Carbohydrate Research, 150 (1986) 199–212
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Streptococcus pneumoniae TYPE XIV POLYSACCHARIDE: SYNTHESIS OF A REPEATING BRANCHED TETRASACCHARIDE WITH DIOXA-TYPE SPACER-ARMS*

PAUL-HENRI AMVAM-ZOLLO AND PIERRE SINAŸ**
Laboratoire de Biochimie Structurale, U.A. 499, U.E.R. de Sciences Fondamentales et Appliquées, B.P. 6759, F-45067 Orléans (France)

(Received December 9th, 1985; accepted for publication, January 1st, 1986)

ABSTRACT

β-Glycosides of 2-acetamido-2-deoxy-D-glucopyranose were synthesised, using either 7-methoxycarbonyl-3,6-dioxa-1-heptanol or 8-azido-3,6-dioxa-1-octanol. Selective β-lactosylation of 7-methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside with hepta-*O*-acetyl-lactosyl-trichloroacetimidate, followed by β-galactosylation of the secondary hydroxyl group with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)trichloroacetimidate, catalytic hydrogenolysis, and *O*-deacetylation, gave 7-methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-6-*O*-(4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-glucopyranoside. Selective β-lactosylation of 8-azido-3,6-dioxaoctyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside with hepta-*O*-acetyl-lactosyl bromide in the presence of silver triflate, followed by condensation with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of silver triflate, catalytic hydrogenolysis, and *O*-deacetylation, gave 8-azido-3,6-dioxaoctyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-6-*O*-(4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-glucopyranoside.

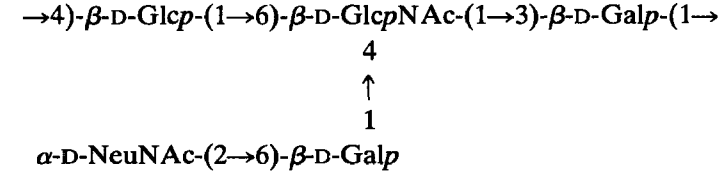
INTRODUCTION

Lancefield[1] characterised two polysaccharide antigens from group-B *Streptococcus*, the group-B polysaccharide common to all strains, and the type-specific polysaccharide that distinguishes four serotypes: Ia, Ib, II, and III. Baker and Kasper[2] demonstrated a significant correlation of low concentrations of the maternal antibody directed against the native type-III polysaccharide with the susceptibility to neonatal group-B streptococcal infection. This is an important finding inasmuch as life-threatening group-B streptococcal infections in neonates have become a major problem throughout many parts of the world[3]. In 1980, Jennings *et al.*[4]

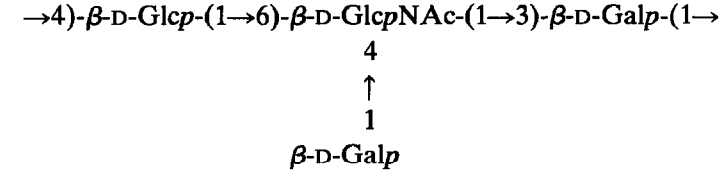
*Dedicated to Professor N. K. Kochetkov.
**To whom enquiries should be sent.

0008-6215/86/$ 03.50

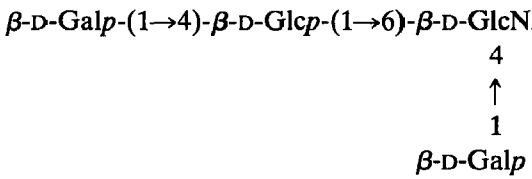
demonstrated that the structure of the native polysaccharide antigen isolated from type-III group-B *Streptococcus* can be represented by the following repeating-unit:



By cleavage of all the acid-labile sialic acid end-groups, the incomplete type-III antigen is obtained which is structurally identical to the *Streptococcus pneumoniae* type XIV polysaccharide[5]:



It has been demonstrated[6] that antisera directed against this pneumococcal polysaccharide were protective against the type-III group-B *Streptococci*. In order to determine the actual size and structure of the antigenic determinant of the incomplete type-III antigen which is responsible for antibody production, we have synthesised the following branched tetrasaccharide harnessed with spacer-arms suitable for conjugation to proteins.



Jennings *et al.*[7] hypothesised that the pentasaccharide resulting from addition of a sialic acid residue may be the antigenic determinant of the native polysaccharide. The free tetrasaccharide has previously been synthesised[8] by a combination of the orthoester[9] and diphenylcyclopropenyl[10] methods.

RESULTS AND DISCUSSION

The preparation of spacer-armed synthetic oligosaccharides that can be coupled to proteins and particles for use as immunogens or immunoabsorbants for affinity chromatography is well known[11]. Important factors in the selection of a particular spacer-arm are its length, its hydrophobicity, and its availability from

cheap chemicals. Lemieux *et al.*[12] developed a C_9 spacer which is frequently used. In order to decrease the hydrophobic interaction with the saturated alkyl chain, a more hydrophilic C_9-amide spacer has been used by Paulsen *et al.*[13]. 2-(2-Methoxycarbonylethylthio)ethyl glycosides (CETE-glycosides) have also been prepared[14]. As diethylene and triethylene glycols are cheap chemicals, we now propose their use as dioxa-type spacer-arms.

Diethylene glycol was converted into 7-methoxycarbonyl-3,6-dioxa-1-heptanol as follows. Treatment of diethylene glycol with benzyl chloride in 1,4-dioxane in the presence of powdered potassium hydroxide for 2 h at 80° gave a mixture of mono- and di-benzyl ethers. The monobenzyl ether **1** was selectively extracted (55%) with water from a solution of the mixture in ethyl acetate. Alkylation of **1** with methyl bromoacetate in tetrahydrofuran at 0° in the presence of sodium hydride gave 66% of liquid methyl 10-phenyl-3,6,9-trioxadecanoate (**2**), catalytic hydrogenolysis (Pd/C) of which in methanol gave an excellent yield of 7-methoxycarbonyl-3,6-dioxa-1-heptanol (**3**).

