A new synthesis of the 3,6-branched hexasaccharide fragment of the cell wall mannan in *Candida albicans*, corresponding to the antigenic factor 4*

*D. A. Argunov,[a] A. A. Karelin,[b] A. A. Grachev,[b] Yu. E. Tsvetkov,[b] and N. E. Nifantiev[b]**

[a] *Higher Chemical College of the Russian Academy of Sciences, 9 Miusskaya pl., 125047 Moscow, Russian Federation*
[b] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (499) 135 8784. E-mail: nen@ioc.ac.ru*

In the context of the project dealing with the synthesis and immunological study of the immunodominant fragments of the cell wall mannan in *Candida albicans*, the hexamannoside fragment corresponding to the antigenic factor 4 was assembled using the scheme [3+2+1]. The yield of the target hexasaccharide with the (α1→3)-glycosidic bond was higher than that obtained according to the previously used assembly scheme [2+2+2].

Key words: *Candida albicans*, mannan, oligomannosides, synthesis.

The present work is part of the project aimed at the synthesis and study of immunological properties of oligosaccharides corresponding to the mannan fragments of the cell wall of the *Candida* fungi. Dimorphic yeastlike fungi *Candida albicans* is part of the normal microflora of skin, mucous membranes, and the gastrointestinal tract. However, immunocompromised people may be vulnerable to superficial and deep candidiases[1]. The *Candida* cell wall is the first to interact with a host organism and is responsible for the antigen response, adhesion, and intercellular interactions.[2]

The cell wall mannan[3] is a (α1→6)-linked chain with side oligomannoside branches attached to its separate mannose residues through (α1→2)-bonds. These branches in turn may contain (α1→2)-, (α1→3)-, (α1→6)-, and (β1→2)-linked mannose residues. It is the side chains that are responsible for the antigen specificity of *Candida*. Earlier, it has been demonstrated that the carriers of the antigenic factor 4 are 3,6-branched structures (for example, structures I and II).[4]

In this communication, we describe a new approach to the synthesis of 3,6-branched hexamannoside 3-aminopropylglycoside **1**. The presence of an amino group in the aglycone allows subsequent covalent bonding to high-molecular-weight carriers and tags of various types.

Earlier,[5] we have obtained hexamannoside **1** by successively joining disaccharide units **4**, **5**, and **2** according to the scheme [2+2+2] (Scheme 1, pathway *a*). However, glycosylation of tetrasaccharide **3** with disaccharide **2** in the final step of the assembly of the carbohydrate chain is accompanied by the formation of a great amount of a by-product containing the (β1→3)-bond (α : β = 2 : 1), which

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—
6
↑
1
α-D-Man*p*
I

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

* Dedicated to Academician of the Russian Academy of Sciences V. N. Charushin on the occasion of his 60th birthday.

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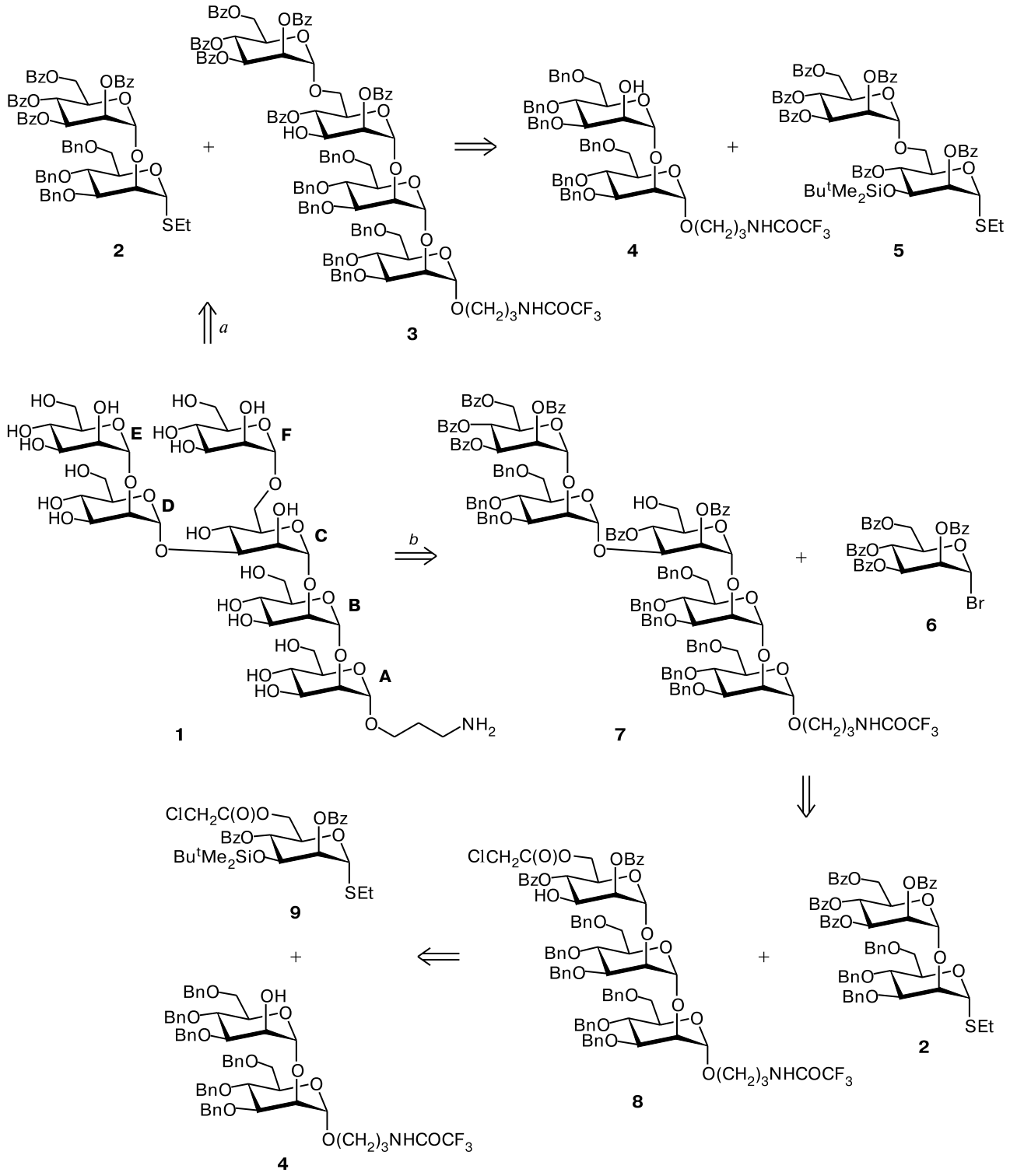
Scheme 1



