How to Improve Chemical Synthesis of Laminaribiose on a Large Scale

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Abstract: Laminaribiose, which is the simplest β-(1,3)-glucan, is one of the most powerful agents able to increase germination. Its chemical synthesis was revised in detail starting from peracylated donors and easily available glucofuranose protected by acetal groups in the presence of appropriated catalyst and/or promoter. We particularly focused our attention on the nature of the Lewis acid generally required in glycosidic couplings. Finally, an interesting scale-up was performed which allowed us to prepare laminaribiose on a kilogram scale.

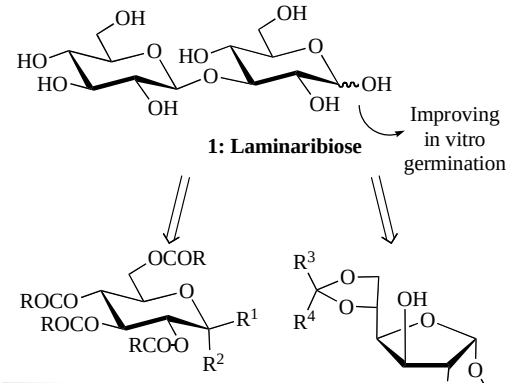
Keywords: Laminaribiose, Glycosylation, Trichloroacetimidates, Thioglycosides, Lewis acid.

INTRODUCTION

Life-expectancy of hydrated pollen is generally lower than 24 hours and its germination ability decreases with time. Consequently, all parameters are likely to improve speed of production of pollinic tubes or their number should favor fecundation. Most of the time, germination of grains, which is highly sensitive to the quality of pollen itself but also to environmental conditions and pH, which can be achieved *in vitro* in solution generally containing boric acid and simple carbohydrates. Amongst the latter, the most oftenly used is sucrose. However, it was shown that other disaccharides present similar or improved properties and laminiaribiose is now recognized as a really powerful germination agent [1]. The simplest β-(1,3)-glucan is available according to various approaches: enzymatic degradation of natural polysaccharides such as curdlan [2-4], enzymatic transglycosylation [5, 6], or chemical synthesis [7-11]. What attracts us to chemical means is the opportunity to enhance value of many intermediates in order to introduce fine structural modulations for further modulations of biological applications. Moreover, we were highly interested in producing large amounts of this disaccharide for direct use on cultures. In this context, we have first reinvestigated glycosidic coupling between various glucopyranosyl donors and glucofuranoses protected by two acetal groups (Fig. **1**), and secondly proposed a scale-up procedure to synthesize laminaribiose on a kilogram scale.

RESULTS AND DISCUSSION

During the second half of the last century, amongst many approaches, two main methods of glycosylation have emerged. The first one relies on the use of thioglycosides which are generally recognized as universal donors [12] and the other one recommends imidates, and more particularly trichloroacetimidates [13]. In order to ensure specific β-glycosylation, peracylated donors **2** and **3** were selected and prepared from the corresponding pentaacyl glucopyranose **5** (Scheme **1**). Trichloroacetimidates **2a** were obtained from **5a** after selective deacetylation using a secondary amine such as morpholine. The preparation of **2b** was best performed after acetolysis in acidic media of the perbenzoyl glucose **5b**. The resulting compound **5c** was further submitted to an activation procedure as described previously to afford the desired donor.



	R	R^1	R^2
2a	Me	H, $OCNHCCl_3$	
2b	Ph	H, $OCNHCCl_3$	
3a	Me	SEt	H
3b	Ph	SEt	H
3c	Ph	H	SEt

	R^3	R^4
4a	Me	Me
4b	C_5H_{10}	

Fig. (1). Retrosynthetic scheme.

While the thioethyl glucoside **3a** was obtained directly from **5a** (Scheme **2**), applications of Ferrier conditions [14] to **5b** yielded exclusively **3c** with a α-anomeric configuration. Consequently, the more reactive β-donor **3b** was synthesized from **3a** by acyl group interconversion in two steps.

