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Synthesis of allyl glycosides for conversion into neoglycoproteins bearing epitopes of mycobacterial glycolipid antigens*

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ABSTRACT

Neoglycoproteins bearing key glycosyl substituents of several glycopeptidolipid antigens of pathogenic *Mycobacterium* species have been synthesized. Allyl glycosides of the terminal 6-deoxyhexose-containing units of the antigens were prepared, with appropriate ether and ester substituents in place. Ozonolysis of the allyl glycosides was then followed by reductive coupling with ε-amino groups of lysine residues in bovine serum albumin, using sodium cyanoborohydride at pH 7.8. The resulting neoglycoproteins emulated the antigenicity of the native molecule in several serological tests.

INTRODUCTION

Current interest in members of the *M. avium* complex (including *M. intracellulare* and *M. scrofulaceum*) stems largely from the occurrence of several serovars as opportunistic pathogens in persons with acquired immunodeficiency syndrome (AIDS)[1]. Other serovars have long been associated with pulmonary infections[2]. Individual serovars of the *M. avium* complex (MAC) are distinguished by highly characteristic surface glycopeptidolipids (GPLs). From some 30 serovars the GPLs of 12 have been examined in detail and the structures of these have been established with a high degree of certainty. Table I shows in shorthand form the structures of the known outer chains of oligosaccharide haptens of GPLs, including those from *M. avium* serovars 2, 4, and 9, which are among those found most frequently in persons with AIDS[1]. The *N*-fatty acyl tetrapeptide core bearing a 3,4-di-*O*-methyl-α-L-rhamnopyranosyl residue glycosidically attached to the *C*-terminal L-alaninol unit is highly conserved, as is the inner 3-*O*-substituted α-L-rhamnopyranosyl-(1→2)-6-deoxy-α-L-talopyranosyl unit of the oligosaccharide chain that is *O*-glycosidically linked to the D-allothreonine residue in this the tetrapeptide (**1**). Antigenic specificity is due to structural variability of the outer

* Dedicated to Professor Grant Buchanan on the occasion of his 65th birthday.
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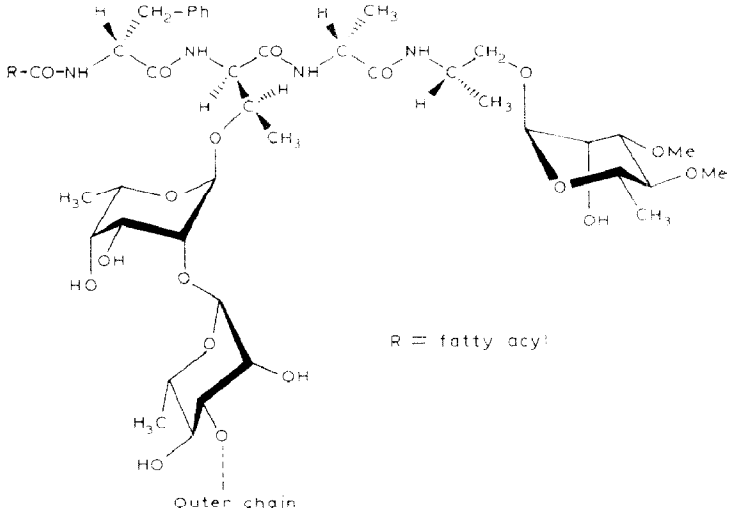
TABLE I

Oligosaccharide haptens of GPLs from *Mycobacterium avium* serovars

Serovar Number	*Structure*[a]
2	2,3-Me_2-α-L-Fuc*p*-[→3)-α-L-Rha*p*-(1→2)-6-deoxy-α-L-Tal*p*-(1→][f]
4	4-Me-α-L-Rha*p*-(1→4)-2-Me-α-L-Fuc*p*
8	3-Me-4,6-Pyr-β-D-Glc*p*
9	4-Ac-2,3-Me_2-α-L-Fuc*p*-(1→4)-β-D-Glc*p*A-(1→4)-2,3-Me_2-α-L-Fuc*p*
12	**3-Me-β-Qui*p*4NLa**-(1→3)-4-Me-α-L-Rha*p*-(1→3)-α-L-Rha*p*
14	α-**Kan*p*4NFo**-(1→3)-2-Me-α-D-Rha*p*-(1→3)-2-Me-α-L-Fuc*p*
17	β-**Qui*p*3NMHB**-(1→3)-4-Me-α-L-Rha*p*-(1→3)-α-L-Rha*p*
19	3,4-Me_2-β-D-Glc*p*A-(1→3)-**2,4-Me_2-α-Eva*p***-(1→3)-α-L-Rha*p*
20	2-Me-α-D-Rha*p*-(1→3)-2-Me-α-L-Fuc*p*
21	4,6-Pyr-β-D-Glc*p*
25	**2-Me-α-Fuc*p*4NAc**-(1→4)-β-D-Glc*p*A-(1→4)-2-Me-α-L-Fuc*p*
26	2,4-Me_2-α-L-Fuc*p*-(1→4)-β-D-Glc*p*A-(1→4)-2-Me-α-L-Fuc*p*

[a] Kan4N = kansosamine (4-amino-4-deoxy-3-*C*-methyl-2-*O*-methylrhamnose), Eva = evalose (3-*C*-methylrhamnose), QuixN = *x*-amino-*x*-deoxy-6-deoxyglucose (quinovose), Ac = acetyl, Fo = formyl, La = lactoyl (configuration unknown), MHB = 2-methyl-3-hydroxybutanoyl (configuration unknown), Me = *O*-methyl, Pyr = pyruvic acetal [4,6-*O*-(1-carboxyethylidene)]. **Bold type** is used for sugars of previously unknown type and with undetermined configurational assignments [enantiomeric (D,L) or anomeric (α,β) for the sugar, or chiral center (R,S) in acyl substituents]. [f] All chains are terminated internally by this sequence in attachment to allothreonine residues in the peptide moiety.



Fatty acyl-CO-NH-(D)-Phe-(D)-alloThr-(D)-Ala-(L)-alaninol-O-sugar
|
O-sugar

1

chain, comprising one, two, or three additional glycosyl units[3]. These outer segments contain a remarkable array of rare sugars as constituents and in previously unknown types of combination. For such unusual structures chemical synthesis of artificial antigens with distinctive sugar units conjugated to protein could furnish potential specific diagnostic agents less expensively and in larger amounts than would be readily accessible by isolation of the natural antigens.

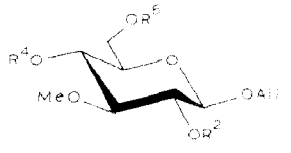
The most widely used method for the attachment of glycosyl units to protein as potential epitopes is that developed by Lemieux and his collaborators[4]. In this method the glycosidically attached 8-methoxycarbonyloctyl linker arm is converted into the hydrazide for generation of the azidocarbonyl derivative, which acylates ε-amino groups of lysine residues in a carrier protein. This approach has been used in the preparation of neoglycoproteins (NGPs) containing one, two or all three of the outer sugar residues of the natural phenolic glycolipid from *M. leprae*[5-7]. These synthetic antigens have been shown to be of high selectivity for the serodiagnosis of leprosy. Since antibodies to this organism may recognize small carbohydrate epitopes containing a 3,6-di-*O*-methyl-β-D-glucopyranose unit, even as a single sugar residue, we have explored the synthesis of NGPs with the smallest number of terminal sugar residues required as specific epitopes for GPLs from *M. avium* serovars. Additional objectives have been: (1) to achieve conjugation to protein via the simplest type of linker arm consistent with binding of the carbohydrate moiety to antibodies, and (2) to retain potentially removable groups such as *O*-acetyl substituents, which would be lost during hydrazide formation from methoxycarbonyloctyl functions in the Lemieux procedure[4]. To these ends we have examined the method of Bernstein and Hall[8] in which ozonolysis of allyl glycosides is followed by a reductive amination step leading to the formation of *N*-glycosyloxyethyllysine residues in the protein. Our results show that this type of NGP is satisfactory for the presentation of glycosyl epitopes to antibodies to mycobacterial species. Two examples are presented of the immunoreactivity/seroreactivity of NGPs, namely those incorporating the terminal disaccharide units of GPLs from serovars 2 and 4.

This paper describes the synthesis of epitopes containing 6-deoxyhexose residues as their allyl glycosides. The synthetic requirements are for the regioselective introduction of methyl ether substituents, with the use of temporary protecting groups whose introduction and subsequent removal must be compatible with retention of the allyl glycosidic substituent. Allyl glycosides are versatile units for oligosaccharide synthesis, providing the innermost residue, which is to be modified for conjugation to protein, and also serving as precursors of glycosyl donors (*e.g.* glycosyl halides or trichloroacetimidates) for the attachment of terminal residues.

