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Stereodirecting Effect of C5-Carboxylate Substituents on the Glycosylation Stereochemistry of 3-Deoxy-D-*manno*-oct-2-ulosonic Acid (Kdo) Thioglycoside Donors: Stereoselective Synthesis of α- and β-Kdo Glycosides

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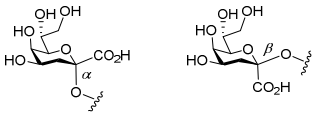
ABSTRACT: The stereodirecting effect of C5-ester functions on the glycosylation stereoselectivity of 3-Deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) ethyl thioglycoside donors is presented. The coupling of 5-*O*-arylcarbonyl or acetyl protected Kdo thioglycosides with acceptors proceeds in an α-selective and high-yielding manner, leading to formation of α-linked Kdo glycosides products. On the other hand, the glycosylation stereoselectivity of the 5-*O*-2-quinolinecarbonyl (Quin) or 4-nitropicoloyl substituted Kdo thioglycoside donors is switchable: (1) the glycosylation of the 5-*O*-Quin carrying Kdo donors with primary glycosyl acceptors shows complete β-stereoselectivity, furnishing the corresponding β-glycosides in good-to-excellent yield. (2) the stereochemical outcome of the secondary acceptors with these Kdo donors is determined mainly by the stereoelectronic nature of the acceptor. Only or predominant α anomeric products are obtained when the Kdo donors couple with the disarmed or highly crowded secondary carbohydrate acceptors, while the selectivity may switch to predominant β in the glycosylation of the 5-*O*-4-nitropicoloyl carrying donor with more reactive secondary alcohols. The synthetic use of the newly developed Kdo donors **1c** and **7b** has been demonstrated by facile preparation of a structurally unique trisaccharide motif **19** which possesses both α- and β-Kdo glycosidic bonds.

INTRODUCTION

3-Deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), a acidic eight-carbon saccharide, is a characteristic component of bacterial lipopolysaccharide (LPS) and capsular polysaccharide (CPS).[1] Kdo glycosides can exist in nature as both α- and β-anomers at C2-positions (Figure 1). Bacterial LPS and CPS are known to be closely connected with the pathogenicity and survival of pathogenic bacteria. Chemical synthesis of Kdo-containing portions of LPSs and CPSs in pure and homogeneous forms has attracted great attention since these molecules will benefit the development of potential carbohydrate-based vaccines or therapeutics against bacterial pathogens.[2] However, the stereoselective and high-yielding synthesis of a Kdo glycoside poses a great challenge in carbohydrate chemistry owing to (1) the lack of C3-participating group which hampers the stereocontrol of product formation, (2) the presence of C1-electron-

withdrawing carboxylic group that heavily deactivates the anomeric center, and (3) the formation of a significant amount of 2,3-dehydro byproduct during glycosylation reaction.[3]

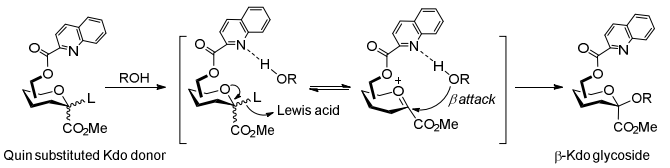
Figure 1. Structures of α- and β-Kdo glycosides



A number of studies have focused on the development of Kdo glycosyl donors for efficient α-glycosylation, including the utility of various anomeric activating groups, such as halides,[4] *N*-phenyltrifluoroacetimidates,[5] glycals,[6] thioglycosides,[7] as well as the installment of C3-stereodirecting auxiliary groups.[8] Among the Kdo donors reported to date, the cyclic 4,5-*O*-isopropylidene acetal or 4,5-di-*O*-*tert*-butyldimethylsilyl (TBS) ether protected Kdo fluorides or *N*-phenyltrifluoroacetimidates exhibited special validity and have been applied to the synthesis of LPS-related α-Kdo-containing carbohydrates.[4a-d,5b] Besides, in the preceding paper,[7e] our group have studied the effect of cyclic 5,7-*O*-acetals on the Kdo glycosylation stereoselectivity and developed an efficient methodology for the assembly of α-Kdo glycoside employing bulky 5,7-*O*-di-*tert*-butylsilylene (DTBS) protected Kdo thioglycosides as glycosyl donors. The approach allows a wide range of acceptors to be used, producing diverse Kdo glycosides including the naturally occurring α-(2→4)-, (2→5)-, and (2→8)-linked Kdo-Kdo dimers in good-to-excellent yields with complete α-stereoselectivity.

