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Total synthesis of the D-acofriose-containing trisaccharide repeating unit of the O-antigen from *Azospirillum brasilense* JM6B2†

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The unique D-acofriose (6-deoxy-3-*O*-methyl-D-mannose) unit present in the target oligosaccharide was synthesized from commercially available D-mannose in six steps with ~50% overall yield. The synthesis was found to be equally successful at the multigram scale (100 mmol). 2-Bromoethyl α-L-fucopyranoside, required at the reducing end, was prepared exclusively *via* H_2SO_4–silica promoted Fischer glycosylation. The influence of the protecting group at the 3-position of the fucosyl moiety on glycosylation at the 4-position was studied and ether protection was found to be essential over ester protection. Further global deprotection gave the target conjugation-ready trisaccharide in 34% overall yield.

Introduction

Selective enrichment of the rhizosphere with adapted microorganisms results in increased plant growth activity and productivity.[1,2] Such plant–microbe associations have been extensively studied with *Azospirillum* bacteria.[3] The surface-located glycopolymers produced by the *Azospirillum* species are key to their rhizosphere adaptation, with lipopolysaccharides (LPSs) playing an important role. The success of the initial stages of bacteria–root association[4,5] is governed by bacterial surface polysaccharides that are involved in the adhesion and adsorption of microorganisms to the roots of plants. Furthermore, the O-specific polysaccharide (OPS) portion of the LPS interacts with the environment and possesses antigenic determinants as part of its structure. Owing to the significant influence of *Azospirillum* species on plant growth activities and productivity, their OPS structures have been the subject of detailed studies. In this context, Fedonenko *et al.*[6] reported the OPS structure of *Azospirillum brasilense* (*A. brasilense*) Jm6B2, which has a unique 3-*O*-methyl-D-rhamnose (D-acofriose) that is not found in other *Azospirillum* species. In continuation of our effort towards the chemical synthesis of biologically relevant bacterial oligosaccharides,[7–9] we herein report the total synthesis of the trisaccharide repeating unit of the OPS from *A. brasilense* Jm6B2 in the form of its 2-aminoethyl glycoside (Fig. 1). Literature studies on D-acofriose reveal that it is also abundant in some *Pseudomonas aeruginosa* species.[10,11] This information prompted us to develop a convenient route for the gram-scale synthesis of D-acofriose from commercially available D-mannose.

Results and discussion

Retrosynthetic analysis for the synthesis of the target trisaccharide **1** revealed that sequential glycosylation of the suitably functionalized fucosyl acceptor **18** with the fully protected D-xylose donor **15** and D-acofriose donor **7** would successfully afford the protected trisaccharide. Since no other amino functionalities are present in the structure, an azidoethyl glycoside at the reducing end was considered ideal; this can be subsequently converted to the corresponding 2-aminoethyl glycoside to facilitate further conjugation. Literature reports indicate that insertion of a sugar at the 3-*O*-position of a fucose moiety significantly reduces the nucleophilicity of the 4-*O*-position, thereby hindering the introduction of another sugar at that position.[11] To address this, we planned to protect the 3-*O*-

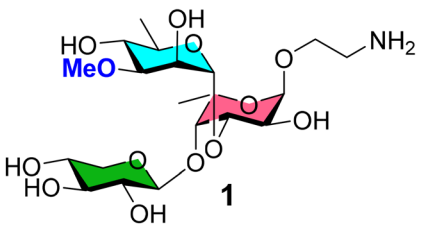

Fig. 1 Structure of the target trisaccharide repeating unit of the OPS from *A. brasilense* Jm6B2 in the form of its 2-aminoethyl glycoside.

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position of the fucosyl moiety with a temporary protecting group and introduce a protected D-xylose moiety at the 4-*O*-position. Furthermore, the removal of the 3-*O*-protection and introduction of the D-acofriose unit would lead to the protected trisaccharide. Finally, the removal of the protecting groups and reduction of the terminal azide to the corresponding amine would furnish the target molecule (Fig. 2).

For the synthesis of D-acofriose, commercially available D-mannose was converted to methyl D-mannoside (**2**) using H_2SO_4–silica in dry MeOH at 65 °C.[12] Our previously developed Fischer glycosylation strategy worked perfectly to give compound **2** in 95% yield. Further reaction with iodine in the presence of PPh_3 using dry DMF[13] as solvent gave the 6-iodo derivative, which was filtered through a silica gel column to remove triphenylphosphine oxide, and the iodo-derivative was hydrogenolyzed using H_2 in the presence of 10% Pd–C[14] to give methyl D-rhamnoside (**3**)[15] in 87% overall yield. Methylation at the 3-*O*-position was successfully achieved *via* stannylene chemistry. The formation of the tin ketal using Bu_2SnO in refluxing toluene followed by reaction with MeI in the presence of Bu_4NI[16] gave methyl 3-*O*-methyl D-acofrioside (**4**) in 73% yield. It is worth noting that the methylation reaction proceeded rather slowly as the low boiling point of MeI limits its availability as an electrophile and excess MeI is required to drive the reaction to completion with 73% yield. Acetolysis of compound **4** afforded the per-*O*-acetylated acofriose derivative **5** in 86% yield. Finally, Zemplen de-*O*-acetylation gave D-acofriose (**6**) in 96% yield (Scheme 1). Following this route, D-acofriose was obtained in 50% overall yield *via* six steps from commercially available D-mannose. All reactions were performed at the gram scale without any alteration in yields. It is worth noting that the current method for the preparation of D-acofriose is significantly superior to the methods reported by Sauvageau *et al.*[17,18] and Brimacombe *et al.*[19] (for the L-isomer). Moreover, it is observed that the process is equally efficient at the 100 mmol scale and therefore may be used for large-scale preparation of D-acofriose. To fulfil the requirement of our D-acofriose donor for total synthesis, the per-*O*-acetylated derivative **5** was reacted with *p*-thiocresol in the presence of $BF_3{\cdot}Et_2O$ at −5 °C to give the corresponding thioglycoside donor **7** in 89% yield (Scheme 1).

The initial challenge with the synthesis of the fucosyl acceptor was to install the reducing end α-glycoside. Therefore, the known thioglycoside donor **8**[20] was activated by NIS and TMSOTf in the presence of 2-bromoethanol. Different solvents at varying temperatures were tested to achieve α-selectivity, but with limited success. Glycosylations in pure CH_2Cl_2 showed no selectivity at 0 °C or −20 °C and only marginal selectivity (3 : 2) at −40 °C (entries 1, 2 and 3, Table 1). Switching to a CH_2Cl_2–Et_2O mixture[21] in varying ratios could achieve only up to a ratio of 2 : 1 of the desired α-glycoside (entries 4–6). Pre-activation of the donor with DMF[22] in CH_2Cl_2 followed by glycosylation with 2-bromoethanol was also tested, but glycoside **9** was obtained only in a 5 : 1 (α/β) ratio (entry 7, Table 1). In addition, we were unable to separate the mixture and use the desired isomer further.

