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Synthesis of a tetra- and a trisaccharide related to an anti-tumor saponin "Julibroside J_{28}" from *Albizia julibrissin*

Bimalendu Roy · Kausikisankar Pramanik · Balaram Mukhopadhyay

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Abstract Simple and convergent synthesis of a tetra- and a trisaccharide portions of an antitumor compound Julibroside J_{28}, isolated from *Albizia julibrissin*, that showed significant *in vitro* antitumor activity against HeLa, Bel-7402 and PC-3M-1E8 cancer cell lines is reported. The tetrasaccharide has been synthesized as its *p*-methoxyphenyl glycoside starting from commercially available D-glucose, L-rhamnose and L-arabinose. The trisaccharide part has been synthesized from commercially available *N*-acetyl D-glucosamine, D-fucose and D-xylose using simple protecting group manipulations. Sulfuric acid immobilized on silica has been used successfully as a Brönsted acid catalyst for the crucial glycosylation steps.

Keywords Saponin · Synthesis · H2SO4-silica · Growth-inhibitory

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B. Roy · B. Mukhopadhyay (✉)
Medicinal and Process Chemistry Division,
Central Drug Research Institute,
Chattar Manzil Palace,
Lucknow, 226 001 Uttar Pradesh, India
e-mail: sugarnet73@hotmail.com

K. Pramanik
Department of Chemistry, Inorganic Chemistry Section,
Jadavpur University,
Jadavpur, Kolkata 700 032, India

Introduction

Development of "Glycomics" has already opened up a new definition of life beyond genome and proteome. It is well understood and recognized that the carbohydrates are responsible for various biological processes beyond its energy storage, structural and protective roles. For long, natural products containing carbohydrates such as saponins, flavonoids have created a great deal of interest as potential drug candidates for many diseases. Most of them are used as folk medicines and their exact properties are not well defined until recently. But the scarcity of these active constituents in the natural sources and difficulty of purification is causing a damaging effect on the development in this area. Therefore, synthetic approach becomes eminent step forward to extrapolate Mother Nature's resources for the betterment of humanity.

Triterpenoid saponins are glycosylated plant secondary metabolites that are found in many major food crops [1], synthesized by numerous plants as part of their normal programme of growth and development. Examples include plants that are exploited as sources of drugs, such as ginseng and liquorice, and also crop plants, such as legumes and oats [2]. Since many saponins have potent antifungal properties and are present in healthy plants in high concentration, these molecules may act as preformed chemical barriers to fungal attack [3]. Despite commercial interest, the genetic machinery required for the elaboration of this important family of plant secondary metabolites is as yet largely uncharacterized. One common feature of all saponins is the presence of a sugar chain attached to the aglycone at the C-3 hydroxyl position. The sugar chains differ substantially between saponins, but are often branched and may consist of up to five sugar units (usually selected from glucose, arabinose, glucuronic acid, xylose or

rhamnose) [4]. An understanding of the glycosylation process, which is believed to be the terminal stage in the saponin biosynthesis, is important since the presence of the C-3 sugar chain is critical for saponin biological activity [5, 6]. To obtain a better understanding of the glycosyltransferases involved and in order to establish the order of events in saponin biosynthesis, synthetic saccharide fragments would be very useful.

The Chinese plant *Alizibia julibrissin* (Leguminosae) is well known as an anti-inflammatory and sedative drug for treating skin ulcers, wounds or swelling and pain of the lungs [7]. Attracted by the medicinal value of the plant, Zhao *et al.* have analyzed different extracts from the bark of *A. julibrissin* and isolated a cytotoxic triterpenoid saponin "Julibroside J_{28}" (Schemes 1 and 2) which showed significant *in vitro* inhibitory activity against human tumor cell lines (HeLa, PC-3M-1E8 and Bel-7402) [8]. In continuation to our constant effort towards synthesis of carbohydrate containing natural products, here we report a simple and convergent route for the synthesis of the tetrasaccharide (**1**) and trisaccharide (**2**) portions of the natural product.

From the retrosynthetic analyses of the target tetrasaccharide, it is evident that the construction of a protected trisaccharide (**II**) and glycosylation with a suitably protected glucoside acceptor (**I**) would provide the target tetrasaccharide. The trisaccharide **II** could be prepared from the orthogonally protected monosaccharide building blocks (**III**, **IV** and **V**) derived from commercially available D-glucose, L-rhamnose and L-arabinose.

Results and discussion

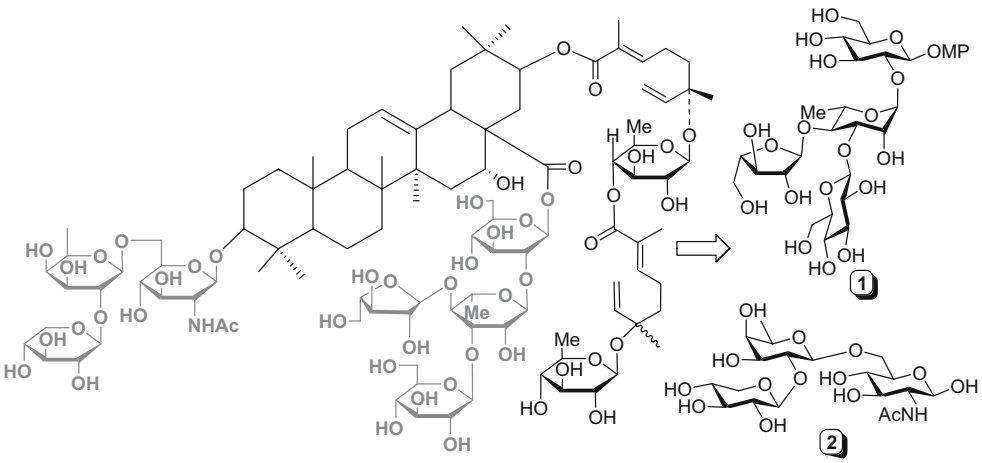
The synthesis starts from known *p*-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**3**) [9, 10]. The choice of the *p*-methoxyphenyl group is to serve as a temporary glycoside that can be converted to other activating glycosides such as trichloroacetimidate or thioglycosides for the final conjugation with the glucoside moiety. Compound **3** was coupled with known arabinofuranosyl donor **4** [11] using *N*-iodosuccinimide in conjunction with sulfuric acid immobilized on silica (H_2SO_4-silica) [12–15][1] to afford the disaccharide **5** in 87% yield. The use of H_2SO_4-silica instead of TfOH or TMSOTf as the acid source for the *N*-iodosuccinimide promoted activation of thioglycoside is particularly beneficial since it is a solid and can be weighed in small quantity. Moreover, it replaces the anxiety of using perchloric acid immobilized on silica ($HClO_4$-silica) as perchloric acid is known to be a potential explosive. Once the disaccharide **5** is in hand, the 2,3-isopropylidene acetal was cleaved with 80% aqueous acetic acid at 80°C to provide the corresponding diol (**6**) in 95% yield. Orthoesterification of the diol **6** with trimethyl orthoacetate and 10-camphorsulfonic acid in dry acetonitrile followed by rearrangement of the orthoester by aqueous work-up with 1N HCl afforded the disaccharide acceptor **7** in 86% yield. *N*-iodosuccinimide and H_2SO_4-silica promoted glycosylation of the disaccharide acceptor **7** with known *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**8**) [16] furnished the trisaccharide (**9**) in 91% yield. The next challenge was to convert the *p*-methoxyphenyl trisaccharide to an activated donor for the final coupling. Oxidative cleavage of the *p*-methoxyphenyl group with ceric ammonium nitrate [17] in acetonitrile-water (9:1) afforded the trisaccharide hemiacetal as anomeric mixture which was then converted to the corresponding trichloroacetimidate donor (**10**) in 78% overall yield using trichloroacetonitrile and DBU [18].

