ORGANIC LETTERS

2002
Vol. 4, No. 11
1863−1866

In Situ Preparation of β-D-1-*O*-Hydroxylamino Carbohydrate Polymers Mediated by Galactose Oxidase

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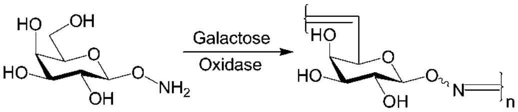
Received March 7, 2002

ABSTRACT



Galactose oxidase produced a C-6 aldehyde in various terminal-containing galactose hydroxylamines for the simultaneous in situ generation of an A–B type condensation for the construction of unique oxime polymers. Molecular weights of the corresponding polymers were determined to be in the range of 4200–8900 g/mol, respectively. This indicates that approximately 20–25 sugar units were incorporated in these unique polymers.

Synthetic or modified natural carbohydrate polymers have a wide variety of practical applications including hydrogels,[1] adsorbents,[2] biorecognition agents,[3] and glycoconjugate assemblies.[4] Carbohydrates conjugated to a variety of materials, including proteins, lipids, and synthetic polymers, have been utilized in diverse scientific disciplines.[5,6] For instance, novel techniques of medical treatments such as artificial organs or drug delivery systems have been developed by ingeniously taking advantage of the recognition processes of carbohydrates in vivo.[7,8] The selective incorporation of sugars and their derivatives into polymers is taking on increased importance, particularly in the preparation of biocompatible and biodegradable materials.[9] The vast majority of in vitro preparation of sugar-containing polymers have utilized glycosidases for polysaccharide synthesis involving unnatural monosaccharide precursors[10] or lipases, esterases, and proteases for poly(sugar ester, amide, or acrylate) synthesis in organic solvents.[11]

The highly selective condensation reactions of ketones or aldehydes with aminooxy, hydrazide, or thiosemicarbazide

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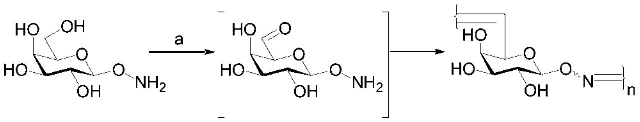
10.1021/ol0258379 CCC: $22.00

groups have been widely used for the conjugation of small and macromolecules. The reactions proceed in aqueous conditions, and their high selectivity negates the requirement for protection of other functional groups on the coupling partners.[12] Galactose oxidase (GO) selectively oxidizes exposed primary hydroxyl groups in nonreducing, terminal galactose and *N*-acetyl galactosamine residues to the corresponding aldehyde.[13] The galactose oxidase/$NaBH_3CN$ method is well-known and widely used as a technique to label cell-surface glycoconjugates.[14] GO also has broad substrate specificity for a variety of galactose- and nongalactose-based compounds.[15] The native reaction on galactose yields a C-6 aldehyde, which has been shown to undergo spontaneous Schiff base formation in the presence of amines.[16] Recently, Dordick and co-workers reported the chemoenzymatic synthesis of sugar-containing polyamines using GO coupled with chemical reduction. GO was first used to selectively oxidize C6−CH_2OH to an aldehyde, and then in a subsequent reaction, polycondensation of the aldehyde with diamines via reductive amination, either in an AA−BB or A−B fashion, afforded the poly(galactose amine)s.[17]

Our approach to synthesizing nonnatural carbohydrate polymers takes advantage of an anomeric hydroxylamino preparation, first reported by Roy and co-workers.[18] In the consideration of the fact that chemoselective ligation of an aldehyde and a hydroxylamine proceeds readily in aqueous solvents for the formation of oximes which are stable in water, we envisioned that the GO oxidation of *β*-D-1-*O*-hydroxylamino galactose (Scheme 1) and related compounds in aqueous buffer solutions would lead to the formation of polysaccharides by selective C6−CH_2OH oxidations and sequential A−B condensations with hydroxylamine moieties. The result would be novel, high molecular weight oxime polymers that are relatively stable to hydrolysis. This methodology would allow enzyme-mediated polymer synthesis under simple and mild conditions and provide products with a relationship to natural carbohydrate polymers in which most are connected from the anomeric carbon.

