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A new Kdo derivative for the synthesis of an inner-core disaccharide of lipopolysaccharides and lopooligosaccharides

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ABSTRACT

Methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-D-*manno*-2-oct-ulopyranoside)onate was found to be a useful new intermediate in the synthesis of an inner-core oligosaccharide of lipooligosaccharides and lipopolysaccharides produced by gram-negative bacteria. This intermediate could be converted to the corresponding glycosyl fluoride and 4,5-diol acceptor with ease. Syntheses of dimeric Kdo, *O*-(sodium 3-deoxy-α-D-*manno*-2-octuropyranosylonate)-(2–4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octuropyranoside)onate, and *O*-(sodium 3-deoxy-α-D-*manno*-2-octuropyranosylonate)-(2–8)-sodium (allyl 3-deoxy-α-D-*manno*-2-octuropyranoside)onate were successfully demonstrated.

1. Introduction

Lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs) are important surface antigens for a wide variety of gram-negative bacteria. LPS is composed of *O*-antigen, a core oligosaccharide (OS), and lipid A, whereas LOS consists of a core OS and lipid A. The core OS moiety of an LPS or LOS consists of a structurally variable region and a conserved inner-core OS. In its mono-, di-, or trimeric form, 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) is a component of the inner core.

Although many syntheses of monomeric Kdo from D-arabinose and D-mannose have been reported, reports pertaining to the synthesis of dimeric Kdo (i.e., Kdo(α2–4)Kdo) are few.[1–7] Because it is difficult to devise a suitable synthon for the synthesis of a Kdo-containing oligosaccharide, a Kdo acceptor has typically been synthesized from Kdo ammonium salt through a complex series of operations, such as selective protection, deprotection, and chromatographic separation of protected products.

To alleviate this problem, we therefore designed and synthesized methyl 7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-D-*manno*-octulosonate as a synthon for oligosaccharides. This compound has an acid-labile isopropylidene group, and a base-sensitive benzoyl group for OH protection, and can be converted to either a donor or an acceptor. The anomeric OH group can be easily converted to a glycosyl fluoride, phosphate, or trichloroacetimidate under standard conditions. After glycosylation or O-alkylation at the anomeric position, acidic hydrolysis would give a 4,5-diol acceptor, whereas base-catalyzed methanolysis would give a 7,8-diol acceptor. In this paper, we report the preparation of this Kdo intermediate from D-mannose by simple operations, and that it could be converted to its corresponding glycosyl donor and acceptor. Finally we demonstrated syntheses of dimeric Kdo, Kdo(α2–4)Kdo and Kdo(α2–8)Kdo, which are a constituent of the conserved inner core of LPSs and LOSs.

2. Results and discussion

Our designed Kdo derivative, 7,8-di-*O*-acyl-4,5-diisopropylidene-D-*manno*-octulosonate was constructed from 5,6-di-*O*-acyl-2,3-*O*-isopropylidene-D-mannofuranose as follows: two-carbon elongation by the Wittig reaction, dihydroxylation of the unsaturated ester, cyclic sulfite formation, and cyclization. The starting materials, 5,6-di-*O*-acetyl (**2**) and 5,6-di-*O*-benzoyl furanose (**3**), were prepared from 1-*O*-acetyl-2,3-*O*-isopropylidene-D-mannofuranose (**1**)[8] by treatment with acetic anhydride and benzoyl chloride, respectively, followed by hydrolysis of the anomeric acetate in 99% and 93% yield (Fig. 1).

To construct the carbon skeleton, two-carbon elongation of 5,6-diacetate **2** and 5,6-dibenzoate **3** was examined. Treatment of 5,6-diacetate **2** with methyl triphenylphosphoranylidene acetate in

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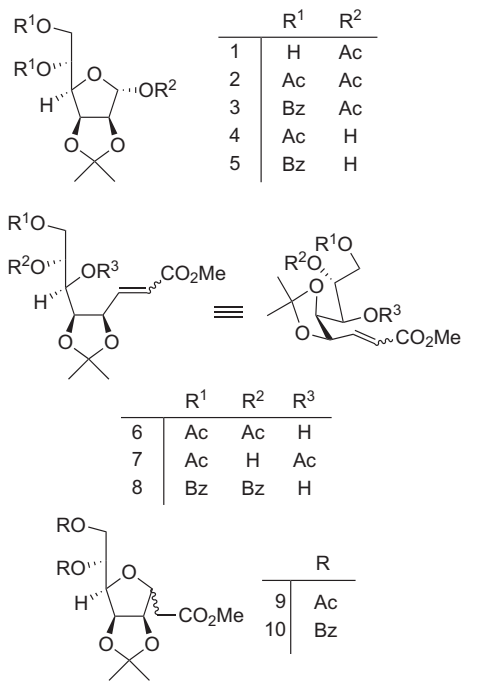
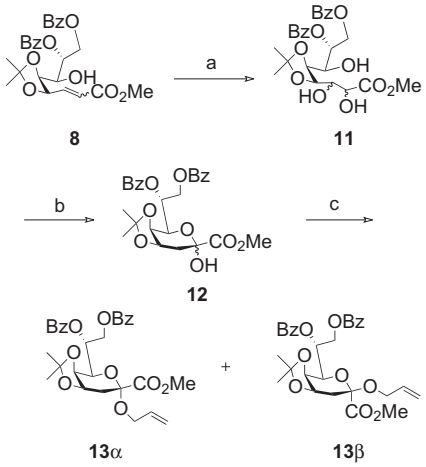


Fig. 1.

toluene at 80 °C afforded a mixture of 7,8-diacetyl unsaturated ester **6** (*E*/*Z*=10/1), 6,8-diacetyl ester **7** from an unexpected acetyl migration, and cyclized product **9**. Although **7** was isolated by silica gel column chromatography, compounds **6** and **9** could not be separated completely. When the reaction was carried out at 110 °C, only the undesired cyclic product **9** was obtained in quantitative yield. Treatment of a mixture of **6** and **9** under Wittig reaction conditions at 110 °C gave the compound **9** convergently. We found that the elongation reaction did not proceed under 80 °C, but migration of the acetyl group from the 7- to 6-position of **6** could not be prevented even when the reaction was carried out at 80 °C. Therefore, to avoid the undesired migration, we abandoned the use of the acetyl group for hydroxyl protection, and instead turned our attention to the benzoyl group. Treatment of benzoyl-protected furanose **3** with 1.2 equiv of Wittig reagent at 55 °C afforded the α,β-unsaturated ester **8** in 97% yield as a mixture of geometric isomers. The *E*/*Z* ratio was 10/1, as determined by comparison of the peak areas of H-2 and H-3 in the ^{1}H NMR spectra. This elongation reaction was also sensitive to temperature. When the reaction was carried at 80 °C, an undesired cyclized product (**10**) was formed in quantitative yield. Using of Horner–Wadsworth–Emmons conditions instead of Wittig conditions also gave **10**. From these results, we selected 7,8-di-*O*-benzoyl-2-octenoate **8** for later studies.

Dihydroxylation of α,β-unsaturated ester **8** with osmium tetraoxide and *N*-methylmorphorine *N*-oxide (NMO) gave triol **11** as a mixture of diastereomers in 96% yield (Scheme 1), along with a small amount of **10** (<3%). To avoid the formation of **10**, it was necessary to add NMO in small portions to the reaction mixture containing the catalyst. The subsequent reaction was carried out without further purification because the newly formed stereocenter would be destroyed later.

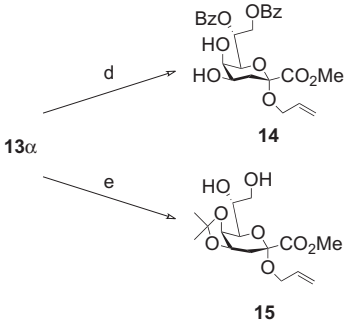
To construct a pyranose ring, the 2- and 3-hydroxyl groups of triol **11** were converted to cyclic sulfites by treatment with thionyl chloride. The sulfites were sequentially treated with DBU in the presence of TMSCl and with 1 M HCl, in accordance with Kuboki's procedure,[9] to give Kdo derivative **12** (Scheme 1) in good yield (82%) with a moderate anomeric ratio (3/1 to 5/1).



Scheme 1. Conditions: (a) OsO_4, NMO, acetone/H_2O, rt, 20 h, 96%; (b) (i)$SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 2 h, (ii) DBU, THF, −78 °C, 0.5 h, and TMSCl, 0 °C, 3.0 h, then 1 M HCl, 10 h, 84%, α/β=3/1; (c) allyl ethyl carbonate, $Pd_2(dba)_3$, dppb, THF, 65 °C, 23 h, 58%.

For the synthesis of dimeric Kdo, a diol acceptor was prepared by O-allylation at the anomeric position of **12**, followed by acid-catalyzed hydrolysis of the 4,5-*O*-isopropylidene group. Compound **12** was treated with allyl ethyl carbonate in the presence of $Pd_2(dba)_3$/dppb to give *O*-allylated **13α** in moderate yield (58%). Although the diastereomeric ratio of starting material **12** was 3/1, only trace amount of β-*O*-allylated **13β** could be detected. The structure of **13α** was determined by X-ray crystal structure analysis (Fig. 2). The pyranose ring was found to have not a chair conformation but rather a boat-like form. However, the molecule did not completely assume the boat configuration because of strain caused by steric repulsion between the methoxy carbonyl group and the methyl group of the 4,5-isopropylidene unit.

