Direct Synthesis of β-Mannans. A Hexameric [→3)-β-D-Man-(1→4)-β-D-Man-(1]$_3$ Subunit of the Antigenic Polysaccharides from *Leptospira biflexa* and the Octameric (1→2)-Linked β-D-Mannan of the *Candida albicans* Phospholipomannan. X-ray Crystal Structure of a Protected Tetramer

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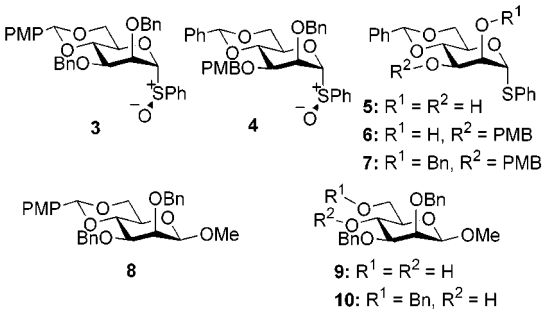
Protocols for the reliable, efficient, diastereoselective synthesis of β-mannopyranosides have only been developed within the past decade. Among the methods now available,[1,2] the direct ones developed in this laboratory from 4,6-*O*-benzylidene protected thiomannosides and their sulfoxides[3] are arguably optimal, owing to the combination of high yield, excellent diastereoselectivity, and the ease of operation that they offer. We now turn to the application of this methodology, an off-shoot of the sulfoxide glycosylation method,[4] to the synthesis of β-mannans and select as first proving grounds a mixed hexasaccharide (**1**) and a homogeneous octamer (**2**).

The mannobiose [→3)-β-D-Man-(1→4)-β-D-Man-(1] was recently characterized as the main repeat unit of the antigenic polysaccharides from *Leptospira biflexa* serovar patoc strain Patoc I.[5] This particular mannan was chosen as target, aside from its potential importance as a genus-specific leptospiral antigen, because of its intriguing, alternating β-(1→3)-β-(1→4)-configuration: the hexamer was selected as being sufficiently long to prove the chemistry. Oligomeric β-1,2-linked mannans such as **2** are found in the *C. albicans* cell wall phosphopeptidomannan.[6] They are immunogenic and elicit specific antibodies in both humans and animals.[7] Furthermore, it has been shown that β-1,2-mannans derived from *C. albicans* phosphopeptidomannans induce TNF[1]-α synthesis from cells of the macrophage lineage and bind to macrophage cell membranes.[8] The octamer **2** was chosen for synthesis as the shortest of a series of phospholipomannans isolated recently and shown to have strong TNF-α inducing properties in vivo and in vitro.[8d,9] The two syntheses together illustrate the ease with which the β-1,2, β-1,3, and β-1,4 mannobiose linkages may now be incorporated into complex oligosaccharides.

The synthesis of **1** began with donor **3**,[10] which was readily prepared from phenyl 1-thio-α-D-mannoside by conversion to the 4,6-*O*-*p*-methoxybenzylidene derivative,[11] followed by dibenzylation and oxidation[3a,e] to a single sulfoxide. Donor **4** was obtained from diol **5**[3a] by selective protection of the equatorial alcohol by treatment with Bu_2SnO,[12] then PMB chloride, followed by benzylation of the residual hydroxy group in **6**, and, eventually, oxidation of **7**. Donor **3** was then converted to the methyl glycoside **8** by activation with Tf_2O at −78 °C in CH_2Cl_2 in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP)[3d] followed by addition of methanol. The *p*-methoxybenzylidene group was removed with camphorsulfonic acid (CSA) in methanol, giving **9**, regioselective monobenzylation of which, with Bu_2SnO and benzyl bromide, afforded the acceptor **10**.



Standard activation of **4** with Tf_2O and TTBP in CH_2Cl_2 at −78 °C, followed by the addition of **10** gave the disaccharide **11** in 88% yield as a separable 11.6/1 β/α mixture,[13] a typical selectivity for the β-mannosylation of the somewhat hindered glucopyranose 4-OH.[3c] Exposure of **11** to DDQ[14] then afforded the alcohol **12** in 83% yield (Scheme 1).

Standard activation of donor **3** followed by addition of **12** afforded the trisaccharide **13** as a 9.0/1 β/α mixture. Selective

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(13) The assignment of stereochemistry in all coupling reactions en route to **1** and **2** was facilitated by our earlier observation[3a] of the characteristic upfield chemical shift (δ 3.1−3.3) of the mannose *H*-5 resonance in 4,6-*O*-benzylidene protected β-mannosides. The corresponding β-mannosides have no such characteristic signal with all ring proton chemical shifts being normal. The assignments were supported by the isolation of the minor α-anomer, which was always lacking in the unusual upfield β-mannoside region. Further confirmation was obtained, resolution permitting, by measurement of the characteristic $^1J_{CH}$ anomeric coupling constants: Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.

