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Stereoselective α-galactofuranosylation and synthesis of di- and tetrasaccharide subunits of cell wall polysaccharides of *Talaromyces flavus*

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ABSTRACT

Stereoselective α-galactofuranosylation employing 2′-carboxybenzyl glycosides as galactosyl donors has been established. The tetrabenzyl-protective group on the galactosyl donor was essential for the α-galactosylation of secondary alcohol acceptors. The present method was successfully applied to the synthesis of di- and tetrasaccharide subunits of cell wall polysaccharides of *Talaromyces flavus*.

Talaromyces flavus is a soil-inhabiting fungus and has been known to suppress Verticulum wilt of eggplant and tomato,[1] and parasitizes *Rhizoctonia solani*[2] and *Sclerotinia sclerotiorum*.[3] Like the cell wall of other fungi,[4] that of *T. flavus* was known to be rich of polysaccharides but contains unusually high proportion of galactofuranose.[5] The water-soluble cell wall polysaccharides were isolated from *T. flavus* and the following structure of the disaccharide repeating unit was identified: →6)-[α-D-Gal*f*-(1→2)]-α-D-Man*p*-(1→ (**A**) (Fig. 1).[6] Interesting structural feature of this polysaccharide is the occurrence of the α-D-galactofuranosyl moiety, which is often a problematic subunit to incorporate stereoselectively. Due to the potential of *T. flavus* as a biocontrol agent and the synthetic challenge for the construction of the α-D-galactofuranosyl linkage, we were interested in the synthesis of the repeating unit **A**. For the construction of the α-D-galactofuranosyl linkages, galactofuranosyl trichloroacetimidates[7] and thiogalactofuranosides[8] have been used as galactofuranosyl donors but they are not generally applicable for α-galactofuranosylation with a range of glycosyl acceptors. Recently, an indirect method employing 2,3-anhydroglucofuranosyl thioglycosides as glycosyl donors has been utilized as alternatives for the synthesis of α-galactofuranosides.[9] We have also reported an example of the direct stereoselective construction of the α-galactofuranosyl linkage employing the 2′-carboxylbenzyl (CB) glycoside method during the total synthesis of immunomodulatory glycolipids, agelagalstatin.[10] Herein, we report the stereoselective construction of the α-galactofuranosyl linkage and synthesis of the disaccharide subunit (**A**, $n = 1$) and the tetrasaccharide subunit (**A**, $n = 2$) of cell wall polysaccharides of *T. flavus* employing the latent-active glycosylation strategy with CB glycosides and 2′-(benzyloxycarbonyl)benzyl (BCB) glycosides.[11]

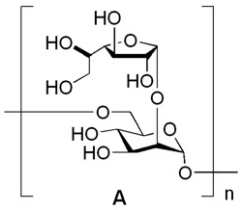

Figure 1. A repeating unit of cell wall polysaccharides of *T. flavus*.

In order to establish the efficient and stereoselective method for the construction of the α-galactofuranosyl linkage, we at first examined galactosyl donors having different protective groups such as 5,6-isopropylidene CB galactoside **1** tribenzoyl CB galactoside **2** and tetrabenzyl CB galactoside **3**. Common starting material **4** was converted into galactosyl donor **3** and intermediates **5** and **6** by the known procedure.[10] Then, benzylation of **5** followed by selective hydrogenolysis of resulting BCB galactoside on Pd/C in the presence of NH_4OAc[11] afforded **1** as shown in Scheme 1. Compound **6** was converted into **2** by the following sequence: (i) benzoylation of the C-3 hydroxy group of **6**, (ii) removal of the TBS group at O-2, (iii) benzylation of the resulting C-2 hydroxyl group, (iv) hydrolysis of the 5,6-isopropylidene group, (v) benzoylation of the resulting 5,6-diol, and (vi) selective hydrogenolysis of the benzyl ester functionality of the BCB moiety. Galactosylations of primary alcohol acceptors **7** and **8** with donors **1**–**3** were carried out

