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Synthesis of spacer-equipped di-, tri-, and the tetrasaccharide fragments of the deacetylated O-PS1 of *Citrobacter gillenii* O9a,9b, and a related pentasaccharide

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Abstract—The title rhamnooligosaccharides [α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-1-*O*-$(CH_2)_5$COOMe, α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-1-*O*-$(CH_2)_5$COOMe, α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-1-*O*-$(CH_2)_5$COOMe, and α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-1-*O*-$(CH_2)_5$COOMe] were synthesized in a stepwise fashion from 5-methoxycarbonylpentyl 4-azido-4,6-dideoxy-2-*O*-benzyl-α-D-mannopyranoside and orthogonally protected 1-thioglycoside glycosyl donors. The amorphous, final products were fully characterized as corresponding per-*O*-acetyl derivatives.
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Keywords: Citrobacter gillenii; Oligosaccharide synthesis; D-4-NAc-rhamnooligosaccharides

1. Introduction

Citrobacter gillenii O9a,9b is one of the *Citrobacter* strains that are found in the intestinal tract of some vertebrates, and may be associated with meningitis, brain abscesses, and neonatal sepsis in humans.[1] A characteristic feature[2] of *C. gillenii* O9a,9b is the presence of two different O-polysaccharides (PS) as parts of the lipopolysaccharide (LPS). One of them (PS2) is a homopolymer composed of α-(1→2)-linked 4-acetamido-4,6-dideoxy-α-D-mannopyranose (*N*-acetyl-D-perosamine, D-Rha *p*4NAc) residues. The other one (PS1) is also built up of *N*-acetyl-D-perosamine, and its tetrasaccharide repeating unit has the structure: →3)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→. The NMR data suggest[2] that the O-PS1 is partially 2-O-acetylated, and that the degree of acetylation of the →3-linked *N*-acetyl-D-perosamine residues is ~70%. Gel-permeation chromatography of products of mild acid hydrolysis of the *C. gillenii* O9a,9b LPS yielded PS1 and PS2 in 11.4% and 18% yield, respectively.

Rabbit antiserum raised against *C. gillenii* O9a,9b bacteria reacted with PS1 in Ouchterlony double immunodiffusion test and immunoblotting. PS2 and LPS from *Vibrio cholerae* O:1, which has a structurally related O-PS, were not reactive with the same sera. It was reasoned[2] that the unreactivity was due to the small molecular size of PS2, and to the different *N*-acyl group present in the O-PS from *V. cholerae* O:1. The structure of the tetrasaccharide repeating unit of PS1 lacking the *O*-acetyl group is the same as the tetrasaccharide part of a pentasaccharide repeating unit from the O-chain PS of *Escherichia hermanii*,[3] the bacteria often isolated from infected wounds. It is noteworthy, in this context, that the serological reactivity of the LPS of *C. gillenii* O9a,9b was not affected by O-deacetylation.[2]

2. Results and discussion

This laboratory has been involved in developing a conjugate vaccine for cholera using synthetic fragments of

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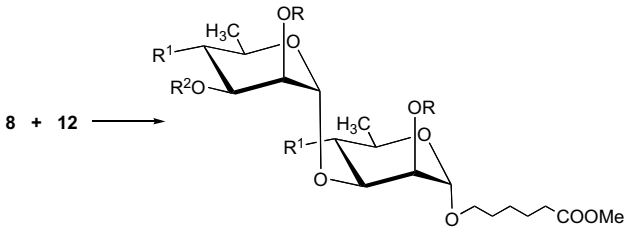
the O-PS of *V. cholerae* O:1 as the antigenic component.[4–9] The considerable structural similarity between the O-PS of *V. cholerae* O:1 and *C. gillenii* O9a,9b lies in the presence of the (1→2)-α-linked D-perosamine in both O-PSs. The two polysaccharides differ in the *N*-acyl groups present, that is, acetyl in *C. gillenii* O9a,9b as compared to 3-deoxy-L-*glycero*-tetronyl group in *V. cholerae* O:1, and in absence of the (1→3)-interglycosidic linkages and *O*-acetyl groups in *V. cholerae* O:1. The usefulness of pure, well-defined fragments of the O-PS of *C. gillenii* O9a,9b as probes for inhibition studies and studies of cross-reactivity as well as our experience in the chemical synthesis of perosamine-containing oligosaccharides[10–15] prompted us to synthesize fragments that mimic partial structures of the O-PS of *C. gillenii* O9a,9b.

Because *O*-acetyl groups are not present in the O-PS of *V. cholerae* O:1, and their presence in the O-PS of *C. gillenii* O9a,9b was found non-essential for serology,[2] we designed the synthetic schemes to yield non-acetylated oligosaccharides (Schemes 1–4). To obtain the tetrasaccharide repeating unit and the two oligosaccharide fragments from which it is formed, we chose a stepwise synthetic strategy starting with the linker (spacer)-equipped glycosyl acceptor **12**. In this way, as the linker chosen can be readily transformed into derivatives suitable for conjugation, the final oligosaccharides were obtained in form amenable to both inhibition/binding studies and preparation of neoglycoconjugates by a variety of protocols.[16–18]

The hitherto unknown compound **12** was obtained from mesylate **1**[19] following a series of known conversions, where a number of minor modifications led to improved yields of desired intermediates and/or made the individual steps experimentally less demanding. Consequently, the known[20,21] azido compound **2** was prepared



	R	R^1	R^2
13	Bn	N_3	Ac
14	Bn	N_3	H
15	Bn	NHAc	H
16	H	NHAc	H
17	Ac	NHAc	Ac

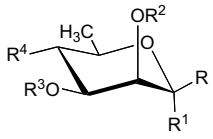
Scheme 1.

by combining advantages of each of the previous two protocols leading to it.[20,21] We used mesylate **1**, recommended by Eis and Ganem[20] but prepared by an improved procedure,[19] and treated it with NaN_3[20] in the presence of a crown ether, as used by Bundle et al.[21] with methyl 6-deoxy-1,2-*O*-isopropylidene-4-*O*-trifluoromethanesulfonyl-α-D-mannopyranoside. In this way, we were able to readily introduce the azido function while bypassing the need to use the better, trifluoromethanesulfonyloxy leaving group,[21] which would be cost prohibitive when working on a large scale. Unlike with the conversion in the absence of the crown ether, only slight discoloration of the reaction mixture occurred during the displacement reaction, and the crystalline azide **2** was obtained consistently in 80–90% yield.

Precursors to glycosyl donors bearing selectively removable protecting groups at position O-2 or O-3, benzyl ethers **3** and **4**, which were required for the synthesis of oligosaccharides containing (1→2)- and (1→3)-linkages, were obtained by selective benzylation of **2** by phase-transfer catalysis and benzylation via stannylation, respectively. The reaction time of the phase-transfer mediated alkylation could be shortened, and the yield of the conversion[22] **2**→**3** + **4** could be improved by using a larger relative amount of the phase-transfer catalyst. The mixture was resolved by column chromatography, and the 2-benzyl ether was now obtained crystalline. Some amount of the 3-benzyl ether **4** was obtained from this reaction but the bulk of this compound was obtained by benzylation of the 2,3-stannylene acetal of **2**. However, instead of applying the original procedure[20] leading to **4**, which required removal of large amount of unchanged BnBr, we employed the benzylation protocol by Nashed[23] (cf. Section 3). Acetolysis of **3** gave the hitherto unknown 1,3-di-*O*-acetyl derivatives **5** (α) and **6** (β). The 1,3-di-*O*-acetyl derivative **5** and its 1,2-O-acetylated analog **7**, prepared as described,[14,21] were subsequently transformed to the corresponding 1-thioglycosides **8** and **10**,[14,21] respectively. The β anomer **9**, formed when **5** was treated with ethanethiol and isolated by chromatography along with **8**, was also fully characterized.

5-Methoxycarbonylpentyl glycoside **12** was prepared from acetate **11** by Zemplén deacetylation. The latter was obtained from **6** and 5-methoxycarbonylpentanol[24] by $SnCl_4$-mediated[25] glycosylation, essentially as described for the preparation of the similar 3-*O*-benzyl derivative.[7] It is worth noting that the conversion **6**→**11** was fast and afforded a high yield (~80%) of the 1,2-*trans* glycoside, even though the reaction could not involve the acetoxonium ion intermediate, which was the case in previous situations involving a participating group at *O*-2.[7,25]

Oligosaccharides **16**, **21**, and **26** were synthesized (Schemes 1–3) by NIS and silver triflate-mediated con-



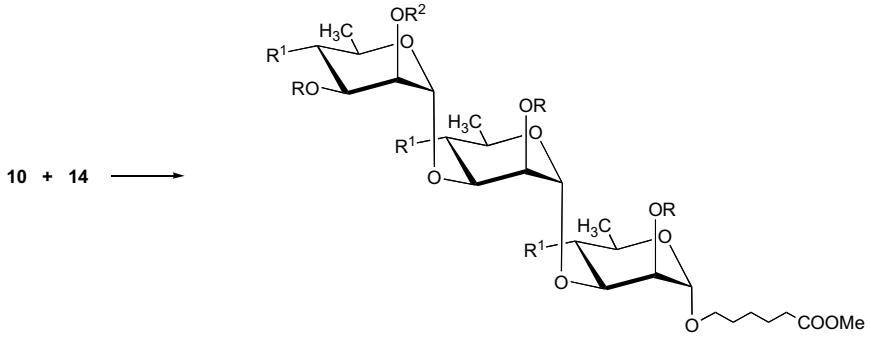
	R	R^1	R^2	R^3	R^4
1	H	OMe	H	H	OMs
2	H	OMe	H	H	N_3
3	H	OMe	Bn	H	N_3
4	H	OMe	H	Bn	N_3
5	H	OAc	Bn	Ac	N_3
6	OAc	H	Bn	Ac	N_3
7	H	OAc	Ac	Bn	N_3
8	H	SEt	Bn	Ac	N_3
9	SEt	H	Bn	Ac	N_3
10	H	SEt	Ac	Bn	N_3
11	H	O COOMe	Bn	Ac	N_3
12	H	O COOMe	Bn	H	N_3

densations of thioglycoside donors **8** or **10**[14,21] with acceptors **12**, **14**, and **19**. The products thus obtained (**13**, **18**, and **23**) were deacetylated (Zemplén), to afford glycosyl acceptors (**14**, **19**, and **24**) for further extension of the oligosaccharide chain. It is noteworthy that the fast-atom-bombardment (FAB) mass-spectrum of compound **13** showed a peak at *m*/*z* 685.4, instead of the expected peak at *m*/*z* 710([M+H]$^+$). It reflected reduction of one azido group to an amino group during the course of mass-spectrometric analysis.[26] The same phenomenon was observed with all compounds containing more than one azido group.

While the one-pot conversion of a single azide group to the *N*-acetamido group by catalytic hydrogenolysis in the presence of Ac_2O and MeOH works well,[27] similar conversions described here with compounds containing multiple azido groups were achieved more cleanly using a two-step process. The azido groups in **14**, **19**, and **24** were first reduced with H_2S in aqueous pyridine,[21,28] and subsequent N-acetylation with acetic anhydride in the presence of methanol gave the acetamido derivatives **15**, **20**, and **25**. Catalytic hydrogenolysis of the foregoing substances then gave the target oligosaccharide fragments **16**, **21**, and **26**.

