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 revisited 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 often used is sucrose. However, it was shown that other disaccharides present similar or improved properties and laminaribiose 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.

![Fig. (1). Retrosynthetic scheme.](image-url)

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.
considered while acetone is cheaper than cyclohexanone, we also con-
trolled crystallization in 90% and 87% yield, respectively. However,
lyzed step from glucose in 1,4-dioxane and isolated by crys-
tals. They were easily prepared on a large scale in one acid cata-
lysis, because they require too many syntheses and purification
steps for molecule having non-pharmaceutical uses. As a
result, we focused our attention on diacetone glucose
because such elaborated compounds 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
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 interest-
ingly reached 32.3/1 and the products were isolated in 88%
overall yield (entry 5). Moreover, the benzoyl groups on
acceptor, compared to acetyl ones, contributed to the success of
the reaction because significant higher yields were obtained
(entries 2/4 and 3/5).

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

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

On another hand, it is important to note that some limita-
tions 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 conse-
quently to favor 1,2-trans couplings, these desired results
could not be easily attained using glucopyranosyl acceptors
resulted from a migration of the 5,6-acetal to the 3,5-
positions, we expected that a more stable cyclohexyldyl
protecting group could favor the target O-3 glycosylation.

GLYCOSYLATION WITH TRICHLOROACETIMI-
DATES

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 sol-
vents are used. After experimentation from 1 equivalent of
acceptor 4 and 1.1 of donor 2 in dichloromethane, we ob-
served that complex mixtures were obtained with boron
trifluoride-etherate. The small excess of donor can be ex-
plained by partial but the inescapable degradation of the imi-
date into the corresponding hemiacetal. When catalyzed by
triethylsilyl trifluoromethanesulfonate (TESOTf), the re-
action gave three main products: the desired coupling disaccha-
ride 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 system-
atically 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 cyclohexyldyl
protecting group could favor the target O-3 glycosylation.

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

**GLYCOSYLATION WITH THIOGLYCOSIDES**

Thioglycosides are interesting donors especially because
they are compatible with many protecting group manipula-
tions, 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-
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 trflate (entry 6) and nearly quantitative yields were obtained with silver trflate (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) trflate 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) trflate (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 1. Glycosylation of 4 with Trichloroacetimidates 2

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor/Acceptor</th>
<th>TESOTf (equiv.)</th>
<th>Yield (%)</th>
<th>6/7/8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2a/4a</td>
<td>0.02</td>
<td>61</td>
<td>0/1/0</td>
</tr>
<tr>
<td>2</td>
<td>2a/4a</td>
<td>0.1</td>
<td>56</td>
<td>4.0/0/1</td>
</tr>
<tr>
<td>3</td>
<td>2a/4b</td>
<td>0.1</td>
<td>62</td>
<td>4.3/0/1</td>
</tr>
<tr>
<td>4</td>
<td>2b/4a</td>
<td>0.1</td>
<td>84</td>
<td>7.3/0/1</td>
</tr>
<tr>
<td>5</td>
<td>2b/4b</td>
<td>0.1</td>
<td>88</td>
<td>32.3/0/1</td>
</tr>
</tbody>
</table>

