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Diastereoselective synthesis of the benzoxazinone acetal glucoside *ent*-GDIMBOA: the first enantiomer of a natural acetal glucoside [1]

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Abstract

The synthesis of (2*S*)-2-β-L-glucopyranosyloxy-4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, the enantiomer of the natural acetal glucoside GDIMBOA from maize, has been achieved by the double diastereoselective L-glucosylation of racemic 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one with 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyl trichloroacetimidate in the presence of excess of boron trifluoride etherate as promoter as well as protecting and equilibrating agent.

Keywords: Acetal glucoside; Benzoxazinone; Glycosylation, stereoselective; Hydroxamic acid GDIMBOA, enantiomer of, synthesis of

1. Introduction

Several acetal glucosides of the 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton have been found to occur as allelo chemicals in different species of *Gramineae* [1,2], *Acanthaceae* [3,4], *Ranunculaceae* [5], and *Scrophulariaceae* [6]. The biological function of such acetal glucosides as possible endogeneous ligands in the plant cell is under investigation [7]. We have reported the isolation of the hydroxamic acids (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GDIBOA) from rye (*Secale cereale L.*) [8] and its 7-methoxy derivative (GDIMBOA) from maize (*Zea mays L.*) [9]. Their aglycon hemiacetals exhibit high bioactivity as plant resistance factors in maize, rye, and wheat against microbial diseases and insects [10], phytotoxins of root exudates from the weed quackgrass [11], and inhibitory agents towards prostate cancer cell lines [12].

The work of Tietze et al. on the synthesis of iridoid glycosides [13,14] showed the stereoselective glycosylation of configurationally labile cyclic hemiacetals, to form acetal glycosides, to be a very difficult undertaking in comparison to the glycosylation of phenols or alcohols with a definite configuration. Blepharin [(2*R*)-2-β-D-glucopyranosyloxy-2*H*-1,4-benzoxazin-3(4*H*)-one], a natural product found in *Blepharis edulis* Pers [15], was the first benzoxazinone glucoside synthesized with single 2-β-diastereoselectivity by an inverse Koenigs–Knorr tech-

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[1] Dedicated to Professor Peter Welzel on the occasion of his 60th birthday.

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nique using an aglycon carrying the bromine function [16]. We have described the synthesis of GDIBOA with single 2-β-diastereoselectivity [17], and, most recently, a general approach to both GDIBOA and GDIMBOA with double (2*R*)-2-β-diastereoselectivity [18], using a Schmidt trichloroacetimidate [19] as the glucosyl donor. The synthesis of enantiomers of natural products is a field of increasing interest because such compounds are used in structural studies and as probes for the elucidation of biological processes, a recent example being *ent*-enterobactin [20].

We describe here the synthesis of (2*S*)-2-β-L-glucopyranosyloxy-4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (*ent*-GDIMBOA) by the double diastereoselective L-glucosylation of racemic 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA).

2. Results and discussion

The glucosylation of such cyclic hemiacetals as DIMBOA offers the challenge to synthesize exclusively only one of the four configurational possibilities, (2*R*)-2-β-, (2*S*)-2-β-, (2*R*)-2-α-, (2*S*)-2-α-, that arise from the connection of two hemiacetals to form an acetal unit. Nevertheless, a total synthesis of a natural acetal glycoside with stereogenic centers in both the aglycon and the glycosidic unit can proceed with high diastereoselectivity, due to the contribution by both partners in the asymmetric induction, as shown for iridoid glycosides [14]. Benzoxazinone glucosides of interest to us offer the same stereochemical challenge, but with a reduced kind of support because no asymmetric induction results from a 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton. Such acetyl-protected glucosyl donors as 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate are known to give exclusively a mixture of both β-glucosides due to neighboring-group assistance by the acetyl groups [21]. The use of an 8-fold excess of boron trifluoride etherate during glucosylation of the racemic aglycon DIMBOA, instead of a catalytic amount, was found to bring about exclusive formation of the natural (2*R*) configuration. The excess of Lewis acid acts as a noncovalent protecting group for the aglycon, a promoter for the glucosyl transfer, and a reagent ensuring an equilibration to the thermodynamically most stable diastereomer with the natural configuration of (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GDIMBOA) [18].

