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Sequential Three- and Four-Substrate Aldol Reactions Catalyzed by Aldolases

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Abstract: This paper describes a useful synthetic strategy based on sequential enzyme-catalyzed aldol addition reactions. The aldol reaction of an acceptor aldehyde with acetaldehyde, catalyzed by 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4), gives a new aldehyde, which subsequently reacts with a second donor substrate, catalyzed by DERA or another aldolase. This sequential aldol reaction proceeds because of the formation of a stable cyclic hemiacetal or hemiketal product. In this way several 6-substituted 2,4,6-trideoxyhexoses have been stereoselectively synthesized via a one-pot double DERA-catalyzed reaction. DERA coupled with fructose-1,6-diphosphate aldolase from rabbit muscle (RAMA, EC 4.1.2.13) in a one-pot reaction gave a variety of 5-deoxyheptuloses, however not in 100% stereoselectivity. The sequential aldol reactions with four substrates were accomplished by coupling DERA with *N*-acetylneuraminic acid aldolase (NeuAc aldolase, EC 4.1.3.3) to give several nine-carbon sialic acid-type sugar derivatives.

Introduction

Aldolases are a group of enzymes which catalyze C−C bond formation, often in a highly stereoselective way. Over 30 aldolases have been identified so far, and several have been used in organic synthesis.[1] The mild reaction conditions, high stereoselectivity, and minimal use of protective group chemistry make the use of aldolases an interesting alternative to the chemical aldol reactions. Most aldolases catalyze the condensation of an aldehyde with a ketone donor, giving a ketone as product. The enzyme 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4)[2-4] is the only aldolase which accepts two aldehydes in the condensation reaction, giving a new aldehyde product. Recent studies show that, in certain DERA-catalyzed reactions, product **2** can be an acceptor substrate for a second aldol condensation catalyzed by DERA[5] or another aldolase,[6] giving products of type **3** (Scheme 1). Thus, DERA and other aldolases can be used in combination for sequential aldol reactions leading to products with multiple chiral centers, starting from simple, nonchiral substrates. This report details the scope of these sequential aldol reactions, catalyzed by DERA[5] or by DERA coupled with fructose-1,6-diphosphate aldolase from rabbit muscle (RAMA, EC 4.1.2.13)[6] or with *N*-acetylneuraminic acid aldolase (NeuAc aldolase, EC 4.1.3.3). The three enzymes DERA, RAMA, and NeuAc aldolase are commercially available and have been shown to accept a wide range of acceptor substrates.[1] The products obtained through the combination of these enzymes are carbohydrate-related compounds with deoxy carbons at various positions and might be useful as chiral building blocks or carbohydrate mimetics. The three

[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1995.

(1) (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamom: Oxford, U.K., 1994; Chapter 4. (b) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 412.

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

(3) Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 741. DERA can be easily prepared from a recombinant *E. coli* (ATCC 86963).

(4) Wong, C.-H.; Garcia-Junceda, E.; Chen, L.; Blanco, O.; Gijsen, H. J. M.; Steensma, D.H. *J. Am. Chem. Soc.* **1995**, *117*, 3333.

(5) Gijsen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 8422.

(6) Gijsen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 2947.

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Scheme 1

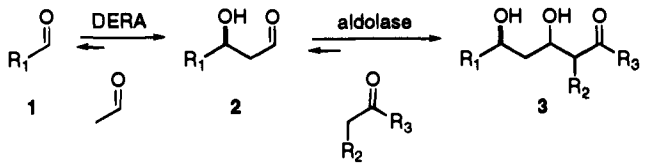
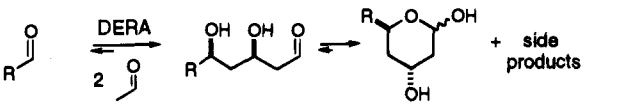
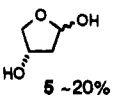
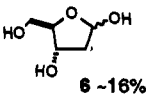


Table 1. Sequential Aldol Reactions Catalyzed by DERA



R		Yield		Ref.
1a	CH_3	**4a** 22%		4
1b	CH_2OH	**4b** <3%[b]		5
			5 ~20%	
1c	CH_2OMe	**4c** 65%		4
1d	CH_2OMOM	**4d** 25%		
1e	CH_2Cl	**4e** 70%		4
1f	CH_2Br	**4f** --		5
1g	CH_2N_3	**4g** 23%		4
1h	$CH(CH_3)_2$	**4h** 13%		
1i	CH_2CH_2COOH	**4i** 80%		4
1j	$CH_2OPO_3^{2-}$	**4b** <3%[c]		
1k	CH_2COOH	**4k** --		
1l	COOH	**4l** <3%		
1m	$CH_2(OMe)_2$	**4m** --		
1n	$CH_2CH_2(OEt)_2$	**4n** --		
1o[a]	$CH(OH)CH_2OPO_3^{2-}$ (D-Glyceraldehyde-3-phospate)	**4o** 75%[c]	 **6** ~16%	

[a] Prepared *in situ* from DHAP.[9] [b] Products **4b** and **5** were isolated as an inseparable mixture together with the starting material. [c] Products were obtained after treatment of the reaction mixture with acid phosphatase.

combinations DERA–DERA, DERA–RAMA, and DERA–NeuAc will be discussed separately below.

Results and Discussion

DERA-Catalyzed Sequential Aldol Reactions. In a previous communication[5] we described the stereoselective formation of a variety of 2,4,6-trideoxyhexoses from different C2-substituted acetaldehydes and two molecules of acetaldehyde via a sequential aldol reaction, catalyzed by DERA. We have further investigated this sequential reaction, and Table 1 summarizes the substrates tested so far and the products formed in the reactions. The enzymatic polymerization stops after two aldol condensations due to the formation of stable cyclic hemiacetals. As soon as a stable cyclic hemiacetal is formed, the concentration of free aldehyde available for a subsequent aldol condensation diminishes and the reaction stops at that stage. This was illustrated by the sequential aldol reaction with glycolaldehyde to give product **4b** in a relatively low yield. After aldol condensation between glycolaldehyde and acetaldehyde product **5** is obtained, which is predominantly in its hemiacetal form, slowing down the formation of **4b** via a second aldol condensation. Protection of the hydroxyl group in glycolaldehyde prevents cyclization after the first aldol condensation and should make it possible to prepare **4b** in a better yield. To test this, the MOM-protected glycolaldehyde **1d** and glycolaldehyde phosphate **1j** were tried as substrates. For **1d**, the yield of the corresponding sequential aldol product **4d** was indeed higher at 25% but was still disappointing if compared with the structurally similar, but much better substrate, methoxyacetaldehyde **1c**. Compound **1j** was expected to be a good substrate for a sequential reaction not only because the intermediate aldehyde **2** cannot cyclize but also because the phosphate group in **1j** resembles the phosphate group in the natural substrate D-glyceraldehyde 3-phosphate (G3P). A negatively charged group in the substrate, mimicking the phosphate group in G3P, has been shown before to give a high yield of product (succinic semialdehyde **1i**).[4] Unfortunately, upon reaction with **1j** only traces of **4b** could be detected, after *in situ* treatment of the phosphorylated product with acid phosphatase. The acid aldehydes **1k,l** also proved to be poor substrates, and only traces of aldol products were observed. A negative charge close to the aldehyde functionality apparently makes the aldehyde a poor acceptor for DERA. The binding of the negatively charged group of these short molecules in the active site of DERA might place the aldehyde group too far from the lysine residue with which it has to form a Schiffs base intermediate.[7] The 1,3-dicarbonyl substrate **1k** might also be a poor substrate because it will tautomerize predominantly to its enolate form. The semiprotected dialdehydes **1m,n**, which would have given interesting products, did not give any sequential aldol product. The reasons for being a good or poor substrate for the sequential aldol reaction are still not fully understood. What makes it more complex is that the initial aldehyde acceptor and the product from the first aldol condensation both have to be reasonable substrates for DERA. Perhaps the substrate specificity of this enzyme will be better understood when the crystal structure of DERA is elucidated.[8]

