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154

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154 Topics in Current Chemistry

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Table of Contents

Synthesis of Oligosaccharides Related to Bacterial O-Antigens

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Table of Contents

The O-polysaccharides of bacterial lipopolysaccharides are in general regular, periodic polymers with diverse structures that contain, in many instances, comparatively rare monosaccharides. The oligosaccharides that constitute the repeating units of these polysaccharide antigens provide a demanding challenge in terms of glycoside synthesis, an objective which is particularly important since these structures act as antigenic determinants which are valuable markers of bacterial infection. Advances in glycoside synthesis together with the ancillary techniques of chromatographic separation and high resolution NMR spectroscopy have permitted rational synthesis of such oligosaccharides to be planned, successfully completed and have in a limited number of instances, even allowed small polysaccharides, representative of the O-polysaccharide, to be synthesized via polymerization of synthetic repeating units. The most intensive synthetic efforts have focused on the O-antigens of *Salmonella*, *Shigella*, and *E. coli*, although increasing attention is being given to the synthesis of antigenic determinants of other Gram-negative pathogens, including those of *Brucella*.

Topics in Current Chemistry, Vol. 154

1 Survey of Synthesized and Structurally Defined O-Antigens

1.1 Introduction

The lipopolysaccharides (LPS) of most Gram-negative bacteria, pathogenic for humans and animals, carry an O-polysaccharide component. Morphologically the colonies of such organisms have a smooth rather than rough appearance. The latter are often associated with LPS that lack this structural component and, as a consequence, the designations smooth (S) and rough (R) indicate the presence or absence of an O-polysaccharide [1]. During the course of bacterial infection, a humoral and cellular response is mounted most often with specificity for antigenic determinants of the O-polysaccharide and, ultimately, immunity to reinfection by the causative bacterium can be established [2]. The antibodies produced to unique cell-surface antigens constitute diagnostic markers by which the infectious agent can be subsequently identified. Consequently, these antigens or their fragments may be used both as diagnostic markers of specific infectious agents and possibly may even serve as vaccines in special circumstances [2]. The lipopolysaccharide molecule itself, due to its toxic lipid A component, is unsuitable for this purpose [3] and hence synthetic variants of the O-polysaccharide antigen become attractive candidates as chemically defined antigens for diagnostic and prophylactic purposes [2].

The advances that have led to the almost routine synthesis of tri- and tetrasaccharides began in the early 1970s and included a new approach to α-glycosides, the halide-ion catalyzed glycosylation [4], and the introduction of improved heavy metal catalysts such as silver trifluoromethanesulfonate (triflate) [5]. Numerous other technical and conceptual advances punctuated the intervening period and reference to these developments are to be found in the cited literature of several reviews [6—9].

1.2 Lipopolysaccharide Structure

A general architecture of LPS has been established based in large part upon extensive studies of *Salmonella* LPS [10]. The picture of LPS that has emerged and been confirmed in general detail for most LPS has three well-defined regions (Fig. 1). The O-specific antigen (also called somatic antigen or O-polysaccharide) is a polysaccharide generally consisting of from 5 to 40 repeating units that may contain between

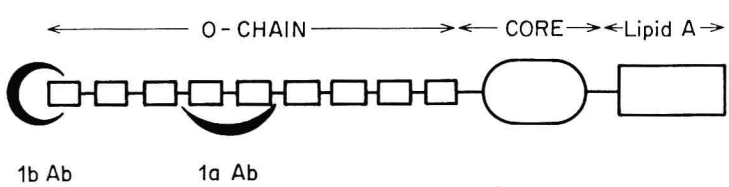


Fig. 1. Schematic representation of the three distinct regions of LPS. The repating unit structure of the O-polysaccharide may be recognized by antibodies with specificities for determinants associated with the terminal non-reducing residues (antibody type 1 b) or internal sequences often spanning more than one repeating sequence (cf. Refs. [72, 73])

one to seven monosaccharides [3, 10]. This component is biosynthesized by addition of single repeating units to the reducing end of the growing O-polysaccharide and then completed polysaccharide is translocated to a specific monosaccharide close to or at the non-reducing terminus of an oligosaccharide core, which is composed of approximately ten monosaccharides. This core is biosynthesized, prior to O-chain attachment, by sequential introduction of monosaccharides to the third region, Lipid-A, a hydrophobic segment which anchors the assembly in the outer membrane of the Gram-negative bacterial cell wall.

