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Enzymatic synthesis of a 6-sialyl lactose analogue using a pH-responsive water-soluble polymer support

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ABSTRACT

The Letter describes a strategy for the enzymatic synthesis of glycans based on a pH-responsive water-soluble polymer. In neutral condition, the polymer is water-soluble and convenient for in-solution enzymatic synthesis, whereas in acidic condition (pH lower than 4.0), the polymer disconnects with the product and becomes insoluble, which can be easily removed. A 6-Sialyl lactose analogue was synthesized as a model reaction using this approach.

Solid supports, such as trityl resins and Controlled-Pore Glass (CPG), are widely used for the synthesis of peptides, DNA, and other polymers or oligomers that are assembled in a linear fashion.[1] There are two distinct advantages of solid-phase synthesis. Firstly, excessive material can be used to drive reactions to completion; Secondly, products are easily harvested by simple filtration. For the synthesis of oligosaccharides, enzymatic approaches are more attractive than traditional chemical methods, which are plagued with multiple steps of protection/deprotection steps as well as poor regio/stereoselectivity.[2] Pioneer works in the solid-phase enzymatic synthesis of carbohydrates were based on various kinds of supports including CPG,[3,4] water-compatible polyacrylamide gel,[5,6] sepharose[7,8] and polyethylene glycol polyacrylamide (PEGA).[9] However, researchers are plagued with such problems as non-linear kinetics, low reactivity, stereo-chemical complexity and analytical difficulty.[10]

Recently, Wong and co-workers designed a thermo-responsive water-soluble polymer support to overcome these problems.[10] In their technique, oligosaccharides were synthesized on the polymer through enzymatic glycosylation in homogenous phase and harvested by elevating the temperature. However, the polymer cannot be recovered by simply raising the temperature of the solution for sialic acid containing glycans, due to its hydrophilic character. To compensate for this drawback, we design herein a pH-responsive polymer using 3-pentene-1,3,4-tricarboxylic acid, cyclic 3,4-anhydride as a switchable linker. A trisaccharide, associated with the infection of flu virus, a 6-sialyl lactose analogue was synthesized in a proof-of-concept experiment (Scheme 1).[11,12]

The polymer modified by 3-pentene-1,3,4-tricarboxylic acid, cyclic 3,4-anhydride was synthesized as described in Scheme 2.[13–15] 3-Pentene-1,3,4-tricarboxylic acid, cyclic 3,4-anhydride was obtained from α-ketoglutaric acid diethyl ester and triethyl 2-phosphonopropionate through Wadsworth–Emmons reaction. *N*-Isopropylacrylamide and acrylic acid *N*-hydroxysuccinimide ester were allowed to polymerize after activation by AIBN. To avoid the crosslink in the final product, an excessive amount of 1,2-ethylenediamine was used. In the second step, the 3-pentene-1,3,4-tricarboxylic acid, cyclic 3,4-anhydride was activated by oxalyl chloride and coupled with the free amine group of the synthesized polymer. Due to the facts that acyl chloride is more reactive than the anhydride and excessive amount of acyl chloride used, the linear product was exclusively formed. The reaction mixture was acidified to pH 2 and purified to afford our desired polymer **7**. The IR spectrum confirmed the structure of polymer **7** (Fig. 1). Red shift of the wave number of stretching vibration (1765.0, 1715.3 cm^{-1}) of the anhydride can be observed because of the dampness of the polymer backbone. When reacted with an amine group in neutral condition, the polymer could generate the same amount of carboxyl groups which make the resulting polymer highly hydrophilic and soluble in water. Moreover, when the pH value becomes lower than 4.0, 3-pentene-1,3,4-tricarboxylic acid, cyclic 3,4-anhydride could be regenerated

