The antituberculosis, antitumor, multibranched dodecafuranoarabinan of *Mycobacterium* species has been assembled from a single n-pentenylfuranoside source†

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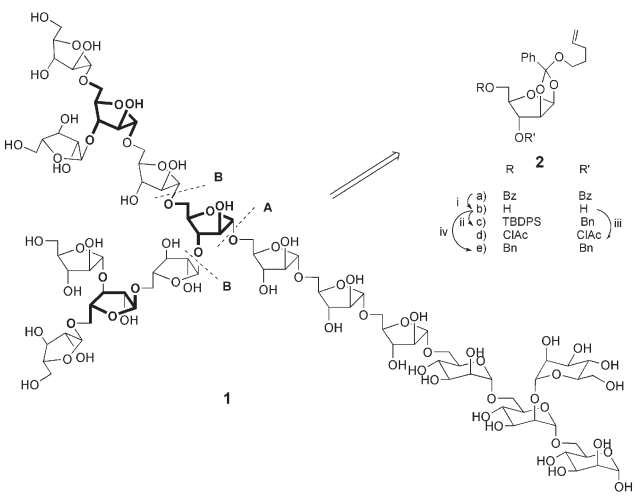
An n-pentenyl furanosyl-1,2-orthoester can function as a donor or be rearranged leading to an n-pentenyl furanoside acceptor which is glycosylated by its progenitor, regioselectively or doubly, thereby enabling rapid fabrication of a multibranched dodecasaccharide, known to possess a wide variety of biological interactions.

The lipoarabinomannan (LAM) glycolipid of *Mycobacterium tuberculosis*,[1] being the seat of the organism's baffling drug resistant capability,[1,2] has long been the focus of intense biological scrutiny.[3] Separate and equally intense interest is being paid to the immunomodulatory arabinomannan, code-named Z-100,[4] isolated from the aoyama B strain of the parasite. Z-100 displays potent activity against (a) tumor growth,[5] (b) opportunistic infections,[6] herpes,[7] and also potentiates antiretrovirals for many HIV variants.[8] Chemists wishing to assist in deconstructing the biological maze presented by these complex lipoarabinomannans, must face the synthetic challenges posed by the unique arabinan array, rendered partially in structure **1** (Scheme 1). Knowledge about the furanosyl donors needed to cope with these challenges is sparse, and it is therefore reasonable to try and import methodology from well established pyranosyl counterparts. However, in view of the higher reactivity of furanose systems,[9] this technology transfer may not be trivial as, indeed, we have recently learned.

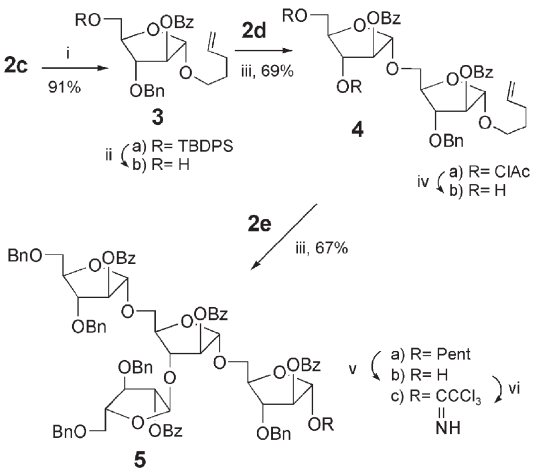


Scheme 1 *Reagents and conditions*: i. NaOMe, rt, 2 h, 75%; ii. a) TBDPSCl, Et_3N, DMAP, 12 h, b) BnBr, NaH, DMF, 84%; iii. $ClAc_2O$, CH_2Cl_2, −10 °C, 73%; iv. BnBr, NaH, DMF, 87%.

The arabinan array **1** features a repeated branched motif, represented by the "bold" units which require a 3,5-diol retron, while the "other" units could conceivably be catenated from a C5-OH free donor. Thus, the entire arabinan component of **1** could be derived ultimately from the readily prepared n-pentenyl orthoester (NPOE) stock **2a**.[10]

Two retrosynthetic plans for **1**, with different levels of convergence, are implied by the dissections A and B (Scheme 1). In option A, a nona-arabinan donor would be delivered to the primary-OH of a tri-arabinan acceptor, while in option B two identical tetra-arabinan donors would be delivered simultaneously in double glycosidation of a tetra-arabino acceptor diol.

