Highly Regio- and Stereoselective Synthesis of Bioactive Oligosaccharides Using 1,2-*O*-Ethylidene-α-D-gluco- and -*β*-D-Mannopyranose as the Acceptors and Acetobromosugars as the Donors via Ortho Ester Intermediates

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This paper presents a new, and effective method for highly regio- and stereoselective synthesis of oligosaccharides with 1,2-trans linkages using 1,2-*O*-ethylidenated gluco- and mannopyranose as the acceptors and simple acetobromosugars as the glycosyl donors through ortho ester formation−rearrangement strategy. Biologically important 3,6-branched oligosaccharides such as the phytoalexin elicitor hexasaccharide and ConA-binding 3,6-branched mannotriose were synthesized readily in high yields by the new method.

It is well-known that cell surface oligosaccharides play important roles in many biological processes,[1] such as cell-to-cell recognition, interaction, and signal transduction, and attachment of pathogen to host cells, etc. For intensive study on the relation between structure and bioactivity, great efforts have been devoted to the development of new, easy, and effective methods for regio- and stereoselective synthesis of oligosaccharides. So far, most of the reported methods[2] involve the use of diverse protecting groups and multiple protection−deprotection steps. It is of particular interest, and a great challenge as well, for synthetic organic chemists to use unprotected or less protected sugars as raw materials for glycosylation since the synthetic procedures would be substantially simplified. In our preceding communication,[3] we reported a new method of glycosylation using unprotected glycosides as the acceptors and acetobromosugars as the donors via ortho ester intermediates, and 1→6 linked oligosaccharides were obtained in satisfactory yields. Also, OH-3-selective glycosylation using partially protected glucose acceptors with free hydroxyl groups at C-2 and C-3 or at C-3 and C-4 was achieved. It was found, however, that glycosylation using unprotected glycosides as the acceptors was rather slow and difficult to monitor due to the poor solubility of the acceptors in the reaction media. 1,2-*O*-Ethylidenated sugars are easily available materials[4] with better solubility, and when OH-3 is blocked, OH-4 and OH-6 showed differences in coupling with acetobromoglucose, giving selectively an ortho ester at O-6.[5] This inspired us to use 1,2-*O*-ethylidenated sugars having OH-3, OH-4, and OH-6 free as the acceptor to synthesize biologically important oligosaccharides.

The phytoalexin elicitor hexasaccharide has been well-known as the basic structure for elicitor activity.[6] It has the same action as the corresponding heptasaccharide which is effective in very low doses—approximately 0.1 pmol per cotyledon[7a]—and gives a half-maximal activity at a concentration of 10 nM.[7b] The high activity, low toxicity, and applicability in a wide range of plant species open a possible use for the oligosaccharides in agriculture as "green pesticide" provided their facile and economical synthesis is available. Several methods have been reported,[5,8] but the procedures were still relatively complex. Here, we present a very effective and practical synthesis of the hexasaccharide based on the ortho ester formation−rearrangement method as shown in Scheme 1.

Coupling of acetobromoglucose **1**[9] (2.5 equiv) with 1,2-*O*-ethylidene-(*R*,*S*)-α-D-glucopyranose[4a] (**2**, 1 equiv) promoted by silver triflate (2.2 equiv) with dichloromethane as the solvent in the presence of 2,4-lutidine (2.4 equiv) at room temperature afforded the diortho ester **3** in a high yield (92%, based on acceptor **2**) as the sole product, and no 4,6- or 3,4-linked diortho ester isomers were

