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SYNTHESIS OF PART OF THE ANTIGENIC REPEATING-UNIT OF *Streptococcus pneumoniae* TYPE II*

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ABSTRACT

Condensation of methyl 4-*O*-acetyl-3-*O*-(2 3 4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride gave a mixture of methyl *O*-[2,3,4,6-tetra-*O*-benzyl-α- (**4**) and -β-D-glucopyranosyl]-(1→2)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)]-4-*O*-acetyl-α-L-rhamnopyranoside (**9**) in 43 7 proportion in 63% yield After chromatographic separation removal of the benzyl and acetyl groups gave methyl *O*-α-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranoside and the β anomer Removal of benzyl groups of **4** was followed by tritylation, acetylation, and detritylation of the α-D-glucopyranosyl group, and finally condensation with benzyl (2,3,4-tri-*O*-benzyl-D-glucopyranosyl chloride)uronate gave a mixture of two tetrasaccharides (**15** and **16**), containing the α- and β-D-glucopyranosyluronic acid groups in the ratio 81 19, and an overall yield of 71% After chromatographic separation, alkaline hydrolysis and hydrogenation of **15** gave methyl *O*-α-D-glucopyranosyluronic acid-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranoside The β-D anomer was obtained by similar treatment of **16** 6-*O*-α-D-Glucopyranosyluronic acid-α,β-D-glucopyranose was synthesized as a model compound

INTRODUCTION

The availability of an antibody of restricted heterogeneity would be of great advantage for the study of antigen–antibody interaction Such antibodies may be induced with an artificial antigen of well-defined chemical structure[1,2] In addition, study of the conformation of the artificial antigen in solution may provide valuable information on the chemical nature of the antigen–antibody interaction

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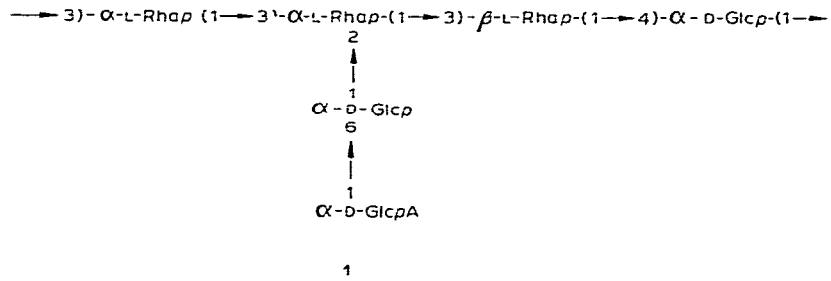
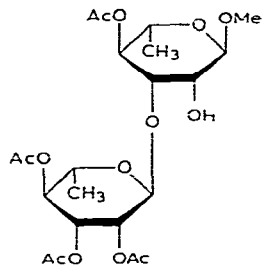
Capsular polysaccharides as models for artificial antigens are of interest because their immunological properties have been extensively investigated[3,4]. Among them, the structure of the repeating unit (**1**) of the capsular polysaccharide of *Streptococcus pneumoniae*, type II (S2) has been established by Kenne *et al.*[5]. The immunodominant group of this antigenic polysaccharide is the α-D-glucopyranosyluronic acid group of the side-chain and our ultimate goal is the total synthesis of the repeating unit **1**. In this report we describe the synthesis of the trisaccharide, methyl *O*-(α-L-rhamnopyranosyl)-(1→3)-[*O*-α-D-glucopyranosyl)-(1→2)]-α-L-rhamnopyranoside (**5**) and of the tetrasaccharide, methyl *O*-(α-L-rhamnopyranosyl)-(1→3)-[*O*-(α-D-glucopyranosyluronic acid)-(1→6)-*O*-(α-D-glucopyranosyl)-(1→2)]-α-L-rhamnopyranoside (**16**), the side-chain of which inhibits 70% of the precipitation of antibodies. In previous communications, we have described the synthesis of a disaccharide[6] and reported briefly on a trisaccharide[7] that are parts of the repeating unit (**1**) of *S. pneumoniae* type II.



RESULTS AND DISCUSSION

A major problem in the synthesis of tetrasaccharide **16** is the presence of two glycosidic bonds in *cis* disposition[8] to OH-2. From the various possible routes, we selected the addition of an α-D-glucopyranosyl group to the partially protected disaccharide methyl 4-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**2**), followed by the addition of an α-D-glucopyranosyluronic acid group to the protected trisaccharide **8**. This route allows for a better understanding of the n.m.r. data of the various intermediates and, consequently, an easier assignment of the anomeric configurations of the oligosaccharides synthesized.

