Carbohydrate Research, 32 (1974) 239–249

SYNTHESIS OF 3- AND 4-*O*-β-D-GALACTOPYRANOSYL-L-RHAMNOSE, AND OF 3-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-L-RHAMNOSE, AND THEIR USE IN PRECIPITIN INHIBITION STUDIES WITH TYPE VII ANTIPNEUMOCOCCAL SERUM*

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(Received July 2nd, 1973; accepted August 27th, 1973)

ABSTRACT

Syntheses of 3- and 4-*O*-β-D-galactopyranosyl-L-rhamnose and of 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-rhamnose are described. Comparison of the inhibitory powers of these three disaccharides with those of a selection of other disaccharides on the precipitin reaction between Type VII antipneumococcal horse serum and Type VII pneumococcal polysaccharide or Tamarind A polysaccharide showed that *O*-D-galactosyl- and *O*-(2-acetamido-2-deoxy-D-glucosyl)-L-rhamnose groups are important serological determinants in the pneumococcal Type VII polysaccharide.

INTRODUCTION

A recent publication[1] described structural studies on the specific Type VII pneumococcal polysaccharide, and related the results to previously published predictions of structure based upon serology[2]. The present paper describes the synthesis of three disaccharides and their use in inhibition studies as further support for the structural features postulated.

RESULTS AND DISCUSSION

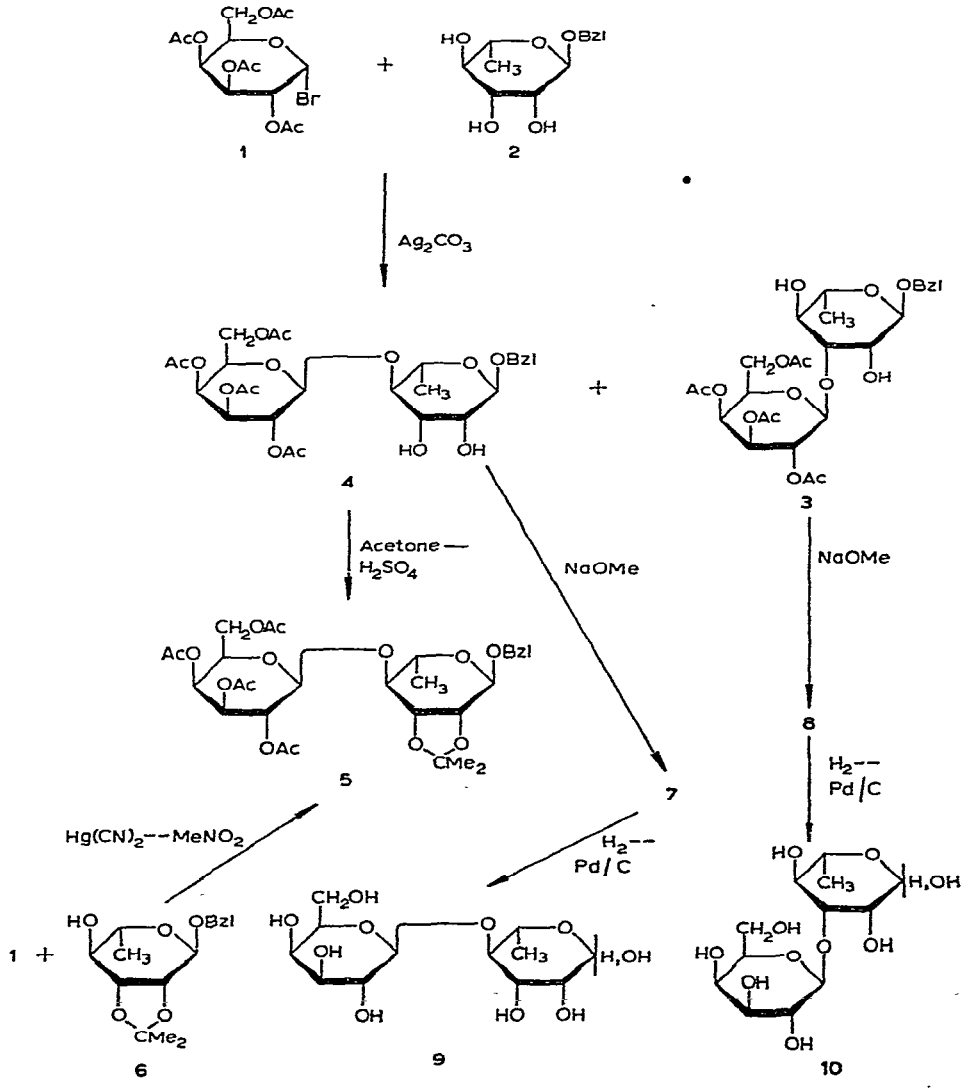
(*a*) *Syntheses.* — Studies on the selective esterification of D-mannosides have shown that the 3-hydroxyl group is consistently more reactive than either the 2- or the 4-hydroxyl groups[3,3a]. This observation suggested to us that, under mild conditions, a similar order of reactivity might prevail on condensing a glycosyl halide with a 6-deoxy-L-mannoside (L-rhamnoside). In this way, the desired (1→3)-linked disaccharides might be obtained.