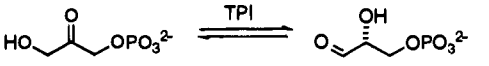
A very good substrate for the DERA sequential aldol condensation appeared to be the natural substrate G3P (**1o**). The reaction of dihydroxyacetone phosphate (DHAP) with acetaldehyde in the presence of triose phosphate isomerase (TPI)[9] and DERA gave, after acid phosphatase treatment, 75% of **4o** (R = $CH(OH)CH_2OH$) and 16% of 2-deoxyribose **6**. The standard amount of DERA (specific activity 189 U/mg)[3] for the sequential aldol reaction (500 U/mmol of substrate) was used for this reaction, which is very high considering G3P to be the natural substrate for DERA. Despite the fact that the initial product 2-deoxyribose 5-phosphate (DRP) is mainly in its hemiacetal form, the high affinity of DRP for DERA and the use of large amounts of enzyme make a second aldol condensation possible, giving product **4o**. A similar addition to a hemiacetal product took place when acetaldehyde was treated with a very large amount of DERA (2500 units/mmol of initial substrate) for a long period of time (2 weeks). Besides a much higher yield of **4a** (64%), compound **7** (~6%), formed after three subsequent aldol condensations, was isolated (Scheme 2). DERA-catalyzed aldol condensation of four substrates was never observed except with acetaldehyde, presumably because acetaldehyde condensed faster with itself, giving **4a**, than with the double aldol condensation product which is predominantly in its hemiacetal form.

Besides acetaldehyde, DERA also accepts propanal and acetone as donor substrates.[2,3] The reactions in Scheme 2 were

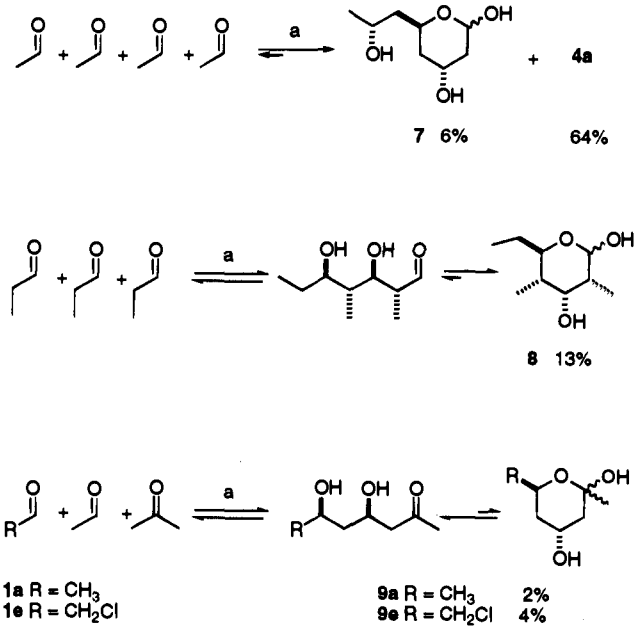
(7) Rosen, O. M.; Hoffee, P.; Horecker, B. L.; Speck, J. C., Jr. *J. Am. Chem. Soc.* **1964**, *86*, 2092.

(8) Crystals from DERA, suitable for X-ray crystallography, have already been obtained: Stura, E. A.; Ghosh, S.; Garcia-Junceda, E.; Chen, L.; Wong, C.-H.; Wilson, I. A. *Proteins*, in press.

(9) TPI isomerases DHAP into G3P, which subsequently reacts as a substrate for DERA:



Scheme 2



[a] 1250−2500 units of DERA/mmol of initial substrate, 2 weeks.

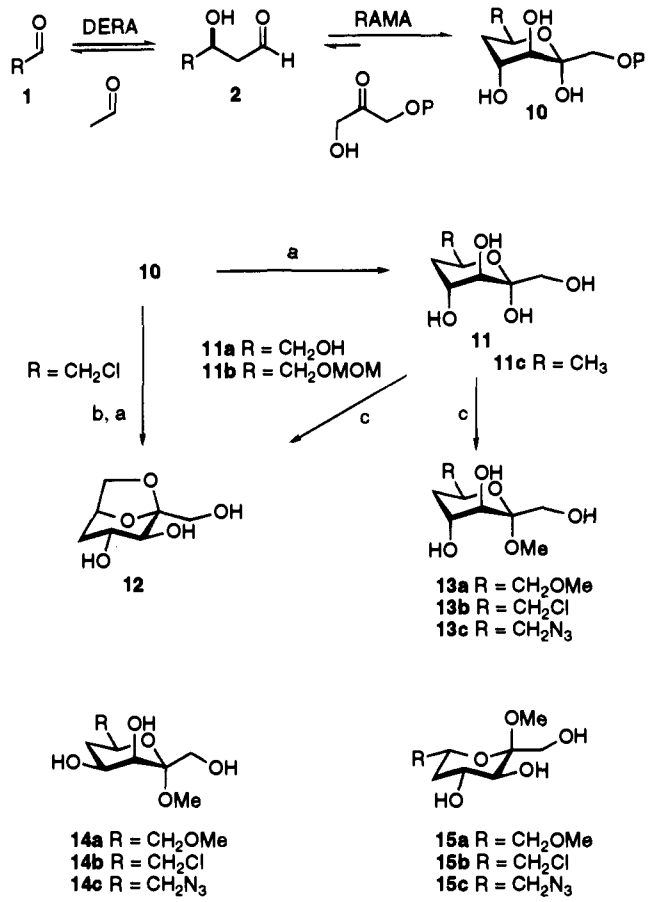
performed to investigate whether sequential aldol reactions were possible with different donor substrates. Since both propanal and acetone react at a much slower (<1%) rate than acetaldehyde,[3] large amounts of DERA and long reaction times were necessary to observe product formation. In this way product **8** was isolated in 13% yield from the sequential aldol reaction between three molecules of propanal. Despite the moderate yield in this process, a molecule with four asymmetric centers was formed stereospecifically from a very simple starting material in a one-pot reaction.

The sequential aldol reaction between acetaldehyde or chloroacetaldehyde with acetaldehyde and acetone gave the products **9a** and **9e** in only 2 and 4% yields, respectively. Due to the very slow rate of reaction with the donor substrate acetone, the competing reaction with acetaldehyde gave the corresponding 2,4,6-trideoxyhexoses **4a,e** as the major products. This could not be prevented by using a 5-fold excess of acetone. The low yield may be attributed to the fact that both **9a** and **9b** are predominantly in their open ring form, as was shown by NMR analysis, thus preventing the reaction from going to completion.

Coupled DERA−RAMA-Catalyzed Sequential Aldol Reactions. When smaller amounts of DERA (250 units/mmol) were used for the double DERA aldol condensation (*vide supra*), the monoaldol product (**2**) could be isolated as the main product,[5] making it possible to add another aldolase whose donor substrate subsequently would react with the product formed from the DERA-catalyzed reaction. We chose RAMA to study this combination since RAMA is readily available and has a broad substrate specificity.[1,6] The natural substrates for RAMA are G3P as acceptor and DHAP as donor to give fructose 1,6-diphosphate (FDP) as the product. As new stereocenters formed by RAMA have the D-*threo* stereochemistry, coupling of DERA and RAMA should lead to product **10** with the stereochemistry as indicated (Scheme 3).