In a separate experiment, known *p*-methoxyphenyl 4,6-benzylidene-β-D-glucopyranoside (**11**) [19, 20] was selectively benzoylated at the 3-position using benzoyl chloride in CH_2Cl_2-pyridine mixture at -50°C which afforded 2,3-di-*O*-benzoyl, 3-*O*-benzoyl, 2-*O*-benzoyl and unreacted **11** in a ratio of 1:5:2:1. The required 3-*O*-benzoyl derivative **12** was isolated in 55% yield after chromatography. The final glycosylation was performed by the activation of trichloroacetimidate donor **10** with H_2SO_4-silica at room temperature to afford the protected tetrasaccharide **13** in 82% yield. Again H_2SO_4-silica proved to be an excellent promoter for trichloroacetimidate activation in oligosaccharide synthesis. Finally, removal of the benzylidene acetal with 80% AcOH at 80°C [21] followed by Zemplén de-*O*-acylation afforded the target tetrasaccharide **1** in 85% yield (Scheme 3). Stereochemistry of all newly formed glycosidic bonds were further ascertained using proton coupled carbon NMR and gave satisfactory results in favour of the desired stereochemistry.

Synthesis of the trisaccharide **2** was first planned by simple one-to-one glycosylation of three different monosaccharide building blocks with orthogonal protection-deprotection route. Thus, known 2-acetamido-2-deoxy-6-*O*-trityl D-glucopyranose (**14**) [22, 23] was per-benzoylated with benzoyl chloride in pyridine and subsequently the trityl group was removed using 90% TFA in CH_2Cl_2 to afford the acceptor **15** in 78% yield. Chloroacetylation of known *p*-tolyl 3,4-*O*-isopropylidene-1-thio-β-D-fucopyranose (**16**) [24, 25] with chloroacetic anhydride in pyridine provided the donor **17** in 89% yield. Glycosylation between acceptor **15** and donor **17** using *N*-

[1] Preparation of H_2SO_4–silica: To a slurry of silica gel (10 g, 200–400 mesh) in dry diethyl ether (50 mL) was added commercially available conc. H_2SO_4 (1 mL) and the slurry shaken for 5 min. The solvent was evaporated under reduced pressure resulting in free flowing H_2SO_4-silica which was dried at 110°C for 3 h and used for the reactions. For use in various carbohydrate reactions see: Rajput *et al.* [12]; Rajput and Mukhopadhyay [13]; Mukhopadhyay [14] and Roy and Mukhopadhyay [15].

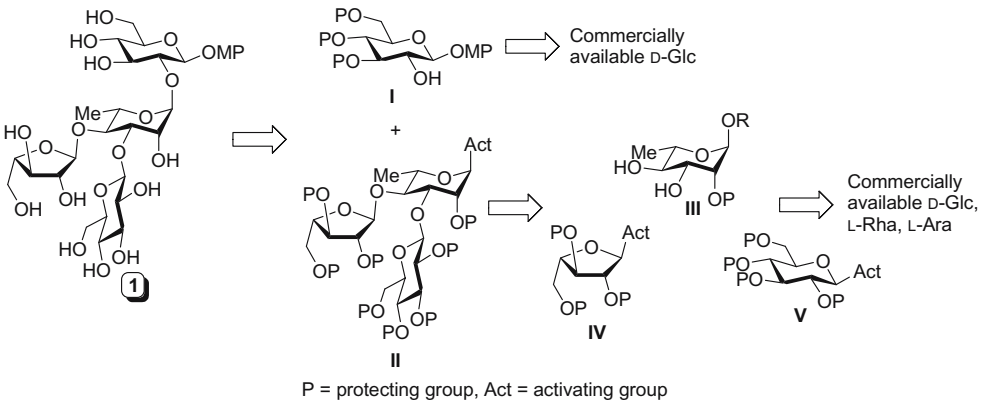
Scheme 1 Structure of "Julibroside J_{28}" and synthetic target oligosaccharides



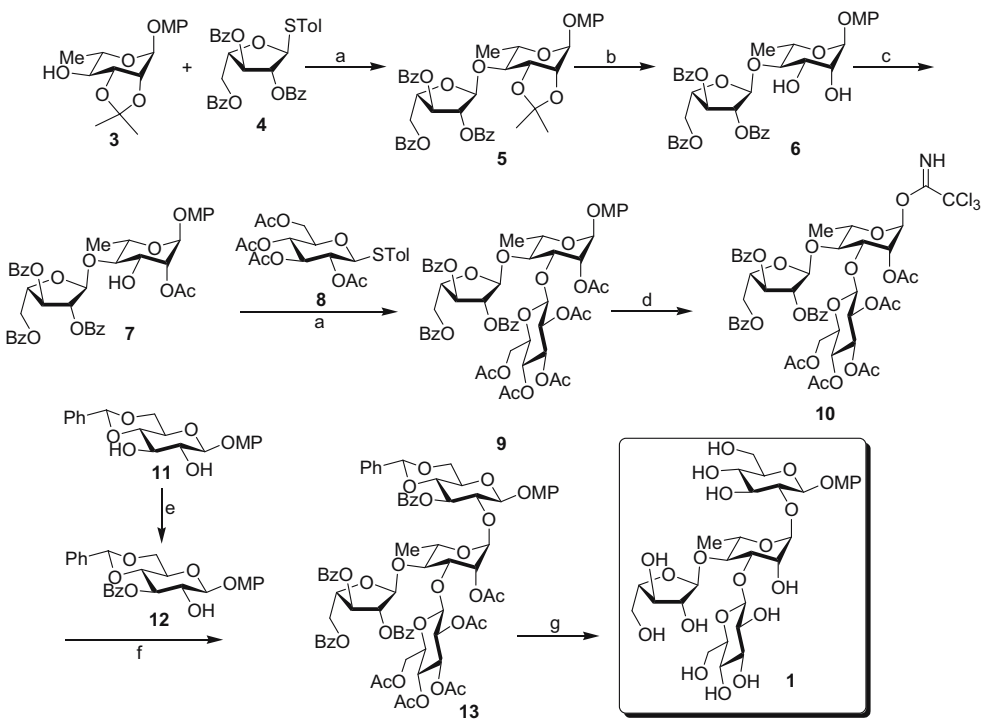
iodosuccinimide and H_2SO_4-silica afforded the disaccharide **18** in 87% yield. But at this stage selective cleavage of the chloroacetyl group with thiourea in methanol–CH_2Cl_2 (2:3) mixture in the presence of 2,4,6-collidine [26] failed to produce the desired disaccharide acceptor **19**. Even after 24 h reflux with 15-fold excess of thiourea only negligible product was evident by TLC and the resulted product was impossible to purify and characterize (Schemes 4, 5). It is worth noting that hydrazine acetate also used to remove the chloroacetyl group but resulted in an inseparable mixture of compounds possibly due to uneven loss of other acyl protections. It may be postulated that there is some unusual steric hindrance arising from the adjacent 4-*O*-benzoyl group of the glucosamine moiety or some hydrogen bonding that stabilizing the chloroacetyl protection.

From the difficulties faced for the selective deprotection of the 2-position of glycosylated D-fucose moiety, it was anticipated that the glycosylation of protected D-xylose-(1→2)-D-fucose disaccharide to the primary hydroxyl of the glucosamine unit may resolve the problem. Thus, known 2,3,4-tri-*O*-acetyl-D-fucopyranose (**20**) [27] was converted into the corresponding *p*-methoxyphenyl glycoside (**21**) in 78% yield. De-*O*-acetylation followed by 3,4-*O*-isopropylidene acetal formation using 2,2-dimethoxy-propane and 10-camphorsulfonic acid in dry acetone afforded the required acceptor **22** in 87% overall yield. *N*-iodosuccinimide and H_2SO_4-silica promoted coupling of known methyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside (**23**)[11] at the 2-position of the D-fucose moiety furnished the disaccharide **24** in 83% yield. The isopropylidene acetal was removed using 80% AcOH at 80°C followed by acetylation of the di-ol with Ac_2O in pyridine to afford disaccharide **25** in 81% yield. Oxidative cleavage of the reducing end *p*-methoxyphenyl glycoside with ceric ammonium nitrate followed by the reaction with trichloroacetonitrile and DBU afforded the corresponding α-trichloroacetimidate donor **26** in 78% yield over two steps. H_2SO_4–silica promoted glycosylation of the trichloroacetimidate donor with glucosamine acceptor **15** went smoothly to furnish the protected trisaccharide **27** in 76% yield. Exclusive formation of the required β-linkage was affirmed by ^1H and ^{13}C NMR. Although neighboring group participation effect is not available in this case, but the reaction condition and the presence of a bulky xylose moiety at the 2-position of the D-fucose drive the reaction to 1,2-*trans* glycosylation. Moreover, activation of the α-trichloroacetimidate also favoured the S_N2 attack of the reactive 6-hydroxy group of the acceptor moiety. Zemplén de-*O*-acylation using NaOMe in MeOH afforded the target trisaccharide **2** in 83% yield.

