Artificial Carbohydrate Antigens. Synthesis and Conformation of a *Shigella flexneri* Trisaccharide Hapten

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The trisaccharide 8-methoxycarbonyloctyl 2-*O*-[2'-acetamido-2'-deoxy-3'-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-α-L-rhamnopyranoside has been synthesised in good yield utilising silver trifluoromethanesulphonate-promoted Königs–Knorr reactions. 3,4-Di-*O*-benzyl-β-L-rhamnopyranose 1,2-(methyl orthoacetate) provided access to the 2-*O*-substituted rhamnoside, 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside. Introduction of the amino-sugar was achieved with tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide in conjunction with silver trifluoromethanesulphonate and 2,4,6-trimethylpyridine. Selective conversion of the 2-deoxy-2-phthalimido glucoside to a 2-acetamido-2-deoxy glucoside blocked at positions C-4 and -6 of the pyranose ring by a benzylidene acetal gave the trisaccharide, following reaction with tri-*O*-acetyl-α-L-rhamnopyranosyl bromide. Proton and ^{13}C n.m.r. evidence indicated that, despite a sterically crowded environment, the orientation of both the 2-acetamido-2-deoxy-glucose and terminal rhamnose residues is dictated by the *exo*-anomeric effect.

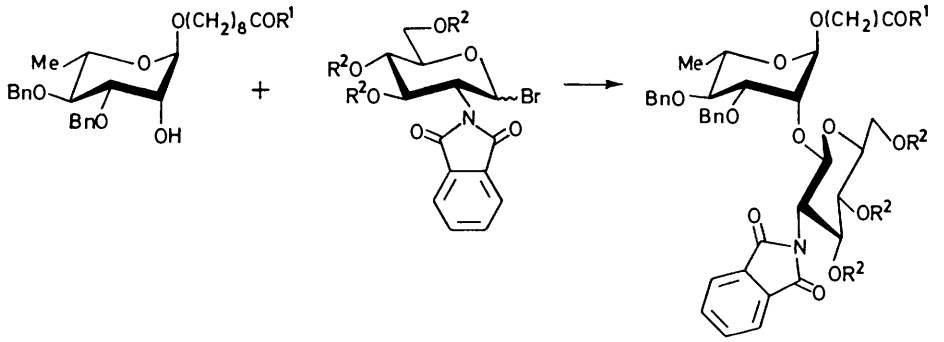
In a previous paper [1] we described the synthesis of three disaccharides that constitute portions of the tetrasaccharide repeating unit, →2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-β-D-GlcNAc*p*-(1→ of the *Shigella flexneri* lipopolysaccharide [2,3] (LPS). We report here the synthesis of a trisaccharide portion, α-L-Rha*p*-(1 → 3)-β-D-GlcNAc*p*-(1 → 2)-α-L-Rha*p*-(1 → R), R = $O(CH_2)_8CO_2CH_3$, of the repeating unit which is also functionalised, as were the disaccharides,[1] for artificial antigen synthesis.[4,5] The reasons for our interest in artificial antigens and especially those rhamnose-containing oligosaccharide repeating units of *S. flexneri* LPS were discussed earlier.[1] However, in the context of this paper two features are worthy of special attention. The conformation about the glycosidic linkages is shown to follow predictions based on the *exo*-anomeric effect.[6] The significance of this observation together with the proportion of 1,2-linkages and their effect on polysaccharide geometry [7] should have profound consequences with respect to the immunodominance within the antigen determinant. Also the use of tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glycosyl bromide (2) is demonstrated with respect to further chain-extension reactions carried out on the amino-sugar following selective removal of the phthalimido-group.

The objective of the synthetic scheme reported here and in our previous paper [1] has been to elaborate oligosaccharides as large as tetrasaccharides on the alcohol 8-methoxycarbonyloctanol,[4] which is used after de-blocking to establish a covalent linkage to protein. The advantages of this method over others have been discussed [4] and demonstrated [4,5,8] elsewhere. The appropriate combination of persistent and temporary blocking groups chosen for this and earlier work [1] is consistent with the ultimate goal, a tetrasaccharide artificial antigen. For this purpose 3,4-di-*O*-benzyl-β-L-rhamnopyranose 1,2-(methyl orthoacetate) was used. This orthoester provided, after a standard orthoester glycosylation reaction [1] with 8-methoxycarbonyloctanol, the acetylated form of the rhamnoside (1). Removal of the 2-*O*-acetate provides a route to 2-*O*-substituted rhamnopyranosides in which C-3 and C-4 of the pyranose ring are protected by ' persistent ' blocking groups. In addition the 1,2-orthoester, 3,4-di-*O*-benzyl-β-L-rhamnopyranose 1,2-(methyl orthoacetate), used to prepare (1) is a suitable intermediate for chain extension when sequent reactions to provide additional 2-*O*-substituted linkages are planned.

