Unusual Stereoselectivity in Sialic Acid Aldolase-Catalyzed Aldol Condensations: Synthesis of Both Enantiomers of High-Carbon Monosaccharides

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Abstract: An inversion of stereoselectivity in aldol condensations catalyzed by sialic acid aldolase (from *Escherichia coli*, Shinko American Inc.) was observed when L-mannose, 6-deoxy-L-mannose, L-talose, 2-deoxy-L-glucose, 2-deoxy-L-rhamnose, *N*-acetyl-L-mannosamine, D-gulose, D-arabinose, and 2-azido-2-deoxy-L-mannose were used as acceptor substrates. In all substrates tested, except the last three, a complete inversion of stereoselectivity was observed; i.e., the C-nucleophile of pyruvate attacks the *re* face of the acceptor carbonyl instead of the *si* face as in the normal case for the enantiomeric substrates. Examination of the product distribution during the course of enzymatic reactions indicates that the stereoselectivity is thermodynamically controlled in nature; i.e., attack on the *re* face would take place if the resulting product would be more stable than the one from the *si* face attack. Both enantiomers of several high-carbon monosaccharides are now accessible via the aldolase reactions. A new practical procedure has also been developed for the preparation of the aldolase products where unreacted pyruvate (usually used in 7-fold excess to drive the reaction) is decomposed with pyruvate decarboxylase to simplify product isolation.

The stereoselectivity of aldol addition reactions based on enzymatic catalysis is usually controlled by the enzymes, not by the substrates,[1] as documented by numerous reactions catalyzed by dihydroxyacetone phosphate-dependent aldolases,[1] sialic acid aldolase,[2] and 2-deoxyribose-5-phosphate aldolase.[3] Recently, we have reported a complete reversal of stereoselectivity in aldol reactions catalyzed by sialic acid aldolase (EC 4.1.3.3, from *Escherichia coli*, supplied by Shinko American Inc.);[4] i.e., the enzymatic reactions with L-mannose and 6-deoxy-L-mannose gave the products enantiomeric to those obtained from the corresponding D-substrates (Scheme I). The aldolase is a Schiff base-forming enzyme; the resulting enamine complex normally attacks the *si* face of the carbonyl group of the acceptor (such as the natural acceptor *N*-acetyl-D-mannosamine and many other sugars[2]) to form a new stereogenic center with *S* configuration.[5] Attack on the *re* face to form a new *R* center occurs in the inverted cases.

In this paper, we report more examples of such inverted stereoselective enzymatic aldol reactions and investigate the course of inversion by using NMR analysis. This unusual stereoselectivity allows the synthesis of several enantiomeric high-carbon sugars. We also report a new practical procedure for the preparation of the aldolase products where unreacted pyruvate (usually used in 7-fold excess) is decomposed with pyruvate decarboxylase to simplify product isolation. Previous procedures[2c,e] for the isolation of sialic acid require repetitive extraction of pyruvic acid with ethyl acetate under acidic conditions, where pyruvate actually exists and stays mainly as a hydrated form in the aqueous phase and makes the isolation of sialic acid difficult.

Table I. Relative Rates of the Sialic Acid Aldolase-Catalyzed Reaction of Pyruvate with Different Sugars

sugar	rel rate	sugar	rel rate
N-acetyl-D-mannosamine	100[a]	D-talose	16
N-acetyl-L-mannosamine	0.8	L-talose	1.0
D-mannose	91	D-gulose	3
L-mannose	2.6	L-gulose	20
2-deoxy-D-glucose	35	D-arabinose	1.2
2-deoxy-L-glucose	1.0	L-arabinose	1.6
2,6-dideoxy-D-glucose	18	2-azido-2-deoxy-D-mannose	13
2-deoxy-L-rhamnose	0.6	2-azido-2-deoxy-L-mannose	0.2

[a] Specific activity = 18 units/mg. One unit = 1 μmol of NeuAc formed per minute. All reactions were carried out at pH 7.5 (0.1 M phosphate) with 10 mM pyruvate and 0.25 M sugar.

Table II. Kinetic Parameters (K_m, V_{max}) for the Sialic Acid Aldolase-Catalyzed Reaction of Pyruvate with D- and L-Mannose[a]

substrate	K_m (M)	V_{max} (units/mg)[b]
D-mannose	2.5	43
L-mannose	0.86	3

[a] For details, see Experimental Section. [b] One unit = 1 μmol of product formed per minute.

Results and Discussion

Sialic Acid Aldolase-Catalyzed Addition Reactions. Several reactions were carried out on the millimolar scale using synthetic (Schemes II-V) and commercially available substrates (Scheme VI). The new *R* stereogenic center (the inverted one) of the $^5C_2$-pyranose products formed in the aldol reactions is diagnosed by the coupling type of H_{3ax}: it is usually a triplet with large coupling constants because of the trans and geminal couplings, $J_{3ax-3eq} = J_{3ax-4} = 12$–$13$ Hz (Figure 1).

Among the substrates tested, the inverted stereoselectivity was observed for L-talose, 2-deoxy-L-glucose, 2-deoxy-L-rhamnose, 2-azido-2-deoxy-L-mannose, and *N*-acetyl-L-mannosamine. The normal stereoselectivity (i.e., formation of a new *S* stereogenic center), however, was observed in the enzymatic reaction with the corresponding enantiomers. Interestingly, the reaction with L-gulose proceeded with the normal stereoselectivity and that with

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(2) (a) Augé, C.; David, S.; Gautheron, C.; Malleron, A.; Cavayé, B. *New. J. Chem.* **1988**, *12*, 733. (b) Augé, C.; Gautheron, C.; David, S.; Malleron, A.; Cavaye, B.; Bouxom, B. *Tetrahedron* **1990**, *46*, 201. (c) Kim, M.-J.; Hennen, W. J.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 6481. (d) Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, C. F.; Gautheron, C. M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, *7*, 499. (e) 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.

(3) Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 741. Barbas, C. F., III; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1990**, *112*, 2013.

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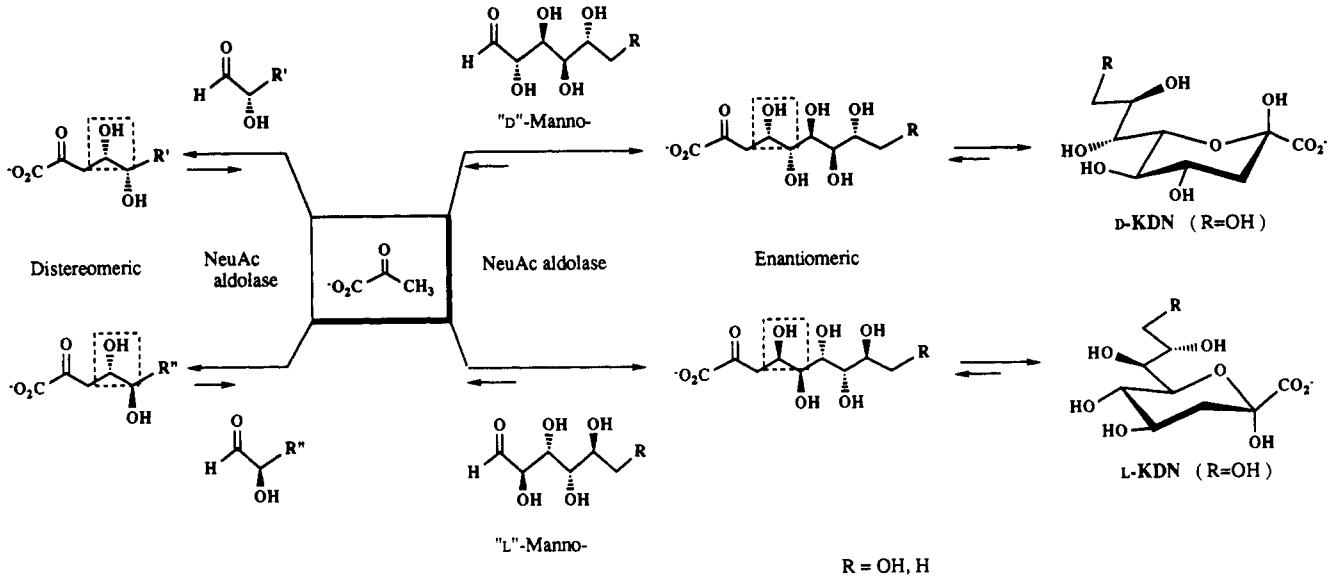
Scheme I. Sialic Acid Aldolase-Catalyzed Addition Reaction with Normal (*si* Face Attack) and Unusual (*re* Face Attack) Stereoselectivity: Synthesis of D- and L-KDN and Their 9-Deoxy Analogs



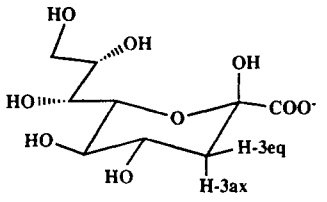
Normal Cases **Exceptional Cases**

D-gulose gave a 5:1 mixture of products in favor of the inverted stereoselectivity. It was known[6] that when D-arabinose was used as a substrate, 3-deoxy-D-*manno*-octulosonic acid (D-KDO) and its 4-epimer (the normal case) were obtained in a 44:56 ratio. We found that the enzymatic reaction with L-arabinose gave L-KDO (the normal case) as the only product.

The possibility of using the crude products from azidonitration[7] of D-glucal was also investigated. When a 1:1 mixture of 2-azido-2-deoxy-D-mannose and 2-azido-2-deoxy-D-glucose was used as a substrate, the manno isomer selectively reacted to give compound **21** as the only product. This provides a direct route to **21** and minimizes the number of purification steps.