Therefore, we expected that application of this glucosidation method to 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyl trichloroacetimidate should give rise to *ent*-GDIMBOA as the first enantiomer of a natural acetal glucoside. The hitherto undescribed 2,3,4,6-tetra-*O*-acetyl-β-L-glucose (**1**) was synthesized from 2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl bromide in good yield (Scheme 1).

From additions of **1** to trichloroacetonitrile catalyzed by potassium carbonate in dichloromethane either 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyl trichloroacetimidate (**2**) or its α isomer **3** have been isolated. Whether the kinetically favored β-imidate **2** or the thermodynamically more stable α-imidate **3** is formed depends on the base and the time of reaction. It has been shown for the case of the enantiomeric 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl trichloroacetimidates that, under the described conditions using potassium carbonate, the ratio of anomers depends on the reaction time [22]. Thus, reaction for 2 h proved to be optimal to give rise to a crude product with the maximum β/α-ratio from which the crystalline β-imidate **2** could be isolated in 50% yield. Prolongation of the reaction time led to the syrupy α-imidate **3** in 72% yield. Though both imidates are suitable for glucosylation, we have only used crystalline **2**.

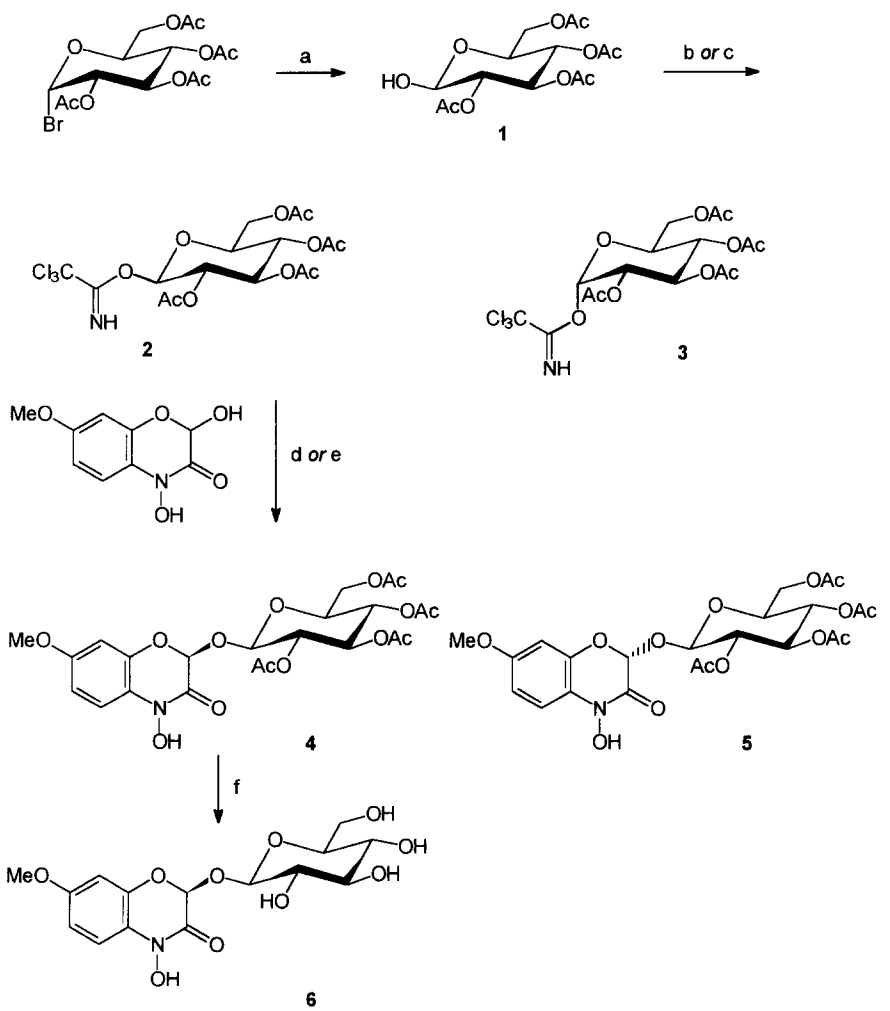
Two protocols for the L-glucosylation of the aglycon DIMBOA have been followed. First, (2*S*)-4-hydroxy-7-methoxy-2-(2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyloxy)-2*H*-1,4-benzoxazin-3(4*H*)-one (**4**), the enantiomer of the tetraacetate of the natural product GDIMBOA [18], was formed exclusively when racemic DIMBOA and **2** reacted in the presence of 8 equivalents of boron trifluoride etherate. The excess of Lewis acid promotes both the initial glucosylation that gives rise to a mixture of (2*S*)-β-L and (2*R*)-β-L acetal glucoside tetraacetates **4** and **5** and the subsequent equilibration of the latter to the thermodynamically more stable isomer **4**. L-glucosylation of DIMBOA with **2** under the same conditions, but using a catalytic amount of boron trifluoride etherate (0.1 equivalent), resulted in a crude reaction product that was shown by TLC to consist of two acetal glucoside tetraacetates. Repeated recrystallization finally led to pure (2*R*)-4-hydroxy-7-methoxy-2-(2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyloxy)-2*H*-1,4-benzoxazin-3(4*H*)-one (**5**).

