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Synthesis, (1→3)-β-D-glucanase-binding ability and phytoalexin-elicitor activity of (*R*)-2,3-epoxypropyl (1→3)-β-D-pentaglucoside

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Abstract—The (1→3)-β-D-pentaglucoside was synthesized as its (*R*)-2,3-epoxypropyl glycoside via 2 + 3 strategy. The disaccharide donor **8** was obtained by 3-selective coupling of **2** with **4**, followed by deallylation, and trichloroacetimidation. Meanwhile, the trisaccharide acceptor **12** was prepared by coupling of **10** with **4**, followed by deacetylation. Condensation of **8** with **12**, followed by epoxidation, and deprotection, gave the target pentaoside. The results of these bioassays demonstrated that the (1→3)-β-D-glucanase was obviously inactivated by **15** with $k_{app} = 3.79 \times 10^{-4} min^{-1}$. At the same time, we found that the **15** was more active as compared to the laminaripentaose in eliciting phytoalexin accumulation in tobacco cotyledon tissue, and it could be kept longer time than laminaripentaose, which indicated it is much more stable than laminaripentaose.

Higher plants have the ability to initiate various defence reactions such as the production of phytoalexins, antimicrobial proteins, reactive oxygen species and reinforcement of the cell wall when they are infected by pathogens such as fungi, bacteria and viruses. If these reactions occur in a timely manner, the infection will not proceed further. However, if the defence reactions occur too late or are suppressed, the infection process will proceed successfully.[1] Thus, it is critically important for plant to detect infecting pathogens effectively and deliver such information intracellularly/intercellularly to activate their defence machinery.

It is believed that the detection of pathogens is mediated by chemical substances secreted/generated by the pathogens. Various types of such compounds (elicitor molecules) including oligosaccharides, (glyco)proteins, (glyco)peptides and lipids, have been shown to induce defence responses in plant cells and their involvement in the detection of (potential) pathogens in plant has been discussed.[2–4] Oligosaccharides derived from fungal and plant cell wall polysaccharides are one class of well characterized elicitors that, in some cases, can induce defence responses at a very low concentration, for example, nanomoles. At the same time, the elicitor activity of oligosaccharides is dependent on the structural properties of oligosaccharide molecules, such as the number of saccharide units, the length of the aglycone chain and the molecule configuration.[5]

However, the elicitor-active oligosaccharides can be hydrolyzed by endo- and exohydrolases from higher plants, and give elicitor-inactive oligosaccharide fragments.[6] Therefore, improving the stability of the elicitor-active oligosaccharides is the key to developing the biological pesticide of oligosaccharides.

The use of epoxyalkyl glycosides as active-site-directed inhibitors has been invaluable in delineating the mechanism of action for a variety of hydrolases, for example, β-D-glucan endo- and exohydrolases.[7,8] The epoxyalkyl glycoside moiety targets the inhibitor to the substrate-binding site and if the length of the alkyl chain is correct, the epoxide group is brought into the vicinity of the catalytic amino acids. Protonation of the epoxide oxygen opens the epoxide ring and results in the formation of