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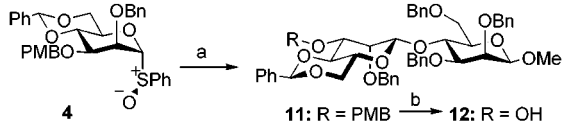
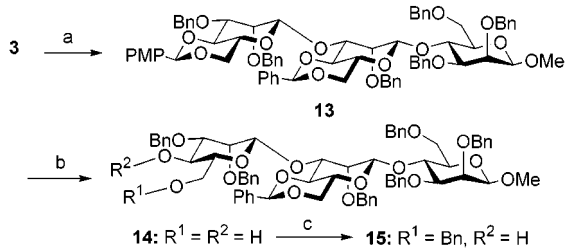
Scheme 1. Typical Formation of a β-1→4 Linkage[a]



[a] Conditions: (a) (i) Tf_2O, TTBP, −78 °C, (ii) **10**, 81% β, 7% α; (b) DDQ, 83%.

Scheme 2. Typical Formation of a β-1→3 Linkage[a]



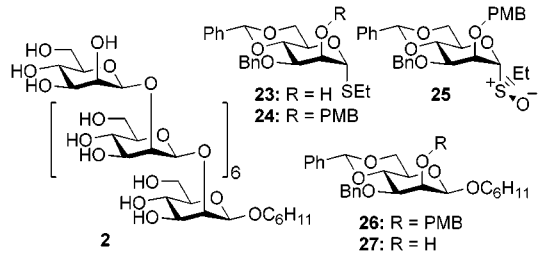
[a] Conditions: (a) (i) Tf_2O, TTBP, CH_2Cl_2, −78 °C, (ii) **12**, 72% β, 8% α; (b) 80% HOAc, 70%; (c) Bu_2SnO, BnBr, 87%.

removal of the *p*-methoxybenzylidene group, in the presence of the benzylidene group, was achieved in 70% yield by exposure to 80% acetic acid at room temperature.[15] Regioselective monobenzylation of diol **14** was accomplished uneventfully, as for the conversion of **9** to **10**, by treatment first with Bu_2SnO and then with benzyl bromide giving **15** in 87% yield (Scheme 2). At this stage each of the various protocols required for oligomer synthesis had been successfully implemented with the result that completion of the synthesis was a matter of careful iteration.

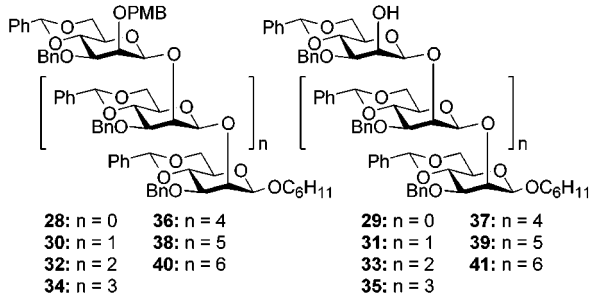
Accordingly, coupling of donor **4** with acceptor **15** gave the tetramer **16** in 70% yield as a separable 7.8/1 β/α mixture and removal of the PMB group provided the next acceptor **17** in 72% yield. Coupling of **17** to the activated donor **3** afforded the pentamer **18** as a 9.0/1 β/α mixture in 80% yield (Scheme 3). Acidolysis of the *p*-methoxybenzylidene group of **18** gave the diol **19** and recovered substrate in 50 and 39% yields, respectively (Scheme 3). Prolonged exposure of **18** to the cleavage conditions resulted in the onset of benzylidene cleavage; it was therefore found expeditious to stop the reaction before completion and recycle the substrate. Treatment of diol **19** with Bu_2SnO and BnBr in the usual manner afforded the acceptor **20** in 62% yield and set the stage for the final coupling. This was accomplished in the standard manner with the sulfoxide donor **21**[3a] to give the hexasaccharide **22** in 70% yield as 9.0/1 β/α mixture. Final deprotection of **22** was achieved by hydrogenolysis (3 atm) over Pd/C giving **1**, with a correct ESI MS, in 85% yield (Scheme 3).

Turning to octamer **2** we reasoned that it could be accessed by a two-step iterative protocol involving β-selective coupling to mannose O2, followed by regioselective deprotection of O2 in the newly introduced residue, and iteration.