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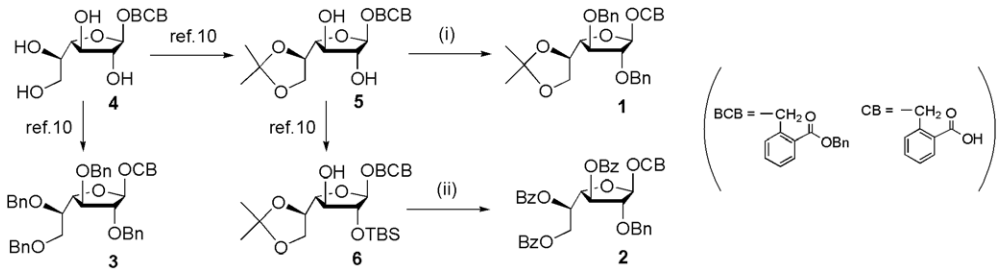
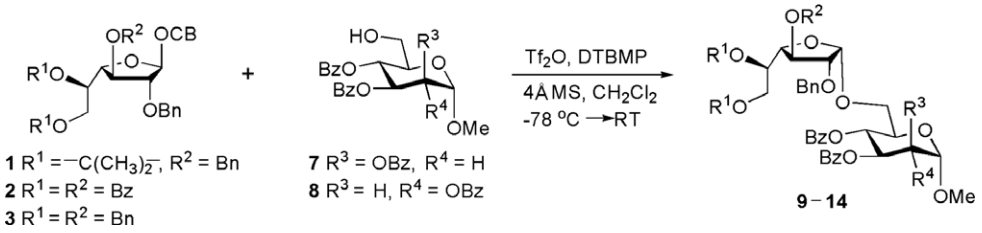


Scheme 1. Reagents and conditions: (i) (a) BnBr, NaH, DMF, RT, 30 min, 84%; (b) H_2, Pd/C, NH_4OAc, CH_3OH/EtOAc, RT, 1 h, 95%; (ii) (a) BzCl, DMAP, pyridine, CH_2Cl_2, RT, 30 min, 98%; (b) *n*-Bu_4NF, THF, RT, 1 h, 96%; (c) BnBr, NaH, DMF, RT, 30 min, 84%; (d) TFA, THF/H_2O, RT, 95%; (e) BzCl, DMAP, pyridine, CH_2Cl_2, RT, 30 min, 98%; (f) H_2, Pd/C, NH_4OAc, CH_3OH/EtOAc, RT, 1 h, 95%.

Table 1
Galactosylation of primary alcohol acceptors **7** and **8** with CB galactosides



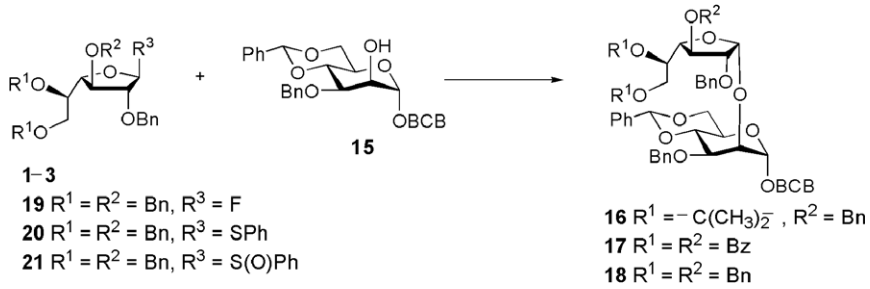
Entry	Donor	Acceptor	Product	Yield[a] (%)	Ratio (α/β)[a]
1	**1**	**7**	**9**	83	α only
2	**1**	**8**	**10**	81	α only
3	**2**	**7**	**11**	86	α only
4	**2**	**8**	**12**	83	α only
5	**3**	**7**	**13**	88	8:1
6	**3**	**8**	**14**	84	7:1

[a] Determined after isolation.

employing Tf_2O as an activator in the presence of 2,6-di-*t*-butyl-4-methylpyridine (DTBMP) as shown in Table 1. Galactosylations of **7** and **8** with the 5,6-*O*-isopropylidene galactosyl donor **1** gave exclusively α-disaccharides **9** and **10**, respectively, in high yield (entries 1 and 2 in Table 1). The galactosylations of **7** and **8** with the tribenzoyl galactosyl donor **2** were also very stereoselective to afford exclusively α-products **11** and **12** (entries 3 and 4). The same galactosylations of **7** and **8** with the tetrabenzyl galactosyl donor **3** were less stereoselctive to provide a mixture of α- and β-anomers of **13** (α/β = 8:1) and **14** (α/β = 7:1), respectively (entries 5 and 6). The result indicates that the galactosylation of the primary alcohol acceptor with the donor **1** or **2** is more stereoselective than that with the donor **3**.