### Table 2. Glycosylation of 4b with Thioglucosides 3

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>L. A. (equiv.)</th>
<th>Yield (%)</th>
<th>6b/7b/8b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*a</td>
<td>3b</td>
<td>TESOTf (0.03)</td>
<td>nr</td>
<td>1/0/1.7</td>
</tr>
<tr>
<td>2*b</td>
<td>3b</td>
<td>TESOTf (0.09)</td>
<td>33</td>
<td>0/1/0</td>
</tr>
<tr>
<td>3*c</td>
<td>3b</td>
<td>Zn(OTf)2 (0.25)</td>
<td>25</td>
<td>7.3/0/1</td>
</tr>
<tr>
<td>4*d</td>
<td>3b</td>
<td>Zn(OTf)2 (0.6)</td>
<td>30</td>
<td>nd</td>
</tr>
<tr>
<td>5*e</td>
<td>3b</td>
<td>Cu(OTf)2 (0.2)</td>
<td>47</td>
<td>7.3/0/1</td>
</tr>
<tr>
<td>6*f</td>
<td>3b</td>
<td>Cu(OTf)2 (0.5)</td>
<td>76</td>
<td>13.3/0/1</td>
</tr>
<tr>
<td>7*g</td>
<td>3b</td>
<td>Sn(OTf)2 (0.1)</td>
<td>54</td>
<td>4.6/0/1</td>
</tr>
<tr>
<td>8*h</td>
<td>3b</td>
<td>Sn(OTf)2 (0.2)</td>
<td>72</td>
<td>2.4/0/1</td>
</tr>
<tr>
<td>9*i</td>
<td>3b</td>
<td>AgOTf (0.1)</td>
<td>41</td>
<td>9/0/1</td>
</tr>
<tr>
<td>10*j</td>
<td>3b</td>
<td>AgOTf (0.15)</td>
<td>85</td>
<td>3.5/0/1</td>
</tr>
<tr>
<td>11*k</td>
<td>3b</td>
<td>AgOTf (0.2)</td>
<td>93</td>
<td>2.0/0/1</td>
</tr>
<tr>
<td>12*l</td>
<td>3b</td>
<td>AgOTf (0.24)</td>
<td>96</td>
<td>3.3/0/1</td>
</tr>
<tr>
<td>13*m</td>
<td>3c</td>
<td>Cu(OTf)2 (1)</td>
<td>63</td>
<td>4.9/0/1</td>
</tr>
<tr>
<td>14*n</td>
<td>3c</td>
<td>Sn(OTf)2 (0.1)</td>
<td>57</td>
<td>4/0/1</td>
</tr>
<tr>
<td>15.o</td>
<td>3c</td>
<td>Sn(OTf)2 (0.1)</td>
<td>86</td>
<td>4.6/0/1</td>
</tr>
<tr>
<td>16*p</td>
<td>3c</td>
<td>AgOTf (0.15)</td>
<td>76</td>
<td>0.8/0/1</td>
</tr>
<tr>
<td>17*q</td>
<td>3c</td>
<td>AgOTf (0.2)</td>
<td>82</td>
<td>1/0/1</td>
</tr>
</tbody>
</table>

Reactions were carried out at (a) 0 °C to RT; (b) 0 °C; (c) -20 °C.
Indeed, we observed that (i) behaviors of cyclohexylidene groups. Moreover, physicochemical the disaccharide performing the last sequence of the procedure starting from charide. Experimentally, best results were obtained by ester groups present on the non reducing part of the disaccharide. On the other hand, it is well known that glycosidic bond are cleaved in acidic media so that we could expect a favorable stabilization impact from the electro-withdrawing bonds. 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.

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 peeling, 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 trifluoroacetic acid (TFA) at 40 °C, and (ii) the resulting product 9 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 the diacetate approach (Scheme 4). Two essential parameters have to be mentioned: (1) the toxicity of trichloroacetoni trile is well known, and (2) it is less volatile and odorant than ethan ethiol. 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 aceton 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, resulting from partial degrada-

![Scheme 3. Deprotection steps to give laminaribiose 1.](image-url)
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.

![Diagram of chemical structures](image)

**Scheme 4.** Improved overall procedure.

Conditions: (a) Cyclohexanone, H$_2$SO$_4$; (b) BzCl, Pyridine, 1,4-dioxane; Ac$_2$O, AcOH, H$_2$SO$_4$, CH$_2$Cl$_2$; Morpholine, Me$_2$CO; (c) Cl$_3$CCN, DBU, Toluene; (d) TMSOTf; TFA, H$_2$O, Me$_2$CO; (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 followed by heating. For column chromatography, Gleduran Si 60 (40-63 μm) Silica Gel was used. $^1$H, $^13$C, $^{15}$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-glucopyranosyl fluoride (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 benzoylated glucose 5b. Acetylation 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 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 Carboptic 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$_2$SO$_4$ in EtOH followed by heating. For column chromatography, Gleduran Si 60 (40-63 μm) Silica Gel was used. $^1$H, $^13$C, $^{15}$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).
then filtered and dried under reduced pressure at 70 °C (0.82 kg, 87%).

3-O-(2,3,4,6-tetra-O-benzyl-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-diazabicyclo[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 to 0 °C and glycosylation 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 due (1 kg in 0.5 L of acetone heated at 40 °C) in an equivalent amount of water (3.5 L) at room temperature, washed with a solution of sodium hydroxide (24 L), was filtered and isolated as a white solid (307 g) in 68% yield. NMR analysis was similar to that already published from water (3.5 L) at room temperature, washed with a

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References