

RAPID SYNTHESIS OF MYCOBACTERIAL MANNAN POLYSACCHARIDE WITH ALKYNE TERMINATED LINKER

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Abstract

Recently, we have found that the synthetic polysaccharides, $\alpha(1,6)$ polymannans, derived from the surface molecules of *Mycobacterium tuberculosis* (Mtb), have potent immune modulatory properties. In the quest to increase the potency of this polysaccharide, a dimerization on the reducing end of the polymer molecules, that would yield an $\alpha(1,6)$ polymannan dimer, is revealed here. The dimer of the polysaccharide would interact stronger with mammalian immune receptors through more multivalent bindings. Thus, we set out to synthesize the dimerized $\alpha(1,6)$ polymannan relying on a cross coupling of the functionalized polysaccharide at its reducing end. A conjugated $\alpha(1,6)$ polymannan with an alkyne linker was achieved in a single chemical transformation, using a tricyclic mannosyl orthoester as a monomer. The synthetic approach relies on regio- and stereocontrolled polymerization. The terminal alkyne of conjugated polymannan facilitates a self-cross-coupling reaction, providing a dimer of $\alpha(1,6)$ polymannan. The dimer of the polysaccharide may be a more potent immune modulatory agent due to the more multivalency binding with human immune receptors such as DC-SIGN and mannose receptor in human immune system. This target molecule may serve as a vaccine adjuvant by increasing immune responses toward a vaccine antigen.

Keywords: Adjuvant, glycan, *Mycobacterium tuberculosis* (Mtb), polymannan, rapid synthesis

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Introduction

Multivalency describes the interactions between two units, such as viruses and host cells, a receptor and a ligand, or between two cell surfaces (Haag, 2015). Multivalency bindings play an important role in biological systems, especially in carbohydrate-protein interactions (Huskens, 2006). Carbohydrate-protein interactions are not as strong as protein-protein interactions, this limitation could be overcome by multivalent interactions (Kiessling *et al.*, 2001; Zeng *et al.*, 2012). Furthermore, multivalent binding not only leads to increase binding affinity but it also leads to increase binding specificity (Lee and Lee, 2000).

Carbohydrates play increasing crucial roles in prophylactic and therapeutic approaches in medicine (Laura *et al.*, 2011; Chan *et al.*, 2015; Kato and Ishiwa, 2015). Lipomannan (LM), the core polysaccharides found on the surface of *Mycobacterium tuberculosis* (Mtb), has shown to be highly reactive pro-inflammatory modulators. LM isolated from different mycobacterial species was found to induce TNF- α , IL-8, IL-12, and apoptosis (Vignal *et al.*, 2003; Dao *et al.*, 2004; Quesniaux *et al.*, 2004; Mishra *et al.*, 2008). LM has shown to induce the expression of matrix metalloproteinase-9 (MMP-9) (Elass *et al.*, 2005). LM plays important roles during interactions between the bacteria and host mammalian cells, especially immune responses (Neyrolles and Guilhot, 2011). However, the applications of the polysaccharides are limited by tedious processes to isolate glycans from a cell culture, which usually results in poor yields and uncertain chemical identities. Genetic engineering to produce the polysaccharides is very difficult because of the post-translation of polysaccharide biosynthesis (Koeller and Wong, 2000). In addition, it is almost impossible to synthesize polysaccharides by a traditional stepwise synthesis because of their macromolecular nature (Fraser-Reid *et al.*, 2008; Sahloul and Lowary, 2015).

To overcome the aforementioned drawbacks, we have aimed to develop a rapid and efficient synthesis for polysaccharide found on the

surface of Mtb, already equipped with an alkyne linker (Figure 1). The synthetic strategy relies on a regio- and stereocontrolled ring opening polymerization. The terminal alkyne is strategically installed to be further utilized as a reactive group in a self-cross-coupling reaction between two reducing ends of the glycan. The cross-coupling would provide a dimer of $\alpha(1,6)$ polymannan (Figure 2). The multivalency binding of the polysaccharide dimer would be evaluated for their immunological properties in the future.

