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Structure of the glycopeptidolipid antigen of serovar 20 of the *Mycobacterium avium* serocomplex, synthesis of allyl glycosides of the outer di- and tri-saccharide units of the antigens of serovars 14 and 20, and serology of the derived neoglycoproteins*,‡

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ABSTRACT

The tetrasaccharide hapten released from the glycopeptidolipid (GPL) antigen of *Mycobacterium avium* serovar 20 has been characterized as *O*-(2-*O*-methyl-α-D-rhamnopyranosyl)-(1→3)-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose. Syntheses are reported of allyl glycosides of the outer disaccharide unit of this hapten, *O*-(2-*O*-methyl-α-D-rhamnopyranosyl)-(1→3)-2-*O*-methyl-α-L-fucopyranose, and also of the outer di- and tri-saccharide units of the GPL antigen of *M. avium* serovar 14, *O*-(*N*-formyl-α-L-kansosaminyl)-(1→3)-2-*O*-methyl-α-D-rhamnopyranose and *O*-(*N*-formyl-α-L-kansosaminyl)-(1→3)-*O*-(2-*O*-methyl-α-D-rhamnopyranosyl)-(1→3)-2-*O*-methyl-α-L-fucopyranose. The key steps in the latter synthesis involve the preparation of allyl 4-azido-4,6-dideoxy-3-*C*-methyl-2-*O*-methyl-α-L-mannopyranoside as a precursor for the *N*-formylkansosamine unit, followed sequentially by conversion into and use of a trichloroacetimidate as glycosyl donor for di- and tri-saccharide formation, *O*-deacylation, reduction, and *N*-formylation. The allyl glycosides, representative of the haptens from both serovars, have been converted into neoglycoproteins (NGPs) and their serological activities have been compared in the light of the structural relationship between them.

INTRODUCTION

Mycobacteria of the *Mycobacterium avium–M. intracellulare–M. scrofulaceum* complex (*M.avium* complex) have long been associated with pulmonary and other organ-localized infections[1] and some serovars are of current interest as opportunistic pathogens in patients with acquired immunodeficiency syndrome (AIDS)[2]. The various *M. avium* serovars are differentiated by antigenic glycopeptidolipids (GPLs) in which the outer segments (1–3 residues) of the oligosaccharide chains contain very unusual sugars, often in previously unknown combinations, and are highly specific in their

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

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interaction with antibodies to the intact serovar[3]. Interaction of these same sugar residues conjugated to protein as neoglycoproteins (NGPs) with antibodies to the parent organism provides a potential basis for the diagnosis of infections and an explanation of the precise chemical requirements for antigenicity. With this in mind we have shown in the accompanying paper[4] that ozonolysis of allyl glycosides followed by reductive coupling[5] is a convenient procedure for the preparation of NGPs as potential agents for serodiagnosis and also for the determination of the specificities of monoclonal antibodies which recognize the organism itself[6]. The use of the allyl group places some restrictions on the other types of protecting groups used in the synthesis of the oligosaccharide haptens, but the allyl glycosides have advantages over the widely used, lipophilic 8-methoxycarbonyloctyl derivatives in ease of manipulation and in permitting the retention of *O*-acetyl groups during conjugation[4] to protein. The reaction or lack of reaction of the NGPs synthesized to date with cognate antibodies appears to be dependent on the carbohydrate epitope and independent of the nature of the linker arm.

In the GPL from *M. avium* serovar 14 the oligoglycosyl haptenic chain has the structure *O*-(*N*-formylkansosaminyl)-(1→3)-*O*-(2-*O*-methyl-α-D-rhamnopyranosyl)-(1→3)-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-6-deoxy-α-L-talopyranosyl[7] (**1**). The unusual branched-chain amino sugar was characterized by conversion into an *N*-acetyl derivative that was chromatographically and spectroscopically indistinguishable from the similarly generated derivative of *N*-[(*R*)-2-methoxypropanoyl]-L-kansosamine[8], which occurs as the immunodominant glycosyl unit of lipo-oligosaccharides from *M. kansasii*[9,10]. Determination of the enantiomeric configurations was achieved for the inner sugar residues of the oligosaccharide by conversion into chiral glycosides[8], but not for the kansosamine unit because 4-acylamino-4-deoxy sugars undergo *N*-deacylation and thence degradation to achiral pyrrolidine or pyrrole derivatives during acid-catalyzed glycosidation[9]. ^{1}H-N.m.r. data for the oligosaccharide suggested that the kansosamine residue had the α-configuration but the available quantities of material were not sufficient to permit confirmation of this assignment by the determination of $^1J_{C,H}$ for the anomeric carbon[11]. For synthesis we have assumed that this terminal sugar residue in the serovar 14 GPL has the same absolute configuration (α-L) as in the *M. kansasii* lipo-oligosaccharide.

In further studies of antigens from *M. avium*[3] the GPL from serovar 20 has now been examined, and its structure has been found to be identical to that of the serovar 14 antigen for the inner portion of the oligosaccharide hapten, lacking only the *N*-formylkansosamine residue. In syntheses of NGPs related to the GPLs of serovars 14 and 20 we have therefore designed a route that generates those portions of the oligosaccharides distal to the →3)-α-L-Rha*p*-(1→2)-6-dTal*p* region, which is common to all GPLs of this group. Syntheses were planned to give residues of 2-*O*-methyl-α-D-rhamnopyranose having differentially removable substituents at O-3 and O-4 so that O-3 could be exposed to function as a glycosyl acceptor for attachment of the terminal *N*-formylkansosamine residue of the serovar 14 hapten. It has been convenient to synthesize the precursor of each of the sugar residues as the allyl glycoside, and from these to generate partially protected glycosyl acceptors or fully substituted glycosyl donors. The serological properties of the oligosaccharide haptens were examined, as neoglycoproteins, in order to define the structural requirements for the endowment of antigenicity on such sugar epitopes and to provide serological tools for the diagnosis of infections.