Scheme 2 Retrosynthetic analysis for the tetrasaccharide (**1**)



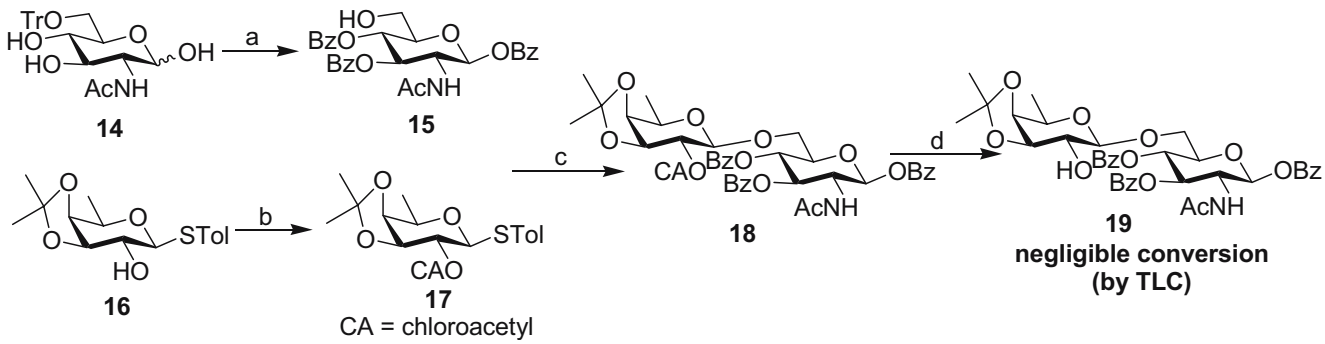
Scheme 3 Synthesis of the tetrasaccharide **1**



Reagents and Conditions: a. NIS, H_2SO_4-silica, CH_2Cl_2, 15°C, 30 min., 87%; b. 80% AcOH, 80°C, 3 h, 95%; c. (i). Trimethyl orthoacetate, CSA, CH_3CN, 45 min. (ii). 1N HCl, aq. work-up, 86%; d. (i). CAN, CH_3CN-H_2O 9:1, rt, 1.5 h, (ii). CCl_3CN, DBU, CH_2Cl_2, 45 min., 78% overall; e. BzCl, Py (2 eqv.), CH_2Cl_2, -50 °C, 3 h, 55%; f. H_2SO_4-silica, CH_2Cl_2, rt, 30 min., 82%; g. (i). 80% AcOH, 80 °C, 3 h, (ii). NaOMe, MeOH, rt, 8 h, 85% overall.

Conclusion

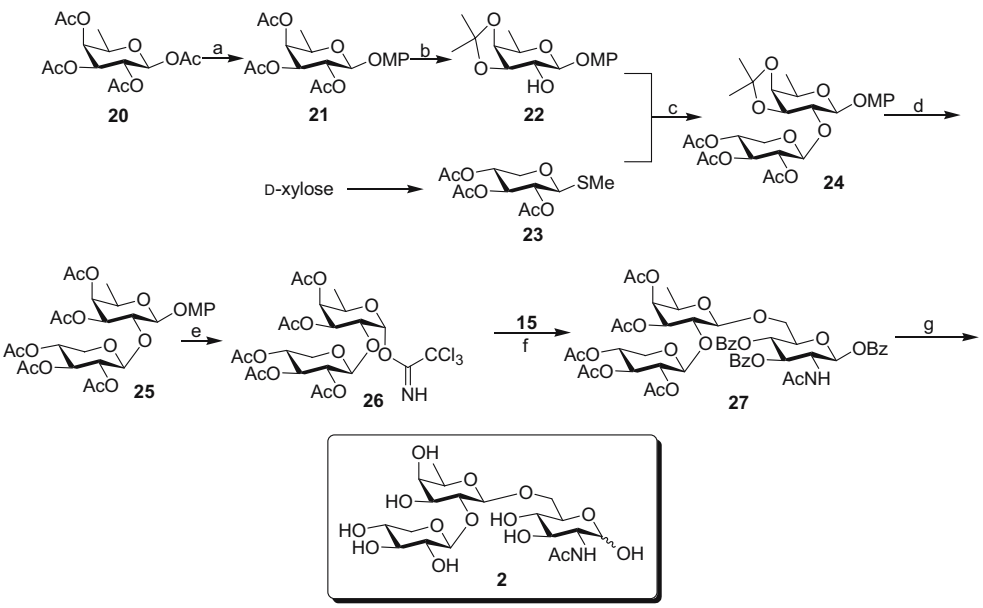
In conclusion, we have synthesized the glycone parts of the triterpenoid saponin "Julibroside J_{28}" isolated from *Albizia julibrissin* in the form of the corresponding *p*-methoxyphenyl glycoside for the tetrasaccharide that can easily be removed and converted into its trichloroacetimidate for glyco-conjugation with various aglycons. For the trisaccharide, we have prepared the fully acylated derivative in which the anomeric benzoate can be removed and used for further conjugation as indented. During the synthetic process, we have utilized H_2SO_4 immobilized on silica as an alternative promoter instead of TfOH or TMSOTf which are toxic, expensive and difficult to handle. The catalyst proved to be general for the activation of thioglycosides (in conjunction with NIS) and trichloroacetimidates. The yields



Reagents and Conditions: a. (i). BzCl, Py, rt, 4 h, (ii). 90% aq. TFA, CH_2Cl_2, 1 h, 78%; b. CA_2O, Py, -20 °C, 45 min., 89%; c. NIS, H_2SO_4-silica, CH_2Cl_2, 15 °C, 45 min, 87%; d. Thiourea, 2,4,6-collidine, MeOH-CH_2Cl_2 (2:3), reflux, 24 h, negligible

Scheme 4 Synthesis of the disaccharide acceptor **19**

Scheme 5 Synthesis of the trisaccharide 2



Reagents and Conditions: a. *p*-methoxyphenol, $BF_3.Et_2O$, CH_2Cl_2, 0 °C, 3h, 78%; b. (i). NaOMe, MeOH, rt, 2h, (ii). 2,2-DMP, CSA, acetone, rt, 1h, 87%; c. NIS, H_2SO_4-silica, CH_2Cl_2, 15 °C, 45 min, 83%; d. (i). 80% AcOH, 80 °C, 2h, (ii). Ac_2O, Py, rt, 2h, 81%; e. (i). CAN, CH_3CN-H_2O, rt, 45 min, (ii). CCl_3CN, DBU, CH_2Cl_2, rt, 1h, 78%; f. H_2SO_4-silica, CH_2Cl_2, rt, 2h; g. NaOMe, MeOH, rt, 6h.

are good to excellent in each glycosylation step and therefore, the total synthetic route is compatible with large scale requirement for further use.

Experimental

General All reagents and solvents were dried prior to use according to standard methods. [28] Commercial reagents were used without further purification unless otherwise stated. Analytical TLC was performed on Silica Gel 60-F_{254} with detection by fluorescence and/or by charring following immersion in a 10% ethanolic solution of sulfuric acid. An orcinol dip, prepared by the careful addition of concentrated sulfuric acid (20 ml) to an ice-cold solution of 3,5-dihydroxytoluene (360 mg) in EtOH (150 ml) and H_2O (10 ml), was used to detect deprotected compounds by charring. Flash chromatography was performed with Silica Gel 60. Optical rotations were measured at the sodium D-line at ambient temperature. ^{1}H NMR and ^{13}C NMR spectra were recorded on a spectrometer at 300 and 75 MHz. In case of the tetrasaccharide, ^{1}H NMR values are denoted as H for reducing end glucose, H′ for rhamnose, H″ for arabinofuranose and H‴ for the non-reducing end glucose. For ^{13}C NMR assignment, similar primes are used.

