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Synthesis of glycomonomers via biocatalytic methods

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Abstract

The demand to develop sustainable polymers has increased in the last decades. Sustainable polymers contain some or all components that are derived from renewable resources. Carbohydrates, or saccharides, are one of the most abundant renewable biomass produced annually. Harnessing carbohydrates for building blocks of polymers can generate novel functional water-soluble materials. One of the examples is called glycopolymers, that are synthetic polymers having pendant saccharide moieties. In this report, we present the synthesis of glycomonomers, the precursors of glycopolymers, by

enzymatic approaches. Some enzymes, like lipases, proteases, glycosidases, and glycosyltransferases, are exploited for this purpose. These enzymes catalyze the formation of ester or glycosidic bonds through transesterification reaction, reverse hydrolysis reaction, transglycosylation reaction, or glycosyl transfer from donors to acceptors.

1. Introduction

1.1 Carbohydrates

Carbohydrates are one of the most abundant natural organic substances on Earth and they mainly serve as structural and functional elements of cellular materials. In general, carbohydrates are defined as biomolecules consisting of carbon (C), hydrogen (H), and oxygen (O) atoms with the molecular formula $C_m(H_2O)_n$ also known as hydrates of carbon (Alén, 2018). According to the IUPAC definition, another term for carbohydrates is saccharides; which includes monosaccharides, oligosaccharides, and polysaccharides as well as their derivative components (Moss, Smith, & Tavernier, 1995).

Monosaccharides are the simplest carbohydrates and they typically contain three to nine carbon atoms. Fig. 1 shows some examples of monosaccharide structures in the chair conformation. Carbohydrates with two or more monosaccharide units linked together by glycosidic bonds are classified as oligo- and polysaccharides (Mcnaught, 1996). Oligosaccharides consist of a small number of repeating monosaccharides, generally between 2 and

Fig. 1 Monosaccharide structures in the chair conformation. The numbers within the structures indicate the position of functionalization by vinyl groups as discussed in Sections 2–4.

Fig. 2 Oligo- and polysaccharide structures in the chair conformation. The numbers within the structures indicate the position of functionalization by vinyl groups as discussed in [Sections 2–4](#).

10 units. In the case of two or three monosaccharides combined, specific terms are used, namely, disaccharides or trisaccharides. [Fig. 2](#) presents some examples of oligo- and polysaccharides.

1.2 Enzymes as biocatalysts in synthetic organic chemistry

Enzymes are catalysts that perform biosynthetic reactions in the cells at a remarkably high rate under mild reaction conditions (temperature, pH, and pressure) ([Blanco & Blanco, 2017](#)). The enzymatic reaction rates are about 10^6 - to 10^{12} -fold of the uncatalyzed reaction, even up to 10^{20} times

Fig. 3 Energy diagram of a chemical reaction with and without enzyme.

in some special cases (Borman, 2004). The energy diagram of a typical chemical reaction with enzymes (*in vivo*) and without enzymes is presented in Fig. 3. In principle, the role of enzymes as biocatalysts is identical to chemical catalysts in terms of speeding up the reaction rate without changing the total free energy from reactants to products. This is achieved by providing an alternative reaction mechanism, which has a lower activation energy than the non-catalyzed reaction. Besides, enzymes show amazing efficiency and specificity as well as the fact that they are non-toxic, natural, and renewable materials in comparison with conventional catalysts (Loos, 2010). As a result, *in vitro* enzymatic reactions have been developed in order to provide chemical synthesis in a green approach.

Probably the first *in vitro* enzymatic reaction was reported in the 1930s for the synthesis of esters catalyzed by lipase in organic solvents (Sym, 1933, 1936). However, it began to gain widespread interest after Zaks and Klivanov published their articles in the 1980s dealing with different lipases as catalysts for various organic reactions in anhydrous solvents (Zaks & Klivanov, 1984, 1985). Since then, biocatalysts in synthetic organic chemistry have been one of the most studied topics with a constantly growing reputation.

Many enzymes are found to be able to work not only with their native substrates but also with unnatural substrates which results in broad variety of materials (Shoda, Uyama, Kadokawa, Kimura, & Kobayashi, 2016).

1.3 Enzymatic synthesis of glycomonomers

The demand to develop sustainable materials and production methods, especially in the field of polymers, has increased in the last decades (Gandini & Lacerda, 2015; Jiang & Loos, 2016). This is reasonable considering that the main components for polymer production are based on fossil resources which have been predicted to be exhausted in the next several hundred years. In addition, the manufacturing of fossil resources often creates environmental issues like global warming, pollution, and dangerous waste. In order to significantly decrease the disadvantages created by the use of fossil resources, the European Union and the United Nations, through their policies and legislation, support the use of eco-friendly materials and simultaneously limit the consumption of environmentally hazardous substances in industry (Satyanarayana, Arizaga, & Wypych, 2009; Zhu, Romain, & Williams, 2016).

Sustainable polymers contain some or all portions of building blocks that are derived from renewable feedstocks (Gandini & Lacerda, 2015; Jiang & Loos, 2016; Zhu et al., 2016). One of the most promising examples of renewable feedstocks is biomass and its derivatives since these resources are produced by nature via photosynthetic pathways. Especially carbohydrates, one of the most abundant biomass, are interesting materials for the production of durable polymers. However, the chemical industry is still only using a small percentage of the accessible carbohydrates (Lichtenthaler & Peters, 2004; Nakajima, Dijkstra, & Loos, 2017), although carbohydrates give rise to a new family of functional water-soluble monomers and polymers. One example for this is glycopolymers, synthetic polymers composed of saccharides as the pendant group (Adharis, Ketelaar, Komarudin, & Loos, 2019; Becer & Hartmann, 2015).

The potential applications of glycopolymers have attracted much research in the last decades starting in the 1970s when synthetic glycopolymers with polyacrylamide backbones could interact with carbohydrate-binding proteins called lectins (Hořejší, Chaloupecká, & Kocourek, 1978; Schnaar & Lee, 1975). Glycopolymers exhibit a high affinity and specificity to lectins, thus mimicking glycolipids and glycoproteins at the cell surface

(Kiessling & Grim, 2013; Miura, Hoshino, & Seto, 2016). Moreover, glycopolymers have been used as inhibitors that prevent the adhesion of pathogens to the cell surface during an infection (Bhatia, Camacho, & Haag, 2016). Other applications of glycopolymers cover, for example, drug delivery (Appelhans, Klajnert-Maculewicz, Janaszewska, Lazniewska, & Voit, 2015; Sunasee, Adokoh, Darkwa, & Narain, 2014; Utama, Jiang, Zetterlund, & Stenzel, 2015), biosensors (Hushegyi, Klukova, Bertok, & Tkac, 2015; Zeng, Qu, & Rehman, 2016), and diseases inhibitors (Bhatia et al., 2016; Branson & Turnbull, 2013).