The structures of **4** and **5** were assigned by spectroscopic methods using H,H COSY, HMQC, and HMBC spectra. Comparison of their NMR spectra indicates significant differences for ^{1}H and ^{13}C chemical shifts at or near to both anomeric centers. Thus, protons at these positions are deshielded for **5** (H-2: δ

5.86; H-1′: δ 4.99) in relation to **4** (H-2: δ 5.77; H-1′: δ 4.92), whereas shielding results for the anomeric carbons of **5** (C-2: δ 94.6; C-1′: δ 95.9) compared with **4** (C-2: δ 97.6; C-1′: δ 101.0). One of the acetyl groups of **5**, most likely the 2-OAc, is extremely (ca. 0.5 ppm) shielded to δ 1.49 in comparison to the four acetyl groups in **4** between δ 1.95 and 2.07.

The CD spectrum of **4** proved to be an exact mirror image to that of GDIMBOA tetraacetate [18]. The CD spectrum of **5** was of most interest because, hitherto, CD spectra have not been described either for the (2*R*)-2-β-L or for the (2*S*)-2-β-D configurations of benzoxazinoid acetal glucosides, despite the fact that a tetraacetate of the latter configuration has been separated during a Blepharin synthesis [16]. We have now found that, in **5**, changing the configuration at C-2 of the benzoxazinone results in a complete alteration of the shape of its CD spectrum in comparison to **4**. The first Cotton effect at 232 nm is a positive one, and the second Cotton effect at 269 nm a negative one. The CD spectrum of this L-glucoside tetraacetate **5** is reminiscent of the CD spectra of natural (2*R*)-2-β-D-benzoxazinoid acetal glucosides but the size of the Cotton effects of **5** is higher. This leads to the conclusion that only the (2*R*) configuration of the acetal glucoside determines the kind of Cotton effects, and that the question of whether the β-glucose unit has the D or L configuration plays no important role in the CD spectrum. Finally, a comparison of the CD spectrum of a hitherto undescribed α-diastereoisomer would be an interesting aim for further investigations.

Deprotection of tetraacetate **4** led to (2*S*)-2-β-L-glucopyranosyloxy-4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**6**) (*ent*-GDIMBOA). The spectroscopic data of this compound were in accordance with those of natural GDIMBOA [9]; the CD



Scheme 1. (a) Ag_2CO_3, H_2O; (b) for **2**: Cl_3CCN, K_2CO_3, CH_2Cl_2, r.t., 2 h; (c) for **3**: Cl_3CCN, K_2CO_3, CH_2Cl_2, r.t., 2 d; (d) for **4**: 8 $BF_3 \cdot Et_2O$, CH_2Cl_2, r.t.; (e) for **5**: 0.1 $BF_3 \cdot Et_2O$, CH_2Cl_2, r.t.; (f) 1. NaOMe, MeOH, 2. Amberlite IR-120.

spectrum of **6** was an exact mirror image of the natural product.

