ACS Catalysis

Research Article

pubs.acs.org/acscatalysis

Minimalist Protein Engineering of an Aldolase Provokes Unprecedented Substrate Promiscuity

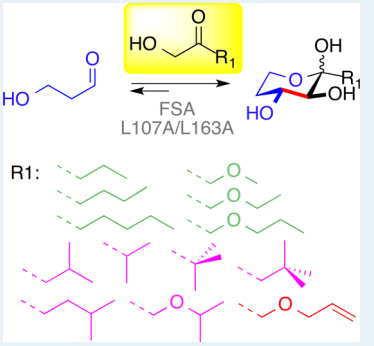
Deniz Güclü,[†,§] Anna Szekrenyi,[‡,§] Xavier Garrabou,[‡] Michael Kickstein,[†] Sebastian Junker,[†] Pere Clapés,*[,‡] and Wolf-Dieter Fessner*[,†]

[†]Institut für Organische Chemie und Biochemie, Technische Universität Darmstadt, Alarich-Weiss-Straße 4, 64287 Darmstadt, Germany

[‡]Instituto de Química Avanzada de Cataluña-IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

Supporting Information

ABSTRACT: Application of aldolases for the asymmetric synthesis of multifunctional chiral products is hampered by their reputed strict nucleophile (=aldol donor) specificity owing to a mechanistic requirement for creating a carbanion nucleophile in aqueous medium. Here we report that a minimalist engineering can extensively broaden the substrate scope of native D-fructose-6-phosphate aldolase (FSA) from *Escherichia coli*, for which hydroxyacetone is the most proficient substrate, to accept an unprecedented wide variety of alternative nucleophiles. By single- or double-space-generating mutations using simple conservative Leu to Ala replacement of active site residues, we found enzyme variants to efficiently convert larger ketols and bioisosteric ether components with up to seven skeletal atoms, including linear and branched-chain structures. All reactions occurred with full retention of the natural D-*threo* diastereospecificity. These FSA variants open new avenues toward the synthesis of novel product families that hitherto were inaccessible by biological catalysis.



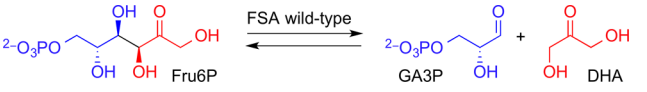
KEYWORDS: *aldol reactions, biocatalysis, carbohydrates, mutagenesis, protein engineering*

INTRODUCTION

The concept of enzyme promiscuity, reflecting on an enzyme's capability to catalyze more than one chemically distinct reaction type by stabilizing different transition states (*catalytic promiscuity*) or to show ambiguity in the conversion of several substrates while involving the same transition state (*substrate promiscuity*, also called *substrate ambiguity*), has aroused substantial interest recently because of its implications in the natural mechanisms for the divergent evolution of new enzyme functions from a common progenitor.[1] Indeed, adaptation of an enzyme to degrade xenobiotics or to convert cognate metabolites may result from only few mutations, even as little as a single replacement in the active site.[2] The utilization of selected enzymes to catalyze reactions on a plethora of non-natural substrates is the core principle for a growing sustainable bioproduction industry,[3] underscoring the notion that some enzymes are catalytically much more flexible than originally assumed.

Aldolases catalyze a highly ordered, stereoselective addition of a carbon nucleophile (the aldol donor), which typically is a ketone enolate or transiently formed enamine equivalent, to a carbonyl electrophile (the aldol acceptor), which typically is an aldehyde. This carboligation process leads to the formation of up to two adjacent chiral centers of known absolute configuration. Whereas most aldolase-type enzymes tolerate a broad variety of non-natural aldehyde electrophiles with good catalytic rates, they generally share high substrate specificity for their nucleophile, as reiteratively documented for a broad variety of distinct aldolases from various sources.[4] Even small structural variations in the nucleophile, such as replacing ethanal for propanal,[5] 1,3-dihydroxyacetone phosphate (DHAP) for a bioisosteric phosphonate,[6] or pyruvate for fluoropyruvate,[7] with specific aldolases resulted in a decrease of activity of up to several orders of magnitude, reflecting the strong influence of steric and electronic factors on the intricate binding environment required to stabilize the highly ordered bisubstrate transition state. Only subtle variations in the nucleophilic substrate structure were found to be permissible, such as from protein engineering attempts[4c] to change the substrate specificity of D-fructose-6-phosphate aldolase (FSA; Scheme 1) from hydroxyacetone (**1**) to dihydroxyacetone (DHA)[8] or to hydroxyethanal,[9] that of transaldolase B^{F178Y}

