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# SYNTHESIS OF A COMMON POLYSACCHARIDE ANTIGEN OF *Pseudomonas aeruginosa* AS THE 6-AMINOHEXYL GLYCOSIDE

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ABSTRACT

The synthesis is described of a tritylated 1,2-*O*-cyanoethylidene derivative (**3**) of the trisaccharide α-D-Rha-(1→2)-α-D-Rha-(1→3)-D-Rha. Triphenylmethylium perchlorate-catalysed polycondensation of **3** in the presence of 6-phthalimidohexyl 2,4-di-*O*-benzoyl-3-*O*-trityl-α-D-rhamnopyranoside followed by deprotection afforded the 6-aminohexyl glycoside of a D-rhamnan corresponding to a common polysaccharide antigen of *Pseudomonas aeruginosa*.

## INTRODUCTION

Of the strains of *Ps. aeruginosa*, ~80% contain[1,2], in the lipopolysaccharide fraction, a common polysaccharide antigen which is a linear D-rhamnan with the trisaccharide repeating-unit →3)-α-D-Rha-(1→2)-α-D-Rha-(1→3)-α-D-Rha-(1→ (**1**).

The synthesis of this polysaccharide as a 6-aminohexyl glycoside[3,4] could provide immunogenic conjugates and immunosorbents for studies of *Pseudomonas* antigens.

## RESULTS AND DISCUSSION

The polysaccharide could be synthesised by polycondensation of a tritylated 1,2-*O*-cyanoethylidene derivative[5] of a trisaccharide corresponding to the repeating unit **1** or its isomer →2)-α-D-Rha-(1→3)-α-D-Rha-(1→3)-α-D-Rha-(1→ (**2**).

The trisaccharide derivatives should bear the glycosyl-donor (1,2-*O*-cyanoethylidene) group in the 3-substituted rhamnose residue, and the glycosyl-acceptor (trityloxy) site should be at position 3 or 2 of the non-reducing rhamnose residue. The derivative **3** was chosen which corresponds to the repeating unit **1**, assuming the equatorial 3-trityloxy group to be more reactive than the axial group at position 2.

The strategy for synthesis of **3** was based on the use of monosaccharide synthons with "permanent" (benzoyl) and "temporary" (acetyl) *O*-protecting

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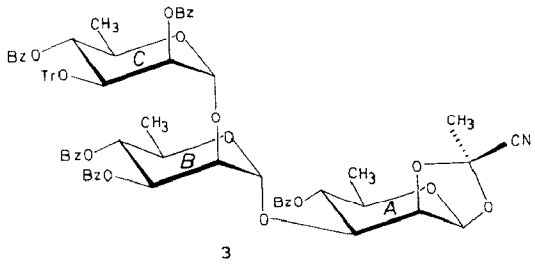
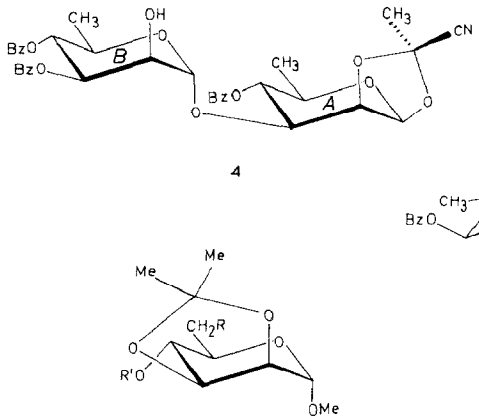


3

groups. The latter can be removed selectively by mild acid-catalysed methanolysis[6]. This approach was exemplified first by the synthesis[7] of a monomer for preparation of the *Shigella flexneri* variant Y O-antigenic polysaccharide and then applied[8] in the synthesis of a monomer precursor for the *Streptococcus pneumoniae* type 14 polysaccharide.

Two approaches to **3** are possible, namely, B + A → BA then C + BA → CBA, or C + B → CB then CB + A → CBA. The former requires the use of the monohydroxy derivative **4** (BA fragment), the L enantiomer (**5**) of which was prepared in moderate yield[7] by competitive formation of the methoxycarbonylethylidene derivative **6** on acid-catalysed deacetylation of the corresponding acetate **7**. Hence, the latter approach was chosen since it involves acid-catalysed deacetylation as the penultimate step, namely, prior to tritylation of a trisaccharide cyanoethylidene derivative.



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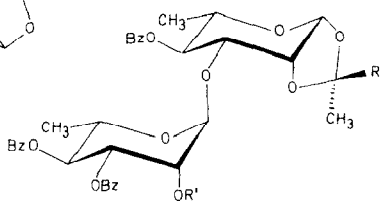


5 R = CN, R' = H

6 R = COOMe, R' = H

7 R = CN, R' = Ac



8 R = OH, R' = H

9 R = OTs, R' = Bz

10 R = I, R' = Bz

11 R = H, R' = Bz

The key D-rhamnose intermediate **12** was synthesised by deoxygenation at C-6 of the D-mannose derivative **8**[9]. Selective monotosylation of **8** followed by benzoylation afforded 81.5% of the crystalline tosylate **9**. Treatment of **9** with

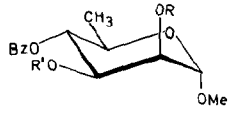
sodium iodide in boiling acetonitrile gave the iodide **10** quantitatively. Hydrogenation of **10** over Raney nickel in the presence of triethylamine gave 81% of the crystalline D-rhamnoside derivative **11**. This synthesis of **11** makes use of readily available and cheap reagents, involves high yields and easy isolation at each step, and does not require dry solvents or expensive reducing agents.

Hydrolysis of **11** with aqueous 90% trifluoroacetic acid afforded the diol **12** quantitatively, which was the precursor of the synthons **13, 18**, and **21** required for the assembly of **3**. The synthesis of **13, 18**, and **21** followed the reaction schemes elaborated for preparation of the L analogues[4,7,10].

Selective benzoylation of **12** with benzoyl chloride–pyridine at 0–5° gave 77% of the 3,4-dibenzoate **13**.

Acetolysis of **12** afforded the triacetate **14**, which was treated with hydrogen bromide–acetic acid to give the rhamnosyl bromide **15**. Reaction of **15** with sodium cyanide in acetonitrile gave 84% of a ~4:1 mixture of *exo*- and *endo*-CN isomers. Only the *exo*-CN isomer **16**, isolated in 67% yield by column chromatography, was employed in subsequent transformations in order to facilitate the interpretation of spectral data. Mild acid-catalysed methanolysis of **16** gave the target synthon **18**.

Reaction of **12** with ethyl orthobenzoate in the presence of toluene-*p*-sulfonic acid gave an orthoester **19**, which was converted regioselectively, by hydrolysis of the orthoester group with aqueous 80% acetic acid[10], into the 2,4-dibenzoate **20**. Acetolysis of **20** gave the diacetate **21**. The n.m.r. spectra of **13, 18**, and **21** and of the intermediates **14, 16, 17**, and **20** (Tables I and II) were identical to those for the corresponding L analogues[4,7,10].