Condensation of methyl 4-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside[6] (**2**) with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride[9] (**3**) in ether–1,2-dimethoxyethane containing silver perchlorate catalyst gave a 4:3:7 mixture of methyl *O*-[2,3,4,6-tetra-*O*-benzyl-α- (**4**) and -β-D-glucopyranosyl]-(1→2)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)]-4-*O*-acetyl-α-L-rhamnopyranoside (**9**) in 63% yield. Compounds **4** and **9** were readily separated by column chromatography on silica gel. At this stage, the anomeric configuration of the D-glucopyranosyl group could only be determined by the mode of synthesis, which leads preferentially to α-D-glycosides, and by the optical rotations $[\alpha]_D$ +24.4°



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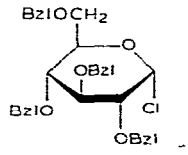

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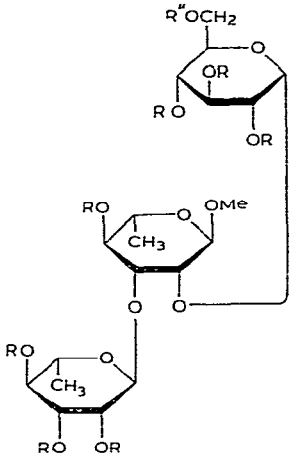
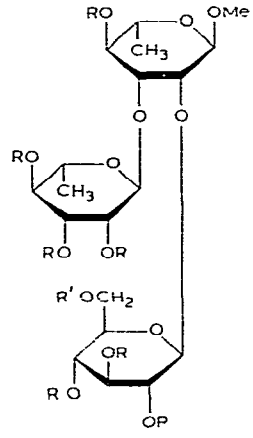


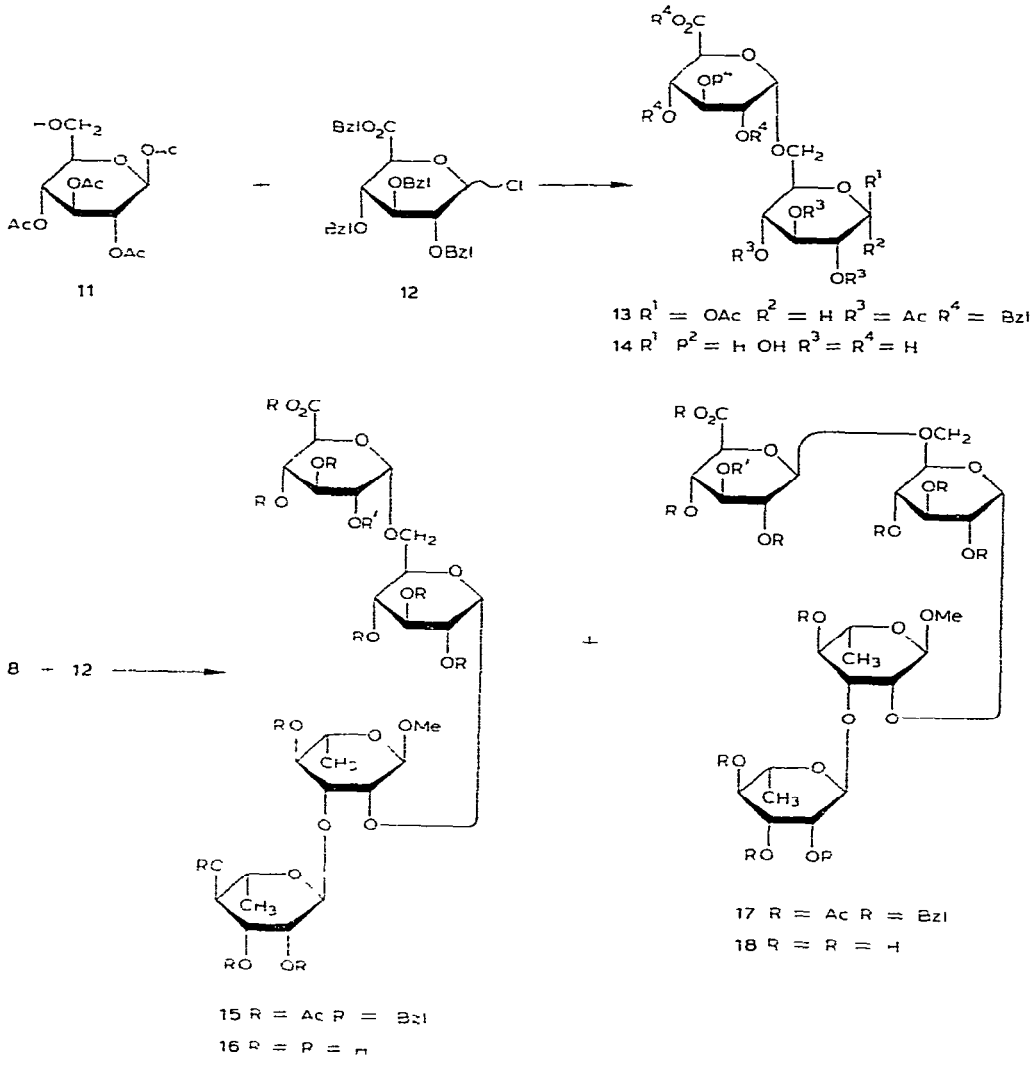
4 R = Ac R = R′ = Bzl
5 R = R′ = R″ = H
6 R = Ac R = R″ = H
7 R = R = Ac R′ = Tr
8 R = R = Ac R = H



