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Facile Syntheses of a Hexasaccharide and a Nonasaccharide Related to the Cell Wall D-Mannan of Yeast *Candida albicans*

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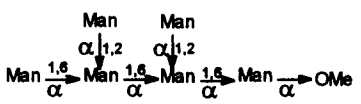
Abstract: A highly efficient strategy for the preparation of D-manno-hexa- and nonasaccharides related to the cell wall D-mannan of yeast *Candida albicans* having α-(1→6)- and α-(1→2)-linkages has been developed using 6-O-acetyl-2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranosyl trichloroacetimidate (7) as the key glycosyl donor in the "Inverse Schmidt" procedure.

Recently, Kanbe and Culter reported that the α-linked oligo-D-mannosyl side-chains of a cell-wall D-mannan of the pathogenic yeast *Candida albicans* is in large part responsible for the binding of yeast cells to the marginal zone of mouse spleen.[1] Stratford also noted the importance of α-linked oligo-D-mannosyl side-chains of the cell wall D-mannan in the mechanism of several types of yeast flocculation.[2] Furthermore, Nelson and co-workers reported that the alkali-released α-linked D-manno-oligosaccharides from *C. albicans* cell-wall D-mannan were potent inhibitors of lymphoproliferation induced by the parent D-mannan.[3, 4] These facts are of interest from the viewpoints of both host-parasite interactions and the biological roles of carbohydrates.

The outer chain moiety of many yeast D-mannans has a long backbone consisting solely of α-(1→6)-linked D-mannopyranose units to which are attached various kinds of side-chains at O-2 in a comb-like structure.[5-7] To elucidate the mechanisms of action of the biological effects of yeast mannans, it is of interest to synthesize different fragments of yeast D-mannans. Here we disclose a highly efficient strategy for the preparation of α-(1→6)-linked D-manno-oligosaccharides containing α-(1→2)-linked D-mannopyranose side-chains using 6-O-acetyl-2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranosyl trichloroacetimidate (7) as the key glycosyl donor in the "Inverse Schmidt" procedure. The syntheses of hexasaccharide **1** and nonasaccharide **2** related to the cell wall D-mannan of yeast *Candida albican* have been presented as typical examples.



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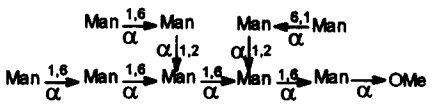

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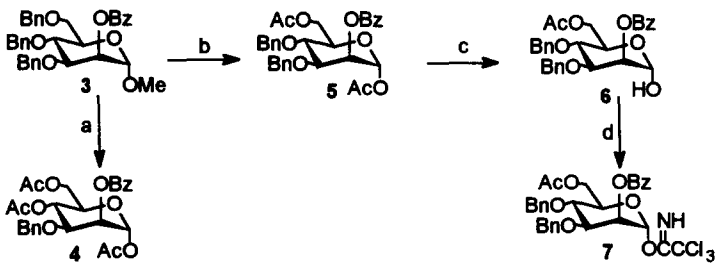
Methyl 2-O-benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**3**) was prepared in 65% overall yield according to the literature[8] using D-mannose as the starting material. Selective acetolysis of **3** using $HOAc/Ac_2O/H_2SO_4$[9] in a ratio of 6: 1: 0.05 gave the corresponding diacetate **5** (Scheme 1). The concentration of H_2SO_4 should be controlled in the acetolysis, otherwise 1,4,6-tri-O-acetyl-2-O-benzoyl-3-O-benzyl-α-D-mannopyranose (**4**) (Scheme 1, path a) was obtained. Compound **5** can be used directly as a glycosyl donor for preparation of some simple disaccharides by the Helferich method. Selective removal of the 1-O-acetyl group of the diacetate **5** was achieved using the conditions designated for selective removal of the 2-O-trichloroacetyl group of 3,4,6-tri-O-acetyl-2-O-trichloroacetyl-β-D-glucopyranosyl chloride.[10] Thus **6** was obtained in nearly quantitative yield by treatment of **5** in anhydrous ether saturated with dry ammonia. Subsequent reaction of **6** with CCl_3CN/DBU in dichloromethane afforded the key glycosyl donor **7**.[11]

