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PREPARATION OF NOVEL PYRANOSYL FLUORIDES OF 3-DEOXY-D-manno-2-OCTULOSONIC ACID (KDO) FEASIBLE FOR SYNTHESIS OF KDO α-GLYCOSIDES

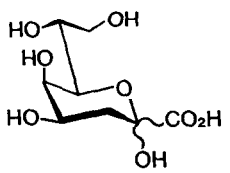
Masahiro Imoto, Naoto Kusunose, Yoshiki Matsuura,[+] Shoichi Kusumoto, and Tetsuo Shiba*
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan
[+]Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan

Summary : α-Ketopyranosyl fluorides, 2 and 3, of 4,5:7,8-di-O-isopropylidene KDO methyl and benzyl esters were prepared and shown to act as effective glycosyl donors. X-Ray structure analysis of 2 and ^{1}H NMR study established the boat ($B_{3,6}$) conformation of the di-O-isopropylidene derivatives of KDO and enabled the assignment of their anomeric configurations.

In the preceding paper[1] we described a new efficient synthetic procedure for 3-deoxy-D-manno-2-octulosonic acid (formerly 2-keto-3-deoxy-D-manno-octonic acid, KDO) (1) which is a characteristic component sugar of cell surface lipopolysaccharide (LPS) and capsular polysaccharides of Gram-negative bacteria.[2,3] In LPS, a pyranosidic KDO moiety is ketosidically bound to the glucosamine residue of the glycolipid part designated lipid A, which is now known to be responsible for most of the biological activities of LPS related to toxicity and immunostimulation.[4] As a new step of our synthetic study on LPS,[4] we are attempting to couple KDO with lipid A part, in order to test the effect of KDO moiety on the biological activity of lipid A. In this communication we describe a preparation of new glycosyl fluorides, methyl and benzyl (3-deoxy-4,5:7,8-di-O-isopropylidene-α-D-manno-2-octulopyranosylfluorid)onate (2 and 3), feasible for the synthesis of KDO glycosides.

Chemical synthesis of KDO glycosides have been intensively studied by the group of Unger and Paulsen.[3,5] In their studies, however, only the chloride or the bromide of peracetylated KDO methyl ester (4 and 5) were used as glycosyl donors. For the purpose of our synthetic study on LPS, a different KDO donor is indispensable which has protecting groups removable without affecting the O-acyl groups present in the lipid A part.

A synthetic intermediate, methyl 3-deoxy-4,5:7,8-di-O-isopropylidene-D-manno-octulosoate (6), described in our preceding communication seemed to be a good starting material for a new pyranosidic donor of KDO, since this compound was expected to exist in a ketopyranose form.[6] Indeed, acetylation of 6 (Ac_2O - 4-dimethylaminopyridine (DMAP) - pyridine in CH_2Cl_2 at room temperature) yielded a single acetate 7 (81%, mp 80-81°C, $[\alpha]_D^{19}$ +74.6°), the α-pyranose structure of which was confirmed by converting it [i) 10:1 mixture of CH_2Cl_2 and aqueous 95%



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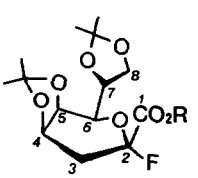
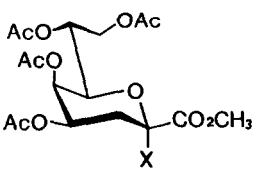
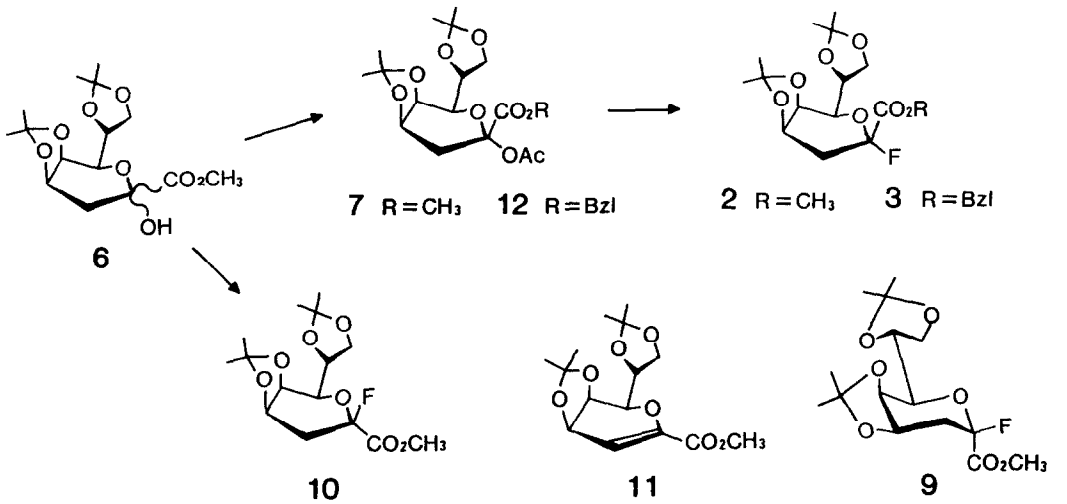


