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Synthesis of 3,6-branched oligomannoside fragments of the mannan from *Candida albicans* cell wall corresponding to the antigenic factor 4

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ABSTRACT

3-Aminopropyl glycosides of 3,6-branched penta- and hexamannoside fragments of the cell wall mannan from *Candida albicans*, corresponding to the antigenic factor 4, have been synthesized. Subsequent coupling of both oligosaccharides with BSA using the squarate procedure provided corresponding neoglycoconjugates.

1. Introduction

The opportunistic pathogenic fungus *Candida albicans* is able to cause severe infection in immunocompromised patients. The main surface antigen of *Candida* responsible for its antigenic specificity is mannan, which represents the carbohydrate part of the cell wall mannoprotein.[1] It has been shown that the determinants of the antigenic factor 4 are oligosaccharide structures **1** and **2** featuring the presence of the 3,6-branching.[2] Oligomannosides corresponding to the antigenic factors 1, 5, 6, 9, and some others have been synthesized[3–13] (for review see Ref. 14). Surprisingly, only one synthesis of an oligosaccharide corresponding to the factor 4, viz. heptasaccharide **2** as the methyl glycoside, has been described.[15] Herewith we report the synthesis of 3-aminopropyl glycosides of branched penta- and hexamannosides related to the structures **1** and **2** and their conjugates with BSA. These products were prepared within a project focused on the synthesis[11,12] and glycoimmunological studies[16] of oligomannoside antigenic determinants of *Candida*.

2. Results and discussion

The protected pentasaccharide **8** was prepared by glycosylation of the known acceptor **7**[11] with branched trisaccharide thioglycoside **6** (Scheme 1). The known 3,6-ditrityl ether **3**[17] was used as a

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man
6
↑
1
α-D-Man*p* **1**

α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man
6
↑
1
α-D-Man*p* **2**

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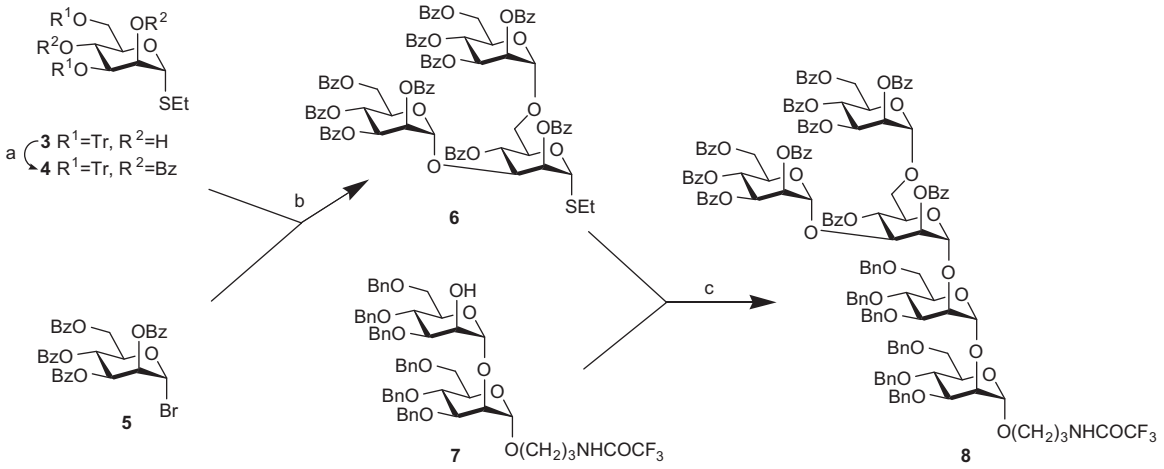
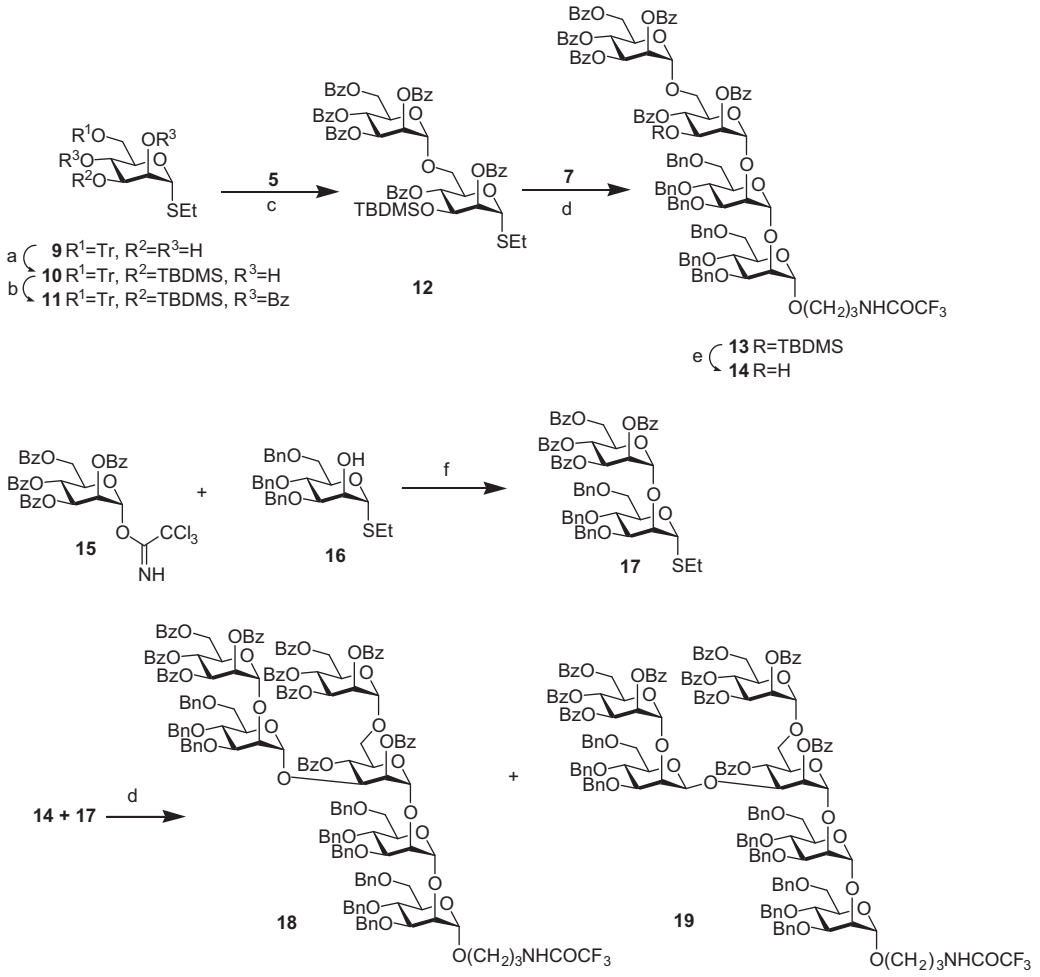
precursor of the 3,6-bis-glycosylated mannose unit. Conventional benzoylation of free hydroxyl groups in **3** followed by Bredereck bis-glycosylation of **4** with mannosyl bromide **5** as described[17] smoothly afforded trisaccharide thioglycoside **6**. Subsequent NIS–TfOH-promoted coupling of **6** and **7** produced target pentamannoside **8**.

Hexasaccharide **18** was assembled from three disaccharide synthetic blocks (Scheme 2). As the target product contains different glycosyl substituents at O-3 and O-6 in the branching point, it was necessary to discriminate between these positions in the corresponding monosaccharide precursor. With this goal in mind, 6-*O*-trityl thioglycoside **9**[18] was subjected to selective silylation[19]



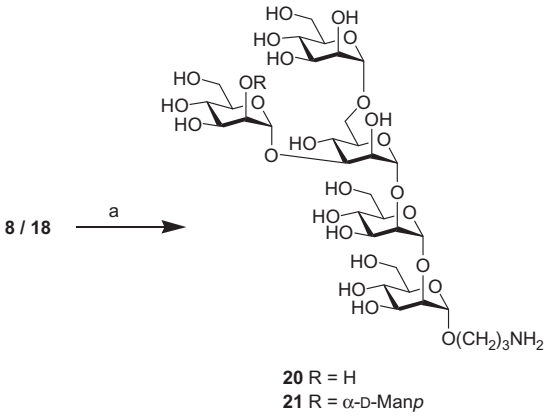
Scheme 1. Synthesis of pentasaccharide **8**. Reagents and conditions: (a) BzCl, pyridine, rt, 65%; (b) AgOTf, CH_2Cl_2, −10 °C, 64%; (c) NIS, TfOH, CH_2Cl_2, −20 to −30 °C, 57%.