Glycomonomers, the precursors of glycopolymers, contain saccharide units that are linked to some polymerizable groups. Currently, vinyl groups are the most exploited ones. The preparation of glycomonomers from saccharides and vinyl containing molecules can be carried out using chemical reactions (Ambrosi, Cameron, Davis, & Stolnik, 2005; Schuster, Mortell, Hegeman, & Kiessling, 1997; Wulff, Schmid, & Venhoff, 1996). However, this approach is time-consuming given that it involves multiple protection steps of hydroxyl groups of saccharides in the course of the reactions. An elegant way to synthesize glycomonomers is by using enzymes as the biocatalysts (Adharis, Vesper, Koning, & Loos, 2018; Albertin, Stenzel, Barner-Kowollik, Foster, & Davis, 2005; Gill & Valivety, 2000; Kloosterman, Roest, Priatna, Stavila, & Loos, 2014). As already mentioned in the previous section, enzymes exhibit an excellent reaction specificity compared to otherwise used chemical catalysts. Therefore, in the enzymatic synthesis of glycomonomers, the vinyl group can specifically be attached to a particular position, among multiple possible positions, of saccharides without involving the tedious protection steps. Some vinyl groups of the synthesized glycomonomers are presented in Fig. 4.

To the best of our knowledge, the first work on the synthesis of vinyl monomers containing saccharide moieties by biocatalytic approaches was published by Klibanov and coworkers in 1986 (Therisod & Klibanov, 1986, 1987). In this work, lipases were used to catalyze the acylation of various monosaccharides. The following sections discuss some of the latest results found in the last two decades. The early findings in this area were summarized in several excellent review articles published in the early 2000 (Gross, Kumar, & Kalra, 2001; Kadereit & Waldmann, 2001; La Ferla, 2002; Wulff et al., 1996). Enzymes from the class of hydrolases (EC 3), such as lipases, proteases, and glycosidases, were mainly used for the bio-catalytically synthesized glycomonomers since they are able to catalyze the formation of ester-, amide-, and glycosidic bonds as well as reverse hydrolysis reactions.

Fig. 4 Some examples of vinyl functionalities of glycomonomers.

2. Lipase-catalyzed synthesis of glycomonomers

Lipases are a subclass of [esterases](#) that, in nature, catalyze the hydrolysis reaction of ester bonds of long-chain triglycerides to diglycerides, monoglycerides, glycerols, and fatty acids ([Rosales, Pazos, & Ángeles Sanromán, 2018](#)). Lipases can be isolated from various origins such as bacteria, fungi, and mammals. *In vitro*, lipases can perform the reverse reaction of its natural functions, meaning that lipases create ester bonds from alcohols and carboxylic acids of substrates in dry organic solvents.

For the case of glycomonomer synthesis, lipases catalyze the transesterification of hydroxyl groups of saccharides with divinyl esters. The transesterification reaction occurs in an equilibrium state. Therefore, in order to shift the equilibrium reaction to the products side, divinyl esters are favored over carboxylic acids as substrates. The esters generate vinyl alcohol as a side product which immediately converts, via tautomerization, to volatile acetaldehyde. [Fig. 5](#) exemplarily shows the lipase-catalyzed synthesis of glycomonomers from glucose and divinyl adipate.

[Table 1](#) summarizes some examples of glycomonomers that were synthesized by lipases. Lipase from *Candida antarctica* was the most frequently used biocatalyst; however, other sources of lipases from *Alcaligenes* sp., *Pseudomonas* sp., and *Thermomyces* sp. were also utilized. These lipases show a good activity in commonly available organic solvents like acetonitrile, pyridine, acetone, DMF, *t*-butanol, and butanone. Nevertheless, working with a particular solvent may be more preferred than the others. For instance, [Cameron et al.](#) reported that by replacing the solvent from acetonitrile to butanone for the synthesis of methyl 6-*O*-methacryloyl- α -D-glucoside, the glycomonomer yield was improved from 70% to 85% and the reaction time was decreased from 5 to 2 days ([Cameron et al., 2008](#)). In general, the lipase-catalyzed transesterification reaction was performed between 1 and 5 days at 30–60 °C to obtain a product yield up to 85%.

Fig. 5 Reaction scheme of the lipase-catalyzed synthesis of glycomonomers ([Albertin, Kohlert, Stenzel, Foster, & Davis, 2004](#)).

Table 1 Lipase-catalyzed synthesis of glycomonomers.

Saccharides	Vinyl group	Vinyl position	Enzyme sources	Reaction condition	Yield (%)	References
1	b	C-6	<i>Candida antarctica</i>	Acetonitrile, 50 °C, 1 day	50	Albertin, Kohlert, et al. (2004)
1	c	C-6	<i>Alcaligenes</i> sp.	Pyridine, 30 °C, 7 days	31	Kitagawa and Tokiwa (1998)
1	e	C-6	<i>Candida antarctica</i>	<i>t</i> -Butanol, 50 °C, 1 day	34	Perin and Felisberti (2018)
2	d	C-6	<i>Candida antarctica</i>	Acetone, 60 °C, 18h	82	Li and Rethwisch (2002)
2	e	C-6	<i>Candida antarctica</i>	Acetonitrile, 50 °C, 5 days	70	Albertin, Stenzel, et al. (2004) and Albertin et al. (2005)
2	e	C-6	<i>Candida antarctica</i>	Butanone, 60 °C, 2 days	85	Cameron et al. (2008)
3	d, e	C-6	<i>Candida antarctica</i>	<i>t</i> -Butanol, 50 °C, 1 day	100 ^a	Kim et al. (2004) and Park, Haam, Lee, Kim, and Kim (2004)
5	e	C-6	<i>Candida antarctica</i>	Acetone, 50 °C, 5 days	74	Granville, Quémener, Davis, Barner-Kowollik, and Stenzel (2007)
6	e	C-6	<i>Candida antarctica</i>	Acetonitrile, 50 °C, 4 days	48	Albertin et al. (2005)
9	b	C-4	<i>Pseudomonas</i> sp.	Pyridine, 30 °C, 7 days	11	Raku and Tokiwa (2003)
11	e	C-1, C-6, C-1,6	<i>Candida antarctica</i>	<i>t</i> -Butanol, 50 °C, 1 day	95 ^a	Perin and Felisberti (2018)
14	b, c	C-6	<i>Candida antarctica</i>	Pyridine, 50 °C, 3 days	70 ^a , 99 ^a	Miura, Ikeda, and Kobayashi (2003) and Miura, Ikeda, Wada, Sato, and Kobayashi (2003)

