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Regiospecific alkaline protease-catalyzed divinyl acyl transesterifications of primary hydroxyl groups of mono- and di-saccharides in pyridine

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Abstract—This paper describes highly selective transesterification reactions, catalyzed by an alkaline protease from *Bacillus subtilis* in pyridine, of several mono- and di-saccharides with divinyl dicarboxylates ranging from 4 to 10 carbon atoms. A series of polymerizable vinyl fatty acid sugar esters were obtained with good selectivity and high yields. Most products had high proportions of the α anomer. The influences of the enzymes, solvents, temperature, and acyl donor chain length on the reaction were studied. Vinyl sugar esters offer a new family of functional water-soluble monomers for preparation of sugar-containing polymers.

Keywords: Monosaccharide; Disaccharide; Vinyl monomer; Enzymatic synthesis; Regioselectivity; Protease

1. Introduction

Synthetic polymers containing sugar branches have attracted considerable interest.[1] They have recently been a focus of intensive research as functional materials for drug delivery systems, biocompatible devices, and biodegradable polymers.[1–3] However, functionalization of polymers with saccharides via chemical routes is complicated and needs specific blocking/deblocking steps. Since Klibanov and co-workers first demonstrated selective monosaccharide acylation catalyzed by lipases in organic media,[4] various studies concerning similar biotransformations have been reported.[5,6] Carbohydrates bearing vinyl acyl esters can be obtained by selective enzyme-catalyzed transformations, and they offer a new family of functional water-soluble monomers for preparation of sugar-containing polymers. Lately, the enzymatic acylation of carbohydrates with vinyl acrylate or trifluoroethyl (meth)acrylate was reported, and the acryloyl carbohydrate obtained was chemically polymerized to yield a sugar-based polyacrylate.[7–9] However, the main polyacrylate chain was not biodegradable.

Divinyl dicarboxylates have higher transesterification reactivity than trihaloethyl carboxylates. They proved very useful acyl donors for synthesis of polymerizable sugar vinyl esters.[10–12] One vinyl ester group of a divinyl dicarboxylate could be used in transesterification reactions and the other as a polymerizable group. The polymer obtained has sugar branches and a poly(vinyl alcohol) main chain, and was shown to be a biodegradable polymer.[13] To the best of our knowledge, most of the research performed in this area have been devoted to the synthesis of vinyl fatty acid esters of glucose,[11–14] whereas di-saccharides or other monosaccharides had rarely been studied.

In this paper, in order to prepare various carbohydrate-containing polymers and investigate their novel application as bio-polyelectrolytes in nano-encapsulation of drug micro-particles, we have extended the methodology for preparing new, polymerizable vinyl long chain fatty acid esters from several monosaccharides, namely

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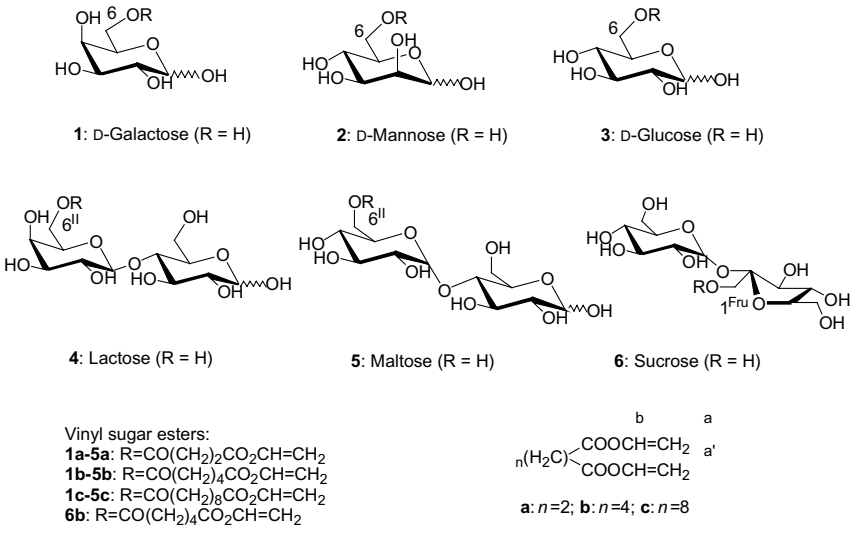

Figure 1. Structures of saccharide substrates and vinyl sugar ester monomers.

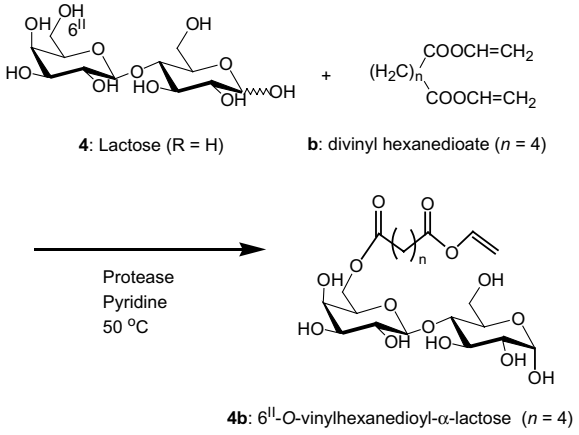

Figure 2. Enzymatic synthesis of vinyl lactose esters **4b** ($n=4$).

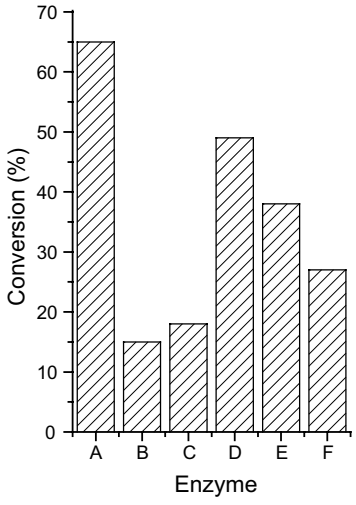

Figure 3. Enzyme screen for transesterification reactions of D-glucose. Experimental conditions: 0.3 mmol glucose, 1.2 mmol divinyl hexanedioate, 50 mg/mL enzyme, 3 mL pyridine. The reaction was carried out at 50 °C for 24 h. Enzyme A: alkaline protease from *B. subtilis*; B: Proteinase from *A. orgzae*; C: Lipase from porcine pancreas (PPL); D: Lipase from hog pancreas (HPL); E: lipase from *C. cylindracea* (CCL); F: Lipozyme®.

D-galactose (**1**), D-mannose (**2**), D-glucose (**3**), and the disaccharides β-D-galactopyranosyl-(1→4)-D-glucose (lactose, **4**), α-D-glucopyranosyl-(1→4)-D-glucose (maltose, **5**), β-D-fructofuranosyl α-D-glucopyranoside (sucrose, **6**) by transesterification reaction with divinyl dicarboxylates (**a**–**c**, $n=2$, 4, 8) (Figs. 1 and 2). A series of polymerizable vinyl fatty acid sugar esters was obtained with good selectivity and high yields. The influence of the enzymes, solvents, temperature, and acyl donor chain length on the reaction was studied.

