Sequential One-Pot Aldol Reactions Catalyzed by 2-Deoxyribose-5-phosphate Aldolase and Fructose-1,6-diphosphate Aldolase

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Several aldolases have been shown to be useful catalysts for asymmetric aldol condensation.[1] The enzymes 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4)[2−5] and fructose-1,6-diphosphate aldolase from rabbit muscle (RAMA, EC 4.1.2.13)[1] have been reported to accept a wide variety of acceptor substrates and were used in the synthesis of a variety of carbohydrate derivatives. In this paper we describe the coupling of the DERA- and RAMA-catalyzed sequential aldol reactions to form a variety of 5-deoxy 7-carbon carbohydrate derivatives. Also it was observed for the first time that the reactions in this enzymatic process were not 100% stereoselective at the reaction center for both enzymes.

The DERA-catalyzed aldol condensation between a substrate aldehyde **1** and acetaldehyde produces a new C2-unsubstituted aldehyde **2** that itself can be a substrate for a subsequent aldol reaction. Especially if this newly formed aldehyde cannot form a stable hemiacetal, a second condensation with acetaldehyde can take place as reported recently.[4] RAMA uses dihydroxyacetone phosphate **3** (DHAP) as a donor substrate and has been shown to accept several C2-unsubstituted aldehydes as acceptor substrates.[1,6] When the two aldolases and the right substrates are combined, the aldehyde formed after the DERA-catalyzed aldol condensation might undergo a second aldol condensation with DHAP catalyzed by RAMA to give a 5-deoxy ketose **4**. Similar to the sequential aldol reaction catalyzed by DERA, the driving force behind this reaction will be the formation of a stable hemiketal product. In Scheme 1 the different reactions that can take place in the reaction mixture are depicted.

To prevent the formation of products of type **5** from only RAMA-catalyzed reactions it will be necessary to use substrates that cannot form stable hemiketals after aldol reaction with DHAP. For initial experiments the substituted acetaldehydes were chosen with R = MeO, N_3, MOMO, and Cl. Hydroxyacetaldehyde (glycolaldehyde) was chosen for the study of a substrate that is capable of forming a stable hemiacetal or ketal after only a DERA- or RAMA-catalyzed reaction, respectively.

If the reaction is continued long enough, an equilibrium might be established with the expected 5-deoxy ketose **4** as the major product. The ratio of the original substrates is of course an important factor. Too much acetaldehyde gives side products **6** derived from a sequential DERA-catalyzed reaction,[4,7] but an excess of both initial acceptor aldehyde and acetaldehyde is necessary to get a high concentration of second acceptor aldehyde **2** for the RAMA-catalyzed reaction. It was found that a ratio of initial acceptor aldehyde:acetaldehyde:DHAP of 2:3:1 gave reasonable yields of 5-deoxy ketoses **4**.

Since all the reactions in Scheme 1 took place, other minor products of these reactions were present in the reaction mixture to some extent. Additionally, NMR analysis of these crude mixtures revealed the presence of small quantities of two other 5-deoxy ketoses which were apparently diastereomers of the main products **4**. These diastereomers were easily separated from the main product by flash chromatography (SiO_2). Further removal of other impurities with Dowex 50(H^+) in methanol, followed by flash chromatography (SiO_2), provided the pure methyl glycosides **7**. Scheme 2 depicts the different products. Yields of products were determined on the basis of their isolated methyl glycosides. Although diastereomers **9** and **10** were observed for all the substrates tested, they could be separated from each other only for methoxyacetaldehyde (**1a**) and azidoacetaldehyde (**1b**). The configuration of products **9** and **10** was determined via extensive NMR homodecoupling experiments.

The substrate glycolaldehyde (**1e**) gave a low yield of product **8e**, as expected because of the formation of product **5** (R = OH), which can form a stable hemiketal. A reasonable yield of **8e** could be obtained by incubating acetaldehyde and glycolaldehyde with DERA for 2 days, before adding RAMA and DHAP. Initial buildup of **2** (R = OH)[4] made the subsequent formation of **8e** in 13% yield possible. The main product formed in the reaction with the substrate chloroacetaldehyde (**1d**) was different from the expected **8d**. Apparently dehydrohalogenation of the initially formed product takes place, giving compound **11** as the final product in 26% yield (Scheme 3). The same anhydro compound was formed from **8c** and **8e** after treatment with MeOH/Dowex 50(H^+).

