Synthesis of Allyl 3-Deoxy-D-*manno*-2-octulopyranosidic Acid 4- and 5-Phosphates

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Allyl glycosides (ketosides) of pyranosidic 3-deoxy-D-*manno*-2-octulosonic acid (Kdo), phosphorylated at either positions 4 or 5 were synthesized with the aim to study the biological properties of phosphorylated Kdo. The α- and β-allyl glycosides prepared from a pyranosidic fluoride of diisopropylidene Kdo were protected at the 7- and 8-positions, and then *O*-phosphorylated at the 4- or 5-position by the phosphoramidite procedure. Separation of the positional isomers followed by deprotection afforded four compounds in pure states.

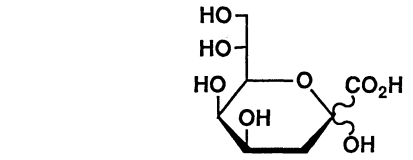
3-Deoxy-D-*manno*-2-octulosonic acid (Kdo) is a common and obligatory constituent of lipopolysaccharides (LPS) of Gram-negative bacteria.[1] It is structurally important as it forms the link between the polysaccharide region and the lipid A component of LPS.[2] Thus, Kdo is located at the reducing terminal of the polysaccharide region and α-ketosidically bound to lipid A.[3] Kdo is also of biological importance, since it modulates the endotoxic activity of lipid A[1,4] and, further, since it appears to be essential in the biosynthesis of LPS, and, thus, for the viability of Gram-negative bacteria.[5-7]

The intracatenarian Kdo-residue is, depending on the bacterial source, substituted by glycosyl or phosphoryl groups.[1,2,7] Kdo phosphate has been found in the LPS of *Bordetella pertussis*,[8] *Vibrio cholerae*,[9] *Bacteroides gingivalis*,[10] *Haemophilus influenzae*,[11] and *Vibrio parahaemolyticus*.[12] In the latter case, Kdo-4-phosphate was found to be present, whereas in the other LPS, Kdo 4-phosphate and/or Kdo 5-phosphate was identified.

As part of our synthetic work related to the elucidation of the biological significance of Kdo residues in LPS[13,14] we report here on the synthesis of α- and β-allyl glycoside (ketoside) of Kdo 4- and 5-phosphates.[15] In analogy to other Kdo derivatives these compounds are regarded as targets for serological studies aiming at the characterization of the immunoreactivity of the Kdo-containing inner core region of LPS.[16,17] The allyl groups were introduced to serve as a link for the preparation of Kdo phosphate containing antigens, i.e. the conjugation with proteins or the copolymerization with acrylamide.[17]

Results and Discussion

In the present study, the α-fluoride of diisopropylidene Kdo **4**, prepared from the Kdo ammonium salt, was employed for the formation of allyl glycosides of Kdo. The same fluoride **4** had been prepared from D-mannose in our previous work and shown to be an efficient pyranosidic glycosyl donor of Kdo.[18] In that work, it was also demonstrated that the fluoride **4** gives a mixture of α- and β-glycosides of Kdo on Lewis acid-catalyzed glycosidation when a lower alcohol is employed as the glycosyl acceptor.[18] Thus, the formation of both α- and β-allyl glycosides of Kdo could be expected in one reaction with the fluoride **4**.



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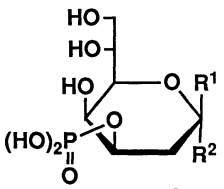
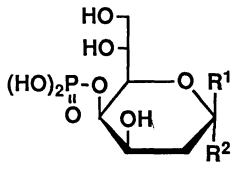
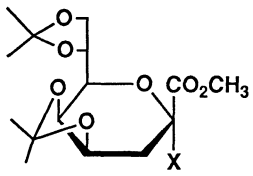




2a,b **3a,b**

a : $R^1 = CO_2H$, $R^2 = OCH_2\text{-}CH{=}CH_2$
b : $R^1 = OCH_2\text{-}CH{=}CH_2$, $R^2 = CO_2H$



4 : X = F **5** : X = OAc

The methyl ester of Kdo, obtained from the ammonium salt, was converted to the 4,5 : 7,8-di-*O*-isopropylidene 2-acetate (**5**) of the pyranose form. Reaction of **5** with hydrogen fluoride in pyridine gave the α-fluoride (**4**) which was identical with the specimen obtained in our previous work.[18] The glycosidation

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reaction with the fluoride using dry allyl alcohol was carried out in the presence of boron trifluoride etherate and *N*,*N*-diisopropylethylamine in dichloromethane.[19] The anomeric mixture of the allyl glycosides obtained was separated by column chromatography on silica gel to give the α-allyl glycoside **6a** (58%) and the β-anomer **6b** (24%). The former was accompanied with a small amount (2.7%) of the glycal **7** which could not be separated at this stage: the ratio of **6a** to **7** was determined spectrometrically by ^{1}H NMR.

The glycosidic configuration of these allyl glycosides was determined by examination of the differences in the chemical shift values of H_{3a} and H_{3e} in ^{1}H NMR spectra.[18,20] The large $\Delta_{3e\text{-}3a}$ value of 0.91 for **6a** assures its α-anomeric configuration, since the values are around the range of 0.6—1.19 for the various known α-anomers of 4,5 : 7,8-di-*O*-isopropylidene derivatives of Kdo. In the case of the other anomer **6b**, the corresponding $\Delta_{3e\text{-}3a}$ value was 0.24 which was close to the range of 0.26—0.28 so far observed for β-anomers.

