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Synthesis and serological characterization of L-*glycero*-α-D-*manno*-heptopyranose-containing di- and tri-saccharides of the non-reducing terminus of the *Escherichia coli* K-12 LPS core oligosaccharide

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Abstract

Synthesis of the title trisaccharide was accomplished by sugar chain extension starting from the non-reducing terminus: coupling of Glc*p*NAc with LD-Hep*p*, then adding Glc*p*-OAll. An alternative route started from the reducing end: coupling of LD-Hep*p* with Glc*p*-OAll, then addition of Glc*p*NAc. In the synthesis of the title disaccharide a modification of the first approach was employed. The allyl glycosides were coupled with cysteamine, activated with thiophosgene and conjugated to bovine serum albumin (BSA). The neoglycoconjugates obtained were used in immunochemical studies of monoclonal and polyclonal antibodies directed against *Escherichia coli* K-12 lipopolysaccharide.

Keywords: Polysaccharides; *Escherichia coli*; Sugar chain extension

1. Introduction

A prerequisite for the synthesis of neoglycoconjugates that can be used in immunochemical studies as haptens, antigens, or immunosorbents is the preparation of appropriate oligosaccharides, often in the form of glycosides that can be subject to subsequent polymerisation or attachment to a macromolecular carrier. One of the popular aglycons utilised to this end is the allyl group.

Allyl glycosides of various oligosaccharides have been used for the preparation of oligosaccharide–acrylamide copolymers, both as such or following the addition of cysteamine and *N*-acryloylation [1]. Alternatively, the cysteamine adducts can be converted into the respective isothiocyanates and then coupled to a protein carrier, e.g., bovine serum albumin (BSA). It is the latter approach that has been used in the preparation of synthetic neoglycoconjugates related to

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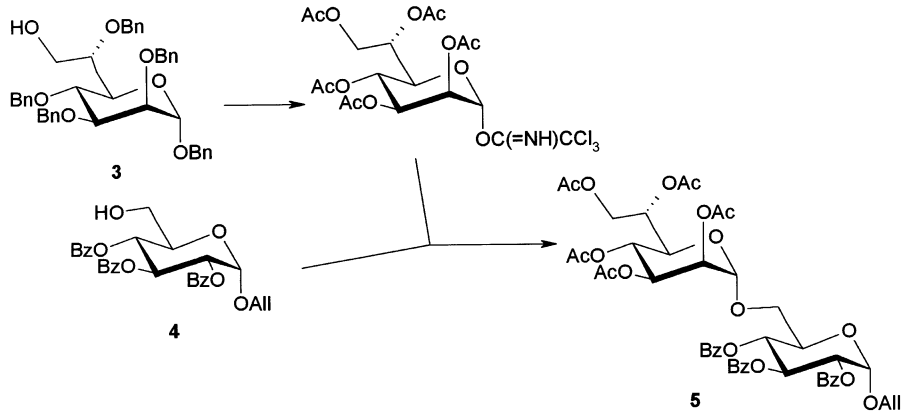


Scheme 1.

the K-12 core of *Escherichia coli* [2] (synthesis of the corresponding oligosaccharide derivatives, viz., L-α-D-Hep*p*-(1→6)-α-D-Glc*p*-*O*-All, L-α-D-Hep*p*-(1→6)-α-D-Glc*p*-(1→2)-α-D-Glc*p*-*O*-All, and L-α-D-Hep*p*-(1→6)-α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-*O*-All has been described in Ref. [3] rather than in Ref. [4], erroneously cited in Ref. [2]).

These glycoconjugates proved to be reactive as immunogens and antigens, although they did not bind to three monoclonal antibodies raised in mice by immunisation with heat-killed *E. coli* K-12 bacteria, and the allyl glycosides of the oligosaccharides did not manifest inhibitory activities in 'deacylated lipopolysaccharide–monoclonal antibody' systems [2].

In the K-12 core, the heptose linked to the Glc*p*-(1→2)-Glc*p*-(1→3)-Glc*p* sequence carries at O-7 a β-*Glcp*NAc substituent in a non-stoichiometric amount [2,5]. For further insight into the immunochemistry of K-12 strains of *E. coli* and in a continuation of our synthetic studies on oligosaccharide fragments of the *E. coli* K-12 core [3,4], here we report the syntheses of allyl glycosides **1** and **2**. Trioside **1** was prepared using two synthetic strategies, which differed in the direction of the chain extension.

β-D-Glc*p*NAc (1→7)-L-α-D-Hep*p*-(1→6)-α-D-Glc*p*-OAll (1)

β-D-Glc*p*NAc (1→7)-L-α-D-Hep*p*-OAll (2)

2. Results

Synthesis of **1** *and* **2**.—The common feature of both approaches to **1** is that the glucosamine unit was introduced by glycosylation with a thioglycoside donor mediated by methyl trifluoromethanesulfonate (MeOTf) [6], while 1-*O*-trichloroacetimidate methodology [7] was applied to create a Hep–Glc linkage.

According to the first reaction (Scheme 1), benzyl 2,3,4,6-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (**3**) [8] was converted into the corresponding peracetylated glycosyl trichloroacetimidate, which was coupled with allyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (**4**) as described earlier [3] using trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) as a catalyst to give the bioside (**5**). In the preparation of the glycosyl acceptor **4**, selective 6-*O*-formylation of allyl α-D-glucopyranoside followed by benzoylation and acid-catalyzed deformylation [9] were used instead of the tritylation–benzoylation–detritylation procedure employed before [3].

