Organic LETTERS

Letter

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

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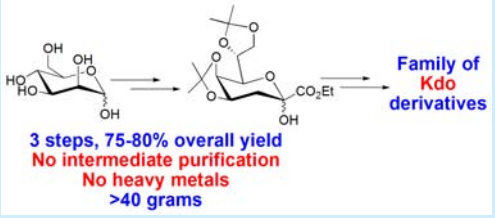
Supporting Information

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.



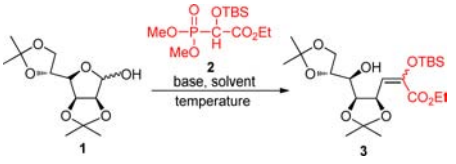
Kdo (3-deoxy-D-manno-2-octulosonic acid), an unusual 8-carbon acidic sugar, is an essential constituent of the cell wall lipopolysaccharides (LPS) of Gram-negative bacteria.[1] The transformation of Kdo to CMP-Kdo, catalyzed by the enzyme CMP-Kdo synthetase (CKS), is a key reaction in the biosynthesis 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

Received: March 28, 2015
Published: April 30, 2015

DOI: 10.1021/acs.orglett.5b00901
Org. Lett. 2015, 17, 2388−2391

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

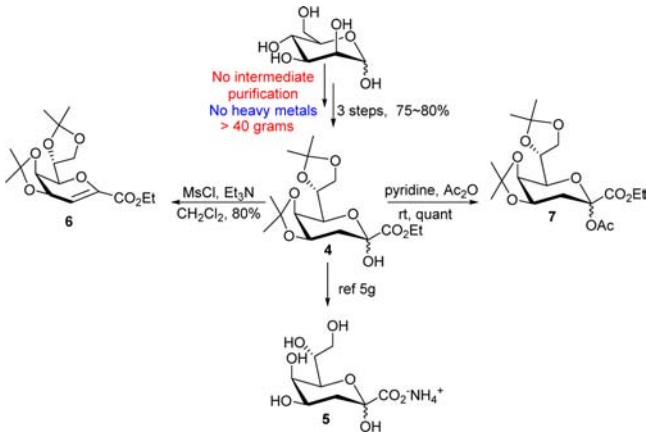
entry	reagent (equiv)	time	yield (%)
1	TBAF (1 equiv)	5 min	67
2	TBAF (1 equiv)[a]	3 h	quant
3	KF (1 equiv)[a]	6 h	73
4	HF·Py (1.5 equiv)	10 h	quant

[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

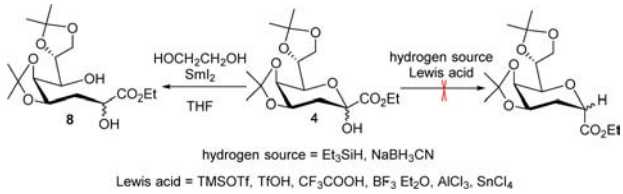


succeeding in the efficient synthesis of Kdo ester **4**, we completed the syntheses of Kdo ammonium salt, 2-acetylated Kdo ester, and Kdo glycal on a large scale. Deprotection and hydrolysis of Kdo ester **4** provided Kdo ammonium salt **5** in almost quantitative yield on the basis of a reported procedure.[5g,12] 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]

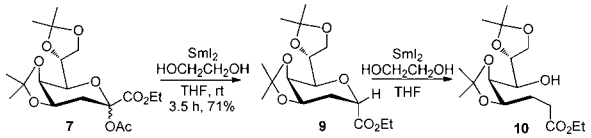
With the large scale synthesis of Kdo ester **4** in hand, we attempted to directly deoxygenate the C-2 hydroxyl group of Kdo ester **4** to finish the synthesis of 2-deoxy-β-Kdo and 8-NH_2-2,8-dideoxy-β-Kdo, both of which are potent inhibitors of CMP-Kdo synthase[4] and have been studied by many groups.[10d,g,15,16] 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**.

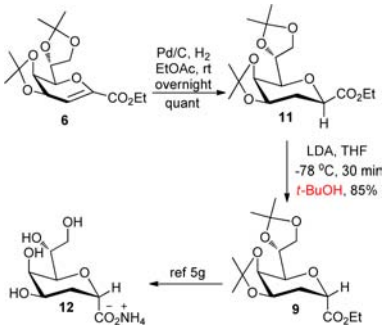
Scheme 3. Reduction with SmI_2



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

DOI: 10.1021/acs.orglett.5b00901
Org. Lett. 2015, 17, 2388−2391

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.

■ ACKNOWLEDGMENTS

This work was supported by the Fundamental Research Funds for the Central Universities (No. GK200902005), Shaanxi Provincial Natural Science Foundation (No. 2010JM2010), the National Natural Science Foundation of China (Nos. 21072122, 21472119), and Ministry of Science and Technology (No. 2012ZX09502-001-001). All of the financial support is gratefully acknowledged.

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DOI: 10.1021/acs.orglett.5b00901
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