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Convergent strategy for the synthesis of *S*-linked oligoxylans

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Arabinoxylans (AX) are a major class of hemicellulose and an important polysaccharide component of lignocellulosic biomass. To utilize the glycan polymer effectively, it is desirable to learn more about the enzymatic hydrolysis of AXs. Well-defined glycans can help to elucidate these processes. Here, we report the efficient synthesis of a mixed *O*- and *S*-linked tetraxylan. This thio-oligosaccharide has been developed as a putative inhibitor of arabinoxylan degrading enzymes used for the saccharification of biomass. Two common approaches for the synthesis of thio-oligosaccharides, either involving 1-thioglycoside donors or thioacceptors, are presented and compared regarding byproduct formation and yields. Both methods have shown to be useful for the synthesis of thiolinkages in oligoxylans assembly. However, the success of the reaction is highly dependent on the "match" between donors and acceptors.

1. Introduction

The search for an environmentally and economically sustainable process for biofuel production has focused on biomass feedstock. Thereby, it is important that the source material does not compete with feed and food production such as waste biomass and biomass grown on degraded and abandoned agricultural lands.[1,2] Arabinoxylan (AX) is one of the major components of feedstocks that are currently being investigated as a source of 2nd generation biofuels.[3,4] AXs are composed of β-(1→4)-D-xylopyranose, partially substituted by α-L-arabinofuranose residues on the second and third position of the xylan residues. Many enzymes are required for efficient AX degradation, and among them, we find endoxylanases, α-L-arabinofuranosidases and to complete the process, β-D-xylosidases.[5] To optimize the production of 2nd generation biofuel a better understanding of the carbohydrate-protein interactions that govern biomass degradation, is needed. A versatile approach for mapping the active site of glycosyl-hydrolases is to utilize enzyme resistant substrates which are competitive inhibitors, like thiooligosaccharides where the oxygen of one or more glycosidic bonds are replaced by a sulfur atom.[6–9]

There are two general approaches for the synthesis of thiooligosaccharides.[10,11] The first approach is a glycosylation with a saccharide thiol as the glycosyl acceptor.[12,13] The second method is an "inverse" glycosylation, using an anomeric thio function, which is introduced first to yield the anomeric thiol or thiolate and then reacted with a saccharide electrophile, to form the inter-*S*-glycosidic linkage through an S_N2 displacement reaction.[8] Both approaches are commonly used, but the latter approach, where the anomeric configuration of the product is more easily controlled has received the most attention. The stereochemistry at the anomeric center is controlled during the formation of the anomeric thiol function and not during the coupling reaction. Furthermore, replacement of oxygen with sulfur to create *S*-thioglycosides presents other synthetic challenges than the mere formation of the thio-linkage itself due to the difference in chemical properties of the two elements. Firstly, sulfur atoms are incompatible with catalytic hydrogenolysis[14], which complicates the use of benzyl ethers as protective groups, and secondly, thiols easily form disulfides, both as glycosyl donors and acceptors.[15]

Using a thiol as the acceptor facilitates the utilization of most of the known *O*-glycosylation methods and allows for the variation of the donor to find the right "match" for the thiol acceptor. However, only glycosylation methods using promoters that are not thiophilic can be employed, which disqualifies some of the *O*-glycosylation methods (e.g. thioglycosides). In the past, mostly anhydrosugars[16] and glycals[17] were used as donors and more recently trichloroacetimidates.[12,13]

The challenges of the second method are the stereospecific introduction of the anomeric sulfur group and its activation into the corresponding thiolate, in addition to the nature of the electrophile. Because they can be selectively cleaved to form the thiol in the presence of ester protecting groups, almost only thiourea salts and thioacetates have been used as intermediates in the synthesis of anomeric thiols.[18,17,19–26]

In this work, we present the effective synthesis of the tetraxylan **1**, which bears a glycosidic thiolinkage on the first glycosidic bond from the non-reducing end by using both mentioned synthetic strategies. Moreover, a comparison between the two mentioned methods for preparing thiolinkages with a special focus on the challenges encountered and main byproducts is also presented.

The length of the target structure is four units as this is the minimal length recognized by endo-β-1,4-xylanases[27], but also a suitable length for studying β-xylosidases, therefore, a possible interesting candidate for the investigation and the characterization of AX degrading enzymes.

