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Short communication

Preparation and copolymerization of a novel carbohydrate containing monomer

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Abstract

A novel carbohydrate containing monomer was prepared by simple reaction of 2-vinyl-4,4′-dimethylazlactone (VDMA) and 1,2;5,6-diisopropylidene-α-D-glucofuranose (DAG). The monomer was easily homopolymerized as well as copolymerized with methyl methacrylate (MMA) to give high molecular weight polymers using free radical conditions. Upon removal of the isopropylidene groups from the polymer, water contact angles decreased for polymeric films of the material, indicating a more hydrophilic surface. The addition of a carbohydrate moiety to MMA copolymers increases its hydrophilicity and allows for potential use of the new polymer as a biomaterial in a variety of applications.

Keywords: Vinyl saccharide; Polymerization; MMA; Contact angle; Deprotection

1. Introduction

In recent years, the desire for polymeric materials which demonstrate increased biocompatibility has grown significantly. Traditional definitions of biocompatibility often make reference to the hydrophilicity of a material, whose originally intended use may or may not have been for a biomaterial application (Ratner, Hoffman, Schoen, & Lemons, 1996). Recently, definitions of biocompatibility have been expanded to include the ability to elicit a desired response specific to the intended application of the material. As polymer research has progressed, new methods for polymer synthesis and characterization have been developed and allow for specific molecular design of polymers intended for biomaterial applications.

One group of monomers intended for use in polymeric biomaterial applications are those containing carbohydrate functionality (Callstrom et al., 1992; Gabius, 1998; Ojala, Gleason, Connelly, Wallis, & Kremer, 1996; Weisz & Schnaar, 1991). Copolymerization allows for the creation of hydrophilic materials containing common monomers (e.g., methyl methacrylate) resulting in polymers with high strength, good coating characteristics, and the ability to form hydrogels (Koch & Yaacoub, 2003; Ren, Zhang, & Liu, 2001; Shantha & Harding, 2002; Wulff & Diederichs, 1998; Wulff, Schmidt, & Zhu, 1999; Yamada, Minoda, Fukuda, & Miyamoto, 2001). Copolymerization of carbohydrate monomers not only increases hydrophilicity, there are potential advantages toward biomaterial applications as well. In many biological systems, carbohydrates serve as receptors for proteins, often as specific molecular recognition agents, allowing for cell binding and proliferation. Moreover, polymers with pendant carbohydrate moieties have been useful in clinical diagnostic applications, and for targeted gene therapies (Gabius, 1998; Kunath, von Harpe, Fischer, & Kissel, 2003). Other applications for polymeric materials containing carbohydrate moieties include cell surface mimics, cell adhesion scaffolds, materials for reduced platelet and protein adsorption, thin film

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coatings, and anticoagulants (Bertozzi, Mukkamala, Chen, Hu, & Baude, 2000; Bulpitt & Aeschlimann, 1999; García-Martín et al., 2000; Morra & Cassinelli, 1999, 1997).

This communication describes the synthesis and characterization of a novel carbohydrate monomer, 1,2;5,6-di-*O*-isopropylidene-3-*O*-(*N*-acryloyl-2-methylalaninate)-α-D-glucofuranose (VDMGlu) and its subsequent copolymerization with methyl methacrylate (MMA). Analysis was conducted to examine monomer and polymer structure, molecular weight, and surface characteristics.

2. Experimental

2.1. Materials

1,2;5,6-Di-*O*-isopropylidene-α-D-glucofuranose (DAG) was obtained from Pfanstiehl (Waukegan, IL); 2-vinyl-4,4′-dimethylazlactone (VDMA) was obtained from SPNE (Princeton, NJ) and was distilled following a previously described technique (Stanek, Heilmann, & Gleason, 2003); and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), butylated hydroxytoluene (BHT), and 2,2′-azobisisobutyronitrile (AIBN) were obtained from Aldrich (Milwaukee, WI). All other materials were reagent grade or higher and used as received.