Analytical data recorded for the known compounds (**3**, **4**, **8**, **11**, **14**, **16**, **20** and **23**) were compared with the literature data and found satisfactory.

p-Methoxyphenyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranoside (5) A mixture of acceptor **3** (1.5 g, 4.8 mmol), donor **4** (2.9 g, 5.8 mmol) and activated MS 4Å (4 g) in dry CH_2Cl_2 (45 ml) was stirred under nitrogen atmosphere for 1 h. After cooling in ice-water bath (10–15°C), NIS (1.6 g, 7 mmol) was added followed by H_2SO_4–silica (50 mg) and the mixture was stirred for 45 min when TLC (*n*-hexane–EtOAc, 2:1) showed complete conversion of the acceptor spot. The mixture was filtered through celite® and the filtrate was diluted with CH_2Cl_2 (30 ml) and washed successively with aq. $Na_2S_2O_3$ (2×75 ml), aq. $NaHCO_3$ (2×75 ml) and brine (75 ml). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure disaccharide **5** as colourless foam (3.2 g, 87%). $[\alpha]_D^{25} + 36°(c\ 1.1,\ CHCl_3)$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.98–7.16 (m, 15 H, ArH), 6.86, 6.69 (2d, 4 H, *p*-MeOC_6H_4), 5.70 (s, 1 H, H-1′), 5.47 (s, 1 H, H-1), 5.45 (s, 1 H, H-2′), 5.41 (d, 1 H, *J* 4.5 Hz, H-3′), 4.67 (dd, 1 H, *J* 3.6 Hz, 12.0 Hz, H-5a′), 4.58 (dd, 1 H, *J* 5.4 Hz, 12.0 Hz, H-5b′), 4.43 (q, 1 H, *J* 4.5 Hz, H-4′), 4.35 (t, 1 H, *J* 6.3 Hz, H-3), 4.21 (d, 1 H, *J* 3.3 Hz, H-2), 3.79 (m, 1 H, H-5), 3.66 (s, 3 H, $C_6H_4OCH_3$), 3.61 (m, 1 H, H-4), 1.49, 1.25 (2 s, 6 H, isopropylidene-CH_3), 1.14 (d, 3 H, *J* 6.0 Hz, C–CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 165.8, 165.4, 165.0 (3 COC_6H_5), 155.0, 150.3, 133.4, 132.8, 130.1, 130.0, 129.9, 129.4, 129.3, 128.5, 128.2, 117.5, 114.6 (ArC), 109.8 (isopropylidene *C*), 104.2 (*C*-1′), 96.1 (*C*-1), 82.0 (*C*-2′),

81.9 (*C*-4′), 78.3 (*C*-3′), 78.2 (*C*-3), 76.4 (*C*-4), 76.1 (*C*-2), 64.9 (*C*-5), 64.0 (*C*-5′), 55.4 ($C_6H_4OCH_3$), 28.0, 26.6 (isopropylidene–CH_3), 17.9 (C-CH_3). HRMS calcd. for $C_{42}H_{46}O_{13}N$ (M+NH_4): 772.2969; found *m/z* 772.2972.

p-Methoxyphenyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1→4)-α-L-rhamnopyranoside (6) To a solution of disaccharide **5** (3 g, 4.0 mmol) in AcOH (40 ml), H_2O (10 ml) was added and the mixture was heated at 80°C for 2 h when TLC (3:1 *n*-hexane–EtOAc) showed complete conversion to a slower running component. Solvents were evaporated *in vacuo* and co-evaporated with toluene. Flash chromatography (5:1 *n*-hexane–EtOAc) of the crude product thus obtained, afforded pure disaccharide **6** as light yellow glass (2.7 g, 95%). $[\alpha]_D^{25} + 48°$(*c* 0.9, *CHCl*$_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 8.02–7.31 (m, 15 H, ArH), 6.98, 6.81 (2d, 4 H, *p*-MeOC_6H_4), 5.66 (s, 1 H, H-1′), 5.64 (d, 1 H, *J* 2.1 Hz, H-3′), 5.46 (bs, 1 H, H-2′), 5.36 (s, 1 H, H-1), 4.78 (dd, 1 H, *J* 3.6 Hz, 12.0 Hz, H-5a′), 4.66 (m, 2 H, H-5b′, H-4′), 4.11 (dd, 1 H, *J* 3.0 Hz, 9.0 Hz, H-3), 4.02 (bs, 1 H, H-2), 3.75 (m, 1 H, H-5), 3.65 (s, 3 H, $C_6H_4OCH_3$), 3.59 (m, 1 H, H-4), 3.21 (bs, 1 H, O*H*), 1.32 (d, 3 H, *J* 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*:166.7, 166.0, 165.5 (3 COC_6H_5), 154.8, 150.3, 133.8, 133.6, 133.0, 130.1, 130.0, 129.9, 129.7, 129.6, 128.9, 128.6, 128.5, 128.3, 117.4, 114.5 (ArC), 107.4 (*C*-1′), 98.1 (*C*-1), 84.2, 80.2, 80.0, 76.6, 71.7, 71.1, 67.0, 63.8 (*C*-5′), 55.5 ($C_6H_4OCH_3$), 17.8 (C–CH_3). HRMS calcd. for $C_{39}H_{42}O_{13}N$ (M+NH_4): 732.2656; found *m/z* 732.2659.

p-Methoxyphenyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1→4)-2-O-acetyl-α-L-rhamnopyranoside (7) To a solution of **6** (2.5 g, 3.5 mmol) in dry CH_3CN (2.5 ml), trimethyl orthoacetate (585 μl, 4.6 mmol) was added followed by CSA (50 mg) and the mixture was allowed to stir at room temperature until complete conversion of the starting material was evident by TLC (3:1 *n*-hexane–EtOAc). After 45 min, the solution was neutralized by Et_3N and evaporated *in vacuo*. The resulting syrupy mass was dissolved in CH_2Cl_2 (30 ml) and washed successively with 1N HCl (3×50 ml) to rearrange the orthoester to corresponding 2-*O*-acetate, followed by aq. $NaHCO_3$ (2× 50 ml) and brine (50 ml). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography (4:1 *n*-hexane–EtOAc) to give pure disaccharide acceptor **7** (2.3 g, 86%) as colourless foam. $[\alpha]_D^{25} + 33°$(*c* 1.1, *CHCl*$_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 7.99–7. 18 (m, 15 H, ArH), 6. 84, 6.66 (2d, 4 H, *p*-MeOC_6H_4), 5.58 (s, 1 H, H-1′), 5.53 (bd, 1 H, *J* 2.1 Hz, H-3′), 5.33 (bs, 1 H, H-2′), 5.19 (s, 1 H, H-1), 5.17 (bs, 1 H, H-2), 4.67 (bd, 1 H, *J* 11. 2 Hz, H-5a′), 4.52 (m, 2 H, H-5b′, H-4′), 4.29 (dd, 1 H, *J* 2.7 Hz, 9.3 Hz, H-3), 3.79 (m, 1 H, H-5), 3.61 (s, 3 H, $C_6H_4OCH_3$), 3.59 (m, 1 H, H-4), 3.49 (bs, 1 H, O*H*), 2.03 (s, 3 H, $COCH_3$), 1.22 (d, 3 H, *J* 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 170.1 ($COCH_3$), 166.4, 165.7, 165.3 (3 COC_6H_5), 155.0, 150.2, 133. 6, 133.5, 132.9, 130.1, 129.9, 129.8, 129.7, 129.0, 128.8, 128.5, 128.3, 128.2, 128.1, 117.6, 114.5 (ArC), 107.4 (*C*-1′), 96.4 (*C*-1), 84.0, 80.2, 79.8, 77.4, 72.4, 69.9, 67.2, 63.7 (*C*-5′), 55.3 ($C_6H_4OCH_3$), 20.9 ($COCH_3$), 17.9 (C-CH_3). HRMS calcd. for $C_{41}H_{44}O_{14}N$ (M+NH_4): 774.2762; found *m/z* 774.2765.