Accordingly, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) was condensed with benzyl α-L-rhamnopyranoside (**2**) in the presence of silver carbonate.

*Issued as NRCC No. 13710.

**NRCC Postdoctorate Fellow, 1972–74.

Fractionation of the resultant products by preparative, thin-layer chromatography (t.l.c.) yielded two new compounds, the n.m.r. spectra of which were consistent with those expected for galactose–rhamnose disaccharides. Only one of the two compounds could be oxidized by periodate, indicating that it had a (1→2)- or (1→4)-linkage. The periodate-resistant product was tentatively assigned the structure of benzyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α-L-rhamnopyranoside (**3**). Reaction of the periodate-susceptible product with acetone in the presence of concentrated sulfuric acid to form an isopropylidene derivative (**5**) strongly favored its formulation as benzyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-α-L-rhamnopyranoside (**4**). This assignment was corroborated by a definitive synthesis of the isopropylidene

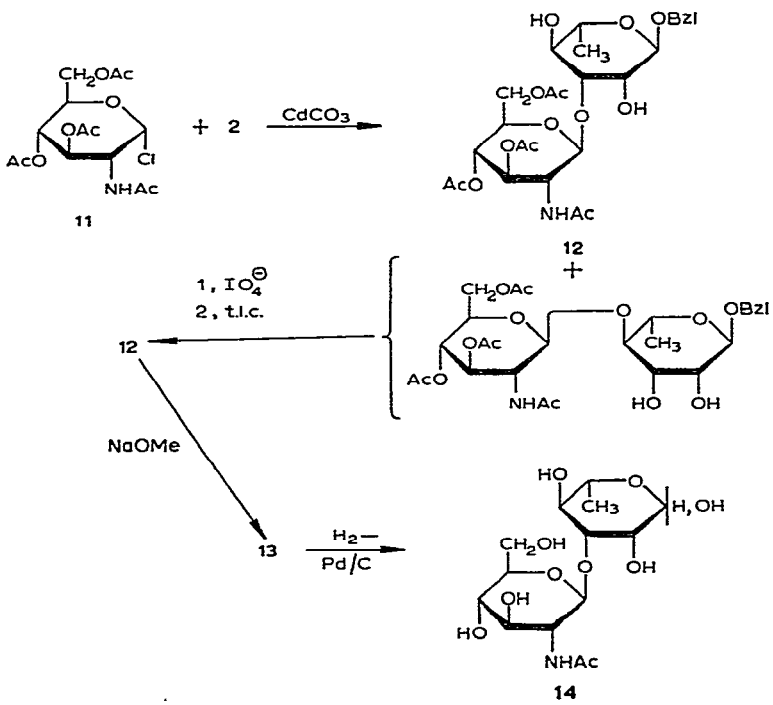


derivative (**5**) by condensation of **1** with benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**6**).

The free disaccharides 3-*O*-β-D-galactopyranosyl-L-rhamnose (**10**) and 4-*O*-β-D-galactopyranosyl-L-rhamnose (**9**) were obtained by deacetylation of the condensation products (**3** and **4**), followed by hydrogenolysis of the benzyl glycosides (**7** and **8**). The n.m.r. spectra of the disaccharides (**9** and **10**) in deuterium oxide showed $J_{1',2'}$ 8.5 and 6.5 Hz, respectively, consistent with β-linkages[4].