substantially lowers the yield of the target α-linked product. This reaction outcome, as well as a reported[6,7] example of the formation of the (β1→3)-bond under nearly the same conditions, suggests that the low stereoselectivity in the (1→3)-glycosylation step can be due not only to the absence of the participating acyl group at the O(2) atom

from donor **2** but also the presence of the 6-*O*-mannoside unit in the tetrasaccharide **3** under glycosylation.[5] In connection with this, we proposed an alternative scheme (Scheme 1, pathway *b*) involving tri-, di-, and monosaccharide units **8**, **2**, and **6**, respectively, with (1→6)-glycosylation in the final step of the assembly of the target hexasaccharide (synthetic scheme [3+2+1]).

Since the final product contains different glycosyl substituents at the O(3) and O(6) atoms in the branch point, we should differentiate between these positions in the corresponding monosaccharide precursor. Thiomannoside **10** we have used earlier[5] (Scheme 2) meets this requirement; however, the trityl group is labile under glycosylation conditions. That is why it was replaced by the chloroacetyl group, which remains intact during glycosylation and can be selectively removed later in the presence of the benzoyl groups. The presence of the 2-*O*-benzoyl group in donor **10** ensures the high α-selectivity of the glycosylation (no β-isomer is usually formed at all).

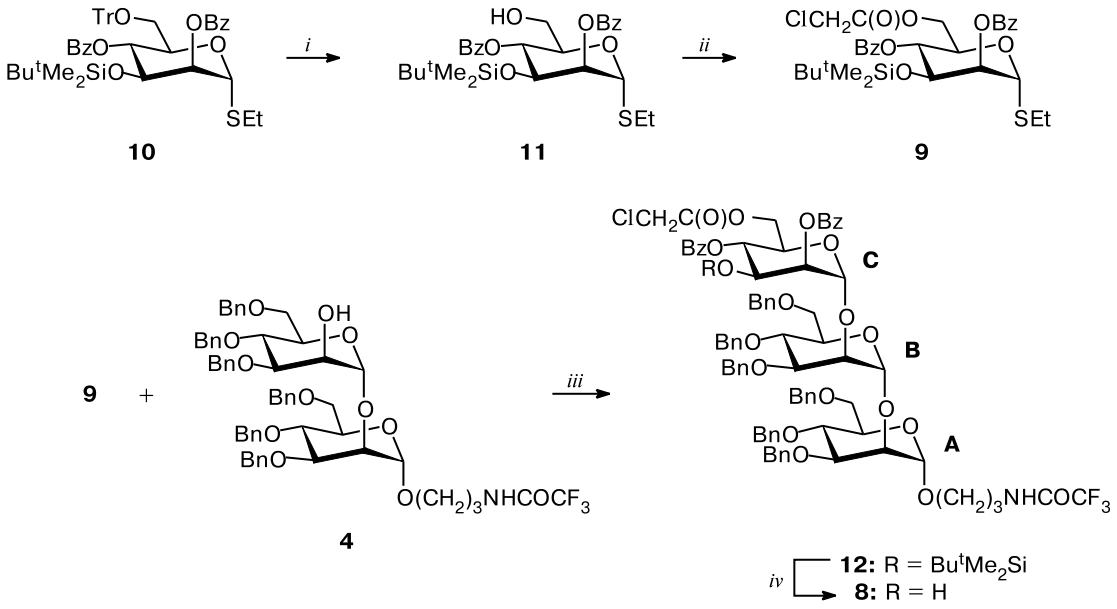
Removal of the trityl protecting group from thioglycoside **10** also having the *tert*-butyl(dimethyl)silyl protection was carried out under the action of iron chloride as described[8] for a similar compound containing the allyloxy group instead of SEt at the anomeric carbon. However, the yield was low (<50%). Fortunately, we found that the trityl group can be removed in 1.5 h under the action of trifluoroacetic acid in chloroform, the *tert*-butyl(dimethyl)silyl protection persisting for several days. This enabled selective removal of the trityl group with the silyl protection remaining virtually intact. Then, the OH group at the C(6) atom in thiomannoside **11** was protected with the chloroacetyl group to give the desired donor **9**.

Glycosylation of disaccharide acceptor **4** (see Ref. 9) with donor **9** proceeded smoothly to give a trisaccharide in high yield (96%). The latter was deprived of its silyl protection under the action of 90% trifluoroacetic acid, being thereby transformed into trisaccharide mannosyl acceptor **8** (see Scheme 2).

The key step of the synthesis of the target hexamannoside **1** (Scheme 3) is glycosylation with donor **2**. Although no sugar substituent is present at the O(6) atom in unit **C**, the glycosylation gave a mixture of α- and β-linked pentasaccharides **13**. However, the α-stereoselectivity of the reaction was appreciably higher (α : β ≈ 6 : 1, NMR data on the intensities of the signals for the anomeric protons showing no overlap with other signals) than that of the earlier described[5] glycosylation of tetrasaccharide **3** (α : β = = 2 : 1). We failed to separate the anomeric pentasaccharides even by HPLC, either in the completely protected (**13**) or dechloroacetylated form (**14**). Nevertheless, because we have shown previously[5] that an anomeric mixture of hexamannosides can be efficiently separated, we used this mixture in the next steps of the synthetic sequence and isolated the target α-linked product after the final glycosylation.