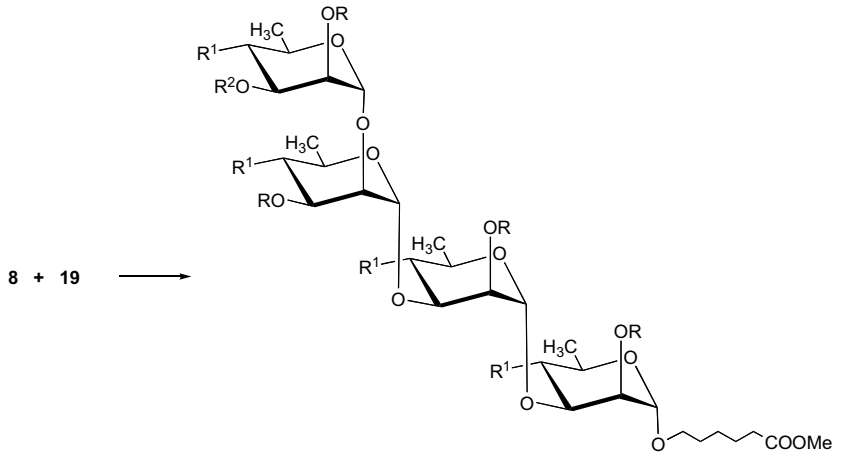
The purpose of making the related pentasaccharide **31** was its use in inhibition and other immunochemical studies with antibodies specific for the O-PS of *C. gillenii* O9a,9b. It was obtained by coupling of donor **8** with tetrasaccharide acceptor **24**, to give fully protected pentasaccharide **28**, followed by conversions (Scheme 4) similar to those described above.

The molecular mass of the deprotected oligosaccharides was verified by mass spectrometry and their structures follow from the mode of synthesis. The



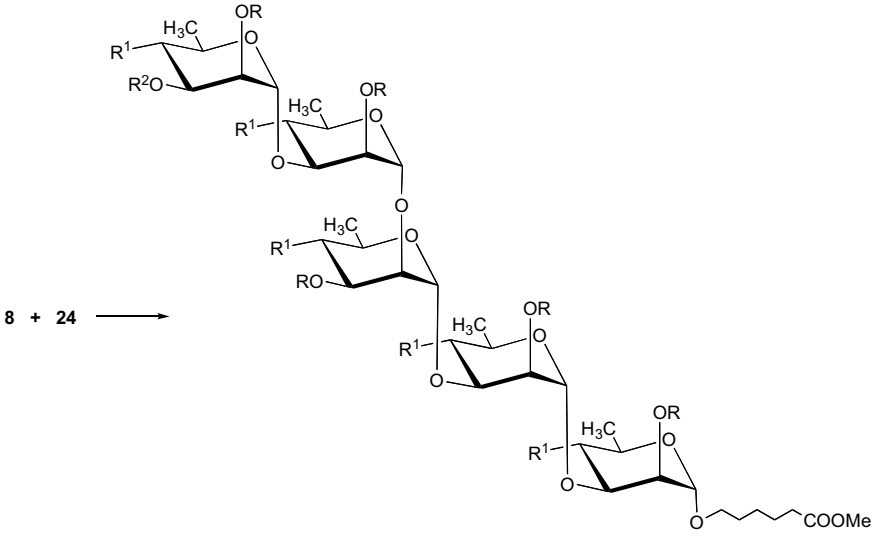
	R	R^1	R^2
18	Bn	N_3	Ac
19	Bn	N_3	H
20	Bn	NHAc	H
21	H	NHAc	H
22	Ac	NHAc	Ac

Scheme 2.



	R	R^1	R^2
23	Bn	N_3	Ac
24	Bn	N_3	H
25	Bn	NHAc	H
26	H	NHAc	H
27	Ac	NHAc	Ac

Scheme 3.



	R	R^1	R^2
28	Bn	N_3	Ac
29	Bn	N_3	H
30	Bn	NHAc	H
31	H	NHAc	H
32	Ac	NHAc	Ac

Scheme 4.

compounds were fully characterized as per-O-acetates **17**, **22**, **27**, and **32** whose NMR data were fully consistent with their structures.

3. Experimental

3.1. General methods

Unless stated otherwise, optical rotations were measured at ambient temperature for solutions in chloroform ($c \sim 1$), with a Perkin–Elmer automatic polarimeter, Model 341 or with a Jasco automatic polarimeter, Model P-2000. All reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 coated glass slides (Whatman or Analtech). Column chromatography was performed by gradient elution from columns of silica gel. Solvent mixtures less polar than those used for TLC were used at the onset of development. NMR spectra of monosaccharide derivatives were measured at 22 °C, at 300 MHz (^{1}H) and 75 MHz (^{13}C), with a Varian Mercury spectrometer. NMR spectra of oligosaccharides were measured at 300 or 600 MHz (^{1}H) and 75 or 150 MHz (^{13}C), with a Varian Mercury or Bruker Avance spectrometers. All assignments were supported by homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. When reporting assignments of NMR signals of oligosaccharides, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycon, and are identified by a Roman numeral superscript in listings of signal assignments. When reporting NMR data, the nuclei belonging to the spacer aglycon are denoted with a prime (′). ^{1}H NMR spectra of some compounds containing NHAc groups showed presence of isomers and/or considerable broadening of signals, and coupling constants in such cases are not reported. When presence of isomers was evident, only resonances characteristic of the more abundant isomer are reported. Liquid chromatography–electron spray-ionization mass spectrometry (ESI-MS) was performed with a Hewlett–Packard 1100 MSD spectrometer. Attempts have been made to obtain correct analytical data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtained within ±0.4%. The $[\alpha]_D$ values reported for such materials may not be quite accurate. Structures of these compounds follow unequivocally from the mode of synthesis and *m*/*z* values found in their mass spectra, and TLC and NMR spectroscopy verified their purity. Palladium-on-charcoal catalyst (5%, ESCAT 103) was a product of Engelhard Industries. 1,2-Di-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**7**) was prepared as described.[21] Before final freeze-drying of the deprotected oligosaccharides **16**, **21**, **26**, and **31**, their solutions in water (HPLC quality) were passed through the Anotop syringe filter (Whatman, 0.2 μm porosity). Solutions in organic solvents other than alcohols were dried with anhydrous Na_2SO_4, and concentrated at <40 °C/2 kPa.

3.2. General procedure for the NIS/AgOTf-mediated glycosylations

A mixture of glycosyl donor (1.3 mmol), acceptor (1 mmol) and finely powdered 4 Å molecular sieves (0.5 g) in CH_2Cl_2 (10–15 mL) was stirred under argon for 15 min. The mixture was cooled to ~10 °C, and solid NIS (1.4 mmol) was added, followed by a solution of AgOTf (0.4 mmol) in toluene (4 mL). The stirring was continued for 3 min at the same temperature, cooling was terminated, and when TLC showed that the reaction was complete (~15 min) the mixture was neutralized with Et_3N, partitioned between CH_2Cl_2 and satd aq $NaHCO_3$, concentrated, and the residue was purified by chromatography.

3.3. Methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (2)

A mixture of methyl 4-*O*-methanesulfonyl-6-deoxy-α-D-mannopyranoside[19] (**1**, 142.2 g, 0.555 mol), NaN_3 (87 g, 1.338 mol), and dicyclohexano-15-crown-5 (3.7 g, 16.8 mmol) in DMF (1 L) was heated with vigorous stirring at 100 °C until TLC (3:1 hexane–acetone) showed that all starting material was consumed. DMF was evaporated from the yellow reaction mixture, the residue was treated with CH_2Cl_2, and the mixture was filtered through a Celite pad. The filtrate was concentrated and the residue was purified by chromatography to give, after crystallization from isopropyl ether–hexane, 95.5 g (85%) of pure **2**, mp 84–85 °C, Ref. 20 mp 83–84 °C, 72% yield. CIMS *m*/*z* 221 $[M+NH_4]^+$.

3.4. Methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (3) and methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (4)

3.4.1. A mixture of **2** (20 g, 98.4 mmol) and Bu_4NBr (2.7 g, 8.375 mmol), CH_2Cl_2 (300 mL), aqueous 20% NaOH (300 mL), and BnBr (12 mL, 98.4 mmol) was vigorously stirred overnight, when TLC (3:1 hexane–EtOAc) showed almost complete conversion of the starting material. The phases were separated, the aqueous phase was extracted with CH_2Cl_2, and the combined organic phase was washed with water and concentrated. Chromatography gave first methyl 4-azido-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (2.95 g, 7.8%), $[\alpha]_D$ +60.4 (*c* 0.9), Ref. 22 $[\alpha]_D$ +65.9 (*c* 2.5). CIMS: *m*/*z* 401 $[M+NH_4]^+$.

Eluted next was the 2-*O*-benzyl derivative **3** (18.97 g, 65%), mp 35–36 °C (from hexane), $[\alpha]_D$ +6.4 (*c* 0.7), Ref. 22 $[\alpha]_D$ +8.54 (*c* 5.2), for amorphous **3** obtained in 56% yield. CIMS: *m*/*z* 311 $[M+NH_4]^+$. Anal. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.53; N 14.33. Found: C, 57.08; H, 6.46; N, 14.36.

Eluted last was the 3-*O*-benzyl derivative **4**, (4.75 g, 16.5%), CIMS: *m*/*z* 311 $[M+NH_4]^+$. ^{1}H NMR data recorded for **3** and **4** agreed with those reported.[20–22]

3.4.2. Bu_2SnO (40.0 g, 164.7 mmol) was added to a solution of **2** (33 g, 162.4 mmol) in MeOH (~1 L) and the mixture was heated under reflux until a clear solution was obtained (~1–2 h). Toluene (~100 mL) was added and solvents were evaporated. After the residue had been dried in a vacuum oven at 40 °C for 2 h, it was dissolved in DMF (200 mL), benzyl bromide (38 mL, 317 mmol) and Bu_4NI (1 g) was added, and the mixture was heated at ~100 °C until TLC showed complete disappearance of the starting material (4–6 h). After concentration, excess of BnBr was evaporated at 60 °C/133 Pa, and the residue was purified by chromatography to give the desired compound **4** as a light-yellow thick oil (40 g, 84%), which was identical with the material described above.

3.5. 1,3-Di-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-(5) and β-D-mannopyranose (6)

A solution of **3** (19.86 g, 67.7 mmol) in Ac_2O–AcOH–H_2SO_4 (50:20:0.01, 480 mL) was kept at room temperature for 45 min, when TLC (40:1 toluene–acetone) showed that the reaction was complete. NaOAc trihydrate was added to neutralize H_2SO_4, and the pH was adjusted to ~7 with aqueous Na_2CO_3 and $NaHCO_3$. The mixture was extracted with dichloromethane to give, after concentration, crystalline residue **5** (23 g, 93%), mp 60–62 °C (from ethanol), $[\alpha]_D$ +49.5 (*c* 0.7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.12 (d, 1H, $J_{1,2} = 2.0$ Hz, H-1), 5.05 (dd, 1H, $J_{2,3} = 3.2$, $J_{3,4} = 10.4$ Hz, H-3), 4.73, 4.55 (2d, 1H each, $^2J = 12.5$ Hz, CH_2Ph), 3.81 (dd, 1H, H-2), 3.74–3.63 (m, 2H, H-4,5), 2.11, 2.06 (2s, 3H each, 2COCH_3), 1.36 (d, 3H, $J_{5,6} = 6.0$ Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 90.93 (C-1), 73.41 (C-2), 72.90 (*C*H_2Ph), 71.85 (C-3), 69.44 (C-5), 62.04 (C-4), 20.95, 20.77 (2CO *C*H_3), 18.39 (C-6). CIMS: *m*/*z* 381 $[M+NH_4]^+$. Anal. Calcd for $C_{17}H_{21}N_3O_6$: C, 56.19; H, 5.83; N, 11.56. Found: C, 56.43; H, 5.82; N, 11.62.

The mother liquor was purified by chromatography, to give first 400 mg of the α-anomer **5**, total yield, 95%.

