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Synthesis of α-lactosyl-(1→3)-L-*glycero*-α-D-*manno*-heptopyranoside, a partial oligosaccharide structure expressed within the lipooligosaccharide produced by *Neisseria gonorrhoeae* strain 15253

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Abstract

The glycosyl donor, hepta-*O*-benzyl-β-lactosyl trichloroacetimidate (**4**) was prepared by treating hepta-*O*-benzyl-lactose with trichloroacetonitrile in the presence of potassium carbonate. The acceptor, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-7,8-dideoxy-α-D-*manno*-oct-7-enopyranoside (**8**) was synthesized by hydrolysis of a 3,4-butane diacetal of methyl L-*glycero*-α-D-*manno*-oct-enopyranoside and subsequent benzylidenation. Glycosidation of the donor **4** with the acceptor **8** in 1,4-dioxane using Me_3SiOTf as a promoter for 1 h at room temperature gave methyl (2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-7,8-dideoxy-α-D-*manno*-oct-7-enopyranoside (**9**) as a major product (59%). The oct-enopyranoside moiety of the trisaccharide **9** was converted to a heptopyranoside (80%) by oxidative cleavage with OsO_4–$NaIO_4$ and subsequent reduction. Hydrogenolysis of the resulting trisaccharide and subsequent acetylation gave the peracetate of α-lactosyl-(1→3)-Hep. Deacetylation of the peracetate afforded the title trisaccharide.

Keywords: Lipooligosaccharide; Lipopolysaccharide; Neisseria; Vaccine; Oligosaccharide

1. Introduction

Lipooligosaccharides (LOS) produced by *Neisseria gonorrhoeae* are important antigenic and immunogenic components of the outer membrane complex. The oligosaccharide (OS) moiety of LOS consists of a structurally variable region and a conserved inner core linked to the lipid A moiety via the KDO bridge.[1–7] Gonococci synthesize this variable region by elongating a conserved core trisaccharide, GlcNAc–Hep[2]–Hep[1] to express two different types of OS elongation (Fig. 1); OS elongates from Hep[1] only or from both Hep[1] and Hep[2].[3–5,7]

In a recent publication, we characterized a murine monoclonal antibody (MAb 2C7) whose epitope resides on some of gonococcal LOS having OS on both Hep[1] and Hep[2].[7] This MAb 2C7-defined epitope requires the OS structure of 15253 LOS as a minimum size, and this 15253 OS has an unusual structure containing two lactosides;[5,7] one lactose is β-(1→4)-linked to Hep[1] of the di-heptose, and the other is α-(1→3)-linked to Hep[2]. This latter structure is not expressed on LOS or LPS produced by other Gram-negative species except for some gonococcal LOS,[7] and its synthesis has not yet been accomplished. To establish a synthetic methodology for the 2C7-defined epitope, we undertook the synthesis of this trisaccharide, α-lactosyl-(1→3)-Hep, a partial OS structure of 15253 LOS.[5]

2. Results and discussion

To accomplish α-lactosylation, we chose to use hepta-*O*-benzyl-β-lactosyl trichloroacetimidate as a

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donor (Scheme 1). This donor was synthesized in three steps (Scheme 1) from *p*-methoxyphenyl β-lactoside (**1**).[8] Benzylation of **1** with NaH and benzyl bromide in DMF gave its hepta-*O*-benzyl ether **2** in 65% yield. This rather moderate yield was improved by benzylating **1** in the presence of tetra-*n*-butyl ammonium iodide, and compound **2** was obtained in 95% yield. Treatment of **2** with ceric(IV) diammonium nitrate gave a known hepta-*O*-benzyl-lactose **3**.[9] Although the melting point of compound **3** was higher than the one that had been reported,[9] the structure of **3** was fully characterized by analyzing its NMR data. We included the ^{1}H and ^{13}C data of **3** in Tables 1 and 2 as a reference because only partial ^{13}C-data[10] had been available.

Treatment of compound **3** with trichloroacetonitrile (CCl_3CN) in the presence of potassium carbonate[11] in CH_2Cl_2 for 96 h at room temperature gave hepta-*O*-benzyl-β-lactosyl trichloroacetimidate (**4**) and its α anomer **5** in 62 and 6.4% yields, respectively. Similar treatment of **3** with CCl_3CN in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene[12] gave the α anomer **5** (α:β 2:1) as a dominant product, although the combined yields (83%) of **4** and **5** were higher than those obtained using potassium carbonate. The anomeric configuration of each anomer was confirmed by both the $J_{1,2}$ value (7.0 and 3.5 Hz for β and α anomer, respectively) and the ^{13}C-signal of C-1 (98.26 and 94.50 ppm for β and α anomer, respectively) of the Glc moiety (Tables 1 and 2). The β-trichloroacetimidate **4** was used as a donor as will be described later.

As an acceptor, we selected methyl 2-*O*-benzyl-4,6-*O*-benzylidene-7,8-dideoxy-α-D-*manno*-oct-7-enopyranoside (**8**) (Scheme 1). Compound **8** bearing a free 3-OH was synthesized as follows. The 3,4-butane diacetal **6**[13] of methyl L-*glycero*-α-D-*manno*-oct-enopyranoside was hydrolyzed in 9:1 TFA–water to give the 2-*O*-benzyl-7,8-dideoxy-*manno*-oct-7-enopyranoside (**7**) as an oil (87%). Treatment of **7** with benzaldehyde dimethylacetal and *p*-toluenesulfonic acid monohydrate in DMF followed by chromatographic purification gave the acceptor **8** as a syrup in 79%. Its 4,6-*O*-benzylidene group was confirmed by the typical downfield ^{13}C shifts[14] for C-4 and C-6 compared with those of **7** (Table 2).

Glycosidation of the donor **4** with the acceptor **8** in 1,4-dioxane[15] using trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf)[16] as a promoter for 1 h at room temperature and subsequent chromatographic purification of the reaction mixture gave methyl (2,3,4,6-tetra-