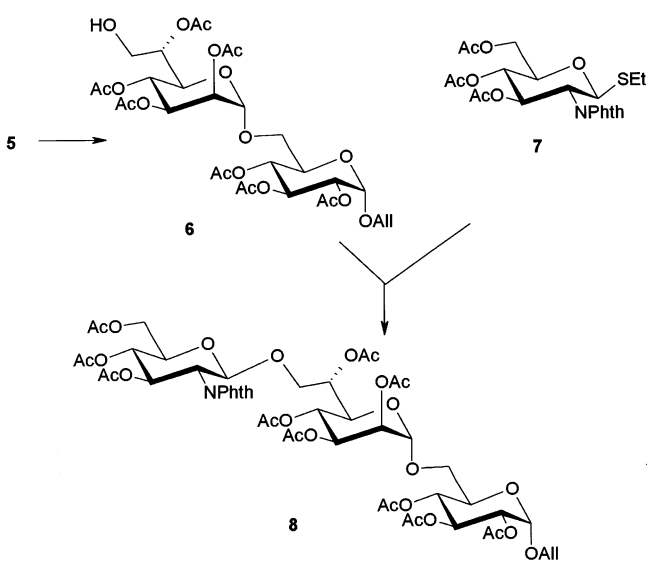
Peracylated allyl bioside **5** was deacylated with Et_3N in MeOH, the product was selectively 7′-*O*-silylated with *tert*-butyldimethylsilyl chloride in pyridine and acetylated. Subsequent desilylation with 40% HF in acetonitrile afforded a disaccharide glycosyl acceptor (**6**) isolated in a yield of 75% over four steps. The relatively high-field position of H-7′a,b (δ 3.79, m) in the ^1H NMR spectrum of

this disaccharide derivative proved that desilylation was not accompanied by acyl migration. On the contrary, conventional desilylation with Bu_4NF in THF gave a complex mixture. Unsatisfactory results were also obtained on Bu_4NF-promoted desilylation of a perbenzoyl analogue of the disaccharide under consideration. It should also be noted that an attempt to prepare 7-*O*-*tert*-butyldimethylsilyl-heptose from benzyl heptoside **3** failed due to the instability of the silyl group under hydrogenolysis conditions (Scheme 2).

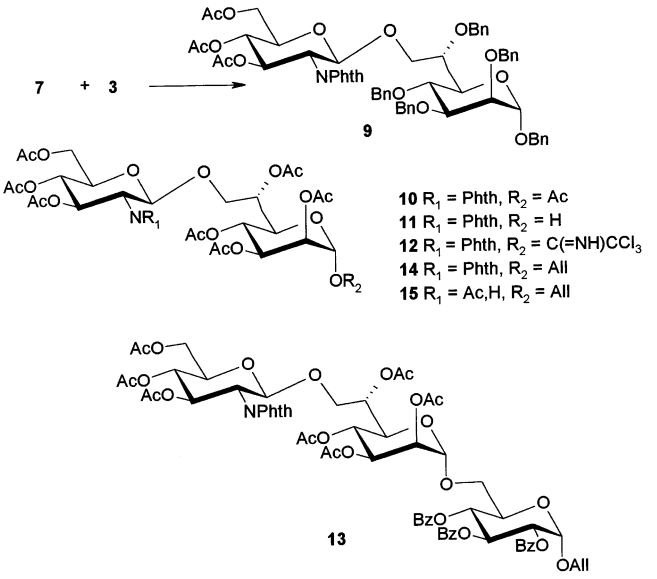
The introduction of the glucosamine residue into the disaccharide acceptor **6** was accomplished successfully using ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**7**) [10] as the glycosyl donor. The MeOTf-mediated glycosylation [6] resulted in a fully protected allyl trioside (**8**) in 70% yield. The newly formed glycosidic bond was β-(1 → 7) as shown by the NMR spectral data: $\delta_{H\text{-}1''}$ 5.39 (d, J 8.4 Hz); $\delta_{C\text{-}7'}$ 67.9.

This trioside was N,O-deprotected in one step by treatment with ethylenediamine in BuOH [11]; this procedure has been especially recommended for N-dephthaloylation of allyl glycosides of oligosaccharides containing 2-deoxy-2-phthalimidosugars. Subsequent N,O-acetylation and O-deacetylation afforded the target trisaccharide **1**.

An alternative glycosylation sequence was also explored. In this case, the heptose derivative **3** served as the first glycosyl acceptor. Its MeOTf-mediated coupling [6] with the donor **7** yielded 74% of the benzyl bioside (**9**).



Scheme 2.

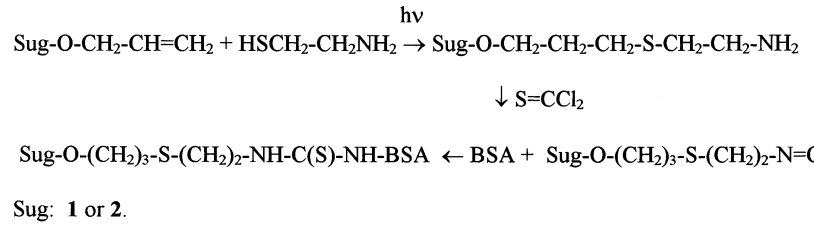


Scheme 3.

This was debenzylated by catalytic hydrogenolysis on Pd/C and the product was acetylated. The thus obtained per-*O*-acetyl disaccharide derivative (**10**) (yield 83% over two steps) was selectively 1-*O*-deacetylated with hydrazinium acetate (cf. [3]) and the product (**11**) was converted into the disaccharide 1-*O*-trichloroacetimidate (**12**) isolated by chromatography on silica gel in 71% yield. The low-field position of the signal for H-1 (δ 6.21, br.s) and the presence of an NH-proton (δ 8.78, s) in the ^{1}H NMR spectrum corroborated its structure (Scheme 3).

To complete the assembly of the trisaccharide, the acceptor **4** was glycosylated with the disaccharide donor **12** in the presence of Me_3SiOTf to yield 78% of the trisaccharide derivative (**13**). ^{1}H NMR chemical shifts for the protons of the GlcNAc and Hep units of this trisaccharide almost coincided with those of the trisaccharide **8**, while the signals for the protons of the Glc unit were predictably shifted downfield.

