Synthesis of Di- and Trisaccharide Congeners of the (1→2)-β-Mannan from the Cell-wall Phosphomannan of *Candida albicans.*

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Introduction

Candida albicans is one of the most common hospital-acquired opportunistic pathogens[1] and is particularly serious for immuno-compromised patients or those undergoing long-term antibiotic treatment (Figure 1a,b). Anti-fungal drugs are only partially effective against this pathogen and immunotherapeutic approaches are currently under consideration. An especially attractive vaccine candidate is the unique (1→2)-β-mannan of the cell wall phosphomannan complex (Figure 1c).

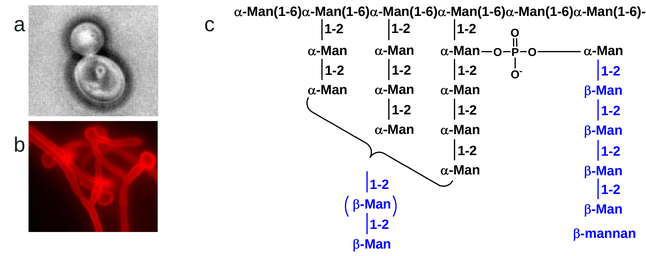
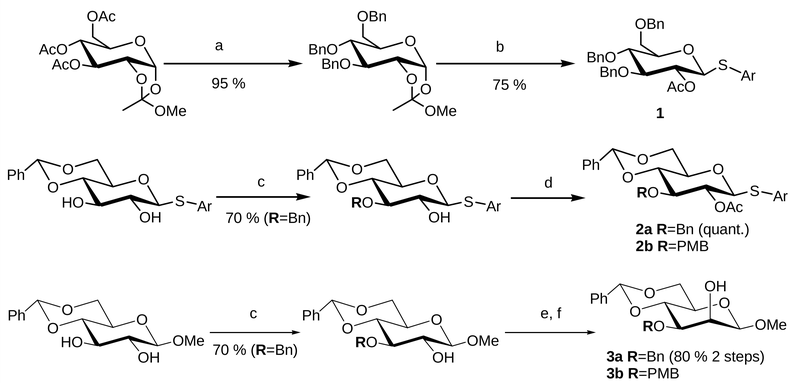


Figure 1. Growth-stages of *C. albicans* a) yeast and b) fungal-stage showing surface binding of rhodamine-labelled mAb specific for (1→2)-β-mannan; c) Cell wall phosphomannan with the (1→2)-β-mannan highlighted in blue.

Two monoclonal antibodies, B6.1 (IgM) and C3.1 (IgG3), raised against the cell-wall phosphomannan of *C. albicans*[2,3] are specific for (1→2)-β-mannan and provide passive protection against *C. albicans* challenge in mice. Remarkably both antibodies exhibit inhibition profiles with 1,2-linked β-mannan homo-oligomers that show di- and trisaccharides to be significantly more effective inhibitors than tetra- up to hexasaccharides.

We have undertaken a project to map out the key polar-contacts of synthetic di- and trisaccharide epitopes of (1→2)-β-mannan with these antibodies. Chemical mapping of the antibody-binding site by functional group replacement involves the use of mono-deoxy and mono-*O*-methyl congeners[4]. A detailed understanding of the key polar-contacts will provide insight into the size and topology of the protective epitope with the potential to guide the design of conjugate vaccines. Here we describe progress towards the chemical synthesis of di- and trisaccharide congeners of (1→2)-β-mannan.