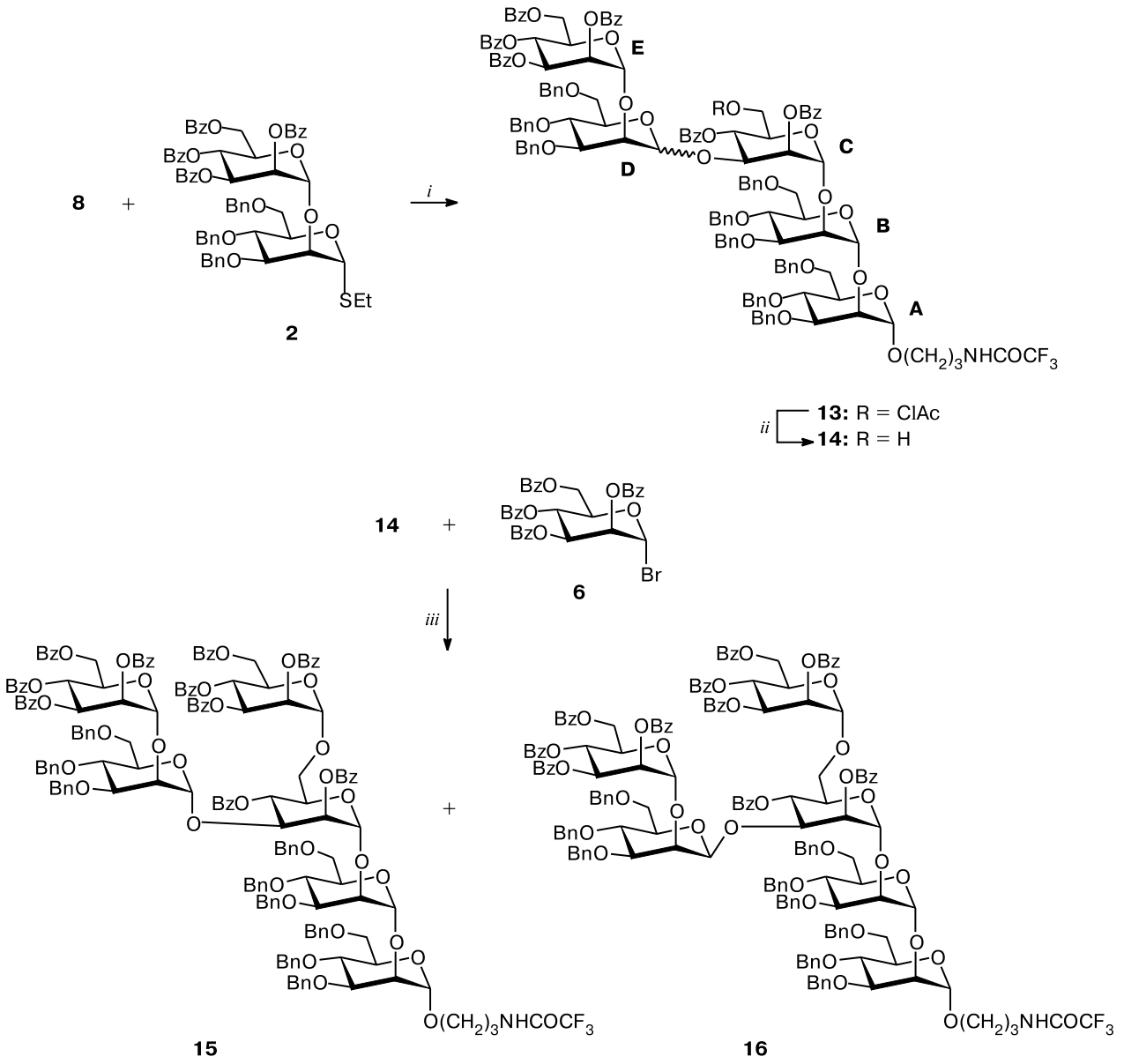
Signal assignment in the NMR spectrum is strongly complicated by the presence of similarly protected benzylated monosaccharide residues and a noticeable amount of

Scheme 2



Reagents, conditions, and yields: *i.* TFA (90% aqueous), $CHCl_3$, 70%; *ii.* Chloroacetyl chloride, pyridine, 95%; *iii.* NIS, TfOH, CH_2Cl_2, −25—−15 °C, 96%; *iv.* TFA (90% aqueous), 76%.

Scheme 3



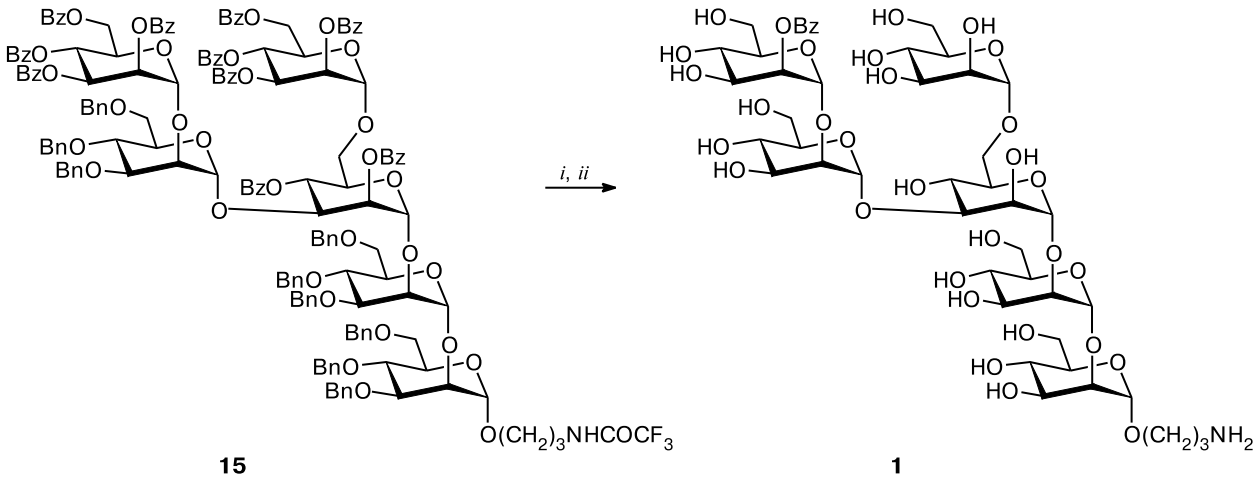
Reagents, conditions, and yields: *i*. NIS, TfOH, CH_2Cl_2, −25—−15 °C, 70%; *ii*. $NH_2C(S)NH_2$, collidine, MeOH, reflux, 88%; *iii*. AgOTf, CH_2Cl_2, −20—−10 °C, 72% for **15** and 10% for **16**.

