Synthesis of a 3,4-Di-*O*-substituted Heptose Structure: A Partial Oligosaccharide Expressed in Neisserial Lipooligosaccharide

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We have synthesized a tetrasaccharide containing a 3,4-dibranched L-*glycero*-D-*manno*-heptose (Hep), β-lactosyl-(1→4)-[L-α-D-Hep-(1→3)]-L-α-D-Hep **19**, by using a mannose (Man) derivative as an acceptor. Prior to the construction of the branched Hep, we confirmed that the 3,4-dibranched Man structure could be synthesized using a 3-branched Man **6** as an acceptor. Glycosylation of the acceptor **6** using hepta-*O*-acetyl-α-lactosyl trichloroacetimidate (**7**) gave the desired 3,4-dibranched structure, β-lactosyl-(1→4)-[α-Man-(1→3)]-α-Man **8**. As expected, β-lactosyl-(1→4)-[L-α-D-Hep-(1→3)]-α-D-Man **14** was also obtained by glycosylating the 4-OH acceptor **13** with **7** in a similar manner. The Man residue of **14** was converted into the Hep unit by Swern oxidation, Grignard reaction, and oxidative cleavage followed by reduction. Thus, we constructed the 3,4-dibranched Hep structure **19** by using the 3-branched Man **13** as an acceptor. The current results demonstrate that the *gauche* orientation of the *O*-3 and *O*-4 units of the Man configuration does not prevent the formation of the 3,4-di-*O*-substituted structure. This approach should provide an alternative method to synthesize the 3,4-dibranched Hep structure expressed in LOS produced by pathogenic Gram-negative bacteria such as the Neisserial and Haemophilus species.

Introduction

Lipooligosaccharides (LOS) produced by *Neisseria gonorrhoeae* are important antigenic and immunogenic components of the outer membrane complex.[1−4] Recently, we characterized an epitope defined by a monoclonal antibody 2C7 that potentially could be developed as a vaccine target against the organism.[5−7] This 2C7-defined epitope is expressed in the oligosaccharide (OS) moiety of LOS produced by strain 15253.[7,8]

As Figure 1 shows, the 15253 OS contains two heptose (Hep) residues, HepI and HepII, within the conserved core. Both of the Hep residues are dibranched; HepI and HepII are branched at *O*-3 and *O*-4 and at *O*-2 and *O*-3, respectively.[8] The 3,4-dibranched Hep, represented by HepI, within 15253 LOS is a common structure among Neisserial LOS[9−12] and it is also expressed in Haemophilus[13−15] and Campylobacter LOS.[16,17] A trisaccharide containing the 3,4-dibranched Hep structure has been synthesized by Oscarson's group.[18] They used a 1,6-anhydro-heptose derivative as an acceptor to build the 3-*O*-substituted Hep, which was then employed to construct the 3,4-dibranched Hep. The authors reported that glycosylation using either an *O*-3- or *O*-4-substituted Hep of $^4C_1$ conformation as an acceptor did not produce the desired 3,4-dibranched structures. In addition, they reported difficulties in synthesizing the 3,4-dibranched mannose (Man) structure,[19] and these unfruitful results were ascribed to steric crowding arising from the *gauche* orientation of the *O*-3 and *O*-4 positions.[18]

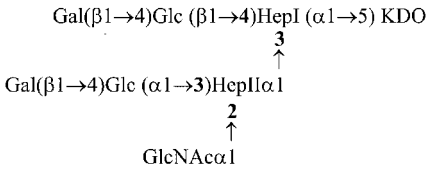

Figure 1. The oligosaccharide structure of LOS produced by *Neisseria gonorrhoeae* strain 15253; Gal: D-galactose; Glc: D-glucose; Hep: L-*glycero*-D-*manno*-heptose; GlcNAc: *N*-acetyl-D-glucosamine; KDO: 2-keto-3-deoxy-D-*manno*-octulosonic acid; the two heptoses are designated as described previously[8]

In our present study, we examined glycosylation reactions for the construction of the 3,4-dibranched Hep structure using a Man derivative of $^4C_1$ form as an acceptor and

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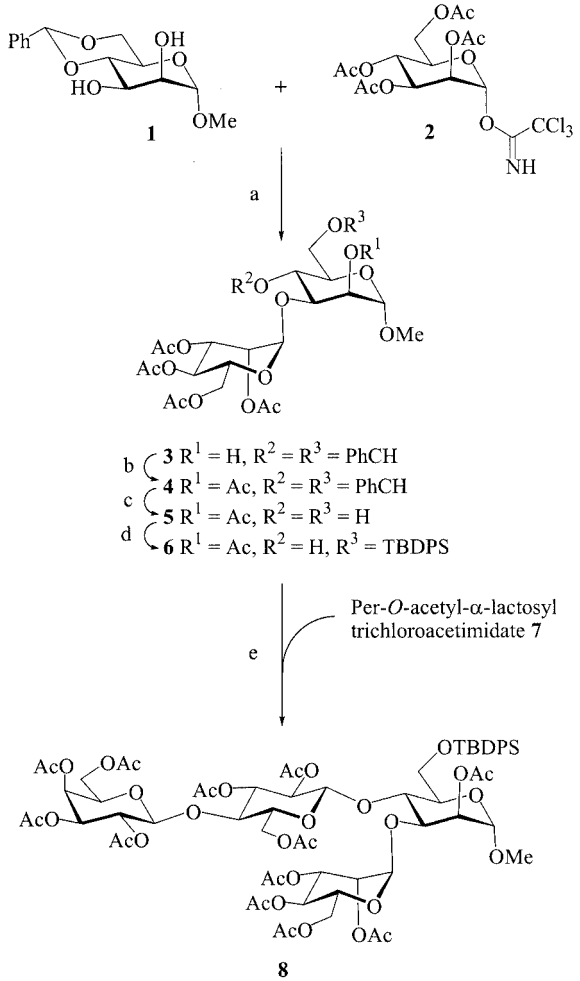
found that the *gauche* orientation of the endocyclic hydroxy groups does not disfavor the formation of the 3,4-dibranched structure. We constructed the 3,4-di-*O*-substituted Hep expressed in 15253 LOS as follows: synthesis of a disaccharide, α-Hep-(1→3)-Man, β-lactosylation at *O*-4 of the Man residue, and subsequent conversion the Man residue to the Hep residue.

Results and Discussion

First of all, we examined whether it is possible to synthesize a 3,4-dibranched Man using a 3-*O*-substituted Man as an acceptor. As Scheme 1 shows, we prepared the acceptor by coupling methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**)[20] and tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**2**).[21] Treatment of **1** with 1.1 molar equivalents of **2** in CH_2Cl_2 in the presence of $BF_3{\cdot}OEt_2$ for 2 h at −10 °C and subsequent chromatographic purification of the reaction mixture gave α-ManII-(1→3)-ManI **3** as a major product (56%). The α-configuration of **3** was established from the $^1J_{C\text{-}1,H\text{-}1}$ value[22−24] (168 Hz) of the ManII residue, which is almost identical to that (169 Hz) of the α-ManI residue, and the linkage site was identified by the distinctive downfield shift (Δδ = 4.9 ppm) of the *C*-3 atom of the ManI (Table 2) and was also confirmed by the HMBC experiment. The minor product obtained from the glycosylation above was found by 2D NMR spectroscopy to be a mixture of Man-(1→2)-Man and Man-(1→2)-[Man-(1→3)]-Man. Although these products were not further characterized, the yields of the disaccharide and the trisaccharide were estimated to be 2.3 and 4.6%, respectively, based on the ratio of integrals of the OCH_3 signals. The α-Man-(1→3)-Man **3** was acetylated with Ac_2O/pyridine to give the 2-*O*-Ac derivative **4** (99%), which was then hydrolyzed in aqueous 70% acetic acid (AcOH) to remove the benzylidene acetal unit (93%). The resulting 4,6-diol **5** was treated with *tert*-butyldiphenylsilyl chloride (TBDPSCl) in DMF to give the 4-OH acceptor **6** (93%). The ^1H and ^{13}C NMR spectroscopic data of products **4**−**6** (Tables 1 and 2) confirmed their structures.



Scheme 1. a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10 °C, 56%; b) Ac_2O, pyridine, 99%; c) AcOH (70% aq.), 60−70 °C, 93%; d) TBDPSCl, imidazole, DMF, 0 °C → room temp., 93%; e) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10 °C, 80%

Glycosylation of the 4-OH acceptor **6** with two molar equivalents of hepta-*O*-acetyl-α-lactosyl trichloroacetimidate (**7**)[25] using $BF_3{\cdot}OEt_2$ as catalyst in CH_2Cl_2 for 2 h at −10 °C, followed by chromatographic purification, gave the desired 3,4-dibranched structure, β-lactosyl-(1→4)-[α-Man-(1→3)]-α-Man **8** in 80% yield. The β-linkage of the Glc residue was assigned from the $^3J_{1,2}$ value (7.8 Hz; Table 1). The downfield shifts of both C-4 (Δδ = 4.3 ppm) and H-4 (Δδ = 0.45 ppm) of the ManI residue (Tables 1 and 2) of **8** and the HMBC experiment verified that the lactose was *O*-4-linked to the ManI residue. The formation of the tetrasaccharide containing the 3,4-di-*O*-substituted Man as a major product showed that glycosylation of the 4-OH group of the 3-*O*-substituted Man acceptor **6** is possible, which demonstrated that the 3,4-*gauche* orientation of Man would not disfavor the formation of the di-*O*-substituted structure, in contrast to a previous finding by Oscarson et al.[18]

After confirming that construction of the 3,4-dibranched Man is possible using the 3-*O*-substituted Man **3** as an acceptor, we synthesized a 3,4-di-*O*-substituted Hep structure expressed in 15253 LOS as follows: synthesis of α-Hep-(1→3)-α-Man, lactosylation of the α-Hep-(1→3)-α-Man, and conversion of the Man residue to the Hep residue (Schemes 2 and 3).

As expected from the glycosylation results of **1** and **2**, treatment of **1** with 1.1 molar equivalents of 2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl trichloroacetimidate (**9**)[26] under conditions similar to those described earlier gave the α-Hep-(1→3)-α-Man **10** in 70% yield and the unreacted acceptor **1** (5%). Although a mixture of two products, presumably Hep-(1→2)-Man and Hep-(1→2)-[Hep-(1→3)]-Man were also obtained, we did not carry out further structural characterization of these two products. The structure of compound **10** was determined by the $^1J_{C\text{-}1,H\text{-}1}$ value of the Hep residue (181 Hz)[18] and the shift of the ^{13}C-3 satellite (Table 2) in a similar manner described for **3**.