Eluted next was a small amount of material that was identified by NMR spectroscopy as the β-anomer **6**, $[\alpha]_D$ −12.7 (*c*, 5.1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.65 (d, 1H, $J_{1,2} = 0.9$ Hz, H-1), 4.83, 4.65 (2d, partially overlapped, $^2J = 12.2$ Hz, CH_2Ph), 4.78 (dd, partially overlapped, $J_{2,3} = 3.2$, $J_{3,4} = 10.5$ Hz, H-3), 4.01 (br d, 1-H, H-2), 3.64 (t, $J = 10.0$ Hz, H-4), 3.44–3.34 (m, 1H, H-5), 2.11, 2.00 (2s, 3H each, 2COCH_3), 1.40 (d, 3H, $J_{5,6} = 6.0$ Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 92.14 (C-1), 74.94 (*C*H_2Ph), 74.32 (C-3), 73.91 (C-2), 71.84 (C-5), 61.81 (C-4), 20.79, 20.63 (2CO *C*H_3), 18.20 (C-6).

3.6. Ethyl 3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-1-thio-α-(8) and β-D-mannopyranoside (9)

Ethanethiol (7.8 mL, 105 mmol) followed by $BF_3{\cdot}Et_2O$ (10.3 mL, 83.7 mmol) was added to a solution of **5**[14,21] (30 g, 82.6 mmol) in dry CH_2Cl_2 (660 mL), which had been stirred with molecular sieves (4 Å, 3 g) for 15 min. The stirring was continued for 20 min at room temperature, when TLC (10:1 hexane–EtOAc) showed that the reaction was complete. After neutralization with satd aq $NaHCO_3$, the product was extracted with CH_2Cl_2, the organic layer was washed with water and concentrated. The residue was purified by chromatography to give first the α-anomer **8** (25 g), $[\alpha]_D$ +123 (*c* 1.6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.30 (d, 1H, $J_{1,2} = 1.2$ Hz, H-1), 5.04 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 10.3$ Hz, H-3), 4.71, 4.54 (2d, 1H each, $^2J = 12.3$ Hz, CH_2Ph), 4.04–3.96 (m, 2H, H-2,5), 3.68 (t, 1H, $J = 10.2$ Hz, H-4), 2.70–2.51 (m, 2H, CH_2CH$_3$), 2.07 (s, 3H, COCH_3), 1.37 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6), 1.27 (t, 3H, $J = 7.3$ Hz, CH$_2$CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 81.53 ($J_{C\text{-}1,H\text{-}1} = 164.2$ Hz, C-1), 76.34 (C-2), 72.71 (C-3), 72.49 (*C*H_2Ph), 67.22 (C-5), 62.84 (C-4), 25.22 (*C*H_2CH$_3$), 20.78 (CO*C*H_3), 18.25 (C-6), 14.74 (CH$_2$*C*H_3). CIMS: *m*/*z* 383 $[M+NH_4]^+$. Anal. Calcd for $C_{17}H_{23}N_3O_4S$: C, 55.87; H, 6.34; N, 11.50; S, 8.77. Found: C, 55.99; H, 6.23; N, 11.54; S, 8.75.

Eluted later was the β-anomer **9** (2.2 g), $[\alpha]_D$ −95 (*c* 1.6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.79 (dd, partially overlapped, $J_{2,3} = 3.2$, $J_{3,4} = 10.4$ Hz, H-3), 4.80, 4.62 (2d, partially overlapped, $^2J \sim 12.0$ Hz, CH_2Ph), 4.60 (d, partially overlapped, $J_{1,2} \sim 1.0$ Hz, H-1), 4.03 (dd, 1H, H-2), 3.65 (t, 1H, H-4), 3.31–3.22 (m, 1H, H-5), 2.71 (q, 2H, $J = 7.4$ Hz, CH_2CH$_3$), 1.98 (s, 3H, COCH_3), 1.40 (d, 3H, $J_{5,6} = 6.0$ Hz, H-6), 1.29 (t, 3H, $J = 7.5$ Hz, CH$_2$CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 84.31 ($J_{C\text{-}1,H\text{-}1} = 150.6$ Hz, C-1), 77.41 (C-2), 76.18 (C-3), 76.08 (*C*H_2Ph), 75.65 (C-5), 62.60 (C-4), 27.00 (*C*H_2CH$_3$), 21.03 (CO*C*H_3), 18.95 (C-6), 15.34 (CH$_2$*C*H_3). CIMS: *m*/*z* 383 $[M+NH_4]^+$. Anal. Calcd for $C_{17}H_{23}N_3O_4S$: C, 55.87; H, 6.34; N, 11.50; S, 8.77. Found: C, 56.17; H, 6.26; N, 11.52; S, 8.76.

The intermediate, mixed fraction (1.85 g) was purified by chromatography, to give more **8** (665 mg, total yield, 85%) and **9** (625 mg, total yield, 9.4%).

3.7. 5-Methoxycarbonylpentyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (12)

$SnCl_4$ (3.5 mL, 29.5 mmol) was added to a solution of **5** (8.25 g, 22.7 mmol) in dry CH_2Cl_2 (200 mL) and the

mixture was stirred for 20 min with exclusion of atmospheric moisture. A solution of 5-(methoxycarbonyl)pentanol[24] (4.3 g, 29.5 mmol) in dry CH_2Cl_2 (30 mL) was added to a slightly yellow reaction mixture and, when the reaction was complete (~40 min, TLC in 40:1 toluene–acetone), the mixture was neutralized with satd aq $NaHCO_3$. The product was extracted with CH_2Cl_2 and, after concentration, chromatography gave 5-methoxycarbonylpentyl 3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**11**, 8.28 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.09 (dd, 1H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 10.3 Hz, H-3), 4.73 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.66, 4.57 (2d, 1H each, ^2J = 12.1 Hz, CH_2Ph), 3.80 (dd, 1H, H-2), 3.67 (s, partially overlapped, COOCH_3), 3.67–3.43 (m, partially overlapped, H-4,5,1′a), 3.37–3.34 (2t, 1H, J = 6.5 Hz, H-1′b), 2.31 (t, 2H, J = 7.3 Hz, H-5′a,b), 2.07 (s, 3H, COCH_3), 1.71–1.51 (m, 4H, H-4′a,b,2′a,b), 1.39–1.31 (m, 5H, H-3′a,b, incl. d, 1.35, $J_{5,6}$ ~ 5.8 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 97.57 ($J_{C\text{-}1,H\text{-}1}$ = 169.1 Hz, C-1), 75.00 (C-2), 73.14 (*C*H_2Ph), 72.53 (C-3), 67.53 (C-1′), 66.82 (C-5), 62.74 (C-4), 51.43 (COO*C*H_3), 33.84 (C-5′), 28.92 (C-2′), 25.58 (C-3′), 24.57 (C-4′), 20.89 (CO*C*H_3), 18.38 (C-6). FABMS: *m*/*z* 450 $[M+H]^+$, 472 $[M+Na]^+$.

A solution of the foregoing acetylated compound **11** (5.66 g, 15.58 mmol) in MeOH (150 mL) was treated with 1 M NaOMe (~5 mL). After 18 h, TLC (5:1 hexane–EtOAc) showed that the deacetylation of **12** was complete. The solution was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, concentrated, and chromatography of the residue gave **12** (4.38 g, 85%), $[\alpha]_D$ +19.4 (*c* 4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.80 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.73, 4.57 (2d, 1H each, ^2J = 11.7 Hz, CH_2Ph), 3.84 (ddd, 1H, $J_{2,3}$ = 3.7, $J_{3,4}$ = 10.1 Hz, H-3), 3.67 (dd, partially overlapped, 1H, H-2), 3.66 (s, partially overlapped, COOCH_3), 3.63, 3.60 (2t, partially overlapped, J = 6.8 Hz, H-1′a), 3.53–3.44 (m, 1H, H-5), 3.36, 3.33 (2t, 1H, H-1′b), 3.26 (t, 1H, J = 9.9 Hz, H-4), 2.60 (d, 1H, $J_{3,OH}$ = 10.3 Hz, OH), 2.30 (t, 2H, J = 7.4 Hz, H-5′a,b), 1.68–1.50 (m, 4H, H-4′a,b,2′a,b), 1.39–1.26 (m, 5H, H-3′a,b, incl. d, 1.31, $J_{5,6}$ = 6.2 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 96.53 (C-1), 77.36 (C-2), 72.84 (*C*H_2Ph), 70.22 (C-3), 67.28 (C-1′), 66.39 (2C, C-4,5), 51.36 (COO*C*H_3), 33.73 (C-5′), 28.85 (C-2′), 25.51 (C-3′), 24.47 (C-4′), 18.24 (C-6). FABMS: *m*/*z* 408 $[M+H]^+$, 430 $[M+Na]^+$. Anal. Calcd for $C_{20}H_{29}N_3O_6$: C, 58.95; H, 7.17; N, 10.31. Found: C, 59.10; H, 7.19; N, 10.23.

3.8. 5-Methoxycarbonylpentyl (3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α- D-mannopyranoside (13)

The glycosyl donor **8** (7.18 g, 19.65 mmol) and acceptor **12** (6.16 g, 15.12 mmol) were coupled as described in Section 3.2, to give after chromatography (10:1→5:1 hexane–EtOAc) **13** (9.385 g, 89%), $[\alpha]_D$ +65 (*c* 0.8). ^{1}H NMR (300 MHz, C_6D_6): δ 5.63 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 10.0 Hz, H-3^{II}), 5.28 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1^{II}), 4.81 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1^{I}), 4.61, 4.48 (2dd, 1H each, ^2J = 11.8 and 12.1 Hz, 2CH_2Ph), 4.22 (dd, 1H, H-2^{II}), 4.14 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.8 Hz, H-3^{I}), ~3.87 (m, partially overlapped, H-5^{II}), 3.81 (t, partially overlapped, H-4^{II}), 3.76 (dd, 1H, H-2^{I}), 3.71–3.55 (m, 2H, H-4^{I},5^{I}), 3.47, 3.44 (2t, 1H, J = 6.4 Hz, H-1′a), 3.38 (s, 3H, COOCH_3), 3.13, 3.09 (2t, 1H, H-1′b), 2.06 (t, 2H, J = 7.4 Hz, H-5′), 1.74 (s, 3H, COCH_3), 1.52–1.42 (m, 2H, H-4′a,b), 1.35–1.09 (m, 10H, H-2′a, b,3′a,b, incl. 2d at 1.24, 1.22, $J_{5,6}$ = 6.1 Hz, H-6^{I,II}). ^{13}C NMR (75 MHz, C_6D_6): δ 100.34 (C-1^{II}), 97.88 (C-1^{I}), 78.39 (C-3^{I}), 78.15 (C-2^{I}), 76.25 (C-2^{II}), 73.72, 73.43 (2*C*H_2Ph), 72.85 (C-3^{II}), 68.64 (C-5^{II}), 68.20 (C-5^{I}), 68.05 (C-1′), 65.53 (C-4^{I}), 63.55 (C-4^{II}), 51.38 (COO*C*H_3), 34.20 (C-5′), 29.60 (C-2′), 26.25 (C-3′), 25.19 (C-4′), 20.79 (CO*C*H_3), 18.97 (C-6^{I}), 18.84 (C-6^{II}). FABMS: *m*/*z* 685.4 $[M+H-2N+2H]^+$, 843.3 $[M+Cs]^+$, 717.1 $[M+Li]^+$. Anal. Calcd for $C_{35}H_{46}N_6O_{10}$: C, 59.14; H, 6.52; N, 11.82. Found: C, 58.95; H, 6.52; N, 11.72.

