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

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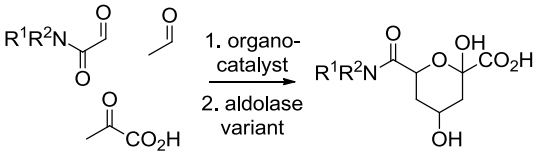

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Development of an organo- and enzyme-catalysed one-pot, sequential three-component reaction

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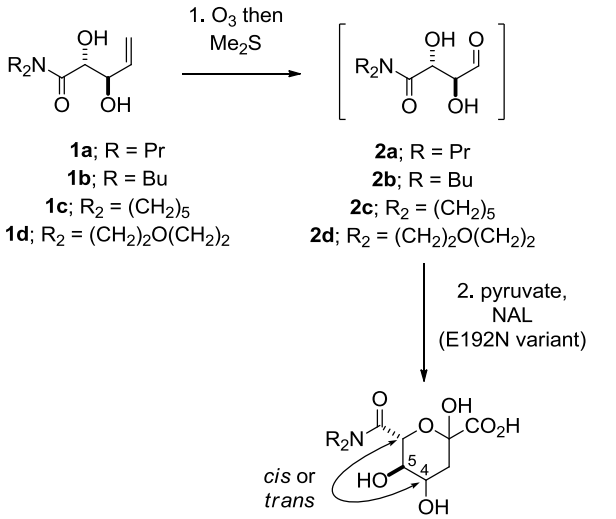
ABSTRACT

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.

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



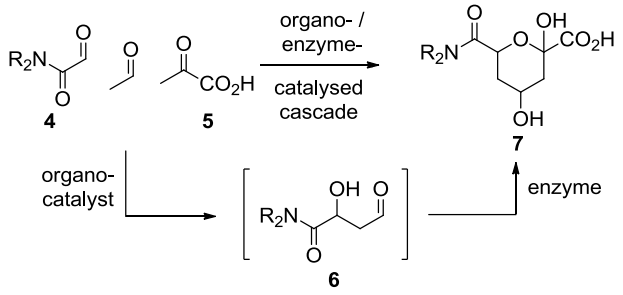
1a; R = Pr
1b; R = Bu
1c; $R_2 = (CH_2)_5$
1d; $R_2 = (CH_2)_2O(CH_2)_2$

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

A drawback of our chemoenzymatic synthesis of the aldol products **3** was that the synthesis of the alkenes **1** was rather lengthy (6 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.

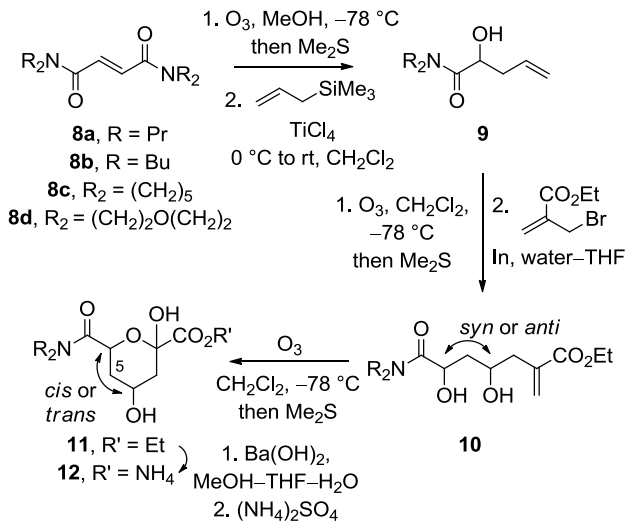


Scheme 2. Overview of the proposed organo- and enzyme-catalysed cascade

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)

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**.

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**.

A coupled enzyme assay was used to determine 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 may be 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.