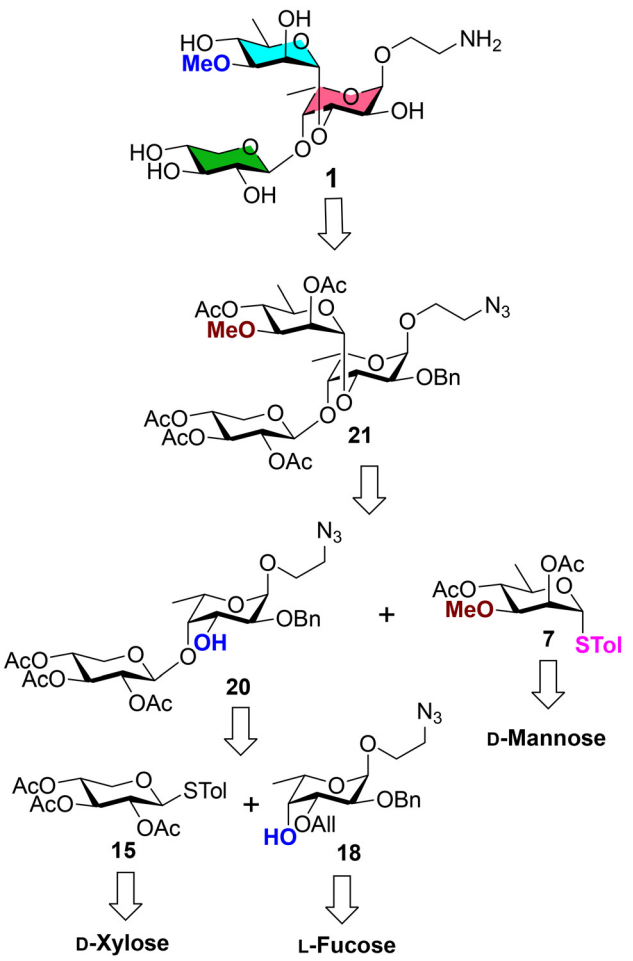
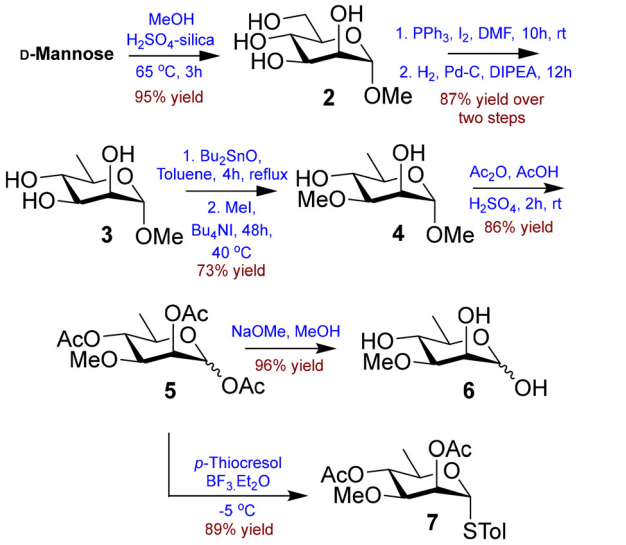


Fig. 2 Retrosynthetic analysis for the synthesis of the target trisaccharide **1**.



Scheme 1 Synthesis of D-acofriose (**6**) and the thioglycoside donor **7** from commercially available D-mannose.

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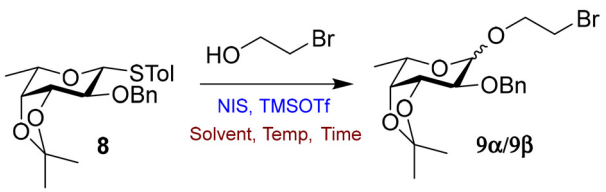
Table 1 Optimization of the α-glycosylation for the synthesis of the reducing end fucosyl moiety **9**

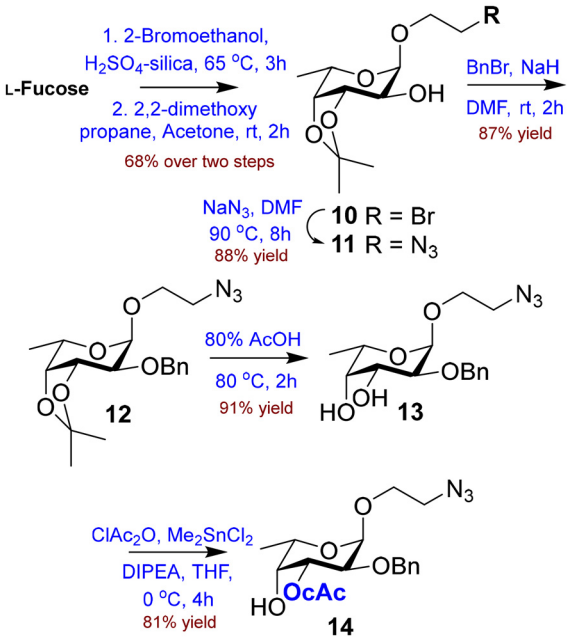


Entry	Solvent	Temp.	Time	9α : 9β [a]	Yield
1	Dry CH_2Cl_2	0 °C	1 h	1 : 1	81%
2		−20 °C	3 h	1 : 1	72%
3		−40 °C	3 h	3 : 2	74%
4	CH_2Cl_2–Et_2O (3 : 2)	−40 °C	3 h	3 : 2	76%
5	CH_2Cl_2–Et_2O (1 : 1)	−40 °C	3 h	3 : 2	75%
6	CH_2Cl_2–Et_2O (1 : 3)	−40 °C	3 h	2 : 1	76%
7	Dry CH_2Cl_2 pre-activation with DMF	−20 °C	3 h	5 : 1	78%

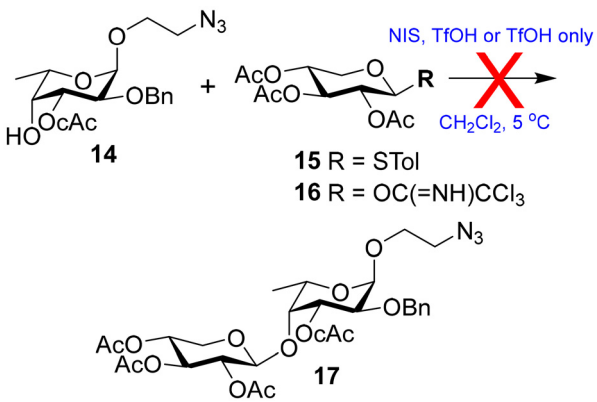
[a] As obtained from the ^{1}H NMR of the mixture.

Finally, we resorted to H_2SO_4–silica promoted Fischer glycosylation[12] of free L-fucose with 2-bromoethanol and successfully achieved the desired glycoside. After removal of excess 2-bromoethanol using diethyl ether, the residue was treated with 2,2-dimethoxypropane in acetone to afford the isopropylidene derivative **10** in 68% yield over two steps.

No further acid catalyst was required as H_2SO_4–silica was present in the mixture and only α-glycoside was isolated in pure form. Furthermore, the terminal bromide was replaced with azide using NaN_3 in DMF[23] at 90 °C to give the azido derivative **11** in 88% yield. The transformation was confirmed by the IR peak at 2104 cm^{-1}. The free 2-*O*-position was benzylated using BnBr and NaH[24] to afford the fully protected derivative **12** in 87% yield. Next, the hydrolysis of the isopropylidene group using 80% aq. AcOH at 80 °C[25] gave diol **13** in 91% yield. Selective chloroacetylation of diol **13** using chloroacetic anhydride in the presence of Me_2SnCl_2 [26,27] gave the 3-*O*-chloroacetate derivative **14** in 81% yield (Scheme 2).



Scheme 2 Synthesis of the fucose acceptors **14**.



Scheme 3 Failed synthesis of disaccharide **17** with fucosyl acceptor **14**.

Next, the glycosylation of the fucosyl acceptor **14** with the known xylose donor **15** [28] was tested through the activation of thioglycoside using NIS in the presence of TfOH.[29] However, the desired disaccharide **17** was not formed; instead, the donor was converted to the corresponding hemiacetal. Changing the donor to the known xylose trichloroacetimidate (**16**)[30] led to the same hemiacetal of the donor instead of the desired disaccharide (Scheme 3). It was assumed that the electron-withdrawing chloroacetate group at the neighbouring 3-*O*-position significantly reduced the nucleophilicity of the 4-*O*-position of acceptor **14**.

