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Note

Synthesis of the 'primer–adaptor' trisaccharide moiety of *Escherichia coli* O8, O9, and O9a lipopolysaccharide

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Abstract—Described is the synthesis of the trisaccharide α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-β-D-Glc*p*NAcO$(CH_2)_8N_3$, the glycan portion of which corresponds to the 'adaptor–primer' moiety linking the O-chain and core oligosaccharide in the lipopolysaccharide of several *Escherichia coli* and *Klebsiella pneumoniae* serotypes. This report represents the first synthesis of this trisaccharide motif, and in the route involved, a key step is a [2+1] coupling of a protected Man*p*-(1→3)-α-D-Man*p* glycosyl donor with a Glc*p*NAc acceptor. The azido group was included in the target to facilitate future preparation of neoglycoconjugates.

Keywords: Lipopolysaccharide; Synthesis; Oligosaccharide; *Escherichia coli*; *Klebsiella pneumoniae*; Neoglycoconjugate

Lipopolysaccharide (LPS) is a critical component of the outer membrane of Gram-negative bacteria and is a potent endotoxin.[1] This glycoconjugate possesses three structural domains: Lipid A (the endotoxic component), a core oligosaccharide, and the O-chain, which contains multiple copies of a repeating unit with one or more monosaccharide residues. In *Escherichia coli* serotypes O8, O9a, and O9, the O-chain repeating unit is a tri-, tetra-, or penta-mannopyranoside, respectively (Fig. 1). These O-chains are linked to the core oligosaccharide via an 'adaptor–primer' trisaccharide[2] with the structure α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-β-D-Glc*p*NAc; the O-chain extends from the 3-hydroxyl group of the terminal mannose unit in this trisaccharide.[3] In LPS, from *Klebsiella pneumoniae* serotypes O3 and O5, the O-chain repeating unit and trisaccharide linkage structures are identical to those of *E. coli* O9 and O8, respectively (Fig. 1).[3]

As a part of a nascent collaboration on LPS biosynthesis in *E. coli* serotype O9a, we needed a derivative of the 'adaptor–primer' trisaccharide that would readily allow the generation of neoglycoconjugates, for example, fluorescently labeled analogues. Surprisingly, this trisaccharide appears not to have been synthesized previously, and we describe herein the first synthesis of this motif as its 8-azidooctyl glycoside. We envisioned that the target trisaccharide **1** (Fig. 2) could be produced from three suitably protected monosaccharide building blocks, **2**–**4**. Reaction of **3** with **4** would provide a disaccharide, which, upon removal of the *p*-methoxyphenyl aglycone and the conversion of the resulting hemiacetal to a glycosyl trichloroacetimidate, could be coupled with **2** thus giving a trisaccharide. With this plan in place, we turned our attention to the preparation of the required building blocks. Glycosyl imidate **3** was obtained as described previously;[4] the synthesis of **2** and **4** was carried out as shown below.

The azidooctyl glycoside **2** was prepared in two steps from furanosyl oxazoline **5**,[5] as illustrated in Scheme 1. Reaction of **5** with *p*-toluenesulfonic acid and 8-azidooctanol in dichloromethane gave a 67% yield of β-glycoside **6**; the stereochemistry at the anomeric center was determined from the magnitude of the $^3J_{1,2}$, which was 8.4 Hz. Protection of the 4,6-diol was achieved upon treatment with α,α-dimethoxytoluene and *p*-toluenesulfonic acid, which gave **2** in 84% yield.

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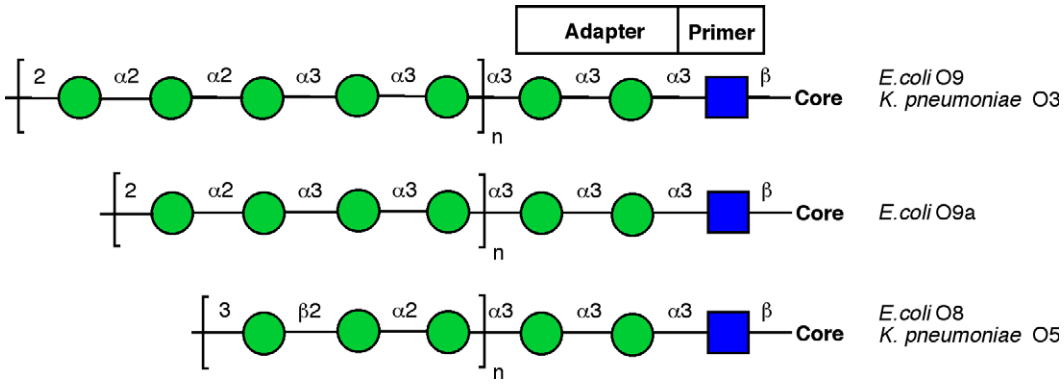


Figure 1. O-chain structures of LPS from *E. coli* serotypes O8, O9a, and O9 and their relationship to those present in *K. pneumoniae* O3 and O5; the 'adaptor–primer' trisaccharide is highlighted. The symbols used are those proposed by the Consortium for Functional Glycomics (www.functionalglycomics.org). Green circles: Man*p*; Blue squares: Glc*p*NAc.

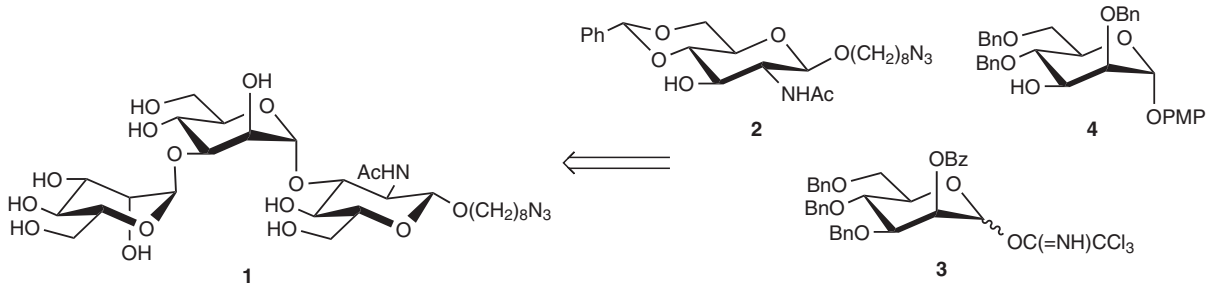
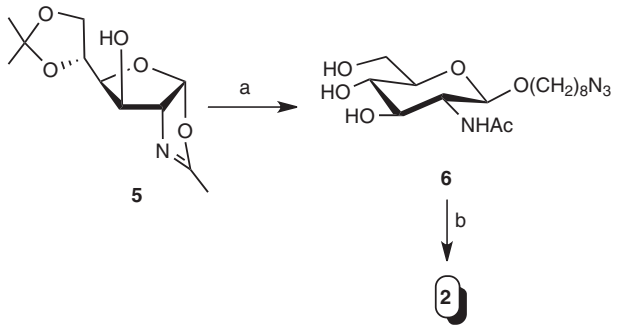