2. Results and discussion

Enzymes derived from a variety of sources, including bacteria, yeasts, and molds exhibit different activities and specificities. Six commercially available enzymes were tested for the transesterification of D-glucose with divinyl hexanedioate in anhydrous pyridine at 50 °C for 24 h. The results were compared as shown in Figure 3. The extent of glucose conversion was determined by GLC. In the absence of enzyme, no substrate conversion was observed. The percent conversion of the substrates catalyzed by the six enzymes ranged from 15% to 70%. The best result was obtained from the alkaline protease from *Bacillus subtilis*. To make this synthesis more practical, we employed the alkaline protease from *B. subtilis*, a crude enzyme that was also an efficient catalyst in the transesterification,[12,15–17] because of its low cost and the possibility of industrial application.

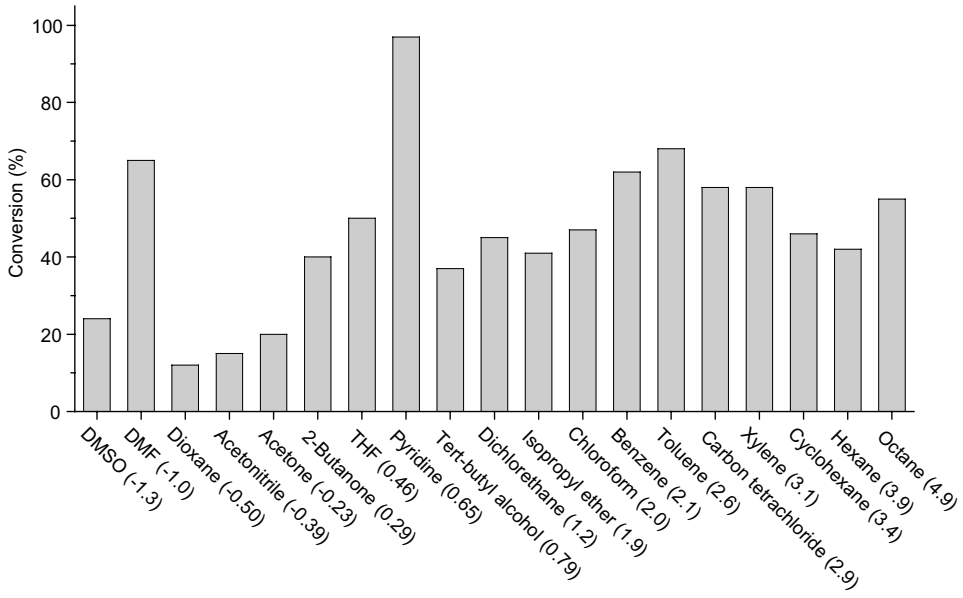

Figure 4. Effect of solvents on conversion of the transesterification reaction. Experimental conditions: 0.1 mmol D-glucose, 0.4 mmol divinyl butanedioate, 50 mg/mL alkaline protease from *B. subtilis*, 1 mL solvent. The reaction was carried out at 50 °C for 48 h.

Previous studies showed that the choice of solvent was important for efficient ester synthesis in an organic medium.[18,19] The solvent plays two important roles. On the one hand, the solvent must maintain enzyme catalytic activity. On the other hand, it must dissolve both carbohydrates and acyl donor substrates. In our study, the formation of the vinyl glucose ester was detected in 19 different solvents under consideration (Fig. 4). *N*,*N*-Dimethylfomamide (DMF), pyridine, and Me_2SO are all hydrophilic organic solvents that can dissolve carbohydrates. In general, enzymatic esterification was not effective in these polar solvents, but the activity of the alkaline protease from *B. subtilis* in anhydrous pyridine for the transesterification described here was the highest. Other polar solvents, such as 1,4-dioxane, acetonitrile, and acetone were not suitable for this protease. In contrast, apolar solvents, such as hexane, octane, cyclohexane, were suitable for ester synthesis, but the reaction was hindered by the low solubility of sugars. As a consequence, synthesis was possible, but yields were low.

The effect of temperature on the catalytic activity of the alkaline protease from *B. subtilis* in the transesterification is shown in Figure 5. The conversion rate at the early stage of reaction (4 h) increased with the increase of the temperature over the range of 16–50 °C and decreased over the range of 50–80 °C. The optimum temperature for the alkaline protease from *B. subtilis* for the vinyl sugar ester synthesis was thus 50 °C, and the protease became gradually inactivated above 50 °C. The conversion of glucose between 16 and 80 °C increased with increasing reaction time and then reached a plateau of complete conversion.

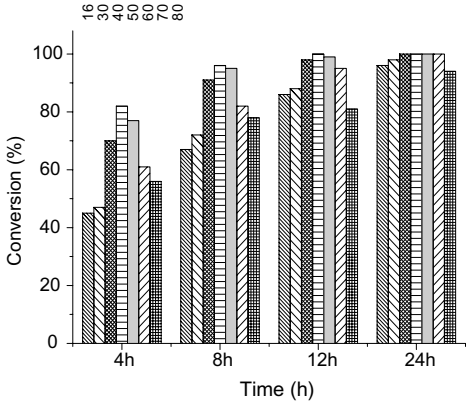

Figure 5. Effect of temperature on conversion level for the transesterification reaction between D-glucose and divinyl butanedioate as catalyzed by alkaline protease from *B. subtilis*. Experimental conditions: 0.5 mmol D-glucose, 2 mmol divinyl butanedioate, 50 mg/mL alkaline protease from *B. subtilis*, 5 mL pyridine.

The position of acylation in the enzymatically prepared vinyl fatty acid sugar esters was verified by ^{13}C NMR according to the general strategy described by Yoshimoto et al.[20] As noted by them, acylation of a hydroxyl group of sugar results in a downfield shift of the peak corresponding to the *O*-acylated carbon and an upfield shift of the peak corresponding to the neighboring carbon. The reactions were very regioselective towards a specific hydroxyl group in the sugar substrate. The acylation of monosaccharides (galactose **1**, mannose **2**, glucose **3**) occurred preferentially at the primary hydroxyl group, whereas lactose (**4**) and maltose (**5**)

Table 1. Highly selective enzymatic synthesis of vinyl fatty acid sugar ester monomers

Entry	Sugar substrate	Acyl donor	Products	Acylation site[a]	Yield[b] (%)	Anomeric ratio (α/β)[c]
1	Galactose	a	**1a**	6	70(0.5d)	100:0
2	Galactose	b	**1b**	6	47(3d)	100:0
3	Galactose	c	**1c**	6	35(5d)	100:0
4	Mannose	a	**2a**	6	85(0.5d)	82:18
5	Mannose	b	**2b**	6	69(4d)	90:10
6	Mannose	c	**2c**	6	43(5d)	91:9
7	Glucose	a	**3a**[12]	6	80(0.5d)	50:50
8	Glucose	b	**3b**[12]	6	53(3d)	100:0
9	Glucose	c	**3c**[12]	6	38(3d)	100:0
10	Lactose	a	**4a**	6^{II}	62(5d)	49:51
11	Lactose	b	**4b**	6^{II}	46(5d)	100:0
12	Lactose	c	**4c**	6^{II}	31(5d)	100:0
13	Maltose	a	**5a**	6^{II}	53(5d)	49:51
14	Maltose	b	**5b**	6^{II}	42(5d)	48:52
15	Maltose	c	**5c**	6^{II}	34(5d)	50:50
16	Sucrose	b	**6b**	1^{Fru}	55(5d)	N

[a] Determined by ^{13}C NMR.
[b] Based on weight of crude samples and as percent of the theoretical.
[c] Determined by NMR and GLC or HPLC.

were specifically acylated at the primary hydroxyl of the non-reducing-end moiety, and the sucrose ester was substituted at the C-1^{Fru} primary hydroxyl group of sucrose **6**.

