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The Synthesis of Blood Group Antigenic A Trisaccharide and Its Biotinylated Derivative

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Abstract: Blood group antigenic A trisaccharide represents the terminal residue of all A blood group antigens and plays a key role in blood cell recognition and blood group compatibility. Herein, we describe the synthesis of the spacered A trisaccharide by means of an assembly scheme that employs in its most complex step the recently proposed glycosyl donor of the 2-azido-2-deoxy-selenogalactoside type, bearing stereocontrolling 3-O-benzoyl and 4,6-O-(di-tert-butylsilylene)-protecting groups. Its application provided efficient and stereoselective formation of the required α-glycosylation product, which was then deprotected and subjected to spacer biotinylation to give both target products, which are in demand for biochemical studies.

Keywords: blood group determinants; carbohydrates; stereoselective glycosylation

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1. Introduction

Since the discovery of the ABO blood group system and the role of carbohydrate residues in blood antigens [1,2], there has been continued interest in developing new synthetic approaches to the assembly of carbohydrate blood group antigen determinants [3–10]. Besides playing an important role in blood cell recognition and blood group compatibility, blood transfusion, and organ transplantation [11–13], A trisaccharide and structurally related compounds can be used as haptens to test the carbohydrate specificities of plant [14] and mammalian lectins [15,16] and serve as a model for conformational and spectral studies [17] of vicinally branched oligosaccharides. A trisaccharide derivatives can also serve as model compounds in the development of new biomedical technologies, since antibodies against this carbohydrate antigen are commercially available.

A trisaccharide represents the minimal terminal fragment of all blood group A antigens. It has a branched structure where the central β-Gal residue is glycosylated with α-fucose at O-2 and with α-galactosamine at O-3 (see Figure 1). Despite numerous works devoted to the synthesis of oligosaccharides related to blood group antigens, there are only a few papers dedicated specifically to the synthesis of A trisaccharide derivatives [4–6,9]. Herein, we report on the assembly of spacered A trisaccharide **1a** and its biotinylated derivative **1b**, making use of the new galactose block **4**, which bears a set of convenient temporary protecting groups permitting selective liberation of HO-groups as well fucosyl donor **5** [18,19] and bicyclic 2-azido-2-deoxy-selenogalactoside **10** [20] containing stereo-controlling O-protecting groups, which favor the required α-(1,2-*cis*)-glycosylation. A bulky 4,6-O-(di-tert-butylsilylene)-protecting group at O-4 and O-6 was used to prevent the formation of undesirable β-glycosylation products [21] while a 3-O-benzoyl group was introduced to provide α-stereocontrol through remote anchimeric participation [22].

Donor **10** was recently proposed [20,23] and has been successfully applied in the

syntheses of complex linear oligosaccharides related to bacterial and fungal antigenic polysaccharides (see a mini-review [23] and references therein). At the same time, there is still little published data on the glycosylation of vicinally branched oligosaccharides by means of 2-azido-2-deoxy-selenogalactoside donors as well as the application of the donors in the regioselective glycosylation of diolic acceptors. The studies discussed below were planned to fill these gaps.

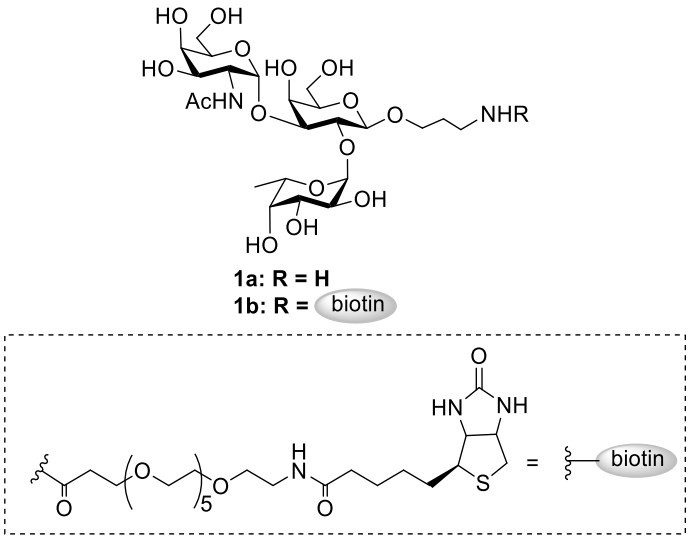
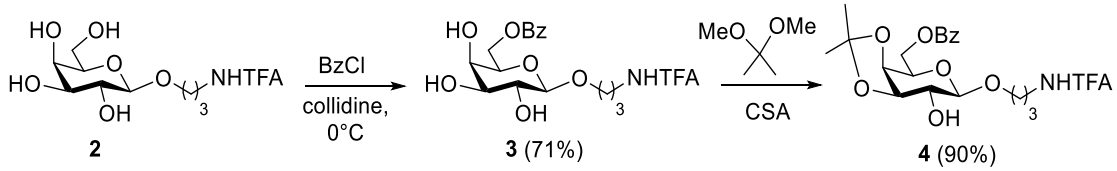


