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The Synthesis of 3-*O*-(β-D-Glucopyranosyl)- and 3-*O*-(β-Laminaribiosyl)-isofagomines, Potent Inhibitors of a 1,3-β-D-Glucan *endo*-Hydrolase

James M. Macdonald,[A] *Maria Hrmova*,[B] *Geoffrey B. Fincher*,[B] *and Robert V. Stick*[A,C]

[A] Chemistry, School of Biomedical and Chemical Sciences M313, University of Western Australia, Crawley WA 6009, Australia.

[B] Faculty of Sciences, School of Agriculture and Wine, University of Adelaide, Glen Osmond SA 5064, Australia.

[C] Author to whom correspondence should be addressed (e-mail: rvs@chem.uwa.edu.au).

The glycosylation of 4,6-*O*-benzylidene-*N*-benzyloxycarbonylisofagomine with a D-glucosyl and a laminaribiosyl trichloroacetimidate has given, after removal of protecting groups, 3-*O*-(β-D-glucopyranosyl)- and 3-*O*-(β-laminaribiosyl)-isofagomines. Also included are similar glycosylations of a related tetrahydrooxazine. 3-*O*-(β-D-Glucopyranosyl)- and 3-*O*-(β-laminaribiosyl)-isofagomines acted as potent inhibitors of a barley 1,3-β-D-glucan *endo*-hydrolase, with ID_{50} values of 7.8 and 3.1 μM, respectively.

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We have recently described the synthesis of 4-*O*-(β-D-glucopyranosyl)- and 4-*O*-(β-cellobiosyl)-isofagomine, compounds **1** and **2** (Diagram 1).[1] While both compounds were effective inhibitors of some *endo*-cellulases, compound **2** was strikingly so (K_i 5 nM) against Cel5A from *Bacillus agaradhaerens*.[2] It occurred to us that similar success could be obtained by glycosylating isofagomine **3** at the C3 hydroxyl group, so generating potential inhibitors for enzymes that hydrolyze 1,3-β-D-glucans. The target molecules were to be 3-*O*-(β-D-glucopyranosyl)- and 3-*O*-(β-laminaribiosyl)-isofagomine, **4** and **5**.

Our synthesis of compounds **1** and **2** profited from the glycosynthase-assisted glycosylation of the isofagomine carbamate **6**.[1] Although a related glycosynthase exists for the construction of the 1,3-β linkage required here,[3] the glycosyl donor is, of necessity, α-laminaribiosyl fluoride, limiting the synthesis to just compound **5**. In any case, we did try such a glycosynthase-assisted approach, but the carbamate **6** did not appear to act as an acceptor for α-laminaribiosyl fluoride (results not shown). Direct chemical synthesis seemed the only viable alternative; with this decision, we also included glycosylation of the oxazine **7** (Diagram 2), to form compounds **8** and **9**, but were aware that similar compounds in the cello-series were only moderate inhibitors of *endo*-cellulases.[1]

Towards compounds **4** and **5**, we converted the isofagomine carbamate **6**[1] into the alcohol **10** (Diagram 3). Subsequent glycosylation of this alcohol with the trichloroacetimidates **11** and **12** gave the glycosides **13** and **14**, respectively. Although the stereochemistry of the newly formed glycosidic linkages could not be confirmed at this stage (owing to the complexity of the NMR spectra brought

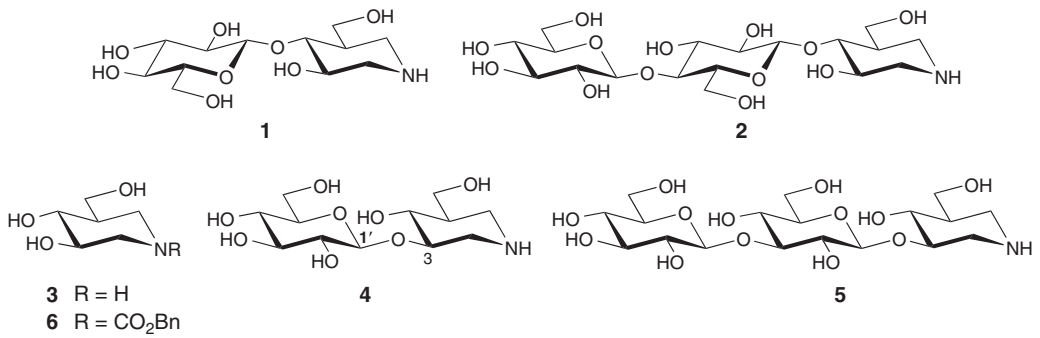

Diagram 1.