Definitive proof of the structures of the two disaccharides was obtained by methylation of the benzyl glycosides (**7** and **8**) and hydrolysis, followed by reduction with borohydride, and acetylation. The *O*-methylalditol acetates thus produced were separated by t.l.c., and characterized by mass spectrometry[5].

The formation of a disaccharide from 2-amino-2-deoxy-D-glucose and benzyl α-L-rhamnopyranoside required conditions more rigorous than those used for the neutral disaccharides. Condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**11**) with benzyl α-L-rhamnopyranoside (**2**) was eventually effected by heating the two compounds in refluxing toluene in the presence of cadmium carbonate and Drierite. Preparative t.l.c. of the resultant products yielded a disaccharide fraction that contained two compounds which could not be separated from each other. The mixture was, therefore, oxidized by periodate to destroy the L-rhamnose moiety of the (1→4)-linked disaccharide, and the periodate-resistant disaccharide (**12**) was then readily purified by t.l.c. Its n.m.r. spectrum was consistent



with that of a 2-acetamido-2-deoxyglucose–rhamnose disaccharide, and the structure of benzyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**12**) was confirmed by methylation analysis, as already described for the neutral disaccharides. Acetate **12** was then *O*-deacetylated with sodium methoxide, and the resulting benzyl glycoside (**13**) was catalytically hydrogenolyzed, to afford the free disaccharide **14**.

(*b*) *Inhibition Studies.* — The previous structural studies[1] showed that the pneumococcal Type VII polysaccharide is branched, and that the branches are terminated by nonreducing, D-galactopyranosyl and 2-acetamido-2-deoxy-D-glucopyranosyl groups in the approximate ratio of 3:1. Time-lapse hydrolysis indicated that these end groups are linked to L-rhamnosyl residues, and, thus, that the latter are the penultimate units in the branches. The definitive identification of the nonreducing end-groups fully confirmed the predictions based upon serology[2], but there was no serological evidence that could be used to confirm or negate the proposed location of L-rhamnose units in the penultimate positions. Inhibition studies with the three disaccharides whose synthesis is reported here have now provided such evidence.

TABLE I

INHIBITION OF PNEUMOCOCCAL TYPE VII POLYSACCHARIDE–TYPE VII ANTISERUM SYSTEM BY NEUTRAL DISACCHARIDES[a]

Inhibitor	*Amount added (μmoles)*	*Antibody N ppt'd (μg)*	*Inhibition (%)*
None	—	640	0
3-*O*-β-D-Galactopyranosyl-L-rhamnopyranose	10	555	13
	20	509	21
	30	538	16
	40	544	15
	50	528	18
	60	499	22
4-*O*-β-D-Galactopyranosyl-L-rhamnopyranose	10	533	17
	20	480	25
	30	475	26
	40	469	27
	50	437	32
	60	402	37
4-*O*-β-D-Galactopyranosyl-D-glucopyranose	20	640	0
	40	619	3.3
	60	576	10.0
	80	560	12.5
	100	560	12.5
	120	586	8.5
Previously reported[2]:			
4-*O*-β-D-Galactopyranosyl-D-glucopyranose	115		13
6-*O*-β-D-Glucopyranosyl-D-glucopyranose	115		8
6-*O*-β-D-Galactopyranosyl-D-glucopyranose	100		29

[a]All results calculated to 1.0 ml of horse 937C Type VII antiserum.

Table I shows the results of inhibition of the homologous Type VII pneumococcal system by 3- and 4-*O*-*β*-D-galactopyranosyl-L-rhamnopyranose, and by 4-*O*-*β*-D-galactopyranosyl-D-glucopyranose (lactose), and, for purposes of comparison, it includes some results with other disaccharides that were reported previously[2]. The data show clearly that the galactosyl-rhamnose disaccharides are much better inhibitors than any of the galactosyl-glucose disaccharides, and confirm the importance of rhamnose as part of the determinant group. At the higher concentrations (50–60 mmolar), it appeared that the (1→4)-linked galactosyl-rhamnose was a better inhibitor than its (1→3) analog; this observation contrasts with the results of the structural studies, which showed that the rhamnose units in the polysaccharide carry substituents at O-3, and, to a minor extent, at O-2, but that none were substituted[1] at O-4. It is possible that, at the higher molar ratios of inhibitor, the disaccharides cover active sites directed towards other parts of the polysaccharide, because, at low molarities (10–20 mmolar), the inhibitory powers of the two galactosyl-rhamnoses are similar.

