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Note

Synthesis of allyl 2-*O*-(α-L-arabinofuranosyl)-6-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside, a unique plant *N*-glycan motif containing arabinose

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Abstract

The synthesis of the trisaccharide allyl 2-*O*-(α-L-arabinofuranosyl)-6-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside is reported. Stereoselective glycosylation at C-6 of a non-protected allyl β-mannoside with the acetylated α-D-mannosyl bromide gave the α-(1→6)-disaccharide as the main product and the crystalline 3,6-branched trisaccharide as minor compound. Further glycosylation of the 2,3 diol (1→6) disaccharide with L-arabinofuranosyl bromide furnished a mixture of 3-*O*- and 2-*O*-α-L-Ara-trisaccharides from which the title compound was isolated.

Keywords: L-Arabinose; Plant *N*-glycan; β-D-Allyl mannoside

Plant *N*-glycosylation exhibits a certain diversity, sharing both common and different features by comparison to animal *N*-glycosylation. In addition to D-Man and D-GlcNAc which are found in all *N*-glycans, extra sugars D-GlcNAc, D-Xyl, L-Fuc, and D-Gal can substitute the inner core, resulting in the diversity of all described plant *N*-glycoproteins.

Despite the fact that an important number of plant *N*-glycans have been fully characterized and shown to only contain the sugar residues listed above, a few authors reported the occurrence of 'exotic' sugars in the composition of some plant *N*-glycoprotein extracts. For instance, in a study on the analysis of secreted glycoproteins of carrot, the presence of L-rhamnose and L-arabinose in *N*-glycosylation was suspected on the basis of radioisotope labeling and enzymatic treatments [1]. Another report mentioned the presence of L-arabinose in equal amount as L-fucose in a glycoprotein of red kidney seedling [2]. A few years latter, while studying the structural diversity of free *N*-glycans in tomato fruit pericarp, Priem et al. [3] eventually described a structure containing α-L-arabinofuranose with the following sequence:

α-D-Man*p*-(1→6)- β-D-Man*p*-(1→4)-β-D-GlcNAc*p*-(1→4)- α,β-DGlcNAc*p*

2 3

↑ ↑

1 1

α-L-Ara*f* α-L-Fuc*p*

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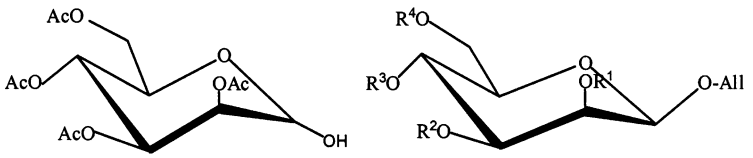


1 **(2-12)**

α-Man(Ac) = 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl

2	R^1, R^2, R^3, R^4 = Ac,	
3	R^1, R^2, R^3, R^4 = H,	
4	R^1, R^3 = H,	R^2, R^4 = α-Man(Ac)
5	R^1, R^2 = H	R^3 = H, R^4 = α-Man(Ac)
6	R^1, R^2 = $C(CH_3)_2$,	R^3 = H, R^4 = α-Man(Ac)
7	R^1, R^2 = $C(CH_3)_2$,	R^3 = Ac, R^4 = α-Man(Ac)
8	R^1, R^2 = H,	R^3 = Ac, R^4 = α-Man(Ac)
9	R^1 = H, R^2 = 2,3,4-tri-*O*-benzoyl-α-L-Ara*f*	R^3 = Ac, R^4 = α-Man(Ac)
10	R^1 = 2,3,4-tri-*O*-benzoyl-α-L-Ara*f*, R^2 = H	R^3 = Ac, R^4 = α-Man(Ac)
11	R^1 = 2,3,4-tri-*O*-benzoyl-α-L-Ara*f*, R^2 = Ac,	R^3 = Ac, R^4 = α-Man(Ac)
12	R^1 = α-L-Ara*f*, R^2 = H,	R^3 = H, R^4 = α-D-Man*p*