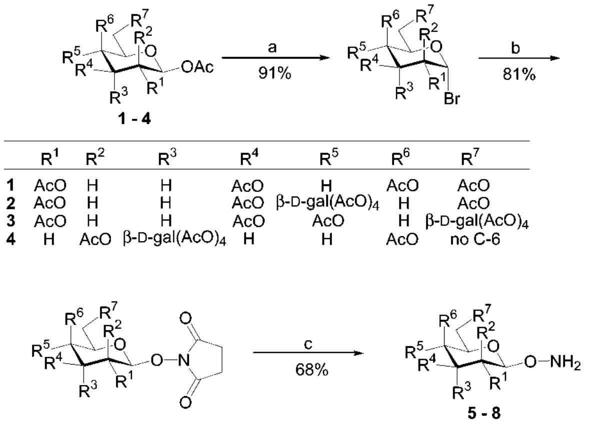
Scheme 1. In-Situ Polymerization[a]



[a] 120 U GO, 1200 U catalase in 10 mL of phosphate buffer (50 mM pH 7.0) containing 3 mg of $CuSO_4{\cdot}5H_2O$ with a continuous stream of air.

The synthesis of the *β*-D-1-*O*-hydroxylamine galactose (GO substrates **5**−**8** for in situ polymerization) moieties is shown in Scheme 2. Previously, *β*-D-1-*O*-hydroxylamine sugars prepared in the same manner have been purified by preparative HPLC.[18b] However, in the present study, an effective way for purification on a relatively large scale was desirable. To this end, ion-exchange columns were found to be inadequate due to stability problems of the products under such conditions.[19] Recrystallization with a variety of solvent systems could only reduce the amount of impurities.[20] Finally, it was found that purification on gram-scale could be achieved by utilizing a gel-filtration column.

Scheme 2. Synthetic Route to *β*-D-1-*O*-hydroxylamino Carbohydrates[a]



	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	AcO	H	H	AcO	H	AcO	AcO
2	AcO	H	H	AcO	β-D-gal(AcO)$_4$	H	AcO
3	AcO	H	H	AcO	AcO	H	β-D-gal(AcO)$_4$
4	H	AcO	β-D-gal(AcO)$_4$	H	H	AcO	no C-6

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
5	OH	H	H	OH	H	OH	OH
6	OH	H	H	OH	β-D-gal(OH)$_4$	H	OH
7	OH	H	H	OH	OH	H	β-D-gal(OH)$_4$
8	H	OH	β-D-gal(OH)$_4$	H	H	OH	no C-6

[a] (a) 33% HBr/AcOH, 6 h. (b) NHS, (1:1, CH_2Cl_2:1 M $NaCO_3$), 24 h. (c) $NH_2NH_2−H_2O$, 12 h.

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(19) Three different types of cation-exchange resin were used; Amberlite IRP-64, Amberlite IRP-69, and NaFion R NRSO XR ion-exchange resin from DuPont Company.

(20) H_2O/MeOH with varying concentrations; H_2O/CH_3CN with varying concentrations.

GO-catalyzed oxidation reactions of β-D-1-*O*-hydroxylamino terminal galactose proceeded readily at room temperature. The corresponding rates of reaction for β-D-1-*O*-hydroxylamino lactose, melibiose, and β-D-gal(1→3)-D-arabinose were found to be slower under the same conditions. As was expected, polymerization took place for all β-D-1-*O*-hydroxylamino compounds; the progress was monitored via TLC. As the generation of the aldehyde proceeded, a distinctly new spot began to materialize at the base of the silica gel TLC plate, while the color concentration of the starting material faded (solvent system *i*-PrOH/H_2O/(28−30%)NH_4OH:7/3/2). For comparison, Figure 1 shows the ^{1}H NMR spectra of the starting material and isolated products from polymerization reactions of **5** and **6**. The spectra of the products show significant differences from those of the starting materials. In addition to completely different display patterns, all the peaks have been considerably broadened. The key features of the spectra are the two signals in the region from 6.90 to 8.00 ppm, which belong to the proton on the oxime carbon. It is possible to distinguish between *syn* and *anti* isomers since the H−C═N shift is upfield in the sterically more compressed form, and the more hindered substitute (*syn* to the OR) is upfield to the less hindered (*anti*).

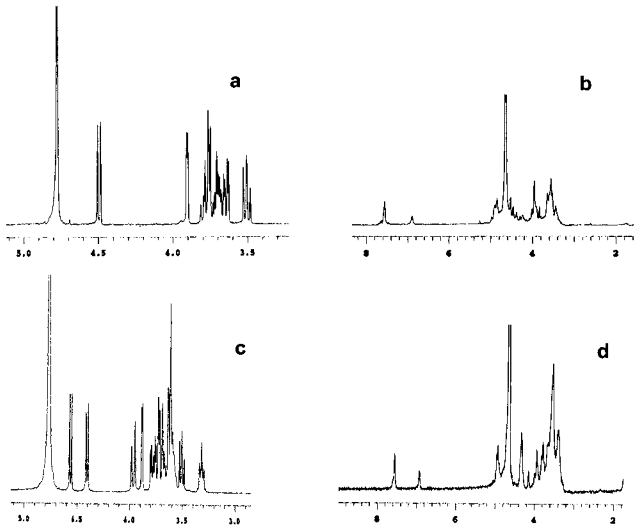

Figure 1. ^{1}H NMR of the starting β-D-1-*O*-hydroxylamino sugars (galactose and lactose) and the resulting polymers. Note the *syn* and *anti* peaks in the 6.0−8.0 ppm region. (a) β-D-1-*O*NH_2 galactose, (b) oxime Gal-polymer, (c) β-D-1-*O*NH_2 lactose, (**d**) oxime lactose-polymer.

