A Simple Synthesis of C-8 Modified 2-Keto-3-deoxy-D-manno-octulosonic Acid (KDO) Derivatives

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A Simple Synthesis of C-8 modified KDO derivatives.

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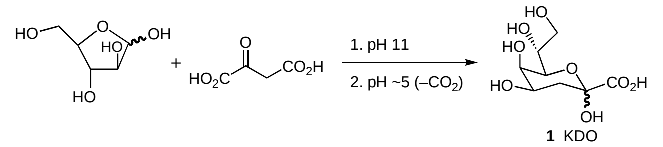
This paper is dedicated to Prof Gerry Pattenden on the occasion of his 70th birthday - thank you for the wonderful insight into chemistry.

Abstract: This paper describes a simple and efficient method to prepare C-8 modified KDO derivatives from C-5 modified arabinose derivatives.

Key words: carbohydrates, ulosonic acids, aldol condensation, benzylation, protecting groups.

The eight carbon acidic sugar 2-keto-3-deoxy-D-*manno*-octulosonic acid (KDO, **1**) is a member of the family of 3-deoxy-2-ulosonic acids. KDO is an essential component of the outer membrane lipopolysaccharide (LPS), or lipo-oligosaccharide (LOS), of Gram-negative bacteria, where it forms the link between the lipid A and polysaccharide components of the LPS.[1,2,3] KDO appears to be unique to Gram-negative bacteria, and since KDO biosynthetic pathway knockout mutants are no longer viable,[1b,4] it is reasonable to assume that small molecule inhibitors of KDO biosynthesis have the potential to act as a new class of antibacterial agent.[3] As part of a program aimed at exploring the binding specificity of enzymes involved in the biosynthesis of KDO and its incorporation into the LPS, we required an efficient and flexible route towards a range of structurally modified KDO derivatives. Specifically, we were interested in preparing C-8 modified KDO derivatives, since of those bacteria that have had their LPS structures elucidated, most have more than one KDO unit present in their core region, with the additional KDO residues usually attached to KDO through the C-4 or C-8 hydroxyl groups.[2]

The chemical synthesis of KDO (**1**) was first reported by Ghalambor and Heath in 1963,[5] following a method developed by Cornforth for the synthesis of sialic acid.[6] The method involved a base-catalysed aldol condensation between D-arabinose and oxalacetic acid, followed by decarboxylation under mildly acidic conditions (Scheme 1). Since this first report there have been numerous papers describing of the synthesis of KDO,[1,7] often involving the use of D-mannose and the addition of a two-carbon unit,[8] or starting from D-arabinose and adding a three-carbon unit.[9]

