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The first synthesis of tetraglucosyl glucitol having phytoalexin-elicitor activity in rice cells based on a sequential glycosylation strategy

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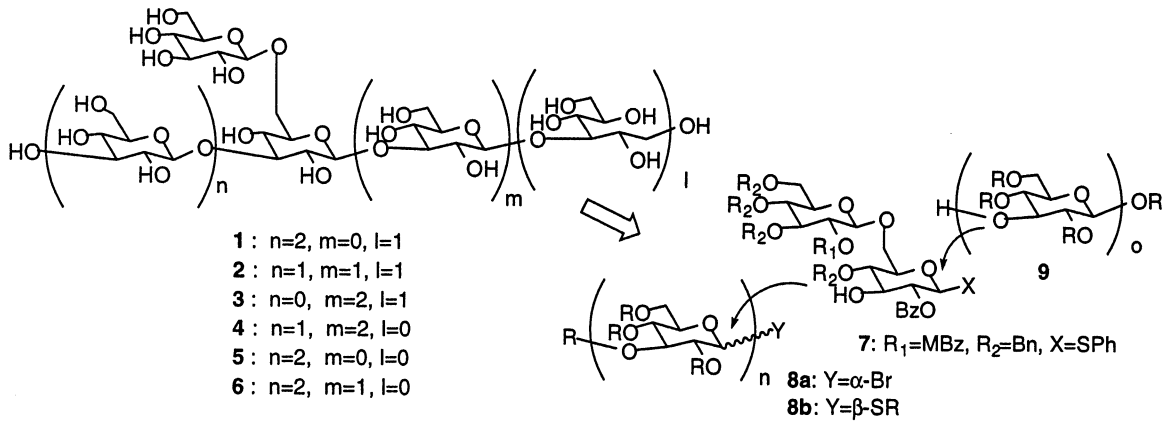
Abstract—We describe a highly convergent approach for the synthesis of the tetraglucosyl glucitol **1** and its derivatives **4**, **5** and **6** which exhibit phytoalexin-elicitor activity in rice cells based on sequential glycosylation.

The production of phytoalexin is one of various defense responses in higher plants. β-Glucan and some of its hydrosates composed of β-(1,6) and β-(1,3) glucose are known to demonstrate the phytoalexin-elicitor activity. For example, hepta-β-D-glucopyranosyl-D-glucitol containing a β-(1,6) pentasaccharide backbone is a well-known elicitor in soybean.[1] Because of the difficulty of the purification and the structural determination of these oligosaccharides, the chemical synthesis of the β-glucan elicitor and related compounds has contributed to the study of their structure–activity relationships.[2]

Recently, Yamaguchi and co-workers have purified and characterized the elicitor-active tetraglucosyl glucitols **1**, **2**, and **3** after partial acid/enzymatic hydrolysis of the cell walls of the rice blast disease fungus *Pyricularia oryzae* (*Magnaporthe grisea*) (Scheme 1). These oligosaccharides contain a β-(1,3) tetrasaccharide backbone with a β-(1,6) branched glucose at different glucose units. The position of a β-(1,6) branched glucose might influence the elicitor activity because the elicitor activity of **1** is much stronger than that of **2** and **3**.[3]



Scheme 1. The structures and synthetic strategy of phytoalexin elicitors in rice cells.

Keywords: β-glucan; phytoalexin elicitor; branched oligosaccharide; sequential glycosylation; thioglyciside.