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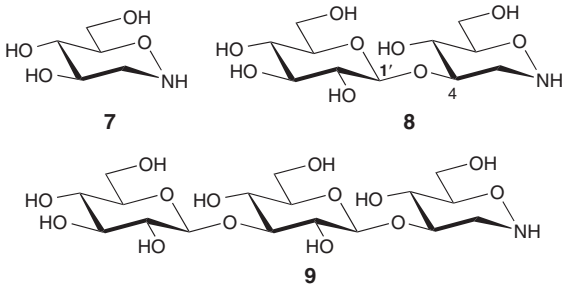
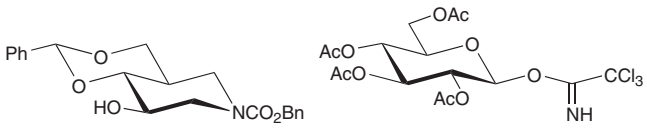


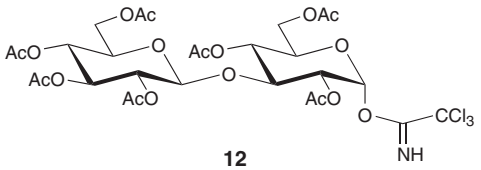
7 **8**

9

Diagram 2.



10 **11**



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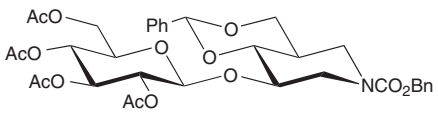

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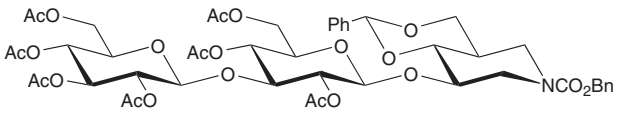

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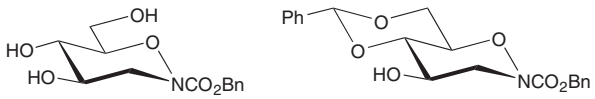
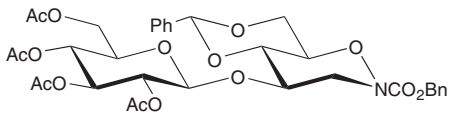
Diagram 3.



15 **16**



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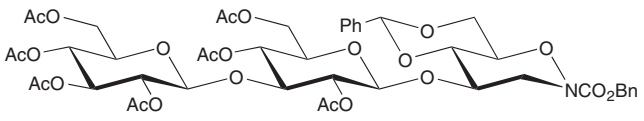

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Diagram 4.

about by the inherent carbamate[1]), a two-step deprotection protocol furnished the desired compounds **4** and **5**. The NMR spectra were now amenable to analysis, with H3 of **4**, a doublet of doublets of doublets, clearly downfield from all other protons directly bound to the isofagomine ring system, and $J_{1',2'}$ having a value of 8.0 Hz. As well, C3 for **4** and **5** resonated at about δ 80, a value some 10 ppm greater than the corresponding resonance in isofagomine itself.

Table 1. ID_{50} values for isofagomine and oxazine derivatives with a barley 1,3-β-D-glucan *endo*-hydrolase

Inhibitor[A]	ID_{50} [μM]
3	>20 mM
4	7.8
5	3.1
7	—[B]
8	678
9	197

[A] Using reduced laminarin as a substrate at the pH optimum (4.75) of the enzyme.[5]
[B] Not determined.

For compounds **8** and **9**, the carbamate **15**[1] was converted into the alcohol **16** (Diagram 4), and subsequent glycosylations as described above gave the new glycosides **17** and **18**. These molecules were not plagued by phenomena associated with restricted rotation (around the N–CO bond) and so direct evidence was available from the associated NMR spectra to establish the nature of the new linkages (1,4-β). Subsequent deprotection provided compounds **8** and **9**.

Finally, the glycosylated derivatives of isofagomine **3** and the tetrahydrooxazine **7** were tested as inhibitors of a barley 1,3-β-D-glucan *endo*-hydrolase (Table 1). While compounds **8** and **9** were ineffective, compounds **4** and **5** showed good inhibition of the hydrolase. In particular, the laminaribiosyl isofagomine **5** is a candidate molecule for generating a binary complex with the barley 1,3-β-D-glucan *endo*-hydrolase in future X-ray crystallographic investigations.[4]

Experimental

General experimental procedures have been given previously.[6]

*Methyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucoside*

Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[7] (5.60 g, 11.4 mmol), methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucoside[8] (3.38 g, 8.77 mmol), and crushed molecular sieves (4 Å, 8 g) in dry CH_2Cl_2 (30 mL) were stirred under Ar (2 h) and then cooled (−20°C). TMSOTf (60 μL) was added, and this mixture stirred (30 min), and then allowed to warm to room temperature and stirred for a further 30 min. Et_3N (1 mL) was added and the mixture was filtered through Celite, washing with EtOAc. The combined filtrate/washings were concentrated, and flash chromatography (EtOAc/petrol, 28 : 72 then 35 : 65) of the residue afforded a colourless solid. Recrystallization gave the title glycoside (5.05 g, 81%) as colourless micro-cubes, mp 184–186°C (EtOAc/petrol; lit.[9] 187–189°C), $[\alpha]_D$ +34.4° (lit.[9] +34.8°).

