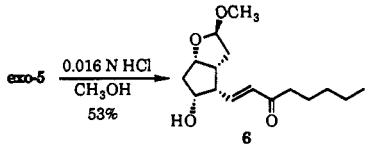
Enone **5** is readily converted to the isomerically pure *exo*-methoxy alcohol **6** upon treatment with 0.016 N HCl in methanol for 3 days at room temperature (eq 5). Since this alcohol has

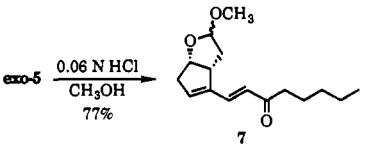


(5)

6

been prepared previously by a biosynthetic approach[12] and subsequently epimerized to the corresponding β-enone, which has been carried on to $PGF_{2\alpha}$ in three routine subsequent steps, this synthesis constitutes one of the more efficient chiral approaches to $PGF_{2\alpha}$.

Enone **5** is also readily converted to a mixture of diastereomeric dienones **7** in 77% yield when treated with HCl in methanol for 3 days (eq 6). Enone **6** also gives dienone **7** in over 90% yield



(6)

7

when treated with 75 and 25 equiv of acetic acid and morpholine, respectively, in 2:1 DME/H_2O for 72 h at 70 °C. Dienone **7** should prove particularly valuable in the synthesis of the C prostaglandins.[13]

Since 12-*epi*-$PGF_{2\alpha}$ (**10**) has previously only been synthesized by a rather tedious process[14] or via a side product arising during the synthesis of $PGF_{2\alpha}$,[15] and apparently nothing is known about its pharmacological properties, we elected to complete its synthesis using readily available enone **5** (Scheme II). Reduction with (*S*)-BINAL-H[16] affords a single product assigned structure **8** in analogy with previous such reductions. Hydrolysis proceeded in 77% yield. The attempted Wittig reaction using sodium dimsyl[17] or KO-*t*-Bu[18] to generate the ylide proved unsuccessful. However, the use of potassium hexamethyldisilazide[10] afforded 12-*epi*-$PGF_{2\alpha}$ (**10**) in 54% yield. Unfortunately, 12-*epi*-$PGF_{2\alpha}$ exhibited limited activity toward blood platelet aggregation ($I_{50} > 1000$ μM against ADP-induced aggregation and $I_{50} = 179$ μM against arachidonic acid induced aggregation).

In conclusion, the controlled, palladium-promoted, one-step, intermolecular insertion of three different alkenes affords a highly efficient synthesis of compound **5**, a valuable intermediate in the formal synthesis of $PGF_{2\alpha}$. This same intermediate affords 12-*epi*-$PGF_{2\alpha}$ in only four steps and 21% overall yield from the readily available chiral starting material **1**.

Acknowledgment. We gratefully acknowledge the National Institutes of Health and the American Heart Association—Iowa Affiliate for financial support; Bristol-Myers Squibb for biological testing; and Johnson Matthey, Inc., and Kawaken Fine Chemicals Co., Ltd., for the palladium acetate.

Supplementary Material Available: Procedures for the synthesis of compounds **5–9** and appropriate spectral data (6 pages). Ordering information is given on any current masthead page.

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A Complete Change of Stereoselectivity in Sialic Acid Aldolase Reactions: A Novel Synthetic Route to the KDO Type of Nine-Carbon L Sugars[1]

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A major synthetic value of enzyme catalysis is its predictable stereoselectivity.[2] A change of stereoselectivity, though very unusual, may occur, however, with different substrate structures,[3] temperatures,[4] or solvents.[5] These selectivity changes are often not very significant, with some exceptions[3] where the enantioselectivity is inverted. In the case of enzymatic aldol reactions, the diastereofacial selectivity for the aldehyde component is often consistent and completely controlled by the enzyme as documented by numerous reactions catalyzed by fructose-1,6-diphosphate aldolase[6] and *N*-acetylneuraminic acid (or sialic acid) aldolase[7] (EC 4.1.3.3). We report here a complete reversal of stereoselectivity in the sialic acid aldolase catalyzed reactions of pyruvate with L-mannose and with 6-deoxy-L-mannose (L-rhamnose) (Scheme I).

