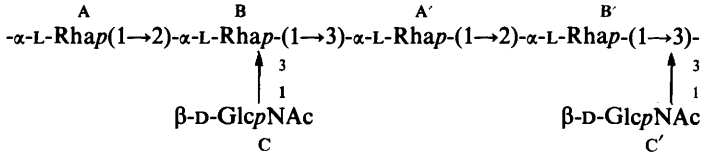
Oligosaccharides Corresponding to the Antigenic Determinants of the β-Haemolytic *Streptococci* Group A. Part 2.† Synthesis and 2D Nuclear Magnetic Resonance Analysis of a Branched Tetrasaccharide Hapten

John S. Andrews and B. Mario Pinto*
Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

The synthesis of a functionalised linear trisaccharide and its use in the synthesis of a branched tetrasaccharide corresponding to a portion of the cell-wall polysaccharide of the β-haemolytic *Streptococci* Group A is described. The acetate groups in the disaccharide, allyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-phthalimido-β-D-glucopyranosyl)-α-L-rhamnopyranoside, were transesterified and the resulting free alcohol groups were protected using 2-(trimethylsilyl)-ethoxymethyl chloride. Deallylation, followed by treatment with *N*,*N*-dimethyl(chloromethylene)-ammonium chloride, afforded the disaccharide chloride. Reaction of this glycosyl donor with allyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside under Königs–Knorr conditions gave the aforementioned linear trisaccharide, allyl 3-*O*-(2'-*O*-benzoyl-4'-*O*-benzyl-3'-*O*-{2''-deoxy-2''-phthalimido-3'',4'',6''-tris-*O*-[2-(trimethylsilyl)ethoxymethyl]-β-D-glucopyranosyl}-α-L-rhamnopyranosyl)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside. Selective removal of the 2'-*O*-benzoate yielded a functionalised trisaccharide for use in the synthesis of the branched tetrasaccharide. Reaction with the glycosyl donor 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide under Königs–Knorr conditions then yielded the fully blocked tetrasaccharide. Transesterification, followed by hydrogenolysis, hydrazinolysis, and selective *N*-acetylation, afforded the pure tetrasaccharide, as its propyl glycoside, for use as a hapten in binding studies and NMR studies.

A programme to synthesize a panel of oligosaccharides corresponding to the antigenic determinants of the β-haemolytic *Streptococci* Group A is in progress.[1] The various oligosaccharides and their corresponding glycoconjugates will be used in inhibition studies, in conjunction with NMR and molecular modelling studies, to identify a surface unique to the *Streptococci* Group A organism. The selected oligosaccharides and/or their complementary monoclonal antibodies will then be used to improve or replace existing immunodiagnostic kits[2] for the detection of *Streptococci* Group A bacteria.

The repeating unit of the *Streptococci* Group A cell-wall polysaccharide is shown below.



Thus far, we have synthesized the disaccharide BC,[1] as its propyl glycoside, and linear A'BC[1] and branched ABC[3] trisaccharides, as their propyl and 8-methoxycarbonyloctyl glycosides. In addition, the synthesis of the disaccharide BC, as its 8-methoxycarbonyloctyl glycoside, has been reported.[4] However, none of these synthetic efforts has afforded a fully functionalised oligosaccharide block that could be used for the elaboration of higher-order structures. It was desirable, therefore, to develop a general synthetic route to higher-order structures. This work describes such a route, in particular the synthesis of a suitably functionalised trisaccharide (A'BC) and its use in the synthesis of a branched tetrasaccharide hapten [AB(C)A'].

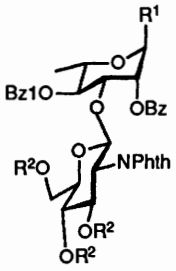
Investigation into the use of the 2-(trimethylsilyl)ethoxymethyl (SEM) group[5] as a viable alcohol-protecting group in carbohydrate chemistry has been carried out in our laboratory.[6] It was discovered that SEM acetals were stable during the removal of benzoate esters and during the isomerisation of the 1-*O*-allyl group and its subsequent hydrolysis. The effect of SEM acetals (on glucosamine) on the reactivity of a glycosyl donor, and its stability during glycosylation reactions, was unknown, however.

We envisaged that it would be possible to synthesize the disaccharide (BC), used in our previous synthesis,[1] with SEM protecting groups at the 3-, 4-, and 6-position of *N*-phthalimido-glucosamine (instead of the previously used acetate or benzyl groups). After manipulation of the disaccharide to give a suitable glycosyl donor, the synthesis of a fully functional linear trisaccharide and subsequent synthesis of the branched tetrasaccharide would then be feasible.

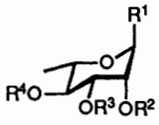
The first stage in the synthesis was the removal of the acetate groups in the disaccharide (**1**)[1] with 3% HCl in methanol to give compound (**2**) in 81% yield. Protection of the alcohol groups as SEM acetals was accomplished by treatment with 2-(trimethylsilyl)ethoxymethyl chloride and the hindered base di-isopropylethylamine,[5] giving compound (**3**) in 70% yield. Isomerisation of the allyl group with tris(triphenylphosphine)rhodium(I) chloride[7,8] gave the prop-1-enyl glycoside (**4**), which was then hydrolysed with mercury(II) chloride–mercury(II) oxide[9] to

† Part 1 is reference 1.

give the epimeric hemiacetals (**5**) in an overall yield of 76%. The epimeric glycosyl chlorides (**6**) were prepared from compounds (**5**) with the Vilsmeier–Haack reagent, *N*,*N*-dimethyl(chloromethylene)ammonium chloride.[10,11] In conjunction with the glycosyl acceptor (**7**)[6] under silver trifluoromethanesulphonate promotion in the presence of 1,1,3,3-tetramethylurea (TMU),[12] the trisaccharide (**10**) was obtained in 81% yield. Selective removal of the 2′-benzoate was accomplished with 0.1M-sodium methoxide to yield a fully functionalised trisaccharide acceptor (**11**) (81% yield) for use in the preparation of the tetrasaccharide (the use of higher concentrations of sodium methoxide resulted in the removal of the SEM groups).



