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Chemical synthesis of the rare D-Fuc3NAc containing tetrasaccharide repeating unit of the *O*-antigenic polysaccharide from *E. coli* O74

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ABSTRACT

Chemical synthesis of the tetrasaccharide repeating unit of the *O*-antigen from *E. coli* O74 is accomplished by a convergent [2 + 2] block synthesis strategy. The challenging rare D-Fuc3NAc has been prepared using DTBP and TIPST mediated deoxygenation reaction. Other monosaccharide synthons are prepared through rational protecting group manipulations and the stereoselective glycosylations are achieved either by the activation of thioglycoside or glycosyl trichloroacetimidate. The target tetrasaccharide is made in the form of its 2-aminoethyl glycoside to facilitate further glycoconjugate formation without affecting the anomeric stereochemistry.

1. Introduction

Escherichia coli (*E. coli*) is a Gram-negative facultative anaerobic rod-shaped bacterium found in the colonic flora of the animals and humans. Based on the immunogenicity of the bacterial surface structures, the species is subdivided into various serotypes. The strains are normally classified as the O-, K- or H-serotypes. *O*-serotypes designate the *O*-antigen or the *O*-polysaccharide (OPS) portion of the lipopolysaccharides (LPS). Whereas, K designates the capsular polysaccharide and H denotes the flagella antigen. Till date the myriad of *E. coli* strains comprised of more than 180 different *O*-antigens and over 100 capsular polysaccharides [1,2]. The pathogenic *E. coli* strains may be further classified into three groups on the basis of the clinical syndromes caused by their respective infections (a) enteric/diarrheal, (b) urinary tract infections and (c) septicemia/meningitis. The *O*-antigens plays a crucial role towards the bacterial adhesion to the hosts and their pathogenesis. Therefore, they are potential targets for the development of synthetic vaccines against these deadly pathogens. Recently, Widmalm and co-workers reported the structure of the *O*-polysaccharide of the strain *E. coli* O74 [3]. It has structural similarity with *E. coli* O2 as both LPS contains rare D-Fuc3NAc unit [4,5]. The strain *E. coli* O74 has shown cross-reactivity with *E. coli* O2. Herein, we report the total chemical synthesis of the tetrasaccharide repeating unit of the *O*-antigen from *E. coli* O74 in the form of its 2-aminoethyl glycoside (**1**, Fig. 1).

2. Results and discussion

It is essential to have a suitable linker installed beforehand at the reducing end of the final oligosaccharide target. The linker will enable the conjugation of required aglycon without hampering the anomeric stereochemistry to form antigenic glycoconjugate for possible vaccine candidate. Therefore, known 2-azido glucose trichloroacetimidate **2** [6] was glycosylated with Cbz protected aminoethanol **3** in the presence of TMSOTf to form the desired α-linked glucoside **4** in 70% isolated yield. Glycosylation reaction at −20 °C using 0.2 equivalents of TMSOTf favoured the 1,2-*cis* glycoside with the α-β ratio of 5:1. The corresponding β-derivative (~15%) was successfully separated by column chromatography. Further, de-*O*-acetylation [7] of compound **4** followed by benzylidene reaction [8] afforded the desired acceptor **6** in 75% yield (Scheme 1).

Synthesis of the Fuc3NAc unit was started with the known 3-azido glucoside **7** [9]. It was subjected to di-*tert*-butylperoxide (DTBP) and Tri-isopropylsilane thiol (TIPST) mediated deoxygenation [10] to afford the corresponding C-6 deoxygenated derivative **8** in 85% yield. Further, the 2-OH was protected with methoxymethyl (MOM) [11] group followed by de-*O*-benzoylation of the 4-OH to give compound **10**. At this point, the equatorial 4-OH was converted to the corresponding triflate using Tf_2O in pyridine. Further reaction with Bu_4NOAc in CH_3CN [12] lead to the axial 4-OAc derivative **11** in 72% yield. Next, deprotection of the MOM using 80% AcOH at 80 °C [13] followed by benzoylation using BzCl in pyridine furnished the desired donor **13** (Scheme 2).

Glycosylation of the acceptor **6** and the donor **13** was accomplished

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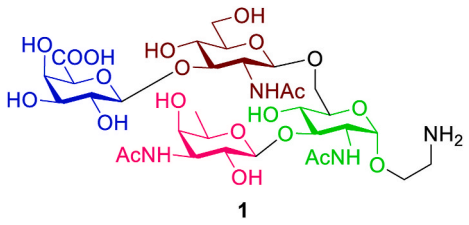


Fig. 1. Structure of the target tetrasaccharide repeating unit of the *O*-antigen from *E. coli* O74.

through activation of the thiophenyl glycoside using *N*-iodosuccinimide (NIS) in the presence of TMSOTf at −4 °C to form the disaccharide **14** in 70% yield. Further, regioselective opening of the benzylidene acetal using trichlorocyanuric acid (TCT) in the presence of $NaBH_4$ [14] afforded the disaccharide acceptor **15** in 78% yield (Scheme 3).

For the synthesis of the disaccharide donor, known benzylidene derivative **16** was reacted with TCT in the presence of $NaBH_4$ to form the derivative **17** in 79% yield through regioselective opening of the benzylidene acetal. Further the primary hydroxyl group of compounds **17** was oxidized using TEMPO in the presence of *bis*-acetoxy iodobenzene (BAIB) [15] to afford the corresponding uronic acid derivative which was subsequently methylated using MeI in the presence of K_2CO_3 [16] to give the methyl ester **18** in 67% overall yield. The thioglycoside of compound **18** was hydrolyzed using NIS in the presence of TMSOTf in moist CH_2Cl_2 [17] and subsequently reacted with trichloroacetonitrile in the presence of DBU [18] to afford the corresponding trichloroacetimidate donor **19** in 65% yield over two steps. Glycosylation of the donor **19** with the known acceptor **20** [19] using TMSOTf gave the disaccharide donor **21** in 75% yield (Scheme 4). Formation of the desired disaccharide **21** was confirmed by the ^1H NMR signal at δ 4.74 ppm with $J_{1,2}$ 7.6 Hz and the ^{13}C NMR signal at δ 100.1 ppm.

Finally, glycosylation between the disaccharide acceptor **15** and the disaccharide donor **21** using NIS in the presence of TMSOTf at −4 °C gave the protected tetrasaccharide **22** in 82% yield. Once the protected tetrasaccharide was in hand, the next challenge was to get the target tetrasaccharide by the modification of the protecting groups. The benzylidene acetal was hydrolyzed using 80% AcOH at 80 °C [20]. Then reaction with ethylene diamine converted the NPhth to the corresponding amine [21] which was subsequently acetylated to get the desired acetamido derivative **23** in 70% overall yield. Next, the azido group was converted to the corresponding acetamido derivative using thioacetic acid [22]. Further, de-*O*-acetylation was achieved using NaOMe in MeOH. Water was added after de-*O*-acetylation to hydrolyze the methyl ester. Finally, catalytic hydrogenation using 10% Pd–C cartridge in a ThalesNano hydrogenation assembly under continuous flow of H_2 afforded the target tetrasaccharide **1** in 63% yield over three steps (Scheme 5).

3. Conclusion

Chemical synthesis of the tetrasaccharide repeating unit of the *O*-antigenic polysaccharide from *E. coli* O74 has been accomplished through a convergent [2 + 2] strategy. Synthetic equivalent of the challenging rare D-Fuc3NAc was derived through rational strategy. This practical synthetic strategy for the preparation of the derivative **13** will be useful for the inclusion of D-Fuc3NAc unit in other oligosaccharides. The final structure has been synthesized as its 2-amino ethyl glycoside. This will allow further glycoconjugate formation without hampering the anomeric stereoselectivity.