$BzlO(CH_2)_2OCH_2CH_2OH$ $RO(CH_2)_2O(CH_2)_2OCH_2CO_2Me$

1 **2** R = Bzl

3 R = H

$HO(CH_2)_2O(CH_2)_2OCH_2CH_2R$

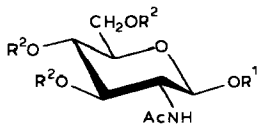
4 R = OTs

5 R = N_3

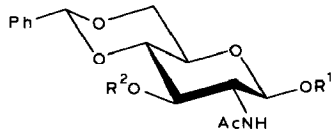
As a planned development of this work will be the synthesis of a sialic acid-containing pentasaccharide, and in order to avoid complications due to the competitive role of the carboxylic group of sialic acid, a non-acidic spacer-arm was elaborated from triethylene glycol, as follows. Triethylene glycol was treated with *p*-toluenesulfonyl chloride in pyridine–dichloromethane to give a mixture of mono- and di-tosyl derivatives. The ditosyl derivative was conveniently removed by crystallisation from ethanol and the monotosyl derivative **4** was transformed into liquid 8-azido-3,6-dioxa-1-octanol (**5**, ~65% from commercial triethylene glycol).

Condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[15] in dichloromethane at room temperature with **3** in the presence of Drierite and mercuric cyanide gave, after chromatography, amorphous 7-methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**6**) in 68% yield. The β configuration of **6** was clear from the $[\alpha]_D$ value of −31° (chloroform). In a routine manner, the glycoside **6** was *O*-deacetylated with methanolic sodium methoxide, the product **8** was treated[16] with α,α-dimethoxytoluene in *N,N*-dimethylformamide in the presence of *p*-toluenesulfonic acid, and the resulting 4,6-acetal **10** was benzylated with benzyl bromide in *N,N*-dimethylformamide, in the presence of barium oxide and barium hydroxide octahydrate, to

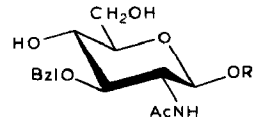
give **12**. The β configurations of **8, 10,** and **12** are apparent from the n.m.r. signals for H-1 [**8** (D_2O): δ 5.08, $J_{1,2}$ 8 Hz; **10** ($CDCl_3$): δ 4.82, $J_{1,2}$ 8 Hz,; **12** ($CDCl_3$): δ 4.98, $J_{1,2}$ 8 Hz]. Benzylation of **10** was accompanied by hydrolysis of the methyl ester of the spacer-arm, so that re-esterification with diazomethane was necessary to obtain pure, crystalline **12** in 73% yield from **8**. Hydrolysis of **12** with aqueous 80% acetic acid for 1 h at 80° gave crystalline **14** in 65% yield.



6 R^1 = M, R^2 = Ac
7 R^1 = Z, R^2 = Ac
8 R^1 = M, R^2 = H
9 R^1 = Z, R^2 = H



10 R^1 = M, R^2 = H
11 R^1 = Z, R^2 = H
12 R^1 = M, R^2 = Bzl
13 R^1 = Z, R^2 = Bzl



14 R = M
15 R = Z

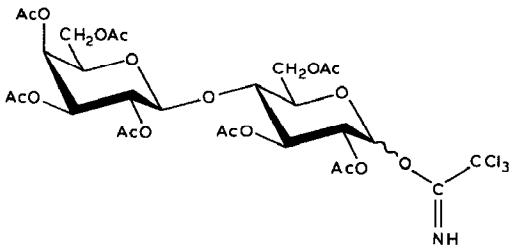
M = 7-methoxycarbonyl-3,6-dioxaheptyl
Z = 8-azido-3,6-dioxaoctyl

Since the reactivity of the primary hydroxyl group of **14** is higher than that of the secondary hydroxyl group, selective β-lactosylation of **14** was attempted. Selective β-lactosylation of the related benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside[17] with hexa-*O*-acetyl-1,2-*O*-(1-*tert*-butoxyethylidene)-α-lactose has been reported[8] to give the expected (1→6)-linked trisaccharide in 49% yield. We selected the trichloroacetimidate procedure[18]. 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose[19] was prepared from the corresponding bromide and treated with trichloroacetonitrile and sodium in dichloromethane to give an αβ-mixture (68:32) of *O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α,β-D-glucopyranosyl]trichloroacetimidate (**16**). When **16** was condensed with the diol **14** for 6 h in dichloromethane in the presence of boron trifluoride etherate, the trisaccharide **17** was isolated in 51% yield after chromatography. The primary hydroxyl group of **14** had been selectively lactosylated, since **17** did not react with trityl chloride in pyridine. No low-field n.m.r. signal for H-1 was apparent, so that β-lactosylation at C-6 had occurred.

