SYNTHESIS OF BACTERIAL ANTIGENIC POLYSACCHARIDES AND THEIR FRAGMENTS

8. PYRIDINIUM PERCHLORATE AS A REAGENT FOR THE DETRITYLATION AND DEACETONATION OF SACCHARIDES

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In the course of a study of the synthesis of fragments of the antigenic polysaccharides of Gram-negative bacteria we encountered the need for a mild method of de-O-tritylating derivatives of oligosaccharides substituted by various functional groups. Condensation of tritylated or detritylated oligosaccharide derivatives under the conditions of Bredereck or Koenigs—Knorr glycosylation with an acetohalogenose also prepared from the oligosaccharide is one of the possible approaches to the stepwise synthesis of polysaccharides containing (1 → 6)-linkages between repeating oligosaccharide units [1].

Extant methods of de-O-tritylation involve hydrogenolysis in the presence of Raney nickel [2] or Pt group catalysts and acid hydrolysis or alcoholysis [3]. Recently, detritylation by sodium in liquid NH_3 has been described [4]. The mildest of these methods, catalytic hydrogenolysis in the presence of Pd or Pt, has not been widely used in the carbohydrate series, since many trityl ethers are stable under the hydrogenolysis conditions. Detritylation by HCl or HBr in organic solvents involves a harsh, difficultly quantifiable reagent, which precludes any possibility of monitoring the course of the reaction and affects the alkylidene protecting group. Dilute AcOH is a sufficiently mild reagent, but it is not always suitable because of the poor solubility of the substrate and the absence of any convenient method for monitoring the course of the reaction; moreover AcOH lacks selectivity and affects the alkylidene protecting group. Nor has alcoholysis of trityl ethers been introduced into synthetic carbohydrate chemistry, apparently because of the risk of affecting the glycosidic linkage. However, a case of detritylation of a di-O-trityl ether of acetylated hexitol during attempted recrystallization from alcohol in the presence of pyridine hydrochloride has been described [5].

We have found that alcoholysis can become an adequately mild method of detritylation of sugar derivatives if pyridinium perchlorate (PP) [6] is used as the acid; this salt is readily accessible, nonhygroscopic, easily quantifiable, and soluble in polar organic solvents. Our chosen alcohol was methanol.

Experimental trials of the de-O-tritylation of simple glycosides (Ia)-(IIIa) revealed that the reaction proceeds readily on heating in nitromethane—methanol, is easily monitored by TLC, and is quenched by addition of a drop of pyridine or by cooling. After evaporation of the reaction mixture the product can be isolated by extraction with $CHCl_3$ or benzene or purified by preparative chromatography on silica gel. Treatment with aqueous $CHCl_3$ can also be used to remove PP.

Since we would subsequently apply this method of detritylation to oligosaccharide derivatives containing alkylidene protecting groups [notably to trityl ether (VIIIb)], we also examined the stability of alkylidene protecting groups under these detritylation conditions. We found that the O-isopropylidene protecting group is completely removed, whereas O-ethylidene is stable. Thus 1,2-O-isopropylidene-3-O-acetyl-4,6-O-ethylidene-α-D-galactopyranose (IVb) is smoothly de-O-acetonated when heated with PP in nitromethane—methanol, to form, after acetylation, tri-O-acetyl derivative (V), identical to an authentic sample prepared by acetylation of 4,6-O-ethylidene-α-D-galactopyranose. This makes possible the selective removal of an isopropylidene protecting group in the presence of an ethylidene group, which could prove useful in synthetic work. Moreover, the deacetonation of (IVb) shows that PP cannot act as a reagent for selective detritylation in the presence of the isopropylidene protecting group.

