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Enzymatic Derivatization of Saccharides and Their Chemical Polymerization

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Abstract. The enzymatic synthesis of sugar-based acetylene and ethylene derivatives as precursors to hydrophilic polymers is described. Propargyl and allyl alcohols have been used as glycosyl acceptors in the transglycosylation reactions of glycosidases with various disaccharides including lactose, maltose, and cellobiose. Reaction of propargyl and allyl alcohols with lactose catalyzed by β-galactosidase resulted in the formation of propargyl-β-D-galactopyranoside and allyl-β-D-galactopyranoside in 42 and 13% yields, respectively. Polymerization of propargyl-β-D-galactopyranoside with $AlBr_3$ in ethanol resulted in the formation of oligomeric poly(acetylenic) species (M_W = 1,300). Free radical polymerization of allyl-β-D-galactopyranoside in DMF or water resulted in poly(ethylenic) species with M_W > 30,000. The combined enzymatic and chemical reactions inherent in these syntheses provide a unique approach in the preparation of hydrophilic polymers containing sugars and their derivatives.

The vast majority of poly(acetylene)s and poly(ethylene)s are hydrophobic and derived from simple monofunctional precursors[1]. These materials have applications as electrical conductors, semiconductors, and magnetic materials for poly(acetylene)s[2], and as cured resins for films and coatings, and structural resins for adhesives and composite matrices for both poly(acetylene)s and poly(ethylene)s[2,3]. Recently, we demonstrated that polymerization of sugar-containing acrylate derivatives led to biodegradable and highly hydrophilic polymers[4]. Incorporation of polyfunctional molecules such as sugars into poly(acetylenic) or poly(ethylenic) homo- or co- polymers may allow the resulting materials to be blended into hydrophilic polymers such as starches or poly(vinyl alcohol, acrylates, etc.). An attachment of unsaturated functional groups to carbohydrates is also important in the construction of artificial polysaccharide T-independent antigens[5]. Such antigens do not require T-cell participation to be recognized by B-lymphocytes[5]. The regioselective synthesis of sugar-based acetylene and ethylene derivatives via chemical routes, however, is difficult due to the multiple number of reactive hydroxyl groups. A substantial number of blocking and deblocking steps are required and the syntheses are tedious , invariably result in low yields[6], and still may provide a mixture of anomers[7]. Unlike chemical catalysts, enzymes are highly selective catalysts and have been used to modify sugars and their derivatives[8]. In the present work, we describe the synthetic strategy

which involves the enzyme-catalyzed attachment of sugars to propargyl or allyl alcohols through a glycosidic linkage followed by chemical oligomerization/polymerization. The high degree of regioselectivity and anomeric selectivity afforded by enzymatic catalysis is applied to modify the sugars prior to polymerization.

RESULTS AND DISCUSSION

Perhaps the most common approach to modify sugars is the lipase- or protease-catalyzed transesterification of primary hydroxyl groups in various unprotected saccharides in anhydrous pyridine or DMF[8,9]. According to this strategy, a propiolic acid ester should be used as an acylating agent in order to produce a sugar-propiolate ester. Unfortunately, propiolic acid and its esters polymerize easily in the presence of bases such as pyridine[10]. Because of this problem, incubation of *O*-methyl-β-D-glucopyranoside with excess ethyl propiolate in pyridine using porcine pancreatic lipase (as well as several other lipases and proteases) results in only trace amounts of the 6-propiolate glucose esters (Blinkovsky and Dordick, unpublished).