Thioglycoside **23**[3c,10] was converted to the 2-*O*-PMB ether **24** and thence to the diastereomerically pure sulfoxide **25**.[3e] Cyclohexanol was arbitrarily chosen to cap the reducing end, and, following activation of **25** with Tf_2O in the presence of TTBP in CH_2Cl_2 at −78 °C, was coupled up to give **26** in excellent yield

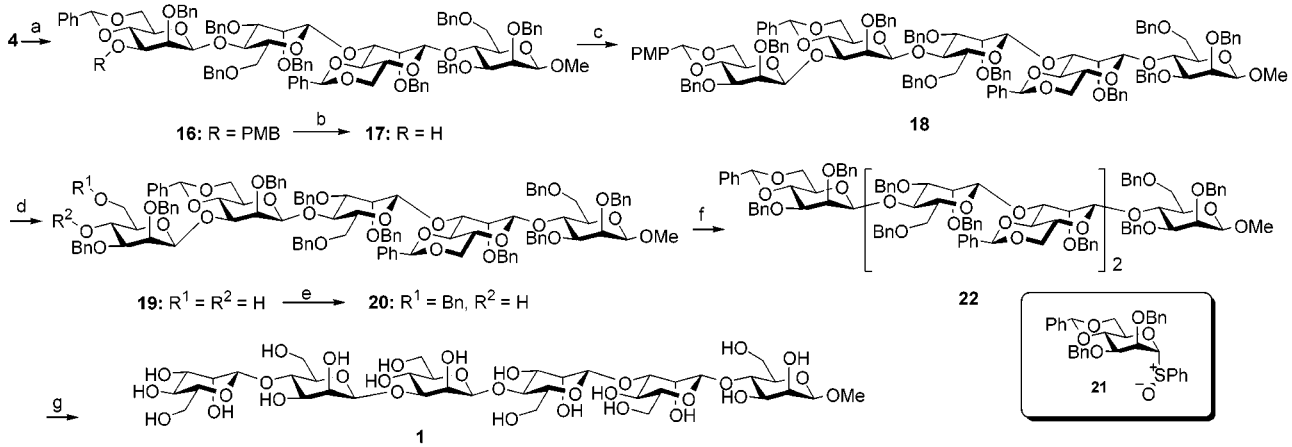


and selectivity. Removal of the PMB group with DDQ then provided alcohol **27**. The first iteration provided disaccharide **28**, which in turn gave acceptor **29**. Subsequent steps proceeded more or less uneventfully with the yields and stereoselectivities presented in Table 1, ultimately leading to the octasaccharide **40** and, after removal of the PMB group, the alcohol **41**.



The yields and selectivities for the first three linkages in **2** are consistent with those reported previously from our laboratory for the formation of simple β-mannosides.[3a] They do, however, fall off at the level of introduction of the third and fourth units before settling down to around 80% and 4−5:1, respectively, thereafter. The early yields and selectivites are certainly superior to the those in the earlier synthesis of the corresponding tetramer using the alternative ulosyl bromide coupling.[9] It seems apparent that longer oligomers should be accessible by this methodology if yields and selectivities of the type in the lower half of Table 1 can be tolerated.

Scheme 3. Completion of the Synthesis of **1**[a]



[a] Conditions: (a) (i) Tf_2O, TTBP, CH_2Cl_2, −78 °C, (ii) **15**, 62% β, 8% α; (b) DDQ, 72%; (c) (i) **3**, Tf_2O, TTBP, CH_2Cl_2, −78 °C, (ii) **17**, 72% β, 8% α; (d) 80% HOAc, 50%; (e) Bu_2SnO, BnBr, 62%; (f) (i) **21**, Tf_2O, TTBP, CH_2Cl_2, −78 °C, (ii) **20**, 63% β, 7% α; (g) H_2, Pd/C, MeOH, 85%.