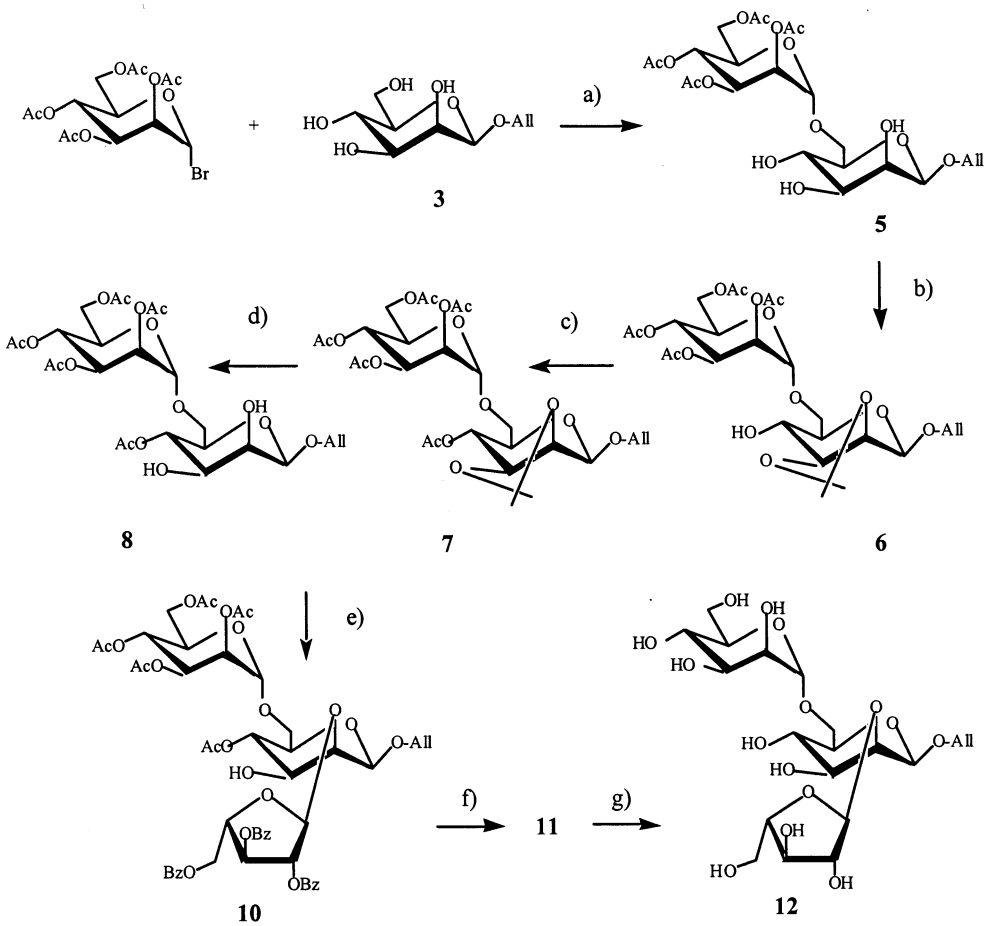
Scheme 1.

Here the L-arabinose replaces the D-xylose often found in the *N*-glycan core of plant glycoproteins. In fact, α-L-Araf and β-D-Xylp exhibit very similar conformational features, and some bacterial hydrolases can either act as α-L-arabinofuranosidases or β-D-xylopyranosidases (see for example [4], Swiss-Prot accession # P49943). These enzymes all belong to the family 43 of glycosylhydrolases based on their HCA plot similarity [5]. Thus, D-xylose- and L-arabinose-containing *N*-glycans might share common building or hydrolytic enzymes, and hence similar biosynthetic and/or hydrolytic pathways.

A practical consequence of the presence of particular motifs in plant glycoproteins is that they are often potent allergens for mammal organisms. The best-known plant carbohydrate allergenic determinants are β-(1 → 2)-linked D-xylopyranose and α-(1 → 3)-linked L-fucopyranose, often as part of *N*-glycoproteins and both absent in mammals [6,7]. Hence, it is reasonable to think that putative arabinose-containing plant glycoproteins would be allergenic.

Here, we describe the synthesis of allyl 2-*O*-(α-L-arabinofuranosyl)-6-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside sharing a common motif with L-arabinose-containing *N*-glycan of tomato. The β-allyl aglycon was chosen to produce a neoglycoprotein which may be used to raise a specific antibody for screening of arabinosylated *N*-glycoproteins in plants. A full ^1H and ^{13}C NMR 1D and 2D spectroscopy analysis was done for all newly described compounds (Scheme 1).

The key compound involved in the synthesis of allyl (2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1 → 6)-β-D-mannopyranoside (**4**)



Scheme 2. (a) CH_3CN, $Hg(CN)_2$–$HgBr_2$ (50%). (b) Acetone, 2,2-dimethoxypropane, H^+ (70%). (c) DCM, Ac_2O, pyridine (97%). (d) CH_3CN, 35% aq soln HBF_4 (96%). (e) Toluene, $Hg(CN)_2$–$HgBr_2$, 2,3,5-tri-*O*-benzoyl-L-Ara*f* bromide (32%). (f) Ac_2O, pyridine, rt (95%). (g) MeO^-Na^+, MeOH, resin H^+ (99%).

was the β-allyl mannoside **3** prepared by reaction of tetraacetate **1** [8] with an excess of allyl bromide in toluene at room temperature in the presence of silver oxide [9], which furnished in high yield (98%) a mixture of acetylated mannoside **2α**,**β** [10] in a 7:13 ratio. Crystallization from diethyl ether–cyclohexane led to the isolation of a major amount ($\geq 60\%$) of the pure **2β** isomer. Chromatography on silica gel allowed the isolation of both pure isomers from the mother liquor. Subsequently, glycosylation of **3** in acetonitrile (obtained by Zemplén method [11] from **2β**) with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide [12] in the presence of $Hg(CN)_2$–$HgBr_2$ gave in 16% yield the allyl 3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**4**) and the target disaccharide allyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**5**) (50%) as major products (Scheme 2). Purification by chromatography furnished pure **4** [21] which eluted first and crystallized from diethyl ether–hexanes after a few weeks at −18 °C. The disaccharide **5** eluted next and was an amorphous solid containing minor contaminants[1] as evidenced by ^{13}C NMR spectroscopy. Interestingly, the yield of **5** was enhanced when conventional washings with saturated aqueous phases were omitted and the mixture directly chromatographed after evaporation in vacuo of the reaction solvent. However, the procedure suffered from the traces of mercury salts easily eliminated in the next step. Thus, treatment of **5** in dry acetone with