Removal of the 4,5-di-*O*-isopropylidene group was accomplished in a few minutes by treatment with aqueous 90% TFA to give 4,5-diol **14** in quantitative yield. Treatment with 80% aqueous acetic acid at 80 °C was also effective for removal of the isopropylidene group but a longer reaction time (>7 h) was required for the reaction to reach completion. The other side, methanolysis of benzoyl esters at 7- and 8-position gave the corresponding 7,8-diol **15** (Scheme 2).



Scheme 2. Conditions: (a) 90% TFA aq, rt, 0.5 h, quant. (b) NaOMe, MeOH, rt, 18 h, quant.

On the other hand, treatment of **12** with DAST[10] afforded glycosyl fluoride **16** as a mixture of anomers in good yield (81%), along with a small amount of an undesired glycal with an α,β-unsaturated ester (11%).[11] The anomeric ratio of **16** was 5/2, and the major isomer was easily isolated by crystallization from ethyl acetate and hexane. The anomeric configuration of the major product was presumed to be α based on a compare with the NMR data of the

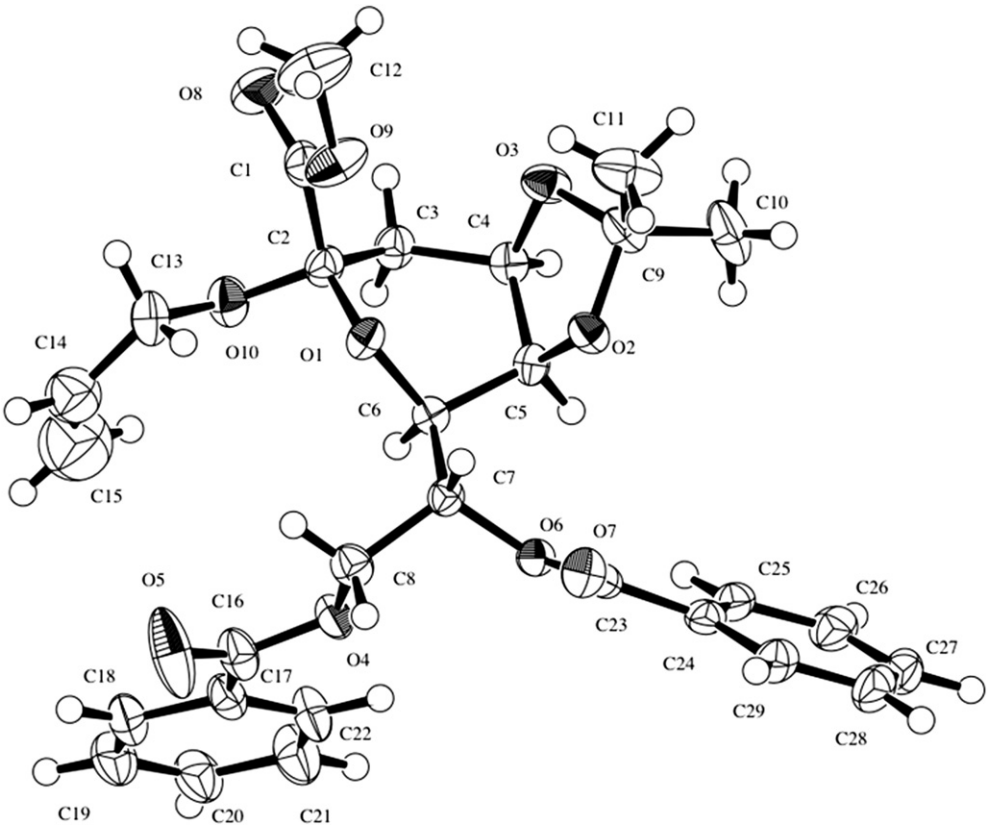


Fig. 2. Crystal structure of **13α**.

corresponding Kdo derivatives **17α** and **17β** reported by Imoto.[12] Table 1 lists data from partial ^{1}H and ^{13}C NMR spectra of fluorides **16α** and **16β** and **17α** and **17β**. In the ^{1}H spectra, clear differences in the chemical shifts at H-3 between major **16** and minor **16** were observed. The difference in chemical shift between the H-3e and the H-3a of major **16** (δ 0.95 ppm) was greater than that of minor **16** (δ 0.14 ppm). Furthermore, the coupling constants of H-3a, H-3e, and F-2 of major **16** were similar to those of compound **17α**, and the chemical shift at C-3 of major **16** was also higher than that of minor **16** in the ^{13}C spectra. Therefore, we presumed the configuration of major **16** to be α (Fig. 3).

Glycosylation of the fluoride donor **16α** with the acceptor **14** in the presence of boron trifluoride etherate was carried out at −20 °C to give α2−4 and β2−4 linked dimeric Kdo **18α** and **18β** in 30% yield and the ratio was 5/1(Scheme 3). However, as Oikawa showed that the addition of tertiaryamine was effective for their glycosylation, the presence of triethylamine increased the yield of **18α**/**β** upto 72 percent.[13] The major and minor product could be separated by silica gel column chromatography. The structure of the major Kdo dimer was determined by analysis of 2D-NMR analysis (COSY, HMQC, and HMBC). From the COSY and HMQC spectra, we were able to identify the endocyclic protons, the hydroxyl proton, and the endocyclic carbon atoms of each residue of **18**. The linkage of the newly formed glycoside was determined by HMBC analysis. Fig. 4 shows part of the COSY and HMBC spectra. The cross peaks in the COSY spectrum (Kdo H-4/H-5 and Kdo H-5/Kdo OH; panel A) and the cross-relay peak in the HMBC spectrum (Kdo H-4/Kdo C-2′; panel B) confirmed that the donor **13α** is linked to the 4-position of the acceptor **14**. The minor component was also formed a 2−4 linkage, which was confirmed in the same manner.

The anomeric configuration of major product **18** was α, which was determined by comparison of the difference in chemical shift between H-3a and H-3b proton in the ^{1}H NMR of each major and

Table 1
Partial ^{1}H and ^{13}C NMR[a] data for glycosyl fluoride derivatives **16α**, **16β**, **17α**, and **17β**

	16α	**16β**	**17α**	**17β**
H-3a	2.08	2.49	1.98	2.14
H-3e	3.04	2.63	3.07	2.42
H-4	4.58	4.66	4.57	4.62
$^2J_{3a,3e}$	15.5	16.0	15.5	16.0
$^3J_{3a,F}$	18.0	32.5	18.0	34.0
$^3J_{3a,4}$	3.0	4.0	3.0	4.0
$^3J_{3e,F}$	4.0	11.0	4.0	10.0
$^3J_{3e,4}$	7.0	2.5	4.0	2.0
C-1	166.0	167.0	166.1	167.5
C-2	107.5	107.1	107.3	107.5
C-3	31.0	30.7	30.7	30.8
$^1J_{C2,F}$	34.5	39.5	35.0	39.5
$^3J_{C4,F}$	7.5	<1.0	8.5	<1.0

[a] Data were acquired in $CDCl_3$ at 25 °C. The ^{1}H and ^{13}C NMR chemical shifts (ppm) were determined by comparing 2D-NMR data (DQF-COSY, HMQC and HMBC), and the *J* couplings (Hz) were obtained by analyzing either the DQF−COSY or 1D NMR spectrum.

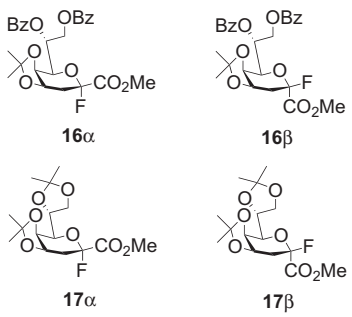
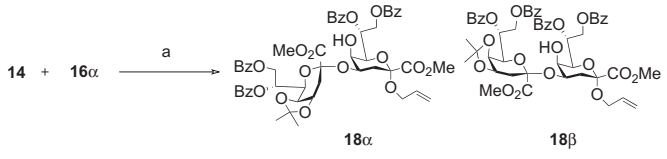


Fig. 3.



Scheme 3. Conditions: (a) $BF_3{\cdot}OEt_2$, Et_3N, MS 5 Å, CH_2Cl_2, 0 °C, 1.5 h, 72% (α/β=5/1).

products was unsatisfactory, the major product was α2–8 linked Kdo dimmer **19**. Minor products could not be determined accurately. In this case, we found that the yield of the Kdo dimer could not be improved even if the reaction performed at −78 °C and the use of excess amount (5 equiv) of boron trifluoride etherate was not suitable because the migration of 4,5-isopropylidene group of the acceptor **15** and other reactions were occurring. Thus further optimization of reaction conditions is necessary.