In order to make sure that the double glycosidation required by option B was feasible, acceptor **3b** was prepared from the NPOE **2a**[10] by routine steps *via* intermediates **2b** and **2c**, followed by rearrangement to **3a** and desilylation (Scheme 2). Coupling of **3b** and NPOE **2d** was chemoselectively executed under the agency of ytterbium triflate ($YbOTf_3$) and *N*-iodosuccinimide (NIS)[11] to give disaccharide **4a** in 69% yield. Removal of the chloroacetate esters with thiourea then gave diol **4b** which underwent double glycosidation by the dibenzylated orthoester **2e**, again using $Yb(OTf)_3$/NIS, to give the branched NPG **5a** in 67% yield.



Scheme 2 *Reagents and conditions*: i. TBDMSOTf; ii. TBAF, THF, rt, 82%; iii. NIS, $Yb(OTf)_3$, 0 °C, 30 min; iv. thiourea, THF, reflux, 5 h, 61%; v. NIS, CH_3CN–H_2O, rt, 74%; vi. Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 70%.

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b4/b413694b/

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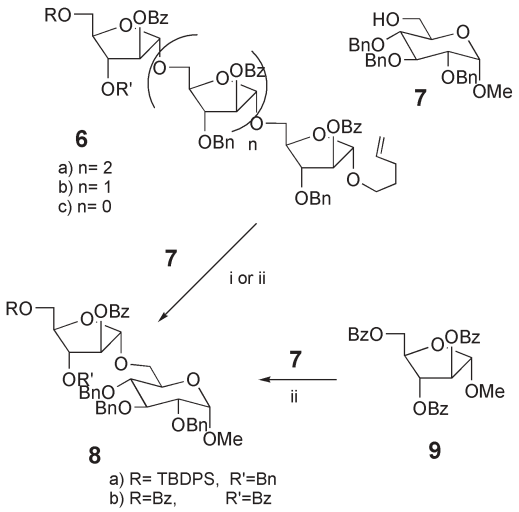


Scheme 3 *Reagents and conditions*: i. NIS, TESOTf, CH_2Cl_2, 0 °C; ii. TESOTf, CH_2Cl_2, 0 °C, 30 min, 20–30%.

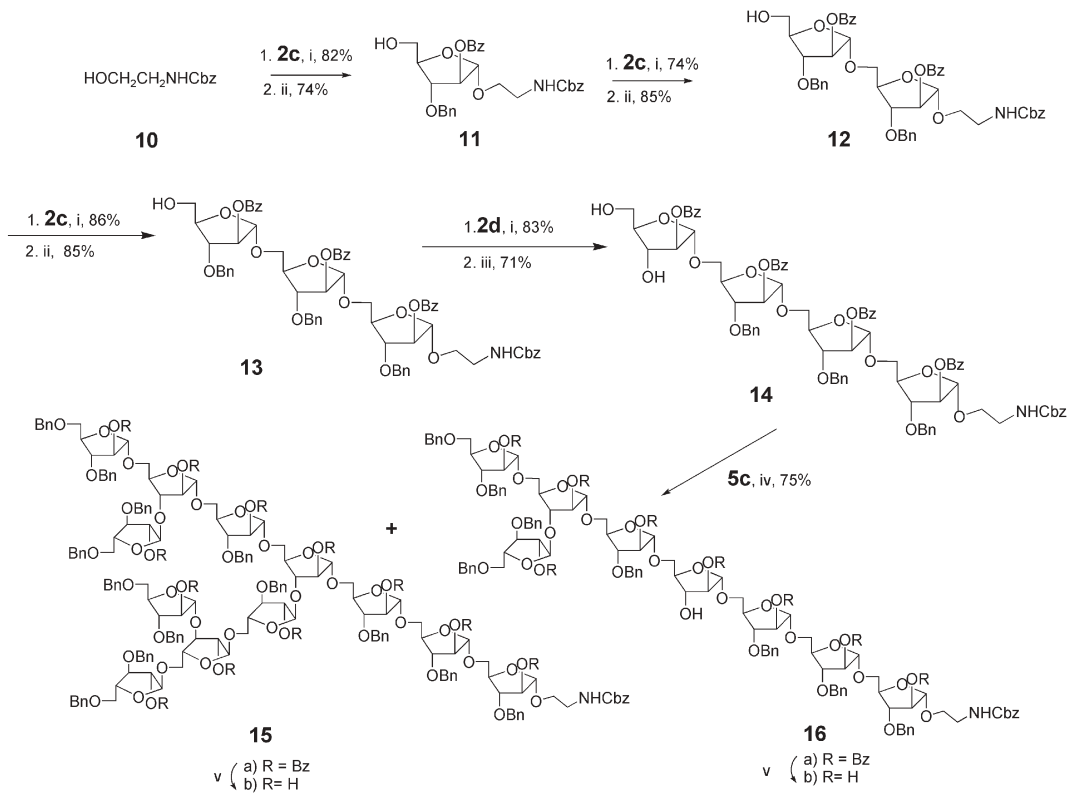
Both disconnections A and B of **1** would entail coupling of an n-pentenyl furanosidyl donor to the acceptor in question and we decided to probe this possibility by coupling recently synthesized[10] tetrasaccharide **6a** to the glucoside acceptor **7** under the agency of TESOTf/NIS (Scheme 3). To our great surprise, the major product turned out to be disaccharide **8a** in which the non-reducing moiety of **6a** had become attached to the acceptor. The same result was obtained when the tri- and di-saccharide analogs **6b** and **6c** were presented to **7**.

