Aldotetroses and C(3)-Modified Aldohexoses as Substrates for *N*-Acetylneuraminic Acid Aldolase: A Model for the Explanation of the Normal and the Inversed Stereoselectivity

Wolfgang Fitz, Jan-Robert Schwark, and Chi-Huey Wong*

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

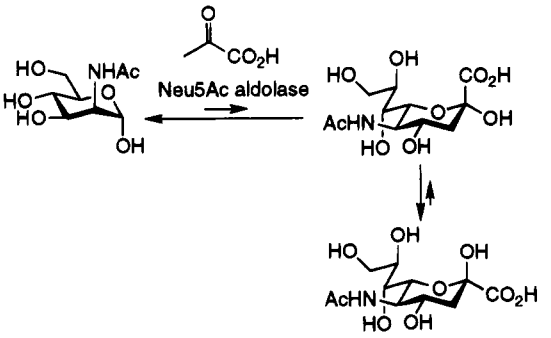
Received January 31, 1995[⊗]

The four stereoisomeric aldotetroses were accepted with different reactivities by *N*-acetylneuraminic acid aldolase. C(3)-modified D-mannose and D-glucose derivatives, respectively, failed to undergo enzymatic aldol addition. Based on the observed reactivities of the tested compounds (about 58), a mechanistic scheme is proposed which relates substrate structure, reactivity and stereochemical outcome observed in Neu5Ac aldolase-catalyzed reactions. The condensation products obtained in the L-erythrose and D-threose reactions are side-chain modified sialic acid and D-KDO derivatives, respectively, of biological interest.

Introduction

The enzyme *N*-acetylneuraminic acid (Neu5Ac) aldolase (E. C. 4.1.3.3) catalyzes the reversible aldol reaction of *N*-acetyl-D-mannosamine (ManNAc) and pyruvate[1] (Scheme 1). The α anomer of ManNAc is the substrate and the less stable α anomer of Neu5Ac is released after the enzymatic reaction.[2] Being a type I aldolase the enzyme first forms a Schiff base with a lysine residue and pyruvate which then reacts with *N*-acetyl-D-mannosamine in a reversible manner. The imidazole group of a histidine residue is presumed to protonate the aldehyde group of the acceptor substrate.[3] With regard to its substrate specificity, Neu5Ac aldolase has been shown to be specific for pyruvate,[4] but flexible to a variety of hexoses and pentoses, both D- and L-sugars, as the acceptor substrate.[4,5] Consequently, the enzyme has been used for the synthesis of various sialic acids, which either are of interest due to the involvement of these compounds in biological recognition processes[6] or which have served as intermediates in the synthesis of polyhydroxylated alkaloids[5k] and C-linked sialosides.[7] In order to shift the overall equilibrium towards product formation an excess of pyruvate is usually provided, when the enzyme is used for synthetic purposes. The relaxed specificity towards the structure of the acceptor component is a general feature displayed by various aldolases, which tend to be only specific for the donor component in the aldol addition reaction.[8] However, in contrast to the strict enzymatic control of the stereochemical course usually encountered with aldolases, the stereochemical outcome of Neu5Ac aldolase-catalyzed reactions depends on the structure of the substrate. With most substrates (e.g. *N*-acetyl-D-mannosamine, D-mannose,[5i] L-allose,[5l] D-lyxose[5f]) the carbonyl group is attacked from the *si* face, which results in the formation of a new stereogenic center with *S* configuration (the sialic acid type). With some substrates (e.g. L-mannose,[5i] D-altrose,[5l] L-xylose[5l]), on the other hand, the stereochemical course of the reaction can be reversed: the enamine attacks the carbonyl component from the *re* face, and the C(4) center of the resulting product has the *R* configuration (the D-KDO type). The stereochemical outcome of the reaction seems to be under thermodynamic control. In all cases, where *re* attack has been shown to occur partially or exclusively, the resulting *R* configured product is the thermodynamically more stable species with the hydroxy group at C(4) in the equatorial position.

Scheme 1. Aldol Condensation Mediated *in Vivo* by Neu5Ac Aldolase



In spite of the large body of experimental data[4,5] from reactions involving both *re* and *si* attack obtained mainly

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1995.

(1) Comb, D. G.; Roseman, S. *J. Am. Chem. Soc.* **1958**, *80*, 497. Comb, D. G.; Roseman, S. *J. Biol. Chem.* **1960**, *235*, 2529. DeVries, G. H.; Binkley, S. B. *Arch. Biochem. Biophys.* **1972**, *151*, 234.

(2) (a) Baumann, W.; Freidenreich, J.; Weisshaar, G.; Brossmer, R.; Friebolin, H. *Biol. Chem.* **1989**, *370*, 141. (b) Deijl, C. M.; Vliegenthart, J. F. G. *Biochem. Biophys. Res. Commun.* **1983**, *111*, 668.

(3) Nees, S.; Schauer, R.; Mayer, F.; Ehrlich, K. *Z. Physiol. Chem.* **1976**, *357*, 839. Barnett, J. E. G.; Corina, D. L.; Rasool, G. *Biochem. J.* **1971**, *125*, 275.

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

(5) (a) Augé, C.; David, S.; Gautheron, C.; Veyrières, A. *Tetrahedron Lett.* **1985**, *26*, 2439. (b) Augé, C.; Gautheron, C. *J. Chem. Soc., Chem. Commun.* **1987**, 859. (c) Augé, C.; David, S.; Gautheron, C.; Malleron, A.; Cavayé, B. *New J. Chem.* **1988**, *12*, 733. (d) Augé, C.; Bouxom, B.; Cavayé, B.; Gautheron, C. *Tetrahedron Lett.* **1989**, *30*, 2217. (e) Schrell, A.; Whitesides, G. M.; *Liebigs Ann. Chem.* **1990**, 1111. (f) Augé, C.; Gautheron, C.; David, S. *Tetrahedron* **1990**, *46*, 201. (g) Gautheron-Le Narvor, C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 7816. (h) Liu, J. L.-C.; Shen, G.-J., Ichikawa, Y.; Rutan, J. F.; Zapata, G.; Vann, W. F.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 3901. (i) Lin, C.-H.; Sugai, T.; Halcomb, R. L.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 10138. (j) David, S.; Malleron, A.; Cavayé, B. *New J. Chem.* **1992**, *16*, 751. (k) Zhou, P.; Salleh, H. M.; Honek, J. F. *J. Org. Chem.* **1993**, *58*, 264. (l) Kragl, U.; Gödde, A.; Wandrey, C.; Lubin, N.; Augé, C. *J. Chem. Soc. Perkin Trans. 1* **1994**, 119.

(6) Schauer, R. *Sialic Acids*; Springer-Verlag, Wien and New York, 1982.

(7) Spevak, W.; Nagy, J. O.; Charych, D. H.; Schaefer, M. E.; Gilbert, J. H.; Bednarski, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 1146.

(8) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier: New York, 1994; pp 195–228.

0022-3263/95/1960-3663$09.00/0

by David's group and ours, the kinetic behavior observed with the different substrates has not been rationalized in general terms. The pathways leading to C(4)-*S*-products and C(4)-*R*-products, respectively, seem to involve transition states of different energy: literature data[5i,5l] suggests that reactions involving *si* face attack (the normal case) proceed faster than those involving *re* face attack. ^{1}H NMR studies of the product distribution during the course of the reactions also indicate that *re* face attack is kinetically disfavored. In cases, where the product resulting from *re* face attack is thermodynamically favored, the product distribution changes during the course of the reaction. With L-mannose, for example, the ratio C(4)-*S*-product versus C(4)-*R*-product has been demonstrated to change from 3:5 after 1.5 h to 1:10 after 7 days.[5i] In this research we aimed at delineating the requirements, which have to be applied to the conformation of the substrates in Neu5Ac aldolase reactions. The lack of enzymatic control of the stereoselectivity obviously indicates that the enzyme can bind the substrates—and, as indicated by the ^{1}H NMR studies, one and the same substrate—in different conformations, which allow the attack of the enamine to occur either from the *re* or the *si* face of the carbonyl group. Our approach to this problem has been focused on the examination of the role of C(2) and C(3) substituents. For this purpose the four stereoisomeric C_4-aldoses and C(3)-modified D-mannose and D-glucose derivatives were tested as substrates of Neu5Ac aldolase.

