# A novel glycosyl donor for chemo-enzymatic oligosaccharide synthesis: 4,6-dimethoxy-1,3,5-triazin-2-yl glycoside

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**A novel activated glycosidic compound, 4,6-dimethoxy-1,3,5-triazin-2-yl β-lactoside (DMT-β-Lac), which can be prepared directly from lactose in water without using any protecting groups, was found to be an efficient glycosyl donor for enzymatic glycosylation catalyzed by an endo-1,4-β-glucanase.**

Enzymatic glycosylation has become an attractive methodology in the field of synthetic carbohydrate chemistry.[1] Enzymes that have been employed are glycosidases,[2–6] phosphorylases,[7,8] glycosyltansferases,[9–11] and glycosynthases.[12] The use of glycosidases possesses several merits: Typical glycosidases are stable, easy to handle, and produced on an industrial scale. In principle, glycosidase-catalyzed transglycosylation reactions require activated glycosidic compounds (glycosyl donors) such as glycosyl fluorides[13] and *p*-nitrophenyl glycosides.[14] However, the preparation of these compounds necessitates the laborious task including the protection and deprotection of the hydroxy groups and the purification of the products.

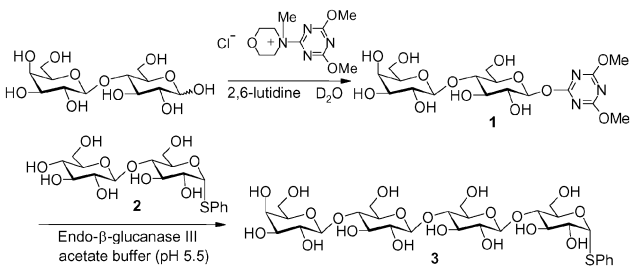
Here we report the synthesis and utility of a novel activated glycosidic compound, 4,6-dimethoxy-1,3,5-triazin-2-yl β-lactoside (DMT-β-Lac) **1**, as a glycosyl donor for enzymatic glycosylation. The compound **1** can be directly prepared starting from lactose in aqueous media without the use of any protection and deprotection of hydroxy groups. An endo-glucanase-catalyzed glycosylation of a thiocellobioside derivative **2** as a glycosyl acceptor has successfully been demonstrated, giving rise to the corresponding tetrasaccharide derivative **3** (Scheme 1).

DMT-β-Lac **1** was prepared as follows: a deuterium oxide (0.5 ml) solution of lactose (α/β = 26/74) (68.5 mg, 0.20 mmol), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMT-MM)[15] (110.7 mg, 0.40 mmol) and 2,6-lutidine (46 μl, 0.40 mmol) was stirred at room temperature for 18 h. After confirming the disappearance of lactose by NMR, the solvent was evaporated and the residue was crystallized from ethanol, giving rise to **1** (58.7 mg, 61%).[16] Among several bases screened (pyridine, 2,6-lutidine, trietylamine, diisopropylethylamine, *N*-methylmorpholine, $NaHCO_3$), 2,6-lutidine gave the best result concerning the yield of **1**.

The $^1$H NMR spectrum of **1** showed a doublet peak at 5.81 ppm derived from the anomeric proton with the coupling constant of 8.06 Hz, indicating that the anomeric configuration of the product is β-type. It is to be noted that the anomeric hydroxy group predominantly attacked the 2-position of DMT-MM because it has a higher acidity compared with other hydroxy groups.[17]

In this reaction, a disubstituted α-lactoside derivative at the 1 and 2 positions of the lactose moiety was formed as the by-product, which can be completely removed by recrystallization.[18] At the early stage of the reaction, the formation of mono-substituted DMT-α-lactoside was observed, which is converted to the disubstituted product. The nucleophilicity of the 2-hydroxy group of the DMT-α-lactoside may be intramolecularly enhanced by the lone-pair on the nitrogen atom of the triazine ring, resulting in the formation of the disubstituted product.

Firstly, enzymatic hydrolysis experiments of **1** were performed in 200 mM acetate buffer (pH 5.5) at 30 °C in order to know whether the DMT glycosidic moiety can be recognized by a glycosidase (Fig. 1). Obviously, almost no glycosidic bond cleavage was observed in the absence of the enzyme, indicating that compound **1** is stable in acetate buffer (open circle in Fig. 1). Interestingly, when endo-β-glucanase III (EGIII) from *Trichoderma reesei*[19] (0.3 wt% for **1**) was added, the compound **1** was found to be hydrolyzed smoothly, giving rise to lactose and 4,6-dimethoxy-1,3,5-triazine derivative (closed circle in Fig. 1). These results clearly indicated that the compound **1** can be accepted by the −2 subsite as well as −1 subsite of the enzyme. The hydrolysis mechanism has not been made clear, however, it is assumed that the glycosidic oxygen atom or the nitrogen atom (1 or 3 position) of the triazine ring is protonated with an acidic amino acid located at the catalytic center of EGIII, affording an oxocarbenium ion or a covalent α-glycosyl-enzyme intermediate. The resulting intermediate is then attached by water to give the hydrolyzate.