Reaction of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide (2) with the partially protected rhamnoside (1) under silver trifluoromethanesulphonate (triflate)-promoted Königs–Knorr conditions in which 2,4,6-trimethylpyridine (collidine) was the proton acceptor,[9] provided the fully blocked disaccharide (3) in 73% yield. The *O*-acetate groups were removed by transesterification in methanol with no evidence of 2'-carboxybenzamide formation. Selective conversion of the phthalimido-function of the disaccharide (4) to an acetamido-function (5) was achieved in 80% yield. The disaccharide (4) was refluxed with 6 equivalents of hydrazine in ethanol and the resultant 2-amino-2-deoxy-glucoside was *N*-acetylated to give the disaccharide (5). The ester function of the 8-methoxycarbonyloctyl aglycon, the maintenance of which until the penultimate step is essential for efficient antigen synthesis, was unreactive toward hydrazine under these conditions. The 4',6'-*O*-benzylidene acetal was introduced to give the selectively blocked disaccharide (6) providing for the introduction of an α-linked rhamnopyranoside residue. This was achieved by a silver triflate-promoted Königs–Knorr reaction between 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide [10] and the disaccharide (6) with *NN*-tetramethylurea as the proton acceptor.[11] The fully blocked trisaccharide was transesterified in methanol solution and subsequently hydrogenated over palladium–charcoal. The 4',6'-*O*-benzylidene acetal which survived this treatment was hydrolysed by trifluoroacetic acid to give the fully de-blocked trisaccharide (8) as a pure syrup in 72% yield after column chromatography. Treatment of the trisaccharide (8) with hydrazine gave the hydrazide derivative (9), the immediate precursor for artificial antigen synthesis.[4,5] Compounds (6)—(9)

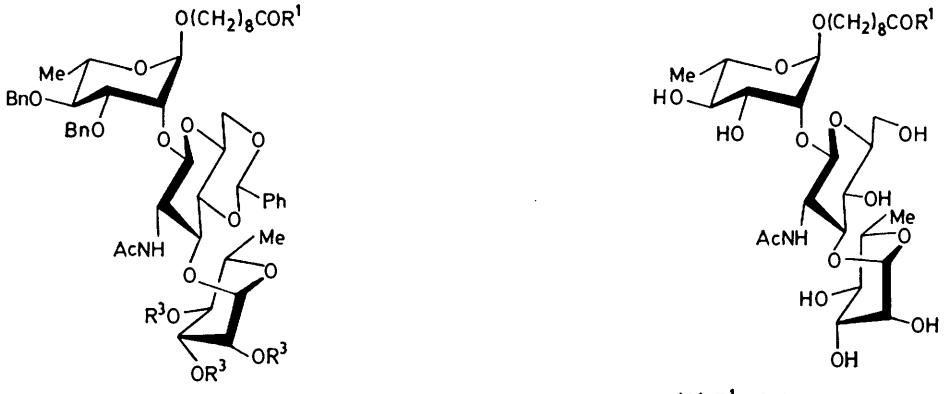
were not crystalline although all were analytically pure syrups giving proton n.m.r. parameters in agreement with the required structures. However it was essential to establish the stereochemistry of the terminal α-L-rhamnopyranoside linkage. This was achieved by ^{13}C n.m.r. spectroscopy, which for a 0.5M-solution of (7) provided a sensitivity of *ca.* 100 : 1. Since only 3 anomeric signals were observed for this compound and no doubling of associated ring carbon resonances was seen, a high degree of purity for (7) is indicated.