9 R = Ac R = Bzl
10 R = R′ = H

for **4** and −13 2° for **9** Compounds **5** and **10** were obtained by hydrogenation of **4** and **9**, respectively, in methanol in the presence of palladium-on-charcoal, followed by treatment of the products with sodium methoxide The anomeric configuration of **5** and **10** was readily established on the basis of their rotations ($[\alpha]_D$ +15 3° for **5** and −31° for **10**) and by comparing their n m r data These were indicative of a *cis*-disposition of H-1 and H-2 of **5** owing to a coupling constant <4 Hz ($J_{1,2}$ 3 5 Hz)*, and a *trans*-disposition of H-1 and H-2 of **10**, the coupling constant being between 6 and 9 Hz ($J_{1,2}$ 7 8 Hz) The trisaccharide **8** having OH-6 free in the α-D-glucopyranosyl group was prepared by *O*-debenzylation of **4** to give **6**, selective

*By convention, H corresponds to the protons of the α-L-rhamnopyranosyl residue substituted at O-2 and O-3, H′ to those of the α-L-rhamnopyranosyl residue, and H″ and H‴ to those of the α-D-glucopyranosyl and α-D-glucopyranosyluronic acid residues, respectively



tritylation followed by acetylation to give **7**, and subsequent removal of the trityl group

In order to evaluate conditions for the synthesis of **15** and **17**, we prepared the disaccharide **13** as a model compound 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranose[10] (**11**) was dissolved in ether–1,2-dimethoxyethane containing silver perchlorate, and treated with benzyl (2,3,4-tri-*O*-benzyl-D-glucopyranosyl chloride)uronate (**12**) The condensation took place almost immediately and, after deprotection, the n m r spectrum of **14** showed that the α-D anomer had been formed preferentially, in 59% yield ($J_{1,2}$ <3.5 Hz) To our knowledge, the only published preparation[11] of isomaltouronic acid was accomplished by oxidation of isomaltose obtained from the acid reversion of D-glucose[12] The synthesis of the tetrasaccharides **15** and **17** was

performed in the manner just described, namely by adding chloride **12** to a solution of **8** in ether containing silver perchlorate as catalyst, and molecular sieves as acid acceptor. Both compounds, **15** and **17**, were separated by column chromatography and obtained in 81 : 19 proportion, in 72% overall yield based on **8**. They showed R_f values of 0.48 and 0.40, and $[\alpha]_D$ values of +51 and +39°, respectively. The anomeric configurations were definitely established on the basis of the n.m.r. data (for **16**, δ 5.07, $J_{1,2}$ 3.5 Hz; for **18**, δ 4.49, $J_{1,2}$ 7.5 Hz), and of the optical rotations ($[\sigma]_D$ +56° for **16** and +5° for **18**) for the deprotected tetrasaccharides. The stability of the chemical shift of the anomeric proton of the σ-D anomer of the glucoside moiety is also of interest: δ 5.04 for **5** and **16**, and 5.03 for **18**.

EXPERIMENTAL

General methods. — Evaporations were conducted *in vacuo* with the bath temperature kept below 40°. Analytical thin-layer chromatography (t.l.c.) was performed on precoated plates of silica gel (Merck 60 F-254) with solvents (v/v) (*A*) 1:1 ethyl acetate–hexane, (*B*) 2:1 ethyl acetate–hexane, (*C*) 1:1 ether–hexane, (*D*) 2:1 ether–hexane, (*E*) 2:1 diisopropyl ether–methanol, and (*F*) 3:3:2 2-propanol–ethyl acetate–water, and detection with the phosphomolybdic reagent; the R_F values were measured on 2.5 × 7.5-cm plates, thickness 0.25 mm. Column chromatography was performed on silica gel (Merck 60F-254, 70–230 mesh for dry column chromatography). N.m.r. spectra were recorded with Varian T-60 or Bruker HX-270 spectrometers with tetramethylsilane or acetone as internal standard; chemical shifts are given in δ values (Me_4Si signal as 0). I.r. spectra were recorded with a Perkin–Elmer Model 237 spectrophotometer. Melting points were determined with a Mettler FP-2 apparatus. The microanalyses were performed by Dr. W. Manser, Zurich, Switzerland, and Galbraith Laboratories, Inc., Knoxville, TN.

