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Intramolecular aglycon delivery for (1 → 2)-β-mannosylation: towards the synthesis of phospholipomannan of *Candida albicans*

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ABSTRACT

A high yielding method for 1,2-*cis*-β-D-mannosylation by intra-molecular aglycon delivery (IAD) through *p*-methoxy benzyl ether/acetal exchange and phenylsulfoxide donor is reported, along with its application in iterative assembly of antigenic (1 → 2)-β-pentamannoside domain of phospholipomannan (PLM) of fungal pathogen *Candida albicans*.

Candida albicans, the most widespread human pathogen responsible for cutaneous and systemic fungal infections, synthesize a unique sequence of (1 → 2)-β-oligomannans on its cell surface that act as adhesin, induce production of pro-inflammatory cytokines and antibodies. One of the key (1 → 2)-β-mannan expressed by *C. albicans* is the membrane-anchored glucosphingolipid (GSL), named phospholipomannan[1] (PLM, Fig. 1) where oligomeric (1 → 2)-β-mannan domain is linked through a unique α-1,2-mannosyl anomeric phosphodiester moiety to a mannose-inositol-phosphoceramide (MIPC) glycolipid anchor, which is embedded in the cell wall of the *C. albicans*. The PLM of *Candida albicans* is highly immunogenic due to its (1 → 2)-β-mannan structural motif, which is absent in human host. When *C. albicans* infects and interacts with the macrophages (the first-line-of-defence cells of the human host), a large amount of soluble PLM fragments (largely (1 → 2)-β-mannans) are rapidly shed by the pathogen resulting in severe pro-inflammatory response[2] (TNF-α production and release) from host cells.[3] Several biochemical[4] and immunological studies[5] have shown that PLM motif provides a scaffold for anchoring of various virulence factors to the cell surface of the pathogen, a mechanism quite similar to that used by the mammalian cells for anchoring various cell-surface proteins and glycans through the glycosylphosphatidylinositol[6] (GPI) anchor. A comparison of the structures of PLM of *C. albicans* (pathogen) and GPI anchor of human cells (host) reveals remarkable biomimetic features (Fig. 1). These include: (a) (1 → 2)-β-mannan in PLM in place of (1 → 2)-α-mannan motif in GPI, (b) (1 → 2)-α-mannose linked to *myo*-inositol in place of (1 → 6)-α glucosamine-inositol motif in GPI; and c) presence of phytoceramide in PLM and glycerolipid in GPI. Perhaps, these remarkable bio-mimetic features represent mechanisms of molecular and evolutionary adaptations between the species. In continuation to our long-term interest in chemistry

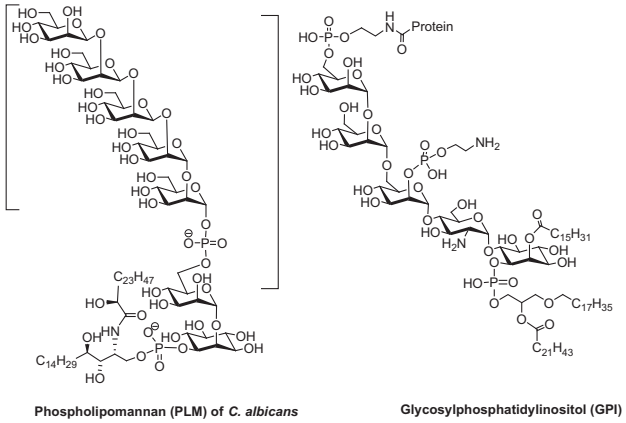

Figure 1. Phospholipomannan of *C. albicans* and GPI anchor of H. sapiens.

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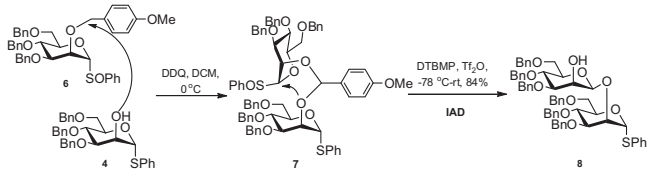
and biology of GPI anchors,[7] we initiated the synthesis of PLM of *C. albicans*, which present substantial opportunities.

Arguably, one of the most challenging problems in carbohydrate chemistry is the construction of thermodynamically unstable (1 → 2)-β-mannoside bond, ubiquitous in several pathogenic microorganisms. First synthesis of β-oligomannosides was reported by Hindsgaul,[8a] and Stork[8b,c] followed by Ogawa and Ito[9] using intramolecular aglycon delivery (IAD) and temporary tethering concept. Later on, a number of other leading groups designed (1 → 2)-β-glycosidations via intermolecular glycosylation using orthogonal functional groups.[10]

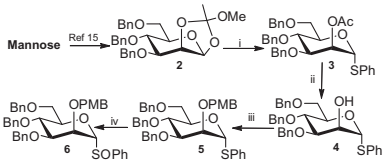
Here, we report the first application of intramolecular glycosylation approach by linkage of the accepting atom to the donor via a bifunctional group (intramolecular aglycon delivery) for the synthesis of (1 → 2)-β-mannosides. Previously, Crich[11] and Seeberger[12] reported the synthesis of (1 → 2)-β, (1 → 3)-β and (1 → 4)-β-mannosides using 4,6-*O*-benzylidene protected mannosyl-1-*O*-sulfoxides or thiomannosides respectively through intermolecular glycosylation. In our approach, we used mannosyl-1-*O*-sulfoxide donor first time in PMB mediated IAD approach which because of good leaving nature gave high yield of (1 → 2)-β-mannosides even without the 4,6-benzylidene group.