3.9. 5-Methoxycarbonylpentyl (4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (14)

De-O-acetylation (Zemplén) of **13** and chromatography (15:1 toluene–EtOAc) gave **14** (4.23 g, 94%), $[\alpha]_D$ +54 (*c* 1.2). ^{1}H NMR (C_6D_6, 300 MHz): δ 5.22 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1^{II}), 4.81 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1^{I}), 4.50, 4.40 (2dd, 2H each, ^2J = 11.9 Hz, 2CH_2Ph), 4.12 (dd, partially overlapped, 1H, $J_{3,4}$ = 3.1, $J_{2,3}$ = 9.7 Hz, H-3^{I}), ~4.11 (ddd, partially overlapped, H-3^{II}), 3.90 (dd, 1H, H-2^{II}), 3.81–3.75 (m, partially overlapped, H-2^{I},5^{II}), 3.72 (t, partially overlapped, J = 9.7 Hz, H-4^{I}), 3.66–3.56 (m, 1H, H-5^{I}), 3.34 (t, partially overlapped, J = 9.9 Hz, H-4^{II}), 3.48, 3.45 (2t, 1H, J = 6.2 Hz, H-1′a), 3.37 (s, 3H, COOCH_3), 3.12, 3.09 (2t, 1H, H-1′b), 2.17 (d, 1H, $J_{3,OH}$ = 10.1 Hz, OH), 2.06 (t, 2H, J = 7.4 Hz, H-5′a,b), 1.52–1.42 (m, 2H, H-4′a,b), 1.37–1.09 (m, 10H, H-2′a,b, 3′a,b, incl. 2d at 1.28, 1.23, $J_{5,6}$ = 6.2 Hz, H-6^{I,II}, in that order). ^{13}C NMR (75 MHz, C_6D_6): δ 100.05 (C-1^{II}), 97.69 (C-1^{I}), 78.96 (C-3^{I}), 78.37 (C-2^{II}), 78.13 (C-2^{I}), 73.56, 73.16 (2*C*H_2Ph), 71.25 (C-3^{II}), 68.17 (C-5^{I}), 68.13 (C-1′), 68.09 (C-5^{II}), 67.03 (C-4^{II}), 65.69 (C-4^{I}), 51.37 (COO*C*H_3), 34.20 (C-5′), 29.59 (C-2′), 26.25 (C-3′), 25.19 (C-4′), 18.04 (C-6^{I}), 18.88 (C-6^{II}). FABMS: *m*/*z* 643.4 $[M+H-2N+2H]^+$, 691.4 $[M+Na]^+$. Anal. Calcd for $C_{33}H_{44}N_6O_9$: C, 59.57; H, 6.63; N, 12.57. Found: C, 59.45; H, 6.76; N, 12.76.

3.10. 5-Methoxycarbonylpentyl (4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (15)

A gentle stream of H_2S was passed through a solution of **14** (500 mg, 0.75 mmol) in 2:1 pyridine–water (5 mL) for 30 min at 40 °C. The mixture was stirred at this temperature for 16 h, when TLC (10:1:0.1 CH_2Cl_2–MeOH–concd NH_4OH) showed that the reaction was complete. A stream of nitrogen was passed through the reaction mixture for 15 min, and the solution was co-concentrated with several co-evaporations of water to remove pyridine. After co-evaporation with toluene, to remove water, the residue was dissolved in MeOH (20 mL) and Ac_2O (1 mL) was added. After 16 h, TLC (3:2 CH_2Cl_2–acetone) showed that the reaction was complete. The mixture was concentrated and chromatography of the residue (2:1→1:1 CH_2Cl_2–acetone) gave **15** (390 mg, 75%), mp 176.5–177 °C (from acetone), $[\alpha]_D$ +35.4 (*c* 0.8). ^{1}H NMR (600 MHz, ~1:1 $CDCl_3$–C_6D_6): δ 5.87 (d, 1H, $J_{4,NH} = 9.8$ Hz, NH^I), 5.18 (d, 1H, $J_{4,NH} = 9.2$ Hz, NH^{II}), 5.00 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1^{II}), 4.80 (d, 1H, $J_{1,2} = 2.0$ Hz, H-1^I), 4.78–4.50 (4d partially overlapped, 2CH_2Ph), 4.25 (q, 1H, $J =$ 9.8 Hz, H-4^I), 4.00 (q, 1H, $J = 9.8$ Hz, H-4^{II}), 3.94 (dd, $J_{2,3} = 3.1$, $J_{3,4} = 10.4$ Hz, H-3^I), 3.70 (dd, $J_{2,3} =$ 3.4 Hz, H-2^{II}), 3.69 (br t, H-2^I), 3.67 (m, 2H, H-3^{II},5^I), 3.60 (m, 1H, 1′a), 3.45 (m, 1H, H-5^{II}), 3.42 (s, 3H, $COOCH_3$), 3.27 (m, 1H, H-1′b), 2.66 (br d, 1H, OH), 2.21–2.12 (m, 2H, H-5′a,b), 1.80, 1.79 (2s, 6H, 2$COCH_3$), 1.59–1.52 (m, 3H, H-4′a,b,2′a), 1.48–1.42 (m, 1H, H-2′b), 1.40–1.29 (m, 1H, H-3′a), 1.27 (d, 3H, $J_{5,6} = 6.0$ Hz, H-6^I), 1.25–1.19 (m, 1H, H-3′b), 1.10 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6^{II}). ^{13}C NMR (150 MHz, ~1:1 $CDCl_3$–C_6D_6): δ 99.26 (C-1^{II}), 97.12 (C-1^I), 77.52 (C-2^{II}), 77.33 (C-2^I), 76.66 (C-3^I), 72.70, 72.01 (2*C*H_2Ph), 69.41 (C-3^{II}), 67.99 (C-5^{II}), 67.84 (C-5^I), 66.85 (C-1′), 53.99 (C-4^{II}), 52.68 (C-4^I), 51.22 ($COOCH_3$), 33.82 (C-5′), 28.62 (C-2′), 26.00 (C-3′), 24.44 (C-4′), 23.13, 23.08 (2$COCH_3$), 18.09 (C-6^I), 17.94 (C-6^{II}). FABMS: *m*/*z* 701 $[M+H]^+$, 723 $[M+Na]^+$. Anal. Calcd for $C_{37}H_{52}N_2O_{11}$: C, 63.41; H, 7.49; N, 4.00. Found: C, 63.44; H, 7.39; N, 3.97.

3.11. 5-Methoxycarbonylpentyl (4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-4,6-dideoxy-α-D-mannopyranoside (16) and 5-methoxycarbonylpentyl (4-acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranoside (17)

A mixture of compound **15** (140 mg) and 5% palladium-on-charcoal catalyst (140 mg) in MeOH (40 mL) was stirred under hydrogen overnight at 45 °C. TLC (5:1 $CHCl_3$–MeOH) showed that the reaction was complete. After filtration and evaporation of the solvent, the residue was eluted from a small column of silica gel using 5:1 $CHCl_3$–MeOH as eluent to give, after freeze-drying and additional drying at 40 °C/133 Pa, **16** (99.5 mg, 96%), $[\alpha]_D$ +8.2 (*c* 1.7, H_2O). Definite signals in the ^{1}H NMR spectrum (600 MHz, D_2O, 50 °C) were at δ 5.18 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{II}), 5.08 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1^I), 4.23 (dd, 1H, $J_{2,3} = 3.0$ Hz, H-2^I), 4.19 (t, $J = 10.3$ Hz, H-4^I), ~4.12 (dd, overlapped, H-3^I), ~4.11 (m, overlapped, H-3^{II},5^{II}), ~4.09 (overlapped, H-4^{II}), ~4.08 (overlapped, H-2^{II}), ~4.07 (m, H-5^I), 3.98–3.94 (m, 4H, H-1′a, incl. s at 3.96 for $COOCH_3$), 3.80, 3.78 (2t, 1H, $J = 6.0$ Hz, H-1′b), 2.67 (t, 2H, $J = 7.5$ Hz, H-5′a,b), 2.30, 2.29 (2s, 6H, 2NHCO*C*H_3), 1.92–1.85 (m, 4H, H-4′a,b,2′a,b), 1.69–1.62 (m, 2H, H-3′a,b), 1.46 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6^I), 1.44 (d, 3H, $J_{5,6} = 5.8$ Hz, H-6^{II}). ^{13}C NMR (D_2O, 50 °C): δ 180.41 (*C*$OOCH_3$), 177.67, 177.41 (2NH*C*OCH_3), 105.26 (C-1^{II}), 102.75 (C-1^I), 79.99 (C-3^I), 72.68 (C-2^{II}), 72.33 (C-2^I) 71.34, 71.27 (C-3^{II},5^{II}), 70.96 (C-1′), 70.60 (C-5^I), 56.04 (C-4^{II}), 55.20 (COO*C*H_3), 55.07 (C-4^{I,II}), 36.72 (C-5′), 31.26 (C-2′), 28.07 (C-3′), 27.13 (C-4′), 25.33, 25.25 (2CO*C*H_3), 19.96, 19.90 (C-6^{I,II}). FABMS: *m*/*z* 543 $[M+Na]^+$. The corresponding per-*O*-acetyl derivative **17** showed $[\alpha]_D$ +58 (*c* 0.9, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.09 (d, 1H, $J_{4,NH} = 9.5$ Hz, NH^I), 5.68 (d, 1H, $J_{4,NH} = 10.0$ Hz, NH^{II}), 5.18 (dd, 1H, $J_{2,3} = 3.1$ Hz, H-2^I), 5.09 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 10.9$ Hz, H-3^{II}), 4.99 (d, 1H, $J_{1,2} = 2.0$ Hz, H-1^{II}), 4.90 (dd, 1H, H-2^{II}), 4.70 (d, 1H, H-1^I), 4.18 (q, partially overlapped, $J = 10.1$ Hz, H-4^{II}), 4.13 (q, partially overlapped, $J = 10.9$ Hz, H-4^I), 4.07 (dd, 1H, H-3^I), 3.88 (m, 1H, H-5^{II}), 3.77 (m, 1H, H-5^I), 3.69–3.66 (m, 4H, H-1′a, incl. 3.67, s, $COOCH_3$), 3.42, 3.40 (2t, 1H, $J = 5.1$ Hz, H-1′b), 2.36 (m, 2H, H-5′a,b), 2.23, 2.12, 2.01, 2.06, 1.96 (5s, 15H, 5$COCH_3$), 1.67 (m, 3H, H-2′a,4′a,b), 1.58 (m, 1H, H-2′b), 1.49 (m, 1H, H-3′a), 1.35 (m, 1H, H-3′b), 1.26 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6^I), 1.23 (d, 3H, $J_{5,6} = 6.3$ Hz, H-6^{II}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 97.97 (C-1^{II}), 97.25 (C-1^I), 73.49 (C-3^I), 70.45 (C-2^I), 69.95 (C-2^{II}), 68.84 (C-5^{II}), 67.95 (C-3^{II}), 67.63 (C-5^I), 66.86 (C-1′), 52.61 (C-4^I), 51.58 (COO*C*H_3), 51.34 (C-4^{II}), 33.86 (C-5′), 28.55 (C-2′), 25.88 (C-3′), 24.29 (C-4′), 23.40, 23.33 (2 NHCO*C*H_3), 21.20, 20.96, 20.81 (3CO*C*H_3), 17.92 (C-6^{II}), 17.75 (C-6^I). ESI-MS (*m*/*z*): 647.3023; calcd for $[M+H]^+$ 6747.3027. Anal. Calcd for $C_{29}H_{46}N_2O_{14}$: C, 53.86; H, 7.17; N, 4.33. Found: C, 54.04; H, 7.26; N, 4.29.

