freeze-thaw method and the stopcock closed. The Pyrex tube was now irradiated by a 450-W medium-pressure Hanovia lamp through Pyrex in a water-cooled bath. The diazo compound was completely decomposed in 6 h as seen by the discharge of the yellow color. The tube was cooled and opened, and the excess reactant was removed. The crude products were well separated by the Hewlett-Packard GC/MS, and the data were processed on HP-ChemStation software. Finally, the crude products were chromatographed and collected by using a 6-ft 10% OV-101/Gas-chrom W-HP 80-100 column on the GOW-MAC GC 580 instrument.

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Supplementary Material Available: Table of ^{1}H NMR, ^{13}C NMR, and IR spectral data and MS and HRMS data for compounds **10–14** and **17**, as well as for all of the addition and insertion products formed from carbenes **2–4** (7 pages). Ordering information is given on any current masthead page.

Synthesis of 3-Deoxy-D-*manno*-2-octulosonic Acid (KDO) and Its Analogs Based on KDO Aldolase-Catalyzed Reactions[1]

Takeshi Sugai, Gwo-Jenn Shen, Yoshitaka Ichikawa, and Chi-Huey Wong*

Contribution from the Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037. *Received September 8, 1992*

Abstract: 3-Deoxy-D-*manno*-2-octulosonic acid (D-KDO) was synthesized from D-arabinose and pyruvate in 67% yield by using KDO aldolase (EC 4.1.2.23) from *Aureobacterium barkerei* strain KDO-37-2 (ATCC 49977). Studies on the substrate specificity of the enzyme with more than 20 natural and unnatural sugars indicate that this enzyme widely accepts trioses, tetroses, pentoses, and hexoses as substrates, especially the ones with the *R* configuration at the 3 position. The substituent on the 2 position had little effect on the aldol reaction. Nine substrates were submitted to the aldol reaction to prepare the products, including D-KDO, 3-deoxy-D-*arabino*-2-heptulosonic acid (D-DAH), 2-keto-3-deoxy-L-gluconic acid (L-KDG), and 3-deoxy-L-*glycero*-L-*galacto*-nonulosonic acid (L-KDN). It appears that the attack of pyruvate took place on the *re* face of the carbonyl group of acceptor substrates, a facial selection complementary to sialic acid aldolase (*si* face attack) reactions. The aldolase products can be converted to aldoses via radical-mediated decarboxylation. For example, decarboxylation of pentaacetyl-KDO and hexaacetylneuraminic acid gave penta-*O*-acetyl-2-deoxy-β-D-*manno*-heptose and penta-*O*-acetyl-4-acetamido-2,4-dideoxy-β-D-*glycero*-D-*galacto*-octose, respectively.

Introduction

3-Deoxy-D-*manno*-2-octulosonic acid (KDO, **1**) is a vital component of the outer membrane lipopolysaccharide of Gram-negative bacteria.[2] Of many chemical[3] and enzymatic (based on KDO-8 phosphate synthase[4a] or sialic acid aldolase[4b]) syntheses developed so far, the Cornforth's method[3a,b] of chemical aldol reaction of D-arabinose and oxalacetic acid has been considered the most practical. The yield, stereoselectivity, and reproducibility of the reaction, however, are not satisfactory although several improved procedures have been reported.[3e,k] Thus, the enzymatic aldol reaction[5] of pyruvate and D-arabinose and analogs catalyzed by KDO aldolase under nearly neutral and mild conditions may be useful for the synthesis of KDO and analogs.

In lipopolysaccharide biosynthesis, the incorporation of KDO[6] consists of two steps: the formation of CMP-KDO by CMP-KDO synthetase[7] (EC 2.7.7.38) and the subsequent coupling with lipid-A precursor.[8] Since the rate-limiting step is the activation of the KDO moiety,[9] inhibitors of CMP-KDO synthetase are potentially

(1) Supported by the NIH (Grant GM44154).

(2) (a) Levin, D. H.; Racker, E. *J. Biol. Chem.* **1959**, *234*, 2532. For a general review for KDO, see: (b) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323.

(3) (a) Ghalambor, M. A.; Levine, E. M.; Heath, E. C. *J. Biol. Chem.* **1966**, *241*, 3207. (b) Hershberger, C.; Davis, M.; Binkley, S. B. *J. Biol. Chem.* **1968**, *243*, 1585. (c) Collins, P. M.; Overend, W. G.; Shing, T. K. M. *J. Chem. Soc., Chem. Commun.* **1981**, *1139*. (d) Danishefsky, S. J.; Pearson, W. H.; Segmuller, B. E. *J. Am. Chem. Soc.* **1985**, *107*, 1280. (e) McNicholas, P. A.; Batley, M.; Redmond, J. W. *Carbohydr. Res.* **1986**, *146*, 219. (f) Imoto, M.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1987**, *28*, 6235. (g) Danishefsky, S. J.; DeNinno, M.; Chen, S. *J. Am. Chem. Soc.* **1988**, *110*, 3929. (h) Itoh, H.; Kaneko, T.; Tanami, K.; Yoda, K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3356. (i) Branchaud, B. P.; Meier, M. S. *J. Org. Chem.* **1989**, *54*, 1320. (j) Enhsen, A.; Schmidt, R. R. *Liebigs Ann. Chem.* **1989**, *69*. (k) Shirai, R.; Ogura, H. *Tetrahedron Lett.* **1989**, *30*, 2263. (l) Esswein, A.; Bets, R.; Schmidt, R. R. *Helv. Chim. Acta* **1989**, *72*, 213. (m) Boons, G. J. P. H.; van der Klein, P. A. M.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 273. (n) Smith, D. B.; Wang, Z.; Schreiber, S. L. *Tetrahedron* **1990**, *46*, 4793. (o) Frick, W.; Krulle, T.; Schmidt, R. R. *Liebigs Ann. Chem.* **1991**, 435. (p) Dondoni, A.; Merino, P. *J. Org. Chem.* **1991**, *56*, 5294. (q) Ramage, R.; MacLeod, A. M.; Rose, G. W. *Tetrahedron* **1991**, *47*, 5625. (r) Martin, S. F.; Zinke, P. W. *J. Org. Chem.* **1991**, *56*, 6600. For other earlier syntheses, see ref 2b.

(4) (a) Bednarski, M. D.; Crans, D. C.; DiCosimo, R.; Simon, E. S.; Stein, P. D.; Whitesides, G. M.; Schneider, M. J. *Tetrahedron Lett.* **1988**, *29*, 427. (b) Augé, C.; Gautheron, C.; David, S.; Malleron, A.; Cavayé, B.; Bouxon, B. *Tetrahedron* **1990**, *46*, 201.

(5) For a review, see: (a) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365. (b) Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, C. F., III; Gautheron, C. M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, 499. For FDP aldolase, (c) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199. (d) Durrwachter, J. R.; Drueckhammer, D. G.; Nozaki, K.; Sweers, H. M.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175. (e) von der Osten, C. H.; Sinskey, A. J.; Barbas, C. F., III; Pederson, R. L.; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1989**, *111*, 3924. (f) Bednarski, M. D.; Simon, E. S.; Bischefberger, N.; Fessner, W.-D.; Kim, M.-J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 627. (g) Straub, A.; Effenberger, F.; Fischer, P. *J. Org. Chem.* **1990**, *55*, 3926. (h) Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A., Jr.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187. For deoxyribose-5-phosphate aldolase (DERA), (i) Barbas, C. F., III; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1990**, *112*, 2013. (j) Chen, L.; Dumas, D. P.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 741. For sialic acid aldolase, (k) Comb, D.; Roseman, S. *J. Am. Chem. Soc.* **1958**, *80*, 497. (l) Augé, C.; David, S.; Gautheron, C.; Malleron, A.; Cavayé, B. *New J. Chem.* **1988**, *12*, 733. (m) Kim, M.-J.; Hennen, W. J.; Sweers, H. M.; Wong, C. H. *J. Am. Chem. Soc.* **1988**, *110*, 6481. (n) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159. (o) Augé, C.; David, S.; Malleron, A. *Carbohydr. Res.* **1989**, *188*, 201. (p) Schrell, A.; Whitesides, G. M. *Liebigs Ann. Chem.* **1990**, 1111. (q) Gautheron-Le Narvor, C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 7817. (r) Kragel, U.; Gygax, D.; Ghisalba, O.; Wandrey, D. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 827. (s) 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. (t) Lin, C.-H.; Sugai, T.; Halcomb, R. L.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 10138. Also see ref 4b.

(6) Rick, D.; Osborn, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 3756.

(7) Ghalambor, M. A.; Heath, E. C. *J. Biol. Chem.* **1966**, *241*, 3216.

(8) Munson, R. S.; Rasmussen, N. S.; Osborn, M. J. *J. Biol. Chem.* **1978**, *252*, 1503.

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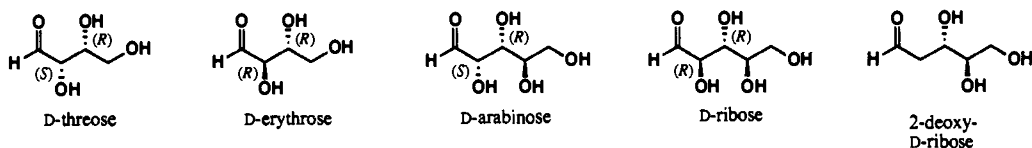
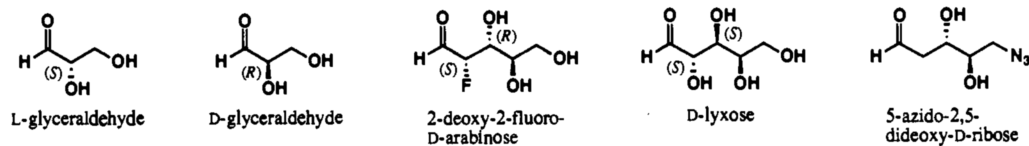
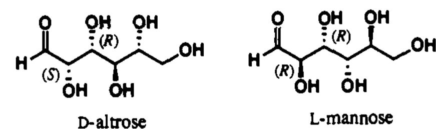
Good substrates



Fair substrates





Poor substrates

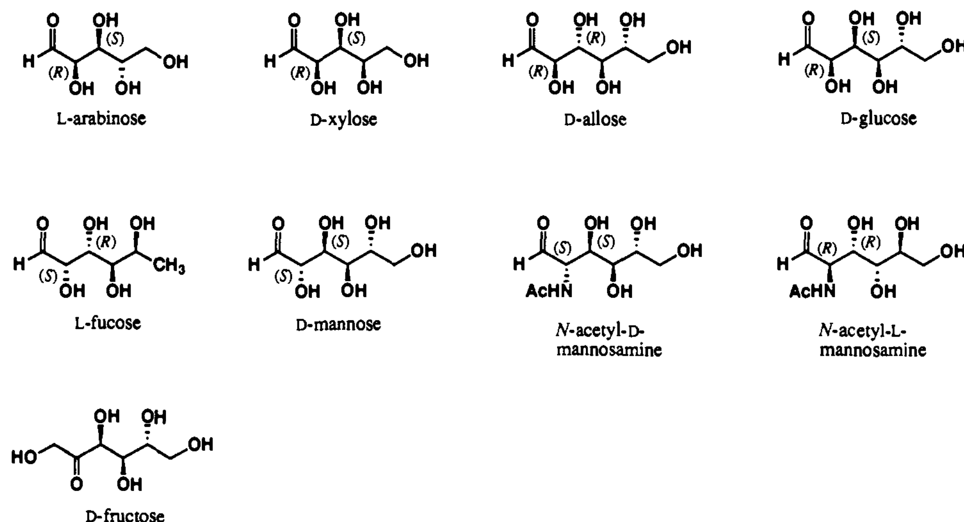


Figure 1. (a) Substrate specificity of KDO aldolase from *A. barkerei* KDO-37-2. (b) Substrate specificity of KDO aldolase from *A. barkerei* KDO-37-2.

useful as antibacterial agents.[10]

In this paper, we report the specificity study of a KDO aldolase and its application to the synthesis of KDO and analogs. We also report the synthesis of high-carbon aldoses via radical-mediated decarboxylation of KDO aldolase products.