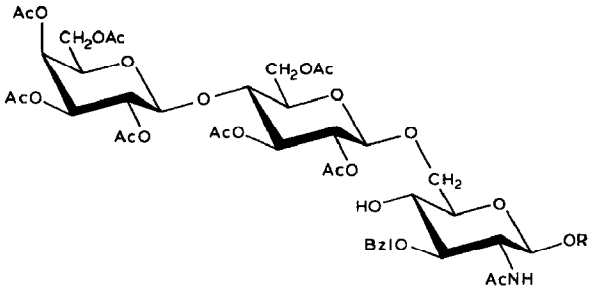
In order to galactosylate the secondary hydroxy group at C-4, Zurabyan *et al.*[8] activated this position through a 2,3-diphenyl-2-cyclopropen-1-yl (CDP) ether. However, we demonstrated[20] that O-4 of a protected 2-amino-2-deoxy-D-glucose residue was rather reactive, provided that appropriate glycosylation conditions were devised, and the trichloroacetimidate procedure has now proved satisfactory. Schmidt and Stumpp[21] reported the preparation of the anomeric mixture *O*-(2,3,4,6-tetra-*O*-acetyl-α,β-D-galactopyranosyl)trichloroacetimidate from 2,3,4,6-tetra-*O*-acetyl-D-galactose. We obtained the two crystalline imidates **19** and **20** in pure form after chromatography. The α-imidate **19** was condensed with **17** for 6 h in dichloromethane in the presence of boron trifluoride etherate to give, after

catalytic hydrogenolysis to remove the protecting benzyl ether, the branched tetrasaccharide **21** in 61% yield. This compares favorably with the 49% yield obtained by the Russian group[8] and indicates that glycosylation of **17** can be achieved without prior activation of the secondary hydroxyl group. *O*-Deacetylation of **21** gave the title tetrasaccharide **23** as an amorphous product in 63% yield. The 400-MHz ^{1}H-n.m.r. spectrum of a solution of **23** in D_2O exhibited four signals due to anomeric protons at δ 4.56–4.60 (reference: external Me_4Si) as doublets with $J_{1,2}$ 7 Hz. No individual assignment has been made but, since the coupling constants were large and the chemical shifts were in the higher part of the field region observed for anomeric protons, these data strongly suggest that all of the sugar residues are β-linked. The 100-MHz ^{1}H-n.m.r. spectrum[5] of *Streptococcus pneumoniae* type XIV showed signals for anomeric protons at δ 4.3–4.9 (reference: internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate) with $J_{1,2}$ 7 Hz.

Condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride in dichloromethane for 20 h at room temperature with 8-azido-3,6-dioxa-1-octanol, in the presence of tetramethylurea and freshly prepared silver triflate[22], gave, after chromatography, crystalline 8-azido-3,6-dioxaoctyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**7**) in 90% yield. The n.m.r. signal of H-1 of **7** in $CDCl_3$ was a doublet having a large coupling constant (δ 4.54, $J_{1,2}$ 9 Hz). In a routine manner, the glycoside **7** was converted, as previously described for the transformation of **6**, into amorphous **9** (76%), crystalline **11** (82%), and crystalline **13** (95%). The β configurations of **9**, **11**, and **13** are apparent from the n.m.r. signals for H-1 [**9** (D_2O): δ 5.00, $J_{1,2}$ 8 Hz; **11** ($CDCl_3$): δ 4.64, $J_{1,2}$ 8 Hz; **13** ($CDCl_3$): δ 4.92, $J_{1,2}$ 8 Hz]. The β configuration of **13** is also clear from the ^{13}C-n.m.r. chemical shift of C-1 in $CDCl_3$ + CD_3OD relative to tetramethylsilane (δ 101.8). Acid hydrolysis of **13** with aqueous 80% acetic acid for 1 h at 80° gave crystalline **15** in 90% yield. When 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide was condensed with the diol **15** in dichloromethane in the presence of tetramethylurea and freshly prepared silver triflate[22], the trisaccharide **18** was isolated, after chromatography, in 41% yield. As unreacted **15** was recovered in 45% yield, the yield of the condensation reaction was ~75%. The primary hydroxyl group had been selectively lactosylated, since **18** did not react with trityl chloride in pyridine. Treatment of **18** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide for 3 days at room temperature in the presence of tetramethylurea and freshly prepared silver triflate[22] gave, after chromatography, the crystalline tetrasaccharide **22** in 31% yield. Unreacted **18** was recovered in 60% yield, and therefore the yield of the galactosylation was ~80%. *O*-Deacetylation of **22**, followed by catalytic hydrogenolysis (Pd/C) in methanol and selective *N*-acetylation of the free amino group, gave the spacer-arm tetrasaccharide **24** in 56% yield. The optical rotation of **24** is almost identical to that of **23**, so that all the sugar residues are also β-linked. The ^{1}H-n.m.r. spectra of **18** and **22** exhibited no low-field signals attributable to an α-anomeric proton.

