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Parallel synthesis of multi-branched oligosaccharides related to elicitor active pentasaccharide in rice cell based on orthogonal deprotection and glycosylation strategy

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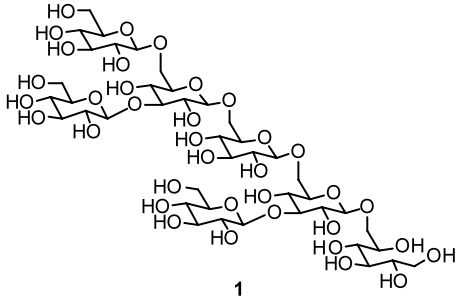
Abstract—We describe a parallel and efficient synthesis of multi-branched oligosaccharides **3a**–**g** based upon the structure of the phytoalexin elicitor active branched pentasaccharide **2**. One-pot sequential orthogonal deprotection of tetrasaccharide **5** with three different protecting groups provided each of seven glycosyl acceptors **4a**–**g**. Glycosylation of the acceptors **4a**–**g**, followed by deprotection provided branched oligosaccharides **3a**–**g**. All the reaction processes from scaffold **5** to **3a**–**g** except for final hydrogenolysis were achieved utilizing an automated synthesizer in a parallel fashion. © 2003 Published by Elsevier Science Ltd.

Branched oligosaccharides play important roles in biological events that occur at cell surfaces.[1] For example, β-glucan hydrosates such as β-(1,6) glucan **1** with two β-(1,3) branching saccharides[2] and β-(1,3) glucan **2** with a β-(1,6) branching saccharide[3] have phytoalexin elicitor activity in soybean and rice, respectively (Fig. 1). The position of the branching glucoses is essential for their biological activity. We have already reported several syntheses[4,5] of related compounds by solid-phase synthesis and one-pot glycosylation in solution-phase, which are based on a sequential coupling strategy using several building blocks. The sequential coupling reactions are effective to form the β-(1,6) linked backbone. However, coupling at the C-3 hydroxy group is more difficult than coupling at the C-6 hydroxyl group due to their steric hindrance.

In order to elucidate structure–activity relationships of **2**, we designed multi-branched oligosaccharides **3a**–**g** varying in the number and position of their branching glucoses (Scheme 1). The synthesis of the branched oligosaccharides **3a**–**g** based on a sequential coupling strategy requires multiple β-(1,3) coupling reactions, which would not be effective for a library synthesis in terms of yields and efficiency. Therefore, we designed an alternative strategy for the synthesis of the branched



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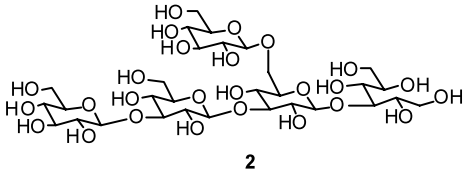

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Figure 1. Structure of phytoalexin elicitors **1** and **2** for soybean and rice.