In summary, the synthesis of **6** (*ent*-GDIMBOA), the first enantiomer of a natural acetal glucoside, has been accomplished by the double diastereoselective L-glucosylation of racemic 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one with trichloroacetimidate **2** in the presence of an 8-fold excess of boron trifluoride etherate serving as promoter as well as protecting and equilibrating agent.

3. Experimental

Starting materials.—Commercial 98% L-(−)-Glucose (mixture of anomers) from Aldrich was peracetylated to 1,2,3,4,5-penta-*O*-acetyl-α-L-glucopyranose [23], which was transformed into 2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl bromide [24]. Racemic 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one was synthesized according to our published method [25].

Analytical methods.—Melting points were determined on a Boetius melting-point apparatus and are corrected. The ^{1}H and ^{13}C NMR spectra were recorded at 500.13 and 125.75 MHz (Bruker DRX-500) and at 199.975 and 50.289 MHz (Varian Gemini 200), respectively (internal Me_4Si as standard in organic solutions; internal sodium 4,4-dimethyl-4-silapentanoate as standard in D_2O). IR spectra were obtained on a Carl Zeiss Jena spectrometer M80. ESI-MS measurements were made on an AMD-402 mass spectrometer with 70-eV EI ionization. CD spectra were recorded with a JASCO J-710 spectrometer. Optical rotations were measured with a semiautomatic polarimeter Polartronic D (Schmidt and Haensch) using the Na-D line. TLC was performed on precoated Silica Gel 60 F_{254} aluminum sheets (Merck); eluents: 1:2 toluene–EtOAc for tetraacetates and 3:1 $CHCl_3$–MeOH for the deprotected glucoside. Column chromatography was performed on Silica Gel 60 (15–25 μm, Merck) with 1:3 toluene–EtOAc, using a Büchi B-580 MPLC system (pressure, 2 bar; flow rate, 13 mL/min; 254-nm detection). Elemental analyses were determined on a Heraeus CHN-O-Rapid analyzer.

2,3,4,6-Tetra-O-acetyl-β-L-glucose (**1**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl bromide (8.22 g, 0.02 mol) and H_2O (0.3 mL) in absolute acetone (15 mL) was added at 0 °C Ag_2CO_3 (4.65 g, 0.017 mol) in small portions over 15 min. After stirring for 30 min the solution was warmed to 60 °C and filtered. The filter cake was washed with absolute acetone (3 × 20 mL). The combined filtrates were evaporated under reduced pressure until most of the solution was filled with crystals. After heating the suspension to dissolve the crystals, an equal volume of absolute diethyl ether and a similar volume of light petroleum were added, and the product crystallized to give 6.3 g (90%) of **1** as colorless crystals; mp 129–131 °C; $[\alpha]_D^{24}$ −23° (*c* 1.0, $CHCl_3$); IR (KBr): ν 1630, 1375, 1237, and 1040 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.26 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.3 Hz, H-3), 5.08 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.89 (dd, 1 H, $J_{1,2}$ 8.1 Hz, H-2), 4.74 (dd, 1 H, $J_{1,OH}$ 8.6 Hz, H-1), 4.25 (dd, 1 H, $J_{5,6b}$ 4.8, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.12 (dd, 1 H, $J_{5,6a}$ 2.4 Hz, H-6a), 3.74 (ddd, 1 H, H-5), 3.64 (d, 1 H, OH), 2.09 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.02 (s, 3 H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.5 (CO), 171.1 (CO), 170.8 (CO), 170.1 (CO), 95.8 (C-1), 73.4 (C-2), 72.8 (C-3), 72.3 (C-5), 68.8 (C-4), 62.4 (C-6), 20.9 (4 × CH_3); EIMS (70 eV): *m/z* (%) 348 (3, M^+), 200 (21), 157 (27), 115 (51), 42 (100). Anal. Calcd for $C_{14}H_{20}O_{10}$: C, 48.27; H, 5.79. Found: C, 48.40; H, 5.89.

