Oligosaccharides Corresponding to Biological Repeating Units of *Shigella flexneri* Variant Y Polysaccharide: Part 3. Synthesis and 2D-Nuclear Magnetic Resonance Analysis of a Heptasaccharide Hapten

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The block synthesis of a heptasaccharide portion of the biological repeating unit, [2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1], of the *Shigella flexneri* variant Y polysaccharide is described. The synthetic strategy relies on the use of the key trisaccharide intermediate α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*, both as a glycosyl acceptor and as a donor. Thus, the trisaccharide bromide in conjunction with the β-D-Glc*p*NPhth-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p* unit under Helferich conditions yielded the blocked heptasaccharide in 86% yield. The latter unit was obtained, in turn, from the key trisaccharide intermediate functioning as an acceptor molecule. Attempts at coupling the tetrasaccharide donor, α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NPhth, with the trisaccharide acceptor α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*, to give the heptasaccharide under a variety of conditions were unsuccessful. The blocked derivatives were synthesized as their allyl glycosides. Removal of the blocking groups, hydrogenation of the allyl group, and *N*-acetylation yielded the heptasaccharide hapten, α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*, as its propyl glycoside, for use in inhibition studies with complementary monoclonal antibodies, and in NMR and X-ray studies. The detailed NMR analysis of the protected and deprotected heptasaccharides by use of two-dimensional NMR techniques is also described.

Molecular recognition processes that are mediated by carbohydrate recognition markers are widespread and range from antibody–antigen interaction to cell–cell recognition and development. We have chosen the study of antibody–antigen interactions using bacterial antigens with which to probe such recognition processes. The work holds promise for defining the requirements of synthetic vaccines and immunodiagnostic reagents in animal and human diseases. In this connection, we have recently described[1,2] a programme to synthesize higher order oligosaccharides corresponding to the biological repeating unit[3] of the lipopolysaccharide *O*-antigen of the bacterium *Shigella flexneri* variant Y.[4] These oligosaccharides were intended to elucidate the nature of the extended conformational surface of possible importance in recognition by antibodies. Knowledge of such surfaces and the associated topographical features would derive from NMR experiments, in conjunction with theoretical calculations.[5,6]

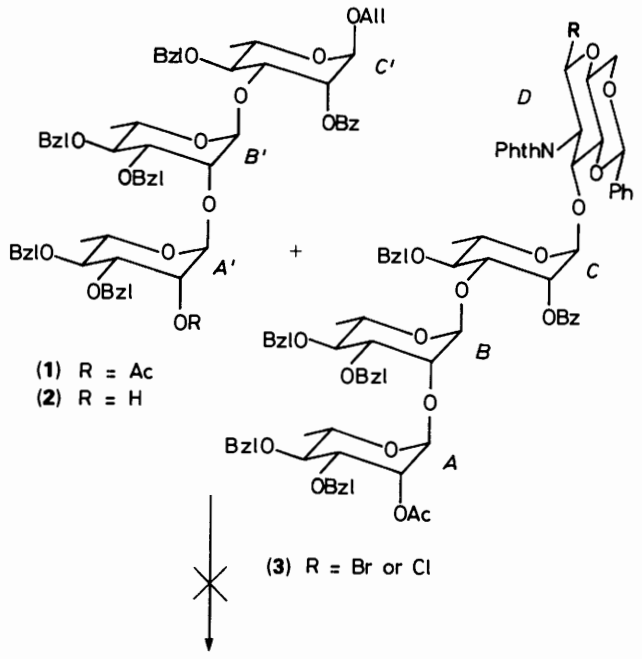
The biological repeating unit[3] of the lipopolysaccharide *O*-antigen of the bacterium *Shigella flexneri* variant Y[4] has the following structure:

[2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1]
A *B* *C* *D*

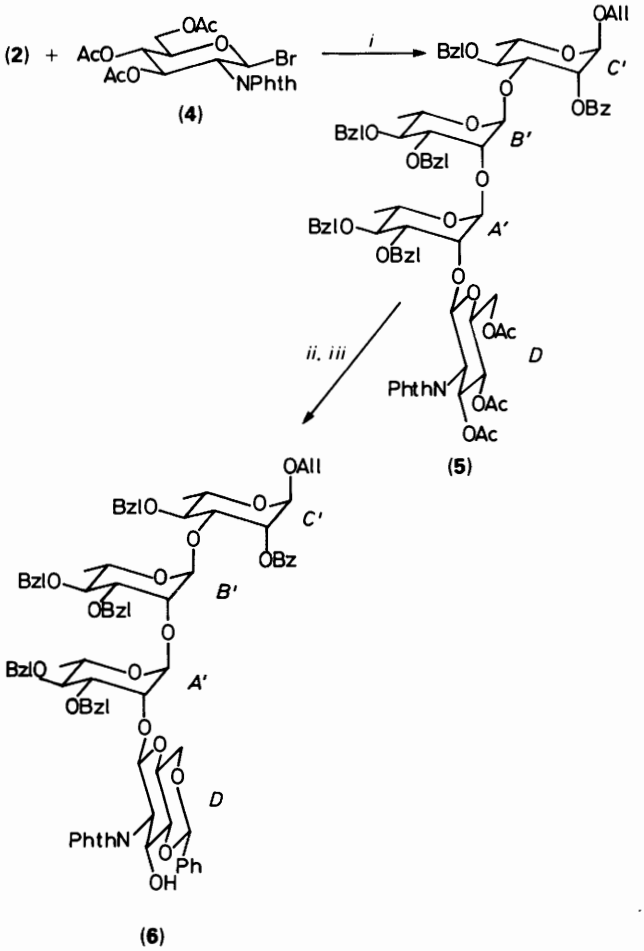
Thus far, we have described the synthesis of pentasaccharide[1] and hexasaccharide[2] haptens comprising the *ABCDA'* and *ABCDA'B'* sequences, respectively. We now report the efficient synthesis of the next homologue of the series, namely the heptasaccharide hapten, *ABCDA'B'C'*, as its propyl glycoside, for use in inhibition assays with complementary monoclonal antibodies,[7] in NMR studies, and in X-ray crystallographic studies of the corresponding antibody–hapten complexes.

