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The First Mimetic of the Transketolase Reaction

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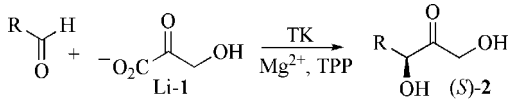
Although the biocatalytic formation of acyclic α,α′-dihydroxy ketones by transketolase is well documented in the literature, there is currently no one-pot chemical synthesis of these dihydroxy ketones available. Here, we report preliminary results of an atom-efficient one-pot synthesis of α,α′-dihydroxy ketones in water by a mimic of the transketolase reaction. The formation of a quaternary ammonium enolate is postulated in this tertiary-amine-mediated carbon–carbon bond-forming reaction.

Transketolase (TK) (EC 2.2.1.1) is an important and versatile enzyme that has been used in stereospecific carbon–carbon bond formation.[1] In vivo TK catalyses the transfer of the C1–C2 hydroxy ketone unit from D-xylulose-5-phosphate to D-ribose-5-phosphate, in a key step of the pentose phosphate pathway, and the enzyme requires magnesium(II) ions as well as thiamine pyrophosphate (TPP) as cofactors.[1] This stereospecific transfer is reversible; however, Srere et al. reported that β-hydroxypyruvic acid is a substrate for TK, donating the two-carbon nucleophilic moiety with the subsequent loss of carbon dioxide, rendering the reaction irreversible.[2] The TK-catalysed condensation of hydroxypyruvate (HPA, **1**) with a variety of aldehydes has been performed successfully in vitro to generate (*S*)-**2** on a preparatively useful scale (Scheme 1).[1] Dihydroxy ketone functionalities are present in several natural products and are also important synthons for further structural elaboration to a range of compounds including keto sugars.[3]



Scheme 1. Transketolase-catalysed reaction using HPA (**1**) to generate dihydroxy ketones (*S*)-**2**.

Although the biocatalytic formation of dihydroxy ketones by TK is well documented in the literature, there is currently no one-pot non-enzymatic synthesis available. Reported syntheses of α,α′-dihydroxy ketones include: a five-step synthetic approach to aromatic and aliphatic dihydroxy ketones starting from 1,4-dioxene and lithiation with *tert*-butyllithium,[4] a strategy including a ruthenium-catalyzed oxidation of allenes,[5] a double hydroxylation of silyl enol ethers with *m*-chloroperbenzoic acid,[6] the utilisation of 1-chloroalkyl *p*-tolyl sulfoxides as hydroxycarbonyl anion equivalents,[7] the approach by Enders et al. to enantiomeric α,α′-dihydroxy ketones using dihydroxyacetone and chiral auxiliaries,[8] and an organoiron-templated route to cyclic species.[9] The lack of concise, direct strategies, presumably stems from the difficulties associated with creating a stable hydroxycarbonyl anion equivalent: umpolung approaches using dithianes or *tert*-butyl hydrazones can result in β-elimination of hydroxide or alkoxide upon generation of the hydroxycarbonyl anion.[10] Herein, we report preliminary results of a remarkable new atom-efficient, one-pot synthesis of racemic α,α′-dihydroxy ketones by a mimetic of the TK reaction in water.

As part of an ongoing research programme to develop TK-catalysed carbon–carbon bond-formation methodologies, a number of high-throughput methods for the screening of libraries of TK mutants are under development. During investigations when screening for the TK reaction between propanal (Scheme 1, R = C_2H_5) and lithium hydroxypyruvate (Li-**1**, LiHPA), at pH = 7.0, a range of different buffers were evaluated. Interestingly, the formation of 1,3-dihydroxypentan-2-one (**2a**, R = C_2H_5) was observed in some control experiments and was therefore investigated further. When carrying out experiments using 3-(4-morpholino)propanesulfonic acid (MOPS, **3**) buffer with TPP and Mg^{2+}, but no TK, **2a** was generated. However, under comparable conditions no reactions were observed when either tris(hydroxymethyl)aminomethane (TRIS, **4**) or glycylglycine (Gly-Gly, **5**) were used (Figure 1). Most surprisingly, the same results were observed using buffers **3**–**5** when both TPP and Mg^{2+} were omitted from the reactions, ruling out

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the mechanistic involvement of TPP and formation of **2a** via the thiazolium ylide.[11] However, these results indicated that MOPS was promoting the carbon–carbon bond-forming reaction.

