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Chemo-enzymatic synthesis of oligosaccharides using a dendritic soluble support

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Abstract

A new soluble support with high loading capacity is described. This support was used for chemical sulfatation and enzymatic synthesis of the trisaccharide Lewis^X.

Keywords: oligosaccharides; chemo-enzymatic synthesis; soluble support; dendritic support.

Besides the development of polymer-supported synthesis of oligosaccharides using purely chemical glycosylation methods,[1] a chemo-enzymatic approach based on the use of both chemical methods and glycosyltransferases has started to develop.[2] In fact, glycosyltransferases have become valuable reagents for glycosylation due to their high regio- and stereoselectivity and increasing availability via recombinant DNA technology.[3] They appear to be quite appropriate reagents for use in solid phase synthesis too. However, enzymatic solid phase synthesis raises extra problems, such as accessibility to the interior of the solid matrix of the enzyme,[4] or for chemo-enzymatic synthesis, compatibility of the support with both aqueous and organic solvents.

A potential solution to these problems would be the use of soluble polymers, which enjoy the advantages of both liquid (for the accessibility) and solid phase (for the purification) syntheses. Although polyethylene glycol (PEG) is among the most studied soluble polymer supports for organic chemistry,[5] it suffers from a severe drawback, that is its very low loading capacity; indeed there are only one or two attachment points per polymer molecule. This problem could be overcome by combining principles of dendrimer chemistry[6] with those of PEG chemistry to produce new PEG supports with expanded functional group capacity.[7]

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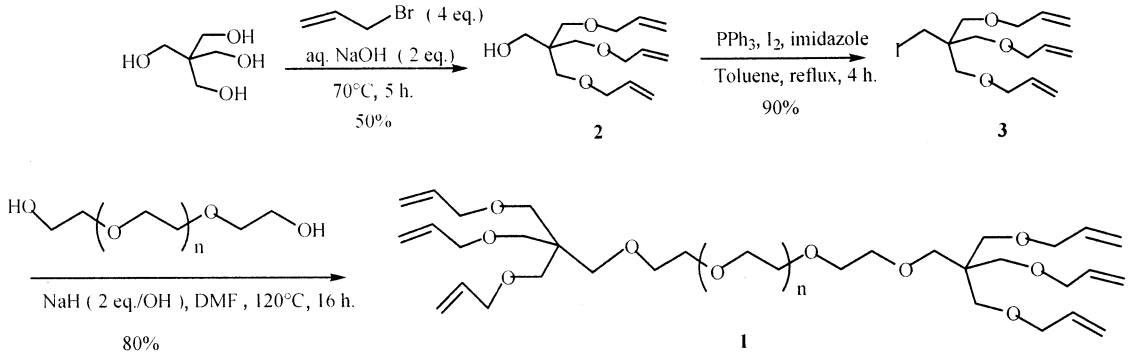
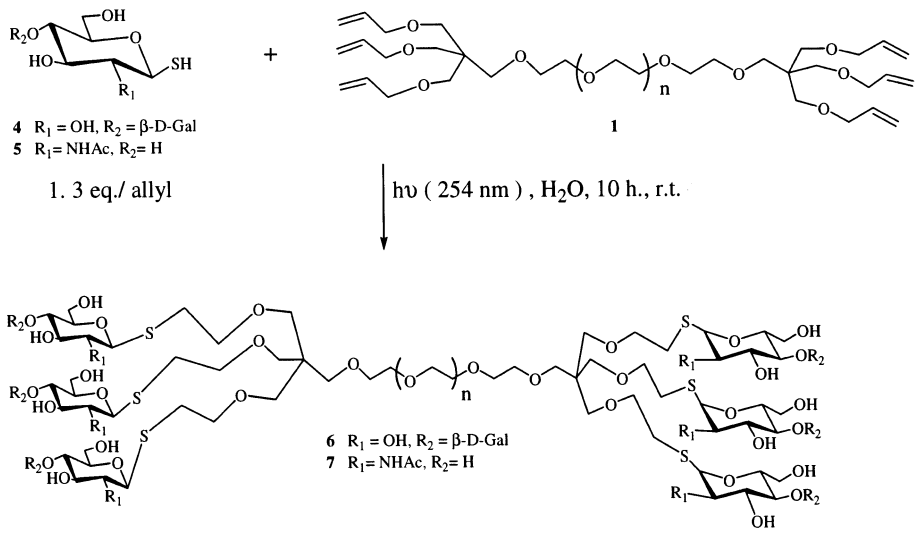
In this paper, we report the use of a new, easily available, PEG derivative **1** with high loading capacity (1 mmol per g) as a soluble support for oligosaccharide enzymatic synthesis. This capacity is similar to that of most of the widely used commercial resins such as the well-known Merrifield resin.[8]