Scheme 1. FSA Catalysis for the Cleavage of D-Fructose 6-Phosphate (Fru6P) into D-Glyceraldehyde 3-Phosphate (GA3P) and Dihydroxyacetone (DHA)



Received: December 14, 2015
Revised: January 29, 2016

DOI: 10.1021/acscatal.5b02805
ACS Catal. XXXX, XXX, XXX−XXX

from DHA to **1**,[10] or that of L-rhamnulose-1-phosphate aldolase from DHAP to DHA.[11]

We present herein an unprecedented, wide expansion of the nucleophilic substrate tolerance of FSA by structure-guided rational protein engineering to tune the substrate binding site for larger nucleophile structures that could potentially carry additional functionalization.

We have found that a single- or double-active-site mutation was sufficient to allow productive binding of a large number of non-natural nucleophilic components, unlocking an entry to a broad variety of chiral products that expand widely beyond the horizon of currently known biocatalysts.

RESULTS AND DISCUSSION

FSA is a class I aldolase that reversibly cleaves D-fructose 6-phosphate (Fru6P) via covalent substrate activation by Schiff base formation at K85 (Figure 1).[13] Wild-type FSA shows the highest activity with **1** and 1-hydroxy-2-butanone (**2**) as aldol nucleophiles[10,14] but tested negative with up to 150 mM of the higher homologous 1-hydroxy-2-pentanone (**3**). To increase the substrate tolerance of wild-type FSA for larger nucleophile structures, mutagenesis was directed at carefully selected active-site residues lining the substrate binding pocket. The crystal structure of FSA had been solved without liganded substrate (PDB entry 1l6w).[13] For better guidance, we have built a model by inserting the Fru6P structure from the substrate-liganded transaldolase B from *E. coli* (PDB entry 3s1v),[12] taking advantage of the very high structural homology among those enzymes (Figure 1A). The model suggests that the aliphatic portion of the preferred nucleophiles **1**/**2** will be in contact with the side chains of L107, A129, L163, and A165 that jointly form a hydrophobic binding pocket at the distal end of the active site. For a mutagenesis to increase the substrate-accessible volume, the selection included both bulky residues (i.e., L107, L163, and A129) that could reduce space restrictions for donor binding and residues that in earlier studies were identified to benefit the overall kinetic competence of the enzyme in the direction of synthesis (i.e., L107 and A129).[9,15] For an evaluation of variants toward a substrate tolerance beyond the C_4 donor **2**, we first selected a series of higher homologous ketols having longer aliphatic chains (**3**–**5**; C_5–C_7).[16] We chose to expand the substrate up to the C_7 ketol as the minimum chain length that allows investigating systematically the effect of branching, using only structures that were constitutional isomers and thus would pose similar requirements in total ligand volume.

In a first round, single-site variants L107A, A129G, and L163A (Figure 1) were assayed against wild-type FSA or A129S variant as a reference, for their ability to catalyze an aldol addition of the 1-hydroxyalkanones (**2**–**5**) (Figure 2A) to 3-hydroxypropanal (**16**), furnishing stable six-membered-ring structures (Table 1, **18**–**21**β,α). Both reaction components were chosen to be prochiral to avoid complications from kinetic enantiomer selectivity. The methylene unit introduced by **16** is a useful reporter for the product stereochemistry by proton