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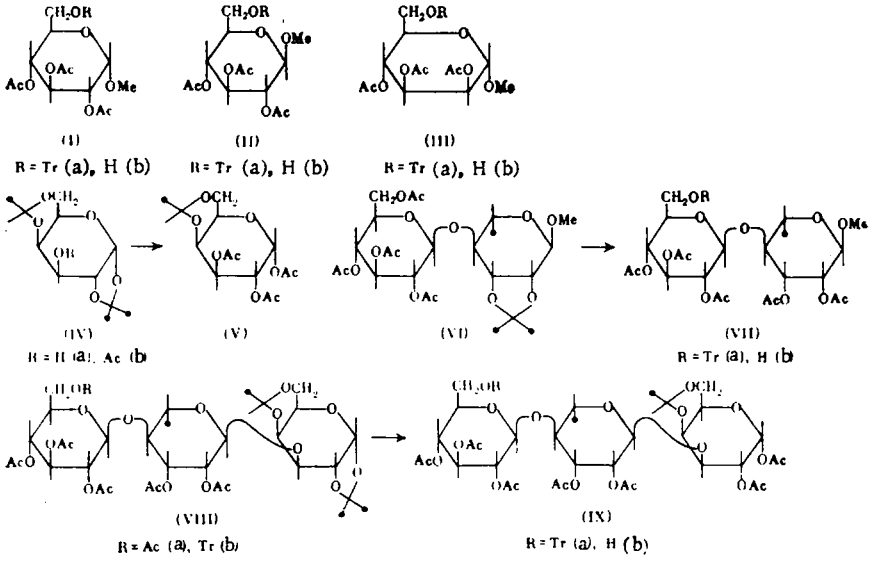
TABLE 1. ^{13}C Chemical Shifts (ppm from tetramethylsilane) in $CDCl_3$ of C-6 of the D-Glucopyranose Residue in Various Derivatives

Pair of compounds	Compound	δ, ppm ±0.05	Shift difference, ppm
I	1. Methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside	62,1	1,0
	2. Methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside	61,1	
II	1. Methyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside	62,05	0,65
	2. Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranoside	61,40	
III	1. (VI)	62,50	0,82
	2. (VIIb)	61,68	
IV	1. (VIIIa)	62,29	0,79
	2. (IXb)	61,50	



We used this property of PP for facile detritylation and deacetonation for the synthesis of bioside (VIIb) and trioside (IXb), which are models for oligosaccharides with free primary hydroxyl group on the D-glucose residue. In the course of the synthesis of these derivatives we used PP as a detritylation reagent and as a deacetonation reagent (removal of the 2,3-O-isopropylidene protecting group from the D-galactose residue).

We synthesized tritylbioside (VIIa) and trityltrioside (IXa) from disaccharide (VI) [7] and trisaccharide (VIIIa) [8] respectively by a four-step synthesis, involving deacetylation with triethylamine in methanol, deacetonation by PP, tritylation, and acetylation. We isolated the crystalline trityl ether (VIIa) and the syrupy trityl ester (IXa) in 67 and 81% yields, respectively. For comparison we synthesized trityl ether (VIIIb) from trisaccharide (VIIIa) by a three-step synthesis. We verified the structures of all three trityl ethers from their PMR spectra. For PMR spectrum of ether (IXa) contains the signals of the aromatic protons of the trityl group, the signals of the seven acetyl groups, one of which lies far upfield as a result of the shielding effect of the trityl group [disaccharide derivative (VIIa) shows the same phenomenon], and the doublet of the CH_3 group of the ethylidene residue and of the CH_3 group of the rhamnose residue. The PMR spectrum of trityl derivative (VIIIb) resembles that of derivative (IXa), except that the signals of the isopropylidene group replace the signals of the two acetyl groups. Detritylation of tritylbioside (VIIa) by PP gave the crystalline disaccharide (VIIb) in 60% yield. Analogous detritylation of tritylbioside (IXa) yielded the syrupy trisaccharide (IXb) in 85% yield.