the β-(1→3)-isomer. That is why the assignment of the signals for the pentasaccharides is incomplete. Because the vicinal coupling constants $J_{1,2}$ in the ^1H NMR spectrum and the chemical shifts of the C(1) atom in the ^{13}C NMR spectrum are not indicative of the configuration of the glycosidic bond in mannosides, the (1→3) bond configuration in compound **13** was determined from the heteronuclear coupling constants $J_{C(1),H(1)}$. All the five constants of the major isomer are in a range of 172—175 Hz; *i.e.*, all glycosidic bonds in the major product have α-configuration (for β-glycosidic bonds, the constants $J_{C(1),H(1)}$ do not exceed 160 Hz).[10]

Removal of the chloroacetyl protection by reflux with thiourea gave a mixture of two isomeric pentasaccharides **14**, which was glycosylated with benzobromomannose **6**. The resulting two hexasaccharides **15** and **16** were separated by HPLC; their yields were 72 and 10%, respectively.

The fully protected hexasaccharide **15** containing only α-linked mannose residues were debenzylated by hydrogenolysis and then the *O*-benzoyl and *N*-trifluoroacetyl

Scheme 4



15 **1**

Reagents and conditions: *i*. H_2, Pd/C, MeOH; *ii*. 1) MeONa, MeOH, 2) NaOH, H_2O, MeOH.

groups were removed by successive treatment with MeONa in methanol and aqueous NaOH (Scheme 4). The target hexasaccharide **1** was obtained in 85% yield and is completely identical with an authentic sample.[5]

As expected, the scheme [3+2+1] for the synthesis of hexamannoside **1** is superior to the previously proposed scheme [2+2+2] because of the substantially higher stereoselectivity of the (1→3)-glycosylation.

Experimental

Solvents were purified according to standard procedures; TfOH, NIS (Acros), AgOTf (Merck), TBDMSCl (Fluka), collidine (Fluka), chloroacetyl chloride (Acros), and thiourea (Reakhim) were used without further purification. Thin-layer chromatography was carried out on Kieselgel 60 F254 plates (Merck); spots were visualized by spraying the plates with a solution of orcinol (180 mg) in a mixture of water (85 mL), H_3PO_4 (10 mL), and ethanol (5 mL) followed by heating at ~150 °C. Column chromatography was carried out on Silica gel 60 (40—63 μm, Merck). For HPLC, a Knauer G27-185 column packed with Silica gel 60 (5 μm) was used. Gel chromatography was carried out on a column (1.5×90 cm) filled with gel TSK HW-40(S) in 0.1 *M* acetic acid; the eluates were analyzed with a Knauer 88 00 flow refractometer.

NMR spectra were recorded at 25 °C on Bruker DRX-500 and Bruker Avance 600 instruments in $CDCl_3$ (δ_C 77.0) for protected sugar derivatives. The residual nondeuterated chloroform (δ_H 7.27) and $CDCl_3$ (δ_C 77.0) were used as the internal standards for ^1H and ^{13}C NMR spectra, respectively. The spectra of free oligosaccharides were recorded in D_2O with acetone (δ_H 2.225, δ_C 31.45) as the internal standard. The signals were assigned by 2D correlation spectroscopic techniques (COSY, TOCSY, ROESY, and HSQC). Elemental analysis was performed at the Laboratory of Analytical Chemistry of the N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences.

High-resolution mass spectra were measured on a Bruker micrOTOF II instrument (ESI) in the positive (capillary voltage 4500 V) or negative ion mode (capillary voltage 3200 V) for the scan range m/z 50—3000 D; external or internal calibration was performed with an Electrospray Calibrant Solution (Fluka). Samples were dissolved in acetonitrile and infused through syringes (flow rate 3 μL min^{-1}, nitrogen as a spraying gas (4 L min^{-1}), interface temperature 180 °C).

Glycosylation was carried out in dehydrated solvents under dry argon. Before the reaction, molecular sieves MS 4 Å were activated in an oil pump vacuum at 180 °C for 2 h.

