Supporting Information for

Substrate Structure-Activity Relationships Guide Rational Engineering of Stereocontrol in Modular Polyketide Synthase Ketoreductases

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General Considerations: Anhydrous tetrahydrofuran (THF) was distilled from sodium and benzophenone, and dichloromethane (DCM) was distilled from calcium hydride. Thin layer chromatography (TLC) was conducted with EMD gel 60 F_{254} pre-coated plates (0.25 mM). Fisher Scientific silica gel 60 (particle size 230-400 μm) was used for flash column chromatography. ^{1}H NMR data were acquired on a Varian Mercury 400 MHz instrument at ambient temperature and are reported in terms of chemical shift (δ ppm), multiplicity, coupling constant, and integration and are referenced downfield from $(CH_3)_4Si$ to the residual solvent peak at 7.26 ppm for $CDCl_3$ as an internal standard. ^{13}C NMR data were acquired on either a Varian Mercury 400 MHz, 500 MHz, or 600 MHz instrument and are reported in terms of chemical shift and referenced to the residual solvent peak at 77.16 ppm for $CDCl_3$ as an internal standard. High-resolution mass spectrometry measurements were obtained by chemical ionization (CI) with a VG analytical ZAB2-E instrument. Characterization of **1**,[1] (*E*)-4-ethylidene-3-methyloxetan-2-one,[2] 2-mercaptoethyl acetate,[3] and acyl oxozolidinone intermediates (*S*)-4-benzyl-3-propionyloxazolidin-2-one,[1, 4-7] (*S*)-4-benzyl-3-((2*S*,3*R*)-3-hydroxy-2-methylpentanoyl)oxazolidin-2-one,[6] (*S*)-4-benzyl-3-((2*R*,3*R*)-3-hydroxy-2-methylpentanoyl)oxazolidin-2-one,[8] and (*S*)-4-benzyl-3-((2*R*,3*S*)-3-hydroxy-2-methylpentanoyl)oxazolidin-2-one[6]) were in accordance with literature reported data.

Protein Expression and Purification: *Bacillus subtilis* glucose dehydrogenase (GDH),[1,9] TylKR1,[1,9] EryKR1,[1,9] AmpKR2,[1,9] RifKR7,[10,11] and their respective point mutants were expressed in *E. coli* BL21(DE3) (the expression plasmid for all proteins was pET28b, except RifKR7, which was in pET28a). Starter cultures (50 mL) were grown to inoculate pre-warmed Luria broth supplemented with 25 mg/L kanamycin. 5 mL of starter culture was added to 1L of Luria broth supplemented with 25 mg/L kanamycin, and cultures were grown at 37 °C until OD_{600}=0.4. When OD_{600}=0.4, the media was cooled to 15 °C and then induced with 0.5 mM IPTG. After 16 h, the proteins were harvested by centrifugation (3,000 x g for 20 min), and the pellets were re-suspended in lysis buffer (100 mM HEPES, 500 mM NaCl, 10% (v/v) glycerol, pH 7.5). The cells were then lysed by sonication on ice and centrifuged (30,000 x g for 45 min) to remove cellular debris. The protein was purified by passing the lysate over a nickel-NTA column equilibrated with lysis buffer. The column was then washed with lysis buffer containing 15 mM imidazole, and the protein was eluted with lysis buffer containing 150 mM imidazole. Final protein concentrations were determined using a Thermo Scientific Nanodrop 1000.

Biocatalytic Assays: Reactions were modified from a method described previously[1] using the following conditions: 200 mL HEPES pH 7.5, 100 mM NaCl, 10% (v/v) glycerol, 200 mM D-glucose, 5 mM $NADP^+$, 5 mM diketide substrate (**1-3**), 0.1 μM GDH, and 10 μM KR in a total volume of 1 mL. For reactions with substrate **3**, 20% (v/v) DMSO was added to solubilize the substrate.[i] After overnight incubation (18 h) at 23 °C, the reactions were extracted with 2 volumes of ethyl acetate and evaporated to dryness. Subsequently, the reactions were diluted in ethanol (for substrate **1**) or running buffer (for substrates **2-3**) and analyzed via chiral chromatography. All reactions were performed in duplicate. Product ratios and percent conversions were determined via HPLC integration.

Chiral Chromatography: Samples were separated using ChiralCel OJ-H or ChiraCel OC-H column (250 x 4.6, Daicel Corporation) on a Beckman Coulter HPLC system with a 20 μL loop and 20 μL injections. Reactions were monitored at 235 nm.

Table S1. HPLC conditions for substrates **1-3**.

Substrate	**Solvent**	**Flow Rate**	**Column**
1	7% ethanol:hexanes	0.8 mL/min	OCH
2	2% ethanol:hexanes	0.8 mL/min	OJH
3[1]	0.25% isopropanol:hexanes	0.8 mL/min	OJH

Table S2. Raw Integrals[a]

KR (Substrate)	B1 (2*R*,3*R*)	A2 (2*S*,3*S*)	B2 (2*S*,3*R*)	A1 (2*R*,3*S*)	Substrate
EryKR1 (**1**)	0	0	9214805	0	1469470
	445484	0	23666537	0	956840
EryKR1 (**2**)	0	1027292	19726215	0	11185389
	0	770858	14191485	0	7968723
EryKR1 (**3**)	0	0	263895	0	1016987
	0	0	476494	0	2024354
TylKR1 (**1**)	21844837	2133405	0	0	0
	18468769	1717771	0	0	0
TylKR1 (**2**)	26440881	5621039	0	1093963	2519523
	15916294	3376531	461772	807726	1484929
TylKR1 (**3**)	2949978	1629280	74063	83256	839345
	3121918	1681017	78026	86137	1178413
AmpKR2 (**1**)	0	233136	366565	10402083	7117744
	0	680406	346325	14444250	10956941
AmpKR2 (**2**)	160639	1120470	2753576	8205203	4241079
	278637	2036427	4758966	15085007	7299425
AmpKR2(**3**)	0	1119893	1247198	8812280	4493291
	0	202073	233738	1603230	1248737
RifKR7 (**1**)	0	15475012	0	0	0

[i] Screens were performed with less DMSO for substrate **3**; however, activity was not observed without a minimum of 20% (v/v) DMSO because an insufficient amount of the substrate was solubilized. Screens with varying amounts of DMSO were also performed with substrates **1-2**; however, because the DMSO interacted with the amide/ester and was difficult to remove, it interfered with the resolution on the chiral HPLC.