By choosing the right C2-substituted acetaldehydes **1**, reactions of these initial substrates with DHAP gave products which could not form a stable hemiacetal, limiting the formation of such products. Under thermodynamically controlled conditions, the major product would then be a 5-deoxyheptulose **10**. This strategy was tested with several C2-substituted acetaldehydes (Table 2). The products were isolated from the reaction

Scheme 3



[a] (a) Acid phosphatase. (b) DERA/RAMA reaction conditions. (c) Dowex 50(H^+).

Table 2. Coupled DERA−RAMA-Catalyzed Sequential Aldol Reactions

initial substrate (RCHO)		product	yield (%) with TPI-activity[6]	yield (%) TPI-inhibited
1a	R = CH_3	**11c**		<17[a]
1b	R = CH_2OH	**12**	13[a]	
1c	R = CH_2OMe	**13a**	45	54
1d	R = CH_2OMOM	**12**	23	
1e	R = CH_2Cl	**12**	26	60[b]
1g	R = CH_2N_3	**13c**	34	61

[a] This yield could only be obtained after incubating **1a** or **1b** with acetaldehyde and DERA for 2 days before adding DHAP and RAMA. [b] Isolated as 45% of **12** and 15% of **13b**.

mixtures after dephosphorylation with acid phosphatase to give **11**, which after treatment with Dowex 50(H^+) in methanol gave **13**. In this way pure, **13a** and **13c** were obtained for R = CH_2-OMe and R = CH_2N_3, respectively, and compound **12** was obtained for R = CH_2OH and CH_2OMOM. For R = CH_2Cl, compound **12** was obtained as the major product, together with some **13b**, via dehydrohalogenation during incubation with the aldolases followed by the phosphatase reaction. Besides products with the stereochemistry shown in **10**, traces of the diastereomers **14** and **15** were also detected for all the substrates tested.[6] Products of type **14** resulted from an inversion of the stereochemistry at C4, formed by RAMA; products of type **15** resulted from an inversion of the stereochemistry at C6, formed by DERA.[10] Product **10** has three hydroxyl groups in axial position, making it thermodynamically disfavored compared to

(10) For experiments to exclude the formation of these products by other enzymes or via chemical aldol/retroaldol reactions, see ref 6.

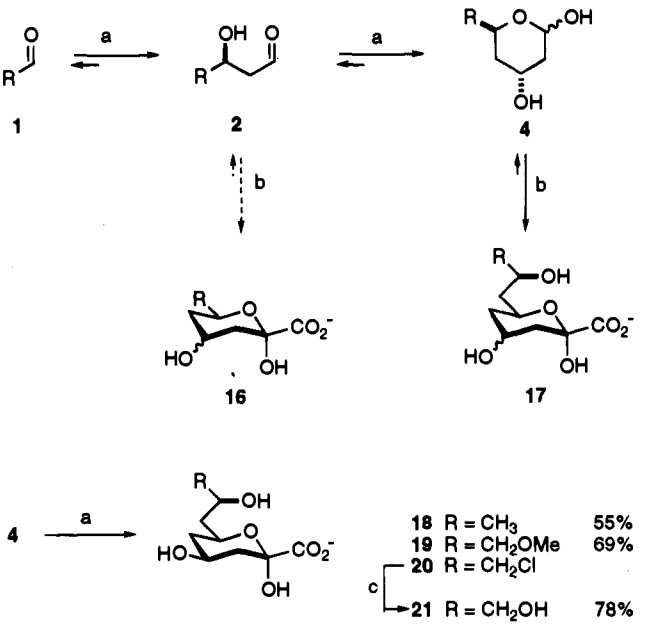
the two diastereomeric derivatives **14** and **15**, which have less 1,3-diaxial interactions than **10**. Since the aldol reactions were reversible and long reaction times (6 days) were used, some minor products with different stereoselectivity in DERA- or RAMA-catalyzed reactions were formed. For R = CH_2OMe, CH_2Cl, and CH_2N_3, the two minor diastereomers were isolated and were pure enough to be characterized by NMR.

By using acetaldehyde as the initial acceptor substrate, no 5-deoxyheptuloses could be isolated. However, the major products isolated appeared to be 2-deoxyribose **6** and **4o**. These products could only be formed via a DERA-catalyzed aldol condensation between G3P and acetaldehyde. This implicated that triose phosphate isomerase (TPI)[11] was present in the reaction mixture, which converts DHAP into G3P. Indeed the RAMA suspension used (Sigma) contains traces of TPI. Also the DERA preparation was shown to contain TPI by incubating DERA with DHAP and acetaldehyde without the addition of extra TPI. This gave **4o** and **6** in 67% and 6% yields, respectively. Although not isolated, G3P will also react with DHAP, catalyzed by RAMA, to give FDP. In order to prevent these side reactions, which also contributed to the low yield of the other entries in Table 2, both enzyme preparations were treated with bromohydroxyacetone phosphate, a specific inhibitor of TPI.[12] By using these TPI-free enzyme preparations, the yield of the reactions could be improved considerably, as was shown for several substrates (Table 2).[13] Acetaldehyde (entry 1), however, still proved to be a very weak substrate. Only after incubation of acetaldehyde with DERA for 2 days before addition of RAMA and DHAP, impure **11c** (R = CH_3) was obtained in 17% yield. The limiting factor in this reaction was probably the DERA-catalyzed reaction, for which acetaldehyde is a weak acceptor substrate. Fortunately, because of this slow reaction with acetaldehyde, products from the other entries in Table 2 were not contaminated with side products resulting from acetaldehyde as initial acceptor.

Coupled DERA–NeuAc Aldolase-Catalyzed Sequential Aldol Reactions. *N*-Acetylneuraminic acid aldolase (NeuAc aldolase, EC 4.1.3.3) catalyzes *in vivo* the reversible condensation of pyruvate with D-*N*-acetylmannosamine to form NeuAc or sialic acid. The enzyme only accepts pyruvate as a donor substrate but is more flexible for the acceptor substrates.[1] NeuAc aldolase seems to have a preference for larger acceptor molecules (five or more C-atoms);[14] therefore in the coupled DERA-NeuAc aldolase reaction the main substrate for NeuAc aldolase would not be the monoaldol product, tetrose **2** , but the double DERA aldol product, hexose **4**, and the expected final product should be **17** (Scheme 4). Removal of product **4** by the NeuAc aldolase-catalyzed reaction would drive the DERA reaction to completion.

Unfortunately, in a one-pot reaction of DERA and NeuAc aldolase, with acetaldehyde, methoxyacetaldehyde, and chloroacetaldehyde as initial acceptors, we did not observe any products of type **17**. Formation of trideoxyhexoses **4a**,**c**,**e** was observed but no subsequent reaction with pyruvate. Although NeuAc aldolase has been shown to exhibit activity at room temperature, the rate of reaction is very slow.[15] On the other hand, DERA was inactivated too fast above room temperature

Scheme 4



[a] (a) Acetaldehyde, DERA. (b) Pyruvate, NeuAc aldolase. (c) Dowex 1-X8-100 (HCO_3^- form).

making the reaction at higher temperature unsuccessful. Incubation of pure **4a**,**c**,**e** with pyruvate and NeuAc aldolase showed that these 2,4,6-trideoxyhexoses were good substrates for NeuAc aldolase. After 4–8 days of incubation at 37 °C, NMR analysis of the crude, lyophilized reaction mixtures showed optimal conversion of substrates to products. During purification via ion-exchange chromatography on Dowex 1-X8-100 (HCO_3^- form), the chloro compound **20** was hydrolyzed to give the hydroxy compound **21** as the isolated product.[16] The stereochemistry of **18**–**21** at C4 is inverted to the normal stereochemistry observed after reaction with NeuAc aldolase. This result is in accordance with earlier findings where, under thermodynamically controlled conditions products with a C4 equatorial hydroxyl group are predominant.[17]

For methoxyacetaldehyde, formation of **19** was examined with the DERA-catalyzed reaction first at room temperature, followed by the addition of NeuAc aldolase and pyruvate at a higher temperature, without isolation of the intermediate product **4**. This process gave a lower yield (29%) of **19**, which was isolated as an inseparable mixture with small amounts of **18** and presumably product **16** (R = CH_2OMe), resulting from NeuAc aldolase-catalyzed reaction between pyruvate and **2** (R = CH_2-OMe). Although the one-pot combination of DERA and NeuAc aldolase was apparently not feasible, combination of the two in sequence provided a facile synthesis of these nine-carbon sugar derivatives, which might be useful mimics of KDO and related structures.