The cross reaction between Tamarind A polysaccharide and Type VII antipneumococcal serum has been shown to be due primarily to the presence of terminal D-galactopyranosyl residues in the polysaccharide[2], with minor participation of D-glucosyl groups, probably[1,2] *β*-(1→4). This cross-reaction therefore offered good possibilities for more-sensitive definition of the active sites directed towards the

TABLE II

INHIBITION OF TAMARIND A POLYSACCHARIDE–TYPE VII ANTIPNEUMOCOCCAL SERUM CROSS-REACTION BY NEUTRAL DISACCHARIDES[a]

Inhibitor	*Amount added (μmoles)*	*Antibody N ppt'd (μg)*	*Inhibition (%)*
None		144	0
3-*O*-*β*-D-Galactopyranosyl-L-rhamnopyranose	5	88.0	39
	15	32.0	78
4-*O*-*β*-D-Galactopyranosyl-L-rhamnopyranose	5	82.8	42
	15	46.8	68
4-*O*-*β*-D-Galactopyranosyl-D-glucopyranose	20	131	9
	30	117	19
Previously reported[2]:			
4-*O*-*α*-D-Glucopyranosyl-D-glucopyranose	115		13
4-*O*-*β*-D-Glucopyranosyl-D-glucopyranose	120		13
6-*O*-*β*-D-Glucopyranosyl-D-glucopyranose	115		24
6-*O*-*α*-D-Galactopyranosyl-D-galactopyranose	20		18
6-*O*-*β*-D-Galactopyranosyl-D-galactopyranose	115		80
	20		60
6-*O*-*α*-D-Galactopyranosyl-D-glucopyranose	115		30
6-*O*-*β*-D-Galactopyranosyl-D-glucopyranose	100		74
	25		58

[a]All results calculated to 1.0 ml of horse 937C Type VII antiserum.

terminal D-galactosyl residues than did the homologous system. The results of inhibition by neutral disaccharides of the Tamarind A–Type VII antipneumococcal system are shown in Table II. Again, the results showed that the two galactosyl-rhamnose disaccharides are far better inhibitors than any of the other disaccharides tested. At concentrations of 15mM, the (1→3)-linked galactosyl-rhamnose was a better inhibitor (78%) than its (1→4)-linked analog (68% inhibition), but it was clear that either linkage could be accommodated in the active site. The results previously published[2] (and included in Table II) proved conclusively that β-linked galactosyl disaccharides are better inhibitors than their α anomers. For this determinant group in the polysaccharide, it therefore appears that the occurrence, contiguously, of galactosyl-rhamnose units joined by β-linkages constitutes the principal requirement of specificity, and that the difference between a (1→3)- or a (1→4)-linkage is of less importance.

Evidence that the 2-acetamido-2-deoxy-D-glucosyl end-groups were also joined to the polysaccharide through rhamnose units was provided by the inhibition results shown in Table III. The conclusions that may be drawn from these data are limited, because (*a*) of the small number of compounds available for testing, and (*b*) there was

TABLE III

INHIBITION OF PNEUMOCOCCAL TYPE VII POLYSACCHARIDE–TYPE VII ANTISERUM SYSTEM BY AMINO SUGARS[a]

Inhibitor	*Amount added (μ moles)*	*Antibody N ppt'd (μg)*	*Inhibition (%)*
None		736	0
2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose	10–80	736	0
3-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-rhamnopyranose	10	672	9
	30	650	12
	60	619	16
	120	587	21
Previously reported[2]:			
2-Acetamido-2-deoxy-D-galactopyranose	365		12
2-Acetamido-2-deoxy-D-glucopyranose	430		21

[a]All results calculated to 1.0 ml of horse 937C Type VII antiserum.