In order to gain more information about the inverted diastereoselectivity for most L-sugar substrates, we measured and compared the relative rates of reaction for 16 carbohydrates (Table I), and the K_m and V_{max} values were determined for D- and L-mannose (Table II) as selective examples.

In general, D-sugars are better substrates than L-sugars except arabinose and gulose where the L enantiomer is a better substrate. The K_m values for the sugars are unusually high as indicated in the mannose (D and L) cases and some other sugars reported previously.[2c] This is consistent with the speculation that the open-chain forms of sugars may be the real substrates, since in general only a small population (<1% by NMR) of aldose sugars exist in the aldehyde form in aqueous solution.



14

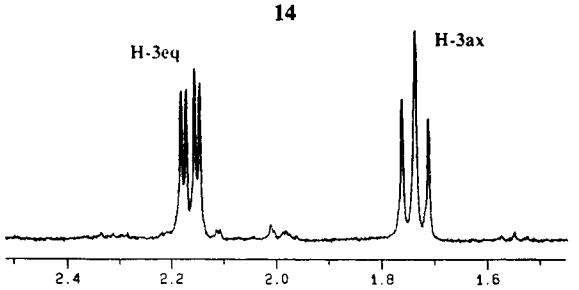


Figure 1. The ^1H NMR spectrum of compound **14** shows the coupling type of H-3ax and H-3eq.

Table III. Product Distribution in the Sialic Acid Aldolase-Catalyzed Reaction between Pyruvate and D-Arabinose

reaction time (h)	D-arabinose consumed (%)	D-KDO:4-epimer ratio
5	10	1:2.55
17	18	1:2.28
23	24	1:2.11
30	28	1:1.96
40	30	1:1.85
45	32	1:1.65
66	38	1:1.54

Table IV. Product Distribution in the Sialic Acid Aldolase-Catalyzed Reaction between Pyruvate and D-Gulose

reaction time (h)	D-gulose consumed (%)	4-eq-OH:4-ax-OH ratio
2	8	1:0.65
3.5	11	1:0.53
5	15	1:0.43
21	26	1:0.39
32	51	1:0.37
72	58	1:0.34
7 days	89	1:0.19

Although it is still not clear why the inverted selectivity takes place, it appears that the product always forms a pyranose ring with the $^2C_5$ conformation in the normal case and the $^5C_2$ conformation in the inverted case, and the OH group of the new stereogenic center tends to be in the equatorial position in both cases.

Further study of the reaction with D-arabinose indicates that the product distribution changes during the course of the reaction. As shown in Table III, the ratio of D-KDO (the inverted product) to the 4-epimer (the normal product) changes from 1:2.55 after 5-h reaction to 1:1.54 after 66-h reaction. This result suggests that the stereoselectivity is thermodynamically controlled, as D-KDO is about 1.9 kcal/mol (calculated by MM2) more stable

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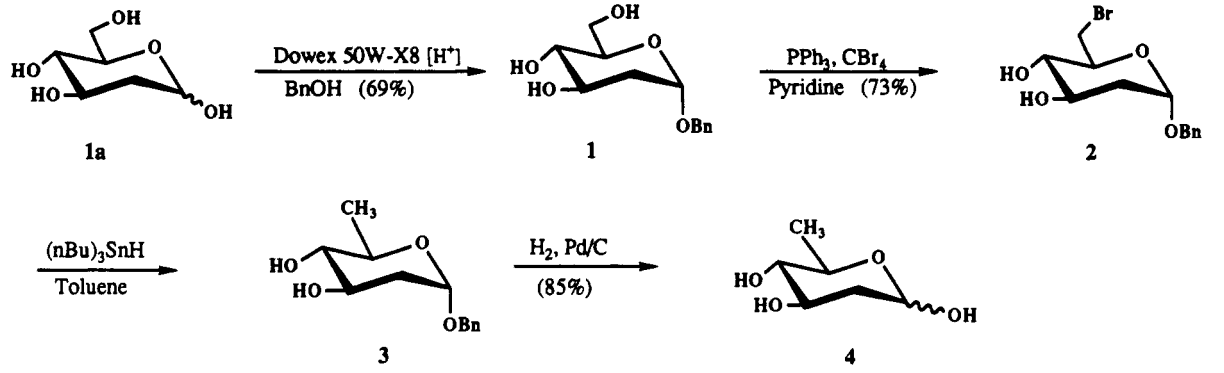
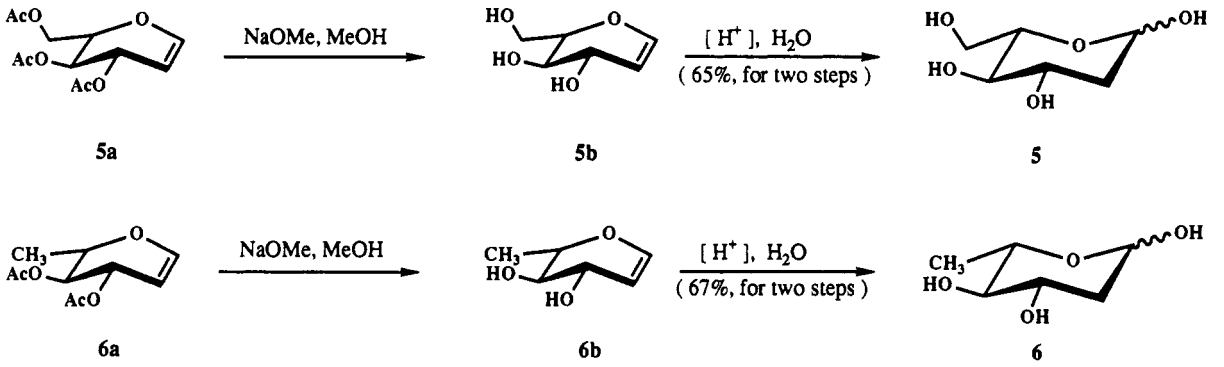
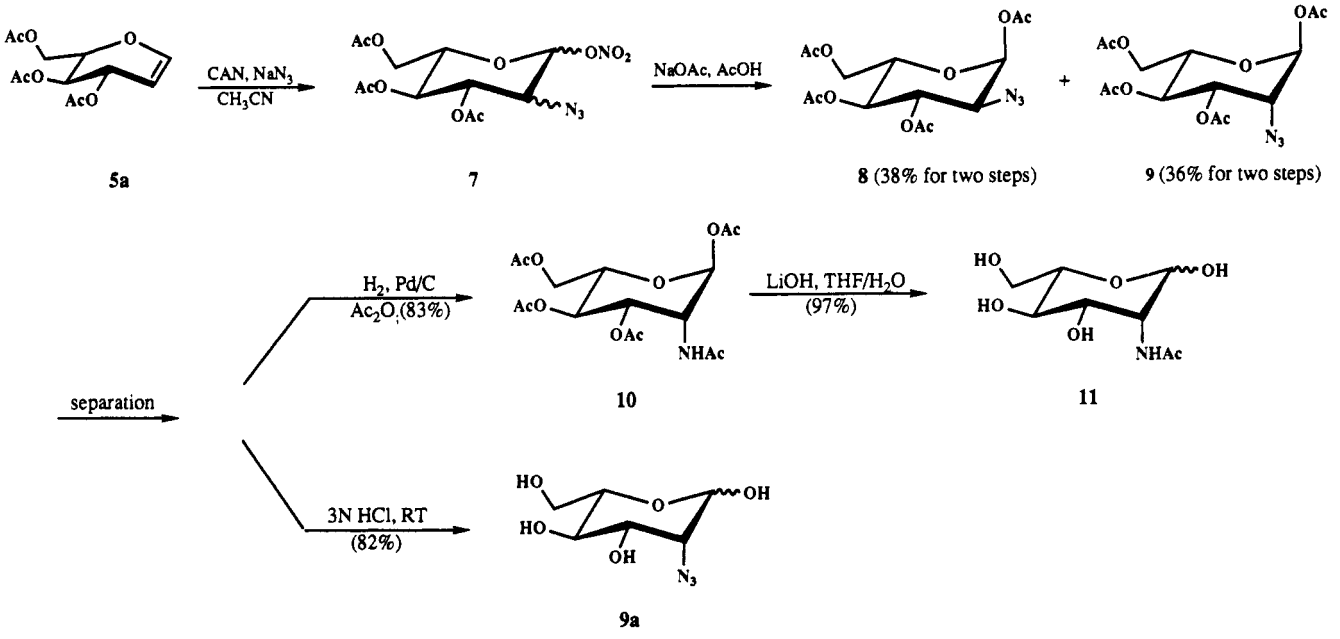
Scheme II. Synthesis of 2,6-Dideoxy-D-glucose **4**



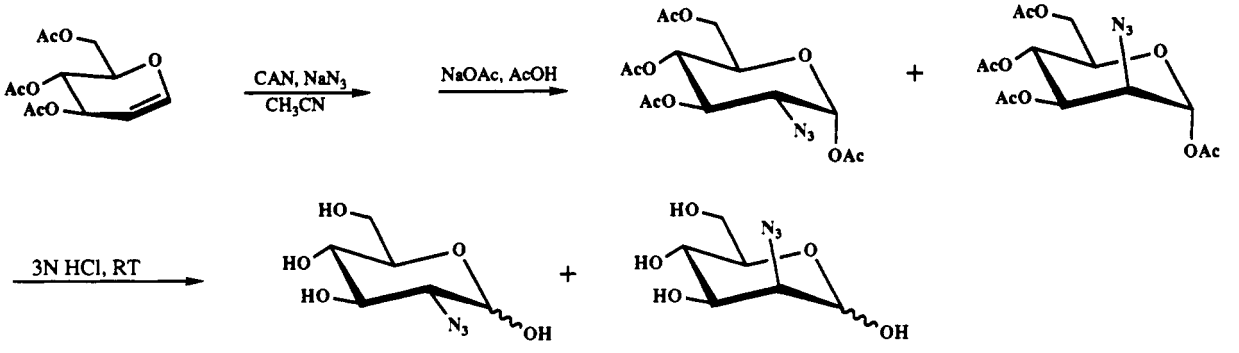
Scheme III. Synthesis of 2-Deoxy-L-glucose **5** and 2-Deoxy-L-rhamnose **6** (2,6-Dideoxy-L-glucose)



