Glycosylation

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Hydrogen Bond Mediated Aglycone Delivery: Synthesis of Linear and Branched α-Glucans**

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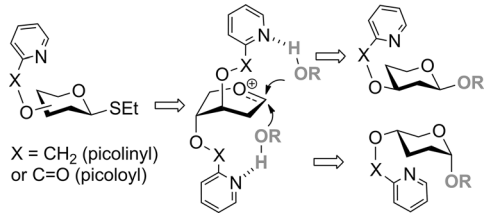
Abstract: *A Hydrogen bond mediated aglycone delivery (HAD) method was applied to the synthesis of α-glucans, which are abundant in nature, but as targets represent a notable challenge to chemists. The synthesis of linear oligosaccharide sequences was accomplished in complete stereoselectivity in all glycosylations. The efficacy of HAD may diminish with the increased bulk of the glycosyl acceptor, and may be an important factor for the syntheses of oligomers beyond pentasaccharides. The synthesis of a branched structure proved more challenging, particularly with bulky trisaccharide acceptors.*

Glycosylation reactions are challenging for chemists because of the necessity to obtain high stereoselectivity of substitutions, and only pure anomers are needed for biological studies or pharmaceutical development.[1] A failure to control stereoselectivity of glycosylations leads to mixtures of 1,2-*cis* and 1,2-*trans* diastereomers, which are hard to separate. As a result, stereocontrolled glycosylation has emerged as an important area of modern glycosciences and inspires many scientists, and has led to many breakthroughs.[2–27] The aim of stereocontrol of glycosylations has been approached in a variety of ways, and the effect of a neighboring acyl participating group has been among the most powerful stereodirecting factors leading to high or even complete 1,2-*trans* selectivity.[28–31] Boons and co-workers, and subsequently Turnbull et al., developed chiral auxiliary group based approaches wherein the participation takes place from the opposite face of the ring, thus leading to 1,2-*cis*-linked products with high stereoselectivity.[32–37]

Previously, our group introduced a 2-*O*-picolinyl (2-pyridylmethyl) participating group which can be used to obtain 1,2-*trans* glycosides. We demonstrated that these reactions proceed via a six-membered ring intermediate which in all cases provided complete 1,2-*trans* (or 1,2-*anti*) selectivity with respect to the 2-*O*-picolinyl group.[38,39] In an attempt to broaden the scope of this method, we placed the picolinyl group at remote positions, C3, C4, and C6. Rather unexpectedly, we obtained very high *syn* stereoselectivities, which was an indication of a different mode of action by which remote picolinyl substituents influence stereoselectivity of glycosylation. To explain the stereoselectivity observed, we acquired experimental evidence consistent with a new concept we call hydrogen bond mediated aglycone delivery (HAD).[40] The nucleophile, the hydroxyl group of the glycosyl acceptor, forms a hydrogen bond with the picolinyl nitrogen atom of the glycosyl donor and is then delivered to form the glycosidic linkage from the same face (*syn*) with respect to that of the picolinyl group (Scheme 1). This reaction typically results in higher rates of glycosylations in comparison to that of the corresponding benzylated glycosyl donors. This difference was particularly evident for experiments performed at low concentrations (5 mM of glycosyl acceptor).[40,41]

Using *O*-picolinyl and structurally similar 4-*O*-picoloyl (2-pyridinecarbonyl; Pico) substituents, we obtained different linkages ranging from relatively accessible 1,2-*trans* glycosides to much more challenging 1,2-*cis* glycosides of the D-gluco,[40] D-manno,[41] and L-rhamno series.[40] Yang and co-workers applied the same principle to 2-quinolinecarbonyl-assisted synthesis of β-arabinofuranosides.[42] As an extension of the earlier study, herein we report the application of HAD to connect multiple 1,2-*cis*-linked residues.

We first approached the synthesis of a linear pentasaccharide connected through α-(1→4)-linkages, thus postulating that 4-*O*-picoloyl could be used both as the stereodirecting group and as the temporary substituent. Picoloyl can be selectively removed with copper(II) acetate,[41,43] reaction conditions under which many protecting groups are stable.[44–49] The synthesis depicted in Scheme 2 began with the glycosylation of the acceptor **2**[50] with the 4-*O*-picoloylated ethyl thioglycoside donor **1**. This reaction was performed in the presence of DMTST (2 equiv) and −30 °C→



Scheme 1. Hydrogen bond mediated aglycone delivery (HAD) through remote picolinyl/picoloyl substituents.