Methyl O-(2,3,4,6-tetra-O-benzyl-σ-D-glucopyranosyl)-(1→2)-O-[(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-(1→3)]-4-O-acetyl-α-L-rhamnopyranoside (**4**). — To a solution of methyl 4-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-σ-L-rhamnopyranosyl)-α-L-rhamnopyranoside[6] (**2**, 985 mg, 2 mmol) in dry 1,2-dimethoxyethane (8 mL) under nitrogen, was added a 0.1M solution of silver perchlorate in ether (35 mL) and powdered 4-Å molecular sieve (1 g). The mixture was stirred for 2–3 h, and then 2,3,4,6-tetra-*O*-benzyl-σ-D-glucopyranosyl chloride[9] (**3**, 1.68 g, 3 mmol) dissolved in ether (5 mL) was added dropwise in the dark. The progress of the reaction was monitored by t.l.c. and, after 15 h, the mixture was filtered on a Celite pad, the solvents were evaporated, and the resulting syrup was chromatographed on silica gel (500 g, solvent *A*). Two fractions having R_F values 0.39 and 0.44 (solvent *D*) corresponding to **4** and **9**, respectively, were recovered, in the ratio of 43:7 (overall yield 63%). The reaction was performed under rigorously anhydrous conditions in order to avoid formation of hydrolytic compounds from **3**, which may be eluted with **9**, none were observed by t.l.c. The fractions corresponding to **4** were evaporated to a syrup, $[\sigma]_D^{20}$ +2.4° (*c* 1.1, chloroform), t.l.c. (*D*) R_F 0.44, ν_{max}^{NaCl} 2940, 1750 (CO), 1370, and 1225 cm^{-1},

^{1}H-n m r (60 MHz $CDCl_3$) δ 7 50–6 95 (m, 20 H, Ph), 5 20–3 30 (m, 25 H), 3 21 (s, 3 H, OMe). 2 11, 2 09, 1 93 and 1 83 (4 s, 4 × 3 H, OAc) and 1 11 and 1 07 (2 d, 2 × 3 H, $J_{5,6} = J_{5',6'} = 5$ 6 Hz, H-6 and -6′)

Anal Calc for $C_{55}H_{66}O_{18}$ C, 65 08, H, 6 55, O, 28 37 Found C, 64 87, H, 6 47, O, 28 31

Methyl O-(*2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl*)-(*1→2*)-[O-(*2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl*)-(*1→3*)]-*4-O-acetyl-α-L-rhamnopyranoside* (**9**) — Compound **9** was obtained as described in the previous paragraph, amorphous, m p 49–50 5°, $[\alpha]_D^{20}$ −13 2° (*c* 1 3, chloroform), t l c (*D*) R_F 0 39, ν_{max}^{KBr} 2940, 1750 (CO), 1460, 1375, and 1230 cm^{-1}. ^{1}H-n m r (60 MHz $CDCl_3$) δ 7 50–7 05 (m 20 H, Ph), 5 53–3 4 (m, 25 H), 3 28 (s, 3 H, OMe), 2 17, 2 11, 1 95, and 1 86 (4 s, 4 × 3 H, OAc), and 1 12 and 1 05 (2 d, 2 × 3 H $J_{5,6} = J_{5',6'} = 6$ Hz, H-6 and -6′)

Anal Calc. for $C_{55}H_{66}O_{18}$ C, 65 08, H, 6 55, O, 28 37 Found C, 65 10, H 6 61, O, 28 24

Methyl O-*α-D-glucopyranosyl*-(*1→2*)-O-[*α-L-rhamnopyranosyl*-(*1→3*)]-*α-L-rhamnopyranoside* (**5**) — Compound **4** (100 mg, 0 98 mmol) was treated with 0 1M sodium methoxide (10 mL) for 10 h at room temperature The solution was passed through a column of Dowex 50 (H^+), and then hydrogenated in the presence of 10% palladium-on-charcoal (60 mg, Fluka) for 5 h at 3 atm. The catalyst was filtered off, and the solution evaporated to give an amorphous powder, m p 157–158 5°, $[\alpha]_D^{20}$ +15 3° (*c* 1 0, water), t l.c. (*F*). R_F 0 55. ν_{max}^{KBr} 3400 (OH), 1455, 1395, 1140, and 1060 cm^{-1}, ^{1}H-n m r (270 MHz, D_2O) δ 5 14 (s, 1 H, H-1), 5 04 (d, 1 H, $J_{1,2}$ 3 5 Hz, H-1″), 4 85 (s, 1 H, H-1′), 3 98–3 39 (m, 14 H), 3 41 (s, 3 H, OMe), and 1 31 and 1 28 (2 d, 2 × 3 H, $J_{5,6} = J_{5',6'} \simeq 5$ 8 Hz, H-6 and -6′)

