Synthetic Methods

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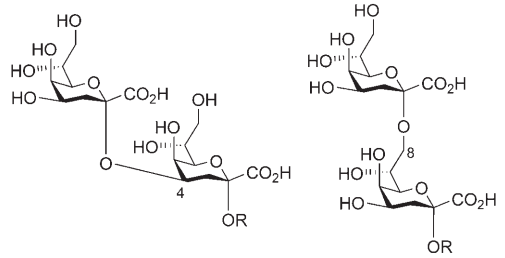
Stereoselective Synthesis of Oligo-α(2,8)-3-deoxy-D-*manno*-2-octulosonic Acid Derivatives

*Hiroshi Tanaka, Daisuke Takahashi, and Takashi Takahashi**

3-Deoxy-D-*manno*-2-octulosonic acid (KDO), a unique sugar component of lipopolysaccharides (LPSs), is an important constituent of the outer membrane of Gram-negative bacteria.[1] KDO is frequently present in the form of α(2,4)- and α(2,8)-linked homooligomers **1** and **2** connected to lipid A in

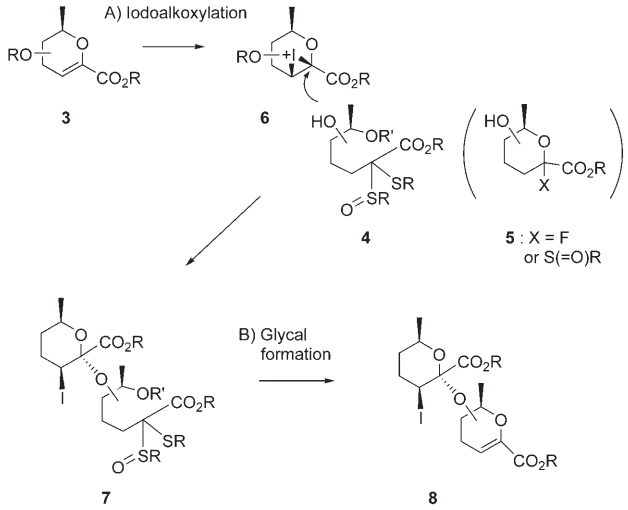


1 **2**

LPSs. These unique di- and oligo-KDO fragments are found only in bacterial cell walls and do not exhibit significant biological activity. Therefore, they could serve as potential antigens in the development of antibacterial vaccines. However, the possibility that such isolated oligosaccharides could be heterogeneous and/or contaminated with antigenic compounds cannot be excluded. Therefore, the chemical synthesis of these ulosonic acid-containing oligosaccharides would circumvent these issues as they relate to vaccine development.

Recent progress in oligosaccharide synthesis has resulted in a number of efficient glycosidation methodologies. However, the glycosidation of ulosonic acids often involves the generation of undesirable β stereoisomers and/or the 2,3-elimination of the donors as a result of the presence of a C1 carboxyl group and the lack of a C3 substituent.[2] In addition, the hydroxy groups, both in the pyran ring and on the endocyclic side chain, are less reactive toward glycosylation. In 1987, Shiba and co-workers reported that α-ketopyranosyl fluoride was an effective donor for the α-selective glycosidation of KDO.[3] Furthermore, in 1989, Achiwa and co-workers reported that the glycal ester underwent glycosyloxyselenation to provide α-linked 3-phenylselenyl-KDO derivatives.[4] The feasibility of these methods was mainly demonstrated by the synthesis of mono-KDO-containing oligosaccharides. However, the coupling of two and three KDO units often resulted in reduced product yields and some loss of selectivity. Notably, the low-nucleophilicity hydroxy groups on KDO units promote significant 2,3-elimination of the donor instead of the coupling reaction. Therefore, an efficient method for the stereoselective synthesis of α-linked KDO dimers and trimers is still needed.

Previously, we investigated the synthesis of oligosaccharides with acyclic glycosyl donors,[5] and reported on the synthesis of 2,3,6-deoxyglycosides by the one-pot cyclization and glycosidation of acyclic glycosyl donors possessing sulfoxide and alkylsulfanyl groups at the pseudo-anomeric position.[6] The acyclic donors were stable to various glycosidation reagents such as Lewis acids and to oxidative reagents. We envisaged that the acyclic saccharide precursors **4** could be used as glycosyl acceptors for iodoalkoxylation of the glycal ester **3**, and could be converted to α-KDO glycosylated products **7** through diaxial opening[4,7] of the iodonium ion **6** without decomposition of the sulfur-based leaving groups (Scheme 1). The hydroxy groups on the acyclic



Scheme 1. Strategy for the synthesis of α-linked oligo-KDO by iodoalkoxylation.

saccharide precursors would be expected to exhibit an enhanced reactivity toward glycosylation in comparison with the corresponding cyclic acceptors **5**, because the opening of the pyran and furan rings of the saccharide units is known to be an effective strategy for improving the reactivity of hydroxy groups toward glycosylation.[8] The coupling products **7** can be converted to the glycal ester **8** for glycosidation. Herein, we describe the synthesis of oligosaccharides containing tri- and di-α(2,8)-KDO units by iodoalkoxylation with an acyclic saccharide precursor.

