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Efficient Large Scale Syntheses of 3-Deoxy-D-manno-2-octulosonic acid (Kdo) and Its Derivatives

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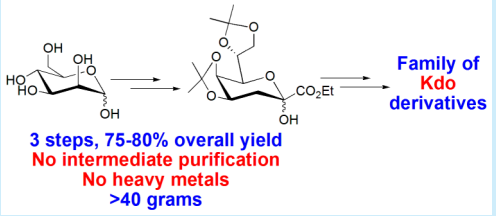
Supporting Information

ABSTRACT: An efficient method to rapidly synthesize 3-deoxy-D-manno-2-octulosonic acid (Kdo) and its derivatives in large scale has been developed. Starting from D-mannose, the di-*O*-isopropylidene derivative of Kdo ethyl ester was prepared in three steps on a scale of more than 40 g in one batch in an overall yield of 75−80% without any intermediate purification. Kdo, Kdo glycal, and 2-acetylated Kdo ester were synthesized quickly in high yield from a di-*O*-isopropylidene derivative of Kdo ethyl ester. 2-Deoxy-β-Kdo ester was obtained with high stereoselectivity via the epimerization of the α-isomer using *t*-BuOH as a proton source.



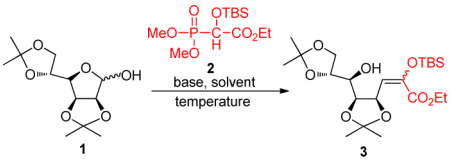
Kdo (3-deoxy-D-manno-2-octulosonic acid), an unusual 8-carbon acidic sugar, is an essential constituent of the cell wall lipopolysaccharides (LPS) of Gram-negative bacteria.[1] The transformation of Kdo to CMP-Kdo, catalyzed by the enzyme CMP-Kdo synthetase (CKS), is a key reaction in the biosynthesis of LPS, which renders CKS a promising pharmaceutical target in the development of new classes of antibiotics.[2] Kdo-containing oligosaccharides have been chemically synthesized for the development of Gram-negative bacteria vaccines.[3] However, the difficulty of Kdo isolation from natural sources results in an extremely high cost ($41/mg, Sigma-Aldrich) of Kdo production, which thereby hampers the extensive exploration of Kdo-related chemistry and biology. As a result, highly efficient syntheses of Kdo and its derivatives are necessary for research on Kdo-containing oligosaccharides and CKS inhibitors based on the Kdo skeleton, such as 2-deoxy-β-Kdo and 8-NH_2-2,8-dideoxy-β-Kdo.[4]

Many chemical and enzymatic syntheses of Kdo and its derivatives have been developed, mainly using D-mannose, D-arabinose, or other small organic molecules as starting materials.[5] These protocols used the Wittig reaction, Horner−Wadsworth−Emmons reaction (HWE reaction), Diels−Alder reaction, ring-closing metathesis, and other metal-mediated reactions to build the skeleton of Kdo. However, most of these methods required multiple steps to construct Kdo and its derivatives. The Cornforth reaction[6a] has been efficiently applied to the synthesis of Kdo by condensation of oxalacetic acid with D-arabinose.[1,6b−d] This method provides a short route to Kdo, but the difficulty in separation of the 4-epimer of Kdo could not be neglected. Moreover, Kdo derivatives could not be rapidly accessed via this method.

We envisioned that the di-*O*-isopropylidene derivative of Kdo ethyl ester **4** might be rapidly synthesized via Horner−Wadsworth−Emmons reaction between protected mannose **1** and phosphate ester[7] **2** followed by desilylation. Then, ester **4** could be employed to finish the syntheses of Kdo, 2-acetylated Kdo, glycal, and 2-deoxy-β-Kdo in short steps.

Isopropylidenation of D-mannose with 2,2-dimethoxypropane and a catalytic amount of TsOH·H_2O gave **1**,[8] which was used directly for the next step without purification (Table 1).

Table 1. Synthesis of Compound 3 via HWE Condensation



entry	base (equiv)[c]	solvent	temp (°C)	yield
1	LiHMDS	toluene	100	72%
2	NaH	toluene	100	trace
3	*t*-BuOK	toluene	100	28%
4	*t*-BuONa	toluene	100	trace
5[a]	*t*-BuOLi	toluene	80	55%
6[a]	*t*-BuOLi	toluene	100	85%
7[a]	*t*-BuOLi	toluene	120	73%
8	*t*-BuOLi	DCE	reflux	56%
9	*t*-BuOLi	1,4-dioxane	reflux	62%
10[b]	*t*-BuOLi	THF	50	88%

[a]1.2 equiv of compound **2** was added. [b]1.3 equiv of compound **2** was added. [c]1.2 equiv of base was used.