Scheme 2. Synthesis of hexasaccharide **19**. Reagents and conditions: (a) TBDMSCl, imidazole, pyridine, rt; (b) BzCl, pyridine, 50 °C, 70% in two steps; (c) AgOTf, CH_2Cl_2, −10 °C, 73%; (d) NIS, TfOH, CH_2Cl_2, −20 to −30 °C, 70% of **13**, 44% of **18**; (e) CF_3CO_2H, $CHCl_3$, rt, 77%; (f) TMSOTf, CH_2Cl_2, −20 °C, 60%.

with *tert*-butyldimethylsilyl chloride to give 3-*O*-silyl derivative **10**. Conventional benzoylation of **10** afforded desirable dibenzoate **11** with different protecting groups at O-3 and O-6. Direct glycosylation of the latter at O-6 with mannosyl bromide **5** under Bredereck conditions produced the necessary (1→6)-linked disaccharide glycosyl donor **12**. NIS–TfOH-promoted coupling of disaccharide blocks **12** and **7** resulted in the formation of tetrasaccharide **13**; subsequent acidic removal of the TBDMS group gave tetrasaccharide acceptor **14**. The third, (1→2)-linked donor block **17** was obtained in acceptable yield (60%) by coupling of 2-OH thioglycoside **16**[20] with imidate **15**.[21] It is noteworthy that AgOTf-promoted glycosylation of **16** with bromide **5** provided disaccharide **17** in much lower yield (~20%) due to the predominant SEt group transfer from acceptor **16** to donor **5**. This side reaction was also observed by TLC in the reaction of compounds **15** and **16** but to a much smaller extent (~20%, TLC). At the final step, NIS–TfOH-promoted glycosylation of **14** with **17** produced a mixture of the target hexasaccharide **18** and its β-anomer **19**, which were isolated in 44% and 14% yield, respectively. The configuration of the (1→3)-glycoside bond in the products **18** and **19** was deduced from corresponding $^1J_{C1,H1}$ coupling constants values (173 Hz for α-anomer **18** and 155 Hz for β-anomer **19**).

Protected oligomannosides **8** and **18** were converted into the corresponding free sugars **20** and **21** in two steps: first benzyl groups were removed by catalytic hydrogenolysis; then O,N-deacylation was achieved by treatment with either the strongly basic anion-exchanger Amberlyst A-26 (OH^-) or with sodium methoxide followed by sodium hydroxide (Scheme 3).



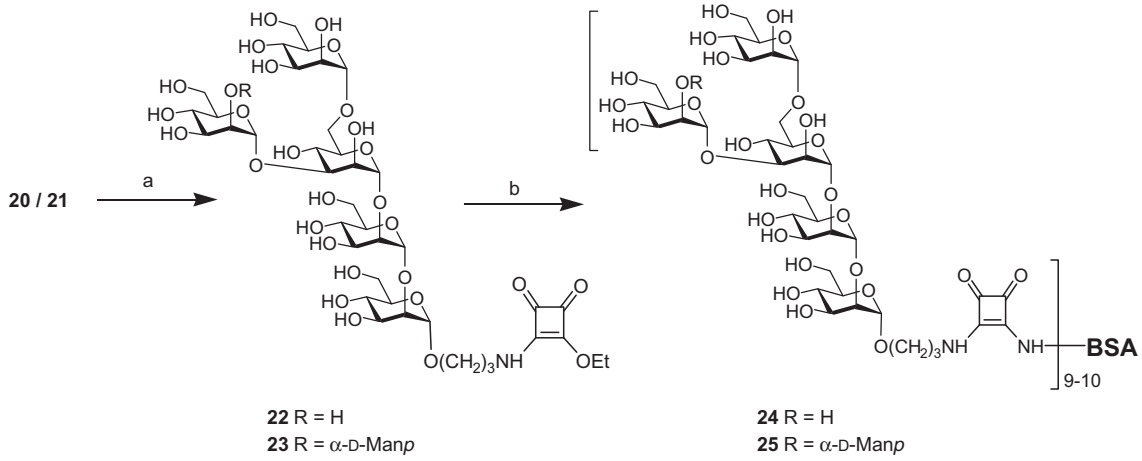
Scheme 3. Synthesis of free oligomannosides **20** and **21**. Reagents and conditions: (a) (1) H_2, Pd/C, MeOH, rt; (2) Amberlyst A-26 (OH^-), water, or MeONa, MeOH then NaOH, aq MeOH, rt, 57% of **20**, 65% of **21**.

Aminopropyl glycosides **20** and **21** were conjugated with BSA using the squarate protocol.[22–25] Reaction of **20** and **21** with diethyl squarate at pH 7 afforded monoamides **22** and **23**, which were then coupled with BSA at pH 9 to produce target conjugates **24** and **25** (Scheme 4). MALDI-TOFMS data showed that **24** and **25** contained on average 10 and 9 oligosaccharide units per protein molecule, respectively. Immunological investigations of obtained oligosaccharides and glycoconjugates thereof are in the progress and will be reported elsewhere.

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 the 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 Finnigan LTQ FT (Thermo Scientific) and MicrOTOF II (Bruker Daltonics) instruments. 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 protected oligosaccharides was performed on a Bio-Beads SX-3 (Bio-Rad Laboratories) column (13 × 450 mm) in toluene. 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.



Scheme 4. Synthesis of oligomannoside–BSA conjugates **24** and **25**. Reagents and conditions: (a) diethyl squarate, water, EtOH, pH 7, rt, 89% of **22**, 80% of **23**; (b) BSA, 350 mM $KHCO_3$, 70 mM $Na_2B_4O_7{\cdot}10H_2O$, pH 9, rt.

3.2. Ethyl 2,4-di-*O*-benzoyl-1-thio-3,6-di-O-trityl-α-D-mannopyranoside (4)

BzCl (0.76 mL, 6.52 mmol) was added to soln of **3** (1.15 g, 1.64 mmol) in pyridine (4 mL). The mixture was kept for 24 h at room temperature, diluted with $CHCl_3$, washed with satd $NaHCO_3$, concentrated, and toluene was evaporated twice from the residue. Column chromatography (8:1 light petroleum–EtOAc) provided **4** (976 mg, 65%) as a colorless foam; $[\alpha]_D$ +11.1 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.29–8.05, 7.37–6.99 (m, 40H, 8Ph-*H*), 6.01 (t, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.37 (s, 1H, H-1), 4.48 (s, 1H, H-2), 4.19 (m, 1H, H-5), 4.08 (dd, $J_{2,3} = 2.6$, $J_{3,4} = 9.7$ Hz, 1H, H-3), 3.14 (m, 2H, H-6_a, H-6_b), 2.52–2.64 (m, 2H, SCH_2CH$_3$), 1.35 (t, 3H, SCH$_2$CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.7, 165.2 (PhCO), 144.0, 143.7, 133.3, 132.8, 130.6–126.0 (Ph), 86.4 (Ph$_3$*C*), 81.6 (C-1), 73.9 (C-2), 71.3 (C-5), 71.2 (C-3), 68.3 (C-4), 62.6 (C-6), 25.4 (SCH_2CH$_3$), 14.8 (SCH$_2$CH_3). Anal. Calcd for $C_{60}H_{52}O_7S$: C, 78.58; H, 5.71. Found: C, 78.51; H, 5.79.

3.3. Ethyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-1-thio-α-D-mannopyranoside (6)

Molecular sieves 4 Å (350 mg) were added to a soln of thioglycoside **4** (93 mg, 101 μmol) and mannosyl bromide **5** (267 mg, 405 μmol) in CH_2Cl_2 (3 mL), the mixture was stirred for 30 min at room temperature, cooled to −20 °C, and then AgOTf (156 mg, 608 μmol) was added. The resulting mixture was stirred at −10 °C until disappearance of **4** (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. The residue was subjected to column chromatography (20:1 toluene–EtOAc) to give **6** (102 mg, 64%) as a colorless foam; $[\alpha]_D$ −36.0 (*c* 0.5, $CHCl_3$). ^1H: NMR (500 MHz, $CDCl_3$): δ 8.30–7.11 (m, 50H, 10Ph-*H*), 6.10 (t, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4^{III}), 5.98 (t, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4^{II}), 5.92 (dd, 1H, $J_{2,3} = 3.2$, $J_{3,4} = 10.1$ Hz, H-3^{III}), 5.89 (t, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4^{I}), 5.79 (dd, 1H, $J_{1,2} = 1.0$, $J_{2,3} = 3.3$ Hz, H-2^{I}), 5.72 (dd, 1H, $J_{1,2} = 1.8$, $J_{2,3} = 2.9$ Hz, H-2^{III}), 5.67 (dd, 1H, $J_{2,3} = 3.0$, $J_{3,4} = 10.1$ Hz, H-3^{II}), 5.61 (s, 1H, H-1^{I}), 5.34 (s, 1H, H-1^{II}), 5.33 (d, 1H, $J_{2,3} = 3.0$ Hz, H-2^{II}), 5.10 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1^{III}), 4.70 (m, 1H, H-5^{I}), 4.60 (dd, 1H, $J_{2,3} = 3.4$, $J_{3,4} = 9.9$ Hz, H-3^{I}), 4.58–4.52 (m, 2H, H-$6_a{}^{II}$, H-$6_a{}^{III}$), 4.49–4.44 (m, 2H, H-5^{II}, H-5^{III}), 4.37–4.30 (m, 2H, H-$6_b{}^{II}$, H-$6_b{}^{III}$), 4.17 (dd, 1H, $J_{5,6} = 6.8$, $J_{6a,6b} = 10.8$ Hz, H-$6_a{}^{I}$), 3.75 (d, 1H, $J_{6a,6b} = 10.8$ Hz, H-$6_b{}^{I}$), 2.83 (m, 2H, SCH_2CH$_3$), 1.43 (t, 3H, SCH$_2$CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1–164.6 (PhCO), 133.6–132.9, 130.1–128.1 (Ph), 99.5 (1C, C-1^{II}), 97.4 (1C, C-1^{III}) 81.8 (1C, C-1^{I}), 76.7 (1C, C-3^{I}), 73.4 (1C, C-2^{I}), 70.2 (1C, C-2^{III}), 70.1 (1C, C-3^{III}), 70.0 (2C, C-5^{I}, C-2^{II}), 69.6 (1C, C-5^{II}), 69.2 (1C, C-3^{II}), 68.8 (1C, C-4^{I}), 68.8 (1C, C-5^{III}), 66.9 (1C, C-6^{I}), 66.5 (1C, C-4^{III}), 66.4 (1C, C-4^{II}), 62.6 (2C, C-6^{II}, C-6^{III}), 25.5 (1C, SCH_2CH$_3$), 14.9 (SCH$_2$CH_3). Anal. Calcd for $C_{90}H_{76}O_{25}S$: C, 68.00; H, 4.82. Found: C, 68.39; H, 5.22

