Orthoester-Based Strategy for Efficient Synthesis of the Virulent Antigenic-1,2-Linked Oligomannans of *Candida albicans*

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Dedicated to the memory, inspiration and teachings of Ray Lemieux, our mentor or grandmentor. The work described herein is possible because of his pioneering studies on 'Replacement reactions at the anomeric center', which led directly to the ready synthesis of glycosyl orthoesters and their use as glycosyl donors.

Abstract: Orthobenzoates of glucose and mannose provide donor and acceptor partners to produce a disaccharide bearing a benzoyl group at the site where *gluco* to *manno* conversion is required, the inverted center being ready to function, iteratively, as the next acceptor for the *gluco n*-pentenyl orthobenzoate to extend the oligomannan.

Key words: *n*-pentenylorthoesters, β-oligomannosides, iterative synthesis, structure verification

The spectrum of diseases[1] caused by *Candida albicans* exceeds that of most other microorganisms, this virulence being attributable to the pathogen's ability to survive in several anatomically distinct sites.[2] The organism's cell wall phospholipomannan, first identified by Shibata and co-workers as a β-1,2-linked oligomannan,[3] is the seat of its activity. Rees' 1971 prediction that such oligomannans would display α-helicity,[4] is fraught with structural and biological implications,[5] fulfilled by observations of Poulain[6] and Bundle[7] on the correlation of periodic biological activity with chain length. Our long-standing interest in glycosylphosphatidylinositols (GPIs),[8] was captured by Poulain's observation that *C. albicans* triggers 'intense signaling and secretory responses' similar to those that are induced by GPI-related glycolipids.[9]

In this paper, we describe an *n*-pentenyl orthoester (NPOE)-based approach that is simple and totally iterative, using identical methodologies for synthetic as well as structure verification protocols. It should be noted that although the orthoester **1** and 2-O-benzoyl NPG **3** are functionally equivalent, the former (a) usually gives much higher yields of *trans*-coupled products,[10,11] and (b) reacts infinitely more quickly as measured by our procedure for comparing relative reactivity of glycosyl donors.[12]

The above biological concerns have induced much activity towards the synthetically defiant[13] β-1,2-mannoside motif. Sinay and co-workers[14] employed the classical Garegg protocol[15] to prepare a tetramer. Elegant procedures developed by Bundle[7] and Crich[16] have afforded structures in the M4-M8 range, and recently Bundle reported detailed ^{1}H NMR analysis of a pentamannan[7a] which confirms the 'compact configuration' predicted by Rees.[4]

The iterative cycle is depicted in Scheme 2. The high yields maintained throughout, have afforded all units, including the 8-mer, in ca 200 mg amounts. The key starting glycosyl orthoesters **1** and **2**, are easily prepared[10] from the corresponding glycoses in five simple, identical steps using 4-pentenol and benzyl alcohol respectively. Treatment of each with ytterbium (III) triflate[17] or TBDMSOTf effects quantitative rearrangement to the *n*-pentenyl and benzyl glycosides **3** and **4** respectively, and debenzoylation of the latter gives the starting acceptor **5**, which constitutes the reducing end unit of the prospective oligomannan

Coupling of **5** with NPOE **1** mediated by TBDMSOTf/NIS gave the glucoside **6** in 92% yield, which carries a single benzoyl group. The exclusive use of benzyl protecting groups elsewhere provided a convenient window for monitoring the upcoming transformations leading to **7** by ^{1}H and ^{13}C NMR spectroscopy (Scheme 1). Thus, employing the Garegg strategy,[14,15] compound **6** was deesterified, oxidized and reduced (with L-selectride as pioneered by Danishefsky[18]) leading to the dimannan **7** in excellent yield.

The iterative protocols,[19] for both synthesis and structure analysis remained constant up to the 8-mer (i.e. **15**, n = 6), Scheme 2, without any need to tamper with protecting groups. As the synthesis progressed the acceptor, generated in the previous step, became more and more precious, and so a generous excess of the easily prepared donor **1** was applied. Normally ketone **14b**, obtained by Swern oxidation,[20] was not isolated but was reduced directly with L-selectride.[21]

The critical use of NPOE **1** as donor gave only a trace, if any, of *cis*-coupled product thereby simplifying purification and monitoring of subsequent transformations. Thus the 5.6 ppm region of the ^{1}H NMR spectra of the coupling products, exemplified for **7**–**10** in Figure 1A characteristically shows a triplet ca 5.4 ppm which is consistent with H2 of the non-reducing end (*gluco*) entity. Upon the debenzoylation to **14a**, the 5–6 ppm window is no longer useful; however, ^{13}C NMR provides excellent resort. Thus as shown in Figure 1C, C2 occurs ca 105 ppm in the *gluco*

