

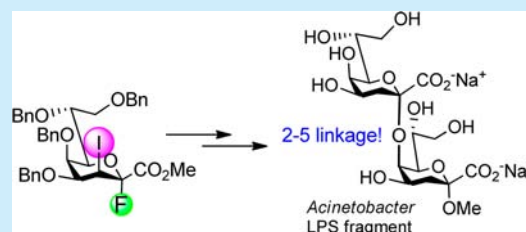
First and Stereoselective Synthesis of an α -(2 \rightarrow 5)-Linked Disaccharide of 3-Deoxy-D-manno-oct-2-ulosonic Acid (Kdo)

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Supporting Information

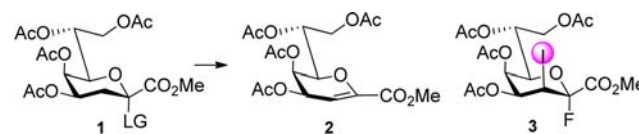
ABSTRACT: Resistance of bacterial pathogens toward antibiotics has revived interest in lipopolysaccharide (LPS) motifs as potential therapeutic targets. The LPS of several pathogenic *Acinetobacter* strains comprises a 4,5-branched Kdo trisaccharide containing an uncommon (2 \rightarrow 5)-linkage. In this contribution the first stereoselective glycosylation method for obtaining an α -Kdo-(2 \rightarrow 5)- α -Kdo disaccharide in good yield is highlighted. The synthetic approach used for accessing this linkage type will allow for future studies of the immunoreactivity associated with this unique bacterial Kdo inner core structure.



Lipopolysaccharides (LPS) are found in the outer membrane of the Gram-negative bacterial cell wall and comprise the conserved Lipid A which is noncovalently anchored to the cell wall via sugar-bound fatty acids. This lipophilic part is further linked to the core region followed by a species-specific polysaccharide, termed O-antigen.¹ The common bacterial sugar 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) is a fundamental component of the LPS core domain, being present in a structurally conserved α -(2 \rightarrow 4)-linked Kdo disaccharide unit.² This basic disaccharide is elongated at position 5 of the proximal Kdo unit by various additional core sugars such as L-glycero-D-manno-heptose, glucose or mannose, resulting in branched structures.³ Investigations of LPS from different *Acinetobacter* strains, however, have revealed the presence of branched Kdo tetra- and trisaccharides containing an unusual α -(2 \rightarrow 5) inter-Kdo linkage. In particular, several *A. baumannii* strains as well as the *A. radioresistans* strain S13 have been found to harbor an α -Kdo-(2 \rightarrow 5)[α -Kdo-(2 \rightarrow 4)] α -Kdo trisaccharide fragment in the inner core region.^{4a-d} An α -(2 \rightarrow 5)-linked Kdo disaccharide was also detected in *Campylobacter lari* strain ATCC 35221.^{4e} *Acinetobacter* strains are known to be responsible for severe nosocomial infections which are difficult to treat due to a pronounced resistance of the bacteria against antimicrobial drugs.⁵

While various routes toward (2 \rightarrow 4)-, (2 \rightarrow 8)-, and also (2 \rightarrow 7)- interconnected Kdo disaccharides have been reported,⁶⁻⁸ a synthetic access toward the (2 \rightarrow 5)-disaccharide via glycosylation has not been achieved thus far. The chemical glycosylation of Kdo glycosyl acceptors at position 5 is hampered by low reactivity due to the axial orientation of the C-5 hydroxyl group, the steric hindrance exerted by the side chain unit, and the deactivating effect of the C-1 ester group. Likewise, Kdo donors (e.g. 1, Scheme 1) usually suffer from considerable degradation during glycosylation reactions since they are prone to elimination reactions that produce glycal esters such as 2.^{2c,9} The elimination is also observed for conformationally constrained Kdo donors equipped with a 4,5-isopropylidene

Scheme 1. Kdo Donors (LG = Leaving Group) and Glycal Ester Formation during Glycosylation