Results and Discussion

Synthesis of Conjugated Polymannan with Alkyne Linker

The synthesis of the $\alpha(1,6)$ polymannan was previously achieved by ring-opening polymerization of mannosyl tricyclic orthoester 1 as a monomer (Yongyat *et al.*, 2010; Wattanasiri *et al.*, 2017). Furthermore, the monomer 1 can be used to provide conjugated $\alpha(1,6)$ polymannan with thiol (Leelayuwapan *et al.*, 2016; Leelayuwapan *et al.*, 2017) and amine (Bunthitsakda *et al.*, 2018) linkers. The synthetic approaches rely on size, regio- and stereocontrolled polymerization. In this study, we focus on the rapid synthesis of conjugated $\alpha(1,6)$ polymannan with an alkyne linker 2. The terminal alkyne of conjugated polymannan would facilitate a self-cross-coupling reaction, which would provide a dimer of $\alpha(1,6)$ polymannan.

Mannosyl tricyclic building block 1 was dried under high vacuum at 80°C overnight, prior to the polymerization reaction. The reaction was performed by premixing monomer 1 with 4-pentyn-1-ol (2) at a molar ratio of 20:3 under inert atmosphere. The reaction was performed at -40°C for 6 h with dried CH₂Cl₂ as a solvent. The polymerization was triggered by the Lewis acid-trimethylsilyl trifluoromethanesulfonate (TMSOTf). The possible mechanism of polymerization with a linker was described in the previous studies (Leelayuwapan *et al.*, 2017). The reaction was

terminated by saturated NaHCO_3 aqueous solution and purified by size exclusion chromatography to give desired polymers 3, 4 and 5 (Figure 1).

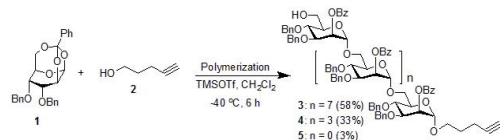


Figure 1. Synthesis of $\alpha(1,6)$ polymannans with alkyne linker 3, 4 and 5

Synthesis of Polysaccharide Dimer

The terminal alkyne is a versatile functional group that can be used further in several fashions, including cross-coupling reactions, cycloaddition reactions, radical reactions, reductive addition reactions and click chemistry (Chinchilla and Nájera, 2014; Liu *et al.*, 2015; Boyarskiy *et al.*, 2016; Huang *et al.*, 2016).

The oxidative homocoupling of terminal alkynes has been significantly investigated over the years (Siemsen *et al.*, 2000). Traditional methodologies for homocoupling of terminal alkynes include Glaser coupling (Glaser, 1869, 1870), Eglinton coupling (Eglinton and Galbraith, 1956), Hay coupling (Hay, 1962), Cadiot-Chodkiewicz coupling (Cadiot and Chodkiewicz, 1969) and Sonogashira coupling (Sonogashira *et al.*, 1975). In this study, the homocoupling reaction of terminal alkyne of $\alpha(1,6)$ polymannan was carried out. The $\alpha(1,6)$ polymannan dimer would have higher affinity with immune target

proteins due to the multivalency binding (Dower *et al.*, 1981; Lee and Lee, 2000; Luo *et al.*, 2009; Wittmann, 2013).

Synthetic polysaccharide 3 was further modified into polysaccharide dimer 6 through homocoupling reaction of the terminal alkyne. In order to examine the optimal reaction conditions, the reaction conditions were experimented as shown in Table 1. We investigated the influence of the catalysts on the formation of polysaccharide dimer 6. The dimerization reaction did not take place with $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (Wang *et al.*, 2010) and $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (Balaraman and Kesavan, 2010) as a catalyst (Entries 1-6, Table 1). The reaction was efficiently catalyzed by the CuI and Pd/C (Kurita *et al.*, 2007) in the presence of DMSO as a solvent under O_2 atmosphere (Entry 7, Table 1). The optimal conditions were shown in Figure 2. The reaction products were purified by size exclusion chromatography to give polysaccharide dimer 6 as the desired product.