NeuAc aldolase is a type I aldolase forming an enamine intermediate with pyruvate, which reversibly reacts with the second substrate *N*-acetylmannosamine to give NeuAc.[8] The enzyme accepts many aldoses as acceptor substrates. In all reactions, the enamine intermediate approaches the *si* face of the incoming aldehyde substrate to form a new stereogenic center of *S* configuration.[7] In the reaction with L-mannose or 6-deoxy-L-mannose (L-rhamnose), however, a single product with a new stereogenic center of *R* configuration generated via *re* face attack was obtained in each case in >80% yield. Both products adopt a $^5C_2$ conformation as indicated by the adjacent transaxial coupling of protons at positions 3, 4, and 5. The enzyme products have the same NMR

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(6) In most cases, the "D" isomer of an α-substituted aldehyde reacts faster than the L isomer, both with *si*-facial selectivity. The Cram–Felkin mode[9] of attack on the "D" aldehyde is therefore proposed for the transition state. Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365. Bednarski, M. D.; Simon, E. S.; Bischefberger, N.; Tessner, W. D.; Kim, M. J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 627. Straub, A.; Effenberger, F.; Fischer, P. *J. Org. Chem.* **1990**, *55*, 3926. Durrwachter, J. R.; Drueckhammer, D. G.; Nozaki, K.; Sweers, H. M.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175. 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. Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, A. J., Jr.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187.
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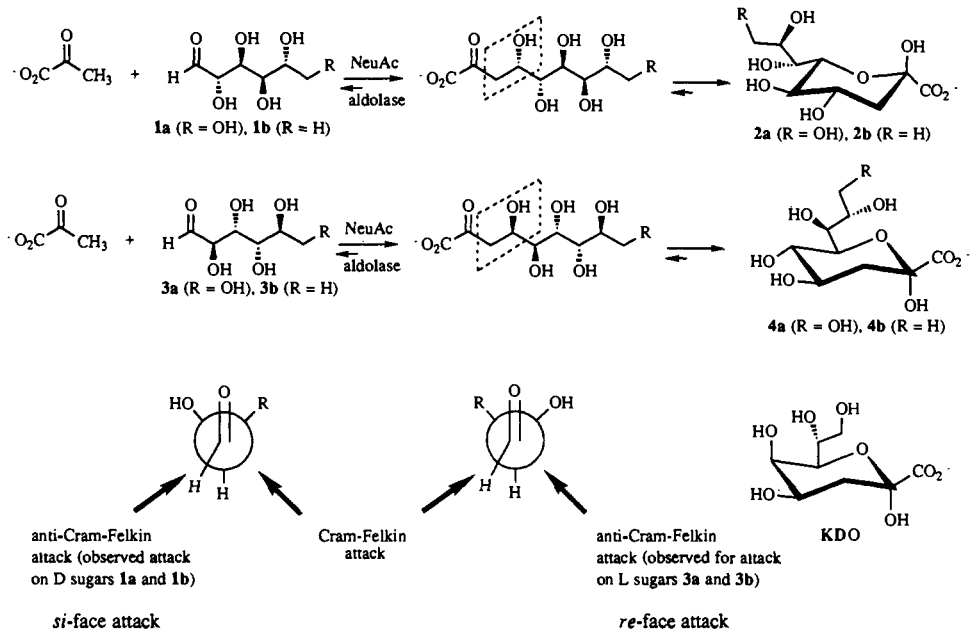
Scheme I



spectra (^1H and ^{13}C) but opposite specific rotations compared to those from the corresponding D substrates (Figure 1). The specific activities for both L sugars are approximately the same as those for their enantiomers (~25 units/mg). These syntheses demonstrate for the first time a complete change of stereoselectivity in enzymatic aldol reactions. In both the D and L sugars, an anti-Cram–Felkin[9] attack on the aldehyde component was observed (Figure 1, bottom).

