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Synthesis of part structures of *Cryptococcus neoformans* serotype C capsular polysaccharide

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ABSTRACT

Cryptococcus neoformans is a fungal pathogen that can cause life-threatening infections in immunocompromised patients. The development of a vaccine based on the capsular polysaccharide of *C. neoformans* is still an open challenge due to the heterogeneity of the capsular polysaccharide and the difficulty of identifying protective epitopes. Therefore, construction of structurally defined part structures of the *C. neoformans* GXM capsule is in great demand. Herein is presented the synthesis of a 3-*O*-naphthalenylmethyl protected trisaccharide thioglycoside building block which is present in *C. neoformans* serotype C polysaccharide. Its property as a donor in a glycosylation reaction with a model acceptor has been evaluated together with its behaviour as an acceptor following removal of the temporary protecting group. The heavily branched hexasaccharide was obtained in good yields and excellent α-selectivity. The frame shifted octasaccharide structural triad motif for serotype C was also prepared following the same building block strategy. For the first time this structural motif, which is the most substituted amongst the four *C. neoformans* serotypes, was prepared. Three synthesized *C. neoformans* serotype C fragments of varying size, from penta-up to octasaccharide, were deprotected and will be included in unique glycoarrays to further investigate the possibility to develop a synthetic vaccine against this pathogen.

1. Introduction

Cryptococcus neoformans is an opportunistic pathogen that causes severe diseases, e.g. cryptococcal meningoencephalitis (cryptococcosis) and death in immunocompromised individuals, including AIDS patients [1,2] and organ transplant recipients [3] or other patients receiving immunosuppressive drugs.

A thick layer of polysaccharides envelops the fungal cells and represents an important virulence factor. The basic structural motif of the major polysaccharide, which comprises 90–95% of the whole capsule, consists of a trimer of α-D-(1 → 3)-mannoses which is substituted at OH-2 with a β-D-glucopyranosyluronic acid (GlcA) residue. Further substitutions of the mannan backbone with β-D-xylopyranosyl residues at OH-2, and/or at OH-4 give rise to four different serotypes A-D (Fig. 1). [4–7].

Although the relative abundance of the triads reported in Fig. 1 allowed for serotyping, the glucurono-xylo-mannan (GXM) polysaccharide is highly heterogeneous and minor amounts of cross-serotype substituted mannoses are found in each serotype. Also, the capsular polysaccharide contains acetyl groups at the 6 positions of the mannan backbone [8] and these are believed to be immunologically relevant at least for serotype A and serotype D. [9]. The degree of *O*-acetylation varies between the serotypes with an average of two acetates per triad for serotype A and serotype D, and lower degree of acetylation for serotype B and C.

To investigate in depth the immunological determinants of the *C. neoformans* capsular polysaccharide for the development of glycoconjugate vaccines, well-defined synthetic oligosaccharide structures are crucial.

Most approaches explored to target the synthesis of part structures of the GXM polysaccharide have required late modification on rather large structures, e.g. introduction of GlcA residues [10–15], introduction of acetyl groups [16], and oxidation of glucose 6-OH. [17]. An attempt based on this type of strategy failed in the preparation of the octasaccharide structural motif of

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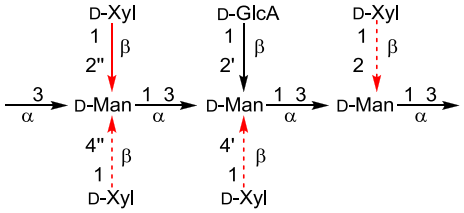

Fig. 1. Suggested structures of *C. neoformans* GXM serotype triads.

Xylose substitution

Serotype	2	2″	4′	4″
A	X	X		
B	X	X	X	
C	X	X	X	X
D		X		

serotype C. [15].

A complementary approach entails the preparation and consecutive assembly of already modified building blocks. [18–25]. In theory, only six building blocks, four disaccharides and two trisaccharides (Fig. 2), are required for the construction of any *C. neoformans* mannan variant.

In previous publications [18–21], we reported the preparation of 3-*O*-allyl protected thioglycoside building blocks and their use in the manufacture of GXM fragments. However, in light of recent findings, showing the incompatibility of the double bond and the thioethyl group in subsequent transformations [23], we are currently re-investigating the synthesis of the desired building blocks replacing the allyl group with the naphthalenylmethyl (NAP) group. [23–25]. Herein we report the preparation of a new building block **V**, and the study of its behaviour as a donor and as an acceptor in glycosylation reactions, targeting serotype C triad structural motifs.

2. Results and discussion

Concurrent introduction of the two xylose moieties appeared to be the most efficient approach for the synthesis of disubstituted thioglycoside **5**. The benzylidene ring on **1** [24] was opened regioselectively with $NaCNBH_3$/HCl [26], providing 2,4-OH acceptor **2** in a 73% yield. The coupling of **2** and **3** [27] was carried out using TMSOTf in the presence of (commercial) acid-washed molecular sieves to prevent orthoester formation, affording compound **4** in a 68% yield (Scheme 1).

