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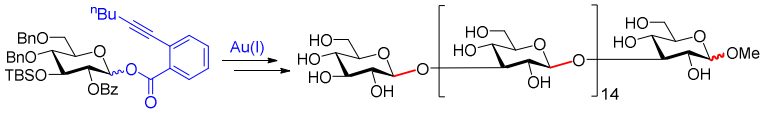
Graphical abstract

A gold(I)-catalyzed highly convergent strategy is described for the efficient synthesis of a β-(1,3)-glucan hexadecasaccharide.



Gold(I)-promoted Synthesis of a β-(1,3)-Glucan Hexadecasaccharide via the Highly Convergent Strategy

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Abstract:

Elucidation of the structure-activity relationships of β-(1,3)-glucans is hampered by the difficulty to isolate the β-(1,3)-glucan polysaccharides from natural sources. We describe a gold(I)-promoted approach for the efficient assembly of a β-(1,3)-glucan hexadecasaccharide via the orthogonal and consecutive activation of thioglycosides and glucosyl *ortho*-hexynylbenzoates in a highly convergent manner. The synthetic hexadecasaccharide serves as the basis for further evaluation of their biological functions.

Keywords: Glycosylation; β-(1,3)-glucan; polysaccharide; gold; glycosyl *ortho*-hexynylbenzoate

Introduction

In nature, β-(1,3)-glucans are widely distributed as the essential constituents of fungi and seeweeds.[1,2] By interaction with the complement receptor type 3 and dectin-1, β-(1,3)-glucans are regarded as an important type of biological response modifiers that are able to stimulate

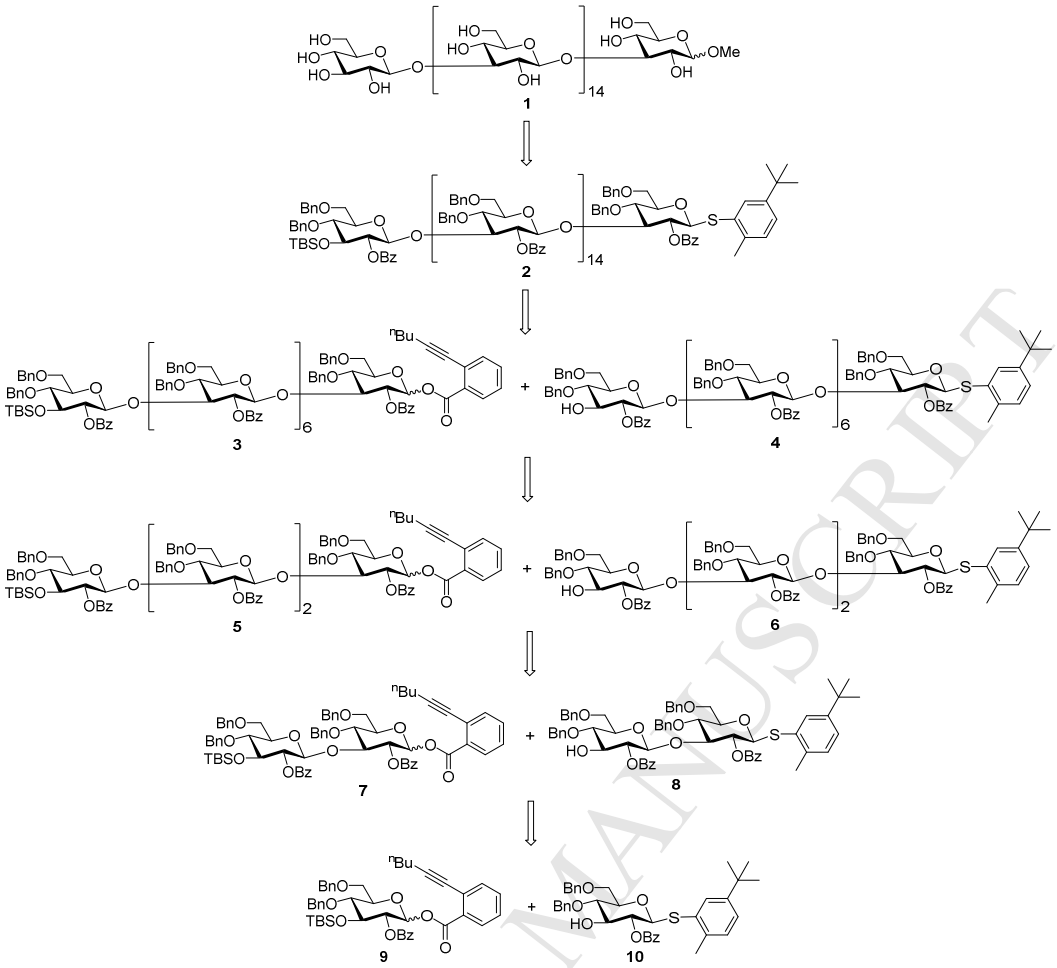
the immune system and exhibit antitumor, antifungal, and antibacterial effects.[3-14] The strong binding between β-(1,3)-glucans and dectin-1 has been explored for searching modulators of innate immunity.[15,16] Glycoconjugate vaccines based on β-(1,3)-glucans can provide protection in mice against fungal pathogens such as *Candida albicans* and *Aspergillus fumigatus*.[17-22] However, biological studies of naturally occurring β-(1,3)-glucans containing short 6-*O*-branched β-glucans or their hydrolysates may lead to contradictory results due to the heterogeneity of natural β-glucans.[23-25] As such, synthetic β-(1,3)-glucans are highly desirable for understanding the mechanism and structure-activity relationships of β-(1,3)-glucans in the interaction with cell surface receptors.