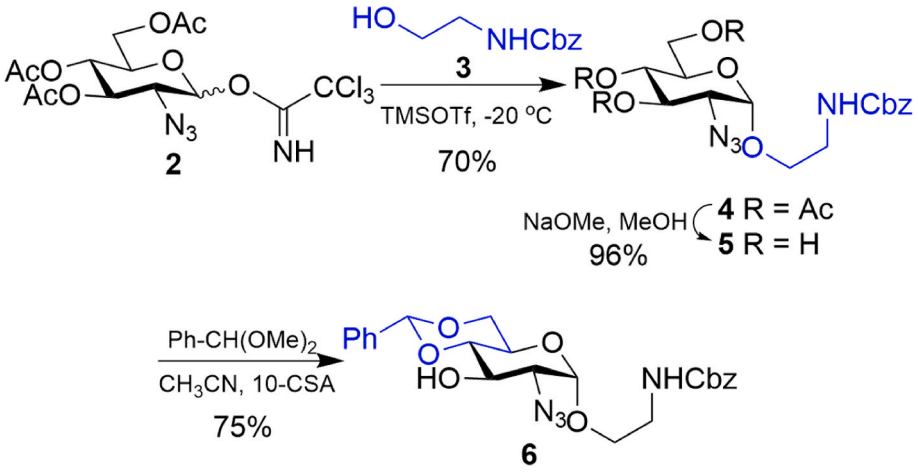
4. Experimental section

4.1. General

All solvents were dried through distillation prior to use according to literature methods [23]. The commercially procured reagents were used without any further purification unless mentioned otherwise. Dichloromethane was dried and distilled over P_2O_5 to make it anhydrous and adequately moisture-free for glycosylation reactions. All reactions were monitored by Thin Layer Chromatography (TLC) on Silica-Gel 60-F_{254} with detection via fluorescence and by charring after immersion in 10% ethanolic solution of sulphuric acid. Flash chromatography was performed with Silica Gel 230–400 mesh. Optical rotations were measured on sodium D-line at ambient temperature. ^1H and ^{13}C NMR were recorded on Bruker Avance 500 MHz spectrometer at 500 MHz and 125 MHz or Jeol 400 MHz at 400 MHz or 100 MHz respectively.

4.2. 2-(Benzyloxycarbonylamino) ethyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranoside (4)

A mixture of the known trichloroacetimidate donor **2** (2.2 g, 5.90 mmol), acceptor **3** (1.15 g, 5.90 mmol) and MS 4 Å (2.5 g) in anhydrous CH_2Cl_2 (25 mL) was stirred under nitrogen atmosphere for 10 min. Then the mixture was cooled to −20 °C, TMSOTf (210 μL, 1.18 mmol) was added and stirred for 2 h when the TLC (*n*-hexane-EtOAc; 2:1) showed complete consumption of the donor. The mixture was filtered through a Celite pad and the filtrate was successively washed with aq. $Na_2S_2O_3$ (2 × 40 mL), aq. $NaHCO_3$ (2 × 40 mL) and brine (40 mL). Organic layer was collected over Na_2SO_4 and evaporated *in vacuo*. The crude syrup thus obtained was purified by flash column chromatography using *n*-hexane-



Scheme 1. Synthesis of the 2-azido glycosyl acceptor **6**.

EtOAc (2:1) to afford pure compound **4** (2.1 g, 70%) and the corresponding β-anomer (450 mg, 15%).

$[\alpha]D^{25} = +87^{\circ}(c1.0,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.35–7.25 (m, 5H, ArH), 5.45 (t, 1H, $J_{2,3}$, $J_{3,4}$ 10.5 Hz, H-3), 5.28 (d, 1H, CH_2Ph), 5.27 (bs, 1H, N*H*Cbz), 5.10 (d, 1H, CH_2Ph), 5.01 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 4.97 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.23 (m, 1H, H-6a), 4.03 (m, 1H, H-6b), 4.00 (m, 1H, H-5), 3.80 (m, 1H, OCH_2), 3.60 (m, 1H, OCH_2), 3.49 (m, 1H, $NHCH_2$), 3.42 (m, 1H, $NHCH_2$), 3.32 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.5 Hz, H-2), 2.07 (s, 3H, $COCH_3$), 2.06 (s, 6H, 2 × $COCH_3$).

^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.4, 169.9, 169.5 (3 × *C*OCH_3), 156.3, 136.3, 128.4, 128.0, 127.9 (ArC), 98.0 (C-1), 68.4 (C-3), 68.1 (C-4), 68.0 (O*C*H_2), 67.7 (C-5), 66.7 (CH_2Ph), 61.7 (C-6), 60.8 (C-2), 40.6 (CH_2NHCbz), 20.5 (3 × CO*C*H_3).

HRMS calcd. for $C_{22}H_{28}N_4O_{10}Na$ $(M + Na)^+$: 531.1703, found: 531.1707.

4.3. 2-(Benzyloxycarbonylamino) ethyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (6)

To a solution of compound **4** (1.5 g, 2.95 mmol) in MeOH (20 mL), NaOMe in MeOH (0.5 M, 5.0 mL) was added and the solution was stirred at room temperature for 2 h till TLC (*n*-hexane-EtOAc, 1:1) showed complete conversion of starting material. The reaction mixture was neutralized with DOWEX 50 W H^+ resin and filtered. The filtrate was evaporated *in vacuo* to get the triol **5** (1.08 g, 96%) as colourless syrup. Further, to a mixture of the triol **5** (1.0 g, 2.60 mmol) in anhydrous CH_3CN, 2,2-dimethoxy benzaldehyde (0.6 mL, 3.9 mmol) was added followed by CSA (25 mg) and the reaction mixture was stirred at room temperature for 3 h till the TLC (*n*-hexane-EtOAc, 1:1) showed complete conversion of starting material to a faster moving spot. The reaction mixture was neutralized with Et_3N and evaporated *in vacuo*. The crude mixture was purified by flash column chromatography using *n*-hexane-EtOAc (1:1) to afford pure acceptor **6** (923 mg, 75%).

$[\alpha]D^{25} = +103^{\circ}(c0.9,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.47–7.29 (m, 10H, ArH), 5.51 (s, 1H, C*H*Ph), 5.49 (bs, 1H, N*H*Cbz), 5.10 (s, 2H, CH_2Ph), 4.86 (bs, 1H, H-1), 4.24 (m, 1H, OCH_2), 4.18 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.79 (m, 2H, H-5, OCH_2), 3.69 (t, 1H, $J_{5,6a}$, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.49 (m, 3H, H-4, H-6b, $NHCH_2$), 3.36 (m, 1H, $NHCH_2$), 3.26 (m, 1H, H-2), 3.14 (bs, 1H, O*H*).

^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.3, 136.7, 136.3, 129.3, 128.4, 128.3, 128.0, 126.2 (ArC), 102.0 (*C*HPh), 98.7 (C-1), 81.6 (C-4), 68.7 (C-3), 68.6 (*C*H_2Ph), 67.7 (C-6), 66.7 (O*C*H_2), 63.0 (C-5), 62.5 (C-2), 40.6 (*C*H_2NHCbz).

HRMS calcd. for $C_{23}H_{26}N_4O_7Na$ $(M + Na)^+$: 493.1699, found: 493.1696.

4.4. Phenyl 3-azido-4-O-benzoyl-3,6-dideoxy-1-thio-β-D-glucopyranoside (8)

A suspension known compound **7** (3.6 g, 9.34 mmol) in *n*-octane (50 mL), DTBP (2.06 mL, 11.21 mmol) was added followed by TIPST (0.2 mL, 0.93 mmol) and the mixture was refluxed under N_2 atmosphere for 17 h till the TLC (*n*-hexane-EtOAc; 3.5:1) showed complete conversion of the starting material to a faster moving spot. The reaction mixture was evaporated *in vacuo* to a syrup and the crude mixture thus obtained was purified by flash column chromatography using *n*-hexane-EtOAc (4:1) to get the pure compound **8** (3.05 g, 85%) as colourless syrup.