Compared to the considerable progress on the synthesis of α-Kdo glycosides, fewer synthetic studies of β-Kdo glycosides have been reported so far. In 2012, Ling et al. discovered that a class of Kdo 1-*C*-arylglycals could undergo β-glycosylation with alcohols when mediated by *N*-iodosuccinimide (NIS) to give 2-iodo-β-D-ketopyranosides.[9] These intermediates were in turn subjected to deiodination and followed by oxidization of the C1-aryl group to give the corresponding β-Kdo glycosides. Later, the Mong group described that the 4,5,7,8-di-*O*-isopropylidene protected Kdo glycal donor showed excellent β-stereoselectivity of glycosylation in a CH_2Cl_2-CH_3CN co-solvent, giving high yields of 2-iodo-β-Kdo glycosides which could be converted into the desired β-Kdo glycosides after reductive removal of the 2-iodide.[10] Gauthier et al. developed the peracetylated Kdo thioglycosides equipped with a 4'-methoxyphenacyl (Phen) group at C1-position with which a series of β-Kdo glycosides were synthesized in good yield but with moderate β-selectivity only. Additionally, the Phen auxiliary group has to be cleaved after the glycosylation, thereby lowering the synthetic efficiency.[11] More recently, Yang et al. reported a Gold(I)-catalyzed synthesis of β-Kdo glycosides using Kdo *ortho*-hexynylbenzoate as donor. Despite the fact that complete β-selectivity was realized in this direct glycosylation reaction, only one pyranosyl alcohol especially no Kdo alcohol was used as acceptor, which limits the application of the method.[12]

Scheme 1. 2-Quinolinecarbonyl-Directed Formation of β-Kdo Glycoside



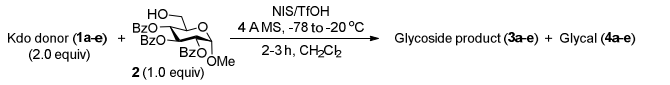
Recently, a facile hydrogen-bond-mediated aglycone delivery (HAD) approach was uncovered by Demchenko and co-workers to synthesize stereoselectively various challenging 1,2-*cis*-pyranosides.[13] Relying on such a concept, our group developed a novel 2-quinolinecarbonyl (Quin)-assisted 1,2-*cis*-furanosylation method for efficient preparation of β-arabino- and α-galactofuranosides.[14] The Quin group, serving as a hydrogen bond acceptor, shows a strong stereocontrolling ability in the condensation of furanosyl thioglycoside donors with a wide range of carbohydrate and non-carbohydrate alcohols. In this work, we sought to expand the Quin-directed stereoselective glycosylation strategy to the synthesis of the relevant Kdo glycoside. As shown by Scheme 1, we envisioned that, in a typical glycosylation course of a Quin substituted Kdo donor, the hydrogen bond tethering between the Quin group and the acceptor is over the sugar ring and thereby would be likely to offer a β-facial selectivity for the attack of the acceptor. As a consequence, a β-Kdo glycoside product would be formed. In order to test the effectiveness of hydrogen bond as a stereocontrolling factor on the construction of β-Kdo glycoside, a series of Kdo donors including 4-, 5-, 7-, and 8-*O*-quinoline-2-carbonylated ethyl thioglycosides **1a-e** (Table 1) were synthesized (see Supporting Information) and their glycosylation behaviors were examined by reaction with model glucosyl acceptor **2**.[15] All glycosylations were run with 2 equiv of the donor and 1 equiv of the acceptor in the presence of NIS (3.0 equiv), catalytic triflic acid (TfOH), and 4 Å molecular sieve (MS) at –78 to –20 °C in CH_2Cl_2 (Table 1). The anomeric configuration of Kdo glycosides with a $^5C_2$ conformation is confirmed based on the coupling constant between the axial proton at C3-position and the ^{13}C carbon at C1-position in the undecoupled ^{13}C NMR spectra. For the α-Kdo anomer, the $^3J_{C\text{-}1/H\text{-}3ax}$ value is less than 1.5 Hz, while for the β-Kdo anomer, the $^3J_{C\text{-}1/H\text{-}3ax}$ is about 5.0-6.0 Hz.[4e,16]

RESULTS AND DISCUSSION

As shown in Table 1, entries 1-5, the reactions of **1a-e** with **2** provided the corresponding glycoside products **3a-e** in satisfactory 75-88% chemical yields but with quite different degree of anomeric selectivity. The coupling of donor **1a** carrying a Quin substituent at the equatorial C4-position showed a poor β-anomeric selectivity (α/β 1:3, entry 1). Gratifyingly, donors **1b**,**c** both with an axially oriented C5-Quin group exhibited excellent stereocontrolling property in the glycosylations with **2** and complete β-stereoselectivities were obtained for the products **3b**,**c** (entries 2 and 3). Furthermore, the reactions of the 7- and 8-*O*-Quin substituted **1d**,**e** displayed no or little β-selectivity, respectively (entries 4 and 5), which might be due to the flexibility of the C7- and C8-positions. Of note is that though

elimination byproducts (**4a-e**) were obtained in 40-53% yield together with the Kdo disaccharides in each case, these byproducts could be conveniently isolated from the expected Kdo glycosides by silica gel column chromatography.