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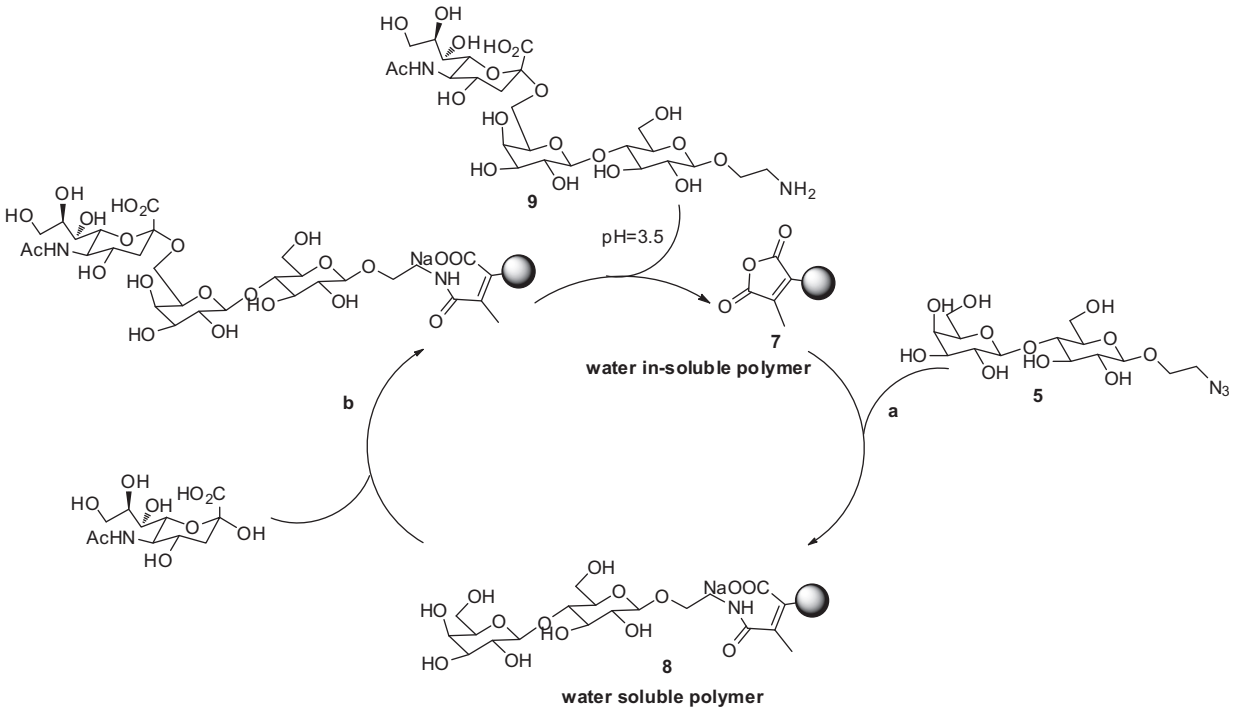
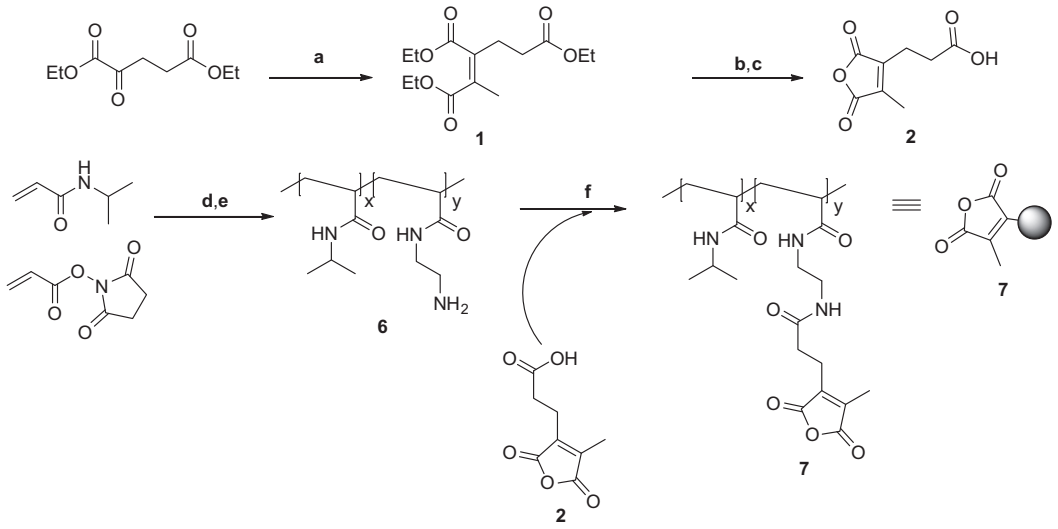