RESULTS AND DISCUSSION

Structural characterization of the triglycosyl alditol hapten of the GPL from serovar 20 of the M. avium *complex.* — Purified GPL of serovar 20 was subjected to β-elimination and the liberated oligoglycosyl alditol was purified by gel filtration chromatography[12] (Sephadex G-15). The sugar composition of the oligosaccharide was established by conventional g.l.c.–m.s. of the alditol acetates on a DB-23 capillary column. 2-*O*-Methylrhamnose, 2-*O*-methylfucose, rhamnose, and 6-deoxytalose were readily identified. F.a.b.-m.s. analysis of the oligoglycosylalditol gave molecular ions at *m/z* 633 (MH^+) and 655 (MNa^+), confirming a mol. wt. of 632. Linkage and sequence analysis was effected through f.a.b.-m.s. of the per-*O*-trideuteriomethylated[13] oligoglycosylalditol and characterization by g.l.c.–m.s. of acetates of the alkylated alditol derivatives, 3,4-di-*O*-CD_3-2-*O*-CH_3-rhamnitol, 4-*O*-CD_3-2-*O*-CH_3-fucitol, 2,4-di-*O*-CD_3-rhamnitol, and 6-deoxy-1,3,4,5-tetra-*O*-CD_3-talitol. The fragment ions shown in Fig. 1, together with a molecular ion at *m/z* 786 (MH^+), confirmed the sequence of natural *O*-methyl sugar units, and led to formulation of the proposed structure. With

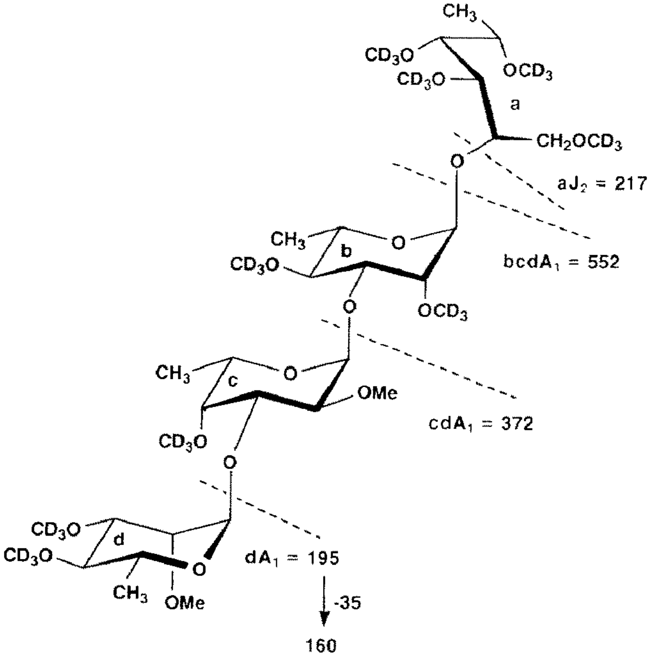
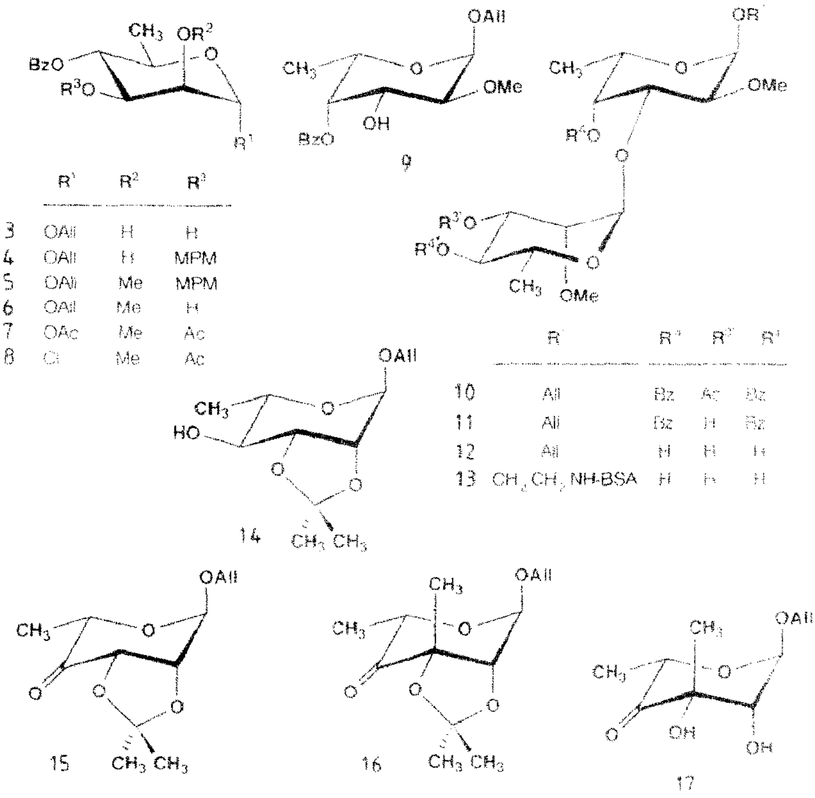


Fig. 1. Molecular ion, MH^+ = 786, and fragment ions in the f.a.b.–m.s. of the pertrideuteriomethylated triglycosylalditol from *M. avium* serovar 20 GPL.

the recognition of the close relationship of the serovar 20 hapten to that of serovar 14, hydrolysis products from the former were converted into *O*-trimethylsilyl derivatives of (*S*)-2- and (*R*)-2-butyl glycosides[14], which were identical (g.l.c.) to those from the latter serovar, thus confirming the presence of sugar residues of the same enantiomeric configurations. ^{1}H-N.m.r. spectroscopy showed resonances at δ 5.25 ($J_{1,2}$ ~ 3.0 Hz), 5.0, and 4.8 (both broad singlets), indicative of glycosyl residues in the α configuration, although not definitive for the rhamnose units. It is to be noted that all other GPLs contain an internal 3-linked α-L-Rha*p*, and that the synthesis of a neoantigen described herein confirms the anomeric configuration of the nonreducing terminal residue. Thus the specific hapten of the GPL antigen of serovar 20 may be proposed as *O*-(2-*O*-methyl-α-D-rhamnopyranosyl)-(1→3)-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose (**2**).

1 α-L-Kan$_p$4Fo-(1→3)-2-Me-α-D-Rha$_p$-(1→3)-2-Me-α-L-Fuc$_p$-(1→3)-α-L-Rha$_p$-(1→2)-6-deoxy-α-L-Tal

2 2 Me-α-D-Rha$_p$-(1→3)-2-Me-α-L-Fuc$_p$-(1→3)-α-L-Rha$_p$-(1→2)-6-deoxy-L-Tal



	R¹	R²	R³
3	OAll	H	H
4	OAll	H	MPM
5	OAll	Me	MPM
6	OAll	Me	H
7	OAc	Me	Ac
8	Cl	Me	Ac

	R	R⁴	R³	R¹
10	All	Bz	Ac	Bz
11	All	Bz	H	Bz
12	All	H	H	H
13	CH_2CH_2NH-BSA	H	H	H

Kan$_p$4NFo = 4,6-dideoxy-4-formamido-3-*C*-methyl-2-*O*-methylmannopyranose (*N*-formylkansosamine)