Synthesis.—Retrosynthetic analysis indicated[1] that the most advantageous disconnections would be at the *C–D* junction since this would yield a key rhamnose trisaccharide, ABC, that could serve both as a glycosyl acceptor and donor in the elaboration of higher order structures. Following this strategy, two synthetic routes to the heptasaccharide were explored. The first route envisaged the coupling of an *A'B'C'* acceptor with an *ABCD* donor. Thus, transesterification of the trisaccharide (**1**)[1] yielded the required acceptor (**2**) in 80% yield. However, numerous attempts to glycosylate compound (**2**) with the tetrasaccharide donors (**3**)[2] under a variety of conditions were totally unsuccessful (Scheme 1). This result was reminiscent of that encountered in the attempted synthesis of the related hexasaccharide[2] using the same tetrasaccharide donors and the glycosyl acceptor unit α-L-Rha*p*-(1→2)-α-L-Rha*p*-OAll.

The second route to the desired compound envisaged the alternative combination between an *ABC* donor and a *DA'B'C'* acceptor. The strategy had precedent in that the same donor had reacted successfully with a *DA'B'* acceptor to afford a hexasaccharide.[2] The *DA'B'C'* acceptor was synthesized in the following manner. The trisaccharide (**2**) was treated with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**4**)[8] in the presence of silver trifluoromethanesulphonate and collidine (2,4,6-trimethyl pyridine) to give the protected *DA'B'C'* tetrasaccharide (**5**) in 88% yield (Scheme 2). Deacetylation, followed by treatment with α,α-dimethoxytoluene and toluene-*p*-sulphonic acid (PTSA) then gave the required benzylidenated tetrasaccharide (**6**) in 75% yield. The crucial reaction to give the heptasaccharide was then investigated. As was the case in the corresponding reaction to give the hexasaccharide,[2] the best glycosylating conditions were found to be when the glycosyl donor was present as its bromide (**7**), and when mercury(II) cyanide was used as a promoter in dichloromethane. Under these reaction conditions, the desired



Scheme 1.



Scheme 2. *Reagents and conditions:* i, CF_3SO_3Ag, collidine, CH_2Cl_2, 60 h, −35 °C to 25 °C; ii, HCl/MeOH, 36 h; iii, PTSA, $(CH_3O)_2CHPh$, $(CH_3)_2NCHO$.

α-linked heptasaccharide (**8**) was obtained in 86% yield (Scheme 3). The following protocol was used for removal of the protective groups. Transesterification, followed by hydrazinolysis of the phthalimido group, selective *N*-acetylation of the resultant amine and, finally, hydrogenolysis of the product in the presence of palladium–charcoal in aqueous acetic acid yielded the deprotected heptasaccharide (**9**). Successive chromatography on silica gel and sephadex LH20 then afforded the analytically pure compound (**9**) in 35% yield.

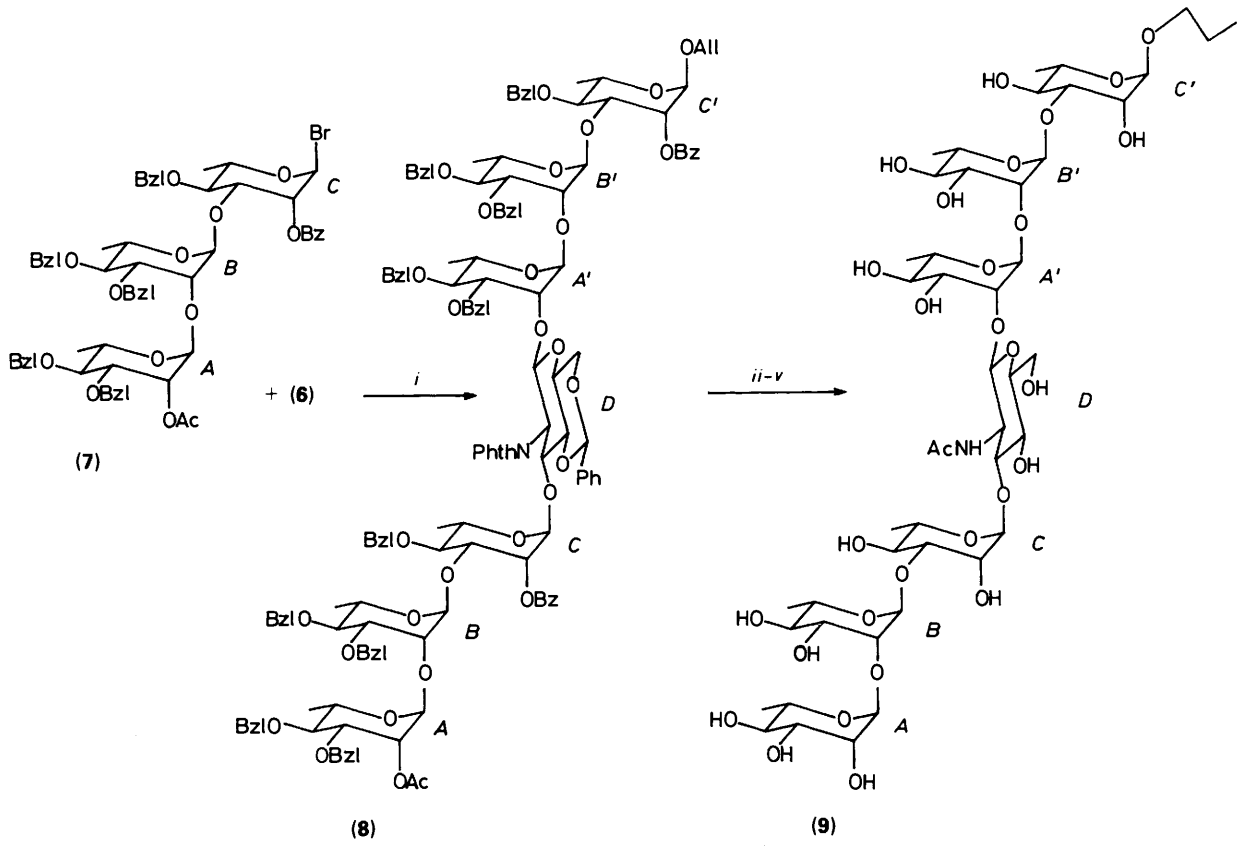
Nuclear Magnetic Resonance Analysis.—The ^1H and ^{13}C NMR spectra of the compounds were in agreement with the assigned structures. Compounds were characterised by means of routine ^1H, $^{13}C\{^1H\}$ and ^{13}C-multiplicity-sorted NMR spectra. ^1H-Homonuclear chemical-shift-correlated (COSY) experiments[9] were carried out on compounds (**8**) and (**9**), and a ^{13}C–^1H chemical-shift-correlated experiment[10] was performed on compound (**8**). In addition, a relayed-COSY experiment[11] was performed on compound (**9**).