The strategy developed for the synthesis of α(2,8)-KDO derivatives **9** and **10** is shown in Scheme 2. These compounds

[*] Dr. H. Tanaka, D. Takahashi, Prof. Dr. T. Takahashi
Department of Applied Chemistry
Graduate School of Science and Engineering
Tokyo Institute of Technology
2-12-1 Ookayama, Meguro, Tokyo 152-8552 (Japan)
Fax: (+81) 3-5734-2884
E-mail: ttak@apc.titech.ac.jp

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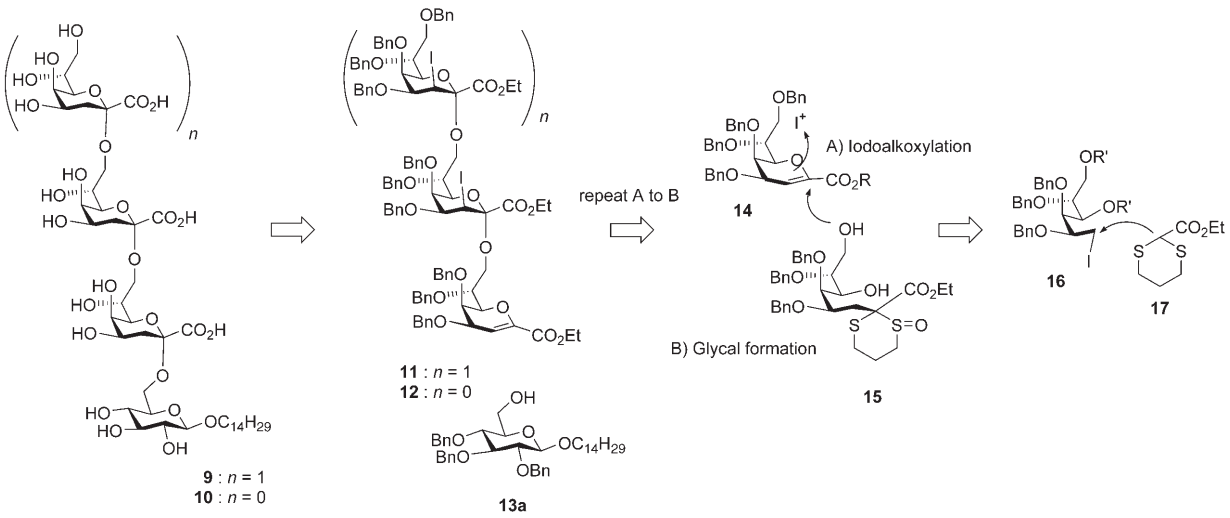
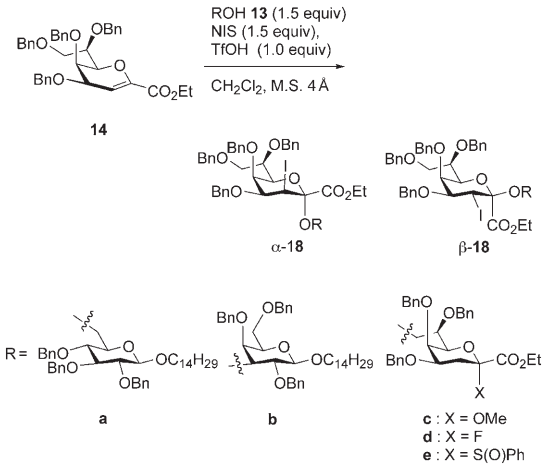


Scheme 2. Strategy for the synthesis of tri- and di-α(2,8)-KDO derivatives **9** and **10**. Bn = benzyl.

could be prepared by the glycosylation of acceptor **13a** with the corresponding glycal esters **11** and **12** attached to α(2,8)-KDO units. The synthesis of the glycal ester **12** could be achieved by the regio- and stereoselective iodoalkoxylation of the glycal ester **14** with diol **15**, which contains alkylsulfanyl and sulfoxide groups at the C2 position, followed by subsequent cyclization and β elimination. Trisaccharide **11** was prepared from **12** by the iodoalkoxylation and β elimination protocol. Benzyl ethers were selected as O-protecting groups to improve the reactivity of the glycal esters **11**, **12**, and **14** toward iodoalkoxylation. The resulting iodide and benzyl ethers can be readily removed by hydrogenolysis. Diol **15** can be prepared by alkylation of dithiane **17**[9] with the corresponding alkyl halide **16**.[5,6,10]

We first investigated the iodoalkoxylation of the glycal ester **15** with alcohols **13** (Scheme 3 and Table 1). Treatment of the benzyl-protected glycal ester **14** and primary alcohols **13a** with NIS and TfOH provided the 3-iodo-α-glycoside α-**18a** in 93% yield with excellent selectivity.[11] Notably,



Scheme 3. Iodoalkoxylation of **14** with various acceptors **13** (see also Table 1) NIS = *N*-iodosuccinimide; TfOH = trifluoromethanesulfonic acid; M.S. = molecular sieves.