Concluding Remarks

It has been demonstrated that the DERA-catalyzed aldol condensation can be coupled with a subsequent aldol reaction catalyzed by other aldolases. In this way a variety of 6-sub-

(11) TPI has a very high specific activity (up to 10 000 U/mg) and is very difficult to remove completely.

(12) De La Mare, S.; Coulson, A. F. W.; Knowles, J. R.; Priddle, J. D.; Offord, R. E. *Biochem. J.* **1972**, *129*, 321.

(13) The amounts of side products **16** and **17** also increased, but this was not a problem for purification of the major products.

(14) Kim, M.-J.; Hennen, W. J.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 6481.

(15) Baumann, W.; Freidenreich, J.; Weisshaar, G.; Brossmer, R.; Friebolin, H. *Biol. Chem.* **1989**, *370*, 141.

(16) Cardillo, G.; Orena, M.; Porzi, G.; Sandri, S. *Synthesis* **1981**, 793.

(17) (a) Augé, C.; Gautheron, C.; David, S. *Tetrahedron* **1990**, *46*, 201. (b) Gautheron-Le Narvor, C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 7816. (c) Lin, C.-H.; Sugai, T.; Halcomb, R. L.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 10138. (d) Kragl, U.; Gödde, A.; Wandrey, C.; Lubin, N.; Augé, C. *J. Chem. Soc., Perkin Trans. 1* **1994**, 119.

stituted 2,4,6-trideoxyhexoses[18] and 7-substituted 5-deoxyheptuloses have been synthesized in a one-pot reaction via DERA–DERA- and DERA–RAMA-catalyzed sequential aldol reactions, respectively. The formation of a stable, cyclic hemiacetal or hemiketal is the driving force for these sequential aldol reactions. This requirement was illustrated by the observation of the low-yield products from glycolaldehyde **1b** as well as the low yield of products of type **9**, which existed only in a small percentage in their hemiketal form. An exception to this is the formation of **4o** from G3P and two molecules of acetaldehyde, which proceeds in excellent yield, presumably due to the high affinity of the intermediate product, 2-deoxyribose 5-phosphate, for DERA. Although it is still not very well understood what the criteria are for being a good substrate, a negatively charged group on a similar position as in G3P makes the substrate react very well.

Sequential four-substrate aldol reactions have been performed by combining DERA with NeuAc aldolase. Although this process has not been possible in a one-pot reaction due to the incompatibility of the reaction conditions for the two enzymes, several sialic acid derivatives have been synthesized in good yields.

The products obtained from the DERA–DERA and DERA–NeuAc sequential aldol reactions contain 1,3-polyol functionalities which are widely distributed in nature. They might be useful building blocks for the synthesis of compounds containing such polyol systems. The 5-deoxyheptuloses obtained from the DERA–RAMA sequential aldol reactions may have interesting physiological properties which need to be investigated further.

Other aldolases can probably be coupled with DERA if they are active under conditions similar to those with DERA (pH, temperature). Three other aldolases with DHAP as the donor substrate,[1] for example, give products with stereochemistry complementary to that of **10**. Care should be taken, however, to remove TPI activity in the enzyme preparations used.

Experimental Section

General Procedure. A Bruker AMX-400 spectrometer was used for 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR spectra. In ^{1}H NMR spectra, signals belonging to protons from α- or β-anomers are indicated with α or β, respectively. Anomeric ratios were determined in D_2O. High-resolution mass spectra (HRMS) were obtained on a VG ZAB-ZSE mass spectrometer in electron impact (EI) or fast atom bombardment (FAB) or with solid probe. Optical rotations were obtained from $CHCl_3$ on a Rudolph research autopol III polarimeter and were measured in H_2O. NeuAc aldolase was obtained from Toyobo; all other chemicals and enzymes, except DERA (from *E. coli* strain DH5α, ATCC 86963),[19] were purchased from Aldrich or Sigma. DHAP was synthesized as described previously.[20]

A. General Procedure for DERA-Catalyzed Reactions. DERA (1000 U) was added to a 20 mL solution containing 100 mM acceptor aldehyde, 300 mM donor aldehyde, 100 mM triethanolamine buffer (pH 7.3), and 1 mM EDTA. The resulting solution was stirred in the dark for 6 days under N_2. The reaction was quenched by addition of 2 vol of acetone, then cooled to 0 °C for 20 min and centrifuged to remove the precipitated protein. After removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography.

From the mixture of products obtained after DERA-catalyzed reaction between glycolaldehyde and acetaldehyde (general procedure), the following characteristic peaks could be identified (^{1}H NMR, D_2O).

2,4-Dideoxy-D-*erythro*-hexapyranose (4b): δ 1.49–1.99 (m, 4H), 3.57–3.68 (m, 2H), 3.96–4.06 (m, 1H, H-5β), 4.33–4.38 (m, 1H, H-3β), 5.13 (dd, J = 10.1, 2.3 Hz, 1H, H-1β).

2-Deoxy-D-*erythro*-pentafuranose (5) (major anomer): δ 2.06 (ddd, J = 14.7, 5.8, 4.2 Hz, 1H), 2.22 (ddt, J = 14.7, 5.6, 1.7 Hz, 1H), 3.8 (d, J = 10.0 Hz, 1H), 4.04 (dd, J = 10.0, 3.7 Hz, 1H), 4.56–4.60 (m, 1H), 5.68 (dd, J = 5.4, 4.4 Hz, 1H).

6-*O*-(Methoxymethyl)-2,4-dideoxy-D-*erythro*-hexose (4d). Using the general procedure, flash chromatography (EtOAc to 10:1 EtOAc/MeOH) gave 99 mg (25%) of **4d** as a mixture of anomers (α:β ratio, 1:5.5). ^{1}H NMR (D_2O): δ 1.56–2.02 (m, 4H), 3.44 (s, 3H), 3.64 (dd, J = 11.0; 7.0 Hz, 1H), 3.73 (dd, J = 11.0, 3.1 Hz, 1H), 4.14–4.22 (m, 1H, H-5β), 4.29 (quint, J = 3.8 Hz, 1H, H-3α), 4.40 (quint, J = 3.0 Hz, 1H, H-3β), 4.46–4.52 (m, 1H, H-5α), 4.74 (d, J = 6.8 Hz, 1H), 4.77 (d, J = 6.8 Hz, 1H), 5.17 (dd, J = 10.1, 2.2 Hz, 1H, H-1β), 5.33 (t, J = 3.1 Hz, 1H, H-1α). ^{13}C NMR (D_2O) (β-anomer): δ 35.04, 40.60, 57.53, 66.99, 72.58, 72,86, 94.38, 98.59. ^{13}C NMR (D_2O) (α-anomer): δ 35.19, 37.72, 65.75, 66.21, 72.58, 72.86, 94.05, 98.59. HRMS for $C_8H_{16}O_5Na$ (M + Na): calcd 215.0895, found 215.0900.

