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Fructose-6-phosphate Aldolase in Organic Synthesis: Preparation of D-Fagomine, *N*-Alkylated Derivatives, and Preliminary Biological Assays

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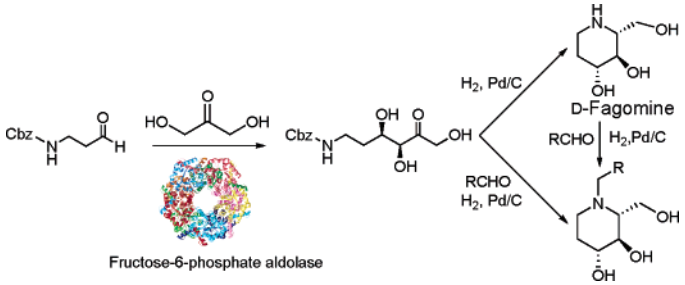
ABSTRACT



D-Fructose-6-phosphate aldolase (FSA) mediates a novel straightforward two-step chemo-enzymatic synthesis of D-fagomine and some of its *N*-alkylated derivatives in 51% isolated yield and 99% de. The key step is the FSA-catalyzed aldol addition of simple dihydroxyacetone (DHA) to *N*-Cbz-3-aminopropanal. The use of FSA greatly simplifies the enzymatic procedures that used dihydroxyacetonephosphate or DHA/esters. Some *N*-alkyl derivatives synthesized elicited antifungal and antibacterial activity as well as enhanced inhibitory activity, and selectivity against *β*-galactosidase and α-glucosidase.

D-Fagomine, (2*R*,3*R*,4*R*)-2-hydroxymethylpiperidine-3,4-diol is a naturally occurring iminosugar that was first isolated from buckwheat seeds of *Fagopyrum esculentum* Moench[1] with remarkable biological properties. This iminosugar has inhibitory activity against mammalian intestinal α-, *β*-glucosidase and α-, *β*-galactosidase.[2,3] Moreover, it appears to have a potent antihyperglycemic effect on streptozotocin-induced diabetic mice and potentiates glucose-induced insulin secretion.[4] Segraves et al.[5] have found that *C*-alkylated 3,4-di-*epi*-fagomines from *Batzella* sp. sponge have a good antimicrobial activity against *Staphylococcus epidermidis*.

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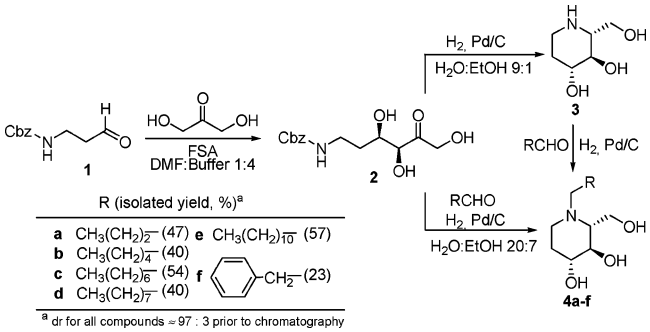
Chemical synthetic approaches of D-fagomine and its derivatives involve cumbersome protection−deprotection reactions and chiral starting materials, and therefore, moderate global yields are achieved.[6,7] Recent syntheses of D-fagomine and other stereoisomers have been described starting from chiral D-serine-derived Garner aldehyde in six to seven steps with global yields around 12%.[8]

Stereodivergent asymmetric chemo-enzymatic methodologies are mostly based on dihydroxyacetone phosphate (DHAP)-dependent aldolases. The key step is the stereoselective aldol addition of DHAP or DHA/arsenate (500 mM) to synthetic equivalents of aminoaldehydes.[9,10] Given the toxicity of arsenate, it would be more attractive to find a system allowing the use of "naked" DHA.[11] Chemical synthesis of DHAP involves several steps in ca. 70% overall yield.[12] Alternatively, enzymatic methods to generate DHAP, which can be coupled with the aldol reaction, have also been described.[13] However, some limitations arising from the lack of compatibility of conditions between the coupled enzymatic reactions and the generation of complex mixtures that make the product separation and purification difficult have been observed.[14]

We report herein a straightforward procedure for the stereoselective synthesis of D-fagomine and *N*-alkylated derivatives using fructose-6-phosphate aldolase (FSA) as biocatalyst and achiral easily accessible starting materials. The key step in this synthetic scheme was the stereoselective aldol addition of simple dihydroxyacetone (DHA) to **1** catalyzed by FSA (Scheme 1). FSA is a novel class I aldolase from *E. coli* related to a novel group of bacterial transaldolases, which catalyzes the aldol addition of DHA to glyceraldehyde-3-phosphate. The cloning and overexpression in *E.* coli DH5α of the gene encoding FSA and the biochemical characterization was carried out for the first time by Schürmann et al.[15] These authors[16] reported aldol additions of either DHA or hydroxyacetone to some hydroxyaldehydes for the synthesis of sugar derivatives. The most interesting feature of FSA is that utilizes DHA instead of either DHAP or DHA/esters which greatly simplifies the chemo-enzymatic strategies to α,*β*-dihydroxyketones.

