

Oligomannan Synthesis Using Ionic
Liquid Supported Glycosylation[§]

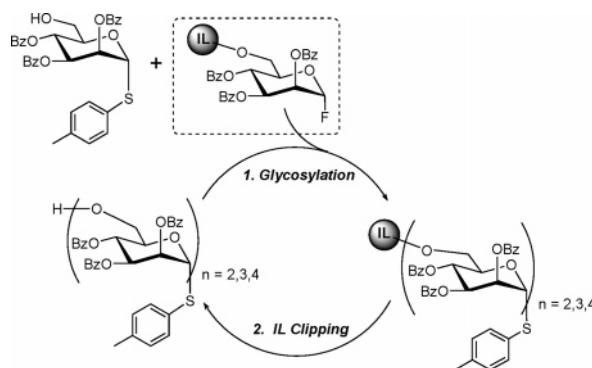
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ABSTRACT



The synthesis of complex oligosaccharides has been a challenge for researchers. Herein, we describe a strategy for the synthesis of an activated oligomannan **1** that employs ionic liquid (IL) support glycosylation methodology on an IL-tagged mannosyl fluoride donor. This method is capable of rapidly producing linear $\alpha(1\rightarrow6)$ oligomannan thio glycosides in a convenient and cost-effective manner without the need of column purification after each glycosylation step.

Carbohydrates play a pivotal role in many different areas of chemistry and biology, as they possess a diversity of functionalities and structures. The efficient synthesis of oligosaccharides has been a challenge of considerable magnitude, as the traditional synthesis is not only enormously time-consuming but also requires purification by chromatography after each glycosylation step.¹ The contemporary challenges in the synthesis of oligosaccharides have led to the development of several new strategies for oligosaccharide synthesis.² Recently developed carbohydrate synthetic techniques are making it possible to create novel immunogenic carbohydrates in sufficient quantities and in pure form. Advances in polymer-supported solid-phase synthesis have also provided an effective way of assembling complex oligosaccharides, and there are some impressive examples of successful automated syntheses.³ The solid-phase method allows the removal of excess reagents by simply washing the resins,

thereby minimizing the chromatographic purifications. Although highly successful, polymer-supported solid-phase synthesis has some serious limitations due to the heterogeneous reaction conditions. This has led to an alternative polymer-supported liquid-phase methodology that restores homogeneous reaction conditions. Some other limitations of polymer-supported synthesis include low loading capacity and limited solubility of the glycosyl donors during the reaction process (which results in poor yields). Expensive

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sugar synthons are also often wasted. The inability to monitor the reaction progress and the lack of good characterization methods to assess the success of the reaction are also disadvantages. At present, high-resolution magic angle spinning NMR spectroscopy is being used to determine the structures of polymer-bound oligosaccharides,^{2a,b} but this is a difficult technique to apply.

Ionic liquids (ILs) have aroused considerable interest over the past decade due to their wide variety of properties. In this regard, they can be used as solvents and reaction supports.⁴ The range of available IL supports makes them compatible with most common reaction conditions. For example, ILs have been used as supports for catalyst/reagent immobilization and for the synthesis of peptides and nucleotides.⁵ IL-supported glycosylation reactions were recently reported utilizing trichloroacetamido and sulfoxide glycosides.⁶ The main features of IL-supported substrates are their solubility properties, as solubility can be turned on and off by varying the anions. Thereafter, IL-supported species can be purified from the reaction mixture by simply washing the product with a nonpolar solvent. Whenever required, the IL support can be clipped off and purified by a simple phase separation technique. In principle, synthesis of almost pure oligosaccharides can be achieved efficiently with minimal column chromatographic purification via this approach. An IL-supported synthesis of disaccharide is illustrated in Figure 1. The IL-tagged glycosyl donor results in an IL-tagged