no cross-reaction that could be used to heighten specificity towards the amino sugar determinant. Nevertheless, the observation that the β-(1→3)-linked 2-acetamido-2-deoxy-D-glucosyl-rhamnose disaccharide inhibited at all levels of concentration, whereas *N*-acetylchitobiose [β-(1→4)-linked 2-acetamido-2-deoxy-D-glucosyl-2-acetamido-2-deoxy-D-glucose] gave no inhibition up to the 80mM level, proved that rhamnose was an important part of this determinant. The possibility that the *O*-(2-acetamido-2-deoxy-D-glucosyl)-rhamnose covers the sites directed towards

galactosyl-rhamnose groups is excluded by the data in Tables I and II, which show that the terminal glucose configuration is not well accommodated in those sites.

The inhibition studies with the three disaccharides whose syntheses are reported here thus support the location of L-rhamnose units in the penultimate positions of the branches, as indicated by the structural studies[1].

EXPERIMENTAL

General. — Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter, and are equilibrium values unless otherwise stated. N.m.r. spectra were recorded with a Varian A60A spectrometer, with tetramethylsilane as the internal standard. Analytical and preparative t.l.c. were performed on glass plates coated with Silica Gel F-254, with the following solvent mixtures (v/v): (*A*) 3:2 ethyl acetate–light petroleum (b.p. 40–60°), (*B*) 3:7 ethyl acetate–light petroleum (b.p. 40–60°), and (*C*) 9:1 chloroform–ethanol. Sugars that contained *O*-benzyl substituents could be detected on thin-layer chromatograms by examination under u.v. irradiation. For general detection of all components, the plates were sprayed with 5% conc. sulfuric acid in ethanol and then heated for 5–10 min at 105°. A Hewlett-Packard Model 402 gas chromatograph with flame-ionization detector was used for gas–liquid chromatography (g.l.c.). The columns were glass U-tubes (150 × 0.3 cm i.d.) packed with 3% ECNSS-M on Gas-Chrom Q (100–120 mesh). Mass spectra were recorded on a Hitachi RMU-6D mass spectrometer.

Benzyl α-L-rhamnopyranoside (**2**). — This compound was prepared from L-rhamnose monohydrate, essentially as described by Brimacombe *et al.*[6], but it was purified by column chromatography (silica gel, ethyl acetate), and recrystallized from ethyl acetate: m.p. 75–76°, $[\alpha]_D^{24}$ −65° (*c* 1.0, water); lit.[6] m.p. 76°, $[\alpha]_D$ −63° (in water); n.m.r. data ($CDCl_3$): τ 2.53 (5 H singlet, aromatic protons), 5.09 (1 H doublet, $J_{1,2}$ 1.5 Hz, H-1), 5.36 (2 H doublet, J 11 Hz, benzylic protons), and 8.70 (3 H doublet, $J_{5,6}$ 9.0 Hz, CH_3).

Benzyl 3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-L-rhamnopyranoside (**3**) *and benzyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-L-rhamnopyranoside* (**4**). — A solution of compound **2** (2.5 g) in chloroform (60 ml) was stirred overnight with silver carbonate (6 g) and Drierite (10 g) in a flask covered with aluminum foil. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide[7] (**1**, 6.0 g), prepared by a general procedure for glycosyl halides[8], was added to the stirred mixture during 1 h. Stirring was continued until examination of the reaction by t.l.c. (solvent *A*) indicated complete reaction of the glycoside **2** (~4 h). The suspension was filtered, the residue was washed with chloroform (2 × 50 ml), and the filtrate and washings were combined, and evaporated under diminished pressure. The syrup was purified by preparative t.l.c. (double development, solvent *A*) to yield compound **3** (510 mg), the faster-moving component, as a partly crystalline powder having $[\alpha]_D^{24}$ −11.0° (*c* 2.0, chloroform); n.m.r. data ($CDCl_3$): τ 2.49 (5 H singlet, aromatic protons), 7.79

(3 H singlet, *O*-Ac), 7.89 (3 H singlet, *O*-Ac), 7.96 (6 H singlet, 2 *O*-Ac), and 8.68 (3 H doublet, $J_{5,6}$ 9.0 Hz, CH_3). The compound did not react with an excess of sodium metaperiodate at room temperature, as shown by t.l.c.