Continued

Table 1 Lipase-catalyzed synthesis of glycomonomers.—cont'd

Saccharides	Vinyl group	Vinyl position	Enzyme sources	Reaction condition	Yield (%)	References
16	b, c	C-6	<i>Candida antarctica</i>	Pyridine, 50 °C, 3 days	7 ^a , 44 ^a	Miura, Ikeda, and Kobayashi (2003) and Miura, Ikeda, Wada, et al. (2003)
17	b	C-6,6'	<i>Candida antarctica</i>	Acetone, 50 °C, 2 days	21	Park, Kim, and Dordick (2000)
18	b	C-6,6'	<i>Candida antarctica</i>	Acetone, 50 °C, 2 days	20	Park et al. (2000)
18	c	C-6'	<i>Pseudomonas</i> sp.	Pyridine, 60 °C, 3 days	19	Miura, Wada, Nishida, Mori, and Kobayashi (2004)
19	c	C-6'	<i>Pseudomonas</i> sp.	Pyridine, 60 °C, 3 days	44	Miura et al. (2004)
20	b	C-6''	<i>Candida antarctica</i>	Pyridine, 60 °C, 3 days	42	Pérez-Victoria and Morales (2007)
20	b	C-6''	<i>Thermomyces lanuginosus</i>	Pyridine, 60 °C, 1 day	75	Pérez-Victoria and Morales (2007)
21	b	C-6	<i>Candida antarctica</i>	Pyridine, 60 °C, 3 days	38	Pérez-Victoria and Morales (2007)
21	b	C-6''	<i>Thermomyces lanuginosus</i>	Pyridine, 60 °C, 1 day	50	Pérez-Victoria and Morales (2007)

^aSubstrate conversion (%), yield data are not available.

The structure of saccharides and vinyl groups correspond to Figs. 1, 2, and 4.

Lipase from *Candida antarctica* selectively catalyzes the monosubstitution of vinyl groups toward a primary alcohol at the C-6 position of monosaccharides with pyranose structures (Albertin, Stenzel, et al., 2004; Granville et al., 2007; Kim et al., 2004; Li & Rethwisch, 2002; Park et al., 2004), at the C-6 position of reduced disaccharides (Miura, Ikeda, & Kobayashi, 2003; Miura, Ikeda, Wada, et al., 2003), and at C-6 or C-6'' positions of trisaccharides (Pérez-Victoria & Morales, 2007). Mono- and disubstitution of vinyl groups was observed when fructose, sucrose, and trehalose were used as the substrates (Park et al., 2000; Perin & Felisberti, 2018). This happened probably because they possess two primary alcohols on the β -position allowing the enzymes to perform two substitutions of vinyl groups in a saccharide unit. Also, this enzyme shows a reaction selectivity toward divinyl esters with different alkyl chain lengths. For example, Miura et al. reported that divinyl sebacate (Fig. 4C) yielded a better substrate conversion than divinyl adipate (Fig. 4B) during the synthesis of glycomonomers (Miura, Ikeda, Wada, et al., 2003). A similar pattern was observed during the enzymatic polymerization of polyesters where this lipase exhibits a preference toward more hydrophobic diols and diesters (Pellis et al., 2018).

A functionalization at the C-4 position of monosaccharide rhamnose with divinyl adipate was reported by Raku and Tokiwa using lipase from *Pseudomonas* species (Raku & Tokiwa, 2003). In that case, a transesterification occurred at the secondary alcohol since no primary alcohol was present in the rhamnose structure. If the disaccharide trehalose—bearing a primary alcohol at the C-6' position—was used as the substrate for that lipase, monosubstitution of vinyl groups selectively occurred in the position of that alcohol (Miura et al., 2004). Moreover, a monosubstitution of trisaccharides with divinyl adipate was observed when lipase from *Thermomyces* sp. catalyzed the reaction (Pérez-Victoria & Morales, 2007).

The following procedure is based on the study by Blanazs and coworkers that generates the highest yield of the synthesized glycomonomers (Cameron et al., 2008). The reaction was catalyzed by lipase from *Candida antarctica* whereas methyl- α -D-glucoside served as the substrate.

2.1 Equipment

- Analytical balance
- Common glass laboratory equipments such as erlenmeyer flask, measuring cylinder, beaker glass, funnel, round-bottom flask, and dropping pipettes
- Temperature controlled shaker

- Rotary evaporator
- Hot plate
- Automated flash chromatography on a Biotage SP1 system with automatic fraction collector and on-board UV detector
- Glass-backed silica-gel plates for thin layer chromatography (TLC)
- Electrospray ionization mass spectrometry (ESI-MS) on a Micromass LCT spectrometer
- Nuclear magnetic resonance (NMR) spectroscopy on a Varian Inova 500 spectrometer

2.2 Materials

- Methyl- α -D-glucoside from Sigma-Aldrich
- Lipase (Novozym 435) from Novozymes A/S
- Vinyl methacrylate from Sigma-Aldrich
- Solvents (butanone, methanol, chloroform, ethanol) from Sigma-Aldrich
- Sulfuric acid from Sigma-Aldrich
- Deuterated methanol (CD_3OD) from Sigma-Aldrich

2.3 Procedure

1. Prepare an erlenmeyer flask containing methyl- α -D-glucoside (8.0 g, 0.041 mol), lipase (4.0 g), vinyl methacrylate (4.4 g, 0.039 mol), and butanone (40 mL).
2. Seal the flask with a rubber septum and shake at 200 rpm and 60 °C for 2 days.
3. Stop the reaction by separating the enzyme from the reaction mixture.
4. Wash the filtrate with methanol (100 mL).
5. Evaporate the solvent of the collected organic phases by rotary evaporator until a yellow-brown syrup is obtained.
6. Purify the product by flash chromatography using the eluent of chloroform/methanol (8:2).
7. Identify the product in the collected fractions by TLC using the same eluent as in the previous step. The TLC plates is immersed in a 20% H_2SO_4 /ethanol solution and heated for spots detection. The product is detected at retention fraction of 0.39.
8. Evaporate the pooled fractions containing the products by rotary evaporator until a clear syrup is obtained.
9. Characterize the product with ESI-MS, ^1H and ^{13}C NMR spectroscopy. CD_3OD is used as the solvent for NMR measurement.