The n.m.r. spectrum of the blocked trisaccharide (7) shows the H-6″ doublet at δ 0.55, an upfield shift of 0.67 p.p.m. This substantial upfield shift was previously observed for 8-methoxycarbonyloctyl 2-acetamido-3-*O*-(tri-*O*-acetyl-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside and its de-*O*-acetylated derivative.[1] It was established that this shift was due to anisotropic shielding which requires that the protons of the 6-deoxy-function are positioned close to the centre of the aromatic nucleus.[1] Not surprisingly, the identical structural unit present in trisaccharide (7) displays the same spectral properties. This data is interpreted as supporting proposals made by Lemieux and Koto,[6] who have predicted, on the basis of the *exo*-anomeric effect, that the torsional angles ϕ and ψ (Figure), defining the conformation of the glycosidic linkage, assume values such that ϕ is fixed and ψ varies to minimise non-bonded interactions. Several conclusions were drawn from the calculations made in these studies. The most salient point is that the preferred conformation



(1) R^1 = OMe (2) R^2 = Ac (3) R^1 = OMe, R^2 = Ac
(4) R^1 = OMe, R^2 = H

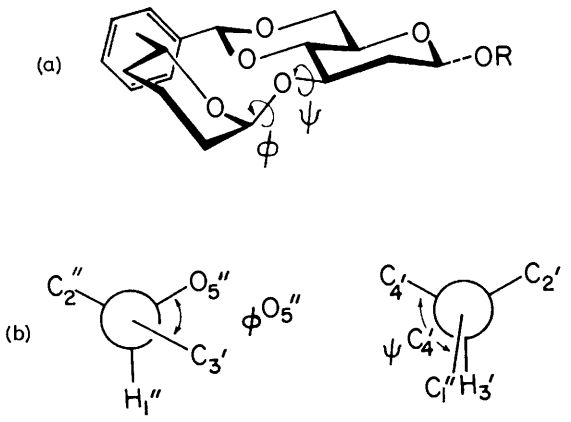
(5) R^1 = OMe, R^2 = H (6) R^1 = OMe, R^2 = H



(7) R^1 = OMe, R^3 = Ac Bn = $PhCH_2$ (8) R^1 = OMe
(9) R^1 = $NHNH_2$

is not the staggered arrangement about the glycosidic oxygen to aglyconic carbon bond (torsion angle ψ), but rather that in which the C(1'')–O(1'') bond almost eclipses the C(3')–H(3') bond (Figure). Molecular models (both Dreiding and space filling) for compound (7) show that with ϕ set at $\sim|60°|$, ψ must assume a value close to $|120°|$ (eclipsed form) in order to provide the required juxtaposition of phenyl ring and 6-deoxy-function. Indeed, unless ϕ is close to $|60°|$ the required overlap of these functions is unlikely. In the predicted conformation the plane of the phenyl ring is required to be perpendicular to that of the mean plane of the fused ring system. Such an arrangement has been shown to exist by *X*-ray analysis of a 4,6-*O*-benzylidene acetal.[12]

Further evidence based on the work of Lemieux



(a) Conformation of the glycosidic linkage for the disaccharide unit, 2'-acetamido-3'-*O*-(tri-*O*-acetyl-α-L-rhamnopyranosyl)-4',6'-*O*-benzylidene 2'-deoxy-β-D-glucopyranoside, and (b) Newman projections of the torsional angles ϕ and ψ. (Only those substituents essential to the arguments presented in the text are shown in this structure.)

and Koto that supports the conformation (Figure) is provided by ^{13}C n.m.r. spectroscopy. Steric compression of the anomeric hydrogen in the eclipsed conformation, such as that depicted in the Figure, has been predicted [7] to cause displacement of the C-1 resonance; such is the case here for the trisaccharide (9) in which C-1'' absorbs at 102.5 p.p.m., compared to C-1, 99.8 p.p.m. and C-1, 101.8 p.p.m., for methyl-α-L-rhamnopyranoside. Thus the predicted conformation appears well substantiated and is further proof of the influence of the *exo*-anomeric effect. That the sterically crowded linkage of compound (7) maintains a ϕ value close to 60° is considered of the utmost significance since this preferred conformation should surely be adopted in the less strained circumstances of the de-blocked trisaccharide (8). Related arguments may be advanced for the conformation of the 2-acetamido-2-deoxy-glucose-to-rhamnose linkage. As represented in the diagrams the disaccharide (5) and trisaccharide (7) could be expected to have the methyl group of the acetamido-function in close proximity to the C-3 benzyl ether. This would appear to be the case since for the de-blocked trisaccharide (8) the chemical shift of the acetamido-methyl group is δ 1.99 p.p.m., whereas, for compounds (5), (6), and (7) the values are 1.83, 1.85, and 1.88 p.p.m. in deuteriated methanol. The shielding is apparent but less marked than that observed for the rhamnose H-6'' protons.