In a typical experiment, a 0.1 M solution of sugar (1 mmol) in a 0.05 M potassium phosphate buffer, pH 7.2, containing 0.01 M dithiothreitol, sodium pyruvate (3 equiv), and 10 units of NeuAc aldolase was incubated at 37 °C (total volume = 10 mL) for 2 days. The reaction was monitored by TLC (PrOH/water = 7:3 v/v). The product was isolated by anion-exchange chromatography on Dowex 1X8-100 ($HCOO^-$ form; 30 × 2 cm) using a gradient of formic acid (0 → 2 M) as eluant. Fractions containing the product were pooled and freeze-dried.[10]

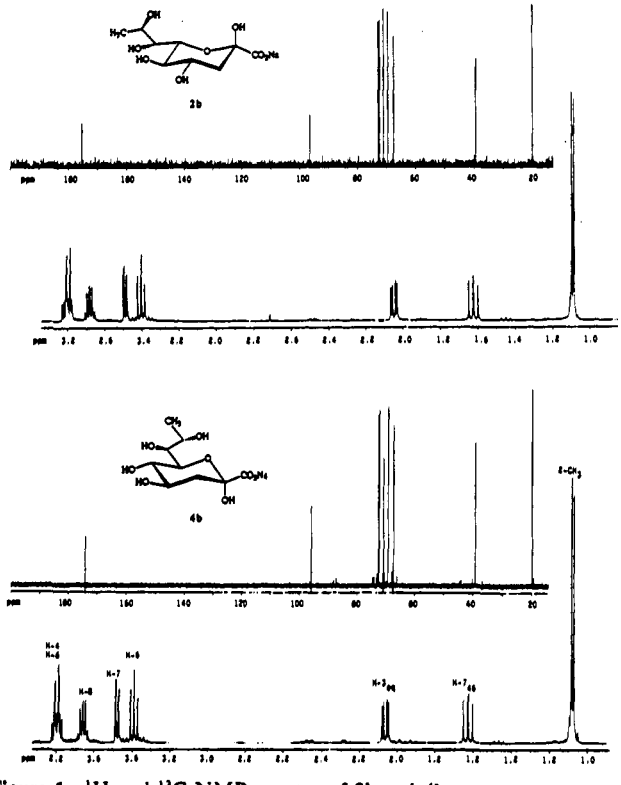


Figure 1. ^1H and ^{13}C NMR spectra of **2b** and **4b**.

These enzyme products have the KDO type of structure and may be useful for the development of compounds as inhibitors of KDO-utilizing enzymes.[11] The unusual stereoselectivity ob-

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(10) 3,9-Dideoxy-L-glycero-L-galactononulosonic acid (L-9-deoxy-KDN) (**4b**): yield 200 mg (80%); ^1H NMR (500 MHz, D_2O) δ 1.08 (d, $J_{8,CH3}$ = 6.5 Hz, CH_3), 1.62 (dd, $J_{3ax,3eq}$ = 13.3 Hz, $J_{3ax,4}$ = 11.5 Hz, H_{3ax}), 2.06 (dd, $J_{3eq,4}$ = 6.7 Hz, H_{3eq}), 3.39 (t, $J_{4,5}$ = $J_{5,6}$ = 9.5 Hz, H-5), 3.675 (dd, $J_{6,7}$ = 0.8 Hz, $J_{7,8}$ = 8.2 Hz, H-7), 3.66 (dd, H-8), 3.8 (dd, H-6), 3.76–3.83 (ddd, H-4); ^{13}C NMR (125 MHz, reference CH_3CN 1.6), 175.3800 (C-1), 96.1195 (C-2), 72.7438 (C-8), 72.3426 (C-6), 70.9565 (C-5), 69.3967 (C-7), 67.4548 (C-4), 39.3253 (C-3), 19.8777 (CH_3); $[\alpha]^{20}_D$ +60° (*c* 1.2, water); HRMS calcd for $C_9H_{15}O_8$ 253.0923, found 253.0923. 3,9-Dideoxy-D-glycero-D-galactononulosonic acid (D-9-deoxy-KDN) (**2b**) was prepared similarly from **1b** (Haskins, W. T.; Hann, R. M.; Hudson, C. S. *J. Am. Chem. Soc.* **1946**, *68*, 628). The physical data (^1H and ^{13}C NMR and HRMS) were identical to those of **4b** except for the specific rotation $[\alpha]^{20}_D$ −60° (*c* 1.2, H_2O)]. In an analogous manner, D- and L-KDN were prepared from D-mannose (**1a**) and L-mannose (**3a**), respectively, and the physical data were identical to the reported values for D-KDN,[7c] again with opposite specific rotation for L-KDN.