*2,4,6-Tri-*O*-acetyl-3-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucosyl Fluoride*

In a screw-capped plastic vial, octa-*O*-acetyl-α-laminaribiose (obtained from the previous glycoside;[9] 500 mg, 0.74 mmol) was treated with HF–pyridine (7 : 3, 1 mL), and the mixture kept for 48 h. The mixture was poured into EtOAc and carefully neutralized by washing with aqueous $NaHCO_3$. The organic layer was subjected to normal workup and flash chromatography (EtOAc/petrol, 2 : 3 then 1 : 1) to afford the *title fluoride* (280 mg, 60%) as a colourless solid. A small portion was recrystallized

to give fine needles, mp 196–198°C (EtOH), $[\alpha]_D$ +11.6° (Found: C 48.9, H 5.5. $C_{26}H_{35}FO_{17}$ requires C 48.9, H 5.5%). δ_H (300 MHz) 1.95, 1.96, 1.99, 2.03, 2.05, 2.07, 2.21 (21 H, 7 s, CH_3), 3.68 (1 H, ddd, *J* 2.3, 4.4, 9.8), 4.00–4.23 (5 H, m), 4.35 (1 H, dd, *J* 4.4, 12.5), 4.63 (d, $J_{1',2'}$ 8.1, H1′), 4.83–4.98 (2 H, m), 5.01–5.16 (3 H, m), 5.63 (dd, $J_{1,2}$ 2.7, $J_{1,F}$ 53, H1). δ_C (125.8 MHz) 20.26, 20.32, 20.50, 20.57, 20.67, 20.69 (7 C, CH_3), 61.29, 61.55 (C6, 6′), 66.65, 67.83 (2 C), 70.01 (1 C, d, $J_{C,F}$ 24), 72.83 (1 C), 75.56 (C3), 100.73 (C1′), 103.98 (d, $J_{1,F}$ 228, C1). *m*/*z* (FAB) 639.1931 $[(M+H)^{+\bullet}$ requires 639.1937].

3-O-*β*-*D*-*Glucopyranosyl*-*α*-*D*-*glucopyranosyl Fluoride (α-Laminaribiosyl Fluoride)*

The above per-acetylated fluoride (90 mg) in MeOH was treated with Na (5 mg). After 1 h, the mixture was neutralized with resin (Dowex-50W, H^+), then filtered, the resin washed with MeOH, and the combined filtrate and washings were concentrated to give the crude title fluoride. The purity of the product was checked by TLC and NMR spectroscopic analysis. δ_H (500 MHz, D_2O) 3.38 (1H, dd, *J* 8.3, 9.4), 3.43 (1H, t, $J \approx 9.4$), 3.47–3.57 (2 H, m), 3.64 (1 H, t, $J \approx 9.7$), 3.75 (1 H, dd, *J* 6.0, 10.3), 3.79–3.92 (4 H, m), 3.94 (1 H, dd, *J* 2.0, 12.4), 3.98 (1H, t, $J \approx 9.5$), 4.75 (d, $J_{1',2'}$ 7.9, H1′), 5.72 (dd, $J_{1,2}$ 2.7, $J_{1,F}$ 53, H1). δ_C (125.8 MHz, D_2O) 60.77, 61.34 (C6, 6′), 67.72, 70.29 (2 C), 71.37 (1 C, d, $J_{C,F}$ 25), 74.08 (1 C), 74.65 (1 C, d, $J_{C,F}$ 3.0), 76.24, 76.68 (2 C), 81.71 (C3), 103.42 (C1′), 108.05 (d, $J_{1,F}$ 223, C1). *m*/*z* (FAB) 345.1192 $[(M+H)^{+\bullet}$ requires 345.1197].

2,4,6-Tri-O-*acetyl-3*-O-*(tetra*-O-*acetyl-β-D-glucopyranosyl)-α-D-glucosyl Trichloroacetimidate* ***12***