The vicinal coupling constants of the ring protons in the monosaccharide units within the oligosaccharides were found to be consistent with a $^4C_1$(D) conformation for the *N*-acetylglucosamine residues and a $^1C_4$(L) conformation for the rhamnopyranosyl residues.

The stereochemical integrity of the newly formed β-glycosidic linkage between the *D*- and the *A'*-unit of compound (**5**) was confirmed by the large vicinal coupling constant (8.5 Hz) found for the anomeric proton resonance. The anomeric configuration of the newly formed *C*–*D* linkage of compound (**8**) was confirmed by examination of the one-bond ^{13}C–^1H coupling constant $^1J_{^{13}C-^1H}$. This value (173 Hz) was found to be consistent with the presence of an α-L configuration for this rhamnopyranosyl residue.[12]

Assignment of the ^1H NMR spectrum of compound (**8**) was made possible by analysis of the COSY spectrum. The chemical-shift values for the individual ring proton signals within overlapped regions were determined from the COSY cross-peak pattern. The reported vicinal coupling constants were determined from clearly distinguishable signals in the one-dimensional ^1H NMR spectrum. The signals were assigned to individual rings based on the following analysis.

Since the COSY experiments readily identified the group of signals of a given ring, it would have sufficed to assign unambiguously one of the signals within this group to a particular ring in order to make the complete assignment of signals to the individual rings. The protocol consisted, then, of the search for these appropriate markers. The signal at δ 5.42 in the spectrum of compound (**8**) was assigned to the 2-H of the *A*-ring, based on the deshielding effect of the 2-*O*-acetyl group. The signals at δ 4.84 and 5.25 were assigned to the 2-Hs of the *C*- and *C'*-ring respectively. The spin system containing the rhamnosyl 2-H at δ 4.84 also contained the most deshielded rhamnosyl 5-H at δ 3.87. This deshielding effect is observed for rhamnosyl 5-Hs of an α-L-rhamnopyranosyl unit when linked to the 3-position of a β-D-*N*-acetylglucosamine unit.[5] In this way the *C*-ring was distinguished from the *C'*-ring. The assignment of the signals of the *D*-ring protons was based on the assignment of the doublet at δ 5.18 (*J* 8.5 Hz) to the β-D-*N*-acetylglucosamine unit. Of the three remaining sets of ring proton signals, two sets of chemical shifts showed severe overlap. These signals were assigned to the *B*- and *B'*-ring and could not be distinguished from one another and, therefore, the assignments may be interchanged. The remaining spin system, containing the rhamnosyl 1-H signal at δ 4.90, was assigned to the *A'*-ring. These assignments were in agreement with those in the spectra of the parent tri- and tetrasaccharide, as well as of previous compounds in this series.[1,2] Following the assignment of the ^1H NMR spectrum of compound (**8**), the assignment of the $^{13}C\{^1H\}$ NMR signals



Scheme 3. *Reagents and conditions:* i, $Hg(CN)_2$, CH_2Cl_2, 60 h, −78 to 25 °C; ii, NaOMe, CH_2Cl_2, 3 days; iii, $N_2H_4 \cdot H_2O$, EtOH, reflux, 16 h; iv, Ac_2O–MeOH, 16 h; v, Pd/C, H_2, aq HOAc (80%), 5 days.

followed directly from the analysis of the ^{13}C–^1H chemical-shift-correlated spectrum.

A similar analysis was carried out for the deblocked heptasaccharide (**9**). The COSY spectrum (Figures 1 and 2) permitted the identification of several of the individual spin systems. However, owing to near chemical-shift degeneracy of three rhamnosyl 3-H signals and two rhamnosyl 4-H signals, it was not possible to establish all of the three sets of signals. A relayed-COSY experiment was thus performed; this experiment gives cross-peaks in the spectrum not only between vicinal ring protons, but also between spins which are not directly coupled. For example, the rhamnosyl 1-H signal at δ 5.11 showed a cross-peak at δ 4.12, indicating the position of the 2-H to which it is directly coupled as well as a cross-peak to a third spin at δ 3.83, indicating the position of the 3-H to which the latter spin is coupled. With the aid of this extra information, all of the signals were assigned to individual spin systems. The sets of signals were then assigned to individual rings based on chemical-shift correlations with those in the spectrum of the analogous synthetic hexasaccharide of this series which comprised an *ABCDA'B'* sequence.[2] The assignment of the spectrum of the hexasaccharide was made unambiguously based on a 2D-NOE. (NOESY) experiment.[2]

The complete assignment of the ^1H NMR spectrum of compound (**9**) represents the first stage in the detailed conformational analysis of (**9**) by quantitative measurement of NOE effects. Such information, in conjunction with molecular mechanics calculations, will be used to infer a model of conformation and, thus, the topographical features of possible importance in recognition by antibodies.

The $^{13}C\{^1H\}$ NMR spectrum was assigned based on chemical-shift correlations of the ring carbon signals (after correction for the different references used in the various studies) with those in the spectra of the natural polymer and various key synthetic sequences.[5,13] The extremely close correlation of the chemical shift data for heptasaccharide (**9**) and the polysaccharide indicates a similar distribution of conformations in solution for these two molecules. The complete attribution of signals in the $^{13}C\{^1H\}$ NMR spectrum will facilitate future experiments on the conformational analysis of the heptasaccharide by way of long-range ^{13}C–^1H correlation experiments.

The heptasaccharide hapten, together with previously synthesized members of the series, are currently being used as inhibitors in immunochemical studies with monoclonal antibodies and polyclonal sera raised against the Y polysaccharide. The results of these studies are expected to reveal the nature and extent of the surfaces and conformational determinants responsible for the molecular basis of antibody specificity.

