tributyltin hydride (70 μL) in benzene (5 mL) were stirred and refluxed under nitrogen for 4 h. Methyl iodide (0.5 mL) was added to the reaction mixture, and reflux was continued overnight. Chromatography on a silica gel column gave (*S*)-(−)-**6**: yield 38 mg (76.7%); $[\alpha]^{20}_D$ −13.57° (*c* 1.0, chloroform).

B. A suspension of dibromoformaldoxime (1.2 g, 6 mmol), (*S*)-(−)-3-butyn-2-ol (0.2 g, 2.8 mmol), and sodium bicarbonate (2.4 g) in dichloromethane (30 mL) were stirred at room temperature until evolution of gas ceased. The slurry was treated with diluted HCl (20 mL) and extracted with dichloromethane (2 × 30 mL). The organic extracts were dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. Column chromatography of the residue gave 0.503 g (89% yield) of (*S*)-(−)-**6**: $[\alpha]^{20}_D$ −14.95° (*c* 1.1, chloroform).

Determination of Enantiomeric Excess of 5, 6, 10, 12. A. The enantiomeric excess of **5** was directly determined by HPLC with a commercially available chiral column (4 mm × 100 mm) packed with α_1-acid glycoprotein (LKB Enantiopac); eluent: sodium phosphate buffer 8 mM, pH = 5, containing NaCl (0.05 M) and 2-propanol (0.5%); flow rate 0.3 mL/min. Retention times (min): 14.8 (*S*) and 19.4 (*R*); peak resolution R_S = 1.73.

B. Chiral alcohols **6**, **10**, and **12** and the corresponding racemic forms were converted into the (*R*)-(+)-MTPA esters,[19] and the ee were determined by integration of the doublets of the methyl group α to the hydroxyl group in the 200-MHz ^{1}H NMR spectrum: **6** *S* form δ 1.74, *R* form δ 1.67; **10** *S* form δ 1.69, *R* form δ 1.61; **12** *S* form δ 1.68, *R* form δ 1.60. ^{1}H NMR spectral data of the (*R*)-(+)-MTPA esters of **6**(*S*) and **6**(*R*), **10**(*S*) and **10**(*R*), **12**(*S*), and **12**(*R*) are reported below.

6(*S*): δ 1.74 (d, 3 H, Me, *J* = 6.1 Hz), 3.60 (b s, 3 H, OMe), 6.14 (s, 1 H, H-4), 6.23 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

6(*R*): δ 1.67 (d, 3 H, Me, *J* = 6.1 Hz), 3.60 (b s, 3 H, OMe), 6.33 (s, 1 H, H-4), 6.23 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

10(*S*): δ 1.69 (d, 3 H, Me, *J* = 6.7 Hz), 2.21 (s, 3 H, Me-3), 3.50 (q, 3 H, OMe, $J_{C,F}$ = 1.3 Hz), 5.87 (s, 1 H, H-4), 6.17 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

10(*R*): δ 1.61 (d, 3 H, Me, *J* = 6.7 Hz), 2.26 (s, 3 H, Me-3), 3.50 (q, 3 H, OMe, $J_{C,F}$ = 1.3 Hz), 6.05 (s, 1 H, H-4), 6.17 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

12(*S*): δ 1.68 (d, 3 H, Me, *J* = 6.1 Hz), 2.35 (d, 3 H, Me-5, *J* = 1.0 Hz), 3.55 (q, 3 H, OMe, $J_{C,F}$ = 1.1 Hz), 5.76 (q, 1 H, H-4, *J* = 1.0 Hz), 6.17 (q, 1 H, CH), 7.30–7.70 (m, 5 H, Ar).

12(*R*): δ 1.60 (d, 3 H, Me, *J* = 6.1 Hz), 2.38 (d, 3 H, Me-5, *J* = 1.0 Hz), 3.55 (q, 3 H, OMe, $J_{C,F}$ = 1.1 Hz), 5.93 (q, 1 H, H-4, *J* = 1.0 Hz), 6.17 (q, 1 H, CH), 7.30–7.70 (m, 5 H, Ar).

Acknowledgment. This work was financially supported by the Ministero della Pubblica Istruzione, National Council of Research (Rome), and by the Biotechnology Action Program of the Commission of the European Communities. We are also indebted with Istituto Donegani S.p.A. for a postdoctoral fellowship to one of us (S.S.).

Registry No. (±)-**1**, 119596-01-9; (*R*)-(+)-**1**, 104164-30-9; (*S*)-(−)-**1**, 119717-13-4; (*S*)-(−)-**1**·HCl, 104182-21-0; **2**, 76596-53-7; **3**, 119619-10-2; **4**, 76596-54-8; (±)-**5**, 119596-02-0; (±)-**5** butyrate ester, 119596-15-5; (±)-**5** hexanoate ester, 119596-16-6; (±)-**5** octanoate ester, 119596-13-3; (±)-**5** dodecanoate ester, 119596-17-7; (±)-**5** hexadecanoate ester, 119596-18-8; (*R*)-(−)-**5**, 119677-65-5; (*S*)-(+)-**5**, 119677-66-6; (*R*)-(+)-**6**, 119596-05-3; (*R*)-**6** (*R*)-(+)-MTPA ester, 119596-08-6; (*S*)-(−)-**6**, 119596-03-1; (*S*)-**6** (*R*)-(+)-MTPA ester, 119596-07-5; **7**, 1423-60-5; (*S*)-(−)-**8**, 2914-69-4; **9**, 55086-61-8; (*S*)-(−)-**10**, 119596-04-2; (*R*)-**10** (*R*)-(+)-MTPA ester, 119596-10-0; (*S*)-**10** (*R*)-(+)-MTPA, 119596-09-7; **11**, 24068-54-0; (*S*)-(−)-**12**, 119596-06-4; (*S*)-**12** (*R*)-(+)-MTPA ester, 119596-11-1; (*R*)-**12** (*R*)-(+)-MTPA ester, 119596-12-2; TBADA, 9031-72-5; 3α,20β-HSDH, 72855-18-6; dibromoformaldoxime, 74213-24-4; *tert*-butylamine, 75-64-9; acetohydroximoyl chloride, 683-58-9; pyruvohydroximoyl chloride, 5471-68-1; 2-bromopropene, 557-93-7; 2,2,2-trifluoroethyl octanoate, 2264-29-1; 2,2,2-trifluoroethyl butyrate, 371-27-7; formate dehydrogenase, 9028-85-7; 2,2,2-trifluoroethyl hexanoate, 2822-57-3; 2,2,2-trifluoroethyl dodecanoate, 70253-78-0; 2,2,2-trifluoroethyl hexadecanoate, 119596-14-4; lipase, 9001-62-1.

Oligosaccharides Corresponding to Biological Repeating Units of *Shigella flexneri* Variant Y Polysaccharide. 2. Synthesis and Two-Dimensional NMR Analysis of a Hexasaccharide Hapten[1]

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The block synthesis of a hexasaccharide 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*, as a glycosyl 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* unit under Helferich conditions yielded the blocked hexasaccharide in 85% yield. 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 disaccharide acceptor, α-L-Rha*p*-(1→2)-α-L-Rha*p*, to give the hexasaccharide 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 hexasaccharide 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*, 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 hexasaccharides by use of two-dimensional NMR techniques is also described.