RESULTS AND DISCUSSION

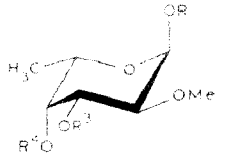
Synthesis of allyl glycosides for conversion into neoglycoproteins. — Attention was turned first to the synthesis of allyl 3,6-di-*O*-methyl-β-D-glucopyranoside (**2**), required for the preparation of an NGP related to the phenolic glycolipid from *M. leprae*. The

phenolic glycolipids are a class of mycobacterial glycolipid antigens different from the GPLs (ref. 3). The advantage of the product from *M. leprae* in the present context is a large body of published information on NGPs. Compound **2** was prepared from allyl 3-*O*-methyl-β-D-glucopyranoside (**4**), which had been generated from 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-β-D-glucopyranose[9] by sequential 1-*O*-deacetylation with hydrazine acetate[10], conversion into the α-D-glucosyl trichloroacetimidate[11], glycosidation (to **3**), and *O*-deacetylation. Acetalation of **4** with 4-methoxybenzaldehyde dimethyl acetal (to **5**), followed sequentially by benzoylation (to **6**), regioselective ring opening[12] (to **7**) with sodium cyanoborohydride in the presence of dry hydrogen chloride, benzoylation (to **8**), and selective removal of the 4-methoxybenzyl substituent with ceric ammonium nitrate, afforded allyl 2,4-di-*O*-benzoyl-3-*O*-methyl-β-D-glucopyranoside (**9**). Methylation at O-6 with diazomethane–boron trifluoride etherate[13] afforded the dimethyl ether **10**, which was then *O*-debenzoylated (to **2**). Ozonolysis of allyl 3,6-di-*O*-methyl-β-D-glucopyranoside (**2**), then coupling to bovine serum albumin by reductive amination,



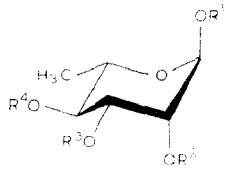
3-Me-β-D-Glc*p* Derivatives

	R^1	R^2	R^4	R^6
2	All	H	H	Me
3	All	Ac	Ac	Ac
4	All	H	H	H
5	All	H	4-MBzd	
6	All	Bz	4-MBzd	
7	All	Bz	H	MPM
8	All	Bz	Bz	MPM
9	All	Bz	Bz	H
10	All	Bz	Bz	Me



2-Me-α-L-Fuc*p* Derivatives

	R^1	R^3	R^6
11	All	Me	H
12	All	Me	Ac
15	All	MPM	H
16	All	MPM	Me
17	All	H	Me
23	All	NPM	H
28	H	Me	Ac
34	CH_2CH_2NH–BSA	Me	Ac
35	CH_2CH_2NH–BSA	Me	H



α-L-Rha*p* Derivatives

	R^1	R^2	R^3	R^4
13	All	3-Ipd		Me
14	All	H	H	Me
19	All	Ac	Ac	Me
20	H	Ac	Ac	Me
27	All	Bz	H	Bz
32	CH_2CH_2NH–BSA	H	H	Me

MBzd = 4-methoxybenzylidene

MPM = 4-methoxyphenylmethyl (4-methoxybenzyl)

NPM = 2-nitrophenylmethyl (2-nitrobenzyl)

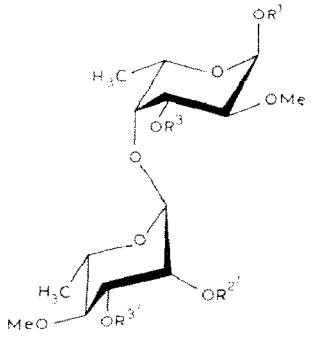
CH_2CH_2NH–BSA = linkage to bovine serum albumin via lysine side chains

gave a neoglycoprotein[8] that was similar in sensitivity and specificity to a previously synthesized NGP when used in the analysis of sera from leprosy patients[5] [details are not reported].

The terminal 2,3-di-*O*-methyl-α-L-fucopyranose residue in the GPL from *M. avium* serovar 9 (ref. 14) is now known to occur as the 4-*O*-acetyl derivative[15]. The existence of the modified structure thus required the conversion of allyl 2,3-di-*O*-methyl-α-L-fucopyranoside[16] (**11**) into the 4-*O*-acetyl derivative (**12**). Both allyl glycosides were submitted to ozonolysis and reductively coupled to bovine serum albumin, using sodium cyanoborohydride at pH 7.8, to give the corresponding NGPs (respectively neo 2 and neo 9), with that from the 4-*O*-acetyl derivative retaining the ester substituent.

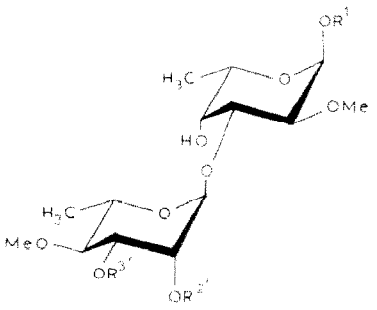
Syntheses of other allyl 6-deoxyhexopyranosides were performed by conventional procedures. Methylation of allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[6] (to **13**) followed by *O*-deisopropylidenation afforded allyl 4-*O*-methyl-α-L-rhamnopyranoside (**14**). Regioselective alkylation of the 3,4-dibutylstannylene derivative of allyl 2-*O*-methyl-α-L-fucopyranoside[16] with 4-methoxybenzyl chloride and tetra-*n*-butylammonium bromide gave the 3-*O*-substituted 4-methoxybenzyl ether (**15**), and methylation (to **16**) followed by treatment with ceric ammonium nitrate[12] furnished allyl 2,4-di-*O*-methyl-α-L-fucopyranoside (**17**). Allyl glycosides **14** and **17** were each converted into the corresponding NGPs, displaying the terminal sugar residues of GPLs from *M. avium* serovars 4 (ref. 15, 17) and 26 (ref. 18), respectively, but no interaction with cognate antibodies to the parent organisms was detected in either case. This is in contrast to the monoglycosyl NGPs which interact with homologous antibodies to serovars 2, 8 and 9 (ref. 15). However, the present results, those in the accompanying paper[19], and unpublished data suggest that a more common requirement is for epitopes to contain a disaccharide unit, as has been indicated in the case of serovar 4 (ref. 15). The NGP containing the *O*-(4-*O*-methyl-α-L-rhamnopyranosyl)-(1→4)-2-*O*-methyl-α-L-fucopyranose unit, attached by the oxynonanoyl (Lemieux) linker arm (Brennan and Chatterjee, unpublished results cited in ref. 15), reacts specifically with monoclonal antibodies to *M. avium* serovar 4, and thereby serves to define the molecular specificity of the antibodies[15].

In the light of these observations we extended these studies to the synthesis of the same disaccharide unit as its allyl glycoside (**18**). Allyl glycosides were used as precursors for both glycosyl residues in **18**. Acetylation of allyl 4-*O*-methyl-α-L-rhamnopyranoside (to **19**) followed by *O*-deallylation by isomerization with tris(triphenylphosphine)-rhodium(I) chloride in the presence of diazabicyclo[2.2.0]octane[20,21] followed by treatment of the resulting 1-propenyl glycoside with mercuric chloride and mercuric oxide[22], gave the reducing sugar (**20**). From this the α-glycosyl trichloroacetimidate[11] was generated by reaction in trichloroacetonitrile in the presence of anhydrous potassium carbonate. Coupling of the trichloroacetimidate with allyl 3-*O*-(4-methoxybenzyl)-2-*O*-methyl-α-L-fucopyranoside (**15**), using trimethylsilyl trifluoromethanesulfonate as catalyst in the presence of tetra-*N*-methylurea, gave the fully substituted disaccharide (**21**) in modest yield (52%). Deprotection, first with ceric ammonium nitrate (to **22**) and then



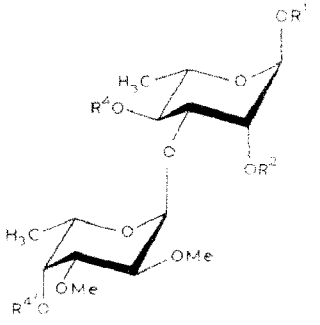
4-Me-α-L-Rhap-(1→4)-2-Me-α-L-Fucp Derivatives

	R^1	R^3	$R^{2'}$	$R^{3'}$
18	All	H	H	H
21	All	MPM	Ac	Ac
22	All	H	Ac	Ac
24	All	NPM	Ac	Ac
31	CH_2CH_2NH-BSA	H	H	H



4-Me-α-L-Rhap-(1→3)-2-Me-α-L-Fucp Derivatives

	R^1	$R^{2'}$	$R^{3'}$
25	All	Ac	Ac
26	All	H	H
33	CH_2CH_2NH-BSA	H	H



2,3-Me_2-α-L-Fucp-(1→3)-α-L-Rhap Derivatives

	R^1	R^2	R^4	$R^{4'}$
29	All	Bz	Bz	Ac
30	All	H	H	H
36	CH_2CH_2NH-BSA	H	H	H

with sodium methoxide to remove *O*-acetyl substituents afforded the desired disaccharide allyl glycoside (**18**). When the synthesis was repeated with the corresponding glycosyl chloride as donor and silver trifluoromethanesulfonate as catalyst in the presence of a large excess of tetramethylurea, the yields of the fully substituted disaccharide **21** were even less satisfactory. On this occasion, however, the presence of the partially deprotected disaccharide **22** in the reaction mixture was recognized, pointing to the susceptibility of the 4-methoxybenzyl ether group to inadvertent cleavage, probably by traces of acid generated during workup. We have observed this behaviour with other 4-methoxybenzyl ethers, especially in contact with silica gel.