6-Methyl-2,4,6,7-tetradeoxy-D-*erythro*-heptose (4h). Using the general procedure, flash chromatography (hexane/EtOAc, 1:2 to 1:3) gave 43 mg (13%) of **4h** as a mixture of anomers (α:β ratio, 1:4). ^{1}H NMR (D_2O) (β-anomer): δ 0.87 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 1.41–1.95 (m, 5H), 3.57 (ddd, J = 1.9, 6.8, 12.0 Hz, 1H), 4.32 (quint, J = 3 Hz, 1H), 5.06 (dd, J = 2.2, 10.2 Hz, 1H). ^{1}H NMR (D_2O) (α-anomer) (characteristic peaks): δ 3.87 (ddd, J = 5.3, 7.6, 7.6 Hz, 1H), 4.19 (quint, J = 4.3 Hz, 1H), 5.21 (t, J = 3.5 Hz, 1H). ^{13}C NMR (D_2O) (β-anomer): δ 19.82, 20.48, 34.35, 35.60, 40.78, 67.51, 78.78, 94.63. ^{13}C NMR (D_2O) (α-anomer): δ 20.09, 20.48, 33.24, 35.87, 38.50, 66.10, 73.21, 93.69. HRMS for $C_8H_{16}O_3Na$ (M + Na): calcd 183.0997, found 183.0977.

2,4-Dideoxy-D-*ribo*-heptose (4o). A solution of 1.5 mmol of DHAP, 4.5 mmol of acetaldehyde, 1000 U of DERA, and 100 U of TPI was stirred at pH 7.3 in the dark for 6 days under N_2. Then the pH was adjusted to 4.7 with aqueous HCl, and 500 U of acid phosphatase was added. After stirring for one night at 37 °C, the reaction mixture was worked up in the standard way. The residue was flash chromatographed (EtOAc/MeOH, 7:1 to 5:1) to give 32 mg (16%) of 2-deoxyribose **6** and 201 mg (75%) of **4o** as a mixture of anomers (α:β ratio, 1:5). ^{1}H NMR (D_2O): δ 1.54–1.98 (m, 4H), 3.58–3.66 (m, 1H), 3.69–3.78 (m, 2H), 3.94 (ddd, J = 11.8, 5.8, 2.1 Hz, 1H, H-5β), 4.21–4.29 (m, 2H, H-3α and H5α), 4.39 (quint, J = 3.0 Hz, 1H, H-3β), 5.12 (dd, J = 10.1, 2.1 Hz, 1H, H-1β), 5.27 (t, J = 3.1 Hz, 1H, H-1α). ^{13}C NMR (D_2O) (β-anomer): δ 34.52, 40.65, 64.75, 67.01, 73.48, 75.98, 94.60. ^{13}C NMR (D_2O) (α-anomer): δ 34.18, 37.89, 64.88, 65.63, 67.37, 75.44, 94.16. HRMS for $C_7H_{14}O_5Na$ (M + Na): calcd 201.0739, found 201.0742.

In a similar experiment but without the addition of TPI and 1500 U of DERA, 6% of **6**, and 67% of **4o** were obtained.

2,4,6,8-Tetradeoxy-D-*ribo*-octose (7). DERA (1250 U) was added to a 10 mL solution containing 3 mmol of acetaldehyde, 100 mM triethanolamine buffer (pH 7.3), and 1 mM EDTA. After 2 days, another 2 mmol of acetaldehyde and 1250 U of DERA were added. After another 12 days, the reaction was worked up as usual to give after flash chromatography 141 mg (64%) of **4a**[4] and 15 mg (6%) of **7** as a mixture of anomers (α:β ratio, 1:6). ^{1}H NMR (major anomer) (D_2O): δ 1.22 (d, J = 6.3 Hz, 1H), 1.48–1.98 (m, 6H), 3.97–4.09 (m, 2H), 4.31–4.35 (m, 1H), 5.11 (dd, J = 10.2, 2.2 Hz, 1H). ^{13}C NMR (D_2O): δ 24.26, 39.03, 40.59, 45.82, 67.25, 67.77, 71.48, 94.39. Characteristic ^{1}H NMR peaks minor anomer (D_2O): δ 1.23 (d, J = 6.3 Hz, 3H), 5.24 (t, J = 3.5 Hz, 1H). HRMS for $C_8H_{16}O_4Na$ (M + Na): calcd 199.0946, found 199.0945.

2,4-Dimethyl-2,4,6,7-tetradeoxy-D-*allo*-heptose (8). Compound **8** was synthesized from 5 mmol of propanal as described above for **7**. Flash chromatography (1:1 EtOAc/hexane) gave 38 mg (13%) of **8** as a mixture of anomers (α:β ratio, 1:5). ^{1}H NMR (β-anomer) (D_2O): δ 0.90 (d, J = 7.3 Hz, 6H), 0.96 (d, J = 7.0 Hz, 3H), 1.37–1.61 (m, 2H), 1.71–1.80 (m, 2H), 3.78 (t, J = 2.8 Hz, 1H), 3.82 (ddd, J = 7.6, 6.6, 2.3 Hz, 1H), 4.67 (d, J = 9.3 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 10.26, 10.38, 12.29, 24.90, 37.01, 38.38, 74.01, 75.83, 97.15. ^{1}H NMR (α-anomer) (D_2O): δ 0.89 (d, J = 7.1 Hz, 3H), 0.92 (d, J = 7.4 Hz, 3H), 1.01 (d, J = 7.2 Hz, 3H), 1.37–1.61 (m, 2H), 1.85–1.93 (m, 1H), 1.98–2.05 (m, 1H), 3.74 (dt, J = 2.5,1.2 Hz, 1H), 4.12 (ddd, J = 8.3, 6.9, 2.8 Hz, 1H), 4.97 (d, J = 3.4 Hz). ^{13}C NMR ($CDCl_3$): δ

(18) These products can be easily oxidized to derivatives of the lactone moiety of mevinic acids, see ref 5.

(19) Lyophilized DERA was used, obtained after sulfate fractionation (40–65%) and dialysis as described in refs 3 and 4.

(20) Jung, S.-H.; Jeong, J.-H.; Miller, P.; Wong, C.-H. *J. Org. Chem.* **1994**, *59*, 7182.

10.10, 10.30, 13.21, 24.74, 32.10, 38.00, 65.71, 75.54, 96.41. HRMS for $C_9H_{18}O_3Na$ (M + Na): calcd 197.1154, found 197.1156.

(4*R*,6*R*)-4,6-Dihydroxy-2-heptanone (9a). DERA (1000 U) was added to a 20 mL solution containing 4 mmol of acetaldehyde, 10 mmol of acetone, 100 mM triethanolamine buffer (pH 7.3), and 1 mM EDTA. The resulting solution was stirred in the dark for 14 days under N_2. During this period, another 1500 U of DERA was added portionwise. Workup as usual gave a residue which was purified by flash chromatography (10:1 EtOAc/hexane to EtOAc) to give 5 mg (2%) of **9a** as a 3:1 mixture of the acyclic and hemiketal product. ^{1}H NMR (D_2O): δ 1.28 (d, J = 6.0 Hz, 3H), 1.50−1.78 (m, 2H), 2.26 (s, 3H), 2.72 (dd, J = 16.5, 8.5 Hz, 1H), 2.81 (dd, J = 16.5, 4.1 Hz, 1H), 3.99 (sext, J = 6.5 Hz, 1H), 4.21-4.30 (m, 1H). ^{13}C NMR (D_2O): δ 24.34, 32.45, 47.02, 52.74, 67.98, 68.13, 216.60. Hemiketal. ^{1}H NMR (D_2O): δ 1.21 (d, J = 5.2 Hz, 3H), 1.38 (s, 3H), 1.50−1.78 (m, 2H), 1.83 (br d, J = 14.6 Hz, 1H), 1.95 (br d, J = 14.6 Hz, 1H), 4.21−4.30 (m, 1H), 4.31−4.40 (m, 1H). ^{13}C NMR (D_2O): δ 22.94, 30.72, 40.11, 40.99, 64.15, 67.12, C(hemiketal) unclear. HRMS for $C_7H_{14}O_3Na$ (M + Na): calcd 169.0841, found 169.0836.