Therefore, the diol **13** was converted to the 3-*O*-allyl derivative **18** in 71% yield *via* stannylene chemistry, using Bu_2SnO in refluxing toluene followed by reaction with AllBr in the presence of Bu_4NI.[31] Finally, glycosylation of the fucosyl acceptor **18** and the xylosyl thioglycoside **15** using NIS in the presence of TfOH[28] at 0 °C gave disaccharide **19** in 81% yield with complete β-selectivity, as confirmed by the peaks at δ 4.74 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1) and 4.68 (d, 1H, $J_{1',2'}$ 6.8 Hz, H-1′) in the ^{1}H NMR spectra and at δ 98.2 (C-1) and 100.5 (C-1′) in the ^{13}C NMR spectra. The HRMS peak at 644.2433 $[M + Na]^+$ further confirmed the successful formation of disaccharide **19**. In contrast to the chloroacetyl group at the 3-*O*-position, the allyl ether did not hamper the nucleophilicity of the 4-*O*-position. Furthermore, the allyl protection was removed using $PdCl_2$ in MeOH[32] to afford the disaccharide acceptor **20** in 77% yield. Final glycosylation of the disaccharide acceptor **20** with the acofriose donor **7** using NIS in the presence of TfOH at 0 °C was uneventful and furnished the protected trisaccharide **21** in 83% yield. The successful formation of the trisaccharide was evident from the peaks at δ 97.5 (C-1), 101.4 (C-1′) and 98.9 (C-1″) in the ^{13}C NMR spectra. The HRMS peak at 848.3061 $[M + Na]^+$ further affirmed the formation of trisaccharide **21**.

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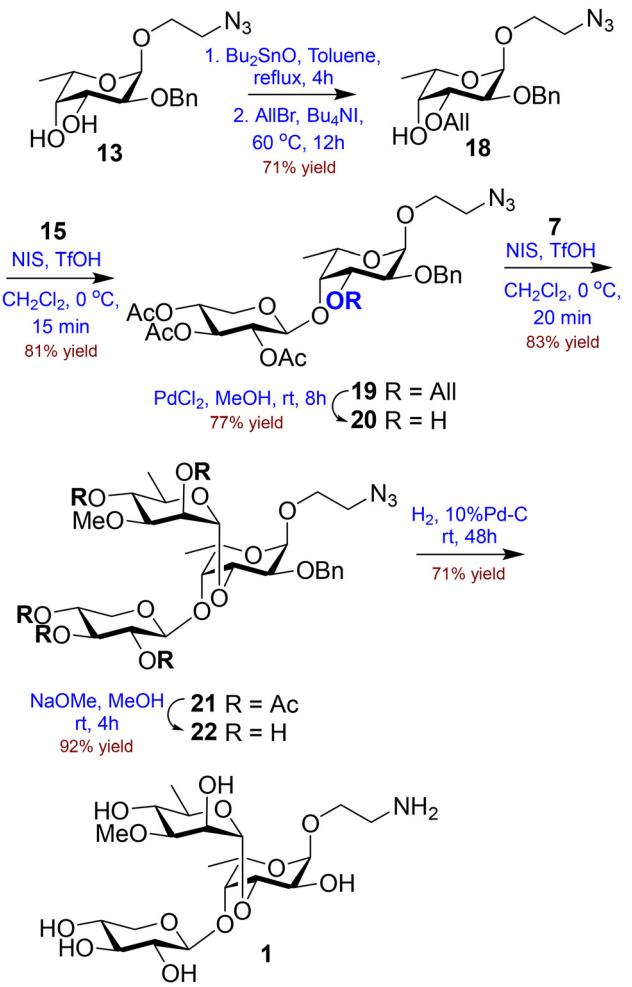

Scheme 4 Synthesis of the target trisaccharide **1**.

Furthermore, Zemplen de-*O*-acetylation[33] using NaOMe in MeOH gave the de-*O*-acetylated trisaccharide **22** in 92% yield. Finally, catalytic hydrogenation using 10% Pd–C[34] in the presence of H_2 gave the target trisaccharide **1** in 71% yield (Scheme 4).

Conclusions

In conclusion, we have successfully accomplished the synthesis of the target trisaccharide repeating unit of the O-antigen from *A. brasilense* JM6B2 in the form of its 2-aminoethyl glycoside. In the process, we have developed a concise route for the preparation of D-acofriose from commercially available D-mannose *via* six steps with ~50% yield. The route has been tested and found to be effective up to the 100 mmol scale and therefore has the potential to be used for large scale preparation. The 1,2-*cis* 2-bromoethyl fucoside was obtained exclusively using H_2SO_4–silica promoted Fischer glycosylation. The target trisaccharide retains the 2-aminoethyl group, allowing for future glycoconjugate formation without affecting the anomeric stereochemistry at the reducing end. The availability of this synthetic variant in its purest possible form will facilitate detailed investigations into the biological roles of this oligosaccharide repeat.

Experimental section

General methods

All solvents and reagents were dried before use according to standard methods.[35] The commercially purchased reagents were used without any further purification unless mentioned otherwise. Dichloromethane was dried and distilled over P_2O_5 to make it anhydrous and moisture-free. All reactions were monitored by thin layer chromatography (TLC) on silica-gel 60-F254 with detection by fluorescence, followed by charring after immersion in a 10% ethanolic solution of H_2SO_4. Flash chromatography was performed with silica gel (100–200 mesh). Optical rotations were measured on the sodium D-line at ambient temperature. IR data were collected on a Bruker Alpha FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz or 500 MHz and 125 MHz, respectively, on a JEOL JNM ECZL 400S or Bruker Avance Neo 400 or Bruker Avance Neo 500 spectrometer. The ^{1}H and ^{13}C peaks were assigned based on ^{1}H–^{1}H COSY and ^{1}H–^{13}C HSQC spectra. In the target trisaccharide **1**, proton signals were denoted as H for the reducing-end L-fucose unit, H′ for D-xylose, and H″ for D-acofriose. Similarly, the carbon signals are denoted as C for the reducing-end L-fucose unit, C′ for the D-xylose, and C″ for the D-acofriose unit. HRMS analysis was performed using a XeVO G2-XS Q-TOF (Waters Corporation) instrument in the +ve electrospray ionization mode.

Synthesis of methyl 6-deoxy-3-*O*-methyl-α-D-mannopyranoside (4). A mixture of compound **3** (2.4 g, 13.5 mmol) and Bu_2SnO (3.40 g, 13.7 mmol) in toluene (20 mL) was refluxed at 110 °C for 4 hours until the solution became clear. The solution was then cooled to room temperature and TBAI (5.0 g, 13.7 mmol) was added followed by MeI (4.2 mL, 67.4 mmol) and the solution was stirred at 40 °C for 24 hours. After 24 hours, the same amount (4.2 mL) of MeI was added again and the solution was stirred under the same conditions for another 24 hours. When TLC (CH_2Cl_2–MeOH; 7 : 1) showed complete conversion of the starting material to a faster-moving spot, the solvent was evaporated under reduced pressure. The crude product thus obtained was charged on a column of silica gel and eluted with CH_2Cl_2–MeOH (10 : 1) to afford the pure compound **4** (1.9 g, 73%) as a yellowish syrup.

$[\alpha]_D^{25}$: + 118 (*c* 0.8, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 3362, 2847, 1248, 1086, 1038, 791.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.60 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 3.95 (dd, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.2 Hz, H-2), 3.55–3.51 (m, 1H, H-5), 3.41 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.4 Hz, H-4), 3.37 (s, 3H, OCH_3), 3.27 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.4 Hz, H-3), 3.26 (s, 3H, OCH_3), 1.22 (d, 3H, $J_{5,6}$ 6.0 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 100.6 (C-1), 81.3 (C-3), 71.2 (C-4), 67.8 (C-5), 66.8 (C-2), 56.9 (OCH_3), 54.7 (OCH_3), 17.5 (C-CH_3).

