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Synthesis of a β1,2-Mannopyranosyl Tetrasaccharide Found in the Phosphomannan Antigen of *Candida albicans*

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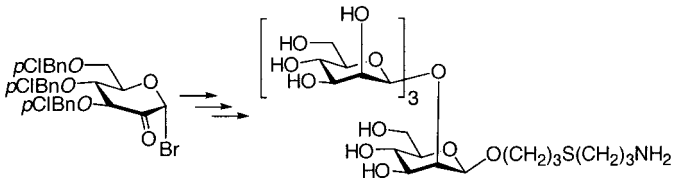
ABSTRACT



11 **1**

The synthesis of a portion of the challenging β1,2-mannosyl polymer found in the cell walls of *Candida albicans* was undertaken to develop a conjugate vaccine against *C. albicans* and to facilitate NMR conformational studies of this unique polysaccharide. The novel approach to the synthesis of tetrasaccharide 1 employed the modified ulosyl bromide 11 as the glycosyl donor which provided high diastereoselectivity. A participating solvent as well as *p*-chlorobenzyl protection facilitated the new approach.

Candida albicans is the most common etiologic agent in candidiasis,[1] an infection commonly affecting immunocompromised patients and those undergoing long-term antibiotic treatment.[2] Recently it has been shown that monoclonal antibodies raised against a portion of the *C. albicans* phosphomannan in rats were protective against subsequent infection.[3] Further studies indicated the active epitope to be a portion of the β1,2-mannan polymer found in the phosphomannan antigen.[4] In addition to their immunochemical interest, it was predicted almost 30 years ago that polymers composed of β1,2-linked mannose or glucose residues would present interesting conformational properties. Conformations of such oligomers that adopt typical glycosidic torsional angles expected for disaccharide elements would be expected to show compact conformations that fold back on themselves and show strong steric interactions between noncontiguous monomer units.[5] The synthesis of **1**, a portion this polymer, was undertaken to provide material for the development of a carbohydrate-based conjugate vaccine against *C. albicans* and to facilitate NMR conformational studies of a portion of this unique polysaccharide.

The rational synthesis of β-mannosides is a longstanding problem in glycoside synthesis, that lacks a general solution, despite several novel approaches.[6] In the construction of large homooligomers such as **1**, the separation of anomeric mixtures would pose a major obstacle to efficient assembly by either block or sequential chain extension reactions. Consequently, only those methods known to give exclusively the β-mannopyranosyl linkage were investigated. In elabora-

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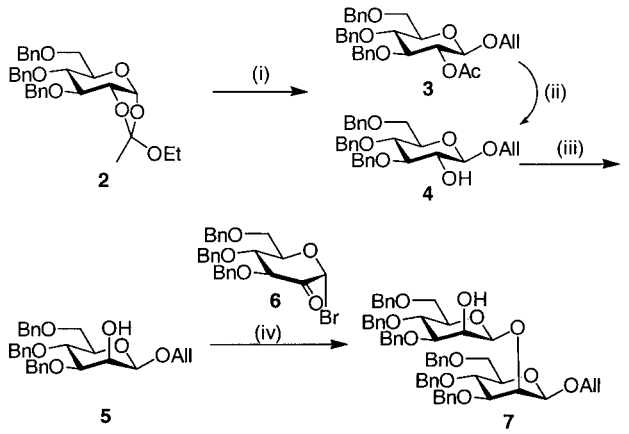
tion of a 1,2 linked polymer, a ulosyl bromide[7] provides first the corresponding ulopyranoside, which can be reduced to afford directly the selectively protected glycosyl acceptor of the next glycosylation step (for example **7**). The glycosyl donor **6** was attractive because of its high diastereoselectivity over both the glycosylation and reduction steps, and the minimization of the number of protecting groups required.

Allyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (**5**) was targeted as a desirable building block for the reducing terminus of the tetrasaccharide. The allyl group served as a persistent anomeric protecting group which could be transformed into a variety of functionalities late in the synthesis to provide a tether via which attachment to protein could be achieved for synthesis of neoglycoconjugates.[8]

Scheme 1



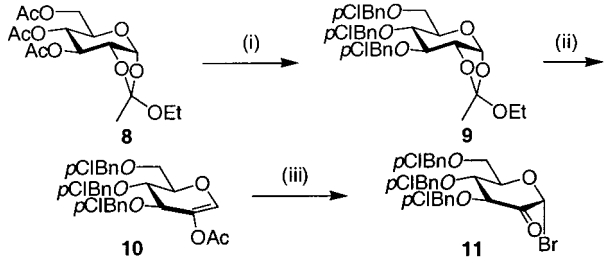
i) Allyl alcohol, BF_3•OEt_2, **3** (43%), **4** (53%). ii) NaOMe, MeOH, quant
iii)a)Ac_2O, DMSO, b) $NaBH_4$, CH_2Cl_2/MeOH (85%).
iv) a)Ag(zeolite), CH_2Cl_2, (78%), b) L-Selectride, THF

Allyl-β-D-mannopyranoside (**5**) could be conveniently synthesized on a large scale starting from the readily accessible ortho ester **2**.[9] Lewis acid promoted glycosylation in allyl alcohol gave high yield of a near 1:1 mixture of the expected product **3** and its deacetylated counterpart **4**. The allyl-β-D-glucopyranoside **3** was treated under Zemplén deacylation conditions to give the desired alcohol **4**. Oxidation of the glucopyranoside (**4**) using acetic anhydride and DMSO and reduction with sodium borohydride gave the mannopyranoside **5** in good yield with high selectivity (Scheme 1).[10]

The synthesis of the first 1,2-linked β-mannosyl unit was accomplished using conditions similar to those employed by Lichtenthaler et al.[11] The ulosyl bromide **6** was synthesized in a similar fashion, although the yields for the thermal rearrangement that converts ortho ester **2** to an acetoxyglycal (cf. **10**) were approximately 65%.

Using silver-exchanged zeolite[12] to promote the glycosylation of **5** by **6**, followed by reduction of the product with L-Selectride gave the desired disaccharide (**7**) in excellent yield after purification. No α-*manno* anomer or *gluco* epimers were isolated from the reaction mixture. It was necessary to use the sterically hindered L-Selectride for this reduction since in contrast to the monosaccharide, the disaccharide gave epimeric mixtures when sodium borohydride was employed.

Scheme 2



i)a) NaOMe, MeOH, b) *p*-chlorobenzyl chloride, NaH, DMF, (96%),
ii) Bromobenzene, pyridine, reflux (85%),
iii) NBS, EtOH, CH_2Cl_2, (91%)

Introducing subsequent β-mannopyranosyl units proved more difficult. The conditions employed for disaccharide synthesis failed to yield significant amounts of trisaccharide. Exploration of different activation protocols led to the use of the soluble promoter, silver triflate, with 2,6-di-*tert*-butyl-4-methylpyridine (DtBMP)as an acid scavenger,[6d] and acetonitrile as a participating solvent. These conditions followed by a similar L-Selectride mediated reduction gave a 40−45% yield of the trisaccharide, and 10% yield of the α-gluco epimer together with a significant portion of the 3,4-di-*O*-benzyl-1,6-anhydro-β-D-mannopyranose.[11] It was hypothesized that stabilization of the protecting groups would disfavor the formation of this anhydro sugar side product and in turn increase the yield of the reaction. The *p*-chlorobenzyl protecting group was explored for this purpose since it has been shown to be more acid stable than the parent benzyl group but to have similar properties.[13] The ulosyl bromide **11** was synthesized in an analogous fashion to the related 3,4,6-tri-*O*-benzyl-α-D-*arabino*-hexopyranos-2-ulosyl bromide **6** used earlier in the disaccharide synthesis.