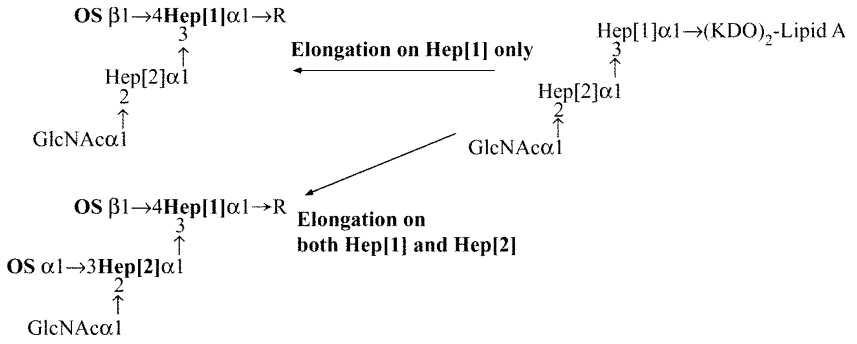
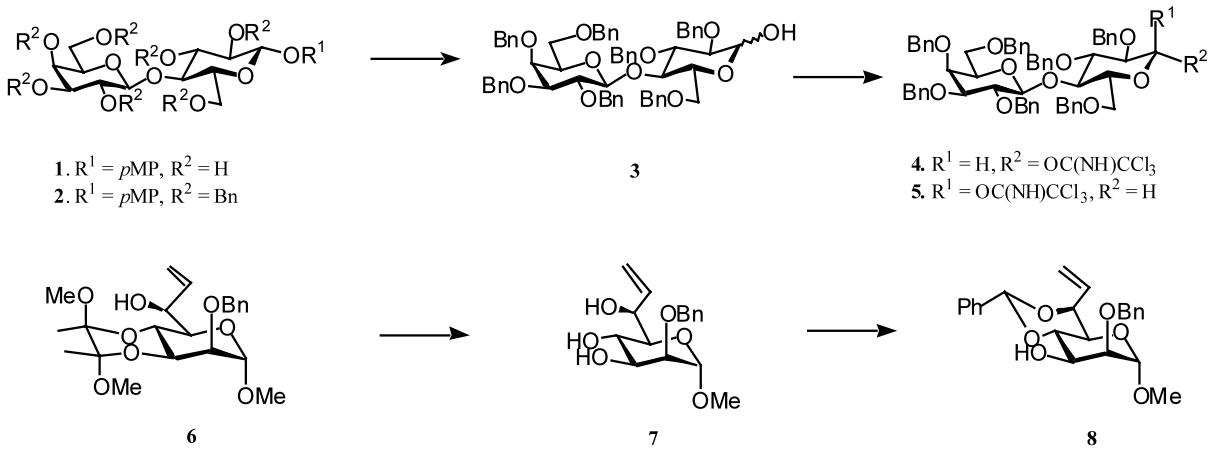


Fig. 1. *N. gonorrhoeae* synthesizes oligosaccharides of two different elongation patterns; R = $(KDO)_2$-Lipid A. The Hep linked to KDO is defined as Hep[1] and the other Hep linked to Hep[1] as Hep[2].



Scheme 1.

Table 1
^{1}H (500 MHz) NMR data for compounds **2**–**5**, **7**, **8**

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8			
2	Glc	4.88	3.69	3.65	3.99	3.52	3.84, 3.81					
	Gal	4.47	3.80	3.46	3.95	3.40	3.57, 3.36					
3 [a]	Glc	5.15	3.50	3.85	3.93	3.98	3.83, 3.53					
	Gal	4.34	3.75	3.35	3.89	3.33	3.53, 3.37					
4	Glc	5.77	3.67	3.67	4.08	3.54	3.85, 3.70					
	Gal	4.45	3.76	3.40	3.91	3.37	3.53, 3.36					
5	Glc	6.42	3.68	3.93	4.77	3.91	3.87, 3.49					
	Gal	4.34	3.75	3.34	3.91	3.35	3.56, 3.37					
7		4.70	3.68	3.78	3.95	3.46	4.46	5.97	5.35, 5.18			
8		4.82	3.81	4.05	4.12	4.05	4.81	6.29	5.65, 5.57			
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6\ (6a)}$	$J_{5,6b}$	$J_{6a,6b}$	$J_{6,7}$	$J_{7,8a}$	$J_{7,8b}$	$J_{8a,8b}$
2	Glc	7.5	8.5	9.0	9.8	2.0	5.0	10.5				
	Gal	8.0	10.0	2.5	n.d.	n.d.	n.d.	n.d.				
3 [a]	Glc	3.5	9.0	8.8	8.5	3.0	n.d.	n.d.				
	Gal	7.0	8.5	n.d.	n.d.	n.d.	n.d.	n.d.				
4	Glc	7.0	n.d.	9.0	9.0	4.0	1.5	11.0				
	Gal	7.5	9.5	2.5	n.d.	n.d.	5.0	10.0				
5	Glc	3.5	9.8	9.5	9.5	3.0	2.0	10.8				
	Gal	7.5	10.0	2.5	n.d.	8.5	5.0	n.d.				
7		1.0	3.5	9.5	9.5	2.5			5.5	17.5	10.5	n.d.
8		1.0	4.0	9.5	9.5	n.d.			5.0	18.0	11.0	n.d.

^{1}H-chemical shifts of **2**–**5**, **7**, **8** were determined by comparatively analyzing the 2D NMR data (DQF-COSY and HMQC), and the coupling constants were obtained from the DQF-COSY experiment.

[a] A mixture of 20(α):1(β): The anomeric protons of the β anomer were as follows; δ 4.61 (d, 1 H, $J_{1^{I},2^{I}}$ 8.5 Hz, H-1^{I}), 4.37 (d, 1 H, $J_{1^{II},2^{II}}$ 8.0 Hz, H-1^{II}); n.d.: not determined. Other protons of **2**–**5**, **7**, **8** are as follows: **2**: δ 7.38–7.14, 7.06–7.30, 6.83–6.79 (aromatic *H*), 5.08, 4.76, 5.02, 4.86, 5.00, 4.58, 4.85, 4.80, 4.75, 4.71, 4.51, 4.40, 4.36, 4.26 (PhCH_2), 3.78 (PhOCH_3); **3**: δ 8.66 [C(N*H*)CCl$_3$], 7.35–7.10 (aromatic *H*), 5.06, 4.70, 4.97, 4.55, 4.86, 4.78, 4.79, 4.76, 4.71, 4.68, 4.56, 4.40, 4.35, 4.25 (PhCH_2); **4**: δ 8.66 [C(N*H*)CCl$_3$], 7.35–7.10 (aromatic *H*), 5.06, 4.70, 4.97, 4.55, 4.86, 4.78, 4.79, 4.76, 4.71, 4.68, 4.56, 4.40, 4.35, 4.25 (PhCH_2); **5**: δ 8.57 [C(N*H*)CCl$_3$], 7.36–7.09 (aromatic *H*), 5.02, 4.74, 4.98, 4.56, 4.81, 4.73, 4.74, 4.69, 4.72, 4.69, 4.51, 4.30, 4.36, 4.25 (PhCH_2); **7**: δ 7.32–7.25 (aromatic *H*), 4.66, 4.58 (PhCH_2), 3.26 (OCH_3); **8**: δ 7.52–7.31 (aromatic *H*), 5.93 (acetal–PhC*H*), 4.74, 4.68 (PhCH_2), 3.38 (OCH_3).