Table 1. ^{1}H NMR spectroscopic data for compounds **1**, **3**−**6**, and **8**

Compound[a]	Residue[b]	H-1 ($^3J_{1,2}$)	H-2 ($^3J_{2,3}$)	H-3 ($^3J_{3,4}$)	H-4 ($^3J_{4,5}$)	H-5 ($^3J_{5,6a}$)	H-6a ($^3J_{5,6b}$)	H-6b ($^2J_{6a,6b}$)
1[c]		4.76	4.05	4.07	3.92	3.82	3.83	4.29
		(1.5)	(3.5)	(9.0)	(9.0)	n.d.	n.d.	n.d.
3[d]	ManI	4.77	4.06	4.17	4.17	3.82	3.87	4.29
		(1.5)	n.d.	n.d.	n.d.	n.d.	(4.4)	(10.4)
	ManII	5.13	5.43	5.38	5.25	4.10	4.15	4.23
		(2.0)	(3.6)	(9.8)	(9.6)	(2.0)	(6.4)	(12.2)
4[c]	Man I	4.67	5.33	4.30	4.06	3.85	3.85	4.30
		(1.5)	(4.0)	(8.5)	(9.5)	n.d.	n.d.	n.d.
	ManII	5.19	5.35	5.19	5.26	4.08	4.11	4.28
		(1.5)	(3.0)	(10.0)	(10.0)	n.d.	(6.0)	(12.8)
5[c]	ManI	4.68	5.20	4.06	4.02	3.62	3.88	3.88
		(1.5)	(3.0)	(9.8)	(9.0)	n.d.	n.d.	n.d.
	ManII	5.19	5.32	5.21	5.26	4.08	4.09	4.27
		(1.5)	(3.0)	(10.0)	(10.0)	(5.0)	(6.5)	(13.0)
6[c]	ManI	4.65	5.18	4.06	4.03	3.57	3.92	3.92
		(1.5)	(3.0)	(9.5)	(9.8)	n.d.	n.d.	n.d.
	ManII	5.20	5.36	5.23	5.26	4.11	4.10	4.27
		(1.5)	(3.0)	(10.0)	(10.0)	n.d.	(6.0)	(12.8)
8[e]	ManI	4.70	5.23	4.13	4.48	3.50	3.87	3.99
		(0.6)	(3.6)	(9.6)	(9.6)	n.d.	(1.8)	(11.8)
	ManII	5.10	5.39	5.24	5.25	4.19	4.13	4.24
		(1.2)	n.d.	(10.2)	(10.2)	(1.8)	(8.4)	(11.4)
	Glc	4.84	4.73	5.03	3.65	3.49	4.25	4.46
		(7.8)	(9.6)	(9.6)	(9.6)	(6.0)	(1.8)	(11.8)
	Gal	4.49	5.11	4.99	5.34	3.88	4.06	4.12
		(7.8)	(10.2)	(3.0)	n.d.	n.d.	(6.0)	(11.8)

[a] Data were acquired in $CDCl_3$ at 20−23 °C. The ^{1}H NMR spectroscopic chemical shifts (ppm) were determined by analyzing 2D NMR spectroscopic data (DQF-COSY, HMQC and HMBC) comparatively, and the *J* couplings (Hz) were obtained by analyzing either the DQF-COSY or 1D NMR spectra. The ^{1}H NMR spectroscopic chemical shifts of other protons are listed in the Exp. Sect. [b] To distinguish between the two Man residues, the one at the reducing end and the other at the non-reducing end are designated as ManI and ManII, respectively. [c] 500 MHz. [d] 400 MHz. [e] 600 MHz. n.d.: not determined.

Table 2. ^{13}C NMR spectroscopic data for compounds **1**, **3**−**6**, and **8**

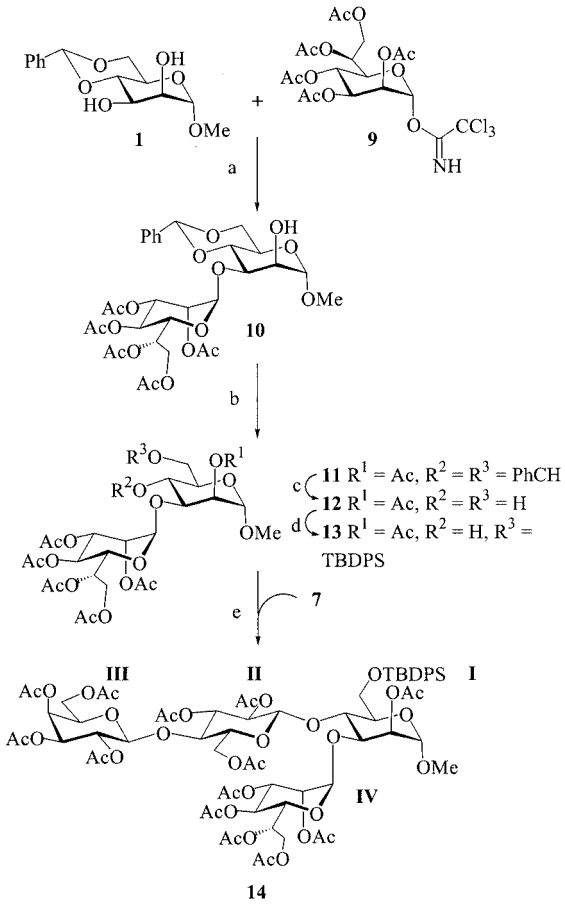
Compound[a]	Residue[b]	C-1	C-2	C-3	C-4	C-5	C-6
1[c]		101.4	70.9	68.7	78.8	63.0	68.9
3[d]	ManI[e]	101.3	70.9	73.6	78.4	63.4	68.7
	ManII[e]	98.4	69.1	69.0	66.3	66.9	69.6
4[c]	ManI	99.7	70.8	70.6	78.9	63.3	68.5
	ManII	98.1	69.1	68.8	65.6	69.2	62.4
5[c]	ManI	98.7	71.2	76.3	67.5	71.9	62.1
	ManII	99.1	69.5	69.0	65.8	69.3	62.4
6[c]	ManI	98.4	70.9	75.2	69.0	71.3	64.1
	ManII	98.9	69.3	69.0	65.8	69.1	62.4
8[e]	ManI	98.6	71.5	72.1	73.3	71.8	60.9
	ManII	98.8	69.5	68.6	66.3	69.1	62.7
	Glc	99.6	72.5	73.4	77.0	73.0	62.4
	Gal	101.3	69.0	71.1	66.7	70.5	60.7

[a] Data were acquired in $CDCl_3$ at 20−23 °C. The ^{13}C NMR spectroscopic chemical shifts (ppm) were determined by analyzing 2D NMR spectroscopic data (DQF-COSY, HMQC and HMBC) comparatively. Only the data for the skeletal carbon atoms are presented, and those for other carbon atoms are listed in the Exp. Sect. [b] The two Man residues are designated as ManI and ManII, as described in Table 1. [c] 125 MHz. [d] 100 MHz. [e] The $^1J_{H\text{-}1,C\text{-}1}$ values are as follows: ManI, 168 Hz; ManII, 169 Hz. [e] 150 MHz.

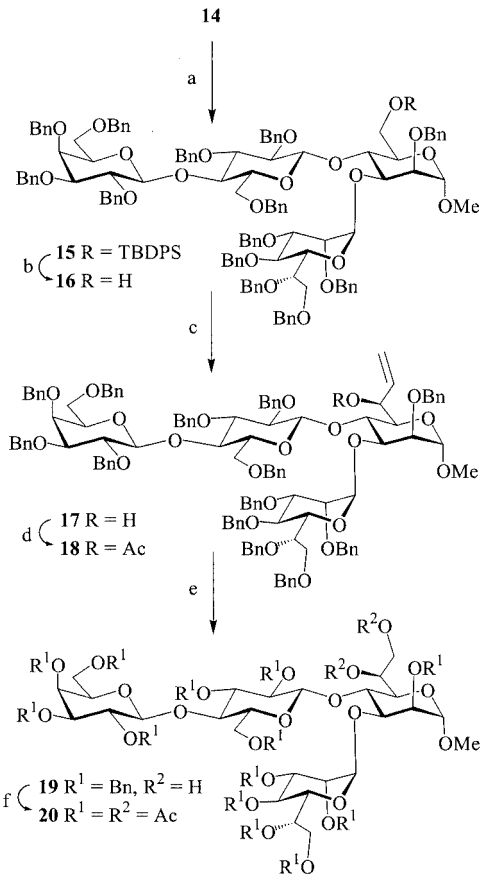
The disaccharide **10** was converted into the 4-OH acceptor **13** in a similar manner described earlier for **3** (Scheme 2): acetylation (89%), hydrolysis (88%), and subsequent protection of the 6-OH group with TBDPSCl (87%). The structures of **11**−**13** were confirmed by their ^{1}H and ^{13}C NMR spectroscopic data, which are shown in Tables 3 and 4.

Glycosylation of the acceptor **13** with two molar equivalents of the α-lactosyl trichloroacetimidate **7** ($BF_3{\cdot}OEt_2$ in CH_2Cl_2 for 3 h at −10 °C and then for 30 min at room temperature) followed by flash column chromatography gave the expected tetrasaccharide **14** in 59% yield and the unreacted acceptor **13** (40%).

Analysis of the HMQC spectrum (Figure 2, panels A and B) identified the exo- and endocyclic protons and carbon atoms of each residue of **14**. Two sets of cross-relay peaks, Hep H-1/Man C-3 and Man H-3/Hep C-1, Glc C-1/Man H-4 and Man C-4/Glc H-1, in the HMBC spectrum (Figure 2, panels C and D) confirmed that the lactosyl moiety is linked to the 4-position of the Man residue. The $^3J_{1,2}$ value (8 Hz) of the Glc residue (Table 3) verified that the lactose is β-linked. In addition, comparison of the $^1J_{C\text{-}1,H\text{-}1}$ values of α-Man-(1→3)-α-Man **3**, α-Hep-(1→3)-α-Man **10**, and the tetrasaccharide **14** showed that the α-Hep residue tends to give large $^1J_{C\text{-}1,H\text{-}1}$ values (over 180 Hz) relative to those of the α-Man residue (168 −175 Hz). In addition, the Man residue of **14** has the largest value of $^1J_{C\text{-}1,H\text{-}1}$ (175 Hz), which indicates that the degree of substitution affects the $^1J_{C\text{-}H,H\text{-}1}$ value.