3.4. 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-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 (8)

Molecular sieves 4 Å (150 mg) were added to a soln of acceptor **7** (52 mg, 50 μmol) and donor **6** (87 mg, 55 μmol) in CH_2Cl_2 (3 mL); the mixture was stirred for 30 min at room temperature and cooled to −10 °C. NIS (23 mg, 100 μmol) was added, the mixture was stirred for 10 min, then the temperature was decreased to −30 °C and TfOH (2 μL, 20 μmol) was added. The reaction mixture was stirred at −20 °C to −30 °C until TLC showed disappearance of **7**. The reaction was quenched with pyridine, diluted with $CHCl_3$, and filtered through a Celite layer. The filtrate was washed with 1 M aq $Na_2S_2O_3$ soln and water, concentrated, and toluene was twice evaporated from the residue. Column chromatography of the residue (15:1 toluene–EtOAc) afforded pentasaccharide **8** (73 mg, 57%) as a colorless foam; $[\alpha]_D$ −17.4 (*c* 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.41, 8.17–6.78 (m, 80H, 16Ph-*H*), 6.80 (m, 1H, N*H*), 6.13 (t, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4^{IV}), 6.12 (t, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4^{V}), 6.02 (t, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4^{III}), 5.99 (dd, 1H, $J_{2,3} = 3.0$, $J_{3,4} = 10.2$ Hz, H-3^{V}), 5.92 (br s, 1H, H-2^{V}), 5.82 (br s, 1H, H-2^{III}), 5.76 (dd, 1H, $J_{2,3} = 2.9$, $J_{3,4} = 10.1$ Hz, H-3^{IV}), 5.42 (s, 1H, H-1^{II}), 5.41 (br s, 1H, H-2^{IV}), 5.37 (s, 1H, H-1^{IV}), 5.12 (s, 1H, H-1^{III}), 5.10 (s, 1H, H-1^{V}), 4.41 (d, 1H, $J = 10.9$ Hz, PhCH_2), 4.88 (s, 1H, H-1^{I}), 4.85 (d, 1H, $J = 11.0$ Hz, PhCH_2), 4.83 (d, 1H, $J = 10.7$ Hz, PhCH_2), 4.71 (dd, 1H, $J_{2,3} = 3.2$, $J_{3,4} = 9.9$ Hz, H-3^{III}), 4.68 (d, 1H, $J = 11.1$ Hz, PhCH_2), 4.65–4.56 (m, 5H, 5PhCH_2), 4.56–4.45 (m, 5H, H-5^{III}, H-5^{IV}, H-$6_a{}^{IV}$, 2 PhCH_2), 4.43 (m, 1H, H-$6_a{}^{V}$), 4.39 (d, 1H, $J = 12.2$ Hz, PhCH_2), 4.36 (m, 1H, H-5^{V}), 4.26 (dd, 1H, $J_{5,6} = 3.7$, $J_{6a,6b} = 12.2$ Hz, H-$6_b{}^{V}$), 4.22 (s, 1H, H-2^{I}), 4.12–4.08 (m, 2H, H-2^{II}, H-$6_b{}^{IV}$), 4.01–3.94 (m, 3H, H-3^{II}, H-4^{II}, H-$6_a{}^{III}$), 3.92–3.86 (m, 2H, H-3^{I}, H-5^{II}), 3.74–3.70 (m, 3H, H-5^{I}, H-$6_a{}^{I}$, H-$6_b{}^{III}$), 3.70–3.60 (m, 3H, H-4^{I}, H-$6_b{}^{I}$, H-$6_a{}^{II}$), 3.59–3.52 (m, 2H, H-$6_b{}^{II}$, OCH_2CH$_2$CH$_2$N), 3.27–3.21 (m, 2H, OCH_2CH$_2$CH$_2$N, OCH$_2$CH$_2$CH_2N), 3.18 (m, 1H, OCH$_2$CH$_2$CH_2N), 1.58 (m, 1H, OCH$_2$CH_2CH$_2$N), 1.49 (m, 1H, OCH$_2$CH_2CH$_2$N); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1–164.7 (PhCO), 138.4–138.0, 133.6–132.8, 130.3–127.4 (Ph), 100.4 (1C, C-1^{II}), 99.3 (2C, C-1^{I}, C-1^{IV}), 99.2 (1C, C-1^{III}), 97.2 (1C, C-1^{V}), 80.0 (1C, C-3^{I}), 78.5 (1C, C-3^{II}), 77.5 (1C, C-2^{II}), 76.1 (1C, C-3^{III}), 75.4 (1C, C-2^{I}), 75.2 (2C, PhCH_2), 75.0 (1C, C-4^{I}), 74.4 (1C, C-4^{II}), 73.3, 72.2, 72.7 (3C, PhCH_2), 72.0 (2C, C-5^{I}, PhCH_2), 71.9 (1C, C-5^{II}), 71.7 (1C, C-2^{III}), 70.7 (1C, C-3^{V}), 70.2 (1C, C-2^{IV}), 70.0 (1C, C-2^{V}), 69.6 (1C, C-5^{IV}), 69.5 (1C, C-3^{IV}), 69.4 (1C, C-6^{I}), 69.3 (1C, C-5^{III}), 68.8 (2C, C-5^{V}, C-6^{II}), 68.0 (1C, C-4^{III}), 66.3 (1C, C-4^{V}), 66.1 (1C, C-4^{IV}), 65.9 (1C, C-6^{III}), 65.6 (1C, OCH$_2$CH$_2$CH$_2$N), 62.5 (1C, C-6^{V}), 61.8 (1C, C-6^{IV}), 37.6 (1C, OCH$_2$CH$_2$CH$_2$N), 27.9 (1C, OCH$_2$CH$_2$CH$_2$N). Anal. Calcd for $C_{147}H_{134}F_3NO_{37}$: C, 68.87; H, 5.27; N, 0.55. Found: C, 68.57; H, 5.58; N, 0.53.