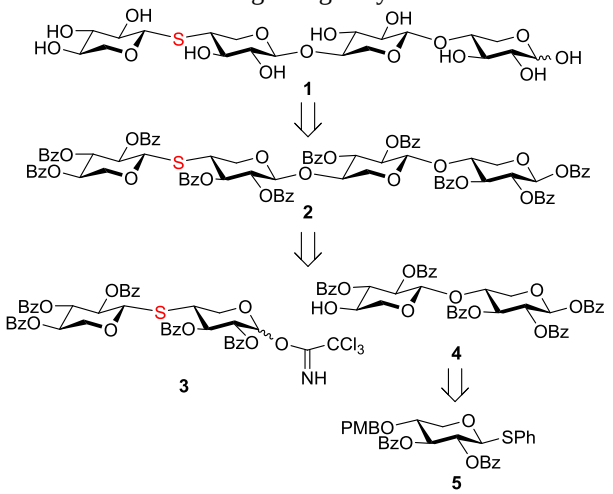


Figure 1: A proposed retrosynthetic strategy for the synthesis of tetraxylan **1**.

2. Results and Discussion

The synthesis of target **1** is based on a 2 + 2 block strategy between the *S*-disaccharide donor **3** and the *O*-disaccharide acceptor **4** (Figure 1). The acceptor **4** can be obtained by the employment of thioglycoside **5**, which can be used as a donor but also converted in the desired acceptor in a few steps. *S*-disaccharide **6** is envisioned to be obtained through a glycosylation reaction between a trifluoroacetimidate donor **7** and thioacceptor **8**, but also by employing acceptor **9** and thiodonor **10** (Figure 2). We decided to initially investigate the first method involving a thiodonor as the nucleophile to produce the thiolinkage.

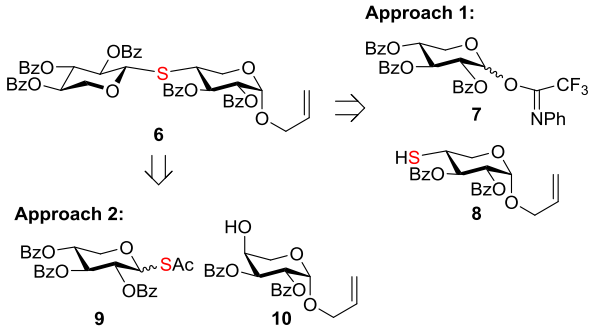
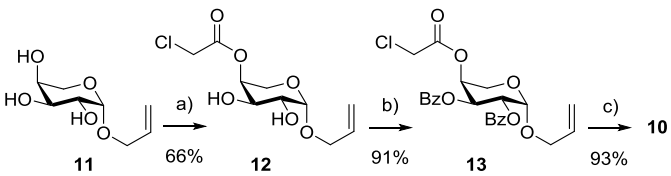


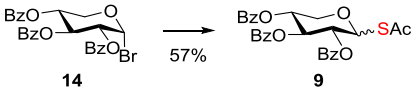
Figure 2: Different approaches for the synthesis of *S*-linked disaccharide **6**.

Acceptor **10** was prepared from allyl β-L-arabinopyranoside **11**, which was first functionalized at C4-OH with a chloroacetyl protecting group followed by benzoylation of the remaining positions (Scheme 1).[28,29] Compound **13** was subsequently deprotected to afford **10**.



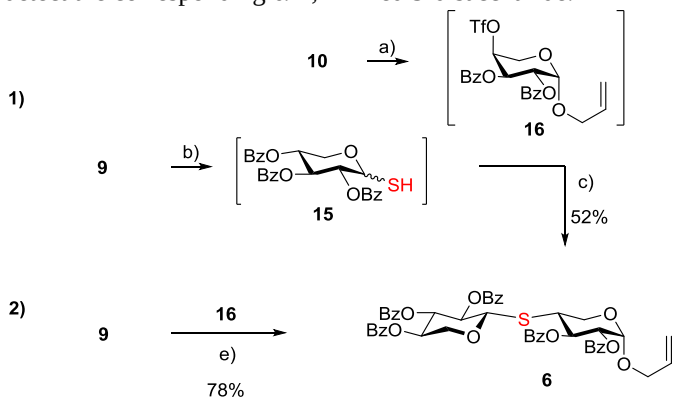
Scheme 1: a) *i.* Trimethyl chloro-orthoacetate, *p*-TsOH, CH_3CN, 40 ˚C, 10 min, *ii.* TFA, CH_3CN, 22 ˚C, 1 h, **b)** BzCl, DMAP, Et_3N, CH_2Cl_2, **c)** thiourea, $NaHCO_3$, TBAI, THF.

