Supporting Information:

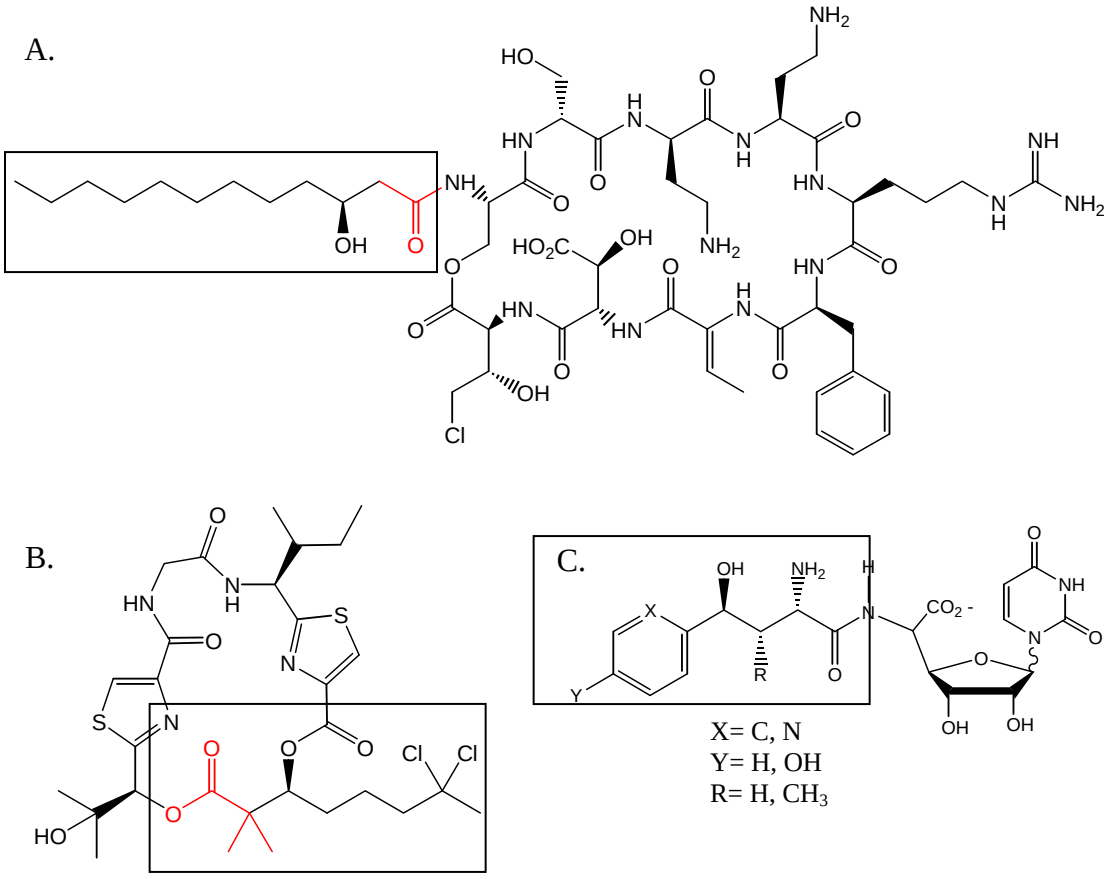


Figure S1. Pharmaceutical targets that could be partially synthesized by enzyme-catalyzed aldol condensations. The portions of each molecule that can be synthesized using a pyruvate aldolase followed by a minimal number of steps are shown with boxes. The pyruvate equivalences are highlighted in red: (A) the 3-hydroxylipoic amides of syringomycin E[1] and other lipodepsipeptide; (B) the dimethyl β-hydroxylactone of lyngbyabellin[2]; and (C) the 2-amino-(4-aryl)-4-hydroxybutyrate chain of nikkomycins.