2.2. VDMGlu synthesis

DAG (20.35 g, 78 mmol), VDMA (10.88 g, 78 mmol) and chloroform (20 mL) were added to a 250-mL round bottom flask. To the reaction mixture was added DBU (0.35 g, 2.3 mmol, 3 mole%) as a catalyst. The mixture quickly homogenized upon slight heating and slowly turned a dark brown upon soft reflux for 18 h. After cooling and concentrating under vacuum, a light-brown syrup resulted. The syrup was taken up into 100 mL of hot ether, and 25 mL of hexane was added. After cooling, a brown layer formed and upon stirring, the product crystallized. The solid product was filtered, washed with hexane, and dried under vacuum. The yield was 30.0 g of a light tan solid (96% by mass balance). NMR analysis was consistent with desired product. (See Fig. 1 for the schematic of the reaction of VDMA and DAG and Fig. 2 for labelled structure of VDMGlu along with the ^{13}C NMR spectra.)

2.3. Polymer synthesis

The following is a representative procedure. VDMGlu (5.0 g, 12.5 mmol), MEK (13 g) and AIBN (0.01 g, 0.061 mmol, 0.2 mass%) were placed into a 50-mL vial and homogenized. (For copolymerization, MMA and VDMGlu were added in varying amounts and the reactions were run at various polymerization times.) The solution was sparged with nitrogen gas for 30 min before being sealed with Teflon tape and capped with an aluminum cap. The vial was placed in a shaking, 65 °C water bath for 18 h, removed and allowed to cool to room temperature, and solid polymer was precipitated by slow addition to stirring isopropyl alcohol. The polymer was vacuum-filtered and dried in a vacuum oven for 20 h. ^1H NMR was consistent with the predicted polymer product.

2.4. Copolymer deprotection

In a 4-dram vial, 0.501 g poly(MMA-co-VDMGlu) (Table 1, Polymer 1) and 5.0 mL of trifluoracetic acid:-water (TFA:H_2O, 9:1, v/v ratio) were added. The polymer slowly dissolved upon stirring for 1 h with an open cover to allow acetone to escape. The solution was poured into 50.0 mL of HPLC grade water and 75 mL of 0.093 M NaOH was added to neutralize the TFA. The solid was vacuum-filtered and washed extensively with HPLC grade water and lyophilized overnight to remove any residual water. The polymer yield was approximately 80%.

2.5. Characterization

GPC analysis was conducted on a HP GPC system with a HP 1074A refractive index detector, an Agilent 35900E analog to digital signal converter with Agilent Technologies software for molecular weight determinations. All samples were dissolved in degassed, HPLC grade tetrahydrofuran (THF) at concentrations of 10 mg/mL.

Proton NMR was conducted on either a Varian Inova 300 MHz, or 500 MHz spectrometer. Chloroform ($CDCl_3$) was used as solvent with TMS as reference. VDMGlu was analyzed by 2D NMR (HMQC) for structural analysis and determination.

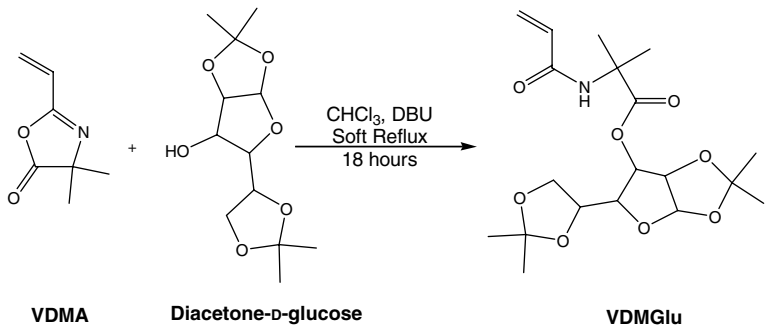

Fig. 1. Preparation of VDMGlu from DAG and VDMA.

