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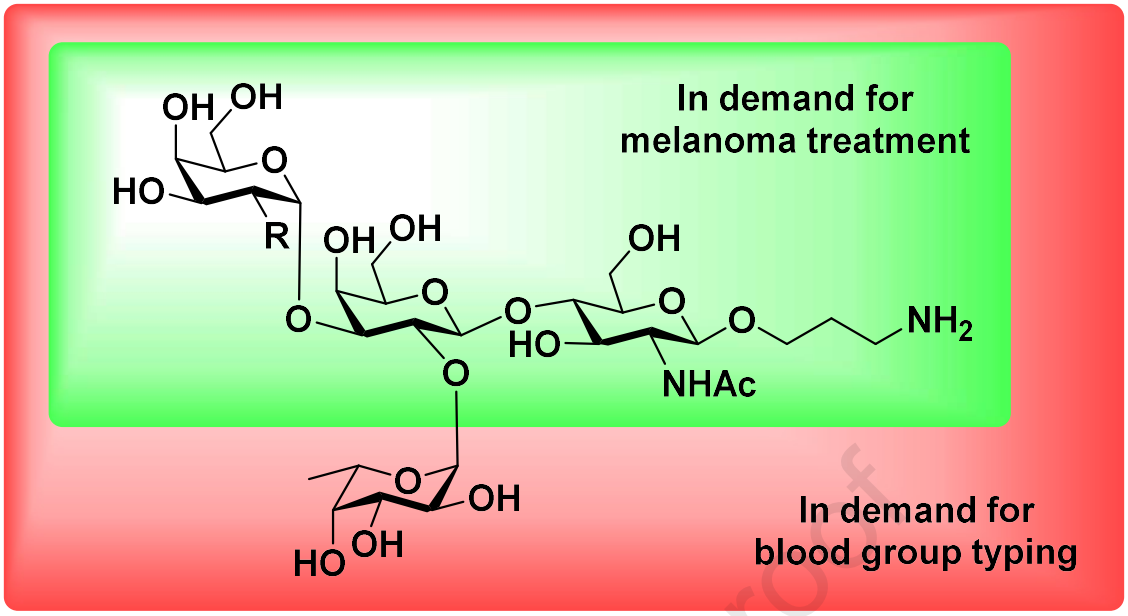

Synthesis of blood group A and B (type 2) tetrasaccharides. A strategy with fucosylation at the last stage

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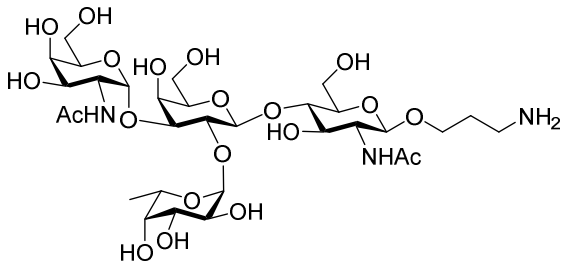
Abstract

The traditionally used strategy for the synthesis of blood group A and B tetrasaccharides includes 2'-O-fucosylation of lactosamine followed by insertion of an α1-3 linked N-acetylgalactosamine or a galactose moiety. Here, we report the synthesis of 3-aminopropyl glycosides of A (type 2) and B (type 2) tetrasaccharides *via* an alternative sequence, i.e. α-galactosaminylation (or α-galactosylation) followed by α-fucosylation. This strategy allows us to synthesize fucose-free trisaccharides GalNAcα1-3Galβ1-4GlcNAc and Galα1-3Galβ1-4GlcNAc, which are promising targets for immunotherapy utilising human natural antibodies against the trisaccharides. The key stage in this scheme was the selective chloroacetylation of the 2`-OH group of βGal in the intermediate trisaccharides having the second (3-OH) unprotected group.The protocol is suitable for multigram syntheses and its further scaling up.

1. Introduction

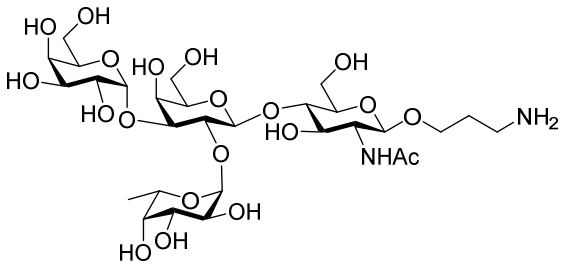
Human histo-blood group oligosaccharides with -Galβ1-4GlcNAc inner core (known as type 2 glycans) dominate on the AB-serologically active glycoproteins and glycolipids of human erythrocytes (RBC). A (type 2) and B (type 2) tetrasaccharides, and H (type 2) trisaccharide terminate N- and O-chains in glycoproteins, as well as glycolipids in composition of practically all endothelial and epithelial tissues, they are also found as free oligosaccharides in body secretions [1],[2]. A (type 2) and B (type 2) tetrasaccharide terminations (Fig.1) are minimal epitopes responsible for A and B antigens recognition by natural antibodies (known as allo-antibodies, or isoagglutinins) [3]. Chemical synthesis of these glycans was described in numerous works [4-14]. Nevertheless, synthetic scheme leading to the tetrasaccharides now are still in demand because of recent advances in diagnostics and therapy. One example of medical application is design of so called kodecytes (blood group O erythrocytes modified with lipophilic derivatives of tetrasaccharides A and B), routinely used in the ABO-typing [15-17]; second example is removal of anti-A and anti-B isoagglutinins from recipient's blood by means of affinity adsorbents (A and B glycans immobilized on Sepharose) in ABO-incompatible organ transplantation [18].

We recently published block synthesis of tetrasaccharides A (type 2) and B (type 2), where the unit GlcNAc was glycosylated by the trichloroacetimidate form of protected trisaccharides A and B [10], an approach that is acceptable and convenient for obtaining various oligosaccharides containing these trisaccharide motifs, but inappropriate for multigram tetrasaccharide synthesis; the same is true for a synthetic scheme based on the use of 1,6-anhydroglucosamine as a key synthetic feature [19].



1a

A_{tetra} (type 2)

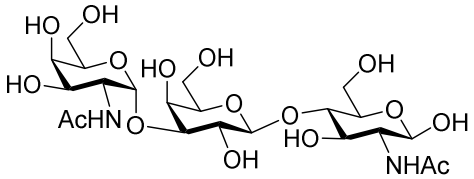


1b

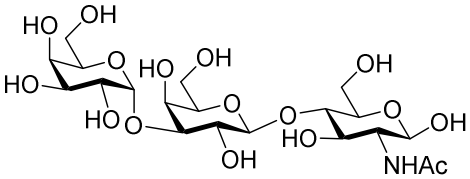
B_{tetra} (type 2)

Fig. 1. Aminopropyl glycosides of A (type 2) **1a** and B (type 2) **1b** tetrasaccharides.

Traditionally, A (type 2) and B (type 2) tetrasaccharides are synthesized *via* galactosaminylation or galactosylation of 3-OH in Gal residue of H (type 2) trisaccharide. This approach was applied in early versions of A (type 2) and B (type 2) tetrasaccharide synthesis [5],[6] and is still in use [7-9],[20],[21]. Alternative scheme, GlcNAc → Galβ1–4GlcNAc → Gal(NAc)α1–3Galβ1–4GlcNAc → Gal(NAc)α1–3(Fucα1–2)Galβ1–4GlcNAc, is at first glance less rational since it requires two fucosylation reactions instead of one – i.e. in the synthesis of each of the tetrasaccharides. However, the alternative scheme has undoubted advantage when large-scale synthesis requires a fucose-free trisaccharide **2b** and this is indeed the case, since glycan **2b** is used to deplete antibodies during xenotransplantation [22] and to improve the fight against nosocomial infections [23].