Experimental

General.—M.p.s were determined with a Fisher–Johns apparatus. ^1H NMR (500.13 MHz) and ^{13}C NMR (125.8 MHz) spectra were recorded with a Bruker AM-500 NMR spectrometer for deuteriochloroform solutions unless otherwise stated. The concentrations of the solutions for the protected compounds were 40 mg ml^{-1} and that for compound (**9**) was 20 mg ml^{-1}. 1D NMR spectra were acquired with 16K data sets for the ^1H NMR and 32K data sets for the ^{13}C NMR spectra. Chemical shifts are given in ppm downfield from Me_4Si. For those spectra measured in deuterium oxide, chemical shifts are given in ppm downfield from 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) (δ O). Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. The NMR spectra of compound (**9**) were measured on a Bruker WM-400 NMR

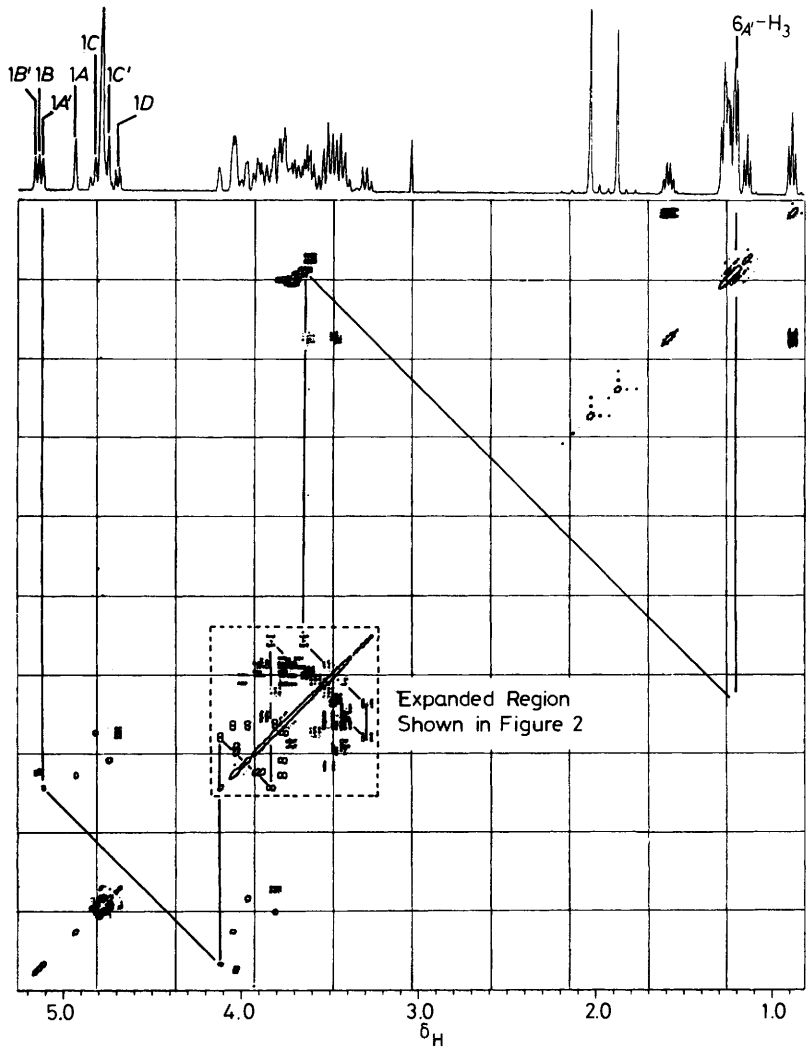


Figure 1. 400 MHz Two-dimensional ^{1}H NMR COSY spectrum of the deprotected heptasaccharide (**9**).

spectrometer operating at 400.13 MHz for ^{1}H and 100.6 MHz for ^{13}C. The ^{1}H homonuclear chemical-shift-correlated (COSY) spectrum of compound (**8**) made use of a 2K × 1K data set with 256 experiments and that of compound (**9**) made use of a 2K × 1K data set with 512 experiments. The ^{13}C–^{1}H chemical-shift-correlated experiment on compound (**8**) made use of a 2K × 1K data set and 128 experiments.

Analytical TLC was performed on precoated aluminium plates with Merck silica gel 60F-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.[14] Purification at each stage was crucial to the outcome of subsequent glycosylation reactions.

Solvents were distilled before use and were dried, as necessary, by literature procedures. Solvents were evaporated under reduced pressure and below 40 °C.