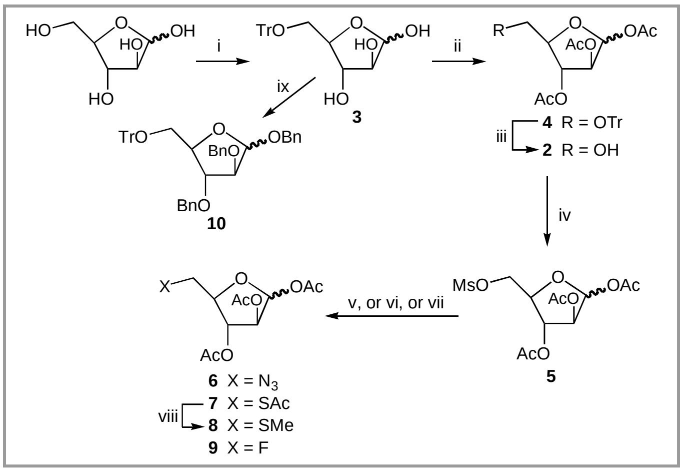


Scheme 1. Cornforth method for the synthesis of KDO

Whilst there are several published procedures towards the synthesis of KDO, many of these methods require the use of expensive reagents or are multi-step approaches. Our aim was to find a single method that utilized relatively cheap reagents and would be flexible enough to allow the preparation of structural analogues of KDO. Towards this end, the method based on the Cornforth procedure, although not giving high yields, appeared attractive to us. In 1986 McNicholas *et al.*[9a] described a modification of the original procedure for the synthesis of KDO, wherein sodium carbonate was used to ensure that an optimal pH 11 was maintained for the aldol condensation. In this way KDO was consistently obtained in yields of ~35%. Subsequently, it was suggested[9b] that the modest yields for KDO may be due to the lack of optimized conditions for the decarboxylation step that follows the aldol condensation. By using a catalytic amount of $NiCl_2$ in the decarboxylation step, the yield of KDO was reported to increase to an acceptable 66%.[9b]

In using the Cornforth method to prepare our target C-8 modified KDO derivatives, we required ready access to C-5 modified arabinose derivatives, since carbon-5 of arabinose becomes carbon-8 of KDO after the aldol condensation. Our target compounds were those in which the normal 5-hydroxyl of arabinose was replaced by –OR, –SR, or –NHR. It was reasoned that, after transformation into the corresponding C-8 modified KDO derivatives, such groups still have the potential to participate in hydrogen bonding interactions with KDO recognising enzymes. In order to introduce these types of groups at C-5, we envisaged preparing a differentially protected arabinose derivative like **2** (Scheme 2). Compounds such as **2** have been described before,[10,11,12] and the approaches generally rely on selectively introducing a temporary protecting group at the 5-hydroxyl, protecting the three remaining hydroxyls, and then removal of the C-5 protecting group. However as noted by others,[10] this proved more challenging than could be inferred from previously reported procedures.

Our initial approach towards a compound like **2** involved attempts at introducing a silyl protecting group onto the C-5 hydroxyl of arabinose. However we found it difficult to get consistent outcomes from this approach, a finding comparable with the observations of others.[10] Ultimately, we settled for a sequence towards **2** involving the use of a trityl ether at C-5 and acetate groups on the other hydroxyls. Accordingly, treatment of D-arabinose with trityl chloride in pyridine gave the known[12] 5-*O*-trityl ether **3** which, without purification, was acetylated (pyridine and acetic anhydride) to give the fully protected compound **4**[12] (Scheme 2). De-*O*-tritylation of **4** was achieved smoothly and efficiently by treatment with 80% acetic acid at 65 ˚C for 1 hour.[12] In this way the key 5-hydroxy arabinose derivative **2** was obtained in 49% yield from arabinose. Importantly, we found that when working on a large scale (>5g of arabinose) the tritylation, acetylation, de-*O*-tritylation sequence could be carried out without any purification of intermediates.



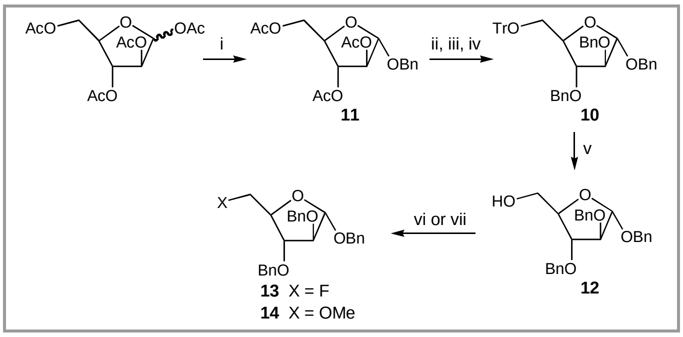
Scheme 2. *Reagents and conditions:* (i) Ph_3CCl, pyridine, RT, 48h, 58%; (ii) Ac_2O, pyridine, RT, 16h, 96%; (iii) 80% aq. AcOH, 65 ˚C, 1h, 88%; (iv) MsCl, CH_2Cl_2, EtN^iPr_2, 0 ˚C to RT, 1h, 97%; (v) NaN_3, DMF, 60 ˚C, 16h, 73%; (vi) KSAc, acetone, 40 ˚C, 72h, 82%; (vii) TBAF or DAST; (viii) $H_2NNH_2{\cdot}HOAc$, DMF, $(CH_3O)_2SO_2$, RT, 4h, 61%; (ix) NaH, BnBr, Bu_4NI, DMF, 0 ˚C to RT.