Scheme 1. Chemo-enzymatic Synthesis of **3** and *N*-Alkylated Derivatives



In this work, after growing and disrupting the *E. coli* cells the enzyme was purified easily by a heat treatment at 75 °C during 40 min, centrifugation, and lyophilization of the supernatant to yield a pale brown powder with 1.7 U mg^{-1}.[17] Further purification steps are not needed since they were not crucial for the activity and stereoselectivity of the enzymatic aldol addition.

Preparation of **1** was carried out by previously described procedures from 3-aminopropanol.[10,18] The FSA-catalyzed aldol addition of DHA to **1** was conducted at 4 °C in boric−borate 50 mM pH 7 buffer containing 20% v/v DMF, furnishing 79% reaction conversion by HPLC after 1 h (69% isolated yield of **2**). Glycylglycine 50 mM pH 7.0 buffer can also be used yielding 82% reaction conversion after 1 h.[11] Hence, FSA readily accepts DHA, and there is no need to in situ generate DHA esters. Furthermore, FSA tolerates organic solvents as other DHAP-dependent aldolases do. D-Fagomine (**3**) was then obtained by selective catalytic reductive amination[10] of **2** (Pd/C, H_2 50 psi) in 89% isolated yield without further purification and 93:7 diastereomeric ratio by NMR: $[\alpha]^{20}_D = +20.4$ (*c* 1.0, H_2O) (lit.[3] $[\alpha]_D^{20} = +19.5$ (*c* 1.0, H_2O)). Further purification by cation-exchange chromatography on CM-sepharose in NH_4^+ form, eluted isocratically with 0.01 M NH_4OH, gave an excellent separation of **3** (83% recovery and de ≥99%) and a minor diastereoisomer identified as D-2,4-di-*epi*-fagomine. This

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(17) One unit of U will synthesize 1 μmol of fructose-6-phosphate from D-glyceraldehyde-3-phosphate and DHA per minute at pH 8.5 (glycylglycine 50 mM buffer) and 30 °C. The maximum specific activity observed for the purified enzyme was 7.13 U mg^{-1}.

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compound arose from the *re*-face attack of the DHA−FSA complex on the aldehyde, similar to that found with D-fructose-1,6-bisphosphate aldolase.[10] *N*-Alkylated derivatives (**4a**−**f**) were easily obtained by catalytic reductive amination (Pd/C, H_2 50 psi) of a mixture of **3** and the corresponding aldehyde. Most interestingly, we carried out the preparation of **4a**−**f** by reductive amination of a mixture of **2** and the aldehyde in a one-pot reaction (Scheme 1). The isolated yields obtained by this procedure were similar to those achieved starting from **3**.

Investigations of the synthetic abilities of FSA in organic synthesis are currently in progress. We have found that this enzyme accepts, among others, *N*-Cbz-glycinal and *N*-Cbz-3-amino-2-hydroxypropanal, but α-alkyl-branched *N*-Cbz-aminoaldehydes were not tolerated as substrates.

Antimicrobial activity of **3** and **4a**−**f** was tested against 15 bacteria and 7 fungi (see the Supporting Information). Compounds **3** and **4a**−**d**,**f** did not show activity at concentrations below 256 μg mL^{-1}, thus indicating much lower potency than that found for antiseptics such as cationic surfactants and biguanides.[19] Interestingly, **4e** gave minimum inhibitory concentrations (MIC)s ranging from 32 to 64 μg mL^{-1} against most of the Gram-positive bacteria tested, whereas MICs of 128−256 μg mL^{-1} were observed for the Gram-negative bacteria. Compound **4e** was also rather potent against fungi such as *Aspergillus repens* (32 μg mL^{-1}) and *Cladosporium cladosporoides* (64 μg mL^{-1}) but less potent (128−256 μg mL^{-1}) against the rest of the fungi tested.