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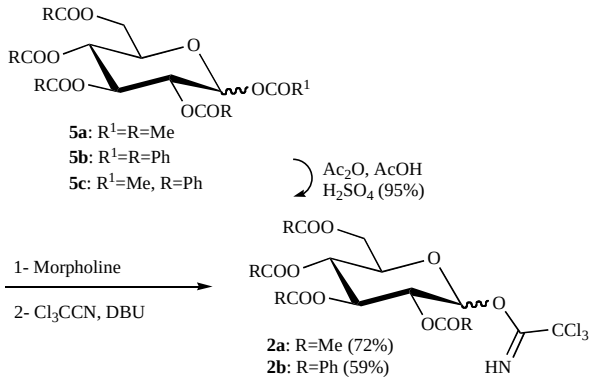
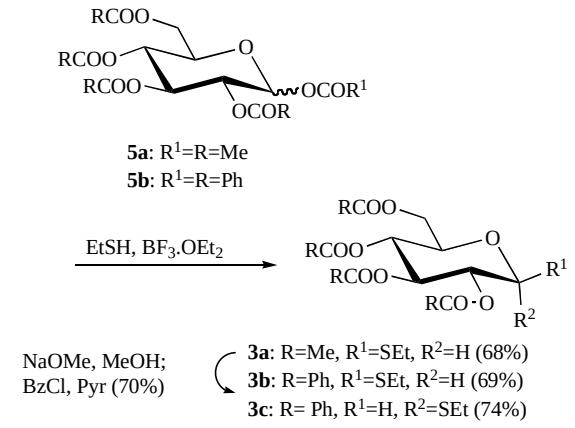


Scheme 1. Synthesis of trichloroacetimidates **2**.

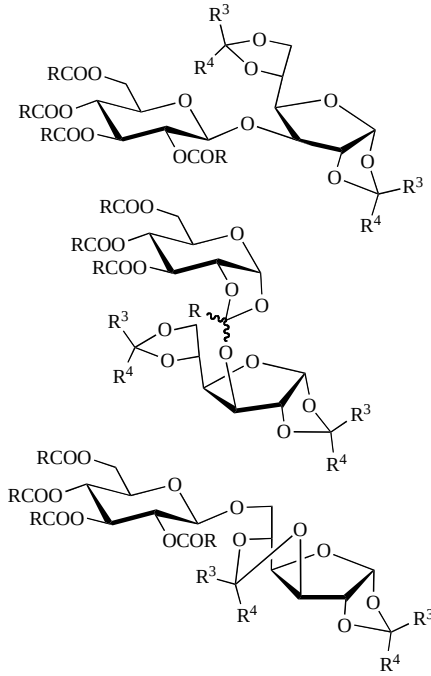


Scheme 2. Synthesis of thioglycosides **3**.

On another hand, it is important to note that some limitations connected with inefficiency of participating protecting groups were recently highlighted for the synthesis of oligo-β-(1,3)-glucans. While acetyl groups at O-2 on the donor species are expected to stabilize intermediate oxonium, to induce nucleophilic attack on the opposite site and consequently to favor 1,2-trans couplings, these desired results could not be easily attained using glucopyranosyl acceptors characterized by a free 3-OH and acyl groups to protect other hydroxyl functions [15]. However this difficulty could be overcome by using acceptors bearing a 4,6-benzylidene group [16,17]. Nevertheless, we thought that such elaborated compounds could not be suitable for a pre-industrial process because they require too many syntheses and purification steps for molecule having non-pharmaceutical uses. As a consequence, we focused our attention on diacetone glucose **4a** and dicyclohexylidene glucose **4b** as glucosyl acceptors. They were easily prepared on a large scale in one acid catalyzed step from glucose in 1,4-dioxane and isolated by crystallization in 90% and 87% yield, respectively. However, while acetone is cheaper than cyclohexanone, we also considered **4b** as an interesting acceptor since the cyclohexylidene acetals are known to be less sensitive than the standard isopropylidene acetals to acidic conditions required in glycosidic couplings [18].

GLYCOSYLATION WITH TRICHLOROACETIMIDATES

Activation of trichloroacetimidates is efficient with only a catalytic amount of an appropriate Lewis acid (L. A.). Most of the time, catalysts which are soluble in organic solvents are used. After experimentation from 1 equivalent of acceptor **4** and 1.1 of donor **2** in dichloromethane, we observed that complex mixtures were obtained with boron trifluoride-etherate. The small excess of donor can be explained by partial but the inescapable degradation of the imidate into the corresponding hemiacetal. When catalyzed by triethylsilyl trifluoromethanesulfonate (TESOTf), the reaction gave three main products: the desired coupling disaccharide **6**, the orthoester **7**, and the precursor of gentiobiose **8** (Fig. **2**) whose ratios were tightly dependent on experimental conditions (Table **1**). The data showed that the orthoester **7** was mainly synthesized with an insufficient amount of the L. A. (entry 1). Nevertheless, the acid catalyzed rearrangement of **7** was performed in situ by simply using 0.1 equivalent of TESOTf so that it was no more isolated. However, we were still disappointed by the presence of a second disaccharide. While the desired precursor of laminaribiose **6** was obtained as the major product, a gentiobiose derivative **8** was systematically present in the reaction mixture (entries 2-5). Since it resulted from a migration of the 5,6-acetal to the 3,5-positions, we expected that a more stable cyclohexyildyl protecting group could favor the target O-3 glycosylation. Indeed, the ratio **6**/**8** slightly increased from 4.0/1 to 4.3/1 using **4b** as an acceptor (entries 2 and 3) but more interestingly reached 32.3/1 and the products were isolated in 88% overall yield (entry 5). Moreover, the benzoyl groups on donor, compared to acetyl ones, contributed to the success of the reaction because significant higher yields were obtained (entries 2/4 and 3/5).