All reactions catalyzed by DERA and RAMA reported previously have been observed or assumed to take place with 100% stereoselectivity, giving products with 3*S* stereochemistry in the case of DERA and 3*S*,4*R* stereochemistry for the RAMA-catalyzed reaction.[1] This stereochemistry will give products **8** with three hydroxyl groups in axial positions. Side product **9** has the inverse stereochemistry at C4, one of the stereocenters formed in the RAMA-catalyzed reaction. Side products **10** has the inverse stereochemistry at C6, formed in the DERA-catalyzed reaction. Both side products **9** and **10** have less 1,3 diaxial interaction than **8** and are thus thermodynamically more stable products. Apparently, since all reactions are reversible and are run for a long time (thermodynamically controlled conditions), some inversion in the normal stereochemistry of both DERA and RAMA takes place. Inversion of the stereochemistry at C4 by other DHAP dependent aldolases has been observed,[8] and similar, but more pronounced, inversion has also been reported for *N*-acetylneuraminic acid aldolase.[9] For DERA and RAMA, this is the first occasion on which the catalyzed reactions have been observed not to proceed fully stereospecifically at the reaction center.

Several experiments were carried out to prove that the inversion of stereochemistry at C4 and C6 was caused by DERA and RAMA, respectively. Incubation of substrates of type **1** or **2** with acetaldehyde and DHAP, without adding aldolases, did not give any detectable products of type **8**, **9**, or **10**. The 3*S*,4*S* stereochemistry in product **9** is the usual stereochemistry

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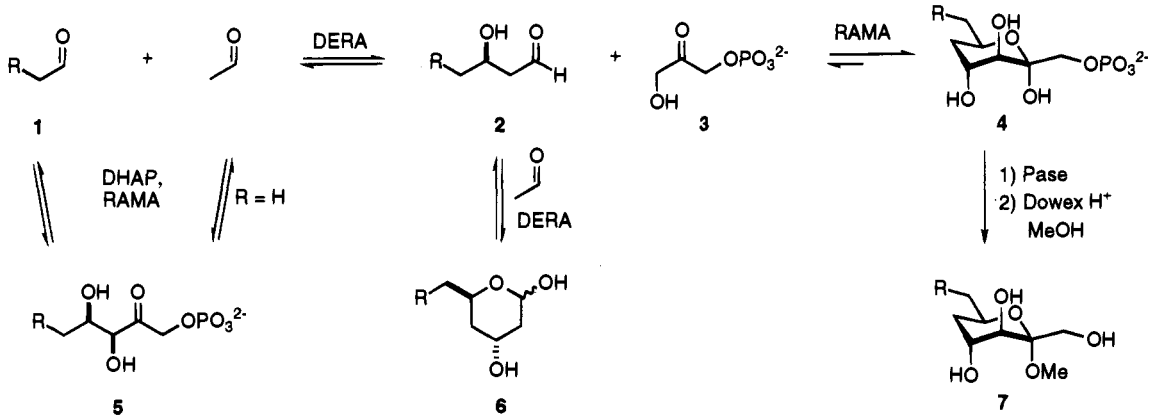
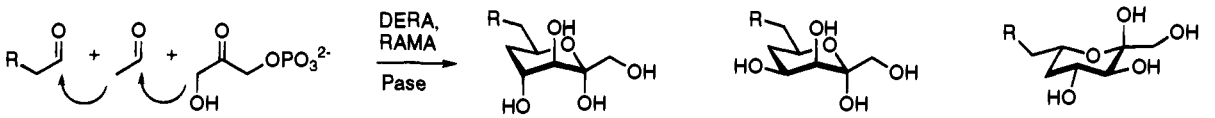
Scheme 1



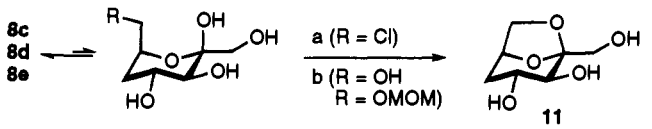
Scheme 2[a]