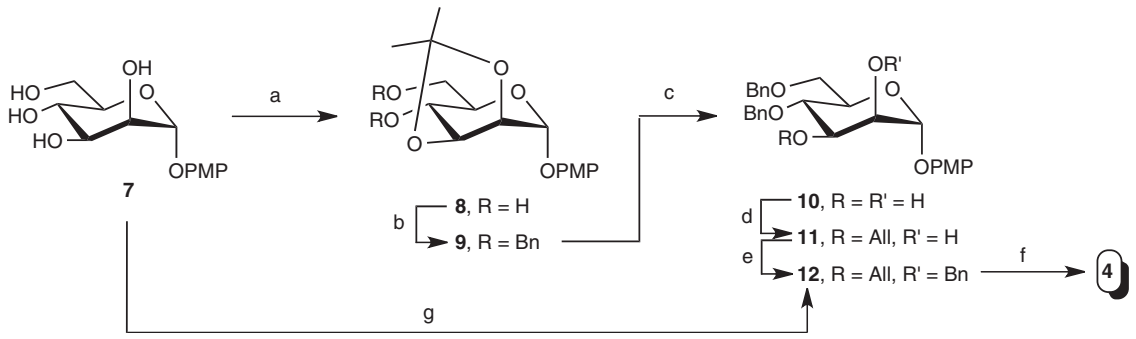
Figure 2. Target trisaccharide (**1**) and the building blocks (**2**–**4**) used for its synthesis.



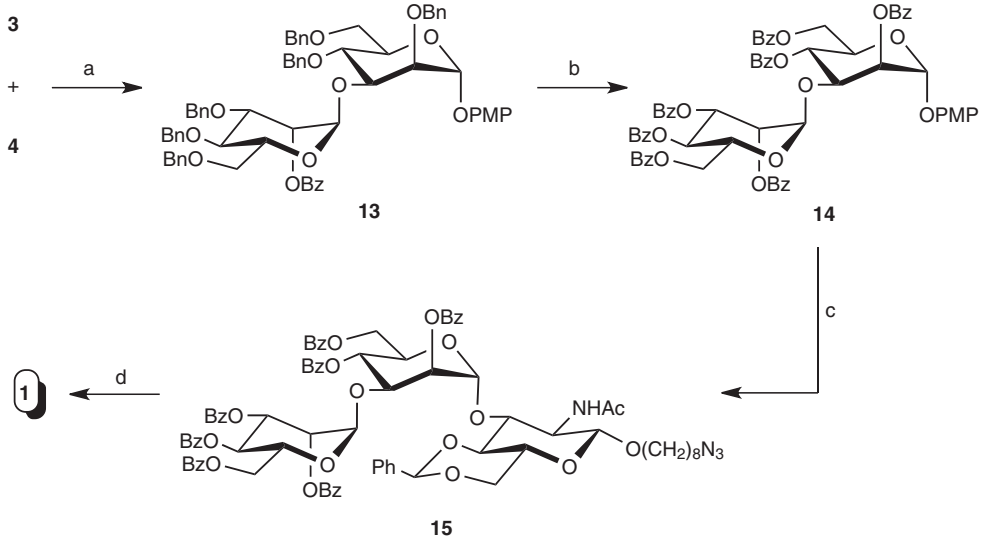
Scheme 1. Reagents and conditions: (a) $HO(CH_2)_8N_3$, *p*-TsOH, rt, 67%; (b) $PhCH(OCH_3)_2$, CH_3CN, *p*-TsOH, 50 °C, 84%.

The synthesis of *p*-methoxyphenyl glycoside acceptor **4** was somewhat more involved than the preparation of **2**, but was nevertheless straightforward (Scheme 2). The known *p*-methoxyphenyl α-D-mannopyranoside **7**[6] was converted into the mono-isopropylidene derivative **8** in 61% yield by a standard approach[7] involving the formation of the 2,3:4,6-di-*O*-isopropyldiene derivative, followed by selective hydrolysis of the more acid labile dioxane acetal protecting O4 and O6. Subsequent benzylation of **8** with NaH and benzyl bromide afforded **9** in near-quantitative yield. Hydrolysis of the isopropylidene ketal in **9** was achieved by heating in 4:1 HOAc–water to give **10** in 92% yield. The resulting diol was then converted, in 84% yield, to the 3-*O*-allyl derivative **11** by the formation of the stannylene acetal[8] and its reaction with allyl bromide and tetra-*n*-butyl ammonium iodide. A comparison of the ^{13}C NMR spectra for **10** and **11** revealed the expected downfield shift of the resonance for C3 upon allylation (71.6 in **10** to 79.6 in **11**). Benzylation of **11** gave **12**, which was then treated with palladium chloride in methanol to remove the allyl group, affording **4** in 77% yield over two steps. It was possible to synthesize alcohol **4** more efficiently, in only three steps, from **7** by treatment first with di-*n*-butyltin oxide and then allyl bromide followed by benzylation. Under these conditions, the fully protected mannopyranoside **12** was produced in 57% overall yield from **7**.

With the required building blocks in hand, trisaccharide **1** was assembled as illustrated in Scheme 3. Reaction of trichloroacetimidate **3**[4] with alcohol **4** at −30 °C in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) resulted in the formation of the expected disaccharide **13** in 86% yield. As the final target molecule possessed an azido group, it was necessary to replace the benzyl ethers in **13** with protecting groups that could, once the trisaccharide was assembled, be removed while leaving the azido



Scheme 2. Reagents and conditions: (a) (i) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH, acetone–DMF (1:4), rt; (ii) HOAc–H_2O (3:1), rt, 61%; (b) NaH, BnBr, DMF, 0 °C→rt, 99%; (c) HOAc–H_2O (4:1), 50 °C, 92%; (d) (i) *n*-Bu_2SnO, toluene, 105 °C; (ii) *n*-Bu_4NI, AllBr, toluene, 105 °C, 84%; (e) NaH, BnBr, DMF, 0 °C→rt, 91%; (f) $PdCl_2$, CH_3OH, rt, 85%; (g) (i) *n*-Bu_2SnO, CH_3OH, 60 °C; (ii) *n*-Bu_4NI, AllBr, toluene, 110 °C, 57%.



