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Asymmetric Self- and Cross-Aldol Reactions of Glycolaldehyde Catalyzed by D-Fructose-6-phosphate Aldolase**

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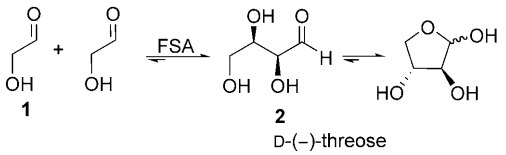
Aldol additions are key chemical reactions for the construction of chiral complex polyhydroxylated molecules.[1–4] Recent developments in direct aldol additions using bio-, organo-, and metal catalysts are promising since these methodologies do not require separate generation of enolate equivalents and thus improve the atom economy of the transformation.[1,2,5–8] Aldehydes have been regarded as highly interesting donors in aldol reactions, because the products formed are themselves aldehydes that can be used in further aldol additions for the construction of complex polyfunctional molecular frameworks.[4] Hence, the direct catalytic cross-aldol reaction of aldehydes constitutes a challenge for these methodologies.[4,9,10] Self- and cross-aldol reactions were achieved by organocatalysis in *N,N*-dimethylformamide (DMF) using simple aliphatic and aromatic aldehydes.[11–14] Self- and cross-aldol additions involving glycolaldehyde derivatives are of paramount interest because they allow access to polyol architectures.[9,13] Organocatalytic self- and cross-aldol additions of free glycolaldehyde failed to provide promising results.[15,16] A successful self-aldol addition was accomplished in DMF, but it was limited to glycolaldehyde derivatives with electron-rich α-alkyloxy or bulky α-silyloxy protecting groups.[13] No further additions were observed on the corresponding aldol adducts, a feature essential for a two-step aldol-based synthesis of carbohydrates.[13] This approach was used to prepare protected hexoses: a direct organocatalytic self-aldol addition was followed by a direct metal-catalyzed aldol addition.[17] In cross-aldol additions, the organocatalyst cannot selectively control the donor and acceptor roles; this is governed by the aldehyde structure and reactivity.[9] Therefore, in the presence of simple aliphatic aldehyde donors[9,13] O-protected glycolaldehyde derivatives act invariably as acceptors, likely because they are kinetically disfavored as donors.[18]

Biocatalytic synthetic strategies for carbohydrates and their analogues require water-soluble polyhydroxyaldehyde derivatives as acceptor substrates for aldolases.[19,20] Multistep strategies have suffered from the laborious and costly isolation of sensitive deprotected hydroxyaldehydes which are usually obtained by chemical means.[21] In addition, the vast majority of reported biocatalytically prepared carbohydrates and related products are ketoses. This is because aldolases specific for aldose-type sugars are scarce in nature; 2-deoxyribose-5-phosphate aldolase (DERA) is a notable exception and actually functions as a deoxysugar aldolase.[22–26] Hence, cross-aldol reactions of aldehydes have been a limited field for biocatalysis, and DERA is the only enzyme known to catalyze the stereoselective cross-aldol addition of acetaldehyde to other aldehydes. However, the low conversion rates of this enzyme with non-phosphorylated, unnatural substrates and its inability to generate two consecutive hydroxylated positions with each newly formed bond limit considerably its scope of applicability. Consequently, the biocatalytic self- and cross-aldol additions of glycolaldehyde are a challenge for the cascade two-step synthesis of carbohydrates.

Recently, we reported the synthesis of iminosugars and other polyhydroxylated compounds catalyzed by D-fructose-6-phosphate aldolase (FSA).[27,28] This aldolase shows an unprecedented tolerance for donor substrates such as dihydroxyacetone (DHA), hydroxyacetone (HA), and 1-hydroxy-2-butanone.[28–30] In the course of our investigations on the catalytic properties of FSA, we discovered a new and unexpected activity of paramount importance: its ability to catalyze the direct stereoselective self-aldol addition of glycolaldehyde (GA) (**1**) to furnish D-(−)-threose (**2**) (Scheme 1).[31] In this reaction, GA (**1**) acts as both the



Scheme 1. FSA-catalyzed self-aldol addition of glycolaldehyde (**1**).

