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SYNTHESIS OF IMMUNOLOGICALLY ACTIVE OLIGOSACCHARIDE DETERMINANTS OF THE *BRUCELLA* A ANTIGEN: UTILIZATION OF INTERMEDIATES DERIVED FROM METHYL 4-AZIDO-4,6-DIDEOXY-α-D-MANNOPYRANOSIDE

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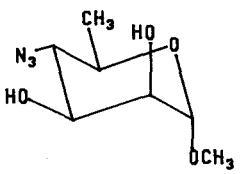
Abstract: A facile, high yield synthesis of methyl 4-azido-4,6-dideoxy-D-mannopyranoside is described in conjunction with an integrated strategy for the synthesis of its 1,2-linked oligosaccharides, which are antigenic determinants of the *Brucella* A antigen.

Structural studies of the *Brucella* A and M antigens[1,2], fifty years after they were first identified[3], has resolved the molecular basis for the inter-relationship of these major cell wall antigens which provide a foundation for serodiagnosis of brucellosis. Both antigens are homopolymers containing 1,2-linked 4-deoxy-4-formamido-α-D-mannopyranosyl residues, a rare sugar, difficult to isolate because of the instability of the aldose form of the amino-sugar[4]. This paper reports the synthesis of the parent monosaccharide at the 10-20 gramme scale and the subsequent utilization of this material in a unified strategy to yield immunologically active di- and trisaccharides that form the basis of an approach towards totally synthetic diagnostic antigens and vaccines.



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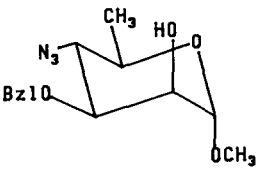

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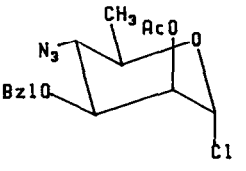

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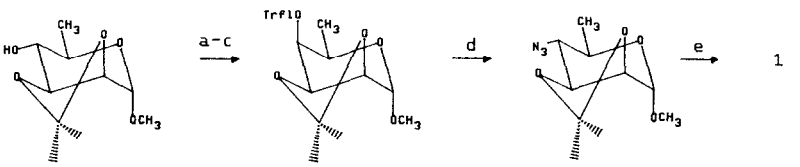
Methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (1) was envisioned as a common precursor to selectively protected alcohols (2) and glycosyl halides (3) that serve respectively as glycosyl acceptors and donors in silver triflate promoted oligosaccharide syntheses. The first obstacle was to derive (1) from a readily available monosaccharide and this was achieved by first transforming D-mannose to the methyl α-D-rhamnopyranoside (4) according to Thiem and Gerken[5]. A more direct route was later developed in which (4) was prepared

directly from methyl 2,3-O-isopropylidene-α-D-mannopyranoside[6] in 65% yield by iodination at C-6 followed by hydrogenation[7]. A double inversion at C-4 accompanied by introduction of an azide substituent provided the target intermediate (1) via a talopyranoside prepared by pyridinium chlorochromate[8] or Swern[9] oxidation, followed by sodium borohydride reduction. Displacement of triflate from the talopyranoside[10] (5) gave the azido derivative (1) 80%, m.p. 79-80° lit.[4] 81.5-82.5°, $[\alpha]_D^{22}$ + 127.2° (*c*, 0.98 in CH_2Cl_2) lit.[4] + 126.9° (in MeOH) after removal of the isopropylidene group from (6). Selective protection for utilization in glycoside synthesis was accomplished by regio-selective acetylation of (1) to give (7) via the 2,3-orthoacetate[11]. This compound which also serves as a glycosyl acceptor for generation of 1,3 linkages present to some extent in the *Brucella* M antigen[2] was benzylated by benzyl trichloroacetimidate[12] under acid catalysis. Transesterification of (8) produced the glycosyl acceptor (2) as an analytically pure syrup, $[\alpha]_D^{22}$ + 141.0° (*c*, 0.46 in CH_2Cl_2) in 62% overall yield from (4). Acetolysis of glycoside (8) gave quantitatively a mixture of anomeric acetates that were smoothly converted by reaction[11] with dichloromethyl methyl ether into the glycosyl chloride (3) 89%, which is suitably protected to direct 1,2-*trans*-glycoside formation and then permits chain extension after glycosylation by removal of the temporary protecting group at O-2. The purity of syrupy glycosyl chloride (3) was established by n.m.r. at 500 MHz.