The thioacetate donor **9** was obtained from the corresponding bromide **14** by reaction with KSAc in DMF (Scheme 2; α:β = 1:4).[16,30] Compound **9** was selectively deprotected with NaSMe, and the resulting crude intermediate **15** was treated with NaH in THF and mixed with the triflate **16**, which was freshly prepared from compound **10** and Tf_2O/pyridine (Scheme 3.1).[31] The glycosylation yielded *S*-disaccharide **6** in 52% yield.



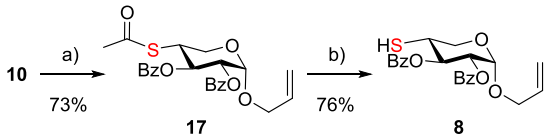
Scheme 2: KSAc, DMF, 22 ˚C, 1 h.

However, the coupling reaction gives a degree of uncertainty about the effective amount of donor and acceptor used in the reaction because their crude mixtures are employed. To investigate alternatives, we decided to vary the approach slightly according to Driguez and co-workers.[32] The method still uses the thioacetate **9** as a glycosidic donor and involves the employment of cysteamine for the removal of the acetate *in situ* and dithioerytritol (DTE) as reducing agent and scavenger (Scheme 3.2).[33,34] The coupling reaction between **9** and the activated acceptor **16** was performed at room temperature for 16 h and yielded merely the β-1,4 linked *S*-disaccharide **6** in 78%. We assume that the **9α** anomer epimerizes during the coupling reaction, since we do not detect the corresponding α-1,4 linked *S*-disaccharide.



Scheme 3: One pot syntheses of *S*-disaccharide 6; 1 a) Tf_2O, pyridine, CH_2Cl_2, 22 ˚C, 30 min, **b)** NaSMe, CH_2Cl_2/MeOH (1:1), **c)** NaH, THF, then **16**, DMF, 0 ˚C , 1 h, **2 d)** cysteamine, DTE, DMF, 22 ˚C, 16 h.

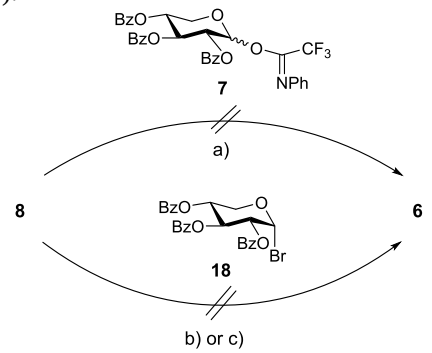
The most typical side reactions in these type of coupling are the elimination of the triflate and the anomerization of the thiol.[35] We observed mainly the elimination of triflic acid from compound **16** as a byproduct. However, we also identified the presence of the disulphide originating from dimerization of the thiol donor **9**.



Scheme 4: a) *i.* Tf_2O, pyridine, CH_2Cl_2, 30 min, *ii.* KSAc, DMF, 22 ˚C, 2 h, **b)** NaSMe, CH_2Cl_2/MeOH (1:1).

Next, we investigated the coupling of thiol acceptor **8** and the imidate **7**. To this end, **10** was treated with Tf_2O and pyridine, followed by reaction with KSAc in DMF[36] and selective

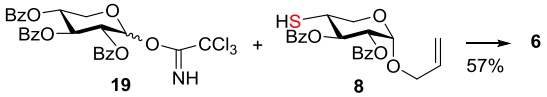
deprotection to thiol **8** with NaSMe[37] (Scheme 4). The coupling reaction to the desired *S*-disaccharide **6** was performed at −35 ˚C in CH_2Cl_2 with TMSOTf as a promoter (Scheme 5).



Scheme 5: a) TMSOTf, CH_2Cl_2, -35 ˚C, **b)** Cs_2CO_3, DMF, 22 ˚C, **c)** AgOTf, 2,4,6-collidine, CH_2Cl_2, -20 °C.