Keywords: combinatorial chemistry; branched oligosaccharides; phytoalexin elicitor; orthogonal deprotection; automated synthesizer.
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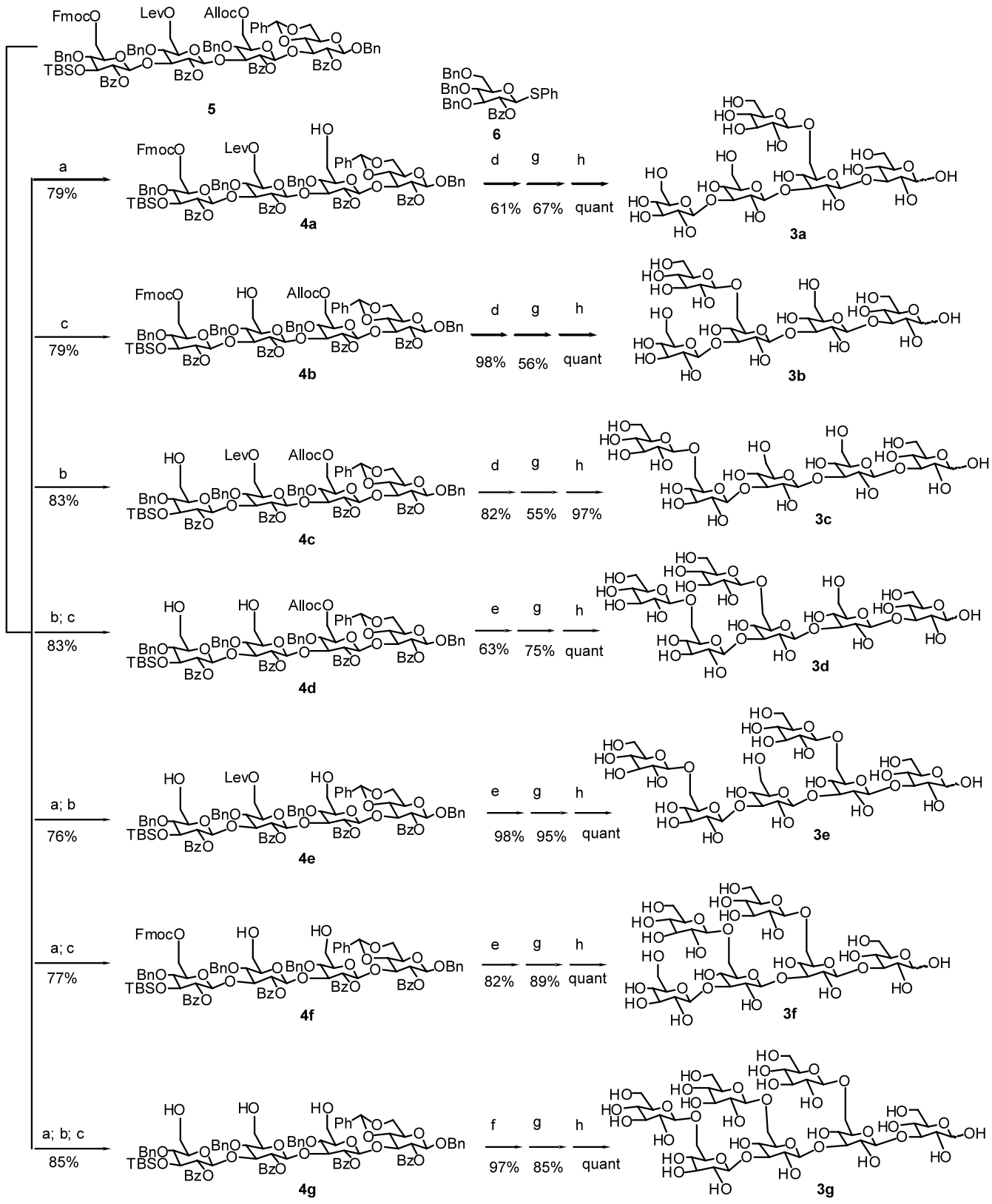
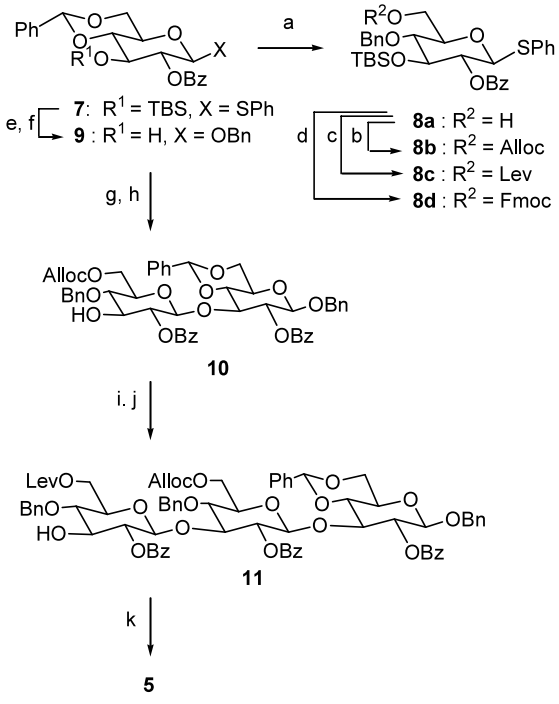


Scheme 1. *Reagents and conditions*: (a) $Pd(PPh_3)_4$, dimedone, THF, rt; (b) piperidine, THF, rt; (c) hydrazine acetate, THF, rt; (d) **6** (2.5 equiv.), DMTST, CH_2Cl_2, rt, then NEt_3, rt; (e) **6** (5.0 equiv.), DMTST, CH_2Cl_2, rt, then NEt_3, rt; (f) **6** (7.5 equiv.), DMTST, CH_2Cl_2, rt, then NEt_3, rt; (g) TBAF, THF, 50°C, then NaOMe, MeOH; (h) H_2, $Pd(OH)_2$, ethyl acetate–MeOH–H_2O.

oligosaccharides **3a**–**g** in a parallel fashion. Herein we report the effective synthesis of mono- to tri-branched oligosaccharides based on orthogonal deprotection and glycosylation strategy.