2a



2b

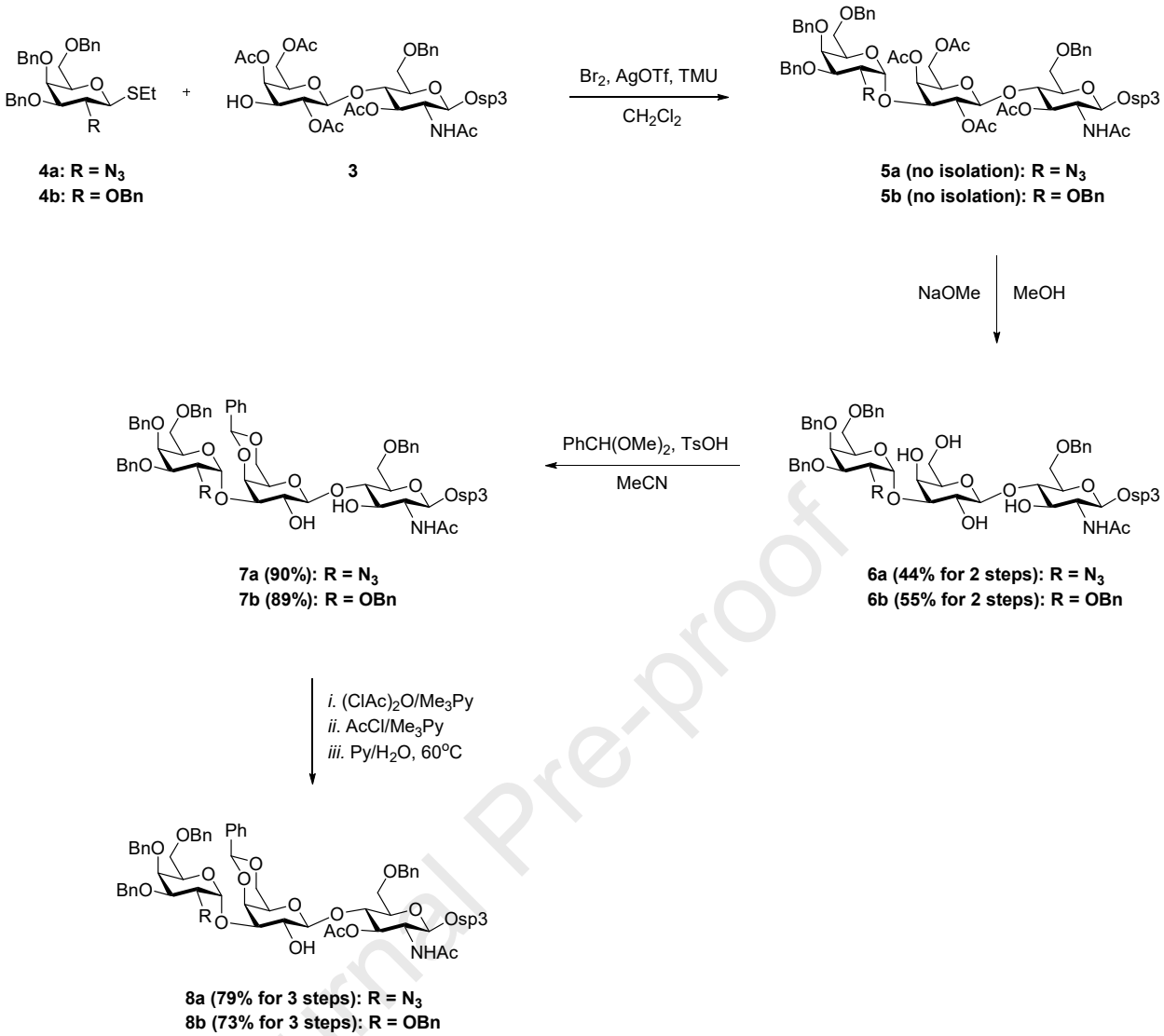
Galili-trisaccharide

Fig. 2. Trisaccharides **2a** and **2b**.

Since anti-GalNAcα1–3Galβ1–4GlcNAc (**2a**) immunoglobulin titers are even higher in humans than against Galα1–3Galβ1–4GlcNAc (**2b**) [24], there is no doubt that the trisaccharide (Fig. 2) will also soon be used in medicine. In this paper, we have demonstrated that the second scheme is not just realizable, but very promising for practical scaling-up.

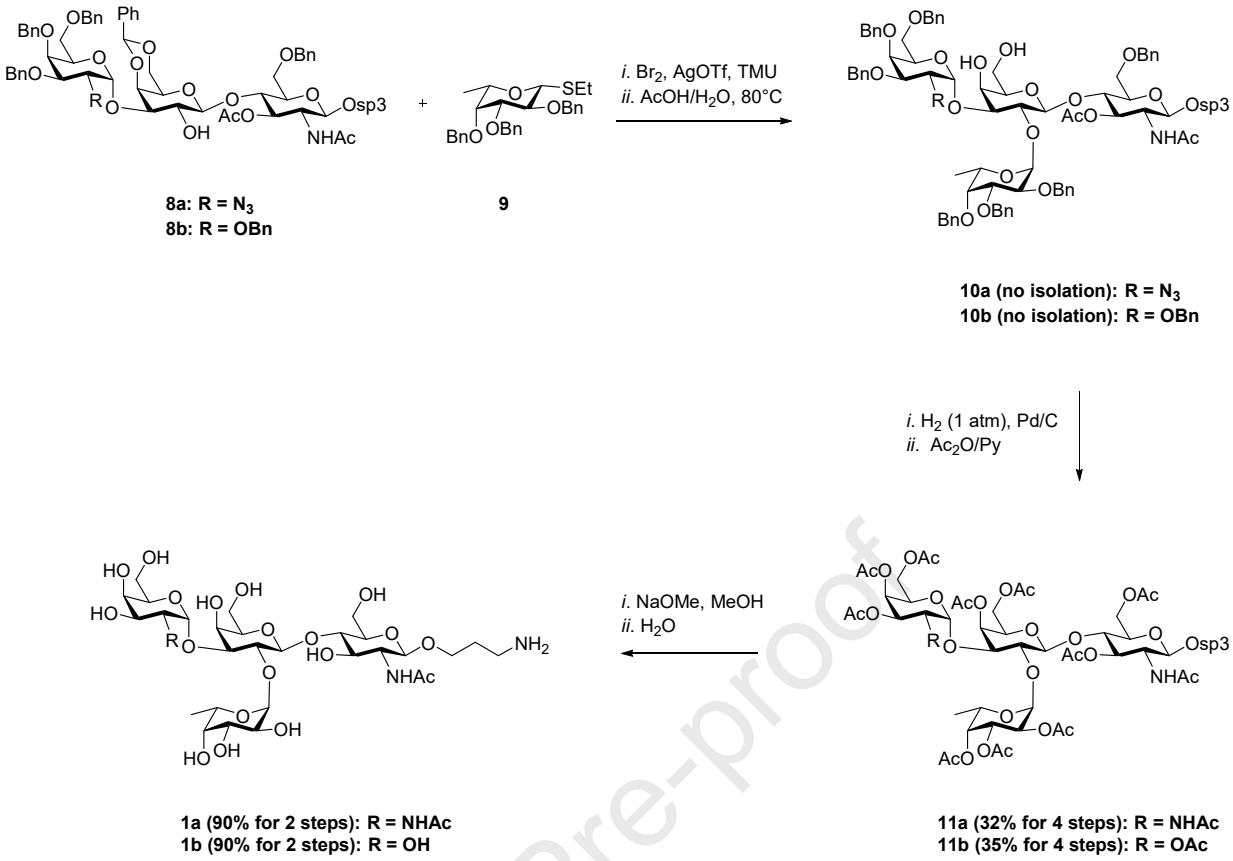
2. Results and discussion

Our synthesis for aminopropyl glycosides of A (type 2) **1a** and B (type 2) **1b** tetrasaccharides is presented on Scheme 1 and 2. In conformity with the schemes, 3`-OH lactosamine **3** (prepared according to literature procedure [25],[26]) was glycosylated with ethyl thioglycosides **4a** and **4b**, with bromine activation and AgOTf as a promoter [25] at r.t. In both cases α-selectivity was high; resulting trisaccharides **5a** and **5b** after partial purification were deacetylated under the classical Zemplen conditions to trisacchrides **6a** and **6b** respectively; after complete conversion, the basic components were removed using cation exchange resin in the PyH^+ form. This form was taken since in some cases it was previously found cation exchangers in the form of H^+ led to the replacement of the spacer by OMe, the latter coming from methanol. Isolated trisaccharides **6a** and **6b** with four OH groups required selective protection before final glycosylation. Therefore, in the next step, the 4`-OH and 6`-OH groups were protected with a benzylidene group leading to diols **7a** and **7b**, where 3-OH must be protected in the presence of 2`-OH. An attempt for di-*O*-acetylation and subsequent selective deacylation of the product under controlled Zemplen conditions was unsuccessful - both 3-*O*Ac and 2'-*O*Ac showed the same deacetylation rates. Selective protection of one of the hydroxyls with bulky silyl groups was also unsuccessful. TBDMS chloride/imidazole in DMF [27] showed low selectivity, while the more bulky TIPS chloride/imidazole in DMF [28] was almost inert to both hydroxyl groups even at 60 °C. Effective and selective protection of 3-OH in the presence of 2`-OH in compounds **7a** and **7b** was achieved using the following path: chloroacetylation-acetylation-dechloroacetylation. Chloroacetic anhydride in the presence of collidine quickly reacted with 2`-OH under selected conditions, in which the second hydroxyl was almost inert. This remaining free 3-OH was then acetylated with acetyl chloride/collidine. Further gentle removal of the chloroacetyl group with a water-pyridine mixture at 60 °C gave the desired glycosyl-acceptor trisaccharides **8a** and **8b**, with similar selectivity and yields (Scheme 1).



Scheme 1. Syntheses of **8a** and **8b** trisaccharide glycosyl acceptors; sp3 = $(CH_2)_3NHCOCF_3$.