Introduction

A program of research designed to probe the interaction of antigens with antibodies at a molecular level is in progress.[1] The approach consists of the synthesis of well-defined, complex carbohydrate antigens and their testing in inhibition reactions with complementary monoclonal antibodies. The objective is to define the molecular specificity of these antibodies in terms of the

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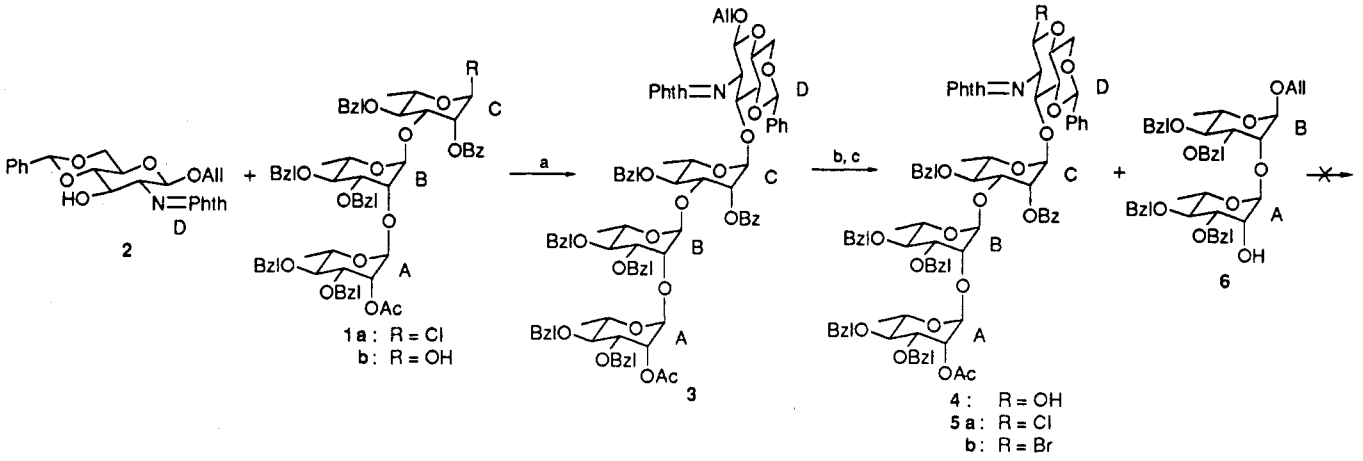
Table I. ^{1}H NMR Data[a,b] for the Ring Protons in the Hexasaccharides 11 and 12

compd	ring	H-1	H-2	H-3	H-4	H-5	H-6
11							
	B′[c]	4.64 (1.8)	3.86	3.63	3.31 (19.0)[d]	3.42	0.99
	A′	4.91 (1.8)	3.85	3.77 (3.0, 9.0)	3.11 (19.5)[d]	3.62	1.18
	D	5.24 (8.5)	4.44	4.79 (19.8)[d]	3.66	3.41	3.56[ax], 3.88[eq]
	C	4.76 (1.8)	4.90	4.08	3.31 (19.0)[d]	3.94	0.72
	B	4.96 (1.8)	3.71 (6.0)[d]	3.77 (3.0, 9.0)	3.13 (19.5)[d]	3.59	1.22
	A	4.93 (1.8)	5.48	3.95	3.39	3.79	1.21
12							
	B′[c]	4.86 (1.8)	3.90	3.82	3.42	3.69	1.26 (6.3)
	A′	5.11 (1.8)	4.11 (4.9)[d]	3.84	3.30 (19.5)[d]	3.70	1.22 (6.3)
	D	4.69 (8.3)	3.81	3.58 (18.2)[d]	3.51	3.41	3.71 3.87
	C	4.82 (1.8)	3.81	3.76	3.50	4.00 (6.2, 9.8)	1.21 (6.3)
	B	5.14 (1.8)	4.03 (1.8, 3.3)	3.89	3.46	3.72	1.29 (6.3)
	A	4.93 (1.8)	4.05 (1.8, 3.5)	3.77	3.41	3.67	1.23 (6.3)

[a] In $CDCl_3$ for 11 and in D_2O for 12. The numbers in parentheses denote coupling constants, in hertz. [b] Other signals 11: δ_H (500.13 MHz) 5.81 (m, 1 H, $CH_2CH{=}CH_2$), 5.51 (s, 1 H, $PhCHO_2$), 5.21 (m, J_{trans} = 17.5 Hz, 1 H, $CH_2CH{=}CH_Z$), 5.14 (m, J_{cis} = 10.5 Hz, 1 H, $CH_2CH{=}CH_EH$), 4.91 and 4.60, 4.77 and 4.51, 4.72 and 4.54, 4.71 and 4.49, 4.66 and 4.45, 4.63 and 4.59, 4.48 and 4.44, 4.29 and 4.08, 4.09 and 3.97 (AB q's, J = 11–12 Hz, OCH_2Ph), 4.05 (m, 1 H, $CH_aH_bCH{=}CH_2$), 3.85 ($CH_aH_bCH{=}CH_2$). Other signals 12: δ_H (400.13 MHz) 3.61 (dt, J = 7.0 and 10.0 Hz, $OCH_aH_bCH_2CH_3$), 3.47 (m, 1 H, $OCH_aH_bCH_2CH_3$), 2.02 (s, 3 H, $NHCOCH_3$), 1.56 (m, 2 H, $OCH_2CH_2CH_3$), 0.87 (t, J = 7.5 Hz, 3 H, $OCH_2CH_2CH_3$). [c] Indicates the ring to which the aglycon is attached. [d] These values are the sums of the individual coupling constants, $J_{AX} + J_{BX}$.

Scheme I[a]



[a] (a) CF_3SO_3Ag, TMU, CH_2Cl_2, 44 h, −78 to 25 °C; (b) $Rh(PPh_3)_3Cl$, EtOH–H_2O (9:1), reflux 18 h; (c) HgO/$HgCl_2$, $(CH_3)_2CO$–H_2O (10:1), 10 days.

number of sugar residues recognized and, more importantly, in terms of the topographical features of the carbohydrate structures involved in recognition. Elucidation of the three-dimensional structure of the oligosaccharides derives from NMR experiments, in conjunction with theoretical calculations.[2,3] Oligosaccharides whose conformations are well established are then used together with the monoclonal antibodies to study molecular recognition processes.

The lipopolysaccharide *O*-antigen of the bacterium *Shigella flexneri* variant Y[4] was chosen for study. The biological repeating unit of this antigen has the following structure:[5]

[2)-α-L-Rhap-(1→2)-α-L-Rhap-(1→3)-α-L-Rhap-(1→3)-β-D-GlcpNAc-(1]

A B C D

Several di-, tri-, and tetrasaccharide fragments[6] as well as polymeric structures[7] corresponding to this unit and to frame-shifted units have been synthesized. In addition, phage-mediated enzymic hydrolysis of the natural polymer has yielded tetra-, octa-, and decasaccharides.[5] Initial immunochemical studies with the lower order oligosaccharides and those obtained by enzymic hydrolysis indicated that the combining sites of several monoclonal antibodies might accommodate sequences larger than four saccharides.[8] Since the appropriate size and sequence of oligosaccharide for detailed studies with these antibodies was not available, we proposed[1] to synthesize penta- to octasaccharides corresponding to portions of the biological repeating unit,[5] with which to probe the aforementioned specificities.