	R	R^3	R^4
6a	Me	Me	Me
6b	Me	C_5H_{10}	
6c	Ph	Me	Me
6d	Ph	C_5H_{10}	

	R	R^3	R^4
7a	Me	Me	Me
7b	Me	C_5H_{10}	
7c	Ph	Me	Me
7d	Ph	C_5H_{10}	

	R	R^3	R^4
8a	Me	Me	Me
8b	Me	C_5H_{10}	
8c	Ph	Me	Me
8d	Ph	C_5H_{10}	

Fig. (2). Structure of products **6**-**8**.

GLYCOSYLATION WITH THIOGLYCOSIDES

Thioglycosides are interesting donors especially because they are compatible with many protecting group manipulations, they can be stored for a long time without possible degradation, and also because their synthesis is shorter than that of trichloroacetimidates. On another hand, their activa-

Table 1. Glycosylation of 4 with Trichloroacetimidates 2

Entry	Donor/Acceptor	TESOTf (equiv.)	Yield (%)	6/7/8
1	**2a/4a**	0.02	61	0/1/0
2	**2a/4a**	0.1	56	4.0/0/1
3	**2a/4b**	0.1	62	4.3/0/1
4	**2b/4a**	0.1	84	7.3/0/1
5	**2b/4b**	0.1	88	32.3/0/1

tion generally requires thiophilic halonium sources, such as *N*-iodosuccinimide (NIS), and a catalytic amount of a Lewis acid likely to weaken the nitrogen-halogen bond and to favor the catching of the resulting cation by the sulfur atom. Considering the previous results, the reactions were first carried out in dichloromethane using 1.1 equivalent of donor **3b**, 1 of acceptor **4b**, 1.1 of NIS, and 0.03-1.0 of a Lewis acid. Once again, the same three main products **6b**, **7b** and **8b** were obtained depending on reaction conditions (Table **2**). Considering the disappointed results with TESOTf (entries 1, 2), we further studied the glycosidation of **3b** with metal triflates. The zinc salt gave poor results (entries 3, 4) as well as the cupric, tin and silver derivatives when used in too small amounts (entries 5, 7, 9). However, the desired disaccharides were obtained in significant increased yield by slightly increasing the ratio of the Lewis acid. Moreover, very interesting selectivity in favor of the laminaribiose precursor **6b** was observed with cupric triflate (entry 6) and nearly quantitative yields were obtained with silver triflate (entries 11, 12). In order to limit the number of overall steps, we also considered the activation of the α-thioglycoside **3c** which was prepared in only two steps but which is also known to be less reactive than its β-counterpart. In this case, an equimolar ratio of cupper(II) triflate was needed to isolate a 4.9/1 mixture of **6b**/**8b** (entry 13). Nevertheless, the best compromise between yield and selectivity was observed for a catalysis with tin(II) triflate (entry 15) and not with the silver salt (entries 16, 17).

Subsequent research of optimum conditions relied on substituting expensive reactive by cheaper ones. We first tried reactions with non metal triflates such as pyridinum, triethyl ammonium and tetrabutyl ammonium and salts. Un-