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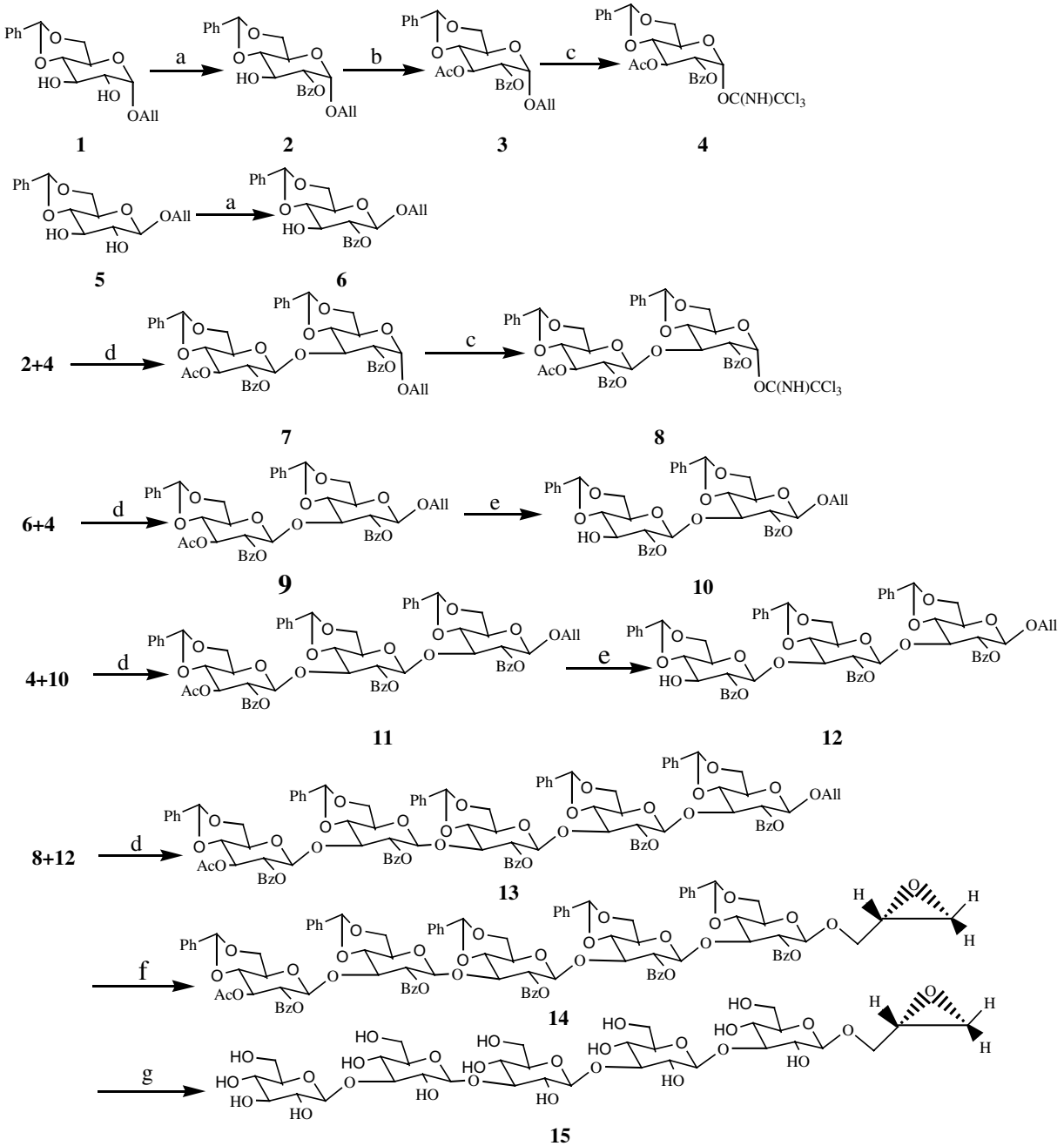
a stable ester linkage between the inhibitor and the catalytic nucleophile. It has been well demonstrated[9] that the chain length of aglycone in the mechanism-based epoxide-bearing inhibitors have a significant effect on their activity.

Structure activity studies with laminarin, laminarin oligomers, high molecular weight β-1,3–1,6 glucans from fungal cell walls and the β-1,6–1,3 heptaglucan showed that the elicitor effects observed in tobacco with β-glucans are specific to linear β-1,3 linkages, with laminaripentaose being the smallest elicitor-active structure.[10] With the aim of improving the stability of elicitor-active laminaripentaose and studying the protein-binding ability to (1→3)-β-D-glucanase, as well as phytoalexin-elicitor activity in tobacco cotyledon tissue, herein we present a very facile and convergent synthesis of (*R*)-2,3-epoxypropyl (1→3)-β-D-pentaglucoside, with benzylidenated glucose derivatives as the key intermediates. It is an analogue of laminaripentaose, where the (*R*)-2,3-epoxypropyl has been introduced at the reducing ends of the aglycones. There are also some reports of synthetic studies on phytoalexin-elicitor oligosaccharides.[11,12]

Retrosynthetic analysis revealed that the best way to synthesize the target **15** was to first prepare the β-(1→3)-linked disaccharide and trisaccharide fragments,



Scheme 1. Conditions and reagents: (a) PhCOCl, CH_2Cl_2, 0 °C; (b) Ac_2O, pyridine, rt; (c) $PdCl_2$, CH_3OH, 40 °C/CCl_3CN, CH_2Cl_2, K_2CO_3, rt; (d) TMSOTf, CH_2Cl_2, 0 °C; (e) HBF_4, THF, rt, 4 h; (f) *m*-CPBA, CH_2Cl_2, rt; (g) 90% HOAc–H_2O, reflux, 3 h; CH_3ONa–CH_3OH, rt.