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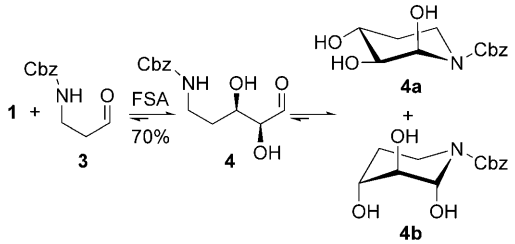
donor and acceptor substrate for FSA. This tolerance for an aldehyde was unprecedented and unexpected for FSA and other related ketose-dependent aldolases (e.g. DHAP aldolases).

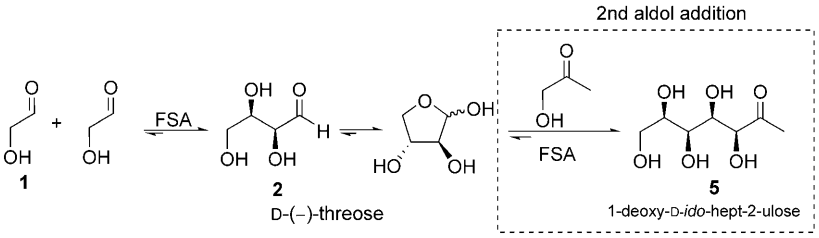
We next explored the ability of FSA to catalyze the direct cross-aldol addition of **1** to other aldehyde acceptors. To this end, aldehyde **3**, a well-known acceptor substrate for FSA,[27] was selected as an example. Interestingly, a preliminary experiment gave 70% conversion of **3** to the cross-aldol adduct **4** (>97% *syn*)[32] as a mixture of the two cyclic isomeric forms **4a** and **4b** (Scheme 2; see the Supporting Information).



Scheme 2. Cross-aldol addition of **1** to **3** catalyzed by FSA. Cbz = benzyloxycarbonyl.

To understand and further exploit this new catalytic potential of FSA for the direct cross-aldol additions of aldehydes, we determined the kinetic parameters for enzymatic aldol reactions with dihydroxyacetone (DHA) and hydroxyacetone (HA) as donors and GA as both donor and acceptor (Table 1). In addition, the kinetic parameters for the retro-aldol reaction with D-fructose-6-phosphate (D-F6P), D-arabinose-5-phosphate (D-Ara-5-P), and D-threose were measured. As the reported kinetic parameters for DHA and HA have significant discrepancies,[29,30] we determined our own parameters for this study (Table 1, entries 1 and 2). The kinetic model for an enzyme-catalyzed dimerization reaction developed by Neuhaus[33] was applied to determine the K_M of GA as the donor (entry 4) as well as the acceptor (Table 1, entry 5) in the self-aldol reaction.

GA and DHA are similar donors in terms of their V_{max} values but GA binds to FSA with higher affinity than DHA, as indicated by its K_M value. Hence, the fact that GA is a better donor substrate than DHA may explain why when DHA and GA are mixed in the presence of FSA, **2** is the major product whereas the expected D-xylulose was not detected.[28] More importantly, a second in situ cross-aldol addition of HA to **2** was indeed possible, furnishing 1-deoxy-D-*ido*-hept-2-ulose (**5**)[28] in 68% yield (Scheme 3), whereas no reaction was detected with either DHA or GA. As judged by the ratio V_{max}/K_M, FSA functions with HA approximately 45 times better than with DHA, which might reflect the different reactivities of these substrates towards aldehyde acceptors.[28–30] Moreover, **2** binds to FSA at both the acceptor and donor sites, with an affinity similar to that of GA as the donor (Table 1, entries 4 and 7); this likely interferes with the trimerization reaction. The formation of **5** illustrates the application of a cascade of two consecutive aldol additions with two different donors catalyzed by FSA. The kinetic parameters for the FSA-catalyzed retro-aldol reactions of some sugar derivatives (Table 1, entries 6–8) indicate that the affinity follows the order D-threose > D-Ara-5-P > D-F6P,



Scheme 3. FSA-catalyzed dimerization of GA (**1**) and a second aldol addition of HA to D-(−)-threose in situ.