3. Protease-catalyzed synthesis of glycomonomers

Proteases, or proteinases/peptidases, naturally hydrolyze peptide bonds of amino acids during the metabolism of proteins in all organisms (Clark & Pazdernik, 2016). Depending on the type of proteases, the hydrolysis reaction may be specific at particular sites of a protein structure or non-specific resulting in individual amino acids. For the *in vitro* reaction, proteases work in a similar approach like lipase; that is catalyzing transesterification of saccharides with divinyl esters during the synthesis of glycomonomers in organic solvents. For example, Lin and coworkers used galactose and several divinyl esters as the substrate for protease (see Fig. 6) (Wu, Wang, Xiao, Lu, & Lin, 2004).

Unlike lipases, proteases are able to produce the highest yield of glycomonomers when monosaccharides are combined with divinyl esters bearing a few methylene groups (Wu et al., 2004), meaning that this enzyme has a good selectivity toward more hydrophilic substrates. Comparable results were reported for disaccharide (Wang, Wu, Wang, & Lin, 2005; Wu et al., 2004) and trisaccharide (Qi, Deshui, Zhichun, Guohua, & Xianfu, 2003; Wu, Chen, Lu, & Lin, 2006) substrates. However, an exception was found when glucose reacted with divinyl adipate (bearing four methylene groups). This resulted in an increased yield compared to the reaction with divinyl succinate (bearing two methylene groups) and divinyl sebacate (bearing eight methylene groups) (Wu et al., 2001). It is interesting to note that some lipases were successfully commercialized in the immobilized state whereas proteases are only available in the liquid or granulate form, which limits their reusability drastically.

Numerous examples of glycomonomer syntheses by proteases are outlined in Table 2 in which the enzymes were mainly obtained from *Bacillus* species, especially *Bacillus subtilis*. Like lipases, proteases also work well in organic solvents especially pyridine and DMF. Protease-catalyzed synthetic reactions are typically performed in 1–7 days with a temperature between 30 °C and 55 °C. The highest yield of the isolated product was reported to be up to 85%.

Fig. 6 Reaction scheme of the protease-catalyzed synthesis of glycomonomers.

Table 2 Protease-catalyzed synthesis of glycomonomers.

Saccharides	Vinyl group	Vinyl position	Enzyme sources	Reaction condition	Yield (%)	References
1	a, b, c	C-6	<i>Bacillus subtilis</i>	Pyridine, 55 °C, 3 days	30, 53, 35	Wu et al. (2001)
1	b	C-6	<i>Bacillus subtilis</i>	DMF, 50 °C, 5 days	80 ^a	Borges, dos Santos, Vieira, and Balaban (2009)
1	e	C-6	<i>Bacillus subtilis</i>	DMF, 45 °C, 1 day	36	Perin and Felisberti (2018)
2	e	C-6	<i>Bacillus subtilis</i>	DMF, 45 °C, 7 days	33	Potier, Bouchu, Gagnaire, and Queneau (2001)
4	a, b, c	C-6	<i>Bacillus subtilis</i>	Pyridine, 50 °C, 0.5/3/5 days	70, 47, 35	Wu et al. (2004)
5	a, b, c	C-6	<i>Bacillus subtilis</i>	Pyridine, 50 °C, 0.5/4/5 days	85, 69, 43	Wu et al. (2004)
7	b	C-2	<i>Bacillus subtilis</i>	DMF, 30 °C, 7 days	55	Raku and Tokiwa (2003)
8	b	C-2	<i>Bacillus subtilis</i>	DMF, 30 °C, 7 days	32	Raku and Tokiwa (2003)
10	b	C-4 (10y), C-5 (10z)	<i>Bacillus subtilis</i>	DMF, 50 °C, 7 days	66	Borges and Balaban (2014)
10	b	C-5 (10z)	<i>Streptomyces</i> sp.	DMF, 30 °C, 7 days	46	Tokiwa et al. (1999)
11	e	C-1, C-6	<i>Bacillus subtilis</i>	DMF, 45 °C, 1 day	60 ^a	Perin and Felisberti (2018)
12	e	C-1, C-6	<i>Bacillus subtilis</i>	DMF, 45 °C, 3 days	33	Potier et al. (2001)

13	a, b, c	C-6'	<i>Bacillus subtilis</i>	Pyridine, 50 °C, 5 days	62, 46, 31	Wang et al. (2005) and Wu et al. (2004)
15	a, b, c	C-6'	<i>Bacillus subtilis</i>	Pyridine, 50 °C, 5 days	53, 42, 34	Wu et al. (2004)
17	b	C-1	<i>Bacillus subtilis</i>	Pyridine, 50 °C, 5 days	55	Wang et al. (2005) and Wu et al. (2004)
17	b	C-1	<i>Bacillus subtilis</i>	DMF, 50 °C, 5 days	90 ^a	Borges and Balaban (2007)
17	e	C-1	<i>Bacillus subtilis</i>	DMF, 45 °C, 3 days	70	de Oliveira and Felisberti (2013) and Potier, Bouchu, Descotes, and Queneau (2000)
17	b	C-1	<i>Bacillus licheniformis</i>	Pyridine, 45 °C, 2 days	13	Park et al. (2000)
18	e	C-6'	<i>Bacillus subtilis</i>	DMF, 45 °C, 3 days	25	Potier et al. (2001)
18	b	C-6'	<i>Bacillus licheniformis</i>	Pyridine, 45 °C, 2 days	22	Park et al. (2000)
20	a, b, c	C-1	<i>Bacillus subtilis</i>	Pyridine, 50 °C, 6 days	55, 48, 30	Qi et al. (2003) and Wu et al. (2006)
20	b	C-1	<i>Bacillus licheniformis</i>	Pyridine, 40 °C, 1 day	65	Pérez-Victoria and Morales (2007)
21	b	C-6'	<i>Bacillus licheniformis</i>	Pyridine, 40 °C, 1 day	55	Pérez-Victoria and Morales (2007)
22	a, b, c	C-2'	<i>Bacillus subtilis</i>	DMF, 50 °C, 5 days	5, 10, 13	Xiao, Wu, Wang, and Lin (2004)

^aSubstrate conversion (%), yield data are not available.

The structure of saccharides and vinyl groups correspond to Figs. 1, 2, and 4.

Protease from *Bacillus subtilis* selectively catalyzes the monosubstitution of vinyl groups to primary alcohols of saccharides at various positions. The substitution was located at the C-6 position of monosaccharides with pyranose structures (Borges et al., 2009; Perin & Felisberti, 2018; Potier et al., 2001; Wu et al., 2001, 2004), at the C-1 or C-6 position of monosaccharides with furanose structures (Perin & Felisberti, 2018; Potier et al., 2001), at the C-6' position of the disaccharides lactose/maltose/trehalose (Potier et al., 2001; Wang et al., 2005; Wu et al., 2004), as well as at the C-1 position of the disaccharide sucrose and trisaccharide raffinose (Borges & Balaban, 2007; de Oliveira & Felisberti, 2013; Potier et al., 2000; Wang et al., 2005; Wu et al., 2004). Besides that, monosubstitution was also found at the secondary alcohol (at the C-2 position) when fucose and β -cyclodextrin served as the substrates (Raku & Tokiwa, 2003; Xiao et al., 2004).