Our new strategy for the synthesis of β-(1,3) linked oligosaccharides **3a**–**g** having tri-, di-, and mono-β-(1,6) branched glucoses involves preparation of seven acceptors **4a**–**g** with mono- to tri-hydroxyl groups from a scaffold **5** by orthogonal deprotection,[6] followed by glycosylation of all hydroxy groups. All branching in **3a**–**g** can be achieved by β-(1,6) glycosylation reaction. In order to demonstrate the applicability of the solution-phase methodology to a library synthesis, the processes for the synthesis of **3a**–**g** from the key intermediate **5** would be achieved utilizing an automated synthesizer in a parallel fashion. For the selective deprotection, we selected three orthogonally removable protecting groups; allyloxycarbonyl (Alloc),[7] 9-fluorenylmethoxycarbonyl (Fmoc),[6d,8] and levulinoyl (Lev)[9] which can be removed by $Pd(PPh_3)_4$ with dimedone, piperidine, and hydrazine acetate, respectively.

The preparation of the building blocks for the synthesis of scaffold **5** is described in Scheme 2. Reductive cleavage of the benzylidene acetal of thioglycoside **7**[8b] provided the primary alcohol **8a**. Protection of the resulting alcohol with the appropriate protecting reagents gave the three glycosyl donors **8b**–**d** each having a different protecting group at the 6 position. The synthesis of **5** is as follows. Glycosylation of benzyl alcohol with thioglycoside **7** in the presence of NIS/TfOH, followed by removal of the TBS group provided glycosyl acceptor **9**. Acceptor **9** was coupled with donor **8b**, followed by deprotection of the TBS group to give disaccharide **10** in 50% yield after recrystallization from hexane–ether. Coupling of disaccharide **10** with donor **8c**, followed by removal of the TBS group afforded trisaccharide **11** in 61% yield. Finally, glycosylation of trisaccharide **11** with donor **8d** gave the key intermediate **5**[10] in 90% yield.



Scheme 2. *Reagents and conditions*: (a) $BH_3{\cdot}NMe_3$, $AlCl_3$, CH_2Cl_2, Et_2O, 90%; (b) AllocCl, Py, CH_2Cl_2, 82%; (c) EDCI, LevOH, CH_2Cl_2, 87%; (d) FmocCl, Py, CH_2Cl_2, 44% (recryst. from methanol); (e) BnOH, NIS, TfOH, CH_2Cl_2, MS4A; (f) HF·Py, Py, 81%, two steps; (g) **8b**, NIS, TfOH, CH_2Cl_2, MS4A; (h) HF·Py, Py, 50%, two steps (recryst. from hexane–ether); (i) **8c**, NIS, TfOH, CH_2Cl_2, MS4A; (j) HF·Py, Py, 61%, two steps; (k) **8d**, NIS, TfOH, CH_2Cl_2, MS4A, 90%.

The orthogonal deprotection of three protecting group (Alloc, Fmoc, or Lev) of **5** was examined (Scheme 1). Treatment of **5** with each of the three deprotection reagents $Pd(PPh_3)_4$ (25 mol%) and dimedone (2.0 equiv.),[11] piperidine (10 equiv.) in THF, and hydrazine acetate (50 equiv.) in THF provided the corresponding monools **4a**–**c** in good yield. The sequential addition of two deprotection reagents; piperidine–hydrazine, Pd reagents–piperidine, and Pd reagents–hydrazine acetate afforded diols **4d**–**f** in good yields. Deprotection of all protecting groups was achieved by sequential addition of the three deprotection reagents to yield triol **4g** in 85% yield. The sequential deprotection reactions proceeded smoothly at room temperature. It should be noted that each of the acceptors **4a**–**g** can be prepared from scaffold **5** by these synthetic protocols. The sequential deprotection reaction is adaptable to an automated synthesizer (L-COS™)[12,13] with computer controlled stirring, reaction temperature, and rate of addition of reagents. Purification was achieved utilizing automated parallel column chromatography (Combi Flash™).[14] Structure determination of acceptors **4a**–**g** was achieved by analysis of ^1H and ^{13}C NMR and mass spectra.