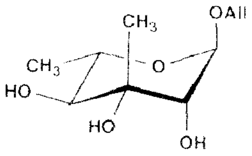
MPM = 4-methoxyphenylmethyl (4-methoxybenzyl)

BSA = bovine serum albumin

Synthesis of neoglycoproteins. NGP related to serovar 20 GPL. — Benzoylation of allyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside[15] followed by controlled acid hydrolysis afforded allyl 4-*O*-benzoyl-α-D-rhamnopyranoside (**3**). Regioselective alkylation[16] of the dibutylstannylene derivative of **3** with 4-methoxybenzyl chloride and tetra-*n*-butylammonium bromide (to **4**) followed by methylation using sodium hydride and methyl iodide[17] in oxolane gave allyl 4-*O*-benzoyl-3-*O*-(4-methoxybenzyl)-2-*O*-methyl-α-D-rhamnopyranoside (**5**). Compound **5** served to furnish both glycosyl acceptor **6** on treatment with ceric ammonium nitrate[18], and acetate **7** for subsequent conversion into glycosyl donor on acetolysis with accompanying deprotection and acetylation at O-3. For the synthesis of the outer disaccharide unit of the GPL from serovar 20, treatment of glycosyl acetate **7** with dichloromethyl methyl ether in the presence of anhydrous zinc bromide[19] gave the corresponding α-glycosyl chloride **8**, which was condensed directly with allyl 4-*O*-benzoyl-2-*O*-methyl-α-L-fucopyranoside[20] (**9**) in the presence of silver triflate to furnish the fully protected disaccharide **10**. ^{1}H-N.m.r. spectra of disaccharide **10** and the products of deprotection **11** and **12** indicated the formation of an α-D-glycosyl linkage with no detectable β anomer, despite the absence of a participating group in the glycosyl donor. Selective *O*-deacetylation of **10** was achieved on treatment with methanolic hydrogen chloride at room temperature giving partially deprotected disaccharide **11**, which was to be required later as glycosyl acceptor in the synthesis of the trisaccharide allyl glycoside **33**. *O*-Debenzoylation of **11** was effected catalytically with sodium methoxide, giving the terminal disaccharide unit of serovar 20 GPL as its allyl glycoside **12**. Ozonolysis of **12** followed by reductive coupling[5] to bovine serum albumin afforded NGP 20 (**13**).

NGPs related to serovar 14 GPL. — In experiments leading to the isolation and characterization[7] of the oligosaccharide hapten of serovar 14 *N*-deformylation has been observed to occur with relative ease. Consequently for the synthesis of the terminal *N*-formylkansosamine residue we followed the general strategy recently reported by Giuliano and Kasperowicz[21], in which the nitrogen function at C-4 was introduced by azide opening of an epoxide, but the conversion of azidodeoxy to deoxyformamido sugar was delayed until the final stages of oligosaccharide assembly. The stereoselectivity of this approach gives it advantages over that used by Yoshimura *et al.*[7] for the synthesis of the *N*-(*R*)-2-methoxypropanoyl derivative of L-kansosamine, and very recently by Bajza and Lipták[22] for synthesis of the phenyl α-thioglycoside of *N*-formyl-L-kansosamine. These syntheses involved reduction of oximes of glycosid-4-uloses to give mixtures of 4-amino-4-deoxy sugars having the *manno* and *talo* configurations.

Syntheses of kansosamine and related branched-chain sugars by Giuliano and Kasperowicz[21] involved stereoselective introduction of the *C*-methyl branching unit into a methyl 6-deoxy-α-L-mannofuranoside derivative. However, for our synthesis the skeleton of the branched sugar was assembled in the pyranose form starting from allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[4] (**14**). Oxidation of **14** with the chromium trioxide–pyridine complex[23] gave the corresponding 4-ulose **15**, and this underwent *C*-methylation on reaction with lithium diisopropylamide and methyl iodide according to the procedure of Klemer *et al.*[2] to give allyl 6-deoxy-2,3-*O*-isopropylidene-3-*C*-me-



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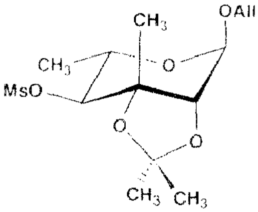

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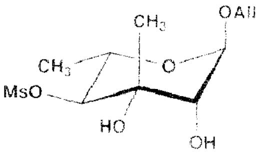

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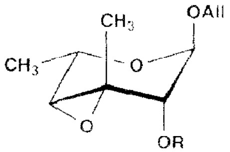
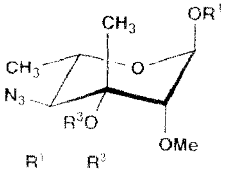
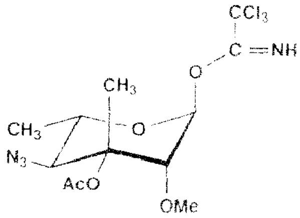


21 R = H
22 R = Me



	R¹	R³
23	All	H
24	Ac	Ac
25	H	Ac



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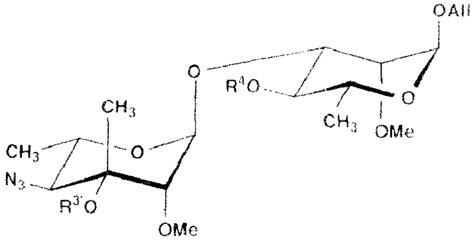
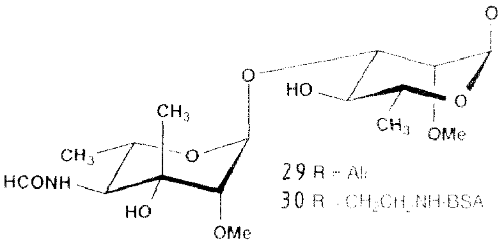