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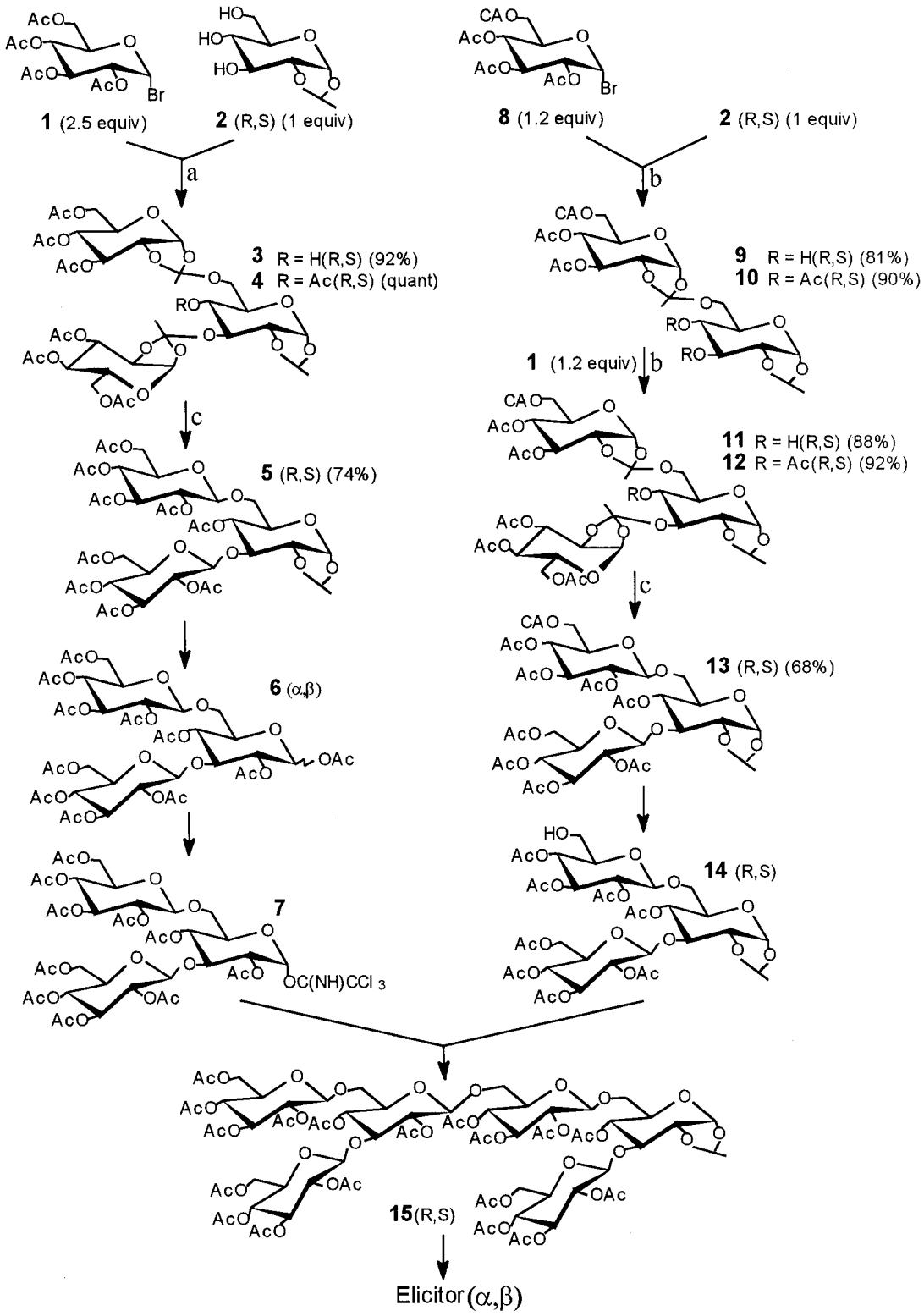
Scheme 1[a]



[a] Conditions and reagents: (a) AgOTf (2.2 equiv), 2,4-lutidine (2.4 equiv), MS 4 Å, CH_2Cl_2, RT, 5 h. (b) AgOTf (1.2 equiv), 2,4-lutidine (1.4 equiv), MS 4 Å, CH_2Cl_2, RT, 5 h. (c) TMSOTf (0.1 equiv), MS 4 Å, CH_2Cl_2, −30 °C, 30−40 min.

detected. Acetylation of **3** with acetic anhydride in pyridine quantitatively gave the diortho ester **4**, and the structure of **4** was unambiguously verified from its 2-D ^{1}H NMR spectrum showing H-1′, H-1″ at δ 5.72, 5.78 ppm and two singlets of 1.75, 1.69 ppm for CH_3CO_3 respectively characteristic for diortho ester. Rearrangement[3,5,10] of **4** catalyzed by TMSOTf (0.1 equiv) furnished the 3,6-branched trisaccharide **5** as a mixture of *R* and *S* isomers in a satisfactory yield, and **5** gave physical data the same as previously reported for the same compounds obtained

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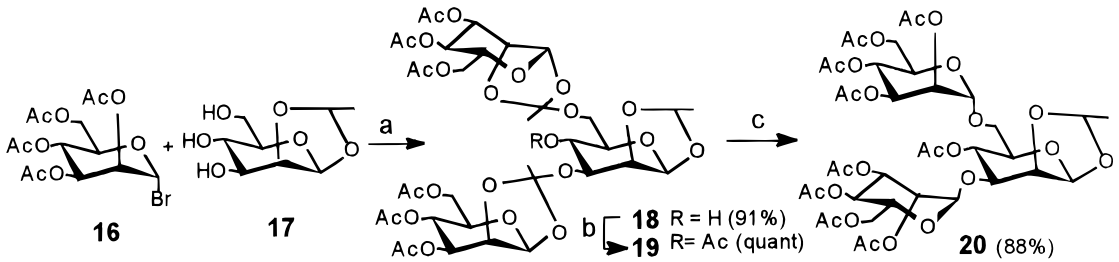
Scheme 2



