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Synthesis and antihyperlipidemic activity of novel glycosyl fructose derivatives☆

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Abstract—A series of novel di and trisaccharide derivatives containing D-fructose moiety at the reducing end have been synthesized and evaluated for their antihyperlipidemic activity in hyperlipidemic hamster model. Among 11 glycosyl fructose derivatives five compounds showed potent antihyperlipidemic activity either by enhancing high-density lipoprotein (HDL) cholesterol concentration and/or lowering triglyceride (TG) level.

Coronary heart disease (CHD) remains the leading cause of death in the industrialized countries.[2] The primary cause of CHD is atherosclerosis, a disease characterized by the deposition of lipids including cholesterol, in the arterial vessel wall, resulting in a narrowing of the vessel passages and ultimately hardening of the vascular system, which may lead to ischemic heart disease, myocardial infarction, and cerebrovascular incidents. The angiographic studies have well established the fact that one of the risk factors for atherosclerotic cardiovascular disease comprises low levels of high-density lipoprotein (HDL) cholesterol concentration and shows an inverse correlation.[3–5] The Framingham Heart Study[6] showed that a 10 mg/dL increase in HDL cholesterol was associated with a 19% decrease in coronary artery disease death and a 12% decrease in all causes of mortality.[7] One of the plausible mechanisms for the protective action of HDL from coronary heart disease may be that HDL collects particles from the cells[8] and other circulating lipoprotein[9] which in turn counteracts the role of LDL cholesterol thus preventing the formation of atherosclerotic lesions.[10,11] The cholesterol rich lipoprotein returns to the liver, where the cholesterol is unloaded. Some of this cholesterol is recycled or converted into bile salts which are ultimately excreted out.[5,11] Therefore, agents that increase HDL cholesterol concentration in the blood and thereby ratio of HDL cholesterol to total cholesterol (H/C) would have promising therapeutic utility as antihyperlipidemic agents.

Lactic acid bacteria present in the body produce an abundant variety of exopolysaccharides (EPSs), which provide an important contribution to human health by acting as prebiotic substrates, nutraceuticals, cholesterol lowering agents or immunomodulants.[12] Most of the fructo oligosaccharides isolated have been put to various pharmaceutical uses such as bifidus factor[13] and anti-cariogenic agents.[14] Among all isolated fructo oligosaccharides, D-raffinose, lactosucrose, and D-melezitose have been used in health food materials as well as food substituents for various animals.[15] They have been used as osteoporosis preventing agents.[16] Sulfated raffinose derivatives have also been reported as antiarteriosclerotic agent.[17] Besides this, lactosucrose and lactulose, used as health food materials,[18] have been tested for lowering serum cholesterol and bile cholesterol in the animal model.[19] However, glycosyl-(1 → 3)-fructose compounds have not been studied for their use in the treatment of metabolic disorders like hypercholesterolemia. As a part of our ongoing research program on the development of carbohydrate derived therapeutic of metabolic disorders, we set out to evaluate the pharmaceutical potentiality of several glycosyl-(1 → 3)-fructose derivatives (**4a**–**i**) as antihyperlipidemic agents. Due to the presence of fructose moiety in the

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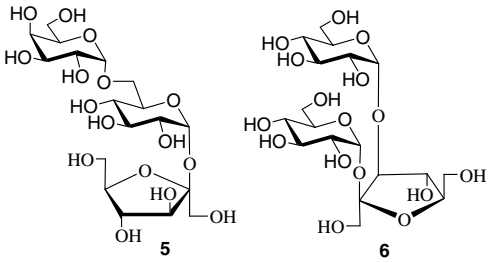
reducing end of D-raffinose (**5**) and D-melezitose (**6**), commercially available fructose oligosaccharides, they have been screened for their antihyperlipidemic activity. The details of synthesis and biological evaluation are reported herein.