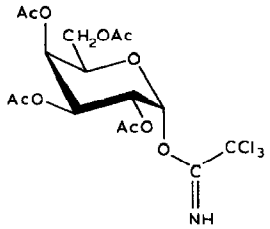


16

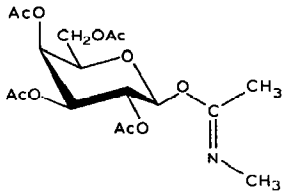


17 R = M

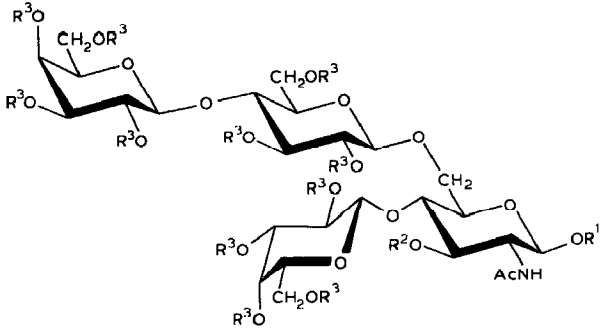
18 R = Z



19



20



21 R^1 = M, R^2 = H, R^3 = Ac

22 R^1 = Z, R^2 = Bzl, R^3 = Ac

23 R^1 = M, R^2 = R^3 = H

24 R^1 = $(CH_2)_2O(CH_2)_2O(CH_2)_2NHAc$, R^2 = R^3 = H

M = 7-methoxycarbonyl-3,6-dioxaheptyl

Z = 8-azido-3,6-dioxaoctyl

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–22° with a Perkin–Elmer Model 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Perkin–Elmer R-32 (90 MHz) or Bruker W-M-400 (400 MHz) spectrometer. ^{13}C-N.m.r. spectra were recorded with a Bruker (15.08 MHz) spectrometer. Purity of products was determined by t.l.c. on Silica Gel 60 F_{254} (Merck) with detection by charring with sulphuric acid. Column chromatography was performed on Silica Gel 60 (Merck, 0.063–0.200 mm) which was used without pre-treatment. Elemental analyses were performed by the Service Central de Micro-Analyse du Centre National de la Recherche Scientifique (Vernaison, France).

7-Phenyl-3,6-dioxa-1-heptanol (**1**). — Benzyl chloride (17 mL) was added dropwise to a solution of diethylene glycol (14.25 mL) in 1,4-dioxane (150 mL), and the mixture was heated at 80° in the presence of powdered potassium hydroxide. After 2 h, the mixture was cooled to room temperature, filtered, and concentrated. A solution of the residue in ethyl acetate was washed with water, dried (Na_2SO_4), and concentrated. The residue was diluted with hexane and extracted with water; the organic phase contained the diethylene glycol dibenzyl ether. Concentration of the aqueous phase gave the monobenzyl ether **1** (16 g, 55%), b.p. 110–120°/1 mmHg.

Anal. Calc. for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22. Found: C, 67.61; H, 8.32.

Methyl 10-phenyl-3,6,9-trioxadecanoate (**2**). — Methyl α-bromoacetate (8 mL) was added dropwise at 0° during 2 h to a mixture of **1** (15 g), oil-free sodium hydride (6 g), and dry tetrahydrofuran (200 mL). After 2 h, dry methanol (1 mL) and then acetic acid (3 mL) were added, the mixture was concentrated, and the residue was eluted from a column of silica gel (200 g) with hexane–ethyl acetate (1:1) to give **2** (13.5 g, 66%), b.p. 110°/5 mmHg. ^{1}H-N.m.r. data ($CDCl_3$): δ 7.30 (s, 5 H, Ph), 4.55 (s, 2 H, PhCH_2), 4.18 (s, 2 H, OCH_2CO), 3.73 (s, 3 H, COOMe).

Anal. Calc. for $C_{14}H_{20}O_5$: C, 62.57; H; 7.51. Found: C, 62.75; H, 7.56.

7-Methoxycarbonyl-3,6-dioxa-1-heptanol (**3**). — A solution of **2** (10 g) in methanol (100 mL) was hydrogenolysed in the presence of 10% Pd/C (1 g) for 18 h, filtered, and concentrated. The residue was distilled *in vacuo* (5 mmHg) at 120–130° to give **3** (6 g, 91%). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.15 (s, 2 H, OCH_2COOMe), 3.74 (s, 3 H, COOMe), 2.76 (s, 1 H, OH).

Anal. Calc. for $C_7H_{14}O_5$: C, 47.19; H, 7.92. Found: C, 47.08; H, 8.06.

*7-Methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**6**). — A solution of **3** (1 g) and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (2.5 g) in dry dichloromethane (10 mL) was stirred for 30 h at room temperature in the presence of anhydrous calcium sulfate (1 g) and mercuric cyanide (3 g). The mixture was then filtered and concentrated. A solution of the residue in chloroform (50 mL) was washed with aqueous 10% potassium iodide, aqueous 5% sodium hydrogencarbonate, and water, dried

(Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (200 g) with ether–methanol (20:1) gave syrupy **6** (1 g, 67.8%), $[\alpha]_D$ −31° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 6.92 (d, 1 H, $J_{NH,2}$ 9 Hz, NH), 4.18 (s, 2 H, OC*H*$_2$COOMe), 3.80 (s, 3 H, COOMe), 1.80–2.10 (m, 12 H, Ac).

Anal. Calc. for $C_{21}H_{33}NO_{13}$: C, 49.70; H, 6.55; N, 2.76. Found: C, 49.46; H, 6.76; N, 2.91.

*7-Methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**8**) — A solution of **6** (1 g) in dry methanol (10 mL) was treated with methanolic M sodium methoxide (1 mL) for 2 h at room temperature, de-ionised with Dowex 50W-X4 (H^+) resin, filtered, and concentrated. The residue was eluted from a column of silica gel (100 g) with chloroform–methanol (7:3) to give amorphous **8** (650 mg, 86.5%), $[\alpha]_D$ −22° (*c* 0.6, methanol). ^{1}H-N.m.r. data (D_2O): δ 5.08 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.75 (s, 2 H, OC*H*$_2$COOMe), 4.28 (s, 3 H, COOMe), 2.52 (s, 3 H, Ac).

Anal. Calc. for $C_{15}H_{27}NO_{10}$: C, 47.24; H, 7.14; N, 3.67. Found: C, 46.28; H, 7.11; N, 3.65.