The intramolecular aglycon delivery method (IAD) has been successfully used for the synthesis of (1 → 3)-β and (1 → 4)-β-mannosides, but the most challenging (1 → 2)-β-mannosides have not been successful by IAD method so far. Keeping in view the challenge, we designed a new strategy for the synthesis of (1 → 2)-β-mannosides via IAD approach where we envisioned that a *p*-methoxybenzyl group at C-2 position of mannosyl residue will participate in both ether/acetal exchange as well as in intra-molecular aglycon delivery for glycosylation. To test the hypothesis, we first synthesized and coupled 2-*O*-*p*-methoxybenzyl-3,4,6-tri-*O*-benzylmannosyl phenyl sulfoxide **6** as donor with suitably protected α-phenyl thiopyranosides **4** as acceptor in the presence of DDQ (1.4 equiv), under anhydrous conditions which afforded the mixed acetals **7** quite smoothly.[9,13,14] Further the mixed acetal was used for glycosylation by performing reaction with triflic anhydride (Tf_2O, 0.95 equiv) in the presence of 2,6-di-tert-butyl-4-methylpyridine (DTBMP, 3.0 equiv) at −78 °C which gave exclusively the desired (1 → 2)-β-mannopyranoside **8** with 84% yield as revealed by spectroscopic studies (Scheme 1). The starting materials **4** and **6** were used in this glycosylation, which in turn were synthesized from corresponding orthoester donor of mannose **2** as shown in Scheme 2.[15,16]

The stereochemistry of compound **8** was determined by ^1H NMR and further confirmed by 2D NMR spectral data analysis. The α- and β-configurations of **8** were assigned by the appearance of anomeric protons as doublets at δ 5.60, 4.58 with coupling constant values of $J_{1,2}$ 1.2 and 6.4 Hz, respectively. The HSQC and HMBC correlation of anomeric proton at δ 5.60 (d, J = 1.2 Hz) with ^{13}C (δ 85.2) indicated the α-orientation whereas the other anomeric proton at δ 4.58 (d, J = 6.4 Hz) with ^{13}C (δ 96.6) confirmed the β-orientation. The relative configuration of **8** was authenticated by NOESY correlation as being compatible with computer modelling in which the close contact of atoms in space calculated were consistent with NOESY correlation. In NOESY correlation H-1 exhibited



Scheme 1. Formation of (1 → 2) β-mannosylation by IAD.



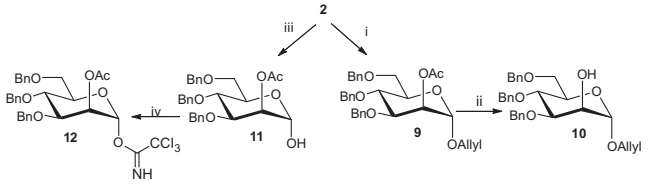
Scheme 2. Synthesis of mannoside fragments **4** and **6**. Reagents and conditions: (i) BF_3Et_2O, PhSH; (ii) NaOMe, MeOH; 90% over 2 steps; (iii) PMBCl, NaH, DMF; (iv) *m*-CPBA, DCM, 85%.

a correlation with H-1′ indicating that the two protons H-1 & H-1′ were situated in a same face and assigned as α-proton with the β-linkage (Fig. 2 of ESI).

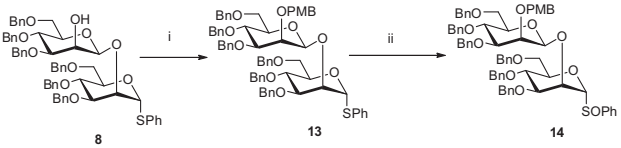
Now we extended our IAD method for the synthesis of final product **1**, the (1 → 2)-β-pentamannoside of PLM. The intermediates were prepared as shown in Schemes 2–5. For this, we started with mannose and converted it into an orthoester donor 2 (Scheme 2) using reported method.[16] The orthoester was successfully converted to an acceptor 10[17] as well as mannosyl TCA donor **12** (Scheme 3).[18]

Having the new IAD method and the key intermediates in hand, the (1 → 2)-β-mannopyranoside **8** was now successfully converted into the corresponding sulfoxide donor **14** (Scheme 4).[19] The synthesis of the second key trisaccharide intermediate **16** was achieved by the coupling of mannopyranosyl donor **12** with the acceptor **10** (Scheme 5), which on further deacetylation by using sodium methoxide provided the alcohol **15**.[20] The α-stereochemistry of the resultant glycoside **15** was established by NMR analysis.