Octa-*O*-acetyl-α-laminaribiose[9] (1.27 g, 1.87 mmol) in CH_2Cl_2 (5 mL) was treated with HBr in AcOH (30% w/v, 2.5 mL) overnight. This mixture was then diluted with ice–water, and normal workup (EtOAc) gave a solid (1.28 g). This solid, in acetone/H_2O (6 : 1, 30 mL), was treated with Ag_2CO_3 (560 mg, 2.0 mmol), and stirred (1 h) with the exclusion of light. The mixture was filtered (silica; washing with EtOAc) and the combined filtrate/washings were concentrated to give a solid. Recrystallization gave, presumably, the hemiacetal (740 mg, 62%) as fine needles, mp 210–213°C (EtOH; lit.[10] 232–234°C). A portion of these needles (520 mg, 0.81 mmol) in dry CH_2Cl_2 (15 mL) was treated with Cl_3CCN (200 μL, 2.0 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1 drop). After 1 h, the mixture was concentrated. Flash chromatography (EtOAc/petrol, 3 : 7 then 4 : 6) of the residue gave the *trichloroacetimidate* **12** [530 mg, 84% from the hemiacetal] as an oil, $[\alpha]_D$ +36.4° (Found: C 42.8, H 4.4, N 1.6. $C_{28}H_{36}Cl_3NO_{18}$ requires C 43.1, H 4.6, N 1.8%). δ_H (500 MHz) 1.97, 1.98, 2.00, 2.05, 2.07, 2.08, 2.10 (21 H, 7 s, CH_3), 3.73 (1 H, ddd, *J* 2.4, 4.6, 10.0), 4.07–4.21 (5 H, m), 4.35 (1 H, dd, *J* 4.6, 12.4), 4.66 (d, $J_{1',2'}$ 8.1, H1′), 4.90 (1 H, dd, *J* 8.1, 9.6), 5.03–5.18 (4 H, m), 6.45 (d, $J_{1,2}$ 3.7, H1), 8.70 (s, NH). δ_C (125.8 MHz) 20.33, 20.42, 20.51, 20.53, 20.61, 20.68 (7 C, CH_3), 61.54, 61.71 (C6, 6′), 67.18, 68.10, 70.21, 71.37, 71.67, 71.73, 72.86, 76.37 (8 C), 90.77 (CCl_3), 93.11 (C1), 100.93 (C1′), 160.57 (C=NH), 169.10, 169.29, 169.55, 170.33, 170.48, 170.64 (7 C, C=O).

*(1*R*,3*R*,6*R*,10*R*)-8-Benzyloxycarbonyl-3-phenyl-8-aza-2,4-dioxabicyclo[4.4.0]decan-10-ol* ***10***

The carbamate **6** (110 mg, 0.39 mmol), $PhCH(OEt)_2$ (102 μL, 0.54 mmol), and camphorsulfonic acid (CSA; 5 mg) in dry $CHCl_3$ (5 mL) were heated under reflux (1 h) and allowed to cool. Et_3N (0.5 mL) was added and then this mixture concentrated. Flash chromatography (EtOAc/petrol, 1 : 4 then 2 : 3) of the residue gave the *benzylidene acetal* **10** (120 mg, 88%) as a colourless foam, $[\alpha]_D$ +2.0° (Found: C 68.0, H 6.1, N 3.7. $C_{21}H_{23}NO_5$ requires C 68.3, H 6.3, N 3.8%). δ_H (300 MHz) 1.87–2.02 (m, H6), 2.34–2.52 (1 H, m), 2.61–2.78, (1 H, m), 2.86–2.98 (1 H, m), 3.45 (1 H, t, *J* 9.1), 3.57 (1 H, t, *J* 11.0), 3.62–3.77 (1 H, m), 3.90–4.20 (2 H, m), 4.30–4.55 (1 H, m), 5.09–5.15 (m, PhCH_2), 5.55 (s, H3), 7.28–7.50 (10 H, m, Ph). δ_C (75.5 MHz) 36.85 (C6), 43.30, 47.91 (C7,9), 67.56, 68.07 (C5, PhCH_2), 68.19 (C10), 84.84 (C1), 101.97 (C3), 126.17, 127.95, 128.15, 128.30, 128.5, 129.18, 136.24, 137.58 (12 C, Ph), 155.02 (C=O). *m*/*z* (FAB) 370.1691 $[(M+H)^{+\bullet}$ requires 370.1654].

*(1*R*,3*R*,6*R*,10*R*)-8-Benzyloxycarbonyl-3-phenyl-10-(tetra*-O-*acetyl-β-D-glucopyranosyl)oxy-8-aza-2,4-dioxabicyclo[4.4.0]decane* ***13***

Tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate **11**[11] (150 mg, 0.30 mmol), the alcohol **10** (90 mg, 0.23 mmol), and crushed molecular sieves (4 Å, 1 g) in dry CH_2Cl_2 (10 mL) were stirred under Ar (2.5 h) and then cooled (−30°C). TMSOTf (30 μL) was added and this mixture stirred (1 h). Et_3N (0.5 mL) was added, and the mixture was allowed to warm to room temperature and then filtered through Celite, washing with EtOAc. The combined filtrate/washings were concentrated, and flash chromatography (EtOAc/petrol, 3 : 7 then 9 : 11) of the residue gave the *glycoside* **13** (150 mg, 91%) as a colourless foam, $[\alpha]_D$ −6.2° (Found: C 60.5, H 6.3, N 1.8. $C_{35}H_{41}NO_{14}$ requires C 60.1, H 5.9, N 2.0%). δ_H (500 MHz) 1.98, 1.98, 1.99 (12 H, 3 s, CH_3), 2.04–2.09 (m, H6), 2.38–2.57 (1 H, m), 2.63–2.83 (1 H, m), 3.39–3.53(1 H, m), 3.60–4.00 (5 H, m), 4.03–4.28 (3 H, m), 4.64–4.72 (1 H, m), 4.87–5.17 (5 H, m), 5.60 (s, H3), 7.31–7.49 (10 H, m, Ph). δ_C (125.8 MHz) δ 20.22, 20.54, 20.58, 20.67 (4 C, CH_3), 37.56, 37.98 (C6), 43.10, 46.56 (C7, 9), 61.54 (C6′), 67.81, 68.08 (C4′, 5, PhCH_2), 71.57, 71.90, 72.78 (C2′, 3′, 5′, 10), 82.72 (C1), 100.88, 101.39 (C1′, 3), 125.93, 128.17, 128.33, 128.60, 128.76, 129.06, 136.08, 137.72 (12 C, Ph), 154.90 (NC=O), 169.29, 170.27, 170.68 (4 C, CH_3C=O). *m*/*z* (FAB) 700.2612 $[(M+H)^{+\bullet}$ requires 700.2605].

