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Synthesis of KDO Using Indium-Mediated Allylation of 2,3:4,5-Di-*O*-isopropylidene-D-arabinose in Aqueous Media

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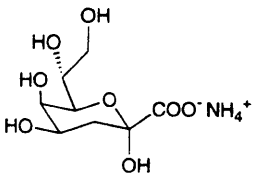
Introduction

3-Deoxy-D-*manno*-2-octulosonic acid (ammonium salt, **1**), KDO, is an integral component of the lipopolysaccharides of Gram-negative bacteria. New syntheses of KDO



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may be useful in developing analogs capable of disrupting the biosynthesis of bacterial cell-wall components, and thereby lead to new antibacterial agents.[1] A number of syntheses of KDO have been described.[2] Here, we describe a new route to KDO based on allylation of 2,3:4,5-di-*O*-isopropylidene-D-arabinose (**2**) with ethyl 2-(bromomethyl)acrylate and indium.[3] The stereochemistry of this reaction is based on the work of Schmid,[4] who reported allylations yielding products having *erythro* selectivity at the carbon with the newly generated hydroxyl group, relative to the hydroxyl group at C-2 of the aldoses, by using acetonide-protected aldoses.

Results and Discussion

A mixture of 2,3:4,5-di-*O*-isopropylidene-D-arabinose (**2**),[5] ethyl 2-(bromomethyl)acrylate,[6] and formic acid in aqueous acetonitrile was stirred at 0 °C, and indium metal was added in one portion.[7] After the mixture was stirred for 1 h at 0 °C and 6 h at ambient temperature, TLC showed complete consumption of the reactants (Scheme 1). Analytical HPLC of the crude product indicated a 2:1 diastereoselectivity. We hypothesized, based on the Felkin–Anh model,[8] that the major component of **3** possessed an *erythro* relationship between the stereocenters at C-4 and C-5. This hypothesis was confirmed by comparison of each deprotected stereoisomer of **3** with product obtained directly from allylation of unprotected arabinose and by conversion of the major component to **1**.

The two diastereomers were separated by flash column chromatography. The *erythro* product was ozonized at −78 °C in methanol to provide α-keto ester **4** in 92% yield. Hydrolysis of the ester and acetonides using 10% aqueous trifluoroacetic acid, followed by neutralization with aqueous ammonia, yielded a white precipitate. Recrystallization from hot aqueous ethanol gave KDO (**1**), which was indistinguishable (mp, ^{1}H NMR spectroscopy, and TLC) from authentic material purchased from Sigma.

Conclusion

This short synthesis of KDO (**1**) in 20% overall yield based on **2** proceeds *via* the key intermediate, diacetonide protected KDO (**4**). The regeneration of the indium, reported by Schmid,[9] should greatly reduce the cost of this synthesis. The intermediate **4** will allow facile glycosidation at the C-4 position, which is a common linkage in KDO-containing oligosaccharides.[1] This synthesis is complementary to the existing nonenzymatic and enzymatic methods of synthesizing KDO.

Experimental Section

Enoates 3. A solution of 2,3:4,5-di-*O*-isopropylidene-D-arabinose (**2**) (120 mg, 0.5 mmol), ethyl 2-(bromomethyl)acrylate (0.10 g, 1.65 mmol), and aqueous formic acid (10%, 0.5 mL) in 150 mL of aqueous acetonitrile (1:1, v/v) was stirred in an ice bath for 10 min before indium metal (Aldrich, 62 mg, 0.55 mmol) was added in one portion. The mixture was stirred for 1 h at 0 °C and for 6 h at ambient temperature. The reaction mixture was filtered, evaporated, and extracted with chloroform (3 × 10 mL). The organic layer was washed with brine (2 × 25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was subjected to flash chromatography on silica gel (CH_2Cl_2:MeOH = 100:1) to give **3** (*erythro:* 72 mg, 42%; *threo:* 32 mg, 19%). HPLC of the crude reaction mixture showed *erythro*:*threo* = 2:1 (RP-18 column, eluent CH_3CN:MeOH = 1:1). *erythro*-**3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24 (d, 1 H, J = 1.6 Hz), 5.70 (d, 1 H, J = 1.2 Hz), 4.19 (qd, 2 H, J = 7.1 and 1.4 Hz), 4.15 (dd, 1 H, J = 8.5 and 6.2 Hz), 4.07 (dt, 1 H, J = 8.2 and 6.0 Hz), 3.97 (dd, 1 H, J = 8.4 and 5.4 Hz), 3.82 (m, 1 H), 3.80 (t, 1 H, J = 8.1 Hz), 3.72 (t, 1 H, J = 7.2 Hz), 3.46 (s, 1 H), 2.85 (dt, 1 H, J = 14.3 and 1.3

(1) For a general review on KDO, see: Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323. For a recent review on KDO-containing oligosaccharides, see: Paulsen, H.; Hoeffgen, E. C.; Brenken, M. *ACS Symp. Ser.* **1993**, *519*, 132.