Products **1a–c**, **3b–c**[12] and **4b–c** (Table 1) were the pure α-pyranose derivatives, as determined by ^{13}C NMR, GLC, or HPLC. The α- and β-D anomeric ratios of the mannose ester products **2a–c** were also higher than that of the starting mannose (78:22). There appear to be few reports on the preparation of α-D sugar esters starting from anomeric mixtures of saccharide substrates by enzyme methods.[21] Most sugar esters reported have been anomeric mixtures.[4,5,14] Degn et al.[22] synthesized some long-chain fatty acid esters of glucose having a high proportion of the α anomer in *tert*-butyl alcohol as solvent, using immobilized lipases from *C. antarctica* and *M. miehei*. However, only when the chain length of acyl donors increased to a certain number (>8), did the enzyme show selective preference for the α-anomer substrate, and no pure α-anomeric products were obtained.

In summary, transesterification reactions with high selectivity catalyzed by an alkaline protease from *B. subtilis* in anhydrous pyridine were investigated and a range of new pure polymerizable, α-anomeric vinyl sugar esters were synthesized with good selectivity and high yields. The reaction conditions, including enzyme selection, solvent, and temperature for the esterification, were optimized. Copolymers of vinyl sugar esters with acrylic acid or allylamine have been prepared and will be reported separately. Studies are in progress concerning copolymer applications as bio-polyelectrolytes in nano-encapsulation of drug micro-particles by layer-by-layer assembly method for controlled release at specific regions.

3. Experimental

3.1. General methods

The alkaline protease from *B. subtilis* (EC 3.4.21.62) was purchased from Wuxi Enzyme Co. Ltd (Wuxi, PR China). Lipase from hog pancreas (EC 3.1.1.3), lipase from *Candida cylindracea* (EC 3.1.1.3), proteinase from *Aspergillus orgzae* (EC 3.4.24.39), and Lipozyme® immobilized from *Mucor miehei* (EC 3.1.1.1), were purchased from Fluka. Lipase from porcine pancreas (EC 3.1.1.3) was from Sigma. Divinyl butanedioate, divinyl hexanedioate, and divinyl decanedioate were produced and purified as described in the patent.[23] D-Galactose, D-mannose, D-glucose, lactose, maltose, sucrose and all other chemicals were of analytical grade. The organic solvents were stored over molecular sieves.

The reaction process was monitored via analyses of samples using thin-layer chromatography (TLC), gas chromatography (GLC) and high-performance liquid chromatography (HPLC). Analytical TLC was performed on silica gel 60 plates using EtOAc–MeOH–H_2O as eluent. Spots were detected by spraying with 10% (v/v) H_2SO_4 in MeOH and heating on a hot plate. GLC was used for the analysis of monosaccharide vinyl esters using a SE-30 capillary column (30 m×0.25 mm). Monosaccharide derivatives were subjected to precolumn derivatization with hexamethyldisilazane and Me_3SiCl. HPLC was carried out by reverse-phase high-performance liquid chromatography (HPLC) using a system equipped with a Waters 2690 Separations Module, an XTerraTM RP-18 Column (5 μm, 150×3.9 mm) and a refractive-index detector (Waters 2410). Infrared spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. The position of

acylation in enzymatically prepared sugar esters was established by ^{13}C NMR (Bruker Avance DMX 500). Me_2SO-d_6 or D_2O were used as solvents and Me_4Si as the internal reference. Mass spectrometry data was obtained on Bruker Esquire-LC for electro-spray (MS-ES) measurements (solvent: methanol; positive mode).

3.2. Synthesis of vinyl esters of galactose, mannose, lactose, maltose, and sucrose

3.2.1. 6-*O*-Vinylbutanedioyl-α-D-galactopyranose (1a). A mixture of D-galactose (1.4 g, 7.8 mmol), divinyl butanedioate (5.3 g, 4 equiv), alkaline protease from *B. subtilis* (1 g, 25 mg/mL), and 40 mL pyridine was shaken at 250 rev./min for 0.5 day at 50 °C. The reactions were terminated by filtering off the enzyme. The pyridine was evaporated. Formation of the sugar ester was confirmed by TLC. The product was isolated by silica gel chromatography with an eluent consisting of EtOAc–MeOH–H_2O (95:10:1, by vol) to give product **1a**; yield: 1.67 g, 70%; colorless crystals; mp 144–146 °C; Pure α-anomeric product; $[\alpha]_D^{25}$ +55 (*c* 0.4, MeOH); IR (KBr): *v* 3405 (v_{O-H}), 1737 ($v_{C=O}$), 1647 ($v_{C=C}$); ^1H NMR (Me_2SO-d_6): δ 7.21 (dd, 1H, $J_{a,b}$ 6.3, $J_{a',b}$ 14.1 Hz, –CH=), 6.25 (d, 1H, $J_{1\text{-OH,H-1}}$ 4.5 Hz, 1-OH of α-D-galactose), 4.94 (s, 1H, H-1 of α-D-galactose), 4.91 (dd, 1H, $J_{a,a'}$ 1.1, $J_{a',b}$ 14.2 Hz, CH_2=), 4.67 (d, 1H, $J_{a,b}$ 6.2 Hz, CH_2=), 4.54 (m, 2H, 3-OH and 4-OH of α-D-galactose), 4.33 (d, 1H, $J_{2\text{-OH,H-2}}$ 6.7 Hz, 2-OH of α-D-galactose), 4.08 (m, 2H, H-6 and H-6′ of α-D-galactose), 3.99 (t, 1H, H-5 of α-D-galactose), 3.66 (s, 1H, H-4 of α-D-galactose), 3.54 (m, 1H, H-3 of α-D-galactose), 3.50 (m, 1H, H-2 of α-D-galactose), 2.7–2.6 (m, 4H, $–CH_2–CH_2–$ of butanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 171.82 (CO), 169.57 (CO), 141.16 (–CH=CH_2), 98.26 (CH_2=CH–), 92.64 (C-1α), 69.19 (C-4α), 68.93 (C-3α), 68.44 (C-2α), 67.52 (C-5α), 64.44 (C-6α), 28.31, 28.22 ($2CH_2$); ESI-MS (*m*/*z*): 345 ($M_{1a}+K^+$), 329 ($M_{1a}+Na^+$), M_{1a} corresponding exactly to the molecular mass of 6-*O*-vinylbutanedioyl-α-D-galactopyranose. Anal. Calcd for $C_{12}H_{18}O_9$: C, 47.06; H, 5.92. Found: C, 47.16; H, 5.96.