Our earlier work described the synthesis of a pentasaccharide comprising the ABCDA sequence.[1] We describe herein the efficient synthesis of a hexasaccharide hapten,

(2) Bock, K.; Josephson, S.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 2* **1982**, 59.
(3) Bock, K. *Pure Appl. Chem.* **1983**, *55*, 605. Cumming, D. A.; Carver, J. P. *Biochemistry* **1987**, *26*, 6664. Homans, S. W.; Dwek, R. A.; Rademacher, T. W. *Biochemistry* **1987**, *26*, 6571.
(4) Kenne, L.; Lindberg, B.; Petersson, K.; Katzenellenbogen, E.; Romanowska, E. *Carbohydr. Res.* **1977**, *56*, 363.
(5) Carlin, N. I. A.; Lindberg, A. A.; Bock, K.; Bundle, D. R. *Eur. J. Biochem.* **1984**, *139*, 189.
(6) Bundle, D. R.; Gidney, M. A. J.; Josephson, S.; Wessel, H.-P. *ACS Symp. Ser.* **1983**, *231*, 49 and references therein; see also ref 7.
(7) Byramova, N. E.; Tsvetkov, Y. E.; Backinowsky, L. V.; Kochetkov. N. K. *Carbohydr. Res.* **1985**, *137*, C8. Kochetkov, N. K.; Byramova, N. E.; Tsvetkov, Y. E.; Backinowsky, L. V. *Tetrahedron* **1985**, *41*, 3363.
(8) Carlin, N. I. A.; Gidney, M. A. J.; Lindberg, A. A.; Bundle, D. R. *J. Immunol.* **1986**, *137*, 2361.

Table II. ^{13}C NMR Data[a,b] for the Ring Carbons in the Hexasaccharides 11 and 12

compd	ring	C-1	C-2	C-3	C-4	C-5	C-6
11							
	B'[c]	97.8 (172)	74.8	79.2	79.6	68.6	17.4
	A'	101.1 (175)	75.7	79.1	80.5	68.5	17.6
	D	100.7 (164)	56.5	74.2	80.1	66.1	67.6
	C	97.2 (175)	73.1	77.64	79.9	77.60	17.2
	B	100.9 (173)	79.1	79.1	80.5	68.5	18.0
	A	98.9 (175)	68.9	77.60	80.0	68.3	18.0
12							
	B'[c]	101.0	81.6	71.4	75.0	71.1	19.4
	A'	103.8	81.6	72.5	75.0	71.9	19.4
	D	104.9	58.4	84.2	71.7	78.6	63.5
	C	103.9	72.8	80.0	74.4	71.9	19.1
	B	103.6	80.7	72.8	74.8	71.4	19.4
	A	105.0	73.3	73.3	74.8	71.9	19.4

[a] In $CDCl_3$ for 11 and in D_2O for 12. The numbers in parentheses denote coupling constants, in hertz. [b] Other signals 11: δ_C (125.8 MHz) 170.0, 169.4, 167.8, 165.0 (carbonyl), 133.8 ($CH_2CH{=}CH_2$), 117.1 ($CH_2CH{=}CH_2$), 101.7 ($PhCHO_2$, $^1J_{^{13}C-^1H}$ = 167 Hz), 75.32, 75.25, 74.9, 74.1, 72.4, 72.0, 71.8, 71.6 (OCH_2Ph), 67.5 ($CH_2CH{=}CH_2$). Other signals 12: δ_C (100.6 MHz) 177.1 ($NHCOCH_3$), 72.8 ($OCH_2CH_2H_3$), 25.0 ($NHCOCH_3$), 24.6 ($OCH_2CH_2CH_3$), 12.5 ($OCH_2CH_2CH_3$). [c] Indicates the ring to which the aglycon is attached.

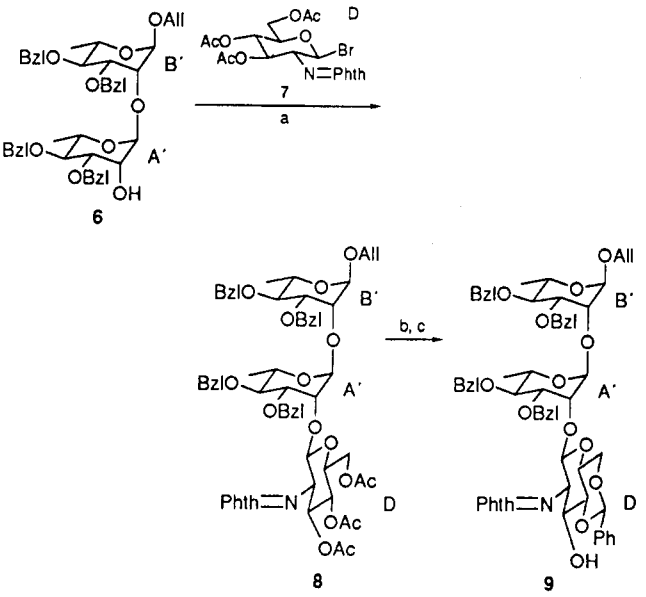
ABCDAB, as its propyl glycoside, for use in binding studies, NMR studies, and in X-ray crystallographic studies of the corresponding antibody–hapten complexes.

Results and Discussion

Synthesis. The original plan of disconnections was designed[1] to ensure that the terminal trisaccharide, α-L-Rhap-(1→2)-α-L-Rhap-(1→3)-α-L-Rhap, could be used as a key intermediate in future glycosylation reactions to give higher order structures. Indeed, this trisaccharide chloride **1** reacted with the disaccharide acceptor, β-D-GlcpNAc-(1→2)-α-L-Rhap, to give a pentasaccharide in our earlier work. In the present work, a similar reaction of **1** with an allyl 2-deoxy-2-phthalimido-β-D-glucopyranosyl acceptor **2**, using silver trifluoromethanesulfonate as promoter and 1,1,3,3-tetramethylurea as base,[9] yielded the protected ABCD tetrasaccharide as an allyl glycoside **3** (Scheme I). Conversion of the latter compound to the corresponding hemiacetals **4** via rhodium(I)-catalyzed isomerization to the prop-1-enyl glycosides[10] and subsequent hydrolysis,[11] followed by treatment with Vilsmeier–Haack reagents[12] then afforded the glycosyl chloride or bromide **5a** or **5b** for future chain extension reactions. However, numerous attempts to link either **5a** or **5b** to an α-L-Rhap-(1→2)-α-L-Rhap-OAll unit **6** under a variety of Königs–Knorr or Helferich conditions were unsuccessful, with significant decomposition of the halide occurring and the desired hexasaccharide being formed in very low yield.

We turned, therefore, to an alternate synthetic strategy, which, nevertheless, conserved our key rhamnose ABC trisaccharide. We envisaged that a disconnection at the C–D linkage might prove to be more fruitful, and that this ABC unit would combine with a DAB acceptor to give the hexasaccharide. This reasoning was based on the knowledge that the glycosylation reaction of an α-L-Rhap-(1→2)-α-L-Rhap moiety and 2,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide to give a DAB unit had precedent,[13] and that **2** combined successfully with **1a** to give an α-L-Rhap-(1→3)-β-D-GlcpN=Phth linkage (see above). Glycosylation of **6** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide **7**[14] in the presence of silver triflate and collidine afforded the protected DAB trisaccharide **8** in 78% yield (Scheme II). Deacetylation, followed by treatment with α,α-dimethoxytoluene and *p*-toluenesulfonic acid then yielded the benzylidenated trisaccharide **9**, the required glycosyl acceptor, in 86% yield. The crucial coupling reaction of the two trisaccharide units to give the hexasaccharide was then investigated. After examination of many glycosylating conditions, the most efficient reaction was found to take place when the glycosyl donor was present as its bromide **10** and when mercury(II) cyanide was used as a promoter in dichloromethane. Indeed, under these reaction conditions, the desired α-linked hexasaccharide **11** was formed in 85% yield (Scheme III). Transesterification, followed by hydrazinolysis of the phthalimido blocking group, selective N-acetylation of the resultant amine, and, finally, hydrogenolysis of the product in the presence of palladium–charcoal in aqueous acetic acid afforded the deprotected hexasaccharide **12**. The crude product was purified by successive chromatography