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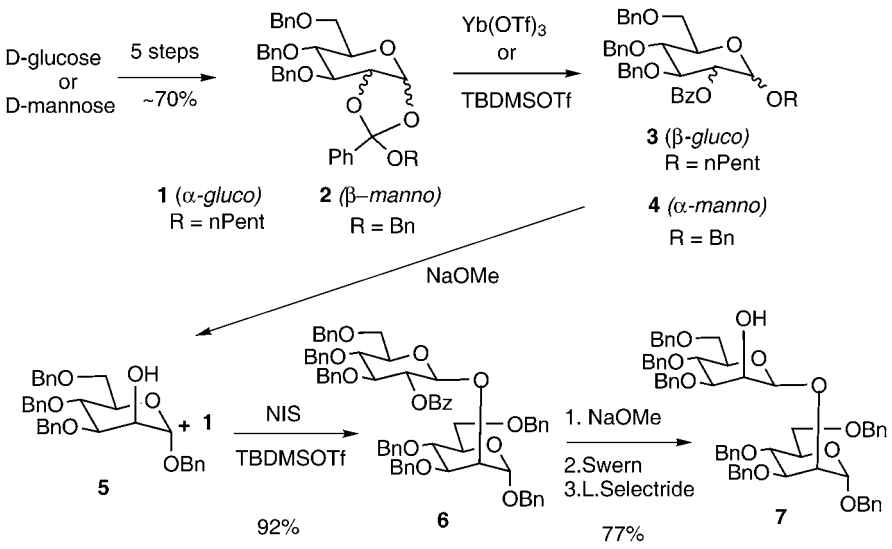
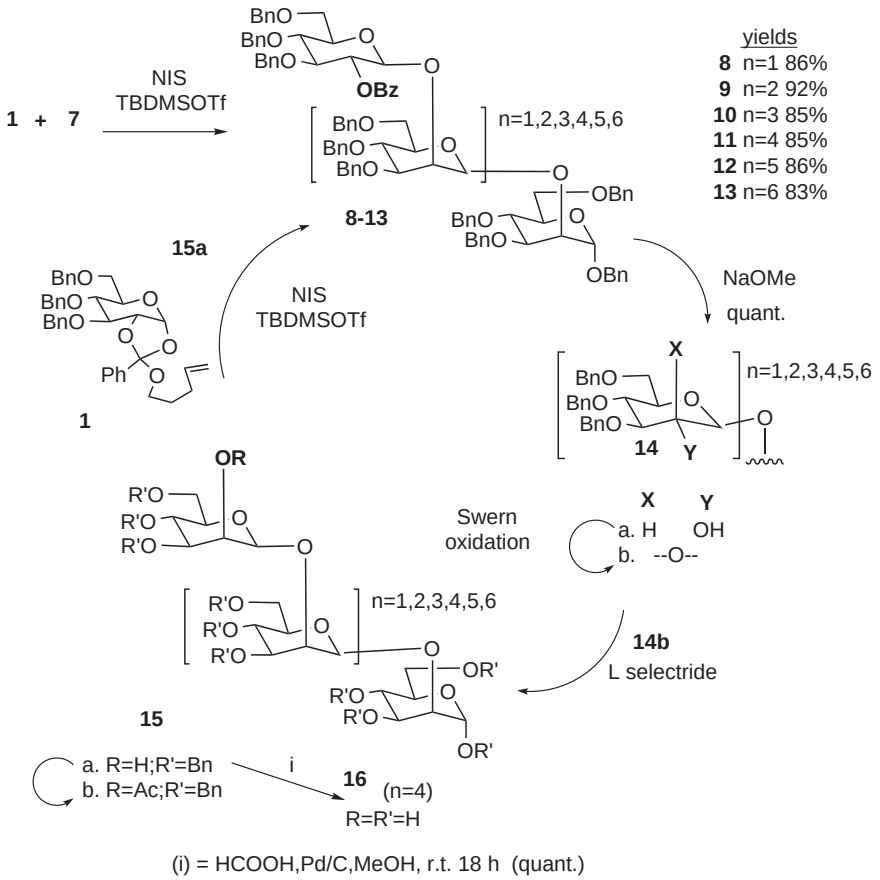


