Chinese Chemical Letters 34 (2023) 107982

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Stereoselective synthesis of the 3,6-branched Fuzi α-glucans up to 15-mer *via* a one-pot and convergent glycosylation strategy

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ARTICLE INFO

Article history:
Received 18 August 2022
Revised 26 October 2022
Accepted 3 November 2022
Available online 6 November 2022

Keywords:
α-D-Glucan
One-pot synthesis
Convergent glycosylation
Stereoselective synthesis
Thioglycoside

ABSTRACT

A family of the 3,6-branched Fuzi α-glucans including the pentasaccharide repeating unit as well as its di- and trimers were efficiently achieved *via* a one-pot and convergent glycosylation strategy. All the protected α-glucans up to 15-mer were assembled with high yields and excellent α-stereoselectivity, which was secured by the synergistic α-directing effects of the TolSCl/AgOTf promotion system and the steric β-facial shielding of bulky saccharide residues linked at the 6-*O*-position of glucosyl donors. Moreover, the 3,6-branched architecture of glycosyl donor was revealed to be more favorable for the α-selective glucosidation of primary hydroxyl group, especially in the case of large oligosaccharide acceptor. The structurally well-defined synthetic α-glucans would be useful for various biological studies.

α-D-glucan made up of iterative α-linked D-glucose units is one of the most abundant natural polysaccharides and is widely expressed by mammals, bacteria, fungi, plants, *etc.* [1,2]. It shows broad structural diversity owing to the different types of α-glucosyl linkages and their specific combination, accordingly exhibiting versatile bioactivities such as antioxidant [3,4], antitumor [5,6], anti-inflammatory [7], and antiviral [8,9] properties. Notably, a lot of natural α-glucans possess strong immunostimulating activity, thus making them potential vaccine adjuvant candidates [2,10–15]. Fuzi, the daughter root of *Aconitum carmichaeli* Debx, is a reputed herbal drug in traditional Chinese medicine for thousands of years and is collected in Chinese and Japanese Pharmacopoeias [16–20]. In 2006, Wu *et al.* isolated a water-soluble polysaccharide (**1**, Fig. 1) from Fuzi, the structure of which was characterized as a branched α-glucan containing an α-(1→6)-linked glucosyl backbone and a single glucose unit branched at C-3 position with one out of every four residues along the main chain. Both the *in vivo* and *in vitro* pharmacological studies showed that the Fuzi α-glucan could promote the murine lymphocyte proliferation induced by concanavalin A or lipopolysaccharide and the splenocyte antibody production in a dose-dependent manner. In addition, no cytotoxicity in cells was observed for Fuzi α-glucan even at a concentration of 100 μg/mL [20].

However, due to the structural heterogeneity, it is difficult to derive a series of homogeneous Fuzi α-glucan oligosaccharides in sufficient amounts and purity as well as comparable structures from natural sources, while these glycans would be very useful for the in-depth exploration of the structure-activity relationship (SAR) and the biological mechanism at molecular level. Chemical synthesis has thus become an important tool to resolve this issue. Stereoselective glucosylation with exclusive 1,2-*cis*-selectivity is a prerequisite for the chemical synthesis of α-glucans, otherwise, very complex products would be generated after repetitive glycosylation cycles. Although many elegant 1,2-*cis*-glycosylation methods have been developed for preparation of α-glucans [21–32], there have been only a few reports about the synthesis of branched α-glucans to date. Demchenko group accomplished the synthesis of a 4,6-branched α-glucan tetrasaccharide by using a hydrogen bond-mediated aglycon delivery method (HAD) to realize α-specific glycosylation [21]. By means of variant activator/additive combinations, the Codée group achieved the stepwise synthesis of a 4,6-branched *Mycobacterium tuberculosis* α-nonaglucan [22]. Very recently, the Seeberger group finished the synthesis of a family of branched starch and glycogen α-glucans *via* automated glycan assembly strategy (AGA) by utilizing the remote anchimeric assistance of 3-*O*- and 6-*O*-acyl groups equipped on thioglucoside donors to favor α-glycosylation [23]. Besides, Boons *et al.* finished