Anal. Calc. for $C_{27}H_{36}O_{14}$: C, 55.47; H, 6.17. Found: C, 55.39; H, 6.16.

The slower-moving component in t.l.c. was compound **4**, also obtained as a partly crystalline powder (485 mg). It had $[\alpha]_D^{24}$ −38.2° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$): τ 2.50 (5 H, singlet, aromatic protons), 7.80 (3 H, singlet, *O*-Ac), 7.89 (3 H, singlet, *O*-Ac), 7.92 (3 H, singlet, *O*-Ac), 7.98 (3 H, singlet, *O*-Ac), and 8.66 (3 H, doublet, $J_{5,6}$ 10 Hz, CH_3). The compound readily reacted with an excess of sodium metaperiodate at room temperature, as shown by t.l.c.

Anal. Calc. for $C_{27}H_{36}O_{14}$: C, 55.47; H, 6.17. Found: C, 55.20; H, 6.13.

Benzyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-L-rhamnopyranoside (**5**). — A mixture of compound **1** (500 mg), mercuric cyanide (250 mg), and benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[3] (**6**, 250 mg) in nitromethane (20 ml) was stirred for 16 h at room temperature. The suspension was filtered, the filtrate evaporated under diminished pressure, and the residual syrup fractionated on preparative t.l.c. plates (solvent *B*) to yield compound **5** (112 mg), This was crystallized from ether, and had m.p. 132–134°, $[\alpha]_D^{24}$ −18.7° (*c* 1.0. chloroform); n.m.r. data ($CDCl_3$): τ 2.52 (5 H, singlet, aromatic protons), 7.81 (3 H, singlet, *O*-Ac), 7.90 (3 H, singlet, *O*-Ac), 7.93 (3 H, singlet, *O*-Ac), 7.98 (3 H, singlet, *O*-Ac), 8.45 (3 H, singlet, isopropylidene CH_3), 8.63 (3 H, singlet, isopropylidene CH_3), and 8.69 (3 H, doublet, $J_{5,6}$ 6.0 Hz, CH_3).

Anal. Calc. for $C_{30}H_{40}O_{14}$: C, 57.69; H, 6.43. Found: C, 57.72; H, 6.47.

When the disaccharide **4** was treated overnight with acetone containing a trace of concentrated sulfuric acid, it yielded a derivative, isolated by t.l.c., that was identical with compound **5**, as determined by m.p., mixed m.p., t.l.c., and i.r. spectrum.

Benzyl 4-O-β-D-galactopyranosyl-α-L-rhamnopyranoside (**7**). — A solution of compound **4** (300 mg) in methanol (10 ml) was treated for 1 h at room temperature with 0.1M sodium methoxide (15 ml), and then de-ionized with Amberlite IR-120 (H^+) ion-exchange resin, and evaporated under diminished pressure. The residual, amorphous material (**7**, 174 mg) had $[\alpha]_D^{24}$ −61.7° (*c* 1.0, acetone); n.m.r. data (acetone-d_6): τ 2.60 (5 H, singlet, aromatic protons), 5.09 (1 H, doublet, $J_{1,2}$ 1.5 Hz, H-1), 5.29 (2 H, doublet, *J* 9.0 Hz, benzylic protons), 5.61 (1 H, doublet, $J_{1',2'}$ 8.5 Hz, H-1'), and 8.71 (3 H, doublet, *J* 9.0 Hz, CH_3). The compound was extremely hygroscopic, and difficult to analyze:

Anal. Calc. for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 53.95; H, 6.75.

A portion (10 mg) of compound **7** was methylated[9] with methyl iodide (0.1 ml) and sodium hydride (20 mg) in *N,N*-dimethylformamide (5 ml). Hydrolysis, with 0.5M sulfuric acid (1.0 ml) for 6 h at 100°, of the product purified by t.l.c. (solvent *A*), followed by reduction with sodium borohydride, and acetylation with acetic anhydride–pyridine furnished a 1:1 mixture (g.l.c.) of two partially methylated alditol acetates. After separation by t.l.c. (solvent *B*), these were identified by mass spectro-

metry as 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-rhamnitol (*m/e* 117 and 203) and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol (*m/e* 45, 117, 161, and 205).