Table 1: Steady-state kinetic parameters of aldol and retro-aldol reactions catalyzed by D-fructose-6-phosphate aldolase.

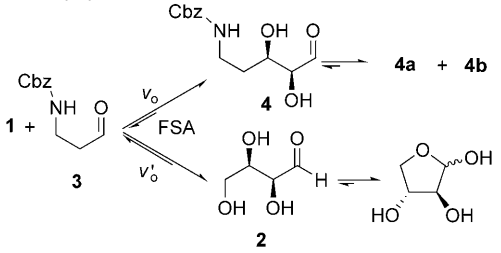
Entry	Compound	K_M [mM]	V_{max} [U[e] mg^{-1} powder]	$(V_{max}/K_M)/10^{-6}$ [min^{-1} mg^{-1}]
1	DHA[a]	32 ± 2	1.46 ± 0.05	46 ± 3
2	HA[a]	17.4 ± 0.5	33.7 ± 0.9	1937 ± 76
3	GA (**1**)[a]	nd[c]	1.5 ± 0.3	nd[c]
4	GA(don.)[b]	0.197 ± 0.045	} 0.22 ± 0.02	1117 ± 274
5	GA(acc.)[b]	62.8 ± 5.5		3.5 ± 0.4
6	D-F6P	9[d]	0.34 ± 0.02	37
7	D-threose (**2**)	0.286 ± 0.017	(1.15 ± 0.04) × 10^{-3}	4.0 ± 0.3
8	D-Ara-5-P	0.561 ± 0.042	0.160 ± 0.012	285 ± 30

[a] Aldol addition to D,L-glyceraldehyde-3-phosphate (D,L-G3P). [b] Self-aldol addition of GA. [c] nd: not determined; the K_M value of GA, as donor, for the reaction GA + D,L-G3P could not be correctly measured, because of the competing self-aldol addition of GA. [d] Data from Ref. [34]. [e] U: µmol consumed per minute. Specific activity of D-fructose-6-phosphate (D-F6P) cleavage = 0.34 ± 0.02 U mg^{-1} FSA powder. (see the Supporting Information for further details)

and, as V_{max}/K_M reflects, D-Ara-5-P is the best substrate in this series. Hence, the metabolic function of FSA, which remains uncertain, may not be restricted to the cleavage of D-F6P; FSA might have a different, even multipurpose function in carbohydrate metabolism that now warrants further investigation.

The kinetic data (Table 1) indicate that the apparent catalytic efficiency for GA is approximately 320-fold better as the donor than as the acceptor. This is of paramount importance since it makes possible to optimize the cross-aldol addition by controlling the concentration of GA during the reaction. We examined in detail the *syn*-selective cross-aldol addition of **1** to **3**. We determined the initial rates of cross-aldol (v_o) and self-aldol (i.e. formation of **2**) (v_o') reactions at different concentrations of [**1**] and [**3**] to shed light on the reaction preferences (Table 2). In good agreement with the K_M value, when [**1**] < 50 mM and with an excess of **3**, $v_o > v_o'$, and the cross-aldol reaction is favored (Table 2).

Table 2: FSA-catalyzed cross-aldol additions of GA (**1**) to **3**. Initial reaction rates of the cross-aldol addition (v_o) and the competing self-aldol addition (v_o') of **1**.