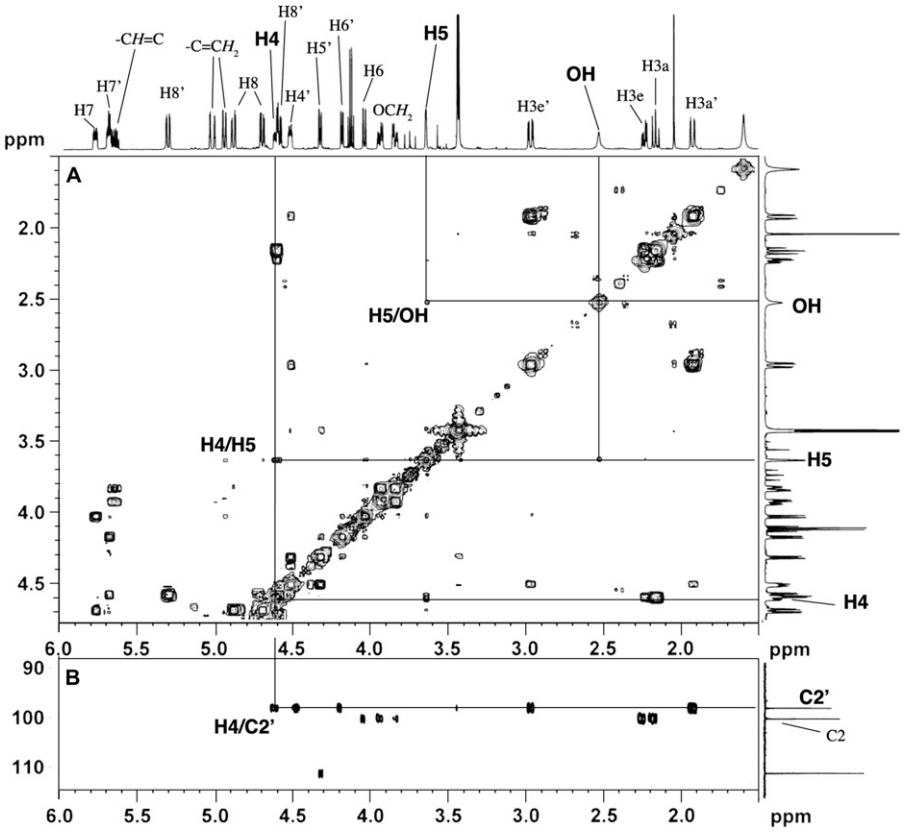
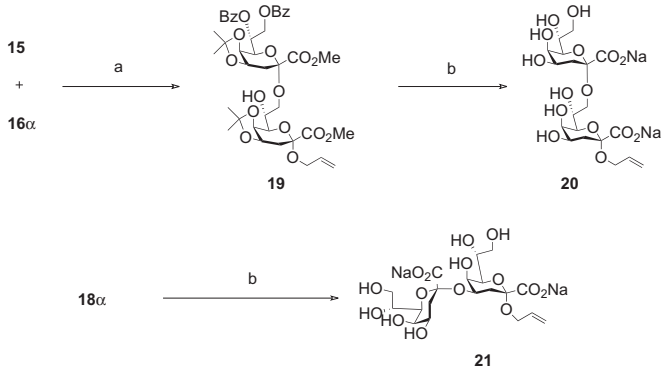


Fig. 4. Partial COSY (panel A) and HMBC (panel B) spectra of compound **18α** in $CDCl_3$ at 25 °C; only partial $^1H/^1H$ cross peaks (A) and cross-relay peak (B) are labeled.

minor product. The difference for the major isomer was δ 1.04 ppm, whereas that for the minor one was δ 0.04 ppm; therefore, the configuration of major product was identified as α and that of minor product was identified as β.

The reaction of 7,8-diol **15** and fluoride **16α** was also given the dimeric Kdo (Scheme 4). The structure of **19** was determined by 2D-NMR described in the above manner. Although the yield of dimeric



Scheme 4. Conditions: (a) $BF_3{\cdot}OEt_2$, Et_3N, MS 5 Å, CH_2Cl_2, (b) 80% TFA aq, CH_2Cl_2, rt, then 1.0 M NaOH, MeOH.

Finally, hydrolysis of the isopropylidene group of Kdo dimers (**18α** and **19**) with aqueous trifluoroacetic acid and subsequent hydrolysis in 0.1 M sodium hydroxide to remove the ester groups afforded fully deprotected dimeric Kdo **20** and **21** as a disodium salt in quantitative yield (Scheme 4). All ^1H and ^{13}C NMR data of synthetic **20** and **21** were consistent with reported data.[14,15]

3. Conclusions

Methyl 7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-D-*manno*-octulosonate **12** was easily converted to glycosyl fluoride and a glycosyl acceptor, and was a useful synthon for the synthesis of dimeric Kdo, *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2–8)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranoside)onate **20** or *O*-(sodium 3-deoxy-α-D-*manno*-2-octuropyranosylonate)-(2–4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octuropyranoside)onate **21**, which are highly conserved disaccharides in the inner-core OS of LPSs and LOSs. Because different conditions can be employed to remove the benzoyl and isopropylidene protecting groups, **12** is able to serve as an acceptor for the synthesis of Kdo(2–4)Kdo and Kdo(2–8)Kdo. The 5-hydroxyl group of compound **18α** can form a connection with several types of monosaccharide and oligosaccharide donors to afford a 4,5-branched core OS. The allyl group at

the reducing end can be functionalized, and can serve as a connection point to a carrier. Therefore, **12** is expected to be a useful intermediate for the synthesis of inner-core OSs containing Kdo as a reducing end residue.

4. Experimental

4.1. General

Optical rotation was measured with a Horiba SEPA-200 or SEPA-500 polarimeter, and melting point (uncorrected) was measured with a Yanagimoto micro melting point apparatus. High-performance liquid chromatography (HPLC) was performed on a Shimadzu HPLC system that consisted of an LC-AD10A pump, SPD-10A UV detector, and a C-R8A recorder. All NMR spectra were recorded at 25 °C on a JEOL JNM-ECP 500 MHz spectrometer or a Brucker Avance II 600 MHz NMR spectrometer. ^{1}H NMR spectra are referenced to internal standards of the residual protonated solvent peaks: δ_H 7.24 ppm for solutions in $CDCl_3$, and δ_H 4.75 ppm for solutions in D_2O, at 25 °C. ^{13}C NMR spectra were recorded at 150 MHz and are referenced to internal $CDCl_3$ (δ_C 77.0 ppm) or to external acetone (δ_C 31.07 ppm). Mass spectrometry (MS) was performed by positive- and negative-mode electrospray ionization on a Thermo Scientific LTQ Orbitrap XL and a Waters LCT Premier spectrometer. For high-precision measurements, the spectra were obtained by scanning voltage over a narrow mass range at 10,000 resolution. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)-MS analysis was performed on an AutoFlex II system from Bruker Daltronics. Elemental analysis was carried out on a Vario EL CUBE and a Vario EL III from Elementar. Silica gel 60 (E. Merck) was used for flash column chromatography (0.040–0.063 mm) and open column chromatography (0.063–0.200 mm). Silica Gel 60 F_{254} (E. Merck) was used for thin-layer chromatography (TLC), and compounds were detected under UV light (254 nm) or by spraying with 10% or 5% H_2SO_4 in MeOH and then heating the plates at 200 °C for 3 min.

*4.1.1. 1-O-Acetyl-5,6-di-O-benzoyl-2,3-O-isopropylidene-α-D-mannofuranose (**3**).* Triethylamine (7.1 mL, 51.2 mmol) and benzoyl chloride (3.3 mL, 28.4 mmol) were added to a solution of 1-*O*-acetyl-2,3-*O*-isopropylidene-α-D-mannofuranose **1** (3.37 g, 12.85 mmol) and 4-*N*,*N*-dimethylaminopyridine (50 mg) in dichloromethane (15 mL) at 0 °C under argon. After stirring for 14 h, the reaction mixture was poured into satd sodium bicarbonate and extracted with dichloromethane. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was crystallized from ethyl acetate and hexane to obtain **3** as a colorless crystal (5.76 g, 95%). Mp 106–107 °C; $[\alpha]_D^{25}$ +26.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.29 (s, 3H, Me), 1.52 (s, 3H, Me), 2.03 (s, 3H, Ac), 4.49 (dd, 1H, $J_{3,4}$=8.0, $J_{4,5}$=7.0 Hz, H-4), 4.64 (dd, 1H, $J_{2,3}$=6.0, $J_{3,4}$=8.0 Hz, H-3), 4.71 (d, 1H, $J_{2,3}$=6.0 Hz, H-2), 4.90 (dd, 1H, $J_{5,6a}$=8.0, $J_{6a,6b}$=12.5 Hz, H-6a), 4.91 (dd, 1H, $J_{5,6b}$=5.6, $J_{6a,6b}$=12.5 Hz, H-6b), 5.74 (ddd, 1H, $J_{4,5}$=7.0, $J_{5,6a}$=8.0, $J_{5,6b}$=5.5 Hz, H-5), 6.22 (s, 1H, H-1), 7.42–8.02 (m, 10H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8 (*C*H$_3$), 24.9 (*C*H$_3$), 26.0 (*C*H$_3$), 63.8 (C-6), 69.6 (C-5), 79.2 (C-4), 80.3 (C-2), 84.7 (C-3), 100.5 (C-1), 113.6 (C_{isop}), 128.3, 128.4, 129.6, 129.7, 129.86, 129.93, 132.9, and 133.1 (Ar), 165.2 (*C*=O), 166.0 (*C*=O), 169.1 (*C*=O). Anal. Calcd for $C_{25}H_{26}O_9$: C, 64.48; H, 5.65. Found C, 64.16; H, 5.89.

