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Synthesis of a heptasaccharide fragment of the mannan from *Candida guilliermondii* cell wall and its conjugate with BSA

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ABSTRACT

The 3-aminopropyl glycoside of a heptasaccharide fragment of the cell wall mannan from *Candida guilliermondii* **18**, which corresponds to the antigenic Factor 9, has been synthesized by a convergent approach based on glycosylation of a tetrasaccharide acceptor with a trisaccharide donor as the key step to give a protected heptasaccharide **17**. Subsequent two-step deprotection of **17** afforded the heptamannoside **18**, which was then conjugated with BSA using the squarate procedure.

1. Introduction

The yeast-like fungi of the genus *Candida* are opportunistic pathogenic microorganisms capable of causing severe infections in immunocompromised patients. The cell surface of *Candida* is the immediate point of contact between the fungus and host, and plays the main role in adhesion and modulation of the immune response. The main surface antigen of *Candida* is mannan, which represents the carbohydrate part of the cell wall mannoprotein.[1] Extensive structural investigations of *Candida* mannans in the past two decades have revealed the details of their structure.[2] These glycopolymers have a comb-like structure with a backbone composed of α-(1→6)-mannan, to which relatively short side chains containing both α- and β-linked mannose residues are attached through α-(1→2)-linkages. These side chains are responsible for the antigenic specificity of *Candida* species. For example, it has been shown that the determinants of antigenic factors 1 and 4 are linear (1→2)-linked[2f] and 3,6-branched α-oligomannosides,[2g] respectively. On the other hand, the determinants of antigenic factors 5 and 6 are represented by linear β-(1→2)-oligomers[2d] and by one or two β-D-Man-(1→2)-residues attached to short α-(1→2)-linked chains,[3] respectively. The former oligomers are bound to the side chains via a phosphodiester bond and are known as the acid-labile mannan.[4] The third type of β-mannose-containing side chains includes the sequence β-D-Man-(1→2)-α-D-Man-(1→3)-α-D-Man and corresponds to the antigenic factor 9. This structural motif has been found in the mannan from *Candida guilliermondii*.[2h] Mannooligosaccharides corresponding to the antigenic factors 1, 4, 5, and 6, and some others have been synthesized[5], while the synthesis of oligosaccharides corresponding to factor 9 has not been, to the best of our knowledge, described so far.

We now report the synthesis of the 3-aminopropyl glycoside of heptamannoside **18** comprising alternating α-(1→2)- and α-(1→3)-linked mannose units capped with a β-(1→2)-linked mannose residue. This oligosaccharide was isolated from *C. guilliermondii* mannan by acetolysis. We have also obtained a conjugate of oligosaccharide **18** with BSA for further immunological investigations. Synthetic yeast oligomannosides and neoglycoconjugates thereof can be used as molecular probes to assess the carbohydrate specificity of mannan-binding proteins[6] in the diagnostic of fungal infections[7] and Crohn's disease,[8] and for the design of vaccines of a new generation.[5f,i,9]

2. Results and discussion

A convergent approach based on glycosylation of a tetrasaccharide glycosyl acceptor with a trisaccharide glycosyl donor as the key step was applied for the preparation of the target heptamannoside. A critical β-mannosylation step was planned at early stages of the assembly of the trisaccharide donor to simplify isolation of the individual β-anomer, because separation of the anomers would be

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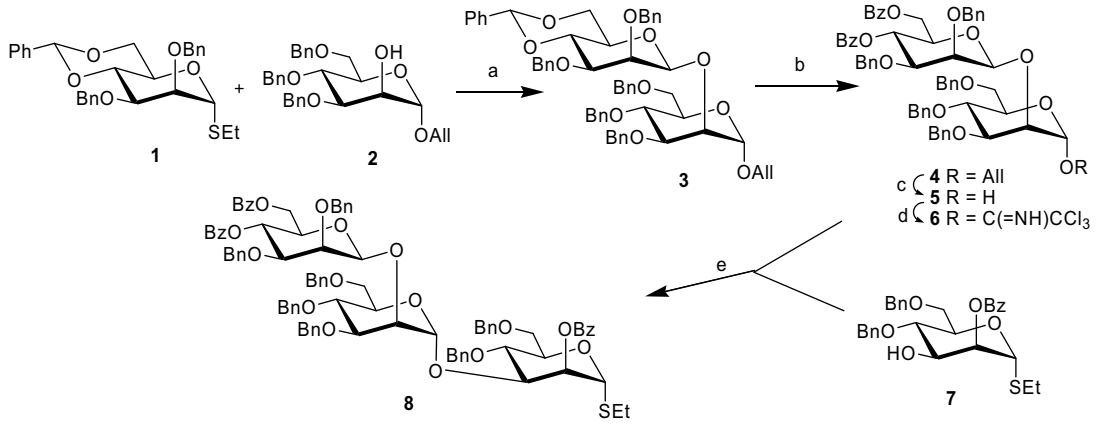
easier for disaccharides than for higher oligosaccharides. Stereoselective construction of β-mannosides is one of the challenges in the glycoside synthesis, and was extensively investigated in the past decade. As a result, diverse approaches to this goal have been developed.[5f–i,10] Glycosylation with easily accessible ethyl thiomannoside **1**[11] under the conditions proposed by Crich et al.[12] has been chosen for the stereoselective introduction of the β-(1→2)-linked mannosyl residue. Glycosylation of acceptor **2**[13] with thioglycoside **1** in the presence of Tf_2O and 1-benzenesulfinylpyrrolidine resulted in the formation of β-disaccharide **3** in 69% yield (Scheme 1). The β-configuration of the nonreducing mannosyl residue in disaccharide **3** was confirmed by the corresponding $^1J_{C1,H1}$ (157 Hz) coupling constant value.

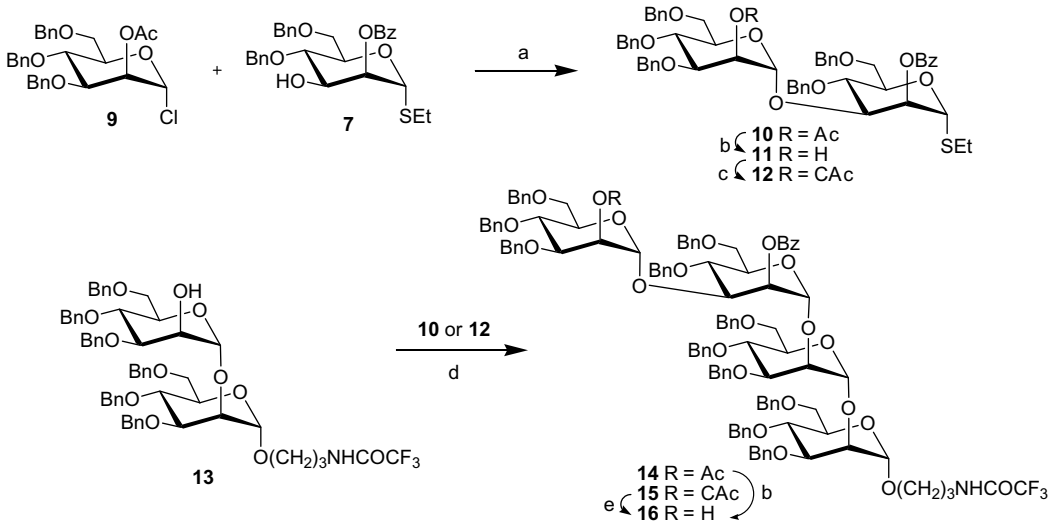
The acid-labile benzylidene group in **3** was replaced by stable benzoyl groups by treatment with aq CF_3CO_2H followed by conventional benzoylation with BzCl in pyridine to produce dibenzoate **4**. Removal of the anomeric allyl group from **4** with $PdCl_2$ and subsequent reaction of hemiacetal **5** with trichloroacetonitrile in the presence of DBU gave disaccharide donor **6**.

Glycosylation of thioglycoside **7**[5j] with trichloroacetimidate **6** resulted in the formation of the trisaccharide thioglycoside **8**, which was used as the donor to construct the nonreducing trisaccharide sequence of the target heptamannoside.