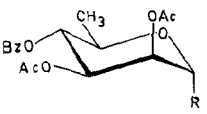


12 R = R' = H

13 R = H, R' = Bz

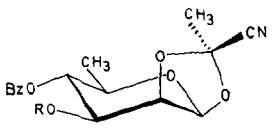
19 R + R' = Ph(EtO)C<

20 R = Bz, R' = H



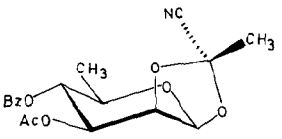
14 R = OAc

15 R = Br



16 R = Ac

18 R = H



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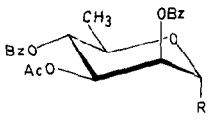
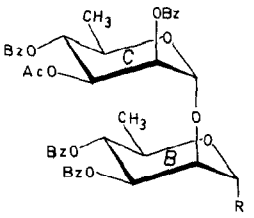


21 R = OAc

22 R = Br

24 R = CN



23 R = OMe

25 R = OAc

26 R = Br

Treatment of **21** with hydrogen bromide–acetic acid afforded the rhamnosyl bromide **22**, which was condensed with the glycosyl-acceptor **13** in acetonitrile in the presence of mercuric cyanide to give the disaccharide derivative **23** contaminated with the rhamnosyl cyanide **24**. This mixture could not be resolved

TABLE I

$^1$H-N.M.R. DATA FOR THE D-MANNOSE DERIVATIVES **9** AND **10** AND D-RHAMNOSE DERIVATIVES

| *Compound* | *Chemical shifts (δ) and coupling constants (Hz)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *H-1* ($J_{1,2}$) | *H-2* ($J_{2,3}$) | *H-3* ($J_{3,4}$) | *H-4* ($J_{4,5}$) | *H-5* ($J_{5,6}$) | *H-6* | *Other signals* |
| **9** | 4.94 s | 4.18 d (5.4) | 4.35 dd (7.5) | 5.10 dd (9.9) | 4.00–4.13 | | 1.33 s, 1.56 s ($Me_2C$), 2.36 s ($CH_3$), 3.41 s (OMe) |
| **10** | 5.03 s | 4.21 d (5.4) | 4.38 dd (7.6) | 5.14 dd (10.0) | 3.88 ddd (2.7) (9.5) | 3.20 dd 3.31 dd ($J_{6,6'}$ 10.8) | 1.37 s, 1.61 s ($Me_2C$), 3.58 s (OMe) |
| **11** | 4.96 s | 4.20 d (5.5) | 4.34 dd (7.9) | 5.13 dd (10.3) | 3.87 dq (6.3) | 1.24 d | 1.36 s, 1.63 s ($Me_2C$), 3.42 s (OMe) |
| **12** | 4.84 d (1.4) | 3.99 ddd (3.5) ($J_{2,4}$ 1.5) | 3.99 dd (10.9) | 5.10 ddd (9.6) | 3.92 dq (6.3) | 1.27 d | 3.40 s (OMe) |
| **13** | 4.81 d (1.8) | 4.28 bs | 5.55–5.65 m | | 4.09 dq (6.3) | 1.33 d | 3.48 s (OMe), 2.41 bs (OH) |
| **14** | 6.08 d (2.1) | 5.31 dd (3.4) | 5.52 dd (10.4) | 5.40 t (10.4) | 4.09 dq (6.2) | 1.29 d | 1.91 s, 2.20 s (3 Ac) |
| **16** | 5.48 d (2.2) | 4.63 dd (4.0) | 5.46 dd (10.0) | 5.33 t (10.0) | 3.72 dq (6.3) | 1.28 d | 1.97 s ($CH_3CCN$), 2.04 s (Ac) |
| **17** | 5.58 d (2.1) | 4.50 d (3.3) | 5.36 dd (9.8) | 5.41 t (9.8) | 3.72 dq (6.4) | 1.36 d | 1.83 s ($CH_3CCN$), 2.04 s (Ac) |
| **18** | 5.45 d (2.3) | 4.60 dd (4.4) | 4.12 ddd (9.1) | 5.11 t (9.1) | 3.67 dq (6.2) | 1.29 d | 1.95 ($CH_3CCN$), 2.76 d ($J_{OH,3}$ 9 Hz, OH) |
| **20** | 4.82 d (1.7) | 5.30 dd (3.5) | 4.22 dd (10.0) | 5.21 t (10.0) | 4.00 dq (6.1) | 1.32 d | 3.47 s (OMe), 2.40 bs (OH) |
| **21** | 6.23 d (2.0) | 5.57 dd (3.4) | 5.64 dd (10.0) | 5.54 t (10.0) | 4.18 dq (6.4) | 1.33 d | 1.87 s, 2.24 s (2 Ac) |
| **24** | 4.77 d (1.7) | 5.95 dd (3.4) | 5.38 dd (10.2) | 5.46 t (10.2) | 3.81 dq (6.1) | 1.43 d | 1.90 s (Ac) |
| **32** | 4.92 d (1.9) | 5.52 dd (3.4) | 5.63 dd (10.0) | 5.47 t (10.0) | 4.10 dq (6.4) | 1.32 d | 1.43 m, 1.70 m (4 $CH_2$), 3.94 dt ($J_{\alpha,\beta}$ 6.5, $J_{\alpha,\alpha'}$ 9.7 Hz, H-α)[a], 3.74 dt ($J_{\alpha',\beta}$ 6.5 Hz, H-α'), 3.71 ($J$ 7.1 Hz, $CH_2N$), 1.84 s (Ac) |
| **33** | 4.95 d (1.9) | 5.38 dd (3.5) | 4.33 ddd (10.0) | 5.28 t (10.0) | 4.05 dq (6.3) | 1.30 d | 1.40 m, 1.72 m (4 $CH_2$), 2.62 d ($J_{OH,3}$ 8.0 Hz, OH), 3.48 dt ($J_{\alpha,\beta}$ 6.4, $J_{\alpha,\alpha'}$ 9.7 Hz, H-α) 3.73 dt ($J_{\alpha',\beta}$ 6.7 Hz, H-α'), 3.69 t ($J$ 7.4 Hz, $CH_2N$) |
| **34** | 4.79 d (2.0) | 4.40 dd (3.0) | 4.17 dd (9.9) | 5.80 t (9.9) | 3.75 dq (6.5) | 1.23 d | 1.38 m, 1.75 m (4 $CH_2$), 3.28 dt ($J_{\alpha,\beta}$ 6.0, $J_{\alpha,\alpha'}$ 9.6 Hz, H-α) 3.54 dt ($J_{\alpha',\beta}$ 6.4 Hz, H-α'), 3.76 t ($J$ 7.2 Hz, $CH_2N$) |

[a]H-α and H-α' refer to the protons of the α-methylene moiety of the 6-phthalimidohexyl group.

