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Assembly of a β-(1 → 3)-glucan laminarihexaose on ionic liquid support

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ABSTRACT

An efficient method for the preparation of β-(1 → 3)-D-glucan laminarihexaose on ionic liquid (IL)-support is described. A β-(1 → 3)-glucan laminarihexaose was rapidly assembled in 15 h in a stereoselective fashion with an average yield of over 90% per step using an optimized combination of glycosylating agents. This ionic liquid support approach provides an efficient and fast means for the assembly of β-(1 → 3)-glucans.

Oligosaccharides are widely studied because they possess a variety of unique structures and functionalities.[1] It is essential to gain access to oligosaccharides in sufficient quantities and in pure form for biological studies.[2] Chemical synthesis of oligosaccharides is still a challenge due to the need of selective protection and deprotection of multiple hydroxyl groups. Moreover, traditional synthesis requires purification by chromatography after each step of glycosylation, which is time-consuming and costly.[3,4] Solid-phase synthesis, which allows convenient product isolation and automation, is one of the most effective ways for assembling complex oligosaccharides.[5] However, it has unavoidable limitations because of the heterogeneous reaction conditions. Therefore, several soluble polymer supports have been used instead in recent years, such as fluorinated labels,[6] hydrophobically assisted switching-phase (HASP) method[7] and more recently ionic liquids (ILs).[8] ILs have attracted growing interest among chemical researchers over the past few years because of their unique and tunable physical and chemical properties. As soluble functional supports, the use of ILs combines the features of solution phase chemistry with the advantages of chromatography-free purification in oligosaccharide synthesis.[9] Several groups have successfully synthesized several classes of oligosaccharides by utilizing ILs as phase-separation tags.[4,10–13]

β-(1 → 3)-Glucans, also known as laminarin polysaccharides, are a family of homopolysaccharides of glucose widespread in nature. They are essential constituents of cell walls in fungi and yeasts as well as major storage polysaccharides in brown seaweeds.[14–16] Because of their immunostimulating, antibacterial and antitumor activities, synthesis of β-(1 → 3)-glucans has attracted intensive interest. And several syntheses of linear β-(1 → 3)-glucans have been published to date.[17–24]

Traditionally, β-(1 → 3)-glucans are synthesized by liquid-phase reaction and purified by chromatography after each step of glycosylation. In 2013, the automated solid phase synthesis of β-(1 → 3)-d-glucododecaoside was described.[5] However, a large excess of donors and expensive promoters was required to facilitate the glycosylation. Herein, we report the first synthesis of Laminarihexaose **1** on ionic liquid support.

Retrosynthetically, we envisioned that the linear synthesis of Laminarihexaose **1** could be accomplished by utilizing just two building blocks, the glycosyl donor **2** and the ionic liquid support **3** (Fig. 1). Thus, the most important factor is the coupling efficiency for each glycosylation step and the control of the anomeric configuration for the newly formed glycosidic bonds, both of which are imperative in order to reduce the complexity of the reaction.

As reported previously, glycosyl acceptors with a free OH group at C-3 are common intermediates for the synthesis of β-(1 → 3)-glucans.[17–22,24] However, when the adjacent hydroxyl groups at C-2 and C-4 are both protected by acyl groups, they may show low reactivity.[19] Moreover, α-glycoside byproducts may be formed with glycosyl donors bearing a participating acyl group at O-2.[25] Therefore, glycosyl acceptors protected with 4,6-*O*-benzylidene and 2-*O*-acyl groups are comparatively more appropriate for the synthesis of such oligosaccharides.[17–22,24] For this work, trichloroacetimidate **2** was envisioned as a suitable building block to prepare β-configured glucans. The anomeric leaving group trichloroacetimidate was chosen for its higher reactivity than