*4.1.2. 5,6-Di-O-benzoyl-2,3-O-isopropylidene-D-mannofuranose (**5**).* 1-*O*-Acetyl-5,6-di-*O*-benzoyl-2,3-*O*-isopropylidene-α-D-mannofuranose **3** (4.69 g, 9.97 mmol) was dissolved in a mixed solvent (25% NH_3 aq/THF/MeOH=1/13/13, 176.5 mL) at 0 °C. The mixture was stirred for 17.5 h, diluted with toluene, and concentrated by evaporation of the solvent. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=3/2) to give **5** (4.19 g, 98%) as a colorless syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (s, 3H, Me), 1.49 (s, 3H, Me), 4.59 (dd, 1H, $J_{1,2}$=4.0, $J_{2,3}$=8.0 Hz, H-2), 4.62–4.67 (m, 2H, H-3 and 4), 4.86–4.89 (m, 2H, H-6a and 6b), 5.45 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.73 (m, 1H, H-5), 7.42–8.03 (m, 10H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.7 (*C*H$_3$), 25.6 (*C*H$_3$), 63.9 (C-6), 69.8 (C-5), 78.2 (C-4), 79.6 (C-3), 85.2 (C-1), 101.21 (C-1), 113.0 (C_{isop}), 128.3, 129.6, 129.7, 129.86, 130.0, 133.0, and 133.1 (Ar), 165.3 (*C*=O), 166.3 (*C*=O). Anal. Calcd for $C_{23}H_{24}O_8$: C, 64.45; H, 5.83. Found C, 64.43; H, 5.66.

*4.1.3. Methyl 7,8-di-O-benzoyl-4,5-O-isopropylidene-2-octenoate (**8**).* Methyl (triphenylphosphoranylidene)acetate (1.67 g, 5.00 mmol) was slowly added to a solution of 5,6-di-*O*-benzoyl-2,3-*O*-isopropylidene-D-mannofuranose **5** (1.87 g, 3.87 mmol) in toluene (44 mL). The mixture was heated at 55 °C and stirred for 21 h. After cooling to room temperature, the reaction solution was directly purified by silica gel column chromatography (dichloromethane/ethyl acetate=5/1) to give an *E*/*Z* mixture of compound **8** (1.85 g, 97%) as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37 (s, 3H, Me), 1.56 (s, 3H, Me), 2.62 (br s, 1H, OH), 3.69 (s, 0.27H, OMe), 3.73 (s, 2.73H, OMe), 3.96 (dd, 1H, $J_{5,6}$=2.5, $J_{6,7}$=8.0 Hz, H-6), 4.42 (dd, 1H, $J_{4,5}$=7.0, $J_{5,6}$=2.5 Hz, H-5), 4.65 (dd, 1H, $J_{7,8a}$=3.0, $J_{8a,8b}$=12.5 Hz, H-8a), 4.81 (dd, 1H, $J_{3,4}$=6.5, $J_{4,5}$=7.0 Hz, H-4), 4.87 (dd, 1H, $J_{7,8b}$=6.0, $J_{8a,8b}$=12.5 Hz, H-8b), 5.48 (ddd, 1H, $J_{6,7}$=8.0, $J_{7,8a}$=3.0, $J_{7,8b}$=6.0 Hz, H-7), 5.99 (d, 0.09H, $J_{2,3}$=11.5 Hz, H-2), 6.12 (d, 0.91H, $J_{2,3}$=15.5 Hz, H-2), 6.57 (d, 0.09H, $J_{2,3}$=11.5 Hz, H-3), 7.11 (d, 0.91H, $J_{2,3}$=15.5 Hz, H-3), 7,42–8.00 (m, 5H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.9 and 26.6 (Me′) 24.8 and 26.9 (Me), 51.6 (OMe′), 51.8 (OMe,), 63.4 (C-8), 64.4 (C-8′), 67.6 (C-6′) 68.3 (C-6), 70.1 (C-7′), 72.8 (C-7), 76.7 (C-4 and C4′), 76.9 (C-5 and C-5′), 109.8 (C_{isop}), 109.2 (C′$_{isop}$), 120.2 (C-3′), 123.6 (C-3), 128.5, 128.6, 129.7, 129.9, 133.2, and 133.5 (Ar), 128.4, 128.5, 129.6, 129.7, 133.0, and 133.3 (Ar′), 143.1 (C-2), 148.6 (C-2′), 166.1 (*C*=O), 166.3 (2*C*=O), 166.4 (*C*=O), 170.6 (C-1) 171.2 (C-1′). Anal. Calcd for $C_{26}H_{28}O_9$: C, 64.45; H, 5.83. Found C, 64.43; H, 5.66.

*4.1.4. Methyl (7,8-di-O-benzoyl-4,5-O-isopropylidene-3-deoxy-D-manno-2-octulopyranoside)onate (**12**).* A solution of compound **8** (2.5 g, 5.1 mmol) in acetone (8.0 mL) was added to a solution of *N*-methylmorpholine *N*-oxide (725 mg, 6.2 mmol) and 1% OsO_4 aq (5.3 mL, 0.21 mmol) in acetone (8.0 mL) and water (8.0 mL) at 0 °C. After being vigorously stirring for 20 h, the mixture was concentrated to remove acetone, and the resulted solution was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane=5/1) to give methyl 7,8-di-*O*-benzoyl-2,3,6-trihydroxyl-4,5-*O*-isopropylidene-2-octanoate (**11**) as a mixture of diastereomers as a colorless syrup (2.5 g, 96%). Diastereomers were not further purified. The diastereomers of methyl 7,8-di-*O*-benzoyl-2,3,6-trihydroxyl-4,5-*O*-isopropylidene-2-octanate **11** (2.1g, 2.0 mmol) were dissolved in dichloromethane (36 mL). Triethylamine (2.8 mL, 20 mmol) was added to the solution, and then thionyl chloride (0.64 mL, 9.0 mmol) was added at 0 °C. After stirring for 2 h, the mixture was poured into satd NH_4Cl aq, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was dissolved in THF (36 mL), and treated with DBU (2.4 mL, 16.2 mmol) at −78 °C. After stirring for 0.5 h, chlorotrimethylsilane (2.0 mL, 15.4 mmol) was added to the mixture, which was warmed to room temperature and stirred for 3.0 h. The mixture was treated with 8.0 mL of 1 M HCl for 10 h, neutralized with satd $NaHCO_3$, and extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by sílica gel column chromatography (hexane/

ethyl acetate=3/2 to 1/1) to afford an α/β mixture of compound **12** as a colorless foam (1.7 g, 84%, major/minor=3/1). Major isomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20 (s, 3H, Me), 1.47 (s, 3H, Me), 1.94 (dd, 1H, $J_{3a,3e}$=14.0, $J_{3a,4}$=5.0 Hz, H-3a), 2.47 (dd, 1H, $J_{3e,4}$=7.5, $J_{3a,3e}$=14.0 Hz, H-3e), 3.82 (s, 3H, OMe), 4.28 (dd, 1H, $J_{4,5}$=6.0, $J_{5,6}$=2.5 Hz, H-5), 4.50 (dd, 1H, $J_{5,6}$=2.5, $J_{6,7}$=7.5 Hz, H-6), 4.52 (ddd, 1H, $J_{3e,4}$=7.5, $J_{3a,4}$=5.0, $J_{4,5}$=6.0 Hz, H-4), 4.75 (dd, 1H, $J_{7,8a}$=5.0, $J_{8a,8b}$=12.0 Hz, H-8a), 4.84 (dd, 1H, $J_{7,8b}$=3.0, $J_{8a,8b}$=12.0 Hz, H-8b), 5.71 (ddd, 1H, $J_{6,7}$=7.5, $J_{7,8b}$=3.0, $J_{7,8a}$=5.0 Hz, H-7), 7.37–7.45 (m, 4H, Ar), 7.52–7.58 (m, 2H, Ar), 7.95–8.03 (m, 4H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.8 (Me) 27.3 (Me), 32.6 (C-3), 53.2 (OMe), 63.1 (C-8), 68.4 (C-6), 69.9 (C-4), 70.8 (C-5), 70.9 (C-7), 94.7 (C-2), 109.5 (C_{isop}), 128.47, 128.51, 129.7, 129.8, 129.6, 132.0, 133.1, and 133.3 (Ar), 169.9 (C-1). Minor isomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (s, 3H, Me), 1.58 (s, 3H, Me), 2.33 (dd, 1H, $J_{3a,3e}$=14.0, $J_{3a,4}$=5.0 Hz, H-3a), 2.49 (dd, 1H, $J_{3a,3e}$=14.0, $J_{3e,4}$=8.0 Hz, H-3e), 3.64 (s, 3H, OMe), 4.41 (dd, 1H, $J_{4,5}$=8.0, $J_{5,6}$=2.5 Hz, H-5), 4.09 (dd, 1H, $J_{5,6}$=2.5, $J_{6,7}$=8.0 Hz, H-6), 4.74 (dd, 1H, $J_{3a,4}$=5.0, $J_{3e,4}$=8.0, $J_{4,5}$=8.0 Hz, H-4), 4.70 (dd, 1H, $J_{7,8a}$=5.0, $J_{8a,8b}$=10.5 Hz, H-8a), 4.87 (dd, 1H, $J_{7,8b}$=2.5, $J_{8a,8b}$=10.5 Hz, H-8b), 5.60 (ddd, 1H, $J_{6,7}$=8.0, $J_{7,8a}$=5.0, $J_{7,8b}$=2.5 Hz, H-7), 7.37–7.45 (m, 4H, Ar), 7.52–7.58 (m, 2H, Ar), 7.95–8.03 (m, 4H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.4 (Me) 26.1 (Me), 31.0 (C-3), 52.8 (OMe), 62.9 (C-8), 60.4 (C-6), 70.3 (C-4), 72.2 (C-5), 71.0 (C-7), 95.9 (C-2), 110.0 (C_{isop}), 128.3, 128.4, 129.9, 130.0, 130.6, 133.1, and 133.2 (Ar), 169.0 (C-1). Anal. Calcd for $C_{26}H_{29}O_{10}$: C, 62.39; H, 5.64. Found C, 62.11; H, 5.67.