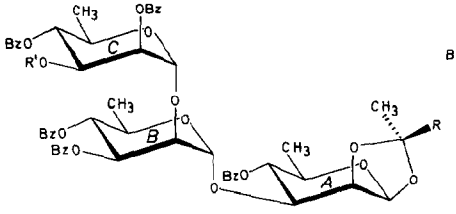
TABLE II

$^{13}$C-N.M.R. DATA FOR THE D-MANNOSE DERIVATIVES **9** AND **10** AND D-RHAMNOSE DERIVATIVES

| *Compound* | *Chemical shifts (p.p.m.)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *C-1* | *C-2* | *C-3* | *C-4* | *C-5* | *C-6* | *Other signals* |
| **9** | 98.3 | 75.8 | 75.8 | 70.3 | 69.0 | 66.7 | 55.3 (OMe), 26.3, 27.7, 110.3 ($Me_2C$) |
| **10** | 98.6 | 76.1 | 75.9 | 74.1 | 68.8 | 4.1 | 56.0 (OMe), 26.4, 27.8, 110.3 ($Me_2C$) |
| **11** | 98.2 | 76.1 | 76.0 | 75.2 | 64.1 | 17.2 | 55.1 (OMe), 25.5, 27.8, 110.0 ($Me_2C$) |
| **12** | 100.8 | 71.1 | 70.5 | 76.3 | 65.8 | 17.7 | 55.2 (OMe) |
| **13** | 100.9 | 69.7 | 72.8 | 71.7 | 66.6 | 17.7 | 55.3 (OMe) |
| **14** | 91.1 | 69.1 | 69.3 | 71.4 | 69.3 | 17.7 | 20.6, 20.8, 20.9 (3 $CH_3CO$) |
| **16** | 97.1 | 78.7 | 69.4 | 70.8 | 70.4 | 17.7 | 20.6 ($CH_3CO$), 26.6, 101.8, 116.8 ($CH_3CCN$) |
| **17** | 98.1 | 78.1 | 70.1 | 70.5 | 70.5 | 17.5 | 20.8 ($CH_3CO$), 26.4, 100.9, 117.2 ($CH_3CCN$) |
| **18** | 97.0 | 80.7 | 70.2 | 74.3 | 70.0 | 17.7 | 26.6, 101.4, 116.8 ($CH_3CCN$) |
| **20** | 98.5 | 73.3 | 69.8 | 75.6 | 66.1 | 17.7 | 55.3 (OMe) |
| **21** | 91.0 | 69.4 | 69.1 | 71.4 | 69.2 | 17.6 | 20.6, 20.8 (2 $CH_3CO$) |
| **32** | 97.7 | 70.9 | 69.4 | 72.2 | 66.7 | 17.7 | 20.7 ($CH_3CO$), 25.7, 26.7, 28.5, 29.3 (4 $CH_2$), 37.9 ($CH_2N$), 68.3 ($CH_2O$) |
| **33** | 97.4 | 73.5 | 69.0 | 75.6 | 66.3 | 17.7 | 25.8, 26.7, 28.6, 29.3 (4 $CH_2$) 38.0 ($CH_2N$), 68.2 ($CH_2O$) |
| **34** | 96.8 | 72.6 | 70.2 | 73.1 | 67.1 | 17.9 | 25.8, 26.8, 28.7, 29.4 (4 $CH_2$), 38.1 ($CH_2N$), 67.6 ($CH_2O$), 86.0 ($CPh_3$) |

by conventional column chromatography and was used as such in the next step, but pure **23** could be isolated by preparative h.p.l.c.

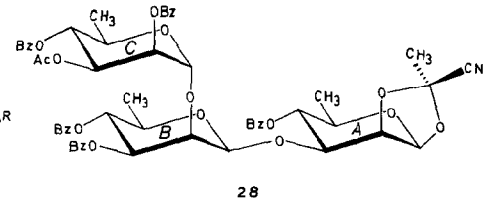
Acetolysis of the mixture of **23** and **24** with acetic anhydride in the presence of sulfuric acid gave a crystalline diacetate **25**, the synthon for the CB fragment, which was easily purified by crystallisation. Treatment of **25** with hydrogen bromide in dichloromethane gave a biosyl bromide **26**, which was coupled with the cyanoethylidene derivative **18** in acetonitrile in the presence of mercuric cyanide and mercuric bromide to give ~80% of a 2.8:1 $\alpha\beta$-mixture (**27** and **28**) of trisaccharide derivatives. The anomers **27** and **28** were isolated by column chromatography and the structures were confirmed by the n.m.r. data (see Experimental). That the glycosidic linkage of the rhamnose unit B was $\alpha$ in **27** and $\beta$ in **28** was shown by the $J_{C\text{-}1,H\text{-}1}$ values, 170 and 158 Hz, respectively.




27 R = CN, R' = Ac

29 R = CN, R' = H

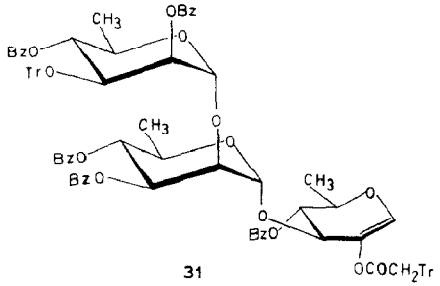
30 R = COOMe, R' = H




28

The acetyl group of residue C in **27** was removed efficiently by mild acid-catalysed methanolysis (*cf.* refs. 6 and 7) to give 69% of **29** together with **27** (23%) and 6% of the methoxycarbonylethylidene derivative **30**. Repeated treatment of recovered **27** increased the yield of **29** to 85%. The location of the unsubstituted hydroxyl group at position 3 of the rhamnose unit C in **29** followed from the upfield shift of the resonance of H-3C in the $^1$H-n.m.r. spectrum and downfield shifts of the resonances of C-2C and C-4C in the $^{13}$C-n.m.r. spectrum upon conversion of **27** into **29**.

Treatment of **29** with triphenylmethylium perchlorate in the presence of 2,4,6-collidine gave 63% of **3**, and 33% of **29** was recovered together with a by-product **31**[11]. The structure of **3** was confirmed by the n.m.r. spectra (see Experimental).

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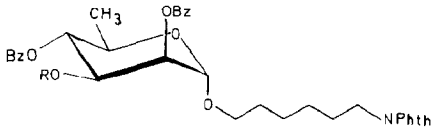
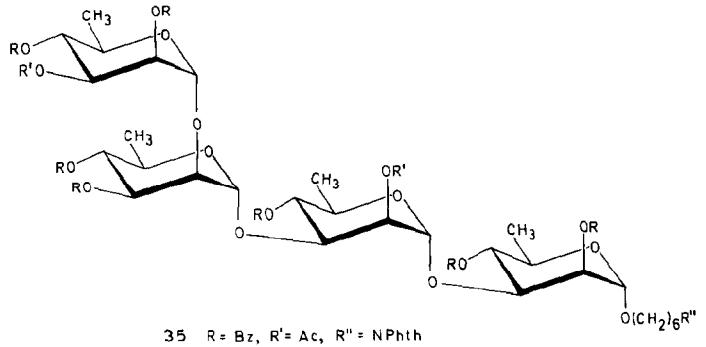



32 R = Ac
33 R = H
34 R = Tr

Starting from the rhamnosyl bromide **22**, the trityl ether **34** (terminator) was prepared which simulates the structure of the rhamnose unit C in **3**. Glycosylation of 6-phthalimidohexanol with **22** in dichloromethane in the presence of mercuric cyanide, mercuric bromide, and molecular sieves gave the glycoside **32** in virtually quantitative yield. Acid-catalysed deacetylation of **32** followed by tritylation of the resulting alcohol **33** with triphenylmethylium perchlorate in the presence of 2,4,6-collidine smoothly gave the trityl ether **34**.

Glycosylation of **34** with the trisaccharide cyanoethylidene derivative **27** was explored first and gave 87% of the crystalline tetrasaccharide derivative **35**. The $^{13}$C-n.m.r. spectrum of **35** contained four signals for anomeric carbons with $J_{C\text{-}1,H\text{-}1}$ values of 168–173 Hz which proved each rhamnose residue to be $\alpha$. Hydrazinolysis of **35** removed the *N*-phthaloyl group to yield a mixture of partially benzoylated amino derivatives that was treated with methanolic sodium methoxide to give the 6-aminohexyl tetrasaccharide-glycoside **36**. On chromatography on Fractogel TSK HW-40(S), **36** gave two peaks, the products in which had the same mobility in t.l.c., identical $^{13}$C-n.m.r. spectra, and almost the same optical rotations. They possibly correspond to neutral and charged forms of **36**.