On the other hand, in the galactosylation of secondary alcohol acceptor **15**,[12] the donor **3** turned out to be more α-selective than the donor **1** or **2** as shown in Table 2. Thus, galactosylations of **15**

Table 2
Galactosylation of secondary alcohol acceptors **15** with various donors



Entry	Donor	Promoters and conditions	Product	Yield[a] (%)	Ratio (α/β)[a]
1	**1**	Tf_2O, DTBMP, 4 Å MS, CH_2Cl_2, −78 °C to RT	**16**	85	3:1
2	**2**	Same as above	**17**	80	5:1
3	**3**	Same as above	**18**	85	α only
4	**19**	$SnCl_2$, $AgClO_4$, THF, −10 °C to RT	**18**	82	1.2:1
5	**20**	$Sn(OTf)_2$, NIS, 4 Å MS, CH_2Cl_2, −78 °C to 0 °C	**18**	84	3:1
6	**21**	Tf_2O, CH_2Cl_2, −78 °C to RT	**18**	93	8:1

[a] Determined after isolation.

with **1** and **2** afforded a mixture of α- and β-anomers of **16** ($\alpha/\beta = 3{:}1$) and **17** ($\alpha/\beta = 5{:}1$), respectively (entries 1 and 2 in Table 2), whereas, the reaction of **3** and **15** was completely stereoselective to provide α-disaccharide **18** exclusively in 85% yield (entry 3).[13] We also examined donors having different anomeric leaving groups such as tetrabenzyl galactosyl fluoride **19**,[14] tetrabenzyl thiogalactoside **20**,[8] and tetrabenzyl galactosyl sulfoxide **21**.[15] Donors **19** and **20** exhibited poor stereoselectivities (entries 4 and 5 in Table 2) while galactosyl sulfoxide **21** showed reasonable stereoselectivity ($\alpha/\beta = 8{:}1$) in its reaction with **15** (entry 6). The results indicated that tetrabenzyl CB galactoside **3** was superior donor for the construction of α-galactofuranosyl linkage in the glycosylation of secondary alcohol acceptor **15**. The origin of the present protective group effect on the outcome of the stereochemistry in the galactofuranosylation is not yet clear.

Based on the above study, the retrosynthesis of target tetrasaccharide **22** and disaccharides **23** and **24** in a suitably protected form of the repeating unit **A** was performed to lead to monosaccharide building blocks **3**, **15**, and **25** as shown in Figure 2. The protective groups in the target tetrasaccharide **22** were chosen after consideration of the future synthesis of a hexasaccharide or an octasaccharide from **22**. Thus, the naphthylmethyl (NAP) group at C-6 of the mannose moiety in the reducing side of the tetrasaccharide **22** would be selectively cleaved to provide the tetrasaccharide acceptor. We also envisioned that coupling of **23** and **24** would provide α-mannosyl tetrasaccharide **22** owing to the steric effect of the bulky furanose ring in the β-side at C-2 of **23** in addition to the anomeric effect.

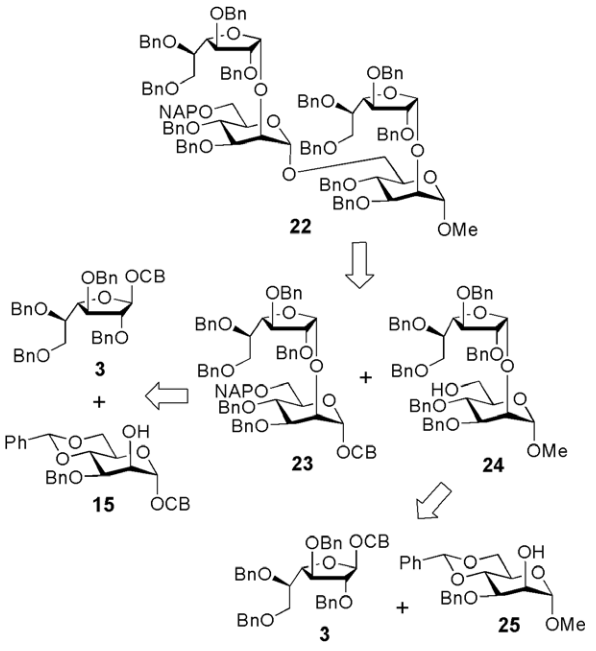
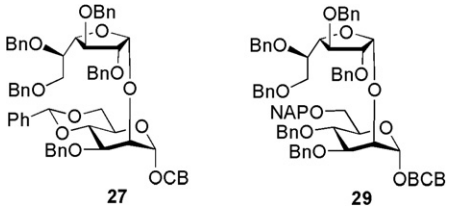
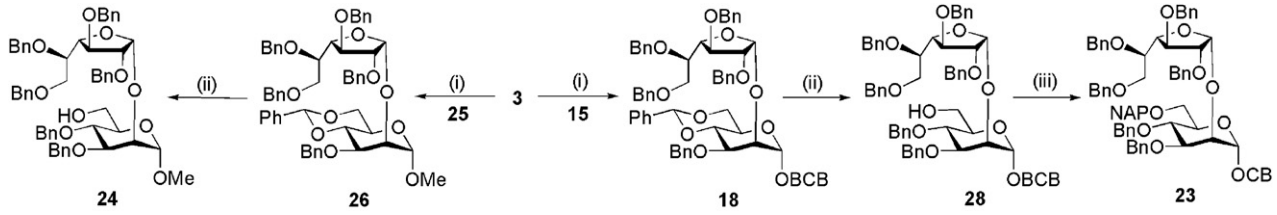