(4*R*,6*S*)-7-Chloro-4,6-dihydroxy-2-heptanone (9e). Starting with 2 mmol of chloroacetaldehyde, 2.2 mmol of acetaldehyde, and 10 mmol of acetone, **9e** was prepared similar to **9a** as a 6:1 mixture of the acyclic and hemiketal product. ^{1}H NMR (D_2O): δ 1.78−1.83 (m, 2H), 2.26 (s, 3H), 2.75 (dd, J = 16.6, 8.5 Hz, 1H), 2.83 (dd, J = 16.6, 4.3 Hz, 1H), 3.64 (dd, J = 11.8, 5.9 Hz, 1H), 3.75 (dd, J = 11.8, 3.4 Hz, 1H), 4.04−4.10 (m, 1H), 4.25−4.35 (m, 1H). ^{13}C NMR (D_2O): δ 32.45, 42.38, 51.54, 52.42, 67.56, 70.92, 216.33. Hemiketal. ^{1}H NMR (D_2O): δ 1.42 (s, 3H), 1.73−1.85 (m, 3H), 1.97 (ddd, J = 14.8, 3.1, 1.9 Hz, 1H), 3.60−3.78 (m, 2H), 4.25−4.35 (m, 1H), 4.43−4.49 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 30.65, 35.04, 39.13, 47.64, 64.79, 65.27, 97.41. HRMS for $C_7H_{13}O_3ClNa$ (M + Na): calcd 203.0451, found 203.0458.

B. General Procedure for Coupled DERA−RAMA-Catalyzed Reactions. To inhibit TPI activity, both the DERA and RAMA enzyme preparations were treated with bromohydroxyacetone phosphate[11] (10 μmol/1000 U of enzyme) for 5 min and then extensively dialyzed using centriprep tybes (Amicon).

DERA (670 U) and RAMA (160 U) were added to a 30 mL solution containing 3 mmol of initial acceptor aldehyde, 4.5 mmol of acetaldehyde, and 1.5 mmol of DHAP. The pH was adjusted to 7.3, and the reaction was stirred at room temperature in the dark under argon. After 2 and 4 days, 80 units of additional RAMA was added. After 6 days, the pH was brought to 4.7 with diluted HCl, 500 μL (250 units) of acid phosphatase (sweet potato, Sigma) was added, and the reaction mixture was stirred overnight at 37 °C. Protein was removed by the addition of 2 vol of acetone followed by centrifugation. The supernatant was concentrated under reduced pressure and prepurified via flash chromatography (SiO_2) to give two fractions which contained in order of elution products of type **11** and mixtures of their diastereomers, respectively. The separate fractions were dissolved in dry methanol, and Dowex 50(H^+) was added. The mixtures were stirred overnight and filtered. Removal of the solvent under reduced pressure and flash chromatography (SiO_2) gave the pure methyl glycosides.

5-Deoxy-D-*arabino*-heptulose (11a). DERA (1000 U, not TPI-inhibited) was added to a 22 mL solution containing 2.5 mmol of glycolaldehyde dimer and 4 mmol of acetaldehyde. The resulting solution was stirred at pH 7.3 for 2 days, after which the reaction was continued as described under the general procedure B. Flash chromatography (EtOAc/MeOH, 7:1 to 5:1) gave 66 mg of crude **11a**. ^{1}H NMR (D_2O): δ 1.60 (br dt, J = 2.6, 14.5 Hz, H5eq), 1.85 (ddd, J = 3.2, 12.5, 14.5 Hz, H5ax), 3.45 (d, J = 11.6 Hz), 3.52−3.75 (m, 4H), 4.10 (q, J = 3.1 Hz, 1H), 4.17−4.25 (m, 1H). ^{13}C NMR (D_2O): δ 30.12, 66.73, 66.94, 67.82, 68.03, 70.29, 100.17. HRMS for $C_7H_{14}O_6$-Na (M + Na): calcd 217.0733, found 217.0739.

2,7-Anhydro-5-deoxy-D-*arabino*-heptulose (12). Treatment of the crude **11a** with Dowex 50(H^+) in MeOH, followed by flash chromatography (EtOAc/MeOH, 8:1 to 4:1), gave 34 mg (13%) of **12**. $[\alpha]_D$ −85° (c = 1.1). ^{1}H NMR (D_2O): δ 1.82 (dddd, J = 1.6, 3.6, 10.9, 13.6 Hz, H5ax), 2.19 (ddd, J = 2.0, 6.6, 13.6 Hz, H5eq), 3.51 (d, J = 8.2 Hz, H3ax), 3.80 (d, J = 12.6 Hz, H1a), 3.84 (d, J = 12.6, H1b), 3.86 (ddd, J = 1.6, 5.1, 7.6 Hz, H7a), 3.91 (ddd, J = 6.6, 8.1, 10.9 Hz, H4ax), 3.97 (d, J = 7.6 Hz, H7b), 4.73−4.77 (m, H6eq). ^{13}C NMR (D_2O): δ 38.93, 62.36, 71.30, 71.84, 76.68, 77.32, 110.14. HRMS for $C_7H_{12}O_5Na$ (M + Na): calcd 199.0582, found 199.0580.

7-*O*-(Methoxymethyl)-5-deoxy-D-*arabino*-heptulose (11b). Using the general procedure B, without inhibition of TPI, flash chromatography (EtOAc/MeOH, 10:1 to 5:1) gave 120 mg of crude **11b**. ^{1}H NMR (D_2O): δ 1.65 (br d, J = 14.6 Hz, H5eq), 1.94 (ddd, J = 3.2, 12.5, 14.6 Hz, H5ax), 3.42 (s, 3H), 3.47 (d, 11.6 Hz, 1H), 3.63−3.74 (m, 4H), 4.14 (q, J = 3.2 Hz, 1H), 4.36−4.43 (m, 1H), 4.73 (s, 2H). ^{13}C NMR (D_2O): δ 30.39, 57.56, 66.45, 66.87, 67.93, 70.24, 72.75, 98.55, 100.37. HRMS for $C_9H_{18}O_7Na$ (M + Na): calcd 261.0950, found 261.0962. Treatment of this mixture with Dowex 50(H^+) in MeOH, followed by flash chromatography (EtOAc/MeOH, 10:1 to 4:1) gave 61 mg (23%) of **12**.