In an attempt to circumvent this problem two other routes to the disaccharide were explored. In one of these, regioselective etherification of the dibutylstannylene derivative of allyl 2-*O*-methyl-α-L-fucopyranoside was carried out with 2-nitrobenzyl bromide and the resulting ether (**23**) was substituted for the 4-methoxybenzyl ether **15** in the disaccharide coupling. Fully protected disaccharide (**24**) was obtained in better yield (70%), and photolytic removal of the nitrobenzyl substituent[23] furnished the partially deprotected disaccharide **22**.

Another projected synthesis of the disaccharide allyl glycoside **18** was predicated on observations of Paulsen *et al.*[24] that regioselective glycosylation at the hydroxyl groups of the 3,4-diol in benzyl penta-*O*-benzyl-β-lactoside may be achieved through the use of soluble silver trifluoromethanesulfonate to give the 3-linked derivative, and of insoluble silver silicate supported on alumina to give the 4-linked isomer. Allyl 2-*O*-methyl-α-L-fucopyranoside, containing a stereochemically similar 3,4-diol group, when condensed with 2,3-di-*O*-acetyl-4-*O*-methyl-α-L-rhamnopyranosyl bromide in dichloromethane in the presence of silver silicate on silica gel gave a major product (69% yield) that was chromatographically and spectroscopically distinguishable from the previously characterized 4-linked disaccharide (**22**), which was isolated as a minor product (~5%). The major product **25** contained an α-L-rhamnopyranosyl linkage, and *O*-deacetylation furnished allyl *O*-(4-*O*-methyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-methyl-α-L-fucopyranoside (**26**), characterized by methylation analysis which afforded *inter alia* the partially methylated alditol acetate derived from 2,4-di-*O*-methylfucose. Similar results were obtained when the condensation was repeated using silver silicate supported on alumina as the catalyst.

The GPL from serovar 2 carries a trisaccharide unit comprised of a single sugar residue, 2,3-di-*O*-methyl-α-L-fucopyranose, attached to the →3)-α-L-Rha*p*-(1→2)-6-deoxy-L-Tal*p* inner chain[25]. In the light of the observations for a number of serovars, although not for serovar 2 (ref. 15), that the terminal disaccharide unit is the minimum required size for the carbohydrate epitope to be recognized by antibodies, the allyl glycoside of the terminal disaccharide segment of the serovar 2 GPL, including the →)-3-α-L-Rha*p* residue, was synthesized. Allyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (**27**) was prepared *via* the route previously used by Josephson and Bundle[26] for the synthesis of similarly substituted glycosides, involving formation of the cyclic 2,3-orthobenzoate followed by benzoylation and regioselective opening of the orthoester. A further quantity of allyl 4-*O*-acetyl-2,3-di-*O*-methyl-α-L-fucopyranoside (**12**) was prepared from allyl 2-*O*-methyl-α-L-fucopyranoside, as described previously for the 4-benzoyl derivative[16], by formation of the cyclic 3,4-orthoacetate followed by regioselective ring opening and methylation with diazomethane–boron trifluoride[13]. Glycoside **12** was treated with selenium dioxide[27] to give 4-*O*-acetyl-2,3-di-*O*-methyl-L-fucopyranose (**28**), and this was converted into the α-trichloroacetimidate. Condensation with **27** using boron trifluoride etherate as catalyst gave the fully acylated disaccharide (**29**), which on *O*-deacylation furnished the desired allyl *O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**30**). Ozonolysis of allyl glycosides and glycobiosides followed by reductive coupling[8] afforded NGPs carrying, in general, 25–30 mol of carbohydrate per mol of bovine serum albumin, which has a total of 59 lysine residues.

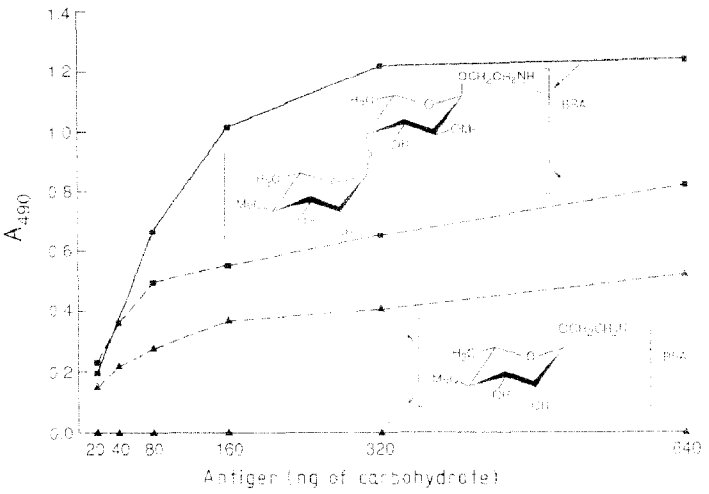


Fig. 1. Comparison of antigenic activities of different concentrations of the glycobiosyl NGP 4 (**31**) and the monoglycosyl NGP 4 (ref. 15) against *M. avium* serovar 4 monoclonal antibody 32B8 (ascites fluid diluted 1:8000, continuous lines) and *M. avium* serovar 4 polyclonal rabbit antibody (serum diluted 1:8000, interrupted lines) in plate ELISA.

Serology. — NGP 4 (**31**), prepared from disaccharide allyl glycoside **18** in parallel with the previously described[15] monoglycosyl NGP containing only the 4-Me-α-L-Rha*p* residue (**32**), was reacted with monoclonal antibody 32B8 to *M. avium* serovar 4. As measured by the plate-ELISA technique, glycobiosyl NGP 4 had the same reactivity (Fig. 1) as the corresponding NGP prepared by Chatterjee and Brennan[15], showing that antibody recognition of this disaccharide epitope is independent of linker arm. The isomeric disaccharide allyl glycoside **26** was also converted into the corresponding NGP 4* (**33**), which failed to react with antibodies to serovar 4. This example of discrimination between correct and incorrect linkage types in similarly constituted disaccharide epitopes provides a further example of the exquisite specificity of monoclonal antibodies in binding to carbohydrate structures.

It has been shown elsewhere[15] that NGP 9 (**34**) bearing the 4-Ac-2,3-Me_2-α-L-Fuc*p* residue, is reactive towards monoclonal antibodies to whole cells of *M. avium* serovar 9, whereas the 2,3-Me_2-α-L-Fuc*p* monoglycosyl NGP 2 (**35**) is unreactive. We have now compared the immunoreactivity of **35** and the glycobiosyl NGP (**36**) prepared from **30**, and the results of analysis are summarized in Table II. Thus, polyclonal antibodies, raised to the whole serovar 2 organism, and relatively specific for the native GPL, do not significantly distinguish between NGPs **35** and **36**. Clearly, then the two NGPs emulate, to some extent at least, the reactivity of the native organism against polyclonal antibodies. Interestingly, we had previously[15] observed that anti-serovar 9 polyclonal antibodies do not recognize the 2,3-Me_2-α-L-Fuc*p* NGP (**35**), an observation attributable to the absence of the 4-Ac-2,3-Me_2-α-L-Fuc*p* residue in the GPL of serovar 2. On the other hand, it was also shown[15] that a prototype anti-serovar 2 monoclonal antibody, MAb 123.4, reacts readily with whole cells of serovar 2, and with the native

TABLE II

Immunoreactivity of neoglycoproteins emulating the epitope of *M. avium* serotype 2[a]

NGP	*Concentration (ng/well)*	A_{490}	
		Rabbit antibody to serovar 2	*Serovar 2-specific MAb 123.4*
2,3-Me_2-α-L-Fuc*p*BSA (**35**)	39.2	1.648	1.139
	9.8	1.364	0.685
	2.5	0.856	0.240
	0.6	0.308	0.071
2,3-Me_2-α-L-Fuc*p*-(1→3)-α-L-Rha*p*-BSA (**36**)	39.2	1.850	0.156
	9.8	1.535	0.041
	2.5	1.001	0.012
	0.6	0.473	0.006
BSA	39.2	0.022	0.006
	9.8	0.007	0.003
	2.5	0.009	0.004
	0.6	0.008	0.010

[a] Conditions for enzyme-linked immunosorbent assay (ELISA) are described in ref. 15. High values of A_{490} show strong interaction of NGP with antibody. The rabbit antibody was diluted 1:250 for the assay, whereas the monoclonal antibody (MAb) was employed as the undiluted culture superantant.