Active-site models have been proposed for various enzymes of interest from a synthetic point of view[9-13] and have proven to be valuable for the subsequent use of these enzymes in organic synthesis. For example, Ziffer[9a-c] proposed a rule for ester hydrolysis catalyzed by the yeast *Rhizopus nigrigans*, and Kazlauskas[10b] elaborated a widely applicable model to predict the reactivity of chiral esters in hydrolysis reactions catalyzed by cholesterol esterase and lipases from *Pseudomonas cepacia* and *Candida rugosa*. For the enantioselectivity in the reduction of ketones by the yeast *Culvaria lunata* a rule was proposed by Prelog,[11a] which was subsequently used for the determination of absolute configurations.[13] Although a crystal structure of alcohol dehydrogenase from horse liver is available, a cubic space model elaborated by Jones[11b] is commonly applied to predict the enantioselectivity of this enzyme, because this model is both reliable and convenient to use. With Neu5Ac aldolase the situation is somewhat complicated by the fact that all steps after Schiff base-formation are reversible under the applied conditions, i.e. the once-formed C(4)-*S*-product can undergo retroaldol reaction to give the substrate, which then can be transformed into the C(4)-*R*-product (and *vice versa*). In view of the important application of Neu5Ac aldolase in the synthesis of modified sialic acids it would nevertheless be useful to have a model, which could be used to predict the reactivity of potential substrates for this enzyme. In conjunction with earlier findings of Brossmer[2a] and of Wandrey and Augé,[5l] the results described in this article allow us to propose a kinetic model, which is an attempt to rationalize the observed reactivity of the variety of aldoses tested so far as substrates of this synthetically useful enzyme.

Results and Discussion

Aldohexoses Modified at C(3) as Substrates of Neu5Ac Aldolase. A survey of the vast body of data collected on the substrate specificity of Neu5Ac aldolase led others[5l] and us to the conclusion that the hydroxy group at C(3) of the acceptor substrate seems to be critical for the enzyme's selectivity. In the D-manno series, for example, the 2-acetamido group and the C(4)-, C(5)-, and C(6)-hydroxyls can be replaced by hydrogen, and the resulting deoxygenated D-mannose derivatives are still fair substrates giving the corresponding deoxygenated sialic acids in good yield.[5f,g,i,j] 3-Deoxy-D-mannose, on the other hand, was reported to be a poor substrate which was only sluggishly converted into a mixture of four unidentified components.[5j] The authors assumed that they had isolated the anomeric mixtures of the furanose forms of the two nonulosonic acids epimeric at C(4). In order to clarify the role of a free hydroxy group at C(3) in the aldolase reaction, we now replaced the C(3)-hydroxyl of D-mannose and D-glucose derivatives, which had previously been demonstrated to be accepted by Neu5Ac aldolase, by azido, amino, bromo, and chloro groups and tested the compounds as substrates. The results can be summarized easily. As indicated by relative rate measurements[5i] none of the substrates in this series, namely 3-azido-3-deoxy-D-mannose (**19**), 3-amino-3-deoxy-D-mannose (**20**), 3-bromo-3-deoxy-D-mannose (**9**), 2,3-diazido-2,3-dideoxy-D-mannose (**16**), 2-azido-3-chloro-2,3-dideoxy-D-mannose (**13**), and 3-bromo-3-deoxy-D-glucose (**6**), were accepted by the enzyme. It becomes clear that a free hydroxyl at the C(3) position of the substrate is a prerequisite for successful aldol reaction.

Aldotetroses as Substrates of Neu5Ac Aldolase. Since previous results[5j] showed that 5-deoxy-*N*-acetyl-D-mannosamine and 5-deoxy-D-mannose are readily transformed into the corresponding C(8) deoxygenated sialic acids it can be concluded that it is not necessary that the pyranose form of the substrate is recognized by the enzyme. This, in turn, should in principle allow aldotetroses to be accepted as substrates by the aldolase.

The reactions with the four stereoisomeric C_4-aldoses were performed on 0.2 mmol scales. In separate experiments, 0.1 M solutions of D-erythrose, L-erythrose (**1**),

(9) Models for esterases: (a) Ziffer, H.; Kawai, K.; Kasai, M.; Imuta, M.; Froussios, C. *J. Org. Chem.* **1983**, *48*, 3017. (b) Kasai, M.; Kawai, K.; Imuta, M.; Ziffer, H. *J. Org. Chem.* **1984**, *49*, 675. (c) Charton, M.; Ziffer, H. *J. Org. Chem.* **1987**, *52*, 2400. (d) Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946. (e) Ohno, M. In *Enzymes in Organic Synthesis: Ciba Foundation Symposium 111*; Clark, R., Porter, S., Eds.; Pitman: London, **1985**; p 171. (f) Mohr, P.; Waespe-Sarcevic, N.; Tamm, C.; Gawronska, K.; Gawronski, J. K. *Helv. Chim. Acta* **1983**, *66*, 2501. (g) Bjorkling, F.; Boutelje, J.; Gatenbeck, S.; Hult, K.; Norin, T.; Szmulik, P. *Tetrahedron*, **1985**, *41*, 1347.

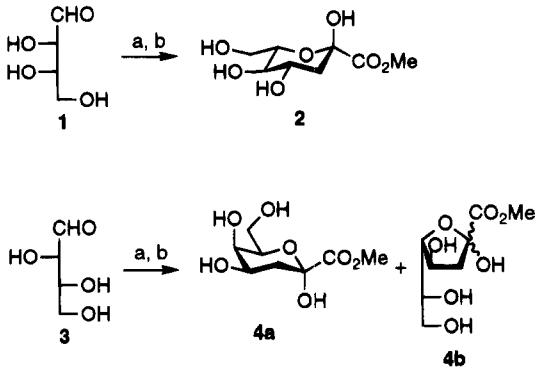
(10) Models for lipases: (a) Hultin, P. G.; Jones, J. B. *Tetrahedron Lett.* **1992**, *33*, 1399. (b) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656. (c) Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129. (d) Guanti, G.; Banfi, L.; Narisano, E. *J. Org. Chem.* **1992**, *57*, 1540. Ehrler, J.; (e) Seebach, D. *Liebigs Ann. Chem.* **1990**, 379. (f) Wimmer, Z. *Tetrahedron* **1992**, *48*, 8431. (g) Johnson, C. R.; Golebiowski, A; McGill, T. K.; Steensma, D. H. *Tetrahedron Lett.* **1991**, *32*, 2597. (h) Ader, U.; Andersch, P.; Berger, M.; Goergens, U.; Seemayer, R.; Schneider, M. *Pure Appl. Chem.* **1992**, *64*, 1165. (i) Kim, M.-J.; Cho, H. *J. Chem. Soc., Chem. Commun.* **1992**, 1411. (j) Oberhauser, T.; Faber, K.; Griengl, H. *Tetrahedron* **1989**, *45*, 1679.