We used ^{13}C NMR to locate the free OH group in the synthetic compounds. We examined the spectra of three pairs of compounds with a free and an acetylated OH group at C-6 of the glucose residue (Table 1). Table 1 shows that the OH group at C-6 of the glucose residue causes a significant and fairly sensitive downfield shift of the signal. The ^{13}C NMR spectrum of trisaccharide (IXb) shows only a signal at 61.50 ppm, implying the presence of the free OH group at C-6 of the glucose residue.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage. Optical rotation was measured on a Perkin—Elmer M-141 polarimeter. All GLC analyses used a Pye Unicam 104 chromatograph with a flame-ionization detector. Spectra were recorded on: PMR: a Varian DA-600 IL, relative to tetramethylsilane (TMS); ^{13}C NMR: a Bruker WP-60, at 15.08 MHz.

Solutions were evaporated under vacuum at 40°C. Analytical TLC was carried out on plates of silica gel (no binder); visualization was by 25% H_2SO_4.

Methyl 2,3,4-Tri-O-acetyl-α-D-glucopyranoside (Ib). To a solution of methyl 6-O-trityl-2,3,4-tri-O-acetyl-α-D-glucopyranoside (266 mg) and PP (360 mg) [6] in dry CH_3NO_2 (18 ml) was added methanol (6 ml); the reaction mixture was heated at 50°C for 1.5 h. The reaction proceeded to completion [TLC, benzene—ether (1:1)]. The solution was warmed at 50°C for a further 0.5 h, cooled to 20°C, and evaporated. The residue was treated with a mixture of benzene and ether to remove the major part of the PP. The filtrate was evaporated, and the residue was chromatographed on a column of silica gel (50 g) in the system benzene—ether (ether concentration rising to 100%). The appropriate fractions were combined and evaporated; the residue (145 mg, 90%) was crystallized from ether—pentane to give the detritylated compound (100 mg, 62.5%), mp 110-111°C; $[\alpha]_D^{20}$ + 13.8° (C 1.2, $CHCl_3$) [9].

Methyl 2,3,4-Tri-O-acetyl-β-D-glucopyranoside (IIb). Methyl 6-O-trityl-2,3,4-tri-O-acetyl-β-D-glucopyranoside (760 mg), PP (515 mg), CH_3NO_2 (2 ml), and methanol (8 ml) were heated at 60°C for 2 h. After evaporation, the residue was extracted with $CHCl_3$ (5 × 8 ml). The extract was evaporated to dryness and the residue was crystallized from ether—pentane to give the detritylated derivative (250 mg, 55.5%), mp 134°C; $[\alpha]_D^{20}$ −19° (C 1.6 $CHCl_3$) [10].

Methyl 2,3,4-Tri-O-acetyl-α-D-mannopyranoside (IIIb). Methyl 6-O-trityl-2,3,4-tri-O-acetyl-α-D-mannopyranoside (532 mg), PP (400 mg), CH_3NO_2 (18 ml), and methanol (8 ml) were heated at 60°C for 2 h. After evaporation, the residue was treated with $CHCl_3$. The filtrate was evaporated and the residue was chromatographed on a column of silica gel (100 g) in the system benzene—ether to give the crystalline detritylated derivative (230 mg, 71.8%), mp 98–99°C (ether—petroleum ether); $[\alpha]_D^{20}$ +57.3° (C 0.59, $CHCl_3$) [11].

1,2-O-Isopropylidene-3-O-acetyl-4,6-O-ethylidene-α-D-galactopyranose (IV). A mixture of 1,2-O-isopropylidene-4,6-O-ethylidene-α-D-galactopyranose (290 mg) [12], dry pyridine (4 ml), and Ac_2O (2 ml) was left at ~20°C for 16 h and then evaporated. The residue was evaporated with toluene and heptane and chromatographed on a column of silica gel (100 g) in the system benzene—ether to give a colorless syrup (330 mg, 95%); $[\alpha]_D^{20}$ +122.5° (C 1.67, $CHCl_3$). Found: C 54.67; H 7.13%. $C_{23}H_{20}O_7$. Calculated: C 54.16; H 6.99%.