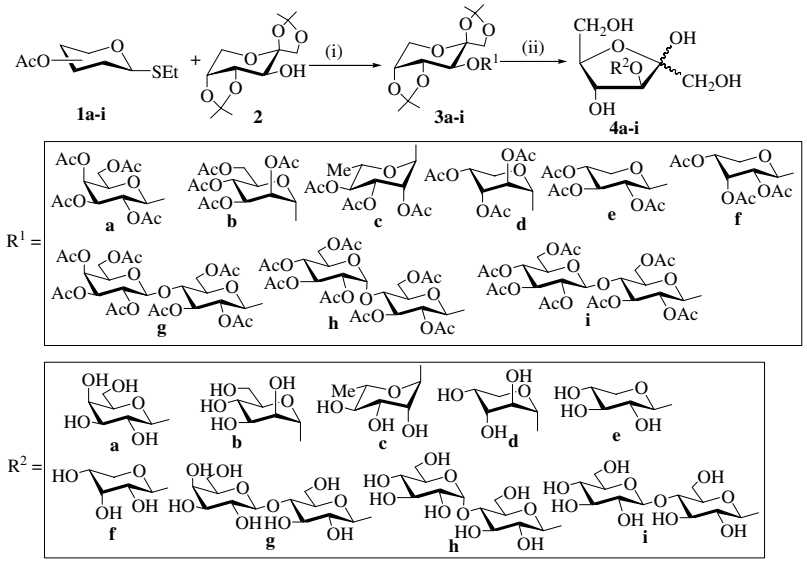
5 **6**

The concise synthesis of compounds **3a**–**i** is outlined in Scheme 1. Compounds **3a**–**i** have been prepared by glycosylation of several per-*O*-acetylated thioglycoside donors[20] (**1a**–**i**) with suitably protected fructose acceptor[21] (**2**) in the presence of *N*-iodosuccinimide (NIS) and $HClO_4$ supported on SiO_2 ($HClO_4$–SiO_2)[22] as glycosyl activator. Removal of isopropylidene acetals using TFA–DCM–H_2O[23] followed by conventional saponification of compounds **3a**–**i** using sodium methoxide furnished deprotected di and trisaccharides **4a**–**i** in excellent yield. All compounds were characterized by IR, mass, NMR, and elemental analysis.[24] Besides a series of synthesized di and trisaccharides, a few commercially available D-fructose containing compounds [D-raffinose (**5**) and D-melezitose (**6**)] have also been evaluated for their antihyperlipidemic activity.

The synthesized as well as commercially available compounds were tested against hyperlipidemia in the high fat diet (HFD) fed hyperlipidemic hamster model against control [i.e., hamsters fed with HFD and given drug vehicle (water) only], by oral administration of 100 mg/kg of the compounds. The results of the biological screening have been summarized in Table 1. Among 11 compounds tested, five compounds (**4a**, **4g**, **4i**, **5**, and **6**) showed potent antihyperlipidemic activity either by increasing HDL cholesterol concentration and/or lowering of TG level and/or increasing HDL cholesterol/total cholesterol ratio (H/C). In order to determine the dose–response activity, compounds **4g** and **4i** were further evaluated at a lower dose (25 mg/kg). In 25 mg/kg dose compound **4g** is equally effective as in the case of 100 mg/kg dose (Table 2).

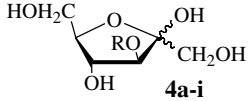
Typical experimental method for glycosylation. To a solution of ethyl per-*O*-acetyl-1-thio-D-glycopyranoside (**1a**–**i**) (1.1 mmol) and 1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**2**) (1.0 mmol) in dry CH_2Cl_2 (5 mL) were added molecular sieves 4 Å (500 mg) and stirred under argon at room temperature for 30 min. The reaction mixture was cooled to 0 °C and *N*-iodosuccinimide (1.2 mmol) was added to it followed by $HClO_4$–SiO_2 (50 mg) and stirred for 1 h at 0 °C. The progress of the reaction was monitored by thin-layer chromatography over silica gel-coated plates. After completion of the reaction, the reaction mixture was diluted with CH_2Cl_2 and filtered through a Celite bed. The filtrate thus obtained was washed with aq $Na_2S_2O_3$, aq $NaHCO_3$, and water. The organic layer was dried (Na_2SO_4) and concentrated to dryness under reduced pressure. The crude reaction mixture was purified over SiO_2 using hexane/EtOAc (4:1) as eluant to furnish desired per-*O*-acetyl-β-D-glycopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**3a**–**i**).