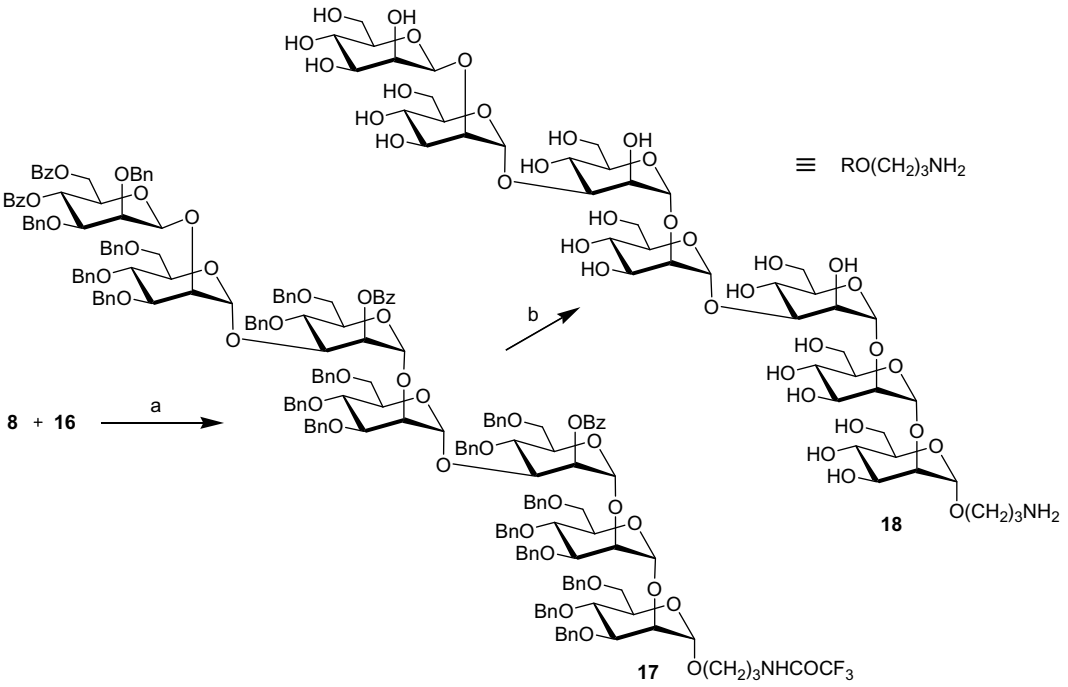
The tetrasaccharide acceptor block was assembled as follows. The α-(1→3)-linked disaccharide **10** was obtained by glycosylation of thiomannoside **7** with the known chloride **9**[14] in the presence of AgOTf (Scheme 2). Subsequent NIS-TfOH-promoted coupling of **10** with our previously described disaccharide acceptor **13**[5j] afforded smoothly tetrasaccharide **14**. Selective removal of an acetyl group in the presence of benzoyl groups is reported to be easily achieved by mild acidic methanolysis.[15] However, the acetyl group at O-2 of the terminal mannose unit in **14** proved to be unusually stable under these conditions, and its removal required a prolonged reaction time and was accompanied by a partial loss of the *N*-trifluoroacetyl and benzoyl groups. As a result, target tetrasaccharide acceptor **16** was obtained in poor yield. To circumvent this difficulty, the acetyl group in thioglycoside **10** was replaced by a chloroacetyl one. Treatment of **10** with HCl in MeOH provided the deacetylated product **11** in an acceptable yield of 54%; its further conventional chloroacetylation afforded the needed disaccharide donor **12**. Coupling of **12** with acceptor **13** in the presence of NIS and TfOH yielded tetrasaccharide **15**; subsequent removal of the chloroacetyl



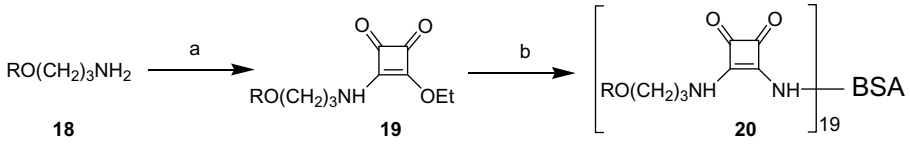
Scheme 1. Synthesis of trisaccharide donor **8**. Reagents and conditions: (a) (i) 1-benzenesulfinyl-pyrrolidine, 2,6-di-*tert*-butylpyridine, Tf_2O, CH_2Cl_2, −78 °C; (ii) **2**, −78 °C→rt, 69%; (b) (i) CF_3CO_2H 90%, $CHCl_3$, rt; (ii) BzCl, pyridine, rt, 71%; (c) $PdCl_2$, MeOH, rt, 67%; (d) CCl_3CN, DBU, CH_2Cl_2, −50 °C, 99%; (e) TMSOTf, CH_2Cl_2, −20 °C, 45%.



Scheme 2. Synthesis of tetrasaccharide acceptor **16**. Reagents and conditions: (a) AgOTf, CH_2Cl_2, –10 °C, 61%; (b) HCl, MeOH, rt, 54% of **11**,% of **16**; (c) CAcCl, pyridine, CH_2Cl_2 rt, 82%; (d) NIS, TfOH, CH_2Cl_2, –20 to –15 °C, 59%; (e) thiourea, 2,4,6-collidine, MeOH, reflux, 83%.



Scheme 3. Synthesis of heptamannoside **18**. Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, –20 to –15 °C, 67%; (b) (i) H_2, Pd/C, MeOH, rt; (ii) Amberlyst A-26 (OH^-), water, rt, 60%.



Scheme 4. Synthesis of heptasaccharide-BSA conjugate **20**. Reagents and conditions: (a) diethyl squarate, water, EtOH, pH 7, rt, 95%; (b) BSA, $KHCO_3$, $Na_2B_4O_7$, water, pH 9, rt, 80%.

group with thiourea proceeded smoothly producing the expected tetrasaccharide acceptor **16** in high yield.

Finally, heptamannoside **17** was obtained by NIS-TfOH-promoted coupling of the trisaccharide donor **8** and the tetrasaccharide acceptor **16** in 67% yield. The protected oligosaccharide **17** was first subjected to hydrogenolysis to remove benzyl groups; then benzoyl and *N*-trifluoroacetyl groups were simultaneously removed by treatment with the anion-exchange resin Amberlyst A-26 (OH^-) to give the target free heptamannoside **18** (Scheme 3).

The squarate procedure[16] was employed for conjugation of heptasaccharide **18** with BSA. First, treatment of **18** with diethyl squarate at pH 7 resulted in the formation of the monosubstituted adduct **19** (Scheme 4). Subsequent reaction of **19** with BSA at pH 9 afforded the target conjugate **20**. According to MALDI-TOFMS data, the conjugate contained on average 19 oligosaccharide copies per protein molecule.

3. Experimental

3.1. General methods

NMR spectra were recorded on Bruker DRX-500 and Bruker AM-300 instruments. Spectra of protected oligosaccharides were measured for solutions in $CDCl_3$, and ^1H NMR chemical shifts were referenced to the residual signal of $CHCl_3$. NMR spectra of free oligosaccharides were measured for solutions in D_2O using acetone (δ_H 2.225, δ_C 31.45) as internal standard. Monosaccharide residues in oligosaccharides are numbered by Roman numerals starting from the reducing end. MALDI-TOF mass spectra were obtained on a Bruker Ultraflex mass spectrometer with 2,5-dihydroxybenzoic acid as the matrix in the positive reflector mode. HRESIMS were obtained on a Finnigan LTQ FT (Thermo Electrom Corp.) instrument. Optical rotations were measured using a JASCO DIP-360 polarimeter at 18–22 °C in $CHCl_3$ in the case of the protected and partially protected derivatives and in water in the case of free oligosaccharides. TLC was performed on Silica Gel 60 F254 plates (E. Merck), and visualization was accomplished using UV light or by charring with 10% H_3PO_4 in EtOH. Column chromatography was carried out on Silica Gel 60 (40–63 μm, E. Merck). Gel-permeation chromatography of free oligosaccharides was performed on a column of TSK HW-40 (S) gel (25 × 800 mm) in 0.1 M AcOH. All reactions involving air- or moisture-sensitive reagents were carried out using dry solvents under dry argon.