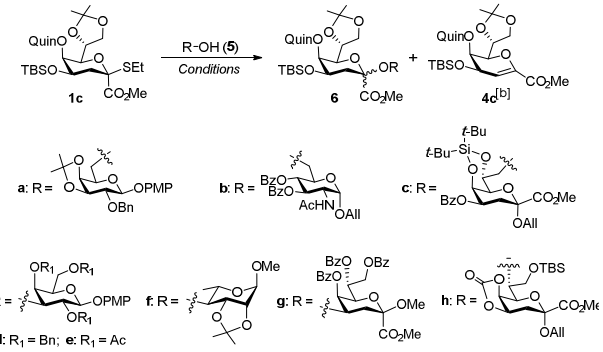
Table 1. Glycosylation of Quin Substituted Kdo Donors (1a-e)[a]



Entry	Donor	Product	Yield[b] (α/β)[c]	Glycal (Yield)[d]
1	**1a**: R_1 = Quin, R_2 = Bn	**3a**: R_1 = Quin, R_2 = Bn	82% (1:3)	**4a**: R_1 = Quin, R_2 = Bn (45%)
2	**1b**: R_1 = Bn, R_2 = Quin	**3b**: R_1 = Bn, R_2 = Quin	85% (β only)	**4b**: R_1 = Bn, R_2 = Quin (40%)
3	**1c**: R_1 = TBS, R_2 = Quin	**3c**: R_1 = TBS, R_2 = Quin	88% (β only)	**4c**: R_1 = TBS, R_2 = Quin (43%)
4	**1d**: R_1 = Quin, R_2 = TBS	**3d**: R_1 = Quin, R_2 = TBS	75% (1:1)	**4d**: R_1 = Quin, R_2 = TBS (53%)
5	**1e**: R_1 = Bn, R_2 = Quin	**3e**: R_1 = Bn, R_2 = Quin	80% (1:3)	**4e**: R_1 = Bn, R_2 = Quin (46%)

[a] Glycosylations were run with donor **1a-e** (2 equiv, 60 mM), acceptor (1 equiv), NIS (3 equiv)/TfOH (0.2 equiv), 4 Å molecular sieves (MS) in anhydrous CH_2Cl_2 at –78 → –20 °C for 2-3 h. [b] Isolated yield based on the acceptor. For entries 1, 4, and 5, the yield is combined yield of α/β-isomers. [c] Determined by ^{1}H NMR of the corresponding isomer mixture. [d] Based on the donor.

Table 2. Stereoselective Glycosylation of 1c with Various Acceptors[a]



Entry	Acceptor	Conditions	Product	Yield[c] (α/β)[d]
1	**5a**	NIS/TfOH, CH_2Cl_2 –78 → –20 °C	**6a**	87% (β only)
2	**5b**	NIS/TfOH, CH_2Cl_2 –78 → 0 °C	**6b**	82% (β only)

3	**5c**	NIS/TfOH, CH_2Cl_2 −78 → −15 °C	**6c**	80% (β only)
4	**5d**	NIS/TfOH, CH_2Cl_2 −78 → −15 °C	**6d**	12% (α only)
5	**5d**	IBr/AgOTf, CH_2Cl_2 −80 °C	**6d**	71% (α only)
6	**5e**	IBr/AgOTf, CH_2Cl_2 −80 °C	**6e**	72% (α only)
7	**5f**	IBr/AgOTf, CH_2Cl_2 −80 °C	**6f**	85% (α only)
8	**5g**	IBr/AgOTf, CH_2Cl_2 −80 °C	**6g**	82% (α only)
9	**5h**	IBr/AgOTf, CH_2Cl_2 −80 °C	**6h**	67% (α only)

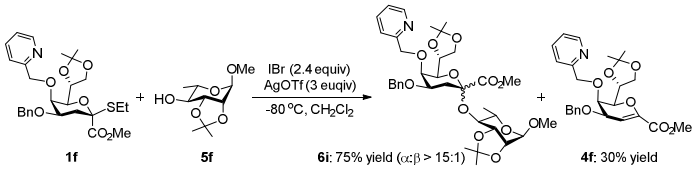
[a] Glycosylations were run with Kdo thioglycoside donor (2 equiv), acceptor (1 equiv), NIS (3 equiv)/TfOH (0.2 equiv) (for entries 1-4) or IBr (2.4 equiv)/AgOTf (3 equiv) (for entries 5-9), 4 Å MS in anhydrous CH_2Cl_2. [b] The yield of **4c** was based on donor **1c** and was about 40-76%. [c] Isolated yield based on the acceptor. [d] Determined by ^{1}H NMR of the corresponding isomer mixture.