The reasoning based on n.m.r. parameters reported here and in a previous paper [1] provides valuable information for attempts to define the conformation of *S. flexneri* oligosaccharide determinants. It is hoped to confirm these predictions based on n.m.r. by *X*-ray studies on blocked and de-blocked 2-acetamido-2-deoxy-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosides.

EXPERIMENTAL

Thin-layer chromatography was performed with Merck precoated silica gel 60 F-254 plates, and compounds were detected by quenching of u.v. fluorescence, and by spraying with 5% sulphuric acid in ethanol and heating. Merck silica gel G60 (70—230 mesh) and redistilled solvents were used for column chromatography. The loading on all columns was 1 : 100 unless otherwise indicated. Skellysolve B refers to hexane supplied by Getty Refining and Marketing Company, Tulsa, Oklahoma. 10% Palladium–charcoal was purchased from Engelhard Industries, Newark, New Jersey. Solvents were purified and dried according to standard procedures.[13] Processed solutions were dried over anhydrous sodium sulphate and solvent removal was achieved at bath temperatures of 40 °C or lower unless otherwise stated. Melting points were determined on a Fisher–Johns apparatus. Optical rotations were measured at 589 nm in a 1-dm cell at room temperature (20—23 °C). ^{13}C and ^{1}H n.m.r. spectra were recorded at 20 and 79.9 MHz, respectively, in the pulsed Fourier-transform mode on a Varian CFT-20 spectrometer. Proton chemical shifts are expressed relative to internal 1% tetramethylsilane for solutions in deuteriochloroform and [$^2H_4$]methanol, and relative to internal sodium [2,2,3,3-$^2H_4$]-3-trimethylsilylpropionate for solutions in deuterium oxide. Carbon-13 shifts are expressed relative to internal Me_4Si in [$^2H_4$]methanol, and to external Me_4Si for deuterium oxide solutions. Assignments of carbon-13 resonances are tentative.

8-Methoxycarbonyloctyl 2-O-(2'-Acetamido-2'-deoxy-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-L-rhamnopyranoside (5). —The dissaccharide (4) [1] (1.4 g, 1.7 mmol) in ethanol (80 cm^3) was boiled with hydrazine hydrate (0.72 g of an 85% solution, 12 mmol) for 2 h. The reaction was monitored by t.l.c. with chloroform–methanol (7 : 1) as solvent. In this system, starting material possessed R_F 0.54 and the free amino-derivative R_F 0.42. The solution was evaporated with ethanol and dried under high vacuum to remove traces of hydrazine. The product was then dissolved in methanol–water (1 : 1; 30 cm^3), acetic anhydride (3 cm^3) added, and the solution stirred at room temperature overnight. Concentration followed by purification on silica gel with chloroform–methanol (7 : 1) gave the pure *disaccharide* (5) (1.0 g, 80%), $[\alpha]_D$ −8.8° (*c* 1.1, MeOH); R_F 0.52 (solvent as above); δ(CD_3OD) 1.10—1.70 [15 H, m, H-6 and $-(CH_2)_6-$], 1.83 (3 H, s, *Me*CONH), 2.28 (2 H, t, CH_2CO), 3.52 (3 H, s, MeO), 7.00—7.80 (10 H, m, aromatic), and 3.15—5.00 (remaining protons); δ_C(CD_3OD) 104.1 (C-1'), 100.4 (C-1), 81.7, 80.7 (2 × CH_2Ph), 77.8 (C-2), 77.8 (C-5'), 76.2 (C-4), 75.8 (C-3'), 73.1 (C-3), 71.8 (C-4'), 69.1 (OCH_2), 68.4 (C-5), 62.6 (C-6'), 57.9 (C-2'), and 18.3 (C-6) (Found: C, 63.45; H, 7.65; N, 1.85. $C_{38}H_{55}NO_{12}$ requires, C, 63.6; H, 7.7; N, 1.95%).