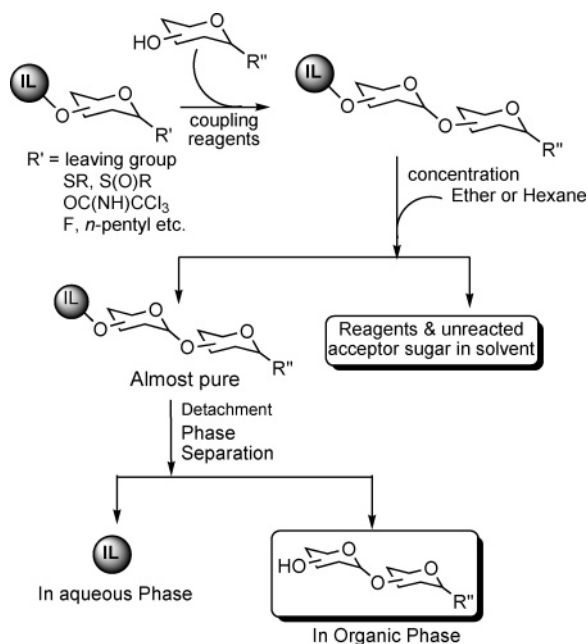


Figure 1. Oligosaccharide synthetic strategy using an IL support.

disaccharide upon coupling with a glycosyl acceptor in an appropriate solvent such as CH_2Cl_2 . Almost pure IL-tagged

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disaccharide can be generated upon concentration of the reaction mixture and washing with a solvent in which the IL-tagged compounds are not soluble (e.g., diethyl ether) to clean out the reagents as well as unreacted acceptor glycoside. A phase separation clipping of IL using a phase transfer catalyst will produce pure disaccharide.

Here, we describe a simple method for synthesizing a homoliner $\alpha(1\rightarrow6)$ oligomannan thioglycoside on an IL support in order to eliminate the excessive use of column chromatographic purification after each glycosylation step. Conventional ^1H NMR at 300 MHz in CDCl_3 , ^{13}C NMR at 75 MHz in CDCl_3 , and HR-ESIMS spectral techniques were also utilized to characterize the products. Previously, the synthesis of linear $\alpha(1\rightarrow6)$ oligomannans was reported using trichloroimidates and allyl glycosides, which required tedious and careful column chromatographic separations after each glycosylation step.⁷

In our approach, the IL support is attached to the donor or acceptor glycoside depending on the requirements of the synthetic manipulation. In the present case, a strategy in which *p*-thiotolyl glycosides acted as acceptors and an IL-tagged fluoride glycoside acted as a donor was utilized for the synthesis of the linear $\alpha(1\rightarrow6)$ tetramannopyranose thioglycoside **1** (Figure 2). The donor glycosyl fluoride was

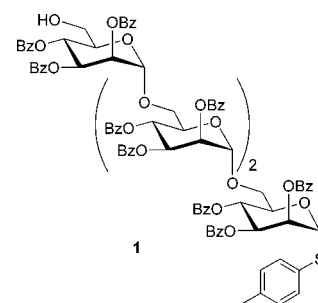


Figure 2. *p*-Thiotolyl $\alpha(1\rightarrow6)$ mannopyranose tetrasaccharide.

attached to the IL support via an ester linkage. Whenever needed, the IL support could be easily detached by stirring with aqueous saturated NaHCO_3 in a $\text{H}_2\text{O}/\text{ether}$ (1:1) biphasic solvent mixture in the presence of phase transfer catalyst.

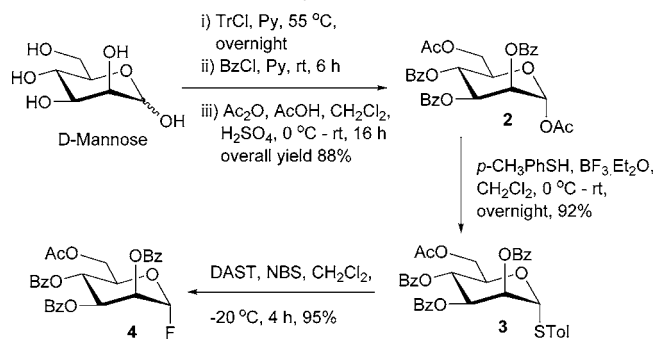
The synthesis of the mannose building blocks **3** and **4** for the construction of the target oligomannan is presented in Scheme 1. The known 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzoyl- α -D-mannopyranoside (**2**) was synthesized in one vessel starting from commercially available D-mannose in an 88% overall yield.⁸ The glycoside **2** was then treated with *p*-thiocresol in the presence of $\text{BF}_3\cdot\text{Et}_2\text{O}$ to produce exclusively the *p*-thiotolyl glycoside **3**. The 300 MHz ^1H NMR spectrum of **3** in CDCl_3 showed the anomeric proton as a doublet at δ 5.69 ppm ($J_{1,2} = 1.2$ Hz), whereas the 75 MHz