Results and Discussion

New Source of KDO Aldolase. KDO aldolase (EC 4.1.2.23) was first reported by Ghalambor and Heath in 1966 as the enzyme responsible for the KDO degradation.[11] After their preliminary investigation on the substrate specificity as well as the μmol scale synthesis of KDO, no synthetic application of this enzyme has been reported, while the related enzyme *N*-acetylneuraminic acid (sialic acid) aldolase has been extensively studied.[4b,5k-t]

Recently, we have identified a Gram-positive bacterium, *Aureobacterium barkerei* strain KDO-37-2, which contains high levels of KDO aldolase. The aldolase activity (10.2 units from a 2-L culture, assayed by Aminoff's method[11,13] based on the degradation of KDO) was 4 times and 8 times higher compared with those from *Escherichia coli* K-12[11] and *Aerobacter cloacae*,[11] respectively. The partially purified enzyme simply obtained by ammonium sulfate precipitation (8.0 units/mL; 0.19 units/mg for degradation of KDO) was used in substrate-specificity studies. For kinetic analysis, this enzyme was purified via DEAE Sepharose and phenyl Sepharose column chromatography to a specific activity of 5.7 units/mg. The K_m and V_{max} for D-arabinose are 1.2 M and 0.73 units/mg, respectively. The unusually high con-

(9) Ray, P. H.; Benedict, C. D.; Grasmuk, H. *J. Bacteriol.* **1981**, *145*, 1273.
(10) (a) Molin, H.; Pring, B. G. *Tetrahedron Lett.* **1985**, *26*, 677. (b) Luthman, K.; Claesson, A.; Jansson, A. M.; Pring, B. G. *Carbohydr. Res.* **1987**, *166*, 233. (c) Claesson, A.; Luthman, K.; Gustafsson, K.; Bondesson, G. *Biochem. Biophys. Res. Commun.* **1987**, *143*, 1063. (d) Norbeck, D. W.; Kramer, J. B. *Tetrahedron Lett.* **1987**, *28*, 773. (e) Claesson, A.; Jansson, A. M.; Pring, B. G.; Hammond, S. M.; Ekström, B. *J. Med. Chem.* **1987**, *30*, 2309. (f) Luthman, K.; Orbe, M.; Waglund, T.; Claesson, A. *J. Org. Chem.* **1987**, *52*, 3777. (g) Claesson, A. *J. Org. Chem.* **1987**, *52*, 4414. (h) Hammond, S. M.; Claesson, A.; Jansson, A. M.; Larsson, L.-G.; Pring, B. G.; Town, C. M.; Ekström, B. *Nature* **1987**, *327*, 730. (i) Pring, B. G.; Jansson, A. M.; Persson, K.; Andersson, I.; Gagner-Michert, I.; Gustafsson, K.; Claesson, A. *J. Med. Chem.* **1989**, *32*, 1069. (j) Andersson, F.; Classon, B.; Samuelsson, B. *J. Org. Chem.* **1990**, *55*, 4699. (k) Liu, Z.; Classon, B.; Garegg, P. J.; Samuelsson, B. *J. Carbohydr. Chem.* **1991**, *10*, 65. (l) Ohrui, H.; Morita, M.; Meguro, H. *Carbohydr. Res.* **1992**, *224*, 319.

(11) Ghalambor, M. A.; Heath, E. C. *J. Biol. Chem.* **1966**, *241*, 3222.
(12) *Bergey's Manual of Systematic Bacteriology*; Holt, J. G., Ed.; Williams & Wilkins: Baltimore, Maryland, 1984. The strain is available from American Type Culture Collection (ATCC 49977).
(13) Aminoff, D. *Biochem. J.* **1961**, *81*, 384.

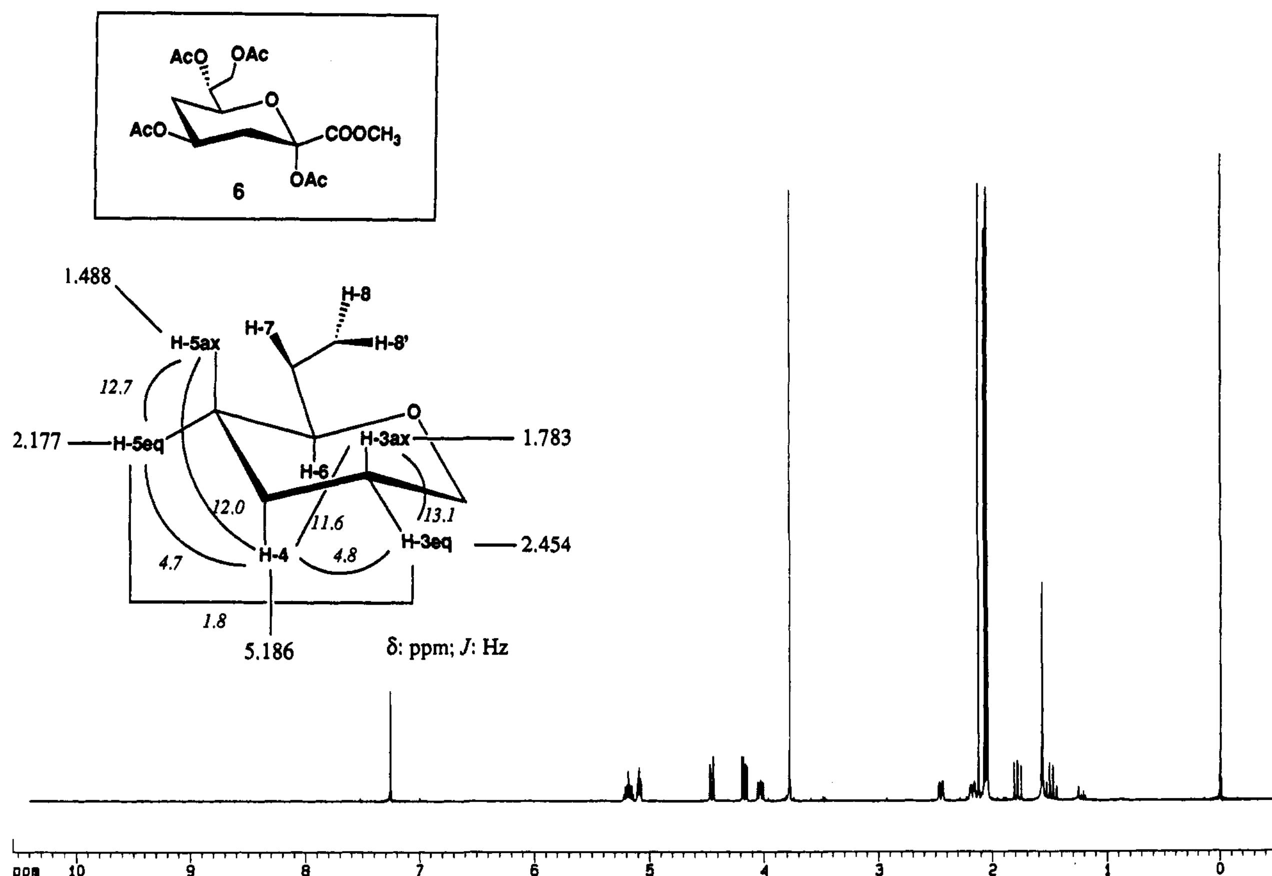


Figure 2. ^1H NMR spectrum of 6, the product from 2-deoxy-D-ribose (400 MHz, $CDCl_3$).

centration of K_m in the condensation compared with that in the course of degradation (6×10^{-3} M for KDO)[11] indicates that the enzyme may accept the open form of aldoses as acceptors in the aldol condensation. The enzymatic reaction favors the cleavage of KDO, with the equilibrium constant K_{eq} = [pyruvate][arabinose]/KDO = 9×10^{-2} M.[3a]

Substrate Specificity. This enzyme exhibits a wide substrate specificity. Several 3–6-carbon sugars were accepted as substrates for the condensation. From the results shown in Table I and Figure 1, the structural requirements of the sugar for this enzyme are as follows. At the 2 position, although the aldolase prefers an *S* configuration, the difference is not significant [examples: between L- and D-glyceraldehyde; D-threose and D-erythrose; D-arabinose, D-ribose, and 2-deoxy-D-ribose]. It is noteworthy that this enzyme also accepts D-ribose as a good substrate (relative $V = 72\%$), while that from *E. coli* or *A. cloacae* poorly accepts this substrate (relative $V < 5\%$).[11] At the 3 position, this enzyme prefers the *R* configuration [examples: comparison between D-arabinose and L-arabinose, D-lyxose and D-xylose]. Hexoses are generally not as good substrates as tetroses and pentoses, even in the case of D-altrose (relative $V = 25\%$) and L-fucose (rate not detectable), both being homoanalogs of the natural substrate D-arabinose. The reason that L-mannose is a better substrate than D-mannose is because the former has the favorable 2*R*, 3*R* configuration and the latter has the unfavorable 2*S*, 3*S* configuration. Finally, neither fluoropyruvate[5m] nor ketohexose was accepted by this enzyme.

Aldol Condensation. The enzymatic synthesis of KDO (Scheme I) on multi-mmol scales using 10 molar excess of pyruvate worked well (e.g. **1** was obtained in 67% yield). The yield of the enzymatic reaction is comparable to the highest one obtained by the modified Cornforth synthesis (66%).[3k] Further crystallization gave KDO ammonium salt monohydrate[14] in 37% overall yield: $[\alpha]^{26}_D$ +40.3° (*c* 2.06, H_2O) [lit.[2b] $[\alpha]^{27}_D$ +42.3° (*c*, 1.7, H_2O), authentic sample from Sigma $[\alpha]^{26}_D$ +40.2° (*c* 2.06, H_2O)]. The ^1H NMR spectrum in D_2O is identical with that of an authentic sample, altough it is complicated by the fact that KDO exists as an anomeric mixture of pyranose and furanose forms and readily cyclizes to the corresponding lactone in aqueous solution.[2b] The crystalline ammonium salt was further converted to pentaacetate methyl ester derivative **2**, whose ^1H NMR spectrum was in good accordance with that reported previously[2b] and clearly shows the $^5C_2$ pyranose conformation.

Encouraged by this result, several substrates with good or fair relative rates were used in the aldol condensation. The reactions with D-ribose and 2-deoxy-D-ribose took place smoothly to give **3** (57% after derivation to **4**) and **5**[15] (47% as **6**), respectively. ^1H NMR spectra of **3**, **4**, **5**, and **6** clearly show a $^5C_2$ pyranose form in both products. The ^1H spectrum of **6** is shown in Figure 2. It is noteworthy that in these cases, even though the relative rates are lower (72% for D-ribose and 71% for 2-deoxy-D-ribose) than that of D-arabinose, TLC analysis of the reaction products showed no starting material left, whereas a substantial amount of starting material always remained in the reaction with D-arabinose. It is suggested that formation of the pyranose form of **3** and **5**, where all substituents are located in the stable orientation, further shifted the equilibrium toward condensation.