Scheme 1



Scheme 2

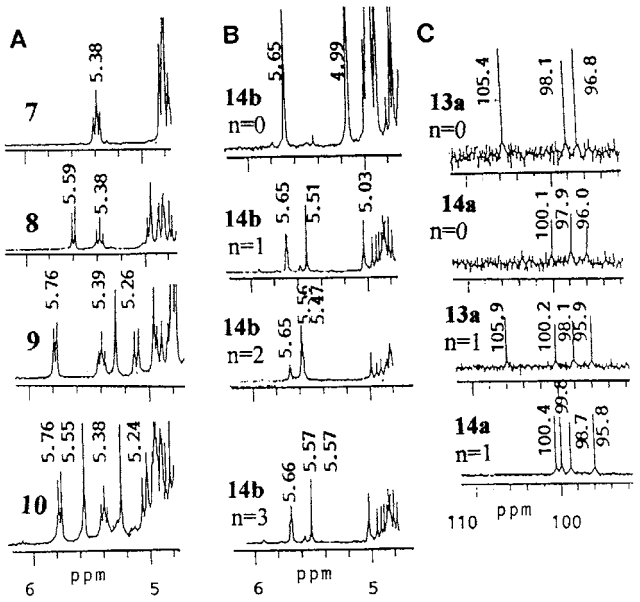


Figure 1 ^{1}H NMR and ^{13}C NMR 'windows' for monitoring transformations of selected substrates.

moiety (**13a**, n = 0 and 1), but ca 100 ppm in the *manno* (**14a**, n = 0 and 1). The stereoselectivity of the L-selectride reduction,[18] could also be easily checked by studying the ^{1}H NMR spectrum of the acetylated products **15b** (n = 0–3) which showed the H2 proton of the newly introduced *manno* units to be shifted downfield to ca 5.6 ppm, Figure 1B.

Interestingly the anomeric proton of the *gluco*-moiety of **7** is ca 4.55 ppm for n = 0 (not shown in Fig 1A) and moves downfield to 5.59 for **8** (n = 1) and 5.76 for both **9** (n = 2) and **10** (n = 3). The spectroscopic data for the 8-mer (**15a**, n = 6) fully support the assigned structure.[22]

Debenzylation is most conveniently carried out by transfer hydrogenation using formic acid. For example **15a** (n = 4) gave **16** quantitatively. Detailed analyses of chemical shifts in protected and deprotected samples of oligomers will be reported in due course.

In summary, once compounds **1** and **5** are in hand, one cycle of the protocol shown in Scheme 2 can be accomplished in 2–3 days, allowing for rigorous characterization of each intermediate. The data in Scheme 2 show that excellent yields are maintained as the array is lengthened. Accordingly, the 9–16-mers of β-1,2-oligomannans, which occur in the cell wall of *C. albicans* should be obtainable by this iterative procedure.

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References

(1) (a) Pugliese, A.; Torre, D.; Baccino, F. M.; Di Perri, G.; Cantamessa, C.; Gerbaudo, L.; Saini, A.; Vidotto, V. *Cell Biochem. Funct.* **2000**, *18*, 235. (b) Bektic, J.; Lell, C. P.; Fuchs, A.; Stoiber, H.; Speth, C.; Lass-Florl, C.; Zepelin, M. B.; Dierich, M. P.; Wurzner, R. *FEMS Immunol. Med. Microbiol.* **2001**, *31*, 65. (c) Cassone, A.; Cauda, R. *Trends Microbiol.* **2002**, *10*, 177.

(2) Calderone, R. A.; Fonzi, W. A. *Trends Microbiol.* **2001**, *9*, 327.

(3) Shibata, N.; Ichikawa, T.; Tojo, M.; Takahashi, M.; Ito, N.; Ohkubo, Y.; Suzuki, S. *Arch. Biochem. Biophys.* **1985**, *243*, 338.

(4) Rees, D. A.; Scott, W. E. *J. Chem. Soc.* **1971**, *469*, 1971.

(5) (a) Nobuyuki, S.; Hisamichi, K.; Kikuchi, T.; Kobayashi, H.; Ikawa, Y.; Suzuki, S. *Biochemistry* **1992**, *31*, 5680. (b) Shibata, N.; Hisamichi, K.; Kobayashi, H.; Suzuki, S. *Arch. Biochem. Biophys.* **1993**, *302*, 113. (c) Cipollo, J. F.; Trimble, R. B.; Rance, M.; Cavanagh, J. *Anal. Biochem.* **2002**, *278*, 52.

(6) Jouault, T.; Lepage, G.; Bernigaud, A.; Trinel, P. A.; Fradin, C.; Wieruszeski, J. M.; Strecker, G.; Poulain, D. *Infect. Immun.* **1995**, *63*, 2378.

(7) (a) Nitz, M.; Chang-Chun, L.; Otter, A.; Cutler, J. E.; Bundle, D. R. *J. Biol. Chem.* **2002**, *277*, 3440. (b) Mitz, M.; Bundle, D. R. *J. Org. Chem.* **2001**, *66*, 8411.