Scheme 3. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −30 °C, 86%; (b) (i) H_2, $Pd(OH)_2$–C, CH_3OH–CH_2Cl_2 (1:1), rt; (ii) BzCl, pyridine, DMAP, rt, 92% over two steps; (c) (i) CAN, CH_3CN–H_2O (4:1); (ii) CCl_3CN, DBU, CH_2Cl_2, rt; (iii) **2**, TMSOTf, CH_2Cl_2, rt, 65% over three steps; (d) (i) CH_3OH–CH_2Cl_2 (4:1), *p*-TsOH, 40 °C; (ii) $NaOCH_3$, CH_3OH–CH_2Cl_2 (2:1), 65% over two steps.

functionality intact. Thus, hydrogenolysis of the benzyl ethers using $Pd(OH)_2$–C as the catalyst provided a disaccharide intermediate that was immediately benzoylated thus affording **14** in 92% yield over two steps. Cleavage of the *p*-methoxyphenyl glycoside in **14** with ceric ammonium nitrate in wet acetonitrile, followed by the reaction with trichloroacetonitrile and DBU, gave the corresponding glycosyl trichloroacetimidate, which, following a rapid purification on silica gel, was immediately reacted with alcohol **2**. The product of this series of transformations was trisaccharide **15**, which was obtained in 65% overall yield. Deprotection of **15** proceeded without difficulty by first acid hydrolysis of the benzylidene acetal and then debenzoylation upon reaction with sodium methoxide. Trisaccharide **1** was obtained in 65% yield over these two deprotection steps. The magnitude of the $^1J_{C1,H1}$ in the mannose residues in **1** were 172.7 and 174.0 Hz, thus confirming the α-stereochemistry.[9]

In summary, we describe herein the first synthesis of the 'primer–adaptor' trisaccharide that, in LPS from a number of *E. coli* and *K. pneumoniae* serotypes, links the O-chain and core oligosaccharide domains. The target, **1**, contains an azido group, which can be conjugated readily to other groups either directly by using 'click' chemistry with a suitable alkyne,[10] or indirectly through a more traditional approach involving reduction to the amine and subsequent amidation. The use of **1** in probing LPS biosynthesis is in progress, and the results of those investigations will be reported separately.

1. Experimental

1.1. General methods

Reactions were carried out in oven-dried glassware. Reaction solvents were distilled from appropriate dry-

ing agents before use. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on Silica Gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by charring with acidified *p*-anisaldehyde solution in EtOH. All column chromatography was performed on silica gel (40–60 μM) or Iatrobeads, which refers to a beaded Silica Gel 6RS-8060 manufactured by Iatron Laboratories (Tokyo). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 22 ± 2 °C. ^{1}H NMR spectra were recorded at 500 MHz or 600 MHz, and chemical shifts are referenced to either TMS (0.0 ppm, $CDCl_3$) or CD_3OD (3.30 ppm, CD_3OD). ^{1}H data are reported as though they were first order. ^{13}C NMR APT spectra[11] were recorded at 125 MHz, and ^{13}C chemical shifts were referenced to internal $CDCl_3$ (77.23 ppm, $CDCl_3$), or CD_3OD (48.9 ppm, CD_3OD). Assignments of resonance in NMR spectra were made on the basis of 2D NMR (^{1}H–^{1}H COSY and HMQC) experiments. The anomeric stereochemistry in the mannose residues in **1** was determined through measurement of the $^1J_{C1,H1}$.[9] In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Organic solutions were concentrated under vacuum at <40 °C. Electrospray-ionization mass spectra were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl.

1.2. 8-Azidooctyl α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

To a solution of compound **15** (152 mg, 0.10 mmol) in 3:1 CH_3OH–CH_2Cl_2 (6 mL) *p*-TsOH was added until pH 3 was obtained. The reaction was stirred at 40 °C for 12 h, and the mixture was neutralized with Et_3N and concentrated. The residue was purified by chromatography (1.5:1 EtOAc–hexane) to afford the deprotected sugar (91 mg, 72%) as a colorless oil. To a solution of the deprotected sugar (91 mg, 0.06 mmol) in 2:1 CH_3OH–CH_2Cl_2 (6 mL) a solution of $NaOCH_3$ in CH_3OH was added until a pH of 11 was obtained. The reaction was stirred at room temperature for 36 h and then neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. Chromatography of the residue on Iatrobeads (3:1 EtOAc–CH_3OH) gave **1** (40 mg, 65% for two steps) as a white solid. R_f 0.43 (2:1 EtOAc–CH_3OH); $[\alpha]_D$ +49.1 (*c* 0.5, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD, δ_H) 5.26 (d, 1H, $J_{1',2'} = 1.9$ Hz, H-1′), 5.05 (d, 1H, $J_{1'',2''} = 1.7$ Hz, H-1″), 4.38 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 4.11 (dd, 1H, $J_{1',2'} = 1.9$ Hz, $J_{2',3'} = 2.8$ Hz, H-2′), 3.95 (dd, 1H, $J_{1'',2''} = 1.7$ Hz, $J_{2'',3''} = 3.3$ Hz, H-2″), 3.90–3.61 (m, 15H, octyl OCH_2, H-3′, H-6a, H-6b, H-6′a, H-6′b, H-6″a, H-6″b, H-3″, H-5″, H-2, H-4, H-4′, NH, and H-5′), 3.55 (dd, 1H, $J_{3'',4''} = 9.7$ Hz, $J_{4'',5''} = 9.7$ Hz, H-4″), 3.50–3.43 (m, 2H, H-3 and octyl OCH_2), 3.29–3.22 (m, 3H, H-5 and octyl CH_2N$_3$), 1.98 (s, 3H, acetyl CH_3), 1.60–1.50 (m, 4H, octyl CH_2), 1.40–1.30 (m, 8H, octyl CH_2); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 103.7 (C-1″, $^1J_{C1,H1} = 172.7$ Hz), 102.7 (C-1, $^1J_{C1,H1} = 159.7$ Hz), 102.3 (C-1′, $^1J_{C1,H1} = 174.0$ Hz), 81.2 (C-5′), 80.0 (C-3′), 77.9 (C-5), 75.1 (C-4), 75.0 (C-5″), 72.6 (C-3), 72.5 (C-3″), 72.1 (C-2″), 71.6 (C-2′), 70.6 (octyl O*C*H$_2$), 69.0 (C-4″), 67.5 (C-2), 63.2 (C-6″), 63.0 (C-6), 62.6 (C-6′), 55.6 (C-4′), 52.5 (octyl *C*H$_2$N$_3$), 30.6 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 30.3 (octyl *C*H$_2$), 29.9 (octyl *C*H$_2$), 27.8 (octyl *C*H$_2$), 27.0 (octyl *C*H$_2$), 23.4 (acetyl *C*H$_3$). ESIMS *m*/*z*: calcd for $[C_{28}H_{50}N_4O_{16}]Na^+$, 721.3114; found, 721.3117.