1,2,3-Tri-O-acetyl-4,6-O-ethylidene-α-D-galactopyranose (V). 4,6-O-Ethylidene-D-galactopyranose (250 mg) was treated with a mixture of pyridine (4 ml) and Ac_2O (2 ml) at ~20°C over 16 h. The solution was evaporated. The residue was dissolved in $CHCl_3$ (20 ml), washed with saturated $NaHCO_3$ solution and water, and dried over $MgSO_4$. The filtrate was evaporated and the residue was evaporated with toluene and heptane to give a crystalline, chromatographically homogeneous residue (390 mg). Recrystallization from ethyl acetate—heptane gave (V) (260 mg, 64.7%), mp 128.5-129.5°C; $[\alpha]_D^{20}$ +157° (C 2.7, $CHCl_3$). PMR spectrum in CCl_4 (δ, ppm): 1.41d (CH_3 of ethylidene residue, J = 5 Hz), 2.01-2.10 (3OAc), 4.71 q (H of ethylidene residue, J = 5 Hz), 5.35 dd (H_{C^2}, $J_{2,3}$ = 7, $J_{2,1}$ = 3.2 Hz), 5.43 d (H_{C3}, $J_{3,2}$ = 7 Hz), 6.43 d (H_{C1}, $J_{1,2}$ = 3.2 Hz). Found: C 51.04; H 6.03%. $C_{14}H_{20}O_9$. Calculated: C 50.60; H 6.07%. Analysis by GLC on a column of ECNSS-M at 190°C showed that the crystalline product was homogeneous and had a retention time of 314 sec, while the mother liquor gave two peaks with retention times of 314 and 425 sec (α- and β-anomers).

1,2,3-Tri-O-acetyl-4,6-O-ethylidene-D-galactopyranose (V) from ,2-O-Isopropylidene-3-O-acetyl-4,6-O-ethylidene-α-D-galactopyranose (IVb). Compound (IIIa) (50 mg) and PP (71 mg) were heated in a mixture of CH_3NO_2 (1 ml) and methanol (0.5 ml) at 50°C for 2 h. The substrate completely disappeared

[R_f 0.8, TLC, methanol—benzene (1:9)] to form a new product with R_f 0.2. Dry pyridine (0.5 ml) was added to the reaction mixture, which was then evaporated to dryness. Pyridine (2 ml) and Ac_2O (1 ml) were added to the residue and the mixture was left at ~20°C overnight. After addition of $CHCl_3$ (20 ml), it was washed with saturated $NaHCO_3$ solution and water, dried over $MgSO_4$, and evaporated. The residue was evaporated with toluene and heptane to give a syrupy residue (50 mg), which on analysis by GLC gave two peaks with retention times of 316 and 425 sec, coinciding with the peaks of the samples from the preceding run.