Ethyl 2,4-di-*O*-benzoyl-3-*O*-[*tert*-butyl(dimethyl)silyl]-1-thio-α-D-mannopyranoside (11). A 90% aqueous solution of trifluoroacetic acid (0.7 mL) was added to a stirred solution of monosaccharide **10** (see Ref. 5) (385 mg, 0.49 mmol) in chloroform (10 mL). The reaction mixture was stirred for 1.5 h, diluted with chloroform, washed with a saturated solution of $NaHCO_3$, and concentrated. Compound **11** was isolated by column chromatography with light petroleum—ethyl acetate as an eluent. The yield was 190 mg (70%), transparent syrup. ^1H NMR (500 MHz, $CDCl_3$), δ: 8.11—8.04, 7.61—7.54, 7.48—7.42 (m, 10 H, 2 Ph); 5.59 (t, 1 H, H(4), $J_{3,4} = J_{4,5} = 9.8$ Hz); 5.46 (br.s, 1 H, H(1)); 5.44 (d, 1 H, H(2), $J_{2,3} = 3.3$ Hz); 4.38 (dd, 1 H, H(3), $J_{2,3} = 3.3$ Hz, $J_{3,4} = 9.8$ Hz); 4.24 (m, 1 H, H(5)); 3.76—3.68 (m, 2 H, H(6a), H(6b)); 2.76—2.62 (m, 2 H, $SC\underline{H}_2Me$); 1.32 (t, 3 H, $SCH_2C\underline{H}_3$); 0.61 (s, 9 H, Me_3CSi); 0.03 (s, 3 H, MeSi); −0.09 (s, 3 H, MeSi). ^{13}C NMR (125.75 MHz), δ: 166.4, 165.9 (PhCO); 133.5, 133.2, 129.9—128.4 (Ph); 82.6 (C(1)); 74.4 (C(2)); 71.4 (C(5)); 70.2 (C(4)); 69.2 (C(3)); 61.6 (C(6)); 25.7 ($S\underline{C}H_2Me$); 25.2 (3 C, $(\underline{C}H_3)_3C$); 17.6 (1 C, $Me_3\underline{C}$); 14.8 ($SCH_2\underline{C}H_3$); −4.8, −5.1 (2 C, MeSi). Found (%): C, 61.36; H, 7.04. $C_{28}H_{38}O_7SSi$. Calculated (%): C, 61.51; H, 7.01.

Ethyl 2,4-di-*O*-benzoyl-3-*O*-[*tert*-butyl(dimethyl)silyl]-6-*O*-chloroacetyl-1-thio-α-D-mannopyranoside (9). Pyridine (110 μL, 1.40 mmol) and chloroacetyl chloride (55 μL, 0.70 mmol) were added to a solution of compound **11** (190 mg, 0.35 mmol) in anhydrous CH_2Cl_2 (4 mL). After 30 min, the reaction mixture

was diluted with chloroform, washed with 1 *M* HCl and a saturated solution of $NaHCO_3$, and concentrated. Compound **9** was isolated by column chromatography with light petroleum—ethyl acetate (10 : 1) as an eluent. The yield was 200 mg (95%), white foam. ^{1}H NMR (600 MHz, $CDCl_3$), δ: 8.18—8.06, 7.66—7.59, 7.54—7.46 (all m, 10 H, 2 Ph); 5.72 (t, 1 H, H(4), $J_{3,4} = J_{4,5} =$ = 9.7 Hz); 5.48 (br.s, 2 H, H(1), H(2)); 4.56 (m, 1 H, H(5)); 4.45 (dd, 1 H, H(6a), $J_{5,6}$ = 5.1 Hz, $J_{6a,6b}$ = 12.0 Hz); 4.38—4.32 (m, 2 H, H(3), H(6b)); 4.11 (d, 1 H, C(O)CH_2Cl, $J_{a,b}$ = 15.0 Hz); 4.07 (d, 1 H, C(O)CH_2Cl, $J_{a,b}$ = 15.0 Hz); 2.80—2.68 (m, 2 H, SC$\underline{H}_2$Me); 1.38 (t, 3 H, $SCH_2C\underline{H}_3$); 0.66 (s, 9 H, Me_3CSi); 0.06 (s, 3 H, MeSi); –0.09 (s, 3 H, MeSi). ^{13}C NMR (150 MHz), δ: 167.0, 165.8, 165.4 (2 Ph$\underline{C}$O, $\underline{C}$(O)CH_2Cl); 133.4, 133.3, 129.8—128.4 (Ph); 82.7 (C(1)); 74.2 (C(2)); 74.2 (C(4)); 69.8 (C(3)); 69.0 (C(5)); 64.5 (C(6)); 40.6 (C(O)$\underline{C}H_2$Cl); 25.8 (S$\underline{C}H_2$Me); 25.2 (3 C, $(\underline{C}H_3)_3C$); 17.6 (1 C, $Me_3\underline{C}$); 14.9 ($SCH_2\underline{C}H_3$); –4.8, –5.1 (2 C, MeSi). Found (%): C, 58.00; H, 6.57. $C_{30}H_{39}ClO_8SSi$. Calculated (%): C, 57.82; H, 6.31.