Scheme IV. Synthesis of 2-Azido-2-deoxy-L-mannose **9a** and *N*-Acetyl-L-mannosamine **11**



Scheme V. Synthesis of a Mixture of 2-Azido-2-deoxy-D-mannose and 2-Azido-2-deoxy-D-glucose



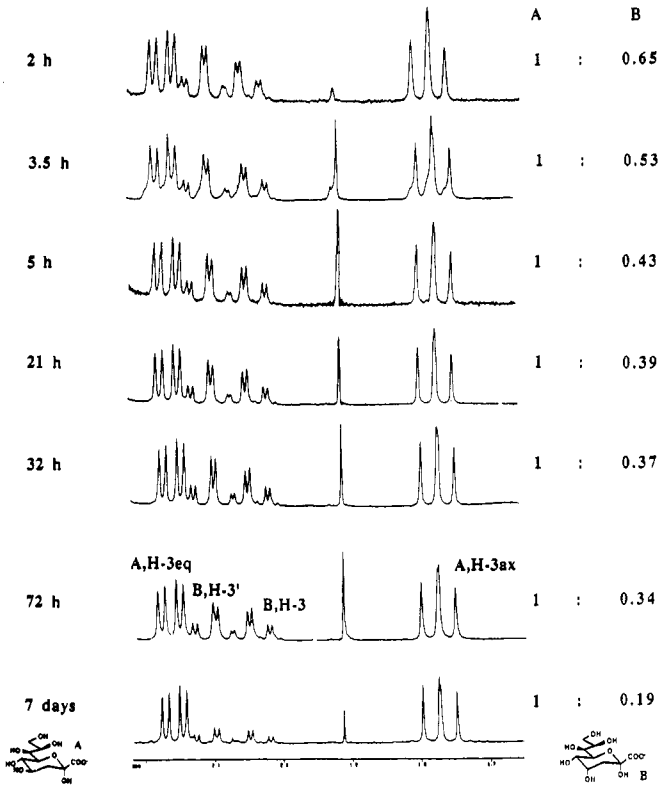


Figure 2. Sialic acid aldolase-catalyzed reaction of pyruvate and D-gulose monitored by ^{1}H NMR analysis. The ratio was determined on the basis of the integration of H-3ax for A and H-3 for B.

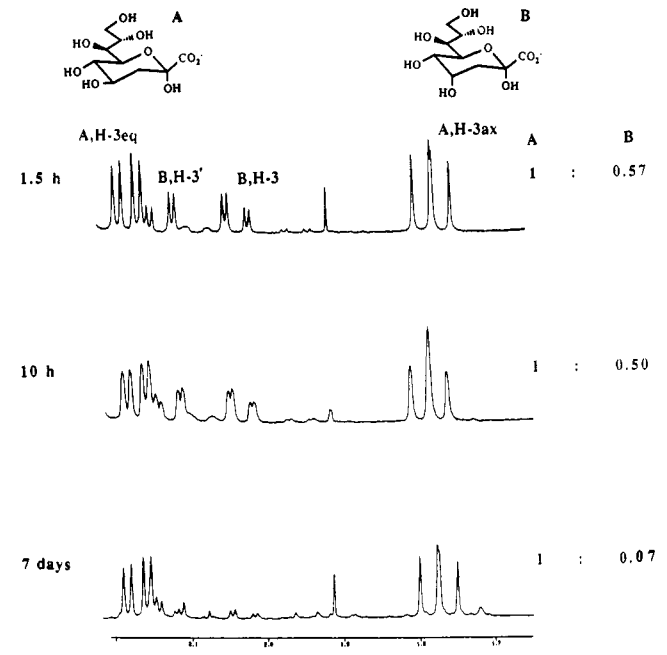


Figure 3. Sialic acid aldolase-catalyzed reaction of pyruvate and L-mannose monitored by ^{1}H NMR analysis. The ratio was determined on the basis of the integration of H-3ax for A and H-3 for B.

than the 4-epimer in which the 4-OH group is in the axial position. A similar situation was observed in the enzymatic reactions with D-gulose (Figure 2, Table IV), L-mannose (Figure 3), and 2-azido-2-deoxy-L-mannose (Figure 4). In all cases, pyruvate was used in excess (10 equiv). When the acceptor was used in excess, however, the reaction more quickly approached equilibrium. For example, in the enzymatic reaction with 7 equiv of L-mannose, a 99:1 mixture of 3-deoxy-L-*glycero*-L-*galacto*-nonulosonic acid (L-KDN, the inverted product) and the 4-epimer was formed in 2 days.

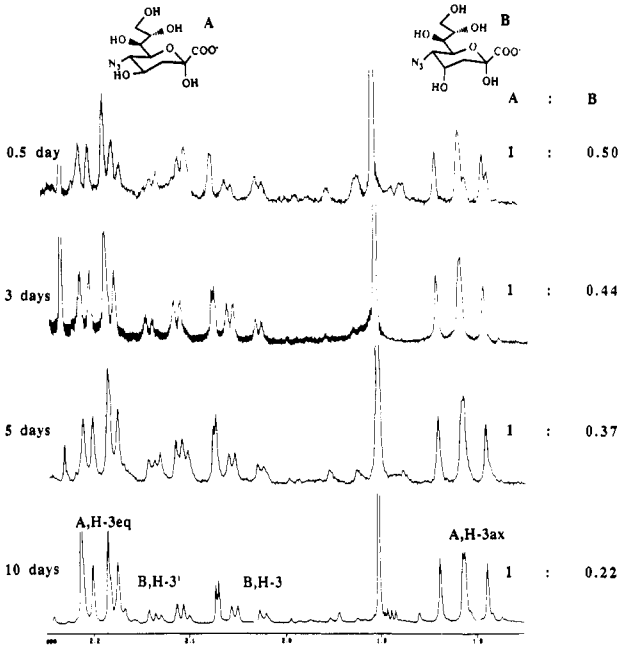


Figure 4. Sialic acid aldolase-catalyzed reaction of pyruvate and 2-azido-2-deoxy-L-mannose monitored by ^{1}H NMR analysis. The ratio was determined on the basis of the integration of H-3ax for A and H-3 for B.

The syntheses of substrates are straightforward. As outlined in Schemes II–V, 2,6-dideoxy-D-glucose **4** was prepared from 2-deoxy-D-glucose via deoxygenation of the primary OH group. 2-Deoxy-L-glucose **5** and 2-deoxy-L-rhamnose **6** (2,6-dideoxy-L-glucose) were prepared from the corresponding glucals via acid-catalyzed hydration.[8] 2-Azido-2-deoxy-L-mannose **9a** and *N*-acetyl-L-mannosamine **11** were prepared from L-glucal via azidonitration.[7]

In conclusion, several enantiomeric high-carbon sugars have been prepared using the sialic acid aldolase reactions. In all examples studied here, though the initial stereoselectivity is controlled by the enzyme, the ultimate product stereochemistry is controlled by the acceptor substrate, following the anti-Cram type of aldol reaction. The compounds prepared in this study may be useful chirons or have interesting biological activities. Of particular interest are L-NeuAc, L-KDO, and L-KDN, the enantiomers of three biologically important monosaccharides.

Experimental Section

Benzyl 2-Deoxy-β-D-glucopyranoside (1). 2-Deoxy-D-glucose (**1a**, 2.5 g, 15.2 mmol) was dissolved in benzyl alcohol (20 mL), and Dowex 50W-X8 (H^+ form, 3.5 g) was added. The reaction solution was stirred at 60 °C for 24 h. Then the resin was filtered off, and the product was purified by flash column chromatography with $CHCl_3$–MeOH (15:1) to give **1** (2.67 g, 10.5 mmol) in 69% yield.

^{1}H NMR (CD_3OD): δ 7.33 (5 H, m), 4.98 (1 H, br d, J_{1-2ax} = 3.5 Hz, H-1), 4.70 (1 H, d, J = 12.0 Hz, benzyl), 4.46 (1 H, d, J = 12.0 Hz, benzyl), 3.88 (1 H, ddd, J_{3-2ax} = 12.0 Hz, J_{3-4} = 9.5 Hz, J_{3-2eq} = 5.0 Hz, H-3), 3.78 (1 H, dd, J_{6a-6b} = 12.0 Hz, J_{6a-5} = 2.5 Hz, H-6a), 3.59 (1 H, ddd, J_{5-4} = 9.5 Hz, J_{5-6b} = 6.0 Hz, J_{5-6a} = 2.5 Hz, H-5), 3.58 (1 H, dd, J_{6b-6a} = 12.0 Hz, J_{6b-5} = 6.0 Hz, H-6b), 3.25 (1 H, t, J_{4-3} = J_{4-5} = 9.5 Hz, H-4), 2.09 (1 H, dd, $J_{2eq-2ax}$ = 13.0 Hz, J_{2eq-3} = 5.0 Hz, H-2eq), 1.63 (1 H, ddd, $J_{2ax-2eq}$ = 13.0 Hz, J_{2ax-3} = 12.0 Hz, J_{2ax-1} = 3.5 Hz, H-2ax). ^{13}C NMR (CD_3OD): δ 139.4, 129.3, 129.0, 138.6, 97.9, 74.3, 73.1, 69.9, 69.7, 62.8, 38.8. HRMS (FAB): calcd for $C_{13}H_{18}O_5Na$ ($M + Na^+$) 277.1052, found 277.1041.