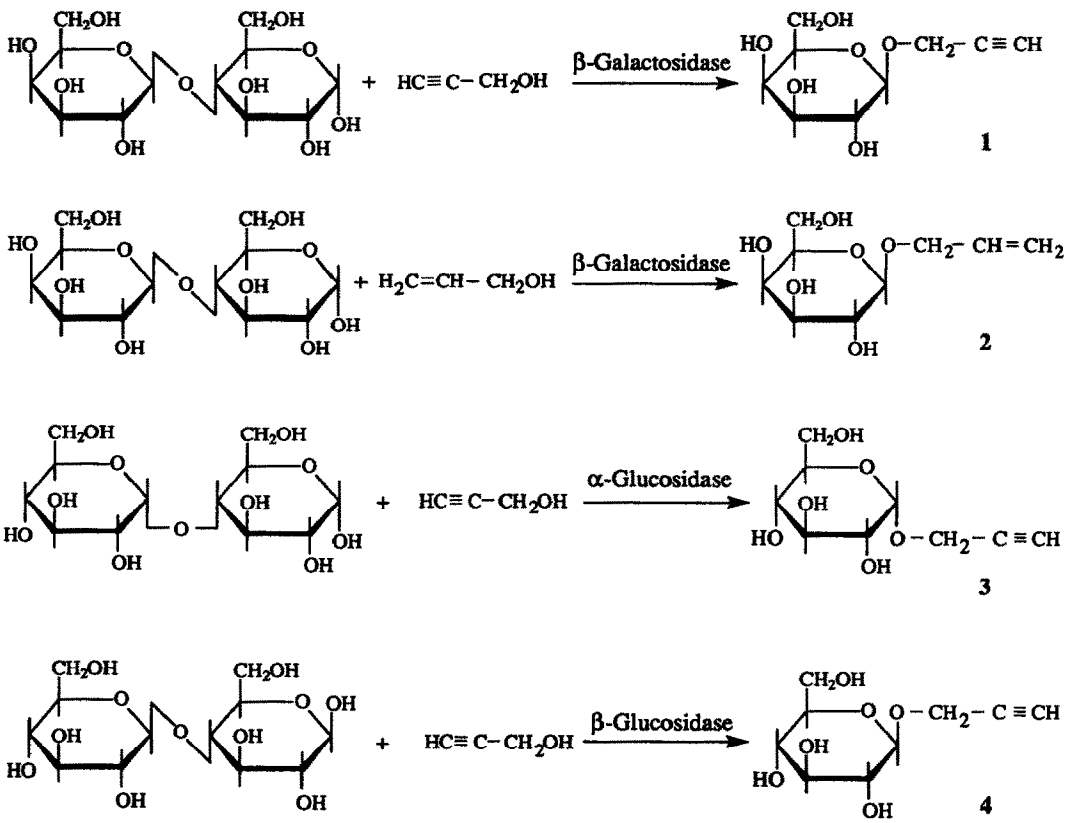
An alternative approach is to attach acetylenic and ethylenic functional groups to sugars via a glycosidic bond using commercially available glycosidases. Such enzymes, in addition to their strong hydrolytic activity, also possess a transferase activity even in aqueous solutions. Scheme 1 illustrates the synthesis of several propargyl and allyl sugar derivatives using specific glycosidases. A particularly instructive synthesis involves the formation of propargyl-β-D-galactopyranoside (**1**) via the β-galactosidase-catalyzed transglycosylation of lactose with propargyl alcohol in aqueous buffer. Following 18 h of incubation, the degree of lactose conversion reached 82% and the reaction was terminated by evaporating off the water. The residue was subjected to silica gel chromatography and 13 g **1** was produced (42% isolated yield); the remainder being free glucose and galactose. The authenticity of **1** as well as the other monosaccharide derivatives was verified by ^{13}C NMR (Table 1).

Table 1. ^{13}C-NMR data[a] for saccharide derivatives.

Compound	Chemical shifts (p.p.m.)								
	C-1	C-2	C-3	C-4	C-5	C-6	$O^aCH_2{}^bC{=}^cCH$ or $O^aCH_2{}^bCH{=}^cCH_2$		
							a	b	c
1	101.4	70.4	73.4	68.2	77.2	60.6	54.8	80.1	75.4
2	103.0	71.8	74.1	69.4	75.5	61.5	70.2	135.0	117.1
3	100.2	71.7	72.6	69.1	72.6	60.6	55.2	80.0	74.7
4	99.6	72.9	73.0	69.6	72.9	61.0	56.5	79.5	75.9

[a]Compound 1, solution in DMSO-d_6; compounds 2, 3, and 4, solutions in D_2O.

