Convergent and Convenient Total Synthesis of Phytoalexin-Elicitor Active Heptasaccharide by One-Pot Sequential Glycosylation

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Convergent and convenient total synthesis of branched hepta-β-glucoside **1** having phytoalexin-elicitor activity was efficiently accomplished by way of two one-pot sequential glycosylation reactions. Trisaccharide **8** was synthesized in high yield by TfOH-catalyzed one-pot glycosylation using three component monosaccharides and subsequent selective deprotection of a 6′-*O*-TBDPS group. The second one-pot glycosylation of trisaccharide **8** with the three monosaccharides smoothly proceeded to afford heptaglucoside **11** stereoselectively in 48% total yield based on monosaccharide **3**. The targeted compound **1** was obtained in high yield after the removal of the protecting groups.

P. Albersheim et al. reported in 1984 that the elicitor-active hexa-β-D-glucopyranosyl-D-glucitol, isolated from the mycelial walls of *Phytophthora megasperma f. sp. Glycinea*, induces antibiotic phytoalexin accumulation in soybeans.[1] Since then, chemical synthesis of phytoalexin elicitor related β-glucans have drawn much attention because of their complex branched structures and biological activities.[2–4]

Recently, several one-pot sequential glycosylation reactions[4,5] for convenient synthesis of linear trisaccharides were reported from our laboratory[6,7] by utilizing orthogonal properties[8] of donor and acceptor glycosides: that is, the combination of glycosyl fluorides (or glycosyl phenylcarbonates) and thioglycosides. Such one-pot procedures certainly reduced the number of laborious and time-consuming purification processes of intermediate saccharides. Therefore, it is important to show its extended usefulness by applying the above-mentioned methods to synthesis of complex branched oligosaccharides besides previously reported linear ones. In this communication, we would like to report convergent and convenient total synthesis of methyl heptaglucoside **1** by one-pot sequential glycosylation.

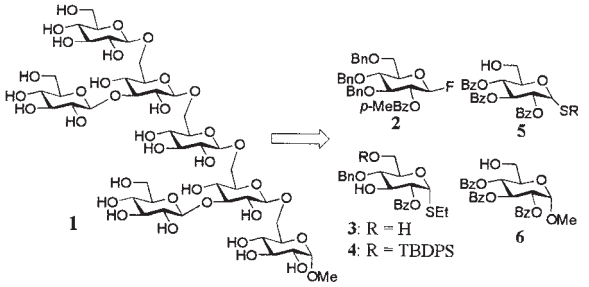
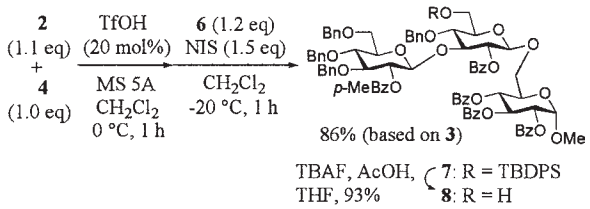


Figure 1. The structure of the hepta-β-D-glucoside **1** and component saccharides employed.

Two one-pot glycosylation reactions were involved in synthetic strategy for hepta-β-glucoside **1** (Scheme 1, 3). According to our previously reported procedure,[7] it was considered that methyl triglucoside **8** should rapidly be constructed by TfOH-catalyzed one-pot glycosylation using three component monosaccharides, **2**, **4**, and **6**. Next, three independent glycosylation reactions, the armed-disarmed glycosylation using a pair of reactivity-tuned thioglucosides[9] (**3** and **5**) as well as orthogonal glycosylation[8] using the combination of glucosyl fluoride **2** and thioglucoside **3** were employed in one-pot for the formation of fully protected heptasaccharide **11**. The stereochemistry of glycosylation reactions was supposed to be controlled by the assist of neighboring effect of 2-*O*-benzoyl protecting group.



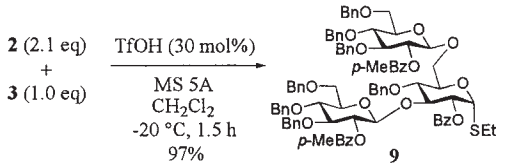
Scheme 1. One-pot synthesis of trisaccharide unit **8**.

Glucosyl fluoride **2** having 2-*O*-*para*-methylbenzoyl group (*p*-MeBz) was prepared easily by treating the corresponding 1-*O*-hydroxyl sugar[10] with diethylaminosulfurtrifluoride (DAST)[11] in CH_2Cl_2. α-Ethylthio glucosides **3** and **4** corresponding to 3, 6-branching positions were synthesized from ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-α-D-glucopyranoside[12] by standard protecting group manipulations.