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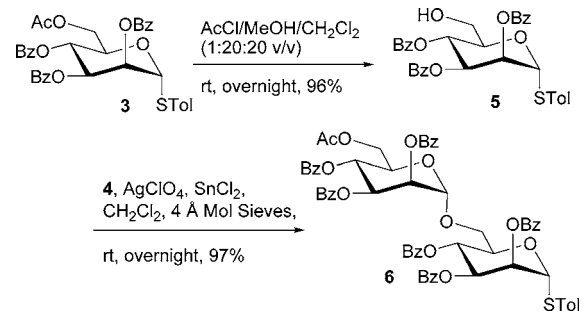
Scheme 1. Synthesis of *p*-Thiotolyl and Fluoride Mannose Glycosides



^{13}C NMR spectrum showed the anomeric carbon signal at δ 86.16 ppm supporting the α -glycoside.⁹ It was further converted to the 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl- α -D-mannopyranosyl fluoride (**4**) using DAST and *N*-bromosuccinimide, in excellent yield. The structure and α -stereochemistry at the anomeric carbon were confirmed by the 300 MHz ^1H NMR spectrum in CDCl_3 , which showed the anomeric proton as a doublet of doublets at δ 5.86 ppm ($J_{\text{H}-1,\text{F}} = 48.7$ Hz, $J_{1,2} = 1.8$ Hz). This was further supported by a signal at δ 104.73 ppm ($J_{\text{C}-1,\text{F}} = 224.0$ Hz) in the ^{13}C NMR spectrum of **4** in CDCl_3 which was attributable to the anomeric carbon.¹⁰

Following the synthesis of the starting mannose glycosides, a prototypical glycosylation reaction utilizing selective anomeric activation of the thioglycosyl acceptor **5**, prepared from thioglycoside **3**, and donor glycosyl fluoride **4** was achieved in high yield (Scheme 2). Selective deacetylation

Scheme 2. Prototypical Selective Glycosylation Reaction



of the thioglycoside **3** was achieved by the reaction with an $\text{AcCl}/\text{MeOH}/\text{CH}_2\text{Cl}_2$ (0.1:20:20 v/v, 12.03 mL/mmol) mixture to produce the acceptor glycoside **5** in 96% yield. The thioglycoside **5** was then coupled with fluoride glycoside **4** in the presence of AgClO_4 and SnCl_2 , yielding the $\alpha(1\rightarrow6)$ disaccharide **6** in 90% yield after the typical workup and

purification by SiO_2 column chromatography. The structure of **6** was confirmed by the 300 MHz ^1H NMR spectrum in CDCl_3 (anomeric protons at δ 5.75 and 5.13 ppm as singlets) and the 75 MHz ^{13}C NMR in CDCl_3 (anomeric carbons at δ 98.01 and 86.75 ppm).