3.5. 3-Aminopropyl α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (20)

$Pd(OH)_2$/C (60 mg) was added to a soln of pentamannoside **8** (61 mg, 24 μmol) in MeOH (2 mL) and EtOAc (1 mL). The mixture was stirred under hydrogen (1 atm) at room temperature for 20 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 12 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 **20**·AcOH (12 mg, 57%) as a white amorphous powder; $[\alpha]_D$ +83.7 (*c* 0.3, H_2O). ^1H NMR (500 MHz, D_2O): δ 5.21 (s, 1H, H-1^{II}), 5.12 (s, 1H, H-1^{IV}), 5.06 (s, 1H, H-1^{I}), 5.01 (s, 1H, H-1^{III}), 4.90 (s, 1H, H-1^{V}), 4.20 (br s, 1H, H-2^{III}), 4.10 (br s, 1H, H-2^{II}), 4.05 (br s, 1H, H-2^{IV}), 4.00–3.95 (m, 2H, H-2^{V}, H-$6_a{}^{III}$), 3.95–3.85 (m, 11H, H-2^{I}, H-3^{I}, H-3^{II}, H-3^{III}, H-3^{IV}, H-4^{III}, H-5^{III}, H-$6_a{}^{I}$, H-$6_a{}^{II}$, H-$6_a{}^{IV}$, H-$6_a{}^{V}$), 3.85–3.80 (m, 2H, H-3^{V}, OCH_2CH$_2$CH$_2$N), 3.80–3.68 (m, 8H, H-4^{I}, H-5^{II}, H-5^{IV}, H-$6_b{}^{I}$, H-$6_b{}^{II}$, H-$6_b{}^{III}$, H-$6_b{}^{IV}$, H-$6_b{}^{V}$), 3.68–3.61 (m, 4H, H-4^{II}, H-4^{IV}, H-4^{V}, H-5^{V}), 3.61–3.56 (m, 2H, H-5^{I}, OCH_2CH$_2$CH$_2$N), 3.11 (m, 2H, OCH$_2$CH$_2$-CH_2N), 1.98 (m, 2H, OCH$_2$CH_2CH$_2$N), 1.90 (s, 3H, CH_3CO$_2$H); ^{13}C NMR (126 MHz, D_2O): δ 103.7 (1C, C-1^{IV}), 103.6 (1C, C-1^{III}), 102.2 (1C, C-1^{II}), 100.7 (1C, C-1^{V}), 99.7 (1C, C-1^{I}), 80.4 (2C, C-2^{I}, C-2^{II}), 79.7 (1C, C-3^{III}), 75.0 (1C, C-5^{II}), 74.8 (1C, C-5^{IV}), 74.3 (1C, C-5^{I}), 74.2 (1C, C-5^{V}), 72.8 (1C, C-5^{III}), 72.0 (1C, C-3^{V}), 71.8 (1C, C-3^{IV}), 71.7 (1C, C-3^{I}), 71.5 (2C, C-2^{IV}, C-2^{V}), 71.4 (1C, C-3^{II}), 71.0 (1C,

C-2^{III}), 68.5, 68.4, 68.3, 68.2 (4C, C-4^{I}, C-4^{II}, C-4^{IV}, C-4^{V}), 67.1 (1C, C-4^{III}), 66.6 (1C, C-6^{III}), 66.4 (1C, $OCH_2CH_2CH_2N$), 62.7, 62.5, 62.4 (4C, C-6^{I}, C-6^{II}, C-6^{IV}, C-6^{V}), 38.9 (1C, $OCH_2CH_2CH_2N$), 28.1 (1C, $OCH_2CH_2CH_2N$). HRESIMS: found *m*/*z* 886.3416 $[M+H]^+$; calcd for $C_{33}H_{60}NO_{26}$ 886.3404.

3.6. Ethyl 2,4-di-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thio-6-*O*-trityl-α-D-mannopyranoside (11)

Trityl chloride (171 mg, 614 μmol) and a catalytic amount of DMAP were added to a soln of ethyl 1-thio-α-D-mannopyranoside (110 mg, 491 μmol) in pyridine (5 mL). The mixture was stirred at 80 °C overnight and cooled to room temperature. Then TBDMSCl (82 mg, 543 μmol) and imidazole (65 mg, 980 μmol) were added, the mixture was kept at room temperature for 16 h and concentrated. Residual pyridine was removed by coevaporation with toluene, the residue was filtered through a silica gel layer in toluene–EtOAc (20:1), and the filtrate was concentrated to give crude **10**. To a solution of **10** in pyridine (4 mL) was added BzCl (230 μL, 1.97 mmol), the resulting mixture was stirred at 50 °C for 16 h, diluted in $CHCl_3$, washed with satd $NaHCO_3$, concentrated and toluene was twice evaporated from the residue. Column chromatography of the residue (15:1 petroleum ether–EtOAc) provided **11** (270 mg, 70%) as white foam; $[\alpha]_D$ +10.3 (*c* 1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.23–7.06 (m, 25H, 5Ph-*H*), 5.72 (t, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.53 (s, 1H, H-1), 5.47 (d, 1H, $J_{2,3} = 3.3$ Hz, H-2), 4.44 (m, 1H, H-5), 4.30 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 9.8$ Hz, H-3), 3.29 (m, 2H, H-6_a, H-6_b), 2.81 (m, 2H, SCH_2CH$_3$), 1.43 (t, 3H, SCH$_2$CH_3), 0.62 (s, 9H, *tert*-Bu–Si), 0.02 (s, 3H, CH_3–Si), −0.17 (s, 3H, CH_3–Si); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 166.0, 164.3 (Ph*C*O), 143.7, 134.5–132.8, 130.5–126.7 (Ph), 82.2 (1C, C-1), 74.6 (1C, C-2), 71.1 (1C, C-5), 70.1 (1C, C-4), 69.8 (1C, C-3), 62.7 (1C, C-6), 25.4 (SCH_2CH$_3$), 25.2 (3C, (CH_3)$_3$C), 14.8 (SCH$_2$CH_3), −4.9, −5.2 (2C, Si-CH_3). Anal. Calcd for $C_{47}H_{52}O_7SSi$: C, 71.54; H, 6.64. Found: C, 71.60; H, 6.69

3.7. Ethyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thio-α-D-mannopyranoside (12)

Molecular sieves 4 Å (500 mg) were added to a soln of **11** (194 mg, 246 μmol) and mannosyl bromide **5** (327 mg, 511 μmol) in CH_2Cl_2 (5 mL), the mixture was stirred for 30 min at room temperature, cooled to −20 °C, and then AgOTf (156 mg, 608 μmol) was added. The resulting mixture was stirred at −10 °C until disappearance of **11** (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. The residue was subjected to silica gel column chromatography (20:1 toluene–EtOAc) and then to gel chromatography to give **12** (202 mg, 73%) as a colorless foam; $[\alpha]_D$ −0.6 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.17–7.23 (m, 30H, 6Ph-*H*), 6.08 (t, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4^{II}), 5.89 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 10.1$ Hz, H-3^{II}), 5.71 (t, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4^{I}), 5.67 (br. s, 1H, H-2^{II}), 5.50 (br. s, 2H, H-2^{I}, H-1^{I}), 5.06 (s, 1H, H-1^{II}), 4.63 (m, 1H, H-5^{I}), 4.53 (dd, 1H, $J_{5,6} = 2.1$, $J_{6a,6b} = 12.2$ Hz, H-$6_a{}^{II}$), 4.42–4.37 (m, 2H, H-3^{I}, H-5^{II}), 4.30 (dd, 1H, $J_{5,6} = 6.8$, $J_{6a,6b} = 12.2$ Hz, H-$6_b{}^{II}$), 4.09 (dd, 1H, $J_{5,6} = 6.8$, $J_{6a,6b} = 10.7$ Hz, H-$6_a{}^{I}$), 3.67 (dd, 1H, $J_{5,6} = 1.6$, $J_{6a,6b} = 10.7$ Hz, H-$6_b{}^{I}$), 2.82 (m, 2H, SCH_2CH$_3$), 1.44 (t, 3H, SCH$_2$CH_3), 0.62 (s, 9H, *tert*-Bu–Si), 0.04 (s, 3H, CH_3–Si), −0.11 (s, 3H, CH_3–Si). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.0–165.1 (6C, Ph*C*O), 133.4–133.0, 130.4–128.3 (*Ph*CO), 97.3 (1C, C-1^{II}), 82.1 (1C, C-1^{I}), 74.2 (1C, C-2^{I}), 70.2 (1C, C-2^{II}), 70.0 (1C, C-4^{I}), 69.9 (2C, C-5^{I}, C-3^{II}), 69.7 (1C, C-3^{I}), 68.7 (1C, C-5^{II}), 67.0 (1C, C-6^{I}), 66.5 (1C, C-4^{II}), 62.5 (1C, C-6^{II}), 25.5 (SCH$_2$CH$_3$), 25.3 (3C, (CH_3)$_3$C), 14.9 (SCH$_2$CH$_3$) −4.9, −5.1 (2C, Si-CH_3). Anal. Calcd for $C_{62}H_{64}O_{16}SSi$: C, 66.17; H, 5.73. Found: C, 66.09; H, 5.95.