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https://doi.org/10.1016/j.cclet.2022.107982
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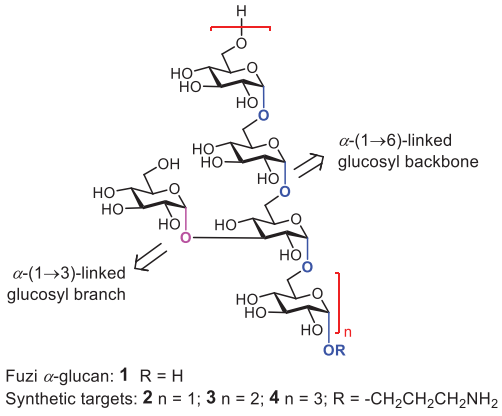


Fig. 1. Structures of the Fuzi α-glucan **1** and its repeating unit monomer **2**, dimer **3**, and trimer **4** as the synthetic targets.

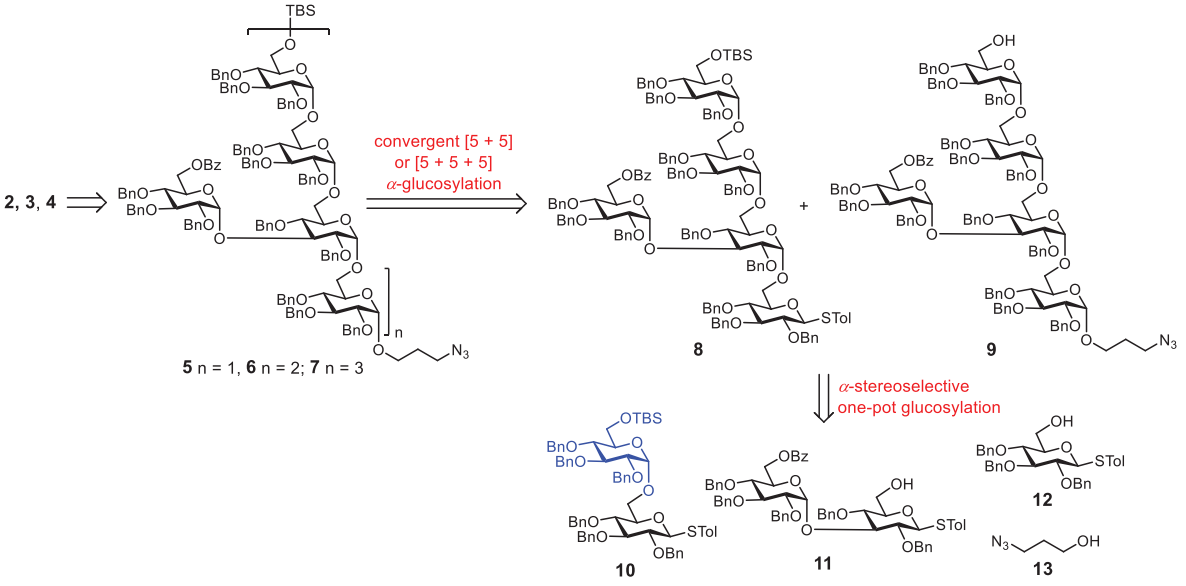
the solid-phase synthesis of a pentasaccharide repeating unit of Fuzi α-glucan by employing the participating effect of the 2-*O*-(*S*)-(phenylthiomethyl)benzyl auxiliary to realize α-selective glucosylation [24].

Despite these progresses, stereoselective synthesis of branched α-glucan still remains challenging as stepwise glycosylation and laborious manipulation of intermediates were usually unavoidable, inevitably decreasing the atom economy and synthetic efficiency. Recently, we have developed a highly α-stereoselective glucosylation method based on the synergistic α-directing properties of the toluenesulfenyl chloride (TolSCl)-silver triflate (AgOTf) promotion system, initially developed by Ye *et al.* [33–35], and the steric β-facial shielding or remote acyl participation of functionalities at the donor 6-*O*-position [36]. This method showed versatile applicability in the efficient and one-pot synthesis of complex α-glucans and oligosaccharides containing α-glucosyl linkages [36–39]. Interestingly, this approach was suitable for the stereoselective construction of the α-aminoglucosidic bond as well [40].