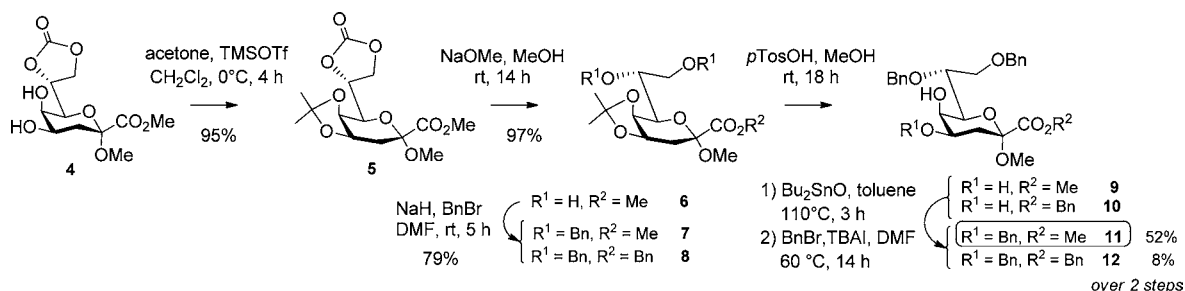


group.^{6c,d} In addition, Kdo donors show low anomeric selectivity and reduced reactivity for glycoside formation due to the deactivating effect of the carboxylic group adjacent to the anomeric center. The challenge met in the construction of a Kdo-(2 \rightarrow 5)-Kdo linkage is reflected in the complete regioselectivity observed during coupling of Kdo donors with 4,5-unprotected Kdo diol acceptors, leading to O-4 substituted products only.^{6,10} Also, previous attempts in the author's laboratory using a peracetylated Kdo phosphite donor and 4-O-protected Kdo acceptor derivatives did not produce any disaccharides.¹¹ Notably, however, glycosylations of the 5-OH group of 4-O-protected Kdo substrates with aldopyranosyl donors (e.g., heptosyl, glucosyl, mannosyl donors) have been successfully implemented, illustrating the difficulty in forming a ketosidic bond to the sterically hindered 5-OH group of a Kdo acceptor.¹²

Recently, we have developed the acetylated 3-iodo-Kdo fluoride donor 3¹³ with a stereodirecting group at position 3. This donor has proven itself capable of α -stereoselective glycosylation reactions to provide several α -Kdo homomers related to *Chlamydia* LPS in high yield. Additionally, the elimination side reaction toward glycal 2 could be significantly suppressed and we surmised that these properties might provide access to the α -(2 \rightarrow 5) Kdo disaccharide motif. For the design of an appropriate Kdo glycosyl acceptor, three

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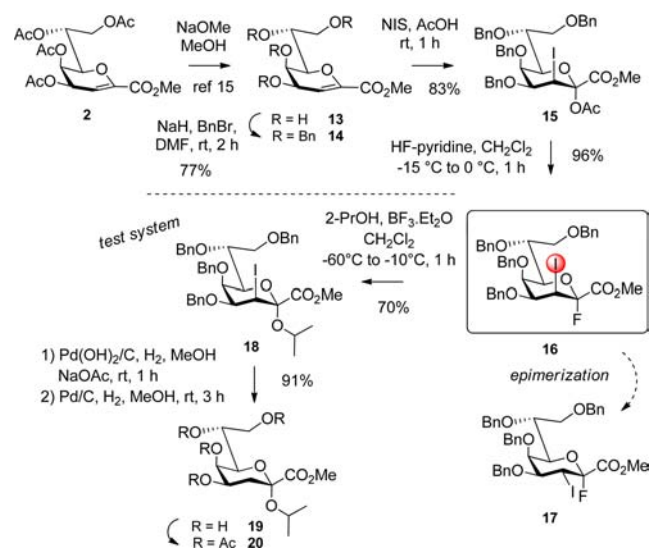
Scheme 2. Synthesis of the 4,7,8-Tri-*O*-benzyl Kdo Acceptor (11)

prerequisites for protecting groups were foreseen: (a) activating groups at remote positions to compensate for the intrinsically low reactivity of HO-5, (b) exclusion of silyl groups that would eventually be cleaved by fluoride species formed during the glycosylation step, and (c) stability toward Bronsted acids. The latter requirement resulted from a previous observation that addition of triethylamine abolished the glycosylation capability of donor 3 (thus, Bronsted acid formed after HF cleavage seems to be the active species involved in the coupling step). Since the disaccharide should serve as a ligand in STD-NMR and crystallographic studies, a methyl aglycon was selected.¹³ Thus, the 4,7,8-tri-*O*-benzyl protected Kdo glycosyl acceptor derivative **11** was prepared starting from the previously described 7,8-*O*-carbonyl derivative **4**¹³ (Scheme 2).

