A Novel and Efficient Synthesis of a Dimeric Le^x Oligosaccharide on Polymeric Support

Tong Zhu and Geert-Jan Boons*

Complex Carbohydrate Research Center
220 Riverbend Road, Athens, Georgia 30602

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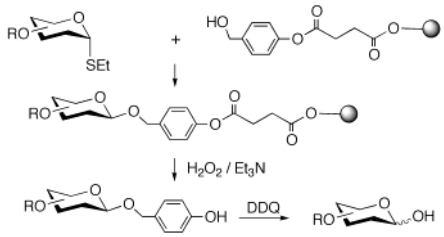
Increased awareness of the biological importance of oligosaccharides and glycoconjugates has stimulated the development of efficient methods for the preparation of these compounds. Improved chemical[1] and enzymatic[2] glycosylation procedures in combination with convergent synthetic strategies make it possible to execute multistep synthetic sequences that give well-defined complex oligosaccharides in reasonable quantities. It is to be expected that further improvements and eventually automation will come from polymer-supported oligosaccharide synthesis. Several relatively large oligosaccharides have been synthesized on polymeric supports[3] although most of these compounds were relatively simple 1,2-trans linked linear homooligomers.

Herein, we report a highly efficient strategy for the polymer-supported synthesis of the dimeric Lewis antigen Lewis^x-Lewis^x (Le^x-Le^x).[3b,d,4] Lewis antigens are an important family of tumor associated antigens that offer promise for the development of cancer vaccines for small cell lung, breast, prostate, lung, colon, stomach, and ovary cancer.[5] These branched compounds contain both α- and β-glycosidic linkages attached to hindered hydroxyls of a glucosamine moiety.

An important requirement for the synthesis of the target compound is the selection of a set of temporary protecting groups and a linker that are compatible with the acid-sensitive fucosidic linkage. Furthermore, the linker and protecting groups should be compatible with the base-sensitive amino protecting group Trichloroethoxycarbonyl (Troc).[6] Glycosyl donors protected with this functionality are highly reactive and offer efficient neighboring group participation to give stereoselective formation of β-glycosides. The Troc group also ensures high glycosyl accepting properties of the C-3 hydroxyl of the glucosamine unit.[4h]

Scheme 1. Concept of New Linker



A strategy was adapted[3f] whereby a polymer-bound Le^x trisaccharide was prepared which could be converted into a glycosyl acceptor by selective removal of a temporary protecting group or into a soluble glycosyl donor by cleavage from the polymeric support followed by activation of the anomeric center. Coupling of the resulting glycosyl donor and acceptor followed by cleavage from the solid support should give the target hexasaccharide. This strategy requires a linker that attaches a saccharide to a polymeric support via the anomeric center of the reducing sugar.

To this end, a novel phenolic ester type linker was developed (Scheme 1). The saccharide is attached to the polymer support by glycosyaltion with the hydroxyl of the acyloxybenzyl linker. Compared to well-established *p*-alkoxybenzyl glycosides, the resulting *p*-acyloxybenzyl glycoside is significantly more stable toward Lewis acidic conditions used in glycosylations. This bond, however, is cleaved within minutes by treatment with hydrogen peroxide/Et_3N. After detachment, a stable *p*-hydroxyl benzyl glycoside is obtained as a single anomer and this feature facilitates purification. Oxidative removal of the *p*-hydroxyl benzyl moiety with DDQ[7] will give a lactol that can be easily converted into a glycosyl donor (e.g. trichloroacetimidate).