*4.1.5. Methyl (allyl 7,8-di-O-benzoyl-4,5-O-isopropylidene-3-deoxy-α-D-manno-2-octulopyranoside)onate (**13α** and **13β**).* To a solution of methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-D-*manno*-2-octulopyranoside)onate **12** (623.2 mg, 1.25 mmol) and $Pd(dba)_2$ (17.9 mg, 0.03 mmol) in THF (10 mL), a solution of 1,4-bis(diphenylphosphino)butane (53.1 mg, 0.13 mmol) and ethyl allyl carbonate (328 μL, 2.49 mmol) in THF was added under argon. The reaction mixture was refluxed for 23 h, cooled to room temperature, and concentrated. Purification of the residue by silica gel column chromatography (toluene/hexane/ethyl acetate=5/1/1) gave 314 mg of compound **13** as a colorless solid (58%). Further recrystallization from ethyl acetate and hexane gave the sample used in X-ray analysis. Crystallographic data for compound **13α** have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 804059. Compound **13α**: mp 116–117 °C; $[\alpha]_D^{25}$ +18.3 (*c* 1.03, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23 (s, 3H, Me), 1.45 (s, 3H, Me), 2.01 (dd, 1H, $J_{3a,3e}$=15.5, $J_{3a,4}$=3.5 Hz, H-3a), 2.76 (dd, 1H, $J_{3e,4}$=4.5, $J_{3a,3e}$=15.5 Hz, H-3e), 3.79 (s, 3H, OMe), 3.84 (dddd, 1H, *J*=12.5, 5.5, 1.5, and 1.5 Hz, O*CH*$_2$), 4.21 (dd, 1H, $J_{5,6}$=1.8, $J_{6,7}$=7.5 Hz, H-6), 4.31 (dddd, 1H, *J*=12.5, 5.5, 1.5, and 1.5 Hz, O*CH*$_2$), 4.32 (dd, 1H, $J_{4,5}$=7.5, $J_{5,6}$=1.8 Hz, H-5), 4.54 (dd, 1H, $J_{3a,4}$=3.5, $J_{3e,4}$=4.5, $J_{4,5}$=7.5 Hz, H-4), 4.72 (dd, 1H, $J_{8a,8b}$=12.3, $J_{7,8a}$=5.0 Hz, H-8a), 4.97 (dddd, 1H, *J*=10.5, 1.5, 1.5, and 1.5 Hz, CH=C*H*$_2$), 5.01 (dd, 1H, $J_{7,8b}$=2.3, $J_{8a,8b}$=12.3 Hz, H-8b), 5.11 (dddd, 1H, *J*=17.0, 1.5, 1.5, and 1.5 Hz, CH=C*H*$_2$), 5.71 (m, 1H, C*H*=CH$_2$), 5.75 (ddd, 1H, $J_{6,7}$=7.5, $J_{7,8a}$=5.0, $J_{7,8b}$=2.3 Hz, H-7), 7.39–7.44 (m, 4H, Ar), 7.52–7.56 (m, 2H, Ar), 7.99–8.03 (m, 4H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.11 (Me), 25.09 (Me), 33.2 (C-3), 52.3 (OMe), 60.4 (O*CH*$_2$–), 63.0 (C-8), 69.4 (C-6), 70.2 (C-4), 70.7 (C-7), 71.6 (C-5), 97.9 (C-2), 109.8 (C_{isop}) 116.9 (*C*H$_2$=), 128.36, 128.41, 129.7, 130.0, 133.0, and 133.5 (Ar), 133.2 (–*C*H=), 165.3 (*C*=O), 166.2 (*C*=O), 169.0 (C-1). Anal. Calcd for $C_{26}H_{29}O_{10}$: C, 64.44; H, 5.97. Found C, 64.56; H, 6.05. Compound **13β**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (s, 3H, Me), 1.51 (s, 3H, Me), 2.17–2.24 (m, 2H, H-3), 3.61 (s, 3H, OMe), 4.05 (dddd, 1H, *J*=12.5, 5.5, 1.5, and 1.5 Hz, O*CH*$_2$), 4.25 (dd, 1H, $J_{5,6}$=2.0, $J_{6,7}$=8.0 Hz, H-6), 4.30 (dddd, 1H, *J*=12.5, 5.5, 1.5, and 1.5 Hz, O*CH*$_2$), 4.32 (dd, 1H, $J_{4,5}$=7.0, $J_{5,6}$=2.0 Hz, H-5), 4.48–4.50 (m, 1H, H-4), 4.77 (dd, 1H, $J_{7,8a}$=5.5, $J_{8a,8b}$=12.0 Hz, H-8a), 4.92 (dd, 1H, $J_{7,8b}$=2.5, $J_{8a,8b}$=12.0 Hz, H-8b), 5.15 (dddd, 1H, *J*=10.5, 1.5, 1.5, and 1.5 Hz, CH=C*H*$_2$), 5.28 (dddd, 1H, *J*=17.0 1.5, 1.5, and 1.5 Hz, CH=C*H*$_2$), 5.71 (ddd, 1H, $J_{6,7}$=8.0, $J_{7,8a}$=5.5, $J_{7,8b}$=2.5 Hz, H-7), 5.91 (m, 1H, C*H*=CH$_2$), 7.40–7.44 (m, 4H, Ar), 7.52–7.56 (m, 2H, Ar), 8.02–8.05 (m, 4H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.2 (Me), 26.9 (Me), 33.6 (C-3), 52.4 (OMe), 63.2 (O*CH*$_2$–), 65.6 (C-8), 70.3 (C-4), 70.8 (C-6), 70.9 (C-7), 71.0 (C-5), 98.5 (C-2), 109.6 (C_{isop}) 117.0 (*C*H$_2$=), 128.36, 128.43, 1323.0, 133.2, and 133.2 (Ar), 133.2 (–*C*H=), 165.3 (*C*=O), 166.2 (*C*=O), 169.0 (C-1).

*4.1.6. Methyl (allyl 7,8-di-O-benzoyl-3-deoxy-α-D-manno-2-octulopyranoside)onate (**14**).* To a solution of methyl (allyl 7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranoside) onate **13** (167.0 mg, 0.31 mmol) in dichloromethane (17 mL) was added 90% aqueous trifluoroacetic acid (1.7 mL) at room temperature. The mixture was stirred for 30 min, and then the solvent was removed by evaporation under an argon stream. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=1/2) to give a colorless syrup (151 mg, 97%). $[\alpha]_D^{25}$ +60.4 (*c* 0.99, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.99 (dd, 1H, $J_{3a,4}$=12.0, $J_{3a,3e}$=12.5 Hz, H-3a), 2.25 (dd, 1H, $J_{3e,4}$=5.0, $J_{3a,3e}$=12.5 Hz, H-3e), 3.78 (s, 3H, OMe), 3.83 (dd, 1H, $J_{4,5}$=3.5, $J_{5,6}$=0.5 Hz, H-5), 3.91 and 4.00 (m, 1H each, O*CH*$_2$), 4.02 (dd, 1H, $J_{5,6}$=0.5, $J_{6,7}$=5.5 Hz, H-6), 4.14 (ddd, 1H, $J_{3a,4}$=12.0, $J_{3e,4}$=5.0, $J_{4,5}$=3.5 Hz, H-4), 4.76 (dd, 1H, $J_{7,8a}$=4.5, $J_{8a,8b}$=12.0 Hz, H-8a), 4.91 (dd, 1H, $J_{7,8b}$=2.5, $J_{8a,8b}$=12.0 Hz, H-8b), 5.03 (dddd, *J*=10.5, 1.5, 1.5 and 1.5 Hz, CH=C*H*$_2$), 5.12 (dddd, *J*=17.0, 1.5, and 1.5 Hz, CH=C*H*$_2$), 5.69–5.76 (m, 1H, C*H*=CH$_2$), 5.75 (ddd, 1H, $J_{6,7}$=5.5, $J_{7,8a}$=4.5, $J_{7,8b}$=2.5 Hz, H-7), 7.41–7.44 (m, 4H, Ar), 7.55–7.59 (m, 2H, Ar), 8.00–8.02 (m, 4H, Ar); ^{13}C NMR 35.1 (C-3), 52.7 (O*CH*$_3$), 63.3 (C-8), 64.6 (O*CH*$_2$), 65.4 (C-4), 65.8 (C-5), 70.3 (C-7), 70.4 (C-6), 99.2 (C-2), 116.5 (=*C*H$_2$), 128.5, 128.6, 128.9,1296, 129.7, 130.0, 133.28, and 133.9 (Ar), 133.3 (–*C*H=), 166.3 (*C*=O), 166.9 (*C*=O), 168.4 (C-1). Anal. Calcd for $C_{26}H_{29}O_{10}$: C, 62.39; H, 5.64. Found C, 62.24; H, 5.70.