Reaction of **4** with acetone in the presence of TMSOTf yielded the 4,5-*O*-isopropylidene derivative **5** in 95% yield after silica gel filtration. Treatment of **5** with sodium methoxide in anhydrous methanol liberated the 7-OH and 8-OH groups (97% of **6**¹⁴), which were subsequently *O*-benzylated using sodium hydride and BnBr in dry DMF. Thus, a mixture of the methyl ester **7** and the benzyl ester **8** was obtained in a total yield of 79%. Cleavage of the 4,5-*O*-acetonide by treatment of the mixture of **7** and **8** with *para*-toluenesulfonic acid in methanol yielded a mixture of the two esters **9** and **10** after filtration through silica gel. Treatment of the mixture with dibutyl tin oxide and subsequent conversion of the tin acetals with BnBr in the presence of tetra-*n*-butylammonium iodide (TBAI) in DMF afforded a 52% yield of methyl ester **11** and 8% of benzyl ester **12** (over 2 steps) after chromatography. The latter product was finally quantitatively transformed into the methyl ester **11** by treatment with stoichiometric amounts of sodium methoxide in MeOH.

The glycosylation reaction of acceptor **11** with the acetylated donor **3** in dry CH₂Cl₂ containing ground molecular sieves (3 Å) and 2 equiv of BF₃·Et₂O did not afford the disaccharide. Instead, unreacted acceptor **11** was isolated, while donor **3** degraded very slowly over a period of several hours. Thus, in order to enhance the reactivity of the glycosyl donor, the armed analogue **16** (Scheme 3) was prepared. De-*O*-acetylation of glycal ester **2**¹⁵ and benzylation of the resulting tetraol **13** using NaH/BnBr in dry DMF provided **14**^{7c} in 77% isolated yield. Notably, destruction of excessive NaH for the workup was performed, after dilution with anhydrous CH₂Cl₂ at 0 °C, by addition of anhydrous MeOH followed by quick extraction and purification. Thus, degradation of the reactive glycal **14** and ester saponification could be avoided. Iodoacetylation using *N*-iodosuccinimide (NIS) in acetic acid was performed at ambient temperature and afforded the 2,3-*trans*-diaxial product **15** in 83% yield.¹⁶ Direct conversion of the anomeric acetate, with an optimized amount of hydrogen fluoride pyridine

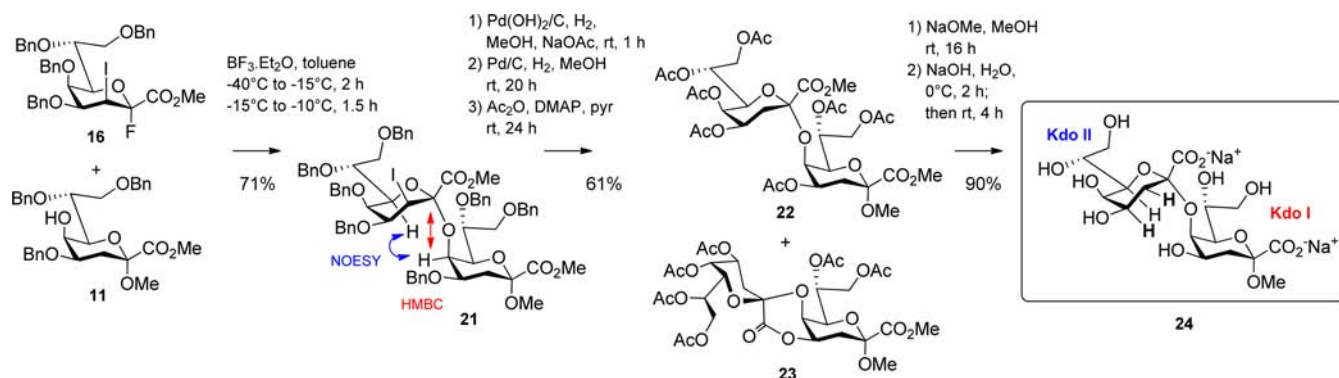
Scheme 3. Preparation of Fluoride Donor (16) and Model Glycosylation