[1] Among the minor compounds expected to be formed during the glycosylation, the 1,3-linked disaccharide showed a faster moving rate than **5** and was isolated pure; the presence of 1,2 and 1,4 linked disaccharides (not isolated) with **5** were supposed to be eliminated during step purification's of **6** and **7**.

dimethoxypropane in large excess (10 fold) catalyzed by dry camphorsulfonic acid afforded in 2 h the 2,3-*O*-acetonide **6** in 70% yield[2]. **6** was isolated pure by chromatography on silica gel with EtOAc–hexanes–Et_3N (1:1:traces) or acetylated with DCM–Ac_2O–pyridine (10:1:1) to give in good yield (68% yield, 2 steps) **7** as a more stable product. The compound **7** was further hydrolyzed at 0 °C (ice–water bath) by aqueous tetrafluoroboric acid in acetonitrile according to the method of Albert et al. [13] affording the 2,3-diol **8** in a quantitative yield. At this stage, attempts towards selective OH-3 acetylation with DCM–Ac_2O–pyridine failed ($\sim$1:1 ratio of OAc-3:OAc-2), so the direct glycosylation of diol **8** with 2,3,5-tri-*O*-benzoyl-L-arabinofuranosyl bromide [14] was performed in dry toluene with $Hg(CN)_2$–$HgBr_2$ as promoters [15]. From the reaction mixture, the 3-*O*- (**9**) and 2-*O*-α-L-arabinofuranosyl (**10**) trisaccharides were identified (total yield 60%, 5:6 ratio) and isolated by chromatography. Subsequent acetylation of **10** with a 10:1:1 mixture of DCM–Ac_2O–pyridine gave **11**, the structure of which was unambiguously determined by 1D and 2D NMR spectroscopy at 400 MHz (100 MHz for ^{13}C NMR) showing the expected (^1H NMR) deshielding effects for α protons belonging to secondary esterified alcohol positions and high field shifts for protons attached to 2-O- and 6-O-glycosylated centers (H-2, H-$6_{a,b}$, 4.11, 3.28, 3.35 ppm compared with 5.35, 4.03, 4.18 ppm for the acetylated β-mannoside **2**). Furthermore, a characteristic feature (^{13}C NMR) was the deshielding effect on the C-2 signal of the β-allyl mannosyl unit confirming the arabinofuranosyl glycosylation at C-2 (C-2, 71.90 ppm for **11** compared with 68.73 for **2**). In addition, small scalar couplings between H-1″, H-2″ and H-3″ belonging to the arabinofuranosyl unit unequivocally ruled out the α-L-configuration (1,2-trans) [16]. Finally, deprotection of **11** by Zemplén trans-esterification [11] procured the title compound namely allyl 2-*O*-(α-L-arabinofuranosyl)-6-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside (**12**), fully analyzed by 1D and 2D NMR spectroscopy in D_2O at 500 MHz for ^1H and 125 MHz for ^{13}C. Comparison of the earlier ^1H NMR data from sample labeled NR5b in Ref. [3] with those of **12** showed an excellent accord for the α-L-arabinofuranosyl moiety (other data were not available from [3] due to close overlap).

In conclusion, we achieved a stereocontrolled synthesis of this new type of oligosaccharide constituting a unique plant *N*-glycan motif containing α-L-arabinose, completing the previously reported syntheses of β-(1→2)-D-xylopyranosyl containing oligosaccharides [17,18]. The β-D-configuration of the allyl aglycon was assigned by comparison with the β-D-mannosyl unit (1→4)-linked to a chitobiosyl moiety observed in the core structure of *N*-glycans. Furthermore, the presence of the allyl group may serve as nascent reactive group for coupling to carriers by a spacer arms in order to initiate antibody production.

1. Experimental

General methods.—Melting points were determined in capillary tubes with a Büchi 535 melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Type 241 polarimeter at 25 °C using a 10 cm cell. All reactions were monitored by thin layer chromatography (TLC) run on aluminum plates precoated with Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany); detection was effected by charring with a 1:15:15 mixture of H_2SO_4–MeOH–water. Flash column chromatography was performed using Silica Gel 60 (0.0063-0.200, E. Merck). 1D and 2D NMR spectra for compounds **1**–**10** were recorded at 30 °C with a Bruker AM300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C using $CDCl_3$ or D_2O as solvents. Assignments of NMR signals were made by first-order analysis of spectra and were supported by DEPT experiments, homo- and heteronuclear two-dimensional correlation spectroscopy (COSY, COSYRCT2, XHCORRD.AU). Spectra for the trisaccharide **11** were recorded on a Bruker AMX 400 spectrometer using $CDCl_3$

[2] Partial hydrolysis of the β-allyl aglycon was observed in non perfect dry conditions.

as solvent. Spectra for the target product **12** were obtained at 45 °C with a VARIAN 500 Unity$^+$ apparatus; total assignments of ^{1}H signals (500 MHz) were made using 1D and 2D spectroscopy supported by 1D TOCSY experiments and ^{13}C signals (125 MHz) were assigned from HMQC 2D spectroscopy. Atom numbering was labeled as prime for the α-(1→6)-mannosyl unit and double prime for the α-L-arabinosyl unit. Mass spectra in the FAB(+) mode were recorded with a Nermag R 10 10C spectrometer.