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

Allyl 2-O-*Benzoyl*-4-O-*benzyl*-3-O-3,4-[*di*-O-*benzyl*-2-O-(3,4-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranoside* (**2**).—A solution of the trisaccharide (**1**)[1] (1.0 g, 0.92 mmol) in 3% methanolic HCl (100.0 cm^3) (prepared by addition of acetyl chloride to anhydrous methanol) was stirred for 18 h under nitrogen. The mixture was neutralized by addition of Rexyn 201 OH^- resin, the resin was removed by filtration, and the filtrate was concentrated to give a syrup. The syrup was dissolved in dichloromethane and the solution was washed with aqueous sodium chloride and dried (Na_2SO_4). Evaporation of the solvent gave a syrup, which was chromatographed with hexane–ethyl acetate (3:1) as eluant. *Compound* (**2**) was obtained as a clear syrup (0.77 g, 80%); $[\alpha]_D^{24}$ − 28.0° (*c* 0.7, CH_2Cl_2); δ_C($CDCl_3$; 125.8 MHz) 17.7, 17.8, and 17.9 (C-$6_{C'}$, -$6_{B'}$, and -$6_{A'}$), 96.2 (C-$1_{C'}$), 100.6 (C-$1_{A'}$), 101.1 (C-$1_{B'}$), 117.3 ($CH_2CH{=}CH_2$), 165.8 (CO), other peaks 67.7, 67.9, 68.2, 68.7, 68.8, 72.1, 72.2, 72.9, 74.2, 75.1, 75.2, 75.3, 78.4, 79.1, 79.4, 80.0, and 80.1; δ_H(500.13 MHz) 1.12, 1.23, and 1.28 (3 × 3 H, 3 × d, *J* 6.1 Hz, $6_{C'}$-, $6_{B'}$-, and $6_{A'}$-H_3), 3.30 (1 H, t, $J_{3,4+4,5}$ 19 Hz, $4_{C'}$-H), 3.45 (1 H, t, $J_{3,4+4,5}$ 19 Hz, $4_{A'}$-H), 3.53 (1 H, t, $J_{3,4+4,5}$ 19 Hz, $4_{B'}$-H), 3.67 (1 H, m, $5_{A'}$-H), 3.73 (1 H, dd, $J_{2,3}$ 2.9, $J_{3,4}$ 9.5 Hz, $3_{B'}$-H), 3.80 (2 H, m, $5_{C'}$- and $_{B'}$-H), 3.85 (1 H, dd, $J_{2,3}$ 3.1, $J_{3,4}$ 9.1 Hz, $3_{A'}$-H), 3.93 (1 H, t, $J_{1,2+2,3}$ 6.0 Hz, $2_{B'}$-H), 4.08 (1 H, m, $W_{\frac{1}{2}}$ 6.75 Hz, $2_{A'}$-H), 4.21 (1 H, dd, $J_{2,3}$ 3.2, $J_{3,4}$ 9.3 Hz, $3_{C'}$-H), 4.92 (1 H, d, $J_{1,2}$ 1.8 Hz, $1_{C'}$-H), 5.02 and 5.03 (2 × 1 H, 2 × d, *J* 1.8 Hz, $1_{B'}$- and $1_{A'}$-H), and 5.38 (1 H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.1 Hz, $2_{C'}$-H) (Found: C, 72.2; H, 6.6. $C_{63}H_{70}O_{14}$ requires C, 71.98; H, 6.71%).

Allyl 2-O-*Benzoyl*-4-O-*benzyl*-3-O-{3,4-*di*-O-*benzyl*-2-O-[3,4-*di*-O-*benzyl*-2-O-(3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-*phthali*-

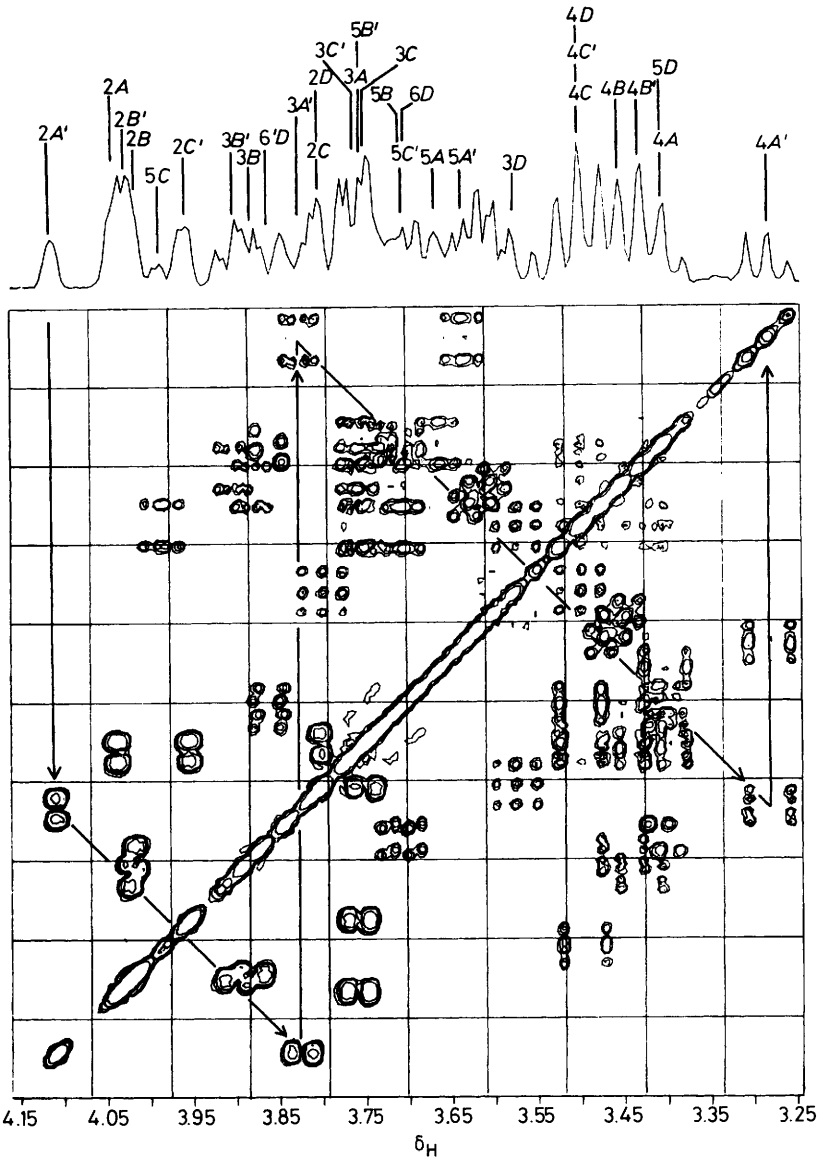


Figure 2. Expanded region of the two-dimensional ^{1}H NMR COSY spectrum of the deprotected heptasaccharide (**9**).