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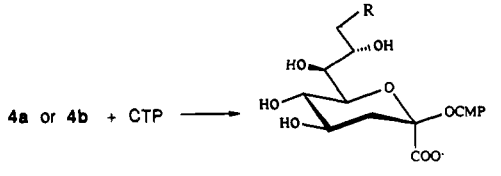

served in this study provides a novel approach to the synthesis of a new class of high-order monosaccharide structures. Given that the enzyme is readily available and highly stable as a free or immobilized form,[7a,b,12] NeuAc aldolase appears to be a useful catalyst for the synthesis of a number of uncommon sugars.

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Observation of Noncovalent Enzyme–Substrate and Enzyme–Product Complexes by Ion-Spray Mass Spectrometry

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The newly developed techniques of electrospray[1] and ion-spray[2] (pneumatically assisted electrospray) mass spectrometry (MS) permit the formation of gas-phase macromolecular ions directly from solution at atmospheric pressure via protonation and ion evaporation. Recently we described for the first time the successful detection of noncovalent receptor–ligand complexes using continuous-introduction ion-spray MS.[3] Here we report that noncovalent enzyme–substrate and enzyme–product complexes of hen egg white lysozyme (HEWL) can also be detected as their protonated, multiply charged species. That the observed complexes are active-site directed and not anomalous aggregates resulting from the electrospray ionization process is supported by several lines of evidence.

Figure 1 depicts a typical ion-spray mass spectrum of HEWL (MW 14305) showing the expected distribution of multiply protonated, multiply charged parent ions.[4] Since ion-spray MS does not generally cause molecular fragmentation, the possibility of detecting noncovalent association complexes between HEWL and its substrates or products may be considered.

HEWL hydrolyzes a hexasaccharide of *N*-acetylglucosamine (NAG_6) with principal cleavage between residues 4 and 5 to produce NAG_4 and NAG_2.[5] From an X-ray structure determination of the enzyme–NAG_3 complex, Perkins et al. propose a model for the lysozyme binding site in which six subsites (designated A–F) can accommodate up to a hexasaccharide substrate domain.[6]

The reaction of HEWL with NAG_6 can be monitored at its optimum pH (NH_4OAc buffer, pH 5)[7] by ion-spray MS, as illustrated in Figure 2, parts A–C. Besides the expected multiply protonated molecule ions at m/z 1789 $(HEWL + 8H)^{8+}$ and 2045 $(HEWL + 7H)^{7+}$, a mass spectrum immediately after mixing (Figure 2A) reveals a new protonated ion at m/z 1943 corresponding to $(HEWL + NAG_6)^{8+}$. A weak ion at m/z 1893 is observed in Figure 2A which increases with time (Figure 2, parts B and C) and represents the $(HEWL + NAG_4)^{8+}$ enzyme–product complex. Binding of HEWL to NAG_2 is almost 100 times weaker (Table I);[8] thus $(HEWL + NAG_2)^{8+}$ is not detected. Slow hydrolysis of NAG_4 and NAG_3 and NAG accounts for the appearance of a weak $(HEWL + NAG_3)^{8+}$ ion at m/z 1868 (Figure 2B).