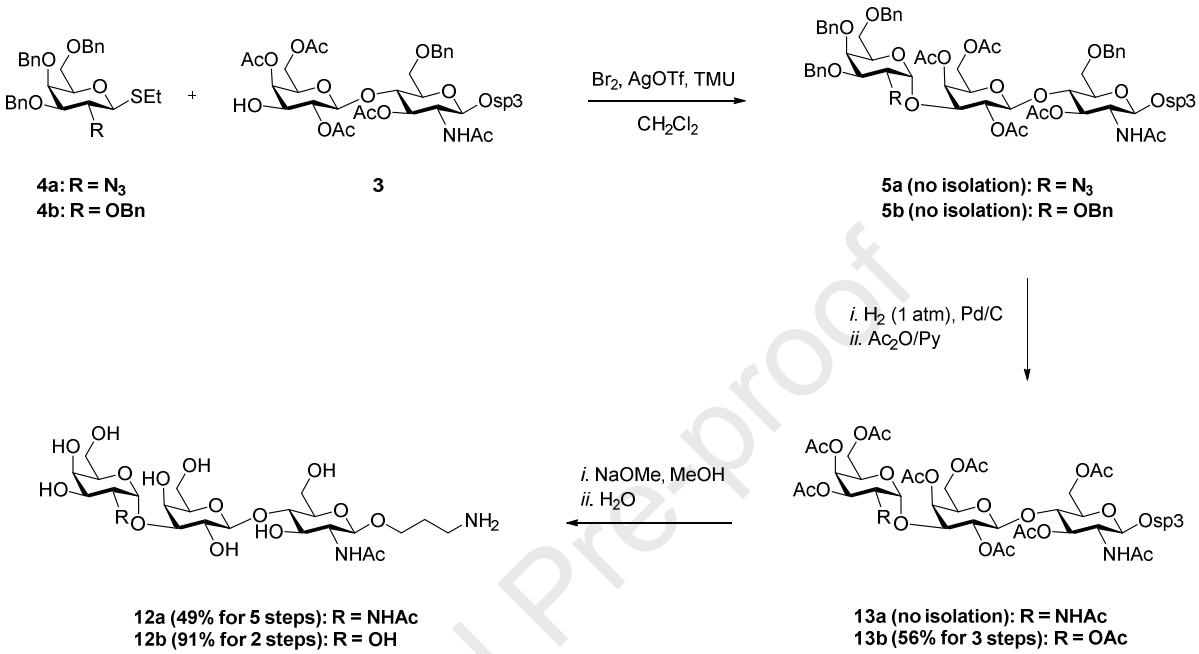
The obtained trisaccharides **8a** and **8b** were fucosylated (Scheme 2) with ethyl thioglycoside **9** with bromine activation and AgOTf as a promoter [25] at r.t. In both cases α-selectivity was high; after remove of benzylidene group, the tetrasaccharides **10a** and **10b** were partially purified by column chromatography. Then, benzyl groups were removed with hydrogenolysis followed by peracetylation to **11a** and **11b** respectively, the products were purified with chromatography and completely deprotected by alkaline hydrolysis. Target tetrasaccharides with aminopropyl linker, **1a** and **1b,** were isolated by ion exchange chromatography on H^+-cationite.



Scheme 2. Syntheses of A (type 2) **1a** and B (type 2) **1b** tetrasaccharides; sp3 = $(CH_2)_3NHCOCF_3$.

The products **5a**-**8a** and **5b**-**8b** are protected trisaccharides **12a** and **12b** respectively (Scheme 3) that are of independent value (in addition to the corresponding fucosylated forms). In particular, **12b** is the well-known *Galili* xeno-antigen, its lipophilic form AGI-134 is being studied as a potential anti-cancer therapeutic [29], while the polymeric form is a potential anti-bacterial therapeutic [23]. The level of natural antibodies against trisaccharide **12a** is even more than that against the *Galili* trisaccharide [30], so, GalNAcα1-3Galβ1-4GlcNAc is also a potential molecule for design of immunotherapeutics. For preparation of **12a** and **12b** (Scheme 3), 3`-OH lactosamine **3** was glycosylated with ethyl thioglycosides **4a** and **4b**, resulting intermediate trisaccharides **5a** and **5b** were partially purified as mentioned earlier (Scheme 1), but instead of deacetylation, benzyl groups were removed by hydrogenolysis followed by peracetylation to **13a**

and **13b** respectively, the products were purified with chromatography and finally deacylated by alkaline hydrolysis. Trisaccarides with aminopropyl linker, **12a** and **12b,** were isolated by ion exchange chromatography on H^+-cationite.



Scheme 3. Syntheses of trisaccharides **12a** and **12b**; sp3 = $(CH_2)_3NHCOCF_3$.

Complete prove of the structure of the target and intermediate oligosaccharides was performed using 1H, 1H-2D-COSY and/or 1H, 13C-2D-HSQC experiments (available in Supporting information).

3. Conclusions

The proposed method for the synthesis of blood groups A and B tetrasaccharides through fucosylation at the last stage uses a minimum of chromatographic procedures. This is fundamentally important, since it opens up a real prospect for scaling this synthesis up for medical purposes not only of tetra-, but also trisaccharides GalNAcβ1-3Gallβ1-4GlcNAc and Galα1-3Gallβ1-4GlcNAc.

4. Experimental

4.1. General procedures

The reactions were performed with the use of commercial reagents (Acros, Aldrich, and Fluka); anhydrous solvents were purified according to the standard procedures.

3-Trifluoroacetamidopropyl *O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside **3** was prepared accordingly to lit. procedure [25]. Ethyl-2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-1-thio-*β*-D-galactopyranoside **4a** was prepared accordingly to lit. procedure [31]. Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-*β*-D-galactopyranoside **4b** was prepared accordingly to lit. procedure [32]. Ethyl 2,3,4-tri-*O*-benzyl-1-thio-*β*-L-fucopyranoside **9** was prepared accordingly to lit. procedure [33].

Column chromatography was performed on silica gel 60 0.040‑0.063 mm (Merck). Solvents were removed in vacuum at 30-40 °C. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminum-backed plates (Merck). The spots of compounds were visualized by dipping a TLC plate into aqueous solution of H_3PO_4 (8%) and subsequent heating (>150 °C). The values of optical rotations were measured on a Perkin Elmer 341 and JASCO P-2000 polarimeters at 25°C.

^{1}H NMR spectra were recorded on a Bruker BioSpin GmbH (700 MHz) spectrometer at 30 °C; chemical shifts (δ, ppm) were referred to solvent residual peak of internal D_2O (δ 4.79), CD_3OD (δ 3.31), $CDCl_3$ (δ 7.27) and $(CD_3)_2SO$ (δ 2.50); coupling constants (J) were measured in Hz. ^{13}C NMR spectra were recorded at 150 MHz. Chemical shifts (δ, ppm) were referred to the peak of internal CD_3OD (δ 49.0), $CDCl_3$ (δ 77.16) and $(CD_3)_2SO$ (δ 39.62). Signals of ^{1}H NMR and ^{13}C NMR spectra were assigned to the corresponding protons using 2D spectroscopy (COSY, HSQC). 1D and 2D NMR spectra pictures are available in Supporting information.

4.2.1 Synthesis of aminopropyl glycoside of A (type 2) tetrasaccharide 1a

*3-Trifluoroacetamidopropyl O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**6a**)*

To ethyl thioglycoside **4a** (11.8 g, 22.7 mmol) solution in abs. dichloromethane (120 mL) was added bromine (1.27 mL, 25.0 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×100 mL) to remove traces of bromine, almost colourless oil of glycosyl bromide was dried in vacuum at r.t.

To disaccharide **3** (12.0 g, 15.1 mmol) solution in abs. dichloromethane (140 mL) were added freshly dried MS-4Å (25 mL) and tetramethylurea (3.40 mL, 27.8 mmol), the mixture stirred for 30 min, then AgOTf (6.40 g, 24.5 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture glycosyl bromide (see above) solution in abs. dichloromethane (70 mL) was added drop-wise under intensive stirring. The reaction was left for 24 h with stirring (TLC control in chloroform/methanol 10:1, *Rf* 0.47). Reaction mixture was neutralized with pyridine (12 mL), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1. The filtrate was poured in 10% aq. solution of $Na_2S_2O_3$ (170 mL), washed with cold sat. $NaHCO_3$ (150 mL), dried with Na_2SO_4/SiO_2.

After filtration, the solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The product **5a** was partially purified with column chromatography on silica gel (from toluene/acetone 5:1 to toluene/acetone 3:1), the fractions containing major product **5a** were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification (contains admixture of tetramethylurea).

The partially purified product **5a** was dissolved in abs. methanol (500 mL), treated with 2M NaOMe (50 mL, 100 mmol) and reaction mixture was left for 2 h. Reaction mixture was neutralized with Dowex 50WX4 (100 g, PyH^+-form) and stirred for 15 min and filtered. Obtained solution was concentrated on rotary evaporator, co-evaporated with ethyl acetate, dried in vacuum. Crude material was diluted with ethyl acetate (360 mL) and product was precipitated by slow addition of n-hexane (360 mL) under intensive stirring, left overnight in fridge for complete crystallization. The crystalline product was collected on glass filter, washed with mixture of n-hexane/ethyl acetate 3:1 and n-hexane subsequently, dried under fume-hood overnight further by vacuum drying.