The products **7**[3q] (3-deoxy-D-*arabino*-2-heptulosonic acid, DAH, 39% as **8**) and **9** (11% as **10**) were also obtained from D-erythrose and D-glyceraldehyde, respectively, indicating that this aldolase-catalyzed condensation is also useful for the synthesis of lower homologs of KDO. The phosphate of **7** (DAHP) plays an important role in the shikimate synthesis pathway in plants and microorganisms.[16]

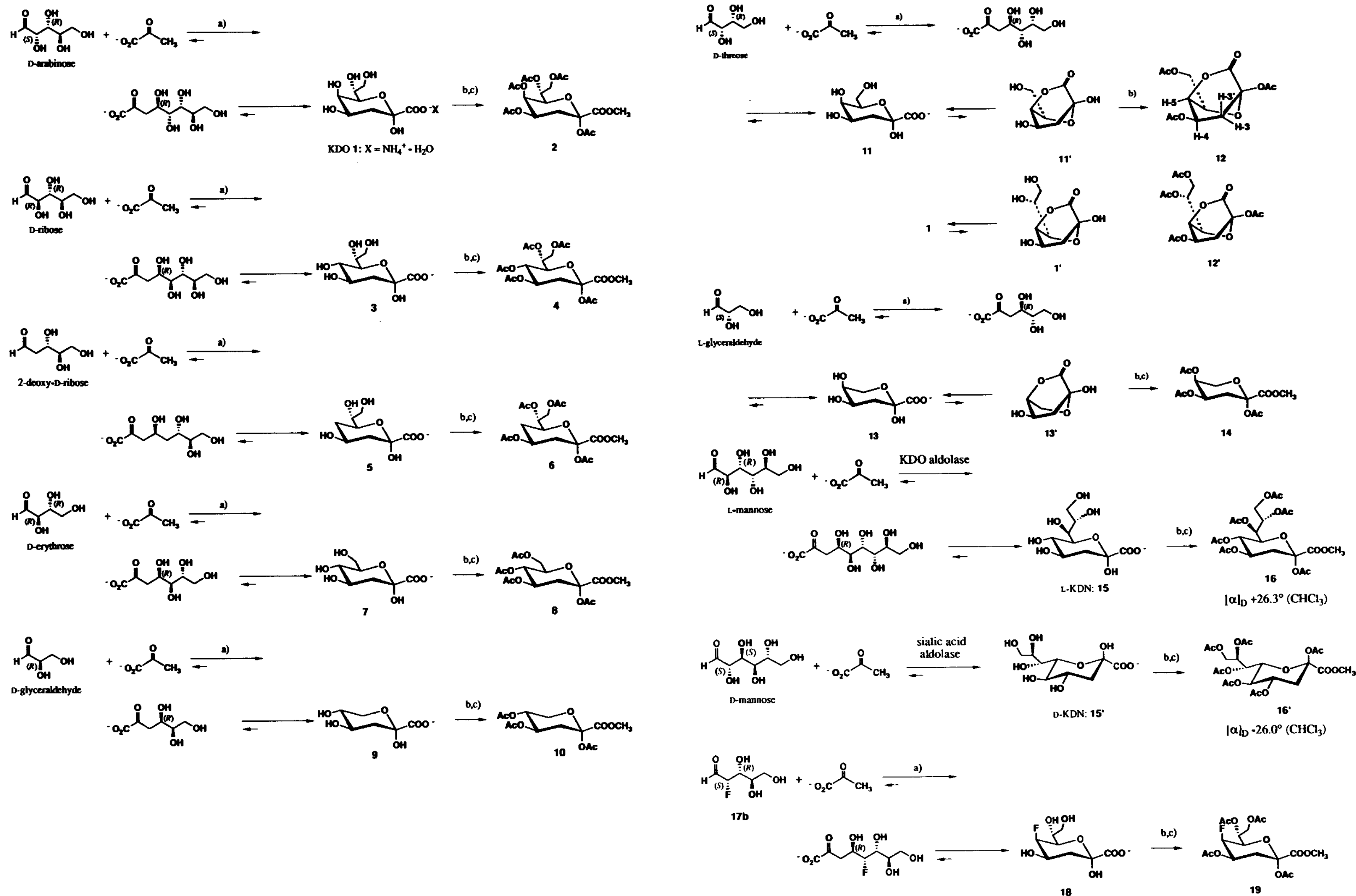
From D-threose, the product **11** was obtained, whose ^1H NMR spectrum was similar to that of KDO. The reaction with L-

(14) Birnbaum, G. I.; Roy, R.; Brisson, J.-R.; Jennings, H. J. *J. Carbohydr. Chem.* **1987**, *6*, 17.

(15) Cherniak, R.; Jones, R. G.; Sen Gupta, D. *Carbohydr. Res.* **1979**, *75*, 39.

(16) Srinivasan, P. R.; Katagiri, M.; Sprinson, D. B. *J. Biol. Chem.* **1959**, *234*, 713.

Scheme I. Aldol Condensation Catalyzed by KDO Aldolase from *A. barkerei* KDO-37-2



[a] Reagents: (a) KDO aldolase; (b) Ac_2O/py, DMAP; (c) CH_2N_2.

Table I. Relative Rates of Several Substrates for KDO Aldolase from *A. barkerei* KDO-37-2

substrate	relative rate[a]	substrate	relative rate[a]
D-arabinose	100	D-altrose	25
D-threose	128	L-mannose	15
D-erythrose	93	L-arabinose	N.D.[b]
D-ribose	72	D-xylose	N.D.[b]
2-deoxy-D-ribose	71	D-allose	N.D.[b]
L-glyceraldehyde	36	D-glucose	N.D.[b]
D-glyceraldehyde	23	D-mannose	N.D.[b]
2-deoxy-2-fluoro-D-arabinose	46	L-fucose	N.D.[b]
D-lyxose	35	*N*-acetyl-D-mannosamine	N.D.[b]
5-azido-2,5-dideoxy-D-ribose	15	*N*-acetyl-L-mannosamine	N.D.[b]
		D-fructose	N.D.[b]

[a] Measured at pH 7.5 with 500 mM sugar and 10 mM pyruvate. For detailed conditions, see experimental. Specific activity based on D-arabinose is 0.2 units/mg; 1 unit = 1 μmol of KDO formed per min. Fluoropyruvate is not acceptable as a donor substrate. [b] Not detectable.

glyceraldehyde afforded **13** (2-keto-3-deoxy-L-gluconic acid, KDG), an enantiomer of D-KDG, whose phosphate (KDGP) is an intermediate in the Entner–Doudoroff pathway.[17] The ^{1}H NMR spectrum of **13** was very complicated (see experimental). To clarify the stereochemistry, preparation of derivatives was attempted; however, the products were still difficult to identify. The only isolable component from **11** was a bicyclic lactone. The structure was determined as **12** (Scheme I) by comparing its ^{1}H NMR spectrum with that of the higher homolog **12′**, which had been obtained from KDO and unambiguously characterized previously.[18] In its ^{1}H NMR spectrum, a long range coupling between H-3 and H-5 (0.6 Hz) indicates that the pyranose form of the product exists as a twisted boat conformation, and all of the coupling constants are consistent with those observed in the case of **12′**. It is interesting that, in the spectra of **11**, **13**, and KDO, a substantial proportion of similar signals was observed, where one of the H-3 signals appears at very low field. From these results, it is assumed that the bicyclic 1 → 5 lactones **1′**, **11′**, and **13′** form at nearly neutral pH. The formation of 1 → 7 lactone is excluded, since those signals were observed in the case of hexulosonate **13′** without any C-7 hydroxy group. The homologs prepared here also proceed through a spontaneous 1 → 5 lactone formation, as already proposed previously for KDO.[19] Compound **13** mainly exists as the $^5C_2$ pyranose form, as indicated in **14**.

The reaction with L-mannose gave **15**[5t] (3-deoxy-L-*glycero*-L-*galacto*-2-nonulosonic acid, L-KDN, 61% as **16**), which is an enantiomer of D-KDN, a component in polysialoglycoprotein and ganglioside of rainbow trout eggs.[20] The optical rotation $[[\alpha]^{25}_D$ $+26.3°$ $(CHCl_3)]$ and ^{1}H NMR spectrum of **16** were in good accordance with those of **16′** $[[\alpha]^{25}_D$ $-26.0°$ $(CHCl_3)]$, which was obtained via reaction with D-mannose catalyzed by sialic acid aldolase,[4b] except for the sign of rotation. The availability of both enantiomers of KDN may make it possible to develop new analogs of sialyl oligosaccharides.[21]

Finally, the aldol reaction with an unnatural sugar containing a fluorine atom was conducted to give **18** (19% of **19**). By comparing the ^{1}H NMR spectra, the proportion of the β-isomer (10.71) of **18** was ca. 1.5 times higher than that of KDO (6.9%), probably due to the absence of furanose and 1 → 5 lactone forms. This result suggests that **18** might be a good substrate for CMP-KDO synthetase, since the enzyme accepts the unstable β-form of KDO as a substrate.[22] We therefore synthesized **18** on a larger scale by combining the use of KDO aldolase and pyruvate decarboxylase,[5t] which made the workup procedure much easier. Preliminary study using **18** as a substrate for CMP-KDO synthetase which had recently been cloned and overexpressed in this group[23] showed that **18** was accepted by the enzyme. The details will be reported elsewhere.

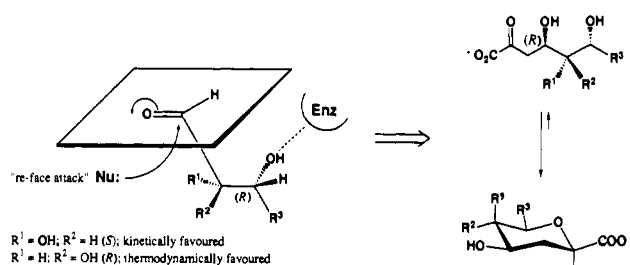


Figure 3. Stereochemistry of the aldol condensation catalyzed by KDO aldolase from *A. barkeri* KDO-37-2.

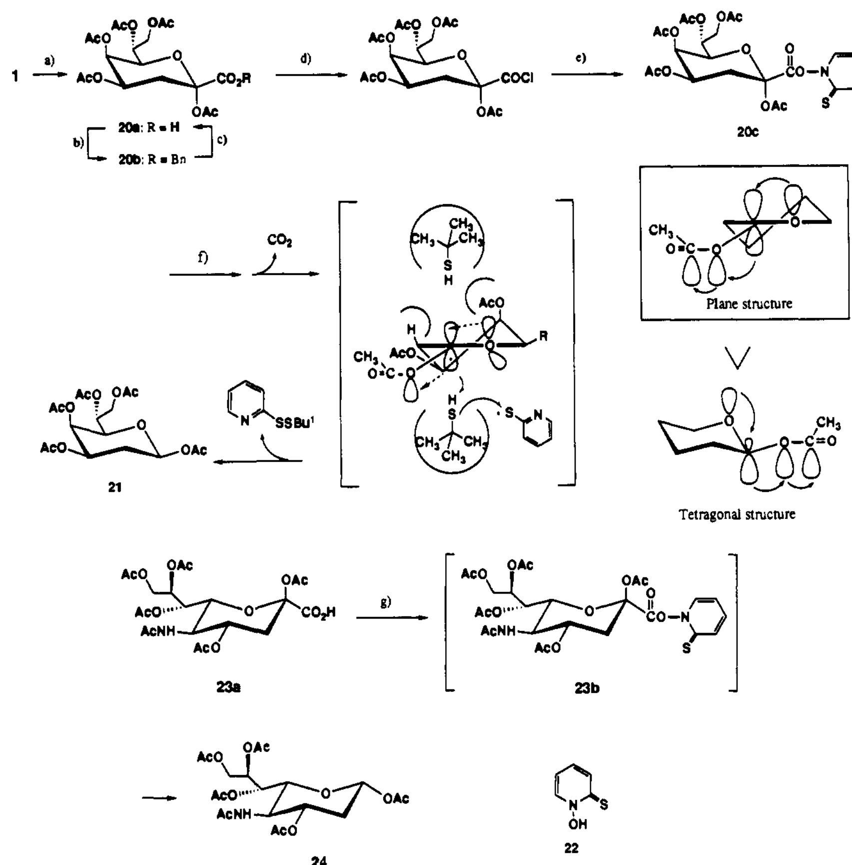
On the basis of the results obtained so far, the stereochemical course of the aldol condensation catalyzed by this KDO aldolase is as follows. The attack of pyruvate always takes place on the *re* face of the carbonyl group of the substrates, a facial selection complementary to sialic acid aldolase reactions (*si* face attack). The stereochemical requirements of substrates and the sterochemical course of the aldol condensation are indicated in Figure 3. It is concluded that in general the enzyme accepts substrates with an *R* configuration at C-3. The substrates with an *S* configuration at C-2 are kinetically favored, while those with an *R* configuration at C-2 are thermodynamically favored to give a better yield.