That the pentenyloxy residue at the reducing end of donors **6a–c** was not involved in these aberrant reactions, was established by showing that the same result was obtained when NIS was omitted from the reaction medium. The implication that a simple acid catalyzed furanosyl transfer was in play was confirmed when the methyl furanoside **9** was also coupled with **7** under the agency of TESOTf to give **8b** (Scheme 3).

The results in Scheme 3 raise several mechanistic questions which will have to be probed; however the immediate message is that an oligomeric n-pentenyl furanoside such as **5a** or **6a**, unlike its n-pentenyl pyranoside counterparts,[12] could not be used as a donor to effect the connections represented by options A or B.

However, n-pentenyl glycosides can be readily converted into trichloroacetimidates[13] which can be activated by the mild Lewis salt $Yb(OTf)_3$.[14] Accordingly, the branched n-pentenyl tetrasaccharide **5a** was subjected to oxidative hydrolysis with NIS/H_2O/CH_3CN,[15] and the resulting furanose, **5b**, was converted into the trichloroacetimidate **5c** using established procedures.[16] By way of confirmation, the ^{13}C NMR spectrum of **5c** showed well resolved signals for four anomeric carbons (106.31, 106.13, 105.42, 103.90) and four carbonyl carbons (165.57, 165.46, 165.37, 165.29).

Option B in Scheme 1 offers the greater level of convergence, and hence was pursued. The linear array was assembled with a view to biological evaluation of the entire arabinan construct. Thus, the protected ethanolamine **10**[17] was used as the first acceptor for an iterative sequence with NPOE donor **2c**, involving couple-deprotect repeats, leading to **11**, **12** and finally acceptor **13** (Scheme 4). The tetrasaccharide diol **14** was then obtained by reaction of **13** with the donor **2d** followed by dechloroacetylation.



Scheme 4 *Reagents and conditions*: i. NIS, $Yb(OTf)_3$, CH_2Cl_2, 0 °C, 30 min; ii. TBAF, THF, 6 h; iii. thiourea, THF–EtOH, reflux, 6 h; iv. TBDMSOTf, Et_2O, rt, 30 min; v. NaOMe, MeOH.

Compound **14** showed clearly resolved signals for four anomeric carbons (106.31, 106.25, 106.19, 105.34) and four carbonyl carbons (166.63, 166.66, 166.35, 166.29).

Diol **14** was treated with 3 equivalents of trichloroacetimidate donor **5c**. The presence of **15a** was evident from MALDI evaluation of the material (calcd. 4198.5; found 4225.7 M + Na^+). However, although the material appeared as a single substance on TLC, treatment with sodium methoxide gave two chromatographically separable compounds that were isolated in 2 : 1 ratio. Their masses, 2974.6 (M + Na^+) and 1995.3 (M + Na^+), were consistent with the dodeca- and octa-saccharides **15b** and **16b**. The ^{13}C NMR spectra of their anomeric regions were not resolved; but confirmation came from COSY analyses which showed thirteen benzyl groups for the former, and nine for the latter. Complete assignments will be reported in the full paper.

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Notes and references

1 M. Gilleron, J. Nigou, B. Cahuzac and G. Puzo, *J. Mol. Biol.*, 1999, **285**, 2147; I. Apostolou, Y. Takahama, C. Belmant, T. Kawano, M. Huerre, G. Marchal, J. Cul, M. Taniguchi, H. Nakauchi, J. J. Fournie, P. Kourilsky and G. Gachelin, *Proc. Natl. Acad. Sci. USA*, 1999, **98**, 5141; M. Gilleron, C. Ronet, M. Mempel, B. Monsarrat, G. Gachelin and G. Puzo, *J. Biol. Chem.*, 2001, **276**, 34896; D. Chatterjee, *Curr. Opin. Biol.*, 1997, **1**, 579; T. L. Lowary, in *Glycoscience: Chemistry and Biology*, B. Fraser-Reid, K. Tatsuta, J. Thiem, Eds.; Springer, Heildelberg, 2001, Vol. **3**, p. 2005.