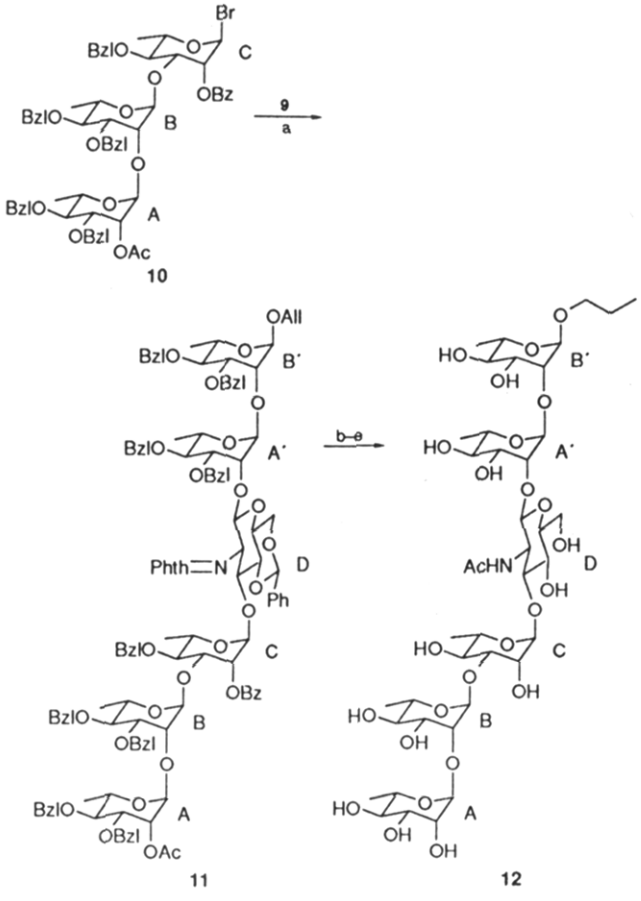
Scheme II[a]



[a] (a) CF_3SO_3Ag, collidine, CH_2Cl_2, 64 h, −78 to 25 °C; (b) HCl/MeOH, 48 h; (c) PTSA, $(CH_3O)_2CHPh$, $(CH_3)_2NCHO$, 36 h.

(9) Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, 13.
(10) Corey, E. J.; Suggs, J. W. *J. Org. Chem.* **1973**, *38*, 3224.
(11) Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1968**, 1903.
(12) Bosshard, H. H.; Mory, R.; Schmid, M.; Zollinger, H. *Helv. Chim. Acta* **1959**, *42*, 1653. Iversen, T.; Bundle, D. R. *Carbohydr. Res.* **1982**, *103*, 29. Lemieux, R. U.; Abbas, S. Z.; Chung, B. Y. *Can. J. Chem.* **1982**, *60*, 58.
(13) Josephson, S.; Bundle, D. R. *Can. J. Chem.* **1979**, *57*, 3073.
(14) Lemieux, R. U.; Takeda, T.; Chung, B. Y. *ACS Symp. Ser.* **1976**, *39*, 90.

Scheme III[a]



[a] (a) $Hg(CN)_2$, CH_2Cl_2, 60 h, −60 to 25 °C; (b) NaOMe, CH_2Cl_2, 3 days; (c) $N_2H_4 \cdot H_2O$, EtOH, reflux, 36 h; (d) Ac_2O/MeOH, 5 h; (e) Pd/C, H_2, HOAc–H_2O (9:1).

on silica gel and sephadex LH20. The product thus obtained in 35% yield was analytically pure.

NMR Analysis. The assigned structures were in accord with their ^1H and ^{13}C NMR spectral data. Compounds were characterized by use of routine ^1H, ^{13}C, $^{13}C\{^1H\}$, and ^{13}C multiplicity sorted spectra. ^1H homonuclear chemical-shift correlated (COSY) experiments[15] were performed on compounds **9**, **11**, and **12**, and a ^{13}C-^1H chemical-shift correlated experiment[16] was performed on compound **11**. In addition, a 2D NOE (NOESY) experiment[17] was performed on **12**.

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 ring and a $^1C_4$(L) conformation for the rhamnopyranosyl units.

The stereochemical integrity of compound **11** was confirmed by examination of the one-bond ^{13}C-^1H coupling constants, $^1J_{^{13}C-^1H}$, for the anomeric carbons. These values (172–175 Hz for the rhamnosyl anomeric carbons and 164 Hz for the glucosamine anomeric carbon) were consistent with an α-L-configuration about the rhamnosyl residues and a β-D-configuration about the glucosamine residue.[18] Assignment of the ^1H NMR spectrum of **11** was facilitated by examination of the COSY spectrum. 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 spectrum of **11**. A detailed account of the attribution of signals to the individual rings follows.

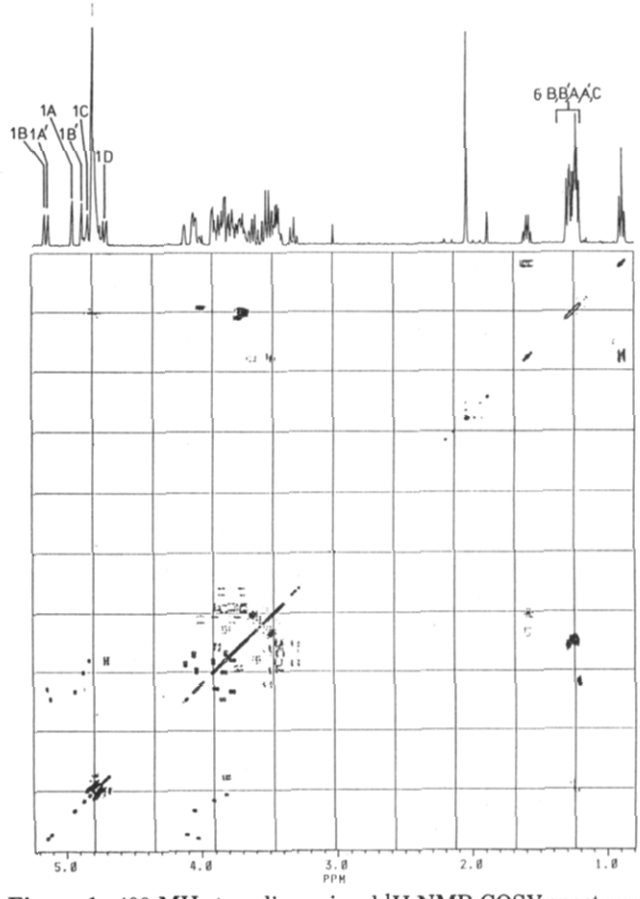


Figure 1. 400-MHz two-dimensional ^1H NMR COSY spectrum of the deprotected hexasaccharide **12**.

Since the COSY experiments readily identified the group of signals of a given ring, it would have sufficed to assign unambigously 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 appropiate markers. The signal at δ 5.48 in the spectrum of **11** was assigned to H-2 of the A ring, based on the deshielding effect of the 2-*O*-acetyl group. Similarly, the signal at δ 4.90 was assigned to H-2 of the C ring (H-2 deshielded by the 2-*O*-benzoyl group). In support of the assignment of the C-ring resonances was the observation that H-5 of the spin system containing the H-2 signal at δ 4.90 was the most deshielded rhamnosyl H-5; this is consistent with the deshielding effect seen for H-5 of an α-L-rhamnopyranosyl unit when it is linked to the 3-position of a β-D-*N*-acetylglucosamine unit.[2] The rhamnosyl H-1 signal at δ 4.64 (the rhamnosyl H-1 signal at highest field) was assigned to the B′ ring based on the expected shielding of H-1 when C-1 is attached to an acyclic aglycon. The assignments of the sets of signals to rings B or A′ of **11** could not be made unambiguously; therefore, these assignments may be interchanged. The assignment of the signals of the D-ring protons was based on the assignment of the doublet at δ 5.24 (J = 8.5 Hz) to H-1 of the β-D-*N*-acetylglucosamine residue. The remaining signals for the D ring were made by tracing the COSY cross-peaks. Following assignment of the ^1H NMR spectrum of **11**, the $^{13}C\{^1H\}$ NMR signals were assigned in a straightforward manner by examination of the ^{13}C–^1H chemical-shift correlated spectrum.

