H37Rv Genome. *Bioscience, Biotechnology, and Biochemistry* **2011,** *75*, 75-81.

73. Layre, E.; Lee, H. J.; Young, D. C.; Jezek Martinot, A.; Buter, J.; Minnaard, A. J.; Annand, J. W.; Fortune, S. M.; Snider, B. B.; Matsunaga, I.; Rubin, E. J.; Alber, T.; Moody, D. B., Molecular profiling of Mycobacterium tuberculosis identifies tuberculosinyl nucleoside products of the virulence-associated enzyme Rv3378c. *Proceedings of the National Academy of Sciences* **2014,** *111*, 2978-2983.

74. Zhao, B.; Lin, X.; Lei, L.; Lamb, D. C.; Kelly, S. L.; Waterman, M. R.; Cane, D. E., Biosynthesis of the Sesquiterpene Antibiotic Albaflavenone in Streptomyces coelicolor A3(2). *Journal of Biological Chemistry* **2008,** *283*, 8183-8189.

75. Zhao, B.; Lei, L.; Vassylyev, D. G.; Lin, X.; Cane, D. E.; Kelly, S. L.; Yuan, H.; Lamb, D. C.; Waterman, M. R., Crystal Structure of Albaflavenone Monooxygenase Containing a Moonlighting Terpene Synthase Active Site. *Journal of Biological Chemistry* **2009,** *284*, 36711-36719.

76. Quaderer, R.; Omura, S.; Ikeda, H.; Cane, D. E., Pentalenolactone Biosynthesis. Molecular Cloning and Assignment of Biochemical Function to PtlI, a Cytochrome P450 of Streptomyces avermitilis. *Journal of the American Chemical Society* **2006,** *128*, 13036-13037.

77. Seo, M.-J.; Zhu, D.; Endo, S.; Ikeda, H.; Cane, D. E., Genome Mining in Streptomyces . Elucidation of the Role of Baeyer−Villiger Monooxygenases and Non-Heme Iron-Dependent Dehydrogenase/Oxygenases in the Final Steps of the Biosynthesis of Pentalenolactone and Neopentalenolactone. *Biochemistry* **2011,** *50*, 1739-1754.

78. You, Z.; Omura, S.; Ikeda, H.; Cane, D. E., Pentalenolactone Biosynthesis. Molecular Cloning and Assignment of Biochemical Function to PtlH, A Non-Heme Iron Dioxygenase of Streptomyces avermitilis. *Journal of the American Chemical Society* **2006,** *128*, 6566-6567.

79. You, Z.; Omura, S.; Ikeda, H.; Cane, D. E.; Jogl, G., Crystal Structure of the Non-heme Iron Dioxygenase PtlH in Pentalenolactone Biosynthesis. *Journal of Biological Chemistry* **2007,** *282*, 36552-36560.

80. You, Z.; Omura, S.; Ikeda, H.; Cane, D. E., Pentalenolactone biosynthesis: Molecular cloning and assignment of biochemical function to PtlF, a short-chain dehydrogenase from Streptomyces avermitilis, and identification of a new biosynthetic intermediate. *Archives of Biochemistry and Biophysics* **2007,** *459*, 233-240.

81. Jiang, J.; Tetzlaff, C. N.; Takamatsu, S.; Iwatsuki, M.; Komatsu, M.; Ikeda, H.; Cane, D. E., Genome Mining in Streptomyces avermitilis : A Biochemical Baeyer−Villiger Reaction and Discovery of a New Branch of the Pentalenolactone Family Tree. *Biochemistry* **2009,** *48*, 6431-6440.

82. Zhu, D.; Seo, M.-J.; Ikeda, H.; Cane, D. E., Genome Mining in Streptomyces . Discovery of an Unprecedented P450-Catalyzed Oxidative Rearrangement That Is the Final Step in the Biosynthesis of Pentalenolactone. *Journal of the American Chemical Society* **2011,** *133*, 2128-2131.

83. Nett, R. S.; Montanares, M.; Marcassa, A.; Lu, X.; Nagel, R.; Charles, T. C.; Hedden, P.; Rojas, M. C.; Peters, R. J., Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. *Nature Chemical Biology* **2017,** *13*, 69-74.

84. Nett, R. S.; Contreras, T.; Peters, R. J., Characterization of CYP115 As a Gibberellin 3-Oxidase Indicates That Certain Rhizobia Can Produce Bioactive Gibberellin A 4. *ACS Chemical Biology* **2017,** *12*, 912-917.

85. Rudolf, J. D.; Dong, L.-B.; Zhang, X.; Renata, H.; Shen, B., Cytochrome P450-Catalyzed Hydroxylation Initiating Ether Formation in Platensimycin Biosynthesis. *Journal of the American Chemical Society* **2018,** *140*, 12349-12353.

