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SYNTHETIC STUDIES

Synthesis of Oligosaccharide Fragments of Mannan from *Candida albicans* Cell Wall and Their BSA Conjugates

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Abstract—3-Aminopropyl glycosides of α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranose, α-*D*-mannopyranosyl-(1⟶3)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranose, and α-*D*-mannopyranosyl-(1⟶2)-[α-*D*-mannopyranosyl-(1⟶3)]-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranose were efficiently synthesized starting from ethyl 2-*O*-acetyl(benzoyl)-3,4,6-tri-*O*-benzyl-1-thio-α-*D*-mannopyranoside, ethyl 4,6-di-*O*-benzyl-2-*O*-benzoyl-1-thio-α-*D*-mannopyranoside, ethyl 4,6-di-*O*-benzyl-2,3-di-O-benzoyl-1-thio-α-*D*-mannopyranoside, and 2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl bromide. The oligosaccharide chains synthesized correspond to the three structural types of side chains of mannan from *Candida albicans* cell wall. A conjugate of the third pentasaccharide with bovine serum albumin was prepared using the squarate method.

Key words: Candida albicans, mannan, neoglycoconjugates, oligomannosides, synthesis

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INTRODUCTION

The *Candida albicans* yeastlike fungi are the opportunistic pathogenic microorganisms capable of causing severe infections in immunodeficient patients [1].[2] The cell surface of *C. albicans* is the first to interact with the host organism. It plays the main role in the pathogen adhesion to the host organism cells and the modulation of immune response [1]. The external surface of *C. albicans* cell wall is enriched with mannoproteins anchored in the matrix of structural polysaccharides. The immunogenic properties of *C. albicans* are in many respects due to mannan, which is the carbohydrate part of mannoproteins. Mannans are highly branched polysaccharides with the backbone built up from (α1–6)-linked mannose residues. The mannan backbone can bear relatively short (α1–2)-oligomannoside side chains in position 2 of mannose residues (structure **A**), which can also bear short (1–4 units) (β1–2)-oligomannoside substituents in position 2 (structure **B**) or α-mannoside residue in position 3 of the terminal mannose unit (structure **C**) [2]. The chains with (α1–3) substitution in the last but one mannose residue (structure **D**) have also been shown to occur (Fig. 1) [3]. Moreover, the (β1–2)-linked chains containing 2–6 mannose units can be attached to the side chains via the phosphodiester fragment [2] (not shown in Fig. 1).

There are reported the syntheses of (α1–2)-linked oligomannoside chains up to the octasaccharide [4, 5], including the solid phase synthetic approach [6], and also the type **C** oligosaccharides with a terminal (α1–3)-linked mannose residue [5, 7].

However, note that, in the papers cited, the target structures were obtained either in the form of free oligosaccharides [4, 7], or as acylated allyl glycosides [5], which substantially complicates their use in the synthesis of neoglycoconjugates. The obtaining of the oligosaccharide sequences **A** and **C**, included as constituents in the more complicated oligosaccharide structures, is also reported [8–11]. No synthesis of the type

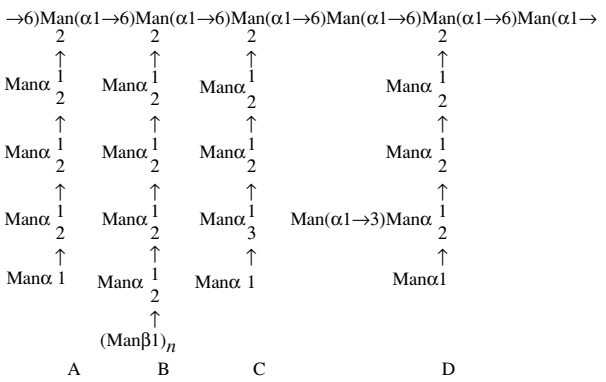

Fig. 1. The structure of mannan from *C. albicans* cell wall.

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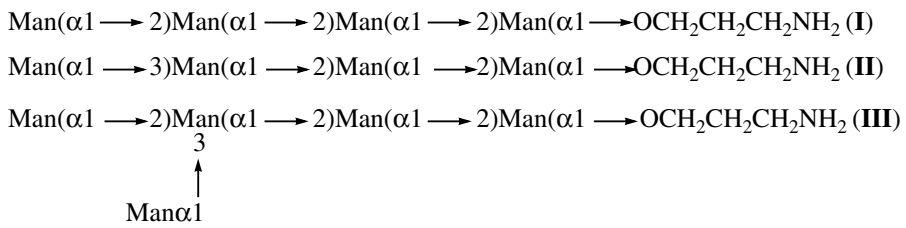
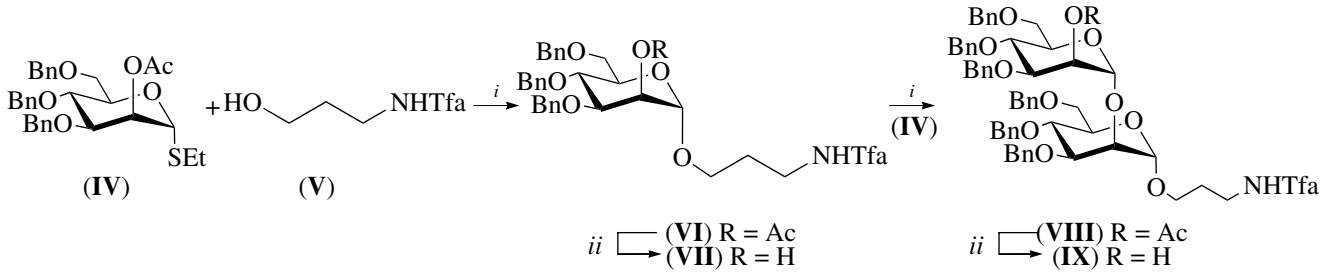
[2] Abbreviations: Bn, benzyl; Bz, benzoyl; NIS, *N*-iodosuccinimide; Tfa, trifluoroacetyl; and Tf, trifluoromethanesulfonyl.





Scheme 1. Reagents: (*i*) NIS, TfOH, molecular sieves 4Å, CH_2Cl_2; (*ii*) MeONa, MeOH.

D branched oligosaccharide structure has yet been reported.

We herein report the synthesis of oligosaccharides (**I**)–(**III**) corresponding to three types of the side chain structures (**A**, **C**, and **D**) of the mannan from *C. albicans* cell wall in the form of 3-aminopropyl glycosides. The presence of the amino group in the aglycones of the synthetic oligosaccharides allows their subsequent covalent binding to high-molecular carriers (synthetic or protein) and labels of various types. In particular, we have obtained a conjugate of oligosaccharide (**III**) with BSA for further immunological studies. Such neoglycoconjugates and molecular probes are valuable tools for glycobiological studies and can be a basis for the creation of vaccines of a new generation.

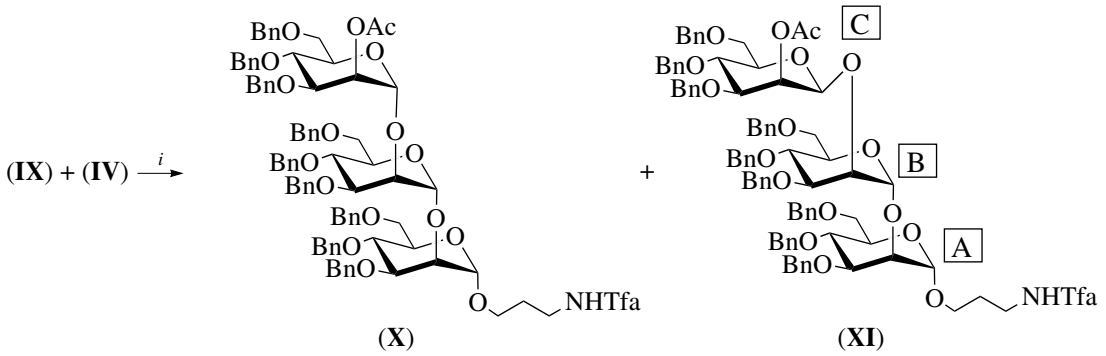
RESULTS AND DISCUSSION

The glycosylating agents bearing permanent protective groups (benzyl or benzoyl) in positions 3, 4, and 6 and an acetyl group in position 2, which provides a high stereoselectivity of α-mannosylation due to participation and can be easily removed from the glycosylation product keeping the other protective groups intact, are usually used for the design of (α1–2)-linked mannooligosaccarides. In this study, we used the well-known thiomannoside (**IV**) as such a glycosylating agent [12].