Polycondensation of **3** was performed by slow addition of its solution to a solution of the terminator **34** and the catalyst (the final molar ratios **3**:**34**:$TrClO_4$ were 10:1:1). Hydrazinolysis of the polymeric products and then treatment with methanolic sodium methoxide gave a mixture of a basic (**37**) and neutral (**38**)



35 R = Bz, R' = Ac, R'' = NPhth

36 R = R' = H, R'' = $NH_2$

polymers that were resolved by cation-exchange chromatography and obtained in yields of 31 and 66%, respectively. High-molecular-weight fractions of **37** and **38** were isolated by gel chromatography on Fractogel TSK HW-40(S).

$[\rightarrow 3)$-α-D-Rha$(1\rightarrow 2)$-α-D-Rha$(1\rightarrow 3)$-α-D-Rha$(1\text{-}]_5\rightarrow 3)$-α-D-RhaO$(CH_2)_6NH_2$

**37**

$[\rightarrow 3)$-α-D-Rha$(1\rightarrow 2)$-α-D-Rha$(1\rightarrow 3)$-α-D-Rha$(1\rightarrow]_n$

**38**

The $^{13}$C-n.m.r. spectra of **37** and **38** were identical in the region of the trisaccharide repeating-unit and almost coincided with that of the natural polysaccharide[2] of *Ps. aeruginosa* (Fig. 1). The spectrum of **37** also contained signals due to the 6-aminohexyl moiety. From the ratio of the integrated intensities of the signals for the repeating unit and the terminal rhamnose moiety, the average d.p. of the high-molecular-weight fraction of **37** was calculated to be 15. Using gel-permeation chromatography on a calibrated column of Fractogel TSK HW-50(S), the weight-average and number-average d.p. for the high-molecular-weight fraction of **38** were estimated to be 36 and 25.5, respectively. The absence from the $^{13}$C-n.m.r. spectra of **37** and **38** of signals for β-D-rhamnopyranose residues, particularly, in the region of 81.0–81.5 p.p.m. (C-3 of the 3-substituted β-rhamnose) proves that the polycondensation was stereospecific. The $[\alpha]_D$ values for **37** and **38** were close to those reported for the *Ps. aeruginosa* polysaccharides[2] and the structurally identical O-specific polysaccharide of *Ps. cerasi*[12].

The data presented here demonstrate that the synthetic polysaccharides **37** and **38** possess the structure of the *Ps. aeruginosa* common polysaccharide antigen. The preparation of artificial antigens from **37** with a 6-aminohexyl spacer-arm will be published elsewhere.

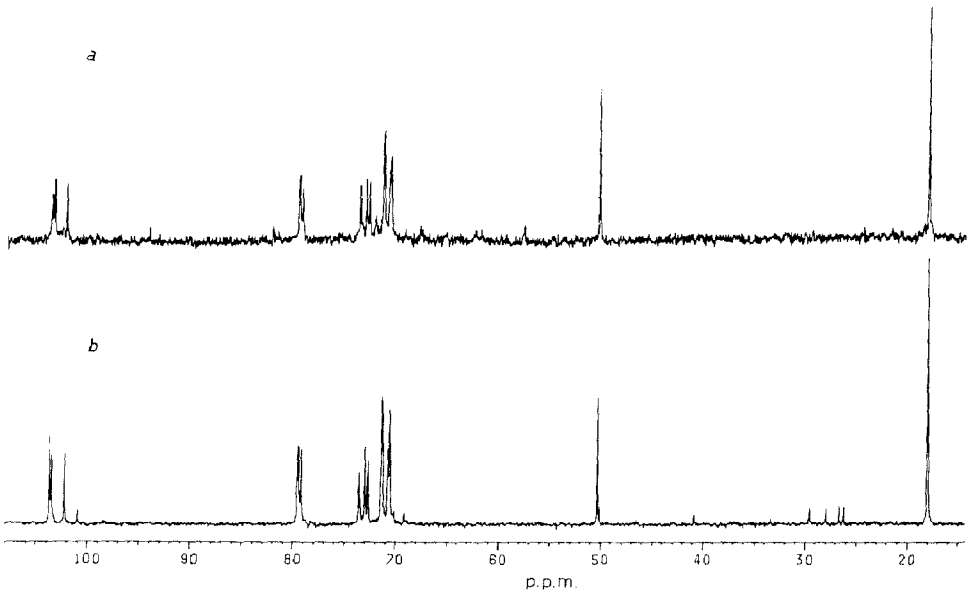



Fig. 1. $^{13}$C-N.m.r. spectra of (a) natural polysaccharide of *Ps. aeruginosa* and (b) synthetic polysaccharide **37** ($D_2O$, internal MeOH, $\delta_{Me_4Si}$ 50.15).

## EXPERIMENTAL

Optical rotations for solutions in chloroform (unless otherwise stated) were recorded with a JASCO DIP-360 polarimeter at 20 ±2°. The $^1$H- and $^{13}$C-n.m.r. spectra were recorded with Bruker WM-250 and AM-300 instruments for solutions in $CDCl_3$ and $D_2O$ [internal $Me_4Si$ and MeOH ($\delta_{Me_4Si}$ 50.15) for protected and deprotected derivatives, respectively]. T.l.c. was performed on Kieselgel 60 $F_{254}$ with u.v. detection or by charring with 70% sulfuric acid. Column chromatography was performed on silica gel L 40/100 μm, (CSSR), using a benzene–ethyl acetate gradient, and gel chromatography was performed on columns of Bio Gel P-4 (−400 mesh, 50 × 2.5 cm, $V_0$ ~80 mL, column *A*) and Fractogel TSK HW-40(S) (75 × 2.5 cm, $V_0$ 120 mL, column *B*) by elution with 0.1M acetic acid at 1 mL/min. The molecular weight of **38** was determined by gel chromatography on a column (70 × 1.5 cm) of Fractogel TSK HW-50(S), using T-10 Dextran (Pharmacia) with a known molecular-weight distribution. The chromatography was monitored using a differential refractometer type 88.00 (Knauer). Solvents were purified as described[13]. Acylated sugar derivatives were converted into the corresponding glycosyl bromides as described[7].

*Methyl 4-O-benzoyl-2,3-O-isopropylidene-6-O-tosyl-α-*D*-mannopyranoside* (**9**). — A solution of toluene-*p*-sulfonyl chloride (27 g, 142 mmol) in pyridine (50 mL) was added during 15 min to a solution of the diol **8**[9] (18.5 g, 79 mmol) in

pyridine (200 mL) with ice-cooling. The mixture was stirred for 5 h with a gradual rise of the temperature to ambient. Following the addition of benzoyl chloride (12 mL), the mixture was stirred overnight, poured into ice-cold aqueous sodium hydrogencarbonate, and stirred for 2 h. The crystalline precipitate was collected, and its solution in chloroform (700 mL) was washed with water, M HCl, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. Crystallisation of the residue from ethanol gave **9** (31.8 g, 81.5%), m.p. 113–114°, $[\alpha]_D$ +40° (*c* 1.2). The n.m.r. data are listed in Tables I and II.

*Anal.* Calc. for $C_{24}H_{28}O_9S$: C, 58.52; H, 5.73. Found: C, 58.85; H, 5.80.