The transformation of this peracylated allyl trioside into the target product **1** was accomplished by the same deprotection–protection procedure as described for the transformation **8** → **1**. The NMR and mass spectra of the both samples of the trioside **1** were identical. As-



Scheme 4.

signments of the signals in the ^{1}H and ^{13}C NMR spectra were made with the use of 1D- and 2D- (TOCSY and HMQC) spectroscopy.

For the synthesis of **2**, peracetylated disaccharide **10** was employed. Its treatment with allyl alcohol in the presence of tin tetrachloride [12] afforded 67% of α-glycoside (**14**). Dephthaloylation and acetylation gave **15** (99%) which was next fully deacetylated to yield 90% of **2**.

Conjugation and serological characterization of **1** *and* **2**.—In order to study the immunoreactive properties of these oligosaccharides, they were derivatized into neoglycoconjugates by cysteamine spacer extension, activation with thiophosgene and subsequent coupling of the resulting isothiocyanate to BSA [2] (Scheme 4). These conjugates will be abbreviated as GlcNAc-Hep-Glc-BSA and as GlcNAc-Hep-BSA, respectively. The amount of ligand present in the conjugates was determined by measuring the amount of protein and glucosamine. For GlcNAc-Hep-Glc-BSA and as GlcNAc-Hep-BSA the analytical values were 123.5 and 74 nmol of ligand per mg BSA, respectively. Three monoclonal antibodies, S31-8, S31-14, and S31-21, and one polyclonal antiserum, described previously [2] were tested in EIA with GlcNAc-Hep-Glc-BSA or GlcNAc-Hep-BSA as solid phase antigens. Another glycoconjugate containing the complete core *E. coli* K-12 LPS was included. The MAbs did not bind to GlcNAc-Hep-Glc-BSA or GlcNAc-Hep-BSA, whereas the polyclonal rabbit antiserum reacted with a titer of 80,000 and 20,000 with GlcNAc-Hep-Glc-BSA and GlcNAc-Hep-BSA, respectively (Table 1). The titer of this antiserum with the conjugate containing the complete core was 320,000.

During the characterization of the epitope specificities of the three K-12 MAs, it could not be determined whether the terminal glucosamine was a part of the epitopes recognized by these antibodies. The data obtained with the glycoconjugates described herein, allowed to answer this question. It is clearly shown that the terminal part of the core oligosaccharide with or without the glucosamine does not constitute any of the epitopes recognized by these MAs. Obviously, they recognize an epitope which requires the complete core region. This is in accordance with observations made with an antibody cross-reacting with the different *E. coli* core types [13]. However, the polyclonal antiserum contains antibody specificities recognizing epitopes in which this terminal glucosamine is included.

3. Experimental

General and serological methods.—Column chromatography was performed on Silica Gel 230–400 or 70–230 mesh (E. Merck). Optical rotations were determined with a Jasco DIP-360 automatic polarimeter (Japan) at 20 ± 2 °C. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini AC-200 (200 MHz) or Bruker AM-500 (500 MHz) spectrometers in $CDCl_3$ solutions with Me_4Si as the internal standard. The spectra of **1** and **2** were recorded on solutions in D_2O with Bruker DRX 600 spectrometer (Research Center Borstel), operating frequencies 600 MHz for ^{1}H NMR and 125.77 MHz for ^{13}C NMR at 27 °C. The resonances were measured relative to internal acetone [$(CH_3)_2CO$, δ_{1H} 2.225, δ_{13C} 31.07]. Mass spectra (LSIMS, positive mode) were obtained with an AMD-604 mass spectrometer.

The glycoconjugates Glc*p*NAc-Hep*p*-Glc*p*-BSA and Glc*p*NAc-Hep*p*-BSA were prepared as described [2]. The conjugates were used as

Table 1
Reactivity in EIA[a] of monoclonal and polyclonal antibodies against *Escherichia coli* K-12 LPS_{deac}–BSA[b] and synthetic glycoconjugates

Antibody	Reactivity against indicated antigen in EIA[c]		
	E.c.K-12 LPS_{deac}–BSA	GlcNAc-Hep-Glc–BSA	GlcNAc-Hep–BSA
MAb S31-8[d]	16	>10,000	>10,000
MAb S31-14	8	>10,000	>10,000
MAb S31-21	16	>10,000	>10,000
Rabbit serum[e]	320,000	80,000	20,000

[a] EIA plates were coated at a concentration of 400 pmol of ligand per mL (50 μL/well).
[b] LPS_{deac} was covalently linked to BSA (LPS_{deac}–BSA) by the glutardialdehyde method [14].
[c] Final concentration (ng/mL) in the case of MAbs or dilution in the case of the rabbit antiserum yielding $OD_{405} > 0.2$.
[d] MAbs from Ref. [2] were obtained after immunization with heat-killed *E. coli* K-12 bacteria.
[e] The serum was obtained from a rabbit (K259 day 58 from Ref. [2]) immunized with heat-killed *E. coli* K-12 bacteria.

solid antigens in an EIA described previously [2]. The monoclonal and polyclonal antibodies were described in the same Ref.

Allyl 2,3,4-tri-O-*acetyl-6*-O-*(2,3,4,6-tetra*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranosyl)*-α-D-*glucopyranoside* (**6**).—Allyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl]-α-D-glucopyranoside (**5**) [3] (70 mg, 0.075 mmol) was dissolved in dry MeOH (5 mL) and triethylamine (0.5 mL) was added. The reaction mixture was stirred for 4 days at room temperature, concentrated, and traces of water were removed by coevaporation with pyridine (twice).

The thus obtained allyl *6-O-(*L-*glycero*-α-D-*manno*-heptopyranosyl)-α-D-glucopyranoside was dissolved in pyridine (3 mL), and *tert*-butyldimethylchlorosilane (30 mg, 0.2 mmol) was added. The mixture was stirred for 3 h at 25 °C (TLC control, 4:1 $CHCl_3$–MeOH), acetic anhydride (1 mL) was added, and stirring was continued for 24 h at room temperature. The mixture was concentrated and the residue was chromatographed on silica gel (9:1 → 2:3 hexane–EtOAc) to give allyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-7-*O*-*tert*-butyldimethylsilyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-α-D-glucopyranoside (60 mg, 0.073 mmol).