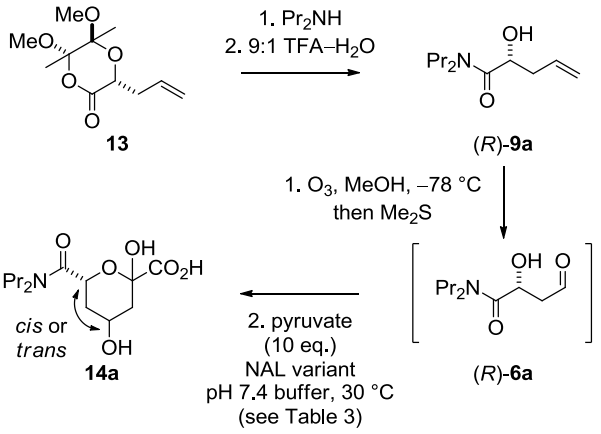
Substrate	R_2	k_{cat} / min^{-1}	K_M / mM	k_{cat}/K_M / min^{-1}mM^{-1-1}
3a[a]	Pr	202 ± 2	0.10 ± 0.01	1960
3b[a]	Bu	61 ± 2	0.11 ± 0.02	580
3c[a]	$(CH_2)_5$	331 ± 6	0.34 ± 0.06	850
3d[a]	$(CH_2)_2O(CH_2)_2$	162 ± 8	0.70 ± 0.20	230
12a	Pr	130 ± 3	0.39 ± 0.04	340
12b	Bu	90 ± 6	0.25 ± 0.04	360
12c	$(CH_2)_5$	95 ± 1	0.57 ± 0.02	167
12d	$(CH_2)_2O(CH_2)_2$	73 ± 2	3.4 ± 0.2	22

[a]See reference 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 aldehyde **2a** with pyruvate was highly stereoselective with the stereochemically complementary variants E192N/T167G (→ >95:<5 *cis*:*trans*-**3a**) and E182N/T167V/S208V (→ <13:>87 *cis*:*trans*-**3a**). Unfortunately, stereocontrol in the condensations of the aldehydes (*R*)- and (*S*)-**6a** was modest in all of the aldolase-catalysed condensations. Evidently, the α-hydroxyl group in the aldehyde **2a** (corresponding to the hydroxyl group at C-5 in **3a**) plays a crucial role in stereocontrol.



Scheme 4. Investigation of the stereoselectivity of reactions of aldehydes **9a** catalysed by NAL variants. The enantiomeric substrate (*S*)-**9a** was also prepared and studied. The stereoselectivity of the aldolase-catalysed condensation was determined in pH 7.4 deuterated sodium phosphate buffer; the structures **6a** and **14a** are drawn in protonated form for convenience.

Table 3. Kinetic and thermodynamic stereoselectivity of aldol reactions catalysed by NAL variants.

Enzyme	Substrate and product		
	2a→**3a** *cis*:*trans*[a] [*re*:*si*[b]]	(*R*)-**6a**→**14a** *cis*:*trans*[a] [*re*:*si*[b]]	(*S*)-**6a**→**14a** *cis*:*trans*[a] [*re*:*si*[b]]
thermodynamic ratio[c]	69:31	74:26	74:26
E192N	79:21 [79:21]	58:42 [42:58]	62:38 [62:38]
E192N/T167G	>95:<5 [>95:<5]	60:40 [40:60]	58:42 [58:42]
E182N/T167V/ S208V	>13:<87 [>13:<87]	29:71 [71:29]	63:27 [63:27]

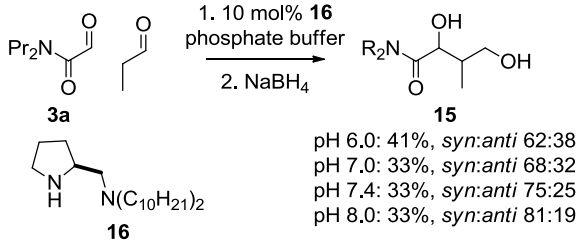
[a]Determined by integration of the 500 MHz ^{1}H NMR spectrum.

[b]Kinetic facial control in the attack on the respective aldehyde.

[c]Ratio after equilibration. After 1 week, a second aliquot of enzyme was added and the reaction incubated for a second week.

2.3 Identification of reaction medium in which both organo- and enzyme catalysis are possible

We investigated the possibility of performing an organocatalysed aldol condensation under the buffered conditions needed for enzymic catalysis. The diamine **16** has been developed as an organocatalyst for asymmetric aldol reactions performed in water.[11] Remarkably, we found that, with 10 mol% **16**, organocatalysed aldol condensation was possible under buffered conditions, and after reduction, the diols **15** were obtained (Scheme 5); in contrast with just 1% **16** at pH 7.0, no product was detected. The diastereoselectivity of the condensation was modest, and varied significantly with pH. The relative configuration of the diols was determined by analysis of diagnostic coupling constants in the corresponding acetonides.



Scheme 5. Study of the organocatalysed condensation of **3a** and propionaldehyde (10 eq.) in aqueous buffer.

