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Synthesis of benzyl protected β-D-GlcA-(1 → 2)-α-D-Man thioglycoside building blocks for construction of *Cryptococcus neoformans* capsular polysaccharide structures

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ABSTRACT

In a project targeting the synthesis of large oligosaccharide structures corresponding to the *Cryptococcus neoformans* GXM capsular polysaccharide, an easy access to thiodisaccharide building blocks comprising a β-linked glucuronic acid moiety and a 6-*O*-acetyl group was required. Several pathways to such building blocks have been investigated, addressing the problem of constructing a β-linked glucuronic acid residue protected with groups that are orthogonal to a primary acetyl group. Two efficient routes have been developed, one using benzoylated glucosyl donors to form the β-linkage followed by a change of protecting groups to benzyls and subsequent introduction of the carboxyl function and the acetyl group. The second route explored the possibility to achieve β-selectivity using glucuronyl donors without acyl protecting groups. BF_3-etherate promoted glycosylations with benzyl (2,3,4-tri-*O*-benzyl-α-D-glucupyranosyl)uronate trichloroacetimidate in the presence of nitrile solvents and at low temperatures reproducibly gave good yields of disaccharides with high β-selectivity. Furthermore, the use of recently reported glucuronyl thioglycoside donors protected with a cyclic 2,4-silylene acetal was found to represent another efficient and completely β-selective way to desired disaccharide building blocks.

In a project aiming at developing glycoconjugate vaccines against the fungi *Cryptococcus neoformans*,[1,2] we are building a library of *Cryptococcus neoformans* oligosaccharide structures ranging from disaccharides up to octadecasaccharides using a building block approach. To accomplish this, access to large quantities of relevant disaccharide building blocks is essential. For the synthesis of *C. neoformans* Serotype A and D structures two variants of disaccharide blocks are required, a β-D-glucopyranosyluronic acid-(1 → 2)-D-mannose type and a β-D-xylopyranosyl-(1 → 2)-D-mannose type (Fig. 1). Of the two glucuronic acid-containing building blocks, the one containing a 6-*O*-acetate (R^1 = Ac) is the major target.

The presence of acetates in target structures severely complicates synthesis since it more or less excludes the use of ester protecting groups as permanent protecting groups. High-yielding methods have been developed for the synthesis of the xylose-containing disaccharide blocks on a large scale and with good reproducibility.[3] The β-xylopyranoside-linkage is constructed using benzoyl-protected xylosyl donors, followed by change of benzoyl to benzyl protecting groups and subsequent introduction of the 6-*O*-acetate. Synthesis of the β-glucuronic acid-containing blocks is however more difficult mainly due to the β-configuration in combination with the notoriously difficult benzylation of glucuronic acid derivatives. Applying the same procedure as for the xylosyl blocks, that is using an acylated glucuronyl donor, gave, after optimization of the benzylation step, acceptable yields of the desired blocks,[4] but there were problems with reproducibility of yields in the benzylation step and the reaction could not be performed on a large scale. Hence, another approach was developed in which a glucosyl donor was employed, and the carboxylic function, as well as the acetate, was introduced after the benzylation step.[5] We here report on further optimization of this route (Schemes 1 and 2) as well as new approaches utilizing non-acyl protected glucuronyl donors (Schemes 3–5) toward the target glucuronic acid-containing disaccharide building blocks.

For reasons relevant to the subsequent use of the disaccharide blocks in the construction of larger *Cryptococcus* structures, we changed the temporary protecting group from the allyl group used so far to a 2-naphtalenylmethyl (NAP) group, starting the synthesis from acceptor **2** instead of **1**. Since large scale synthesis was the target we decided to also change the glucosyl donor used from the bromide **3** to the trichloroacetimidate donor **4**, allowing the use of catalytic amount of promoter and avoiding the use of large

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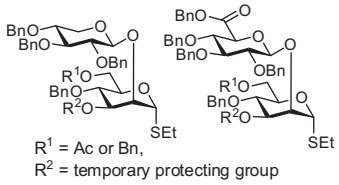
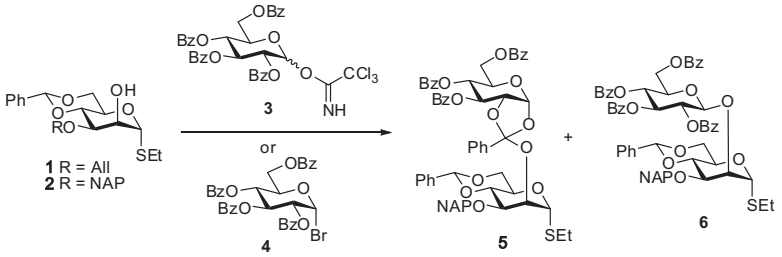


Figure 1. Required *C. neoformans* disaccharide building blocks.

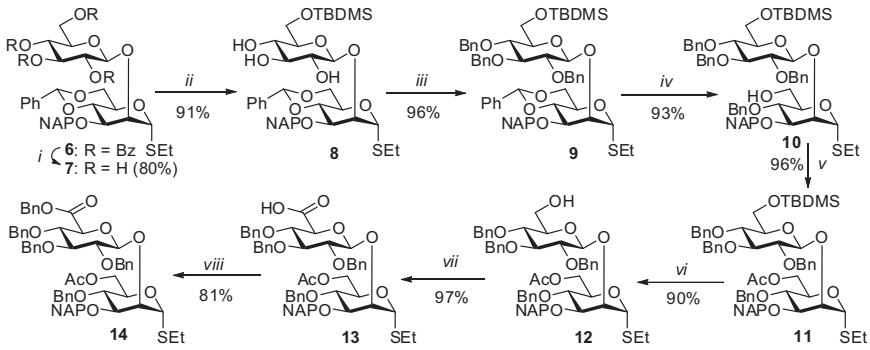
amounts of silver salts. Initially, this caused problems since the orthoester **5** was the product rather than the glycoside **6**. The standard solution to this problem, that is, using more acidic conditions (including acid-washed molecular sieves) and longer reaction times to allow any formed orthoester to rearrange in situ, worked well and after optimization (Scheme 1, Table 1) reproducible yields of ~90% of the β-linked disaccharide **6** were obtained even on 10 g scale glycosylations.

The continued transformation of **6** essentially followed the earlier published procedure[5] but by changing the order of some reaction steps and through optimization of each step it was possible to improve the overall yield in the synthesis of the target building block **14** from 15% up to a 40% overall yield over the eight steps and perform the synthesis on a gram scale (Scheme 2).The major part of this improvement in overall yield originated from the optimization of the benzylation step of compound **8**, where a 6 → 4 silyl migration was observed. By running the benzylation at 0 °C instead of at room temperature the migration could be prevented and the yield in this step raised to 97% from the 67% earlier reported.

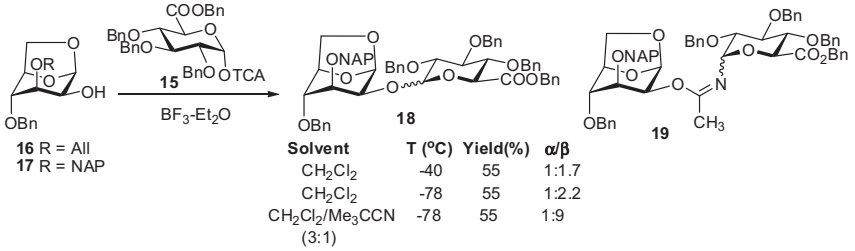
Although efficient, a drawback of this pathway is the amount of steps (eight) required at the disaccharide level, why an alternative approach using already benzylated glucuronic acid donors has been pursued in parallel. Initial experiments were performed with the known α-trichloroacetimidate donor **15**,[6] which methyl ester equivalent, using BF_3-etherate as promoter, is reported to give good yields of β-linked product (S_N2-type glycosylation) in spite of the lack of a 2-*O*-participating group.[7] We have earlier used this approach successfully in the synthesis of β-linked glucuronosyl ester drug metabolites.[6] However, initial results were quite



Scheme 1. Glycosylations with benzoylated glucosyl donors. Reaction conditions see Table 1.



Scheme 2. Transformation of disaccharide **6** into target structure **14**. Key: Reagents and conditions: (i) NaOMe, MeOH; (ii) TBDMSCl, pyridine, DMAP; (iii) NaH, BnBr, DMF, 0 → 20 °C; (iv) Et_3SiH, $PhBCl_2$, CH_2Cl_2, −78 °C; (v) Ac_2O, pyridine; (vi) TBAF·3 H_2O, THF; (vi) TEMPO, BAIB, CH_2Cl_2/H_2O; (vii) Cs_2CO_3, BnBr, DMF, 0 → 20 °C.



Scheme 3. Glycosylations with 1,6-anhydro acceptors.