It should be noted that the biosynthetic assembly involves polymerization of a specific repating unit structure, which is termed the *biological repating unit* as opposed to the *chemical repating unit* [11]. The latter term refers to the repeating unit structure elucidated by analytical techniques, which rely on chemical fragmentation of the polysaccharide and thus reflects the relative resistance of inter-residue glycosidic bonds to cleavage. Consequently, structural analysis of O-polysaccharide rarely yields information on the biological repeating unit, a structural feature that is generally difficult to establish. The antigenic specificity of chemical repeating units can differ appreciably from those of the biological sequence [2], a fact which may have considerable relevance to the planning of synthetic targets and strategies.

1.3 *Salmonella* O-Polysaccharides Structures

The structures of the O-antigens of *Salmonella* serogroups A, B, D, and E were amongst the first to be studied structurally [11] and biosynthetically [12, 13]. These serogroups also comprise by far the largest clinically important group of *Salmonella* responsible for gastrointestinal infections [2].

A linear sequence of Man-Rha-Gal is to be found in the repeating units of all O-polysaccharides belonging to these serogroups, A–E. The repeating units of serogroups A, B, and D are closely related (Table 1) and share a common linear Man-Rha-Gal sequence, which is branched at *O*-3 of the mannose residue by a 3,6-dideoxyhexose, the stereochemistry of which defines the A, B, and D_1 serogroups. These tetrasaccharide repeating sequences are presented together with the original literature reporting the structural elucidation in Table 1. In this regard it is to be noted that the erroneous determination of the configuration of rhamnopyranose as β in structures belonging to serogroup B was never corrected in the original literature and this was perpetuated in several reviews. In both the serogroup A and D polysaccharides, this linkage was correctly assigned as an α-L-rhamnopyranose despite the difficulty of unambiguously identifying this feature. A similar error was made for the equally problematic mannopyranosyl linkage but this was corrected following the results of enzymatic and biosynthetic studies [17, 19]. Thus, it is now clear that in all antigens of the serogroups A, B, and D_1 both mannose and rhamnose residues are involved as α linkages [20]. The exception is the serogroup D_2 where the mannose residue not only adopts the β configuration but also is substituted by galactose at *O*-6 instead of *O*-2. Elaboration upon these serogroup A, B, and D structures by the action of a phage-induced glucosyl transferase, introduces branching on the galactose residue of the main chain at either position 4 or 6 [10]. Since this modification occurs after polymerization, not every repeating unit is glucosylated [10, 14]. Despite this additional level of complexity

Table 1. *Salmonella* serogroups A, B, and D

Salmonella Group (O Facotrs)	Biological Repeating Units	References to Structural Studies	References to Synthetic Work
	Group A		
S. paratyphi (1, 2, 12)	[2)-α-D-Man*p*(1 → 4)-α-L-Rhap(1 → 3)-α-D-Galp(1] 3 4 ↑ ↑ 1 1 α-D-Par*p* α-D-Glc*p*	14	26–28, 33, 34, 37
	Group B		
S. typhimurium (1, 4, 5, 12)	[2)-α-D-Man*p*(1 → 4)-α-L-Rha*p*(1 → 3)-α-D-Galp(1] 3 4/6 ↑ ↑ 1 1 α-D-Abe*p* α-Glc*p*	15–19	30–32, 34–36, 38
S. bredeney (1, 5, 12)	2-*O*-Ac-α-D-Abe*p*		40
	Group D_1		
S. typhi (9, 12)	[2)-α-D-Man*p*(1 → 4)-α-L-Rha*p*(1 → 3)-α-D-Gal*p*(1] 3 4 ↑ ↑ 1 1 α-D-Tyv*p* α-D-Glc*p*	19, 21	24, 28, 29 33, 34, 37
	Group D_2		
S. strasbourg (9), 12_3, 46)	[6)-β-D-Man*p*(1 → 4)-α-L-Rha*p*(1 → 3)-α-D-Gal*p*(1] 3 4 ↑ ↑ 1 1 α-D-Tyv*p* α-D-Glc*p*	21–23	25, 39

the principal antigenic specificity involves the 3,6-dideoxyhexose moieties, for which the term *immunodominant* was originally introduced [11]. Thus, most synthetic approaches address this major antigenic feature [32–36], although attempts, principally by Kotchetkov and co-workers [39], have been made to incorporate the second branch point involving α-glucosylation of the main chain galactose residue (Table 1). Serogroup B structures are known in which *O*-2 of the 3,6-dideoxy-α-D-*xylo*-hexopyranose residue carries an *O*-acetyl group [14]. This structural feature radically alters the antigenic specificity (O-factor 4 changes to O-factor 5) of antibodies directed toward the branching residue but the intrinsic difficulty of introducing and maintaining an *O*-acetate throughout de-protection stages has limited attempts to synthesize determinants with O-factor 5 specificity [40].