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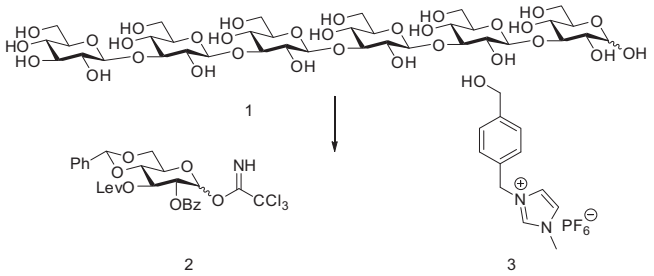
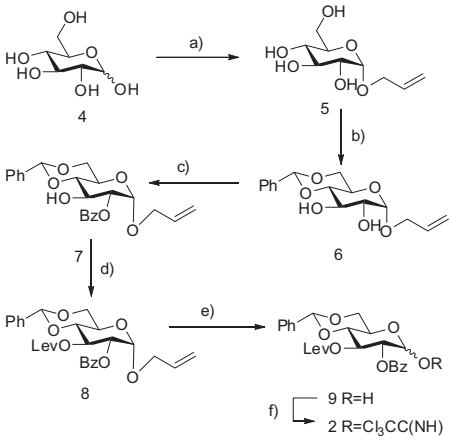
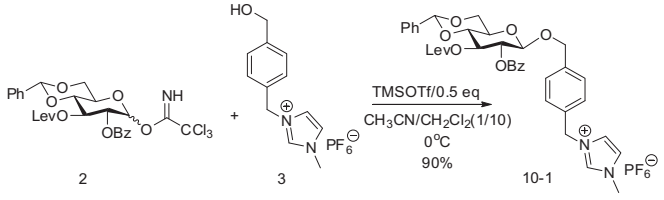


Fig. 1. Retrosynthetic analysis of Laminarihexaose **1**.



Scheme 1. Synthesis of glycosylating agent **2**. a) allyl alcohol, acetyl chloride, 0–70 °C, 53%; b) benzaldehyde dimethylacetal, TsOH·H_2O, anhyd DMF, 60 °C, 39%; c) Benzoyl chloride, imidazole, CH_2Cl_2, 74%; d) levulinic acid, EDC·HCl, 4-dimethylaminopyridin, 0 °C, quant.; e) $PdCl_2$, $CH_3COOH/CH_3COONa/H_2O$, 76%; f) trichloroacetonitrile, Cs_2CO_3, CH_2Cl_2, Ar, 96%.



Scheme 2. Synthesis of **10**–**1**.

thioglycoside and for its successful application in IL supported oligosaccharide synthesis.[4] The benzoyl ester at C2 position was utilized to ensure the stereoselectivity of β-glucosidic linkages.[17,18] As described above, benzylidene acetal was introduced for the protection of the C4 and C6 hydroxyl groups,[15,22,23] while levulinyl (Lev) group was chosen for the temporary protection of the C3 hydroxyl group based on its stability to acids and the mild conditions required for its removal.[26]

The versatility of functionalized ionic liquid in the synthesis of various oligosaccharides has recently been demonstrated.[4,10,11] In this study, modified ionic liquid support **3** was selected since it has been successfully employed in the synthesis of carbohydrates in our group.[4] IL support using α,α′-dioxyxylyl diether as the linker has several advantages: a) it is stable in the common activation conditions for trichloroacetimidate and readily removable by hydrogenolysis; b) it can be coupled via an ether or *O*-glycosidic linkage; c) it can be prepared from commercially available α,α′-dibromo-p-xylene.[4]

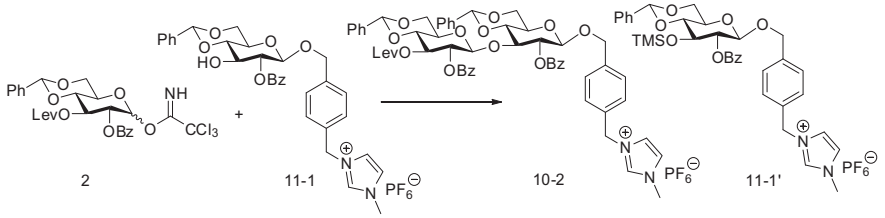
Building block **2** was synthesized in six steps starting from d-glucose (Scheme 1). Intermediate **5**, **6** were prepared according to previously reported procedures.[21] Selective esterification of the C2 hydroxyl group with benzoyl chloride afforded **7** in 74% yield.[21] And the C3 hydroxyl group was then protected with a temporary protecting group levulinyl (Lev) to give the key precursor **8**.[26] Removal of the allyl ether with $PdCl_2$ was smoothly conducted in a buffered solution of acetate. Followed treatment with trichloroacetonitrile finally afforded trichloroacetimidate building block **2** as anticipated.[27]