O-benzyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-7,8-dideoxy-α-D-*manno*-oct-7-enopyranoside (**9**) as a major product (59%) (Scheme 2). The structure of **9** was determined by NMR spectroscopy. Anomeric carbons and protons of **9** were confirmed by the HMQC experiment (Fig. 2, Panel B), and other carbons and protons were identified by analyzing its 2D NMR data (DQF-COSY, HMQC, and HMBC). The ^{13}C shift of the C-1 (at 97.25 ppm) and $J_{1,2}$ value (4.0 Hz) of the Glc residue (Tables 3 and 4) confirmed that the lactosyl moiety was α-linked to the oct-enopyranoside (Oct). The H-3, H-4 and C-3 signals of the Oct moiety of the trisaccharide **9** were detected at lower fields than those of the acceptor **8** (Tables 1 and 2), which showed that the Glc component is linked to the O-3 position of the Oct moiety. Also, the cross-relay peaks, H-1(Glc)/C-3(Oct) and C-3(Oct)/H-1(Glc), in the HMBC spectrum (Fig. 2, Panels C and D) confirmed the linkage site.

A minor component obtained in the above glycosidation reaction was found to be a mixture of a β anomer **10** (Scheme 2) and an unidentified product by ^{1}H and ^{13}C NMR. Neither gel permeation chromatography (Biobeads S-X1) nor flash-column chromatography separated these two products. Therefore, the β anomer **10** was characterized as its corresponding heptoside **12**, which will be described below.

The Oct moiety of **9** was converted to the heptopyranoside by oxidative cleavage with OsO_4–$NaIO_4$ and subsequent reduction with $NaBH_4$ as described previously,[13] and compound **11** was obtained in 80% yield after chromatographic purification. Periodate oxidation of the diol formed from compound **9** was slow compared with methyl *manno*-oct-7-enopyranoside[13] due to steric crowding, and warming the reaction mixture (30–35 °C) was necessary for completion of the oxidation. The structure of **11** was confirmed by analyzing its NMR spectral data. As Fig. 3 shows, the ^{13}C satellites

of the vinyl carbons (C-7 and C-8 of **9**, Panel A) were absent in the ^{13}C spectrum of **11** (Panel B), and the DEPT and 2D NMR data (Tables 3 and 4) confirmed conversion of C-7 to a primary carbon. The β anomer of **9** was characterized as its corresponding heptoside **12** by treating the mixture of **10** and the unidentified compound in a similar manner as described for **9** and subsequent chromatographic purification. The yield of **10** was calculated to be 5.9% based on the isolated yield of **12**. The C-1 and H-1 chemical shifts and $J_{1,2}$ (7.5 Hz) of the Glc residue (Tables 3 and 4) showed that the lactosyl moiety of **12** was β-linked, and the HMBC experiment (data not shown) also confirmed the linkage site.

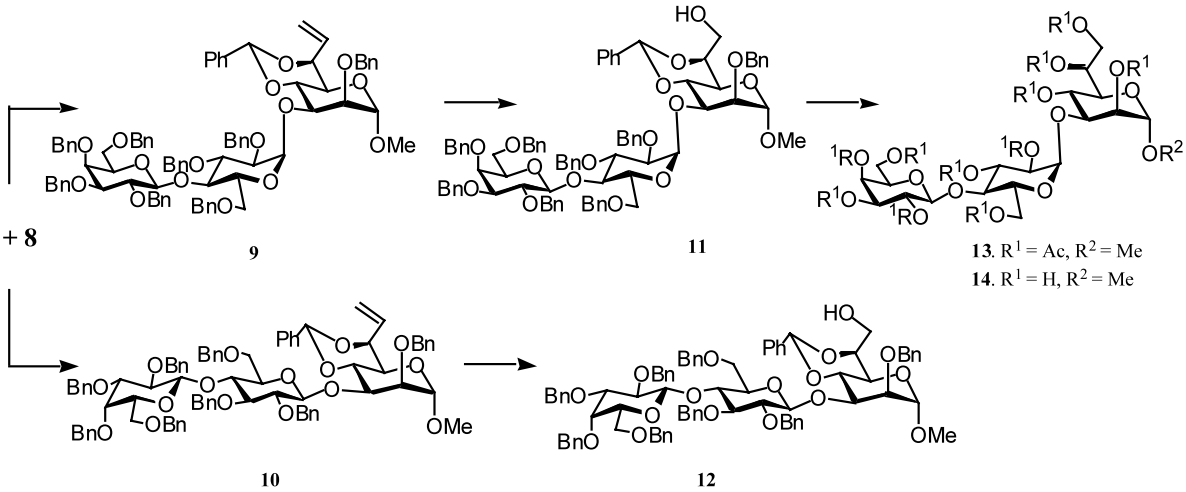
Hydrogenolysis of **11** with Pd/C in DMF followed by acetylation with pyridine and acetic anhydride in the presence of 4-(dimethylamino)pyridine gave methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→3)-2,4,6,7-tetra-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranoside (**13**) as a colorless amorphous solid (73%). Deacetylation of **13** with NaOMe in MeOH followed by chromatographic purification gave the desired trisaccharide **14** (85%).

Table 2
^{13}C (125 MHz) NMR data for compounds **2**–**5**, **7**, **8**

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
2	Glc	102.88	81.67	83.10	76.94	75.45	68.48		
	Gal	103.04	80.07	82.67	73.67	73.15	68.21		
3	Glc	91.46	79.24	80.09	76.76	70.47	68.25		
	Gal	103.05	80.07	82.56	73.87	73.26	68.37		
4	Glc	98.26	80.28	82.81	75.89	76.01	67.56		
	Gal	102.67	79.86	82.48	73.67	73.06	68.14		
5	Glc	94.50	78.33	79.61	75.73	76.14	67.45		
	Gal	102.77	79.78	82.44	73.64	73.04	68.09		
7		98.71	77.93	71.60	68.08	73.25	70.54	138.12	115.75
8 [a]		99.18	78.43	69.17	74.48	65.58	74.15	131.52	120.58

^{13}C-chemical shifts of **2**–**5**, **7**, **8** were determined by comparatively analyzing the 2D NMR data (HMQC and DQF-COSY).

