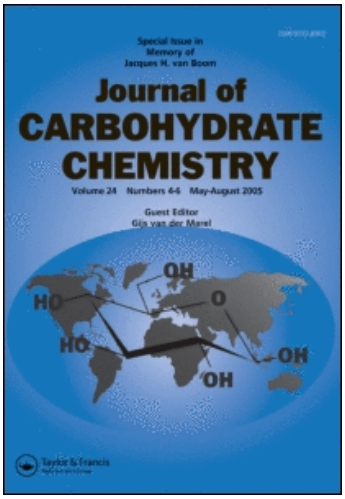

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A New Approach to the Ribosylribitol Intermediate for the Synthesis of Haemophilus Influenzae Type B Oligosaccharides

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A NEW APPROACH TO THE RIBOSYLRIBITOL INTERMEDIATE FOR THE SYNTHESIS OF HAEMOPHILUS INFLUENZAE TYPE B OLIGOSACCHARIDES

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ABSTRACT

The 3-O-allyl-2,5-di-O-benzoyl-β-**D**-ribofuranosyl trichloroacetimidate **9** was obtained in good yield after eleven steps from glucose. This imidate has proved to be an excellent precursor for the synthesis of ribosylribitol **13**, which is the key monomer unit for the synthesis of Haemophilus influenzae type b PRP oligosaccharides.

INTRODUCTION

Haemophilus influenzae type b (Hib) is known as a major cause of meningitis and other invasive infectious diseases in children. It has been shown that a vaccine against these diseases can be prepared using either the capsular polysaccharide polyribosylribitol phosphate (PRP) or its oligosaccharide fragments coupled to a carrier protein.[1-4] Therefore,

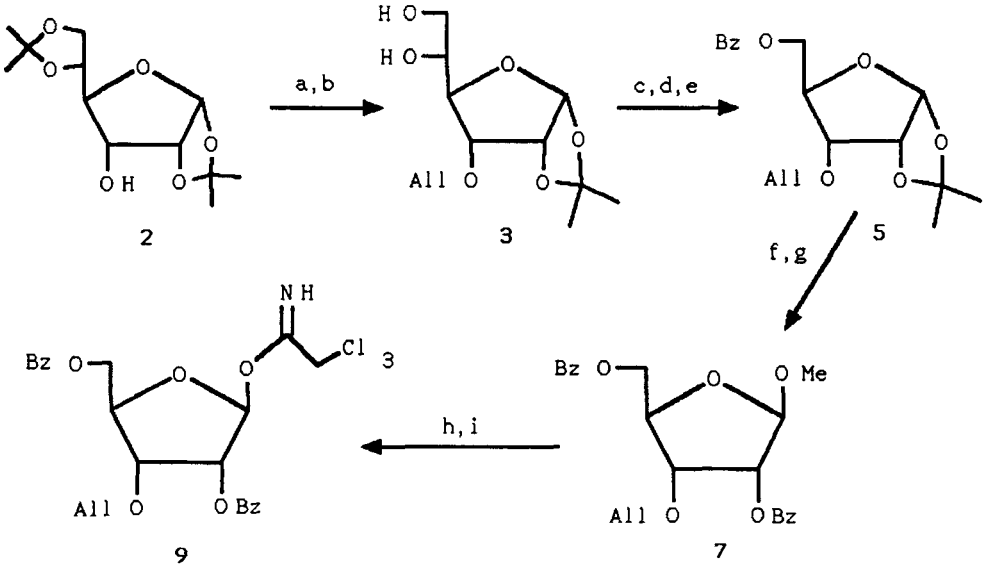
a synthetic oligosaccharide could in principle be used for vaccine evaluation and production.

In PRP oligosaccharides the disaccharide repeating units 1-O-β-D-ribofuranosyl-D-ribitol are linked from C-3 of ribose to C-5 of ribitol by a phosphodiester bond. Several workers have already reported their efforts in the preparation of this basic unit[5-10] and the synthesis of oligomeric fragments using either solution or solid phase techniques.[9-12]

We now report a new approach to ribofuranosyl donor **9** starting from D-glucose, and its use for the synthesis of the disaccharide basic unit **13**.

RESULTS AND DISCUSSION

The following scheme outlines the synthesis leading to the key ribofuranosyl donor **9** starting from D-glucose.