The synthesis of *p*-thiotolyl $\alpha(1\rightarrow6)$ mannopyranose tetrasaccharide **1** utilizing the IL-tagged mannopyranosyl fluoride donor is illustrated in Scheme 3. The reaction sequence started from glycosyl fluoride **4**, which was selectively deacetylated by the reaction with an $\text{AcCl}/\text{MeOH}/\text{CH}_2\text{Cl}_2$ mixture to give the glycosyl fluoride **7**. The treatment of **7** with chloroacetyl chloride in dry CH_2Cl_2 in the presence of dry pyridine resulted in a glycoside **8** with 95% yield. The glycoside **8** was then reacted overnight with *N*-methylimidazole in dry CH_3CN at reflux to obtain the IL-tagged mannopyranosyl fluoride. The chloride anion was exchanged with a hexafluorophosphino anion by the addition of KPF_6 to the reaction mixture and heating the total mixture overnight at reflux.¹¹ The reaction was cooled to room temperature and concentrated in vacuo to a solid, which was then suspended in CHCl_3 and filtered. The filtrate was concentrated to a solid which was purified by washing with diethyl ether. The resulting solid IL-tagged glycosyl fluoride **9** was dried in vacuo. The ^1H NMR spectrum of **9** in CDCl_3 showed it to be almost pure; this was further supported by the HR-ESIMS analysis.

Next, the IL-supported glycosyl donor **9** was treated with acceptor glycoside **5** in the presence of the promoters AgClO_4 and SnCl_2 . It resulted in only 55% yield of the desired disaccharide **10** after workup and purification by washing with diethyl ether. The glycosylation reaction between **5** and **9** was then performed with the coupling reagents Cp_2HfCl_2 and AgClO_4 in dry CH_2Cl_2 . It produced the IL-tagged disaccharide **10** in 88% yield after purification by washing with ether, as described earlier for compound **9**. The 300 MHz ^1H NMR spectrum of IL-tagged disaccharide **10** in CDCl_3 showed the anomeric protons at δ 5.71 and 5.10 ppm ($J_{1,2} = 1.4$ Hz) as a singlet and a doublet, respectively. In the 75 MHz ^{13}C NMR spectrum of **10**, the anomeric carbons were observed at δ 98.17 and 86.82 ppm, supporting the 1,2-*trans* glycosylation.

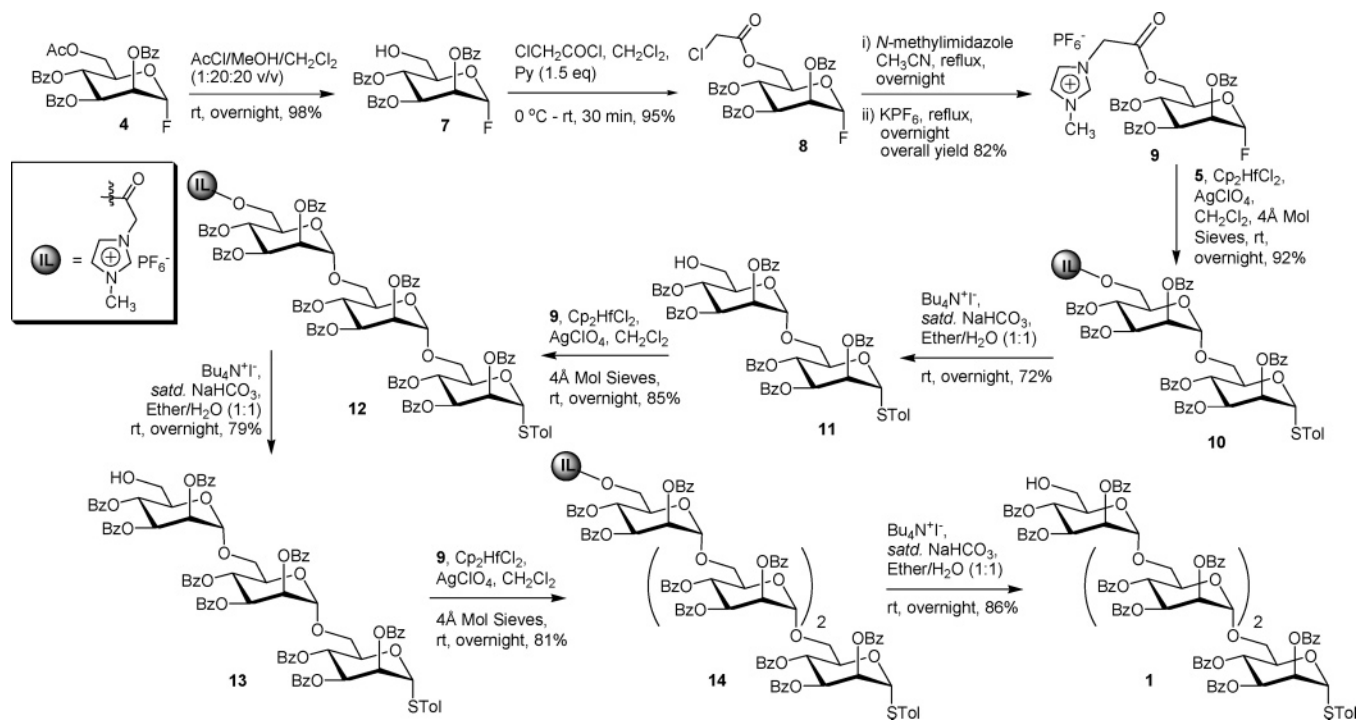
The IL tag on disaccharide **10** was removed by stirring it with saturated aqueous NaHCO_3 and the phase transfer catalyst $\text{Bu}_4\text{N}^+\text{I}^-$ in an ether/ H_2O (1:1) mixture at room temperature. Concentration of the ether layer gave almost pure disaccharide **11** as shown by the ^1H and ^{13}C NMR spectra in CDCl_3 . It was further used for a glycosylation reaction without purification as an acceptor saccharide.