[a] The tertiary carbon of the benzylidene acetal was determined by the HMBC experiment. n.d.: not determined. Other carbons of **2**–**5**, **7**, **8** are as follows: **2**: δ 155.31, 151.73, 139.17, 139.12, 138.84, 138.61, 138.57, 138.49, 138.17, 128.49–127.24, 118.5, 114.59 (aromatic *C*), 75.58, 75.40, 75.29, 74.83, 73.52, 73.19, 72.70 (Ph*C*H_2), 54.90 (PhO*C*H_3); **3**: δ 139.42, 139.20, 138.92, 138.70, 138.31, 138.27, 138.17, 129.19–125.46 (aromatic *C*), 75.56, 75.35, 74.86, 73.67, 73.56, 73.26, 72.72 (Ph*C*H_2); **4**: δ 161.29 [*C*(NH)CCl$_3$], 139.00, 138.94, 138.66, 138.49, 138.23, 138.15, 138.09, 128.36–127.11 (aromatic *C*), 90.99 [C(NH)*C*Cl$_3$], 75.26, 75.01, 74.68, 73.38, 73.01, 72.62 (Ph*C*H_2); **5**: δ 161.15 [*C*(NH)CCl$_3$], 139.11, 139.04, 138.71, 138.51, 138.24, 138.12, 137.98, 128.34–127.03 (aromatic *C*), 91.38 [C(NH)*C*Cl$_3$)], 75.38, 75.17, 74.67, 73.39, 73.12, 73.06, 72.52 (Ph*C*H_2); **7**: δ 137.71, 128.60, 128.05, 127.97 (aromatic *C*), 73.54 (Ph*C*H_2), 54.90 (O*C*H_3); **8**: δ 137.90, 137.34, 129.10–126.43 (aromatic *C*), 95.85 (acetal–Ph*C*H), 73.42 (Ph*C*H_2), 55.15 (O*C*H_3).



Scheme 2.

Table 3
^{1}H (500 MHz) NMR data for compounds **9**, **11**–**14**

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8					
9	Oct [a]	4.72	3.80	4.31	4.51	4.09	4.74	6.26	5.60, 5.53					
	Glc	5.44	3.44	3.88	3.84	3.81	3.82, 3.60							
	Gal	4.29	3.70	3.32	3.88	3.30	3.52, 3.33							
11	Hep	4.69	3.81	4.28	4.57	4.16	4.31	4.10–4.17						
	Glc	5.41	3.43	3.88	3.82	3.82	3.80, 3.61							
	Gal	4.29	3.70	3.32	3.88	3.31	3.51, 3.32							
12	Hep	4.67	3.82	4.26	4.50	4.11	4.33	4.17–4.09						
	Glc	4.55	3.41	3.52	3.98	3.17	3.65, 3.46							
	Gal	4.43	3.72	3.38	3.88	3.31	3.49, 3.33							
13	Hep	4.75	5.23	4.15	5.32	3.92	5.16	4.30, 4.22						
	Glc	5.11	4.68	5.31	3.68	3.99	4.45, 4.18							
	Gal	4.49	5.13	4.96	5.36	3.93	4.13, 4.06							
14 [b]	Hep	4.63	3.96	3.74	3.93	3.50	3.94	3.64, 3.60						
	Glc	5.12	3.50	3.80	3.56	3.83	3.79, 3.74							
	Gal	4.35	3.44	3.55	3.81	3.62	3.69, 3.64							
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6(6a)}$	$J_{5,6b}$	$J_{6a,6b}$	$J_{6,7}$	$J_{6,7b}$	$J_{7a,7b}$	$J_{7,8a}$	$J_{7,8b}$	$J_{8a,8b}$
9	Oct [a]	1.0	4.5	9.5	10.3	6.0	4.5		n.d.			17.5	11.0	n.d.
	Glc	4.0	9.8	10.5	n.d.	n.d.	n.d.	n.d.						
	Gal	7.5	9.3	2.5	n.d.	n.d.	n.d.	n.d.						
11	Hep	1.0	3.5	10.0	10.0	6.0			n.d.	n.d.	n.d.			
	Glc	4.0	9.8	n.d.	n.d.	n.d.	n.d.	10.0						
	Gal	7.5	9.5	2.5	n.d.	n.d.	n.d.	n.d.						
12	Hep	1.5	3.0	10.0	10.0	n.d			n.d.	n.d.	n.d.			
	Glc	7.5	n.d	9.0	9.0	4.0	1.5	11.0						
	Gal	8.0	9.8	2.5	n.d	n.d	n.d.	10.5						
13	Hep	1.5	3.5	10.0	10.0	2.0			5.5	7.0	11.3			
	Glc	3.5	10.0	10.0	10.0	1.5	6.0	12.0						
	Gal	8.0	10.5	3.5	n.d.	4.0	8.0	11.0						
14 [b]	Hep	1.0	3.5	9.8	10.0	1.0			6.5	5.5	11.0			
	Glc	4.0	10.0	10.0	10.3	2.0	4.5	12.0						
	Gal	7.5	10.0	3.0	n.d.	8.0	4.0	11.8						

^{1}H-chemical shifts were determined by comparatively analyzing the 2D NMR data (DQF-COSY, HMQC and HMBC) except for **14** (DQF-COSY and HMQC), and the coupling constants were obtained from the DQF-COSY experiment. n.d., not determined.

[a] The oct-enopyranoside residue is expressed as Oct.

[b] The chemical shifts are in D_2O. Other protons of **9**, **11**–**14** are as follows: **9**: δ 7.38–7.05 (aromatic *H*), 5.80 (acetal–PhC*H*), 4.95, 4.54, 4.95, 4.75, 4.85, 4.65, 4.71, 4.61, 4.68, 4.64, 4.52, 4.35, 4.50, 4.31, 4.32, 4.21 (PhCH_2), 3.36 (OCH_3); **11**: δ 7.37–6.96 (aromatic *H*), 5.70 (acetal–PhC*H*), 4.94, 4.54, 4.86, 4.62, 4.72, 4.62, 4.68, 4.63, 4.51, 4.35, 4.50, 4.32, 4.32, 4.21 (PhCH_2), 3.34 (OCH_3); **12**: δ 7.40–7.05 (aromatic *H*), 5.78 (acetal–PhC*H*), 5.02, 4.63, 4.94, 4.52, 4.77, 4.74, 4.73, 4.56, 4.73, 4.68, 4.65, 4.43, 4.32, 4.29, 4.22 (PhCH_2), 3.31 (OCH_3); **13**: δ 3.36 (OCH_3), 2.24–1.97 (OAc); **14**: δ 3.27 (OCH_3).