Table 1: Iodoalkoxylation of **14** with various acceptors **13** (Scheme 3).

Entry	Acceptor	Product	*T* [°C]	Yield [%]	α/β[a]
1	**13a**	**18a**	−50	93	>95:5
2	**13b**	**18b**	−50 to −40	77	>95:5
3	**13c**	**18c**	−50 to −20	73	92:8
4	**13d**	**18d**	–	–	–

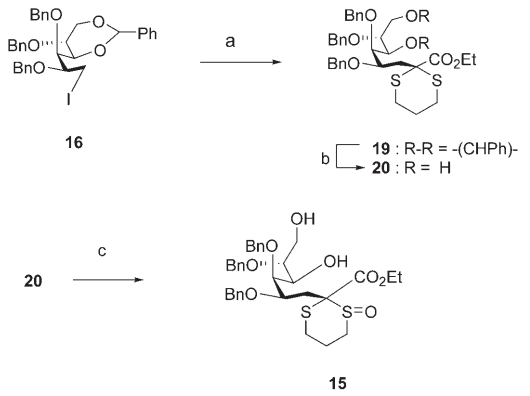
[a] The α/β ratio was estimated by HPLC analysis with refractive index detection.

although iodoalkoxylation of glycals in the synthesis of α-linked 2-deoxyglycosides is known to proceed under mildly oxidative conditions,[12] the use of a stoichiometric amount of TfOH was critical for the iodoalkoxylation of glycal ester **14** to proceed. Glycosylation at the C3 secondary alcohol moiety of galactoside **13b** proceeded smoothly to provide disaccharide α-**18b** in excellent yield and selectivity.[13]

Next, the glycosylation of KDO derivatives **13c–e** at the C8 position was examined. The methyl glycoside **13c** was converted to the corresponding α-glycoside α-**18c** in 73% yield with α/β = 92:8.[11] On the other hand, glycosyl sulfoxide **13e** was difficult to prepare because of its instability. The glycosyl fluoride **13d** decomposed under these reaction conditions. These results suggest that, although the iodoalkoxylation of glycal esters would be effective for the synthesis of α-linked KDO derivatives, it would be difficult to design cyclic glycal precursors that are amenable to iodoalkoxylation.

We then examined the synthesis of the α(2,8)-KDO derivatives **9** and **10**. The preparation of the acyclic building blocks **15** is shown in Scheme 4. Treatment of iodide **16**[14] with dithiane **17** in the presence of NaH in DMF provided the alkylated product **19**, which was followed by removal of the acetal of **19** under acidic conditions to afford diol **20** in 71% overall yield. Oxidation of thioether **20** provided sulfoxide **15** in good yield.

Iodoalkoxylation and glycal formation is shown in Scheme 5. Treatment of the glycal ester **14** and 1.5 equivalents of diol **15** in the presence of NIS/TfOH at −50 to −20 °C



Scheme 4. Reagents and conditions: a) **17**, NaH, *t*BuOH (cat.), DMF; b) CSA, MeOH, THF, 71% (based on **16**); c) *m*-CPBA, CH_2Cl_2, 95%. DMF = *N*,*N*-dimethylformamide; CSA = 10-camphorsulfonic acid; CPBA = chloroperbenzoic acid.

provided α-glycoside **21** in 89% yield. These results indicate that the opening of the pyran ring of the KDO derivative effectively improved the coupling yield of the C8 hydroxy group, and that sulfoxide and alkylsulfanyl groups represent suitable protecting groups for the linear building blocks at the pseudo-anomeric position for the iodoalkoxylation. The sequential activation of the two leaving groups with $(COCl)_2$ and AgOTf in CH_2Cl_2 afforded the glycal ester **12** bearing an α-linked KDO unit as a single diastereomer. AgOTf played a crucial role in increasing the yield of the glycal ester **12**. Iodoalkoxylation of **12** with 1.5 equivalents of **15** followed by glycal formation provided the tri-KDO derivative **11** in good total yield with excellent selectivity. Glycosylation of the primary alcohol **13a** with glycal esters **11** and **12** proceeded stereoselectively to provide α-glycosides **22** and **23** in good yields with excellent selectivity. Removal of the iodide and cleavage of all the benzyl ether protecting groups, followed by acetylation, provided the peracetyl-protected tri- and tetrasaccharides **24** and **25** in 53 and 71% yield, respectively. Hydrolysis of the ester groups in **24** and **25** provided tri- and di-α(2,8)-KDO-containing oligosaccharides **9** and **10** in good yields. The α linkages of the KDO units of **24** and **25** were confirmed by analysis of their ^{1}H NMR spectra on the basis of reported empirical rules.[11]