After investigating the effect of the substituting position of Quin group on the glycosylation selectivity, we next surveyed the glycosylation reaction of the C5-Quin substituted donor **1c** with diverse glycosyl acceptors. For this purpose, a set of representative alcohols (**5a**-**h**, Table 2) were synthesized. As summarized in Table 2, we were pleased to find that, upon treatment with NIS/TfOH (cat.) in CH_2Cl_2, primary sugar alcohols, including galactose (Gal) 6-OH acceptor **5a**, *N*-acetylglucosamine (GlcNAc) 6-OH acceptor **5b**[7e] as well as Kdo 8-OH acceptor **5c**[7e] coupled very well to donor **1c**, giving rise to the corresponding disaccharides **6a**-**c** in 80-87% yields as exclusively β-isomers (entries 1-3). However, we met great difficulties in the reaction of the secondary 2,4,6-tri-*O*-benzylated Gal alcohol **5d**[17] with **1c**. In the event, only 12% yield of the desired disaccharide **6d** was obtained under the NIS/TfOH promotion and to our surprise the glycosylation stereochemistry completely reversed to α (Table 2, entry 4). Our explanation to the reversal of the selectivity is that due to the steric hindrance of the secondary hydroxyl group of the acceptor, there is probably no hydrogen bond between the Quin group and the acceptor but the remote participation of the carbonyl or the sp^2-hybridized nitrogen of the Quin group becomes the stereodirecting factor in the glycosylation, thus resulting in an α-linked product. In fact, a similar remote neighboring group participating phenomenon of the axial 4-*O*-carboxylate ester groups in Gal donors had been observed by Boons et al., which led to preferential formation of 1,2-*cis*-α-galactosides.[18] To improve the glycosylation efficiency of the secondary alcohol **5d** with **1c**, a number of thioglycoside promoters, such as NIS/sliver triflate (AgOTf), iodobromide (IBr)/AgOTf, and the one-electron oxidizing agent tris(4-bromophenyl)ammoniumyl hexachloroantimonate (TBPA)[19] were screened. Consequently, we found that when the glycosylation was catalyzed with IBr (2.4 equiv)/AgOTf (3.0 equiv) reagent system in CH_2Cl_2 at –80 °C, previously utilized by Oscarson and co-workers for the activation of Kdo ethyl thioglycosides,[7d] the product **6d** was obtained in an elevated 71% yield as α-anomer only (Table 2, entry 5). Then, the optimized reaction conditions were further applied to the coupling of other secondary

glycosyl nucleophiles, including the disarmed peracetylated Gal alcohol **5e**,[20] rhamnose (Rha) 4-OH acceptor **5f**,[21] Kdo 4- and 7-OH acceptors **5g**,**h**.[22] As a result, the reaction of these substrates with **1c** proceeded smoothly to yield, respectively, disaccharides **6e**,**f**, (2→4)- and (2→7)-linked Kdo disaccharides **6g**,**h** in good yield and also as a single α-ketoside (Table 2, entries 6-9).

In order to verify the remote participation effect of the sp^2-hybridized nitrogen at the C5-position, we carried out a glycosylation between 4-*O*-Bn-5-*O*-picolinyl (Pic) substituted Kdo thioglycoside donor **1f** and the secondary Rha alcohol **5f** under the activation of IBr/AgOTf in CH_2Cl_2. As shown in Scheme 2, the reaction still exhibits high α-stereoselectivity, affording disaccharide product **6i** in 75% yield as a separable α/β (>15:1) mixture. This provides an indication that the participation of the Pic's nitrogen on the donor's anomeric center may exist.[23]

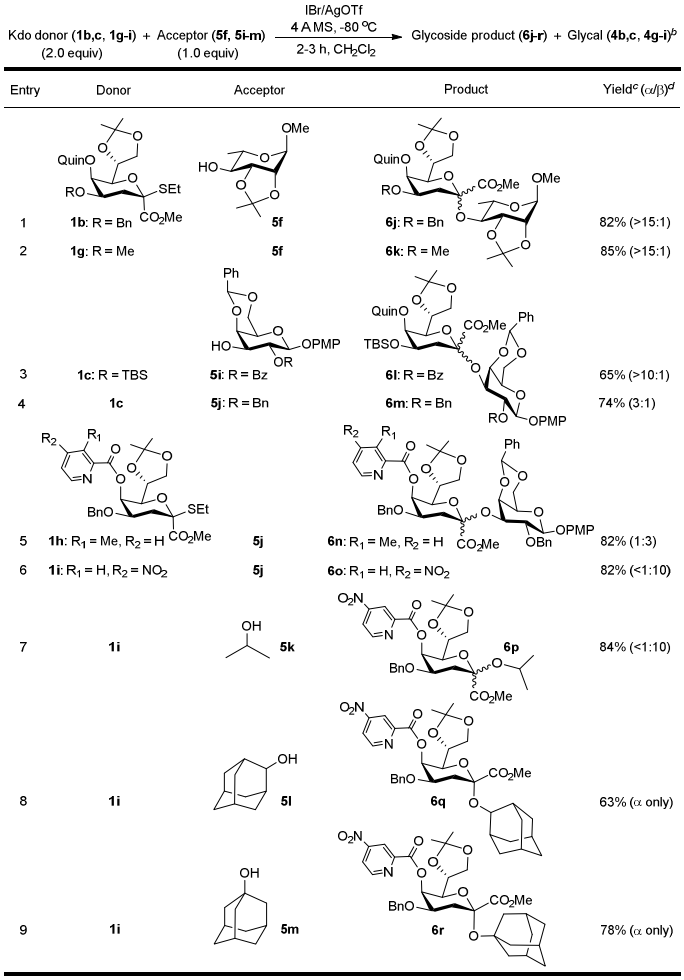
Scheme 2. Glycosylation of 5-*O*-Pic Substituted Kdo Donor 1f with Secondary Acceptor 5f