Scheme 1. The enzymatic synthesis of a 6-sialyl lactose analogue using a pH-responsive water-soluble polymer support. Reagents and conditions: (a) $Pd/C/H_2$ 4 atm (100% crude); (b) NmCSS, 2,6SiaT, $MgCl_2$, CTP, pH 8.0 (35%).



Scheme 2. Synthesis of the pH-responsive polymer support **7**: Reagents and conditions: (a) Triethyl 2-phosphonopropionate, NaH, THF (91%); (b) 2 M KOH, EtOH; (c) 1 M HCl (74% two steps); (d) AIBN, DMF; (e) 1,2-ethylenediamine (80% two steps); (f) oxalyl chloride, Et_3N (60%).

and the resulting polymer appears insoluble in water which could be easily removed by centrifugation or filtration. With a functional polymer in hand, lactose bearing a linker with a terminal azido group was synthesized (Scheme 3). Compound **3** was obtained following the standard condition of Schmidt's trichloroacetimidate glycosylation reaction[16] or synthesized from per-acetylated lactose directly. The azido group was introduced via S_N2 substitution. After deprotection, the azido group of compound **5** was converted into the amine group by catalytic hydrogenation. Finally, the amine group was coupled with the anhydride group on the polymer **7** to give the lactose containing polymer **8**. The length of the linker (14 atoms) probably ensured the dynamics and mobility of the lactose as a free acceptor.

The trisaccharide, 6-sialyl lactose, was enzymatically synthesized on the polymer scaffold using 2,6SiaT as previously reported.[17] The donor, CMP-sialic acid was synthesized in situ using CMP-sialic acid synthase, NmCSS. Briefly, polymer **8** was allowed to incubate with sialic acid and CTP at 25 °C (pH 8.0) for 12 h with brief agitation. The reaction was then quenched by boiling, and small molecules (sialic acid, CMP, CMP-sialic acid) were removed by dialysis (MWCO = 3500). The resulting solution was acidified carefully, in which the pH was kept constant at 3.5 during the dropwise addition of the acid. After the pH was steady as monitored by pH meter, the mixture was incubated for 6 h at 25 °C and centrifuged to remove the insoluble polymer. Trisaccharide **9** which has a terminal amine group was obtained by lyophilization with a yield of 35%. The terminal amine functionality allows further conjugating of this trisaccharide to carbohydrate microarray or to form other important glycoconjugate.