2,3,4,6-Tetra-O-acetyl-β-L-glucopyranosyl trichloroacetimidate (**2**).—A solution of **1** (3.0 g, 8.6 mmol) and trichloroacetonitrile (2.5 mL, 25 mmol) in absolute CH_2Cl_2 (20 mL) was treated with finely powdered K_2CO_3 (2.0 g, 14.4 mmol). The solution was stirred for 2 h at room temperature and diluted with absolute CH_2Cl_2 (80 mL). The K_2CO_3 was filtered off and the filtrate was concentrated in vacuo to a total volume of 15 mL and filtered over silica with 1:1 CH_2Cl_2–diethyl ether. The solvent was removed in vacuo. The remaining syrup was dissolved in absolute diethyl ether from which the product crystallized to yield 2.1 g (50%) of **2** as colorless crystals; mp 148–150 °C; $[\alpha]_D^{24}$ −5.3° (*c* 1.0, $CHCl_3$); R_f 0.65 (Et_2O); IR (KBr): ν 1757, 1679, 1370, 1239, and 1039 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.71 (s, 1 H, NH), 5.87 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 5.31–5.18 (m, 3 H, H-2, H-3, H-4), 4.31 (dd, 1 H, $J_{5,6b}$ 4.3, $J_{6a,6b}$ 12.5 Hz, H-6b), 4.15 (dd, 1 H, $J_{5,6a}$ 2.5 Hz, H-6a), 3.91 (ddd, 1 H, H-5), 2.08 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.01 (s, 3 H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.1 (CO), 170.6 (CO), 169.8 (CO), 169.4 (CO), 161.4 (C=NH), 96.0 (C-1), 90.8 (CCl_3), 73.2 (C-3), 73.1 (C-5), 70.6 (C-2), 68.4 (C-4), 62.0 (C-6), 21.2 (CH_3), 21.1 (2 × CH_3), 21.0 (CH_3); EIMS (70 eV): *m/z* (%) 491 (1, M^+), 456 (1), 347 (3), 287 (3), 200 (6), 42 (100). Anal. Calcd for $C_{16}H_{20}Cl_3NO_{10}$: C,

39.01; H, 4.09; N, 2.84. Found: C, 38.80; H, 4.30; N 2.97.

2, 3, 4, 6 - Tetra - O - acetyl - α - L - glucopyranosyl trichloroacetimidate (**3**).—A solution of **1** (3.0 g, 8.6 mmol) and trichloroacetonitrile (2.5 mL, 25 mmol) in absolute CH_2Cl_2 (20 mL) was treated with finely powdered K_2CO_3 (2.0 g, 14.4 mmol). The solution was stirred at room temperature for 48 h and worked up as described above for compound **2**, to yield 3.05 g (72%) of colorless syrupy **3** which can be used for glucosylations without further purifications; $[\alpha]_D^{24}$ −96.0° (*c* 1.0, $CHCl_3$); R_f 0.74 (Et_2O); IR (film): ν 1757, 1678, 1370, 1233, and 1040 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 8.69 (s, 1 H, NH), 6.53 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.54 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.8 Hz, H-3), 5.16 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 5.11 (dd, 1 H, H-2), 4.35–4.02 (m, 3 H, H-6a, H-6b, H-5), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 1.99 (s, 3 H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.0 (CO), 170.4 (CO), 170.3 (CO), 169.9 (CO), 161.2 (C=NH), 93.4 (C-1), 91.1 (CCl_3), 70.5 (C-2), 70.3 (C-3), 70.2 (C-5), 68.3 (C-4), 61.8 (C-6), 21.1 ($2\times CH_3$), 21.0 (CH_3), 20.9 (CH_3); EIMS (70 eV): *m/z* (%) 456 (57, $M^+ - Cl$), 431 (72, $M^+ -$ AcOH), 331 (84), 115 (92), 42 (100). Anal. Calcd for $C_{16}H_{20}Cl_3NO_{10}$: C, 39.01; H, 4.09; N, 2.84. Found: C, 39.26; H, 4.25; N 2.76.

The synthesis of an α-trichloroacetimidate by anomerization of the β-trichloroacetimidate has been previously described for the enantiomeric D-glucose derivatives [22].