2,3,4,6-Tetra-O-*acetyl*-D-*mannopyranose* (**1**). Per-*O*-acetyl mannose α,β (5.65 g, 14.49 mmol) obtained from mannose by conventional acetylation (Ac_2O, C_5H_5N, rt) was dissolved in 1:4 DMF–CH_3CN (60 mL) and treated with NH_2NH_2, CH_3CO_2H [19] (1.47 g, 15.97 mmol). After 15 h at rt the soln was concd in vacuo. The residue was extracted with CH_2Cl_2, washed with water, then dried (Na_2SO_4) and the organic phase concd. The solid was purified by column chromatography (1:1, EtOAc–hexanes) to yield amorphous **1** [8,19] (4.3 g, 85%) as a quite pure α anomer (α,β, 99:1); $[\alpha]_D$ +19.8° (*c* 1, $CHCl_3$); R_f 0.24 (1:1, EtOAc–hexanes); ^{1}H and ^{13}C NMR (see Ref. [20]).

Allyl 2,3,4,6-tetra-O-*acetyl*-α,β-D-*mannopyranoside* (**2α,β**). To a soln of **1** (1.5 g, 4.31 mmol) and Ag_2O (3 g, 12.93 mmol) in toluene (30 mL) was added allyl bromide (1 mL, 11.82 mmol) over 2 h. The mixture was stirred for 4 days at rt, then filtered on a pad of silica gel (10 g); the gel was washed with EtOAc and the organic phases concd in vacuo. The solid (yield 98%) was purified by column chromatography (2:1, hexanes–EtOAc); the α anomer was eluted first followed by the β-allyl mannoside **2β** (the β anomer was also obtained pure by crystallization (diethyl ether–hexanes) from the reaction mixture with a ≥32% yield).

Pure product **2α** [10]: yield 701 mg (42%); R_f 0.22 (2:1, cyclohexane–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.95, 2.00, 2.07, 2.11 (4 s, CH_3COO), 3.98 (m, 1 H, H-5), 4.00 (m, 1 H, OCH_2–CH=CH_2), 4.07 (dd, 1 H, $J_{5,6b}$ 2.59, $J_{6a,6b}$ 12.25 Hz, H-6b), 4.15 (m, 1 H, OCH_2–CH=CH_2), 4.23 (dd, 1 H, $J_{5,6a}$ 5.18 Hz, H-6a), 4.83 (d, 1 H, $J_{1,2}$ 1.65 Hz, H-1), 5.20 (m, 1 H, –CH=CH_2), 5.22 (dd, 1 H, $J_{2,3}$ 3.30 Hz, H-2), 5.25 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.90 Hz, H-4), 5.27 (m, 1 H, –CH=CH_2), 5.34 (dd, 1 H, H-3), 5.86 (m, 1 H, –CH=CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.67–20.80 (4CH_3COO), 62.46 (C-6), 66.07, 68.42, 68.94, 69.48 (C-2, C-3, C-4, C-5), 68.47 (OCH$_2$CH=CH_2), 96.43 (C-1), 118.17 (–CH=CH$_2$), 132.80 (–CH=CH_2), 169.66–170.55 (4CH_3COO).

Anomer **2β** [10]: 884 mg (53%), crystallized from cyclohexane–diethyl ether; mp 106–107 °C; R_f 0.16 (2:1, cyclohexane–EtOAc); $[\alpha]_D$ −46° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.96, 2.01, 2.06, 2.16 (4 s, CH_3COO), 3.55 (m, 1 H, H-5), 3.90–4.24 (2 m, 2 H, OCH_2–CH=CH_2), 4.03 (dd, 1 H, $J_{5,6b}$ 2.49, $J_{6a,6b}$ 12.30 Hz, H-6b), 4.18 (dd, 1 H, $J_{5,6a}$ 5.54 Hz, H-6a), 4.59 (broad d, 1 H, $J_{1,2} < 1$ Hz, H-1), 4.94 (dd, 1 H, $J_{2,3}$ 3.32, $J_{3,4}$ 10.00 Hz, H-3), 5.13 (t, 1 H, $J_{4,5} = J_{3,4}$ 10.00 Hz, H-4), 5.17–5.28 (2 m, 2 H, –CH=CH_2), 5.35 (broad dd, 1 H, H-2), 5.68–5.81 (m, 1 H, –CH=CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.45–20.76 (4CH_3COO), 62.40 (C-6), 65.99 (C-4), 68.73 (C-2), 70.05 (–OCH$_2$CH=CH_2), 70.97 (C-3), 72.21 (C-5), 97.19 (C-1), 117.97 (–CH=CH$_2$), 132.95 (–CH=CH_2), 169.48, 169.91, 170.25, 170.56 (4CH_3COO).