(**1**) $R^1 = OCH_2CH{=}CH_2$, R^2 = Ac
(**2**) $R^1 = OCH_2CH{=}CH_2$, R^2 = H
(**3**) $R^1 = OCH_2CH{=}CH_2$, R^2 = SEM
(**4**) $R^1 = OCH{=}CHCH_3$, R^2 = SEM
(**5**) R^1 = OH, R^2 = SEM
(**6**) R^1 = Cl, R^2 = SEM

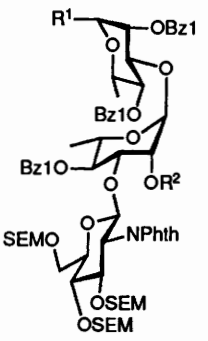


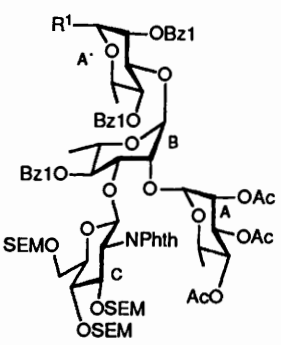
(**7**) $R^1 = OCH_2CH{=}CH_2$ R^2, = R^4 = Bzl, R^3 = H
(**8**) R^1 = Br, $R^2 = R^3 = R^4$ = Ac
(**9**) R^1 = Cl, R^2 = Ac, $R^3 = R^4$ = Bzl

NPhth = *N*- Phthalimido
SEM = $CH_2OCH_2CH_2SiMe_3$

The synthesis of the tetrasaccharide was then attempted under a variety of glycosylating conditions with either 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**8**)[13] or 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride (**9**)[14] as glycosyl donors. The most successful reaction was that with the bromide (**8**) and a mixture of mercury(II) cyanide and mercury(II) bromide as Lewis acid promoters, the tetrasaccharide (**12**) being formed in 48% yield. The poor yields of tetrasaccharide obtained in all the reactions could be due to the presence of a very hindered trisaccharide alcohol (**11**). In support of this hypothesis, removal of the 2′-benzoate from the trisaccharide (**10**) takes typically 50–60 h in 0.1M-NaOMe as compared with 2–3 h for its removal from the monosaccharide, allyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-[2-(trimethylsilyl)ethoxymethyl]-α-L-rhamnopyranoside[6] (in 0.15M-NaOMe). The steric hindrance is presumably due to the presence of the large SEM groups.

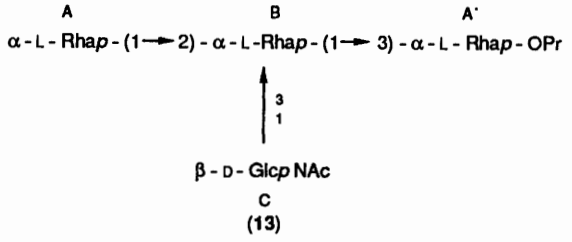
Deprotection of the tetrasaccharide (**12**) by successive (i) removal of SEM and acetate groups with 3% HCl in methanol,[1,6] (ii) palladium-catalysed hydrogenolysis of the benzyl and allyl groups, (iii) removal of the phthalimido group with hydrazine monohydrate, and (iv) selective *N*-acetylation of the resulting free amine with acetic anhydride in methanol afforded the tetrasaccharide as its propyl glycoside (**13**). Successive chromatography on silica gel and Sephadex LH20 then afforded the analytically pure compound (**13**) in 67% yield. Owing to the hygroscopic nature of the compound, a satisfactory combustion microanalysis result was not obtained despite several attempts. Therefore, a plasma desorption mass spectrum[15] was obtained as a confirmation of composition. The peak appearing at *m*/*z* 724.5 was assigned to the M^+ ion of the sodium salt of compound (**13**). $M + Na^+$ ions are commonly observed in plasma desorption mass spectra, particularly of compounds containing labile hydrogens or anionic moieties.[16]





(**10**) $R^1 = OCH_2CH{=}CH_2$, R^2 = Bz
(**11**) $R^1 = OCH_2CH{=}CH_2$, R^2 = H

(**12**) $R^1 = OCH_2CH{=}CH_2$



(**13**)

NMR Analysis.—The assigned structures were in accord with their ^1H and ^{13}C NMR spectral data. Compounds were characterised by use of routine ^1H, ^{13}C, and $^{13}C\{^1H\}$ NMR spectra. ^1H-Homonuclear chemical-shift-correlated (COSY) experiments[17] were performed on compounds (**3**), (**10**), (**12**), and (**13**). The chemical-shift values for individual ring-proton signals within a multiplet were obtained from the COSY cross-peak pattern. Individual vicinal coupling constants were determined from separated signals in the one-dimensional ^1H NMR spectra. ^{13}C–^1H chemical-shift-correlated experiments[18] were performed on compounds (**10**) and (**12**). Only data for the ring protons and carbons are given in the Experimental section for most compounds. Complete data for compounds (**10**), (**12**), and (**13**) are given in Tables 1 and 2.

The stereochemical integrity of the tetrasaccharide (**12**) was confirmed by examination of the one-bond ^{13}C–^1H coupling constants, $^1J(^{13}C–^1H)$, for the anomeric carbons. The values for the rhamnosyl anomeric carbons of rings A′ and B (169 and 168 Hz) were consistent with the presence of an α-L-configuration about the rhamnosyl residues.[19] Both values obtained for the remaining rhamnosyl and *N*-acetylglucosamine anomeric carbons were found to be unusually high, 177 Hz and 165 Hz, respectively. The expected value for the *N*-acetylglucosamine

Table 1. ^{1}H NMR data[a,b] for the ring protons in the trisaccharide (**10**) and tetrasaccharides (**12**) and (**13**).

Compound (Ring)	1-H	2-H	3-H	4-H	5-H	6-H
(10)						
A′	4.76 (1.9)[c]	3.68 (8.6, 3.4)	4.13	3.64	3.64 (6.0)	1.29
B	5.26 (1.5)	5.60 (1.5, 3.2)	4.22 (3.2, 9.5)	3.46 (19.0)[c]	3.76 (9.5, 6.3)	1.08 (6.3)
C	5.42 (7.7)	4.20 (7.8, 10.5)	4.27 (10.8, 8.5)	3.54	3.33 (10.0, 3.2, 1.8)	3.55, 3.63
(12)						
A′	4.77	3.65	4.03 (3.2, 9.2)	3.57	3.64	1.18 (6.9)
B	5.02	4.28	4.08	3.34 (19.2)[c]	3.68	1.04 (6.9)
C	5.40	4.30	4.36 (10.5, 7.7)	3.49	3.73	3.73, 3.70
A	5.41	5.41	5.32	5.02	3.87	0.98 (7.0)
(13)						
A′	4.74 (1.7)	3.96 (1.7, 3.1)	3.76	3.50	3.71	1.25 (6.1)
B	5.09 (1.6)	4.25 (1.6, 3.0)	3.94 (3.0, 9.9)	3.49	3.76	1.25 (6.1)
C	4.70 (8.5)	3.70	3.51	3.42	3.42	3.743 and 3.89 (12.0)
A	5.14 (1.7)	3.99	3.741 (3.3, 9.7)	3.39	3.63	1.22 (6.1)