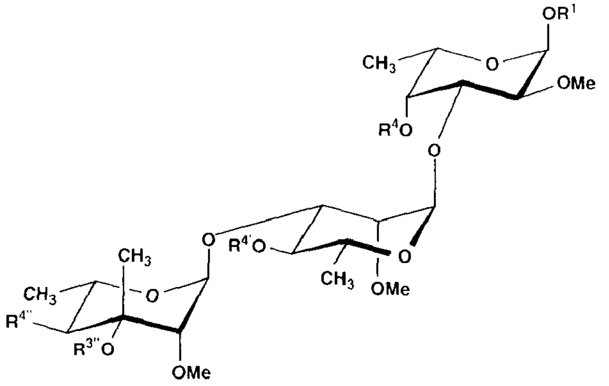
	R⁴	R³
27	Bz	Ac
28	H	H



29 R = All
30 R = CH_2CH_2NH-BSA

thyl-α-L-*lyxo*-hexopyranosid-4-ulose (**16**). Klemer *et al.* reported that reduction of the corresponding methyl glycoside with lithium aluminum hydride affords a product with the *talo* configuration, whereas *O*-deisopropylidenation of the D-enantiomer followed by reduction with sodium borohydride gives stereoselectively methyl 6-deoxy-3-*C*-methyl-α-D-mannopyranoside. In our hands *O*-deisopropylidenation of **16** (to **17**) followed by reduction with sodium borohydride gave roughly equal proportions of *manno* and *talo* epimers, but reduction of **17** with tetramethylammonium triacetoxyborohydride[25] gave allyl 6-deoxy-3-*C*-methyl-α-L-mannopyranoside (**18**) with high stereoselectivity and in good yield. Presumably, as reported by Evans *et al.*[25] for the reduction of acyclic *β*-hydroxyketones, the formation of the *anti* 2,4-diol is favoured by ligand exchange at the axial 2-OH group, followed by intramolecular hydride delivery. Sequential *O*-isopropylidenation and methanesulfonylation of **18** (to **19**) followed by removal of the isopropylidene group gave **20**, which was treated with base to give the 3,4-epoxide **21** and then methylated at O-2 to give **22**. Epoxide opening proceeded sluggishly on heating **22** with sodium azide in *N,N*-dimethylformamide at 110° but afforded regioselectively the kansosamine precursor allyl 4-azido-4,6-dideoxy-3-*C*-methyl-2-*O*-methyl-α-L-mannopyranoside (**23**) in 54% yield. The ^{1}H-N.m.r. data were in accord with the assigned structure.

For the synthesis of di- and tri-saccharides related to the characteristic haptenic oligosaccharides of the *M. avium* serovar 14 GPL, allyl glycoside **23** was first subjected to mild treatment with acetic anhydride–sulfuric acid to give the α-glycosyl acetate (**24**) with accompanying acetylation of the tertiary hydroxyl group. An attempt to transform **24** into the corresponding glycosyl chloride by reaction with dichloromethyl methyl ether in the presence of anhydrous zinc bromide failed; only hemiacetal was detected. Instead, selective 1-*O*-deacetylation of **24** with hydrazine acetate[26] followed by reaction of the hemiacetal (**25**) with trichloroacetonitrile in the presence of 1,5-diazabicyclo[5.4.0]undec-5-ene gave the α-glycosyl trichloroacetamidate **26**, which was used without extensive purification. Disaccharide synthesis was achieved by reaction of the trichloroacetimidate with allyl 4-*O*-benzoyl-2-*O*-methyl-α-D-rhamnopyranoside (**6**) in the presence of boron trifluoride etherate to give the fully substituted disaccharide **27**, which was then *O*-deacylated catalytically with sodium methoxide to give **28**. ^{1}H- And ^{13}C-n.m.r. data showed that glycosylation had occurred stereoselectively, and, although not individually assigned, the two anomeric carbon resonances of **27** observed at δ 96.64 ($^1J_{C,H}$ 168.4 Hz) and 93.49 ($^1J_{C,H}$ 165.4 Hz) were indicative of α-glycosidic linkages[11]. Azido disaccharide **28** was reduced with hydrogen sulfide and then acylated by reaction with ethyl formate to give the terminal disaccharide unit of serovar 14 GPL as its allyl glycoside **29**. Ozonolysis of **29** followed by reductive coupling afforded NGP 14-2 (**30**).



	R^1	R^4	R$^{4'}$	R$^{3''}$	R$^{4'''}$
31	All	Bz	Bz	Ac	N$_3$
32	All	H	H	H	N$_3$
33	All	H	H	H	HCONH
34	CH$_2$CH$_2$NH-BSA	H	H	H	HCONH

For the synthesis of the complete outer trisaccharide unit disaccharide **11** was glycosylated, as for **6**, by reaction with the trichloroacetimidate **26** in the presence of boron trifluoride etherate to give fully substituted product **31**. The n.m.r. data for this trisaccharide derivative were in full accord with the assigned structure, with anomeric carbon resonances at δ 98.89 ($^1J_{C,H}$ 170.9 Hz), 95.10 ($^1J_{C,H}$ 167.7 Hz), and 96.05 ($^1J_{C,H}$ 167.3 Hz) characteristic of α-glycosidic linkages, showing again that glycosylation with **26** had taken place with high stereoselectivity despite the absence of a participating group at O-2. *O*-Deacylation (to **32**), followed by reduction and *N*-formylation as for **29** furnished the terminal trisaccharide segment of the GPL from serovar 14 as its allyl glycoside **33**. Ozonolysis of **33** followed by reductive coupling afforded NGP 14-3 (**34**).

Serology. — NGP 20, representative of the distal disaccharide of the GPL from serovar 20, and NGPs 14-2 and 14-3, containing the outer di- and tri-glycosyl units of the GPL of serovar 14, respectively, were amenable to conventional polyacrylamide gel electrophoresis (PAGE)[6]. It was possible therefore to use the Western blotting technique as well as plate ELISA to examine their interaction with antibodies. Monoclonal

TABLE I

Summary of the reactivity of NGP 14-2 (and 14-3) and NGP 20 against polyclonal and monoclonal antibodies[a]

Antibody	*Reactivity*					
	Plate ELISA			*Western Blot*		
	Neo 14-2	*Neo 14-3*	*Neo 20*	*Neo 14-2*	*Neo 14-3*	*Neo 20*
Polyclonal rabbit anti-serovar 14	+ +	+ +		+ + +	+ + +	
Polyclonal rabbit anti-serovar 20			+ + +			+ +
Pre-immune serum						
Monoclonal anti-GPL 14 (MAb 6B6)	+ + +	+ + +		+ + +	+ + +	
Monoclonal anti-GPL 14 (MAb 1F3)	–			+ +		
Monoclonal anti-GPL 14 (MAb 8B3)	+ +	–		+ +		
Monoclonal anti-GPL 14 (MAb 9A6)	+ +	+ +		+ +	+ +	

[a] Assay conditions and examples of other NGPs are described in ref. 6. MAbs to GPL 20 were not generated.

antibodies (MAbs) were raised to the GPL of serovar 14, and polyclonal rabbit serum was raised against whole cells of serovar 14 (ref. 7). Rabbit anti-serovar 20 was raised similarly but MAbs were not generated to GPL 20. NGP 14-2 (**30**) and NGP 14-3 (**34**) reacted equally well with the monoclonal and homologous polyclonal antibodies but failed to react with polyclonal anti-serovar 20. NGP 20 (**13**) on Western blotting reacted readily with rabbit anti-serovar 20 but did not react with anti-GPL 14 or anti-serovar 14 antibodies. The reactivity patterns of these antibodies and of other MAbs to GPL 14 are summarized in Table I. It is noteworthy that MAbs 6B6 and 9A6 recognize NGPs 14-2 and 14-3 equally well, whereas MAbs 1F3 and 8B3 recognize only NGP 14-2. Yet other MAbs (not shown) were reactive by Western blot only.