*Methyl 4-O-benzoyl-6-deoxy-6-iodo-2,3-O-isopropylidene-α-D-mannopyranoside* (**10**). — A stirred mixture of **9** (33 g, 67 mmol) and anhydrous sodium iodide (40 g, 266 mmol) in acetonitrile (450 mL) was boiled under reflux for 5 h and then concentrated to dryness. A suspension of the residue in chloroform (0.5 L) was washed with water and the organic layer was concentrated to give **10** (30.1 g, ~100%), m.p. 159–161° (from ethanol), $[\alpha]_D$ +10.5° (*c* 1.85). The n.m.r. data are listed in Tables I and II.

*Anal.* Calc. for $C_{17}H_{21}IO_6$: C, 45.55; H, 4.72. Found: C, 45.47; H, 5.11.

*Methyl 4-O-benzoyl-2,3-O-isopropylidene-α-D-rhamnopyranoside* (**11**). — Hydrogen was bubbled through a mixture of **10** (30.1 g, 67 mmol), Raney nickel (15 mL), and triethylamine (19 mL) in methanol (600 mL) for 5 h. The catalyst was collected and washed with methanol, and the combined filtrate and washings were concentrated to dryness. A solution of the residue in chloroform (400 mL) was washed with M HCl, aqueous sodium hydrogencarbonate, and water, then concentrated. The residue was crystallised from methanol to give **11** (17.6 g, 81%), m.p. 99–101°, $[\alpha]_D$ +4.6° (*c* 3); lit.[14] for the L isomer, m.p. 101–102°, $[\alpha]_D$ −3.1°. The n.m.r. data are listed in Tables I and II.

*Methyl 4-O-benzoyl-α-D-rhamnopyranoside* (**12**). — To a solution of **11** (6 g, 18.5 mmol) in chloroform (75 mL) was added aqueous 90% trifluoroacetic acid (6 mL), and the mixture was kept at ambient temperature for 2 h, then washed with water, aqueous sodium hydrogencarbonate, and water, and concentrated to dryness to give **12** as a syrup that crystallised slowly and was used without purification. Crystallisation of a portion from ethyl acetate–hexane afforded **12** with m.p. 112–114°, $[\alpha]_D$ +97° (*c* 1.6); lit.[14] for the syrupy L isomer, $[\alpha]_D$ −82.3°. The n.m.r. data are listed in Tables I and II.

*Methyl 3,4-di-O-benzoyl-α-D-rhamnopyranoside* (**13**). — To a stirred solution of **12** (5.1 g, 18 mmol) in dichloromethane (25 mL) and pyridine (10 mL) at 0–5° was added dropwise a solution of benzoyl chloride (2.3 mL, 19.8 mmol) in dichloromethane (10 mL) during 15 min. The mixture was worked-up conventionally to give, after crystallisation from ethyl acetate–hexane, **13** (3.45 g). Chromatography of the mother liquor afforded more **13** (total yield, 5.37 g, 77.5%), m.p. 159–163°, $[\alpha]_D$ −27° (*c* 1.9); lit.[7] for the L isomer, m.p. 155–160°, $[\alpha]_D$ +26°. The n.m.r. data are listed in Tables I and II.

*1,2,3-Tri-O-acetyl-4-O-benzoyl-α-D-rhamnopyranose* (**14**). — To a solution of

**12** (prepared from 9.66 g of **11**) in acetic anhydride (30 mL), at 5–7°, was added a solution of conc. sulfuric acid (0.5 mL) in acetic anhydride (16 mL). After 2 h, the mixture was poured into ice–water and stirred for 3 h, the precipitate (partially solidified) was separated, and a solution in chloroform (300 mL) was washed with water, aqueous sodium hydrogencarbonate, and water, then concentrated to afford syrupy **14** (11.3 g, 95.5%) which was used without purification. Crystallisation of a portion from ethyl acetate–heptane gave **14** with m.p. 113–115°, $[\alpha]_D$ +28° (*c* 1.6); lit.[7] for the L isomer, m.p. 115–117°, $[\alpha]_D$ −28°. The n.m.r. data are listed in Tables I and II.

*3-O-Acetyl-4-O-benzoyl-1,2-O-[1-(exo- and* endo*-cyano)ethylidene]-β-*D*-rhamnopyranose* (**16** *and* **17**). — To a solution of the glycosyl bromide **15**, obtained from **14** (6.55 g, 14 mmol), in acetonitrile (40 mL) was added sodium cyanide (3.5 g, 70 mmol). The mixture was stirred for 72 h at 20°, filtered through Celite, diluted with chloroform (500 mL), washed with water, and concentrated. Column chromatography of the residue gave the *exo*-isomer **16** (3.4 g, 67%) and the *endo*-isomer **17** (0.88 g, 17%).

Compound **16** had m.p. 126–128° (from ethyl acetate–hexane), $[\alpha]_D$ −44° (*c* 2.2); lit.[7] for the L isomer, m.p. 115–117°, $[\alpha]_D$ +47.5°.

Compound **17** had m.p. 173–176° (from ethyl acetate–hexane), $[\alpha]_D$ −129° (*c* 3.5); lit.[7] for the L isomer, m.p. 180°, $[\alpha]_D$ +116.7°. The n.m.r. data for **16** and **17** are listed in Tables I and II.

*4-O-Benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-β-*D*-rhamnopyranose* (**18**). — To a solution of **16** (3.4 g, 9.4 mmol) in chloroform (10 mL) and methanol (40 mL) was added, with cooling, acetyl chloride (1.9 mL), and the mixture was kept at 20° for 2 h 40 min. The mixture was neutralised with aqueous 10% sodium acetate, concentrated to one-third volume, and diluted with chloroform, and the organic layer was washed with aqueous sodium hydrogencarbonate and water, then concentrated. Crystallisation of the residue from ethyl acetate–heptane gave **18** (1.92 g). Chromatography of the mother liquor afforded more **18** (total yield, 2.44 g, 81%), m.p. 151–153°, $[\alpha]_D$ +15° (*c* 2.2); lit.[7] for the L isomer, m.p. 155–157°, $[\alpha]_D$ −13.2°. The n.m.r. data are listed in Tables I and II.

*Methyl 2,4-di-O-benzoyl-α-*D*-rhamnopyranoside* (**20**). — To a solution of **12**, prepared from **11** (4.18 g, 13 mmol), in acetonitrile (20 mL) was added triethyl orthobenzoate (4.35 mL, 19.5 mmol) and toluene-*p*-sulfonic acid monohydrate (50 mg). After 16 h, pyridine (2 mL) was added, the solvent was evaporated, and a solution of the resulting orthoester **19** in aqueous 80% acetic acid was stored at 20° for 30 min, then concentrated. A solution of the residue in chloroform (150 mL) was washed with water, then concentrated. Column chromatography of the residue afforded **20** (3.90 g, 78%), isolated as a syrup, $[\alpha]_D$ −59° (*c* 3.2); lit.[10] for the L isomer, $[\alpha]_D$ +63.2°. The n.m.r. data are listed in Tables I and II.

*1,3-Di-O-acetyl-2,4-di-O-benzoyl-α,β-*D*-rhamnopyranose* (**21**). — Acetolysis of **20** (7.5 g, 19.4 mmol), as described for preparation of **14**, gave **21** (7.87 g, 89%), isolated as a syrup, $[\alpha]_D$ −67° (*c* 1.8); lit.[4] for the L isomer, $[\alpha]_D$ +51.3°. The n.m.r. data are listed in Tables I and II.