[a] Chemical shifts (±0.01 ppm) in $CDCl_3$ for compounds (**10**) and (**12**) and in D_2O for (**13**). The numbers in parentheses denote coupling constants, in Hz (±0.1 Hz). [b] Other signals: (**10**) δ_H −0.27 (9 H, s, $SiMe_3$), −0.07 (1 H, m, $CH^aH^bSiMe_3$), −0.01 (18 H, s, 2 × $SiMe_3$), 0.27 (1 H, m, $CH^aH^bSiMe_3$), 0.82 and 0.93 (2 × 2 H, m, 2 × CH_2SiMe_3), 2.99 (2 H, m, $CH_2CH_2SiMe_3$), 3.38 (1 H, m, $CH^aH^bCH_2SiMe_3$), 3.50 ($CH^aH^bCH_2SiMe_3$), 3.60 ($CH_2CH_2SiMe_3$), 3.86 (1 H, m, $OCH^aH^bCH{=}CH_2$), 4.08 (1 H, m, $OCH^aH^bCH{=}CH_2$), 4.32 and 4.50 (AB qs, J 12.0 Hz, OCH_2Ph), 4.34 (2 H, t, OCH_2Ph or $OCH_2OCH_2CH_2SiMe_3$), 4.62 (2 H, s, $OCH_2OCH_2CH_2SiMe_3$) or OCH_2Ph), 4.64 and 5.10 (AB qs, J 12.0 Hz, OCH_2Ph), 4.70 and 4.72 (AB qs, J 6.3 Hz, $OCH_2OCH_2CH_2SiMe_3$), 4.72 (2 H, s, OCH_2Ph or $OCH_2OCH_2CH_2SiMe_3$), 5.10 (1 H, m, J_{cis} 10.5 Hz, $OCH_2CH{=}CH^ZH^E$), 5.18 (1 H, m, J_{trans} 17.5 Hz, $OCH_2CH{=}CH^ZH^E$), and 5.80 (1 H, m, $OCH_2CH{=}CH_2$). For compound (**12**) δ_H −0.27 (9 H, s, $SiMe_3$), −0.13 (1 H, m, $CH^aH^bSiMe_3$), −0.01 (18 H, s, 2 × $SiMe_3$), 0.28 (1 H, m, $CH^aCH^bSiMe_3$), 0.80 and 0.90 (2 × 2 H, m, 2 × CH_2SiMe_3), 3.00 and 3.39 (2 × 2 H, m, 2 × $CH_2CH_2SiMe_3$), 3.57 ($CH^aH^bCH_2SiMe_3$), 3.67 ($CH^aH^bCH_2SiMe_3$), 3.87 (1 H, m, $OCH^aH^bCH{=}CH_2$), 4.08 (1 H, m, $OCH^aH^bCH{=}CH_2$), 4.16 and 4.29 (AB qs, J 12.0 Hz, OCH_2Ph), 4.42 and 4.45 (AB qs, J 7.0 Hz, $OCH_2OCH_2CH_2SiMe_3$), 4.53 and 4.77 (AB qs, J 11.5 Hz, OCH_2Ph), 4.61 (2 H, s, OCH_2Ph or $OCH_2OCH_2CH_2SiMe_3$), 4.68 and 4.71 (AB qs, J 6.0 Hz, $OCH_2OCH_2CH_2SiMe_3$), 4.77 (2 H, s, $OCH_2OCH_2CH_2SiMe_3$ or OCH_2Ph), 5.10 (1 H, m, J_{cis} 10.5 Hz, $OCH_2CH{=}CH^ZH^E$), 5.18 (1 H, m, J_{trans} 17.5 Hz, $OCH_2CH{=}CH^ZH^E$), and 5.79 (1 H, m, $OCH_2CH{=}CH_2$). For compound (**13**) δ_H 0.90 (3 H, t, OCH_2CH_2Me), 1.57 (2 H, m, OCH_2CH_2Me), 3.47 and 3.64 (2 H, AB qs, OCH_2Et), and 1.99 (3 H, s, $COMe$). [c] These values are the sum of the individual coupling constants, $J_{AX} + J_{BX}$.

Table 2. ^{13}C NMR data[a,b] for the ring carbons in the trisaccharide (**10**) and tetrasaccharide (**12**).

Compound (Ring)	C-1	C-2	C-3	C-4	C-5	C-6
(10)						
A′	96.8	77.2	77.8	80.9	68.3	17.9
B	98.6	72.4	79.4	79.5	68.0	17.9
C	99.0	56.0	79.8	77.1	74.6	66.8
(12)						
A′	96.5 (169)[c]	77.6	77.8	80.2	68.2	17.3[d]
B	99.6 (168)	78.1	80.9	79.4	68.5[e]	17.7[d]
C	99.1 (165)	56.0	79.8	*f*	74.4	66.9
A	100.8 (177)	69.8	69.2[e]	71.5	66.7	17.8[d]

[a] Chemical shifts (±0.1 ppm) in $CDCl_3$. [b] Other signals: For compound (**10**) δ_C −1.7, −1.42, and 1.36 (3 × SiMe), 17.2, 18.0, and 18.1 (3 × CH_2SiMe_3), 65.0, 65.6, and 66.3 (3 × CH_2CH_2Me), 67.7 ($OCH_2CH{=}CH_2$), 72.7, 74.4, and 75.3 (3 × OCH_2Ph), 95.7, 96.5, and 97.2 (3 × $OCH_2OCH_2CH_2SiMe_3$), 116.8 ($OCH_2CH{=}CH_2$), 133.5 ($OCH_2CH{=}CH_2$), and 165.7 ($OCOPh$). For compound (**12**) δ_C −1.8 ($SiMe_3$), −1.4 (2 × $SiMe_3$), 17.1 (CH_2SiMe_3), 18.1 (2 × CH_2SiMe_3), 20.8 (2 × $OCOMe$), 21.1 ($OCOMe$), 64.9, 65.7, and 66.3 (3 × $CH_2CH_2SiMe_3$), 67.8 ($OCH_2CH{=}CH_2$), 72.5, 74.4, and 75.3 (3 × OCH_2Ph), 95.2, 96.5, and 97.1 (3 × $OCH_2OCH_2CH_2SiMe_3$), 116.9 ($OCH_2CH{=}CH_2$), 133.5 ($OCH_2CH{=}CH_2$), 165.7, 169.7, 169.9, and 170.14 (NCO and $OCOMe$). [c] Values in parentheses are the $^1J(^{13}C{-}^1H)$ coupling constants in Hz (±1 Hz). [d,e] Assignments may be interchanged. [f] Obscured by $CDCl_3$ peaks.