Scheme 1. Enzymatic preparation of propargyl and allyl monosaccharides

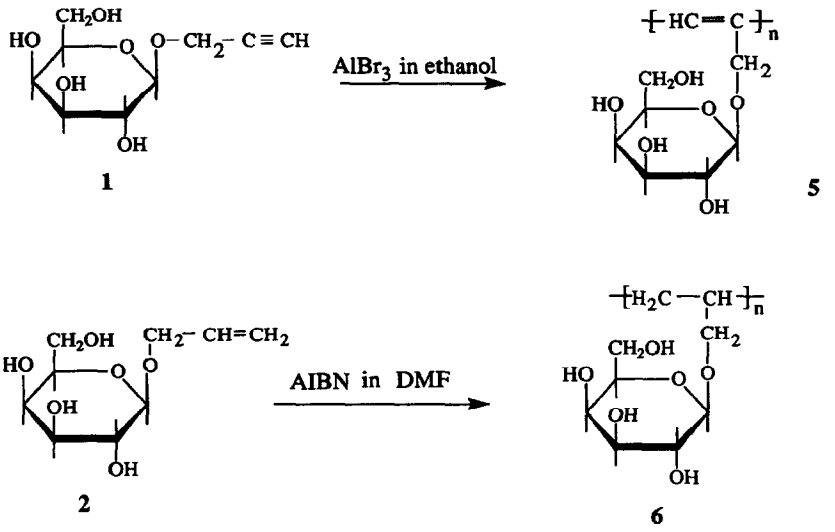


Allyl alcohol is a less reactive acceptor of the β-galactosyl residue in comparison with propargyl alcohol. After 18 h of incubation, 4.0 g of allyl-β-D-galactopyranoside (2) was obtained (13% isolated yield). Propargyl derivatization of α- and β- D-glucopyranoside is also possible using maltose and cellobiose as substrates for α- and β-glucosidase, respectively, to give 3 and 4. Although hydrolysis was the predominant reactions in both cases, isolated yields of the α– and β– propargyl glucosides were ca. 10%. In all cases, only the 1-position of the saccharides were derivatized and with the anomeric specificity of the enzyme employed.

Having established that enzymes can selectively attach triple- and double- bonded groups onto sugars, it was of interest to perform polymerization experiments to incorporate the sugars into poly(acetylene) and poly(ethylene) derivatives. To that end, polymerizations of 1 and 2 were examined using conventional techniques. Polymerization of 1 was attempted by both free radical initiating and cationic catalysts (Table 2). Free radical initiators --AIBN and benzoyl peroxide -- were ineffective. $AlBr_3$ in absolute ethanol was by far the most efficient catalyst. Following 48 h of incubation of 1 with

$AlBr_3$, 2.8 g of oligomeric species were isolated (Scheme 2). GPC analysis indicated an oligomeric distribution with M_w=1,300 and M_n=1,100 for the predominant oligomer. The resulting mixture of

Scheme 2. Polymerization of enzyme-derived monomers



oligomers was dialyzed against water to give 1.95 g of water-soluble, highly hygroscopic propargyl galactopyranoside oligomer (**5**). The triple bond present in **1** (2113 cm^{-1} in FTIR) was replaced by double bonds (1646 cm^{-1}) in **5**. The nickel-based catalyst in Table 2-- dicarbonylbis(triphenylphosphine)nickel -- resulted in the formation of higher molecular weight polymers with M_w = 37,400 and M_n = 36,800, albeit with yields under 5%. Free radical initiators were found to be effective catalysts in the polymerization of **2** containing a double bond in its structure. Both AIBN in DMF and 2,2'-azobis-(2-amidinopropane) (ABAP) in water catalyzed the formation of higher molecular weight water-soluble and highly hygroscopic products (**6**) (M_w=36,000 and M_n=28000, M_w=31000 and M_n=26000, respectively) with yields of 66 and 39%, respectively.