A solution of HF in acetonitrile (350 μL, 50 μL of 40% aq HF in 1 mL of acetonitrile) was added to a solution of the above silyl ether in 2 mL of acetonitrile and the mixture was stirred for 1 h at room temperature. It was then diluted with $CHCl_3$, washed with aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. The residue, practically pure **6** (40 mg, 75% with respect to **5**), $[\alpha]_D$ +69.4° (*c* 0.5, $CHCl_3$), was used in subsequent transformations without additional purification.

Allyl 2,3,4-tri-O-*acetyl-6*-O-*[2,3,4,6-tetra*-O-*acetyl-7*-O-*(3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)*-L-glycero-α-D-manno-*heptopyranosyl]-α*-D-*glucopyranoside* (**8**).—A solution of acceptor **6** (40 mg, 0.056 mmol) and ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **7** (35 mg, 0.073 mmol) in dry ether (3 mL) was stirred with 4 Å molecular sieves under Ar for 1 h. Then methyl trifluoromethanesulfonate (50 μl) was added and stirring was continued for 24 h at room temperature. An additional 5 mg of the glycosyl donor and 60 μL of the promoter were added and stirring was continued for another 48 h. One drop of pyridine was added to destroy the promoter, and the mixture was filtered and concentrated. Column chromatography of the residue (7:3 → 1:1 toluene–EtOAc) afforded **8** (45 mg, 70%); $[\alpha]_D$ +77.5° (*c* 1, $CHCl_3$). ^1H NMR data: δ 7.9–7.1 (m, 4 H, Ph), 5.90 (m, 1 H, –CH=), 5.76 (dd, 1 H, $J_{3,2}$ 10.6, $J_{3,4}$ 9.1 Hz, H-3″), 5.55 (dd, 1 H, $J_{4,3}$ 9.3, $J_{4,5}$ 10 Hz, H-4), 5.50 (dd, 1 H, $J_{3,2}$ 10.2, $J_{3,4}$ 9.3 Hz, H-3), 5.39 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1″), 5.36 (m, 1 H, $=CH_2$), 5.28 (dd, 1 H, $J_{3,2}$ 3.4, $J_{3,4}$ 10.4 Hz, H-3′), 5.23 (m, 2 H, H-2′,

=CH_2), 5.19 (t, 1 H, $J_{4,3} = J_{4,5} = 10.4$ Hz, H-4′), 5.17 (ddd, 1 H, $J_{6,5}$ 1.8, $J_{6,7a}$ 4.4, $J_{6,7b}$ 4.7 Hz, H-6′), 5.14 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.07 (dd, 1 H, $J_{4,3}$ 9.1, $J_{4,5}$ 10.2 Hz, H-4″), 4.99 (dd, 1 H, $J_{2,1}$ 3.8, $J_{2,3}$ 10.2 Hz, H-2), 4.82 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1′), 4.30 (dd, 1 H, $J_{6b,5}$ 4.3, $J_{6a,6b}$ 12.2 Hz, H-6″b), 4.29 (dd, 1 H, $J_{2,1}$ 8.4, $J_{2,3}$ 10.6 Hz, H-2″), 4.23 (m, 1 H, $-OCH_2$), 4.19 (dd, 1 H, $J_{6a,5}$ 2.5, $J_{6b,6a}$ 12.2 Hz, H-6″a), 4.10 (dd, 1 H, $J_{5,4}$ 10.4, $J_{5,6}$ 1.8 Hz, H-5′), 4.05 (m, 1 H, $-OCH_2$), 4.02 (ddd, 1 H, $J_{5,4}$ 10, $J_{5,6a}$ 2, $J_{5,6b}$ 7.4 Hz, H-5), 3.92 (ddd, 1 H, $J_{5,4}$ 10.2, $J_{5,6a}$ 2.5, $J_{5,6b}$ 4.3 Hz, H-5″), 3.82 (dd, 1 H, $J_{6b,5}$ 7.4, $J_{6b,6a}$ 11.5 Hz, H-6b), 3.76, 3.75 (2 dd, 2 H, $J_{7a,6}$ 4.4, $J_{7a,7b}$ 11, $J_{7b,6}$ 4.7 Hz, H-7′a,b), 3.44 (dd, 1 H, $J_{6a,5}$ 2, $J_{6a,6b}$ 11.5 Hz, H-6a), 2.13, 2.12, 2.07, 2.05, 2.03, 2.02, 1.99, 1.95, 1.91, 1.86 (10 s, 30 H, CH_3CO). ^{13}C NMR data: δ 134.1–129.5 (C, Ph, =CH–), 117.95 (CH_2=), 99.34 (C-1″), 97.71 (C-1′), 94.52 (C-1), 71.80 (C-5″), 71.71 (C-2), 70.43 (C-3″), 69.38 (C-2′), 69.19 (C-3′), 69.09, 69.00 (C-3, 4), 68.77 (C-4″), 68.54 ($-OCH_2$), 67.98 (C-5′), 67.9 (C-7′), 67.79 (C-6′), 66.27 (C-6), 64.80 (C-4′), 62.08 (C-6″), 54.47 (C-2″), 20.83–20.27 (7 C, CH_3CO). LSIMS (+): Calcd for $C_{50}H_{61}NO_{28}$ + Na: *m/z* 1146.2. Found: *m/z* 1146.0.