[a] Conditions and reagents: (a) AgOTf (2.2 equiv), lutidine (2.4 equiv), CH_2Cl_2, MS 4 Å, RT, 5 h. (b) Ac_2O, pyridine (dry). (c) TMSOTf (0.1 equiv), CH_2Cl_2, MS 4 Å, −30 °C, 30 min.

by another method.[5] Here we present a one-step, highly regio- and stereoselective synthesis of diortho ester, and its successful rearrangement to the trisaccharide. In contrast, when the glycosylation was carried out under similar conditions but in the absence of lutidine (not through ortho ester intermediate), a complex mixture was obtained. Conversion of **5** to fully acetylated trisaccharide **6** and subsequent preparation of trisaccharide donor **7** were carried out according to the reported procedure.[5]

The trisaccharide acceptor **14** was prepared in a different way since its 3- and 6-positions needed to bear substituent-different glucose residues. Thus condensation of 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-α-D-glucopyranosyl bromide[11] (**8**, 1.2 equiv) with the triol **2** (1 equiv) under similar conditions as described for the preparation of **3** furnished the ortho ester **9** in a good yield (81%). Some diortho ester was formed when excess **8** was used. The structure of **9** was confirmed from its acetylation (giving **10**), and the 2-D ^{1}H NMR spectrum of **10** gave H-3 at δ 5.22 ppm and H-4 at δ 4.95 ppm, characteristic for 6-linked ortho ester. Coupling of **9** with **1** selectively afforded the 3,6-branched diortho ester **11** in a high yield (88%). Acetylation of **11** (giving **12**) followed by rearrangement resulted in the trisaccharide **13**, which gave physical data the same as reported[5] for the same compound prepared in another way. Dechloroacetylation of **13** (yielding **14**), and condensation of **14** with the donor **7** were carried out as previously described[5] affording the hexasaccharide **15**, which can be readily converted to the glucohexaose elicitor.

Encouraged by the success in the facile synthesis of the phytoalexin elicitor using naked 1,2-*O*-ethylidene-α-D-glucopyranose as the glycosyl acceptor, our attention turned to the synthesis of biologically important 3,6-branched mannotriose using 1,2-*O*-ethylidene-*β*-D-mannopyranose[4a,b] (**17**) as the acceptor. It is well-known that αMan*p*(1→6)[αMan*p*(1→3)]Man*p* is present in all asparagine-linked oligosaccharides, and it is the major ConA-binding epitope on oligomannose-type carbohydrates.[12] The reported syntheses of the trisaccharide involve the use of different protective groups and lengthy reaction routes[13] leading to the product in low yields. Employing the new developed ortho ester formation−rearrangement strategy, the trisaccharide was readily synthesized through coupling acetobromomannose **16** (2.4 equiv, prepared according to the procedure for acetobromoglucose[9]) with the triol **17** (1 equiv) in the presence of AgOTf (2.2 equiv), 2,4-lutidine (2.4 equiv) followed by rearrangement as shown in Scheme 2.

Again, a facile, one-step synthesis of the diortho ester **18** was achieved, and no 4-substitution was found. The structure of **18** was unambiguously verified from its ^{1}H NMR spectrum showing two characteristic signals at δ 5.54 and 5.50 ppm for H-1′ and H-1″, and also from its acetylation product **19** giving a new triplet at δ 5.12 ppm for H-4, a clear indication of 3,6-branched glycosylation. TMSOTf-catalyzed rearrangement[3,5,10] of **19** gave the 3,6-branched manno-trisaccharide **20** in a high yield, and its 2-D ^{1}H NMR spectrum gave a clear indication of 3,6-branched glycosylation. Compounds **18**, **19**, and **20** were white crystals enabling their separation and purification easily. The ortho ester **18** is a very important intermediate; modifications at both nonreducing[14] and reducing[15] ends will give a facile route to synthesis of complex oligosaccharides.

In summary, here we present a very effective regio- and stereoselective glycosylation method using 1,2-*O*-ethylidenated glucose and mannose as the glycosyl acceptors and simple acetobromosugars as the glycosyl donors through ortho ester formation−rearrangement. Selective 6-linked disaccharide, and 3,6-branched homo- or heterotrisaccharide with 1,2-*trans*-glycosidic linkages were readily synthesized by the new strategy. This approach, in terms of yields, simplicity, and efficiency, will be a useful method for the synthesis of oligosaccharides. Application of the new method to the synthesis of naturally occurring complex oligosaccharides is in process.