To further investigate the structures of **5** and **6**, degradation of the polymers was studied using β-galactosidase in aqueous buffer. Within 4 h, 38% of the polymer's galactose moieties were released and this value increased slowly to 52% after 43 h. No galactose release was observed in the absence of enzyme. The relatively fast degradation of ca. one-third of the galactose moieties in **5** is consistent with the exoglycosidase activity of the β-galactosidase from *Aspergillus oryzae* [11]. The polymer (M_w = 1,300), on average, contains 6 galactose side-units linked together through a poly(propargyl alcohol) backbone. Treatment of **5** with β-galactosidase results in the facile hydrolysis of the terminal two galactose residues, and hence ca. one-third degradation. The slow hydrolysis of remaining galactose residues is presumably due to sterically hindered

positions of these galactose residues in the short polymer **5**. The degradation of **6** proceeded slowly and less than 10% of the polymer was degraded to free galactose in 8 h using β-galactosidase. Perhaps the larger size of **6** limited its facile degradation by the exogalactosidase employed.

Table 2. Catalysts Used in the Oligomerization of **1**.

Catalyst	Catalyst Conc. (mM)	Conc. of **1** (M)	Solvent	Reaction Time (days)	T(°C)	Conversion (%)[a]	M_w (kdal)
AIBN[b,12]	4	0.45	DMF	7	60	0	-
AIBN	4	0.20	EtOH	7	60	0	-
Benzoyl peroxide[12]	50	0.45	DMF	7	60	0	-
$[(C_6H_5)_3P]_2Ni(CO)_2$[b,13]	10	0.50	EtOH	14	22	3	37.4
$AlBr_3$[14]	3.7	0.20	EtOH	2	30	95	1.3

[a]Conversion determined by GPC on the basis of disappearance of **1**. [b]AIBN -- 2,2-azobisisobutironitrile. The reaction was performed under N_2.

In summary, sugar-based acetylenic and ethylenic derivatives have been prepared by enzymatic synthesis. β-Galactosidase catalyzes the selective glycosidation of galactose in the 1-position with both propargyl alcohol and allyl alcohol resulting in only the β-anomer without the need for blocking and deblocking steps. α- and β-Glucosidases are less effective in the transfer of glucose to propargyl alcohol using maltose and cellobiose, respectively as glucosyl donors. The selective monomers are then oligomerized/polymerized using conventional chemical catalysts. The polymeric materials are water soluble and highly hydrophilic, and should be suitable for applications involving hydrophilic polymers. Furthermore, upon light chemical crosslinking, the polymers are expected to be highly water absorbent. Potential uses of such a material include water absorbents plastics, biocompatible polymers, and hydrogels. The double bonds present in an acetylenic sugar oligomer may be used for further chemical modification to achieve specific functionalities. The observed degradation of **5** and **6** with β-galactosidase indicates that the galactose residues can be biodegraded by microorganisms with both exo- and endo- glycosidase activity, and this represents over 80%, by weight, of the polymer/oligomer. The incorporation of these novel sugar oligomers into existing polymers is now in progress.

EXPERIMENTAL

General. β-Galactosidase from *Aspergillus oryzae* (type XI), α-glucosidase from rice (type V), and β-glucosidase from almonds were obtained from Sigma Chemical Co. (St. Louis, MO) and were used for synthetic reactions without further purification. All polymerization catalysts were obtained from Aldrich Chemical Co. (Milwaukee, WI) with the exception of 2,2-azobisisobutyronitrile (AIBN) and 2,2'-azobis-(2-amidinopropane) (ABAP) which were purchased from Polysciences (Warington, PA). All other compounds

