Carbohydrate-Centered PAMAM Dendrimers for Growing Liver Cells

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Abstract

Glycodendrimers are a fairly new area of polymer synthesis offering an array of possible applications in the field of biomaterials and tissue engineering. In this study a galactose-centered polyamidoamine (PAMAM) dendrimer was synthesized for use in the potential application of tissue engineering, specifically as scaffolds for growing liver cells. Synthesis of the dendrimer can be summarized by the following; conversion of carbohydrate hydroxyls to halides, followed by the two-step Gabriel synthesis, and finishing with the iterative two-step reaction of PAMAM synthesis. Products were examined using the analytical methods of FTIR, H and C-NMR, and MALDI-TOF-MS.

1.0 Introduction

Bioengineering studies are taking an ever increasing precedence in global research today. Stem cell research, genetics, and tissue engineering are several fields receiving major focus. Tissue engineering, in specific, remains an emerging, rapidly expanding field that melds new age studies with engineering principles in an attempt to mimic or control biology through means of achieving specific biological responses. In this research, focus is being directed on the liver due its vital role in the body and continued high demand in the medical field. Liver organ shortage continues to be a major contributor to disease related deaths each year, accounting for more than 27,000 deaths in the US alone. With over 17,000 people still on the liver transplant waiting list (only about 5,000 cadaveric livers become available for transplant each year)(American Liver Foundation, 2002-2003)[1] and liver related disease such as hepatitis expected to rise in coming years, the need for alternative sources of liver tissue will only increase in demand.

Carbohydrate centered polyamidoamine (PAMAM) dendrimers will be studied for their potential application in tissue engineering. It is believed that the advantageous properties of dendrimers combined with the integration of carbohydrate derivatives will provide a support material that will enable high cell-scaffold interactions, thereby increasing cell survivability and functionality. Dendrimers have a variety of properties that can be utilized towards applications in tissue engineering including strictly controlled geometries and possession of vacant cavities near the core for possible use in drug (Twyman, 1999;[2] Kohle, 2003;[3] Beezer, 2003)[4] and DNA (Wang, 2000;[5] Aulenta, 2003)[6] delivery. They also possess a large quantity of functional groups that allow for high reactivity as well as manipulation of certain physical properties such as solubility and toxicity (Jevprasesphant, 2003)[7]. In the present contribution, a galactose-centered PAMAM dendrimer, referred to as an octopus glycoside (Rockendorf and Lindhorst, 2001)[8], was synthesized for future use as a scaffold material in growing liver cells.

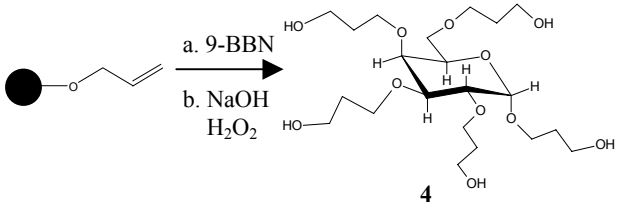
2.0 Experimental

Step1: Acetyl chloride is added to allyl alcohol at 0°C. D-galactose is then added and the solution is heated to 70° for 2.5 hours under reflux to form **2** (Lindhorst, 2000)[9]. The solution is neutralized with sodium bicarbonate, filtered over a celite bed, and concentrated under vacuum. *Step2*: **2** is added to a 33% aqueous NaOH solution, followed by the addition of tetrabutylammonium bromide (TBABr) (5 equivs.). Allyl bromide is added to the solution dropwise at 80°C and the reaction is carried out for 4.5 hours under reflux. Toluene is then added to the mixture and the phases separated. The organic phase is washed thoroughly with DI water and concentrated under vacuum to get **3**. *Step3*: To a solution of **3** in anhydrous THF, 9-borabicyclononane (9-BBN) is added at 0°C. The reaction is then run at room temperature for 1 hour under nitrogen. 35% aqueous hydrogen peroxide and 3M NaOH are then added and the solution stirred at room temperature overnight. Diethyl ether is added and the phases

separated, with the aqueous phase being washed three times with equal volume portions of diethyl ether. The organic phases are combined, dried with anhydrous magnesium sulfate, filtered, and concentrated under vacuum to get **4**. *Step4*: Triphenylphospine (PPh_3) and carbon tetrabromide ($CCBr_4$) are added to a solution of **4** in anhydrous THF. The reaction is carried out in the dark at room temperature overnight, filtered and concentrated. The product is purified with flash chromatography. *Step5*: Potassium phthalimide is added to **5** in DMF. The solution is stirred for 5 days at room temperature, filtered, and concentrated under high vacuum. *Step6*: Step 5 product in dissolved in anhydrous THF, to which hydrazine is added. The reaction is carried out overnight at room temperature under nitrogen then diluted with methanol, filtered, and concentrated under vacuum to get **6**. *Step7*: Methyl acrylate is added to **6** in methanol. Reaction is carried out in the dark for 3 days, and then concentrated. *Step8*: Ethylenediamine is added to **7** in methanol at 0°C, mixed for 5 days at room temperature, and then concentrated under vacuum.