Scheme 2. a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10 °C, 70%; b) Ac_2O, DMAP, pyridine, 89%; c) AcOH (70% aq.), 70 °C, 93%; d) TBDPSCl, imidazole, DMF, room temp., 87%; e) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10 °C → room temp., 59%



Scheme 3. a) 1. MeOH/Et_3N/water (2:1:1, v/v/v), room temp.; 2. BnBr, NaH, DMF, molecular sieves (4 Å), DMF, 0 °C → room temp., 69%; b) TBAF, THF, room temp. → 60 °C, 89%; c) 1. $(COCl)_2$, DMSO/THF, −60 °C, then Et_3N; 2. $CH_2{=}CHMgBr$, THF, −70 °C, 78%; d) Ac_2O, DMAP, pyridine, 82%; e) 1. OsO_4/$NaIO_4$, Et_2O/water (1:1, v/v), 30−35 °C; 2. $NaBH_4$, DMF/MeOH (2:1, v/v), 80%; f) 1. H_2, Pd/C, EtOH/AcOH/water (7:5:5, v/v/v); 2. Ac_2O, DMAP, pyridine, 84%

The target compound, β-lactosyl-(1→4)-[α-Hep-(1→3)]-α-Hep **17** was synthesized by converting the Man residue of **14** to the Hep unit (Scheme 3) as described previously.[27] Compound **14** was deacetylated in a mixture of triethylamine/MeOH/water, and the resulting mixture was benzylated with NaH/benzyl bromide (BnBr) in DMF. Purification by flash column chromatography gave the perbenzylated product **15** in 69%. Although complete removal of the TBDPS group of **15** did not occur when it was treated with tetrabutylammonium fluoride (TBAF) in THF for 2 days at room temperature, treatment of the reaction mixture at 60 °C for 14 h provided the de-*O*-silylated product **16** in 89% yield. Oxidation of **16** with oxalyl chloride and dimethyl sulfoxide (DMSO) in THF at −60 °C and subsequent Grignard reaction using vinylmagnesium bromide[27,28] gave a tetrasaccharide **17** (78%) containing the oct-7-enopyranoside moiety at the reducing end.

The presence of the vinyl function of **17** was confirmed by the distinctive chemical shifts of the corresponding protons (H-7, H-8a, and H-8b) and carbons (C-7 and C-8) (Tables 5 and 6) as reported previously.[27,29]

Compound **17** was acetylated with acetic anhydride/pyridine and then oxidative cleavage of the acetate **18** with OsO_4/$NaIO_4$, followed by reduction of the resulting aldehyde with $NaBH_4$, gave the tetrasaccharide **19** (80% yield) having a Hep residue at the reducing end. Finally, hydrogenation of **19** over Pd/C, followed by acetylation, provided the peracetylated β-lactosyl-(1→4)-[α-Hep-(1→3)]-α-Hep **20** in 84%. The structures of **17**−**20** were confirmed by their NMR spectroscopic data, which are presented in Tables 5 and 6. The stereochemistry of the C-6 center at the reducing end of the Hep residue was determined as described previously to be the L form.[10,27,29]

As described earlier, Oscarson et al. reported that glycosylation of neither the 4-OH group of the 3-*O*-substituted Hep nor the 3-OH group of the 4-*O*-substituted Hep was successful,[18] and similar results had been experienced in synthesizing the 3,4-di-*O*-substituted Man structure.[19] The poor glycosylation results were ascribed to steric factors arising from the *gauche*-oriented OH units at the 3 and 4 positions of the Hep moiety. Our present results clearly indicate, however, that the *gauche*-oriented secondary OH groups do not prevent the formation of the di-*O*-substituted product. This observation is also supported by a paper[30] that appeared during the final stages of preparation of this

Table 3. ^{1}H NMR spectroscopic data for compounds **10**−**16**

Compound[a]	Residue	H-1 ($^3J_{1,2}$)	H-2 ($^3J_{2,3}$)	H-3 ($^3J_{3,4}$)	H-4 ($^3J_{4,5}$)	H-5 ($^3J_{5,6a}$)	H-6a ($^3J_{5,6b}$)	H-6b ($^2J_{6a,6b}$)	H-7a ($^3J_{6,7a}$)	H-7b ($^3J_{6,7b}$)	($^2J_{7a,7b}$)
10[b]	Man	4.76	4.05	4.11	4.20	3.82	3.88	4.28			
		(1.5)	(3.0)	(10.0)	(9.5)	(10.0)	(4.5)	(10.0)			
	Hep	5.39	5.42	5.34	5.29	4.15	5.22	−	4.24	4.24	
		(1.5)	(3.5)	(10.5)	(9.5)	(1.5)	−	−	(6.5)	n.d.	n.d.
11[c]	Man	4.67	5.32	4.22	4.07	3.85	3.85	4.30			
		(1.2)	(4.0)	n.d.	(9.2)	n.d.	n.d.	n.d.			
	Hep	5.30	5.39	5.16	5.30	4.20	5.22	−	4.21	4.29	
		(1.6)	(3.2)	(10.4)	(10.2)	n.d.	−	−	n.d.	(6.8)	
12[c]	Man	4.65	5.21	4.02	4.02	3.60	3.87	3.87			
		(1.6)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
	Hep	5.34	5.33	5.15	5.31	4.23	5.25	−	4.27	4.27	
		n.d.	(2.8)	(10.4)	(10.4)	(1.6)	−	−	(6.4)	n.d.	n.d.
13[b]	Man	4.60	5.18	4.00	4.04	3.55	3.91	3.91			
		n.d.	(2.5)	(10.0)	(10.0)	n.d.	(4.5)	n.d.			
	Hep	5.43	5.38	5.19	5.32	4.23	5.25	−	4.23	4.30	
		n.d.	(3.0)	(10.0)	(10.5)	n.d.	−	−	n.d.	(7.0)	(11.0)
14[b]	Man	4.70	5.22	4.08	4.51	3.52	3.87	3.98			
		n.d.	(3.5)	(10.0)	(10.0)	n.d.	(1.0)	(10.0)			
	Hep	5.22	5.43	5.23	5.32	4.28	5.22	−	4.18	4.28	
		(1.5)	(2.5)	(10.5)	(10.5)	n.d.	−	−	(7.0)	n.d.	(11.0)
	Glc	4.83	4.70	5.02	3.64	3.49	4.26	4.45			
		(8.0)	(9.5)	(9.5)	(12.0)	n.d.	(2.0)	(11.5)			
	Gal	4.48	5.10	5.00	5.34	3.89	4.05	4.13			
		(8.5)	(10.0)	(3.5)	n.d.	(7.5)	(6.5)	(11.5)			
15[b]	Man	4.55	3.73	4.18	4.65	3.38	3.75	4.22			
		n.d.	(3.5)	(10.0)	n.d.	n.d.	n.d.	n.d.			
	Hep	5.63	4.28	3.91	4.24	3.84	4.12	−	3.74	3.85	
		n.d.	(2.5)	(9.0)	(10.0)	n.d.	−	−	(6.5)	n.d.	n.d.
	Glc	4.79	3.22	3.39	3.86	3.35	3.62	3.78			
		(8.0)	(8.0)	(9.0)	(9.0)	(4.5)	n.d.	(11.0)			
	Gal	4.25	3.69	3.25	3.88	3.20	3.30	3.52			
		(8.0)	(10.0)	(3.0)	n.d.	(5.0)	(9.0)	(9.0)			
16[b]	Man	4.47	3.70	4.14	4.30	3.40	3.68	3.90			
		n.d.	(3.0)	(10.0)	n.d.	n.d.	n.d.	n.d.			
	Hep	5.67	4.28	3.92	4.24	3.78	4.08	−	3.69	3.80	
		n.d.	(3.0)	(9.5)	(9.5)	n.d.	−	−	(6.5)	n.d.	n.d.
	Glc	4.48	3.23	3.57	3.88	3.43	3.68	3.79			
		(8.0)	(8.5)	(8.5)	(9.0)	n.d.	n.d.	n.d.			
	Gal	4.26	3.70	3.25	3.87	3.22	3.30	3.50			
		(8.0)	(9.5)	(3.5)	n.d.	(5.0)	(9.0)	(9.0)			

[a] Data were acquired in $CDCl_3$ at 20−23 °C. The ^{1}H NMR spectroscopic chemical shifts (ppm) were determined by analyzing 2D NMR spectroscopic data (DQF-COSY, HMQC and HMBC) comparatively, and the *J* couplings (Hz) were obtained by analyzing either the DQF-COSY or 1D NMR spectra. The ^{1}H NMR spectroscopic chemical shifts of other protons are listed in the Exp. Sect. [b] 500 MHz. [c] 400 MHz. n.d.: not determined.

manuscript: a similar 3,4-dibranched structure, α-Hep-(1→3)-[β-Glc-(1→4)]-Hep was constructed after synthesizing the 4-*O*-substituted Hep. The fact that glycosylation using the α-Hep-(1→3)-α-Man acceptor **10** gave the corresponding tetrasaccharide in lower yield than the one with the α-Man-(1→3)-α-Man acceptor **3** may indicate that the orientation of the endocyclic carbon atoms of a Hep residue could lead to steric crowding, which would affect the outcome of the glycosylation reaction. Comparative conformational analyses of an *O*-3 or *O*-4-substituted Hep with those of the Man counterparts may provide some insights into whether the presence of the extra exocyclic carbon would lead to steric crowding.

Thus, we synthesized the 3,4-dibranched Hep structure present as a partial structure of the OS moiety in 15253 LOS as follows: (1) synthesis of a α-Hep-(1→3)-α-Man containing a free 4-OH group at the Man residue; (2) β-lactosylation of the 4-OH, followed by elongation of the Man to Hep by Swern oxidation, Grignard reaction, and oxidative cleavage followed by $NaBH_4$ reduction. Our approach, which uses a 3-*O*-substituted Man derivative of $^4C_1$ conformation as an acceptor, should provide an alternative approach to build the 3,4-di-*O*-substituted Hep structure expressed in LOS produced by pathogenic Gram-negative bacteria.

