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A NEW APPROACH TO THE SYNTHESIS OF A DIMERIC FRAGMENT OF THE CAPSULAR POLYSACCHARIDE OF HAEMOPHILUS INFLUENZAE type b

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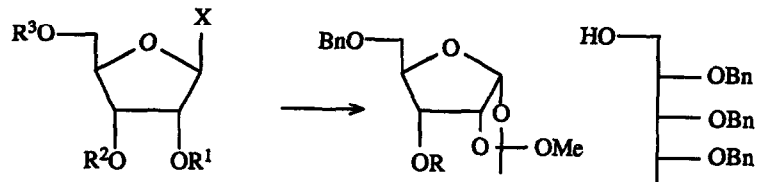
Abstract: The synthesis of the monomeric fragment of the *Haemophilus influenzae* type b (HIb) polysaccharide starting from orthoacetate 7 is described. The construction of the dimer is also reported.

The poly-(ribosyl-ribitol-phosphate) (PRP) 1 capsular polysaccharide isolated from the *Haemophilus influenzae* type b bacteria has recently generated interest because of its possible use as a vaccine for infantile meningitis caused by the HIb bacteria[1].

Recently Van Boom et al[2] reported the synthesis of dimeric and trimeric fragments of PRP. The trimeric PRP attached to the carrier protein via a glycinamide linker was found to elicit a promising immunogenic response. Soon after our laboratory reported a somewhat similar approach to a tetrameric PRP fragment with a linker derived from a representative part of the polysaccharide itself[3].

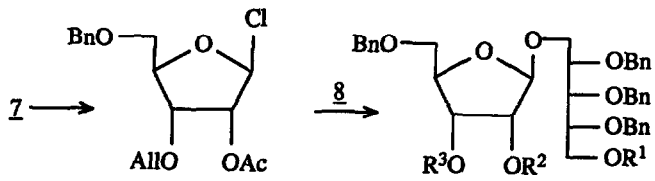
Both approaches had some common features. The ribosyl-ribitol moeity was constructed by a Koenigs-Knorr glycosidation of ribose tetraacetate with a suitably protected ribitol. After hydrolysis of the acetate groups, the resulting triol was differentiated by the use of Markiewicz's protecting group[4] to simultaneously mask the 3' and 5' hydroxy functions. The 2'-hydroxyl was then benzyloxymethylated ; desilylation followed by selective protection of the primary hydroxyl group gave the required free 3'-hydroxyl which could then be coupled with the 5-OH of another ribosyl-ribitol unit through a phosphate moeity.

We now wish to report a new synthesis of the ribosyl- ribitol unit starting with the orthoacetate 7 as key intermediate since we believe it offers several advantages over the reported syntheses. The orthoester 7 has a dual purpose; it allows the simultaneous blocking of the 1' and 2' hydroxy groups thus effectively distinguishing between the 2' and 3' hydroxy groups. It can then be readily transformed to a 1'-chlororiboside by the action of chlorotrimethylsilane[5]. Our sequence also keeps protecting group manipulations on the ribosyl-ribitol unit to a minimum. Furthermore any non-participating hydroxy groups can be benzylated thus simplyfying the deprotection



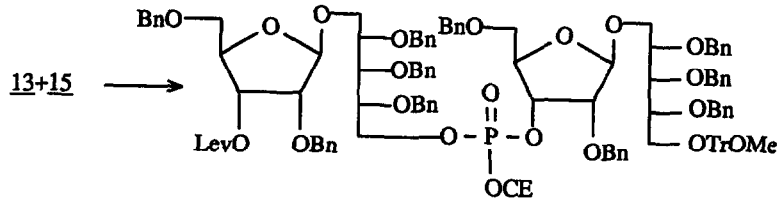
2. X=OMe, R^1=R^2=CMe_2, R^3=H
3. X=OMe, R^1=R^2=CMe_2, R^3=Bn
4. X=OAc, R^1=R^2=Ac, R^3=Bn
5. X=Cl, R^1=R^2=Ac, R^3=Bn
6. R=Ac
7. R=All

8



9. R^1=R^3=All, R^2=Ac
10. R^1=R^3=All, R^2=Bn
11. R^1=R^3=H, R^2=Bn
12. R^1=MeOTr, R^2=Bn, R^3=H
13. R^1=MeOTr, R^2=Bn, R^3=P(OCE)($NiPr_2$)
14. R^1=MeOTr, R^2=Bn, R^3=Lev
15. R^1=H, R^2=Bn, R^3=Lev

OCE=OCH_2CH_2CN, Lev=levulinyl, All=allyl



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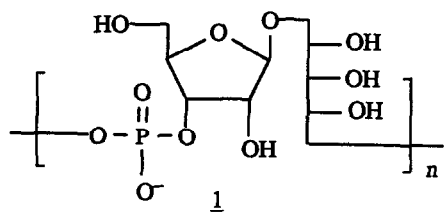

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of the final compounds.

