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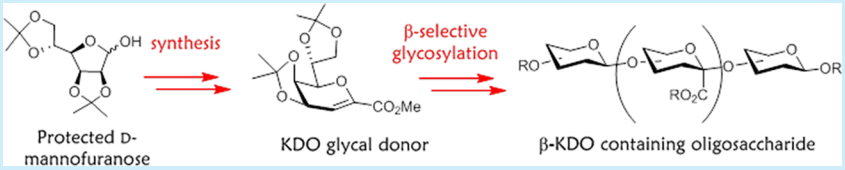
Preparation of a Protected 3-Deoxy-D-*manno*-oct-2-ulosonate Glycal Donor for the Synthesis of β-KDO-Containing Oligosaccharides

Tapan Kumar Pradhan,[†] Chun Cheng Lin,[‡] and Kwok-Kong Tony Mong*[,†]

[†]Applied Chemistry Department, National Chiao Tung University, 1001 Ta Hsueh Road, Taiwan

[‡]Chemistry Department, National Tsing Hua University, Guang Fu Road, Taiwan

Supporting Information



ABSTRACT: A practical method for the synthesis of KDO glycal donors was developed. The prepared KDO donors exhibited excellent disastereoselectivity of glycosylation in a CH_2Cl_2−CH_3CN solvent mixture, which was found to be associated with the isopropylidene protection at the C-4 and C-5 hydroxyls. The synthetic use of the KDO donor was demonstrated in the preparation of β-KDO-containing oligosaccharides.

Glycosides of 3-deoxy-D-*manno*-oct-2-ulosonoic acid (KDO) are prominent in bacteria.[1] Natural KDO glycosides are present in α- and β-anomeric configurations. α-KDO glycosides are found in the core oligosaccharides of lipopolysaccharides (LPS) in Gram-negative bacteria, whereas β-KDO glycosides commonly occur in capsular polysaccharides (CPSs) of Gram-positive and Gram-negative bacteria.[2,3] Recent studies have shown that the reducing-end terminus of CPSs from various pathogenic bacteria, including *Escherichia coli*[4] and *Neisseria meningitides*,[5] is composed of a β-KDO-containing oligosaccharide linker.[6] The CPSs of pathogenic bacteria play defensive roles in the prevention of phagocytosis and complement-mediated killing of the immune system, indicating their potential as new therapeutic targets.[3a,7−9]

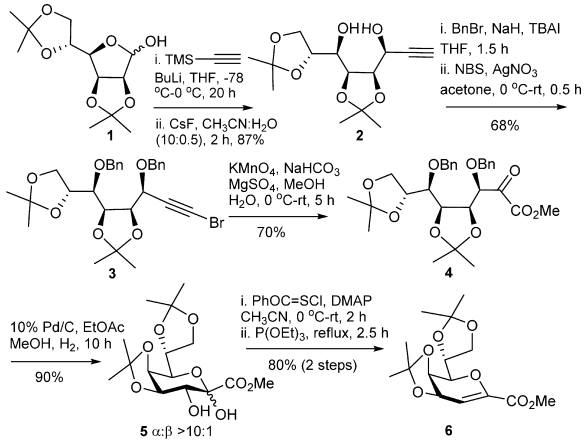
The chemical synthesis of KDO-containing oligosaccharides is nontrivial. As the KDO sugar is not commercially available, a practical method is needed to provide the starting material required for the preparation of the KDO-glycosyl substrates that are used to assemble the oligosaccharides. More challenging is the absence of a C-3 hydroxyl function in the KDO sugar which can be used to control the stereochemistry of glycosylation through neighboring group participation (NGP).[10]

The first synthesis of a KDO glycoside can be traced back to 1978.[8a] Later studies mainly focused on the synthesis of an α-KDO glycoside.[11] Although an increasing number of β-KDO-containing oligosaccharides have been identified in pathogenic bacteria,[4−6] glycosylation methods for the synthesis of β-KDO glycosides remain scarce.[12−14] Therefore, a practical synthetic route to stereoselective KDO glycosyl donors and β-KDO-containing oligosaccharides is highly desirable.