The key reaction, HWE condensation between **1** and phosphate ester **2** in the presence of different bases (including LiHMDS, NaH, *t*-BuOK, *t*-BuONa, and *t*-BuOLi, entries 1−5, respectively), was investigated. The condensation worked smoothly to provide the desired product **3** in 88% isolated yield (entry 10) when *t*-BuOLi as the base[9] and THF as the solvent were used. Phosphate ester **2**, which is commercially

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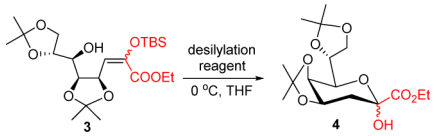
available, was readily synthesized according to Horne's procedure[7c] using ethyl glyoxylate hydrate as starting material.

As an important intermediate, Kdo ethyl ester **4** and Kdo methyl ester have been widely used in the field of Kdo-related synthetic chemistry. Many methods have been reported to synthesize those Kdo esters.[10,5c,j] With HWE product **3** in hand, we explored the deprotection of the TBS group under different conditions (Table 2) to complete the synthesis of the

Table 2. Optimization of the Desilylation Conditions for 3



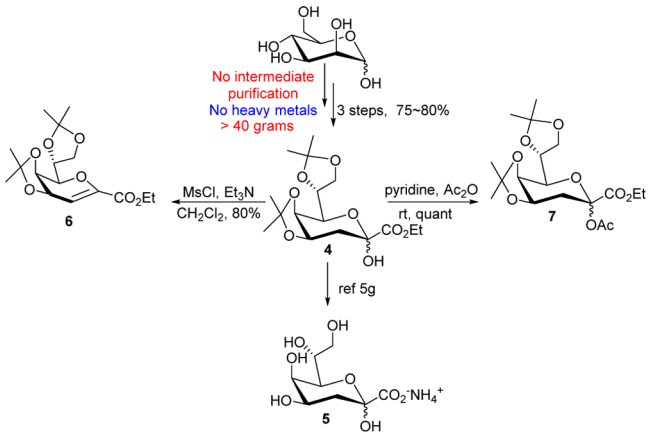
entry	reagent (equiv)	time	yield (%)
1	TBAF (1 equiv)	5 min	67
2	TBAF (1 equiv)[a]	3 h	quant
3	KF (1 equiv)[a]	6 h	73
4	HF·Py (1.5 equiv)	10 h	quant

[a]20% AcOH aqueous was added.

key intermediate-di-*O*-isopropylidene derivative of Kdo ethyl ester **4**. Treatment of **3** with TBAF or KF[11a] gave the ester **4** only in moderate yield, but a quantitative yield was achieved when HF·Py[11b] or the combined system of TBAF with 20% AcOH aqueous solution[5c,11c] was used. Deprotection under more acidic conditions led to more side reactions.

As the desilylation conditions were now well established, we examined the synthesis of **4** directly from D-mannose without intermediate purification. As expected, **4** was prepared on a scale of more than 40 g in one batch in 75−80% overall yield. Compared to those reported methods, our current approach can be used to efficiently synthesize **4** on a large scale starting from cheap material and reagents without intermediate purification and heavy metal pollution (Scheme 1). After

Scheme 1. Synthesis of Kdo and Kdo Derivates from 4

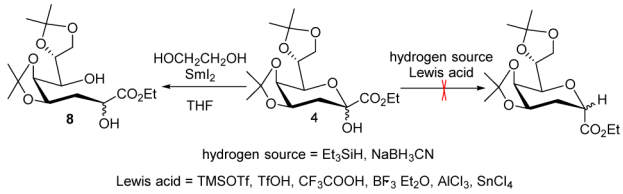


succeeding in the efficient synthesis of Kdo ester **4**, we completed the syntheses of Kdo ammonium salt, 2-acetylated Kdo ester, and Kdo glycal on a large scale. Deprotection and hydrolysis of Kdo ester **4** provided Kdo ammonium salt **5** in almost quantitative yield on the basis of a reported procedure.[5g,12] Treatment of compound **4** with MsCl/Et_3N[13] provided the Kdo glycal **6** in 80% yield, which has been widely used as a donor for construction of Kdo glycoside.[3e,14]

Acylation of Kdo ester **4** in the presence of pyridine/Ac_2O led to 2-acetylated Kdo ester **7** in quantitative yield, which also can be used in the glycosidations of Kdo derivatives.[15]