Moreover, the factors that influence the glycosylation stereoselectivity of the secondary acceptor were studied in more detail and the results were summarized in Table 3. We first investigated the steric effect of the C4-protecting group of the Kdo donor on the glycosylation. Thus, the 4-*O*-Bn and Me substituted donors **1b** and **1g** were reacted with Rha alcohol **5f** under the promotion of IBr (2.4 equiv)/AgOTf (3 equiv) in CH_2Cl_2. As a result, similar to the glycosylation of the 4-*O*-TBS substituted donor **1c** with **5f** (Table 2, entry 7), the two glycosylations still afforded glycoside products with high α-selectivity (Table 3, entries 1 and 2). Next, we examined the influence of the acceptor alcohol and the substituents on the C5-*N*-heterocyclic ring of the donor. Similar to the reactions of the 2,4,6-tri-*O*-Bn/Bz protected Gal 3-OH alcohol **5d**/**e** with donor **1c** where only α-anomeric products were formed (Table 2, entries 5 and 6), the reaction of the 2-*O*-Bz-4,6-*O*-benzylidene protected Gal 3-OH substrate **5i**[24] with **1c** also provided high α-selectivity (Table 3, entry 3, α/β >10:1), but the reaction of the 2-*O*-Bn-4,6-*O*-benzylidene protected counterpart **5j**[25] with **1c** showed a significant drop in α-selectivity (Table 3, entry 4, α/β 3:1). In contrast, when the conformationally locked **5j** was condensed with the 5-*O*-3-methylpicoloyl or 4-nitropicoloyl substituted donors **1h**,**i**, the glycosylation stereoselectivity reversed, yielding the disaccharide products **6n**,**o** in good yields with low or excellent β-selectivities (Table 3, entry 5, α/β 1:3 and entry 6, α/β <1:10, respectively). Finally, we further assessed the effect of the acceptor on the stereochemical outcome by reaction of the 5-*O*-4-nitropicoloyl carrying **1i** with alcohols **5k**-**m**. As outlined in Table 3, entry 7, the reaction of **1i** with small isopropanol **5k** showed excellent β-selectivity, delivering the corresponding β-linked Kdo glycoside **6p** in good yield. However, in the couplings of **1i** with more sterically hindered adamantanols **5l**,**m**, only α-anomeric products were observed (Table 3, entries 8 and 9). Taken together, these experiments reveal that (1) the electronic and steric nature of the secondary acceptors is the

crucial factor affecting the glycosylation stereoselectivity. The decrease in steric hindrance of the secondary alcohols is beneficial to the improvement of the β-anomeric product formation, while the glycosylation of disarmed or highly crowded acceptors tends to form the products with high or complete α-selectivity. (2) the bulkiness at the C4 position of Kdo donor has no effect on the diastereoselectivity of the glycosylation. But the electron-withdrawing substituent on the C5-*N*-heterocyclic ring of the donor may elevate the β-selectivity. High β-stereocontrol can be achieved in the couplings between the C5-4-nitropicoloylated donor **1i** and the secondary acceptors with relatively low steric hinderance.

Table 3. Glycosylation of Various Secondary Acceptors with Kdo Donors[a]

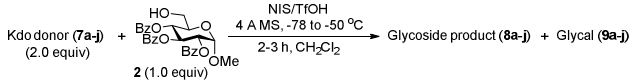


[a] Glycosylations were run using Kdo thioglycoside donor (2 equiv) and acceptor (1 equiv) under the activation of IBr (2.4 equiv)/AgOTf (3 equiv) in anhydrous CH_2Cl_2 at –80 °C for 2-3 h. [b] The yields of glycals **4b**,**c**, **4g-i** were based on the corresponding donors **1b**,**c**, **1g-i** and were about 30-45%. [c] Combined yield of α/β-isomers based on the acceptor. [d] Determined by ^{1}H NMR of the corresponding isomer mixture.

We continued to study the remote participating effect of carbonyl group at C5-position on the glycosylation selectivity. Thus, a set of 5-*O*-arylcarbonyl or acetyl substituted Kdo thioglycoside substrates **7a-g** were prepared and reacted with model compound **2** under the catalysis of NIS/TfOH (cat.) in CH_2Cl_2 (Table 4). As expected, these donors exerted good-to-excellent α-stereoselectivities in all glycosylations with **2**, affording the corresponding disaccharide products **8a-g** as sole or major α-anomers in satisfactory yields (Table 4, entries 1-7). These experiments obviously illustrate the strong α-directing ability of the C5-acyl substitutions. In general, the glycosylations of 5-*O*-arylcarbonylated donors **7a-d** led to higher α-anomeric selectivity than that of 5-*O*-acetylated donor **7e** (Table 4, entries 1-4 vs entry 5). In addition, the electron density of the ketone functionality significantly affects the stereochemical outcome. For instance, compared to the benzoyl (Bz) group (**7b**), the more electron-rich *p*-methoxybenzoyl group (**7c**) provided a much greater α-stereocontrol, enhancing the α/β ratio from 15:1 to 25:1 (entry 2 vs entry 3), whereas the more electron-deficient *p*-nitrobenzoyl substituent (**7d**) brought a lower α-selectivity (α/β 12:1, entry 4 vs α/β 15:1, entry 2). Also examined was the influence of the C4-protecting group on the reaction. As depicted by entries 6 and 7 in Table 4, the couplings of 4-*O*-Bn and Nap (2-naphthylmethyl) donors **7f**,**g** with **2** were much less stereoselective (α/β 6:1 and 8:1, respectively) than that of 4-*O*-TBS counterparts **7a-e** (Table 4, entries 6, 7 vs entries 1-5). To further understand the impact of the substituting position of acyl group on the reaction, we evaluated the glycosylating behaviors of donors **7h-j** carrying a Bz moiety at C4-, C7-, and C8-position, respectively. As indicated in Table 4, the reaction of the 4-*O*-benzoylated **7h** with **2** showed slight α-stereocontrol (α/β 2:1, entry 8), but in contrast the reactions of the 7- and 8-*O*-benzoylated **7i**,**j** favored production of β-anomers (α/β 1:5, entries 9, 10).