3-Trifluoroacetamidopropyl (2,4-di-*O*-benzoyl-3-*O*-[*tert*-butyl(dimethyl)silyl]-6-*O*-chloroacetyl-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 (12). Molecular sieves MS 4 Å (400 mg) were added to a solution of acceptor **4** (see Ref. 9) (207 mg, 0.20 mmol) and thiomannoside **9** (185 mg, 0.30 mmol) in anhydrous CH_2Cl_2 (4 mL). The mixture was stirred for 30 min and then NIS (135 mg, 0.6 mmol) was added at –15 °C. After 10 min, the mixture was cooled to –30 °C and TfOH (6 μL, 60 μmol) was added. The temperature was maintained within –25 to –10 °C throughout the reaction. After 45 min, the reaction mixture was neutralized with pyridine (10 μL), diluted with chloroform, and filtered through Celite. The filtrate was washed with a small amount of 1 *M* HCl and a saturated solution of $NaHCO_3$ and concentrated. Compound **12** was isolated by column chromatography with toluene—ethyl acetate (10 : 1) as an eluent. The yield was 308 mg (96%), transparent syrup. ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.19, 8.00, 7.63—7.12, 6.95 (all m, 40 H, Ph); 6.80 (br.s, 1 H, NH); 5.64 (t, 1 H, $H(4)_C$, $J_{3,4} = J_{4,5}$ = 9.6 Hz); 5.48 (br.s, 1 H, $H(2)_C$); 5.22 (d, 1 H, $H(1)_B$, $J_{1,2}$ = 1.4 Hz); 5.04 (d, 1 H, $H(1)_C$, $J_{1,2}$ = 1.7 Hz); 4.90 (d, 1 H, $H(1)_A$, $J_{1,2}$ = = 1.5 Hz); 4.85 (d, 1 H, PhC$\underline{H}_2$, J = 10.9 Hz); 4.81 (d, 1 H, PhC$\underline{H}_2$, J = 10.9 Hz); 4.64—4.49 (m, 10 H, PhC$\underline{H}_2$); 4.43 (dd, 1 H, $H(3)_C$, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz); 4.23 (m, 1 H, $H(5)_C$); 4.21—4.18 (m, 2 H, $H(6a)_C$, $H(6b)_C$); 4.05 (m, 1 H, $H(2)_B$); 4.03 (d, 1 H, C(O)CH_2Cl, J = 15.1 Hz); 4.00 (br.s, 1 H, $H(2)_A$); 3.99—3.91 (m, 3 H, $H(3)_B$, $H(5)_B$, C(O)CH_2Cl); 3.83—3.78 (m, 2 H, $H(3)_A$, $H(4)_B$); 3.74—3.62 (m, 7 H, $H(4)_A$, $H(5)_A$, $H(6a)_A$, $H(6b)_A$, $H(6a)_B$, $H(6b)_B$, OC$\underline{H}_2CH_2CH_2$N); 3.41 (m, 1 H, $OCH_2CH_2C\underline{H}_2$N); 3.31—3.22 (m, 2 H, OC$\underline{H}_2CH_2CH_2$N, $OCH_2CH_2C\underline{H}_2$N); 1.72 (m, 2 H, $OCH_2C\underline{H}_2CH_2$N); 0.63 (s, 9 H, Bu^{t}Si); 0.03 (s, 3 H, MeSi); –0.12 (s, 3 H, MeSi). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 166.9, 166.5, 165.4 (3 C, 2 Ph$\underline{C}$O, $\underline{C}$(O)CH_2Cl); 138.2—138.0, 133.3, 129.9—127.5 (Ph); 100.7 (1 C, $C(1)_B$); 99.2 (1 C, $C(1)_C$); 99.0 (1 C, $C(1)_A$); 79.5 (1 C, $C(3)_A$); 79.0 (1 C, $C(3)_B$); 76.6 (1 C, $C(2)_B$); 75.8 (1 C, $C(2)_A$); 75.1 (2 C, Ph$\underline{C}H_2$); 74.8 (1 C, $C(4)_A$); 74.6 (1 C, $C(4)_B$); 73.3 (2 C, Ph$\underline{C}H_2$); 72.5 (2 C, $C(2)_C$, Ph$\underline{C}H_2$); 72.1 (2 C, $C(5)_A$, Ph$\underline{C}H_2$); 72.0 (1 C, $C(5)_B$); 69.5 (2 C, $C(5)_C$, $C(6)_A$); 69.3 (2 C, $C(4)_C$, $C(6)_B$); 68.6 (1 C, $C(3)_C$); 65.8 (1 C, O$\underline{C}H_2CH_2CH_2$N); 64.3 (1 C, $C(6)_C$); 40.6 (1 C, C(O)$\underline{C}H_2$Cl); 37.9 (1 C, $OCH_2CH_2\underline{C}H_2$N); 28.2 (1 C, $OCH_2\underline{C}H_2CH_2$N); 25.3 (3 C, Bu^t); –4.9, –5.1 (2 C, MeSi). Found (%): C, 64.29; H, 6.43; N, 0.96. $C_{87}H_{97}ClF_3NO_{20}Si$. Calculated (%): C, 65.42; H, 6.12; N, 0.88. Found: m/z 1613.6347 $[M + NH_4]^+$. $C_{87}H_{101}ClF_3N_2O_{20}Si$. Calculated: $[M + NH_4]$ = 1613.6352.

3-Trifluoroacetamidopropyl (2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-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). Trisaccharide **12** (288 mg, 0.18 mmol) was dissolved in 90% trifluoroacetic acid (6 mL). The resulting solution was stirred for 2 h and concentrated. The residue was concentrated with toluene. Compound **8** was isolated by column chromatography with toluene—ethyl acetate (6 : 1) as an eluent. The yield was 200 mg (76%), syrup. ^{1}H NMR (600 MHz, $CDCl_3$), δ: 8.19, 8.04, 7.71—7.15, 6.98 (all m, 40 H, Ph); 6.80 (br.s, 1 H, NH); 5.56 (s, 1 H, $H(2)_C$); 5.54 (t, 1 H, $H(4)_C$, $J_{3,4} = J_{4,5}$ = 9.9 Hz); 5.30 (s, 1 H, $H(1)_B$); 5.09 (s, 1 H, $H(1)_C$); 4.94—4.90 (m, 2 H, $H(1)_A$, PhC$\underline{H}_2$); 4.84 (d, 1 H, PhC$\underline{H}_2$, J = 10.8 Hz); 4.69—4.53 (m, 10 H, PhC$\underline{H}_2$); 4.45 (m, 1 H, $H(3)_C$); 4.41—4.34 (m, 2 H, $H(5)_C$, $H(6a)_C$); 4.21 (dd, 1 H, $H(6b)_C$, $J_{5,6}$ = 4.3 Hz, $J_{6a,6b}$ = = 11.8 Hz); 4.08 (d, 1 H, $ClCH_2CO$, J = 14.9 Hz); 4.06—4.03 (m, 2 H, $H(2)_A$, $H(2)_B$); 4.02 (d, 1 H, C(O)CH_2Cl, J = 14.9 Hz); 4.01—3.97 (m, 2 H, $H(3)_B$, $H(5)_B$); 3.89 (t, 1 H, $H(4)_B$, $J_{3,4}$ = = $J_{4,5}$ = 9.5 Hz); 3.84 (m, 1 H, $H(3)_A$); 3.78 (t, 1 H, $H(6a)_B$, $J_{5,6} = J_{6a,6b}$ = 9.8 Hz); 3.77—3.68 (m, 6 H, $H(4)_A$, $H(5)_A$, $H(6a)_A$, $H(6b)_A$, $H(6b)_B$, OC$\underline{H}_2CH_2CH_2$N); 3.46 (m, 1 H, $OCH_2CH_2C\underline{H}_2$N); 3.37—3.27 (m, 2 H, OC$\underline{H}_2CH_2CH_2$N, $OCH_2CH_2C\underline{H}_2$N), 1.78 (m, 2 H, $OCH_2C\underline{H}_2CH_2$N). ^{13}C NMR (150 MHz, $CDCl_3$), δ: 166.9, 166.8, 165.7 (3 C, 2 Ph$\underline{C}$O, $\underline{C}$(O)CH_2Cl); 138.3—138.0, 133.7, 133.6, 130.0—127.5 (Ph); 100.6 (1 C, $C(1)_B$); 99.1 (2 C, $C(1)_A$, $C(1)_C$); 79.7 (1 C, $C(3)_A$); 78.8 (1 C, $C(3)_B$); 77.0 (1 C, $C(2)_B$); 75.8 (1 C, $C(2)_A$); 75.1 (2 C, Ph$\underline{C}H_2$); 74.9 (1 C, $C(4)_A$); 74.6 (1 C, $C(4)_B$); 73.4 (1 C, Ph$\underline{C}H_2$); 73.3 (1 C, Ph$\underline{C}H_2$); 72.8 (1 C, $C(2)_C$); 72.5 (1 C, Ph$\underline{C}H_2$); 72.4 (1 C, Ph$\underline{C}H_2$); 72.2 (2 C, $C(5)_A$, $C(5)_B$); 70.1 (1 C, $C(4)_C$); 69.6 (1 C, $C(6)_B$); 69.3 (1 C, $C(6)_A$); 69.0 (1 C, $C(5)_C$); 68.8 (1 C, $C(3)_C$); 65.9 (1 C, O$\underline{C}H_2CH_2CH_2$N); 64.1 (1 C, $C(6)_C$); 40.6 (1 C, C(O)$\underline{C}H_2$Cl); 38.0 (1 C, $OCH_2CH_2\underline{C}H_2$N); 28.2 (1 C, $OCH_2\underline{C}H_2CH_2$N). Found (%): C, 64.97; H, 5.69; N, 0.93. $C_{81}H_{83}ClF_3NO_{20}$. Calculated (%): C, 65.60; H, 5,64; N, 0.94. Found: m/z 1504.5047 $[M + Na]^+$. $C_{81}H_{83}ClF_3NNaO_{36}$. Calculated: [M + Na] = 1504.5041.