These results show that synthesized determinants of serovar 14 and serovar 20 are serologically active, and that polyclonal antibodies effect a clear discrimination between them. Two of the MAbs to NGPs 14 bear out this conclusion. It is apparent, however, for serovar 14 as for serovar 2 (ref. 4), that different MAbs raised to the GPL vary in their reactivity and that definitions of their specificities will require a greater range of compounds of known structure. There is no simple explanation for the reactivities of some MAbs to NGP 14-2 but not to NGP 14-3, which contains an additional sugar residue of the natural GPL. Nevertheless these investigations provide important evidence for the absolute configuration of the terminal *N*-formylkansosamine residue of GPL 14, which was not previously established but was assumed by analogy with the related sugar from *M. kansasii* lipo-oligosaccharide[9,10] to be the α-L enantiomer. The ready interaction between NGP 14-3 and anti-serovar 14 antibodies is a compelling argument that this assumption was correct.

EXPERIMENTAL

General experimental methods, including specifications for drying solvents, chromatographic procedures, and details of the spectroscopic equipment used, were as described in the preceding paper[4]. Methods for the preparation of glycosyl donors and neoglycoproteins, and immunological procedures, are likewise reported there[4].

Purification and analysis of GPL-20. — The purification of the specific GPL from serovar 20 and fundamental observations on its sugar composition were reported previously[27]. Protocols for the isolation of the oligosaccharide hapten as the triglycosylalditol and structural elucidation using g.l.c.-m.s., f.a.b.-m.s., and ^{1}H-n.m.r. spectroscopy have been described[7] in relation to the oligosaccharide hapten from serovar 14 GPL.

*Allyl 4-*O*-benzoyl-α-D-rhamnopyranoside* (**3**). — Benzoyl chloride (3 mL, 26 mmol) was added dropwise to a stirred solution of allyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside[15] (4.9 g, 20 mmol) in dry pyridine (50 mL) at 0°, and the mixture was stirred overnight. Water (30 mL) and chloroform (50 mL) were added, and the organic layer was washed successively with ice-cold 2M hydrochloric acid (3 × 50 mL), satd. aq. sodium hydrogencarbonate (3 × 70 mL), and satd. brine, dried, and concentrated to give allyl 4-*O*-benzoyl-2,3-*O*-isopropylidene-α-D-rhamnopyranoside as a chromatographically pure light yellow syrup (7.02 g), which was used directly. The syrup in 6:4 acetic acid–water was boiled for 2.5 h under reflux. The solvent was evaporated, and three further evaporations after additions of toluene removed the final traces of acetic acid. The syrupy residue was chromatographed on silica gel (2:1 light petroleum–ethyl acetate) to give crystalline **3** (5.63 g, 91%), which after recrystallization from ether–light petroleum had m.p. 57–59° and $[\alpha_D]$ +99° (*c* 1.13)*; lit[28] m.p. 52–54° and $[\alpha]_D$ −80° ($CHCl_3$) for the L enantiomer; ^{1}H-n.m.r.: δ 8.05–7.42 (m, 5 H, Ph-H), 5.08 (t, 1 H, $J_{3,4}=J_{4,5}$ 9.4 Hz, H-4), 4.91 (s, 1 H, H-1), 3.38 (d, 1 H, *J* 6.4 Hz, exchangeable with D_2O, O*H*), 3.13 (d, 1 H, exchangeable with D_2O, O*H*), and 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6).

Anal. Calc. for $C_{16}H_{20}O_6$: C, 62.32; H, 6.53. Found: C, 62.43; H, 6.53.

Allyl 4-O-benzoyl-3-O-(4-methoxybenzyl)-α-D-rhamnopyranoside (**4**). — A solution of **3** (5.185 g, 16.8 mmol) in freshly distilled benzene (150 mL) containing dibutyltin oxide (5.03 g, 1.2 equiv.) was boiled for 2 h under reflux with continuous removal of water. The solution was concentrated to 100 mL, tetrabutylammonium bromide (6.51 g, 1.2 equiv.) and 4-methoxybenzyl chloride (2.73 mL, 1.2 equiv.) were added, and the solution was boiled for a further 12 h and then concentrated. A solution of the residual syrup in ether (150 mL) was filtered to remove a solid residue, and the combined filtrate and washings with ether were washed with water, dried, and concentrated. The syrup was chromatographed on silica gel (1:1 ether–light petroleum) to give **4** (6.66 g, 92.4%), $[\alpha]_D$ +8.2° (*c* 1.0); ^{1}H-n.m.r.: δ 7.99–6.64 (m, 9 H, Ph-H), 5.29 (t, 1 H, $J_{3,4}=J_{4,5}$ 9.7 Hz, H-4), 4.93 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.56, 4.43 (2 d, 2 H, J_{AB} 11.7 Hz, OC*H*$_2$Ph), 3.72 (s, 3 H, OC*H*$_3$), and 1.22 (d, 3 H, $J_{5,6}$ 6.28 Hz, H-6).

Anal. Calc. for $C_{24}H_{28}O_7$: C, 67.27; H, 6.58. Found: C, 66.86; H, 6.49.

Allyl 4-O-benzoyl-3-O-(4-methoxybenzyl)-2-O-methyl-α-D-rhamnopyranoside (**5**). — Sodium hydride (392 mg, 1.4 equiv.) was added to a solution of **4** (3.0 g, 7 mmol) in dry oxolane (75 mL), the mixture was stirred for 30 min at room temperature and cooled to 0°, methyl iodide (1.75 mL) was added dropwise, and the mixture was then stirred for 12 h at room temperature. Methanol (2 mL) was added and the reaction mixture was concentrated. A solution of the residue in ethyl acetate was washed with water, dried, and concentrated, and the resulting syrup was chromatographed on silica gel (5:1 light petroleum–ethyl acetate) to give **5** (2.17 g, 70%), $[\alpha]_D$ +19.0° (*c* 1.97); ^{1}H-n.m.r.: δ 8.01–6.66 (m, 9 H, Ph-H), 5.37 (t, 1 H, $J_{3,4}=J_{4,5}$ 9.7 Hz, H-4), 4.91 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.58, 4.46 (2 s, 2 H, J_{AB} 11.7 Hz, OC*H*$_2$Ph), 3.74, 3.54 (2 s, 6 H, OC*H*$_3$), and 1.23 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); exact mass: calc. for $C_{25}H_{30}O_7$ + H + thioglycerol, 871.4151; found, 871.4129.