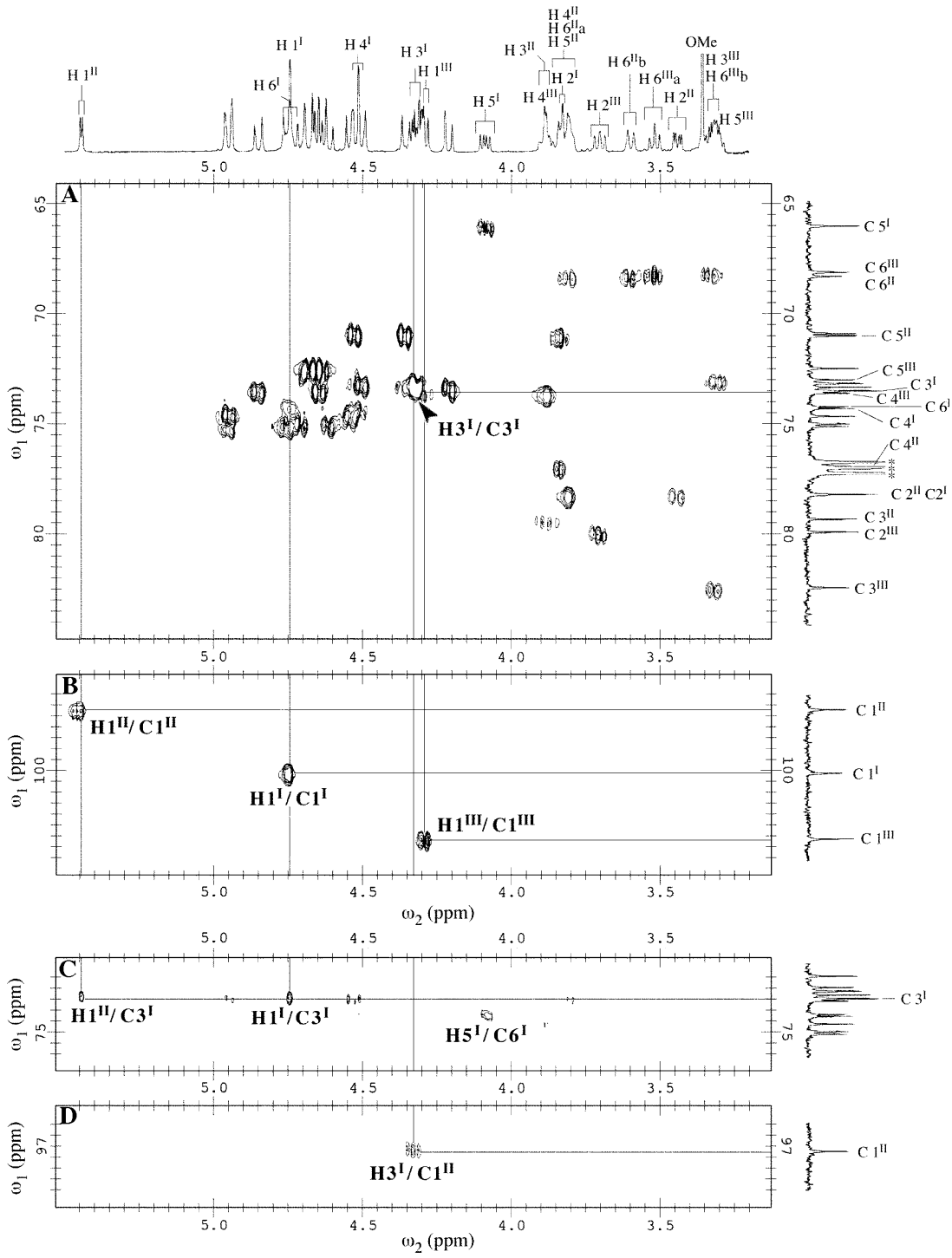


Fig. 2. Parts of HMQC (A, B) and HMBC (C, D) spectra of compound **9**. The part of HMBC spectrum (C) was plotted at a higher contour level to eliminate other relay peaks due to $^{3}J_{C,H}$ and $^{2}J_{C,H}$. *, $CDCl_3$.

In the present study, we used the 4,6-*O*-benzylidene *manno*-oct-enopyranoside **8** as the acceptor, instead of Hep derivatives containing unprotected 3-OH.[17–19] Because compound **8** can be prepared from the precursor **6** for the synthesis of L-*glycero*-*manno*-heptoside,[13] our approach would provide an alternative method to synthesize 3-*O*-substituted heptoside.

Glycosidation of hepta-*O*-benzyl-β-lactosyl trichloroacetimidate (**4**) with the *manno*-oct-enopyranoside **8**, conversion of the oct-enopyranoside moiety of the trisaccharide into the heptoside, and subsequent removal of protecting groups gave α-lactosyl-(1→3)-Hep, a partial oligosaccharide sequence expressed in LOS produced by *N. gonorrhoeae* 15253.

3. Experimental

General methods.—Optical rotations were measured with a HORIBA SEPA-200 polarimeter. Melting points were measured with a YANAGIMOTO micro melting-point apparatus and are uncorrected. Elemental analyses were carried out by using a Perkin–Elmer 2400 Series II CHNS/O Analyzer. High-resolution mass

Table 4
^{13}C (125 MHz) NMR data for compounds **9**, **11**–**14**

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
9	Oct [a]	100.14	78.21	73.54	74.31	66.06	74.24	131.51	120.45
	Glc	97.25	78.21	79.32	76.95	71.02	68.33		
	Gal	103.13	79.92	82.42	73.62	73.02	68.14		
11	Hep	100.28	78.31	73.57	73.75	65.78	74.24	59.23	
	Glc	97.26	78.16	79.34	77.00	71.04	68.38		
	Gal	103.14	79.93	82.46	74.24	73.04	68.16		
12	Hep	99.98	76.44	75.99	72.93	65.97	74.24	59.25	
	Glc	102.16	81.67	83.33	76.22	75.48	67.81		
	Gal	102.55	79.84	82.43	73.65	72.93	68.06		
13	Hep	100.00	70.57	72.99	66.47	68.17	66.67	61.74	
	Glc	96.64	70.89	68.69	75.83	68.97	62.07		
	Gal	100.24	68.79	70.96	66.67	70.47	60.74		
14 [b]	Hep	101.27	70.20	79.61	65.77	71.43	69.12	63.27	
	Glc	100.78	71.93	71.92	78.68	71.60	60.31		
	Gal	103.27	71.43	73.00	69.03	75.82	61.51		

^{13}C-chemical shifts were determined by comparatively analyzing the 2D NMR data (DQF-COSY, HMQC and HMBC) except for **14** (DQF-COSY and HMQC). n.d., not determined.

[a] The oct-enopyranoside residue is expressed as Oct.

[b] The chemical shifts are in D_2O. Other carbons of **9**, **11**–**14** are as follows: **9**: δ 139.39, 139.03, 138.78, 138.49, 138.45, 138.34, 138.18, 138.13, 138.74, 129.02–126.36 (aromatic *C*), 95.82 (acetal–Ph*C*H), 75.12, 75.01, 74.67, 73.53, 73.35, 73.18, 72.49, 70.91 (Ph*C*H$_2$), 54.88 (O*C*H$_3$); **11**: δ 139.38, 139.04, 138.81, 138.49, 138.47, 138.18, 138.14, 137.40, 129.25–126.40 (aromatic *C*), 75.12, 75.01, 74.67, 73.62, 73.37, 73.21, 72.50, 70.93 (Ph*C*H$_2$), 54.99 (O*C*H$_3$); **12**: δ 139.05, 138.80, 138.74, 138.56, 138.51, 138.22, 138.09, 137.57, 128.34–127.03 (aromatic *C*), 96.27 (acetal–Ph*C*H), 75.11, 74.99, 74.70, 74.64, 73.34, 73.26, 72.93, 72.59 (Ph*C*H$_2$), 55.02 (O*C*H$_3$); **13**: δ 170.25, 170.65, 170.46, 170.39, 170.35, 170.25, 170.18, 170.07, 169.26, 169.20, 168.99 (*C*OCH$_3$), 55.20 (O*C*H$_3$), 20.77–20.49 (CO*C*H$_3$); **14**: δ 55.18 (O*C*H$_3$).

