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Development of an organo- and enzyme-catalysed one-pot, sequential three-component reaction

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ABSTRACT

A one-pot, three-component process is described which involves both organo- and enzyme-catalysed carbon–carbon bond-forming steps. In the first step, an organocatalyst catalyses the aldol reaction between acetaldehyde and a glyoxylamide. After dilution with additional aqueous buffer, and addition of pyruvate and an aldolase enzyme variant, a second aldol reaction occurs to yield a final product. Crucially, it was possible to develop a reaction in which both the organo- and enzyme-catalysed reactions could be performed in the same aqueous buffer system. The reaction described is the first example of a one-pot, three-component reaction in which the two carbon–carbon bond-forming processes are catalysed using the combination of an organocatalyst and an enzyme.

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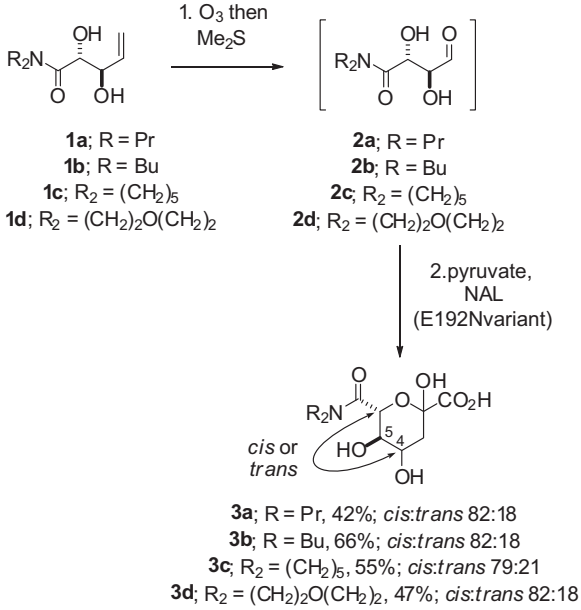
1. Introduction

Cascade reactions can enable the highly efficient synthesis of complex organic molecules from multiple components, reducing the number of work-up and purification steps required.[1] Many combinations of catalyst types have been exploited in bicatalytic processes including pairs of organocatalysts;[2a,b] pairs of enzymes;[2c,d] organometallic and organo-catalysts;[2e,f] and organometallic catalysts and enzymes.[2g] In addition, an aldolase variant has been used to catalyse two sequential aldol reactions to yield a precursor of the statin side-chain.[3] The combination of organocatalysts and enzymes is, however, rare.[4] Here, we report the first example of a three-component reaction in which two carbon–carbon bond-forming steps are catalysed using the specific combination of an organocatalyst and an enzyme.

We have previously exploited directed evolution in the discovery of aldolases with modified, synthetically-useful properties.[5] Wild type *N*-acetylneuraminic acid lyase (NAL) catalyses the reversible aldol condensation between pyruvate and *N*-acetyl mannosamine to give *N*-acetylneuraminic acid. However, we discovered that the E192N variant of NAL can accept a range of alternative aldehydes **2**; thus, ozonolysis of the alkenes **1** (→**2**), and condensation with pyruvate, gave the aldol products **3** (Scheme 1).[5c] We have also used



1a; R = Pr
1b; R = Bu
1c; $R_2 = (CH_2)_5$
1d; $R_2 = (CH_2)_2O(CH_2)_2$

2a; R = Pr
2b; R = Bu
2c; $R_2 = (CH_2)_5$
2d; $R_2 = (CH_2)_2O(CH_2)_2$

3a; R = Pr, 42%; *cis*:*trans* 82:18
3b; R = Bu, 66%; *cis*:*trans* 82:18
3c; $R_2 = (CH_2)_5$, 55%; *cis*:*trans* 79:21
3d; $R_2 = (CH_2)_2O(CH_2)_2$, 47%; *cis*:*trans* 82:18

Scheme 1. Aldolase reaction catalysed by an aldolase variant.

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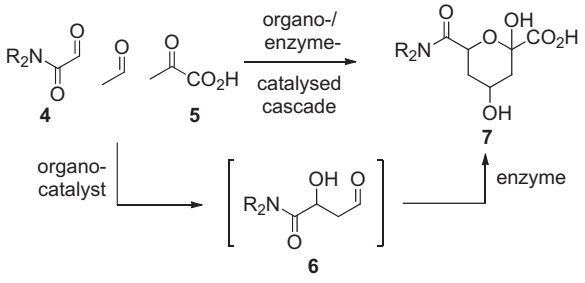
directed evolution to create stereochemically complementary aldolases that enable the stereoselective synthesis of both (4*R*)- and (4*S*)-configured products **3**.[5d]

A drawback of our chemoenzymatic synthesis of the aldol products **3** was that the synthesis of the alkenes **1** was rather lengthy (six steps from ribonolactone).[5c,6] We envisaged, however, that it might be possible to develop a three-component synthesis of similar products (such as **7**) in a single pot (Scheme 2). Thus, it was hoped that organocatalysed condensation[7] between a glyoxylamide **4** and acetaldehyde would yield an aldol product **6**. The structure of complexes between the E192N NAL variant, pyruvate and a substrate analogue suggested that a hydroxyl group α to the aldehyde may not be essential for binding.[8] Accordingly, it was hoped that the aldol products **6** would be viable substrates for the E192N variant, enabling the synthesis of the final products **7** by enzymic addition of pyruvate. The condensation of the aldehyde **4**, acetaldehyde and pyruvate (**5**), to give **7**, would constitute the first example of a three-component reaction in which two carbon–carbon bond-forming steps were catalysed using the specific combination of an organocatalyst and an enzyme.

