Unprecedented Asymmetric Aldol Reactions with Three Aldehyde Substrates Catalyzed by 2-Deoxyribose-5-phosphate Aldolase

Harrie J. M. Gijsen and Chi-Huey Wong*

Department of Chemistry, The Scripps Research Institute 10666 North Torrey Pines Road, La Jolla, California 92037

Received June 16, 1994

The enzyme 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4) catalyzes the reversible aldol reaction of acetaldehyde and D-glyceraldehyde 3-phosphate to form 2-deoxyribose 5-phosphate. DERA has been overexpressed in *Escherichia coli*, and large quantities of the enzyme can easily be obtained.[1,2] As reported previously, DERA accepts a wide variety of acceptor substrates and has been proven useful in organic synthesis.[1-3] We report here a new type of enzymatic aldol reaction catalyzed by DERA using three achiral C2 aldehydes as substrates. The reactions start with a stereospecific addition of acetaldehyde to a substituted acetaldehyde to form a 3-hydroxy-4-substituted-butyraldehyde, which reacts subsequently with another acetaldehyde to form a 2,4-dideoxyhexose derivative also in a stereospecific manner. These enzymatic products are useful chiral synthons of HMG-CoA reductase inhibitors and 1,3-polyol systems.

In the course of exploiting more acceptor substrates for DERA, using relatively large amounts of the enzyme, we noticed the formation of a 2,4,6-trideoxyhexose, identified as **1**. This product is the result of a double aldol condensation of three acetaldehyde molecules, catalyzed by DERA. The product was confirmed to have the 3*R*,5*R* configuration via oxidation of **1** with bromine[4] to the corresponding known lactone **2**[5,6] (Scheme 1), in agreement with the stereoselectivity in DERA-catalyzed reactions.[1-3]

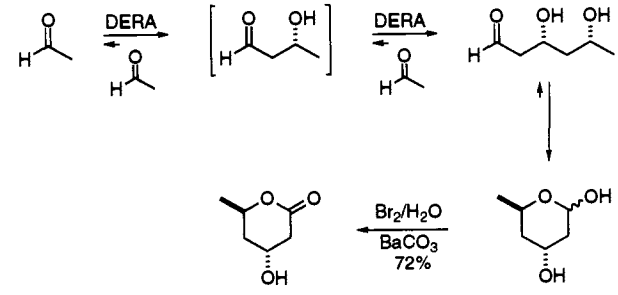
This discovery led us to further examine a variety of C2 substituted acetaldehydes as initial acceptor substrate and acetaldehyde as donor to form the corresponding 6-substituted 2,4-dideoxyhexoses. The results are summarized in Table 1.

In all of the reactions, except that of **3**, no significant amounts of single aldol reaction products (C4 compounds) were found. Products arising from a third coupling with acetaldehyde could not be detected. After the second condensation, products cyclize predominantly to the hemiacetal form, diminishing the concentration of free aldehyde available for a third condensation. Inhibition of further aldol condensations once a stable cyclic hemiacetal has been formed is also illustrated by the low yield of dideoxyhexose **3** formed after reaction between hydroxyacetaldehyde and acetaldehyde (Scheme 2). Hydroxyacetaldehyde (glycolaldehyde) appears predominantly in its dimer (hemiacetal) form **7**, and the product formed after the first aldol reaction, **8**, is capable of forming a five-membered-ring hemiacetal (**8a**).

Most of the products obtained from substrates studied previously formed a hemiacetal after the first aldol condensation, explaining why no products from a double aldol reaction have been isolated previously.[8] Also the use of smaller amounts of DERA decreases the amount of product arising from a second aldol reaction. When the reaction between chloroacetaldehyde and acetaldehyde was performed with half the amount of DERA (250 units/mmol of chloroacetaldehyde), 43% of a monoaldol product was isolated,[9] in addition to 13% of trideoxyhexose **5**.

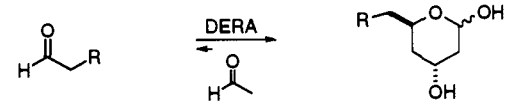
The lactones obtained after oxidation of the aldol products are derivatives of the lactone moiety in mevinic acids, potent HMG-CoA reductase inhibitors which can be used as cholesterol-lowering agents.[10] This two-step synthesis from extremely simple starting materials should be a useful addition to the syntheses of this chiral lactone functionality.[10,11] Treatment of the lactone products with Amberlite IRA 400(OH) in methanol leads to