carbon is 160–162 Hz.[19] The unexpectedly high values obtained may be explained by the fact that the glycosidic linkages between rings B–C and B–A′ are strained due to steric hindrance. It has been found that $^{13}C{-}^1H$ couplings are directly proportional to the degree of s-character in the anomeric carbon–hydrogen bond.[20] We propose that the increased bond strain causes the anomeric carbon to be more sp^2-like in nature thus giving rise to a greater $^{13}C{-}^1H$ coupling. The values of 177 Hz and 165 Hz are still consistent, therefore, with an α-L-configuration about the rhamnosyl residue, and a β-D-configuration about the *N*-acetylglucosamine residue, respectively. Indeed, examination of the $^{13}C{-}^1H$ coupling constants in the spectrum of the deprotected tetrasaccharide (**13**) revealed 'normal' values of 169, 169, and 170 Hz for the rhamnosyl anomeric carbons and a value of 162 Hz for the *N*-acetylglucosamine anomeric carbon.

A COSY spectrum of the deprotected tetrasaccharide (**13**) was used to identify the individual spin systems (see Figures 1 and 2). The ring-proton signals for the *N*-acetylglucosamine ring were clearly identified by their characteristic coupling constants and splitting patterns. The signal at δ 4.74 was assigned to 1-H of the A′-ring, based on the expected shielding of 1-H when C-1 is attached to an acyclic aglycone. The signal at δ 5.14 was assigned to 1-H of the A ring based on chemical-shift

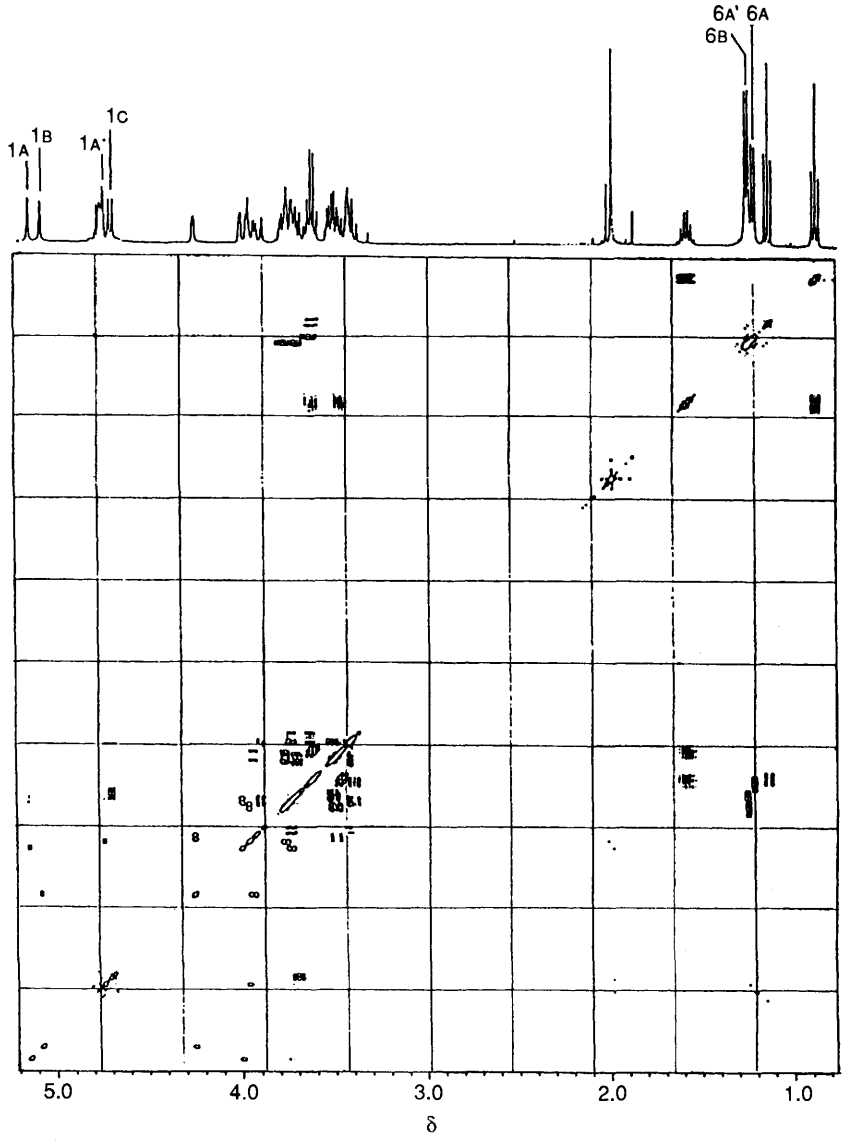


Figure 1. Two-dimensional ^{1}H NMR COSY spectrum of the deprotected tetrasaccharide (**13**).

correlations with the spectra of previously synthesized related structures,[1-4] and the natural polymer.[21] Having assigned these marker signals to the A and A′ rings, the unambiguous assignment of all the A and A′ ring-proton signals was achieved by tracing the COSY cross-peak patterns (see Figure 2). The remaining proton signals were then assigned to the B-ring. Similarly, chemical-shift correlations of the ^{13}C NMR signals in the spectrum of compound (**13**) with those in the spectra of related structures [1-4] permitted assignment of most of the signals.

The complete assignment of the ^{1}H NMR spectrum of compound (**13**) is a necessary prerequisite to the study of the conformational preferences of this hapten. These studies will mainly take the form of difference NOE experiments to furnish information on H–H contacts. Such information will be used, in conjunction with molecular modelling studies, to infer a model of hapten conformation and to identify possible topographical surfaces unique to the β-haemolytic *Streptococci* Group A cell-wall polysaccharide.

Experimental

General Procedures.—^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded with a Bruker WM-400 NMR spectrometer. 1D NMR spectra were acquired with 32K data sets for both ^{1}H and ^{13}C spectra. Spectra were measured in deuteriochloroform (40 mg/ml) for the protected compounds and in deuterium oxide (20 mg/ml) for the deprotected compound; the chemical shifts are given downfield from Me_4Si and sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS), respectively. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. The ^{1}H homonuclear chemical-shift-correlated (COSY) spectra were acquired with 2K × 1K data sets with 512 experiments. The ^{13}C–^{1}H chemical-shift spectra were acquired with 2K × 1K data sets and 128 experiments.