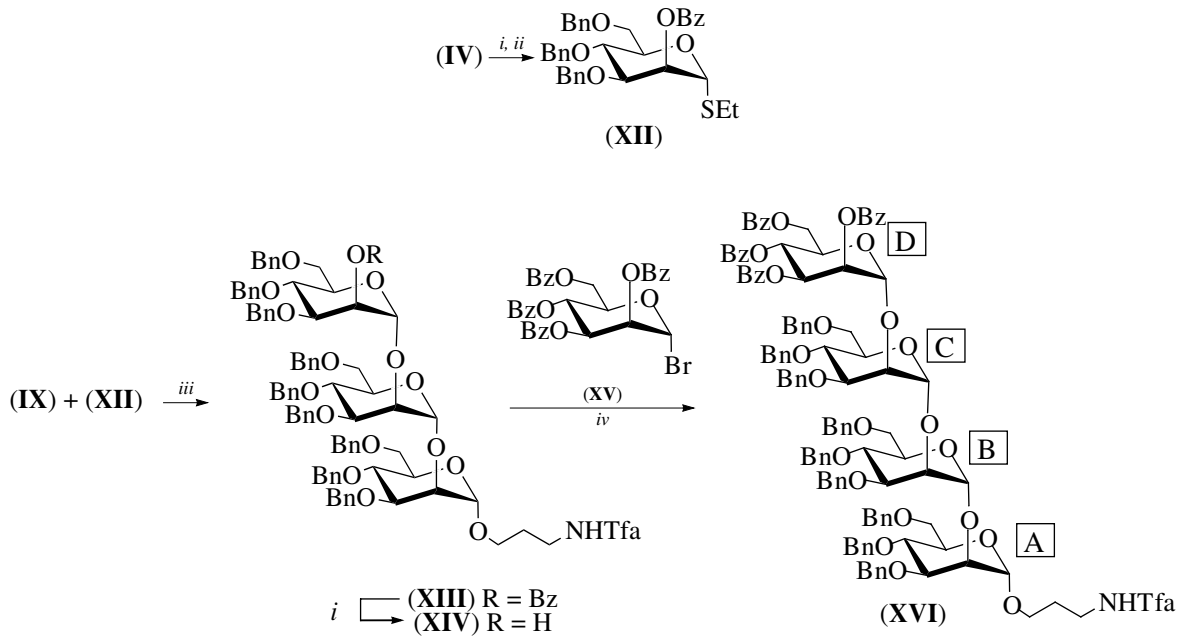
Glycosylation of 3-trifluoroacetamidopropanol (**V**) with thioglycoside (**IV**) in the presence of NIS and TfOH smoothly resulted in glycoside (**VI**), which was deacetylated to give monosaccharide glycosyl acceptor (**VII**) (Scheme 1). The repetition of the operations of glycosylation and deacetylation yielded disaccharide acceptor (**IX**).

Structures of the glycosylation products, first of all, the configuration of the newly formed glycoside bonds, were established on the basis of NMR spectroscopy. It is known that, in the case of mannose residues, unlike other monosaccharides, the vicinal coupling constant $J_{1,2}$ and the chemical shifts of C1 in ^{1}H and ^{13}C NMR spectra, respectively, do not characterize the configuration of glycoside bonds. At the same time, the chemical shift of H5 can be a rather reliable indicator of the anomeric configuration of mannosides [13]. The signals of H5 of the glycosylation products (**VI**) and (**VIII**) are exhibited in the region of δ 3.70–4.00 ppm, which is in a good agreement with the data for 3,4,6-tri-*O*-bensylated α-mannosides [6, 14], whereas the H5 protons of β-linked mannose residues resonate in the region of [delta] 3.30–3.40 ppm [14]. It should be noted that the presence of similarly protected benzylated monosaccharide units in disaccharides (**VIII**) and (**IX**) and trisaccharides (see below) results in a significant overlap of the signals of ring protons H3–H6 of mannose units in the ^{1}H NMR spectra, substantially complicating their reliable assignment. Therefore, when reporting the NMR data for (1–2)-linked oligosaccharides (see the Experimental section), we give the full assignment of signals only for the terminal nonreducing monosaccharide residue that was introduced upon glycosylation or was deacetylated to obtain a new glycosyl acceptor. Only the data for the anomeric H and C atoms are given for the other monosaccharide units.

Glycosylation of disaccharide acceptor (**IX**) with thiomannoside (**IV**) resulted in a mixture of the expected α-linked trisaccharide (**X**) and its β-anomer at the nonreducing unit C (**XI**) with the latter compound prevailing (Scheme 2). In the case of trisaccharide (**X**), the signal of H5 of the terminal nonreducing mannose residue is at δ 3.96 ppm, which confirms its α-configuration, whereas in the case of derivative (**XI**), the signal of H5 of the β-connected mannose residue is shifted upfield (δ 3.33 ppm). Taking into consideration a pro-



Scheme 2. Reagents: (*i*) NIS, TfOH, molecular sieves 4Å, CH_2Cl_2.



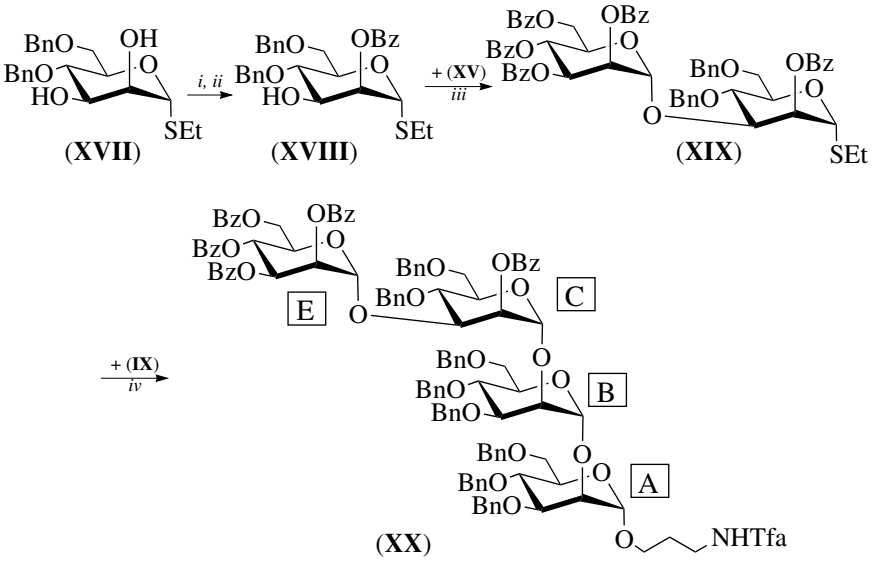
Scheme 3. Reagents: (*i*) MeONa, MeOH; (*ii*) BzCl, Py; (*iii*) NIS, TfOH, molecular sieves 4Å, CH_2Cl_2; (*iv*) AgOTf, molecular sieves 4Å, CH_2Cl_2.

nounced tendency of mannopyranose glycosyl donors to stereoselective α-glycosylation even in the case of nonparticipating group at O2, e.g., glycosyl [4, 5, 8–10], this result appeared to be rather unexpected.

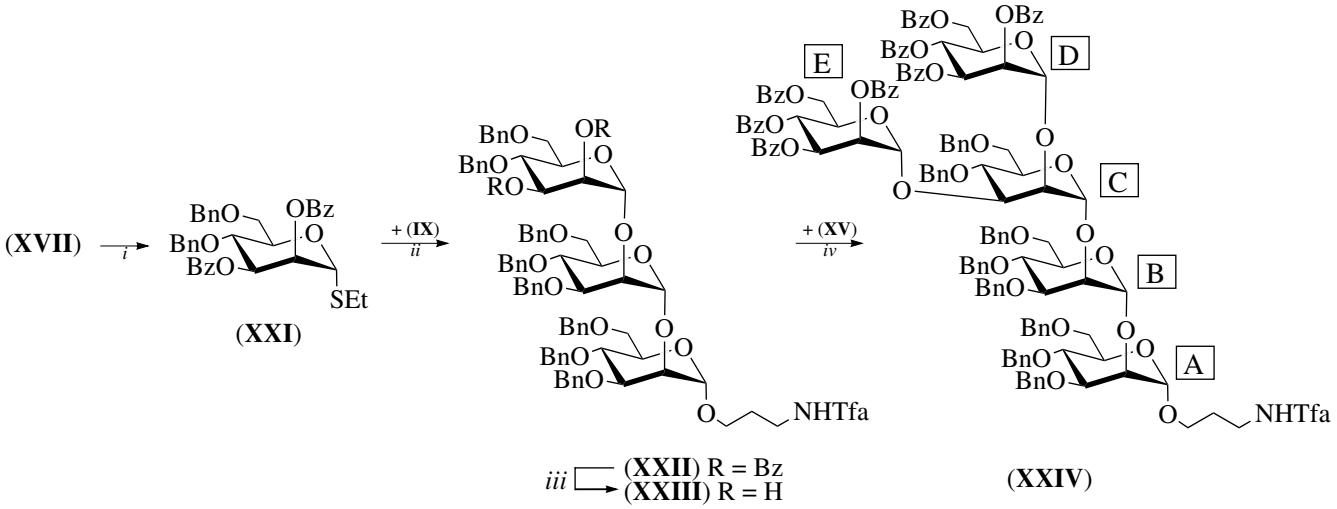
We have substituted benzoyl group in mannosyl donor (**IV**) for acetyl group and obtained thioglycoside (**XII**) to increase α-stereoselectivity of mannosylation of disaccharide acceptor (**IX**) [15] (Scheme 3). In fact, the use of benzoylated derivative (**XII**) allowed us to minimize the formation of β-anomer; the target α-trisaccharide (**XIII**) was obtained in 64% yield. The higher stereoselectivity of mannosylation in the case of donor (**XII**) can be explained by a lesser tendency of phenyl-substituted 1,2-dioxolenium intermediate to isomerisation as compared with methyl-substituted analogue in the case of acetylated donor (**IV**).