complex at $-5-0$ °C, afforded the α -fluoride **16** ($J_{\text{H-3eq,F-ax}}$ 5.1 Hz) selectively and in excellent yield (96%).^{7b} Increased amounts of HF-pyridine and temperatures above 0 °C, however, resulted in epimerization of the 3-iodo substituent, and an inseparable mixture of the desired *D*-glycero-*D*-talo donor **16** and the *D*-glycero-*D*-galacto-epimer **17** was obtained.

To evaluate its glycosylation properties, donor **16** was converted in 70% yield into the 2-propyl glycoside **18** using 2 equiv of 2-propanol under BF₃·Et₂O-promotion in CH₂Cl₂. TLC-analysis of the glycosylation mixture indicated a pronounced reactivity of **16**, which was already activated at -60 °C. In comparison, no reaction of acetylated donor **3** with 2-propanol had been observed below ambient temperature.¹³ Dehalogenation was performed by catalytic hydrogenation using Pd(OH)₂/C (20%) in the presence of NaOAc in MeOH; it was followed by workup (extraction with sodium thiosulfate and water to remove iodo impurities). The ensuing debenzylation over Pd/C (10%) gave **19** in 91% yield (2 steps). Notably, dehalogenation also led to partial cleavage of the benzyl groups. *O*-Acetylation of **19** afforded the known glycoside **20**, and comparison with published data confirmed the assignment of the α -anomeric configuration.¹³

When applying these optimized glycosylation conditions to the coupling of donor **16** with 4,7,8-tri-*O*-benzyl acceptor **11**, trace amounts of product **21** could be isolated (Scheme 4). Furthermore, large amounts of unreacted acceptor **11** and hydrolyzed donor were present. Surprisingly, ¹H NMR analysis revealed the presence of a second 2→5-linked disaccharide

Scheme 4. Glycosylation and Deprotection towards the α -(2 \rightarrow 5) Kdo Disaccharide (24)

bearing an equatorially oriented 3-iodo substituent resulting from epimerization. Optimization of the glycosylation conditions, however, allowed for suppression of the 3-iodo-epimerization when toluene was used as the solvent. Still, the isolated yield was quite low ($\sim 30\%$). On the one hand, at low temperature (-40°C), incomplete conversion of acceptor **11** was observed, while at elevated temperatures (0°C) extensive degradation of the product occurred. Stability tests of the isolated product **21** revealed that it was unstable toward $\text{BF}_3 \cdot \text{Et}_2\text{O}$ at temperatures above -10°C . However, working at high dilution and keeping the temperature between -40°C to -10°C (warming up to -10°C was, however, essential) afforded **21** in a good yield (71%) as the α -anomer only. In addition, only very minor formation of the glycal ester **14** was observed and no β -anomeric product was detected. For workup, $\text{BF}_3 \cdot \text{Et}_2\text{O}$ had to be neutralized by dropwise addition of diluted triethylamine at low temperature followed by quick extraction and purification to secure a high isolated yield. This procedure was reproducible without the need for a large excess of donor **16** (only 1.5 equiv were used).

Broad signal shapes in the ^1H NMR spectrum (600 MHz, in CDCl_3) of **21** complicated the analysis and assignments. This line broadening suggests a high steric load and a rigid structure of **21**. Thus, the sample was subjected to high-temperature measurements¹⁷ in d_8 -toluene to confirm the presence of the (2 \rightarrow 5) linkage. Indeed, the HMBC spectrum showed a cross-correlation between H-5 and C-2' (Figure 1) and the assignment was further supported by a NOESY signal between H-5 and H-6' (see Supporting Information).