3.12. 5-Methoxycarbonylpentyl (2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (18)

Reaction of **10**[14,21] (2.2 g, 6.02 mmol) and **14** (3.125 g, 4.67 mmol) as described in Section 3.2 gave, after chro-

matography (20:1→5:1 hexane–EtOAc), the fully protected trisaccharide **18** (4.275 g, 95%), $[\alpha]_D$ +79.6 (*c* 1.2). ^{1}H NMR (600 MHz, C_6D_6): δ 5.87 (dd, 1H, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.1 Hz, H-2^{III}), ~5.25 (d, partially overlapped, H-1^{III}), ~5.24 (d, partially overlapped, H-1^{II}), 4.81 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1^{I}), 4.74–4.39 (6d, partially overlapped, 6H, 3C H_2Ph), 4.22 (dd, 1H, $J_{2,3}$ = 3.1, $J_{3,4}$ = 9.7 Hz, H-3^{II}), 4.13 (dd, partially overlapped, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.2 Hz, H-3^{I}), 4.12 (dd, partially overlapped, H-2^{II}), 4.10 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3^{III}), 3.94 (m, 1H, H-5^{III}), 3.85–3.77 (m, 3H, H-5^{II},2^{I},4^{II} in that order), 3.69 (t, 1H, *J* = 9.9 Hz, H-4^{I}), 3.63 (t, 1H, *J* = 9.9 Hz, H-4^{III}), 3.59 (m, partially overlapped, H-5^{I}), 3.47, 3.46 (2 t, *J* = 6.7 Hz, 1H, H-1′a), 3.36 (s, 3H, $COOCH_3$), 3.12, 3.11 (2t, 1H, H-1′b), 2.05 (t, 2H, *J* = 7.3 Hz, H-5′a,b), 1.65 (s, 3H, $COCH_3$), 1.49–1.44 (m, 2H, H-4′a,b), 1.36–1.29 (m, partially overlapped, H-2′a,b), 1.31 (d, partially overlapped, $J_{5,6}$ = 5.9 Hz, H-6^{III}), 1.25 (d, $J_{5,6}$ = 6.1 Hz, H-6^{I}), 1.20 (d, $J_{5,6}$ = 6.0 Hz, H-6^{II}), 1.18–1.11 (m, 2H, H-3′a,b). ^{13}C NMR (150 MHz, C_6D_6): δ 100.93 (C-1^{III}), 100.04 (C-1^{II}), 97.32 (C-1^{I}), 80.17 (C-3^{I}), 79.71 (C-3^{II}), 78.34 (C-2^{I}), 77.80 (C-2^{II}), 76.93 (C-3^{III}), 73.02, 72.98, 72.02 (3CH$_2$Ph), 68.81 (C-5^{II}) 68.54 (C-5^{III}), 68.22 (C-1′), 67.99 (C-5^{I}), 67.95 (C-2^{III}), 65.26 (C-4^{I}), 65.19 (C-4^{II}), 64.96 (C-4^{III}), 51.39 (COOCH$_3$), 34.18 (C-5′), 29.61 (C-2′), 26.28 (C-3′), 25.18 (C-4′), 20.73 (COCH$_3$), 19.09, 19.06 (2C) (C-6$^{I–III}$). FABMS: *m*/*z* 946 $[M+H-2N+2H]^+$. Anal. Calcd for $C_{48}H_{61}N_9O_{13}$: C, 59.31; H, 6.33; N, 12.97. Found: C, 59.47; H, 6.34; N, 12.89.

3.13. 5-Methoxycarbonylpentyl (4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (19)

Deacetylation of **18** (5.45 g, 5.6 mmol) as described for **13** gave, after chromatography and crystallization, **19** (3.75 g, 98%), mp 37–40 °C (from isopropyl ether), $[\alpha]_D$ +76 (*c* 1.9). ^{1}H NMR (600 MHz, C_6D_6): δ 5.31 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1^{III}), 5.26 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1^{II}), 4.82 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1^{I}), 4.59–4.34 (6d, partially overlapped, 6H, 3CH_2Ph), 4.24 (dd, 1H, $J_{2,3}$ = 3.1 Hz, H-2^{III}), 4.21 (dd $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.8 Hz, H-3^{II}), ~4.14 (dd, partially overlapped, H-2^{II}), 4.13 (dd, partially overlapped, $J_{2,3}$ = 3.1, $J_{3,4}$ = 9.9 Hz, H-3^{I}), 3.90 (m, partially overlapped, H-5^{III}), 3.88 (dd, partially overlapped, $J_{3,4}$ = 9.8 Hz, H-3^{III}), 3.84–3.80 (m, 2H, H-2^{I},5^{II}), 3.77 (t, 1H, *J* = 9.9 Hz, H-4^{II}), 3.70 (t, *J* = 9.9 Hz, H-4^{I}), 3.60 (m, 1H, H-5^{I}), 3.51 (t, 1H, *J* = 9.9 Hz, H-4^{III}), 3.47, 3.45 (2t, 1H, *J* = 6.6 Hz, H-1′a), 3.36 (s, 3H, $COOCH_3$), 3.12, 3.10 (2t, 1H, H-1′b), 2.05 (t, 2H, *J* = 7.4 Hz, H-5′a,b), 1.50–1.43 (m, 2H, H-4′a,b), 1.36–1.29 (m, 5H, H-2′a,b,6^{III}), 1.26 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6^{I}), 1.20 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6^{II}), 1.17–1.12 (m, 2H, H-3′a,b). ^{13}C NMR (150 MHz, C_6D_6): δ 102.61 (C-1^{III}), 100.01 (C-1^{II}), 97.44 (C-1^{I}), 80.01 (C-3^{I}), 79.35 (C-3^{II}), 79.01 (C-3^{III}), 78.36 (C-2^{I}), 77.96 (C-2^{II}), 73.08, 73.00, 71.93 (3CH$_2$Ph), 68.71 (C-5^{II}), 68.34 (C-5^{III}), 68.19 (C-1′), 68.05 (C-5^{I}), 67.83 (C-2^{III}), 65.32 (C-4^{I}), 65.27 (C-4^{II}), 64.59 (C-4^{III}), 51.38 (COOCH$_3$), 34.19 (C-5′), 29.61 (C-2′), 26.27 (C-3′), 25.19 (C-4′), 19.08, 19.05, 19.04 (C-6$^{I–III}$). FABMS: *m*/*z* 904 $[M+H-2N+2H]^+$, 952 $[M+Na]^+$. Anal. Calcd for $C_{46}H_{59}N_9O_{12}$: C, 59.41; H, 6.39; N, 13.55. Found: C, 59.49; H, 6.55; N, 13.55.

3.14. 5-Methoxycarbonylpentyl (4-acetamido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (20)

Reduction of **19** (1 g, 1.075 mmol) with H_2S followed by N-acetylation, as described for **14**, gave **20** (0.9 g, 86%). ^{1}H NMR (600 MHz, CD_3OD at 45 °C): δ 5.06 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1^{II}), 4.93 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1^{III}), 4.80 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1^{I}), 4.79–4.41 (6d, partially overlapped, 6H, 3CH_2Ph), 4.18–4.13 (m, 2H, H-4^{I,II}), 4.01–3.97 (m, 4H, H-2^{III},4^{III},3^{I,II} in that order), 3.88 (m, 1H, H-5^{II}), 3.82 (m, partially overlapped, H-5^{III}), 3.79 (br t, partially overlapped, H-2^{I}), 3.78 (br t, partially overlapped, H-2^{II}), 3.72 (m, 1H, H-5^{I}), 3.69–3.63 (m, 5H, H-1′a, 3^{III}, incl. s, 3.64, $COOCH_3$), 3.42, 3.41 (2t, 1H, *J* = 6.1 Hz, H-1′b), 2.33 (t, 2H, *J* = 7.3 Hz, H-5′a,b), 1.97, 1.96, 1.88 (3s, 3H each, 3$COCH_3$), 1.66–1.61 (m, partially overlapped, H-4′a,b), 1.61–1.56 (m, partially overlapped, H-2′a,b), 1.43–1.38 (m, 2H, H-3′a,b), 1.18, 1.15, 1.13 (3d, 3H each, $J_{5,6}$ 6.2 Hz, H-6$^{I–III}$, in that order). ^{13}C NMR (150 MHz, CD_3OD, 45 °C): δ 103.45 (C-1^{III}), 100.58 (C-1^{II}), 98.93 (C-1^{I}), 79.38 (C-2^{II}), 78.85 (C-2^{I}), 78.11, 77.97, 77.91 (C-3$^{I–III}$), 74.05, 74.01, 72.07 (3CH$_2$Ph), 69.74 (C-5^{II}), 69.54 (C-5^{III}), 68.96 (C-5^{I}), 68.59 (C-1′), 68.40 (C-2^{III}), 53.90, 53.83 (C-4^{I,II}), 53.12 (C-4^{III}), 51.99 (COOCH$_3$), 34.68 (C-5′), 30.09 (C-2′), 26.81 (C-3′), 25.69 (C-4′), 23.18, 22.99, 22.94 (3NHCOCH$_3$), 18.54, 18.46, 18.28 (C-6$^{I–III}$). FABMS: *m*/*z* 984.5 $[M+Li]^+$, 1000.50 $[M+Na]^+$. FAB HRMS: *m*/*z* 1000.4783 (100%); calcd for $C_{52}H_{71}N_3NaO_{15}$, 1000.4783.

3.15. 5-Methoxycarbonylpentyl (4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-4,6-dideoxy-α-D-mannopyranoside (21) and 5-methoxycarbonylpentyl (4-acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranoside (22)

Compound **20** (0.75 g) was treated with hydrogen, as described for **15**. Elution of the crude product from a small

silica gel column (5:1→3:2 CH_2Cl_2–MeOH) gave compound **21** (345 mg, 64%) $[\alpha]_D$ +78.9 (*c* 3.7, H_2O). Structurally significant signals in the ^1H NMR spectrum (D_2O at 50 °C, 600 MHz) were at δ 5.13 (br d, 2H, H-$1^{II,III}$), 5.02 (br d, 1H, H-1^{I}), 4.21 (br dd, 1H, H-2^{I}), 3.93, 3.92, 3.79, 3.75 (4t, 2H, $J = 6.5$ Hz, H-1′a,b), 2.62 (t, 2H, $J = 7.4$ Hz, H-5′a,b), 2.27. 2.26, 2.25 (3br s, 12H, 3NHCOCH_3), 1.43–1.41 (m, H-$6^{I–III}$). Structurally significant signals in the ^{13}C NMR spectrum (D_2O at 50 °C, 600 MHz) were at δ 105.25, 105.13 (C-$1^{II,III}$), 102.75 (C-1^{I}), 52.11 (COO*C*H_3), 36.86 (C-5′), 31.31 (C-2′), 28.09 (C-3′), 27.16 (C-4′), 25.35, 25.32, 25.25 (3NHCOC*H*$_3$), 20.03, 19.98, 19.95 (C-$6^{I,III}$). FABMS: *m*/*z* 708.5 $[M+H]^+$, 730.5 $[M+Na]^+$. The corresponding per-*O*-acetyl derivative **22** showed $[\alpha]_D$ +50 (*c* 1.2, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 6.38 (d, 1H, $J_{4,NH} = 9.5$ Hz, NH^{I}), 5.83 (d, 1H, $J_{4,NH} = 9.9$ Hz, NH^{III}), 5.80 (d, 1H, $J_{4,NH} = 10.0$ Hz, NH^{II}), 5.14 (dd, 1H, $J_{1,2} = 1.9$ Hz, H-2^{I}), 5.05 (dd, 1H, $J_{2,3} = 2.6$, $J_{3,4} = 10.8$ Hz, H-3^{III}), 5.02 (dd, 1H, $J_{1,2} = 2.0$, $J_{2,3} = 3.3$ Hz, H-2^{II}), 4.92–4.90 (m, 3H, H-2^{III},$1^{III,II}$ in that order), 4.70 (d, 1H, $J_{1,2} = 1.9$ Hz, H-1^{I}), 4.16 (t, partially overlapped, $J = 10.0$ Hz, H-4^{III}), 4.15–4.04 (m, partially overlapped, H-$4^{I,II}$,H-3^{I} in that order), 3.87 (dd, partially overlapped, H-3^{II}), 3.83 (m, partially overlapped, H-5^{I}), ~3.77 (m, partially overlapped, H-5^{III}), ~3.75 (m, partially overlapped, H-5^{I}), 3.69–3.65 (m, 4H, H-1′a, incl. 3.68, s, $COOCH_3$), 3.42, 3.40 (2t, 1H, $J = 5.4$ Hz, H-1′b), 2.36 (m, 2H, H-5′a,b), 2.19, 2.17, 2.14 (3s, 9H, 3NHCOCH_3), 2.05, 2.02, 2.00, 1.97 (4s, 12H, 4COCH_3), 1.74–1.25 (4m, 6H, H-4′a,b,2′b,3′a,3′b in that order), 1.25, 1.23, 1.21 (3d, 9H, $J_{5,6} = 6.2$ Hz, H-$6^{I,II,III}$ in that order). ^{13}C NMR (150 MHz, $CDCl_3$): δ 98.44 (C-1^{II}), 97.85 (C-1^{III}), 97.05 (C-1^{I}), 73.63 (C-3^{I}), 72.76 (C-3^{II}), 70.53 (C-2^{I}), 70.37 (C-2^{II}), 69.49 (C-2^{III}), 68.82 (C-5 ^{III}), 68.44 (C-5^{II}), 68.05 (C-3^{III}), 67.55 (C-5^{I}), 68.84 (C-1′), 52.37 (C-4^{I}), 52.14 (C-4^{II}), 51.49 (COO*C*H_3), 51.02 (C-4^{III}), 33.78 (C-5′), 28.44 (C-2′), 25.76 (C-3′), 24.24 (C-4′), 23.26, 23.15, 21.12, 20.95, 20.88, 20.67 (7CO*C*H_3), 17.75, 17.66 (3C, C-$6^{I–III}$). ESI-MS (*m*/*z*): 914.3589; calcd for $C_{39}H_{61}N_3O_{19}K$: 914.3536. Anal. Calcd for $C_{39}H_{61}N_3O_{19}$: C, 53.48; H, 7.02; N, 4.80. Found: C, 53.76; H, 6.98; N, 4.56.