and solvents were of the highest purity commercially available. Organic solvents were dried overnight using 3Å molecular sieves (Linde (Danbury, CT)). Melting point measurements were uncorrected. ^{13}C NMR spectra were recorded on a Brüker WM 360 MHz instrument with TMS as internal reference and DMSO-d_6 or D_2O as solvent. FTIR spectrum was recorded on a Brüker IFS 113V instrument with the sample as a KBr pellet. Differential scanning calorimetry was performed on a DuPont Instruments DSC 2910. Optical rotations were measured at 589 nm (sodium line) at 25°C in a Jasco DIP-360 optical polarimeter. Enzymatic reactions were followed by TLC. Oligomerization/polymerization reactions were followed by analytical gel permeation chromatography using refractive index detection (Waters (Milford, MA), model 410 refractive index detector) with Ultrahydrogel columns of 120 and 250 Å in series (molecular weight range from 100 to 80,000 daltons). Poly(ethylene glycol) standards were used with molecular weights of 1,100, 12,500, 20,000, and 50,000 daltons purchased from Polysciences. The mobile phase was 0.1 M $NaNO_3$ at a flow rate of 1 ml/min.

Enzymatic Synthesis of Propargyl-β-D-Galactopyranoside (**1**). A solution of 50 g lactose in 100 mL sodium acetate buffer (20 mM, pH 4.5) was prepared and 25 mL propargyl alcohol were added. The reaction was initiated by the addition of 0.1 g β-galactosidase and the mixture stirred at 100 rpm and 25°C. The reaction was stopped by filtering off the residual solid enzyme particles and the filtrate was dried under rotary vacuum to give a yellowish oil. The oil was subjected to silica gel chromatography (4 x 60 cm) with 0.5 L ethyl acetate followed by 0.7 L ethyl acetate:ethanol (4:1). The fractions corresponding to **1** were collected and dried to give a light yellow powder (13 g, 42% isolated yield): mp 118-119°C; thermal decomposition above 250°C (via DSC); $[\alpha]_D^{25}$=-39.8 (c1.8, H_2O). Anal. Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.42; O, 44.04. Found: C, 49.49; H, 6.42; O, 44.09. For ^{13}C-NMR data see Table 1.

Enzymatic Synthesis of Allyl-β-D-Galactopyranoside (**2**). A solution of 50 g lactose in 100 mL sodium acetate buffer (20 mM, pH 4.85) was prepared and 25 mL allyl alcohol were added. The reaction was initiated by the addition of 0.14 g β-galactosidase and the mixture stirred at 100 rpm and 25°C. The reaction was stopped by filtering off the residual solid enzyme particles and the filtrate was dried under rotary vacuum to give a white oil. The oil was subjected to silica gel flash chromatography with ethyl acetate:methanol:water (72:10:3). The fractions corresponding to **2** were collected and dried to give a white powder (4.0 g, 13% isolated yield): mp 102-103°C; $[\alpha]_D^{25}$= -11.2 (c2, H_2O). Anal. Calcd. for $C_9H_{16}O_6$: C, 49.09; H, 7.27; O, 43.64. Found: C, 49.19; H, 7.19; O, 43.62. For ^{13}C-NMR data see Table 1. Allyl-β-D-galactopyranoside was described earlier[15,16]. For comparison, it was found: mp 102-103°C; $[\alpha]_D^{20}$= -11 (c2, H_2O); ^{13}C-NMR data: 103.3, 72.1, 74.4, 69.7, 75.9, 61.9, 70.6, 135.4, 117.5, respectively.

Enzymatic Synthesis of Propargyl-α-D-Glucopyranoside (**3**). A solution of 12.5 g maltose in 25 mL sodium acetate buffer (20 mM, pH 4.85) was prepared and 6.25 mL propargyl alcohol were added. The reaction was initiated by the addition of 0.5 ml (ca. 50 units) α-glucosidase and the mixture stirred at 100 rpm and 25°C. The reaction was stopped by drying under rotary vacuum. The residue was subjected to silica gel flash chromatography with ethyl acetate:methanol:water (72:5:4). The fractions corresponding to **3** were collected and dried to give a white powder (0.78 g, 10% isolated yield): mp 77-79°C; $[\alpha]_D^{25}$=+95.9 (c1.8,

H_2O). Anal. Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.42; O, 44.04. Found: C, 49.22; H, 6.51; O, 44.23. For ^{13}C-NMR data see Table 1.