GPL, but not with the GPL after base treatment, which presumably removes *O*-acetyl groups. The present results show that this MAb reacts with monoglycosyl NGP (**35**) but barely with glycobiosyl NGP (**36**). As pointed out earlier[15] the reactivity patterns of MAbs often defy ready explanation, but this may be due to a lack of work with antigens of known structure, necessary for probing the specific requirements for interaction. In this instance, the surprising contrast between the reactivity of the MAb with **35** and its virtual lack of reactivity with **36** parallels the contrasting reactivities of the MAb with the above-mentioned native and *O*-deacetylated GPLs. It is tempting to suggest that in the reaction of MAb 123.4 with naturally derived and synthetic antigens containing the 2,3-Me_2-α-L-Fuc*p* terminal residue, the absence of hydroxyl groups on the aglycone (linker arm in **35** or acetylated Rha*p* residue in the native GPL) may be a more important factor for effective antigen–antibody binding than the specific presence of *O*-acetyl groups.

EXPERIMENTAL

General methods. — The following solvents were dried and purified by distillation over the reagents specified in parentheses: dichloromethane (phosphorus pentaoxide), oxolane (lithium aluminum hydride), methanol (calcium hydride), and benzene (so-

dium). Chromatographic solvents were purchased already dried and glass-distilled. Molecular sieves were activated and potassium carbonate was dried by heating to 400°. Evaporations were conducted under diminished pressure at <40°. T.l.c. was conducted on plates coated with Silica Gel $60F_{254}$ (Merck). Preparative separations were performed on circular plates coated with silica gel (1 mm or 4 mm thickness), using a "Chromatotron" Model 7924T (Harrison Research, Palo Alto, CA). Optical rotations were measured with a Perkin–Elmer 141 polarimeter, for solutions in $CHCl_3$ at ~20° unless otherwise stated. N.m.r. spectra were recorded with a Bruker AM 300 spectrometer, for solutions in chloroform-*d* unless otherwise stated. N.m.r. spectra are reported in sufficient detail only to substantiate chemical changes effected. Except in the initial descriptions of glycosides resonances for allyl groups are not cited individually since all showed δ_H 6.05–5.90 (m, 1 H, $CH=CH_2$), 5.36–5.20 (m, 2 H, $CH=CH_2$), and 4.20–3.90 (m, 2 H, OCH_2). G.l.c. was performed with a Perkin–Elmer Sigma 3B chromatograph, using a 15 m wide-bore capillary fused-silica column coated with DB-225. For g.l.c.–m.s. the column was attached by a jet separator to a VG Micromass 16F mass spectrometer, which was operated with an inlet temperature of 250°, an ionization potential of 70 eV, and an ion-source temperature of ~250°. Microanalyses were carried out by Guelph Chemical Laboratories, Ltd.

The homogeneities of compounds, in cases where inadequate quantities were available for combustion analysis, were established by t.l.c. in the solvent systems used for their preparative separations, and by their ^{1}H-n.m.r. spectra. Their identities were supported by accurate mass determinations carried out by fast atom bombardment mass spectrometry in a thioglycerol (TG) matrix, performed by Dr. Henrianna Pang and her associates at the University of Toronto Carbohydrate Research Centre.

Glycosyl donors. — In method A, glycosyl α-trichloroacetimidates were prepared by reaction of the anomerically unsubstituted sugar with trichloroacetonitrile in dry dichloromethane by stirring with anhydrous potassium carbonate for 24 h or longer, until t.l.c. showed complete conversion. The desired trichloroacetimidate[27] was obtained by filtration and concentration. ^{1}H-N.m.r. spectroscopy confirmed the anomeric configuration of the product, which was used directly without further purification. In method B similar reaction mixtures containing 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) as catalyst[28] were stirred for 30–60 min at room temperature. Purification of the typically dark coloured product by chromatography on silica gel (1:1 light petroleum–diethyl ether) removed DBU and furnished trichloroacetimidate of adequate purity (t.l.c. and ^{1}H-n.m.r.) for use in glycosylation.

Neoglycoproteins. — The procedure of Bernstein and Hall[5] was applicable to the synthesis of all NGPs. Allyl glycoside (~10 mg) in methanol (30 mL) was ozonized for 10 min at −78°, then treated with dimethyl sulfide (15 μL) for 2 h, and concentrated to a syrup. The product (~9 mg) and bovine serum albumin (15 mg) in 0.2M sodium phosphate buffer (2 mL, pH 7.8) were treated with sodium cyanoborohydride (15 mg) for 72 h at 37°. The resulting solution was passed through a column of Sephadex G-25 equilibrated in, and eluted with, 0.2M phosphate buffer of pH 7.8, and carbohydrate-rich fractions were dialyzed against distilled water and freeze-dried to give the NGP

(~ 18 mg). Analysis of carbohydrate was by the phenol–sulfuric acid method[29] using the allyl glycoside as reference standard.

Immunological procedures. — Details of the application of plate-ELISA and Western blot analysis to NGPs have been described in detail[15] for other *M. avium*-related NGPs and for NGPs emulating the sugar determinants of the *M. leprae* glycolipid[6,30]. Original sources for the preparation of polyclonal rabbit antibodies to whole bacteria have been described by Tsang *et al.*[31], the sources of the monoclonal antibodies used in this research have been reported[15], and new antibodies were prepared as described[15].

*Allyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-*D*-glucopyranoside* (**3**). — 1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-β-D-glucopyranose[9] (1.0 g 2.76 mmol) in dry *N,N*-dimethylformamide (10 mL) was stirred with hydrazine acetate (317 mg, 1.2 equiv.) for 15 min at 50°. Ethyl acetate (30 mL) was added and the mixture washed with satd. aq. sodium chloride (3 × 30 mL), dried, and concentrated to syrupy 2,4,6-tri-*O*-acetyl-3-*O*-methyl-D-glucopyranose (784 mg, 89%) which was used without further purification. Reaction of the hemiacetal (0.77 g, 2.40 mmol) with trichloroacetonitrile (1.5 mL) and 1,5-diazabicyclo[5.4.0]undec-5-ene (0.39 mL) in dry dichloromethane (10 mL) (method B) furnished 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranosyl trichloroacetimidate (837 mg, 75%) as a syrup, which was used directly for glycosylation; ^{1}H-n.m.r.: δ 8.67 (s, 1 H, N*H*), 6.51 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1α), 3.49 (s, 3 H, OCH_3), 2.12 (3 H) and 2.07 (6 H) (2 s, 3 OCOCH_3). A solution of the trichloroacetimidate (0.83 g, 1.78 mmol) and allyl alcohol (0.4 mL) in dichloromethane (15 mL) was stirred for 1 h over 4A molecular sieves, boron trifluoride etherate (0.45 mL) was added, and stirring was continued for 1 h. The solution was filtered through Celite, and the combined filtrate and washings were washed with satd. aq. sodium hydrogencarbonate and then water, dried, and concentrated. Chromatography of the residue on silica gel (1:1 light petroleum–diethyl ether) furnished allyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranoside (**3**) (464 mg, 69%), which crystallized from ether and had m.p. 63–65°; $[\alpha]_D$ −38.7° (*c* 0.6); ^{1}H-n.m.r.: δ 4.51 (d, 1 H, $J_{1,2}$ 7.86 Hz, H-1β), 3.52 (s, 3 H, OCH_3), 2.08, 2.05, and 2.04 (3 s, 9 H, OCOCH_3).

Anal. Calc. for $C_{16}H_{24}O_9$: C, 53.33; H, 6.71. Found: C, 53.48; H, 6.89.

The anomeric α-D-glucoside (82 mg, 13%) was also isolated and characterized by its ^{1}H-n.m.r. spectrum [δ 5.08 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1α)]. Compound **3** was also prepared from the corresponding glycosyl bromide but in significantly lower yield and with less stereoselectivity.

*Allyl 3-*O*-methyl-β-*D*-glucopyranoside* (**4**). — A catalytic quantity of M methanolic sodium methoxide was added to **3** (326 mg, 0.9 mmol) in dry methanol. After 16 h sodium ions were removed by treatment of the solution with Dowex 50W-X8 resin, and the filtrate was concentrated to a syrup which crystallized from ether to give **4** (183 mg, 86%), m.p. 65–66°; $[\alpha]_D$ −54° (*c* 1.43, MeOH); ^{1}H-n.m.r.: δ 4.33 (d, 1 H, $J_{1,2}$ 7.62 Hz, H-1), 3.66 (s, 3 H, OCH_3).