Sinaÿ et al. described the synthesis of a β-KDO-containing disaccharide through Wittig condensation of a mannose-derived aldehyde and a glycosyl phosphonate, but only the *E*-isomeric product could be used for β-glycoside formation.[12] Ling et al. prepared 1-*C*-aryl β-KDO glycoside analogues from a 1-*C*-arylglycal, yet dearomatization was needed to obtain the natural KDO glycosides; thus, elaboration to oligosaccharide synthesis would be tedious.[13] Herein, we report a synthetic route for the preparation of β-selective KDO glycal donors that can be used for the synthesis of simple β-KDO glycosides and bacterial-related β-KDO-containing oligosaccharides.

Our synthetic route to the various KDO glycal donors started with the diacetonide-protected mannofuranose **1**, which was prepared via known procedures that included the addition of lithiated trimethylsilylacetylide and desilylation to give the alkyne derivative **2** (Scheme 1).[15] Subsequent benzylation of the hydroxyl groups and bromination transformed **2** into the

Scheme 1. Synthesis of KDO Glycal 6



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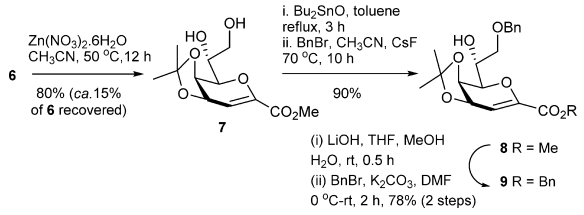
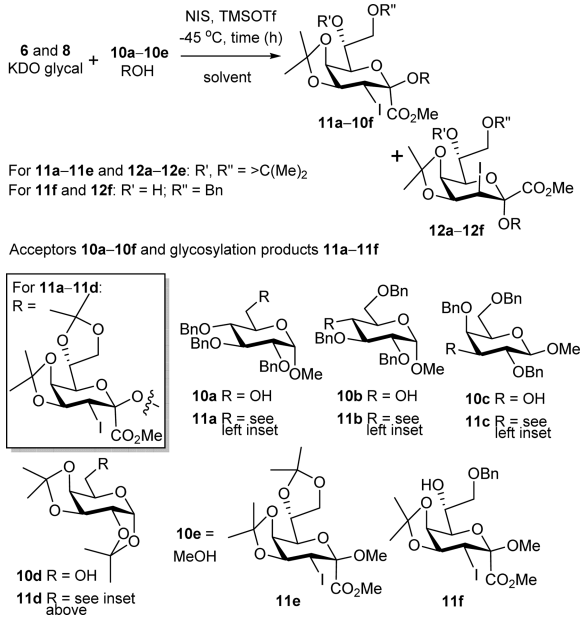
bromoalkyne derivative **3**. The bromoalkyne **3** underwent oxidative cleavage with $KMnO_4$ in methanol−H_2O to provide the methyl α-keto ester **4** in 70% yield.[16] Deprotection of the benzyl ether functions in **4** by hydrogenolysis freed the C-4 and C-6 hydroxyl groups, followed by instantaneous cyclization of the C-6 hydroxyl group with the C-2 ketone function to produce the hemiacetal **5**.[17] Of note is that a prolonged hydrogenolysis reaction time led to the migration of the acetonide function. Finally, subjecting the hemiacetal **5** to the Corey−Winter procedure[18] furnished a KDO glycal **6**,[14,19] which was employed as a donor for β-glycoside synthesis. Besides the synthesis of the glycal **6**, other KDO-glycals **7**−**9** were prepared for the glycosylation studies (Scheme 2).