Anal. Calc. for $C_{25}H_{30}O_7$: C, 67.85; H, 6.83. Found: C, 67.43; H, 6.88.

Allyl 4-O-benzoyl-2-O-methyl-α-D-rhamnopyranoside (**6**). — A solution of **5** (0.57 g, 1.29 mmol) and ceric ammonium nitrate (1.42 g, 2 equiv.) in 9:1 acetonitrile–water (30 mL) was stirred for 3 h at room temperature. The reaction mixture was diluted with chloroform (100 mL) and washed with water (2 × 40 mL), the aqueous layer was extracted, and combined organic layers were washed with satd. aq. sodium hydro-

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* Optical rotations were measured in $CHCl_3$, and n.m.r. spectra in $CDCl_3$, unless otherwise specified.

gencarbonate and satd. brine, dried, and concentrated. The residual syrup was chromatographed on silica gel (5:1 light petroleum–ethyl acetate) to yield **6** as a syrup (316 mg, 76%), $[\alpha]_D$ +47.0° (*c* 1.03); ^{1}H-n.m.r.: δ 8.07–7.42 (m, 5 H, Ph-H), 5.08 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ 9.8 Hz, H-4), 4.97 (s, 1 H, H-1), 3.52 (s, 3 H, OCH_3), and 1.24 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6).

Anal. Calc. for $C_{17}H_{22}O_6$: C, 63.34; H, 6.87. Found: C, 63.40; H, 6.75.

1,3-Di-O-acetyl-4-O-benzoyl-2-O-methyl-α-D-rhamnopyranose (**7**). — A solution of **6** (1.33 g, 4.14 mmol) in 100:40:1 acetic anhydride–acetic acid–sulfuric acid (20 mL) was stirred at room temperature for 1 h. The reaction mixture was poured with stirring into cold aq. 10% sodium hydrogencarbonate (200 mL), stirring was continued for 0.5 h, then the mixture was extracted with chloroform (2 × 30 mL), dried, and concentrated. The resulting syrup was chromatographed on silica gel (2:1 light petroleum–ethyl acetate, to afford **7** (0.93 g, 85%) as a syrup, $[\alpha]_D$ +30.5° (*c* 0.56); ^{1}H-n.m.r.: δ 8.03–7.43 (m, 5 H, Ph-H), 6.20 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.53 (s, 3 H, OCH_3), 2.19, 1.98 (2 s, 6 H, $OCOCH_3$), and 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6).

Anal. Calc. for $C_{18}H_{22}O_8$: C, 59.01; H, 6.05. Found: C, 59.40; H, 6.04.

Allyl O-(3-O-acetyl-4-O-benzoyl-2-O-methyl-α-D-rhamnopyranosyl)-(1→3)-4-O-benzoyl-2-O-methyl-α-L-fucopyranoside (**10**). — A solution of **7** (0.915 g, 2.5 mmol), dichloromethyl methyl ether (0.77 mL), and anhydrous zinc bromide (20 mg) in dry dichloromethane (10 mL) was stirred under argon for 0.5 h. T.l.c. showed complete disappearance of starting material with formation of a single product. Evaporation under vacuum removed solvent and excess reagent, the residue was dissolved in dry dichloromethane, the solution was filtered through glass wool, and the filtrate containing glycosyl chloride **8** [δ_H 6.20 (d, $J_{1,2}$ 1.2 Hz, H-1)] was used directly in the next step.

A solution of **9** (ref. 20) (0.7 g, 2.15 mmol), silver triflate (0.98 g), and 1,1,3,3-tetramethylurea (0.467 mL) in dry dichloromethane (10 mL) was stirred under argon with powdered molecular sieves 4A (1 g) for 1 h. The mixture was cooled to −78°, a solution of **8** (~850 mg, 2.48 mmol) in dry dichloromethane (10 mL) was added dropwise, and the mixture was stirred at room temperature for 22 h, at which time more tetramethylurea (0.1 mL) and silver triflate (0.183 g) were added and stirring was continued for a further 24 h. The mixture was filtered through Celite, the residue was washed with dichloromethane, and the combined filtrate and washings were washed successively with satd. aq. sodium hydrogencarbonate (2 × 30 mL), water (2 × 30 mL), and satd. aq. sodium chloride, dried, and concentrated. The residual syrup was chromatographed on silica gel (2:1 light petroleum–ethyl acetate) to give **10** (1.12 g, 83%) as a foam $[\alpha]_D$ −108° (*c* 1.15); ^{1}H-n.m.r.: δ 8.17–7.38 (m, 10 H, Ph-H), 5.52 (d, 1 H, J 2.4 Hz, H-4), 5.39–5.11 (m, 6 H, H-1,1',3',4',$=CH_2$), 3.53, 3.42 (2 s, 6 H, OCH_3), 1.86 (s, 3 H, $OCOCH_3$), 1.22, and 1.20 (2 d, 6 H, $J_{5,6}$ 6.5, 7.0 Hz, H-6,6').

Anal. Calc. for $C_{33}H_{40}O_{12}$: C, 63.04; H, 6.41. Found: C, 63.36; H, 6.56.

Allyl O-(4-O-benzoyl-2-O-methyl-α-D-rhamnopyranosyl)-(1→3)-4-O-benzoyl-2-O-methyl-α-L-fucopyranoside (**11**). — Compound **10** (400 mg, 0.63 mmol) was kept in methanolic 1.5% hydrogen chloride (5 mL) for 20 h. The solution was neutralized with Amberlite IR-45 resin (OH^- form, washed with methanol), the filtrate was concentrated, and the residue was chromatographed on silica gel (15:1 chloroform–acetone) to give **11** (328 mg, 88%) as a foam, $[\alpha]_D$ −152° (*c* 1.21); ^{1}H-n.m.r.: δ 8.14–7.41 (m, 10 H, Ph-H), 5.39–5.14 (m, 4 H, H-1,1', =CH_2), 4.98 (t, 1 H, $J_{3',4'}$ = $J_{4',5'}$ 9.8 Hz, H-4'), 4.29–3.61 (m, 8 H, H-2,3,5,2',3',5', OCH_2), 3.52, 3.44 (2 s, 6 H, OCH_3), 2.35 (d, 1 H, exchangeable with D_2O, OH), 1.23, 1.20 (2 d, 6 H, $J_{5,6}$ 6.3, 6.6 Hz, H-6,6'); exact mass: calc. for $C_{31}H_{38}O_{11}$ + H, 587.2492; found, 587.2492.