Chemical synthesis of β-(1,3)-glucans has aroused much interest in carbohydrate community aiming at development of immunomodulatory agents and antifungal vaccines.[26-30] To access the β-(1,3)-glucan polysaccharides, various approaches were developed to produce linear deca-,[27] undeca-,[28] dodeca-,[27] trideca-,[28] hexadeca-,[29] and branched heptadecasaccharides[29] in solution phase. Recently, Seeberger and coworkers reported the automated solid-phase synthesis of dodeca- and branched tridecasaccharides for identifying antibody epitopes.[30] However, synthesis of long β-(1,3)-glucans with more than 15 glucose units is very rare. Hence, efficient assembly strategies are highly pursued for the synthesis of ultralong β-(1,3)-glucan polysaccharides.

In terms of the synthesis of β-(1,3)-glucans, the solution-phase convergent [n + n] strategy is usually considered as one of the most efficient approaches for the block assembly of β-(1,3)-glucans from the perspective of symmetry. Based on this strategy, at least four glycosylation steps are required for the assembly of a β-(1,3)-glucan hexadecasaccharide skeleton. Nevertheless, in the solution-phase synthesis of the β-(1,3)-glucan hexadecasaccharide via the glycosylation with the trichloroacetimidates and ethyl

thioglycosides as donors reported by H. Tanaka and coworkers, extension of the sugar chain did not go above tetrasaccharide donors.[29] Here, we report the gold(I)-promoted highly convergent [8 + 8] synthesis of a β-(1,3)-glucan hexadecasaccharide based on the orthogonal and consecutive activation of thioglycosides and glucosyl *ortho*-hexynylbenzoates.

As depicted in Scheme 1, the β-(1,3)-glucan hexadecasaccharide **1** can be derived from odourless 5-*tert*-butyl-2-methyl phenyl thioglucoside **2** that might avoid glycoside transfer,[31] in which the orthogonal TBS group is attached to the 3-OH position of the non-reducing end. The benzoyl groups installed at the 2-OH positions of the glucose units can ensure the stereoselective formation of β-glucosides via the neighboring-group participation effect. Based on the highly convergent strategy, the hexadecasaccharide **2** is dissected into octasaccharide *ortho*-hexynylbenzoate[32] **3** and thioglycoside **4**, which could be constructed by coupling of tetrasaccharide *ortho*-hexynylbenzoate **5** and thioglycoside **6** followed by functional group transformations. Similarly, tetrasaccharide building blocks **5** and **6** are further divided into disaccharide *ortho*-hexynylbenzoate **7** and thioglycoside **8** that can be obtained by glycosylation of monosaccharide *ortho*-hexynylbenzoate **9** and thioglycoside **10** and subsequent functional group transformations.