$[\alpha]D^{25} = +63(c1.0,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.05–7.34 (m, 10H, ArH), 4.88 (t, 1H, $J_{3,4}$, $J_{4,5}$ 10.0 Hz, H-4), 4.58 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 3.69 (m, 2H, H-3, H-5), 3.48 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.0 Hz, H-2), 2.77 (bs, 1H, O*H*), 1.29 (d, 3H, $J_{5,6}$ 5.0 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.2 (*C*OPh), 133.5, 133.3, 130.7, 129.8, 129.1, 128.5 (Ar*C*), 88.1 (C-1), 75.2 (C-5), 73.3 (C-4), 71.4 (C-2), 67.4 (C-3), 17.7 (C-6).

HRMS calcd. for $C_{19}H_{19}N_3O_4SNa$ $(M + Na)^+$: 408.0994, found: 408.0991.

4.5. Phenyl 3-azido-4-O-benzoyl-3,6-dideoxy-2-O-methoxymethyl-1-thio-β-D-glucopyranoside (9)

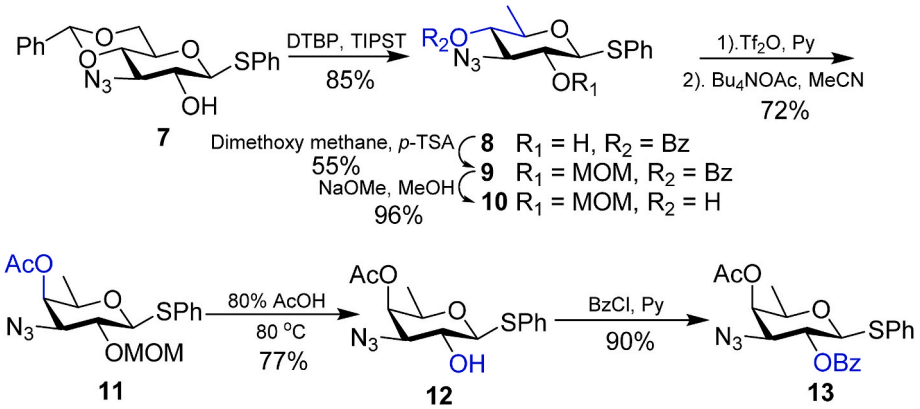
To a solution of compound **8** (3.0 g, 7.80 mmol) in anhydrous CH_2Cl_2 (25 mL), 2,2-dimethoxymethane (1.4 mL, 15.60 mmol) was added followed by *p*-TsOH (295.5 mg, 1.60 mmol) and the mixture was allowed to stir under reflux for 12 h. The reaction mixture was neutralized with Et_3N and evaporated *in vacuo*. The residue was purified by flash column chromatography using *n*-hexane-EtOAc (7:1) to get pure compound **9** (1.9 g, 55%) as light-yellow syrup. Starting material **8** (980 mg, ~33%) was recovered which was subjected to further reaction.

$[\alpha]D^{25} = +38(c0.9,\ CHCl_3)$

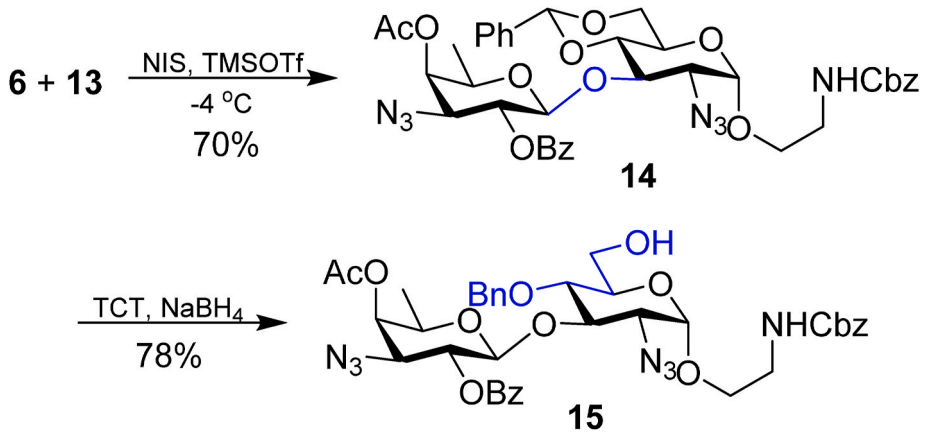
^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.08–7.25 (m, 10H, Ar*H*), 4.95 (m, 2H, H-4, OCH_2), 4.86 (d, 1H, $J_{Ha\text{-}Hb}$ 5.0 Hz, OCH_2), 4.66 (d, 1H, $J_{1,2}$10.0 Hz, H-1), 3.65 (m, 2H, H-3, H-5), 3.53 (m, 1H, H-2), 3.52 (s, 3H, OCH_3), 1.27 (d, 3H, $J_{5,6}$ 6.0 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.2 (*C*OCH_3), 133.5, 132.9, 132.1, 129.7, 129.1, 128.9, 127.8 (Ar*C*), 97.9 (O*C*H_2), 87.6 (C-1), 76.3 (C-2), 74.8 (C-3), 73.6 (C-4), 68.3 (C-5), 56.8 (O*C*H_3), 17.6 (C-6).

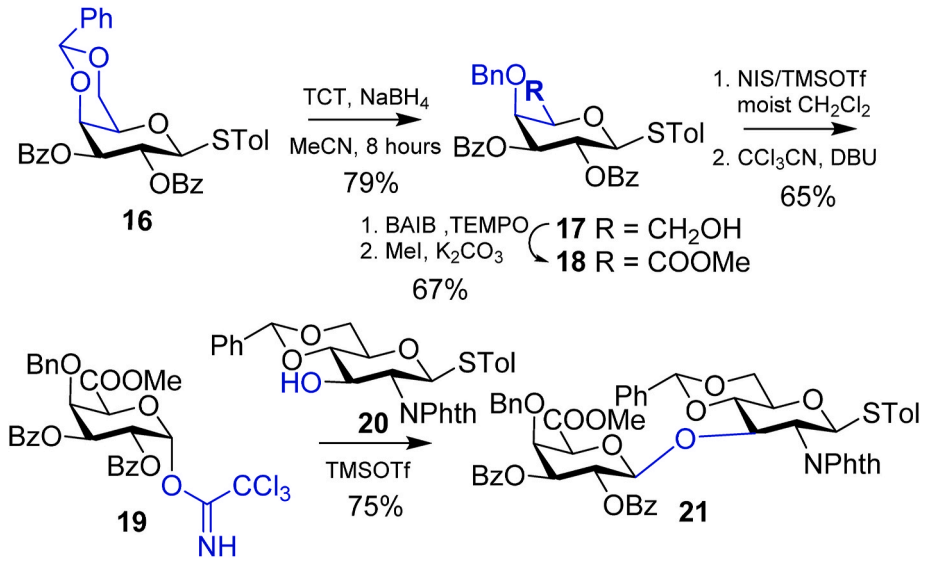
HRMS calcd. for $C_{21}H_{23}N_3O_5SNa$ $(M + Na)^+$: 452.1256, found: 452.1251.



Scheme 2. Synthesis of the 3-azido glycosyl donor **13**.



Scheme 3. Synthesis of the disaccharide acceptor **15**.



Scheme 4. Synthesis of the disaccharide donor **21**.

4.6. Phenyl 3-azido-3,6-dideoxy-2-O-methoxymethyl-1-thio-β-D-glucopyranoside (10)

To a solution of compound **9** (2.1 g, 4.90 mmol) in MeOH (20 mL), NaOMe in MeOH (0.5 M, 5.0 mL) was added and the solution was allowed to stir at room temperature for 2 h till TLC (*n*-hexane-EtOAc, 2:1) suggested complete conversion of starting material to a slower moving spot. The reaction mixture was neutralized with DOWEX 50 W H^+ resin, filtered and the solvent was evaporated *in vacuo* to get pure compound **10** (1.52 g, 96%) as colourless syrup.