	470323	13728778	0	0	423268
RifKR7 (**2**)	0	29886089	0	0	19664
	0	23706392	0	0	0
RifKR7(**3**)	0	3116725	0	0	312597
	0	2641128	0	0	272361
EryKR1 L1810A (**1**)	0	3137430	16358138	0	918228
	0	2813529	15661836	0	0
EryKR1 L1810A (**2**)	0	5950354	56160	0	1204395
	0	10242816	94210	0	3212731
EryKR1 L1810A (**3**)	0	53401072	2536301	0	663022
	0	62088435	2993430	0	34747
EryKR1 L1810Q (**1**)	0	0	9123300	0	9046702
	0	0	10045090	0	4257797
EryKR1 L1810Q (**2**)	0	369268	2692324	0	24450448
	0	725168	5129830	0	50835477
EryKR1 L1810Q (**3**)	0	0	0	0	5603593
	0	0	0	0	7945139
EryKR1 L1810H (**1**)	0	0	14625754	0	12109326
	0	0	7162129	0	26735080
EryKR1 L1810H (**2**)	0	2459384	6180311	0	30721895
	0	1677371	4225340	0	22531554
EryKR1 L1810H (**3**)	0	143477	36591	0	3284704
	0	312835	75581	0	10402151
EryKR1 D1758A (**1**)	0	309678	2606111	0	8477010
	0	67129	1745908	0	6492309
EryKR1 D1758A (**2**)	0	1678939	4785579	0	3860198
	0	1097329	2579759	0	1498447
EryKR1 D1758A (**3**)	0	1186544	2040109	0	13186106
	0	302256	520754	0	5240244
EryKR1	0	7971651	222041	0	293265
D1758A/L1810A (**1**)	0	11659601	315671	0	46163
EryKR1	0	10383537	205691	0	0
D1758A/L1810A (**2**)	0	10410108	159205	0	0
EryKR1	0	10172575	61221	0	1765948
D1758A/L1810A (**3**)	0	8275590	46515	0	1160051
TylKR1 Q2341A (**1**)	2829087	7321732	0	249473	934677
	237764	853823	0	94584	160447
TylKR1 Q2341A (**2**)	2474351	9135642	0	139776	491284
	2646322	9946057	0	177420	473748
TylKR1 Q2341A (**3**)	738336	3590165	39036	32632	872262
	167537	806373	8947	5459	394570
TylKR1 D2288A (**1**)	2552532	4276178	0	0	9252685
	422285	778821	0	0	1848816
TylKR1 D2288A (**2**)	3097373	4924275	93937	992609	138423
	4975899	7976143	154425	1674712	698819
TylKR1 D2288A (**3**)	2657296	7856715	101167	1074630	4845702
	433856	1288805	19890	178583	892564
TylKR1	2973482	10808684	0	0	0
D2288A/Q2341A (**1**)	132245	648518	0	0	0
TylKR1	2852703	1396516	174480	118822	16245
D2288A/Q2341A (**2**)	1628102	8013208	105788	74232	0
TylKR1	114927	736027	4925	6057	20281
D2288A/Q2341A (**3**)	197083	1300438	10192	10598	100943
RifKR7 S1474A (**1**)	638489	17411698	0	0	0
	19455	1990373	0	0	0

RifKR7 S1474A (**2**)	50070	1188219	0	92692	128670
	44112	10960346	0	0	131237
RifKR7 S1474A (**3**)	8493	3656946	0	14788	334108
	0	2590593	0	8609	58653
AmpKR2 Q2892A (**1**)	20112	832644	125251	538268	1658679
	45268	8457910	905308	3135670	888721
AmpKR2 Q2892A (**2**)	62429	3539596	857291	1299603	252877
	213733	10430590	2133083	3583612	110071
AmpKR2 Q2892A (**3**)	0	1608340	79324	1346671	644891
	0	2973851	152171	2393756	1808179

[a] Integrations are reported in arbitrary units.

Table S3. Product ratios (averaged over duplicate runs)