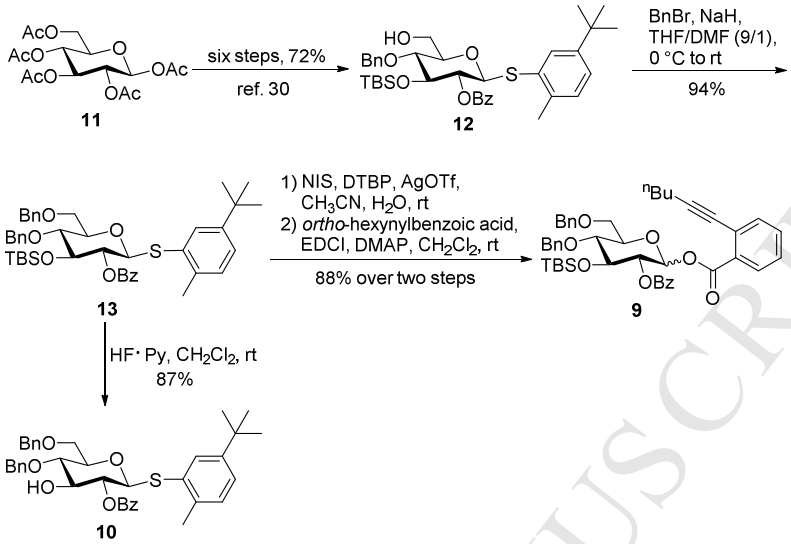


Scheme 1. Retrosynthetic analysis of the β-(1,3)-glucan hexadecasaccharide **1**.

Result and discussion

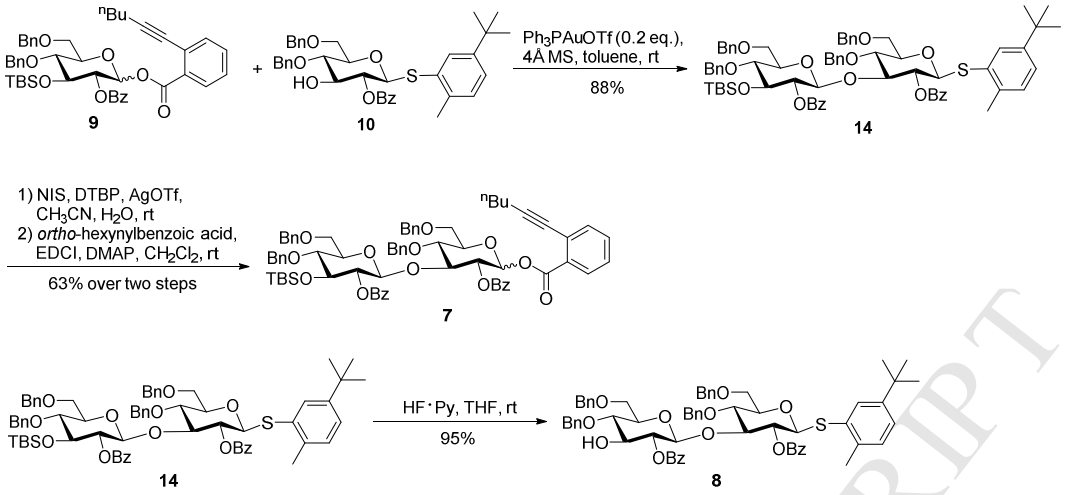
Starting from the commercially available β-D-glucose pentaacetate **11**, alcohol **12** was prepared in 72% overall yield over six steps according to the literature procedures (Scheme 2).[30] Benzylation of alcohol **12** with benzyl bromide in the presence of sodium hydride in a mixture of THF and DMF gave thioglycoside **13** in 94% yield. Subjection of thioglycoside **13** to an NIS/DTBP/AgOTf system in acetonitrile and water led to the formation of the corresponding hemiacetal, which was condensed with *ortho*-hexynylbenzoic acid under the promotion of EDCI and DMAP to provide glucosyl *ortho*-hexynylbenzoate **9** in 88% yield over two steps. Exposure of **13** to HF□pyridine in dichloromethane resulted in the cleavage of

the TBS group, affording glucosyl acceptor **10** in 87% yield without migration of the 2-benzoyl group into the 3-OH position.