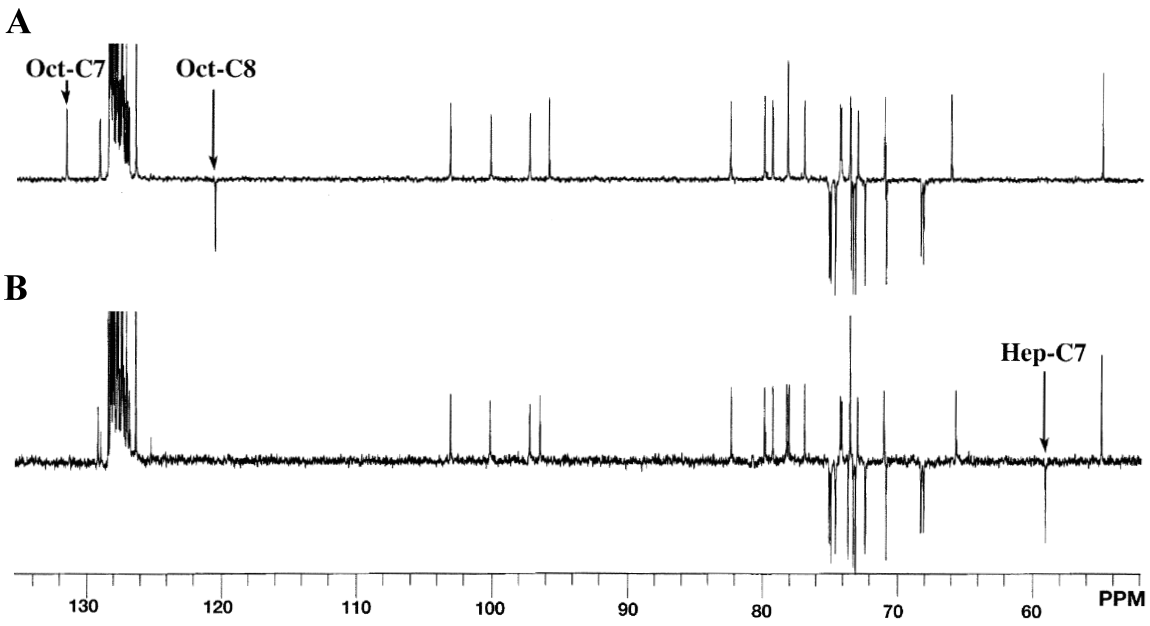


Fig. 3. DEPT spectra of **9** (A) and **11** (B). Of the exocyclic carbons, only C-7 and C-8 are labeled.

spectra were obtained with a JEOL JMS-600H mass spectrometer as described previously.[13] NMR spectra [^{1}H (500 MHz) and ^{13}C (125 MHz), JEOL JNM-ECP 500] were recorded in $CDCl_3$ or D_2O. Me_4Si was used as an internal standard for $CDCl_3$ and CH_3CN (δ 1.95 for ^{1}H; δ 119.58, 1.30 for ^{13}C) for D_2O. 2D NMR data were acquired at rt (20–23 °C) and processed in a similar manner as described previously;[2,5,7,13] DQF-COSY spectra data: digital resolutions in ω_1 and ω_2 after zero filling were in the range of 1.11–3.14 Hz/point; HMQC and HMBC spectra data: digital resolutions in ω_1 and ω_2 after zero filling were in the ranges of 10–25 and 1.66–2.95 Hz/point, respectively. Silica Gel 60 (E. Merck) was used for flash column (0.040–0.063 mm) and open-column (0.063–0.20 mm) chromatography. Silica Gel 60 F_{254} (E. Merck) was used for thin-layer chromatography, and compounds were detected under UV light or by spraying 10% concd H_2SO_4 in MeOH and then by heating the plates at 120 °C for 5 min. For gel chromatography, we used two gel columns packed with Biobeads S-X1 (Bio-Rad, 200-400 mesh, 3 × 90 cm, elution with toluene) and Bio-Gel P-2 (Bio-Rad, < 400 mesh, 1.6 × 6.0 cm, elution with deionized water). The P-2 column was used for purification of an unprotected oligosaccharide, and to monitor its elution, we used a refractrometer (ERC-7522, Erma CR. Inc., Tokyo) connected to an ÄKTA explorer 10S (Amasham Pharmacia, Uppsala, Sweden). *p*-Methoxyphenyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**1**) was prepared according to the literature procedure;[20] mp 271–272 °C; $[\alpha]_D^{23}$ −20.8° (*c* 0.5, DMF), lit.[8] $[\alpha]_D$ −20° (*c* 0.5, DMF).

p-*Methoxyphenyl (2,3,4,6-tetra*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**2**).—A solution of **1** (6.0 g, 13.4 mmol) in DMF (240 mL) was added dropwise to a chilled (0 °C) mixture of NaH (60% in oil 7.5 g, 187.3 mmol; prewashed with hexane) in DMF (60 mL). After stirring the mixture for 30 min at 0 °C, Bu_4NI (3.0 g) was added and the reaction mixture was stirred for 1 h. Benzyl bromide (16.7 mL, 140.7 mmol) was added, and the mixture was stirred for 1.5 h at 0 °C and then overnight at rt. The mixture was quenched with water (100 mL) and extracted with EtOAc (300 mL). The aqueous solution was extracted with EtOAc (2 × 150 mL), and the combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated to give a white solid. Chromatographic purification (5:4:1→2:2:1 hexane–toluene–EtOAc) gave a crystalline residue which was recrystallized from EtOAc hexane to give **2** (13.8 g, 95%) as fine needles. Benzylation of **1** with NaH–BnBr in the absence of Bu_4NI gave **2** in 65% yield. mp 118–120 °C; $[\alpha]_D^{20}$ −14° (*c* 1, $CHCl_3$); Anal. Calcd for $C_{68}H_{70}O_{12}$ (1079.29): C, 75.67; H, 6.54. Found: C, 75.82; H, 6.52.