KR (Substrate)	% B1 (2*R*,3*R*)	%A2 (2*S*,3*S*)	% B2 (2*S*,3*R*)	% A1 (2*R*,3*S*)
EryKR1 (**1**)[a]	1	0	99	0
EryKR1 (**2**)	0	5	95	0
EryKR2 (**3**)	0	0	100	0
TylKR1 (**1**)[b]	91	9	0	0
TylKR1 (**2**)	79	17	1	4
TylKR1 (**3**)	63	34	1.5	1.5
AmpKR2 (**1**)[c]	0	3	94	3
AmpKR2 (**2**)	1	9	22	68
AmpKR2 (**3**)	0	10	11	79
RifKR7 (**1**)	1.6	98.4	0	0
RifKR7 (**2**)	0	100	0	0
RifKR7 (**3**)	0	100	0	0
EryKR1 L1810A (**1**)	0	84	16	0
EryKR1 L1810A (**2**)	0	95	5	0
EryKR1 L1810A (**3**)	0	99	1	0
EryKR1 L1810Q (**1**)	0	0	100	0
EryKR1 L1810Q (**2**)	0	12	88	0
EryKR1 L1810Q (**3**)	0	0	0	0
EryKR1 L1810H (**1**)	0	0	100	0
EryKR1 L1810H (**2**)	0	28	72	0
EryKR1 L1810H (**3**)	0	80	20	0
EryKR1 D1758A (**1**)	0	7	93	0
EryKR1 D1758A (**2**)	0	28	72	0
EryKR1 D1758A (**3**)	0	37	63	0
EryKR1 D1758A/L1810A (**1**)	0	97	3	0
EryKR1 D1758A/L1810A (**2**)	0	98	2	0
EryKR1 D1758A/L1810A (**3**)	0	99.5	0.5	0
TylKR1 Q2341A (**1**)	24	71	0	5
TylKR1 Q2341A (**2**)	21	78	0	1
TylKR1 Q2341A (**3**)	17	81	1	1
TylKR1 D2288A (**1**)	36	64	0	0
TylKR1 D2288A (**2**)	34	54	10	2
TylKR1 D2288A (**3**)	23	67	1	8
TylKR1 D2288A/ Q2341A (**1**)	19	81	0	0
TylKR1 D2288A/ Q2341A (**2**)	16	82	1	1
TylKR1 D2288A/ Q2341A (**3**)	13	86	0.5	0.5
RifKR7 S1474A (**1**)	2	98	0	0
RifKR7 S1474A (**2**)	0.4	98.6	0	1
RifKR7 S1474A (**3**)	0.1	99.5	0	0.4
AmpKR2 Q2892A (**1**)	1	61	8	30
AmpKR2 Q2892A (**2**)	1	63	14	22
AmpKR2 Q2892A (**3**)	0	54	2	44

There were deviations from previously published results[1] with **1**: [a] Product ratio reported previously: 100% B2 product. [b] Product ratio reported previously: 89% B1 product, 7.9% A2 product, 2.7% A1 product. [c] Product ratio reported previously: 1.3% A2 product, 93.2% A1 product, 5.5% B2 product.

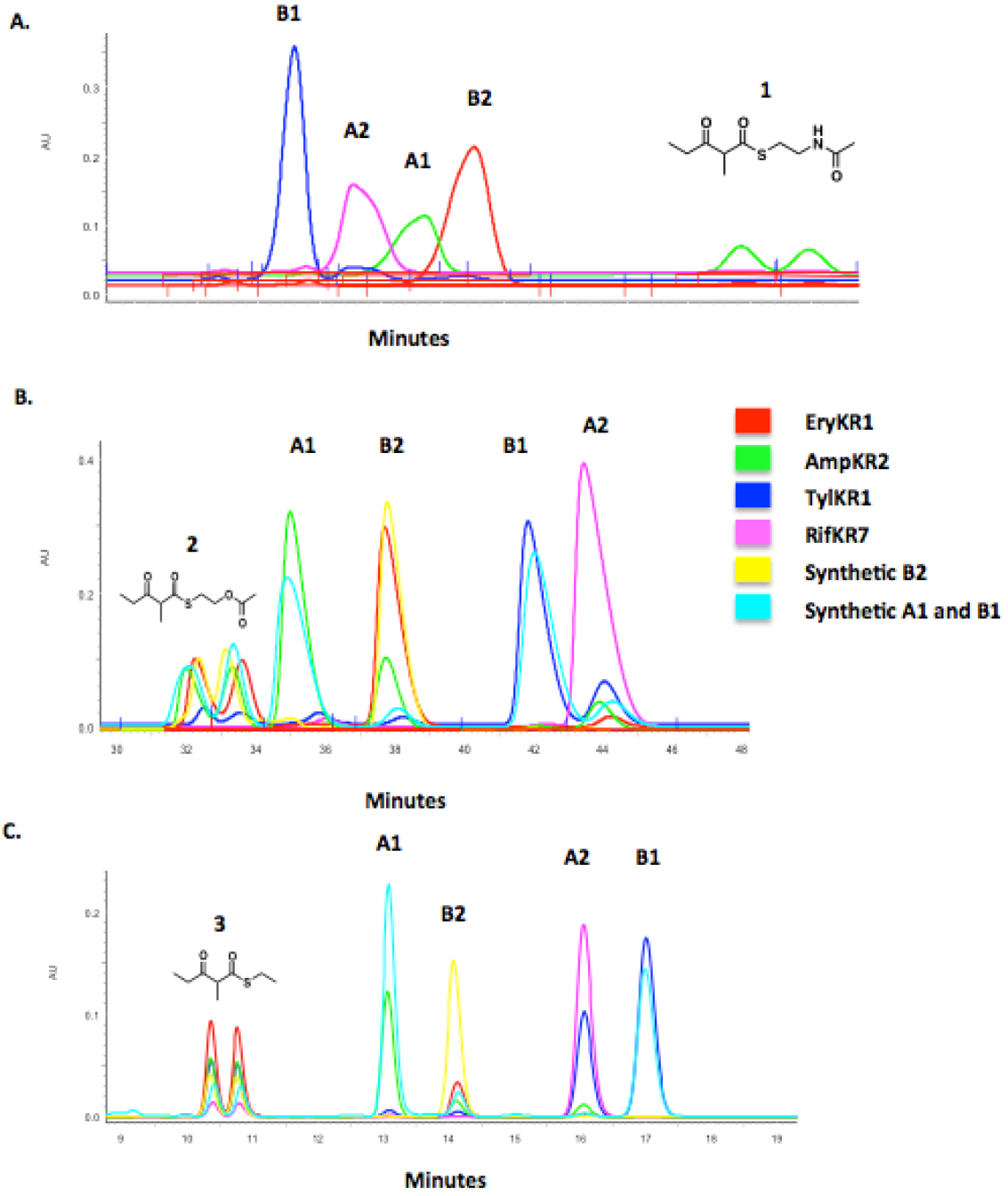


Figure S1. Chiral chromatograms of reactions with **1, 2** and **3** and synthetic standards (see synthetic methods section). Standards were spiked with substrates **2** and **3** confirm the peak alignment. The elution order of the reduction products of **1** was reported previously.[1]