3.8. 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-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 (13)

Molecular sieves 4 Å (150 mg) were added to a soln of acceptor **7** (72 mg, 70 μmol) and donor **12** (87 mg, 77 μmol) in CH_2Cl_2 (3 mL); the mixture was stirred for 30 min at room temperature and cooled to −10 °C. NIS (35 mg, 150 μmol) was added, the mixture was stirred for 10 min, then the temperature was decreased to −25 °C and TfOH (3 μL, 30 μmol) was added. The reaction mixture was stirred at −20 °C to −30 °C until TLC showed disappearance of starting **7**. 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 $Na_2S_2O_3$ soln and water, concentrated, and toluene was twice evaporated from the residue. Column chromatography of the residue (10:1 toluene–EtOAc) afforded **13** (103 mg, 70%) as a colorless foam; $[\alpha]_D$ +2.3 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.23, 7.99, 7.93, 7.81, 7.59–7.16, 6.88 (60H, 12Ph-*H*), 6.74 (m, 1H, N*H*), 6.06 (t, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4^{IV}), 5.93 (dd, 1H, $J_{2,3} = 3.1$, $J_{3,4} = 10.1$ Hz, H-3^{IV}), 5.86 (br s, 1H, H-2^{IV}), 5.81 (t, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4^{III}), 5.56 (br s, 1H, H-2^{III}), 5.36 (s, 1H, H-1^{II}), 5.09 (s, 1H, H-1^{IV}), 5.02 (s, 1H, H-1^{III}), 4.86 (s, 1H, H-1^{I}), 4.84 (d, 1H, *J* = 10.7 Hz, PhCH_2), 4.81 (d, 1H, *J* = 10.7 Hz, PhCH_2), 4.79 (d, 1H, *J* = 11.2 Hz, PhCH_2), 4.67–4.56 (m, 6H, PhCH_2), 4.54 (m, 1H, H-3^{III}), 4.50–4.45 (m, 2H, H-5^{III}, PhCH_2), 4.40 (m, 2H, PhCH_2), 4.31 (dd, 1H, $J_{5,6} = 2.1$, $J_{6,6} = 12.2$ Hz, H-$6_a{}^{IV}$), 4.26 (m, 1H, H-5^{IV}), 4.20–4.16 (m, 2H, H-2^{I}, H-$6_b{}^{IV}$), 4.08 (br s, 1H, H-2^{II}), 3.97–3.83 (m, 5H, H-3^{I}, H-3^{II}, H-4^{II}, H-5^{II}, H-$6_a{}^{III}$), 3.72–3.66 (m, 2H, H-5^{I}, H-$6_a{}^{I}$), 3.66–3.59 (m, 4H, H-4^{I}, H-$6_a{}^{II}$, H-$6_a{}^{III}$, H-$6_b{}^{I}$), 3.56 (m, 1H, OCH_2CH$_2$CH$_2$N), 3.47 (m, 1H, H-$6_b{}^{II}$), 3.27–3.21 (m, 2H, OCH_2CH$_2$CH$_2$N, OCH$_2$CH$_2$CH_2N), 3.17 (m, 1H, OCH$_2$CH$_2$CH_2N), 1.59 (m, 1H, OCH$_2$CH_2CH$_2$N), 1.49 (m, 1H, OCH$_2$CH_2CH$_2$N), 0.67 (s, 9H, *tert*-Bu–Si), 0.07 (s, 3H, CH_3–Si), −0.10 (s, 3H, CH_3–Si); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.0–164.9 (6C, Ph*C*O), 138.4–138.2, 133.4–132.9, 130.0–127.5 (Ph), 100.7 (1C, C-1^{II}), 99.3 (2C, C-1^{I}, C-1^{III}), 97.2 (1C, C-1^{IV}), 79.8 (1C, C-3^{I}), 78.8 (1C, C-3^{II}), 76.7 (1C, C-2^{II}), 75.9 (1C, C-2^{I}), 75.2 (2C, PhCH_2), 75.0 (1C, C-4^{I}), 74.4 (1C, C-4^{II}), 73.3 (2C, PhCH_2), 72.7 (1C, C-2^{III}), 72.6 (1C, PhCH_2), 72.0 (1C, C-5^{I}), 71.9 (1C, PhCH_2), 71.7 (1C, C-5^{II}), 70.6 (1C, C-3^{IV}), 70.1 (1C, C-2^{IV}), 69.4 (2C, C-4^{III}, C-6^{I}), 69.2 (1C, C-5^{III}), 69.0 (1C, C-3^{III}), 68.7 (1C, C-5^{IV}), 68.5 (1C, C-6^{II}), 66.3 (1C, C-4^{IV}), 66.1 (1C, C-6^{III}), 65.6 (1C, OCH_2CH$_2$CH$_2$N), 62.5 (1C, C-6^{IV}), 37.6 (1C, OCH$_2$CH$_2$CH_2N), 27.9 (1C, OCH$_2$CH_2CH$_2$N) 25.4 (3C, (CH_3)$_3$C), −4.8, −5.1 (2C, Si-CH_3). Anal. Calcd for $C_{119}H_{122}F_3NO_{28}Si$: C, 68.08; H, 5.86; N, 0.67. Found: C, 68.10; H, 6.15; N, 0.65.

3.9. 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-benzoyl-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 (14)

Tetramannoside **13** (85 mg, 41 μmol) was dissolved in $CHCl_3$ (2 mL) and 90% aq CF_3COOH (0.5 mL) was added. The mixture was kept until TLC showed disappearance of starting **13**, diluted with $CHCl_3$, washed with water, satd $NaHCO_3$, and concentrated. Column chromatography of the residue (7:1 toluene–EtOAc) provided **14** (65 mg, 77%) as a foam; $[\alpha]_D$ +12.0 (*c* 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 8.27, 8.03–7.93, 7.83, 7.61–7.14, 6.91 (60H, 12Ph-*H*), 6.71 (m, 1H, N*H*), 6.08 (t, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4^{IV}), 5.98 (dd, 1H, $J_{2,3} = 3.1$, $J_{3,4} = 10.1$ Hz, H-3^{IV}), 5.85 (br s, 1H, H-2^{IV}), 5.79 (t, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4^{III}), 5.64 (br s, 1H, H-2^{III}), 5.35 (s, 1H, H-1^{II}), 5.11 (s, 1H, H-1^{III}), 5.04 (s, 1H, H-1^{IV}), 4.92–4.88 (m, 2H, H-1^{I}, PhCH_2), 4.81 (d, 1H, *J* = 10.8 Hz, PhCH_2), 4.77 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.68–4.55 (m, 6H, PhCH_2), 4.54–4.45 (m, 5H,

H-3^{III}, H-5^{III}, 3PhCH_2), 4.29 (dd, 1H, $J_{5,6}$ = 1.7, $J_{6a,6b}$ = 12.2 Hz, H-6$_a^{IV}$), 4.15–4.10 (m, 2H, H-2^{I}, H-5^{IV}), 4.09–4.03 (m, 2H, H-2^{II}, H-6$_b^{IV}$), 4.01–3.89 (m, 4H, H-3^{II}, H-4^{II}, H-5^{II}, H-6$_a^{III}$), 3.87 (dd, 1H, $J_{2,3}$ = 2.7, $J_{3,4}$ = 8.8 Hz, H-3^{I}), 3.73–3.58 (m, 8H, H-4^{I}, H-5^{I}, H-6$_a^{I}$, H-6$_a^{II}$, H-6$_a^{III}$, H-6$_b^{I}$, H-6$_b^{II}$, OCH_2CH$_2$CH$_2$N), 3.34–3.27 (m, 2H, OCH_2CH$_2$CH$_2$N, OCH$_2$CH$_2$CH_2N), 3.15 (m, 1H, OCH$_2$CH$_2$CH_2N), 1.70–1.54 (m, 2H, OCH$_2$CH_2CH$_2$N); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.9, 165.9, 165.3, 165.1 (6C, PhCO), 138.4–138.1, 133.6–132.9, 130.1–127.5 (Ph), 100.5 (1C, C-1^{II}), 99.3 (2C, C-1^{I}, C-1^{III}), 97.3 (1C, C-1^{IV}), 79.9 (1C, C-3^{I}), 78.8 (1C, C-3^{II}), 77.2 (1C, C-2^{II}), 75.5 (1C, C-2^{I}), 75.1 (2C, PhCH_2), 75.0 (1C, C-4^{I}), 74.4 (1C, C-4^{II}), 73.4 (1C, PhCH_2), 73.3 (1C, PhCH_2), 72.9 (1C, C-2^{III}), 72.5 (1C, PhCH_2), 72.3 (1C, PhCH_2), 72.1 (2C, C-5^{I}, C-5^{II}), 70.3 (1C, C-3^{IV}), 70.1 (1C, C-2^{IV}), 70.0 (1C, C-4^{III}), 69.5 (1C, C-6^{I}), 69.2 (1C, C-3^{III}), 69.0 (1C, C-5^{III}), 68.9 (2C, C-5^{IV}, C-6^{II}), 66.4 (1C, C-4^{IV}), 65.8 (1C, C-6^{III}), 65.7 (1C, OCH_2CH$_2$CH$_2$N), 62.2 (1C, C-6^{IV}), 37.7 (1C, OCH$_2$CH$_2$CH_2N), 28.1 (1C, OCH$_2$CH$_2$CH$_2$N). Anal. Calcd for $C_{113}H_{108}F_3NO_{28}$: C, 68.37; H, 5.48; N, 0.71. Found: C, 68.08; H, 5.43; N, 0.69.