3.2.2. 6-*O*-Vinylhexanedioyl-α-D-galactopyranose (1b). D-Galactose (1.4 g, 7.8 mmol), divinyl hexanedioate (6.1 g, 4 equiv), alkaline protease from *B. subtilis* (1 g, 25 mg/mL), 40 mL pyridine, 50 °C, 3 days. Eluent: EtOAc–MeOH–H_2O (95:10:1, by vol); yield: 0.96 g, 47%; colorless crystals; mp 116–118 °C; Pure α-anomeric product; $[\alpha]_D^{25}$ +58 (*c* 0.4, MeOH); IR (KBr): *v* 3394 (v_{O-H}), 1750, 1732 ($v_{C=O}$), 1647 ($v_{C=C}$); ^1H NMR (Me_2SO-d_6): δ 7.22 (dd, 1H, $J_{a,b}$ 6.3, $J_{a',b}$ 14.0 Hz, –CH=), 6.24 (d, 1H, $J_{1\text{-OH,H-1}}$ 4.6, 1-OH of α-D-galactose), 4.93 (t, 1H, *J* 3.9, H-1 of α-D-galactose), 4.89 (dd, 1H, $J_{a,a'}$ 1.1, $J_{a',b}$ 13.9 Hz, CH_2=), 4.65 (dd, 1H, $J_{a,a'}$ 1.1, $J_{a,b}$ 5.7 Hz, CH_2=), 4.54 (m, 2H, 3-OH and 4-OH of α-D-galactose), 4.33 (d, 1H, $J_{2\text{-OH,H-2}}$ 6.5 Hz, 2-OH of α-D-galactose), 4.05 (m, 2H, H-6 and H-6′ of α-D-galactose), 3.99 (t, 1H, H-5 of α-D-galactose), 3.66 (s, 1H, H-4 of α-D-galactose), 3.55 (m, 1H, H-3 of α-D-galactose), 3.50 (m, 1H, H-2 of α-D-galactose), 2.44 (t, 2H, *J* 6.5 Hz, $–CH_2–(CO_2–CH=CH_2)$), 2.31 (t, 2H, *J* 6.5 Hz, $–CH_2–(CO_2$-galactose)), 1.56 (m, 4H, other 2 CH_2 of hexanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.20 (CO), 169.79 (CO), 140.75 (–CH=CH_2), 97.58 (CH_2=CH–), 92.14 (C-1α), 68.78 (C-4α), 68.46 (C-3α), 67.96 (C-2α), 67.12 (C-5α), 63.66 (C-6α), 32.51, 32.17, 23.22, 22.95 ($4CH_2$); ESI-MS (*m*/*z*): 373 ($M_{1b}+K^+$), 357 ($M_{1b}+Na^+$), M_{1b} corresponding exactly to the molecular mass of 6-*O*-vinylhexanedioyl-α-D-galactopyranose.

3.2.3. 6-*O*-Vinyldecanedioyl-α-D-galactopyranose (1c). D-Galactose (1.4 g, 7.8 mmol), divinyl decanedioate (7.9 g, 4 equiv), alkaline protease from *B. subtilis* (1 g, 25 mg/mL), 40 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (95:10:1, by vol); yield: 1.06 g, 35%; colorless crystals; mp 94–95 °C; Pure α-anomeric product; $[\alpha]_D^{25}$ +72 (*c* 0.5, H_2O); IR (KBr): *v* 3394 (v_{O-H}), 1750, 1732 ($v_{C=O}$), 1647 ($v_{C=C}$); ^1H NMR (Me_2SO-d_6): δ 7.22 (dd, 1H, $J_{a,b}$ 6.2, $J_{a',b}$ 13.9 Hz, –CH=), 6.24 (d, 1H, $J_{1\text{-OH,H-1}}$ 4.6 Hz, 1-OH of α-D-galactose), 4.93 (m, 1H, H-1 of α-D-galactose), 4.89 (d, 1H, $J_{a',b}$ 13.9 Hz, CH_2=), 4.65 (d, 1H, $J_{a,b}$ 6.1 Hz, CH_2=), 4.54 (m, 2H, 3-OH and 4-OH of α-D-galactose), 4.34 (d, 1H, $J_{2\text{-OH,H-2}}$ 6.8 Hz, 2-OH of α-D-galactose), 4.05 (m, 2H, H-6 and H-6′ of α-D-galactose), 3.98 (t, 1H, H-5 of α-D-galactose), 3.65 (s, 1H, H-4 of α-D-galactose), 3.55 (m, 1H, H-3 of α-D-galactose), 3.50 (m, 1H, H-2 of α-D-galactose), 2.41 (t, 2H, *J* 7.3 Hz, $–CH_2–(CO_2–CH=CH_2)$), 2.26 (t, 2H, *J* 7.1 Hz, $–CH_2–(CO_2$-galactose)), 1.52, 1.25 (m, 12H, other CH_2 of decanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.42 (CO), 169.42 (CO), 140.75 (–CH=CH_2), 97.51 (CH_2=CH–), 92.14 (C-1α), 68.77 (C-4α), 68.47 (C-3α), 67.96 (C-2α), 67.12 (C-5α), 63.55 (C-6α), 32.89, 32.51, 28.02, 27.98, 27.87, 27.78, 23.90, 23.52 ($8CH_2$); ESI-MS (*m*/*z*): 429 ($M_{1c}+K^+$), 413 ($M_{1c}+Na^+$), M_{1c} corresponding exactly to the molecular mass of 6-*O*-vinyldecanedioyl-α-D-galactopyranose.

3.2.4. 6-*O*-Vinylbutanedioyl-D-mannopyranose (2a). D-Mannose (1.4 g, 7.8 mmol), divinyl butanedioate (5.3 g, 4 equiv), alkaline protease from *B. subtilis* (1 g, 25 mg/mL), 40 mL pyridine, 50 °C, 0.5 day, eluent: EtOAc–MeOH–H_2O (150:10:1, by vol); yield: 2.02 g, 85%; tan amorphous solid; a mixture of α and β anomers; $[\alpha]_D^{25}$ +15.7 (*c* 0.6, MeOH); IR (KBr): *v* 3405 (v_{O-H}), 1737 ($v_{C=O}$), 1647 ($v_{C=C}$); ^1H NMR (Me_2SO-d_6): δ 7.21 (dd, 1H, $J_{a,b}$ 6.1, $J_{a',b}$ 14.0 Hz, –CH=), 6.36 (s, 0.6H, 1-OH of α-D-mannose), 6.25 (d, 0.4H, $J_{1\text{-OH, H-1}}$ 8.1, 1-OH of β-D-mannose), 4.91–4.85 (m, 2.7H, H-1 of D-mannose, one H of CH_2= and 4-OH of D-mannose), 4.63

(d, 1H, $J_{a,b}$ 5.7 Hz, CH_2=), 4.60 (d, 1H, 3-OH of D-mannose), 4.52 (m, 1H, 2-OH of D-mannose), 4.29 (d, 1H, *J* 10.0 Hz, H-6 of D-mannose), 3.99 (m, 1H, H-6′ of D-mannose), 3.70 (m, 1H, H-5 of D-mannose), 3.53 (m, 2H, H-3, H-2 of D-mannose), 3.22 (m, 1H, H-4 of D-mannose), 2.7–2.3 (m, 4H, –CH_2–CH_2– of butanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.36 (CO), 170.07 (CO), 141.61 (–CH=CH_2), 98.71 (CH_2=CH–), 94.74 (C-1α), 94.60 (C-1β), 74.54 (C-5β), 74.01 (C-3β), 70.92 (C-5α), 72.07 (C-2β), 71.88 (C-2α), 70.92 (C-3α), 67.71 (C-4α), 67.31 (C-4β), 64.77 (C-6α,β), 28.81, 28.72 (2CH_2); ESI-MS (*m*/*z*): 345 (M_{2a}+K^+), 329 (M_{2a}+Na^+), M_{2a} corresponding exactly to the molecular mass of 6-*O*-vinylbutanedioyl-D-mannopyranose.