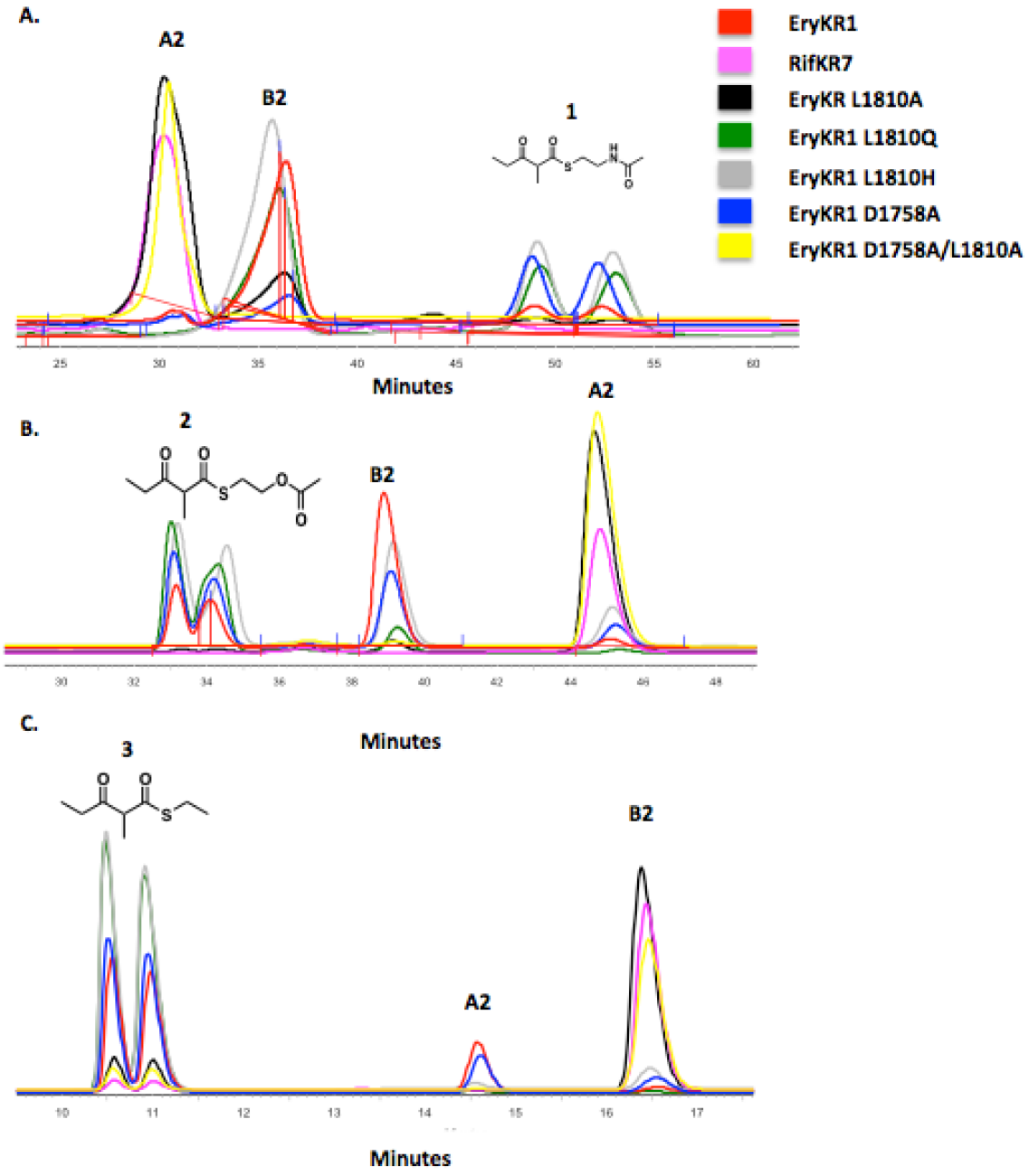


Figure S2. Chiral chromatograms of EryKR1 mutant assays. Unmutated EryKR1 and RifKR7 are shown for reference