Table 1. β-1→2 Linkage Yields and Selectivities in the Synthesis of **2**

acceptor	β-glycoside (% yield)	α-glycoside (% yield)	β/α ratio	freed alcohol (% yield)
$C_6H_{11}OH$	**26-*β*** (77)	**26-α** (0)	β only	**27** (85)
27	**28-*β*** (94)	**28-α** (0)	β only	**29** (97)
29	**30-*β*** (89)	**30-α** (9)	9.9/1	**31** (91)
31	**32-*β*** (77)	**32-α** (20)	3.9/1	**33** (85)
33	**34-*β*** (69)	**34-α** (16)	4.3/1	**35** (80)
35	**36-*β*** (68)	**36-α** (15)	4.5/1	**37** (85)
37	**38-*β*** (64)	**38-α** (13)	4.9/1	**39** (81)
39	**40-*β*** (64)	**40-α** (14)	4.5/1	**41** (71)

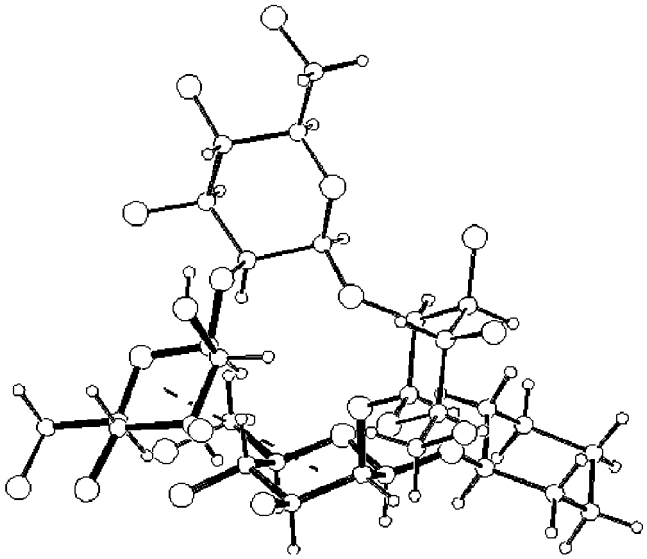
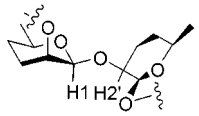


Figure 1. X-ray crystallographic structure of **33** with all nonessential groups removed for clarity and rings 1 and 4 emphasized.

Tetrasaccharide **33** gave suitable crystals for X-ray analysis (Figure 1). Examination of this structure provides a possible explanation for the fall off in yield and selectivity midway through the synthesis. The tetramer approximates to a compact helical structure with the fourth residue obliquely above the first as judged from the distance (5.68 Å) between the centroids of planes defined by C2−C3−C5−O5 in the first and fourth residues as well as the angle (34.1°) between those planes. Early computational studies predicted that 1,2-β-mannans would have crumpled conformations to alleviate steric interactions between remote residues.[16] Although the calculations were for fully deprotected mannans and the structure presented is protected, the parallels are close. Structure **33** is a collapsed irregular helix with approximately three residues per turn with the calculations predicting between 2 and 4.

Table 2. Glycosidic Bond Torsion Angles in Tetrasaccharide **33**[a]



	Cyc−a	a−b	b−c	c−d
φ (H1−C1−O−C2′)	43.7	44.4	18.3	31.3
ψ (C1−O−C2′-H2′)	−15.3	10.7	−8.7	13.6

[a] Where a and d are the sugars at the reducing and nonreducing ends of the chain, respectively.

Although all linkages are relatively hindered, it is at the stage of introduction of the fourth one that severe hindrance from distant residues first comes into play.[17] All subsequent glycosidic bond forming reactions will suffer from the same type of interaction if the collapsed helical structure is maintained, as is expected. The falloff in anomeric selectivity with chain length in the synthesis of **1** (Schemes 1−3) is much less marked, consistent with the more open, less hindered nature of β-1,3 and β-1,4 linkages.

Table 2 shows the φ and ψ torsion angles in **33**, from which it is apparent that all linkages are nonideal and that the helix is disordered. The partially eclipsed bonds presumably arise from minimization of steric interactions between noncontiguous residues, which are obviously more important in the protected mannan. The variation in torsion angles accounts for the irregularity of the helical structure.

Deprotection of **41** was achieved by hydrogenolysis (3 atm) over Pd/C in methanol for 3 days giving the pure octamer **2**, with a correct ESI MS, in 89% yield. Although we have not yet conducted any detailed conformational analysis of **2**, its ^{1}H NMR spectrum at 500 MHz exhibits 7 distinct anomeric protons, which serves to illustrate the irregular nature of its predicted crumpled helical conformation, aside from any obvious fraying at the ends.

Acknowledgment. We thank the NIH (GM 57335) for support of this work.

Supporting Information Available: ^{1}H and ^{13}C NMR for **1**, **2**, **8**−**20**, **22**, **26**−**37**, **39**, and **41** (PDF), the complete structure of **33** (PDB), and tables of bond angles and lengths for **33** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) It is noteworthy that the introduction of the fourth residue proved the most difficult in Bundle's synthesis of the tetramer.[9]