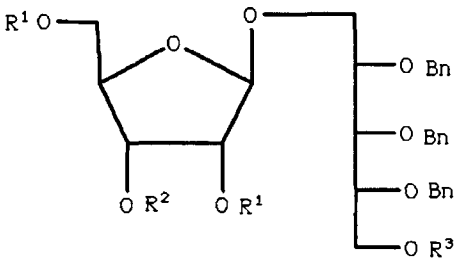


Synthesis of the imidate **9** a. AllBr, NaH, DMF; b. AcOH, 24 h; c. $NaIO_4$, aqueous dioxane; d. $NaBH_4$, EtOH; e. BzCl, Py; f. MeOH, H^+, 50 °C; g. BzCl, Py; h. Cl_2CHOCH_3, $ZnCl_2$, H_2O; i. Cl_3CCN, K_2CO_3.

Oxidation[13] of 1,2;5,6-di-*O*-isopropylidene-**D**-glucofuranose **1** with RuO_2/IO_4^- followed by reduction with sodium borohydride gave **2** in a 76 % yield. Allylation (allyl bromide/sodium hydride in *N*,*N*-dimethylformamide) and subsequent partial hydrolysis in acetic acid gave the diol **3** in 87 % yield. Periodate oxidation in dioxane-water followed by sodium borohydride reduction and benzoylation afforded the ribose derivative **5** in a 80 % yield. The 1,2-*O*-isopropylidene group was removed by methanolysis to give a mixture of furanosides **6** which were benzoylated at HO-2 to give **7**.

Treatment of **7** in dichloromethane with dichloromethyl methyl ether[14] in the presence of moist zinc chloride as catalyst, followed by imidation, using trichloroacetonitrile and potassium carbonate,[15] gave 'ribofuranosyl donor' **9** (62 %).

Condensation of **9** with 5-*O*-allyl-2,3,4-tri-*O*-benzyl-**D**-ribitol[9] with trimethylsilyl triflate as a catalyst afforded the disaccharide **10** (80 %).[16] The β-D-configuration at the anomeric linkage was firmly established[6] by NMR spectroscopy that showed *inter alia* a signal at δ 104.8 ppm corresponding to C-1 and a singlet at δ 5.04 ppm for H-1.



10 R^1 = Bz R^2, R^3 = All

11 R^1 = Bn R^2, R^3 = All

12 R^1 = Bn R^2, R^3 = H

13 R^1 = Bn R^2 = H R^3 = DMTr

The obtained disaccharide **10** was subjected to the reaction sequence debenzoylation (sodium methoxide), benzylation (benzyl chloride/sodium hydride in *N*,*N*-dimethylformamide), and deallylation (palladium chloride in acetic acid) to give the diol **12** in 41 % yield.

Dimethoxytritylation of **12** with dimethoxytrityl chloride in dichloromethane-pyridine afforded the key compound **13** in 60 % yield. The use of **13** as a monomer for the synthesis of Hib capsular polysaccharide fragment using either solid-phase

TABLE 1. ^{13}C NMR Spectral Data[c] for Compounds 3-13

	Ribose					Ribitol				
compd	C1	C2	C3	C4	C5	C1	C2[a]	C3[a]	C4[a]	C5
3	104.1	77.5	76.5	79.0	70.8	63.1[b]				
4	103.6	77.5	76.3	78.5	59.9					
5	104.2	77.3	78.1	76.4	63.4					
6	108.1	72.5	78.5	78.5	64.7					
7	106.0	73.9	77.3	78.8	64.4					
8α	99.2	74.6	77.2	78.6	65.0					
8β	95.8	72.0	76.7	79.3	64.1					
9	102.9	72.3	73.4	80.4	64.5					
10	104.8	73.9	77.7	78.7	65.1	66.7	77.4	77.5	78.1	69.4
11	105.2	79.4	78.5	80.2	71.5	67.1	77.9	78.3	78.6	69.9
12	104.6	81.4	71.5	82.7	71.3	66.7	77.5	78.5	78.7	61.5
13	104.7	81.7	72.0	83.1	71.8	67.5	78.2	78.9		63.6

a. The assignment may be reversed
b. C-6 of the allose derivative
c. Protective groups are as follows:

Allyl CH_2O 71.2-71.9 ppm CH_2= 117.5-118.9 ppm (ribose) 116.5 ppm (ribitol) CH= 133.5-134.2 ppm
Isopropylidene CH_3 26.1-26.8 $(Me)_2C$ 112.5-113.2
Bz CO 165.3-166.3 ppm
Bn CH_2 71.3-73.5 ppm
DMTr CH_3O 55.12ppm, Ph 113.0, 136.3 and 158.4

or soluble polymeric supported synthesis will be reported in a separate paper.