$[\alpha]D^{25} = +47(c0.0,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.50–7.28 (m, 5H, Ar*H*), 4.92 (d, 1H, $J_{Ha\text{-}Hb}$ 6.5 Hz, OCH_2), 4.86 (d, 1H, $J_{Ha\text{-}Hb}$ 6.5 Hz, OCH_2), 4.60 (d, 1H, $J_{1,2}$ 9.0 Hz, H-1), 3.53 (s, 3H, OCH_3), 3.38 (m, 3H, H-2, H-3, H-5), 3.17 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.0 Hz, H-4), 2.74 (bs, 1H, O*H*), 1.33 (d, 3H, $J_{5,6}$ 6.0 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 133.3, 131.7, 128.9, 127.6 (Ar*C*), 97.9 (OCH_2), 87.6 (C-1), 77.2 (C-3), 76.1 (C-4), 74.1 (C-2), 70.8 (C-5), 56.8 (OCH_3), 17.7 (6-CH_3).

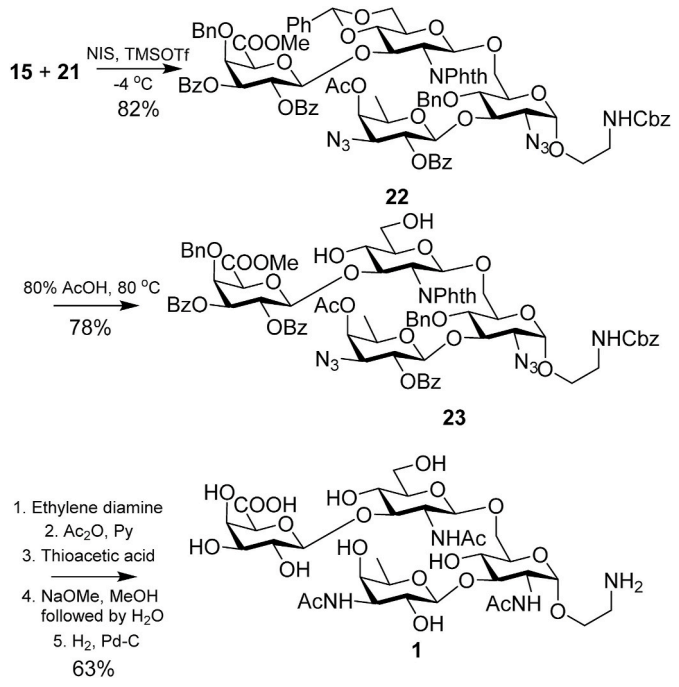
HRMS calcd. for $C_{14}H_{19}N_3O_4SNa$ $(M + Na)^+$: 348.0994, found: 348.0997.

4.7. Phenyl 4-O-acetyl-3-azido-3-deoxy-2-O-methoxymethyl-1-thio-β-D-fucopyranoside (11)

To a solution of compound **10** (1.50 g, 4.60 mmol) in anhydrous CH_2Cl_2 (25 mL), pyridine (1.84 mL, 23.0 mmol) was added followed by Tf_2O (1.51 mL, 9.2 mmol) and the solution was stirred at 0 °C for 2 h when TLC (*n*-hexane-EtOAc; 3.5:1) showed complete conversion of the starting material to a faster moving spot. The reaction mixture was successively washed with H_2O (30 mL), ice cold dilute HCl (30 mL, 0.5 N), aq. $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was collected over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to get the corresponding triflate derivative as reddish oil. The obtained derivative was then dissolved in MeCN (20 mL), Bu_4NOAc (2.77 g, 9.2 mmol) was added and allowed to stir for 14 h at 25 °C till TLC (*n*-hexane-EtOAc, 3.5:1) showed complete conversion. The mixture was evaporated under reduced pressure and residue was dissolved in CH_2Cl_2 (30 mL). Resulting solution was washed with H_2O (2 × 30 mL) and the organic layer was collected over anhydrous Na_2SO_4, filtered and concentrated. The crude product thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (5:1) as eluent to get pure compound **11** (1.22 g, 72%) as light-yellow syrup.

$[\alpha]D^{25} = +71^{\circ}(c0.8,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.55–7.27 (m, 5H, Ar*H*), 5.28 (d, 1H,



Scheme 5. Synthesis of the target tetrasaccharide **1**.

$J_{3,4}$ 2.5 Hz, H-4), 4.92 (d, 1H, $J_{Ha\text{-}Hb}$ 6.5 Hz, OCH_2), 4.83 (d, 1H, $J_{Ha\text{-}Hb}$ 6.5 Hz, OCH_2), 4.60 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 3.73 (m, 2H, H-2, H-5),3.51 (s, 3H, OCH_3), 3.46 (dd, 1H, $J_{2,3}$ 0.0 Hz $J_{3,4}$ 3.0 Hz, H-3), 2.16 (s, 3H, $COCH_3$), 1.19 (d, 3H, $J_{5,6}$ 6.5 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3 ($COCH_3$), 133.4, 131.8, 128.8, 127.6 (Ar*C*), 98.1 (OCH_2), 88.1 (C-1), 73.6 (C-2), 73.4 (C-5), 71.4 (C-4), 65.0 (C-3), 56.7 (OCH_3), 20.6 ($COCH_3$), 16.6 (6-CH_3).

HRMS calculated for $C_{16}H_{21}N_3O_5SNa$ $(M + Na)^+$: 390.1100, found: 390.1103.

4.8. Phenyl 4-O-acetyl-3-azido-3-deoxy-1-thio-β-D-fucopyranoside (12)

To a solution of compound **11** (1.2 g, 3.26 mmol) in 50% aq. AcOH (20 mL), catalytic amount of H_2SO_4 at was added and the solution was allowed to stir at 100 °C for 3 h when TLC (*n*-hexane-EtOAc, 3.5:1) showed complete consumption of starting material to a slower moving spot. The reaction mixture was evaporated and co-evaporated with toluene. The obtained crude residue was then purified by flash column chromatography using *n*-hexane-EtOAc (3:1) as eluent to get pure compound **12** (815 mg, 77%) as light yellowish syrup.

$[\alpha]D^{25} = +58^{\circ}(c0.9,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.51–7.21 (m, 5H, ArH), 5.14 (d, 1H, $J_{3,4}$ 3.0 Hz, H-4), 4.51 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 3.71 (m, 2H, H-2, H-5), 3.51 (dd, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.0 Hz, H-3), 2.77 (bs, 1H, O*H*), 2.07 (s, 3H, $COCH_3$), 1.15 (d, 3H, $J_{5,6}$ 6.0 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3 ($COCH_3$), 132.5, 131.8, 128.9, 128.1 (ArC), 89.2 (C-1), 73.8 (C-5), 70.8 (C-4), 68.3 (C-2), 64.4 (C-3), 20.5 ($COCH_3$), 16.6 (6-CH_3).

HRMS calcd. for $C_{14}H_{17}N_3O_4SNa$ $(M + Na)^+$: 346.0837, found: 346.0834.

4.9. Phenyl 4-O-acetyl-3-azido-2-O-benzoyl-3-deoxy-1-thio-β-D-fucopyranoside (13)

To a solution of compound **12** (750 mg, 2.31 mmol) in pyridine (5 mL), benzoyl chloride (0.56 mL, 4.62 mmol) was added and the reaction mixture was stirred for 2 h at rt till TLC (*n*-hexane-EtOAc; 3:1) showed complete conversion of the staring material to a faster moving spot. The reaction mixture was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with HCl (0.5 N, 30 mL), aq. $NaHCO_3$ (30 mL) and brine (30 mL). Organic layer was separated and dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product thus obtained was purified by flash column chromatography using *n*-hexane-EtOAc (3:1) to afforded pure compound **13** (1.01 g, 90%) as white amorphous mass.

