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# Polymerization of mannosyl tricyclic orthoesters for the synthesis of α(1–6) mannopyranan—the backbone of lipomannan

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ABSTRACT

Tuberculosis (TB) remains a major health problem worldwide. Understanding the interactions between the surface components of *Mycobacterium tuberculosis* (*Mtb*), the main causative agent of TB, with host immune response will be critical for developments of effective treatments and prevention of TB. Chemically defined mimics of the bacterial envelope components serve as important tools for biological studies of the bacterial interactions with mammalian hosts. We report here a rapid synthetic approach utilizing mannosyl tricyclic orthoesters as monomers for regio- and stereo-controlled polymerizations to generate α(1–6) mannopyranan—the backbone of lipomannan. The polymerizations generated multiple glycosidic bonds in a single chemical transformation in regio- and stereo-selective manners. TMSOTf is the optimum catalyst to promote the selective and high yielding polymerization when compared with other Lewis acids. In addition, the monomers 3,4-*O*-benzyl-β-D-mannopyranose 1,2,6-orthobenzoate (**1**) and 3,4-*O*-benzyl-β-D-mannopyranose 1,2,6-orthopivalate (**2**) can be synthesized in multiple-gram scale and in a rapid fashion. Characterizations by GPC and NMR indicate the identity of α(1–6) mannopyranan with DPn (degree of polymerization) = 20.



## 1. Introduction

After numerous years of being relatively dormant, infectious tuberculosis (TB) has re-emerged as a life-threatening global health problem.[5,6] TB treatment requires long term usage of antibiotics which often is not affordable by patients in third world countries.[7] Occurrences of multidrug resistant tuberculosis (MDR-TB) and extensive drug resistant tuberculosis (XD-TB), resulting from inadequate therapy, contribute to more epidemic concerns.[8,9] Although, vaccination by BCG, the only available vaccine against TB, creates protection in newborns, BCG fails to defend against reactivation of pulmonary TB in adults.[6]

One third of the world population is infected with TB. This chronic disease has continuously created major social, economic, and medical problems for humans, especially in the third world countries.[10–12] Treatment of TB is complicated by the exceptionally complex envelope of *Mycobacterium tuberculosis* (*Mtb*), the major causative agent of tuberculosis (TB).[13]

Lipomannan (LM) along with other glycolipids including phosphatidylinositol mannosides (PIMs), lipoarabinomannan (LAM), and mannan capped lipoarabinomannan (ManLAM) are important interspersed molecules that comprise the biologically and physiologically unique envelope of *Mtb*.[1,11,14–16] The pathogenic bacteria have sophisticatedly evolved to modulate host immune systems and survive in mammalian hosts. LM is one of the macromolecules that are implicated during the infectious, virulent, and survival events of *Mtb* in host mammalian cells.[17,18]

LMs are composed of α(1–6) mannopyranan as the backbone of the molecule.[19] The reducing end of LM is equipped with phosphatidylinositol mannoside (Fig. 1). The mannoside backbone is further branched at numerous C-2 positions with a single unit of α-mannopyranose. The mannopyranan consists of approximately 20–25 mannose residues.[1–3]

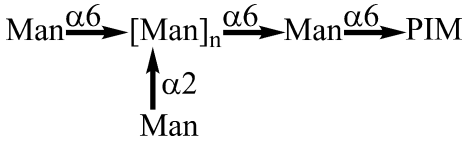


**Figure 1.** [1–4]Structural feature of lipomannan (LM): LM is the extended structure of PIM by having α(1–6) mannopyranan elongated at the non reducing end of PIM. The mannoside backbone is mannosylated at numerous C-2 positions with a single unit of α-mannopyranose. Approximately, 20–25 mannose residues make up the mannopyranan.

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Understanding of LM interactions with the host immune system will lead to effective treatments and prevention of TB. Chemically defined LM structure will facilitate its biological studies. However, many of these studies are impeded by the limited amount of naturally occurring oligosaccharides that are also often difficult to isolate in their pure state. Chemical syntheses offer potential methods to supply well-defined structures for detailed immunological studies.[20–22] Nevertheless, its macromolecular nature makes the synthesis of LM impossible to achieve by traditional stepwise syntheses.

Apart from traditional stepwise synthetic methods, manual and automated solid phase syntheses are employed, as more favorable methods for the synthesis of large oligosaccharides, because these protocols take much shorter time for chemical processing.[23,24] Nonetheless, solid phase syntheses are still limited by the large size of synthetic targets such as LM or LAM.

The nature of the numerous repeating units directs us toward utilization of polymeric reactions. In spite of many biological roles of LM, rarely are there accounts for a one-step synthesis of selective protected poly- or oligo-mannosides. To serve this purpose, we have designed and synthesized tricyclic orthoesters of mannose to be used as monomers for the ring-opening polymerization toward D-mannopyranan. Controlled polymerizations of a monosaccharide monomer can create several glycosidic bonds in a single chemical reaction, and give desired products in significant amounts. We report here the application of controlled polymerizations of D-mannopyranose tricyclic orthoesters for the synthesis of α(1–6) mannopyranan—the backbone of LM and LAM.

## 2. Results and discussion

### 2.1. Design and synthesis of monosaccharide monomers

There are several reports on the synthesis of polysaccharides by ring-opening polymerizations of various building blocks, for example, 3-*O*-benzyl-β-L-arabinofuranose 1,2,5-orthopivalate,[25] 3,6-di-*O*-benzyl-α-D-glucopyranose 1,2,4-orthopivalate,[26,27] 3-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose 1,2,4-orthopivalate,[27] 3-*O*-benzyl-6-deoxy-α-D-glucopyranose 1,2,4-orthopivalate,[28] and 3-*O*-benzyl-α-D-xylopyranose 1,2,4-orthopivalate.[29] Hori and Nakatsubo reported that the ring-opening polymerization of 3-*O*-benzyl-β-L-arabinofuranose 1,2,5-orthopivalate by using $BF_3{\cdot}Et_2O$ as a catalyst gave the stereoregular polysaccharide (1→5)-α-L-arabinofuranan with DPn = 91.[25] The benzyl group at the 3-*O* position and the pivaloyl group at the 2-*O* position are necessary for stereoregularity and regioregularity in the synthesis of arabinofuranan.[25] Moreover, stereoregular polysaccharides of glucose were synthesized by ring-opening polymerization of 3,6-di-*O*-benzyl-α-D-glucopyranose 1,2,4-orthopivalate or 3-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose 1,2,4-orthopivalate. Similar to the synthesis of arabinofuranan, the 3-*O*-benzyl group and 2-*O*-pivaloyl group of glucopyranose play a significant role in the stereospecificity and regiospecificity of the resulting polymer.[30,31] The resulting polymer contained only (1→4)-glycosidic bonds, but not (1→2)-bonds.[31]

In addition to the substituents at 2-*O* and 3-*O* positions, the protecting group (R) of the $-CH_2OR$ at the *C*6-position affected the stereo- and regio-regularity of the resulting polymer due to the electronic effect of the protecting group.[28] Therefore, the alkyl group at the orthoester carbon should be an electron-donating or a slightly withdrawing group such as pivaloyl group. Moreover, types of initiators and temperatures also affect the regioregularity of resulting polymers.[25–27]