3.2. Allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (3)

Molecular sieves 4 Å (400 mg) were added to a soln of thioglycoside **1** (201 mg, 0.408 mmol), 1-benzenesulfinylpyrrolidine[12] (88 mg, 0.45 mmol), and 2,6-di-*tert*-butylpyridine (180 μL, 0.49 mmol) in CH_2Cl_2 (4 mL). The mixture was cooled to −78 °C, stirred for 40 min, then Tf_2O (83 μL, 0.49 mmol) was added followed by a soln of acceptor **2** (200 mg, 0.408 mmol) in CH_2Cl_2

(4 mL). The resulting mixture was stirred at −78 °C for 30 min, and was then allowed to warm to room temperature. The mixture was diluted with $CHCl_3$, filtered through a Celite layer, the filtrate was washed with satd $NaHCO_3$ and water, and the solvent was evaporated. Column chromatography of the residue (20:1 toluene–EtOAc) gave **3** (258 mg, 69%) as a colorless foam; $[\alpha]_D$ −36.7 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 7.58–7.05 (m, 30H, 6Ph), 5.88 (m, 1H, $OCH_2CH{=}CH_2$), 5.59 (s, 1H, PhC*H*), 5.26 (d, 1H, *J* 17.2 Hz, $OCH_2CH{=}CH_2$), 5.18 (d, 1H, *J* 10.3 Hz, $OCH_2CH{=}CH_2$), 5.09 (d, 1H, *J* 11.7 Hz, $PhCH_2$), 4.96 (s, 1H, H-1^I), 4.85 (d, 2H, *J* 11.4 Hz, $PhCH_2$), 4.78 (d, 1H, *J* 10.8 Hz, $PhCH_2$), 4.67 (d, 1H, *J* 12.6 Hz, $PhCH_2$), 4.64 (s, 1H, H-1^{II}), 4.62 (d, 1H, *J* 12.6 Hz, $PhCH_2$), 4.59 (d, 1H, *J* 12.1 Hz, $PhCH_2$), 4.52 (d, 1H, *J* 11.2 Hz, $PhCH_2$), 4.45 (d, 1H, *J* 12.1 Hz, $PhCH_2$), 4.32 (d, 1H, *J* 10.9 Hz, $PhCH_2$), 4.29 (br s, 1H, H-2^I), 4.24–4.20 (m, 3H, H-6^{II}_a, H-4^{II}, $OCH_2CH{=}CH_2$), 4.03 (d, 1H, $J_{2,3}$ 3.0 Hz, H-2^{II}), 4.00 (m, 1H, $OCH_2CH{=}CH_2$), 3.97 (m, 1H, H-3^I), 3.91 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^I), 3.88 (t, 1H, $J_{5,6} = J_{6,6}$ 10.2 Hz, H-6^{II}_b), 3.78 (m, 1H, H-5^I), 3.72 (m, 1H, H-6^I_a), 3.67 (m, 1H, H-6^I_b), 3.58 (dd, $J_{2,3}$ 3.1, $J_{3,4}$ 9.9 Hz, H-3^{II}), 3.32 (m, 1H, H-5^{II}); ^{13}C NMR (126 MHz, $CDCl_3$): δ 138.4–137.6. 133.7, 133.6, 129.1–127.4, 126.1, 125.3 (Ph, $OCH_2CH{=}CH_2$), 117.6 (1C, $OCH_2CH{=}CH_2$), 101.4 (1C, $J_{C1,H1}$ = 157 Hz, C-1^{II}), 100.4 (1C, PhCH), 96.5 (1C, $J_{C1,H1}$ 168 Hz, C-1^I), 78.4 (1C, C-4^{II}), 78.1 (1C, C-3^I), 77.2 (1C, C-3^{II}), 76.2 (1C, C-2^{II}), 75.0, 74.9 (2C, $PhCH_2$), 74.3 (1C, C-4^I), 73.3 (1C, $PhCH_2$), 72.8 (1C, C-2^I), 71.8 (1C, $PhCH_2$), 71.5 (1C, C-5^I), 70.7 (1C, $PhCH_2$), 69.0 (1C, C-6^I), 68.5 (1C, C-6^{II}), 68.1 (1C, $OCH_2CH{=}CH_2$), 67.7 (1C, C-5^{II}). Anal. Calcd for $C_{57}H_{60}O_{11}$: C, 74.33; H, 6.57. Found: C, 74.17; H, 6.73.

3.3. Allyl 4,6-di-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (4)

90% aq CF_3CO_2H (1 mL) was added to a soln of **3** (407 mg, 0.442 mmol) in $CHCl_3$ (8 mL). After 30 min, the mixture was diluted with $CHCl_3$, washed with satd $NaHCO_3$ and water, and the solvent was evaporated. The residue was dissolved in pyridine (1 mL), BzCl (0.31 mL, 2.6 mmol) was added and the mixture was allowed to stand overnight. The mixture was diluted with $CHCl_3$, washed with satd $NaHCO_3$ and water, concentrated, and the residual pyridine was removed by coevaporation with toluene. Column chromatography of the residue (15:1 toluene–EtOAc) provided **4** (328 mg, 71%) as a white solid; $[\alpha]_D$ −41.7 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 7.78–7.03 (m, 35H, 7Ph), 5.84 (m, 1H, $OCH_2CH{=}CH_2$), 5.74 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4^{II}), 5.23 (dd, 1H, *J* 17,2, *J* 1.4 Hz, $OCH_2CH{=}CH_2$), 5.15 (d, 1H, *J* 10.4 Hz, $OCH_2CH{=}CH_2$), 5.10 (d, 1H, *J* 11.7 Hz, $PhCH_2$), 4.96 (d, 1H, $J_{1,2}$ 1,5 Hz, H-1^I), 4.87 (d, 1H, *J* 11.9 Hz, $PhCH_2$), 4.84 (d, 1H, *J* 12.3 Hz, $PhCH_2$), 4.74 (d, 1H, *J* 10.8 Hz, $PhCH_2$), 4.71 (s, 1H, H-1^{II}), 4.59 (d, 1H, *J* 12.1 Hz, $PhCH_2$), 4.54 (dd, $J_{5,6}$ 3.2, $J_{6,6}$ 12.0 Hz, H-6^{II}_a), 4.53 (d, 1H, *J* 12.7 Hz, $PhCH_2$), 4.44 (d, 1H, *J* 12.1 Hz, $PhCH_2$), 4.41–4.36 (m, 3H, H-2^I, H-6^{II}_b, $PhCH_2$), 4.28 (d, 1H, *J* 12.7 Hz, $PhCH_2$), 4.27 (d, 1H, *J* 10.8 Hz, $PhCH_2$), 4.17 (m, 1H, $OCH_2CH{=}CH_2$), 4.03 (d, 1H, $J_{2,3}$ 2.9 Hz, H-2^{II}), 3.98–3.92 (m, 2H, H-3^I, $OCH_2CH{=}CH_2$), 3.88 (t, 1H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4^I), 3.84 (m, 1H, H-5^{II}), 3.70 (m, 1H, H-5^I), 3.76 (dd, 1H, $J_{5,6}$ 4.3, $J_{6,6}$ 9,6 Hz, H-6^I_a), 3.67 (dd, 1H, $J_{5,6}$ 1.9, $J_{6,6}$ 9.6 Hz, H-6^I_b), 3.62 (dd, $J_{2,3}$ 2.9, $J_{3,4}$ 9.7 Hz, H-3^{II}); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.2, 165.3 (PhCO), 138.8–137.6. 133.7, 133.6, 133.0, 132.8, 129.8, 129.6, 128.4–127.4, 126.1, 127.2 (Ph, $OCH_2CH{=}CH_2$), 117.5 (1C, $OCH_2CH{=}CH_2$), 99.3 (1C, C-1^I), 96.1 (1C, C-1^{II}), 77.9 (1C, C-3^I), 77.7 (1C, C-3^{II}), 74.8, 74.3 (2C, $PhCH_2$), 74.0 (1C, C-4^I), 73.6 (1C, C-2^{II}), 73.3 (1C, $PhCH_2$), 72.7 (1C, C-5^{II}), 71.8 (1C, C-2^I), 71.4 (1C, C-5^I), 70.3, 70.1 (2C, $PhCH_2$), 69.1 (1C, C-6^I), 69.0 (1C, C-4^{II}), 68.1 (1C, OCH_2CHCH_2), 63.9 (1C, C-6^{II}). Anal. Calcd for $C_{64}H_{64}O_{13}$: C, 73.83; H, 6.20. Found: C, 73.69; H, 6.20.