*(1*R*,3*R*,6*R*,10*R*)-8-Benzyloxycarbonyl-3-phenyl-10-[(tetra*-O-*acetyl-β-D-glucopyranosyl)-(1→3)*-O-*(tri*-O-*acetyl-β-D-glucopyranosyl)]-oxy-8-aza-2,4-dioxabicyclo[4.4.0]decane* ***14***

The trichloroacetimidate **12** (150 mg, 0.19 mmol) and the alcohol **10** (60 mg, 0.15 mmol) were treated as for **11** and **10** previously to give, after flash chromatography (EtOAc/petrol, 9 : 11 then 6 : 4), the *glycoside* **14** (110 mg, 75%) as an opaque film, $[\alpha]_D$ −17.4° (Found: C 57.1, H 6.0, N 1.3. $C_{47}H_{57}NO_{22}$ requires C 57.1, H 5.8, N 1.4%). δ_H (500 MHz) 1.95, 1.97, 1.97, 1.98, 2.04 (21 H, 5 s, CH_3), 2.08–2.18 (m, H6), 2.35–2.56 (1 H, m), 2.59–2.83 (1 H, m), 3.35–4.25 (12 H, m), 4.31–4.37 (1 H, m), 4.49–4.51 (2 H, m), 4.80–5.17 (7 H, m), 5.57 (s, H3), 7.28–7.49 (10 H, m, Ph). δ_C (125.8 MHz) 20.20, 20.34, 20.41, 20.43, 20.50, 20.60 (7 C, CH_3), 37.55, 37.91 (C6), 43.03, 46.49 (C7, 9), 61.56, 61.72 (C6′, 6″), 67.70, 67.92, 67.95 (4 C), 70.95, 71.58, 71.87, 72.84 (6 C), 78.75 (C3′), 82.50, 82.61 (C1), 100.76, 101.17 (C1′, 1″, 3), 125.83, 128.03, 128.25, 128.52, 128.90, 136.06, 137.72 (12 C, Ph), 154.84 (NC=O), 168.70, 168.96, 169.15, 169.24, 170.24, 170.34, 170.65 (7 C, CH_3C=O). *m*/*z* (FAB) 988.3436 $[(M+H)^{+\bullet}$ requires 988.3450].

*(3*R*,4*R*,5*R*)-3-(β-D-Glucopyranosyl)oxy-4-hydroxy-5-(hydroxymethyl)-piperidine [3*-O-*(β-D-Glucopyranosyl)isofagomine] 4*

The glycoside **13** (75 mg) was treated with aqueous AcOH (14 M, 3 mL) and heated to 70°C (3 h). The mixture was allowed to cool and was then concentrated. Flash chromatography (EtOAc/petrol, 6 : 4, then MeOH/EtOAc, 1 : 9) of the residue gave an oil (63 mg), presumably a diol. A mixture of this oil and NaOH (290 mg) in MeOH/H_2O (2 : 1, 4.5 mL) was heated under reflux (2 h) and then allowed to cool. The reaction mixture was concentrated, taken up in H_2O (2 mL), and brought to pH 5 by the addition of HCl (2 M). The mixture was applied to a column of cation-exchange resin (Dowex 50W-X2, H^+), washed with water, and then eluted with aqueous NH_3 (3 M). The eluate was concentrated, taken up in H_2O (1 mL), applied to an anion-exchange column (Sephadex-DEAE A-25), and eluted with H_2O. Freeze-drying of the eluate afforded the D-glucosyl isofagomine **4** (31 mg, 94%) as a colourless, amorphous solid, $[\alpha]_D$ −18.1° (H_2O). δ_H (600 MHz, D_2O) 1.70–1.72 (m, H5), 2.44–2.51 (2 H, m, H2, 6), 3.12 (dd, $J_{5,6}$ 3.4, $J_{6,6}$ 13.2, H6), 3.29 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.4, H2′), 3.29–3.30 (m, H2), 3.40 (dd, *J* 9.2, 9.8, H4′), 3.45–3.51 (m, H3′, 4, 5′), 3.66 (dd, $J_{5,H}$ 6.5, $J_{H,H}$ 11.5, 1H, CH_2O), 3.71 (ddd, *J* 5.0, 9.0, 10.7, H3), 3.73 (dd, $J_{5',6'}$ 6.0, $J_{6',6'}$ 12.4, H6′), 3.81 (dd, $J_{5,H}$ 3.4, 1H, CH_2O), 3.92 (dd, $J_{5',6'}$ 2.2, H6′), 4.55 (d, H1′). δ_C (150.9 MHz, D_2O) 44.98 (C5), 46.59 (C6), 47.60 (C2), 61.08 (CH_2O), 61.37 (C6′), 70.26 (C4′), 72.32 (C4), 73.57 (C2′),

76.29, 76.61 (C3′,5′), 80.33 (C3), 101.57 (C1′). *m/z* (FAB) 310.1512 [(M + H)$^{+\bullet}$ requires 310.1502].