(15) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.
(16) Wilde, F. A.; Bolton, P. H. *J. Magn. Reson.* **1984**, *59*, 343.
(17) Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 1304.
(18) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293. Bock, K.; Pedersen, C. *Acta Chem. Scand.* **1975**, *B29*, 258.

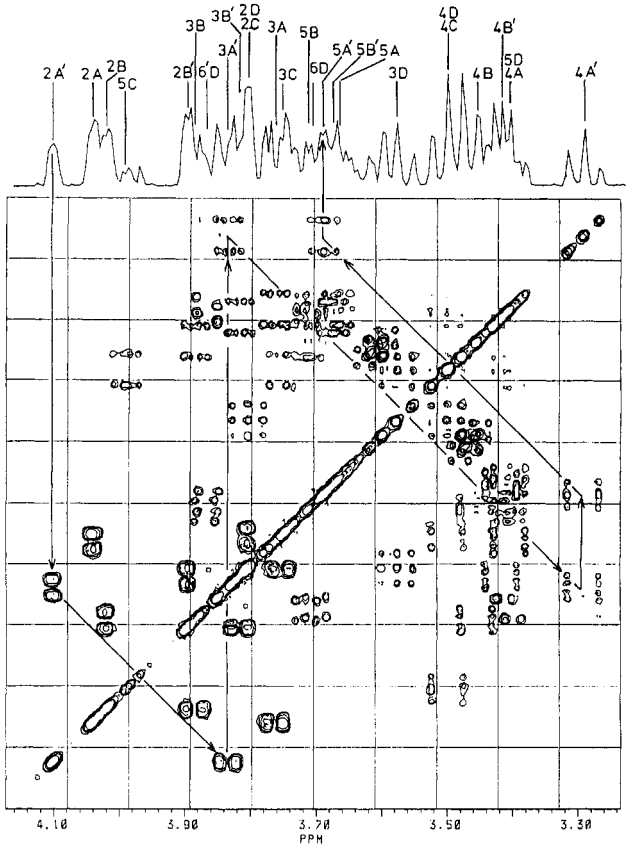


Figure 2. Expanded region of the two-dimensional ^1H NMR COSY spectrum of the deprotected hexasaccharide **12**.

A similar analysis was performed for the spectrum of the deprotected hexasaccharide **12**. Thus, a COSY spectrum permitted the identification of the individual spin systems (Figures 1 and 2). The ring-proton signals for the *N*-acetylglucosamine ring were again clearly identified by their characteristic vicinal coupling constants. The identification of the signals of the individual rhamnosyl units was initially attempted based on chemical-shift correlations with those in the spectra of the natural polymer[2,19] and of various synthetic sequences.[2] On the basis of chemical-shift correlations alone, however, all signals could not be assigned unambiguously; for example, the ring-proton signals for rings B and A′ could not be distinguished from one another. Therefore, the final assignments were made by means of a NOESY experiment.[17]

Since it is well recognized that substantial NOE effects are observed between an anomeric proton and a proton on the aglycon across the glycosidic linkage,[2,3] the signals of the anomeric protons served as suitable markers with which to establish interresidue connectivities. The NOESY spectrum of **12** showed a cross-peak between the H-1_D resonance (δ 4.69) and a rhamnosyl H-2 signal at δ 4.11, suggesting assignment of the latter signal to the A′ ring. The assignment of the remaining A′-ring resonances readily followed from the COSY spectrum. The H-$1_{A'}$ signal (δ 5.11) was used, in turn, as a reporter group in the NOESY experiment to assign the H-2 signal (δ 3.90) of the B′ ring, and the remaining B′-ring resonances were assigned as above. Only two sets of rhamnosyl resonances remained to be assigned, namely those of rings A and B. The H-3_C signal was used as a marker for this purpose. The unambiguous assignment of the signals of the C ring followed from the COSY spectrum and the fact that the H-5 signal of this residue (δ 4.00) could be readily identified. (H-5 of an α-L-rhamnosyl unit is deshielded by 0.25 ppm when linked to the 3-position of β-D-*N*-acetylglucosamine.[2,19]) The observation of an NOE effect between H-3_C (δ 3.76) and the rhamnosyl H-1 resonating at δ 5.14 sufficed to identify the latter resonance as the H-1_B signal, and the remaining signals of the B ring via the COSY spectrum. The remaining set of signals was assigned, therefore, to the A ring.

The complete assignment of the ^1H NMR spectrum of **12** is a necessary prerequisite to the detailed conformational analysis of **12** 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 $^{13}C\{^1H\}$ 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.[2,19] The extremely close correlation of the chemical-shift data for **12** and the polysaccharide indicates a similar distribution of conformations in solution for these two molecules.

The hexasaccharide hapten is one of a panel of inhibitors currently in use in immunochemical studies with monoclonal antibodies and polyclonal sera raised against the Y-polysaccharide. The results of these studies are expected to shed light on the molecular and stereochemical basis of antibody specificity.

Experimental Section

General. Melting points are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded in deuteriochloroform unless otherwise stated. For those spectra measured in deuterium oxide, chemical shifts are given in ppm downfield from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra.

Analytical thin-layer chromatography (TLC) was performed on precoated aluminum 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% sulfuric 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.[20] 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.

Experimental Procedures. Allyl 4,6-*O*-Benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (2). Allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside, prepared in an analogous manner to that described[21] for the corresponding *tert*-butyl glycoside, was acetalated by means of a previously published procedure[22] to give the title compound **2**. Crystals of **2** were obtained from dichloromethane–hexane: mp 173–175 °C; $[\alpha]^{24}_D$ −37.0° (*c* 1.4, CH_2Cl_2); NMR ($CDCl_3$) δ_H (400.13 MHz) 5.58 (s, 1 H, PhC*H*O_2), 5.33 (d, $J_{1,2}$ = 8.5 Hz, 1 H, H-1), 4.67 (dd, $J_{3,4}$ = 8.7 Hz, $J_{2,3}$ = 10.5 Hz, 1 H, H-3), 4.41 (dd, $J_{6,6'}$ = 10.4 Hz, $J_{5,6'}$ = 4.5 Hz, 1 H, H-6′), 4.30 (dd, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 8.5 Hz, 1 H, H-2), 3.86 (t, $W_{1/2}$ = 21 Hz, 1 H, H-6), 3.65 (m, 2 H, H-4, H-5); δ_C (100.6 MHz) 117.6 ($CH_2CH{=}\mathit{C}H_2$), 102.0 (Ph*C*HO_2), 98.1 (C-1),

(19) Jansson, P. E.; Kenne, L.; Wehler, T. *Carbohydr. Res.* **1987**, *166*, 271.

(20) Bundle, D. R.; Iversen, T.; Josephson, S. *Am. Laboratory* **1980**, *12*, 93.

(21) Wessel, H.-P.; Iversen, T.; Bundle, D. R. *Carbohydr. Res.* **1984**, *130*, 5.

(22) Bundle, D. R.; Josephson, S. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2736.

82.3 (C-4), 70.1 (C-3), 68.73, 68.70 (C-5, $CH_2CH{=}CH_2$), 66.2 (C-6), 56.6 (C-2). Anal. Calcd for $C_{24}H_{23}O_7N$: C, 65.90; H, 5.26; N, 3.20. Found: C, 65.76; H, 5.32; N, 3.22.