86. Dong, L.-B.; Zhang, X.; Rudolf, J. D.; Deng, M.-R.; Kalkreuter, E.; Cepeda, A. J.; Renata, H.; Shen, B., Cryptic and Stereospecific Hydroxylation, Oxidation, and Reduction in Platensimycin and Platencin Biosynthesis. *Journal of the American Chemical Society* **2019,** *141*, 4043-4050.

87. Wang, N.; Rudolf, J. D.; Dong, L.-B.; Osipiuk, J.; Hatzos-Skintges, C.; Endres, M.; Chang, C.-Y.; Babnigg, G.; Joachimiak, A.; Phillips, G. N.; Shen, B., Natural separation of the acyl-CoA ligase reaction results in a non-adenylating enzyme. *Nature Chemical Biology* **2018,** *14*, 730-737.

88. Dong, L.-B.; Rudolf, J. D.; Shen, B., A Mutasynthetic Library of Platensimycin and Platencin Analogues. *Organic Letters* **2016,** *18*, 4606-4609.

89. Sowden, R. J.; Yasmin, S.; Rees, N. H.; Bell, S. G.; Wong, L.-L., Biotransformation of the sesquiterpene (+)-valencene by cytochrome P450cam and P450BM-3. *Organic & Biomolecular Chemistry* **2005,** *3*, 57-57.

90. Janocha, S.; Zapp, J.; Hutter, M.; Kleser, M.; Bohlmann, J.; Bernhardt, R., Resin Acid Conversion with CYP105A1: An Enzyme with Potential for the Production of Pharmaceutically Relevant Diterpenoids. *ChemBioChem* **2013,** *14*, 467-473.

91. Janocha, S.; Bernhardt, R., Design and characterization of an efficient CYP105A1-based whole-cell biocatalyst for the conversion of resin acid diterpenoids in permeabilized Escherichia coli. *Applied Microbiology and Biotechnology* **2013,** *97*, 7639-7649.

92. Takamatsu, S.; Xu, L.-H.; Fushinobu, S.; Shoun, H.; Komatsu, M.; Cane, D. E.; Ikeda, H., Pentalenic acid is a shunt metabolite in the biosynthesis of the pentalenolactone family of metabolites: hydroxylation of 1-deoxypentalenic acid mediated by CYP105D7 (SAV_7469) of Streptomyces avermitilis. *The Journal of Antibiotics* **2011,** *64*, 65-71.

93. Bleif, S.; Hannemann, F.; Lisurek, M.; von Kries, J. P.; Zapp, J.; Dietzen, M.; Antes, I.; Bernhardt, R., Identification of CYP106A2 as a Regioselective Allylic Bacterial Diterpene Hydroxylase. *ChemBioChem* **2011,** *12*, 576-582.

94. Girhard, M.; Machida, K.; Itoh, M.; Schmid, R. D.; Arisawa, A.; Urlacher, V. B., Regioselective biooxidation of (+)-valencene by recombinant E. coli expressing CYP109B1 from Bacillus subtilis in a two-liquid-phase system. *Microbial Cell Factories* **2009,** *8*, 36-36.

95. von Bühler, C.; Le-Huu, P.; Urlacher, V. B., Cluster Screening: An Effective Approach for Probing the Substrate Space of Uncharacterized Cytochrome P450s. *ChemBioChem* **2013,** *14*, 2189-2198.

96. Ewen, K. M.; Hannemann, F.; Khatri, Y.; Perlova, O.; Kappl, R.; Krug, D.; Hüttermann, J.; Müller, R.; Bernhardt, R., Genome Mining in Sorangium cellulosum So ce56. *Journal of Biological Chemistry* **2009,** *284*, 28590-28598.

97. Schifrin, A.; Litzenburger, M.; Ringle, M.; Ly, T. T. B.; Bernhardt, R., New Sesquiterpene Oxidations with CYP260A1 and CYP264B1 from Sorangium cellulosum So ce56. *ChemBioChem* **2015,** *16*, 2624-2632.

98. Ly, T. T. B.; Schifrin, A.; Nguyen, B. D.; Bernhardt, R., Improvement of a P450-Based Recombinant Escherichia coli Whole-Cell System for the Production of Oxygenated Sesquiterpene Derivatives. *Journal of Agricultural and Food Chemistry* **2017,** *65*, 3891-3899.

99. Rinkel, J.; Litzenburger, M.; Bernhardt, R.; Dickschat, J. S., An Isotopic Labelling Strategy to Study Cytochrome P450 Oxidations of Terpenes. *ChemBioChem* **2018,** *19*, 1498-1501.