3.10. Ethyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (17)

Molecular sieves 4 Å (140 mg) were added to a soln of thioglycoside **16** (43 mg, 87 μmol) and imidate **15** (78 mg, 105 μmol) in CH_2Cl_2 (2 mL). The mixture was stirred for 30 min at room temperature, cooled to −50 °C, and then TMSOTf (6.8 μL, 35 μmol) was added. The resulting mixture was stirred at −20 °C until disappearance of starting **16** (TLC monitoring), quenched with a drop of triethylamine, diluted with $CHCl_3$, and filtered through a Celite layer. The filtrate was washed with water and concentrated. The residue was subjected first to column chromatography (20:1 toluene–EtOAc) and then to gel chromatography to give **17** (56 mg, 60%) as a colorless foam. $[\alpha]_D$ +2.0 (*c* 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17–7.83, 7.37–6.99 (m, 35H, Ph-*H*), 6.11 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4^{II}), 6.01 (dd, 1H, $J_{2,3}$ = 2.7, $J_{3,4}$ = 9.9 Hz, H-3^{II}), 5.94 (br s, 1H, H-2^{II}), 5.51 (s, 1H, H-1^{I}), 5.28 (s, 1H, H-1^{II}), 4.92 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.79–4.63 (m, 5H, H-5^{II}, H-6$_a^{II}$, 3PhCH_2), 4.63–4.56 (m, 2H, PhCH_2), 4.48 (dd, 1H, $J_{5,6}$ = 4.8, $J_{6a,6b}$ = 12.6 Hz, H-6$_b^{II}$), 4.22 (m. 1H, H-5^{I}), 4.16 (br s, 1H, H-2^{I}), 4.09 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4^{I}), 3.92 (dd, 1H, $J_{2,3}$ = 2.0, $J_{3,4}$ = 9.3 Hz, H-3^{I}), 3.86 (dd, 1H, $J_{5,6}$ = 4.8, $J_{6a,6b}$ = 10.7 Hz, H-6$_a^{I}$), 3.77 (m, 1H, H-6$_b^{I}$), 2.65–2.52 (m, 2H, SCH_2CH$_3$), 1.35 (t, 3H, SCH$_2$CH_3); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 166.2, 165.5, 165.3, 165.1 (PhCO), 138.4, 138.3, 133.3, 133.0, 129.9–127.5 (Ph), 99.4 (1C, C-1^{II}), 83.7 (1C, C-1^{I}), 80.1 (1C, C-3^{I}), 78.2 (1C, C-2^{I}), 75.2 (1C, PhCH_2), 75.0 (1C, C-4^{I}), 73.2 (1C, PhCH_2), 72.6 (1C, PhCH_2), 72.1 (1C, C-5^{I}), 70.4 (1C, C-2^{II}), 70.1 (1C, C-3^{II}), 69.4 (1C, C-5^{II}), 69.1 (1C, C-6^{I}), 67.0 (1C, C-4^{II}), 63.1 (1C, C-6^{II}), 25.5 (SCH_2CH$_3$), 14.9 (SCH$_2$CH_3). Anal. Calcd for $C_{63}H_{60}O_{14}S$: C, 70.51; H, 5.64. Found: C, 70.46; H, 5.54.

3.11. 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-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 (18) and 3-trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-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 (19)

Molecular sieves 4 Å (100 mg) were added to a soln of acceptor **14** (58 mg, 29 μmol) and donor **17** (47 mg, 44 μmol) in CH_2Cl_2 (3 mL); the mixture was stirred for 30 min at room temperature and cooled to −10 °C. NIS (15 mg, 66 μmol) was added, the mixture was stirred for 10 min, then the temperature was decreased to −25 °C and TfOH (1 μL, 10 μmol) was added. The reaction mixture was stirred at −20 °C to −25 °C until TLC showed disappearance of starting **14**. 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 $Na_2S_2O_3$ soln and water, concentrated, and toluene was twice evaporated from the residue. Column chromatography of the residue (10:1 toluene–EtOAc) afforded **18** (38 mg, 44%) and **19** (12 mg, 14%) as a colorless foam.

Compound **18**: $[\alpha]_D$ −4.5 (*c* 0.8, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29, 8.09, 7.98–7.90, 7.84–7.74, 7.61–6.93, 6.83 (95H, 19Ph-*H*), 6.73 (m, 1H, N*H*), 6.06 (t, 1H, $J_{3,4} = J_{4,5}$ = 10.0 Hz, H-4^{VI}), 6.03–5.95 (m, 3H, H-3^{VI}, H-4^{III}, H-4^{V}), 5.87–5.82 (m, 2H, H-2^{VI}, H-3^{V}), 5.75 (br s, 2H, H-2^{III}, H-2^{V}), 5.38 (s, 1H, H-1^{II}), 5.35 (s, 1H, H-1^{IV}), 5.13 (s, 1H, H-1^{III}), 4.98 (s, 1H, H-1^{VI}), 4.91–4.87 (m, 2H, H-1^{I}, PhCH_2), 4.82 (d, 1H, *J* = 11.4 Hz, PhCH_2), 4.79–4.73 (m, 3H, H-1^{V}, 2 PhCH_2), 4.71 (dd, 1H, $J_{2,3}$ = 2.9, $J_{3,4}$ = 9.6 Hz, H-3^{III}), 4.66–4.54 (m, 7H, PhCH_2), 4.52–4.41 (m, 6H, H-5^{III}, 5PhCH_2), 4.34–4.28 (m, 3H, H-6$_a^{VI}$, 2PhCH_2), 4.28–4.19 (m, 2H, H-5^{V}, H-5^{VI}), 4.19–4.13 (m, 4H, H-2^{I}, H-4^{IV}, H-6$_a^{V}$, H-6$_b^{VI}$), 4.12 (br s, 1H, H-2^{II}), 4.01–3.88 (m, 5H, H-3^{II}, H-4^{II}, H-5^{II}, H-5^{IV}, H-6$_a^{V}$), 3.88–3.79 (m, 4H, H-2^{IV}, H-3^{I}, H-3^{IV}, H-6$_a^{III}$), 3.72–3.52 (m, 10H, H-4^{I}, H-5^{I}, H-6$_a^{I}$, H-6$_b^{I}$, H-6$_a^{II}$, H-6$_b^{II}$, H-6$_a^{IV}$, H-6$_b^{IV}$, H-6$_b^{III}$, OCH_2CH$_2$CH$_2$N), 3.29–3.18 (m, 2H, OCH_2CH$_2$CH$_2$N, OCH$_2$CH$_2$CH_2N), 3.09 (m, 1H, OCH$_2$CH$_2$CH_2N), 1.58 (m, 1H, OCH$_2$CH_2CH$_2$N), 1.51 (m, 1H, OCH$_2$-CH_2CH$_2$N); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.0–164.7 (PhCO), 139.0–138.0, 133.6–132.9, 130.2–127.0 (Ph), 100.5 (1C, C-1^{II}), 100.4 (1C, C-1^{IV}), 99.4 (1C, C-1^{III}), 99.2 (1C, C-1^{I}), 99.1 (1C, C-1^{V}), 97.4 (1C, C-1^{VI}), 79.9 (1C, C-3^{I}), 79.3 (1C, C-3^{IV}), 78.9 (1C, C-3^{II}), 76.9 (2C, C-2^{II}, C-2^{IV}), 75.4 (1C, C-2^{I}), 75.2 (2C, PhCH_2), 75.0 (1C, PhCH_2), 74.8 (1C, C-4^{I}), 74.4 (1C, C-4^{II}), 74.1 (1C, C-3^{III}), 73.7 (1C, C-4^{IV}), 73.3 (2C, PhCH_2), 73.0 (1C, PhCH_2), 72.8 (1C, C-5^{IV}), 72.6 (1C, PhCH_2), 72.4 (1C, PhCH_2), 72.1 (2C, C-5^{I}, PhCH_2), 72.0 (1C, C-5^{II}), 71.9 (1C, C-2^{III}), 70.5 (1C, C-3^{VI}), 70.1 (2C, C-2^{V}, C-2^{VI}), 69.9 (1C, C-3^{V}), 69.4 (1C, C-6^{I}), 69.3 (1C, C-6^{II}), 69.2 (1C, C-5^{III}), 69.0 (1C, C-4^{III}), 68.8 (2C, C-5^{V}, C-5^{VI}), 68.1 (1C, C-6^{IV}), 66.4 (2C, C-4^{V}, C-4^{VI}), 65.8 (1C, C-6^{III}), 65.6 (1C, OCH_2CH$_2$CH$_2$N), 62.4 (1C, C-6^{VI}), 61.9 (1C, C-6^{V}), 37.6 (1C, OCH$_2$CH$_2$CH_2N), 28.0 (1C, OCH$_2$CH_2CH$_2$N). Anal. Calcd for $C_{174}H_{162}F_3NO_{42}$: C, 69.75; H, 5.45; N, 0.47. Found: C, 69.88; H, 5.70; N, 0.52.