Yield of **6a** for two steps: 44% (7.20 g, 6.62 mmol). White crystalline material; *Rf* 0.40 (chloroform/ethyl acetate/methanol, 9:3:2); $[\alpha]_D$ could not be measured precisely owing to low solubility of the compound in polar as well nonpolar solvents;

^{1}H NMR (CD_3OD, characteristic signals): δ 1.75-1.87 (m, 2H, $OCH_2CH_2CH_2N$), 1.98 (s, 3H, $NHC(O)CH_3$), 4.32 (d, 1H, $J_{1,2}$ 7.7, H-1^{II}), 4.40 (d, 1H, $J_{1,2}$ 8.4, H-1^{I}), 5.11 (d, 1H, $J_{1,2}$ 3.6, H-1^{III}); ^{13}C NMR (CD_3OD, characteristic signals): 22.6 ($NHC(O)CH_3$), 29.7 ($OCH_2CH_2CH_2N$), 96.2 (C-1^{III}), 102.7 (C-1^{I}), 104.9 (C-1^{II}); ESI MS: m/z Calcd $[C_{53}H_{64}F_3N_5O_{16}]H^+$: 1084.4. Found: 1084.4

(^{1}H, ^{13}C, COSY and HSQC NMR spectra pictures can be found in Supporting information)

*3-Trifluoroacetamidopropyl O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-O-(4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**7a**)*

Trisaccharide **6a** (7.20 g, 6.62 mmol) from previous experiment was suspended in dry acetonitrile (120 mL), then $PhCH(OMe)_2$ (3.60 mL, 24.0 mol) and solution of $TsOH \cdot H_2O$ (150 mg, 0.80 mmol) in dry acetonitrile (5 mL) were added. Reaction mixture was stirred overnight at

r.t. The reaction was neutralised with pyridine (12 mL). Diethyl ether (360 mL) was added under stirring, and formed crystalline product was collected on glass filter, washed with diethyl ether, n-hexane, dried in vacuum.

Yield of **7a**: 90% (6.95 g, 5.95 mmol). White crystalline material; *Rf* 0.65 (chloroform/ethyl acetate/methanol, 9:3:2); $[\alpha]_D$ +36° (*c* 0.7, $CHCl_3$);

^{1}H NMR ($CDCl_3$, characteristic signals): δ 1.74-1.89 (m, 2H, $OCH_2CH_2CH_2N$), 2.01 (s, 3H, $NHC(O)CH_3$), 4.64 (d, 1H, $J_{1,2}$ 8.4, H-1^{I}), 5.11 (d, 1H, $J_{1,2}$ 3.5, H-1^{III}), 5.52 (s, 1H, PhC*H*), 6.34 (d, 1H, $J_{2,NH}$ 6.8, N*H*$COCH_3$); ^{13}C NMR ($CDCl_3$, characteristic signals): δ 23.5 ($NHC(O)CH_3$), 28.5 ($OCH_2CH_2CH_2N$), 97.1 (C-1^{III}), 100.9 (PhCH), 101.1 (C-1^{I}), 103.7 (C-1^{II}); ESI HRMS: m/z Calcd $[C_{60}H_{68}F_3N_5O_{16}]H^+$: 1172.4686. Found: 1172.4623.

(^{1}H, ^{13}C and HSQC spectra pictures can be found in Supporting information)

*3-Trifluoroacetamidopropyl O-(2-azido-3,4,6-tri-O-benzyl-2-deoxy- α-D-galactopyranosyl)-(1→3)-O-(4,6-O-benzylidene- β-D-galactopyranosyl)-(1→4)-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy- β-D-glucopyranoside (**8a**)*

To powder of trisaccharide **7a** (6.95 g, 5.95 mmol) from previous experiment was added solution of chloro acetic anhydride (3.10 g, 18.1 mmol) and sym-Collidine (16 mL, 120 mmol) in abs. dichloromethane (40 mL) at 0°C under intensive stirring. The mixture was stirred for 1.5 h. The reaction was quenched with methanol (15 mL), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Resulting oil was dissolved in abs. dichloromethane (90 mL) followed by addition of sym-Collidine (18.0 mL, 137 mmol) and acetyl chloride (9.0 mL, 126 mmol). Reaction mixture was left for 24 h at r.t. Reaction was quenched with methanol (40 mL), concentrated on rotary evaporator, co-evaporated with toluene. The residue was diluted with toluene (200 mL), stirred

for 15 min, collidinium salt was collected on glass filter, washed few times with toluene. The filtered solution was concentrated on rotary evaporator, dried in vacuum.

The residue was dissolved in pyridine (200 mL), water (100 mL) was added and the mixture was warmed at 60 °C for 24 h. The mixture was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

The product **8a** was purified with column chromatography on silica gel (chloroform/acetone, from 5:1 to 3:1, 0.5% pyridine) , the fractions containing major product were collected, concentrated on rotary evaporator (not to dryness). Concentrated solution was diluted with ethyl acetate (100 mL), n-hexane (300 mL) was added drop-wise under stirring to precipitate pure trisaccharide **8a** and the mixture was left overnight in fridge for complete crystallization. Solid product was collected on glass filter, washed with little portion of n-hexane/ethyl acetate 3:1, pure n-hexane, dried in vacuum.

Yield of **8a** for three steps: 79% (5.70 g, 4.69 mmol). White crystalline material; *Rf* 0.50 (chloroform/methanol, 10:1); $[\alpha]_D$ +25° (*c* 0.3, $CHCl_3$);

^{1}H NMR ($CDCl_3$, characteristic signals): δ 1.48-1.56 (m, 1H, OCH_2CHHCH_2N), 1.59-1.68 (m, 1H, OCH_2CHHCH_2N), 1.99, 2.15 (2s, 6H, $OC(O)CH_3$ and $NHC(O)CH_3$), 3.07-3.14 (m, 1H, OCH_2CH_2CHHN), 3.34-3.40 (m, 1H, $OCHHCH_2CH_2N$), 4.10 (ddd, 1H, $J_{1,2}\approx J_{2,3}\approx J_{2,NH}$ 9.0, H-2^I), 4.18 (d, 1H, $J_{1,2}$ 7.6, H-1^{II}), 4.39 (d, 1H, $J_{1,2}$ 8.4, H-1^I), 5.09 (d, 1H, $J_{1,2}$ 3.5, H-1^{III}), 5.22 (dd, 1H, *J* 9.0, *J* 10.5, H-3^I), 5.51 (s, 1H, PhC*H*), 6.44 (d, 1H, $J_{2,NH}$ 8.9, N*H*$COCH_3$); ^{13}C NMR ($CDCl_3$, characteristic signals): δ 21.0, 23.4 ($OC(O)CH_3$ and $NHC(O)CH_3$), 28.4 ($OCH_2CH_2CH_2N$), 37.9 ($OCH_2CH_2CH_2N$), 53.8 (C-2^I), 97.1 (C-1^{III}), 101.1 (PhCH), 101.9 (C-1^I), 103.4 (C-1^{II}); ESI HRMS: m/z Calcd $[C_{62}H_{70}F_3N_5O_{17}]H^+$: 1214.4792. Found: 1214.4781. (^{1}H, ^{13}C, COSY and HSQC NMR spectra pictures can be found in Supporting information)

3-Trifluoroacetamidopropyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-O-[2,3,4-tri-O-acetyl-α-L-fucopyranosyl-(1→2)]-O-(4,6-di-O-acetyl-

β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside

(11a)

To ethyl thioglycoside **9** (4.34 g, 9.08 mmol) solution in abs. dichloromethane (50 mL) was added bromine (0.52 mL, 10.0 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×50 mL) to remove traces of bromine, almost colourless oil of glycosyl bromide was dried in vacuum at r.t.

To trisaccharide **8a** (5.50 g, 4.53 mmol) solution in abs. dichloromethane (100 mL) were added freshly dried MS-4Å (40 mL) and tetramethylurea (1.2 mL, 10.0 mmol), the mixture stirred for 30 min, then AgOTf (2.32 g, 9.0 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture glycosyl bromide (see above) solution in abs. dichloromethane (50 mL) was added drop-wise under intensive stirring. The reaction was left for 24 h with stirring (TLC control in ethyl acetate/toluene 3:1, *Rf* 0.50). Reaction mixture was neutralized with pyridine (7 mL), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1.

Filtered solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue was dissolved in acetic acid (200 mL), water (50 mL) was added under stirring and the mixture was warmed at 80 °C for 3 h (TLC control in chloroform/propan-2-ol 10:1, *Rf* 0.37). The mixture was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Crude product **10a** was partially purified with column chromatography on silica gel (from chloroform/propan-2-ol 20:1 to 15:1, 0.5% pyridine) , the fractions containing major product were collected (TLC control in chloroform/propan-2-ol 10:1+0.5% pyridine), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification (contains admixture of tetramethylurea).

Partially purified **10a** was dissolved in methanol (300 mL), then 10% Pd/C (4.0 g) was added and reaction mixture was degassed in vacuum further by filling with H_2 (1 atm) three times. The reaction was left under H_2 (1 atm) with stirring for 12 h. Acetic anhydride (15 mL) was added to reaction mixture under intensive stirring and the mixture was additionally stirred for 15 min. The solution was degassed in vacuum further by filling with H_2 (1 atm) three times. The reaction was left under H_2 (1 atm) with stirring for 24 h. Conversion was controlled by TLC (propan-2-ol/ethyl acetate/water 2:3:1, product *Rf* 0.34). Reaction mixture was filtered through Celite on glass filter, solid material was washed with methanol, portion of 50% aq. methanol and resulting solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue was dissolved in pyridine (100 mL) and Ac_2O (50 mL), reaction mixture was left at 40 °C for 48 h. Reaction mixture was concentrated on rotary evaporator, co-evaporated with toluene.