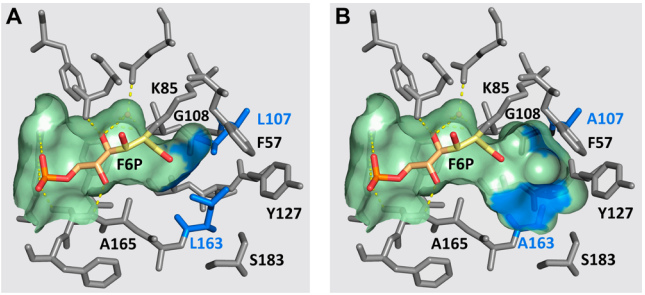


Figure 1. (A) Illustration of the substrate binding based on the X-ray crystal structure of wild-type FSA (PDB 1l6w) and its L107A/L163A variant. Active-site residues that are lining the substrate pocket are shown as light gray sticks. The inner surface of the cavity is frontally sliced for better inspection; the cavity opens to the left toward bulk solvent. The substrate is modeled by fitting the Fru6P structure from the highly similar complex of transaldolase (PDB 3s1v)[12] to form a Schiff base complex with K85. Substrate parts corresponding to electrophilic and nucleophilic moieties are shown in light orange and yellow, respectively. (B) Representation of the L107A/L163A double-site mutant showing the increased binding pocket that will take up the aliphatic substrate portion of extended nucleophile analogues (**3**–**15**). Figures were prepared using PyMOL.

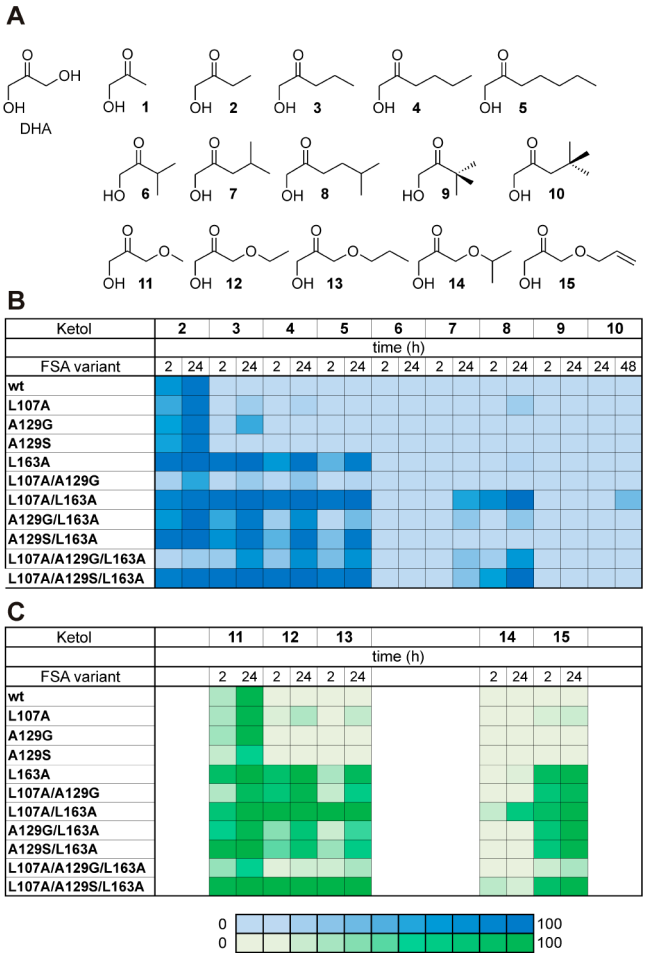
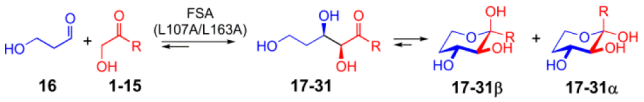


Figure 2. (A) Panel of substrates that can replace DHA with wild-type FSA (**1**, **2**), as well as non-natural substrate analogues (**3**–**15**)[16] considered for probing the substrate promiscuity of FSA variants. Structures are grouped in three series comprised of linear and branched-chain ketols and DHA monoalkyl ethers. (B) Variant screening for conversion of 1-hydroxy-2-alkanones **2**–**10**. (C) Data for 1,3-dihydroxypropanone monoalkyl ethers **11**–**15**. Mixtures of 100 mM ketol and 150 mM **16** were reacted in triethanolamine buffer (50 mM, pH 8, 0.2 mL) in the presence of 0.1 mg of enzyme, and the progress was monitored by HPLC. Ketols **11**–**14** are bioisosteric with **3**–**5** and **8**, respectively, and these columns are vertically aligned for better comparison.