Scheme 2. Synthesis of KDO Glycal Substrates 7−9



With the KDO glycal substrates in hand, we established a β-selective glycosylation method for the synthesis of β-KDO glycosides (Scheme 3, Table 1). In the beginning, the

Scheme 3. β-Selective Glycosylation Method for KDO Glycal Donors 6 and 8



glycosylation of the methyl glucosyl acceptor **10a** with KDO glycal **6** was conducted in CH_2Cl_2 using *N*-iodosuccinimide (NIS, 1.5 equiv) and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 1.5 equiv) as the promoters (entry 1).[20] The reaction produced the desired diastereomer **11a** in 50% yield (isolated) with a moderate dr of 5:1, but ca. 20% of **6** was recovered even when the reaction time was prolonged to 18 h. Nevertheless, 2.0 equiv of NIS (with respect to **6**) boosted the reaction yield to 70%, though with no improvement in stereoselectivity (entry 2). A $^2C_5$ conformation was assigned to the iodo-substituted

Table 1. Glycosylation of 10a−f with KDO Glycal Esters 6 and 8

					11a−g	
entry	donor, acceptor	NIS, TMSOTf (equiv)	time (h)	solvent (A or B)[a]	(%)	dr
1	**6**, **10a**	1.5, 1.5	18	A	**11a**, 50	5:1[b]
2	**6**, **10a**	2.0, 1.5	3	A	**11a**, 70	5:1[b]
3	**6**, **10a**	2.0, 1.5	3	B	**11a**, 68	>20:1[b,c]
4	**6**, **10b**	2.0, 1.5	6	B	**11b**, 65	>20:1[c]
5	**6**, **10c**	2.0, 1.5	3	B	**11c**, 72	14:1[c]
6	**6**, **10d**	2.0, 1.5	1	B	**11d**, 74	13:1[c]
7	**6**, **10d**	2.0, 1.5	1	A	**11d**, 80	6:1[c]
8	**6**, **10e**	2.0, 1.5	12	B	**11e**, 74	13:1[c]
9	**8**, **10e**	2.0, 1.5	18	B	**11f**, 75	6:1[d]

[a]A is CH_2Cl_2 and B is 1:2 (v/v) CH_2Cl_2/CH_3CN mixture. [b]The dr ratios were obtained by HPLC analysis (integral of H-3′). [c]The dr ratios were determined on the basis of the ^{1}H NMR spectra of the deiodinated compounds of the diastereomers **11** and **12**, i.e., **13a−f** (refer to the corresponding ^{1}H NMR spectra in the Supporting Information). [d]The dr ratio of **11f** was determined by isolation of the diastereomers.

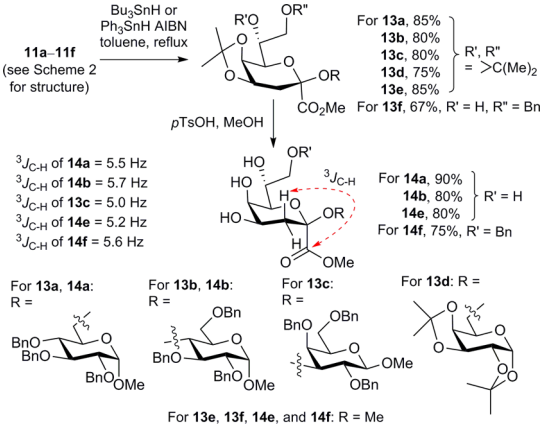
octulosonyl saccharide in **11a** on the base of the $^3J_{H\text{-}3ax'/H\text{-}4'}$ value of 8.4 Hz (indicating the axial position of H-4′).[21]

In the literature, nitrile solvents have long been regarded as a means to promote α-glycosylation for sialyl donors,[22] but the stereodirecting effect of the nitrile solvent on KDO donors remains controversal.[14,23] In the present study, we repeated the glycosylation of **10a** in a 1:2 CH_2Cl_2/CH_3CN mixture (entry 3). This improved the dr to 20:1 in favor of **11a**.

