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Aldolase-Catalysed Stereoselective Synthesis of Fluorinated Small Molecules

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Abstract

The introduction of fluorine has been widely exploited to tune the biological functions of small molecules. Indeed, around 20% of leading drugs contain at least one fluorine atom. Yet, despite profound effects of fluorination on conformation, there is only a limited toolkit of reactions that enable stereoselective synthesis of fluorinated compounds. Aldolases are useful catalysts for the stereoselective synthesis of bioactive small molecules; however, despite fluoropyruvate being a viable nucleophile for some aldolases, the potential of aldolases to control the formation of fluorine-bearing stereocentres has largely been untapped. Very recently, it has been shown that aldolase-catalysed stereoselective carbon–carbon bond formation with fluoropyruvate as nucleophile enable the synthesis of many α-fluoro β-hydroxy carboxyl derivatives. Furthermore, an understanding of the structural basis for the stereocontrol observed in these reactions is beginning to emerge. Here, we review the application of aldolase catalysis in the stereocontrolled synthesis of chiral fluorinated small molecules, and highlight likely areas for future developments.

Introduction

The aldol reaction has facilitated the construction of many complex organic molecules (including many polyketide natural products), and is a cornerstone of modern synthetic organic chemistry.[1-4] Up to two new stereogenic centres are installed during the formation of the new carbon–carbon bond, and, in many variants of the aldol reaction, control of stereochemical configuration is possible. Aldolases have been shown to be synthetically-valuable catalysts for the conversion

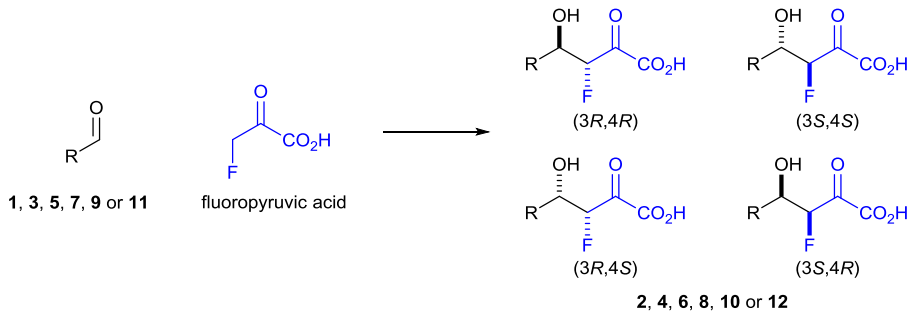
of their substrates into aldol products in high yield and with high stereocontrol under mild conditions.[5,6] For example, an efficient and scalable aldolase-catalysed process has been developed for the enantioselective synthesis of precursors of the side chain found in the statin drugs.[7] Furthermore, protein engineering has been shown to be valuable for increasing further the synthetic value of aldolase enzymes, for example by broadening the range of substrates accepted or by modifying the stereochemistry of carbon–carbon bond formation.[8-14]

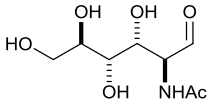
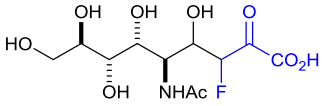
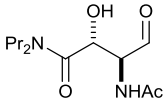
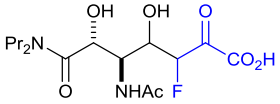
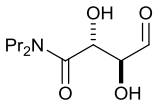
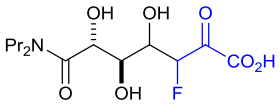
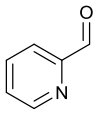
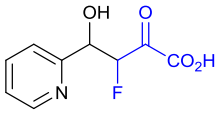
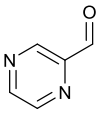
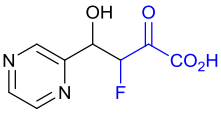
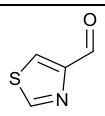
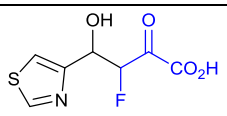
Recently, aldolases have emerged as a useful class of catalysts for controlling the formation of fluorine-bearing stereocentres.[15●,16●●] Approaches for the synthesis of fluorinated small molecules are important because fluorination can tune exquisitely small molecule conformation and biological activity.[17] Specifically, it has been shown that the controlled installation of fluorine-bearing stereocentres is possible through stereoselective formation of an adjacent carbon–carbon bond. In sharp contrast, reactions between metal enolates of fluoroacetate derivatives general exhibit poor stereoselectivity.[18,19] Although aldolases had previously been shown to accept substrates with remote fluorine atoms,[20,21] this review focuses on examples in which carbon–carbon bond formation leads to a new fluorine-bearing stereocentre. Such biocatalytic syntheses of fluorinated small molecules will be contrasted with a recent biomimetic approach that exploits organocatalysis.[22●●] Particular emphasis is placed on aldolase-catalysed reactions involving fluoropyruvate as nucleophile.