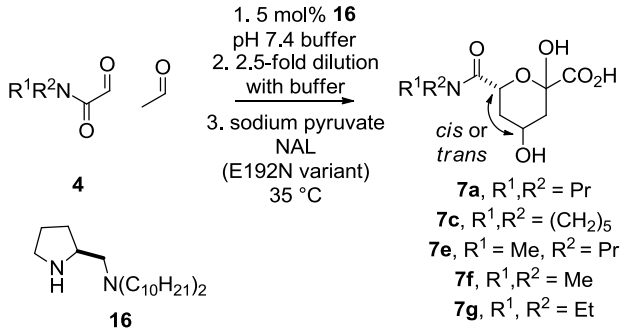
With an organocatalysed aldol reaction in hand that operated under appropriately buffered conditions, we investigated the effect of various additives on the activity of the E192N enzyme. In each case, we assayed enzyme activity by monitoring condensation of the aldehyde **2a** and pyruvate to give **3a** (Scheme 1): the formation of **3a** was assayed by periodic cleavage and condensation with thiobarbituric acid.[12] At the concentrations of propionaldehyde and the glyoxylaldehyde **4a** needed for aldol condensation, the enzyme was still fully active. However, in the presence of the concentration of the organocatalyst **16** that had corresponded to a 10 mol% loading (see Scheme 5), the activity of the enzyme was very significantly impaired.

We decided, therefore, to develop a one-pot, three component process in which the reaction mixture was diluted after the organocatalysed step (Scheme 6). In order to gain insight into the stereoselectivity of the overall process, we determined the enantiomeric excess of both the intermediate aldehyde **6a** (R = Pr) and the final product **7a**. Thus, **6a** and **7a** were converted into the diol **17** (by reduction with $NaBH_4$) and the lactone **18** (by oxidative cleavage, lactonisation and dehydration) respectively. The diol **17** was found to be essentially racemic (<5% ee), and, at completion, the lactone **18** was also racemic (<5% ee).

3. Determination of the scope of the one-pot, three component reaction

Our investigations into the scope of the one-pot, three-component reaction are summarized in Scheme 6 and Table 4. The glyoxylamides **4** were reacted with acetaldehyde (10 eq.) in the presence of 5 mol% **16** in pH 7.4 buffer for 20 hr. Given the lack of enantioselectivity in the organocatalysed step, and the poor diastereoselectivity of the aldolase-catalysed reactions of the aldehydes 6, we made no attempt to ensure that the aldolase-catalysed reactions proceeded under kinetic control. Thus, after ~2.5-fold dilution with buffer, and addition of sodium pyruvate and the E192N variant, the reaction mixture was incubated for 70 hr at 35 °C. The yield and stereoselectivity of the process was determined for a range of substrates (Table 4). The reaction was successful with a range of glyoxylamides **4**, and 40-51% yield of products were obtained. In common with similar aldolase-catalysed reactions with extended reaction times,[5c,d] it is likely that the diastereoselectivity of the one-pot reactions was

thermodynamically controlled. Our studies demonstrate the value of the specific combination of organo- and enzymic catalysis in three-component reactions leading to heterocyclic products.



Scheme 6. Organo- and enzyme-catalysed condensation of the glyoxylamides **4**, acetaldehyde and pyruvate to give products **7** (see Table 4).

Table 4. One-pot, three-component reactions (see Scheme 6).

Substrate	R^1,R^2	Yield[a] **7** / %	*cis*:*trans*[b]
4a	Pr,Pr	40	78:22[c]
4c	-$(CH_2)_5$-	48	66:34
4e	Me,Pr	48	57:43
4f	Me,Me	43	71:29
4g	Et,Et	51	61:39

[a]Based on the limiting reactant **4**. Products were purified by preparative HPLC.

[b]Determined by integration of the 500 MHz ^{1}H NMR spectrum of the purified product.

[c]The product had <5% ee.

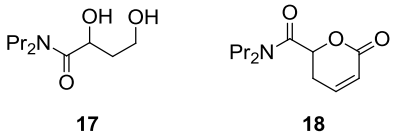


Figure 1. Derivatives used to determine the enantioselectivity of the one-pot process: the diol **17** and the lactone **18** were derived from the aldehyde intermediate **6a** and the product **7a** respectively.