Anal. Calc. for $C_{31}H_{38}O_{11}$: C, 63.47; H, 6.52. Found: C, 62.98; H, 6.55.

Allyl O-*(2-*O*-methyl-α-*D*-rhamnopyranosyl)-(1→3)-2-*O*-methyl-α-*L*-fucopyranoside* (**12**). — Compound **11** (90 mg, 0.56 mmol) was treated with M sodium methoxide (0.4 mL) overnight at room temperature, the solution was neutralized by passage through a column of Amberlite IR-120 (H$^+$) resin, and the eluate was concentrated to a syrup. This was chromatographed on silica gel (19:1 dichloromethane–methanol) to furnish **12** (50 mg, 91%) as a syrup, $[\alpha]_D$ −53° (*c* 1.06); ^{1}H-n.m.r.: δ 5.35–5.21 (m, 3 H, H-1', =CH_2), 5.02 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.46, 3.45 (2 s, 6 H, OCH_3), 1.30, 1.28 (2 d, 6 H, $J_{5,6}$ 6.3 Hz, H-6,6'); exact mass: calc. for $C_{17}H_{30}O_9$ + H, 379.1968; found, 379.1954.

Anal. Calc. for $C_{17}H_{30}O_9$: C, 53.95; H, 7.99. Found: C, 54.25; H, 7.51.

*Allyl 6-deoxy-2,3-*O*-isopropylidene-3-*C*-methyl-α-*L-lyxo-*hexopyranosid-4-ulose* (**16**). — Chromium trioxide (28.5 g, 280 mmol) was added to pyridine (46 mL, 580 mmol) in dichloromethane (650 mL) and the mixture was stirred for 15 min. A solution of **14** (14.8 g, 60 mmol) in dry dichloromethane (50 mL) and acetic anhydride (26.8 mL) were added sequentially and the mixture was stirred for 20 min. The reaction was terminated by pouring onto a short column of silica gel covered with ethyl acetate and the column was eluted with ethyl acetate. The eluate was concentrated and the residue was subjected to several cycles of toluene addition and evaporation to ensure complete removal of acetic anhydride and pyridine. The resulting syrup was chromatographed on silica gel (5:1 light petroleum–ethyl acetate) to yield **15** (10.4 g, 71%) as a syrup. ^{1}H-n.m.r.: δ 5.00 (s, 1 H, H-1), 1.49, 1.37 [2 s, 6 H, C(CH_3)$_2$], 1.40 (d, 3 H, $J_{5,6}$ 6.8 Hz, H-6).

n-Butyllithium, 2.5M in hexane (29.7 mL, 74 mmol), was added to diisopropylamine (10.4 mL, 74 mmol) in dry oxolane (200 mL) under argon at −78° and stirring was continued for 15 min, then **15** (10.37 g, 42.8 mmol) in dry oxolane (50 mL) was added. The mixture was stirred for 1 h at −78°, methyl iodide (22.4 mL, 157 mmol) and hexamethylphosphoramide (11 mL) were added, and the mixture was stirred at room temperature overnight. Aqueous 10% ammonium chloride (170 mL) was added, the organic layer was separated, the aqueous layer was extracted twice with chloroform, and the combined organic layers were dried and concentrated. The residual syrup was chromatographed on silica gel (18:1 light petroleum–ethyl acetate) to furnish **16** (8.72 g, 79%) as a syrup, $[\alpha]_D$ −113° (*c* 0.86); ^{1}H-n.m.r.: δ 5.08 (s, 1 H, H-1), 4.37 (q, 1 H, $J_{5,6}$ 6.7 Hz, H-5), 4.10 (d, 1 H, $J_{2,3}$ 1.0 Hz, H-2), 1.51, 1.41 [2 s, 3 H and 6 H, CCH_3, C(CH_3)$_2$], and 1.37 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6).

Anal. Calc. for $C_{13}H_{20}O_5$: C, 60.92; H, 7.86. Found: C, 60.61; H, 8.19.

*Allyl 6-deoxy-3-*C*-methyl-α-*L-lyxo-*hexopyranosid-4-ulose* (**17**). — A solution of **16** (2.97 g, 11.6 mmol) in 1:1 acetic acid–water (70 mL) was heated for 19 h at 80°. The solution was concentrated, the residue was subjected to several cycles of toluene additions and evaporations to ensure complete removal of acetic acid, and the resulting syrup was chromatographed on silica gel (1:1 hexane–ethyl acetate) to give **17** (1.9 g, 77%), $[\alpha]_D$ $-129°$ (c 1.43); ^{1}H-n.m.r.: δ 5.00 (s, 1 H, H-1), 4.56 (q, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 4.00 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-2), 1.58 (s, 3 H, CCH_3), 1.34 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6).

Anal. Calc. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.14. Found: C, 55.58; H, 7.62.

Allyl 6-deoxy-3-C-methyl-α-L-mannopyranoside (**18**). — A solution of **17** (1.49 g, 6.88 mmol) in dry acetonitrile (20 mL) was added to tetramethylammonium triacetoxyborohydride (9.73 g, 5 equiv.) and anhydrous acetic acid (4.2 mL, 10 equiv.) in dry acetonitrile, and the solution was kept overnight. Excess hydride was destroyed by the addition of Amberlite IR-120 (H^+) resin until gas evolution ceased and the solution was clear. The reaction mixture was filtered and the combined filtrate and washings with methanol were concentrated, the residue was subjected to several cycles of additions of methanol and toluene, then evaporation, to ensure complete removal of residual boric and acetic acids, and the resulting syrup was chromatographed on silica gel (19:1 chloroform–methanol) to give **18** (1.26 g, 84%), $[\alpha]_D$ $-63°$ (c 1.88, methanol); ^{1}H-n.m.r.: δ 4.84 (s, 1 H, H-1), 3.66 (m, 1 H, H-5), 3.62 (s, 1 H, H-2), 3.50 (d, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 1.36 (s, 3 H, CCH_3), and 1.31 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6).

Anal. Calc. for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 54.81; H, 8.02.