After the synthesis of glycosyl donor **2**, we attempted to attach it to the modified ionic liquid support **3**, which was prepared according to the previously described procedures..[4] Generally, donor **2** and acceptor **3** were dissolved in dry CH_3CN/CH_2Cl_2 at 0 °C, and 0.5 equiv of trimethylsilyl trifluoromethanesulfonate (TMSOTf) was added as a promoter of the glycosylation reaction.[4] The crude product was obtained by washing with saturated aqueous $NaHCO_3$ and brine to remove all the water-soluble impurities. Further purification of the product was carried out by concentrating to form syrup, redissolving in CH_2Cl_2 and precipitating with isopropyl ether. Thereafter, the solvent was removed partially by rotary evaporation *in vacuo* until the remaining solution was about double to the original CH_2Cl_2 volume and white precipitate appeared, which was immediately collected by centrifugation to afford the product **10**–**1** (Scheme 2).

Following the attachment of a sugar to the IL support, the IL-supported monosaccharide **10**–**1** was undergone de-levulinoylation by treatment with $N_2H_4·H_2O$ and CH_3COOH in THF. The reaction mixture was diluted with CH_2Cl_2, washed with 1 M HCl aq., saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and then filtered. Thus, the acceptor **10**–**2** was obtained after simple evaporation *in vacuo*, which was sufficiently pure for further reactions.

Unfortunately, the glycosylation of **11**–**1** with donor **2** was unsuccessfully under the same condition as in Scheme 2. The C3 hydroxyl group of **11**–**1** was blocked by TMS ether (yield: 30%) and the expected IL-supported disaccharide **10**–**2** was obtained only in a low yield (60%). (Scheme 3). Therefore, we performed optimization study on the coupling condition to increase the reaction yield (Table 1).

As shown in Table 1, when triethylsilyl trifluoromethanesulfonate (TESOTf) was used as the promoter in place of TMSOTf, the TMS ether byproduct **11-1′** was not observed, but the yield was still low (Table 1, entry 2). Gratefully, we found that $BF_3·Et_2O$ was an appropriate promoter to increase the glycosylation yield,

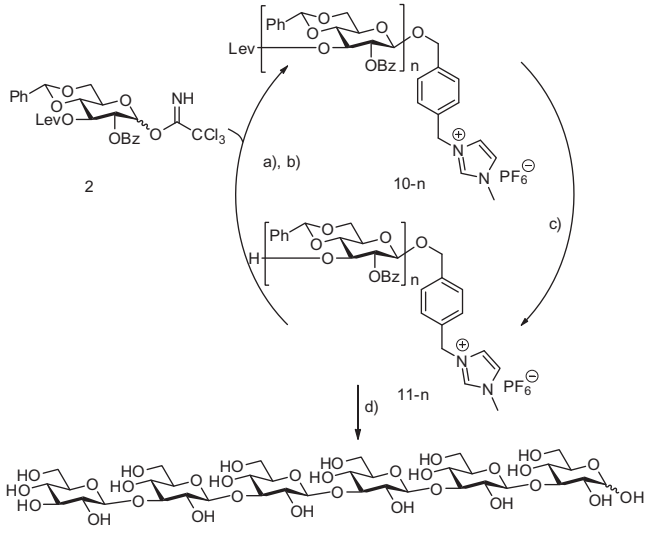


Scheme 3. Synthesis of disaccharide **10**–**2**.