Removal of benzoyl group in trisaccharide (**XIII**) resulted in acceptor (**XIV**). Since we did not plan the further extension of the oligosaccharide chain, we used a more available benzobromomannose (**XV**) as the mannosyl donor at the final step rather than thioglycoside (**IV**). The reaction of bromide (**XV**) with acceptor (**XIV**) in the presence of silver triflate resulted in the target tetrasaccharide (**XVI**) in 68% yield (Scheme 3). The α-configuration of the terminal mannose unit D unambiguously followed from the low-field position of the H5 signal (δ 4.65 ppm) in the ^{1}H NMR spectrum of (**XVI**).

The [2 + 2] scheme was used to synthesize tetrasaccharide (**II**). Glycosylation of disaccharide acceptor (**IX**) with the derivative of (α1–3)-mannobiose was the key step of the synthesis. The known 4,6-di-*O*-benzyl thioglycoside (**XVII**) [16], which was selectively ben-



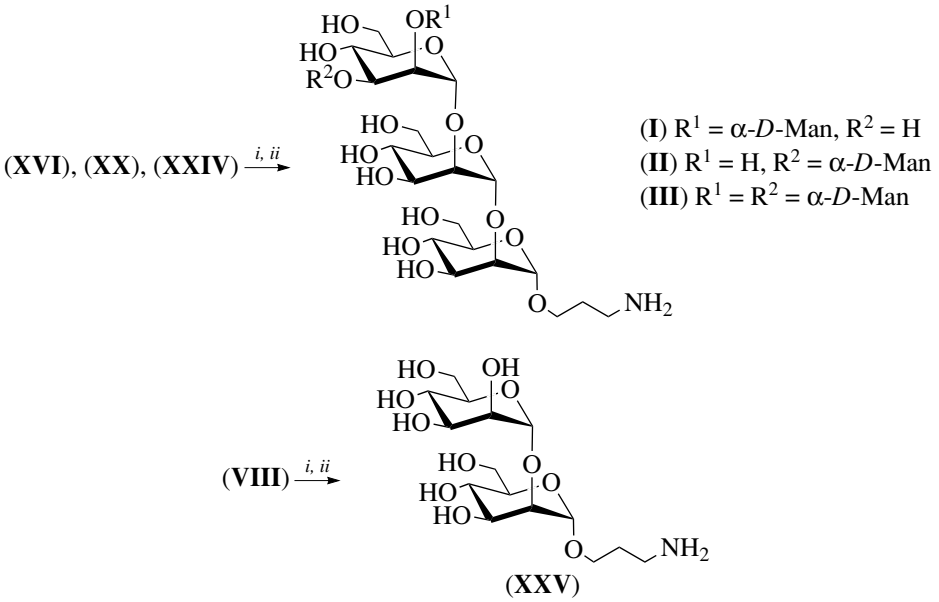
Scheme 4. Reagents: (*i*) $PhC(OMe)_3$, camphorsulfonic acid, MeCN; (*ii*) 80% aqueous AcOH; (*iii*) AgOTf, molecular sieves 4Å, CH_2Cl_2; (*iv*) NIS, TfOH, molecular sieves 4Å, CH_2Cl_2.



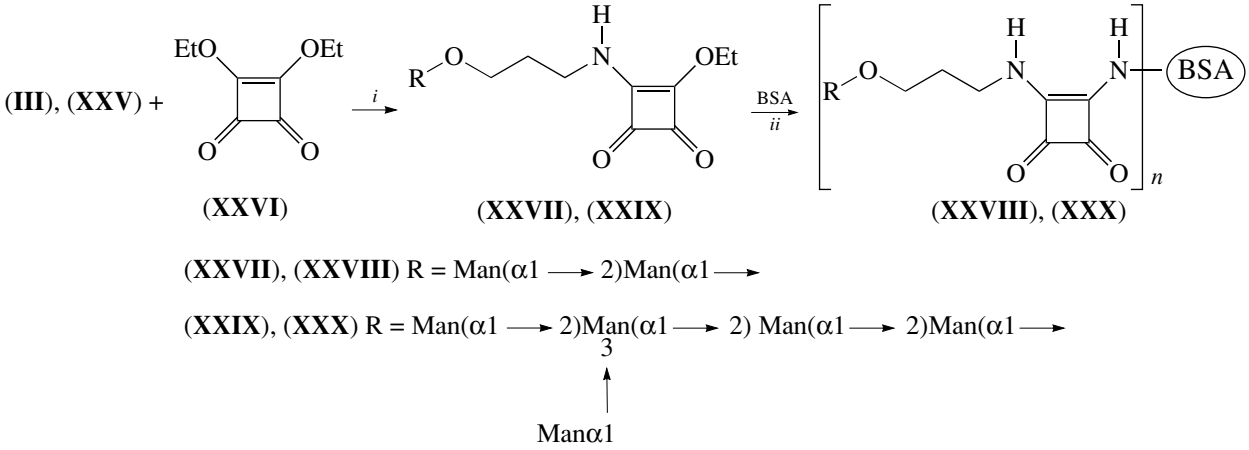
Scheme 5. Reagents: (*i*) BzCl, Py; (*ii*) NIS, TfOH, molecular sieves 4Å, CH_2Cl_2; (*iii*) MeONa, MeOH; (*iv*) AgOTf, molecular sieves 4Å, CH_2Cl_2.

zoylated at O2 via the intermediate formation of cyclic 2,3-orthobenzoate (Scheme 4), was the starting compound in the synthesis of (α1–3)-linked dimannoside block. The reaction of the resulting monobenzoate (**XVIII**) with bromide (**XV**) in the presence of silver triflate resulted in disaccharide thioglycoside (**XIX**) in 64% yield, which can be directly used in the subsequent glycosylation. The low-field position of the H5 signal δ 4.50 ppm) of tetra-*O*-benzoylated nonreducing mannose unit (the future residue E) in the ^{1}H NMR spectrum of (**XIX**) proved its α-configuration. Glycosylation of disaccharide acceptor (**IX**) with thioglycoside (**XIX**) in the presence NIS and TfOH resulted in the target tetrasaccharide (**XX**) in 65% yield. The α -configuration of the resulting glycoside bond was confirmed by the chemical shift of H5 (δ 4.15 ppm) of the monosaccharide unit C in the ^{1}H NMR spectrum of (**XX**).

Diol (**XVII**) was benzoylated to give dibenzoate (**XXI**), which was used as the precursor of the 2,3-bisglycosylated mannose residue in the pentasaccaride (**III**) (Scheme 5). The reaction of thioglycoside (**XXI**) with disaccharide acceptor (**IX**) in the presence of NIS and TfOH resulted in trisaccharide derivative (**XXII**) in 95% yield, with the α-configuration of the terminal mannoside bond being confirmed by the chemical shift (δ 4.10 ppm) of H5 of the ring C in the ^{1}H NMR spectrum. The subsequent debenzoylation of (**XXII**)



Scheme 6. Reagents: (*i*) H_2, $Pd(OH)_2$/C, MeOH; (*ii*) Amberlyst A-26 (OH^-), water.



Scheme 7. Reagents: (*i*) Et_3N, 50% aqueous ethanol; (*ii*) borate buffer, pH 9.0.

resulted in diol (**XXIII**), which was bisglycosylated with benzobromomannose (**XV**) to give the target protected pentasaccaride (**XXIV**) in 85% yield.

The protected oligosaccharides (**XVI**), (**XX**), and (**XXIV**) were first subjected to hydrogenolysis to remove benzyl groups; then benzoyl and *N*-trifluoroacetyl groups were simultaneously removed by the treatment with anion exchanger Amberlyst A-26 (OH^-) to give the target free oligosaccharides (**I**)–(**III**) (Scheme 6). Similarly, protected derivative (**VIII**) was converted into free 3-aminopropyl glycoside (**XXV**), which was used for obtaining of the model conjugate with BSA.

The conjugation of derivative (**XXV**) with BSA was performed by the squarate method [17–19]. At the first step, the reaction of oligosaccharide (**XXV**) with diethyl squarate (**XXVI**) at pH 7 resulted in monosubstituted adduct (**XXVII**) (Scheme 7).The ^{1}H NMR spectrum of the carbohydrate part of (**XXVII**) was practically indistinguishable from that of starting disaccharide (**XXV**) except for the signal of H1 of the mannose unit A, which appeared as two closely located singlets of half intensity, which is likely due to the isomerism of the vinylogous amide group in the squarate fragment [17, 19]. The same reason caused the doubling of the signals of the spacer propyl group and the ethoxy group. The subsequent reaction of derivative (**XXVII**) with free amino groups of BSA proceeded at pH 9 and resulted in conjugate (**XXVIII**). Under the same conditions, pentasaccharide (**III**) was transformed to monoamide (**XXIX**), which was coupled

with BSA to give conjugate (**XXX**). According to MALDI TOF mass spectrometry [18] (Fig. 2), conjugates (**XXVIII**) and (**XXX**) contained, on the average, 20 oligosaccharide residues (n = 20) at an initial oligosaccharide–BSA molar ratio of 20 : 1.

Thus, 3-aminopropyl glycosides of the oligosaccharides corresponding to the three types of side chains of the mannoprotein from *C. albicans* cell wall were synthesized. Conjugates of disaccharide (**XXV**) and pentasaccharide (**III**) with BSA were obtained using the squarate method.