1.3. 8-Azidooctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (2)

To a solution of compound **6** (201 mg, 0.54 mmol) in CH_3CN (4 mL) α,α-dimethoxytoluene (0.13 mL, 0.97 mmol) and *p*-TsOH (5 mg, 0.01 mmol) were added at room temperature. The mixture was stirred for 2 h at 50 °C, quenched by the addition of Et_3N, and then concentrated. The residue was purified by chromatography (10:1 CH_2Cl_2–CH_3OH) to afford **2** (208 mg, 84%) as a white solid. R_f 0.56 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ −45.1 (*c* 0.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.52–7.48 (m, 2H, Ar), 7.38–7.34 (m, 3H, Ar), 5.75 (d, 1H, $J = 5.8$ Hz, NH), 5.56 (s, 1H, ArC*H*), 4.73 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 4.34 (dd, 1H, $J_{5,6a} = 4.9$ Hz, $J_{6a,6b} = 10.4$ Hz, H-6a), 3.18 (dd, 1H, $J_{3,4} = 9.4$ Hz, $J_{4,5} = 9.4$ Hz, H-4), 3.88 (ddd, 1H, $J = 6.6, 6.6, 9.6$ Hz, octyl OCH_2), 3.80 (dd, 1H, $J_{5,6b} = 10.4$ Hz, $J_{6a,6b} = 10.4$ Hz, H-6b), 3.58–3.42 (m, 4H, H-5, octyl OCH_2, H-3, H-2), 3.26 (t, 2H, $J = 6.9$ Hz, octyl CH_2N$_3$), 2.05 (s, 3H, acetyl CH_3), 1.70–1.54 (m, 4H, octyl CH_2), 1.40–1.30 (m, 8H, octyl CH_2); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 171.7 (*C*═O), 137.1 (Ar), 129.2 (Ar), 128.3 (Ar × 2), 126.3 (Ar × 2), 101.9 (Ar*C*H), 100.7 (C-1), 81.7 (C-2), 71.1 (C-4), 70.0 (octyl O*C*H$_2$), 68.6 (C-6), 66.3 (C-5), 59.0 (C-3), 51.4 (octyl *C*H$_2$N$_3$), 29.5 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 29.1 (octyl *C*H$_2$), 28.8 (octyl *C*H$_2$), 26.6 (octyl *C*H$_2$), 25.8 (octyl *C*H$_2$), 23.6 (acetyl *C*H$_3$). ESIMS *m*/*z*: calcd for $[C_{23}H_{34}N_4O_6]Na^+$, 485.2371; found, 485.2373.

1.4. *p*-Methoxyphenyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (4)

Monosaccharide **12** (600 mg, 1.01 mmol) was dissolved in CH_3OH (60 mL) and $PdCl_2$ (600 mg, 0.34 mmol) was added. The reaction mixture was stirred for 3 h before being filtered and concentrated. The crude product was purified by chromatography (4:1 hexane–

EtOAc) to give **4** (477 mg, 85%) as a colorless oil. R_f 0.24 (4:1 hexane–EtOAc); $[\alpha]_D$ +49.2 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.25 (m, 15H, Ar), 7.01–6.98 (m, 2H, Ar), 6.81–6.77 (m, 2H, Ar), 5.54 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1), 4.88 (d, 1H, $J = 11.1$ Hz, PhCH_2), 4.82 (d, 1H, $J = 11.7$ Hz, PhCH_2), 4.66 (d, 1H, $J = 11.7$ Hz, PhCH_2), 4.65 (d, 1H, $J = 11.1$ Hz, PhCH_2), 4.57 (d, 1H, $J = 11.1$ Hz, PhCH_2), 4.48 (d, 1H, $J = 11.1$ Hz, PhCH_2), 4.19 (ddd, 1H, $J_{2,3} = J_{3,4} =$ 3.8 Hz, $J_{3,3\text{-OH}} = 9.2$ Hz, H-3), 3.94 (dd, 1H, $J_{1,2} = 1.0$ Hz, $J_{2,3} = 3.8$ Hz, H-2), 3.91–3.88 (m, 1H, H-5), 3.86–3.80 (m, 2H, H-4, H-6a), 3.77 (s, 3H, OCH_3), 3.71 (dd, 1H, $J_{5,6b} = 1.8$ Hz, $J_{6a,6b} = 11.0$ Hz, H-6b), 2.38 (d, 1H, $J_{3,3\text{-OH}} = 9.2$ Hz, 3-OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.3 (Ar), 138.4 (Ar), 138.3 (Ar), 137.6 (Ar), 128.6 (Ar), 128.4 (Ar × 2), 128.3 (Ar × 2), 128.1 (Ar × 2), 127.9 (Ar × 4), 127.7 (Ar × 3), 127.5 (Ar), 117.8 (Ar × 2), 114.6 (Ar × 2), 96.1 (C-1), 78.2 (C-2), 76.5 (C-4), 74.8 (Ph*C*H$_2$), 73.3 (Ph*C*H$_2$), 73.0 (Ph*C*H$_2$), 71.7 (C-3), 71.5 (C-5), 69.0 (C-6), 55.7 (O*C*H$_3$). ESIMS *m*/*z*: calcd for $[C_{34}H_{36}O_7]Na^+$, 579.2353; found, 579.2349.

1.5. 8-Azidooctyl 2-acetamido-2-deoxy-β-D-glucopyranoside (6)

To a solution of oxazoline **5** (245 mg, 1 mmol) in a mixture of CH_2Cl_2 (1 mL) and 8-azidooctanol (0.5 mL) anhyd *p*-TsOH (142 mg) was added. The mixture was stirred at room temperature for 5 h and then quenched by the addition basic ion-exchange resin. After gravity filtration of the mixture, the filtrate was concentrated, and the crude product was purified by chromatography (10:1 CH_2Cl_2–CH_3OH) to afford **6** (265 mg, 67%) as a white solid. R_f 0.82 (5:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ −18.5 (*c* 0.9, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.38 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 3.90–3.84 (m, 2H, octyl OCH_2, H-6a), 3.67 (dd, 1H, $J_{5,6b} = 5.7$ Hz, $J_{6a,6b} = 10.4$ Hz, H-6b), 3.62 (dd, 1H, $J_{1,2} = 8.4$ Hz, $J_{2,3} = 10.5$ Hz, H-2), 3.48–3.42 (m, 2H, octyl OCH_2, H-3), 3.34–3.22 (m, 4H, octyl CH_2N$_3$, H-5, H-4), 1.96 (s, 3H, acetyl CH_3), 1.60–1.50 (m, 4H, octyl CH_2), 1.40–1.30 (m, 8H, octyl CH_2); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 173.6 (*C*=O), 102.7 (C-1), 78.0 (C-4), 76.1 (C-3), 72.2 (C-5), 70.6 (octyl O*C*H$_2$), 62.9 (C-6), 57.5 (C-2), 52.5 (octyl *C*H$_2$N$_3$), 30.6 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 30.3 (octyl *C*H$_2$), 29.9 (octyl *C*H$_2$), 27.8 (octyl *C*H$_2$), 27.0 (octyl *C*H$_2$), 23.0 (acetyl *C*H$_3$). ESIMS *m*/*z*: calcd for $[C_{16}H_{30}N_4O_6]Na^+$, 397.2058; found, 397.2058.