Standard deacylation of the readily available ortho ester **8**,[9] followed by Williamson ether synthesis with *p*-chlorobenzyl chloride gave the *p*-chlorobenzyl protected ortho ester

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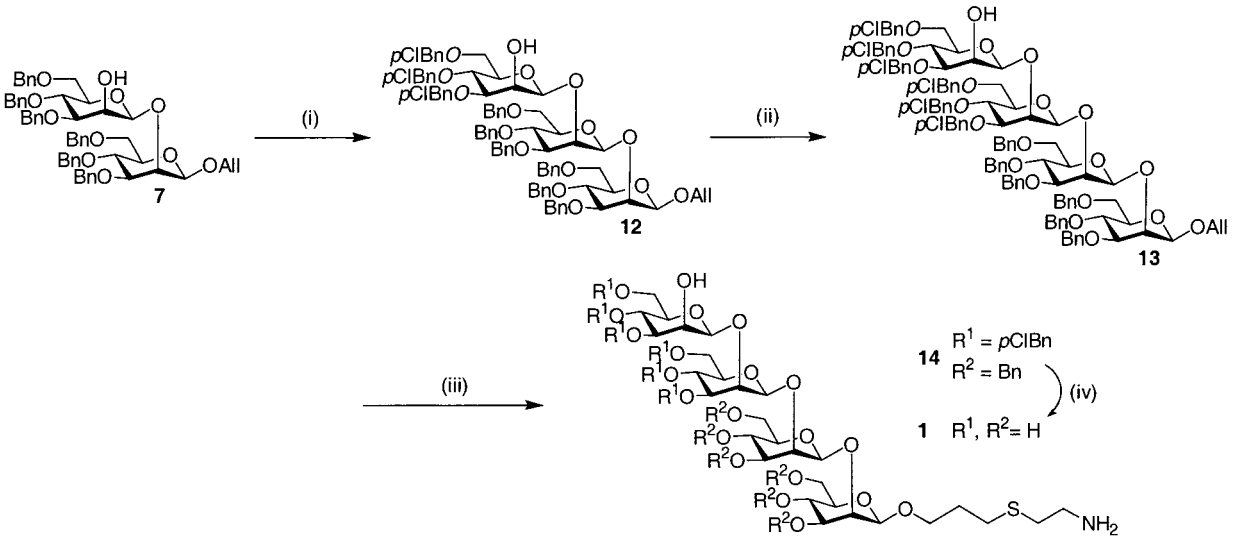
Scheme 3



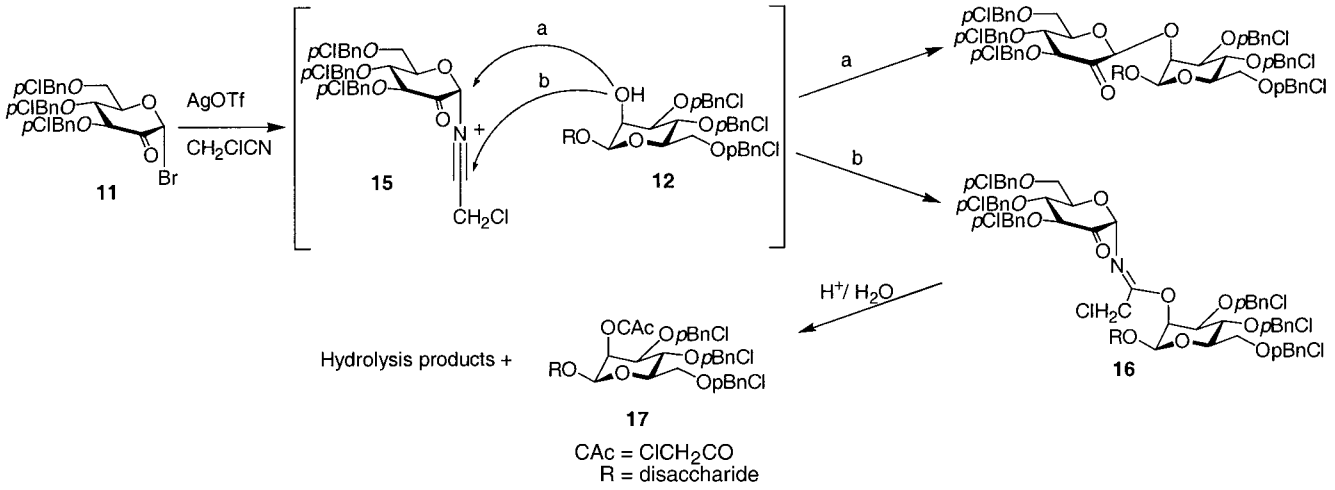
i) a) AgOTf, DtBMP, CH_3CN, **11**, b)L-Selectride, THF, -78 C, 65% ii) a) AgOTf, DtBMP, $(CH_3)_3CCN$, **11**, b) L-Selectride, THF, -78 C, 48% iii) $HSCH_2CH_2NH_3Cl$, $MeOH/CH_2Cl_2$, 365 nm, 74% iv) Na, NH_3/THF, 77%

(**9**). Subsequent thermal rearrangement in bromobenzene gave a high yield of the acetoxyglycal (**10**). Further treatment with *N*-bromosuccinimide and ethanol in dichloromethane gave the desired ulosyl bromide (**11)** (Scheme 2).