*4.1.7. Methyl (allyl 4,5-O-isopropylidene-3-deoxy-α-D-manno-2-octulopyranoside)onate (**15**).* To a solution of methyl (allyl 7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranoside) onate **13α** (210.0 mg, 0.38 mmol) was added 28% sodium methoxide (50 μL) at room temperature. After stirring for 18 h, the mixture was neutralized by addition of the acetic acid, diluted with toluene, and evaporated. The residue was purified by silica-gel column chromatography (EtOAc as an eluent) to give compound **15** as colorless syrup (129 mg, quant.) $[\alpha]_D^{25}$ +43.8 (*c* 1.3, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.25 (s, 3H, Me), 1.36 (s, 3H, Me), 1.90 (dd, 1H, $J_{3a,4}$=3.4, $J_{3a,3e}$=12.5 Hz, H-3a), 2.63 (dd, 1H, $J_{3e,4}$=4.6, $J_{3a,3e}$=12.5 Hz, H-3e), 3.69 (dd, 1H, $J_{7,8a}$=3.6, $J_{8a,8b}$=11.4 Hz, H-8a), 3.75 (s, 3H, OMe), 3.76 (dddd, 1H, *J*=12.5, 5.6, 1.1, and 1.5 Hz, –O*CH*$_2$), 3.77 (dd, 1H, $J_{5,6}$=2.0, $J_{6,7}$=7.9 Hz, H-6), 3.82 (dd, 1H, $J_{7,8b}$=1.8, $J_{8a,8b}$=11.4 Hz, H-8b), 3.97 (ddd, 1H, $J_{6,7}$=7.9, $J_{7,8a}$=3.6, $J_{7,8b}$=1.8 Hz, H-7), 4.05 (dddd, 1H, *J*=12.5, 1.5, 1.5, and 1.5 Hz, –O*CH*$_2$), 4.29 (dd, 1H, $J_{4,5}$=7.3, $J_{5,6}$=2.0 Hz, H-5), 4.47 (dd, 1H, $J_{3a,4}$=3.4, $J_{3e,4}$=4.6, $J_{4,5}$=7.3 Hz, H-4), 5.10 (ddd, 1H, *J*=17.0, 3.0, 1.4 Hz, CH=C*H*$_2$), 5.20 (dd, 1H, *J*=10.5, 1.6 Hz, CH=C*H*$_2$), 5.79 (dddd, 1H, –C*H*=CH$_2$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.1 (*C*H$_3$), 24.8 (*C*H$_3$), 31.9 (C-3), 51.4 (OMe), 63.0 (C-8), 70.9 (C-5), 63.2 (O*CH*$_2$–), 69.1 (C-4), 68.9 (C-7), 69.7 (C-6), 96.4 (C-2), 108.4(C_{isop}), 116.1 (*C*H$_2$=), 132.8 (–*C*H=), 168.3 (C-1). ESI-HRMS calcd for $C_{15}H_{24}O_8$: 333.1549 $[M+H]^+$. Found 333.1551.

*4.1.8. Methyl (7,8-di-O-benzoyl-4,5-O-isopropylidene-3-deoxy-D-manno-2-octulopyranosylfluoride)onate (**16**).* Methyl 7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-D-*manno*-2-octulosonate **12** (717 mg, 1.43 mmol) was treated with (diethylamino)sulfur trifluoride (DAST, l.9 mL, 14.3 mmol) for 30 min at 0 °C. Dry methanol was added into the mixture to quench excess DAST, and then the mixture was diluted with dichloromethane and satd $NaHCO_3$. The

organic solution was separated from the mixture and the aqueous layer was extracted with dichloromethane. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. Purification of the residue by silica gel chromatography (hexane/ethyl acetate=3/2) gave compound (**16**) as a mixture of anomers in 81% yield (581 mg, α/β=3/1). The α-isomer was able to be isolated by recrystallization from ethyl acetate and hexane. Compound **16α**: colorless crystal, mp 117–118 °C, $[\alpha]_D^{25}$ −1.63 (*c* 1.00, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24 (s, 3H, Me), 1.43 (s, 3H, Me), 2.08 (ddd, 1H, $J_{3a,4}$=3.0, $J_{3a,F}$=18.0, $J_{3a,3e}$=15.5 Hz, H-3a), 3.04 (ddd, 1H, $J_{3e,4}$=4.0, $J_{3e,F}$=7.0, $J_{3a,3e}$=15.5 Hz, H-3e), 3.83 (s, 3H, OMe), 4.35 (dd, 1H, $J_{5,6}$=2.0, $J_{6,7}$=7.5 Hz, H-6), 4.39 (dd, 1H, $J_{4,5}$=7.5 Hz, H-5), 4.58 (ddddd, 1H, $J_{3a,4}$=3.0, $J_{3e,4}$=4.0, $J_{3a,F}$=18.0, $J_{3e,F}$=7.0, $J_{4,5}$=7.5 Hz, H-4), 4.69 (dd, 1H, $J_{7,8a}$=5.0, $J_{8a,8b}$=12.5 Hz, H-8a), 5.03 (dd, 1H, $J_{7,8b}$=2.0, $J_{8a,8b}$=12.5 Hz, H-8b), 5.75 (ddd, 1H, $J_{6,7}$=7.5, $J_{7,8a}$=5.0, $J_{7,8b}$=2.0 Hz, H-7), 7.39–7.44 (m, 4H, Ar), 7.52–7.56 (m, 2H, Ar), 7.99–8.03 (m, 4H, Ar). ^{13}C NMR(125 MHz, $CDCl_3$): δ 24.8 (Me), 25.7 (Me), 31.0 (C-3), 53.1 ($COCH_3$), 62.9 (C-8), 69.5 (C-4), 70.1 (C-7), 71.1 (C-5), 71.2 (C-6), 107.5 (C-2), 110.0 (C_{isop}), 128.4, 128.46, 128.51, 129.7, 130.0, and 133.0 (Ar), 165.2 (*C*=O), 166.0 (C-1), 166.1 (*C*=O). Anal. Calcd for $C_{26}H_{29}O_{10}$: C, 62.15; H, 5.42. Found C, 61.85; H, 5.70. Compound **16β**: colorless syrup, ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28 (s, 3H, Me), 1.54 (s, 3H, Me), 2.27 (ddd, 1H, $J_{3a,4}$=4.0, $J_{3a,F}$=32.5, $J_{3a,3e}$=16.0 Hz, H-3a), 2.42 (ddd, 1H, $J_{3e,4}$=2.5, $J_{3e,F}$=11.0, $J_{3a,3e}$=16.0 Hz, H-3e), 3.69 (s, 3H, OMe), 4.50 (dd, 1H, $J_{4,5}$=8.5, $J_{5,6}$=1.5 Hz, H-5), 4.20 (dd, 1H, $J_{5,6}$=1.5, $J_{6,7}$=7.5 Hz, H-6), 4.66 (ddddd, 1H, $J_{3a,4}$=4.0, $J_{3a,F}$=32.5, $J_{3e,4}$=2.5, $J_{3e,F}$=11.0, $J_{4,5}$=8.5 Hz, H-4), 4.75 (dd, 1H, $J_{7,8a}$=5.0, $J_{8a,8b}$=12.5 Hz, H-8a), 4.90 (dd, 1H, $J_{7,8b}$=2.5, $J_{8a,8b}$=12.5 Hz, H-8b), 5.69 (ddd, 1H, $J_{6,7}$=7.5, $J_{7,8a}$=5.0, $J_{7,8b}$=2.5 Hz, H-7), 7.41–7.45 (m, 4H, Ar), 7.53–7.58 (m, 2H, Ar), 8.00–8.04 (m, 4H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.3 (Me), 25.6 (Me), 30.7 (C-3), 53.0 ($COCH_3$), 62.7 (C-8), 68.5 (C-4), 70.7 (C-7), 70.8 (C-5), 71.5 (C-6), 107.1 (C-2), 110.0 (C_{isop}), 128.3, 128.4, 129.6, 129.7, 129.9, 133.0, and 133.3 (Ar), 165.2 (*C*=O), 166.0 (*C*=O), 167.3 (C-1). Anal. Calcd for $C_{26}H_{29}O_{10}$: C, 62.15; H, 5.42. Found C, 61.85; H, 5.70.