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HRMS calcd for $C_8H_{16}O_5Na$ $[M + Na]^+$: 215.0895; found: 215.0891.

Synthesis of acetyl 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-α/β-D-mannopyranoside (5). To a solution of compound **4** (1.9 g, 9.9 mmol) dissolved in CH_2Cl_2 (7.8 mL), Ac_2O (3.6 mL) was added followed by AcOH (1.3 mL) and conc. H_2SO_4 (120 μL). The solution was stirred at room temperature for 2 hours until TLC (*n*-hexane–EtOAc; 5 : 2) showed complete conversion of the starting material to a faster-moving spot. The solution was poured carefully into a solution of Na_2CO_3 (2.5 g) in 30 mL ice-cold water and stirred well with a glass rod. CH_2Cl_2 (20 mL) was added and the mixture was extracted twice. The organic layer was further washed with $NaHCO_3$ (30 mL) and brine solution (30 mL), separated, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated *in vacuo*. The crude residue thus obtained was purified by column chromatography using *n*-hexane–EtOAc (3 : 1) to give an anomeric mixture (α/β; 5 : 2) of compound **5** (2.6 g, 86%) as a colourless gel.

α-Product

$[\alpha]_D^{25}$: +29 (*c* 0.8, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 2853, 1745, 1252, 1083, 1041, 786.

^1H NMR (400 MHz, $CDCl_3$) δ: 6.02 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 5.31 (dd, 1H, $J_{1,2}$ 2.0 Hz, $J_{2,3}$ 2.8 Hz, H-2), 5.01 (t, 1H, $J_{3,4}$, $J_{4,5}$ 10.0 Hz, H-4), 3.90–3.80 (m, 1H, H-5), 3.60 (dd, 1H, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 10 Hz, H-3), 3.35 (s, 3H, OCH_3), 2.14, 2.13, 2.09 (3s, 3 × 3H, 3 × $COCH_3$), 1.20 (d, 3H, $J_{5,6}$ 6.4 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1 ($COCH_3$), 169.9 ($COCH_3$), 168.4 ($COCH_3$), 91.0 (C-1), 76.8 (C-3), 71.9 (C-4), 68.8 (C-5), 66.9 (C-2), 57.8 (OCH_3), 20.9 (2) ($COCH_3$), 20.8 ($COCH_3$), 17.5 (C-CH_3).

HRMS calcd for $C_{13}H_{20}O_8Na$ $[M + Na]^+$: 327.1056; found: 327.1052.

Synthesis of 6-deoxy-3-*O*-methyl-D-mannopyranose (D-acofriose) (6). To a solution of compound **5** (2 g, 6.3 mmol) in MeOH (20 mL), NaOMe in MeOH (0.5M, 2 mL) was added and the solution was stirred at room temperature for 3 hours. Excess NaOMe was neutralized using DOWEX® 50 W H^+ resin. The mixture was filtered and the filtrate was evaporated *in vacuo* to afford compound **6** (1.2 g, 96%) as an anomeric mixture. Analytical data showed a satisfactory match with the data reported in the literature.[18]

Large scale (100 mmol) preparation of D-acofriose (6) from D-mannose. Commercially available D-mannose (18 g, 0.1 mol) was suspended in dry MeOH (200 mL) and stirred at 65 °C for 30 minutes. H_2SO_4–silica (5 g) was added and the stirring was continued at 65 °C for 4 hours. The mixture was neutralized with Et_3N (5 mL) and filtered. The filtrate was evaporated *in vacuo* and the residue was used for the next reaction without any further purification. The residue was dissolved in dry DMF (70 mL) and PPh_3 (31.5 g, 0.12 mol) was added followed by dropwise addition of I_2 (15 g, 0.12 mol in 50 mL DMF). The brown coloured solution was allowed to stir at room temperature for 12 hours and the reaction was monitored by TLC (CH_2Cl_2–MeOH; 9 : 1). Solvents were evaporated and the residue was triturated with EtOAc on an ice-bath. It was filtered through a layered pad of Celite® and silica gel (100–200 mesh). The filtrate was evaporated and the residue was dissolved in MeOH (75 mL). 10% Pd–C (5 g) was added followed by DIPEA (20 mL, 0.11 mol) and the mixture was shaken in a Paar hydrogenation assembly at 3 atm H_2 for 12 hours. The mixture was filtered through a pad of Celite® and the filtrate was evaporated *in vacuo*. The residue was suspended in dry toluene (100 mL), Bu_2SnO (25 g, 0.1 mmol) was added and the mixture was stirred under reflux for 5 hours. The solution was cooled to room temperature and TBAI (37 g, 0.1 mmol) was added followed by MeI (37 mL, 0.6 mol). The mixture was stirred at 40 °C for 24 hours. Another portion of MeI (18.5 mL, 0.3 mol) was added and the stirring continued for 24 hours. The reaction progress was monitored by TLC (CH_2Cl_2–MeOH; 7 : 1). The solvents were evaporated and the residue was dissolved in CH_2Cl_2 (80 mL), Ac_2O (36 mL) was added followed by AcOH (13 mL) and conc. H_2SO_4 (1.2 mL) and the solution was stirred at room temperature for 3 hours. Then the solution was carefully poured into a solution of Na_2CO_3 (25 g) in 250 mL ice water and stirred well with a glass rod. CH_2Cl_2 (70 mL) was added and the organic layer was extracted. The process was repeated one more time with CH_2Cl_2 (30 mL). The combined organic layer was further washed with aq. $NaHCO_3$ (100 mL) and brine solution (100 mL), separated, dried over anhydrous Na_2SO_4 (25 g), and filtered and the solvent was evaporated *in vacuo*. The syrupy residue was purified by column chromatography to remove the tin salts. The light-yellow syrup obtained was redissolved in MeOH (100 mL). Freshly prepared NaOMe in MeOH (0.5 M, 10 mL) was added and the solution was stirred at room temperature for 3 hours. Excess NaOMe was neutralized with DOWEX® 50 W H^+ and filtered and the filtrate was evaporated *in vacuo* to give D-acofriose (8.4 g, 47%) as an off-white sticky mass. It was dissolved in H_2O (40 mL) and lyophilized to obtain a white amorphous powder.

Synthesis of tolyl 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-1-thio-α-D-mannopyranoside (7). To a solution of compound **5** (2.6 g, 8.5 mmol) in dry CH_2Cl_2 (20 mL), *p*-thiocresol (1.4 g, 11.1 mmol) was added and the mixture was stirred at −5 °C for 15 min. To the cooled solution, $BF_3{\cdot}Et_2O$ (2.1 mL, 17.1 mmol) was added and the solution was stirred at the same temperature for 4 hours until TLC (*n*-hexane–EtOAc; 2 : 1) indicated complete conversion of the starting material to a faster-moving spot. The solution was then diluted with CH_2Cl_2 (10 mL) and washed successively with water (30 mL), saturated $NaHCO_3$ solution (2 × 30 mL) and brine (30 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated *in vacuo* and the crude product thus obtained was purified by column chromatography using *n*-hexane–EtOAc (3 : 1) to afford the pure compound **7** (2.8 g, 89%) as a white amorphous powder.

$[\alpha]_D^{25}$: +62 (*c* 0.9, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 2973, 2860, 1740, 1374, 1211, 1040, 804.