(11) Models for alcohol dehydrogenase: (a) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119. (b) Jones, J. B.; Jakovac, I. J. *Can. J. Chem.* **1982**, *160*, 19. (c) Dodds, D. R.; Jones, J. B. *J. Am. Chem. Soc.* **1988**, *110*, 577. (d) Hansch, C.; Bjorkroth, J. P. *J. Org. Chem.* **1986**, *51*, 5461. (e) Lemiere, G. L.; van Osselar, T. A.; Lepoivre, J. A.; Alderweireldt, F. C. *J. Chem. Soc., Perkin Trans. 2* **1982**. 1123. (f) Dutler, H.; Branden, C.-I. *Bioorg. Chem.* **1981**, 101.

(12) Models for chymotrypsin: Cohen, S. G. *Trans. N. Y. Acad. Sci.* **1969**, *31*, 705. Hein, G. E.; Neimann, C. *J. Am. Chem. Soc.* **1962**, *84*, 4487. Model for arene dioxygenase: Ziffer, H.; Kabuto, K.; Gibson, D. T.; Kobal, V. M.; Jerina, D. M. *Tetrahedron* **1977**, *33*, 2491.

(13) VanMiddlesworth, F.; Sih, C. J. *Biocatalysis* **1987**, *1*, 117.

Scheme 2.[a] Neu5Ac Aldolase with l-Erythrose (1) and D-Threose (3)



[a] (a) pyruvate, Neu5Ac aldolase; (b) MeOH, Dowex 50W-X8 [H^+].

D-threose (**3**), and L-threose, 10 equiv of pyruvate, and 10 units of enzyme were incubated at 37 °C for four days. ^1H NMR analysis of the reaction mixtures showed that no reaction had occurred with D-erythrose and L-threose under these conditions. Tetroses **1** and **3**, on the other hand, were accepted as substrates (Scheme 2). In order to circumvent the tedious separation of the product from excess pyruvate, the product-containing fractions obtained from Bio Gel-P2 chromatography of the reaction mixture were freeze dried, and the residue was resuspended in methanol and treated with Dowex 50W-X8 [H^+]. After 12 h at room temperature esterification was complete. Simple silica gel chromatography gave the heptulosonic acid methyl esters. This procedure should provide a useful alternative to the decomposition of excess pyruvate with pyruvate decarboxylase,[5i] especially when the aldolase reaction is performed on a small scale. The methyl esters **2** and **4** were isolated in 23 and 18% yields, respectively.

The *S* configuration of the new stereogenic center in sialic acid methyl ester **2**, the product of the L-erythrose (**1**) reaction, was concluded from the coupling between H-3_{ax} and H-4 (J_{3ax-4} = 11.7 Hz) in the ^1H NMR spectrum of **2**. The adjacent transaxial coupling constants of H-4, H-5 and H-6 ($J_{4-5} = J_{5-6}$ = 9.3 Hz) show that the product forms a pyranose ring in $^2C_5$ conformation. The reaction followed the normal stereochemical course, with the enamine attacking the aldehyde group from the *si* face. With D-threose (**3**), on the other hand, exclusively the product resulting from *re* attack was isolated. The obtained D-KDO derivative was isolated as a mixture of pyranose **4a** (α anomer) and furanose **4b** (α and β anomers) forms with **4a** being the predominant species. The structure of methyl ester **4a** was delineated from the coupling between H-3_{ax}, H-3_{eq}, H-4, and H-5 (J_{3ax-4} = 11.5 Hz, J_{3eq-4} = 5.7 Hz, J_{4-5} = 3.0 Hz) observed in the ^1H NMR spectrum of **4**. Being side-chain shortened sialic acid or D-KDO derivatives, respectively, compounds **2** and **4** should prove to be of biological interest. They can also be used for the synthesis of novel C-glycosides. Tetroses **1** and **3** are the smallest substrates, which have to date been demonstrated to be accepted by the aldolase.

No reaction was observed with D-erythrose and L-threose under the applied conditions. This is an interesting result considering that L-mannose[5i] and D-glucose[4,5f] have been shown to be accepted as poor substrates by Neu5Ac aldolase. Regarding the difference in size of the C_4-aldoses compared to the natural substrate, it seemed reasonable to assume that these compounds were only weakly bound by the enzyme. Since the binding of the acceptor substrates in general is rather poor (e.g. K_m (ManNAc) = 0.7 M)[4] and the reactions with the aldotetroses were performed at 0.1 M substrate concentration, the low reactivity observed with L-erythrose (**1**) and D-threose(**3**) or lacking reactivity of D-erythrose and L-threose under the applied conditions might originate from dilution phenomena. Relative rate measurements with D-erythrose and L-threose at 0.5 M concentration indeed showed that these compounds are weak substrates of the aldolase (Table 1).

Table 1. Relative Rates of the Neu5Ac Aldolase-Catalyzed Reaction of Pyruvate with Aldotetroses

sugar	rel rate
N-acetyl-D-mannosamine	100[a]
D-erythrose	0.3
L-erythrose (**1**)	4.5
D-threose (**3**)	3.7
L-threose	0.3

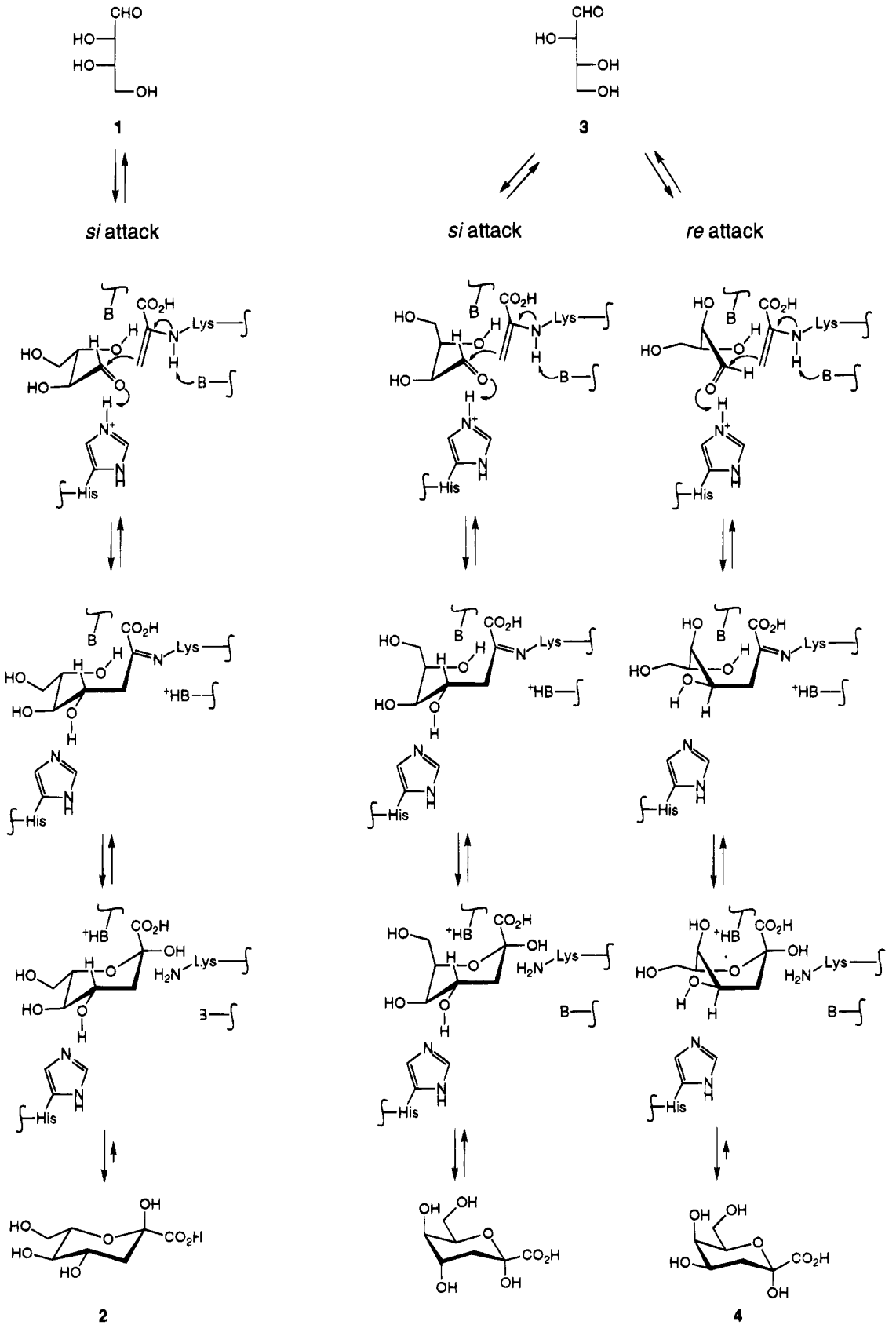
[a] Specific activity = 18 units/mg. One unit = 1 μmol of product formed per minute. All reactions were carried out at pH 7.5 (0.1 M phosphate) with 10 mmol pyruvate. *N*-Acetyl-D-mannosamine was measured at 0.25 M concentration; the C_4-aldoses were used at 0.5 M concentration.