5,7-Dideoxy-D-*arabino*-heptulose (11c). DERA (800 U, not TPI-inhibited) was added to a 20 mL solution containing 6 mmol of acetaldehyde. The resulting solution was stirred at pH 7.3 for 2 days, after which the reaction was continued as described under the general procedure B. Flash chromatography (EtOAc/MeOH, 8:1 to 5:1) gave 65 mg (70% pure, <17% yield) of crude **11c**, which we were not able to purify further. ^{1}H NMR (D_2O): δ 1.19 (d, J = 6.3 Hz, 3H), 1.71 (dddd, J = 0.9, 2.8, 2.8, 14.8 Hz, H5eq), 1.81 (ddd, J = 3.1, 11.7, 14.8 Hz, H5ax), 3.38 (d, J = 11.5 Hz, H1a), 3.67 (dd, J = 0.6, 3.3 Hz, H3eq), 3.68 (d, J = 11.5 Hz, H1b), 4.04−4.09 (m, 1H), 4.27−4.32 (m, 1H). ^{13}C NMR (D_2O): δ 22.69, 35.82, 63.76, 66.80, 67.43, 70.52, 100.52. HRMS for $C_7H_{14}O_5Na$ (M + Na): calcd 201.0739, found 201.0733.

Methyl 7-*O*-Methyl-5-deoxy-D-*arabino*-heptuloside (13a). Using the general procedure B on a 1 mmol scale, flash chromatography (EtOAc/MeOH, 8:1 to 5:1, for glycosides and EtOAc/MeOH, 12:1 to 10:1, for methyl glycosides) gave 120 mg (54%) of **13a**. $[\alpha]_D$ +63° (c = 2.5). ^{1}H NMR (D_2O): δ 1.59 (dddd, J = 0.9, 2.5, 2.6, 14.6 Hz, H5eq), 1.87 (ddd, J = 3.5, 12.5, 14.6 Hz, H5ax), 3.31, (s, 3H), 3.40 (s, 3H), 3.52 (dd, J = 6.5, 11.0 Hz, H7a), 3.55 (dd, J = 3.6, 11.0 Hz, H7b), 3.65 (d, J = 12.3 Hz, H1a), 3.69 (d, J = 12.3 Hz, H1b), 3.69 (d, J = 3.2 Hz, H3eq), 4.01 (q, J = 3 Hz, H4eq), 4.11−4.18 (m, H6ax). ^{13}C NMR (D_2O): δ 30.39, 50.15, 60.09, 60.82, 66.55, 67.57, 70.17, 77.18, 103.74. HRMS for $C_9H_{18}O_6Na$ (M + Na): calcd 245.1001, found 245.1009.

A 3:1 mixture (38 mg) of the diastereomers **14a** (13%) and **15a** (4%) was obtained. Repeated chromatography gave pure samples of them:

Methyl 7-*O*-Methyl-5-deoxy-D-*lyxo*-heptuloside (14a). $[\alpha]_D$ +70° (c = 0.4). ^{1}H NMR (D_2O): δ 1.54 (q, J = 12.2 Hz, H5ax), 1.66 (dddd, J = 0.9, 2.6, 5.1, 12.5 Hz, H5eq), 3.24 (s, 3H), 3.36 (s, 3H), 3.50 (dd, J = 4.0, 6.2 Hz, H7a=b), 3.67 (s, H1a+b), 3.76 (d, J = 3.1 Hz, H3eq), 3.88−3.96 (dddd, J = 2.6, 4.0, 6.2, 12 Hz, H6ax), 4.12 (ddd, J = 3.1, 5.1, 11.9 Hz, H4ax). ^{13}C NMR (D_2O): δ 30.90, 49.94, 59.42, 60.73, 67.81, 69.22, 70.85, 76.96, 104.34. HRMS for $C_9H_{18}O_6Na$ (M + Na): calcd 245.1001, found 245.0995.

Methyl 7-*O*-Methyl-5-deoxy-L-*xylo*-heptuloside (15a). $[\alpha]_D$ −91° (c = 0.15). ^{1}H NMR (D_2O): δ 1.42 (q, J = 12.1 Hz, H5ax), 1.95 (ddd, J = 2.1, 5.1, 12.6 Hz, H5eq), 3.26 (s, 3H), 3.38 (s, 3H), 3.52 (d, J = 6.2, 10.9 Hz, H7a), 3.54 (d, J = 9.5 Hz, H3ax), 3.56 (dd, J = 3.2, 10.9 Hz, H7b), 3.75 (s, H1a+b), 3.89−3.98 (m, H4ax + 6ax). ^{13}C NMR (D_2O): δ 36.54, 50.51, 60.78, 62.95, 70.34, 70.40, 75.12, 76.66, 102.83. HRMS for $C_9H_{18}O_6Na$ (M + Na): calcd 245.1001, found 245.0994.

Methyl 7-Chloro-5,7-dideoxy-D-*arabino*-heptuloside (13b). Using the general procedure B, flash chromatography (EtOAc/MeOH, 10:1 to 4:1, for glycosides and EtOAc/MeOH, 10:1, for methyl glycosides) gave 119 mg (45%) of **12** and 54 mg (15%) of **13b**. $[\alpha]_D$ +67° (c = 1.4). ^{1}H NMR (D_2O): δ 1.69 (br t, J = 2.4, 14.5 Hz, H5eq), 1.95 (ddd, J = 3.5, 12.3, 14.5 Hz, H5ax), 3.32 (s, 3H), 3.62 (dd, J = 7.3, 11.9 Hz, H7a), 3.68 (s, H1a+b), 3.69 (d, J = 4 Hz, H3eq), 3.74 (dd, J = 3.2, 11.9 Hz, H7b), 4.02 (q, J = 3 Hz, H4eq), 4.12−4.19 (m, H6ax). ^{13}C NMR (D_2O): δ 31.94, 49.41, 50.18, 59.99, 67.49, 68.11, 70.33, 104.11. HRMS for $C_8H_{15}O_5ClNa$ (M + Na): calcd 249.0506, found 249.0518.

Diastereomers **14b** and **15b** were obtained in 7% (25 mg) and 3% (10 mg, impure) yields, respectively.

Methyl 7-Chloro-5,7-dideoxy-D-*lyxo*-heptuloside (14b). $[\alpha]_D$ +63° (c = 0.7). ^{1}H NMR (D_2O): δ 1.67 (q, J = 12 Hz, H5ax), 1.79 (ddd,

J = 2.4, 4.9, 12.4 Hz, H5eq), 3.35 (s, 3H), 3.63 (dd, J = 7.2, 11.9 Hz, H7a), 3.72 (s, H1a+b), 3.73 (dd, J = 3.3, 11.9 Hz, H7b), 3.79 (d, J = 3.0 Hz, H3eq), 3.98 (dddd, J = 2.9, 2.9, 7.1, 11.8 Hz, H6ax), 4.14 (ddd, J = 3.1, 5.0, 11.9 Hz, H4ax). ^{13}C NMR (D_2O): δ 32.38, 49.01, 50.02, 59.36, 67.87, 69.05, 72.26, 104.57. HRMS for $C_8H_{15}O_5ClNa$ (M + Na): calcd 249.0506, found 249.0517.

Methyl 7-Chloro-5,7-dideoxy-L-*xylo*-heptuloside (15b). ^{1}H NMR (D_2O): δ 1.51 (q, J = 12 Hz, H5ax), 2.06 (ddd, J = 2.1, 5.1, 12.7 Hz, H5eq), 3.30 (s, 3H), 3.52−3.78 (m, 5H), 3.94 (ddd, J = 5.0, 9.4, 11.5 Hz, H4ax), 3.97−4.05 (m, H6ax). ^{13}C NMR (D_2O): δ 37.85, 48.96, 50.59, 62.83, 70.34, 71.59, 75.06, 102.92. HRMS for $C_8H_{15}O_5ClNa$ (M + Na): calcd 249.0506, found 249.0512.

