Automated synthesis of lipomannan backbone α(1-6) oligomannoside *via* glycosyl phosphates: glycosyl tricyclic orthoesters revisited†‡

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Glycosyl tricyclic orthoesters provide a versatile basis for the efficient generation of glycosyl phosphates, which are used in the automated synthesis of lipomannan backbone α(1-6) hexamannoside.

Mycobacterium tuberculosis (Mtb) cell surface lipomannan (LM) and lipoarabinomannan (LAM) are considered to be the virulent factors in tuberculosis pathogenesis.[1] These oligosaccharides have become the target of chemical syntheses to generate the defined molecular tools for subsequent biological studies.[2] We have recently completed the chemical syntheses of Mtb phosphatidylinositol mannosides (PIMs), as well as a LAM oligosaccharide in the solution phase.[3] To facilitate access to these molecules, an automated synthesis would be convenient. As the initial target for this synthetic campaign, we selected α(1-6) oligomannosides,[4] which constitute the backbone of the LM and LAM complexes.

To achieve the regio- and diastereoselective automated assembly of the α(1-6) oligomannosides in a rapid fashion, a mannose building block such as **1** would be ideal (Fig. 1). Glycosyl trichloroacetimidates or dibutyl phosphates allow for fast glycosylations by Lewis acid activation.[5] A C-2 ester serves as the stereo-directing group, and an orthogonal C-6 fluorenylmethoxycarbonyl (Fmoc) group can be rapidly cleaved for glycan chain elongation events. Although a number of approaches can be adopted for the preparation of **1**, a synthetic route *via* mannosyl orthoester **2** should be the most efficient. The 1,2-*O*-orthoester would serve as a temporary protecting group for the C-1 and C-2 hydroxyl groups. Upon differentiation of the remaining hydroxyls, the glycosyl 1,2-*O*-orthoester can either be selectively hydrolysed to the hemiacetal for subsequent installation of the anomeric trichloroacetimidate[4] or ring opened by dibutyl phosphate to afford the glycosylphosphate directly.[6]

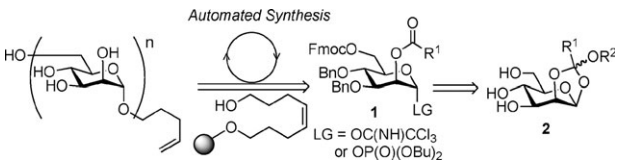


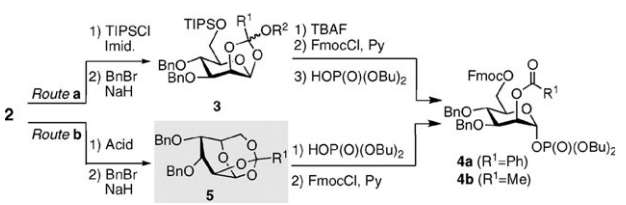
Fig. 1 Retrosynthetic analysis of the automated synthesis of α(1-6) oligomannosides.

Due to the base-labile nature of the Fmoc group, a robust route for its placement in **2** calls for the temporary protection of the C-6 hydroxyl with a triisopropylsilyl (TIPS) group,[7] followed by benzylation of the C-3 and C-4 hydroxyls (Scheme 1, route **a**). Subsequent removal of the TIPS group is necessary before installation of the Fmoc. While such a route is practically applicable,[8] the temporary protection of the C-6 hydroxyl is costly and not atom-efficient.

To develop a more efficient process, we turned our attention to a glycosyl tricyclic orthoester where all three oxygen atoms in the orthoester were derived from the same monosaccharide unit. Although this class of glycosyl orthoester has been known for more than three decades,[9] there are few examples of its application to oligosaccharide synthesis.[10,11]

We planned to access glycosyl tricyclic orthoesters by the simple acidic treatment of glycosyl 1,2-orthoesters.[12] These suitably protected tricyclic orthoesters would be selectively opened by dibutyl phosphate to furnish glycosyl phosphates, allowing for the direct protection of the remaining hydroxyl group.

We evaluated the formation of mannosyl tricyclic orthoesters from their corresponding 1,2-orthoesters (Table 1).[13] Camphorsulfonic acid (CSA) was found to be the optimal acid catalyst for inducing the *trans*-orthoesterification of mannosyl 1,2-*O*-orthoesters. Interestingly, the substituents of mannosyl orthoesters were found to have a profound effect on the efficiency of the acid-induced cyclization. In the case of mannosyl orthobenzoates **2a** and **2b**, the change of one



Scheme 1 Plausible synthetic routes for the preparation of mannosyl phosphates **4a** and **4b** *via* 1,2-*O*-orthoester **3** (route **a**) or tricyclic orthoester **5** (route **b**).