The obtained results allow some speculation about the mechanistic course of Neu5Ac aldolase reactions. Our mechanistic scheme is based on four assumptions: (1) Severe geometrical constraints are applied on pyruvate, which is covalently bound to the enzyme and cannot be replaced by any analogs. (2) The position of the aldehyde group of the acceptor substrate is defined by its interaction with a histidine residue. (3) The C(3) hydroxy group of the acceptor substrate is hydrogen-bonded to a basic residue. (4) The proposed substrate conformation must account for the stereochemical course observed. The emerging picture is shown in Scheme 3. It appears that in the case of the formation of the product with the *R* configuration at C(4), the formed intermediate has a boat-like conformation (D-threose (**3**) case), which makes this pathway kinetically disfavored. With L-erythrose (**1**), on the other hand, the thermodynamically more stable product with the *S* configuration at C(4) is formed through the kinetically favored pathway via a chairlike conformation (Figure 1a). The proposed scheme thus offers a simple explanation for the change in product distribution encountered in reactions, which finally lead to the C(4)-*R*-product, during the course of the reaction. The formation of the C(4)-*S*-product being kinetically preferred, this product is formed in the beginning of the reaction until equilibration occurs and the C(4)-*R*-product predominates (Figure 1b). Considering the structures of the intermediates, the model also accounts for the lower reactivity observed with substrates which are transformed into C(4)-*R*-configured products.

Conclusion

Together with earlier findings[2a,4,5] the results obtained in this work allow some general conclusions to be drawn concerning the active site of the aldolase. Table 2 gives an overview over aldoses tested so far as substrates of Neu5Ac aldolase. It appears that a free hydroxy group at C(3) is essential for successful aldol reaction. Sugars

Scheme 3. Proposed Mechanism for Neu5Ac Aldolase Reactions with L-Erythrose (1) and D-Threose (3)



with a C(3)-*S*-configuration (compare Table 2, C = OH) are attacked from their *si* face to give the C(4)-*S*-configured product. This is the normal case as observed with the natural substrate *N*-acetyl-D-mannosamine. Sugars with a C(3)-*R*-configuration (D = OH in Table 2) can be attacked from their *re* face to give partially or exclusively the C(4)-*R*-configured product. We suggest that the lower reactivity encountered with this second type of substrates is due to boatlike transition states, as illustrated in Scheme 3 and Figure 1. Since in all cases observed so far the more stable product is formed, it is reasonable to assume that the stereochemical outcome

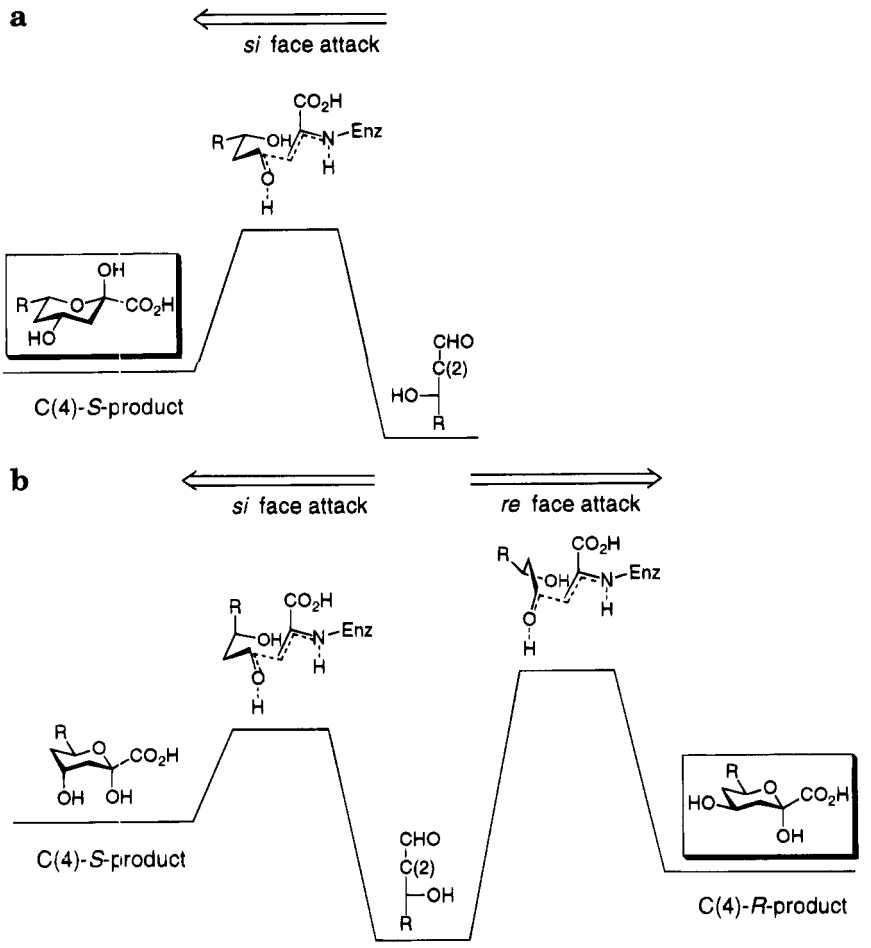


Figure 1. Kinetic scheme for Neu5Ac aldolase reactions. (a) Reactions giving C(4)-*S*-products. (b) Reactions giving C(4)-*R*-products.

of the reactions is thermodynamically controlled. The configuration at C(2) is also critical for the reactivity of the substrates. An equatorial position of the C(3) hydroxymethyl substituent in the intermediate leading to the thermodynamically preferred stereoisomer of the product was found to be favorable for the reactions with the aldotetroses to proceed. The observation that L-erythrose (**1**) is a better substrate than L-threose indicates that in *si* face attack, a C(2)-*S*-configuration (B = H in Table 2) seems to be favored, other things being equal. This is in accord with the data summarized in Table 2 showing that the 2-acetamido group of the natural substrate ManNAc can be replaced by polar, apolar, big, or small substituents as long as the *S* configuration at C(2) is maintained. 2-*N*-(benzyloxycarbonyl)-D-mannosamine,[5k] 2-deoxy-2-phenyl-D-mannose,[5f] 2-azido-2-deoxy-D-mannose,[5i] and 2-deoxy-D-mannose[5i] are all good substrates. D-Glucose[4,5f] and 2-deoxy-2-fluoro-D-glucose,[5h] on the other hand, are poor substrates, and *N*-acetyl-D-glucosamine[4] is not accepted as a substrate at all. It appears that the C(2)-residue is not involved in binding but is critical for steric reasons. In *re* face attack, a pseudoaxial position (A = OH) of the C(2)-hydroxyl seems to be preferred (D-threose (**3**) versus D-erythrose). This effect seems to be most pronounced with the tetroses; as indicated in Table 2, C_6-sugars and C_5-sugars of differing configuration at C(2) vary less in their reactivity (D-altrose versus D-allose[4,5l], D-arabinose versus D-ribose[5d,5l]). Finally, it can be seen from the data in Table 2 that C_6-aldoses are better substrates than C_5- and C_4-sugars. Nevertheless, it appears that the substitution pattern at C(4), C(5), and C(6) is highly flexible; apart from one exception – 5-*O*-methyl-D-mannose, which has been found to be a poor substrate – residues attached to these positions are not important.