*Methyl 2-O-(3-O-acetyl-2,4-di-O-benzoyl-α-D-rhamnopyranosyl)-3,4-di-O-benzoyl-α-D-rhamnopyranoside* (**23**). — A mixture of **13** (2.66 g, 6.9 mmol) and mercuric cyanide (2.27 g, 9 mmol) was dried at $2 \times 10^{-3}$ mmHg for 4 h. A solution of the bromide **22**, prepared from **21** (4.11 g, 9 mmol), in benzene (25 mL) was lyophilised followed by drying for 2 h. A solution of **22** in acetonitrile (25 mL) was added[15] dropwise to a suspension of **13** and mercuric cyanide in acetonitrile (10 mL) under argon. After 16 h, the mixture was concentrated, chloroform (200 mL) was added to the residue, and the solution was washed with aqueous potassium bromide and water, then concentrated. Column chromatography of the residue gave an 8:1 mixture of **23** and **24** which was used without purification. A pure sample of **23**, obtained by preparative h.p.l.c. on "Silasorb 600" (10 μ) using 95:5 benzene–ethyl acetate, was amorphous and had $[\alpha]_D$ −102° (*c* 1.2). N.m.r. data: $^1$H, δ 1.35 (d, 3 H, $J_{6,5}$ 6.2 Hz), 1.42 (d, 3 H, $J_{6,5}$ 6.2 Hz, H-6,6'), 1.90 (s, 3 H, Ac), 3.50 (s, 3 H, OMe), 4.12 (dq, 1 H, H-5'), 4.27 (dq, 1 H, H-5), 4.31 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.88 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.10 (bs, 1 H, H-1'), 5.50 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, H-4'), 5.65 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 5.75–5.81 (m, 3 H, H-2',3,3'); $^{13}$C, δ 99.9 (C-1'), 99.5 (C-1), 76.5 (C-2), 71.9 (C-4,4'), 71.2 (C-3), 70.5 (C-2'), 69.0 (C-3'), 67.7 (C-5'), 66.9 (C-5), 17.7, 17.8 (C-6, C-6'), 55.2 (OMe), 20.7 ($CH_3CO$). The $^1$H-n.m.r. data for **24** are given in Table I.

*1-O-Acetyl-2-O-(3-O-acetyl-2,4-di-O-benzoyl-α-D-rhamnopyranosyl)-3,4-di-O-benzoyl-α-D-rhamnopyranose* (**25**). — To a solution of the mixture of **23** and **24** (9.3 mmol of **23**) in acetic anhydride (40 mL) was added a solution of conc. sulfuric acid (0.4 mL) in acetic anhydride (40 mL) at 0°. The mixture was kept for 1.5 h at 0° and 2 h at room temperature, poured into ice–water, and stirred for 3 h. The crystalline precipitate was separated, washed with water, dried *in vacuo*, and recrystallised from ethanol to give **25** (6.29 g, 83%), m.p. 115–120°, $[\alpha]_D$ −89° (*c* 1.3). N.m.r. data: $^1$H, δ 1.35 (d, 3 H, $J_{6,5}$ 6.2 Hz), 1.41 (d, 3 H, $J_{6,5}$ 6.3 Hz, H-6,6'), 1.90, 2.25 (2 s, each 3 H, 2 Ac), 4.22 (dq, 1 H, H-5'), 4.28 (dq, 1 H, H-5), 4.31 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 5.11 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 5.50 (ddd, 1 H, $J_{4',2'}$ 1.4, $J_{4',3'}$ 11.2, $J_{4',5'}$ 9.5 Hz, H-4'), 5.69 (t, 1 H, $J_{3,4} = J_{4,5}$ = 10.0 Hz, H-4), 5.72–5.82 (m, 3 H, H-2',3,3'), 6.27 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1); $^{13}$C, δ 99.5 (C-1'), 92.3 (C-1), 74.8 (C-2), 71.8, 71.4 (C-4,4'), 70.8 (C-3), 70.4 (C-2), 69.6 (C-3'), 68.9 (C-5), 68.0 (C-5'), 17.5, 17.7 (C-6, C-6'), 20.7, 21.1 (2 $CH_3CO$).

*Anal.* Calc. for $C_{44}H_{42}O_{15}$: C, 65.18; H, 5.22. Found: C, 65.10; H, 5.30.

*O-(3-O-Acetyl-2,4-di-O-benzoyl-α-D-rhamnopyranosyl)-(1→2)-O-(3,4-di-O-benzoyl-α- and -β-D-rhamnopyranosyl)-(1→3)-4-O-benzoyl-1,2-O-[1-(exo-cyano)-ethylidene]-β-D-rhamnopyranose* (**27** *and* **28**). — To a stirred solution of **18** (1.90 g, 5.95 mmol), mercuric cyanide (1.50 g, 5.95 mmol), and mercuric bromide (1.08 g, 3 mmol) in acetonitrile (15 mL) was added a solution of the glycosyl bromide **26**, prepared from **25** (4.83 g, 5.95 mmol), in acetonitrile (30 mL). The reagents were dried in the same manner as described for the synthesis of **23**. After 16 h, the mixture was worked-up as usual. Column chromatography afforded amorphous **27** (3.58 g, 56%), $[\alpha]_D$ −111.5° (*c* 1.4), and amorphous **28** (1.26 g, 20%), $[\alpha]_D$ −143.5° (*c* 1.9). The n.m.r. data are listed in Tables III and IV.

*O-(2,4-Di-O-benzoyl-α-D-rhamnopyranosyl)-(1→2)-O-(3,4-di-O-benzoyl-α-D-rhamnopyranosyl)-(1→3)-4-O-benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-β-D-rhamnopyranose* (**29**). — To a solution of **27** (3.69 g, 3.45 mmol) in chloroform (7 mL) was added methanol (20 mL) and acetyl chloride (0.8 mL). The mixture was kept for 4 h at room temperature, then neutralised with aqueous sodium hydrogencarbonate, and extracted with chloroform (3 × 30 mL). The combined extracts were washed with water and concentrated, and the residue was subjected to column chromatography, to yield **27** (0.86 g, 23%), amorphous **29** (2.45 g, 69%), $[\alpha]_D$ −99° (*c* 2.2), and amorphous **30** (230 mg, 6%), $[\alpha]_D$ −100° (*c* 2.4). The n.m.r. data for **29** and **30** are listed in Tables III and IV.

TABLE III

$^1$H-N.M.R. DATA OF TRISACCHARIDE DERIVATIVES (δ IN P.P.M., *J* IN Hz)