The reaction scope of the 4-*O*-TBS-5-*O*-Bz protected Kdo donor **7b** was further investigated. The glycosylations of **7b** with the primary sugar alcohols **5a-c** proceeded in an α-selective and high-yielding manner, furnishing disaccharide glycosides **10a**,**b**, and (2→8)-linked Kdo dimer **10c** in excellent yield (85-89%) and high α-selectivity (Table 5, entries 1-3). Furthermore, **7b** was also proved to be an efficient glycosyl donor in the IBr/AgOTf-promoted couplings with sterically demanding secondary Gal (**5e**), Rha (**5f**), and Kdo (**5n**) acceptors and the corresponding disaccharide products **10e**,**f**, and **10n** were isolated in 75%, 91%, and 71% yield with complete α-selectivity (Table 5, entries 4-6, respectively). However, in the IBr/AgOTf-catalyzed reaction of Kdo alcohol **5o**,[7e] a difficult glycosyl acceptor because of the poor nucleophilicity of its 5-OH group, only a small amount of the desired α-(2→5)-bound Kdo-Kdo disaccharide **10o** was isolated (entry 7). To our delight, the yield of **10o** was improved to 50% (entry 8) when the coupling was carried out upon activation with TBPA (1.2 equiv) in CH_3CN, which was previously employed by us in the condensation of the 5,7-*O*-DTBS protected Kdo thioglycosides with Kdo 5-OH alcohol.[7e]

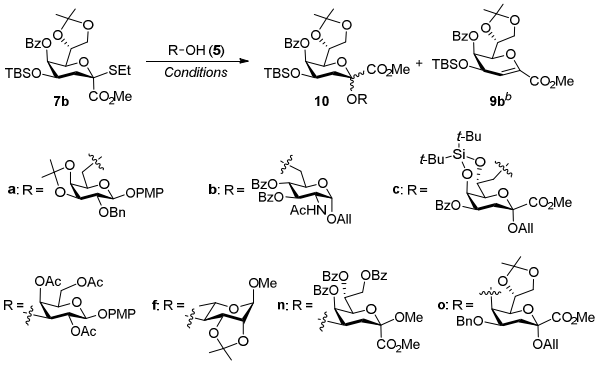
Table 4. Glycosylation of Kdo Donors (7a-j)[a]



Entry	Donor	Product	Yield[b] (α/β)[c]	Glycal (Yield)[d]
1	**7a**: R_1 = TBS, R_2 = 2-naphthoyl	**8a**: R_1 = TBS, R_2 = 2-naphthoyl	87% (25:1)	**9a**: R_1 = TBS, R_2 = 2-naphthoyl (36%)
2	**7b**: R_1 = TBS, R_2 = Bz	**8b**: R_1 = TBS, R_2 = Bz	86% (15:1)	**9b**: R_1 = TBS, R_2 = Bz (36%)
3	**7c**: R_1 = TBS, R_2 = 4-methoxybenzoyl	**8c**: R_1 = TBS, R_2 = 4-methoxybenzoyl	88% (25:1)	**9c**: R_1 = TBS, R_2 = 4-methoxybenzoyl (36%)
4	**7d**: R_1 = TBS, R_2 = 4-nitrobenzoyl	**8d**: R_1 = TBS, R_2 = 4-nitrobenzoyl	84% (12:1)	**9d**: R_1 = TBS, R_2 = 4-nitrobenzoyl (34%)
5	**7e**: R_1 = TBS, R_2 = Ac	**8e**: R_1 = TBS, R_2 = Ac	84% (10:1)	**9e**: R_1 = TBS, R_2 = Ac (37%)
6	**7f**: R_1 = Bn, R_2 = Bz	**8f**: R_1 = Bn, R_2 = Bz	91% (6:1)	**9f**: R_1 = Bn, R_2 = Bz (34%)
7	**7g**: R_1 = Nap, R_2 = Bz	**8g**: R_1 = Nap, R_2 = Bz	87% (8:1)	**9g**: R_1 = Nap, R_2 = Bz (37%)
8	**7h**	**8h**	93% (2:1)	**9h** (25%)
9	**7i**: R_1 = Bz, R_2 = Bn	**8i**: R_1 = Bz, R_2 = Bn	82% (1:5)	**9i**: R_1 = Bz, R_2 = Bn (43%)
10	**7j**: R_1 = Bn, R_2 = Bz	**8j**: R_1 = Bn, R_2 = Bz	88% (1:5)	**9j**: R_1 = Bn, R_2 = Bz (33%)

[a] Glycosylations were run using Kdo thioglycoside donor (2 equiv) and acceptor (1 equiv) under the activation of NIS (3 equiv)/TfOH (0.2 equiv) in anhydrous CH_2Cl_2 at –78 → –50 °C for 2-3 h. [b] Combined yield of α/β-isomers based on the acceptor. [c] Determined by ^{1}H NMR of the corresponding isomer mixture. [d] Based on the donor.