Experimental Section

General Methods. *N*-Acetylneuraminic acid aldolase (EC 4.1.3.3) was obtained from Toyobo. D-Erythrose, L-erythrose, D-threose, and L-threose were purchased from Sigma and purified by silica gel chromatography prior to use. For flash chromatography silica gel 60 (230–400 mesh) from Mallinckrodt was used. All reagents and solvents used were of the highest available purity. ^{1}H NMR spectra were recorded at 400 or 500 MHz, respectively, using $CHCl_3$ (δ = 7.26), HDO (δ = 4.80) or CHD_2OD (δ = 3.35) as internal reference. ^{13}C NMR spectra were run at 100 MHz using $CDCl_3$ (δ = 77.00) or CH_3CN (δ = 1.60) as internal reference. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded under fast-atom bombardment (FAB) conditions.

Substrate Synthesis. The chemical synthesis of the C(3)-modified hexoses followed established procedures.[14,15] Scheme 4 illustrates the synthesis of the substrates **6**, **9**, and **13**. The substrates **16**, **19** and **20** were synthesized as shown in Scheme 5. The required starting materials **5**, **10**, **14** and **17** were prepared as previously described.[14a,16] All substrates were characterized by ^{1}H NMR and HRMS.

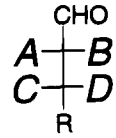
Preparation of the Triflates. At −15 °C a solution of the alcohol in CH_2Cl_2 (10 mL/mmol) and pyridine (4 equiv) is

(14) (a) Binkley, R. W; Ambrose, M. G.; Hehemann, D. H. *J. Org. Chem.* **1980**, *45*, 4387. (b) Hall, L. D.; Miller, D. C. *Carbohydr. Res.* **1976**, *47*, 299. (c) Evans, M. E. *Carbohydr. Res.* **1972**, *21*, 473. (d) Baker, B. R.; Haines, A. H. *J. Org. Chem.* **1963**, *28*, 438.

(15) Maiti, S. N.; Singh, M. P.; Micetich, R. G. *Tetrahedron Lett.* **1986**, *27*, 1423.

(16) Guthrie, R. D.; Murphy, D. *J. Chem. Soc.* **1963**, 5288. Ali, Y.; Richardson, A. C. *Carbohydr. Res.* **1967**, *5*, 441.

Table 2. Survey of Aldoses Tested as Substrates of Neu5Ac Aldolase



A	B	C	D	examples	reactivity[a]	face of attack
NHAc	H	OH	H	*N*-acetyl-D-mannosamine[b]	+ + +	*si*
				6-*O*-acetyl-*N*-acetyl-D-mannosamine[b]	+ + +	*si*
				6-*O*-lactyl-*N*-acetyl-D-mannosamine[c]	+ + +	*si*
				6-*O*-(dimethylphosphinyl)-*N*-acetyl-D-mannosamine[c]	+ + +	*si*
				6-deoxy-6-fluoro-*N*-acetyl-D-mannosamine[c]	+ + +	*si*
				6-deoxy-6-azido-*N*-acetyl-D-mannosamine[c]	+ + +	*si*
				5-deoxy-*N*-acetyl-D-mannosamine[d]	+ + +	*si*
				5-*O*-methyl-*N*-acetyl-D-mannosamine[e]	+	*si*
OH	H	OH	H	D-mannose[b,e,f]	+ + +	*si*
				6-*O*-acetyl-D-mannose[b]	+ + +	nd
				L-gulose[f]	+ + +	*si*
				D-talose[f]	+ + +	*si*
				L-allose[g]	+ + +	*si*
				D-rhamnose[h]	+ + +	*si*
				5-deoxy-D-mannose[d]	+ + +	*si*
				4-deoxy-D-mannose[e]	+ + +	*si*
				4,6-dideoxy-4,6-difluoro-D-talose[c]	+ + +	*si*
				D-lyxose[b,e]	+ +	*si*
				L-erythrose	+ +	*si*
H	H	OH	H	2-deoxy-D-glucose[b,e,f]	+ + +	*si*
				2-deoxy-D-galactose[b]	+ + +	nd
				2,6-dideoxy-D-glucose[f]	+ + +	*si*
NHCbz	H	OH	H	*N*-Cbz-D-mannosamine[i]	+ + +	*si*
N_3	H	OH	H	2-azido-2-deoxy-D-mannose[f,j]	+ + +	*si*
Ph	H	OH	H	2-deoxy-2-*C*-phenyl-D-mannose[e]	+ + +	*si*
OH	H	H	H	3-deoxy-D-mannose[d]	+	
OH	H	N_3		3-azido-3-deoxy-D-mannose	−	
OH	H	NH_2	H	3-amino-3-deoxy-D-mannose	−	
OH	H	Br	H	3-bromo-3-deoxy-D-mannose	−	
N_3	H	N_3	H	2,3-diazido-2,3-dideoxy-D-mannose	−	
N_3	H	Cl	H	2-azido-3-chloro-2,3-dideoxy-D-mannose	−	
H	OH	OH	H	D-glucose[b,j]	+ +	*si*
				L-fucose[b]	+ +	nd
				L-arabinose[f,g]	+ +	*si*
				D-xylose[e,g]	+ +	*si*
				L-threose	+	nd
H	F	OH	H	2-deoxy-2-fluoro-D-glucose[c]	+ +	*si*
H	NHAc	OH	H	*N*-acetyl-D-glucosamine[b]	−	
H	OH	Br	H	3-bromo-3-deoxy-D-glucose	−	
OH	H	H	OH	D-altrose[g]	+ +	*re*[k]
				D-arabinose[f,g,j]	+ +	*re*[k]
				L-xylose[g]	+	*re*[k]
				D-threose	+ +	*re*
H	OH	H	OH	L-mannose[f]	+ +	*re*
				L-rhamnose[h]	+ +	*re*
				D-allose[b]	+	nd
				L-talose[f]	+ +	*re*
				D-gulose[f]	+ +	*re*[k]
				D-ribose[g]	+ +	*re*[k]
				D-erythrose	+	nd
H	NHAc	H	OH	*N*-acetyl-L-mannosamine[f]	+ +	*re*
H	N_3	H	OH	2-azido-2-deoxy-L-mannose[f]	+	*re*[k]
H	H	H	OH	2-deoxy-L-glucose[f]	+ +	*re*
				2-deoxy-L-rhamnose[f]	+ +	*re*