The CF-252 plasma desorption mass spectrum was obtained on a BIN-10K instrument from BIO-ION Nordic (Uppsala, Sweden). The sample was prepared in a solution of aq. methanol and electrosprayed onto aluminium foils. The spectrum was acquired and the mass was assigned using the BIO-ION data system, based upon the PDP 11/73 processor. The experimental masses were obtained by determination of the time centroid of each peak above the baseline and by comparison of these with the times of flight of H^+ and Na^+ peaks appearing in the

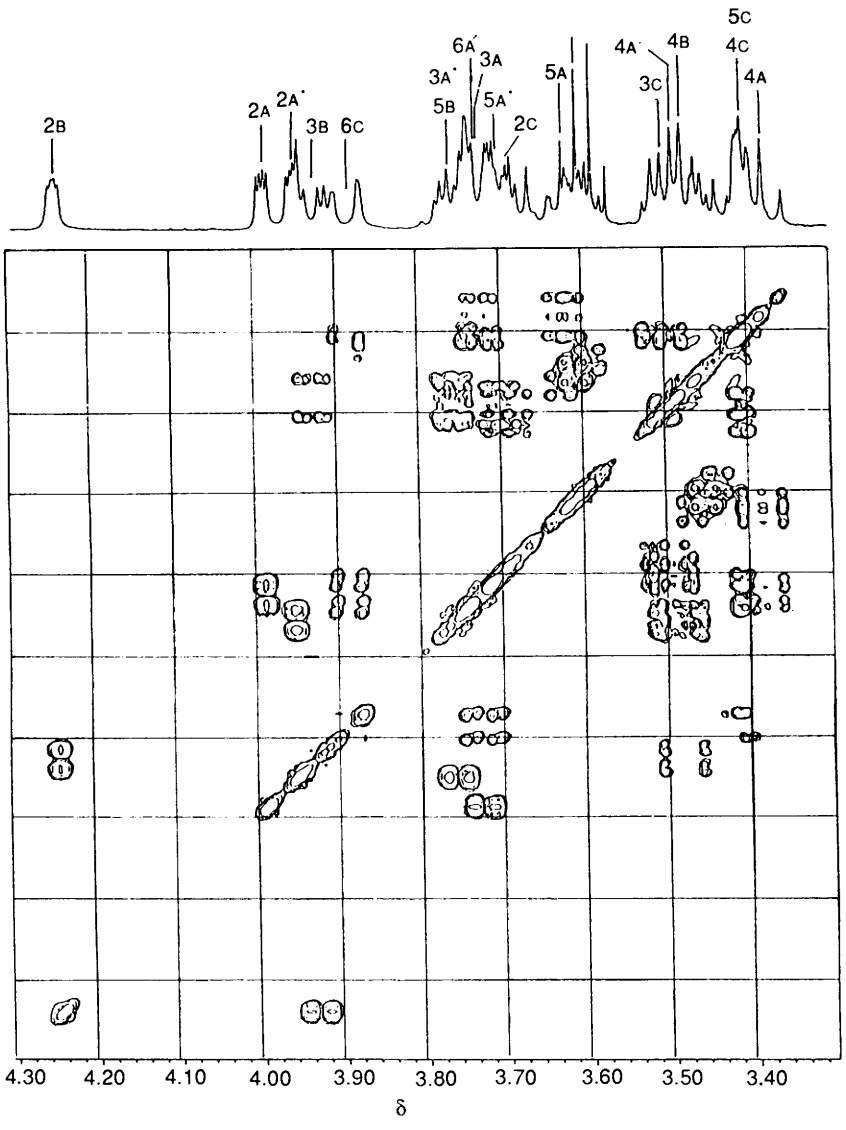


Figure 2. Expanded region of the two-dimensional ^{1}H NMR COSY spectrum of the deprotected tetrasaccharide (13).

spectrum. Mass accuracy is approximately ± 1 amu in the mass range 500–1 000 amu.

Optical rotations were measured on a Perkin-Elmer P22 spectropolarimeter.

Analytical TLC was performed on pre-coated aluminium foil plates with Merck silica gel 60-F_{254} as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with 10% sulphuric acid in ethanol and heated at 150 °C. All compounds were purified by medium-pressure column chromatography on Kieselgel 60 (230–400 mesh) according to a published procedure.[22] High-performance liquid chromatography (HPLC) was performed at 4 MPa on a Waters Associates Prep LC/system 500 instrument with two Prep PAK-500 silica gel normal-phase columns and a refractive-index detector.

Solvents were distilled before use and were dried, as necessary, by literature procedures. Work-up of solutions involved evaporation under reduced pressure below 40 °C.

Reactions performed under nitrogen were carried out in deoxygenated solvents. Transfers under nitrogen were effected by means of standard Schlenk-tube techniques.

Specific Procedures.—*Allyl* 2-O-*Benzoyl*-4-O-*benzyl*-3-O-(2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**2**).—A solution of the disaccharide (**1**)[4] (2.2 g, 2.7 mmol) in methanolic HCl (3%; 80 ml) [prepared by treating anhydrous methanol (80 ml) with acetyl chloride (4.6 ml)] was stirred under nitrogen for 10 h. TLC [hexane–ethyl acetate–methanol (4:4:0.5)] indicated that the starting material had been consumed. The reaction mixture was neutralised with triethylamine and the solvent was removed to give a syrup and triethylammonium chloride. The mixture was dissolved in ethyl acetate and washed with distilled water to remove the salt, and dried (Na_2SO_4). Evaporation of the solvent gave a syrup, which was chromatographed with hexane–ethyl acetate–methanol [(4:4:0.5) R_f 0.26] as eluant. The *title compound* (**2**) was obtained as a white foam (1.53 g, 81%); $[\alpha]_D^{22}$ −89.5° (*c* 1.05 in CH_2Cl_2); δ_H 1.12 (3 H, d, $J_{5,6}$ 6.3 Hz, 6-H_3), 3.36 (1 H, t, $J_{3,4}$ + $J_{4,5}$ = 18.0 Hz, 4-H), 3.45 (1 H, t, $J_{3',4'}$ + $J_{4',5'}$ = 19.0 Hz, 4′-H), 3.50 (1 H, ddd, $J_{4',5'}$ 9.75, $J_{5',6'a}$ 2.0, $J_{5',6'b}$ 5.0 Hz, 5′-H), 3.59 (1 H, dd, $J_{5',6'b}$ 5.0, $J_{6'b,6'a}$ 12.5 Hz, 6′-H^b), 3.73 (1 H, dq, $J_{4,5}$ 9.0, $J_{5,6}$ 6.3 Hz, 5-H), 3.88 (1 H, dd, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ 12.5 Hz, 6′-H^a), 4.04 (1 H, dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 11.0 Hz, 2′-H), 4.15 (1 H, dd, $J_{2,3}$ 3.3, $J_{3,4}$ 9.5 Hz, 3-H), 4.18 (1 H, dd, $J_{2',3'}$ 11.0, $J_{3',4'}$ 9.0 Hz, 3′-H), 4.76 (1 H, s, $J_{1,2}$ 1.8 Hz, 1-H), 5.53 (1 H, d, $J_{1',2'}$ 8.0 Hz, 1′-H), and 5.68 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.3 Hz, 2-H); δ_C 17.9 (C-6), 56.8 (C-2′), 61.8