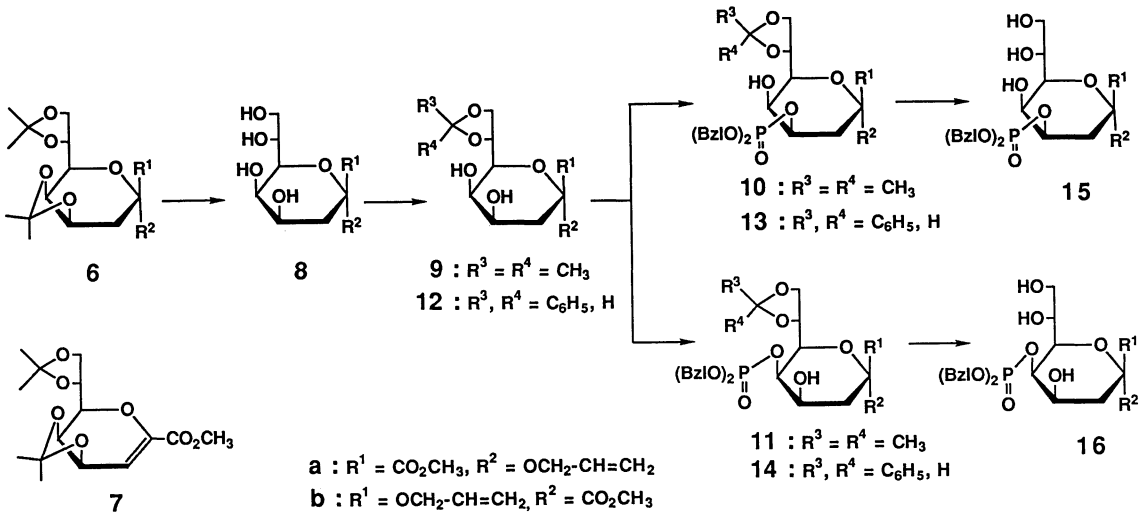
Introduction of the phosphate moiety was first examined at the 4- or 5-position of the α-allyl glycoside. Thus, both isopropylidene groups of **6a** were once removed to give the free α-allyl glycoside methyl ester **8a**, whose 7- and 8-hydroxyl groups were again protected by selective monoisopropylidenation. When **8a** was treated with 2-methoxypropene in the presence of (+)-camphor-10-sulfonic acid (CSA) at −35 °C, TLC analysis indicated that a smooth reaction had occurred to give a monoisopropylidene derivative as the main product. This compound was assumed to be the desired 7,8-*O*-isopropylidene **9a** as judged from the result of our previous work.[14] However, the yield of purified **9a** was poor largely due to the low recovery after silica-gel column chromatography. Therefore, the crude monoisopropylidene derivative was used for the subsequent phosphorylation reaction, without any chromatographic purification.

Subsequently, the nonselective monophosphorylation of the vicinal diol function of the crude **9a** obtained above was attempted expecting that separation of the products will give both of the required 4- and 5-phosphates (**10a** and **11a**). In order to avoid the formation of a 4,5-cyclic phosphate, the phosphoramidite procedure was applied to this monophosphorylation.[21] Phosphitylation of the crude monoisopropylidene derivative **9a** with dibenzyl diisopropylphosphoramidite[22-24] and 1*H*-tetrazole at −30 °C in a mixture of dichloromethane and acetonitrile. The latter solvent was used to improve the solubility of 1*H*-tetrazole. Direct oxidation with *m*-chloroperbenzoic acid (mCPBA), without isolation of the *O*-bis(benzyloxy)phosphino derivatives, afforded a mixture of two products. Their structures were unequivocally determined as 4- and 5-phosphate of Kdo after separation by careful examination of their ^{1}H NMR spectra, where each proton signal was clearly assigned by analysis of the H–H COSY spectra. The product whose H-4 signal appeared at lower field with splitting due to the coupling with phosphorus,[25] was assigned to the 4-*O*-[bis(benzyloxy)phosphinoyl] derivative **10a**. Similarly, based on comparison of chemical shift values and H–P coupling, the other compound was the 5-*O*-[bis(benzyloxy)phosphinoyl] derivative **11a**. Both position isomers were, thus, obtained. However, their total yield was only 21% after the two-step conversion from **8a**, owing mainly to the competitive formation of the corresponding 4,5-bisphosphate and to the insufficient stability of the protecting isopropylidene group.

In order to improve the yield of the phosphorylated products, the use of a benzylidene group was next examined for the protection of the 7- and 8-hydroxyl groups. Treatment of **8a** with α,α-dimethoxytoluene and CSA at room temperature resulted in a diastereomeric mixture of the 7,8-*O*-benzylidene derivative **12a** which gave a single spot on TLC but which showed two signals (total 1H), both attributed to the benzylidene CH in its ^{1}H NMR spectrum. Reaction of this benzylidene derivative **12a** with the dibenzyl phosphoramidite and 1*H*-tetrazole was carried out as described above. The progress of the reaction was monitored by TLC and the amidite reagent was added to complete the conversion. Subsequent oxidation with mCPBA gave a mixture of products which gave two spots on silica gel TLC (R_f 0.77 and 0.54; chloroform–acetone, 9 : 1). After their separation by silica-gel column chromatography, ^{1}H NMR analysis revealed that the faster moving compound was yet a mixture composed of 5-phosphate **14a** and a diastereomer of 4-phosphate **13a**. The slower-moving compound was the other diastereomer of the 4-phosphate.

For preparative purposes, the benzylidene group of the phosphorylation product was removed with trifluoroacetic acid (TFA), prior to the separation. The debenzylidenated product was subjected to preparative HPLC on a reversed phase column to give the dibenzyl esters of 4- and 5-phosphates (**15a** and **16a**) which were identical with the products obtained by TFA treatment of the isopropylidene derivatives **10a** and **11a**, respectively. The yield was much improved by the latter route via the 7,8-*O*-benzylidene derivatives.

Cleavage of the benzyl esters of the phosphate moiety was effected quite smoothly with trimethylsilyl (TMS) bromide in dichloromethane at room temperature.[26] The hydrogenolytic removal of the benzyl group had to be avoided here because the allyl group was to be retained. The final alkaline hydrolysis of the methyl ester, and purification by HPLC on an anion-exchange resin, afforded the deprotected α-allyl glycoside 4- and 5-phosphate (**2a** and **3a**) as their ammonium salt. The purity and the structure of each product were confirmed by ^{1}H NMR and high-resolution negative FAB-mass spectra. Additional evidence excluding the possibility of mutual contamination of 4- and 5-phosphate, which may have resulted from the phosphate migration or



insufficient separation, was also obtained by analytical ion-pair HPLC on a reversed phase column.

The β-allyl glycoside of the Kdo methyl ester **6b** was also converted to the corresponding 4- and 5-phosphate (**2b** and **3b**) in the same way via 7,8-*O*-benzylidene derivative (**12b**).