3.16. 5-Methoxycarbonylpentyl (3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (23)

Reaction of **8** (1.8 g, 5 mmol) and **19** (3.75 g, 3.86 mmol) as described in Section 3.2 gave, after chromatography (15:1→5:1 hexane–EtOAc), the protected tetrasaccharide **23** (4.45 g, 94%), mp 112.5–113.5 °C (EtOH–EtOAc), $[\alpha]_D$ +37.2 (*c* 1.1). ^1H NMR (600 MHz, C_6D_6): δ 5.53 (dd, 1H, $J_{2,3} = 3.1$, $J_{3,4} = 10.1$ Hz, H-3^{IV}), 5.36 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^{IV}), 5.32 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1^{III}), 5.26 (d, 1H, $J_{1,2} = 1.4$ Hz, H-1^{II}), 4.82 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1^{I}), 4.61–4.05 (8d, partially overlapped, 4*C*H_2Ph), 4.49 (br t, H-2^{III}), 4.27 (dd, partially overlapped, H-3^{II}), 4.15 (dd, 1H, $J_{2,3} = 3.0$, $J_{3,4} = 9.6$ Hz, H-3^{I}), ~4.13 (m, 2H, H-$2^{II,IV}$), 4.00 (dd, 1H, $J_{2,3} = 2.8$, $J_{3,4} = 9.8$ Hz, H-3^{III}), 3.92–3.81 (m, partially overlapped, H-$5^{IV,III,II}$ in that order), 3.82 (m, H-2^{I},4^{IV}), 4.81 (t, partially overlapped, H-4^{II}), 4.76 (t, partially overlapped, H-4^{III}), 3.74 (t, partially overlapped, H-4^{I}), 3.60 (m, 1H, H-5^{I}), 3.48, 3.46 (2t, 1H, $J = 6.4$ Hz, H-1′a), 3.36 (s, 3H, $COOCH_3$), 3.13, 3.11 (2t, 1H, H-1′b), 2.05 (t, 2H, $J = 7.3$ Hz, H-5′a,b), 1.70 (s, 3H, $COCH_3$), 1.50–1.45 (m, 2H, H-4′a,b), 1.36–1.30 (m, 2H, H-2′), 1.30 (d, partially overlapped, H-6^{IV}), 1.29 (d, partially overlapped, H-6^{III}), 1.25 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6^{I}), 1.22 (d, 3H, $J_{5,6} = 6.0$ Hz, H-6^{II}), 1.17–1.14 (m, 2H, H-3′a,b). ^{13}C NMR (150 MHz, C_6D_6): δ 102.19 (C-1^{III}), 100.06 (C-1^{II}), 99.59 (C-1^{IV}), 97.36 (C-1^{I}), 80.11 (C-3^{I}), 79.43 (C-3^{III}), 79.01 (C-3^{II}), 78.53 (C-2^{I}), 77.90 (C-2^{II}), 75.52 (C-2^{IV}), 73.29 (C-2^{III}), 73.12, 73.09 (2*C*H_2Ph), 73.01 (C-3^{IV}), 72.97, 72.78 (2*C*H_2Ph), 68.98 (C-5^{III}), 68.77 (C-5^{II}), 68.71 (C-5^{IV}), 68.20 (C-1′), 68.01 (C-5^{I}), 65.51 (C-4^{II}), 65.34 (C-4^{I}), 65.10 (C-4^{III}), 63.52 (C-4^{IV}), 51.37 (COO*C*H_3), 34.18 (C-5′), 29.62 (C-2′), 26.28 (C-3′), 25.19 (C-4′), 20.79 (CO*C*H_3), 19.05, 19.03 18.89 (4C, C-$6^{I–IV}$). FABMS: *m*/*z* 1207.6 $[M+H-2N+2H]^+$, 1255 $[M+Na]^+$. Anal. Calcd for $C_{61}H_{76}N_{12}O_{16}$: C, 59.40; H, 6.21; N, 13.63. Found: C, 59.43; H, 6.11, N, 13.54.

3.17. Methoxycarbonylpentyl (4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (24)

Deacetylation of **23** (4.27 g, 3.46 mmol) as for **13**, except that the reaction was started at 50 °C, to solubilize the starting material, gave pure **24** (4.03 g, 97%), which crystallized on standing but could not be crystallized from common solvents, mp 53–57 °C, $[\alpha]_D$ +10.0 (*c* 0.4). ^1H NMR (600 MHz, C_6D_6): δ 5.30 (br d, partially overlapped, H-1^{IV}), 5.29 (d, partially overlapped, H-1^{III}), 5.26 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{II}), 4.83 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1^{I}), 4.63–4.01 (8d, partially overlapped, 4C H_2Ph), 4.45 (br dd, 1H, H-2^{III}), 4.25 (dd, 1H, $J_{2,3} = 3.0$, $J_{3,4} = 9.8$ Hz, H-3^{II}), 4.14 (dd, 1H, $J_{2,3} = 3.1$, $J_{3,4} = 10.0$ Hz, H-3^{I}), 4.11 (dd, 1H, $J_{2,3} = 3.0$ Hz, H-2^{II}), 4.05 (dd, 1H, $J_{2,3} = 3.6$, $J_{3,4} = 9.9$ Hz, H-3^{IV}), 3.97 (dd, 1H, $J_{2,3} = 3.5$, $J_{3,4} = 9.7$ Hz, H-3^{III}), 3.86–3.81 (m, partially overlapped, H-$5^{II,III}$), 3.82 (dd, partially overlapped, H-2^{I}), 3.80 (t, partially overlapped, $J = 9.8$ Hz, H-4^{II}), ~3.74 (m, partially overlapped, H-5^{IV}), 3.72 (m, partially overlapped, H-4^{I},2^{IV}), 3.62–3.56

(m, 2H, H-5^{I},4^{III}), 3.49, 3.47 (2t, 1H, $J = 6.5$ Hz, H-1′a), 3.37 (s, partially overlapped, $COOCH_3$), 3.35 (t, partially overlapped, $J = 10.0$ Hz, H-4^{IV}), 3.15, 3.13 (2t, 1H, H-1′b), 2.06 (t, 2H, $J = 7.4$ Hz, H-5′a,b), 1.51–1.46 (m, 2H, H-4′a,b), 1.38–1.32 (m, partially overlapped, H-2′a,b), 1.31–1.21 (4d, partially overlapped, H-6$^{I–IV}$), 1.20–1.13 (m, 2H, H-3′a,b). ^{13}C NMR (150 MHz, C_6D_6): δ 102.22 (C-1^{III}), 100.10 (C-1^{II}), 98.96 (C-1^{IV}), 97.33 (C-1^{I}), 80.16 (C-3^{I}), 79.20 (C-3^{III}), 78.86 (C-3^{II}), 78.49 (C-2^{I}), 77.88 (C-2^{II}), 77.41 (C-2^{IV}), 73.11, 73.06, 72.81, 72.66 (4CH_2Ph), 72.97 (C-2^{III}), 71.10 (C-3^{IV}), 68.79. 68.77 (C-5^{II,III}), 68.23 (C-1′), 68.21 (C-5^{IV}), 68.00 (C-5^{I}), 67.03 (C-4^{IV}), 65.67 (C-4^{II}), 65.33 (C-4^{I}), 65.10 (C-4^{III}), 51.43 ($COOCH_3$), 34.20 (C-5′), 29.61 (C-2′), 26.28 (C-3′), 25.19 (C-2′), 19.01, 18.95, 18.75 (4C, C-6$^{I–IV}$). FABMS: m/z 1165.6 $[M+H-2N+2H]^+$. Anal. Calcd for $C_{59}H_{74}N_{12}O_{15}$: C, 59.48; H, 6.26; N, 14.11. Found: C, 59.50; H, 6.27; N, 13.83.

3.18. 5-Methoxycarbonylpentyl (4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-acetamido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (25)

Treatment of **24** (1.42 g, 1.19 mmol) as for compound **19** but in pyridine and water (3:1), to assist solubility of the starting material, gave the corresponding intermediate *tetrakis*-amine (1 g). FABMS: m/z 1087.62 $[M+H]^+$, 1109.61 $[M+Na]^+$.

A solution of the foregoing amine was N-acetylated, as described for preparation of **15**, to give after chromatography **25** (1.05 g, 70% from **24**), $[\alpha]_D$ +30.0 (c 0.2). Structurally significant ^{1}H NMR (600 MHz, C_6D_6) signals were at δ 7.62, 6.73, 5.86, 5.48 (4br s, NH$^{I–IV}$), 5.21, 5.10, 5.07 (3br s, H-1$^{II–IV}$), 4.80 (br s, H-1^{I}), 4.37–4.28 (m, 4H, H-4$^{I–IV}$), 3.60–3.58, 3.24–3.22 (2m, 2H, H-1′a,b), 3.38 (s, 3H, $COOCH_3$), 2.12–2.09 (m, 2H, H-5′a,b), 2.00, 1.99, 1.87, 1.43 (4s, 4NHCOCH_3), 1.46, 1.31, 1.24, 1.23 (4d, H-6$^{I–IV}$). The spectrum taken in CD_3OD showed peaks at δ 5.08, 5.01, 4.96 (3d, 1H each, H-1$^{II–IV}$), 4.80 (d, 1H, $J_{1,2} = 1.9$ Hz, H-1^{I}), 4.82–4.36 (8d, 1H, each, 4CH_2Ph), 4.15 (br t, partially overlapped, H-4^{I}), 3.78 (br dd, 1H, H-2^{I}), 3.64 (s, partially overlapped, $COOCH_3$), 3.42, 3.40 (2t, 1H, $J = 6.2$ Hz, H-1′b), 2.43 (t, 2H, $J = 7.3$ Hz, H-5′a,b), 1.99, 1.98, 1.96, 1.93 (4s, 3H each, 4NHCOCH_3), 1.18, 1.17, 1.12, 1.10 (4d, 12H, $J_{5,6} \sim 6.3$ Hz, H-6$^{I–IV}$). Structurally significant ^{13}C NMR (150 MHz, C_6D_6) signals were at δ 101.20, 99.40, 97.84 (br, C-1$^{II–IV}$), 96.89 (br, C-1^{I}), 51.03 ($COOCH_3$), 23.27, 23.16. 23.09, 22.96 ($NHCOCH_3$). The spectrum taken in CD_3OD showed peaks at δ 102.19, 100.66 (3C) (C-1$^{II–IV}$), 98.94 (C-1^{I}), 74.25, 74.00, 73.92, 72.75 (4CH_2Ph), 68.57 (C-1′), 52.06 ($COOCH_3$), 23.30, 23.25, 22.91, 22.88 (4NHCOCH_3), 18.68, 18.67, 18.57, 18.26 (C-6$^{I–IV}$). FABMS: m/z 1278 $[M+Na]^+$.