Synthesis of Decarboxylated Analogs. It is obvious that decarboxylation of KDO and analogs will give aldose derivatives. The aldodeoxyheptose structure is particularly interesting, since a number of heptoses are widely distributed in nature,[24] some of which play important roles in metabolic pathways. Barton's radical-mediated decarboxylation[25] of the penta-*O*-acetyl derivative **20a**[26] obtained from the corresponding benzyl ester **20b** seems to be the most straightforward route to the desired heptose derivative **21**.

There has recently been growing interest in the synthesis of physiologically active carbohydrate- and nucleic acid-related compounds via anomeric radical intermediates. It appears to us that a radical-mediated reaction stabilized by both electron-withdrawing and electron-donating groups (capto–dative effect[28])

(17) Entner, N.; Doudoroff, M. *J. Biol. Chem.* **1952**, *196*, 853.
(18) Charon, D.; Auzanneau, F.-I.; Mérienne, C.; Szabó, L. *Tetrahedron Lett.* **1987**, *23*, 1393.
(19) Menton, L. D.; Morris, E. R.; Rees, D. A.; Thon, D.; Bociek, S. M. *Carbohydr. Res.* **1980**, *80*, 295.
(20) Nadano, D.; Iwasaki, M.; Endo, S.; Kitajima, K.; Inoue, S.; Inoue, Y. *J. Biol. Chem.* **1986**, *261*, 11550. Song, Y.; Kitajima, K.; Inoue, S.; Inoue, Y. *J. Biol. Chem.* **1991** *266*, 21929.
(21) Ichikawa, Y.; Look, G. C.; Wong, C.-H. *Anal. Biochem.* **1992**, *202*, 215.
(22) Kohlbrenner, W. F.; Fesik, S. W. *J. Biol. Chem.* **1985**, *260*, 14695.
(23) Shen, G.-J.; Wong, C.-H. Manuscript in preparation.
(24) Schaffer, R. *The Carbohydrates, Chemistry and Biochemistry*, 2nd ed.; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1980; Vol. 1A, Chapter 2.
(25) (a) Barton, D. H. R.; Crich, D.; Motherwell, W. B. *J. Chem. Soc., Chem. Commun.* **1983**, 939. (b) Barton, D. H. R.; Crich, D.; Motherwell, W. D. *Tetrahedron* **1985**, *41*, 3901. (c) Barton, D. H. R.; Hervé, Y.; Potier, P.; Thierry, J. *Tetrahedron* **1987**, *43*, 4297. (d) Ahmad-Junan, S. A.; Whiting, P. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1160. (e) Crich, D.; Lim, L. B. L. *J. Chem. Soc., Perkin Trans. I* **1991**, 2205. (f) Crich, D.; Lim, L. B. L. *J. Chem. Soc., Perkin Trans. I* **1991**, 2209.
(26) (a) Unger, F. M.; Stix, D.; Schulz, G. *Carbohydr. Res.* **1980**, *80*, 191. (b) Nakamoto, S.; Achiwa, K. *Chem. Pharm. Bull.* **1987**, *35*, 4537. (c) Auzanneau, F.-I.; Charon, D.; Szabó, L. *Carbohydr. Res.* **1990**, *201*, 337.
(27) For reviews, see: (a) Giese, B. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 969. (b) Jasperse, C. P.; Curran, D. P.; Fevig, T. L. *Chem. Rev.* **1991**, *91*, 1237.

Scheme II. Decarboxylation of KDO and Sialic Acid



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22

^{a}Reagents: (a) Ac_2O, DMAP/pyridine; (b) Cs_2CO_3, BnBr/DMF; (c) H_2, Pd-C/EtOH; (d) $(COCl)_2$/toluene; (e) **22**, DMAP/pyridine/toluene; (f) *t*-BuSH, $h\nu$; (g) $Me_3N(CH_2)_3N{=}C{=}NEt$ (WSCI)-Cl, **22** (5 equiv), *t*-BuSH, DMAP, Et_3N, $MS4A/CH_2Cl_2$, $h\nu$.

at the anomeric position [–C$^{\bullet}$(OAc)O-type] is rare (only a few related examples [eg. –C$^{\bullet}$(CO_2Me)O-type,[29] –C$^{\bullet}$(CHF_2)O-type[30]] are known), while examples in the case of a simple anomeric radical [–C$^{\bullet}$(H or R)O-type][31] and the one bearing two electron-donating oxygen atoms [–C$^{\bullet}$(OR)$_2$-type][25e,f,32] have been extensively studied. The radical intermediate was formed by the thermal decomposition of the thiohydroxamate **20c** generated in situ from the corresponding acid chloride and **22** in the presence of azobis[isobutyronitrile] (AIBN). The subsequent trapping with tributyltin hydride resulted in only a disappointing (less than 2%) yield of **21**. The yield was, however, dramatically improved to 68% by irradiation with white light in the presence of *tert*-butyl mercaptan.[25b,d-f]

(28) For a review, see: Viehe, H. G.; Janousek, Z.; Merényi, R.; Stella, L. *Acc. Chem. Res.* **1985**, *18*, 148.

(29) (a) Schmidt, R.; Christan, R.; Zbiral, E. *Tetrahedron Lett.* **1988**, *29*, 3643. (b) Myrvold, S.; Reimer, L. M.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 1861.

(30) Motherwell, W. B.; Ross, B. C.; Tozer, M. J. *Synlett* **1989**, 68.

(31) (a) Adlington, R. M.; Baldwin, J. E.; Basak, A.; Kozyrod, R. P. *J. Chem. Soc., Chem. Commun.* **1983**, 944. (b) Baumberger, F.; Vasella, A. *Helv. Chim. Acta* **1983**, *66*, 2210. (c) Giese, B.; Dupuis, J. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 622. (d) Giese, B.; Dupuis, J. *Tetrahedron Lett.* **1984**, *25*, 1349. (e) Keck, G. E.; Enholm, E. J.; Yates, J. B.; Wiley, M. R. *Tetrahedron Lett.* **1985**, *41*, 4079. (f) Blattner, R.; Ferrier, R. J.; Renner, R. *J. Chem. Soc., Chem. Commun.* **1987**, 1007. (g) Giese, B.; Hoch, M.; Lamberth, C.; Schmidt, R. R. *Tetrahedron Lett.* **1988**, *29*, 1375. (h) Hart, D. J.; Seely, F. L. *J. Am. Chem. Soc.* **1988**, *110*, 1631. (i) Ghosez, A.; Gobel, T.; Giese, B. *Chem. Ber.* **1988**, *121*, 1807. (j) Barton, D. H. R.; Gero, S. D.; Quiclet-Sire, B.; Samadi, M. *J. Chem. Soc., Chem. Commun.* **1988**, 1372. (k) Araki, Y.; Endo, T.; Tanji, M.; Nagasawa, J.; Ishido, Y. *Tetrahedron Lett.* **1988**, *29*, 351. (l) Giese, B.; Linker, T.; Muhn, R. *Tetrahedron* **1989**, *45*, 935. (m) Lopez, J. C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1989**, *111*, 3450. (n) Haraguchi, K.; Tanaka, H.; Miyasaka, T. *Tetrahedron Lett.* **1990**, *31*, 227. (o) Barton, D. H. R.; Géro, L. F.; Robert-Gero, M.; Quiclet-Sire, B.; Samadi, M. *J. Med. Chem.* **1992**, *35*, 63.

(32) Kahne, D.; Yang, D.; Lim, J. J.; Miller, R.; Paguaga, E. *J. Am. Chem. Soc.* **1988**, *110*, 8716.

The ^{1}H NMR spectrum of **21** clearly shows the exclusive β-anomer (δ 5.75, dd, $J_{1,2eq}$ = 3.0 Hz, $J_{1,2ax}$ = 10.0 Hz, H-1), indicating that the abstraction of a hydrogen atom from *tert*-butyl mercaptan took place at the bottom side of the six-membered ring. The proposed mechanism for the exclusive formation of the β-isomer is as follows. The stable conformer of the radical intermediate which is stabilized by both the electron-donating and electron-withdrawing effects is supposed to be in a plane form as depicted in Scheme II, which allows the maximum interaction between the one-electron p orbital and the lone pair electrons on the adjacent ring oxygen. *tert*-Butyl mercaptan is easily accessible from the bottom side, while the approach from the top side is sterically hindered by the hydrogen and acetoxy groups. This explanation in terms of kinetic control is well matched with the thermodynamic stability of the β-product.

The radical process was also applied to the synthesis of the decarboxylated analog of *N*-acetylneuraminic acid. It turned out, however, that all attempts to synthesize the acyl chloride resulted in a complex mixture, even from the fully protected peracetate form **23a**[33] of sialic acid, because the N*H*Ac proton still has a substantial reactivity toward chlorinating reagents. The direct formation of thiohydroxamate **23b** was also found to be difficult because of the inherent steric hindrance around the carbonyl group in the starting material. Through an extensive examination of the reaction conditions, it was found that the combination of ethyl[(diethylamino)propyl]carbodiimide hydrochloride (WSCI-Cl, 1.5 equiv) and excess **22** (5.0 equiv) worked well for the in situ formation and degradation of the thiohydroxamate, to give **24** (27% yield from **23a**). This condition has the advantage that the reaction can be carried out in one step. The newly formed product was exclusively an α-anomer where the OAc group is located in the equatorial orientation, consistent with the result

(33) Haverkamp, J.; Van Halbeek, H.; Dorland, L.; Vliegenthart, J. F. G.; Pfeil, R.; Schauer, R. *Eur. J. Biochem.* **1982**, *122*, 305.

obtained in the decarboxylation of the KDO derivative.

Conclusion

KDO aldolase-catalyzed condensation has been demonstrated to be effective for the synthesis of KDO and analogs. The reactions are stereospecific with formation of a new *R* stereocenter at C-3 from D-arabinose and related substrates. Decarboxylation of the aldolase products provides a new route to heptose and octose derivatives.

Experimental Section

General Procedures. Optical rotations were measured on a Perkin-Elmer 241 spectrophotometer. UV and visible spectra were recorded on a Beckmann DU-70 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 500 MHz on Bruker AMX-400 and AMX-500 spectrometers. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer under fast-atom bombardment (FAB) conditions. Column chromatography was carried out with silica gel of 70–230 mesh. Preparative TLC was carried out on Merck Art. 5744 (0.5 mm).

Isolation of the Microorganism. *A. barkerei* containing high levels of KDO aldolases was selected with the S medium containing 0.25% of the synthetic KDO mixture[3e,k] as the carbon source (20 mL) in serum bottles (158 mL) and incubated at 37 °C for 2 days with shaking (250 rpm). The bottles which showed turbidity were transferred to the same fresh medium. After several transfers, the cultures were plated on the S medium agar plates (1.5% agar) containing 0.25% of the synthetic KDO mixture. The isolated colonies were transferred to the liquid medium as described above. To confirm the utilization of KDO, the disappearance in the medium was monitored by TLC as described in the synthesis of KDO. The cultures which showed the utilization of KDO were harvested by centrifugation and resuspended in 50 mM phosphate buffer (pH 7.0). The cell suspension was incubated with 1% (w/v) of authentic KDO (from Sigma) at 37 °C overnight to confirm the degradation of KDO by TLC. The cultures were then replated on LB agar plates to ensure the purity of the culture.