Cleavage of the benzyl esters of the 5-phosphate **16b** proceeded satisfactorily with TMS bromide followed by sodium hydroxide to give the 5-phosphate of the β-allyl glycoside **3b**. In contrast, cleavage of the benzyl esters of the corresponding 4-phosphate **15b** with TMS bromide gave a mixture of more polar compounds, which lacked the glycosidic allyl group as judged from the positive reaction on TLC with the 2,3,5-triphenyl-2*H*-tetrazolium chloride reagent for reducing sugars.[27] In the NMR spectrum of **15b**, the signals of H-7 and H-8 appeared as rather broad peaks, most likely indicating the presence of hydrogen bonds between the bis(benzyloxy)phosphinoyl group and the two hydroxyl groups at 7- and 8-positions. Such hydrogen bonding is sterically not possible if the pyranoside ring of **15b** exists in a normal $^5C_2$ conformation, where the C7–C8 side chain and the bis(benzyloxy)phosphinoyl group are in a 1,3-diequatorial relation to each other. The ring of **15b** was therefore assumed to be distorted from the chair to $^{0,4}B$ or similar conformation, in which the benzylated phosphate moiety comes also very close to the glycosidic position. We thus anticipated that the glycosidic allyl group was cleaved by direct intramolecular attack of the acidic 4-*O*-phosphono group after the removal of the benzyl esters with TMS bromide.[28] In order to avoid such possible assistance of the free hydroxyl groups to the undesirable conformational change, the hydroxyl groups were acetylated prior to the reaction with TMS bromide. In fact, the reaction of the resultant peracetate with TMS bromide gave a single product, which, on alkaline hydrolysis, gave the desired 4-phosphate **2b** with the intact β-allyl group. The structures and the purities of both 4- and 5-phosphates of the β-allyl glycoside (**2b** and **3b**) were also confirmed in the same way as their α-anomers.

The synthetic phosphates of pyranosidic Kdo with α- and β-linked allyl groups are now being subjected to serological and other biological studies. In addition, the present work has also provided a synthetic procedure applicable to the future chemical synthesis of LPS which contains phosphorylated Kdo(s) in its inner core region.

Experimental

^{1}H NMR spectra were measured on a Varian XL-100-15 spectrometer (100 MHz) or a JEOL JNM-GSX 270, 400, or 500 spectrometer for $CDCl_3$ solutions unless otherwise noted. The chemical shifts are given in δ values either from TMS as the internal standard in $CDCl_3$ solutions or from sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the external standard in D_2O solutions. High-resolution negative FAB-mass spectra were recorded on a JEOL SX-102 mass spectrometer with glycerol as the matrix, where cluster peaks of glycerol served as standards for calculation. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. High-performance liquid chromatography (HPLC) was carried out with a Shimadzu LC-6A liquid chromatography apparatus with a column specified: The peaks were detected by the absorbance at 220 nm. Analytical ion-pair HPLC was carried out on a Cosmosil 5C18 column (4×250 mm) with a solvent system composed of 0.05 M (1M=1 mol dm^{-3}) *n*-Bu_4NHSO_4 and 0.05 M $(NH_4)_2SO_4$ in 3% CH_3CN at a flow rate of 1.0 ml min^{-1}. Peaks were detected at 210 nm. Kieselgel 60 (E. Merck), 0.040—0.063 mm, was used for silica-gel column chromatography at medium pressure (2—4 kg cm^{-2}). Organic solutions were dried over $MgSO_4$ and evaporated in vacuo.

Methyl 2-*O*-Acetyl-3-deoxy-4,5 : 7,8-di-*O*-isopropylidene-α-D-*manno*-2-octulosonate (5). Kdo ammonium salt (4.00 g, 15.7 mmol) was dissolved in water (15 ml) and passed through a column of Amberlite IRCG-120 (H^+ form, 40×150 mm). The column was eluted with water, and the acidic eluate was combined and lyophilized. A solution of the lyophilizate in MeOH (150 ml) was treated with an ethereal solution of CH_2N_2. The residue obtained by evaporation of the solvent was suspended in dry acetone (300 ml). After the addition of *p*-

toluenesulfonic acid monohydrate (600 mg, 3.15 mmol), the mixture was stirred at room temperature for 13 h and then neutralized by addition of Na_2CO_3 (677 mg, 6.30 mmol). The insoluble materials were filtered off, the filtrate evaporated, and the residue was dissolved in dry CH_2Cl_2 (80 ml). To the solution cooled in an ice bath were added pyridine (9.8 ml, 125 mmol), acetic anhydride (5.9 ml, 63 mmol), and 4-dimethylaminopyridine (766 mg, 6.27 mmol). The mixture was stirred at room temperature for 1 h, diluted with AcOEt (1.2 l) and worked up as usual. The crude product was subjected to column chromatography on silica gel (180 g; $CHCl_3$–acetone, 50 : 1) to give syrup; yield 4.30 g (73%). $[\alpha]_D^{18}$ +74.2° (*c* 1.12, $CHCl_3$). ^1H NMR (100 MHz) δ=4.70—3.90 (5H, m, H-4, 5, 7, 8, 8′), 3.78 (3H, s, OCH_3), 3.60 (1H, dd, *J*=8 and 2 Hz, H-6), 2.72 (1H, dd, *J*=16 and 3 Hz, H-3_{eq}), 2.10 (3H, s, $COCH_3$), 2.08 (1H, dd, *J*=16 and 3 Hz, H-3_{ax}), 1.46, 1.41. 1.39, 1.32 (each 3H, s, isopropylidene CH_3). Found: C, 54.51; H, 7.05%. Calcd for $C_{17}H_{26}O_9$: C, 54.54; H, 700%.