Experimental Section

General: Optical rotations and melting points (uncorrected) were measured using a HORIBA SEPA-200 polarimeter and a YANAG-

Table 4. ^{13}C NMR spectroscopic data for compounds **10**−**16**

Compound[a]	Residue	C-1	C-2	C-3	C-4	C-5	C-6	C-7
10[b]	Man[c]	101.2	71.0	73.2	78.7	63.3	68.7	
	Hep[c]	98.6	69.8	68.8	64.9	69.2	67.0	61.5
11[d]	Man	99.5	70.6	70.2	79.0	63.1	68.5	
	Hep	98.2	69.0	68.9	64.3	68.8	66.9	61.1
12[b]	Man	98.6	70.8	74.4	67.7	71.9	61.6	
	Hep	98.8	69.3	69.1	64.3	68.8	67.0	61.4
13[b]	Man	98.5	69.4	73.7	70.3	70.7	64.5	
	Hep	98.9	70.6	69.2	64.5	68.9	67.2	61.5
14[b]	Man[e]	98.5	71.6	71.9	73.3	71.9	60.9	
	Hep[e]	99.1	69.5	68.8	65.0	68.8	67.2	61.2
	Glc[e]	99.5	72.4	73.4	77.1	73.1	62.4	
	Gal[e]	101.4	69.0	71.1	66.7	70.6	60.8	
15[b]	Man	99.6	77.8	73.6	75.1	72.6	61.6	
	Hep	98.9	75.7	80.2	74.5	71.9	74.5	70.1
	Glc	103.5	82.4	83.3	76.7	75.2	68.5	
	Gal	102.4	79.7	82.2	73.5	72.6	67.8	
16[b]	Man	99.6	77.1	73.4	74.9	72.3	60.7	
	Hep	98.9	75.4	79.9	74.4	71.9	75.0	68.9
	Glc	103.5	82.4	83.1	76.7	75.0	68.9	
	Gal	102.4	79.8	82.2	73.4	72.5	67.8	

[a] Data were acquired in $CDCl_3$ at 20−23 °C. The ^{13}C NMR spectroscopic chemical shifts (ppm) were determined by analyzing 2D NMR spectroscopic data (DQF-COSY, HMQC and HMBC) comparatively. Only the data for the skeletal carbon atoms are presented, and those for other carbon atoms are listed in the Exp. Sect. [b] 125 MHz. [c] The $^1J_{H\text{-}1,C\text{-}1}$ values of **10**: Man, 171 Hz; Hep, 181 Hz. [d] 100 MHz. [e] The $^1J_{H\text{-}1,C\text{-}1}$ values of **14**: Man, 175 Hz; Hep, 182 Hz; Glc, 165 Hz; Gal, 161 Hz.

IMOTO micro melting point apparatus, respectively. High-resolution fast-atom bombardment mass spectrometry (HR-FABMS) was carried out as described previously.[29] Elemental analyses were performed using a Perkin−Elmer 2400 Series II CHNS/O Analyzer. NMR spectra [400 MHz (JEOL JNM-A400), 500 MHz (JEOL JNM-ECP 500), and 600 MHz (JEOL JNM-A600)] were recorded in $CDCl_3$ using tetramethylsilane as the internal standard, and 2D NMR spectroscopic data (DQF-COSY, HMQC, and HMBC) were processed in a manner similar to that described previously.[7,27,29] Silica gel 60 (E. Merck) was used for flash column (0.040−0.063 mm) and open column (0.063−0.200 mm) chromatography. Silica gel 60 F_{254} (E. Merck) was used for thin-layer chromatography (TLC), and compounds were detected under UV light or by spraying with 10% concd. sulfuric acid in methanol and then heating the plates at 120 °C for 5 min. The L- and D-*glycero*-D-*manno*-heptoses were analyzed by high-performance anion-exchange chromatography (HPAEC) as reported previously.[11,29] Glycosylation reactions were carried out under argon using dry solvents. The glycosylation mixture was filtered through Celite, and the filtrate was washed with water, dried over $MgSO_4$ and concentrated to a syrup under diminished pressure below 40 °C, unless otherwise stated. Methyl α-D-mannoside was purchased from Sigma Chemical Co., and the following compounds were prepared according to the literature procedures: methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**; m.p. 155−156 °C, $[\alpha]_D^{23}$ = +64 (*c* = 2.0, $CHCl_3$), {ref.[31] m.p. 146−147 °C, $[\alpha]_D$ = +64.3 (*c* = 2.1, $CHCl_3$)}), 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**2**; $[\alpha]_D^{23}$ = +51 (*c* = 1.0, $CHCl_3$), {ref.[21] $[\alpha]_D^{23}$ = +53 (*c* = 1.01, $CHCl_3$)}), 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**7**; $[\alpha]_D^{26}$ = +47.4 (*c* = 4.2, $CHCl_3$), {ref.[25] $[\alpha]_D$ = +48 (*c* = 4.2, $CHCl_3$)}), and 2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl trichloroacetimidate (**9**; $[\alpha]_D^{21}$ = +25.5 (*c* = 1.0, $CHCl_3$), {ref.[26] $[\alpha]_D^{20}$ = +20.0 (*c* = 1.0, $CHCl_3$)}).

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-4,6-*O*-benzylidene-α-D-mannopyranoside (3): A solution of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 (0.8 M, 1.0 mL, 0.80 mmol) was added dropwise at −10 °C to a mixture of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**; 1.00 g, 3.54 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**2**; 1.95 g, 3.96 mmol) in CH_2Cl_2 (10 mL) containing 4-Å molecular sieves (2.5 g). After stirring at −10 °C for 2 h, triethylamine was added to the reaction mixture, which was then extracted with CH_2Cl_2. Flash column chromatography (CH_2Cl_2/acetone, 23:2 → 9:1) gave the title compound **3** (1.21 g, 56%) as a syrup, which was crystallized from EtOAc/hexane. M.p. 219−220 °C. $[\alpha]_D^{22}$ = +60 (*c* = 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$; data for the ring and the exocyclic protons are listed in Table 1): δ = 1.99 (s, 3 H, COC*H*$_3$), 2.05 (s, 3 H, COC*H*$_3$), 2.09 (s, 3 H, COC*H*$_3$), 2.10 (s, 3 H, COC*H*$_3$), 3.39 (s, 3 H, OC*H*$_3$), 5.59 (s, 1 H, C*H*-Ph), 7.41−7.43 (m, 5 H, aromatic *H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$; data for the skeletal carbon atoms are listed in Table 2): δ = 20.56 (CO*C*H$_3$), 20.66 (CO*C*H$_3$), 20.71 (CO*C*H$_3$), 54.9 (O*C*H$_3$), 101.4 (*C*H-Ph), 1256.0, 128.1, 128.8, 137.2 (aromatic *C*), 169.65 (*C*OCH$_3$), 169.70 (*C*OCH$_3$), 170.1 (*C*OCH$_3$), 170.7 (*C*OCH$_3$) ppm. $C_{28}H_{36}O_{15}$ (612.58): calcd. C 54.90, H 5.92; found C 54.66, H 5.85.

A minor product (200 mg) was found to be a mixture of the following products by 2D NMR spectroscopy: methyl (2,3,4,6-tetra-*O*-acetyl-D-mannopyranosyl)-(1→2)-4,6-*O*-benzylidene-α-D-mannopyranoside and methyl (2,3,4,6-tetra-*O*-acetyl-D-mannopyranosyl)-(1→2)-[2,3,4,6-tetra-*O*-acetyl-D-mannopyranosyl-(1→3)]-4,6-*O*-benzylidene-α-D-mannopyranoside (2.3% and 4.6%, respectively). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.93−2.15 (each s, COC*H*$_3$), 7.32−7.50 (m, aromatic *H*) ppm; ManII-(1→2)-ManI: δ = 3.39 (s, 3 H, OC*H*$_3$), 3.78 (m, 1 H, H-5^I), 3.90 (m, 1 H, H-4^I), 4.03 (m, 1 H, H-2^I), 4.10 (m, 1 H, H-5^{II}), 4.13 (m, 1 H, H-3^I), 4.15 (m, 1 H, H-6^{II}a), 4.23 (m, 1 H, H-6^{II}b), 4.26 (m, 1 H, H-6^{I}a), 4.79 (d, $^3J_{1^I,2^I}$ = 1.0 Hz, 1 H, H-1^I), 5.13 (d, $^3J_{1^{II},2^{II}}$ = 2.0 Hz, 1 H, H-1^{II}), 5.25 (m, 1 H, H-4^{II}), 5.38 (dd, $^3J_{2^{II},3^{II}}$ = 3.4 Hz, $^3J_{3^{II},4^{II}}$ = 10.3 Hz, 1 H, H-3^{II}), 5.43 (m, 1 H, H-2^{II}), 5.63 (s, 1 H, C*H*-Ph) ppm; {ManII-(1→2)-[ManIII-(1→3)]-ManI}: δ = 3.38 (s, 3 H, OC*H*$_3$), 3.77 (m, 1 H, H-5^I), 3.89 (m, 1 H, H-6^{I}a), 3.95 (m, 1 H, H-5^{III}), 4.04 (m, 1 H, H-2^I), 4.08 (m, 1 H, H-5^{II}), 4.09 (m, 1 H, H-6^{III}a), 4.15 (m, 1 H, H-6^{II}a), 4.17 (m, 1 H, H-4^I), 4.25 (m, 1 H, H-6^{III}b), 4.26 (m, 2 H, H-3, H-6^{I}b), 4.27 (m, 1 H, H-6^{II}b), 4.74 (d, $^3J_{1^I,2^I}$ = 1.5 Hz, 1 H, H-1^I), 5.18 (m, 1 H, H-4^{III}), 5.19 (s, 1 H, H-1^{II}), 5.21 (m, 1 H, H-3^{III}), 5.27 (d, $^3J_{1^{III},2^{III}}$ = 2.0 Hz, 1 H, H-1^{III}), 5.29 (m, 1 H, H-4^{II}), 5.32 (dd, $^3J_{2^{III},3^{III}}$ = 2.9 Hz, 1 H, H-2^{III}), 5.42 (m, 1 H, H-2^{II}), 5.43 (m, 1 H, H-3^{II}), 5.66 (s, 1 H, C*H*-Ph) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 20.6−20.8 (CO*C*H$_3$), 126.0−137.2 (aromatic *C*), 169.3−170.6 (*C*OCH$_3$) ppm; ManII-(1→2)-ManI: δ = 55.0 (O*C*H$_3$), 62.7 (C-6^{II}), 63.4 (C-5^I), 66.4 (C-4^{II}), 68.51 (C-6^I), 68.7 (C-3^I), 69.02 (C-5^{II}), 69.04 (C-3^{II}), 69.17 (C-2^{II}), 78.3 (C-2^I), 78.9 (C-4^I), 99.8 (C-1^{II}), 100.5 (C-1^I), 102.1 (*C*H-Ph) ppm; {ManII-(1→2)-[ManIII-(1→3)]-ManI}: δ = 54.8 (O*C*H$_3$), 62.5 (C-6^{III}), 62.8 (C-6^{II}), 63.8 (C-5^I), 66.3 (C-4^{III}), 66.5 (C-4^{II}), 68.3 (2 C, C-3^{III}, C-6^I), 68.51 (C-3^{II}), 69.02 (C-5^{III}), 69.04 (C-5^{II}), 69.15 (C-2^{II}), 69.3 (C-2^{III}), 77.1 (C-3^I), 77.3 (C-2^I), 78.8 (C-4^I), 97.4 (C-1^{II}), 98.2 (C-1^{III}), 100.5 (C-1^I), 101.3 (*C*H-Ph) ppm.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (4): A solution of **3** (2.74 g, 4.47 mmol) in pyridine/Ac_2O (3:1, v/v, 4 mL) was stirred at room temp. for 16 h. The solution was quenched with water (20 mL), stirred for 1 h, and then extracted with CH_2Cl_2 (2 ×