(2S)-4-Hydroxy-7-methoxy-2-(2,3,4,6-tetra-O-acetyl-β-L-glucopyranosyloxy)-2H-1,4-benzoxazin-3(4H)-one (**4**).—To a stirred suspension of 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (105 mg, 0.5 mmol) and **2** (492 mg, 1.0 mmol) in absolute CH_2Cl_2 (20 mL) at 20 °C was added $BF_3 \cdot Et_2O$ (0.5 mL, 4.0 mmol) with a syringe. Immediately, the suspension turned into a light-yellow solution. Monitored by TLC (Kieselgel 60, Merck, eluent: 2:1 EtOAc–toluene), the mixture was stirred at room temperature in an argon atmosphere until the reaction was completed. A 20-mL portion of water was added, and, after 5 min, the organic layer was separated, dried, and evaporated in vacuo. The remaining oil was dissolved in diethyl ether from which the product crystallized. Recrystallization of the crude product thus obtained from aq EtOH yielded 205 mg (76%) of pure **4** as colorless needles; mp 190–192 °C; $[\alpha]_D^{21}$ −35.0° (*c* 1.0, $CHCl_3$); CD $\Delta\varepsilon_{234}$ −17.1, $\Delta\varepsilon_{291}$ +7.3 (*c* 0.7, $CHCl_3$); IR (KBr): ν 1752, 1670, 1510, 1370, and 1228 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 9.41 (s, 1 H, NOH), 7.27 (d, 1 H, $J_{5,6}$ 8.8 Hz, H-5), 6.64 (dd, 1 H, $J_{6,8}$ 2.8 Hz, H-6), 6.61 (d, 1 H, H-8), 5.77 (s, 1 H, H-2), 5.18 (dd, 1 H, $J_{3',4'}$ 9.7, $J_{3',2'}$ 9.2 Hz, H-3'), 5.01 (dd, 1 H, $J_{4',5'}$ 9.8 Hz, H-4'), 4.92 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.86 (dd, 1 H, H-2'), 4.22 (dd, 1 H, $J_{6'a,5'}$ 2.1, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 4.18 (dd, 1 H, $J_{6'b,5'}$ 4.1 Hz, H-6'b), 3.75 (s, 3 H, OCH_3), 3.70 (ddd, 1 H, H-5'), 2.07 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 1.96 (s, 3 H, OAc), 1.95 (s, 3 H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3 (CO), 170.8 (CO), 170.0 (CO), 169.9 (CO), 158.0 (C-7), 154.5 (C-3), 141.8 (C-8a), 120.3 (C-4a), 114.8 (C-5), 109.3 (C-6), 104.2 (C-8), 101.0 (C-1'), 97.6 (C-2), 72.9 (C-3'), 72.8 (C-5'), 71.3 (C-2'), 68.4 (C-4'), 62.1 (C-6'), 56.1 (CH_3O), 21.1 (CH_3), 20.9 ($2\times CH_3$), 20.8 (CH_3); ESIMS: *m/z* (%) 564 (100, $M^+ + Na$), 353 (64), 194 (97), 169 (57). Anal. Calcd for $C_{23}H_{27}NO_{14}$: C, 51.02; H, 5.03; N, 2.59. Found: C, 51.31; H, 5.23, N, 2.45.