(C-6'), 67.5 (C-5), 71.3 (C-4'), 71.8 (C-3'), 72.6 (C-2), 76.3 (C-5'), 79.1 (C-3), 80.2 (C-4), 96.5 (C-1), and 99.3 (C-1') (Found: C, 64.3; H, 5.7; N, 2.0. $C_{37}H_{39}NO_{12}$ requires C, 64.27; H, 5.70; N, 2.03%).

Allyl 2-O-*Benzoyl*-4-O-*benzyl*-3-O-{2'-*deoxy*-2'-*phthalimido*-3',4',6'-*tris*-O-[2-(*trimethylsilyl*)*ethoxymethyl*]-β-D-*glucopyranosyl*}-α-L-*rhamnopyranoside* (**3**).—A mixture of the disaccharide (**2**) (1.77 g, 2.56 mmol), di-isopropylethylamine (3.1 ml, 17.8 mmol), and SEMCl (2.1 ml, 11.9 mmol) in anhydrous dichloromethane (4 ml) was stirred under nitrogen at room temperature for 55 h. The reaction mixture was diluted with dichloromethane and washed successively with 0.5M-HCl (× 2), water, saturated aq. sodium hydrogen carbonate, and aq. sodium chloride. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup, which was chromatographed with hexane–ethyl acetate [(4:1) R_f 0.35] to yield the *title compound* (**3**) as a white foam (1.93 g, 70%); $[\alpha]_D^{22}$ +8.8° (*c* 1.29 in CH_2Cl_2); δ_H 1.09 (3 H, d, $J_{5,6}$ 6.0 Hz, 6-H_3), 3.45 (1 H, t, $J_{3,4}$ + $J_{4,5}$ = 18.0 Hz, 4-H), 3.49–3.76 (9H, complex m, 3.53 [$OCH_2OCH_2CH_2Si$], 3.54 [$J_{3',4'}$ + $J_{4',5'}$ = 19.0 Hz, 4'-H], 3.58 [5'-H], 3.60 [$OCH_2OCH_2CH_2Si$], 3.68 [5-H], 3.73 [6'-H^a], 3.76 [6'-H^b], 4.18 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, 3-H), 4.20 (1 H, dd, $J_{1',2'}$ 7.5, $J_{2',3'}$ 11.0 Hz, 2'-H), 4.32 (1 H, dd, $J_{2',3'}$ 11.0, $J_{3',4'}$ 8.0 Hz, 3'-H), 4.90 (1 H, d, $J_{1,2}$ 1.6 Hz, 1-H), 5.44 (1 H, dd, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.5 Hz, 2-H), and 5.45 (1 H, d, $J_{1',2'}$ 7.5 Hz, 1'-H); δ_C 17.9 (C-6), 56.2 (C-2'), 67.3 (C-6'), 68.3 (C-5), 73.0 (C-2), 74.7 (C-5'), 79.4 (C-4 and -3), 79.9 (C-3'), 96.6 (C-1), and 99.3 (C-1') (Found: C, 61.1; H, 7.5; N, 1.4. $C_{55}H_{81}NO_{15}Si_3$ requires C, 61.14; H, 7.56; N, 1.30%).

2-O-*Benzoyl*-4-O-*benzyl*-3-O-{2'-*deoxy*-2'-*phthalimido*-3',4',6'-*tris*-O-[2-(*trimethylsilyl*)*ethoxymethyl*]-β-D-*glucopyranosyl*}-L-*rhamnopyranose* (**5**).—Tris(triphenylphosphine)-rhodium(I) chloride (0.166 g, 0.179 mmol) and 1,4-diazabicyclo[2.2.2]octane (0.086 g, 0.768 mmol) were added to a solution of the allyl glycoside (**3**) (1.93 g, 1.786 mmol) in ethanol–water (9:1; 70 ml) and the mixture was refluxed lightly for 12 h under nitrogen. The solvent was removed by evaporation to give a dark brown residue, which was taken up in ethyl acetate and filtered through a short column of silica gel. Removal of the solvent gave a light brown foam (**4**), which was dissolved in 90% aqueous acetate (98 ml) and the solution was stirred. Yellow mercury(II) oxide (0.387 g, 1.78 mmol) was added followed by the dropwise addition, during 5 min, of a solution of mercury(II) chloride (0.392 g, 1.78 mmol) in 90% aqueous acetone (5 ml). The mixture was stirred for 12 h, the solvent was evaporated off, and the resulting residue was dissolved in ethyl acetate and filtered through Celite. The filtrate was washed successively with aq. potassium iodide (× 2), aq. sodium thiosulphate (× 2), and water (× 2). The organic layer was dried (Na_2SO_4), the solvent was evaporated off, and the resulting yellow foam was chromatographed with hexane–ethyl acetate [(2:1) R_f 0.25] as eluant. The title compound (**5**) was obtained as a white foam (1.42 g, 76%) and was used directly in the next step; δ_H 1.08 (3 H, d, $J_{5,6}$ 6.2 Hz, 6-H_3), 3.45 (1 H, t, $J_{3,4}$ + $J_{4,5}$ = 18.4 Hz, 4-H), 3.49–3.70 (6 H, complex m, 4'- and 5'-H, 2 × $OCH_2OCH_2CH_2Si$), 3.70 (1 H, dd, $J_{5',6'b}$ 4.5, $J_{6'a,6'b}$ 11.5 Hz, 6'-H^b), 3.79 (1 H, dd, $J_{5',6'a}$ 1.8, $J_{6'a,6'b}$ 11.5 Hz, 6'-H^a), 3.91 (1 H, m, $J_{4,5}$ 9.3, $J_{5,6}$ 6.2 Hz, 5-H), 4.19 (1 H, dd, $J_{1',2'}$ 8.5, $J_{2',3'}$ 11.0 Hz, 2'-H), 4.25 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.2 Hz, 3-H), 4.33 (1 H, dd, $J_{2',3'}$ 11.0, $J_{3',4'}$ 8.5 Hz, 3'-H), 5.26 (1 H, br s, 1-H), 5.45 (1 H, d, $J_{1',2'}$ 8.5 Hz, 1'-H), and 5.48 (1 H, dd, $J_{1,2}$ 1.9, $J_{2,3}$ 3.5 Hz, 2-H).