Much of the current knowledge of the biosynthesis of bacterial O-polysaccharide assembly was derived from early studies of the polysaccharides of *Salmonella* serogroup E [13]. This group consists of a series of four closely related structures belonging to the serogroups $E_1 \rightarrow E_4$ (Table 2). The basic main chain sequence of three monosaccharides, Man-Rha-Gal, seen in the D_2 serogroup is the structural element of all four polysaccharides; however, in serogroups E_1, E_2 and E_3, E_4 the variation oc-

curs by changes in the configuration and position of glycosidic linkages rather than via branching by distinct hexose residues. The polysaccharides of serogroups E_1 and E_2 are amongst the simplest of the *Salmonella* O-polysaccharides and, consequently, attempts to prepare synthetic polysaccharides involved these structures [48–50]. The work which formed the basis for the approach is referenced in Table 2 and covers the synthesis of tri- and tetrasaccharide determinants of the E_1–E_4 structures prepared for the most part as biological repeating units [50–56]. *Salmonella* membrane preparations have also been used to polymerize trisaccharide repeating units, prepared using glycosyl transferase and nucleoside diphosphate sugars [58].

Table 2. *Salmonella* serogroup E

Salmonella Group (O Factors)	Biological Repeating Units	References to Structural Studies	References to Synthetic Work
	Group E_1		
S. anatum (3, 10)	[6)-β-D-Man*p*(1 → 4)-α-L-Rha*p*(1 → 3)-α-D-Gal*p*(1]	41–43	34, 50, 51, 54, 58–61, 64
	Group E_2		
S. newington (3, 15)	[6)-β-D-Man*p*(1 → 4)-α-L-Rha*p*(1 → 3)-β-D-Gal*p*(1]	41, 42, 44	48–51, 54, 58, 60, 62, 63
	Group E_3		
S. minneapolis (3, (15), 34)	[6)-β-D-Man*p*(1 → 4)-α-L-Rha*p*(1 → 3)-β-D-Gal*p*] 4 ↑ 1 α-D-Glc*p*	41, 45	55
	Group E_4		
S. senftenberg (1, 3, 19)	[6)-β-D-Man*p*(1 → 4)-α-L-Rha*p*(1 → 3)-α-D-Gal*p*(1] 6 ↑ 1 α-D-Glc*p*	46, 47	56, 57

Fragments of the basic repeating unit structures of the serogroups A–E have been prepared [59–63], in one case as an allyl glycoside, which was subsequently co-polymerized with acrylamide to provide a highly active antigen [61, 62]. The synthesis of a disaccharide representative of the *Salmonella* serogroup C antigen in which abequose is linked α-1,3 to α-L-rhamnopyranose has also been reported [64].

1.4 *Shigella* O-Polysaccharides

Shigella flexneri LPSs comprise a large family of interrelated O-polysaccharide structures, the simplest of which belongs to the Y variant polysaccharide (Table 3). This has a tetrasaccharide repeating unit containing three L-rhamnose residues and

Table 3. *Shigella flexneri* O-antigens

Shigella flexneri O-Factor	Biological Repeating Unit	References to Structural Studies	References to Synthetic Work
	Variant Y structure		
—: 3, 4	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1]	65, 68	74, 80, 83, 88
	Variant X structure		
—: 7, 8	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 3 ↑ 1 α-D-Glc*p*	66, 67	84–87
V: 7, 8	5a structure [2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 3 ↑ 1 α-D-Glc*p*	66, 67	84–87
	5b structure		
V: 7, 8	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 3 3 ↑ ↑ 1 1 α-D-Glc*p* α-D-Glc*p*	66, 67	84–87
	1a structure		
I: 4	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 4 ↑ 1 α-D-Glc*p*	66, 67	81
	1b structure		
I: 6	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 2 4 ↑ ↑ Ac 1 α-D-Glc*p*	66, 67	81