Typical experimental method for deprotection. To a solution of per-*O*-acetyl-β-D-glycopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**3a**–**i**) (1.0 mmol) in CH_2Cl_2 (5.0 mL) were added trifluoroacetic acid (10.0 mL) and water (5.0 mL). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction (TLC), the reaction mixture was concentrated under reduced pressure and co-evaporated with toluene (3 × 20 mL) to furnish desired per-*O*-acetyl-β-D-glycopyranosyl-(1 → 3)-D-fructose. To a solution of the crude product in dry methanol



Scheme 1. (i) Dry DCM, MS 4 Å, NIS, $HClO_4$–SiO_2, 0 °C; (ii) (a) TFA–DCM–H_2O, rt; (b) MeOH, NaOMe, rt.

Table 1. Effect of compounds (100 mg/kg body weight) on biochemical parameters in hyperlipidemic hamsters

Compound	 **4a-i**	$[\alpha]_D^{25}$ (*c* 1.0, H_2O)	TG (mM)	CHOL (mM)	HDL (mM)	GLY (mM)	FFA (μM)	H/C
Vehicle			14.31 ± 3.53	11.56 ± 1.6	3.1 ± 0.61	0.84 ± 0.21	723.93 ± 96.53	0.27
4a	R = β-D-Galactopyranosyl	+3.3	6.88 ± 1.29 (−52%)***	17.59 ± 2.48 (+52%)*	3.60 ± 0.72 (+16%)	0.82 ± 0.11 NC	450.00 ± 37.58 (−38%)**	0.21 (−22%)
4b	R = α-D-Mannopyranosyl	+6.0	10.16 ± 3.44 (−29%)	15.26 ± 3.65 (+32%)	4.33 ± 0.90 (+40%)	0.66 ± 0.24 (−21%)	510.70 ± 96 (−30%)	0.28 (+5%)
4c	R = 6-Deoxy-α-L-mannopyranosyl	−2.7	14.17 ± 2.99 NC	8.82 ± 3.62 (−24%)	3.10 ± 0.40 NC	1.11 ± 0.27 (+32%)	833.12 ± 155 (+15%)	0.35 (+30%)
4d	R = α-D-Arabinopyranosyl	−11.4	15.26 ± 6.86 (+6%)	12.04 ± 6.65 (+4%)	3.49 ± 0.47 (+13%)	1.00 ±0.24 (+19%)	708.69 ± 233 NC	0.29 (+7%)
4e	R = β-D-Xylopyranosyl	−5.4	10.08 ± 3.28 (−30%)	12.00 ± 4.52 NC	3.26 ± 0.70 (+5%)	0.61 ±0.28 (−28%)	692.34 ± 125.0 (−4%)	0.27 NC
4f	R = β-D-Ribopyranosyl	−6.6	13.09 ± 3.10 (−9%)	8.90 ± 2.56 (−23%)	3.14 ± 0.4 NC	0.99 ± 0.17 (+18%)	924.84 ± 220 (+28%)	0.35 (+31%)
4g	R = β-D-Galactopyranosyl (1 → 4)-β-D-glucopyranosyl	+2.1	7.61 ± 5.72 (−47)*	10.55 ± 6.38 (−9%)	3.55 ± 0.96 (+15%)	0.75 ± 0.11 (−11%)	670.62 ± 116 (−8%)	0.34 (+25%)
4h	R = α-D-Glucopyranosyl (1 → 4)-β-D-glucopyranosyl	+5.7	12.76 ± 6.73 (−11%)	11.38 ± 3.14 NC	3.43 ± 0.58 (+11%)	0.82 ± 0.12 NC	726.74 ± 148 NC	0.30 (+12%)
4i	R = β-D-Glucopyranosyl (1 → 4)-β-D-glucopyranosyl	−18.9	6.09 ± 3.22 (−58%)***	9.79 ± 2.24 (−15%)	3.40 ± 0.62 (+10%)	0.81 ± 0.19 (−4%)	726.03 ± 108 NC	0.34 (+29%)
5	α-D-Galactopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-D-fructose	+123	8.06 ± 3.33 (−44%)	7.55 ± 2.42 (−35%)	3.41 ± 0.44 (+10%)	0.86 ± 0.16 NC	540.49 ± 73 (−25%)	0.45 (+66%)
6	α-D-glucopyranosyl-(1 → 3)-β-D-fructofuranosyl-α-D-glucopyranoside	+88.2	6.98 ± 1.73 (−51%)***	13.0 ± 1.68 (+12%)	4.95 ± 0.60 (+59%)***	0.71 ± 0.54 (−15%)	510.70 ± 105.05 (−30%)	0.38 (+41%)