Since D-fagomine was reported to have inhibitory activity on α-glucosidase,[2,3] different chemical modifications have been introduced to improve its activity and selectivity. Goujon et al. synthesized α-1-*C*-substituted derivatives with or without an *N*-butyl group, which exhibited higher inhibitory activity than the parent compound against α-glucosidase from rice.[7] On the other hand, the substitution of the piperidine ring for an azepane ring decreased the inhibitory activity against both enzymes.[20]

Compounds **3** and **4a**−**f** were inhibitors of α-D-glucosidase from rice and β-D-galactosidase from bovine liver (Table 1), whereas no inhibition was observed against α-D-glucosidase from baker's yeast, β-D-glucosidase from almond, α-D-mannosidase from jack beans, and α-L-rhamnosidase from *Penicillium decumbens*. Interestingly, among the *N*-alkylated derivatives, **4d** and **4e** were the best inhibitors against α-D-glucosidase, whereas **4e** was a good inhibitor for the β-galactosidase. As shown in Table 1, the activity of **4a**−**f** against α-glucosidase increased with the aliphatic chain length up to nine carbon atoms (**4d**). Increasing the number of carbon atoms to 12 (**4e**) or including an aromatic moiety (**4f**) did not improve or caused a drastic decrease on the inhibitory activity, respectively. Kinetic studies indicated that active compounds behaved as competitive inhibitors of α-D-glucosidase from rice. These results suggested that **3** may interact with the subsite −1 of the two catalytic sites reported for α-glucosidase family II[21] and the *N*-alkyl substituent with a convenient length may fit into subsite +1, but not with the phenylethyl substitution.

Table 1. Inhibition of Glycosidases by Compounds **3** and **4a**−**f**

	α-D-glucosidase[a]		β-D-galactosidase[b]	
compd	K_i[c] (μM)	IC_{50} (μM)	K_i[d] (μM)	IC_{50} (μM)
3	9.3 ± 0.8	13.2	35.9 ± 3.9	30.4
4a	126 ± 3.6	151	NI[e]	
4b	73.3 ± 2.0	61.4	NI[e]	
4c	27.3 ± 2.8	35.3	242 ± 14	203
4d	14.9 ± 1.2	18.1	140 ± 18	108
4e	16.4 ± 2.1	20.7	9.3 ± 0.7	6.0
4f	143 ± 5.4	159	691 ± 18	416

[a] From rice. An IC_{50} value of 350 μM has been reported for compound **3**.[3] The reason may lie in the different substrate used to evaluate the glycosidase activity in the literature report (i.e., a disaccharide) and the present work (i.e., *p*-nitrophenyl glucopyranoside). [b] From bovine liver. [c] Competitive inhibition. [d] Noncompetitive. [e] No inhibition.

Inhibition of β-D-galactosidase from bovine liver by **3** and **4a**−**f** is also depicted in Table 1. For **4a**−**e**, the higher the hydrophobicity the lower the IC_{50} values, **4e** being the most active one. Inhibitory activity of **4e** was similar to that reported for the D-galacto isomer of **3**[22] and higher than that found for **3** or its α-1-*C*-ethyl derivative.[23] Active compounds inhibited this enzyme in a noncompetitive manner, with a K_i value for **4e** 4-fold lower than that for **3**.

Finally, cytotoxicity of **3** and **4a**−**f** was estimated by determination of their hemolytic and protein denaturation effects on human erythrocytes.[24] The results showed that they did not have activity, with the exception of **4e** (see the Supporting Information). The HC_{50} (i.e., concentration that induces the hemolysis of 50% of the cells) for compound **4e** was 138 ± 8 mg mL^{-1}, whereas the denaturation index (DI, i.e., hemoglobin denaturation induced by the compound) was 5 ± 1. These values compare with commercial decyl-glucoside (HC_{50} 252 ± 6 mg mL^{-1} and DI 14.2).[24]

In summary, D-fagomine can be obtained stereoselectively in two chemo-enzymatic asymmetric steps using inexpensive achiral DHA and **1** as the starting materials and FSA from *E. coli* as biocatalyst in 51% isolated yield and ≥99% de. The *N*-alkylated derivatives of **3** were obtained for the first time in one pot reaction from a mixture containing **2** and the corresponding aldehyde. Preliminary results indicated that **4e** elicited antifungal and antibacterial activity with slight selectivity against Gram-positive bacteria. Overall, alkylation of the nitrogen did not strongly improve the activity but increased the selectivity toward glucosidase and galactosidase

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inhibition. Compound **4d**, which was not cytotoxic in the red blood cell test, exhibited good inhibitory activity and higher specificity against family II α-glucosidase than that of the parent **3**.

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Supporting Information Available: Experimental preparations, analytical data, biological data, and ^{1}H and ^{13}C NMR spectra for all compounds; COSY, HSQC, and NOE spectra for compound **4a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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