3.4. 4,6-Di-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-D-mannopyranose (5)

$PdCl_2$ (54 mg, 0.304 mmol) was added to a soln of **4** (316 mg, 0.304 mmol) in dry MeOH (3 mL). The mixture was vigorously stirred until disappearance of starting **4** (TLC monitoring). The mixture was diluted with EtOAc, filtered through a silica gel layer, the filtrate was made neutral with NEt_3, and the solvent was evaporated. The residue was subjected to column chromatography (5:1 toluene–EtOAc) to give **5** (204 mg, 67%) as an α,β-mixture in a ratio of ~3:1; $[\alpha]_D$ −46.8 (*c* 1, $CHCl_3$). ^1H NMR for α-**5** (500 MHz, $CDCl_3$): δ 7.98–7.03 (m, 35H 7 Ph), 5.75 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4^{II}), 5.34 (s, 1H, H-1^I), 5.08 (d, 1H, *J* 11.8 Hz, $PhCH_2$), 4.88–4.82 (m, 2H, $PhCH_2$), 4.74 (d, 1H, *J* 10.8 Hz, $PhCH_2$), 4.71 (d, 1H, H-1^{II}), 4.57 (m, 1H, H-6^{II}_a), 4.55–4.43 (m, 3H, $PhCH_2$), 4.42–4.35 (m, 3H, H-2^I, H-6^{II}_b, $PhCH_2$), 4.29 (d, 1H, *J* 11.1 Hz, $PhCH_2$), 4.09(d, 1H, $J_{2,3}$ 2.7 Hz, H-2^{II}), 4.03–3.96 (m, 2H, H-3^I, H-5^I), 3.82 (m, 1H, H-5^{II}), 3.76 (t, 1H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^I), 3.63–3.58 (m, 3H, H-3^{II}, H-6^I_a, H-6^{II}_b); ^{13}C (126 MHz, $CDCl_3$): δ 166.1, 165.3 (PhCO), 138.2–137.5, 133.1, 132.8, 129.8–129.6, 128.4–127.2 (Ph), 99.4 (1C, C-1^{II}), 91.8 (1C, C-1^I), 77.9 (1C, C-3^{II}), 77.3 (1C, C-3^I), 74.6 (1C, $PhCH_2$), 74.2 (2C, C-4^I, $PhCH_2$), 73.5 (1C, C-2^{II}), 73.4 (1C, $PhCH_2$), 72.7 (1C, C-5^{II}), 72.1 (1C, C-2^I), 71.4 (1C, C-5^I), 70.4, 70.3 (2C, $PhCH_2$), 69.4 (1C, C-6^I), 69.0 (1C, C-4^{II}), 63.8 (1C, C-6^{II}). Anal. Calcd for $C_{61}H_{60}O_{13}$: C, 73.18; H, 6.04. Found: C, 73.03; H, 6.25.

3.5. 4,6-Di-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (6)

To a soln of **5** (69 mg, 0.069 mmol) and trichloroacetonitrile (69 μL, 0.69 mmol) in CH_2Cl_2 (2 mL) was added a catalytic amount of DBU at −50 °C. After being stirred for 20 min, the mixture was allowed to warm up to room temperature and the residue was subjected to column chromatography (10:1 toluene–EtOAc) to give **6** (78 mg, 99%) as a white foam; $[\alpha]_D$ −26.6 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.46 (s, 1H, NH), 7.98–7.06 (m, 35 H, 7 Ph), 6.37 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1^I), 5.74 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4^{II}), 5.08 (d, 1H, *J* 11.8 Hz, $PhCH_2$), 4.88 (d, 1H, *J* 11.3 Hz, $PhCH_2$), 4.84 (d, 1H, *J* 11.8 Hz, $PhCH_2$), 4.81 (s, 1H, H-1^{II}), 4.78 (d, 1H, *J* 10.8 Hz, $PhCH_2$), 4.60–4.56 (m, 2H, H-6^{II}_a, $PhCH_2$), 4.56–4.52 (m, 2H, H-2^I, $PhCH_2$), 4.45 (d, 2H, *J* 11.8 Hz, $PhCH_2$), 4.41 (dd, 1H, $J_{5,6}$ 6.2, $J_{6,6}$ 12.0 Hz, H-6^{II}_b), 4.36 (d, 1H, *J* 10.8 Hz, $PhCH_2$), 4.29 (d, 1H, *J* 12.6 Hz, $PhCH_2$), 4.11 (d, 1H, $J_{2,3}$ 2.7 Hz, H-2^{II}), 4.03 (t, 1H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^I), 3.98–3.93 (m, 2H, H-3^I, H-5^{II}), 3.90 (m, 1H, H-5^{II}), 3.74 (dd, 1H, $J_{5,6}$ 3.9, $J_{6,6}$ 11.0 Hz, H-6^I_a), 3.68 (dd, 1H, $J_{5,6}$ 1.6, $J_{6,6}$ 11.0 Hz, H-6^I_b), 3.64 (dd, $J_{2,3}$ 3.0, $J_{3,4}$ 9.7 Hz, H-3^{II}); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 165.4 (PhCO), 160.5 (C=NH), 138.6–137.5, 133.1, 132.8, 129.8–129.6, 128.4–127.3 (Ph), 99.3 (1C, C-1^{II}), 95.4 (1C, C-1^I), 77.7 (1C, C-3^{II}), 76.9 (1C, C-3^I), 75.0, 74.3 (2C, $PhCH_2$), 74.2 (1C, C-5^I), 73.4 (1C, C-2^{II}), 73.3 (2C, C-4^I, $PhCH_2$), 72.8 (1C, C-5^{II}), 70.4, 70.1 (2C, $PhCH_2$), 69.9 (1C, C-2^I), 69.0 (1C, C-4^{II}), 68.6 (1C, C-6^I), 63.9 (1C, C-6^{II}). Anal. Calcd for $C_{63}H_{60}Cl_3NO_{13}$: C, 66.06; H, 5.28; N, 1.22. Found: C, 65.81; H, 5.27; N, 1.32.

3.6. Ethyl 4,6-di-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (8)

Molecular sieves 4 Å (150 mg) were added to a soln of thioglycoside acceptor **7** (35 mg, 0.068 mmol) and donor **6** (78 mg, 0.68 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred for 30 min at room temperature, cooled to −50 °C, and then TMSOTf (4.6 μL, 0.024 mmol) was added. The resulting mixture was stirred at –15

to −20 °C until disappearance of starting **7** (TLC monitoring), quenched with triethylamine, diluted with $CHCl_3$, and filtered through a Celite layer. The filtrate was washed with water and concentrated. Column chromatography of the residue (20:1 toluene–EtOAc) afforded **8** (46 mg, 45%) as a colorless foam; $[\alpha]_D$ −17.5 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09–6.95 (m, 50H, 10Ph), 5.57 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4^{III}), 5.54 (br s, 1H, H-2^{I}), 5.44 (s, 1H, H-1^{I}), 5.33 (s, 1H, H-1^{II}), 5.03 (d, 1H, *J* 11.6 Hz, PhC*H*$_2$), 4.75–4,62 (m, 7H, PhC*H*$_2$), 4.57–4.45 (m, 4H, PhC*H*$_2$), 4.39 (dd, 1H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.9 Hz, H-3^{I}) 4.33 (dd, 1H, $J_{5,6}$ 3.2, $J_{6,6}$ 12.0 Hz H-6$^{III}_a$), 4.25–4.18 (m, 4H, H-2^{II}, H-4^{I}, H-5^{I}, PhC*H*$_2$), 4.15 (d, 2H, *J* 10.1 Hz, PhC*H*$_2$), 4.13 (dd, 1H, $J_{5,6}$ 5.2, $J_{6,6}$ 12.0 Hz, H-6$^{III}_b$), 4.03 (s, 1H, H-1^{III}), 3.93 (m, 1H, H-5^{II}), 3.91(br s, 1H, H-2^{III}), 3.87 (t, 1H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4^{II}) 3.84 (dd, 1H, $J_{5,6}$ 3.2, $J_{6,6}$ 10.6 Hz, H-6$^{I}_a$), 3.76 (dd, $J_{2,3}$ 3.1, $J_{3,4}$ 8.9 Hz, H-3^{II}), 3.71–3.66 (m, 3H, H-6$^{I}_b$, H-6$^{II}_a$, H-6$^{II}_b$), 3.11 (dd, $J_{2,3}$ 3.0, $J_{3,4}$ 9.8 Hz, H-3^{III}), 2.73 (m, 1H, H-5^{III}), 2.65 (m, 2H, SC*H*$_2$CH$_3$), 1.30 (t, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 165.7, 165.4 (Ph*C*O), 139.0–138.0, 133.4–133.0, 130.2–127.3, 126.1–125.9 (Ph), 99.1 (1C, $J_{C1,H1}$ 155 Hz, C-1^{III}), 99.1 (1C, $J_{C1,H1}$ 170 Hz, C-1^{II}), 82.6 (1C, $J_{C1,H1}$ 168 Hz, C-1^{I}) 77.6 (1C, C-3^{II}), 76.9 (1C, C-3^{III}), 76.1 (1C, C-3^{I}), 75.5 (1C, PhC*H*$_2$), 74.7–74.1 (C-2^{I}, C-4^{I}, PhC*H*$_2$), 73.8–73.4 (C-4^{II}, PhC*H*$_2$), 73.3 (1C, C-5^{II}), 72.2 (1C, C-5^{I}), 72.0 (3C, C-2^{II}, C-2^{III}, C-5^{III}), 70.1, 69.7 (2C, PhC*H*$_2$), 68.7 (1C, C-4^{III}), 68.6 (2C, C-6^{I}, C-6^{II}), 63.5 (1C, C-6^{III}), 25.6 (1C, S*C*H$_2$CH$_3$), 14.9 (1C, SCH$_2$*C*H$_3$). Anal. Calcd for $C_{90}H_{90}O_{18}S$: C, 72.46; H, 6.08. Found: C, 72.10; H, 6.23.