Benzyl 3-O-β-D-galactopyranosyl-α-L-rhamnopyranoside (**8**). — Compound **3** (325 mg) was deacylated as described for **4**. The product (**8**, 172 mg) had $[\alpha]_D^{24}$ −48.1° (*c* 1.0, acetone); n.m.r. data (acetone-d_6): τ 2.54 (5 H, singlet, aromatic protons), 5.10 (1 H, doublet, $J_{1,2}$ 1.5 Hz, H-1), 5.33 (2 H, doublet, *J* 9.0 Hz, benzylic protons), 5.77 (1 H, doublet, $J_{1',2'}$ 6.5 Hz, H-1'), and 8.74 (3 H, doublet, *J* 8.0 Hz, CH_3).

Anal. Calc. for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 54.48; H, 6.63.

A portion of compound **8** was methylated, the ether hydrolyzed, the product reduced, and the alditol acetylated as described for compound **7**. The products were separated by t.l.c. (solvent *B*), and identified by mass spectrometry as 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methyl-L-rhamnitol (*m/e* 117, 131, and 233) and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol (*m/e* 45, 117, 161, and 205).

4-O-β-D-Galactopyranosyl-L-rhamnose (**9**). — A solution of compound **7** (110 mg) in 95% ethanol (20 ml) was shaken with hydrogen at atmospheric pressure in the presence of palladium black (20 mg) for 18 h. The catalyst was removed by filtration, and the filtrate was evaporated to a syrup that solidified on trituration with ethyl acetate, to give compound **9** (67 mg); $[\alpha]_D^{24}$ −4.2° (*c* 2.0, ethanol); n.m.r. data (D_2O, external Me_4Si): τ 4.80 (2/3 H doublet, $J_{1,2}$ 2.5 Hz, H-1 of α-L form), 5.04 (1/3 H doublet, $J_{1,2}$ 1.0 Hz, H-1 of β-L form), 5.38 (1 H doublet, $J_{1',2'}$ 8.5 Hz, H-1'), and 8.68 (3 H, doublet, CH_3).

Anal. Calc. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 43.62; H, 6.66.

G.l.c. of the per(trimethylsilyl)ated disaccharide[10] **9** at 180° showed a single peak having R_T 2.34 (relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol as unity).

3-O-β-D-Galactopyranosyl-L-rhamnose (**10**). — Compound **8** (110 mg) was hydrogenolyzed as described for compound **7**. The product (**10**, 63 mg) had $[\alpha]_D^{24}$ +8.5° (*c* 2.0, ethanol); n.m.r. data (D_2O): τ 4.80 (2/3 H, doublet, $J_{1,2}$ 2.5 Hz, H-1 of α-L form), 5.04 (1/3 H, doublet, $J_{1,2}$ 1.0 Hz, H-1 of β-L form), 5.75 (1 H, doublet, $J_{1',2'}$ 6.5 Hz, H-1'), and 8.69 (3 H, doublet, CH_3).

Anal. Calc. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 44.31; H, 6.51.

G.l.c. of the per(trimethylsilyl)ated disaccharide at 180° gave a single peak having R_T 3.36 (relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol as unity).

Benzyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**12**). — A mixture of compound **2** (2.0 g), 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[11] (**11**, 4.5 g), cadmium carbonate (2.5 g), and Drierite (10 g) in toluene (120 ml) was heated under reflux, with stirring, until t.l.c. (solvent *C*) indicated complete reaction of **2** (~1 h). The inorganic residue was removed by filtration, and washed with chloroform (2 × 75 ml), and the filtrate and washings were combined and evaporated under diminished pressure. A solution of the residue in aqueous ethanol was treated overnight with an excess of sodium metaperiodate. Preparative t.l.c. then yielded the periodate-resistant product (**12**,

435 mg). After crystallization from ether, it had m.p. 140–142°, $[\alpha]_D^{24}$ −21.8° (*c* 1.0, chloroform); n.m.r. data ($CDCl_3$): τ 2.49 (5 H, singlet, aromatic protons), 7.92–7.99 (12 H, singlet, 4 *O*-Ac), and 8.73 (3 H, doublet, $J_{5,6}$ 14.0 Hz, CH_3).