Experimental Section

For general remarks, see ref 4b. All of the described ortho esters and the oligosaccharides containing 1,2-*O*-ethylidenated sugar residues were obtained as *R*,*S* mixtures which were used directly in the next reaction. *R*,*S* isomer assignment was based on the assignment in the literature[4a] for 1,2-*O*-ethylidene sugar derivatives. Purification of the mixtures usually gave one isomer (*R* or *S*), but isomerization for the pure isomer occurred upon a long-term storage and during NMR determination.

Diortho ester (3). To a stirred mixture of 2,3,4,6-tetra-*O*-α-D-glucopyranosyl bromide (**1**, 1020 mg, 2.5 mmol), 2,4-lutidine (276 μL, 2.4 mmol), 1,2-*O*-ethylidene(*R*,*S*)-α-D-gluco-

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pyranose **2** (206 mg, 1 mmol), and 4 Å molecular sieves (0.5 g) in dichloromethane (20 mL) under nitrogen atmosphere was added silver triflate (565 mg, 2.2 mmol) in a dark room, and the reaction was carried out at room temperature and monitored by TLC (1:1.5 petroleum ether/ethyl acetate). After completion of the reaction, the mixture was partitioned between dichloromethane and water, the organic phase was washed with 10% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, and brine, then dried over anhydrous Na_2SO_4, concentrated, and subjected to column chromatography with 1:1 petroleum ether/ethyl acetate as the eluent, giving the diortho ester **3** in a high yield (*R*,*S*, 92%, 796 mg, based on acceptor **2**) as the sole product. Further purification by column chromatography (1/1 petroleum ether/ethyl acetate) gave *S* isomer: $[\alpha]_D^{20}$ +40.6° (*c* 0.9, $CHCl_3$); ^{1}H NMR: δ (*S*) 5.80 (d, 1 H, $J_{1'',2''}$ = 5.3 Hz, H-1″), 5.71 (d, 1 H, $J_{1',2'}$ = 5.2 Hz, H-1′), 5.50 (d, 1 H, $J_{1,2}$ = 5.6 Hz, H-1), 5.50 (q, 1 H, *J* = 4.9 Hz, CH_3CH), 5.19 (t, 1 H, *J* = 2.9 Hz, H-3″), 5.15 (t, 1 H, *J* = 2.8 Hz, H-3′), 4.90 (2 dd, 2 H, $J_{2',3'} = J_{2'',3''}$ = 2.7 Hz, $J_{3',4'} = J_{3'',4''}$ = 9.6 Hz, H-4′, 4″), 4.52−4.50 (m, 1 H, H-2″), 4.37−4.35 (m, 1 H, H-2′), 4.20−4.19 (m, 4 H, H-6′, 6″), 4.09 (t, 1 H, *J* = 5.6 Hz, H-2), 3.95−3.92 (m, 2 H, H-5′, 5″), 3.86 (dd, 1 H, $J_{2,3}$ = 5.6 Hz, $J_{3,4}$ = 8.0 Hz, H-3), 3.77−3.75 (m, 2 H, H-6), 3.75−3.68 (m, 1 H, H-5), 3.55 (t, 1 H, *J* = 8.0 Hz, H-4), 2.11, 2.10, 2.10, 2.10, 2.10, 2.09 (6 s, 18 H, 6 CH_3CO), 1.83, 1.72 (2 s, 6 H, 2 CH_3CO_3), 1.37 (d, 3 H, *J* = 4.9 Hz, CH_3CH). Anal. Calcd for $C_{36}H_{50}O_{24}$: C, 49.89; H, 5.81. Found: C, 50.02; H, 5.90.

Diortho ester (4). Treatment of compound **3** (700 mg, 0.8 mmol) with Ac_2O/pyridine (2/3 mL/mL) furnished compound **4** (720 mg) in almost quantitative yield. Purification by column chromatography (2/1 petroleum ether/ethyl acetate) gave *R* isomer: $[\alpha]_D^{20}$ +11.8° (*c* 1.3, $CHCl_3$); ^{1}H NMR: δ (*R*) 5.78 (d, 1 H, $J_{1'',2''}$ = 5.2 Hz, H-1″), 5.72 (d, 1 H, $J_{1',2'}$ = 5.2 Hz, H-1′), 5.54 (d, 1 H, $J_{1,2}$ = 5.0 Hz, H-1), 5.19−5.16 (m, 2 H, H-3′, 3″), 5.07 (q, 1 H, *J* = 4.8 Hz, CH_3CH), 5.04−5.00 (m, 1 H, H-4), 4.92−4.88 (bd, 2 H, $J_{4',5'} = J_{4'',5''}$ = 9.4 Hz, H-4′, 4″), 4.41−4.39 (m, 1 H, H-2″), 4.36−4.34 (m, 1 H, H-2′), 4.19−4.17 (m, 4 H, H-6′, 6″), 4.04−4.03 (m, 1 H, H-3), 3.99−3.98 (m, 1 H, H-2), 3.93−3.85 (m, 3 H, H-5, 5′, 5″), 3.58 (dd, 1 H, $J_{5,6a}$ = 3.2 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6_a), 3.53 (dd, 1 H, $J_{5,6b}$ = 4.6 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6_b), 2.14, 2.13, 2.10, 2.09, 2.09, 2.09, 2.09 (7 s, 21 H, 7 CH_3CO), 1.75, 1.69 (2 s, 6 H, 2 CH_3CO_3), 1.49 (d, 3 H, *J* = 4.8 Hz, CH_3CH). Anal. Calcd. for $C_{38}H_{52}O_{25}$: C, 50.22; H, 5.77. Found: C, 50.14; H, 5.91.