DOI: 10.1021/acscatal.5b02805
ACS Catal. XXXX, XXX, XXX–XXX

Table 1. Preparative Synthesis of Aldol Products Using Catalysis by FSA Variant L107A/L163A for Addition of Artificial Nucleophiles to Nonchiral 3-Hydroxypropanal[a]



nucleophile	product	R	isolated yield (%)
1	**17**	CH_3	87[b]
2	**18**	CH_2CH_3	89[b]
3	**19**	$(CH_2)_2CH_3$	75
4	**20**	$(CH_2)_3CH_3$	76
5	**21**	$(CH_2)_4CH_3$	50
6	**22**	$CH(CH_3)_2$	*c*
7	**23**	$CH_2CH(CH_3)_2$	28
8	**24**	$(CH_2)_2CH(CH_3)_2$	25
9	**25**	$C(CH_3)_3$	*d*
10	**26**	$CH_2C(CH_3)_3$	25
11	**27**	CH_2OCH_3	89
12	**28**	$CH_2OCH_2CH_3$	82
13	**29**	$CH_2O(CH_2)_2CH_3$	28
14	**30**	$CH_2OC(CH_3)_2$	30
15	**31**	$CH_2OCH_2CH{=}CH_2$	45

[a]Mixtures of 150 mM ketol and 100 mM **16** were reacted in GlyGly buffer (50 mM, pH 8.5, 10 mL) in the presence of 15 mg of variant L107A/L163A, and products were isolated by silica gel chromatography. In each case, dr > 98% was assessed by ^{1}H NMR analysis. [b]Identical products have been isolated using wild-type FSA (see ref 10). [c]No conversion. [d]Yield not determined.

NMR spectroscopy.[10] All nucleophiles larger than **2** were only partially converted by variants designed for increased substrate space (Figure 2B); compound **4** was accepted only by the Leu mutants, and the largest probe **5** was converted by the L163A variant only. The L163A variant consistently showed the highest rates with all nucleophiles, causing complete conversion of **2** and **3** already during the initial 2 h reaction phase and nearing complete conversion within 24 h. For the second round, we constructed all double-site combinations starting with the most successful L163A variant, and the potentially beneficial L107A/A129G combination. All variants showed good to excellent activity with **2**–**4**, while **5** was converted only by those incorporating at least the L163A replacement. Remarkably, only the L107A/L163A mutant achieved high conversion already after short reaction times. In a third stage, all positive mutations were combined into triple-site variants. Only the variant L107A/A129S/L163A showed excellent activity with **3**–**5**, practically indistinguishable from its L107A/L163A progenitor, whereas that incorporating the A129G mutation displayed considerably lower activity. Any combination of the A129G with the L107A mutation has a detrimental effect on catalysis, possibly because of higher backbone mobility arising from placing a Gly residue next to the void created by the L163 replacement. In comparison, combination of the A129G with the L163A exchange is practically neutral.

As an increasing steric challenge for substrate binding, we further tested a set of isomeric ketols that contained a single (**6**–**8**) or complete terminal branching (**9** and **10**). All substrate analogues having at least one methylene group between the carbonyl and the branch point (**7**, **8**, and **10**) were converted by those FSA variants, incorporating the L163A mutation with at least one additional space-generating variation, albeit at substantially reduced reaction rates in comparison to the corresponding straight-chain isomers (Figure 2B). Branching directly adjacent to the ketone (**6**, **9**) was not tolerated by any of the FSA variants. Failure to deliver an aldol product most likely is caused by steric interference during one of the early steps in catalysis.[12,17] Although an increasing level of alkyl branching, up to the neopentyl situation (**10**), leads to a more compact size and reduced molecular volume for this substrate part, it appears that linear chains benefit from their higher conformational flexibility in adapting to the binding cavity created by mutagenesis. Generally, variant L107A/L163A seems to be the superior catalyst design among the variations tested.