Scheme 1



2 **1**

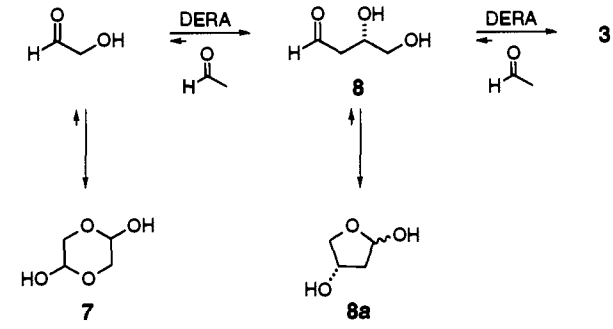
Table 1. Tandem Aldol Condensations Catalyzed by DERA[a]

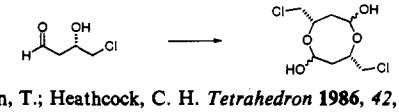


R	yield, %	R	yield, %
H	20 (**1**)	Cl	70 (**5**)
OH	<3[b] (**3**)	Br	
OMe	65 (**4**)	N_3	23 (**6**)

[a] Reactions were conducted in 20 mL of solution containing 100 mM triethanolamine, 1 mM EDTA, 300 mM acetaldehyde, 100 mM acceptor, and 1000 units of DERA.[7] The mixture was stirred at room temperature in the dark under argon for 6 days. Protein was removed by addition of 2 volumes of acetone followed by centrifugation. The supernatant was concentrated under reduced pressure and purified via flash chromatography (SiO_2). [b] About 20% of mono aldol condensation product **8a** was isolated together with starting material and dideoxyhexose **3** as an inseparable mixture; see Scheme 2.

Scheme 2





(10) Rosen, T.; Heathcock, C. H. *Tetrahedron* **1986**, *42*, 4909.

(1) Barbas, C. F., III; Wang, Y.-F.; Wong, C.-H, *J. Am. Chem. Soc.* **1990**, *112*, 2013.

(2) Garcia-Junceda, E.; Chen, L.; Blanco, O.; Steensma, D. H.; Wong, C.-H. Submitted. The recombinant *E. coli* strain was deposited at the American Type culture collection (ATCC #86963).

(3) Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 741.

(4) Morrison, I. M.; Perry, M. B. *Can. J. Biochem.* **1966**, *44*, 1115.

(5) $[\alpha]_D$ +35° (c = 0.7, $CHCl_3$), compared to $[\alpha]_D$ +23.1° (c = 1.0, $CHCl_3$) for the product in 78% ee, synthesized by Bennet et al.[6]

(6) Bennet, F.; Knight, D. W.; Fenton, G. *J. Chem. Soc., Perkin Trans. 1* **1991**, 131.

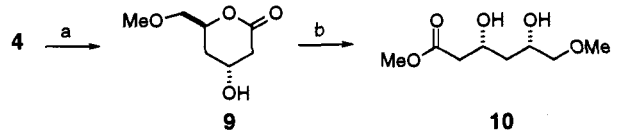
(7) Lyophilized DERA was used, obtained after ammonium sulfate fractionation (40–65%) and dialysis, as described in ref 3.

(8) A small amount of a double aldol condensation product was isolated from the DERA-catalyzed reaction between propanal and an azido analog of D-glyceraldehyde; see ref 3.

(9) The monoaldol product was isolated in dimeric form as a mixture of four diasteromers:

0002-7863/94/1516-8422$04.50/0 © 1994 American Chemical Society

Scheme 3[a]



9 **10**

[a] (a) Br_2, $BaCO_3$, H_2O, 68%, (b) MeOH, Amberlite IRA 400(OH), 96%.

open-chain *syn*-1,3-diol compounds. This is demonstrated for the transformation of dideoxyhexose **4**, which was converted via lactone **9** into the *syn*-3,5-dihydroxy ester **10** in 65% overall yield (Scheme 3). Products like **10** might be used in the synthesis of 1,3-polyol systems, which are widely distributed in nature, particularly in the skipped-polyol polyene macrolides.[12]

(11) McCaque, R.; Olivo, H. F.; Roberts, S. M. *Tetrahedron Lett.* **1993**, *34*, 3785 and references therein.

As can be seen from Table 1, not all substituted acetaldehydes are equally well accepted as substrates for DERA. Further studies are in progress to determine the scope of the DERA-catalyzed double aldol condensation.

Acknowledgment. We thank the NIH (GM 44154) and Quest International for financial support of this research.

Supplementary Material Available: Experimental details for the preparation of **2**, **9**, and **10** and ^1H and ^{13}C NMR spectral data for compounds **1–6**, **8a**, **9**, and **10** (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(12) Omura, S.; Tanaka, H. *Macrolide Antibiotics: Chemistry, Biology and Practice*; Omura, S., Ed.; Academic Press: New York, 1984; pp 351–401.