The three saccharide building blocks **1**,[8] **4**, and **7**[9] and linker modified MPEG **2** were used for the assembly of the target hexasaccharide. The temporary protecting groups 9-fluorenylmethoxycarbonyl (Fmoc) and diethylisopropylsilyl (DEIPS)[10] of these building blocks can be cleaved under very mild conditions without affecting the linker and the Troc protecting group. Methoxypoly(ethylene glycol) (MW 5000) MPEG was chosen as the polymeric support to take advantage of its solubility in many organic solvents. However, the workup procedure involves precipitation of MPEG by addition of diethyl ether or *tert*-butyl methyl ether. Thus, excess of reagents and other side products can easily be removed by washing of the MPEG precipitate.[11]

Coupling of **1** with **2** in the presence of NIS/TMSOTf[12] gave formation of immobilized **3** (Scheme 2). Only 1.1 equiv of donor **1** was required to achieve complete conversion of the polymeric acceptor. No self-condensation of **1** was observed which was expected due to the much greater reactivity of the benzylic alcohol of **2** compared to the C-4 hydroxyl of **1**. The polymer-bound monosaccharide **3**, bearing a free C-4 hydroxyl, was immediately used in a subsequent glycosylation without workup and purification by precipitation. Thus, addition to the reaction mixture of another amount of NIS/TMSOTf and galactosyl donor **4** gave, after standard workup and purification by precipitation, im-

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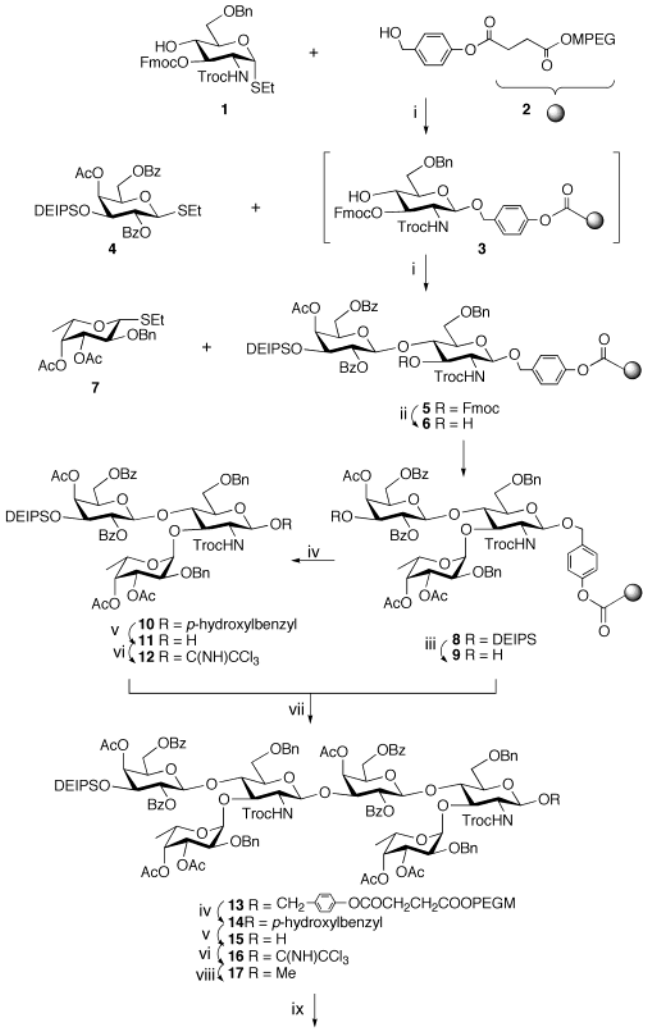
Scheme 2.[a] Synthesis of Le^x-Le^x Hexasaccharide **14**



Galβ(1-4)(Fucα(1−3))GlcNAcβ(1-3)Galβ(1-4)(Fucα(1−3))GlcNAcOMe (**18**)

[a] Reagents and conditions: (i) NIS, TMSOTf, MS 4 Å, 0 °C, DCM; (ii) Et_3N, DCM; (iii) TBAF, HOAc, THF; (iv) H_2O_2, Et_3N, THF; (v) DDQ, DCM, H_2O; (vi) CCl_3CN, DBU, DCM; (vii) TMSOTf, MS 4 Å; (viii) MeOH, TMSOTf, DCM, MS 3 Å; (ix) a. TBAF, HOAc, THF; b. Zn, AcOH then Ac_2O, Py; c. $Pd(OAc)_2$, H_2, EtOH, AcOH; d. NaOMe, MeOH.