In conclusion, an efficient synthesis of tri-α(2,8)-KDO derivatives based on an iterative glycosidation strategy has been developed. Iodoalkoxylation of the glycal esters **11**, **12**, and **14** proceeded in a stereoselective manner to provide α-linked KDO derivatives in good yields. The acyclic building block **15** was found to be an effective glycosyl acceptor and was converted to the glycal ester for glycosidation. The opening of the pyran ring effectively improved the reactivity of the C8 hydroxy group. The glycal esters **11** and **12** bearing KDO units constitute potentially useful building blocks for the synthesis of α-KDO-conjugated oligosaccharides.

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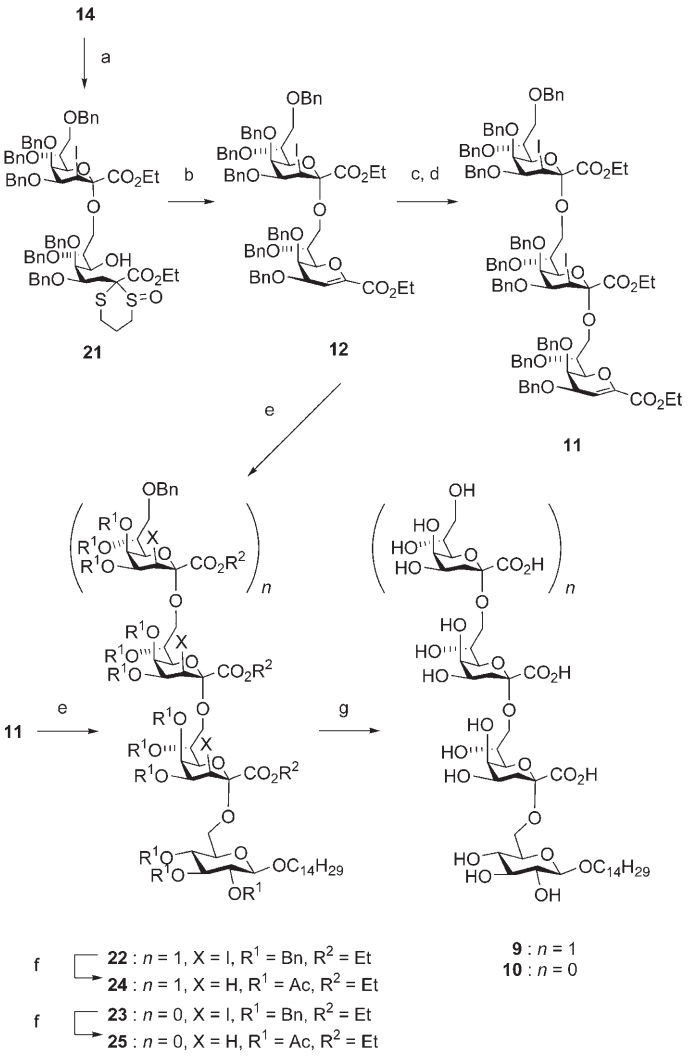

Scheme 5. Reagents and conditions: a) **15** (1.5 equiv), NIS, TfOH, M.S. 4 Å, CH_2Cl_2, −50 to −20°C, 89%; b) $(COCl)_2$, AgOTf, M.S. 4 Å, CH_2Cl_2, −30 to 0°C, 77%, α/β = > 95:5; c) **15** (1.5 equiv), NIS, TfOH, M.S. 4 Å, CH_2Cl_2, −50 to −20°C, 68%; d) $(COCl)_2$, AgOTf, M.S. 4 Å, CH_2Cl_2, −30 to 0°C, 85%, α/β = > 95:5; e) **13a** (1.5 equiv), NIS, TfOH, M.S. 4 Å, CH_2Cl_2, −50°C to room temperature, 80%, α/β = > 95:5 for **23**, 55%, α/β = 86:14 for **22**; f) 1. $Pd(OH)_2$, *n*-BuOH, MeOH, ethyl acetate; 2. Ac_2O, Py, CH_2Cl_2, DMAP, 71% for **25**, 53% for **24**; g) LiOH, $MeOH/H_2O$, 50°C, quantitative for **10**, 89% for **9**. Py = pyridine; DMAP = 4-dimethylaminopyridine.

Keywords: carbohydrates · glycosides · glycosylation · oligosaccharides · synthetic methods

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