Benzyl 6-Bromo-2,6-dideoxy-β-D-glucopyranoside (2). A solution of triphenylphosphine (4.5 g, 17.3 mmol, 2.2 equiv) in pyridine (30 mL) was added dropwise to a cooled solution of **1** (2.0 g, 7.9 mmol) and CBr_4 (3.1 g, 9.4 mmol) in pyridine (30 mL) at 0 °C. The mixture was then heated at 50 °C for 10 h. After cooling, methanol (5 mL) was added dropwise, and the mixture was concentrated. The product was purified by flash

(8) (a) Gervay, J.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*, 5448. (b) Sabesan, S.; Neira, S. *J. Org. Chem.* **1991**, *56*, 5468.

column chromatography with $CHCl_3$–MeOH (32:1) to give 1.82 g (yield 73%) of **2**.

^1H NMR (CD_3OD): δ 7.20 (5 H, m), 4.87 (1 H, br d, J_{1-2ax} = 3.5 Hz, H-1), 4.63 (1 H, d, J = 12.0 Hz, benzyl), 4.38 (1 H, d, J = 12.0 Hz, benzyl), 3.77 (1 H, ddd, J_{3-2ax} = 11.5 Hz, J_{3-4} = 9.0 Hz, J_{3-2eq} = 5.0 Hz, H-3), 3.67 (1 H, dd, J_{6a-6b} = 10.5 Hz, J_{6a-5} = 2.0 Hz, H-6a), 3.64 (1 H, ddd, J_{5-4} = 9.0 Hz, J_{5-6b} = 6.5 Hz, J_{5-6a} = 2.0 Hz, H-5), 3.46 (1 H, dd, J_{6b-6a} = 10.5 Hz, J_{6b-5} = 6.5 Hz, H-6b), 3.13 (1 H, t, $J_{4-3} = J_{4-5}$ = 9.0 Hz, H-4), 2.01 (1 H, dd, $J_{2eq-2ax}$ = 13.0 Hz, J_{2eq-3} = 5.0 Hz, H-2eq), 1.55 (1 H, ddd, $J_{2ax-2eq}$ = 13.0 Hz, J_{2ax-3} = 11.5 Hz, J_{2ax-1} = 3.5 Hz, H-2ax). ^{13}C NMR (CD_3OD): δ 139.0, 129.3, 129.1, 128.7, 97.7, 75.3, 73.1, 69.8, 69.7, 38.7, 34.6. HRMS (FAB): calcd for $C_{13}H_{17}O_4NaBr$ (M + Na^+) 339.0208, found 339.0218.

Benzyl 2,6-Dideoxy-β-D-glucopyranoside (3). A solution of Bu_3SnH (1.0 g, 3.6 mmol, 1 mL) in toluene (10 mL) was added dropwise to a gently refluxing solution of **2** (0.76 g, 2.40 mmol) in toluene (15 mL) over 10 min, and then the mixture was refluxed for 10 h. After cooling, the mixture was concentrated and the residue was chromatographed on a flash column with $CHCl_3$–MeOH (29:1) to give crude **3**, which was acetylated with Ac_2O (10 mL), pyridine (10 mL), and a catalytic amount of (*N*,*N*-dimethylamino)pyridine (DMAP). The product was purified by flash chromatography with toluene–EtOAc (15:1) to give the corresponding peracetate, which was treated with NaOMe (2 mL, 1 N solution) in MeOH (20 mL) for 0.5 h at room temperature. The mixture was neutralized by adding Dowex 50W-X8 (H^+), then the resin was filtered off, and the filtrate was concentrated to give **3**.

^1H NMR ($CDCl_3$): δ 7.28 (5 H, m), 4.89 (1 H, br d, J_{1-2ax} = 3.5 Hz, H-1), 4.62 (1 H, d, J = 12.0 Hz, benzyl), 4.43 (1 H, d, J = 12.0 Hz, benzyl), 3.83 (1 H, ddd, J_{3-2ax} = 11.5 Hz, J_{3-4} = 9.0 Hz, J_{3-2eq} = 5.0 Hz, H-3), 3.64 (1 H, dq, J_{5-4} = 9.0 Hz, J_{5-6} = 6.0 Hz, H-5), 2.97 (1 H, t, $J_{4-3} = J_{4-5}$ = 9.0 Hz, H-4), 2.09 (1 H, dd, $J_{2eq-2ax}$ = 13.0 Hz, J_{2eq-3} = 5.0 Hz, H-2eq), 1.63 (1 H, ddd, $J_{2ax-2eq}$ = 13.0 Hz, J_{2ax-3} = 11.5 Hz, J_{2ax-1} = 3.5 Hz, H-2ax), 1.25 (3 H, d, J_{6-5} = 6.0 Hz, 6-CH_3). ^{13}C NMR ($CDCl_3$): δ 137.6, 128.4, 127.9, 127.7, 96.5, 77.9, 69.1, 68.8, 67.8, 37.7, 17.8. HRMS (FAB): calcd for $C_{13}H_{18}O_4Na$ (M + Na^+) 261.1103, found 261.1116.

2,6-Dideoxy-D-glucopyranose (4). A solution of **3** (0.56 g, 2.3 mmol) in 60% aqueous AcOH (25 mL) was hydrogenated over 100 mg of 10% palladium on charcoal under atmospheric pressure. After 12 h, the solution was filtered and the filtrate was concentrated in vacuo. Flash column chromatography with $CHCl_3$–MeOH (10:1) yielded 0.29 g (2.0 mmol) of **4** (85% yield). β-Isomer: ^1H NMR (CD_3OD) δ 5.12 (1 H, br d, J_{1-2ax} = 3.5 Hz, H-1), 3.75 (1 H, ddd, J_{3-2ax} = 12.0 Hz, J_{3-4} = 9.0 Hz, J_{3-2eq} = 5.0 Hz, H-3), 3.18 (1 H, dq, J_{5-4} = 9.0 Hz, J_{5-6} = 6.5 Hz, H-5), 2.85 (1 H, t, $J_{4-3} = J_{4-5}$ = 9.0 Hz, H-4), 1.96 (1 H, dd, $J_{2eq-2ax}$ = 13.0 Hz, J_{2eq-3} = 5.0 Hz, H-2eq), 1.50 (1 H, ddd, $J_{2ax-2eq}$ = 13.0 Hz, J_{2ax-3} = 12.0 Hz, J_{2ax-1} = 3.5 Hz, H-2ax), 1.14 (3 H, d, J_{6-5} = 6.5 Hz, 6-CH_3); ^{13}C NMR (CD_3OD) δ 92.6, 79.2, 73.2, 68.7, 39.8, 18.3; HRMS (FAB) calcd for $C_6H_{12}O_4Na$ (M + Na^+) 171.0633, found 171.0640.

2-Deoxy-L-glucopyranose (5). A solution of tri-*O*-acetyl-L-glucal (**5a**, 1.0 g, 3.7 mmol) and NaOMe (2 mL, 1 N solution) in MeOH (15 mL) was stirred for 0.5 h at room temperature. After the mixture was neutralized by adding Dowex 50W-X8 (H^+), the resin was filtered off, and the filtrate was concentrated.

The crude product was dissolved in a dilute H_2SO_4 solution (pH 1). After 24 h, the reaction solution was neutralized with 1 N NaOH and then freeze-dried. The product was purified by flash column chromatography with $CHCl_3$–MeOH (5:1) to give **5** (0.39 g; overall yield for these two steps: 65%). ^1H NMR for the α-isomer (CD_3OD): δ 4.78 (1 H, dd, J_{1-2ax} = 10.0 Hz, J_{1-2eq} = 2.0 Hz, H-1), 3.85 (1 H, dd, J_{6a-6b} = 12.5 Hz, J_{6a-5} = 2.0 Hz, H-6a), 3.67 (1 H, dd, J_{6b-6a} = 12.5 Hz, J_{6b-5} = 6.0 Hz, H-6b), 3.54 (1 H, ddd, J_{3-2ax} = 12.0 Hz, J_{3-4} = 9.0 Hz, J_{3-2eq} = 5.0 Hz, H-3), 3.22 (1 H, ddd, J_{5-4} = 9.0 Hz, J_{5-6b} = 6.0 Hz, J_{5-6a} = 2.0 Hz, H-5), 3.15 (1 H, t, $J_{4-3} = J_{4-5}$ = 9.0 Hz, H-4), 2.13 (1 H, dd, $J_{2eq-2ax}$ = 12.5 Hz, J_{2eq-3} = 5.0 Hz, J_{2eq-1} = 2.0 Hz, H-2eq), 1.46 (1 H, ddd, $J_{2ax-2eq}$ = 12.5 Hz, J_{2ax-3} = 12.0 Hz, J_{2ax-1} = 10.0 Hz, H-2ax). ^{13}C NMR (CD_3OD): δ 95.1, 78.1, 73.0, 72.6, 63.0, 41.8. HRMS (FAB): calcd for $C_6H_{12}O_5Na$ (M + Na^+) 187.0582, found 187.0582.

2,6-Dideoxy-L-glucopyranose (6). The reaction procedure was similar to that described above (from compound **5a** to **5**). The final product was purified by flash column chromatography with $CHCl_3$–MeOH (9:1) to give **6** in 67% overall yield from di-*O*-acetyl-L-rhamnal, **6a**.

The ^1H and ^{13}C NMR data are identical with those of compound **4**, which is an enantiomer of **6**. HRMS (FAB) calcd for $C_6H_{12}O_4Na$ (M + Na^+) 171.0633, found 171.0640.

