

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

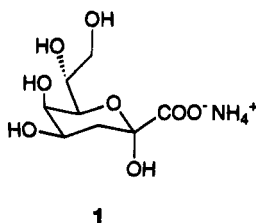
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Introduction

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



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.

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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, ¹H 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 \times 10 mL). The organic layer was washed with brine (2 \times 25 mL), dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was subjected to flash chromatography on silica gel (CH₂Cl₂: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 CH₃CN:MeOH = 1:1). *erythro*-3: ¹H NMR (400 MHz, CDCl₃) 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

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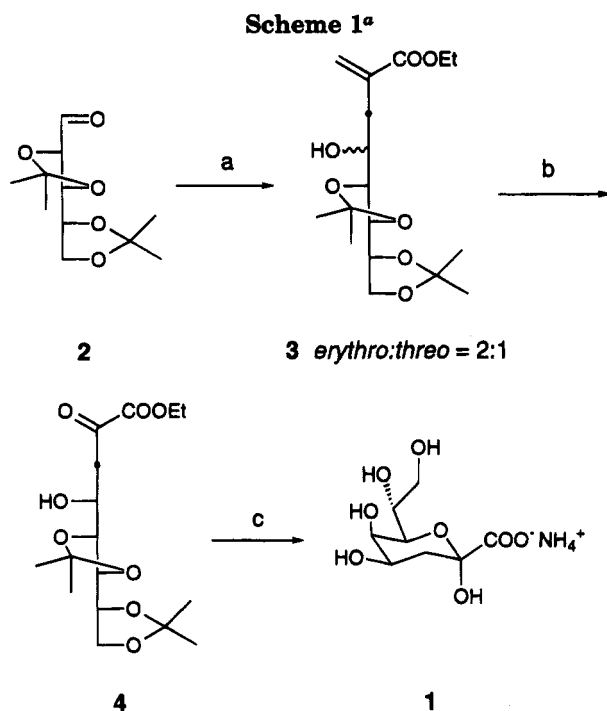
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^a (a) In, ethyl α -(bromomethyl)acrylate, 10% formic acid, aqueous CH_3CN , 61%; (b) O_3 , MeOH, -78°C ; Me_2S , MeOH, -78°C to rt, 92%; (c) aqueous TFA; NH_4OH , 55%.

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 ($\text{M} + \text{Na}$)⁺; HRMS calcd for $\text{C}_{17}\text{H}_{28}\text{O}_7$ ($\text{M} + \text{Na}$)⁺ 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 \times 10 mL). The organic layer was washed with brine (2 \times 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 ($\text{M} + \text{Na}$)⁺; HRMS calcd for $\text{C}_{16}\text{H}_{26}\text{O}_8$ ($\text{M} + \text{Na}$)⁺ 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.