Methyl (3-Deoxy-4,5 : 7,8-di-*O*-isopropylidene-α-D-*manno*-2-octulopyranosyl fluorid)onate (4). To a solution of **5** (390 mg, 1.04 mmol) in pyridine (0.65 ml) cooled to −70 °C was added 70% HF–pyridine (3.0 ml) and CH_2Cl_2 (1.2 ml). The mixture was stirred at −20 °C for 10 min and at 0 °C for 10 min, and then diluted with AcOEt (70 ml). The solution was washed successively with aq solutions of 10% KF (3 times), saturated NaCl, saturated $NaHCO_3$, and NaCl and then dried. The residue obtained after evaporation of the solvent was purified by silica-gel column chromatography (10 g; $CHCl_3$–acetone, 20 : 1); yield 231 mg (66%). Mp 121—122 °C. The product was identified with the compound obtained previously.[18] $[\alpha]_D^{18}$ +11.9° (*c* 1.06, $CHCl_3$). ^1H NMR (270 MHz) δ=4.57 (1H, m, H-4), 4.43—4.35 (2H, m, H-5, 7), 4.15 (1H, dd, *J*=8.9, 5.9 Hz, H-8), 4.08 (1H, dd, *J*=8.9, 4.0 Hz, H-8′), 3.81 (3H, s, OCH_3), 3.67 (1H, ddd, *J*=8.5, 2.2, 2.2 Hz, H-6), 3.06 (1H, ddd, *J*=15.6, 3.7, $J_{3e,F}$=3.7 Hz, H-3_{eq}), 1.98 (1H, ddd, *J*=15.6, 2.8, $J_{3a,F}$=18.4 Hz, H-3_{ax}), 1.44, 1.39, 1.38, 1.33 (each 3H, s, isopropylidene CH_3). Found: C, 53.91; H, 6.96%. Calcd for $C_{15}H_{23}O_7F$: C, 53.89; H, 6.93%.

Methyl (Allyl 3-Deoxy-4,5 : 7,8-di-*O*-isopropylidene-α-D-*manno*-2-octulopyranosid)onate (6a) and Methyl (Allyl 3-Deoxy-4,5 : 7,8-di-*O*-isopropylidene-β-D-*manno*-2-octulopyranosid)onate (6b). To an ice-cooled solution of **4** (1.85 g, 5.53 mmol) in dry CH_2Cl_2 (40 ml) were successively added allyl alcohol (489 μl, 7.19 mmol), *N*,*N*-diisopropylethylamine (963 μl, 5.53 mmol) and BF_3 etherate (1.43 ml, 11.6 mmol) under N_2. The solution was stirred for 5 min at 0 °C, then cooled to −70 °C and *N*,*N*-diisopropylethylamine (1.5 ml, 8.6 mmol) was added.[29] The mixture was diluted with CH_2Cl_2 (200 ml), washed with saturated aq NaCl solution, and dried. Separation by silica-gel column chromatography (160 g; $CHCl_3$–acetone, 70 : 1) gave **6a** and **6b** as colorless syrup.

6a, yield 1.20 g (58%). This fraction was contaminated with small amount of **7** (**6a** : **7**=20 : 1 as judged from ^1H NMR data). $[\alpha]_D^{18}$ +36.5° (*c* 1.03, $CHCl_3$). ^1H NMR (500 MHz) δ=5.86 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.26 and 5.16 (each 1H, m, $-OCH_2-CH=C\underline{H}_2$), 4.52 (1H, ddd, *J*=7.4, 4.0, 3.0 Hz, H-4), 4.39 (1H, ddd, *J*=7.9, 6.4, 4.6 Hz, H-7), 4.29 (1H, dd, *J*=7.4, 1.8 Hz, H-5), 4.16 (1H, dd, *J*=8.7, 6.4 Hz, H-8), 4.15 (1H, m, $-OC\underline{H}_2-CH=CH_2$), 3.99 (1H, dd, *J*=8.7, 4.6 Hz, H-8′), 3.82 (1H, m, $-OC\underline{H}_2-CH=CH_2$), 3.76 (3H, s, OCH_3), 3.60 (1H, dd, *J*=7.9, 1.8 Hz, H-6), 2.82 (1H, dd, *J*=15.5, 4.0 Hz, H-3_{eq}), 1.91 (1H, dd, *J*=15.5, 3.0 Hz, H-3_{ax}), 1.43, 1.41, 1.38, 1.32 (each 3H, s, isopropylidene CH_3).

6b, yield 490 mg (24%). $[\alpha]_D^{18}$ +4.0° (*c*, 1.01, $CHCl_3$). ^1H NMR (400 MHz) δ=5.89 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.25 and 5.12 (each 1H, m, $-OCH_2-CH=C\underline{H}_2$), 4.50 (1H, ddd, *J*=7.5, 4.2, 4.2 Hz, H-4), 4.32 (1H, ddd, *J*=8.2, 6.2, 4.2 Hz, H-7), 4.29 (1H, dd, *J*=7.5, 2.0 Hz, H-5), 4.25 (1H, dd, *J*=8.7, 4.2 Hz, H-8), 4.22 (1H, m, $-OC\underline{H}_2-CH=CH_2$), 4.12 (1H, dd, *J*=8.7, 6.2 Hz, H-8′), 3.96 (1H, m, $-OC\underline{H}_2-CH=CH_2$), 3.77 (3H, s, OCH_3), 3.51 (1H, dd, *J*=8.2, 2.0 Hz, H-6), 2.29 (1H, dd, *J*=15.4, 4.2 Hz, H-3_{eq}), 2.05 (1H, dd, *J*=15.4, 4.2 Hz, H-3_{ax}), 1.50, 1.42, 1.38, 1.36 (each 3H, s, isopropylidene CH_3). Found: C, 57.94; H, 7.60%. Calcd for $C_{18}H_{28}O_8$: C, 58.05; H, 7.58%.

Methyl (Allyl 3-Deoxy-7,8-*O*-isopropylidene-α-D-*manno*-2-octulopyranosid)onate (9a). Trifluoroacetic acid (TFA) (6 ml) containing 5% of water was added to an ice-cooled solution of **6a** (680 mg, 1.83 mmol) in CH_2Cl_2 (70 ml), and the mixture stirred at the same temperature for 1 h. Evaporation under reduced pressure after addition of toluene was repeated three times followed by lyophilization from dioxane gave methyl (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**8a**) as resinous solid (yield 530 mg, 99%).