4-*O*-Acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1,2-*O*-(*R*,*S*)ethylidene-α-D-glucopyranose (5). To a stirred mixture of sugar−sugar ortho ester **4** (580 mg, 0.64 mmol) and 4 Å molecular sieves (0.2 g) in dichloromethane (10 mL) was added TMSOTf (12 μL, 0.1 equiv) under nitrogen atmosphere at −30 °C, and the reaction was monitored by TLC (1:1 petroleum ether/ethyl acetate). After completion of the reaction, triethylamine (20 μL) was added to the mixture. The mixture was filtered, and the filtrate was washed with CH_2Cl_2. The combined solution was washed with 1 N HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (2 × 10 mL), then dried over anhydrous Na_2SO_4, concentrated. The residue was subjected to column chromatography with 1:1 petroleum ether/ethyl acetate as the eluent, giving the product **5** (*R*,*S*, 376 mg, 74%) as an amorphous solid. The physical data and ^{1}H NMR spectrum of compound **5** were the same as reported for the same compound prepared by another way.[5] Anal. Calcd for $C_{38}H_{52}O_{25}$: C, 50.22; H, 5.77. Found: C, 50.48; H, 5.58.

3′,4′-Di-*O*-acetyl-6′-*O*-chloroacetyl-α-D-glucopyranose 1′,2′-(1,2-*O*-Ethylidene-α-D-glucopyranos-6-yl Orthoacetate) (9). To a stirred mixture of 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-α-D-glucopyranosyl bromide **8** (530 mg, 1.2 mmol), 2,4-lutidine (160 μL, 1.2 mmol), 1,2-*O*-ethylidene-(*R*,*S*)-α-D-glucopyranose **2** (206 mg, 1 mmol), and 4 Å molecular sieves (0.5 g) in anhydrous dichloromethane (20 mL) under nitrogen atmosphere was added silver triflate (283 mg, 1.1 mmol) in a dark room, and the reaction was carried out at room temperature and monitored by TLC (1:1.5 petroleum ether/ethyl acetate). After completion of the reaction, the mixture was partitioned between dichloromethane and water, the organic phase was washed with saturated aqueous $NaHCO_3$ and 10% $Na_2S_2O_3$, and concentrated, dried, then subjected to column chromatography with 1:1.5 petroleum ether/ethyl acetate as the eluent, giving the disaccharide ortho ester **9** in a good yield (*R*,*S*, 462 mg, 81%, based on acceptor **2**); Further purification by column chromatography (1/1.5 petroleum ether/ethyl acetate) gave *R* isomer: $[\alpha]_D^{20}$ +21.4° (*R*, *c* 1.0, $CHCl_3$); ^{1}H NMR: δ (*R*) 5.74 (d, 1 H, $J_{1',2'}$ = 5.2 Hz, H-1′), 5.55 (d, 1 H, $J_{1,2}$ = 5.1 Hz, H-1), 5.19 (t, 1 H, *J* = 2.7 Hz, H-3′), 5.13 (q, 1 H, *J* = 4.8 Hz, CH_3CH), 4.90 (dd, 1 H, $J_{3',4'}$ = 2.7 Hz, $J_{4',5'}$ = 8.9 Hz, H-4′), 4.42−4.40 (m, 1 H, H-2′), 4.32−4.30 (m, 2 H, H-6′), 4.13 (s, 2 H, $ClCH_2CO$), 4.04 (t, 1 H, *J* = 5.1 Hz, H-2), 4.00−3.90 (m, 2 H, H-3, 4), 3.88−3.85 (m, 1 H, H-5′), 3.76 (d, 2 H, $J_{5,6}$ = 3.2 Hz, H-6), 3.66−3.62 (m, 1 H, H-5), 2.12, 2.10 (2 s, 6 H, 2 CH_3CO), 1.74 (s, 3 H, CH_3CO_3), 1.48 (d, 3 H, *J* = 4.8 Hz, CH_3CH). Anal. Calcd for $C_{22}H_{31}O_{15}Cl$: C, 46.28; H, 5.47. Found: C, 46.41; H, 5.77.