Enzymatic Synthesis of Propargyl-β-D-Glucopyranoside (**4**). Propargyl alcohol (6.25 ml) was added to a suspension of 5 g cellobiose in 25 mL sodium acetate buffer (20 mM, pH 4.85). The glucosylation was initiated by the addition of 50 mg (ca. 40 units) β-glucosidase and the mixture stirred at 100 rpm and 25°C. The reaction was stopped by drying under rotary vacuum. The residue was subjected to silica gel flash chromatography with ethyl acetate:methanol:water (72:5:4). The fractions corresponding to **4** were collected and dried to give a white powder (0.72 g, 9% isolated yield): mp 59-60°C; $[\alpha]_D^{25}$=-54.5 (c1.8, H_2O). Anal. Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.42; O, 44.04. Found: C, 49.35; H, 6.49; O, 44.20. For ^{13}C-NMR data see Table 1. Propargyl-β-D-glucopyranoside was described earlier[17]. For comparison, it was found: mp 55-56°C; $[\alpha]_D^{20}$= -51.3 (c2, H_2O); ^{13}C-NMR data were not shown.

Synthesis of Propargyl-β-D-Galactopyranoside Oligomer (**5**). Several chemical catalysts were screened for their abilities to catalyze the oligomerization of **1**. The reaction with $AlBr_3$ was the most successful. To that end, 3.0 g **1** was dissolved in 60 mL absolute ethanol. The reaction was initiated by addition of 63 mg $AlBr_3$ and the mixture stirred at 200 rpm and 30°C. The reaction was terminated after 48 h and the ethanol removed by rotary evaporation resulting in 2.8 g of a pale yellow powder. The powder was dissolved in water and dialyzed against a total of 1 L of water with a 1,000 molecular weight cut-off (MWCO) dialysis membrane for 24 h. The retentate was freeze dried to give 1.95 g of a pale yellow powder (65% isolated yield): softening point 183-190°C via both visual determination using a melting point method and a DSC method; $[\alpha]_D^{25}$=+103.7 (c1.2, H_2O); elemental analysis could not be performed due to the extremely hygroscopic nature of the polymer.

Synthesis of Allyl-β-D-Galactopyranoside Polymers (**6**). AIBN and ABAP were used as initiators for polymerization of **2**. A solution of 1.0 g of **2** in 5 ml of DMF was prepared, and 0.1% (w/w) AIBN was added. The polymerization proceeded at 65°C under nitrogen for 14 h. The reaction was terminated by precipitating the polymer with acetone, and the white solids were washed with acetone, dissolved in water and dialyzed against a total of 1 L of water with a 1,000 MWCO dialysis membrane for 24 h. The retentate was freeze dried to give 0.66 g of a white powder (66% isolated yield): softening point 221-231°C via visual determination using a melting point method; $[\alpha]_D^{25}$=+7.6 (c1.4, H_2O). The polymerization reaction with ABAP was performed at 55°C in water under conditions identical to those for AIBN. The polymerization yielded 0.38 g of a white powder (38% isolated yield): softening point 213-222°C via visual determination using a melting point method; $[\alpha]_D^{25}$=+2.4 (c2.0, H_2O).

Enzymatic Degradation of **5** and **6**. 10 mg of **5** or **6** was dissolved in 0.25 mL sodium acetate buffer (20 mM, pH 4.5) containing 0.4 mg dialysed β-galactosidase. The enzyme was dialyzed against aqueous buffer with 3,000 MWCO dialysis membrane to remove low molecular weight oligosaccharides that are present in the commercial enzyme preparation. The reaction was performed at 30°C without shaking. At specified

times, a 25 μL aliquot was removed and free galactose was assayed using the *o*-toluidine reducing sugar assay available from Sigma.

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