EXPERIMENTAL

The procedures for solvent purification are similar to those described in [20]. TfOH, camphorsulfonic acid, ethyl orthobenzoate, diethyl squarate (Fluka), NIS (Acros), and AgOTf (Merck) were used without additional purification. ^{1}H and ^{13}C NMR spectra were registered on Bruker DRX-500 and Bruker AM-300 spectrometers at 25°C in $CDCl_3$ in the case of the protected derivatives and in D_2O in the case of free oligosaccharides. The signals in the NMR spectra were assigned using COSY, TOCSY, ROESY, and HSQC two-dimensional correlation spectroscopy experiments. The designations of monosaccharide residues used in the assigning of NMR spectra, are shown in schemes. MALDI-TOF spectra were registered on a Bruker Ultraflex mass spectrometer with the double time-of-flight analyzer in a linear mode with registration of positive ions. The source had panoramic delay of ion extraction, and an accelerating voltage of 20 kV were used. 2,5-Dihydroxybenzoic acid was used as a matrix. The values of optical rotation were measured on a Polyarimetr Universal'nyi PU-7 polarimeter (Russia) at 18–22°C in chloroform in the case of the protected and partially protected derivatives and in water in the case of free oligosaccharides at c = 1%. TLC was carried out on silica gel 60 (Merck) precoated plates. The spots were visualized by spraying with 10 vol % orthophosphoric acid in ethanol and the subsequent heating at ~150°C. Column chromatography was carried out on silica gel 60 (0.040–0.063 mm) (Merck). For gel chromatography, a TSK HW-40 (S) column (1.5 × 90 cm) in 0.1 M acetic acid was used; the eluate was analyzed by a Knauer 88 00 flow-through refractometer. Molecular sieves 4Å (Fluka) were activated before reaction by heating at 200°C for 2 h in a vacuum (1 mm Hg). Glycosylation was achieved in anhydrous solvents in the atmosphere of dry argon.

A general procedure for glycosylation with thioglycosides (procedure A). Molecular sieves 4Å were added to a solution of a glycosyl acceptor and a glycosyl donor in dichloromethane; the mixture was stirred for 30 min at room temperature and cooled to the temperature in a range from –10 to –15°C. NIS was added, and, after 10 min, the temperature of the reaction mixture was decreased to a value from –30 to –35°C and TfOH was added. Then the temperature of the reaction mixture was maintained within a range from –25°C to –30°C. After the starting glycosyl acceptor was exhausted (according to TLC), the reaction mixture was quenched with pyridine, diluted with chloroform, and filtered through a Celite layer. The filtrate was washed with 1 M sodium thiosulfate and water, the solvent was removed, and the residue was twice coevaporated with toluene.

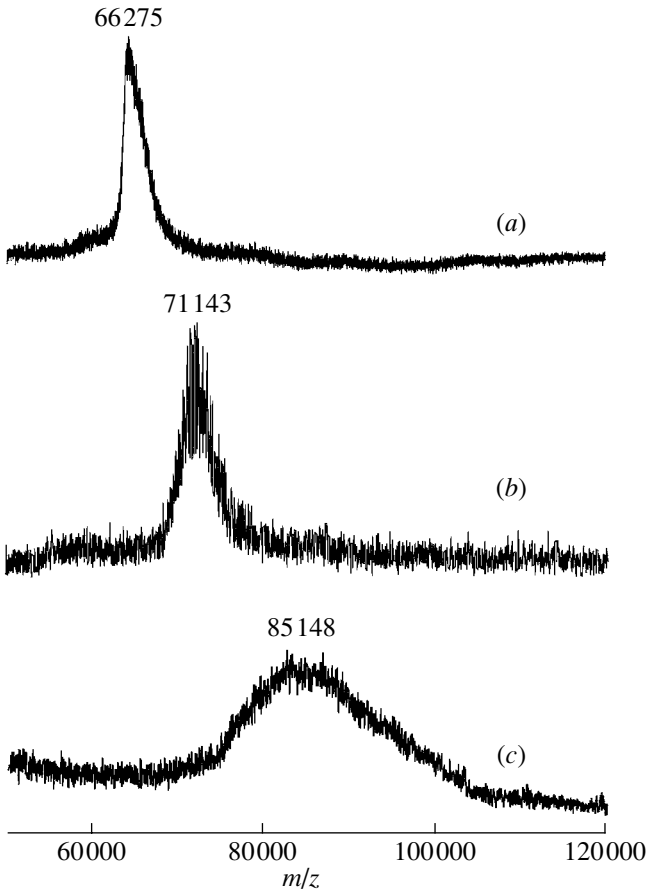


Fig. 2. MALDI TOF mass spectra of (*a*) initial BSA and conjugates (*b*) (**XXVIII**) and (*c*) (**XXX**).

A general procedure for deacylation (procedure B). A 1 M sodium methylate in MeOH was added a solution of a starting compound in anhydrous methanol in the amount necessary to provide the final concentration of MeONa of 0.05–0.1 M. After the deacylation was over (TLC monitoring); cation exchange resin KU-2 (H^+) was added to the reaction mixture until the neutral reaction, then the cation exchanger was filtered off and washed with MeOH, and the filtrate was concentrated in a vacuum.

A general procedure for glycosylation with benzobromomannose (procedure C). Molecular sieves 4Å were added to a solution of a glycosyl acceptor and benzobromomannose in dichloromethane, the mixture was stirred for 30 min at room temperature, cooled to the temperature in a range from –40 to –50°C, and

AgOTf was added. The resulting mixture was stirred at a temperature within a range from –25 to –30°C until the disappearance of the starting glycosyl acceptor (TLC monitoring), quenched with pyridine, diluted with chloroform, and filtered through a Celite layer. The filtrate was washed with 1 M sodium thiosulfate and water, the solvent was removed, and the residue was twice coevaporated with toluene.

3-Trifluoroacetamidopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside (VI). 3-Trifluoroacetamidopropanol (**V**) (3.45 g, 20.2 mmol) was glycosylated with thioglycoside (**IV**) (5.40 g, 10.1 mmol) in 30 ml of dichloromethane in the presence of molecular sieves 4Å (6 g), NIS (4.55 g, 20.2 mmol) and TfOH (33 μl, 0.37 mmol) according to procedure A. Column chromatography (8 : 1 toluene–ethyl acetate) resulted in 4.10 g (62%) of mannoside (**VI**) as a syrup; R_f 0.57 (3 : 2 toluene–ethyl acetate); $[\alpha]_D$ = +40.4°; ^{1}H NMR: 2.18 (3 H, c, CH_3CO), 4.85 (1 H, br s, H1), 5.35 (1 H, br s, H2), 3.94 (1 H, dd, $J_{3,2}$ 3.3 Hz, $J_{3,4}$ 8.8 Hz, H3), 3.83 (H4), 3.79 (H5), 3.74 (2 H6), 4.45–4.90 (6 H, a cluster of d, 3 PhCH_2), 7.10–7.42 (15 H, m, arom.). The spectrum also contained the signals of 3-trifluoroacetamidopropyl aglycone: 1.89 (2 H, m, $CH_2CH_2CH_2$), 3.42 and 3.51 (2 H, 2 m, CH_2N), 3.57 and 3.83 (2 H, 2 m, CH_2O);[3] ^{13}C NMR: 21.0 (CH_3CO), 170.4 (CH_3CO), 98.0 (C1), 68.6 (C2), 78.2 (C3), 74.2 (C4), 71.9 (C5), 69.0 (C6). The spectrum also contained the signals of 3-trifluoroacetamidopropyl aglycone: 28.2 ($CH_2CH_2CH_2$), 38.0 (CH_2N), 66.2 (CH_2O).[4] Found, %: C 64.80, H 6.45. Calculated for $C_{34}H_{38}O_7F_3N$, %: C 64.86, H 6.08.

3-Trifluoroacetamidopropyl 3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside (VII). Mannoside (**VI**) (4.10 g, 6.3 mmol) was deacetylated with 1 M MeONa (3 ml) in MeOH (30 ml) according to procedure B. Column chromatography (3 : 1 toluene–ethyl acetate) resulted in 2.40 g (61%) of acceptor (**VII**) as amorphous substance; R_f 0.29 (3 : 1 toluene–ethyl acetate); $[\alpha]_D$ +40.6°; ^{1}H NMR: 3.64 (3 H, m, H5, 2 H6), 3.74 (2 H, m, H3, H4), 3.93 (1 H, br s, H2), 4.42–4.78 (6 H, a cluster of d, 3 PhCH_2), 4.78 (1 H, br s, H1), 7.08–7.32 (15 H, m, arom.).

3-Trifluoroacetamidopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1⟶2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside (VIII). Acceptor (**VII**) (2.40 g, 3.98 mmol) was glycosylated with thioglycoside (**IV**) (3.20 g, 5.97 mmol) in dichloromethane (25 ml) in the presence of molecular sieves 4Å (4.50 g), NIS (2.69 g, 12.0 mmol), and TfOH (180 μl, 1.99 mmol) according to procedure A. Column chromatography (10 : 1 toluene–ethyl acetate) led to 3.50 g (82%) of mannobioside (**VIII**) as a syrup; R_f 0.55 (3 : 1 toluene–ethyl acetate); $[\alpha]_D$ = +20.5°; ^{1}H NMR: 2.17 (3 H, s, CH_3CO), 4.92 (1 H, br s, H1-A), 5.08 (1 H, br s, H1-B), 5.55 (1 H, br s, H2-B), 3.99 (H3-B), 3.80 (H4-B), 4.00 (H5-B), 3.72, 3.78 (2 H6-B), 4.45–4.92 (12 H, a cluster of d, 6 PhCH_2), 7.18–7.40 (30 H, m, arom.); ^{13}C NMR: 21.1 (CH_3CO), 170.0 (CH_3CO), 98.9 (C1-A), 99.6 (C1-B), 68.7 (C2-B), 78.0 (C3-B), 74.3 (C4-B), 71.9 (C5-B), 69.5 (C6-B). Found, %: C 67.68, H 6.19. Calculated for $C_{61}H_{66}O_{13}F_3N$, %: C 67.95, H 6.17.