Allyl β-D-*mannopyranoside* (**3**). The acetylated product **2β** (800 mg, 2.06 mmol) deprotected under classical conditions by using Zemplén reagent [11] (MeO^-Na^+, MeOH, rt) afforded **3** (453 mg) in a quantitative yield. The solid obtained after stirring the soln with Amberlite H^+ resin for 5 min and concn under reduced pressure was a colorless syrup: $[\alpha]_D$ −55° (*c* 1, water); R_f 0.31 (9:1, CH_3CN–water); ^{1}H NMR (300 MHz, D_2O): δ 3.22 (m, 1 H, H-5), 3.43 (t, 1 H, $J_{4,5} = J_{3,4}$ 940 Hz, H-4), 3.50 (dd, 1 H, $J_{2,3}$ 3.12 Hz, H-3), 3.60 (dd, 1 H, $J_{5,6b}$ 6.40, $J_{6a,6b}$ 12.21 Hz, H-6b), 3.79 (dd, 1 H, $J_{5,6a}$ 2.32 Hz, H-6a), 3.86 (broad dd, 1 H, $J_{1,2} < 1$ Hz, H-2), 4.04–4.10 and 4.20–4.27 (2 m, 2 H, OCH_2–CH=CH_2), 4.56 (broad d, 1 H, H-1), 5.12–5.26 (2 m, 2 H, –CH=CH_2), 5.77–5.91 (m, 1 H, –CH=CH_2); ^{13}C NMR (75 MHz, D_2O): δ 61.46 (C-6), 67.20 (C-4), 70.43 (OCH$_2$CH=CH_2), 70.90 (C-2), 73.35 (C-3), 76.58 (C-5), 99.21 (C-1), 118.74 (–CH=CH$_2$), 133.86 (–CH=CH_2).

Allyl 6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**5**). A soln of **3** (99 mg, 0.45 mmol) in dry CH_3CN (10 mL) was stirred for 2 h with 4 Å molecular sieves. To the mixture were added successively $Hg(CN)_2$ (190 mg, 0.75 mmol), a catalytic amount of $HgBr_2$ and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide [12] (267 mg, 0.65 mmol); the latter, added in two fractions, was poured into the flask with a 2 h interval between additions. The soln was stirred at rt for 15 h, then deposited at the top of a column containing 5 g of silica gel and filtered; the column was washed successively with EtOAc (100 mL) and CH_3CN (50 mL). The organic soln was concd, giving a solid crude mixture (420 mg). Column chromatography (EtOAc, silica gel 50 g) of the solid first eluted the trisaccharide allyl 3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**4**) (60 mg, 0.07 mmol, 16% yield) followed by the disaccharide **5** with minor contaminants (130 mg, 0.24 mmol, 50% yield). Trisaccharide **4** crystallized from diethyl ether–hexanes after few weeks at −18 °C; mp 175–176 °C; $[\alpha]$ +22° (*c* 1, $CHCl_3$); FABMS: $(M+Na)^+$ 903; R_f 0.59 (EtOAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.63–20.83 (8*C*H_3COO), 62.46, 62.81 (C-6″, C-6′), 65.35, 66.03, 66.34 (3 *C*H), 66.68 (C-6), 68.36, 68.95, 69.04, 69.24, 69.46, 69.62 (6 *C*H), 69.73 (O*C*H_2 CH=CH_2), 70.45, 74.68 (2 *C*H), 84.69 (C-3), 99.35, 98.04, 99.70 (C-1, C-1′, C-1″), 118.19 (–CH=*C*H_2), 133.40 (–*C*H=CH_2), 169.77–170.51 (8CH_3*C*OO).

Compound **5** failed to crystallize; R_f 0.30 (4:1, EtOAc–acetone); $[\alpha]_D$ −33° (*c* 1, $CHCl_3$); FABMS: $(M+Na)^+$ 573; ^1H NMR (300 MHz, $CDCl_3$): δ 1.95, 2.00, 2.05, 2.10 (4 s, CH_3COO), 3.30–4.35 (11 H, H-2, H-3, H-4, H-5, H-5′, H-6a, H-6b, H-6′a, H-6′b, OCH_2–CH=CH_2), 4.56 (d, 1 H, $J_{1,2}<1$ Hz, H-1), 4.91 (d, 1 H, $J_{1',2'}$ 1.20 Hz, H-1′), 5.15–5.34 (m, 5 H, H-2′, H-3′, H-4′, –CH=CH_2), 5.86 (m, 1 H, –C*H*=CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.82–20.93 (4*C*H_3COO), 62.37 (C-6′), 67.25 (C-6), 69.99 (O*C*H_2–CH=CH_2), 65.80, 66.98, 68.45, 69.47, 69.67, 70.82, 73.54, 74.86 (C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 98.05, 98.30 (C-1, C-1′), 118.15 (–CH–*C*H_2), 133.54 (–*C*H=CH_2), 169.71–170.89 (4CH_3*C*OO).