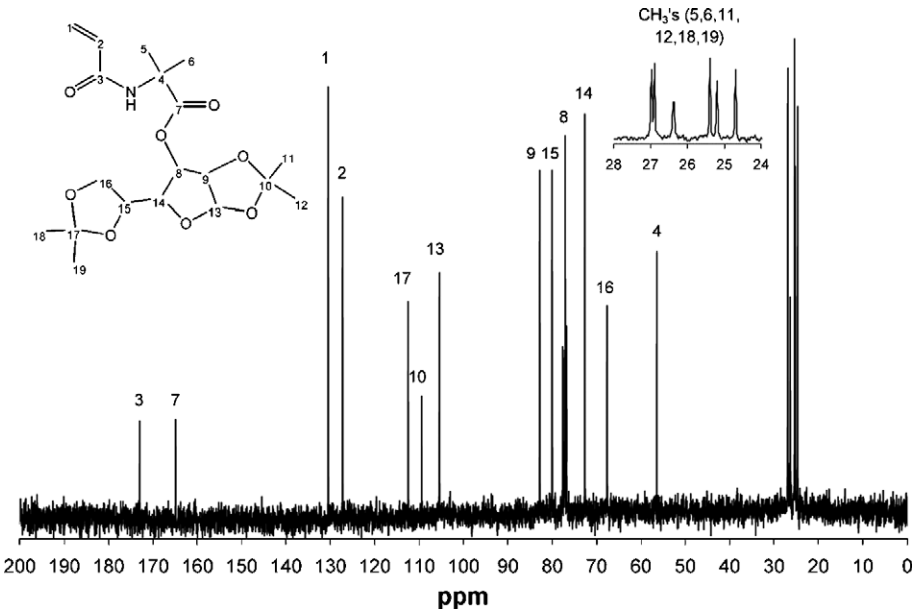

Fig. 2. ^{13}C NMR of VDMGlu taken from $CDCl_3$.

Table 1
Molecular weight data comparing similar polymerization of VDMGlu and VDMA

Polymer	M_w (kg mol^{-1})	M_n (kg mol^{-1})	PDI
p(VDMGlu)	60.5	24.5	2.47
p(VDMGlu)	61.6	25.4	2.42
p(VDMA)	119	39.1	3.05
p(VDMA)	121	35.5	3.42

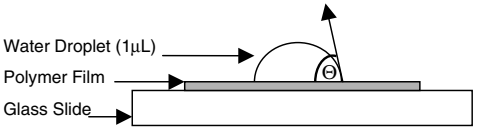

Fig. 3. Schematic of water contact angle measurements where the angle is measured from the surface of the polymer to the tangent of the intersection of the water droplet and the polymer film.

Water contact angle measurements were conducted using a simple optical goniometer to view water droplets on polymer surfaces coated on glass microscope slides. Water droplets (1 μL) were added to the surface of the polymer thin film and the angle was measured as the tangent to the interface of the droplet and the film (see Fig. 3). Ten droplets were placed on the surface and the water contact angles were measured and averaged.

3. Results and discussion

This communication describes a new member of a class of carbohydrate containing monomers, 1,2;5,6-di-*O*-isopropylidene-3-*O*-(*N*-acryloyl-2-methylalaninate)-α-D-glucofuranose (VDMGlu), which was readily prepared in high yield. Proton, carbon and HMQC NMR experiments confirmed the structure of the molecule. Fig. 1 shows the carbon-13 spectra of VDMGlu as well as its chemical structure, along with appropriately labelled peaks corresponding to the labelled carbons in the structure.

3.1. Molecular weight analysis

Molecular weight data obtained from GPC analysis of the homopolymers of VDMA and VDMGlu are shown in Table 1. The data show VDMA homopolymers having higher molecular weight (M_n) and greater PDI than the homopolymers of VDMGlu, when prepared under similar polymerization conditions. This is due to a number of factors, including steric hindrance. Steric hindrance at the site of the propagating radical in the homopolymerization of VDMGlu (large, bulky carbohydrate group) results in a lower rate of polymerization than VDMA. Moreover, the lower molecular weight of poly(VDMGlu) compared to poly(VDMA) is a likely result of chain transfer caused by hydrogen atoms on carbons adjacent to ethereal oxygens.