mido-β-D-*glucopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranosyl*}-α-L-*rhamnopyranoside* (**5**).—A mixture of trisaccharide (**2**) (0.307 g, 0.293 mmol), silver trifluoromethanesulphonate (0.222 g, 0.865 mmol), and collidine (0.12 cm^3, 0.91 mmol) in anhydrous dichloromethane (2.0 cm^3) was stirred under nitrogen with 4 Å molecular sieves for 0.5 h in a Schlenk tube fitted with a dropping funnel equipped with a cooling jacket. A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**4**)[8] in dichloromethane (2.0 cm^3) was stirred under nitrogen with 4 Å molecular sieves for 0.5 h and then transferred under nitrogen pressure to the dropping funnel *via* a cannula, and the flask was rinsed with additional portions of dichloromethane (2 × 1.5 cm^3). The cooled (−78 °C) solution of compound (**4**) was added dropwise (30 min) to the cooled (−35 °C) solution of trisaccharide (**2**). The dropping funnel was rinsed with additional portions of dichloromethane (2 × 1.5 cm^3). The mixture was allowed to warm to room temperature and was stirred in the dark under nitrogen for 60 h. The solids were removed by filtration and the filtrate was washed successively with 1M hydrochloric acid, aqueous sodium hydrogen carbonate, and aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and the solvent was removed by evaporation to give a light yellow syrup, which was chromatographed, with hexane–ethyl acetate (2:1) as eluant, R_f 0.33, to give *compound* (**5**) as a clear syrup (0.382 g, 88%), $[\alpha]_D$ − 4.6° (*c* 0.55, CH_2Cl_2); δ_C($CDCl_3$; 125.8 MHz) 17.6, 17.8, and 18.0 (C-$6_{C'}$, -$6_{B'}$, and -$6_{A'}$), 20.60 and 20.64 (3 × OCO*C*H$_3$), 54.6 (C-2_D), 61.4 (C-6_D), 68.2 (*C*H$_2$CH=CH$_2$), 71.6, 72.6, 74.3, 74.8, and 75.4 (Ph*C*H$_2$), 67.7, 68.45, 68.48, 68.8, 70.1, 71.3, 72.9, 76.5, 77.7, 78.19, 78.26, 79.0, 80.0, 80.3, and 80.6 (ring C–Hs), 96.2 (C-$1_{C'}$), 99.9 (C-1_D), 100.91 and 101.03 (C-$1_{B'}$, and -$1_{A'}$), 117.4 (CH$_2$*C*H=*C*H$_2$), and 165.8, 169.5, 170.1, and 170.5 (carbonyl); δ_H(500.13 MHz) 1.02, 1.08, and 1.21 (3 × 3 H, 3 × d, $J \approx 6.2$ Hz, $6_{C'}$-, $6_{B'}$-, and $6_{A'}$-H$_3$), 1.89, 1.997, and 1.998 (3 × 3 H, 3 × s, OCOMe), 2.99, 3.02, and 3.46 (3 × 1 H, 3 × t, $J_{AX+BX} \approx 19$ Hz, $4_{C'}$-, $4_{B'}$-, and $4_{A'}$-H), 3.50 (1 H, m, $J_{5,6a+5,6b} \approx 5.5$, $J_{5,4}$ 10.0 Hz, 5_D-H), 3.58–3.63 (4 × 1 H, m, $3_{B'}$-, $3_{A'}$-, $5_{B'}$-, and $5_{A'}$-H), 3.66–3.69 (2 × 1 H, m, $2_{B'}$- and $2_{A'}$-H), 3.70–3.78 (2 × 1 H, m, $5_{C'}$-, 6_D-H$_b$), 4.40 (1 H, dd, $J_{1,2}$ 8.5, $J_{2,3}$ 10.8 Hz, 2_D-H), 4.83, 4.85, and 4.86 (3 × 1 H, 3 × d, $J \approx 1.8$ Hz, $1_{C'}$-, $1_{B'}$-, and $1_{A'}$-H), 5.11 (1 H, t, $J_{3,4+4,5} \approx 19.2$ Hz, 4_D-H), 5.27 (1-H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.2 Hz, $2_{C'}$-H), 5.31 (1 H, d, $J_{1,2}$ 8.5 Hz,

Table 1. ^{1}H NMR data[a,b] for the ring protons in the heptasaccharides (**8**) and (**9**).

Compound (ring)	1-H	2-H	3-H	4-H	5-H	6-H
(8)						
C'[c]	4.82	5.25 (2.0, 3.2)	4.07	3.42 (19.0)[d]	3.71 (6.2, 9.5)	1.18 (6.2)
B'	4.82	3.66 (4.8)[d]	3.53	3.02 (19.0)[d]	3.51	0.96 (6.2)
A'	4.90 (1.8)	3.80 (5.0)[d]	3.57 (3.0, 9.4)	3.250 (19.0)[d]	3.36	0.93 (6.2)
D	5.18 (8.5)	4.38 (8.5, 10.8)	4.74	3.59 (19.5)[d]	3.38	3.84_{eq}, 3.49_{ax}
C	4.70 (1.8)	4.84	4.02 (3.5, 9.5)	3.245 (19.0)[d]	3.87	0.77 (6.2)
B	4.82	3.61	3.49	2.98 (19.0)[d]	3.54	1.07 (6.2)
A	4.87 (1.8)	5.42 (2.0, 3.2)	3.88	3.33 (19.0)[d]	3.73 (6.2, 9.5)	1.15 (6.2)
(9)						
C'[c]	4.74 (1.8)	3.96	3.76	3.50	3.71	1.257 (6.2)
B'	5.15 (1.8)	4.03	3.91	3.43	3.77	1.263 (6.2)
A'	5.11 (1.8)	4.12 (4.8)[d]	3.83	3.28 (19.5)[d]	3.64	1.20 (6.2)
D	4.69 (8.6)	3.81	3.58	3.50	3.40	3.71, 3.86
C	4.82 (1.8)	3.81	3.75	3.50	3.99	1.20 (6.2)
B	5.13 (1.8)	4.02	3.88	3.45	3.72	1.29 (6.2)
A	4.93 (1.8)	4.05	3.76	3.40	3.67	1.23 (6.2)

[a] Chemical shifts (±0.01 ppm) in $CDCl_3$ for (**8**) and in D_2O for (**9**). The numbers in parentheses denote coupling constants in Hz (±0.1 Hz). [b] Other signals for (**8**): δ_H (500.13 MHz) 3.99 and 4.06 (2 × 1 H, 2 Xm, $OCH_2CH{=}CH_2$, H_a and H_b), 3.87 and 4.00, 3.99 and 4.21, 4.28 and 4.33, 4.34 and 4.56, 4.35 and 4.42, 4.38 and 4.59, 4.42 and 4.65, 4.43 and 4.63, 4.48 and 4.66, 4.54 and 4.85 (20 × 1 H, ABqs, *J* 9.5–12.4 Hz, OCH_2Ph), 5.11 (1 H, dddd, J_{cis} 10.5 Hz, $OCH_2CH{=}CHH$, *cis*), 5.21 (1 H, dddd, J_{trans} 17.5 Hz, $OCH_2CH{=}CHH$, *trans*), 5.44 (1 H, s, $PhCHO_2$), 5.81 (1 H, m, $OCH_2CH{=}CH_2$); Other signals for (**9**): δ_H (400.13 MHz) 0.88 (3 H, t, *J* 7.5 Hz, OCH_2CH_2Me), 2.02 (3 H, s, NHCOMe), 3.46 and 3.62 (2 × 1 H, 2 Xm, OCH_2CH_2Me, H_a and H_b). [c] Indicates the ring to which the aglycone is attached. [d] These values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$.