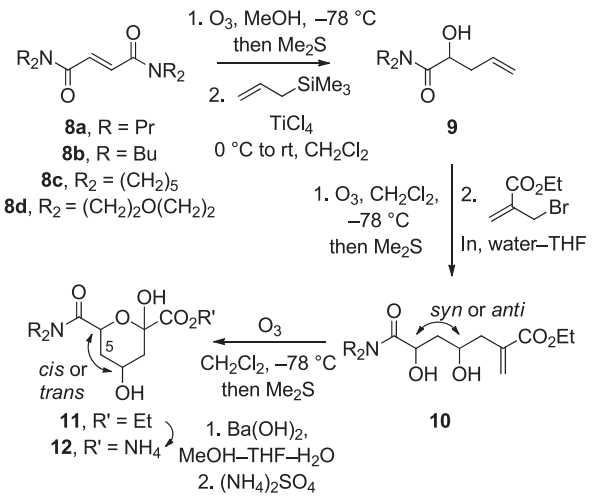


Scheme 2. Overview of the proposed organo- and enzyme-catalysed cascade.

2. Development of a viable three-component reaction

2.1. Synthesis and evaluation of potential products of aldolase-catalysed reactions

We first determined whether the compounds **7** were viable substrates for retro-aldol reactions catalysed by variants of NAL. The synthesis of the ammonium salts, **12**, of the carboxylic acids, **7**, is described in Scheme 3 and Table 1.



Scheme 3. Synthesis of the potential enzyme substrates **12** (see Table 1).

Table 1
Synthesis of the potential enzyme substrates **12** (see Scheme 3)

Substrate	Yield, **9**/%	Yield, **10**/% [anti/syn[a]]	Yield, **11**[b]/% [cis/trans[a]]	Yield, **12**/% [cis/trans[a]]
8a	91[c]	24 [70:30]	59 [95:5]	>98 [95:5]
8b	46	12 [80:20]	62 [95:5]	>98 [95:5]
8c	30	33 [68:32]	27 [88:12]	>98 [90:10]
8d	60	18 [74:26]	49 [78:22]	83 [78:22]

[a] Determined by 500 MHz ^{1}H NMR spectroscopic analysis of the isolated products.
[b] Column chromatography gave mixtures that were enriched in the cis anomers. The relative configuration of the products was determined by analysis of diagnostic geminal coupling constants.
[c] Yield from the isolated glyoxylamide derived from **8a**.

Ozonolysis of the fumaric amides **8**, and allylation by treatment with allyltrimethylsilane and titanium tetrachloride, gave the racemic homoallylic alcohols **9**. Ozonolysis of the homoallylic alcohols **9**, and indium-mediated condensation with ethyl α-(bromomethyl) acrylate, gave the diols **10** with modest *anti* diastereoselectivity.[9] Finally, ozonolysis of the diastereomeric mixtures of the diols **10** gave the diastereomeric esters **11**, which were at least partially separable by column chromatography. Ester hydrolysis and cation exchange, gave the ammonium salts **12**. The relative configuration of the major diastereomers of the diols **10** was determined by careful analysis of diagnostic geminal coupling constants in their derivatives **11**.

A coupled enzyme assay was used to assess the efficiency of aldolase-catalysed cleavage of the substrates.[5b] In this assay, aldolase-catalysed cleavage to yield pyruvate would be followed by lactate dehydrogenase-catalysed reduction; the concomitant oxidation of NADH being detected spectroscopically by monitoring the change in absorption at 340 nm. The steady state kinetic parameters for the cleavage of **3a**–**d** and **12a**–**d** by the E192N NAL variant are presented in Table 2. Crucially, the aldols **12a**–**d** were all substrates for the E192N NAL variant, albeit with up to ~10-fold reduced k_{cat}/K_M relative to the substrates **3a**–**d**, which bear an additional hydroxyl group at C-5.

Table 2
Steady state kinetic parameters for enzyme-catalysed cleavage by the E192N NAL variant

Substrate	R_2	k_{cat}/min^{-1}	K_M/mM	k_{cat}/K_M/min^{-1} mM^{-1}
3a[a]	Pr	200±2	0.10±0.01	1960
3b[a]	Bu	61±2	0.11±0.02	580
3c[a]	$(CH_2)_5$	330±6	0.34±0.06	850
3d[a]	$(CH_2)_2O(CH_2)_2$	160±8	0.70±0.20	230
12a	Pr	130±3	0.39±0.04	340
12b	Bu	90±6	0.25±0.04	360
12c	$(CH_2)_5$	95±1	0.57±0.02	167
12d	$(CH_2)_2O(CH_2)_2$	73±2	3.4±0.2	22

[a] See Ref. 5b.

2.2. Effect of removal of an α-hydroxyl group on the stereoselectivity of aldolase-catalysed reactions

The effect of removing the α-hydroxyl group from the aldehyde substrate **2a** on the stereoselectivity of aldolase-catalysed reactions was investigated. Both enantiomers of **9a** were prepared using an established chiral relay approach:[10] reaction of **13** with dipropylamine, and deprotection, gave the corresponding homoallylic alcohols **9a**, which were ozonolysed to give the aldehydes **6a** (Scheme 4).

The stereoselectivity of the aldolase-catalysed condensations of three substrates—the aldehyde **2a**, and the enantiomeric aldehydes (*R*)- and (*S*)-**6a**—was determined by ^{1}H NMR spectroscopy in deuterated sodium phosphate buffer (pH 7.4) (Table 3). We investigated the stereocontrol exerted by the E192N variant, as well as two stereochemically complementary variants that we had previously discovered using directed evolution: E192N/T167G and E182N/T167V/S208V.[5d] As expected, the condensation of the

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