With the key alcohol **2** in hand, we turned our attention to introducing alternative functionality at C-5, and opted for a strategy involving displacement of a good leaving group. Thus, exposure of **2** to methanesulfonyl chloride gave the mesylate **5** (97%) which, upon treatment with sodium azide in *N*,*N*-DMF afforded the known[10,11] 5-azido derivative **6** (Scheme 2). We felt that an azide would provide us maximum flexibility towards preparing a range of KDO derivatives with nitrogen-based functionality, since the azide group could be reduced after the aldol condensation, allowing the introduction of both amine and amide functionality at C-8 in KDO.

For the introduction of a sulfur functionality at C-5, the mesylate **5** was treated with potassium thioacetate in acetone to give the 5-thioacetyl derivative **7**[12] in 82% yield. Selective unmasking of the thioacetate group in **7** was achieved by treatment with hydrazine acetate,[13] and then *in situ* exposure to dimethyl sulfate gave the thiomethyl derivative **8** in 61% yield.

At this stage we felt that introducing a fluorine at C-5 would be beneficial for our studies, since fluorine is detectable by nmr and would therefore provide us with an excellent "spectroscopic handle" for studies involving interactions between our KDO derivatives and KDO-recognising enzymes. Additionally, fluorine is known to have similar electronic properties to hydroxyl groups but is unable to participate in hydrogen bonding interactions,[14] and is therefore an excellent functionality to utilize in studies aimed at exploring ligand−protein interactions. Unfortunately, we found introduction of fluorine to be quite problematic. Attempted fluoride displacement of the mesylate group in **5** using tetrabutylammonium fluoride (TBAF)[15] in our hands gave a complex mixture of products from which the desired 5-fluoro arabinose derivative **9** was obtained in poor yield. A common reagent for converting alcohols directly into fluorides is diethylamino sulfur trifluoride (DAST). Unfortunately however, treatment of **2** with DAST in dichloromethane,[16] gave poor (~15%) yields of the desired product. It has been reported that changing the solvent to diglyme would improve the outcome of this reaction,[10] but in our hands this was not the case. Our overall analysis of the attempted introduction of fluoride was that the acetate protecting groups were too labile for this type of transformation, since some of the components we isolated from these reactions contained fewer acetate groups than expected.

Dissatisfied with this approach, we investigated an alternative strategy involving the use of the more stable benzyl ether protecting groups. Accordingly, the 5-*O*-trityl ether **3** was treated with sodium hydride and benzyl bromide in *N*,*N*-DMF. Unfortunately, but perhaps not unexpectedly, this resulted in a highly complex reaction mixture from which the desired tri-*O*-benzyl ether **10** was obtained in modest (<40%) and highly variable yield. Interestingly, one of the products from this benzylation reaction appeared, by inspection of the ^1H nmr spectrum, to contain four benzyl ether groups in addition to the 5-*O*-trityl ether, possibly due to sodium hydride mediated reduction of the acyclic aldehyde form of the sugar.[17] After considerable experimentation we opted for a longer sequence of reactions that proved far more reliable and efficient overall. Accordingly, acetylation of arabinose (Ac_2O/pyridine) followed by treatment with $SnCl_4$ and benzyl alcohol[18] gave the α-glycoside **11** in 86% yield (Scheme 3). Removal of the acetate groups, tritylation of the C-5 hydroxyl, and then benzylation of the remaining two hydroxyls gave the desired arabinoside **10** in 30% yield from **11**. Removal of the trityl group in **10** proceeded as expected to give the target alcohol **12** in 72% yield (Scheme 3).