Fluoropyruvate as a nucleophile in aldolase-catalysed reactions

The range of nucleophiles accepted by most aldolase enzymes tends to be rather limited.[6,21,23] Although fluoroacetone has been shown to be a competent nucleophilic substrate for deoxyribose-5-phosphate aldolase (DERA), carbon–carbon bond formation does not occur from the fluorine-bearing carbon, and thus a new fluorine-bearing stereocentre is not formed.[25] However, despite some reports to the contrary,[20,24] fluoropyruvate has been shown to be a viable substrate for several Class I lysine-dependent pyruvate aldolases (Table 1). In such reactions, there are four possible stereoisomeric products, and the stereocontrol exerted by the aldolase in each case is indicated in Table 1.

Table 1: Aldolase-catalysed reactions involving fluoropyruvate as nucleophile



Aldolase	Substrate[a]	Product[a]	(3*R*:4*R*) : (3*S*:4*S*) : (3*R*:4*S*) : (3*S*:4*S*)	Ref.
NAL	 **1**	 **2**	90 : 0 : 0 : 10[b]	[27]
NAL (E192N)	 **3**	 **4**	40 : 0 : 50 : 10	[15●]
NAL (E192N)	 **5**	 **6**	60 : 0 : 40 : 0	
HBPA[c]	 **7**	 **8**	5 : 0 : 95 : 0	[16●●]
	 **9**	 **10**	0 : 0 : 100 : 0	
	 **11**	 **12**	5 : 0 : 95 : 0	

[a]Drawn in open chain form. [b]The (3*R*,4*R*)-configured product predominated initially, and equilibrated to a 45:55 mixture of (3*R*,4*R*)- and (3*S*,4*R*)-configured products.[27]

The kinetic ratio stated is taken from reference 15●. [c]A wide range of other heteroaromatic aldehydes was also accepted.

In pioneering work, it was shown that fluoropyruvate was consumed in (wild-type and variant) 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase-catalysed reactions with 2-pyridine carboxaldehyde[26] and D-glyceraldehyde 3-phosphate (Causey CP, PhD thesis, Duke University, 2007); however, the outcome of these reactions was not reported. More recently, *N*-acetyl neuraminic acid lyase (NAL) has been exploited in the synthesis of fluorinated mechanistic probes for sialidases and sialyltransferases.[27] Initially, the NAL-catalysed reaction between fluoropyruvate and *N*-acetyl mannosamine (**1**) yielded (under kinetic control) mainly (3*R*,4*R*)-**2**. However, after extended reaction times, equilibration occurred to give a mixture of (3*R*,4*R*)- and (3*S*,4*R*)-configured products.[15●,27]

Directed evolution has previously been exploited to modify the substrate specificity of NAL towards aldehydes such as **3** and **5**.[28] With fluoropyruvate in place of pyruvate, the E192N variant still accepted the aldehydes **3** and **5** as substrates;[15●] in each case, approximately equimolar amounts of the (3*R*,4*R*)- and (3*R*,4*S*)-configured products (**4** and **6**) were obtained.