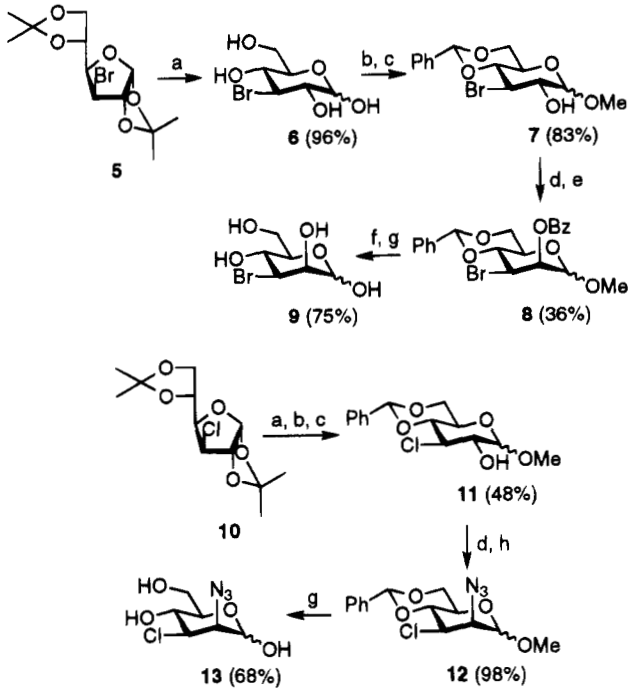
[a] + + +, good substrate; + +, weak substrate; +, very weak substrate; −, no substrate; nd, not determined. [b] Reference 4. [c] Reference 5h. [d] Reference 5j. [e] Reference 5f. [f] Reference 5i. [g] Reference 5l. [h] Reference 5g. [i] Reference 5k. [j] Reference 5d. [k] Partial *re* attack only.

treated with trifluoromethanesulfonic anhydride (1.2 equiv). After 5 min the reaction mixture is allowed to warm to room temperature and stirred until the reaction is complete (5−45 min, as indicated by TLC). Addition of saturated $NaHCO_3$ solution, extraction with CH_2Cl_2, drying of the combined organic layers over $MgSO_4$, and coevaporation with toluene (three times with 5 mL/mmol each) affords the product, which is used without further purification.

Displacement of the Triflyl Groups by Azide, Benzoate, or Bromide. A solution of the triflate and 2.5 equiv of the nucleophile (sodium azide, sodium benzoate, or tetrabutylammonium bromide) in DMF (5 mL/mmol) is stirred at 80 °C until the reaction is complete (8−20 h, as indicated by TLC). DMF is removed in vacuo and EtOAc and water are added (10 mL/mmol each). The aqueous layers are extracted with EtOAc, the combined extracts dried over $MgSO_4$, concentrated, and purified by silica gel chromatography to yield the azido- or bromo-deoxysugars or benzoyl protected sugars, respectively.

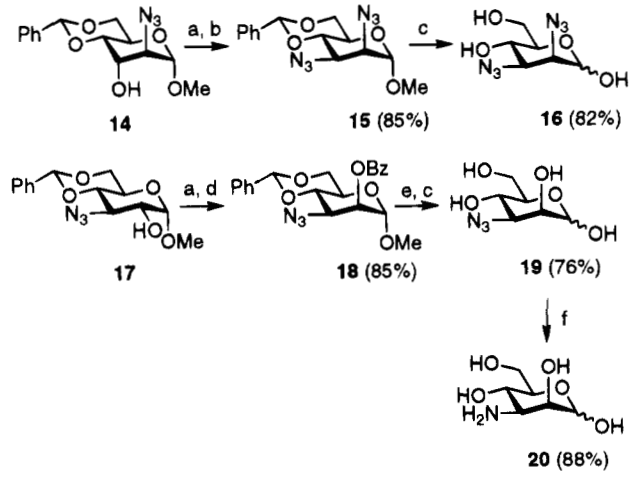
Deprotection of the Methyl 4,6-Benzylidene Glycosides. The protected sugar is refluxed for 20 h in 1.5 N HCl (50 mL/mmol). Concentration of the mixture in vacuo followed by silica gel chromatography affords the pure product.

Scheme 4.[a] Synthesis of Substrates 6, 9, and 13



[a] (a) Dowex 50W-X8 [H^+], H_2O, 40 °C; (b) 1 N HCl, MeOH, 35 °C; (c) α,α-dimethoxytoluene, camphorsulfonic acid, DMF, 70 °C; (d) Tf_2O, pyridine, −15 °C → rt; (e) BzONa, DMF, 80 °C; (f) 1% NaOH, MeOH, 40 °C; (g) 1.5 N HCl, 100 °C; (h) NaN_3, DMF, 80 °C.

Scheme 5.[a] Synthesis of Substrates 16, 19, and 20



[a] (a) Tf_2O, pyridine, −15 °C → rt; (b) NaN_3, DMF, 80 °C; (c) 1.5 N HCl, 100 °C; (d) BzONa, DMF, 80 °C; (e) 1% NaOH, MeOH, 40 °C; (f) $SnCl_2$, MeOH, 65 °C.

3-Bromo-3-deoxy-D-glucose (6). A mixture of **5** (2.85 g, 8.8 mmol) and Dowex 50W-X8 [H^+] (10 g) in water (20 mL) was stirred at 40 °C for 18 h. Filtration, evaporation of the solvent in vacuo and silica gel chromatography (eluting with CH_2Cl_2/MeOH 7:1, followed by CH_2Cl_2/MeOH 3:1) afforded **6** (2.06 g, 96%) as a colorless oil. R_f 0.10 (EtOAc). ^1H NMR (400 MHz, CD_3OD) δ 5.11 (d, $1H_\alpha$, J = 3.5), 4.49 (d, $1H_\beta$, J = 7.5), 4.14 (t, $1H_\alpha$, J = 10.2), 3.87 (dd, $1H_\beta$, J = 11.8, 2.4), 3.85−3.7 (m, $2H_\alpha$ and $2H_\beta$), 3.70 (dd, $1H_\alpha$, J = 11.8, 5.5), 3.63 (dd, $1H_\alpha$, J = 10.3, 3.5), 3.61 (dd, $1H_\beta$, J = 10.5, 9.8), 3.58 (dd, $1H_\alpha$, J = 10.5, 9.8), 3.37 (dd, $1H_\beta$, J = 10.2, 7.5), 3.30 (m, $1H_\beta$); ratio α anomer:β anomer = 6:4. HRMS calcd for $C_6H_{11}BrO_5Na$ ($M + Na^+$) 264.9688; found: 264.9680.

Methyl 4,6-Benzylidene-3-bromo-3-deoxy-D-glucoside (7). A solution of **6** (1.71 g, 7.1 mmol) in MeOH (60 mL), containing 1 N HCl was stirred at 35 °C for 24 h. The solvent was evaporated in vacuo and the residue dissolved in DMF (50 ml), treated with α,α-dimethoxytoluene (2.7 mL, 17.8 mmol) and camphorsulfonic acid (0.36 g, 1.5 mmol), and stirred at 70 °C for 2 h. The solvent was evaporated, and the residue was taken up in saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic extracts were dried smf concentrated in vacuo and the residue esd chromatographed on silica gel (eluting with CH_2Cl_2/MeOH 15:1) to afford **7** (2.01 g, 83%) as a white solid. R_f 0.67 (EtOAc/hexane 1:1). ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (dd, 2 H, J = 7.8, 2.2), 7.39 (m, 3 H), 5.58 (s, $1H_\beta$), 5.57 (s, $1H_\alpha$), 4.82 (d, 1 H, J = 3.8, $1H_\alpha$), 4.37 (dd, $1H_\beta$, J = 10.5, 5.0), 4.35 (d, $1H_\beta$, J = 7.5), 4.30 (dd, $1H_\alpha$, J = 10.1, 4.6), 4.14 (t, $1H_\alpha$, J = 10.3), 3.99 (t, $1H_\beta$, J = 10.3), 3.78 (t, $1H_\beta$, J = 10.4), 3.6−3.8 (m, $3H_\alpha$ and $2H_\beta$), 3.60 (s, $3H_\beta$), 3.48 (m, $1H_\alpha$ and $1H_\beta$), 3.48 (s, $3H_\alpha$), 2.66 (d, $1H_\beta$, J = 2.6), 2.41 (d, $1H_\alpha$, J = 9.1); ratio α anomer:β anomer = 3:7. MS calcd for $C_{14}H_{17}BrO_5Cs$ ($M + Cs^+$) 477/479; found: 477/479.