3′,4′-Di-*O*-acetyl-6′-*O*-chloroacetyl-α-D-glucopyranose 1′,2′-(3,4-Di-*O*-acetyl-1,2-*O*-ethylidene-α-D-glucopyranos-6-yl Orthoacetate) (10). To a solution of **9** (100 mg, 0.18 mmol) in anhydrous dichloromethane (5 mL) and pyridine (5 mL) was added 3 mL of Ac_2O dropwise. The mixture was stirred at room temperature for 3 h, at the end of which time the reaction was complete as indicated by TLC (2/1 petroleum ether/ethyl acetate). Ice water was added, and the mixture was diluted with CH_2Cl_2. The organic layer was combined, dried, and concentrated to give **10** (*R*,*S*, 106 mg, 90%) as a syrup. Further purification by column chromatography (2/1 petroleum ether/ethyl acetate) gave *S* isomer: $[\alpha]_D^{20}$ +39.7° (*S*, *c* 1.0, $CHCl_3$); ^{1}H NMR: δ (*S*) 5.73 (d, 1 H, $J_{1',2'}$ = 5.2 Hz, H-1′), 5.61 (q, 1 H, *J* = 4.9 Hz, CH_3CH), 5.60 (d, 1 H, $J_{1,2}$ = 4.7 Hz, H-1), 5.22 (t, 1 H, *J* = 5.7 Hz, H-3), 5.17 (t, 1 H, *J* = 2.7 Hz, H-3′), 4.95 (dd, 1 H, $J_{3,4}$ = 5.7 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 4.89 (dd, 1 H, $J_{3',4'}$ = 2.7 Hz, $J_{4',5'}$ = 8.9 Hz, H-4′), 4.33−4.30 (m, 3 H, H-2′, 6′), 4.22 (dd, 1 H, $J_{1,2}$ = 4.7 Hz, $J_{2,3}$ = 5.7 Hz, H-2), 4.11 (s, 2 H, $ClCH_2CO$), 3.95−3.88 (m, 2 H, H-5, 5′), 3.62 (dd, 1 H, $J_{5,6a}$ = 2.8 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6_a), 3.57 (dd, 1 H, $J_{5,6b}$ = 4.6 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6_b), 2.12, 2.10, 2.09, 2.06 (4s, 12 H, 4 CH_3CO), 1.70 (s, 3 H, CH_3CO_3), 1.33 (d, 3 H, *J* = 4.9 Hz, CH_3CH).

Diortho ester (11). To a stirred mixture of compound **9** (189 mg, 0.33 mmol) and 4 Å molecular sieves (0.5 g), in anhydrous dichloromethane (15 mL), was added acetobromoglucose (148 mg, 0.36 mmol) and 2,4-lutidine (45 μL, 0.39 mmol). The mixture was stirred at room temperature for 1 h under nitrogen atmosphere, and then silver triflate (92 mg, 0.36 mmol) was added in a dark room. The reaction was carried out at room temperature and monitored by TLC (1:1 petroleum ether/ethyl acetate). After the completion of the reaction, the mixture was filtered and the filtrate was treated by the same method as described for the preparation of compound **9**. After column chromatography with 1:1 petroleum ether/ethyl acetate as the eluent, compound **11** (*R*,*S*, 260 mg) was obtained in a yield of 88%. Further purification by column chromatography (1/1 petroleum ether/ethyl acetate) gave *R* isomer: $[\alpha]_D^{20}$ +34.6° (*c* 0.15, $CHCl_3$); ^{1}H NMR: δ (*R*) 5.78 (d, 1 H, $J_{1'',2''}$ = 5.2 Hz, H-1″), 5.72 (d, 1 H, $J_{1',2'}$ = 5.2 Hz, H-1′), 5.50 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 5.19−5.16 (m, 2 H, H-3′, 3″), 5.09 (q, 1 H, *J* = 4.9 Hz, CH_3CH), 4.90 (m, 2 H, H-4′, 4″), 4.64−4.44 (m, 1 H, H-2″), 4.40−4.38 (m, 1 H, H-2′), 4.31−4.29 (m, 2 H, H-6″), 4.21−4.19 (m, 2 H, H-6′), 4.12 (s, 2 H, $ClCH_2CO$), 3.97−3.93 (m, 4 H, H-2, 3, 4, 5″), 3.80−3.72 (m, 3 H, H-5′, 6), 3.74−3.71 (m, 1 H, H-5), 2.11, 2.10, 2.09 (5 s, 15 H, 5 CH_3CO), 1.81, 1.73 (2 s, 6 H, 2 CH_3CO_3), 1.48 (d, 3 H, *J* = 4.9 Hz, CH_3CH). Anal. Calcd for $C_{36}H_{49}O_{24}Cl$: C, 47.98; H, 5.48. Found: C, 47.80; H, 5.36.