4. Summary

In summary, we have shown that the combination of organo- and enzymic catalysis may be exploited in one-pot reactions with two carbon–carbon bond formation steps. Crucially, it was possible to identify buffered aqueous conditions under which both of the catalysed steps were possible. A range of glyoxylamides **3** were condensed with both acetaldehyde and pyruvate to give the corresponding heterocyclic products **7**. Unfortunately, in this specific case, the stereoselectivity of the overall process was poor, both because the organocatalysed step was not enantioselective, and because the aldolase-catalysed reactions of the intermediate aldehydes **6** (lacking an α-hydroxy group) were poorly diastereoselective. Nonetheless, this study demonstrated the possibility of combining organo- and enzymatic catalysis in bicatalytic chemistry: the combination of organo- and enzymatic catalysis is powerful, and is likely to find further application in one-pot reactions involving two or more carbon–carbon bond-forming steps.

5. Experimental section

The glyoxylaldehyde **3g** (13.5 mg, 95.5 μmmol) was dissolved in buffer (308 μL, pH 7.4, 20 mM potassium phosphate), and was added to the diamine **16** (1.81mg, 4.76 μmol). Acetaldehyde (54μL, 0.955 mmol) was added and reaction mixture was stirred at room temperature for 20 hr, diluted with a pH-adjusted solution (pH 7.4) of sodium pyruvate (105 mg, 0.955 mmol) in buffer (115 μL, pH 7.4, 20 mM potassium phosphate), the E192N aldolase variant (416 μL, 6.98 mg/mL in pH 7.4, 20 mM potassium phosphate) added, and incubated at 35 ºC for 70 hr. The pH of the reaction mixture was lowered to pH 2 by addition of an aqueous solution of 2M formic acid, and after 5 min, raised to pH 7 by addition of an aqueous solution of 2M NH_4OH. The reaction mixture was filtered through Celite, and purification by preparative HPLC (gradient elution: 0:100→30:70 1% TFA in H_2O–1% TFA in MeCN) over 30 min gave the product **7g** (13.2 mg, 51%; *cis*:*trans* 61:39) as a colourless film, ν_{max}/cm^{-1} (liquid film) 3369, 2979, 1761, 1627, 1462 and 1202 cm^{-1}; δ_H (500 MHz, D_2O) 5.13 (0.20H, dd, *J* 9.2 and 3.4 Hz, 6-$H_{trans(maj)}$), 5.07 (0.20H, dd, *J* 6.4 and 4.2 Hz, 6-$H_{trans(min)}$), 4.84 (0.53H, dd, *J* 11.7 and 1.6 Hz, 6-$H_{cis(maj)}$), 4.56-4.51 (0.28H, m, 6-$H_{cis(min)}$ and 4-$H_{trans(min)}$), 4.26 (0.20H, br s, 4-$H_{trans\ (maj)}$), 4.14-4.04 (0.53H, m, 4-$H_{cis(maj)}$), 3.94-3.84 (0.08H, m, 4-$H_{cis(min)}$) 3.40-3.06 (2H, m, NCH_2), 2.15-1.48 (2H, m, H-5 and H-3), 1.12-1.02 (3H, m, CH_3), 0.96 (3H, t, *J* 6.8 Hz, CH_3); δ_C (100 MHz, D_2O, *cis(maj)* only) 163.2 (amide or 1-C), 67.2 and 62.7 (6-C and 4-C), 42.5, 14.2, 39.1 and 35.3 (5-C, 3-C and NCH_2), 13.4 and 11.7 (CH_3); *m/z* (ES^-) 260.1 (100%, $[M–H]^-$); HRMS (EI) $M–H^-$, found 260.1152. $C_{11}H_{20}NO_6$ requires 260.1140. Correlation of the ^{1}H NMR spectrum with that of the acid **7a** allowed the identification of the anomers of the *cis* and *trans* diastereoisomers. The ratio of the species were determined by the integration of the following signals: 5.13 ppm (*trans(maj)*), 5.07 ppm (t*rans(min)*), 4.14-4.04 ppm (*cis(maj)*), 3.94-3.84 ppm (*cis(min)*). Analysis by 500 MHz ^{1}H NMR spectroscopy revealed that a 61:39 mixture of *cis* and *trans* diastereomers was present and that the *cis* isomer existed as an 87:13 mixture of anomers and the *trans* isomer existed as a 50:50 mixture of anomers.

Acknowledgments

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