3.19. 5-Methoxycarbonylpentyl (4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-4,6-dideoxy- α-D-mannopyranosyl)-(1→3)-4-acetamido-4,6-dideoxy-α-D-mannopyranoside (26) and 5-methoxycarbonylpentyl (4-acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-acetamido-3-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranoside (27)

Hydrogenolysis of **25** (960 mg) and processing as described for **16**, gave the deprotected, amorphous tetrasaccharide **26** in virtually theoretical yield, $[\alpha]_D$ +62.4 (c 6.6, H_2O). ^{1}H NMR (600 MHz, D_2O at 50 °C): δ 5.27 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^{III}), 5.20 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{II}), 5.15 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{IV}), 5.05 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{I}), 4.33 (br dd, 1H, H-2^{II}), 4.25 (br t, 1H, H-2^{I}), ~4.23 (m, partially overlapped, H-3^{III}), ~4.19 (m, partially overlapped, H-3^{II}), 4.12 (m, partially overlapped, H-3^{I}), ~4.09 (m, partially overlapped, H-2^{IV}), 4.03 (br dd, 1H, H-2^{III}), 3.96 (m, 4H, H-1′a, incl. s, 3.96 $COOCH_3$), 3.80, 3.78 (2t, 1H, $J = 6.1$ Hz, H-1′b), 2.66 (t, 2H, $J = 7.4$ Hz, H-5′a,b), 2.31, 2.30, 2.29, 2.28 (4s, 3H each, 4NHCOCH_3), 1.92–1.85 (m, 4H, H-2′a,b4′a,b), 1.69–1.62 (m, 2H, H-3′a,b), 1.46–1.45 (m, 12H, H-6$^{I–IV}$). ^{13}C NMR (150 MHz, D_2O at 50°C): δ 105.47 (C-1^{II}), 105.21 (C-1^{1V}), 103.90 (C-1^{III}), 102.72 (C-1^{I}), 81.60 (C-2^{III}), 80.52 (C-3^{I}), 80.02 (C-3^{II}), 72.58 (C-2^{IV}), 72.39 (C-2^{I}), 72.20 (C-2^{II}), 70.98 (C-1′), 52.08 ($COOCH_3$), 36.70 (C-5′), 31.23 (C-2′), 28.04 (C-3′), 27.10 (C-4′), 25.43, 25.34, 25.30, 25.27 (4NHCOCH_3), 20.20, 20.05, 19.90, 19.86 (C-6$^{I–IV}$). FABMS: m/z 917.5 $[M+Na]^+$. The corresponding per-*O*-acetyl derivative **27** showed $[\alpha]_D$ +67 (c 1.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.98 (d, 1H, $J_{4,NH} = 9.3$ Hz, NH^{IV}), 6.38 (d, 1H, $J_{4,NH} = 9.8$ Hz, NH^{I}), 5.82 (br d, 2H, NH^{II,III}), 5.16–5.13 (m, 3H, H-2^{I},2^{IV},3^{IV}, in that order), 5.06 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 8.7$ Hz, H-3^{III}), 4.95 (br dd, 1H, H-2^{II}), 4.90 (d, 1H, $J_{1,2} = 2.0$ Hz, H-1^{II}), 4.88 (d, 1H, $J_{1,2} = 2.8$ Hz, H-1^{III}), 4.86 (br d, 1H, $J_{1,2} \sim 1$ Hz, H-1^{IV}), 4.71 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{I}), 4.20 (br q, 1H, H-4^{IV}), 4.13 (br q, 1H, H-4^{I}), 4.04–3.99 (m, 3H, H-3^{II},4^{III},3^{I}, in that order), 3.94–3.81 (m, 4H, H-4^{II}, 5^{II},2^{III},5^{IV}, in that order), 3.75 (m, partially overlapped, H-5^{I}), 3.73–3.65 (m, 5H, H-5^{III},1′a, incl. 3.68, s, $COOCH_3$), 3.42, 3.40 (2t, 1H, $J = 5.2$ Hz, H-1′b), 2.36 (t, 2H, $J = 7.0$ Hz, H-5′), 2.19, 2.14, 2.13, 2.08, 2.07, 2.03, 2.01, 1.98, 1.97 (9s, 3H each, 9$COCH_3$), 1.71–1.61 (m, 3H, H-4′a,b,2′a), 1.60–1.55 (m, H-2′b), 1.45, 1.38 (2 m, 2H, H-3′a,b), 1.29, 1.25, 1.23, 1.20 (4d,

$J_{5,6} = 1.2$ Hz, H-6^{IV,I,III,II} in that order). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 99.55 (C-1^{III}), 98.55 (C-1^{II}), 97.85 (C-1^{IV}), 97.06 (C-1^{I}), 74.59 (C-3^{I}), 74.21 (C-2^{III}), 72.04 (C-3^{II}), 70.95 (C-2^{II}), 69.71 (C-3^{III}), 70.55, 68.88 (C-2^{I,IV}), 68.81 (C-3^{IV}), 68.56 (C-5^{III}), 68.50 (C-5^{IV}), 68.19 (C-5^{II}), 67.46 (C-5^{I}), 66.81 (C-1′), 52.74 (C-4^{II}), 52.30 (2C, C-4^{I,III}), 51.58 (COO*C*H$_3$), 50.88 (C-4^{IV}), 33.80 (C-5′), 28.48 (C-2′), 25.77 (C-3′), 24.21 (C-4′), 23.49, 23.36, 23.26, 23.16 (4NHCO*C*H$_3$), 21.14, 21.01, 20.91, 20.79, 20.78 (5CO*C*H$_3$), 18.07, 17.78, 17.74, 17.73 (C-6$^{I–IV}$). ESI-MS (*m*/*z*): 1105.4944 [M+H]$^+$; calcd for $C_{49}H_{77}N_4O_{24}$: 1105.4928. Anal. Calcd for $C_{49}H_{76}N_4O_{24}$: C, 53.25; H, 6.93; N, 5.07. Found: C, 53.07; H, 6.97; N, 5.00.

3.20. 5-Methoxycarbonylpentyl (3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (28)

Condensation of the glycosyl donor **8** (1 g, 2.73 mmol) and acceptor **24** (2.61 g, 2.19 mmol), performed as described in Section 3.2, afforded pure (TLC, NMR) **28** (2.37 g, 73%), $[\alpha]_D$ +49.8. ^{1}H NMR (600 MHz, C_6D_6): δ 5.55 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 10.1$ Hz, H-3^{V}), 5.39 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^{III}), 5.36 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^{IV}), 5.28 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^{V}), 5.24 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^{II}), 4.81 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^{I}), 4.61–4.36 (m, 11H, 5C*H*$_2$Ph, incl. br dd, 4.46, H-2^{III}), 4.27 (dd, 1H, $J_{2,3} = 3.1$, $J_{3,4} = 9.9$ Hz, H-3^{II}), 4.22 (dd, $J_{2,3} = 3.0$, $J_{3,4} = 10.1$ Hz, H-3^{IV}), 4.19 (dd, H-2^{V}), 4.13 (dd, partially overlapped, $J_{2,3} = 3.2$, $J_{3,4} = 9.9$ Hz, H-3^{I}), ~4.11 (dd, partially overlapped, H-2^{II}), 4.00 (dd, 1H, $J_{2,3} = 2.9$, $J_{3,4} = 9.9$ Hz, H-3^{III}), 3.89 (dd, partially overlapped, H-2^{IV}), 3.87 (m, partially overlapped, H-5^{III,II,IV}, in that order), ~3.82 (m, partially overlapped, H-2^{I}), 3.80 (t, partially overlapped, H-4^{II}), 3.79 (t, partially overlapped, H-4^{V}), ~3.73 (m, partially overlapped, H-5^{V}), 3.71 (t, partially overlapped, H-4^{IV}), 3.69 (t, partially overlapped, H-4^{I}), 3.66 (t, partially overlapped, H-4^{III}), 3.58 (m, partially overlapped, H-5^{I}), 3.48, 3.46 (2t, 1H, $J = 6.5$ Hz, H-1′a), 3.37 (s, 3H, COOCH$_3$), 3.14, 3.12 (2t, 1H, H-1′b), 2.05 (t, 2H, $J = 7.3$ Hz, H-5′a,b), 1.72 (s, 3H, COCH$_3$), 1.50–1.45 (m, 2H, H-4′a,b), 1.37–1.31 (m, partially overlapped, H-2′a,b, incl. 1.33, d, $J_{5,6} = 6.2$ Hz, H-6^{III}), 1.28 (d, $J_{5,6} = 6.1$ Hz, H-6^{IV}), 1.24 (d, partially overlapped, $J_{5,6} = 6.2$ Hz, H-6^{I}), 1.23, (d, partially overlapped, H-6^{V}), 1.22 (d, partially overlapped, H-6^{II}). ^{13}C NMR (150 MHz, C_6D_6): δ 102.21 (C-1^{III}), 100.26 (C-1^{V}), 100.08 (C-1^{II}), 99.60 (C-1^{IV}), 97.38 (C-1^{I}), 80.18 (C-3^{I}), 79.04 (C-3^{III}), 78.96 (C-3^{II}), 78.47 (C-2^{I}), 77.87 (C-2^{II}), 77.45 (C-2^{IV}), 77.43 (C-3^{IV}), 76.21 (C-2^{V}), 73.85 (C-2^{III}), 73.73, 73.10, 73.07 (3*C*H$_2$Ph), 72.94 (2C, *C*H$_2$Ph, C-3^{V}), 72.67 (*C*H$_2$Ph), 69.27 (C-5^{IV}), 68.82, 68.78 (C-5^{II,III}), 68.70 (C-5^{V}), 68.24 (C-1′), 68.02 (C-5^{I}), 65.62 (C-4^{II}), 65.56 (C-4^{III}), 65.37, 65.34 (C-4^{I,IV}), 63.46 (C-4^{V}), 51.38 (COO*C*H$_3$), 34.19 (C-5′), 29.62 (C-2′), 26.30 (C-3′), 25.20 (C-4′), 20.79 (CO*C*H$_3$), 19.09 (C-6^{III}), 19.05 (C-6^{IV}), 19.03, 18.97 (C-6^{I,IV}), 18.75 (C-6^{II}). FABMS: *m*/*z* 1468.7 [M+H−2N+2H]$^+$, 1516.6 [M+Na]$^+$. Anal. Calcd for $C_{74}H_{91}N_{15}O_{19}$: C, 59.47; H, 6.14; N, 14.06. Found: C, 59.30; H, 6.21; N, 13.93.