(2) For chemical syntheses, see: (a) Coutrot, Ph.; Grison, C.; Tabyaoui, M. *Tetrahedron Lett.* **1993**, *34*, 5089. (b) Dondoni, A.; Merino, P. *J. Org. Chem.* **1991**, *56*, 5294. (c) Smith, D. B.; Wang, Z.; Schreiber, S. L. *Tetrahedron* **1990**, *46*, 4793. (d) Esswein, A.; Betz, R.; Schmidt, R. R. *Helv. Chim. Acta* **1989**, *72*, 213. (e) Branchaud, B. P.; Meier, M. S. *J. Org. Chem.* **1989**, *54*, 1320. (f) Danishefsky, S. J.; DeNinno, M. P.; Chen, S. *J. Am. Chem. Soc.* **1988**, *110*, 3929. (g) Danishefsky, S. J.; Pearson, W. H.; Segmuller, B. E. *J. Am. Chem. Soc.* **1985**, *107*, 1280. For enzymatic syntheses, see: (h) Sugai, T.; Shen, G. J.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 413. (i) 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. (j) Augé, C.; Gautheron, C.; David, S.; Malleron, A.; Cavayé, B.; Bouxom, B. *Tetrahedron* **1990**, *46*, 201.

(3) For allylations of aliphatic aldehydes and ketones, see: (a) Chao, L. C.; Rieke, R. D. *J. Org. Chem.* **1975**, *40*, 2253. (b) Petrier, C.; Einhorn, J.; Luche, J. L. *Tetrahedron Lett.* **1985**, *26*, 1449. (c) Einhorn, C.; Luche, J. L. *J. Organomet. Chem.* **1987**, *322*, 177. (d) Araki, S.; Ito, H.; Butsugan, Y. *J. Org. Chem.* **1988**, *53*, 1831. (e) Araki, S.; Ito, H.; Butsugan, Y. *Synth. Commun.* **1988**, *18*, 453. (f) Wu, S.; Huang, B.; Gao, X. *Synth. Commun.* **1990**, *20*, 1279. (g) Li, C. J.; Chan, T. H. *Tetrahedron Lett.* **1991**, *32*, 7017. For allylations of aldoses, see: (h) Schmid, W.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 6674. (i) Chan, T. H.; Li, C. J. *J. Chem. Soc., Chem. Commun.* **1992**, 747. (j) Kim, E.; Gordon, D. M.; Schmid, W.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 5500. (k) Gordon, D. M.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 7937.

(4) Binder, W. H.; Prenner, R. H.; Schmid, W. *Tetrahedron* **1994**, *50*, 749.

(5) Zinner, H.; Wittenburg, E.; Rembarz, G. *Chem. Ber.* **1959**, *92*, 1614.

(6) Villieras, J. and Rambaud, M. *Org. Synth.* **1987**, *66*, 220.

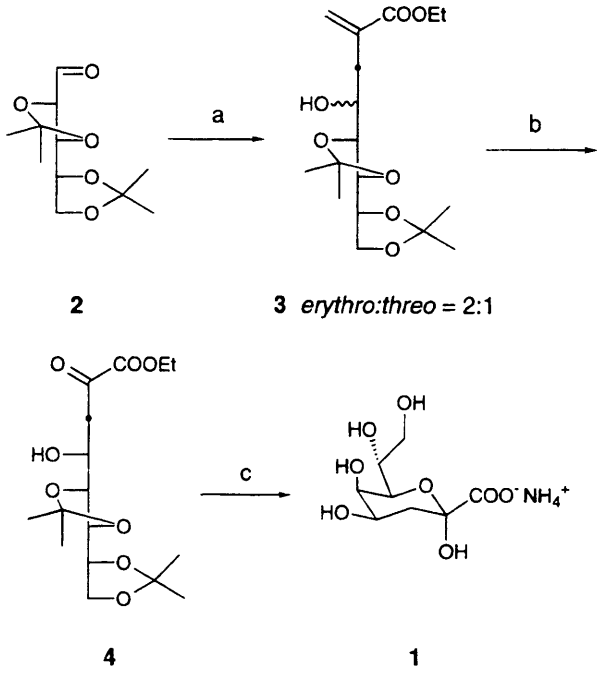
(7) Vasella applied similar methodology in the syntheses of Neu5Ac. (a) Csuk, R.; Hugener, M.; Vasella, A. *Helv. Chim. Acta* **1988**, *71*, 609. (b) Baumberger, F.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 1205.