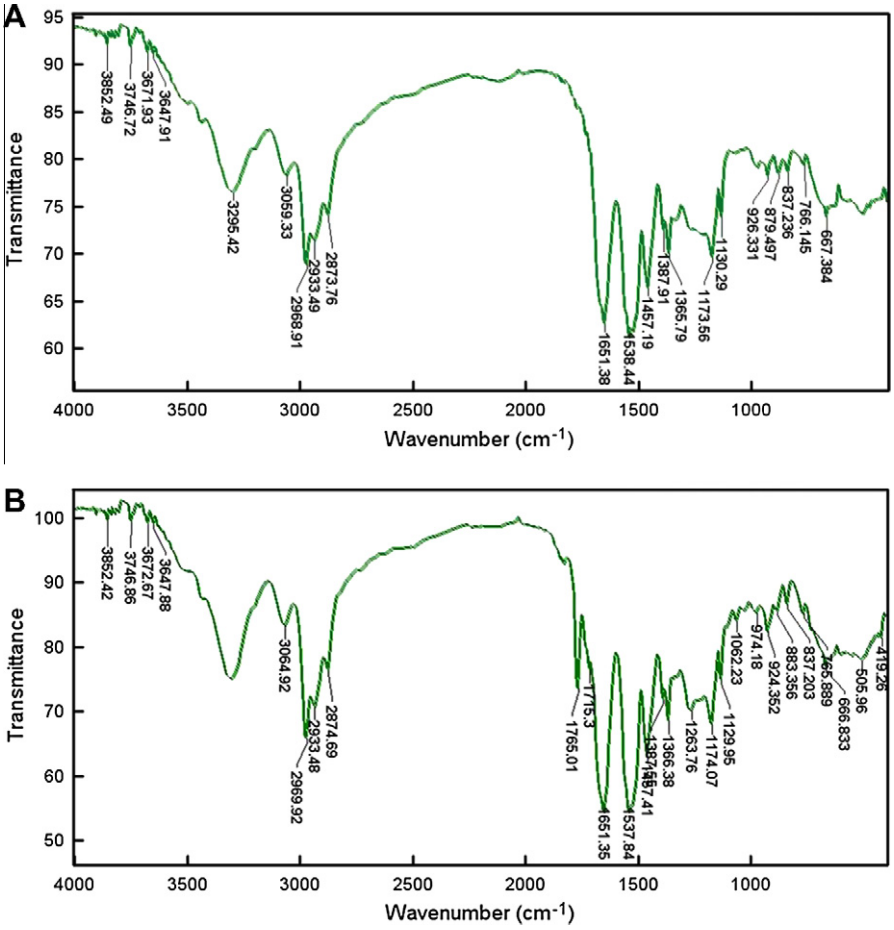
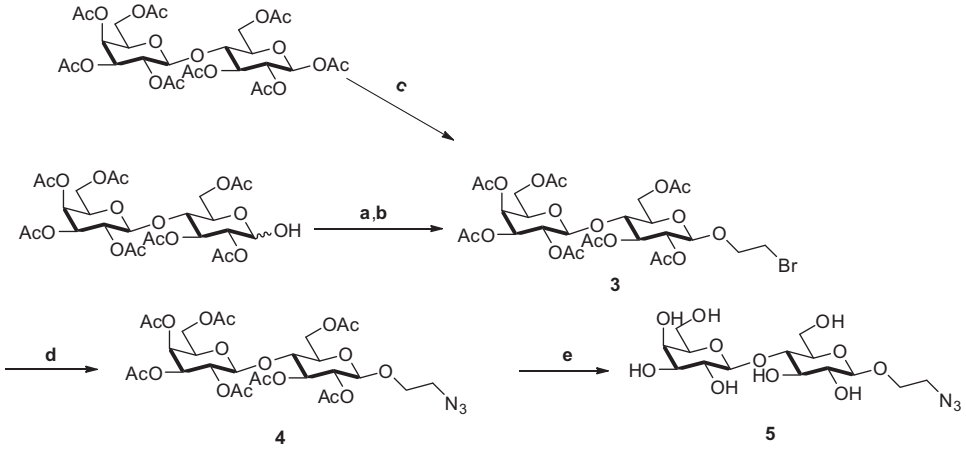


Figure 1. The IR spectrum of polymer **6** (A) and **7** (B).



Scheme 3. Synthesis of the lactose acceptor bearing a linker with a terminal azido group. Reagents and conditions: (a) DBU, CCl_3–CN, CH_2Cl_2; (b) TMSOTf, $HOCH_2CH_2Br$ (65% two steps); (c) $BF_3{\cdot}OEt_2$, $HOCH_2CH_2Br$ (60%); (d) NaN_3, DMF (100%); (e) MeONa, MeOH (85%).

In summary, a strategy for the enzymatic carbohydrate synthesis using a pH responsive water soluble polymer was established, which may be potentially applied for the synthesis of other biologically important glycans. In a model reaction, we synthesized a sialic acid containing trisaccharide and a total yield of 35% was obtained which is much higher than traditional organic methods (8%).[18] The moderate yield compared to the enzymatic method in liquid phase (98%)[17] is partly due to the fact that oligosaccharide also degrades mildly. Optimization of this technique and its application to the synthesis of more carbohydrates of interest is currently underway.

Acknowledgments

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Supplementary data

Supplementary data (detail procedures for the preparation of key intermediates, characterized data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.076.

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