Figure 1. Target spacered A trisaccharide derivatives **1a** and **1b**

2. Results and Discussion

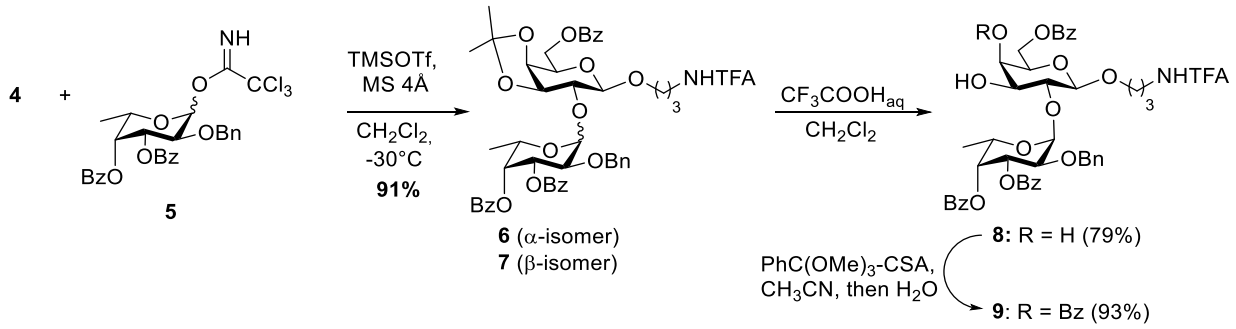
Key steps in the synthesis of spacered A trisaccharide **1a** were the regio- and stereoselective building of three glycosidic bonds. While the formation of a β-glycoside bond is a straightforward task, α-glycosylation by fucosyl and galactosamine donors requires the careful selection of protective groups and experimental conditions effective for α-glycoside bond formation [24]. To promote the desired stereoselectivity, a new type of galactosyl acceptor **4** was easily synthesized from tetraol **2** [25] through the introduction of 3,4-O-isopropylidene and 6-O-benzoyl groups, which can be selectively removed in the presence of other protecting O-substituents (Scheme 1).



Scheme 1. Synthesis of galactosyl acceptor **4**.

To form the fucosyl block, donor **5** was used. This compound contains two benzoyl protecting groups at O-3 and O-4, which, despite reducing the donor's activity, provide effective α-directing glycosylation stereocontrol through remote anchimeric participation [18,19]. Fucosylation of galactoside **4** proceeded stereoselectively, giving an inseparable mixture of α-isomer **6** and βisomer **7** (Scheme 2) in the ratio ~9:1 with a yield of 91%. The anomeric configurations of the Fuc units in disaccharides **6** and **7** were confirmed by the characteristic values of the corresponding C-1 signals in ^{13}C NMR spectra and $J_{1,2}$ constants in the 1H NMR spectra (for **6**: 95.5 ppm and 3.4 Hz; for **7**: and 103.3 ppm and 8.0 Hz, respectively). The individual α-isomer was purified after the removal of the O-isopropylidene group, which gave the desired diol **8** in a 79% yield. The value of coupling constant $J_{1,2}$ (3.6 Hz) confirmed the α-configuration of the Fuc unit in **8**. In addition

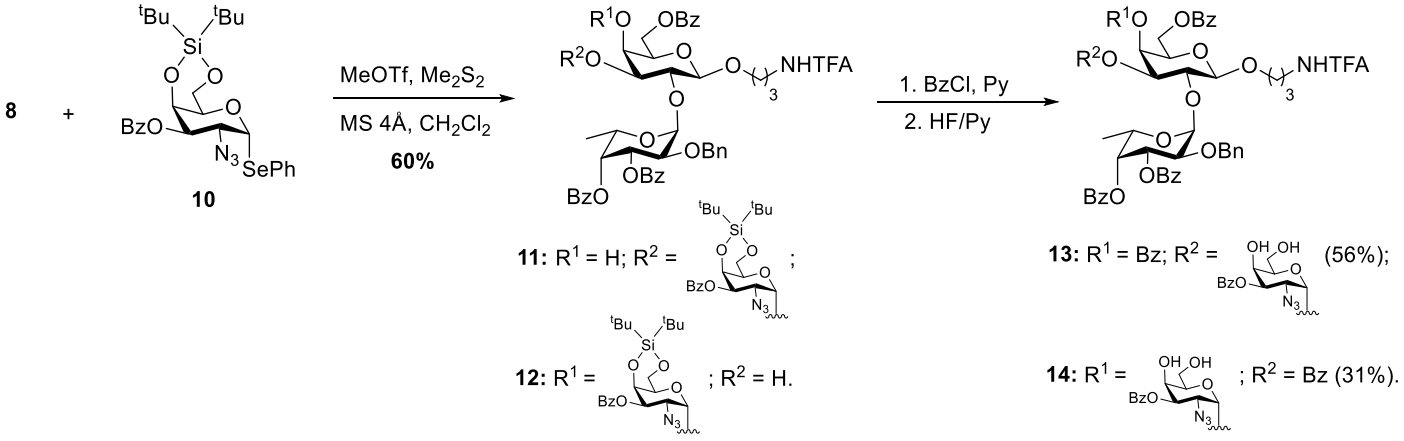
to diol **8,** its monohydroxy-derivative **9** was prepared by treatment with trimethyl orthobenzoate [26] followed by hydrolytic opening of the 3,4-O-orthobenzoic intermediate in an overall yield of 93% (Scheme 2). The presence of the 4-O-benzoyl group was confirmed by a downfield shift of the H-4Gal signal by 1.77 ppm between the spectra for compounds **8** and **9**.



Scheme 2. Synthesis of disaccharides **8** and **9**.

The last step in the assembly of the trisaccharide A backbone was the glycosylation of disaccharide **8** with 2-azido-2-deoxy-galactosyl donor **10**, which was prepared via azidophenylselenylation of a triacetylgalactal [23,27] and subsequent selective protection. The glycosylation α-stereoselectivity of donors of this type can be regulated by the reaction solvent [28] and specially selected types of O-protective groups [20–23,29,30].