In addition to a mere size increase by carbon chain extension, we next explored the set of engineered FSA for their flexibility toward ketol nucleophiles with modified chemical functionality (Figure 2C). We tested a series of DHA ether derivatives **11**–**14** analogous to the alkanones,[16] and also included the allyl compound **15** to facilitate specific postsynthetic modifications. We chose the ether series for several reasons: (i) increased carbonyl electrophilicity, (ii) the ability to gain dipolar contacts to the donor binding site at similar conformational flexibility, (iii) their rather high chemical stability, and (iv) their frequent occurrence as a structural unit in many important natural products and bioactive drugs. Methyl ether **11** as the smallest member showed good reaction rates for all variants, even for wild-type FSA. The latter was somewhat surprising, because the isosteric alkanone **3** is no substrate for wild-type FSA. Higher homologous linear ethers **12** and **13** were converted well by all double-site (or higher) variants, with a reactivity pattern similar to that observed for the alkanone series, but were not tolerated by wild-type FSA. Isopropyl ether **14** was also converted, although at reduced reaction rates that did not yield complete conversion within 24 h.

Initial rates (v_0) measured for the top variants from each generation indicate that the ethers appeared to be somewhat more reactive than the corresponding alkanones (Figure 3). This was confirmed by a direct competition experiment using an equimolar mixture of ketols **3**/**11** for addition to **16** catalyzed by the L107A/L163A variant. Reaction monitoring by in situ NMR analysis revealed the ether **11** indeed showed about 1 order of magnitude higher initial reactivity than **3** (i.e.,

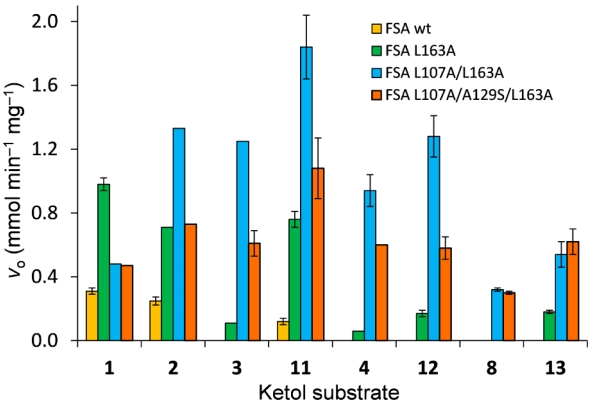


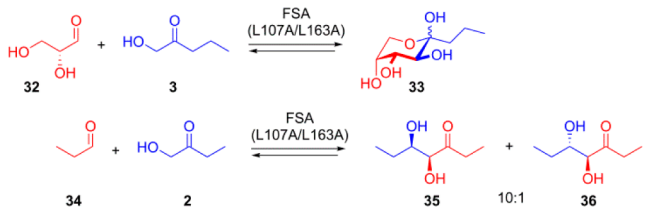
Figure 3. Comparison of initial rates for top FSA variants with nonnatural substrates. Mixtures of 100 mM ketol and 150 mM **16** in triethanolamine buffer (50 mM, pH 8, 1 mL) were reacted in the presence of 60–200 μg of enzyme, and progress was monitored by HPLC (<10% conversion). Data are the mean of at least triplicate experiments ± standard error of the mean.

DOI: 10.1021/acscatal.5b02805
ACS Catal. XXXX, XXX, XXX−XXX

1.8×10^{-4} vs 2.2×10^{-5} mmol min^{-1} mg^{-1}; see Figure S4 in the Supporting Information) and yielded a higher overall competitive conversion (89% vs 17%).

Wild-type FSA had previously been demonstrated to catalyze a highly D-*threo* stereoselective carboligation, practically irrespective of structural variations in the hitherto known nucleophilic (DHA, **2**, **3**, glycolaldehyde) and electrophilic aldol components.[10,14,18] To assess the level of stereoselectivity in the carboligation with non-native nucleophiles **2**−**15**, products **18**−**31** were isolated (25−89% yield) from preparative reactions catalyzed by the L107A/L163A variant (Table 1). Analysis of crude reaction mixtures by HPLC and NMR analysis revealed that only a single type of stereoisomer was formed in all cases (see pp S22−S57 in the Supporting Information). Because both aldol substrates were chosen to be prochiral for simplicity, unambiguous configurational determination of the aldol products required correlation with an established chirality. We note that products **19**−**31** were hitherto inaccessible by biocatalytic routes and yet are unknown from natural sources for comparison. The assignment of the absolute D-*threo* configuration created by catalysis of variant L107A/L163A was therefore confirmed by the analogous addition of **3** to enantiopure D-glyceraldehyde (**32**) for an internal chiral reference, which furnished the expected adduct as a single diastereomer (Scheme 2 and Figure S16 in the