Methyl 2-Benzoyl-4,6-benzylidene-3-bromo-3-deoxy-β-D-mannoside (8). Following the general procedures, the alcohol **7** (177 mg, 0.51 mmol) was sulfonylated and the resulting triflate treated with sodium benzoate to give the above compound (80 mg, 36%). R_f 0.67 (EtOAc/hexane 1:1). ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 ((dd, 2H, J = 7.9, 0.8), 7.3−7.65 (m, 8H), 5.79 (dd, 1H, J = 3.2, 1.1), 5.68 (s, 1H), 4.63 (d, 1H, J = 1.1), 4.38 (dd, 1H, J = 10.5, 4.9), 4.32 (dd, 1H, J = 10.8, 3.2), 4.07 (dd, 1H, J = 10.8, 9.1), 3.92 (dd, 1H, J = 10.4, 10.3), 3.54 (ddd, 1H, J = 10.3, 9.1, 4.9), 3.49 (s, 3 H). HRMS calcd for $C_{21}H_{21}BrO_6Cs$ ($M + Cs^+$) 580.9576; found: 580.9576.

3-Bromo-3-deoxy-D-mannose (9). A mixture of **8** (63 mg, 0.14 mmol) and NaOH (100 mg, 2.5 mmol) in MeOH (10 mL) was stirred at 40 °C for 3 h. After neutralization with Dowex 50W-X8 [H^+], the resin was filtered off and the solvent evaporated in vacuo. Hydrolysis according to the general procedures afforded the bromosugar (26 mg, 75%). R_f 0.13 (EtOAc). ^1H NMR (400 MHz, D_2O) δ 5.17 (s, $1H_\alpha$), 4.96 (s, $1H_\beta$), 4.40 (dd, $1H_\alpha$, J = 10.3, 2.9), 4.25 (dd, $1H_\beta$, J = 10.5, 2.9), 4.07 (m, 1 H_α and $1H_\beta$), 3.75−3.95 (m, $4H_\alpha$ and $3H_\beta$), 3.48 (ddd, $1H_\beta$, J = 9.7, 5.8, 3.2); ratio α anomer:β anomer = 7 : 3. HRMS calcd for $C_6H_{11}BrO_5Na$ ($M + Na^+$) 264.9688; found: 264.9699.

Methyl 4,6-Benzylidene-3-chloro-3-deoxy-D-glucoside (11). Compound **10** (3.35 g, 12.1 mmol) was converted into the above compound (1.76 g, 48%) using the same procedures as described for **7**. R_f 0.90 ($CHCl_3$/MeOH 5:1). ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (dd, 2H, J = 7.9, 2.2), 7.38 (m, 3H), 5.57 (s, $1H_\beta$), 5.56 (s, $1H_\alpha$), 4.84 (d, 1H, J = 4.0), 4.38 (dd, $1H_\beta$, J = 10.0, 5.0), 4.35 (d, $1H_\beta$, J = 7.5), 4.31 (dd, $1H_\alpha$, J = 10.1, 5.5), 4.09 (t, $1H_\alpha$, J = 10.0), 3.95 (t, $1H_\beta$, J = 10.0), 3.3−3.9 (m, $4H_\alpha$ and $4H_\beta$), 3.60 (s, $3H_\beta$), 3.48 (s, $3H_\alpha$), 2.67 (d, $1H_\beta$, J = 2.4), 2.40 (d, $1H_\alpha$, J = 9.2); ratio α anomer:β anomer = 3:7. HRMS calcd for $C_{14}H_{18}ClO_5$ ($M + H^+$) 301.0843; found: 301.0853.

Methyl 2-Azido-4,6-benzylidene-3-chloro-2,3-dideoxy-D-mannoside (12). From the alcohol **11** (102 mg, 0.34 mmol) the above compound (108 mg, 98%) was synthesized using the general procedures. R_f 0.72 (EtOAc/hexane 1:1). ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (dd, 2H, J = 8.0, 2.1), 7.38 (m, 3H), 5.59 (s, $1H_\alpha$), 5.55 (s, $1H_\beta$), 4.77 (d, $1H_\alpha$, J = 3.5), 4.51 (dd, $1H_\alpha$, J = 10.5, 3.5), 4.49 (d, $1H_\beta$, J = 1.3), 4.31 (dd, $1H_\beta$, J = 10.6, 4.9), 4.25 (m, $1H_\alpha$), 4.12 (dd, $1H_\beta$, J = 10.4, 3.5), 4.01 (dd, $1H_\alpha$, J = 3.5, 1.5), 3.97 (dd, $1H_\beta$, J = 3.5, 1.3), 3.95 (m, $1H_\alpha$), 3.75−3.85 (m, $2H_\alpha$ and $2H_\beta$), 3.55 (s, $3H_\beta$), 3.41 (s, $3H_\alpha$), 3.36 (ddd, $1H_\beta$, J = 10.5, 10.0, 4.9); ratio α anomer:β anomer = 3 : 7. HRMS calcd for $C_{14}H_{16}ClN_3O_4Na$ ($M + Na^+$) 348.0727; found: 348.0741.

2-Azido-3-chloro-2,3-dideoxy-D-mannose (13). Deprotection of **12** (75 mg, 0.23 mmol) as described in the general procedures afforded **30** (35 mg, 68%). R_f 0.37 (EtOAc). ^1H NMR (400 MHz, CD_3OD) δ 5.20 (br s, $1H_\alpha$), 4.99 (d, $1H_\beta$, J = 1.3), 4.44 (dd, $1H_\alpha$, J = 9.8, 3.4), 4.14 (dd, $1H_\beta$, J = 9.7, 3.4), 3.99 (m, $1H_\alpha$), 3.86 (dd, $1H_\beta$, J = 11.8, 2.2), 3.82 (m, $1H_\beta$), 3.65 - 3.8 (m, $4H_\alpha$ and $2H_\beta$), 3.57 (t, $1H_\beta$, J = 9.7); ratio α anomer:β anomer = 7:3. HRMS calcd for $C_6H_{10}ClN_3O_4Na$ ($M + Na^+$) 246.0258; found: 246.0268.

Methyl 4,6-Benzylidene-2,3-diazido-2,3-dideoxy-α-D-mannoside (15). Using the general procedures, the alcohol

14 (1.54 g, 5.0 mmol) was converted into the above compound (1.41 g, 85%). R_f 0.83 (EtOAc/hexane 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, 2H J = 7.4, 1.8), 7.36 (m, 3H), 5.65 (s, 1H), 4.70 (d, 1H, J = 1.4), 4.25–4.4 (m, 2H), 4.14 (dd, 1H, J = 10.3, 3.5), 4.04 (m, 1H), 3.89 (dd, 1H, J = 3.4, 1.4), 3.85 (m, 1H), 3.39 (s, 3H). MS calcd for $C_{14}H_{16}N_6O_4Cs$ (M + Cs^+) 465; found: 465.

2,3-Diazido-2,3-dideoxy-D-mannose (16). According to the general procedures, **15** (198 mg, 0.60 mmol) was converted into **16** (112 mg, 82%). R_f 0.39 (EtOAc). ^{1}H NMR (400 MHz, D_2O) δ 5.26 (d, $1H_\alpha$, J = 1.7), 5.07 (d, $1H_\beta$, J = 1.3), 4.13 (dd, $1H_\beta$, J = 3.7, 1.3), 4.09 (dd, $1H_\alpha$, J = 3.7, 1.7), 4.00 (dd, $1H_\alpha$, J = 9.8, 3.7), 3.45–3.9 (m, $4H_\alpha$ and $5H_\beta$); ratio α anomer:β anomer = 6:4. ^{13}C NMR (100 MHz, D_2O) α anomer: δ 93.33, 74.29, 67.92, 65.07, 64.26, 62.63. HRMS calcd for $C_6H_{11}N_6O_4$ (M + H^+) 231.0842; found: 231.0842.