To a solution of **8a** (295 mg, 1.01 mmol) in dry acetone (40 ml) cooled to −35 °C were added 2-methoxypropene (193 μl, 2.02 mmol) and (+)-camphor-10-sulfonic acid (CSA) (23 mg, 0.10 mmol). The mixture was stirred at that temperature for 5 min, neutralized by addition of aq $NaHCO_3$ solution, and filtered. The filtrate was diluted with $CHCl_3$ (300 ml), worked up as usual, and evaporated to give colorless syrup; yield 286 mg (85%). This compound was used without purification for the subsequent reaction. ^1H NMR (270 MHz) δ=5.89 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.28 and 5.18 (each 1H, m, $-OCH_2-CH=C\underline{H}_2$), 4.40 (1H, ddd, *J*=7.9, 6.2, 4.9 Hz, H-7), 4.17 (1H, dd, *J*=8.7, 6.2 Hz, H-8), 4.13 (1H, m, H-4), 4.10—3.80 (4H, m, H-5, 8′, and $-OC\underline{H}_2-CH=CH_2$), 3.78 (3H, s, OCH_3), 3.57 (1H, dd, *J*=7.9, 1.2 Hz, H-6), 2.19 (1H, ddd, *J*=12.9, 5.2, 0.7 Hz, H-3_{eq}), 1.91 (1H, dd, *J*=12.9, 11.4 Hz, H-3_{ax}), 1.41 and 1.37 (each 3H, s, isopropylidene CH_3).

Methyl [Allyl 4-*O*-[Bis(benzyloxy)phosphinoyl]-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-2-octulopyranosid]onate (10a) and Methyl [Allyl 5-*O*-[Bis(benzyloxy)phosphinoyl]-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-2-octulopyranosid]-onate (11a). A mixture of **9a** (62 mg, 0.19 mmol) and 1*H*-tetrazole (30 mg, 0.43 mmol) in dry CH_2Cl_2 (9 ml) and dry CH_3CN (0.9 ml) was cooled to −35 °C under Ar atmosphere. A solution of dibenzyl diisopropylphosphoramidite[22] (128 mg, 0.372 mmol) in dry CH_2Cl_2 (500 μl) was added to the mixture, and the whole mixture was stirred at −30 °C for 2 h. Stirring was further continued at −30 °C for 5 min after addition of a solution of mCPBA (96 mg, 0.56 mmol) in CH_2Cl_2 (2 ml). Then the mixture was diluted with CH_2Cl_2 (60 ml), washed with 10% aq $NaHSO_3$ solution, and worked up as usual. The product was subjected to separation by preparative HPLC under the following conditions and lyophilized: Cosmosil 5C18 (8×250 mm), 60—80% CH_3CN (linear gradient, 1% min^{-1}), 3 ml min^{-1}.

10a, yield 14 mg (13% from **8a**) as colorless solid. Retention time in HPLC, 9.9 min. $[\alpha]_D^{26}$ +47.1° (*c* 1.00, $CHCl_3$). ^1H NMR (270 MHz) δ=7.4—7.25 (10H, aromatic), 5.85 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.27 (1H, m, $-OCH_2-CH=C\underline{H}_2$), 5.21—5.01 (5H, m, $-OC\underline{H}_2-CH=CH_2$, benzyl CH_2), 4.87 (1H, broad m, H-4), 4.34 (1H, ddd, *J*=8.7, 6.2, 4.5 Hz, H-7), 4.08 (1H, dd, *J*=8.7, 6.2 Hz, H-8), 3.96—3.89 (4H, m, H-5, 8′, $-OC\underline{H}_2-$

CH=CH$_2$), 3.75 (3H, s, OCH$_3$), 3.55 (1H, dd, J=8.7, 1.7, 0.5 Hz, H-6), 2.32 (1H, ddd, J=12.4, 4.2, 0.5 Hz, H-3$_{eq}$), 2.03 (1H, dd, J=12.1, 12.4 Hz, H-3$_{ax}$), 1.38 and 1.24 (each 3H, s, isopropylidene CH$_3$). Found: C, 58.05; H, 6.43%. Calcd for $C_{29}H_{37}O_{11}P\cdot 0.4H_2O$: C, 58.07; H, 6.35%.

11a, yield 10 mg (9% from **8a**) as colorless solid. Retention time in HPLC, 11.2 min. $[\alpha]_D^{26}$ +21.0° (c 1.00, CHCl$_3$). ^{1}H NMR (270 MHz) δ=7.35 (10H, aromatic), 5.87 (1H, m, -OCH$_2$-CH=CH$_2$), 5.28 and 5.18 (each, 1H, m, -OCH$_2$-CH=CH$_2$), 5.15—5.05 (4H, benzyl CH$_2$), 4.81 (1H, m, H-5), 4.27 (1H, ddd, J=8.4, 6.2, 4.4 Hz, H-7), 4.20 (1H, ddd, J=12.1, 4.9, 2.5 Hz, H-4), 4.11 (1H, dd, J=8.6, 6.2 Hz, H-8), 3.97—3.93 (3H, m, H-8′, -OCH$_2$-CH=CH$_2$), 3.77 (3H, s, OCH$_3$), 3.60 (1H, ddd, J=8.4, 3.7, 0.9 Hz, H-6), 2.24 (1H, ddd, J=13.0, 4.9, 0.7 Hz, H-3$_{eq}$), 1.87 (1H, dd, J=13.0, 12.1 Hz, H-3$_{ax}$), 1.36 and 1.23 (each 3H, s, isopropylidene CH$_3$). Found: C, 58.21; H, 6.62%. Calcd for $C_{29}H_{37}O_{11}P\cdot 0.4H_2O$: C, 58.07; H, 6.35%.