Table 2. Glycosylation of 4b with Thioglucosides 3

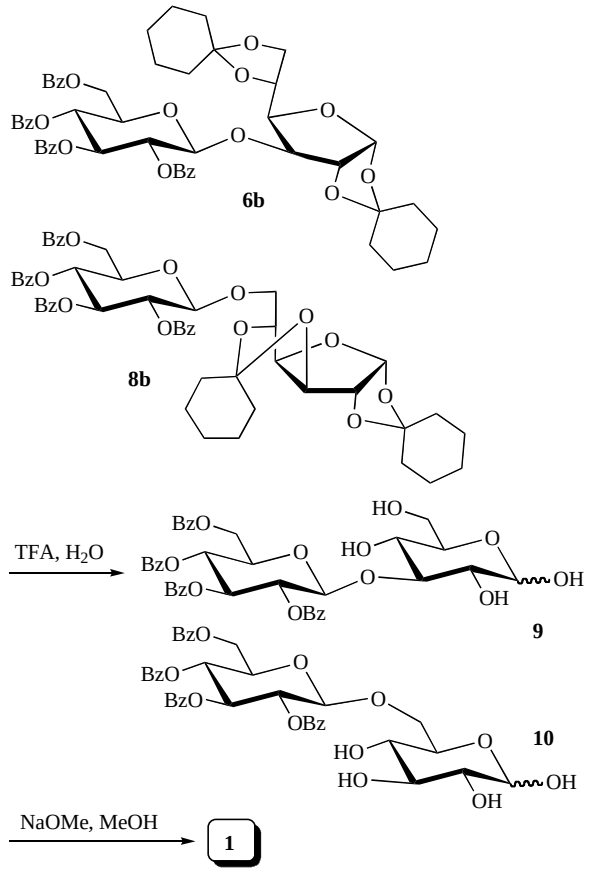
Entry	Donor	L. A. (equiv.)	Yield (%)	6b/7b/8b
1[a]	**3b**	TESOTf (0.03)	nr	
2[a]	**3b**	TESOTf (0.09)	33	1/0/1.7
3[a]	**3b**	$Zn(OTf)_2$(0.25)	25	0/1/0
4[a]	**3b**	$Zn(OTf)_2$ (0.6)	30	nd
5[a]	**3b**	$Cu(OTf)_2$ (0.2)	47	7.3/0/1
6[a]	**3b**	$Cu(OTf)_2$ (0.5)	76	13.3/0/1
7[b]	**3b**	$Sn(OTf)_2$ (0.1)	54	4.6/0/1
8[b]	**3b**	$Sn(OTf)_2$ (0.2)	72	2.4/0/1
9[a]	**3b**	AgOTf (0.1)	41	9/0/1
10[b]	**3b**	AgOTf (0.15)	85	3.5/0/1
11[b]	**3b**	AgOTf (0.2)	93	2.0/0/1
12[b]	**3b**	AgOTf (0.24)	96	3.3/0/1
13[b]	**3c**	$Cu(OTf)_2$ (1)	63	4.9/0/1
14[c]	**3c**	$Sn(OTf)_2$ (0.1)	57	4/0/1
15[b]	**3c**	$Sn(OTf)_2$ (0.1)	86	4.6/0/1
16[b]	**3c**	AgOTf (0.15)	76	0.8/0/1
17[b]	**3c**	AgOTf (0.2)	82	1/0/1

Reactions were carried out at (a) 0 °C to RT; (b) 0 °C; (c) -20 °C.

fortunately, the orthoester was the only product isolated in 18%, 31% and 72% yield, respectively. Moreover, a decrease of reactivity resulted from the use of *N*-bromosuccinimide (NBS) instead of NIS, so that yields were lower than 70%. Finally, we could observe only minor effects of solvent on the studied reaction by carrying out the glycosylations in toluene or tetrahydrofuran as solvents. Consequently, these parameters were not retained for subsequent improvements of the overall procedure.

DEPROTECTION STEPS

Our approach requires two deprotection steps: a hydrolysis under acidic conditions for the acetal groups and a transesterification, preferentially in basic media, for the ester groups. At this stage, it is important to remind that unprotected β-(1,3)-glucans are sensitive to β-elimination, or pealing, under basic conditions so that Zemplen transesterification should be best performed in the presence of acetal functions. On the other hand, it is well known that glycosidic bonds are cleaved in acidic media so that we could expect a favorable stabilization impact from the electro-withdrawing ester groups present on the non reducing part of the disaccharide. Experimentally, best results were obtained by performing the last sequence of the procedure starting from the disaccharide **6b** bearing both benzoyl and cyclohexylidene groups. Moreover, physicochemical behaviors of **6b** and the partially deprotected disaccharide **9** were considered with a particular attention (Scheme **3**). Indeed, we observed that (i) **6b** slowly dissolved in a 1:1 mixture of water and trifluo-roacetic acid (TFA) at 40 °C, and (ii) the resulting product **9** slowly crystallized out from



Scheme 3. Deprotection steps to give laminaribiose **1**.