*7-methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**10**). — Compound **8** (600 mg), α,α-dimethoxytoluene (263 mg), and *p*-toluenesulfonic acid monohydrate (7 mg) were placed in a 25-mL round-bottomed flask; this was then attached to a Büchi evaporator, rotated, evacuated, and lowered into a water bath at 65° so that *N,N*-dimethylformamide refluxed in the vapor duct. After 1 h, a short-path, evaporation adaptor was fitted between the flask and the vapor duct, and the *N,N*-dimethylformamide was evaporated, the temperature of the water bath being raised to 100°. When no more *N,N*-dimethylformamide distilled off, the flask was cooled and removed from the evaporator. The residual solution was diluted with chloroform (50 mL), washed with aqueous 5% sodium hydrogencarbonate and water, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (100 g) with chloroform–methanol (10:1) gave **10** (620 mg, 84%), m.p. 186–187° (from ethanol), $[\alpha]_D$ −69° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60–7.20 (m, 5 H, Ph), 7.00 (d, 1 H, $J_{NH,2}$ 7 Hz, NH), 5.54 (s, 1 H, C*H*Ph), 4.82 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.15 (s, 2 H, OC*H*$_2$COOMe), 3.75 (s, 3 H, COOMe), 2.02 (s, 3 H, Ac).

Anal. Calc. for $C_{22}H_{31}NO_{11}$: C, 56.28; H, 6.66; N, 2.98. Found: C, 56.27; H, 6.66; N, 2.89.

*7-Methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**12**). — A solution of **10** (500 mg) in dry *N,N*-dimethylformamide (5 mL) was stirred for 3 h at room temperature in the presence of barium oxide (980 mg), barium hydroxide octahydrate (270 mg), and freshly distilled benzyl bromide (0.2 mL). The mixture was diluted with chloroform (50 mL), washed successively with ice-cold aqueous 60% acetic acid, aqueous saturated sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was dried *in vacuo*, dissolved in methanol (10 mL), and esterified with ethereal diazomethane. The solution was concentrated and the residue eluted from

a column of silica gel (30 g) with ether–methanol (10:1) to give **12** (435 mg, 73%), m.p. 180–181° (from ethanol), $[\alpha]_D$ −17° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.50–7.20 (m, 10 H, 2 Ph), 6.80 (d, 1 H, $J_{NH,2}$ 8 Hz, NH), 5.56 (s, 1 H, C*H*Ph), 4.98 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.80 (AB system, 2 H, OCH_2Ph), 3.70 (s, 3 H, COOMe), 1.92 (s, 3 H, Ac).

Anal. Calc. for $C_{29}H_{37}NO_{10}$: C, 62.24; H, 6.66; N, 2.50. Found: C, 62.16; H, 6.54; N, 2.35.

*7-Methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**14**). — Compound **12** (200 mg) was stirred for 1 h at 80° with aqueous 80% acetic acid (5 mL). After being cooled, the mixture was concentrated and the residue was eluted from a column of silica gel (20 g) with chloroform–methanol (9:1) to give **14** (109.5 mg, 65%), m.p. 70–71° (from ethyl acetate–hexane–pentane), $[\alpha]_D$ −21° (*c* 2.3, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.30 (s, 5 H, Ph), 6.54 (d, 1 H, $J_{NH,2}$ 8 Hz, NH), 4.80 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.76 (s, 2 H, CH_2Ph), 4.10 (s, 2 H, OCH_2COOMe), 3.72 (s, 3 H, COOMe), 1.92 (s, 3 H, Ac).

Anal. Calc. for $C_{22}H_{33}NO_{10}$: C, 56.04; H, 7.06; N, 2.97. Found: C, 56.10; H, 6.90; N, 2.95.

O-[*2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-α,β-*D*-glucopyranosyl]trichloroacetimidate* (**16**). — A solution of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose (211 mg) in dry dichloromethane (3 mL) was stirred for 3 h at room temperature in the presence of trichloroacetonitrile (0.3 mL) and sodium (12 mg). The mixture was then filtered and concentrated. The residue was eluted from a column of silica gel (20 g) with ethyl acetate–hexane (1:1) to give amorphous **16** (223 mg, 86%). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.75 (s, NH), 8.70 (s, NH), 6.48 (d, $J_{1,2}$ 4 Hz, H-1α), 5.90 (d, $J_{1,2}$ 7 Hz, H-1β), 2.20–1.90 (m, 21 H, Ac). An elemental analysis on this anomeric mixture was not performed.

O-*(2,3,4,6-Tetra-*O*-acetyl-α- and -β-*D*-galactopyranosyl)trichloroacetimidate* (**19** *and* **20**). — A solution of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose (2 g) in dry dichloromethane was stirred for 4 h at room temperature in the presence of trichloroacetonitrile (6 mL) and sodium (207 mg). The mixture was then filtered and concentrated. The residue was eluted from a column of silica gel (80 g) with ethyl acetate–hexane (1:1) to give, first, **19** (1.1 g, 39%), m.p. 122–123° (from benzene–hexane), $[\alpha]_D$ +115.5°; ν_{max}^{KBr} 3330 (NH), 1760 (C=O), and 1683 cm^{-1} (C=N). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.65 (s, 1 H, NH), 6.60 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.40 (m, 2 H, H-2,3), 2.00 and 2.16 (2 s, 12 H, 4 Ac).

Anal. Calc. for $C_{16}H_{20}Cl_3NO_{10}$: C, 39.00; H, 4.09; Cl, 21.58; N, 2.84. Found: C, 39.20; H, 4.09; Cl, 21.69; N, 2.81.

Next eluted was the β-imidate **20** (1.27 g, 45%), m.p. 146–147° (from benzene–hexane), $[\alpha]_D$ +17° (*c* 2, chloroform); ν_{max}^{KBr} 3330 (NH), 1760 (C=O), and 1700 cm^{-1} (C=N). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.70 (s, 1 H, NH), 5.85 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), 5.60–5.30 (m, 2 H, H-2,4), 5.10 (dd, 1 H, $J_{3,4}$ 4, $J_{4,5}$ 9 Hz, H-3), 4.30–4.00 (m, 3 H, H-5,6,6'), 2.18, 2.02, and 2.00 (3 s, 12 H, 4 Ac).