3.7. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (10)

Molecular sieves 4 Å (2.0 g) were added to a soln of a thioglycoside **7** (716 mg, 1.41 mmol) and chloride **9** (1.10 g, 2.15 mmol) in CH_2Cl_2 (8 mL), the mixture was stirred for 30 min at room temperature, cooled to –50 °C, and then AgOTf (830 mg, 3.22 mmol) was added. The resulting mixture was stirred at –15 to –20 °C until disappearance of **7** (TLC monitoring), quenched with triethylamine, diluted with $CHCl_3$, and filtered through a Celite layer. The filtrate was washed with 1 M aq $Na_2S_2O_3$, water, and the solvent was evaporated. Column chromatography of the residue (20:1 toluene–EtOAc) provided **10** (846 mg, 61%) as a colorless foam; $[\alpha]_D$ +27.6 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03–7.05 (m, 30H, 6 Ph-H), 5.52 (br s, 1H, H-2^{I}), 5.42 (s, 1H, H-1^{I}), 5.36 (br s, 1H, H-2^{II}), 5.17 (s, 1H, H-1^{II}), 4.75 (d, 2H, *J* 11.0 Hz, PhC*H*$_2$), 4.71 (d, 1H, *J* 12.5 Hz, PhC*H*$_2$), 4.68 (d, 1H, *J* 12.9 Hz, PhC*H*$_2$), 4.54 (d, 1H, *J* 10.8, PhC*H*$_2$), 4.48 (d, 2H, *J* 12.3 Hz, PhC*H*$_2$), 4.41 (m, 2H, PhC*H*$_2$), 4.32 (d, 1H, *J* 11.1 Hz, PhC*H*$_2$), 4.25–4.18 (m, 3H, H-3^{I}, H-4^{I}, H-5^{I}), 3.89 (m, 2H, H-4^{II}, H-6$^{I}_a$), 3.82 (m, 2H, H-3^{II}, H-5^{II}), 3.71 (d, 1H, $J_{6,6}$ 10.8, H-6$^{I}_b$), 3.62 (dd, 1H, $J_{5.6}$ 3.1, $J_{6.6}$ 10.7 Hz, H-6$^{II}_a$), 3.56 (d, 1H, $J_{6.6}$ 10.7 Hz, H-6$^{II}_b$), 2.61 (m, 2H, SC*H*$_2$CH$_3$), 2.08 (s, 3H, C*H*$_3$CO), 1.26 (t, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2 (CH3*C*O), 165.6, (Ph*C*O), 138.6, 138.3, 137.9. 133.2, 129.8, 128.9–127.3 (Ph), 99.6 (1C, C-1^{II}), 82.2 (1C, C-1^{I}), 77.7 (2C, C-3^{I}, C-3^{II}), 75.2 (1C, PhC*H*$_2$), 75.0 (1C, C-4^{I}), 74.5 (1C, PhC*H*$_2$), 74.1 (1C, C-2^{I}), 73.9 (1C, C-4^{II}), 73.4 (2C, 2 PhC*H*$_2$), 72.3 (1C, C-5^{II}), 72.0 (1C, C-5^{I}), 71.8 (1C, PhC*H*$_2$), 69.0 (1C, C-2^{II}), 68.8 (1C, C-6^{I}), 68.3 (1C, C-6^{II}), 25.7 (1C, S*C*H$_2$CH$_3$), 21.0 (1C, *C*H$_3$CO), 15.0 (1C, SCH$_2$*C*H$_3$). Anal. Calcd for $C_{58}H_{62}O_{12}S$: C, 70.85; H, 6.36. Found: C, 70.20; H, 6.48.

3.8. Ethyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (11)

Anhyd HCl in MeOH (1 M, 1.6 mL), obtained by adding AcCl (114 μL, 1.6 mmol) to chilled MeOH (1.5 mL) was added to a soln of **10** (233 mg, 0.237 mmol) in dry CH_2Cl_2 (0.4 mL). The resulting mixture was kept for 3 days at ambient temperature, diluted with $CHCl_3$, washed with satd $NaHCO_3$ and water, and concentrated. Column chromatography of the residue (10:1 toluene–EtOAc) gave **11** (120 mg, 54%) as a colorless foam; $[\alpha]_D$ +38.6 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02–7.08 (m, 30H, 6Ph), 5.53 (dd, 1H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.1 Hz, H-2^{I}), 5.42 (d, 1H, $J_{1,2}$ 1.9, H-1^{I}), 5.19 (s, 1H, H-1^{II}), 4.73–4.62 (m, 4H, PhC*H*$_2$), 4.56–4.42 (m, 6H, PhC*H*$_2$), 4.24 (dd, 1H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.1 Hz H-3^{I}), 4.19 (m, 1H, H-5^{I}), 4.15 (t, 1H, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4^{I}) 3.91–3.83 (m, 3H, H-6$^{I}_a$, H-2^{II} H-4^{II}), 3.79 (m, 1H, H-5^{II}), 3.73–3.67 (m, 2H, H-6$^{I}_b$, H-3^{II}), 3.59 (dd, 1H, $J_{5,6}$ 3.4, $J_{6,6}$ 10.9 Hz, H-6$^{II}_a$), 3.56 (d, 1H, $J_{5,6}$ 1.8, $J_{6,6}$ 10.7 Hz, H-6$^{II}_b$), 2.61 (m, 2H, SC*H*$_2$CH$_3$), 1.27 (t, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.6, (Ph*C*O), 138.5 138.3, 137.9. 133.1, 129.8, 128.9–127.3 (Ph), 101.4 (1C, C-1^{II}), 82.3 (1C, C-1^{I}), 79.6 (1C, C-3^{II}) 77.9 (1C, C-3^{I}), 75.1 (1C, PhC*H*$_2$), 75.0 (1C, C-4^{I}), 74.5 (1C, PhC*H*$_2$), 74.2 (1C, C-2^{I}), 73.9 (1C, C-4^{II}), 73.4 (2C, 2 PhC*H*$_2$), 72.0 (3C, C-5^{I}, C-5^{II}, PhC*H*$_2$), 68.9 (1C, C-6^{I}), 68.8 (1C, C-2^{II}), 68.4 (1C, C-6^{II}), 25.6 (1C, S*C*H$_2$CH$_3$), 14.9 (1C, SCH$_2$*C*H$_3$). Anal. Calcd for $C_{56}H_{60}O_{11}S$: C, 71.47; H, 6.43. Found: C, 71.23; H, 6.68.