In the first place, synthesis of trisaccharide unit **8** was carried out according to our previously reported one-pot procedure:[7] that is, TfOH-catalyzed glycosylation[13] of thioglucoside **4** having a free hydroxyl group at C-3 with glucosyl fluoride **2** to form the corresponding disaccharide, which in turn was followed by glycosylation of methyl glucoside **6** using NIS-TfOH promoter system[14] to give the corresponding silylated trisaccharide **7** in high yield (86% based on **4**). The desired trisaccharide unit **8** was obtained in 93% yield after selective deprotection of the *tert*-butyldiphenylsilyl (TBDPS) group on treatment with tetra-*n*-butylammonium fluoride (TBAF) in the presence of acetic acid.

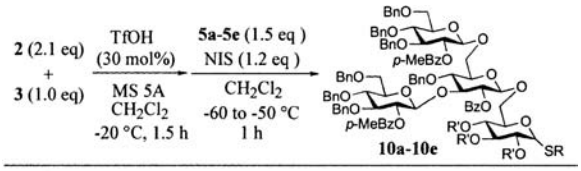
The second one-pot glycosylation of ''4-units'' was studied in detail. In the first step, the double glycosylation of diol **3** having thioglycosidic linkage with 2 molar amount of **2** was attempted in the presence of 30 mol% of TfOH and molecular sieves 5A (MS 5A), and terminal branched trisaccharide **9** was afforded directly in excellent yield (Scheme 2). Next, the armed-disarmed glycosylation with thus formed trisaccharide **9** was examined using several disarmed thioglycoside acceptors by the promotion of NIS-TfOH at low temperature (-60 to $-50\,^{\circ}C$). The desired tetrasaccharide **10a**, however, was not obtained when β-phenylthio glycoside **5a** was used as an acceptor since self-coupling of **5a** exclusively took place, and trisaccharide **9** was recovered. Tetraglucoside **10b** was also obtained in poor yield even though less activated β-glycoside **5b** having *p*-ClBz group

was used. On the other hand, it was found that tetrasaccharide **10c** was obtained in 51% yield by only changing the anomeric group from β-phenylthio to α-ethylthio. It should be noted that the α-ethylthio glycoside **5c** might have been stabilized by the anomeric effect,[15] and that it has lower reactivity than the corresponding β-phenylthio glycoside **5a**. After screening several protective groups, the desired tetraglucoside **10e** was synthesized in high yield (total 76% yield based on **3**) when *p*-CF_3Bz protected α-ethylthio glucoside **5e** was used (Entry 5).



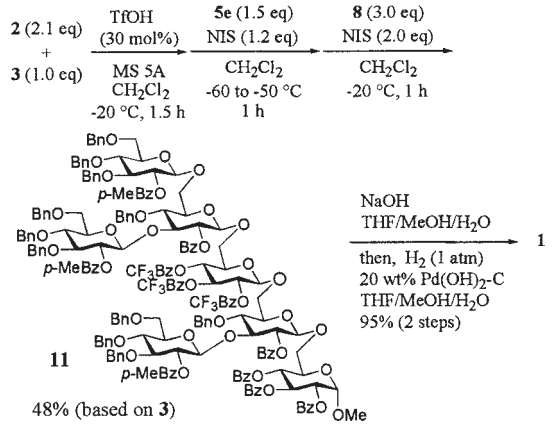
Scheme 2. Double glycosylation of diol **3** with **2**.

Table 1. The second one-pot glycosylation of four units



Entry	Thioglycoside	Yield /%
1	**5a**: R = Ph (β), R' = Bz	Not Detected
2	**5b**: R = Ph (β), R' = *p*-ClBz	30
3	**5c**: R = Et (α), R' = Bz	51
4	**5d**: R = Et (α), R' = *p*-ClBz	71
5	**5e**: R = Et (α), R' = *p*-CF_3Bz	76

Based on the above results, one-pot heptasaccharide synthesis using four building blocks was attempted as shown in Scheme 3. First, TfOH-catalyzed double glycosylation of **3** with **2**, followed by the armed-disarmed coupling with *p*-CF_3Bz-protected **5e** afforded tetraglucoside **10e** as a major product, which was confirmed by TLC monitoring. Next, the glycosylation of the above mentioned trisaccharide unit **8** with **10e** was tried by successively adding NIS. As a result, four glycosidic linkages were formed sequentially in one-pot manners and fully protected heptaglucoside **11** was obtained stereoselectively in 48% yield (based on **3**). Finally, the protected **11** was converted to the final product **1** in 95% yield by saponification of Bz groups and subsequent hydrogenolysis.[16]



Scheme 3. One-pot synthesis of heptasaccharide **11**.

It is noted that convergent and convenient total synthesis of methyl hepta-β-glucoside **1** having phytoalexin-elicitor activity was accomplished by one-pot sequential glycosylation reactions. Fully protected heptasaccharide **11** was rapidly assembled in only three steps from the component monosaccharides by two one-pot reactions. It is also noted that the significant reactivity difference was observed between α-ethylthio- and β-phenylthio-glucosides, which indicated that reactivity tuning of thioglycoside donors is controlled by their anomeric configurations.