^1H NMR (400 MHz, $CDCl_3$) δ: 7.34 (d, 2H, Ar*H*), 7.11 (d, 2H, Ar*H*), 5.56 (dd, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.35 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 5.03 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4), 4.33–4.23 (m, 1H, H-5), 3.57 (dd, 1H $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.6 Hz, H-3), 3.36 (s, 3H,

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OCH_3), 2.32 (s, 3H, $SC_6H_4CH_3$), 2.12, 2.11 (2s, 2 × 3H, 2 × COCH_3), 1.21 (d, 3H, $J_{5,6}$ 6.4 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3 (*C*OCH$_3$), 170.1 (*C*OCH$_3$), 138.1, 132.3, 130.0, 129.8, 86.4 (C-1), 77.5 (C-3), 72.6 (C-4), 69.6 (C-2), 67.7 (C-5), 57.7 (OCH$_3$), 21.1 (COCH$_3$), 21.0 (COCH$_3$), 20.9 ($SC_6H_4CH_3$), 17.3 (C-*C*H$_3$).

HRMS calcd for $C_{18}H_{24}O_6SNa$ [M + Na]$^+$: 391.1191; found: 391.1185.

Synthesis of 2-bromoethyl 3,4-*O*-isopropylidene-α-L-fucopyranoside (10). A suspension of L-fucose (3.0 g, 18.3 mmol) in 2-bromoethanol was stirred at 65 °C for 15 minutes. H_2SO_4–silica (100 mg) was then added and the mixture was stirred at the same temperature for 3 hours. The resulting solution was cooled to room temperature and Et_2O (50 mL) was added and shaken well, causing the product to precipitate while excess 2-bromoethanol was dissolved in the ether layer. The ether layer was decanted and the process was repeated with Et_2O (20 mL). The residue was dried under vacuum for 30 minutes before being suspended in dry acetone (20 mL). 2,2-Dimethoxypropane (3.4 mL, 27.4 mmol) was added and the mixture was stirred at room temperature for 2 hours until TLC (*n*-hexane–EtOAc; 2 : 1) showed complete conversion to a faster running spot. The reaction mixture was quenched with Et_3N (1.5 mL), the solvent was evaporated and the residue was purified by column chromatography using *n*-hexane–EtOAc (3 : 1) to afford the pure compound **10** (3.9 g, 68%) as a colourless syrup.

$[\alpha]_D^{25}$: −52 (*c* 0.8, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.78 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 4.17–4.12 (m, 2H, H-5, H-3), 3.99 (dd, 1H, $J_{3,4}$ 6.0 Hz, $J_{4,5}$ 2.4 Hz, H-4), 3.97–3.92 (m, 1H, OCH_{2a}), 3.81–3.75 (m, 1H, OCH_{2b}), 3.74–3.70 (m, 1H, H-2), 3.49–3.43 (m, 2H, CH_2Br), 2.76 (d, 1H, *J* 6.4 Hz, O*H*), 1.43 (s, 3H, isopropylidene-CH_3), 1.27 (s, 3H, isopropylidene-CH_3), 1.23 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 109.1 (isopropylidene-*C*), 97.8 (C-1), 75.9 (C-3), 75.5 (C-4), 69.2 (C-2), 67.9 (O*C*H$_2$), 64.2 (C-5), 30.6 (*C*H$_2$Br), 27.8 (isopropylidene-*C*H$_3$), 25.9 (isopropylidene-*C*H$_3$), 16.2 (C-*C*H$_3$).

HRMS calcd for $C_{11}H_{19}BrO_5Na$ [M + Na]$^+$: 333.0314; found: 333.0317.

Synthesis of 2-azidoethyl 3,4-*O*-isopropylidene-α-L-fucopyranoside (11). Compound **10** (3.9 g, 12.4 mmol) was dissolved in DMF (12 mL) and NaN_3 (4.0 g, 62 mmol) was added to it. The reaction mixture was stirred at 90 °C for 8 hours until TLC (*n*-hexane–EtOAc; 3 : 2) showed complete conversion of the starting material to a slower running spot. The solvent was evaporated and the residue was diluted with EtOAc (20 mL) and washed with brine solution (3 × 30 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and filtered; the solvent was evaporated under reduced pressure and the crude product thus obtained was purified by column chromatography using *n*-hexane–EtOAc (2 : 1) to afford the pure compound **11** (3.0 g, 88%) as a colourless syrup.

$[\alpha]_D^{25}$: −39 (*c* 0.9, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 2991, 2933, 2104, 1381, 1211, 1059, 861.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.86 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 4.23 (t, 1H, $J_{2,3}$, $J_{3,4}$ 6.4 Hz, H-3), 4.21–4.16 (m, 1H, H-5), 4.08 (dd, 1H, $J_{3,4}$ 6.4 Hz, $J_{4,5}$ 2.0 Hz, H-4), 4.00–3.95 (m, 1H, OCH_{2a}), 3.82 (dd, 1H, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 6.4 Hz, H-2), 3.72–3.67 (m, 1H, OCH_{2b}), 3.52–3.46 (m, 1H, C$H_{2a}N_3$), 3.43–3.37 (m, 1H, C$H_{2b}N_3$), 2.43 (bs, 1H, O*H*), 1.51 (s, 3H, isopropylidene-CH_3), 1.36 (s, 3H, isopropylidene-CH_3), 1.33 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 109.2 (isopropylidene-*C*), 97.8 (C-1), 75.9 (C-3), 75.5 (C-4), 69.2 (C-2), 67.1 (O*C*H$_2$), 64.3 (C-5), 50.7 (*C*H$_2$N$_3$), 27.7 (isopropylidene-*C*H$_3$), 25.9 (isopropylidene-*C*H$_3$), 16.3 (C-*C*H$_3$).

HRMS calcd for $C_{11}H_{19}N_3O_5Na$ [M + Na]$^+$: 296.1222; found: 296.1225.

Synthesis of 2-azidoethyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranoside (12). Compound **11** (3.0 g, 11.0 mmol) was dissolved in DMF (15 mL) and the solution was cooled to 0 °C. After 15 minutes, NaH (925 mg, 38.5 mmol, 50% dispersion in mineral oil) was added followed by the addition of BnBr (2.0 mL, 16.8 mmol), and the reaction mixture was stirred at room temperature for 2 hours until TLC (*n*-hexane–EtOAc; 4 : 1) showed complete conversion of the starting material to a faster-moving spot. Excess NaH was quenched with MeOH (5 mL) and the solvent was evaporated under reduced pressure. The residue was diluted with EtOAc and washed with brine solution (3 × 30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, and filtered and the solvent was evaporated *in vacuo*. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (4 : 1) to furnish the pure compound **12** (3.5 g, 87%) as a colourless syrup.

$[\alpha]_D^{25}$: −57 (*c* 0.7, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 3068, 2927, 2108, 1513, 1151, 1073, 742.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.27 (m, 5H, Ar*H*), 4.84 (d, 1H, *J* 12.4 Hz, CH_2Ph), 4.77 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 4.73 (d, 1H, *J* 12.4 Hz, CH_2Ph), 4.37 (dd, 1H, $J_{2,3}$ 7.6 Hz, $J_{3,4}$ 5.6 Hz, H-3), 4.23–4.17 (m, 1H, H-5), 4.09 (dd, 1H, $J_{3,4}$ 5.6 Hz, $J_{4,5}$ 2.8 Hz, H-4), 3.89–3.83 (m, 1H, OCH_{2a}), 3.61–3.54 (m, 3H, OCH_{2b}, C$H_{2a}N_3$, H-2), 3.37–3.31 (m, 1H, C$H_{2b}N_3$), 1.44 (s, 3H, isopropylidene-CH_3), 1.37 (s, 3H, isopropylidene-CH_3), 1.35 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 138.4, 128.4, 127.9, 127.7, 108.8 (isopropylidene-*C*), 97.5 (C-1), 76.2 (C-2), 76.1 (C-4), 75.7 (C-3), 72.5 (*C*H$_2$Ph), 67.0 (O*C*H$_2$), 63.6 (C-5), 50.6 (*C*H$_2$N$_3$), 28.2 (isopropylidene-*C*H$_3$), 26.3 (isopropylidene-*C*H$_3$), 16.3 (C-*C*H$_3$).