Methyl 3-Azido-2-benzoyl-4,6-benzylidene-3-deoxy-α-D-mannoside (18). As described above, the alcohol **17** (500 mg, 1.63 mmol) was sulfonylated and the resulting triflate treated with sodium benzoate to afford **18** (1.41 g, 85%). R_f 0.67 (EtOAc/hexane 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, 2H, J = 8.0, 0.9), 7.3–7.65 (m, 8H), 5.74 (s, 1H), 5.39 (dd, 1H, J = 3.0, 1.5), 4.82 (d, 1H, J = 1.5), 4.34 (m, 1H), 4.20 (dd, 1H, J = 10.5, 8.4), 4.14 (dd, 1H, J = 10.5, 3.2), 4.00 (m, 1H), 3.91 (t, 1H, J = 10.0), 3.44 (s, 3H).

3-Azido-3-deoxy-D-mannose (19). Compound **18** (187 mg, 0.45 mmol) was debenzoylated by treatment with NaOH (320 mg, 8 mmol) in MeOH (30 mL) at 40 °C for 3 h. The pH was adjusted to 6 by addition of Dowex 50W-X8 [H^+], the resin filtered off, and the solvent evaporated in vacuo. Acidic hydrolysis of the remaining protecting groups was performed as described in the general procedures to give **19** (71 mg, 76%). R_f 0.13 (EtOAc). ^{1}H NMR (400 MHz, D_2O) δ 5.15 (s, 1H), 4.91 (s, $1H_\beta$), 4.02 (m, $1H_\alpha$ and $1H_\beta$), 3.81 (t, $1H_\alpha$, J = 9.9), 3.75–3.9 (m, $3H_\alpha$ and $3H_\beta$), 3.72 (dd, $1H_\alpha$, J = 9.9, 2.9), 3.56 (dd, $1H_\beta$, J = 10.2, 3.1), 3.47 (ddd, $1H_\beta$, J = 9.8, 5.7, 2.2); ratio α anomer:β anomer = 4:1. HRMS calcd for $C_6H_{11}N_3O_5Na$ (M + Na^+) 228.0596; found: 228.0596.

3-Amino-3-deoxy-D-mannose (20). A mixture of **19** (55 mg, 0.27 mmol) and tin(II) chloride dihydrate (90 mg, 0.40 mmol) in MeOH (5 mL) was refluxed for 4 h and filtered through a short Celite column. The solvent was evaporated in vacuo and the residue chromatographed on silica gel (eluting with $CHCl_3$/MeOH 4:1, followed by $CHCl_3$/MeOH 1:1) to give **20** (42 mg, 88%) as a colorless oil. R_f 0.16 (MeOH) ^{1}H NMR (400 MHz, D_2O) δ 5.19 (d, $1H_\alpha$, J = 1.8), 4.97 (d, $1H_\beta$, J = 1.1), 4.08 (dd, $1H_\beta$, J = 3.3, 1.0), 4.06 (dd, $1H_\alpha$, J = 3.3, 1.8), 3.91 (ddd, $1H_\alpha$, J = 9.7, 5.8, 2.2), 3.8 - 3.9 (m, $3H_\alpha$ and $3H_\beta$), 3.56 (dd, $1H_\alpha$, J = 10.2, 3.2), 3.50 (ddd, $1H_\beta$, J = 9.7, 5.8, 2.3), 3.45 (dd, $1H_\beta$, J = 10.6, 3.2); ratio α anomer:β anomer = 6:4. HRMS calcd for $C_6H_{13}NO_5Na$ (M + Na^+) 202.0691; found: 202.0691.

Enzymatic Reactions. To a 0.1 M solution of the C_4-aldose (0.2 mmol) and sodium pyruvate (10 equiv) in 0.05 M potassium phosphate buffer (pH 7.4) containing 1 mM dithiothreitol was added 3 units of Neu5Ac aldolase. The reaction mixture was incubated at 37 °C with occasional shaking. Progress was monitored by TLC (i-PrOH/1 M NH_4OAc, 7:3). After 4 d a small aliquot was withdrawn for ^{1}H NMR analysis. The mixture was then directly applied to a Bio Gel-P2 column (1.5 × 40 cm) and eluted at 4 °C with water at a flow rate of 2 mL/h. Fractions containing the product were lyophilized; the residue was suspended in MeOH (10 mL) and treated with Dowex 50W-X8 [H^+] (200 mg, prewashed with MeOH). After 12 h at rt the mixture was filtered through Celite, MeOH was removed under reduced pressure, and the residue was chromatographed on silica gel (eluting with 5% MeOH in CH_2Cl_2 followed by 10% MeOH in CH_2Cl_2) to give the heptulosonic acid methyl esters.

Methyl 3-Deoxy-L-*arabino*-heptulosonate (2): 10.2 mg, 23%. ^{1}H NMR (500 MHz, D_2O) δ 3.93 (ddd, 1H, J = 11.7, 9.2, 5.1), 3.82 (d, 1H, J = 10.2), 3.79 (m, 1H), 3.79 (s, 3H), 3.77 (d, 1H, J = 10.0), 3.39 (t, 1H, J = 9.3), 2.24 (dd, 1H, J = 13.2, 5.1), 1.82 (dd, 1H, J = 13.2, 11.7). ^{13}C NMR (100 MHz, D_2O) δ 172.15, 95.92, 74.73, 71.28, 69.05, 61.28, 54.23, 39.23. HRMS calcd for $C_8H_{14}O_7Na$ (M + Na^+): 245.0637; found 245.0637.

Methyl 3-Deoxy-D-*lyxo*-heptulosonate (4): 8.0 mg, 18%. ^{1}H NMR (400 MHz, D_2O) pyranose **4a** (α anomer, 62%) δ 4.13 (ddd, 1H, J = 11.5, 5.7, 3.0), 4.09 (m, 1H), 3.90 (br d, 1H, J = 3.0), 3.84 (s, 3H), 3.77 - 3.88 (m, 2H), 2.07 (dd, 1H, J = 13.0, 11.5), 2.01 (dd, 1H, J = 13.0, 5.7); furanose **4b** (α anomer, 20%) δ 2.72 (dd, 1H, J = 14.2, 7.5), 2.13 (dd, 1H, J = 14.2, 4.0); furanose **4b** (β anomer, 18%) δ 2.49 (dd, 1H, J = 13.7, 6.4), 2.44 (dd, 1H, J = 13.7, 6.6). ^{13}C NMR (100 MHz, D_2O) **4a** δ 172.15, 96.09, 73.71, 67.81, 66.06, 62.10, 54.14, 33.70. HRMS calcd for $C_8H_{14}O_7Na$ (M + Na^+): 245.0637; found 245.0626.

Relative Rate Measurements. The relative rates for the aldolase reactions were measured by the determination of the pyruvate concentration profile during the reaction compared with the one of the natural substrate. The reactions were performed at 37 °C in 0.1 M phosphate buffer (0.5 mL, pH 7.5) containing 0.4 units of enzyme, 10 mM pyruvate, and 0.25 M C(3)-modified hexoses or 0.5 M tetroses, respectively. Periodically, a small sample was withdrawn and the amount of unreacted pyruvate determined through a lactate dehydrogenase assay.[5i]

Acknowledgment. This work was financially supported by the NIH (GM44145). J.-R.S. thanks the Deutsche Forschungsgemeinschaft and W.F. thanks the Swiss National Science Foundation for providing postdoctoral fellowships.

Supplementary Material Available: Copies of ^{1}H NMR spectra of **2**, **4**, **6–9**, **11–13**, **15**, **16**, and **18–20** (14 pages). This material is contained in 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.

JO950184D