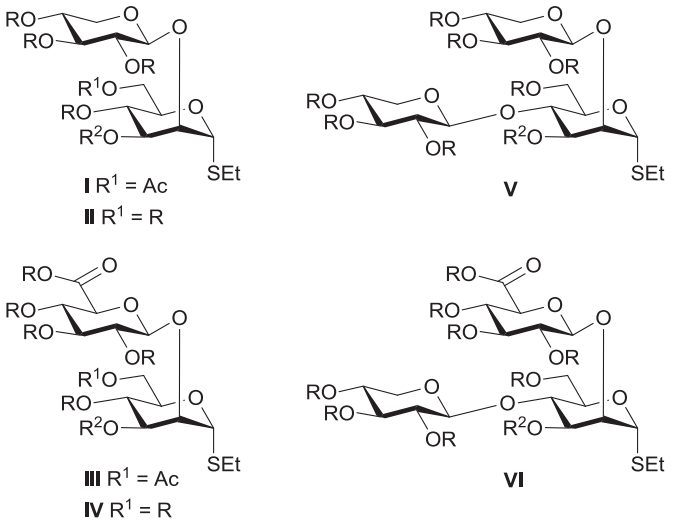

Fig. 2. Desired thioglycoside building blocks. R = persistent protecting group, orthogonal to the acetyl group; R^2 = temporary protecting group, orthogonal to R and acetyl groups.

The benzoate groups, which were required to ensure the β-selectivity in the glycosylation reaction, were then exchanged with benzyl groups giving building block **5** in an 80% yield.

For the purpose of preparing nonacetylated serotype C structures, this last step was unnecessary and **4** could have been used directly. However, owing to the large heterogeneity of the GXM capsular polysaccharide, minor quantities of this trisaccharide are also present in serotype A or D. Therefore, a 2,4-di-xylose trisaccharide building block, that only presents protecting groups compatible with acetyl groups and could be used in the synthesis of any GXM fragment, was selected.

The benzyl protected building block **5** was tested as a donor in a dimethyl(methylthio)sulfonium [DMTST]-promoted glycosylation reaction with a model mannose acceptor **6**. [19]. The reaction proceeded smoothly and afforded the spacer equipped tetrasaccharide **7** in an 84% yield with complete α-selectivity (Scheme 2). This is in agreement with results previously obtained with the analogous 3-*O*-allyl thioglycoside derivative. [19].

The removal of the NAP protecting group by reaction with DDQ in a mixture of CH_2Cl_2/t-BuOH proceeded in a 70% yield giving acceptor **8** allowing the preparation of 2,3,4-tri-*O*-glycosylated structures. Thus, acceptor **8** was reacted with disaccharide thioglycoside **9** [24], again using DMTST as a promoter. Hexasaccharide **10** was obtained with complete α-selectivity in an excellent 93% yield. The anomeric configurations were unambiguously confirmed by the one bond $^1H-^{13}C$ coupling constant values (only the mannose backbone coupling constants are reported for clarity: $^1J_{C\text{-}H}$ 173 Hz, $^1J_{C\text{-}H}$ 174 Hz, $^1J_{C\text{-}H}$ 170 Hz).

We then turned our attention to the preparation of the octasaccharide structural motif for serotype C. This is the largest and most branched structural motif of the four serotypes (A-D), having two disubstituted mannoses in the triad (Fig. 1). As mentioned earlier, the synthesis of this heavily branched structure is still an open challenge. A previous synthetic attempt by Zao and Kong [15] based on the introduction of the GlcA moiety on a heptasaccharide acceptor at the end of the synthetic pathway, a strategy proven successful for formation of a serotype B heptasaccharide [14], failed for type C.

First the spacer equipped trisaccharide **14** was prepared following the same sequence of reactions described above for building block **5** (Scheme 3). Regioselective benzylidene ring opening on **11** [25] (→**12**), followed by coupling with the trichloroacetimidate donor **3** (→**13**), and final exchange of benzoates with benzyl groups gave the desired derivative **14** in a 46% overall yield.

The standardised sequence of reactions for the structure elongation, NAP removal-DMTST promoted glycosylation, was applied to **14** affording first acceptor **15** in an 82% yield and then α-linked pentasaccharide **16** in an 82% yield after coupling with donor **9**. [24]. (Scheme 4).

Consecutive DDQ-promoted cleavage of the orthogonal protecting group gave the new acceptor **17** (68%) ready for the final glycosylation with trisaccharide thioglycoside **18** [25]. This glycosylation, for the first time, afforded the serotype C octasaccharide **19** together with its β anomer **20** in a 3:1 ratio and a 74% yield.

The incomplete α-selectivity of the glycosylation reaction is in contrast to our previous findings when the same thioglycoside donor **18** was used for preparing serotype B heptasaccharide structural motif. The difference between the two acceptors used