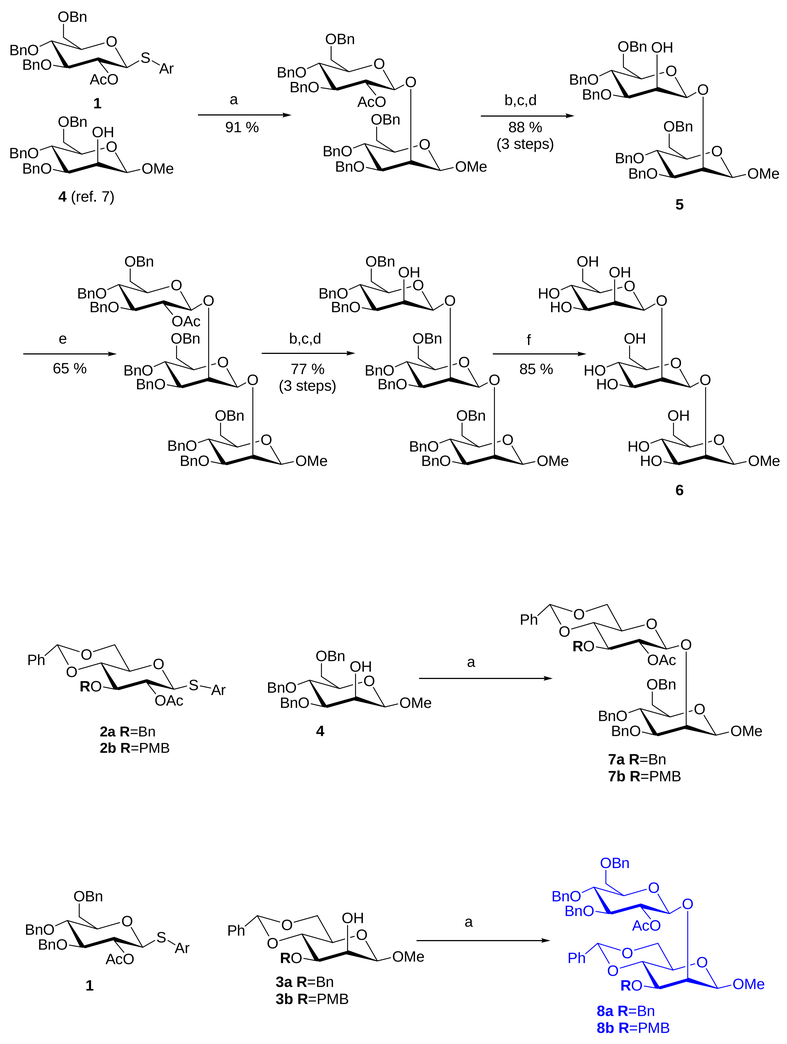
Scheme 1: The synthetic strategy relies on the use of thio-glucoside donors (**1, 2a** and **2b**) with neighbouring participation to afford the β-glycosylation product with β-mannose acceptors (**3a**, **3b** and **4**).



Reagents and Conditions: a) KOH, BnBr, THF (ref 5); b) *p*ClPhSH, BF_3:OEt_2, DCM; c) NaH, $CuCl_2$, BnBr (or PMBCl), TBAI, THF, reflux (ref. 6); d) Ac_2O, Pyridine; e) DMSO, Ac_2O; f) $NaBH_4$, DCM-MeOH, 0 °C. (Ar = *para*-chloro-phenyl).

Scheme 2: Thio-glucoside donors (**1**, **2a** or **2b**) were reacted with β-mannose acceptors (**3a**, **3b** or **4**) to yield the β-glucoside intermediates with high selectivity.

Deprotection under Zemplén conditions followed by oxidation-reduction afforded the (1→2)-β-mannoside product.



Reagents and Conditions: a) NIS, TfOH, DCM, -78 °C; b) NaOMe, MeOH; c) DMSO, Ac_2O; d) L-Selectride, THF, -78 °C; e) **1**, NIS, TfOH, DCM, -78 °C; f) 10 % Pd/C, MeOH. (Ar = *para*-chloro-phenyl).