3.2.5. 6-*O*-Vinylhexanedioyl-D-mannopyranose (2b). D-Mannose (1.4 g, 7.8 mmol), divinyl hexanedioate (6.1 g, 4 equiv), alkaline protease from *Bacillus subtilis* (1 g, 25 mg/mL), 40 mL pyridine, 50 °C, 4 days, eluent: EtOAc–MeOH–H_2O (150:10:1, by vol); yield: 1.8 g, 69%; tan amorphous solid; a mixture of α and β anomers; $[\alpha]_D^{25}$ +14.1 (*c* 0.6, MeOH); IR (KBr): 3390 (ν_{O-H}), 1740 ($\nu_{C=O}$), 1647 ($\nu_{C=C}$); ^{1}H NMR (Me_2SO-d_6): δ 7.21 (dd, 1H, $J_{a,b}$ 6.3, $J_{a',b}$ 14.0 Hz, –CH=), 6.36 (d, 0.7H, $J_{1\alpha\text{-OH},H\text{-}1\alpha}$ 4.4 Hz, 1-OH of α-D-mannose), 6.25 (d, 0.3H, $J_{1\beta\text{-OH},H\text{-}1\beta}$ 8.3 Hz, 1-OH of β-D-mannose), 4.91–4.85 (m, 2.6H, H-1 of D-mannose, one H of CH_2= and 4-OH of D-mannose), 4.65 (d, 1H, $J_{a,b}$ 5.7 Hz, CH_2=), 4.61 (d, 1H, 3-OH of D-mannose), 4.55 (m, 1H, 2-OH of D-mannose), 4.29 (d, 1H, *J* 10.2 Hz, H-6 of D-mannose), 3.99 (m, 1H, H-6′ of D-mannose), 3.70 (m, 1H, H-5 of D-mannose), 3.53–3.51 (m, 2H, H-3, H-2 of D-mannose), 3.29 (m, 1H, H-4 of D-mannose), 2.44 (t, 2H, *J* 6.3 Hz, –CH_2–(CO_2–CH=CH_2)), 2.31 (t, 2H, *J* 6.9 Hz, –CH_2–(CO_2-mannose)), 1.56 (m, 4H, other 2 CH_2 of hexanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 173.35 (CO), 170.87 (CO), 141.80 (–CH=CH_2), 98.63 (CH_2=CH–), 94.73 (C-1α), 94.62 (C-1β), 74.52 (C-5β), 74.03 (C-3β), 70.92 (C-5α), 72.05 (C-2β), 71.85 (C-2α), 70.92 (C-3α), 67.69 (C-4α), 67.32 (C-4β), 64.82 (C-6α,β), 33.60, 33.25, 24.32, 24.02 (4CH_2); ESI-MS (*m*/*z*): 373 (M_{2b}+K^+), 357 (M_{2b}+Na^+), M_{2b} corresponding exactly to the molecular mass of 6-*O*-vinylhexanedioyl-D-mannopyranose. Anal. Calcd for $C_{14}H_{22}O_9$: C, 50.30; H, 6.63. Found: C, 50.22; H, 6.56.

3.2.6. 6-*O*-Vinyldecanedioyl-D-mannopyranose (2c). D-Mannose (1.4 g, 7.8 mmol), divinyl decanedioate (7.9 g, 4 equiv), alkaline protease from *B. subtilis* (1 g, 25 mg/mL), 40 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (150:10:1, by vol); yield: 1.3 g, 43%; tan amorphous solid; a mixture of α and β anomers; $[\alpha]_D^{25}$ +13.4 (*c* 0.6, MeOH); IR (KBr): *v* 3466, 3335 (ν_{O-H}), 1742 ($\nu_{C=O}$), 1648 ($\nu_{C=C}$); ^{1}H NMR (Me_2SO-d_6): δ 7.18 (dd, 1H, $J_{a,b}$ 6.0, $J_{a',b}$ 14.1 Hz, –CH=), 6.30 (s, 0.7H, 1-OH of α-D-mannose), 6.20 (d, 0.3H, $J_{1\beta\text{-OH},H\text{-}1\beta}$ 8.1 Hz, 1-OH of β-D-mannose), 4.91–4.85 (m, 2.7H, H-1 of D-mannose, one H of CH_2= and 4-OH of D-mannose), 4.59–4.49 (m, 3H, one H of CH_2= and 2-OH, 3-OH of D-mannose), 4.22 (d, 1H, *J* 10.2 Hz, H-6 of D-mannose), 3.94 (m, 1H, H-6′ of D-mannose), 3.65 (m, 1H, H-5 of D-mannose), 3.49 (m, 2H, H-3, H-2 of D-mannose), 3.23 (m, 1H, H-4 of D-mannose), 2.36 (t, 2H, *J* 7.3 Hz, –CH_2–(CO_2–CH=CH_2)), 2.22 (t, 2H, *J* 6.9 Hz, –CH_2–(CO_2-mannose)), 1.47, 1.20 (m, 12H, other CH_2 of decanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 173.57 (CO), 171.00 (CO), 141.80 (–CH=CH_2), 98.53 (CH_2=CH–), 94.74 (C-1α), 94.61 (C-1β), 74.55 (C-5β), 74.04 (C-3), 70.94 (C-5α), 72.05 (C-2β), 71.85 (C-2α), 70.94 (C-3α), 67.71 (C-4α), 67.34 (C-4β), 64.79 (C-6α,β), 34.02, 33.59, 29.11, 29.06, 28.96, 28.85, 24.99, 24.59 (8CH_2); ESI-MS (*m*/*z*): 429 (M_{2c}+K^+), 413 (M_{2c}+Na^+), M_{2c} corresponding exactly to the molecular mass of 6-*O*-vinyldecanedioyl-D-mannopyranose.