$[\alpha]D^{25} = +97^{\circ}(c0.9,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.07–7.25 (m, 10H, ArH), 5.45 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.0 Hz, H-2), 5.35 (d, 1H, $J_{3,4}$ 3.0 Hz, H-4), 4.85 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 3.89 (m, 1H, H-5), 3.75 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.0 Hz, H-3), 2.18 (s, 3H, $COCH_3$), 1.25 (d, 3H, $J_{5,6}$ 6.0 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3 ($COCH_3$), 165.1 (*C*OPh), 133.4, 132.6, 132.4, 129.8, 129.1, 128.7, 128.4, 127.9 (Ar*C*), 86.9 (C-1), 73.9 (C-5), 71.0 (C-4), 68.7 (C-2), 63.1 (C-3), 20.5 ($COCH_3$), 16.5 (6-CH_3).

HRMS calcd. for $C_{21}H_{21}N_3O_5SNa$ $(M + Na)^+$: 450.1100, found: 450.1105.

4.10. 2-(Benzoyloxycarbonylamino) ethyl 4-O-acetyl-3-azido-2-O-benzoyl-3-deoxy-β-D-fucopyranosyl-(1 → 3)-2-azido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (14)

A mixture of the donor **13** (590 mg, 1.38 mmol), acceptor **6** (500 mg, 1.06 mmol) and activated MS 4Å (1.0 g) in dry CH_2Cl_2 (15 mL) was stirred under N_2 atmosphere for 15 min. Then NIS (405 mg, 1.8 mmol) was added and the mixture was cooled to −5 °C. TMSOTf (50 μL, 0.28 mmol) was added and the mixture was allowed to stir for 10 min at −5 °C till TLC (*n*-hexane-EtOAc; 2:1) suggested the complete consumption of the donor. The reaction mixture was neutralized by Et_3N (0.5 mL) and filtered through a pad of Celite®. The filtrate was diluted with CH_2Cl_2 (15 mL) and washed successively with saturated aq. $Na_2S_2O_3$ (2 × 25 mL), saturated aq. $NaHCO_3$ (2 × 25 mL) and H_2O (25 mL). The organic layer was collected, dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude mixture thus obtained was purified by flash column chromatography using *n*-hexane-EtOAc (2:1) to get pure disaccharide compound **14** (588 mg, 70%) as colourless amorphous mass.

$[\alpha]D^{25} = +83^{\circ}(c0.8,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.10–7.25 (m, 15H, Ar*H*), 5.56 (s, 1H, C*H*Ph), 5.47 (t, 1H, $J_{1',2'}$, $J_{2',3'}$, 9.5 Hz, H-2′), 5.24 (bs, 1H, H-4′), 5.07 (s, 2H, CH_2Ph), 5.00 (bs, 1H, N*H*Cbz), 4.79 (m, 2H, H-1, H-1′), 4.22 (dd, $J_{5,Ha}$ 4.0 Hz, $J_{H\text{-}6a,\ H\text{-}6b}$ 9.0 Hz, H-6a), 4.09 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.68 (m, 6H, H-3′, H-4, H-5, H-5′, OCH_2, H-6b), 3.41 (m, 2H, OCH_2, $NHCH_2$), 3.27 (m, 2H, H-2, $NHCH_2$), 2.18 (s, 3H, $COCH_3$), 1.07 (d, 3H, $J_{5,6}$ 6.5 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz):δ 170.5 ($COCH_3$), 165.1 (*C*OPh), 156.3, 137.0, 136.3, 133.3, 129.7, 129.5, 129.1, 128.5, 128.3, 128.1, 126.1 (Ar*C*), 102.1 (C-1′), 101.5 (*C*HPh), 98.7 (C-1), 80.4 (C-5), 77.7 (C-3), 71.0 (C-5′), 70.5 (C-2′), 68.6 (C-4′), 67.8 (C-6), 66.8 (CH_2Ph), 62.9 (OCH_2), 62.6 (C-2), 62.1 (C-4), 40.6 ($NHCH_2$), 20.7 ($COCH_3$), 16.1 (6-CH_3).

HRMS calculated for $C_{38}H_{41}N_7O_{12}Na$ $(M + Na)^+$: 810.2711, found: 810.2707.

4.11. 2-(Benzoyloxycarbonylamino) ethyl 4-O-acetyl-3-azido-2-O-benzoyl-3-deoxy-β-D-fucopyranosyl-(1 → 3)-2-azido-4-O-benzyl-2-deoxy-α-D-glucopyranoside (15)

To a solution of compound **14** (550 mg, 0.70 mmol) in acetonitrile (15 mL) at 0 °C, $NaBH_4$ (264 mg, 7.0 mmol) was added followed by TCT (1.03 g, 5.57 mmol). The reaction mixture was allowed to stir for 8 h at

rt till TLC (*n*-hexane-EtOAc; 3:1) showed complete conversion of staring material to a slower moving spot. The excess $NaBH_4$ was quenched by EtOAC (10 mL) and evaporated under reduced pressure. The crude product was purified by flash column chromatography using *n*-hexane-EtOAc (1:1) to get the pure disaccharide acceptor **15** (414 mg, 75%) as white amorphous mass.

$[\alpha]D^{25} = +114^{\circ}(c0.8,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.11–7.25 (m, 15H, Ar*H*), 5.44 (t, 1H, $J_{1',2'}$, $J_{2',3'}$ 9.5 Hz, H-2′), 5.32 (bs, 1H, H-4′), 5.18 (s, 1H, N*H*Cbz), 5.10 (m, 3H, 3 × CH_2Ph), 4.96 (d, 1H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.81 (bs, 1H, H-1), 4.59 (d, 1H, $J_{Ha,Hb}$ 10.5 Hz, CH_2Ph), 4.21 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.0 Hz, H-3), 3.74 (m, 6H, H-3′, H-4, H-5, H-5′, H-6a, H-6b), 3.48 (m, 3H, OCH_2, $NHCH_2$), 3.33 (bs, 1H, $NHCH_2$), 3.05 (dd, $J_{1,2}$ 3.0 Hz, $J_{2,3}$ 10.0 Hz, H-2), 2.17 (s, 3H, $COCH_3$), 1.16 (d, 3H, $J_{5,6}$ 6.0 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3 (*C*OCH_3), 165.3 (*C*OPh), 156.3, 138.0, 133.3, 129.8, 129.3, 128.4, 128.2, 128.1, 128.0, 127.8 (Ar*C*), 100.8 (C-1′), 97.9 (C-1), 77.4 (C-3), 75.6 (C-2′), 75.1 (*C*H_2Ph), 71.1 (C-4′), 71.0 (C-5′), 70.7 (C-4), 70.3 (C-5), 67.7 (*C*H_2Ph), 66.7 (OCH_2), 63.1 (C-2), 62.0 (C-6), 40.6 ($NHCH_2$), 20.5 ($COCH_3$), 16.1 (6-*C*H_3).

HRMS calcd. for $C_{38}H_{43}N_7O_{12}Na$ $(M + Na)^+$: 812.2867, found: 812.2862.

4.12. *p-Tolyl 2,3-di-O-benzoyl-4-O-benzyl-1-thio-β-D-galactopyranoside (17)*

To a solution of compound **16** (3.0 g, 5.15 mmol) in acetonitrile (20 mL) $NaBH_4$ (1.94 g, 51.4 mmol) was added at 0 °C and the mixture was stirred for 5 min and then TCT (7.6 g, 41.2 mmol) was added. The reaction mixture was stirred for 8 h at rt till TLC (*n*-hexane-EtOAc; 2:1) showed complete conversion of staring material to a slower moving spot. The reaction mixture was quenched by EtOAC (10 mL) and evaporated under reduced pressure. The final residue was purified by flash column chromatography using *n*-hexane-EtOAc (2:1) to afford the pure compound **15** (2.37 g, 75%) as amorphous mass.