Allyl 2,3-O-isopropylidene-6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**6**). To a soln of disaccharide **5** (100 mg, 0.18 mmol) in dry acetone (5 mL) was added 2,2 dimethoxypropane (0.22 mL, 1.80 mmol) and a catalytic amount of camphorsulfonic acid. The reaction was monitored by TLC; after 2 h the initial product (R_f 0.10, EtOAc) disappeared totally and a major reaction product was present (R_f 0.63, EtOAc); the soln was neutralized with Et_3N. Conventional work up was performed; the organic phase was evaporated and gave a solid **6** which failed to crystallize. The product was purified by column chromatography (EtOAc–hexanes–Et_3N traces); yield 70% (75 mg); $[\alpha]_D$ −3° (*c* 1, $CHCl_3$); FABMS: $[M+Na]^+$ 613; ^1H NMR (300 MHz, $CDCl_3$): δ 1.37, 1.54 (2CH_3, iso), 1.96, 1.99, 2.08, 2.12 (4 s, CH_3COO), 3.43 (m, 1 H, H-5), 3.77 (m, 1 H, H-5′), 3.79 (2 dd, 2 H, H-6a, H-6b), 4.08 (m, 2 H, H-3, H-4), 4.10–4.22 (m, 2 H, H-6′a, H-6′b), 4.19–4.44 (m, 2 H, OCH_2 CH=CH_2), 4.21 (dd, 1 H, H-2), 4.79 (d, $J_{1,2}$ 2.25 Hz, H-1), 4.84 (d, 1 H, $J_{1',2'}$ 1 Hz, H-1′), 5.21 (dd, 1 H, H-2′), 5.23 (t, 1 H, H-4′), 5.30 (dd, 1 H, H-3′), 5.90 (m, 1 H, –C*H*=CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.66–20.84 (4 s, *C*H_3COO), 26.11, 27.69 (2CH_3, iso) (4*C*H_3COO), 62.37 (C-6′), 66.15 (C-4′), 67.12 (C-6), 68.42 (C-4), 68.98 (C-3′), 69.42 (C-2′), 69.54 (C-5′), 69.94 (–O*C*H_2 CH=CH_2), 73.77 (C-5), 74.37 (C-2), 80.17 (C-3), 96.69 (C-1′), 97.20 (C-1), 110.93 (*C*–$(CH_3)_2$), 118.26 (–CH=*C*H_2), 133.53 (–*C*H=CH_2), 169.69–170.66 (4CH_3*C*OO).

Allyl 2,3-O-isopropylidene-4-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**7**). Compound **6** (59 mg, 0.1 mmol) in dry DCM (3 mL) was acetylated by conventional method with Ac_2O (0.5 mL) and C_5H_5N (0.5 mL) for 24 h at rt; then TLC (1:1, EtOAc–hexanes) showed that the starting material had completely disappeared (R_f 0.18). The excess of reagent was quenched by adding MeOH, then the soln was concd under diminished pressure by co-evaporation with toluene. The solid (R_f 0.32) was purified by silica gel chromatography (1:1, EtOAc–hexanes) and gave pure **7** which failed to crystallize. $[\alpha]_D$ −13° (*c* 1, $CHCl_3$);

FABMS: $[M+Na]^+$ 655; ^1H NMR (300 MHz, $CDCl_3$): δ 1.30, 1.49 (2 s, CH_3, iso), 1.90–2.10 (5 s, CH_3COO), 3.40 (dd, 1 H, $J_{5,6b}$ 2.78, $J_{6a,6b}$ 10.27 Hz, H-6b), 3.52 (m, 1 H, H-5), 3.73 (dd, 1 H, $J_{5,6a}$ 7.92 Hz, H-6a), 3.99–4.08 (m, 2 H, H-5′, H-6b), 4.14 and 4.37 (m, 2 H, OCH_2CH=CH$_2$), 4.14–4.24 (m, 3 H, H-2, H-3, H-6′a), 4.73 (d, 1 H, $J_{1',2'} \leq 1$ Hz, H-1′), 4.76 (d, 1 H, $J_{1,2}$ 1.76 Hz, H-1), 4.91 (dd, 1 H, $J_{3,4}$ 6.75, $J_{4,5}$ 9.69 Hz, H-4), 5.13 (dd, 1 H, $J_{2',3'}$ 3.25 Hz, H-2′), 5.18 and 5.25 (m, 2 H, –CH=CH_2), 5.20 (t, 1 H, $J_{2',3} = J_{3',4'}$ 9.30 Hz, H-4′), 5.27 (dd, 1 H, H-3′), 5.87 (m, 1 H, –CH=CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.51–20.83 (5CH$_3$COO), 26.02, 27.30 (2CH$_3$ iso), 60.19 (C-6′), 65.90 (C-4′), 67.12 (C-6), 68.45 (C-4), 68.86 (C-3′), 69.28 (C-2′), 70.19 (OCH$_2$CH=CH$_2$), 70.31 (C-5′), 72.59 (C-5), 74.28 (C-2), 76.92 (C-3), 96.58 (C-1′), 96.80 (C-1), 111.11 (C(CH$_3$)$_2$, iso), 118.66 (–CH=CH$_2$), 133.32 (–CH=CH$_2$), 169.46–170.91 (5CH$_3$$C$OO).