TG, triglyceride; CHOL, cholesterol; HDL, high-density lipoprotein; GLY, glycerol; FFA, free fatty acid; H/C, HDL cholesterol/total cholesterol. Values are means ± SD of eight hamsters in each group. NC, no change as compared to HFD fed group.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 2. Effect of compounds (25 mg/kg body weight) on biochemical parameters in hyperlipidemic hamsters

Compound		TG	CHOL	HDL	GLY	FFA	H/C
Vehicle		14.31 ± 3.53	11.56 ± 1.6	3.1 ± 0.61	0.84 ± 0.21	723.93 ± 96.53	0.27
4g	R = β-D-Galactopyranosyl (1 → 4)-β-D-glucopyranosyl	7.96 ± 3.04 (−44%)*	11.02 ± 1.83 (−5%)	3.28 ± 0.53 (+5%)	0.70 ± 0.21 (−17%)	571.63 ± 82.29 (−21%)	0.30 (+11%)
4i	R = β-D-Glucopyranosyl (1 → 4)-β-D-glucopyranosyl	10.23 ± 5.05 (−29%)	10.97 ± 3.6 (−5%)	2.64 ± 0.56 (−15%)	0.77 ± 0.17 (−8%)	648.52 ± 178.26 (−10%)	0.24 (−11%)

TG, triglyceride; CHOL, cholesterol; HDL, high-density lipoprotein; GLY, glycerol; FFA, free fatty acid; H/C, HDL cholesterol/total cholesterol. Values are means ± SD of eight hamsters in each group.
* $P < 0.05$.

(10.0 mL) was added sodium methoxide (50 mg) and the reaction mixture was stirred at room temperature for 4 h. After completion of the reaction (TLC), the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered, and concentrated to dryness under reduced pressure. The crude reaction mixture was purified over Sephadex LH-20 using MeOH/H_2O (4:1) as eluant to furnish desired β-D-glycopyranosyl-(1 → 3)-D-fructose (**4a**–**i**).

Biological assay. Male golden Syrian hamsters weighing 120–130 g were divided into hyperlipidemic and hyperlipidemic plus compound treated groups. Each group consisted of the eight animals. Hyperlipidemia was produced by feeding with high fat diet (HFD). Hyperlipidemic hamsters had a free access to the HFD and water ad lib during the entire period of the experiment. The test samples were given orally at dose of 100 mg/kg using water as a drug vehicle from day 4 to day 10 (7 days) in the HFD hamsters. Normal hamsters fed with HFD and given drug vehicle (water) only served as control animals. Body weight and diet intake of each animal group were recorded daily to check the effect of the drug on food intake and body weight of the animals.

At the end of experiment, that is, on the 10th day, the blood of the nonfasted animals was withdrawn in two sets of tubes in which one set contains 120 μL NaF (4.5 mg/mL) and tubes were cooled to 0 °C for 15 min. The cold plasma was separated and analysis of the plasma without NaF was performed on the same day for measuring triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL) concentrations using commercially available enzymatic diagnostic kits. Similarly the plasma containing NaF was assayed for glycerol (GLY) and free fatty acid (FFA) concentration using Synchron CX-5 Clinical System Beckmann Coulter Instrument. The data were analyzed for its significance on Prism Software.