Glycosylation with this donor, in the presence of silver triflate, and 2,6-di-*tert*-butyl-4-methyl pyridine in acetonitrile followed by reduction with L-Selectride gave a 60−65% yield of the desired trisaccharide (**12**) (Scheme 3) along with 15% of the corresponding α-gluco epimer, which was easily separated by column chromatography.

Attempted introduction of the fourth *β*-mannopyranosyl residue was met by the observation of an interesting and unexpected product. Using the same conditions as those employed to make the trisaccharide, up to 20% of the unexpected 2-*O*-acetyl trisaccharide acceptor was isolated. We hypothesized that this product must result from attack of the acceptor on the nitrile carbon of the proposed α-nitrilium intermediate (e.g., **15**) (path b),[14] instead of reaction at the anomeric center (path a) of the donor. This postulate was supported by the isolation of the chloroacetylated acceptor (e.g., **17**) when chloroactonitrile was employed as the solvent (Scheme 4). The resulting imidate intermediate (**16**) hydrolyses to give the chloroacetylated acceptor (**17).** Although reaction at the nitrile carbon of nitrilium intermediates has been seen with water and carboxylic acids,[15] to the authors knowledge no acylated glycosyl acceptors have previously been isolated as a result of using nitriles as a participating solvent. In this case the resulting side product must be favored due to a sterically hindered acceptor as well as the electron-deficient nitrilium uloside (e.g., **15**). When

Scheme 4



the sterically more hindered pivaloyl nitrile was chosen as the solvent, the glycosidic linkage was synthesized in 48% yield to give the desired tetrasaccharide **13** and 10% of the α-gluco epimer along with small amounts of the trisaccharide pivaloyl ester also being formed. Further studies into the solvent effects are ongoing.

A terminal amine was chosen as a versatile handle from which glycoconjugates could be readily generated.[16] This was installed via a photoaddition of 2-aminoethanthiol to the allyl glycoside giving **14**. Using longwave ultraviolet irradiation, 365 nm, and a quartz vessel, it was possible to carry out this addition in the presence of the aromatic protecting groups. Previous reports of this photoaddition used shortwave UV irradiation, conditions under which the benzyl and chlorobenzyl protecting groups were labile, resulting in a complex mixture of products.[17]

Deprotection was accomplished using dissolving metal conditions at −78 °C. It was necessary to minimize the reaction time for this reduction as the thioether proved labile to reduction resulting in the propyl glycoside

Despite the repetitive structure of the homooligomer **1** the majority of the resonances in the ^{1}H NMR spectrum of **1** are well resolved at 600 MHz, with only H-5s and H-6s resonances exhibiting extensive overlap. Complete assignment of the ^{1}H NMR was possible through GCOSY and GTOCSY experiments aided by TROESY[18] experiments to confirm the order of the ring systems. The presence of four β-mannopyranosyl residues was confirmed by heteronuclear $^1J_{C,H}$ coupling constants, that fell within the range 160.1−163.4 Hz.[19] The order of the chemical shifts of the ring systems agree well with the NMR data published for manno-oligosaccharides previously isolated from *C. albicans* phosphomannan.[20] TROESY experiments[18] indicate a potentially interesting tertiary structure as long-range contacts between the nonreducing terminus (fourth residue) and the second residue are evident. More detailed investigations of the conformation of this unique polysaccharide are ongoing.

The tetrasaccharide **1** has been coupled to a protein carrier, and the glycoconjugate will be used to immunize mice. The resulting polyclonal antibodies will be tested for anti-*Candida* activity.

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Supporting Information Available: Spectroscopic, analytical data and experimental procedures for compounds **1**, **7**, **12**−**14** are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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