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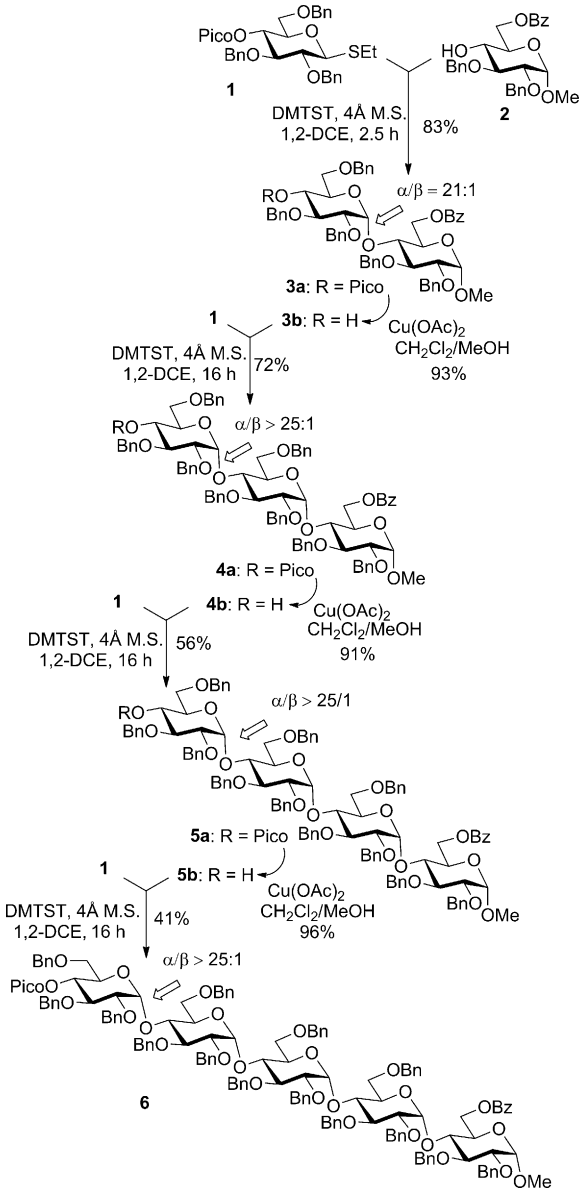


Scheme 2. Pentasaccharide synthesis by sequential picoloyl-mediated glycosylation/deprotection. Bz = benzoyl, 1,2-DCE = 1,2-dichloroethane, DMTST = dimethyl(methylthio)sulfonium triflate, M.S. = molecular sieves.

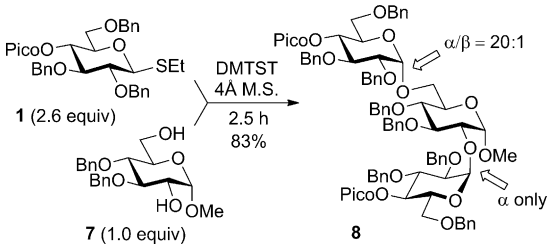
RT, conditions which became standard for the HAD approach. As a result, the disaccharide **3a** was obtained in 83% yield and with a high stereoselectivity of $\alpha/\beta = 21{:}1$. The diastereomers were separated using column chromatorgraphy, and the anomerically pure **3a** was treated with $Cu(OAc)_2$ to give the 4′-OH acceptor **3b**. The latter was glycosylated with **1** under standard HAD glycosylation conditions to afford the trisaccharide **4a** in 72% yield and complete α-stereoselectivity, which is conservatively reported as $\alpha/\beta >$ 25:1 in Scheme 2. Depicoloylation of **4a** gave the glycosyl acceptor **4b**, which was coupled with **1** to afford the tetrasaccharide **5a** in 56% yield and complete α-selectivity. Subsequent depicoloylation gave the acceptor **5b** and coupling with **1** produced the pentasaccharide **6** in 41% yield with complete α-selectivity.

Evidently, over the course of this synthesis, the deprotection efficiency remains high (yields 91–93%), whereas glycosylation yields drop steadily. We explain this by the increased bulk of the acceptor and the inability of the glycosyl donor to maintain the strong hydrogen bonding essential for rapid reactions and high stereoselectivity. Our attempt to push the reaction by adding more DMTST resulted in higher yields, but the stereoselectivity dropped. We believe that this decrease in stereoselectivity is because excess DMTST blocks the picoloyl nitrogen atom and makes it unavailable to perform HAD.[40] Nevertheless, the stereoselectivities remained complete, and the synthesis wherein the same substituent can be used both for controlling the stereoselectivity and as the selectively removable protecting group, offers a useful strategy for streamlining the synthesis of 1→4-linked α-glucans.