p-Methoxyphenyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1→4)-2-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (9) A mixture of disaccharide acceptor **7** (1.5 g, 2.0 mmol), donor **8** (1.2 g, 2.6 mmol) and activated MS 4Å (2 g) in dry CH_2Cl_2 (25 ml) was stirred under nitrogen atmosphere for 1 h. After cooling in a ice-water bath (10–15°C), NIS (702 mg, 3.1 mmol) was added followed by H_2SO_4–silica (25 mg) and the mixture was stirred for 45 min when TLC (*n*-hexane–EtOAc, 2:1) showed complete conversion of the acceptor spot. The mixture was filtered through celite® and the filtrate was diluted with CH_2Cl_2 (30 ml) and washed successively with aq. $Na_2S_2O_3$ (2×50 ml), aq. $NaHCO_3$ (2×50 ml) and brine (50 ml). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure trisaccharide **9** as colourless foam (2.0 g, 91%). $[\alpha]_D^{25} + 49°$(*c* 1.0, *CHCl*$_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 8.11–7. 02 (m, 15 H, ArH), 7.00, 6.83 (2d, 4 H, *p*-MeOC_6H_4), 5.73 (s, 1 H, H-1′), 5.68 (bd, 1 H, *J* 3.6 Hz, H-3′), 5.38 (bs, 1 H, H-2′), 5.32 (m, 2 H, H-1, H-2), 5.30 (t, 1 H, *J* 9.6 Hz, H-4″), 5.08 (d, 1 H, *J* 7.8 Hz, H-1″), 5.07 (t, 1 H, *J* 9.6 Hz, H-3″), 4.93 (dd, 1 H, *J* 7.8 Hz, 9.6 Hz, H-2″), 4.85 (dd, 1 H, *J* 2.1 Hz, 11.2 Hz, H-5a′), 4.74-4.66 (m, 2 H, H-4′, H-5b′), 4.51 (m, 1 H, H-3), 4.25 (m, 2 H, H-6a″, H-6b″), 3.96–3.88 (m, 3 H, H-4, H-5, H-5″), 3.78 (s, 3 H, $C_6H_4OCH_3$), 2.14, 2.13, 2.11, 1.89, 1.48 (5 s, 15 H, 5 $COCH_3$), 1.34 (d, 3 H, *J* 6.0 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) i: 170.6, 170.1, 169.7, 169.5, 168.6 (5 $COCH_3$), 166.0, 165.6, 165.5 (3 COC_6H_5), 155.1, 150.1, 133. 6, 133.5, 133.1, 130.1, 129.7, 129.6, 129.5, 128.8, 128.7, 128.5, 128.3, 128.1, 117.8, 114.5 (ArC), 105.8 (*C*-1′), 99.7 (*C*-1″), 96.6 (*C*-1), 82.3, 82.0, 78.0, 76.5, 74.4, 72.7, 72.2, 71.6, 71.4, 69.1, 67.2, 63.6 (*C*-5′), 61.7 (*C*-6″), 55.5 ($C_6H_4OCH_3$), 20.8, 20.6, 20.5, 20.4(2) (5 $COCH_3$), 18.1 (C-CH_3). HRMS calcd. for $C_{55}H_{62}O_{23}N$ (M+NH_4): 1104.3713; found *m/z* 1104.3717.

p-Methoxyphenyl 3-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranoside (12) To a suspension of compound **11** (3.0 g, 8 mmol) in dry CH_2Cl_2 (30 ml), pyridine (4 ml) was added and the mixture was stirred at −50°C under nitrogen atmosphere for 30 min. Then BzCl (1.0 ml, 8.8 mmol)

was added and stirring continued at −50°C for 3 h. MeOH (2 ml) was added to quench the reaction and the solvents were evaporated *in vacuo*. The crude product was purified by flash chromatography to give pure **12** (2.1 g, 55%) as white foam. $[\alpha]_D^{25} + 79°(c\ 1.2,\ CHCl_3)$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.10-7.31 (m, 10 H, ArH), 7.05, 6.83 (2d, 4 H, *p*-MeOC_6H_4), 5.56 (t, 1 H, *J* 9.3 Hz, H-3), 5.54 (s, 1 H, C*H*C_6H_5), 5.04 (d, 1 H, *J* 7.5 Hz, H-1), 4.40 (dd, 1 H, *J* 4.8 Hz, 10.5 Hz, H-6a), 4.00 (*t*, 1 H, *J* 9.3 Hz, H-4), 3.92–3.83 (m, 2 H, H-2, H-6b), 3.78 (s, 3 H, $C_6H_4OCH_3$), 3.66 (m, 1 H, H-5). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 165.4 (3 *C*OC_6H_5), 154.6, 149.6, 135.5, 132.4, 132.1, 128.9, 128.7, 128.4, 128.1, 127.8, 127.2, 127.1, 126.9, 125.0, 124.9, 117.6, 113.4 (ArC), 101.7 (*C*HC_6H_5), 100.3 (*C*-1), 77.1, 73.2, 72.2, 67.4, 65.4 (*C*-6), 54.4 ($C_6H_4OCH_3$). HRMS calcd. for $C_{27}H_{30}O_8N$ (M+NH_4): 496.1971; found *m*/*z* 496.1973.

p-Methoxyphenyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1→4)-2-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-v-glucopyranosyl)-α-L-rhamnopyranosyl-(1→2)- 3-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranoside (13) To a solution of compound **9** (1.8 g, 1.65 mmol) in CH_3CN–H_2O (9:1, 30 ml) was added CAN (1.8 g, 3.3 mmol) and the mixture was stirred at room temperature for 45 min. The solvents were evaporated and the residue was dissolved in CH_2Cl_2 (30 ml) and washed with H_2O (2×50 ml); organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. It was re-dissolved in dry CH_2Cl_2 (20 ml), CCl_3CN (500 μl, 5.0 mmol) was added followed by DBU (270 μl, 1.8 mmol) and the mixture was stirred at room temperature for 1 h. After that, solvents were evaporated and the residue was charged directly to a flash column and eluted with *n*-hexane–EtOAc (2:1) to afford pure trichloroacetimidate donor **10** (1.4 g, 78%). Next, a mixture of acceptor **12** (480 mg, 1.0 mmol), trisaccharide donor **10** (1.1 g, 1.0 mmol) and activated MS 4Å (1 g) in dry CH_2Cl_2 (15 ml) was stirred under nitrogen atmosphere for 1 h. H_2SO_4-silica (25 mg) was added and the mixture was stirred for 30 min when TLC (*n*-hexane–EtOAc, 2:1) showed complete consumption of the acceptor. The mixture was filtered through celite® and the filtrate was diluted with CH_2Cl_2 (20 ml) and washed successively with aq. $NaHCO_3$ (2×30 ml) and brine (30 ml). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography using *n*-hexane–EtOAc (2:1) to afford pure protected tetrasaccharide **13** (1.2 g, 82%) as colourless foam. $[\alpha]_D^{25} + 66°(c\ 1.0,\ CHCl_3)$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.07–7.26 (m, 25 H, ArH), 7.03, 6.79 (2d, 4 H, OC_6H_4OMe), 5.64 (t, 1 H, *J* 9.0 Hz, H-3), 5.65 (s, 1 H, H-1′), 5.54 (bd, 1 H, *J* 3.6 Hz, H-2″), 5.51 (s, 1 H, C*H*Ph), 5.44 (s, 1 H, H-1″), 5.12 (bt, 2 H, *J* 9.6 Hz, H-3″′, H-4‴), 5.07 (d, 1 H, *J* 7.8 Hz, H-1‴), 4.95 (m, 2 H, H-1, H-2‴), 4.81 (m, 2 H, H-3″, H-4″), 4.64 (m, 3 H, H-2, H-5a″, H-5b″), 4.41 (dd, 1 H, *J* 1.8 Hz, 6.3 Hz, H-3′), 4.17 (m, 4 H, H-4′, H-5, H-6a‴, H-6b‴), 3.99–3.78 (m, 4 H, H-5′, H-5‴, H-6a, H-6b), 3.71 (s, 3 H, $C_6H_4OCH_3$), 2.06, 2.02, 1.86, 1.85, 1.56 (5 s, 15 H, 5 COCH_3), 1.33 (d, 3 H, *J* 6.3 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.7, 169.9, 169.4 (2), 168.6 (5×*C*OCH_3), 166.1, 165.6, 165.5, 165.3 (4×*C*OC_6H_5), 155.6, 150.8, 136.7, 133.6, 133.4, 133.3, 133.1, 130.1, 129.9, 129.8, 129.7, 129.6, 129.0, 128.9, 128.5, 128.3, 128.2, 126.1, 118.1, 114.6 (ArC), 105.9 (C-1″), 101.5 (*C*HPh), 101.2 (C-1‴), 99.4 (C-1), 98.3 (C-1′), 82.3, 81.8, 78.4, 78.2, 74.1, 73.8, 72.8, 71.4, 71.3, 71.1, 68.6, 68.3, 67.5, 66.3, 63.8 (C-6), 61.0 (C-6″), 55.6 ($C_6H_4OCH_3$), 22.7, 20.7, 20.6, 20.5, 18.1 (5×CO*C*H_3), 14.1 (C-*C*H_3). HRMS calcd. for $C_{75}H_{80}O_{29}N$ (M+NH_4): 1458.4816; found *m*/*z* 1458.4818.