(2R)-4-Hydroxy-7-methoxy-2-(2,3,4,6-tetra-O-acetyl-β-L-glucopyranosyloxy)-2H-1,4-benzoxazin-3(4H)-one (**5**).—The glucosylation method described for **4** was followed in principle, with DIMBOA (53 mg, 0.25 mmol) and **2** (246 mg, 0.5 mmol) in CH_2Cl_2 (10 mL); however, only 3.6 mg (0.025 mmol) of $BF_3 \cdot Et_2O$ in the form of 250 μL of a 0.1 M solution in CH_2Cl_2 was used. Recrystallization (five times) of the solid crude product thus obtained from aq EtOH yielded 7 mg (5%) of **5** as colorless needles; mp 204–206 °C; CD $\Delta\varepsilon_{232}$ +18.9, $\Delta\varepsilon_{269}$ −6.88 (*c* 0.68, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 9.4 (s, 1 H, NOH), 7.32 (d, 1 H, $J_{5,6}$ 8.6 Hz, H-5), 6.69–6.66 (m, 2 H, H-6, H-8), 5.86 (s, 1 H, H-2), 5.20 (dd, 1 H, $J_{3',2'}$ 9.4, $J_{3',4'}$ 9.3 Hz, H-3'), 5.08 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, H-4'), 4.99 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.88 (dd, 1 H, H-2'), 4.25 (dd, 1 H, $J_{6'b,5'}$ 4.8, $J_{6'b,6'a}$ 12.4 Hz, H-6'b), 4.14 (dd, 1 H, $J_{6'a,5'}$ 2.1 Hz, H-6'a), 3.80 (s, 4 H, CH_3O, H-5'), 2.10 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 1.96 (s, 3 H, OAc), 1.49 (s, 3 H, OAc); ^{13}C NMR (125 MHz, CD_3COCD_3): δ 170.8 (CO), 170.2 (CO), 169.9 (CO), 169.2 (CO), 157.8 (C-7), 154.9 (C-3), 141.8 (C-8a), 123.3 (C-4a), 114.9 (C-5), 109.3 (C-6), 105.0 (C-8), 95.9 (C-1'), 94.6 (C-2), 73.2 (C-3'), 72.9 (C-5'), 71.4 (C-2'), 69.2 (C-4'), 62.6 (C-6'), 56.1 (CH_3O), 20.6 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 19.8 (CH_3); ESIMS: *m/z* (%) 564 (55, $M^+ + Na$), 353 (100), 194 (35), 169 (52).

(2S)-2-β-L-Glucopyranosyloxy-4-hydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (**6**).—To a solution of **4** (54 mg, 0.1 mmol) in absolute MeOH (10 mL) was added sodium methoxide (5 mg, 0.09 mmol). After stirring the mixture at room temperature

for 30 min the solution was neutralized by the addition of an ion-exchange resin [Amberlite IR-120 (H^+)]. The resin was then filtered off, and the solvent removed in vacuo. The residue was chromatographed over silica gel (Kieselgel 60, 60–200 μm, Merck) with 3:2 $CHCl_3$–MeOH to yield 32 mg (86%) of **6** as colorless crystals; mp 166–168 °C; $[\alpha]_D^{24}$ −68.0° (*c* 1.0, H_2O); CD $\Delta\varepsilon_{230}$ −19.6, $\Delta\varepsilon_{285}$ +8.77 (*c* 0.8, H_2O); IR (KBr): ν 3450, 2900, 1640, 1510, and 1075 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 7.33 (d, 1 H, $J_{5,6}$ 9.3 Hz, H-5), 6.78 (s, 2 H, H-6, H-8), 5.97 (s, 1 H, H-2), 4.83 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 3.88 (dd, 1 H, $J_{6'a,5'}$ 1.7, $J_{6'a,6'b}$ 12.3 Hz, H-6′a), 3.80 (s, 3 H, CH_3O), 3.69 (dd, 1 H, $J_{6'b,5'}$ 5.5 Hz, H-6′b), 3.51 (dd, 1 H, $J_{3',2'}$ 9.2, $J_{3',4'}$ 9.4 Hz, H-3′), 3.47 (m, 1 H, H-5′), 3.35 (dd, 1 H, $J_{4',5'}$ 9.5 Hz, H-4′), 3.25 (dd, 1 H, H-2′); ^{13}C NMR (125 MHz, D_2O): δ 161.3 (C-7), 160.8 (C-3), 145.6 (C-8a), 125.3 (C-4a), 119.0 (C-5), 113.7 (C-6), 108.1 (C-8), 106.5 (C-1′), 101.5 (C-2), 80.8 (C-5′), 79.8 (C-3′), 77.2 (C-2′), 73.6 (C-4′), 65.0 (C-6′), 60.2 (CH_3O); ESIMS: *m/z* 396 (M + Na, 100). Anal. Calcd for $C_{15}H_{19}NO_{10}$: C, 48.26; H, 5.13; N, 3.75. Found: C, 48.40; H, 5.36, N, 3.68.

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