1.6. *p*-Methoxyphenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (8)

To a mixture of **7**[6] (3.27 g, 11.3 mmol) in 4:1 acetone–DMF (70 mL), dimethoxypropane (24.5 mL, 200 mmol) was added, and the solution was stirred for 4 days. TLC (EtOAc) showed that all starting material had been consumed and was converted to a spot, believed to correspond to the 2,3:4,6-di-*O*-isopropylidene derivative of **7**. The mixture was then concentrated, and the resulting residue was treated with 25% aq HOAc (12 mL), and stirred for 14 h. At that time, a mixture of mono- and diisopropylidene derivatives was observed by TLC, and the reaction was quenched by the addition of Et_3N. After the concentration of the solution, the crude product was purified by chromatography (gradient elution hexane→EtOAc) to give **8** (2.25 g, 61%) as a solid. R_f 0.22 (1:1 hexane–EtOAc); $[\alpha]_D$ +67.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.00–6.97 (m, 2H, Ar), 6.85–6.82 (m, 2H, Ar), 5.67 (s, 1H, H-1) 4.38 (d, 1H, $J_{2,3} = 5.6$ Hz, H-2) 4.31 (dd, 1H, $J_{2,3} = 5.6$ Hz, $J_{3,4} = 6.2$ Hz, H-3), 3.84–3.76 (m, 7H, OCH_3, H-4, H-5, H-6a, H-6b), 2.80 (d, 1H, $J_{4,4\text{-OH}} =$ 3.9 Hz, 4-OH), 2.01 (dd, 1H, $J_{6a,6\text{-OH}} = J_{6b,6\text{-OH}} =$ 1.9 Hz, 6-OH), 1.57 (s, 3H, (CH_3)$_2$C), 1.41 (s, 3H, (CH_3)$_2$C); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 155.1 (Ar), 149.7 (Ar), 117.7 (Ar × 2), 114.6 (Ar × 2), 109.8 ((CH$_3$)$_2$*C*), 96.4 (C-1), 78.3 (C-3), 75.5 (C-2), 70.2 (C-5), 69.4 (C-4), 62.1 (C-6), 55.5 (O*C*H$_3$), 27.9 ((*C*H$_3$)$_2$C), 26.1 ((*C*H$_3$)$_2$C). ESIMS *m*/*z*: calcd for $[C_{16}H_{22}O_7]Na^+$, 349.1258; found, 349.1256.

1.7. *p*-Methoxyphenyl 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-α-D-mannopyranoside (9)

Diol **8** (1.92 g, 5.88 mmol) was dissolved in DMF (40 mL), and the solution was cooled to 0 °C. Sodium hydride (60% in oil, 688 mg, 16.5 mmol) was added, and the mixture was stirred for 20 min, after which time BnBr (1.7 mL, 14.3 mmol) was added. The solution was stirred for 16 h and warmed to room temperature. The reaction mixture was quenched by the addition of CH_3OH and partitioned between H_2O and CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated, and the resulting crude product was purified by chromatography (5:1 hexane–EtOAc) to give **9** (2.94 g, 99%) as a white solid. R_f 0.50 (4:1 hexane–EtOAc); $[\alpha]_D$ +78.8 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.33–7.24 (m, 10H, Ar), 7.06–7.02 (m, 2H, Ar), 6.80–6.78 (m, 2H, Ar), 5.67 (d, 1H, $J_{1,2} = 0.9$ Hz, H-1), 4.90 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.59 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.57 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.48 (dd, 1H, $J_{2,3} = J_{3,4} = 5.8$ Hz, H-3), 4.45 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.37 (dd, 1H, $J_{1,2} = 0.9$ Hz, $J_{2,3} = 5.8$ Hz, H-2), 3.97–3.93 (m, 1H, H-5), 3.75 (s, 3H, OCH_3), 3.71–3.67 (m, 3H, H-4, H-6a, H-6b), 1.56 (s, 3H, (CH_3)$_2$C), 1.43 (s, 3H, (CH_3)$_2$C); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.1 (Ar), 138.3 (Ar), 138.2 (Ar), 128.3 (Ar × 2), 128.2 (Ar × 2), 127.9 (Ar × 2), 127.6 (Ar × 2), 127.4 (Ar × 2), 118.1 (Ar × 2), 114.6 (Ar × 2), 109.6 ((CH$_3$)$_2$*C*), 96.6 (C-1), 78.8 (C-3), 75.9

(C-4), 75.6 (C-2), 73.3 (Ph*C*H_2), 72.9 (Ph*C*H_2), 69.13 (C-5), 69.11 (C-6), 55.6 (O*C*H_3), 28.0 (($C(CH_3)_2$C), 26.4 (($C(CH_3)_2$C). ESIMS *m*/*z*: calcd for $[C_{30}H_{34}O_7]Na^+$, 529.2197; found, 529.2199.

1.8. *p*-Methoxyphenyl 4,6-di-*O*-benzyl-α-D-mannopyranoside (10)

Compound **9** (2.94 g, 5.80 mmol) was dissolved in 80% aqueous HOAc (50 mL), heated to 50 °C, and stirred for 22 h. The reaction mixture was then concentrated, and the resulting crude product was purified by chromatography (1:1 hexane–EtOAc) to give **10** (2.49 g, 92%) as a foamy solid. R_f 0.43 (1:1 hexane–EtOAc); $[\alpha]_D$ +106.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.35–7.25 (m, 10H, Ar), 7.02–6.98 (m, 2H, Ar), 6.82–6.79 (m, 2H, Ar) 5.47 (d, 1H, $J_{1,2} = 1.5$ Hz, H-1), 4.73 (d, 1H, $J = 11.4$ Hz, PhCH_2), 4.66 (d, 1H, $J = 11.4$ Hz, PhCH_2), 4.62 (d, 1H, $J = 11.9$ Hz, PhCH_2), 4.49 (d, 1H, $J = 11.9$ Hz, PhCH_2), 4.14–4.08 (m, 2H, H-2, H-3), 3.91–3.76 (m, 6H, H-4, H-5, H-6a, OCH_3), 3.68 (dd, 1H, $J_{5,6b} = 1.8$ Hz, $J_{6a,6b} = 11.0$ Hz, H-6b), 2.51 (d, 1H, $J_{2,2\text{-OH}} = 2.3$ Hz, 2-OH), 2.38 (d, 1H, $J_{3,3\text{-OH}} = 2.8$ Hz, 3-OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.1 (Ar), 138.3 (Ar), 138.0 (Ar), 128.6 (Ar × 2), 128.3 (Ar × 2), 128.0 (Ar × 4), 127.7 (Ar × 2), 117.7 (Ar × 2), 114.6 (Ar × 2), 98.4 (C-1), 75.7 (C-4), 74.7 (Ph*C*H_2), 73.5 (Ph*C*H_2), 71.6 (C-3), 71.4 (C-2), 71.0 (C-5), 68.7 (C-6), 55.6 (O*C*H_3). ESIMS *m*/*z*: calcd for $[C_{27}H_{30}O_7]Na^+$, 489.1884; found, 489.1885.