p-Methoxyphenyl α-L-arabinofuranosyl-(1→4)-3-O-(β-D-glucopyranosyl)-α-L-rhamnopyranosyl-(1→2)- β-D-glucopyranoside (1) To a solution of compound **13** (1.0 g, 0.7 mmol) in AcOH (16 ml), H_2O (4 ml) was added and the suspension was stirred at 80°C for 3 h when TLC (1:1 *n*-hexane–EtOAc) showed complete conversion of the starting material to a slower running component. Solvent were evaporated *in vacuo* and co-evaporated with toluene. The resulting syrupy mass was dissolved in dry MeOH followed by NaOMe and stirred at room temperature for 8 h. The solution was neutralized with DOWEX 50 W H^+ resin, filtered and evaporated to afford pure target tetrasaccharide **1** (432 mg, 85%) as white foam. $[\alpha]_D^{25} + 23°(c\ 1.0,\ H_2O)$; ^{1}H NMR ($D_2O$, 300 MHz) δ: 6.97, 6.86 (2d, 4 H, ArH), 5.17 (bs, 1 H, H-1″), 5.07 (s, 1 H, H-1′), 5.06 (d, 1 H, *J* 6.3 Hz, H-1‴), 4.47 (d, 1 H, *J* 6.6 Hz, H-1), 3.64 (s, 3 H, $C_6H_4OCH_3$), 1.13 (d, 3 H, *J* 6.0 Hz, C-CH_3). ^{13}C NMR (D_2O, 75 MHz) δ: 153.3, 149.8, 116.3, 113.9 (ArC), 108.6 (C-1″), 102.8 (C-1), 99.5 (C-1‴), 98.0 (C-1′), 82.4, 80.8, 78.9, 77.4, 76.2, 75.4, 75.2, 75.0, 74.7, 72.2, 68.8, 68.5, 66.6, 60.1 (C-6‴), 59.4 (C-6), 54.6, 47.6 ($C_6H_4OCH_3$), 15.7 (C-*C*H_3). HRMS calcd. for $C_{30}H_{46}O_{20}Na$ (M+Na): 749.2480; found *m*/*z* 749.2482.

Benzoyl 2-acetamido-2-deoxy-3,4-di-O-benzoyl-β-D-glucopyranose (15) To a cold solution (Ice-bath) of compound **14** (2 g, 4.3 mmol) in dry pyridine (30 ml) was added BzCl (1.8 ml, 15.5 mmol) and the solution was allowed to stir for 4 h with slow warming up to room temperature. Then MeOH (3 ml) was added to quench the reaction and solvents were evaporated *in vacuo*. The resulting semi-solid mass was dissolved in CH_2Cl_2 (50 ml) and washed successively with ice cold 1N HCl (2×50 ml), aq. $NaHCO_3$ (2×50 ml) and brine (50 ml). Organic layer was collected, dried (Na_2SO_4) and evaporated. The residue was dissolved in CH_2Cl_2 (30 ml) followed by addition of 90% aq. TFA (10 ml) and allowed to stir at room temperature till TLC

showed complete conversion of the starting material to a slower running spot (1 h). The solution was diluted with CH_2Cl_2 (20 ml) and washed with H_2O (2×50 ml), aq. $NaHCO_3$ (2×50 ml) and brine (50 ml). Organic layer was collected, dried (Na_2SO_4) and purified by flash chromatography after evaporation to afford pure **15** (1.8 g, 78%) as white amorphous powder. $[\alpha]_D^{25} + 106°(c\ 1.0,\ CHCl_3)$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.20–7. 36 (m, 15 H, ArH), 6.60 (d, 1 H, *J* 3.6 Hz, N*H*), 6.07 (d, 1 H, *J* 8.7 Hz, H-1), 5.91 (dd, 1 H, *J* 9.9 Hz, 10.8 Hz, H-3), 5.70 (t, 1 H, *J* 9.9 Hz, H-4), 4.84 (dt, 1 H, *J* 3.6 Hz, 8.7 Hz, 9.9 Hz, H-2), 4.15 (m, 1 H, H-5), 3.79 (dd, 1 H, *J* 2.1 Hz, 12.9 Hz, H-6a), 3.71 (dd, 1 H, *J* 3.9 Hz, 12.9 Hz, H-6b), 1.83 (s, 3 H, NHCOC*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.3 (NH*C*OCH$_3$), 167.4, 165.7, 164.4 (3 *C*OC$_6$H$_5$), 134.0, 133.7, 133.6, 129.9, 129.8, 128.8, 128.5, 128.4 (ArC), 91.5 (*C*-1), 72.8, 71.1, 68.5, 60.9 (*C*-6), 51.8 (*C*-2), 22.8 (NHCO*C*H$_3$). HRMS calcd. for $C_{29}H_{31}O_9N_2$ (M+NH_4): 551.2030; found *m*/*z* 551.2032.

p-Tolyl 2-O-chloroacetyl-3,4-O-isopropylidene-1-thio-β-D-fucopyranoside (17) A solution of compound **16** (2.5 g, 8 mmol) in dry CH_2Cl_2 (25 ml) and pyridine (5 ml) was cooled to −20°C for 30 min. Chloroacetic anhydride (760 mg, 9.6 mmol) was added and the solution was stirred at the same temperature for 45 min. After addition MeOH (2 ml) to quench the reaction, solvents were evaporated and the residue was purified through flash using 4:1 *n*-hexane–EtOAc as eluent to afford pure **17** (2.75 g, 89%) as light yellow foam. $[\alpha]_D^{25} + 72°(c\ 1.1,\ CHCl_3)$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.35, 7.08 (2d, 4 H, ArH), 4.95 (dd, 1 H, *J* 7.2 Hz, 10.2 Hz, H-2), 4.46 (d, 1 H, *J* 7.2 Hz, H-1), 4.11 (dd, 1 H, *J* 10.2 Hz, 5.4 Hz, H-3), 4.09 (s, 2 H, COC*H*$_2$Cl), 4.01 (dd, 1 H, *J* 2.1 Hz, 5.4 Hz, H-4), 3.83 (m, 1 H, H-5), 2.34 (s, 3 H, SC$_6$H$_5$C*H*$_3$), 1.49, 1.32 (2 s, 6 H, isopropylidene–C*H*$_3$), 1.41 (d, 3 H, *J* 6.6 Hz, C-C*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 165.8 (*C*OCH$_2$Cl), 138.0, 133.3, 129.7 (ArC), 110.4 (isopropylidene *C*), 85.5 (*C*-1), 77.2, 76.4, 73.4, 72.8, 40.8 (CO*C*H$_2$Cl), 27.8, 26.6 (isopropylidene–*C*H$_3$), 21.3 (SC$_6$H$_5$*C*H$_3$), 16.9 (C-*C*H$_3$). HRMS calcd. for $C_{18}H_{27}O_5N_2ClS$ (M+NH_4): 404.1298; found *m*/*z* 404.1295.