*Allyl 4-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**8**). To a soln of compound **7** (50 mg, 0.84 mmol) in CH_3CN (5 mL) at 0 °C (ice–water bath) was added tetrafluoroboric acid (35% in water) according to the method of Albert et al. [13]; after quantitative reaction (TLC, 10 min) Et_3N was added and the solvent co-evaporated with toluene. Compound **8** was then purified on a short column of silica gel (4:1, EtOAc–cyclohexane) and gave a solid (45 mg, 96%); $[\alpha]_D$ −4° (*c* 1, $CHCl_3$); R_f 0.42 (EtOAc); FABMS: $[M+Na]^+$ 615; ^1H NMR (300 MHz, $CDCl_3$): δ 1.96, 1.99, 2.08, 2.09, 2.12 (5, CH_3COO), 3.50 (m, 1 H, H-5′), 3.54 (m, 1 H, H-5), 3.60 (dd, 1 H, $J_{6a,6b}$ 10.80, $J_{5,6b}$ 3.30 Hz, H-6b), 3.77 (dd, 1 H, $J_{5,6a}$ 7.10 Hz, H-6a), 3.99 (broad dd, 1 H, $J_{2,3}$ 3.25 Hz, H-2), 4.06 (dd, 1 H, $J_{3,4}$ 9.65 Hz, H-3), 4.11 (dd, 1 H, $J_{5',6'b}$ 2.51, $J_{6'a,6'b}$ 12.17 Hz, H-6′b), 4.16 (m, 1 H, OCH_2CH=CH$_2$), 4.23 (dd, 1 H, $J_{5',6'a}$ 5.60 Hz, H-6′a), 4.35 (m, 1 H, OCH_2CH=CH$_2$), 4.55 (d, 1 H, $J_{1,2} < 1$ Hz, H-1), 4.83 (d, 1 H, $J_{1',2'}$ 1.35 Hz, H-1′), 4.98 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.65 Hz, H-4), 5.20–5.35 (m, 2 H, –CH=CH_2), 5.90 (m, 1 H, –CH=CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.75–20.87 (5CH$_3$COO), 62.41 (C-6′), 66.18 (C-4′), 66.73 (C-6), 68.53 (C-3), 68.95 (C-3′), 69.52 (C-2′), 69.86 (C-4), 69.91 (OCH$_2$CH=CH$_2$), 70.81 (C-2), 72.44 (C-5), 72.69 (C-5′), 97.17 (C-1′), 98.24 (C-1), 118.25 (–CH=CH$_2$), 133.39 (–CH=CH$_2$), 169.66–170.76 (5CH$_3$$C$OO).

*Allyl 2-*O*-(2,3,5-tri-*O*-benzoyl-α-*L*-arabinofuranosyl)-4-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**10**). To a soln of disaccharide **8** (35 mg, 0.06 mmol) in dry toluene (3 mL) and $Hg(CN)_2$ (30 mg, 0.13 mmol) was slowly added 2 mL of a soln of 2,3,5-tri-*O*-benzoyl-L-arabinofuranosyl bromide [14,15] (240 mg, 0.43 mmol, 10 mL toluene). The mixture was stirred at rt for 20 min, then MeOH (0.2 mL) was added to quench the excess of bromide. The soln was poured into a funnel and washed with a satd aq soln of KBr and then distilled water; the organic layers were isolated and dried (Na_2SO_4). The soln was then concd in vacuo and the solid chromatographed on silica gel with 1:1 EtOAc–hexanes as eluents; the two trisaccharides 2-*O*- and 3-*O*-α-L-Araf were isolated (60% yield), the first moving product (3-O-linked) **9** (17 mg, R_f 0.33, 1:1, EtOAc–hexanes) followed by the 2-*O*-α-L-Araf isomer **10** (20 mg, R_f 0.27) as an amorphous compound.

9 ^{13}C NMR (75 MHz, $CDCl_3$, selected data): 97.15, 99.40, 105.80 (C-1, C-1′, C-1″).

10 ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.60–20.80 (5CH$_3$COO), 62.34, 63.42, 67.00 (C-6′, C-5″, C-6), 66.14, 67.27, 67.56, 68.49, 68.96, 69.45, 69.99, 73.02, 75.81, 77.41, 81.51, 82.93 (C-2, 2′, 2″; C-3, 3′,3″; C-4, 4′, 4″; C-5, 5′, OCH$_2$CH=CH$_2$), 96.24, 98.03 (C-1, C-1), 103.15 (C-1″), 118.42 (–CH=CH$_2$), 128.32–133.71 (C–Ar, –CH=CH$_2$), 165.02–166.32 (3 C$_6$H$_5$$C$OO),169.66–170.64 (5CH$_3$$C$OO).

*Allyl 2-*O*-(2,3,5-tri-*O*-benzoyl-α-*L*-arabinofuranosyl)-3,4-di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**11**). Compound **10** was acetylated with Ac_2O and C_5NH_5 at rt (overnight) and **11** was isolated by conventional workup, then purified on a short column of silica gel; R_f 0.40 (1:1, EtOAc–hexanes); yield 95%; ^1H NMR (400 MHz, $CDCl_3$): δ 1.66, 1.70, 1.77, 1.79, 1.85, 2.07 (6 s, CH_3COO), 3.28 (dd, 1 H, $J_{5,6b}$ 2.60, $J_{6a,6b}$ 10.50 Hz, H-6b), 3.35 (m, 1 H, H-5), 3.59 (dd, 1 H, $J_{5,6a}$ 7.20 Hz, H-6a), 3.83 (m, 3 H, H-6′b, H-5′, –CH_2CH=CH$_2$), 4.01