Anal Calc for $C_{19}H_{34}O_{14}$ 1 5 H_2O C, 44 44, H, 7 26 Found C, 44 23, H, 7 09

Methyl O-*β-D-glucopyranosyl*-(*1→2*)-O-[*α-L-rhamnopyranosyl*-(*1→3*)]-*α-L-rhamnopyranoside* (**10**) — Compound **10** was obtained in amorphous form as described for **5**, m p 156–158°, $[\alpha]_D^{20}$ −31° (*c* 0 9, water), t l c (*F*) R_F 0 57, ν_{max}^{KBr} 3400 (OH), 1640 1075, and 980 cm^{-1}, ^{1}H-n m r (270 MHz, D_2O). δ 5 05 (d, 1 H, $J_{1,2}$ 1 16 Hz, H-1), 4 96 (d, 1 H, $J_{1,2}$ 1 16 Hz, H-1′), 4 58 (d, 1 H, $J_{1,2}$ 7 8 Hz, H-1″), 4 07–3 32 (m, 14 H), 3 41 (s, 3 H, OMe), and 1 3 (d, 2 × 3 H, $J_{5,6} = J_{5',6'} =$ 5 5 Hz)

Anal Calc for $C_{19}H_{34}O_{14}$ 0 75 H_2O C, 45 64, H, 7 15 Found C, 45 86, H, 7 14

Methyl O-*α-D-glucopyranosyl*-(*1→2*)-O-[(*2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl*)-(*1→3*)-*4-O-acetyl-α-L-rhamnopyranoside* (**6**) — Compound **4** (600 mg, 0 59 mmol) was dissolved in methanol (25 mL) and hydrogenated in the presence of 10% palladium-on-charcoal (600 mg) for 4 h at 3 atm The catalyst was filtered off and the methanol evaporated to give **6** (375 mg, 9%), which was crystallized from 2-propanol–ethyl acetate, m p 111–112°, $[\alpha]_D^{20}$ +18 6° (*c* 1 0, methanol), t l c (*F*) R_F 0 81, ν_{max}^{KBr} 3450 (OH), 1750 (CO), 1377, 1225, and 1050 cm^{-1}, ^{1}H-n m r (60 MHz, $CDCl_3$) δ 5 30 –3 45 (m, 17 H). 3.32 (s 3 H, OMe), 2 1, 2 03, and 2 0 (3 s, 6 H, and

2 × 3 H, OAc), and 1 21 and 1 08 (2 d, 2 × 3 H, $J_{5,6} = J_{5',6'} \simeq 5$ Hz, H-6 and -6')

Anal Calc for $C_{27}H_{42}O_{18}$ C, 49 54, H, 6 47 Found C, 49 26, H, 6 49

Methyl O-(*2,3,4-tri-O-acetyl-6-O-triphenylmethyl-α-D-glucopyranosyl*)-(*1→2*)-O-[(*2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl*)-(*1→3*)]-*4-O-acetyl-α-L-rhamnopyranoside* (**7**) — Compound **6** (240 mg 0 37 mmol) was dissolved in dry pyridine (2 mL), and recrystallized chlorotriphenylmethane (106 mg, 0 38 mmol) was added The mixture was heated under nitrogen in a sealed tube overnight at 80° The reaction was monitored by t l c (R_F 0 72, *E*) Then, acetic anhydride (0 1 mL, 7 8 mmol) was added at 0° and, after 3 h at this temperature. the mixture was kept overnight at room temperature It was then poured into ice–water (30 mL), and the solution was extracted with chloroform (3 × 10 mL) The extracts were washed with water (10 ml), sodium hydrogencarbonate (10 mL), and water (10 mL), dried (magnesium sulfate), and evaporated to give 295 mg (78%) of **7** as an amorphous powder, m p 95–98°, $[\alpha]_D^{20}$ +55 2° (*c* 1 0, chloroform), t l c (*A*) R_t 0 42, ν_{max}^{KBr} 2960, 1750 (CO), 1375, 1225, and 1050 cm^{-1} ^{1}H-n m r (60 MHz, $CDCl_3$) δ 7 50–7 00 (m, 15 H, Ph), 5 60–3 10 (m, 17 H), 3 32 (s, 3 H, OMe), 2 1. 2 02, 2 0, 1 96, 1 89, 1 73 and 1 68 (7 s, 7 × 3 H, OAc), and 1 19 and 1 12 (2 d 2 × 3 H, $J_{5,6} = J_{5',6'} = 5$ 2 Hz, H-6 and -6')