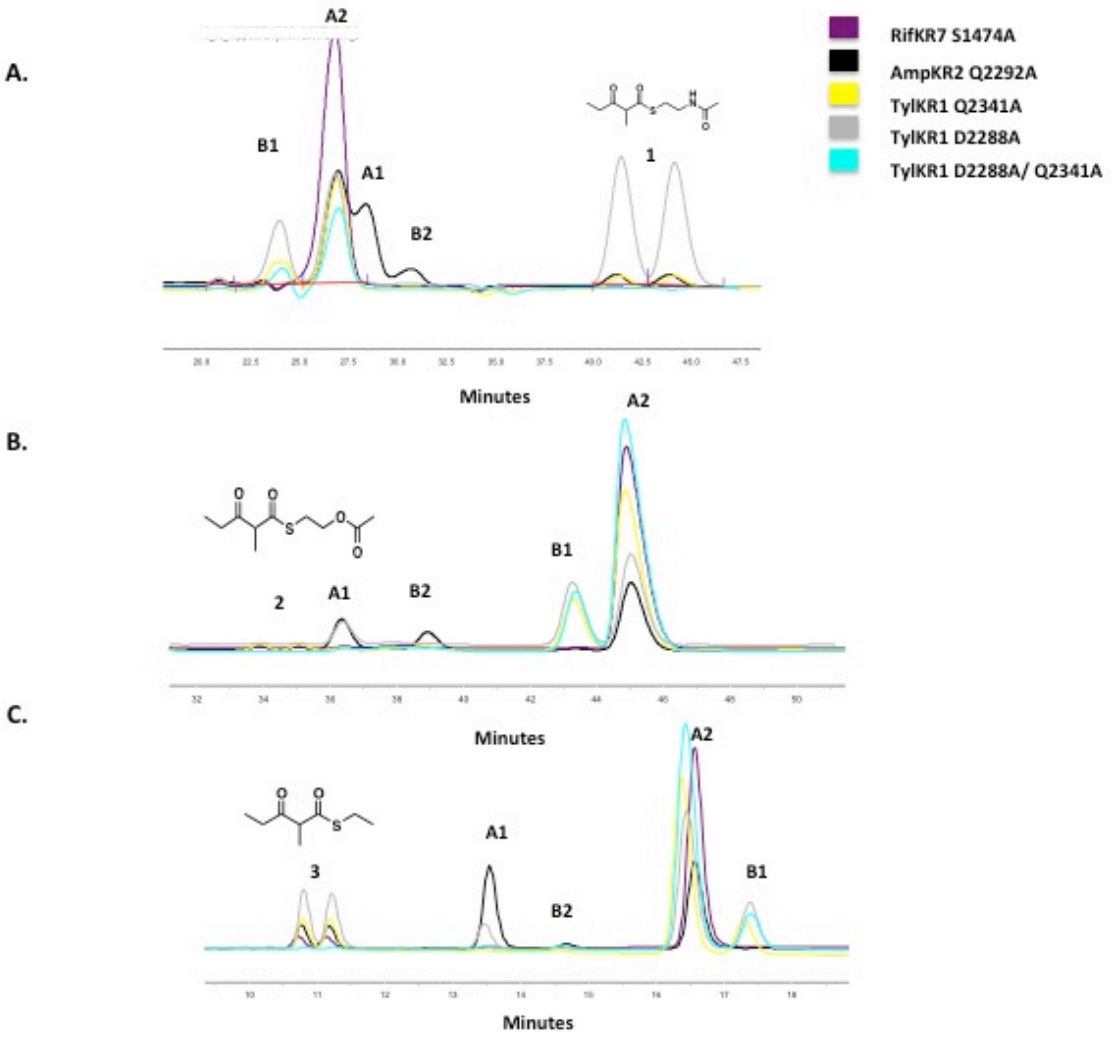


Figure S3. Chiral chromatograms of AmpKR2, RifKR7, and TylKR1 mutant assays.

Table S4. Summary of Ketoreductase Assays

KR	Substrate	[%] Conversion[a]	[%] *de*[b]	[%] *ee*[c]	Absolute Configuration[d]
EryKR1	**1**	91[e]	98	98	2*S*, 3*R*
	2	65	90	90	2*S*, 3*R*
	3	25	100	100	2*S*, 3*R*
TylKR1	**1**	100	1	83	2*R*, 3*R*
	2	93	91	57	2*R*, 3*R*
	3	82	93	25	2*R*, 3*R*
AmpKR2	**1**	60[f]	93	88	2*R*, 3*S*
	2	75	84	35	2*R*, 3*S*
	3	67	80	57	2*R*, 3*S*
RifKR7	**1**	99	97	97	2*S*, 3*S*
	2	100	100	100	2*S*, 3*S*
	3	91	100	100	2*S*, 3*S*
EryKR1 L1810A	**1**	98	69	69	2*S*, 3*S*
	2	99	91	91	2*S*, 3*S*
	3	80	98	98	2*S*, 3*S*
EryKR1 L1810Q	**1**	60	100	100	2*S*, 3*R*
	2	10	76	76	2*S*, 3R
	3	0	n/a	n/a	n/a
EryKR1 L1810H	**1**	62	100	100	2*S*, 3*R*
	2	24	43	43	2*S*, 3*R*
	3	4	61	61	2*S*, 3*S*
EryKR1 D1758A	**1**	24	86	86	2*S*, 3*R*
	2	67	44	44	2*S*, 3*R*
	3	17	26	26	2*S*, 3*R*
EryKR1 D1758A/ L1810A	**1**	98	95	95	2*S*, 3*S*
	2	100	97	97	2*S*, 3*S*
	3	87	99	99	2*S*, 3*S*
TylKR1 Q2341A	**1**	90	89	42	2*S*, 3*S*
	2	96	97	56	2*S*, 3*S*
	3	77	97	63	2*S*, 3*S*
TylKR1 D2288A	**1**	41	100	27	2*S*, 3*S*
	2	97	76	8	2*S*, 3*S*
	3	69	80	34	2*S*, 3*S*
TylKR1 D2288A/ Q2341A	**1**	100	100	66	2*S*, 3*S*
	2	100	96	63	2*S*, 3*S*
	3	96	97	71	2*S*, 3*S*
RifKR7 S1474A	**1**	100	100	95	2*S*, 3*S*
	2	98	98	99	2*S*, 3*S*
	3	95	99	99	2*S*, 3*S*
AmpKR2 Q2892A	**1**	92	24	22	2*S*, 3*S*
	2	98	21	25	2*S*, 3*S*
	3	79	7	7	2*S*, 3*S*

[a] Conversion of total reduced product. [b] *syn:anti* or *anti:syn de* depends on the major product. For wild type EryKR1, AmpKR2, EryKR1 L1810Q, EryKR1 L1810H (with **1** and **2**), and EryKR1 D1758A the *syn: anti de* was calculated. For RifKR7, TylKR1, EryKR1 L1810H (with **3**), EryKR1 D1758A, EryKR1 D1758A/L1810A, RifKR7 S1474A, AmpKR2 Q2892A, TylKR1 Q2341A, TylKR1 D2288A, and TylKR1 D2288A/Q2341A, the *anti:syn de* was calculated. [c] Enantiomeric excess is calculated for the excess of the major product stereoisomer over all four stereosiomers. [d] Absolute configuration of the major product. [e] Reported previously as 66% conversion.[1] [f] Reported previously as 78% conversion.[1]

Site-Directed Mutagenesis:

Site-directed mutagenesis of EryKR1 was performed with the QuikChange method on a template consisting of the DNA encoding EryKR1 inserted into pET28b.[1] The following primers were used (mutagenic sequences in red):

1) for L1810A: 5'-gcctttggtgcaccgggt**gc**cggcgggtatgcgccaggcaac-3' and
   5'-gttgcctggcgcatacccc**gc**cggcacccggtgcaccaaaggc-3'
2) for L1810Q: 5'-gcctttggtgcaccgggtc**ag**ggcgggtatgcgccaggcaac-3' and
   5'-gttgcctggcgcatacccgcc**ct**gacccggtgcaccaaaggc-3'
3) for L1810H: 5'-gcctttggtgcaccgggtc**a**cggcgggtatgcgccaggcaac-3' and
   5'- gttgcctggcgcatacccgccg**t**gacccggtgcaccaaaggc-3'
4) for D1758A: 5'-gcggcggcaaccttggatg**c**cggcaccgtcgatactctg-3' and
   5'-cagagtatcgacggtgccg**g**catccaaggttgccgccgc-3'

For the double mutant D1758A/L1810A, EryKR1 L1810A in pET28b was used as template.

Site-directed mutagenesis of TylKR1 was performed with the QuikChange method on a template consisting of the DNA encoding TylKR1 inserted into pET28b.[1] The following primers were used (mutagenic sequences in red):

1) for Q2341A: 5'-acatggggcaacgccggc**gc**gggtgcgtacgccgccgccaa-3' and
   5'-ttggcggcggcgtacgcaccc**gc**gccggcgttgccccatgt-3'
2) for D2288A: 5'-ttccacaccgccgggattctggacg**c**cgcggtgatcgacacgctg-3' and
5'- cagcgtgtcgatcaccgcg**g**cgtccagaatcccggcggtgtggaa

For the double mutant D2288A/Q2341A, the TylKR1 Q2341A in pET28b was used as template.

Site-directed mutagenesis of RifKR7 was performed with the QuikChange method on a template consisting of the DNA encoding RifKR7 inserted into pET28a.[10,11] The following primers were used (mutagenic sequence in red):

1) for S1474A: 5'-agcatcttcatgggtgccggc**g**ccggtggttacgcggcagcgaat-3' and
   5'-attcgctgccgcgtaaccaccgg**c**gccggcacccatgaagatgct-3'

Site-directed mutagenesis of AmpKR2 was performed with the QuikChange method on a template consisting of the DNA encoding AmpKR2 inserted into pET28b. The following primers were used (mutagenic sequences in red):

1) for Q2892A: 5'-tctggggcagcggtggc**gc**gcccggctacgccgccgccaa-3' and
   5'- ttggcggcggcgtagccgggc**gc**gccaccgctgccccaga-3'

All mutations were confirmed by DNA sequencing.

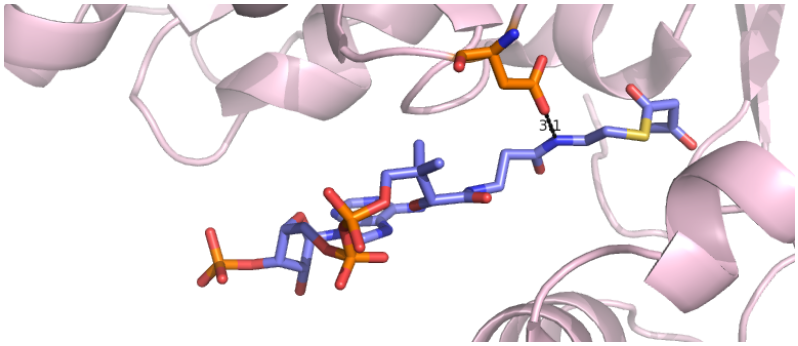


Figure S4. Image of the homologous aspartate residue hydrogen bonding with the first amide of the phosphopantetheinyl moiety of acetylacetyl-CoA (analogous to the amide in substrate **3**) in the structure of (*R)*-3-hydroxybutyryl-CoA dehydrogenase from *Ralstonia eutropha* (PDB: 4N5M).[11]

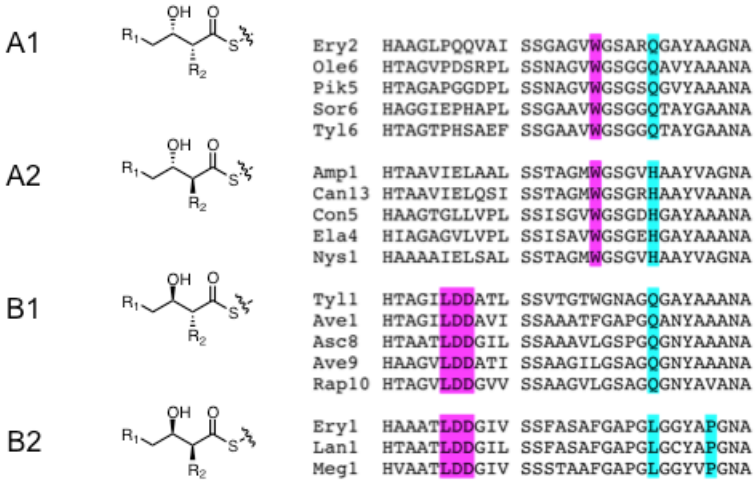
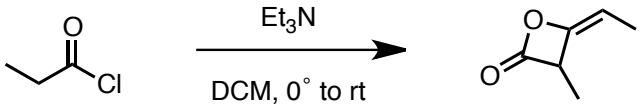
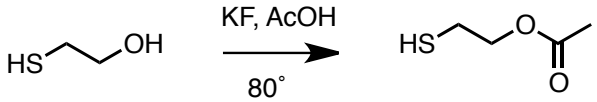