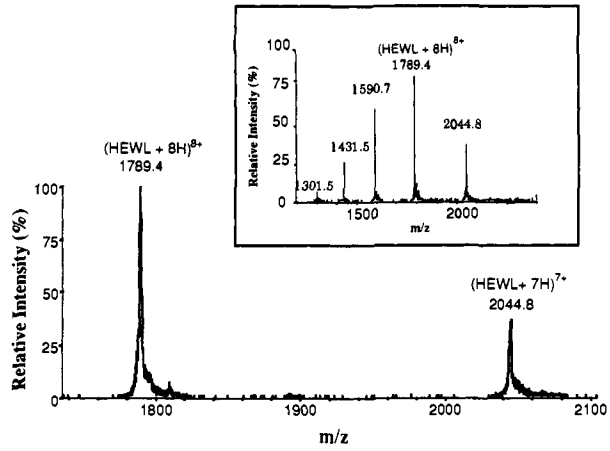

Figure 1. Ion-spray mass spectrum of HEWL recorded on a Sciex TAGA 6000E triple quadrupole mass spectrometer. HEWL was infused (1 μg/μL in 10 mM NH_4OAc buffer, pH 5.0) at 2 μL/min through the ion-spray interface. The instrument was calibrated using the multiply charged ions of HEWL. Unit mass resolution was adjusted to give an approximately 30% valley on the singly charged ions of PEG 2000. The spectrum is an averaged sum of 10 scans from m/z 1200 to 2400 at a scan rate of 2 s/scan. The envelope of multiply protonated, multiply charged ions ranges from the $(M + 7H)^{7+}$ to the $(M + 11H)^{11+}$ charge states of HEWL (figure inset). The mass-to-charge region between the 8+ and 7+ charge states (main figure) is the region of interest for detecting noncovalent enzyme–substrate and enzyme–product complexes described in this work.

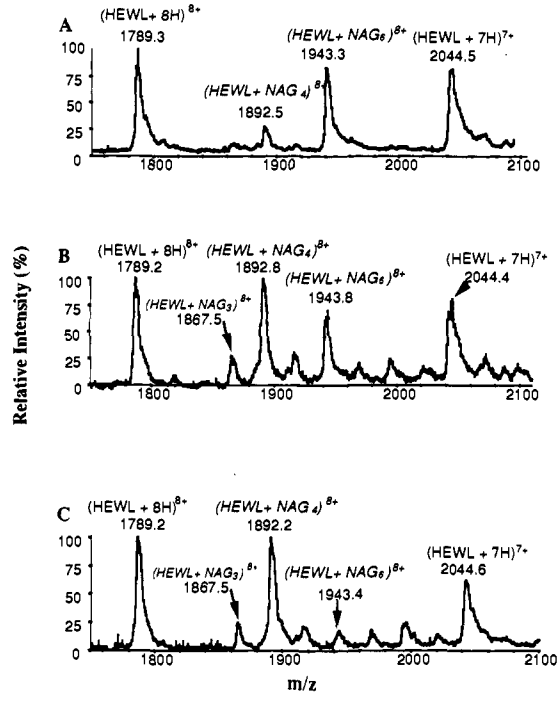

Figure 2. An on-line ion-spray MS time-course study for the hydrolysis of NAG_6 by HEWL at room temperature over 60 min. A mixture of HEWL (3.17×10^{-5} M) and NAG_6 (4.41×10^{-4} M) in buffer (10 mM NH_4OAc, pH 5.0) was infused through the ion-spray interface at 2 μL/min. Mass spectra were obtained as 1 min (panel A), 10 min (panel B), and 60 min (panel C).

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