Methyl (Allyl 7,8-*O*-Benzylidene-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (12a). To a solution of **8a** (50 mg, 0.17 mmol) in dry THF (1 ml) were added α,α-dimethoxytoluene (50 μl, 0.33 mmol) and CSA (5.5 mg, 24 μmol). The mixture was stirred at room temperature for 2 h and neutralized with aq NaHCO$_3$. After usual work up, the product was purified by silica-gel column chromatography (10 g; CHCl$_3$–acetone, 10 : 1) to give colorless solid; yield 37 mg (56%). $[\alpha]_D^{18}$ +58.8° (c, 1.02, CHCl$_3$). ^{1}H NMR (270 MHz) δ=7.9—7.35 (5H, aromatic), 5.93 and 5.82 (total 1H, s, benzylidene CH), 5.88 (1H, m, -OCH$_2$-CH=CH$_2$), 5.34—5.14 (2H, m, -OCH$_2$-CH=CH$_2$), 4.70—3.80 (7H, m, H-4, 5, 7, 8, 8′, -OCH$_2$-CH=CH$_2$), 3.78 (3H, s, OCH$_3$), 3.7—3.6 (1H, m, H-6), 2.30—2.15 (1H, m, H-3$_{eq}$), 1.94—1.84 (1H, m, H-3$_{ax}$). Found: C, 59.96; H, 6.27%. Calcd for $C_{19}H_{24}O_8$: 59.99; H, 6.36%.

Methyl [Allyl 4-*O*-[Bis(benzyloxy)phosphinoyl]-3-deoxy-α-D-*manno*-2-octulopyranosid]onate (15a) and Methyl [Allyl 5-*O*-[Bis(benzyloxy)phosphinoyl]-3-deoxy-α-D-*manno*-2-octulopyranosid]onate (16a). A suspension of **12a** (58 mg, 0.15 mmol) and 1*H*-tetrazole (21 mg, 0.31 mmol) in dry CH$_2$Cl$_2$ (2 ml) and dry CH$_3$CN (0.2 ml) was cooled to −35 °C under Ar atmosphere. To this cooled mixture was added a solution of dibenzyl diisopropylphosphoramidite (80 mg, 0.23 mmol) in dry CH$_2$Cl$_2$ (270 μl) with stirring. After 2 h, a solution of the amidite (40 mg, 0.12 mmol) was added and stirring was further continued for 1 h. After addition of mCPBA (61 mg, 0.35 mmol), the mixture was stirred for 5 min and then worked up as described above for the preparation of **10a** and **11a**. Column chromatography on silica gel (4 g; CHCl$_3$–acetone, 12 : 1) gave a mixture of **13a** and **14a** as solid, which was dissolved in CH$_2$Cl$_2$ (3 ml) and treated with TFA (150 μl) containing 5% of water under ice-cooling for 30 min. The mixture was worked up as described above and the products were separated by HPLC: Cosmosil 5C18 (16.7×250 mm), 40—50% CH$_3$CN (linear gradient, 1% min^{-1}), 8.0 ml min^{-1}.

15a: Resinous solid; yield 32 mg (38%). Retention time in HPLC under above conditions, 13.8 min. $[\alpha]_D^{20}$ +58.2° (c 1.00, CHCl$_3$). ^{1}H NMR (270 MHz) δ=7.35 (10H, aromatic), 5.85 (1H, m, -OCH$_2$-CH=CH$_2$), 5.25 and 5.16 (each 1H, m, -OCH$_2$-CH=CH$_2$), 5.10—4.98 (4H, benzyl CH$_2$), 4.70 (1H, broad m, H-4), 4.14 (1H, m, H-5), 4.02 (1H, m, H-7), 3.96 and 3.91 (each 1H, m, -OCH$_2$-CH=CH$_2$), 3.81 (1H, m, H-8), 3.75 (3H, s, OCH$_3$), 3.75 (1H, m, H-8′), 3.62 (1H, dd, J=7.2, 1.0 Hz, H-6), 2.25—2.05 (2H, m, H-3$_{ax\ and\ eq}$). Found: C, 55.79; H, 6.16%. Calcd for $C_{26}H_{33}O_{11}P\cdot 0.4H_2O$: C, 55.79; H, 6.09%.

16a: Resinous solid; yield 17 mg (20%). Retention time in HPLC, 15.8 min. $[\alpha]_D^{20}$ +59.6° (c 1.10, CHCl$_3$). ^{1}H NMR (400 MHz) δ=7.27 (10H, aromatic), 5.79 (1H, m, -OCH$_2$-CH=CH$_2$), 5.20 and 5.08 (each 1H, m, -OCH$_2$-CH=CH$_2$), 5.05—4.95 (4H, benzyl CH$_2$), 4.74 (1H, dd, J=2.4 Hz, $J_{H,P}$=9.4 Hz, H-5), 4.13 (1H, m, J=12.4, 4.6, 2.4, 2.4 Hz, H-4), 3.94 and 3.82 (each 1H, m, -OCH$_2$-CH=CH$_2$), 3.82—3.75 (2H, m, H-7, 8), 3.70 (3H, s, OCH$_3$), 3.67—3.62 (2H, m, H-6, 8′) 2.15 (1H, dd, J=12.4, 4.6 Hz, H-3$_{eq}$), 1.75 (1H, dd, J=12.4, 12.4 Hz, H-3$_{ax}$). Found: C, 55.99; H, 6.22%. Calcd for $C_{26}H_{33}O_{11}P\cdot 0.3H_2O$: C, 55.97; H, 6.07%.

Allyl 3-Deoxy-4-*O*-phosphono-α-D-*manno*-2-octulopyranosidonic Acid Diammonium Salt (2a). Compound **15a** (30 mg, 54 μmol) was dissolved in dry CH$_2$Cl$_2$ (2 ml) and treated with TMS bromide (43 μl, 0.33 mmol) at room temperature for 10 min. After evaporation of the mixture, the residue was lyophilized from dioxane and suspended in water (1.5 ml). The mixture was stirred with 1 M NaOH (216 μl, 216 μmol) at room temperature for 35 min and then neutralized with acetic acid, and lyophilized. The crude product was purified by HPLC: TSK-GEL DEAE-5PW (21.5×150 mm), 0.2 M ammonium formate, 7.5 ml min^{-1}. The peak with retention time of 8.4 min was collected and lyophilized to give a resinous solid; yield 19 mg (91%). Retention time in the ion-pair HPLC, 7.3 min. $[\alpha]_D^{15}$ +57.7° (c 1.00, H$_2$O). ^{1}H NMR (400 MHz, D$_2$O) δ=5.95 (1H, m, -OCH$_2$-CH=CH$_2$), 5.34 and 5.21 (each 1H, m, -OCH$_2$-CH=CH$_2$), 4.50 (1H, broad m, H-4), 4.18 (1H, broad, s, H-5), 3.97—3.90 (3H, m, H-7, 8, -OCH$_2$-CH=CH$_2$), 3.82 (1H, m, -OCH$_2$-CH=CH$_2$), 3.65—3.60 (2H, m, H-6, 8′), 2.16 (1H, dd, J=12.7, 4.4 Hz, H-3$_{eq}$), 1.92 (1H, dd, J=12.7, 12.7 Hz, H-3$_{ax}$). High resolution FAB-MS (negative). Found: m/z 357.0592. Calcd for $C_{11}H_{18}O_{11}P$: M−H, 357.0587.