EXPERIMENTAL

General procedures. Optical rotations were measured with a POLAMAT A automatic polarimeter for 1 % solutions in

chloroform at 25 °C. NMR spectra were recorded with a BRUKER AC-250F. ^{1}H and ^{13}C assignments were made on the basis of homo- and heteronuclear correlation experiments. NMR spectra were recorded in deuteriochloroform as the solvent with tetramethylsilane as the internal reference. All compounds characterized were purified by column chromatography using one of the following solvent system: A, dichloromethane-acetone; B, hexane-ethyl acetate; C, toluene-acetone.

3-*O*-Allyl-1,2-*O*-isopropylidene-α-D-allofuranose (3). Compound **2** (3.7 g, 14.2 mmol) was dissolved in dry *N,N*-dimethylformamide (15 mL) and sodium hydride (0.37 g, 15.5 mmol) was added in small portions. The stirred reaction mixture was cooled to 0 °C and allyl bromide (1.35 mL, 15.6 mmol) was added dropwise within approximately 30 min. Stirring was continued for 30 min at 0 °C and, thereafter, overnight at room temperature. Dry methanol (10 mL) was added slowly in order to destroy the excess of sodium hydride. Then the reaction mixture was concentrated. The residue was taken up in dichloromethane (50 mL) and washed with water (3 x 20 mL). The organic layer was dried, concentrated and dissolved in acetic acid (56 mL) and water (14 mL) and stirred at room temperature for 16 h. TLC (10:1 dichloromethane-acetone) showed the disappearance of the starting material. The mixture was concentrated and coconcentrated with toluene (3 x 10 mL) to afford **3** (3.2 g, 87 %) as a syrup: $[\alpha]_D$ +112.5° (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.96 (m, 1H, -CH=), 5.78 (d, 1H, H-1), 5.31 (m, 2H, H_2C=), 4.66 (t, 1H, H-2), 1.7 and 2.6 (s,-OH 5,6), $J_{1,2}$ = 4.08.

Anal. Calcd for $C_{13}H_{20}O_7$: C, 60.71; H, 5.99. Found: C, 60.73; H, 5.93.

3-*O*-Allyl-1,2-*O*-isopropylidene-α-D-ribofuranose (4). To a solution of **3** (2.8 g, 10.8 mmol) in dioxane (24 mL) was added sodium periodate (2.3 g, 10.8 mmol) and water (24 mL). The resulting suspension was stirred in the dark for 20 h, concentrated and extracted with ethyl acetate (4 x 10 mL). The combined extracts were dried, filtered and concentrated. To a solution of the residue in ethanol (40 mL) was added sodium borohydride (0.3 g, 7.9 mmol) in small portions at 0 °C.

After 1 h the mixture was concentrated and coevaporated with methanol (3 x 10 mL). The residue was dissolved in dichloromethane (50 mL), washed with water (3 x 5 mL), dried and concentrated to yield **4** (2.1 g, 84 %) as a syrup: $[\alpha]_D$ + 122.9° (c 1, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.96 (m, 1H, -CH=), 5.29 (m, 2H, H_2C=), 4.62 (t, 1H, H-2), 3.62 and 3.92 (m, 2H, H-5), 3.05 (s, 1H, OH), $J_{1,2}$= 3.90 Hz.

Anal. Calcd for $C_{11}H_{18}O_5$: C, 57.38; H, 7.88. Found: C, 57.04; H, 8.06.

3-*O*-Allyl-5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose (5). To a solution of **4** (3 g, 13 mmol) in pyridine (14 mL), cooled to 0 °C, was added benzoyl chloride (2 mL, 17.2 mmol) and the reaction mixture was stirred overnight. The solvent was evaporated *in vacuo* and the residue was dissolved in dichloromethane, washed with saturated $NaHCO_3$ solution, with water, dried and concentrated to yield **5** (4.1 g, 95 %) as a syrup: $[\alpha]_D$ 78.4° (c 1 chloroform); ^{1}H NMR δ 5.95 (m, 1H, -CH=), 5.81 (d, 1H, H-1), 5.26 (m, 1H, H_2C=), 4.58 and 4.33 (m, 2H, H-5), 4.58 (t, 1H, H-2), $J_{1,2}$ = 4.10 .