Preparation of the Enzyme. The incubation was carried out according to the reported procedure with a slight modification.[11] The ingredients of the medium were as follows: NH_4Cl (5 g), K_2SO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (200 mg), $CaCl_2$ (20 mg), $FeSO_4 \cdot 7H_2O$ (1 mg), yeast extract (1 g), $Na_2HPO_4 \cdot 7H_2O$ (10 g), and KH_2PO_4 (3 g) in distilled water (1 L), at pH 7.2. To 50 mL of this medium in a 100-mL Erlenmeyer flask were added D-glucose (40% solution in water, 25 μL) and KDO[3e,k] (100 mg, 0.2%), and a loopful of *A. barkerei* KDO-37-2 was inoculated. The flask was shaken at 250 rpm on a gyrorotary shaker at 30 °C for 16 h. The seed culture thus obtained was poured into 1950 mL of the same incubation medium containing KDO (3.9 g). The mixture was divided and poured into two 2.8-L Erlenmeyer flasks. The flasks were shaken at 250 rpm at 30 °C for 24 h. The growth of microorganisms was estimated by OD at 600 nm to be 1.90. The cells were harvested at 10 000 × g for 30 min at 4 °C and washed with 50 mM potassium sodium phosphate buffer (pH 7.5). The collected cells were then resuspended in the same buffer solution (20 mL) and disrupted by French-pressure apparatus (at 16 000 lb/in.). The cell debris was removed by centrifugation at 23 000 × g for 1 h at 4 °C to give the supernatant (ca. 20 mL) as the crude enzyme preparation. The enzyme activity was determined to be 1.45 units/mL for the degradation of KDO.[13] Ammonium sulfate precipitation between 45% and 75% saturation was collected and dialyzed in phosphate buffer (2 L; 100 mM containing 1 mM of dithiothreitol) to give partially purified enzyme (13.5 mL, 1.73 units/mL for KDO degradation).[5m]

Kinetic Measurements. The rates for aldolase-catalyzed reactions were obtained by measuring the amount of remaining pyruvate.[5m] The reactions were carried out in 0.1 M phosphate buffer (pH 7.5) containing varied concentrations of pyruvate (2.0, 3.33, 5, and 10 mM) and varied concentrations of D-arabinose (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 aliquot (25–100 μL) was withdrawn and mixed with an assay solution (1.4 mL) containing 0.1 M phosphate (pH 7.5) buffer, 0.3 mM NADH, and 20–30 units of L-lactate dehydrogenase. The decrease in absorbance at 340 nm was measured and converted into the amount of the unreacted pyruvate using 6220 M^{-1} cm^{-1} for the molecular absorbance of NADH. The kinetic parameters were obtained from the Lineweaver–Burk plots.

For the relative rate measurements, the concentrations of pyruvate (fluoropyruvate) and sugar were fixed at 10 mM and 0.5 M, respectively. Other conditions were the same as above.

Ammonium 3-Deoxy-α-D-*manno*-2-octulosonate Monohydrate (KDO Ammonium Salt Monohydrate, 1). D-Arabinose (250 mg, 1.67 mmol), sodium pyruvate (1.83 g, 16.7 mmol), dithiothreitol (1.5 mg), NaN_3 (2% solution in water, 100 μL), $NaHPO_4 \cdot 7H_2O$ (53 mg), and KH_2PO_4 (13 mg) were added to the KDO aldolase (5.1 units, 10 mL). The pH was adjusted to 7.5, and the mixture was stirred under N_2 at 30 °C for 3 days. The product was purified by treatment with a Dowex-1 resin column (bicarbonate form) eluted with a linear gradient of from 0 to 0.25 M ammonium bicarbonate. KDO ammonium salt was further purified by a BioGel P-2 column. The fraction eluted with H_2O containing KDO was collected and its total amount was estimated to be 1.11 mmol (67%) by Aminoff's assay.[13] The residue after lyophilization was recrystallized from aqueous ethanol to give colorless plates (168 mg, 37% from D-arabinose): mp 123–125 ° C dec (lit.[3b] mp 121–123 °C, authentic sample from Sigma mp 123–125 °C dec); $[\alpha]^{26}_D$ +40.3° (*c* 2.06, water) [lit.[3b] $[\alpha]^{27}_D$ +42.3° (*c* 1.7, water), authentic sample from Sigma $[\alpha]^{26}_D$ +40.2° (*c* 2.03, water)]. Its ^{1}H NMR spectrum in D_2O was identical with that of an authentic sample: ^{1}H-NMR (D_2O) for α-pyranose form (64.1%) δ 1.863 (H-3eq $J_{3eq,3ax}$ = 13.0 Hz, $J_{3eq,4}$ = 5.5 Hz, $J_{3eq,5}$ = 1.0 Hz), 1.951 (H-3ax, $J_{3ax,4}$ = 12.0 Hz); for β-pyranose form (6.9%) δ 2.373 (H-3eq, $J_{3eq,3ax}$ = 11.7 Hz, $J_{3eq,4}$ = 5.0 Hz, $J_{3eq,5}$ = 1.0 Hz), 1.735 (H-3ax, $J_{3ax,4}$ = 11.7 Hz); for furanose form (19.5%) δ 2.275 (H-3eq, $J_{3eq,3ax}$ = 13.5 Hz, J_{3eq4} = 3.0 Hz), 2.351 (H-3ax, $J_{3ax,4}$ = 7.5 Hz); for lactone form (9.5%) δ 2.053 (H-3eq, $J_{3eq,3ax}$ = 14.0 Hz, $J_{3eq,4}$ = 3.0 Hz), 2.562 (H-3ax, $J_{3ax,4}$ = 7.5 Hz). A small portion was converted to pentaacetate methyl ester derivative **2**: ^{1}H NMR ($CDCl_3$) δ 1.994 (3 H, s, acetyl), 1.998 (3 H, s, acetyl), 2.045 (3 H, s acetyl), 2.108 (3 H, s, acetyl), 2.139 (3 H, s, acetyl), 2.201 (1 H, dd, $J_{3ax,4}$ = 12.0 Hz, $J_{3ax,3eq}$ = 13.0 Hz, H-3ax), 2.245 (1 H, dd, $J_{3eq,4}$ = 6.0 Hz, $J_{3eq,3ax}$ = 13.0 Hz, H-3eq), 3.810 (3 H, s, $COOCH_3$), 4.113 (1 H, dd, $J_{8',7}$ = 12.5 Hz, $J_{8',8}$ = 12.5 Hz, H-8'), 4.173 (1 H, dd, $J_{6,5}$ = 1.3 Hz, $J_{6,7}$ = 9.5 Hz, H-6), 4.475 (1 H, dd, $J_{8,7}$ = 4.0 Hz, $J_{8,8'}$ = 12.5 Hz, H-8), 5.220 (1 H, ddd, $J_{7,8}$ = 4.0 Hz, $J_{7,6}$ = 9.5 Hz, $J_{7,8'}$ = 12.5 Hz, H-7), 5.322 (1 H, ddd, $J_{4,5}$ = 3.0 Hz, $J_{4,3eq}$ = 6.0 Hz, $J_{4,3eq}$ = 6.0 Hz, $J_{4,3ax}$ = 12.0 Hz, H-4), 5.385 (1 H, dd, $J_{5,6}$ = 1.3 Hz, $J_{5,4}$ = 3.0 Hz, H-5). The ^{1}H NMR spectrum was in good accordance with that reported previously.[2b]

Methyl 2,4,5,7,8-Penta-*O*-acetyl-3-deoxy-α-D-*altro*-2-octulosonate (4). In the same manner as described for the preparation of **1**, the product **3** (as ammonium salt) was prepared from D-ribose (0.33 mmol): ^{1}H NMR (D_2O) δ 1.773 (1 H, dd, $J_{3ax,4}$ = 11.9 Hz, $J_{3ax,3eq}$ = 13.0 Hz, H-3ax), 2.148 (1 H, dd, $J_{3eq,4}$ = 5.1 Hz, $J_{3eq,3ax}$ = 13.0 Hz, H-3eq), 3.500 (1 H, dd, $J_{5,4}$ = 9.1 Hz, $J_{5,6}$ = 10.0 Hz, H-5), 3.745 (1 H, dd, $J_{8,7}$ = 7.3 Hz, $J_{8,8'}$ = 12.1 Hz, H-8), 3.789 (1 H, dd, $J_{8',7}$ = 3.7 Hz, $J_{8',8}$ = 12.1 Hz, H-8'), 3.809 (1 H, dd, $J_{6,7}$ = 2.8 Hz, $J_{6,5}$ = 10.0 Hz, H-6), 3.901 (1 H, ddd, $J_{4,3eq}$ = 5.1 Hz, $J_{4,5}$ = 9.1 Hz, $J_{4,3ax}$ = 11.9 Hz, H-4), 4.004 (1 H, dd, $J_{7,6}$ = 2.8 Hz, $J_{7,8'}$ = 3.7 Hz, $J_{7,8}$ = 7.3 Hz, H-7). This was converted to **4** by the successive treatment with acetic anhydride/pyridine/DMAP (see also the preparation of **20b**) and etherial diazomethane solution. The product was purified with silica gel preparative TLC to afford **4** (87.7 mg, 57% from D-ribose) as an oil: $[\alpha]^{25}_D$ +70.9° (*c* 0.81, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.010 (1 H, dd, $J_{3ax,4}$ = 11.6 Hz, $J_{3ax,3eq}$ = 13.5 Hz, H-3ax), 2.030 (3 H, s, acetyl), 2.050 (3 H, s, acetyl), 2.064 (3 H, s, acetyl), 2.105 (3 H, s, acetyl), 2.154 (3 H, s, acetyl), 2.559 (1 H, dd, $J_{3eq,4}$ = 5.2 Hz, $J_{3eq,3ax}$ = 13.5 Hz, H-3eq), 3.793 (3 H, s, $COOCH_3$), 4.084 (1 H, dd, $J_{6,7}$ = 3.2 Hz, $J_{6,5}$ = 10.3 Hz, H-6), 4.241 (1 H, dd, $J_{8,7}$ = 7.0 Hz, $J_{8,8'}$ = 12.0 Hz, H-8), 4.415 (1 H, dd, $J_{8',7}$ = 4.0 Hz, $J_{8',8}$ = 12.0 Hz, H-8'), 5.110 (1 H, dd, $J_{5,4}$ = 9.3 Hz, $J_{5,6}$ = 10.3 Hz, H-5), 5.169 (1 H, ddd, $J_{7,6}$ = 3.2 Hz, $J_{7,8'}$ = 4.0 Hz, $J_{7,8}$ = 7.0 Hz, H-7), 5.271 (1 H, ddd, $J_{4,3eq}$ = 5.2 Hz, $J_{4,5}$ = 9.3 Hz, $J_{4,3ax}$ = 11.6 Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 20.52, 20.56, 20.56, 20.67, 20.67, 35.47, 53.12, 61.23, 68.33, 68.96, 69.85, 71.98, 96.66, 166.21, 167.94, 169.52, 169.85, 169.89, 170.38. HRMS (M + Cs^+) calcd $C_{19}H_{26}O_{13}Cs$ 595.0428, found 595.0428.