Allyl 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-glucopyranoside (3). A mixture of allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**) (0.062 g, 0.14 mmol), silver trifluoromethanesulfonate (0.120 g, 0.468 mmol), 1,1,3,3-tetramethylurea (0.06 mL, 0.5 mmol), and 4A molecular sieves in anhydrous dichloromethane (2 mL) was stirred under an atmosphere of N_2 for 40 min. The mixture was then cooled to −78 °C and a solution of the glycosyl chloride **1a** (0.479 g, 0.447 mmol) in anhydrous dichloromethane (3 mL), previously stirred with 4A molecular sieves for 40 min under N_2 and cooled to −78 °C, was added under N_2 by means of a double-tipped needle. The flask was rinsed with an additional portion of solvent (4.0 mL), and the contents were transferred as before. The mixture was allowed to warm gradually to room temperature, and stirring was continued for 20 h. TLC (hexane–ethyl acetate, 2:1) indicated the presence of some starting materials. Additional portions of 1,1,3,3-tetramethylurea (0.025 mL, 0.21 mmol) and silver trifluoromethanesulfonate (0.054 g, 0.21 mmol) were added, and stirring was continued for 24 h. The solids were removed by filtration, and the filtrate was washed successively with sodium hydrogen carbonate solution, sodium chloride solution, and water and dried (Na_2SO_4). Evaporation of the solvent yielded a syrup that was chromatographed with hexane–ethyl acetate (2.5:1) as eluant. The title compound **3** was obtained as a clear glass (0.146 g, 70%): $[\alpha]^{28}_D$ −4.2° (*c* 2.0, CH_2Cl_2); NMR ($CDCl_3$) δ_H (400.13 MHz) 5.59 (s, 1 H, PhC*H*O_2), 5.48 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.0 Hz, 1 H, H-2_A), 5.30 (d, $J_{1,2}$ = 8.2 Hz, 1 H, H-1_D), 4.93 (m, 2 H, H-1_A, H-1_B), 4.84 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.2 Hz, H-2_C), 4.68 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1_C), 4.07 (dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.4 Hz, 1 H, H-3_C), 3.96 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.4 Hz, 1 H, H-3_A), 3.63 (dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.2 Hz, 1 H, H-3_B), 3.42 (m, 1 H, H-5_A), 3.40, 3.32, 3.29 (t's, $J_{AX} + J_{BX} \approx 19$ Hz, 3 × 1 H, H-4_C, H-4_B, H-4_A), 2.12 (s, 3 H, OCOC*H*$_3$), 1.23, 0.98 (d's, *J* = 6.1 Hz, 2 × 3 H, H_3-6_B, H_3-6_A), 0.68 (d, *J* = 6.1 Hz, 3 H, H_3-6_C); δ_C (100.6 MHz) 169.9, 164.8 (carbonyl), 117.4 ($CH_2CH{=}CH_2$), 102.0 (PhCHO$_2$), 100.8 ($^1J_{^{13}C\text{-}^1H}$ = 171 Hz, C-1_B), 98.9 ($^1J_{^{13}C\text{-}^1H}$ = 170 Hz, C-1_A), 98.0 ($^1J_{^{13}C\text{-}^1H}$ = 163 Hz, C-1_D), 97.5 ($^1J_{^{13}C\text{-}^1H}$ = 167 Hz, C-1_C), 56.3 (C-2_D), 21.0 (OCO*C*H$_3$), 17.9, 17.4, 17.2 (C-6_A, C-6_B, C-6_C), other peaks 80.5, 80.1, 79.9, 79.8, 79.2, 77.7, 77.6, 75.2, 75.1, 75.0, 74.9, 74.1, 73.1, 72.0, 71.7, 70.1, 68.9, 68.8, 68.6, 68.4, 67.8, 66.5. Anal. Calcd for $C_{86}H_{89}O_{21}N$: C, 70.24; H, 5.96; N, 0.95. Found: C, 70.30; H, 6.18; N, 1.05.

A mixture of hemiacetals **1b** (0.264 g, 0.251 mmol) was also isolated and recycled.

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-glucopyranose (4). Tris(triphenylphosphine)rhodium(I) chloride (42.5 mg, 0.045 mmol) was added to a solution of the tetrasaccharide **3** (0.213 g, 0.145 mmol) in ethanol–water (9:1) (50 mL), and the mixture was refluxed under an atmosphere of N_2 for 18 h. Removal of the solvent left a residue that was dissolved in ethyl acetate and filtered through a short column of silica gel. The solvent was removed, and the resulting oil was dissolved in 90% aqueous acetone (35 mL). Yellow mercuric oxide (0.128 g, 0.591 mmol) was added, followed by the dropwise addition, over 2 min, with stirring of mercuric chloride (2.0 mL of 8.2% solution, 0.6 mmol) in acetone–water (10:1) and the dropwise addition of 90% aqueous acetone (15 mL) over 2 min. The mixture was stirred for 10 days, the solvent was evaporated, and the resulting syrup was dissolved in ethyl acetate. After filtration through Celite, the filtrate was washed with saturated potassium iodide solution, sodium thiosulphate solution, and sodium chloride solution. The organic layer was dried (Na_2SO_4), the solvent was evaporated, and the residue was dissolved in ethyl acetate. Filtration through a short column of silica gel, followed by concentration of the filtrate, gave a syrup that was chromatographed with hexane–ethyl acetate (1.5:1) as eluant. The title compound **4** was obtained as a clear colorless syrup (0.138 g, 67%). The product was obtained as a highly biased anomeric mixture. NMR data is given only for the major β-anomer since the signals of the α-anomer could not be assigned unambiguously; NMR ($CDCl_3$) δ_H (400.13 MHz) 5.58 (s, 1 H, PhC*H*O_2), 5.48 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, 1 H, H-2_A), 5.44 (dd, $J_{1,OH} + J_{1,2}$ = 17.0 Hz, 1 H, H-1_D), 4.92 and 4.91 (d's, $J_{1,2} \approx 1.8$ Hz, 2 H, H-1_A, H-1_B), 4.82 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2_C), 4.69 (d, $J_{1,2} \approx 1.8$ Hz, 1 H, H-1_C), 4.24 (dd, $J_{1,2}$ = 8.7 Hz, $J_{2,3}$ = 10.3 Hz, 1 H, H-2_D), 4.04 (dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, 1 H, H-3_C), 3.94 (dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.8 Hz, 1 H, H-3_A), 3.90 (m, 1 H, H-5_C, 3.79 (m, 1 H, H-5_A), 3.61 (dd, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 9.5 Hz, 1 H, H-3_B), 3.39 (m, 2 H, H-5_B, H-4_A), 3.28 (t, $J_{AX+BX} \approx 19.5$ Hz, 2 H, H-4_C, H-4_D), 3.02 (d, $J_{1,OH}$ = 8.4 Hz, 1 H, O*H*), 2.12 (s, 3 H, OCOC*H*$_3$), 1.21, 0.89 (d's, J = 6.1 Hz, 2 × 3 H, H_3-6_B, H_3-6_A), 0.65 (d, *J* = 6.1 Hz, 3 H, H_3-6_C); δ_C (100.6 MHz) 170.0, 165.0 (carbonyl), 102.0 (PhCHO$_2$), 100.9 (C-1_B), 99.0 (C-1_A), 97.4 (C-1_C), 93.5 (C-1_D), 58.3 (C-2_D), 21.0 (OCO*C*H$_3$), 18.0, 17.4, 17.2 (C-6_A, C-6_B, C-6_C), other peaks 80.5, 80.1, 79.9, 79.8, 79.2, 77.8, 77.6, 75.3, 74.9, 74.8, 74.2, 73.1, 72.1, 71.7, 69.0, 68.7, 68.4, 67.8, 66.7. Anal. Calcd for $C_{83}H_{85}O_{21}N$: C, 69.64; H, 5.91; N, 0.98. Found: C, 69.39; H, 6.23; N, 1.10.