$[\alpha]D^{25} = +47(c1.0,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.95–7.05 (m, 19H, Ar*H*), 5.85 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.0 Hz, H-2), 5.35 (d, 1H, $J_{2,3}$ 10.0 Hz, H-3), 4.87 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 4.75 (d, 1H, $J_{Ha,Hb}$ 11.5 Hz, CH_2Ph), 4.47 (d, 1H, $J_{Ha,Hb}$ 11.5 Hz, CH_2Ph), 4.16 (s, 1H, H-4), 3.91 (m, 1H, H-6a), 3.76 (m, 1H, H-5), 3.61 (m, 1H, H-6b), 2.31 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz) δ: 165.9 (*C*OPh), 165.2 (*C*OPh), 138.1, 137.3, 133.4, 133.1, 133.0, 129.8, 129.7, 129.6, 129.5, 128.9, 128.7, 128.4, 128.3, 128.1, 127.9 (Ar*C*), 86.8 (C-1), 78.9 (C-5), 76.0 (C-3), 74.6 (C-4), 73.7 (*C*H_2Ph), 68.5 (C-2), 61.9 (C-6), 21.1 (*C*H_3).

HRMS calcd. for $C_{34}H_{32}O_7SNa$ $(M + Na)^+$: 607.1766, found: 607.1762.

4.13. *Methyl (p-tolyl-2,3-di-O-benzoyl-4-O-benzyl-1-thio-β-D-galactopyranoside) uronate (18)*

Compound **17** (2 g, 3.42 mmol) was dissolved in biphasic mixture of CH_2Cl_2–H_2O (2:1, 18 mL), cooled to 0 °C and BAIB (2.75 g, 8.5 mmol) was added followed by TEMPO (107 mg, 0.68 mmol) and the mixture was stirred vigorously at room temperature for 40 min. The reaction mixture was quenched with $Na_2S_2O_3$ (10% w/v 10 mL) and diluted with CH_2Cl_2 and organic layer was separated. The aqueous phase was acidified to pH 1 with 1 mL HCl and washed with EtOAc (3 × 40). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product thus obtained was dried under vacuum. The mass was dissolved in DMF (20 mL), K_2CO_3 (1.4 g, 10.26 mmol) was added followed by MeI (971 mg, 6.84 mmol) and the mixture was allowed to stir overnight. TLC (*n*-hexane-EtOAc; 2:1) showed complete conversion of starting material to a faster moving spot. The mixture was diluted with EtOAc (60 mL) and it was washed with H_2O (2 × 40 mL). The organic layer was collected, dried over Na_2SO_4 and evaporated *in vacuo*. The crude product thus obtained was purified by flash column chromatography using *n*-hexane-EtOAc (2:1) to afford pure compound **18** (1.41 g, 67%) as amorphous mass.

$[\alpha]D^{25} = +41^{\circ}(c0.8,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.96–7.05 (m, 19H, Ar*H*), 5.84 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.0 Hz, H-2), 5.40 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.0 Hz, H-3), 4.86 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 4.66 (d, 1H, $J_{Ha,Hb}$ 11.5 Hz, CH_2Ph), 4.59 (m, 1H, H-4), 4.51 (d, 1H, $J_{Ha,Hb}$ 11.5 Hz, CH_2Ph), 4.36 (m, 1H, H-5), 3.71 (s, 3H, $COOCH_3$), 2.30 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz) δ: 167.5 (*C*$OOCH_3$), 165.7 (*C*OPh), 165.0 (*C*OPh), 138.3, 137.3, 133.7, 133.4, 133.1, 129.8, 129.6, 129.5, 128.7, 128.4, 128.2, 128.1, 127.7, 127.5 (Ar*C*), 86.8 (C-1), 77.1 (C-5), 75.4 (C-3), 75.1 (C-4), 74.7 (*C*H_2Ph), 67.8 (C-2), 52.4 (*C*$OOCH_3$), 21.1 (*C*H_3).

HRMS calcd. for $C_{35}H_{32}O_8SNa$ $(M + Na)^+$: 635.1716, found: 635.1714.

4.14. *2-(Benzoyloxycarbonylamino) ethyl 2,3-di-O-benzoyl-4-O-benzyl-β-D-galactopyranoside) uronate (1 → 3)-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (21)*

To a solution of compound **18** (1.4 g, 2.29 mmol) in CH_2Cl_2:H_2O (20 mL: 0.5 mL) was added NIS (673.5 mg, 2.98 mmol) followed by TMSOTf (83 μL, 0.46 mmol). The mixture was stirred at room temperature for 20 min when TLC (*n*-hexane-EtOAc; 2:1) showed complete conversion of the starting material to a slower moving spot. The mixture was washed with $Na_2S_2O_3$ (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL) and H_2O (25 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue obtained was purified by flash column chromatography using *n*-hexane-EtOAc (3:1) to get pure hemiacetal as reddish syrup. The product was dissolved in anhydrous CH_2Cl_2 (15 mL) and trichloro acetonitrile (1.15 mL, 11.5 mmol) was added followed by DBU (0.34 mL, 2.29 mmol). The resultant solution was allowed to stir at room temperature for 2 h when TLC (*n*-hexane-EtOAc; 3:1) suggested complete conversion of the starting material to a faster moving spot. The mixture was evaporated under reduced pressure and the brown residue was purified by flash column chromatography using *n*-hexane-EtOAc (3:1) to afford the corresponding trichloroacetimidate derivative **19** (967 mg, 65%) as light reddish syrup. A mixture of the trichloroacetimidate derivative **19** (950 mg, 1.46 mmol), known acceptor **20** (570 mg, 1.13 mmol) and MS 4 Å (1.0 g) in anhydrous CH_2Cl_2 (10 mL) was stirred under nitrogen atmosphere for 10 min. Then the mixture was cooled to −5 °C and TMSOTf (0.065 mL, 0.29 mmol) was added and stirred for 12 h till the TLC (*n*-hexane-EtOAc; 2:1) showed that the complete consumption of the donor. The reaction mixture was neutralized by Et_3N (0.1 mL) and filtered through a Celite pad. The filtrate was successively washed with aq. $Na_2S_2O_3$ (2 × 15 mL) and aq. $NaHCO_3$ (2 × 15 mL) and brine (15 mL). Organic layer was separated and collected over Na_2SO_4 and evaporated *in vacuo*. The crude syrup thus obtained was purified by flash column chromatography using *n*-hexane-EtOAc (2:1) to get the pure disaccharide donor **21** (730 mg, 65%).

$[\alpha]D^{25} = +90^{\circ}(c0.8,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.79–6.89 (m, 28H, Ar*H*), 5.59 (dd, 1H, $J_{1',2'}$ 7.6 Hz, $J_{2',3'}$ 10.0 Hz, H-2′), 5.54 (s, 1H, C*H*Ph), 5.47 (d, 1H, $J_{1,2}$ 10.8 Hz, H-1), 5.01 (dd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{3',4'}$ 2.8 Hz, H-3′), 4.74 (d, 1H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.71 (m, 1H, H-6a), 4.53 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.43 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.8 Hz, H-2), 4.36 (m, 2H, H-6b, CH_2Ph), 4.28 (bs, 1H, H-4′), 4.03 (t, 1H, $J_{3,4}$, $J_{4,5}$ 10.8 Hz, H-4), 3.86 (t, 1H, $J_{2,3}$, $J_{3,4}$ 10.8 Hz, H-3), 3.72 (m, 1H, H-5), 3.59 (m, 1H, H-5′), 3.52 (s, 3H, $COOCH_3$), 2.29 (s, 3H, S–$C_6H_5CH_3$).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 167.3 (*C*OOMe), 165.6, 164.3 (2 × *C*OPh), 138.5–122.5 (Ar*C*), 102.1 (*C*HPh), 100.1 (C-1′), 83.9 (C-1), 81.4 (C-4), 77.2 (C-6), 74.7 (C-4′), 74.6 (C-3), 74.2 (*C*H_2Ph), 73.3 (C-5′), 70.3 (C-3′), 69.8 (C-5), 68.8 (C-2′), 53.8 (C-2), 52.1 (COO*C*H_3), 21.1 (S–*C*H_3).

HRMS calcd. for $C_{56}H_{49}NO_{14}SNa$ $(M + Na)^+$: 1014.2771, found: 1014.2775.