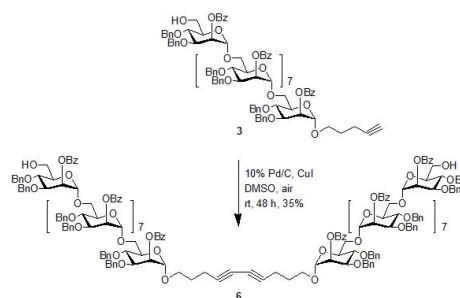


Figure 2. Synthesis of polysaccharide dimer 6

Table 1. Optimization of homocoupling reaction of conjugated $\alpha(1,6)$ polymannan with pentyne linker

Entry	Catalyst	Base	Solvent	Temperature	Time (h)	Yield (%) ^b
1	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	Et_3N	Toluene	rt	16	—
2	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	Et_3N	Toluene	60°C	6	—
3	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	Et_3N	Toluene	60°C	16	—
4	$\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$	Piperidine	CH_2Cl_2	rt	16	—
5	$\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$	Piperidine	CH_2Cl_2	50°C	6	—
6	$\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$	Piperidine	CH_2Cl_2	50°C	16	—
7	Pd/C-CuI	—	DMSO	rt	48	35

^b Isolated yields after size exclusion chromatography.

— Indicates unobserved product(s).

Our results showed that the oxidative homocoupling of terminal alkynes **3** could be carried out using 10%Pd/C and CuI as a catalyst system, and air as an oxidant. The general mechanism of Pd-catalyzed cross-coupling reaction consists of 3 steps which are 1) oxidative addition of Pd(0) to Pd(II); 2) transmetalation of copper acetylide to Pd(II) to generate a dialkynyl Pd(II) intermediate; and 3) reductive elimination to form the C_{sp}–C_{sp} bond (Batsanov *et al.*, 2005; Jana *et al.*, 2011). The presence of Cu(II) facilitates the homocoupling of terminal alkynes by mediating the alkynyl transfer from a Cu-alkynyl complex to Pd(II) via transmetalation and accelerating the reoxidation of Pd(0) to Pd(II) (Batsanov *et al.*, 2005).

Characterization of The Polymer Products

The synthetic $\alpha(1,6)$ polymannan with alkyne linker can be identified by NMR spectra. The ¹H and ¹³C NMR spectra of compound **3** and peak assignments are shown in Figure 3. The major peaks of anomeric protons and anomeric carbons are found at 5.04 ppm and 98.6 ppm, respectively. These peaks confirm the high regio- and stereoregularity of the synthetic compound **3**.

The regioselectivity of the $\alpha(1,6)$ glycosidic bonds was determined by anomeric protons and C-2 protons. The ¹H NMR integration ratio of anomeric protons was equal to C-2 protons. This evidence indicated the composition of the (1,6)glycosidic linkages.

The stereoregularity of the α -glycosidic bonds was determined by coupling constants between the anomeric carbon and the anomeric proton (*J*_{C1,H1}). The *J*_{C1,H1} of polymer **3** was 172 Hz, which was closely similar to the typical *J*_{C1,H1} of an α -mannoside (Podlasek *et al.*, 1996; Yu *et al.*, 2012).

The repeating units of polymer **3** were calculated by the integration ratio between the second methylene group (C1OCH₂CH₂–) from anomeric carbon and C-2 protons of each unit of the mannose (Figure 3(a)). The ¹H NMR spectra indicated 9 mannose repeating units in the polymer chain. The molecular weight of compound **3** was confirmed by MALDI-TOF analysis (Supporting Information).

The MALDI-TOF spectra showed a series of peaks separated by an interval of 446 m/z corresponding to the molecular weight of monomer **1**.

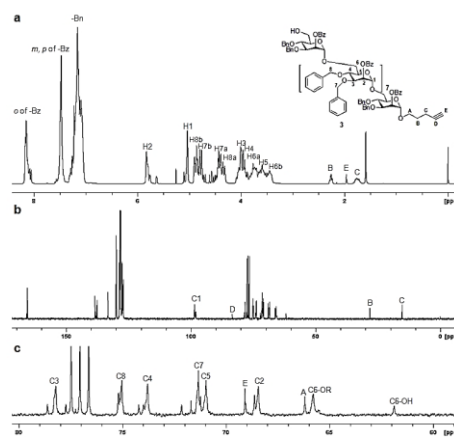


Figure 3. NMR spectra of $\alpha(1,6)$ polymannan with pentyne linker **3** (CDCl₃ as solvent): (a) 300 MHz ¹H NMR spectrum; (b) and (c) 75 MHz ¹³C NMR spectrum