Scheme 2. Absolute D-*threo* Diastereospecificity for FSA Variant L107A/L163A Using Enantiopure D-Glyceraldehyde 32 and Observation of Incomplete Specificity upon Addition to Propanal 34[a]



[a]Mixtures of 150 mM ketol and 100 mM aldehyde were reacted in GlyGly buffer (50 mM, pH 8.5, 10 mL) upon catalysis by variant L107A/L163A, and products were isolated by silica gel chromatography.

Supporting Information). Also, exemplary addition of **2** to propanal as a simple, nonfunctionalized aliphatic acceptor furnished the corresponding product, demonstrating the broad utility of the method. However, stereoselectivity was incomplete with a 10:1 ratio for *threo*/*erythro* configuration (Scheme 2 and Figure S17 in the Supporting Information),[10,19] which may originate from a less specific binding orientation of the unsubstituted aliphatic chain.

A critical mutation of the engineered FSA is the L163A replacement that creates additional space in a direction where an enlargement of the substrate apparently can be well accommodated in a rather extended conformation, while the L107A mutation seems to require a less favorable bent orientation of the ketol. In the L107A/L163A double-site variant, the contiguous extra void now expanding to second-sphere protein residues (e.g., F57, Y127, A164, and S183; see Figure 1B) enables binding of ketols with chain lengths (at least) up to C_7. It is noteworthy that in comparison to the activity of wild-type FSA with **2** (i.e., the best substrate together with **1**) the engineered L107A/L163A variant is equally efficient with the branched-chain C_7 ketol **8** and even more efficient with the linear C_7 ketol **5**. This points out that the expanded substrate binding space, which plausibly will be filled with water molecules in the native state, does not interfere with the overall kinetics of substrate binding and release. We interpret the success of our design strategy to be due to the fact that the catalytic machinery remained intact; in particular, the mutations did not interfere with (i) the covalent substrate activation by Schiff base formation at K85 or (ii) the catalytic function of acid−base residue Tyr131; (iii) in addition, it did not induce a disturbing change or switch of the H-bonding network.[20]

Mutagenic creation of a larger cavity extending from the donor-binding pocket may be expected to destabilize the rigid hydrophobic core surrounding the FSA active site (T_m = 87.0 °C).[21] Interestingly, the more hidden L107A modification (T_m = 90.4 °C) actually leads to protein *stabilization*, as judged by differential scanning fluorimetry, whereas the stability of the L163A variant (T_m = 78.4 °C) substantially decreased (Figure 4

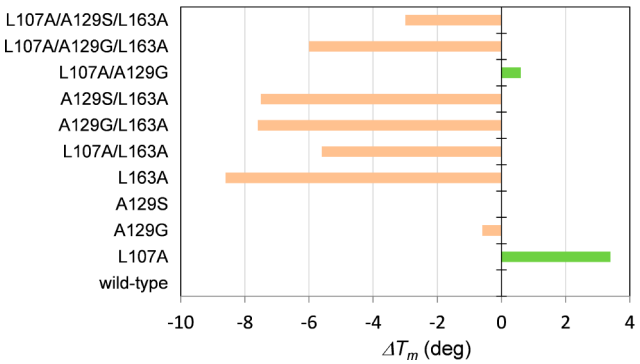


Figure 4. Changes in the unfolding transition temperature (ΔT_m) in reference to wild-type FSA. A negative ΔT_m value indicates a destabilizing mutation, and a positive ΔT_m value shows stabilization.

and Table S4 in the Supporting Information). Both consequences seem independent, as in the combined L107A/L163A mutant (T_m = 81.4 °C) the effect apparently is additive. Considering that engineered enzymes are often compromised by low stability,[22] which hampers their practical use, the outcome for the new FSA variants is significant, since they retain high stability for preparative applications.