Allyl 3-O-(2'-O-*Benzoyl*-4'-O-*benzyl*-3'-O-{2''-*deoxy*-2''-*phthalimido*-3'',4'',6''-*tris*-O-[2-(*trimethylsilyl*)*ethoxymethyl*]-β-D-*glucopyranosyl*}-α-L-*rhamnopyranosyl*)-2,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (**10**).—Oxalyl chloride (0.06 ml, 0.685 mmol) was added to a stirred solution of DMF (0.055 ml, 0.685 mmol) in anhydrous dichloromethane (0.5 ml) and the reaction mixture was stirred under nitrogen for 5 min. The solvent was evaporated off under reduced pressure and the white salt was dried *in vacuo* for 1 h. The *N*,*N*-dimethyl(chloromethylene)ammonium chloride was then dissolved in anhydrous dichloromethane (2 ml) containing pyridine (0.06 ml), and a solution of the hemiacetals (**5**) (0.143 g, 0.137 mmol) in anhydrous dichloromethane (1 ml) was transferred to the flask under nitrogen by means of a cannula. The flask was rinsed with additional portions of solvent which was then transferred as before. The mixture was stirred under nitrogen for 2 h, and then the reaction was quenched by the addition of cold aq. sodium hydrogen carbonate (10 ml). The organic layer was diluted with dichloromethane and washed successively with 0.5M-hydrochloric acid, aq. sodium hydrogen carbonate, and aq. sodium chloride. The organic layer was dried over anhydrous potassium carbonate and the solvent was evaporated off to give the disaccharide chloride (**6**) as a dark brown syrup, which was then dried *in vacuo* for 5 h.

A mixture of allyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**7**)[6] (0.036 g, 0.094 mmol), silver trifluoromethanesulphonate (0.042 g, 0.164 mmol), TMU (0.03 ml, 0.233 mmol), and 4 Å molecular sieves in anhydrous dichloromethane (1 ml) was stirred in the dark under nitrogen for 0.5 h in a Schlenk tube fitted with a dropping funnel which was equipped with a cooling jacket. A solution of the glycosyl chloride (**6**) in anhydrous dichloromethane (1 ml), previously stirred with 4 Å molecular sieves for 0.5 h under nitrogen, was transferred to the dropping funnel by means of a cannula. The flask was rinsed with additional portions of anhydrous dichloromethane (3 × 0.5 ml) which were then transferred as before. The solution of the glycosyl chloride (**6**) was cooled to −78 °C (acetone–solid CO_2) and added dropwise, during 10 min, to the cooled (−78 °C) mixture containing compound (**7**). The dropping funnel was rinsed with additional portions of anhydrous dichloromethane (2 × 0.5 ml). The mixture was stirred under nitrogen in the dark and slowly allowed to attain room temperature. After 40 h, TLC [hexane–ethyl acetate (3:1)] indicated the reaction to be complete. The solids were removed by filtration and the filtrate was diluted with dichloromethane and washed successively with 0.2M-hydrochloric acid, aq. sodium hydrogen carbonate, and aq. sodium chloride. The organic layer was dried (Na_2SO_4) and the solvent was evaporated off to yield a syrup, which was chromatographed with hexane–ethyl acetate (4:1) as eluant (R_f 0.40). The *title compound* (**10**) was obtained as a white foam (0.106 g, 81%); δ_H: see Table 1; δ_C: see Table 2 (Found: C, 63.95; H, 7.3; N, 1.3. $C_{75}H_{103}NO_{19}Si_3$ requires C, 64.03; H, 7.38; N, 1.0%).

Allyl 2,4-*Di*-O-*benzyl*-3-O-(4'-O-*benzyl*-3'-O-{2''-*deoxy*-2''-*phthalimido*-3'',4'',6''-*tris*-O-[2-(*trimethylsilyl*)*ethoxymethyl*]-β-D-*glucopyranosyl*}-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyranoside* (**11**).—A sample of compound (**10**) (0.439 g, 0.312 mmol) was dissolved in freshly prepared 0.1M-NaOMe (5 ml) and the solution was stirred at room temperature under nitrogen for 47 h. TLC [hexane–ethyl acetate (3:1)] showed most of the starting material to have been consumed and the presence of both a relatively highly polar side-product (removal of SEM groups; R_f (0.0) and the desired product (R_f 0.38). The reaction mixture was worked up by a quench in ice-cold 0.2M-HCl, extraction with dichloromethane (4 × 15 ml), and successive washes with aq. sodium hydrogen carbonate and aq. sodium chloride, followed by drying (Na_2SO_4). The solvent was removed to yield a light yellow syrup, which was purified by column chromatography with hexane–ethyl acetate [(3:1) R_f 0.38] as eluant to yield the *title compound* (**11**) as a white foam (0.330 g, 81%); $[\alpha]_D^{22}$ −3.7° (*c* 1.29 in CH_2Cl_2); δ_H 1.06 (3 H, d,

$J_{5',6'}$ 6.1 Hz, 6'-H_3), 1.30 (3 H, d, $J_{5,6}$ 6.0 Hz, 6-H_3), 3.27 (1 H, br s, 2'-OH), 3.46–3.77 (12 H, complex m, 2-, 4-, 4'-, 4''-, 5-, 5'-, 5''-, 6''-H^a, and 6''-H^b, $OCH_2OCH^aH^bCH_2Si$, and $OCH_2OCH_2CH_2$-Si), 3.83 (1 H, m, $OCH^aH^bCH{=}CH_2$), 3.95 (1 H, dd, $J_{2,3}$ 3.1, $J_{3,4}$ 9.0 Hz, 3-H), 4.09 (2 H, complex m, $J_{2',3'}$ 3.4, $J_{3',4'}$ 9.1 Hz, 3'-H and $OCH^aH^bOCH{=}CH_2$), 4.19 (1 H, br s, 2'-H), 4.25–4.35 (2 H, complex m, [$J_{1'',2''}$ 8.1, $J_{2'',3''}$ 9.5 Hz, 2''- and 3''-H), 4.77 (1 H, d, $J_{1,2}$ 1.8 Hz, 1-H), 5.21 (1 H, s, 1'-H), and 5.40 (1 H, d, $J_{1'',2''}$ 8.0 Hz, 1''-H); δ_C 17.7 (C-6'), 17.84 (C-6), 55.7 (C-2''), 66.9 (C-6''), 67.6 and 67.7 ($OCH_2CH{=}CH_2$ + C-5'), 68.2 (C-5), 69.8 (C-2'), 74.4 (C-5'' + OCH_2Ph), 77.5 (C-4''), 77.8 (C-2 + -3), 79.0 (C-4'), 79.8 (C-3''), 80.8 (C-4), 82.9 (C-3'), 96.7 (C-1), 98.1 (C-1''), and 101.1 (C-1') (Found: C, 62.7; H, 7.6; N, 1.3. $C_{68}H_{99}NO_{18}Si_3$ requires C, 62.69; H, 7.66; N, 1.08%).