Anal. Found: C, 39.07; H, 4.24; Cl, 21.57; N, 3.02.

*7-Methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside* (**17**). — A solution of **14** (1 g) and imidate **16** (1.65 g) was stirred in dry dichloromethane (5 mL) at 0°, and a solution of boron trifluoride etherate (0.3 mL) in dry dichloromethane (1 mL) was added dropwise during 30 min. After 6 h, the mixture was diluted with dichloromethane (20 mL), washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (150 g) with toluene–ethyl acetate (1:1) to give by-products, and then with ethyl acetate–acetone (3:2) to give the amorphous trisaccharide **17** (1.18 g, 51.5%), $[\alpha]_D$ −23° (*c* 1.4, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.30 (s, 5 H, Ph), 6.59 (d, 1 H, $J_{NH,2}$ 7 Hz, NH), 4.76 (s, 2 H, CH_2Ph), 4.10 (s, 2 H, OCH_2COOMe), 3.72 (s, 3 H, COOMe), 3.20 (1 H, OH), 2.20–2.00 (m, 21 H, 7 OAc), 1.95 (s, 3 H, NAc).

Anal. Calc. for $C_{48}H_{67}NO_{27}$: C, 52.89; H, 6.19; N, 1.28. Found: C, 52.65; H, 6.27; N, 1.22.

*7-Methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-6-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside* (**21**). — A solution of **17** (900 mg) and the α-imidate **19** (813 mg) in dry dichloromethane (10 mL) was stirred at room temperature and a solution of boron trifluoride etherate (0.25 mL) in dry dichloromethane (1 mL) was added dropwise during 30 min. After 6 h, the mixture was worked-up as previously described. The residue was eluted from a column of silica gel (100 g) with ethyl acetate–acetone (3:2) to give a tetrasaccharide fraction, a solution of which in methanol (5 mL) was hydrogenolysed in the presence of 10% Pd/C (150 mg) for 16 h, filtered, and concentrated. The residue was eluted from a column of silica gel (50 g) with ether–methanol (4:1) to give amorphous **21** (517 mg, 61%), $[\alpha]_D$ −8° (*c* 0.67, chloroform). ^{1}H-N.m.r. data: δ 6.50 (d, 1 H, $J_{NH,2}$ 8 Hz, NH), 4.15 (s, 2 H, OCH_2COOMe), 3.75 (s, 3 H, COOMe), 2.12, 2.04, 1.98 (m, 36 H, 12 OAc), 1.96 (s, 3 H, NAc).

Anal. Calc. for $C_{55}H_{79}NO_{36}$: C, 49.66; H, 5.98; N, 1.05. Found: C, 49.15; H, 5.98; N, 1.05. Found: C, 49.15; H, 5.92; N, 1.04.

*7-Methoxycarbonyl-3,6-dioxaheptyl 2-acetamido-2-deoxy-4-*O*-β-*D*-galactopyranosyl-6-*O*-(4-*O*-β-*D*-galactopyranosyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**23**). — A solution of **21** (700 mg) in dry methanol (10 mL) was treated with methanolic M sodium methoxide (2 mL) for 3 h at room temperature, de-ionised with Dowex 50W-X4 (H^+), filtered, and concentrated. The residue was eluted from a column of silica gel (50 g) with methanol–ethyl acetate–water (7:2:1) to give amorphous **23** (187.5 mg, 63%), $[\alpha]_D$ −12° (*c* 0.7, methanol–water). ^{1}H-N.m.r. data (400 MHz, D_2O): δ 4.56–4.00 (4 d, 4 H, *J* ~7–8 Hz, β-anomeric protons), 4.10 (s, OCH_2COOMe), 3.70 (s, 3 H, COOMe), 2.00 (s, 3 H, Ac).

Anal. Calc. for $C_{33}H_{56}NO_{25} \cdot 4\ H_2O$: C, 42.17; H, 6.97; N, 1.49. Found: C, 42.20; H, 6.89; N, 1.43.

8-Azido-3,6-dioxa-1-octanol (**5**). — A solution of *p*-toluenesulfonyl chloride (1.26 g) in anhydrous dichloromethane (5 mL) was dropwise added, at 0°, to a solution of triethylene glycol (1 g) in pyridine (5 mL). After 4 h, the mixture was poured into ice-cold water and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with water, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (50 g) with chloroform–methanol (20:1) gave, first, a crystalline ditosylate derivative which was not investigated further, and then syrupy 8-*O*-tosyl-3,6-dioxa-1,8-octanediol (1.5 g, 75%). When this reaction was conducted on a large scale, the ditosyl derivative was conveniently separated from the monotosyl derivative by crystallisation (ethyl acetate–hexane). A solution of 8-*O*-tosyl-3,6-dioxa-1,8-octanediol (20 g) in *N,N*-dimethylformamide (20 mL) was stirred for 2 h at 80° in the presence of sodium azide (8.5 g). The mixture was cooled to room temperature, filtered, and concentrated. A solution of the residue in chloroform was washed with water, dried (Na_2SO_4), and concentrated. *N,N*-Dimethylformamide was first distilled from the residue at 100°/10 mmHg, followed by **5** at 150°/10 mmHg. A second distillation gave pure **5** (7.5 g, 65%), b.p. 60°/3 × 10^{-3} mmHg; ν_{max}^{film} 3500 (OH) and 2120 cm^{-1} (N_3).

Anal. Calc. for $C_6H_{13}N_3O_3$: C, 41.13; H, 7.48; N, 23.99. Found: C, 41.34; H, 7.54; N, 23.61.