2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-β-L-mannopyranosyl Nitrate (7). A solution of **5a** (1.50 g, 5.5 mmol) in CH_3CN (30 mL) was added dropwise to a solid mixture of NaN_3 (0.54 g, 8.3 mmol, 1.5 equiv) and ammonium cerium(IV) nitrate (CAN, 9.1 g, 16.5 mmol, 3 equiv) at ca. −20 °C. The suspension was stirred vigorously under N_2. After 7 h, the starting material disappeared on TLC, and the solution was poured into ice–water and extracted with EtOAc. The combined extracts were successively washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The compound was chromatographed on a flash silica gel column with EtOAc–toluene (1:12) to give 1.57 g of the azido compound. The ^1H NMR spectrum showed at least three isomers (gluco and manno types which contained their α and β isomers). The major manno-type product was characterized: ^1H NMR ($CDCl_3$) δ 6.22 (1 H, d, J_{1-2} = 2.0 Hz, H-1), 5.40 (1 H, t, $J_{4-5} = J_{4-3}$ = 9.5 Hz, H-4), 5.25 (1 H, dd, J_{3-4} = 9.5 Hz, J_{3-2} = 4.0 Hz, H-3), 4.28 (1 H, dd, J_{6a-6b} = 13.0 Hz, J_{6a-5} = 5.0 Hz, H-6a), 4.21 (1 H, dd, J_{2-3} = 4.0 Hz, J_{2-1} = 2.0 Hz, H-2), 4.11 (1 H, ddd, J_{5-4} = 9.5 Hz, J_{5-6a} = 5.0 Hz, J_{5-6b} = 2.5 Hz, H-5), 4.11 (1 H, dd, J_{6b-6a} = 13.0 Hz, J_{6b-5} = 2.5 Hz, H-6b), 2.12 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 2.07 (3 H, s, acetyl); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.6, 169.2, 97.0, 71.0, 70.2, 64.6, 61.2, 58.6, 20.4, 20.3, 20.2; HRMS (FAB) calcd for $C_{12}H_{16}N_4O_{10}Na$ (M + Na^+) 399.0674, found 399.0670.

2-Azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-L-glucopyranose (8) and 2-Azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-L-mannopyranose (9). The mixture mentioned above was used subsequently without further purification. Two equivalents of NaOAc (0.60 g, 7.3 mmol) was added to a solution of **7** (1.37 g, 3.6 mmol) in acetic acid (30 mL), and the reaction mixture was heated at 100 °C overnight. After cooling, the reaction mixture was diluted with EtOAc (30 mL), then successively washed with water, aqueous $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was chromatographed on a flash column with EtOAc–toluene (1:5) to give two products. The less polar isomer was identified as gluco-type compound **8** (0.78 g), and the more polar isomer was identified as manno-type compound **9** (0.75 g).

Compound **8**: ^1H NMR ($CDCl_3$) δ 6.30 (1 H, d, J_{1-2} = 3.5 Hz, H-1), 5.46 (1 H, t, $J_{3-2} = J_{3-4}$ = 10.0 Hz, H-3), 5.12 (1 H, t, $J_{4-5} = J_{4-3}$ = 10.0 Hz, H-4), 4.30 (1 H, dd, J_{6a-6b} = 12.5 Hz, J_{6a-5} = 4.0 Hz, H-6a), 4.08 (1 H, ddd, J_{5-4} = 10.0 Hz, J_{5-6a} = 4.0 Hz, J_{5-6b} = 2.5 Hz, H-5), 4.06 (1 H, dd, J_{6b-6a} = 12.5 Hz, J_{6b-5} = 2.5 Hz, H-6b), 3.68 (1 H, dd, J_{2-3} = 10.0 Hz, J_{2-1} = 3.5 Hz, H-2), 2.20 (3 H, s, acetyl), 2.11 (3 H, s, acetyl), 2.08 (3 H, s, acetyl), 2.05 (3 H, s, acetyl); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.0, 169.4, 168.4, 89.8, 70.6, 69.6, 67.6, 61.3, 60.2, 20.8, 20.6, 20.6, 20.4; HRMS (FAB) calcd for $C_{14}H_{19}N_3O_9$ (M + Na^+) 396.1019, found 396.1023.

The manno-type isomer **9** was further purified by recrystallization (prisms from diethyl ether): mp 130–131 °C; $[\alpha]^{25}_D$ −82.1° (*c* 1.12, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.11 (1 H, d, J_{1-2} = 2.0 Hz, H-1), 5.39 (1 H, t, $J_{4-5} = J_{4-3}$ = 9.5 Hz, H-4), 5.36 (1 H, dd, J_{3-4} = 9.5 Hz, J_{3-2} = 3.0 Hz, H-3), 4.24 (1 H, dd, J_{6a-6b} = 12.5 Hz, J_{6a-5} = 4.5 Hz, H-6a), 4.07 (1 H, dd, J_{6b-6a} = 12.5 Hz, J_{6b-5} = 2.5 Hz, H-6b), 4.03 (dd, J_{2-3} = 3.0 Hz, J_{2-1} = 2.0 Hz, H-2), 4.02 (1 H, ddd, J_{5-4} = 9.5 Hz, J_{5-6a} = 4.5 Hz, J_{5-6b} = 2.5 Hz, H-5), 2.16 (3 H, s, acetyl), 2.11 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 2.05 (3 H, s, acetyl); ^{13}C NMR ($CDCl_3$) δ 170.7, 170.0, 169.3, 168.2, 91.3, 70.7, 70.5, 65.2, 61.7, 60.4, 20.9, 20.7, 20.6, 20.5; HRMS (FAB) calcd for $C_{14}H_{19}N_3O_9Na$ (M + Na^+) 396.1019, found 396.1023.

2-Azido-2-deoxy-L-mannopyranose (9a). A solution of **9** (541 mg, 1.45 mmol) in 3 N aqueous hydrochloric acid (100 mL) was warmed until the solution became clear, and then the solution was stirred at room temperature. After 10 h, 1-butanol was added and the solution was evaporated. The residue was chromatographed on a flash column with $CHCl_3$–MeOH (4:1) to give 0.244 g of **9a** (82% yield).

$[\alpha]^{25}_D$: −13.6° (*c* 0.98, H_2O). ^1H NMR for the major β-isomer (CD_3OD): δ 4.90 (1 H, d, J_{1-2} = 1.5 Hz, H-1), 4.02 (1 H, dd, J_{3-4} = 9.5 Hz, J_{3-2} = 4 Hz, H-3), 3.80 (1 H, dd, J_{2-3} = 4 Hz, J_{2-1} = 1.5 Hz, H-2), 3.79 (1 H, dd, J_{6a-6b} = 12 Hz, J_{6a-5} = 2 Hz, H-6a), 3.73 (1 H, ddd, J_{5-4} = 9.5 Hz, J_{5-6b} = 6 Hz, J_{5-6a} = 2 Hz, H-5), 3.67 (1 H, dd, J_{6b-6a} = 12 Hz, J_{6b-5} = 6 Hz, H-6b), 3.58 (t, $J_{4-3} = J_{4-5}$ = 9.5 Hz, H-4). ^{13}C NMR (CD_3OD): δ 93.8, 74.1, 72.2, 68.9, 66.6, 62.8. HRMS (FAB): calcd for $C_6H_{11}N_3O_5Na$ 228.0596, found 228.0596.

2-Acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-L-mannopyranose (10). A solution of **9** (0.60 g, 1.62 mmol) in Ac_2O (20 mL) was hydrogenated over 70 mg of 10% palladium on charcoal under atmospheric pressure. After 10 h, the solution was filtered and the filtrate was concentrated in vacuo. Flash column chromatography with EtOAc–toluene (2.5:1) gave 0.52 g of **10** (83% yield).

^1H NMR ($CDCl_3$): δ 6.03 (1 H, d, J_{1-2} = 1.5 Hz, H-1), 5.34 (1 H, dd, J_{3-4} = 10.0 Hz, J_{3-2} = 5.0 Hz, H-3), 5.18 (1 H, t, $J_{4-5} = J_{4-3}$ = 10.0 Hz, H-4), 4.65 (1 H, ddd, J_{5-4} = 10.0 Hz, J_{5-6a} = 5.0 Hz, J_{5-6b} = 2.0 Hz, H-5), 4.29 (1 H, dd, J_{6a-6b} = 12.5 Hz, J_{6a-5} = 5 Hz, H-6a), 4.06 (1 H, dd, J_{2-3} = 5.0 Hz, J_{2-1} = 1.5 Hz, H-2), 4.05 (1 H, dd, J_{6b-6a} = 12.5 Hz, J_{6b-5} = 2.0 Hz, H-6b), 2.18 (3 H, s, acetyl), 2.10 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 2.01 (3 H, s, acetyl). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.1, 170.0, 169.6, 168.0, 91.7, 73.4, 70.1, 65.5, 62.1, 49.3, 23.2, 20.8, 20.7, 20.7, 20.6. HRMS (FAB): calcd for

$C_{16}H_{23}NO_{10}Na$ (M + Na^+) 412.1220, found 412.1224.