1.9. *p*-Methoxyphenyl 3-*O*-allyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (11)

Diol **10** (900 mg, 1.93 mmol) was dissolved in toluene (60 mL) and *n*-Bu_2SnO (0.72 g, 2.89 mmol) was added. The reaction mixture was heated to 105 °C and stirred for 4.5 h, then cooled for 30 min before *n*-Bu_4NI (820 mg, 2.22 mmol) and AllBr (2.0 mL, 23.1 mmol) were added. The solution was then heated to 105 °C again and stirred for 16 h. The reaction mixture was then cooled and concentrated, and the crude product was purified by chromatography (3:1 hexane–EtOAc) to give **11** (821 mg, 84%) as an oil. R_f 0.44 (2:1 hexane–EtOAc); $[\alpha]_D$ +128.2 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.33–7.22 (m, 10H, Ar), 7.03–7.00 (m, 2H, Ar), 6.82–6.79 (m, 2H, Ar), 6.02–5.97 (m, 1H, CH_2=C*H*), 5.53 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1) 5.37 (d, 1H, $J = 17.2$ Hz, CH_2=CH), 5.24 (d, 1H, $J = 13.3$ Hz, CH_2=CH), 4.84 (d, 1H, $J = 10.8$ Hz, PhCH_2), 4.62 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.54 (d, 1H, $J = 10.8$ Hz, PhCH_2), 4.46 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.30–4.20 (m, 3H, H-2, OCH_2CH=CH_2 × 2), 3.98–3.91 (m, 3H, H-3, H-4, H-5), 3.77–3.75 (m, 4H, OCH_3, H-6a), 3.67 (d, 1H, $J_{6a,6b} = 10.9$ Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.1 (Ar), 138.3 (Ar), 138.2 (Ar), 134.5 (*C*H=CH_2),128.4 (Ar × 2), 128.3 (Ar × 2), 128.0 (Ar × 2), 127.8 (Ar × 2), 127.7 (Ar), 127.5 (Ar), 117.8 (Ar × 2), 117.4 (CH=*C*H_2), 114.6 (Ar × 2), 98.2 (C-1), 79.6 (C-3), 75.2 (Ph*C*H_2), 74.2 (C-4), 73.4 (Ph*C*H_2), 71.6 (C-5), 71.0 (O*C*H_2CH=CH_2), 68.8 (C-6), 68.5 (C-2), 55.6 (O*C*H_3). ESIMS *m*/*z*: calcd for $[C_{30}H_{34}O_7]Na^+$, 529.2197; found, 529.2197.

1.10. *p*-Methoxyphenyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (12)

1.10.1. From 11. Alcohol **11** (821 mg, 1.62 mmol) was dissolved in DMF (40 mL), and the solution was cooled to 0 °C. NaH (60% in oil, 150 mg, 3.73 mmol) was added, and the mixture was stirred for 20 min at which point BnBr (0.4 mL, 3.37 mmol) was added. The solution was stirred for 24 h, warmed to room temperature, and then CH_3OH was added. After concentration, the crude product was purified by chromatography (6:1 hexane–EtOAc) to give **13** (877 mg, 91%) as an oil. R_f 0.44 (4:1 hexane–EtOAc); $[\alpha]_D$ +65.2 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.44–7.43 (m, 2H, Ar), 7.36–7.24 (m, 13H, Ar), 6.98–6.96 (m, 2H, Ar), 6.80–6.78 (m, 2H, Ar), 6.02–5.96 (m, 1H, CH_2=C*H*), 5.48 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1), 5.37 (dd, 1H, $J = 3.5$ Hz, $J = 17.3$ Hz, CH_2=CH), 5.21 (dd, 1H, $J = 1.7$ Hz, $J = 10.4$ Hz, CH_2=CH), 4.92 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.85 (d, 1H, $J = 12.5$ Hz, PhCH_2), 4.80 (d, 1H, $J = 12.5$ Hz, PhCH_2), 4.65 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.56 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.47 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.19–4.17 (m, 2H, OCH_2CH=CH_2 × 2), 4.07 (dd, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.01–3.96 (m, 2H, H-2, H-3), 3.90 (ddd, 1H, $J_{4,5} = 9.5$ Hz, $J_{5,6a} = 4.9$ Hz, $J_{5,6b} = 1.9$ Hz, H-5), 3.80 (dd, 1H, $J_{5,6a} = 4.9$ Hz, $J_{6a,6b} = 11.0$ Hz, H-6a), 3.76 (s, 3H, OCH_3), 3.71 (dd, 1H, $J_{5,6b} = 1.9$ Hz, $J_{6a,6b} = 11.0$ Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_H) 154.9 (Ar), 150.3 (Ar), 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 135.0 (*C*H=CH_2), 128.4 (Ar), 128.3 (Ar × 3), 128.2 (Ar × 3), 128.0 (Ar), 127.8 (Ar × 2), 127.7 (Ar × 3), 127.6 (Ar), 127.4 (Ar), 117.8 (Ar × 2), 116.7 (CH=*C*H_2), 114.6 (Ar × 2), 97.2 (C-1), 79.7 (C-3), 75.1 (Ph*C*H_2), 74.8 (C-4), 74.6 (C-2), 73.3 (Ph*C*H_2), 72.8 (Ph*C*H_2), 72.3 (C-5), 71.3 (O*C*H_2CH=CH_2), 69.2 (C-6), 55.6 (O*C*H_3). ESIMS *m*/*z*: calcd for $[C_{37}H_{40}O_7]Na^+$, 619.2666; found, 619.2671.

1.10.2. From 7. *p*-Methoxyphenyl glycoside **7**[6] (1.35 g, 4.72 mmol) was dissolved in CH_3OH (135 mL), *n*-Bu_2SnO (1.3 g, 5.22 mmol) was added, and the reaction mixture was heated to 60 °C. After cooling, the mixture was concentrated to dryness and redissolved in toluene (250 mL). *n*-Bu_4NI (2.1 g, 5.69 mmol) and AllBr (6.1 mL, 70.5 mmol) were added, and the reaction mixture was heated to 110 °C and stirred for 22 h before being concentrated. The crude product was purified by

chromatography (EtOAc) to give *p*-methoxyphenyl 3-*O*-allyl-α-D-mannopyranoside (1.14 g) as an oil. The NMR spectra of this compound matched those previously reported.[12] This material was dissolved in DMF (35 mL), and the solution was cooled to 0 °C. NaH (60% in oil, 890 mg, 22.0 mmol) was added, followed 20 min later by BnBr (2.1 mL, 17.6 mmol). After stirring for 24 h while warming to room temperature, CH_3OH was added, and the solution was concentrated. Purification of the residue by chromatography (6:1 hexane–EtOAc) gave **12** (1.35 g, 57% over 2 steps) as an oil.