It is known that equatorial hydroxyl groups are usually more reactive than axial ones [31,32]. Based on this assumption, we suggest that a regioselective 3-O-glycosylation of diol **8** would be possible. However, the reaction between disaccharide **8** and donor **10** yielded an inseparable mixture of products (Scheme 3). Presumably, it consisted of regioisomers **11** and **12** in a 2.8:1 ratio (NMR data), which were formed via (1→3)- and (1→4)-glycosylation, respectively.

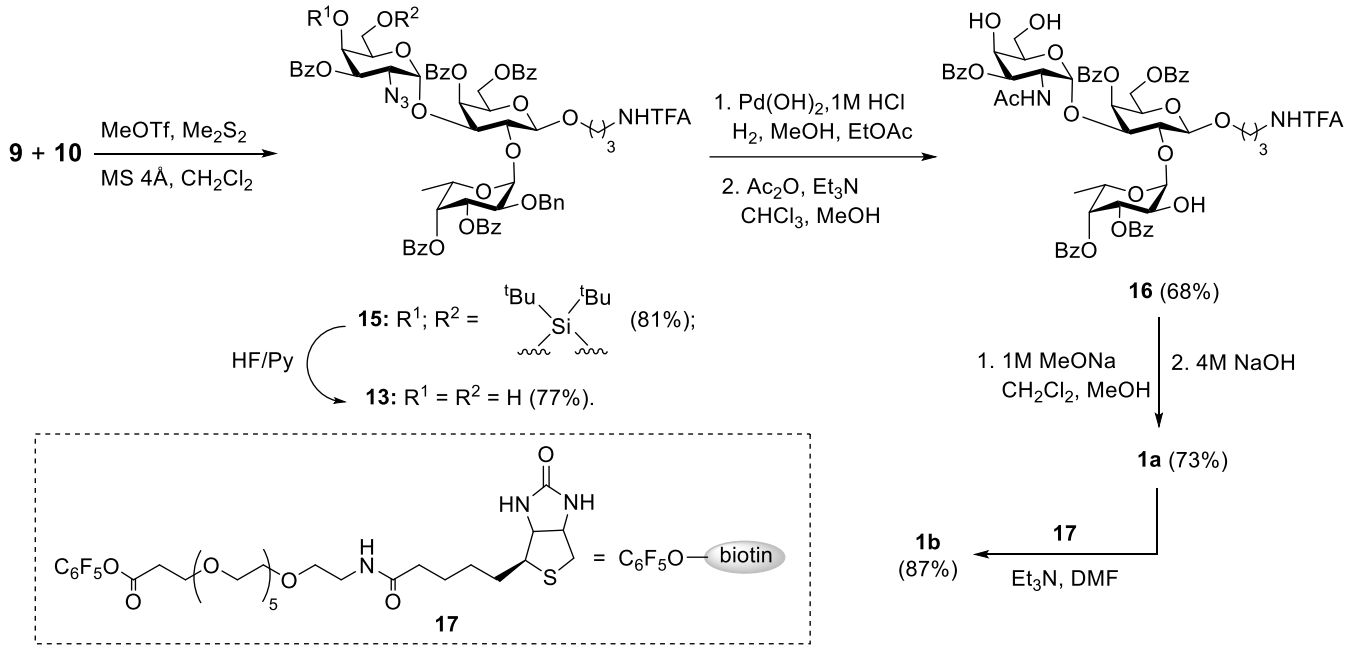


Scheme 3. Glycosylation of disaccharide **8** with donor **10**.

To check our assumption, we treated the glycosylation products with BzCl in Py and then removed the 4,6-O-(di-tert-butylsilylene)-protection with HF/Py. As result, we obtained two separate compounds: α-3-O- and α-4-O-glycosylation products **13** and **14**, which were identified by NMR spectroscopy. In particular, the formation of α-glycoside bonds was confirmed by coupling constant $J_{1,2}$ for the GalN-unit (3.8 and 3.6 Hz in ^{1}H NMR spectra for **13** and **14**, respectively). Regioselectivity of the glycosylation reaction was confirmed by comparing the downfield signals H-3Gal and H-4Gal relative to each other in the ^{1}H NMR spectra (**13**: 4.24 ppm for H-3Gal; 5.99 ppm for H-4Gal; **14**: 5.42 ppm

for H-3Gal; 4.46 ppm for H-4Gal) and by downfield signals C-3Gal of **13** (73.2 ppm) and C-4Gal of **14** (74.5 ppm) in the ^{13}C NMR spectra.

As an alternative method to conduct 3-O-glycosylation with donor **10,** we used monohydroxy-acceptor **9** (Scheme 4). As expected, the coupling of compounds **9** and **10** was stereoselective and gave the desired trisaccharide **15** in an 81% yield, contaminated by traces of isomeric product that was formed due to the migration of a benzoyl group in **9** from O-4 to O-3 in the galactose unit during the reaction. Further removal of the di-tert-butylsilylene-group by HF/Py solution and chromatography purification gave the individual diol **13**. The α-configuration of the glycoside bond at the GalN-unit of **13** was confirmed by the characteristic value of the corresponding coupling constant $J_{1,2}$ (3.8 Hz) in the ^1H NMR spectrum. Hydrogenolysis of **13** to remove the 2-O-benzyl group at the fucosyl residue and reduce the azide substituent to an amine and subsequent N-acetylation resulted in the formation of trisaccharide **16** in an overall yield of 68%. Its saponification gave the target spacered A trisaccharide **1a** (73%), which was then treated with the biotin derivative bearing an activated ester group **17** [33] to give the glycoconjugate **1b**.



Scheme 4. Assembly of the A trisaccharide backbone and preparation of target compound **1a** and its biotinylated derivative **1b**.

3. Materials and Methods

3.1. General Information

All reagents were purchased at Sigma-Aldrich unless otherwise noted. MeCN and CH_2Cl_2 were distilled over P_2O_5 and CaH_2. MeOH was distilled over $Mg(OMe)_2$. Anhydrous pyridine and DMF were commercial (Sigma-Aldrich). Molecular sieves AW-300 MS (4Å) were crushed and activated before reaction for 5 min at 400–500 °C in vacuo. Amberlite IR-120 (hydrogen form, Fluka) was washed with 1M aq. HCl, H_2O, acetone, and dried.

Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F254 (Merck). TLC plates were inspected under UV light (λ = 254 nm) and developed with treatment by a mixture of 15% H_3PO_4 and orcinol (1.8 g/L) in EtOH–H_2O (5: 95, *v*/*v*) followed by heating. Flash chromatography was performed on a Buchi Reveleris X2 system using Buchi FlashPure EcoFlex cartridges (irregular 40–63 μm silica). Column chromatography was performed with silica gel 60 (40–63 μm, E. Merck).

Gel-filtration was performed on a TSK-40 HW(S) column (420 × 25 mm) and Sephadex G-15 column (400 × 17 mm) by elution with 0.1 M AcOH in water at a flow rate of 0.5 mL·min^{-1} with a RI detector.

NMR spectra were recorded on Bruker Fourier 300HD (300 MHz), Bruker AV400 (400 MHz), or Bruker AV600 (600 MHz) spectrometers at temperatures denoted on the spectra. The resonance assignment in ^{1}H and ^{13}C NMR spectra was performed using 2D-experiments (COSY, HSQC). Chemical shifts are reported in ppm referenced to tetramethylsilane as a standard for 1H and solvent signal (δ = 77.16 for $CDCl_3$) for ^{13}C. ^{1}H-NMR spectra in D_2O were registered with water suppression using a presaturation pulse sequence. See all NMR spectra in Supplementary Materials.

High-resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The measurements were performed in positive ion mode (interface capillary voltage −4500 V) or in negative ion mode (3200 V); mass range from *m*/*z* 50 to *m*/*z* 3000 Da; external or internal calibration was made with an electrospray calibrant solution (Fluka). A syringe injection was used for solutions in a mixture of acetonitrile and water (50:50 *v*/*v*, flow rate 3 μL·min^{-1}). Nitrogen was applied as a dry gas; interface temperature was set at 180 °C.

Glycosylation reactions were carried out in anhydrous solvent. Powdered molecular sieves were activated for 2 h at 180 °C in vacuo using an oil pump before use in the reaction.

*3.2. Synthesis of Compounds **4**, **8**, **9**, **13, 14, 1a** and **1b***

3.2.1. 3-Trifluoroacetamidopropyl 3,4-*O*-Isopropylidene-6-*O*-Benzoyl-β-D-Galactopyranoside (**4**)

To a stirred solution of galactoside **2** (312.5 mg, 0.94 mmol) in collidine (1.4 mL), BzCl (120 μL, 1.03 mmol, 1.1 eq.) was added. The mixture was stirred at 0 °C for 2 h, quenched with dimethylaminopropylamine (DMAPA), diluted with EtOAc, washed with 1 M aq. HCl, sat. aq. $NaHCO_3$, the combined organic phase was dried by filtration through Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2:MeOH (0→8%), giving 291.9 mg (71 %) of benzoylated monosaccharide **3**. It was dissolved on 2,2-dimethoxypropane (5 mL) and CSA (73.5 mg) was added. The mixture was stirred at RT for 1.5 h, quenched with Et_3N, and co-evaporated with toluene in vacuo. The residue was purified by flash chromatography (toluene:ethyl acetate 20→50%), giving 286.8 mg (90 %; summary yield 64%) of monosaccharide **4**. $[\alpha]_D^{16}$ + 29.5 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 1.36 and 1.53 (both s, on 3H, 2 CH_3), 1.82–1.91 (m, 2H, $OCH_2C\underline{H_2}CH_2N$), 2.44 (s, 1H, OH), 3.49 (qd, 2H, *J* = 2.7 Hz, 6.1 Hz; $OCH_2CH_2C\underline{H_2}N$), 3.57 (t, 1H, *J* = 7.7 Hz; H-2), 3.68–3.76, and 3.92–3.98 (both m, on 1H, $OC\underline{H_2}CH_2CH_2N$), 4.09–4.13 (m, 1H, H-3), 4.13–4.18 (m, 1H, H-5), 4.21–4.24 (m, 2H, H-1, H-4), 4.60–4.63 (m, 2H, H-6), 7.42–8.05 (m, 5H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 26.3, 28.0 (2 CH_3), 28.3 ($OCH_2\underline{C}H_2CH_2N$), 38.3 ($OCH_2CH_2\underline{C}H_2N$), 63.6 (C-6), 69.0 ($O\underline{C}H_2CH_2CH_2N$), 71.4 (C-5), 73.3 (C-4), 73.5 (C-2), 79.0 (C-3), 102.4 (C-1), 110.7 ($\underline{C}Me_2$), 128.5, 129.6, 133.3, 166.3 (Ph), 166.3 ($\underline{C}OPh$). HRMS (ESI) m/z: found $[M + NH_4]^+$ 495.1941, $C_{21}H_{26}F_3NO_8$ calcd for $[M + NH_4]^+$ 495.1949.

3.2.2. 3-Trifluoroacetamidopropyl 2-*O*-Benzyl-3,4-Di-*O*-Benzoyl-α-L-Fucopyranosyl-(1→2)-6-*O*-Benzoyl-β-D-Galactopyranoside (**8**)

A carefully dried mixture of donor **5** (389.8 mg, 0.64 mmol, 1.45 eq.) and galactosyl acceptor **4** (211.6 mg, 0.44 mmol, 1 eq.) was dissolved in CH_2Cl_2 (6 mL) and molecular sieves 4 Å (600 mg) were added. The mixture was cooled to −30 °C and TMSOTf (8 μL, 44 μmol, 0.1 eq.) was added. The mixture was stirred and warmed up to ambient temperature for 3 h and then quenched with one drop of Et_3N. The mixture was filtered through a Celite pad with CH_2Cl_2, and the filtrate was evaporated in vacuo. The residue was purified by flash chromatography (toluene:ethyl acetate 0→10%), giving 369.2 mg (91%) of disaccharide **6** with inseparable minor quantity (~11%) of β-isomer **7**. ^{1}H NMR (600 MHz, $CDCl_3$, inter alia): 4.38 (d, 1H, *J* = 8.0 Hz; H-1Gal of **7**), 4.43 (d, 1H, *J* = 8.2 Hz; H-1Gal of **6**),

4.99 (d, 1H, $J_{1,2}$ = 8.02 Hz; H-1Fuc of **7**), 5.63 (d, 1H, *J* = 3.4 Hz; H-1Fuc of **6**). ^{13}C NMR (150 MHz, $CDCl_3$, inter alia): 95.5 (C-1Fuc of **6**), 101.3 (H-1Gal of **6**), 102.7 (H-1Gal of **7**), and 103.3 (C-1Fuc of **7**).

To the disaccharide mixture (369.2 mg, 0.4 mmol) in CH_2Cl_2 (4 mL) was added 90% aq. TFA (400 μL). After 15 min, the mixture was diluted with CH_2Cl_2, washed with sat. aq. $NaHCO_3$, the organic phase was dried by filtration through Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography ($CHCl_3$: acetone 0→10%), giving 278 mg (79%; summary yield 72 %) of disaccharide **8**. $[\alpha]_D^{17}$ −95.1 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): 1.18 (d, 3H, *J* = 6.5 Hz; CH_3-Fuc), 1.83–1.97 (m, 2H, $OCH_2C\underline{H_2}CH_2N$), 2.73 (s, 1H, OH-4Gal), 3.39–3.46, and 3.53–3.60 (both m, on 1H, $OCH_2CH_2C\underline{H_2}N$), 3.64–3.73 (m, 2H, $OC\underline{H}HCH_2CH_2N$, H-2Gal), 3.76 (dd, 1H, *J*= 3.0; 9.0 Hz; H-3Gal), 3.86 (t, 1H, *J* = 6.4 Hz; H-5Gal), 3.99–4.05 (m, 2H, $OCH\underline{H}CH_2CH_2N$, H-4Gal), 4.18 (dd, 1H, *J* = 3.5; 10.3 Hz; H-2Fuc), 4.33 (s, 1H, OH-3Gal), 4.38 (d, 1H, *J* = 7.8 Hz; H-1Gal), 4.50 (q, 1H, *J* = 6.6 Hz; H-5Fuc), 4.59–4.62 (m, 1H, H-6Gal), 4.64 and 4.72 (AB system, *J* = 11.4 Hz; $C\underline{H_2}$-Ph), 5.16 (d, 1H, *J* = 3.6 Hz; H-1Fuc), 5.65 (d, 1H, *J* = 3.3 Hz; H-4Fuc), 5.74 (dd, 1H, *J* = 3.2; 10.3 Hz; H-3Fuc), 7.24–7.64 (m, 20H, 4 Ph). ^{13}C NMR (150 MHz, $CDCl_3$): 16.1 (C-6Fuc), 28.4 ($OCH_2\underline{C}H_2CH_2N$), 38.6 ($OCH_2CH_2\underline{C}H_2N$), 63.0 (C-6Gal), 66.1 (C-5Fuc), 67.9 (C-4Gal), 68.8 ($O\underline{C}H_2CH_2CH_2N$), 71.0 (C-3Fuc), 72.0 (C-4Fuc), 72.3 (C-5Gal), 73.1 (C-3Gal), 74.2 ($\underline{C}H_2$-Ph), 74.3 (C-2Fuc), 81.9 (C-2Gal), 100.6 (C-1Fuc), 102.0 (C-1Gal), 128.3–136.3 (4 Ph), 157.1 ($\underline{C}OCF_3$), 165.7, 165.8, 166.3 (3 $\underline{C}$OPh). HRMS (ESI) m/z: found $[M + Na]^+$ 904.2764, $C_{45}H_{46}F_3NO_{14}$ calcd for $[M + Na]^+$ 904.2763.

3.2.3. 3-Trifluoroacetamidopropyl 2-*O*-Benzyl-3,4-di-*O*-Benzoyl-α-L-Fucopyranosyl-(1→2)-4,6-Di-*O*-Benzoyl-β-D-Galactopyranoside (**9**)

To a stirred solution of disaccharide **8** (278 mg, 0.32 mmol) in CH_3CN (3 mL) was added trimethyl orthobenzoate (181 μL, 1.06 mmol, 3.3 eq.) and catalytic amounts of CSA (15 mg) up to pH <7. At the end of the reaction according to TLC, H_2O (50 μL) was added. The mixture was diluted with CH_2Cl_2, washed with sat. aq. $NaHCO_3$, the organic phase was dried by filtration through Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (toluene:ethyl acetate 0→20%), giving 294.3 mg (93%) of disaccharide **9**. $[\alpha]_D^{28}$ −92.3 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 1.19 (d, 3H, *J* = 6.5 Hz; CH_3-Fuc), 1.91–2.00 (m, 2H, $OCH_2C\underline{H_2}CH_2N$), 3.49–3.57 (m, 2H, $OCH_2CH_2C\underline{H_2}N$), 3.71–3.77 (m, 1H, H-2Gal), 3.80–3.87 (m, 1H, $OC\underline{H}HCH_2CH_2N$), 4.01–4.09 (m, 2H, $OCH\underline{H}CH_2CH_2N$, H-3Gal), 4.10–4.17 (m, 2H, H-5Gal, H-2Fuc), 4.40 (dd, 1H, *J* = 5.8, 11.28 Hz; H-6aGal), 4.49–4.67 (m, 5H, H-5Fuc, H-6bGal, H-1Gal, $C\underline{H_2}$-Ph), 5.10 (d, 1H, *J* = 3.6 Hz; H-1Fuc), 5.66 (d, 1H, *J* = 3.3 Hz; H-4Fuc), 5.73 (dd, 1H, *J* = 3.2; 10.4 Hz; H-3Fuc), 5.77 (d, 1H, *J* = 3.4 Hz; H-4Gal), 7.03–8.18 (m, 25H, 5 Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 16.1 (C-6Fuc), 28.3 ($OCH_2\underline{C}H_2CH_2N$), 39.0 ($OCH_2CH_2\underline{C}H_2N$), 62.3 (C-6Gal), 65.9 (C-5Fuc), 69.5 (C-4Gal), 69.7 ($O\underline{C}H_2CH_2CH_2N$), 71.0 (C-3Fuc), 71.73 (C-5Gal), 72.0 (C-4Fuc), 72.5 (C-3Gal), 73.5 (C-2Fuc), 73.9 ($\underline{C}H_2$-Ph), 81.7 (C-2Gal), 100.8 (C-1Fuc), 102.3 (C-1Gal), 128.3–136.4 (5Ph), 165.6, 165.8, 165.9, 166.1 (4 $\underline{C}$OPh). HRMS (ESI) *m*/z: found $[M + NH_4]^+$ 1003.3472, $C_{52}H_{50}F_3NO_{15}$ calcd for $[M + NH_4]^+$ 1003.3471.

3.2.4. 3-Trifluoroacetamidopropyl 2-Azido-3-O-Benzoyl-2-Deoxy-α-D-Galactopyranosyl-(1→3)-[2-*O*-Benzyl-3,4-Di-*O*-Benzoyl-α-L-Fucopyranosyl-(1→2)]-4,6-Di-*O*-Benzoyl-β-D-Galactopyranoside (**13)** and 3-Trifluoroacetamidopropyl 2-Azido-3-O-Benzoyl-2-Deoxy-α-D-Galactopyranosyl-(1→4)-[2-*O*-Benzyl-3,4-Di-*O*-Benzoyl-α-L-Fucopyranosyl-(1→2)]- 4,6-Di-*O*-Benzoyl-β-D-Galactopyranoside (**14**)

A: A carefully dried mixture of donor **10** (29.4 mg, 50 μmol, 1.1 eq.) and disaccharide acceptor **8** (39.7 mg, 45 μmol) was dissolved in CH_2Cl_2 (1 mL) and molecular sieves 4 Å (100 mg) were added. After 10 min, MeOTf (27 μL, 0.25 mmol, 5.5 eq.) and Me_2S_2 (22 μL,0.25 mmol, 7.5 eq.) were added. The mixture was stirred for 2 h at room temperature and quenched with one drop of Et_3N. The mixture was filtered through a Celite pad with CH_2Cl_2 and the filtrate was washed with sat. aq. $NaHCO_3$. The organic phase was separated, and the solvent was evaporated in vacuo. The residue was purified by flash

chromatography (toluene:ethyl acetate 0→10%), giving 35.5 mg of an inseparable mixture of trisaccharides **11** and **12**. To a solution of purified mixture in Py (0.3 mL) was added BzCl (7 μL, 54 μmol, 2 eq.) while stirring. After 30 min, the mixture was diluted with EtOAc, washed with 1 M aq. HCl, sat. aq. $NaHCO_3$, the combined organic phase was dried by filtration through Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (toluene:ethyl acetate 0→10%). To a solution of the obtained compound in Py (0.5 mL) was added 40% aq. HF (83 μL). After 10 min, the mixture was diluted with EtOAc, washed with 1 M aq. HCl, sat. aq. $NaHCO_3$, the combined organic phase was dried by filtration through Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (toluene: ethyl acetate 20→50%), giving 19.3 mg (34%) of trisaccharide **13** and 10.3 mg (19%; purity 85%) of trisaccharide **14**. **13**: $[\alpha]_D^{20}$ +10.4 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 1.21 (d, 3H, *J* = 6.4 Hz; CH_3-Fuc), 1.92–1.99 (m, 2H, OCH_2 C*H*$_2$$CH_2N$), 3.41–3.60 (m, 2H, $OCH_2CH_2CH_2$N), 3.72–3.85 (m, 3H, H-6aGalN, OC*H*HCH_2CH_2N), 3.89 (dd, 1H, *J* = 3.7; 10.9 Hz, H-2GalN), 4.01–4.09 (m, 2H, H-2Gal, OCH*H*CH_2CH_2N), 4.10–4.19 (m, 2H, H-5Gal, H-2Fuc), 4.24 (dd, 1H, *J* = 3.4; 9.6 Hz, H-3Gal), 4.32 (d, 1H, *J* = 2.8 Hz; H-4GalN), 4.35–4.41 (m, 2H, H-5GalN, H-6aGal), 4.55–4.65 (m, 3H, H-1Gal, H-5Fuc, H-6bGal), 4.72–4.82 (m, 2H, C*H*$_2$-Ph), 5.26 (d, 1H, *J* = 3.7 Hz, H-1Fuc), 5.44 (dd, 1H, *J* = 2.9; 10.9 Hz; H-3GalN), 5.54 (d, 1H, *J* = 3.8 Hz, H-1GalN), 5.61–5.67 (m, 2H, H-4Fuc, H-3Fuc), 5.99 (d, 1H, *J* = 3.4 Hz, H-4Gal), 7.05–8.20 (m, 30H, 6Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 16.2 (C-6Fuc), 28.5 (OCH_2CH_2CH_2N), 38.4 (OCH_2CH_2 CH_2N), 57.3 (C-2GalN), 62.17 (C-6Gal), 62.9 (C-6GalN), 64.6 (C-4Gal), 65.6 (C-5Fuc), 68.52 (C-4GalN), 69.1 (OC$H_2CH_2CH_2N$), 70.1 (C-5GalN), 70.9 (C-5Gal, C-3Fuc), 71.4 (C-3GalN), 72.1 (C-4Fuc), 73.0 (C-2Fuc), 73.2 (C-3Gal), 73.5 (***C***H_2-Ph), 77.4 (C-2Gal), 93.5 (C-1GalN), 99.1 (C-1Fuc), 103.3 (C-1Gal), 127.7–137.9(6Ph), 164.9, 165.9, 166.0, 166.1, 166.4 (5 COPh). **14**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.21 (d, 3H, *J* = 6.5 Hz; CH_3-Fuc), 1.95–2.00 (m, 2H, OCH_2C*H*$_2CH_2N$), 3.33 (dd, 1H, *J* = 4.1; 12.2 Hz; H-6aGalN), 3.39 (dd, 1H, *J* = 4.5; 12.2 Hz; H-6bGalN), 3.44–3.60 (m, 2H, $OCH_2CH_2CH_2$N), 3.79–3.85 (m, 1H, OC*H*HCH_2CH_2N), 4.00–4.20 (m, 8H, OCH*H*CH_2CH_2N, H-2GalN, H-2Gal, H-2Fuc, H-5Gal, H-5GalN, C*H*$_2$-Ph), 4.44 (d, 1H, *J* = 3.0 Hz; H-4GalN), 4.46 (d, 1H, *J* = 2.9 Hz; H-4Gal), 4.66–4.80 (m, 4H, H-1Gal, H-5Fuc, H-6Gal), 5.14 (d, 1H, *J*=3.6 Hz; H-1GalN), 5.42 (dd, 1H, *J* = 2.9; 10.1 Hz; H-3Gal), 5.50 (dd, 1H, *J* = 2.9; 11.0 Hz; H-3GalN), 5.54 (d, 1H, *J* = 3.5 Hz; H-1Fuc), 5.60–5.67 (m, 2H, H-4Fuc, H-3Fuc), 6.77–8.20 (m, 30H, 6Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 15.9 (C-6Fuc), 28.5 (OCH_2CH_2CH_2N), 37.8 (OCH_2CH_2CH_2N), 58.3 (C-2GalN), 62.2 (C-6Gal), 63.0 (C-6GalN), 65.6 (C-5Fuc), 67.7 (OC$H_2CH_2CH_2N$), 68.9 (C-4GalN), 69.7 (C-5GalN), 70.3 (C-3Fuc), 71.9 (C-3GalN), 72.1 (C-4Fuc), 72.2 (CH_2-Ph), 72.3 (C-5Gal), 72.6 (C-2Fuc), 74.5 (C-4Gal), 75.8 (C-3Gal), 97.2 (C-1Fuc), 99.3 (C-1GalN), 102.3 (C-1Gal), 127.6-133.9 (6Ph), 165.5, 165.9 (2 COPh). HRMS (ESI) m/z: found $[M + NH_4]^+$ 1294.4324, $C_{65}H_{63}F_3N_4O_{20}$ calcd for $[M + NH_4]^+$ 1294.4326.

B: A carefully dried mixture of donor **10** (84.8 mg, 0.14 mmol, 1.2 eq.) and disaccharide acceptor **9** (118 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (2 mL) and molecular sieves 4 Å (200 mg) were added. After 10 min, MeOTf (98.7 μL, 0.9 mmol, 7.5 eq.) and Me_2S_2 (79.7 μL,0.9 mmol, 7.5 eq.) were added. The mixture was stirred for 2 h at room temperature and quenched with one drop of Et_3N. The mixture was filtered through a Celite pad with CH_2Cl_2 and the filtrate was washed with sat. aq. $NaHCO_3$. The organic phase was separated, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (toluene:ethyl acetate 0→20%), giving 137.6 mg (81%) of trisaccharide **15** (contaminated by traces of (1→4)-glycosylation product). To a solution of the purified compound in Py (2 mL) was added 40% aq. HF (333 μL). After 10 min, the mixture was diluted with EtOAc, washed with 1 M aq. HCl, sat. aq. $NaHCO_3$, the combined organic phase was dried by filtration through Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (toluene:ethyl acetate 20→50%), giving 117.9 mg (77%; summary yield 62%) of trisaccharide **13**. All spectral characteristics w completely identical to the compound **13** obtained by method **A**.

3.2.5. 3-Aminopropyl
2-Acetamido-2-Deoxy-α-D-Galactopyranosyl-(l→3)-[(α-L-Fucopyranosyl)-(l→2)]-β-D-Galactopyranoside (1a)

To a solution of the trisaccharide **13** (83.8 mg, 66 μmol) in EtOAc (2 mL) and MeOH (1mL) were added 1 M HCl (50 μL) and $Pd(OH)_2/C$ (100 mg) after the flask was filled with hydrogen. The reaction mixture was stirred for 3 h at RT. Then, the reaction mixture was filtered on a glass filter through a Celite pad and concentrated in vacuo. The crude material was dissolved in $CHCl_3$:MeOH (2 mL in ratio 1:1), then Et_3N (27 μL, 0.19 mmol) and Ac_2O (12 μL, 0.13 mmol) were added. After completing the reaction, the mixture was concentrated in vacuo and purified by flash chromatography ($CHCl_3$:MeOH 0→10%), giving 57.3 mg (68%) of trisaccharide **16**. One M MeONa (100 μL) was added to a solution of the purified compound in MeOH:CH_2Cl_2 (0.8 mL in ratio 3:1). The mixture was left for 2 h, then 4 M NaOH (50 μL) was added and left overnight. The base was neutralized by 1 M aq. HCl and the resulting solution was concentrated. The residue was purified by gel-permeation chromatography (TSK HW-40 (S), 0.1 M AcOH) giving 19.4 mg (73%) of trisaccharide **1a**. All NMR and HRMS data corresponded to the literature data [9].

3.2.6. 3-(21-Biotinamino-4,7,10,13,16,19-Hexaoxagenicaminoamino)-Propyl
2-Acetamido-2-Deoxy-α-D-Galactopyranosyl-(l→3)-[(α-L-Fucopyranosyl)-(l→2)]-β-D-Galactopyranoside (1b).

To a solution of trisaccharide **1a** (0.5 mg, 0.85 μmol) in DMF (100 μL) was added a 0.0062 M solution of biotin-activated ester **17** (20 μL, 1.28 μmol, 1.5 eq.) and Et_3N (15 μL, 0.1 mmol). After 20 min, the mixture was concentrated in vacuo, after which the residue was purified by gel-permeation chromatography (G-15, 0.1 M AcOH) to give 0.85 mg (87%) biotinylated conjugate **1b**. ^1H NMR (600 MHz, D_2O, characteristic signals): oligosaccharide fragment: 1.24 (d, 3H, J = 6.6 Hz; CH_3-Fuc), 1.93 (s, 1H, NH*Ac*), 4.55 (d, 1H, J = 7.8 Hz; H-1Gal), 5.20 (d, 1H, J = 3.7 Hz; H-1GalN), 5.32 (d, 1H, J = 3.8 Hz; H-1Fuc); biotin fragment: 1.42–1.78 (m, 6H, H-b, H-c, H-d), 2.30 (t, 2H, J = 7.3 Hz; H-a), 2.81 (d, 1H, J = 13.0 Hz; H-h`), 3.02 (dd, 1H, J = 5 Hz; 13.1 Hz; H-h), 3.34–3.38 (m, 1H, H-e), 4.63 (dd, 1H, J = 5.0 Hz; 7.9 Hz; H-g); linker: 1.83–1.89 (m, 2H, $OCH_2C\mathit{H_2}CH_2N$), 2.54 (t, 2H, J = 6.1 Hz; $OCH_2CH_2C\mathit{H_2}N$). ^{13}C NMR characteristic signals derived from (^1H, ^{13}C) HSQC spectrum (D_2O): oligosaccharide fragment: 16.7 ($\mathit{C}H_3$-Fuc), 26.4 (NH*Ac*), 92.7 (C-1GalN), 98.8 (C-1Fuc), 102.7 (C-1Gal); biotin fragment: 26.4 (C-c), 29.1 (C-b, C-d), 36.7 (C-a), 56.8 (C-e), 61.6 (C-g); linker: 29.8 ($OCH_2\mathit{C}H_2CH_2N$), 37.4 ($OCH_2CH_2\mathit{C}H_2N$). HRMS (ESI) m/z: found $[M + Na]^+$ 1170.5197, $C_{48}H_{85}N_5O_{24}S$ calcd for $[M + Na]^+$ 1170.5178.

4. Conclusions

The synthesis of spacered A trisaccharide derivatives **1a** and **1b** was performed using a 2-azido-2-deoxy-selenogalactoside glycosyl donor bearing stereo-controlling 3-O-benzoyl and 4,6-O-(di-tert-butylsilylene)-protecting groups, showing once again the efficacy of this α-glycosylation agent for the assembly of even vicinally branched oligosaccharide chains. At the same time, we observed rather poor applicability of donor **10** for the regioselective glycosylation of diolic acceptor **8**. Obtained trisaccharide **1b** is being used in the coating of magnetic nanobeads for glycobiological applications to be described in due course.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/molecules26195887/s1, Copies of NMR spectra of compounds **4**, **6**–**9**, **13**, **14**, **1a**, and **1b**.

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