Figure S5. Sequence alignment of KRs indicating conserved fingerprint residues. Residues corresponding to hydroxyl stereochemistry (the conserved W of A-type KRs and the LDD of B-type KRs)[12-14] are highlighted in magenta. Residues corresponding to α-substituent stereochemistry are highlighted in cyan.[14]

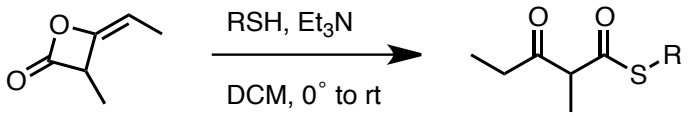
Synthetic Methods:



(E)-4-ethylidene-3-methyloxetan-2-one. Dichloromethane (150 mL) and triethylamine (18.72 mL, 66 mmol, 1 eq) were added over 3Å molecular sieves to a flame-dried flask and cooled to 0°C. Propionyl chloride (19.02 mL, 66 mmol, 1 eq) was added dropwise at 0 °C over a period of 90 minutes. The reaction was then stirred at room temperature for 16 h, concentrated under vacuum, and filtered over celite to remove the triethylammonium chloride salt, affording a yellow oil (2.5 g, 33%). The characterization was in agreement with reported literature data.[2]



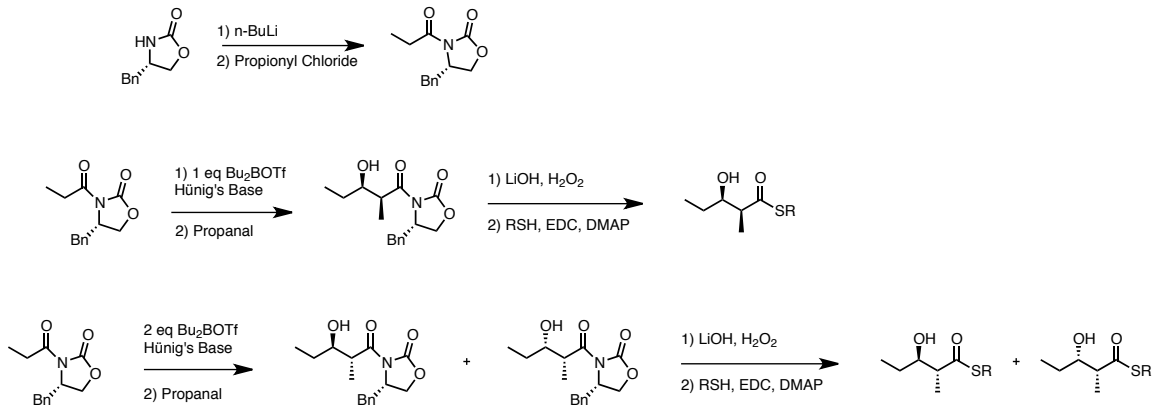
2-mercaptoethylacetate. According to a modified literature procedure,[5] 2-mercaptoethanol (0.65 mL, 7.6 mmol, 1 eq) and potassium flouride (0.93 g, 7.6 mmol, 1 eq) were dissolved in acetic acid (15 mL) and heated at 80 ˚C for 16 h. The reaction was diluted with water (50 mL) and extracted with ethyl acetate (2 x 100 mL). The organic layer was then washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, evaporated to dryness, and filtered over a plug of silica with 20% ethyl acetate: hexanes to afford a yellow oil (312 mg, 28%). The characterization was in agreement with literature reported data.[3]



General Synthetic Route for α-Methyl, β-Ketoacyl Thioester Substrates (1-3):
To a flame-dried flask, methyl diketene dimer (427 mg, 3.8 mmol, 1 eq) and thiol (3.8 mmol, 1 eq) were added to dichloromethane (30 mL) at 0 °C. Catalytic triethylamine was added, and the reaction warmed to room temperature and stirred for 16 h. The reaction was then washed with saturated NaCl (1 x 30 mL), extracted in ethyl acetate (EtOAc) (2 x 50 mL) and concentrated under vacuum to afford the crude product. The crude products were purified via dry flash column chromatography (silica; for **1**, 50% EtOAc:hexanes; for **2**, 30% EtOAc:hexanes; and for **3**, 10% EtOAc:hexanes). An additional semi-preparative HPLC purification step was required for **3** to remove co-eluting impurities: Varian Microsorb-MV C18 column (250 x 4.6 mm, 5 µm particle size, 100 Å pore size) with a matching Metaguard column, 15-35% B over 30 minutes at 1 mL/min, with mobile phases consisting of water with 0.1% TFA (solvent A) and methanol with 0.1% TFA (solvent B).

(R,S)-ethyl 2-methyl-3-oxopentanethioate (1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (q, J=7, 1H), 2.91 (q, J=7, 3H), 2.68-2.43 (m, 2H), 1.37 (d, J=7, 3H), 1.26 (t, J=7, 3H), 1.05 (t, J=7, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 205.30, 196.71, 61.14, 35.70, 23.66, 14.44, 13.54, 7.57. HRMS (CI) (*m/z*) $[M+H]^+$: calcd. for $C_8H_{14}O_2S$: 175.0793, found 175.0792.

(R,S) 2-methyl((2-methyl-3-oxopentanoyl)thio)ethyl acetate (2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (td, J=6.5 Hz, J=1.3 Hz, 2H), 3.73 (q, J=7 Hz, 1H), 3.14 (t, J=6.5Hz, 2H), 2.65-2.44 (m, 2H), 2.05 (s, 3H), 1.38 (d, 3H), 1.06 (t, 3H, J=7 Hz). ^{13}C NMR (400 MHz, $CDCl_3$) δ 204.79, 195.80, 170.45, 62.27, 60.91, 34.63, 27.81, 20.57, 13.43, 7.49. HRMS (CI) (*m/z*) $[M+H]^+$: calcd. for $C_{10}H_{17}O_4S$: 233.0850, found 233.0848.