Crude product **11a** was purified with column chromatography on silica gel (from pure ethyl acetate to ethyl acetate/ propan-2-ol 20:1), the fractions containing major product were collected (TLC control in ethyl acetate/ propan-2-ol 50:1 and chloroform/ propan-2-ol 9:1), concentrated on rotary evaporator, co-evaporated with toluene, co-evaporated with chloroform, dried in vacuum.

Yield of **11a** for four steps: 32% (1.91 g, 1.46 mmol). White solid material; *Rf* 0.24 (n-hexane/acetone, 1:2); $[\alpha]_{546}$ -22° (*c* 1, $CHCl_3$), lit. [19] -22°;

^{1}H spectrum was identical to early described in lit. [10], [19]; ^{1}H NMR ($CDCl_3$): δ 1.17 (d, 3H, $J_{5,6}$ 6.5, H-6^{III}), 1.77–1.84 (m, 1H, OCH_2CHHCH_2N), 1.86–1.94 (m, 1H, OCH_2CHHCH_2N), 1.95, 1.98, 1.98, 2.00, 2.07, 2.09, 2.09, 2.12, 2.12, 2.14, 2.14, 2.16 (12s, 36H, 10 $OC(O)CH_3$, 2 $NHC(O)CH_3$), 3.24–3.31 (m, 1H, OCH_2CH_2CHHN), 3.58–3.67 (m, 2H, OCH_2CH_2CHHN, $OCHHCH_2CH_2N$), 3.69 (ddd, 1H, $J_{4,5}$ 9.6, $J_{5,6a}$ 4.6, $J_{5,6b}$ 1.9, H-5^{I}), 3.75 (dd, 1H, $J_{1,2}$ 7.2, $J_{2,3}$ 9.1, H-2^{II}), 3.82 (dd, 1H, $J_{5,6a}$ 6.8, $J_{5,6b}$ 6.8, H-5^{II}), 3.86 (dd, 1H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.6, H-4^{I}), 3.87 (dd, 1H, $J_{2,3}$ 9.1, $J_{3,4}$ 3.2, H-3^{II}), 3.91–3.95 (m, 1H, $OCHHCH_2CH_2N$), 3.98 (ddd, 1H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.1,

$J_{2,NH}$ 8.9, H-2^{I}), 4.05–4.15 (m, 4H, H-6a^{II}, H-6b^{II}, H-6a^{IV}, H-6b^{IV}), 4.18 (dd, 1H, $J_{5,6a}$ 6.0, $J_{5,6b}$ 6.0, H-5^{IV}), 4.32 (dd, 1H, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 12.2, H-6a^{I}), 4.42 (d, 1H, $J_{1,2}$ 7.2, H-1^{II}), 4.44 (q, 1H, $J_{5,6}$ 6.5, H-5^{III}), 4.46–4.49 (m, 1H, H-2^{IV}), 4.50 (d, 1H, $J_{1,2}$ 8.1, H-1^{I}), 4.55 (dd, 1H, $J_{5,6b}$ 1.9, $J_{6a,6b}$ 12.2, H-6b^{I}), 5.02 (dd, 1H, $J_{2,3}$ 11.3, $J_{3,4}$ 3.0, H-3^{IV}), 5.09 (dd, 1H, $J_{2,3}$ 10.1, $J_{3,4}$ 9.2, H-3^{I}), 5.12 (dd, 1H, $J_{2,3}$ 11.1, $J_{3,4}$ 3.3, H-3^{III}), 5.22 (d, 1H, $J_{1,2}$ 3.5, H-1^{IV}), 5.28 (dd, 1H, $J_{1,2}$ 3.7, $J_{2,3}$ 11.1, H-2^{III}), 5.30 (br. d, 1H, $J_{3,4}$ 3.3 H-4^{III}), 5.38 (br. d, 1H, $J_{3,4}$ 3.2, H-4^{II}), 5.45 (br. s, 1H, H-4^{IV}), 5.51 (d, 1H, $J_{1,2}$ 3.7, H-1^{III}), 5.76 (d, 1H, $J_{2,NH}$ 8.9, NHAc^{I}), 6.17 (d, 1H, $J_{2,NH}$ 7.9, NHAc^{IV}), 7.30–7.36 (m, 1H, NHC(O)CF_3).

(^{1}H NMR spectrum picture can be found in Supporting information)

*3-Aminopropyl O-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-O-[α-L-fucopyranosyl-(1→2)]-O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**1a**)*

Tetrasaccharide **11a** (1.91 g, 1.46 mmol) was dissolved in abs. methanol (115 mL), treated with 2M NaOMe (5.62 mL, 11.3 mmol) and reaction mixture was left for 2 h. The mixture was concentrated in vacuum (not to dryness) and diluted with water (115 mL), reaction mixture was left overnight.

Target deprotected tetrasaccharide was isolated with cation-exchange chromatography on Dowex 50X4-400 (H+) by subsequent elution with water, 1M pyridine water solution and 1M aqueous ammonia, fractions containing the carbohydrate were collected, concentrated in vacuum and freeze dried twice.

Yield of **1a** for two steps: 90% (1.03 g, 1.31 mmol). White solid material; $[\alpha]_D$ +26° (*c* 1, H_2O) lit. [19] +26°;

^{1}H spectrum was identical to early described in lit. [7], [19]; ^{1}H NMR (D_2O+TFA, characteristic signals): δ 1.29 (d, 3H, $J_{5,6}$ 6.6, FucMe), 1.94-2.03 (m, 2H, $OCH_2CH_2CH_2N$), 2.08, 2.09 (2s, 6H,

2 NHC(O)CH_3), 3.12 (t, 2H, *J* 6.7, $OCH_2CH_2CH_2N$), 4.53, 4.63 (2d, 2H, $J_{1,2}$ 8.5, $J_{1,2}$ 7.7, H-1^{I} and H-1^{II}), 5.22, 5.39 (2d, 2H, $J_{1,2}$ 3.8, $J_{1,2}$ 4.1, H-1^{III} and H-1^{IV}).

(^{1}H NMR spectrum picture can be found in Supporting information)

4.2.2 Synthesis of aminopropyl glycoside of B (type 2) tetrasaccharide 1b

*3-Trifluoroacetamidopropyl O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**6b**)*

To ethyl thioglycoside **4b** (15.8 g, 27.0 mmol) solution in abs. dichloromethane (150 mL) was added bromine (1.53 mL, 29.7 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×100 mL) to remove traces of bromine, almost colourless oil of glycosyl bromide was dried in vacuum at r.t.

To disaccharide **3** (14.3 g, 18.0 mmol) solution in abs. dichloromethane (180 mL) were added freshly dried MS-4Å (45 mL) and tetramethylurea (3.9 mL, 32.4 mmol), the mixture stirred for 30 min, then AgOTf (7.63 g, 29.7 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture glycosyl bromide (see above) solution in abs. dichloromethane (90 mL) was added drop-wise under intensive stirring. The reaction was left for 24 h with stirring (TLC control in chloroform/methanol 10:1, *Rf* 0.36). Reaction mixture was neutralized with pyridine (15 mL), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1.

The filtrate was poured in 10% aq. solution of $Na_2S_2O_3$ (180 mL), washed with cold sat. $NaHCO_3$ (170 mL), dried with Na_2SO_4/SiO_2. After filtration, the solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The product **5b** was partially purified with column chromatography on silica gel (from ethyl acetate/n-hexane 1:1 to ethyl acetate/n-hexane 7:1), the fractions containing major product **5b** were collected, concentrated on

rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification (contains admixture of tetramethylurea).

Partially purified product **5b** was dissolved in abs. methanol (900 mL), treated with 2M NaOMe (90 mL, 180 mmol) and reaction mixture was left for 4 h at r.t. Reaction mixture was neutralized with Dowex 50WX4 (180 g, PyH^+-form) and stirred for 15 min and filtered. Obtained solution was concentrated on rotary evaporator, co-evaporated with ethyl acetate, dried in vacuum. Crude material was diluted with ethyl acetate (400 mL), suspension was warmed up to boiling, acetonitrile (~250 mL) was added under reflux to complete dissolving of the crystals. Clear solution was cooled down and left overnight in fridge for complete crystallization. The crystalline product was collected on glass filter, washed with cold ethyl acetate and n-hexane subsequently, dried under fume-hood overnight further by vacuum drying.

Yield of **6b** for two steps: 55% (11.3 g, 9.90 mmol). White crystalline material; *Rf* 0.18 (chloroform/methanol, 10:1); $[\alpha]_D$ could not be measured precisely owing to low solubility of the compound in polar as well nonpolar solvents;

^{1}H NMR ($(CD_3)_2SO$, characteristic signals): δ 1.67-1.74 (m, 2H, $OCH_2CH_2CH_2N$), 1.81 (s, 3H, $NHC(O)CH_3$), 3.15-3.27 (m, 2H, $OCH_2CH_2CH_2N$), 4.24 (d, 1H, $J_{1,2}$ 7.5, H-1^I or H-1^{II}), 5.19 (d, 1H, $J_{1,2}$ 3.5, H-1^{III}), 7.74 (d, 1H, $J_{2,NH}$ 8.3, $NHCOCH_3$), 9.34 (br. t, 1H, $NHCOCF_3$); ^{13}C NMR ($(CD_3)_2SO$, characteristic signals): δ 22.9 ($NHC(O)CH_3$), 28.4 ($OCH_2CH_2CH_2N$), 36.5 ($OCH_2CH_2CH_2N$), 92.5 (C-1^{III}), 100.8, 103.9 (C-1^I and C-1^{II}); ESI HRMS: m/z Calcd $[C_{60}H_{71}F_3N_2O_{17}]H^+$: 1149.4778. Found: 1149.4742.