Unlike aldose sugars, the assignment of anomeric configuration for KDO glycosides is nontrivial because of the lack of an anomeric proton. Unger et al. employed the coupling constant between the axial proton at C-3 and the ^{13}C carbon at the C-1 position ($^3J_{C\text{-}1/H\text{-}3ax}$) for assignment of the anomeric configuration of a KDO glycoside with a $^5C_2$ conformation.[24] A $^3J_{C\text{-}1/H\text{-}3ax}$ value of ca. 5.0−6.0 Hz indicates a β-configuration, while a value of ≤1.0 Hz indicates an α-configuration.

Thus, glycoside **11a** was reduced with triphenyl (or tributyl) tin hydride and azo-bis(isobutyro)nitrile (AIBN)[25] to produce the deoxy derivative **13a** (Scheme 4). However, the ^{13}C NMR spectrum of **13a** was indiscernible, probably because it adopted a non-$^5C_2$ conformation (as evidenced by a $^3J_{H\text{-}3ax'/H\text{-}4'}$ value of

Scheme 4. De-iodination of 11a−f and Removal of the Isopropylidene Functions of the Deoxy Derivatives 13a,b,e,f



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≤6.0 Hz).[25] Accordingly, we removed the isopropylidene functions of **13a** to form the glycoside **14a** that gave a $^{3}J_{C\text{-}1/H\text{-}3ax}$ value of ≥5.0 Hz (see Table SII of the Supporting Information), thus confirming a β-configuration.

Thereafter, the cosolvent system described in entry 3 was used for the glycosylations of the other acceptors **10b−e**. The reactions of **10b−e** with the glycal donor **6** produced the expected β-linked glycosides **11b−e** with high dr (≥13:1) and good yield (>65%) (entries 4−8). To confirm the nitrile solvent effect, the glycosylation of **10d** was repeated in CH_2Cl_2, which yielded **11d** at a much lower 6:1 dr (entries 6 and 7).

To probe the influence of the isopropylidene protection on the stereochemistry, the glycal donor **8** bearing a single 4,5-*O*-isopropylidene group was used for the glycosylation of alcohol **10e** (entry 9). The diastereoselectivity achieved with **8** decreased significantly (compared with **6**, in entry 9), yet an acceptable dr of 6:1 for the desired disastereomer **11f** was obtained. Interestingly, the replacement of the 4,5-*O*-isopropylidene in **6** with a benzyl ether function eroded the selectivity of the glycosylation.[26] Taken together, the results indicate a β-stereodirecting effect for the 4,5-*O*-isopropylidene protecting group in KDO glycal donors.

To confirm the β-anomeric configurations of the glycosides **11b−f**, the same procedures including the deiodination and deprotection of the isopropylidene functions as used for **11a** were performed (Scheme 4). Among the deoxy derivatives **13b−f**, only **13c** gave a $^{3}J_{C\text{-}1/H\text{-}3ax}$ value of 5.0 Hz in the coupled ^{13}C NMR experiment. With the exception of **13d**,[27] the other deoxy derivatives underwent deprotection of the isopropylidene functions to afford the glycosides **14b**, **14e**, and **14f**. Eventually, these glycosides gave $^{3}J_{C\text{-}1/H\text{-}3ax}$ values of ≥5.0 Hz (see Table SI of the Supporting Information), thus confirming a β-configuration.