Compound **19**: $[\alpha]_D$ −18.6 (*c* 0.8, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13, 8.04, 8.02–7.96, 7.90, 7.86, 7.78–7.72, 7.70, 7.59–7.52, 7.49–7.00, 6.89 (95 H, Ph-H), 6.71 (m, 1H, N*H*), 6.12 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4^{VI}), 6.04–5.95 (m, 3H, H-2^{VI}, H-3^{VI}, H-4^{V}), 5.84 (br s, 1H, H-2^{V}), 5.77 (br s, 1H, H-2^{III}), 5.63 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4^{III}), 5.54 (dd, 1H, $J_{2,3}$ = 3.0, $J_{3,4}$ = 10.2 Hz, H-3^{V}) 5.37 (s, 1H, H-1^{II}), 5.15 (s, 1H, H-1^{III}), 5.09 (s, 1H, H-1^{VI}), 5.02 (s, 1H, H-1^{V}), 4.89 (s, 1H, H-1^{I}), 4.88–4.77 (m, 4H, PhCH_2), 4.77–4.68 (m, 2H, PhCH_2), 4.68–4.65 (m, 2H, H-1^{IV}, PhCH_2), 4.63–4.59 (m, 3H, H-3^{III}, 2PhCH_2), 4.59–4.47 (m, 6H, H-5^{V}, H-6^{V}, 4PhCH_2), 4.47–4.39 (m, 6H, H-5^{III}, H-5^{VI}, H-6$_a^{VI}$, 3PhCH_2), 4.37 (d, 1H, *J* = 12.2 Hz, PhCH_2), 4.26 (dd, 1H, $J_{5,6}$ = 3.0, $J_{6a,6b}$ = 12.0 Hz, H-6$_b^{VI}$), 4.20–4.13 (4H, H-2^{I}, H-2^{II}, H-2^{IV}, PhCH_2), 4.01 (dd, 1H, $J_{2,3}$ = 2.3, $J_{3,4}$ = 8.6 Hz, H-3^{II}), 3.98–3.90 (m, 4H, H-4^{IV}, H-5^{II}, H-6$_a^{III}$, H-6$_b^{V}$), 3.89–3.84 (m, 2H, H-3^{I}, H-4^{II}), 3.71–3.56 (9H, H-3^{IV}, H-4^{I}, H-5^{I}, H-6$_a^{I}$, H-6$_b^{I}$, H-6$_a^{II}$, H-6$_b^{III}$, H-6$_a^{IV}$, H-6$_b^{IV}$), 3.56–3.50 (m, 2H, H-6$_b^{II}$, OCH_2CH$_2$CH$_2$N), 3.40 (m, 1H, H-5^{IV}), 3.24–3.18 (m, 2H, OCH_2CH$_2$CH$_2$N, OCH$_2$CH$_2$CH_2N), 3.06 (m, 1H, OCH$_2$CH$_2$CH_2N), 1.58 (m, 1H, OCH$_2$CH_2CH$_2$N), 1.49 (m, 1H, OCH$_2$CH_2CH$_2$N); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.2–164.9 (PhCO), 138.6–138.0, 133.3–132.6, 130.7–127.2 (Ph), 100.7 (1C, C-1^{II}), 99.4 (2C, C-1^{III}, C-1^{V}), 99.3 (1C, C-1^{I}), 97.8 (1C, C-1^{IV}), 97.7 (1C, C-1^{VI}), 82.3 (1C, C-3^{IV}), 79.9 (1C, C-3^{I}), 79.2 (1C, C-3^{II}), 77.2 (2C, C-2^{I}, C-2^{IV}), 76.1 (1C, C-5^{IV}), 75.7 (1C, C-2^{II}), 75.1 (4C, C-4^{I}, 3PhCH$_2$), 74.7 (2C, C-4^{II}, C-4^{IV}), 73.4 (3C, PhCH_2), 73.3 (1C, PhCH_2), 72.7 (1C, PhCH_2), 72.3 (2C, C-3^{III}, PhCH_2), 72.1 (2C, C-5^{I}, C-5^{II}), 70.9 (1C, C-3^{V}), 70.8 (1C,

C-3^{VI}), 70.5 (2C, C-2^{V}, C-2^{VI}), 69.7 (1C, C-6^{IV}), 69.5 (2C, C-5^{III}, C-6^{I}), 69.2 (1C, C-2^{III}), 69.1 (1C, C-5^{VI}), 68.9 (1C, C-6^{II}), 68.4 (1C, C-5^{V}), 67.3 (1C, C-4^{III}), 66.5 (1C, C-6^{III}), 66.4 (1C, C-4^{VI}), 66.0 (2C, C-4^{V}), 65.6 (1C, O*C*H$_2$CH$_2$CH$_2$N), 62.7 (1C, C-6^{VI}), 62.5 (1C, C-6^{V}), 37.6 (1C, OCH$_2$CH$_2$*C*H$_2$N), 28.0 (1C, OCH$_2$*C*H$_2$CH$_2$N). Anal. Calcd for $C_{174}H_{162}F_3NO_{42}$: C, 69.75; H, 5.45; N, 0.47. Found: C, 69.62; H, 5.37; N, 0.49.

3.12. 3-Aminopropyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (21)

Pd(OH)$_2$/C (90 mg) was added to a soln of hexamannoside **18** (85 mg, 28 μmol) in MeOH (2 mL) and EtOAc (1 mL). The mixture was stirred under H_2 (1 atm) at room temperature for 20 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 MeOH (4 mL) and 1 M MeONa (0.4 mL) was added. The mixture was kept for 3 h, water (0.5 mL) was added, and after 24 h the solvents were evaporated. The residue was subjected to gel chromatography, appropriate fractions were collected, and lyophilized to give **21**·AcOH (19 mg, 65%) as a white amorphous powder. $[\alpha]_D$ +77.0 (*c* 0.3, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 5.38 (s, 1H, H-1^{IV}), 5.23 (s, 1H, H-1^{II}), 5.17 (s, 1H, H-1^{I}), 5.04 (s, 1H, H-1^{V}), 5.01 (s, 1H, H-1^{III}), 4.92 (s, 1H, H-1^{VI}), 4.21 (br s, 1H, H-2^{III}), 4.10 (br s, 2H, H-2^{II}, H-2^{IV}), 4.07 (br s, 1H, H-2^{V}), 4.00–3.97 (m, 3H, H-2^{VI}, H-3^{IV}, H-6$_a^{III}$), 3.97–3.81 (m, 14H, H-2^{I}, H-3^{I}, H-3^{II}, H-3^{III}, H-3^{V}, H-3^{VI}, H-4^{III}, H-5^{III}, H-6$_a^{I}$, H-6$_a^{II}$, H-6$_a^{IV}$, H-6$_a^{V}$, H-6$_a^{VI}$, OC*H*$_2$CH$_2$CH$_2$N), 3.80–3.69 (m, 9H, H-5^{II}, H-5^{IV}, H-5^{V}, H-6$_b^{I}$, H-6$_b^{II}$, H-6$_b^{III}$, H-6$_b^{IV}$, H-6$_b^{V}$, H-6$_b^{VI}$), 3.69–3.56 (m, 8H, H-4^{I}, H-4^{II}, H-4^{IV}, H-4^{V}, H-4^{VI}, H-5^{I}, H-5^{VI}, OC*H*$_2$CH$_2$CH$_2$N), 3.11 (m, 2H, OCH$_2$CH$_2$C*H*$_2$N), 1.99 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N), 1.90 (s, 3H, C*H*$_3$COOH); ^{13}C NMR (126 MHz, D_2O): δ 103.8 (1C, C-1^{V}), 103.5 (1C, C-1^{III}), 102.1 (2C, C-1^{II}, C-1^{IV}), 100.7 (1C, C-1^{VI}), 99.7 (1C, C-1^{I}), 80.2 (4C, C-2^{I}, C-2^{II}, C-2^{IV}, C-3^{III}), 75.0 (1C, C-5^{II}), 74.7 (2C, C-5^{IV}, C-5^{V}), 74.3 (1C, C-5^{VI}), 74.2 (1C, C-5^{I}), 72.8 (1C, C-5^{III}), 72.0 (1C, C-3^{V}), 71.8 (1C, C-3^{VI}), 71.7 (1C, C-3^{I}), 71.5 (2C, C-2^{VI}, C-3^{II}), 71.4 (2C, C-2^{V},C-3^{IV}), 71.0 (1C, C-2^{III}), 68.6–68.2 (5C, C-4^{I}, C-4^{II}, C-4^{IV}, C-4^{V}, C-4^{VI}), 67.1 (1C, C-4^{III}), 66.6 (1C, C-6^{III}), 66.4 (1C, O*C*H$_2$CH$_2$CH$_2$N), 62.7–62.4, (5C, C-6^{I}, C-6^{II}, C-6^{IV}, C-6^{V}, C-6^{VI}), 38.9 (1C, OCH$_2$CH$_2$*C*H$_2$N), 28.1 (1C, OCH$_2$*C*H$_2$CH$_2$N). HRESIMS: found *m*/*z* 1048.3931 [M+H]$^+$; calcd for $C_{39}H_{70}NO_{31}$ 1048.3932.