*4.1.9. Methyl O-[methyl (7,8-di-O-benzoyl-4,5-O-isopropylidene-3-deoxy-D-manno-2-octulopyranosyl)onate]-(2–4)-(allyl 7,8-di-O-benzoyl-3-deoxy-α-D-manno-2-octulopyranoside)onate (**18**).* A mixture of methyl (allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulopyranoside)-onate **14** (124.0 mg, 0.25 mmol), methyl (7,8-di-*O*-benzoyl-4,5 -*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranosylfluoride) onate **16α** (248.9 mg, 0.50 mmol), and molecular sieves 5 Å (300.0 mg) was suspended in dichloromethane (8.0 mL). The reaction mixture was stirred for 1 h and then cooled to −20 °C. Triethylamine (69 μL, 0.50 mmol) was added, and boron trifluoride etherate (187 μL, 1.49 mmol) was dropwise to the mixture. After stirring for 1.5 h, the reaction was quenched by addition of satd sodium hydrogen carbonate. The reaction mixture was filtered through Celite and the filtrate was extracted twice with dichloromethane. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The brownish residue was purified by silica gel column chromatography (toluene/ethyl acetate=2/1) to give compounds **18** as a mixture of anomeric isomers (175.3 mg, 72%). The α-linked product was isolated by flash chromatography. α-isomer **18α**: $[\alpha]_D^{25}$ +49.5 (*c* 1.00, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.20 (s, 3H, Me), 1.38 (s, 3H, Me), 1.93 (dd, 1H, $J_{3a',4'}$=2.4, $J_{3a',3e'}$=15.6 Hz, H-3a′), 2.16 (dd, 1H, $J_{3a,4}$=11.6, $J_{3a,3e}$=12.6 Hz, H-3a), 2.24 (dd, 1H, $J_{3e,4}$=5.0, $J_{3a,3e}$=12.6 Hz, H-3e), 2.53 (br, 1H, 5-OH), 2.97 (dd, 1H, $J_{3e',4'}$=3.6, $J_{3a',3e'}$=15.6 Hz, H-3e′), 3.43 (s, 3H, OMe′), 3.44 (s, 3H, OMe), 3.64 (br s, 1H, H-5), 3.84 (dddd, 1H, *J*=13.0, 4.9, 1.6, and <0.2 Hz, $-OCH_2$), 3.94 (dddd, 1H, *J*=13.0, 5.4, 1.4, and <0.2 Hz, $-OCH_2$), 4.03 (dd, 1H, $J_{5,6}$=1.0, $J_{6,7}$=9.1 Hz, H-6), 4.18 (dd, 1H, $J_{5',6'}$=1.8, $J_{6',7'}$=7.5 Hz, H-6′), 4.32 (dd, 1H, $J_{4',5'}$=7.8, $J_{5',6'}$=1.8 Hz, H-5′), 4.51 (ddd, 1H, $J_{3a',4'}$=2.4, $J_{3e',4'}$=3.6, $J_{4',5'}$=7.8, H-4′), 4.59 (dd, 1H, $J_{7',8a'}$=4.6, $J_{8a',8b'}$=12.5 Hz, H-8a′), 4.61 (dd, 1H, $J_{3a,4}$=11.6, $J_{3e,4}$=5.0, $J_{4,5}$=2.8 Hz, H-4), 4.69 (dd, 1H, $J_{7,8a}$=4.2, $J_{8a,8b}$=12.4 Hz, H-8a), 4.88 (dd, 1H, $J_{7,8b}$=2.5, $J_{8a,8b}$=12.4 Hz, H-8b), 4.94 (dddd, 1H, *J*=17.0, 1.5, 1.5, and 1.5 Hz, CH=CH_2), 5.01 (dd, 1H, *J*=10.5, 1.5, 1.5, and 1.5 Hz, CH=CH_2), 5.30 (dd, $J_{7',8b'}$=2.5, $J_{8a',8b'}$=12.5 Hz, H-8b′), 5.68 (ddd, 1H, $J_{6',7'}$=7.5, $J_{7',8a'}$=4.6, $J_{7',8b'}$=2.5 Hz, H-7′), 5.65 (m, 1H, −C*H*=CH_2), 5.77 (ddd, 1H, $J_{6,7}$=9.1, $J_{7,8a}$=4.2, $J_{7,8b}$=2.5 Hz, H-7), 7.34–7.44 (m, 8H, Ar, Ar′), 7.47–7.56 (m, 4H, Ar, Ar′), 7.93–8.08 (m, 8H, Ar, Ar′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.1 (CH_3), 26.7 (CH_3), 32.75 (C-3′), 32.70 (C-3), 52.6 (OMe), 52.7 (OMe′), 62.8 (C-8′), 63.2 (C-8), 65.9 (C-5), 52.6 (OCH_2−), 70.1 (C-4), 59.6 (C-7), 69.3 (C-6), 70.4 (C-4′), 71.7 (C-6′), 70.4 (C-7′), 70.8 (C-5′), 99.3 (C-2′), 99.0 (C-2), 116.1 (CH_2=), 128.37, 128.40, 128.43, 128.6, 129.7, 129.8, 133.0, 133.3, 133.7, 165.0, 165.3, 166.0 (Ar, Ar′), 133.3 (−*C*H=), 165.0, 165.3, 166.0 (*C*=O), 168.4 (C-1), 169.7 (C-1′). Anal. Calcd for $C_{52}H_{54}O_{19}$: C, 63.54; H, 5.54. Found C, 63.33; H, 5.58. Compound **18β**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.11 (s, 3H, Me), 1.12 (s, 3H, Me), 2.10 (dd, 1H, $J_{3a,4}$=3.5, $J_{3a,3e}$=15.5 Hz, H-3a), 2.11 (dd, 1H, $J_{3a',4'}$=not determined, $J_{3a',3e'}$=13.0 Hz, H-3a′), 2.15 (dd, 1H, $J_{3e',4'}$=4.0, $J_{3a',3e'}$=13.0 Hz, H-3e′), 2.34 (dd, 1H, $J_{3e,4}$=4.0, $J_{3a,3e}$=15.5 Hz, H-3e), 3.60 (s, 3H, OMe'), 3.76 (s, 3H, OMe), 3.92 (dddd, 1H, *J*=13.0, 5.0, 1.5, and 1.5 Hz, $-OCH_2$), 4.02 (dddd, 1H, *J*=13.0, 5.0, 1.5, and 1.5 Hz, $-OCH_2$), 4.10 (dd, 1H, $J_{5',6'}$=2.5, $J_{6',7'}$=8.0 Hz, H-6′), 4.20 (d, 1H, $J_{4,5}$=7.5, $J_{5,6}$=1.5 Hz, H-5), 4.21 (dd, 1H, $J_{4',5'}$=7.8, $J_{5',6'}$=2.5 Hz, H-5′), 4.30 (dd, 1H, $J_{5,6}$=1.5, $J_{6,7}$=9.0 Hz, H-6), 4.41 (ddd, 1H, $J_{3a',4'}$=not determined, $J_{3e',4'}$=4.0, $J_{4',5'}$=7.8 Hz, H-4′), 4.49 (dd, 1H, $J_{3a,4}$=3.5, $J_{3e,4}$=4.0, $J_{4,5}$=7.5 Hz, H-4), 4.69 (dd, 1H, $J_{7',8a'}$=5.5, $J_{8a',8b'}$=12.5 Hz, H-8a′), 4.74 (dd, 1H, $J_{7,8a}$=4.0, $J_{8a,8b}$=12.5 Hz, H-8a), 4.81 (dd, $J_{7',8b'}$=2.5, $J_{8a',8b'}$=12.5 Hz, H-8b′), 4.92 (dd, 1H, $J_{7,8b}$=2.5, $J_{8a,8b}$=12.5 Hz, H-8b), 4.98 (dddd, 1H, *J*=17.0, 1.5, 1.5, and 1.5 Hz, CH=CH_2), 5.07 (dddd, 1H, *J*=10.5, 1.5, 1.5, and 1.5 Hz, CH=CH_2), 5.49 (ddd, 1H, $J_{6',7'}$=8.0, $J_{7',8a'}$=5.5, $J_{7',8b'}$=2.5 Hz, H-7′), 5.72 (m, 1H, −C*H*=CH_2), 5.85 (ddd, 1H, $J_{6,7}$=9.0, $J_{7,8a}$=4.0, $J_{7,8b}$=2.5 Hz, H-7), 7.36–7.44 (m, 8H, Ar, Ar′), 7.49–7.56 (m, 4H, Ar, Ar′), 8.00–8.08 (m, 8H, Ar, Ar′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.7 (CH_3), 25.1 (CH_3), 32.8 (C-3′), 33.3 (C-3), 52.1 (OMe′), 52.2 (OMe), 62.2 (C-8′), 63.0 (C-8), 64.0 (C-5), 64.5 (OCH_2−), 67.6 (C-4), 69.6 (C-7), 69.88 (C-6), 69.90 (C-4′), 70.2 (C-6′), 70.7 (C-7′), 72.0 (C-5′), 96.7 (C-2′), 98.8 (C-2), 116.2 (CH_2=), 128.37, 128.41, 128.47, 128.54, 129.4, 129.61, 129.68, 129.71, 129.72, 129.8, 130.0, 130.0, 132.9, 133.0, 133.4, and 133.5 (Ar, Ar′), 133.1 (−*C*H=), 165.2, 165.5, 165.9, 166.2 (*C*=O), 167.6 (C-1), 169.6 (C-1′). ESI-HRMS calcd for $C_{52}H_{54}O_{19}$: 983.3338 $[M+H]^+$. Found: 983.3335.