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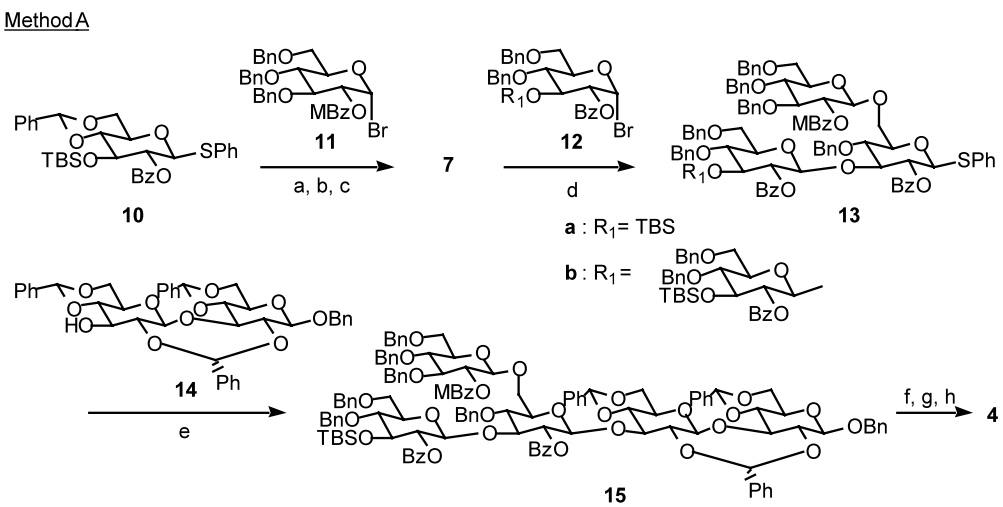
Synthetic studies of the soybean elicitor having a β-(1,6) backbone have been reported by many research groups.[2a,4,9] We have already developed one-pot sequential glycosylation[5] to synthesize the soybean elicitor.[6] Elongation of the β-(1,6) backbone could be achieved by glycosylation of a reactive primary alcohol at 6 position. However, the synthesis of β-(1,3) linked backbone should require repeat glycosylation of less reactive secondary alcohol at 3 position.[7] Herein we wish to report the total synthesis of rice elicitor **1** and the related oligosaccharide **4**, **5** and **6** having β-(1,3) backbone based on sequential glycosylation.

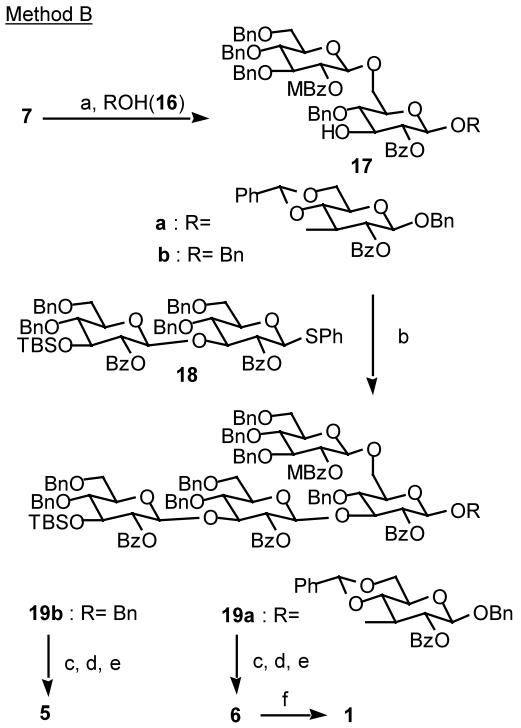
Our synthetic strategy for the phytoalexin elicitor **1** and derivatives **4**, **5** and **6** is illustrated in Scheme 1. 3-Hydroxy thioglycoside **7** having a branched glucose was designed as a key intermediate, which can be connected with various oligosaccharide units at the C1 and C3 hydroxyl groups independently by sequential glycosylation. A phenylthio group was chosen as a leaving group for **7** because it is one of the most powerful leaving groups. Furthermore, it is stable to the conditions of activation for several other leaving groups such as glycosyl bromide.[5a] It is possible to elongate the β-(1,3) glucan chain in two ways using the key intermediate **7**. Method A: chemoselective glycosylation of intermediate **7** with glycosyl bromide **8a**, followed by activation of the resulting thioglycoside to couple glycosyl acceptor **9**. Method B: site-selective glycosylation of acceptor **9** with thioglycoside **7** followed by glycosylation of the resultant alcohol in **7** with thioglycoside **8b**.[8] For the success of the Method A, it is necessary that the glycosyl donor prepared by glycosylation of **7** would have the high reactivity enough to form the β-(1,3) linkage. On the other hand, in Method B the oligosaccharide resulting from the first glycosylation would be used as the glycosyl acceptor for the second glycosylation. However, the acceptor for the first glycosylation should be more reactive than **7**.