Methyl 7-Azido-5,7-dideoxy-D-*arabino*-heptuloside (13c). Using the general procedure B on a 1 mmol scale, flash chromatography (EtOAc/MeOH, 10:1, for glycosides and EtOAc for methyl glycosides) gave 143 mg (61%) of **13c**. $[\alpha]_D$ +40° (c = 1.1). ^{1}H NMR (D_2O): d 1.59 (br d, J = 14.4 Hz, H5eq), 1.88 (ddd, J = 3.5, 12.4, 14.4 Hz, H5ax), 3.31 (s, 3H), 3.36−3.41 (m, H7a+b), 3.64 (d, J = 12.3 Hz, H1a), 3.66 (d, J = 2.5 Hz, H3eq), 3.68 (d, J = 12.3 Hz, H1b), 3.99 (q, J = 3 Hz, H4eq), 4.07−4.14 (m, H6ax). ^{13}C NMR (D_2O): δ 31.38, 50.12, 56.60, 60.03, 67.42, 67.45, 70.25, 103.86. HRMS for $C_8H_{15}O_5N_3Na$ (M + Na): calcd 256.0909, found 256.0907.

Diastereomers **14c** and **15c** were obtained in 5% (12 mg) and 3% (10 mg, ~70% pure) yields, respectively.

Methyl 7-Azido-5,7-dideoxy-D-*lyxo*-heptuloside (14c). $[\alpha]_D$ +59° (c = 0.7). ^{1}H NMR (D_2O): δ 1.60 (q, J = 12 Hz, H5ax), 1.69 (ddd, J = 2.6, 5.1, 12.5 Hz, H5eq), 3.28 (s, 3H), 3.39 (d, J = 5.6 Hz, 2H), 3.69 (s, 2H), 3.76 (d, J = 3.1 Hz, H3eq), 3.92 (ddt, J = 2.6, 5.6, 11.7 Hz, H6ax), 4.12 (ddd, J = 3.1, 5.1, 11.8 Hz, H4ax). ^{13}C NMR (D_2O): δ 31.95, 49.97, 56.39, 59.46, 67.84, 69.10, 71.57, 104.42. HRMS for $C_8H_{15}O_5N_3Na$ (M + Na): calcd 256.0909, found 256.0915.

Methyl 7-Azido-5,7-dideoxy-L-*xylo*-heptuloside (15c). ^{1}H NMR (D_2O): δ 1.44 (q, J = 12 Hz, H5ax), 1.98 (ddd, J = 2.0, 5.1, 12.6 Hz, H5eq), 3.29 (s, 3H), 3.37−3.44 (m, H7a+b), 3.55 (d, J = 9.4 Hz, H3eq), 3.73 (d, J = 11.9 Hz, H1a), 3.77 (d, J =11.9 Hz, H1b), 3.92 (ddd, J = 5.1, 9.4, 11.4 Hz, H4eq), 3.93−4.00 (m, H6ax). ^{13}C NMR (D_2O): δ 37.54, 50.51, 56.16, 62.83, 70.36, 71.06, 74.97, 103.46. HRMS for $C_8H_{15}O_5N_3Na$ (M + Na): calcd 256.0909, found 256.0918.

C. General Procedure for NeuAc Aldolase Reactions. NeuAc aldolase (50 U) was added to a 10 mL solution containing 100 mM **4**, 1 M of sodium pyruvate, and 50 mM KPi. The mixture was shaken slowly at pH 7.6 and 37 °C. The reaction was monitored by making NMR spectra of crude 300 μL lyophilized aliquots. When the ratio of product to starting material did not change further (4−8 days), the reaction was concentrated under vacuum. The product was isolated by anion-exchange chromatography on Dowex 1-X8 (HCO_3^- form; 70 × 2.5 cm), using a gradient of ammonium bicarbonate (0−0.2 M) as the eluent. Fractions containing the product were pooled, lyophilized, deionized with Dowex 50W-X8, (H^+), and again lyophilized. Finally, Bio Gel P-2 chromatography was applied to obtain the pure compound.

3,5,7,9-Tetradeoxy-L-*arabino*-2-nonulosonic Acid (18). Using the general procedure C, **18** was obtained in 55% yield. $[\alpha]_D$ +40° (c = 1.1). ^{1}H NMR (D_2O): δ 1.17 (d, J = 6.3 Hz, 3H), 1.28 (q, J = 12 Hz, 1H), 1.57−1.66 (m, 2H), 1.77−1.85 (m, 1H), 2.08 (dddd, J = 2, 2, 4.5, 12.4 Hz, 1H), 2.18 (ddd, J = 1.7, 4.7, 12.7 Hz, 1H), 3.94−4.03 (m, 1H), 4.07−4.16 (m, 2H). ^{13}C NMR (D_2O): δ 24.62, 41.41, 41.69, 45.60, 65.87, 67.57, 70.89, 98.01, 175.82. HRMS for $C_9H_{16}O_6Na$ (M + Na): calcd 243.0845, found 243.0854.

9-*O*-Methyl-3,5,7-trideoxy-L-*arabino*-2-nonulosonic Acid (19). Using the general procedure C, **19** was obtained in 69% yield. $[\alpha]_D$ +34° (c = 3.0). ^{1}H NMR (D_2O): δ 1.27 (q, J = 12 Hz, 1H), 1.60 (dd, J = 11.6, 12.7 Hz, 1H), 1.63−1.79 (m, 2H), 2.03−2.10 (m, 2H), 3.37 (s, 3H), 3.41 (dd, J = 7.1, 10.7 Hz, 1H), 3.49 (dd, J = 3.3, 10.7 Hz, 1H), 3.96−4.13 (m, 3H). ^{13}C NMR (D_2O): δ 40.62, 41.43, 42.27, 60.74, 66.50, 69.72, 70.13, 78.34, 99.19, 179.29. HRMS for $C_{10}H_{18}O_7Na$ (M + Na): calcd 273.0950, found 243.0945.

9-Chloro-3,5,7,9-tetradeoxy-L-*arabino*-2-nonulosonic Acid (20). After concentration of the crude reaction mixture, the following data were obtained for **20** (sodium salt). ^{1}H NMR (D_2O): δ 1.32 (q, J = 11.9 Hz, 1H), 1.63 (dd, J = 11.8, 12.6 Hz, 1H), 1.75−1.95 (m, 2H), 2.06−2.14 (m, 2H), 3.63 (dd, J = 5.9, 11.8 Hz, 1H), 3.74 (dd, J = 3.2, 11.8 Hz, 1H), 4.04−4.17 (m, 3H). ^{13}C NMR (D_2O): δ 41.30, 41.47, 42.27, 51.72, 66.50, 69.95, 70.70, 99.24, 179.34. After anion-exchange chromatography on Dowex 1-X8 (HCO_3^- form; 70 × 2.5 cm), **20** was converted into **21**, which was isolated in 78% yield.

3,5,7-Trideoxy-L-*arabino*-2-nonulosonic Acid (21). $[\alpha]_D$ +30° (c = 0.3). ^{1}H NMR (D_2O): δ 1.28 (q, J = 12 Hz, 1H), 1.61 (t, J = 12 Hz, 1H), 1.65−1.80 (m, 2H), 2.05−2.13 (m, 2H), 3.49 (dd, J = 6.6, 11.8 Hz, 1H), 3.60 (dd, J = 3.8, 11.8 Hz, 1H), 3.84-3.90 (m, 1H), 4.05−4.14 (m, 2H). ^{13}C NMR (D_2O): δ 40.37, 41.36, 42.18, 66.41, 67.66, 70.23, 71.53, 99.14, 178.53. HRMS for $C_9H_{16}O_7Cs$ (M + Cs): calcd 368.9950, found 368.9962.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **4d**,**h**,**o**, **7**, **8**, **9a**,**e**, **12**, **13a**−**c**, **14a**−**c**, **15a**, **18**, **19**, and **21** (35 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of this 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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