Benzoyl 2-O-chloroacetyl-3,4-O-isopropylidene-β-D-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-3,4-di-O-benzoyl-β-D-glucopyranose (18) A mixture of acceptor **15** (1.5 g, 2.8 mmol), donor **17** (1.3 g, 3.4 mmol) and activated MS 4Å (2 g) in dry CH_2Cl_2 (25 ml) was stirred under nitrogen atmosphere for 1 h. After cooling in a ice-water bath (10–15°C), NIS (920 mg, 4.1 mmol) was added followed by H_2SO_4-silica (40 mg) and the mixture was stirred for 45 min when TLC (*n*-hexane–EtOAc, 3:1) showed complete conversion of the acceptor spot. The mixture was filtered through celite® and the filtrate was diluted with CH_2Cl_2 (30 ml) and washed successively with aq. $Na_2S_2O_3$ (2×50 ml), aq. $NaHCO_3$ (2×50 ml) and brine (50 ml). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure trisaccharide **18** (1.9 g, 87%) as colourless foam. $[\alpha]_D^{25} + 47°(c\ 1.0,\ CHCl_3)$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.17–7.26 (m, 15 H, ArH), 6.54 (d, 1 H, *J* 3.6 Hz, N*H*), 5.94 (d, 1 H, *J* 8.7 Hz, H-1), 5.77 (t, 1 H, *J* 9.6 Hz, H-3), 5.65 (t, 1 H, *J* 9.6 Hz, H-4), 5.58 (d, 1 H, *J* 5.4 Hz, H-1′), 4.75 (dt, 1 H, *J* 3.6 Hz, 8.7 Hz, 9.6 Hz, H-2), 4.50 (dd, 1 H, *J* 1.5 Hz, 9.6 Hz, H-3′), 4.37 (bd, *J* 5.7 Hz, H-4′), 4.26 (m, 1 H, H-5), 4.08–3.99 (m, 2 H, H-2′, H-5′), 3.73 (m, 2 H, H-6a, H-6b), 3.71 (d, 2 H, *J* 3.0 Hz, OCOC*H*$_2$Cl), 1.79 (s, 3 H, NHCOC*H*$_3$), 1.42, 1.31 (isopropylidene–C*H*$_3$), 1.19 (d, 3 H, *J* 6.3 Hz, C-C*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.8 (NH*C*OCH$_3$), 167.2, 164.9, 164.0 (3 *C*OC$_6$H$_5$), 133.8, 133.5, 133.4, 130.4, 129.9, 129.8, 129.7, 129.1, 128.8, 128.4, 117.8 (ArC), 108.9 (isopropylidene-*C*), 97.4 (*C*-1′), 91.3 (*C*-1), 72.7, 71.4, 71.1, 70.5, 70.1, 68.9, 63.8, 62.7 (*C*-6), 51.8 (*C*-2), 42.3 (O*C*H$_2$Cl), 26.0, 24.4 (isopropylidene–*C*H$_3$), 22.8 (NHCO*C*H$_3$), 15.7 (C-*C*H$_3$). HRMS calcd. for $C_{40}H_{46}O_{14}N_2Cl$ (M+NH_4): 813.2638; found *m*/*z* 813.2640.

p-Methoxyphenyl 3,4-O-isopropylidene-β-D-fucopyranoside (22) To a solution of compound **20** (2.0 g, 6 mmol) in dry CH_2Cl_2 (20 ml) was added *p*-methoxyphenol (1.1 g, 9 mmol) followed by $BF_3.OEt_2$ (1.5 ml, 12 mmol) at 0°C and the mixture was stirred at the same temperature for 3 h. After complete conversion, the solution was diluted with CH_2Cl_2 (20 ml) and washed successively with H_2O (2×50 ml), aq. $NaHCO_3$ (2×50 ml) and brine (50 ml). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure *p*-methoxyphenyl 2,3,4-tri-*O*-acetyl-β-D-fucopyranoside (21) (1.9 g, 78%) as colourless syrup.

To a methanolic solution of compound **21** (1.8 g, 4.5 mmol), NaOMe was added and stirred at room temperature for 2 h. The solution was then neutralized with DOWEX 50 W H^+ resin and filtered. Solvents were evaporated *in vacuo* and the residue was suspended in dry acetone (20 ml). 2,2-DMP (820 μl, 6.75 mmol) was added followed by CSA (25 mg) and the mixture was stirred at room temperature until TLC (3:1 *n*-hexane–EtOAc) showed complete conversion to a faster moving spot (1 h). After neutralization with Et_3N, solvents were evaporated *in vacuo* and the crude product was purified by flash chromatography (4:1 *n*-hexane–EtOAc) to afford compound **22** (1.2 g, 87%) as colourless glass. $[\alpha]_D^{25} + 54°(c\ 1.2,\ CHCl_3)$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.95, 6.78 (2d, 4 H, *p*-MeOC$_6$*H*$_4$), 4.59 (d, 1 H, *J* 8.4 Hz, H-1), 4.09 (dd, 1 H, *J* 5.7 Hz, 8.4 Hz, H-2), 4.00 (dd, 1 H, *J* 1.8 Hz, 5.7 Hz, H-3), 3.88 (m, 1 H, H-5), 3.76 (s, 3 H, C$_6$H$_4$OC*H*$_3$), 3.73 (bs,

1 H, H-4), 1.56, 1.37 (2 s, 6 H, isopropylidene–CH_3), 1.40 (d, 3 H, *J* 6.3 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 155.4, 151.2, 118.8, 114.4 (ArC), 109.8 (isopropylidene *C*), 96.1 (*C*-1), 78.9, 76.1, 73.0, 69.1, 55.5 ($C_6H_4OCH_3$), 28.2, 26.3 (isopropylidene *C*H_3), 16.6 (C-*C*H_3). HRMS calcd. for $C_{16}H_{26}O_6N$ (M+NH_4): 328.1760; found *m/z* 328.1762.

p-Methoxyphenyl 2,3,4-tri-O-acetyl-β-D-xylopyranosyl-(1→2)- 3,4-O-isopropylidene-β-D-fucopyranoside (24) A mixture of acceptor **22** (1.0 g, 3.2 mmol), donor **23** (1.45 g, 3.8 mmol) and activated MS 4Å (2 g) in dry CH_2Cl_2 (25 ml) was stirred under nitrogen atmosphere for 1 h. After cooling in a ice-water bath (10–15°C), NIS (1.0 g, 4.6 mmol) was added followed by H_2SO_4–silica (40 mg) and the mixture was stirred for 45 min when TLC (*n*-hexane–EtOAc, 2:1) showed complete conversion of the acceptor spot. The mixture was filtered through celite® and the filtrate was diluted with CH_2Cl_2 (25 ml) and washed successively with aq. $Na_2S_2O_3$ (2×50 ml), aq. $NaHCO_3$ (2×50 ml) and brine (50 ml). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure disaccharide **24** (1.5 g, 83%) as white foam. $[\alpha]_D^{25} + 76°$(*c* 0.9, *CHCl*$_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 6.94, 6.76 (2d, 4 H, *p*-MeOC_6H_4), 5.08 (t, 1 H, *J* 7.2 Hz, H-3′), 4.97 (d, 1 H, *J* 5.4 Hz, H-1′), 4.88 (t, 1 H, *J* 7.2 Hz, H-4′), 4.87 (dd, 1 H, *J* 5.4 Hz, 7.2 Hz, H-2′), 4.67 (d, 1 H, *J* 8.1 Hz, H-1), 4.23 (dd, 1 H, *J* 2.1 Hz, 7.2 Hz, H-5a′), 4.10 (t, 1 H, *J* 8.1 Hz, H-2), 3.97 (dd, 1 H, *J* 1.8 Hz, 5.4 Hz, H-3), 3.85 (m, 2 H, H-4, H-5), 3.74 (s, 3 H, $OC_6H_5OCH_3$), 3.45 (dd, 1 H, *J* 2.1 Hz, 5.4 Hz, H-5b′), 2.08, 2.05, 1.94 (3 s, 9 H, 3×COCH_3), 1.53, 1.33 (2 s, 6 H, isopropylidene–CH_3), 1.39 (d, 3 H, *J* 6.6 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 169.5 (2), 169.1 (3×*C*OCH_3), 155.3, 151.5, 118.7, 114.4 (ArC), 109.9 (isopropylidene *C*), 100.6 (*C*-1′), 99.4 (*C*-1), 79.1, 78.5, 76.1, 70.3, 70.2, 68.7, 68.5, 61.2, 55.4 ($OC_6H_5OCH_3$), 28.0, 26.4 (isopropylidene *C*H_3), 20.7, 20.6, 20.5 (3×CO*C*H_3), 16.6 (C-*C*H_3). HRMS calcd. for $C_{27}H_{40}O_{13}N$ (M+NH_4): 586.2500; found *m/z* 586.2503.