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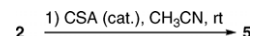
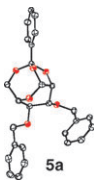
Table 1 Formation of mannosyl tricylic orthoesters *via* 1,2-*O*-orthoesters[a]



Entry	Mannosyl 1,2-orthoester	Tricyclic orthoester	Yield (%)
1	**2a** (R^1 = Ph; R^2 = Me)	**5a** (R^1 = Ph)	85
2	**2b** (R^1 = Ph; R^2 = All)	**5a**	95
3	**2c** (R^1 = Me; R^2 = Me)	**5b** (R^1 = Me)	40
4	**2d** (R^1 = Me; R^2 = All)	**5b**	95



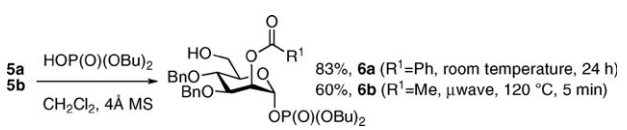
[a] All *trans*-orthoesterifications were performed in the presence of 4 Å MS with 10 mol% CSA. The completion of the reaction required 24 h for entries 1 and 3, and 12 h for entries 2 and 4. The crude tricyclic orthoester was subjected directly to benzylation without purification.

orthoester substituent from *O*-methyl to *O*-allyl not only shortened the reaction time, but also improved the chemical yield (Table 1, entries 1 and 2). Similarly, in contrast to mannosyl allyl orthoacetate **2d**, the cyclization of mannosyl methyl orthoacetate **2c** was rather sluggish using CSA as the acid catalyst. X-Ray crystallography clearly shows the presence of the tricyclic system in **5a** (Table 1).‡

With the desired mannosyl tricyclic orthoesters in hand, we proceeded to investigate their ring opening with dibutyl phosphate (Scheme 2). In contrast to mannosyl 1,2-orthoesters,[6] mannosyl tricyclic orthoesters were found to be less reactive towards dibutyl phosphate.[14] Although the addition of excess dibutyl phosphate (6 equiv.) was required to complete the conversion of **5a** to **6a** in 24 h using CH_2Cl_2 as the solvent, the reaction proceeded cleanly with complete regioselectivity to give mannosyl phosphate **6a** in an 83% isolated yield. Surprisingly, mannosyl tricyclic orthoacetate **5b** was completely inert to dibutyl phosphate at room temperature. Microwave irradiation at 120 °C for 5 min was necessary to achieve the conversion of **5b** to **6b**.

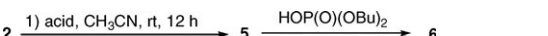
We further expanded this approach to generate xylosyl and glucosyl phosphates *via* their corresponding tricylic orthoesters (Table 2).[13] The direct formation of xylosyl tricylic orthoester **5c** from 1,2-*O*-orthoester **2e** was effectively induced by CSA, although **2f** and glucosyl 1,2-*O*-orthoester **2g** required milder acidic agents such as silica gel or acid-washed molecular sieves (AW-MS). The subsequent ring openings of tricylic orthoesters **5c–5e** by dibutyl phosphate proceeded regioselectively to give the corresponding glycosyl phosphates, **6c–6e**, in good to excellent yield.

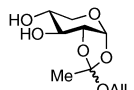
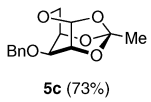
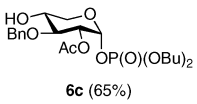
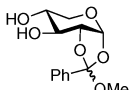
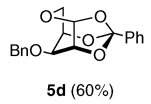
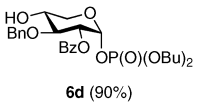
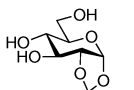
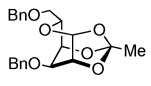
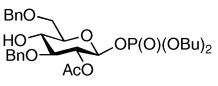
Having established suitable procedures for the formation of tricylic mannosyl orthoesters and their transformation into mannosyl phosphates, we focused our efforts on the preparative scale-up of these procedures in order to supply the automation campaign (Scheme 3). Mannosyl allyl 1,2-*O*-orthobenzoate **2b** was readily prepared from D-mannose in 4 steps on a 50 mmol scale. After a short silica gel filtration to remove the methylbenzoate by-product, **2b** was subjected to a CSA-induced cyclization. Without purification, the intermediate tricylic orthoester was benzylated to give crystalline **5a** in 70–80% yield in 6 steps. Ring opening of **5a** with dibutyl phosphate was routinely performed on a 10 mmol scale, and the resulting C-6 hydroxyl group was capped with Fmoc to give target building block **4a** (Scheme 3).



Scheme 2 Ring opening of mannosyl tricylic orthoesters with dibutyl phosphate.