In conclusion, a series of novel glycosyl-D-fructose derivatives have been synthesized and shown to be effective HDL elevating and TG lowering agents. Hence, these compounds may be considered as potential antihyperlipidemic agent. Further optimization of better antihyperlipidemic agents derived from carbohydrates is under progress.

Acknowledgments

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2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**3b**): colorless oil (yield 74%); $[\alpha]_D^{25}$ − 5.4 (*c* 1.0, $CHCl_3$); IR (neat): 3023, 2937, 1749, 1374, 1219, 1143, 1083, 1052, 979, 761, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.36 (br s, 2H), 5.30–5.26 (m, 2H), 4.36–4.24 (m, 2H), 4.21–4.19 (m, 2H), 4.14–4.12 (m, 2H), 4.07–3.96 (m, 2H), 3.78–3.74 (m, 2H), 2.16, 2.09, 2.05, 1.98 (4s, 12 H, 4COCH_3), 1.52, 1.45 (2s, 6H, (CH_3)$_2$), 1.34 (s, 6H, (CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 170.1 (3C), 109.9, 109.7, 104.2, 97.4, 77.3, 76.3, 74.1, 72.6, 70.0, 69.8, 69.2, 66.3, 63.2, 60.8, 28.4, 26.8, 26.6 (2C), 21.2, 21.0, 20.9 (2C); ESI-MS: 613 [M+Na]; Anal. Calcd. $C_{26}H_{38}O_{15}$ (590): C, 52.88; H, 6.49%; found: C, 52.62; H, 6.75%.

2,3,4-Tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl-(1 → 3)-1,2:4, 5-di-*O*-isopropylidene-D-fructopyranose (**3c**): colorless oil (yield 76%); $[\alpha]_D^{25}$ − 47.4 (*c* 1.0, $CHCl_3$); IR (neat): 2990, 2938, 1747, 1375, 1222, 1140, 1083, 1052, 763, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.36–5.30 (m, 1H), 5.25–5.18 (m, 1H), 5.15–5.03 (m, 1H), 4.87–4.83 (m, 1H), 4.26–4.17 (m, 3H), 4.05–3.76 (m, 3H), 3.70–3.64 (m, 2H), 2.14, 2.04, 1.95 (3s, 9H, 3COCH_3), 1.52,1.46 (2s, 6H, (CH_3)$_2$), 1.35 (s, 6H, (CH_3)$_2$), 1.22 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.2 (2), 109.4, 109.2, 104.4, 98.7, 77.5, 76.3, 74.1, 72.1, 71.4, 70.5, 69.6, 67.9, 61.5, 28.6, 26.9 (2C), 26.3, 21.2 (2C), 21.0, 17.7; ESI-MS: 555 [M+Na]; Anal. Calcd. $C_{24}H_{36}O_{13}$ (532): C, 54.13; H, 6.81%; found: C, 53.88; H, 7.10%.

2,3,4-Tri-*O*-acetyl-α-D-arabinopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**3d**): colorless oil (yield 76%); $[\alpha]_D^{25}$ − 33.3 (*c* 1.0, $CHCl_3$); IR (neat): 3022, 2991, 2938, 1748, 1374, 1221, 1067, 768, 689 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.23–5.21 (m, 2H), 5.08–5.06 (m, 1H), 4.59–4.56 (m, 1H), 4.48–4.45 (m, 1H), 4.29–4.20 (m, 2H), 4.07–4.01 (m, 2H), 3.94–3.87 (d, *J* = 11.2 Hz, 1H), 3.73–3.59 (m, 2H), 3.52–3.46 (d, *J* = 11.9 Hz, 1H), 2.12, 2.08, 2.00 (3s, 9H, 3COCH_3), 1.51, 1.44, 1.40, 1.33 (4s, 12H, 2(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 170.4, 169.6, 109.6, 105.1, 104.6, 99.7, 74.4, 73.6, 72.2, 70.6 (2C), 69.7, 68.2, 63.9, 60.4, 28.4, 26.8 (2C), 26.0, 21.3, 21.1, 21.0; ESI-MS: 541 [M+Na]; Anal. Calcd. $C_{23}H_{34}O_{13}$ (518): C, 53.28; H, 6.61%; found: C, 53.02; H, 6.90%.