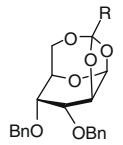
In brief, the previous studies of cationic ring-opening polymerizations of glycosyl tricyclic orthoesters suggested that the regio- and stereo-selectivity of the resulting polymer could be achieved. The pattern of protecting groups on the hydroxyls of building blocks heavily influences both the polymerization efficiency and the selectivity of the resulting polymers. The regioselectivity was created by the preferential attacks of a Lewis acid at different oxygen atoms bonding with orthoester carbon. The stereoselectivity mainly took place by the unique geometry of the orthoester intermediate that favors the incoming nucleophile of the monomer in one direction during propagation steps. After careful consideration, we decided to take advantage of the unique geometry of the mannosyl 1,2,6-tricyclic orthoester (Fig. 2). The protecting groups on mannose were manipulated to temporarily mask C-2 hydroxyl and to be suitable for tricyclic formation. The orthogonal protecting group on C-2 allows further mannosylation on the LM backbone to generate branching of α-single mannose units.

For a rapid synthesis of oligo- and poly-mannosides, tricyclic orthoester building blocks of mannose are suitable structural features for ring-opening polymerizations. The building blocks—3,4-*O*-benzyl-β-D-mannopyranose 1,2,6-orthobenzoate (**1**) and 3,4-*O*-benzyl-β-D-mannopyranose 1,2,6-orthopivalate (**2**) were synthesized and utilized as monomers in ring-opening polymerizations. The monomers **1** and **2** contain a highly strained tricyclic structure which is readily susceptible for ring-opening polymerizations upon activation with Lewis acids.

The C-3 and C-4 hydroxyl groups of both building block monomers **1** and **2** were protected with an electron donating group, *O*-benzyl ether. The 1-, 2- and 6-*O* positions are masked in the form of tricyclic orthobenzoate and orthopivalate in building blocks **1** and **2**, respectively. During the ring-opening polymerization, the 3-*O* benzyl group has a considerable effect on chemical activity because the benzyl group is an electron-donating group. The electron donating group is responsible for stabilizing the dioxolenium intermediate, resulting in the formation of a glycosidic bond via $S_N2$ attack of the next monomer, after the activation by Lewis acid initiators. A possible mechanism of cationic ring-opening polymerizations of mannosyl 1,2,6-tricyclic orthoester, that results in regio- and stereo-controlled products, is summarized in Scheme 1.

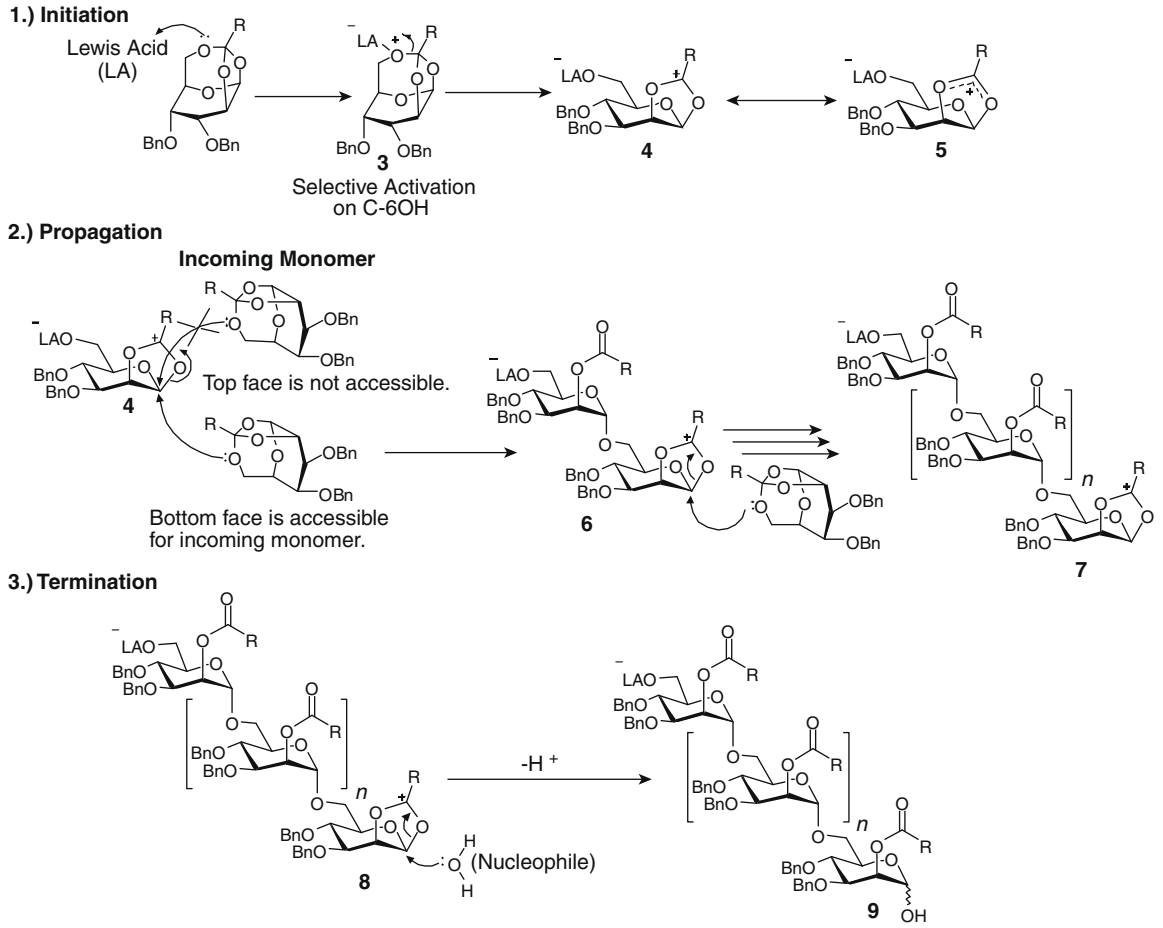
The 4-*O* benzyl group plays an important role in the regioregularity of the resulting polymer because it has a high electron-donating property. The electron donating group contributes to the high electron density of C-6 oxygen of the monomers. It has been shown that when treated with $BF_3{\cdot}Et_2O$ and allyl alcohol in $CH_2Cl_2$, the C–O bond between the orthoester carbon and the C-6 oxygen atom is cleaved.[4] The fact that the C-6 oxygen atom attaches to the C-6 methylene carbon, also makes the C-6 oxygen atom less sterically hindered and facilitates the binding to a Lewis acid, when compared with C-2 and C-1 oxygen atoms. Therefore, we envision that during the initiation step of polymerization of monomers **1** and **2** (Scheme 1), the Lewis acid will preferably coordinate with the C-6 oxygen atom. The Lewis acid will weaken the bond between the C-6 oxygen atom and the orthoester carbon. Consequently, the C–O orthoester carbon–oxygen bonds will be selectively cleaved at the C-6 oxygen atom. The cleavage may be assisted by the delocalization of the lone pair electrons from both C-1 and C-2 oxygen atoms. In a similar system to monomers **1** and **2**, Kamitakahara et al. proposed that in the initiation step of cationic ring-opening polymerization of α-D-glucopyranose 1,2,4-orthopivalate derivatives, the resulting dioxolenium ion is the more likely intermediate.[27]