HRMS calcd for $C_{18}H_{25}N_3O_5Na$ [M + Na]$^+$: 386.1692; found: 386.1688.

Synthesis of 2-azidoethyl 2-*O*-benzyl-α-L-fucopyranoside (13). A suspension of compound **12** (3.5 g, 9.6 mmol) in 80% AcOH (30 mL) was stirred at 80 °C for 2 hours until TLC (*n*-hexane–EtOAc; 1 : 2) showed complete conversion of the starting material to a slower-moving spot. The solvents were evaporated *in vacuo* and co-evaporated with toluene to ensure complete removal of AcOH. The crude product thus obtained was purified by column chromatography using *n*-hexane–EtOAc (1 : 4) to afford the pure compound **13** (2.8 g, 91%) as a colourless syrup.

$[\alpha]_D^{25}$: −18 (*c* 0.7, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 3430, 2918, 2104, 1090, 1040, 742.

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^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.31 (m, 5H, Ar*H*), 4.81 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.67 (s, 2H, CH_2Ph), 4.05–3.98 (m, 2H, H-5, H-3), 3.85 (m, 1H, H-4), 3.83–3.79 (m, 1H, OCH_{2a}), 3.73 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.6 Hz, H-2), 3.53–3.47 (m, 2H, OCH_{2b}, C$H_{2a}N_3$), 3.39–3.34 (m, 1H, C$H_{2b}N_3$), 2.55 (d, 1H, *J* 2.8 Hz, O*H*), 2.37 (bs, 1H, O*H*), 1.30 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 138.2, 128.7, 128.2, 97.1 (C-1), 76.5 (C-2), 72.9 (*C*H_2Ph), 71.6 (C-4), 69.3 (C-5), 66.9 (O*C*H_2), 65.9 (C-3), 50.8 (*C*H_2N_3), 16.2 (C-*C*H_3).

HRMS calcd for $C_{15}H_{21}N_3O_5Na$ $[M + Na]^+$: 346.1379; found: 346.1383.

Synthesis of 2-azidoethyl 2-*O*-benzyl-3-*O*-chloroacetyl-α-L-fucopyranoside (14). To a solution of compound **13** (500.0 mg, 1.5 mmol) in dry THF (7 mL), Me_2SnCl_2 (17.0 mg, 0.075 mmol) and DIPEA (0.5 mL, 3.0 mmol) were added and the solution was cooled to 0 °C and stirred for 15 minutes. Chloroacetic anhydride (325.0 mg, 1.9 mmol) was added and the solution was stirred at the same temperature for 4 hours until TLC (*n*-hexane–EtOAc; 1 : 1) showed complete conversion of the starting material to a faster running spot. The reaction mixture was then quenched with 3% aqueous HCl (5 mL) and extracted with ethyl acetate (2 × 15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered, and the solvents were evaporated *in vacuo* and purified by column chromatography using *n*-hexane–EtOAc (1 : 1) to furnish the pure compound **14** (486.0 mg, 81%) as a white amorphous mass.

$[\alpha]_D^{25}$: −72 (*c* 0.7, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 3476, 2918, 2102, 1752, 1158, 1040, 752.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35–7.25 (m, 5H, Ar*H*), 5.25 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 2.8 Hz, H-3), 4.78 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.65 (d, 1H, *J* 12.4 Hz, CH_2Ph), 4.60 (d, 1H, *J* 12.4 Hz, CH_2Ph), 4.10–4.03 (m, 2H, H-5, CH_{2a}Cl), 3.99 (d, 1H, *J* 14.8 Hz, CH_{2b}Cl), 3.94–3.89 (m, 2H, H-4, H-2), 3.81–3.75 (m, 1H, OCH_{2a}), 3.58–3.52 (m, 1H, OCH_{2b}), 3.51–3.44 (m, 1H, C$H_{2a}N_3$), 3.42–3.35 (m, 1H, C$H_{2b}N_3$), 2.81 (bs, 1H, O*H*), 1.22 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.8 (*C*OCH_2Cl), 138.1, 128.5, 128.0, 127.9, 97.5 (C-1), 74.5 (C-3), 73.3 (C-4), 73.1 (*C*H_2Ph), 70.3 (C-2), 66.9 (O*C*H_2), 65.7 (C-5), 50.6 (*C*H_2N_3), 40.9 (*C*H_2Cl), 16.0 (C-*C*H_3).

HRMS calcd for $C_{17}H_{22}ClN_3O_6Na$ $[M + Na]^+$: 422.1095; found: 422.1092.

Synthesis of 2-azidoethyl 3-*O*-allyl-2-*O*-benzyl-α-L-fucopyranoside (18). A suspension of compound **13** (500.0 mg, 1.5 mmol) and Bu_2SnO (548.0 mg, 2.2 mmol) in dry toluene (15 mL) was stirred at 110 °C for 4 hours until the solution became clear. After completion of the reaction, the solvent was evaporated *in vacuo* and the crude product was kept under vacuum for 1 hour. The residue was dissolved in dry toluene (20 mL) and TBAI (812.0 mg, 2.2 mmol) was added followed by the addition of allyl bromide (0.2 mL, 2.3 mmol) and the reaction mixture was stirred at 60 °C for 12 hours. After completion of the reaction (monitored by TLC in *n*-hexane–EtOAc; 1 : 1), the reaction mixture was diluted with EtOAc and washed with brine solution (2 × 30 mL). The organic layer was then separated and dried over anhydrous Na_2SO_4, and the solvent was evaporated *in vacuo* and the crude product was purified by column chromatography using *n*-hexane–EtOAc (3 : 2) to provide the pure compound **18** (387.0 mg, 71%) as a yellowish syrup.

$[\alpha]_D^{25}$: −81 (*c* 0.7, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 3323, 2915, 2101, 1691, 1540, 1270, 1058, 743.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.26 (m, 5H, Ar*H*), 6.00–5.90 (m, 1H, All*CH*), 5.32 (dd, 1H, J_{gem} 1.6 Hz, J_{trans} 17.2 Hz, AllCH_{2a}), 5.2 (dd, 1H, J_{gem} 1.6 Hz, J_{cis} 10.4 Hz, AllCH_{2b}), 4.8 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.75 (d, 1H, $J_{1,2}$ 2.8 Hz, H-1), 4.64 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.28–4.18 (m, 2H, OCH_2All), 3.98 (q, 1H, $J_{5,6}$ 6.8 Hz, H-5), 3.86 (d, 1H, $J_{3,4}$ 2.4 Hz, H-4), 3.84–3.77 (m, 3H, OCH_{2a}, H-2, H-3), 3.60–3.51 (m, 2H, OCH_{2b}, C$H_{2a}N_3$), 3.37–3.30 (m, 1H, C$H_{2b}N_3$), 2.58 (bs, 1H, O*H*), 1.29 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 138.4, 134.6 (All*C*H), 128.2, 127.7, 127.5, 117.1 (All*C*H_2), 97.7 (C-1), 77.1 (C-2), 75.1 (C-3), 73.2 (*C*H_2Ph), 71.3 (O*C*H_2All), 70.0 (C-4), 66.6 (O*C*H_2), 65.5 (C-5), 50.5 (*C*H_2N_3), 16.0 (C-*C*H_3).

HRMS calcd for $C_{18}H_{25}N_3O_5Na$ $[M + Na]^+$: 386.1692; found: 386.1688.