The stereocontrolled synthesis of fluorinated molecules has been shown to be viable with the related enzyme *trans*-*o*-hydroxybenzylidene pyruvate hydratase aldolase (HBPA). HBPA catalyses the reactions between fluoropyruvate and many aromatic and heteroaromatic aldehydes (e.g. **7**, **9** and **11**) to yield the corresponding aldol

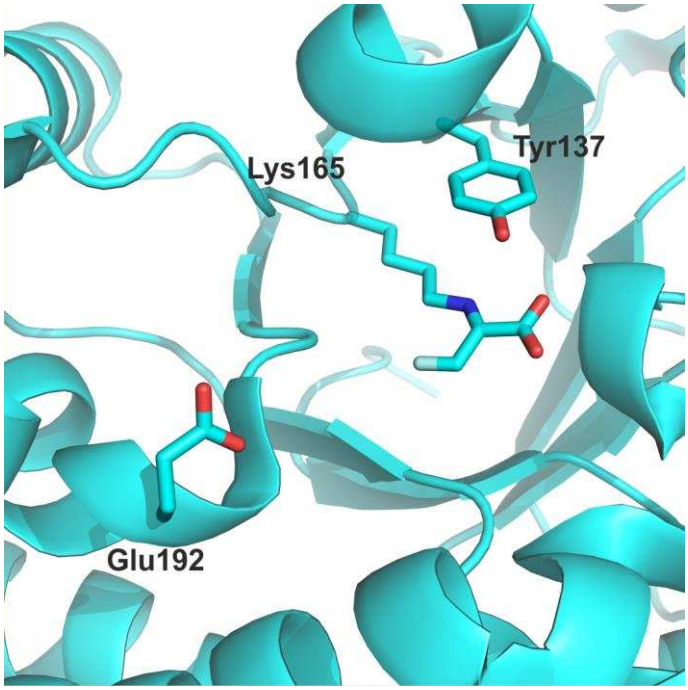
adducts (e.g. **8**, **10** and **12**) (see Table 1 for selected examples).[16●●] In each case, high diastereoselectivity (83:17 to >98:<2) and excellent enantioselectivity (>98% ee) was observed. In contrast to HBPA-catalysed reactions with pyruvate as nucleophile, dehydration of the fluorinated aldol adducts (such as **8**, **10** and **12**) did not occur. It is possible that the introduced fluorine atom replaces the hydrogen atom that would otherwise be removed during the enzyme-catalysed dehydration step.

Origin of stereocontrol in NAL-catalysed reactions involving fluoropyruvate

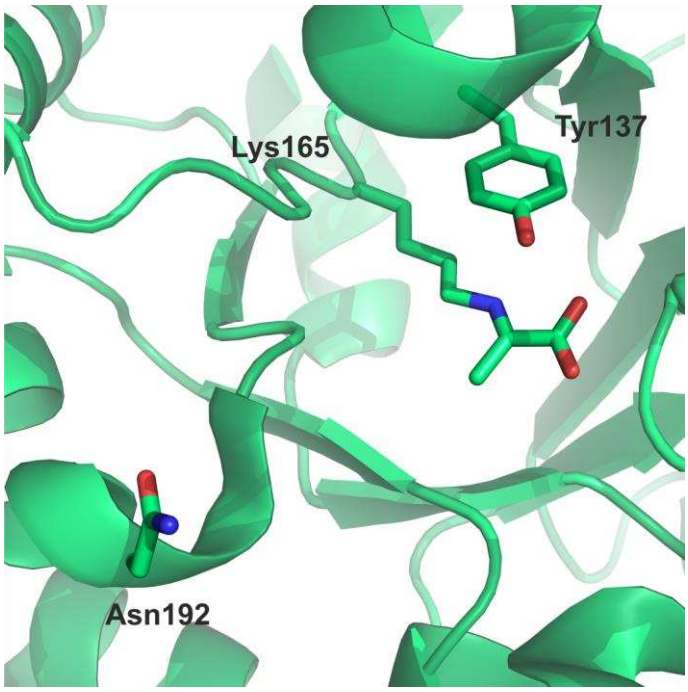
Some insights into the structural basis of stereocontrol in NAL-catalysed reactions involving fluoropyruvate have been gleaned using protein crystallography (Figure 1).[15●] Notably, for all combinations of substrate and NAL variant studied, 3*R*-configured products were selectively obtained under kinetic control (Table 1). The structure of *S. aureus* NAL, whose properties have been shown to be extremely similar to those of *E. coli* NAL, has been determined in complex with fluoropyruvate (Panel A). The formation of a *Z*-configured enamine was observed: reaction of this intermediate via the face that is presented to aldehyde substrates would lead to the formation of 3*R*-configured products (Panel C). In the case of the E192N variant of NAL in complex with pyruvate, it is notable that the enamine presents the same face to substrates, and that 3*R*-configured products are also obtained (Panel B). The HBPA-catalysed reactions between fluoropyruvate and many aldehydes also yield 3*R*-configured products (Table 1); unfortunately, the structure of HBPA has not yet been determined, preventing a more general understanding of the structural basis of stereochemistry of aldolase-catalysed reactions involving fluoropyruvate.