IL-tagged trisaccharide **12** was synthesized by the coupling reaction between acceptor disaccharide **11** and glycoside **9**, in a similar manner to the synthesis of disaccharide **10**. The workup and simple washing purification protocol with diethyl ether produced almost pure IL-tagged trisaccharide **12**. The 300 MHz ^1H NMR spectrum of **12** in CDCl_3 showed three anomeric protons at δ 5.78, 5.20, and 4.80 ppm as singlets.

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Scheme 3. IL-Supported Assembly of Homolinear $\alpha(1\rightarrow6)$ Tetramannopyranose



The presence of three anomeric carbons at δ 98.24, 97.66, and 86.86 ppm was observed in the 75 MHz ^{13}C NMR spectrum of **12** in CDCl_3 . The IL support on **12** was clipped off by the phase separation technique, as described earlier for **11**, yielding donor trisaccharide **13** in almost pure form as evidenced from NMR studies. It was used for a further glycosylation reaction without purification. The trisaccharide **13** was glycosylated with donor IL fluoride **8** to give IL-tagged tetrasaccharide **14** in 81% yield after purification by simple washing with ether. The presence of four anomeric protons in the 300 MHz ^1H NMR spectrum of **14** in CDCl_3 at δ 5.65, 5.20, 4.94, and 4.80 ppm as singlets supported the formation of the tetrasaccharide. The IL support from the tetrasaccharide **14** was removed as described for **11** to produce the almost pure tetrasaccharide **1** in 86% yield. The 300 MHz ^1H NMR spectrum of **1** in CDCl_3 showed the four anomeric protons at δ 5.68, 5.22, 5.01, and 4.81 ppm as singlets. In the 75 MHz ^{13}C NMR spectrum of **1** in CDCl_3 , the four anomeric carbons were observed: δ 98.30, 98.13, 97.78, and 86.86 ppm. Other signals in the ^1H NMR spectrum of **1** were not well-resolved at 300 MHz, but the total number of protons was found to correspond to the molecular formula. The final structural confirmation was obtained by FABMS analysis of tetrasaccharide **1**, which showed a peak at 2043.3

$[\text{M} + \text{Na}]^+$ corresponding to the molecular formula $\text{C}_{115}\text{H}_{96}\text{O}_{32}\text{SNa}$.

In conclusion, we have reported a successful, efficient, and cost-effective synthesis of a tetrasaccharide on an IL support with minimum column chromatographic purification as an alternative to solid-phase polymer-supported synthesis. To our knowledge, this is the first report of a tetrasaccharide synthesis using an ionic liquid support and may be a very useful technique to produce oligosaccharides on a large scale. Currently, other complex oligosaccharides are being synthesized using this technique and will be reported in the near future.

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Supporting Information Available: Experimental procedures and characterization data of all new compounds are available. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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