Mixture of 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)-(2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-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 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)-(2,4-di-*O*-benzoyl-6-*O*-chloroacetyl-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 MS 4 Å (370 mg) were added to a solution of acceptor **8** (177 mg, 0.12 mmol) and donor **2** (see Ref. 5) (193 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (4 mL). The mixture was stirred for 35 min and then NIS (81 mg, 0.36 mmol) was added at –15 °C. After 20 min, the mixture was cooled to –35 °C and TfOH (3.5 μL, 0.036 mmol) was added. The reaction temperature was maintained within –25 to –15 °C. After 1 h, the reaction mixture was neutralized with pyridine (10 μL), diluted with chloroform, and filtered through Celite. The filtrate was washed with a small amount of 1 *M* HCl and a saturated solution of $NaHCO_3$ and concentrated. Compound **13** was isolated by column chromato-

graphy with toluene—ethyl acetate (10 : 1) as an eluent. The yield was 208 mg (70%), white foam. ^{1}H NMR (600 MHz, $CDCl_3$), δ: 8.22—7.80, 7.70—7.00 (both m, Ph); 6.80 (br.s, 1 H, NH); 6.07 (t, 1 H, $H(4)_E$, $J_{3,4} = J_{4,5} = 10$ Hz); 5.88 (m, 1 H, $H(3)_E$); 5.75 (br.s, 1 H, $H(2)_E$); 5.74 (t, 1 H, $H(4)_C$, $J = 10$ Hz); 5.70 (br.s, 1 H, $H(2)_C$); 5.38 (s, 1 H, $H(1)_D$, $J_{C(1),H(1)} = 172$ Hz); 5.32 (s, 1 H, $H(1)_B$, $J_{C(1),H(1)} = 172$ Hz); 5.29 (s, $H(1)_\beta$); 5.18 (s, $H(1)_\beta$); 5.15 (s, 1 H, $H(1)_C$, $J_{C(1),H(1)} = 175$ Hz); 5.08 (s, $H(1)_\beta$); 4.94 (s, 1 H, $H(1)_A$, $J_{C(1),H(1)} = 173$ Hz); 4.72 (br.s, 1 H, $H(1)_E$, $J_{C(1),H(1)} = 172$ Hz). ^{13}C NMR (150 MHz, $CDCl_3$), δ: 166.9—164.7 (Ph<u>C</u>O, <u>C</u>(O)CH_2Cl); 138.9—138.0, 133.7—132.9, 130.1—127.1 (Ph); 100.5 ($C(1)_B$); 100.4 ($C(1)_D$); 99.7 ($C(1)_\beta$); 99.5 ($C(1)_\beta$); 99.2 ($C(1)_C$); 99.1 ($C(1)_E$); 99.0 ($C(1)_A$); 97.9 ($C(1)_\beta$); 79.3 ($C(2)_D$); 75.7 ($C(2)_A$); 72.0 ($C(2)_C$); 70.2 ($C(2)_E$); 70.1 ($C(3)_E$); 69.1 ($C(5)_E$); 68.8 ($C(4)_C$); 66.5 ($C(4)_E$); 65.8 (O<u>C</u>$H_2CH_2CH_2N$); 64.1 ($C(6)_C$); 61.8 ($C(6)_E$); 40.6 (C(O)<u>C</u>H_2Cl); 37.9 (OCH_2CH_2<u>C</u>H_2N); 28.2 (OCH_2<u>C</u>H_2CH_2N). Found (%): C, 68.50; H, 5.43; N, 0.50. $C_{142}H_{137}ClF_3NO_{34}$. Calculated (%): C, 68.38; H, 5,54; N, 0.56. Found: *m/z* 2509.9003 $[M + NH_4]^+$. $C_{142}H_{141}ClF_3N_2O_{34}$. Calculated: $[M + NH_4] = 2509.9001$.