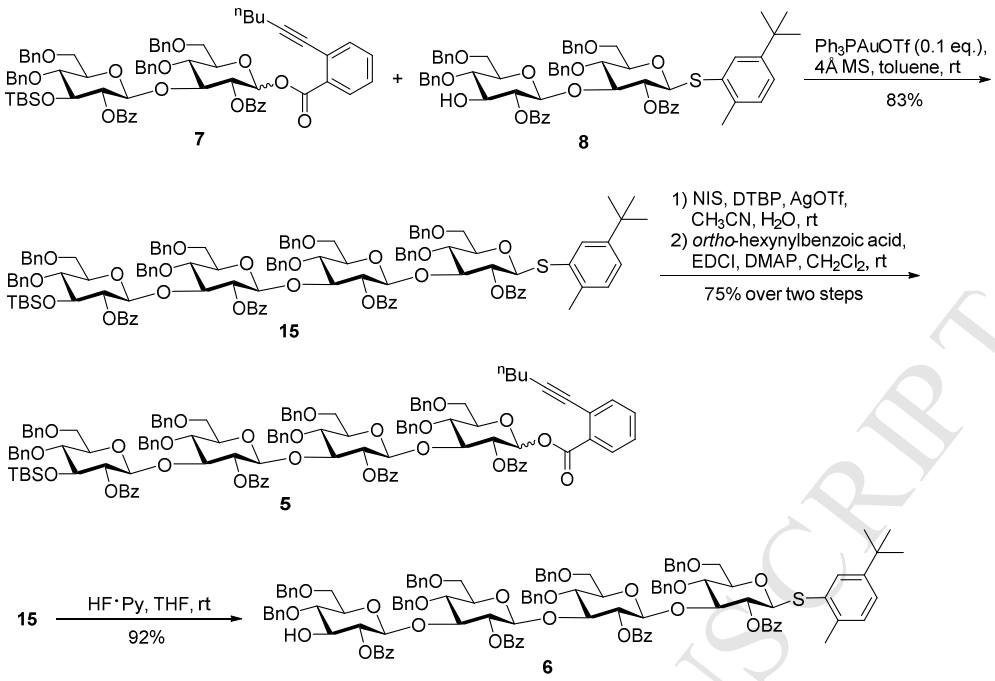
Scheme 2. Synthesis of the monosaccharide building blocks **9** and **10**.

At the outset, PPh_3AuOTf (0.2 eq.)-catalyzed glycosylation of *ortho*-hexynylbenzoate **9** with glucosyl acceptor **10** was performed in dichloromethane at room temperature to give the desired β-linked disaccharide **14** in 70% yield, as determined by analysis of the coupling constants between H-1 and H-2 of the corresponding glucoses ($^3J_{H1,H2}$ = 8.0, 10.0 Hz, respectively). Interestingly, by replacing dichloromethane with toluene, the coupling gave a cleaner reaction and the glycosylation yield was improved to 88% due to the solvent effects[33] (Scheme 3). Compound **14** was then transformed into disaccharide *ortho*-hexynylbenzoate **7** in 63% yield over two steps via selective removal of the anomeric thiol ether with NIS/DTBP/AgOTf and subsequent condensation with *ortho*-hexynylbenzoic acid. Removal of the TBS group in **14** with HF□pyridine in dichloromethane generated disaccharide acceptor **8** in a moderate 67% yield accompanied by the recovery of starting material **14** in 11% yield. However, treatment of **14** with HF□pyridine in THF proceeded smoothly to afford disaccharide acceptor **8** in an excellent 95% yield.