	1b structure		
I: 6	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 2 4 ↑ ↑ Ac Ac 1 α-D-Glc*p*	66, 67	—
	2a structure		
II: 3, 4	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 4 ↑ 1 α-D-Glc*p*	59, 60	—
	2b structure		
II: 7, 8	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 3 4 ↑ 1 1 α-D-Glc*p* α-D-Glc*p*	66, 67	—
	3a structure		
III: 6, 7, 8	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 3 ↑ 1 α-D-Glc*p*	66, 67	—
	3b structure		
III: 3, 4, 6	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 2 ↑ Ac	66, 67	—
	4a structure		
IV: 3, 4	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 6 ↑ 1 α-D-Glc*p*	66, 67	—
	4b structure		
IV: 6	[2)α-L-Rha*p*(1 → 2)α-L-Rha*p*(1 → 3)α-L-Rha*p*(1 → 3)β-D-GlcNAc*p*(1] 2 6 ↑ ↑ Ac 1 α-D-Glc*p*	66, 67	—

2-acetamido-2-deoxy-D-glucose and is the basis for a series of elaborations involving α-glucosylation and *O*-acetylation [65–68]. The serological classification and identification of specific O-factors is less well established than the Kauffman-White scheme [69] for *Salmonella*. Recently, the basis of the previously proposed O-factor scheme for *Shigella flexneri* [70] has been questioned [71–73]. Nevertheless, the structural details of these polysaccharides are well established and several extensive synthetic approaches have been mounted, centered mainly upon the simplest Y variant repeating unit [74–83, 88] — and more recently extended to include the X, 5a, and 5b structures [84–87]. In addition, structural variations designed to probe features of the antibody combining site have been the subject of recent studies in this area [89–91].

A synthetic *S. flexneri* Y antigen containing ten repeating units has been prepared [88].

The *S. flexneri* serotype 6 structure is in fact unrelated to the other *S. flexneri* repeating units and should be classified separately [20]. One of the two proposed structures [92, 93] has been the target of a synthetic study which provided a branched tetrasaccharide structure [94] and separate work gave a disaccharide monoacetate structure [95].

The O-polysaccharides of *Shigella dysenteriae* are structurally very distinct from those of the *S. flexneri* [20] and have been the subject of synthetic studies in which a branched pentasaccharide of the serotype 2 antigen was prepared [96].

1.5 *E. coli* and *Klebsiella* O-Polysaccharides

Although the O-antigens of these organisms provide a rich variety of complex structures, the synthesis of antigenic determinants of only a few structures has been attempted. The synthesis of the 3,6-dideoxy-α-L-*xylo*-hexopyranose-containing structure of *E. coli* 0111 has been attempted but only to the trisaccharide level of complexity [97]. A very elegant synthesis of *E. coli* 075 antigen has been reported [98] and the oligomannose determinant of the 09 antigen [99] was also successfully prepared.

Even fewer structures corresponding to *Klebsiella* O-antigens have been synthesized although elements of the O-7 LPS have been prepared [100].

Finally, a synthesis of the determinant of the enterobacterial common antigen which is not a true O-antigen has been reported [101].

1.6 Other O-Polysaccharides

The O-antigens of *Brucella* species are homopolymers of α-1,2-linked 4,6-dideoxy-4-formamido-D-mannopyranose residues in which there may be α-1,3 linkages [102, 103]. The *Yersinia* 0:9 antigen is a similar 1,2-linked homopolymer but contains no 1,3 linkages. A related structure *N*-acylated by 3-deoxy-L-glycero-tetronic acid in place of the formate group is produced by *Vibrio cholerae* Inaba and Ogawa strains [104]. These structures have been the subject of synthetic work [105–107].

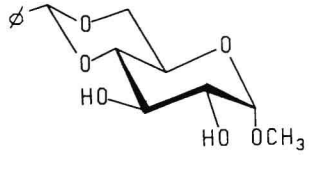
O-Antigens from *Aeromoanas salmonicida* [108] and *Pseudomanas* [109] have also been the subject of synthetic efforts [110, 111].