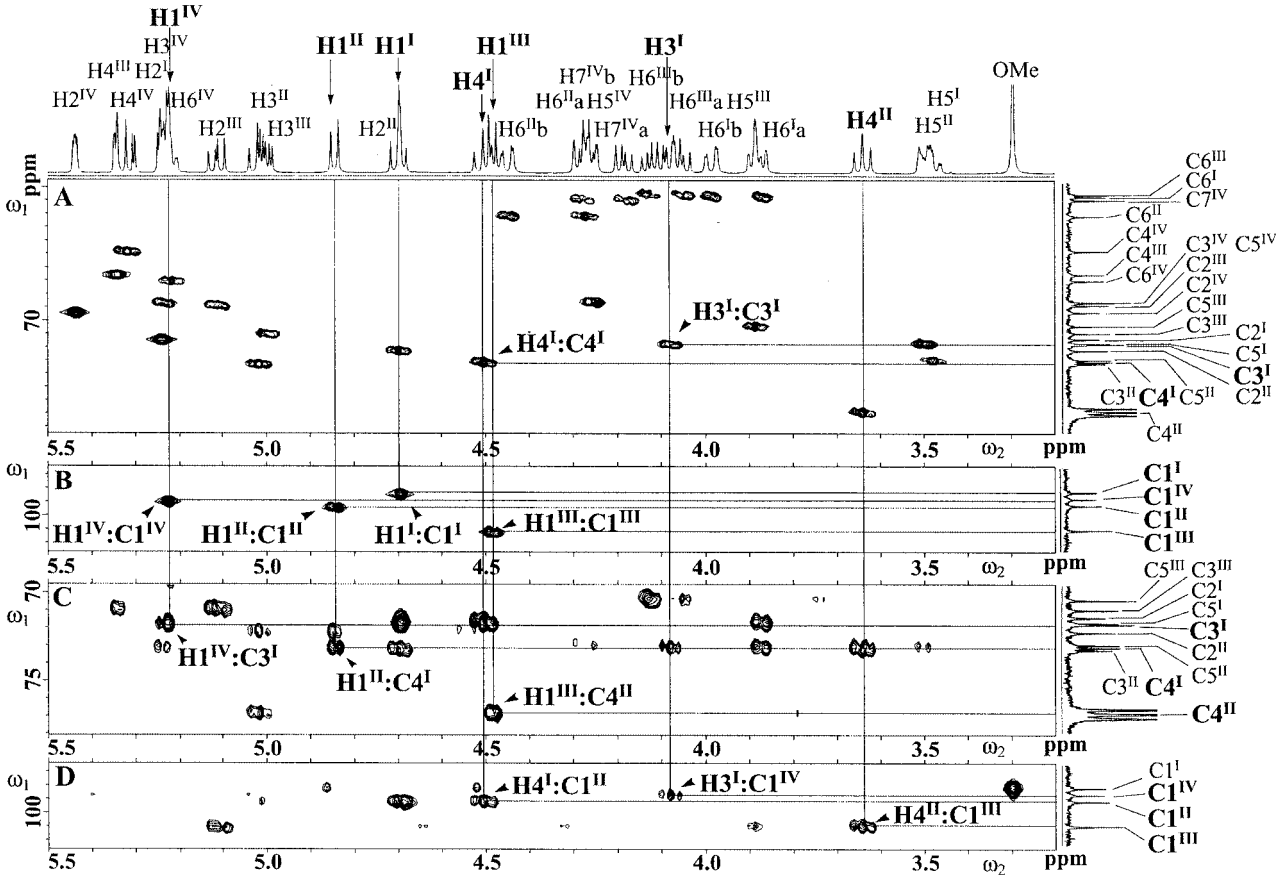


Figure 2. Partial HMQC (panels A and B) and HMBC (panels C and D) spectra of compound **14** recorded in $CDCl_3$ at 25 °C; only partial $^{13}C/^{1}H$ cross-peaks (A and B) and cross-relay peaks (C and D) are labeled; each Roman numeral in panels A−D corresponds to that of **14** as shown in Scheme 2

30 mL). The extracts were washed with aqueous saturated $NaHCO_3$ and water, dried ($MgSO_4$), and filtered and then the filtrate was concentrated. The residual syrup was purified by flash column chromatography (CH_2Cl_2/acetone, 10:1) to give the title compound **4** (2.90 g, 99%) as a white solid, which was crystallized from EtOAc/hexane. M.p. 176−177 °C. $[\alpha]_D^{23}$ = +51.1 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 1): δ = 1.97−2.24 (5 s, 15 H, 5 COC*H*$_3$), 3.39 (s, 3 H, OC*H*$_3$), 5.61 (s, 1 H, C*H*-Ph), 7.31−7.42 (m, 15 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 2): δ = 20.5 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 20.71 (CO*C*H$_3$), 20.73 (2 C, 2 CO*C*H$_3$), 55.0 (O*C*H$_3$), 101.3 (*C*H-Ph), 125.9−137.0 (aromatic *C*), 169.7 (*C*OCH$_3$), 169.8 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 170.4 (*C*OCH$_3$), 170.6 (*C*OCH$_3$) ppm. $C_{30}H_{38}O_{16}$ (654.62): calcd. C 55.04, H 5.85; found C 55.02, H 6.00.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-α-D-mannopyranoside (5): A solution of compound **4** (2.80 g, 4.27 mmol) in aqueous 70% AcOH (AcOH/H_2O, 7:3, v/v, 80 mL) was stirred at 60−70 °C for 4 h. The reaction mixture was concentrated by co-evaporating with toluene and MeOH. The residual oil was diluted with EtOAc (100 mL), washed with aqueous saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/acetone, 4:1) to give the title compound **5** (2.06 g, 93%) as a foam. $[\alpha]_D^{23}$ = +58.3 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 1): δ = 1.99−2.17 (5 s, 15 H, 5 COC*H*$_3$), 3.37 (s, 3 H, OC*H*$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 2): δ = 20.6−20.8 (5 C, CO*C*H$_3$), 55.0 (O*C*H$_3$), 169.8 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 170.3 (*C*OCH$_3$), 170.4 (*C*OCH$_3$), 170.7 (*C*OCH$_3$) ppm. $C_{23}H_{34}O_{16}$ (566.51): calcd. C 48.76, H 6.05; found C 48.58, H 6.06.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranoside (6): A solution of *tert*-butyldiphenylsilyl chloride (1.20 g, 4.35 mmol) in DMF (4 mL) was added at 0 °C to a mixture of compound **5** (2.05 g, 3.63 mmol) and imidazole (0.54 g, 7.98 mmol) in DMF (8 mL). The mixture was stirred at 0 °C for 2 h and then at room temp. for 18 h. The mixture was quenched with water (50 mL) and extracted with EtOAc (4 × 50 mL). Purification by flash column chromatography (hexane/EtOAc, 2:1 → 3:2) gave the title compound **6** (2.71 g, 93%) as a foam. $[\alpha]_D^{23}$ = +39.3 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 1): δ = 1.10 [s, 9 H, C(C*H*$_3$)$_3$], 1.99−2.16 (5 s, 15 H, 5 COC*H*$_3$), 2.78 (d, $^3J_{4\text{-OH,H-4}}$ = 2.0 Hz, 1 H, 4-O*H*), 3.30 (s, 3 H, OC*H*$_3$), 7.39−7.73 (m, 10 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 2): δ = 19.1 [*C*(CH$_3$)$_3$], 20.49 (CO*C*H$_3$), 20.58 (CO*C*H$_3$), 20.67 (CO*C*H$_3$), 20.74 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 26.8 [C(*C*H$_3$)$_3$], 54.7 (O*C*H$_3$), 127.7−135.6 (aromatic *C*), 169.8 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 170.0 (*C*OCH$_3$), 170.4 (*C*OCH$_3$), 170.6 (*C*OCH$_3$) ppm. $C_{39}H_{52}O_{16}$ (804.92): calcd. C 58.20, H 6.51; found C 57.96, H 6.48.