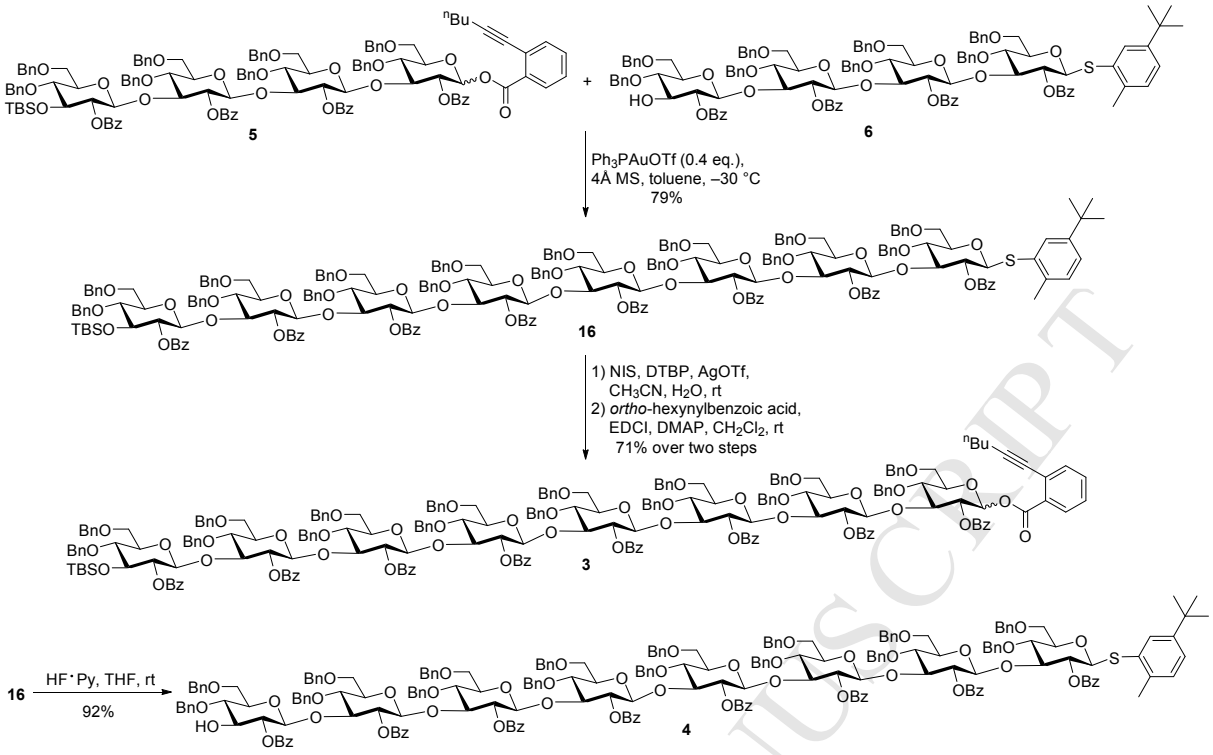
Scheme 3. Synthesis of the disaccharide building blocks **7** and **8**.

The [2 + 2] glycosylation of **7** with **8** catalyzed by PPh_3AuOTf (0.1 eq.) was originally investigated in dichloromethane at room temperature, producing β-linked tetrasaccharide **15** in only 41% yield. Exhilaratingly, when toluene was used as solvent, the PPh_3AuOTf (0.1 eq.)-catalyzed glycosylation of **7** with **8** resulted in a very clean reaction, furnishing the β-linked tetrasaccharide **15** in 83% yield (Scheme 4). The β-glycosidic linkages of tetrasaccharide **15** were confirmed by analysis of the coupling constants between C-1 and H-1 of the corresponding glucoses ($^1J_{C1,H1}$ = 156.0, 162.0, 162.5 Hz, respectively). In a manner similar to the functional group transformations from **14** to **7**, compound **15** was converted into tetrasaccharide *ortho*-hexynylbenzoate **5** in 75% yield over two steps. The TBS group in **15** was removed using HF□pyridine in THF to provide tetrasaccharide acceptor **6** in 92% yield.



Scheme 4. Synthesis of the tetrasaccharide building blocks **5** and **6**.

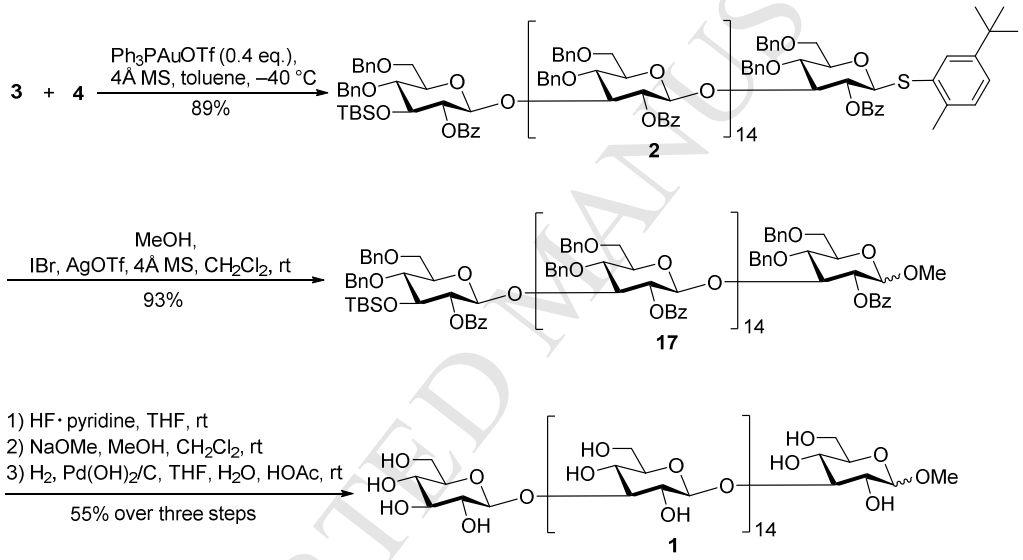
Coupling of tetrasaccharide *ortho*-hexynylbenzoate **5** with tetrasaccharide acceptor **6** under the promotion of PPh_3AuOTf (0.2-0.5 eq.) in toluene at room temperature afforded the β-linked octasaccharide **16** in around 55% yield with the concomitant hydrolysis of donor **5**. By lowering the temperature to –30 °C, the PPh_3AuOTf (0.4 eq.)-promoted [4 + 4] glycosylation of **5** with **6** provided the β-linked octasaccharide **16** as the only anomer in 79% yield (Scheme 5). The β-glycosidic linkages of octasaccharide **16** were confirmed by analysis of the coupling constants between C-1 and H-1 of the corresponding glucoses ($^1J_{C1,H1}$ = 160.0, 161.0, 161.5 Hz, respectively). Steps similar to the **15** → **5** conversion were then employed to prepare octasaccharide *ortho*-hexynylbenzoate **3** from **16** (71% over two steps). Cleavage of the TBS group in 1**6** with HF□pyridine in THF produced octasaccharide acceptor **4** in 92% yield.