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References and Notes

1 J. K. Sharp, B. Valent, and P. Albersheim, *J. Biol. Chem.*, **259**, 11312 (1984); J. K. Sharp, M. McNeil, and P. Albersheim, *J. Biol. Chem.*, **259**, 11321 (1984).
2 W. Birberg, P. Fügedi, P. J. Garegg, and Å. Pilotti, *J. Carbohydr. Chem.*, **8**, 47 (1989); N. Hong, Y. Nakahara, and T. Ogawa, *Proc. Jpn. Acad.*, **69**, 55 (1993); J. P. Lorentzen, B. Helpap, and O. Lockhoff, *Angew. Chem., Int. Ed. Engl.*, **30**, 1681 (1991); S. Aldington and S. C. Fry, *Adv. Bot. Res.*, **19**, 1 (1993); C. M. Timmers, A. Gijsbert, G. A. van der Marel, H. Jacques, and J. H. van Boom, *Chem. Eur. J.*, **1**, 161 (1995); K. C. Nicolaou, N. Watanabe, J. Li, J. Pastor, and N. Winssinger, *Angew. Chem., Int. Ed.*, **37**, 1559 (1998); W. Wang and F. Kong, *J. Org. Chem.*, **64**, 5091 (1999); R. Geurtsen, F. Côté, M. G. Hahn, and G.-J. Boons, *J. Org. Chem.*, **64**, 7828 (1999); O. J. Plante, E. R. Palmacci, and P. H. Seeberger, *Science*, **291**, 1523 (2001).
3 R. Verduyn, M. Douwes, P. A. M. van der Klein, E. M. Mösinger, G. A. van der Marel, and J. H. van Boom, *Tetrahedron*, **49**, 7301 (1993).
4 One-pot syntheses in this field; H. Yamada, T. Harada, and T. Takahashi, *J. Am. Chem. Soc.*, **116**, 7919 (1994); H. Yamada, H. Takimoto, T. Ikeda, H. Tsukamoto, T. Harada, and T. Takahashi, *Synlett*, **2001**, 1751.
5 Review: K. M. Koeller and C.-H. Wong, *Chem. Rev.*, **100**, 4465 (2000).
6 K. Takeuchi, T. Tamura, and T. Mukaiyama, *Chem. Lett.*, **2000**, 124.
7 H. Jona, K. Takeuchi, and T. Mukaiyama, *Chem. Lett.*, **2000**, 1278.
8 O. Kanie, Y. Ito, and T. Ogawa, *J. Am. Chem. Soc.*, **116**, 12073 (1994).
9 G. H. Veeneman and J. H. van Boom, *Tetrahedron Lett.*, **31**, 275 (1990); Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov, and C.-H. Wong, *J. Am. Chem. Soc.*, **121**, 734 (1999).
10 K. Takeuchi, T. Tamura, and T. Mukaiyama, *Chem. Lett.*, **2000**, 122.
11 Wm. Rosenbrook, Jr., D. A. Riley, and P. A. Lartey, *Tetrahedron Lett.*, **26**, 3 (1985); G. H. Posner and S. R. Haines, *Tetrahedron Lett.*, **26**, 5 (1985).
12 K. Takeo, K. Maki, Y. Wada, and S. Kitamura, *Carbohydr. Res.*, **245**, 81 (1993).
13 T. Mukaiyama, H. Jona, and K. Takeuchi, *Chem. Lett.*, **2000**, 696.
14 G. H. Veeneman, S. H. van Leeuwen, and J. H. van Boom, *Tetrahedron Lett.*, **31**, 1331 (1990); P. Konradsson, U. E. Udodong, and B. Fraser-Reid, *Tetrahedron Lett.*, **31**, 4313 (1990).
15 R. Geurtsen, D. S. Holmes, and G.-J. Boons, *J. Org. Chem.*, **62**, 8145 (1997); E. Juaristi and G. Cuevas, *Tetrahedron*, **48**, 5019 (1992).
16 Spectroscopic data for compound **1** are in fully agreement with those reported by J. H. van Boom et al.[3] Selected ^{1}H NMR (600 MHz, D_2O, 285 K, δ = TMS); δ 4.34 (1H, d, J = 8.4 Hz), 4.37 (3H, t, J = 8.4 Hz), 4.58 (1H, d, J = 7.8 Hz), 4.59 (1H, d, J = 7.8 Hz), 4.63 (1H, d, J = 3.6 Hz) (anomeric positions); Selected ^{13}C NMR (125 MHz, D_2O, δ = TMS); δ 99.01, 102.19, 102.41, 102.45, 102.52, 102.62, 102.65 (anomeric positions); ESI-HRMS: $[M+NH_4]^+$ calculated for $C_{43}H_{78}NO_{36}$, 1184.4304; found 1184.4308; $[\alpha]^{20}_D = -3.98^\circ$ (*c* 1.0, H_2O); FT-IR (KBr): 764, 1033, 3410 cm^{-1}.