## Synthesis of 4,6-Dideoxy-4-formamido- $\alpha$ -D-mannose containing Tri-, Tetra-, and Penta-saccharides, Antigenic Determinants of the *Brucella* A and M Antigens<sup>†</sup>

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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  $\alpha$ -1,2 linked 4,6-dideoxy-4-formamido- $\alpha$ -D-mannose homo-oligomers as well as structures containing  $\alpha$ -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<sup>2</sup> and recently the structure of the related Brucella M antigen was also elucidated.<sup>3</sup> Both polysaccharides are homopolymers of 4,6-dideoxy-4-formamido-D-mannose. Whilst the A antigen contains this rare monosaccharide as an exclusively  $\alpha$ -1,2 linked homopolymer, the M antigen is a linear polymer composed of pentasaccharide repeating units containing one  $\alpha$ -1,3 and four  $\alpha$ -1,2 linkages. A high yield synthesis of 4,6-dideoxy-4-formamido- $\alpha$ -D-mannose oligosaccharides in combination with strategies for the covalent attachment of these structures to proteins or solid supports<sup>4</sup> 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.<sup>5</sup> This approach has proven to be especially useful in block synthesis, involving oligosaccharides as glycosyl donors.<sup>5</sup> 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- $\alpha$ -D-mannose containing oligosaccharides. A high yield synthesis of methyl 4-azido-4,6dideoxy- $\alpha$ -D-mannopyranoside from D-mannose<sup>6</sup> and its demonstrated potential<sup>7</sup> in conventional Königs–Knorr reactions provide ready access to a versatile intermediate that may be converted<sup>8</sup> via its 1,2-di-O-acetyl-4-azido-3-O-benzyl-4,6dideoxy- $\alpha$ -D-mannose derivative to the *S*-ethyl glycoside (1).

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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  $\alpha$ -linked products (Scheme 2). This was easily demonstrated by measuring the C(1)–H(1) coupling constants (170—172 Hz).<sup>10</sup> The method was also tested for the synthesis of spacer-arm linked oligosaccharides and it was found that 8-methoxycarbonyloctanol glycosides<sup>4</sup> were as easily accessible as the corresponding methyl glycosides (Scheme 2). Using the outlined strategy, the trisaccharide methyl glycosides (10) and (11) containing only  $\alpha$ -1,2 or one

 $\alpha$ -1,2 and one  $\alpha$ -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<sup>11</sup> 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 <sup>1</sup>H and <sup>13</sup>C respectively. Finally it should be noted

that the strategy reported here for the synthesis of  $\alpha$ -1,2linked 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.<sup>12</sup>

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## References

- 1 G. C. Alton, L. M. Jones, and D. E. Pietz, Laboratory Techniques in Brucellosis, 2nd Edn., W.H.O. Monogr. Ser. No. 55, 1975.
- 2 M. Caroff, D. R. Bundle, M. B. Perry, and J. W. Cherwonogrodzky, Infect. Immun., 1984, 46, 384.
- 3 D. R. Bundle, J. W. Cherwonogrodzky, and M. B. Perry, *Biochemistry*, in the press.

- 4 R. U. Lemieux, D. R. Bundle, and D. A. Baker, J. Am. Chem. Soc., 1975, 97, 4076.
- 5 H. Lönn, Carbohydr. Res., 1985, 139, 105.
- 6 D. R. Bundle, M. Gerken, and T. Peters, *Carbohydr. Res.*, in the press.
- 7 D. R. Bundle and M. Gerken, Third European Symposium on Carbohydrates, Grenoble, 1985, p. 207.
- 8 R. J. Ferrier and R. H. Furneaux, Carbohydr. Res., 1976, 52, 63.
- 9 F. Weygand, H. Ziemann, and H. J. Bestmann, *Chem. Ber.*, 1958, **91**, 2534.
- 10 K. Bock and C. Pedersen, Acta Chem. Scand., Ser. B, 1975, 29, 258.
- 11 B. C. McKusick and M. E. Hermes, Org. Synth., 1961, 41, 14; G. Moffat, M. V. Newton, and G. J. Papenmeier, J. Org. Chem., 1962, 27, 4058.
- 12 J. W. Redmond, *FEBS Lett.*, 1975, **50**, 147; L. Kenne, B. Lindberg, P. Unger, B. Gustafsson, and T. Holme, *Carbohydr. Res.*, 1983, **111**, 289.