(^{1}H, ^{13}C and HSQC NMR spectra pictures can be found in Supporting information)

*3-Trifluoroacetamidopropyl O-(2,3,4,6-tetra-O-benzyl- α-D-galactopyranosyl)-(1→3)-O-(4,6-O-benzylidene- β-D-galactopyranosyl)-(1→4)-2-acetamido-6-O-benzyl-2-deoxy- β-D-glucopyranoside (**7b**)*

Trisaccharide **6b** (11.2 g, 9.78 mmol) was suspended in dry acetonitrile (150 mL), then $PhCH(OMe)_2$ (4.5 mL, 30.0 mol) and solution of $TsOH \cdot H_2O$ (180 mg, 0.98 mmol) in dry acetonitrile (3 mL) were added. Reaction mixture was stirred overnight at r.t. The reaction was neutralised with pyridine (5 mL). Diethyl ether (300 mL) was added under stirring, and formed crystalline product was collected on glass filter, washed with diethyl ether, n-hexane, dried in vacuum.

Yield of **7b**: 89% (10.8 g, 8.70 mmol). White crystalline material; *Rf* 0.30 (chloroform/methanol, 10:1); $[\alpha]_D$ +24° (*c* 0.9, $CHCl_3$);

^{1}H NMR ($(CD_3)_2SO$, characteristic signals): δ 1.66-1.76 (m, 2H, $OCH_2CH_2CH_2N$), 1.81 (s, 3H, $NHC(O)CH_3$), 3.15-3.27 (m, 2H, $OCH_2CH_2CH_2N$), 5.33 (d, 1H, $J_{1,2}$ 3.5, H-1^{III}), 5.64 (s, 1H, PhC*H*), 7.75 (d, 1H, $J_{2,NH}$ 8.3, $NHCOCH_3$), 9.34 (br. t, 1H, $NHCOCF_3$); ^{13}C NMR ($(CD_3)_2SO$, characteristic signals): δ 22.9 ($NHC(O)CH_3$), 28.4 ($OCH_2CH_2CH_2N$), 36.5 ($OCH_2CH_2CH_2N$), 91.8 (C-1^{III}), 99.7 (Ph*C*H), 100.9, 103.4 (C-1^{I} and C-1^{II}); ESI HRMS: m/z Calcd $[C_{67}H_{75}F_3N_2O_{17}]H^+$: 1237.5091. Found: 1237.5033.

(^{1}H, ^{13}C, COSY and HSQC NMR spectra pictures can be found in Supporting information)

*3-Trifluoroacetamidopropyl O-(2,3,4,6-tetra-O-benzyl- α-D-galactopyranosyl)-(1→3)-O-(4,6-O-benzylidene- β-D-galactopyranosyl)-(1→4)-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy- β-D-glucopyranoside (**8b**)*

To powder of trisaccharide **7b** (10.2 g, 8.24 mmol) was added solution of chloro acetic anhydride (4.23 g, 24.7 mmol) and sym-Collidine (22.0 mL, 165 mmol) in abs. dichloromethane (55 mL) under intensive stirring. The mixture was stirred for 3 h at 0°C. The reaction was quenched with methanol (20 mL), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Resulting oil was dissolved in abs. dichloromethane (107 mL) followed by addition of sym-Collidine (25 mL, 190 mmol) and acetyl chloride (10.0 mL, 140 mmol). Reaction mixture was left for 24 h at r.t. Reaction was quenched with methanol (40 mL), concentrated on rotary evaporator, co-evaporated with toluene. The residue was diluted with toluene (200 mL), stirred for 15 min, collidinium salt was collected on glass filter, washed few times with toluene. The filtered solution was concentrated on rotary evaporator, dried in vacuum.

The residue was dissolved in pyridine (200 mL), water (100 mL) was added and mixture warmed at 60 °C for 24 h. The mixture was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Crude product **8b** was purified with column chromatography on silica gel (chloroform/acetone, from 5:1 to 3:1, 0.5% pyridine), the fractions containing major product were collected, concentrated on rotary evaporator (not to dryness). Concentrated solution was diluted with ethyl acetate (100 mL), n-hexane (100 mL) was added drop-wise under stirring to precipitate pure trisaccharide **8b** and the mixture was left overnight in fridge for complete precipitation.

Solid product was collected on glass filter, washed with little portion of n-hexane/ethyl acetate 1:1, n-hexane/ethyl acetate 3:1, pure n-hexane, dried in vacuum.

Yield of **8b** for three steps: 73% (7.74 g, 6.05 mmol). White crystalline material; *Rf* 0.45 (n-hexane/chloroform/propan-2-ol, 4:2:1); $[\alpha]_D$ +16° (*c* 1.2, $CHCl_3$);

^{1}H NMR ($CDCl_3$, characteristic signals): δ 1.47-1.71 (m, 1H, OCH_2CHHCH_2N), 1.98, 2.09 (2s, 6H, $OC(O)CH_3$ and $NHC(O)CH_3$), 2.91-3.024 (m, 1H, OCH_2CH_2CHHN), 5.14 (d, 1H, $J_{1,2}$ 3.2, H-1^{III}), 5.28 (dd, 1H, $J_{2,3} \approx J_{3,4}$ 9.8, H-3^{I}), 5.42 (s, 1H, PhC*H*), 5.72 (d, 1H, $J_{2,NH}$ 7.1, $NHCOCH_3$); ^{13}C NMR ($CDCl_3$, characteristic signals): 21.1, 23.5 ($OC(O)CH_3$ and $NHC(O)CH_3$), 28.3 ($OCH_2CH_2CH_2N$), 38.2 ($OCH_2CH_2CH_2N$), 73.4 (C-3^{I}), 94.6 (C-1^{III}), 101.5 (PhCH); ESI HRMS: m/z Calcd $[C_{69}H_{77}F_3N_2O_{18}]H^+$: 1279.5196. Found: 1279.5111.

(^{1}H, ^{13}C, COSY and HSQC NMR spectra pictures can be found in Supporting information)

*3-Trifluoroacetamidopropyl O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→3)-O-[2,3,4-tri-O-acetyl-α-L-fucopyranosyl-(1→2)]-O-(4,6-di-O-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**11b**)*

To ethyl thioglycoside **9** (5.78 g, 12.1 mmol) solution in abs. dichloromethane (75 mL) was added bromine (0.69 mL, 13.3 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×50 mL) to remove traces of bromine, almost colourless oil of glycosyl bromide was dried in vacuum at r.t.

To **8b** (7.74 g, 6.05 mmol) solution in abs. dichloromethane (150 mL) were added freshly dried MS-4Å (75 mL) and tetramethylurea (1.6 mL, 13.3 mmol), the mixture stirred for 30 min, then AgOTf (3.11 g, 12.1 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture glycosyl bromide (see above) solution in abs. dichloromethane (75 mL) was added drop-wise under intensive stirring. The reaction was left for 24 h with stirring (TLC control in ethyl acetate/toluene 3:1, *Rf* 0.50). Reaction mixture was neutralized with pyridine (6 mL), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1.

Filtered solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue was dissolved in acetic acid (200 mL), water (50 mL) was added under stirring and the mixture was warmed at 80 °C for 3 h (TLC control in pure ethyl acetate, *Rf* 0.45). The mixture was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Crude product **10b** was partially purified with column chromatography on silica gel (from toluene/ethyl acetate 1:1 to pure ethyl acetate), the fractions containing major product were collected (TLC control in pure ethyl acetate), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification (contains admixture of tetramethylurea).

The product **10b** was dissolved in methanol (300 mL), then 10% Pd/C (3.0 g) was added and reaction mixture was degassed in vacuum further by filling with H_2 (1 atm) three times. The reaction was left under H_2 (1 atm) with stirring for 48 h at r.t. Conversion was controlled by TLC (propan-2-ol/ethyl acetate/water 2:3:1, product *Rf* 0.35). Reaction mixture was filtered through Celite on glass filter, solid material was washed with methanol, portion of 50% aq. methanol and resulting solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue was dissolved in pyridine (100 mL) and Ac_2O (50 mL), reaction mixture was left at 40 °C for 24 h. Reaction mixture was concentrated on rotary evaporator, co-evaporated with toluene.