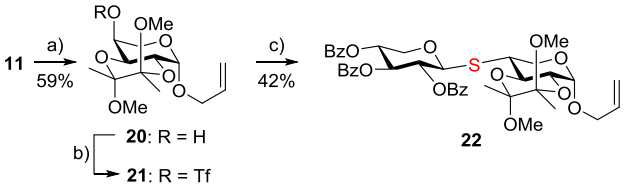
We envisioned the successful use of *N*-phenyl trifluoroacetimidate **7**, due to its lower propensity to undergo side reactions and rearrangements to acetamide during glycosylations[38] compared to trichloroacetimidates. Unfortunately, no *S*-linked disaccharide **6** was detected in the reaction mixture. One of the major problems was the dimerization of the acceptor into the corresponding disulphide, which was found to be stable to several reducing agents. We investigated the possibility of reducing disulphides to thiol acceptors both before the glycosylation and *in situ* using $NaCNBH_3$, $P(OCH_3)_3$, DTT, $P(n\text{-}Bu)_3$, or PPh_3. However, conversion to the thiol **8** was only obtained employing PPh_3, and when this was added to the glycosylation reaction, the yield of disaccharide **6** was still unsatisfactory (< 25%). Another byproduct isolated in the reaction mixture was the glycal and hydrolyzed donor. This result was found to be consistent with previous reports from other groups.[39] Changing the ratio of acceptor to donor, the concentration, the solvent as well as the promoter did not lead to the formation of **6**. We concluded that the *N-phenyl* trifluoroacetimidate **7** was not applicable under these conditions.

Cao *et al*. reported the successful synthesis of an *S*-linked heparan sulfate trisaccharide employing halides as donors, where acetimidate donors failed.[40] Inspired by these results, glycosylation between bromide **18** and thioacceptor **8** was performed using two different promoter systems (Scheme 5). Disappointingly, neither reaction afforded disaccharide **6**.



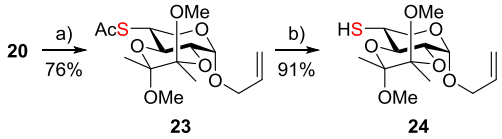
Scheme 6: TMSOTf, CH_2Cl_2, -35 ˚C**.**

We hypothesized that the reactivity between the chosen donors **7** or **18** and acceptor **8** was poorly matched. Therefore, we were prompted to try a different donor with higher reactivity like trichloracetimidates, as the group of Pinto *et al*. showed the applicability of these in the synthesis of *S*-linked glycans.[12] Using trichloracetimidate **19** to glycosylate acceptor **8** afforded **6** in 57% yield (Scheme 6). The choice of the trichloroacetimidate donor seems to be critical for this reaction, as it appears to favor the glycosylation to **6** over the dimerization of the thiol **8**.



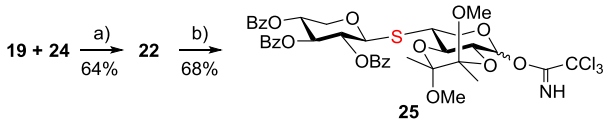
Scheme 7: a) 2,3-butanedione, $CH(OMe)_3$, CSA, MeOH, 22 ˚C, **b)** Tf_2O, pyridine, CH_2Cl_2, 22 ˚C, **c) 9**, **21**, cysteamine, DTE, DMF, 22 ˚C.

We believed it would be of interest to investigate not only the possible influence of different protecting groups but also the possibility of extending the reaction to other substrates. Therefore, acceptor **20** and triflate **21** were obtained from the allyl protected L-arabinose **11** (Scheme 7). The acid-catalyzed reaction of **11** with 2,3-butanedione in methanol allowed protection of the *trans*-diequatorial hydroxyl groups on the 2- and 3-positions with a cyclic butane diacetal to afford compound **20** in 59% yield. The yield of the reaction between triflate **21** and thioacetate **9** affording **22** was not as high as it was for the analogue thiol acceptor **16**. We can hypothesize that due to the diacetal, the transition state of the more strained **21** is disfavored compared to **16**. Also side reactions such as the elimination of the triflate group in **21** as well as the corresponding disulphide dimer of **9** were observed.



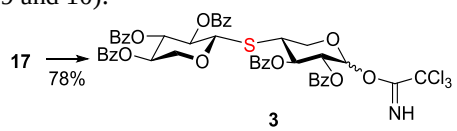
Scheme 8: a) *i.* Tf_2O, pyridine, CH_2Cl_2, *ii.* KSAc, DMF, 22 ˚C, **b)** NaOMe, MeOH.

The thioacceptor **24** was obtained from **20** in three steps (Scheme 8). The epimerization of **20** to thioacetate **23** was performed with Tf_2O and pyridine, followed by KSAc in DMF.[36] The acetate **23** was converted to the corresponding thiol **24** under Zémplen conditions in 91% yield. Glycosylation of thioacceptor **24** with trichloroacetimidate **19** afforded **22** in a good yield of 64% (Scheme 9).