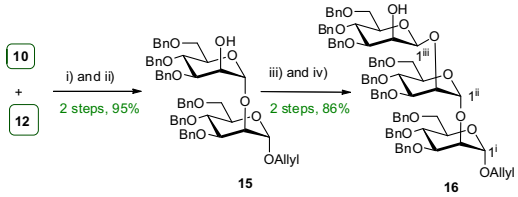
To construct the required trisaccharide **16**, the disaccharide **15** was coupled with donor **6** using our intra-molecular aglycon deliv-



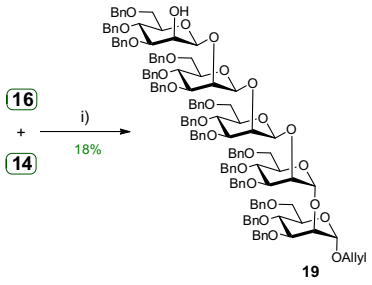
Scheme 3. Synthesis of mannoside fragments 10 and 11. Reagents and conditions: (i) BF_3Et_2O, AllOH; (ii) NaOMe, MeOH; 90% over 2 steps; (iii) *p*-TSA, DCE; (iv) Tri chloro acetonitrile, K_2CO_3, DCM, 90% over 2 steps.



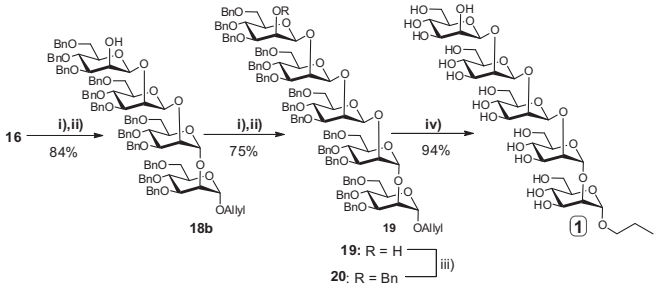
Scheme 4. Synthesis of β-dimannoside donor 14. Reagents and conditions: (i) PMBCl, NaH, DMF; (ii) *m*-CPBA, DCM, 96%, over 2 steps.



Scheme 5. Synthesis of β-trimannoside acceptor 16. Reagents and conditions: (i) TMSOTf, DCM; (ii) NaOMe, MeOH; (iii) **6**, DDQ, 1.5 equiv; (iv) DTBMP, Tf_2O, DCM, −78 °C to rt.



Scheme 6. Synthesis of β-pentamannoside by 2+3 coupling. Reagents and conditions: (i) DDQ, (1.5 equiv); (ii) DTBMP, Tf_2O, DCM, −78 °C to rt.



Scheme 7. Iterative synthesis of β-pentamannoside 1. Reagents and conditions: (i) **6**, DDQ, (1.5 equiv); (ii) DTBMP, Tf_2O, DCM, −78 °C to rt; (iii) BnBr, NaH, DMF; (iv) 20, Pd/C 10%, THF, MeOH, H_2, 94%.

ery (IAD) condition and the desired product obtained in 86% yield. The stereochemistry of **16** having α- and β-anomeric linkages was determined by HSQC NMR. Bundle et al. also reported the synthesis of compound **16** but by a different synthetic strategy.[21]

To further confirm the stereochemistry at the anomeric position of trisaccharide **16**, disaccharide **15** was then converted to the trisaccharides **17** and **18** by 1,2-α-glycosylation with **12** (Scheme 5b, of ESI) and then compared with trisaccharide **16** (HSQC given in ESI).

Finally, the key synthetic step to construct (1 → 2)-β-pentamannoside via 2+3 glycosylation with the mannosyl donor **14** and the acceptor trimannoside **16** was tried, which gave the desired pentamannoside **19** in only low 18% yield; not satisfactory for the total synthesis of PLM (Scheme 6).

Therefore, we modified our approach to construct the (1 → 2)-β-pentamannoside **19** by an iterative synthetic strategy, wherein the trimannoside **16** was first converted into tetra-mannoside **18b** and subsequently into pentamannoside **19** using the donor **6** as shown in Scheme 7. The (1 → 2)-β-pentamannoside **19** was further converted into perbenzylated pentamannoside **20**. The stereochemistry of (1 → 2)-β-pentamannoside **20** was confirmed by NMR and HSQC experiment. Finally, controlled global hydrogenolysis with Pd/C afforded the target (1 → 2)-β-pentamanan **1** in 94% isolated yield.

In summary, we have designed a high yielding method for (1 → 2)-β-mannosylation employing IAD via PMB ether/acetal intermediate and also achieved the first synthesis of (1 → 2)-β-pentamannoside domain of phospholipomannan (PLM) of *Candida albicans*.

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Supplementary data

Supplementary data (spectral data for all compounds **1**–**20**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.03.099.

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