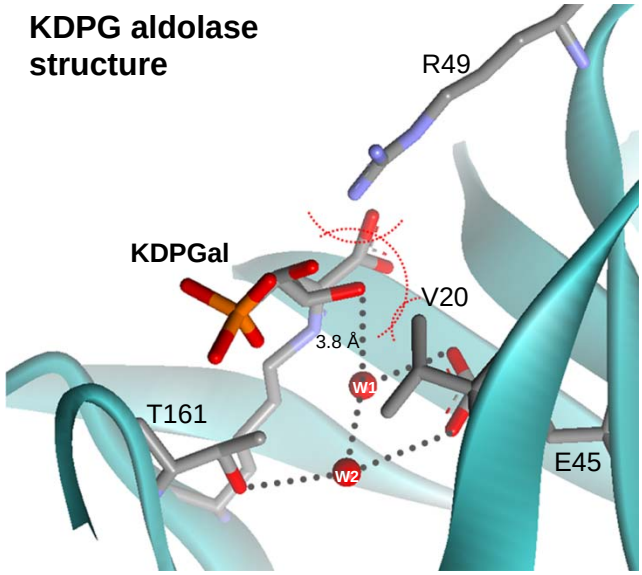


Figure S2. Model of KDPGal bound to *E. coli* KDPG aldolase. The *galacto*-configuration is disfavored because the C4 hydroxyl makes unfavorable van der Waals contacts with R49 and V20 (shown as dashed red lines). Furthermore, W1 is poorly situated to facilitate proton transfer (3.8 Å and a proton abstraction angle of 76°).

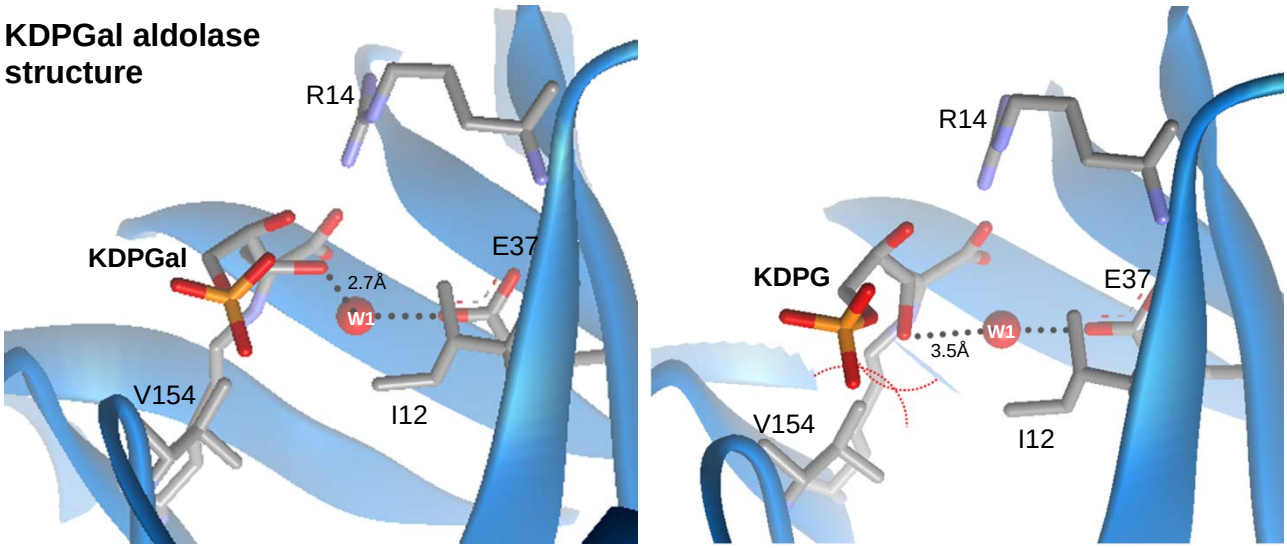
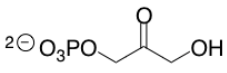
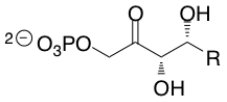
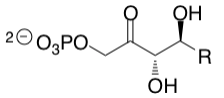
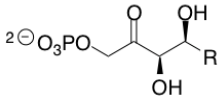
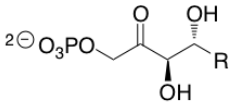
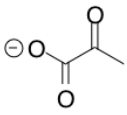
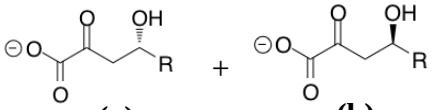
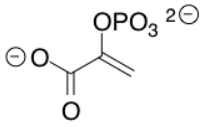
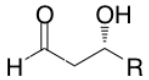
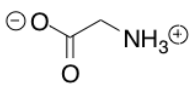
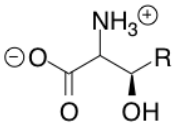