Crude product **11b** was purified with column chromatography on silica gel (from pure ethyl acetate to ethyl acetate/ propan-2-ol 50:1), the fractions containing major product were collected (TLC control in ethyl acetate/ propan-2-ol 50:1 and chloroform/ propan-2-ol 9:1), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Prepurified product **11b** was dissolved in ethyl acetate (100 mL). To resulting solution was added n-hexane drop-wise under stirring till light turbidity appeared, after crystallization started, addition of n-hexane (to whole solution volume 400 mL) was continued, the mixture allowed to crystallize for 12 h. Crystalline material was collected on glass filter, washed with n-hexane/ethyl acetate 3:1, pure n-hexane. Recrystallization procedure was repeated with the same amount of solvents, obtained crystalline material was dried in vacuum.

Yield of **11b** for four steps: 35% (2.78 g, 2.12 mmol). White crystalline material; *Rf* 0.35 (chloroform/propan-2-ol, 9:1); $[\alpha]_{546}$ -11° (*c* 1, $CHCl_3$), lit. [19] -11°;

^{1}H spectrum was identical to early described in lit. [10], [19]; ^{1}H NMR ($CDCl_3$): δ 1.16 (d, 3H, $J_{5,6}$ 6.5, H-6^{III}), 1.78–1.84 (m, 1H, OCH_2CHHCH_2N), 1.86–1.90 (m, 1H, OCH_2CHHCH_2N), 1.91, 1.97, 1.99, 1.99, 2.07, 2.07, 2.07, 2.08, 2.10, 2.11, 2.15, 2.16 (12s, 36H, 11 $OC(O)CH_3$, 1 $NHC(O)CH_3$), 3.24–3.31 (m, 1H, OCH_2CH_2CHHN), 3.56 (dd, 1H, $J_{1,2}$ 7.6, $J_{2,3}$ 10.0, H-2^{II}), 3.57–3.61 (m, 1H, $OCHHCH_2CH_2N$), 3.61–3.66 (m, 1H, OCH_2CH_2CHHN), 3.78 (dd, 1H, $J_{5,6a}$

6.6, $J_{5,6b}$ 6.6, H-5^{II}), 3.79–3.81 (m, 1H, H-5^{I}), 3.88 (dd, 1H, $J_{2,3}$ 10.0, $J_{3,4}$ 2.8, H-3^{II}), 3.91 (dd, 1H, $J_{3,4}$ 8.8, $J_{4,5}$ 8.2, H-4^{I}), 3.92–3.94 (m, 1H, OCH*H*CH_2CH_2N), 4.02 (ddd, 1H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.3, $J_{2,NH}$ 8.8, H-2^{I}), 4.06–4.13 (m, 3H, H-6a^{II}, H-6b^{II}, H-6a^{IV}), 4.24 (dd, 1H, $J_{5,6b}$ 6.9, $J_{6a,6b}$ 11.2, H-6b^{IV}), 4.39 (d, 1H, $J_{1,2}$ 7.6, H-1^{II}), 4.39–4.43 (m, 2H, H-6a^{I}, H-5^{III}), 4.44–4.48 (m, 2H, H-6b^{I}, H-5^{IV}), 4.49 (d, 1H, $J_{1,2}$ 7.7, H-1^{I}), 5.05 (dd, 1H, $J_{2,3}$ 11.1, $J_{3,4}$ 3.4, H-3^{III}), 5.07 (dd, 1H, $J_{2,3}$ 9.3, $J_{3,4}$ 8.8, H-3^{I}), 5.21 (dd, 1H, $J_{1,2}$ 3.7, $J_{2,3}$ 11.1, H-2^{III}), 5.25 (br. d, 1H, $J_{3,4}$ 3.4, H-4^{III}), 5.32–5.36 (m, 2H, H-1^{IV}, H-2^{IV}), 5.39–5.44 (m, 2H, H-4^{II}, H-3^{IV}), 5.45 (d, 1H, $J_{1,2}$ 3.7, H-1^{III}), 5.60 (br. d, 1H, $J_{3,4}$ 2.7, H-4^{IV}), 5.86 (d, 1H, $J_{2,NH}$ 8.8, NHAc^{I}), 7.39–7.44 (m, 1H, NHC(O)CF_3);

(^{1}H NMR spectrum picture can be found in Supporting information)

*3-Aminopropyl O-(α-D-galactopyranosyl)-(1→3)-O-[α-L-fucopyranosyl-(1→2)]-O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**1b**)*

Tetrasaccharide **11b** (2.50 g, 1.98 mmol) was dissolved in abs. methanol (150 mL), treated with 2M NaOMe (7.50 mL, 15 mmol) and reaction mixture was left for 2 h. The mixture was concentrated in vacuum (not to dryness) and diluted with water (150 mL), reaction mixture was left overnight.

Target deprotected tetrasaccharide was isolated with cation-exchange chromatography on Dowex 50X4-400 (H+) by subsequent elution with water, 1M pyridine water solution and 1M aqueous ammonia, fractions containing the carbohydrate were collected, concentrated in vacuum and freeze dried twice.

Yield of **1b** for two steps: 90% (1.33 g, 1.78 mmol). White solid material; ^{1}H spectrum was identical to early described in lit. [7], [19]; $[\alpha]_D$ -10° (*c* 1, H_2O), lit. [19] -10°;

^{1}H NMR (D_2O+TFA, characteristic signals): δ 1.29 (d, 3H, $J_{5,6}$ 6.6, FucMe), 1.97-2.05 (m, 2H, $OCH_2CH_2CH_2N$), 2.11 (s, 3H, NHC(O)CH_3), 3.14 (t, 2H, *J* 7.0, $OCH_2CH_2CH_2N$), 4.55, 4.67 (2d, 2H, $J_{1,2}$ 8.5, $J_{1,2}$ 7.7, H-1^{I} and H-1^{II}), 5.30, 5.38 (2d, 2H, $J_{1,2}$ <2.0, $J_{1,2}$ 4.2, H-1^{III} and H-1^{IV}).

(^{1}H NMR spectrum picture can be found in Supporting information)

4.2.3 Synthesis of trisaccharide 12a

*3-Aminopropyl O-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**12a**)*

To ethyl thioglycoside **4a** (3.76 g, 7.24 mmol) solution in abs. dichloromethane (40 mL) was added bromine (0.41 mL, 8.0 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×30 mL) to remove traces of bromine, almost colourless oil of glycosyl bromide was dried in vacuum at r.t.

To disaccharide **3** (3.82 g, 4.82 mmol) solution in abs. dichloromethane (50 mL) were added freshly dried MS-4Å (8 mL) and tetramethylurea (1.08 mL, 8.87 mmol), the mixture stirred for 30 min, then AgOTf (2.04 g, 7.82 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture glycosyl bromide (see above) solution in abs. dichloromethane (20 mL) was added drop-wise under intensive stirring. The reaction was left for 24 h with stirring (TLC control in chloroform/methanol 10:1, *Rf* 0.47). Reaction mixture was neutralized with pyridine (4 mL), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1. The filtrate was poured in 10% aq. solution of $Na_2S_2O_3$ (60 mL), washed with cold sat. $NaHCO_3$ (50 mL), dried with Na_2SO_4/SiO_2.

After filtration, the solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The product **5a** was partially purified with column chromatography on silica gel (from toluene/acetone 5:1 to toluene/acetone 3:1), the fractions containing major product **5a** were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification (contains admixture of tetramethylurea).

The partially purified product **5a** was dissolved in methanol (300 mL), then 10% Pd/C (4.0 g) was added and reaction mixture was degassed in vacuum further by filling with H_2 (1 atm) three

times. The reaction was left under H_2 (1 atm) with stirring for 12 h. Acetic anhydride (15 mL) was added to reaction mixture under intensive stirring and the mixture was additionally stirred for 15 min. The solution was degassed in vacuum further by filling with H_2 (1 atm) three times. The reaction was left under H_2 (1 atm) with stirring for 24 h. Conversion was controlled by TLC (propan-2-ol/ethyl acetate/water 2:10:1). Reaction mixture was filtered through Celite on glass filter, solid material was washed with methanol, portion of 50% aq. methanol and resulting solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue was dissolved in pyridine (100 mL) and Ac_2O (50 mL) and left at 40 °C for 48 h. Reaction mixture was concentrated on rotary evaporator, co-evaporated with toluene.

Crude product **13a** (chloroform/methanol 10:1, *Rf* 0.36)was partially purified with column chromatography on silica gel (from pure ethyl acetate to ethyl acetate/propan-2-ol 20:1), the fractions containing major product were collected, concentrated on rotary evaporator, co-evaporated with toluene, co-evaporated with chloroform, dried in vacuum. The product **13a** contains admixture of tetramethylurea, but being amorphous solid cannot be completely purified with crystallization [34].

Partially purified trisaccharide **13a** was dissolved in abs. methanol (230 mL), treated with 2M NaOMe (11.2 mL, 22.6 mmol) and reaction mixture was left for 2 h. The mixture was concentrated in vacuum (not to dryness) and diluted with water (230 mL), reaction mixture was left overnight.

Target deprotected trisaccharide was isolated with cation-exchange chromatography on Dowex 50X4-400 (H+) by subsequent elution with water, 1M pyridine water solution and 1M aqueous ammonia, fractions containing the carbohydrate were collected, concentrated in vacuum and freeze dried twice.