Table 5. α-Selective Glycosylation of 7b with Various Acceptors[a]

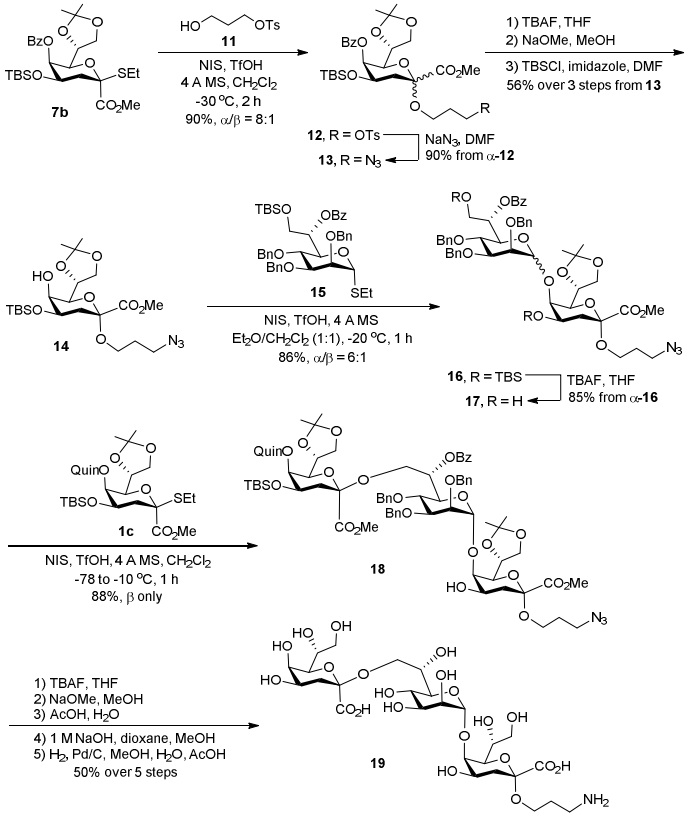


Entry	Acceptor	Conditions	Product	Yield[c] (α/β)[d]

1	**5a**	NIS/TfOH, CH_2Cl_2 –78 → –50 °C	**10a**	85% (12:1)
2	**5b**	NIS/TfOH, CH_2Cl_2 –78 → –50 °C	**10b**	86% (>15:1)
3	**5c**	NIS/TfOH, CH_2Cl_2 –78 °C	**10c**	89% (>15:1)
4	**5e**	IBr/AgOTf, CH_2Cl_2 –80 °C	**10e**	75% (α only)
5	**5f**	IBr/AgOTf, CH_2Cl_2 –80 °C	**10f**	91% (α only)
6	**5n**	IBr/AgOTf, CH_2Cl_2 –80 °C	**10n**	71% (α only)
7	**5o**	IBr/AgOTf, CH_2Cl_2 –80 °C	**10o**	-
8	**5o**	TBPA, CH_3CN –30 → –20 °C	**10o**	50% (α only)

[a] Glycosylations were run using Kdo thioglycoside donor (2 equiv) and acceptor (1 equiv) under the activation of NIS (3 equiv)/TfOH (0.2 equiv) (for entries 1-3) or IBr (2.4 equiv)/AgOTf (3 equiv) (for entries 4-7) in CH_2Cl_2 or TBPA (1.2 equiv) in CH_3CN (for entry 8). [b] The yield of **9b** was based on donor **1c** and was about 40-60%. [c] Isolated yield based on the acceptor. For entries 1-3, the yield is combined yield of α/β-isomers. [d] Determined by ^1H NMR of the corresponding isomer mixture.

Scheme 3. Synthesis of β-Kdo-(2→7)-α-Hep-(1→5)-α-Kdo Trisaccharide (19)