| *Residue* | *Atom* | *Compound* | | | | |
|---|---|---|---|---|---|---|
| | | **27** | **28** | **29** | **30** | **3** |
| Rha A | H-1 | 5.49 d | 5.47 d | 5.48 d | 5.46 d | 5.44 d |
| | ($J_{1,2}$) | (2.2) | (2.2) | (2.2) | (2.2) | (2.2) |
| | H-2 | 4.71 dd | 4.66 dd | 4.70 dd | 4.69 dd | 4.68 dd |
| | ($J_{2,3}$) | (4.1) | (4.2) | (4.0) | (4.2) | (4.1) |
| | H-3 | 4.22 dd | 4.38 dd | 4.19 dd | 4.13 dd | 4.08 dd |
| | ($J_{3,4}$) | (9.8) | (8.4) | (9.7) | (10.0) | (9.8) |
| | H-4 | 5.36 t | 5.21 t | 5.34 t | 5.39 t | 5.28 t |
| | ($J_{4,5}$) | (9.8) | (8.4) | (9.7) | (10.0) | (9.8) |
| | H-5 | 3.70 dq | 3.83 dq | 3.71 dq | 3.67 dq | 3.66 dq |
| | ($J_{5,6}$) | (6.2) | (5.5) | (6.2) | (6.2) | (6.3) |
| | H-6 | 1.31 d | 1.36 d | 1.40 d | 1.33 d | 1.29 d |
| | C-$CH_3$ | 2.06 s | 1.80 s | 2.05 s | 1.89 s | 2.05 s |
| Rha B | H-1 | 5.19 d | 4.86 d | 5.18 d | 5.16 d | 4.94 d |
| | ($J_{1,2}$) | (1.6) | (0.9) | (1.6) | (1.7) | (1.4) |
| | H-2 | 4.04 dd | 4.47 dd | 3.99 dd | 4.01 dd | 3.77 dd |
| | ($J_{2,3}$) | (3.3) | (2.8) | (3.2) | (3.4) | (3.5) |
| | H-3 | 5.77 dd | 5.50 dd | 5.74 dd | 5.78 dd | 5.71 dd |
| | ($J_{3,4}$) | (10.0) | (10.0) | (10.0) | (10.0) | (10.2) |
| | H-4 | 5.62 t | 5.34 t | 5.55 t | 5.54 t | 5.36 t |
| | ($J_{4,5}$) | (10.0) | (10.0) | (10.0) | (10.0) | (10.2) |
| | H-5 | 4.35 dq | 3.59 dq | 4.34 dq | 4.36 dq | 4.29 dq |
| | ($J_{5,6}$) | (6.3) | (5.5) | (6.3) | (6.3) | (6.4) |
| | H-6 | 1.43 d | 0.81 d | 1.31 d | 1.31 d | 1.42 d |
| Rha C | H-1 | 4.72 d | 5.06 d | 4.69 d | 4.70 d | 4.59 d |
| | ($J_{1,2}$) | (1.5) | (1.5) | (1.7) | (1.7) | (1.9) |
| | H-2 | 5.60 dd | 5.86 dd | 5.43 dd | 5.45 dd | 4.43 dd |
| | ($J_{2,3}$) | (3.3) | (3.5) | (3.5) | (3.5) | (3.1) |
| | H-3 | 5.69 dd | 5.90 dd | 4.41 m | 4.43 dd | 4.22 dd |
| | ($J_{3,4}$) | (10.0) | (9.6) | (9.8) | (10.0) | (9.9) |
| | H-4 | 5.41 t | 5.46 t | 5.24 t | 5.24 t | 5.74 t |
| | ($J_{4,5}$) | (10.0) | (9.6) | (9.8) | (10.0) | (9.9) |
| | H-5 | 4.06 dq | 4.86 dq | 4.10 dq | 4.08 dq | 3.77 dq |
| | ($J_{5,6}$) | (6.2) | (5.5) | (6.1) | (6.3) | (6.3) |
| | H-6 | 1.09 d | 1.32 d | 1.15 d | 1.12 d | 1.05 d |

TABLE IV

$^{13}$C-N.M.R. DATA OF TRISACCHARIDE DERIVATIVES ($\delta$ IN P.P.M.)[a]

| *Residue* | *Atom* | *Compound* | | | | |
|---|---|---|---|---|---|---|
| | | **27** | **28** | **29** | **30** | **3**[b] |
| Rha A | C-1 | 97.1 | 97.2 | 97.1 | 97.4 | 96.9 |
| | C-2 | 80.4 | 79.0 | 80.4 | 80.1 | 80.4 |
| | C-3 | 78.0 | 73.5 | 78.3 | 78.7 | 78.9 |
| | C-4 | 72.0 | 71.2 | 72.1 | 72.4 | 71.8 |
| | C-5 | 70.1 | 70.2 | 70.4 | 70.0 | 70.2 |
| | C-6 | 17.5 | 17.7 | 17.6 | 17.6 | 17.7 |
| Rha B | C-1 | 101.9 | 97.9 | 102.1 | 101.9 | 102.4 |
| | C-2 | 77.1 | 75.3 | 77.8 | 77.8 | 77.6 |
| | C-3 | 70.5 | 73.5 | 70.5 | 70.6 | 72.7 |
| | C-4 | 71.5 | 71.8 | 71.8 | 72.0 | 72.2 |
| | C-5 | 68.1 | 71.6 | 68.1 | 67.7 | 68.0 |
| | C-6 | 17.7 | 16.9 | 17.7 | 17.7 | 17.7 |
| Rha C | C-1 | 99.4 | 98.8 | 99.6 | 99.5 | 99.6 |
| | C-2 | 70.3 | 70.6 | 72.9 | 72.9 | 71.9 |
| | C-3 | 68.8 | 69.6 | 68.7 | 68.7 | 70.0 |
| | C-4 | 71.7 | 72.2 | 75.2 | 75.2 | 70.0 |
| | C-5 | 67.7 | 67.0 | 67.3 | 67.2 | 67.7 |
| | C-6 | 17.7 | 17.4 | 17.7 | 17.9 | 17.9 |
| | $CH_3$ | 26.5 | 26.4 | 26.5 | 23.6 | 26.6 |
| | C | 101.8 | 101.6 | 101.9 | 107.9 | 101.9 |
| | CN ($COOCH_3$) | 117.0 | 116.9 | 117.0 | 52.5 | 117.1 |

[a]Assignment of signals which differ by <0.5 p.p.m. may be interchanged. [b]Assigned using selective heteronuclear $^{13}C\{^1H\}$ resonance.

Repeated treatment of the recovered **27** gave more **29** (0.57 g; total yield, 3.02 g, 85%).

*O-(2,4-Di-O-benzoyl-3-O-trityl-α-D-rhamnopyranosyl)-(1→2)-O-(3,4-di-O-benzoyl-α-D-rhamnopyranosyl)-(1→3)-4-O-benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-β-D-rhamnopyranose* (**3**). — To a solution of **29** (2.11 g, 2.05 mmol) and 2,4,6-collidine (0.37 mL, 2.8 mmol) in dichloromethane (25 mL) was added portionwise triphenylmethylium perchlorate (700 mg, 2.05 mmol) during 2 h. The mixture was then diluted with chloroform (100 mL), washed with water, and concentrated. Column chromatography of the residue gave **29** (0.7 g, 33%), amorphous **3** (1.59 g, 61%), $[\alpha]_D$ −61° (*c* 2), and amorphous **31** (110 mg, 3.6%), $[\alpha]_D$ −30° (*c* 1.4). The n.m.r. data for **3** are listed in Tables III and IV.

Tritylation of the recovered **29** yielded more **3** (0.44 g; total yield, 2.03 g, 78%).

*6-Phthalimidohexyl 3-O-acetyl-2,4-di-O-benzoyl-α-D-rhamnopyranoside* (**32**). — A mixture of 6-phthalimidohexanol (140 mg, 0.67 mmol), mercuric cyanide (170

mg, 0.67 mmol), mercuric bromide (130 mg, 0.36 mmol), and molecular sieves 3A (1 g) in dichloromethane (10 mL) was stirred for 2 h. A solution of the glycosyl bromide **22**, prepared from **21** (310 mg, 0.68 mmol), in dichloromethane (5 mL) was added. The mixture was stirred for 2 h, filtered through Celite, washed with aqueous potassium bromide and water, and concentrated. Column chromatography of the residue gave amorphous **32** (360 mg, 98%), $[\alpha]_D$ −48° (*c* 2.5); lit.[4] for the L isomer, $[\alpha]_D$ +49.7°. The n.m.r. data are listed in Tables I and II.