Allyl 2,4-*Di*-O-*benzyl*-3-O-(4'-O-*benzyl*-3'-O-{2'''-*deoxy*-2'''-*phthalimido*-3''',4''',6'''-*tris*-O-[2-(*trimethylsilyl*)-*ethoxymethyl*]-β-D-*glucopyranosyl*}-2'-O-(2'',3'',4''-*tri*-O-*acetyl*-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyrano side* (**12**).—A mixture of the trisaccharide alcohol (**11**) (0.048 g, 0.037 mmol), $Hg(CN)_2$ (0.029 g, 0.112 mmol), $HgBr_2$ (0.403 g, 0.112 mmol), and 4Å molecular sieves in anhydrous dichloromethane (1 ml) was stirred in the dark under nitrogen for 0.5 h in a Schlenk tube fitted with a dropping funnel which was equipped with a cooling jacket. A solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**8**)[13] in anhydrous dichloromethane (1 ml), previously stirred with 4Å molecular sieves for 0.5 h under nitrogen, was transferred to the dropping funnel under nitrogen by means of a cannula. The flask was rinsed with additional portions of anhydrous dichloromethane (3 × 0.5 ml) and these portions were transferred as before. The solution of the glycosyl bromide was cooled to −78 °C (acetone–solid CO_2) and added dropwise, during 10 min, to the cooled (−78 °C) mixture containing compound (**11**). The dropping funnel was rinsed with additional portions of anhydrous dichloromethane (2 × 0.5 ml). The mixture was stirred under nitrogen in the dark and slowly allowed to attain room temperature. After 24 h, TLC [hexane–ethyl acetate (3:1)] indicated the reaction to be complete. The solids were removed by filtration and the filtrate was diluted with dichloromethane and washed successively with aq. potassium iodide and aq. sodium chloride. The organic layer was dried (Na_2SO_4) and the solvent was evaporated off to yield a syrup, which was chromatographed with hexane–ethyl acetate (3:1) as eluant, R_f 0.35. The *title compound* (**12**) was obtained as a clear syrup (0.028 g, 48%); $[\alpha]_D^{22}$ −10.6° (*c* 0.9 in CH_2Cl_2); δ_H: see Table 1; δ_C: see Table 2 (Found: C, 60.9; H, 7.1; N, 0.8. $C_{80}H_{115}NO_{25}Si_3$ requires C, 61.01; H, 7.36; N, 0.89%).

Propyl 3-O-[3'-O-(2''-*Acetamido*-2''-*deoxy*-β-D-*glucopyranosyl*)-2'-O-(α-L-*rhamnopyransoyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranoside* (**13**).—The protected tetrasaccharide (**12**) (0.037 g, 0.0235 mmol) was dissolved in 3% HCl in methanol (3 ml) and the solution was stirred under nitrogen at room temperature. TLC [hexane–ethyl acetate–methanol (2:3:1)] after 14 h showed the reaction to be complete. The reaction mixture was worked up by dilution with methanol and neutralisation with Rexyn 201 (OH^-) beads. After filtration, the solution was concentrated to give an amorphous solid, which was chromatographed on silica gel with hexane–ethyl acetate–methanol [(0.2:4:0.5) R_f 0.27] to yield the partially deprotected tetrasaccharide (acetate and SEM groups removed) (0.019 g, 76%). This was then dissolved in a mixture of 80% aq. acetic acid (2.5 ml) and 90% ethanol (1 ml) and hydrogenolysed over 10% palladium–carbon (0.027 g) at a hydrogen pressure of 51 psi for 65 h. The mixture was filtered through Celite and concentrated to give an amorphous solid; the acetic acid was removed by repeated co-distillation with 100% ethanol. The solid residue was dissolved in 100% ethanol (2.5 ml) containing 98% hydrazine monohydrate (0.001 ml) and the solution was refluxed under nitrogen. After 14 h TLC [ethyl acetate–methanol–water (7:2:0.5)] indicated the reaction to be complete (R_f 0.1). The solids were removed by filtration through Celite and the solvent was removed to give a yellow glass, which was dissolved in methanol (1.5 ml) containing acetic anhydride (0.15 ml) and the solution was stirred for 1.5 h. Excess of acetic anhydride was removed at 30 °C by repeated co-distillation with methanol and the acetic acid formed was removed in the usual manner to give a white amorphous solid, which was purified by column chromatography on silica gel with ethyl acetate–methanol–water (7:2:1) as eluant. (R_f 0.37). The resulting clear syrup was then further purified by gel filtration on Sephadex LH20 with methanol as eluant. The *title compound* (**13**) was obtained as a clear glass [0.011 g, 67%, based on protected tetrasaccharide used (**12**)]; $[\alpha]_D^{25}$ −52.3° (*c* 0.77 in water); δ_H: see Table 1; δ_C: 12.6 ($OCH_2CH_2CH_3$), 19.3 and 19.4 (C-6, −6', and -6''), 24.8 (OCH_2CH_2Me), 25.1 ($NHCOCH_3$), 58.7 (C-2'''), 63.8 (C-6'''), 71.5 and 71.9 (C-5 and -5''), 72.1 (C-5'), 72.5, 72.8, 72.85, and 72.9 (C-2, -2', -2'', C-4''', and OCH_2Et), 74.0 (C-4''), 74.6 (C-4'), 74.9 (C-4), 76.7 (C-3'''), 78.6 (C-5'''), 79.1 and 80.4 (C-3 and -3'), 82.4 (C-3''), 102.4 [$^1J(^{13}C,^1H)$ 169 Hz, C-1]), 103.8 [$^1J(^{13}C,^1H)$ 170 Hz, C-1''], 104.4 [$^1J(^{13}C,^1H)$ 169 Hz, C-1'], 105.2 [$^1J(^{13}C,^1H)$ 162 Hz, C-1'''], and 177.5 (NH*C*OMe) (PD-MS Found: *m/z* 724 $(M\text{Na})^+$. $C_{29}H_{51}NNaO_{18}$ requires *m/z* 724).

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