3.21. 5-Methoxycarbonylpentyl (4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (29)

Deacetylation of **28** (2.25 g, 1.505 mmol) as described for **12**, except that 1:2 MeOH–toluene mixture was used as solvent, gave **29** (2.05 g, 94%), $[\alpha]_D$ +69.2 (*c* 2.4). ^{1}H NMR (600 MHz, C_6D_6): δ 5.37 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{III}), 5.36 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{IV}), 5.24 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{II}), 5.20 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1^{V}), 4.81 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{I}), 4.60–4.21 (10d, partially overlapped, 5C*H*$_2$Ph), 4.46 (br t, 1H, H-2^{III}), 4.27 (dd, 1H, $J_{2,3} = 3.1$, $J_{3,4} = 9.9$ Hz, H-3^{II}), 4.18 (dd, 1H, $J_{2,3} = 3.0$, $J_{3,4} = 10.2$ Hz, H-3^{IV}), 4.12 (dd, 1H, $J_{2,3} = 3.2$, $J_{3,4} = 9.9$ Hz, H-3^{I}), 4.10 (dd, 1H, H-2^{II}), 4.02 (dd, 1H, $J_{2,3} = 3.6$, $J_{3,4} = 9.8$ Hz, H-3^{V}), 3.99 (dd, 1H, $J_{3,4} = 9.9$ Hz, H-3^{III}), 3.88–3.82 (m, 5H, H-2^{IV},2-^{V},5^{III},5^{IV},5^{II}, in that order), 3.81 (br t, 1H, H-2^{I}), 3.80 (t, 1H, $J = 10.0$ Hz, H-4^{II}), 3.74 (t, 1H, $J = 10.0$ Hz, H-4^{IV}), 3.68 (t, partially overlapped, $J = 10.0$ Hz, H-4^{I}), 3.66 (t, partially overlapped, $J = 10.0$ Hz, H-4^{III}), 3.59 (m, 2H, H-5^{V},5^{I}, in that order), 3.48, 3.46 (2 t, 1H, $J = 6.6$ Hz, H-1′a), 3.36 (s, 3H, COOCH$_3$), 3.30 (t, 1H, $J = 9.9$ Hz, H-4^{V}), 3.14, 3.12 (2t, 1H, H-1′b), 2.05 (t, 2H, $J = 7.2$ Hz, H-5′a,b), 1.50–1.45 (m, 2H, H-4′a,b), 1.37–1.31 (m, partially overlapped, H-2′a,b), 1.31 (d, partially overlapped, $J_{5,6} = 6.2$ Hz, H-6^{III}), 1.30 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6^{IV}), 1.23 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6^{I}), 1.22 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6^{V}), 1.21 (d, 3H, $J_{5,6} = 6.1$ Hz, H-6^{II}), 1.18–1.13 (m, 2H, H-3′a,b). ^{13}C NMR (C_6D_6, 150 MHz): δ 102.19 (C-1^{III}), 100.04 (C-1^{II}), 99.86 (C-1^{V}), 99.34 (C-1^{IV}), 97.34 (C-1^{I}), 80.34 (C-3^{I}), 79.05 (C-3^{III}), 78.97 (C-3^{II}), 78.44 (C-2^{I}), 78.30 (C-2^{V}), 77.92 (C-3^{IV}), 77.83 (C-2^{II}), 77.38 (C-2^{IV}), 73.83 (C-2^{III}), 73.54, 73.07, 73.02, 72.68, 72.62 (5*C*H$_2$Ph), 71.28 (C-3^{V}), 69.17 (C-5^{IV}), 68.83, 68.76 (C-5^{II,III}), 68.25 (C-1′), 68.10 (C-5^{V}), 67.99 (C-5^{I}), 66.86 (C-4^{V}), 65.58 (C-4^{II}), 65.52 (C-4^{IV}), 65.50 (C-4^{III}), 65.30 (C-4^{I}), 51.41 (COO*C*H$_3$), 34.19 (C-5′), 29.62 (C-2′), 26.29 (C-3′), 25.19 (C-4′), 19.07, 19.04, 19.02 (2C),

18.78 (C-6^{I-V}). FABMS: *m/z* 1426.6 $[M+H-2N+2H]^+$, 1474.5 $[M+Na]^+$. Anal. Calcd for $C_{72}H_{89}N_{15}O_{18}$: C, 59.53; H, 6.18; N 14.46. Found: C, 59.56; H, 6.25; N, 14.57.

3.22. 5-Methoxycarbonylpentyl (4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-acetamido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-benzyl-4,6- dideoxy-α-D-mannopyranoside (30)

Reduction of **29** (1.935 g, 1.33 mmol) in 3:1 pyridine–H_2O was performed as described for **24**. After work-up and chromatography (EtOAc–MeOH 5:1 to 3:1), the intermediate *pentakis*-amine {(: *m/z* 1322.67 $[M+H]^+$) was N-acetylated, as described above for similar conversions, and chromatography (CH_2Cl_2–acetone 1:1→2:3) gave **30** (1.5 g, 74%), $[\alpha]_D$ +31.2 (*c* 1.8). FAB-MS: *m/z* 1532.8 $[M+H]^+$, 1554.8 $[M+Na]^+$. Structurally significant resonances in the ^{1}H NMR (600 MHz, CD_3OD at 45 °C) were at δ 5.15, 5.08, 5.07, 5.02 (4d, 1H each, $J_{1,2} \sim 1.6$ Hz, H-1^{II-V}), 4.79 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1^I), 4.80–4.41 (10d, partially overlapped, 5CH_2Ph), 3.64 (s, 3H, $COOCH_3$), 2.33 (t, 2H, $J = 7.5$ Hz, H-5′), 1.98, 1.97, 1.95, 1.92, 1.89 (5s, 3H each, 5NHCOCH_3), 1.19, 1.18, 1.14, 1.30, 1.12 (5d, partially overlapped, H-6^{I-V}). Structurally significant resonances in the ^{13}C NMR (150 MHz, CD_3OD at 45 °C) were at δ 102.20, 100.92, 100.28, 100.16 (C-1^{II-V}), 99.12 (C-1^I), 74.28, 74.15, 74.02, 73.96, 73.19 (5CH_2Ph), 68.78 (C-1′), 52.14 ($COOCH_3$), 23.50, 23.44, 23.21, 23.06, 23.03 (5NHCOCH_3), 18.90, 18.77, 18.73, 18.72, 18.46 (C-6^{I-V}).

3.23. 5-Methoxycarbonylpentyl (4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-acetamido-4,6-dideoxy- α-D-mannopyranosyl)-(1→3)-(4-acetamido-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-4,6-dideoxy-α-D-mannopyranoside (31) and 5-methoxycarbonylpentyl (4-acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-(4-acetamido-3-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-(4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→3)-4-acetamido-2-*O*-acetyl-4,6-dideoxy-α-D-mannopyranoside (32)

Hydrogenolysis of **30** (1.4 g, 0.91 mmol), performed as described for **25** gave, after freeze-drying, the deprotected pentasaccharide **31** in virtually theoretical yield, $[\alpha]_D$ +79.2 (*c* 5.1, H_2O). Structurally significant resonances in the ^{1}H NMR spectrum (600 MHz, D_2O at 50 °C) were at 5.26 (d, 1H, $J_{1,2} = 1.4$ Hz, H-1^{III}), 5.22, 5.19, 5.15 (3d, $J_{1,2} \sim 1.6$ Hz, H-$1^{II,IV,V}$), 5.05 (d, 1H, $J_{1,2} = 1.5$ Hz, H-1^I), 4.24 (dd, 1H, $J_{2,3} = 3.1$ Hz, H-2^I), 4.04 (d, 1H, $J_{2,3} = 3.0$ Hz, H-2^{III}), 3.95 (m, 4H, H-1′a, incl. s, $COOCH_3$), 3.80, 3.78 (2t, 1H, H-1′b), 2.66 (t, 2H, $J = 7.6$ Hz, H-5′a,b), 2.31, 2.30, 2.28 (3s, 15H, 5NHCOCH_3), 1.91–1.84 (m, 4H, H-2′a,b,4′a,b), 1.68–1.60 (m, 2H, H-3′a,b), 1.48–1.44 (5d, partially overlapped, H-6^{I-V}). ^{13}C NMR (150 MHz, D_2O at 50 °C): δ 105.26, 105.22, 105.07 (C-$1^{II,IV,V}$), 103.84 (C-1^{III}), 102.71 (C-1^I), 81.48 (C-2^{III}), 80.56, 80.25, 79.67 (C-$3^{I,II,IV}$), 70.99 (C-1′), 52.06 ($COOCH_3$), 36.69 (C-5′), 31.21 (C-2′), 28.01 (C-3′), 25.08 (C-4′), 25.41, 25.29, 25.28, 25.34, 25.22 (5NHCOCH_3), 20.13, 20.04, 19.94, 19.87, 19.83 (C-6^{I-V}). FABMS: *m/z* 1082.6 $[M+H]^+$, 1104.6 $[M+Na]^+$. The corresponding per-*O*-acetyl derivative **32** showed $[\alpha]_D$ +57 (*c* 1.1, $CHCl_3$). ESI-MS (*m/z*): 1351.6146 $[M+NH_4]^+$, calcd for $C_{59}H_{95}N_6O_{29}$: 1351.6143. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.33 (br d, 1H, NH^{IV}), 6.39 (d, 1H, $J_{4,NH} = 10.1$ Hz, NH^I), 5.98 (br s, 1H, NH^{II}), 5.86 (br d, 1H, NH^{III}), 5.78 (d, 1H, $J_{4,NH} = 9.9$ Hz NH^V), 5.18 (br dd, 1H, H-2^{IV}), 5.15 (dd, 1H, $J_{1,2} = 1.9$, $J_{2,3} = 3.2$ Hz, H-2^I), 5.07 (dd, partially overlapped, H-3^V), ~5.07 (d, partially overlapped, H-1^V), ~5.06 (dd, partially overlapped, H-3^{III}), 4.95 (dd, 1H, $J_{1,2} = 2.0$, $J_{2,3} = 3.1$ Hz, H-2^V), 4.92 (br t, 1H, H-2^{II}), 4.90 (d, 1H, $J_{1,2} = 2.0$ Hz, H-1^{II}), 4.84 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1^{IV}), 4.83 (br d, 1H, $J_{1,2} \sim 3$ Hz, H-1^{III}), 4.71 (d, 1H, $J_{1,2} = 1.9$ Hz, H-1^I), 4.16 (t, partially overlapped, $J \sim 10.0$ Hz, H-4^V), 4.14 (t, partially overlapped, H-4^{IV}), 4.12 (t, partially overlapped, $J = 10.4$ Hz, H-4^I), 4.04–3.97 (m, 4H, H-3^{IV},3^{II},4^{III},3^I, in that order), 3.96–3.89 (m, 3H, H-5^V,4^{II},5^{II}), 3.86 (br t, 1H, H-2^{III}), 3.80 (m, 1H, H-5^{IV}), 3.74 (m, 1H, H-5^I), 3.70–3.65 (m, 5H, H-5^{III},1′a, incl. 3.68, s $COOCH_3$), 3.41, 3.40 (2 t, 1H, $J = 4.9$ Hz, H-1′b), 2.37 (t, 2H, $J = 6.9$ Hz, H-5′a,b), 2.22–1.97 (10s, 33H, 11$COCH_3$), 1.71–1.63 (m, 3H, H-2′a,4′a,b), 1.61–1.54 (m, 1H, H-2′b), 1.53–1.46 (m, 1H, H-3′a), 1.37–1.30 (m, 1H, H-3′b), 1.28–1.20 (6d, 15H, partially overlapped, $J_{5,6} \sim 6.2$ Hz, H-6^{I-V}). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 99.60 (C-1^{III}), 98.58 (C-1^{II}), 97.81 (br, C-1^{IV}), 97.31 (C-1^V), 97.02 (C-1^I), 74.77 (br, C-3^I), 73.64 (br, C-2^{III}), 72.05 (br, C-3^{II}), 71.47 (br, C-3^{IV}), 71.03 (C-2^{II}), 70.59 (C-2^I), 69.96 (C-3^{III}), 69.87 (C-2^{IV}), 69.57 (C-2^V), 68.57 (C-5^V), 68.48 (C-5^{III}), 68.39 (C-5^{IV}), 68.09 (C-5^{II}), 68.00 (C-3^V), 67.45 (C-5^I), 66.72 (C-1′), 52.79 (C-4^{II}), 52.40 (C-4^{III}), 52.26 (2C, C-$4^{I,IV}$), 51.56 ($COCH_3$), 51.19 (C-4^V), 33.79 (C-5′), 28.47 (C-2′), 25.72 (C-3′), 24.16 (C-4′), 23.46, 23.37, 23.33 (2C), 23.13 (5NHCOCH_3), 21.07, 21.04, 20.99, 20.93, 20.73 (5COCH_3), 18.08, 17.83, 17.76, 17.70, 17.64 (C-6^{I-V}). Anal. Calcd for $C_{59}H_{91}N_5O_{29}$: C, 53.11; H, 6.87; N, 5.25. Found: C, 52.99; H, 6.99; N, 5.01.

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