3.2.7. 6^{II}-*O*-Vinylbutanedioyl-lactose (4a). Lactose (3.6 g, $C_{12}H_{22}O_{11}\cdot H_2O$, 10 mmol), divinyl butanedioate (6.8 g, 4 equiv), alkaline protease from *B. subtilis* (2 g, 20 mg/mL), 100 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (90:10:5, by vol); yield: 2.90 g, 62%; yellow syrupy solid; a mixture of α and β anomers; $[\alpha]_D^{25}$ +51.5 (*c* 0.5, MeOH); IR (KBr): *v* 3398 (*v*O–H), 1740 (*v*C=O), 1647 (*v*C=C); ^{1}H NMR (Me_2SO-d_6): δ 7.22 (dd, 1H, $J_{a,b}$ 6.2, $J_{a',b}$ 14.0 Hz, –CH=), 6.67 (br s, 0.5H, 1β-OH of lactose), 6.35 (br s, 0.5H, 1α-OH of lactose), 5.17 (br s, 1H, 2′α-OH and 2′β-OH of lactose), 4.93–4.90 (m, 2H, 2β-OH, H-1α of lactose and one H of CH_2=), 4.81 (s, 1H, 3′α-OH and 3′β-OH of lactose), 4.66 (d, 1H, $J_{a,b}$ 5.9 Hz, CH_2=), 4.57–4.54 (m, 1.5H, 2α-OH, 3β-OH, 6β-OH of lactose), 4.45–4.42 (m, 1H, 4′α-OH and 4′β-OH of lactose), 4.33 (m, 1H, 3α-OH, 6α-OH of lactose), 4.25–4.17 (m, 1.5H, H-1β, H-1′α, H-1′β of lactose), 4.17–4.11 (m, 2H, H_a-6′ and H_b-6′ of lactose), 3.82 (d, 0.5H, *J* 6.3 Hz, H_b-6β of lactose), 3.72–3.20 (m, other α or β H of lactose), 3.18 (0.5H, H-2α of lactose), 2.97 (0.5H, H-2β of lactose), 2.7–2.52 (m, 4H, –CH_2–CH_2– of butanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 171.26 (CO), 169.10 (CO), 140.70 (–CH=CH_2), 97.78 (CH_2=CH–), 103.09 (C-1′), 96.18 (C-1β), 91.51 (C-1α), 80.76 (C-4α), 80.33 (C-4β), 74.34 (C-5β), 74.17 (C-3β), 74.04 (C-2β), 72.36 (C-3′), 71.87 (C-5′), 71.66 (C-5α), 70.78 (C-3α), 69.81 (C-2′), 69.36 (C-2α), 67.71 (C-4′), 63.06 (C-6′), 60.04 (C-6α), 59.78 (C-6β), 28.08, 27.78 (2CH_2); ESI-MS (*m*/*z*): 491 (M_{4a}+Na^+), M_{4a} corresponding exactly to the molecular mass of 6^{II}-*O*-vinylbutanedioyl-lactose. Anal. Calcd for $C_{18}H_{28}O_{14}$: C, 46.15; H, 6.03. Found: C, 46.02; H, 6.06.

3.2.8. 6^{II}-*O*-Vinylhexanedioyl-α-lactose (4b). Lactose (7.2 g, $C_{12}H_{22}O_{11}\cdot H_2O$, 20 mmol), divinyl hexanedioate (15.8 g, 4 equiv), alkaline protease from *B. subtilis* (4 g,

20 mg/mL), 200 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (100:10:5, by vol); yield: 4.56 g, 46%; yellow solid; mp 98–101 °C; pure α-anomeric product; $[\alpha]_D^{25}$ +38 (*c* 0.4, MeOH); IR (KBr): *v* 3390 (v_{O-H}), 1742 ($v_{C=O}$), 1647 ($v_{C=C}$); ^{1}H NMR (Me_2SO-d_6): δ 7.22 (dd, 1H, $J_{a,b}$ 5.7, $J_{a',b}$ 13.1 Hz, –CH=), 6.33 (s, 1H, 1α-OH of lactose), 5.15 (s, 1H, 2′α-OH of lactose), 4.89 (m, 2H, H-1α of lactose and one H of CH_2=), 4.86 (s, 1H, 3′α-OH of lactose), 4.78 (s, 1H, 2α-OH of lactose), 4.65 (d, 1H, $J_{a,b}$ 5.5 Hz, CH_2=), 4.56 (d, 1H, $J_{4'\alpha\text{-OH},H\text{-}4'\alpha}$ 6.1 Hz, 4′α-OH of lactose), 4.43 (m, 1H, 3α-OH of lactose), 4.24 (m, 2H, 6α-OH and H-1′ of lactose), 4.18, 4.09 (2H, H_a-6′α and H_b-6′α of lactose), 3.73 (m, 2H, H-5α and H-4′α of lactose), 3.63, 3.58, 3.56 (m, 4H, H_a-6α, H_b-6α, H-5′α and H-3α of lactose), 3.33 (m, HDO and H-2′α of lactose), 3.39 (m, 1H, H-4α of lactose), 3.17 (m, 1H, H-2α of lactose), 2.45 (m, 2H, –CH_2–(CO_2–CH=CH_2)), 2.34 (m, 2H, –CH_2–(CO_2-lactose)), 1.57 (m, 4H, other 2 CH_2 of hexanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.21 (CO), 169.85 (CO), 140.80 (–CH=CH_2), 97.60 (CH_2=CH–), 103.12 (C-1′), 91.55 (C-1α), 80.70 (C-4α), 72.34 (C-3′), 71.92 (C-5′), 71.71 (C-5α), 70.79 (C-3α), 69.83 (C-2′), 69.23 (C-2α), 67.82 (C-4′), 62.95 (C-6′), 60.01 (C-6α), 32.44, 32.21, 23.14, 23.01 (4CH_2); ESI-MS (*m*/*z*): 519 (M_{4b}+Na^+), M_{4b} corresponding exactly to the molecular mass of 6^{II}-*O*-vinylhexanedioyl-α-lactose.

3.2.9. 6^{II}-*O*-Vinyldecanedioyl-α-lactose (4c). Lactose (3.6 g, $C_{12}H_{22}O_{11}\cdot H_2O$, 10 mmol), divinyl decanedioate (10.2 g, 4 equiv), alkaline protease from *B. subtilis* (2 g, 20 mg/mL), 100 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (120:10:5, by vol); yield: 1.71 g, 31%; yellow solid; mp 124–127 °C; pure α-anomeric product; $[\alpha]_D^{25}$ +42 (*c* 0.4, MeOH); IR (KBr): *v* 3389 (v_{O-H}), 1747 ($v_{C=O}$), 1647 ($v_{C=C}$); ^{1}H NMR (Me_2SO-d_6): δ 7.21 (dd, 1H, $J_{a,b}$ 6.2, $J_{a',b}$ 13.9 Hz, –CH=), 6.34 (d, 1H, $J_{1\alpha\text{-OH},H\text{-}1\alpha}$ 4.1 Hz, 1α-OH of lactose), 5.16 (s, 1H, 2′α-OH of lactose), 4.90–4.87 (m, 3H, H-1α and 3′α-OH of lactose, one H of CH_2=), 4.79 (d, 1H, $J_{2\alpha\text{-OH},H\text{-}2\alpha}$ 4.1 Hz, 2α-OH of lactose), 4.65 (d, 1H, $J_{a,b}$ 5.6 Hz, CH_2=), 4.56 (d, 1H, $J_{4'\alpha\text{-OH},H\text{-}4'\alpha}$ 6.9 Hz, 4′α-OH of lactose), 4.44 (t, 1H, $J_{3\alpha\text{-OH},H\text{-}3\alpha}$ 5.5 Hz, 3α-OH of lactose), 4.22 (s, 2H, 6α-OH and H-1′ of lactose), 4.17–4.07 (2H, H_a-6′α and H_b-6′α of lactose), 3.73 (m, 2H, H-5α and H-4′α of lactose), 3.62, 3.58–3.54 (m, 4H, H_a-6α, H_b-6α, H-5′α and H-3α of lactose), 3.33 (m, HDO and H-2′α of lactose), 3.29 (m, 1H, H-4α of lactose), 3.17 (m, 1H, H-2α of lactose), 2.41 (t, 2H, *J* 7.2 Hz, –CH_2–(CO_2–CH=CH_2)), 2.30 (t, 2H, *J* 7.1 Hz, –CH_2–(CO_2-lactose)), 1.53, 1.26 (m, 12H, other CH_2 of decanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.42 (CO), 169.97 (CO), 140.78 (–CH=CH_2), 97.53 (CH_2=CH–), 103.08 (C-1′), 91.55 (C-1α), 80.60 (C-4α), 72.32 (C-3′), 71.91 (C-5′), 71.72 (C-5α), 70.78 (C-3α), 69.82 (C-2′), 69.22 (C-2α), 67.80 (C-4′), 62.82 (C-6′), 59.97 (C-6α), 32.81, 32.55, 28.04, 28.00, 27.92, 27.80, 23.81, 23.55 (8CH_2); ESI-MS (*m*/*z*): 575 (M_{4c}+Na^+), M_{4c} corresponding exactly to the molecular mass of 6^{II}-*O*-vinyldecanedioyl-α-lactose.