*Benzyl 2,3,4,6-tetra-*O*-benzyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-*L*-glycero-α-*D*-manno-heptopyranoside* (**9**).—A solution of benzyl 2,3,4,6-tetra-*O*-benzyl-*L*-*glycero*-α-*D*-*manno*-heptopyranoside **3** (688 mg, 1.0 mmol) and ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside **7** (600 mg, 1.25 mmol) in dry Et_2O (30 ml) was stirred with 4 Å molecular sieves at room temperature for 1 h under Ar and methyl trifluoromethanesulfonate (700 μL, 6.2 mmol) was added. Stirring was continued for 24 h at room temperature. One drop of pyridine was added to destroy the catalyst, and the mixture was filtered and concentrated. Column chromatography of the residue (toluene → 4:1 toluene–EtOAc) afforded **9** (800 mg, 74%) as a syrup, $[\alpha]_D$ + 30.4° (*c* 1.46, $CHCl_3$). ^1H NMR data: δ 7.8–7.0 (m, 29 H, Ph), 5.8 (dd, 1 H, $J_{3,2}$ 10,5, $J_{3,4}$ 9.4 Hz, H-3′), 5.42 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1′), 5.17 (t, 1 H, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4′), 4.9 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.8-3.6 (m, 21 H, H-2,3,4,5,6,7a,7b,2′,5′,6′a,6′b, 5 × CH_2Ph), 2.04, 2.03, 1.85 (3s, 9 H, 3 × CH_3CO). ^{13}C NMR data: δ 98.0 (C-1′), 96.6 (C-1), 80.3 (C-3), 74.1, 72.1, 71.9, 70.7, 68.9 (C-2,4,5,6,4′,5′), 68.8 (C-7), 62.0 (C-6′), 54.6 (C-2′). LSIMS (+): Calcd for $C_{62}H_{63}NO_{16}$ + Na: *m/z* 1100. Found: *m/z* 1100.

*2,3,4,6-Tetra-*O*-acetyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-*L*-glycero-α-*D*-manno-heptopyranose* (**11**).—A solution of **9** (800 mg, 0.74 mmol) in a 1:1 MeOH–EtOAc mixture (50 mL) was hydrogenolyzed over 10% palladium-on-charcoal for 64 h. The mixture was filtered through Celite and concentrated. The residue was conventionally acetylated with acetic anhydride in pyridine (1:1) for 16 h at ambient temperature. The mixture was concentrated, toluene was added, and the solution was concentrated to dryness. Column chromatography of the residue (4:1 → 1:1 hexane–EtOAc) afforded 480 mg of a mixture of 1,2,3,4,6-penta-*O*-acetyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-*L*-*glycero*-α,β-*D*-*manno*-heptopyranoses (**10**). Hydrazinium acetate (30 mg, 0.32 mmol) was added to a solution of this product (200 mg, 0.23 mmol) in DMF (3 mL). The mixture was stirred for 2 h at room temperature, diluted with EtOAc, and washed twice with water. The organic layer was dried ($MgSO_4$) and concentrated. Column chromatography of the residue (7:3 → 2:3 hexane–EtOAc) afforded the title compound as a syrup (108 mg, 52%); $[\alpha]_D$ + 0.7° (*c* 0.5, $CHCl_3$). LSIMS (+): Calcd for $C_{35}H_{41}O_{20}N$ + Na: *m/z* 818. Found: *m/z* 818.

*Allyl 2,3,4-tri-*O*-benzoyl-6-*O*-[2,3,4,6-tetra-*O*-acetyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-*L*-glycero-α-*D*-manno-heptopyranosyl]-α-*D*-glucopyranoside* (**13**).—Potassium carbonate (0.3 g) and trichloroacetonitrile (400 μL, 4 mmol) were added to a solution of **11** (130 mg, 0.14 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred for 16 h and filtered through a layer of silica gel. The sorbent was washed with CH_2Cl_2 (20 mL), hexane (50 mL), and the syrupy 2,3,4,6-tetra-*O*-acetyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-*L*-*glycero*-α,β-*D*-*manno*-heptopyranosyl tri-

chloroacetimidate (**12**) was eluted with 4:1 → 1:1 toluene–EtOAc, yield 110 mg (71%); $[\alpha]_D$ + 31° (*c* 0.5, $CHCl_3$). ^1H NMR data: δ 8.78 (s, 1 H, NH), 7.84 and 7.73 (2 m, 4 H, Ph), 6.21 (br. s, 1 H, H-1), 5.71 (dd, 1 H, $J_{3,2}$ 10.7, $J_{3,4}$ 9.2 Hz, H-3′), 5.43 (dd, 1 H, $J_{2,1}$ 1.8, $J_{2,3}$ 3.2 Hz, H-2), 5.40 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1′), 5.35 (dd, 1 H, $J_{3,2}$ 3.3, $J_{3,4}$ 10 Hz, H-3), 5.31 (t, 1 H, $J_{4,3} = J_{4,5} = 10.1$ Hz, H-4), 5.22 (m, 1 H, H-6), 5.13 (dd, 1 H, $J_{4,3}$ 9.3, $J_{4,5}$ 10.1 Hz, H-4′), 4.30 (m, 1H, H-5), 4.29 (m, 1 H-6′b), 4.22 (dd, 1 H, $J_{2,1}$ 8.4, $J_{2,3}$ 10.7 Hz, H-2′), 4.15 (dd, 1 H, $J_{6a,5}$ 2.3, $J_{6a,6b}$ 12.2 Hz, H-6′a), 3.87 (ddd, 1 H, $J_{5,4}$ 10.2, $J_{5,6a}$ 2.4, $J_{5,6b}$ 4.2 Hz, H-5′), 3.80 (dd, 1 H, $J_{7b,6}$ 7.3, $J_{7b,7a}$ 11.4 Hz, H-7b), 3.71 (dd, 1 H, $J_{7a,6}$ 6.8, $J_{7a,7b}$ 11.4 Hz, H-7a), 2.18, 2.13, 2.02, 1.99, 1.98, 1.96, 1.84 (7 s, 21 H, CH_3CO). ^{13}C NMR data: δ 199.26 (C-1′), 94.40 (C-1), 71.71 (C-5′), 70.62 (C-3′), 70.49 (C-5), 68.94 (C-3), 68.52 (C-4′), 67.96 (C-7), 67.83 (C-2), 67.40 (C-6), 64.21 (C-4), 61.84 (C-6′), 54.38 (C-2′).