Allyl 3-Deoxy-5-*O*-phosphono-α-D-*manno*-2-octulopyranosidonic Acid Diammonium Salt (3a). Compound **16a** (32 mg, 58 μmol) was treated successively with TMS bromide (48 μl, 0.35 mmol) and 1 M NaOH (174 μl, 174 μmol) as described above for **2a**. The crude product was purified by HPLC with the same column and solvent that used for **2a**: The peak with retention time of 9.6 min with a flow rate of 8.0 ml min^{-1} was collected and lyophilized to give resinous solid: Yield 18 mg (80%). Retention time in the ion-pair HPLC, 16.6 min. $[\alpha]_D^{15}$ +52.5° (c 1.00, H$_2$O). ^{1}H NMR (400 MHz, D$_2$O) δ=5.95 (1H, m, -OCH$_2$-CH=CH$_2$), 5.32 and 5.21 (each 1H, m, -OCH$_2$-CH=CH$_2$), 4.49 (1H, dd, J=2.6 Hz, $J_{H,P}$=9.2 Hz, H-5), 4.14 (1H, ddd, J=12.5, 4.8, 2.6 Hz, H-4), 3.96—3.92 (2H, m, H-7, 8), 3.90 and 3.82 (each 1H, m, -OCH$_2$-CH=CH$_2$), 3.63 (1H, dd, J=12.1, 7.1 Hz, H-8′), 3.59 (1H, d, J=8.2 Hz, H-6), 2.05 (1H, dd, J=12.5, 4.8 Hz, H-3$_{eq}$), 1.86 (1H, dd, J=12.5, 12.5 Hz, H-3$_{ax}$). High resolution FAB-MS (negative). Found: m/z 357.0552. Calcd for $C_{11}H_{18}O_{11}P$: M−H, 357.0587. Found: C, 29.31; H, 6.79; N, 6.19%. Calcd for $C_{11}H_{25}O_{11}PN_2\cdot 3H_2O$; C, 29.60; H, 7.00; N, 6.28%.

Methyl (Allyl 7,8-*O*-Benzylidene-3-deoxy-β-D-*manno*-2-octulopyranosid)onate (12b). An ice-cooled solution of **6b** (470 mg, 1.26 mmol) in CH$_2$Cl$_2$ (40 ml) was treated with 95% TFA (170 μl) and worked up as described above for the preparation of **8a** to yield **8b** (yield 366 mg, 99%) as resinous solid.

Compound **12b** was prepared from **8b** (144 mg, 0.49 mmol) with action of α,α-dimethoxytoluene (98 μl, 0.65 mmol) and CSA (8.5 mg, 37 μmol) under the same conditions described

above for **12a**. The product was purified by silica-gel column chromatography (20 g; $CHCl_3$–acetone, 10 : 1) to give colorless solid; yield 120 mg (64%). $[\alpha]_D^{20}$ +51.7° (*c* 1.00, $CHCl_3$). ^1H NMR (270 MHz) δ=7.47—7.36 (5H, aromatic), 5.90 and 5.81 (total 1H, s, benzylidene CH), 5.87 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.25 and 5.15 (each 1H, m, $-OCH_2-CH=C\underline{H}_2$), 4.56—3.66 (10H, m, H-4, 5, 7, 8, 8′, OCH_3, $-OC\underline{H}_2-CH=CH_2$), 3.59 (1H, dd, *J*=8.5, 1.2 Hz, H-6), 2.48 (1H, m, H-3_{eq}), 1.96 (1H, m, H-3_{ax}). Found: C, 59.94; H, 6.34%. Calcd for $C_{19}H_{24}O_8$: C, 59.99; H, 6.36%.

Methyl [Allyl 4-*O*-[Bis(benzyloxy)phosphinoyl]-3-deoxy-β-D-*manno*-2-octulopyranosid]onate (15b) and Methyl [Allyl 5-*O*-[Bis(benzyloxy)phosphinoyl]-3-deoxy-β-D-*manno*-2-octulopyranosid]onate (16b). To a cooled suspension of **12b** (142 mg, 0.37 mmol) and 1*H*-tetrazole (65 mg, 0.93 mmol) in dry CH_2Cl_2 (6 ml) and dry CH_3CN (0.6 ml) were added two portions of a solution of dibenzyl diisopropylphosphoramidite (194 mg, 0.56 mmol, and 38 mg, 0.11 mmol) in dry CH_2Cl_2 under the same conditions as described for the synthesis of **15a** and **16a**. Then mCPBA (122 mg, 0.71 mmol) was added to the mixture, and the mixture worked up as described above. Column chromatography on silica gel (7 g; $CHCl_3$–acetone, 12 : 1) gave a mixture of **13b** and **14b** as syrup, which was dissolved in CH_2Cl_2 and treated with aq TFA. The products were separated by HPLC: Cosmosil 5C18 (16.7×250 mm), 55—70% CH_3CN (linear gradient, 1% min^{-1}), 8.0 ml min^{-1}.