Anal Calc for $C_{52}H_{62}O_{21}$ C, 61 05 H, 6 11, O, 32 84 Found C 60 93 H, 6 12, O, 32 58

Methyl O-(*2,3,4-tri-O-acetyl-α-D-glucopyranosyl*)-(*1→2*)-[(*2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl*)-(*1→3*)-*4-O-acetyl-α-L-rhamnopyranoside* (**8**) — Compound 7 (195 mg, 0 19 mmol) was dissolved in methanol (5 mL) and hydrogenated in the presence of 10% palladium-on-charcoal The catalyst was filtered off and the solvent evaporated to give 139 mg (94%) of 7, which was crystallized from ethyl acetate–hexane, m p 99–100 7°, $[\alpha]_D^{25}$ +48 6° (*c* 0 8. chloroform), t l c (*B*) R_F 0 31 ν_{max}^{KBr} 3525 (OH), 1750 (CO), 1375, 1230, and 1050 cm^{-1}, ^{1}H-n m r (60 MHz, $CDCl_3$) δ 5 70–3 55 (m, 17 H), 3 31 (s, 3 H, OMe), 2 15–1 90 (m, 21 H, OAc), and 1 22 and 1 18 (2 d, 2 × 3 H, $J_{5,6} = J_{5',6'} = 6$ Hz, H-6 and -6')

Anal Calc for $C_{33}H_{48}O_{21}$ C, 50 77, H, 6 20, O, 43 03 Found C. 50 70 H, 6 30, O, 42 93

Benzyl (*2,3,4-tri-O-benzyl-D-glucopyranosyl chloride*)*uronate* (**12**) — This compound was obtained by use of a modification of the method proposed by Pravdic and Keglević[13], dry ether replacing acetic acid After 4 days, the solution was evaporated, and the residue treated by several additions and evaporations of benzene The residual syrup was used without further purification (estimated yield from t l c data 100%), t l c (*D*) R_F 0 50 and 0 57

1,2,3,4-Tetra-O-acetyl-6-O-(benzyl 2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate)-β-D-glucopyranose (**13**) — 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranose[10] (**11**, 174 mg, 0 5 mmol) was dissolved in ether (5 mL) containing 1,2-dimethoxyethane (0 5 mL) A 0 1M solution (5 mL) of silver perchlorate and chloride **12** (344 mg, 0.6 mmol) dissolved in ether (1 mL) were added in the dark After 2 h, the insoluble material was filtered off and the solvent evaporated Chromatography of the residue

on silica gel (100 g solvent *A*) gave 260 mg (59%) of amorphous **13**, m p 46–47 5°, $[\alpha]_D^{20}$ +16 9° (*c* 1 2, chloroform), t l c (*A*) R_F 0 71, ν_{max}^{KBr} 3040, 2940, 1760 (CO), 1500 and 1460 cm^{-1}, ^{1}H-n m r. (60 MHz. $CDCl_3$) δ 7 4–7 0 (m, 20 H, Ph), 5 68 (d. 1 H $J_{1,2}$ 8 Hz. H-1), 5 30–3 33 (m, 19 H), and 1 99. 1 95. and 1 87 (3 s, 6 H. and 2 × 3 H, OAc)

Anal Calc for $C_{48}H_{52}O_{16}$ C, 65 15, H 5 92 O, 28 93 Found C, 65 03, H. 5 94 O. 29 04