2-Acetamido-2-deoxy-L-mannopyranose (*N*-Acetyl-L-mannosamine) (11). A solution of aqueous LiOH (0.3 M, 20 mL) was added dropwise to a solution containing **10** (0.52 g, 1.34 mmol) in THF–H_2O (3:1, 22 mL). As determined by TLC, the reaction was complete within 30 min. Dowex 50W-X8 (H^+) was added to neutralize the solution, then the resin was filtered off, and the filtrate was concentrated. The product was purified by flash column chromatography with $CHCl_3$–MeOH (3.5:1) to obtain 0.29 g (1.3 mmol) of **11** (97% yield). Further purification was carried out with Bio Gel P-2 chromatography using water as eluent. ^1H NMR for the major β-isomer (CD_3OD): δ 4.95 (1 H, d, J_{1-2} = 1.5 Hz, H-1), 4.23 (1 H, dd, J_{2-3} = 4.5 Hz, J_{2-1} = 1.5 Hz, H-2), 3.95 (1 H, dd, J_{3-4} = 10.0 Hz, J_{3-2} = 4.5 Hz, H-3), 3.80 (3 H, dd, J_{6a-6b} = 12.0 Hz, J_{6a-5} = 4.5 Hz, H-6a), 3.72 (1 H, m, H-5), 3.72 (1 H, dd, J_{6b-6a} = 12.0 Hz, J_{6b-5} = 2.0 Hz, H-6b), 3.55 (1 H, t, J_{4-5} = J_{4-3} = 10.0 Hz, H-4), 1.91 (3 H, s, acetyl). ^{13}C NMR (CD_3OD): δ 174.0, 94.9, 73.4, 70.6, 68.4, 62.2, 55.1, 22.6. The ^1H and ^{13}C NMR spectra were identical with those of an authentic sample of the D-enantiomer from Sigma. HRMS (FAB): calcd for $C_8H_{15}NO_6Na$ (M + Na^+) 224.0797, found 244.0797.

Enzymatic Reactions. A 0.1 M solution of sugar (1 mmol) in a 0.05 M potassium phosphate buffer (pH 7.4) containing 1 mM dithiothreitol, sodium pyruvate (10 equiv), and 10 units of NeuAc aldolase (Shinko American Inc. NAL-301) was incubated at 37 °C (total volume = 10 mL) for 3 days. The reaction was monitored by TLC (*i*-PrOH–H_2O = 7:3 v/v). The product was isolated by anion-exchange chromatography on Dowex 1-X8 (HCO_3^- form; 20 × 2.5 cm) using a gradient of ammonium bicarbonate (0 → 0.2 M) as the eluent. Fractions containing the products were pooled, freeze-dried, deionized with Dowex 50W-X8 (H^+), and again freeze-dried. Finally, Bio Gel P-2 chromatography was applied to obtain the pure compound.

3-Deoxy-D-*glycero*-L-*altro*-nonulosonic acid (12): $[\alpha]^{25}_D$ −35.6° (*c* 0.89, H_2O); ^1H NMR (D_2O, HOD = 4.80 ppm) δ 3.98 (1 H, ddd, J_{8-9a} = 7.0 Hz, J_{8-9b} = 5 Hz, J_{8-7} = 2.5 Hz, H-8), 3.95 (1 H, ddd, J_{4-3ax} = 12.5 Hz, J_{4-5} = 9.0 Hz, J_{4-3eq} = 5.0 Hz, H-4), 3.93 (1 H, dd, J_{7-6} = 3.5 Hz, J_{7-8} = 2.5 Hz, H-7), 3.85 (1 H, dd, J_{6-5} = 9.5 Hz, J_{6-7} = 3.5 Hz, H-6), 3.69 (1 H, dd, J_{9b-9a} = 11.5 Hz, J_{9b-8} = 5.0 Hz, H-9b), 3.65 (1 H, dd, J_{9a-9b} = 11.5 Hz, J_{9a-8} = 7 Hz, H-9a), 3.56 (1 H, t, J_{5-6} = J_{5-4} = 9.5 Hz, H-5), 2.21 (1 H, dd, $J_{3eq-3ax}$ = 12.5 Hz, J_{3eq-4} = 5.0 Hz, H-3eq), 1.75 (1 H, t, $J_{3ax-3eq}$ = J_{3ax-4} = 12.5 Hz, H-3ax); ^{13}C NMR (D_2O + CD_3CN) δ 176.9, 96.7, 74.4, 72.2, 71.6, 71.1, 69.4, 63.2, 39.3; HRMS (FAB) calcd for $C_9H_{16}O_9Na$ (M + Na^+) 291.0692, found 291.0698.

3-Deoxy-L-*glycero*-D-*altro*-nonulosonic acid (13): $[\alpha]^{25}_D$ +32.1° (*c* 0.41, H_2O). The ^1H and ^{13}C NMR data are identical with those of compound **12**, which is an enantiomer of **13**. HRMS (FAB): calcd for $C_9H_{16}O_9Na$ (M + Na^+) 291.0692, found 291.0680.

3-Deoxy-L-*glycero*-D-*galacto*-nonulosonic acid (14): $[\alpha]^{25}_D$ −28.6° (*c* 0.22, H_2O); ^1H NMR (D_2O) δ 3.96 (1 H, br d, J_{7-8} = 6.0 Hz, H-7), 3.91 (1 H, ddd, J_{4-3ax} = 12.5 Hz, J_{4-5} = 9.5 Hz, J_{4-3eq} = 5.0 Hz, H-4), 3.82 (1 H, dd, J_{8-9a} = J_{8-7} = 6.0 Hz, J_{8-9b} = 3.5 Hz, H-8), 3.74 (1 H, d, J_{6-5} = 9.5 Hz, H-6), 3.67 (1 H, dd, J_{9b-9a} = 12.0 Hz, J_{9b-8} = 3.5 Hz, H-9b), 3.57 (1 H, t, J_{5-4} = J_{5-6} = 9.5 Hz, H-5), 3.55 (1 H, dd, J_{9a-9b} = 12.0 Hz, J_{9a-8} = 6.0 Hz, H-9a), 2.17 (1 H, dd, $J_{3eq-3ax}$ = 12.5 Hz, J_{3eq-4} = 5.0 Hz, H-3eq), 1.74 (1 H, t, $J_{3ax-3eq}$ = J_{3ax-4} = 12.5 Hz, H-3ax); ^{13}C NMR (D_2O + CD_3CN) δ 177.1, 96.9, 73.7, 73.6, 70.9, 69.3, 68.9, 62.5, 39.5; HRMS (FAB) calcd for $C_9H_{16}O_9$ (M − H^+) 267.0716, found 267.0729.

3-Deoxy-L-*manno*-octulosonic Acid (L-KDO) (15). The specific rotation and spectral data were obtained after **15** had been converted to its ammonium salt: $[\alpha]^{25}_D$ −37.2° (*c* 0.68, H_2O) [lit.[9] for D-KDO, $[\alpha]^{27}_D$ +42.3° (*c* 1.7, H_2O); authentic sample from Sigma, $[\alpha]^{25}_D$ +40.1° (*c* 2.1, H_2O)]. Since KDO has an axial 5-OH group, it is known that it exists as a mixture of pyranose and furanose forms and readily cyclizes to the corresponding lactone.[9] ^1H and ^{13}C NMR data of the predominant form: ^1H NMR (D_2O) δ 4.03 (1 H, ddd, J_{4-3ax} = 13.0 Hz, J_{4-3eq} = 5.5 Hz, J_{4-5} = 3.0 Hz, H-4), 4.00 (1 H, m, H-5), 3.87 (1 H, dt, J_{7-8b} = 3.0 Hz, J_{7-6} = J_{7-8a} = 5.5 Hz, H-7), 3.84 (1 H, m, H-6), 3.78 (1 H, dd, J_{8b-8a} = 12.0 Hz, J_{8b-7} = 3.0 Hz, H-8b), 3.60 (1 H, dd, J_{8a-8b} = 12.0 Hz, J_{8a-7} = 5.5 Hz, H-8a), 2.00 (1 H, t, $J_{3ax-3eq}$ = J_{3ax-4} = 13.0 Hz, H-3ax), 1.86 (1 H, dd, $J_{3eq-3ax}$ = 13.0 Hz, J_{3eq-4} = 5.5 Hz, H-3eq); ^{13}C NMR (D_2O + CD_3CN) δ 177.3, 96.8, 72.0, 71.3, 67.0, 66.6, 63.4, 34.0. ^1H and ^{13}C NMR spectra were identical with those of D-KDO from Sigma. HRMS (FAB): calcd for $C_8H_{14}O_8Na$ (M + Na^+) 261.0586, found 261.0591.

3,5-Dideoxy-D-*arabino*-D-*glycero*-nonulosonic acid (16): $[\alpha]^{25}_D$ −37.2° (*c* 0.44, H_2O); ^1H NMR (D_2O) δ 4.13 (1 H, m, H-4), 4.10 (1 H, dt, J_{6-5ax} = 12.0 Hz, J_{6-5eq} = J_{6-7} = 2.0 Hz, H-6), 3.77 (1 H, dd, J_{9a-9b} = 12.0 Hz, J_{9a-8} = 3.0 Hz, H-9a), 3.72 (1 H, ddd, J_{8-7} = 9.0 Hz, J_{8-9a} = 3.0 Hz, H-8), 3.56 (1 H, dd, J_{9b-9a} = 12.0 Hz, J_{9b-8} = 6.5 Hz, H-9b), 3.41 (1 H, dd, J_{7-8} = 3.5 Hz, J_{7-6} = 1.5 Hz, H-7), 2.03 (1 H, ddd, $J_{3eq-3ax}$ = 12.5 Hz, J_{3eq-4} = 4.5 Hz, $J_{3eq-5eq}$ = 1.5 Hz, H-3eq), 1.82 (1 H, b dt, $J_{5eq-5ax}$ = 12.0 Hz, J_{5eq-6} = J_{5eq-4} = 2.0 Hz, H-5eq), 1.56 (1 H, dt, J_{5ax-4} = 11.5 Hz, J_{5ax-6} = $J_{5ax-5eq}$ = 12.0 Hz, H-5ax), 1.49 (1 H, t, $J_{3ax-3eq}$ = J_{3ax-4} = 12.0 Hz, H-3ax); ^{13}C NMR (D_2O + CD_3CN) δ 177.8, 97.4, 73.2, 71.3, 68.6, 64.5, 63.5, 40.3, 35.7; HRMS (FAB) calcd for $C_9H_{16}O_8Na$ (M + Na^+) 275.0743, found 275.0754.