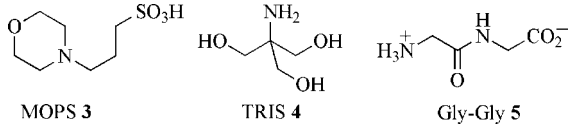
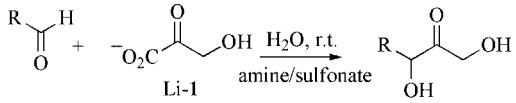


Figure 1. Buffers used in initial screening experiments.

MOPS (**3**) was then used with alternative aldehydes in preparative reactions at pH = 7. Using propanal and Li-**1** with **3**, **2a** was isolated in 21–35% yield depending on the reaction time (Table 1, Entries 1, 2). The use of glycolaldehyde and the aromatic acceptor benzaldehyde gave the α,α′-dihydroxy ketones **2b** and **2c** in 63% and 25% yields, respectively (Table 1, Entries 3, 4). No side products resulting from the addition of a second aldehyde unit were observed using any of the aldehydes in the presence of MOPS after 72 h. The highest yields were observed using 1 equiv. of MOPS.

Table 1. Use of different aldehydes and sulfonate or amine.[a]



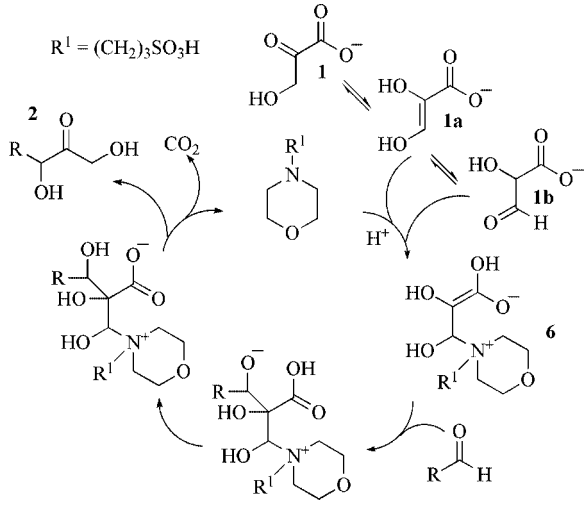
2a R = C_2H_5
2b R = CH_2OH
2c R = Ph

Entry	Sulfonate or amine	*t* [h]	Yield (%) **2**[b]	Side products
1	MOPS (**3**)	5	**2a** (21)	0
2	MOPS (**3**)	72	**2a** (35)	0
3	MOPS (**3**)	72	**2b** (63)	0
4	MOPS (**3**)	72	**2c** (25)	0
5	*N*,*N*′-dimethylpiperazine	5	**2a** (26)	8
6	*N*-methylmorpholine	5	**2a** (27)	9
7	*N*-methylpiperidine	5	**2a** (8)	7
8	morpholine	5	0	0
9	piperidine	5	0	0
10	triethylamine	5	0	0
11	sodium 1-decanesulfonate	5	0	0
12	*N*-methylmorpholine	5	**2a** (35)[c]	0

[a] Aldehyde (3 equiv.), Li-**1** (1 equiv.), amine/sufonate (1 equiv.) in water at 50 mM, room temp. [b] Yield of isolated product after purification by chromatography. [c] Aldehyde (1 equiv.), Li-**1** (1 equiv.), amine (1 equiv.) in water at 50 mM, room temp.