The data in Table 2 for copolymers of MMA and VDMGlu indicate that with increasing mole fraction of the carbohydrate monomer, the number average molecular weight systematically decreases. In addition, the polydispersity

Table 2
Molecular weight data for poly(MMA-co-VDMGlu) copolymer prepared at short reaction time (2 h)

Trial	F_{VDMGlu} [a]	M_n [b] (kg mol^{-1})	PDI [b]
1	0.05	31.3	1.77
2	0.11	25.8	3.09
3	0.28	21.1	3.14
4	0.68	17.6	3.64

[a] Determined from ^1H NMR.
[b] Determined by GPC using polystyrene standards.

increased when the composition changes to greater amounts of VDMGlu. For the MMA-VDMGlu copolymer system, "penultimate unit effect (PUE)" described by several researchers may help to explain the increased polydispersity shown in Table 2. For copolymers of the carbohydrate monomers, the PUE (i.e., influence of the second to last monomer on polymerization progress) results from the large structure of the carbohydrate moiety (Fukuda, Ma, Kubo, & Inagaki, 1991; Heuts, Gilbert, & Maxwell, 1997). Therefore, at higher mole fraction of VDMGlu, the molecular weight decreases and polydispersity increases because of chain transfer reactions occur more readily than at low mole fraction of VDMGlu.

3.2. Carbohydrate deprotection

Removal of the isopropylidene groups from the carbohydrate containing polymers is an important step in unmasking potential sites for biomolecular recognition. Isopropylidene groups on these protected carbohydrates were readily and quickly removed, under relatively mild acidic conditions, using dilute trifluoroacetic acid. However, reaction times longer than 1 h resulted in polymer degradation and are to be avoided. As shown in Fig. 4, the ^{1}H NMR spectra indicate the removal of the isopropylidene groups was achieved. The key indication of efficient deprotection was the disappearance of the methyl resonances of the isopropylidene groups between 1.2 and 1.6 ppm. The resulting peak at approximately 1.4 ppm is a result of the dimethyl functionality between the amide–ester linkage of the ring opened VDMA.

The molecular weight data shown in Table 3 indicates an increase in molecular weight following the deprotection step. It is hypothesized that the resulting increase in molecular weight shown in Table 3 may be attributed to an associative effect through hydrogen bonding between the carbohydrate moieties. Previous work by Wulff et al. has shown aggregation upon freeze drying of deprotected poly(vinyl saccharides) resulting in increased molecular weight (Wulff, Bellmann, Schmid, & Podzimek, 1997). Although we initially considered aggregation due to hydrogen bonding as a possible pathway to molecular weight

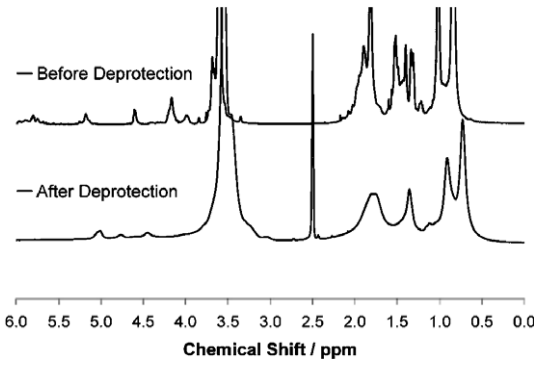

Fig. 4. ^{1}H NMR of before and after deprotection of poly(MMA-co-VDMGlu) copolymer with mole fraction of VDMGlu = 5%.