Scheme 1



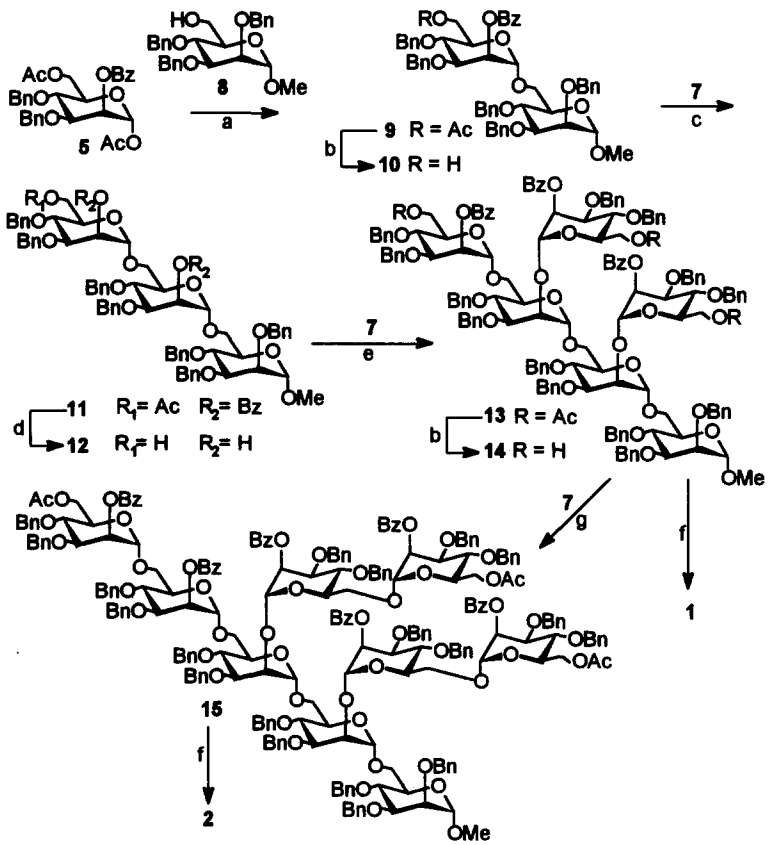
Reagents and conditions: (a) $HOAc/Ac_2O/H_2SO_4$ = 6/1/0.5 (v/v), RT, 16 h, 95% (b) $HOAc/Ac_2O/H_2SO_4$ = 6/1/0.05 (v/v), RT, 16 h, 90% (c) anhydrous ether saturated with dry ammonia, RT, 24 h, 97%.(d) CCl_3CN (1.2 equiv.), DBU (0.2 equiv.), 0°C, 2 h, 92%.

As shown in Scheme 2, the disaccharide **9** was prepared by the Helferich reaction using **5** as the glycosyl donor and methyl 2,3,4-tri-O-benzyl-α-D-mannopyranoside (**8**) as the acceptor. Selective removal of the acetyl group of **9** using a methanol solution containing 0.5% HCl gave the glycosyl acceptor **10** quantitatively. The trisaccharide **11** was prepared using the "Inverse Schmidt" strategy. Thus the glycosyl acceptor **10** and the catalyst TMSOTf were mixed first in dry CH_2Cl_2, and after stirring for 15 min, the glycosyl donor **7** was added dropwise within 30 min in order to get a high yield. The ^1H NMR spectrum of **11** showed one acetyl signal (δ 1.98), one methyl signal (δ 3.25) and two H-2 signal at downfield (δ 5.75, 5.78), confirming the structure of **11**. Removal of the acetyl group and the benzoyl groups of the trisaccharide **11** with a catalytic amount $NaOCH_3$ in methanol gave quantitatively the glycosyl accepter **12** having free 6''-OH, 2'-OH, and 2''-OH groups respectively. "Inverse Schmidt" coupling of the triol **12** with **7** afforded the hexasaccharide **13** in 84% yield. The ^1H NMR spectrum[11] of **13** showed three acetyl signals (δ 1.93,1.96, and 1.98), one methyl signal (δ 3.25) and three downfield H-2 signals (δ 5.67, 5.76, and 5.86), characteristic of the structure of the hexasaccharide **13**. Deprotection of **13** gave the title mannohexatose **1**. We are gratified to find that selective tri-deacetylation of **13** using the same conditions as that used for selective deacetylation of **9** also afforded in nearly quantitative yield the glycosyl accepter **14** having three 6-OH groups. The fully protected nonasaccharide **15** was smoothly obtained using the "Inverse Schmidt" method again. The ^1H NMR data of **15** contained structurally characteristic information, i.e. three acetyl signals (δ 1.91,1.93, and 1.95), one methyl

signal (δ 3.20) and six downfield H-2 signals (δ 5.68, 5.75, 5.76, 5.77, 5.80 and 5.82). Deprotection of a nonasaccharide **15** gave the target mannononatose **2**.

In summary, we have successfully developed a highly efficient strategy for the preparation of D-manno-oligosaccharides i.e. reiteration of selective deacetylation of **9** or **11** followed by "Inverse Schmidt" coupling with **7** can afford α-(1→6)-linked tri- tetra-, and even higher oligosachharides, while Zemplén deacylation of the α-(1→6)-linked tri- tetra- oligosachharides followed by "Inverse Schmidt" coupling with **7** can afford comb-like or dendritical manno-oligosaccharide containing α-(1→6) and α-(1→2) linkages.