During the propagation step of polymerization (Scheme 1), the next unit of monomer can approach the intermediate **4** by attacking at the C-1 anomeric carbon. The most nucleophilic and most accessible nucleophile is the C-6 oxygen atom on the incoming monomer. Because of the unique geometry of the intermediate **4**, the next monomer will favorably attack at the bottom face of the




**1**: R = $C_6H_5$
**2**: R = $C(CH_3)_3$

**Figure 2.** The structures of tricyclic orthoester mannoside building blocks **1** and **2**.




**Scheme 1.** A possible mechanism of cationic ring-opening polymerizations of mannosyl 1,2,6-tricyclic orthoester that results in regio- and stereo-controlled products.

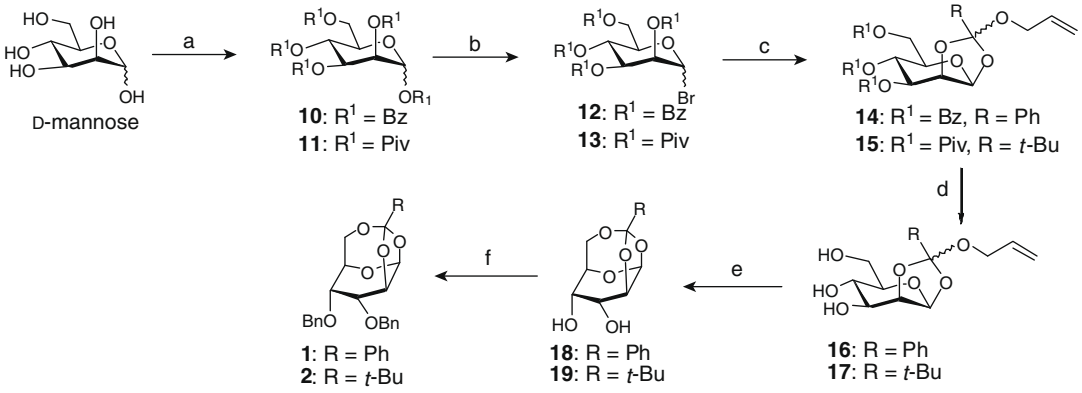
anomeric carbon. Consequently, the incoming nucleophilic oxygen is likely to form an α glycosidic bond with the anomeric carbon. Therefore, the stereospecificity in the polymannoside product is achieved in concurrence with the reformation of the acyl protecting group on the C-2 hydroxyl of the penultimate mannoside unit. The acyl protecting group on the C-2 oxygen is orthogonal to the permanent 3-*O* and 4-*O* benzyl ether protecting groups. This allows for further selective mannosylations on the C-2 oxygen. The propagation reactions will keep adding more mannose building blocks on the growing chain of the polymer. And finally, the polymerization will be terminated by attacks of nucleophiles other than the monomer.

## 2.2. Synthesis of mannoside building blocks

With the ultimate goal of being able to prepare the building blocks in large scale and a rapid fashion, we set out to develop a synthetic route that is robust and scalable. The synthesis of monomers **1** and **2** was developed based on a previously published report.[32] We found that some chemical reagents used in the previous report were not suitable for a high humidity climate in a country like Thailand. We successfully develop a new synthetic route that would not be affected by humid conditions. The overall redesigned synthesis of monomers **1** and **2** is illustrated in Scheme 2. We have developed a short synthetic route that requires only six chemical transformations, in which only two column purifications are necessary. Moreover, the same chemical conditions can be applied to produce both building blocks **1** and **2** in multiple-gram scales.

The synthetic routes for building blocks **1** and **2** are different only in the first step of global protection of hydroxyl groups in the native mannose. The native mannose sugar was globally protected with benzoate esters (Bz) and pivalate esters (Piv) for the synthesis of building blocks **1** and **2**, respectively.

We observed reactivity difference in the orthoester bicyclic formation. The conversion yields from **12** to **14** and **13** to **15** were




**Scheme 2.** Synthesis of building blocks **1** and **2**. Reagents and conditions: (a) BzCl, pyridine, 0 °C to rt, 12 h or PivCl, pyridine, reflux, 24 h; (b) HBr/HOAc (33%), acetic anhydride, 24 h (c) allyl alcohol, lutidine, 83% (three steps) for **14**, 42% (three steps) for **15**; (d) KOH, $H_2O$, MeOH, THF, rt, 24 h; (e) 0.05 equiv CSA, $CH_2Cl_2$, 1 h; (f) BnBr, NaH, DMF, 0 °C to rt, 12 h, 37% (three steps) for **1** and 56% (three steps) for **2**.

monitored. We found that the conversion from **12** to **14** was significantly more effective than the conversion from **13** to **15**. This difference highlights the influence of the substituent (R, Scheme 2) on the orthoester carbocation stabilization. The presence of the phenyl substituent on **12** resulted in the better conversion. Both phenyl and *t*-butyl groups can donate electrons to stabilize the carbocation. The phenyl group mainly donates electrons by delocalizing electrons from the aromatic ring to the benzylic carbocation. The *t*-butyl group inductively donates electrons to the orthoester carbocation. In addition, the *t*-butyl substituent is considered to be bulkier; consequently, it is more difficult for the attack of allyl alcohol. We also observed similar effects of the substituent groups (R) during the polymerization reaction (Scheme 1).

To globally remove the acyl protecting groups, which are benzoyl and pivaloyl groups, we initially performed typical transesterification reactions which relied on the basic sodium methoxide, generated in situ from the reaction between solid sodium and methanol. Under the transesterification conditions, benzoyl groups were partially removed only at elevated temperatures. The amounts of NaOMe used, from catalytic (5%) to stoichiometric at reflux temperature (THF/MeOH ratio of 1:1), failed to remove the acyl protecting groups. The starting material was gradually decomposed by basic and heated conditions. Even though, we attempted to perform the reactions under inert atmosphere, we suspected that NaOMe was degraded under the highly humid conditions in Thailand.

We then turned to hydrolysis reactions to remove the acyl protecting groups. Moisture in the laboratory atmosphere was irrelevant during hydrolysis setup because water is one of the reagents used in this reaction. The acyl groups of the bicyclic orthoesters **14** and **15** were successfully removed after treatment with KOH in $H_2O$ at rt. Compounds **16** and **17** were obtained in almost quantitative yield. After organic solvent/aqueous extraction, compounds **16** and **17** were used in the next step without further purifications.

The overall synthesis yields of building blocks **1** and **2** from D-mannose were 31% and 24%, respectively. The $^1H$ and $^{13}C$ NMR spectra of compound **1** synthesized by the developed route are similar to the previously published data.[32] The monomer **2** was characterized extensively by 1D and 2D NMR and the anomeric doublet resonance of **2** was at 5.67 ppm ($J$ = 5.8 Hz).