2 R=CH_3 3 R=Bzl



4 X=Cl 5 X=Br
8 X=OAc

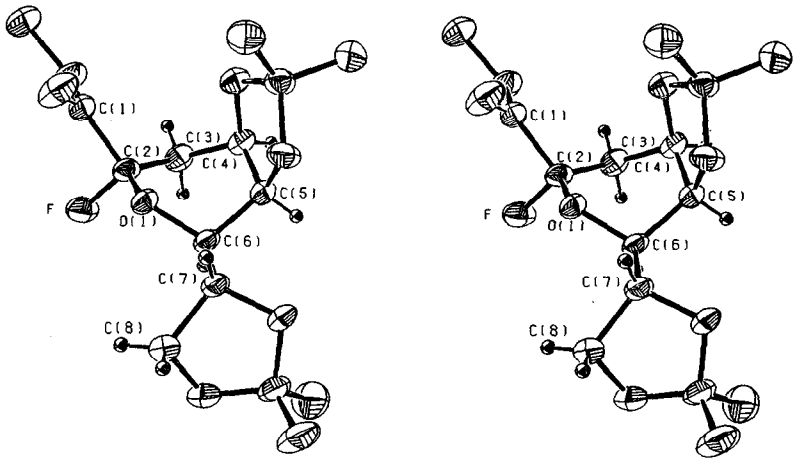


TFA at 0°C for 30 min; ii) Ac_2O–DMAP–pyridine in CH_2Cl_2 at 0°C for 4h] into the known peracetyl KDO methyl ester (8) (94%, mp 156–157°C).[6,7]

Activation of the ketosidic position as fluoride was next attempted, because glycosyl fluorides are recently known to be very effective for glycosidation in many cases.[8–12] On treatment with 50% hydrogen fluoride–pyridine (at 0°C for 4 h),[11] the acetate 7 was converted into a single crystalline fluoride 2 [58%, mp 121–123°C, $[\alpha]_D^{19}$ +12.1° (c 1.05, $CHCl_3$)]. ^1H NMR data given in Table indicated that the fluoride 2 has the same stereochemistry as the acetate (7) which has the α-configuration at C-2. However, the relatively small $J_{F,\ H-3}$ values seemed to suggest the absence of a 1,3-diaxial relation between them. These data could be reasonably explained by assuming a boat ($B_{3,6}$) conformation as shown in the scheme, but the possibility of a β-fluoride structure in a chair form like 9 could not be completely excluded.

Therefore, the single crystal X-ray analysis was undertaken to establish the stereochemistry of the fluoride 2. The result of the analysis showed the α-fluoride structure in a boat conformation as to the pyranose ring in accord with the NMR analysis described above. This conformation seems to be caused by the presence of the 4,5-O-isopropylidene ring. The stereochemical parameters concerning the pyranose ring are given in the note[13] together with the crystallographic data. The other di-O-isopropylidene derivatives (6 and 7) are also concluded to have similar boat conformations as judged from the NMR data given in Table.[14]

The direct transformation of the hydroxyl group in 6 into fluoride was also attempted but proved to be unsatisfactory, a different single fluoride 10 [mp 62–63°C, $[\alpha]_D^{19}$ +2.5° (c 1.30, $CHCl_3$)] being obtained only by use of 2-fluoro-1-methylpyridinium tosylate[15] (with Et_3N in CH_2Cl_2 at −20°C) in a poor yield (33%) because of the formation of glycal 11 as a by-product. The new fluoride 10 was concluded to be the β-fluoride in a similar boat form. It exhibited almost the same coupling constant values as those of 2 except $J_{3a,F}$ and $J_{3e,F}$. The large $J_{3a,F}$ value (33.9 Hz) showed the axial orientation of the F atom. When diethylaminosulfur trifluoride (DAST)[16] was used as the fluorinating reagent, only the glycal (11) was obtained even at −75°. Reaction of 6 with triphenylphosphine, diethyl azodicarboxylate, and triethyloxonium tetrafluoroborate[10] gave a complex mixture from which no fluoride was obtained.