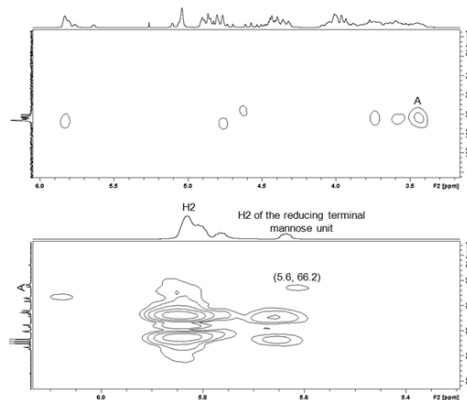


Figure 4. HMBC spectrum of $\alpha(1,6)$ polymannan with pentyne linker **3** (CDCl₃ as solvent): (a) the correlation between the anomeric carbon of reducing terminal mannose unit and the methylene protons located next to the hydroxyl end of the pentyne linker; (b) the correlation between the H2 of reducing terminal mannose unit and the methylene carbon located next to the hydroxyl end of the pentyne linker

The covalent linkage between the polymer and the pentyne linker were confirmed by the HMBC spectrum (Figure 4). We observed the correlation between the anomeric carbon of reducing terminal mannose unit and the methylene protons located next to the hydroxyl end of the pentyne linker as shown in Figure 4(a). At the same time, the correlation between the H2 of reducing terminal mannose unit and the methylene carbon located next to the hydroxyl end of the pentyne linker was observed (Figure 4(b)).

The ^1H and ^{13}C NMR spectra of the polysaccharide dimer 6 and peak assignments are shown in Figure 5. The major peaks of anomeric protons and anomeric carbons are found at 5.04 ppm and 98.6 ppm, respectively. The peaks confirm the high regio- and stereoregularity of the synthetic dimer 6.

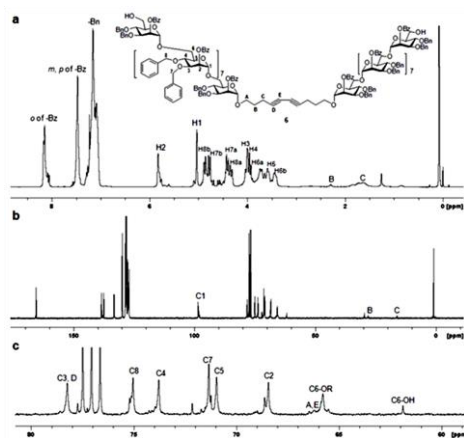


Figure 5. NMR spectra of polysaccharide dimer 6 (CDCl_3 as solvent): (a) 300 MHz ^1H NMR spectrum; (b) and (c) 75 MHz ^{13}C NMR spectrum

The terminal alkyne ($-\text{C}\equiv\text{CH}$) of polymer 3 was converted into the quaternary carbon ($-\text{C}\equiv\text{C}-$) of synthetic dimer 6 after the oxidative homocoupling of terminal alkynes. Therefore, the terminal alkyne proton located at the end of the linker ($-\text{C}\equiv\text{CH}$) was not observed as shown in Figure 4a. Moreover, the ^{13}C NMR signal of the quaternary carbons ($-\text{C}\equiv\text{C}-$) of dimer 6 are shifted up-field to 66.4

ppm, when compared to the tertiary carbon ($-\text{C}\equiv\text{CH}$) of glycan 3 (at 69.1 ppm). This up-field shifts of quaternary carbons of dimer are also reported previously (Cahiez *et al.*, 2007; Kurita, *et al.*, 2007; Wang, *et al.*, 2010; Guoli *et al.*, 2015). These evidences confirm the formation of compound 6.

The repeating units of the synthetic dimer 6 were calculated by the integration ratio between the second methylene group from anomeric carbon (assigned as B in NMR spectra) and anomeric protons (Figure 5(a)). The ratio is around 4 to 18 which corresponds to the dimer of two polymer chains with 9 repeating mannose units. The molecular weight of compound 6 was confirmed by MALDI-TOF (Supporting Information). The MALDI-TOF spectra showed a series of peaks separated by an interval of 446 m/z corresponding to the molecular weight of monomer 1.