### 2.3. Polymerization

The monosaccharide monomers **1** and **2** were treated with a Lewis acid type initiator under inert atmosphere to set off the cationic ring-opening polymerization. We have screened several Lewis acids, including diethyl etherated boron trifluoride ($BF_3{\cdot}Et_2O$) and triphenylmethyl tetrafluoroborate ($Ph_3CBF_4$), that were successfully used to trigger cationic ring-opening polymerization of several glycosyl tricyclic orthoesters, as previously reported.[25,26] In addition, trimethylsilyl trifluoromethanesulfonate (TMSOTf), a typical promoter for glycosylation reactions, was also introduced as an initiator. The concentration of the catalyst was kept constant at 5 mol % (0.05 equiv).

The polymerizations were carried out at different temperatures ranging from −40 °C to room temperature (rt, 27 °C) to see the temperature effects on the selectivity and yield of the polymer products. The reactions were mainly done in dichloromethane which has proven to be the best solvent for all polymerization conditions carried out in previous studies.[26] In order to observe and account for all the products resulting from polymerizations, the polymer crude products were characterized by gel permeation chromatography (GPC), $^1H$ and $^{13}C$ in 1D and 2D NMR spectroscopy, and optical rotation. The representative results of polymerizations under different reaction conditions are highlighted in Table 1.

The Lewis acid catalysts, the temperatures, and the substituents (R, Scheme 2) on the orthoester carbon strongly influenced the yields, and the regio- and stereo-chemistry of the products from polymerizations. The majority of the polymerization reactions initiated by $Ph_3CBF_4$ and $BF_3{\cdot}Et_2O$ as catalyst resulted in decompositions of the monomers (Table 1). In addition, there were large

**Table 1**
Overall results of polymerization reactions carried out at different experimental conditions

| Condition No. | Monomer | Catalyst | Temp (°C) | Observations |
|---|---|---|---|---|
| 1 | 1 | $BF_3{\cdot}Et_2O$ | rt | Oligosaccharides |
| 2 | 1 | $BF_3{\cdot}Et_2O$ | 0 | Oligosaccharides |
| 3 | 1 | $BF_3{\cdot}Et_2O$ | −10 | Decomposed monomer |
| 4 | 1 | $BF_3{\cdot}Et_2O$ | −40 | Oligosaccharides |
| 5 | 1 | $Ph_3CBF_4$ | rt | Decomposed monomer |
| 6 | 1 | TMSOTf | rt | Oligosaccharides |
| 7 | 1 | TMSOTf | 0 | Oligosaccharides |
| **8** | **1** | **TMSOTf** | **−40** | **Polysaccharides** |
| 9 | 2 | $BF_3{\cdot}Et_2O$ | rt | Oligosaccharides |
| 10 | 2 | $BF_3{\cdot}Et_2O$ | 0 | Oligosaccharides |
| 11 | 2 | $BF_3{\cdot}Et_2O$ | −10 | Oligosaccharides |
| 12 | 2 | $BF_3{\cdot}Et_2O$ | −40 | Oligosaccharides |
| 13 | 2 | $Ph_3CBF_4$ | rt | Decomposed monomer |
| 14 | 2 | $Ph_3CBF_4$ | −10 | Decomposed monomer |
| 15 | 2 | $Ph_3CBF_4$ | −40 | Decomposed monomer |
| 16 | 2 | TMSOTf | rt | Oligosaccharides |
| 17 | 2 | TMSOTf | 0 | Oligosaccharides |
| 18 | 2 | TMSOTf | −40 | Oligosaccharides |

portions of the monomers left when treated with $Ph_3CBF_4$. In a few cases, oligosaccharides were observed along with decomposed monomers. The $BF_3{\cdot}Et_2O$ catalyst, when compared with TMSOTf, was less effective especially when the orthopivalate **2** was used as a monomer. The $^1H$ and $^{13}C$ NMR of the oligosaccharides isolated from polymerizations with $BF_3{\cdot}Et_2O$ mostly showed mixtures of several products which are not useful for our goal of synthesizing the regio- and stereo-controlled poly- or oligo-mannosides. The better polymerization yields were obtained when TMSOTf was used as a catalyst.

In general, TMSOTf gave larger polymer products with the desired chemical selectivity than the polymerizations initiated by $BF_3{\cdot}Et_2O$. The decompositions of monomers by treatment with TMSOTf were less than when $BF_3{\cdot}Et_2O$ was used. More importantly, we observed better regio- and stereo-controlled products as well as narrower molecular weight distributions in the case of TMSOTf. In order to account for this difference, we used GAUSSIAN03 to perform quantum chemistry studies using the density functional theory method at the B3LYP/6-311+g(d,p) level. The binding energies between the Lewis acid catalysts and the monomer **1** at either C-6 oxygen or C-2 oxygen atoms were assessed. The calculations showed that $BF_3{\cdot}Et_2O$ indiscriminately binds to the monomer at either C-6 oxygen or C-2 oxygen atoms. In contrast, TMSOTf selectively activates the monomer at the C-6 oxygen atom.

The initial reaction temperatures affected the molecular weight distributions of the polymer products (Table 2). We found that the molecular weight distributions were narrower if the polymerization reactions were carried out at lower temperatures (−40 °C or −10 °C). Higher temperature can also increase the probability of attaining the undesired regioisomers. Therefore, for the purpose of our synthesis of preparing chemically well-defined structures, −40 °C was the optimal temperature.

Polymerizations of the monomer **1**, with the phenyl substituent (R, Scheme 2) on the orthoester carbon, gave more uniform polymer products with the desired regio- and stereo-selectivity (Table 2). We observed that most of the oligomers and polymers, resulting from the polymerizations with the monomer **2**, were more heterogeneous. For example the major product of reaction condition No. 17 (Table 2) was polymannosides with ~16 repeating units, but the polydispersity of this product was 2.09, which implied that the products contain polymers in many different sizes. In a separate study (data not shown), if the protecting group on C-4 oxygen of the monomer is an electron withdrawing group such as benzoyl (OBz), the polymerization resulted in decomposed monomer and small oligosaccharides. This result illustrates the important role of the 4-*O* benzyl group as an electron donating group to promote the polymer chain growth, as part of our monomer design. The overall polymerization results can be distilled into the proposed mechanism shown in Scheme 1.

The optimal polymerization conditions are listed in condition No. 8 in Table 1. The orthobenzoate monomer **1** was efficiently converted into the most homogeneous polymer products upon activation with TMSOTf at −40 °C in $CH_2Cl_2$. The yield of the polymer products from this condition was almost quantitative (98%). The GPC chromatogram of the products from this reaction is shown in Figure 4 as a dominant and narrow distribution curve. These polymer products were extensively characterized and confirmed to possess the desired regio- and stereo-chemistry (described in detail in the next section). The high conversion yield, and the highly homogenous polymer product make condition No. 8 a prime method to access the α(1–6) mannopyranan, the backbone of LM, in substantial quantity.