Compound **1** was prepared by alkylation of PEG 6000 with the pentaerythritol derivative **3** (Scheme 1) in 80% isolated yield after dialysis.[9] Compound **3** was readily prepared according to literature procedures[10,11] in two steps from pentaerythritol. NMR spectroscopy showed nearly complete conversion of the PEG to compound **1** with less than 5% of free hydroxyl groups.[12] In fact, we choose allyl functional groups in view of using the readily available thioglycosides derivatives for grafting sugars through the smooth, high yielding, radical coupling of the thiol group onto double bonds.[13] This linkage has the advantage of being resistant to most chemical conditions.[14] Furthermore, it can be rapidly cleaved by thiophilic reagents[15] or in an electrochemical procedure.[16]



Scheme 1.

First, lactose was installed on the new PEG support **1** using 1-thiolactose **4**.[17] The radical coupling onto compound **1** was carried under 254 nm lamp irradiation (Scheme 2) using 0.05 M



Scheme 2.

solutions of **1** in water and 1.3 equiv. of sugar per allyl group.[18] Purification was easily carried out by dialysis. Subsequent cleavage using mercury(II) trifluoroacetate in the presence of barium carbonate afforded, after gel filtration, 0.77 mmol of lactose from 1 g of support. Alternatively, sugar can be cleaved by electrohydrolysis in an one-compartment three-electrode cell under potentiostatic control in acetonitrile containing water (5%) and 0.2 M lithium tetrafluoroborate using woven carbon or platinum as working electrode.[16]

Then we tried the same conditions as for lactose, the coupling of 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside **5**.[19] We obtained after dialysis and similar cleavage using mercury(II) trifluoroacetate, 0.67 mmol of *N*-acetylglucosamine per gram of PEG. These results were confirmed by mass spectrometry. Indeed, MALDI-TOF analysis showed that the compound **6** contains an average of 5.5 lactose residues/mol of PEG and 4 GlcNAc residues/mol of PEG for compound **7**.[20]

Our support was then tested for chemical manipulations. In view of the synthesis of sulfated oligosaccharides as ligands of selectins,[21] we tried the sulfatation of compound **6** using the stannylene methodology.[22] In fact, the dried compound **6** was dissolved in MeOH and treated with dibutyltin oxide (3 equiv./lactose) one night at reflux. After careful evaporation, sulfatation was performed using SO_3–NEt_3 complex (1.2 equiv./lactose) in THF. Cleavage using mercury(II) trifluoroacetate afforded a (2.7:1) mixture of 3′-sulfated lactose[23] and lactose from which the sulfated derivative could be easily separated using anionic exchange resin.

Then, we tested the ability of the new support toward enzymatic glycosylations. First, enzymatic galactosylation of **7** (1.5 g) was carried out using bovine milk β(1–4) galactosyltransferase (2 U) and UDP–glucose/UDP–glucose 4-epimerase.[24] The reaction could be easily followed using ^{1}H NMR analysis or alternatively by TLC after chemical cleavage of an aliquot. After 3 days, the reaction was still incomplete as judged by the presence of both *N*-acetyllactosamine and *N*-acetyl glucosamine. So, we took advantage of the use of supported synthesis: after a simple dialysis, the support was recycled using fresh enzymes (0.4 U of GalTase). Then, cleavage in the usual way gave pure *N*-acetyllactosamine without contamination with *N*-acetyl glucosamine. In this way, we obtained 230 mg of *N*-acetyllactosamine per gram of support. Having in hand a small quantity of recombinant Fuc TIII (0.2 U), and although *N*-acetyllactosamine is known to be a poor substrate for Fuc TIII[25] we tested the fucosylation of an aliquot of **7** (100 mg after galactosylation). After 3 days, cleavage by mercury salts as described previously afforded a 1.5/1 (without recycling) mixture of Lewis^X trisaccharide and *N*-acetyllactosamine (18 mg). NMR data were identical with those reported.[26]

In summary, it was clearly demonstrated that a high loading capacity of polyethyleneglycol can be useful for the enzyme-assisted synthetic strategy. Studies are in progress to extend the use of this PEG derivative to various chemo-enzymatic syntheses.

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