The synthesis of intermediate **7** is shown in Scheme 2. Reductive cleavage of acetal **10**[9] (90%), followed by glycosylation at the 6 position with glycosyl bromide **11**[10] (84%) and deprotection of the silyl ether provided the key intermediate **7** in 79% yield. We first examined Method A to synthesize **1** and **4**. Selective activation of glycosyl bromides **12a**[10] and **12b**[10] in the presence of thioglycoside **7** was achieved using silver triflate to afford branched saccharide **13a** and **13b** in 68 and 43% yields. In the next glycosylation a triacetal disaccharide **14**[11] was chosen as an acceptor because using the cyclic acetal group we could utilize a highly reactive hydroxyl group at the C3 position. Coupling of thioglycoside **13a** and acceptor **14** was accomplished in the presence of NIS/TfOH to provide pentasaccharide **15** in 83% yield as a single product. In order to synthesize elicitor **1**, glycosylation of monosaccharide **16a**[12] (see Scheme 3) with tetrasaccharide donor **13b** was examined. However, the coupling reaction of the two units **13b** and **16a** did not proceed in the presence of NIS/TfOH. Thin layer chromatography (TLC) analysis showed the thioglycoside **13b** decomposed under the conditions. This result indicates that the tetrasaccharide donor **13b** would not be reactive enough to glycosylate the acceptor **16a**. Deprotection of the silyl ether in **15** and hydrolysis of the esters, followed by hydrogenolysis of the benzyl ethers and benzylidene acetals afforded the fully deprotected pentasaccharide **4** in 38% overall yield. Analysis of the ^{13}C NMR spectra of **4** indicated that the four glycosidic linkages except for that in the reduced end were formed with β configuration.[13]

We next applied Method B to the synthesis of rice elicitors **1** and **6**, as shown in Scheme 3. We envisaged that the reactivity of a benzyl alcohol and the C3-OH in a monoglucose unit is higher than that of the secondary hydroxyl in **7**, thus allowing for a site-selective glycosylation. Coupling of thioglycoside **7** and acceptor **16a**[12] or benzyl alcohol **16b** was achieved in the presence of NIS/TfOH to afford the corresponding oligosaccha-



Scheme 2. *Reagents and conditions*: (a) $BH_3{\cdot}NMe_3$, $AlCl_3$, CH_2Cl_2, Et_2O, 0°C, 1 h, 90%; (b) **11**, AgOTf, toluene, CH_2Cl_2, 4 Å MS, –40°C, 84%; (c) 40% aq. HF, CH_3CN, 12 h, 79%; (d) **12**, AgOTf, toluene, CH_2Cl_2, 4 Å MS, –40°C, 10 min, 68% (for **13a**), 43% (for **13b**); (e) **7**, NIS, TfOH, CH_2Cl_2, 4 Å MS, –20°C, 35 min, 83%; (f) 40% aq. HF, CH_3CN, 12 h; (g) NaOMe, MeOH, 4 h; (h) $Pd(OH)_2$, MeOH, H_2O, H_2 (1 atm), 6 h, 37% (three steps).



Scheme 3. *Reagents and conditions*: (a) NIS, TfOH, CH_2Cl_2, 4 Å MS, 0°C, 69% (for **17a**), 87% (for **17b**); (b) NIS, TfOH, CH_2Cl_2, 4 Å MS, 0°C, 10 min, 76% (for **19a**), 87% (for **19b**); (c) 40% aq. HF, CH_3CN, 12 h; (d) NaOMe, MeOH, 4 h; (e) $Pd(OH)_2$, MeOH, H_2O, H_2, 6 h, 38% (for **6**), 48% (for **5**) (three steps); (f) $NaBH_4$, MeOH–H_2O, 84%.

rides **17a** and **17b** in 69 and 87% yields. It should be noted that self-condensation of **7** was not observed in the reaction. Sequential glycosylation with disaccharide donor **18**[14] carried out in the presence of NIS/TfOH provided penta- or tetrasaccharide **19a** and **19b** in 76 and 87% yields, respectively. The deprotection of **19a** and **19b** was accomplished by removal of the ester groups, followed by hydrogenolysis of the benzyl ether to afford the fully deprotected penta- and tetrasaccharides **6** and **5** in 38 and 48% yields. Reduction of pentasaccharide **6** was achieved by treatment with sodium borohydride in water to afford the reported glucitol **1** in 84% yield. The analytical data (^1H NMR, MS, HPLC) of the synthetic glucitol **1**[15] were identical with those of the isolated material.