[**1**], [**3**] [mM, mM]	v_o cross-aldol [μmol min^{-1}]	v_o' auto-aldol [μmol min^{-1}]	v_o/v_o'
10, 100	0.37	0.04	9.3
25, 100	0.15	0.06	2.3
50, 100	0.09	0.11	0.8
25, 25	0.07	0.06	1.1
50, 50	0.04	0.11	0.4
100, 50	0.03	0.12	0.2

On the other hand, the higher the concentration of **1**, the faster the rate v_o' achieved regardless of the concentration of **3**. In practice, it is convenient to keep [**1**] < 5 mM in the reaction mixture to ensure a saturation concentration of **1** as the donor and well under saturation levels as the acceptor, therefore resulting in a high ratio of cross- to self-aldol reaction rates. We conducted a set of experiments (see the Supporting Information) in which a solution of **1** was added with a syringe pump in order to approximate a constant concentration of **1;** the rate of the cross-aldol reaction was found to be much higher than that of the self-aldol (Figure 1). Moreover, the D-(−)-threose generated reacted further, at much a slower rate (as expected according to its V_{max}/K_M) to furnish **4a** and **4b** under equilibrium conditions. These conditions can be generalized for other acceptor aldehydes; however, the outcome will depend upon the substrate quality (i.e. the K_M value should be lower than that of **1** as the acceptor) and the tendency for cyclization of the adduct formed (e.g. **4a** and **4b**).

In further examples (Scheme 4) the FSA catalyst showed exquisite *syn* stereoselectivity and good to excellent conversions, and provided the products in yields between 28 and 68% under nonoptimized reaction conditions and purification procedures. L-Lactaldehyde (**6**), easily obtained by a previously described methodology,[35,36] furnished 5-deoxy-L-xylose (**7**) in a single step. In this case, $v_o/v_o' = 1.24$ at a concentration of 25 mM for each substrate, indicating that **6** is a relatively good acceptor. Furthermore, we observed that when a mixture of **2** and **6** (2 equiv) was treated with FSA, **7** was obtained, indicating that both equilibrium thermodynamics and kinetics favor **7** and that **2** acts as donor substrate as well (i.e. by means of a retro-aldol/aldol sequence).

Racemic **8** was also accepted yielding the iminosugar cyclic derivatives **9a** and **9b** as major products; the non-cyclized isomer was not detected by NMR spectroscopy. In this case, quantitative conversion was achieved in the first tests indicating that **8** is a good acceptor (see the Supporting Information). Removal of the Cbz protecting group of **9**

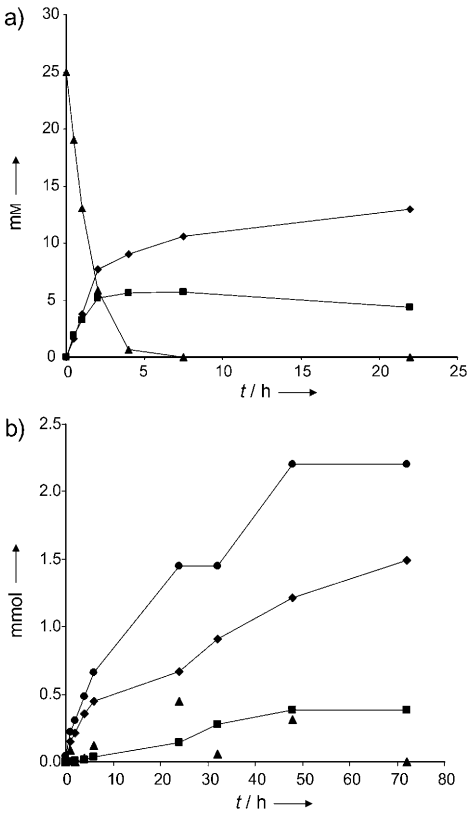
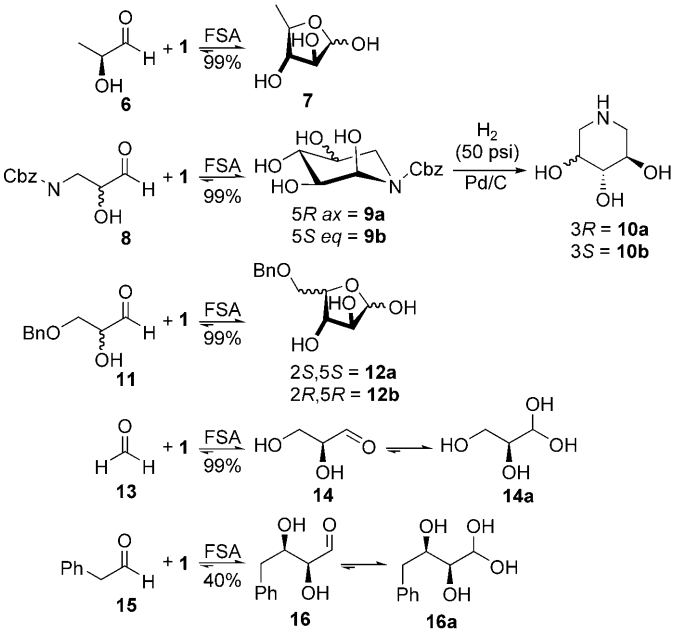