8-*Methoxycarbonyloctyl* 2-O-(2′-*Acetamido*-4′,6′-O-*benzylidene*-2′-*deoxy*-β-D-*glucopyranosyl*)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (6).—The dissaccharide (5) (790 mg, 1.1 mmol) and αα-dimethoxytoluene (300 mg, 2.0 mmol) in dry acetonitrile (40 cm³) containing toluene-*p*-sulphonic acid (20 mg) was rotated under reduced pressure on the rotary evaporator at a temperature of 60 °C and a pressure such as to avoid excessive evaporation of solvent. After 1 h the acetonitrile was evaporated off, and the residue, dissolved in methylene chloride, was extracted with saturated sodium hydrogencarbonate solution and water. The syrup was purified on silica gel [Skellysolve B–ethyl acetate (1 : 3)] to give the pure *disaccharide* (6) (830 mg, 94% yield), $[\alpha]_D$ −39.5° (*c* 1.1, $CHCl_3$); R_F 0.58 (solvent as above); δ($CDCl_3$) 1.10—1.80 [15 H, m, H-6 and $-(CH_2)_6-$], 1.55 (3 H, s, *Me*CONH), 2.27 (2 H, t, CH_2CO), 3.62 (3 H, s, OMe), 5.50 (1 H, s, C*H*Ph), 6.70—7.30 (15 H, m, aromatic), and 3.10—4.90 (remaining protons); δ_C(CD_3OD) 104.5 (C-1′), 102.6 (C*H*Ph), 100.4 (C-1), 82.5 (C-4′), 81.6, 80.6 (2 × CH_2Ph), 78.1 (C-2), 76.2 (C-4), 73.3 (C-3), 72.1 (C-5′), 69.4 (C-3′), 69.1 (OCH_2), 68.2 (C-5), 67.3 (C-6′), 58.2 (C-2′), and 18.2 (C-6) (Found: C, 67.15; H, 7.35; N, 1.7. $C_{45}H_{59}NO_{12}$ requires C, 67.05; H, 7.4; N, 1.75%).

8-*Methoxycarbonyloctyl* 2-O-[2′-*Acetamido*-3′-O-(2″,3″,4″-*tri*-O-*acetyl*-α-L-*rhamnopyranosyl*)-4′,6′-O-*benzylidene*-2′-*deoxy*-β-D-*glucopyranosyl*]-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (7).—The selectively blocked disaccharide (6) (800 mg, 1.0 mmol) was dissolved in dichloromethane (30 cm³) together with silver trifluoromethanesulphonate (540 mg, 2.1 mmol) and *NN*-tetramethylurea (1 cm³, 8.3 mmol). The stirred solution was cooled to −40 °C under dry nitrogen and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide [10] (750 mg 2.1 mmol) in dichloromethane solution (10 cm³) was added dropwise. The mixture was allowed to warm to room temperature overnight and then filtered. Following extraction with saturated sodium hydrogencarbonate and water, the concentrated syrup was purified on silica gel with Skellysolve B–ethyl acetate (1 : 2) to give the pure *trisaccharide* (7) (780 mg, 73% yield), $[\alpha]_D$ −34.3° (*c* 1.2, $CHCl_3$); R_F 0.69 (solvent as above); δ(CD_3OD) 0.55 (3 H, d, $J_{5.6}$ 6.1 Hz, H-6″), 1.22 (3 H, d, $J_{5.6}$ 5.6 Hz, H-6), 0.90—1.65 [12 H, m, $-(CH_2)_6-$], 1.88 (3 H, s, *Me*CONH), 1.94 (3 H, s, OAc), 1.95 (3 H, s, OAc), 2.07 (3 H, s, OAc), 2.27 (2 H, t, CH_2CO), 3.62 (3 H, s, OMe), 5.63 (1 H, s, C*H*Ph), 6.80—7.50 (15 H, m, aromatic), and 3.20—5.20 (remaining protons); δ_C(CD_3OD) 103.6 (C-1′), 102.7 (C*H*Ph), 100.2 (C-1), 98.6 (C-1″), 81.5, 80.5 (CH_2Ph), 80.1 (C-4′), 77.7 (C-2), 77.4 (C-3′), 76.0 (C-4), 72.9 (C-3), 72.1 (C-5′), 71.4 (C-4″), 69.8 (C-3″), 69.2 (OCH_2), 69.0 (C-2″), 68.1 (C-5), 67.1 (C-6′), 67.1 (C-5″), 57.7 (C-2′), 18.2 (C-6), and 17.0 (C-6″) (Found: C, 63.5; H, 7.15; N, 1.35. $C_{57}H_{75}NO_{19}$ requires C, 63.5; H, 7.0; N, 1.3%).