Table 5. ^{1}H NMR spectroscopic (500 MHz) data for compounds **17**−**20**

Compound[a]	Residue[b]	H-1 ($^3J_{1,2}$)	H-2 ($^3J_{2,3}$)	H-3 ($^3J_{3,4}$)	H-4 ($^3J_{4,5}$)	H-5 ($^3J_{5,6a}$)	H-6a ($^3J_{5,6b}$)	H-6b ($^2J_{6a,6b}$)	H-7a ($^3J_{6,7a}$)	H-7b ($^3J_{6,7b}$)	($^2J_{7a,7b}$)	H-8a ($^3J_{7,8a}$)	H-8b ($^3J_{7,8b}$)	($^2J_{8a,8b}$)
17	Oct	4.46	3.68	4.13	4.37	3.35	4.55	−	5.63	−	−	4.96	4.98	
		(1.5)	(3.0)	(9.5)	(10.0)	n.d.[c]	−	−	(5.0)	−	−	(17.0)	(11.0)	n.d.
	Hep	5.58	4.30	3.92	4.24	3.77	4.08	−	3.68	3.79				
		n.d.	(3.0)	(9.5)	(9.0)	n.d.	−	−	(6.0)	n.d.	(9.3)			
	Glc	4.62	3.23	3.60	3.93	3.46	3.72	3.80						
		(8.0)	(9.0)	(9.0)	(9.0)	n.d.	n.d.	n.d.						
	Gal	4.27	3.69	3.23	3.87	3.21	3.28	3.49						
		(7.5)	n.d.	(3.0)	n.d.	(4.5)	(9.0)	(9.0)						
18	Oct	4.58	3.69	3.92	4.22	3.46	5.67	−	5.70	−	−	4.96	5.05	
		(1.5)	(3.0)	(9.5)	(9.5)	n.d.	−	−	−	−	−	(16.5)	(10.0)	(1.5)
	Hep	5.50	4.27	4.09	4.24	3.77	4.06	−	3.64	3.77				
		(<1.0)	(3.5)	(9.5)	(9.5)	n.d.	−	−	(6.0)	n.d.	(9.5)			
	Glc	4.33	3.23	3.60	3.78	3.31	3.23	3.63						
		(7.5)	(8.5)	(9.0)	(9.3)	(5.0)	n.d.	(12.0)						
	Gal	4.29	3.72	3.31	3.89	3.25	3.24	3.48						
		(7.5)	(10.0)	(2.5)	n.d.	(5.0)	(10.0)	(10.5)						
19	HepI	4.44	3.70	4.12	4.35	3.42	4.04	−	3.25	3.39				
		(<1.0)	(2.5)	(10.0)	(10.0)	n.d.	−	−	n.d.	(5.0)	(11.5)			
	HepII	5.58	4.30	3.92	4.24	3.78	4.08	−	3.67	3.78				
		(<1.0)	n.d.	(9.0)	(9.0)	n.d.	−	−	(7.0)	n.d.	(9.5)			
	Glc	4.59	3.24	3.61	3.91	3.45	3.71	3.80						
		(8.0)	(9.0)	(9.0)	(9.0)	(4.5)	n.d.	(10.5)						
	Gal	4.28	3.71	3.25	3.87	3.21	3.32	3.51						
		(8.0)	n.d.	(2.5)	n.d.	(4.5)	(9.0)	(9.0)						
20	HepI	4.68	5.20	4.02	3.99	3.76	5.27	−	4.19	4.32				
		(1.5)	(3.5)	(9.5)	(9.5)	n.d.	−	−	(3.0)	(6.0)	(11.0)			
	HepII	5.30	5.42	5.14	5.30	4.21	5.21	−	4.27	4.17				
		(1.5)	(3.5)	(10.0)	(10.0)	n.d.	−	−	n.d.	n.d.	n.d.			
	Glc	4.38	4.66	5.03	3.71	3.54	4.44	4.25						
		(8.0)	(9.5)	(9.5)	(9.5)	(2.5)	(3.0)	(11.5)						
	Gal	4.49	5.10	4.99	5.32	3.89	4.03	4.13						
		(8.0)	(10.0)	(3.5)	n.d.	n.d.	n.d.	n.d.						

[a] Data were acquired in $CDCl_3$ at 25 °C. The ^{1}H NMR spectroscopic chemical shifts (ppm) were determined by analyzing 2D NMR spectroscopic data (DQF-COSY, HMQC and HMBC) comparatively, and the *J* couplings (Hz) were obtained by analyzing either the DQF-COSY or 1D NMR spectra. The ^{1}H NMR spectroscopic chemical shifts of other protons are listed in the Exp. Sect. [b] The oct-7-enopyranoside residue is expressed as Oct. The two heptoses are designated as HepI and HepII, as described previously.[8] [c] n.d.: not determined.

Methyl (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)]-2-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranoside (8): A solution of 10% $BF_3{\cdot}OEt_2$ in CH_2Cl_2 (0.65 mmol, 1.64 mL) was added dropwise at −10 °C to a mixture of **6** (2.60 g, 3.23 mmol) and the donor **7** (5.04 g 6.45 mmol) in CH_2Cl_2 (32 mL) containing 4-Å molecular sieves (3 g). After stirring at −10 °C for 2 h, the reaction mixture was treated with triethylamine (0.2 mL) and then extracted with CH_2Cl_2 (50 mL). Purification by flash column chromatography (CH_2Cl_2/acetone, 19:1 → 23:2 → 10:1), followed by crystallization from CH_2Cl_2/hexane, gave the title compound **8** (4.27 g, 80%). M.p. 114−115 °C. $[\alpha]_D^{23}$ = +40 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 1): δ = 1.11 [s, 9 H, C(CH_3)$_3$] 1.88, 1.97, 1.98, 2.04, 2.06, 2.06, 2.07, 2.11, 2.15, 2.18, 2.21 (s, 36 H, 12 COCH_3), 3.30 (s, 3 H, OCH_3), 7.39−7.80 (m, 10 H, aromatic *H*) ppm. ^{13}C NMR (150 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 2): δ = 19.4 [*C*(CH$_3$)$_3$], 20.5−21.1 (CO*C*H$_3$), 26.6 [C(*C*H$_3$)$_3$], 54.7 (O*C*H$_3$) 127.5, 128.2, 129.8, 130.3, 132.1, 133.4, 135.2, 136.1 (aromatic *C*), 169.3 (*C*OCH$_3$), 169.6 (*C*OCH$_3$), 169.7 (*C*OCH$_3$), 169.8 (*C*OCH$_3$), 170.07 (*C*OCH$_3$), 170.14 (*C*OCH$_3$), 170.3 (*C*OCH$_3$), 170.4 (*C*OCH$_3$), 170.7 (*C*OCH$_3$) ppm. $C_{65}H_{86}O_{33}Si$ (1423.44): calcd. C 54.85, H 6.09; found C 54.66, H 5.85.

Methyl (2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-4,6-*O*-benzylidene-α-D-mannopyranoside (10): A solution of 10% $BF_3{\cdot}OEt_2$ in CH_2Cl_2 (400 μL, 0.16 mmol) was added at −10 °C to a stirred mixture of compound **9** (890 mg, 1.58 mmol), **1** (404 mg, 1.43 mmol), and 4-Å molecular sieves (1 g) in dry CH_2Cl_2 (8 mL). After stirring at −10 °C for 2 h, the mixture was quenched with triethylamine (3 drops) and then extracted with CH_2Cl_2 (50 mL). After purifying twice by flash column chromatography (CH_2Cl_2/acetone, 10:1, toluene/acetone, 3:1), the title compound **10** was obtained (685 mg, 70%) together with the unreacted acceptor **1** (20 mg, 5%). Although a mixture (130 mg) of two additional products, presumably Hep-(1→2)-Man and Hep-(1→2)-[Hep-(1→3)]-Man was obtained, their structural characterization was not carried out any further. **Compound 10:** $[\alpha]_D^{24}$ = +45.4 (*c* = 0.50, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 3): δ = 1.98 (s, 3 H, COCH_3), 2.02 (s, 3 H, COCH_3), 2.06 (s, 3 H, COCH_3), 2.09 (s, 3 H, COCH_3), 2.13, (s, 3 H, COCH_3), 3.38 (s, 3 H, OCH_3), 5.59 (s, 1 H, C*H*-Ph), 7.27−7.41 (m, 5 H, aromatic *H*) ppm. ^{13}C NMR

Table 6. ^{13}C NMR spectroscopic (125 MHz) data for compounds **17**−**20**

Compound[a]	Residue[b]	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
17	Oct	99.5	77.0	73.4	75.1	73.9	69.0	137.9	115.2
	Hep	98.9	75.5	79.9	75.0	71.8	75.0	69.8	
	Glc	103.4	82.4	83.1	76.6	75.0	68.8		
	Gal	102.4	79.8	82.1	73.5	72.5	67.8		
18	Oct	99.2	75.5	73.9	74.4	73.4	72.2	133.1	118.4
	Hep	99.2	76.4	80.1	74.6	71.9	75.1	69.8	
	Glc	102.8	82.5	83.1	77.0	75.4	68.9		
	Gal	102.6	79.8	82.5	73.3	72.6	67.8		
19	HepI	99.7	76.8	73.4	75.1	72.9	64.4	67.2	
	HepII	98.9	75.4	79.9	74.4	71.8	75.0	69.8	
	Glc	103.4	82.3	83.1	76.6	74.9	68.9		
	Gal	102.4	79.8	82.2	73.4	72.6	67.8		
20	HepI	98.7	71.0	72.3	72.0	69.2	68.0	62.0	
	HepII	99.4	69.3	69.0	64.8	69.1	67.2	61.4	
	Glc	99.2	72.2	73.4	77.3	73.1	62.6		
	Gal	101.4	69.1	71.2	66.7	70.6	60.8		

[a] Data were acquired in $CDCl_3$ at 25 °C. The ^{13}C NMR spectroscopic chemical shifts (ppm) were determined by analyzing 2D NMR spectroscopic data (DQF-COSY, HMQC and HMBC) comparatively. Only the data for the skeletal carbon atoms are presented, and those for other carbon atoms are listed in the Exp. Sect. [b] The octenopyranoside residue is expressed as Oct. The two heptoses are designated as HepI and HepII, as described previously.[8]

(125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 4): δ = 20.6 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 54.9 (O*C*H$_3$), 101.4 (*C*H-Ph), 126.0, 128.1, 128.9, 137.2 (aromatic *C*), 169.5 (*C*OCH$_3$), 169.7 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 170.3 (*C*OCH$_3$), 170.4 (*C*OCH$_3$) ppm. $C_{31}H_{40}O_{17}$ (684.64): calcd. C 54.38, H 5.89; found C 54.55, H 6.15.

Methyl (2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (11): Compound **10** (600 mg, 0.876 mmol) was acetylated with pyridine/Ac_2O (3:1, v/v, 2 mL) in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) over 16 h. Usual workup and subsequent purification by flash column chromatography (CH_2Cl_2/acetone, 15:1) gave the title compound **11** (569 mg, 89%). M.p. 190−193 °C (recrystallized from hexane/EtOAc). $[\alpha]_D^{24}$ = +23.9 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 3): δ = 1.96 (s, 3 H, COC*H*$_3$), 2.05 (s, 3 H, COC*H*$_3$), 2.09 (s, 3 H, COC*H*$_3$), 2.09 (s, 3 H, COC*H*$_3$), 2.17 (s, 3 H, COC*H*$_3$), 2.25 (s, 3 H, COC*H*$_3$), 3.39 (s, 3 H, OC*H*$_3$), 5.60 (s, 1 H, C*H*-Ph), 7.30−7.40 (m, 5 H, aromatic *H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 4): δ = 20.57 (CO*C*H$_3$), 20.62 (CO*C*H$_3$), 20.67 (CO*C*H$_3$), 20.71 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 54.9 (O*C*H$_3$), 101.3 (*C*H-Ph), 125.8, 127.9, 128.8, 136.8 (aromatic *C*), 169.51 (*C*OCH$_3$), 169.54 (*C*OCH$_3$), 169.7 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 170.1 (*C*OCH$_3$), 170.2 (*C*OCH$_3$) ppm. $C_{33}H_{42}O_{18}$ (726.68): calcd. C 54.54, H 5.83; found C 54.44, H 5.82.