Proteases from two resources can provide different reaction selectivity and this was observed when L-arabinose was used as the substrate. In solution, L-arabinose possesses two isomers that exhibit different structures, either furan- or pyran-like molecules. Protease from *Bacillus subtilis* catalyzed the transesterification of L-arabinose on both isomers (Borges & Balaban, 2014) while protease from *Streptomyces* sp. only worked with the furanose isomer (Tokiwa et al., 1999) under similar reaction condition.

Wu et al. reported the synthesis of a mannose-based glycomonomer using protease from *Bacillus subtilis* (Wu et al., 2004). The isolated product yield was remarkably high, up to 85%. The following section describes the experimental procedure for preparation of 6-O-vinylbutanedioyl-D-mannopyranose according to literature.

3.1 Equipment

- Analytical balance
- Common glass laboratory equipments such as conical flask, measuring cylinder, beaker glass, funnel, round-bottom flask, and dropping pipettes
- Temperature controlled shaker
- Rotary evaporator
- Glass column loaded with silica gel
- Glass-backed silica-gel plates for thin layer chromatography (TLC)
- Electrospray ionization mass spectrometry (ESI-MS) on a Bruker Esquire-LC

- Infrared (IR) spectroscopy on a Nicolet Nexus FTIR 670 spectrophotometer
- Nuclear magnetic resonance (NMR) spectroscopy on a Bruker Avance DMX500 spectrometer

3.2 Materials

- Protease from Wuxi Enzyme Co. Ltd. (Wuxi, PR China)
- D-Mannose from Sigma-Aldrich
- Divinyl butanedioate from Sigma-Aldrich
- Solvents (pyridine, methanol, ethyl acetate) from Sigma-Aldrich
- Sulfuric acid from Sigma-Aldrich
- DMSO- d_6 from Sigma-Aldrich

3.3 Procedure

1. Prepare a conical flask containing D-mannose (1.4 g, 7.8 mmol), protease (1 g, 25 mg mL⁻¹), divinyl butanedioate (5.3 g, 4 equiv), and pyridine (40 mL).
2. Seal the flask with a rubber septum and shake at 250 rpm and 50 °C for 12 h.
3. Stop the reaction by filtering off the enzyme.
4. Evaporate the pyridine by rotary evaporator.
5. Isolate the product by column chromatography using the eluent of ethyl acetate/methanol/H₂O (150:10:1).
6. Analyze the product formation by TLC using the same eluent as in the previous step. The TLC plates is sprayed by 10% H₂SO₄/methanol solution and heated for spots detection.
7. Evaporate the pooled fractions containing the products by rotary evaporator until tan amorphous solid is formed.
8. Characterize the product with ESI-MS, IR, ¹H and ¹³C NMR spectroscopy. DMSO- d_6 is used as the solvent for NMR measurement.

4. Glycosidase-catalyzed synthesis of glycomonomers

Glycosidases, or glycosyl hydrolases, are carbohydrate-active enzymes that *in vivo* catalyze the hydrolytic degradation of glycosidic bonds in carbohydrates (Kwan, 2017). Considering their versatility, glycosidases have been utilized extensively in industrial processes. For instance, glycosidases are applied as biocatalysts during the degradation of biomass (cellulose and other

plant-derived polysaccharides) yielding mono- and oligosaccharides for biofuels and biobased chemical manufacturing (Kumar, Barrett, Delwiche, & Stroeve, 2009; Olofsson, Bertilsson, & Lidén, 2008; Pérez, Muñoz-Dorado, De La Rubia, & Martínez, 2002).

For the synthesis of glycomonomers, glycosidases catalyze the mono-substitution of vinyl groups at the anomeric carbon (the C-1 position) of saccharides. Depending on the enzyme type, the linkage of the vinyl group may be on the α or β -position of the anomeric carbon which results in anomerically pure products. Glycosidases provide a different location for the attachment of vinyl groups compared to lipases and proteases which usually functionalize the saccharides at their primary alcohols. It was reported that functionalization at the primary alcohol of monosaccharides could be a disadvantage because the resulted glycopolymers were found to lose their activity with saccharide-binding proteins (Granville et al., 2007).

Harnessing glycosidases for the glycomonomer synthesis is done via two approaches, namely, thermodynamically controlled reversed hydrolysis and kinetically controlled transglycosylation. Both methodologies were exploited in the past with their advantages and limitations summarized elsewhere (Van Rantwijk, Van Woudenberg-, & Sheldon, 1999). The following paragraphs discuss briefly the differences between these two methods for the synthesis of saccharide-vinyl monomers listed in Table 3.

4.1 Thermodynamically controlled reverse hydrolysis

Hydrolysis reactions of saccharides by glycosidases are performed under equilibrium conditions. In principle, reverse hydrolysis reactions are used for the synthesis of glycomonomers by reacting monosaccharides with vinyl-based alcohols. Nevertheless, a small amount of water is still required to maintain the enzyme activity and to increase the solubility of the monosaccharides in the reaction mixture. Consequently, this method is limited to monosaccharide substrates since oligo- and polysaccharides can be hydrolyzed by glycosidases in the presence of water.

Fig. 7 shows an example of the thermodynamically controlled reverse hydrolysis of glucose and 2-hydroxyethyl acrylate catalyzed by β -glucosidase derived from almonds (Millqvist-Fureby et al., 1998). Some vinyl-based alcohols (Fig. 4F–K) were tested as well, but the obtained glycomonomer yields were rather low (up to 21%) although highly concentrated glucose solutions were used to shift the equilibrium reaction to the product side. In order to improve the product yield, Kloosterman et al. optimized the

Table 3 Glycosidase-catalyzed synthesis of glycomonomers.