*8-Azido-3,6-dioxaoctyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**7**). — A mixture of **5** (3 g), 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (10.5 g), freshly prepared silver triflate (7 g), tetramethylurea (2 mL), and anhydrous dichloromethane (20 mL) was stirred for 20 h at room temperature in the dark. The mixture was then diluted with dichloromethane (100 mL), filtered, washed with water, cold aqueous 10% sulphuric acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (250 g) with ether–methanol (20:1) gave **7** (7.9 g, 90%), m.p. 69–70° (from ethyl acetate–ether), $[\alpha]_D$ −29° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 6.80 (d, 1 H, $J_{NH,2}$ 10 Hz, NH), 4.54 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), 2.10–1.80 (m, 12 H, 4 Ac).

Anal. Calc. for $C_{20}H_{32}N_4O_{11}$: C, 47.61; H, 6.39; N, 11.10. Found: C, 47.48; H, 6.17; N, 10.89.

*8-Azido-3,6-dioxaoctyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**9**). — A solution of **7** (500 mg) in dry methanol (10 mL) was treated with methanolic M sodium methoxide (1 mL) for 2 h at room temperature, de-ionised with Dowex 50W-X4 (H^+) resin, filtered, and concentrated. The residue was eluted from a column of silica gel (50 g) with chloroform–methanol (6:3) to give amorphous **9** (285 mg, 76%), $[\alpha]_D$ −23° (*c* 1, methanol). ^{1}H-N.m.r. data (D_2O): δ 5.00 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 2.49 (s, 3 H, Ac).

Anal. Calc. for $C_{14}H_{26}N_4O_8$: C, 44.44; H, 6.93; N, 14.81. Found: C, 44.65; H, 7.00; N, 14.56.

*8-Azido-3,6-dioxaoctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**11**). — Compound **9** (300 mg), α,α-dimethoxytoluene (133 mg), *N,N*-

dimethylformamide (6 mL), and *p*-toluenesulphonic acid monohydrate (7 mg) were placed in a 25-mL, round-bottomed flask and processed as previously described for the preparation of **10**. Elution of the product from a column of silica gel (50 g) with chloroform–methanol (9:1) gave **11** (304 mg, 82%), m.p. 193–194° (from ethanol), $[\alpha]_D$ −76° (*c* 1.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60–7.20 (m, 5 H, Ph), 6.71 (d, 1 H, $J_{NH,2}$ 7 Hz, NH), 5.50 (s, 1 H, C*H*Ph), 4.64 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 1.98 (s, 3 H, Ac).

Anal. Calc. for $C_{21}H_{30}N_4O_8$: C, 54.07; H, 6.48; N, 12.01. Found: C, 54.24; H, 6.34; N, 12.14.

*8-Azido-3,6-dioxaoctyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**13**). — A solution of **11** (600 mg) in dry *N,N*-dimethylformamide (5 mL) was stirred for 2 h at room temperature in the presence of barium oxide (1.5 g), barium hydroxide octahydrate (290 mg), and freshly distilled benzyl bromide (0.3 mL). The excess of benzyl bromide was then eliminated by the addition of methanol (1 mL) and stirring for 1 h. The mixture was diluted with chloroform (30 mL), washed successively with ice-cold aqueous 60% acetic acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue crystallised from ethanol to give **13** (679 mg, 95%), m.p. 188–189°, $[\alpha]_D$ −7° (*c* 1.5, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60–7.20 (m, 10 H, 2 Ph), 5.95 (d, 1 H, $J_{NH,2}$ 8 Hz, NH), 4.92 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 1.88 (3 H, s, Ac).

Anal. Calc. for $C_{28}H_{36}N_4O_8$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.75; H, 6.41; N, 10.09.

*8-Azido-3,6-dioxaoctyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**15**). — Compound **13** (150 mg) was stirred for 1 h at 80° with aqueous 80% acetic acid (5 mL). After being cooled, the mixture was concentrated, and the residue was crystallised from ethanol to give **15** (113 mg, 90%), m.p. 129–130°, $[\alpha]_D$ −7° (*c* 1.1, methanol). N.m.r. data: ^{1}H (CD_3OD + $CDCl_3$), δ 7.30 (s, 5 H, Ph), 4.74 (d, 1 H, $J_{1,2}$ 7 Hz, H-1), 1.84 (s, 3 H, Ac); ^{13}C, δ 165.3 (CO), 101.8 (C-1), 55.2 (C-2), 23.8 ($NHCOCH_3$).

Anal. Calc. for $C_{21}H_{32}N_4O_8$: C, 53.83; H, 6.88; N, 11.96. Found: C, 53.81; H, 6.81; N, 11.89.

*8-Azido-3,6-dioxaoctyl 3-*O*-benzyl-2-deoxy-6-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside* (**18**). — A mixture of **15** (2 g), 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide (3 g), tetramethylurea (3 mL), freshly prepared silver triflate (3.2 g), and dry dichloromethane (20 mL) was stirred in the dark for 7 h at −10°, then allowed to attain room temperature, and stirred for 24 h. The mixture was diluted with dichloromethane (200 mL), filtered, washed with water, cold 0.1M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (200 g) with toluene–ethyl acetate (1:1) gave side-products; further elution with ethyl acetate–acetone (4:1) gave amorphous **18**

(1.9 g, 41%), $[\alpha]_D$ −16° (*c* 0.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.30 (s, 5 H, Ph), 6.22 (d, 1 H, $J_{NH,2}$ 8 Hz, NH), 2.12–2.00 (m, 21 H, 7 OAc), 1.92 (s, 3 H, NAc).

Anal. Calc. for $C_{47}H_{66}N_4O_{25}$: C, 51.93; H, 6.12; N, 5.15. Found: C, 51.72; H, 6.27; N, 5.43.