The thus obtained biosyl trichloroacetimidate **12** (110 mg, 0.11 mmol) and allyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **4** (60 mg, 0.11 mmol) were stirred in dry CH_2Cl_2 (10 ml) with 4 Å molecular sieves under Ar for 1 h. A solution of trimethylsilyl trifluoromethanesulfonate (3 μL, 0.016 mmol) in CH_2Cl_2 was added to a mixture at −30 °C and stirring was continued for 2 h at −30 to −15 °C. One drop of pyridine was added to destroy the catalyst, and the mixture was filtered and concentrated. Column chromatography of the residue (4:1 → 2:5 toluene–EtOAc) afforded the title compound **13** (120 mg, 78%); $[\alpha]_D$ + 42.5° (*c* 1,$CHCl_3$). ^1H NMR data: δ 8.0–7.2 (m, 19 H, Ph), 6.21 (t, 1 H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 5.95–5.87 (m, 1 H, =CH–), 5.78 (dd, 1 H, $J_{3,2}$ 10.6, $J_{3,4}$ 9.0 Hz, H-3″), 5.56 (dd, 1 H, $J_{4,3}$ 9.6, $J_{4,5}$ 10.2 Hz, H-4), 5.44 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.38 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1″), 5.38 (dd, 1 H, $J_{2,1}$ 3.6, $J_{2,3}$ 10 Hz, H-2), 5.34 (dd, 1 H, $J_{3,2}$ 3.5, $J_{3,4}$ 10.1 Hz, H-3′), 5.26 (dd, 1 H, $J_{2,1}$ 1.3, $J_{2,3}$ 3.5 Hz, H-2′), 5.18 (t, 1 H, $J_{4,3} = J_{4,5} = 10.1$ Hz, H-4′), 5.14 (t, 1 H, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4″), 5.14 (dt,1 H, $J_{6,5}$ 1.8, $J_{6,7a} = J_{6,7b} = 6.9$ Hz, H-6′), 4.82 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1′), 4.32 (ddd, 1 H, $J_{5,4}$ 10.2, $J_{5,6a}$ 1.8, $J_{5,6b}$ 6.6 Hz, H-5), 4.3 (dd, 1 H, $J_{2,1}$ 8.4, $J_{2,3}$ 10.6 Hz, H-2″), 4.27 (dd, 1 H, $J_{6a,5}$ 4.8, $J_{6a,6b}$ 12.3 Hz, H-6a′), 4.17 (dd, 1 H, $J_{6b,5}$ 2.4, $J_{6b,6a}$ 12.3 Hz, H-6b″), 4.11 (dd, 1 H, $J_{5,4}$ 10.1, $J_{5,6}$ 1.8 Hz, H-5′), 3.88 (ddd, 1 H, $J_{5,4}$ 10.1, $J_{5,6a}$ 4.8, $J_{5,6b}$ 2.4 Hz, H-5″), 3.86 (dd, 1 H, $J_{6b,5}$ 6.6, $J_{6b,6a}$ 11.3 Hz, H-6b), 3.75 (d, 2 H, $J_{7a,6} = J_{7b,6} = 6.9$ Hz, H-7′a,b), 3.54 (dd, 1 H, $J_{6a,5}$ 1.8, $J_{6a,6b}$ 11.3 Hz, H-6a), 2.12, 2.10, 2.03, 1.96, 1.95, 1.91, 1.86 (7s, 21 H, CH_3CO). ^{13}C NMR data: δ 134.0–123.4 (Ph, $-CH_2CH=$), 117.85 ($-CH=CH_2$), 99.26 (C-1″), 97.62 (C-1′), 94.94 (C-1), 71.82 (C-5″), 71.78 (C-2), 70.69 (C-3), 70.56 (C-3″), 69.43 (C-4), 69.38 (C-2′), 69.13 (C-3′), 68.90 (C-5), 68.84 (C-4″), 68.82 ($-OCH_2-$), 68.09 (C-5′), 68.02 (C-7′), 67.76 (C-6′), 66.48 (C-6), 64.88 (C-4′), 62.11 (C-6″), 54.46 (C-2″), 20.82–20.34 (7 C, CH_3CO). LSIMS (+): Calcd for $C_{65}H_{67}NO_{28}$ + Na: *m*/*z* 1332.35. Found: *m*/*z* 1331.9.

Allyl 6-O-[7-O-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-L-glycero-α-D-manno-heptopyranosyl]-α-D-glucopyranoside (**1**).—Ethylenediamine (5 mL) was added to a solution of **13** (177 mg, 0.13 mmol) in *n*-butanol (25 ml) under Ar and the mixture was stirred for 20 h at 90 °C. After the reaction was completed (TLC control in 1:1:1 *n*-BuOH–EtOH–25% aq NH_3, one ninhydrin-positive spot, no UV absorption), the mixture was concentrated, and coevaporated twice with a mixture EtOH–toluene. The residue was dissolved in a 1:1 mixture of Py–Ac_2O (50 mL), and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine was added. After 4 h at room temperature, the mixture was concentrated to dryness. Toluene was added to and distilled from the residue, which was then chromatographed on silica gel with a gradient (0 → 3%) of MeOH in $CHCl_3$ to give allyl 2,3,4-tri-*O*-acetyl-6-*O*-[2,3,4,6-tetra-*O*-acetyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-L-*glycero*-α-D-*manno*-heptopyranosyl]-α-D-glucopyranoside (120 mg, 89%); $[\alpha]_D$ + 32.7° (*c* 1, $CHCl_3$). ^1H NMR data: δ 5.95 (d, 1 H, *J* 8.4 Hz, NH), 5.90 (m, 1 H, –CH=), 5.52 (t, 1 H, $J_{3,4} = J_{3,2} = 9.9$ Hz, H-3), 5.39 (dd, 1 H, $J_{3,2}$ 10.2, $J_{3,4}$ 9.6 Hz, H-3″), 5.36 (m, 1 H, $=CH_2$), 5.31 (m, 1 H, H-6′), 5.30 (dd, 1 H, *J* 10.2, 8.9 Hz, H-4′), 5.26 (m, 1 H, H-3′), 5.25 (m, 2H, H-2′, $=CH_2$), 5.14 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.05 (t, 1 H, $J_{4,3} = J_{4,5} = 9.8$ Hz, H-4″), 4.96 (dd, 1 H, $J_{4,5}$ 10.2, $J_{4,3}$ 9.5 Hz, H-4), 4.91 (dd, 1 H, $J_{2,1}$ 3.8,