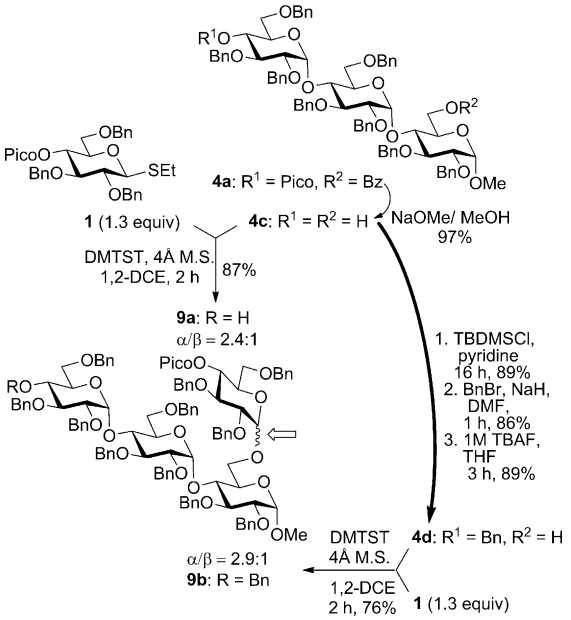
Subsequently, we turned our attention to investigating whether this methodology would offer viable access to branched oligosaccharides, which are common in many mammalian and bacterial systems. In particular, we became interested in synthesizing the trisaccharide repeating unit of *Lactobacillus* spp. G-77, consisting of primary 1→6 and secondary 1→2 α-gluco linkages.[51–53] Concomitant α-glucosylation of the 2,6-diol acceptor **7**[54] with an excess of **1** (2.6 equiv) under standard HAD conditions afforded the branched trisaccharide **8** in 83% yield and a nearly complete stereoselectivity (Scheme 3).

Another target which has drawn our attention is the 4,6-branched glycogen-like fungal cell wall α-glucan motif from *Pseudallescheria boydii*, which showed potential activity towards fungal phagocytosis and activation of innate immune responses.[55,56] This structure inspired other synthetic work in the area, and chiral auxiliary assisted synthesis by Boons and co-workers has emerged.[57] For our synthesis of the 4,6-branching motif we adapted **4a** which was obtained en route to **6**. The compound **4a** was treated with 2 M NaOMe in MeOH to affect concomitant removal of 6-*O*-benzoyl and 4″-*O*-picoloyl protecting groups (Scheme 4). The resulting 4″,6-diol acceptor **4c** was glycosylated with **1** (1.3 equiv) under standard HAD conditions to obtain the branched tetrasaccharide **9a** in 87% yield. The coupling was entirely regioselective at the primary position and no side products resulting from glycosylation of 4″-hydroxy group were obtained. In contrast to all other glycosylations with **1**, this glycosylation gave poor stereoselectivity ($\alpha/\beta = 2.4{:}1$), but the pure α-linked tetrasaccharide **9a** could still be isolated in 61% yield.

It occurred to us that having an additional hydroxy group in the acceptor unit may result in the formation of other



Scheme 3. Synthesis of the trisaccharide **8** representing the repeating unit by *Lactobacillus* spp. G-77.



Scheme 4. Low selectivity observed in the synthesis of the branched α-glucans **9a** and **9b**. DMF = *N*,*N*-dimethylformamide, TBAF = tetra-*n*-butylammonium fluoride, TBDMS = *tert*-butyldimethylsilyl, THF = tetrahydrofuran.

hydrogen bonds which would cause slower reactions and, possibly, lower stereoselectivity. To rule out this possibility, we protected the 4″-hydroxy group of **4c** by sequential 6-*O*-silylation, 4″-*O*-benzylation, and desilylation, as shown on the bypass route in Scheme 4, to obtain the 6-OH acceptor **4d** in 88% yield overall. Coupling of **1** with **4d** under HAD conditions produced the tetrasaccharide **9b** in 76% yield. Unfortunately, the stereoselectivity of this coupling was still low ($\alpha/\beta = 2.9{:}1$) and represented only a marginal improvement. The observed low stereoselectivity from both mono-ol and diol acceptors led us to conclude that the presence of the other hydroxy group has no prevailing effect on the stereoselectivity of HAD. Arguably, other factors, such as double stereodifferentiation, or simply low facial accessibility of the 6-hydroxy group of the trisaccharide acceptors **4c** or **4d** with **1** was responsible for this result.