Table 2. ^{13}C NMR data[a,b] for the ring carbons in the heptasaccharides (**8**) and (**9**)

Compound (ring)	C-1	C-2	C-3	C-4	C-5	C-6
(8)						
C'[c]	96.1 (168)	72.9	78.3	79.9	67.6	17.2
B'	100.94	76.0	78.5	80.11	68.54	17.4
A'	100.86	74.87	79.2	79.7	68.6	17.6
D	100.7	56.4	74.2	80.07	66.0	68.50
C	97.2 (173)	73.2	77.7	79.9	67.6	17.7
B	100.94	79.0	77.5	80.6	68.54	17.9
A	98.9 (173)	68.9	77.6	80.0	68.3	17.9
(9)						
C'[c]	102.2	71.7	80.2	74.5	71.0	19.3
B'	103.4	80.9	72.6	74.8	71.3	19.3
A'	103.7	81.4	72.3	75.0	71.8	19.2
D	104.8	58.4	84.2	71.7	78.6	60.1
C	103.9	72.7	79.9	74.3	71.8	19.1
B	103.5	80.7	72.6	74.8	71.3	19.3
A	105.0	72.7	73.3	74.7	71.8	19.5

[a] Chemical shifts (± 0.1 ppm) in $CDCl_3$ for (**8**) and in D_2O for (**9**). The numbers in parentheses denote coupling constants in Hz (±1.0 Hz). [b] Other signals for (**8**): δ_C (125.8 MHz) 21.1 ($OCOCH_3$), 68.2 ($OCH_2CH{=}CH_2$), 71.8 (2 overlapping signals), 72.0, 72.5, 74.1, 74.3, 74.8, 74.93, 75.2 and 75.4 (OCH_2Ph), 101.7 [$^1J(^{13}C{-}^1H)$ 167 Hz, $PhCHO_2$], 117.4 ($OCH_2CH{=}CH_2$), and 164.9, 165.7, and 170.1 (carbonyls); other signals for (**9**): δ_C (100.6 MHz) 12.5 ($OCH_2CH_2CH_3$), 24.7 (OCH_2CH_2Me), 25.0 ($NHCOCH_3$), 72.4 (OCH_2CH_2Me), and 177.0 (NHCOMe). [c] Indicates the ring to which the aglycone is attached.

1_D-H), 5.85 (1 H, m, $OCH_2CH{=}CH_2$), and 5.99 (1-H, dd, $J_{3,4}$ 9.2, $J_{2,3}$ 10.8 Hz, 3_D-H) (Found: C, 67.6; H, 6.34; N, 1.1. $C_{83}H_{89}NO_{23}$ requires C, 67.88; H, 6.11; N, 0.95%).

Allyl-2-O-*Benzoyl*-4-O-*benzyl*-3-O-{3,4-*di*-O-*benzyl*-2-O-[3,4-*di*-O-*benzyl*-2-O-(4,6-O-*benzylidene*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranosyl*}-α-L-*rhamnopyranoside* (**6**).—A sample of the tetrasaccharide (**5**) (0.247 g, 0.169 mmol) was dissolved in 3% methanolic HCl (12 cm^3) [prepared by treating anhydrous methanol (100 cm^3), with acetyl chloride (5.7 cm^3)] and the solution was stirred under nitrogen for 36 h, then neutralized by the addition of Rexyn 201 OH^- resin beads; the resin was removed by filtration, and the solvent was removed by evaporation. The resulting syrup was taken up in dichloromethane and washed with aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup, which was dissolved in freshly distilled *N*,*N*-dimethylformamide (25 cm^3) containing PTSA (10 mg). To this solution was added α,α-dimethoxytoluene (1.0 cm^3, 6.7 mmol) and the mixture was kept under partial vacuum at 50 °C on a rotary evaporator for 40 h. The solution was neutralized with triethylamine and the solvent was removed by evaporation to give a syrup. The syrup was dissolved in dichloromethane and the solution was washed successively with aqueous sodium hydrogen carbonate and aqueous sodium chloride. The organic layer was dried (Na_2SO_4) and the filtrate was concentrated to give a syrup, which was chromatographed with hexane–ethyl acetate (2 : 1) as eluant, R_F 0.26. *Compound* (**6**) was obtained as a clear syrup 0.180 g, 74.6%); $[\alpha]_D^{22}$ − 8.4° (*c* 1.8, CH_2Cl_2); δ_C ($CDCl_3$; 125.8 MHz) 17.67, 17.74, and 18.0 (C-$1_{C'}$, -$1_{B'}$, and -$1_{A'}$), 56.6 (C-2_D), 68.2 ($CH_2CH{=}CH_2$), 68.5 (C-6_D), 71.8, 72.6, 74.3, 74.9, and 75.4 ($PhCH_2$), 67.7, 68.2, 68.56, 68.57, 72.9, 76.2, 77.2, 77.6, 78.3, 78.5, 79.3, 79.9, 80.2, 80.7, and 82.0 (ring C-Hs), 96.2 (C-$1_{C'}$), 100.84, 100.91, and 100.99 (C-$1_{B'}$, -$1_{A'}$, and -1_D), 101.9 ($PhCHO_2$), 117.4 ($CH_2CH{=}CH_2$), 133.7 ($CH_2CH{=}CH_2$), and 165.8 (OCOPh); δ_H (500.13 MHz) 1.01, 1.13, and 1.21 (3 × 3 H, 3 × d, J ≈ 6.2 Hz, $6_{C'}$-, $6_{B'}$-, and $6_{A'}$-H_3), 3.02, 3.07, and 3.46 (3 × 1 H, 3 × t, J_{AX+BX} ≈ 19 Hz, $4_{C'}$-, $4_{B'}$-, and $4_{A'}$-H), 3.40 (1 H, dt, $J_{5,6ax}$ 5.0, $J_{5,6ax+4,5}$ ≈ 19,5 Hz, 5_D-H), 3.52–3.64 (6 H, m, $3_{B'}$-, $3_{A'}$-, $5_{B'}$-, $5_{A'}$-, and 4_D-H and m, $J_{5,6}$ 6.2, $J_{4,5}$ 9.5 Hz, $5_{C'}$-H), 3.90 (1 H, dd, $J_{5,6eq}$ 5.0, 6_D-H_{ax}), 3.68 and 3.71 (2 × 1 H, 2 × t, J_{AX+BX} ≈ 5.0 Hz, $2_{B'}$-, and $2_{A'}$-H), 3.75 (1 H, $J_{6eq,6ax}$ 10.3 Hz, 6_D-H_{eq}), 4.11 (1 H, dd, $J_{2,3}$ 3.2, $J_{3,4}$ 9.3 Hz, $3_{C'}$-H), 4.35 (1 H, dd, $J_{1,2}$ 8.6, $J_{2,3}$ 10.4 Hz, 2_D-H), 4.82 (1 H, t, $J_{2,3+3,4}$ ≈ 19.5 Hz, 3_D-H), 4.855, 4.860, and 4.875 (3 × 1 H, 3 × d, *J* ≈ 1.8 Hz, $1_{C'}$-, $1_{B'}$-, and $1_{A'}$-H), 5.24 (1 H, d, $J_{1,2}$ 8.6 Hz, 1_D-H), 5.29 (1-H, dd, $J_{1,2}$ 2.3, $J_{2,3}$ 3.2 Hz, $2_{C'}$-H), 5.48 (1-H, s, $PhCHO_2$), and 5.85 (1-H, m, $OCH_2CH_2{=}CH_2$)

