

Communication

Synthesis of *N,N*-Dimethylaminopropyl Derivative of A Blood Sugar Antigen

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Abstract: Gold nanoparticles (AuNPs) are a promising tool for drug delivery due to their unique chemical properties that make them biocompatible and easy to functionalize. However, when AuNPs are introduced into biological systems, they are coated by the so-called protein corona (PC), which affects their biodistribution and limits their therapeutic efficacy. The functionalization of AuNPs with endogenous carbohydrates can be a possible strategy to reduce immune recognition, thus enhancing their biocompatibility and circulation time. Suitable candidates for this approach are the ABO blood sugar antigens, di- and tri-saccharides that represent the terminal portion of some glycolipids and glycoproteins present on the surface of human red blood cells and other tissues. In this work, we illustrate the synthesis of trisaccharide antigen A derivative, whose last step is worthy of investigation. During the final hydrogenolysis reaction, intended to remove protecting groups, an unexpected side reaction occurred, the isolated product bearing an *N,N*-dimethyl moiety on the anomeric propyl linker. This side reaction might be ascribed to the in situ formation of formaldehyde and successive imine formation and reduction. The obtained compound can be used as a monomeric control compound in biochemical and structural biology studies involving ABO blood sugar antigens.

Keywords: glyco-gold nanoparticles; blood sugar antigens; glycosylation; glycoderivatives; oligosaccharides



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1. Introduction

Gold nanoparticles (AuNPs) are the most promising nanomaterials for medical endeavours, considering their inertness, distinctive nanoscale characteristics and in vivo biocompatibility [1–4]. AuNPs can be easily prepared with controlled size, geometry, and surface properties, and the gold core can react with thiols to form covalent bonds, thus allowing for quick and robust functionalization with a variety of bioactive molecules and/or drugs [5,6]. AuNPs are a very promising tool for drug delivery, but when placed in biological fluids, they are selectively coated by proteins, forming the so-called protein corona (PC). This side effect impacts biodistribution by triggering immune system recognition and, consequently, reducing the circulation time, thus obstructing the NPs from reaching their therapeutic target [7,8]. A possible solution to overcome this limitation is the functionalization of the AuNP surface with endogenous species recognized as self-antigens by the immune system. In this regard, endogenous carbohydrates are suitable candidates, as they could enhance the biocompatibility of AuNPs and consequently increase the immune tolerance and their circulation time. These systems, known as glyco-gold nanoparticles (GAuNPs), comprise AuNPs with the surface Au atoms covalently attached

to thiols of thiol-terminated oligosaccharides [9]. Glycans enable AuNPs to achieve more colloidal stability and biocompatibility while reducing unspecific interactions with proteins in the media. For this reason, the saccharide coating can be used to create stealth nanomaterials [10].

Since the seminal work by Penadès in 2001 [11], gold nanoparticles decorated with carbohydrates or glycoderivatives (glyco-gold nanoparticles, GAuNPs) have garnered increasing attention for their potential in biochemical and biomedical nanotechnology [12]. GAuNPs are valuable tools for studying carbohydrate-mediated interactions in biochemical and physiological processes [13,14] and for investigating the multivalency effects of sugars [11]. Additionally, they serve as promising platforms for vaccine development [15,16]. The introduction of various thiol ligands on the same gold nanoplatfrom further enables the creation of multifunctional nanosystems [17,18]. Carbohydrate-based compounds and glycoderivatives, ligands of GAuNPs, have attracted increasing interest in drug development, particularly as anti-diabetic [19–22], anti-viral [23], anti-bacterial [24–26], anti-inflammatory [27,28], and antitumor agents [29–31]. Their potential to overcome the inherent limitations of natural carbohydrates as drugs, such as low stability under physiological conditions and limited drug-like properties [32], makes them promising candidates in drug discovery and development.

Promising candidates for the development of stealth AuNPs are the ABO blood sugar antigens, endogenous oligosaccharides (di- and tri-saccharides) present on the surface of human red blood cells and other tissues, where they compose the terminal portion of some glycolipids and glycoproteins [33,34].

The H(O) antigen is a disaccharide in which L-fucose is α -(1-2)-linked to D-galactose, while the A and the B antigens are trisaccharides in which the H antigen is α -(1-3)-linked at the non-reducing end of *N*-acetyl-D-galactosamine or of a D-galactose residue, respectively. In our research, we focused on the synthesis of derivatives of ABO blood sugar epitopes for later AuNPs functionalization (Figure 1). An aminopropyl linker was introduced at the anomeric position of the core D-galactosyl unit in order to condense the antigens to the carboxylic moiety at one end of a heterobifunctional COOH-PEG-SH linker, which ensures a correct spatial separation between the Au core and the sugar ligands.

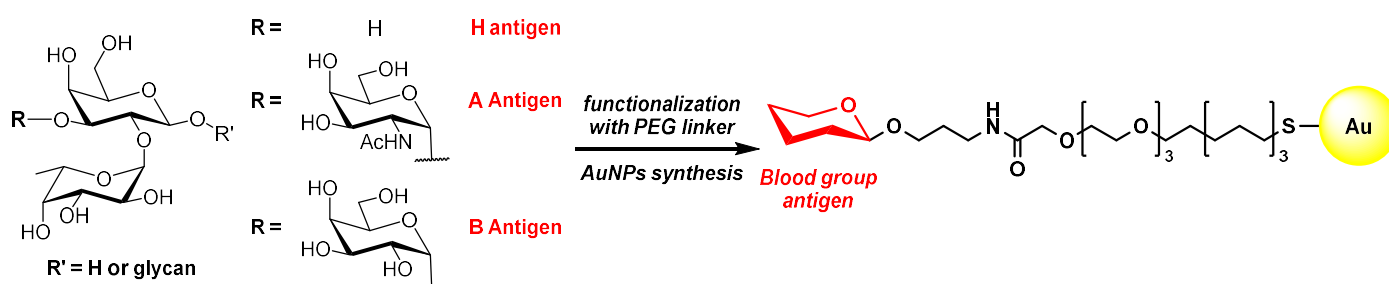
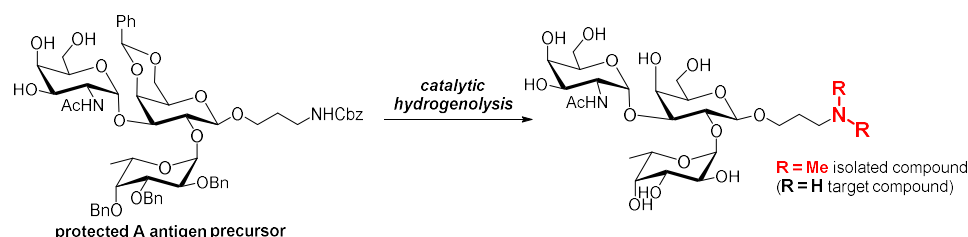


Figure 1. Structures of ABO blood sugar antigens and general structure of the sugar-functionalized target AuNPs.

In our work on synthesizing blood group sugar derivatives, we encountered an unusual side reaction during the final deprotection step of the protected precursor of the A antigen derivative. Specifically, the terminal amino group on the aminopropyl linker underwent a dimethylation transformation, and this side reaction is worthy of investigation (Scheme 1). The isolated *N,N*-dimethylamino compound was fully characterized using ^1H -, ^{13}C -NMR and HRMS analysis. This derivative is particularly interesting as it can serve as a monomeric control ligand in biochemical and structural biology studies involving blood A antigen derivative, including GAuNPs decorated with ABO blood sugar antigens.

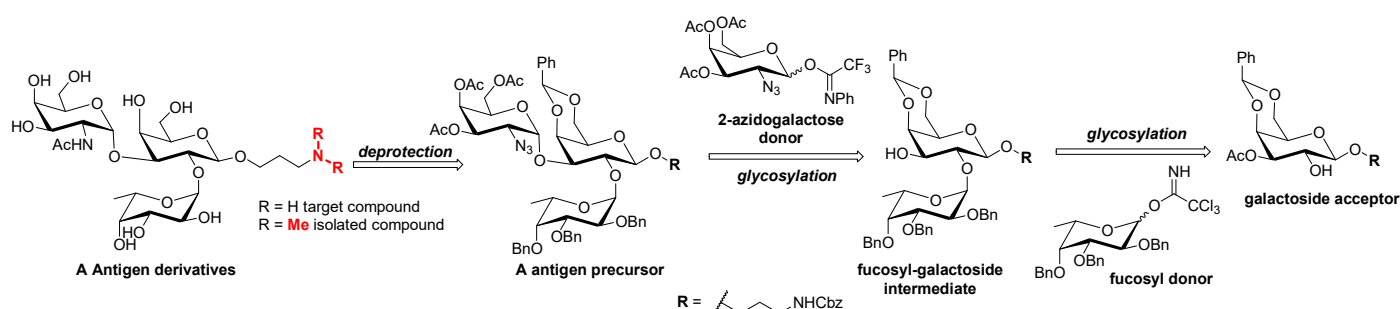


Scheme 1. Synthesis of the A antigen derivative target compound (R = H) and the isolated side compound (R = Me).

2. Results and Discussion

2.1. Retrosynthesis

The synthesis of the target A blood antigen derivative (Scheme 2) involved the preparation of its protected precursor (A antigen precursor), synthesized through a glycosylation reaction with a L-fucosyl- α -1,2-D-galactosyl disaccharide acceptor and a 3,4,6-tri-O-acetyl-2-azido-2-deoxy-D-galactopyranosyl-N-phenyl-trifluoroacetimidate donor. The core fucosyl-galactosyl disaccharide was obtained from 3-O-acetyl-4,6-O-benzylidene- β -D-galactopyranoside acceptor and 2,3,4-tri-O-benzyl- α , β -L-fucopyranosyl-1-O-trichloroacetimidate donor. The following removal of the acetyl-protecting group on the C-3 position of the galactose unit delivered the hydroxyl for the last glycosylation step.



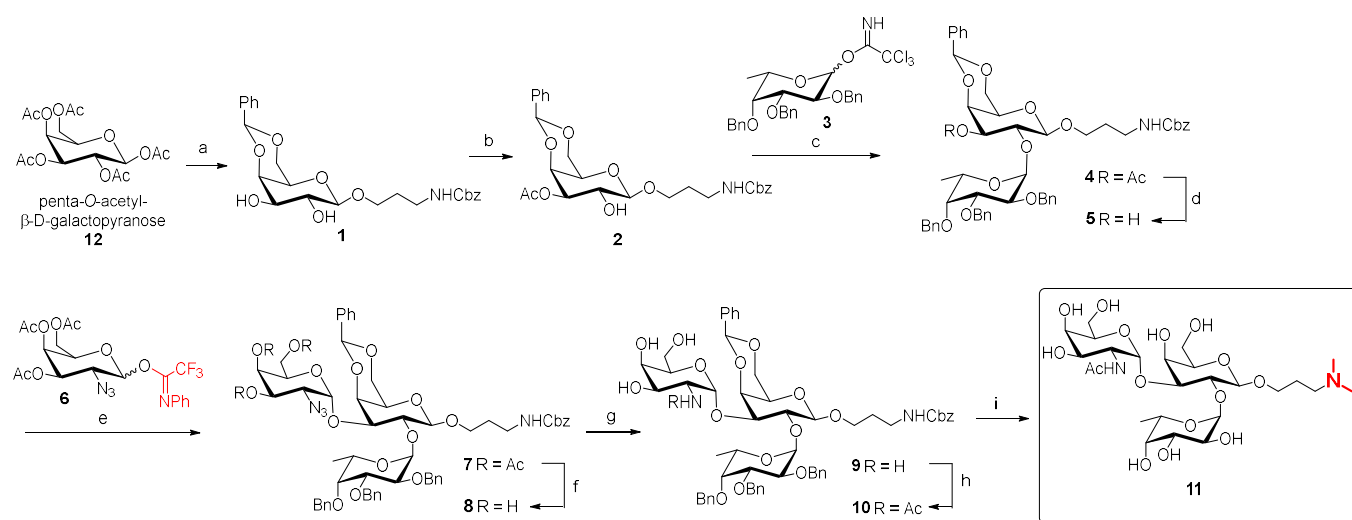
Scheme 2. Retrosynthetic approach for the preparation of A antigen derivatives.

2.2. Synthesis of Trisaccharide Antigen A Derivative 5

The first synthetic phase was the synthesis of β -D-galactopyranoside acceptor **2**. It was accomplished by a regioselective acetylation of C-3 hydroxyl of benzylidene-protected glucopyranoside **1**, involving the hindered base *sym*-collidine and acetyl chloride (Scheme 3). Compound **1** was synthesized following previously reported procedures [35,36]. The choice of the Cbz protecting group on the aminopropyl linker was based on its stability under the conditions required for the reduction and transformation of the azido group in the 2-azidogalactose unit constituting the trisaccharide intermediate **8**. Consequently, the Cbz group can undergo late-stage deprotection during the final hydrogenolysis reaction.

Galactoside acceptor **2** was the starting material for the synthesis of the Cbz-protected aminopropyl α -L-fucopyranosyl- β -D-galactopyranoside derivative **4** [36], by means of a glycosylation reaction with 2,3,4-tri-O-benzyl-L-fucopyranosyl trichloroacetimidate donor **3**, synthesized as reported by Schmidt and co-workers [37]. In order to minimize the formation of the undesired β -glycosidic linkage, Et₂O was the solvent of choice [38], but some DCM was also necessary to facilitate the solubilization of acceptor **2** whose poor solubility could be ascribed to a marked polarity conferred by the Cbz protecting group. The extreme reactivity of donor **9** prompted us to adopt an inverse glycosylation procedure [39], in which a solution of the donor was added dropwise to a mixture constituted by the acceptor and 20% mol of TMSOTf as a promoter at -20°C . The desired α -glycoside was obtained in

82% yield. Deacetylation on position C-3 was carried out, yielding compound **5** (Scheme 3). The subsequent glycosylation with 3,4,6-tri-O-acetyl-2-azido-2-deoxy-galactopyranosyl imidate donor **6** [40] furnished compound **7** [36] in a 40% yield. The NMR spectra showed a complete absence of the β -anomer; this can be explained by the coordinating effect of acetyl groups in positions C-4, C-3 and C-6 that favour a 1,2-cis attack [38]. The last steps of the synthetic pathway for preparing the trisaccharide A antigen derivative involved an initial deacetylation reaction, yielding an intermediate **8** in 72% yield. This reaction was followed by the conversion of the azide into the acetamide group, whose transformation was carried out in two steps: first, Staudinger reduction in the azido group to amine functionality using polymer bound-PPh₃, followed by a chemoselective N-acetylation procedure with Ac₂O in MeOH which afforded compound **10**, with a final yield of 45% over two steps (Scheme 3).

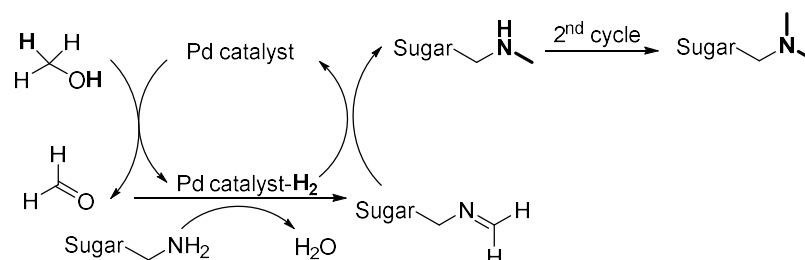


Scheme 3. Reagents and conditions: (a) Refs. [35,36]; (b) AcCl, *sym*-coll, DCM, -20°C , 72%; (c) TMSOTf, Et₂O/DCM, MS 4A, -20°C , 82%; (d) MeONa, MeOH, rt, quant.; (e) TMSOTf, Et₂O, -20°C , 40%; (f) NaOMe, MeOH, 72%; (g) PPh₃ polymer bound, THF:H₂O 3:2, 50°C ; (h) Ac₂O, MeOH, 45% over two steps; (i) Pd(OH)₂/C, H₂ atm, MeOH:H₂O 3:2, 77%.

The final reaction of the synthesis involved a hydrogenolysis reaction on intermediate **10** to remove all protecting groups and deliver the target A antigen trisaccharide derivative (Scheme 2). The reaction was carried out under H₂ atmosphere in an MeOH/H₂O mixture using Pd(OH)₂/C as a catalyst. After a 6-day reaction, ¹H and ¹³C NMR, along with high-resolution mass spectrometry analyses, clearly indicated that the isolated product was not the desired target compound bearing the terminal aminopropyl linker but the rather the *N,N*-dimethyl side product **11** (Scheme 3). To understand the formation of this side product, the literature suggests several explanations. N-alkylation of amines during hydrogenolysis/hydrogenation reactions is a reported side reaction. Benoiton et al. proposed that N-alkylation can be attributed to the in situ formation of aldehydes from the respective alcohols used in the solvent mixture, likely due to the Pd-catalyzed oxidation with residual oxygen traces even after solvent degassing. These aldehydes then react with amino groups to form imines, which are subsequently reduced under catalyzed hydrogenation conditions [41]. Filira et al. associated the N-alkylation of amines with prolonged reaction times [42]. In our case, both hypotheses could be valid: the 6-day duration of the deprotection step, and the use of methanol in the solvent mixture, led to the complete formation of the *N,N*-dimethyl derivative of compound **11**.

The observed side reaction, amine alkylation, can be rationalized as an example of the so-called “Borrowing Hydrogen (BH)” or “Hydrogen Autotransfer” reactions [43,44]. These reactions provide an effective method to synthesize alkylamines by employing various

metal catalysts, using amines and alcohols as starting materials [45]. BH reactions between primary amines and alcohols allow the synthesis of alkylamines while avoiding the use of toxic alkylating agents such as alkyl halides or alkyl sulfonates. Based on the literature, the reaction mechanism leading to the formation of the *N,N*-dimethylamino derivative **11** (Scheme 4) was hypothesized [44]. Typically, these reactions enable the controlled formation of monoalkylated amine compounds. However, in our case, it is hypothesized that extended hydrogenolysis reaction times resulted in a second alkylation, ultimately producing the dimethylated product.



Scheme 4. Hypothesized mechanism explaining the formation of compound X.

To avoid the formation of the *N,N*-dialkylamino side product and, thus, deliver the desired aminopropyl ABO group antigen derivatives, as well as other amino-functionalized carbohydrate derivatives, the hydrogenation/hydrogenolysis reactions should be carried out using a mixture of the non-oxidation prone tert-butanol and water as solvent or ensuring a thorough removal of oxygen from the reaction mixture. Nevertheless, the isolated *N,N*-dimethylaminopropyl derivative of A blood sugar antigen represents an important and useful compound. It can be utilized as a monomeric control ligand in multivalency studies involving GAuNPs decorated with A blood sugar antigen, as well as in potential biochemical or structural biology studies aimed at deepening the understanding of interactions between relevant biological counterparts [46–48].

3. Materials and Methods

3.1. General Remarks

All the commercial chemicals were purchased from Merck© (Darmstadt, Germany) or Thermo Fisher Scientific© (Waltham, MA, USA). All the chemicals were used without further purification. All the required anhydrous solvents were dried with molecular sieves for at least 24 h prior to use. Thin layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ plates (Merck©, Darmstadt, Germany) with detection under UV light when possible or by charring with a solution of (NH₄)₆Mo₇O₂₄ (21 g), Ce(SO₄)₂ (1 g), concentrated H₂SO₄ (31 mL) in water (500 mL), or with an ethanol solution of ninhydrin. For some reactions, High-Performance Thin Layer Chromatography (HPTLC) (Merck© precoated 60 F254 plates 0.20 mm) was used and correspondently pursued. Flash-column chromatography was performed on silica gel 230–400 mesh (Merck©, Darmstadt, Germany) or using the Isolera Flash Chromatography System (Biotage Sweden AB™, Uppsala, Sweden). ¹H and ¹³C NMR spectra were recorded at 25 °C, unless otherwise stated, with a Bruker© Avance TM NEO 400 MHz (Billerica, MA, USA) (400 MHz for ¹H and 100.6 MHz for ¹³C).

Chemical shift assignments, reported in parts per million, were referenced to the corresponding solvent peaks. Low-resolution mass analysis was recorded in negative or positive mode on a Thermo© Finnigan LCQ Advantage (Waltham, MA, USA) equipped with an ESI source. High-resolution mass spectra (HR-MS) were acquired on a Synapt G2-Si QToF mass spectrometer (Waters™, Milford, MA, USA) equipped with a Zspray ESI-probe for electrospray ionization in full scan mode.

3.2. Synthetic Procedures and Characterizations

3.2.1. *N*-(Benzyloxycarbonyl)aminopropyl-3-*O*-acetyl-4,6-*O*-benzylidene- β -D-galactopyranoside (**2**)

Compound **1** (2.52 g, 5.48 mmol) was dissolved in dry DCM (55 mL, 0.1 M), and sym-collidine (2.9 mL, 21.92 mmol) was added under a nitrogen atmosphere. The solution was cooled down to $-20\text{ }^{\circ}\text{C}$, and acetyl chloride was added dropwise. The reaction was monitored by TLC (Hex:EtOAc 3:7). After 2 h, the reaction was quenched with MeOH (3 mL) at $-20\text{ }^{\circ}\text{C}$ and diluted with EtOAc. The organic phase was washed with HCl 4.8% ($2 \times 50\text{ mL}$), sat. NaHCO_3 (50 mL) and brine (50 mL) and dried over anhydrous Na_2SO_4 . The crude product was purified by column chromatography (Hex:EtOAc 3:7), affording compound **2** (1.988 g, 72%). ^1H NMR (400 MHz, CDCl_3) δ 7.53–7.45 (m, 2H, H_{arom}), 7.38–7.27 (m, 8H, H_{arom}), 5.50 (s, 1H, H-7), 5.14 (d, $J = 5.9\text{ Hz}$, 1H, NH), 5.09 (s, 2H, H-11), 4.84 (dd, $J = 10.2, 3.6\text{ Hz}$, 1H, H-3), 4.38 (d, 1H, H-4), 4.33 (m, 1H, H-1), 4.29 (d, $J = 1.7\text{ Hz}$, 1H, H-6_a), 4.07–3.95 (m, 3H, H-6_b, H-8_a, H-2), 3.60 (m, 1H, H-8_b), 3.53–3.44 (m, 2H, H-10_a, H-5), 3.26 (m, 1H, 10_b), 2.14 (s, 3H, $-\text{COCH}_3$), 1.84 (m, 1H, H-9_a), 1.74 (m, 1H, H-9_b) ppm. ^{13}C NMR (101 MHz, CDCl_3) δ 171.2 ($-\text{CH}_3\text{COO}$), 137.8 ($-\text{NHCOOCH}_2\text{Ph}$), 129.1–126.4 ($\text{C}_{\text{q Arom}}$), 103.2 (C-4), 101.1 (C-7), 74.2 (C-3), 73.5 (C-1), 69.2 (C-6), 68.4 (C-2), 66.9 (C-11), 66.9 (C-8), 66.6 (C-5), 37.7 (C-10), 29.2 (C-9), and 21.3 ($-\text{COCH}_3$). $\text{C}_{26}\text{H}_{31}\text{NO}_9$; calcd. mass: 501.20; ESI-MS found m/z 502.45 $[\text{M} + \text{H}]^+$.

3.2.2. *N*-(Benzyloxycarbonyl)aminopropyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-(2',3',4'-tri-*O*-benzyl- α -L-fucopyranosyl)- β -D-galactopyranoside (**4**)

Acceptor **2** (300 mg, 0.598 mmol) was co-evaporated with toluene and left in under a vacuum overnight. After 12 h, the acceptor and activated 4 Å molecular sieves were suspended in dry DCM and dry Et_2O (1:4, 6 mL) under a Ar atmosphere; the mixture was stirred for 1 h. Then, the solution was cooled down to $-20\text{ }^{\circ}\text{C}$, and TMSOTf (11.56 μL , 0.060 mmol) was added dropwise. Then, a previously prepared solution of donor **3** (865 mg, 1.495 mmol) in Et_2O (15 mL) was added dropwise to the reaction mixture. The reaction was monitored by HPTLC (Hex:EtOAc 6:4), and after 2 h and 30 min, the reaction was neutralized with Et_3N . The mixture was diluted with DCM, and the molecular sieves were filtered over celite. The crude product was purified by column chromatography to afford compound **4** (453 mg, 82%). ^1H NMR (400 MHz, CDCl_3) δ 7.55–7.20 (m, 25H, H_{arom}), 5.49 (s, 1H, H-7), 5.30 (m, 1H, NH), 5.27 (d, $J_{1',2'} = 3.5\text{ Hz}$, 1H, H-1'), 5.06 (s, 2H, H-11), 5.04 (d, $J_{3,4} = 3.5\text{ Hz}$, 1H, H-3), 4.99–4.60 (m, 6H, $-\text{CH}_2\text{Ph}$), 4.48 (d, $J_{1,2} = 7.7\text{ Hz}$, 1H, H-1), 4.36 (d, $J_{3,4} = 3.5\text{ Hz}$, 1H, H-4), 4.30 (m, H, H-6_a), 4.22 (m, 1H, H-5'), 4.12 (M, 1H), 4.08–3.99 (m, 2H, H-2', H-6_b), 3.95 (m, 1H, H-8_a), 3.88 (dd, $J_{3',2'} = 10.3\text{ Hz}$, $J_{3',4'} = 2.7\text{ Hz}$, 2H, H-3'), 3.69 (d, $J_{3',4'} = 2.7\text{ Hz}$, 1H, H-4'), 3.57 (m, 1H, H-8_b), 3.47 (s, 1H, H-5), 3.27 (m, 2H, H-10), 1.86 (s, 3H, $-\text{COCH}_3$), 1.76 (m, 2H, H-9), 1.12 (d, $J_{6',5'} = 6.7\text{ Hz}$, 3H, H-6') ppm. ^{13}C NMR (101 MHz, CDCl_3) δ 170.8 ($-\text{CH}_3\text{COO}$), 156.6 ($-\text{NHCOOCH}_2\text{Ph}$), 139.0–137.8 ($\text{C}_{\text{q Arom}}$), 129.1–126.4 (C_{Arom}), 102.2 (C-1), 101.0 (C-7), 97.9 (C-1'), 79.6 (C-3'), 77.9 (C-4'), 76.8 (C-2'), 74.9–73.2 ($-\text{CH}_2\text{Ph}$), 74.7 (C-3), 73.7 (C-4), 72.6 (C-2), 69.1 (C-6), 67.1 (C-5'), 66.6 (C-8), 66.3 (C-5), 37.9 (C-10), 29.9 (C-9), 21.2 ($-\text{COCH}_3$), and 16.8 (C-6'). $\text{C}_{53}\text{H}_{59}\text{NO}_{13}$; calcd. mass: 917.40; ESI-MS found m/z 918.61 $[\text{M} + \text{H}]^+$.

3.2.3. *N*-(Benzyloxycarbonyl)aminopropyl-4,6-*O*-benzylidene-2-*O*-(2',3',4'-tri-*O*-benzyl- α -L-fucopyranosyl)- β -D-galactopyranoside (**5**)

Compound **4** (493 mg, 0.537 mmol) was dissolved in MeOH (5 mL, 0.1 M) and NaOMe (2.9 mg, 0.054 mmol) was added. The reaction was monitored by TLC (Hex:EtOAc 5:5) and stirred overnight. After 23 h, the reaction mixture was turned into a white suspension. The solid precipitate was dissolved in DCM, and the solution was diluted with MeOH and quenched with Amberlite IR-120 (H^+ form) until pH = 6/5. The resin was filtered, and

the solvent was evaporated to afford compound **5** (454 mg, quantitative yield). ^1H NMR (400 MHz, CDCl_3) δ 7.60–7.12 (m, 25H, H_{arom}), 5.56 (s, 1H, H-7), 5.36 (m, $J = 6.6$ Hz, 1H, NH), 5.13 (d, $J = 3.6$ Hz, 1H, H-1'), 5.06 (s, 2H, H-11), 4.96 (d, $J = 11.6$ Hz, 1H, $-\text{CH}_2\text{Ph}$), 4.86–4.70 (m, 4H, CH_2Ph), 4.65 (d, $J = 11.6$ Hz, 1H, $-\text{CH}_2\text{Ph}$), 4.34 (d, $J = 6.9$ Hz, 1H, H-1), 4.29 (d, $J = 12.5$ Hz, 1H, H-6_b), 4.21 (d, $J = 2.7$ Hz, 1H, H-4), 4.13–4.03 (m, 3H, H-5', H-6_a, H-2'), 4.01–3.88 (m, 2H, H-3', H-8_b), 3.87–3.73 (m, 2H, H-2, H-3), 3.66 (d, 1H, H-4'), 3.58 (dt, $J = 5.8$ Hz, 1H, H-8_a), 3.41 (m, 1H, H-5), 3.29 (m, $J = 6.6$ Hz, 2H, H-10), 1.78 (m, $J = 5.8$ Hz, 2H, H-9), 1.10 (d, $J = 6.6$ Hz, 3H, H-6'). ^{13}C NMR (101 MHz, CDCl_3) δ 156.7 ($-\text{NHCOOCH}_2\text{Ph}$), 138.8–137.0 (C_{qArom}), 129.2–126.7 (C_{Arom}), 102.0 (C-1), 101.5 (C-7), 99.8 (C-1'), 79.7 (C-3'), 78.5 (C-2), 77.6 (C-4'), 77.1 (C-2'), 75.6 (C-4), 74.9–73.0 ($-\text{CH}_2\text{Ph}$), 73.2 (C-3), 69.4 (C-6), 67.5 (C-5'), 66.7 (C-5), 66.5 (C-11), 66.4 (C-8), 38.0 (C-10), 29.4 (C-9), 16.9 (C-6'). $\text{C}_{51}\text{H}_{57}\text{NO}_{12}$; calcd. mass: 875.39; ESI-MS found m/z 875.53 $[\text{M} + \text{H}]^+$.

3.2.4. *N*-(Benzyloxycarbonyl)aminopropyl-2-*O*-(2',3',4'-tri-*O*-benzyl- α -L-fucopyranosyl)-3-*O*-(3'',4'',6''-tri-*O*-acetyl-2-azido-2-deoxy- α -D-galactopyranosyl)-4,6-*O*-benzylidene- β -D-galactopyranoside (**7**)

Acceptor **5** (150 mg, 0.171 mmol) and donor **6** (186 mg, 0.370 mmol) were co-evaporated with toluene and left under a vacuum pump overnight. After 12 h, the acceptor and the donor were dissolved in dry Et_2O (5.5 mL, 0.1 M) under a Ar atmosphere. Then, the solution was cooled down to -20°C and TMSOTf (6.6 μL , 0.0342 mmol) was added dropwise. The reaction was monitored by TLC (Hex:EtOAc 4:6 + Et_3N). After 7 h, the reaction was cooled down to -10°C and neutralized with Et_3N (1 mL). The crude product was purified by column chromatography (Hex:EtOAc 5:5 to 4:6) to afford compound **7** (81 mg, 40%). ^1H NMR (400 MHz, CDCl_3) δ 7.54 (m, 2H, H_{arom}), 7.26 (m, 23H, H_{arom}), 5.55 (s, 1H, H-7), 5.31 (m, 4H, H-1', H-1'', H-3'', NHCbz), 5.21 (dd, $J = 1.32$ Hz, $J = 3.37$ Hz, 1H, H-4''), 5.09–5.07 (m, 3H, CH_2Ph , H-11), 4.93 (d, $J = 11.6$ Hz, 1H, CH_2Ph), 4.86–4.71 (m, 3H, CH_2Ph), 4.62 (d, $J = 11.6$ Hz, 1H, CH_2Ph), 4.39 (d, $J = 7.7$ Hz, 1H, H-1), 4.37–4.23 (m, 4H, H-4, H-6_b, H-5'', H-5'), 4.14–4.03 (m, 3H, H-2', H-2, H-6_a), 3.99–3.88 (m, 2H, H-8_b, H-3'), 3.88–3.79 (m, 2H, H-6_a'', H-3), 3.71 (d, $J = 3.72$, 1H, H-4'), 3.62–3.51 (m, 2H, H-2'', H-8_a), 3.45–3.32 (m, 2H, H-5, H-6_b''), 3.24 (m, 2H, H-10), 2.11 (s, 3H, $-\text{OCOCH}_3$), 2.03 (s, 3H, $-\text{OCOCH}_3$), 1.95 (s, 3H, $-\text{OCOCH}_3$), 1.81–1.68 (m, 2H, H-9), 1.12 (d, $J = 6.5$ Hz, 3H, H-6'). ^{13}C NMR (101 MHz, CDCl_3) δ 170.5, 169.9, 169.6 (3x $-\text{OCOCH}_3$), 156.6 ($-\text{NHCOOBn}$), 139.4–137.6 (C_{qArom}), 129.0–126.2 (C_{Arom}), 102.0 (C-1), 100.8 (C-7), 98.3 (C-1'), 94.1 (C-1''), 80.5 (C-3'), 77.5 (C-4'), 77.0 (C-3), 76.1 (C-2'), 74.8 (CH_2Ph), 74.2 (CH_2Ph), 72.8 (CH_2Ph), 73.2 (C-2), 71.9 (C-4), 69.3 (C-6), 68.7 (C-3''), 68.1 (C-4''), 67.5 (C-5''), 66.7 (C-5'), 66.6 (C-11), 66.2 (C-5), 66.1 (C-8), 62.8 (C-6''), 57.8 (C-2''), 37.7 (C-10), 29.6 (C-9), 20.9 ($-\text{OCOCH}_3$), 20.8 ($-\text{OCOCH}_3$), 20.8 ($-\text{OCOCH}_3$), 16.9 (C-6'). $\text{C}_{63}\text{H}_{72}\text{N}_4\text{O}_{19}$; calcd. mass: 1188.48; ESI-MS found m/z : 1188.74 $[\text{M} + \text{H}]^+$, 1211.28 $[\text{M} + \text{Na}]^+$.

3.2.5. *N*-(Benzyloxycarbonyl)aminopropyl-2-*O*-(2',3',4'-tri-*O*-benzyl- α -L-fucopyranosyl)-3-*O*-(2-azido-2-deoxy- α -D-galactopyranosyl)-4,6-*O*-benzylidene- β -D-galactopyranoside (**8**)

Compound **7** (98 mg, 0.0825 mmol) was dissolved in dry MeOH (2 mL, 0.05 M). Then, NaOMe (3 mg, 0.0495 mmol) was added. The reaction was followed by TLC (DCM:MeOH 95:5). The reaction mixture was stirred at r.t. for 3 h, then diluted with MeOH and quenched with Amberlite IR-120 (H^+ form) until pH 6. The resin was filtered off, and the solvent was evaporated. The obtained crude was purified by flash chromatography (DCM:MeOH 100:0 to 9:1, control TLC EtOAc:MeOH 95:5) to afford compound **8** (63 mg, 72%). ^1H NMR (400 MHz, CD_3OD) δ 7.69–7.06 (m, 25H, H_{arom}), 5.67–5.63 (m, 1H, H-7), 5.32 (d, $J_{1'-2'} = 3.3$ Hz, 1H, H-1'), 5.29 (d, $J_{1''-2''} = 3.6$ Hz, 1H, H-1''), 5.04 (s, 1H, H-11), 4.98 (d, $J = 12.2$ Hz, 1H, CH_2Ph), 4.85 (d, $J = 11.3$ Hz, 1H, CH_2Ph), 4.73–4.57 (m, 4H, CH_2Ph), 4.55 (d, $J = 2.9$ Hz, 1H, H-4), 4.50–4.41 (m, 2H, H-1, H-5'), 4.26–4.14 (ddd, $J_{6,5} = 12.2$, $J_{6a,6b} = 1.1$ Hz,

2H, H-6_a, H-6_b), 4.06–3.90 (m, 8H, H-3', H-2', H-2, H-3, H-3'', H-8_b, H-5'', H-4'), 3.60–3.51 (m, 3H, H-8_a, H-6_b'', H-5), 3.45 (d, $J = 3.6$ Hz, 1H, H-4''), 3.43 (d, $J_{2'',1''} = 3.6$ Hz, 1H, H-2''), 3.26–3.16 (m, 3H, H-6_a'', H-10), 1.83–1.71 (m, 2H, H-9), 1.14 (d, $J_{6',5'} = 6.6$ Hz, 3H, H-6'). ¹³C NMR (101 MHz, CD₃OD) δ 158.5 (-NHCOOBn), 140.4–139.6 (C_{qArom}), 129.6–127.1 (C_{Arom}), 103.3 (C-1), 101.6 (C-7), 99.4 (C-1'), 94.1 (C-1''), 80.9 (C-3'), 79.2 (C-4'), 77.2 (C-2'), 76.2 (CH₂Ph), 76.0 (C-2), 74.9 (CH₂Ph), 74.1 (C-3), 73.3 (CH₂Ph), 73.2 (C-5'), 72.2 (C-4), 71.1 (C-4''), 70.3 (C-6), 69.1 (C-3''), 68.1 (C-5'), 68.0 (C-8), 67.7 (C-5), 67.3 (C-11), 63.3 (C-6''), 61.4 (C-2''), 39.1 (C-10), 30.8 (C-9), 17.0 (C-6'). C₅₇H₆₆N₄O₁₆; calcd. mass: 1062.45; ESI-MS found m/z : 1063.01 [M + H]⁺, 1086.34 [M + Na]⁺.

3.2.6. *N*-(Benzyloxycarbonyl)aminopropyl-2-*O*-(2',3',4'-tri-*O*-benzyl- α -L-fucopyranosyl)-3-*O*-(2-*N*-acetamido- α -D-galactopyranosyl)-4,6-*O*-benzylidene- β -D-galactopyranoside (**10**)

Compound **8** (79 mg, 0.0744 mmol) was dissolved in a mixture of THF/H₂O 3:2 (5 mL, 0.015 M). Then, polymer-bound PPh₃ was added. The reaction was stirred at 50 °C for 4 days (TLC EtOAc:MeOH 9:1). The mixture was filtered over celite and solvent was evaporated. The obtained crude compound **9** was dissolved in dry MeOH/Ac₂O 10:1 (3.74 mL, 0.02 M). The reaction was stirred under Ar atmosphere overnight at r.t. After 18 h TLC (EtOAc:MeOH 9:1 + 0.1% NH₄OH) showed complete conversion. The solvent was evaporated and the crude compound was purified by flash chromatography (DCM:MeOH 100:0 to 9:1). The mixed fractions were further purified by reversed phase C18 column using biotage system (gradient H₂O/CH₃CN) to afford compound **10** (36 mg, 45%). ¹H NMR (400 MHz, CD₃OD) δ 7.50–7.43 (m, 2H, H_{arom}), 7.41–7.19 (m, 23H, H_{arom}), 5.57 (s, 1H, H-7), 5.42 (d, $J_{1',2'} = 3.1$ Hz, 1H, H-1'), 5.14 (d, $J_{1'',2''} = 3.7$ Hz, 1H, H-1''), 5.06 (s, 2H, H-11), 4.99 (d, $J = 12.1$ Hz, 1H, CH₂Ph), 4.89 (d, $J = 11.2$ Hz, 1H, CH₂Ph), 4.75 (dd, $J = 11.2$, 4.1 Hz, 2H, CH₂Ph), 4.66–4.53 (m, 2H, CH₂Ph), 4.52–4.46 (m, 3H, H-5', H-4, H-1), 4.28 (dd, $J_{2'',3''} = 11.1$, $J_{2'',1''} = 3.7$ Hz, 1H, H-2''), 4.23–4.12 (ddd, $J_{6-5} = 12.6$, $J_{6a,6b} = 1.6$ Hz, 1H, H-6_a, H-6_b), 4.07–4.04 (m, 2H, H-3', H-2'), 4.03–3.95 (m, 4H, H-2, H-3, H-4', H-8_b), 3.84 (dd, $J = 7.5$, 2.4 Hz, 1H, H-5''), 3.70 (dd, $J_{3'',2''} = 11.1$, $J_{3'',4''} = 2.9$ Hz, 1H, H-3''), 3.60–3.50 (m, 3H, H-8_a, H-6_b'', H-5), 3.45 (d, $J_{4'',3''} = 2.9$ Hz, 1H, H-4''), 3.26–3.18 (m, 2H, H-10), 3.14–3.09 (m, 1H, H-6_a''), 1.83–1.75 (m, 2H, H-9), 1.64 (s, 3H, -NHCOCH₃), 1.20 (d, $J_{6',5'} = 6.6$ Hz, 3H, H-6'). ¹³C NMR (101 MHz, CD₃OD) δ 174.0 (-NHCOCH₃), 158.7 (-NHCOOBn), 140.3–139.6 (C_{qArom}), 129.9–127.5 (C_{Arom}), 103.1 (C-1), 102.0 (C-7), 98.9 (C-1'), 93.0 (C-1''), 80.5 (C-3'), 79.1 (C-4'), 77.0 (C-2'), 76.3 (CH₂Ph), 75.8 (C-2), 75.1 (CH₂Ph), 73.1 (CH₂Ph), 73.0 (C-5''), 72.4 (C-3), 72.0 (C-4), 70.6 (C-4''), 70.3 (C-6), 69.7 (C-3''), 68.3 (C-8), 68.2 (C-5'), 67.7 (C-5'), 67.3 (C-11), 63.4 (C-6''), 50.9 (C-2''), 39.2 (C-10), 31.0 (C-9), 22.5 (-NHCOCH₃), 16.9 (C-6'). C₅₉H₇₀N₂O₁₇; calcd. mass: 1078.47; ESI-MS found m/z : 1079.69 [M + H]⁺, 1102.33 [M + Na]⁺.

3.2.7. *N,N*-Dimethylaminopropyl-[2-*O*-(α -L-fucopyranosyl)-3-*O*-(2-deoxy-2-acetamido- α -D-galactopyranosyl)]- β -D-galactopyranoside **11**

Compound **10** (60 mg, 0.0556 mmol) was dissolved in a mixture of MeOH and H₂O (7.5 mL, 3:2), then 3 drops of AcOH were added. The solution was degassed under vacuum for 10 min, then Pd(OH)₂/C (20% in weight) was added. The reaction was stirred under H₂ atmosphere. After 6 days TLC (EtOAc:AcOH:MeOH:H₂O 4:2:3:1) showed complete disappearance of the starting material. Pd(OH)₂/C was filtered off and the solvent was evaporated to afford the *N,N*-dimethylated side product **11** (25 mg, 77%). ¹H NMR (400 MHz, D₂O) δ 5.28 (d, $J = 3.4$ Hz, 1H, H-1'), 5.17 (d, $J = 3.8$ Hz, 1H, H-1''), 4.56 (d, $J = 7.8$ Hz, 1H, H-1), 4.35 (q, $J = 8.4$, 6.7 Hz, 1H, H-5'), 4.29–4.19 (m, 4H, H-3', H-2''), 4.01–3.93 (m, 4H, H-7_a, H-3'', H-7_b), 3.92–3.86 (m, 1H), 3.84–3.70 (m, 9H, H-2, H-2', H-4'', H-4'), 3.69–3.64 (m, 1H), 3.29 (m, 2H, H-9), 2.91 (s, 6H, -N(CH₃)₂), 2.07 (m, 2H, H-8), 2.04 (s, 3H, -NHCOCH₃), 1.24 (d, $J = 6.6$, 3H, H-6'). ¹³C NMR (101 MHz, D₂O) δ 174.8, 136.9,

129.9, 128.8, 126.3, 101.6, 101.3, 101.2, 98.8, 91.4, 91.3, 75.5, 74.9, 74.1, 73.1, 72.7, 71.8, 71.1, 70.2, 69.7, 68.5, 67.7, 67.8, 67.4, 67.4, 67.3, 66.9, 66.2, 62.9, 62.5, 61.3, 61.0, 55.7, 55.5, 49.5, 42.8, 24.6, 22.0, 21.3, 15.4. HRMS (ESI+) m/z calculated for $[C_{25}H_{46}N_2O_{15}+H]^+$ 615.2971, found 615.2959; calculated for $[C_{25}H_{46}N_2O_{15}+Na]^+$ 637.2790; found 637.2777.

Supplementary Materials: NMR spectra of synthesized compounds (Page 2); HRMS spectrum of compound 11 (Page 9).

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