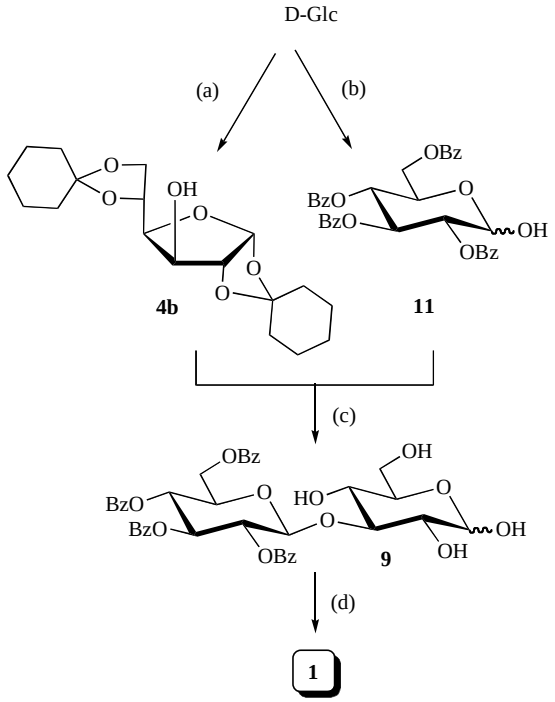
slowly crystallized out from this solution at 20 °C. It resulted from these observations that crystallization limits the break of the interglycosidic linkage. Consequently, the removal of cyclohexylidene and benzoyl groups allowed us to isolate the desired products in 71% and 68%, respectively, without any chromatographic purification. Moreover, a recrystallization of a mixture of **9** and **10** from methanol yielded pure laminaribiose intermediate **9** since the gentiobiose derivative **10** is highly soluble in methanol. Consequently, according to the targeted application, a further purification step can be added to obtain pure laminaribiose without any traces of gentiobiose.

SCALE-UP

A pre-industrial process generally requires attention to many non chemical parameters, such as cost of reactants, equipments and their maintenance, purification and depollution steps, but also purity, which has to be adapted to the desired application, the visual aspect of the final product, such an aspect having an impact on consumers on so on sale. On the assumption that we have defined agrofurnitures as the main domain of applications for laminaribiose, specifications can now be drawn: (i) it is better to prepare a solid compound and (ii) the presence of gentiobiose as well as glucose does not represent a limitation for the targeted biological properties. Nevertheless, the analytical specifications have to be defined and respected for all batches. In this context, we further improved the procedure for a laminaribiose possibly containing less than 10% of gentiobiose and glucose.

With these data in mind, we finally develop a kilogram-scale synthesis of laminaribiose using the trichloroacetimidate approach (Scheme **4**). Two essential parameters have to be mentioned: (1) the toxicity of trichloroacetonitrile is well known, and (2) it is less volatile and odorant than ethanethiol. Subsequently, we focused our attention on the scale-up parameters. More precisely, all solvents were adapted to industrial constraints. For instance, perbenzoylation of glucose was carried out in 1,4-dioxane in the presence of a minimum amount of pyridine required to quenched the released hydrochloric acid. We also reinvestigated the activation and coupling processes themselves. On the laboratory scale, the trichloroacetimidate **2b** was purified by flash chromatography. However, on a larger scale, the synthesis of donor **2b** and its coupling to acceptor **4b** were achieved according to a one-pot procedure without neither isolation nor purification of **2b**. This approach required first to isolate the acetal intermediate **11** [19] by simple crystallization. With this compound in hand, both activation and glycosidic reactions were performed in toluene using only 0.05 equivalent of DBU for the deprotonation of **11** and 0.15 equivalent of TMSOTf for the coupling with **4b**. It is interesting to note that DBU was preferred to an inorganic base such as potassium carbonate since it contributes to improve reaction time thanks to homogeneity of the reaction mixture. In practice, fine purification step further occurred after removal of the acetal protections under the assistance of aqueous trifluoroacetic acid in the presence of acetone so that **9** was isolated in a 71% yield over the last three steps. Zemplen transesterification finally afforded laminaribiose **1** in a 42% yield over the all process. Its purity was analyzed by HPLC and was greater than 90%. The by-products were identified as D-glucose and gentiobiose, resulted from partial degrada-

tion of the donor and protecting group migration on the acceptor, respectively, but which have no detrimental effects for the targeted germination use. Nevertheless, increased purity was obtained by adding a recrystallization step from a water/ethanol mixture.



Scheme 4. Improved overall procedure.