Figure 1: Structural basis of stereocontrol in NAL-catalysed reactions involving fluoropyruvate. Panel A: *S. aureus* NAL in complex with fluoropyruvate (PDB: 5a8g). Panel B: E192N variant of *E. coli* NAL in complex with pyruvate (PDB: 2WKJ). Panel C: Reaction of the top face (as drawn in all Panels) of a *Z*-configured enamine intermediate would yield (3*R*)-configured aldol products.

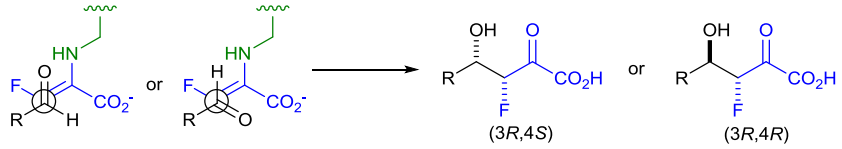
A



B



C

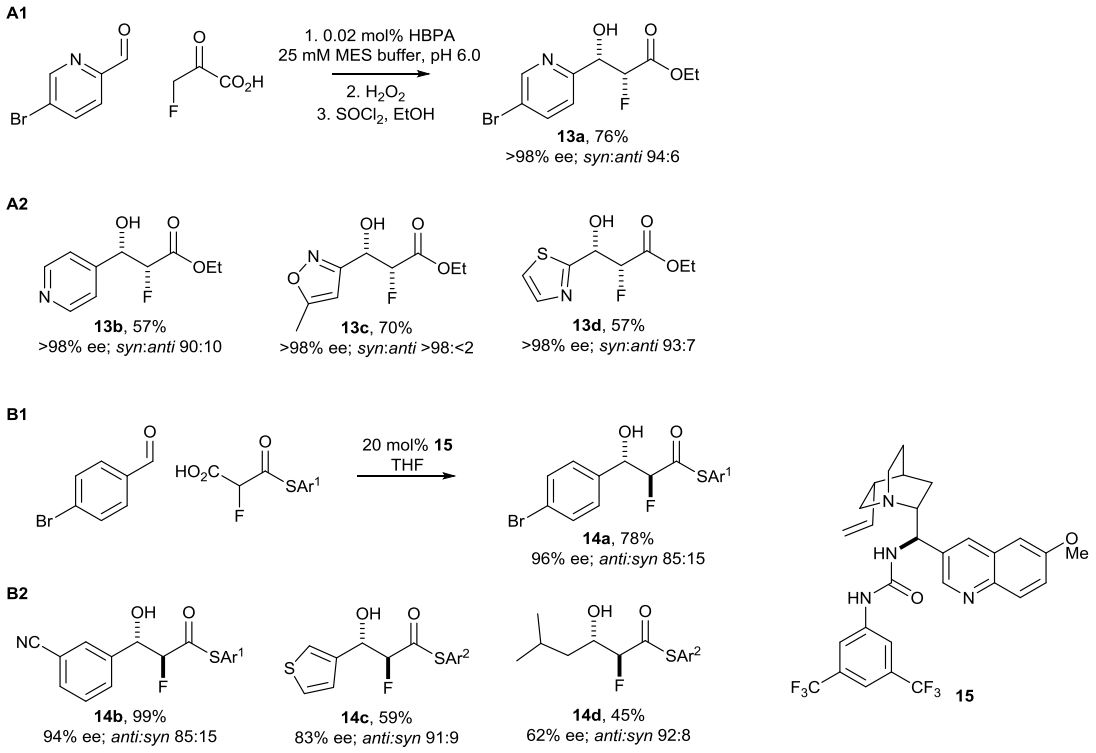


Applications of biocatalysis and biomimetic chemistry in the stereocontrolled synthesis of fluorinated molecules

The broad substrate specificity of HPBA has been exploited in the enantioselective synthesis of fluorinated building blocks (Figure 2, Panel A).[16●●] Thus, reaction between an aldehyde (27.5 mM) and fluoropyruvate (25 mM), catalysed by 0.02 mol% HPBA was followed by oxidative decarboxylation and esterification. For a broad range of aromatic and heteroaromatic aldehyde substrates, the corresponding α-fluoro β-hydroxy carboxylic esters **13** were obtained in >98% ee and good diastereoselectivity (syn:*anti* 83:17 to >98:<2). Because HPBA accepts simple (het)aromatic aldehydes as substrates, the reaction products may be easily

converted into building blocks with molecular properties and features suitable for drug discovery applications. [16●●]

Figure 2: Enantioselective aldol reactions with masked fluoroacetate derivatives. Panel A: HPBA-catalysed reaction between aldehydes and fluoropyruvate, followed by oxidative decarboxylation and esterification, yields *syn*-configured α-fluoro β-hydroxy carboxylic esters **13**. An exemplar reaction (Panel A1) and selected other examples (Panel A2) are shown. Panel B: Organocatalysed reaction between aldehydes and fluoromalonic acid halfthioesters yields *anti*-configured α-fluoro β-hydroxy carboxylic thioesters **14**. An exemplar reaction (Panel B1) and selected other examples (Panel B2) are shown. Ar^1, *p*-methoxyphenyl; Ar^2, *o*-fluorophenyl.



This aldolase-catalysed synthetic approach is complemented by a recent organocatalytic approach that yields related *anti*-configured products.[22●●]