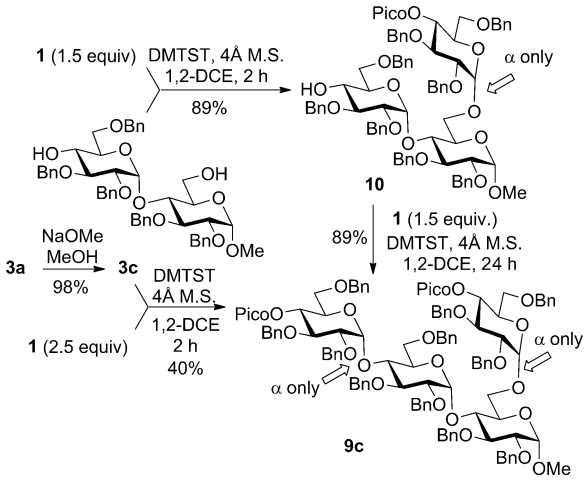
Since the stereoselectivity was poor in the final step of the synthesis of **9a**, we decided to investigate whether other classes of glycosyl donors would provide superior selectivity in comparison to that obtained with **1**. Disappointingly, and somewhat surprisingly, glycosyl donors of all other common series, including aryl thioglycosides and O/S-imidates, gave lower stereoselectivity. For glycosyl donors with nitrogen-containing leaving groups (O,S-imidates) we relate reduced stereoselectivity to the fact that the glycosyl acceptor may form hydrogen bonds with the leaving group rather than with the picoloyl nitrogen atom. The origin of lower stereoselectivity observed within the *S*-phenyl/tolyl series remains elusive. It may be related to the observed lower reactivity of these glycosyl donors in comparison to that of *S*-ethyl donors in the presence of DMTST. This lower reactivity may lead to DMTST interfering with picololyl and hence blocking the nitrogen atom from forming the hydrogen bond with the glycosyl acceptor. The full experimental account of this work will be presented elsewhere.

To reduce the influence of the steric bulk which might have prevented us from getting high stereoselectivity for the synthesis of **9a** and **9b**, we altered our strategy as follows. The disaccharide **3a** was treated with 2 M NaOMe in methanol to affect concomitant debenzoylation and depicoloylation to afford the 4′,6-diol **3c** (Scheme 5). The coupling of **3c** with **1** under HAD conditions proceeded regioselectively, and most importantly, stereoselectively, thus providing the trisaccharide **10** in 89% yield as a pure α-anomer. Subsequent glycosylation of the 4′-hydroxy group in **10** with **1** was also successful and the branched tetrasaccharide **9c** was obtained in 89% yield with complete α-stereoselectivity. We also attempted glycosylation of the diol acceptor **3c** with excess **1** (2.5 equiv). In this case, we also achieved complete stereoselectivity for the formation of both glycosidic linkages, but the tetrasaccharide **9c** was obtained in only 40% yield. All attempts to push the reaction with the use of extra DMTST led to higher yields albeit scrambled stereoselectivity.

In conclusion, we focused the HAD method on the synthesis of α-glucans, which are abundant in nature, but as targets represent a notable challenge to chemists. The synthesis of linear oligosaccharide sequences was accomplished with complete stereoselectivity in all glycosylations ranging from a disaccharide up to a pentasaccharide. The efficacy of HAD may diminish with the increased bulk of the glycosyl acceptor, and may be an important factor to consider when attempting syntheses of longer oligosaccharide sequences. The synthesis of the branched structure was proven more challenging, particularly with bulky trisaccharide acceptors.

Experimental Section

General procedure for glycosylation in the presence of DMTST (commonly referred to as "HAD conditions"): A mixture of a glycosyl donor (0.13–0.15 mmol for mono- or 0.26 mmol for diglycosylations), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4 Å, 200 mg) in $(ClCH_2)_2$ (2.6 mL or 26 mL for 10× dilution) was



Scheme 5. Stepwise and one-pot synthesis of the branched tetrasaccharide **9c**.

stirred under argon for 1 h. The mixture was cooled to −30 °C and DMTST (0.26–0.50 mmol) was added. The resulting mixture was warmed to RT over a period of 1 h and was stirred at RT for the time specified in the schemes. Triethylamine (0.3 mL) was added and the resulting mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 (10 mL), the solid was filtered off, and the residue was washed sucessively with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (10 mL) and water (3 × 10 mL). The organic phase was separated, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (5% ethyl acetate/hexane gradient elution). Anomeric ratios (or anomeric purity) were determined by comparison of the integral intensities of relevant signals in the ^{1}H NMR spectra.

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