mobilized disaccharide **5**. The NMR spectrum indicated clean formation of **5** and no production of truncated or incomplete glycosylated structures. To the best of our knowledge, this glycosylation sequence represents the first example of a one-pot multistep glycosylation sequence performed on polymeric support. Next, the Fmoc of **5** was removed by the treatment with nonnucleophilic base Et_3N in DCM to give **6**. No cleavage of disaccharide from the polymer support was observed. The revealed hydroxyl of **6** was glycosylated with fucosyl donor **7** in the presence of NIS/TMSOTf to give trisaccharide **8**. The NMR spectrum indicated the formation of only one anomer. It is noteworthy that only a very small amount of trisaccharide was formed when the amino function was protected as phthaloyl (Phth) instead of Troc. It is well-known that the reactivity of a hydroxyl adjacent to Phth is reduced due to steric hindrance.[4h]

The polymer-bound trisaccharide **8** was split into two pools, A and B (ratio, A/B = 1/4). Pool A was treated with acetic acid buffered tetrabutylammonium fluoride (TBAF) to remove the DEIPS group to give the trisaccharide acceptor **9**. Under these conditions a TBDMS group could not be removed and the use of other desilylating reagents resulted in concomitant cleavage of the fucoside or linker. The protected Le^x trisaccharide of pool B was released from the polymeric support by treatment with hydrogen peroxide in the presence of Et_3N in DCM to give, after silica gel column chromatography, trisaccharide **10** in an overall yield of 35% (based on initial loading of polymer). Oxidative removal of the anomeric *p*-hydroxy-benzyl moiety of **10** with DDQ gave within minutes lactol **11**. Next, **11** was converted into trichloracetimidate **12** by treatment with trichloroacetonitrile in the presence of a catalytic amount of DBU (α/β, 1/1, overall yield 75%).[13] Next, trisaccharide donor **12** (1.5 equiv) was coupled with polymer-bound trisaccharide acceptor **9** in the presence of TMSOTf.[13b] The reaction was repeated to achieve complete consumption of immobilized acceptor and quantitative formation of **13**. Precipitation of **13** with diethyl ether did not pose any problems and the NMR spectrum indicated a pure polymer-bound compound. Finally, the protected Le^x-Le^x hexasaccharde **14** was obtained in overall yield of 20% (based on loading) by cleavage of **13** from the polymeric support using standard conditions. Compound **14** was conveniently converted into methyl glycoside **17** by oxidative removal of the anomeric *p*-hydroxybenzyl followed by conversion of the resulting lactol **15** into the trichloroacetimidate **16** which was glycosylated with methanol in the presence of TMSOTf. Deprotection of **17** was easily accomplished by a five-step procedure. The DEIPS group was removed by treatment with buffered TBAF. Next, the N-Troc was cleaved by treatment with activated Zn in acetic acid and the resulting amino functionality was acetylated with acetic anhydride. Finally, target hexasaccharide **18** was obtained by removal of the benzyl ethers by catalytic hydrogenation over Pd followed by deacylation with NaOMe in methanol. The NMR and mass spectroscopy data of **18** were in agreement with the proposed structure.

In conclusion, we have described a highly efficient synthesis of Le^x-Le^x hexasaccharide on MPEG. This compound, which is branched and contains both α- and β-glycosidic linkage, is one of the most complex saccharides ever synthesized on polymeric support. A key feature of the approach is a novel phenolic ester linker that is compatible with the hydroxyl protecting groups Fmoc and DEIPS and the amino protecting group Troc. This set of protecting groups and linker can be used for the synthesis of acid-sensitive oligosaccharides that contain fucosides and amino sugars. Cleavage of the linker gives an oligosaccharide that can easily be converted into a glycosyl donor for further synthesis. A one-pot multistep glycosylation sequence was employed to minimize the number of protecting group manipulations.

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Supporting Information Available: Schemes for preparation of compounds **2** and **4**, 500 MHz ^{1}H NMR spectra of **4**, **10**, **14**, and **18**, ^{1}H NMR and ^{13}C NMR spectral data of **10**, **14**, and **18**, general procedure for NIS/TMSOTf mediated glycosylation on MPEG, general procedure for the cleavage of product from MPEG, and procedures for the deprotection of **14** to give **18** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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