Scheme 5. Synthesis of the octasaccharide building blocks **3** and **4**.

With octasaccharide *ortho*-hexynylbenzoate **3** and octasaccharide acceptor **4** in hand, we commenced to couple them into the β-(1,3)-glucan hexadecasaccharide **1** (Scheme 6). Exhilaratingly, [8 + 8] coupling of **3** with **4** promoted by PPh_3AuOTf (0.4 eq.) in toluene at –40 °C proceeded smoothly to furnish β-linked hexadecasaccharide skeleton **2** as the single anomer in 89% yield. The desired β-glycosidic linkages of hexadecasaccharide **2** were confirmed by analysis of the coupling constants between C-1 and H-1 of the corresponding glucoses ($^1J_{C1,H1}$ = 156.5, 160.5, 161.0, 161.5, 162.0, 162.5, 163.0 Hz, respectively). Unexpectedly, glycosylation of **2** with methanol under the promotion of NIS and TfOH afforded compound **17** as a mixture of α-methylated and β-methylated anomers in only 51% yield. With the assistance of IBr and AgOTf, the glycosylation yield was improved to 93% albeit accompanied by the formation of almost equal amount of α/β anomers ($^1J_{C1,H1}$ = 174.0, 159.0, 159.6, 160.2, 157.2 Hz, respectively). Similarly, glycosylation of monosaccharide **13** with methanol promoted by IBr and AgOTf gave a mixture of the desired methyl glycoside in

89% yield (β/α = 3:1), testifying that the neighboring-group participation effect did not play a decisive role in this type of glycosylation reactions.[34] Global deprotection of **17** involving removal of the TBS group with HF□pyridine, saponification with sodium methoxide in methanol and dichloromethane, hydrogenolysis of the benzyl groups over $Pd(OH)_2/C$ in a mixture of THF, water and acetic acid, provided hexadecasaccharide **1** in 55% yield over three steps. The structure of **1** was confirmed by analysis of ^{1}H NMR spectrum and MALDI-TOF. The ^{1}H NMR spectrum of synthetic **1** was found to be in good agreement with those reported in the literature.[29,30,35]



Scheme 6. Synthesis of the β-(1,3)-glucan hexadecasaccharide **1**.

Conclusion

In conclusion, we have described the gold(I)-promoted synthesis of a β-(1,3)-glucan hexadecasaccharide via the orthogonal and consecutive activation of thioglycosides and glucosyl *ortho*-hexynylbenzoates. The highly convergent synthetic approach required only four glycosylation steps for procurement of the β-(1,3)-glucan hexadecasaccharide skeleton starting from the monosaccharide building blocks. The couplings with glucosyl *ortho*-hexynylbenzoates as donors and PPh_3AuOTf as promoter in toluene proved to be very

efficient for the construction of β-(1,3)-glucan polysaccharides. The synthetic approach described here lays the foundation for further biological evaluation of β-(1,3)-glucan polysaccharides.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/

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Highlights：

- Efficient synthesis of a β-(1,3)-glucan hexadecasaccharide.
- Orthogonal and consecutive activation of thioglycosides and glucosyl *ortho*-hexynylbenzoates
- Gold(I)-catalyzed highly convergent approach in toluene.