### 2.4. Polymer characterizations

The homogeneity of polymerization products were verified by gel permeation chromatography (GPC), and $^1H$ and $^{13}C$ NMR (Figs. 3 and 4). Among the products obtained from polymerization conditions listed in Table 1, the polymer products obtained from condition No. 8 were the most chemically defined structures with precise regio- and stereo-regulation. The $^1H$ (600 MHz), $^{13}C$ (150 MHz) NMR spectra and GPC chromatogram of the product from condition No. 8 are shown in Figures 3 and 4. The NMR spectra show only one major peak of anomeric proton at 4.88 ppm, and of anomeric carbon at 98.65 ppm, which indicate the high regio- and stereo-regularity of the polymer products. In similar fashion to the anomeric proton, $^1H$ NMR shows distinct peaks of the ring protons of the chemical shift from 5.8 to 3.2 ppm.

The regioselectivity of the 1→6 glycosidic bonds is confirmed by $^1H$ NMR of C-2 proton at the lower field peak of 5.68 ppm. This C-2 proton resonance shows up as a distinct major peak. The integrated area is equal to the anomeric peak, which indicates one proton each. The benzoyl ester protecting group on the C-2 hydroxyl group withdraws electrons from the C-2 carbon and thus, sends the C-2 proton downfield. If the polymer were to consist of the other possible linkage of 1→2 glycosidic bond, there would not be a downfield peak with integration equal to one proton.

**Table 2**
GPC analysis of polymerization products obtained from reaction conditions listed in Table 1

| Condition No.[a] | All products[b] | | | | Major product[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | [d] $M_w$ | $M_n$ | DPn | $M_w/M_n$ | $M_w$ | $M_n$ | DPn | $M_w/M_n$ | % Yield |
| 1 | 2566 | 1339 | 3.0 | 1.92 | 3378 | 2278 | 5.1 | 1.48 | 92 |
| 2 | 1282 | 998 | 2.2 | 1.28 | 1491 | 1370 | 3.1 | 1.09 | 89 |
| 3 | 270 | 267 | 0.6 | 1.01 | Not analyzed (decomposed monomer) | | | | |
| 4 | 2140 | 1612 | 3.6 | 1.33 | 2249 | 1919 | 4.3 | 1.17 | 96 |
| 5 | 978 | 198 | 0.4 | 4.94 | Not analyzed (decomposed monomer) | | | | |
| 6 | 9878 | 2687 | 6.0 | 3.68 | 10,520 | 5102 | 11.4 | 2.06 | 94 |
| 7 | 6767 | 2756 | 6.2 | 2.46 | 6973 | 3707 | 8.3 | 1.88 | 97 |
| **8** | **11,580** | **5417** | **12.1** | **2.14** | **11,845** | **8809** | **19.7** | **1.34** | **98** |
| 9 | 2371 | 950 | 2.2 | 2.50 | 3012 | 1962 | 4.6 | 1.54 | 76 |
| 10 | 723 | 531 | 1.2 | 1.36 | 866 | 812 | 1.9 | 1.07 | 74 |
| 11 | 1077 | 545 | 1.3 | 1.98 | 1383 | 1160 | 2.7 | 1.19 | 72 |
| 12 | 1573 | 1090 | 2.6 | 1.44 | 1726 | 1457 | 3.4 | 1.18 | 93 |
| 16 | 7178 | 2061 | 4.8 | 3.48 | 7771 | 3932 | 9.2 | 1.98 | 92 |
| 17 | 12,514 | 2125 | 5.0 | 5.89 | 13,984 | 6700 | 15.7 | 2.09 | 89 |
| 18 | 4849 | 2410 | 5.7 | 2.01 | 5089 | 3643 | 8.5 | 1.40 | 95 |

[a] Reaction conditions are listed in Table 1 by condition No.
[b] The reaction mixtures were directly analyzed without extraction or precipitation.
[c] Separated by GPC.
[d] $M_w$ = weight average molecular weight; $M_n$ = number average molecular weight; DPn = degree of polymerization; $M_w/M_n$ = polydispersity index.

The stereoselectivity of the α glycosidic bonds is confirmed by the value of NMR *J* coupling constant between the anomeric carbon and the anomeric proton ($J_{C1,H1}$). The typical $J_{C1,H1}$ values of α mannoside is 171 Hz and β mannoside is lower at 159 Hz.[33] The C–H NMR coupled spectra of the polymer products from condition No. 8 is shown as 1-D spectra in Figure 3C. The $J_{C1,H1}$ of the polymers is 170 Hz, which closely matches the typical $J_{C1,H1}$ of α glycosidic bonds in mannosides. If the polymer were to consist of other possible linkages of β glycosidic bonds, the $J_{C1,H1}$ would be significantly lower.

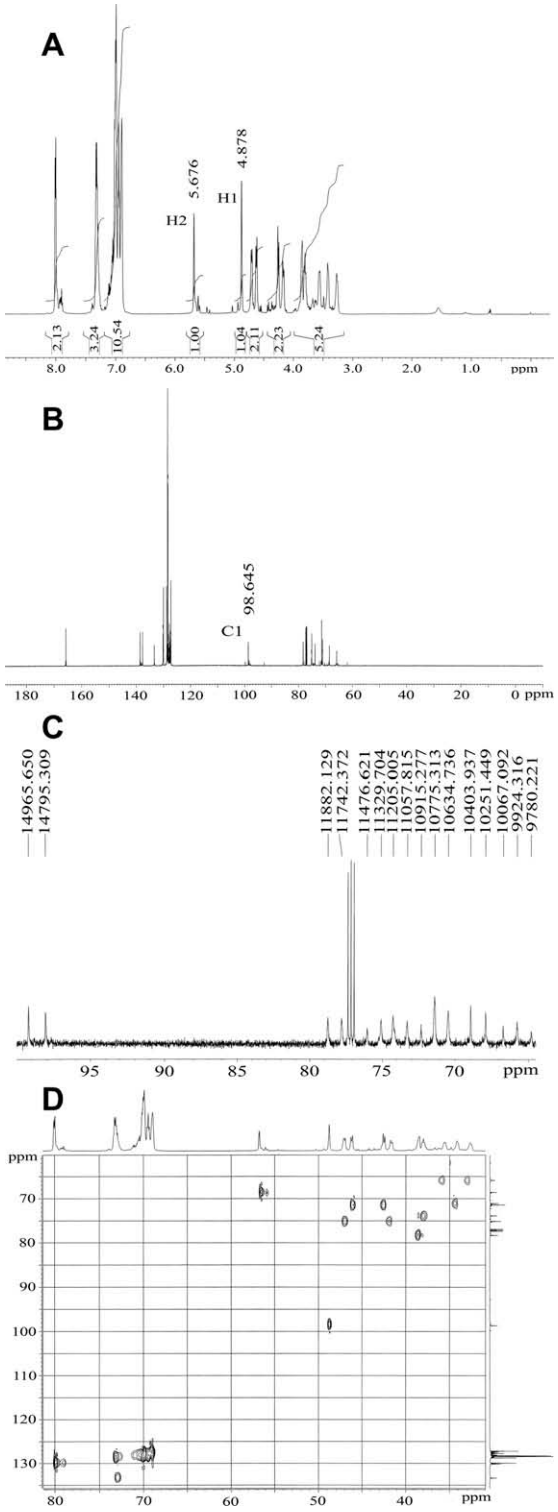



**Figure 3.** NMR spectra of polymer products generated in condition no. 8 of Table 1: (A) $^{1}H$ NMR, (B) $^{13}C$ NMR, (C) $^{13}C$–$^{1}H$ coupled $^{13}C$ NMR, and (D) HMQC 2D NMR.