CONCLUSION

The unprecedented realization of wide substrate promiscuity of an aldolase for the nucleophilic component provides insight into the malleability of protein active sites, even in the case of aldolases that are generally perceived to have strict specificity.[4,23] This is highly relevant to our understanding of enzyme evolution and should improve the general knowledge of substrate−protein interactions for future endeavors in protein engineering. The modular nature of the enzymatic aldol synthesis is amenable to considerable structural variation, previously only for the electrophilic component but now also for the nucleophilic component, which allows the targeting of a largely extended range of product structures in a combinatorial fashion. We have shown that this critical feature in synthetic versatility allows the rapid, protecting group free construction of unnatural carbohydrate analogues, exemplified by **17**−**31**. We anticipate that the FSA mutants will be useful for the asymmetric synthesis also of more generic, non-carbohydrate

DOI: 10.1021/acscatal.5b02805
ACS Catal. XXXX, XXX, XXX−XXX

chiral building block structures (e.g., **35**) that expand widely beyond the horizon of currently known biocatalysts. The designed FSA variants are expected to stimulate innovative biotechnological applications, including the stereoselective access to novel product families that hitherto have not been readily available for pharmaceutical study.

EXPERIMENTAL SECTION

General Procedure for Enzymatic Syntheses. Lyophilized FSA L107A/L163A variant (15 mg) was added to a solution (10 mL total reaction volume) containing the respective ketol (150 mM) and aldehyde components (100 mM) in glycyl-glycine buffer (50 mM, pH 8.5), and the resulting mixture was incubated at room temperature with monitoring at regular intervals by TLC (chloroform/methanol 5/1). Depending on the rate of aldehyde consumption, completed reactions were worked up after 24−48 h by lyophilization of the crude reaction mixture. The residue was purified by silica gel column chromatography using chloroform/methanol (15/1 to 5/1) as eluent to provide the pure aldol products.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.5b02805.

Materials, general procedures, protein expression and purification, differential scanning fluorimetry, enzymatic aldol reactions, compound characterization, and NMR spectra (PDF)

AUTHOR INFORMATION

Corresponding Authors

*E-mail for P.C.: pere.clapes@iqac.csic.es.

*E-mail for W.-D.F.: fessner@tu-darmstadt.de.

Author Contributions

[§]These authors contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by the Bundesministerium für Bildung und Forschung (BMBF grant 0315775B PT-J to W.-D.F.) and the Ministerio de Economía y Competitividad (MINECO) (grant CTQ2012-31605 to P.C.), within the transnational Eurotrans-Bio framework, as well as by student exchange funds from the DAAD (grant PPP-50749958 to W.-D.F.), Acciones Integradas (MINECO; grant AIB2010DE-00405 to P.C.), and COST action CM1303 *Systems Biocatalysis*.

REFERENCES

(1) (a) O'Brien, P. J.; Herschlag, D. *Chem. Biol.* **1999**, *6*, R91−R105. (b) Khersonsky, O.; Tawfik, D. S. *Annu. Rev. Biochem.* **2010**, *79*, 471−505. (c) Pandya, C.; Farelli, J. D.; Dunaway-Mariano, D.; Allen, K. N. *J. Biol. Chem.* **2014**, *289*, 30229−30236.

(2) Toscano, M. D.; Woycechowsky, K. J.; Hilvert, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 3212−3236.

(3) Nobeli, I.; Favia, A. D.; Thornton, J. M. *Nat. Biotechnol.* **2009**, *27*, 157−167.

(4) (a) Fessner, W.-D. In *Enzyme Catalysis in Organic Synthesis*, 3rd ed.; Drauz, K., Groger, H., May, O., Eds.; Wiley-VCH: Weinheim, Germany, 2011; Vol. 2, pp 857−917. (b) Brovetto, M.; Gamenara, D.; Saenz Méndez, P.; Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346−4403. (c) Windle, C. L.; Müller, M.; Nelson, A.; Berry, A. *Curr. Opin. Chem. Biol.* **2014**, *19*, 25−33. (d) Clapés, P. In *Biocatalysis in Organic Synthesis*; Faber, K., Fessner, W.-D., Turner, N. J., Eds.; Georg Thieme Verlag: Stuttgart, Germany, 2015; Vol. 2, pp 31−92.

(5) Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 741−748.