Experimental Section

General Information

All chemicals used were reagent grade and used as supplied except where stated. Dichloromethane (CH_2Cl_2) was obtained from a solvent purification system (PureSolv MD 5, Innovative Technology). All the reactions were performed in oven-dried glassware under an inert atmosphere, unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). The compounds were visualized by staining with a cerium sulfate-ammonium molybdate (CAM) solution. Size exclusion chromatography was performed on Merck Sephadex G-15.

All new compounds were characterized by NMR spectroscopy (^1H , ^{13}C NMR) and MALDI-TOF or ESI analysis. NMR spectra were recorded on a Bruker AVANCE III (300 MHz). NMR chemical shifts (δ) are reported in ppm with chemical shift referenced to internal standards (CDCl_3 , δ 7.26 ppm for ^1H and δ 77.0 ppm for ^{13}C ; D_2O , δ 4.79 ppm for ^1H), and coupling constants (J) are reported in Hz. Splitting patterns are indicated as s, singlet; d, doublet; and m, multiplet for ^1H NMR data. Broad peaks are denoted by br before the chemical shift multiplicity. MALDI-TOF was

measured using JEOL JMS-S3000 SpiralTOF. The data was interpreted using JEOL MS Tornado. The mass spectra were measured using a Bruker micro-TOF mass spectrometer. The data was interpreted by Bruker Compass Data Analysis 4.3.

One-Pot Polymerization and Termination

Tricyclic mannosyl orthoester monomer 1 (200 mg, 0.46 mmol) was dried in a glass chamber under high vacuum at 80°C for 16 h. Dried CH_2Cl_2 (680 μL) and 4-pentyn-1-ol (2) (6.9 mg, 0.069 mmol, 0.15 equiv) were transferred into a reaction flask under inert atmosphere. A catalytic amount of TMSOTf (4 μL , 0.022 mmol, 0.05 equiv) was added to the reaction flask at -40°C (dry ice–acetonitrile bath). The reaction solution was constantly stirred and the reaction temperature was kept at -40°C for 6 h under an inert atmosphere. The reaction mixture was terminated with NaHCO_3 , extracted with CH_2Cl_2 (3 $\times$), dried over $\text{Na}_2\text{SO}_4(\text{s})$, and concentrated *in vacuo*. The crude products were purified by size exclusion chromatography using $\text{MeOH}:\text{CH}_2\text{Cl}_2$ (3:7 v/v) as the eluent to obtain compound 3 (120.8 mg, 58%); compound 4 (69.9 mg, 33%); compound 5 (6.1 mg, 3%).

Protected $\alpha(1,6)$ polymannan with pentyne linker 3 (9 mannose repeating units, 120.8 mg, 58%): The atom labels were assigned according to Figure 3. ^1H NMR (300 MHz, CDCl_3) δ 1.66–1.85 (m, 2H, $-\text{CH}_2\text{C}\equiv\text{CH}$, C), 1.93–1.99 (t, 1H, $-\text{C}\equiv\text{CH}$, E), 2.20–2.33 (m, 2H, $\text{C1OCH}_2\text{CH}_2^-$, B), 3.20–3.43 (m, 2H, $\text{C1OCH}_2\text{CH}_2^-$, A), 3.20–4.16 (m, 45H, H3, H4, H5, H6), 4.24–4.37 (m, 9H, $\text{C4OCH}_2\text{aPh}$), 4.37–4.54 (m, 9H, $\text{C3OCH}_2\text{aPh}$), 4.66–4.82 (m, 9H, $\text{C3OCH}_2\text{bPh}$), 4.82–4.97 (m, 9H, $\text{C4OCH}_2\text{bPh}$), 4.97–5.17 (bs, 9H, H1), 5.58–5.92 (bs, 9H, H2), 6.96–7.34 (m, 90H), 7.36–7.60 (m, 27H), 7.99–8.24 (m, 18H); ^{13}C NMR (75 MHz, CDCl_3) δ 15.3 ($-\text{CH}_2\text{C}\equiv\text{CH}$, C), 28.2 ($\text{C1OCH}_2\text{CH}_2^-$, B), 61.8 (C6–OH), 65.9 (C6–OC1), 66.2 (C1OCH_2^- , A), 68.6 (C2), 69.1 ($-\text{C}\equiv\text{CH}$, E), 71.0 (C5), 71.3 ($\text{C3OCH}_2\text{Ph}$), 73.8 (C4), 75.0 ($\text{C4OCH}_2\text{Ph}$), 78.2 (C3), 83.4 ($-\text{C}\equiv\text{CH}$, D), 98.5 (C1), 127.1, 127.3, 127.4, 127.6, 127.7, 128.2, 128.4, 128.7, 130.0, 133.3, 137.6, 137.9, 138.3,

138.6, 165.6. MALDI-TOF (m/z): $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{248}\text{H}_{242}\text{O}_{55}\text{Na}$, 4122.6029; Found: 4125.1293.