Conditions: (**a**) Cyclohexanone, H_2SO_4; (**b**) BzCl, Pyridine, 1,4-dioxane; Ac_2O, AcOH, H_2SO_4, CH_2Cl_2; Morpholine, Me_2CO; (**c**) Cl_3CCN, DBU, Toluene; **4b**, TMSOTf; TFA, H_2O, Me_2CO; (**d**) MeONa, MeOH (overall yield: 42%)

CONCLUSIONS

An efficient chemical synthesis of laminaribiose was developed starting from D-glucose. Increasing the amounts of reactants allowed us to identify more precisely all products and by-products obtained through the all process as well as the main physico-chemical parameters that impacted it. Consequently, many efforts dealt with the glycosidic coupling to yield the targeted disaccharide. During this study, we observed that the reactivity of peracylated thioglucopyranosides could be easily modulated by varying the nature of the Lewis catalyst. Indeed, triflate salts from amines or pyridine were too weak and gave only the corresponding orthoester. Using highly reactive silyl triflates resulted in acetal migration in the acceptor followed by competitive glycosylation with this new species. Nevertheless, interesting results were obtained with metal triflate, and more especially silver triflate whose impact is also linked to the stereochemistry of the donor used. We have finally preferred the trichloroacetimidate approach particularly because we could run through several synthetic and purification steps one after the other, notably thanks to the use of benzoyl protecting groups on the donor. It is interesting to note that removal under reduced pressure of liquids such as solvents or methyl benzoate was a critical point for further efficiency of biological tests. In conclusion, the proposed overall procedure avoided all chromatographic purification steps and, even if it was initially designed for agrofurnitures, it can be easily extended for more fine applications thanks to highly selective crystallization.

EXPERIMENTAL PART

General Methods

HPLC analysis were performed on a Dionex DX 300 instrument using pulsed amperometric detector ED 40, a Carbopac PA1 column (4 x 250 mm) eluting with gradient of X and Y at 1.0 mL/min where X is a150 mM aqueous solution of sodium hydroxide, and Y a mixture of a 500 mM aqueous solution of sodium acetate and a150 mM aqueous solution of sodium hydroxide: isocratic A for 8 min, then enrichment with B over 20 min until 100 of B. Thin layer chromatography (TLC) analyses were conducted on E. Merck 60 F_{254} Silica Gel non activated plates and compounds were revealed using a 5% solution of H_2SO_4 in EtOH followed by heating. For column chromatography, Geduran Si 60 (40-63 μm) Silica Gel was used. ^{1}H, ^{13}C, ^{31}P, ^{19}F, HMQC and COSY NMR spectra were recorded on a Bruker ARX 400 spectrometer at 400 MHz for ^{1}H, 100 MHz for ^{13}C. Chemical shifts are given in δ-units (ppm).

Synthetic Procedure

1,2:5,6-di-O-cyclohexylidene-α-D-glucofuranose (4b)

To a suspension of D-glucose (1 kg, 5.55 mol) in anhydrous 1,4-dioxane (0.77 L) and cyclohexanone (1.31 L, 12.60 mol) was added dropwise sulphuric acid (262 mL, 4.88 mol). After completion of the reaction under vigorous stirring, dilution in water (8 L) induced precipitation of 4b which was washed with a 5% aqueous solution of sodium bicarbonate and dried under reduced pressure (1.5 kg, 61%).

2,3,4,6-tetra-O-benzoyl-D-glucopyranose (11)

A solution of D-glucose (1 kg, 5.55 mol) in pyridine (2.6 L) was heated under reflux for 30 min. After cooling at room temperature, the solution was diluted with 1,4-dioxane (8 L) and benzoyl chloride (4.16 L, 35.57 mol) was added dropwise over 2 h. After stirring for 18 h, water heated at 60 °C (30 L) was added to the reaction mixture and the solid was filtered. The later was then washed with a basic aqueous solution (40 L of water containing 1 kg of sodium carbonate) heated at 60 °C and then with hot water until neutralization. The resulting solid was subsequently dried at 50 °C for 48 h, and finally recrystallized from ethyl acetate to give perbenzoylated glucose **5b**. Acetolysis of the later compound (1 kg, 1.43 mol) was further performed in dichloromethane (6 L) and using acetic anhydride (2.68 L, 28.54 mol) and acetic acid (0.82 L, 14.27 mol) in the presence of sulphuric acid (80 mL, 1.50 mol) which was added dropwise. The reaction was stirred at room temperature for 4 h, then neutralized by adding triethylamine (218 mL, 1.57 mol), and concentrated under reduced pressure at 50 °C. To the resulting crude oil was added water (20 L) and stirring was maintained overnight. After filtration, it was washed with a 5% aqueous solution of sodium bicarbonate (20 L) and finally dried under reduced pressure at 70 °C. The last step consisted in the selective deacetylation of **5c** (1 kg, 1.57 mol) by morpholine (412 mL, 4.70 mol) in acetone (3 L) at 35 °C for 3 h. After cooling at 20 °C, the reaction mixture was diluted with at least 3 L of acetone and the desired product **11** crystallized out. It was

then filtered and dried under reduced pressure at 70 °C (0.82 kg, 87%).

