Synthesis of 4,6-Dideoxy-4-formamido-α-D-mannose containing Tri-, Tetra-, and Penta-saccharides, Antigenic Determinants of the *Brucella* A and M Antigens†

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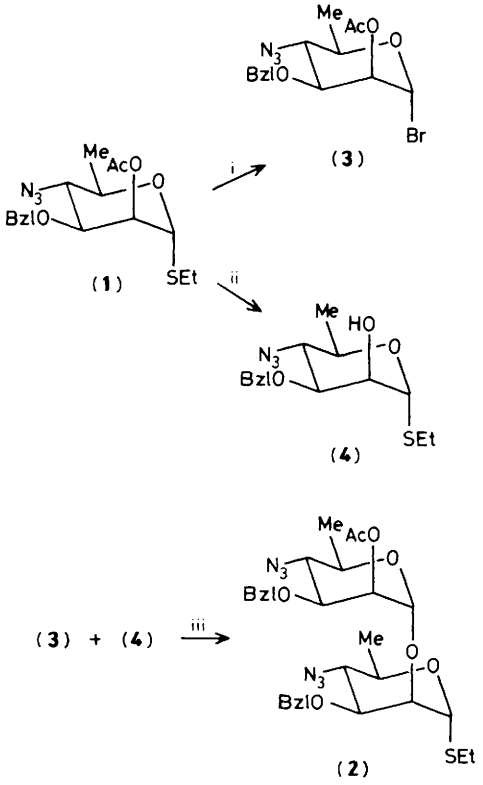
A synthetic strategy based upon the use of thioglycosides to generate 1,2-*trans*-glycosidic linkages has been developed and has permitted efficient synthesis of pentameric α-1,2 linked 4,6-dideoxy-4-formamido-α-D-mannose homo-oligomers as well as structures containing α-1,3 linkages.

Diagnosis of brucellosis often relies upon serological detection of host antibodies to a cell wall polysaccharide, the *Brucella* A antigen, since isolation and culture of the causative organism is difficult.[1] The structural analysis of this antigen was reported by this laboratory[2] and recently the structure of the related *Brucella* M antigen was also elucidated.[3] Both polysaccharides are homopolymers of 4,6-dideoxy-4-formamido-D-mannose. Whilst the A antigen contains this rare monosaccharide as an exclusively α-1,2 linked homopolymer, the M antigen is a linear polymer composed of pentasaccharide repeating units containing one α-1,3 and four α-1,2 linkages. A high yield synthesis of 4,6-dideoxy-4-formamido-α-D-mannose oligosaccharides in combination with strategies for the covalent attachment of these structures to proteins or solid supports[4] permits the use of synthetic, well defined compounds as substitutes for polysaccharides of bacterial origin in serological tests and eventually as vaccines.

To achieve this goal, a method was chosen that takes advantage of methyl trifluoromethanesulphonate promoted glycosylations, using 1-thio sugars as glycosyl donors.[5] This approach has proven to be especially useful in block synthesis, involving oligosaccharides as glycosyl donors.[5] The promising results of those studies led us to the design of the *S*-ethyl glycosides (**1**) and (**2**) as key intermediates, for the synthesis of 4,6-dideoxy-4-formamido-α-D-mannose containing oligosaccharides. A high yield synthesis of methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside from D-mannose[6] and its demonstrated potential[7] in conventional Königs–Knorr reactions provide ready access to a versatile intermediate that may be converted[8] *via* its 1,2-di-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannose derivative to the *S*-ethyl glycoside (**1**).



Scheme 1. *Reagents and conditions:* i, Br_2 in dichloromethane, room temp., 1 h, quantitative yield; ii, NaOMe in methanol, room temp., 4 h, quantitative yield; iii, dichloromethane, AgOTf, −30 °C, 1 h, 75% yield after chromatography. (Tf = CF_3SO_2; Bzl = $PhCH_2$).