3.2.10. 6^{II}-*O*-Vinylbutanedioylmaltose (5a). Maltose (3.6 g, $C_{12}H_{22}O_{11}\cdot H_2O$, 10 mmol), divinyl butanedioate (6.8 g, 4 equiv), alkaline protease from *B. subtilis* (2 g, 20 mg/mL), 100 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (150:30:10, by vol); yield: 2.48 g, 53%; yellow solid; a mixture of α and β anomers; $[\alpha]_D^{25}$ +105 (*c* 0.6, MeOH); IR (KBr): *v* 3389 (v_{O-H}), 1737 ($v_{C=O}$), 1647 ($v_{C=C}$); ^{1}H NMR (Me_2SO-d_6): δ 7.22 (dd, 1H, $J_{a,b}$ 6.2, $J_{a',b}$ 14.0 Hz, –CH=), 6.71 (d, 0.5H, *J* 6.3 Hz, 1β-OH of maltose), 6.38 (d, 0.5H, *J* 4.3 Hz, 1α-OH of maltose), 5.58 (d, 1H, *J* 3.7 Hz, 2′β-OH and 2′α-OH of maltose), 5.52 (d, 0.5H, *J* 1.7 Hz, 3β-OH of maltose), 5.36 (d, 0.5H, *J* 2.0 Hz, 3α-OH of maltose), 5.21 (t, 1H, *J* 5.1 Hz, 4′β-OH and 4′α-OH of maltose), 5.06 (d, 1H, *J* 4.1 Hz, 3′α-OH and 3′β-OH of maltose), 5.00 (m, 1H, H-1′α and H-1′β of maltose), 4.97 (d, 0.5H, *J*3.1 Hz, H-1α of maltose), 4.93–4.91 (m, 1.5H, 2β-OH of maltose and one H of CH_2=), 4.66 (m, 1.5H, the other H of CH_2= and 2α-OH of maltose), 4.53 (t, 0.5H, *J* 5.7 Hz, 6β-OH of maltose), 4.42 (t, 0.5H, *J* 5.5 Hz, 6α-OH of maltose), 4.33–4.29 (m, 1.5H, H_a-6′α, H_a-6′β and H-1β of maltose), 4.02 (m, 1H, H_b-6′α, H_b-6′β of maltose), 3.72–3.65 (m, 3H, H_a-6β, H-5′α, H-5′β, H-5α, H_a-6α and H_b-6α of maltose), 3.60–3.50 (m, 1.5H, H-3′α, H-3′β and H-3β of maltose), 3.28–3.17 (m, 3H, H-4β, H-4α, H-2′β, H-2′α, H-2α and H-5β of maltose), 3.08–3.03 (m, 1H, H-4′α and H-4′β of maltose), 2.96 (m, 0.5H, H-2β of maltose), 2.7–2.51 (m, 4H, –CH_2–CH_2– of butanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 171.89 (CO), 169.67 (CO), 141.22 (–CH=CH_2), 98.32 (CH_2=CH–), 101.13 (C-1′), 96.80 (C-1β), 92.12 (C-1α), 81.19 (C-4α), 80.63 (C-4β), 76.52 (C-3β), 75.18 (C-5β), 74.26 (C-2β), 73.20 (C-3′), 72.95 (C-3α), 72.40 (C-2′), 71.82 (C-2α), 70.59 (C-5′), 70.41 (C-5α), 69.97 (C-4′), 64.10 (C-6′), 60.76 (C-6α), 60.64 (C-6β), 28.34, 28.19 (2CH_2); ESI-MS (*m*/*z*): 491 (M_{5a}+Na^+), M_{5a} corresponding exactly to the molecular mass of 6^{II}-*O*-vinylbutanedioylmaltose.

3.2.11. 6^{II}-*O*-Vinylhexanedioylmaltose (5b). Maltose (3.6 g, $C_{12}H_{22}O_{11}\cdot H_2O$, 10 mmol), divinyl hexanedioate (7.9 g, 4 equiv), alkaline protease from *B. subtilis* (2 g, 20 mg/mL), 100 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (170:30:10, by vol); yield: 2.08 g, 42%; yellow solid; a mixture of α and β anomers; $[\alpha]_D^{25}$ +118 (*c* 0.6, MeOH); IR (KBr): *v* 3390 (v_{O-H}), 1742 ($v_{C=O}$), 1647 ($v_{C=C}$); ^{1}H NMR (Me_2SO-d_6): δ 7.22 (dd, 1H, $J_{a,b}$ 6.3, $J_{a',b}$ 14.0 Hz, –CH=), 6.71 (d, 0.5H, *J* 6.5 Hz, 1β-OH of maltose), 6.38 (d, 0.5H, *J* 4.6 Hz, 1α-OH of maltose), 5.58 (t, 1H, *J* 6.4 Hz, 2′β-OH and 2′α-OH of maltose), 5.52 (d, 0.5H, *J* 2.7 Hz, 3β-OH of maltose),

5.36 (d, 0.5H, *J* 2.7 Hz, 3α-OH of maltose), 5.20 (t, 1H, *J* 5.7 Hz, 4′β-OH and 4′α-OH of maltose), 5.05 (d, 1H, *J* 5.0 Hz, 3′α-OH and 3′β-OH of maltose), 5.00 (m, 1H, H-1′α and H-1′β of maltose), 4.96 (d, 0.5H, *J* 3.1 Hz, H-1α of maltose), 4.91–4.89 (m, 1.5H, 2β-OH of maltose and one H of CH_2=), 4.66 (m, 1.5H, the other H of CH_2= and 2α-OH of maltose), 4.53 (t, 0.5H, *J* 5.7 Hz, 6β-OH of maltose), 4.42 (t, 0.5H, *J* 5.5 Hz, 6α-OH of maltose), 4.33–4.26 (m, 1.5H, H_a-6′α, H_a-6′β and H-1β of maltose), 4.02 (m, 1H, H_b-6′α, H_b-6′β of maltose), 3.71–3.64 (m, 3H, H_a-6β, H-5′α, H-5′β, H-5α, H_a-6α and H_b-6α of maltose), 3.60–3.50 (m, 1.5H, H-3′α, H-3′β and H-3β of maltose), 3.27–3.17 (m, 3H, H-4β, H-4α, H-2′β, H-2′α, H-2α and H-5β of maltose), 3.05–3.03 (m, 1H, H-4′α and H-4′β of maltose), 2.96 (m, 0.5H, H-2β of maltose), 2.46–2.35 (m, 4H, two –CH_2–CO_2), 1.56 (m, 4H, other 2 CH_2 of hexanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.75 (CO), 170.33 (CO), 141.27 (–CH=CH_2), 98.12 (CH_2=CH–), 101.11 (C-1′), 96.77 (C-1β), 92.10 (C-1α), 81.21 (C-4α), 80.67 (C-4β), 76.50 (C-3β), 75.18 (C-5β), 74.23 (C-2β), 73.19 (C-3′), 72.94 (C-3α), 72.50 (C-2′), 71.80 (C-2α), 70.60 (C-5′), 70.41 (C-5α), 70.04 (C-4′), 63.65 (C-6′), 60.77 (C-6α), 60.66 (C-6β), 32.95, 32.71, 23.71, 23.52 (4CH_2); ESI-MS (*m*/*z*): 519 (M_{5b}+Na^+), M_{5b} corresponding exactly to the molecular mass of 6^{II}-*O*-vinylhexanedioylmaltose. Anal. Calcd for $C_{20}H_{32}O_{14}$: C, 48.39; H, 6.50. Found: C, 48.45; H, 6.56.