3-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-D-glucopyranose (9)

To a solution of **11** (1 kg, 1.68 mol) in toluene (3 L) were added trichloroacetonitrile (843 mL, 7.62 mol) and 1,8-diazbicyclo[5,4,0]undecene (DBU). After stirring for 1 h at room temperature, the reaction mixture was diluted with toluene (2.2 L) and cooled at 0 °C and glycosyltion reaction could occur by adding successively acceptor **4b** (519 g, 1.52 mol) and TMSOTf (41.5 mL, 0.23 mol). The reaction was monitored by TLC and quenched by adding triethylamine (31.8 mL, 0.23 mol). The resulting mixture was filtered and finally concentrated under reduced pressure. Subsequent Zemplen transesterification was performed on the later residue (1 kg in 0.5 L of acetone heated at 40 °C) in a equivolumic mixture (3 L) of water and trifluoroacetic acid. After stirring at 40 °C for 3 days, the target product 8 was precipitated from water (3.5 L) at room temperature, washed with a 5% aqueous solution of sodium hydrogenocarbonate (20 L), water (2x20 L), and a 1:1 mixture of heptane and toluene. The dried product **9** was thus isolated in 71% yield. TLC (9:1 CH_2Cl_2/MeOH) 0.6; ^{13}C NMR (d^5-pyridine), δ (ppm) 166.3, 166.2, 166.1, 166.0, 165.9, 165.7, 165.6 (CO); 98.5 (C-1aβ); 93.6 (C-1aα); 84.7 (C-3aα); 75.8 (C-3aβ); 86.5 (C-3aβ); 69.6 (C-4aβ or C-4aα); 77.8 (C-5aβ); 101.7 (C-2bα); 74.1 (C-3bα or C-3bβ); 73.2 (C-2aα); 73.0 (C-5aα or C-2bα or C-2bβ); 72.9 (C-2bα or C-2bβ or C-5aα); 72.0 (C-5bα, C-5bβ); 70.3 (C-4bα or C-4bβ); 65.5 (C-4aα or C-4aβ); 63.2 (C-6bα, C-6bβ); 62.5, 62.4 (C-6aα, C-6aβ); ^1H NMR, (d^5-pyridine) δ (ppm) 8.30-7.09 (m, 40 H, C_6H_5); 6.32 (d, H-2bα or H-1bβ, $J_{1,2}$ 8.0 Hz); 6.58 (t, H-3bα or H-3bβ, $J_{1,2}$, $J_{2,3}$ 9.5 Hz); 6.52 (t, H-3bα or H-3bβ, $J_{1,2}$, $J_{2,3}$ 9.5 Hz); 6.23-6.10 (m, H-1bα or H-1bβ, H-2bα, H-2bβ, H-4bα, H-4bβ); 5.69 (d, H-1aα, $J_{1,2}$ 3.4 Hz), 5.22 (d, H-1aβ, $J_{1,2}$ 7.4 Hz); 4.94 (dd, H-6'bα, H-6'bβ, $J_{6,6'}$ 12.1 Hz, $J_{5,6'}$ 2.7 Hz); 4.88-4.69 (m, H-3aα, H-5aα, H-6bα, H-6bβ, H-5bβ); 4.57-4.52 (m, H-6aα, H-6aβ, H-5bα); 4.45 (t, H-3aβ, $J_{2,4}$, $J_{3,4}$ 9.1 Hz); 4.34-4.26 (m, H-6'aα, H-6'aβ); 4.20-4.13 (m, H-4aα, H-4aβ); 4.10-4.03 (m, H-2aα, H-2aβ); 3.97-3.93 (m, H-5aβ); HRMS $[C_{39}H_{38}O_{15}+Na]^+$: calcd 781.2108, found 781.2114; $[C_{39}H_{38}O_{15}+K]^+$: calcd 797.1848, found 797.1842.