(8) Chérest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, 2199. Anh, N. T. *Top. Curr. Chem.* **1980**, *88*, 146. Reetz, M. T. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 556.

(9) Prenner, R. H.; Binder, W. H.; Schmid, W. *Liebigs Ann. Chem.* **1994**, 73.

0022-3263/94/1959-3714$04.50/0

Scheme 1[a]



[a] (a) In, ethyl α-(bromomethyl)acrylate, 10% formic acid, aqueous CH_3CN, 61%; (b) O_3, MeOH, −78 °C; Me_2S, MeOH, −78 °C to rt, 92%; (c) aqueous TFA; NH_4OH, 55%.

Hz), 2.35 (ddd, 1 H, J = 14.4, 9.3 and 0.7 Hz), 1.41 (s, 3 H), 1.37 (s, 3 H), 1.35 (s, 3 H), 1.33 (s, 3 H), 1.29 (t, 3 H, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.51, 137.35, 127.25, 110.08, 109.43, 83.05, 80.72, 76.44, 71.11, 67.67, 60.76, 36.45, 26.94, 26.39, 25.18, 14.16. MS m/z 367 $(M + Na)^+$; HRMS calcd for $C_{17}H_{28}O_7$ $(M + Na)^+$ 367.1733, found 367.1736.

Diacetonide-Protected KDO 4. Ozone was bubbled through a solution of enoate **3** (*erythro* diastereomer, 50 mg, 0.15 mmol) in CH_3OH (10 mL) at −78 °C for 15 min. The reaction was purged with O_2 for 5 min, and Me_2S (0.5 mL) was added. The reaction was warmed to room temperature and stirred for 2 h until TLC (hexane:ethyl acetate = 1:1) showed complete decomposition of the ozonide. The mixture was concentrated and extracted with diethyl ether (3 × 10 mL). The organic layer was washed with brine (2 × 10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give **4** (46 mg, 92%): ^1H NMR (400 MHz, $CDCl_3$) δ 4.31 (q, 2 H, J = 7.1 Hz), 4.23 (m, 1 H), 4.19 (dd, 1 H, J = 8.5 and 6.0 Hz), 4.05 (dt, 1 H, J = 8.6 and 8.4 Hz), 3.99 (dd, 1 H, J = 8.5 and 5.3 Hz), 3.80 (t, 1 H, J = 7.4 Hz), 3.72 (dd, 1 H, J = 8.4 and 7.6 Hz), 3.62 (s, 1 H), 3.25 (dd, 1 H, J = 16.4 and 4.9 Hz), 3.06 (dd, 1 H, J = 16.4 and 7.8 Hz), 1.37 (t, 3 H, J = 7.1 Hz), 1.34 (s, 12 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.49, 160.90, 110.32, 109.75, 82.82, 80.83, 76.35, 69.02, 67.98, 62.46, 43.41, 26.82, 26.64, 26.43, 25.13, 14.03; MS m/z 369 $(M + Na)^+$; HRMS calcd for $C_{16}H_{26}O_8$ $(M + Na)^+$ 369.1525, found 369.1521.

Ammonium Salt of KDO (1). A solution of **4** (35 mg) in 10 mL of 10% aqueous trifluoroacetic acid was stirred at 80 °C for 20 min. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Aqueous ammonia (0.5 mL) was added, and a white precipitate appeared. The mixture was concentrated *in vacuo* and recrystallized from hot aqueous ethanol to give the ammonium salt of KDO (**1**, 14 mg, 55%): mp 121−123 °C (lit. mp 122−124 °C, authentic sample from Sigma 121−123 °C); TLC (MeOH:$CHCl_3$:H_2O = 10:10:3) R_f = 0.55 (this material co-spots with authentic material from Sigma); ^1H NMR (400 MHz, D_2O) δ 4.50−4.40 (m), 4.10−3.95 (m), 3.90−3.50 (m). The assignment of the C-3 protons for different stereoisomers are as follows: for α-pyranose form (79%) δ 1.91 (t, 1 H, J = 12.4 Hz), 1.82 (dd, 1 H, J = 12.8 and 5.5 Hz); for furanose form (6%) δ 2.30, 2.24; for lactone form (15%) δ 2.51 (dd, 1 H, J = 14.1 and 6.5 Hz), 2.01 (dd, 1 H, J = 14.1 and 3.1 Hz).

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Supplementary Material Available: HPLC chromatogram of crude reaction mixture of **3** and NMR and mass spectra of **1**, **3**, and **4** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.