Table 1
Optimization of the glycosylation conditions.

Entry	Solvent	Promoter (equiv)	Temp (°C)	Yield[b] (%)
1	CH_2Cl_2	TMSOTf (0.5)	0	66
2	CH_2Cl_2	TESOTf (0.5)	0	61
3	CH_2Cl_2	$BF_3{\cdot}Et_2O$ (0.5)	0	90
4	CH_2Cl_2	$BF_3{\cdot}Et_2O$ (0.3)	0	69
5	CH_2Cl_2	$BF_3{\cdot}Et_2O$ (0.1)	0	23
6	CH_3CN/CH_2Cl_2[a]	$BF_3{\cdot}Et_2O$ (0.5)	0	84
7	CH_2Cl_2	$BF_3{\cdot}Et_2O$ (0.5)	−20	–
8	CH_2Cl_2	$BF_3{\cdot}Et_2O$ (0.5)	r.t.	85

[a] CH_3CN/CH_2Cl_2, 1:10(v/v).
[b] Yield of **10–2**; the byproduct was **11-1′** (yield: 30%) in entry 1; **11-1′** was not found in entries 2–8.



Scheme 4. IL-supported assembly of β-(1 → 3)-glucan laminarihexaose. a) glycosylation; b) purification; c) removal of Lev group; d) deprotection.

without the formation of TMS ether (Table 1, entries 3–8). ^{1}H NMR analysis indicated that the coupling reaction was highly efficient when 0.5 equiv of $BF_3{\cdot}Et_2O$ was used (Table 1, entry 3). As donor **2** and acceptor **11–1** were both easily soluble in CH_2Cl_2, co-solvent CH_3CN was no longer needed for the glycosylation reaction (Table 1, entry 6). Moreover, the reaction went efficiently when the reaction temperature was between 0 °C and room temperature (Table 1, entry 8). Thus, following the optimized conditions in Table 1 entry 3, IL-supported disaccharide **10–2** was prepared, with its structure and purity confirmed by NMR and MS analysis. The glycosylation condition for IL-supported synthesis was finally determined as follows: 2.0 equiv of trichloroacetimidate donor with 0.5 equiv of $BF_3{\cdot}Et_2O$ as promoter in dry CH_2Cl_2 at 0 °C for 0.5 h. The same post-treatment and precipitation purification technique was used as in the preparation of **10–1**.

Following the routes illustrated in Scheme 4, β-(1 → 3) glucan laminarihexaose **10–6** was synthesized with glycosyl trichloroacetimidates **2** as donors in consecutive glycosylation reactions. The structure of **10–6** was supported by ^{1}H and ^{13}C NMR spectra and further confirmed by MALDI-TOF-MS analysis, which showed a m/z at 2425.7410 $[M-PF_6]^+$. Details of each step are listed in Table 2. Hexamer **10–6** was prepared in average yields of 90–94% per step. The short reaction times, about 2.5 h per monomer addition, allowed the synthesis of **10–6** in 53.9% overall yield within 15 h. In comparison with the previously published liquid phase synthesis of β-(1 → 3)-glucan oligosaccharides, our method is much more faster.[15] Moreover, the reaction time is similar to the automated solid-phase synthesis method for oligosaccharides, while the equivalents of glycosyl donors (2 equiv) and promoter (0.5 equiv) used were much less.[5]

The purity of **10–6** was analyzed by HPLC as in our previous work.[4] Due to the unique properties of ionic liquid species, mobile phases of 0.1% trifluoroacetic acid (TFA) in acetonitrile and water were used.[11] As shown in Fig. 2, the glycosylation and purification