Synthesis of 2-azidoethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1 → 4)-3-*O*-allyl-2-*O*-benzyl-α-L-fucopyranoside (19). A mixture of fucosyl acceptor **18** (350.0 mg, 0.96 mmol), xylosyl donor **15** (513.0 mg, 1.34 mmol) and activated MS 4 Å (1.0 g) in dry CH_2Cl_2 (10 mL) was stirred under a N_2 atmosphere for 20 minutes. NIS (389.0 mg, 1.73 mmol) was added and the reaction mixture was stirred again under a N_2 atmosphere at 0 °C for 15 minutes. After that, TfOH (27 μL, 0.30 mmol) was added and the mixture was stirred at 0 °C for 15 minutes until TLC (*n*-hexane–EtOAc; 3 : 2) showed complete consumption of the acceptor. The mixture was filtered through Celite® and the filtrate was successively washed with $Na_2S_2O_3$ solution (2 × 15 mL), saturated $NaHCO_3$ solution (20 mL) and brine solution (20 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The yellowish syrup thus obtained was purified by column chromatography using *n*-hexane–EtOAc (2 : 1) to furnish the pure disaccharide **19** (485.0 mg, 81%) as a colourless syrup.

$[\alpha]_D^{25}$: +121 (*c* 0.8, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 2936, 2103, 1740, 1638, 1369, 1218, 1086, 1037, 733.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.25 (m, 5H, Ar*H*), 6.0–5.90 (m, 1H, All*CH*), 5.32 (dd, 1H, J_{gem} 1.2 Hz, J_{trans} 17.2 Hz, AllCH_{2a}), 5.17 (dd, 1H, J_{gem} 1.2 Hz, J_{cis} 10.4 Hz, AllCH_{2b}), 5.09 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 6.4 Hz, H-3′), 5.01 (dd, 1H, $J_{1',2'}$ 6.8 Hz, $J_{2',3'}$ 6.4 Hz, H-2′), 4.95–4.90 (m, 1H, H-4′), 4.87 (d, 1H, *J* 12.8 Hz, CH_2Ph), 4.74 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 4.68 (d, 1H, $J_{1',2'}$ 6.8 Hz, H-1′), 4.66 (d, 1H, *J* 12.8 Hz, CH_2Ph), 4.40 (dd, 1H, J_{gem} 12.4 Hz, $J_{4',5'}$ 4.0 Hz, H-5′a), 4.23 (m, 1H, OCH_{2a}All), 4.12 (m, 1H, OCH_{2b}All), 3.96 (q, 1H, $J_{5,6}$ 6.8 Hz, H-5), 3.87 (s, 1H, H-4), 3.83–3.77 (m, 3H, H-3, H-2, OCH_{2a}), 3.64–3.53 (m, 2H, OCH_{2b}, C$H_{2a}N_3$), 3.43 (dd, 1H, J_{gem} 12.4 Hz, $J_{4',5'}$ 6.4 Hz, H-5′b), 3.35–3.30 (m, 1H, C$H_{2b}N_3$), 2.09 (s, 3H, COCH_3), 2.08 (s, 3H, COCH_3), 1.95 (s, 3H, COCH_3), 1.23 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

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^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9 ($COCH_3$), 169.8 ($COCH_3$), 169.3 ($COCH_3$), 138.8, 134.9 (All*C*H), 128.2, 127.6, 127.5, 116.7 (AllCH_2), 100.5 (C-1′), 98.2 (C-1), 78.2 (C-4), 76.9 (C-3), 75.0 (C-2), 73.5 (CH_2Ph), 71.1 (OCH_2All), 69.7 (C-3′), 69.6 (C-2′), 68.4 (C-4′), 66.8 (OCH_2), 66.2 (C-5), 61.0 (C-5′), 50.6 (CH_2N_3), 20.8 ($COCH_3$), 20.7 ($COCH_3$), 20.6 ($COCH_3$), 16.3 (C-CH_3).

HRMS calcd for $C_{29}H_{39}N_3O_{12}Na$ [M + Na]$^+$: 644.2431; found: 644.2433.

Synthesis of 2-azidoethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1 → 4)-2-*O*-benzyl-α-L-fucopyranoside (20). To a solution of compound **19** (450 mg, 0.73 mmol) in MeOH (10 mL), $PdCl_2$ (65.0 mg, 0.37 mmol) was added and the solution was stirred at room temperature for 8 hours until TLC (*n*-hexane–EtOAc; 3 : 2) showed complete conversion of the starting material to a slower running spot. The solvent was removed under reduced pressure and the crude product was immediately charged into a column of silica gel and eluted with *n*-hexane–EtOAc (2 : 3) to afford the pure compound **20** (325.0 mg, 77%) as a colourless syrup.

$[\alpha]_D^{25}$: +73 (*c* 0.8, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 3342, 2105, 1742, 1373, 1216, 1082, 1040, 753.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38–7.23 (m, 5H, Ar*H*), 5.18 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.2 Hz, H-3′), 5.04–4.96 (m, 2H, H-2′, H-4′), 4.83 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.68 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.60 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.49 (d, 1H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.15 (dd, 1H, J_{gem} 11.6 Hz, $J_{4',5'}$ 5.2 Hz, H-5′a), 4.03–3.96 (m, 2H, H-5, H-3), 3.76 (d,1H, $J_{4,5}$ 2.8 Hz, H-4), 3.73–3.68 (m, 1H, OCH_{2a}), 3.57 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.0 Hz, H-2), 3.51–3.44 (m, 2H, OCH_{2b}, C$H_{2a}N_3$), 3.36–3.25 (m, 2H, C$H_{2b}N_3$, H-5′b), 3.21 (d, *J* 8.8 Hz, O*H*), 2.02 (s, 6H, 2 × COCH_3), 2.01 (s, 3H, COCH_3), 1.17 (d, 3H, $J_{5,6}$ 6.8 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1 ($COCH_3$), 169.6 ($COCH_3$), 169.1 ($COCH_3$), 138.6, 128.2, 127.8, 127.6, 102.2 (C-1′), 97.8 (C-1), 83.5 (C-4), 76.9 (C-2), 73.3 (CH_2Ph), 71.9 (C-3′), 71.4 (C-2′), 68.5 (C-4′, C-3), 66.8 (OCH_2), 65.7 (C-5), 62.5 (C-5′), 50.6 (CH_2N_3), 20.6 ($COCH_3$), 20.5 (2 × $COCH_3$), 16.0 (C-CH_3).

HRMS calcd for $C_{26}H_{35}N_3O_{12}Na$ [M + Na]$^+$: 604.2118; found: 604.2114.

Synthesis of 2-azidoethyl 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-α-D-mannopyranosyl-(1 → 3)-2-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-α-L-fucopyranoside (21). A mixture of the disaccharide acceptor **20** (300.0 mg, 0.52 mmol), acofriosyl donor **7** (269.0 mg, 0.73 mmol) and activated MS 4 Å (1.0 g) in dry CH_2Cl_2 (10 mL) was stirred under a N_2 atmosphere for 30 minutes. NIS (211.5 mg, 0.94 mmol) was added and the reaction mixture was stirred again under a N_2 atmosphere at 0 °C for 30 minutes. Subsequently, TfOH (14 μL, 0.16 mmol) was added and the mixture was stirred at 0 °C for another 20 minutes until TLC (*n*-hexane–EtOAc; 2 : 3) confirmed complete consumption of the acceptor. The mixture was filtered through Celite® and the filtrate was successively washed with $Na_2S_2O_3$ solution (15 mL × 2), saturated $NaHCO_3$ solution (30 mL) and brine solution (30 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The crude product thus obtained was purified by column chromatography using *n*-hexane–EtOAc (2 : 3) to afford the pure trisaccharide **21** (355.0 mg, 83%) as a yellowish syrup.