(8) (a) Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1985**, *111*, 8540. (b) Roberts, C.; Madsen, R.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 1546. (c) Madsen, R.; Udodong, U. E.; Roberts, C.; Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 1554. (d) Campbell, A. S.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 10387. (e) Arasappan, A.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 2401.

(9) Trinel, P.-A.; Plancke, Y.; Gerold, P.; Jouault, T.; Delplace, F.; Schwarz, R. T.; Streckers, G.; Poulain, D. *J. Biol. Chem.* **1999**, *274*, 30520.

(10) Mach, M.; Schlueter, U.; Mathew, F.; Fraser-Reid, B.; Hazen, K. C. *Tetrahedron* **2002**, *58*, 7345.

(11) Kochetkov, N. K.; Khorlin, A. Y.; Boschkov, A. F. *Tetrahedron* **1967**, *23*, 693.

(12) Wilson, B. G.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 317.

(13) Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155; *Angew Chem.*, **1982**, *94*, 184.

(14) Fradin, C.; Jovanlt, T.; Mallet, A.; Mallet, J. M.; Camas, D.; Sinay, P.; Poulain, D. J. *J. Leukocyte Biol.* **1996**, *60*, 81.

(15) Boren, H. B.; Ekborg, G.; Iklind, K.; Garegg, P. J.; Pilotti, A.; Swan, C. G. *Acta Chem. Scand.* **1973**, *27*, 2639.

(16) (a) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217. (b) Crich, D.; Li, H.; Yao, Q.; Wink, D. J.; Sommer, R. D.; Rheingold, A. L. *J. Am. Chem. Soc.* **2001**, *123*, 5826.

(17) Jayaprakash, K. N.; Radhakrishnan, K. V.; Schlueter, U.; Fraser-Reid, B. *Tetrahedron Lett.* **2002**, *43*, 6953.

(18) Wang, Z.-G.; Zhang, X.; Visser, M.; Live, D.; Zatorsky, A. M.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 1728.

(19) Typical procedure for preparation of **9** by reaction of **15a** with **1**: The acceptor, trisaccharide **15a** (n = 1, 1.40 g, 1 mmol) and the donor (**1**, 4.36 g, 7 mmol) were separately rotovaporised twice with dry toluene, and then dried for 8 h under vacuum (0.5 mm Hg). To a solution of the acceptor in 15 mL of dry CH_2Cl_2 were added freshly activated molecular sieves (4 g, 3 A beads, 8–12 mesh), NIS re-crystallized from hot CH_2Cl_2 and cold hexane and dried under vacuum (0.5 mm Hg, over night, 1.35 g, 6 mmol) followed by TBDMSOTf (0.1 mL, 0.4 mmol) at 10 ºC (ice bath). To this solution was added a solution of NPOE in CH_2Cl_2 drop-wise over 10 min, and then the ice bath was removed. After 10

min TLC (7:3, hexane–ethyl acetate) showed that all of the acceptor had been consumed. The mixture was diluted with CH_2Cl_2 (200 mL), and washed with 10% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and brine. The dried (Na_2SO_4) material was concentrated and flash chromatography (7:1& ndash;4:1, hexane–ethyl acetate) afforded 1.78 g (92%) of **9** (n = 2). R_f = 0.5 (7:3 hexane–ethyl acetate).

(20) Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.

(21) Nitz, M.; Purse, B. W.; Bundle, D. R. *Org. Lett.* **2000**, *2*, 2939.

(22) For **15a** n = 6: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45–6.77 (m, 125 H), 5.56–5.52 (broad 6 H), 5.19 (s, 1 H), 4.98 (s, 1 H), 4.91–4.22 (m, 51 H), 4.12–3.56 (m, 49 H). ^{13}C NMR (300 MHz, $CDCl_3$): δ = 139.4, 139.2, 139.1, 139.0, 138.9, 138.6, 138.4, 138.3, 138.1, 138.0, 137.9, 137.0, 128.7, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.1, 127.1, [8 anomeric carbons comes at (100.9, 100.8, 100.5, 100.3, 100.1, 99.9, 98.7, 95.7 (α anomeric carbon)]. 83.3, 82.0, 81.9, 81.8, 81.7, 79.8, 78.1, 75.7, 75.5, 75.3, 75.1, 74.9, 74.7, 74.4, 74.1, 74.0, 73.6, 73.5, 73.2, 73.1, 73.0, 72.8, 72.7, 71.3, 70.3, 70.2, 70.1, 70.0, 69.9, 69.7, 69.6, 69.5, 69.4, 68.5, 67.6. FAB-MS (*m/z*) 3696 (M + Cs).