*(3*R*,4*R*,5*R*)-3-[(β-*D*-Glucopyranosyl)-(1→3)*-O*-(β-*D*-glucopyranosyl)]oxy-4-hydroxy-5-(hydroxymethyl)piperidine [3-O-(β-Laminaribiosyl)isofagomine] **5***

The pseudo-trisaccharide **14** (90 mg) was treated as for **13** previously to give, after flash chromatography (EtOAc/petrol, 3 : 1, then MeOH/EtOAc, 1 : 19), first an oil (80 mg), presumably a diol, and then the laminaribiosyl isofagomine **5** (40 mg, 93%) as a colourless, amorphous solid, $[\alpha]_D$ −23.4° (H_2O). δ_H (500 MHz, D_2O) 1.67–1.71 (m, H5), 2.38–2.48 (2 H, m, H2, 6), 3.08 (dd, $J_{5,6}$ 4.3, $J_{6,6}$ 13.0, H6), 3.28 (dd, $J_{2,2}$ 5.0, $J_{2,3}$ 12.5, H2), 3.41 (1H, dd, *J* 9.1, 9.8), 3.36 (1H, dd, *J* 8.0, 9.4), 3.44–3.55 (6 H, m), 3.63–3.78 (5 H, m), 3.81 (1 H, dd, *J* 3.5, 11.5), 3.90–3.96 (2 H, m), 4.59, 4.75 (2 d, *J* 7.9, 8.0, H1′,1″). δ_C (125.8 MHz, D_2O) 45.35 (C5), 46.74 (C6), 47.84 (C2), 61.22, 61.34, 61.37 (C6′, 6″, CH_2O), 68.79 (C4′), 70.25 (C4″), 72.56 (C4), 73.38, 74.10 (C2′, 2″), 76.21, 76.67 (C3‴, 5′, 5″), 80.71 (C3), 84.84 (C3′), 101.30, 103.47 (C1′, 1″). *m/z* (FAB) 472.2042 [(M + H)$^{+\bullet}$ requires 472.2030].

*(1*R*,5*R*,6*S*,8*R*)-3-Benzyloxycarbonyl-8-phenyl-3-aza-2,7,9-trioxabicyclo[4.4.0]decan-5-ol **16***

The carbamate **15**[1] (580 mg, 2.0 mmol) was treated with $PhCH(OEt)_2$ (520 μL, 2.8 mmol) and CSA (20 mg) in dry $CHCl_3$ (20 mL) as for **6** previously to give, after flash chromatography (EtOAc/petrol, 1 : 4 then 2 : 3), the *benzylidene acetal* **16** (610 mg, 81%) as a colourless solid. A small portion was recrystallized to give colourless needles, mp 153–156°C (EtOAc/petrol), $[\alpha]_D$ +54.3° (Found: C 65.0, H 5.8, N 4.0. $C_{20}H_{21}NO_6$ requires C 64.7, H 5.7, N 3.8%). δ_H (500 MHz) 3.06 (br s, OH), 3.23 (dd, *J* 10.4, 13.5, H4), 3.65 (1 H, t, *J* 9.2), 3.71 (1 H, t, *J* 10.2), 3.80 (1 H, dd, *J* 4.8, 9.8), 3.82–3.89 (1 H, m), 4.36–4.44 (2 H, m), 5.18–5.24 (2 H, m), 5.57 (s, H8), 7.33–7.50 (10 H, m, Ph). δ_C (125.8 MHz) 50.63 (C4), 66.23 (1 C), 66.53 (C10), 68.19 ($PhCH_2$), 74.02 (1 C), 82.63 (C1), 102.10 (C8), 126.15, 128.11, 128.32, 128.42, 128.56, 129.38, 135.42, 136.61 (12 C, Ph), 155.06 (C=O). *m/z* (FAB) 372.1447 [(M + H)$^{+\bullet}$ requires 372.1447].