$[\alpha]_D^{25}$: +135 (*c* 0.9, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 2872, 2101, 1744, 1371, 1216, 1082, 1041, 746.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36–7.28 (m, 5H, Ar*H*), 5.48 (s, 1H, H-2″), 5.24–5.17 (m, 2H, H-3′, H-1″), 5.11 (t, 1H, $J_{1',2'}$, $J_{2',3'}$ 8.0 Hz, H-2′), 5.0–4.9 (m, 2H, H-4′, H-4″), 4.78 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.66 (d, 1H, $J_{1,2}$ 2.8 Hz, H-1), 4.62–4.53 (m, 2H, CH_2Ph, H-1′), 4.34 (dd, 1H, J_{gem} 11.2 Hz, $J_{4',5'}$ 4.4 Hz, H-5′a), 4.08 (bd, 1H, $J_{2,3}$ 10.0 Hz, H-3), 4.04–3.92 (m, 2H, H-5, H-5″), 3.82 (d, 1H, $J_{1,2}$ 2.8 Hz, $J_{2,3}$ 10.0 Hz, H-2), 3.8–3.7 (m, 3H, H-4, OCH_{2a}, H-3″), 3.64–3.48 (m, 2H, OCH_{2b}, C$H_{2a}N_3$), 3.43 (s, 3H, OCH_3), 3.39–3.29 (m, 2H, C$H_{2b}N_3$, H-5′b), 2.11 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3), 2.07 (s, 6H, 2 × COCH_3), 2.05 (s, 3H, COCH_3), 1.24 (d, 3H, $J_{5,6}$ 6.4 Hz, C-CH_3) 1.18 (d, 3H, $J_{5'',6''}$ 6.0 Hz, C-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1 ($COCH_3$), 170.0 ($COCH_3$), 169.9 ($COCH_3$), 169.8 ($COCH_3$), 169.4 ($COCH_3$), 138.3, 128.4, 128.0, 127.8, 101.4 (C-1′), 98.9 (C-1″), 97.5 (C-1), 79.8 (C-4), 77.2 (C-3″), 76.5 (C-2), 73.2 (CH_2Ph), 73.0 (C-4′), 72.3 (C-3), 71.2 (C-3′), 71.1 (C-2′), 69.2 (C-4″), 68.1 (C-2″), 66.9 (OCH_2), 66.6 (C-5), 66.2 (C-5″), 61.6 (C-5′), 57.9 (OCH_3), 50.6 (CH_2N_3), 21.0 ($COCH_3$), 20.9 ($COCH_3$), 20.8 ($COCH_3$), 20.7 (2 × $COCH_3$), 17.5 (C-CH_3), 16.2 (C-CH_3).

HRMS calcd for $C_{37}H_{51}N_3O_{18}Na$ [M + Na]$^+$: 848.3065; found: 848.3061.

Synthesis of 2-azidoethyl 6-deoxy-3-*O*-methyl-α-D-mannopyranosyl-(1 → 3)-2-*O*-benzyl-4-*O*-(β-D-xylopyranosyl)-α-L-fucopyranoside (22). To a solution of compound **21** (300 mg, 0.36 mmol) in MeOH (5 mL), NaOMe in MeOH (0.5 mL, 0.5M) was added and the solution was stirred at room temperature for 4 hours. The solution was neutralized with DOWEX 50 W H+ resin and the resin was filtered off with a pad of cotton. The solvents were evaporated to obtain compound **22** (206.0 mg, 92%) as colourless foam.

$[\alpha]_D^{25}$: + 98 (*c* 0.7, $CHCl_3$).

IR (cm^{-1}, $CHCl_3$) ν: 3432, 2921, 2105, 1092, 1037, 743.

^{1}H NMR (500 MHz, CD_3OD) δ: 7.42–7.28 (m, 5H, Ar*H*), 5.13 (s, 1H, H-1″), 4.81 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.70 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.64 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.31 (d, 1H, $J_{1',2'}$ 6.5 Hz, H-1′), 4.11–4.06 (m, 4H, H-3, H-2″, H-5′a, H-5), 3.95–3.88 (m, 3H, H-4″, H-2, H-5″), 3.82–3.78 (m, 1H, OCH_{2a}), 3.56–3.45 (m, 8H, H-4′, H-4, H-3′, OCH_{2b}, C$H_{2a}N_3$, OCH_3), 3.42–3.37 (m, 1H, C$H_{2b}N_3$), 3.37–3.29 (m, 2H, H-2′, H-3″), 3.15 (t, 1H, J_{gem}, $J_{4',5'}$ 11.0 Hz, H-5′b), 1.32 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3), 1.24 (d, 3H, $J_{5'',6''}$ 6.0 Hz, C-CH_3).

^{13}C NMR (125 MHz, CD_3OD) δ: 138.5, 128.1, 127.8, 127.5 (Ar*C*), 104.8 (C-1′), 101.5 (C-1″), 97.4 (C-1), 80.3 (C-4), 79.4 (C-2), 76.5 (C-4″), 76.4 (C-3″), 74.1 (C-2′), 72.4 (C-3), 72.3 (CH_2Ph), 71.5 (C-4′), 69.9 (C-3′), 68.8 (C-5″), 67.1 (C-2″), 66.9 (OCH_2), 66.8 (C-5), 65.4 (C-5′), 56.1 (OCH_3), 50.4 (CH_2N_3), 16.6 (C-CH_3), 15.2 (C-CH_3).

HRMS calcd for $C_{27}H_{41}N_3O_{13}Na$ [M + Na]$^+$: 638.2537; found: 638.2532.

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2-Aminoethyl 6-deoxy-3-*O*-methyl-α-D-mannopyranosyl-(1 → 3)-4-*O*-(β-D-xylopyranosyl)-α-L-fucopyranoside (1). To a solution of compound **22** (50 mg, 0.08 mmol) in MeOH (5.0 mL), 10% Pd–C (25 mg) was added and the mixture was stirred at room temperature under a H_2 atmosphere for 48 hours. It was filtered through a pad of Celite® and the filtrate was evaporated *in vacuo*. The residue was dissolved in H_2O (2 mL) and lyophilized. The pure compound **1** (30.0 mg, 71%) was obtained as a white amorphous mass.

$[\alpha]_D^{25}$: +102 (*c* 0.4, CH_3OH).

^{1}H NMR (400 MHz, CD_3OD) δ: 5.08 (s, 1H, H-1″), 4.91 (s, 1H, H-1), 4.32 (d, 1H, $J_{1',2'}$ 7.2 Hz, H-1′), 4.20 (s, 1H, H-2″), 4.11–4.01 (m, 3H, H-3, H-5, H-5′a), 3.99–3.93 (m, 3H, H-5″, OCH_{2a}, H-4″), 3.89 (m, 1H, H-2), 3.80–3.74 (m, 1H, OCH_{2b}), 3.50–3.46 (m, 6H, H-4′, H-4, H-3′, OCH_3), 3.34–3.28 (m, 4H, H-3″, CH_2N, H-2′), 3.16 (t, 1H, J_{gem}, $J_{4',5'}$ 10.8 Hz, H-5′b), 1.36 (d, 3H, $J_{5,6}$ 6.4 Hz, C-CH_3), 1.26 (d, 3H, $J_{5'',6''}$ 6.0 Hz, C-CH_3).

^{13}C NMR (100 MHz, CD_3OD) δ: 104.3 (C-1′), 101.6 (C-1″), 98.6 (C-1), 79.9 (C-4), 79.3 (C-2), 75.7 (C-3″), 73.6 (2C, C-2′, C-4″), 70.8 (C-4′), 69.4 (C-3′), 69.0 (C-5″), 68.5 (C-3), 67.5 (C-5), 65.5 (C-2″), 64.9 (C-5′), 62.5 (OCH_2), 56.0 (2C, OCH_3, CH_2N), 16.6 (C-CH_3), 15.0 (C-CH_3).

HRMS calcd for $C_{20}H_{37}NO_{13}Na$ $[M + Na]^+$: 522.2163; found: 522.2157.

Author contributions

SM performed the experiments and collected analytical data. BM and SM contributed equally towards the analysis of the data and preparation of the manuscript and its associated content.

Data availability

Necessary data are available in the ESI† associated with the manuscript.

Conflicts of interest

There are no conflicts to declare.

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