**Scheme 1** One-step synthesis of DMT-β-Lac **1** from lactose in aqueous solution, and transglycosylation with α-thiophenyl cellobioside **2** catalyzed by EGIII.

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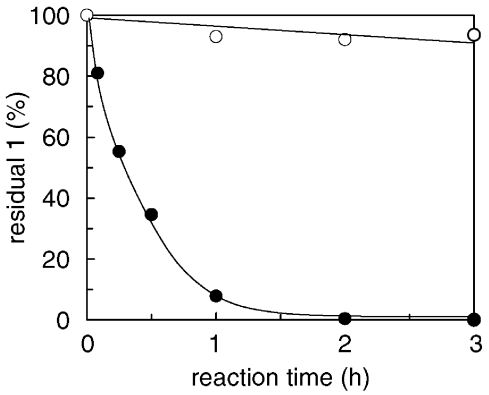


**Fig. 1** Time-course of consumption of **1**. ○: no enzyme, ●: in the presence of EGIII.

Having been encouraged by these new findings, we tried to synthesize an oligosaccharide by the reaction of DMT-β-Lac **1** as glycosyl donor and an appropriate cellobiose derivative as glycosyl acceptor. When the donor substrate **1** was mixed with α-thiophenyl cellobioside (Cel-α-SPh)[20] **2** as glycosyl acceptor in the presence of EGIII (0.3 wt% for **1**) in 200 mM acetate buffer (pH 5.5) at 30 °C (donor/acceptor ratio = 1/1), the corresponding tetrasaccharide derivative (Lac-Cel-α-SPh) **3** was obtained in 66%.[21] When the reaction was carried out under the condition of donor/acceptor ratio 2.5/1, the yield of **3** on the basis of the acceptor increased to 95% (Fig. 2).

The $^{1}$H-NMR spectrum of the resulting tetrasaccharide **3** showed a doublet signal at around $\delta$ 4.4 ppm ascribable to the H-1″ proton with the coupling constant value of 8.0 Hz. The $^{13}$C-NMR showed a signal due to the C4′ at around 78 ppm. All of these data clearly indicated that the glycosylation proceeded in a regio- and stereo-selective manner, affording a β-1,4 glycoside.

One of the most significant characteristics of the present glycosylation reaction is that the glycosyl donor **1** can be prepared in water starting from the corresponding free sugar. This fact prompted us to investigate a one-pot procedure without isolating the synthetic intermediate **1**. When lactose was treated with DMT-MM (2 equiv.) and 2,6-lutidine (2 equiv.) followed by the addition of the acceptor **2** and EGIII enzyme (0.3 wt% for lactose), the corresponding tetrasaccharide was directly obtained in 32% yield (on the basis of lactose) (Fig. 3).

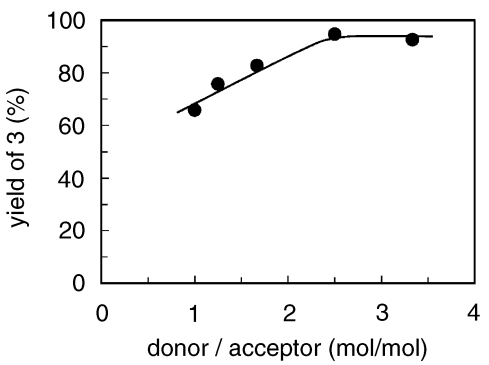


**Fig. 2** Effect of donor/acceptor ratio on yield of **3**.

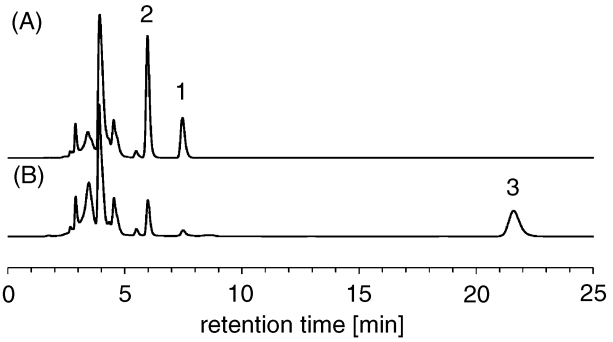


**Fig. 3** HPLC chart of reaction mixture for one-pot synthesis of **3** from lactose. (A) $t$ = 0, (B) $t$ = 7 h, after addition of **2** and EGIII.