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.75. Found: C, 50.89; H, 7.30.

*Allyl 4,6-*O*-(4-methoxybenzylidene)-3-*O*-methyl-β-*D*-glucopyranoside* (**5**). — A solution of **4** (6.09 g, 26 mmol), 4-methoxybenzaldehyde dimethyl acetal (6.7 mL, 39

mmol), and *p*-toluenesulfonic acid (49 mg, 0.26 mmol) in *N,N*-dimethylformamide (40 mL) was heated for 2 h at 50° under reduced (aspirator) pressure, to ensure removal of methanol. Solid sodium hydrogencarbonate was added, the solution was concentrated at 80° under reduced pressure to ~10 mL, then poured into a mixture of ice (25 g) and saturated aqueous sodium hydrogencarbonate (50 mL), and stirred. The precipitate which separated was dried and recrystallized from dichloromethane–light petroleum to yield **5** (7.53 g, 82%), m.p. 146–147°; $[\alpha]_D$ −45.8° (*c* 0.79); ^{1}H-n.m.r.: δ 7.40–6.88 (m, 4 H, Ph-H), 5.50 (s, 1 H, C*H*Ph), 4.45 (d, 1 H, $J_{1,2}$ 7.61 Hz, H-1), 3.80, 3.65 (2 s, 6 H, OCH_3), and 2.50 (br. s, 1 H, exchanged with D_2O).

Anal. Calc. for $C_{31}H_{32}O_7$: C, 61.35; H, 6.86. Found: C, 61.11; H, 6.78.

*Allyl 2-O-benzoyl-4,6-O-(4-methoxybenzylidene)-3-O-methyl-β-*D*-glucopyranoside* (**6**). — Benzoyl chloride (7.3 mL, 63 mmol) was added slowly to a solution of **5** (7.4 g, 21 mmol) in pyridine (50 mL) at 0°, the solution was allowed to reach room temperature, and after 2 h was poured into ice–water and stirred for 1 h. The mixture was extracted with dichloromethane and the extract was concentrated. Recrystallization of the crystalline residue afforded **6** (8.22 g, 86%), m.p. 173–174°; $[\alpha]_D$ −16° (*c* 1.04); ^{1}H-n.m.r.: δ 8.07–6.89 (m, 9 H, Ph-H), 5.54 (s, 1 H, C*H*Ph), 4.69 (d, 1 H, $J_{1,2}$ 7.78 Hz, H-1), 3.80, and 3.51 (2 s, 6 H, OCH_3).

Anal. Calc. for $C_{25}H_{28}O_8$: C, 65.78; H, 6.18. Found: C, 65.84; H, 6.10.

*Allyl 2-O-benzoyl-3-O-methyl-6-O-(4-methoxybenzyl)-β-*D*-glucopyranoside* (**7**). — Trifluoroacetic acid (12.6 mL, 164 mmol) in dry *N,N*-dimethylformamide was added dropwise with stirring to a solution of **5** (7.5 g, 16.4 mmol) and sodium cyanoborohydride (5.15 g, 82 mmol) in dry *N,N*-dimethylformamide (50 mL) containing 3A molecular sieves, and stirring was continued for 18 h at room temperature. The filtered solution was poured into ice–saturated aqueous sodium hydrogencarbonate, the mixture was extracted several times with dichloromethane, and the extract was dried and concentrated. The residue was chromatographed on silica gel (light petroleum–ethyl acetate) to give **7** (5.26 g, 70%) as a syrup, $[\alpha]_D$ −12.5° (*c* 1.28); ^{1}H-n.m.r.: δ 8.08–6.89 (m, 9 H, Ph-H), 4.57 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.58, 4.52 (ABq, 2 H, *J* 11.6 Hz, OC*H*$_2$Ph), 3.81, and 3.49 (2 s, 6 H, OCH_3).

Anal. Calc. for $C_{25}H_{30}O_8$: C, 65.49; H, 6.59. Found: C, 65.81; H, 6.87.

*Allyl 2,4-di-O-benzoyl-3-O-methyl-6-O-(4-methoxybenzyl)-β-*D*-glucopyranoside* (**8**). — Benzoylation of **7** (1.39 g, 3.0 mmol) as in the synthesis of compound **6** followed by work up and chromatography on silica gel (dichloromethane–ethyl acetate mixtures) gave **7** (1.57 g, 93%) which after crystallization from dichloromethane–hexane had m.p. 90–91°; $[\alpha]_D$ −8.1° (*c* 1.1); ^{1}H-n.m.r.: δ 8.20–6.76 (m, 14 H, Ph-H), 5.33 (dd, 1 H, $J_{2,3}$ 7.76, $J_{2,3}$ 9.28 Hz, H-2), 5.33 (dd, 1 H, $J_{3,4} = J_{4,5}$ ~9.52 Hz, H-4), 4.70 (d, 1 H, H-1), 4.44 (s, 2 H, OCH_2Ph), 3.74, and 3.37 (2 s, 6 H, OCH_3).

Anal. Calc. for $C_{32}H_{34}O_8$: C, 68.31; H, 6.09. Found: C, 68.06; H, 5.98.

*Allyl 2,4-di-O-benzoyl-3,6-di-O-methyl-β-*D*-glucopyranoside* (**10**). — Ceric ammonium nitrate (2.52 g, 4.6 mmol) was added to **8** (1.3 g, 2.3 mmol) in 9:1 acetonitrile–water (12 mL) and the solution was stirred for 4 h at room temperature, diluted with dichloromethane, washed successively with aq. sodium hydrogencarbonate, aq. sodium

chloride, and water, dried, and concentrated. The residue was chromatographed on silica gel (9:1 light petroleum–ethyl acetate) to give syrupy allyl 2,4-di-*O*-benzoyl-3-*O*-methyl-β-D-glucopyranoside (**9**) (1.01 g, 98%), ^{1}H-n.m.r.: δ 8.08–7.46 (m, 10 H, Ph-H), 5.33 (dd, 1 H, $J_{1,2}$ 7.70, $J_{2,3}$ 9.24 Hz, H-2), 5.30 (dd, 1 H, $J_{3,4} = J_{4,5}$ ~9.0 Hz, H-4), 4.74 (d, 1 H, H-1), 3.88 (dd, 1 H, H-3), 3.40 (s, 3 H, OCH_3), and 2.51 (br. s, 1 H, O*H*).

A solution of the just-described syrupy **9** (808 mg, 1.8 mmol) in dry dichloromethane (10 mL) was stirred at 0° with boron trifluoride etherate (25 μL), and diazomethane in dichloromethane was added until a faint yellow color persisted. After 1.5 h the solution was diluted with dichloromethane, filtered, washed with aq. sodium hydrogencarbonate and water, dried, and concentrated. Chromatography of the residue on a silica gel plate (3:1 light petroleum–ethyl acetate) using the Chromatotron afforded **10** (543 mg, 66%) which was crystallized from ethyl acetate–hexane, m.p. 109–111°; $[\alpha]_D$ −19° (*c* 1.33); ^{1}H-n.m.r.: δ 8.09–7.36 (m, 10 H, Ph-H), 4.71 (d, 1 H, $J_{1,2}$ 7.78 Hz, H-1), 3.38, and 3.34 (2 s, 6 H, OCH_3).

Anal. Calc. for $C_{24}H_{26}O_8$: C, 65.76; H, 6.18. Found: C, 65.68; H, 6.15.

*Allyl 3,6-di-*O*-methyl-β-*D*-glucopyranoside* (**2**). — Molar sodium methoxide in methanol (3 mL) was added to a solution of **10** (1.0 g, 2.2 mmol) and the solution was kept for 24 h at room temperature. The solution was neutralized by treatment with dry Amberlite IR-120 (H^+) resin, filtered, and concentrated to a syrup. This was chromatographed on a silica gel plate (9:1 dichloromethane–acetone) using the Chromatotron to yield **1** (333 mg, 59%) as a syrup, $[\alpha]_D$ −40.5° (*c* 1.52); ^{1}H-n.m.r.: δ 4.31 (d, 1 H, $J_{1,2}$ 7.77 Hz, H-1), 3.67, and 3.41 (2 s, 6 H, OCH_3); ^{13}C: δ 101.7 (C-1), 85.53 (C-3), 72.40 (C-6), 60.58, and 59.47 (2 OCH_3).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.11. Found: C, 52.92; H, 8.32.