then connect them at the C-3 of the glucose residue of the trisaccharide backbone. The previous studies[13] indicated that in (1→3)-glucosylation the glycosyl bond originally present in either donor or acceptor controlled the stereoselectivity of the forthcoming bond, that is, the newly formed glycosidic linkage had the opposite anomeric configuration of that of either the donor or acceptor. In addition, some reports[14,15] revealed that with 4, 6-*O*-benzylidenated glucose derivatives as either donor or acceptor, β-linked oligosaccharides are readily obtained. Thus, in the present research, the benzylidenated glucose derivatives were applied as the key intermediates. As outlined in Scheme 1, **1** and **5**, obtained from 4,6-*O*-benzylidenation of allyl α,β-D-glucopyranoside, were monobenzoylated to afford **2** and **6** in high yield (75%). Acetylation of **2** with acetic anhydride in pyridine furnished **3** in high yield (95%). Deallylation of **3** with $PdCl_2$ in methanol,[16] followed by trichloroacetimidation with Cl_3CCN, gave **4**. The **4** was coupled with the acceptor **2** or **6** in the presence of TMSOTf to afford a unique disaccharide **7** or **9** in 53% yield. Deallylation of **7**, followed by trichloroacetimidation, again gave **8**. Deacetylation of **9** with HBF_4 gave the disaccharide acceptor **10** in satisfactory yield (80%). The **4** was again coupled with the acceptor **10** in the presence of TMSOTf to give **11**. Compound **11** was deacetylated with HBF_4 to gave **12**. Then coupling of **8** with the trisaccharide acceptor **12** gave **13** as the sole product in 82% yield. The reaction of **13** with *m*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane at room temperature gave the corresponding **14**. Finally, deprotection of **14** in turn in 90% HOAc–H_2O and sodium methoxide–methanol solution gave the target **15**. The analytical data (^{1}H NMR, ^{13}C NMR, ESI-MS, anal. found) of the synthetic **15**[17] was identical with those of the isolated material. Epoxidation of **13** introduced new chiral centres at C-2 of the aglycone. In the case, the major isomer was isolated and purified by column chromatography on silica gel, and the ^{1}H NMR spectrum indicated that C-2 of the aglycone was *R* configuration (*R*:*S* = 5:1). It indicated that the anomeric proton of this major compound **15** (C-2 *R*) resonates at higher field (δ = 3.09) than the minor diastereoisomer (C-2 *S*, δ = 3.24).

Seeing from the above-mentioned synthetic route, the method was simple and practical, and it should be possible to apply the process to large-scale synthesis of **15**.

The results of these bioassays, shown in Table 1, demonstrated that the (1→3)-β-D-glucanase was obviously inactivated by **15** with $k_{app} = 3.79 \times 10^{-4}$ min^{-1}. At the same time, we found that the **15** was more active as compared to the laminaripentaose (purchased from Fluka Chemical Company) in eliciting phytoalexin accumulation in tobacco cotyledon tissue, and it could be kept longer time than laminaripentaose, which indicated it is much more stable than laminaripentaose. The biological activity of **15** fell at late time points, which was possible because the **15**–hydrolase complex was unstable, so the **15** would been hydrolyzed gradually by hydrolases.

Table 1. Binding and phytoalexin-elicitor activity of (*R*)-2,3-epoxypropyl (1→3)-β-D-pentaglucoside (**15**)

Substance	k_{app} (min^{-1})[a]	Biological activity (EC_{50}, μmol/L)[b]		
		22 h	44 h	66 h
Laminaripentaose		90	500	900
15	3.79×10^{-4}	50	80	160

[a] Value was means of at least three independent determinations.
[b] Determined by measuring the absorbance (*A*) at 285 nm.

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17. Analytical data for **15**: syrup; $[\alpha]_D$ +86.3 (*c* 1.1, $CHCl_3$); ^{1}H NMR (D_2O, 400 MHz) δ 5.24 (1H, d, $J_{1,2}$ 3.5 Hz, α-H-1^I), 4.62–4.54 (5H, m, H-1$^{V,\ IV,\ III,\ II}$), 3.92–3.85 (5H, m), 3.75–3.33 (19H, m), 3.25–3.19 (6H, m), 3.09 (1H, m, –C*H*(O)CH_2), 2.76–2.68 (2H, m, –CH(O)C*H*$_2$); ^{13}C NMR (D_2O, 75 MHz) δ 103.8, 103.65 (C-1^1, 1^5), 103.4 (3C) (C-1^2, 1^3, 1^4), 85.4 (C-3^1), 85.2 (C-3^2), 85.0 (2C) (C-3^3, 3^4), 6.8 (C-5^5), 76.45, 76.4 (5C) (C-3^5, 5^1, 5^2, 5^3, 5^4), 74.3 (C-2^5), 74.1 (3C) (C-2^2, 2^3, 2^4), 73.6 (C-2^1), 70.4 (C-4^5), 69.0, 68.9 (4C) (C-4^1, 4^2, 4^3, 4^4), 61.55 (5C) (C-6^1, 6^2, 6^3, 6^4, 6^5), 50.4 (–*C*H(O)CH_2), 44.2, 44.1 (–CH(O)*C*H$_2$); ESI-MS *m*/*z* (%) 907 [M+Na]$^+$; Anal. Calcd for $C_{33}H_{56}O_{27}$: C, 44.80; H, 6.33. Found: C, 44.67; H, 6.40.