Methyl 4-O-(2,3,4-Tri-O-acetyl-6-O-trityl-β-D-glucopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (VIIa). To a solution of (VI) (1 g) in methanol (8 ml) was added a 1 N solution (0.1 ml) of sodium methoxide in methanol. The mixture was left at ~20°C overnight and then deionized with IR-120 cation-exchange resin (H^+ form). The filtrate was evaporated and the residue was evaporated with ethyl acetate and methanol to give a white powder with R_f 0.3 [methanol—benzene (3:7)], which was dissolved in a mixture of CH_3NO_2 (2.5 ml) and methanol (10 ml). After addition of PP (650 mg) the mixture was heated at 80°C for 5.5 h. The reaction mixture contained (TLC) traces of the substrate and a new product with R_f 0.20. Methanol (1 ml) was added and the mixture was heated for a further 1 h. After addition of dry pyridine (1 ml), it was evaporated to dryness; pyridine (5 ml) freshly distilled from P_2O_5 and trityl chloride (1.03 g, 3.6 mmole) were added, and the mixture was heated at 70°C for 2.5 h. A single product was formed with R_f 0.6 [methanol—benzene (3:7)]. After addition of Ac_2O (2 ml) to the solution (cooled to ~20°C), the mixture was left at ~20°C overnight and then poured into ice water. The solid precipitate was separated, washed with water, dissolved in $CHCl_3$ (30 ml), washed with 1 N HCl, saturated $NaHCO_3$ solution, and water, dried over $MgSO_4$, and evaporated. The residue was washed several times with heptane at 20°C and crystallized from absolute alcohol to give (VIIa) (870 mg). The mother liquor gave more crystalline (VIIa) (100 mg, 67%), mp 182-183°C; $[\alpha]_D^{20}$ −15.33° (C 1.5, $CHCl_3$). PMR spectrum in $CDCl_3$ (δ, ppm): 7.1-7.5 m (trityl protons, 15H), 1.67 s (OAc), 1.91-2.11 (4 OAc), 1.5 d (CH_3 group of rhamnose residue, J = 6 Hz), 3.36 s (OMe). Found: C 63.58; H 6.13%. $C_{42}H_{48}O_{15}$. Calculated: C 63.63; H 6.10%.

Methyl-4-O-(2,3,4-Tri-O-acetyl-β-D-glucopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranoside (VIIb). To a solution of (VIIa) (140 mg) in a mixture of CH_3NO_2 (1 ml) and methanol (5 ml) was added PP (39 mg). The mixture was heated at 80°C for 6 h, while the reaction was followed by TLC. After evaporation to dryness, the residue was treated with $CHCl_3$ (5 ml). The filtrate was evaporated and the residue was chromatographed on a column of silica gel (100 g) in the system benzene—ether (ether concentration rising to 100%) to give syrupy (VIIb) (90 mg, 91%), $[\alpha]_D^{20}$ −42.9° (C 2.21, $CHCl_3$), which crystallized on treatment with absolute ether and petroleum ether. Recrystallization from absolute ether—petroleum gave (VIIb) (60 mg, 63%), mp 109-114°C. Found: C 50.78; H 6.74%. $C_{23}H_{34}O_{15}$. Calculated: C 50.18; H 6.22%.

4,6-O-Ethylidene-1,2-O-isopropylidene-3-O-[4-O(2,3,4-tri-O-acetyl-6-O-trityl-β-D-glucopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranosyl]-α-D-galactopyranose (VIIIb). Trisaccharide (VIIIa) (700 mg, 0.87 mmole) was treated with a solution of triethylamine (3.6 ml) in methanol (70 ml) at ~20°C over 16 h. The mixture was evaporated to dryness and then evaporated several times with methanol. The residue was dissolved in pyridine (15 ml) freshly distilled from P_2O_5 and trityl chloride (470 mg, 1.75 mmole) and the mixture was heated at 80°C for 4 h. The deacetylated trisaccharide [R_f 0.2, benzene—methanol (7:3)] completely disappeared to form a substance with R_f 0.65. After addition of Ac_2O (5 ml) at 0°C, the mixture was left at ~20°C for 48 h and then evaporated. The residue was treated with water and $CHCl_3$ and the aqueous layer was extracted with $CHCl_3$. The chloroform extracts were washed with saturated $NaHCO_3$ solution and water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on a column of silica gel (100 g) in the system benzene—ether (ether concentration rising to 50%) to give (VIIIb) (540 mg), chromatographically homogeneous (R_f 0.65) and (VIIIb) (130 mg), slightly contaminated with a compound with R_f 0.55. The yield was 63%; $[\alpha]_D^{20}$ −5.09° (C 3, $CHCl_3$). PMR spectrum in $CDCl_3$ (δ, ppm): 7.2-7.45 m (15H, trityl group), 5.97 d (H_C^1 $J_{1,2}$ = 3.6 Hz), 2.14, 2.12, 2.08, 2.00, and 1.72 (5OAc), 1.15-1.52 m (CH_3 groups of the rhamnose residue, ethylidene and isopropylidene groups, 12H).