4.15. 2-(Benzoyloxycarbonylamino) ethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-3-(2,3-di-O-benzoyl-4-O-benzyl-β-D-galactopyranoside)uronate-β-D-glucopyranosyl-(1 → 6)-2-azido-4-O-benzyl-2-deoxy-3-(4-O-acetyl-3-azido-2-O-benzoyl-3-deoxy-β-D-fucopyranoside)-α-D-glucopyranoside (22)

To a solution of acceptor **15** (400 mg, 0.51 mmol), donor **21** (654 mg, 0.66 mmol) and MS 4 Å (1.0 g) in anhydrous CH_2Cl_2 (10 mL) was stirred under nitrogen atmosphere for 10 min. Then NIS (194.4 mg, 0.86 mmol) was added and the mixture was cooled to −40 °C followed by addition of TMSOTf (0.03 mL, 0.13 mmol). The reaction was allowed to stir for 10 min till the TLC (*n*-hexane-EtOAc; 1:1) showed complete consumption of the donor. The reaction mixture was neutralized by Et_3N (0.1 mL) and filtered through a pad of Celite. The filtrate was successively washed with aq. $Na_2S_2O_3$ (2 × 15 mL), aq. $NaHCO_3$ (2 × 15 mL) and brine (15 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated in reduced pressure. The crude syrup was purified by flash column chromatography using *n*-hexane-EtOAc (1:1) to afford the pure tetrasaccharide **22** (545 mg, 65%) as a colourless amorphous mass.

$[\alpha]D^{25} = +131^{\circ}(c0.8,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.10–7.07 (m, 38H, ArH), 5.60 (t, 1H, $J_{1'',2''}$ $J_{2'',3''}$ 8.5 Hz, H-2″), 5.52 (s, 1H, CHPh), 5.28 (m, 3H, NHCbz, H-2‴, H-4‴), 5.08 (m, 5H, H-1′, H-3″, 3 × CH_2Ph), 4.85 (d, 1H, $J_{1''',2'''}$ 7.5 Hz, H-1‴), 4.74 (m, 2H, H-1″, H-3′), 4.65 (d, 1H, $J_{Ha,Hb}$ 10.0 Hz, CH_2Ph), 4.52 (d, 1H, $J_{Ha,Hb}$ 11.5 Hz, CH_2Ph), 4.47 (d, 1H, $J_{1,2}$ 2.5 Hz, H-1), 4.37 (m, 2H, H-2′, CH_2Ph), 4.28 (m, 2H, H-4″, 6-H_a), 4.03 (m, 2H, H-3, H-4′), 3.97 (m, 2H, 6-H_a', 6-H_b), 3.84 (m, 1H, H-5″), 3.72 (m, 1H, H-5‴), 3.64 (m, 3H, H-4, H-3‴, H-5′), 3.49 (s, 3H, $COOCH_3$), 3.43 (m, 2H, OCH_2, 6-H_b'), 3.29 (m, 1H, $NHCH_2$), 3.17 (m, 2H, OCH_2, $NHCH_2$), 3.07 (m, 1H, H-5), 2.91 (dd, 1H, $J_{1,2}$ 10.0 Hz $J_{2,3}$ 3.5 Hz, H-2), 2.14 (s, 3H, $COCH_3$), 1.02 (d, 3H, $J_{5,6}$ 6.5 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.2 ($COCH_3$), 167.2 ($COOCH_3$), 165.6, 165.3, 164.3 (3 × COPh), 156.2 (NHCO), 137.8, 137.2, 137.0, 136.5, 133.6, 133.4, 132.5, 129.8, 129.7, 129.4, 129.3, 129.2, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.6, 126.0 (Ar*C*), 102.1 (CHPh), 100.6 (C-1‴), 100.2 (C-1″), 98.5 (C-1′), 97.5 (C-1), 81.5 (C-4′), 77.2 (C-3), 76.1 (C-3′), 75.8 (C-2′), 74.7 (C*H*$_2$Ph), 74.6 (C-5) 74.6 (C-6) 74.6 (C*H*$_2$Ph), 74.1 (C-3″), 73.3 (C-4), 71.1 (C-4‴), 70.5 (C-5‴), 70.2 (C-2‴), 69.7 (C-2″), 68.8 (C-4″), 67.8 (C-6′), 67.2 (OCH_2), 66.6 (C*H*$_2$Ph), 66.4 (C-5′), 62.8 (C-2), 62.0 (C-3‴), 54.5 (C-2), 52.0 ($COOCH_3$), 40.4 ($NHCH_2$), 20.6 ($COCH_3$), 16.0 (CH_3).

HRMS calcd. for $C_{87}H_{84}N_8O_{26}Na$ $(M + Na)^+$: 1679.5394, found: 1679.5391.

4.16. 2-(Benzoyloxycarbonylamino) ethyl 2-deoxy-2-phthalimido-3-(2,3-di-O-benzoyl-4-O-benzyl-β-D-galactopyranoside)uronate)-β-D-glucopyranosyl-(1 → 6)-2-azido-4-O-benzyl-2-deoxy-3-(4-O-acetyl-3-azido-2-O-benzoyl-3-deoxy-β-D-fucopyranoside)-α-D-glucopyranoside (23)

The compound **22** (500 mg, 0.302 mmol) was dissolved in 80% aq. AcOH (AcOH 9 mL; H_2O 1 mL) and the reaction mixture was allowed to stir at 80 °C for 4 h until TLC (*n*-hexane-EtOAc; 1:1) showed complete conversion of staring material to a slower moving spot. The solvents were evaporated and co-evaporated with toluene under reduced pressure to remove residual AcOH. The crude mixture was purified by flash column chromatography using *n*-hexane-EtOAc (1:2) to get pure compound **23** (370 mg, 78%) as amorphous mass.

$[\alpha]D^{25} = +123^{\circ}(c0.0,\ CHCl_3)$

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.11–6.98 (m, 38H, ArH), 5.76 (t, 1H, $J_{1'',2''}$ $J_{2'',3''}$ 9.0 Hz, H-2″), 5.28 (m, 3H, H-2‴, H-3″, H-4‴), 5.06 (m, 2H, 2 × CH_2Ph), 4.91 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 4.87 (bs, 1H, NHCbz), 4.83 (d, 1H, $J_{1''',2'''}$ 7.5 Hz, H-1‴), 4.64 (m, 3H, H-1″, H-3′, CH_2Ph), 4.55 (d, 1H, $J_{Ha,Hb}$ 10.5 Hz, CH_2Ph), 4.47 (m, 3H, H-1, H-4″, CH_2Ph), 4.37 (bs, 1H, H-5″), 4.20 (t, 1H, $J_{1',2'}$, $J_{2',3'}$ 9.5 Hz, H-2′), 3.99 (m, 2H, H-3, 6-H_a), 3.86 (m, 2H, 6-H_a', 6-H_b), 3.72 (m, 2H, H-4′, H-5‴), 3.68 (s, 3H, $COOCH_3$), 3.65 (m, 1H, H-3‴), 3.54 (m, 2H, OCH_2, H-5), 3.40 (m, 2H, H-5′, 6-H_b'), 3.29 (m, 1H, $NHCH_2$), 3.16 (m, 2H, H-4, $NHCH_2$), 3.00 (m, 1H, OCH_2), 2.86 (m, 1H, H-2), 2.14 (s, 3H, $COCH_3$), 1.03 (d, 3H, $J_{5,6}$ 6.5 Hz, 6-CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.1 ($COCH_3$), 166.9 ($COOCH_3$), 165.6, 165.2, 164.4 (3 × COPh), 156.2 (NHCO), 137.7, 136.9, 133.5, 133.3, 132.6, 130.6, 129.7, 129.6, 129.3, 129.2, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6 (Ar*C*), 100.6 (C-1‴, C-1″), 98.2 (C-1′), 97.5 (C-1), 82.1 (C-3′), 77.2 (C-3), 75.7 (OCH_2), 74.9 (C*H*$_2$Ph), 74.7 (C-4″), 74.5 (C*H*$_2$Ph), 73.5 (C-5″), 73.3 (C-3″), 71.1 (C-2‴), 70.8 (C-4‴), 70.5 (C-4′), 70.2 (C-5‴), 69.7 (C-5), 69.3 (C-2″), 67.6 (C-5′, C-6′), 67.2 (C-4), 66.6 (C*H*$_2$Ph), 63.1 (C-6), 62.8 (C-2), 62.0 (C-3‴), 54.6 (C-2′), 52.7 ($COOCH_3$), 40.4 ($NHCH_2$), 20.5 ($COCH_3$), 16.0 (CH_3).