*Allyl 4-*O*-acetyl-2,3-di-*O*-methyl-α-*L*-fucopyranoside* (**12**). — (*A*). Conventional acetylation of allyl 2,3-di-*O*-methyl-α-L-fucopyranoside[16] afforded **12** as a syrup, $[\alpha]_D$ −166° (*c* 1.4); ^{1}H-n.m.r.: δ 5.04 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.49, 3.41 (2 s, 6 H, OCH_3), 2.17 (s, 3 H, OCOCH_3), and 1.14 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6).

Anal. Calc. for $C_{11}H_{20}O_5$: C, 56.92; H, 8.80. Found: C, 57.24; H, 8.09.

(*B*). A further quantity of **12** was prepared, as described for the corresponding benzoate[16], by reaction of a mixture of allyl 2-*O*-methyl-α-L-fucopyranoside (2.02 g, 9.3 mmol), trimethyl orthoacetate (2.24 mL), and *p*-toluenesulfonic acid (50 mg) in acetonitrile (20 mL), followed by regioselective hydrolysis to give allyl 4-*O*-acetyl-2-*O*-methyl-α-L-fucopyranoside (1.91 g, 79%) as a syrup, $[\alpha]_D$ −126° (*c* 1.3); ^{1}H-n.m.r.: δ 5.23 (d, 1 H, $J_{3,4(or\ 4,5)}$ 3.67 Hz, H-4), 5.07 (d, 1 H, $J_{1,2}$ 3.50 Hz, H-1), 3.48 (s, 3 H, OCH_3), 2.17 (s, 3 H, OCOCH_3), and 1.13 (d, 3 H, $J_{5,6}$ 6.56 Hz, H-6). Methylation of this compound with diazomethane in dichloromethane[16] afforded **12** (1.44 g, 75%) as a syrup with the same optical rotation and ^{1}H-n.m.r. spectrum.

*Allyl 2,3-*O*-isopropylidene-4-*O*-methyl-α-*L*-rhamnopyranoside* (**13**). — Allyl α-L-rhamnopyranoside was prepared from L-rhamnose by heating in allyl alcohol with Amberlite IR-120 (H^+) resin for 16 h at 60°, followed by chromatography on silica gel (19:1 chloroform–methanol). For this product $[\alpha]_D$ −75° (*c* 1.0) was recorded consistently, at variance with the literature[32] $[\alpha]_D$ value of −56°. Isopropylidenation as

described[6] furnished allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside having $[\alpha]_D$ −35° (*c* 1.0) (lit.[6] $[\alpha]_D$ −35°). Sodium hydride (5 g) was added to the latter compound (11.3 g, 46 mmol) in oxolane (50 mL), the mixture was stirred for 30 min at room temperature then cooled to 0°, methyl iodide (8 mL) was added, and the mixture was allowed to reach room temperature over 3 h. Excess hydride was destroyed by addition of methanol, solvents were evaporated, the residue was dissolved in chloroform, and the solution was washed twice with satd. aq. sodium chloride and once with water, dried, and concentrated. The resulting syrup (11.9 g), containing only traces of impurities (t.l.c. in 10:1 chloroform–methanol), was used without purification in the next step. A small sample was chromatographed on silica gel (1:1 light petroleum–diethyl ether) to furnish an analytical sample of **13**, $[\alpha]_D$ −48.3° (*c* 1.0); ^{1}H-n.m.r.: δ 5.00 (s, 1 H, H-1), 3.53 (s, 3 H, OCH_3), 1.55, 1.35 [2 s, 6 H, (CH_3)$_2$C], and 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6).

Anal. Calc. for $C_{13}H_{22}O_5$: C, 60.47; H, 8.53. Found: C, 60.32; H, 8.56.

Allyl 4-O-methyl-α-L-rhamnopyranoside (**14**). — A solution of **13** (11.65 g, 45 mmol) in 3:2 acetic acid–water (50 mL) was stirred for 30 min at room temperature, and then concentrated with repeated additions of toluene. The residue was chromatographed on silica gel (1:1 light petroleum–ethyl acetate) to give **14** (8.05 g, 80% from allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside) as a syrup, $[\alpha]_D$ −78.5° (*c* 1.0, methanol); ^{1}H-n.m.r.: δ 4.80 (s, 1 H, H-1), 3.57 (s, 3 H, OCH_3), and 1.23 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6).

Anal. Calc. for $C_{10}H_{18}O_5$: C, 55.05; H, 8.26. Found: C, 54.61; H, 7.89.

Allyl 3-O-(4-methoxybenzyl)-2-O-methyl-α-L-fucopyranoside (**15**). — A solution of allyl 2-*O*-methyl-α-L-fucopyranoside[16] (3.51 g, 16 mmol) in benzene containing dibutyltin oxide (4.0 g) was boiled for 2 h under reflux with continuous removal of water. After concentration to ~75 mL, 4-methoxybenzyl chloride (4.4 mL) and tetra-*n*-butylammonium bromide (5.2 g) were added, and and the mixture was boiled under reflux overnight and then concentrated. The residue was chromatographed on silica gel (10:1 then 2:1 light petroleum–ethyl acetate) to give **15** (4.68 g, 86%) as a syrup, $[\alpha]_D$ −105° (*c* 0.99); ^{1}H-n.m.r.: δ 7.30–6.87 (m, 4 H, Ph-H), 4.99 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.73, 4.58 (ABq, 2 H, *J* 11.1 Hz, OCH_2Ph), 3.80, 3.52 (2 s, 6 H, OCH_3), and 1.27 (d, 3 H, $J_{5,6}$ 6.62 Hz, H-6).

Anal. Calc. for $C_{18}H_{26}O_6$: 63.89; H, 7.74. Found: 64.36; H, 8.05.

Allyl 3-O-(4-methoxybenzyl)-2,4-di-O-methyl-α-L-fucopyranoside (**16**). — Sodium hydride (1 g) was added to **15** (2.3 g, 10.5 mmol) in oxolane (27 mL), the mixture was stirred for 30 min at room temperature then cooled to 0°, methyl iodide (2.7 mL) was added, and the mixture was allowed to reach room temperature during 3 h. Workup as for **13** furnished **16** (2.10 g, 88%) as a syrup, $[\alpha]_D$ −186° (*c* 1.02); ^{1}H-n.m.r.: δ 7.29–6.88 (m, 4 H, Ph-H), 4.99 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.73, 4.61 (ABq, 2 H, *J* 11.4 Hz, OCH_2Ph), 3.80, 3.59, 3.52 (3 s, 9 H, OCH_3), and 1.21 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6).

Anal. Calc. for $C_{19}H_{28}O_6$: C, 64.75; H, 8.00. Found: C, 64.44; H, 8.14.

Allyl 2,4-di-O-methyl-α-L-fucopyranoside (**17**). — A solution of **16** (100 mg) and ceric ammonium nitrate (300 mg) in 9:1 acetonitrile–water (1 mL) was kept for 3.5 h at room temperature, then diluted with dichloromethane, washed with satd. aq. sodium

hydrogencarbonate, dried, and concentrated. The residue was chromatographed on silica gel (1:3 then 1:1.5 benzene–ethyl acetate) to give **17** (65 mg, 98%) which after crystallization from ether–hexane had m.p. 70°; $[\alpha]_D$ −169° (*c* 0.98); ^{1}H-n.m.r.: δ 5.03 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.62, 3.47 (2 s, 6 H, OCH_3), and 1.26 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6). A sample was hydrolyzed, reduced with sodium borohydride, and acetylated to give a partially methylated alditol acetate that on examination by g.l.c.–m.s. gave characteristic fragment ions for a 6-deoxy-2,4-di-*O*-methylhexitol triacetate.

Anal. Calc. for $C_{11}H_{20}O_5$: C, 56.88; H, 8.67. Found: C, 56.84; H, 8.90.

*Allyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-*L*-rhamnopyranoside* (**19**). — Conventional acetylation of **14** (3.48 g) afforded **19** (4.49 g, 93%) as a syrup, $[\alpha]_D$ −54° (*c* 1.2); ^{1}H-n.m.r.: δ 4.72 (s, 1 H, H-1), 3.48 (s, 3 H, OCH_3), 2.14, 2.05 (2 s, 6 H, OCOCH_3), and 1.33 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); exact mass: calc. for $C_{14}H_{22}O_7$ + H + TG, 411.1689; found, 411.1662.

Anal. Calc. for $C_{14}H_{22}O_7$: C, 55.62; H, 7.34. Found: C, 55.62; H, 7.43.