3.2.12. 6^{II}-*O*-Vinyldecanedioylmaltose (5c). Maltose (3.6 g, $C_{12}H_{22}O_{11}\cdot H_2O$, 10 mmol), divinyl decanedioate (10.2 g, 4 equiv), alkaline protease from *B. subtilis* (2 g, 20 mg/mL), 100 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (190:30:10, by vol); yield: 1.88 g, 34%; yellow solid; A mixture of α and β anomers; $[\alpha]_D^{25}$ +122 (*c* 0.6, MeOH); IR (KBr): *v* 3395 (v_{O-H}), 1740 ($v_{C=O}$), 1647 ($v_{C=C}$); ^{1}H NMR (Me_2SO-d_6): δ 7.22 (dd, 1H, $J_{a,b}$ 6.2, $J_{a',b}$ 14.0 Hz, –CH=), 6.70 (d, 0.5H, *J* 6.1 Hz, 1β-OH of maltose), 6.32 (d, 0.5H, *J* 4.3 Hz, 1α-OH of maltose), 5.56 (d, 1H, *J* 3.7 Hz, 2′β-OH and 2′α-OH of maltose), 5.51 (d, 0.5H, *J* 1.8 Hz, 3β-OH of maltose), 5.35 (d, 0.5H, *J* 2.0 Hz, 3α-OH of maltose), 5.20 (t, 1H, *J* 5.1 Hz, 4′β-OH and 4′α-OH of maltose), 5.07 (d, 1H, *J* 4.1 Hz, 3′α-OH and 3′β-OH of maltose), 5.01 (m, 1H, H-1′α and H-1′β of maltose), 4.96 (d, 0.5H, *J* 3.1 Hz, H-1α of maltose), 4.93–4.90 (m, 1.5H, 2β-OH of maltose and one H of CH_2=), 4.67 (m, 1.5H, the other H of CH_2= and 2α-OH of maltose), 4.52 (t, 0.5H, *J* 5.7 Hz, 6β-OH of maltose), 4.41 (t, 0.5H, *J* 5.5 Hz, 6α-OH of maltose), 4.33–4.28 (m, 1.5H, H_a-6′α, H_a-6′β and H-1β of maltose), 4.04 (m, 1H, H_b-6′α, H_b-6′β of maltose), 3.72–3.66 (m, 3H, H_a-6β, H-5′α, H-5′β, H-5α, H_a-6α and H_b-6α of maltose), 3.60–3.50 (m, 1.5H, H-3′α, H-3′β and H-3β of maltose), 3.28–3.17 (m, 3H, H-4β, H-4α, H-2′β, H-2′α, H-2α and H-5β of maltose), 3.08–3.03 (m, 1H, H-4′α and H-4′β of maltose), 2.96 (m, 0.5H, H-2β of maltose), 2.41–2.30 (m, 4H, two –CH_2–CO_2), 1.53, 1.26 (m, 12H, other CH_2 of decanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.32 (CO), 169.87 (CO), 140.82 (–CH=CH_2), 97.50 (CH_2=CH–), 101.15 (C-1′), 96.70 (C-1β), 92.08 (C-1α), 81.15 (C-4α), 80.60 (C-4β), 76.55 (C-3β), 75.25 (C-5β), 74.25 (C-2β), 73.15 (C-3′), 72.90 (C-3α), 72.51 (C-2′), 71.85 (C-2α), 70.65 (C-5′), 70.44 (C-5α), 70.05 (C-4′), 63.85 (C-6′), 60.75 (C-6α), 60.60 (C-6β), 32.81, 32.55, 28.04, 28.00, 27.92, 27.80, 23.81, 23.55, (8CH_2); ESI-MS (*m*/*z*): 575 (M_{5c}+Na^+), M_{5c} corresponding exactly to the molecular mass of 6^{II}-*O*-vinyldecanedioylmaltose.

3.2.13. 1^{Fru}-*O*-Vinylhexanedioylsucrose (6b). Sucrose (6.84 g, $C_{12}H_{22}O_{11}$, 20 mmol), divinyl hexanedioate (15.8 g, 4 equiv), alkaline protease from *B. subtilis* (4 g, 20 mg/mL), 200 mL pyridine, 50 °C, 5 days, eluent: EtOAc–MeOH–H_2O (100:10:5, by vol); yield: 5.46 g, 55%; yellow solid; $[\alpha]_D^{25}$ +58 (*c* 0.5, MeOH); IR (KBr): *v* 3362 (v_{O-H}), 1740 ($v_{C=O}$), 1648 ($v_{C=C}$); ^{1}H NMR (D_2O): δ 7.15 (dd, 1H, $J_{a,b}$ 6.3, $J_{a',b}$ 14.0 Hz, –CH=), 5.36 (d, 1H, H-1 of sucrose), 4.92 (d, 1H, $J_{a',b}$ 13.9 Hz, CH_2=), 4.70 (br, HDO and the other H of CH_2=), 4.30–3.30 (br m, 13H, other H of sucrose), 2.43–2.35, 1.62 (m, 8H, 4 CH_2 of hexanedioyl part); ^{13}C NMR (D_2O): δ 176.50 (CO), 174.51 (CO), 142.12 (–CH=CH_2), 100.26 (CH_2=CH–), 103.32 (C-2′), 93.61 (C-1), 82.54 (C-5′), 77.72 (C-3′), 74.43 (C-4′), 73.51 (C-3), 73.51 (C-5), 71.95 (C-2), 70.20 (C-4), 63.60 (C-1′), 63.04 (C-6′), 61.11 (C-6), 32.28, 32.14, 23.50, 23.31 (4CH_2); ESI-MS (*m*/*z*): 519 (M_{6b}+Na^+), M_{6b} corresponding exactly to the molecular mass of 1^{Fru}-*O*-vinylhexanedioylsucrose. Anal. Calcd for $C_{20}H_{32}O_{14}$: C, 48.39; H, 6.50. Found: C, 48.31; H, 6.52.

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