Methyl (2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-2-*O*-acetyl-α-D-mannopyranoside (12): A mixture of compound **11** (460 mg, 0.633 mmol) in 70% AcOH (3.5 mL) was stirred at 70 °C for 4 h. After cooling down, the mixture was concentrated to a syrup, which was purified by flash column chromatography (CH_2Cl_2/acetone, 2:1) to give the title compound **12** (355 mg, 88%). M.p. 194−195 °C (recrystallized from hexane/EtOAc). $[\alpha]_D^{24}$ = +45.3 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 3): δ = 1.98 (s, 3 H, COC*H*$_3$), 2.02 (s, 3 H, COC*H*$_3$), 2.06 (s, 3 H, COC*H*$_3$), 2.11 (s, 3 H, COC*H*$_3$), 2.14 (s, 3 H, COC*H*$_3$), 2.17 (s, 3 H, COC*H*$_3$), 2.66 (br. s, 1 H, O*H*), 3.37 (s, 3 H, OC*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 4): δ = 20.5 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 20.61 (CO*C*H$_3$), 20.62 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 54.8 (O*C*H$_3$), 169.5 (*C*OCH$_3$), 169.96 (*C*OCH$_3$), 170.00 (*C*OCH$_3$), 170.1 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 170.3 (*C*OCH$_3$) ppm. $C_{26}H_{38}O_{18}$ (638.57): calcd. C 48.90, H 6.00; found C 48.64, H 5.95.

Methyl (2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-2-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranoside (13): Compound **12** (250 mg, 0.391 mmol) was treated with *tert*-butyldiphenylsilyl chloride (122 μL, 0.469 mmol) in dry DMF (2 mL) in the presence of imidazole (67 mg, 0.978 mmol) for 20 h at room temp. After terminating the reaction by adding water (3 mL), the mixture was extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated to a residue that was purified by flash column chromatography (hexane/EtOAc, 2:1 → 1:1). The title compound **13** was obtained as a syrup (297 mg, 87%). $[\alpha]_D^{24}$ = +34.3 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 3): δ = 1.09 [s, 9 H, C(C*H*$_3$)$_3$], 1.98 (s, 3 H, COC*H*$_3$), 2.05 (s, 3 H, COC*H*$_3$), 2.07 (s, 3 H, COC*H*$_3$), 2.14 (s, 3 H, COC*H*$_3$), 2.17 (s, 3 H, COC*H*$_3$), 2.17 (s, 3 H, COC*H*$_3$), 2.59 (d, $^3J_{4\text{-OH,H-4}}$ = 3.5 Hz, 1 H, 4-O*H*), 3.28 (s, 3 H, OC*H*$_3$), 7.40−7.72 (m, 10 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 4): δ = 19.1 [*C*(CH$_3$)$_3$], 20.56 (CO*C*H$_3$), 20.63 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 26.8 [C(*C*H$_3$)$_3$], 54.8 (O*C*H$_3$), 127.8, 127.90, 129.93, 130.1, 132.6, 132.9, 135.5, 135.6 (aromatic *C*), 169.8 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 170.0 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 170.4 (*C*OCH$_3$), 170.6 (*C*OCH$_3$) ppm. $C_{42}H_{56}O_{18}Si$ (876.97): calcd. C 57.52, H 6.44; found C 57.30, H 6.45.

Methyl (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-[2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)]-2-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranoside (14): A solution of 10% $BF_3{\cdot}OEt_2$ in CH_2Cl_2 (250 μL, 0.10 mmol) was added to a stirred mixture of **13** (285 mg, 0.325 mmol), **7** (761 mg, 0.975 mmol), and 4-Å molecular sieves (1 g) in dry CH_2Cl_2 (7 mL) at −10 °C. After stirring for 3 h at −10 °C and then for 30 min at room temp., the reaction mixture was quenched with triethylamine (4 drops) and then diluted with CH_2Cl_2 (40 mL). The mixture was worked up as described for compound **8** and purified by flash column chromatography (CH_2Cl_2/acetone, 15:1 → 10:1) to give the title compound **14** (288 mg, 59%) and the unreacted acceptor **13** (114 mg, 40%). M.p. 186−188 °C (crystallized from EtOH). $[\alpha]_D^{20}$ = +35.6 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 3): δ = 1.11 [s, 9 H, C(C*H*$_3$)$_3$], 1.89 (s, 3 H, COC*H*$_3$), 1.97 (s, 3 H, COC*H*$_3$), 1.97 (s, 3 H, COC*H*$_3$), 2.04 (s, 3 H, COC*H*$_3$), 2.05 (s, 3 H, COC*H*$_3$), 2.06 (s, 3 H, COC*H*$_3$), 2.06 (s, 3 H, COC*H*$_3$), 2.06 (s, 3 H, COC*H*$_3$), 2.09 (s, 3 H, COC*H*$_3$), 2.15 (s, 3 H, COC*H*$_3$), 2.15 (s, 3 H, COC*H*$_3$), 2.18 (s, 3 H, COC*H*$_3$), 2.23 (s, 3 H, COC*H*$_3$), 3.30 (s, 1 H, OC*H*$_3$), 7.39−7.79 (m, 5 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 4): δ = 19.5 [*C*(CH$_3$)$_3$], 20.5 (CO*C*H$_3$), 20.52 (CO*C*H$_3$), 20.57 (CO*C*H$_3$), 20.62 (CO*C*H$_3$), 20.66 (CO*C*H$_3$), 20.71 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 21.2 (CO*C*H$_3$), 26.7 [C(*C*H$_3$)$_3$], 54.9

(OCH_3), 127.5, 128.1, 129.8, 130.2, 131.9, 133.3, 135.0, 136.0 (aromatic *C*), 169.05 ($COCH_3$), 169.1 ($COCH_3$), 169.47 ($COCH_3$), 169.53 ($COCH_3$), 169.65 ($COCH_3$), 169.70 ($COCH_3$), 169.91 ($COCH_3$), 169.96 ($COCH_3$), 169.98 ($COCH_3$), 170.10 ($COCH_3$), 170.11 ($COCH_3$), 170.17 ($COCH_3$) ppm. $C_{65}H_{86}O_{33}Si$ (1423.44): calcd. C 54.58, H 6.13; found C 54.87, H 6.33.

Methyl (2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-[2,3,4,6,7-penta-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)]-2-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranoside (15): Compound **14** (346 mg, 0.231 mmol) was treated with a mixture of MeOH/triethylamine/H_2O (2:1:1, v/v/v, 8 mL) for 24 h at room temp. The reaction mixture was concentrated, by co-evaporating with toluene and MeOH, to a residue that was dried overnight in vacuo over P_2O_5. The dried residue was benzylated with benzyl bromide (427 μL, 3.60 mmol) and NaH (60% in paraffin liquid, 240 mg, 6.01 mmol) in dry DMF (8 mL) in the presence of 4-Å molecular sieves (0.2 g) for 30 min at 0 °C and then for 7 h at room temp. The reaction was quenched with water (10 mL) and then filtered through Celite. The filtrate was extracted with Et_2O (4 × 20 mL) and then the combined extracts were washed with brine (4 × 20 mL) and dried. Purification by flash column chromatography (hexane/EtOAc, 10:1 → 5:1) gave the title compound **15** as syrup (338 mg, 69%). $[\alpha]_D^{24} = +19.6$ (c = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 3): δ = 0.98 [s, 9 H, $C(CH_3)_3$], 3.20 (s, 3 H, OCH_3), 4.21, 4.29 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.22, 4.39 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.34, 4.44 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.34, 4.87 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.46, 4.53 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.50, 4.74 (d, ^2J = 10.0 Hz, 1 H each, CH_2-Ph), 4.51, 4.82 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.51, 4.95 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.62−4.72 (m, 4 H, 2 CH_2-Ph), 4.62, 4.74 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.70, 5.01 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.75 (s, 2 H, CH_2-Ph), 7.03−7.34 (m, 71 H, aromatic *H*), 7.71−7.75 (m, 4 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 4): δ = 19.4 [$C(CH_3)_3$], 26.6 [$C(CH_3)_3$], 54.5 (OCH_3), 71.4 (CH_2-Ph), 71.8 (CH_2-Ph), 72.48 (CH_2-Ph), 72.48 (CH_2-Ph), 72.50 (CH_2-Ph), 72.7 (CH_2-Ph), 72.8 (CH_2-Ph), 73.0 (CH_2-Ph), 73.3 (CH_2-Ph), 74.7 (CH_2-Ph), 75.1 (CH_2-Ph), 75.2 (CH_2-Ph), 75.4 (CH_2-Ph), 126.6−139.7 (aromatic *C*) ppm. $C_{133}H_{142}O_{22}Si$ (2120.62): calcd. C 54.38, H 5.89; found C 54.55, H 6.15.

Methyl (2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-[2,3,4,6,7-penta-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)]-2-*O*-benzyl-α-D-mannopyranoside (16): Compound **15** (205 mg, 0.097 mmol) was treated with tetrabutylammonium fluoride (1 M in THF, 1.5 mL, 1.5 mmol) in dry THF (5 mL) for 2 days at room temp. and then at 60 °C for 14 h. The reaction mixture was diluted with water (5 mL) and then extracted with Et_2O (5 × 10 mL). The combined extracts were washed with brine and dried ($MgSO_4$). Flash column chromatography (hexane/EtOAc, 2:1) gave the title compound **16** as a syrup (162 mg, 89%). $[\alpha]_D^{24} = +19.6$ (c = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 3): δ = 2.00 (br. s, 1 H, 6-O*H*), 3.23 (s, 3 H, OCH_3), 4.22, 4.30 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.23, 4.44 (d, ^2J = 11.5 Hz, 2 H, 1 H each, CH_2-Ph), 4.36, 4.88 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.44, 4.52 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.44−4.52 (m, 2 H, CH_2-Ph), 4.48, 4.80 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.50, 4.68 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.52, 4.93 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.63−4.77 (m, 2 H, CH_2-Ph), 4.63, 5.04 (d, ^2J = 10.5 Hz, 1 H each, CH_2-Ph), 4.67−4.72 (m, 4 H, 2 CH_2-Ph), 4.74 (s, 2 H, CH_2-Ph), 7.03−7.31 (m, 65 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 4): δ = 54.6 (OCH_3), 71.7 (CH_2-Ph), 72.35 (CH_2-Ph), 72.40 (CH_2-Ph), 72.42 (CH_2-Ph), 72.8 (CH_2-Ph), 72.9 (CH_2-Ph), 73.1 (CH_2-Ph), 73.3 (CH_2-Ph), 74.4 (CH_2-Ph), 74.6 (CH_2-Ph), 75.2 (CH_2-Ph), 75.3 (CH_2-Ph), 75.4 (CH_2-Ph), 137.9−139.5, 126.6−128.3 (aromatic *C*) ppm. $C_{117}H_{124}O_{22}$ (1882.22): calcd. C 54.38, H 5.89; found C 54.55, H 6.15.