3.9. Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (12)

To a soln of disaccharide **11** (93 mg, 0.10 mmol) in dry CH_2Cl_2 (2 mL) was added pyridine (32 μL, 0.40 mmol) followed by chloroacetyl chloride (16 μL, 0.20 mmol). After 6 h, the mixture was diluted with $CHCl_3$ and washed successively with 1 M HCl, satd $NaHCO_3$ and water. The solvent was evaporated and the residue was chromatographed (15:1 toluene–EtOAc) to give **12** (83 mg, 82%) as a yellowish foam; $[\alpha]_D$ +28.6 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03–7.05 (m, 30H, 6Ph), 5.50 (dd, 1H, $J_{1,2}$ 1.4, $J_{2,3}$ 2.7 Hz, H-2^{I}), 5.40 (d, 1H, $J_{1,2}$ 1.4, H-1^{I}), 5.36 (br s, 1H, H-2^{II}), 5.17 (d, 1H, $J_{1,2}$ 1.4, H-1^{II}), 4.72 (d, 2H, *J* 11.1 Hz, PhC*H*$_2$), 4.68 (d, 1H, *J* 10.0 Hz, PhC*H*$_2$), 4.64 (d, 1H, *J* 12.1 Hz, PhC*H*$_2$), 4.57 (d, 1H, *J* 10.9, PhC*H*$_2$), 4.49 (d, 1H, *J* 11.9 Hz, PhC*H*$_2$), 4.47 (d, 1H, *J* 11.1 Hz, PhC*H*$_2$), 4.41 (m, 2H, PhC*H*$_2$), 4.31 (d, 1H, *J* 11.1 Hz, PhC*H*$_2$), 4.25–4.19 (m, 3H, H-3^{I}, H-4^{I}, H-5^{I}), 4.07 (d, 1H, *J* 15.2, ClC*H*$_2$CO), 4.01 (d, 1H, *J* 15.2, ClC*H*$_2$CO), 3.90 (dd, 1H, $J_{5.6}$ 2.2, $J_{6,6}$ 10.8 Hz, H-6$^{I}_a$), 3.87–3.80 (m, 3H, H-3^{II}, H-4^{II}, H-5^{II}), 3.71 (d, 1H, $J_{6,6}$ 10.9, H-6$^{I}_b$), 3.63 (dd, 1H, $J_{5,6}$ 2.4, $J_{6,6}$ 10.7 Hz, H-6$^{II}_a$), 3.58 (d, 1H, $J_{6,6}$ 10.7 Hz, H-6$^{II}_b$), 2.61 (m, 2H, SC*H*$_2$CH$_3$), 1.27 (t, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.6 (ClCH$_2$*C*O), 165.6, (Ph*C*O), 138.4, –137.6. 133.2, 129.8, 128.5–127.3 (Ph), 99.1 (1C, C-1^{II}), 82.3 (1C, C-1^{I}), 77.6 (1C, C-3^{II}), 77.4 (1C, C-3^{I}), 75.2 (1C, PhC*H*$_2$), 75.0 (1C, C-4^{I}), 74.5 (1C, PhC*H*$_2$), 74.0 (1C, C-2^{I}), 73.7 (1C, C-4^{II}), 73.4 (2C, 2 PhC*H*$_2$), 72.3 (1C, C-5^{II}), 72.1 (2C, C-5^{I}, PhC*H*$_2$), 70.7 (1C, C-2^{II}), 68.8 (1C, C-6^{I}), 68.2 (1C, C-6^{II}), 40.7 (1C, ClC*H*$_2$CO), 25.6 (1C, S*C*H$_2$CH$_3$), 14.9 (1C, SCH$_2$*C*H$_3$). Anal. Calcd for $C_{58}H_{61}ClO_{12}S$: C, 68.46; H, 6.04. Found: C, 68.26; H, 6.18.

3.10. 3-Trifluoroacetamidopropyl 3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (15)

Molecular sieves 4 Å (700 mg) were added to a soln of acceptor **13** (300 mg, 0.290 mmol) and donor **12** (360 mg, 0.344 mmol) in CH_2Cl_2 (2 mL); the mixture was stirred for 30 min at room temperature and cooled to −15 °C. NIS (155 mg, 0.644 mmol) was added, the mixture was stirred for 10 min, then the temperature was decreased to −35 °C and TfOH (11 μL, 0.12 mmol) was added. The reaction mixture was stirred at −25 °C to −30 °C until TLC showed disappearance of starting **13**. The reaction was quenched with pyridine (19 μL, 0.24 mmol), diluted with $CHCl_3$,

and filtered through a Celite layer. The filtrate was successively washed with 1 M aq $Na_2S_2O_3$ soln, 1 M HCl and water, and concentrated. Column chromatography of the residue (9:1 toluene–EtOAc) afforded **15** (406 mg, 59%); colorless foam, $[\alpha]_D$ +16.7 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.03–6.85 (m, 60H, 12 Ph), 5.55 (br s, 1H, H-2^{III}), 5.43 (br s, 1H, H-2^{IV}), 5.22 (s, 2H, H-1^{II}, H-1^{IV}), 5.10 (s, 1H, H-1^{III}), 4.91 (s, 1H, H-1^{I}), 4.86 (d, 1H, *J* 10.8 Hz, PhCH_2), 4.78 (d, 1H, *J* 10.8 Hz, PhCH_2), 4.71–4.44 (m, 16H, PhCH_2), 4.39–4.31 (m, 4H, H-3^{III}, PhCH_2), 4.22 (d, 1H, *J* 12.6 Hz, PhCH_2), 4.18 (t, 1H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4^{III}), 4.08 (d, 1H, *J* 15.3 Hz, ClCH_2CO), 4.02 (br s, 1H, H-2^{II}), 4.00 (d, 1H, *J* 15.3 Hz, ClCH_2CO), 3.99–3.89 (m, 5H, H-2^{I}, H-3^{II}, H-4^{IV}, H-5^{III}, H-5^{II}), 3.87 (dd, 1H, $J_{2,3}$ 2.8, $J_{3,4}$ 8.5 Hz, H-3^{IV}), 3.83 (m, 1H, H-5^{IV}), 3.80–3.69 (m, 5H, H-3^{I}, H-4^{II}, H-6^{II}_a, H-6^{II}_b, H-6^{III}_a), 3.69–3.64 (m, 4H, H-4^{1}, H-5^{1}, H-6^{I}_a, H-6^{I}_b), 3.60 (m, 1H, OCH_2CH_2CH_2N), 3.54 (d, 2H, *J* 10.8 Hz, H-6^{III}_b, H-6^{IV}_a), 3.47 (d, 1H, $J_{6,6}$ 10.6 Hz, H-6^{IV}_b), 3.36 (m, 1H, OCH_2CH_2CH_2N), 3.26–3.15 (m, 2H, OCH_2CH_2CH_2N, OCH_2CH_2CH_2N), 1.70 (m, 2H, OCH_2CH_2CH_2N). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.6 (ClCH_2CO), 165.4, (PhCO), 138.5–137.6, 133.2, 129.8, 129.0, 128.5–127.4 (Ph), 100.7, (1C, C-1^{II}), 99.2 (1C, C-1^{III}), 99.1 (1C, C-1^{IV}), 98.9 (1C, C-1^{I}), 79.5 (1C, C-3^{I}), 78.9 (1C, C-3^{II}), 77.8 (1C, C-3^{IV}), 76.9 (1C, C-3^{III}), 76.5 (1C, C-2^{II}), 75.3–75.0 (C-2^{I}, C-4^{II}, PhCH_2), 74.8–74.5 (C-4^{I}, C-4^{III}, PhCH_2), 73.6 (1C, C-4^{IV}), 73.4–73.2 (PhCH_2), 72.3–71.8 (C-2^{III}, C-5^{I}, C-5^{II}, C-5^{III}, C-5^{IV}, PhCH_2), 70.8 (1C, C-2^{IV}), 69.9 (1C, C-6^{II}), 69.3 (1C, C-6^{I}), 68.8 (1C, C-6^{III}), 68.0 (1C, C-6^{IV}), 65.8 (1C, OCH_2CH_2CH_2N), 40.8 (1C, ClCH_2CO), 38.0 (1C, OCH_2CH_2CH_2N), 28.0 (1C, OCH_2CH_2CH_2N). Anal. Calcd for $C_{115}H_{119}ClF_3NO_{24}$: C, 69.35; H, 6.02; N, 0.70. Found: C, 69.02; H, 6.04; N, 0.78.

3.11. 3-Trifluoroacetamidopropyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (16)

Tetrasaccharide **15** (379 mg, 0.191 mmol) was dissolved in a mixture of MeOH (10 mL) and $CHCl_3$ (2 mL), then 2,4,6-collidine (27 μL, 0.20 mmol) and thiourea (77 mg, 1.01 mmol) were added. The mixture was boiled under reflux for 3 days, cooled, and taken to dryness. A soln of the residue in $CHCl_3$ was washed with 1 M HCl and satd $NaHCO_3$, and concentrated. The residue was purified by column chromatography (5:1 toluene–EtOAc) to provide **16** (304 mg, 83%) as a white foam; $[\alpha]_D$ +24.5 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.20–7.09 (m, 60 H, 12 Ph), 5.70 (br s, 1H, H-2^{III}), 5.37 (s, 1H, H-1^{II}), 5.32 (s, 1H, H-1^{IV}), 5.27 (s, 1H, H-1^{III}), 5.04 (s, 1H, H-1^{I}), 4.96 (d, 1H, *J* 10.9 Hz, PhCH_2), 4.91 (d, 1H, *J* 10.8 Hz, PhCH_2), 4.88 (d, 1H, *J* 11.3 Hz, PhCH_2), 4.81 (d, 1H, *J* 11.1 Hz, PhCH_2), 4.78–4.57 (m, 16H, PhCH_2), 4.49–4.56 (m, 2H, H-3^{III}, PhCH_2), 4.38 (d, 1H, *J* 11.5 Hz, PhCH_2), 4.25 (t, 1H, $J_{3,4} = J_{4,5}$ 7.3 Hz, H-4^{III}), 4.22 (br s, 1H, H-2^{II}), 4.14–4.00 (m, 6H, H-2^{I}, H-2^{IV}, H-3^{II}, H-4^{IV}, H-5^{II}, H-5^{III}), 3.95–3.91 (m, 2H, H-3^{I}, H-5^{IV}), 3.91–3.78 (m, 9H, H-3^{IV}, H-4^{I}, H-4^{II}, H-5^{I}, H-6^{I}_a, H-6^{I}_b, H-6^{II}_a, H-6^{II}_b, H-6^{III}_a), 3.74 (m, 1H, OCH_2CH_2CH_2N), 3.70–3.66 (m, 2H, H-6^{III}_b, H-6^{IV}_a), 3.61 (d, 1H, $J_{6,6}$ = 9.4 Hz, H-6^{IV}_b), 3.44–3.34 (m, 3H, OCH_2CH_2CH_2N, 2 OCH_2CH_2CH_2N), 1.86 (m, 2H, OCH_2CH_2CH_2N); ^{13}C NMR (126 MHz, $CDCl_3$): δ 138.9–138.0, 129.9, 128.3–127.4 (Ph), 101.8 (1C, C-1^{IV}), 100.7, (1C, C-1^{II}), 99.2 (1C, C-1^{III}), 99.0 (1C, C-1^{I}), 79.7 (1C, C-3^{IV}), 79.6 (1C, C-3^{I}), 79.1 (1C, C-3^{II}), 77.3 (1C, C-3^{III}), 76.4 (1C, C-2^{II}), 75.4–74.9 (C-2^{I}, C-4^{I}, C-4^{II}, PhCH_2), 74.7 (1C, C-4^{III}), 74.5 (1C, PhCH_2), 73.9 (1C, C-4^{IV}), 73.7–73.3 (PhCH_2), 72.5 (1C, C-2^{III}), 72.3–71.8 (C-5^{I}, C-5^{II}, C-5^{III}, C-5^{IV}, PhCH_2), 70.0 (1C, C-6^{II}), 69.5 (1C, C-6^{I}), 69.1 (1C, C-6^{III}), 69.0 (1C, C-2^{IV}), 68.7 (1C, C-6^{IV}), 65.3 (1C, OCH_2CH_2CH_2N), 37.4 (1C, OCH_2CH_2CH_2N), 28.3 (1C, OCH_2CH_2CH_2N). Anal. Calcd for $C_{113}H_{118}ClF_3NO_{23}$: C, 70.87; H, 6.21; N, 0.73. Found: C, 70.88; H, 6.38; N, 0.78.

3.12. 3-Trifluoroacetamidopropyl 4,6-di-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (17)

Molecular sieves 4 Å (100 mg) were added to a soln of **16** (30 mg, 0.016 mmol) and **8** (43 mg, 0.029 mmol) in CH_2Cl_2 (2 mL), and the resulting mixture was stirred for 30 min at room temperature. After cooling to −15 °C, NIS (13 mg, 0.058 mmol) was added, and, after 10 min, the temperature of the reaction mixture was decreased to −35 °C, and then TfOH (1 μL, 0.01 mmol) was added. The stirring was continued for further 5 h at −15 °C to −20 °C. The reaction was quenched with a drop of pyridine, diluted with $CHCl_3$, and filtered through a Celite layer. The filtrate was washed with 1 M aq $Na_2S_2O_3$ and water, concentrated, and toluene was twice evaporated from the residue. Chromatographic purification of the residue (10:1 toluene–EtOAc) produced **17** (36 mg, 67%) as a colorless foam; $[\alpha]_D$ −2.3 (*c* 1, $CHCl_3$). ^1H (500 MHz, $CDCl_3$): δ 8.21–6.92 (m, 110H, 22Ph), 5.63 (br s, 1H, H-2^{III}), 5,55 (br s, 1H, H-2^{V}), 5.52 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4^{VII}), 5.39 (s, 1H, H-1^{IV}), 5.33 (s, 1H, H-1^{VI}), 5.22 (s, 1H, H-1^{II}), 5.17 (s, 1H, H-1^{III}), 5.02 (m, 2H, H-1^{V}, PhCH_2), 4.91 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1^{I}), 4.84–4.76 (m, 3H, PhCH_2), 4.71–4.39 (m, H-3^{V}, PhCH_2), 4.39–4.35 (m, 2H, H-3^{III}, PhCH_2), 4.30–4.24 (m, 2H, H-6^{VII}_a, PhCH_2), 4.20–4.03 (m, H-2^{II}, H-2^{VI}, H-4^{III}, H-4^{V}, H-5^{V}, H-6^{VII}_b, PhCH_2), 4.03–3.83 (m, 12H, H-1^{VII}, H-2^{I}, H-2^{IV}, H-2^{VII}, H-3^{II}, H-3^{IV}, H-4^{IV}, H-4^{VI}, H-5^{II}, H-5^{III}, H-5^{IV}, H-5^{VI}), 3.78–3.69 (m, 4H, H-3^{I}, H-3^{VI}, H-4^{II}, H-6^{I}), 3.68–3.48 (m, 13H, H-4^{I}, H-5^{I}, H-6^{I}_b, H-6^{II}_a, H-6^{II}_b, H-6^{III}_a, H-6^{III}_b, H-6^{IV}_a, H-6^{IV}_b, H-6^{V}_a, H-6^{VI}_a, H-6^{VI}_b, OCH_2CH_2CH_2N), 3.44 (d, 1H, *J* 10.8 Hz, H-6^{V}_b), 3.31 (m, 1H, OCH_2CH_2CH_2N), 3.20–3.14 (m, 2H, OCH_2CH_2CH_2N, OCH_2CH_2CH_2N), 3.01 (dd, $J_{2,3}$ 2.8, $J_{3,4}$ 9.7 Hz, H-3^{VII}), 2.61 (m, 1H, H-5^{VII}), 1.63 (m, 2H, OCH_2CH_2CH_2N); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 165.9, 165.4, 165.4, 165.1 (4C, PhCO), δ 139.0–137.7. 133.1, 132.7, 129.9–127.0, 125.6 (Ph), 101.8 (1C, C-1^{II}), 100.5 (1C, C-1^{IV}), 99.7 (1C, C-1^{V}), 99.2 (1C, C-1^{III}), 98.9 (2C, C-1^{I}, C-1^{VII}), 98.7 (1C, C-1^{VI}), 79.5 (1C, C-3^{I}), 79.2 (1C, C-3^{II}), 77.8 (2C, C-3^{IV}, C-3^{VI}), 77.2 (1C, C-3^{III}), 76.8 (1C, C-3^{VII}), 75.8 (1C, C-2^{II}), 75.5–74.6 (C-2^{I}, C-3^{V}, C-4^{I}, C-4^{II}, C-4^{III}, C-4^{V}, PhCH_2), 74.6-74.0 (C-4^{IV}, PhCH_2), 73.7–72.8 (C-2^{IV}, C-2^{VI}, C-4^{VI}, PhCH_2), 72.5–71.4 (C-2^{III}, C-2^{V}, C-2^{VII}, C-5^{I}, C-5^{II}, C-5^{III}, C-5^{IV}, C-5^{V}, C-5^{VI}, C-5^{VII}, PhCH_2), 70.0 (2C, C-6^{1}, PhCH_2), 69.5 (PhCH_2), 69.3 (1C, C-6^{II}), 68.8 (1C, C-6^{III}), 68.7–68.2 (4C, C-4^{VII}, C-6^{IV}, C-6^{V}, C-6^{VI}), 65.8 (1C, OCH_2CH_2CH_2N), 63.4 (1C, C6^{VII}), 38.0 (1C, OCH_2CH_2CH_2N), 28.0 (1C, OCH_2CH_2CH_2N). Anal. Calcd for $C_{201}H_{202}F_3NO_{41}$: C, 72.18; H, 6.09; N, 0.42. Found: C, 72.23; H, 6.24; N, 0.46.