Saccharides	Vinyl group	Vinyl position	Enzyme	Reaction condition	Yield (%)	References
1	f, g, h, i, j, k	C-1 β	β -Glucosidase from almonds	Citrate buffer pH 5.2, 37 °C, 1–3 days	16, 3, 3, 21, 18, 14	Millqvist-Fureby, MacManus, Davies, and Vulfson (1998)
1	f, g, h	C-1 β	β -Glucosidase from almonds	Water/dioxane, 50 °C, 1 day	41, 43, 21	Kloosterman, Roest, et al. (2014)
1	f	C-1 β	Cellulase from <i>Trichoderma reesei</i>	Citrate buffer pH 5.0, 50 °C, 3 days	5	Kloosterman, Brouwer, and Loos (2014)
1	f	C-1 α	Amyloglucosidase from <i>Aspergillus niger</i>	Acetate buffer pH 5.0, 55 °C, 5 days	N/A	Kloosterman, Jovanovic, Brouwer, and Loos (2014)
1	l, m, n	C-1 β	β -Glucosidase from almonds	Water, 50 °C, 1 h	16, 12, 18	Adharis, Vesper, et al. (2018)
4	g, o	C-1 β	β -Galactosidase from <i>Escherichia coli</i>	Phosphate buffer pH 7, 40 °C, 1.5 days	58, 39	Gill and Valivety (2000)
4	g	C-1 β	β -Galactosidase from <i>Aspergillus niger</i>	Phosphate buffer pH 7, 40 °C, 1.5 days	43	Gill and Valivety (2000)
4	o	C-1 β	β -Galactosidase from <i>Aspergillus oryzae</i>	Phosphate buffer pH 7, 40 °C, 1.5 days	60	Gill and Valivety (2000)
4	g	C-1 β	β -Galactosidase from <i>Sulfolobus solfataricus</i>	Phosphate buffer pH 6.5, 75 °C, 1.5 h	75–80	Santin, Rosso, Sada, and Peluso (1996)
15	f, g, h	C-1 α	Amylase from <i>Bacillus stearothermophilus</i>	Acetate buffer pH 5, 55 °C, 4 days	5	Kloosterman, Jovanovic, et al. (2014)

N/A = Not available, the product was not isolated.

The structure of saccharides and vinyl groups correspond to [Figs. 1, 2, and 4](#).

Fig. 7 Thermodynamically controlled reverse hydrolysis reaction catalyzed by β -glucosidase.

reaction time, the composition of alcohols and water, as well as adding an organic solvent into the reaction mixtures (Kloosterman, Roest, et al., 2014). As a result, the synthesized glycomonomers were obtained in a higher yield (up to 41%) than in the previous report.

In addition, less product yield was generated when 4-hydroxybutyl acrylate was used rather than 2-hydroxyethyl acrylate as the substrate in both studies, revealing the enzyme has preferences toward hydrophilic alcohols. To overcome the issue with hydrophobic alcohols, Gill and Valivety proposed to conduct the enzymatic reaction in plasticized glass phases using β -galactosidase from *E. coli*, *A. niger*, and *A. oryzae* (Gill & Valivety, 2000). In their study, the highest yield up to 71% was achieved by mixing saccharides/alkyl glycoside/dopant with a small amount of buffer, followed by melting the reaction mixture and finally adding alcohols and enzymes.

The synthetic procedure reported by Kloosterman et al. is suitable for both hydrophilic and hydrophobic vinyl-containing alcohols which result in moderate yield of glycomonomers (Kloosterman, Roest, et al., 2014). In addition, they utilize cheap starting materials as the substrates for β -glucosidase in a simple experimental method. Therefore, the mentioned procedure in the next paragraphs refers to that reference.

4.1.1 Equipment

- Analytical balance
- Common glass laboratory equipments such as round-bottom flasks, measuring cylinder, beaker glass, funnel, and dropping pipettes
- Stir/hot plate
- Rotary evaporator
- Glass column loaded with silica gel
- Aluminum sheet silica gel 60/kieselguhr for thin layer chromatography (TLC)
- Electrospray ionization mass spectrometry (ESI-MS) on a Thermo Scientific LTQ Orbitrap mass spectrometer
- Nuclear magnetic resonance (NMR) spectroscopy on a 400 MHz Varian VXR spectrometer

4.1.2 Materials

- D-Glucose from Sigma-Aldrich
- 2-Hydroxyethyl acrylate and 2-hydroxyethyl methacrylate from Sigma-Aldrich
- 4-Hydroxybutyl acrylate from ABCR Chemicals
- β -Glucosidase from Sigma-Aldrich
- Dioxane from Acros Organics
- Other solvents (chloroform, methanol, ethanol, sulfuric acid) from Lab-Scan
- Deuterated water (D_2O) from Sigma-Aldrich

4.1.3 Procedure

1. In a round-bottom flask, prepare D-glucose solution (0.7 g of glucose in 1 mL water).
2. Put the flask in a hotplate equipped with waterbath.
3. Add 2-hydroxyethylacrylate/2-hydroxyethylmethacrylate/4-hydroxybutyl acrylate (12 mL) and dioxane (1 mL).
4. Start the reaction by adding the enzyme solution (70 mg of β -glucosidase in 1 mL water).
5. Close the flask and stir the reaction mixture at 200 rpm and 50 °C for 1 day.
6. Identify the product formation by TLC using the eluent of chloroform/methanol (4/1).
7. Spray the TLC plates by 5% H_2SO_4 /ethanol solution and heated for spots detection. 2-(β -Glucosyloxy)-ethyl acrylate, 2-(β -glucosyloxy)-ethyl methacrylate, and 4-(β -glucosyloxy)-butyl acrylate are detected at retardation factor of 0.55, 0.59, and 0.69, respectively.
8. Isolate the product by column chromatography using the same eluent as in the previous step.
9. Evaporate the pooled fractions containing the products by rotary evaporator until a yellowish syrup is obtained.
10. Characterize the product with ESI-MS, 1H and ^{13}C NMR spectroscopy. D_2O is used as the solvent for NMR measurement.

4.2 Kinetically controlled transglycosylation

In the synthesis of glycomonomers by kinetically controlled transglycosylation, glycosidases catalyze the reaction between saccharides working as glycosyl donors, and vinyl-based alcohols serving as glycosyl acceptors. The enzymatic reaction is carried out in aqueous solutions. As shown in Fig. 8, water acts not only as the solvent, but also as a competing reactant

Fig. 8 Competing transglycosylation and hydrolysis catalyzed by β -glucosidase.

to glycosyl acceptors in the transglycosylation reaction. Therefore, it is crucial to optimize the time required for the reaction, as short reaction times may provide a low substrate conversion while hydrolysis of the synthesized glycomonomers is likely to occur during the longer reaction times.

In contrast to the previous approach, oligo- and polysaccharides can be used as the substrate of glycosidases in this method. For example, cellulase from *Trichoderma reesei* catalyzed the formation of 2-(β -glucosyloxy)-ethyl acrylate from cellulose (Kloosterman, Brouwer, et al., 2014). In addition, the same glycomonomer with the acrylate unit linked at the α -position was prepared from starch using amyloglucosidase from *Aspergillus niger* (Kloosterman, Jovanovic, et al., 2014). Moreover, maltogenic amylase from *Bacillus stearothermophilus* was able to catalyze the synthesis of maltose-based glycomonomers from starch (Kloosterman, Jovanovic, et al., 2014). A disadvantage was the low glycomonomers yield of less than 5%.