Scheme 3. *Reagents and conditions:* (i) $SnCl_4$, BnOH, CH_3CN, 1h, 86%; (ii) aq. NaOH (1M), CH_3OH, RT, 16h, 72%; (iii) Ph_3CCl, pyridine, RT, 48h, 52%; (iv) NaH, BnBr, Bu_4NI, DMF, 0 ˚C to RT, 16h, 81%; (v) 80% aq. AcOH, 65 ˚C, 1h, 72%; (vi) DAST, CH_2Cl_2, 0 ˚C to RT, 3h, 58%; (vii) NaH, CH_3I, DMF, 16h, 81%.

To see if changing protecting groups would indeed result in an improved outcome, the alcohol **12** was exposed to DAST. To our delight the desired 5-fluoro derivative **13** was obtained in a modest 58% yield, a significant improvement over our results with acetate protecting groups. Attempts to increase this yield by altering the reaction temperature and the equivalents of DAST failed to significantly improve the outcome. Having prepared the alcohol **12**, we felt we should exploit the robust nature of the benzyl ethers by introducing a methyl ether at C-5. Thus, treatment of **12** with sodium hydride and methyl iodide proceeded smoothly, and gave the methyl ether **14** in 81% yield (Scheme 3).

Each of the C-5 modified arabinose derivatives was deprotected in the standard way. Acetate protecting groups were removed by treatment with dilute sodium hydroxide (1M) in methanol, whilst benzyl ethers were removed by hydrogenolysis. Interestingly, deprotection of the 5-thioacetyl derivative **7** gave the known 5-thio-D-arabinose,[12] as a mixture of the pyranose and furanose forms in a 3:1 ratio, respectively. Attempted deprotection of the ester groups in the 5-thiomethyl derivative **8** resulted in extensive decomposition, even when dilute aqueous lithium hydroxide and shorter reaction times were employed.

Having successfully prepared a range of C-5 modified arabinose derivatives we next explored their condensation with oxalacetic acid to give KDO derivatives. Initially we followed the method reported by McNicholas *et al*,[9a] and, using arabinose itself as a model compound, obtained the ammonium salt of KDO in ~30% yield after ion-exchange chromatography on Amberlite CG-400 (HCO_3^-) resin. As expected, the product obtained in this way is a complex mixture of components, due to both the lack of stereoselectivity of the aldol condensation, giving a mixture of epimers at C-4, as well as the fact that reducing sugars exist as a mixture of α- and β-anomers in both pyranose and furanose forms. Since this aldol condensation is conducted in the absence of chelation control, the stereochemical outcome of the enolate addition to the aldehyde will fit the Felkin-Ahn model,[19] where the incoming nucleophile preferentially attacks from the sterically less-hindered (*Re*) face of the aldehyde (Figure 1).

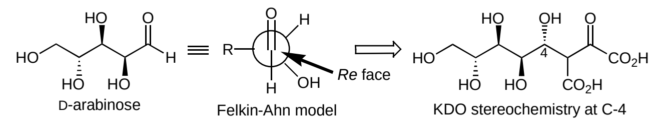
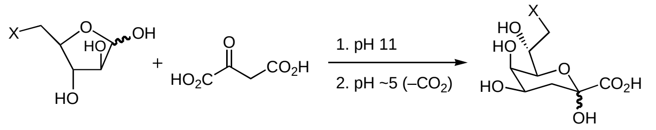


Figure 1. Diagram showing the preferential *Re*-face attack on D-arabinose, giving the desired KDO stereochemistry at C-4.

In this way we, as well as others,[1a,9a,9b] have observed that the stereochemical outcome for the condensation between arabinose and oxalacetic acid appears to favour the formation of KDO over 4-*epi*-KDO, with a ratio of KDO to 4-*epi*-KDO typically around 4 to 1. Purification of our KDO product using Dowex 1x8 (200-400 mesh) anion exchange chromatography following the method reported by Kragl *et al.*[20] resulted in separation of KDO from 4-*epi*-KDO, allowing complete assignment of their respective ^1H nmr spectra (see Supporting Information).