3-Trifluoroacetamidopropyl 3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1⟶2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside (IX). Dimannoside (**VIII**) (1.50 g, 1.39 mmol) was deacetylated with 1 M MeONa (0.5 ml) in MeOH (5 ml) according to method B. Column chromatography (5 : 1 toluene–ethyl acetate) resulted in 955 mg (66%) of acceptor (**VII**) as a syrup; R_f 0.44 (3 : 1 toluene–ethyl acetate); $[\alpha]_D$ +25.6°; ^{1}H NMR: 4.79 (1 H, br s, H1-A), 4.94 (1 H, br s, H1-B), 4.00 (1 H, br s, H2-B), 3.74 (H3-B), 3.66 (H4-B), 3.83 (H5-B), 3.62 (2 H6-B), 4.36 - 4.74 (12 H, a cluster of d, 6 PhCH_2), 7.05–7.27 (30 H, m, arom.); ^{13}C NMR: 98.7 (C1-B), 101.6 (C1-B), 68.1 (C2-B), 79.3 (C3-B), 74.2 (C4-B), 71.8 (C5-B), 69.4 (C6-B).

3-Trifluoroacetamidopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl- and -β-*D*-mannopyranosyl-(1⟶2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1⟶2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosides (X) and (XI). Glycosylation of acceptor (**XI**) (65 mg, 0.062 mmol) with thioglycoside (**IV**) (57 mg, 0.106 mmol) in dichloromethane (6 ml) in the presence of MS 4Å (50 mg), NIS (48 mg, 0.214 mmol), and TfOH (3.9 μl, 0.044 mmol) was performed according to method A. Column chromatography (10 : 1 toluene–ethyl acetate) resulted in 27 mg (28%) of α-isomer (**X**) and 40 mg (42%) of β-isomer (**XI**). Compound (**X**), a syrup; R_f 0.58 (4 : 1 toluene–ethyl acetate); $[\alpha]_D$ = +32.2°; ^{1}H NMR: 2.15 (3 H, c, CH_3CO), 4.95 (1 H, br s, H1-A), 5.20 (1 H, br s, H1-B), 5.07 (1 H, br s, H1-C), 5.55 (1 H, br s, H2-C), 4.02 (H3-C), 3.93 (H4-C), 3.96 (H5-C), 3.72 and 3.78 (2 H6-C), 4.33–4.89 (18 H, a cluster of d, 9 PhCH_2), 7.15–7.39 (45 H, m, arom.); ^{13}C NMR: 21.0 (CH_3CO), 170.5 (CH_3CO), 99.0 (C1-A), 100.7 (C1-B), 99.3 (C1-C), 68.8 (C2-C), 78.0 (C3-C), 74.2 (C4-C), 72.2 (C5-C), 69.9 (C6-C). Compound (**XI**), a syrup; R_f 0.40 (4 : 1 toluene–ethyl acetate); $[\alpha]_D$ –3.1°; ^{1}H NMR: 2.11 (3 H, c, CH_3CO), 4.99 (1 H, br s, H1-A), 5.15 (1 H, d, $J_{1,2}$ 3.1 Hz, H1-B), 4.75 (1 H, br s, H1-C), 5.53 (1 H, d, $J_{2,3}$ 2.3 Hz, H2-C), 3.44 (1 H, dd, $J_{3,4}$ 9.2 Hz, H3-C), 3.78 (H4-C), 3.33 (1 H, m, H5-C), 3.69 (2 H, H6-C), 4.23–4.90 (18 H, a cluster of d, 9 PhCH_2), 7.13–7.44 (45 H, m, arom.); ^{13}C NMR: 21.0 (CH_3CO), 170.5 (CH_3CO), 99.2 (C1-A), 100.3 (C1-B),

[3] Similar signals with negligible alterations in chemical shifts (±0.1 ppm) were present in the spectra of all protected oligosaccharides described below. Therefore, the signals of 3-trifluoroacetamidopropyl group are not given in the further assignment of ^{1}H NMR spectra.

[4] Similar signals (±0.5 ppm) were present in the spectra of all protected oligosaccharides described below. Therefore, the signals of 3-trifluoroacetamidopropyl group are not given in the further assignment of ^{13}C NMR spectra.

96.1 (C1-C), 68.1 (C2-C), 80.1 (C3-C), 74.4 (C4-C), 75.4 (C5-C), 69.2 (C6-C). Found, %: C 69.37, H 6.35. Calculated for $C_{88}H_{94}O_{18}F_3N$, %: C 69.97, H 6.27.

Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl-1-thio-α-*D*-mannopyranoside (XII). A solution of thioglycoside (**IV**) (200 mg, 0.373 mmol) in MeOH (4 ml) was treated with 1 M MeONa (0.2 ml) according to procedure B. The resulting monohydroxy derivative was dissolved in pyridine (4 ml), and benzoyl chloride (0.13 ml, 1.11 mmol) was added. When benzoylation was over, the reaction mixture was poured in ice-cold water; the product was extracted with chloroform (100 ml). The extract was washed with the saturated sodium hydrocarbonate and water, the solvent was removed, and the residue was twice coevaporated with toluene. Column chromatography of the residue (25 : 1 toluene–ethyl acetate) resulted in 200 mg (90%) of benzoate (**XII**) as a syrup; R_f 0.86 (4 : 1 toluene–ethyl acetate); $[\alpha]_D$ +27.3°; ^{1}H NMR: 1.29 (3 H, t, J 7.5 Hz, CH_2CH_3), 2.65 (2 H, m, CH_2CH_3), 3.75 (1 H, d, $J_{6,6'}$ 10.8 Hz, H6), 3.92 (1 H, dd, $J_{6',5}$ 3.8 Hz, H6'), 4.02 (1 H, dd, $J_{3,2}$ 3.0 Hz, $J_{3,4}$ 9.2 Hz, H3), 4.13 (1 H, t, $J_{4,5}$ 9.7 Hz, H4), 4.49–4.89 (6 H, a cluster of d, 3 PhCH_2), 5.43 (1 H, br s, H1), 5.69 (1 H, br s, H2), 7.19–8.17 (20 H, m, arom.).

3-Trifluoroacetamidopropyl 3,4,6-tri-*O*-benzyl-2-*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 (XIII). Acceptor (**IX**) (1.75 g, 1.69 mmol) was glycosylated with thioglycoside (**XII**) (1.50 g, 2.51 mmol) in dichloromethane (20 ml) in the presence of molecular sieves 4Å (3 g), NIS (1.12 g, 4.98 mmol), and TfOH (60 μl, 0.68 mmol) by procedure A. Column chromatography (15 : 1 toluene–ethyl acetate) resulted in 1.70 g (64%) of trimannoside (**XIII**) as a syrup; R_f 0.43 (6 : 1 toluene–ethyl acetate); $[\alpha]_D$ +16.3°; ^{1}H NMR: 4.98 (1 H, d, $J_{1,2}$ 1.5 Hz, H1-A), 5.27 (1 H, d, $J_{1,2}$ 1.5 Hz, H1-B), 5.16 (1 H, d, $J_{1,2}$ 1.6 Hz, H1-C), 5.80 (1 H, br s, H2-C), 4.15 (H3-C), 4.15 (H4-C), 4.03 (H5-C), 3.76 and 3.82 (2 H6-C), 4.42–4.93 (18 H, a cluster of d, 9 PhCH_2), 7.10–8.17 (50 H, m, arom.); ^{13}C NMR: 99.1 (C1-A), 100.8 (C1-B), 99.5 (C1-C), 69.1 (C2-C), 78.0 (C3-C), 74.4 (C4-C), 72.3 (C5-C), 70.0 (C6-C), 165.5 (Ph*C*O). Found, %: C 71.90, H 6.38. Calculated for $C_{93}H_{96}O_{18}F_3N$, %: C 71.02, H 6.15.

3-Trifluoroacetamidopropyl 3,4,6-tri-*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 (XIV). A solution of trimannoside (**XIII**) (1.65 g, 1.05 mmol) in MeOH (20 ml) was treated with 1 M MeONa (1.0 ml) according to procedure B. Column chromatography (10 : 1 toluene–ethyl acetate) resulted in 950 mg (62%) of acceptor (**XIV**) as white foam; R_f 0.50 (4 : 1 toluene–ethyl acetate); $[\alpha]_D$ = +33.4°; ^{1}H NMR: 4.76 (1 H, d, $J_{1,2}$ 1.4 Hz, H1-A), 5.04 (1 H, d, $J_{1,2}$ 1.2 Hz, H1-B), 4.91 (1 H, d, $J_{1,2}$ 1.5 Hz, H1-C), 3.95 (H2-C), 3.76 (1 H, dd, $J_{3,2}$ 2.9 Hz, $J_{3,4}$ 9.1 Hz, H3-C), 3.53 (H4-C), 3.78 (H5-C), 3.46 (H6-C), 4.31–4.70 (18 H, a cluster of d, 9 PhCH_2), 7.00–7.25 (45 H, m, arom.); ^{13}C NMR: 98.6 (C1-A), 100.6 (C1-B), 101.5 (C1-C), 68.1 (C2-C), 79.0 (C3-C), 74.8 (C4-C), 71.7 (C5-C), 69.1 (C6-C). Found, %: C 69.58, H 6.15. Calculated for $C_{86}H_{92}O_{17}F_3N$, %: C 70.33, H 6.31.