Acknowledgements

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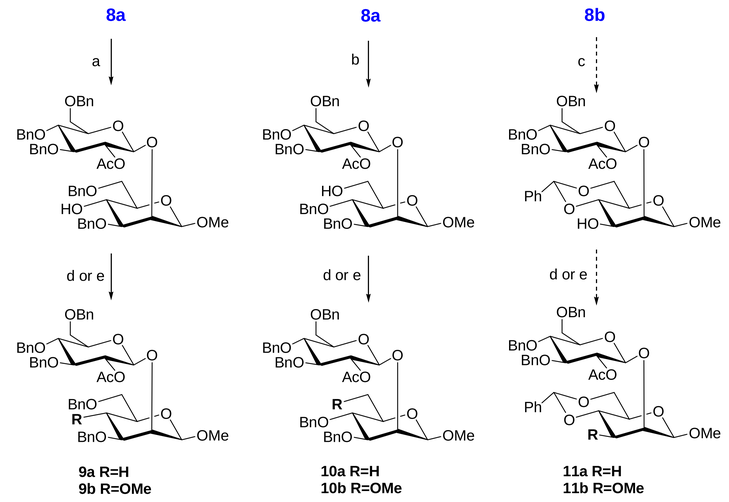
Scheme 3: The strategy for synthesis of the (1→2)-β-mannan congeners relies on the regioselective deprotection of intermediates (**7a,b** and **8a,b**) to access each hydroxyl group. Methylation or Barton deoxygenation will provide the mono-*O*-methyl or mono-deoxy congeners respectively.



Reagents and Conditions: a) $NaCNBH_3$, HCl, Et_2O, THF, 3Å ms, 0 °C; b) BH_3:THF, $Cu(OTf)_2$ (ref. 8); c) DDQ, DCM; d) CH_3I, NaH, THF; e) i) Dithiocarbonyl diimidazole, $PhCH_3$, 110 °C; ii) Bu_2SnO, AIBN, $PhCH_3$, 110 °C; (Ar = *para*-chloro-phenyl).

Summary

The synthesis of disaccharides 7**a** and **8a** has been secured. Mono-deoxy congener **9a** has been synthesized and current efforts are focused on the remaining congeners. The acceptor **5** allows for preparation of trisaccharide congeners modified at the non-reducing residue. Regioselective protection and deprotection allows for ready access to prepare each mono-deoxy or mono-*O*-methyl congener.

Inhibition data from ELISA will be collected when the syntheses of these compounds are completed. The inhibitory activities of these compounds will be used to define the size and topology of the epitope's contact surface with the binding sites of the two protective monoclonal antibodies.

The epitope binding motif for trisaccharide **6** with these antibodies will also be defined by saturation transfer difference NMR (STD-NMR).

References

1. Centers for Disease Control and Prevention. (1996) National nosocomial infections surveillance (NNIS) report, data summary from October 1986-April 1996. *Am. J. Infect. Control*, 24, 380-388.
2. Han, Y.; Riesselman, M.H.; Cutler, J.E. *Infect. Immun.* **2000,** *68*, 1649-1654.
3. Nitz, M.; Ling, C.C.; Otter, A.; Cutler, J.E.; Bundle, D.R. *J. Biol. Chem.* **2002,** *277*, 3440-3446.
4. Nikrad, P.V.; Beierbeck, H.; Lemieux, R.U. *Can. J. Chem.* **1992,** *70*, 241.
5. Lichtenthaler, F.W.; Schneider-Adams, T. *J. Org. Chem.* **1994,** *59*, 6728-6734.
6. Eby, R.; Webster, K.T.; Schuerch, C. *Carb. Res.* **1984**, *129*, 111-120.
7. Nitz, M.; Purse, B.W.; Bundle, D.R. *Org. Lett.* **2000**, *2*, 2939-2942.
8. Shie, C.R.; Tzeng, Z.H.; Kulkarni, S.S.; Uang, B.J.; Hsu, C.Y.; Hung, S.C. *Angew. Chem. Int Ed.* **2005**, *44*, 2-5.