Yield of **12a** for five steps: 49% (1.50 g, 2.34 mmol). White solid material; $[\alpha]_{546}$ +128° (*c* 0.3, MeCN-H_2O, 1:1), lit. [34] +128°;

^{1}H spectrum was identical to early described in lit. [34]; ^{1}H NMR (D_2O+TFA, characteristic signals): δ 1.97-2.05 (m, 2H, $OCH_2CH_2CH_2N$), 2.10, 2.11 (2s, 6H, 2 $NHC(O)CH_3$), 3.14 (t, 2H, *J* 7.0, $OCH_2CH_2CH_2N$), 4.57-4.60 (m, 2H, H-1^I and H-1^{II}), 5.14 (d, 1H, $J_{1,2}$ 3.7, H-1^{III}).
(^{1}H NMR spectrum picture can be found in Supporting information)

4.2.4 Synthesis of trisaccharide 12b (Aminopropyl glycoside of *Galili* trisaccharide)

*3-Trifluoroacetamidopropyl O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**13b**)*

To ethyl thioglycoside **4b** (4.11 g, 7.02 mmol) solution in abs. dichloromethane (40 mL) was added bromine (0.40 mL, 7.72 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×30 mL) to remove traces of bromine, almost colourless oil of glycosyl bromide was dried in vacuum at r.t.
To disaccharide **3** (3.72 g, 4.68 mmol) solution in abs. dichloromethane (50 mL) were added freshly dried MS-4Å (12 mL) and tetramethylurea (1.0 mL, 8.42 mmol), the mixture stirred for 30 min, then AgOTf (2.0 g, 7.72 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture glycosyl bromide (see above) solution in abs. dichloromethane (25 mL) was added drop-wise under intensive stirring. The reaction was left for 24 h with stirring (TLC control in chloroform/methanol 10:1, *Rf* 0.36). Reaction mixture was neutralized with pyridine (4 mL), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1.
The filtrate was poured in 10% aq. solution of $Na_2S_2O_3$ (60 mL), washed with cold sat. $NaHCO_3$ (50 mL), dried with Na_2SO_4/SiO_2. After filtration, the solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The product **5b** was partially purified

with column chromatography on silica gel (from ethyl acetate/n-hexane 1:1 to ethyl acetate/n-hexane 7:1), the fractions containing major product **5b** were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification (contains admixture of tetramethylurea).

Partially purified product **5b** was dissolved in methanol (300 mL), then 10% Pd/C (4.0 g) was added and reaction mixture was degassed in vacuum further by filling with H_2 (1 atm) three times. The reaction was left under H_2 (1 atm) with stirring for 24 h. Conversion was controlled by TLC (propan-2-ol/ethyl acetate/water 2:10:1). Reaction mixture was filtered through Celite on glass filter, solid material was washed with methanol, portion of 50% aq. methanol and resulting solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue was dissolved in pyridine (100 mL) and Ac_2O (50 mL), left at 40 °C for 48 h. Reaction mixture was concentrated on rotary evaporator, co-evaporated with toluene.

Crude product **13b** was purified with column chromatography on silica gel (from pure ethyl acetate to ethyl acetate/ propan-2-ol 20:1), the fractions containing major product were collected, concentrated on rotary evaporator, co-evaporated with toluene and dried in vacuum. Pure product **13b** was precipitated from concentrated ethyl acetate solution with excess of n-hexane, filtered, dried in vacuum.

Yield of **13b** for three steps: 56% (2.81 g, 2.61 mmol). White crystalline material; *Rf* 0.45 (chloroform/propan-2-ol, 10:1); $[\alpha]_D$ +29° (*c* 1.2, $CHCl_3$);

^{1}H NMR ($CDCl_3$): δ 1.75–1.84 (m, 1H, OCH_2CHHCH_2N), 1.86–1.92 (m, 1H, OCH_2CHHCH_2N), 1.95, 1.97, 2.06, 2.06, 2.06, 2.08, 2.10, 2.11, 2.14, 2.15 (10s, 30H, 9 $OC(O)CH_3$, 1 $NHC(O)CH_3$), 3.21–3.30 (m, 1H, OCH_2CH_2CHHN), 3.52–3.59 (m, 1H, $OCHHCH_2CH_2N$), 3.59–3.66 (m, 2H, OCH_2CH_2CHHN, H-5^{I}), 3.77 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 8.4, H-4^{I}), 3.82 (dd, 1H, $J_{5,6a} \approx J_{5,6b}$ 6.7, H-5$^{II/III}$), 3.86 (dd, 1H, $J_{3,4}$ 3.0, $J_{2,3}$ 10.3, H-3^{II}), 3.88-3.92 (m, 1H, $OCHHCH_2CH_2N$), 3.99-4.24 (m, 7H, H-2^{I}, H-5$^{III/II}$, H-6a^{I}, H-6a^{II}, H-6b^{II}, H-6a^{III}, H-6b^{III}), 4.42 (d, 1H, $J_{1,2}$ 7.7, H-1^{I}), 4.45 (d, 1H, $J_{1,2}$ 7.8, H-1^{II}), 4.51 (dd, 1H, $J_{5,6}$ 2.6, $J_{6a,6b}$ 11.8, H-6b^{I}),

5.06 (dd, 1H, $J_{2,3} \approx J_{3,4}$ 8.7, H-3^{I}), 5.11 (dd, 1H, $J_{3,4}$ 3.2, $J_{2,3}$ 10.8, H-3^{III}), 5.16 (dd, 1H, $J_{1,2}$ 7.9, $J_{2,3}$ 10.2, H-2^{II}), 5.22-5.30 (m, 2H, H-1^{III}, H-2^{III}), 5.34 (d, 1H, $J_{3,4}$ 2.3, H-4^{II}), 5.45 (d, 1H, $J_{3,4}$ < 2.5, H-4^{III}), 5.92 (d,1H, $J_{2,NH}$ 8.9, N*H*COCH$_3$), 7.41 (br.t, 1H, NHCOCF$_3$); ^{13}C NMR ($CDCl_3$): 20.7, 20.7, 20.7, 20.7, 20.8, 20.8, 20.9, 20.9, 21.0, 23.4 (9 OC(O)*C*H$_3$, 1 NHC(O)*C*H$_3$), 28.6 (OCH$_2$*C*H$_2$CH$_2$N), 37.5 (OCH$_2$CH$_2$*C*H$_2$N), 53.5 (C-2^{I}), 61.2, 61.4 (C-6^{II}, C-6^{III}), 62.2 (C-6^{I}), 64.8 (C-4^{II}), 66.3 (C-2^{III}), 67.0, 67.0, 67.3 (O*C*H$_2$CH$_2$CH$_2$N, C-3^{III}, C-5$^{II/III}$), 67.8 (C-4^{III}), 70.0 (C-2^{II}), 71.0 (C-5$^{III/II}$), 72.6 (C-3^{I}), 72.9 (C-3^{II}), 73.2 (C-5^{I}), 75.4 (C-4^{I}), 93.7 (C-1^{III}), 101.2 (C-1^{II}), 101.5 (C-1^{I}), 169.1, 169.8, 170.0, 170.2, 170.3, 170.3, 170.5, 170.5, 170.8, 170.8 (9 O*C*(O)CH$_3$, 1 NH*C*(O)CH$_3$); ESI MS: m/z Calcd $[C_{43}H_{59}F_3N_2O_{26}]^+$: 1076.3. Found: 1076.8. (^{1}H, ^{13}C, COSY and HSQC NMR spectra pictures can be found in Supporting information)

*3-Aminopropyl O-(α-D-galactopyranosyl)-(1→3)-O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**12b**)*

Trisaccharide **13b** (2.81 g, 2.61 mmol) was dissolved in abs. methanol (230 mL), treated with 2M NaOMe (11.2 mL, 22.6 mmol) and reaction mixture was left for 2 h. The mixture was concentrated in vacuum (not to dryness) and diluted with water (230 mL), reaction mixture was left overnight.

Target deprotected trisaccharide was isolated with cation-exchange chromatography on Dowex 50X4-400 (H+) by subsequent elution with water, 1M pyridine water solution and 1M aqueous ammonia, fractions containing the carbohydrate were collected, concentrated in vacuum and freeze dried twice.

Yield of **12b** for two steps: 91% (1.42 g, 2.36 mmol). White solid material; $[\alpha]_D$ +54° (*c* 1, H_2O), lit. [7] +54°;

^{1}H spectrum was identical to early described in lit. [7] ^{1}H NMR (D_2O+TFA, characteristic signals): δ 1.94-2.05 (m, 2H, OCH$_2$C*H$_2$*CH$_2$N), 2.10 (s, 3H, NHC(O)C*H$_3$*), 3.13 (t, 2H, *J* 6.8,

$OCH_2CH_2CH_2N$), 4.57, 4.59 (2d, 2H, $J_{1,2}$ 8.1, $J_{1,2}$ 7.9, H-1^{I} and H-1^{II}), 5.19 (d, 1H, $J_{1,2}$ 3.5, H-1^{III}).

(^{1}H NMR spectrum picture can be found in Supporting information)

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Highlights

- Synthesis of type 2 A and B glycans with fucosylation as last step is practical.
- The strategy includes preparation of *Galili*-trisaccharide as key intermediate.
- Chloroacetylation distinguishes 2`-OH/3-OH groups in intermediate trisaccharides.

Declaration of interests

☑The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: