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Synthesis of *Staphylococcus aureus* type 5 capsular polysaccharide repeating unit using novel L-FucNAc and D-FucNAc synthons and immunochemical evaluation

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ABSTRACT

Staphylococcus aureus is a major cause of nosocomial infections. Glycoconjugates of type 5 and 8 capsular polysaccharides have been investigated for vaccine application. The proposed structure of type 5 polysaccharide is: →4-β-D-ManNAcA-(1→4)-α-L-FucNAc(3OAc)-(1→3)-β-D-FucNAc-(1→. The stereocontrolled insertion of these three glycosydic bonds is a real synthetic challenge. In the present paper we report the preparation of two novel versatile L- and D-fucosamine synthons from commercially available starting materials. In addition we applied the two building blocks to the synthesis of type 5 trisaccharide repeating unit. The immunochemical properties of the synthesized trisaccharide were assessed by competitive ELISA and by immunodot blot analysis using sera of mice immunized with type 5 polysaccharide conjugated to CRM_{197}. The results suggest that although the type 5 *S. aureus* trisaccharide is recognized by specific anti polysaccharide antibodies in dot blot, structures longer than the trisaccharide may be needed in order to significantly compete with the native type 5 polymer in the binding with sera from mice immunized with *S. aureus* type 5 polysaccharide–CRM_{197} conjugate.

1. Introduction

Staphylococcus aureus is a major cause of nosocomial infections, the most frequent and serious of which is bacteremia and its complications such as endocarditis, arthritis, and osteomyelitis in hospitalized patients.[1] *S. aureus* accounts approximately for one fourth of the isolates in patients affected by bacteremia, which is often associated with high mortality due to the spreading antibiotic resistance.[2]

Based on the structure of the capsular polysaccharide, 12 types have been isolated. However type 5 and 8 comprise the majority of clinical isolates, and thereby represent an important target for the development of a conjugate vaccine.[3] An unadjuvanted bivalent vaccine composed of *S. aureus* capsular polysaccharides types 5 and 8 chemically conjugated to the recombinant non-toxic mutant of *Pseudomonas aeruginosa* exotoxin A (rEPA), showed to confer 60% protection for 10 months,[4] however in further efficacy trials it failed to induce long lasting protection in end stage renal disease patients who are unusually susceptible to *S. aureus* bacteremia and represent an important target population.[5]

The structure of type 5 repeating unit was first determined by Moreau et al.,[6] then revised by Vann et al.[7] and ultimately by Jones,[8] who proposed the trisaccharide structure →4-β-D-ManNAcA-(1→4)-α-L-FucNAc(3OAc)-(1→3)-β-D-FucNAc-(1→.

To our best knowledge, the synthesis of this structure has never been described and represent a challenge for carbohydrate chemistry: not only it is composed of three very unusual sugars, but it also presents a β-linkage to the ManNAcA (*N*-acetyl-mannuronic acid) unit, an α glycosidic bond to the 3-OAc L-FucNAc (3-*O*-acetyl *N*-acetyl-L-fucosamine) unit, and finally a β-linkage between the D-FucNAc (*N*-acetyl-D-fucosamine) and the following sugar residue.

D-FucNAc has been also found as constituent of the O-chain of *Pseudomonas aeruginosa* lipopolysaccharide antigens.[9] The synthesis of related benzyl protected glycosides has been reported by Horton et al,[10] who used as starting material 2-acetamido-2-deoxy-D-glucose in a strategy based on the inversion of the hydroxyl group in position 4 and C-6 deoxygenation.

L-FucNAc, besides being a component of the O-chain of the antigenic lipopolysaccharide of *Pseudomonas aeruginosa*, has been obtained from the polysaccharides of certain enteric bacteria[11] and by acidic hydrolysis of *Pneumococcus* type 5[12] and type 12[13] capsular polysaccharides. Its preparation by azidonitration of 3,4-di-*O*-acetyl-L-fucal was first reported by Anisuzzaman et al.[14]

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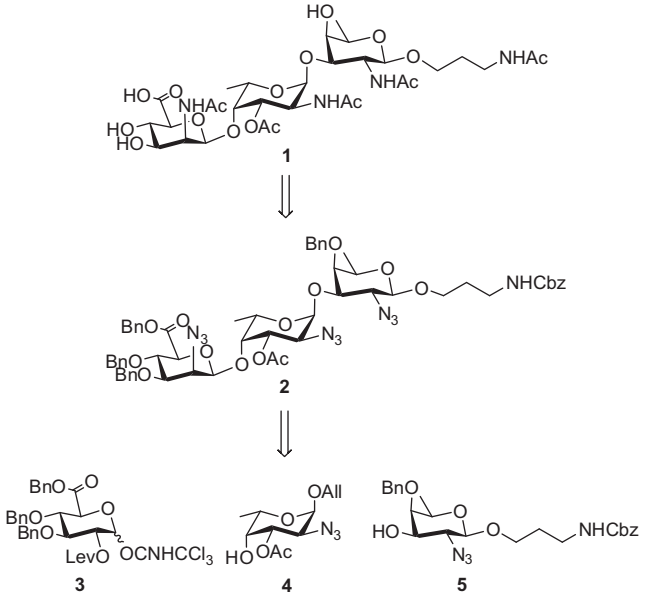


Scheme 1. Retrosynthetic analysis of trisaccharide **1**

In the present paper we disclose the synthesis of the two novel versatile L-FucNAc and D-FucNAc synthons **4** and **5** (Scheme 1), respectively, and their application for assembling *S. aureus* type 5 trisaccharide repeating unit. The immunochemistry of the trisaccharide was evaluated by competitive ELISA and immunodot blot analysis using sera of mice vaccinated with *S. aureus* type 5 polysaccharide–CRM_{197} conjugate.[15]

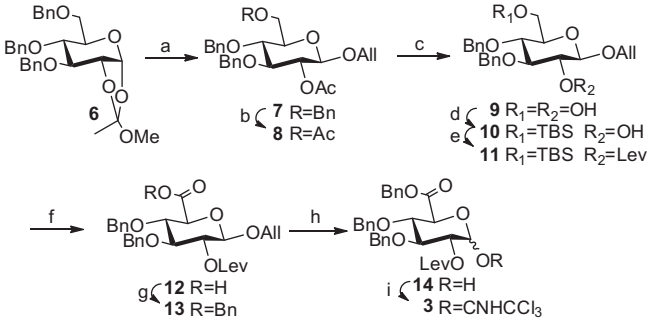
2. Results and discussion

2.1. Synthesis of monosaccharide building blocks and assembling of *S. aureus* type 5 trisaccharide repeating unit

Our retrosynthetic approach to target molecule **1** is outlined in Scheme 1. Since very few preparations of L- and D-FucNAc building blocks have been previously reported, we designed and synthesized the new precursors **4** and **5** as key intermediates. On the other hand, the choice of a suitable synthon for the ManNAcA unit was not trivial. The β-linkage is notoriously difficult to be installed in mannosides, moreover the problem is increased by the presence of a carboxylic group, which further deactivates the glycosyl donor. Though some methods have been reported for the insertion of β-ManNAcA building blocks, the outcome of the reaction is often unsatisfactory and strictly dependent on the substituents in the donor.[16] Very recently, an improved mannopyranosyl uronic acid donor for β- glycosylation has been reported by van der Marel and coworkers.[17] Another option to introduce a β- mannosyl linkage is the glycosylation with a glucopyranosyl donor, followed by epimerization at C-2 to get the *manno* configuration.[18] In our synthetic strategy we pursued the latter approach, and we employed the 2-*O*-levulinoyl glucuronate donor **3** as a precursor of the β-ManNAcA unit.

Azides were chosen to mask *N*-acetyl groups of the three carbohydrate units with the purpose of favouring the formation of the α- glycosidic bond between L- and D-FucNAc units, which represented an additional challenge. Moreover, azides would deliver the amine functions during the hydrogenolytic debenzylation of trisaccharide **2**.

The D-FucNAc unit carries an anomerically-linked aminopropyl tether N-protected as a benzyl carbamate (Cbz)[19] for possible conjugation to a carrier protein.



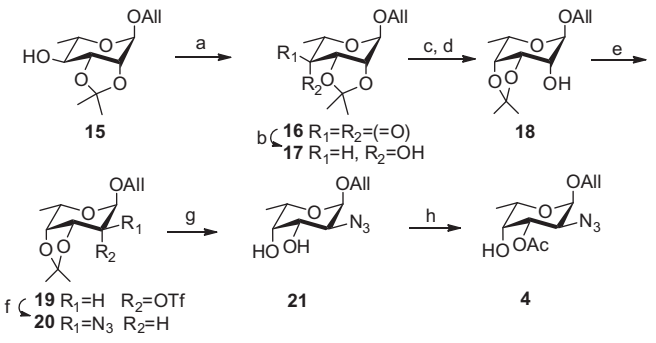
Scheme 2. Synthesis of NAcManA building block **3**. Reagents and conditions: (a) AllOH, TMSOTf; then Ac_2O, py, 88%; (b) $ZnCl_2$, AcOH, 76%; (c) NaOMe/MeOH, 85%; (d) TBSCl, Im, DMF, 94%; (e) LevOH, DCC, DMAP, CH_2Cl_2, 80%; (f) CrO_3, H_2SO_4, $(CH_3)_2CO/H_2O$; g. BnBr, $NaHCO_3$, CH_2Cl_2, 65% (2 steps); (h) $PdCl_2$, NaOAc, AcOH; (i) CCl_3CN, DBU, CH_2Cl_2, 66% (2 steps).

The glucuronic donor **3** was prepared by glycosylation of allyl alcohol with the known orthoester **6**.[20] The reaction afforded a mixture of the allyl 2-*O*-acetyl and allyl 2-*O*-hydroxy derivatives, as reported in literature,[20] so acetylation of the reaction crude was needed to provide **7** in 88% yield (Scheme 2). Following acetolysis of the 6-*O*-benzyl ether at C-6 with $ZnCl_2$ in acetic acid (76% yield) gave the 6-*O*-acetylated derivative **8**.

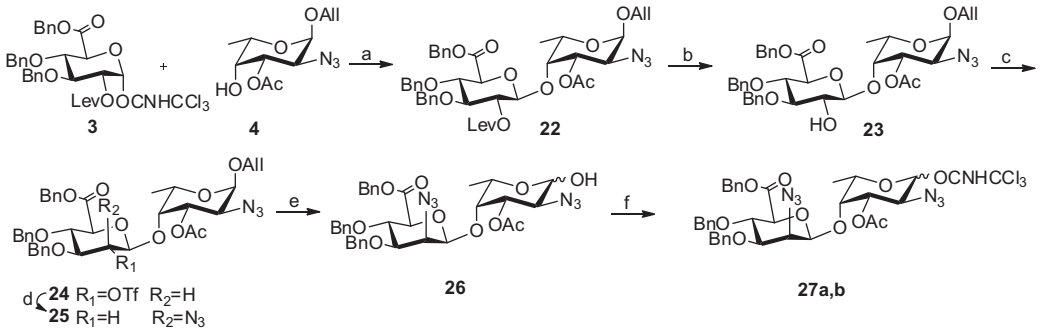
Deacetylation by Zemplen transesterification and subsequent selective silylation of the primary alcohol **9** using *t*-butyldimethylsilyl chloride (TBSCl) and imidazole provided compound **10** (94% yield). At this stage orthogonal protection at C-2 of **10** was inserted by reaction with levulinic acid, *N,N′*-dicyclohexylcarbodiimide and 4-dimethylaminopyridine (80% yield).[21]

The 6-O-desilylation and C-6 oxidation of **11** were achieved one-pot under Jones conditions,[22] followed by mild benzylation with BnBr and $NaHCO_3$ to provide the glucuronic derivative **13** in 65% yield over two steps. Deallylation of the anomeric position by reaction with $PdCl_2$ and subsequent treatment of the foregoing 1-hydroxyl derivative with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) led to donor **3**, almost exclusively as α- anomer.

The preparation of acceptor **4** required a new reaction sequence (Scheme 3), and we envisaged L-rhamnose as suitable starting material, which could be converted into L-FucNAc synthon by sequential epimerization at C-4 and C-2. Accordingly, compound **15**,[23] easily obtained from commercial L-rhamnose, was epimerized at C-4 by Swern oxidation and subsequent reduction with $NaBH_4$ to afford the *talo* derivative **17** in excellent yield (94%) with complete stereoselectivity.[24]



Scheme 3. Synthesis of L-FucNAc building block **4**. Reagents and conditions: (a) $(COCl)_2$; DMSO, CH_2Cl_2; (b) $NaBH_4$, EtOH, 94% (2 steps); (c) 90% AcOH; (d) $CH_3C(OCH_3)_2CH_3$, *p*-TsOH, 82% (2 steps); (e) Tf_2O, py, CH_2Cl_2; (f) $TBAN_3$, Tol, 64% (2 steps); (g) 80% AcOH, 86%; (h) AcCl, py, CH_2Cl_2, 99%.



Scheme 4. Synthesis of disaccharide donor **27a,b**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −10 °C, 61%; (b) $H_2NNH_2{\cdot}AcOH$, CH_2Cl_2/MeOH, 86%; (c) Tf_2O, py, CH_2Cl_2; (d) $TBAN_3$, Tol, 70%; (e) $PdCl_2$, MeOH, 78%; (f) CCl_3CN, DBU, CH_2Cl_2, 98%.

A triplet at 3.49 ppm in the ^{1}H NMR spectrum of **17** with J = 5.6 Hz assigned to H-4 (to be compared with $J_{3,4}$ = 7.1 Hz in precursor **15**) confirmed the axial orientation of C-4 hydroxyl and the inversion of configuration. The shift of the isopropylidene protection from position 2,3 to 3,4 was achieved by hydrolysis in aqueous acetic acid, followed by isopropylidene re-insertion, making the C-2 hydroxyl group of the resulting **18** available for the inversion of configuration (82% yield over two steps). The treatment of **18** with trifluoromethanesulfonic anhydride (Tf_2O) in CH_2Cl_2 in the presence of pyridine, followed by nucleophilic displacement with freshly prepared tetrabutylammonium azide furnished the 2-azido L-fucoside **20** in 64% yield. A doublet of doublet at 3.37 ppm with $J_{2,3}$ = 10.7 Hz and $J_{1,2}$ = 3.7 Hz assigned to H-2 confirmed the *fuco* configuration.

Finally, isopropilydene removal by acidic hydrolysis with 80% aqueous acetic acid to give **21** (86% yield), and regioselective acetylation of C-3 hydroxyl group by dropwise addition of acetyl chloride in dichloromethane-pyridine at 0 °C provided the acceptor **4** in nearly quantitative yield.

The synthesis of compound **1** was based on a (2+1) strategy, where disaccharide donor **27** would be reacted with the D-Fuc (D-Fucose) acceptor **5**. To this end disaccharide **22** was assembled by glycosylation of **4** with **3**, using TMSOTf a as promoter at −10 °C (Scheme 4).

The optimization of the reaction conditions was a very challenging task, since the 3-*O*-acetyl group of **4** was prone to partial migration from C-3 to C-4 under the acidic conditions of the glycosylation. While low temperature showed to slow down this side reaction, the glycosylation proceeded in sluggish way leading to decomposition of the donor **3**. Final conditions were the best compromise to drive the reaction toward the desired product, obtained in reasonable 61% yield. The β-*manno* configuration was then introduced by selective removal of the levulinoyl group and inversion at C-2 through O-triflylation and nucleophilic displacement of the O-triflate with tetrabutylammonium azide to obtain **25** in 70% yield. The β-configuration of the mannuronic unit was evidenced by a singlet at 4.44 ppm in the ^{1}H NMR spectrum and a signal at 101.03 ppm ($J_{C\text{-}1,H\text{-}1}$ = 157 Hz) in the ^{13}C NMR spectrum.[25]

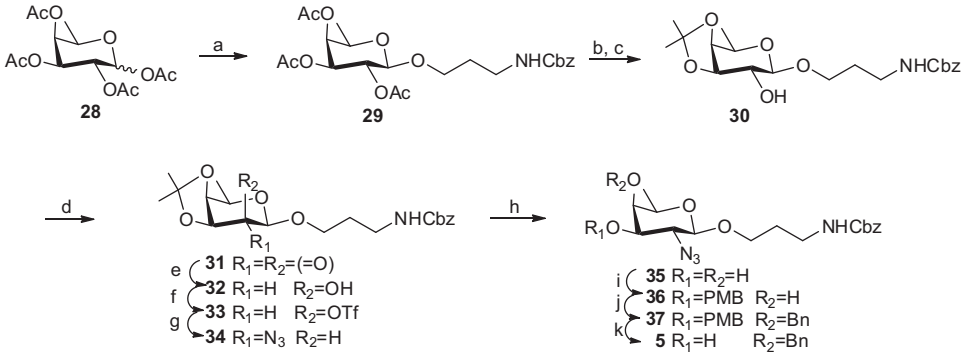
After cleavage of the anomeric allyl protection by $PdCl_2$, compound **26** was converted into the disaccharide trichloroacedimidate **27a,b** by reaction with trichloroacetonitrile and DBU. The α/β mixture of trichloroacedimidates **27a,b** could be resolved by chromatography and the reactivity of the two epimers was studied separately.

The synthesis of D-FucNAc acceptor **5** commenced with glycosylation of benzyl *N*-(3-hydroxypropyl)carbamate with D-Fuc peracetate to give **36** in 72% yield (Scheme 5).

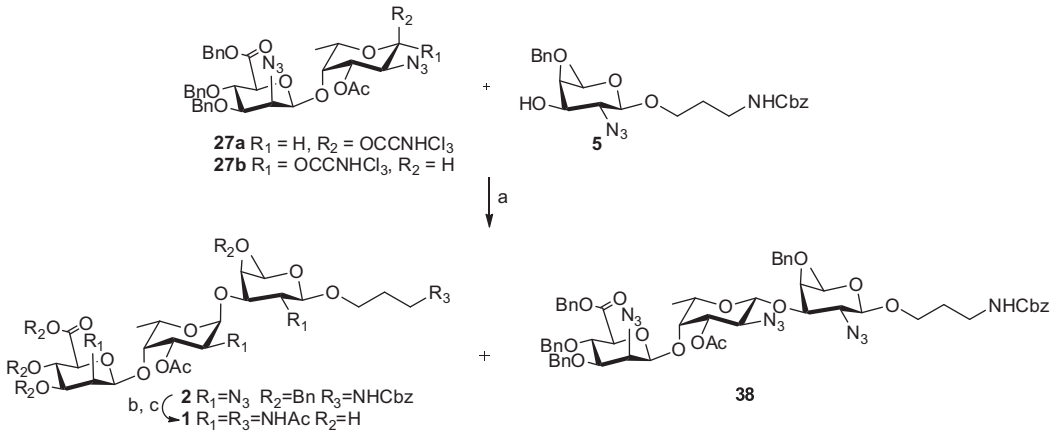
A doublet at 5.21 ppm and $J_{1,2}$ = 7.8 Hz in the ^{1}H NMR spectrum was attributed to H-1 and confirmed the β- configuration of the newly formed glycosidic bond. Deacetylation of **28** and following introduction of the isopropylidene protection afforded compound **30**, which was very efficiently inverted at C-2 by Swern oxidation and subsequent reduction with $NaBH_4$, leading stereoselectively to the *talo* configuration in **32** (87% yield, over two steps).[24]

O-triflylation at C-2 followed by displacement with sodium azide provided in high yield (90%) the 2-azido D-fucoside **34**, which possessed a β-anomeric configuration, as proved in the ^{1}H NMR spectrum by a doublet at 4.17 ppm with $J_{1,2}$ = 8.6 Hz assigned to H-1.

After removal of the isopropylidene protective group, the unfavoured introduction of a benzyl protective group at the axial C-4



Scheme 5. Synthesis of D-FucNAc acceptor **5**. Reagents and conditions: (a) $HO(CH_2)_3NHCbz$, $BF_3{\cdot}Et_2O$, 72%; (b) NaOMe/MeOH; (c) $CH_3C(OCH_3)_2CH_3$, *p*-TsOH, 87% (2 steps); (d) $(COCl)_2$, DMSO; CH_2Cl_2; (e) $NaBH_4$, EtOH, 87%; (f) Tf_2O, py, CH_2Cl_2; (g) NaN_3, DMF, 90%; (h) 90% AcOH, 98%; (i) $BuSn_2O$, PMBBr, TBAI, Tol, 76%; (j) BnBr, NaOH, 18-crown-6, THF/H_2O, 99%; (k) DDQ, CH_2Cl_2/H_2O, 94%.



Scheme 6. Synthesis of trisaccharide **1**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −10 °C, 65%; (b) 10% Pd-C, MeOH/H_2OAcOH; (c) Ac_2O, MeOH/H_2O, 40%.

hydroxyl required the temporary protection of the C-3 hydroxyl group as *p*-methoxybenzyl ether (PMB) to give **35**.[25]

Benzylation at C-4 was achieved in excellent yield using BnBr and NaOH as base in the presence of crown ether in THF.[25] Other methods, such as BnBr–NaH or BnBr–NaOH in DMF, produced benzylation also of the amide function. After selective oxidative cleavage of PMB by use of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), compound **37** furnished the key compound **5** in very good overall yield.

Glycosylation of D-Fuc acceptor **5** with the α disaccharide **27a,** in the presence of TMSOTf as promoter, at -20 °C (Scheme 6) led to a mixture of the desired trisaccharide **2** and the product of β-linkage formation **38** in a ratio ~2.3:1 (Table 1, entry 1). The stereochemical orientation of the newly formed anomeric center was determined by ^{1}H NMR: whereas the α- product **2** presented a doublet at 5.29 ppm with a $J_{1,2}$ = 3.8 Hz, the β-anomer **38** showed a doublet at 4.40 ppm with $J_{1,2}$ = 8.3 Hz. When the temperature was raised up to −10 °C, only very little improvement was achieved (entry 2), while using diethyl ether as solvent instead of dichloromethane gave very surprisingly the predominant formation of the β trisaccharide (**2**:**38** = 1:2, entry 3). The use of β donor **27b** and TMSOTf as promoter led to a mixture **2**:**38** ≈ 2.3:1 at −20 °C and 2.8:1 at −10 °C (entry 4 and 6, respectively). $BF_3{\cdot}Et_2O$ was then added as promoter at 0 °C, to favour the displacement of β-trichloroacetidimoyl group of **27b** by the acceptor **5** via S_N2 process.[26] Nevertheless, the reaction stereoselectivity was not improved, and once more a mixture **2**:**38** = 2.4:1 was obtained (entry 5). Eventually, since our primary purpose was a preliminary antigenic evaluation of trisaccharide **1**, we deemed the yield in entries 2 and 6 acceptable and no further investigation of this challenging glycosylation was performed at this stage.

Conversion of the azide groups to acetamide and simultaneous deprotection of trisaccharide **2** was carried out by hydrogenation in flow chemistry using a 10% Pd-C cartridge, followed by treatment with acetic anhydride in MeOH. The anomeric region of the deprotected trisaccharide **1** in the ^{1}H NMR was in a good agreement with reported NMR data,[8] except for the D-FucNAc unit which carries a *N*-acetamidopropyl chain as aglycon in the synthesized repeating unit. When we tried to selectively convert the azides of **2** into amines without concomitant removal of the Cbz protection in the linker, the lactamization of the mannuronic unit was the prevalent reaction either under Staudinger conditions (trimethylphosphine and aqueous NaOH in THF) or by treatment with H_2S in pyridine–water.

2.2. Immunochemical analyses

The immunochemical properties of the synthesized trisaccharide **1** were assessed by competitive ELISA and immunodot blot analysis. For this purpose, *S. aureus* type 5 capsular polysaccharide (SA5) was purified adapting a procedure described in literature.[4a,27] After depolymerization and sizing of the capsular polysaccharide, the obtained fragments were oxidized in order to introduce an aldehyde group and coupled to CRM_{197} via direct reductive amination.[28] The glycoconjugate, formulated with aluminium hydroxide as adjuvant, was then used for immunization of CD1 mice. Sera containing anti-SA5 capsular polysaccharide IgG antibodies, as determined by ELISA assay, were used for competitive ELISA and dot blot.

As shown in Figure 1, in competitive ELISA on plates coated with SA 5 polysaccharide, the native polysaccharide at a starting concentration of 4.5 mg/ml fully inhibited the binding of sera from mice immunized with SA5-CRM_{197} conjugate, whereas the synthetic repeating unit did not exhibit significant inhibition (≈15%) at the same concentration. The synthetic trisaccharide **1** was then tested in dot blot (Fig. 2), and while no staining was observed for the negative control (the non *S. aureus* correlated β-glucan polysaccharide laminarin[29]), antibodies directed against SA5 recognized the native polysaccharide used as positive control (SA5) as well as compound **1**, although apparently with lower intensity.

3. Conclusions

We report here the synthesis and the antigenic evaluation of the repeating unit of the type 5 *S. aureus* polysaccharide, which to date has never been described. Our synthetic goal was achieved by exploiting the versatility of two novel synthons for L-FucNAc and D-FucNAc residues (**4** and **5**, respectively), synthesized from cheap commercially available starting materials.

A (2+1) strategy based on glycosylation of the D-FucNAc acceptor **5** with the protected β-ManNAcA-(1→4)-α-L-FucNAc(3OAc) disaccharide donor **27a,b** was applied.

The β-mannuronate of disaccharide **27a,b** was in turn obtained by coupling the L-FucNAc synthon **4** and the β- glucopyranosyl donor **3**, followed by epimerization at C-2 of the β-*gluco* unit.

Glycosylation of **5** with **27a,b** proved a moderate α stereoselectivity allowing the synthesis of the desired trisaccharide **2**, which after deprotection provided the target molecule **1**. The spectroscopic characterization of trisaccharide **1** confirmed previous structure assignment.[8]

The immunochemical properties of trisaccharide **1** were evaluated by competitive ELISA and immunodot blot analysis. While dot blot indicated that a fragment as small as a single repeating unit is recognized by anti *S. aureus* type 5 polysaccharide sera, competitive ELISA result suggested that longer structures are necessary

Table 1
Tested conditions for glycosylation of acceptor **5**[a]

Entry	Donor	Solvent	Promoter	Temperature (°C)	α/β = **2:38**
1	**27a**	CH_2Cl_2	TMSOTf	−20	2.3:1
2	**27a**	CH_2Cl_2	TMSOTf	−10	2.8:1
3	**27a**	Et_2O	TMSOTf	−10	1:2
4	**27b**	CH_2Cl_2	TMSOTf	−20	2.3:1
5	**27b**	CH_2Cl_2	$BF_3{\cdot}Et_2O$	0	2.4:1
6	**27b**	CH_2Cl_2	TMSOTf	−10	2.8:1

[a] Ratios of trisaccharides **2** and **38** were determined by NMR analysis of isolated products. Yields were comprised in the range 63–70%.

to efficiently inhibit the binding between the native polymer and *S. aureus* type 5 polysaccharide–CRM_{197} conjugate.

These findings highlight that synthetic glycans from *S. aureus* type 5 capsular polysaccharide could be useful structures to be studied as potential candidates for an anti *S. aureus* vaccine, however fragments longer than the single trisaccharide repeating unit would be needed.

Our conclusions are in line with results reported for other bacterial polysaccharides, as it has been observed that while in certain cases small synthetic fragments are sufficient to elicit antibodies that efficiently bind to the native polysaccharide,[30] other examples show that longer structures are required.[31]

4. Experimental section

4.1. General methods

All chemicals were of reagent grade, and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 F254 (Sigma–Aldrich); after examination under UV light, compounds were visualized by heating with 10% (v/v) ethanolic H_2SO_4. In the work up procedures, organic solutions were washed with the amounts of the indicated aqueous solutions, then dried with anhydrous Na_2SO_4, and concentrated under reduced pressure at 30–50 °C on a water bath. Column chromatography was performed on Silica Gel 60 (Sigma Aldrich, 0.040–0.063 nm) or using pre-packed silica cartridges RediSep (Teledyne-Isco, 0.040–0.063 nm) SiliaSep HP (Silicycle, 0.015–0.040 nm) or Supelco (Sigma Aldrich, spherical silica 0.040–0.075 nm). Unless otherwise specified, a gradient 0 → 100% of the elution mixture was applied in a Combiflash Rf (Teledyne-Isco) instrument. ^{1}H NMR spectra were measured at 400 MHz with a Bruker Avance III 400 spectrometer; δ_H values are reported in ppm, relative to internal Me_4Si (δ_H = 0.00, $CDCl_3$ and CD_3OD); solvent peak for D_2O was calibrated at 4.79 ppm. ^{13}C NMR spectra were measured at 100 MHz with a Bruker Avance^{III} 400 spectrometer; δ_C values are reported in ppm relative to the signal of $CDCl_3$ (δ_C = 77.0, $CDCl_3$), CD_3OD (δ_C = 49.0, CD_3OD) or internal acetone (δ_C = 30.9, D_2O). Assignments of NMR signals were made by homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometer. Assignment of ^{13}C NMR spectra of some compounds was aided by comparison with spectra of related substances reported previously from this laboratory or elsewhere.

When reporting assignments of NMR signals, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycon, and are identified by a Roman numeral superscript in listings of signal assignments. Nuclei associated with the linker are denoted with a prime. Exact masses were measured by electron spray ionization cut-off spectroscopy, using a Q-Tof *micro* Macromass (Waters) instrument. Structures of these compounds follow unequivocally from the mode of synthesis, NMR data and *m/z* values found in their mass spectra, and their purity was verified by TLC and NMR spectroscopy. Palladium chloride catalyst was purchased from Sigma–Aldrich. Hydrogenation reactions were performed in a continuous flow reactor H-Cube (Thalesnano) instrument, using packed catalyst cartridges CatCart. Laminarin polysaccharide was purchased from Sigma–Aldrich.

4.1.1. Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside 7

3,4,6-Tri-*O*-benzyl-[1,2-*O*-(1-methoxyethylidene)]-α-D-glucopyranoside **6** (22.6 g, 0.045 mol) was dissolved under nitrogen atmosphere in anhydrous allyl alcohol (47.5 ml). The solution was cooled to 0 °C and TMSOTf (1.8 ml, 0.01 mmol) was slowly added. The mixture was reacted at room temperature for 3 h, when TLC (7:3 cyclohexane/EtOAc) showed the formation of a ~1:1 (v/v) mixture of the allyl 2-*O*-acetyl and allyl 2-O-hydroxy derivatives, as reported in literature.[20] The residue was dissolved in 4:1 (v/v) acetic anhydride/pyridine (300 ml), and the mixture was stirred 3 h at room temperature (TLC, 7:3 cyclohexane/EtOAc), when it was concentrated and purified on silica gel (9:1 cyclohexane/EtOAc), to give compound **7** (21.2 g, 88% yield) as a pale yellow oil. $[\alpha]_D^{23}$ = +4.8 (*c* 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35–7.16 (m, 15H, Ph), 5.90–5.80 (m, 1H, CH=), 5.26–5.14 (m, 2H, CH_2=), 5.03 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.03 Hz, H-2), 4.79, 4.67 (2 d, 2H, ^2J = 11.4 Hz, CH_2Ph), 4.78, 4.55 (d, 2H, ^2J = 11.9 Hz, CH_2Ph), 4.62, 4.55 (2 d, 2H, ^2J = 11.9 Hz, CH_2Ph), 4.41 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.36–4.31 (m, 1H, CH_{2a}), 4.10–4.04 (m, 1H, CH_{2b}), 3.76–3.64 (m, 4H, H-3,4,6), 3.50–3.46 (m, 1H, H-5), 1.96 (s, 3H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.40 (CO), 138.05, 137.99, 137.76 (Ar), 133.71 (CH=), 128.31, 128.25, 127.90, 127.71, 127.61, 127.50, 127.40 (Ar), 116.84 (CH_2=), 99.75 (C-1), 82.86, 77.88 (C-3,4), 75.04 (C-5), 74.91, 74.91 (CH_2Ph), 73.36 (CH_2Ph), 72.98 (C-2), 69.43 (CH_2), 68.60 (C-6), 20.81 (CH_3CO). ESI HR-MS ($C_{32}H_{36}O_7$): *m/z* = ([*M*+Na]$^+$ found 555.2353; calcd 555.2359).

4.1.2. Allyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranoside 8

Zinc chloride (5 g, 0.037 mol), freshly melted under a nitrogen atmosphere, was dissolved in acetic acid (88 ml). A solution of compound **7** (5.85 g, 0.011 mol) in acetic anhydride (175 ml) was then added, and the mixture was stirred at room temperature for 12 h when TLC (2:1 cyclohexane/EtOAc) showed complete reaction. The mixture was then diluted with H_2O (100 ml) and partitioned with EtOAc (150 ml). The organic layer was separated and washed with aq $NaHCO_3$ (×4). Combined organic layers were concentrated, and the crude residue was purified on silica gel (17:3 cyclohexane/EtOAc) to give compound **8** (4.0 g, 76% yield) as pale yellow oil. $[\alpha]_D^{23}$ = −20.7 (*c* 0.20, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35–7.26 (m, 10H, Ph), 5.85–5.78 (m, 1H, CH=), 5.27–5.16 (m, 2H, CH_2=), 5.03 (dd, 1H, $J_{1,2}$ = 8.3, $J_{2,3}$ = 8.6 Hz, H-2), 4.83, 4.69 (2 d, 2H, ^2J = 10.9 Hz, CH_2Ph), 4.80, 4.57 (2 d, 2H, ^2J = 11.2 Hz, CH_2Ph), 4.42 (d, 1H, H-1), 4.37 (dd, 1H, $J_{6a,6b}$ = 11.9, $J_{5,6a}$ = 2.0 Hz, H-6a), 4.33–4.28 (m, 1H, CH_{2a}), 4.21 (dd, 1H, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.07–4.00 (m, 1H, CH_{2b}), 3.72–3.62 (m, 2H, H-3,4), 3.54–3.51 (m, 1H, H-5), 2.04 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.76, 169.52 (CO), 137.93, 137.46 (Ar), 133.61

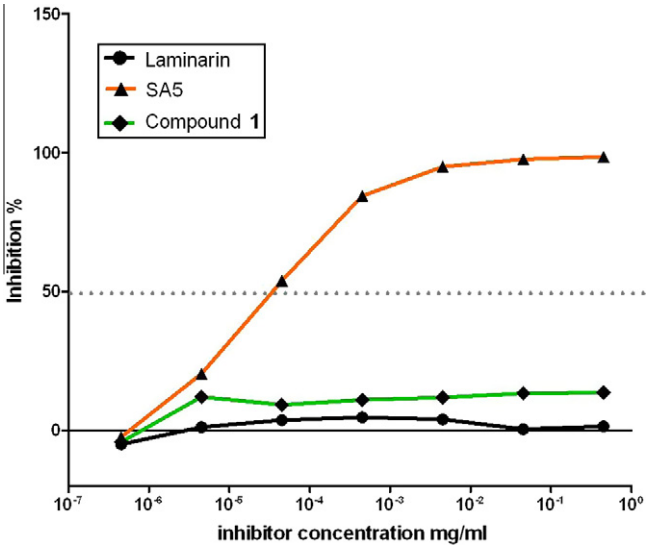


Figure 1. Competitive ELISA analysis of *S. aureus* type 5 polysaccharide ($IC_{50} = 3.4 \times 10^{-5}$ mg/ml) and synthetic trisaccharide **1** at a starting inhibitor concentrantion of 4.5 mg/ml and dilutions thereof. Laminarin was used as negative control.

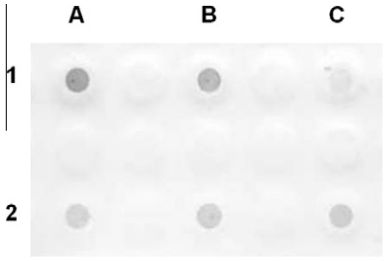


Figure 2. Immunodot blot assay with anti SA5 sera. A1: SA5 (100 μg); B1: SA5 (10 μg); C1: laminarin (100 μg, negative control); A2, B2 and C2: trisaccharide **1** (50 μg each).

(CH=), 129.32, 128.88, 128.52, 128.46, 128.15, 128.07 (Ar), 117.21 (CH_2=), 99.72 (C-1), 82.98, 77.41 (C-3,4), 75.12, 75.04 (CH_2Ph), 72.97 (C-2,5), 69.71 (CH_2), 62.78 (C-6), 20.89, 20.84 (CH_3CO). ESI HR-MS ($C_{27}H_{32}O_8$): *m/z* ($[M+Na]^+$ found 507.1983; calcd 507.1995).

4.1.3. Allyl 3,4-di-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-β-D-glucopyranoside 10

To a solution of compound **8** (4.0 g, 8.25 mol) in MeOH (150 ml), a solution of NaOMe (1.8 g) in MeOH (20 ml) was added. The mixture was stirred at 50 °C for 12 h, when TLC (2:1 cyclohexane/EtOAc) showed the reaction was complete. The mixture was neutralized with Dowex H^+, then filtered and the solvent evaporated. The crude mixture was chromatographed (cyclohexane/EtOAc) to afford compound **9** (2.8 g, 85% yield).

4.1.4. Allyl 3,4-di-*O*-benzyl-β-D-glucopyranoside 9

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38–7.23 (m, 10H, Ph), 5.98–5.88 (m, 1H, CH=), 5.34–5.20 (m, 2H, CH_2=), 4.93, 4.85 (2 d, 2H, ^2J = 11.2 Hz, CH_2Ph), 4.87, 4.68 (2 d, 2H, ^2J = 11.5 Hz, CH_2Ph), 4.38–4.34 (m, 1H, CH_{2a}), 4.35 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.15–4.10 (m, 1H, CH_{2b}), 3.86 (dd, 1H, $J_{6a,6b}$ = 12.0, $J_{5,6a}$ = 2.4 Hz, H-6a), 4.24 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, H-6b), 3.63–3.51 (m, 3H, H-2,3,4), 3.39–3.36 (m, 1H, H-5), 2.50 (br s, 1H, OH), 2.04 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 138.48, 137.88 (Ar), 133.64 (CH=), 128.45 128.02, 127.89, 127.73 (Ar), 118.04 (CH_2=), 101.80 (C-1), 84.23, 77.20 (C-2/3/4), 75.31 (C-5), 75.14, 75.03 (CH_2Ph), 74.58 (C-2/3/4), 70.51 (CH_2), 61.88 (C-6). ESI HR-MS ($C_{23}H_{28}O_6$): *m/z* = ($[M+Na]^+$ found 423.1769 calcd 423.1784).

To a solution of **9** (4.75 g, 11.86 mmol) in dry DMF (95 ml), imidazole (1.21 g, 13.05 mmol) was added, under a nitrogen atmosphere. The mixture was cooled down to 0 °C and *t*-butyldimethylsilyl chloride (1.97 g, 17.79 mmol) was added. The mixture was slowly warmed up to room temperature and stirred for 2 days, when TLC (3:1 cyclohexane/EtOAc) showed that the reaction was complete. The mixture was diluted with H_2O (50 ml), and partitioned with EtOAc (100 ml × 2). Combined organic layers were washed with aq $NaHCO_3$, and concentrated. The crude residue was purified on silica gel (4:1 cyclohexane/EtOAc), to give compound **10** (5.77 g, 94% yield) as pale yellow oil. $[\alpha]_D^{23} = -15.5$ (*c* 0.99, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39–7.26 (m, 10H, Ph), 5.99–5.89 (m, 1H, CH=), 5.33–5.20 (m, 2H, CH_2=), 4.89, 4.86 (2 d, 2H, ^2J = 11.2 Hz, CH_2Ph), 4.85, 4.68 (2 d, 2H, ^2J = 11.2 Hz, CH_2Ph), 4.37–4.32 (m, 1H, CH_{2a}), 4.30 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.15–4.09 (m, 1H, CH_{2b}), 3.87 (dd, 1H, $J_{6a,6b}$ = 11.5, $J_{5,6a}$ = 1.8 Hz, H-6a), 3.83 (dd, 1H, H-6b), 3.63–3.56 (m, 2H, H-3,4), 3.52 (dd, 1H, $J_{2,3}$ = 7.9 Hz, H-2), 3.32–3.29 (m, 1H, H-5), 2.30 (br s, 1H, OH-2), 1.26 (s, 9H, $(CH_3)_3$CSi), 0.07 (s, 3H, CH_3Si), 0.06 (s, 3H, CH_3Si). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 138.58, 138.27 (Ar), 133.88 (CH=), 128.43 128.40, 127.96, 127.74, 127.71 (Ar), 117.79 (CH_2=), 101.37 (C-1), 84.40, 77.28 (C-3,4), 76.05 (C-5), 75.18, 74.96 (CH_2Ph), 74.60 (C-2), 69.85 (CH_2), 62.17 (C-6), 25.84 ($(CH_3)_3$CSi), 5.41 (CH_3Si), 5.10 (CH_3Si). ESI HR-MS ($C_{29}H_{42}O_6Si$): *m/z* = ($[M+Na]^+$ found 537.2654; calcd 514.2648).

4.1.5. Allyl 3,4-di-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-2-*O*-levulinoyl-β-D-glucopyranoside 11

Compound **10** (5.77 g, 11.21 mmol) was dissolved in dry CH_2Cl_2 (250 ml), then dicyclohexyl carbodiimide (3.0 g, 14.57 mmol), levulinic acid (1.69 g, 14.57 mmol), and dimethyl aminopyridine (1.78 g, 15.57 mmol) were added. The mixture was stirred overnight at room temperature (TLC, 2:1 cyclohexane/EtOAc). After filtration, the solvent was evaporated and the crude was purified by column chromatography (cyclohexane/EtOAc), to obtain compound **11** (5.88 g, 85%) as a pale yellow oil. $[\alpha]_D^{23} = -39.5$ (*c* 1.08, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35–7.25 (m, 10H, Ph), 5.88–5.81 (m, 1H, CH=), 5.25–5.14 (m, 2H, CH_2=), 4.96 (dd, 1H, $J_{1,2}$ = 8.4, $J_{2,3}$ = 8.6 Hz, H-2), 4.81, 4.67 (2 d, 1H, ^2J = 10.2 Hz, CH_2Ph), 4.80, 4.70 (2 d, 2H, ^2J = 11.3 Hz, CH_2Ph), 4.39 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.29–4.24 (m, 1H, CH_{2a}), 4.11–4.03 (m, 1H, CH_{2b}), 3.88–3.81 (m, 2H, H-6), 3.71–3.63 (m, 2H, H-3,4), 3.30–3.28 (m, 1H, H-5), 2.70–2.67 (m, 2H, $COCH_2$), 2.57–2.51 (m, 2H, CH_2COO), 2.15 (s, 3H, CH_3CO), 1.60 (s, 9H, $(CH_3)_3$CSi), 0.06 (s, 3H, CH_3Si) 0.05 (s, 3H, CH_3Si). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 206.31 (CO), 171.48 (COO), 138.30, 138.17 (Ar), 133.98 (CH=), 128.77, 128.45, 128.35, 128.02, 127.94, 127.83, 127.65 (Ar), 117.03 (CH_2=), 99.54 (C-1), 82.77, 77.66 (C-3,4), 79.94 (C-5), 75.05, 75.02 (CH_2Ph), 73.55 (C-2), 69.26 (CH_2), 62.01 (C-6), 37.90 (CH_2CO), 29.88 (CH_3CO), 28.01 ($COOCH_2$)), 25.88 (($CH_3)_3$CSi), 5.40 (CH_3Si), 5.06 (CH_3Si). ESI HR-MS ($C_{34}H_{48}O_8Si$): *m/z* = ($[M+Na]^+$ found 635.3047; calcd 635.3016).

4.1.6. (Allyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-β-D-glucopyranosid) uronate 12

To a solution of **11** (4.68 g, 7.64 mmol) in acetone (230 ml) was added Jones' reagent at 0 °C [prepared by slow addition of H_2SO_4 (5.5 ml) to a solution of CrO_3 (3.05 g, 30.56 mmol) in H_2O (40 ml) at 0 °C] and the mixture was stirred for 12 h at room temperature. 2-Propanol was then added until the colour of the solution turned deep green/blue, then the mixture was filtered through Celite and evaporated. The crude was chromatographated on silica gel (1% AcOH/EtOAc) to give compound **12** (3.69 g, 94%) as a pale yellow oil. $[\alpha]_D^{23} = -45.5$ (*c* 1.90, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.30–7.24 (m, 10H, Ph), 5.86–5.82 (m, 1H, CH=), 5.27–5.16 (m, 2H, CH_2=), 5.04 (dd, 1H, $J_{1,2}$ = 7.3, $J_{2,3}$ = 8.2 Hz, H-2), 4.77–

4.62 (m, 4H, CH_2Ph), 4.56 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.34–4.28 (m, 1H, CH_{2a}), 4.09–4.04 (m, 1H, CH_{2b}), 4.04 (d, 1H, $J_{4,5}$ = 8.4 Hz, H-5), 3.94 (dd, 1H, $J_{3,4}$ = 8.8, $J_{4,5}$ = 8.4 Hz, H-4), 3.72 (dd, 1H, $J_{2,3}$ = 8.3 Hz, H-3), 2.71–2.68 (m, 2H, CH_2CO), 2.54–2.45 (m, 2H, CH_2COO), 2.17 (s, 3H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 206.41 (CO), 172.66 (C-6), 171.33 (COO), 137.72, 137.23 (Ar), 133.21 (CH=), 128.38, 128.28, 128.02, 127.87, 127.83, 127.67, 127.61 (Ar), 117.54 (CH_2=), 99.67 (C-1), 81.13 (C-3), 78.39 (C-4), 74.78, 74.56 (CH_2Ph), 74.13 (C-5), 73.13 (C-2), 69.98 (CH_2), 37.70 (CH_2CO), 29.70 (CH_3CO), 27.76 (CH_2COO). ESI HR-MS ($C_{28}H_{32}O_9$): ([M+Na]$^+$ found 535.1921; calcd 535.1944).

4.1.7. Benzyl (allyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-β-D-glucopyranosid)uronate 13

To a solution of compound **12** (3.64 g, 7.10 mmol) in dry DMF (37 ml), $NaHCO_3$ (1.79 g, 21.3 mmol) and benzyl bromide (4.2 mL, 35.5 mmol) were added. The mixture was stirred at room temperature for 4 h, monitoring by TLC (1:1 cyclohexane/EtOAc). The solvent was then evaporated, and the obtained residue was dissolved in EtOAc (50 mL) and washed with aq $NaHCO_3$. Combined organic layers were again concentrated, and the crude mixture was purified on silica gel (6:1 cyclohexane/EtOAc) to give compound **13** (2.8 g, 65% yield). White crystals from EtOAc: mp 44–45 °C. $[\alpha]_D^{23}$ = −47.7 (*c* 0.67, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.32–7.10 (m, 15H, Ph), 5.89–5.78 (m, 1H, CH=), 5.26–5.15 (m, 2H, CH_2=), 5.18 (s, 2H, CH_2Ph), 5.05 (dd, 1H, $J_{1,2}$ = 7.6, $J_{2,3}$ = 9.1 Hz, H-2), 4.84, 4.52 (2 d, 2H, ^2J = 11.4 Hz, CH_2Ph), 4.75, 4.73 (2 d, 2H, ^2J = 10.7 Hz, CH_2Ph), 4.53 (d, 1H, H-1), 4.32–4.27 (m, 1H, CH_{2a}), 4.07–4.02 (m, 1H, CH_{2b}), 3.98–3.85 (m, 2H, H-4,5), 3.68 (m, 1H, H-3), 2.70–2.67 (m, 2H, $COCH_2$), 2.55–2.40 (m, 2H, CH_2COO), 2.15 (s, 3H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 206.16 (CO), 171.31 (COO), 168.19 (C-6), 137.93, 137.59, 136.00 (Ar), 133.41 (CH=), 128.57, 128.51, 128.34, 128.31, 128.15, 127.90, 127.83, 127.77, 127.72 (Ar), 117.46 (CH_2=), 100.01 (C-1), 81.82 (C-3), 79.12 (C-4/5), 74.96, 74.84 (CH_2Ph), 74.61 (C-4/5), 73.18 (C-2), 69.93 (CH_2), 67.35 (CH_2Ph), 37.79 (CH_2CO), 29.81 (CH_3CO), 27.85 (CH_2COO). ESI HR-MS ($C_{35}H_{38}O_9$): m/z = ([M]$^+$ found 602.2532; calcd 602.2516).

4.1.8. Benzyl (3,4-di-*O*-benzyl-2-*O*-levulinoyl-α-D-glucopyranosyl trichloroacetimidate) uronate 3

To a solution of compound **13** (2.4 g, 4.4 mmol) in 20:1 (v/v) AcOH/H_2O (134 ml), AcONa (22 g) and $PdCl_2$ (1.8 g, 12.0 mmol) were added. The mixture was stirred at room temperature for 24 h, when TLC (1:1 cyclohexane/EtOAc) showed that the reaction was complete. The mixture was diluted with EtOAc and filtered over celite. The recovered solution was washed with aq $NaHCO_3$, and combined organic layers were concentrated. The crude product was chromatographed (cyclohexane/EtOAc), to give compound **14** (1.9 g, 77% yield) as a colourless oil mainly in α-configuration.

4.1.9. Benzyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-α-D-glucopyranosyl uronate 14

^1H NMR ($CDCl_3$, 400 MHz): δ = 7.33–7.12 (m, 15H, Ph), 5.43 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1), 5.14 (s, 2H, CH_2Ph), 4.87 (dd, 1H, $J_{1,2}$ = 3.3, $J_{2,3}$ = 9.3 Hz, H-2), 4.77, 4.74 (2 d, 2H, ^2J = 11.0 Hz, CH_2Ph), 4.75, 4.48 (2 d, 2H, ^2J = 11.0 Hz, CH_2Ph), 4.51 (d, 1H, $J_{4,5}$ = 9.1 Hz, H-5), 4.06 (dd, 1H, $J_{2,3}$ = 9.3, $J_{3,4}$ = 9.0 Hz, H-3), 3.84 (dd, 1H, H-4), 3.63 (br s, 1H, OH), 2.72–2.39 (m, 4H, CH_2CH_2), 2.14 (s, 3H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 206.88 (CO), 171.96 (COO), 169.13 (C-6), 138.14, 137.58, 134.89, 128.46, 128.36, 128.27, 128.21, 127.74, 127.64, 127.58 (Ar), 90.38 (C-1), 79.10 (C-4), 78.57 (C-3), 75.22, 74.81 (CH_2Ph), 73.17 (C-2), 70.52 (C-5), 67.23 (CH_2Ph), 37.81 (CH_2CO), 29.66 (CH_3CO), 27.78 (CH_2COO). ESI HR-MS ($C_{32}H_{34}O_9$): m/z = ([M+K]$^+$ found 601.1792; calcd 601.1840).

To a solution of compound **14** (1.9 g, 3.4 mmol) in dry CH_2Cl_2, trichloroacetonitrile (1.02 ml, 10 mmol) and DBU (51 µl, 0.34 mmol) were added, under a nitrogen atmosphere. The mixture was stirred at room temperature for 4 h, when TLC (1:1 cyclohexane/EtOAc) showed complete reaction. The solvent was then evaporated, and the crude residue was chromatographed (cyclohexane/EtOAc, containing 0.5% Et_3N), to give compound **3** (1.58 g, 66%) as a colourless oil. $[\alpha]_D^{23}$ = −7.6 (*c* 1.10, $CHCl_3$).

^1H NMR ($CDCl_3$, 400 MHz): δ = 7.34–7.10 (m, 15H, Ph), 6.53 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 5.16 (s, 2H, CH_2Ph), 5.10 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2), 4.81, 4.76 (2 d, 2H, ^2J = 11.4 Hz, CH_2Ph), 4.71, 4.45 (2 d, 2H, ^2J = 10.6 Hz, CH_2Ph), 4.46 (d, 1H, $J_{4,5}$ = 10.0 Hz, H-5), 4.10 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 3.90 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 2.68–2.40 (m, 4H, CH_2CH_2) 2.14 (s, 3H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 205.90 (CO), 171.73 (COO), 168.08 (C-6), 160.60 (C=N), 137.83, 137.24, 134.78, 128.96, 128.53, 128.48, 128.34, 128.15, 127.94, 127.90, 127.84, 127.78 (Ar, CCl_3), 93.43 (C-1), 78.90, 78.47 (C-4, C-3), 75.40, 75.35 (CH_2Ph), 72.83 (C-5), 71.90 (C-2), 67.48 (CH_2Ph), 37.57 (CH_2CO), 29.73 (CH_3CO), 27.46 ($COOCH_2$). ESI HR-MS ($C_{34}H_{33}Cl_3NO_9$): m/z = ([M+K]$^+$ 704.0936; calcd 744.0965); ([M+Na]$^+$ found 728.1135; calcd 728.1197).

4.1.10. Allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside 15

Compound **15** was prepared modifying a procedure described in literature.[23] A suspension of rhamnose monohydrate (20 g, 108 mmol) in AllOH (150 ml) was refluxed for 2 h in presence of Dowex H^+ (6 g), monitoring by TLC (1:1 CH_2Cl_2/acetone). The mixture was filtered, and the filtrate was concentrated to be used for the next step.

To a solution of the foregoing allyl rhamnoside (108 mmol) in 1:1 (v/v) 2,2-dimethoxypropane-acetone (160 ml), $BF_3{\cdot}Et_2O$ (0.5 ml) was added, and the mixture was stirred for 1 h, at which time TLC (2:1 cyclohexane/EtOAc) showed that the reaction was complete. The mixture was neutralized with triethylamine (0.5 ml), filtered and the filtrate was concentrated. Chromatography of the residue (4:1 cyclohexane/EtOAc) gave the product 15 (19.3 g, 73% over two steps), whose structure was confirmed by NMR (spectra not reported in literature).[22] ^1H NMR ($CDCl_3$, 400 MHz): δ = 5.89–5.86 (m, 1H, CH=), 5.29–5.17 (m, 2H, CH_2=), 4.96 (s, 1H, H-1), 4.30–4.17 (m, 2H, CH_{2a}, incl. d, 4.15, $J_{2,3}$ = 5.5 Hz, H-2), 4.06 (dd, 1H, $J_{3,4}$ = 7.1 Hz, H-3), 4.02–3.97 (m, 1H, CH_{2b}), 3.74–3.65 (m, 1H, H-5), 3.38–3.43 (ddd, 1H, $J_{4,5}$ = 9.3, $J_{4,OH}$ = 5.4 Hz, H-4), 2.64 (d, 1H, OH-4), 1.53 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.29 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 133.53 (CH=), 117.80 (CH_2=), 109.44 (C(CH_3)$_2$), 96.18 (C-1), 86.03 (C-3), 75.77 (C-2), 74.42 (C-4), 67.89 (CH_2), 65.89 (C-5), 27.92, 26.08 (2 CH_3), 17.41 (C-6).

4.1.11. Allyl 4-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside 16

To a mixture of oxalyl chloride (10.2 ml, 122 mmol) in CH_2Cl_2 (50 ml), DMSO (17 ml, 244 mmol) in CH_2Cl_2 (50 ml) was added drop-wise at −78 °C. After stirring at the same temperature for 30 min, the sugar (15 g, 61 mmol) dissolved in CH_2Cl_2 (150 ml) was added drop-wise and stirring was continued for 30 min at −78 °C. Di-*i*-propylethylamine (43 ml, 244 mmol) was added drop-wise and stirring was continued for 12 h at room temperature. After TLC (2:1 cyclohexane/EtOAc) showed complete reaction, the crude mixture was washed with 10% $Na_2S_2O_3$, and the organic layers concentrated to be used for the next step. A small portion (50 mg) of the crude material was purified on silica gel (cyclohexane/EtOAc) to be used for characterization. ^1H NMR ($CDCl_3$, 400 MHz): δ = 5.94–5.86 (m, 1H, CH=), 5.34–5.24 (m, 2H, CH_2=), 5.02 (s, 1H, H-1), 4.50–4.46 (m, 2H, H-2,3), 4.33–4.20 (m, 2H, H-5, CH_{2a}), 4.11–4.06 (m, 1H, CH_{2b}), 1.53 (s, 3H, CH_3), 1.40 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.37 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 204.67 (CO), 132.90 (CH=), 118.26 (CH_2=), 111.36 (C(CH_3)$_2$),

95.91 (C-1), 78.74 (C-2), 75.89 (C-3), 69.98 (C-5), 68.14 (CH_2), 26.69, 25.44 (2 CH_3), 15.80 (C-6). ESI MS ($C_{12}H_{18}O_5$): *m/z* = found ($[M+H]^+$ 243.1260; calcd 243.1232).

The foregoing cheto compound **16** (62 mmol) was dissolved in EtOH (250 ml), and $NaBH_4$ (122 g, 62 mmol) was added portion wise at 0 °C. The mixture was stirred for 1 h when TLC (4:1 cyclohexane/EtOAc) showed the reaction was complete. The mixture was filtered and the filtrate concentrated and purified on silica gel (99:1 → 7:3 cyclohexane/EtOAc) to give the *talo* sugar **17** (14.1 g, 94%), as colourless sirup. $[\alpha]_D^{23} = -53.0$ (*c* 0.25, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.90–5.80 (m, 1H, CH=), 5.27–5.14 (m, 2H, CH_2=), 5.02 (s, 1H, H-1), 4.18–4.11 (m, 2H, CH_{2a}, incl. t, 4.15, J = 6.5 Hz, H-3), 4.01–3.95 (m, 2H, CH_{2b}, incl. d, 4.00, $J_{2,3}$ = 6.0 Hz, H-2), 3.83–3.78 (m, 1H, H-5), 3.49 (t, 1H, J = 5.6 Hz, H-4), 2.23 (d, 1H, $J_{4,OH}$ = 6.0 Hz, OH-4), 1.52 (s, 3H, CH_3), 1.32 (s, 3H, CH_3). 1.26 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 133.53 (CH=), 117.56 (CH_2=), 109.05 ($C(CH_3)_2$), 96.43 (C-1), 73.09 (C-2), 72.78 (C-3), 68.01 (CH_2), 66.64 (C-5), 64.25 (C-4), 25.64, 25.08 (2 CH_3), 16.48 (C-6). ESI HR-MS ($C_{12}H_{20}O_5$): *m/z* = found ($[M+Na]^+$ 267.1166; calcd 267.1208).

4.1.12. Allyl 6-deoxy-3,4-*O*-isopropylidene-α-L-talopyranoside 18

A solution of talopyranoside **17** (1 g, 0.61 mmol) in 10% AcOH (10 ml) was stirred at 50 °C for 5 h, monitoring by TLC (1:1 cyclohexane/EtOAc). The mixture was concentrated co-evaporating with toluene, then the residue was re-dissolved in 1:1 (v/v) 2,2-dimethoxypropane-acetone (20 ml), and *p*-toluensulfonic acid (100 mg) was added. After stirring for 30 min, TLC (2:1 cyclohexane/EtOAc) showed that the reaction was complete, and the mixture was neutralized with triethylamine and concentrated. Chromatography of the residue (cyclohexane/EtOAc) yielded 800 mg of syrupy product **18** (80%). $[\alpha]_D^{23} = -16.5$ (*c* 2.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.99–5.89 (m, 1H, CH=), 5.34–5.18 (m, 2H, CH_2=), 4.81 (d, 1H, $J_{1,2}$ = 5.4 Hz, H-1), 4.52 (dd, 1H, $J_{2,3}$ = 3.5, $J_{3,4}$ = 7.5 Hz, H-3), 4.29–4.24 (m, 1H, CH_{2a}), 4.12 (dd, 1H, $J_{4,5,}$ = 2.0 Hz, H-4), 4.08–4.03 (m, 1H, CH_{2b}), 3.88–3.83 (m, 1H, H-5), 3.71–3.74 (m, 1H, H-2), 2.37 (br s, 1H, OH-2), 1.53 (s, 3H, CH_3), 1.37 (s, 3H, CH_3). 1.27 (d, 3H, $J_{5,6,}$ = 6.5 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 134.28 (CH=), 117.19 (CH_2=), 109.89 ($C(CH_3)_2$), 99.91 (C-1), 76.10 (C-4), 73.51 (C-3), 68.72 (C-2), 68.49 (CH_2), 65.09 (C-5), 26.01, 25.16 (2 CH_3), 15.80 (C-6). ESI HR-MS ($C_{12}H_{20}O_5$): *m/z* = found ($[M+Na]^+$ 267.1158; calcd 267.1208).

4.1.13. Allyl 2-azido-2-deoxy-3,4-*O*-isopropylidene-α-L-fucopyranoside 20

To a solution of compound **18** (2.5 g, 12.2 mmol) in 6:1 (v/v) CH_2Cl_2–pyridine (28 ml), Tf_2O (2.5 ml, 15 mmol) was added at 0 °C. The mixture was stirred for 30 min at room temperature, when TLC (5:1 cyclohexane/EtOAc) showed the reaction was complete. The mixture was washed with iced aq $NaHCO_3$, and the combined organic layers were concentrated at 30 °C, then at the high vacuum pump to be used for the next step.

4.1.13.1. Allyl 6-deoxy-3,4-O-isopropylidene-2-O-trifluoromethansulfonyl-α-L-talopyranoside 19.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.93–5.83 (m, 1H, CH=), 5.39–5.22 (m, 2H, CH_2=), 5.00 (d, 1H, $J_{1,2}$ = 5.7 Hz, H-1), 4.82 (dd, 1H, $J_{2,3}$ = 3.1, H-2), 4.64 (dd, 1H, $J_{3,4}$ = 7.4 Hz, H-3), 4.28–4.23 (m, 1H, CH_{2a}), 4.19 (dd, 1H, $J_{4,5}$ = 2.0 Hz, H-4), 4.08–4.02 (m, 1H, CH_{2b}), 3.88–3.85 (m, 1H, H-5), 1.53 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.26 (d, 3H, $J_{5,6,}$ = 6.7 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 133.41 (CH=), 118.36 (q, CF_3), 116.79 (CH_2=), 111.28 ($C(CH_3)_2$), 95.81 (C-1), 83.41 (C-2), 75.54 (C-4), 71.56 (C-3), 68.75 (CH_2), 65.62 (C-5), 25.86, 25.26 (2 CH_3), 15.45 (C-6). ESI HR-MS ($C_{13}H_{19}F_3O_7S$): *m/z* = found ($[M+H_2O+H]^+$ 395.1354; calcd 395.0987).

The foregoing triflate **19** was re-dissolved in toluene (25 ml), and tetrabutylammonium azide (8.5 g, 30 mmol) was added. The resulting suspension was stirred for 12 h at 60 °C, when TLC (6:1 cyclohexane/EtOAc) showed the reaction was complete. The mixture was concentrated and purified on silicagel (99:1 → 9:1 cyclohexane/EtOAc) to yield 1.75 g of product **20** as a sirup (64%). $[\alpha]_D^{23} = -48.1$ (*c* 0.25, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.94–5.85 (m, 1H, CH=), 5.33–5.19 (m, 2H, CH_2=), 4.86 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 4.36 (dd, 1H, $J_{2,3}$ = 8.9, $J_{3,4}$ = 5.2 Hz, H-3), 4.30–4.10 (m, 2H, CH_{2a}, H-5), 4.06 (dd, 1H, $J_{4,5}$ = 2.5 Hz, H-4), 4.03–3.98 (m, 1H, CH_{2b}), 3.32 (dd, 1H, H-2), 1.52 (s, 3H, CH_3), 1.35–1.33 (m, 6H, CH_3, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 133.24 (CH=), 117.74 (CH_2=), 109.37 ($C(CH_3)_2$), 96.80 (C-1), 75.56 (C-4), 73.57 (C-3), 68.57 (CH_2), 63.49 (C-5), 61.16 (C-2), 28.37, 26.26 (2 CH_3), 16.24 (C-6). ESI HR-MS ($C_{12}H_{19}N_3O_4$): *m/z* = found ($[M-N_2+H]^+$ 242.1332; calcd 242.1392). FT-IR: 2108.78 cm^{-1} (N_3).

4.1.14. Allyl 2-azido-2-deoxy-α-L-fucopyranoside 21

A solution of fucopyranoside **20** (3.5 g, 13 mmol) in 9:1 (v/v) AcOH–H_2O (50 ml) was stirred for 6 h at 50 °C, when TLC (1:1 cyclohexane/EtOAc) showed the reaction was complete. The mixture was concentrated co-evaporating with toluene, and the residue was purified on silicagel (cyclohexane/EtOAc) to afford 2.55 g of product **21** (86%). White crystals from EtOAc, mp 87–88 °C. $[\alpha]_D^{23} = -37.0$ (*c* 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.99–5.87 (m, 1H, CH=), 5.39–5.20 (m, 2H, CH_2=), 4.96 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.23–4.17 (m, 1H, CH_{2a}), 4.11–3.95 (m, 3H, CH_{2b}, H-5, incl. dd, 4.08, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 3.82 (dd, 1H, H-4), 3.47 (dd, 1H, H-2), 2.93 (br s, 2H, OH-3,4), 1.29 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 133.35 (CH=), 117.69 (CH_2=), 97.03 (C-1), 71.82 (C-4), 68.62, 68.58 (C-3, CH_2), 65.84 (C-5), 60.09 (C-2), 16.05 (C-6). ESI HR-MS ($C_9H_{15}N_3O_4$): *m/z* = found ($[M-N_2+H]^+$ 202.1029; calcd 202.1079).

4.1.15. Allyl 3-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranoside 4

To a solution of diol **21** (500 mg, 2.18 mmol) in 4:1 (v/v) dichloromethane-pyridine (25 ml), a solution of AcCl (185 μl, 2.62 mmol) in dichloromethane (5 ml) was added drop-wise at 0 °C. After stirring for 3 h at the same temperature, the reaction was complete (TLC, 1:1 cyclohexane/EtOAc). The mixture was partitioned with aq $NaHCO_3$, and combined organic layers were filtered and concentrated. The residue was chromatographed (99:1 → 4:1 cyclohexane/EtOAc) to give 585 mg of product **4** as a sirup (99%). $[\alpha]_D^{23} = -65.6$ (*c* 2.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.98–5.88 (m, 1H, CH=), 5.38–5.21 (m, 3H, CH_2=, incl. dd, 5.32, $J_{2,3}$ = 10.9, $J_{3,4}$ = 2.9 Hz, H-3), 4.99 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.24–4.19 (m, 1H, CH_{2a}), 4.12–4.09 (m, 2H, $CH_{2b,}$ H-5), 3.68 (dd, 1H, H-2), 3.94 (d, 1H, H-4), 2.22–2.14 (m, 4H, CH_3CO, OH-4), 1.28 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.95 (CO), 133.34 (CH=), 117.91 (CH_2=), 97.13 (C-1), 71.23 (C-3), 70.92 (C-4), 70.14 (CH_2), 65.73 (C-5), 57.14 (C-2), 20.98 (CH_3CO), 15.93 (C-6). ESI HR-MS ($C_{11}H_{17}N_3O_5$): *m/z* = found 244.2132 ($[M-N_2+H]^+$; calcd 244.1185).

4.1.16. 3-(Benzyloxycarbonyl)aminopropyl 2,3,4-tri-*O*-acetyl-β-D-fucopyranoside 29

To a solution of fucose peracetate **28** (24 g, 72 mmol) and benzyl *N*-(3-hydroxypropyl)carbamate (22.5 g, 108 mmol) in CH_2Cl_2 (100 ml), $BF_3{\cdot}Et_2O$ (13.3 ml, 108 mmol) was added at 0 °C. The mixture was stirred at room temperature for 2 h, when TLC (3:2 cyclohexane/EtOAc) showed that the reaction was complete. After neutralization with triethylamine (13.3 ml), the crude mixture was concentrated and purified on silica gel (6:1 → 1:1 cyclohexane/EtOAc) to give 26.1 g of **29** (72%). $[\alpha]_D^{23} = -31.9$ (*c* 3.90, $CHCl_3$). ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.40–7.29 (m, 5H, Ph), 5.22 (d, 1H,

$J_{3,4}$ = $J_{4,5}$ = 3.5 Hz, H-4), 5.18 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.5 Hz, H-2), 5.13–5.08 (m, 3H, NH, CH_2Ph), 5.00, (dd, 1H, H-3), 4.42 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 3.98–3.92 (m, 1H, H-1′a), 3.82–3.73 (m, 1H, H-5), 3.59–3.54 (m, 1H, H-1′b), 3.38–3.20 (m, 2H, H-3′), 2.16, 2.02, 1.98 (3 s, 3H each, 3 CH_3), 1.86–1.74 (m, 2H, H-2′), 1.22 (d, 3H, $J_{5,6}$ = 6.7 Hz, H-6). ^{13}C NMR (CD_3OD, 100 MHz): δ = 170.69, 170.22, 169.69, 156.58 (4 CO), 136.66, 128.47, 128.03 (Ph), 100.97 (C-1), 71.27 (C-3), 70.18 (C-4), 69.20 (C-2), 68.87 (C-5), 67.46 (C-1′), 66.52 (CH_2), 38.29 (C-3′), 29.44 (C-2′), 20.67, 20.62 (3 CH_3), 16.00 (C-6). ESI HR-MS ($C_{23}H_{31}NO_{10}$): m/z = found ([M+Na]$^+$ 504.1874; calcd 504.1846).

4.1.17. 3-(Benzyloxycarbonyl)aminopropyl 3,4-*O*-isopropylidene–D-fucopyranoside 30

To a solution of 3-(benzyloxycarbonyl)aminopropyl D-fucopyranoside **29** (20.9 g, 43 mmol) in MeOH (100 ml), 1 M NaOMe in MeOH was added until pH was 10. The mixture was stirred for 2 h, then TLC (1:1 cyclohexane/EtOAc) showed the reaction was complete. The mixture was neutralized with Dowex H$^+$, filtered and the filtrate was concentrated.

The residue was re-dissolved in 2,2-dimethoxypropane (100 ml), to which *p*-TsOH (1.2 g) was added. After 1 h (TLC, 1:1 toluene/EtOAc or 4:1 CH_2Cl_2/acetone) the reaction went to completion. The mixture was concentrated and chromatography of the residue (3:1 CH_2Cl_2/acetone) gave 14.8 g of product **30** (87%). $[\alpha]_D^{23}$ = −13.5 (*c* 0.40, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.37–7.15 (m, 5H, Ph), 5.14 (br t, 1H, J = 4.9 Hz, NH), 5.09 (s, 2H, CH_2Ph), 4.11 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.04 (dd, 1H, $J_{2,3}$ = 10.5, $J_{3,4}$ = 5.4 Hz, H-3), 3.98 (dd, 1H, $J_{4,5}$ = 2.1 Hz, H-4), 3.97–3.94 (m, 1H, H-1′a), 3.87–3.81 (m, 1H, H-5), 3.63–3.57 (m, 1H, H-1′b), 3.52 (d, 1H, J = 7.0 Hz, H-2), 3.49–3.42 (m, 1H, H-3a′), 3.29–3.21 (m, 1H, H-3b′), 3.00 (s, 1H, OH-2), 1.89–1.67 (m, 2H, H-2′), 1.49 (s, 3H, CH_3), 1.40 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6), 1.31 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.66 (CO), 136.59, 129.04, 128.48, 128.21, 128.10 (Ph), 109.83 (*C*(CH_3)$_2$), 102.27 (C-1), 78.93 (C-3), 76.68 (C-4), 73.48 (C-2), 69.14 (C-5), 66.87 (C-1′), 66.65 (CH_2), 38.87 (C-3′), 29.58 (C-2′), 28.20, 26.33 (CH_3), 16.54 (C-6). ESI HR-MS ($C_{20}H_{29}NO_7$): m/z = found ([M+H]$^+$ 396.2017; calcd 396.2022).

The structure of the compound **30** was confirmed by acetylation. ^{1}H NMR showed a shift of H-2 to 4.83 ppm (t, J = 8.0 Hz).

4.1.18. 3-(Benzyloxycarbonyl)aminopropyl 6-deoxy-3,4-*O*-isopropylidene–D-talopyranoside 32

To a mixture of oxalyl chloride (6 ml, 70.8 mmol) in CH_2Cl_2 (40 ml), DMSO (10 ml, 141 mmol) in CH_2Cl_2 (40 ml) was added drop-wise at −78 °C. After stirring at the same temperature for 30 min, compound **30** (14 g, 35.4 mmol) dissolved in CH_2Cl_2 (20 ml) was added drop-wise and stirring was continued for 30 min at −78 °C. DIPEA (24 ml) was gently dropped, and the mixture was warmed up to room temperature. After stirring for 3 h TLC (4:1 CH_2Cl_2/acetone) showed the reaction was complete. The crude mixture was washed with 10% NaS_2O_3, and the organic layers were concentrated to afford compound **31**. $[\alpha]_D^{23}$ = −10.3 (*c* 1.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.33–7.15 (m, 5H, Ph), 5.04–5.01 (m, 3H, CH_2Ph, NH), 4.73 (s, 1H, H-1), 4.37 (d, 1H, $J_{3,4}$ = 5.7 Hz, H-3), 4.33 (dd, 1H, $J_{4,5}$ = 1.8 Hz, H-4), 4.09–4.04 (m, 1H, H-5), 3.90–3.84 (m, 1H, H-1′a), 3.65–3.60 (m, 1H, H-1′b), 3.32–3.21 (m, 2H, H-3′), 1.66–1.60 (m, 2H, H-2′), 1.38 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.37 (s, 3H, CH_3), 1.32 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 198.96 (CO), 156.53 (CONH), 136.42, 128.53, 128.44, 128.16, 127.94 (Ph), 111.07 (*C*(CH_3)$_2$), 99.15 (C-1), 80.38 (C-4), 77.62 (C-3), 69.20 (C-5), 67.26 (C-1′), 66.80 (CH_2), 59.52 (C-2), 37.68 (C-3′), 29.33 (C-2′), 27.14, 25.98 (CH_3), 16.40 (C-6). ESI HR-MS ($C_{20}H_{27}NO_7$): m/z = found ([M+Na]$^+$ 394.1887; calcd 394.1866).

The foregoing compound **31** was re-dissolved in dry EtOH (80 ml), to which $NaBH_4$ (10.5 g) was added at 0 °C. After 30 min TLC (3:1 CH_2Cl_2/acetone) showed the reaction was complete and one product was formed. The mixture was diluted with CH_2Cl_2 (100 ml) and washed with brine. Combined organic layers were concentrated and purified on silica gel (1:1 cyclohexane/EtOAc) to give 11.3 g of product **32** (81%). $[\alpha]_D^{23}$ = −20.3 (*c* 1.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.36–7.26 (m, 5H, Ph), 5.35 (br t, 1H, J = 5.0 Hz, NH), 5.09 (s, 2H, CH_2Ph), 4.44 (s, 1H, H-1), 4.21 (t, 1H, J = 5.2 Hz, H-3), 4.04 (dd, 1H, $J_{3,4}$ = 6.0, $J_{4,5}$ = 2.1 Hz, H-4), 3.96–3.90 (m, 1H, H-1′a), 3.83–3.72 (m, 2H, H-2,5), 3.64–3.59 (m, 1H, H-1′b), 3.41–3.27 (m, 2H, H-3′), 2.47 (d, H, $J_{2,OH}$ = 8.4 Hz, OH-2), 1.86–1.75 (m, 2H, H-2′), 1.59 (s, 3H, CH_3), 1.39 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.36 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.53 (CO), 136.74, 128.51, 128.43, 128.09, 127.94 (Ph), 109.79 (*C*(CH_3)$_2$), 99.78 (C-1), 74.16 (C-4), 73.82 (C-3), 68.45 (C-5), 67.39 (C-1′), 66.41 (C-2), 66.34 (CH_2), 38.45 (C-3′), 29.48 (C-2′), 25.57, 25.26 (CH_3), 16.53 (C-6). ESI HR-MS ($C_{20}H_{29}NO_7$): m/z = found ([M+Na]$^+$ 396.2025; calcd 396.2022).

4.1.19. 3-(Benzyloxycarbonyl)aminopropyl 2-azido-2-deoxy-3,4-*O*-isopropylidene–D-fucopyranoside 34

To a solution of compound **32** (4.4 g, 11.1 mmol) in 6:1 (v/v) CH_2Cl_2/pyridine (60 ml), Tf_2O (2.8 ml, 16.7 mmol) was added at 0 °C. The mixture was stirred for 30 min at room temperature, at which time TLC (1:1 cyclohexane/EtOAc) showed that the reaction was complete. The mixture was washed with iced aq $NaHCO_3$, and the combined organic layers were concentrated at 30 °C.

4.1.19.1. 3-(Benzyloxycarbonyl)aminopropyl 6-deoxy-3,4-*O*-isopropylidene-2-*O*-trifluoromethansulfonyl–D-talopyranoside 33.

^{1}H NMR1H NMR ($CDCl_3$, 400 MHz): δ = 7.28–7.13 (m, 5H, Ph), 5.18 (br t, J = 5.0 Hz, 1H, NH), 5.06 (s, 2H, CH_2Ph), 4.83 (t, 1H, J = 5.7 Hz, H-3), 4.36 (s, 1H, H-1), 4.32 (d, 1H, $J_{3,4}$ = 5.7 Hz, H-2), 3.99 (dd, 1H, $J_{3,4}$ = 6.0, $J_{4,5}$ = 2.7 Hz, H-4), 3.97–3.93 (m, 1H, H-1′a), 3.84–3.79 (m, 1H, H-5), 3.60–3.54 (m, 1H, H-1′b), 3.42–3.35 (m, 1H, H-3′a), 3.27–3.19 (m, 1H, H-3′b), 1.77–1.68 (m, 2H, H-2′), 1.58 (s, 3H, CH_3), 1.44 (d, 3H, $J_{5,6}$ = 6.7 Hz, H-6), 1.36 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.58 (CO), 136.75, 129.04, 128.48, 128.22, 127.95 (Ph), 118.48 (q, CF_3), 110.82 (*C*(CH_3)$_2$), 97.58 (C-1), 78.79 (C-3), 72.74 (C-4), 71.06 (C-2), 69.80 (C-5), 66.97 (C-1′), 66.47 (CH_2), 37.63 (C-3′), 29.38 (C-2′), 25.65, 25.02 (CH_3), 16.23 (C-6). ESI HR-MS ($C_{21}H_{28}NF_3O_9S$): m/z = found ([M+Na]$^+$ 550.1346; calcd 550.1335).

The foregoing residue was dissolved in DMF (15 ml), to which NaN_3 (4.3 g, 66.6 mmol) was added. The mixture was stirred for 6 h at 60 °C, when TLC (1:1 cyclohexane/EtOAc) showed a new spot had been formed. The mixture was concentrated and purified on silica gel (7:3 cyclohexane/EtOAc) to give 4.2 g of product **34** as a sirup (90%). $[\alpha]_D^{23}$ = −10.1 (*c* 1.60, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38–7.30 (m, 5H, Ph), 5.18 (br t, 1H, J = 5.6 Hz, NH), 5.09 (s, 2H, CH_2Ph), 4.17 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 4.00–3.95 (m, 1H, H-1′a), 3.95 (d, 1H, $J_{3,4}$ = 5.1, $J_{4,5}$ = 2.1 Hz, H-4), 3.90 (dd, 1H, $J_{2,3}$ = 8.2 Hz, H-3), 3.85–3.79 (m, 1H, H-5), 3.63–3.58 (m, 1H, H-1′b), 3.43–3.27 (m, 3H, H-3′, incl. t, 3.37, J = 8.1 Hz, H-2), 1.88–1.83 (m, 2H, H-2′), 1.55 (s, 3H, CH_3), 1.40 (d, 3H, $J_{5,6}$ = 6.8 Hz, H-6), 1.36 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.48 (CO), 136.66, 128.49, 128.13 (Ph), 110.22 (*C*(CH_3)$_2$), 101.41 (C-1), 77.30 (C-3), 75.47 (C-4), 69.05 (C-5), 67.38 (C-1′), 66.53 (CH_2), 65.12 (C-2), 38.25 (C-3′), 29.38 (C-2′), 28.26, 26.20 (CH_3), 16.51 (C-6). ESI HR-MS ($C_{20}H_{28}NO_4$): m/z = found ([M+H]$^+$ 421.2131; calcd 421.2187); found ([M+Na]$^+$ 443.1950; calcd 450.1907); found ([M+K]$^+$ 459.1695; calcd 459.1646).

4.1.20. 3-(Benzyloxycarbonyl)aminopropyl 2-azido-2-deoxy–D-fucopyranoside 35

A solution of compound **34** (4.2 g, 10 mmol) in 9:1 (v/v) AcOH/H_2O (50 ml) was stirred at 60 °C for 5 h, when TLC (1:1 cyclohex-

ane/EtOAc) showed complete reaction. The mixture was concentrated and purified on silica gel (cyclohexane/EtOAc) to afford 3.7 g of deprotected product **35** (98%), as colourless syrup. $[\alpha]_D^{23} = -39.3$ (*c* 0.72, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.36–7.29 (m, 5H, Ph), 5.20 (br t, 1H, *J* = 5.7 Hz, NH), 5.09 (s, 2H, CH_2Ph), 4.22 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1), 4.00–3.95 (m, 1H, H-1′a), 3.67 (d, 1H, $J_{3,4} = J_{4,5}$ = 1.6 Hz, H-4), 3.70–3.66 (m, 1H, H-1′b), 3.58–3.54 (m, 1H, H-5), 3.46 (t, 1H, *J* = 8.1 Hz, H-2), 3.39–3.29 (m, 3H, H-3,3′), 2.89 (br s 1H, OH-4), 2.58 (br s, 1H, OH-3), 1.87–1.81 (m, 2H, H-2′), 1.31 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.52 (CO), 136.62, 128.48, 128.04, 127.33 (Ph), 102.04 (C-1), 73.35 (C-3), 70.84 (C-4), 70.54 (C-5), 67.52 (C-1′), 66.58 (CH_2), 64.03 (C-2), 38.24 (C-3′), 29.47 (C-2′), 16.02 (C-6). ESI HR-MS ($C_{17}H_{24}N_4O_6$): *m/z* = found ([*M*+Na]$^+$ 403.1593; calcd 403.1594); found ([*M*+K]$^+$ 419.1280; calcd 419.1333).

4.1.21. 3-(Benzyloxycarbonyl)aminopropyl 2-azido-2-deoxy-3-*O*-*p*-methoxybenzyl–D-fucopyranoside 36

A solution of diol **35** (3.5 g, 9.2 mmol) and Bu_2SnO (3.46 g, 13.8 mmol) in toluene (50 ml) containing pre activated 4 Å MS was stirred under reflux for 1 h. Then temperature was decreased to 60 °C and PMBBr (2 ml, 13.8 mmol) was added, followed by TBAI (5.09 g, 13.8 mmol). After stirring for 3 h the reaction was complete (TLC 1:1 cyclohexane/EtOAc). The mixture was filtered and concentrated. The residue was chromatographed (cyclohexane/EtOAc) to give 3.45 g of product **36** (76%). $[\alpha]_D^{23} = -65.1$ (*c* 2.05, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.37–6.90 (m, 9H, Ar), 5.27 (br t, 1H, *J* = 5.0 Hz, NH), 5.09 (s, 2H, CH_2Ph^{Cbz}), 4.62 (s, 2H, CH_2Ph^{PMB}), 4.15 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.99–3.94 (m, 1H, H-1′a), 3.81 (s, 3H, OCH_3), 3.69 (br t, 1H, *J* = 2.5 Hz, H-4), 3.62–3.54 (m, 2H, H-1′b, incl. dd, $J_{2,3}$ = 10.0 Hz, H-2), 3.49–3.44 (m, 1H, H-5), 3.41–3.26 (m, 3H, H-3,3′), 2.40 (br s, 1H, OH-4), 1.86–1.80 (m, 2H, H-2′), 1.33 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 159.61 (C_q), 156.49 (CO), 136.75, 129.68, 129.15, 128.44, 128.02, 127.96, 114.02 (Ar), 101.72 (C-1), 79.23 (C-3), 71.68 (CH_2^{PMB}), 70.25 (C-5), 68.08 (C-4), 67.53 (C-1′), 66.46 (CH_2^{Cbz}), 62.31 (C-2), 55.27(OCH_3), 38.39 (C-3′), 29.32 (C-2′), 16.30 (C-6). ESI HR-MS ($C_{25}H_{32}N_4O_7$): *m/z* = found ([*M*+H]$^+$ 501.2357; calcd 501.2349); found ([*M*+Na]$^+$ 523.2188; calcd 523.2169); found ([*M*+K]$^+$ 539.1921; calcd 539.1908).

4.1.22. 3-(Benzyloxycarbonyl)aminopropyl 2-azido-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-2-deoxy–D-fucopyranoside 37

The substrate **36** (1.5 g, 3 mmol) was dissolved in THF (25 mol) containing 0.5% of water. Then finely powdered NaOH (0.6 g, 15 mmol) was added, followed by BnBr (0.5 ml, 4.5 mmol) and a catalytic amount of 18-crown-6. The mixture was stirred at room temperature for 3 d when TLC (3:2 cyclohexane/EtOAc) showed a new spot had been formed. The mixture was neutralized with 5% HCl, then partitioned with water. Combined organic layers were dried over $NaSO_4$, filtered and the filtrated was concentrated. Chromatography of the residue (cyclohexane/EtOAc) yielded 1.76 g of syrupy product **36** (99%). $[\alpha]_D^{23} = -77.2$ (*c* 0.85, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.36–6.90 (m, 14H, Ar), 5.29 (br t, 1H, *J* = 4.8 Hz, NH), 5.08 (s, 2H, CH_2Ph^{Cbz}), 4.91, 4.64 (2 d, 2H, ^2J = 11.5 Hz, CH_2Ph^{Bn}), 4.62 (s, 2H, CH_2Ph^{PMB}), 4.13 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.98–3.93 (m, 1H, H-1′a), 3.81 (s, 3H, OCH_3), 3.77 (dd, 1H, $J_{2,3}$ = 10.1 Hz, H-2), 3.59–3.53 (m, 1H, H-1′b), 3.40 (d, 1H, $J_{3,4} = J_{4,5}$ = 2.5 Hz, H-4), 3.41–3.34 (m, 1H, H-5), 3.33–3.25 (m, 3H, H-3,3′), 1.84–1.78 (m, 2H, H-2′), 1.17 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 159.42 (C_q), 156.52 (CO), 136.15, 136.78, 130.26, 129.63, 129.51, 128.46, 128.22, 128.02, 127.94, 127.73, 114.73 (Ar), 101.97 (C-1), 80.72 (C-3), 74.73 (C-4), 74.63 (CH_2^{Bn}), 72.45 (CH_2^{PMB}), 70.07 (C-5), 67.60 (C-1′), 66.45 (CH_2^{Cbz}), 62.88 (C-2), 55.28 (OCH_3), 38.48 (C-3′), 29.70 (C-2′), 16.80 (C-6). ESI HR-MS ($C_{32}H_{38}N_4O_7$): *m/z* = found ([*M*+Na]$^+$ 629.2373; calcd 629.2378).

4.1.23. 3-(Benzyloxycarbonyl)aminopropyl 2-azido-4-*O*-benzyl-2-deoxy–D-fucopyranoside 5

To a solution of the 3-*O*-PMB protected sugar **37** (1.5 g, 2.5 mmol) in CH_2Cl_2 (20 ml) moistened with water (2 ml), DDQ (0.74 mg, 3.3 mmol) was added and the mixture was stirred for 2 h (TLC, 1:1 cyclohexane/EtOAc). The mixture was partitioned with 10% sodium thiosulfate. Combined organic layers were concentrated and purified on silica gel (cyclohexane/EtOAc) to afford 1.13 g of product **5** (94%). White crystals from EtOAc: mp 86–87 °C. $[\alpha]_D^{23} = -3.5$ (*c* 1.70, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.38–7.26 (m, 10H, Ph), 5.19 (br t, 1H, *J* = 4.8 Hz, NH), 5.09 (s, 2H, CH_2Ph^{Cbz}), 4.81, 4.70 (2 d, 2H, ^2J = 11.3 Hz, CH_2Ph^{Bn}), 4.18 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 3.99–3.93 (m, 1H, H-1′a), 3.63–3.57 (m, 1H, H-1′b), 3.57–3.44 (m, 4H, H-2,3,4,5), 3.41–3.25 (m, 2H, H-3′), 2.25 (br s, 1H, OH-3), 1.86–1.80 (m, 2H, H-2′), 1.27 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 156.49 (CO), 131.87, 130.00, 129.63, 129.59, 128.46, 128.25, 128.13, 128.04, 127.99 (Ar), 102.02 (C-1), 74.38 (C-4), 75.94 (CH_2^{Bn}), 72.97 (C-3), 70.31 (C-5), 67.51 (C-1′), 66.50 (CH_2^{Cbz}), 64.55 (C-2), 38.29 (C-3′), 29.40 (C-2′), 16.83 (C-6). ESI HR-MS ($C_{24}H_{30}N_4O_6$): *m/z* = found ([*M*+H]$^+$ 471.2230; calcd 471.2244); found ([*M*+Na]$^+$ 493.2032; calcd 493.2063); = found ([*M*+K]$^+$ 509.1833; calcd 509.1802).

The structure of **5** was further confirmed by acetylation of 3-OH which shifted H-3 to 4.61 ppm (dd, $J_{2,3}$ = 10.3, $J_{3,4}$ = 2.4 Hz).

4.1.24. Allyl 3-*O*-acetyl-2-azido-4-*O*-(benzyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-β-D-glucopyranosyl uronate)-2-deoxy-α-L-fucopyranoside 22

TMSOTf (27 μl, 0.15 mmol) dissolved in CH_2Cl_2 (100 μl) was slowly added at -10 °C to a mixture of acceptor **4** (400 mg, 1.47 mmol) and donor **3** (1.8 g, 2.54 mmol) in CH_2Cl_2 (7 ml). The mixture was stirred at the same temperature for 2 h, then temperature was slowly allowed to go 0 °C, monitoring by TLC (4:1 toluene/EtOAc). After 4 h the reaction was quenched with TEA (27 μl), and the mixture was concentrated and purified on silica gel (99:1 → 93:7 toluene/EtOAc) to afford 735 mg of disaccharide **22** as a sirup (61%). $[\alpha]_D^{23} = -31.3$ (*c* 1.2, $CHCl_3$).

^1H NMR ($CDCl_3$, 400 MHz): δ = 7.34–7.09 (m, 15H, Ph), 5.94–5.89 (m, 1H, CH═), 5.33–5.19 (m, 2H, CH_2═), 5.15 (s, 2H, CH_2Ph), 5.13 (m, 1H, H-2^{II}), 5.05 (dd, 1H, $J_{2,3}$ = 11.3, $J_{3,4}$ = 2.7 Hz, H-3^I), 4.87 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1^I), 4.75, 4.70 (2 d, 2H, ^2J = 11.0 Hz, CH_2Ph), 4.71, 4.45 (2 d, 2H, ^2J = 11.2 Hz, CH_2Ph), 4.37 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1^{II}), 4.15 (m, 1H, CH_{2a}), 4.05 (d, 1H, $J_{3,4}$ = 2,7 Hz, H-4^I), 4.03–3.91 (m, 3H, CH_{2b}, H-5^I,4^{II}), 3.86 (d, 1H, $J_{4,5}$= 9.7 Hz, H-5^{II}), 3.75 (dd, 1H, H-2^I), 3.66 (dd, 1H, $J_{2,3}$ = 9.1, $J_{3,4}$ = 9,1 Hz, H-3^{II}), 2.72–2.48 (m, 4H, CH_2CH_2), 2.15 (s, 3H, CH_3CO), 2,00 (s, 3H, CH_3CO), 1.19 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6^I). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 206.12 (CO), 171.34 (CH_2COO), 170.76 (CH_3CO), 167.83 (C-6^{II}), 137.92, 137.47, 134.67 (Ar), 133.38 (CH═), 128.67, 128.62, 128.33, 127.82, 127.71 (Ar), 117.62 (CH_2═), 101.64 (C-1^{II}), 96.91 (C-1^I), 81.73 (C-3^{II}), 79.35 (C-4^{II}), 75.77 (C-4^I), 75.06 (*C*H_2Ph), 74.91 (*C*H_2Ph), 74.64 (C-5^{II}), 73.21 (C-2^{II}), 70.14 (C-3^I), 68.52 (CH_2), 67.55 (*C*H_2Ph), 65.46 (C-5^I), 56.95 (C-2^I), 37.92 (*C*H_2CO), 29.77 (*C*H_3CO), 27.88 (*C*H_2COO), 20.80 (*C*H_3CO), 16.04 (C-6^I). ESI HR-MS ($C_{43}H_{49}N_3O_{13}$): *m/z* = ([*M*+H]$^+$ found 816.3300, calcd 816.3344); ([*M*+Na]$^+$ found 838.3083 calcd 838.3163).

4.1.25. Allyl 3-*O*-acetyl-2-azido-4-*O*-(benzyl 3,4-di-*O*-benzyl-β-D-glucopyranosyl uronate)-2-deoxy-α-L-fucopyranoside 23

To a solution of disaccharide **28** (1.4 g, 1.71 mmol) in 20:1 (v/v) CH_2Cl_2/MeOH (20 ml), hydrazine acetate (185 mg, 2.06 mmol) was added and the mixture was stirred for 2 h (TLC, 4:1 toluene/EtOAc, 4:1 cyclohexane/acetone). The mixture was concentrated and puri-

fied on silica gel (99:1 → 1:1 toluene/EtOAc) to give 1.10 g of desired product **23** (86%). $[\alpha]_D^{23} = -48.7$ (*c* 1.35, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.33–7.08 (m, 15H, Ph), 5.87 (m, 1H, CH═), 5.35–5.21 (m, 2H, CH_2═), 5.15 (s, 2H, CH_2Ph), 5.11 (dd, 1H, $J_{2,3}$ = 11.1, $J_{3,4}$ = 3.2 Hz, H-3^I), 4.94 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1^I), 4.90, 4.82 (2 d, 2H, ^2J = 11.4 Hz, CH_2Ph), 4.74, 4.46 (2 d, 2H, ^2J = 10.8 Hz, CH_2Ph), 4.28 (d, 1H, $J_{1,2}$= 7.9 Hz, H-1^{II}), 4.18 (m, 1H, CH_{2a}), 4.11 (d, 1H, H-4^I), 4.07–4.01 (m, 2H, CH_{2b}, H-5^I), 3.85 (m, 2H, H-4^{II},5^{II}), 3.78 (dd, 1H, H-2^I), 3.69 (dd, 1H, $J_{2,3}$ = 8.2 Hz, H-2^{II}), 3.58–3.44 (m, 1H, H-3^{II}), 2.01 (s, 3H, CH_3CO), 1.28 (d, 3H, $J_{5,6}$ = 6.7 Hz, H-6^I). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.63 (CO), 168.20 (C-6^{II}), 138.37, 137.62, 134.65 (Ar), 133.15 (CH═), 128.56, 128.32, 128.24, 128.13, 127.67, 127.62 (Ar), 117.83 (CH_2═), 104.07 (C-1^{II}), 96.79 (C-1^I), 83.63 (C-3^{II}), 78.99 (C-4^{II}/5^{II}), 76.23 (C-4^I), 75.18 (CH_2Ph), 74.95 (CH_2Ph), 74.59 (C-4^{II}/5^{II}), 74.36 (C-2^{II}), 70.01 (C-3^I), 68.58 (CH_2), 67.38 (CH_2Ph), 65.81 (C-5^I), 57.10 (C-2^I), 20.73 (CH_3CO), 15.91 (C-6^I). ESI HR-MS ($C_{38}H_{43}N_3O_{11}$): *m/z* = ([*M*+Na]$^+$ found 740.2664, calcd 740.2795); ([*M*+K]$^+$ found 756.2496, calcd 756.2536).

4.1.26. Allyl 3-*O*-acetyl-2-azido-4-*O*-(benzyl 2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-mannopyranosyl uronate)-2-deoxy-α-L-fucopyranoside 25

To a solution of disaccharide **23** (900 mg, 1.26 mmol) in 6:1 (v/v) CH_2Cl_2/pyridine (20 ml), Tf_2O (320 μl, 1.89 mmol) was added at 0 °C. The mixture was stirred at room temperature for 30 min, at which time the reaction was complete (TLC, 1:1 cyclohexane/EtOAc). The mixture was washed with iced aq $NaHCO_3$, and the combined organic layers were concentrated at 30 °C and used for the next step.

4.1.26.1. Allyl 3-*O*-acetyl-2-azido-4-*O*-(benzyl 3,4-di-*O*-benzyl-2-*O*-trifluomethansulfonyl-β-D-glucopyranosyl uronate)-2-deoxy-α-L-fucopyranoside 24.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.33–7.02 (m, 15H, Ph), 5.90 (m, 1H, CH═), 5.35–5.20 (m, 2H, CH_2═), 5.16 (s, 2H, CH_2Ph), 5.12 (dd, 1H, $J_{2,3}$ = 11.1, $J_{3,4}$ = 3.2 Hz, H-3^I), 4.94 (d, 1H, $J_{1,2}$ = 3,5 Hz, H-1^I), 4.85, 4.79 (2 d, 2H, ^2J = 10.6 Hz, CH_2Ph), 4.73 (dd, 1H, $J_{1,2}$= 8.2, $J_{2,3}$ = 9.1 Hz, H-2^{II}), 4.64, 4.46 (2 d, 2H, ^2J = 10.7 Hz, CH_2Ph), 4.57 (d, 1H, H-1^{II}), 4.19 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4^I), 4.19–4.15 (m, 1H, CH_{2a}), 4.11–4.01 (m, 2H, CH_{2b}, H-5^I), 3.98 (dd, 1H, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.4 Hz, H-4^{II}), 3.90 (d, 1H, H-5^{II}), 3.76 (dd, 1H, H-3^{II}), 3.70 (dd, 1H, H-2^I), 2.03 (s, 3H, CH_3CO), 1.25 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6^I).

The foregoing triflate was re-dissolved in toluene (20 ml), and tetrabutilammonium azide (1.07 g, 3.78 mmol) was added. The mixture was stirred at 60 °C for 3 h, when TLC (1:1 cyclohexane/EtOAc) showed a new spot had been formed.

The crude mixture was concentrated and purified on silica gel (99:1 → 4:1 toluene/EtOAc) to yield 625 mg of product **25** (70%). $[\alpha]_D^{23} = -17.4$ (*c* 1.65, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.28–7.02 (m, 15H, Ph), 5.83 (m, 1H, CH═), 5.28–5.14 (m, 2H, CH_2═), 5.15, 5.09 (2 d, 2H, ^2J = 11.7 Hz, CH_2Ph), 5.03 (dd, 1H, $J_{2,3}$ = 11.2, $J_{3,4}$ = 3.2 Hz, H-3^I), 4.85 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1^I), 4.71, 4.43 (2 d, 2H, ^2J = 10.8 Hz, CH_2Ph), 4.63 (s, 2H, CH_2Ph), 4.44 (s, 1H, H-1^{II}), 4.11 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4^I), 4.12–4.07 (m, 1H, CH_{2a}), 4,01–3.94 (m, 4H, CH_{2b}, H-2^{II},4^{II},5^I), 3.80 (dd, 1H, H-2^I), 3.66 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-5^{II}), 3.48 (dd, 1H, $J_{2,3}$ = 3.5, $J_{3,4}$ = 9.4 Hz, H-3^{II}), 1.97 (s, 3H, CH_3CO), 1.10 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6^I). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.81 (CO), 167.48 (C-6^{II}), 137.72, 137.18, 134.76 (Ar), 133.24 (CH═), 128.72, 128.60, 128.29, 128.15, 127.83, 127.75 (Ar), 117.85 (CH_2═), 101.03 (C-1^{II}), 96.85 (C-1^I), 79.44 (C-3^{II}), 75.81 (C-4^I), 75.42 (C-4^{II}/5^I/2^{II}), 75.38 (CH_2Ph), 75.20 (C-5^{II}), 72.19 (CH_2Ph), 69.75 (C-3^I), 68.67 (CH_2), 67.54 (CH_2Ph), 65.27 (C-4^{II}/5^I/2^{II}), 61.00 (C-4^{II}/5^I/2^{II}), 56.89 (C-2^I), 20.81 (CH_3CO), 16.33 (C-6^I). ESI HR-MS ($C_{38}H_{42}N_6O_{10}$): *m/z* = ([*M*+Na]$^+$ found 765.2771, calcd 765.2860); ([*M*+K]$^+$ found 781.2612, calcd 781.2599).

4.1.27. 3-*O*-Acetyl-2-azido-4-*O*-(benzyl 2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-mannopyranosyl uronate)-2-deoxy-α-L-fucopyranosyl trichloroacetimidate 27a

To a solution of disaccharide **25** (380 mg, 0.5 mmol) in dry MeOH (20 ml), $PdCl_2$ (120 mg, 0.8 mmol) was added under nitrogen atmosphere. The mixture was stirred for 1 d, when TLC (7:3 cyclohexane/EtOAc) showed two new spots formed. The mixture was filtered, and the filtrate purified on silicagel (4:1 cyclohexane/EtOAc) to give 290 mg (78%) of product **26**, as α,β mixture.

4.1.27.1. 3-*O*-Acetyl-2-azido-4-*O*-(benzyl 2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-mannopyranosyl uronate)-2-deoxy-L-fucopyranose 26.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.34–7.09 (m, Ph), 5.30 (s, $J_{1,2}$ = 3.5 Hz, H-1$^I{}_\alpha$), 5.21, 5.15 (2 d, ^2J 12.0 Hz, CH_2Ph), 5.12–5.08 (m, H-3$^I{}_\beta$, incl. dd, 5.10, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.3 Hz, H-3$^I{}_\alpha$), 4.79, 4.46 (2 d, ^2J = 10.7 Hz, CH_2Ph), 4.70 (s, CH_2Ph), 4.54 (d, $J_{1,2}$ = 8.4 Hz, H-1$^I{}_\beta$), 4.49 (s, H-1^{II}), 4.24 (m, H-5$^I{}_\alpha$), 4.19 (d, H-4$^I{}_\alpha$), 4.14 (d, $J_{3,4}$ = 1.9 Hz, H-4$^I{}_\beta$), 4.09–4.03 (m, H-2^{II},4^{II}), 3.95 (dd, $J_{2,3}$ = 11.1 Hz, H-2$^I{}_\alpha$), 3.83–3.71 (m, H-5^{II},2$^I{}_\beta$), 3.66 (m, H-5$^I{}_\beta$), 3.56 (dd, $J_{2,3}$ = 9.3, $J_{3,4}$ = 3.6 Hz, H-3^{II}), 2.06 (s, CH_3CO), 1.24 (d, $J_{5,6}$ = 6.4 Hz, H-6$^I{}_\beta$), 1.17 (d, $J_{5,6}$ = 6.6 Hz, H-6$^I{}_\alpha$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.86 (CO), 167.52 (C-6^{II}), 137.73, 137.18, 134.77, 129.46, 128.75, 128.63, 128.32, 128.19, 127.86 (Ar), 101.03 (C-1^{II}), 96.06 (C-1$^I{}_\beta$), 92.11 (C-1$^I{}_\alpha$), 79.44 (C-3^{II}), 75.73 (C-4$^I{}_\alpha$), 75.43 (C-4^{II}/2^{II}), 75.42 (CH_2Ph), 75.22 (C-5^{II}), 72.64 (C-4$^I{}_\beta$), 72.19 (CH_2Ph), 70.04 (C-5$^I{}_\beta$), 69.88 (C-3$^I{}_\alpha$), 69.642 (C-3$^I{}_\beta$), 67.58 (CH_2Ph), 65.38 (C-5$^I{}_\alpha$), 61.78 (C-2$^I{}_\beta$), 60.97 (C-4^{II}/2^{II}), 57.60 (C-2$^I{}_\alpha$), 20.85 (CH_3CO), 16.59, 16.46 (C-6$^I{}_{\alpha,\beta}$). ESI HR-MS ($C_{35}H_{38}N_6O_{10}$): *m/z* = ([*M*+Na]$^+$ found 725.2487, calcd 725.2547); ([*M*+K]$^+$ found 741.2251. calcd 741.2286).

To a solution of the disaccaride **26** (280 mg, 0.4 mmol) in CH_2Cl_2 (10 ml), CCl_3CN (400 μl, 4 mmol) was added, followed by DBU (60 μl, 0.04 mmol). The mixture was stirred for 4 h, when TLC (1:1 cyclohexane/EtOAc) showed that the reaction was complete. The mixture was concentrated and purified on silica gel (4:1 cyclohexane/EtOAc) to give 330 mg of product (98%).

4.1.27.2. First eluted: 3-*O*-Acetyl-2-azido-4-*O*-(benzyl 2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-mannopyranosyl uronate)-2-deoxy-α-L-fucopyranosyl trichloroacetimidate 27a (33%).

$[\alpha]_D^{23} = -31.4$ (*c* 0.65, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.69 (s, 1H, NH), 7.37–7.01 (m, 15H, 3 Ph), 6.38 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1^I), 5.21, 5.15 (2 d, 2H, ^2J = 11.9 Hz, CH_2Ph), 5.10 (dd, 1H, $J_{2,3}$ = 11.1, $J_{3,4}$ = 3.3 Hz, H-3^I), 4.79, 4.47 (2 d, 2H, ^2J = 10.5 Hz, CH_2Ph), 4.73, 4.69 (2 d, 2H, ^2J = 12.3 Hz, CH_2Ph), 4.50 (s, 1H, H-1^{II}), 4.29 (d, 1H, $J_{4,5}$ = 3.0 Hz, H-4^I), 4.23–4.16 (m, 2H, H-5^I, incl. dd, 4.17, H-2^I), 4.06 (t, 1H, J = 9.5 Hz, H-4^{II}), 4.01 (d, 1H, $J_{2,3}$ = 3.4 Hz, H-2^{II}), 3.74 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-5^{II}), 3.56 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3^{II}), 2.06 (s, 3H, CH_3CO), 1.20 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6^I). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.68 (CO), 167.38 (C-6^{II}), 160.72 (C═NH), 137.62, 137.06, 134.66, 128.66, 128.54, 128.23, 128.13, 127.79, 127.76 (Ar), 100.93 (C-1^{II}), 94.73 (C-1^I), 80.78 (C-3^{II}), 75.32 (CH_2Ph), 75.15 (C-4^{II},5^{II}), 75.00 (C-4^I), 72.19 (CH_2Ph), 69.94 (C-3^I), 67.93 (C-5^I), 67.52 (CH_2Ph), 60.93 (C-2^{II}), 56.27 (C-2^I), 20.78 (CH_3CO), 16.38 (C-6^{II}). ESI HR-MS ($C_{37}H_{38}Cl_3N_7O_{10}$): *m/z* = found ([M+Na]$^+$ 868.1572; calcd 868.1643).

4.1.27.3. Second eluted: 3-*O*-Acetyl-2-azido-4-*O*-(benzyl 2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-mannopyranosyl uronate)-2-deoxy-β-L-fucopyranosyl trichloroacetimidate 27b (65%).

$[\alpha]_D^{23} = -57.1$ (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.63 (s, 1H, NH), 7.29–7.02 (m, 15H, 3 Ph), 5.50 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1^I), 5.14, 5.08 (2 d, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.73, 4.40 (2 d, 2H, ^2J = 10.9 Hz, CH_2Ph), 4.66, 4.60 (2 d, 2H, ^2J = 11.9 Hz, CH_2Ph), 4.54 (dd, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.5 Hz, H-3^I), 4.44 (s, 1H, H-1^{II}), 4.08 (d, 1H, $J_{3,4}$ = $J_{4,5}$= 3.2 Hz, H-4^I), 4.06 (d, 1H,

$J_{2,3}$ = 10.4 Hz, H-2^{II}), 4.00 (t, 1H, J = 9.5 Hz, H-4^{II}), 3.96 (dd, 1H, H-2^{I}), 3.74–3.71 (m, 1H, H-5^{I}), 3.74 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-5^{II}), 3.49 (dd, 1H, $J_{3,4}$ = 4.6 Hz, H-3^{II}), 1.99 (s, 3H, CH_3CO), 1.20 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6^{I}). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.66 (CO), 167.39 (C-6^{II}), 160.09 (C=NH), 137.77, 137.64, 137.03, 134.68, 129.42, 128.93, 128.67, 128.55, 128.23, 128.13, 127.76, 127.19 (Ar), 100.85 (C-1^{II}), 96.64 (C-1^{I}), 79.30 (C-3^{II}), 75.34 (CH_2Ph, C-4^{II}), 75.16 (C-5^{II}), 74.83 (C-4^{I}), 72.49 (C-3^{I}), 71.95 (CH_2Ph), 70.81 (C-5^{I}), 67.52 (CH_2Ph), 60.58 (C-2^{II}), 59.78 (C-2^{I}), 20.69 (CH_3CO), 16.38 (C-6^{II}). ESI HR-MS ($C_{37}H_{38}Cl_3N_7O_{10}$): m/z = found ([M+Na]$^+$ 868.1622; calcd 868.1643).