Reaction between an aldehyde and a fluoromalonic acid halfthioesters (2-3 eq.), catalyzed by 20 mol% **15**, yielded α-fluoro β-hydroxy carboxylic thioesters **14** in 62-99% ee and good diastereoselectivity (*anti*:*syn* 75:25 to 95:5). Remarkably, the approach was successful with aliphatic aldehydes as well as aromatic and heteroaromatic aldehydes; however, the enantioselectivities are generally lower, and the catalytic loadings higher, than the HPBA-catalysed approach. Nonetheless, the two approaches yield different diastereomeric series of products, highlighting the potential for complementarity between biocatalysis and organocatalysis.

Conclusions and future perspectives

Fluoropyruvate is a viable nucleophilic substrate for several Class I pyruvate aldolases, offering the potential to control the formation of fluorine-bearing stereocentres by carbon–carbon bond formation. Until recently, however, demonstrated synthetic utility has been limited to NAL-catalysed reactions between fluoropyruvate and sugars. Yet, there are glimpses that aldolases may have much greater value in the stereoselective synthesis of fluorinated molecules. First, some wild-type aldolases in addition to HBPA may accept a very broad range of aldehydes as electrophiles, which might be further broadened by directed evolution. Second, aldolase-catalysed reaction involving fluoropyruvate are generally stereoselective, and the structural basis for this stereoselectivity is beginning to emerge. The broad substrate specificity and excellent stereoselectivity of HBPA is particularly exciting since it enables the synthesis of many fluorinated building blocks with exquisite stereocontrol.

Acknowledgements

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•• of outstanding interest

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It was demonstrated that HBPA catalyses the highly stereoselective (>98% ee and up to >98:<2 diastereoselectivity) synthesis of α-fluoro β-

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