2,3,4,6-Tetra-O-*benzyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*benzyl*-D-*glucopyranose* (**3**).—Ceric(IV) diammonium nitrate (21.1 g, 38.4 mmol) was added to a chilled (0 °C) solution of **2** (13.8 g, 12.8 mmol) in 4:1:1 MeCN–toluene–water (150 mL), and the mixture was stirred for 3 h at 0 °C. Ethyl acetate (200 mL) was added to the reaction mixture, and the aqueous solution was extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with water (3 × 150 mL) and brine (2 × 150 mL), dried ($MgSO_4$), filtered and concentrated to give a dark red syrup. Chromatographic purification (1:7→1:6→1:5 EtOAc–toluene) and subsequent crystallization (EtOAc–hexane) gave **3** as needles (5.87 g, 47%). Compound **3** was found to be an anomeric mixture (α:β 20:9) by ^{1}H NMR spectroscopy; mp 115–116 °C; $[\alpha]_D^{25}$ +16° (*c* 1, $CHCl_3$), lit.[9] mp 102–105 °C; $[\alpha]_D$ +10° (*c* 1.2, $CHCl_3$).

2,3,4,6-Tetra-O-*benzyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranosyl trichloroacetimidate* (**4**).—Trichloroacetonitrile (CCl_3CN, 4.8 mL, 47.8 mmol) was added to a stirred suspension of **3** (2.51 g, 2.58 mmol) and potassium carbonate[11] (4.6 g) in CH_2Cl_2 (30 mL) at rt, and after stirring for 96 h, the mixture was filtered and concentrated to a syrup. TLC (2:3:1 hexane–toluene–EtOAc, containing 1% Et_3N) showed the presence of two new products and unreacted **3**. Chromatography (4:2:1 hexane–toluene–EtOAc, containing 1% Et_3N) of the syrup gave the β-imidate **4** (3.28 g, 62%, syrup). TLC (2:3:1 hexane–toluene–EtOAc, containing 1% Et_3N): R_f 0.63; $[\alpha]_D^{22}$ +13° (*c* 1, $CHCl_3$).

Eluted next was the α-imidate **5** (0.35 g, 6.4%, syrup): TLC (2:3:1 hexane–toluene–EtOAc, containing 1% Et_3N): R_f 0.53; $[\alpha]_D^{22}$ +30° (*c* 1, $CHCl_3$).

Further elution gave the starting material **3** (0.73 g, 17%): TLC (2:3:1 hexane–toluene–EtOAc, containing 1% Et_3N): R_f 0.20.

Compound **3** (410 mg, 2.58 mmol) in CH_2Cl_2 (3 mL) was also treated with CCl_3CN (0.42 mL, 4.20 mmol) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.25 mL, 0.08 mmol) at 0 °C for 6 h. The use of DBU gave the α anomer **5** as a major product (α:β 2:1), although the combined yields (83%) of **4** and **5** were higher than those obtained with potassium carbonate.

Methyl 2-O-*benzyl-7,8-dideoxy-α*-D-manno-*oct-7-enopyranoside* (**7**).—Compound **6** (1.73g, 4.08 mmol) was hydrolyzed in 9:1 TFA–water (10 mL) for 15 min at rt, and the hydrolysate was concentrated to an oil which was purified by chromatography (1:1→2:1 EtOAc–hexane) to give **7** (1.10 g, 87%) as an oil; $[\alpha]_D^{22}$ −1° (*c* 1, $CHCl_3$); FAB-HRMS: Calcd for $C_{16}H_{23}O_6$ [M + H], 311.1495; Found: 311.1527.

Methyl 2-O-*benzyl-4,6*-O-*benzylidene-7,8-dideoxy-α*-D-manno-*oct-7-enopyranoside* (**8**).—A solution of *p*-TsOH·H_2O (0.30 mg, 1.59 mmol) in DMF (20 mL) was

added to a stirred solution of **7** (4.92 g, 15.9 mmol) and benzaldehyde dimethylacetal (7.13 mL, 47.6 mmol) in DMF (55 mL) at rt. The reaction mixture was warmed to 40–45 °C and stirred for 16 h. After addition of Et_3N (2 mL), the reaction mixture was concentrated to a syrup. The syrup was dissolved in EtOAc, and the solution was washed with satd $NaHCO_3$ and brine, dried, and concentrated to give a syrup. Purification by flash-column chromatography (1:4 EtOAc–hexane) gave the 4,6-*O*-benzylidene compound **8** (4.97 g, 79%) as a syrup; $[\alpha]_D^{23}$ −81.4° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{23}H_{26}O_6$ (398.45): C, 69.33; H, 6.58; Found: C, 69.35; H, 6.37.

Methyl (2,3,4,6-tetra-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→3)-2*-O-*benzyl-4,6*-O-*benzylidene-7,8-dideoxy-α*-D-manno-*oct-7-enopyranoside* (**9**).—A solution of Me_3SiOTf (1.1 mL, 0.06 mmol) in 1,4-dioxane (20 mL) was added dropwise at rt to a stirred mixture of **8** (0.58 g, 1.28 mmol), **4** (1.70 g, 1.52 mmol) and molecular sieves 4 Å (1.0 g) in 1,4-dioxane (12 mL). The mixture was stirred for 1 h at rt, treated with Et_3N (0.3 mL) and filtered through Celite. The filtrate was concentrated to a syrup which was dissolved in CH_2Cl_2 (30 mL). The organic solution was washed with water (20 mL), and the aqueous solution was extracted with CH_2Cl_2 (2 × 15 mL). The combined extracts were washed with aq satd $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated to give a syrup. Elution of a flash column with 2:3:6 Et_2O–hexane–toluene gave a product of R_f 0.54. This product was found to be a mixture of a β-(1→3) linked trisaccharide and an unidentified product by ^1H NMR spectroscopy, and neither flash-column chromatography nor gel chromatography (Biobeads S-X1) separated the two products. The above β anomer was therefore characterized after osmylation of the mixture (311 mg) as will be described below. Further elution gave **9** as a syrup (1.03 g, 59%). TLC (1:2:1 EtOAc–hexane–toluene): R_f 0.50; $[\alpha]_D^{24}$ +17.9° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{84}H_{88}O_{16}$ (1353.31): C, 74.54; H, 6.55; Found: C, 74.27; H, 6.40. The acceptor **8** was also recovered (0.13 g, 25%), and the hydrolyzed donor was not further characterized.