3.13. 3-(3,4-Dioxo-2-ethoxycyclobut-1-enylamino)propyl α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (22)

Diethyl squarate (1.6 μL, 10.7 μmol) and Et_3N (1.2 μL) were added to a soln of pentasaccharide **20** (8.1 mg, 8.9 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 **22** (8 mg, 89%) as a white amorphous powder; $[\alpha]_D$ +65.3 (*c* 0.5, H_2O), ^{1}H NMR (500 MHz, D_2O): δ 5.19 (s, 1H, H-1^{II}), 5.13 (s, 1H, H-1^{IV}), 5.04–5.01 (m, 2H, H-1^{I}, H-1^{III}), 4.91 (s, 1H, H-1^{V}), 4.75 (m, 2H, OC*H*$_2$CH$_3$), 4.21 (br s, 1H, H-2^{III}), 4.10 (br s, 1H, H-2^{II}), 4.06 (br s, 1H, H-2^{IV}), 4.03–3.98 (m, 2H, H-2^{V}, H-6$_a^{III}$), 3.95–3.82 (m, 12H, H-2^{I}, H-3^{I}, H-3^{II}, H-3^{III}, H-3^{IV}, H-3^{V}, H-4^{III}, H-5^{III}, H-6$_a^{I}$, H-6$_a^{II}$, H-6$_a^{IV}$, H-6$_a^{V}$), 3.79 (m, 1H, OC*H*$_2$CH$_2$CH$_2$N), 3.78–3.68 (m, 8H, H-5^{II}, H-5^{IV}, H-6$_b^{I}$, H-6$_b^{II}$, H-6$_b^{III}$, H-6$_b^{IV}$, H-6$_b^{V}$, OCH$_2$CH$_2$C*H*$_2$N), 3.68–3.63 (m, 5H, H-4^{I}, H-4^{II}, H-4^{IV}, H-4^{V}, H-5^{V}), 3.63–3.56 (m, 3H, H-5^{I}, OC*H*$_2$CH$_2$CH$_2$N, OCH$_2$CH$_2$C*H*$_2$N), 1.94 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N), 1.45 (m, 3H, OCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, D_2O): δ 103.7 (1C, C-1^{IV}), 103.6 (1C, C-1^{III}), 102.3 (1C, C-1^{II}), 100.8 (1C, C-1^{V}), 99.8, 99.6 (1C, C-1^{I}), 80.3 (1C, C-2^{I}), 79.9 (1C, C-2^{II}), 79.7 (1C, C-3^{III}), 74.9 (1C, C-5^{II}), 74.8 (1C, C-5^{IV}), 74.3 (1C, C-5^{I}), 74.2 (1C, C-5^{V}), 72.9 (1C, C-5^{III}), 72.1 (2C, C-3^{V}, OCH$_2$CH$_3$), 71.8 (2C, C-2^{IV}, C-3^{I}), 71.5 (3C, C-2^{V} C-3^{II}, C-3^{IV}), 71.0 (1C, C-2^{III}), 68.4 (2C, C-4^{I}, C-4^{II}), 68.3 (1C, C-4^{V}), 68.2 (1C, C-4^{IV}), 67.1 (1C, C-4^{III}), 66.5 (1C, C-6^{III}), 66.3, 66.2 (1C, O*C*H$_2$CH$_2$CH$_2$N), 62.6, 62.5, 62.4 (4C, C-6^{I}, C-6^{II}, C-6^{IV}, C-6^{V}), 43.4, 43.1 (1C, OCH$_2$CH$_2$*C*H$_2$N), 30.7, 30.5 (1C, OCH$_2$*C*H$_2$CH$_2$N), 16.6 (1C, OCH$_2$*C*H$_3$). HRESIMS: found *m*/*z* 1032.3341 [M+Na]$^+$; calcd for $C_{39}H_{63}NNaO_{29}$ 1032.3383.

3.14. 3-(3,4-Dioxo-2-ethoxycyclobut-1-enylamino)propyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (23)

Compound **23** was obtained from amine **21** (8.1 mg, 7.5 μmol) and diethyl squarate (1.3 μL, 9.1 μmol) in the presence of Et_3N (1.1 μL) as described above for **22**. Yield 7 mg (80%); $[\alpha]_D$ +68.3 (*c* 0.5, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 5.38 (s, 1H, H-1^{IV}), 5.19 (s, 1H, H-1^{II}), 5.05–5.03 (m, 2H, H-1^{I}, H-1^{V}), 5.02 (s, 1H, H-1^{III}), 4.92 (s, 1H, H-1^{VI}), 4.75 (m, 2H, OC*H*$_2$CH$_3$), 4.21 (br s, 1H, H-2^{III}), 4.09 (br s, 2H, H-2^{II}, H-2^{IV}), 4.06 (m, 1H, H-2^{V}), 4.02–3.97 (m, 3H, H-2^{VI}, H-3^{IV}, H-6$_a^{III}$), 3.95–3.82 (m, 13H, H-2^{I}, H-3^{I}, H-3^{II}, H-3^{III}, H-3^{V}, H-3^{VI}, H-4^{III}, H-5^{III}, H-6$_a^{I}$, H-6$_a^{II}$, H-6$_a^{IV}$, H-6$_a^{V}$, H-6$_a^{VI}$), 3.81 (m, 1H, OC*H*$_2$CH$_2$CH$_2$N), 3.79–3.71 (m, 9H, H-5^{II}, H-5^{IV}, H-5^{V}, H-6$_b^{I}$, H-6$_b^{II}$, H-6$_b^{IV}$, H-6$_b^{V}$, H-6$_b^{VI}$, OCH$_2$CH$_2$C*H*$_2$N), 3.70 (m, 1H, H-6$_b^{III}$), 3.69–3.60 (m, 7H, H-4^{I}, H-4^{II}, H-4^{IV}, H-4^{V}, H-4^{VI}, H-5^{VI}, OCH$_2$CH$_2$C*H*$_2$N), 3.60–3.53 (m, 2H, H-5^{I}, OC*H*$_2$CH$_2$CH$_2$N), 1.94 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N), 1.44 (m, 3H, OCH$_2$C*H*$_3$); ^{13}C NMR (126 MHz, D_2O): δ 103.7 (1C, C-1^{V}), 103.6 (1C, C-1^{III}), 102.3 (1C, C-1^{II}), 102.2 (1C, C-1^{IV}), 100.9 (1C, C-1^{VI}), 99.9, 99.7 (1C, C-1^{I}), 80.4 (1C, C-2^{I}), 80.2 (1C, C-3^{III}), 80.1 (2C, C-2^{II}, C-2^{IV}), 75.0 (1C, C-5^{II}), 74.8 (2C, C-5^{IV}, C-5^{V}), 74.4 (1C, C-5^{I}), 74.2 (1C, C-5^{VI}), 72.9 (1C, C-5^{III}), 72.2 (3C, C-3^{V}, C-3^{VI}, OCH$_2$CH$_3$), 71.9 (1C, C-2^{VI}), 71.5 (4C, C-2^{V}, C-3^{I}, C-3^{II}, C-3^{IV}), 71.1 (1C, C-2^{III}), 68.6 (3C, C-4^{I}, C-4^{IV}, C-4^{VI}), 68.3 (2C, C-4^{II}, C-4^{V}), 67.1 (1C, C-4^{III}), 66.6 (1C, C-6^{III}), 66.4, 66.2 (1C, O*C*H$_2$CH$_2$CH$_2$N), 62.7–62.3 (5C, C-6^{I}, C-6^{II}, C-6^{IV}, C-6^{V}, C-6^{VI}), 43.4, 43.2 (1C, OCH$_2$CH$_2$*C*H$_2$N), 30.7, 30.6 (1C, OCH$_2$*C*H$_2$CH$_2$N), 16.6 (1C, OCH$_2$*C*H$_3$). HRESIMS: found *m*/*z* 1194.3940 [M+Na]$^+$; calcd for $C_{45}H_{73}NNaO_{34}$ 1194.3912.

3.15. BSA–pentasaccharide conjugate (24)

A soln of **22** (8 mg, 7.3 μmol) and BSA (16 mg, 0.240 μmol) in 2 mL of the buffer soln (350 mM $KHCO_3$ and 70 mM $Na_2B_4O_7$·10H_2O, pH 9) was kept for three 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 **24** (18 mg, 98%) as a white amorphous powder. MALDI-TOFMS showed a broad peak with a maximum at *m*/*z* 76121 (~10 pentasaccharide residues per BSA molecule).

3.16. BSA–hexasaccharide conjugate (25)

Conjugate **25** (13 mg, 95%) was obtained similarly from **23** (7 mg, 5.5 μmol) and BSA (12 mg, 0.181 μmol). MALDI-TOFMS showed a broad peak with a maximum at *m*/*z* 75955 (~9 hexasaccharide residues per BSA molecule).

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