The synthesis of the branched oligosaccharides **3a**–**g** from **4a**–**g** is illustrated in Scheme 1. Complete glycosylation of the glycosyl acceptors **4a**–**g** with thioglycoside **6** in the presence of dimethyl(methylthio)sulfonium triflate (DMTST) provided the corresponding penta- to hepta-saccharides in high yield (61–98%). Treatment of the reaction mixtures with triethylamine for neutralization resulted in removal of the Fmoc group. Sequential deprotection of the TBS group and the acyl protecting groups (55–95%), followed by hydrogenolysis of the benzyl ethers and the benzylidene acetal afforded the deprotected oligosaccharides **3a**–**g** in good yield (97% quant). Analysis of the ^{13}C NMR spectra of **3a**–**g**[15,16] indicated that the all glycosidic linkages except for that in the reduced end were formed with the β-configuration.[17] All reaction processes from **5** to **3a**–**g** except for the final hydrogenolysis were achieved utilizing an automated synthesizer in a parallel fashion.

In summary we have demonstrated an efficient synthesis of multi-branched oligosaccharides **3a**–**g** by orthogonal deprotection and glycosylation strategy. This methodology is effective for the synthesis of branched oligosaccharides having a common backbone with various branching saccharides at different positions. Multi-

ple deprotection reactions and glycosylation of resulting hydroxyl groups are attractive protocols to achieve utilizing an automated synthesizer. The phytoalexin elicitor activity of these oligosaccharides is currently being explored along with the application of the one-pot deprotection method to other oligosaccharide libraries.

Acknowledgements

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10. Spectral data of **5**: ^{1}H NMR (270 MHz, $CDCl_3$): δ −0.41 (s, 3H), −0.14 (s, 3H), 0.72 (s, 9H), 2.00 (s, 3H), 2.18 (t, 2H, J=6.6 Hz), 2.39 (t, 2H, J=6.6 Hz), 3.29–3.71 (m, 13H), 3.88 (dd, 1H, J=6.9, 6.9 Hz), 4.04–4.59 (m, 20H), 4.72–4.93 (m, 6H), 5.02 (dd, 1H, J=7.9, 8.3 Hz), 5.19 (dd, 1H, J=10.6, 10.6 Hz), 5.24 (br-d, 1H, J=10.2 Hz), 5.34 (br-d, 1H, J=17.5 Hz), 5.51 (s, 1H), 5.92 (ddt, 1H, J=10.2, 17.5, 5.9 Hz), 7.04–7.61 (m, 45H, Ar), 7.72 (d, 2H, J=8.6 Hz), 7.77 (d, 2H, J=9.2 Hz), 7.93 (d, 2H, J=7.3 Hz), 7.98 (d, 2H, J=7.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ −4.6, −4.1, 17.7, 25.6, 27.6, 29.7, 37.7, 46.7, 66.3, 66.5, 68.5, 70.1, 70.2, 71.7, 72.8, 73.1, 73.2, 73.7, 74.0, 74.2, 74.6, 74.8, 75.0, 75.4, 77.2, 77.3, 78.7, 78.9, 79.0, 79.3, 97.7, 98.3, 99.4, 99.9, 102.0, 119.0, 120.0, 125.2×2, 126.6, 127.7×2, 127.8, 127.9, 128.0, 128.1, 128.3×2, 128.4, 128.5, 128.8, 128.9, 129.2, 129.3, 129.6×2, 129.7, 129.9, 130.2, 131.6, 133.2, 133.7, 136.7, 137.3, 137.5, 137.9, 138.2, 141.3, 143.3, 143.4, 154.8, 154.9, 164.3, 164.5, 164.7, 165.5, 172.3; IR (KBr) 2998, 2996, 2892, 2800, 1720, 1255, 1985, 769 cm^{-1}. Anal. calcd for $C_{117}H_{120}O_{31}Si$: C, 68.54; H, 5.90. Found: C, 65.55; H, 6.01%.
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12. Experimental procedure for the sequential selective deprotection of **5** utilizing an automated synthesizer (L-COS™): Seven reaction vessels 1–7 were placed on the L-COS™. A solution of **5** (35 mg, 0.017 mmol) in THF (0.5 mL) was added to each of the reaction vessels 1–7 at room temperature. For removal of the Alloc groups, two solutions of dimedone (5 mg, 36.0 μmol) in THF (0.2 mL) and $Pd(PPh_3)_4$ (5 mg, 4.3 μmol) in THF (0.2 mL) were added to each of the reaction vessels 1–4 at room temperature. The reaction mixtures were stirred at the same temperature for 15 min. For removal of the Fmoc group, a solution of piperidine (20 μL, 0.20 mmol) in THF (0.2 mL) was added to each of the reaction vessels 2, 3, 5, and 6 at room temperature. The reaction mixtures were stirred at the same temperature for 15 min. For removal of the Lev group, a solution of hydrazine (29 μL, 0.93 mmol)–acetic acid (73 μL, 1.28 mmol) in THF (0.2 mL) was added to each of the reaction vessels 3, 4, 6, and 7 at the same temperature. After stirring at the same temperature for 30 min, the reaction mixtures were auto-