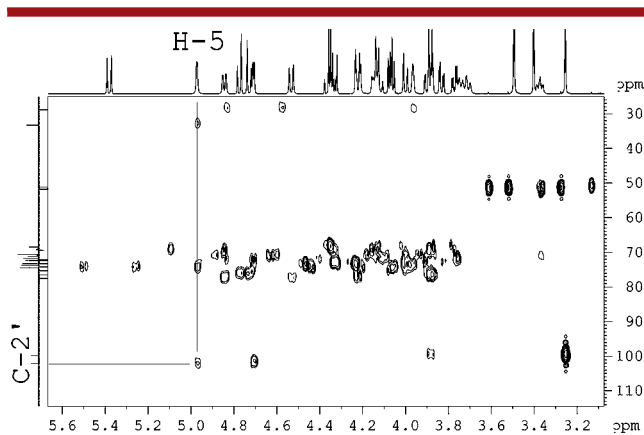


Figure 1. HMBC spectrum (d_8 -tol, 323 K) of disaccharide **21**.

The dehalogenation/debenzylation sequence of **21** using $\text{Pd}(\text{OH})_2/\text{C}$ - NaOAc and Pd/C in MeOH under an atmosphere of H_2 yielded an impure product, which was *O*-acetylated and purified by normal-phase HPLC providing a mixture of **22** together with the 1' \rightarrow 4 lactone **23** in 61% yield (3 steps).

NMR characterization of **22** supported the assignment of the α -anomeric configuration as indicated by the characteristic chemical shifts of H-3' $_{ax}$ and H-3' $_{eq}$ and the low-field shift of H-4'.¹⁸ Analysis of the side products revealed that partial cleavage of the glycosidic bond had occurred. This was further supported by LC-MS studies showing that glycoside hydrolysis (and lactone formation) had already occurred during the treatment with $\text{Pd}(\text{OH})_2/\text{C}$ - NaOAc . Deacetylation of the $\sim 10:1$ mixture of **22** and **23** using sodium methoxide and subsequent methyl ester hydrolysis with aq NaOH afforded **24** containing a trace impurity. To obtain the pure target disaccharide, the mixture of **22** and **23** was partially separated by normal-phase HPLC, providing a pure fraction of **22** (1.2 mg from 9.9 mg of a 10:1 mixture of **22/23**, 12%), which was globally deprotected to afford the α -(2 \rightarrow 5)-linked Kdo disaccharide **24**.

The NMR spectroscopic data for **24** (recorded in D_2O) were in close agreement with the related Kdo units present in the branched Kdo-tetrasaccharide.^{4a-d} The proton signals of H-4' and H-6' of Kdo II (see Scheme 4) were substantially shifted to lower field (4.26 and 3.90 ppm, respectively), presumably due to the deshielding effect of the O-4 group of Kdo I. Similarly, the equatorial proton H-3' $_{eq}$ at the distal Kdo II unit was also shifted to lower field (2.24 ppm), which is most likely attributable to the fixed orientation of the lone pairs at O-5 of the glycosidic bond. These effects have been published for the branched Kdo tetrasaccharide isolated from *A. baumannii* strain NCTC 10303.^{4a} Since these features are also seen in the α -(2 \rightarrow 5)-linked disaccharide, the additional, lateral α -(2 \rightarrow 4)-linked Kdo unit has thus only a limited impact on the NMR characteristics of the α -(2 \rightarrow 5)-unit (except for ^{13}C NMR shift differences at C-4 and C-5 for the branched Kdo moiety, respectively). Carbon-5 of Kdo I revealed a significant shift to low field (74.2 ppm), confirming the site of attachment of the second Kdo unit (which was again proven by an HMBC correlation from H-5 to carbon C-2').

In conclusion, we have developed a stereoselective route toward α -(2 \rightarrow 5)-interconnected Kdo residues using an armed 3-iodo-Kdo fluoride donor displaying high reactivity and α -anomeric stereoselectivity. The NMR data for the deprotected disaccharide corresponding to fragments of *Acinetobacter* LPS

favorably match the assignments made for the native oligosaccharides.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic procedures for **5–12**, **14–19**, and **21–24**; characterization data; and NMR spectra. 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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