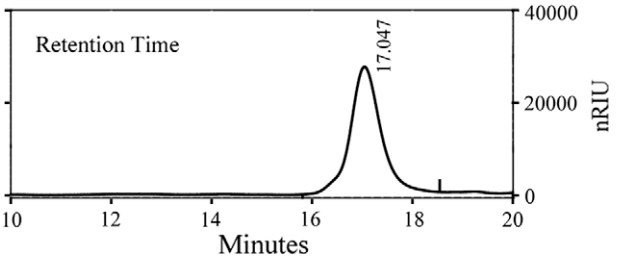



**Figure 4.** GPC chromatogram of polymer products generated in condition No. 8 of Table 1.

The GPC chromatogram (Fig. 4) of major products from polymerization condition No. 8 (Table 1) shows a narrow molecular weight distribution in a single peak. The polydispersity index of the polymer products is 1.34, which signifies a uniform molecular weight distribution. Since the products are very similar in the number of repeating units, they will be useful as the backbone polysaccharides for further α mannosylation at the C-2 hydroxyl groups. The C-2 α mannosylated α(1–6) mannopyranan is the core component of the LM, LAM, and ManLAM.

The optical activity of the polymer obtained from condition No. 8 is highly positive with an alpha rotation of +54.40° (*c* 0.1, 1 mg/mL in $CHCl_3$). The melting point range of this polymer is narrow, from 100.3 °C to 106.8 °C. Both high optical activity and a narrow range of melting point further support the chemical selectivity found in the polymer products.

## 3. Conclusion

The building blocks 3,4-*O*-benzyl-β-D-mannopyranose 1,2,6-orthobenzoate (**1**) and 3,4-*O*-benzyl-β-D-mannopyranose 1,2,6-orthopivalate (**2**) were synthesized. Compounds **1** and **2** were utilized as monomers in ring-opening polymerizations, for rapid syntheses of α(1–6) mannopyranan, the backbone of lipomannan (LM). LM is among important interspersed molecules that compose the unique envelope of *Mtb*.

The synthetic protocols of both building blocks were developed to be robust and scalable to prepare the monomers in a large scale and a rapid fashion. The polymerization reactions can be carried out with a simple basic organic synthesis set up. Tricyclic orthoesters **1** and **2** were successfully prepared in six steps with multiple-gram scale and high yielding chemical reactions. The new synthetic routes are not affected by the highly humid conditions in Thailand. The same reaction conditions were applied to produce both building blocks **1** and **2**.

The polymerizations of monomers **1** and **2** were optimized at different conditions. The regio- and stereo-selectivity along with the conversion efficiency were optimal when TMSOTf was used as a catalyst with building block **1** as a monomer. Characterizations by gel permeation chromatography, 1D and 2D NMR spectroscopy, and optical rotation indicate the identity of α(1→6) glycosidic bonds present in the polymer products. The α(1–6) mannopyranan product serves as the backbone of the synthesis of lipomannan, which is part of the envelope of major causative agent of tuberculosis.

## 4. Experimental sections

### 4.1. General information

All chemicals used were reagent grade and used as supplied except where noted. All reactions were performed in oven-dried glassware under an inert atmosphere unless noted otherwise.

Dichloromethane ($CH_2Cl_2$) was dried over calcium hydride ($CaH_2$) prior to use. Lutidine was treated by potassium hydroxide (KOH) and allyl alcohol was treated with potassium carbonate ($K_2CO_3$) prior to use. The monomers used for polymerizations were dried by using a kügelrohr apparatus (Büchi GKR-51). Analytical thin layer chromatography (TLC) was performed on Merck Silica Gel 60 F254 plates (0.25 mm). Compounds were visualized by staining with cerium sulfate-ammonium molybdate (CAM) solution or phosphomolybdic acid (PMA) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230–400 mesh).

All new compounds were characterized by NMR spectroscopy ($^1H$, $^{13}C$ NMR and 2D NMR for some key intermediates), high resolution mass spectrometry (HRMS), optical rotation activity, and melting point. NMR spectra were recorded on a Varian Gemini 2000 (200 MHz), Bruker AVANCE 400 (400 MHz), and Bruker AVANCE 600 (600 MHz), in $CDCl_3$ with chemical shift reference to internal standards $CDCl_3$ (7.26 ppm for $^1H$ and 77.0 ppm for $^{13}C$). Splitting patterns are indicated as s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublet; m, multiplet for $^1H$ NMR data. NMR chemical shifts ($\delta$) are reported in ppm and coupling constants ($J$) are reported in Hz. High resolution mass spectral (HRMS) analyses were performed by the MS-service at CRI. Peaks are reported as $m/z$. Optical rotations were measured at 26 °C using a JASCO P-1020 polarimeter. Melting points were measured in capillaries on a BÜCHI apparatus (Model BÜCHI 535). Average molecular weights ($M_W$ and $M_N$) of the polymer were analyzed by gel permeation chromatography (GPC/Agilent) using HPLC grade chloroform as an eluent. The eluent flow rate was kept constant at 0.5 ml/min. The temperature of the column was maintained at 40 °C while the detector was maintained at 35 °C. Calibration curves were generated by using polystyrene standards (Shodex standard) with molecular weights of $3.9 \times 10^6$, $6.29 \times 10^5$, $6.59 \times 10^4$, $9.68 \times 10^3$, and $1.30 \times 10^3$ g/mol. The samples were dissolved and diluted with chloroform (0.5 mg/ml) and filtered before injection. The GPC analysis system was equipped with a universal styrene-divinylbenzene copolymer column (PLgel Mixed-C, 300 × 7.5 mm, 5 μm), a differential refractometer detector (AGILENT/RI-G1362A), an online degasser (AGILENT/G1322A), an autosampler (AGILENT/G1329A), a thermostatted column compartment (AGILENT/G1316A) and a quaternary pump (AGILENT/G1311A).