3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*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*-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside (XVI). Acceptor (**XIV**) (157 mg, 0.107 mmol) was glycosylated with bromide (**XV**) (106 mg, 0.161 mmol) in dichloromethane (4 ml) in the presence of molecular sieves 4Å (50 mg) and AgOTf (47 mg, 0.118 mmol) according to procedure C. The product was isolated by column chromatography (10 : 1 toluene–ethyl acetate) to yield 150 mg (68%) of tetrasaccharide (**XVI**) as a syrup; R_f 0.58 (4 : 1 toluene–ethyl acetate); $[\alpha]_D$ +56.2°; ^{1}H NMR: 5.03 (1 H, br s, H1-A), 5.29 (1 H, br s, H1-B), 5.40 (1 H, br s, H1-C), 5.19 (1 H, br s, H1-D), 5.96 (1 H, br s, H2-D), 6.07 (1 H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.0 Hz, H3-D), 6.21 (1 H, t, H4-D), 4.65 (H5-D), 4.32 and 4.57 (2 H6-D), 4.47–5.01 (18 H, a cluster of d, 9 PhCH_2), 7.10–8.16 (65 H, m, arom.); ^{13}C NMR: 99.2 (C1-A), 101.1 (C1-B), 100.5 (C1-C), 99.0 (C1-D), 70.6 (C2-D), 70.2 (C3-D), 66.9 (C4-D), 69.5 (C5-D), 62.5 (C6-D), 165.1, 165.3, 165.5, and 166.1 (4 Ph*C*O). Found, %: C 70.25, H 5.67, N 0.78. Calculated for $C_{120}H_{118}O_{26}F_3N$, %: C 70.40, H 5.81, N 0.68.

Ethyl 4,6-di-*O*-benzyl-2-*O*-benzoyl-1-thio-α-*D*-mannopyranoside (XVIII). Methyl orthobenzoate (3.2 ml, 18.8 mmol) and the catalytic amount of camphorsulfonic acid were added to a solution of diol (**XVII**) (2.4 g, 5.9 mmol) in acetonitrile (15 ml). The mixture was kept for 30 min, then 80% acetic acid was added. After 10 min, the reaction mixture was diluted with chloroform and washed with saturated sodium hydrocarbonate and water. The solvent was removed, and the residue was chromatographed (5 : 1 toluene–ethyl acetate) to give 1.53 g (51%) of (**XVIII**); $[\alpha]_D$ = +46.3°; ^{1}H NMR: 1.26 (3 H, t, J 7.4 Hz, SCH_2CH_3), 2.62 (2 H, m, SCH_2CH_3), 3.73 (1 H, d, $J_{6,6'}$ 9.9 Hz, H6), 3.89 (1 H, dd, $J_{6',5}$ 3.6 Hz, H6'), 4.01 (1 H, t, $J_{4,5}$ 9.5 Hz, H4), 4.14 (1 H, dd, $J_{3,2}$ 2.9 Hz, $J_{3,4}$ 9.4 Hz, H3), 4.17 (1 H, m, H5), 4.49, 4.60, 4.69, and 4.82 (4 H, 4 d, J 11.0–12.0 Hz, 2 PhCH_2), 5.39 (2 H, br s, H1, H2), 7.20–8.04 (15 H, m, arom.). Found, %: C 68.66, H 6.49. Calculated for $C_{29}H_{32}O_6S$, %: C 68.48, H 6.34.

Ethyl 2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-benzoyl-1-thio-α-*D*-mannopyranoside (XIX). Thioglycoside (**XVIII**) (135 mg, 0.266 mmol) was glycosylated with bromide (**XV**) (263 mg, 0.40 mmol) in dichlo-

romethane (3 ml) in the presence of molecular sieves 4Å (300 mg) and AgOTf (102 mg, 0.4 mmol) according to procedure C. The product was isolated by column chromatography (3 : 1 petroleum ether–ethyl acetate) to give 186 mg (64%) of (**XIX**) as white foam; $[\alpha]_D$ –17.5°; ^{1}H NMR: 1.31 (3 H, t, *J* 7.2 Hz, SCH_2CH_3), 2.67 (2 H, m, SCH_2CH_3), 3.79 (1 H, d, $J_{6,6'}$ 11.0 Hz, H6-C), 3.99 (1 H, dd, $J_{6',5}$ 3.2 Hz, H6'-C), 4.29 (1 H, m, H5-C), 4.35 (1 H, t, $J_{4,5}$ 9.2 Hz, H4-C), 4.38 (2 H, m, H3-C, H6-E), 4.50 (1 H, m, H5-E), 4.57, 4.73, 4.77, and 5.02 (4 H, 4 d, *J* 11.5–12.0 Hz, 2 $PhCH_2$), 4.60 (1 H, dd, H6'-E), 5.47 (1 H, br s, H1-E), 5.52 (1 H, br s, H1-C), 5.70 (1 H, br s, H2-C), 5.80 (1 H, br s, H2-E), 5.82 (1 H, dd, $J_{3,2}$ 2.8 Hz, $J_{3,4}$ 10.1 Hz, H3-E), 6.09 (1 H, t, $J_{4,5}$ 9.8 Hz, H4-E), 7.17–8.25 (35 H, m, arom.); ^{13}C NMR: 14.9 (SCH_2CH_3), 25.7 (SCH_2CH_3), 82.3 (C1-C), 74.2 (C2-C), 79.2 (C3-C), 75.0 (C4-C), 72.1 (C5-C), 68.8 (C6-C), 99.7 (C1-E), 70.2 (C2-E), 70.3 (C3-E), 66.3 (C4-E), 69.6 (C5-E), 62.7 (C6-E), 165.1, 165.4, 166.0, and 166.1 (5 PhCO). Found, %: C 69.38, H 5.59. Calculated for $C_{63}H_{58}O_{15}S$, %: C 69.60, H 5.38.

3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1⟶3)-4,6-di-*O*-benzyl-2-*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 (XX). Acceptor (**IX**) (84 mg, 0.081 mmol) was glycosylated with thioglycoside (**XIX**) (106 mg, 0.098 mmol) in dichloromethane (3 ml) in the presence of molecular sieves 4Å (350 mg), NIS (44 mg, 0.0196 mmol), and TfOH (2.9 μl, 0.032 mmol) by procedure A. The product was isolated by column chromatography (5 : 1 toluene–ethyl acetate) to yield 109 mg (65%) of (**XX**); $[\alpha]_D$ +0.45°; ^{1}H NMR: 4.93 (1 H, br s, H1-A), 5.24 (1 H, br s, H1-B), 5.18 (1 H, br s, H1-C), 5.72 (1 H, br s, H2-C), 4.49 (H3-C), 4.35 (1 H, t, *J* 9.6 Hz, H4-C), 4.15 (H5-C), 3.62 (H6-C), 3.83 (1 H, dd, $J_{6,6'}$ 11.0 Hz, $J_{6,5}$ 3.2 Hz, H6'-C), 5.42 (1 H, br s, H1-E), 4.41–5.04 (16 H, a cluster of d, 8 $PhCH_2$), 7.00–8.25 (65 H, m, arom.); ^{13}C NMR: 99.0 (C1-A), 100.8 (C1-B), 99.8 (C1-E), 99.3 (C1-C), 72.3 (C2-C), 78.9 (C3-C), 74.5 (C4-C), 72.2 (C5-C), 68.9 (C6-C), 165.1, 165.6, and 165.8 (5 PhCO). Found, %: C 70.34, H 5.83, N 0.66. Calculated for $C_{120}H_{116}O_{27}F_3N$, %: C 69.93, H 5.67, N 0.68.

Ethyl 4,6-di-*O*-benzyl-2,3-di-*O*-benzoyl-1-thio-α-*D*-mannopyranoside (XXI). Benzoyl chloride (0.61 ml, 5.2 mmol) was added to a solution of diol (**XVII**) (352 mg, 0.87 mmol) in pyridine (4 ml). The reaction mixture was stirred for 50 min at room temperature, diluted with chloroform, and washed with a saturated sodium hydrocarbonate and water. The solvent was removed; the residue was twice coevaporated with toluene. The product was isolated by column chromatography (10 : 1 toluene–ethyl acetate) to yield 458 mg (86%) of dibenzoate (**XXI**); $[\alpha]_D$ –16.6°; ^{1}H NMR: 1.34 (3 H, t, *J* 7.3 Hz, CH_2CH_3), 2.70 (2 H, m, CH_2CH_3), 3.80 (1 H, d, $J_{6,6'}$ 9.9 Hz, H6), 4.01 (1 H, dd, $J_{6',5}$ 9.9 Hz, H6'), 4.38 (1 H, m, H5), 4.43 (1 H, t, $J_{4,5}$ 9.7 Hz, H4), 4.57 (2 H, d, *J* 11.8 Hz, $PhCH_2$), 4.68 (1 H, d, *J* 10.9 Hz, $PhCH_2$), 4.80 (1 H, d, *J* 11.9 Hz, $PhCH_2$), 5.51 (1 H, br s, H1), 5.70 (1 H, dd, $J_{3,2}$ 3.1 Hz, $J_{3,4}$ 8.5 Hz, H3), 5.74 (1 H, br s, H2), 7.07–8.22 (20 H, m, arom.).