Protected $\alpha(1,6)$ polymannan with pentyne linker 4 (5 mannose repeating units, 69.9 mg, 33%): The atom labels were assigned according to Figure 3. ^1H NMR (300 MHz, CDCl_3) δ 1.68–1.90 (m, 2H, $-\text{CH}_2\text{C}\equiv\text{CH}$, C), 1.93–2.01 (t, 1H, $-\text{C}\equiv\text{CH}$, E), 2.21–2.34 (m, 2H, $\text{C1OCH}_2\text{CH}_2^-$, B), 3.32–3.48 (m, 2H, $\text{C1OCH}_2\text{CH}_2^-$, A), 3.32–4.20 (m, 25H, H3, H4, H5, H6), 4.25–4.42 (m, 5H, $\text{C4OCH}_2\text{aPh}$), 4.42–4.68 (m, 5H, $\text{C3OCH}_2\text{aPh}$), 4.68–4.82 (m, 5H, $\text{C3OCH}_2\text{bPh}$), 4.82–4.97 (m, 5H, $\text{C4OCH}_2\text{bPh}$), 4.97–5.20 (bs, 5H, H1), 5.58–5.91 (bs, 5H, H2), 6.83–7.37 (m, 50H), 7.37–7.64 (m, 15H), 7.99–8.30 (m, 10H); ^{13}C NMR (75 MHz, CDCl_3) δ 15.3 ($-\text{CH}_2\text{C}\equiv\text{CH}$, C), 28.2 ($\text{C1OCH}_2\text{CH}_2^-$, B), 61.8 (C6–OH), 65.9 (C6–OC1), 66.2 (C1OCH_2^- , A), 68.6 (C2), 69.1 ($-\text{C}\equiv\text{CH}$, E), 71.0 (C5), 71.3 ($\text{C3OCH}_2\text{Ph}$), 73.8 (C4), 75.0 ($\text{C4OCH}_2\text{Ph}$), 78.2 (C3), 83.4 ($-\text{C}\equiv\text{CH}$, D), 98.5 (C1), 127.1, 127.3, 127.4, 127.6, 127.7, 128.2, 128.4, 128.7, 130.0, 133.3, 137.6, 137.9, 138.3, 138.6, 165.6. ESI (m/z): $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{140}\text{H}_{138}\text{O}_{31}\text{Na}$, 2337.9112; Found: 2338.9106.

Protected $\alpha(1,6)$ polymannan with pentyne linker 5 (2 mannose repeating units, 6.1 mg, 3%): The atom labels were assigned according to Figure 3. ^1H NMR (300 MHz, CDCl_3) δ 1.70–1.91 (m, 2H, $-\text{CH}_2\text{C}\equiv\text{CH}$, C), 1.91–2.01 (t, 1H, $-\text{C}\equiv\text{CH}$, E), 2.21–2.36 (m, 2H, $\text{C1OCH}_2\text{CH}_2^-$, B), 3.34–3.60 (m, 2H, $\text{C1OCH}_2\text{CH}_2^-$, A), 3.60–4.17 (m, 10H, H3, H4, H5, H6), 4.36–4.68 (m, 10H, $\text{C4OCH}_2\text{aPh}$ and $\text{C3OCH}_2\text{aPh}$), 4.68–4.97 (m, 10H, $\text{C3OCH}_2\text{bPh}$ and $\text{C4OCH}_2\text{bPh}$), 5.00–5.15 (bs, 2H, H1), 5.53–5.78 (bs, 2H, H2), 7.01–7.42 (m, 20H), 7.42–7.65 (m, 6H), 8.00–8.19 (m, 4H); ^{13}C NMR (75 MHz, CDCl_3) δ 15.3 ($-\text{CH}_2\text{C}\equiv\text{CH}$, C), 28.2 ($\text{C1OCH}_2\text{CH}_2^-$, B), 61.8 (C6–OH), 65.9 (C6–OC1), 66.2 (C1OCH_2^- , A), 68.6 (C2), 69.1 ($-\text{C}\equiv\text{CH}$, E), 71.0 (C5), 71.3 ($\text{C3OCH}_2\text{Ph}$), 73.8 (C4), 75.0 ($\text{C4OCH}_2\text{Ph}$), 78.2 (C3), 83.4 ($-\text{C}\equiv\text{CH}$, D), 98.5 (C1), 127.1, 127.3, 127.4, 127.6, 127.7, 128.2, 128.4, 128.7, 130.0, 133.3, 137.6,