Figure 1. Progress of the FSA-catalyzed direct aldol addition of GA (**1**) to **3**. a) Initial amount of reactants [**1**] = [**3**] = 25 mM; amounts of cross-aldol product **4** (◆), self-aldol product **2** (■), and **1** (▲) in solution. b) Total **1** added 2.2 mmol, **3** = 2 mmol; amounts of cross-aldol product **4** (◆), self-aldol product **2** (■), **1** added by a syringe pump (●). See the Supporting Information for details.



Scheme 4. FSA-catalyzed aldol additions of **1** and selected aldehydes. Conversions of the enzyme-catalyzed reaction are given under the reaction arrows.

followed by reductive amination led to the 1-deoxyiminosugars **10a** and **10b**, which are of interest as glycosidase inhibitors.[37–39] 3-Benzyloxy-D,L-glyceraldehyde (**11**) was a good substrate and furnished a mixture of 5-*O*-benzyl-protected precursors of L-xylose and D-arabinose (**12a** (67%) and **12b** (33%), respectively). Reaction with formaldehyde (**13**) furnished L-glyceraldehyde (**14**) quantitatively; v_o/v_o' was 2.22 at substrate concentrations of 25 mM, as expected since **13** is an excellent substrate in enzymatic aldol additions.[40,41] Phenylacetaldehyde (**15**) was also a substrate with a good v_o/v_o' ratio (i.e. 1.39 at [**15**] = [**1**] = 25 mM). Interestingly, a single aldol addition was observed in all the examples under the reaction conditions employed; this is also of great value for a two-step biocatalytic aldol addition for the stereoselective synthesis of carbohydrates.

In conclusion, we have discovered a new general activity of FSA, unprecedented for ketose-dependent aldolases. As a result, a new biocatalytic strategy based on FSA for the direct *syn* cross-aldol addition of glycolaldehyde to aldehydes in aqueous media has been developed. As the high affinity of GA for FSA as a donor is higher than that as an acceptor, cross-aldol reactions are possible with good to poor aldehyde acceptors if the concentration of GA in the reaction is kept low. Significantly, the control of the nucleophile and the electrophilic acceptor depend on FSA regardless of the absolute configuration and the intrinsic relative reactivity of the aldehydes, in contrast to what occurs in organocatalytic reactions. Hence, in the cross-aldol additions of GA to the aldehydes selected, the former acted invariably as the donor. The fact that aldol adducts are inert to further GA additions is advantageous. Therefore, new synthetic strategies can be easily devised through in situ or enzyme-catalyzed two-step aldol cascade reactions. In fact, by FSA catalysis we have efficiently generated various aldehydes that were previously used in reported enzymatic aldol reactions.[21,42–45] Structure-based site-directed mutagenesis of the FSA active center and exploitation of directed evolution to modify its donor or acceptor substrate tolerance and the stereochemical outcome of the reaction can expand the synthetic possibilities. Work in this direction is currently in progress in our lab.

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