$J_{2,3}$ 10.1 Hz, H-2), 4.87 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1′), 4.84 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1″), 4.25 (m, 1 H, $-CH_2O-$), 4.24 (m, 1 H, H-6″b), 4.15 (m, 1 H, H-5′), 4.14 (dd, 1 H, $J_{6a,5}$ 2.4, $J_{6a,6b}$ 12.2 Hz, H-6″a), 4.08 (m, 1 H, H-5), 4.06 (m, 1 H, $-CH_2O-$), 3.88 (dd, 1 H, $J_{7b,6}$ 6.7, $J_{7b,7a}$ 11.7 Hz, H-7′b), 3.84 (dd, 1 H, $J_{7a,6}$ 7.8, $J_{7a,7b}$ 12.2 Hz, H-7′a), 3.76 (m 2 H, H-6b, H-5″), 3.68 (ddd, 1 H, $J_{2,NH}$ 8.4, $J_{2,1}$ 8.3, $J_{2,3}$ 10.5 Hz, H-2″), 3.51 (dd, 1 H, $J_{6a,5}$ 2.0, $J_{6a,6b}$ 11.3 Hz, H-6a), 2.17, 2.11, 2.09, 2.07, 2.06, 2.04, 2.02, 2.01, 2.00, 1.97, 1.95 (11 s, 33 H, CH_3CO). ^{13}C NMR data: δ 133 ($-CH=$), 118 ($=CH_2$), 100.5 (C-1″), 98.0 (C-1′), 94.5 (C-1), 72.1 (C-3″), 71.7 (C-5″), 70.8 (C-2), 70.1 (C-3), 69.5 (C-2′), 69.4 (C-4), 69.1 (C-6′), 68.5 (C-4″), 68.4 ($-CH_2O-$, C-5′), 68.1 (C-5), 67.3 (C-3′), 67.1 (C-7′), 66.6 (C-6), 64.8 (C-4′), 62.0 (C-6″), 55.4 (C-2″). LSIMS (+): Calcd for $C_{44}H_{61}NO_{27}$ + Na: m/z 1058.15. Found: m/z 1057.9.

Triethylamine (1 mL) was added to a solution of the peracetylated trisaccharide (120 mg) in dry MeOH (9 mL). After 48 h at room temperature, the mixture was concentrated, dissolved in water, filtered through Celite and concentrated to give **1** (70 mg, 98%); $[\alpha]_D$ + 33.5° (c 1, H_2O), R_f 0.3 (10:3:2, n-BuOH:MeOH:H_2O). ^1H NMR data: δ 6.00 (m, 1 H, $-CH=$), 5.38 (m, 1 H, $=CH_2$), 5.29 (m, 1 H, $=CH_2$), 4.97 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.88 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1′), 4.57 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1″), 4.23 (ddt, 1 H, $-CH_2O-$), 4.17 (ddd, $J_{6,5}$ 1.5, $J_{6,7a}$ 4.0, $J_{6,7b}$ 8.5 Hz, H-6′), 4.11 (ddt, 1 H, $-CH_2O-$), 4.00 (dd, 1 H, $J_{7b,6}$ 8.3, $J_{7b,7a}$ 10.9 Hz, H-7′b), 3.96 (dd, 1 H, $J_{2,1}$ 1.7, $J_{2,3}$ 3.4 Hz, H-2′), 3.93 (dd, 1 H, $J'_{6,5}$ 2.0, $J_{6b,6a}$ 8.3 Hz, H-6″b), 3.90 (m, 1 H, H-6b), 3.87 (t, 1 H, $J_{4,3} = J_{4,5} = 9.8$ Hz, H-4′), 3.86 (m, 1 H, H-5), 3.80 (dd, 1 H, $J_{3,2}$ 3.6, $J_{3,4}$ 9.8 Hz, H-3′), 3.80 (m, 1 H, H-7′a), 3.76 (dd, 1 H, $J_{6a,5}$ 1.5, $J_{6a,6b}$ 8.3 Hz, H-6″a), 3.74 (dd, $J_{2,1}$ 8.6, $J_{2,3}$ 10.4 Hz, H-2″), 3.72 (t, 1 H, $J_{3,2} = J_{3,4} = 9.8$ Hz, H-3), 3.70 (dd, 1 H, $J_{6a,5}$ 2.3, $J_{6a,6b}$ 9.8 Hz, H-6a), 3.58 (dd, 1 H, $J_{2,1}$ 3.8, $J_{2,3}$ 9.8 Hz, H-2), 3.58 (dd, 1 H, H-7′a), 3.56 (m, 1 H, H-3″), 3.49 and 3.48 (m, 2 H, H-4,4″), 3.46 (m, 1 H, H-5″), 2.07 (s, 3 H, CH_3CO). ^{13}C NMR data: δ 133.8 ($-CH=$), 118.1 ($=CH_2$), 102.8 (C-1″), 100.9 (C-1′), 98.9 (C-1), 77.2 (C-5″), 75.1 (C-3″), 74.8 (C-3), 72.8 (C-5′), 72.8 (C-2), 70.1 (C-7′), 72.5 (C-3′), 71.4 (C-5), 71.1 (C-4), 71.1 (C-2′), 73.1 (C-4″), 68.4 (C-6′), 68.6 ($-CH_2O-$), 67.2 (C-4′), 62.1 (C-6), 66.5 (C-6″), 56.8 (C-2″), 22.0 (CH_3CO). LSIMS (+): Calcd for $C_{24}H_{41}NO_{17}$ + Na: m/z 638.05. Found: m/z 638.0.