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 Bromide (5b). Oxalyl bromide (0.23 mL) was added to a solution of *N*,*N*-dimethylformamide (0.12 mL, 1.5 mmol) in anhydrous dichloromethane (2 mL) under N_2, and the mixture was stirred for 5 min. The solvent was removed in vacuo, and the resulting white solid was dried for 0.5 h. The *N*,*N*-dimethyl(bromomethylene)ammonium bromide was then dissolved in anhydrous dichloromethane (3 mL), and a solution of the hemiacetals **4** (0.131 g, 0.091 mmol) in anhydrous dichloromethane (3 mL) was added under N_2 by means of a double-tipped needle. The flask was rinsed with additional portions of solvent (2 × 2 mL), and these were transferred as before. The mixture was stirred under N_2 for 5 h and was then quenched by addition of cold saturated sodium hydrogen carbonate solution (15 mL). The organic layer was diluted, washed with sodium hydrogen carbonate solution and sodium chloride solution, and dried (Na_2SO_4). Evaporation of the solvent gave the glycosyl bromide **5b** (0.128 g, 94%), which was dried and used directly in glycosylation reactions.

Allyl 2-*O*-(3,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (6). The title compound **6** was prepared in an analogous manner to that described[13] for the corresponding 8-(methoxycarbonyl)octyl glycoside and was obtained as a solid. Recrystallization from hexane afforded white needles: mp 100.0 °C; $[\alpha]^{25}_D$ −32.5° (*c* 0.4, CH_2Cl_2); NMR ($CDCl_3$) δ_H (400.13 MHz) 5.09 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1_A), 4.89 (dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 11.0 Hz, 1 H, H-3_B), 4.76 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1_B), 4.14, 4.03 (m's, 2 × 1 H, H-2_B and H-2_A), 3.89 (dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.2 Hz, 1 H, H-3_A), 3.81 (m, 1 H, H-5_A), 3.69 (m, 1 H, H-5_A), 3.47 (t, $J_{3,4} + J_{4,5}$ = 19 Hz, 1 H, H-4_A), 3.39 (t, $J_{3,4} + J_{4,5}$ = 19 Hz, 1 H, H-4_B), 1.30 (d, *J* = 6.1 Hz, 6 H, H_3-6_B, H_3-6_A); δ_C (100.6 MHz) 117.0 ($CH_2CH{=}CH_2$), 100.7 (C-1_A), 97.9 (C-1_B), 75.10, 75.08, 72.1, 72.0, (OCH$_2$Ph), 67.5 ($CH_2CH{=}CH_2$), 80.3, 80.0, 79.7, 79.4, 74.6, 68.7, 67.9, 67.8 (CH), 17.9, 17.7 (C-6_A, C-6_B). Anal. Calcd for $C_{43}H_{50}O_9$: C, 72.65; H, 7.09. Found: C, 72.98; H, 6.98.

Allyl 2-*O*-(2-*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (8). A mixture of allyl 2-*O*-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (**6**) (0.317 g, 0.446 mmol), silver trifluoromethanesulfonate (0.364 g, 1.42 mmol), and collidine (0.20 mL, 1.5 mmol) in anhydrous dichloromethane (2.0 mL) was stirred under N_2 with 4A molecular sieves for 0.5 h in a flask 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 (**7**)[11] (0.530 g, 1.06 mmol) in dichloromethane was stirred under N_2 with 4A molecular sieves for 0.5 h, and then transferred under N_2 to the dropping funnel using a double-tipped needle. The flask was rinsed with additional portions of dichloromethane (2 × 1.5 mL) and transferred as before. The glycosyl bromide solution **7** was cooled to −78 °C and added dropwise, over a period of 20 min, to the cooled solution (−35 °C) of the allyl glycoside **6**. The dropping funnel was rinsed with additional portions of dichloromethane (2 × 1.5 mL) and added to the alcohol solution.

The mixture was allowed to warm to room temperature and was stirred in the dark, under N_2, for 64 h. The solids were removed by filtration, and the filtrate was washed successively with hydrochloric acid solution (1 M), sodium hydrogen carbonate, and sodium chloride solution. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup, which was chromatographed with hexane–ethyl acetate (1:1) as eluant; the title compound **8** was obtained as a clear colorless syrup (0.379 g, 78%): $[\alpha]^{27}_D$ 0.9° (*c* 1.1, CH_2Cl_2); NMR ($CDCl_3$) δ_H (500.13 MHz) 6.00 (dd, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 10.8 Hz, 1 H, H-3_D), 5.31 (d, $J_{1,2}$ = 8.5 Hz, H-1_D), 5.10 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.2 Hz, 1 H, H-4_D), 4.87 (d, $J_{1,2}$ = 1.8 Hz, H-$1_A'$), 4.62 (d, $J_{1,2} \approx$ 1.8 Hz, 1 H, H-$1_B'$), 4.40 (dd, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 10.8 Hz, 1 H, H-2_D), 4.06 (dd, $J_{5,6'}$ = 3.5 Hz, $J_{6,6'}$ = 12.3 Hz, 1 H, H-6′$_D$), 3.80 (m, 1 H, H-$2_A'$ or H-$2_B'$), 3.76 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, 1 H, H-$3_B'$), 3.72 (m, 2 H, H-6_D, H-$2_A'$ or H-$2_B'$), 3.61 (m, 3 H, H-$3_A'$, H-$5_A'$, H-$5_B'$), 3.47 (ddd, $J_{5,6}$ = 2.5 Hz, $J_{5,6'}$ = 3.5 Hz, $J_{4,5}$ = 10.2 Hz, 1 H, H-5_D), 3.11, 3.03 (t's, J_{AX} + $J_{BX} \approx$ 19 Hz, 2 × 1 H, H-$4_A'$, H-$4_B'$), 2.01, 1.97, 1.89 (s's, 3 × 3 H, OCOCH_3), 1.21 (d, $J_{5,6}$ = 6.1 Hz, 3 H, H_3-$6_B'$), 1.14 (d, $J_{5,6}$ = 6.2 Hz, 3 H, H_3-$6_A'$); δ_C (125.8 MHz) 133.7 (CH_2CH=CH_2), 117.1 (CH_2CH=CH_2), 101.3 (C-$1_A'$), 100.0 (C-1_D), 97.8 (C-$1_B'$), 80.7, 80.5, 79.2, 78.8, 77.7, 76.3, 71.35, 70.1, 68.8, 68.4, 67.58 (ring CH's), 75.4, 75.0, 72.6, 71.32 (OCH_2Ph), 67.59 (CH_2CH=CH_2), 61.4 (C-6_D), 54.6 (C-2_D), 20.69, 20.64, 20.59 (OCOCH_3), 18.0, 17.6 (C-$6_A'$, C-$6_B'$). Anal. Calcd for $C_{63}H_{69}NO_{18}$: C, 67.07; H, 6.16; N, 1.24. Found: C, 66.78, H, 6.19; N, 1.38.