To demonstrate the utility of these methods in the synthesis of Kdo-containing oligosaccharides, we targeted the assembly of trisaccharide **19** (Scheme 3). This trisaccharide motif, possessing a linear β-Kdo-(2→7)-α-Hep-(1→5)-α-Kdo backbone, corresponds to the part structure of the LPS from marine bacterium *Alteromonas macleodii* ATCC 27126^{T}.[26] Its unique structural features include: (1) it comprises both α- and β-Kdo glycosidic linkages, which is rarely found in natural LPS. (2) the L-*glycero*-D-*manno*-heptopyranose (Hep) residue attaches to the inert 5-OH of the Kdo unit, which brings extra difficulty in creating the trisaccharide.[6b] Here, we decided to adopt the newly developed Kdo donors **1c** and **7b** as key building blocks for the construction of the β- and α-Kdo linkages, respectively. As shown by Scheme 3, the target compound, equipped with an α-propyl amino linker at the reducing end, would be synthesized by a stepwise approach. The amino linker will facilitate the subsequent application in neoglycoconjugate preparation. Thus, the synthesis of **19** started with the glycosylation between the α-directing donor **7b** and alcohol **11** according to the aforementioned conditions to furnish the glycoside **12** in 90% yield as a separable α/β (8:1) diastereomixture. Pure α-**12** was easily converted to azide **13** (90% yield), which was then subjected to selective removal of the 5-*O*-Bz protecting group. However, attempted deprotection under basic conditions such as sodium methoxide (NaOMe) in MeOH and sodium hydroxide (NaOH) in THF/H_2O led to simultaneous cleavage of C4- and C5-protecting groups. In the end, **13** was successfully converted to the required Kdo 5-OH alcohol **14** via a three-step sequence: (i) deprotection of 4-*O*-TBS with tetrabutylammonium fluoride (TBAF) in THF, (ii) Zemplén deacylation of 5-*O*-benzoate group with NaOMe in MeOH, and (iii) re-introduction of a TBS group on C4 with TBSCl and imidazole in DMF, delivering **14** in 56% yield for three steps. This Kdo derivative was then condensed with Hep thioglycoside building block **15** in Et_2O/CH_2Cl_2 (1:1) co-solvent under NIS (2 equiv)/TfOH (cat.) activation at –20 °C for 1 h to generate the challenging (1→5)-linked Hep-Kdo disaccharide **16** as a separable mixture of α/β-anomers (α/β 6:1, 86% combined yield). The α-configuration of the newly formed Hep glycoside was confirmed by the coupling constant between C1 and H1 of the Hep residue ($^{1}J_{C1/H1}$ = 170.4 Hz).[27] Subsequent removal of the C4- and C7'-TBS groups of the resulting α-**16** upon treatment with TBAF in THF gave cleanly disaccharide alcohol **17** (85% yield). Then, using the same NIS/TfOH-mediated protocol, **17** was coupled readily and stereospecifically with the β-directing donor **1c** and produced the desired trisaccharide **18** in a good 88% yield (β only) without formation of α-anomeric byproduct. The global deprotection of **18** was fulfilled in the following order: removal of the TBS group with TBAF in THF, cleavage of the isopropylidene functionalities with acetic acid (AcOH) in water, removal of all the ester groups via hydrolysis with NaOMe in MeOH, release of the carboxylic group via saponification with NaOH in a dioxane/MeOH (3:1) co-solvent, and finally deprotection of the Bn ethers and reduction of the azide to amine by catalytic hydrogenolysis over Pd/C. Purification of the deprotected trisaccharide was performed using gel filtration chromatography on Sephadex LH-20 column with water as eluent to produce **19** as a white solid in 50% yield over five steps. The structure of trisaccharide **19** was verified through the use of NMR and ESIMS spectral analysis. The α- and β-configurations of the reducing and nonreducing end Kdo units in **19** were determined on the basis of the undecoupled ^{13}C NMR analysis ($^{3}J_{C-1/H-3ax}$ = 0 and 6.0 Hz,

respectively).[16] Further support for the structure came from the high-resolution MS data, which provided an $(M + Na)^+$ signal at m/z 730.2383 (calcd 730.2376).

CONCLUSIONS

In conclusion, the tuning effect of carboxylate ester groups at C5-position on the glycosylation stereoselectivity of Kdo thioglycoside donors has been investigated. On one hand, the glycosylations of the Kdo thioglycosides having 5-*O*-benzoate or acetate groups with various acceptors are found to be highly α-selective, affording α-glycoside products in good yields. On the other hand, the glycosylation stereochemistry of the 5-*O*-Quin or 4-nitropicoloyl substituted Kdo donors is switchable: (1) exclusive β-selectivity is realized when the 5-*O*-Quin Kdo donor reacts with primary sugar alcohols. (2) the glycosylation selectivity of the secondary acceptors mainly depends on the stereoelectronic effect of the acceptor. Only or predominant α anomeric products are obtained when these donors glycosylate with the secondary acceptors possessing low nucleophilicity, while the selectivity is likely to switch to predominant β when the 5-*O*-4-nitropicoloyl carrying donor reacts with more reactive secondary alcohols. By combined use of the donors **1c** and **7b**, we have accomplished the stereocontrolled preparation of the trisaccharide **19** with an unusual structure. Currently, we are exploring the use of the novel donors to promote the synthesis of complex Kdo-containing carbohydrates.

ASSOCIATED CONTENT

Supporting Information. Experimental details, ^1H and ^{13}C NMR spectra for all new compounds, and 2D NMR spectra for **18** and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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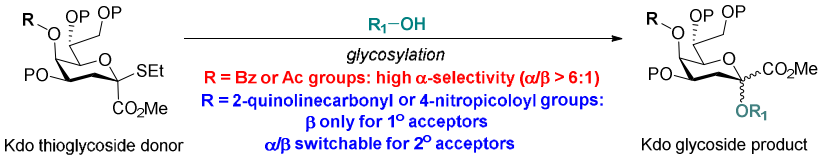
R
OP OP
O
PO
O
SEt
CO2Me
Kdo thioglycoside donor
R1−OH
glycosylation
R = Bz or Ac groups: high α-selectivity (α/β > 6:1)
R = 2-quinolinecarbonyl or 4-nitropicoloyl groups:
β only for 1° acceptors
α/β switchable for 2° acceptors
R
OP OP
O
PO
O
CO2Me
OR1
Kdo glycoside product

TOC