In conclusion, we have described the first synthesis of the tetraglucosyl glucitol **1** and derivatives **4**, **5** and **6** having phytoalexin-elicitor activity for rice based on a sequential glycosylation protocol. Our key intermediate **7** allows for easy access to β-(1,3) linked oligosaccharides **4**, **5** and **6** with a β-(1,6) branched glucose in two ways. The phytoalexin-elicitor activity of these oligosaccharides is currently being explored.

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11. The glycosyl acceptor **14** was prepared by the hydrolysis (NaOMe, MeOH) of benzyl 2,4,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-laminaribioside followed by the 2,2′:4,6:4′,6′-tri-*O*-benzylidenation ($PhCH(OMe)_2$, CSA, DMF), see: Wang, L.-X.; Sakairi, N.; Kuzuhara, H. *J. Carbohydr. Chem.* **1991**, *10*, 349–361. For a related benzylidanation procedure, see: Sakairi, N.; Okazaki, Y.; Furukawa, J.; Kuzuhara, H.; Nishi, N.; Tokura, S. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 679–683.
12. The glycosyl acceptor **16a** was prepared by the glycosylation of BnOH (NIS, TfOH, CH_2Cl_2, 4 Å MS) with phenylthio glucoside **10**, followed by the removal of the silyl ether (HF·Py, Py).
13. Selected physical data **4**: ^{13}C NMR (67.8 MHz, D_2O): $\delta = 94.7$ (α anomer of the reduced end), 98.4 (β anomer of the reduced end), 105.2–105.5 (other β anomer). Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307–3330.
14. The glycosyl donor **18** was prepared by the coupling of glycosyl bromide **12a** and phenylthio 2-*O*-benzoyl-4,6-dibenzyl-β-D-glucoside, which was prepared from the 6-*O*-benzylation (BnBr, TBAI, then NaH, 0°C, 1 h) followed by the removal of the silyl ether (40% aq. HF, CH_3CN) as donor and acceptor, respectively (AgOTf, CH_2Cl_2, PhMe, 4 Å MS).
15. **1**: $[\alpha]_D^{27} = -17.5$ ($c = 0.21$ in H_2O); ^1H NMR (800 MHz, D_2O): $\delta = 3.27$ (dd, $J = 9.22$, 8.14 Hz, 1H), 3.31 (dd, $J = 8.1$, 9.3 Hz, 1H), 3.34 (dd, $J = 9.5$, 9.5 Hz, 1H), 3.36 (dd, $J = 9.5$, 9.5 Hz, 1H), 3.40–3.49 (m, 7H), 3.50 (dd, $J = 8.2$, 9.0 Hz, 1H), 3.51 (dd, $J = 9.5$, 9.5 Hz, 1H), 3.55 (dd, $J = 8.3$, 9.0 Hz, 1H), 3.60–3.70 (m, 7H), 3.73 (dd, $J = 8.4$, 8.9 Hz, 1H), 3.74 (dd, $J = 9.1$, 9.6 Hz, 1H), 3.80 (dd, $J = 2.7$, 12.0 Hz, 1H), 3.83–3.88 (m, 5H), 3.97 (dt, $J = 3.5$, 6.4 Hz, 1H), 4.00 (dd, $J = 1.5$, 6.6 Hz, 1H), 4.18 (dd, $J = 2.1$, 11.4 Hz, 1H), 4.44 (d, $J = 7.9$ Hz, 1H), 4.64 (d, $J = 8.0$ Hz, 1H), 4.70 (d, 7.9 Hz, 1H), 4.75 (d, 8.0 Hz, 1H); ^{13}C NMR (99.6 MHz, D_2O): $\delta = 61.0$, 61.1, 62.2, 62.9, 68.4×2, 68.6, 69.7, 69.9×2, 70.3, 71.1, 73.0, 73.4×2, 73.5, 73.8, 74.6, 75.9×2, 76.2×2, 76.3, 79.1, 84.2, 84.6, 102.7×2, 103.1, 103.2; IR (solid): $\nu = 3364.9$, 1616.2, 1456.2, 1080.9 cm^{-1}; HRMS (ESI-TOF): calcd for $[C_{30}H_{34}O_{26}+Na^+]$ 853.2796, found 853.2796.