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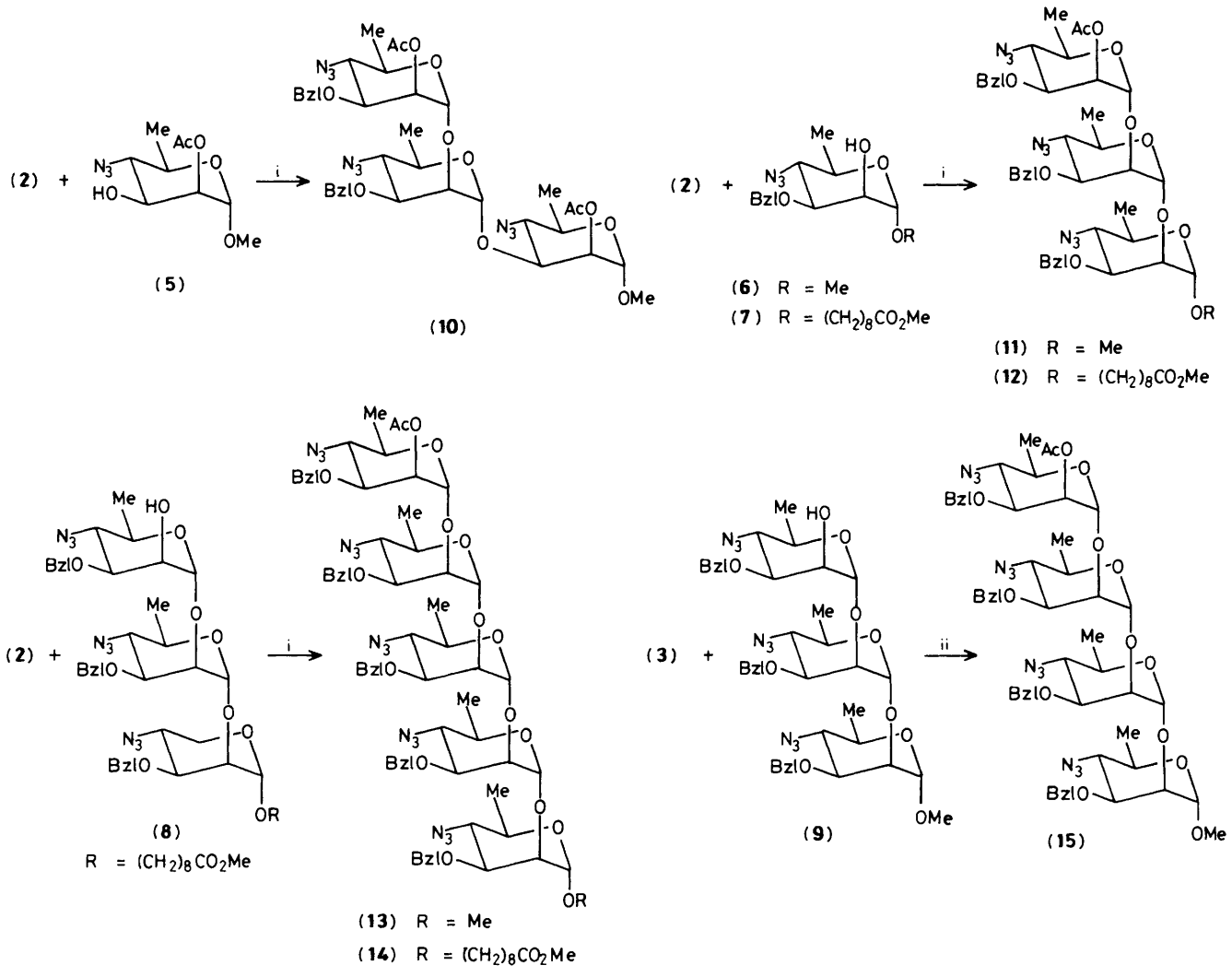

Scheme 2. *Reagents and conditions:* i, dichloromethane, MeOTf, room temp., 12—48 h, 60—80% yield after chromatography; ii, dichloromethane, AgOTf, room temp. 5 h, 80% yield after chromatography.

Whereas (**1**) represents a monosaccharide synthon, (**2**) is a disaccharide building block, thus enabling chain elongation by either one or two sugar moieties. The disaccharide donor (**2**) is obtained from (**1**) by converting it under very mild conditions into the bromide (**3**)[9] which is subsequently coupled to the 2-*O*-deacetylated derivative (**4**), using silver trifluoromethanesulphonate as a catalyst (Scheme 1).

The disaccharide donor (**2**) has been coupled to different aglycones (**5**)—(**9**) in dichloromethane at room temperature with promotion by methyl trifluoromethanesulphonate. The reaction time is typically overnight to two days, resulting in yields of 60—80% based upon the aglycone. Although lacking neighbouring group participation at the C-2-position, (**2**) formed exclusively α-linked products (Scheme 2). This was easily demonstrated by measuring the C(1)–H(1) coupling constants (170—172 Hz).[10] The method was also tested for the synthesis of spacer-arm linked oligosaccharides and it was found that 8-methoxycarbonyloctanol glycosides[4] were as easily accessible as the corresponding methyl glycosides (Scheme 2). Using the outlined strategy, the trisaccharide methyl glycosides (**10**) and (**11**) containing only α-1,2 or one α-1,2 and one α-1,3 linkage were synthesized. In analogous fashion the spacer-arm linked trisaccharide (**12**) and pentasaccharides (**13**) and (**14**) were synthesized (Scheme 2). To obtain even numbered oligosaccharides, the bromide (**3**) was coupled to different de-*O*-acetylated oligosaccharides, *e.g.* coupling of (**3**) with (**9**) under silver trifluoromethanesulphonate catalysis in dichloromethane at room temperature gave the tetrasaccharide (**15**) in 80% yield.

Deprotection of the oligosaccharides was achieved in a 4-step process. Deacetylation gave derivatives containing only azido functions and benzyl ethers as protective groups. The azido groups were easily converted into amino functions by mild H_2S reduction. Treatment of the amines with methyl formate at room temperature[11] provided the corresponding formamido compounds in quantitative yield. Subsequent catalytic hydrogenation, using 5% palladium on charcoal, led to the fully deblocked oligosaccharides. In all cases the syntheses of the blocked oligosaccharides were performed on a gram scale and the products were fully characterized by analytical data and n.m.r. spectra recorded at 500 and 125 MHz for ^1H and ^{13}C respectively. Finally it should be noted

that the strategy reported here for the synthesis of α-1,2-linked oligosaccharides of 4-amino-4,6-dideoxy-D-mannose is suited, in principle, to the synthesis of antigenic determinants of the *Vibrio cholerae* O-antigen, which differs from that of *Brucella* only in its *N*-acyl moiety.[12]

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