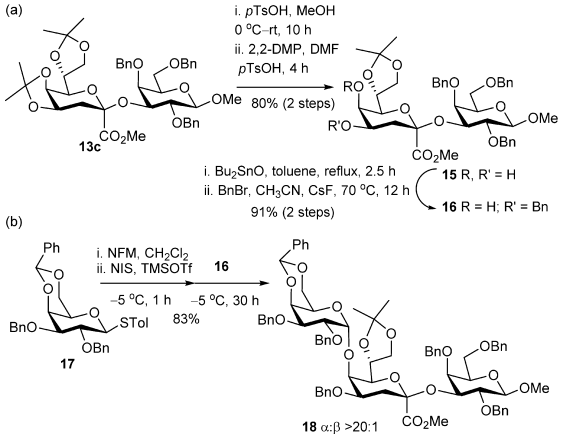
It should be noted that other than the $^{3}J_{C\text{-}1/H\text{-}3ax}$ value, we obtained the difference of the chemical shift ($\Delta\delta$) between the axial and equatorial H-3 protons of **13c**, **14a**, **14b**, **14e**, and **14f** (and trisaccharide **18**) in $CDCl_3$, which varied from 0.12 to 0.81 ppm (see Table SI in S38 of the Supporting Information). These data clearly reveal the influence of the acceptor structure on the $\Delta\delta$ of the H-3 geminal protons. As shown in the present study, a great caution should be taken when employing $\Delta\delta$ to determine the anomeric configuration of a KDO glycoside.[28]

After establishing conditions for the β-selective glycosylation, the KDO glycal donors were employed for the synthesis of protected β-KDO-containing oligosaccharides, which to the best of our knowledge, is unprecedented.

The first synthetic target was the protected α-Gal-(1→5)-β-KDO-(2→3)-Gal trisaccharide **18**. This trisaccharide unit is part of the CPS in the cell wall of *Rhizobium fredii*.[1b] The synthetic route to **18** started from the disaccharide **13c**, which was treated with *p*-TsOH in MeOH to remove the isopropylidene groups (Scheme 5a). Selective protection of the C7- and C8-hydroxyls then furnished the disaccharide intermediate **15** (Scheme 5a). Subsequent benzylation of the C4-hydroxyl in **15** with dibutyltin oxide (Bu_2SnO) followed by benzyl bromide gave the desired acceptor **16**, which was coupled with the thiogalactosyl donor **17** using the *N*-formylmorpholine (NFM)-modulated glycosylation method to produce the target trisaccharide **18** with high α-selectivity (Scheme 5b).[29] The α- and β-configurations of the nonreducing end Gal and KDO residues in **18** were confirmed by the $^{1}J_{H\text{-}1''/C\text{-}1''}$ value of 173 Hz and the $^{3}J_{C\text{-}1'/H\text{-}3'ax}$ value of 5.2 Hz, respectively.

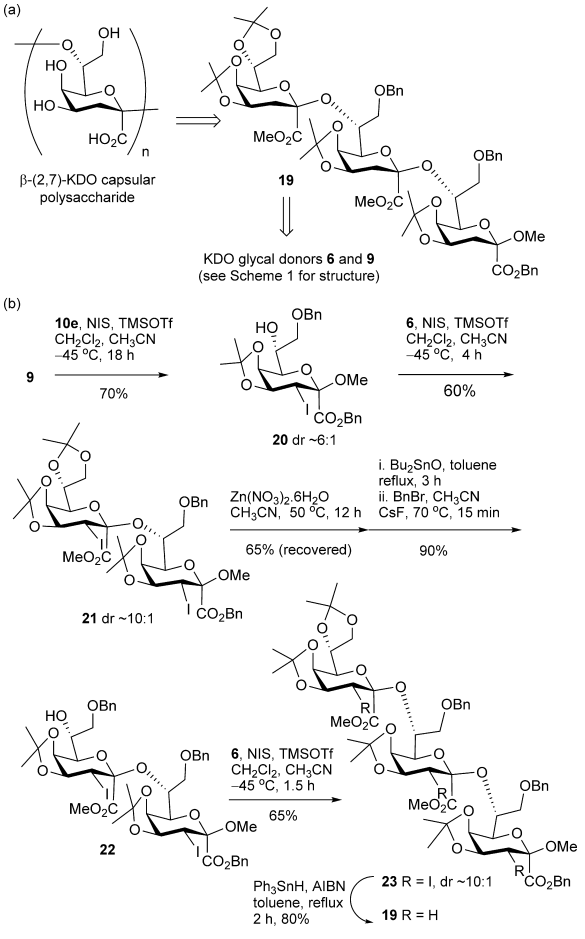
Encouraged by the synthesis of **18**, we synthesized a protected β-(2→7)-KDO trisaccharide **19** from the glycal donors **6** and **9**