HRMS calcd. for $C_{80}H_{80}N_8O_{26}Na$ $(M + Na)^+$: 1591.5081, found: 1591.5084.

4.17. 2-Aminoethyl 2-acetamido-2-deoxy-3-(β-D-galactopyranosyluronate)-β-D-glucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-3-(3-acetamido-3-deoxy-β-D-fucopyranoside)-α-D-glucopyranoside (1)

The compound **23** (350 mg, 0.22 mmol) was dissolved in *n*-butanol (8 mL), ethylene diamine (0.20 mL) was added and the reaction mixture was stirred at 110 °C for 20 h till TLC (CH_2Cl_2–MeOH; 1:1) suggested the complete conversion of the starting material. The solvents were evaporated *in vacuo* and co-evaporated with toluene. The resultant residue was dissolved in pyridine (5 mL) and Ac_2O (5 mL) and the mixture was allowed to stir at 50 °C for 14 h. Then the solvents were evaporated under reduced pressure and co-evaporated with toluene to remove pyridine. The residual syrup was directly dissolved in CH_2Cl_2 (25 mL) and washed with H_2O (25 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated and dried *in vacuo*. Then the residue was dissolved in thioacetic acid (8 mL) and left for 4 days in dark. Solvents were evaporated and co-evaporated with toluene under reduced pressure. The residue thus obtained was dissolved in MeOH (10 mL) and NaOMe in MeOH (1 mL, 0.5 N) added. The reaction mixture was stirred at room temperature for 4 h till TLC (CH_2Cl_2–MeOH; 10:1) suggested complete consumption of the starting materials. In the mixture the excess NaOMe was neutralized by DOWEX 50 W H+ resin. The mixture was filtered through cotton and the solvents were evaporated *in vacuo*. Thus, syrupy mass obtained, was dissolved in MeOH and hydrogenolyzed using 10% Pd–C cartridge in a ThalesNano flow hydrogenation assembly with a flow rate of 1 mL/min and atmospheric pressure of hydrogen. Complete hydrogenation was achieved in four cycles, the solvents were evaporated under reduced pressure. Finally, the residue was dissolved in water and washed with CH_2Cl_2 (10 mL) to remove the organic impurities. The aqueous layer was collected and lyophilized to furnish the pure target tetrasaccharide **1** (103 mg, 56%) as colourless amorphous mass.

$[\alpha]D^{25} = +29^{\circ}(c0.7,\ MeOH)$

^{1}H NMR (CD_3OD, 500 MHz) δ: 4.93 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.61 (d, 1H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.54 (d, 1H, $J_{1'',2''}$ 7.5 Hz, H-1″), 4.53 (d, 1H, $J_{1''',2'''}$ 7.5 Hz, H-1‴), 2.05, 1.95, 1.93 (3s, 9H, 3 × $NHCOCH_3$), 1.26 (d, 3H, $J_{5''',6'''}$ 7.0 Hz, H-6‴).

^{13}C NMR (CD_3OD, 125 MHz) δ: 172.8, 172.4, 172.3 (3 × $NHCOCH_3$),

104.8 (C-1‴), 103.3 (C-1′, C-1″), 100.8 (C-1), 78.4, 78.0, 76.5, 75.9, 75.6, 74.2, 73.4, 71.6, 71.5, 70.7, 70.1, 69.7, 67.7, 66.8, 66.2, 62.9, 61.0, 55.2, 53.8, 40.1, 29.2, 21.5, 21.3, 21.2 (3 × NHCO*C*H$_3$), 15.5 (C-6‴).

HRMS calculated for $C_{32}H_{54}N_4O_{21}Na$ $(M + Na)^+$: 853.3178, found: 853.3175.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2021.108366.

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Supporting Information

Copies of the ^{1}H and ^{13}C NMR spectra of all new compounds are given in the Supporting Information.

References

[1] F. Scheutz, T. Cheasty, D. Woodward, H.R. Smith, APMIS 112 (2004) 569–584.
[2] F. Ørskov, I. Ørskov, Can. J. Microbiol. 38 (1992) 699–704.
[3] K. Hanna, M. Jonsson, A. Weintraub, G. Widmalm, Carbohydr. Res. 344 (2009) 1592–1595.
[4] F. Ørskov, I. Ørskov, B. Jann, K. Jann, E. Müller-Seitz, O. Westphal, Acta Pathol. Microbiol. Scand. 71 (1967) 339–358.
[5] P.-E. Jansson, H. Lennholm, B. Lindberg, U. Lindquist, S.B. Svenson, Carbohydr. Res. 161 (1987) 273–279.
[6] S.M. Rele, S.S. Iyer, S. Baskaran, E.L. Chaikof, J. Org. Chem. 69 (2004) 9159–9170.
[7] G. Zemplén, Ber. Deutch. Chem. Ges. 59 (1926) 1254–1266.
[8] J. Barbat, J. Gelas, D. Horton, Carbohydr. Res. 219 (1991) 115–121.
[9] J. Wang, H.-N. Chen, H. Chang, C.T. Tanifum, H.-H. Liu, P.G. Czyrca, C.-W. T Chang, J. Med. Chem. 48 (2005) 6271–6285.
[10] (a) H.-S. Dang, B.P. Roberts, J. Sekhon, T.M. Smit, Org. Biomol. Chem. 1 (2003) 1330–1341;
(b) A. Chaudhury, B. Mukhopadhyay, RSC Adv. 10 (2020) 4942–4948.
[11] E. Mensah, N. Camasso, W. Kaplan, P. Nagorny, Angew. Chem. 52 (2013) 12932–12936.
[12] (a) R. Lattrell, G. Lohaus, Justus Liebigs Ann. Chem. (1974) 901–920;
(b) R. Albert, K. Dax, R.W. Link, A.E. Stutz, Carbohydr. Res. 118 (1983) C5–C6.
[13] F.B. LaForge, J. Am. Chem. Soc. 55 (1933) 3040–3048.
[14] M. Tatina, S.K. Yousuf, D. Mukherjee, Org. Biomol. Chem. 10 (2012) 5357–5360.
[15] (a) L.J. van den Bos, J.D.C. Codee, J.C. van der Toorn, T.J. Boltje, J.H. van Boom, H.S. Overkleeft, G.A. van der Marel, Org. Lett. 6 (2004) 2165–2168;
(b) A. Mitra, B. Mukhopadhyay, Eur. J. Org Chem. (2019) 4869–4878;
(c) M. Bera, B. Mukhopadhyay, Carbohydr. Res. (2020) 107955.
[16] A.N. Cheallaigh, S. Oscarson, Can. J. Chem. 94 (2016) 940–960.
[17] M.S. Motawia, J. Marcussen, B.L. Möller, J. Carbohydr. Chem. 9 (1995) 1279–1294.
[18] R.R. Schmidt, Angew Chem. Int. Ed. Engl. 25 (1986) 212–235.
[19] J.C. Lee, W.A. Greemberg, C.-H. Wong, Nat. Protoc. 1 (2006) 3143–3152.
[20] P.A. Gent, R. Gigg, J. Chem. Soc., Perkin Trans. 1 (1974) 1446–1455.
[21] O. Kanie, S.C. Crawley, M.M. Palcic, O. Hindsgaul, Carbohydr. Res. 243 (1993) 139–164.
[22] Y. Nakahara, T. Ogawa, Carbohydr. Res. 292 (1996) 71–81.
[23] D.D. Perrin, W.L. Amarego, D.R. Perrin, Purification of Laboratory Chemicals, Pergamon, London, 1996.