2 Synthesis of *Salmonella* Oligosaccharides

2.1 *Salmonella* Serogroups A, B, C, and D

Chemical synthesis of disaccharide elements of these oligosaccharides marks the beginning of systematic synthetic studies of the antigenic determinants of bacterial O-antigens [24–31]. The 3,6-dideoxyhexoses, which are the immunodominant monosaccharides of each serogroup, were an intrinsic synthetic challenge since their synthesis in a form suitable for use as glycosyl donors was a prerequisite to successful synthesis. Early synthetic approaches used in the identification of the 3,6-dideoxyhexoses [112–114] were judged unsuitable for subsequent incorporation into synthetic strategies for disaccharide synthesis. Recently, *p*-nitrobenzoylation of 3,6-dideoxyhexoses synthesized from 1,4-lactones [115, 116] shows that the reducing monosaccharide may indeed be a useful starting point for synthesis. Numerous syntheses of the 3,6-dideoxyhexoses have been reported [112–114, 117–123, and references cited therein] but the most frequently adopted routes for subsequent exploitation in glycosylation reactions have used a common methyl hexopyranoside precursor in which the 3-deoxy function is first introduced followed by deoxygenation at C-6, or starting from a 6-deoxyhexose the deoxygeneration at C-3 is the final step in the preparation of starting material.

Typical examples of these two approaches are to be seen in syntheses of 3,6-dideoxy-D-*arabino*-hexose [29, 33, 121] and 3,6-dideoxy-D-*xylo*-hexose [35, 118, 122]. Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (*1*) is converted in a one-flask reaction and



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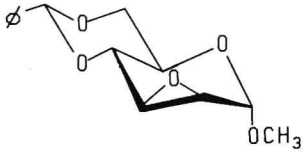

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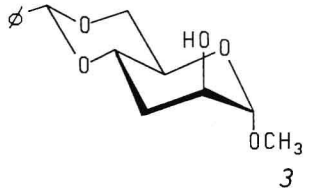

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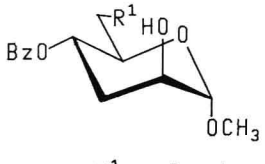
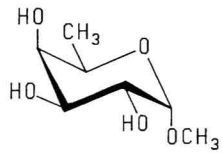


R^1 = Br *4*

R^1 = H *5*

high yield to the 2,3-anhydro-compound (*2*) via the 2-*O*-tosylate and reduction by $LiAlH_4$ affords the 3-deoxy-mannopyranoside (*3*) [124] from which a 6-bromo-deoxy derivative (*4*) is easily prepared by the Hanessian-Hullar reaction [125]. Reduction affords methyl 4-O-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside (*5*) [29, 33]. Methyl 6-deoxy-α-D-galactopyranoside (*6*) may be generated by either the deoxygenation of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose followed by a Fischer

glycosylation [35] or from methyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactopyranoside [122]. Subsequent formation of the *R* and *S* 3,4-*O*-benzylidene acetals (*7a* and *7b*) followed by *N*-bromosuccinimide actel opening gave the 3-bromo-deri-



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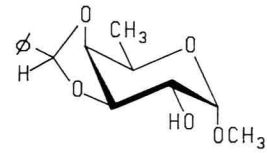
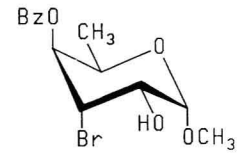


7a
7b



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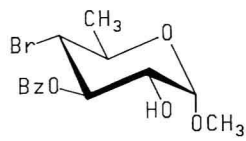

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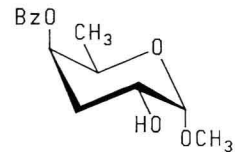

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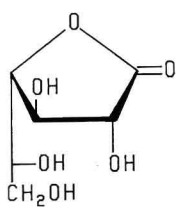
vative (*8*) and, in the case of the α-series (cf. Ref. [35, 126]), also methyl 3-*O*-benzoyl-4-bromo-α-D-glucopyranoside (*9*). Reduction provides the target monosaccharide glycoside (*10*). Severyl convenient syntheses of paratose, 3,6-dideoxy-*ribo*-hexose, using the aforementioned approaches, have been described [32, 33, 37], and as well direct conversion of methyl β-D-glucopyranoside to the 3,6-dichloro-3,6-dideoxy-allopyranoside by chlorosulfation [120] or the bromo analogue with triphenylphosphine, bromine, and imidazole [122] are practical routes, together with recent modification of the chlorosulfation approach [119].