2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**3e**): yellow oil (yield 77%); $[\alpha]_D^{25}$ − 41.1 (*c* 1.0, $CHCl_3$); IR (neat): 3022, 2338, 1753, 1375, 1221, 1069, 889, 767, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.15–5.11 (m, 1H), 4.95–4.89 (m, 2H), 4.67–4.60 (m, 2H,), 4.42–4.41 (d, *J* = 1.6 Hz, 1H), 4.24–4.20 (m, 1H), 3.98–3.81 (m, 3H), 3.75–3.65 (m, 2H), 3.60–3.59 (m, 1H), 2.06, 2.04 (2s, 9H, 3COCH_3), 1.52, 1.45, 1.35, 1.33 (4s, 12H, 2 (CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 169.8, 169.6, 109.4, 109.0, 102.4, 99.9, 71.3, 70.9, 70.5 (2C), 70.1, 69.4, 68.7, 61.4 (2C), 26.9, 26.2, 25.8, 24.4, 21.1, 21.0 (2C); ESI-MS: 541 [M+Na]; Anal. Calcd. $C_{23}H_{34}O_{13}$ (518): C, 53.28; H, 6.61%; found: C, 53.0; H, 6.86%.

2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**3f**): yellow oil (yield 78%); $[\alpha]_D^{25}$ − 52.5 (*c* 1.0, $CHCl_3$); IR (neat): 2990, 2937, 1749, 1375, 1222, 1074, 979, 771 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.36–5.30 (m, 1H), 5.18–5.13 (m, 1H), 4.94–4.92 (m, 1H), 4.64–4.59 (dd, *J* = 7.8 and 2.6 Hz,