Laminaribiose (1)

To a suspension of 8 (1 kg, 1.32 mol) in methanol (20 L) was added a 10% solution of sodium methylate in methanol (60 mL). After 3 h at room temperature, the reaction mixture was filtered and the resulting solution neutralized with IR-120 resin (H^+-form). After another filtration step, methanol was partly removed until a solution of 8 L was obtained. The desired disaccharide, which crystallized by adding acetone (32 L), was filtered and isolated as a white solid (307 g) in 68% yield. NMR analysis was similar to that already published [20].

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REFERENCES

[1] Yvin JC, Cruz F, Tran Thanh K, Van Le B. Composition and process for the stimulation and germination of grains of pollen. Euro PCT 98/00555. 1998.

[2] Villa TG, Phaff HJ, Notario V. A method for preparing laminaribiose by using yeast endo-(1,3)-β-D-glucanase. Carbohydr Res 1979; 74: 369-370.

[3] Kusama S, Kusakabe I, Zama M, Murakami K, Yasui T. Enzymatic preparation of crystalline laminaribiose from curdlan. Agric Biol Chem 1984; 48(6): 1433-40

[4] Wang LX, Sakairi N, Kuzuhara H. Peracetylated laminaribiose: preparation by specific degradation of curdlan and its chemical conversion into N-acetylhyalobiuronic acid. Carbohydr Res 1991; 219:133-48.

[5] Moreau V, Viladot JL, Samain E, Planas A, Driguez H. Design and chemoenzymatic synthesis of thiooligosaccharide inhibitors of 1,3:1,4-β-D-glucanases. Bioorg Med Chem 1996; 4(11): 1849-55.

[6] Viladot JL, Moreau V, Planas A, Driguez H. Transglycosylation activity of Bacillus 1,3-1,4-β-D-glucan 4-glucanohydrolases. Enzymatic synthesis of alternate 1,3-1,4-β-D-glucooligosaccharides. J Chem Soc Perkin Trans 1 1997(16); 2383-7.

[7] Koenigs W, Knorr E. Ueber einige Derivate des Traubenzuckers und der Galactose. Chem Ber 1901; 34(1): 957-81.

[8] Freudenberg K, von Oertzen K. Synthese der 3- und der 5-(β-Glucosido)-glucose. Justus Liebigs Ann Chem 1951; 574: 37-54.

[9] Bächli P, Percival EGV. The synthesis of laminaribiose and proof of its identity with laminaribiose isolated from laminarin. J Chem Soc 1952; 224: 1243-6.

[10] Takeo K. A convenient synthesis of laminarabiose and some of its glycosides. Carbohydr Res 1979; 77: 245-51.

[11] Takeo K. Synthesis of benzyl α- and β-sophorosides, and of benzyl α-laminarabioside. Carbohydr Res 1980; 86(1): 151-7.

[12] Zhang Z, Ollmann IR, Ye X-S, Wischnat R, Baasov T, Wong C-H. Programmable One-Pot Oligosaccharide Synthesis. J Am Chem Soc 1999; 121(4); 734-53.

[13] Schmidt RR, Kinzy W. Anomeric-oxygen activation for glycoside synthesis: the trichloroacetimidate method. Adv Carbohydr Chem Biochem 1994; 50: 21-123.

[14] Ferrier R, Furneaux RH. Synthesis of 1,2-trans-related 1-thioglycoside esters. Carbohydr Res 1976; 52(1): 63-8.

[15] See for example Zeng Y, Ning J, Kong F. Remote control of α- or β-stereoselectivity in (1,3)-glucosylations in the presence of a C-2 ester capable of neighboring-group participation. Carbohydr Res 2003; 338(4): 307-11.

[16] Takeo K, Maki K, Wada Y, Kitamura S. Synthesis of the laminara-oligosaccharide methyl β-glycosides of dp 3–8. Carbohydr Res 1993; 245(1): 81-96.

[17] Jamois F, Ferrières V, Guégan JP, Yvin JC, Plusquellec D, Vetvicka V. Glucan-like synthetic oligosaccharides: Iterative synthesis of linear oligo-β-(1,3)-glucans and immunostimulatory effects. Glycobiology 2005; 15: 393-407.

[18] van Heeswijk WAR, Goedhart JB, Vliegenthart. Acid-catalysed hydrolysis of 1,2-O-alkylidene-α-D-glucofuranoses. Carbohydr Res 1977; 58(2): 337-44.

[19] Sabesan S, Neira S. Synthesis of glycosyl phospahtes and azides. Carbohydr Res 1992; 223: 169-85.

[20] Hoffma RE, Christofides JC, Davies DB. Scope and limitations of simple ^{13}C NMR method of structural análisis of carbohydrates: Glucodisaccahrides. Carbohydr Res 1986; 153: 1-16.

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