Final elution with chloroform–methanol (9:1) gave unreacted **15** (900 mg, 45%), m.p. 129–130° (from ethanol).

*8-Azido-3,6-dioxaoctyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-6-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside* (**22**). — A mixture of **18** (1.5 g), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.6 g), tetramethylurea (0.6 mL), freshly prepared silver triflate (650 mg), and dry dichloromethane (10 mL) was stirred for 5 h in the dark at 0°, allowed to attain room temperature, and then stirred for 3 days. The mixture was diluted with dichloromethane (200 mL), filtered, washed with water, cold 0.1M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (200 g) with toluene–ethyl acetate (1:1) gave side-products; further elution with ethyl acetate–acetone (4:1) gave tetrasaccharide **22** (600 mg, 31%), m.p. 94–95° (from ethyl acetate–ether), $[\alpha]_D$ −13.5° (*c* 1.3, chloroform). ^{1}H-N.m.r. data: δ 7.30 (s, 5 H, Ph), 6.32 (d, 1 H, $J_{NH,2}$ 8 Hz, NH), 2.20–1.80 (m, 36 H, 12 Ac).

Anal. Calc. for $C_{61}H_{84}N_4O_{34}$: C, 51.69; H, 5.97; N, 3.95. Found: C, 51.69; H, 6.16; N, 3.76.

Further elution with chloroform–methanol (9:1) gave unreacted **18** (900 mg, 60%).

*8-Acetamido-3,6-dioxaoctyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-6-*O*-(4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-glucopyranoside* (**24**). — A solution of **22** (150 mg) in dry methanol (3 mL) was treated with methanolic M sodium methoxide (0.2 mL) for 2 h at room temperature, de-ionised with Dowex 50W-X4 (H^+) resin, filtered, and concentrated. The residue was dried *in vacuo*, suspended in methanol (10 mL), hydrogenolysed in the presence of 10% Pd/C (100 mg) for 20 h, and filtered. Acetic anhydride (0.2 mL) was added to the filtrate. After 1 h, the solution was concentrated, and the residue was eluted from a column of silica gel (10 g) with methanol–chloroform–water (8:2:1) to give amorphous **24** (48.5 mg, 56%), $[\alpha]_D$ −13° (*c* 0.5, methanol). ^{1}H-N.m.r. data (D_2O): δ 5.10–4.80 (m, 4 H, anomeric protons, β linkages), 2.52 and 2.50 (2 s, 6 H, 2 NAc).

Anal. Calc. for $C_{34}H_{60}N_2O_{24} \cdot 6\ H_2O$: C, 41.29; N, 2.83. Found: C, 41.25; N, 2.89.

REFERENCES

1 R. C. Lancefield, *J. Exp. Med.*, 37 (1933) 571–595.
2 C. J. Baker and D. L. Kasper, *N. Engl. J. Med.*, 294 (1976) 753–756.
3 C. J. Baker, *J. Infect. Dis.*, 136 (1977) 137–149.

4 H. J. JENNINGS, K.-G. ROSELL, AND D. L. KASPER, *Can. J. Biochem.*, 58 (1980) 112–120.
5 B. LINDBERG, J. LÖNNGREN, AND D. A. POWELL, *Carbohydr. Res.*, 58 (1977) 177–186.
6 G. W. FISCHER, G. H. LOWELL, M. M. CUMRINE, AND J. W. BASS, *J. Exp. Med.*, 148 (1978) 776–786.
7 H. J. JENNINGS, C. LUGOWSKI, AND K.-G. ROSELL, *Abstr. Pap. Int. Symp. Carbohydr. Chem., Xth, Sydney*, 1980, Th. 11.
8 S. E. ZURABYAN, V. A. NESMEYANOV, AND A. YA. KHORLIN, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 6 (1976) 1421–1423.
9 N. K. KOCHETKOV, A. J. KHORLIN, AND A. F. BOCHKOV, *Tetrahedron*, 23 (1967) 693–707; N. K. KOCHETKOV, A. F. BOCHKOV, T. A. SOKOLOVSKAYA, AND V. J. SNYATKOVA, *Carbohydr. Res.*, 16 (1971) 17–27.
10 A. YA. KHORLIN, V. A. NESMEYANOV, AND S. E. ZURABYAN, *Carbohydr. Res.*, 43 (1975) 69–77.
11 C. P. STOWELL AND Y. C. LEE, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 225–281.
12 R. U. LEMIEUX, D. R. BUNDLE, AND D. A. BAKER, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.
13 H. PAULSEN, J.-C. JACQUINET, AND W. RUST, *Carbohydr. Res.*, 104 (1982) 195–219.
14 J. DAHMÉN, T. FRÈJD, G. GRÖNBERG, T. LAVE, G. MAGNUSSON, AND G. NOORI, *Carbohydr. Res.*, 118 (1983) 292–301.
15 D. HORTON, *Methods Carbohydr. Chem.*, 6 (1972) 282–285.
16 M. E. EVANS, *Carbohydr. Res.*, 21 (1972) 473–475.
17 J.-C. JACQUINET, J.-M. PETIT, AND P. SINAŸ, *Carbohydr. Res.*, 38 (1974) 305–311.
18 R. R. SCHMIDT AND J. MICHEL, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 731–732.
19 C. S. HUDSON AND R. SAYRE, *J. Am. Chem. Soc.*, 38 (1916) 1867–1873.
20 J.-C. JACQUINET AND P. SINAŸ, *Carbohydr. Res.*, 46 (1976) 138–142.
21 R. R. SCHMIDT AND M. STUMPP, *Justus Liebigs Ann. Chem.*, (1983) 1249–1256.
22 R. N. HASZELDINE AND J. M. KIDD, *J. Chem. Soc.*, (1954) 4228–4232.