#### 4.1.1. 1,2,3,4,6-Penta-*O*-benzoyl-D-mannose (10)

The first step is global protection of hydroxyl groups in the native mannose. First, the native mannose sugar (5 g, 28 mmol) was globally protected with benzoate esters (Bz). Benzyl chloride (42 ml, 331 mmol, 12 equiv) was added dropwise to the sugar starting material suspended in pyridine (101 ml, 1.24 mol, 45 equiv) at 4 °C. The reaction mixture was allowed to stir for 12 h at rt. The reaction mixture was filtered to remove suspended solid. The filtrate was concentrated in vacuo and extracted by EtOAc and 1 N HCl. The residual solid was extracted by $H_2O/CH_2Cl_2$ and 1 N HCl/$CH_2Cl_2$. The combined organic layer was washed with saturated $NaHCO_3$ solution (3×), dried over $Na_2SO_4$(s), concentrated in vacuo and placed under high vacuum for at least 1 h. NMR spectra for compound **10** are the same as reported in the literature.[32]

#### 4.1.2. 1,2,3,4,6-Penta-*O*-pivaloyl-D-mannose (11)

To a solution of native mannose sugar (5 g, 28 mmol) in pyridine (101 ml, 1.24 mol, 45 equiv), pivaloyl chloride (41 ml, 331 mmol, 12 equiv) was added dropwise at 4 °C. The reaction mixture was allowed to stir and refluxed for 24 h to yield **11**. The reaction mixture was filtered to remove suspended solid. The filtrate was concentrated in vacuo and extracted by EtOAc and 1 N HCl. The residual solid was extracted by $H_2O/CH_2Cl_2$ and 1 N HCl/$CH_2Cl_2$. The combined organic layer was washed with saturated $NaHCO_3$ solution (3×), dried over $Na_2SO_4$(s), concentrated in vacuo and placed under high vacuum for at least 1 h. $R_f$ 0.62 (hexanes/EtOAc = 4:1); $[\alpha]_D^{rt}$ = +35.1909 (*c* 1.1 mg/ml, $CHCl_3$); mp = 147.2–151.8 °C; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 1.08–1.34 (m, 45H), 3.78–3.90 (m, 1H), 4.10–4.27 (m, 2H), 5.11–5.21 (m, 1H), 5.40–5.55 (m, 2H), 5.82 (d, $J$ = 1.0 Hz, 1H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 26.49, 27.04, 38.78, 39.12, 61.67, 64.49, 68.20, 69.35, 71.17, 90.72, 175.53, 176.56, 176.78, 177.35, 177.99; HRMS-ESI ($m/z$): $[M+Na]^+$ calculated for $C_{31}H_{52}O_{11}Na$, 623.3402; Found: 623.3411.

#### 4.1.3. 3,4,6-tri-*O*-Benzoyl-α-D-mannopyranose 1,2-orthobenzoate (14) and 3,4,6-tri-*O*-pivaloyl-α-D-mannopyranose 1,2-orthopivalate (15)

Without further purifications, compounds **10** and **11** were treated with acetic anhydride (21 ml, 221 mmol, 8 equiv) and a solution of 33% HBr in AcOH (142 ml, 828 mmol, 30 equiv). The reaction mixture was allowed to stir at rt for 24 h and extracted with ice-cold water and $CH_2Cl_2$ (3×). The combined organic layer was dried over $Na_2SO_4$(s) and concentrated in vacuo. The crude product obtained from this step was further dried under high vacuum for at least 4 h, without purifications, and was treated with 2,6-lutidine (20 ml, 166 mmol, 6 equiv) and allyl alcohol (57 ml, 828 mmol, 30 equiv). The reaction mixture was stirred at rt for 20 h and concentrated in vacuo. The crude product was co-evaporated with toluene (3×), dried under high vacuum, and washed with water (3×). The combined organic layer was dried over $Na_2SO_4$(s), and concentrated in vacuo. The crude product was purified by flash silica column chromatography (hexanes/EtOAc) to obtain bicyclic orthoesters **14** (14 g, 83%, three steps) and **15** (7 g, 42%, three steps) as foamy syrups. The side products obtained from this reaction were intermediates of compounds **12** and **13**. They each have an OH group at the anomeric carbon. These compounds can be converted to **12** and **13** via reaction condition b (Scheme 2). NMR spectra for compound **14** are the same as reported in the literature.[32] Characterizations of compound **15** are as the following: $R_f$ 0.62 (hexanes/EtOAc = 7:3); $[\alpha]_D^{rt}$ = −31.1048 (*c* 1.0 mg/ml, $CHCl_3$); mp = 107.2–110.0 °C; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.05–1.20 (m, 36H), 3.61–3.66 (m, 1H), 3.91–4.10 (m, 3H), 4.20–4.26 (m, 1H), 4.75 (dd, $J$ = 5.2, 3.0 Hz, 1H), 4.95–5.0 (m, 1H), 5.06–5.12 (m, 1H), 5.21–5.28 (m, 1H), 5.44–5.52 (m, 2H), 5.77–5.88 (m, 1H); $^{13}C$ NMR (80 MHz, $CDCl_3$) $\delta$ 25.68, 26.93, 27.02, 27.09, 38.70, 38.76, 38.86, 39.46, 61.66, 63.91, 64.38, 70.52, 71.77, 75.69, 97.40, 115.49, 127.84, 134.37, 176.16, 177.89, 178.22; HRMS-ESI ($m/z$): $[M+Na]^+$ calculated for $C_{29}H_{48}O_{10}Na$, 579.3140; Found: 579.3140.

#### 4.1.4. α-D-Mannopyranose 1,2,6-orthoesters (18 and 19)

To bicyclic orthoesters **14** and **15**, THF (339 ml for **14**, 154 mL for **15**), MeOH (678 ml for **14**, 308 mL for **15**) and $H_2O$ (28 ml for **14**, 13 mL for **15**) in the ratio of 12:24:1 were added at rt. KOH (5 g, 89 mmol for **14**, 3 g, 53 mmol for **15**, 4 equiv) in $H_2O$ solution was prepared and added to the reaction mixture. After stirring for 24 h at rt, the resulting mixture was extracted with $CH_2Cl_2$ and water in a separatory funnel (3×), dried over $Na_2SO_4$(s), concentrated in vacuo, and dried under high vacuum. Compounds **16** and **17** were obtained in quantitative yield without further purifications. To obtain tricyclic orthoesters **18** and **19**, CSA (266 mg, 1.2 mmol for **16**, 135 mg, 0.6 mmol for **17**, 0.05 equiv) was added to a solution of compounds **16** and **17** in $CH_2Cl_2$ (687 ml for **16**, 348 mL for **17**). The reaction mixture was stirred at rt for 1–2 h and quenched by the addition of triethylamine (0.24 ml, 1.7 mmol for **16**, 0.12 mL, 0.9 mmol for **17**, 0.075 equiv). The crude product was concentrated in vacuo and placed under high vacuum for at least 1 h to give compounds **18** and **19** as white solids. NMR

spectra for compound **18** are the same as reported in the literature.[32] Characterizations of compound **19** are as the following: $R_f$ 0.55 ($CH_2Cl_2$/MeOH = 9:1); $[\alpha]_D^{rt}$ = +5.2588 (*c* 0.85 mg/ml, $CHCl_3$); mp = 131.0–131.6 °C; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.001 (s, 9H), 3.67 (dd, *J* = 8.0, 2.2 Hz, 1H), 3.85–4.10 (m, 4H), 4.36 (dd, *J* = 6.0, 2.2 Hz, 1H), 5.76 (d, *J* = 5.8 Hz, 1H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 24.72, 27.18, 38.26, 69.80, 70.53, 70.59, 72.02, 76.42, 80.18, 99.67; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calculated for $C_{11}H_{18}O_6Na$, 269.0996; Found: 269.0996.