6-O-(α-D-Glucopyranosyluronic acid)-α,β-D-glucopyranose (**14**) — Compound **13** (50 mg 0 56 mmol) was dissolved in a mixture of methanol (7 mL) and triethylamine (5 mL) After being kept for 15 h at room temperature, the solution was evaporated. and the residual triethylamine removed by evaporation of ethanol from the residue The resulting syrup was dissolved in methanol (5 mL) and hydrogenated in the presence of 10% palladium-on-charcoal (50 mg) at 3 atm After filtration and evaporation of the solvents, the crystalline residue was recrystallized from ethanol to give 16 mg of **14** (83%), m.p 186–188°. $[\alpha]_D^{20}$ +71° (*c* 0 8, water), t l c (*F*) R_F 0 36 ν_{max}^{KBr} 3380 (OH), 1727 (CO), 1425, 1260, and 1040 cm^{-1}. ^{1}H-n m r (270 MHz, D_2O) δ 5 23 (d 0 3 H, $J_{1,2}$ 3 75 Hz, H-1α of α-D-glucopyranose anomer), 4 96 (d, 0 7 H, $J_{1,2}$ 3 42 Hz, H-1′α of β-D-glucopyranose anomer), 4 94 (d, 0 3 H $J_{1,2}$ 2 8 Hz, H-1′α of α-D-glucopyranose anomer), 4 67 (d, 0 7 H, $J_{1,2}$ 7 86 Hz, H-1β of β-D-glucopyranose anomer), 4 07–3 46 (m, 8 H, H-2 -2′, -3, -3′, -4, -4′, -5, and -5′). 3 27 (d. 1 H. $J_{6a,6b}$ 8 5 Hz. H-6a), and 3 24 (d, 1 H, H-6b)

Anal Calc for $C_{12}H_{20}O_{11}$ H_2O C, 40 22 H, 6 18 Found C, 40 00, H, 5 87

Methyl O-(*benzyl 2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate*)-(*1→6*)-O-(*2 3,4-tri-O-acetyl-α-D-glucopyranosyl*)-(*1→2*)-[O-(*2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl*)-(*1→3*)]-*4-O-acetyl-α-L-rhamnopyranoside* (**15**) — Trisaccharide **8** (78 mg 0.1 mmol) was dissolved under nitrogen in a solution of 0 1M silver perchlorate in ether (3 mL) containing 1 2-dimethoxyethane (0 3 mL) Powdered 4-Å molecular sieves (150 mg) were added and the mixture was stirred for 2 h at room temperature Then, chloride **12** (86 mg, 0 15 mmol) dissolved in ether (1 mL) was added at one time. and the stirring was continued for 4 h. The solid material was filtered off, the solvents were evaporated, and the residue was chromatographed on silica gel (100 g, 5 3, v/v, ethyl acetate–hexane) to give 75 mg of **15** and 18 mg of **17** (ratio 81 19, overall yield 71%) Compound **15** was recrystallized from methanol, m p 173–174°, $[\alpha]_D^{20}$ +57° (*c* 1 1, chloroform). t l c (*A*) R_F 0 48, ν_{max}^{KBr} 2940, 1750 (CO), 1460, 1375, 1230, and 1050 cm^{-1}. ^{1}H-n m r (270 MHz, $CDCl_3$) δ 7 28–6 89 (m, 20 H, Ph), 5 40–3 40 (m, 22 H), 3 32 (s, 3 H, OMe), 2 12, 2 11, 2 06, 2 02, 2 01, 1 9, and 1 84 (7s, 7 × 3 H. OAc), and 1 22 and 1 16 (2 d, 2 × 3 H, $J_{5,6} = J_{5',6'}$ = 6 01 Hz, H-6 and -6′)

Anal Calc for $C_{67}H_{80}O_{27}$ C, 61 09, H, 6 12; O, 32 79 Found C, 60 95, H 6 20. O, 32 59

Methyl O-(*benzyl 2,3,4-tri-O-benzyl-β-D-glucopyranosyluronate*)-(*1→6*)-O-(*2,3,4-tri-O-acetyl-α-D-glucopyranosyl*)-(*1→2*)-O-[(*2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl*)-(*1→3*)]-*4-O-acetyl-α-L-rhamnopyranoside* (**17**) — Compound **17**, obtained

as described in the preceding paragraph, was amorphous (m p 77 6–78 8°), $[\alpha]_D^{20}$ +32° (*c* 1 0, chloroform), t l c (*A*) R_F 0 40, ν_{max}^{KBr} 2940, 1750, 1460, 1375, 1230, and 1050 cm^{-1} ^{1}H-n m r (270 MHz, $CDCl_3$) δ 7 29–6 87 (m, 20 H, Ph), 5 44–3 42 (m 22 H), 3 23 (s, 3 H, OMe), 2 13, 2 11, 2 06, 2 05, 1 99, 1 94, and 1 92 (7 s 7 × 3 H, OAc), 1 22 (d, 3 H, $J_{5,6}$ 5 99 Hz, H-6), and 1 14 (d, 3 H, $J_{5,6}$ 6 45 Hz, H-6′)

Anal Calc for $C_{67}H_{80}O_{27}$ C, 61 09, H, 6 12, O 32 79 Found C, 60 99, H, 6 22, O, 32 58