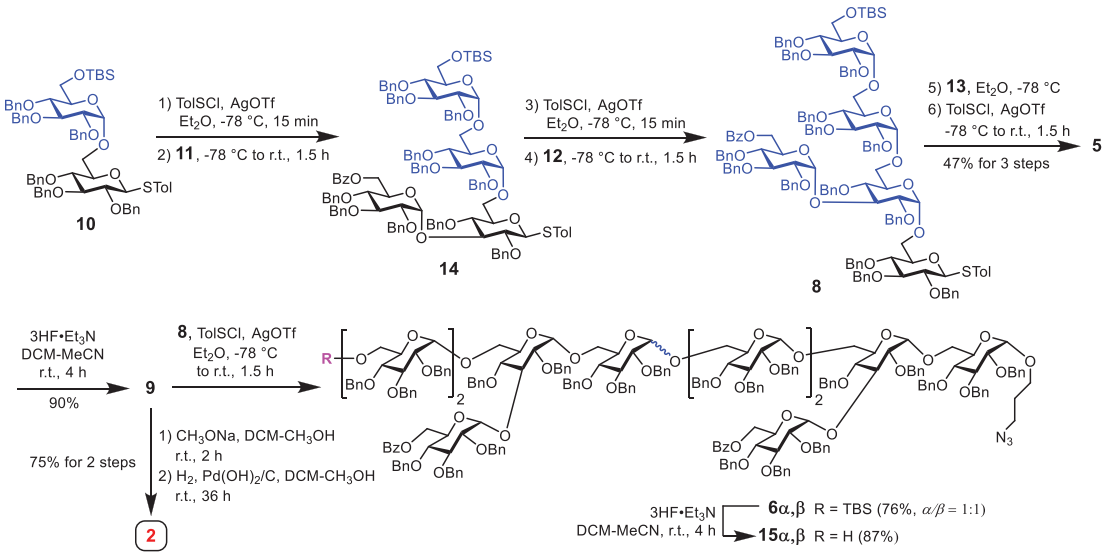
Encouraged by the robustness of our α-glucosylation method, we here designed and accomplished the chemical synthesis of a series of structurally defined Fuzi α-glucans including the pentasaccharide repeating unit **2** as well as its dimer **3** and trimer **4** (Fig. 1), in the hope of facilitating the further biological study. These synthetic targets contain iterative α-glucosyl linkages up to 15 mer and the α-(1→3) branches, thus representing a notable synthetic challenge.

As outlined in Scheme 1, a one-pot and convergent glycosylation strategy was envisioned for the target molecules **2–4**. Pentasaccharide **5** as the fully protected precursor of **2** could be sequentially assembled from thioglycoside donors/acceptors **10–12** and 3-azido-1-propanol **13** *via* preactivation-based iterative one-pot glycosylation [33–39,41–48]. Each coupling should be α-stereoselective due to the steric β-shielding effect exerted by the bulky sugar residue at glycosyl donor 6-*O*-position, such as the nonreducing end monosaccharide unit (labelled in blue color) in **10**. Decasaccharide **6** and pentadecasaccharide **7** as the protected forms of **3** and **4**, respectively, were initially attempted to be stereoselectively constructed *via* a convergent [5 + 5] or [5 + 5 + 5] strategy, both of which would utilize pentasaccharides **8** and **9** as building blocks. In turn, thioglucoside **8** could be concisely assembled from **10** to **12** *via* a three-component one-pot glycosylation.