1a: R = OMe	**8a**: 45%	**9a**: 9%	**10a**: 3%
1b: R = N_3	**8b**: 34%	**9b**: 3%	**10b**: 2%
1c: R = OMOM	**8c**: 23%	**9c**: < 2%	**10c**: < 1%
1d: R = Cl	**8d**: < 5%	**9d**: < 3%	**10d**: < 2%
1e: R = OH	**8e**: 13%	**9e**: < 1%	**10e**: < 1%

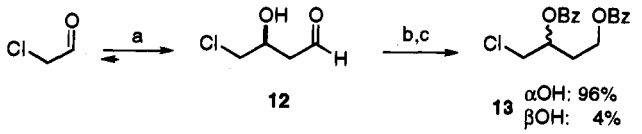
[a] Reactions were run for 6 days at pH 7.3 with 3 mmol of **1**, 4.5 mmol of acetaldehyde, 1.5 mmol of DHAP, 670 units of DERA, and 320 units (added in 3 portions) of RAMA in 30 mL total volume. Yields of products are based on isolated methyl glycosides or **11**.

Scheme 3[a]



[a] (a) Enzymatic Incubation; (b) Dowex 50(H^+), MeOH.

Scheme 4[a]



[a] (a) Acetaldehyde, DERA; (b) $NaBH_4$; (c) BzOCl, pyridine.

formed by tagatose-1,6-diphosphate aldolase (TDP-aldolase). To exclude the possibility that **9** was formed by other contaminating enzymes like TDP-aldolase,[10] the two aldol reactions were carried out separately. Product **12** was isolated after DERA-catalyzed aldol condensation of **1d** with acetaldehyde (Scheme 4).[4] After incubation of **12** with DHAP and RAMA,[10] a mixture of diastereomers similar to that from the one-pot reaction was obtained. Incubation of **12** with DHAP and crude DERA did not give any detectable products of type **8**, **9**, or **10**.

The stereoselectivity of the DERA-catalyzed reaction was tested further by reducing product **12**, followed by benzoylation of the obtained diol to give **13** (Scheme 4). The same product was synthesized as a racemic mixture by reaction of 3-butenol with NaOCl followed by benzoylation. Separation of the two enantiomers on HPLC, using a Daicel OD-H column, showed the presence of 96% of the 3*S* enantiomer and 4% of the 3*R* enantiomer, corresponding to an ee of 92%, indicating that at least for some substrates the DERA-catalyzed aldol condensation is not 100% selective. RAMA-catalyzed condensations between substrates of type **2** (Scheme 1) with the 3*R* stereochemistry are thermodynamically much more favorable as has been shown by the selective formation of products of type **10**, when racemic **2** is used as substrate.[6]

Finally, chemical inversion of the stereochemistry at C4 and C6 via retro-aldol/aldol reactions was excluded as follows. Product **12** and the crude phosphate-product mixture obtained from **2a** were kept under reaction conditions in the absence of enzymes for 1 week. After isolation and derivatization, the ee of **12** and the ratio of compounds **8a**, **9a** and **10a** had not changed.

In summary, this report describes a new multienzyme system for sequential aldol addition reactions. Due to the reversible formation of thermodynamically highly disfavored products of type **8** and the long reaction times, some inversion of the usual stereochemistry of both DERA and RAMA takes place. Since the side products are only a minor fraction of the major product, the present method of coupling the DERA- and RAMA-catalyzed aldol condensation provides a good, one-pot method for making 5-deoxy ketose derivatives **8**, using simple starting materials. Further studies are in progress to improve the yield of these reactions and to couple DERA with other aldolases.

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Supplementary Material Available: Detailed Procedure for the synthesis of the methyl glycosides of **8**, **9** and **10**. ^1H and ^{13}C NMR spectra for compounds **8a,b**, **9a,b**, and **10a,b** (methyl glycosides) and **8c**, **8e**, **11**, and **13** (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(10) Contamination of RAMA with TDP-aldolase can be excluded since, except for FDP-aldolase, no other DHAP dependent aldolases are present in mammalian tissues.