*2,3-Di-*O*-acetyl-4-*O*-methyl-*L*-rhamnopyranose* (**20**). — A mixture of **19** (3.61 g, 12 mmol), tris(triphenylphosphine)rhodium(I) chloride (256 mg), and 1,4-diazabicyclo[2.2.2]octane (1.28 g) in 7:3:1 ethanol–toluene–water (100 mL) was boiled for 6 h under reflux then evaporated to dryness, and the residue was extracted with dichloromethane. The extract was washed successively with water, cold M hydrochloric acid, aq. sodium hydrogencarbonate, and water, dried, and concentrated. To a solution of the residue in 1:1 acetone–water (75 mL) was added mercuric oxide (1.28 g) followed by a solution of mercuric chloride (1.28 g) in 9:1 acetone–water (20 mL). The suspension was stirred for 30 min at room temperature, solids were removed, the filtrate was concentrated, and a solution of the syrupy residue in dichloromethane was washed successively with water, aq. potassium iodide, and water, dried, and concentrated. The residue was chromatographed on silica gel (9:1 dichloromethane–acetone) to give crystalline **20** (2.22 g, 71%), m.p. 93–94°, $[\alpha]_D$ −9.2° (*c* 1.0); ^{1}H-n.m.r.: δ 5.11 (d, 1 H; $J_{1,2}$ 3.5 Hz, H-1α), 3.48 (s, 3 H, OCH_3), 2.14, 2.06 (2 s, 6 H, OCOCH_3), and 1.33 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6).

Anal. Calc. for $C_{11}H_{18}O_7$: C, 50.38; H, 6.92. Found: C, 50.54; H, 6.67.

*Allyl O-(2,3-di-*O*-acetyl-4-*O*-methyl-α-*L*-rhamnopyranosyl)-(1→4)-3-*O*-(4-methoxybenzyl)-2-*O*-methyl-α-*L*-fucopyranoside* (**21**). — α-Trichloroacetimidate (240 mg, 0.59 mmol; from **20** by method A) in dichloromethane (5 mL) was added to a solution of **15** (100 mg, 0.30 mmol) in dichloromethane (2 mL) containing 4A molecular sieves (0.5 g), and the mixture was stirred for 30 min. Trimethylsilyl trifluoromethanesulfonate (20 μL) in dichloromethane containing tetra-*N*-methylurea (18 μL) was added and the mixture was stirred overnight. Reaction was terminated by the addition of solid sodium hydrogencarbonate, and the solids were removed by filtration through Celite and washed with dichloromethane. The combined filtrate and washings were washed successively with water, aq. sodium hydrogencarbonate, and water, dried, and concentrated. The residual syrup was chromatographed (19:1 then 9:1 dichloromethane–ethyl acetate) on a silica gel plate (Chromatotron) to yield **21** (90 mg, 52%) as a syrup, $[\alpha]_D$ −124° (*c* 3.3); ^{1}H-n.m.r.: δ 7.32–6.84 (m, 4 H, Ph-H), 4.99 (d, 1 H, $J_{1,2}$ 3.56 Hz, H-1), 4.77 (s, 1 H,

H-1'), 3.79, 3.54, 3.42 (3 s, 9 H, OCH_3), 2.13, 2.07 (2 s, 6 H, $OCOCH_3$), 1.20, and 1.06 (2 d, 6 H, $J_{5,6}$ 5.6, 6.2 Hz, H-6,6'); exact mass: calc. for $C_{30}H_{42}O_{12}$ + H + TG, 691.2999; found, 691.3023.

Allyl O-*(2,3-di-*O*-acetyl-4-*O*-methyl-*α*-*L*-rhamnopyranosyl)-(1→4)-2-*O*-methyl-*α*-*L*-fucopyranoside* (**22**). — A solution of **21** (79 mg, 0.14 mmol) and ceric ammonium nitrate (150 mg) in 9:1 acetonitrile–water (10 mL) was stirred for 15 min at room temperature. Workup as for **17**, followed by chromatography (18:1 chloroform–acetone) on a silica gel plate (Chromatotron), furnished **22** (45 mg, 71%) as a syrup, $[\alpha]_D$ −134° (*c* 1.5); ^{1}H-n.m.r.: δ 5.02 (d, 1 H, $J_{1,2}$ 3.14 Hz, H-1), 4.78 (s, 1 H, H-1'), 3.50, 3.49 (2 s, 6 H, OCH_3), 2.14, 2.07 (2 s, 6 H, $OCOCH_3$), 1.34, and 1.25 (2 d, 6 H, $J_{5,6}$ 6.2, 6.5 Hz, H-6,6'); exact mass: calc. for $C_{31}H_{54}O_{11}$ + Na + TG, 593.2244; found, 593.2228.

Anal. Calc. for $C_{31}H_{54}O_{11}$: C, 54.54; H, 7.41. Found: C, 54.39; H, 7.16.

Allyl O-*(4-*O*-methyl-*α*-*L*-rhamnopyranosyl)-(1→4)-2-*O*-methyl-*α*-*L*-fucopyranoside* (**18**). — A solution of **22** (51 mg, 0.11 mmol) in 0.5M sodium methoxide in methanol (2 mL) was kept for 0.5 h at room temperature, neutralized by treatment with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give **18** (38 mg, 92%) as a chromatographically homogeneous syrup (t.l.c. in 1:1 light petroleum–ethyl acetate), $[\alpha]_D$ −72° (*c* 3.4, MeOH); ^{1}H-n.m.r.: δ 5.00 (d, 1 H, $J_{1,2}$ 3.59 Hz, H-1), 4.81 (d, 1 H, $J_{1',2'}$ 1.64 Hz, H-1'), 3.58, 3.49 (2 s, 6 H, OCH_3), 1.34, and 1.21 (2 d, 6 H, $J_{5,6}$ 6.3, 6.5 Hz, H-6,6'); exact mass: calc. for $C_{15}H_{26}O_7$ + Na + TG, 509.2033; found, 509.2020.

Allyl 2-O-*methyl-3-*O*-(2-nitrobenzyl)-α-L-fucopyranoside* (**23**). — Allyl 2-*O*-methyl-α-L-fucopyranoside (568 mg, 2.6 mmol) was converted into the dibutylstannylene derivative as described for the synthesis of **15** and treated with 2-nitrobenzyl bromide (1.38 g). The reaction mixture was worked up in a similar manner, and the product chromatographed successively on silica gel columns with 20:1 chloroform–methanol and 1:1 petroleum–ethyl acetate to give **23** (592 mg, 64%) as a syrup, $[\alpha]_D$ −172° (*c* 0.7); ^{1}H-n.m.r.: δ 8.04–7.42 (m, 4 H, Ph-H), 5.16, 5.02 (ABq, 2 H, *J* 14.4 Hz, OCH_2Ph), 5.03 (d, 1 H, $J_{1,2}$ 3.63 Hz, H-1), 3.47 (s, 3 H, OCH_3), and 1.32 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6).

Anal. Calc. for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.56; N, 3.96. Found: C, 57.54; H, 6.55; N, 3.88.

Allyl O-*(2,3-di-*O*-acetyl-4-*O*-methyl-*α*-*L*-rhamnopyranosyl)-(1→4)-2-*O*-methyl-3-*O*-(2-nitrobenzyl)-*α*-*L*-fucopyranoside* (**24**) *and conversion into allyl* O-*(2,3-di-*O*-acetyl-4-*O*-methyl-*α*-*L*-rhamnopyranosyl)-(1→4)-2-*O*-methyl-*α*-*L*-fucopyranoside* (**22**). — α-Trichloroacetimidate (234 mg, 0.58 mmol; from **20** by method A) in dichloromethane (5 mL) was added to a solution of **23** (174 mg, 0.49 mmol) in dichloromethane (5 mL) containing 4A molecular sieves and the mixture was stirred for 30 min. Trimethylsilyl trifluoromethanesulfonate (19.5 μL) was added and the mixture was stirred overnight. Reaction was terminated, the mixture was worked up as described for the synthesis of **21**, and chromatography on silica gel (9:1 dichloromethane–acetone) afforded **24** (205 mg, 70%) as a syrup, $[\alpha]_D$ −175° (*c* 0.6); ^{1}H-n.m.r.: δ 8.10–7.42 (m, 4 H, Ph-H), 5.03 (d, 1 H, $J_{1,2}$ 3.58 Hz, H-1), 4.79 (d, 1 H, $J_{1',2'}$ 0.93 Hz, H-1'), 3.51, 3.42 (2 s, 6 H, OCH_3), 2.13, 2.08 (2 s, 6 H, $OCOCH_3$), 1.26, and 0.89 (2 d, 6 H, $J_{5,6}$ 6.6, 6.2 Hz, H-6,6'); exact mass:

calc. for $C_{28}H_{39}NO_{13}$ + Na + TG, 728.2564; found, 728.2549.

A solution of **24** (120 mg, 0.2 mmol) in methanol (100 mL) was placed in a Rayonet reactor and irradiated at 300 nm for 5 h, the solution was concentrated, and the residue was chromatographed on silica gel (1:1 light petroleum–ethyl acetate) to yield **22** (69 mg, 75%), $[\alpha]_D$ −134° (*c* 1.5), which was chromatographically and spectroscopically indistinguishable from the previously prepared sample.