Figure 2. Retrosynthesis of tetrasaccharide **22**.

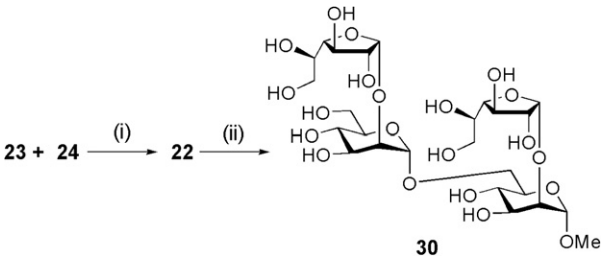
Synthesis commenced with galactosylations of **15** and **25** with the tetrabenzyl galactosyl donor **3** to provide exclusively α-disaccharides **18** and **26**, respectively, both in 85% yield as shown in Scheme 2. Anomeric carbon chemical shifts at δ 100.3 of **18** and δ 100.2 of **26** and the H1′–H2′ coupling constant, $J_{H1'-H2'} = 4.4$ Hz for both **18** and **26** clearly indicated that the newly generated galactosyl linkage of the disaccharides is in the α-configuration.[16] Then, the reductive cleavage of the benzylidene group of **26** with BH_3·THF/Bu_2BOTf[17] afforded disaccharide acceptor **24** in 78% yield. Coupling of acceptor **24** and disaccharide donor **27**, which was obtained from **18** by selective hydrogenolysis, by glycosylation reaction employing Tf_2O, however, failed to provide a desired tetrasaccharide. We, therefore, cleaved the conformationally disarming benzylidene group[18] of the compound **18** with BH_3·THF/Bu_2BOTf to obtain compound **28**. Then, we treated compound **28** with naphthylmethyl (NAP) bromide in the presence of NaH in order to make NAP-protected BCB disaccharide **29**. Unexpectedly, however, not only **29** (20%) but also NAP-protected CB disaccharide **23** (40%) was generated. It is quite unusual that the BCB group was converted into the CB group under the present reaction condition since the BCB group was very stable during the protection of hydroxy groups with benzyl, NAP, and PMB groups in other sugar.[10–12] This unexpected result, however, led us to save one step in the synthetic sequence by obtaining **23** directly from **28** without preparation of the intermediate **29**. Thus, when the reaction was conducted by addition of a small amount of H_2O (26 μL, 0.02 mmol) to a mixture of **28**, NAP bromide (24 mg, 0.109 mmol), and NaH (5.8 mg, 0.146 mmol) in DMF at 50 °C. The desired NAP-protected CB disaccharide **23** was obtained 70% yield as shown in Scheme 2. Under this reaction condition, the more nucleophilic alkoxide of **28** would attack NAP bromide while the hydroxide would hydrolyze the benzyl ester of the BCB moiety to give the NAP-protected CB glycoside **23** (Scheme 3).