#### 4.1.5. 3,4-*O*-Benzyl-α-D-mannopyranose 1,2,6-orthoesters (1 and 2)

To a solution of tricyclic orthoesters **18** and **19** in DMF (183 ml for **18**, 93 mL for **19**), BnBr (14 ml, 115 mmol for **18**, 7 mL, 58 mmol for **19**, 5 equiv) and NaH (8 g, 115 mmol for **18**, 4 g, 58 mmol for **19**, 5 equiv) were added at 0 °C. The reaction mixture was gradually warmed up to rt during 12 h. The reaction mixture was extracted with $Et_2O$ and water (3×), washed with brine, and dried over anhydrous $Na_2SO_4$(s). The combined organic layer was concentrated in vacuo, and purified by flash silica column chromatography (hexanes/EtOAc) to obtain compounds **1** (3.8 g, 37%, three steps) and **2** (2.8 g, 56%, three steps) as white solids. NMR spectra for compound **1** are the same as reported in the literature.[32] Characterizations of compound **1** are as the following: $R_f$ 0.55 (hexanes/EtOAc = 4:1); $[\alpha]_D^{rt}$ = −1.4133 (*c* 1.5 mg/ml, $CHCl_3$); mp = 83.2–84.0 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.02–1.05 (m, 9H), 3.64–3.71 (m, 2H), 3.92–3.98 (m, 1H), 4.06–4.11 (m, 2H), 4.36–4.40 (m, 1H), 4.62 (d, *J* = 11.6 Hz, 1H), 4.76 (d, *J* = 11.6 Hz, 1H), 4.823 (s, 2H), 5.67 (d, *J* = 5.8 Hz, 1H) 7.26–7.44 (m, 10H); $^{13}$C NMR (80 MHz, $CDCl_3$) $\delta$ 24.67, 38.17, 70.12, 72.22, 72.91, 74.28, 77.52, 79.18, 99.31, 126.76, 127.70, 127.76, 127.90, 128.37, 128.43, 138.21; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calculated for $C_{25}H_{30}O_6Na$, 449.1935; Found: 449.1940.

### 4.2. Polymerizations

A monomer was dried in Kügelrohr under high vacuum at 90 °C for 24 h. Dichloromethane was distilled from $CaH_2$. The solvent was transferred under inert atmosphere. To a solution of monomer in the solvent at the desired temperature (0 °C by ice-water bath, −10 °C by ice-acetone bath, and −40 °C by dry ice-acetonitrile bath), a catalytic amount of initiator ($BF_3{\cdot}Et_2O$, $Ph_3CBF_4$, or TMSOTf) was added and kept at the desired temperature for 2 h. The reaction mixture was kept at nearly the desired temperature in the refrigerator or freezer for 12 h. The reaction mixture was diluted with $CH_2Cl_2$, washed with saturated aqueous $NaHCO_3$ (3×), dried over anhydrous $Na_2SO_4$(s), and concentrated in vacuo. The crude product was dissolved in a minimum amount of $CH_2Cl_2$ and added pentane until there was a white precipitate. The precipitate was allowed to settle overnight. After the mother liquor was removed, the precipitated polymer was dried under high vacuum to give a white powder.

#### 4.2.1. Product from condition 6 in Table 1

$[\alpha]_D^{rt}$ = +56.78 (*c* 0.1, 1 mg/ml, $CHCl_3$); mp = 105.0–111.8 °C; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 3.30–4.22 (m, 6H), 4.24–4.67 (m, 3H), 4.70–5.20 (m, 3H), 5.73–5.88 (m, 1H), 6.83–7.67 (m, 17H), 7.92–8.30 (m, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 68.56, 71.07, 71.41, 73.87, 75.11, 78.29, 98.65 (br s), 127.16, 127.42, 127.72, 127.93, 128.223, 128.40, 128.74, 129.93, 130.05, 133.37, 137.62, 138.55, 165.58.

#### 4.2.2. Product from condition 7 in Table 1

$[\alpha]_D^{rt}$ = +45.47 (*c* 0.1, 1 mg/ml, $CHCl_3$); mp = 98.2–109.1 °C; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 3.43–4.13 (m, 5H), 4.22–5.32 (m, 5H), 5.58–5.98 (m, 1H), 6.92–7.64 (m, 10H), 8.0–8.3 (m, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 65.91, 68.58, 71.10, 71.46, 73.89, 75.17, 78.35, 98.69 (br s), 128.41, 130.17, 137.70, 138.64, 165.68.

#### 4.2.3. Product from condition 8 in Table 1

$[\alpha]_D^{rt}$ = +54.40 (*c* 0.1, 1 mg/ml, $CHCl_3$); mp = 100.3–106.8 °C; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 3.23–4.00 (m, 5H), 4.10–4.45 (m, 2H), 4.53–4.76 (m, 2H), 4.88 (s, 1H), 5.68 (br s, 1H), 6.87–7.15 (m, 10H), 7.20–7.50 (m, 3H), 7.88–8.05 (m, 2H); $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 65.86, 68.53, 71.06, 71.30, 71.39, 73.86, 75.09, 75.24, 78.27, 98.65, 127.17, 127.22, 127.40, 127.44, 127.47, 127.52, 127.65, 127.69, 127.74, 127.83, 127.90, 128.08, 128.80, 128.15, 128.21, 128.23, 128.26, 128.31, 128.32, 128.38, 128.40, 128.45, 128.48, 128.56, 128.59, 128.62, 128.65, 128.72, 129.89, 129.94, 129.97, 130.06, 130.09, 130.13, 133.36, 137.64, 138.57, 165.63.

#### 4.2.4. Product from condition 12 in Table 1

$[\alpha]_D^{rt}$ = +24.10 (*c* 1.0, 10 mg/ml, $CHCl_3$); mp = 56.8–68.1 °C; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 1.12–1.28 (s, 9H), 3.28–4.12 (m, 5H), 4.30–5.02 (m, 5H), 5.31–5.52 (m, 1H), 7.10–7.40 (s, 10H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 27.33, 39.05, 62.18, 65.73, 67.92, 69.86, 70.95, 71.50, 71.90, 73.81, 74.20, 75.17, 78.18, 78.79, 52.60, 98.06 (br s), 128.35, 138.10, 177.50, 177.74.

#### 4.2.5. Product from condition 16 in Table 1

$[\alpha]_D^{rt}$ = +40.19 (*c* 1.0, 10 mg/ml, $CHCl_3$); mp = 63.3–80.4 °C; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 1.08–1.33 (m, 9H), 3.15–4.13 (m, 5H), 4.18–5.05 (m, 6H), 5.32–5.58 (br s, 1H), 6.95–7.41 (m, 10H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 27.22, 38.91, 65.53, 67.44, 70.60, 71.26, 73.60, 74.85, 78.97, 83.16, 98.25 (br s), 128.18, 137.83, 138.50, 177.14.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.014.

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