In order to improve the product yield, activated saccharides can be used as the glycosyl donors rather than native saccharides. Activated saccharides usually contain aryl or vinyl groups which are much better leaving groups in transglycosylation reaction than glycosyl units of natural sugars (Chiffolleau-Giraud, Spangenberg, & Rabiller, 1997; van Woudenberg-, Van Belle, Van Rantwijk, & Sheldon, 1998). For instance, Santin et al. reported *p*-nitrophenyl β -D-galactopyranoside as the substrate of β -galactosidase from *Sulfolobus solfataricus* with an isolated glycomonomer yield of about 75% (Santin et al., 1996). Another way to improve the product yield is by adding cosolvents like organic solvents (Bridiau, Issaoui, & Maugard, 2010;

Giacomini, Irazoqui, Gonzalez, Batista-viera, & Brena, 2002; Zeuner, Riisager, Mikkelsen, & Meyer, 2014) or ionic liquids (Bayón, Cortés, Berenguer, & Hernáiz, 2013; Kaftzik, Wasserscheid, & Kragl, 2002; Lang, Kamrat, & Nidetzky, 2006) to increase the enzyme-substrate interactions. For example, the yield of glucosyl-(meth)acrylamide monomers was improved from 16% to 68% upon addition of the ionic liquid BMIMPF₆ and replacing the native cellobiose with activated glucose (Adharis, Vesper, et al., 2018). This report comprehensively studied the synthesis of glycomonomers by providing either native or activated saccharides as the substrate for the enzyme. Furthermore, different solvent systems, either single or mixture solvents, were utilized and its influence into the product yields were investigated. Hence, the synthetic procedure mentioned in the following section is based on this literature.

4.2.1 Equipment

- Analytical balance
- Common glass laboratory equipments such as round-bottom flasks, measuring cylinder, beaker glass, funnel, and dropping pipettes
- Stir/hot plate
- Falcon tube
- Centrifuges on a Thermo Scientific Heraeus Labofuge 400 R
- Rotary evaporator
- Glass column loaded with silica gel
- Aluminum sheet silica gel 60/kieselguhr for thin layer chromatography (TLC)
- Electrospray ionization mass spectrometry (ESI-MS) on a Thermo Scientific LTQ Orbitrap mass spectrometer
- Nuclear magnetic resonance (NMR) spectroscopy on a 400 MHz Varian VXR spectrometer

4.2.2 Materials

- Cellobiose from Acros Organics
- *N*-Hydroxyethyl acrylamide from Sigma-Aldrich
- *N*-Hydroxyethyl methacrylamide and *N*-hydroxybutyl methacrylamide were synthesized according to literature (Rijcken et al., 2005)
- β -Glucosidase from Sigma-Aldrich
- *p*-Nitrophenyl β -D-glucopyranoside (*p*-NPG) from Alfa Aesar

- 1-*N*-Butyl-3-methylimidazolium hexafluorophosphate (BMIMPF₆) from Alfa Aesar
- Other solvents (chloroform, methanol, ethanol, sulfuric acid) from Avantor
- Deuterated water (D₂O) from Sigma-Aldrich

4.2.3 Procedure

1. In a round-bottom flask, prepare cellobiose solution (0.5 g of cellobiose in 2.25 mL water) or *p*-NPG solution (0.50 g in 4.50 mL water).
2. Put the flask in a hotplate equipped with waterbath.
3. Add *N*-hydroxyethyl acrylamide (0.65 g, 5.65 mmol), *N*-hydroxyethyl methacrylamide (0.75 g, 5.80 mmol), or *N*-hydroxybutyl methacrylamide (0.9 g, 5.72 mmol).
4. Start the reaction by adding the enzyme solution (50 mg of β -glucosidase in 0.25 mL water).
5. Close the flask and stir the reaction mixture at 200 rpm and 50 °C for 1 h.
6. Identify the product formation by TLC using the eluent of chloroform/methanol (4/1).
7. Spray the TLC plates by 5% H₂SO₄/ethanol solution and heated for spots detection. *N*-(β -Glycosyloxy)-ethyl acrylamide, *N*-(β -glycosyloxy)-ethyl methacrylamide, and *N*-(β -glycosyloxy)-butyl methacrylamide are detected at retardation factor of 0.30, 0.35, and 0.37, respectively.
8. Evaporate the water in the reaction mixture by rotary evaporator.
9. Precipitate the unreacted cellobiose and side products by addition of methanol into the reaction mixture.
10. Separate the supernatant by centrifugation (4500 rpm, 10 min, 4 °C).
11. Concentrate the supernatant by evaporating the solvent in rotary evaporator.
12. Isolate the product by column chromatography using the same eluent as in the previous step.
13. Evaporate the pooled fractions containing the products by rotary evaporator until a yellowish syrup is obtained.
14. Characterize the product with ESI-MS, ¹H and ¹³C NMR spectroscopy. D₂O is used as the solvent for NMR measurement.

Note: In the case of using the ionic liquid BMIMPF₆ as cosolvent (30 v%), centrifugation of the reaction mixture is required after the reaction is stopped. The aim of this step is to separate the ionic liquid phase and the aqueous phase that contains the product.

5. Glycosyltransferase-catalyzed synthesis of glycomonomers

Glycosyltransferases are enzymes that catalyze the formation of glycosidic linkages by transferring glycosyl units from glycosyl donors to glycosyl acceptors (Gloster, 2014; Lairson, Henrissat, Davies, & Withers, 2008). In the preparation of saccharide-vinyl macromonomers (Table 4), various glucose-based monomers were utilized as the glycosyl acceptors, and either natural saccharides or glucosyl phosphates were used as the glycosyl donors. However, the number of glycosyltransferases used for this purpose was limited, probably because the isolation process of these enzymes on the practical scale is rather challenging (Shoda et al., 2016).

Cyclodextrin glycosyltransferase from *Bacillus macerans* catalyzes the synthesis of 2-(β -maltooligooxy)-ethyl (meth)acrylate macromonomers (Kloosterman, Van Dijk, et al., 2014). In that reaction, α -cyclodextrin and 2-(β -glucosyloxy)-ethyl (meth)acrylate are served as the glycosyl donor and acceptor, respectively. The glycomonomers contained 1–15 glucosyl units that are linked through α -(1,4)-glycosidic bonds while the β -linkage of the vinyl group remained intact after the coupling reaction.