Anal. Calcd for $C_{18}H_{22}O_6$: C, 64.66; H, 6.63. Found: C, 64.69; H, 6.28.

Methyl 3-*O*-Allyl-5-*O*-benzoyl-α,β-D-ribofuranoside (6). A solution of **5** (2 g, 6 mmol) in methanol (5 mL), trifluoroacetic acid (4 mL) and water (0.5 mL) was stirred at 50 °C. When TLC (system A, 20:1) showed the absence of **5** (R_F 0.65) the solution was concentrated and residual solvent coevaporated with toluene. The residue was dissolved in dichloromethane and washed succesively with water, saturated $NaHCO_3$ solution, again with water, dried and solvent evaporated to afford **6** (1.6 g, 86 %) as an α, β mixture. **6** β: $[\alpha]_D$ -3.9° (c 1 chloroform); ^{1}H NMR ($CDCl_3$) δ 5.85 (m, 1H, -CH=), 5.25 (m, 2H, H_2C=), 4.91 (s, 1H, H-1), 3.33 (s, 3H, $-OCH_3$).

Anal. Calcd for $C_{16}H_{22}O_6$(α, β): C, 62.33; H, 6.54. Found: C, 62.33; H, 7.00.

Methyl 3-*O*-Allyl-2,5-di-*O*-benzoyl-α,β-D-ribofuranoside (7). Compound **6** (1.6 g, 5.2 mmol) was benzoylated as for **5** to give **7** in 92 % yield. Pure β $[\alpha]_D$ +29.1° (c 1 chloro-

form); ^{1}H NMR δ 5.78 (m, 1H, -CH=), 5.48 (d, 1H, H-2), 5.15 (m, 1H, H_2C=), 5.03 (s, 1H, β H-1), 3.35 (s, 3H, $-OCH_3$), $J_{2,3}$ = 4.17.

Anal. Calcd for $C_{23}H_{24}O_7$: C, 66.98; H, 5.86. Found: C, 67.18; H, 5.80.

3-O-Allyl-2,5-di-O-benzoyl-α,β-D-ribofuranose (8). To a solution of **7** (1 g, 2.4 mmol) in dichloromethane (10 mL) was added zinc chloride (20 mg), water (50 μL) and dichloromethyl methyl ether (0.2 mL). The mixture was stirred at room temperature for 8 h. TLC (system A, 20:1) indicated an almost complete conversion of **7** into **8** (R_F 0.60). The mixture was washed with saturated $NaHCO_3$ solution, with water, dried and concentrated. Column chromatography (system C, 4:1) of the residue gave **8** (0.63 g, 65 %) as a syrup: ^{1}H NMR ($CDCl_3$) δ 5.77 (m, 1H, CH=), 5.60 (s, 1H, β H-1), 5.52 (d, 1H, $J_{2,3}$ = 3.0, H-2), 5.34 (m, 2H, H_2C=).

Anal. Calcd for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 66.26; H, 5.44.

3-O-Allyl-2,5-di-O-benzoyl-β-D-ribofuranosyl Trichloroacetimidate (9). To a solution of **8** (2.6 g, 6.5 mmol) in dry dichloromethane (20 mL) and trichloroacetonitrile (3 mL, 30 mmol) was added freshly fused potassium carbonate (0.9 g, 6.5 mmol) and the mixture was stirred for 5 h. When TLC (system B, 3:1) showed the conversion of **8** into two new compounds **9** α, β with R_F 0.8 and 0.85, the mixture was filtered through celite and concentrated to give **9** α, β (3.4 g, 95 %) as a syrup α/β 1/8: ^{1}H NMR δ 8.63 (s, 1H, =NH, β), 8.51 (s, 1H, =NH, α), 6.48 (s, 1H, H-1, β), δ 6.67 (s, 1H, H-1, α), 5.73 (d, 1H, $J_{2,3}$ = 4.30, H-2, β).