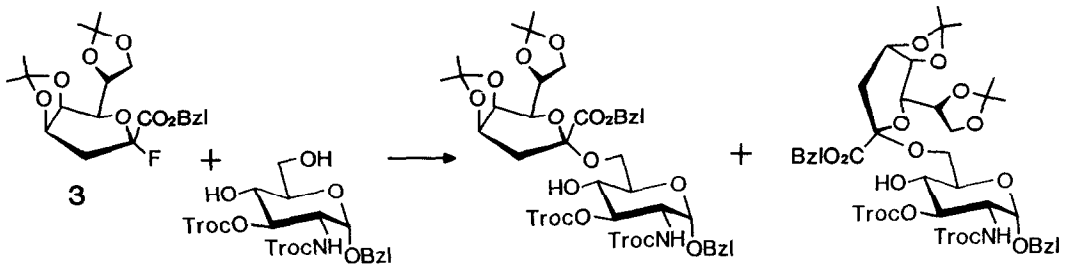
Stereoview of KDO α-Fluoride 2

Feasibility of a KDO fluoride in a glycosidation reaction was next examined. α-Fluoride benzyl ester **3** (mp 144°C dec) was prepared via **12** and subjected to a boron trifluoride-catalyzed condensation[10] with a model glucosamine derivative **13** (in dry CH_2Cl_2 in the presence of 1.5 equivalent of $BF_3 \cdot Et_2O$ and 1.0 equivalent of Et_3N at 0°C for 10 min). Purification with a silica gel column ($CHCl_3$ - acetone 30:1) afforded the α(2-6) bound disaccharide **14** as the main product (60%, mp 85-88°C) and a trace amount of the corresponding β(2-6) disaccharide **15** (2%). The anomeric configurations of these KDO glycosides could be assigned by comparison of their ^1H NMR spectra with the data given in Table. Thus, large differences (0.60 - 1.09 ppm) between the chemical shift values of C-3 methylene protons are observed in α-anomers (**2**, **3**, **6**, **7**, and **14**) as compared to the corresponding values (0.26 - 0.28 ppm) in β-anomers (**10** and **15**) This is a characteristic feature of boat form 4,5:7,8-di-O-isopropylidene derivatives of KDO and can be regarded as a criterion of anomeric configurations. In case of the chair form derivatives of KDO, on the contrary, similar large chemical shift differences of these methylene protons were observed not in α- but in β-anomers.[3]

Table. Chemical Shift and Coupling Constant Values in ^1H NMR Spectra of 4,5:7,8-Di-O-isopropylydene Derivatives of KDO (ppm from TMS and Hz in $CDCl_3$ at 360 or 400 MHz).

	α-Anomers					β-Anomers	
	2	**3***	**6**	**7**	**14**	**10**	**15***
H-3a	1.98	1.99	1.90	2.08	1.88	2.14	2.04
H-3e	3.07	3.04	2.50	2.72	3.07	2.42	2.30
H-4	4.57		4.51	4.55	4.56	4.62	
H-5	4.39		4.26	4.36	4.22	4.50	
H-6	3.67		3.89	3.60	3.19	3.53	
H-7	4.39		4.35	4.40	4.31	4.34	
H-8	4.08		3.98	3.86	4.07	4.07	
	4.15		4.08	4.11	4.22	4.11	
$J_{3a,3e}$	15.6		14.3	15.6	15.9	16.2	
$J_{3a,4}$	2.8		4.9	3.1	2.4	4.0	
$J_{3e,4}$	3.7		6.7	3.4	3.4	2.1	
$J_{4,5}$	7.6		6.4	7.9	7.6	8.2	
$J_{5,6}$	2.1		2.1	1.8	1.8	1.8	
$J_{6,7}$	8.5		8.2	8.5	10.1	8.2	
$J_{3a,F}$	18.6					33.9	
$J_{3e,F}$	3.4					10.4	

* at 100 MHz



Troc = CCl_3CH_2OCO

Consequently the α-fluorides of 4,5:7,8-di-O-isopropylidene derivative obtained in this study proved to be efficient glycosyl donors of KDO. They are stable and can be stored at room temperature without decomposition. The reaction with the fluoride affords α-ketosidic linkage of KDO, which occurs in bacterial LPS, with a high anomeric selectivity. The reaction proceeds in a short time under mild conditions. Utilization of the fluoride 3 in syntheses of part structures of LPS will be reported soon elsewhere.

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6. The conformations of **6** and **7** were determined later by comparison of their NMR data with those of the fluoride **2** derived from **7** (see text below and Table).
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13. Crystallographic data: Orthorhombic $P2_12_12_1$, a= 8.517(1)Å, b=18.465(2)Å, c= 10.908(1)Å, Z= 4, R= 0.056 for 1371 significant reflections. Bond lengths (Å): C(2)-C(3)= 1.491(9), C(3)-C(4)= 1.506(9), C(4)-C(5)= 1.535(8), C(5)-C(6)= 1.526(8), C(6)-O(1)= 1.454(7), O(1)-C(2)= 1.381(7). Bond angles (°): C(2)-C(3)-C(4)= 112.3(5), C(3)-C(4)-C(5)= 112.0(5), C(4)-C(5)-C(6)= 111.3(5), C(5)-C(6)-O(1)= 108.1(4), C(6)-O(1)-C(2)= 113.8(4), O(1)-C(2)-C(3)= 115.1(5). Tortion angles (°): O(1)-C(2)-C(3)-C(4)= -34.7(7), C(2)-C(3)-C(4)-C(5)= 50.9(7), C(3)-C(4)-C(5)-C(6)= -9.5(7), C(4)-C(5)-C(6)-O(1)= -46.2(6), C(5)-C(6)-O(1)-C(2)= 67.0(6), C(6)-O(1)-C(2)-C(3)= -24.7(7). The e.s.d. in parenthesis is in the unit of the least significant digit.
14. The NMR data given for **6** are those obtained with a freshly prepared $CDCl_3$ solution of the pure α-anomer whose preparation was described in the preceding paper (ref. 1).
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