4.1.28. 3-(Benzyloxycarbonyl)aminopropyl 3-*O*-(3-*O*-acetyl-2-azido-4-*O*-[benzyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2-deoxy-α-L-fucopyranosyl)-2-azido-4-*O*-benzyl-2-deoxy–D-fucopyranoside 2

To a mixture of acceptor **5** (158 mg, 0.34 mmol) and disaccharide donor **27a**/b (140 mg, 0.165 mmol) in CH_2Cl_2 (4 ml), TMSOTf (3 μl, 0.017 mmol) was added at -10 °C. The mixture was stirred for 30 min, when the donor was consumed (TLC, 3:2 cyclohexane/EtOAc). The mixture was neutralized with triethylamine (3 μl) and concentrated. Chromatography of the residue (99:1 → 9:1 →1:1 cyclohexane/EtOAc) afforded a mixture 2.4:1 of α- and -trisaccharides.

4.1.28.1. First eluted was compound 2 (94 mg, 50%). $[\alpha]_D^{23}$ = −22.3 (*c* 1.20, $CHCl_3$). ^{1}H NMR^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.36–7.10 (m, 25H, Ph), 5.29 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1^{II}), 5.20, 5.15 (2 d, 2H, ^2J = 11.9 Hz, CH_2Ph^{Bn}), 5.12–5.08 (m, 3H, NH, CH_2Ph^{Cbz}), 5.05 (dd, 1H, $J_{2,3}$= 11.2, $J_{3,4}$ = 3.0 Hz, H-3^{II}), 4.88, 4.68 (2 d, 2H, ^2J = 12.0 Hz, CH_2Ph^{Bn}), 4.78, 4.46 (2 d, 2H, ^2J = 10.7 Hz, CH_2Ph^{Bn}), 4.72, 4.68 (2 d, 2H, ^2J = 11.9 Hz, CH_2Ph^{Bn}), 4.46 (s, 1H, H-1^{III}), 4.20 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1^{I}), 4.12 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4^{II}), 4.06 (t, 1H, J = 9.7 Hz, H-4^{III}), 4.01 (d, 1H, $J_{2,3}$ = 3.6 Hz, H-2^{III}), 4.00–3.95 (m, 1H, H-1′a), 3.89–3.80 (m, 3H, H-2^{I,II},5^{II}), 3.72 (d, 1H, $J_{4,5}$ = 9.9 Hz, H-5^{III}), 3.64–3.53 (m, 1H, H-1′b), 3.54 (dd, 1H, $J_{2,3}$ = 3.6, $J_{3,4}$ = 9.3 Hz, H-3^{III}), 3.52–3.46 (m, 2H, H-5^{I}, incl. dd, 3.47, $J_{2,3}$ = 9.3, $J_{3,4}$ = 3.6 Hz, H-3^{I}), 3.42 (d, 1H, $J_{3,4}$ = $J_{4,5}$ =2.3, 1H, H-4^{I}), 3.39–3.29 (m, 2H, H-3′), 1.89–1.82 (m, 2H, H-2′), 1.58 (s, 3H, CH_3CO), 1.29 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6^{I}), 1.10 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6^{II}). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.74 (CO), 167.43, (C-6^{III}), 156.48 (CONH), 137.84, 137.62, 137.19, 136.68, 134.75, 128.77, 128.63, 128.44, 128.32, 128.17, 128.03, 127.95, 127.84, 127.26 (Ar), 102.51 (C-1^{I}), 101.01 (C-1^{III}), 99.26 (C-1^{II}), 79.46 (C-3^{III}), 78.84 (C-3^{I}), 77.32 (C-4^{I}), 75.65 (C-4^{II}), 75.41 (C-4^{III}), 75.25 (C-5^{III}), 75.00 (CH_2^{Bn}), 72.19, 70.87 (CH_2^{Bn}), 69.11 (C-5^{I}), 67.96 (C-3^{II}), 67.61 (CH_2^{Bn}), 67.43 (C-1′), 66.50 (CH_2^{Cbz}), 63.61 (C-5^{II}), 61.00 (C-2^{III}), 56.64 (C-2^{I}), 56.66 (C-2^{II}), 38.13 (C-3′), 29.49 (C-2′), 20.82 (CH_3CO), 16.97 (C-6^{I}), 16.48 (C-6^{II}). ESI HR-MS ($C_{59}H_{66}N_{10}O_{15}$): m/z = found ([M+H]$^+$ 1155.4786; calcd 1155.4787).

4.1.28.2. Second eluted was compound: 3-(Benzyloxycarbonyl)aminopropyl 3-*O*-(3-*O*-acetyl-2-azido-4-*O*-[benzyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2-deoxy-β-L-fucopyranosyl)-2-azido-4-*O*-benzyl-2-deoxy-β-D-fucopyranoside 38 (28 mg, 15%). $[\alpha]_D^{23}$ = −32.6 (*c* 0.25, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.40–7.10 (m, 25H, Ph), 5.23 (br t, 1H, J = 4.5 Hz, NH), 5.20, 5.14 (2 d, 2H, ^2J = 11.9 Hz, CH_2Ph^{Bn}), 5.08 (s, 2H, CH_2Ph^{Cbz}), 5.00, 4.70 (2 d, 2H, ^2J = 11.1 Hz, CH_2Ph^{Bn}), 4.78, 4.45 (2 d, 2H, ^2J = 10.3 Hz, CH_2Ph^{Bn}), 4.70, 4.67 (2 d, 2H, ^2J = 11.2 Hz, CH_2Ph^{Bn}), 4.60 (dd, 1H, $J_{2,3}$= 10.3, $J_{3,4}$ = 2.9 Hz, H-3^{II}), 4.51 (s, 1H, H-1^{III}), 4.40 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1^{II}), 4.12 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1^{I}), 4.11 (d, 1H, $J_{3,4}$ = 2.0 Hz, H-4^{II}), 4.10–4.03 (m, 2H, H-2^{III} incl. t, 4.05, J = 9.5 Hz, H-4^{III}), 3.99–3.93 (m, 1H, H-1′a), 3.92 (dd, 1H, $J_{2,3}$ = 10.8 Hz, H-2^{II}), 3.77 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2^{I}), 3.73 (d, 1H, $J_{4,5}$ = 9.8 Hz, H-5^{III}), 3.67 (dd, 1H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.3 Hz, H-3^{III}), 3.66–3.48 (m, 4H, H-1′b,4^{I},5^{II} incl. dd, 3.51, $J_{2,3}$ = 9.5, $J_{3,4}$ = 3.7 Hz, H-3^{I}), 3.44–3.27 (m, 2H, H-3′,5^{I}), 1.84–1.81 (m, 2H, H-2′), 1.60 (s, 3H, CH_3CO), 1.27 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6^{II}), 1.15 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6^{I}). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.89 (CO), 167.53 (C-6^{III}), 156.49 (CONH), 138.16, 137.74, 137.15, 136.77, 134.75, 128.75, 128.63, 128.44, 128.32, 128.18, 127.95, 127.84, 127.67 (Ar), 101.99 (C-1^{I}), 101.12 (C-1^{III}), 100.44 (C-1^{II}), 80.00 (C-3^{III}), 79.45 (C-3^{I}), 75.52 (C-4^{I}), 75.40 (CH_2^{Bn}, C-4^{III}), 75.23 (C-5^{III}), 74.74 (CH_2^{Bn}), 74.37 (C-4^{II}), 73.41 (C-3^{II}), 72.08 (CH_2^{Bn}), 70.46 (C-5^{I}), 70.09 (C-5^{II}), 67.58 (CH_2^{Bn}, C-1′), 66.45 (CH_2^{Cbz}), 62.66 (C-2^{III}), 60.89 (C-2^{I}), 60.49 (C-2^{II}), 38.37 (C-3′), 29.68 (C-2′), 20.81 (CH_3CO), 16.69 (C-6^{II}), 16.58 (C-6^{I}). ESI HR-MS ($C_{59}H_{66}N_{10}O_{15}$): m/z = found ([M+H]$^+$ 1155.4757; calcd 1155.4787).

4.1.29. 3-Acetamidopropyl 2-acetamido-3-*O*-(3-*O*-acetyl-2-acetamido-4-*O*-[2-acetamido-2-deoxy-β-D-mannopyranosyl uronate]-2-deoxy-α-L-fucopyranosyl)-2-deoxy–D-fucopyranoside 1

Trisaccharide **2** (5 mg, 0.004 mmol) was dissolved in 9:1 (v/v) MeOH/H_2O (5 ml) and flown for 18 h in a H-Cube Thales-Nano hydrogenator, over a Pd/C 10% cartridge at room temperature and atmospheric pressure. The solvent was then evaporated, and the crude material was dissolved in 4:1 (v/v) Ac_2O–MeOH (1 ml). After stirring overnight, the mixture was concentrated, and purification of the residue on a G10 PD MiniTrap™ GE Healthcare cartridge afforded 1.35 mg of the final trisaccharide **1** (40%). $[\alpha]_D^{23}$ = +24.0 (*c* 0.10, H_2O). ^{1}H NMR (D_2O, 323 K, 400 MHz): δ = 5.00–4.98 (m, 2H, H-1^{II},3^{II}), 4.70 (br s, 1H, H-1^{III}), 4.65 (br s, 1H, H-2^{III}), 4.43 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1^{I}), 4.08–4.4 (m, 3H), 3.88–3.66 (m, 6H), 3.62–3.55 (m, 3H), 3.82–3.55 (m, 2H), 2.19, 2.04, 2.02, 1.96, 1.86 (5 s, 15H, CH_3CO), 1.75–1.68 (m, 2H, H-2′), 1.28 (d, 3H, $J_{5,6}$ = 6.9 Hz, H-6^{I}), 1.25 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6^{II}). ESI HR-MS ($C_{31}H_{50}N_4O_{17}$): m/z = found ([M+H]$^+$ 751.3278; calcd 751.3249).

4.2. Immunochemical analysis

4.2.1. Preparation of *S. aureus* type 5 (SA5)-CRM$_{197}$ glycoconjugate

Purified *S. aureus* type 5 capsular polysaccharide[4a,27] was dissolved in distilled water at 2 mg/ml. Acetic acid was added to a final concentration of 2% (v/v) and the reaction kept at 90 °C for 3 h. The solution was then neutralized with 1 M NaOH, and the hydrolyzed saccharide was purified on a gel-filtration column (performed on an Akta™ system (G&E Healthcare) using a S300 Sephacryl resin (G&E Healthcare) eluting with 10 mM NaPi/10 mM NaCl pH 7.2 buffer). The saccharide was detected at 215 nm. Pooled fractions were dialyzed against distilled water using a 1 kDa membrane (SpectraPor). The depolymerized saccharide was dissolved in distilled water at 2 mg/ml. $NaIO_4$ was added at a saccharide:$NaIO_4$ ratio of 1:1 (w/w) and the reaction kept at room temperature for 1-2 h in the dark. The solution was then dialyzed against distilled water using a 1 kDa membrane (SpectraPor).

The oxidized saccharide was dissolved in a 200 mM NaPi/1 M NaCl pH 7.2 buffer at a concentration of 10 mg/ml. CRM$_{197}$ was added to the solution at a saccharide:protein ratio of 4:1 (w/w) and $NaBH_3CN$ (Aldrich) added at a saccharide:$NaCNBH_3$ ratio of 2:1 (w/w). The solution was kept at 37 °C for 48 h.

The conjugate was purified by gel-filtration chromatography (performed on an Akta™ system G&E Healthcare) using a S300 Sephacryl resin (G&E Healthcare) with a 10 mM NaPi/10 mM NaCl pH 7.2 buffer).

The glycoconjugate was characterized by SDS–PAGE using 3–8% Tris–Acetate gels (NuPAGE, Invitrogen). Total saccharide in the conjugate was determined by HPAEC-PAD analysis and protein content by MicroBCA potein assay kit (Thermo Scientific).

4.2.2. Immunizations

Animal experimental guidelines set forth by the Novartis Animal Care Department were followed in the conduct of all animal studies.

A group of eight female CD1 mice (5–6 week old) were immunized on days 1 and 14 with 2 μg of conjugated carbohydrate antigen formulated with Aluminium hydroxyde as adjuvant. All immunizations were performed by administering a 200 μl of vaccine via intraperitoneal route. Adjuvant alone was used for negative control groups. Sera were collected on days 0 (before the first immunization), 15 (two weeks after the first immunization) and 22 (eight days after the second immunization).

Pool serum obtained after the second immunization was analyzed by ELISA assay. Anti-capsular IgG antibody titers were calculated by interpolating an optical density (OD) of 1 as cutoff and expressed in ELISA units (EU). Sera showing anti-SA5 IgG titers (6400 EU) were used for competitive ELISA and dot blot.

4.2.3. Competitive ELISA

The protocol described above was followed to prepare SA5-coated plates. The plate was designed to contain (a) a blank column with TPBS alone, without serum and inhibitors, and (b) a column with serum alone, without inhibitors (b0); the other columns contained both, the serum and the inhibitors, including SA5 and the not correlated polysaccharide laminarin as positive and negative controls, respectively.

The different competitors (SA5 and compound **1**) were prediluted to obtain the starting concentration of 4.5 mg/ml and eight 10-fold dilutions were performed on the plate.

The competitors at different concentrations were mixed with an equal volume of a fixed dilution of anti-SA5 immune serum, followed by 2 h incubation at 37 °C. After addition of 100 μl/well of 1:10,000 in TPBS antimouse IgG alkaline phosphatase conjugated (Sigma–Aldrich), the plates were incubated for 1 h at 37 °C. Plates were then developed for 30 min at rt with 100 μl per well of 1 mg/ml *p*-nitrophenyl phosphate disodium (Sigma–Aldrich) in 1 M diethanolamine (pH 9.8) and read at 405 nm with a microplate spectrophotometer (Biorad). All OD lectures were subtracted from the mean value of the blank column (b). The inhibition percentage was expressed as follows:

$$\%\text{inhibition} = [(B0 - ODx)/B0] \times 100,$$

where B0 is the mean values of the b0 column (serum without inhibitor) and ODx is the optical density corresponding to each inhibitor concentration. IC_{50} was defined as the inhibitor concentration resulting in 50% inhibition of the main reaction. Fitting of inhibition curves and calculation of IC_{50} values were performed on the Graphpad Prism software using the variable slope model (Graphpad Prism Inc.).

4.2.4. Immunodot blot analysis

Trisaccharide **1** (50 μg), *S. aureus* type 5 polysaccharide (100 and 10 μg, positive control) and laminarin (100 μg, negative control) were deposed onto the cellulose membrane using the vacuum system for 30 min. Subsequently, the membrane were washed three times with 20 ml of PBS with 0.05% Tween 20 (TPBS) at pH 7.2 and then, blocked with 5% bovine serum albumin (Fraction V, Sigma–Aldrich) in TPBS for 1 h at room temperature. After three washing with 20 ml of TPBS, 20 ml of anti SA5-CRM_{197} polyclonal immune mouse sera pre-diluted in TPBS were placed on the membrane at the dilution of 1:100 and incubated for 1 h at room temperature. The membrane was washed again with TPBS and 20 ml of 1:1000 of anti-mouse IgG alkaline phosphatase conjugated (Sigma–Aldrich) were added and incubated for 30 min at room temperature. Finally, the membrane was developed at room temperature with 20 ml of colorimetric AP substrate reagent kit (Biorad).

Supplementary data

Supplementary data (copies of all described ^{1}H NMR and ^{13}C NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.048.

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