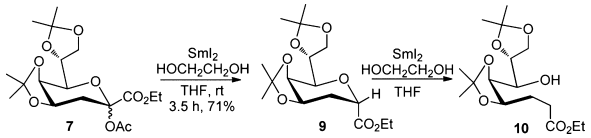
With the large scale synthesis of Kdo ester **4** in hand, we attempted to directly deoxygenate the C-2 hydroxyl group of Kdo ester **4** to finish the synthesis of 2-deoxy-β-Kdo and 8-NH_2-2,8-dideoxy-β-Kdo, both of which are potent inhibitors of CMP-Kdo synthase[4] and have been studied by many groups.[10d,g,15,16] When Et_3SiH or $NaBH_3CN$ with different Lewis acids was applied to the direct deoxygenation,[17] no desired product was formed (Scheme 2). Deoxygenation of **4** with SmI_2[15,16d,18] afforded the ring-opened product **8** instead of the desired product with a 1:1 ratio of the *R* and *S* configuration at the C-2 position.

Scheme 2. Direct Deoxygenation of 4



The deoxygenation of 2-acetylated Kdo ester **7** in the presence of Et_3SiH or $NaBH_3CN$ with different Lewis acids did not give the desired product either. When compound **7** was treated with SmI_2 in the presence of ethylene glycol,[16d] 2-deoxy-β-Kdo **9** was obtained in 71% yield along with 13% of the α-isomer (Scheme 3). Careful control of the reaction conditions was necessary. Otherwise, over-reduction would occur to form compound **10**.

Scheme 3. Reduction with SmI_2



Considering the low stereoselectivity, the low solubility of SmI_2 in THF, and the strict oxygen-free conditions, the SmI_2-mediated deoxygenation of 2-acetylated Kdo ester was not suitable for the large scale synthesis of 2-deoxy-β-Kdo. We exploited Burke's epimerization protocol to afford the β-isomer.[16g] As we mentioned above, Kdo glycal **6** was synthesized on a large scale. The quantitative hydrogenation of **6** gave 2-deoxy-α-Kdo **11** on more than a 10-g scale in the presence of a catalytic amount of 10% Pd/C (Scheme 4). Epimerization of the α-isomer **11** to the β-isomer **9** was explored under different conditions. A 3:1 ratio of the β and α isomers was obtained when 1.2 equiv of LDA was used as the base and aqueous NH_4Cl was the proton source;[16g] the ratio was increased to 5:1 with *t*-BuOH as the proton source. Surprisingly, only a trace amount of the β-isomer was obtained when AcOH was used as a proton source. The epimerization did not favor the β-isomer when LiTMP, *t*-BuOLi, or NaOMe was used as the base. Gratifyingly, the ratio of β/α was greatly increased to 10:1 when 2.5 equiv of LDA as the base and *t*-BuOH as a proton source were used. Finally, 2-deoxy-β-Kdo **9** was prepared in 85% yield on a scale of 10 g under the

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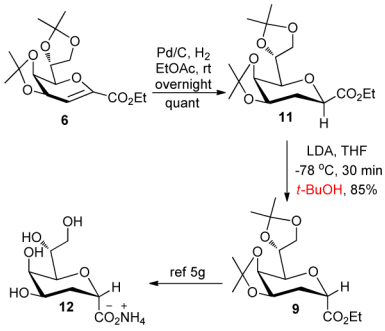
Scheme 4. Synthesis of 2-β-Kdo 9 via the Epimerization of α-Isomer 11



optimized conditions. The ammonium salt of 2-deoxy-β-Kdo was also synthesized according to the reported procedure.[5g]

With the compound **9** in hand, we proceeded directly to the synthesis of 8-NH_2-2,8-dideoxy-β-Kdo ethyl ester,[5h] which will be used in the further investigation of CKS inhibitors in our laboratory.

In summary, we have developed an efficient route for the large-scale synthesis of Kdo and its derivatives. Without intermediate purification and heavy metal pollution, Kdo ester **4** was prepared in three steps starting from D-mannose. Kdo, Kdo glycal, and 2-acetylated Kdo ester were rapidly afforded in high yield from **4**. Highly stereoselective synthesis of 2-deoxy-β-Kdo was achieved in 85% yield via epimerization. Finally, 8-NH_2-2,8-dideoxy-β-Kdo ester was prepared according to the reported procedure. Further studies on the CKS inhibitors based on 2-deoxy-β-Kdo and 8-NH_2-2,8-dideoxy-β-Kdo are in progress in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization data and NMR spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b00901.

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Notes

The authors declare no competing financial interest.

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