Methyl (2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-[2,3,4,6,7-penta-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)]-2-*O*-benzyl-7,8-dideoxy-α-D-*manno*-oct-7-enopyranoside (17): Dimethyl sulfoxide (197 μL, 2.78 mmol) in THF (3 mL) was added to a solution of oxalyl chloride (121 μL, 1.39 mmol) in THF (1 mL) at −60 °C. After stirring the mixture for 20 min, a solution of **16** (262 mg, 0.139 mmol) in dry THF (5 mL) was added at −60 °C. The reaction mixture was stirred for 1 h at −60 °C and then for 2.5 h at −50 °C. After adding triethylamine (0.78 mL, 5.56 mmol), the mixture was warmed to room temp. and stirred for 30 min. The mixture was then cooled to −70 °C and vinylmagnesium bromide (1 M in THF, 1.39 mL, 1.39 mmol) was added. The mixture was stirred for 2 h at −70 °C and then for 1 h at −50 °C. The reaction was terminated by the addition of EtOH (2 mL) and, after adding saturated aqueous NH_4Cl (10 mL), the mixture was warmed to room temp. The mixture was extracted with Et_2O (3 × 20 mL), and the combined extracts were washed sequentially with 5% sodium hypochlorite in water (2 × 20 mL) and brine (2 × 20 mL) and then dried ($MgSO_4$). Flash column chromatography (hexane/EtOAc, 4:1 → 3:1) gave the title compound **17** as a syrup (207 mg, 78%). $[\alpha]_D^{20} = +21.9$ (c = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 5): δ = 3.16 (s, 3 H, OCH_3), 4.31, 4.22 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.42, 4.23 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.52, 4.43 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.67, 4.49 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.39−4.77 (m, 10 H, 5 CH_2-Ph), 4.80, 4.49 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.88, 4.34 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.93, 4.52 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 5.06, 4.67 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 7.03−7.31 (m, 65 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 6): δ = 54.5 (OCH_3), 71.4 (CH_2-Ph), 71.8 (CH_2-Ph), 72.38 (CH_2-Ph), 72.42 (CH_2-Ph), 72.8 (CH_2-Ph), 72.9 (CH_2-Ph), 73.1 (CH_2-Ph), 73.3 (CH_2-Ph), 74.4 (CH_2-Ph), 74.6 (CH_2-Ph), 75.1 (CH_2-Ph), 75.30 (CH_2-Ph), 75.34 (CH_2-Ph), 126.6−128.3, 137.8−139.6 (aromatic *C*) ppm. $C_{119}H_{126}O_{22}$ (1908.26): calcd. C 74.90, H 6.66; found C 74.64, H 6.59.

Methyl (2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-[2,3,4,6,7-penta-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)]-6-*O*-acetyl-2-*O*-benzyl-7,8-dideoxy-α-D-*manno*-oct-7-enopyranoside (18): Compound **17** (454 mg, 0.24 mmol) was treated with pyridine/Ac_2O (2:1, v/v, 3 mL) and a catalytic amount of DMAP for 15 h at room temp. The mixture was co-evaporated with toluene to give a residue that was diluted with CH_2Cl_2 (10 mL) and washed with water (5 mL). The aqueous solution was extracted with CH_2Cl_2 (2 × 5 mL). The combined extracts were washed sequentially with 10% $NaHCO_3$ (5 mL) and water (5 mL), and then dried ($MgSO_4$). Purification by flash column chromatography (hexane/EtOAc, 7:3) gave the title compound **18** (380 mg, 82%) as a syrup. $[\alpha]_D^{25} = +30$ (c = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 5): δ = 2.03 (s, 3 H, $COCH_3$), 3.21 (s, 3 H, OCH_3), 4.18, 4.27 (d, ^2J = 12.0 Hz, 1 H each, CH_2-

Ph), 4.28, 4.36 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.36, 4.90 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.40, 4.43 (d, ^2J = 12.5 Hz, 1 H each, CH_2-Ph), 4.42, 4.53 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.49, 4.80 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.52, 4.61 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.52, 4.93 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.75−4.77 (m, 4 H, 2 CH_2-Ph), 4.63, 4.68 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.71, 5.04 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.75, 4.81 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 7.35−7.03 (m, 65 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 6): δ = 21.1 (CO*C*H$_3$), 54.7 (O*C*H$_3$), 71.4 (*C*H$_2$-Ph), 72.0 (*C*H$_2$-Ph), 72.4 (*C*H$_2$-Ph), 72.5 (*C*H$_2$-Ph), 72.6 (*C*H$_2$-Ph), 72.89 (*C*H$_2$-Ph), 72.90 (*C*H$_2$-Ph), 73.3 (*C*H$_2$-Ph), 74.4 (*C*H$_2$-Ph), 74.6 (*C*H$_2$-Ph), 75.2 (*C*H$_2$-Ph), 75.2 (*C*H$_2$-Ph), 75.3 (*C*H$_2$-Ph), 128.3−126.7 (aromatic *C*), 137.9, 138.1, 138.27, 138.39, 138.44, 138.5, 138.7, 138.8 (2 C), 139.0, 139.1, 139.3, 139.4 (aromatic *C*), 170.1 (*C*OCH$_3$) ppm. HR-FABMS: calcd. for $C_{121}H_{128}O_{23}Na$ [M + Na]$^+$: 1971.8744; found 1971.8718.

Methyl (2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-[2,3,4,6,7-penta-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)]-6-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (19): A solution of osmium tetraoxide in H_2O (1% w/v; 1 mL) containing sodium periodate (0.32 g, 1.5 mmol) was added to a mixture of **18** (292 mg, 0.150 mmol) in Et_2O/water (1:1, v/v, 8 mL). After stirring for 8 h at room temp., sodium periodate (0.32 g, 1.5 mmol) was added, and the reaction mixture was stirred vigorously for 3 days at 30−35 °C. The mixture was extracted with Et_2O (3 × 10 mL) and the combined extracts were then washed with water (2 × 15 mL), dried ($MgSO_4$), and concentrated. After re-dissolving the residue in DMF/MeOH (2:1, v/v, 15 mL), the mixture was treated with $NaBH_4$ (90 mg, 2.4 mmol). The mixture was diluted with Et_2O (15 mL) and washed with water (10 mL). The aqueous solution was extracted with Et_2O (2 × 10 mL). Purification by flash column chromatography (hexane/toluene/EtOAc, 2:2:1) gave the title compound **19** (229 mg, 80%) as a syrup. $[\alpha]_D^{22}$ = +28 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 5): δ = 3.18 (s, 3 H, OC*H*$_3$), 4.41, 4.22 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.23, 4.32 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.34, 4.88 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.41, 4.45 (d, ^2J = 12.5 Hz, 1 H each, CH_2-Ph), 4.44, 4.53 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.48, 4.80 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.49, 4.69 (d, ^2J = 12.5 Hz, 1 H each, CH_2-Ph), 4.52, 4.93 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.64, 4.66 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.66, 4.81 (d, ^2J = 12.0 Hz, 1 H each, CH_2-Ph), 4.69, 5.06 (d, ^2J = 11.5 Hz, 1 H each, CH_2-Ph), 4.74, 4.77 (d, ^2J = 11.0 Hz, 1 H each, CH_2-Ph), 4.75 (m, 2 H, CH_2-Ph), 7.31−7.00 (m, 65 H, aromatic *H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are shown in Table 6): δ = 54.5 (O*C*H$_3$), 71.4 (*C*H$_2$-Ph), 71.7 (*C*H$_2$-Ph), 72.4 (2 C, 2 *C*H$_2$-Ph), 72.77 (*C*H$_2$-Ph), 72.81 (*C*H$_2$-Ph), 73.1 (*C*H$_2$-Ph), 73.3 (*C*H$_2$-Ph), 74.4 (*C*H$_2$-Ph), 74.6 (*C*H$_2$-Ph), 74.9 (*C*H$_2$-Ph), 75.1 (*C*H$_2$-Ph), 75.3 (*C*H$_2$-Ph), 123.9−126.6, 137.8, 137.9, 138.1, 138.3, 138.5 (2 C), 139.56, 138.66, 138.67, 138.8, 139.0 (2 C), 139.1 (aromatic *C*) ppm. HR-FABMS: calcd. for $C_{118}H_{126}O_{23}Na$ [M + Na]$^+$: 1933.8588; found 1933.8594.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-[2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)]-2,6,7-tri-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranoside (20): Compound **19** (53 mg, 0.028 mmol) was hydrogenated in the presence of 10% Pd/C (53 mg) in EtOH/AcOH/water (7:5:5, v/v/v, 1.7 mL) at 0.9 MPa overnight. The mixture was filtered through Celite and the filtrate was concentrated to dryness. The residue was treated overnight at room temp. with pyridine/Ac_2O (2:1, v/v, 0.9 mL) containing a catalytic amount of DMAP. The solution was co-evaporated with toluene to give a residue, which was purified by flash column chromatography (EtOAc/hexane, 3:1) to yield the title compound **20** (32 mg, 84%). $[\alpha]_D^{24}$ = +8.0 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$; data for the ring and the exocyclic protons are shown in Table 5): δ = 1.98 (s, 3 H, COC*H*$_3$), 2.01 (s, 3 H, COC*H*$_3$), 2.04 (s, 3 H, COC*H*$_3$), 2.05 (s, 3 H, COC*H*$_3$), 2.07 (s, 3 H, COC*H*$_3$), 2.08 (s, 3 H, COC*H*$_3$), 2.09 (s, 3 H, COC*H*$_3$), 2.14 (s, 3 H, COC*H*$_3$), 2.15 (s, 3 H, COC*H*$_3$), 2.18 (s, 3 H, COC*H*$_3$), 2.21 (s, 3 H, COC*H*$_3$), 2.23 (s, 3 H, COC*H*$_3$), 3.34 (s, 3 H, OC*H*$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$; data for the skeletal carbon atoms are listed in Table 6): δ = 20.59 (CO*C*H$_3$), 20.66 (CO*C*H$_3$), 20.69 (CO*C*H$_3$), 20.73 (CO*C*H$_3$), 20.77 (CO*C*H$_3$), 20.80 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 21.2 (CO*C*H$_3$), 169.4 (*C*OCH$_3$), 169.6 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 170.0 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 170.3 (*C*OCH$_3$), 170.4 (*C*OCH$_3$) ppm. HR-FABMS: calcd. for $C_{57}H_{78}O_{38}Na$ [M + Na]$^+$: 1393.4069; found 1393.4102.

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