(6) (a) Fessner, W.-D.; Sinerius, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 209−212. (b) Arth, H. L.; Fessner, W.-D. *Carbohydr. Res.* **1997**, *305*, 313−321.

(7) Watts, A. G.; Withers, S. G. *Can. J. Chem.* **2004**, *82*, 1581−1588.

(8) Castillo, J. A.; Guérard-Hélaine, C.; Gutiérrez, M.; Garrabou, X.; Sancelme, M.; Schürmann, M.; Inoue, T.; Hélaine, V.; Charmantray, F.; Gefflaut, T.; Hecquet, L.; Joglar, J.; Clapés, P.; Sprenger, G. A.; Lemaire, M. *Adv. Synth. Catal.* **2010**, *352*, 1039−1046.

(9) (a) Szekrenyi, A.; Soler, A.; Garrabou, X.; Guerard-Helaine, C.; Parella, T.; Joglar, J.; Lemaire, M.; Bujons, J.; Clapés, P. *Chem. - Eur. J.* **2014**, *20*, 12572−12583. (b) Szekrenyi, A.; Garrabou, X.; Parella, T.; Joglar, J.; Bujons, J.; Clapés, P. *Nat. Chem.* **2015**, *7*, 724−729.

(10) Rale, M.; Schneider, S.; Sprenger, G. A.; Samland, A. K.; Fessner, W.-D. *Chem. - Eur. J.* **2011**, *17*, 2623−2632.

(11) Garrabou, X.; Joglar, J.; Parella, T.; Bujons, J.; Clapés, P. *Adv. Synth. Catal.* **2011**, *353*, 89−99.

(12) Lehwess-Litzmann, A.; Neumann, P.; Parthier, C.; Lüdtke, S.; Golbik, R.; Ficner, R.; Tittmann, K. *Nat. Chem. Biol.* **2011**, *7*, 678−684.

(13) Thorell, S.; Schürmann, M.; Sprenger, G. A.; Schneider, G. *J. Mol. Biol.* **2002**, *319*, 161−171.

(14) Samland, A. K.; Rale, M.; Sprenger, G. A.; Fessner, W.-D. *ChemBioChem* **2011**, *12*, 1454−1474.

(15) Gutierrez, M.; Parella, T.; Joglar, J.; Bujons, J.; Clapés, P. *Chem. Commun.* **2011**, *47*, 5762−5764.

(16) Güclü, D.; Rale, M.; Fessner, W.-D. *Eur. J. Org. Chem.* **2015**, *2015*, 2960−2964.

(17) Samland, A. K.; Sprenger, G. A. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 253−264.

(18) Garrabou, X.; Castillo, J. A.; Guérard-Hélaine, C.; Parella, T.; Joglar, J.; Lemaire, M.; Clapés, P. *Angew. Chem., Int. Ed.* **2009**, *48*, 5521−5525.

(19) Products reported in ref 10 for related reactions catalyzed by wild-type FSA also seem to contain similar levels of diastereoisomer formation.

(20) (a) Tittmann, K. *Bioorg. Chem.* **2014**, *57*, 263−280. (b) Stellmacher, L.; Sandalova, T.; Leptihn, S.; Schneider, G.; Sprenger, G. A.; Samland, A. K. *ChemCatChem* **2015**, *7*, 3140−3151. (c) Sautner, V.; Friedrich, M. M.; Lehwess-Litzmann, A.; Tittmann, K. *Biochemistry* **2015**, *54*, 4475−4486.

(21) (a) Eriksson, A.; Baase, W.; Zhang, X.; Heinz, D.; Blaber, M.; Baldwin, E.; Matthews, B. *Science* **1992**, *255*, 178−183. (b) Buckle, A. M.; Henrick, K.; Fersht, A. R. *J. Mol. Biol.* **1993**, *234*, 847−860.

(22) (a) Bloom, J. D.; Arnold, F. H. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 9995−10000. (b) Tokuriki, N.; Tawfik, D. S. *Curr. Opin. Struct. Biol.* **2009**, *19*, 596−604.

(23) Clapés, P.; Garrabou, X. *Adv. Synth. Catal.* **2011**, *353*, 2263−2283.

DOI: 10.1021/acscatal.5b02805
ACS Catal. XXXX, XXX, XXX−XXX