8*Methoxycarbonyloctyl* 2-O-[2′*Acetamido*-2′-*deoxy*-3′-O-(α-L-*rhamnopyranosyl*)-β-D-*glucopyranosyl*]-α-L-*rhamnopyranoside* (8).—The trisaccharide (7) (700 mg, 0.65 mmol) was dissolved in methanol (50 cm³) containing a catalytic amount of sodium. After 14 h the solution was de-ionised and concentrated. The residue was homogeneous by t.l.c. [single spot, R_F 0.37 chloroform–methanol (7 : 1)] and contained no *O*-acetyl groups (^{1}H n.m.r.) The residue was dissolved in acetic acid (50 cm³) and hydrogenated over 10% palladium–charcoal (0.5 g) at 505 kPa for 3 h. Filtration and evaporation provided a residue which was dissolved in 90% aqueous trifluoroacetic acid [14] (20 cm³) at 0 °C and left for 45 min. Evaporation and co-distillation with ethanol. followed by purification on silica gel gave the pure de-blocked *trisaccharide* (8) (320 mg, 72% yield), $[\alpha]_D$ −46.4° (*c* 1.1, water); R_F 0.40 (dichloromethane–methanol 3 : 1); δ(D_2O; 85 °C) 0.90—1.60 [18 H, m, H-6, H-6″ and $-(CH_2)_6-$], 1.99 (3 H, s, *Me*CONH), 2.25 (2 H, t, CH_2CO), 3.58 (3 H, s, OMe), 4.67 (1 H, d, $J_{1,2}$ 8.1 Hz, H-1 2-acetamido-2-deoxyglucose residue), 4.82 (2 H, br s, H-1 and H-1″), and 3.00—3.90 (16 H, m, remaining protons); δ_C(CD_3OD) 103.7 (C-1′), 102.9 (C-1″), 100.3 (C-1), 83.6 (C-3′), 80.6 (C-2), 77.7 (C-5′), 74.1 (C-4), 73.7 (C-4′), 72.4 (C-4″), 72.1 (C-3), 72.1 (C-3″), 70.4 (C-2″), 70.2 (C-5″), 69.7 (OCH_2), 68.5 (C-5), 62.4) C-6′), 56.9 (C-2′), 18.0 (C-6), and 17.8 (C-6″).

8-*Hydrazinocarbonyloctyl* 2-O-[2′-*Acetamido*-2′-*deoxy*-3′-O-[α-L-*rhamnopyranosyl*)-β-D-*glucopyranosyl*]-α-L-*rhamnopyranoside* (9).—The de-blocked trisaccharide (8) (200 mg, 0.29 mmol) was dissolved in ethanol (5 cm³) to which 85% hydrazine hydrate (0.5 g) was added. The solution was stirred for 48 h, then evaporated and dried under high vacuum. Purification on silica gel with ethyl acetate–methanol–water (5 : 3 : 2) gave the pure *hydrazide* (9) (180 mg, 90%), $[\alpha]_D$ −47.8° (*c* 1.2, water); R_F 0.53 (solvent same as above); δ(D_2O; 85 °C) 1.00—1.80 [18 H, m, H-6, H-6″, and $-(CH_2)_6-$], 2.09 (3 H, s, *Me*CONH), 2.23 (2 H, t, CH_2-CO), 4.78 (1 H, d, $J_{1,2}$ 8.0 Hz, H-1 2-acetamido-2-deoxy-glucose residue), 4.94 (2 H, br s, H-1 and H-1″), and 3.30—4.00 (16 H, remaining protons); δ_C(D_2O) 103.2 (C-1′), 102.5 (C-1″), 99.8 (C-1), 82.4 (C-3′), 80.0 (C-2), 77.1 (C-5′), 73.5 (C-4), 73.1 (C-4′), 71.9 (C-4″), 71.8 (C-3), 71.4 (C-3″), 70.1 (C-2″), 69.9 (C-5″), 69.4 (OCH_2), 69.3 (C-5), 61.9 (C-6′), 56.9 (C-2′), 17.8 (C-6), and 17.8 (C-6″) (Found: C, 50.8; H, 7.6; N, 6.4. $C_{29}H_{53}N_3O_{15}$ requires C, 50.95; H, 7.8; N, 6.15%).

We wish to thank Mr. J. Christ for technical assistance.

[8/2041 *Received*, 24*th November*, 1978]

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