p-Methoxyphenyl 2,3,4-tri-O-acetyl-β-D-xylopyranosyl-(1→2)- 3,4-di-O-acetyl-β-D-fucopyranoside (25) A suspension of compound **24** (1.4 g, 2.5 mmol) in 80% aq. AcOH (20 ml) was stirred at 80°C for 2 h to remove the isopropylidene acetal. After evaporation of the solvents, the residue was dissolved in pyridine (10 ml) followed by addition of Ac_2O (10 ml) and the solution was stirred at room temperature for 2 h. Solvents were evaporated and co-evaporated with toluene and the crude product thus obtained was purified by flash chromatography (3:1 *n*-hexane–EtOAc) to afford pure **25** (1.2 g, 81%) as colourless glass. $[\alpha]_D^{25} + 81°$(*c* 1.1, *CHCl*$_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 6.98, 6.78 (2d, 4 H, *p*-MeOC_6H_4), 5.19 (bd, *J* 1.8 Hz, H-4), 5.08 (t, 1 H, *J* 7.2 Hz, H-3′), 5.02 (dd, 1 H, *J* 1.8 Hz, 7.2 Hz, H-3), 4.91–4.75 (m, 4 H, H-1, H-1′, H-2′, H-4′), 4.26 (dd, 1 H, *J* 1.8 Hz, 5.4 Hz, H-5a′), 4.05 (dd, 1 H, *J* 7.2 Hz, 8.1 Hz, H-2), 3.85 (m, 1 H, H-5), 3.78 (s, 3 H, $OC_6H_5OCH_3$), 3.43 (dd, 1 H, *J* 2.1 Hz, 5.4 Hz, H-5b′), 2.13, 2.08 (2), 2.05, 1.94 (5 s, 15 H, 5×COCH_3), 1.29 (d, 3 H, *J* 6.6 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 170.1, 169.7, 169.5, 169.4, 169.0 (5×*C*OCH_3), 155.5, 151.3, 118.8, 114.4 (ArC), 101.2 (*C*-1′), 99.9 (*C*-1), 74.2, 73.3, 70.5, 70.4 (2), 69.0, 68.6, 61.4, 55.4 ($OC_6H_5OCH_3$), 20.6, 20.5, 20.4, 20.3, 20.2 (5×CO*C*H_3), 16.1 (C-*C*H_3). HRMS calcd. for $C_{28}H_{40}O_{15}N$ (M+NH_4): 630.2398; found *m/z* 630.2396.

Benzoyl 2,3,4-tri-O-acetyl-β-D-xylopyranosyl-(1→2)- 3,4-di-O-acetyl-β-D-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-3,4-di-O-benzoyl-β-D-glucopyranose (27) To a solution of compound **25** (1.1 g, 1.8 mmol) in CH_3CN–H_2O (9:1, 20 ml) was added CAN (2.0 g, 3.6 mmol) and the mixture was stirred at room temperature for 45 min. The solvents were evaporated and the residue was dissolved in CH_2Cl_2 (30 ml) and washed with H_2O (2×50 ml); organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. It was re-dissolved in dry CH_2Cl_2 (20 ml), CCl_3CN (540 μl, 5.4 mmol) was added followed by DBU (300 μl, 2 mmol) and the mixture was stirred at room temperature for 1 h. After that, solvents were evaporated and the residue was charged directly to a flash column and eluted with *n*-hexane–EtOAc (2:1) to afford pure trichloroacetimidate donor **26** (915 mg, 78%). Next, a mixture of **26** (900 mg, 1.4 mmol), acceptor **15** (750 mg, 1.4 mmol) and MS 4Å (1 g) in dry CH_2Cl_2 (15 ml) was stirred under nitrogen atmosphere for 1 h. H_2SO_4–silica (15 mg) was added and the mixture was stirred at room temperature for 2 h. The mixture was filtered through celite® after neutralization with Et_3N. The solvents were evaporated and the residue was purified through flash chromatography (2:1 *n*-hexane–EtOAc) to afford pure trisaccharide **27** (1.1 g, 76%) as white foam. $[\alpha]_D^{25} + 41°$(*c* 1.1, *CHCl*$_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 8.22–7.36 (m, 15 H, ArH), 6.52 (d, 1 H, *J* 3.6 Hz, N*H*), 5.96 (d, 1 H, *J* 8.4 Hz, H-1), 5.79 (t, 1 H, 9.3 Hz, H-4), 5.75 (t, 1 H, *J* 9.3 Hz, H-3), 5.25 (dd, 1 H, *J* 1.8 Hz, 7.8 Hz, H-3′), 5.21 (bs, 1 H, H-4′), 5.02 (t, 1 H, *J* 8.4 Hz, H-4″), 4.97 (d, 1 H, *J* 6.0 Hz, H-1′), 4.87 (m, 2 H, H-2″, H-3″), 4.64 (d, 1 H, 6.3 Hz, H-1″), 4.20 (m, 1 H, H-5), 4.16 (m, 3 H, H-2, H-2′, H-6^a), 3.88 (dd, 1 H, *J* 3.3 Hz, 10.5 Hz, H-6b), 3.81 (m, 2 H, H-5′, H-5a″), 3.41 (dd, 1 H, *J* 5.4 Hz, 10.2 Hz, H-5b″), 2.15, 2.03 (3), 2.00 (3 s, 15 H, 5×COCH_3), 1.73 (s, 3 H, NHCOCH_3), 0.98 (d, 3 H, *J* 6.3 Hz, C-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 169.6, 169.5 (2), 169.3, 169.2, (5×*C*OCH_3), 169.1 (NH*C*OCH_3), 167.6, 164.9, 164.3 (3×*C*OPh), 133.9, 133.6, 133.5, 130.0, 129.9, 129.7, 128.8, 128.7, 128.5 (ArC), 101.1 (C-1″), 98.4 (C-1′), 91.3 (C-1), 74.6, 71.5 (2), 70.3, 69.8, 69.1,

68.4, 68.3, 66.4, 64.4, 61.3, 52.0 (C-2), 22.9 ($NHCOCH_3$), 20.7 (4), 20.5 ($5\times COCH_3$), 15.6 (C-CH_3). HRMS calcd. for $C_{50}H_{59}O_{22}N_2$ ($M+NH_4$): 1039.3560; found *m/z* 1039.3563.

β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranose(2) To a solution of protected trisaccharide **27** (920 mg, 0.9 mmol) in dry MeOH (25 ml), NaOMe (25 mg) was added and the solution was stirred at room temperature for 6 h. After completion, the solution was neutralized with DOWEX 50 W H^+ resin, filtered and evaporated to a semi-solid mass. It was then partitioned between H_2O and CH_2Cl_2 to remove methyl benzoate. The water layer was collected and freeze-dried to furnish pure trisaccharide **2** (370 mg, 83%) as white amorphous powder. HRMS calcd. for $C_{19}H_{33}O_{14}NNa$ ($M+Na$): 522.1799; found *m/z* 522.1801.

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Supporting Information Available Experimental details and Copies of ^1H and ^{13}C spectra of all new compounds.

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