3-Trifluoroacetamidopropyl 4,6-di-*O*-benzyl-2,3-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 (XXII). Glycosylation of acceptor (**IX**) (122 mg, 0.118 mmol) with thioglycoside (**XXI**) (108 mg, 0.176 mmol) was carried out in dichloromethane (3 ml) in the presence of molecular sieves 4Å (250 mg), NIS (79 mg, 0.35 mmol), and TfOH (3 μl, 0.035 mmol) according to procedure A. Column chromatography (5 : 1 toluene–ethyl acetate) yielded 177 mg (95%) of (**XXII**); $[\alpha]_D$ +8.5°; ^{1}H NMR: 4.96 (1 H, d, $J_{1,2}$ 1.7 Hz, H1-A), 5.23 (1 H, d, $J_{1,2}$ 1.6 Hz, H1-B), 5.17 (1 H, d, $J_{1,2}$ 1.7 Hz, H1-C), 5.82 (1 H, dd, $J_{2,3}$ 3.2 Hz, H2-C), 5.84 (1 H, dd, $J_{3,4}$ 9.2 Hz, H3-C), 4.35 (1 H, t, H4-C), 4.10 (H5-C), 3.58 (1 H, dd, $J_{6,5}$ 1.4 Hz, $J_{6,6'}$ 11.0 Hz, H6-C), 3.78 (1 H, dd, $J_{6',5}$ 3.2 Hz, H6'-C), 4.38–4.89 (16 H, a cluster of d, 8 $PhCH_2$), 7.07–8.10 (50 H, m, arom.); ^{13}C NMR: 99.0 (C1-A), 100.7 (C2-B), 99.4 (C1-C), 70.8 (C2-C), 72.5 (C3-C), 73.2 (C4-C), 72.1 (C5-C), 68.8 (C6-C). Found,%: C 69.82, H 6.32, N 0.88. Calculated for $C_{93}H_{94}O_{19}F_3N$, %: C 70.40, H 5.97, N 0.88.

3-Trifluoroacetamidopropyl 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 (XXIII). Compound (**XXII**) (177 mg, 0.112 mmol) was debenzoylated by treatment with 1 M MeONa (0.2 ml) in MeOH (2 ml) according to procedure B. Column chromatography (3 : 1 toluene–ethyl acetate) resulted in 100 mg (61%) of diol (**XXIII**); $[\alpha]_D$ +34.7°; ^{1}H NMR: 4.85 (1 H, d, $J_{1,2}$ 1.6 Hz, H1-A), 5.12 (1 H, d, $J_{1,2}$ 1.5 Hz, H1-B), 5.09 (1 H, br s, H1-C), 3.90 (H2-C), 3.65 (H3-C), 4.24, 4.80 (16 H, a cluster of d, 8 $PhCH_2$), 7.11–7.29 (40 H, m, arom.); ^{13}C NMR: 98.7 (C1-A), 100.7 (C1-B), 101.7 (C1-C). Found, %: C 69.28, H 6.91, N 0.83. Calculated for $C_{79}H_{86}O_{17}F_3N$, %: C 68.83, H 6.29, N 1.02.

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⟶2)]-4,6-di-*O*-benzyl-α-*D*-mannopyranosyl-(1⟶2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1⟶2)-3,4,6-*O*-benzyl-α-*D*-mannopyranoside (XXIV). Diol (**XXIII**) (85 mg, 0.058 mmol) was glycosylated with bromide (**XV**) (114 mg, 0.174 mmol) in dichloromethane (4 ml) in the presence of molecular sieves 4Å (350 mg) and AgOTf (53 mg, 0.21 mmol) according to procedure B.

The product was isolated by column chromatography (10 : 1 toluene–ethyl acetate) to yield 126 mg (85%) of pentasaccaride (**XXIV**); $[\alpha]_D$ +5.4°; ^{1}H NMR: 4.96 (1 H, br s, H1-A), 5.18 (1 H, br s, H1-B), 5.61 (1 H, br s, H1-C), 5.07 (1 H, br s, H1-D), 5.88 (1 H, br s, H2-D), 6.12 (1 H, dd, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 10.3 Hz, H3-D), 6.31 (1 H, t, $J_{4,5}$ 10.3 Hz, H4-D), 4.97 (H5-D), 4.42 and 4.70 (2 H6-D), 5.47 (1 H, br s, H1-E), 6.07 (1 H, br s, H2-E), 6.26 (1 H, dd, $J_{2,3}$ 2.3 Hz, $J_{3,4}$ 10.4 Hz, H3-E), 6.40 (1 H, t, $J_{4,5}$ 10.0 Hz, H4-E), 4.80 (H5-E), 4.75 (2 H6-E), 4.37–4.93 (16 H, a cluster of d, 8 PhCH_2), 6.80–8.20 (80 H, m, arom.); ^{13}C NMR: 99.0 (C1-A), 100.8 (C1-B), 99.4 (C1-C), 98.2 (C1-D), 70.8 (C2-D), 70.4 (C3-D), 66.4 (C4-D), 69.4 (C5-D), 62.7 (C6-D), 100.6 (C1-E), 70.7 (C2-E), 70.9 (C3-E), 66.3 (C4-E), 69.9 (C5-E), 62.6 (C6-E). Found, %: C 69.50, H 5.52, N 0.55. Calculated for $C_{147}H_{138}O_{35}F_3N$, %: C 69.63; H 5.49; N 0.55.

A general procedure for removal of protective groups (procedure D). Palladium hydroxide on carbon (20%, Aldrich) in the amount equal to the mass of oligomannoside to be deprotected was added to the solution of the protected oligosaccharide in methanol. A mixture was stirred for 16 h in the atmosphere of hydrogen at room temperature and then filtered through a Celite layer, the catalyst was carefully washed with methanol, and the combined filtrates were concentrated. The residue was dissolved in water and treated with anion exchange resin Amberlyst A-26 (OH^-) (Fluka) for 16 h. Then the resin was filtered off, and the filtrate was concentrated. Free oligosaccharides (**I**)–(**III**) were isolated by gel chromatography on a TSK HW-40 (S) column in the form of acetates and were lyophilized from water.

3-Aminopropyl α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranoside (I) was obtained from the protected tetrasaccharide (**XVI**) according to procedure D in 87% yield; $[\alpha]_D$ +74.3°; ^{1}H NMR: 5.30 and 5.27 (2 H, 2 c, H1-B, H1-C), 5.09 (1 H, c, H1-A), 5.04 (1 H, c, H1-D), 4.10 and 4.09 (2 H, 2 br s, H2-B, H2-C), 4.07 (1 H, 2 br s, H2-D), 3.95 (H2-A), 3.94 (H3-B, H3-C), 3.89 (H3-C), 3.84 (H3-D), 3.62–3.70 (H4-A, H4-B, H4-C, H4-D), 3.73–3.79 (H5-B, H5-C, H5-D), 3.61 (H5-A), 3.72–3.79 (H6-A, H6-B, H6-C, H6-D), 3.86–3.92 (H6'-A, H6'-B, H6'-C, H6'-D), 3.85 and 3.59 (CH_2O), 3.12 (2 H, m, CH_2N), 1.97 (2 H, m, $CH_2CH_2CH_2$); ^{13}C NMR: 103.4 (C1-D), 101.8 (×2) (C1-B, C1-C), 99.4 (C1-A), 80.1, 79.9, and 79.7 (C2-A, C2-B, C2-C), 74.5 and 74.4 (×2), 74.1 (C5-A, C5-B, C5-C, C5-D), 71.5 (C3-D), 71.4 and 71.2 (×3) (C2-D, C3-A, C3-B, C3-C), 68.4, 68.3, 68.2, and 68.0 (C4-A, C4-B, C4-C, C4-D), 62.2, 62.3, and 62.4 (×2) (C6-A, C6-B, C6-C, C6-D), 66.2 (CH_2O), 38.7 (CH_2N), 27.8 ($CH_2CH_2CH_2$).

3-Aminopropyl α-*D*-mannopyranosyl-(1⟶3)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranoside (II). Derivative (**XX**) was deprotected according to procedure D to give free tetrasaccharide (**II**) in 90% yield; $[\alpha]_D$ = +57.2°; ^{1}H NMR: 5.26 (1 H, s, H1-B), 5.12 (1 H, s, H1-E), 5.07 (1 H, s, H1-A), 5.01 (1 H, s, H1-C), 4.20 (1 H, br s, H2-C), 4.09 (1 H, br s, H2-B), 4.05 (1 H, br s, H2-E), 3.93 (H2-A), 3.92 (H3-B, H3-C), 3.87 (H3-A), 3.86 (H3-E), 3.73 (H4-C), 3.66 (H4-A), 3.64 (H4-B), 3.62 (H4-E), 3.72–3.79 (H5-B, H5-C, H5-E), 3.58 (H5-A), 3.69–3.77 (H6-A, H6-B, H6-C, H6-E), 3.85–3.92 (H6'-A, H6'-B, H6'-C, H6'-E), 3.82 and 3.57 (CH_2O), 3.10 (2 H, m, CH_2N), 1.97 (2 H, m, $CH_2CH_2CH_2$); ^{13}C NMR: 103.3 (×2) (C1-C, C1-E), 101.9 (C1-B), 99.4 (C1-A), 80.0 (C2-A), 79.7 (C2-B), 79.1 (C3-C), 74.5 (×3) (C5-B, C5-C, C5-E), 74.1 (C5-A), 71.5, 71.3, and 71.2 (×2) (C2-E, C3-A, C3-B, C3-E), 70.8 (C2-C), 68.3, 68.1 (×2) (C4-A, C4-B, C4-E), 67.4 (C4-C), 62.4, 62.3, and 62.2 (×2) (C6-A, C6-B, C6-C, C6-E), 66.2 (CH_2O), 38.6 (CH_2N), 27.8 ($CH_2CH_2CH_2$).