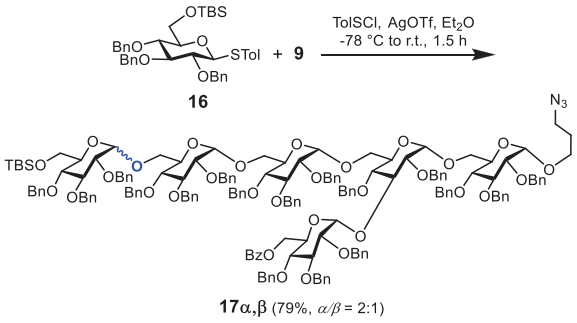
The synthesis of **2** (Scheme 2) commenced with facile preparation of compounds **10** and **12** according to the reported procedures [36,49]. Disaccharide **11** containing a preassembled α-(1→3) glucosidic bond was obtained by the regioselective ring opening of a benzylidene protected precursor (see the Supporting information for details) [36]. Thereafter, the iterative one-pot coupling reactions between **10** – **13** were carried out through preactivation of glycosyl donor using TolSOTf, *in situ* generated from TolSCl and AgOTf, in diethyl ether at −78 °C for 15 min, which was followed by addition of acceptor at the same temperature, and the reaction mixture was subsequently warmed up to room temperature and stirred for another 15 min to finish the glycosylation. The amount of each building block was pivotal for the smooth assembly of pentasaccharide **5**. At first, 1.0 equiv. of donor **10** and 0.9 equiv. of **11** were utilized, and thereafter, 0.8 equiv. of **12** and 0.7 equiv. of **13** were sequentially added after activation of the resultant donors **14** and **8**, respectively. Finally, the four-component one-pot glycosylation reactions afforded the linker equipped **5** in a 47% overall yield. All of its glycosyl linkages were identified as α-configuration by the small anomeric coupling constants in ^{1}H NMR spectrum ($\leq$3.6 Hz). No other anomeric isomers were positively isolated or identified by NMR analysis, indicating that all the above three steps glycosylation were virtually α-specific, possibly due to the synergistic α-directing effects of the TolSCl/AgOTf promotion system and the



Scheme 1. Initial retrosynthetic analysis of target α-glucans **2–4**.



Scheme 2. Stereoselective and one-pot synthesis of α-pentaglucan **2** and the attempted assembly of decasaccharide **6** *via* a convergent [5 + 5] glycosylation.



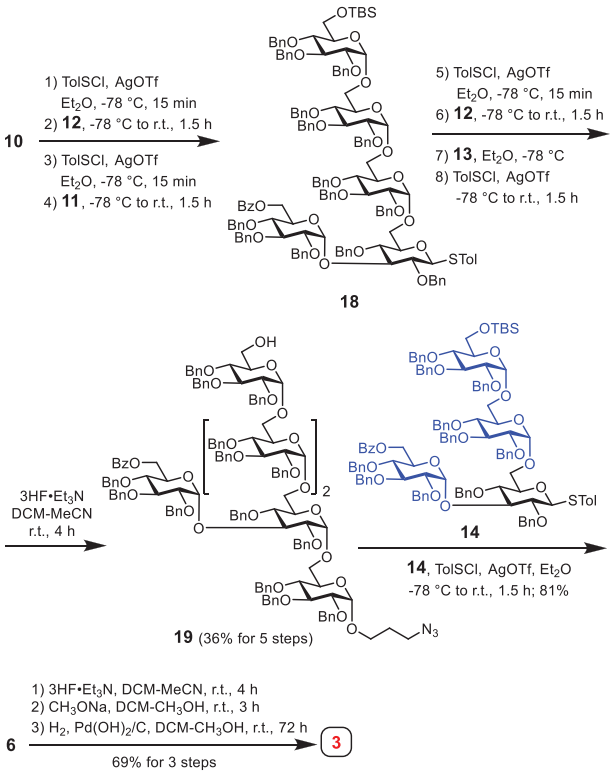
Scheme 3. Attempted synthesis of **17** *via* [1 + 5] glycosylation.

steric β-shielding effect of the bulky mono-, di-, and tetrasaccharide motifs (labelled in blue color) in glycosyl donors **10**, **14**, and **8**, respectively. Thereafter, removal of the *tert*–butyldimethylsilyl (TBS) group in **5** by using 3HF·Et_3N resulted in the pentasaccharide **9** in 90% yield. Finally, deprotection of the benzoyl (Bz) group under Zemplén condition (CH_3ONa/CH_3OH) was followed by removal of all the benzyl (Bn) groups and reduction of the azido group *via* $Pd(OH)_2$-catalyzed hydrogenolysis to deliver the target α-glucan **2** in 75% overall yield after purification on a G10 column and lyophilization. The structure of **2** was rigorously characterized with 1D, and 2D NMR and HR MS data. However, on the other hand, the convergent glycosylation between pentasaccharides **8** and **9** under the same reaction conditions gave compound **6**, unexpectedly, with a low stereospecificity ($\alpha:\beta = 1:1$), albeit in a 76% yield. Desilylation of the mixture with 3HF·Et_3N led to **15*α*** and **15*β***, which could be readily separated and characterized.