Scheme 9: a) TMSOTf, CH_2Cl_2, -40 ˚C, **b)** *i.* $Pd(PPh_3)_4$, AcOH, 60 ˚C, *ii.* CCl_3CN, DBU, CH_2Cl_2.

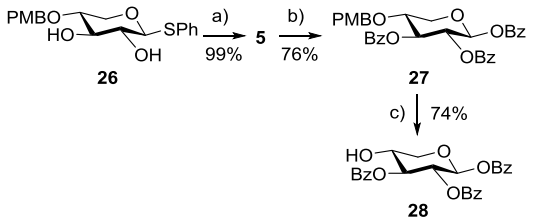
The anomeric position of the reducing end of the *S*-disaccharides **6** and **22** was deprotected with Pd(0). Allyl deprotection of **22** at 40 ˚C did not proceed to completion. Therefore, the reaction was performed at 60 ˚C for 15 min affording the hemiacetal in 86%. The deallylated structures were then converted in the corresponding trichloroacetimidates **25** and **3** under standard conditions (Scheme 9 and 10).[41,42]



Scheme 10: *i.* $Pd(PPh_3)_4$, AcOH, 40 ˚C, *ii.* CCl_3CN, K_2CO_3, CH_2Cl_2.

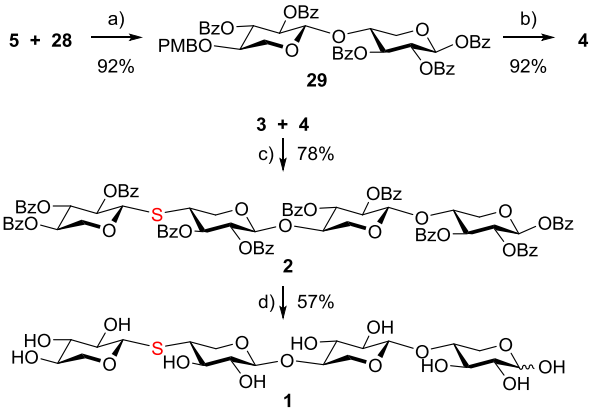
The synthesis of the *O*-disaccharide acceptor **4** starts with benzoylation of diol **26**, which was prepared following

literature procedures (Scheme 11).[43–46] The anomeric thioacetal of **5** was hydrolyzed and benzoylation afforded compound **27** in 70% yield[47]. The 4-position was deprotected with DDQ to provide acceptor **28** in 74% yield.[48]



Scheme 11: a) BzCl, pyridine, **b)** *i*. NBS, acetone/H_2O (10:1), *ii*. BzCl, Et_3N, CH_2Cl_2, **c)** DDQ, CH_2Cl_2/H_2O (9:1).

The coupling between thioglycoside donor **5** and acceptor **28** afforded **29** in 92% yield by employing the promoter system NIS/TMSOTf. The PMB group of **29** was removed to gain acceptor **4**, which was reacted with the *S*-disaccharide donor **3** in CH_2Cl_2 and TMSOTf affording tetrasaccharide **2** in 78% yield. Glycosylation with disaccharide **25** resulted in an α/β-ratio of 1:1 and was not pursued further. The final step to gain *S*-linked tetraxylan **1** was global deprotection under basic conditions.



Scheme 12: a) NIS, TMSOTf, CH_2Cl_2, -35 °C, **b)** DDQ, CH_2Cl_2/H_2O (9:1), **c)** TMSOTf, CH_2Cl_2, MS 4Å, -30 °C, **d)** 1 M NaOH, MeOH.

Different strategies for the assembly of *S*-linked disaccharides have been investigated, both involving 1-thioglycoside donors and thioacceptors. In the latter strategy, the leaving group of the donor proved to be critical for the success of the coupling. When the procedure involved 1-thioglycosides, the protecting groups present on the acceptor was shown to influence the stability of the C4-triflate acceptor greatly and therefore also affect the yield of the coupling reaction. In both routes, side-products were determined, where disulphide formation and elimination of the triflate were observed as the major challenges. Both approaches have shown to be useful for the synthesis of the thiolinkages in oligoxylans assembly. However, the method involving 1-thioglycosides was found to be often preferable due to the minor amounts of byproducts obtained in the reaction mixture.

Target **1** has been synthesized by a 2+2 block-strategy, which involves the final coupling of an *S*-disaccharide and an *O*-disaccharide. The synthesis of other *S*-linked oligoxylans, as well as their application as enzyme inhibitors, is undergoing in our laboratories.

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