Table 3
Molecular weight comparison of before and after acid wash of poly(MMA-co-VDMGlu)

Copolymer[a]	M_n[b] (kg mol^{-1})	PDI[b]
Before deprotection	27	2.9
After deprotection	67	2.4

[a] poly(MMA-co-VDMGlu) with 20 mass% VDMGlu.
[b] Determined by GPC using polystyrene standard (see Section 2).

increase, it is also possible that this is due to covalent cross-linking of the polymer chains. Under conditions of acid catalyzed hydrolysis of isopropylidene groups, an increase in polymer molecular weight was observed. Possible mechanisms may include an acid-catalyzed intramolecular aldol reaction, or anhydro ring formation followed by intramolecular reaction. Thus Uryu et al. (Uryu, Kitano, Tachikawa, Ito, & Matsuzaki, 1978) have reported that 5,6-anydro-1,2-*O*-isopropylidene-alpha-D-glucofuranose may be polymerized under cationic conditions. These possibilities will be explored in the future in our laboratory. Through such effects, the resulting larger structures are more readily excluded in GPC analysis, resulting in an observed increase in molecular weight. In addition, similar acidic treatment of pure PMMA (MW = 15 kg mol^{-1}) was conducted and NMR and GPC data indicate no polymer degradation, therefore no loss of methoxy groups from MMA to form methacrylic acid residues was observed.

Following the deprotection step, polymer hydrophilicity was investigated using static water contact angle measurements. Water contact angle measurements are a useful characterization tool to quickly and qualitatively determine the hydrophilicity of a polymer coating. The contact angle of a variety of glycopolymers has been examined previously (Klein, Kunz, & Guderjahn, 1995; Morra & Cassinelli, 1999; Wulff et al., 1999), and therefore adopted for study of the carbohydrate containing copolymers in this communication. Fig. 3 depicts the typical view of a water droplet on thin films of the VDMGlu copolymer.

The data shown in Table 4 indicated that copolymer films containing VDMGlu were hydrophobic, described by the large water contact angle. Pure PMMA films also exhibit hydrophobic water contact angles of approximately 66° (Kwok, Leung, Lam, Wu, & Neumann, 1998). The decreased hydrophobicity of the MMA-VDMGlu

Table 4
Contact angle values for p(MMA-co-VDMGlu) and deprotected p(MMA-co-VDMGlu) coatings on glass microscope slides

Polymer	Contact angle, Θ[a] (degrees)	Standard deviation
PMMA (Kwok et al., 1998)	66.1	3.5
p(M-co-VG)[b]	62.8	2.3
p(M-co-deproVG)	59.0	3.5
p(VG)	0[c]	0[c]

[a] Angle from polymer surface, and an average of 10 measurements.
[b] Mole percent VG as determined by ^{1}H NMR is 15%.
[c] Dissolved p(VG) coating on glass microscope slide.

copolymer is likely due to the small mole fraction of carbohydrate monomer. In addition, following deprotection, contact angle once again decreased, which indicated an increase in hydrophilicity. Interestingly, poly(VDMGlu) is completely wetted by water droplets, resulting in no water contact angle. This indicated that even in the protected from, poly(VDMGlu) is a hydrophilic polymer, and the addition of carbohydrate containing monomers to a copolymer of MMA will result in increased hydrophilicity.

4. Conclusions

We have described the synthesis and copolymerization of a new carbohydrate containing monomer, 1,2;5,6-di-*O*-isopropylidene-3-*O*-(*N*-acryloyl-2-methylalaninate)-α-D-glucofuranose (VDMGlu), using a simple and efficient preparation method. The ability of VDMGlu to undergo copolymerization with methyl methacrylate demonstrates that incorporation of carbohydrate monomers into copolymeric biomaterials can be readily accomplished. The resulting copolymers contained lower mole fraction of VDMGlu than the monomer feed predicted, based on NMR results, indicating greater amounts of MMA in the copolymers. In addition, isopropylidene groups were easily removed under acidic conditions, resulting in an increase in the hydrophilicity of the polymer film based on static contact angle measurements. Through this work, the preparation of new carbohydrate monomers and the ability to control the amount of carbohydrate in the copolymer may prove useful in a wide variety of biomaterials applications.

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