Methyl 2,4,7,8-Tetra-*O*-acetyl-3,5-dideoxy-α-D-*manno*-2-octulosonate (6). In the same manner as **3**, the product **5** (as ammonium salt) was prepared from 2-deoxy-D-ribose (0.33 mmol): ^{1}H NMR (D_2O) δ 1.400 (1 H, ddd, $J_{5ax,4}$ = 11.9 Hz, $J_{5ax,6}$ = 11.9 Hz, $J_{5ax,5eq}$ = 12.3 Hz, H-5ax), 1.591 (1 H, dd, $J_{3ax,4}$ = 12.1 Hz, $J_{3ax,3eq}$ = 12.7 Hz, H-3ax), 2.009 (1 H, dddd, $J_{5eq,3eq}$ = 1.8 Hz, $J_{5eq,6}$ = 2.2 Hz, $J_{5eq,4}$ = 4.6 Hz, $J_{5eq,5ax}$ = 12.3 Hz, H-5eq), 2.094 (1 H, ddd, $J_{3eq,5eq}$ = 1.8 Hz, $J_{3eq,4}$ = 4.6 Hz, $J_{3eq,3ax}$ = 12.7 Hz, H-3eq), 3.398 (1 H, dd, $J_{8,7}$ = 7.1 Hz, $J_{8,8'}$ = 11.8 Hz, H-8), 3.588 (1 H, dd, $J_{8',7}$ = 4.1 Hz, $J_{8',8}$ = 11.8 Hz, H-8'), 3.786 (1 H, ddd, $J_{7,8'}$ = 4.1 Hz, $J_{7,6}$ = 4.6 Hz, $J_{7,8}$ = 7.1 Hz, H-7), 3.945 (1 H, ddd, $J_{6,5eq}$ = 2.2 Hz, $J_{6,7}$ = 4.6 Hz, $J_{6,5ax}$ = 11.9 Hz, H-6), 4.112 (1 H, dddd, $J_{4,3eq}$ = 4.6 Hz, $J_{4,5eq}$ = 4.6 Hz, $J_{4,5ax}$ = 11.9 Hz, $J_{4,3ax}$ = 12.1 Hz, H-4). This was converted to **6** (62.2 mg, 47% from 2-deoxy-D-ribose): $[\alpha]^{25}_D$ +86.0° (*c* 0.56, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.488 (1 H, ddd, $J_{5ax,4}$ = 12.0 Hz, $J_{5ax,6}$ = 12.0 Hz, $J_{5ax,5eq}$ = 12.7 Hz, H-5ax), 1.783 (1 H, dd, $J_{3ax,4}$ = 11.6 Hz, $J_{3ax,3eq}$ = 13.1 Hz, H-3ax), 2.045 (3 H, s, acetyl), 2.054 (3 H, s, acetyl), 2.070 (3 H, s, acetyl), 2.123 (3 H, s, acetyl), 2.177 (1 H, dddd, $J_{5eq,3eq}$ = 1.8 Hz, $J_{5eq,6}$ = 2.2 Hz, $J_{5eq,4}$ = 4.7 Hz, $J_{5eq,5ax}$ = 12.7 Hz, H-5eq), 2.454 (1 H, ddd, $J_{3eq,5eq}$ = 1.8 Hz, $J_{3eq,4}$ = 4.8 Hz, $J_{3eq,3ax}$ = 13.1 Hz, H-3eq), 3.782 (3 H, s, $COOCH_3$), 4.034 (1 H, ddd, $J_{6,5eq}$ = 2.2 Hz, $J_{6,7}$ = 7.6 Hz, $J_{6,5ax}$ = 12.0 Hz, H-6), 4.169 (1 H, dd, $J_{8,7}$ = 5.1 Hz, $J_{8,8'}$ = 12.2 Hz, H-8), 4.457 (1 H, dd, $J_{8',7}$ = 2.8 Hz, $J_{8',8}$ = 12.2 Hz, H-8'),

5.093 (1 H, ddd, $J_{7,8'}$ = 2.8 Hz, $J_{7,8}$ = 5.1 Hz, $J_{7,6}$ = 7.6 Hz, H-7), 5.186 (1 H, dddd, $J_{4,5eq}$ = 4.7 Hz, $J_{4,3eq}$ = 4.8 Hz, $J_{4,3ax}$ = 11.6 Hz, $J_{4,5ax}$ = 12.0 Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 20.56, 20.56, 20.73, 20.96, 32.21, 36.03, 52.96, 61.82, 65.72, 69.00, 71.96, 97.61, 167.02, 167.96, 169.81, 170.06, 170.32. HRMS (M + Cs^+) calcd $C_{17}H_{24}O_{11}Cs$ 537.0373, found 537.0373.

Methyl 2,4,5,7-Tetra-*O*-acetyl-3-deoxy-α-D-*arabino*-2-heptulosonate (8). 7: ^1H NMR (D_2O) δ 1.773 (1 H, dd, $J_{3ax,4}$ = 11.8 Hz, $J_{3ax,3eq}$ = 13.0 Hz, H-3ax), 2.180 (1 H, dd, $J_{3eq,4}$ = 5.1 Hz, $J_{3eq,3ax}$ = 13.0 Hz, H-3eq), 3.433 (1 H, dd, $J_{5,4}$ = 9.2 Hz, $J_{5,6}$ = 9.5 Hz, H-5), 3.744 (1 H, ddd, $J_{6,7}$ = 3.5 Hz, $J_{6,7'}$ = 3.5 Hz, $J_{6,5}$ = 9.5 Hz, H-6), 3.807 (1 H, m, H-7), 3.812 (1 H, m, H-7′), 3.930 (1 H, ddd, $J_{4,3eq}$ = 5.1 Hz, $J_{4,5}$ = 9.2 Hz, $J_{4,3a}$ = 11.8 Hz, H-4). **8** (50.0 mg, 39% from 0.33 mmol of D-erythrose): $[\alpha]^{25}_D$ +54.0° (*c* 0.50, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.034 (3 H, s, acetyl), 2.053 (3 H, s, acetyl), 2.087 (3 H, s, acetyl), 2.087 (1 H, dd, $J_{3ax,4}$ = 11.4 Hz, $J_{3ax,3eq}$ = 13.6 Hz, H-3ax), 2.173 (3 H, s, acetyl), 2.658 (1 H, dd, $J_{3eq,4}$ = 5.2 Hz, $J_{3eq,3ax}$ = 13.6 Hz, H-3eq), 3.808 (3 H, s, $COOCH_3$), 4.058 (1 H, dd, $J_{6,7}$ = 2.3 Hz, $J_{6,7'}$ = 4.3 Hz, $J_{5,6}$ = 10.2 Hz, H-6), 4.100 (1 H, $J_{7,6}$ = 2.3 Hz, $J_{7,7'}$ = 12.4 Hz, H-7), 4.355 (1 H, $J_{7',6}$ = 4.3 Hz, $J_{7',7}$ = 12.4 Hz, H-7′); ^{13}C NMR ($CDCl_3$) δ 20.65, 20.76, 20.76, 20.84, 35.58, 53.31, 61.69, 68.16, 68.37, 71.51, 97.29, 166.41, 168.43, 169.61, 170.13, 170.77. HRMS (M + Cs^+) calcd $C_{16}H_{22}O_{11}Cs$ 523.0216, found 523.0216.

Methyl 2,4,5-Tri-*O*-acetyl-2-keto-3-deoxy-α-D-galactonate (10). 9: ^1H NMR (D_2O) δ 1.795 (1 H, dd, $J_{3ax,4}$ = 11.6 Hz, $J_{3ax,3eq}$ = 13.1 Hz, H-3ax), 2.176 (1 H, dd, $J_{3eq,4}$ = 5.1 Hz, $J_{3eq,3ax}$ = 13.1 Hz, H-3eq), 3.60–3.65 (2 H, m), 3.77–3.91 (2 H, m). **10** (11.0 mg, 11% from 0.33 mmol of D-glyceraldehyde): $[\alpha]^{25}_D$ +31.8° (*c* 1.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.948 (1 H, dd, $J_{3ax,4}$ = 11.2 Hz, $J_{3ax,3eq}$ = 13.5 Hz, H-3ax), 2.055 (3 H, s, acetyl), 2.059 (3 H, s, acetyl), 2.170 (3 H, s, acetyl), 2.618 (1 H, dd, $J_{3eq,4}$ = 5.2 Hz, $J_{3eq,3ax}$ = 13.5 Hz, H-3eq), 3.629 (1 H, dd, $J_{6ax,5}$ = 10.6 Hz, $J_{6ax,6eq}$ = 11.3 Hz, H-6ax), 3.809 (3 H, s, $COOCH_3$), 4.149 (1 H, dd, $J_{6eq,5}$ = 5.7 Hz, $J_{6eq,6ax}$ = 11.3 Hz, H-6eq), 5.049 (1 H, ddd, $J_{5,6eq}$ = 5.7 Hz, $J_{5,4}$ = 9.5 Hz, $J_{5,6ax}$ = 10.6 Hz, H-5), 5.320 (1 H, ddd, $J_{4,3eq}$ = 5.2 Hz, $J_{4,5}$ = 9.5 Hz, $J_{4,3ax}$ = 11.2 Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 20.67, 20.72, 20.89, 35.81, 53.25, 62.17, 67.66, 68.49, 96.80, 166.96, 168.50, 169.84, 170.05. HRMS (M + Cs^+) calcd $C_{13}H_{18}O_9Cs$ 451.0005, found 451.0005.

2,4,7-Tri-*O*-acetyl-3-deoxy-α-D-*lyxo*-2-heptulosonic Acid 1 → 5 Lactone (12). 11: ^1H NMR (D_2O) δ 1.90–1.98 (m, H-3 of the major component, α-pyranose form); a minor pair of H-3 protons from **11′** δ 2.072 (dd, $J_{3,4}$ = 3.1 Hz, $J_{3,3'}$ = 14.2 Hz, H-3), 2.576 (dd, $J_{3',4}$ = 7.3 Hz, $J_{3',3}$ = 14.2 Hz, H-3′); another minor pair of H-3 protons from the furanose form δ 2.301 (dd, *J* = 7.0, 13.4 Hz), 2.384 (dd, *J* = 7.0, 13.4 Hz); 3.60–3.95 (m), 3.95–4.20 (m), 4.48–4.52 (m). **12** (1.9 mg): ^1H NMR ($CDCl_3$) δ 2.096 (3 H, s, acetyl), 2.127 (3 H, s, acetyl), 2.180 (3 H, s, acetyl), 2.339 (1 H, ddd, $J_{3,5}$ = 0.6 Hz, $J_{3,4}$ = 2.4 Hz, $J_{3,3'}$ = 14.9 Hz, H-3), 2.972 (1 H, dd, $J_{3',4}$ = 9.4 Hz, $J_{3',3}$ = 14.9 Hz, H-3′), 4.180 (1 H, ABX type, $J_{6,7}$ = 5.6 Hz, $J_{6,7'}$ = 9.9 Hz, H-6), 4.28–4.35 (2 H, m, ABX type, H-7, H-7′), 4.904 (1 H, d, $J_{5,4}$ = 2.0 Hz, H-5), 5.164 (1 H, ddd, $J_{4,5}$ = 2.0 Hz, $J_{4,3}$ = 2.4 Hz, $J_{4,3'}$ = 9.4 Hz, H-4). HRMS (M + Cs^+) calcd $C_{13}H_{16}O_9Cs$ 448.9849, found 448.9858. **12′**: ^1H-NMR ($CDCl_3$)[16] δ 2.10 ($J_{3ax,3eq}$ = 15.0 Hz, $J_{3eq,4}$ = 2.5 Hz, $J_{3ax,4}$ = 9.0 Hz, H-3eq), 2.80 (H-3ax).