Although satisfied that we could prepare and isolate pure KDO, the poor yield was a significant limitation of this approach, especially given that many of our arabinose derivatives were not trivial to prepare. In an attempt to optimize this reaction, we repeated the condensation between arabinose and oxalacetic acid using the $NiCl_2$ modification reported by Shirai and Ogura,[9b] and obtained a 60% yield of KDO, as a ~5:1 epimeric mixture at C-4. Whilst delighted with this improved chemical yield, a limitation with this method (as indeed with all previous reports of preparing KDO using this approach) is the use of a molar excess of arabinose. Since in our strategy the arabinose derivative is the more difficult coupling partner to prepare, the aldol condensation was repeated using a molar excess of oxalacetic acid. In this way, KDO could be routinely obtained in ~65% yield based on arabinose. Having established appropriate reaction conditions[21] for the synthesis of KDO, we turned our attention to using the C-5 modified arabinose derivatives in the aldol condensation with oxalacetic acid. The results are summarized in Table 1, and clearly show that this approach represents an efficient and flexible synthesis of C-8 modified KDO derivatives **15**. The KDO derivatives **15** were all obtained along with their 4-*epi* analogues, with the ratio of **15** to 4-*epi*-**15** typically better than 5 to 1 (as determined by ^1H nmr). Reduction of the azide group in **15** (X = N_3) to an amine (**15**, X = NH_2) was accomplished by hydrogenation.

Table 1 Synthesis of C-8 modified KDO derivatives



15

	Yield (%) of **15**
X = OH	65%
X = OMe	84%
X = N_3	87%
X = SH	67%

In conclusion, we have shown that C-8 modified KDO derivatives can be prepared efficiently from readily available starting materials. We are currently investigating simple ways to improve the stereochemical outcome of the aldol condensation between structurally modified arabinose derivatives and oxalacetic acid. We are also using the C-8 modified KDO derivatives described herein as probes for KDO utilizing proteins. These investigations will be described in due course.

Acknowledgment

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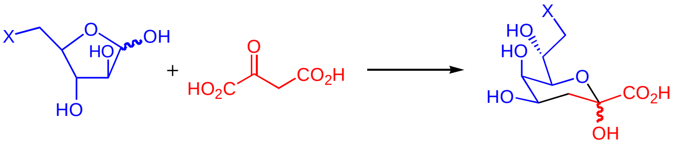
(21) General procedure for the synthesis of KDO and C-8 modified derivatives: D-Arabinose (500mg, 3.3 mmol) was added to a solution of sodium carbonate (860mg, 8.1 mmol) in water (8 mL). Oxalacetic acid (525mg, 4.0 mmol) was added portionwise over 5 min, and the solution adjusted to pH 11 using NaOH (10M). After stirring for 2h at RT the solution was acidified to pH 5 using AcOH, $NiCl_2$ (7.5mg, 0.03 mmol) added, and the mixture heated at 50 ˚C for 1h. After cooling to RT the reaction was neutralized (to pH 8) with ammonia and the KDO isolated by column chromatography using CG-400 (HCO_3^-) resin, washing first with water and then eluting with ammonium hydrogen carbonate solution (0.5M). The eluant was concentrated under reduced pressure, and then freeze-dried. The lyophilized residue was purified using reversed-phase (C_{18}) silica gel with water as the mobile phase. Fractions containing KDO can be visualized as bluish-grey spots on silica gel (t.l.c. plates) using $CHCl_3$:MeOH:H_2O (5:5:1) as the mobile phase, and staining with anisaldehyde–sulfuric acid dip.



Synthesis of C-8 modified KDO derivatives.

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