The synthesis of the orthoacetate 7 began with the known protected ribofuranoside 2[6], which was converted to the benzyl ether 3 (NaH, BnBr, nBu$_4$NI, THF)[7] (bp 130-135°C/0.1 mmHg). Acid hydrolysis (80% aq. HOAC, 80°C)followed by acetylation (Ac_2O, Py, DMAP, CH_2Cl_2) of the hydroxyl groups afforded the triacetate 4 The unstable chlororiboside 5 obtained by bubbling dry HCl in an ice-cold methylene chloride solution of 4 was treated with *N,N*-dimethylformamide dimethylacetal[8] to give orthoester 6 in good yield. The 3'-O-acetyl was hydrolysed (cat. NaOMe, MeOH) and reaction with NaH/allyl bromide/THF completed the synthesis of the desired intermediate ($[\alpha]^{22}_D$ +95.7°, *c* 2.29/$CHCl_3$) . It is worthy to note that in the above sequence no chromatographic purification was performed except on the orthoacetate 7. The synthesis of the ribitol 8 portion was straightforward; conversion of 2,3,4-tri-O-benzyl-D-ribose diethyl dithioacetal[9] to its allyl ether followed by hydrolysis of the dithioacetal ($HgCl_2$, HgO, acetone/H_2O) to the aldehyde and subsequent reduction ($NaBH_4$, MeOH) yielded the desired compound ($[\alpha]^{22}_D$ -23.2° *c* 0.11/$CHCl_3$).

With both intermediates in hand , the construction of the ribosyl-ribitol unit was achieved as follows. Treatment of the orthoester 7 with Me_3SiCl in methylene chloride gave the chlororiboside which was then coupled with the ribitol 8 in the presence of silver perchlorate[10] to give the β-ribosyl-ribitol unit 9 ($[\alpha]^{22}_D$ -18.2° *c* 1.29/$CHCl_3$) in 80% yield. The 2'-O-acetyl was hydrolysed (cat. NaOMe, MeOH) the resulting alcohol was then converted[7] to benzyl ether 10. Deallylation by Corey's method[11] (1. $(Ph_3P)_3RhCl$, DABCO, EtOH, reflux; 2. $HgCl_2$, HgO, acetone/H_2O) provided the 3',5 diol 11 in moderate yield. Differentiation of the diol was achieved by monomethoxytritylation (MeOTrCl, Py, DMAP, CH_2Cl_2) giving 12. Treatment of 12 with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite[12] yielded the two diastereomeric phosphoramidites 13 . Since it was to be coupled with another ribosyl-ribitol unit at the 5-position , the 3'-hydroxyl group of 12 was transformed into the levulinyl ester[13] 14 and cleaving the monomethoxytrityl group by the action of 3% trichloroacetic acid in methylene chloride gave the required intermediate 15. The tetrazole catalysed coupling[14] of 13 with 15 followed by iodine oxidation to give the tetrasaccharide 16 as two diastereomers ($[\alpha]^{22}_D$ -15.19° *c* 5.59/$CHCl_3$) proceeded in excellent yield. All the compounds described gave satisfactory NMR spectra c(^1H, ^{13}C ^{31}P)[15].

In order to characterise the dimer 1 (n=2) it was fully deprotected as follows; the levulinyl ester was removed by the action of hydrazine in pyridine-acetic acid buffer[13]. After detritylation,the resulting diol was treated with ammonium hydroxide to decyanoethylate the phosphate. Hydrogenolysis of the benzyl groups (10% Pd/C, MeOH) followed by ion exchange gave the dimer 1 (n=2) as the sodium salt. The physical data (^1H, ^{13}C, ^{31}P, Hi.Res FAB MS) was identical to that reported[3].

The synthesis of larger fragments of the polysaccharide by solution or by solid-phase approach will be reported in due course.

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15. Typical 200 or 300 MHz ^{1}H and 75.4 MHz ^{13}C data (H-1/C-1 of **7**, **9-15** in $CDCl_3$) (ppm): **7**, 5.9(d, J=4Hz)/104.2; **9**, 4.9(s)/105.2; **10**, 5.1(s)/105.4; **11**, 5.0(s)/104.8; **12**, 5.0(s)/104.6; **13**, 5.0(d, J=1Hz), 5.1(d, J=1Hz)/105.4, 105.6; **14**, 5.0(d, J=2Hz); **15**, 5.0(d, J=2Hz)/106.1. Compounds **13** and **16** also gave the expected ^{31}P resonances: **13**, 149.8 ppm; **16**, -1.3, -1.7ppm.

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