Scheme 2



Reagents and conditions: (a) **8** (1.3 equiv.), $BF_3{\cdot}OEt_2$ (1.4 equiv.), CH_2Cl_2, RT, 85%. (b) methanol/0.5%HCl, RT., 18 h, 95%. (c) **7** (1.3 equiv.), CH_2Cl_2, TMSOTf (0.1 equiv.), 0°C, 1 h, 94%. (d) CH_3OH/catalytic amount $NaOCH_3$, 96%. (e) **7** (4.5 equiv.), CH_2Cl_2, TMSOTf (0.2 equiv.), 0°C, 1 h, 84%. (f) i. CH_3OH/catalytic amount $NaOCH_3$, ii. H_2, Pd/C 10%, EtOAc, RT, 1 h, 95% for **1** and 93% for **2**.(g) **7** (6.0 equiv.), CH_2Cl_2, TMSOTf (0.2 equiv.), 0°C, 1 h, 66%.

ACKNOWLEDGEMENT

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11. All new compounds gave satisfactory elemental analysis results. Selected physical data for some key compounds are as follows: For **5**: mp: 102-104°C; $[\alpha]_D$+3.2° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 400MHz): δ 8.20-7.20 (m, 15H, 3Ph*H*), 6.20 (d, 1H, $J_{1,2}$ = 2.1 Hz, H-1), 5.62 (dd, 1H, $J_{1,2}$ = 2.1 Hz, $J_{2,3}$ = 2.9 Hz, H-2), 4.89, 4.60 (ABq, 2H, J = 10.7 Hz, PhCH_2), 4.83, 4.61 (ABq, 2H, J = 11.9 Hz, PhCH_2), 4.34 (m, 2H, H-6, 6'), 4.11 (m, 1H, H-5), 3.96 (m, 2H, H-3,4), 2.10, 2.06 (2s, 6H, 2COCH_3). Anal. Calcd for $C_{31}H_{32}O_9$: C, 67.88; H, 5.84. Found: C, 67.95, H, 5.79. For **7**: mp: 91-93°C; $[\alpha]_D$+16.9° (*c* 2.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 400MHz): δ 8.72 (s, 1H, OC(N*H*)CCl_3), 8.10-7.10 (m, 15H, 3Ph*H*), 6.36 (d, 1H, $J_{1,2}$ = 2.1 Hz, H-1), 5.73 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 2.1 Hz, H-2), 4.90, 4.64 (ABq, 2H, J = 10.8 Hz, PhCH_2), 4.82, 4.61 (ABq, 2H, J = 11.3 Hz, PhCH_2), 4.35 (dd, 1H, $J_{6,6'}$ = 11.9 Hz, $J_{5,6}$ 1.7 Hz, H-6), 4.30 (dd, 1H, $J_{6,6'}$ = 11.9 Hz, $J_{5,6'}$ = 3.4 Hz, H-6'), 4.17 (dd, 1H, $J_{2,3}$ = 2.1 Hz, $J_{3,4}$ = 8.7 Hz, H-3), 4.1 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.7 Hz, H-4), 4.02 (m, 1H, $J_{5,6}$ = 1.7 Hz, $J_{5,6'}$ = 3.4 Hz, $J_{4,5}$.= 8.7 Hz, H-5), 2.03 (s, 3H, COCH_3). Anal. Calcd for $C_{31}H_{30}O_8Cl_3N$: C, 57.19; H, 4.61. Found: C, 57.42; H, 4.60. For **13**: $[\alpha]_D$+19.5° (*c* 2.2, $CHCl_3$); ^1H NMR($CDCl_3$, 400MHz): δ 8.06-7.09 (m, 80H, 16Ph*H*), 5.86, 5.76, 5.67 (3t, 3H, 3H-2, geminal to BzO), 3.25 (s, 3H, OCH_3), 1.98, 1.97, 1.93 (3s, 9H, 3COCH_3); ^{13}C NMR($CDCl_3$, 400MHz): δ 170.7, 170.6, 170.5 (3CH_3*C*O), 165.2 (2*C*OPh), 165.0 (*C*OPh), 138.5-133.1 (quaternary C), 130.1-127.2 (Ph*C*H), 99.59, 99.57. 99.10, 98.91. 98.72, 98.70(6 C-1), 54.7(O*C*H_3), 21.06-20.8(3CO*C*H_3). Anal. Calcd for $C_{155}H_{160}O_{37}$: C, 71.21; H, 6.12. Found: C, 71.37; H, 6.09. For **1**: ESMS Calcd for $C_{37}H_{64}O_{31}$: 1004.88[M]. Found: 1003[M-H]$^+$. For **15**: $[\alpha]_D$+20.3° (*c* 1.1, $CHCl_3$); ^1H NMR($CDCl_3$, 400MHz): δ 5.82 5.80 5.77 5.76 5.75 5.68 (6t, 6H, 6H-2, geminal to BzO), , 3.20 (s, 3H, OCH_3), 1.95, 1.93, 1.91 (3s, 9H, 3COCH_3). Anal. Calcd for $C_{236}H_{238}O_{55}$: C, 71.70; H, 6.02. Found: C, 71.76; H, 6.00. For **2**: ESMS Calcd for $C_{55}H_{94}O_{46}$: 1491.30[M]. Found: 1490[M-H]$^+$.