(Found: C, 70.5; H, 6.1; N, 0.9. $C_{84}H_{87}NO_{20}$ requires C, 70.52; H, 6.13; N, 0.98%).

Allyl 3-O-{2-O-[2-O-(3-O-{3-O-[2-O-(2-O-*Acetyl*-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*]-2-O-*benzoyl*-4-O-*benzyl*-α-L-*rhamnopyranosyl*}-4,6-O-*benzylidene*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*]-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranosyl*}-2-O-*benzyl*-4-O-*benzoyl*-α-L-*rhamnopyranoside* (**8**).—In a Schlenk tube fitted with a dropping funnel equipped with a cooling jacket, a mixture of tetrasaccharide (**6**) (0.169 g, 0.118 mmol) and mercury(II) cyanide (0.0546 g, 0.216 mmol) in anhydrous dichloromethane (2.0 cm^3) was stirred under nitrogen with 4 Å molecular sieves for 0.5 h. A sample of the glycosyl bromide (**7**)[2] (0.305 g, 0.290 mmol) in anhydrous dichloromethane (2.0 cm^3) was stirred under nitrogen with 4 Å molecular sieves for 0.5 h and then transferred *via* a cannula under nitrogen pressure to the dropping funnel. The cooled (−78 °C) solution of compound (**7**) was added dropwise (during 30 min) to the cooled (−78 °C) solution of tetrasaccharide (**6**). After 60 h the solids were removed by filtration and the filtrate was washed successively with aqueous sodium hydrogen carbonate and aqueous sodium chloride. The solvent was removed by evaporation and the resulting syrup was purified by chromatography with hexane–ethyl acetate (2:1) as eluant, R_F 0.41. *Compound* (**8**) was obtained as a clear syrup (0.251 g, 86.2%), $[\alpha]_D^{22}$ − 3.3° (*c* 1.5, CH_2Cl_2) (Found: C, 71.2; H, 6.05; N, 0.45. $C_{146}H_{153}NO_{34}$ requires C, 71.12; H, 6.25; N, 0.57%); NMR ($CDCl_3$): see Tables 1 and 2.

Propyl 3-O-{2-O-[2-O-(2-*Acetamido*-2-*deoxy*-3-O-{3-O-[2-O-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyranosyl*]-α-L-*rhamnopyranosyl*}-β-D-*glucopyranosyl*)-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyranosyl*}-α-L-*rhamnopyranoside* (**9**).—A sample of the fully blocked heptasaccharide (**8**) (0.141 g, 0.0571 mmol) was dissolved in sodium methoxide (1M; 5 cm^3), with the addition of dichloromethane (0.5 cm^3) to effect complete dissolution. The mixture was stirred under nitrogen for 3 days and then neutralized by the dropwise addition of methanolic HCl (3%). The precipitated salt was removed by filtration and the filtrate was evaporated to dryness, giving a clear syrup, which was dissolved in absolute ethanol (20 cm^3) which contained hydrazine hydrate (100%) (0.03 cm^3, 0.62 mmol). The mixture was refluxed under nitrogen for 16 h and the solvent was evaporated to yield a clear syrup, which was dried *in vacuo* for 24 h. The syrup was then dissolved in methanol (15 cm^3) and acetic anhydride (1.5 cm^3) was added. The mixture was left under nitrogen at room temperature for 16 h. Removal of solvent, and co-evaporation of the residue with absolute ethanol (3 × 20 cm^3), gave a syrup, which was dissolved in 80% aqueous acetic acid (15 cm^3) and hydrogenolysed over 10% palladium–carbon (175 mg) at a hydrogen pressure of 52 psi for 5 days. The solids were removed by filtration through Celite, and the filtrate was concentrated to give a clear, light-brown syrup, which was chromatographed on a silica gel column with ethyl acetate–methanol–water (7:2:1) as eluant, R_F 0.15. The clear syrup was then further purified by gel filtration on Sephadex LH20 with methanol as eluant. Removal of solvent gave *compound* (**9**) as a clear syrup (22.9 mg, 35.2%); $[\alpha]_D^{26}$ −168° (*c* 0.25, water) (Found: C, 49.7; H, 7.0; N, 1.3. $C_{47}H_{81}NO_{30}$ requires C, 49.59; H, 7.11; N, 1.23%); NMR (D_2O): see Tables 1 and 2.

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