1.11. *p*-Methoxyphenyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (13)

To a solution of **3**[4] (96 mg, 0.17 mmol) and **4** (63 mg, 0.11 mmol) in CH_2Cl_2 (10 mL) 4 Å molecular sieves (300 mg) was added. The mixture was stirred for 0.5 h at room temperature and then cooled to −30 °C before TMSOTf (16 μL of a 10% (v:v) solution in CH_2Cl_2, 8.7 μmol) was added dropwise. After TLC indicated that the reaction was complete, the reaction mixture was quenched by the addition of Et_3N. The solution was then diluted with CH_2Cl_2 (30 mL) and filtered through Celite. The filtrate was concentrated, and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to afford **13** (106 mg, 86%) as a colorless oil. R_f 0.41 (2:1 hexane–EtOAc); $[\alpha]_D$ +28.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 8.10–8.07 (m, 2H, Ar), 7.60–7.56 (m, 1H, Ar), 7.42–7.20 (m, 32H, Ar), 6.97–6.94 (m, 2H, Ar), 6.81–6.71 (m, 2H, Ar), 5.79 (dd, 1H, $J_{1',2'} = 1.9$ Hz, $J_{2',3'} = 3.0$ Hz, H-2′), 5.48 (d, 1H, $J_{1,2} = 2.0$ Hz, H-1), 5.42 (d, 1H, $J_{1',2'} = 1.9$ Hz, H-1′), 4.92 (d, 1H, $J = 11.1$ Hz, PhCH_2O), 4.84–4.64 (m, 6H, PhCH_2O), 4.58–4.52 (m, 5H, PhCH_2O), 4.47 (d, 1H, $J = 11.9$ Hz, PhCH_2O), 4.40 (dd, 1H, $J_{2,3} = 3.1$ Hz, $J_{3,4} = 9.3$ Hz, H-3), 4.19–4.14 (m, 2H, H-4, H-3′), 4.08–4.05 (m, 3H, H-2, H-4′, H-5′), 3.91–3.87 (m, 1H, H-5), 3.82–3.75 (m, 6H, H-6a, H-6′a, H-6′b, OCH_3), 3.68 (dd, 1H, $J_{5,6b} = 1.9$ Hz, $J_{6a,6b} = 11.1$ Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.5 (*C*=O), 155.0 (Ar), 150.4 (Ar), 138.7 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 137.98 (Ar), 137.97 (Ar × 2), 133.1 (Ar), 130.0 (Ar), 128.5 (Ar × 2), 128.4 (Ar × 2), 128.35 (Ar × 2), 128.32 (Ar × 2), 128.31 (Ar), 128.27 (Ar × 2), 128.10 (Ar × 2), 128.07 (Ar × 2), 128.04 (Ar × 2), 127.82 (Ar × 2), 127.80 (Ar × 2), 127.7 (Ar × 2), 127.64 (Ar × 2), 127.59 (Ar × 2), 127.56 (Ar × 2), 127.52 (Ar × 2), 127.49 (Ar × 2), 117.9 (Ar × 2), 114.6 (Ar × 2), 99.9 (C-1), 96.5 (C-1′), 78.6 (C-3), 78.1 (C-3′), 77.4 (C-4), 75.2 (Ph*C*H_2O), 75.1 (Ph*C*H_2O), 75.0 (C-2), 74.5 (C-4′), 73.6 (Ph*C*H_2O), 73.4 (Ph*C*H_2O), 72.5 (C-5, C-5′), 72.4 (Ph*C*H_2O), 71.6 (Ph*C*H_2O), 69.4 (C-6′), 69.2 (C-2′), 69.0 (C-6), 55.7 (O*C*H_3). ESIMS *m*/*z*: calcd for $[C_{68}H_{68}O_{13}]Na^+$, 1115.4552; found, 1115.4560.

1.12. *p*-Methoxyphenyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (14)

To a solution of compound **13** (298 mg, 0.27 mmol) in CH_2Cl_2 (13 mL) and CH_3OH (13 mL) 20% $Pd(OH)_2$–C (60 mg) was added at room temperature. The reaction was stirred under a positive pressure of hydrogen for 12 h. The resulting mixture was filtered through Celite and concentrated. BzCl (0.51 mL, 4.10 mmol) and 4-(dimethylamino)pyridine (20 mg) were added to the crude residue in pyridine (5 mL). The solution was stirred for 12 h and quenched by the addition of CH_3OH and concentrated. Chromatography (2:1 hexane–EtOAc) of the residue gave **14** (297 mg, 92% over two steps) as a white solid. R_f 0.48 (2:1 hexane–EtOAc); $[\alpha]_D$ −33.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 8.26–8.23 (m, 2H, Ar), 8.14–8.10 (m, 2H, Ar), 8.10–8.06 (m, 2H, Ar), 8.02–8.00 (m, 2H, Ar), 7.88–7.84 (m, 2H, Ar), 7.78–7.76 (m, 2H, Ar), 7.74–7.70 (m, 2H, Ar), 7.68–7.64 (m, 1H, Ar), 7.58–7.46 (m, 7H, Ar), 7.42–7.36 (m, 5H, Ar), 7.34–7.28 (m, 6H, Ar), 7.22–7.20 (m, 2H, Ar), 7.04–7.00 (m, 2H, Ar), 6.78–6.74 (m, 2H, Ar), 6.08–5.98 (m, 2H, H-4, H-4′), 5.87 (dd, 1H, $J_{1,2} = 1.9$ Hz, $J_{2,3} = 3.5$ Hz, H-2), 5.72 (dd, 1H, $J_{2',3'} = 3.3$ Hz, $J_{3',4'} = 10.0$ Hz, H-3′), 5.70 (d, 1H, $J_{1,2} = 1.9$ Hz, H-1), 5.42 (d, 1H, $J_{1',2'} = 1.9$ Hz, H-1′), 5.36 (dd, 1H, $J_{1',2'} = 1.9$ Hz, $J_{2',3'} = 3.3$ Hz, H-2′), 4.84 (dd, 1H, $J_{2,3} = 3.5$ Hz, $J_{3,4} = 9.7$ Hz, H-3), 4.66–4.62 (m, 1H, H-5′), 4.60 (dd, 1H, $J_{5,6'a} = 2.4$ Hz, $J_{6'a,6'b} = 12.4$ Hz, H-6′a), 4.54–4.51 (m, 1H, H-5), 4.49–4.44 (m, 2H, H-6a, H-6′b), 4.47 (dd, 1H, $J_{5,6b} = 4.1$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.03 (*C*=O), 166.01 (*C*=O), 165.9 (*C*=O), 165.4 (*C*=O), 165.1 (*C*=O), 164.65 (*C*=O), 164.62 (*C*=O), 155.3 (Ar), 149.5 (Ar), 133.6 (Ar), 133.3 (Ar × 2), 133.22 (Ar × 2), 133.21 (Ar × 2), 132.9 (Ar × 2), 132.8 (Ar × 2), 130.0 (Ar), 129.95 (Ar), 129.75 (Ar × 2), 129.72 (Ar × 2), 129.64 (Ar × 2), 129.58 (Ar × 2), 129.53 (Ar × 2), 129.1 (Ar × 3), 128.9 (Ar × 2), 128.8 (Ar × 2), 128.7 (Ar × 2), 128.4 (Ar × 2), 128.3 (Ar), 128.29 (Ar × 2), 128.26 (Ar × 2), 128.22 (Ar × 2), 128.1 (Ar × 2), 117.8 (Ar × 2), 114.6 (Ar × 2), 99.6 (C-1′), 96.3 (C-1), 76.2 (C-3), 71.6 (C-2), 70.1 (C-2′), 69.7 (C-5), 69.4 (C-5′), 69.2 (C-3′), 68.3 (C-4), 66.4 (C-4′), 62.9 (C-6), 62.5 (C-6′), 55.5 (O*C*H_3). ESIMS *m*/*z*: calcd for $[C_{68}H_{56}O_{19}]Na^+$, 1199.3308; found, 1199.3306.