Finally, as already noted, simple and elegant lactone chemistry [115, 116] starting from the readily available 1,4-galactonolactone (*11*) and HBr/HOAc yields crystalline



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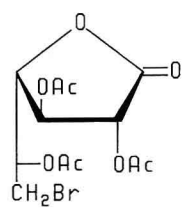

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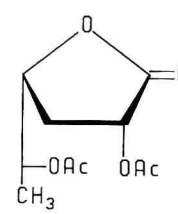

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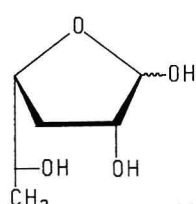
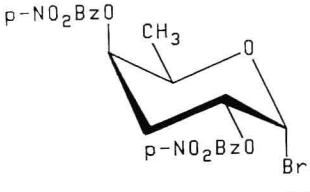


14

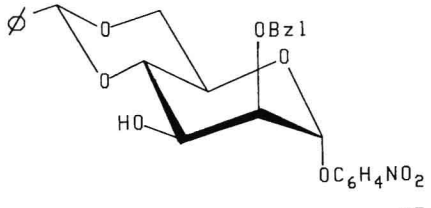
6-bromo-6-deoxylactone (*12*) from which the 3,6-dideoxy-1,4-lactones (*13*) are obtained by hydrogenolysis in the presence of triethylamine, via a β-elimination reaction that provides a 2,3-unsaturated lactone, which is reduced to *13*. The deacetylated 3,6-dideoxy-1,4-lactone is then reduced under controlled conditions to give the 3,6-dideoxy-D-*xylo*-hexose (*14*). This compound was utilized further as its 2,4-di-*O*-*p*-nitrobenzoyl glycosyl bromide [36]. Since acetylated lactones undergo this β-elimination, tyvelose may in principale be prepared by HBr/HOAc bromination of the 3-deoxy-D-*arabino*-hexono-1,4-lactone obtained from either D-*manno*- or D-*glucono*-1,4-lactones [115, 116].

The branching 3,6-dideoxyhexoses are exclusively α-linked to the main chain mannose residues of the O-polysaccharide and for paratose and abequose this requires the formation of a 1,2-*cis*-glycopyranoside bond [8]. Thus, glycosyl halides with the *ribo* and *xylo* configuration are required with a nonparticipating group at O-2. In the case of tyvelose, 3,6-dideoxy-D-*arabino*-hexopyranose, glycosyl halides with participating protecting groups lead to the desired 1,2-*trans*-glycosidic bond formation. Thus, 2,4-di-*O*-benzoyl α-D-*arabino*-hexopyranosyl bromide [29] or chloride [33, 34, 37] have been used in the synthesis of the serogroup D_1 O-factor 9 oligosaccharides. Generally, abequose and paratose methyl glycopyranosides have been dibenzylated or the respective 4-*O*-benzoates may be monobenzylated at *O*-2 employing neutral- or acid-catalyzed benzylation condictions [30, 32, 33, 35–37]. Direct conversion of the protected methyl pyranosides to glycosyl halides has employed hydrogen chloride or hydrogen bromide [27, 31], dichloro- or dibromomethyl methyl ether [33, 35] or chloro- or bromotrimethylsilane [34, 37]. Alternately, the less direct aqueous hydrolysis of the methyl glycoside, followed by formation of the 1-*O*-nitrobenzoates, has been recommended to yield cleaner glycosyl bromide preparations [34].

Employing glycosyl donors of the above type and a simple monosaccharide acceptor such as methyl 2-O-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside or the



16



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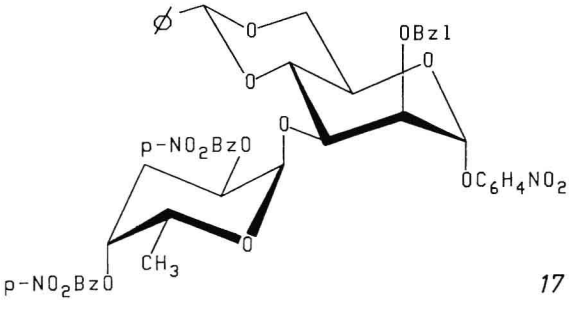

17