Methyl 2,4,5-Tri-*O*-acetyl-2-keto-3-deoxy-α-L-gluconate (14). **13** (L-KDG): ^1H NMR (D_2O) A major pair of H-3 protons from the α-pyranose form δ 1.873 (dd, $J_{3eq,4}$ = 5.2 Hz, $J_{3eq,3ax}$ = 13.0 Hz, H-3eq), 1.984 (dd, $J_{3ax,4}$ = 11.9 Hz, $J_{3ax,3eq}$ = 13.0 Hz, H-3ax); a minor pair of H-3 protons from the lactone δ 2.051 (dd, $J_{3,4}$ = 3.2 Hz, $J_{3,3'}$ = 14.1 Hz, H-3), 2.521 (dd, $J_{3',4}$ = 7.5 Hz, $J_{3',3}$ = 14.1 Hz, H-3′); a minor H-3 proton ($^2C_5$ β-pyranose form is suggested) δ 2.167 (dd, $J_{3,4}$ = 4.0 Hz, $J_{3,3'}$ = 13.7 Hz), in this case the H-3′ proton could not be specified by overlapping of the signals; another minor pair of H-3 protons from the furanose form δ 2.284 (dd, *J* = 6.4, 13.1 Hz), 2.341 (dd, *J* = 6.4, 13.1 Hz), 3.60–4.10 (m), 4.15–4.20 (m), 4.30–4.40 (m). **14** (2.0 mg): ^1H NMR ($CDCl_3$) δ 2.034 (3 H, s, acetyl), 2.150 (3 H, s, acetyl), 2.152 (3 H, s, acetyl), 2.288 (1 H, d, $J_{3ax,4}$ = 10.1 Hz, H-3ax), 2.292 (1 H, dd, $J_{3eq,5}$ = 0.4 Hz, $J_{3eq,4}$ = 7.0 Hz, H-3eq), 3.830 (3 H, s, $COOCH_3$), 3.999 (1 H, dd, $J_{6eq,5}$ = 1.5 Hz, $J_{6eq,6ax}$ = 13.2 Hz, H-6eq), 4.092 (1 H, dd, $J_{6ax,5}$ = 2.0 Hz, $J_{6ax,6eq}$ = 13.2 Hz, H-6ax), 5.251 (1 H, dddd, $J_{5,3eq}$ = 0.4 Hz, $J_{5,6eq}$ = 1.5 Hz, $J_{5,6ax}$ = 2.0 Hz, $J_{5,4}$ = 2.7 Hz, H-5), 5.313 (1 H, ddd, $J_{4,5}$ = 2.7 Hz, $J_{4,3eq}$ = 7.0 Hz, $J_{4,3ax}$ = 10.1 Hz, H-4). HRMS (M + Na^+) calcd $C_{13}H_{18}O_9Na$ 341.0849, found 341.0849.

Methyl 2,4,5,7,8,9-Hexa-*O*-acetyl-3-deoxy-β-L-*glycero*-L-*galacto*-nonulosonate (16). **15** (L-KDN): ^1H NMR (D_2O) δ 1.773 (1 H, dd, $J_{3ax,4}$ = 11.8 Hz, $J_{3ax,3eq}$ = 12.9 Hz, H-3ax), 2.168 (1 H, dd, $J_{3eq,4}$ = 5.1 Hz, $J_{3eq,3ax}$ = 11.8 Hz, H-3eq), 3.579 (1 H, dd, $J_{5,4}$ = 9.3 Hz, $J_{5,6}$ = 9.9 Hz, H-5), 3.654 (1 H, dd, $J_{9,8}$ = 6.3 Hz, $J_{9,9'}$ = 11.8 Hz, H-9), 3.766 (1 H, ddd, $J_{8,9'}$ = 2.6 Hz, $J_{8,9}$ = 6.3 Hz, $J_{8,7}$ = 9.0 Hz, H-8), 3.831 (1 H, dd, $J_{7,6}$ = 1.1 Hz, $J_{7,8}$ = 9.0 Hz, H-7), 3.873 (1 H, dd, $J_{9',8}$ = 2.6 Hz, $J_{9',9}$ = 11.8 Hz, H-9′), 3.925 (1 H, dd, $J_{6,7}$ = 1.1 Hz, $J_{6,5}$ = 9.9 Hz, H-6), 3.971 (1 H, ddd, $J_{4,3eq}$ = 5.1 Hz, $J_{4,5}$ = 9.3 Hz, $J_{4,3ax}$ = 11.8 Hz, H-4). **16** (108.3 mg, 61% from 0.33 mmol of L-mannose): $[\alpha]^{25}_D$ +26.3° (*c* 1.14, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.084 (1 H, dd, $J_{3ax,4}$ = 11.6 Hz, $J_{3ax,3eq}$ = 13.6 Hz, H-3ax), 2.013 (3 H, s, acetyl), 2.024 (3 H, s, acetyl), 2.040 (3 H, s, acetyl), 2.069 (3 H, s, acetyl), 2.115 (3 H, s, acetyl), 2.157 (3 H, s, acetyl), 2.625 (1 H, dd, $J_{3eq,4}$ = 5.3 Hz, $J_{3eq,3ax}$ = 13.6 Hz, H-3eq), 3.790 (3 H, s, $COOCH_3$), 4.141 (1 H, dd, $J_{9,8}$ = 5.8 Hz, $J_{9,9'}$ = 12.6 Hz, H-9), 4.186 (1 H, dd, $J_{6,7}$ = 2.3 Hz, $J_{6,5}$ = 10.3 Hz, H-6), 4.440 (1 H, dd, $J_{9',8}$ = 2.5 Hz, $J_{9',9}$ = 12.6 Hz, H-9′), 4.975 (1 H, dd, $J_{5,4}$ = 9.6 Hz, $J_{5,6}$ = 10.3 Hz, H-5), 5.150 (1 H, ddd, $J_{8,9'}$ = 2.5 Hz, $J_{8,9}$ = 5.8 Hz, $J_{8,7}$ = 6.3 Hz, H-8), 5.264 (1 H, ddd, $J_{4,3eq}$ = 5.3 Hz, $J_{4,5}$ = 9.6 Hz, $J_{4,3ax}$ = 11.6 Hz, H-4), 5.396 (1 H, dd, $J_{7,6}$ = 2.3 Hz, $J_{7,8}$ = 6.3 Hz, H-7); ^{13}C NMR ($CDCl_3$) δ 20.46, 20.48, 20.58, 20.58, 20.67, 35.32, 53.06, 61.67, 66.68, 67.21, 68.57, 70.00, 71.27, 97.14, 165.91, 168.03, 169.46, 169.57, 169.80, 169.96, 170.41. HRMS (M + Cs^+) calcd $C_{22}H_{30}O_{15}Cs$ 667.0639, found 667.0639. **16′**: $[\alpha]^{25}_D$ −26.0° (*c* 1.00, $CHCl_3$). The ^1H NMR spectrum was identical with that of **16**.

2-Deoxy-2-fluoro-D-arabinose (17b). To a solution of tribenzoate **17a** (available from Pfanstiehl Co., 500 mg, 1.08 mmol) in ethanol (5 mL) was added 10 N NaOH aqueous solution (485 μL, 1.5 equiv for each OBz group, total of 4.5 equiv) at room temperature. After 15 min, H_2O (10 mL) and ethanol (5 mL) were added and the mixture was stirred and heated to 50 °C to dissolve the precipitated sodium benzoate. The mixture was further stirred for 1 h at room temperature. After the ethanol was evaporated in vacuo, the residue was dissolved in H_2O and Dowex 50W-X8 (H^+ form) was added to acidify the mixture. The precipitated benzoic acid was filtered off, and the filtrate was treated with Dowex 1-X8 (HCO_3^- form) filtered, and then concentrated in vacuo to give **17b** as a colorless syrup (153 mg, 94%): ^1H NMR (D_2O) δ 3.60–4.20 (4 H, m), 4.337 (ddd, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 9.3 Hz, $J_{2,F}$ = 51.8 Hz, H-2 of β-anomer), 4.666 (ddd, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 9.5 Hz, $J_{2,F}$ = 49.5 Hz, H-2 of α-anomer), 4.763 (dd, $J_{1,F}$ = 3.3 Hz, $J_{1,2}$ = 7.7 Hz, H-1 of β-anomer), 5.434 (dd, $J_{1,F}$ = 1.5 Hz, $J_{1,2}$ = 3.7 Hz, H-1 of α-anomer). This anomeric mixture was used in the next step without further purification.

Methyl 2,4,7,8-Tetra-*O*-acetyl-3,5-dideoxy-5-fluoro-α-D-*manno*-2-octulosonate (19). **18**: ^1H NMR (D_2O) δ 1.814 (dd, $J_{3ax,3eq}$ = 12.4 Hz, $J_{3ax,4}$ = 12.4 Hz, H-3ax of β-anomer), 1.988 (1 H, ddd, $J_{3eq,5}$ = 0.8 Hz, $J_{3eq,4}$ = 5.6 Hz, $J_{3eq,3ax}$ = 12.9 Hz, H-3eq of α-anomer), 2.058 (1 H, dd, $J_{3ax,4}$ = 11.8 Hz, $J_{3ax,3eq}$ = 12.9 Hz, H-3ax of α-anomer), 2.461 (ddd, $J_{3eq,5}$ = 0.8 Hz, $J_{3eq,4}$ = 5.3 Hz, $J_{3eq,3ax}$ = 12.4 Hz, H-3eq of β-anomer), 3.663 (1 H, dd, $J_{8,7}$ = 5.4 Hz, $J_{8,8'}$ = 12.1 Hz, H-8), 3.828 (1 H, dd, $J_{8',7}$ = 2.4 Hz, $J_{8',8}$ = 12.1 Hz, H-8′), 3.80–3.95 (2 H, m), 4.182 (1 H, dddd, $J_{4,5}$ = 2.4 Hz, $J_{4,3eq}$ = 5.6 Hz, $J_{4,3ax}$ = 11.8 Hz, $J_{4,F}$ = 30.5 Hz, H-4), 4.957 (1 H, ddd, $J_{5,3eq}$ = 0.8 Hz, $J_{5,4}$ = 2.4 Hz, $J_{5,F}$ = 50.9 Hz, H-5). **19** (25.3 mg, 18% from 0.33 mmol of **17b**): $[\alpha]^{25}_D$ +96.4° (*c* 2.53, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.043 (3 H, s, acetyl), 2.067 (3 H, s, acetyl), 2.131 (3 H, s, acetyl), 2.137 (3 H, s, acetyl), 2.271 (1 H, dd, $J_{3ax,4}$ = 11.5 Hz, $J_{3ax,3eq}$ = 13.3 Hz, H-3ax), 2.319 (1 H, dd, $J_{3eq,4}$ = 5.9 Hz, $J_{3eq,3ax}$ = 13.3 Hz, H-3eq), 3.805 (3 H, s, $COOCH_3$), 4.073 (1 H, dd, $J_{6,7}$ = 9.5 Hz, $J_{6,F}$ = 27.8 Hz, H-6), 4.154 (1 H, dd, $J_{8,7}$ = 3.5 Hz, $J_{8,8'}$ = 12.5 Hz, H-8), 4.601 (1 H, dd, $J_{8',7}$ = 2.2 Hz, $J_{8',8}$ = 12.5 Hz, H-8′), 4.827 (1 H, dd, $J_{5,4}$ = 2.1 Hz, $J_{5,F}$ = 50.9 Hz, H-5), 5.240 (1 H, dddd, $J_{4,5}$ = 2.1 Hz, $J_{4,3eq}$ = 5.9 Hz, $J_{4,3ax}$ = 11.5 Hz, $J_{4,F}$ = 21.3 Hz, H-4), 5.288 (1 H, ddd, $J_{7,8'}$ = 2.2 Hz, $J_{7,8}$ = 3.5 Hz, $J_{7,6}$ = 9.5 Hz, H-7); ^{13}C NMR ($CDCl_3$) δ 20.56, 20.56, 20.71, 20.83, 30.60, 53.18, 61.46, 66.45, (d, $J_{C,F}$ = 17.8 Hz), 67.89 (d, $J_{C,F}$ = 4.1 Hz), 69.60 (d, $J_{C,F}$ = 18.2 Hz), 83.02 (d, $J_{C,F}$ = 186.2 Hz), 97.04, 166.49, 167.75, 169.14, 170.18, 170.20. HRMS (M + Cs^+) calcd $C_{17}H_{23}O_{11}FCs$ 555.0279, found 555.0288.