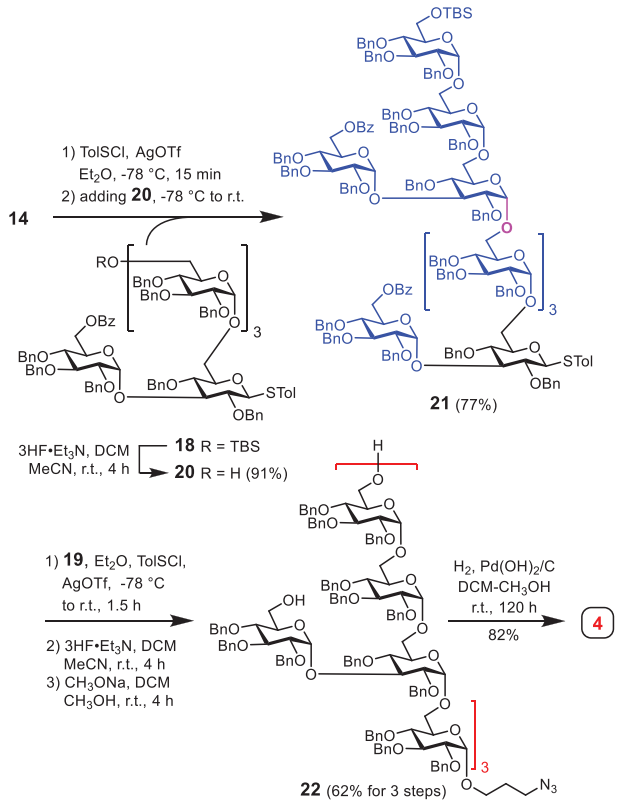
It was found in our previous study that by means of the same α-glycosylation method the convergent coupling between bulky oligosaccharide donor and acceptor afforded the α-(1→3) and α-(1→4) glucosidic linkages with exclusive α-selectivity [36,39]. In this context, we next conducted the glycosylation between monosaccharide donor **16** [36] and pentasaccharide acceptor **9** to examine whether the low α-selectivity for **6** in Scheme 2 was resulted from the steric hindrance of bulky acceptor, as shown in Scheme 3. Likewise, a bad but expected stereoselectivity was produced for compound **17** (79%, $\alpha:\beta = 2:1$) as well. Presumably, the mismatch between large building blocks, such as the low facial accessibility of the hydroxyl group in bulky **9** to the reactive glycosyl oxocarbenium resulted from activation of donor **16** or **8**, was probably responsible for the aforementioned low stereoselectivity. It is worth noting that the α-selectivity of glucosylation reactions diminished with the increasing size of glycosyl acceptor was a common problem for the reported chemical synthesis of α-glucans [21,50]. All the above discoveries extensively indicated the notable synthetic challenge for stereoselective construction of an α-(1→6) glucosyl linkage, particularly *via* convergent glycosylation method and the use of bulky oligosaccharide substrates.

At this stage, we abandoned the initial [5 + 5] glycosylation strategy for compound **6** and decided to exploit other types of glycosyl donors with an aim to get a better α-selectivity in comparison of that obtained with donors **8** and **16**. Hung and co-workers [51] have ingeniously revealed that when two large groups were simultaneously situated at the 3,6-*O*-positions of a 2-azido protected glucosaminosyl donor, a complete α-selectivity was afforded for related glycosylation reactions owing to the combined steric β-facial shielding property of these large substituents. Inspired by the finding, we then envisaged an alternative [4 + 6] convergent glycosylation strategy (Scheme 4) for accessing to **6** by using tetrasaccharide donor **14** and hexasaccharide acceptor **19** as building blocks, hoping to make use of the remote anchimeric assistance of the saccharide residues installed at the 3-*O*- and 6-*O*-positions of the glycosylating unit of donor **14**.