The structure of MOPS suggested that a heterocyclic or sulfonic acid moiety may be required to promote the reaction. Accordingly, a range of secondary and tertiary amines and sulfonic acids were screened for propanal conversion with Li-**1** to 1,3-dihydroxypentan-2-one (**2a**), in an attempt to elucidate the functionality required for dihydroxy ketone formation. The reactions were carried out in water, and to avoid complications due to the addition of a buffer, no buffer was added. The use of *N*,*N*′-dimethylpiperazine, *N*-methylmorpholine and *N*-methylpiperidine led to the formation of **2a** (Table 1, Entries 5–7). Morpholine, piperidine, triethylamine and sodium 1-decanesulfonate (Table 1, Entries 8–11) generated no **2a**. This demonstrated that the reaction required the addition of a cyclic tertiary amine. When morpholine was used, the secondary amine reacted with the aldehyde alone generating the enamine. The formation of side-products resulting from an aldol addition of **2a** to propanal was also observed using cyclic tertiary amines other than MOPS (Table 1, Entries 5–7). This implied that buffering the reaction at near neutral conditions by the sulfonate group reduces the basicity of the reaction medium and stops unwanted side reactions. However, upon reducing the equivalents of aldehyde used from three to one with *N*-methylmorpholine, **2a** was cleanly formed with no side-products in 35% yield, and the reaction proceeded more rapidly than in the MOPS-buffered solution (Table 1, Entry 12). The lower yield of **2a** when using *N*-methylpiperidine indicated that a second heteroatom at the 4-position in the ring may be important mechanistically.

From the acceptors used in the transformation, the reaction proceeds with an aliphatic, aromatic and hydroxylated aliphatic aldehyde. However, changing the donor molecule had a significant impact on product formation. Although both lithium and potassium hydroxypyruvate reacted to give the dihydroxy ketone product in comparable yields, pyruvic acid (free acid and sodium salt) failed to give any reaction under the above conditions. The reaction mimics the biological transketolase reaction with respect to the substrates and product, however does not involve the use of TPP and therefore mechanistically operates by a different pathway. Based upon all observations, a mechanism for the reaction has been proposed (Scheme 2) involving tertiary amine catalysis and quaternary amine enolate formation which has previously been described, for example, in cyclopropanation reactions.[12]



Scheme 2. Proposed mechanism of the formation of **2** using MOPS.

In aqueous alkaline media the enolisation and tautomerisation of **1** has been studied.[13] Thus, the conjugate

addition of the tertiary amine to the enol **1a** of **1**, or tautomerisation to tartronate semialdehyde (**1b**) and subsequent addition of amine to the aldehyde moiety will generate the enolate **6**. From observations to date, the improvement in yield in the presence of a second heteroatom suggests that some stabilisation of the nitrogen enolate **6** or other intermediates through hydrogen bonding may be important. Addition of the aldehyde, proton transfer, then elimination of the tertiary amine and decarboxylation will, after conversion of the enol to the ketone tautomer, give **2** and should release the tertiary amine at the end of the reaction. This postulated mechanism, reminiscent of the Baylis–Hillman reaction, is further substantiated by the lack of observed reactivity with pyruvic acid and sodium pyruvate, which will undergo tautomerisation less readily. Detailed studies into the mechanistic role of the tertiary amine and catalytic function in the reaction cycle have yet to be fully established.

In summary, a one-pot tertiary-amine-mediated carbon–carbon bond-forming reaction in water has been observed that is chemoselective for hydroxypyruvate as a donor group. Interestingly, the observed donor substrate tolerance to date is analogous to that observed with transketolase. We are currently exploring the scope of this reaction since in principle it should be possible to use the tertiary amine in catalytic quantities, and it has significant synthetic potential for the construction of keto sugars and keto sugar analogues. This new methodology to racemic substrates is complementary to current approaches using TK to generate α,α′-dihydroxy ketones enantioselectively, and further mechanistic studies will be performed.

Experimental Section

Representative Experimental Procedure: Li-**1** (3 mmol), propanal (9 mmol) and the tertiary amine (3 mmol) were stirred in water (60 mL) at room temp. for the time specified. The water was removed in vacuo and the dry compound loaded onto a flash silica chromatography column [EtOAc/petroleum ether (boiling range 40–60 °C), 4:1] to give the products indicated (**2a**–**2c**) which were characterised by ^{1}H and ^{13}C spectroscopy, high-resolution mass spectrometry and IR spectroscopy.[1e,4] The identity of the inseparable mixture of isomeric side products (leading to superimposed NMR spectra) was confirmed by MS. ESMS: *m*/*z* (%) = 194 (88) $[M + NH_4]^+$, 159 (100) $[M - H_2O]^+$. HRMS: *m*/*z* calcd. for $C_8H_{17}O_4$ $[MH]^+$ 177.11268, found 177.11291.

Acknowledgments

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