Allyl O-*(2,3-di*-O-*acetyl-4*-O-*methyl*-α-L-*rhamnopyranosyl)-(1→3)-2*-O-*methyl*-α-L-*fucopyranoside* (**25**). — Conventional acetylation of **20** afforded 1,2,3-tri-*O*-acetyl-4-*O*-methyl-α-L-rhamnopyranose[33]; ^{1}H-n.m.r.: δ 5.95 (s, 1 H, H-1), 3.50 (s, 3 H, OCH_3), 2.15, 2.13, 2.05 (3 s, 9 H, OCOCH_3), and 1.33 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6). α-Glycosyl bromide (278 mg, 0.86 mmol; from the preceding compound by treatment with hydrogen bromide in acetic acid for 3 h at 0°) in dichloromethane (5 mL) was added at −30° to a solution of allyl 2-*O*-methyl-α-L-fucopyranoside (168 mg, 0.77 mmol) in dichloromethane (5 mL) in the presence of silver silicate on silica gel[24] (150 mg) and 4A molecular sieves (0.2 g). The mixture was stirred overnight at room temperature, solids were removed by filtration, and the combined filtrate and washings were washed with water, dried, and concentrated. The residue was chromatographed on silica gel (1:1 light petroleum–diethyl ether) to furnish **25** (259 mg, 73%) as a syrup, $[\alpha]_D$ −102°(*c* 1.2); ^{1}H-n.m.r.: δ 5.00 (d, 1 H, $J_{1,2}$ 3.67 Hz, H-1), 4.89 (s, 1 H, H-1′), 3.48, 3.47 (2 s, 6 H, OCH_3), 2.15, 2.06 (2 s, 6 H, OCOCH_3), 1.33, and 1.28 (2 d, 6 H, $J_{5,6}$ 6.17, 6.59 Hz, H-6,6′); exact mass: calc. for $C_{21}H_{34}O_{11}$ + Na + TG, 593.2244; found, 593.2228. Further elution of the column afforded **22** (25 mg) having chromatographic mobility and a ^{1}H-n.m.r. spectrum indistinguishable from those of the previously synthesized sample.

A similar condensation using silver silicate on alumina[34] afforded **25** in 69% yield together with **22** as a minor product.

Allyl O-*(4*-O-*methyl*-α-L-*rhamnopyranosyl)-(1→3)-2*-O-*methyl*-α-L-*fucopyranoside* (**26**). — *O*-Deacetylation of **25** (84 mg, 0.18 mmol) as described for the synthesis of **18** afforded **26** (61 mg, 89%) as a syrup, chromatographically homogeneous by t.l.c. (1:1 light petroleum–ethyl acetate), $[\alpha]_D$ −180° (*c* 1.5, MeOH); ^{1}H-n.m.r.: δ 5.01 (d, 1 H, $J_{1,2}$ 3.59 Hz, H-1), 4.81 (d, 1 H, $J_{1',2'}$ 1.64 Hz, H-1′), 3.58, 3.49 (2 s, 6 H, OCH_3), 1.35, and 1.22 (2 d, 6 H, $J_{5,6}$ 6.33, 6.54 Hz, H-6,6′); exact mass: calc. for $C_{17}H_{30}O_{11}$ + Na + TG, 509.2033; found, 509.2033.

A sample of **26** was per-*O*-methylated in oxolane using sodium hydride and methyl iodide. Hydrolysis of the methylated derivative, followed by reduction with sodium borohydride and acetylation, gave the acetates of 2,3,4-tri-*O*-methylrhamnitol and 2,4-di-*O*-methylfucitol which were identified by g.l.c.–mass spectrometry.

Allyl 2,4-di-O-*benzoyl*-α-L-*rhamnopyranoside* (**27**). — Allyl α-L-rhamnopyranoside (5.0 g, 24.5 mmol) in benzene (60 mL) containing trimethyl orthobenzoate (10 mL) was heated under reflux in a Dean–Stark apparatus to remove traces of water, and the mixture was concentrated to ~50 mL. *p*-Toluenesulfonic acid (22 mg) was added to the cooled solution, which was stirred for 1 h. Acid was neutralized by the addition of triethylamine and the mixture was concentrated. Benzoyl chloride (2.2 mL) was added to the residue in pyridine (40 mL) at 0° and the mixture was kept for 1 h at room

temperature. Excess benzoyl chloride was destroyed, solvents were evaporated, the residue was dissolved in dichloromethane, and the solution was washed with water, dried, and concentrated. A solution of the residual syrup in 4:1 acetic acid–water (30 mL) was stirred at room temperature for 30 min, and then concentrated by repeated evaporations with toluene. This residue was chromatographed on silica gel (8:1 light petroleum–diethyl ether) to give **27** (7.69 g, 76%) as a syrup, $[\alpha]_D$ +41° (*c* 3.7); ^{1}H-n.m.r.: δ 8.13–7.44 (m, 10 H, Ph-H), 5.41 (dd, 1 H, $J_{2,3}$ 3.50 Hz, H-2), 5.01 (d, 1 H, $J_{1,2}$ 1.43 Hz, H-1), 4.34 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), and 1.31 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6).

Anal. Calc. for $C_{28}H_{24}O_7$: C, 66.99; H, 5.83. Found: C, 66.74; H, 6.12.

Allyl O-(4-O-acetyl-2,3-di-O-methyl-α-L-fucopyranosyl)-(1→3)-2,4-di-O-benzoyl-α-L-rhamnopyranoside (**29**). — A suspension of **12** (741 mg, 2.7 mmol), selenium dioxide (332 mg), and acetic acid (230 μL) in 1,4-dioxane was heated for 1 h under reflux. The filtrate and washings were evaporated, with additions of toluene, and the residue was chromatographed on silica gel (2:1 light petroleum–ethyl acetate) to give **28** (428 mg, 68%), ^{1}H-n.m.r.: δ 5.38, 5.29 (2 d, 1 H, $J_{1,2}$ 10.8, 3.46 Hz, H-1β and H-1α), 3.63, 3.54, 3.42 (3 s, 6 H, 2 OCH_3 of α- and β-anomers), 2.18, 2.17 (2 s, 3 H, OCOCH_3 of α- and β-anomers), 1.21, and 1.15 (2 d, 3 H, $J_{5,6}$ 6.4, 6.5 Hz, H-6 of α- and β-anomers); exact mass: calc. for $C_{10}H_{18}O_6$ + Na, 257.1001; found, 257.1005. Glycosyl α-trichloroacetimidate (128 mg, 0.42 mmol; from **28** by method B) in dichloromethane (5 mL) was added with stirring to **27** (118 mg, 0.29 mmol) in dichloromethane containing 4A molecular sieves (0.5 g), and after 1 h boron trifluoride etherate (55 μL) was added. When t.l.c. showed complete disappearance of the imidate (30 min) the mixture was filtered, and the combined filtrate and washings were washed with water, dried, and concentrated. The residue was chromatographed on silica gel (3:1 light petroleum–ethyl acetate) to give **29** (131 mg, 73%) as a syrup, $[\alpha]_D$ −4.8° (*c* 2.3); ^{1}H-n.m.r.: δ 8.14–7.43 (m, 10 H, Ph-H), 5.13 (d, 1 H, $J_{1,2}$ 2.32 Hz, H-1), 5.06 (d, 1 H, $J_{1',2'}$ 3.52 Hz, H-1'), 3.17, 2.80 (2 s, 6 H, OCH_3), 2.07 (s, 3 H, OCOCH_3), 1.31, and 0.95 (2 d, 6 H, $J_{5,6}$ 6.21, 6.49 Hz, H-6,6'); exact mass: calc. for $C_{33}H_{40}O_{12}$ + Na + TG, 759.2662; found, 759.2665.

Allyl O-(2,3-di-O-methyl-α-L-fucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**30**). — A solution of **29** (120 mg, 0.19 mmol) in methanolic 0.33M sodium methoxide (3 mL) was heated overnight at 50°, neutralized by treatment with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was chromatographed on silica gel (1:1 light petroleum–ethyl acetate) to give **30** (64 mg, 88%) as a syrup, $[\alpha]_D$ −132° (*c* 2.5, MeOH); ^{1}H-n.m.r.: δ 5.12 (d, 1 H, $J_{1',2'}$ 3.51 Hz, H-1'), 4.82 (d, 1 H, $J_{1,2}$ 1.18 Hz, H-1), 3.56, 3.50 (2 s, 6 H, OCH_3), 1.33, and 1.31 (2 d, 6 H, $J_{5,6}$ 6.38, 6.86 Hz, H-6,6'); exact mass: calc. for $C_{17}H_{30}O_9$ + Na + TG, 509.2033; found, 509.1988.

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