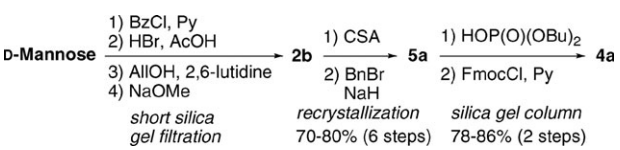
Table 2 Generation of xylosyl and glucosyl phosphates *via* tricylic orthoesters[a]



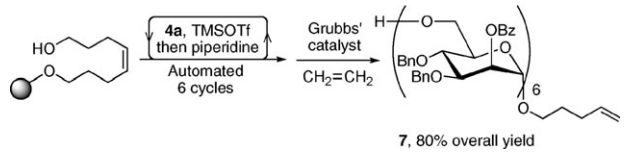
Entry	Glycosyl 1,2-orthoester	Tricylic orthoester (yield)[c]	Glycosyl phosphate (yield)[d]
1	 **2e**	 **5c** (73%)	 **6c** (65%)
2	 **2f**	 **5d** (60%)	 **6d** (90%)
3[b]	 **2g**	 **5e** (50%)	 **6e** (90%)

[a] CSA (10 mol%) (entry 1), silica gel (entry 2) and 4 Å AW-MS (entry 3) were used as acid catalysts. [b] In entry 3, CH_3CN was used instead of CH_2Cl_2. [c] Yields indicated for **5** are two-step yields, after benzylation. [d] The ring openings of **5** to **6** by dibutyl phosphate were performed as described for the conversion of **5a** to **6a** in Scheme 2.

With a large quantity of mannosyl phosphate **4a** secured, we assembled α(1-6) oligomannosides by an automated synthesis. For each cycle, a single glycosylation was performed using 5 equiv. of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as an activator and 5 equiv. of building block **4a** at −15 °C for 45 min. Removal of the Fmoc group was efficiently conducted using piperidine. Six-fold repetition of the glycosylation–deprotection sequence, followed by cleavage of the oligosaccharide from the resin,[15] furnished the desired hexasaccharide, **7**, in an 80% isolated yield (Scheme 4). The HPLC spectrum of the crude material after the cleavage showed oligosaccharide **7** as a major peak, with a negligible amount of impurities (Fig. 2). Purification was performed by simple column chromatography.



Scheme 3 Scaled-up preparation of **4a** *via* a tricylic mannosyl orthoester.



Scheme 4 Automated assembly of α-(1,6) hexa-mannoside **7**.

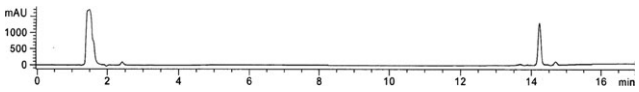


Fig. 2 HPLC trace of crude hexasaccharide **7**.

In summary, the pursuit of an automated synthesis of LM backbone α(1-6) hexa-mannoside led us to identify glycosyl tricyclic orthoesters as unique synthetic intermediates for the preparation of functional carbohydrate building blocks. The simple acid-catalyzed *trans*-orthoesterification of glycosyl 1,2-orthoesters allows the direct formation of glycosyl tricyclic orthoesters. These orthoesters can be efficiently converted to glycosyl dibutyl phosphates, as exemplified by mannose, xylose and glucose.

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Notes and references

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9. (*a*) A. F. Bochkov, I. V. Obruchnikov and N. K. Kochetkov, *Izv. Akad. Nauk. SSSR, Ser. Khim.*, 1971, 1291; (*b*) A. F. Bochkov, V. I. Snyatkova, Y. V. Voznyi and N. K. Kochetkov, *Zh. Obshch. Khim.*, 1971, **41**, 2776; (*c*) A. F. Bochkov, Y. V. Voznyi, V. N. Chernetskii, V. M. Dashunin and A. V. Rodionov, *Izv. Akad. Nauk. SSSR, Ser. Khim.*, 1975, 420.
10. Prandi *et al.* have reported the CSA-catalyzed *trans*-orthoesterification of 1,2-orthoarabinose to the tricyclic 1,2,5-orthoester. These tricyclic orthoesters were reacted with thiols to give thioglycosides, which were used in the syntheses of oligoarabinofuranosides. See: (*a*) S. Sanchez, T. Bamhaoud and J. Prandi, *Tetrahedron Lett.*, 2000, **41**, 7447; (*b*) T. Bamhaoud, S. Sanchez and J. Prandi, *Chem. Commun.*, 2000, 659. Glycosyl phosphates are currently easier to use for automated synthesis than thioglycosides because glycosyl phosphates can be activated under homogeneous reaction conditions with a single activator, such as TMSOTf.
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12. The acid-induced *trans*-orthoesterification of glycosyl 1,2-orthoesters has been described in the literature; see refs. 9, 10 and 11*a*. However, we were unable to reproduce the results of Bochkov *et al.* (see ref. 9*c*), reporting that *para*-toluenesulfonic acid can induce the *trans*-orthoesterification of **2g** to the corresponding tricyclic orthoester in 48% yield (our result: 26%). Hiranuma *et al.* (ref. 11*a*) reported the formation of a mannosyl tricyclic orthoester using pyridine as the solvent and pyridium trifluoromethanesulfonate as the acid catalyst. However, these conditions are less practical for scale-up.
13. 1,2-Orthoesters were prepared in four steps from D-mannose, D-glucose or D-xylose in good yield. See ESI†.
14. Tricyclic orthoesters have been used as protecting groups for carboxylic acids, and they require fairly vigorous conditions for cleavage. See: (*a*) E. J. Corey and N. Raju, *Tetrahedron Lett.*, 1983, **24**, 5571; (*b*) M. A. Blaskovich and G. A. Lajoie, *J. Am. Chem. Soc.*, 1993, **115**, 5021.
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