Methyl (2,3,4,6-tetra-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→3)-2*-O-*benzyl-4,6*-O-*benzylidene*-L-glycero-α-D-manno-*heptopyranoside* (**11**).—A solution of OsO_4 in water (1%, w/v, 3.0 mL) containing $NaIO_4$ (4.8 g, 22.4 mmol) was added to a chilled mixture of **9** (1.00 g, 0.74 mmol) in 2:1 Et_2O–water (24 mL). The mixture was stirred vigorously at 30–35 °C for 3 days and diluted with Et_2O (20 mL) and water (20 mL). The aqueous solution was extracted with Et_2O (2 × 15 mL), and the combined extracts were washed with water (3 × 30 mL) and concentrated to give a syrup. $NaBH_4$ (0.28 g, 7.40 mmol) was added to a chilled solution (0 °C) of the syrup in 1:1 1,4-dioxane–MeOH (30 mL), and the mixture was warmed to rt and stirred for 16 h. The mixture was extracted with EtOAc (50 mL), and the aqueous solution was extracted with EtOAc (2 × 20 mL). The combined extracts were washed with water (2 × 40 mL) and brine (40 mL), dried ($MgSO_4$), and concentrated to a syrup. Purification by flash-column chromatography (1:1:2 EtOAc–hexane–toluene) gave **11** (0.80 g, 80%) as a syrup; $[\alpha]_D^{22}$ +32° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{83}H_{88}O_{17}$ (1357.60): C, 73.43; H, 6.53; Found: C, 73.22; H, 6.47.

Methyl (2,3,4,6-tetra-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-(2,3,6-tri*-O-*benzyl-β*-D-*glucopyranosyl)-(1→3)-2*-O-*benzyl-4,6*-O-*benzylidene*-L-glycero-α-D-manno-*heptopyranoside* (**12**).—The mixture (311 mg) containing the β anomer **10** and the unidentified product was oxidized and then reduced in a similar manner as described for the synthesis of **11**. Purification of the reaction mixture by flash-column chromatography (3:1 toluene–EtOAc) gave **12** as a syrup (103 mg); $[\alpha]_D^{24}$ +9° (*c* 1, $CHCl_3$).

Methyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1→3)-1,2,4,6,7-penta*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranoside* (**13**).—A suspension of **11** (238 mg, 0.18 mmol) and Pd/C (10%, 350 mg) in DMF (4 mL) was stirred vigorously for 10 days under a hydrogen atmosphere. The mixture was filtered through Celite, and the filtrate was concentrated to a syrup. The syrup, dried over P_2O_5, was acetylated with pyridine and Ac_2O (2 mL each) with a catalytic amount of 4-(dimethylamino)pyridine at rt overnight. The reaction solution was co-evaporated with toluene several times to a residue. The residue was dissolved in EtOAc (5 mL), and the organic solution was washed with water (5 mL). The aqueous solution was extracted with EtOAc (5 mL), and the combined extracts were washed with brine (5 mL), dried ($MgSO_4$), filtered and concentrated to a syrup. Purification by flash-column chromatography of the syrup (2:1 EtOAc–hexane) gave **13** as a colorless amorphous solid (129 mg, 73%); $[\alpha]_D^{22}$ +46° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{42}H_{58}O_{28}$ (1010.90): C, 49.90; H, 5.78; Found: C, 49.65; H, 5.68.

Methyl (β-D-*galactopyranosyl)-(1→4)-(α*-D-*glucopyranosyl)-(1→3)*-L-glycero-α-D-manno-*heptopyranoside* (**14**).—Compound **13** (21 mg, 20.8 μmol) was treated with 20 mM NaOMe in MeOH (2.0 mL) for 2 h, and the solution was neutralized with Amberlite IR-120 (H^+), filtered and concentrated to a residue. The residue was purified by gel chromatography [Bio-Gel P-2] to give **14** (9.7 mg, 85%) as a white powder; $[\alpha]_D^{25}$ +124° (*c* 0.5, water); FAB-HRMS: Calcd for $C_{20}H_{37}O_{17}$ [M + H], 549.2031; Found: 549.2043.

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References

1. Gibson, B. W.; Webb, J. W.; Yamasaki, R.; Fisher, S. J.; Burlingame, A. L.; Mandrell, R. E.; Schneider, H.; Griffiss, J. M. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 17–21.
2. Yamasaki, R.; Bacon, B. E.; Nasholds, W.; Schneider, H.; Griffiss, J. M. *Biochemistry* **1991**, *30*, 10566–10575.
3. Kerwood, D. E.; Schneider, H.; Yamasaki, R. *Biochemistry* **1992**, *31*, 12760–12768.
4. John, C. M.; Griffiss, J. M.; Apicella, M. A.; Mandrell, R. E.; Gibson, B. W. *J. Biol. Chem.* **1991**, *266*, 19303–19311.
5. Yamasaki, R.; Kerwood, D. E.; Schneider, H.; Quinn, K. P.; Griffiss, J. M.; Mandrell, R. E. *J. Biol. Chem.* **1994**, *269*, 30345–30351.
6. John, C. M.; Schneider, H.; Griffiss, J. M. *J. Biol. Chem.* **1999**, *274*, 1017–1025.
7. Yamasaki, R.; Koshino, H.; Kurono, S.; Nishinaka, Y.; McQuillen, D. P.; Kume, A.; Gulati, S.; Rice, P. A. *J. Biol. Chem.* **1999**, *274*, 36550–36558.
8. Kuyama, H.; Nukada, T.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1993**, *34*, 2171–2174.
9. Koto, S.; Morishima, N.; Shichi, S.; Haigoh, H.; Hirooka, M.; Okamoto, M.; Higuchi, T.; Shimizu, K.; Hashimoto, Y.; Irisawa, T.; Kawasaki, H.; Takahashi, Y.; Yamazaki, M.; Mari, Y.; Kudo, K.; Ikegaki, T.; Suzuki, S.; Zen, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 3257–3274.
10. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629–5647.
11. Schmidt, R. R.; Michel, J.; Michael, R. *Liebigs Ann. Chem.* **1984**, 1343–1357.
12. Numata, M.; Sugimoto, M.; Koike, K.; Ogawa, T. *Carbohydr. Res.* **1987**, *163*, 209–225.
13. Yamasaki, R.; Takajyo, A.; Kubo, H.; Matsui, T.; Ishii, K.; Yoshida, M. *J. Carbohydr. Chem.* **2001**, *20*, 171–180.
14. Salanski, P.; Descotes, G.; Bouchu, A.; Queneau, Y. *J. Carbohydr. Chem.* **1998**, *17*, 129–142.
15. Demchenko, A.; Stauch, T.; Boons, G. J. *Synlett* **1997**, *7*, 818–820.
16. Schmidt, R. R.; Grudler, G. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 781–782.
17. Boons, G. J. P. H.; Hoogerhout, P.; Poolman, J. T.; van der Marel, G. A.; van Boom, J. H. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 303–308.
18. Grzeszczyk, B.; Zamojski, A. *Carbohydr. Res.* **1994**, *262*, 49–57.
19. Garegg, P. J.; Oscarson, S.; Tiden, A. K. *Carbohydr. Res.* **1990**, *200*, 475–480.
20. Zang, Z.; Magnusson, G. *Carbohydr. Res.* **1996**, *925*, 41–55.