Anal. Calc. for $C_{27}H_{37}NO_{13}$: C, 55.62; H, 6.40; N, 2.40. Found: C, 54.88; H, 6.35; N, 2.62.

Benzyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**13**). — Deacetylation of compound **12** (400 mg) as described for **4** yielded compound **13** (236 mg), which was crystallized from chloroform–ethanol; m.p. 119–121°; $[\alpha]_D^{24}$ −14.8° (*c* 1.0, ethanol); n.m.r. data (D_2O): τ 2.29 (5 H, singlet, aromatic protons), 4.91 (1 H doublet, $J_{1,2}$ 2.2 Hz, H-1), 5.65 (1 H, doublet, $J_{1',2'}$ 7.0 Hz, H-1'), 7.83 (3 H, singlet, *N*-Ac), and 8.69 (3 H, doublet, $J_{5,6}$ 15.0 Hz, CH_3).

Anal. Calc. for $C_{20}H_{31}NO_{10}$: C, 53.92; H, 7.01; N, 3.14. Found: C, 53.61; H, 6.81; N, 3.08.

A portion (10 mg) of compound **13** was methylated with methyl iodide and sodium hydride in *N,N*-dimethylformamide as described for compounds **7** and **8**. Hydrolysis of the methylated product gave two compounds, one of which was the same as an authentic sample of 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucose in t.l.c. (solvent *C*). Reduction of the hydrolyzate and acetylation of the product yielded a 1:1 mixture (by t.l.c.) of two alditol derivatives. Separation by t.l.c. (solvent *B*), and analysis by mass spectrometry, confirmed the identity of the deoxyalditol derivative as 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methyl-L-rhamnitol (*m/e* 117, 131, and 233).

3-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-rhamnose (**14**). — Hydrogenolysis of compound **13** (180 mg) as described for **7** yielded compound **14** (114 mg); $[\alpha]_D^{24}$ −3.2° (*c* 2.0, ethanol); n.m.r. data (D_2O, external Me_4Si): τ 4.80 (2/3 H, doublet, $J_{1,2}$ 2.5 Hz, H-1 of α-L form), 5.04 (1/3 H, doublet, $J_{1,2}$ 1.0 Hz, H-1 of β-L form), 5.74 (1 H, doublet, $J_{1',2'}$ 6.5 Hz, H-1'), 7.91 (3 H, singlet, *N*-Ac), and 8.70 (3 H, doublet, CH_3).

Anal. Calc. for $C_{14}H_{25}NO_{10}$: C, 45.77; H, 6.86; N, 3.81. Found: C, 45.55; H, 7.17; N, 3.65.

Inhibition studies. — Inhibition studies were conducted according to the general directions of Kabat and Mayer[12]. All operations were performed at 0–4°. Aliquots (0.25 ml) of Type VII antipneumococcal horse serum (horse 937C) were used in all tests. To determine equivalence points, increasing quantities of the two antigens (pneumococcal Type VII polysaccharide[1] and Tamarind A polysaccharide[13,14]) (5 mg) in water (10 ml) were simultaneously added to the antiserum, and the total volumes were adjusted to 0.75 ml. Antibody nitrogen in the precipitates was determined by use of the Folin–Ciocalteu reagent[15] as described by Kabat and Mayer[12]. The quantities of antigens required for maximal precipitation of antibody N (equivalence points) were 450 μg/ml of antiserum for the pneumococcal Type VII polysaccharide, and 400 μg/ml of antiserum for the Tamarind A polysaccharide.

In the inhibition tests, increasing amounts of the inhibitors, dissolved in water, were added to the antiserum, and the mixtures were kept for 2 h. The solution of

antigen was then added in the amount needed to give maximal precipitation, and the mixtures were adjusted to the same volume (0.80 ml) with water. The results are given in Tables I to III.

ACKNOWLEDGMENTS

The authors are grateful to Dr. M. Heidelberger for samples of the Type VII antipneumococcal horse serum and the Tamarind A polysaccharide, and to Mr. H. Seguin, N.R.C., for the microanalyses.

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