matically subjected to flash chromatography in parallel fashion, eluting with hexane: ethyl acetate (1: 4) to give the seven tetrasaccharides **4a** (26.4 mg, 0.013 mmol, 79% yield), **4b** (26.3 mg, 0.014 mmol, 79% yield), **4c** (25.8 mg, 0.014 mmol, 83% yield), **4d** (24.4 mg, 0.014 mmol, 83% yield), **4e** (22.7 mg, 0.013 mmol, 76% yield) **4f** (24.4 mg, 0.013 mmol, 77% yield), **4g** (24.0 mg, 0.015 mmol, 85% yield), respectively.

13. L-COS™ is purchased from Moritex Corporation, Japan.
14. Combi Flash™ automated column chromatograph with ten parallel columns is purchased from Isco, Incorporation.
15. Compound **3c**: $[\alpha]_D^{24} = -12.3$ (*c* 0.96, H_2O); Selected ^{13}C NMR (67.8 MHz, D_2O, 30°C): δ 94.7, 98.3, 105.2–105.5; HRMS (ESI-TOF) calcd for $C_{30}H_{52}O_{26}Na$ ($M+Na^+$): 851.2645, found: 851.2675. Compound **3d**: $[\alpha]_D^{23} = -10.3$ (*c* 0.88, H_2O); Selected ^{13}C NMR (67.8 MHz, D_2O, 30°C): δ 94.6, 98.3, 105.2–105.5; HRMS (ESI-TOF) calcd for $C_{36}H_{67}O_{31}$ ($M+H^+$): 1008.3619, found 1008.3597. Compound **3e**: $[\alpha]_D^{23} = -15.1$ (*c* 0.91, H_2O); Selected ^{13}C NMR (67.8 MHz, D_2O, 30°C): δ 94.7, 98.4, 105.2–105.5; HRMS (ESI-TOF) calcd for $C_{36}H_{67}O_{31}$ ($M+H^+$): 1008.3619, found 1008.3654. Compounds **3f**: $[\alpha]_D^{20} = -14.7$ (*c* 0.74, H_2O); Selected ^{13}C NMR (67.8 MHz, D_2O, 30°C): δ 94.7, 98.4, 105.2–105.5; HRMS (ESI-TOF) calcd for $C_{36}H_{67}O_{31}$ ($M+H^+$): 1008.3619, found 1008.3635. Compounds **3g**: $[\alpha]_D^{20} = -13.4$ (*c* 1.0, H_2O); Selected ^{13}C NMR (67.8 MHz, D_2O, 30°C): δ 94.7, 98.4, 105.2–105.5; HRMS (ESI-TOF) calcd for $C_{42}H_{73}O_{36}$ ($M+H^+$): 1170.4147, found 1170.4194.
16. Analytical data (^1H and ^{13}C NMR, and MS) of pentaglycosides **3a** and **3b** were identical with those of our previous reported authentic samples, see Ref. 5a.
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