Crucial coupling of **23** and **24** was carried out by slow addition of Tf_2O to a solution of **23**, **24**, and DTBMP in CH_2Cl_2 at −40 °C to afford exclusively desired α-tetrasaccharide **22**[19] in 65% yield. Deprotection of all benzyl groups and a NAP group of **22** by hydrogenolysis employing $Pd(OH)_2$ catalyst gave fully deprotected tetrasaccharide subunit **30**[20] as a methyl glycoside. The stereochemistry at newly generated anomeric center of the tetrasaccharide **30** was determined unequivocally on the basis of the one bond C1–H1 coupling constant: $J_{C1'-H1'} = 170.2$ Hz.[21]



Scheme 2. Reagents and conditions: (i) DTBMP, 4 Å MS, Tf_2O, CH_2Cl_2, −78 °C to 0 °C, 1 h, 85% for both **18** and **26**; (ii) BH_3 · THF, Bu_2BOTf, THF, 0 °C to RT, 30 min, 78% for **24** and 81% for **28**; (iii) NAPBr, NaH, *n*-Bu_4NI, H_2O (trace), DMF, 50 °C, 1 h, 70%.



Scheme 3. Reagents and conditions: (i) DTBMP, 4 Å MS, Tf_2O, CH_2Cl_2, −78 °C to 0 °C, 1 h, 65%; (ii) $Pd(OH)_2$, H_2, CH_3OH/CH_2Cl_2, RT, 95%.

Acknowledgements

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13. *A typical procedure for α-galactosylation*: A solution of the acceptor **15** (521 mg, 0.89 mmol) and DTBMP (456 mg, 2.22 mmol) in CH_2Cl_2 (10 mL) in the presence of 4 Å molecular sieves was stirred for 10 min at room temperature and cooled to −78 °C. To the resulting solution was added Tf_2O (187 μL, 1.11 mmol) and subsequently was added dropwise a solution of donor **3** (500 mg, 0.74 mmol) in CH_2Cl_2 (10 mL). After stirring at −78 °C for further 1 h, the reaction mixture was allowed to warm to 0 °C over 1 h, and quenched with saturated aqueous $NaHCO_3$ (1 mL).
14. Galactosyl fluoride **19** was prepared by fluorination of 2,3,5,6-tetra-*O*-benzyl-D-galactofuranose[7a] with diethylaminosulfur trifluoride (DAST).
15. Galactosy sulfoxide **21** was prepared by the oxidation of thiogalactoside **20** with mCPBA.
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19. *The procedure for the coupling of* **23** *and* **24**: A solution of the donor **23** (170 mg, 0.147 mmol), the acceptor **24** (174 mg, 0.191 mmol), and DTBMP (73 mg, 0.353 mmol) in CH_2Cl_2 (5 mL) in the presence of 4 Å molecular sieves was stirred for 10 min at room temperature and cooled down to −40 °C. Then, to this solution was added dropwise a diluted solution of Tf_2O (30 μL, 0.176 mmol) in CH_2Cl_2 (1 mL) at −40 °C. After being stirred at −40 °C for further 30 min, the reaction mixture was allowed to warm up to 0 °C over 1 h, and quenched with saturated aqueous $NaHCO_3$ (1 mL).
20. *Compound* **30**: Colorless oil; R_f = 0.48 (MeOH); $[\alpha]_D^{20}$ + 3.0 (c 0.8, MeOH); ^{1}H NMR (400 MHz, MeOD) δ 3.26–3.28 (m, 3H), 3.35 (s, 3H), 3.51–3.57 (m, 5H), 3.58–3.64 (m, 5H), 3.65–3.69 (m, 3H), 3.70–3.76 (m, 4H), 3.79 (dd, *J* = 9.6, 3.2 Hz, 1H), 4.14-4.20 (m, 2H), 4.97 (d, *J* = 4.4 Hz, 1H), 5.02 (d, *J* = 4.8 Hz, 1H), 5.04 (d, *J* = 1.2 Hz, 1H), 5.21 (d, *J* = 1.2 Hz, 1H);^{13}C NMR (100 MHz, CD_3OD) δ 49.9, 55.6, 62.9, 64.7, 64.8, 67.8, 68.7, 69.1, 69.3, 72.1, 72.6, 72.8, 73.0, 74.4, 74.86, 74.93, 75.1, 78.7, 78.8, 81.7, 81.9, 83.1, 83.2, 99.9, 101.1, 104.6, 104.8; HRMS: $[M+Na]^+$ Calcd for $C_{25}H_{44}O_{21}Na$: 703.2273. Found: 703.2266.
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