Methyl O-(*α*-D-*glucopyranosyluronic acid*)-(*1→6*)-O-*α*-D-*glucopyranosyl*-(*1→2*)-[O-*α*-L-*rhamnopyranosyl*-(*1→3*)]-*α*-L-*rhamnopyranoside* (**16**) — Compound **15** (62 mg, 0 047 mmol) was *O*-deacetylated in 0 5M sodium methoxide solution for 18 h at room temperature, and the solution was passed through a column of Dowex 50 (H^+), and then *O*-debenzylated by hydrogenation in methanol in the presence of 10% palladium-on-charcoal for 4 h The catalyst was filtered off and the solvent evaporated to give 29 mg (93%) of **16**, amorphous (m p 156–160°), $[\alpha]_D^{20}$ +56° (*c* 0 8 water) t l c (*F*) R_F 0 3, ν_{max}^{KBr} 3420 (OH), 2940, 1730 (CO), and 1050 cm^{-1}, ^{1}H-n m r (270 MHz, D_2O) δ 5 11 (d, 1 H, $J_{1,2}$ 1 1 Hz, H-1), 5 07 (d, 1 H $J_{1,2}$ 3 5 Hz, H-1‴), 5 04 (d, 1 H, $J_{1,2}$ 3 4 Hz, H-1″) 4 84 (s, 1 H H-1′), 4 18–3 46 (m, 18 H) 3 41 (s 3 H, OMe), 1 31 (d, 3 H $J_{5,6}$ 5 69 Hz, H-6), and 1 30 (d, 3 H, $J_{5,6}$ 5 93 Hz, H-6′)

Anal Calc for $C_{25}H_{42}O_{20}$ H_2O C, 44 11 H 6 51 Found C 44 26 H, 6 66

Methyl O-(*β*-D-*glucopyranosyluronic acid*)-(*1→6*)-O-*α*-D-*glucopyranosyl*-(*1→2*)-O-[*α*-L-*rhamnopyranosyl*-(*1→3*)]-*α*-L-*rhamnopyranoside* (**18**) — Compound **17** (32 mg, 0 024 mmol) was *O*-deacetylated and *O*-debenzylated as described for the preparation of **16**, to give amorphous **18**, yield 14 2 mg (92%) m p 169–172° $[\alpha]_D^{20}$ +5° (*c* 0 9, water), t l c (*F*) R_F 0 3 ν_{max}^{KBr} 3415 (OH), 2940, 1740 (CO), and 1060 cm^{-1}, ^{1}H-n m r (270 MHz, D_2O) δ 5 12 (s 1 H H-1) 5 03 (d 1 H $J_{1,2}$ 3 5 Hz, H-1″), 4 82 (s, 1 H, H-1′), 4 49 (d, 1 H $J_{1,2}$ 7 5 Hz, H-1‴), 4 03–3 38 (m, 18 H), 3 41 (s, 3 H, OMe) 1 30 (d, 3 H, $J_{5,6}$ 5 5 Hz, H-6) and 1 28 (d, 3 H $J_{5,6}$ 6 6 Hz, H-6′)

Anal Calc for $C_{25}H_{42}O_{20}$ 0 5 H_2O C, 44 71 H, 6 45 Found C, 44 73, H, 6 54

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REFERENCES

1 G Koehler and C Milstein *Nature (London)* 256 (1975) 495–497
2 C Milstein and G Koehler, in E Haber and R M Krause (Eds), *Antibodies in Human Diagnosis and Therapy*, Raven Press, New York 1977 pp 271–284

3 M HEIDELBERGER, *Fortschr Chem Org Naturst*, 18 (1960) 501–536
4 D G BRAUN AND J C JATON, *Curr Top Microbiol Immunol*, 66 (1974) 29–76
5 L KENNE, B LINDBERG, AND S SVENSSON, *Carbohydr Res*, 40 (1979) 69–75
6 D SCHWARZENBACH AND R W JEANLOZ, *Carbohydr Res*, 81 (1980) 323–329
7 D SCHWARZENBACH AND R W JEANLOZ, *Carbohydr Res*, 77 (1979) c5–c7
8 K IGARASHI, *Adv Carbohydr Chem Biochem*, 34 (1977) 243–283
9 J R POUGNY, M A M NASSR, N NAULET, AND P SINAY, *Nouv J Chim*, 2 (1978) 389–395
10 E A TALLEY, *Methods Carbohydr Chem*, 2 (1963) 337–340
11 M HEIDELBERGER, N ROY, AND C P J GLAUDEMANS, *Biochemistry*, 8 (1969) 4822–4824
12 M L WOLFROM AND A THOMPSON, *Methods Carbohydr Chem*, 1 (1963) 316–318
13 N PRAVDIC AND D KEGLEVIC, *Tetrahedron*, 21 (1965) 1897–1901