1.13. 8-Azidooctyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (15)

To a solution of compound **14** (120 mg, 0.10 mmol) in 4:1 CH_3CN–H_2O (6.4 mL) ceric ammonium nitrate

(392 mg, 0.71 mmol) was added at room temperature. The mixture was stirred for 2 h, diluted with EtOAc and washed with water. The organic layer was dried ($MgSO_4$), filtered, concentrated and purified by chromatography (2:1 hexane–EtOAc) to afford the reducing sugar (89 mg, 82%) as a colorless oil. This material was dissolved in CH_2Cl_2 (2 mL) and DBU (3 μL, 0.02 mmol), and CCl_3CN (0.13 mL, 0.12 mmol) was added. The reaction mixture was stirred at room temperature for 12 h before the solution was concentrated. The resulting residue was purified by chromatography (2:1 hexane–EtOAc) to afford the imidate (83 mg, 82%) as a colorless oil. The imidate and compound **2** (24 mg, 0.05 mmol) were dried on a high-vacuum pump for 2 h and then dissolved in CH_2Cl_2 (2 mL), and 4 Å molecular sieves (100 mg) were added. The mixture was stirred for 0.5 h at room temperature before TMSOTf (12 μL of a 10% (v:v) solution in CH_2Cl_2, 6.8 μmol) was added dropwise. The reaction mixture was stirred at room temperature for 12 h and quenched by the addition of Et_3N. The solution was then diluted with CH_2Cl_2 (30 mL) and filtered through Celite. The filtrate was concentrated and purified by chromatography (2:1 hexane–EtOAc) to afford **15** (50 mg, 65%) as a colorless oil. R_f 0.35 (2:1 hexane–EtOAc); $[\alpha]_D$ −57.1 (*c* 0.7, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 8.20–8.17 (m, 2H, Ar), 8.10–8.02 (m, 6H, Ar), 7.86–7.83 (m, 2H, Ar), 7.70–7.68 (m, 4H, Ar), 7.66–7.62 (m, 2H, Ar), 7.58–7.46 (m, 9H, Ar), 7.40–7.34 (m, 7H, Ar), 7.30–7.23 (m, 6H, Ar), 7.22–7.16 (m, 2H, Ar), 6.02 (dd, 1H, $J_{2'',3''} = 10.0$ Hz, $J_{3'',4''} = 10.0$ Hz, H-3″), 5.96–5.92 (m, 2H, H-4′, NH), 5.75 (dd, 1H, $J_{1',2'} = 1.9$ Hz, $J_{2',3'} = 3.4$ Hz, H-2′), 5.66 (dd, 1H, $J_{1'',2''} = 2.9$ Hz, $J_{2'',3''} = 10.0$ Hz, H-2″), 5.64 (s, 1H, ArC*H*), 5.62 (d, 1H, $J_{1',2'} = 1.9$ Hz, H-1′), 5.36–5.32 (m, 2H, H-1″, H-4″), 4.72 (dd, 1H, $J_{5',6'a} = 3.6$ Hz, $J_{6'a,6'b} = 11.5$ Hz, H-6′a), 4.62–4.54 (m, 3H, H-6″a, H-3′, H-1), 4.50–4.42 (m, 2H, H-6′b, H-5′), 4.38–4.32 (m, 2H, H-5″, H-6a), 4.24 (dd, 1H, $J_{3,4} = 9.7$ Hz, $J_{4,5} = 9.7$ Hz, H-4), 4.10 (dd, 1H, $J_{5'',6''b} = 2.6$ Hz, $J_{6''a,6''b} = 12.5$ Hz, H-6″b), 3.86–3.74 (m, 4H, H-6b, octyl OCH_2, H-2, H-3), 3.46 (ddd, 1H, $J = 6.8$, 6.8, 9.7 Hz, octyl OCH_2), 3.34 (ddd, 1H, $J_{4,5} = 9.7$ Hz, $J_{5,6a} = 5.0$ Hz, $J_{5,6b} = 9.7$ Hz, H-5), 3.26 (t, 1H, $J = 6.9$ Hz, octyl CH_2N_3), 2.06 (s, 3H, acetyl CH_3), 1.62–1.56 (m, 4H, octyl CH_2), 1.40–1.30 (m, 8H, octyl CH_2); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 170.2 (*C*═O), 166.8 (*C*═O), 166.0 (*C*═O), 165.6 (*C*═O × 2), 165.0 (*C*═O), 164.85 (*C*═O), 164.7 (*C*═O), 136.8 (Ar), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 133.18 (Ar), 132.87 (Ar), 132.85 (Ar), 130.0 (Ar × 2), 129.98 (Ar × 2), 129.93 (Ar × 2), 129.85 (Ar × 2), 129.76 (Ar × 2), 129.68 (Ar × 2), 129.62 (Ar), 129.3 (Ar), 129.23 (Ar), 129.19 (Ar), 129.07 (Ar), 128.99 (Ar), 128.8 (Ar × 2), 128.6 (Ar × 2), 128.5 (Ar × 2), 128.4 (Ar), 128.39 (Ar × 2), 128.38 (Ar × 2), 128.32 (Ar × 2), 128.30 (Ar × 2), 128.2 (Ar × 2), 128.1 (Ar × 2), 128.0 (Ar × 2), 126.0 (Ar × 2), 101.1 (Ar*C*H), 100.9 (C-1), 99.6 (C-1″), 97.7 (C-1′), 82.0 (C-2), 76.1 (C-3′), 75.3 (C-4), 71.6 (C-2′), 70.1 (C-4″), 69.8 (octyl O*C*H_2), 69.6 (C-5″), 69.5 (C-2″), 69.3 (C-4′), 68.6 (C-6), 68.5 (C-5′), 66.3 (C-3″), 65.9 (C-5), 64.0 (C-6′), 62.1 (C-6″), 55.6 (C-3), 51.5 (octyl *C*H_2N_3), 29.5 (octyl *C*H_2), 29.2 (octyl *C*H_2), 29.1 (octyl *C*H_2), 28.8 (octyl *C*H_2), 26.7 (octyl *C*H_2), 25.8 (octyl *C*H_2), 23.4 (acetyl *C*H_3). ESIMS *m*/*z*: calcd for $[C_{84}H_{82}N_4O_{23}]Na^+$, 1537.5262; found, 1537.5268.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.08.020.

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