*(1*R*,5*R*,6*S*,8*R*)-3-Benzyloxycarbonyl-8-phenyl-5-(tetra-*O*-acetyl-β-*D*-glucopyranosyl)oxy-3-aza-2,7,9-trioxabicyclo[4.4.0]decane **17***

The trichloroacetimidate **11** (160 mg, 0.32 mmol) and the alcohol **16** (100 mg, 0.27 mmol) were treated as for **11** and **10** previously to give, after flash chromatography (EtOAc/petrol, 2 : 8 then 1 : 2), the *glycoside* **17** (150 mg, 82%) as a colourless foam, $[\alpha]_D$ +23.9° (Found: C 57.9, H 5.6, N 2.0. $C_{34}H_{39}NO_{15}$ requires C 58.2, H 5.6, N 2.0%). δ_H (500 MHz) 1.94, 1.97, 1.98, 1.99 (12 H, 4 s, CH_3), 3.22 (dd, $J_{4,4}$ 13.7, $J_{4,5}$ 10.4, H4), 3.49 (ddd, *J* 2.4, 4.0, 9.7, H5′), 3.74 (1 H, t, *J* 9.8), 3.79 (dt, *J* 3.9, *J* ≈ 8.7, H1), 3.86 (1 H, t, *J* 8.9), 3.92–3.99 (2 H, m), 4.10 (1 H, dd, *J* 4.0, 12.4), 4.36 (1 H, dd, *J* 4.0, 9.6), 4.40 (dd, $J_{4,5}$ 5.5, H4), 4.67 (d, $J_{1',2'}$ 8.0, H1′), 4.97 (dd, $J_{2',3'}$ 9.4, H2′), 5.07 (t, $J_{3',4'} \approx J_{4',5'}$ 9.6, H4′), 5.12 (t, H3′), 5.20 (s, $PhCH_2$), 5.60 (s, H8), 7.31–7.46 (m, 10 H, Ph). δ_C (125.8 MHz) 20.40, 20.46, 20.49, 20.58 (4 C, CH_3), 48.96 (C4), 61.42 (C6′), 66.53 (C10), 67.80 (C4′), 68.35 ($PhCH_2$), 71.19, 71.88, 72.68, 73.68, 74.48 (C2′, 3′, 5, 5′, 6), 80.62 (C1), 100.17, 101.64 (C1′, 8), 125.94, 128.14, 128.21, 128.51, 128.61, 129.25, 135.32, 136.70 (12 C, Ph), 155.02 (NC=O), 169.12, 169.20, 170.17, 170.54 (4 C, CH_3C=O). *m/z* (FAB) 702.2401 [(M + H)$^{+\bullet}$ requires 702.2398].

*(1*R*,5*R*,6*S*,8*R*)-3-Benzyloxycarbonyl-8-phenyl-5-[(tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1→3)-*O*-(tri-*O*-acetyl-β-*D*-glucopyranosyl)]oxy-3-aza-2,7,9-trioxabicyclo[4.4.0]decane **18***

The trichloroacetimidate **12** (240 mg, 0.31 mmol) and the alcohol **16** (90 mg, 0.24 mmol) were treated as for **11** and **10** previously to give, after flash chromatography (EtOAc/petrol, 4 : 6 then 6 : 4), the *glycoside* **18** (170 mg, 73%) as a colourless glass, $[\alpha]_D$ +3.2° (Found: C 55.5, H 5.6, N 1.6. $C_{46}H_{55}NO_{23}$ requires C 55.8, H 5.6, N 1.4%). δ_H (500 MHz) 1.96, 1.98, 1.98, 1.98, 1.99, 2.01, 2.04 (21 H, 7 s, CH_3), 3.21 (dd, $J_{4,4}$ 13.6, $J_{4,5}$ 10.1, H4), 3.44–3.50 (1 H, m), 3.64 (1 H, ddd, *J* 2.4, 4.2, 9.3), 3.70–3.94 (5 H, m), 3.98 (1 H, dd, *J* 2.4, 12.3), 4.02 (1 H, dd, *J* 2.4, 12.5), 4.06 (1 H, dd, *J* 4.1, 12.3), 4.31–4.40 (3 H, m), 4.53, 4.54 (2 d, *J* 8.1, 8.1, H1′,1″), 4.86, 4.89 (2 dd, *J* 8.1, 9.5, *J* 8.1, 9.6, H2′, 2″), 4.92, 5.04, 5.11 (3 t, *J* 9.7, 9.5, 9.4, H3″, 4′, 4″), 5.20 (s, $PhCH_2$), 5.59 (s, H8), 7.30–7.46 (10 H, m, Ph). δ_C (125.8 MHz) 20.25, 20.38, 20.47, 20.55, 20.63, 20.67 (7 C, CH_3), 48.96 (C4), 61.59, 61.69 (C6′, 6″), 66.52 (C10), 67.74, 67.94 (2 C), 68.35 ($PhCH_2$), 70.97, 71.63, 71.96, 72.60, 72.85, 73.53, 74.55 (9 C), 78.71 (C3′), 80.59 (C1), 100.20, 100.81, 101.57 (C1′, 1″, 8), 125.95, 128.11, 128.18, 128.53, 128.63, 129.21, 135.35, 136.77 (12 C, Ph), 155.08 (NC=O), 168.66, 168.99, 169.19, 169.27, 170.28, 170.38, 170.64 (7 C, CH_3C=O). *m/z* (FAB) 990.3271 [(M + H)$^{+\bullet}$ requires 990.3243].