Deprotection of **8** (20 mg) as described for **13** afforded the target allyl trioside **1** (10 mg), whose NMR spectra were identical with those listed above.

Allyl 2,3,4,6-tetra-O-*acetyl*-7-O-*(3,4,6-tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl)*-L-glycero-α-D-manno-*heptopyranoside* (**14**).—To a solution of **10** (106 mg) in freshly distilled CH_2Cl_2 (3 mL) molecular sieves 3 Å (172 mg) were added and the mixture was stirred under argon at room temperature. After 30 min the mixture was cooled (ice–water) and a solution of $SnCl_4$ (0.3 mL of a solution made of 0.5 mL $SnCl_4$ in 5 mL CH_2Cl_2) was added. After 1 h, allyl alcohol (0.017 mL) was added and stirring was continued for 16 h. Solid $NaHCO_3$ and $Na_2SO_4 \cdot 10H_2O$ were added and the suspension was stirred overnight, then filtered through a Celite pad. The pad was washed with ethanol and acetone, the filtrate was concentrated and the residue was chromatographed with 1:1 hexane–EtOAc 1:1 to yield **14**, 70 mg (67%); $[\alpha]_D$ + 36.5° (c 1.43, $CHCl_3$). ^1H NMR data: δ 5.85 (m, 1 H, $-CH=$), 5.76 (dd, 1 H, $J_{3,2}$ 10.7, $J_{3,4}$ 9.1Hz, H-3′), 5.42 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1′), 5.32 (dd, 1 H, $J_{3,2}$ 3.5, $J_{3,4}$ 10.2 Hz, H-3), 5.26–5.18 (m, 5 H, H-2,4,6, $CH_2=$), 5.16 (dd, 1 H, $J_{4,3}$ 9.2, $J_{4,5}$ 10.1Hz, H-4′), 4.83 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.30 (dd, 1 H, $J_{6a,5}$ 4.8, $J_{6a,6b}$ 12.2 Hz, H-6′a), 4.29 (dd, 1 H, $J_{2,1}$ 8.4, $J_{2,3}$ 10.7 Hz, H-2′), 4.21 (dd, 1 H, $J_{6b,5}$ 2.4, $J_{6b,6a}$ 12.2 Hz, 6′b), 4.17 (m, 2 H, H-5, CH*H*O), 3.92 (ddd, 1 H, $J_{5,4}$ 10.1, $J_{5,6a}$ 4.8, $J_{5,6b}$ 2.4 Hz, H-5′), 3.88 (dd, 1 H, $J_{7a,6}$ 8.3, $J_{7a,7b}$ 11.1 Hz, H-7a), 3.89 (m, 1 H, CH*H*O), 3.73 (dd, 1 H, $J_{7b,6}$ 6.2, $J_{7b,7a}$ 11.1 Hz, H-7b), 2.13 (× 2), 2.03, 2.01, 1.96 (× 2), 1.85 (5 s, 21 H, $7CH_3CO$). ^{13}C NMR data: δ 99.2 (C-1′), 96.2 (C-1), 71.9, 70.6, 69.7, 69.4, 68.8, 67.9, 67.8 (C-2, 3, 4, 5, 6, 3′, 4′, 5′), 67.5 (C-7), 62.1 (C-6′), 54.6 (C-2′). LSIMS (+): Calcd for $C_{38}H_{45}NO_{20}$ + Na: m/z 858. Found: m/z 858.

Allyl 7-O-*(2*-*deoxy*-2-*acetamido*-β-D-*glucopyranosyl)*-L-glycero-α-D-manno-*heptopyranoside* (**2**).—De-phthaloylation and sub-

sequent acetylation of **14** (90 mg) was performed as for **13**. The peracetyl derivative **15** was obtained (80 mg). ^{13}C NMR data: δ 133.3 (–CH=), 119 (CH_2=), 100.7 (C-1′), 97.1 (C-1), 73.3, 72.5, 70.1, 69.9, 68.9 (× 2), 67.6, 65.6 (C-2, 3, 4, 5, 6, 3′, 4′, 5′), 69.0 (CH_2O), 67.3 (C-7), 62.6 (C-6′), 54.6 (C-2′).

To a solution of **15** (80 mg) in MeOH (2 mL) $NaHCO_3$ (220 mg) was added and the suspension was stirred overnight, then filtered and the filtrate was concentrated. The residue was chromatographed on a Sephadex G-10 column with water. After concentration and lyophilization 39 mg (90%) of **2** was obtained, $[\alpha]_D$ + 12.6° (*c* 0.56, H_2O); ^1H NMR data: δ 4.89 (d, 1 H, H-1), 4.53 (d, 1 H, H-1′), 4.12 (t, 1 H, $J_{6,7a}$ 5.7, $J_{6,7b}$ 7.7 Hz, H-6), 3.92 (dd, 1 H, $J_{7a,6}$ 7.7, $J_{7a,7b}$ 10.5 Hz, H-7a), 3.91 (m, 2 H, H-2,4′), 3.88 (H-6a′), 3.82 (t, 1 H, $J_{4,3}$ 9.6, $J_{4,5}$ 9.8 Hz, H-4), 3.74 (H-3), 3.73 (H-7b), 3.71 (H-6b′), 3.69 (dd, 1 H, $J_{2,1}$ 8.7, $J_{2,3}$ 8.9 Hz, H-2′), 3.55 (H-5), 3.53 (H-3′), 3.43 (H-5′). ^{13}C NMR data: δ 102.3 (C-1′), 99.9 (C-1), 76.8 (C-5′), 74.8 (C-3′), 72.3 (C-5), 72.1 (C-3), 71.9 (C-7), 71.1 (C-2,4), 67.8 (C-6), 66.9 (C-4), 61.6 (C-6′), 56.3 (C-2′). LSIMS (+): Calcd for $C_{18}H_{31}NO_{12}$ + Na: *m*/*z* 476.1744. Found: *m*/*z* 476.1757.

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