Scheme 5. Synthesis of α-Gal-(1→5)-β-KDO-(2→3)-Gal Trisaccharide 18



(Scheme 6a) as found in the CPS of *Sinorhizobium meliloti*.[1a] First, the KDO glycal **9** was coupled with the alcohol **10e** to

Scheme 6. Synthesis of β-(2→7)-KDO Trisaccharide



produce the 3-iodo β-glycoside **20** with a dr of 6:1 (Scheme 6b). The subsequent glycosylation of **20** with the KDO glycal **6** gave the diiodo-substituted β-KDO-(2→7)-KDO disaccharide **21** in 60% isolated yield with a dr of ca.10:1 (estimated from TLC). Treatment of the disaccharide **21** with a dilute $Zn(NO_3)_2$ solution at 50 °C selectively removed the 7,8-*O*-isopropylidene group and produced a diol intermediate, which underwent

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benzylation of the C-8 hydroxyl to produce the disaccharide acceptor **22**. Iterative glycosylation of **22** with the KDO glycal **6** afforded the triiodo-substituted β-KDO trisaccharide **23** in 65% isolated yield with a high dr of >10:1 (estimated from TLC). The trisaccharide **23** was reduced by treatment with Ph_3SnH and AIBN to furnish the target β-KDO trisaccharide **19**. The $^3J_{C\text{-}1/H\text{-}3ax}$ values of the internal KDO glycosidic bonds in **19** were found to be 6.1 and 4.4 Hz, confirming a β-anomeric configuration.

In summary, we describe a practical synthetic route for the preparation of KDO glycals, which are efficient glycosyl donors in the synthesis of β-KDO glycosides and oligosaccharides. The reported synthetic method provides access to these valuable oligosaccharide compounds.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for preparation of KDO glycals **6**–**9**, glycosides **11a**–**f**, **13a**–**f**, **14a**,**b**,**e**,**f**, **15**, and **16**, β-KDO-containing oligosaccharides **18** and **19**, and relevant NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: tmong@mail.nctu.edu.tw.

Notes

The authors declare no competing financial interest.

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(20) We initially used TfOH acid promoters as described in ref 11c (and ref 22a), but the β-selectivity of glycosylation was lower than that obtained from the use of TMSOTf.
(21) The $^3J_{H\text{-}3ax'/H\text{-}4'}$ coupling constants for glycosides **11c** and **11f** were also obtained ($\geq$ 8.0 Hz), which supported a $^5C_2$ conformation. Accordingly, such $^2C_5$ conformation was assigned to other 3-iodo-substituted KDO-glycosides including **11b**,**d**–**e** and **20**–**23**. See: Birnbaum, G. I.; Roy, R.; Brisson, J.-R.; Jennings, H. J. *J. Carbohydr. Chem.* **1987**, *6*, 17–39.
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(26) We examined the glycosylation of **10a** with a 4,5-di-*O*-benzyl-7,8-*O*-isopropylidene-protected KDO glycal donor, but glycosylation products in ca. 1:1 dr were obtained (unpublished).
(27) The configuration of **13d** was inferred from comparison with a similar isopropylidene-protected β-KDO glycoside in the literature. For a boat $B_{2,5}$ conformation, the $\Delta\delta$(s) of $\geq$0.6 ppm and $\leq$0.28 ppm suggest α- and β-anomeric configurations, respectively: Imoto, M.; Kusunose, N.; Matsuura, Y.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1987**, *28*, 6277–6280.
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