Figure S3. Stereoselectivity in *E. coli* KDPGal aldolase. (A) Structure of *galacto*-sugar bound to KDPGal aldolase.[11] Unlike KDPG aldolase, KDPGal aldolase does not have a W2 binding pocket. Consequently, W1 and Glu37 are positioned deeper in the active site and closer to R14. Only in the *galacto*-configuration where O4 points towards R14 can this oxygen reach W1 to accomplish proton transfer (distance 2.7 Å and proton abstraction angle of 114°). (B) Model of *gluco*-sugar bound to KDPGal aldolase. The *gluco*-configuration is disfavored because the distance and geometry of the O4 water W1 interaction are poor (3.5 Å and 80° angle of proton abstraction). Also the lower pocket occupied by the O4 of the *gluco*-sugar is destabilized by steric clashes with V154 (shown as dashed red lines) and the pocket is entirely hydrophobic.

Table S1. Some important aldolases for synthetic applications.

	Donor nucleophile	Names of aldolases	Products	References
1	Dihydroxyacetone phosphate/ Dihydroxyacetone 	FBP FSA[a]		Choi 2001[3] Schurmann 2001[4]
		TBP		Hall 2002[5] & Lowkam 2010[6]
		RhuA		Kroemer 2003[7]
		FucA		Joerger 2000[8]
2	Pyruvate / Phosphoenol-pyruvate 	KDG	 **(a)** + **(b)**	Theodossis 2004[9]
		KDPG	**(a)**	Allard 2001[10]
		KDPGal	**(b)**	Walters 2008[11]
		NeuAc	**(a)**	Izard 1994[12]
		BphI	**(b)**	Baker 2011[13]
		HpaI	**(b)**	Wang 2005[14]
		KDO8	**(b)**	Radaev 2000[15]
3	Acetaldehyde 	DERA		Hiene 2004[16]

4	Glycine 	TA		Kielkopf 2002[17]

[a] FSA uses dihydroxyacetone as its nucleophilic substrate.
FBP, fructose-1,6-bisphosphate aldolase; FSA, fructose-6-phosphate aldolase; TBP, tagatose-1,6-bisphosphate aldolase; RhuA, rhamnulose-1-phosphate aldolase; FucA, fuculose-1-phosphate aldolase; KDG, 2-keto-3-deoxygluconate aldolase; KDPG, 2-keto-3-deoxy-6-phosphogluconate aldolase; KDPGal, 2-keto-3-deoxy-phosphogalactonate aldolase; KDO8, 3-deoxy-D-manno-octulosonate 8-phosphate synthase; NeuAc, N-acetylneuraminic acid lyase or D-sialic acid aldolase; DERA, 2-deoxyribose-5-phosphate aldolase; TA, L-threonine acetaldehyde-lyase; BphI is a catabolic aldolase part of the polychlorinated biphenyls degradation pathway; HpaI is a catabolic aldolase in the hydroxyphenylacetate pathway

Table S2

	T161		G162		S184	
	KHO	KHPB	KHO	KHPB	KHO	KHPB
Number of plasmids transformed	160	120	>200	>200	>200	>200
Number of colonies grown	2	3	3	0	3	0
Number of unique sequences	2	1	2	-	1	-

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