*(4*R*,5*S*,6*R*)-4-(β-*D*-Glucopyranosyl)oxy-5-hydroxy-6-hydroxymethyl-3,4,5,6-tetrahydro-1,2(2*H*)-oxazine **8***

The glycoside **17** (110 mg) was treated as for **13** previously to give, after flash chromatography (EtOAc/petrol, 4 : 6, then MeOH/EtOAc 1 : 9), first an oil (75 mg), presumably a diol, and then the D-glucosyl oxazine **8** (31 mg, 64%) as a colourless, amorphous solid, $[\alpha]_D$ −29.3° (H_2O). δ_H (600 MHz, D_2O) 3.11 (dd, $J_{3,3}$ 13.2, $J_{3,4}$ 10.7, H3), 3.31 (dd, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.4, H2′), 3.42 ($J_{3',4'}$ 9.1, $J_{4',5'}$ 9.8, H4′), 3.47–3.53 (m, H3′, 5′), 3.61 (dd, $J_{3,4}$ 5.3, H3), 3.66 (t, $J_{4,5} \approx J_{5,6}$ 8.9, H5), 3.72 (dd, $J_{5',6'}$ 6.3, $J_{6',6'}$ 12.4, H6′), 3.75–3.80 (2 H, m, H6, CH_2O), 3.91–3.97 (2 H, m, H6′, CH_2O), 4.01 (ddd, H4), 4.59 (d, H1′). δ_C (150.9 MHz, D_2O) 48.89 (C3), 60.11 (CH_2O), 61.36 (C6′), 68.34 (C5), 70.23 (C4′), 73.48 (C2′), 76.18 (C3′), 76.64 (C5′), 77.17 (C4), 84.11 (C6), 101.26 (C1′). *m/z* (FAB) 312.1277 [(M + H)$^{+\bullet}$ requires 312.1295].

*(4*R*,5*S*,6*R*)-4-[(β-*D*-Glucopyranosyl)-(1→3)-*O*-(β-*D*-glucopyranosyl)]oxy-5-hydroxy-6-hydroxymethyl-3,4,5,6-tetrahydro-1,2(2*H*)-oxazine **9***

The pseudo-trisaccharide **18** (110 mg) was treated as for **13** previously to give, after flash chromatography (EtOAc/petrol, 8 : 2, then MeOH/EtOAc, 1 : 19), first an oil (90 mg), presumably a diol, and then the laminaribiosyl oxazine **9** (36 mg, 66%) as a colourless, amorphous solid, $[\alpha]_D$ –39.6° (H_2O). δ_H (500 MHz, D_2O) 2.98 (dd, $J_{3,3}$ 13.3, $J_{3,4}$ 10.6, H3), 3.36 (1 H, dd, *J* 7.9, 9.4), 3.42 (1 H, dd, *J* 8.9, 9.8), 3.44–3.64 (8 H, m), 3.70–3.78 (4 H, m), 3.90–3.97 (4 H, m), 4.62, 4.76 (2 d, *J* 7.9, 8.0, H1′, 1″). δ_C (125.8 MHz, D_2O) 50.85 (C3), 60.46 (CH_2O), 61.36, 61.37 (C6′, 6″), 68.81, 69.05 (C4′, 5), 70.24 (C4″), 73.36, 74.09 (C2′, 2″), 76.20, 76.26, 76.66 (C3″, 5′, 5″), 78.51 (C4), 84.12, 84.74 (C3′, 6), 100.99, 103.45 (C1′, 1″). *m/z* (FAB) 474.1859 [(M + H)$^{+\bullet}$ requires 474.1823].

Enzyme Inhibition

Enzyme Isolation and Purity

Barley 1,3-β-D-glucan *endo*-hydrolase isoenzyme GII was purified from a homogenate of 14-day-old seedlings as described.[5] The purity of the enzyme was assessed by SDS–PAGE, where a single protein band was detected at high protein loadings, and by NH_2-terminal amino acid sequence analysis.[12]

Determination of ID_{50} Values of the Isofagomine and Oxazine Derivatives

The ID_{50} (inhibitory dose at 50%) values were determined by incubating 0.5 pkat* of the purified enzyme at 37°C in 50 mM sodium acetate buffer (pH 4.75) containing 160 μg/mL BSA, and 0.1% (w/v)

* The unit picokatal (pkat) refers to one-trillionth of a katal (kat), where a katal is a unit of catalytic activity equal to one mole of product formed (or substrate consumed) per second, as of the amount of enzyme that catalyses the transformation of one mole of substrate per second.

reduced *Laminaria digitata* laminarin (Sigma Chemical Co., St Louis, MO, USA). The ID_{50} values were estimated using concentrations of inhibitors that were 0.2–3 times the ID_{50} values, at five concentrations, in duplicate. The residual enzyme activity was monitored reductometrically.[13,14] Substrate hydrolysis never exceeded 10% of the initial substrate concentrations. Standard deviation values for assays were within the range 6–9%. The ID_{50} values were calculated using non-linear regression analyses[15] and the GraFit program.[16] One unit of enzyme activity is defined as 1 μmol glucose equivalents released per min and one unit corresponds to 16.67 nkat.

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