*6-Phthalimidohexyl 2,4-di-*O*-benzoyl-α-*D*-rhamnopyranoside* (**33**). — A solution of **32** (360 mg, 0.56 mmol) in chloroform (1 mL) and methanol (2.5 mL) was treated with acetyl chloride (0.11 mL) for 5 h at 20°. After the usual work-up and column chromatography, amorphous **33** (330 mg, 98%) was obtained, $[\alpha]_D$ −33° (*c* 2.6); lit.[4] for the L isomer, $[\alpha]_D$ +31.4°. The n.m.r. data are listed in Tables I and II.

*6-Phthalimidohexyl 2,4-di-*O*-benzoyl-3-*O*-trityl-α-*D*-rhamnopyranoside* (**34**). — A solution of **33** (200 mg, 0.33 mmol) in dichloromethane (4 mL) was treated with triphenylmethylium perchlorate (220 mg, 0.67 mmol) in the presence of 2,4,6-collidine (96 μL, 0.73 mmol). The mixture was diluted with chloroform (100 mL), washed with water, and concentrated. Column chromatography of the residue gave amorphous **34** (250 mg, 89%), $[\alpha]_D$ −21° (*c* 2.6). The n.m.r. data are listed in Tables I and II.

*6-Phthalimidohexyl O-(3-*O*-acetyl-2,4-di-*O*-benzoyl-α-*D*-rhamnopyranosyl)-(1→2)-*O*-(3,4-di-*O*-benzoyl-α-*D*-rhamnopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4-*O*-benzoyl-α-*D*-rhamnopyranosyl)-(1→3)-2,4-di-*O*-benzoyl-α-*D*-rhamnopyranoside* (**35**). — Using the vacuum technique (see below), **34** (200 mg, 0.237 mmol) was condensed with **27** (250 mg, 0.234 mmol) in dichloromethane (3 mL) in the presence of triphenylmethylium perchlorate (8 mg, 0.023 mmol) for 18 h. Pyridine (1 drop) and chloroform (50 mL) were added, and the mixture was washed with water and concentrated. Column chromatography of the residue yielded **35** (340 mg, 87%), m.p. 176–178° (from ethanol), $[\alpha]_D$ −96° (*c* 2.1). $^{13}$C-N.m.r. data: δ 97.4 ($J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1), 99.1 ($J_{C\text{-}1,H\text{-}1}$ 168 Hz), 99.7 ($J_{C\text{-}1,H\text{-}1}$ 168 Hz), 99.9 ($J_{C\text{-}1,H\text{-}1}$ 173 Hz) (C-1′,1″,1‴).

*6-Aminohexyl O-α-*D*-rhamnopyranosyl-(1→2)-O-α-*D*-rhamnopyranosyl-(1→3)-O-α-*D*-rhamnopyranosyl-(1→3)-α-*D*-rhamnopyranoside* (**36**). — A mixture of **35** (200 mg, 0.12 mmol) and hydrazine hydrate (0.5 mL) in ethanol (5 mL) was boiled under reflux for 5 h, then concentrated. Excess of hydrazine hydrate was co-evaporated with 1-butanol from the residue which was then treated with methanolic 0.5M sodium methoxide (3 mL) for 3 h at 40–45°. The mixture was neutralised with acetic acid and concentrated. Gel chromatography of the residue on column *B* gave fraction 1 (20 mg), retention vol. 200 mL, $[\alpha]_D$ +89° (*c* 1.1, methanol); and fraction 2 (40 mg), retention vol. 230 mL, $[\alpha]_D$ +87° (*c* 2.1, methanol). Fractions 1 and 2 had identical $^{13}$C-n.m.r. spectra: δ 103.5 (C-1′,1‴), 102.1 (C-1″), 100.9 (C-1), 79.5, 79.1 (C-3,3′,2″), 73.4, 73.3, 72.9, 72.6 (C-4,4′,4″,4‴), 71.3 (C-2,2′,3″,2‴,3‴), 70.4 (C-5′,5″,5‴), 70.1 (C-5), 69.0 ($CH_2O$), 40.7 ($CH_2N$), 29.6, 27.9, 26.7, 26.2 (4 $CH_2$), 17.9 (C-6,6′,6″,6‴).

*α*-D-*Rhamnans* **37** *and* **38**. — In one limb of a tuning-fork-shaped (⅄) tube was placed a solution of **34** (79 mg, 0.094 mmol) in benzene (1 mL), in the other a solution of triphenylmethylium perchlorate (32 mg, 0.094 mmol) in nitromethane (0.4 mL), and the solutions were lyophilised. Benzene (1 mL) was distilled to the first limb and the remainder was lyophilised. In a separate flask, **3** (1.193 g, 0.94 mmol) was twice lyophilised from benzene (5 mL). Dichloromethane (1 and 5 mL, respectively) was distilled into the reaction tube and into the flask with **3** (lyophilisation of the reactants, as well as distillation from $CaH_2$ of benzene and dichloromethane, was carried out at $10^{-3}$ mmHg). The tube was filled with dry argon and sealed with a septum, and the solutions of **34** and catalyst were mixed. Using a syringe, the solution of **3** was introduced portionwise (0.5 mL in each 2 h) into the above mixture, and the solution was kept for 18 h at room temperature. Trifluoroacetic acid (90%, 2 mL) was added; after 0.5 h, the mixture was diluted with chloroform (100 mL), washed with water and aqueous sodium hydrogencarbonate, and concentrated. Column chromatography (benzene → 2:3 ethyl acetate–benzene) of the residue gave fractions that did not contain TrCN and TrOH and which were combined and concentrated. The residue was treated with hydrazine hydrate (2 mL) in boiling ethanol (20 mL) for 9 h, the solvent was evaporated, and an excess of hydrazine hydrate was co-evaporated with 1-butanol from the residue which was then treated with methanolic 0.2M sodium methoxide for 5 h at 40–45°. The mixture was neutralised with acetic acid and concentrated. Gel chromatography of the residue on column *A* separated the carbohydrate and non-carbohydrate products. The mixture of **37** and **38** in 0.1M acetic acid (8 mL) was applied onto a column of Dowex 50-X2 ($H^+$) resin (5 mL) which was eluted with water (200 mL) to give, after concentration, the neutral polysaccharide **38** (272 mg, 66%). Gel chromatography on column *B* then gave the high-molecular-weight fraction (66 mg, 16%) of **38** (elution interval from 120–140 mL), $[\alpha]_D$ +92° (*c* 2.2, water); lit. for the rhamnans of *Ps. aeruginosa* and *Ps. cerasi*, $[\alpha]_D$ +82° and +89.1°[12].

The resin column was eluted with M ammonia (200 mL) and the eluate was concentrated to give the basic polysaccharide **37** (135 mg, 31%). Fractionation of **37** on column *B* afforded the high-molecular-weight fraction (35 mg, 8%) of **37** (elution interval from 135–160 mL), $[\alpha]_D$ +89.5° (*c* 1.8, water). $^{13}$C-N.m.r. data: **38**, *δ* 103.5 (C-1C), 103.3 (C-1A), 102.1 (C-1B), 79.4, 79.1 (C-3A,2B,3C), 73.5 (C-4B), 72.9 (C-4C), 72.6 (C-4A), 71.2 (C-2A,3B,2C), 70.6 (C-5B), 70.5 (C-5A,5C), 17.9 (C-6A,6B, 6C). The $^{13}$C-n.m.r. spectrum of **37** (Fig. 1) contained signals for the 6-aminohexyl rhamnoside moiety at *δ* 100.9 (C-1), 70.0 (C-5), 69.1 ($CH_2O$), 40.7 ($CH_2N$), 29.5, 27.9, 26.6, 26.2 (4 $CH_2$).

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