Mixture of 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)-(2,4-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 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)-(2,4-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). Thiourea (47 mg, 0.62 mmol) and collidine (10 μL, 0.075 mmol) were added to a solution of pentasaccharides **13** (154 mg, 0.062 mmol) in anhydrous methanol (8 mL). The mixture was refluxed for 24 h. After the reaction was completed, the solvent was removed *in vacuo*. The residue was suspended in chloroform; the undissolved precipitate was filtered off and washed with chloroform. The filtrate was washed with 1 *M* HCl and a saturated solution of $NaHCO_3$ and concentrated. Compound **14** was isolated by column chromatography with toluene—ethyl acetate (5 : 1) as an eluent. The yield was 122 mg (81%), white foam. ^{1}H NMR (600 MHz, $CDCl_3$), δ: 8.20—7.79, 7.68—7.02 (both m, Ph); 6.86 (br.s, 1 H, NH); 6.1 (t, 1 H, $H(4)_E$, $J_{3,4} = J_{4,5} = 9.9$ Hz); 5.91 (dd, 1 H, $H(3)_E$, $J_{2,3} = 2.9$ Hz, $J_{3,4} = 9.9$ Hz); 5.78 (br.s, 1 H, $H(2)_E$); (br.s, 1 H, $H(2)_C$); 5.58 (t, 1 H, $H(4)_C$, $J_{3,4} = J_{4,5} = 9.7$ Hz); 5.41 (s, 1 H, $H(1)_D$); 5.32 (s, 1 H, $H(1)_B$); 5.25 (s, $H(1)_\beta$); 5.23 (s, 1 H, $H(1)_C$); 5.17 (s, $H(1)_\beta$); 4.93 (br.s, 1 H, $H(1)_A$); 4.76 (s, 1 H, $H(1)_E$). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 166.0—164.74 (Ph<u>C</u>O); 138.9—137.8, 133.7—132.5, 130.6—126.8 (Ph); 100.4 ($C(1)_B$); 100.3 ($C(1)_D$); 99.6 ($C(1)_\beta$); 99.2 ($C(1)_C$); 99.0 ($C(1)_E$); 98.9 ($C(1)_A$); 97.5 ($C(1)_\beta$); 76.0 ($C(2)_B$); 72.0 ($C(2)_C$); 70.2 ($C(2)_E$); 70.0 ($C(3)_E$); 66.6 ($C(4)_E$); 65.8 (O<u>C</u>$H_2CH_2CH_2$N); 62.0 ($C(6)_E$); 61.4 ($C(6)_C$); 37.9 (OCH_2CH_2<u>C</u>H_2N); 28.1 (OCH_2<u>C</u>H_2CH_2N). Found (%): C, 69.59; H, 5.78; N, 0.65. $C_{140}H_{136}F_3NO_{33}$. Calculated (%): C, 69.55; H, 5,67; N, 0.58. Found: *m/z* 2433.9284 $[M + NH_4]^+$. $C_{140}H_{140}F_3N_2O_{33}$. Calculated: $[M + NH_4] = 2433.9285$.

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 (15) 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 (16). Molecular sieves MS 4 Å (165 mg) were added to a stirred solution of an anomeric mixture of pentasaccharides **14** (109 mg, 0.045 mmol) and benzobromomannose **6** (45 mg, 0.068 mmol) in anhydrous CH_2Cl_2 (4 mL). The mixture was stirred for 40 min and solid-state AgOTf (26 mg, 0.10 mmol) was added at −40 °C. The reaction temperature was maintained within −20 to −15 °C. After 1.5 h, the reaction mixture was neutralized with a drop of Et_3N, diluted with chloroform, and filtered through Celite. The filtrate was washed with a small amount of 1 *M* HCl and a saturated solution of $NaHCO_3$, concentrated, and subjected to column chromatography (toluene—ethyl acetate, 8 : 1). Then isomeric compounds **15** and **16** were separated by HPLC in light petroleum—acetone (2 : 1). The yields of products **15** and **16** were 97 mg (72%) and 14 mg (10%), respectively. Their NMR spectra are identical with the literature data.[5]

<u>Compound **15**.</u> Found (%): C, 68.55; H, 5.29; N, 0.56. $C_{174}H_{162}F_3NO_{42}$. Calculated (%): C, 69.75; H, 5,45; N, 0.47. Found: *m/z* 3012.0839. $[M + NH_4]^+$. $C_{174}H_{166}F_3N_2O_{42}$. Calculated: $[M + NH_4] = 3012.0862$.

<u>Compound **16**.</u> Found (%): C, 69.66; H, 5.36; N, 0.47. $C_{174}H_{162}F_3NO_{42}$. Calculated (%): C, 69.75; H, 5,45; N, 0.47. Found: *m/z* 3012.0872 $[M + NH_4]^+$. $C_{174}H_{166}F_3N_2O_{42}$. Calculated: $[M + NH_4] = 3012.0862$.

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 (1). A catalyst Pd/C (90 mg) was added to a stirred solution of protected hexasaccharide **15** (90 mg, 0.030 mmol) in methanol—ethyl acetate (1 : 1, 2 mL). The mixture was stirred under hydrogen at room temperature for 20 h. After the reaction was completed, the reaction mixture was diluted with methanol and filtered through Celite. The filtrate was concentrated and the residue was dissolved in methanol (3.5 mL). Then a solution of 1 *M* MeONa in methanol (0.3 mL) was added. After 40 min, a drop of water was added and the mixture was left for 16 h, neutralized with acetic acid, and concentrated. Product **1** was isolated by gel chromatography in the form of acetate and lyophilized from water. The yield was 26.5 mg (85%).

The NMR spectra of compound **1** are fully identical with those published earlier.[5] Found: *m/z* 1048.3913 $[M + H]^+$. $C_{39}H_{69}NO_{31}$. Calculated: $[M + H] = 1048.3926$.

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