3,5,9-Trideoxy-D-*arabino*-D-*glycero*-nonulosonic acid (17): $[\alpha]^{25}_D$ −24.2° (*c* 0.25, H_2O); ^1H NMR (D_2O) δ 4.12 (1 H, ddt, J_{4-3eq} = 5.0 Hz, J_{4-5eq} = 2.5 Hz, J_{4-3ax} = J_{4-5ax} = 12.0 Hz, H-4), 4.07 (1 H, dt, J_{6-5ax} = 12.0 Hz, J_{6-5eq} = J_{6-7} = 2.5 Hz, H-6), 3.86 (1 H, dq, J_{8-7} = J_{8-9} = 6.5 Hz, H-8), 3.32 (1 H, dd, J_{7-8} = 6.5 Hz, J_{7-6} = 3.0 Hz, H-7), 2.06 (1 H, ddd, $J_{3eq-3ax}$ = 12.0 Hz, J_{3eq-4} = 5.0 Hz, J_{3eq-5} = 2.0 Hz, H-3eq), 1.85 (1 H, dt, $J_{5eq-5ax}$ = 12.0 Hz, J_{5eq-6} = J_{5eq-4} = 2.5 Hz, H-5eq), 1.53 (1 H, q, J_{5ax-4} = J_{5ax-5} = $J_{5ax-5eq}$ = 12.0 Hz, H-5ax), 1.51 (1 H, t, $J_{3ax-3eq}$ = J_{3ax-4} = 12.0 Hz, H-3ax), 1.18 (d, J_{9-8} = 6.5 Hz, H-9); ^{13}C NMR (D_2O + CD_3CN) 177.8, 97.3, 77.2, 69.3, 67.5, 64.6, 40.3, 35.8, 18.6; HRMS (FAB) calcd for $C_9H_{16}O_7Na$ (M + Na^+) 259.0794, found 259.0799.

3,5-Dideoxy-L-*arabino*-L-*glycero*-nonulosonic acid (18): $[\alpha]^{25}_D$ +35.8° (*c* 0.27, H_2O). The ^1H and ^{13}C NMR data are identical with those of compound **16**. HRMS (FAB): calcd for $C_9H_{16}O_8Na$ (M + Na^+) 275.0743, found 275.0751.

3,5,9-Trideoxy-L-*arabino*-L-*glycero*-nonulosonic acid (19): $[\alpha]^{25}_D$ +22.1° (*c* 0.19, H_2O). The ^1H and ^{13}C NMR data are identical with those of compound **17**. HRMS (FAB): calcd for $C_9H_{16}O_7Na$ (M + Na^+) 259.0794, found 259.0799.

5-Acetamido-3,5-dideoxy-β-L-*glycero*-L-*galacto*-2-nonulosonic acid (*N*-acetyl-L-neuraminic acid) (20): $[\alpha]^{25}_D$ +27.6° (*c* 0.17, H_2O) [(lit.[10] for D-NeuAc, $[\alpha]^{21}_D$ −29° (H_2O)]; ^1H NMR (D_2O) δ 4.03 (1 H, ddd, J_{4-3ax} = 12.0 Hz, J_{4-5} = 10.0 Hz, J_{4-3eq} = 5.5 Hz, H-4), 4.01 (1 H, dd, J_{6-5} = 10.0 Hz, J_{6-7} = 1.0 Hz, H-6), 3.90 (1 H, t, J_{5-6} = J_{5-4} = 10.0 Hz, H-5), 3.82 (1 H, dd, J_{9a-9b} = 12.0 Hz, J_{9a-8} = 2.5 Hz, H-9a), 3.73 (1 H, ddd, J_{8-7} = 9.0 Hz, J_{8-9b} = 6.0 Hz, J_{8-9a} = 2.5 Hz, H-8), 3.60 (1 H, dd, J_{9b-9a} = 12.0 Hz, J_{9b-8} = 6.0 Hz, H-9b), 3.51 (1 H, dd, J_{7-8} = 9.0 Hz, J_{7-6} = 1.0 Hz, H-7), 2.26 (1 H, dd, $J_{3eq-3ax}$ = 12.0 Hz, J_{3eq-4} = 5.5 Hz, H-3eq), 2.03 (3 H, s, acetyl), 1.84 (1 H, t, $J_{3ax-3eq}$ = J_{3ax-4} = 12.0 Hz, H-3ax); ^{13}C NMR (D_2O + CD_3CN) δ 175.3, 174.1, 95.9, 70.8, 70.6, 68.7, 67.2, 63.6, 52.5, 39.4, 22.5. The ^1H and ^{13}C NMR spectra were identical with those of an authentic D-NeuAc from Pfanstiehl Co. HRMS (FAB): calcd for $C_{11}H_{18}NO_9Na$ (M + Na^+) 331.2572, found 331.2579.

5-Azido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulosonic Acid (21). A solution of 2-azido-2-deoxy-D-glucose (120 mg, 0.585 mmol), 2-azido-2-deoxy-D-mannose (120 mg, 0.585 mmol), sodium pyruvate (650 mg, 5.91 mmol), dithiothreitol (2 mg), NaN_3 (5 mg), and sialic acid aldolase (30 units) in doubly-distilled H_2O (4.0 mL) was adjusted to pH 7.4 with phosphate buffer. The resulting solution was incubated at 37 °C for 3 days. The ^1H NMR of the reaction mixture indicated almost complete consumption of the azidomannose derivative and the presence of **21** as the only product. Compound **21** was isolated and characterized, and the NMR (^1H and ^{13}C) data were the same as reported.[6,11]

Improved Enzymatic Preparation of *N*-Acetyl-D-neuraminic Acid. A solution containing *N*-acetyl-D-mannosamine monohydrate (1.89 g, 7.5 mmol), sodium pyruvate (5.89 g, 52.5 mmol), NaN_3 (25 mg), phosphate buffer (0.1 M, 2.5 mL, pH 7.5), and water (35 mL) was adjusted to pH 7.5. NeuAc aldolase (Shinko American Inc., 10 units) was added, and the mixture was stirred at 25 °C for 2 days. More enzyme (5 units) was added, and the mixture was further stirred for an additional 2 days until ~80% of ManNAc was consumed as determined by ^1H NMR analysis [*N*-acetyl signals of ManNAc at δ 2.04 and 2.08 (1:1, two anomers) in D_2O and of NeuAc at δ 2.04]. The mixture was adjusted to pH 2.0 by the addition of Dowex 50W-X8 (H^+ form, 20–50 mesh) and incubated for 1 h to denature the enzyme. The mixture was then adjusted to pH 6.5 by adding concentrated aqueous ammonia solution.

Bakers' yeast was pretreated separately as follows: Bakers' yeast (Sigma type II, YSC-2, 15 g) was suspended in cold water (200 mL), and the mixture was stirred at 4 °C overnight. The cells were harvested by centrifugation at 10000*g* (8500 rpm) for 30 min at 4 °C and washed twice with cold water (100 mL). This procedure is absolutely necessary to remove all of the polar acidic materials prior to use.

The collected cells (35 mL) containing pyruvate decarboxylase were resuspended in water (40 mL) and then added to the aldol product solution obtained above. After antifoam AF emulsion (Dow-Corning-Nakaraitesque, diluted to a 10% aqueous solution, 0.4 mL) was added, the mixture was stirred with bubbling of air (1000 mL/min). The pH was kept between 5.8 and 6.5 by the occasional addition of Dowex 50W-X8 (H^+ form, 20–50 mesh). The consumption of pyruvate was determined by lactate dehydrogenase assay for the remaining pyruvate. The