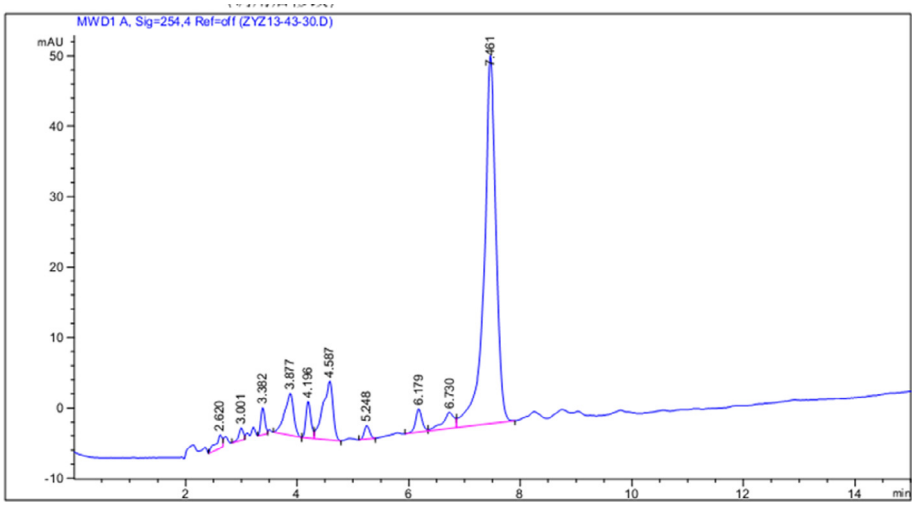


Fig. 2. HPLC anlysis of prepared **10–6** following ionic liquid supported purification. Mobile phase A: 0.1% TFA in water, B: 0.1% (TFA) in acetonitrile, flow rate: 1 mL/min by 20–50% B, UV absorbance at 254 nm, on an Agilent SB-C18 column.

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Table 2
IL-supported assembly of β-(1 → 3)-glucan laminarihexaose.

n	Operation[b]	Product	Time[a] (min)	Recovery (%)
1	A and B	10–1	60 + 30 + 30	90
	C	11–1	30	>99
2	A and B	10–2	60 + 30 + 30	90
	C	11–2	30	>99
3	A and B	10–3	60 + 30 + 30	90
	C	11–3	30	>99
4	A and B	10–4	60 + 30 + 30	93
	C	11–4	30	>99
5	A and B	10–5	60 + 30 + 30	94
	C	11–5	30	>99
6	A and B	10–6	60 + 30 + 30	90
	C	11–6	30	>99
Total			900	53.9

[a] The time includes the preparation time before glycosylation (60 min),the time of glycosylation (30 min), and the time of purification (30 min).The time for removal of Lev protecting groups is about 30 min.
[b] A:glycosylation. B: purification. C: removal of Lev group.

cycle gave product **10–6** in 70.8% purity, with retention time at 7.46 min.

Eventually, the target Laminarihexaose **1** was obtained after deprotection (Scheme 4, d)). The Lev and Bz esters were removed easily with sodium methoxide, followed by successful cleavage of benzylidene group with a CH_3COOH/H_2O (9:1, v/v) system at 70 °C. After removal of the remaining IL support through catalytic hydrogenolysis, the fully deprotected β-(1 → 3)-glucan Laminarihexaose **1** was finally achieved. The analytical data (^1H and ^{13}C NMR spectra) of **1** were identical to that reported for the natural laminarihexaose, with its structure also confirmed by HRMS (ESI-FT-ICR) analysis (*m*/*z* calcd for $C_{36}H_{62}NaO_{31}$ $[M+Na]^+$ 1013.3173, found 1013.3166).[22]

Conclusions

In summary, a rapid synthesis of β-(1 → 3)-glucan on ionic liquid support was developed. The optimal glycosylating promoter was screened. Glycosyl trichloroacetimidate **2** and ionic liquid supported **3** were shown to be a suitable combination for the stereocontrolled synthesis of **10–6** with an average yield of more than 90% per step. No chromatography separation was needed. Protected glucan **10–6** was deprotected in three steps to obtain the target linker-equipped β-(1 → 3)-glucan Laminarihexaose **1**. Our method for the efficient synthesis of large oligosaccharides on IL support could be a very useful technique to produce oligosaccharides on a large scale.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.03.035.

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