Larger Scale Synthesis of 18. Fluorosugar **17b** (340 mg, 2.25 mmol), sodium pyruvate (2.074 g, 28.9 mmol), dithiothreitol (1.7 mg), NaN_3 (2.3 mg), and phosphate buffer (pH 7.5, 50 mM, 1.12 mL) were added to the enzyme solution (3.0 mL, 24 units). After the pH was adjusted to 7.5, the volume was made up to 10.0 mL. The mixture was stirred under N_2 at room temperature for 7 days. The pH was lowered to 2.5 by addition of Dowex 50W-X8 (H^+ form), and the mixture was kept at 0 °C for 1 h. The precipitate was removed by centrifugation at 23 000 × g for 1 h at 4 °C. Before the anion-exchange-resin treatment, the excess pyruvate was removed as follows. The mixture was diluted to 80 mL, and the pH was adjusted to 6.5 by the addition of 2 N aqueous ammonia solution. The antifoam (Antifoam AF emulsion, Dow-Corning Nakaraitesque, 10% emulsion in water, 0.32 mL) and pyruvate decarboxylase (Sigma P 6810, 0.2 mL, 12.5 units) were added, and the mixture was stirred at room temperature with bubbling of N_2 (1.5 L/min). The pH was monitored and occasionally adjusted between 6.0 and 6.5, by addition of Dowex 50W-X8 (H^+ form). The decarboxylase was periodically added to the mixture (0.2 mL each) at an interval of 30 min, to avoid the denaturation which is caused by the rapid formation of acetaldehyde. The total amount of the enzyme was 3.2 mL (200 units).

The reaction mixture was further stirred overnight. Then the mixture was centrifuged, and the supernatant was diluted to 100 mL and applied to a column of Dowex 1-X8 (20-50 mesh, bicarbonate form, bed volume of 100 mL). The pH of the eluent and washings was readjusted to 5.5, and the resulting solution was again applied to the same column to ensure the adsorption of the desired product. After the column was washed with water, the desired product was eluted with a linear gradient of from 0 to 0.3 M ammonium bicarbonate. The product was further purified on a BioGel P-2 column (bed volume of 20 mL) to give 192 mg (33%) of **18**. The ^{1}H NMR spectrum was identical with that of the sample mentioned above.

Benzyl 2,4,5,7,8-Penta-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulosonate (20b). A suspension of KDO ammonium salt monohydrate (160 mg, 0.59 mmol), acetic anhydride (3 mL), pyridine (3 mL), and 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 2 mg) was stirred overnight at room temperature. Ice-cooled water was added, and the mixture was stirred for 30 min. After dilution with water, the pH of the mixture was adjusted to 3.5 by addition of Dowex 50W-X8 (H^+ form). The resin was filtered off, and the filtrate was concentrated in vacuo. The residue was diluted with a mixture of chloroform and toluene, and the solvent was evaporated. This procedure was repeated three times to remove traces of water. The residue was dissolved in anhydrous DMF. Benzyl bromide (161 mg, 0.94 mmol), Cs_2CO_3 (390 mg, 1.20 mmol), and tetrabutylammonium iodide (33 mg) were added, and the mixture was stirred for 4 h at room temperature under N_2. The mixture was diluted with 0.5 N ice-cooled hydrochloric acid and extracted twice with a mixture of diethyl ether and toluene (1:1). The organic layer was successively washed with water, saturated aqueous $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed over silica gel (20 g). Elution with hexane/diethyl ether (2:1-1:1) afforded **15b**, which was recrystallized from diethyl ether to give 220 mg (70%) as colorless plates: mp 102-103 °C (lit.[26b] mp 98-99 °C); $[\alpha]^{26}_D$ +293° (*c* 1.0, $CHCl_3$) [lit.[26b] $[\alpha]^{25}_D$ +91.9° (*c* 0.9, $CHCl_3$). Its ^{1}H NMR spectrum ($CDCl_3$) was in good accordance with that reported previously. HRMS (M + Na^+) calcd 561.1584, found 561.1602.

2,4,5,7,8-Penta-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulosonic Acid (20a). A mixture of **20b** (220 mg, 0.41 mmol) and Pd-C (10%, 55 mg) in ethanol (3 mL) was vigorously stirred under H_2 at room temperature for 1 h. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was recrystallized from diethyl ether to give **20a** (177 mg, 97%) as fine needles: mp 132-133 °C; $[\alpha]^{25}_D$ +374° (*c* 0.88, $CHCl_3$). Its ^{1}H NMR spectrum (C_6D_6) was identical with that reported previously.[26a]

1,3,4,6,7-Penta-*O*-acetyl-2-deoxy-β-D-*manno*-heptose (21). To a solution of acid chloride prepared from **20a** (30 mg, 0.067 mmol) in toluene was added dropwise a solution of *N*-hydroxythiopyridone (**22**) (11 mg, 0.09 mmol) and DMAP (2 mg) in toluene (0.5 mL) and pyridine (0.3 mL) at room temperature under N_2 in the dark. After the reaction mixture was stirred for 10 min, *tert*-butyl mercaptan (0.5 mL) was added and the mixture was irradiated with white light (tungsten lamp, 100 W) at room temperature. After the reaction mixture was stirred for 10 min, N_2 was introduced to the mixture under a slightly reduced pressure to remove residual *tert*-butyl mercaptan for 30 min. Usual workup and purification by silica gel preparative TLC [developed with hexane/Et_2O (1:1)] afforded **21** (18.5 mg, 68%) as an oil: $[\alpha]^{22}_D$ +36.8° (*c* 1.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.000-2.150 (2 H, m, H-2ax, H-2eq), 2.010 (6 H, s, acetyl), 2.082 (3 H, s, acetyl), 2.119 (3 H, s, acetyl), 2.137 (3 H, s, acetyl), 3.882 (1 H, dd, $J_{5,4}$ = 1.5 Hz, $J_{5,6}$ = 10.0 Hz, H-5), 4.115 (1 H, dd, $J_{7',6}$ = 4.5 Hz, $J_{7',7}$ = 12.5 Hz, H-7′), 4.437 (1 H, dd, $J_{7,6}$ = 2.5 Hz, $J_{7,7'}$ = 12.5 Hz, H-7), 5.073 (1 H, ddd, $J_{3,4}$ = 3.0 Hz, $J_{3,2eq}$ = 5.0 Hz, $J_{3,2ax}$ = 12.5 Hz, H-3), 5.165 (1 H, ddd, $J_{6,7}$ = 2.5 Hz, $J_{6,7'}$ = 4.5 Hz, $J_{6,5}$ = 10.0 Hz, H-6), 5.303 (1 H, dd, $J_{4,5}$ = 1.5 Hz, $J_{4,3}$ = 3.0 Hz, H-4), 5.748 (1 H, dd, $J_{1,2eq}$ = 3.0 Hz, $J_{1,2ax}$ = 10.0 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 20.59, 20.59, 20.65, 20.65, 20.84, 30.35, 62.26, 63.84, 67.32, 67.90, 71.62, 91.67, 168.60, 169.60, 169.83, 170.30, 170.54. HRMS (M + Cs^+) calcd $C_{17}H_{24}O_{11}Cs$ 537.0373, found 537.0359.

4-Acetamido-1,3,6,7,8-penta-*O*-acetyl-2,4-dideoxy-α-D-*glycero*-D-*galacto*-octose (24). A 25-mL two-necked flask equipped with a septum, a micro-scale Dean-Stark trap which was filled with 4-Å molecular sieves, and a reflux condenser was used as the reaction vessel. A mixture of **23a** (35.0 mg, 0.07 mmol), DMAP (12.3 mg, 1.5 equiv), **22** (41.0 mg, 5.0 equiv), and triethylamine (19 μL) in CH_2Cl_2 (1 mL) was placed in the flask described above. To this was successively added a solution of WSCI-Cl (20 mg) in CH_2Cl_2 (1 mL) and *tert*-butyl mercaptan (0.5 mL). The mixture was stirred and irradiated with white light (tungsten lamp, 100 W) at room temperature for 5 h. The reaction was worked up in a similar manner as described above. The crude product was purified by silica gel preparative TLC [developed with ethyl acetate/tetrahydrofuran (1:1)] to give **24** (8.7 mg, 27% from **23a**) as an oil: $[\alpha]^{22}_D$ +21.3° (*c* 2.87, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.908 (3 H, s, *N*-acetyl), 1.915 (1 H, ddd, $J_{2ax,1}$ = 10.3 Hz, $J_{2ax,3}$ = 11.5 Hz, $J_{2ax,2eq}$ = 12.4 Hz, H-2ax), 2.043 (3 H, s, *O*-acetyl), 2.051 (3 H, s, *O*-acetyl), 2.102 (3 H, s, *O*-acetyl), 2.107 (3 H, s, *O*-acetyl), 2.134 (3 H, s, *O*-acetyl), 2.219 (1 H, ddd, $J_{2eq,1}$ = 2.1 Hz, $J_{2eq,3}$ = 4.9 Hz, $J_{2eq,2ax}$ = 12.4 Hz, H-2eq), 3.764 (1 H, dd, $J_{5,6}$ = 2.4 Hz, $J_{5,4}$ = 10.4 Hz, H-5), 4.023 (1 H, dd, $J_{8,7}$ = 5.5 Hz, $J_{8,8'}$ = 12.6 Hz, H-8), 4.062 (1 H, ddd, $J_{4,NH}$ = 10.0 Hz, $J_{4,3}$ = 10.3 Hz, $J_{4,5}$ = 10.4 Hz, H-4), 4.389 (1 H, dd, $J_{8',7}$ = 2.6 Hz, $J_{8',8}$ = 12.6 Hz, H-8′), 5.127 (1 H, ddd, $J_{7,8'}$ = 2.6 Hz, $J_{7,8}$ = 5.5 Hz, $J_{7,6}$ = 7.3 Hz, H-7), 5.058 (1 H, ddd, $J_{3,2eq}$ = 4.9 Hz, $J_{3,4}$ = 10.3 Hz, $J_{3,2ax}$ = 11.5 Hz, H-3), 5.190 (1 H, d, $J_{NH,4}$ = 10.0 Hz, NH), 5.391 (1 H, dd, $J_{6,7}$ = 7.3 Hz, $J_{6,5}$ = 2.4 Hz, H-6), 5.646 (1 H, dd, $J_{1,2eq}$ = 2.1 Hz, $J_{1,2ax}$ = 10.3 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 20.70, 20.70, 20.75, 20.83, 20.83, 23.15, 35.09, 49.22, 61.98, 67.11, 70.23, 70.23, 73.67, 91.19, 168.75, 169.90, 170.12, 170.36, 170.59, 170.88. HRMS (M + Cs^+) calcd $C_{20}H_{29}O_{12}NCs$ 608.0744, found 608.0750.

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The Endocyclic Restriction Test: Determination of the Transition-Structure Geometry for the Transfer of Oxygen from *N*,*N*-Dialkylhydroxylamines to Triarylphosphines

Mitchell L. Kurtzweil, Dekai Loo, and Peter Beak*

Contribution from the Department of Chemistry, University of Illinois, Urbana, Illinois 61801.
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Abstract: The transfers of oxygen from nitrogen to phosphorus in the conversions of **1** to **2** and **3** to **4** are shown by kinetic, solvent-labeling, and double-labeling criteria to be intramolecular reactions. This information in conjunction with the stabilities of **13** and **14** is taken to rule out the mechanisms of classic linear S_N2 substitutions at oxygen or nitrogen, biphilic insertion, or a radical chain reaction and to favor reactions via a 10-P-5 species (**18**). These results appear to provide the first experimental demonstration that oxygen can be transferred at an oblique angle.

Steps in which an oxygen atom is transferred to phosphorus are involved in a number of reactions, including deoxygenations of a variety of substrates.[1] Our interest in evaluating the geometries of substitutions at heteroatoms by use of the endocyclic

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