3.13. 3-Aminopropyl β-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside, acetate (18)

$Pd(OH)_2$/C (20%, 40 mg) was added to a soln of heptamannoside **17** (82 mg, 0.025 mmol) in MeOH (3 mL). The mixture was stirred under H_2 (1 atm) at room temperature for 16 h and then filtered through a Celite layer. The catalyst was carefully washed with MeOH, and the combined filtrates were concentrated. The residue was dissolved in water (2 mL) and treated with anion-exchange resin Amberlyst A-26 (OH^-) (1.5 mL) for 16 h. The resin was filtered off, and the filtrate was concentrated. The residue was subjected to gel chromatography, appropriate fractions were collected, and lyophilized to give **18**·AcOH (18 mg, 60%) as a white amorphous powder; $[\alpha]_D$ +68.4 (*c* 1, water). ^1H NMR (500 MHz, D_2O): δ 5,38

(s, 1H, H-1^{IV}), 5.28 (s, 1H, H-1^{II}), 5.25 (1H, H-1^{VI}), 5.08 (s, 1H, H-1^{I}), 5.03 (s, 1H, H-1^{V}), 5.02 (s, 1H, H-1^{III}), 4.76 (s, 1H, H-1^{VII}), 4.27 (br s. 1H, H-2^{VI}), 4.22 (d, 1H, $J_{2,3}$ 2.0 Hz, H-2^{V}), 4.21 (d, 1H, $J_{2,3}$ 2.2 Hz, H-2^{III}), 4.10 (br s, 2H, H-2^{II}, H-2^{IV}), 4.04 (d, 1H, $J_{2,3}$ 2.9 Hz, H-2^{VII}), 3.99 (dd, 1H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.6 Hz, H-3^{IV}), 3.97–3.84 (m, 13H, H-2^{I}, H-3^{I}, H-3^{II}, H-3^{III}, H-3^{V}, H-3^{VI}, 7 H-6), 3.83 (m, 1H, OCH_2CH$_2$CH$_2$N), 3.81–3.62 (m, 19H, H-3^{VII}, H-4^{I}, H-4^{II}, H-4^{III}, H-4^{IV}, H-4^{V}, H-4^{VI}, H-5^{II}, H-5^{III}, H-5^{IV}, H-5^{V}, H-5^{VI}, 7 H-6), 3.61–3.54 (m, 3H, H-4^{VII}, H-5^{I}, OCH_2CH$_2$CH$_2$N), 3.37 (m, 1H, H-5^{VII}), 3.11 (m. 2H, OCH$_2$CH$_2$CH_2N), 1.97 (m, 2H, OCH$_2$CH_2CH$_2$N), 1.90 (s, 3H, CH_3CO$_2$H). ^{13}C NMR (126 MHz, D$_2$O): δ 103.5 (2C, C-1^{III}, C-1^{V}), 102.0 (1C, C-1^{II}), 101.9 (1C, C-1^{IV}), 101.3 (1C, C-1^{VI}), 99.9 (1C, C-1^{VII}), 99.5 (1C, C-1^{I}), 80.0 (1C, C-2^{I}), 79.7 (2C, C-2^{II}, C-2^{IV}), 79.4 (2C, C-3^{III}, C-3^{V}), 78.2 (1C, C-2^{VI}), 77.5 (1C, C-5^{VII}), 74.5 (5C, C-5^{II}, C-5^{III}, C-5^{IV}, C-5^{V}, C-5^{VI}), 74.0 (1C, C-3^{VII}), 73.9 (1C, C-5^{I}), 71.9 (1C, C-2^{VII}), 71.3 (1C, C-3^{I}), 71.2 (1C, C-3^{IV}), 71.1 (1C, C-3^{II}), 70.9 (1C, C-3^{VI}), 70.7 (2C, C-2^{III}, C-2^{V}), 68.2 (3C, C-4^{II}, C-4^{IV}, C-4^{VI}), 68.1 (1C, C-4^{I}), 67.9 (1C, C-4^{VII}), 67.3 (1C, C-4^{V}), 67.2 (1C, C-4^{III}), 66.2 (1C, OCH_2CH$_2$CH$_2$N), 62.4–61.8 (7C, 7 C-6), 38.8 (1C, OCH$_2$CH$_2$CH_2N), 28.0 (1C, OCH$_2$CH_2CH$_2$N), 24.6 (1C, CH_3CO$_2$H); HRESIMS: found *m/z* 1210.4446 [M+H]$^+$; calcd for $C_{45}H_{80}NO_{36}$ 1210.4460.

3.14. 3-(3,4-Dioxo-2-ethoxycyclobut-1-enylamino)propyl β-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (19)

Diethyl squarate (1.5 μL, 10.1 μmol) and Et$_3$N (0.5 μL) were added to a soln of heptasaccharide **18** (8.0 mg, 6.7 μmol) in 50% aq EtOH (2 mL); the mixture was kept for 24 h at room temperature and then concentrated. The residue was dissolved in water and applied on a Sep-Pak C-18 cartridge. The cartridge was washed with water (10 mL); then the product was eluted with aq MeOH in 2-mL portions, increasing the concentration of MeOH from 5 to 20%. Concentration of the eluate and subsequent lyophilization from water afforded **19** (8 mg, 95%) as a white amorphous powder; $[\alpha]_D$ +56.5 (*c* 0.5, water). ^{1}H NMR (selected assignments for the spacer and squarat moieties, 500 MHz, D$_2$O): δ 4.75 (q, 2H, *J* 7.2 Hz, OCH_2CH$_3$), 3.81 (m, 1H, OCH_2CH$_2$CH$_2$N), 3.72 (m, 1H, OCH$_2$CH$_2$CH_2N), 3.59 (m, 2H, OCH_2CH$_2$CH$_2$N, OCH$_2$CH$_2$CH_2N), 1.92 (m, 2H, OCH$_2$CH_2CH$_2$N), 1.44 (t, 3H, OCH$_2$CH_3); ^{13}C NMR (selected assignments for the spacer and squarat moieties, 126 MHz, D$_2$O): δ 72.1 (1C, OCH_2CH$_3$), 66.4, 66.2 (1C, OCH_2CH$_2$CH$_2$N), 43.1, 43.3 (1C, OCH$_2$CH$_2$CH_2N), 30.7, 30.5 (1C, OCH$_2$CH_2CH$_2$N), 16.4 (1C, OCH$_2$CH_3); the carbohydrate parts of the ^{1}H and ^{13}C NMR spectra of **19** were essentially the same as those in the spectra of the starting 3-aminopropyl glycoside **18.**

3.15. BSA—heptasaccharide conjugate (20)

A sol of **19** (3.7 mg, 7.6 μmol) and BSA (3.2 mg, 0.048 μmol) in 2 mL of the buffer soln (350 mM KHCO$_3$ and 70 mM $Na_2B_4O_7{\cdot}10H_2O$, pH 9) was kept for 7 days at room temperature. The resulting mixture was subjected to gel chromatography on a Sephadex G-15 column (350 × 25 mm) in water to give, after lyophilization, conjugate **20** (3.0 mg, 80%) as a white amorphous powder. MALDI-TOFMS showed a broad peak with a maximum at *m/z* 91640.

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