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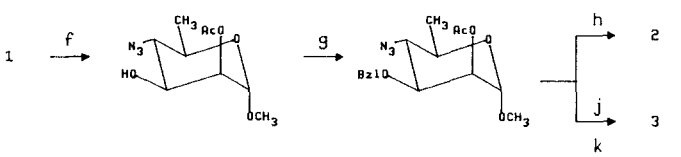
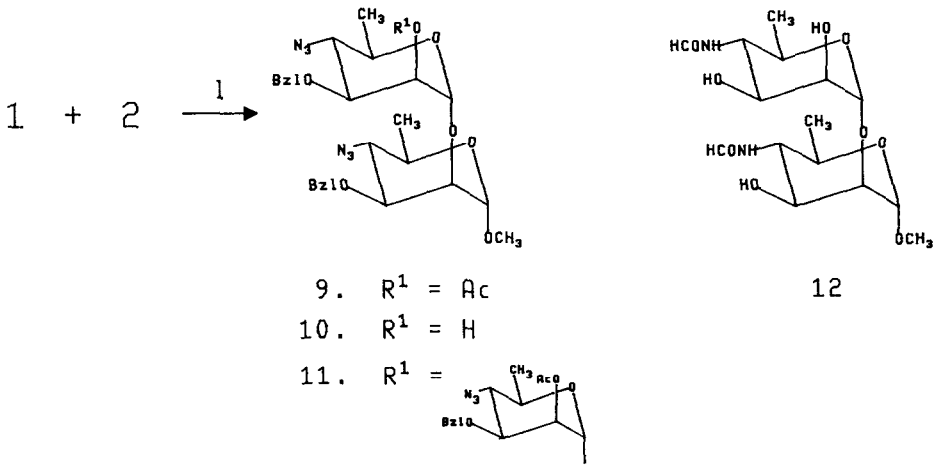


7 8

[a] PCC, powdered molecular sieve, CH_2Cl_2; [b] $NaBH_4$ in MeOH; [c] $(CF_3SO_2)_2O$ (1.17 eq) in CH_2Cl_2 containing pyridine; [d] KN_3 in DMF, 18-crown-6, room temp 2 h; [e] CF_3CO_2H:H_2O 9:1, 10 min 0°; [f] (i) $(EtO)_3CMe$ in DMF/H^+; (ii) 80% aqueous HOAc; [g] $Cl_3CCNHOCH_2C_6H_4$ in CCl_4/cyclohexane CF_3SO_3H; [h] NaOMe in MeOH; [j] Ac_2O:HOAc:H_2SO_4 100:40:1, room temp 3 h; [k] Cl_2HCOMe, $ZnBr_2$ in CH_2Cl_2, room temp 1 h.

SCHEME 1

Glycosyl chloride (3) effectively glycosylated the protected alcohol (2) in a silver triflate promoted reaction to yield the disaccharide (9) 85% $[\alpha]_D^{22}$ + 99.3° (*c*, 0.94 in CH_2Cl_2), and it was equally efficient in chain extension reactions with the selectively deprotected disaccharide (10) yielding the trisaccharide (11) 83% $[\alpha]_D^{22}$ + 82.9° (*c*, 1.0 in CH_2Cl_2). The disaccharide was deprotected in two steps by transesterification and hydrogenolysis, and the N-formylated disaccharide (12) $[\alpha]_D^{22}$ + 39.3° (*c*, 0.5 in MeOH), obtained after a mixed anhydride reaction with its free amino derivative. This disaccharide was an inhibitor of the binding of *Brucella* A antigen by both infected cow sera and monoclonal antibodies.



9. R^1 = Ac
10. R^1 = H
11. R^1 =

12

[1] CF_3SO_3Ag, 4A molecular sieve in CH_2Cl_2, -30° 1 h and 3 h to room temp.

SCHEME 2

Syntheses of extended oligomers utilizing the strategy outlined here in combination with methodologies for covalent attachment to protein provide ligands and synthetic antigens that are being applied to problems of serodiagnosis and development of superior reagents for bacterial detection. This includes work in progress to characterize the antibody combining site fragment (Fab) derived from monoclonal antibodies that bind the *Brucella* A antigen and which is the subject of X-ray diffraction studies.

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