Accordingly, **19** was firstly prepared from compounds **10–13** through a TolSCl/AgOTf promoted five-component one-pot glycosylation and subsequent removal of the TBS group of the resultant hexasaccharide intermediate by a similar method used for synthesis of **9**. After purification with silica gel column chromatography, **19** was obtained with a 36% overall yield and all of its glucosyl linkages were identified as α-configuration by the anomeric coupling constants in ^{1}H NMR spectrum ($\leq$ 3.6 Hz). It is noteworthy that the pentasacharide intermediate **18** could also be employed as a pivotal substrate for assembly of the target pentadecasaccharide later on. Thereafter, the convergent condensation between acceptor **19** and 3,6-branched donor **14** was performed under the same TolSCl/AgOTf activation conditions and to our delight, the desired decasaccharide **6** was generated stereospecifically in a high 81% yield. Next, following the same deprotection procedures described



Scheme 4. One-pot and convergent synthesis of α-decaglucan **3**.



Scheme 5. One-pot and convergent synthesis of α-pentadecaglucan **4**.

for preparation of **2**, compound **6** was readily converted into the target α-glucan **3** in 69% overall yield.

Encouraged by the high yield and excellent α-selectivity of the [4 + 6] glycosylation, we next planned to assemble the target pentadecasaccharide **4** *via* a [4 + 5 + 6] convergent method (Scheme 5). The TBS group in **18** was firstly removed by treatment with 3HF·Et_3N to liberate the related C6‴-OH. As expected, preactivation-based glycosylation of **20** with donor **14** resulted in the nonasaccharide **21** in an isolated 77% yield together with small amount of the β-isomer (α:β > 15:1) that was only detected by MALDI-MS. Subsequently, thioglucoside **21** possessing a 3,6-branched architecture (labelled in blue color) around its reducing end glucose unit was directly employed as the glycosyl donor to couple with hexasaccharide acceptor **19** in the presence of TolSCl/AgOTf, and the desired pentadecasaccharide **22** was smoothly generated in 62% yield after *in situ* deprotection of the TBS and Bz groups *via* the aforementioned general manipulations. Finally, $Pd(OH)_2$-catalyzed hydrogenolysis to remove all the Bn groups and reduce the azido group concomitantly was followed by purification on a G25 column and lyophilization to deliver the target α-pentadecaglucan **4** in 82% yield, and its structure was verified with NMR and HR MS data.

In summary, we reported the efficient and systematic synthesis of a series of 3,6-branched Fuzi α-glucans including the pentasaccharide repeating unit as well as its di- and trimers *via* a convergent and one-pot glycosylation strategy. All the protected α-glucans up to 15-mer were obtained in high yields and excellent α-stereoselectivity due to the combined α-directing effects of the TolSCl/AgOTf activation system and the bulky saccharide residues at the glycosyl donor 6-*O*-position to exert the steric β-shielding effect. Moreover, the glucosyl donors carrying large saccharide residues at the 3-*O*- and 6-*O*-positions of the glycosylating unit were revealed to be favorable for stereoselective construction of the α-(1→6) glycosyl linkages, especially with bulky oligosaccharide acceptors. Compared to the reported and typically used stepwise glycosylation methods for synthesis of α-glucans, our one-pot and convergent glycosylation approach obviated a number of glycosylation cycles and laborious intermediates manipulation, significantly increasing the synthetic efficiency. The synthetic α-glucans and their derivatives, which could be easily obtained by elaboration of the anomeric amino group of **2–4**, would be very useful for in-depth biological studies such as the novel vaccine adjuvant development and SAR elucidation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 22177060), the Young Scholars Program of Shandong University (No. 2018WLJH41), the Open Projects Fund of Shandong Key Laboratory of Carbohydrate Chemistry and Glycobiology, Shandong University (No. 2021CCG06), and the Central Government Guide Local Science and Technology Development Funds (No. YDZX20203700002579). We thank Haiyan Sui and Xiaoju Li from State Key Laboratory of Microbial Technology in Shandong University for NMR measurements.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.107982.

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