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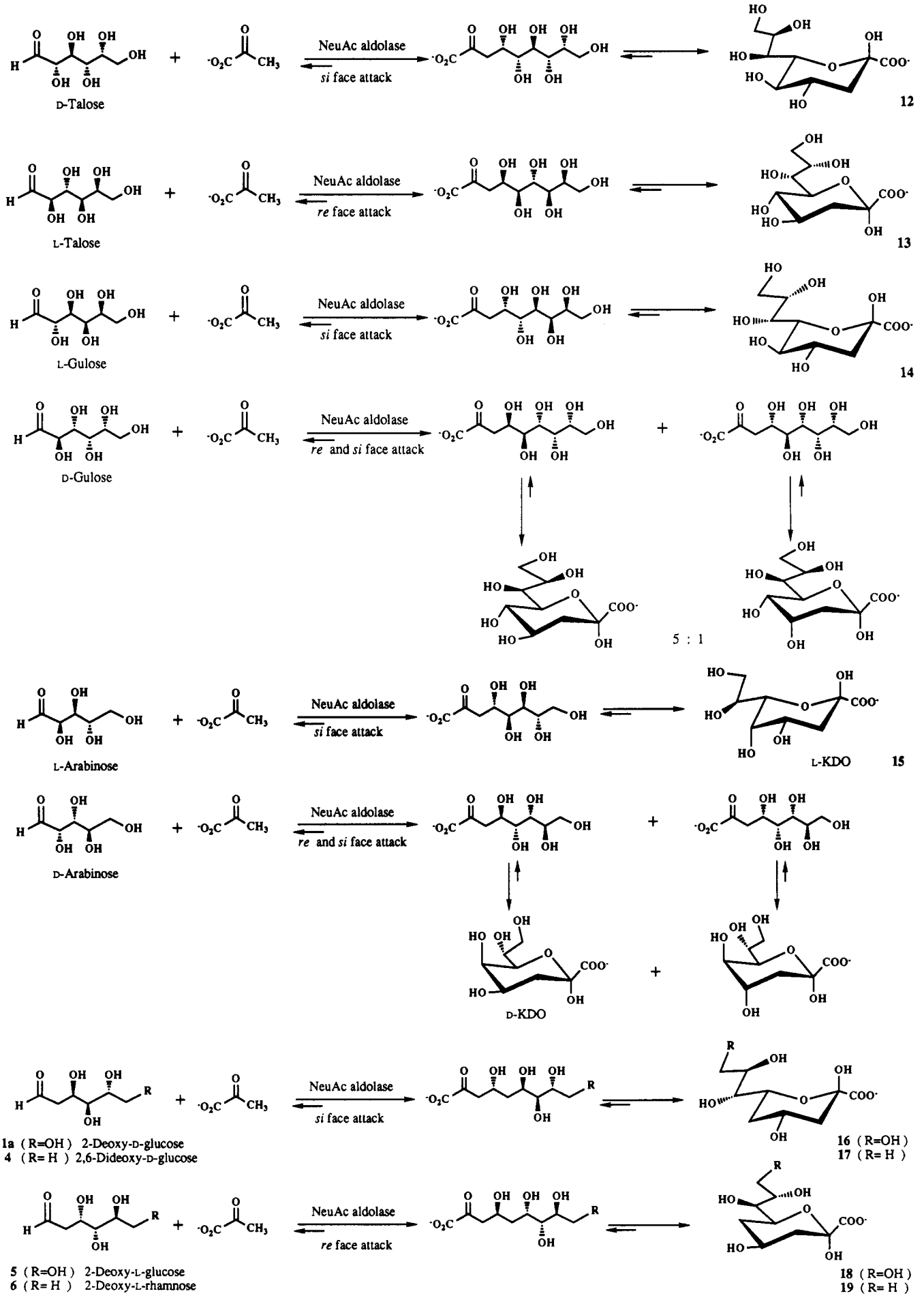
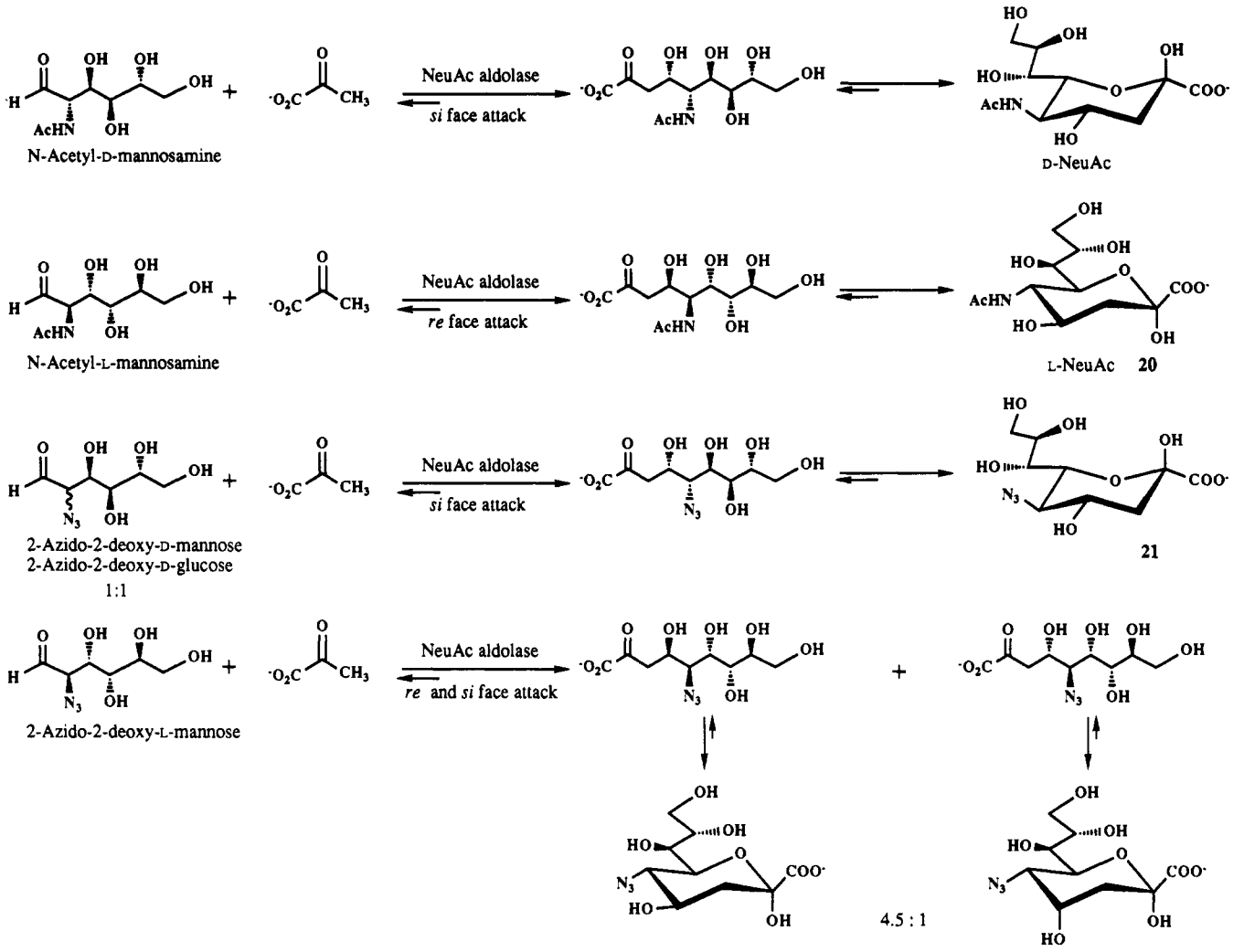
Scheme VI. Sialic Acid Aldolase-Catalyzed Aldol Condensations with Normal and Unusual Stereoselectivity: Synthesis of Enantiomeric High-Carbon Sugars



Scheme VI (continued)



NMR spectrum of the sample taken after 6 h showed a complete decomposition of pyruvate (δ 2.36 for $CH_3COCO_2^-$ and δ 1.52 for $CH_3C(OH)_2CO_2^-$ in D_2O).

The yeast cells were removed by centrifugation at 10000*g* (8500 rpm) for 30 min at 4 °C, and the cells were washed twice with water (100 mL). The pH of the combined extract was adjusted to 2.3 by the addition of Dowex 50W-X8 (H^+ form), and the solution was concentrated in vacuo. The residue was suspended in 50% aqueous methanol (200 mL) and left to stand overnight at 4 °C. The precipitated material was removed by centrifugation at 10000*g* (8500 rpm) for 30 min at 4 °C, and the residue was washed twice with 50% aqueous methanol (100 mL).

The solution was concentrated in vacuo, and the residue was diluted with water (400 mL). Then the pH was adjusted to 5.5 by the addition of concentrated aqueous ammonia solution. To this mixture was added Dowex 1-X8 (HCO_2^- form, 20–50 mesh, 100 mL of the bed volume). After washing with water, the resin was eluted with 2 M formic acid (400 mL). Concentration of the eluent afforded a crystalline product, which was washed successively with methanol and diethyl ether to give pure NeuAc (987 mg, 3.2 mmol; 42.6% yield based on D-ManNAc): $[\alpha]^{25}_D$ −34.0° (*c* 2.09, H_2O) [authentic NeuAc from Pfanstiehl Co., $[\alpha]^{20}_D$ −32° (*c* 2.0, H_2O)]. The NMR spectrum of the product in D_2O was identical with that of authentic NeuAc. A similar result was obtained with the use of commercially available pyruvate decarboxylase (EC 4.1.1.1, Sigma P6810) instead of yeast.

A larger scale synthesis was carried out starting from a mixture of *N*-acetyl-D-mannosamine (23.4 g) and *N*-acetyl-D-glucosamine (4.9 g), which was obtained by the base-catalyzed epimerization[12] of *N*-acetyl-D-glucosamine. In this case, the decarboxylation procedure was slightly modified as follows: The pH of the reaction mixture was adjusted to 6.0 by the addition of 2 N NaOH solution after the denaturation of NeuAc aldolase. The subsequent procedure was the same as described above. Finally, more than 10 g of NeuAc could be isolated simply by recrystallization, without any treatment with anion-exchange resin. After the removal of precipitates by treatment with 50% aqueous methanol, the concentrated residue was dissolved in 75% aqueous acetic acid (100 mL), and the solution was left to stand overnight at 4 °C. The resulting thick suspension was diluted with cold 75% aqueous acetic acid (100 mL) and centrifuged at 10000*g* (8500 rpm) for 30 min at 4 °C. The precipitated NeuAc was washed successively with cold 75% aqueous acetic acid (100 mL) and cold methanol (100 mL) by the use of centrifugation. The precipitates were collected by filtration and washed with cold methanol and diethyl ether to give 12.1 g of NeuAc. For a larger scale synthesis, the aldolase can be used in an immobilized form.[2e]

Kinetic Measurements. The rates for aldolase-catalyzed reactions were obtained by measuring the amount of remaining pyruvate.[2c] The reactions were carried out in 0.1 M phosphate buffer (pH 7.5) containing 0.4 unit of the enzyme, varied concentrations of pyruvate (2.0, 2.5, 3.33, 5, and 10 mM), and varied concentrations of carbohydrates (0.2, 0.25, 0.33, and 0.50 M) in 0.5 mL of solution. Each solution was incubated at 37 °C. Periodically, a small volume (20–50 μL) was withdrawn and mixed with an assay solution (1.4 mL) containing 0.1 M phosphate (pH 7.5), 0.3 mM NADH, and 20–30 units of L-lactate dehydrogenase. The decrease in absorbance at 340 nm was measured, and the amount of the unreacted pyruvate was determined using 6220 M^{-1} cm^{-1} as the molar absorbance of NADH. The kinetic parameters were obtained from the Lineweaver–Burk plots.

For the relative rate measurements, the concentrations of pyruvate and sugar were fixed at 10 mM and 0.25 M, respectively. Other conditions were the same as the above.

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