137.9, 138.3, 138.6, 165.6. ESI (m/z): $[M+Na]^+$ calculated for $C_{59}H_{60}O_{13}Na$, 999.3924; Found: 999.3900.

Dimerization of polysaccharide

The synthetic alkyne **3** (45 mg, 0.009 mmol) was dissolved in dimethylsulfoxide (2 mL). CuI (257.1 mg, 1.35 mmol) and 10% Pd/C (22.5 mg, 0.225 mmol) were added, and the reaction mixture was stirred in open atmospheric air at room temperature for 48 h. The mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was washed sequentially with 0.1 N sodium thiosulfate solution (10 mL) and H_2O (5×20 mL), dried over $Na_2SO_4(s)$, and concentrated *in vacuo*. The crude product was purified by size exclusion chromatography using $MeOH:CH_2Cl_2$ (3:7 v/v) as the eluent to obtain compound **6** (18 mannose repeating units, 25.3 mg, 35%): The atom labels were assigned according to Figure 5. 1H NMR (300 MHz, $CDCl_3$) δ 1.43-1.70 (m, 4H, $-CH_2C \equiv CH$, C), 2.20-2.38 (m, 4H, $C1OCH_2CH_2-$, B), 3.31-3.53 (m, 4H, $C1OCH_2CH_2-$, A), 3.31-4.19 (m, 90H, H3, H4, H5, H6), 4.25-4.38 (m, 18H, $C4OCH_{2a}Ph$), 4.38-4.54 (m, 18H, $C3OCH_{2a}Ph$), 4.71-4.83 (m, 18H, $C3OCH_{2b}Ph$), 4.83-4.97 (m, 18H, $C4OCH_{2b}Ph$), 4.97-5.16 (bs, 18H, H1), 5.57-5.92 (bs, 18H, H2), 6.93-7.35 (m, 180H), 7.35-7.63 (m, 54H), 8.01-8.23 (m, 36H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.2 ($-CH_2C \equiv CH$, C), 28.2 ($C1OCH_2CH_2-$, B), 61.9 ($C6-OH$), 65.8 ($C6-OC1$), 66.2 ($C1OCH_2-$, A), 66.4 ($-C \equiv C-$, E), 68.4 (C2), 70.9 (C5), 71.3 ($C3OCH_2Ph$), 73.8 (C4), 75.0 ($C4OCH_2Ph$), 78.2 (C3 and $-C \equiv CH$, D), 98.6 (C1), 127.1, 127.3, 127.5, 127.6, 127.8, 128.2, 128.4, 128.7, 129.9, 133.3, 137.5, 138.5, 165.5. MALDI-TOF (m/z): $[M+Na]^+$ calculated for $C_{496}H_{482}O_{110}Na$, 8220.2013; Found: 8224.9378.

Conclusions

The $\alpha(1,6)$ polymannan dimer **6** with high regio- and stereoregularity can be prepared in only 2 steps which are 1) controlled polymerization, using a tricyclic mannosyl orthoester **1** and 4-pentyn-1-ol (**2**); and 2) homocoupling of

terminal alkynes catalyzed by Pd/C and CuI. These reactions can be carried out with a simple preparation and gave a reasonable yield. The chemical characteristics of $\alpha(1,6)$ polymannan dimer **6** was verified by NMR spectra and MALDI-TOF. The synthetic dimer **6** would be utilized further for future biological investigations to determine the effect of the glycan dimer.

Acknowledgements

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