15b: Resinous solid; yield 55 mg (27%). Retention time in HPLC under above conditions, 9.7 min. $[\alpha]_D^{18}$ +41.7° (*c* 1.06, $CHCl_3$). ^1H NMR (400 MHz) δ=7.30—7.24 (10H, aromatic), 5.75 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.15 and 5.06 (each 1H, m, $-OCH_2-CH=C\underline{H}_2$), 5.05—4.90 (4H, benzyl CH_2), 4.21 (1H, broad m, H-4), 4.15 (1H, m, $-OC\underline{H}_2-CH=CH_2$), 4.10 (1H, broad s, H-5), 3.92 (1H, broad m, H-7), 3.88—3.78 (2H, H-8, $-OC\underline{H}_2-CH=CH_2$), 3.72 (1H, m, H-8′), 3.66 (3H, s, OCH_3), 3.31 (1H, d, *J*=8.4 Hz, H-6), 2.41 (1H, dd, *J*=12.5, 4.8 Hz, H-3_{eq}), 2.17 (1H, dd, *J*=12.5, 12.5 Hz, H-3_{ax}). Found: C, 56.11; H, 6.03%. Calcd for $C_{26}H_{33}O_{11}P$: C, 56.52; H, 6.02%.

16b: Resinous solid; yield 68 mg (33%). Retention time in HPLC, 15.8 min. $[\alpha]_D^{18}$ +40.5° (*c* 1.10, $CHCl_3$). ^1H NMR (400 MHz) δ=7.30—7.25 (10H, aromatic), 5.78 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.18 and 5.09 (each 1H, m, $-OCH_2-CH=C\underline{H}_2$), 5.05—4.95 (4H, benzyl CH_2), 4.66 (1H, dd, *J*=2.3 Hz, J_{HP}=8.7 Hz, H-5), 4.15 and 3.85 (each 1H, m, $-OC\underline{H}_2-CH=CH_2$), 3.75—3.72 (2H, m, H-8,8′), 3.71 (3H, s, OCH_3), 3.74—3.62 (2H, m, H-4,7), 3.53 (1H, dd, *J*=9.0, 0.9 Hz, H-6), 2.43 (1H, dd, *J*=12.7, 4.7 Hz, H-3_{eq}), 1.85 (1H, dd, *J*=12.7, 12.7 Hz, H-3_{ax}). Found: C, 56.25; H, 6.20%. Calcd for $C_{26}H_{33}O_{11}P$: C, 56.52; H, 6.02%.

Allyl 3-Deoxy-5-*O*-phosphono-α-D-*manno*-2-octulopyranosidonic Acid Diammonium Salt (3b). Compound **16b** (49 mg, 89 μmol) was treated with TMS bromide (70 μl, 0.53 mmol) and 1 M NaOH, and the crude product was purified by HPLC with the same column and solvent that used for **2a**: The peak with retention time of 10.8 min at an elution rate of 8.0 ml min^{-1} was collected and lyophilized to give resinous solid; yield 25 mg (71%). Retention time in ion-pair HPLC, 13.4 min. $[\alpha]_D^{15}$ +26.0° (*c* 1.00, H_2O). ^1H NMR (400 MHz, D_2O) δ=5.91 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.30 and 5.20 (each 1H, m, $-OCH_2-CH=C\underline{H}_2$), 4.41 (1H, broad d, $J_{H,P}$=8.3 Hz, H-5), 4.19 and 3.94 (each 1H, m, $-OC\underline{H}_2-CH=CH_2$), 3.92 (1H, m, H-7), 3.83—3.80 (2H, m, H-8,8′), 3.77 (1H, m, H-4), 3.75 (1H, d, *J*=9.2 Hz, H-6), 2.40 (1H, dd, *J*=12.1, 4.4 Hz, H-3_{eq}), 1.87 (1H, dd, *J*=12.4, 12.1 Hz, H-3_{ax}). High resolution FAB-MS (negative). Found: *m/z* 337.0595. Calcd for $C_{11}H_{18}O_{11}P$: M−H, 357.0587.

Allyl 3-Deoxy-4-*O*-phosphono-β-D-*manno*-2-octulopyranosidonic Acid Diammonium Salt (2b). To a solution of **15b** (45 mg, 81 mmol) in dry CH_2Cl_2 (1.0 ml) were added pyridine (51 μl, 0.65 mmol), acetic anhydride (31 μl, 0.33 mmol), and 4-dimethylaminopyridine (4.0 mg, 33 μmol). The mixture was stirred at room temperature for 1 h and worked up as usual. The acetylated product (54 mg) was dissolved in dry CH_2Cl_2 and treated with TMS bromide (42 μl, 0.32 mmol) and worked up as described above. The crude product (40 mg) was dissolved in MeOH (2 ml) without purification. To this solution was added 1 M NaOH (400 μl) under ice-cooling, and the mixture was stirred at room temperature for 1 h.

The crude product was purified by HPLC under the same conditions (elution rate, 7.0 ml min^{-1}) as described for **2a**. The peak with retention time of 7.9 min was collected and lyophilized to give a resinous solid; yield 25 mg (79%). Retention time in ion pair HPLC, 7.0 min. $[\alpha]_D^{15}$ +52.5° (*c* 1.00, H_2O). ^1H NMR (400 MHz, D_2O) δ=5.91 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 5.30 and 5.19 (each, 1H, m, $-OCH_2-CH=C\underline{H}_2$), 4.19 (1H, m, $-OC\underline{H}_2-CH=CH_2$), 4.10 (1H, broad s, H-5), 4.06 (1H, broad m, H-4), 3.95 (1H, m, $-OC\underline{H}_2-CH-CH_2$), 3.91 (1H, ddd, *J*=8.8, 4.4, 1.7 Hz, H-7), 3.87 (1H, dd, *J*=12.1, 4.4 Hz, H-8), 3.73 (1H, dd, *J*=12.1, 1.7 Hz, H-8′), 3.63 (1H, d, *J*=8.8 Hz, H-6), 2.50 (1H, dd, *J*=12.3, 4.6 Hz, H-3_{eq}), 1.88 (1H, dd, *J*=12.3, 12.3 Hz, H-3_{ax}). High resolution FAB-MS (negative). Found: *m/z* 357.0601. Calcd for $C_{11}H_{18}O_{11}P$: M−H, 357.0587.

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28) This explanation is based on the assumption that the boat-like conformation favorable for the hydrogen bonding was retained even after cleavage of the benzyl esters. However, we can not exclude the possibility that the allyl group was lost during the reaction with TMS bromide under not yet understood participation of the phosphate moiety.

29) Addition of *N*,*N*-diisopropylethylamine at higher temperature resulted in considerable cleavage of the isopropylidene groups.