Diortho ester (12). As described for the preparation of compound **10**, acetylation of compound **11** (200 mg, 0.22 mmol) with Ac_2O/pyridine/CH_2Cl_2 (3/5/5 mL/mL) furnished acetylated diortho ester trisaccharide **12** (*R*,*S*, 190 mg, 92%); Further purification by column chromatography (1/1 petroleum ether/ethyl acetate) gave *R* isomer: $[\alpha]_D^{20}$ +14.4° (*c* 1.5, $CHCl_3$); ^{1}H NMR: δ (*R*) 5.78 (d, 1 H, $J_{1'',2''}$ = 5.2 Hz, H-1″), 5.71 (d, 1 H, $J_{1',2'}$ = 5.3 Hz, H-1′), 5.54 (d, 1 H, $J_{1,2}$ = 5.0 Hz, H-1), 5.19−5.16 (m, 2 H, H-3′, 3″), 5.07 (q, 1 H, *J* = 4.8 Hz, CH_3CH), 4.99 (bd, 1 H, $J_{4,5}$ = 9.6 Hz, H-4), 4.91 (t, 1 H, *J* = 2.3 Hz, H-4″),

4.89 (t, 1 H, J = 2.2 Hz, H-4′), 4.40−4.38 (m, 1 H, H-2″), 4.36−4.34 (m, 1 H, H-2′), 4.30−4.28 (m, 2 H, H-6″), 4.18 (d, 2 H, $J_{5',6'}$ = 4.0 Hz, H-6′), 4.11 (s, 2 H, ClCH_2CO), 4.05−4.03 (m, 1 H, H-3), 3.99−3.97 (m, 1 H, H-2), 3.92−3.86 (m, 3 H, H-5, 5′, 5″), 3.57 (dd, 1 H, $J_{5,6a}$ = 3.1 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6_a), 3.53 (dd, 1 H, $J_{5,6b}$ = 4.6 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6_b), 2.13, 2.12, 2.10, 2.10, 2.09, 2.09 (6 s, 18 H, 6 CH_3CO), 1.75, 1.69 (2 s, 6 H, 2 CH_3CO$_3$), 1.49 (d, 3 H, J = 4.8 Hz, CH_3CH). Anal. Calcd for $C_{38}H_{51}O_{25}Cl$: C, 48.39; H, 5.45. Found: C, 48.12; H, 5.38.

4-*O*-Acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-*β*-D-glucopyranosyl)-1,2-*O*-(*R*,*S*)-ethylidene-α-D-glucopyranose (13). As described for the preparation of compound **5**, rearrangement of compound **12** (116 mg, 0.12 mmol) with catalytic amount of TMSOTf (2 μL, 0.1 equiv) in anhydrous dichloromathane at −30 °C furnished trisaccharide **13** (*R*,*S*, 78 mg, 68%) as a syrup. Compound **13** showed physical data and ^{1}H NMR spectrum the same as reported.[5] Anal. Calcd for $C_{38}H_{51}O_{25}Cl$: C, 48.39; H, 5.45. Found: C, 48.16; H, 5.31.

Diortho ester (18). As described for the preparation of compound **3**, to a mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **16** (1016 mg, 2.4 mmol), 2,4-lutidine (280 μL, 2.4 mmol), 1,2-*O*-ethylidene-(*R*,*S*)-*β*-D-mannopyranose **17** (208 mg, 1 mmol), and 4 Å molecular sieves (0.5 g) in dichloromethane (20 mL) under nitrogen atmosphere was added silver triflate (570 mg, 2.2 mmol) in a dark room, and the reaction was carried out at room temperature and monitored by TLC (1:1.5 petroleum ether/ethyl acetate). After completion of the reaction treatment of the mixture as described for the preparation of compound **3**, the diortho ester **18** was obtained in a yield of 91% (*R*,*S*, 788 mg, based on acceptor **17**) as crystals. Further purification by column chromatography (1/1 petroleum ether/ethyl acetate) gave *R* isomer: $[\alpha]_D$ +5.2° (*c* 1.5, $CHCl_3$); mp 110−112 °C; ^{1}H NMR: δ_H (*R*) 5.54, 5.50 (2 d, J = 2.7 Hz, H-1′, 1″), 5.30−5.15 (m, 6 H, H-1, 3′, 3″, 4′, 4″, CH_3CH), 4.75, 4.63 (2 dd, $J_{2',3'}$ = 3.8 Hz, $J_{2'',3''}$ = 3.9 Hz, H-2′, 2″), 4.23−4.13 (m, 4 H, H-6′, 6″), 4.06 (dd, 1 H, $J_{1,2}$ = 2.4 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 3.84−3.68 (m, 6 H, H-3, 4, 5′, 5″, 6), 3.40−3.35 (m, 1 H, H-5), 2.11, 2.10, 2.07, 2.07, 2.06, 2.05 (6 s, 18 H, 6 CH_3CO), 1.85, 1.74 (2 s, 6 H, 2 CH_3CO), 1.47 (d, 3 H, J = 5.0 Hz, CH_3CH). Anal. Calcd. for $C_{36}H_{50}O_{24}$: C, 49.89; H, 5.81. Found: C, 49.96; H, 5.89.