In conclusion, a novel glycosyl compound, 4,6-dimethoxy-1,3,5-triazin-2-yl β-lactoside, can be prepared directly from lactose and recognized by an endo-glucanase for the first time. It is also to be noted that the present glycosylating process is the first example of chemo-enzymatic glycosylation, where both of the glycosyl donor synthesis[22] and the successive glycosylation can be achieved without any protecting and deprotecting steps in water. This new methodology would be an efficient and a practical tool for the construction of oligosaccharide moieties in glycotechnology.

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## Notes and references

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16 $^{1}$H NMR of DMT-β-Lac (500 MHz, $D_2O$); $\delta$ 5.81 (1H, d, H-1, $J_{1,2}$ = 8.06 Hz), 4.36 (1H, d, H-1′, $J_{1',2'}$ = 8.61 Hz), 3.91 (6H, OMe), 3.86–3.81 (2H, m, H-6$^{a}$, H-4′), 3.74–3.61 (7H, m, H-3, H-4, H-5, H-6$^{b}$, H-5′, H-6′$^{a}$, H-6′$^{b}$), 3.60–3.50 (2H, m, H-2, H-3′), 3.45 (1H, t, H-2′). $^{13}$C NMR of DMT-β-Lac (125 MHz, $D_2O$); $\delta$ 173.3 and 171.9 (3C, triazine), 102.9 (1C, C-1′), 96.8 (1C, C-1), 77.5 (1C, C-4), 75.5 (1C, C-5), 75.3 (1C, C-5′), 74.0 (1C, C-3), 72.5 (1C, C-3′), 71.6 (1C, C-2), 70.9 (1C, C-2′), 68.5 (1C, C-4′), 61.0 (1C, C-6′), 59.6

(1C, C-6), 55.9 (2C, OMe). MALDI-TOF MS; 504.7 ([M + $Na^+$]; calc. 504.4).
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18 $^1$H NMR of disubstituted by-product (500 MHz, $D_2O$); $\delta$ 6.73 (1H, d, H-1, $J_{1,2}$ = 3.53 Hz), 5.25 (1H, dd, H-2), 4.42 (1H, d, H-1′, $J_{1',2'}$ = 7.77Hz), 3.87, 3.80 (12H, OMe). $^{13}$C NMR of disubstituted by-product (125 MHz, $D_2O$); $\delta$ 173.1, 173.0, 172.2 and 171.3 (6C, triazine), 102.8 (1C, C-1′), 92.4 (1C, C-1), 75.1 (1C, C-2), 55.8, 55.7 (4C, OMe). MALDI-TOF MS; 643.9 ([M + $Na^+$]; calc. 643.5).
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20 The compound **2** was found to be the best acceptor for an EGIII-catalyzed lactosylation using β-lactosyl fluoride as glycosyl donor, which will be reported elsewhere.
21 $^1$H NMR of tetrasaccharide derivative **3** (500 MHz, $D_2O$); $\delta$ 7.47 and 7.29 (5H, Ph), 5.52 (1H, d, H-1, $J_{1,2}$ = 5.43 Hz), 4.44 and 4.43 (2H, d, H-1′, H-1″, $J$ = 8.04, 7.97 Hz), 4.33 (1H, d, H-1‴, $J_{1''',2'''}$ = 7.80), 4.22 (1H, H-5), 3.90–3.50 (20H, m, sugar-H), 3.43 (1H, H-2‴), 3.25 (2H, H-2′, H-2″). $^{13}$C NMR of tetrasaccharide derivative **3** (125 MHz, $D_2O$); $\delta$ 132.8, 132.3, 129.4 and 128.3 (6C, Ph), 102.9 (1C, C-1‴), 102.3 (2C, C-1′, C-1″), 88.9 (1C, C-1), 78.6 (1C, C-4), 78.2 and 78.0 (2C, C-4′, C-4″), 75.3 (1C, C-5‴), 74.8 (2C, C-5′, C-5″), 74.1 and 74.0 (2C, C-3′, C-3″), 72.9 and 72.8 (2C, C-2′, C-2″), 72.5 (1C, C-3‴), 72.1 (1C, C-3), 71.4 (1C, C-5), 70.9 (2C, C-2, C-2‴), 68.5 (1C, C-4‴), 61.0 (1C, C-6‴), 59.9, 59.8 and 59.7 (3C, C-6, C-6′, C-6″). MALDI-TOF MS; 782.1 ([M + $Na^+$]; calc. 781.8).
22 The direct synthesis of *p*-nitrophenyl glycosides from free sugars using Mitsunobu reagent in organic solvents was reported. S. Shoda, A. Kobayashi, and S. Takahashi, 2006, PCT Int. Appl. WO 2006038440.

 