Scheme S1. Route for synthetic standards.

General Synthetic Route for α-Methyl, β-Hydroxy Standards.
Standards were synthesized using the method detailed previously by Piasecki *et al.*[1] Briefly, the (2*S*,3*R*)-thioester standards were synthesized via an Evans *syn* selective aldol reaction with (4*S*)-Benzyl-3-propionyl-2-oxozolidinone and propionaldehyde.[4,5] The (2*R*, 3*R*)- and (2*R*, 3*S*)-thioester standards were synthesized as a mixture of diasteromers via the *anti* selective aldol reaction described by Heathcock *et al.* with (4*S*)-Benzyl-3-propionyl-2-oxozolidinone and propionaldehyde. The Heathcock adol reaction uses conditions identical to the classic Evans *syn* selective aldol reaction, except that two equivalents of dibutylboron triflate are added and results in a mixture of diastereomers: an *anti* product with the same hydroxyl stereochemistry the Evans *syn* product and the non-Evans *syn* product.[7] Subsequently, the chiral auxiliary was cleaved and the α-methyl β-hydroxy acids were subjected to a Steiglich-type esterification with the appropriate thiol as described by Boddy *et al.*[6] The characterizations of the acyl oxazolidinone intermediates were in agreement with literature reported data.[1,4-8]

(2R,3S)-S-ethyl 3-hydroxy-2-methylpentanethioate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (m, 1H), 2.88 (q, J=7, 2H), 2.72-2.66 (m, 1H), 2.38 (br s, 1H), 1.52-1.39 (m, 2H), 1.26 (t, J=7, 3H), 1.21 (d, J=7, 3H), 0.97 (t, J=7, 3H). ^{13}C NMR δ 204.54, 73.58, 52.69, 27.15, 23.36, 14.79, 11.45, 10.51. ^{13}C (500 MHz, $CDCl_3$): 204.22, 73.43, 52.63, 26.98, 23.14, 14.56, 11.25, 10.29 HRMS (CI) (*m/z*) $[M+H]^+$ calcd. for $C_8H_{17}O_2S$: 177.0949, found 177.0952.

(2S,3R)-S-ethyl 3-hydroxy-2-methylpentanethioate and ***(2R,3R)-S-ethyl 3-hydroxy-2-methylpentanethioate*** were isolated as an inseparable mixture of diasteromers (4:3 *syn:anti* dr). ^{1}H NMR (400 MHz, $CDCl_3$) δ *syn* diasteromer: 3.82 (m, 1H), 2.89 (q, J=7, 2H), 2.72-2.66 (m, 1H), 1.52-1.39 (m, 2H), 1.26 (t, J=7, 3H), 1.21 (d, J=7, 3H), 0.97 (t, J=7, 3H). ^{13}C (400 MHz, $CDCl_3$): 204.26, 75.20, 52.80, 27.10, 24.00, 13.52, 11.54, 9.99δ *anti* diasteromer: 3.61 (m, 1H), 2.87 (q, J=7, 2H), 2.72-2.66 (m. 1H), 1.57-1.46 (m, 2H), 1.26 (t, J=7, 3H), 1.23 (d, J=3, 2H), 0.98 (t, J=7, 3H). ^{13}C (400 MHz, $CDCl_3$): 204.22, 75.57, 53.32, 27.77, 25.95, 13.11, 10.40. 9.89. HRMS (CI) (*m/z*) $[M+H]^+$: calcd. for $C_8H_{17}O_2S$: 177.0949, found 177.0954.

2-(((2R,3S)-3-hydroxy-2-methylpentanoyl)thio)ethyl acetate ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.32 (t, J=7, 2H), 3.88-3.81 (m, 1H), 3.15 (t, J=7, 2H), 2.76-2.69 (m, 1H), 2.08 (s, 3H), 1.54-1.40 (m, 2H), 1.22 (2, J=7, 3H), 0.98 (t, J=7, 3H). ^{13}C (600 MHz, $CDCl_3$): 202.78, 170.54, 73.34, 62.41, 53.40, 27.41, 29.56, 20.59, 13.70, 10.12. HRMS (CI) (*m/z*) $[M+H]^+$: calcd. For $C_{10}H_{18}O_4S$: 235.1104, found 235.1106.

2-(((2S,3R)-3-hydroxy-2-methylpentanoyl)thio)ethyl acetate and ***2-(((2R,3R)-3-hydroxy-2-methylpentanoyl)thio)ethyl acetate*** were isolated as an inseparable mixture of diasteromers (3:4 *syn: anti* dr). (400 MHz, $CDCl_3$) δ *syn* diasteromer 4.32 (t, J=7, 2H), 3.86-3.81 (m, 1H), 3.15 (t, J=7, 2H), 2.76-2.69 (m, 1H), 2.29 (br d, J=4 HZ, 1H), 2.08 (s, 3H), 1.54-1.40 (m, 2H), 1.22 (d J=7, 3H), 0.98 (t, J=7, 3H). ^{13}C (400 MHz, $CDCl_3$): 204.48, 170.37, 73.20, 62.25, 53.45, 29.39, 27.50, 20.38, 14.44, 9.90 (400 MHz, $CDCl_3$). δ *anti* diasteromer: 4.18 (t, J=7, 3H), 3.68-3.61 (m, 1H), 3.13 (t, J=7, 2H), 2.78-2.69 (m, 1H), 2.24 (br d, J=7.6), 2.06 (s, 3H), 1.63-1.51 (m, 2H), 1.23 (d J=7, 3H), 0.98 (t, J=7, 3H). ^{13}C (600 MHz, $CDCl_3$): 204.48, 170.34, 74.64, 62.25, 53.70, 28.14, 27.24, 20.34, 14.55, 9.33. HRMS (CI) (*m/z*) $[M+Na]^+$: calcd. for $C_{10}H_{18}O_4S$: 257.08180, found 257.0821.

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