The molecular weights of the resulting polymers were determined by HPLC size exclusion chromatography using dextran standards.[21] As summarized in Table 1, the resulting polymer from the reaction of β-D-1-*O*-hydroxylamino lactose **6** (entry 2) in the phosphate buffer (pH 7.0) has a molecular weight (MW) of 7800. The corresponding reaction of β-D-1-*O*-hydroxylamino galactose **5** (entry 1), however, gave a polymer with a lower molecular weight (MW) of 4200. Since it is known that acidic conditions promote oxime formation, we performed selective GO reactions in a buffer at pH 5.5 only to obtain lower molecular weight polymers. This was attributed to the fact that optimal GO oxidation conditions are conducted in a 50 mM phosphate buffer at pH 7.0. However, slightly improved results were obtained by stirring the resulting mixture in an acidic solution (0.2 M HOAc) for a prolonged time (24 h), entries 7 and 8 (Table 1).

Table 1. GO-Mediated Polymerization of β-D-1-*O*-hydroxylamino Sugars

entry	monomer	yield (%)	M_w	M_w/M_n[d]
1[a]	1-ONH2 Gal	62	4200	1.7
2[a]	1-*O*NH_2 Lac	44	7800	1.9
3[a]	1-*O*NH_2 Mel	12	4500	2.2
4[a]	1-*O*NH_2-Gal-Ara	47	7300	2.4
5[b]	1-*O*NH_2 Gal	51	4000	1.9
6[b]	1-*O*NH_2 Lac	38	7500	1.8
7[c]	1-*O*NH_2 Gal	60	5100	2.0
8[c]	1-*O*NH_2 Lac	42	8900	2.1

[a] Reactions conducted in phosphate buffer (50 mM, pH 7.0). [b] Reactions conducted in phosphate buffer (50 mM, pH 5.5). [c] Resulting polymers from method a were stirred in 0.20 M acetic acid for 24 h. [d] Polydispersity where M_n is the number average molecular weight.

These novel C-6 oxime-linked polymers show a fair amount of monomeric carbohydrate unit polymerization. For instance, in the galactose example (entry 1), the resulting polymer contains a MW of 4200, which corresponds to the polymerization of 24 galactose units. For lactose (entry 2), the resulting MW of the polymer is 7800, which translates to 23 monomeric lactose units. The number of substituted monomeric units increases when the resulting polymers are allowed to stir in 0.20 M acetic acid (entries 7 and 8) from 23 to 26 for both entries. These values indicate the same number of monomeric units form the oxime-linked polymer regardless of monosaccharide or disaccharide carbohydrates. HPLC of the retentate material gave the calculated M_w/M_n (polydispersity) values indicated in Table 1. If polymers of higher molecular weights are desired, the number of condensing chains must be smaller, so that the polymerization reaction rate is slower and can become similar to the thermal polymerization rate, thus broadening the polydispersity. Polymers were subjected to decomposition conditions[22] and were found to be stable at pH 2, 25 °C (1 M HOAc/NaOAc) and at pH 11, 25 °C (1 M Na_2HPO_4).

In summary, we have demonstrated that GO-mediated selective oxidation of β-D-1-*O*-hydroxylamino galactose and other β-D-1-*O*-hydroxylamino sugars containing a galactose terminus resulted in the formation of carbohydrate polymers, which contain oxime linkages between the C-6 of galactose

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[22] Polymers were heated to 100 °C at pH 2.0 and 11 for 2 h. At pH 7.0 the polymer did not decompose when heated.

and the anomeric carbon, either of the same galactose moiety or of other sugar units (D-lactose, β-gal-D-arabinose, and D-melibiose). The GO-mediated polymerization, under environmentally benign conditions, provides a unique and convenient way for the synthesis of nonnatural polysaccharides with novel structures, closely related to those found in nature.

Acknowledgment. P.R.A. acknowledges a summer 2001 graduate fellowship from Wayne State University and P.G.W. acknowledges a research grant from Hercules Incorporated.

Supporting Information Available: Experimental details for the syntheses of compounds in Scheme 2, steps a−c, as well as the reaction conditions for the GO-mediated polymerization. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0258379