*4.1.10. Methyl O-[methyl (7,8-di-O-benzoyl-3-deoxy-α-D-manno-2-octulopyranosyl)onate]-(2–8)-(allyl 4,5-O-isopropylidene-3-deoxy-α-D-manno-2-octulopyranoside)onate (**19**).* A mixture of methyl (allyl 4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranoside)onate **15** (50.0 mg, 0.15 mmol), methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranosylfluoride) onate **16α** (113 mg, 0.22 mmol), and molecular sieves 5 Å (150 mg) was suspended in dichloromethane (3.5 mL). The reaction mixture was stirred for 15 min and then cooled to −78 °C. Triethylamine (30 μL, 0.22 mmol) was added, and boron trifluoride etherate (135 μL, 1.1 mmol) was dropwise to the mixture. The reaction temperature was warmed to −15 °C. After stirring for 1.5 h, the reaction was quenched by addition of satd sodium hydrogen carbonate. The reaction mixture was filtered through Celite and the filtrate was extracted twice with dichloromethane. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The brownish residue was purified by gel permeation chromatography (BioRad S-X3, toluene/ethyl acetate=3/1) purified by silica gel column chromatography (toluene/ethyl acetate=2/1), silica gel column chromatography (ethyl acetate), and preparative thin-layer chromatography to give compounds **19** as a colorless syrup (19 mg, 16%). $[\alpha]_D^{25}$ +32.6 (*c* 0.96, $CHCl_3$), ^{1}H NMR (600 MHz,

$CDCl_3$): δ 1.15 (s, 3H, Me), 1.21 (s, 3H, Me), 1.32 (s, 3H, Me), 1.38 (s, 3H, Me), 1.76 (dd, 1H, $J_{3a,4}$=3.0, $J_{3a,3e}$=15.2 Hz, H-3a), 1.93 (dd, 1H, $J_{3a',4'}$=3.6, $J_{3a',3e'}$=15.0 Hz, H-3a′), 2.61 (dd, 1H, $J_{3e,4}$=4.4, $J_{3a,3e}$=15.2 Hz, H-3e), 2.63 (dd, 1H, $J_{3e',4'}$=5.0, $J_{3a',3e'}$=15.0 Hz, H-3e′), 3.58 (dd, 1H, $J_{7,8a}$=3.1, $J_{8a,8b}$=10.4 Hz, H-8a), 3.66 (dd, 1H, $J_{7,8b}$=not determined, $J_{8a,8b}$=10.4 Hz, H-8b), 3.64 (dddd, 1H, J=12.5, 5.2, 2.6, and 1.5 Hz, −OCH_2), 3.66 (s, 3H, OMe′), 3.73 (s, 3H, OMe), 3.94 (dddd, 1H, J=12.5, 5.2, 3.2, and 1.5 Hz, −OCH_2), 4.12 (br s, 1H, H-5), 3.33 (dd, 1H, $J_{5,6}$=1.9, $J_{6,7}$=8.5 Hz, H-6), 4.17 (dd, 1H, $J_{5',6'}$=2.0, $J_{6',7'}$=7.4 Hz, H-6′), 4.24 (dd, 1H, $J_{4',5'}$=7.0, $J_{5',6'}$=2.0 Hz, H-5′), 4.45 (ddd, 1H, $J_{3a',4'}$=3.6, $J_{3e',4'}$=5.0, $J_{4',5'}$=7.0 Hz, H-4′), 4.67 (dd, 1H, $J_{7',8a'}$=5.8, $J_{8a',8b'}$=12.3 Hz, H-8a′), 4.37 (dd, 1H, $J_{3a,4}$=3.0, $J_{3e,4}$=4.4, $J_{4,5}$=7.4 Hz, H-4) 5.08 (ddd, 1H, J=10.6, 2.6, 1.6, and 1.5 Hz, CH=CH_2), 5.18 (dd, 1H, J=17.0, 3.2, 1.6, and 1.5 Hz, CH=CH_2), 4.92 (dd, 1H, $J_{7',8b'}$=2.6, $J_{8a',8b'}$=12.3 Hz, H-8b′), 5.68 (ddd, 1H, $J_{6',7'}$=7.4, $J_{7',8b'}$=2.6, $J_{7',8a'}$=5.8 Hz, H-7′), 5.75 (dddd, 1H J=17.0, 10.6, 5.2, 5.2 Hz, −CH=CH_2), 3.92 (ddd, 1H, $J_{6,7}$=8.5, $J_{7,8a}$=3.1 Hz, $J_{7,8b}$=not determined, H-7), 7.34–7.44 (m, 4H, Ar′), 7.47–7.56 (m, 2H, Ar′), 7.93–8.08 (m, 4H, Ar′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.2, 24.3, 23.4, 25.9 (CH_3), 31.8 (C-3′), 32.80 (C-3), 51.1 (OMe), 51.7 (OMe′), 61.2 (C-8′), 66.0 (C-8), 63.6 (C-5), 64.0 (OCH_2−), 66.9 (C-4), 72.5 (C-7), 71.6 (C-6), 68.9 (C-4′), 69.7 (C-6′), 70.3 (C-7′), 71.0 (C-5′), 95.9 (C-2′), 97.5 (C-2), 108.2, 108.8, 117.0 (CH_2=), 127.32, 127.37, 127.44, 128.65, 128.71, 129.1, 131.9, 132.0 (Ar′), 133.1 (−CH=), 164.8, 166.9 (C=O), 168.7 (C-1), 169.5 (C-1′). ESI-HRMS for $C_{41}H_{50}O_{17}$: 837.2936 $[M+Na]^+$. Found 837.2933.

*4.1.11. O-(Sodium 3-deoxy-α-D-manno-2-octulopyranosylonate)-(2–8)-sodium (allyl 3-deoxy-α-D-manno-2-octulopyranoside)onate (**20**)*[1416]. To a solution of methyl *O*-[methyl (7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2–8)-(allyl 4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranoside)onate **19** (19.0 mg, 23 μmol) in dichloromethane (1.0 mL) was added aqueous 80% trifluoroacetic acid (220 μL) at 0 °C. After stirring for 1 h, the solvent was removed by evaporation under an argon stream. The residue was dissolved in methanol (1.0 mL), and then 1.0 M sodium hydroxide (250 μL, 250 μmol) was added at room temperature. After stirring for 8 h, the mixture was concentrated under argon gas. The residue was purified by gel filtration chromatography (Biogel P-2) to give compound **20** as colorless powder (8.1 mg, 65%). ^{1}H NMR (600 MHz, D_2O): δ 1.71 (dd, 1H, $J_{3a,4}$=12.5, $J_{3a,3e}$=12.5 Hz, H-3a) 1.71 (dd, 1H, $J_{3'a,4'}$=12.5 Hz, $J_{3'a,3'e}$=12.5, H-3′a), 1.94 (dd, 1H, $J_{3'e,4'}$=4.0, $J_{3'a,3'e}$=12.5 Hz, H-3′e), 1.96 (dd, 1H, $J_{3e,4}$=4.0, $J_{3a,3e}$=12.5 Hz, H-3e), 3.47–3.58 (m, 3H), 3.78–3.97 (m, 5H), 5.16 (d, 1H, J=11.0 Hz, =CH_2), 5.26 (d, 1H, J=17.5 Hz, =CH_2), 5.84–5.91 (m, 1H, −CH=). ^{13}C NMR (125 MHz, D_2O): δ 175.9 (C-1′), 175.3 (C-1), 133.8 (−CH=), 117.5 (=CH_2), 100.6 (C-2), 100.1 (C-2′), 71.7 (C-6′), 71.3 (C-6), 69.3 (C-7′), 67.7 (C-7), 66.3 (C-5), 66.2 (C-5′), 66.03 (C-4), 65.95 (C-4′), 64.9 (C-8) 64.3 (OCH_2−), 63.1 (C-8′), 34.1 (C-3′), 33.9 (C-3).

*4.1.12. O-(Sodium 3-deoxy-α-D-manno-2-octulopyranosylonate)-(2–4)-sodium (allyl 3-deoxy-α-D-manno-2-octulopyranoside)onate (**21**)*[15]. To a solution of methyl *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene- 3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2–4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulopyranoside)onate **18α** (10.0 mg, 0.01 mmol) in dichloromethane (3.0 mL) was added aqueous 80% trifluoroacetic acid (450 μL) at room temperature. After stirring for 30 min, the solvent was removed by evaporation under an argon stream to give crude compound, which was not subjected to further purification. The crude compound was dissolved in methanol (10 mL), and then 0.1 M sodium hydroxide (679 μL, 0.067 mmol) was added at room temperature. After stirring for 5 h, the mixture was concentrated by evaporation. The residue was purified by gel filtration chromatography (Biogel P-2) to give compound **21** as colorless powder in quantitative yield. ^{1}H NMR (500 MHz, D_2O): δ 1.68 (dd, 1H, $J_{3a,4}$=12.5, $J_{3a,3e}$=12.5 Hz, H-3a) 1.83 (dd, 1H, $J_{3'a,4'}$=12.5 Hz, $J_{3'a,3'e}$=12.5, H-3′a), 1.92 (dd, 1H, $J_{3'e,4'}$=4.0, $J_{3'a,3'e}$=12.5 Hz, H-3′e), 2.04 (dd, 1H, $J_{3e,4}$=4.0, $J_{3a,3e}$=12.5 Hz, H-3e), 3.45–4.06 (m, 13H), 5.13 (d, 1H, J=11.0 Hz, =CH_2), 5.25 (d, 1H, J=17.5 Hz, =CH_2), 5.84–5.92 (m, 1H, −CH=). ^{13}C NMR (125 MHz, D_2O): δ 176.0 (C-1′), 175.2 (C-1), 134.0 (−CH=), 117.3 (=CH_2), 100.1 (C-2), 99.3 (C-2′), 72.3 (C-6′), 71.5 (C-6), 69.9 (C-7′), 69.5 (C-7), 68.6 (C-4), 66.2 (C-5′), 65.9 (C-4′), 64.21 (C-5), 64.15 (OCH_2−), 63.1 (C-8 and 8′), 34.5 (C-3′), 33.2 (C-3).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2011.06.039. These data include MOL files and InChIKeys of the most important compounds described in this article.

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