4,6-O-Ethylidene-1,2-di-O-acetyl-3-O-[4-O-(2,3,4-tri-O-acetyl-6-O-trityl-β-D-glucopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranosyl]-D-galactopyranose (IXa). Trisaccharide (VIIIa) (260 mg) was treated with triethylamine (0.9 ml) in methanol (18 ml) at ~20°C over 16 h. The residue (180 mg) was dissolved in a mixture of methanol (4 ml) and CH_3NO_2 (4 ml); after addition of PP (145 mg), the mixture was heated at 75°C for 4 h and evaporated to dryness. The residue was dissolved in pyridine (8 ml) freshly distilled from P_2O_5. After addition of trityl chloride (140 mg), the mixture was left at ~20°C for 16 h and then, after addition of more trityl chloride (40 mg), heated at 60°C for 1 h, whereupon the substrate had

completely disappeared. After addition of Ac_2O (2.5 ml) at 20°C, the mixture was left at ~20°C for 16 h, poured into saturated $NaHCO_3$ solution (40 ml) at 0°C, and extracted with $CHCl_3$ (2 × 20 ml). The extract was washed with saturated $NaHCO_3$ solution (4 × 20 ml) and water, dried over $MgSO_4$, and evaporated. The residue was a mixture [TLC, benzene—methanol (9:1)] of four compounds, one of which with R_f 0.3, the major product, was chromatographed on a column of silica gel (50 g; 40-100 μ) in the system benzene—ether (ether concentration rising to 50%) to give derivative (IXa) (220 mg) with R_f 0.3 [benzene—methanol (9:1)], slightly contaminated with a compound with R_f 0.15. The yield was 81%; $[\alpha]_D^{20}$ +24.4° (C 3.33, $CHCl_3$). PMR spectrum in $CDCl_3$ (δ, ppm): 1.36-1.49 (CH_3 groups of rhamnose and ethylidene residues, 6H), 1.67, 1.95, 1.98, 2.00, 2.08 (7OAc), 6.33 d (H_{C1} of the galactose residue, $J_{1,2}$ = 3.5 Hz), 7.1-7.5 m (trityl protons, 15H).

4,6-O-Ethylidene-1,2-di-O-acetyl-3-O-[4-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-2,3-di-O-acetyl-α-L-rhamnopyranosyl]-D-galactopyranose (IXb). To a solution of trityl derivative (IXa) (130 mg, 0.124 mmole) in methanol (4 ml) and CH_3NO_2 (2 ml) was added PP (94 mg, 0.795 mmole). The mixture was heated at 70°C for 4.5 h and then evaporated to dryness. The residue was dissolved in $CHCl_3$ (50 ml), washed with water, and dried over Na_2SO_4. Chromatography on a column of silica gel (100 g; 40-100 μ) in the system benzene—ether (ether concentration rising to 100%) gave derivative (IXb) (69 mg, 85%), R_f 0.55 [benzene—methanol (8:2)]; $[\alpha]_D^{20}$ +17.9° (C 1.72, $CHCl_3$). PMR spectrum in $CDCl_3$ (δ, ppm): 1.25-1.46 (CH_3 groups of rhamnose and ethylidene residues, 6H), 2.08, 1.95, 1.98, and 2.0 (7OAc), 6.36 d (H_{C^1} of galactose residue, $J_{1,2}$ = 3.5 Hz).

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CONCLUSIONS

1. We have proposed a new method for the de-O-tritylation and de-O-acetonation of sugar derivatives by pyridinium perchlorate.

2. We have used this method for the synthesis of trisaccharide derivatives that are models for the stepwise synthesis of polysaccharides constructed from repeating oligosaccharide units.

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