3-Aminopropyl α-*D*-mannopyranosyl-(1⟶2)-[α-*D*-mannopyranosyl-(1⟶3)]-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranoside (III) was obtained from the protected pentasaccaride (**XXIV**) by procedure D; yield 85%; $[\alpha]_D$ +58.8°; ^{1}H NMR: 5.25 (1 H, s, H1-C), 5.23 (1 H, s, H1-B), 5.15 (1 H, s, H1-E), 5.10 (1 H, s, H1-D), 5.06 (1 H, s, H1-A), 4.22 (1 H, br s, H2-C), 4.07 (1 H, br s, H2-B), 4.03 (1 H, br s, H2-E), 3.98 (1 H, br s, H2-D), 3.92 (H2-A), 4.02 (H3-C), 3.90 (H3-B), 3.85 (H3-A), 3.82 (H3-D), 3.73 (H3-E), 3.77 (H4-C), 3.60–3.67 (H4-A, H4-B, H4-D, H4-E), 3.71–3.77 (H5-B, H5-C, H5-D), 3.65 (H5-E), 3.56 (H5-A), 3.68–3.76 (H6-A, H6-B, H6-C, H6-D, H6-E), 3.82–3.89 (H6'-A, H6'-B, H6'-C, H6'-D, H6'-E), 3.82 and 3.56 (CH_2O), 3.09 (2 H, m, CH_2N), 1.95 (2 H, m, $CH_2CH_2CH_2$); ^{13}C NMR: 103.3 (C1-E), 102.8 (C1-D), 101.8 (C1-B), 101.7 (C1-C), 99.3 (C1-A), 80.1 (C2-A), 79.8 (C2-B), 78.5 (C2-C), 78.3 (C3-C), 74.8, 74.6, 74.5, 74.4 (C5-B, C5-C, C5-D, C5-E), 74.0 (C5-A), 71.7, 71.5, 71.3 (×2), and 71.1 (×2) (C2-D, C2-E, C3-A, C3-B, C3-D, C3-E), 68.3, 68.1, and 67.9 (×3) (C4-A, C4-B, C4-C, C4-D, C4-E), 62.4 and 62.2 (×2), and 62.1 (×2) (C6-A, C6-B, C6-C, C6-D, C6-E), 66.2 (CH_2O), 38.6 (CH_2N), 27.8 ($CH_2CH_2CH_2$).

3-Aminopropyl α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranoside (XXV)[bold] was obtained from disaccharide (**VIII**) by procedure D; yield 95%; $[\alpha]_D$ = +53°; ^{1}H NMR: 5.10 (1 H, s, H1-A), 5.02 (1 H, s, H1-B), 4.07 (1 H, br s, H2-B), 3.98 (1 H, br s, H2-A), 3.90 (H3-A), 3.84 (H3-B), 3.68 (H4-A), 3.61 (H4-B), 3.77 (H5-B), 3.62 (H5-A), 3.70–3.79 (H6-A, H6-B), 3.88–3.92 (H6'-A, H6'-A), 3.61, 3.85 (CH_2O), 3.13 (2 H, m, CH_2N), 1.99 (2 H, m, $CH_2CH_2CH_2$); ^{13}C NMR: 103.5 (C1-B), 99.4 (C1-A), 79.9 (C2-A), 74.5 (C5-B),

74.1 (C5-A), 71.5 (C3-B), 71.4 (C3-A), 71.1 (C2-B), 68.8 (×2) (C4-A, C4-B), 62.2 and 62.4 (H6-A, H6-B), 66.2 (CH_2O), 38.7 (CH_2N), 27.8 ($CH_2CH_2CH_2$).

3-(3,4-Dioxo-2-ethoxycyclobut-1-enylamino)propyl α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranoside (XXVII). Diethyl squarate (**XXVI**) (4 μl, 0.027 mmol) and triethylamine (3 μl) were added to a solution of disaccharide (**XXV**) (10 mg, 0.022 mmol) in 50% aqueous ethanol (0.8 ml); the mixture was kept for 16 h at room temperature and then concentrated in a vacuum. 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 aqueous methanol in 2-ml portions, increasing the concentration of methanol from 5 to 20%. Concentration of the eluate and the subsequent lyophilization resulted in 13 mg (90%) of monoamide (**XXVII**); ^{1}H NMR: 1.43 (3 H, m, OCH_2CH_3), 1.92 (2 H, m, $OCH_2CH_2CH_2N$), 3.60, 3.72 (2 H, 2 m, $OCH_2CH_2CH_2N$), 3.56, 3.81 (2 H, 2 m, $OCH_2CH_2CH_2N$), 4.70 and 4.74 (2 H, 2 q, *J* 7.2 Hz, OCH_2CH_3), 5.04 and 5.06 (1 H, 2 s, H1-A). The carbohydrate part of the spectrum was practically the same as that in the spectrum of the starting 3-aminopropyl glycoside (**XXV**), except for the signal of H1-A; ^{13}C NMR: 16.0 and 16.3 (1 C, OCH_2CH_3), 30.3 and 30.4 (1 C, $OCH_2CH_2CH_2N$), 43.1 and 43.2 (1 C, $OCH_2CH_2CH_2N$), 66.1 and 66.2 (1 C, $OCH_2CH_2CH_2N$), 71.9 (1 C, OCH_2CH_3), 174.7 (cyclobutenedione); the carbohydrate part of the spectrum was practically the same as that in the spectrum of the starting 3-aminopropyl glycoside (**XXV**).

BSA–disaccharide conjugate (XXVIII). A solution of monoamide (**XXVII**) (4 mg, 7.6 μmol) and BSA (25 mg, 0.37 μmol) in 6 ml of the buffer solution (250 ml of water, 8.8 g of $KHCO_3$, 6.7 g of $Na_2B_4O_7 \cdot 10H_2O$, pH 9) was kept for 3 days at room temperature. The conjugate was isolated by gel chromatography on a Sephadex G-15 column in water and lyophilized from water to give 17 mg (59%) of conjugate (**XXVIII**). For MALDI TOF mass spectrum see Fig. 2.

3-(3,4-Dioxo-2-ethyxycyclobut-1-enylamino)propyl α-*D*-mannopyranosyl-(1⟶2)-[α-*D*-mannopyranosyl-(1⟶3)]-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranosyl-(1⟶2)-α-*D*-mannopyranoside (XXIX)[bold]. Diethyl squarate (**XXVI**) (4 μl, 0.027 mmol) was added to a solution of pentasaccaride (**III**) (9.8 mg, 0.011 mmol) in 50% aqueous ethanol (1 ml). The resulting mixture was kept for 16 h at room temperature. Then triethylamine (3 μl) was added; after 5 h the solvents were removed. The residue was dissolved in 2 ml of water and applied onto a Sep-Pak C-18 cartridge. The cartridge was washed with water (10 ml); the product was eluted with a gradient of methanol (5% ⟶ 20%) in water. The eluate was concentrated; the residue was lyophilized from water to give 9.3 mg (84%) of adduct (**XXIX**), $[\alpha]_D$ +45.0°. ^{1}H NMR: 1.44 (3 H, m, OCH_2CH_3), 1.92 (2 H, m, $OCH_2CH_2CH_2N$), 3.52 and 3.73 (2 H, 2 m, $OCH_2CH_2CH_2N$), 3.57 and 3.81 (2 H, 2 m, $OCH_2CH_2CH_2N$), 4.71 and 4.75 (2 H, 2 q, *J* 7.0 Hz, OCH_2CH_3), 5.02 and 5.04 (1 H, 2 s, H1-A); the carbohydrate part of the spectrum was practically the same as that in the spectrum of the starting 3-aminopropyl glycoside (**III**), except for signal H1-A; ^{13}C NMR: 16.4 and 16.5 (1 C, OCH_2CH_3), 30.5 and 30.7 (1 C, $OCH_2CH_2CH_2N$), 43.0 and 43.2 (1 C, $OCH_2CH_2CH_2N$), 66.2 and 66.3 (1 C, OCH (1 C, $OCH_2CH_2CH_2N$), 71.9 and 72.0 (1 C, OCH_2CH_3), 99.5 and 99.6 (1 C, C1-A), 174.7, 178.3, 184.5, and 190.3 (cyclobutenedione); the carbohydrate part of the spectrum was practically the same as that in the spectrum of the starting 3-aminopropyl glycoside (**III**), except for signal C1-A.

BSA–pentasaccaride conjugate (XXX). A solution of derivative (**XXIX**) (4.5 mg, 4.5 μmol) and BSA (15.2 mg) in 3 ml of the buffer solution (pH 9) was kept for 2 days at room temperature. The conjugate was isolated by gel chromatography on a Sephadex G-15 column in water and lyophilized to give 9 mg (46%) of conjugate (**XXX**). For MALDI TOF mass spectrum see Fig. 2.

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