Allyl 2-*O*-(2-*O*-(4,6-Di-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (9). A solution of the trisaccharide **8** (0.234 g, 0.296 mmol) was dissolved in methanolic HCl (12 mL) [prepared by treating anhydrous methanol (100 mL) with acetyl chloride (5.7 mL)] and stirred under N_2 at room temperature for 48 h, after which TLC (hexane–ethyl acetate, 1:1) indicated the reaction was complete. The mixture was neutralized by addition of Rexyn 201 OH^- resin beads. The resin beads were removed by filtration, and the filtrate was concentrated to give a syrup. The syrup was taken up in dichloromethane, and the solution was washed with sodium chloride solution, dried (Na_2SO_4), and concentrated to give a syrup, which was then dried in vacuo. The dried syrup was dissolved in dry, freshly distilled *N,N*-dimethylformamide (25 mL) containing α,α-dimethoxytoluene (0.50 mL, 3,3 mmol). *p*-Toluenesulfonic acid (10 mg) was added, and the mixture was stirred under partial vacuum for 36 h on a rotary evaporator. The mixture was neutralized with triethylamine, and the solution was concentrated to give a syrup. the syrup was dissolved in dichloromethane and washed successively with aqueous sodium hydrogen carbonate solution and aqueous sodium chloride solution. The organic layer was dried (Na_2SO_4) and evaporated to dryness to give a yellow syrup, which was chromatographed with hexane–ethyl acetate (1:1) as eluant. The title compound **9** was obtained as a clear colorless syrup (0.229 g, 86%): $[\alpha]^{22}_D$ −8.4° (*c* 1.85, CH_2Cl_2); NMR ($CDCl_3$) δ_H (500.13 MHz) 5.48 (s, 1 H, PhCHO_2), 5.23 (d, $J_{1,2}$ = 8.4 Hz, 1 H, H-1_D), 4.89 (d, $J_{1,2}$ = 1.9 Hz, 1 H, H-$1_A'$), 4.81 (dd, $J_{3,4}$ = 9.0 Hz, $J_{2,3}$ = 10.7 Hz, 1 H, H-3_D), 4.61 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-$1_B'$), 4.34 (dd, $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = 10.7 Hz, 1 H, H-2_D), 3.88 (dd, $J_{5,6_{eq}}$ = 5.0 Hz, $J_{6_{eq},6_{ax}}$ = 10.3 Hz, 1 H, H_{eq}-6_D), 3.75 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.4 Hz, 1 H, H-$3_B'$), 3.82, 3.71 (t's, J_{AX} + $J_{BX} \approx$ 5.0 Hz, 2 × 1 H, H-$2_A'$, H-$2_B'$), 3.58 (m, 5 H, H-3_A, H-4_D, H-$5_B'$, H-$5_A'$, H_{ax}-6_D), 3.36 (dt, $J_{5,6_{ax}}$ + $J_{4,5} \approx$ 19.5 Hz, $J_{5,6_{eq}}$ = 5.0 Hz, 1 H, H-5_D), 3.12 (t, J_{AX} + $J_{BX} \approx$ 19.5 Hz, 2 H, H-$4_B'$, H-$4_A'$) 1.20 (d, $J_{5,6}$ = 6.2 Hz, 3 H, H_3-$6_B'$), 1.17 (d, $J_{5,6}$ = 6.3 Hz, 3 H, H_3-$6_A'$); δ_C (125.8 MHz) 133.8 (CH_2CH=CH_2), 117.2 (CH_2CH=CH_2), 101.9 (PhCHO_2), 101.2 (C-$1_A'$), 100.9 (C-1_D), 97.8 (C-$1_B'$), 82.0, 80.7, 80.6, 79.4, 79.1, 77.7, 75.9, 68.5, 68.2, 67.7 (ring CH's), 75.4, 75.1, 72.6, 71.7 (OCH_2Ph), 68.4 (C-6_D), 67.6 (CH_2CH=CH_2), 56.6 (C-2_D), 18.0, 17.6 (C-$6_A'$, C-$6_B'$). Anal. Calcd for $C_{64}H_{67}NO_{15}$: C, 70.51; H, 6.19; N, 1.28. Found: C, 70.41; H, 6.40; N, 1.04.

Allyl 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-rhamnopyranoside (11). A mixture of the trisaccharide **9** (0.229 g, 0.210 mmol) and mercury(II) cyanide (0.104 g, 0.413 mmol) in anhydrous dichloromethane (2.0 mL) was stirred under N_2 with 4A molecular sieves for 0.5 h in a Schlenk tube fitted with a dropping funnel equipped with a cooling jacket. A sample of the trisaccharide bromide **10** (0.537g, 0.482 mmol), prepared in analogous fashion to **5b**, in anhydrous dichloromethane (2.0 mL) was stirred under N_2 with 4A molecular sieves for 0.5 h and then transferred, via cannula, to the dropping funnel. The flask was rinsed with additional portions of dichloromethane (2 × 1.5 mL) and transferred as before. The cooled (−70 °C) glycosyl bromide solution was added dropwise (over 30 min) to the cooled (−60 °C) alcohol solution, rinsing the dropping funnel with additional dichloromethane (2 × 1.0 mL). The mixture was allowed to warm to room temperature gradually. After 60 h TLC (hexane–ethyl acetate, 2:1) indicated the reaction was complete. The solids were removed by filtration and the filtrate diluted and washed successively with aqueous sodium hydrogen carbonate solution, and aqueous sodium chloride solution. The organic layer was dried (Na_2SO_4), and the solvent was removed by evaporation to give a light yellow syrup, which was purified by silica gel chromatography, using hexane–ethyl acetate (2:1) as eluant, to give the title compound **11** as a white foam: R_f 0.61 (0.381 g, 85%); $[\alpha]^{22}_D$ 7.3° (*c* 2.2, CH_2Cl_2). Anal. Calcd for $C_{126}H_{133}O_{29}N$: C, 71.20; H, 6.31; N, 0.66. Found: C, 71.15; H, 6.50; N, 0.55. NMR ($CDCl_3$) see Tables I and II.

Propyl 2-*O*-(2-*O*-(2-Acetamido-2-deoxy-3-*O*-(3-*O*-(2-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnoyranosyl)-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (12). A sample of the fully blocked hexasaccharide (0.289 g, 0.136 mmol) **11** was dissolved in sodium methoxide (1 M, 16 mL) with the addition of dichloromethane (1.5 mL) to complete dissolution. The mixture was stirred under N_2 for 3 days and then neutralized by the dropwise addition of methanolic HCl (3%). The precipitated salt was removed by filtration, and the filtrate evaporated to dryness giving a clear colorless syrup. The syrup was dissolved in absolute ethanol (30 mL), which contained hydrazine hydrate 100% (0.07 mL, 1.4 mmol). The mixture was refluxed under N_2 for 36 h, and the solvent was removed by evaporation, giving a clear syrup, which was dried in vacuo for 10 h to remove traces of hydrazine hydrate. The syrup was then dissolved in methanol (16 mL) to which was added acetic anhydride (2.0 mL). The mixture was let stand under N_2 at room temperature for 5 h. Solvent removal and codistillation of the residue with absolute ethanol (3 × 20 mL) gave a syrup, which was chromatographed with hexane–ethyl acetate–methanol (3.5:1.0:0.5) as eluant, R_f 0.3. The syrup was dissolved in 80% aqueous acetic acid (15 mL) and hydrogenated over 10% palladium–carbon (210 mg) at a hydrogen pressure of 52 psi for 4 days. The solids were removed by filtration through Celite, and the solvent was evaporated to give a clear light brown syrup, which was chromatographed with ethyl acetate–methanol–water (6:3:1) as eluant, R_f 0.15. The clear colorless syrup was then further purified by gel filtration with Sephadex LH20 using methanol as eluant. Solvent removal gave the title compound **12** as a clear glass (47.6 mg, 35%); $[\alpha]^{26}_D$ −73.1° (*c* 0.46, H_2O). Anal. Calcd for $C_{41}H_{71}O_{26}N$: C, 49.54; H, 7.20; N, 1.41. Found C, 49.36; H, 7.05; N, 1.26. NMR (D_2O): see Tables I and II.

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