Diortho ester (19). Acetylation of compound **18** (610 mg, 0.7 mmol) according to the standard method gave the compound **19** (*R*,*S*, 632 mg) in almost quantative yield. Purification by column chromatography (2/1 petroleum ether/ethyl acetate) gave *R* isomer as crystals: $[\alpha]_D$ +0.4° (*c* 0.1, $CHCl_3$); mp 135−138 °C; ^{1}H NMR: δ_H (*R*) 5.49, 5.43 (2 d, $J_{1',2'}$, $J_{1'',2''}$ = 2.8 Hz, 2.6 Hz, H-1′, 1″), 5.30−5.16 (m, 6 H, H-1, 3′, 3″, 4′, 4″, CH_3CH), 5.12 (t, 1 H, J = 9.6 Hz, H-4), 4.66 (dd, 1 H, $J_{1'',2''}$ = 2.9 Hz, $J_{2'',3''}$ = 3.9 Hz, H-2″), 4.58 (dd, 1 H, $J_{1',2'}$ = 2.8 Hz, $J_{2',3'}$ = 4.0 Hz, H-2′),4.24−4.08 (m, 5 H, H-2, 6′, 6″), 4.01 (dd, 1 H, $J_{2,3}$ = 4.6 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.75−3.65 (m, 2 H, H-5′, 5″), 3.60 (d, 2 H, $J_{6a,6b}$ = 4.5 Hz, H-6), 3.55−3.50 (m, 1 H, H-5), 2.12, 2.10, 2.07, 2.06, 2.06, 2.05, 2.04 (7 s, 21 H, 7 CH_3CO), 1.77, 1.71 (2 s, 6 H, 2 CH_3CO$_3$), 1.49 (d, 3 H, J = 4.9 Hz, CH_3CH). Anal. Calcd. for $C_{38}H_{52}O_{25}$: C, 50.22; H, 5.77. Found: C, 50.01; H, 5.94.

4-*O*-Acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-1,2-*O*-(*R*,*S*)ethylidene-*β*-D-mannopyranose (20). As described for the preparation of compound **5**, rearrangement of compound **19** (260 mg, 0.28 mmol) with catalytic amount of TMSOTf (5 μL, 0.1 equiv) in anhydrous dichloromathane at −30 °C furnished trisaccharide **20** (*R*,*S*, 228 mg, 88%). Further purification by column chromatography (1/1 petroleum ether/ethyl acetate) gave *R* isomer as crystals: $[\alpha]_D$ +16.9° (*c* 0.15, $CHCl_3$); mp 177−178 °C; ^{1}H NMR: δ_H (*R*) 5.36−5.21 (m, 8 H, H-1, 2′, 3′, 3″, 4, 4′, 4″, CH_3CH), 5.14 (dd, 1 H, $J_{1'',2''}$ = 1.9 Hz, $J_{2'',3''}$ = 3.1 Hz, H-2″), 4.98 (d, 1 H, $J_{1'',2''}$ = 1.9 Hz, H-1″), 4.78 (d, 1 H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.34−4.27 (m, 4 H, H-2, 5′, 6″), 4.10−4.01 (m, 3 H, H-5″, 6′), 3.91 (dd, 1 H, $J_{2,3}$ = 3.8 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.80 (dd, 1 H, $J_{5,6a}$ = 6.3 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6_a), 3.62−3.56 (m, 2 H, H-5, 6_b), 2.16, 2.15, 2.15, 2.11, 2.09, 2.06, 2.06, 2.00, 1.98 (9 s, 27 H, 9 CH_3CO), 1.53 (d, 3 H, J = 5.0 Hz, CH_3CH). Anal. Calcd. for $C_{38}H_{52}O_{25}$: C, 50.22; H, 5.77. Found: C, 50.07; H, 5.81.

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