1H), 4.39–4.38 (m, 1H), 4.31–4.18 (m, 2H), 4.08–4.01 (m, 1H), 3.98–3.93 (m, 1H), 3.90–3.89 (m, 1H), 3.84–3.82 (m, 1H), 3.79–3.73 (m, 1H), 3.70–3.68 (m, 1H), 2.12 (s, 6H, 2COCH_3), 2.02 (s, 3H, COCH_3), 1.53, 1.46, 1.41, 1.34 (4s, 12 H, 2 (CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.0 (2C), 109.4, 109.1, 102.3, 98.9, 71.3, 70.3, 70.2, 68.5, 68.0, 66.1, 65.9, 62.1, 61.7, 26.9 (2C), 26.2, 25.7, 21.2 (2C), 21.0; ESI-MS: 541 [M+Na]; Anal. Calcd. $C_{23}H_{34}O_{13}$ (518): C, 53.28; H, 6.61%; found: C, 53.48; H, 6.90%.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-1,2:4,5-di-O-isopropylidene-D-fructopyranose (**3g**): yellow oil (yield 72%); $[\alpha]_D^{25}$ − 25 (*c* 1.0, $CHCl_3$); IR (neat): 3022, 2933, 1752, 1654, 1584, 1373, 1222, 1066, 909, 770 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.34–5.33 (d, *J* = 2.9 Hz, 1H), 5.19–5.06 (m, 2H), 4.98–4.93 (m, 1H), 4.68–4.63 (dd, *J* = 7.9 Hz, 1H), 4.68–4.46 (m, 2H), 4.43–4.38 (m, 1H), 4.20–4.14 (m, 2H), 4.12–4.07 (m, 2H), 4.05–3.96 (m, 3H), 3.89–3.84 (m, 3H), 3.79–3.74 (dd, *J* = 4.2 and 9.9 Hz, 1H), 3.70–3.66 (m, 2H), 2.15 (s, 3H, COCH_3), 2.06 (s, 6H, 2COCH_3), 2.04 (s, 9H, 3 COCH_3), 1.96 (s, 3H, COCH_3), 1.51, 1.46, 1.36, 1.33 (4s, 12H, 2 (CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7 (2C), 170.5 (2C), 170.2, 170.0, 169.5, 109.3, 108.9, 104.0, 101.5, 101.4, 78.8, 76.7, 75.8, 73.7, 73.5, 73.1, 72.2, 71.3, 71.0, 69.5, 67.0, 62.5, 61.3, 61.2, 60.7, 26.9, 26.7, 26.5, 26.3, 21.1 (3C), 20.9 (4C); ESI-MS: 901 [M+Na]; Anal. Calcd. $C_{38}H_{54}O_{23}$ (878): C, 51.93; H, 6.19%; found: C, 51.68; H, 6.50%.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (3 h): yellow oil (yield 75%); $[\alpha]_D^{25}$ + 7.2 (*c* 1.0, $CHCl_3$); IR (neat): 1753, 1372, 1227, 1043, 767 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.41–5.36 (m, 2H), 5.34–5.30 (m, 1H), 5.25–5.20 (dd, *J* = 10.8 and 3.3 Hz, 1H), 5.10–5.00 (dd, *J* = 9.8, 9.8 Hz, 1H), 4.92–4.81 (m, 2H), 4.72–4.64 (t, *J* = 7.6, 7.6 Hz, 1H), 4.61–4.56 (dd, *J* = 7.9 and 3.0 Hz, 1H), 4.42–4.36 (m, 2H), 4.30–4.17 (m, 4H), 4.14–3.94 (m, 3H), 3.92–3.78 (m, 1H), 3.75–3.64 (m, 2H), 2.10 (s, 3H, COCH_3), 2.04, 2.02, 2.00 (3s, 18H, 6 COCH_3), 1.53, 1.51, 1.45 (3s, 12H, 2 (CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9 (2C), 170.3 (2C), 170.2, 170.0, 169.5, 109.3, 109.0, 104.0, 102.2, 100.2, 79.3, 76.0, 73.3 (2 C), 72.7, 72.0, 70.4 (2 C), 70.0 (2 C), 69.7, 68.8, 68.4, 63.3, 61.4, 60.7, 26.8, 26.7 26.4 (2 C), 21.2 (2 C), 21.1 (2C), 20.9 (3C); ESI-MS: 901 [M+Na]; Anal. Calcd. $C_{38}H_{54}O_{23}$ (878): C, 51.93; H, 6.19%; found: C, 51.65; H, 6.50%.

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (3i): yellow oil (yield 73%); $[\alpha]_D^{25}$ − 1.8 (*c* 1.0, $CHCl_3$); IR (neat): 3023, 1753, 1373, 1225, 1094, 767 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.22–5.05 (m, 3H), 5.00–4.87 (m, 2H), 4.66–4.62 (d, *J* = 8.1 Hz, 1H), 4.52–4.46 (m, 2H), 4.40–4.32 (m, 2H), 4.20–4.17 (m, 1H), 4.12–4.10 (m, 1H), 4.06–4.01 (m, 2H), 3.96–3.93 (m, 2H), 3.89–3.77 (m, 2H), 3.68–3.54 (m, 3H), 2.12, 2.08 (2s, 6H, 2COCH_3), 2.03 (s, 6H, 2COCH_3), 2.01 (s, 6H, 2COCH_3), 1.98 (s, 3H, COCH_3), 1.50, 1.45, 1.36, 1.33 (4s, 12 H, 2 (CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8 (2C), 170.5, 170.1, 169.9, 169.6, 169.4, 109.9, 109.4, 104.0, 101.6, 101.2, 78.8, 75.8, 73.7, 73.2 (2C), 73.1, 72.6 (2C), 72.4, 72.3, 68.3 (2C), 62.4, 62.0, 61.4, 28.2, 26.7, 26.4, 26.2, 21.2 (3C), 20.8 (4C); ESI-MS: 901 [M+Na]; Anal. Calcd. $C_{38}H_{54}O_{23}$ (878): C, 51.93; H, 6.19%; found: C, 51.70; H, 6.47%.