(dd, 1 H, $J_{5',6'a}$ 5.15, $J_{6'a,6'b}$ 12.30 Hz, H-6′a), 4.08 (m, 1 H, –CH_2CH=CH$_2$), 4.11 (broad dd, 1 H, $J_{1,2} \ll 1$, $J_{2,3}$ 3.25 Hz, H-2), 4.30 (m, 1 H, H-4″), 4.35 (dd, 1 H, $J_{4'',5''}$ 5.37, $J_{5''a,5''b}$ 11.54 Hz, H-5″b), 4.39 (broad d, 1 H, H-1), 4.49 (dd, 1 H, $J_{4'',5''a}$ 3.24 Hz, H-5″a), 4.56 (d, 1 H, $J_{1',2'}$ 1.60 Hz, H-1′), 4.75 (dd, 1 H, $J_{3,4}$ 10.09 Hz, H-3), 4.88 (m, 1 H, –CH=CH_2), 4.95 (dd, 1 H, $J_{2',3'}$ 3.32 Hz, H-2′), 5.01 (m, 2 H, H-4, H-4′), 5.03 (m, 1 H, –CH=CH_2), 5.06 (dd, 1 H, $J_{3',4'}$ 9.97 Hz, H-3′), 5.27 (broad dd, 1 H, $J_{3'',4''}$ 4.10 Hz, H-3″), 5.39 (broad d, 1 H, $J_{1'',2''} < 1$ Hz, H-2″), 5.49 (broad d, 1 H, H-1″), 5.58 (m, 1 H, –CH=CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6–20.7 (6CH$_3$COO), 62.27 (C-6′), 64.06 (C-5″), 65.99 (C-4′), 67.20 (C-4, C-6), 68.72 (C-5′), 69.01 (C-3′), 69.40 (C-2′), 70.04 (OCH$_2$CH=CH$_2$), 71.90 (C-2, C-3), 73.27 (C-5), 77.76 (C-3″), 81.34 (C-2″), 82.17 (C-4″), 97.15 (C-1′), 99.03 (C-1), 105.97 (C-1″), 118.07 (–CH=CH$_2$), 128.25–133.47 (–CH=CH$_2$, Ar.), 165.12–166.45 (3 C$_6$H$_5$$C$OO), 169.63–170.01 (6CH$_3$$C$OO).

*Allyl 2-*O*-(α-*L*-arabinofuranosyl)-6-*O*-(α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**12**). The esterified derivative **11** was stirred overnight with MeONa in MeOH (6 equiv), the soln was neutralized with ion-exchange resin H^+ (Amberlite IR 120), filtered, diluted with distilled water; the aq soln was extracted twice with $CHCl_3$ then freeze-dried to give **12** (10 mg) as an amorphous solid; R_f 0.26 (8:2, CH_3CN–water); $[\alpha]_D$ +8° (*c* 1, water); ^{1}H NMR (500 MHz, D_2O, 45 °C): δ 3.52 (m, 1 H, H-5), 3.63 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.05 Hz, H-4), 3.67 (dd, 1 H, $J_{2,3}$ 3.10 Hz, H-3), 3.70 (m, 2 H, H-4′, H-5′), 3.72 (dd, 1 H, $J_{4'',5''b}$ 6.03, $J_{5''a,5''b}$ 12.25 Hz), 3.77 (dd, 1 H, $J_{5',6'b}$ 5.48, $J_{6'a,6'b}$ 12.06 Hz, H-6′b), 3.81 (dd, 1 H, $J_{4'',5''a}$ 3.48 Hz, H-5″a), 3.82 (dd, 1 H, $J_{5,6b}$ 2.01, $J_{6a,6b}$ 11.33 Hz, H-6b), 3.83 (dd, 1 H, H-3′), 3.90 (dd, 1 H, $J_{5',6a}$ 2.02 Hz, H-6′a), 3.93 (dd, 1 H, $J_{5,6a}$ 5.66 Hz, H-6a), 3.96 (dd, 1 H, $J_{2'',3''}$ 3.11, $J_{3'',4''}$ 5.48 Hz, H-3″), 401 (dd, 1 H, $J_{1',2'}$ 1.82, $J_{2',3'}$ 3.47 Hz, H-2′), 4.19 (broad dd, 1 H, H-2), 4.20–4.23 (m, 2 H, H-2″, H-4″), 4.17–4.22 and 4.34–4.39 (m, 2 H, OCH$_2$CH=CH$_2$), 4.76 (broad d, 1 H, $J_{1,2} \leq 1$ Hz, H-1), 4.92 (d, 1 H, H-1′), 5.35 (d, 1 H, $J_{1,2} \leq 1$ Hz, H-1″), 5.26–5.38 (m, 2 H, –CH=CH_2), 5.96–6.04 (m, 1 H, –CH=CH$_2$); ^{13}C NMR (125 MHz, D_2O, 45 °C): δ 61.28 (C-6′), 61.56 (C-5″), 66.68 (C-6), 67.08 (C-4), 67.48 (C-3), 70.29 (C-2′), 70.65 (C-3′), 70.94 (OCH$_2$CH=CH$_2$), 72.65 (C-5′), 73.03 (C-4′), 74.83 (C-2, C-5), 76.94 (C-3″), 81.14 (C-2″), 84.69 (C-4″), 99.86 (C-1′), 100.08 (C-1), 108.03 (C-1″), 118.28 (–CH=CH$_2$), 134.00 (–CH=CH$_2$).

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