5-O-Allyl-1-O-(3-O-allyl-2,5-di-O-benzoyl-β-D-ribofuranosyl)-2,3,4-tri-O-benzyl-D-ribitol (10). Compound **9** (3.1 g, 5.6 mmol) and 5-O-allyl-2,3,4-tri-O-benzyl-D-ribitol[9] (1.7 g, 3.7 mmol) were dried in a high vacuum system for 2 h. Dry dichloromethane (20 mL) and molecular sieves 4Å were added and the mixture was stirred at room temperature for 30 min. The reaction was cooled to 0 °C and trimethylsilyl triflate (0.17 mL, 0.94 mmol) was added under an argon atmosphere.

After several minutes, TLC (system C, 10-1) showed a new spot with R_F 0.52. Triethylamine was added and the mixture was filtered, washed with water, dried and concentrated. Column chromatography of the mixture afford **10** (2.5 g, 80 %). ^{1}H NMR ($CDCl_3$) δ 5.43 (d, 1H, $J_{2',3'}$ = 4.3, H-2'), 5.04 (s, 1H, H-1').

Anal. Calcd for $C_{51}H_{54}O_{11}$: C, 72.66; H, 6.45. Found: C, 72.54; H, 6.23.

5-*O*-Allyl-1-*O*-(3-*O*-allyl-2,5-di-*O*-benzyl-β-D-ribofuranosyl)-2,3,4-tri-*O*-benzyl-D-ribitol (11). To a solution of **10** (1.2 g, 1.5 mmol) in methanol (4 mL) was added sodium methoxide in methanol (1 % w/v) to pH 9. After 12 h, the mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. The resulting syrup was dissolved in dry *N*,*N*-dimethylformamide (15 mL) and sodium hydride (0.22 g, 9.2 mmol) was added in small portions. The stirred reaction mixture was cooled to 0 °C and benzyl chloride (0.41 mL, 3.6 mmol) was added dropwise within approximately 30 min. Stirring was continued for 30 min at 0 °C and, thereafter, overnight at room temperature. Dry methanol (10 mL) was added slowly in order to destroy the excess of sodium hydride. Then the reaction mixture was concentrated. The residue was taken up in dichloromethane (50 mL) and washed with water (3 x 20 mL). The organic layer was dried and concentrated to afford **11** (1.0 g, 85 %) as a syrup: ^{1}H NMR ($CDCl_3$) δ 5.02 (s, 1H, H-1'), 3.94 (dd, 1H, H-3'), 3.84 (d, 1H, H-2').

Anal. Calcd for $C_{51}H_{58}O_9$: C, 75.15; H, 7.17. Found: C, 75.45; H, 7.20.

1-*O*-(2,5-Di-*O*-benzyl-β-D-ribofuranosyl)-2,3,4-tri-*O*-benzyl-D-ribitol (12). To a solution of **11** (3.3 g, 4 mmol) in acetic acid (15.7 mL) and water (0.8 mL) was added palladium chloride (0.36 g, 2 mmol) and sodium acetate (0.33 g) and the mixture was stirred for 24 h. TLC showed the reaction to be complete, and the mixture was concentrated, residual solvent coevaporated with toluene. The residue was dissolved in dichloromethane, washed with water, dried and concentrated. Column chromatography (system C, 20:1) of the

residue afforded **12** (1.8 g, 60 %) as a syrup: ^{1}H NMR ($CDCl_3$) δ 5.02 (s, 1H, H-1'), 4.15 (dd, 1H, H-3'), 3.82 (d, 1H, H-2').

Anal. Calcd for $C_{45}H_{50}O_9$: C, 73.54; H, 6.85. Found: C, 73.60; H, 6.99.

5-O-Dimethoxytrityl-1-O-(2,5-di-O-benzyl-β-D-ribofuranosyl)-2,3,4-tri-O-benzyl-D-ribitol (13). To a solution of the disaccharide **12** (1.2 g, 1.5 mmol) in anhydrous dichloromethane (10 mL) was added molecular sieves 4Å (0.5 g), dry pyridine (0.27 mL), and dimethoxytrityl chloride (0.64 g, 1.8 mmol). After 5 min, the reaction mixture was filtered, concentrated and residual solvent coevaporated with toluene. Column chromatography (system C, 20:1) of the residue yielded **13** as a syrup (1.0 g, 60 %). ^{1}H NMR ($CDCl_3$) δ 4.97 (s, 1H, H-1'), 3.72 (s, 6H, CH_3O).

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