2 For some pertinent citations see: D. C. Alexander, J. R. W. Jones, T. Tan, J. M. Chen and J. Liu, *J. Biol. Chem.*, 2004, **274**, 18824–18833 and references cited therein.

3 P. J. Brennan, *Tuberculosis*, 2003, 1; B. Hamasur, M. Haile, A. Pawlowski, U. Schroder, A. Williams, G. Hatch, G. Hall, P. Marsh, G. Kallenius and S. B. Svenson, *Vaccine*, 2003, **21**, 25–26.

4 H. Kobatake, T. Suekane, Y. Murakami, S. Niwa, A. Okahira and H. Kushida, *Yakugaku-Zasshi*, 1981, **101**, 713–722.

5 Y. Hayashi, T. Ebina, F. Suzuki and N. Ishida, *Microbiol. Immunol.*, 1981, **25**, 305–316; H. Sasaki, M. Kobayashi, Y. Emori, O. Ohya, Y. Hayashi and K. Nomoto, *Biotherapy*, 1997, **10**, 139; Y. Emori, H. Sasaki, Y. Hayashi and K. Nomoto, *Biotherapy*, 1996, **9**, 249–272; M. Mukai, S. Kubota, S. Morita and A. Akanuma, *Cancer*, 1995, **75**, 2276–2280; M. Kobayashi, R. B. Pollard and F. Suzuki, *Anti-Cancer Drugs*, 1997, **8**, 156–153; Y. Hayashi, H. Sasaki, Y. Emori and K. Nomoto, *Biotherapy*, 1993, **7**, 63–69.

6 Y. Luo, X. Chen, T. M. Downs, W. C. DeWolf and M. A. O'Donnell, *J. Immunol.*, 1999, **162**, 2399–2405; H. Oka, Y. Emori, O. Ohya, N. Kobayashi, H. Sasaki, Y. Tanaka, Y. Hayashi and K. Nomoto, *Immunol. Lett.*, 1999, **70**, 109–117; H. Oka, Y. Shiraishi, H. Sasaki, K. Yoshinaga, Y. Emori and M. Takei, *Biol. Pharm. Bull.*, 2003, **26**, 1336–1341; H. Oka, Y. Emori, H. Sasaki, Y. Shiraishi, K. Yoshinaga and T. Kurimoto, *Microbiol. Immunol.*, 2002, **46**, 343–351; H. Oka, H. Sasaki, Y. Shiraishi, Y. Emori, K. Yoshinaga and M. Takei, *Biol. Pharm. Bull.*, 2004, **27**, 82–88.

7 M. Kobayashi, D. N. Herndon, R. B. Pollard and F. Suzuki, *Immunol. Lett.*, 1994, **40**, 199–205.

8 J. S. James, *AIDS Treatment News*, Feb. 23, 2001.

9 *Monosaccharides: Their Chemistry and Their Roles in Natural Products*, P. M. Collins, R. J. Ferrier, John Wiley & Sons, New York, 1995.

10 J. Lu and B. Fraser-Reid, *Org. Lett.*, 2004, **6**, 3051–3054.

11 K. N. Jayaprakash, K. V. Radhakrishnan and B. Fraser-Reid, *Tetrahedron Lett.*, 2002, **43**, 6953; K. N. Jayaprakash and B. Fraser-Reid, *Synlett*, 2004, 301.

12 See for example: R. Madsen, U. E. Udodong, C. Roberts, D. R. Mootoo, P. Konradsson and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1995, **117**, 1554–1565; J. R. Merritt, E. Naisang and B. Fraser-Reid, *J. Org. Chem.*, 1994, **59**, 4443–4449.

13 K. N. Jayaprakash, *Org. Lett.*, in press.

14 M. Adinolfi, A. Iadonisi and M. Schiattarella, *Tetrahedron Lett.*, 2003, **44**, 6479.

15 B. Fraser-Reid, U. E. Udodong, Z. Wu, H. Ottoson, R. Merritt, C. S. Rao, C. Roberts and R. Madsen, *Synlett*, 1992, 927–942.

16 T. G. Mayer and R. R. Schmidt, *Eur. J. Org. Chem.*, 1999, 1153.

17 A. Campbell and B. Fraser-Reid, *Bioorg. Med. Chem.*, 1994, **2**, 1209–1219.