In another example, a cellooligosaccharide-based glycomonomer was prepared from 2-(β -glucosyloxy)-ethyl methacrylate and α -D-glucose 1-phosphate (Wang et al., 2017). Cellodextrin phosphorylase from *Clostridium thermocellum* (CtCdP) was utilized as the biocatalyst. The synthesized

Table 4 Glycosyltransferase-catalyzed synthesis of glycomonomers.

Saccharides	Vinyl group	Vinyl position	Enzyme	Reaction condition	Yield (%)	References
23	f, g	C-1 β	Cyclodextrin glycosyltransferase from <i>Bacillus macerans</i>	Tris buffer pH 6, 60 °C, 1.5 h	38	Kloosterman, Van Dijk, and Loos (2014)
24	f, g, h, l, m	C-1 β	Cellodextrin phosphorylase from <i>Clostridium thermocellum</i>	HEPES buffer pH 7.5, 45 °C, 3 days	65, 67, 58, 63, 70	Adharies, Petrović, Özdamar, Woortman, and Loos (2018) and Wang, Niu, Sawada, Shao, and Serizawa (2017)

The structure of the saccharides and vinyl groups correspond to Figs. 2 and 4.

Fig. 9 Enzymatic synthesis of cellobiosaccharide-based glycomonomers catalyzed by CtCdP ($m = 1$ or 2 , $A = O$ or NH , and $R = H$ or CH_3).

macromonomer possesses an average number of nine glucosyl units which are coupled by β -(1,4)-glycosidic bonds. A disadvantage is that the solubility issue of the product hinders the enzyme ability to extend the number of glucosyl units further. Recently, the versatility of this enzyme with some unnatural substrates was reported by us (Adharis, Petrović, et al., 2018). In this work, several types of vinyl glucosides bearing (meth)acrylate/(meth)acrylamide functionalities were applied as the glucosyl acceptors (Fig. 9) and the isolated yields were about 65%. Interestingly, these vinyl glucosides were synthesized from renewable materials following an enzymatic approach in aqueous solutions, thus, providing an environmentally friendly process. The following procedure is described on the basis to this report.

5.1 Equipment

- Analytical balance
- Falcon tube
- Common glass laboratory equipments such as measuring cylinder, beaker glass, and dropping pipettes
- Eppendorf thermomixer comfort
- Centrifuges on a Thermo Scientific Heraeus Labofuge 400 R
- Aluminum sheet silica gel 60/kieselguhr for thin layer chromatography (TLC)
- Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) on a Voyager DE-PRO instrument from Applied Biosystems
- Electrospray ionization mass spectrometry (ESI-MS) on a Thermo Scientific LTQ Orbitrap mass spectrometer
- Nuclear magnetic resonance (NMR) spectroscopy on a 400 MHz Varian VXR spectrometer
- Size exclusion chromatography (SEC) on an Agilent Technologies 1260 Infinity from PSS
- Wide-angle X-ray diffraction (WAXD) on a Bruker D8 Advance diffractometer

5.2 Materials

- α -D-Glucose 1-phosphate disodium salt hydrate from Sigma-Aldrich
- Cellobiose from Acros Organics
- Solvents (ethanol, isopropyl alcohol, *n*-butanol) from Avantor
- Sulfuric acid from Sigma-Aldrich
- Deuterated water (D₂O) and sodium deutoxide (NaOD) from Sigma-Aldrich
- 2-(β -Glucosyloxy)-ethyl acrylate (G-EA), 2-(β -glucosyloxy)-ethyl methacrylate (G-EMA), 4-(β -glucosyloxy)-butyl acrylate (G-BA), *N*-(β -glucosyloxy)-ethyl acrylamide (G-EAAM), and *N*-(β -glucosyloxy)-ethyl methacrylamide (G-EMAAM) were synthesized following the literature (Adharis, Vesper, et al., 2018; Kloosterman, Roest, et al., 2014)
- Cellodextrin phosphorylase from *Clostridium thermocellum* (CtCdP) was isolated according to the literature (Petrović, Kok, Woortman, Ćirić, & Loos, 2015)

5.3 Procedure

1. Prepare a falcon tube containing α -Glc1P (1.82 g, 6.0 mmol) and 30 mL HEPES buffer 500 mM, pH 7.5.
2. Add G-EA (83.4 mg, 0.3 mmol), G-EMA (87.6 mg, 0.3 mmol), G-BA (96 mg, 0.3 mmol), G-EAAM (83.1 mg, 0.3 mmol), G-EMAAM (87.3 mg, 0.3 mmol), or cellobiose (102.7 mg, 0.3 mmol) into the reaction mixture.
3. Add the CtCdP solution (250 μ L).
4. Put the tube on a temperature controlled shaker at 600 rpm and 45 °C for 72 h.
5. Observe the formation of the products after few hours as indicated from changing a transparent solution to a white turbid solution.
6. Identify the product in the reaction mixture by TLC using eluent of *n*-butanol/isopropyl alcohol/H₂O (1/2.5/1.5).
7. Spray the TLC plates by 5% H₂SO₄/ethanol solution and heated for spots detection. The product is detected at retardation factor of 0.63.
8. Isolate the product by centrifugation at 4500 rpm and 4 °C for 20 min.
9. Wash the precipitates at least three times with Milli-Q water.
10. Lyophilize the product in a freeze-drier at -45 °C and 0.01 mbar for overnight.
11. Characterize the product with MALDI-Tof MS, ¹H NMR spectroscopy, SEC, and WAXD. 4% NaOD in D₂O is used as the solvent for NMR measurement.

6. Summary

Various types of saccharide-vinyl monomers have been successfully synthesized using enzymes as the biocatalyst. In organic solvents, lipases and proteases catalyzed transesterification of hydroxyl groups of saccharides with divinyl esters. Depending on the saccharide types and enzyme sources, mono- and disubstitution of vinyl groups were observed at several locations in a saccharide unit. Furthermore, an excellent selectivity was displayed by glycosidases in which the synthesized glycomonomers were characterized to be anomerically pure and monofunctional. Both thermodynamically controlled reversed hydrolysis and kinetically controlled transglycosylation approaches were used to generate these glycomonomers. Moreover, glycosyltransferases catalyzed the synthesis of saccharide-vinyl macromonomers by transferring the glycosyl units from donors to acceptors.

A well-defined structure of glycomonomers provides a good starting point for the investigation of the interaction of glycopolymers with sugar-binding proteins (Kießling & Grim, 2013; Yilmaz & Becer, 2015). Therefore, further experiments will be aimed to prepare glycopolymers by controlled radical polymerization techniques such as atom-transfer radical polymerization, reversible addition-fragmentation chain transfer polymerization, nitroxide-mediated radical polymerization, and cyanoxyl-mediated free radical polymerization.

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