3.0 Results and Discussion

Fischer glycosylation is first undertaken to react the more stable hydroxyl located off the anomeric carbon of galactose, taking advantage of the carbocation forming ability of this carbon by reaction with an acid. A color change of clear to yellow is observed over the course of reaction, with a yellow syrup acquired as the product [90+% yield]. To obtain like chain ends, the remaining hydroxyls are reacted in a phase transfer catalysis reaction (perallylation) with allyl bromide. Adequate washing of the organic phase with water is necessary to ensure complete removal of TBABr from the product [74% yield]. FTIR: [C=C, 1540; no OH band signifies complete conversion] MALDI: [379.1015]



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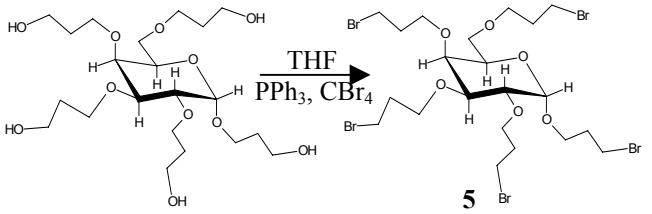
Scheme 0: Hydroboration/oxidation



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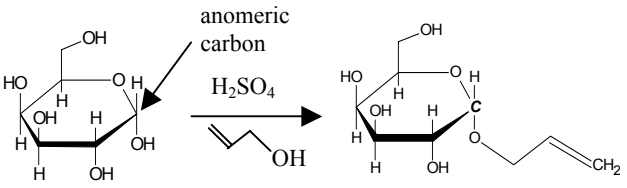
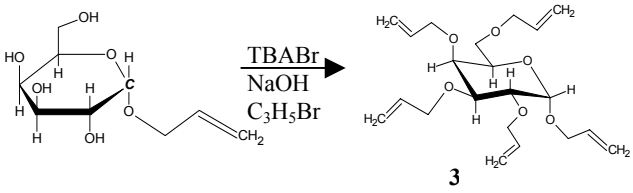
Scheme 4: Halogenation



1 **2**

Scheme 1: Fischer glycosylation



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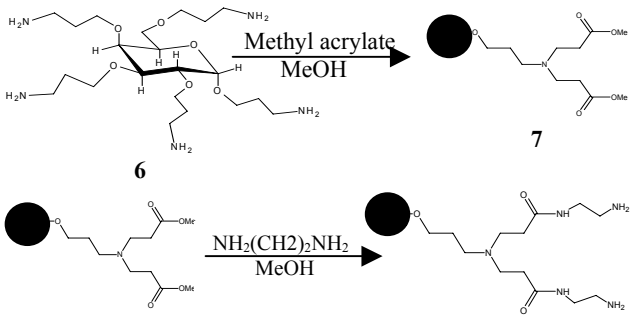
Scheme 2: Perallylation

9-borabicylononane (9-BBN) was used in the hydroboration reaction, as borane-THF complex was found to form a gel when used. Crosslinking is thought to occur with the use of borane as a result of both **3** and borane having multiple reactive sites. Crosslinking is prevented with the use of 9-BBN, as it is capable of reacting with only a single molecule of **3**. A light yellow syrup was attained as a product [60% yield]. FTIR: [OH, 3200-3600; absence of C=C peak] MALDI: [475.1863]

Halogenation was accomplished through the Appel reaction [65% yield]. (Appel, 1975)[10] FTIR: [HBr, 540; no OH peak]; MALDI: [784.2253]. The final conversion of bromine to amine is done in two steps. (Gibson and Bradshaw, 1968)[11] The first

step is nucleophilic substitution of bromide with phthalimide (KPhthN) [89% yield]. FTIR: [C=O, 1750; N, 3450 sharp] The second step is hydrazinolysis, resulting in the replacement of KPhthN with an amine [60% yield]. FTIR: [N, 3400 broad; no C=O peak]

The final steps are those of the repetitive PAMAM synthesis. The first step is the exhaustive Michael addition of methyl acrylate to **6**. The second step is reaction with excess ethylenediamine to obtain the desired amido-amine. These two reactions can be repeated in sequence to obtain higher generations of the dendrimer. Only one arm is depicted due to space constraints.



Scheme 0: PAMAM synthesis

Ongoing work includes surface modification with galactose and short peptide chains (e.g. RGD) to improve cellular interaction, as galactose is known to be a specific binding ligand for the asialoglycoprotein receptor of hepatocytes, i.e. liver cells. The dendrimer will then be crosslinked into gel form for use in cellular studies. Cell functions such as albumin synthesis and p450 activity will be performed through ELISA and EROD assays. Cytotoxicity of the scaffold material will also be studied.

4.0 References

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