Allyl 6-deoxy-2,3-O-isopropylidene-3-C-methyl-4-O-methylsulfonyl-α-L-mannopyranoside (**19**). — A solution of **18** (2.89 g, 13.2 mmol), *p*-toluenesulfonic acid (0.05 g), and 2,2-dimethoxypropane (20 mL) in dry acetone (30 mL) was kept for 1 h at room temperature. The reaction mixture was neutralized by the addition of triethylamine and concentrated, and methanesulfonyl chloride (5 mL) was added dropwise to the residue in dry pyridine (20 mL) at 0°. The solution was kept for 3 h at room temperature, water (5 mL) was added slowly, and the reaction mixture was diluted with chloroform (200 mL). The organic layer was washed with water, dried, and concentrated to a syrup. Crystallization from light petroleum–ethyl acetate gave **19** (3.4 g, 76%), m.p. 107–108°, $[\alpha]_D$ $-56°$ (c 1.2). A further quantity (0.23 g, 6%) of **19** was obtained from the mother liquors by chromatography on silica gel (light petroleum–ethyl acetate); ^{1}H-n.m.r.: δ 5.03 (s, 1 H, H-1), 4.55 (d, 1 H, $J_{4,5}$ 10 Hz, H-4), 3.19 (s, 3 H, CH_3SO_2), 1.58 (s, 3 H, CCH_3), 1.42, 1.38 (2 s, 6 H, CCH_3), and 1.33 (d, 3 H, $J_{5,6}$ 5.5 Hz, H-6).

Anal. Calc. for $C_{14}H_{24}O_7S$: C, 49.98; H, 7.19; S, 9.53. Found: C, 50.12; H, 6.96; S, 9.21.

Allyl 6-deoxy-3-C-methyl-4-O-methylsulfonyl-α-L-mannopyranoside (**20**). — A solution of **19** (3.38 g, 10.05 mmol) in methanolic 2% hydrogen chloride (90 mL) was kept at room temperature for 72 h. The solution was neutralized by the addition of sodium carbonate (3.2 g), the mixture was filtered, and the filtrate was concentrated to a residue, which was chromatographed on silica gel (1:1 hexane–ethyl acetate) to yield **20** (2.48 g, 92%), and recovered **19** (0.32 g). Compound **20** had $[\alpha]_D$ $-87.5°$ (c 1.31); ^{1}H-n.m.r.: δ 4.87 (s, 1 H, H-1), 4.51 (d, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.20 (s, 3 H, CH_3SO_2), 1.40 (s, 3 H, CCH_3), and 1.34 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6).

Anal. Calc. for $C_{11}H_{20}O_7S$: C, 44.58; H, 6.80. Found: C, 44.48; H, 7.01.

Allyl 3,4-anhydro-6-deoxy-3-C-methyl-2-O-methyl-α-L-talopyranoside (**22**). — A mixture of **20** (2.48 g, 8.38 mmol) and powdered potassium hydroxide (0.73 g) in dry methanol (75 mL) was stirred for 20 min at room temperature. The residue from evaporation of the solvent was extracted with dichloromethane, and the extract was washed with water (5 × 75 mL), dried, and concentrated to yield chromatographically pure allyl 3,4-anhydro-6-deoxy-3-*C*-methyl-α-L-talopyranoside (**21**, 1.38 g, 83%), $[\alpha]_D$ −115° (*c* 3.1); ^{1}H-n.m.r.: δ 4.56 (s, 1 H, H-1), 3.55 (d, 1 H, $J_{2,OH}$ 11.3 Hz, H-2), 3.04 (s, 1 H, H-4), 2.46 (d, 1 H, exchangeable with D_2O, O*H*), 1.48 (s, 3 H, H-3′), and 1.31 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6); exact mass: calc. for $C_{10}H_{16}O_4$ + Na + TG, 331.1191; found, 331.1205.

Sodium hydride (122 mg) was added to **21** (0.512 g, 2.56 mmol) in dry oxolane and the mixture was stirred under argon for 20 min. The mixture was cooled to 0°, methyl iodide (1 mL) was added, and the mixture was stirred for 2 h at room temperature. Excess hydride was destroyed by the addition of methanol, the solution was concentrated, and the residue was chromatographed on silica gel (10:1 chloroform–acetone) to furnish **22** (0.52 g, 96%), $[\alpha]_D$ −95° (*c* 1.08); n.m.r. data: ^{1}H, δ 4.67 (s, 1 H, H-1), 3.53 (s, 3 H, OC*H*$_3$), 1.49 (s, 3 H, CC*H*$_3$), and 1.33 (d, 3 H, $J_{5,6}$ 5.2 Hz, H-6).

Anal. Calc. for $C_{11}H_{18}O_4$: C, 61.66; H, 8.46. Found: C, 61.44; H, 8.84.

Allyl 4-azido-4,6-dideoxy-3-C-methyl-2-O-methyl-α-L-mannopyranoside (**23**). — A mixture of **22** (0.765 g, 3.57 mmol), sodium azide (2.33 g, 10 equiv.) and tetraethylammonium chloride (2.95 g, 5 equiv.) in *N,N*-dimethylformamide (10 mL) was heated with stirring for 20 h at 110°. Water (75 mL) was added to the cooled mixture, followed by extraction with dichloromethane (3 × 30 mL), and the extract was washed once with water, dried, and concentrated to a residue for chromatography on silica gel (7:1 light petroleum-ethyl acetate). This yielded **23** (0.50 g, 54%), $[\alpha]_D$ −92° (*c* 1.17); i.r.: ν_{max} 2100 cm^{-1}; ^{1}H-n.m.r.: δ 4.91 (s, 1 H, H-1), 3.50 (s, 3 H, OC*H*$_3$), 1.38 (s, 3 H, CC*H*$_3$), and 1.29 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6). An exact mass determination in a thioglycerol matrix gave *m/z* 340.1744, corresponding to the value 340.1775 calculated for an amino compound ($C_{11}H_{21}NO_4$ + Na + TG) formed by reductive cleavage of the azide **23** (exact mass: calc. for $C_{11}H_{19}N_3O_4$ + Na + TG, 366.1751) in the spectrometer[29].

Anal. Calc. for $C_{11}H_{19}N_3O_4$: C, 51.35; H, 7.44; N, 16.33. Found: C, 51.17; H, 6.53; N, 16.02.

1,3-Di-O-acetyl-4-azido-4,6-dideoxy-3-C-methyl-2-O-methyl-α-L-mannopyranose (**24**). — Acetic anhydride (0.875 mL) containing 2% of sulfuric acid was added to **23** (190 mg, 0.74 mmol) in acetic anhydride (5 mL), and this reaction mixture was stirred for 15 min. It was then poured with stirring into cold, saturated aqueous sodium hydrogencarbonate (25 mL), stirring was continued for 20 min, and the mixture was extracted with dichloromethane (3 × 30 mL). The organic extract was washed with water (2 × 20 mL), dried, and concentrated, and the residue was chromatographed on silica gel (5:1 light petroleum–ethyl acetate) to yield **24** (0.22 g, 81%) as a solid, m.p. 71–75°, $[\alpha]_D$ −76° (*c* 0.85); ^{1}H-n.m.r.: δ 6.15 (d, 1 H, $J_{1,2}$ 1.4 Hz), 3.43 (s, 3 H, OC*H*$_3$), 2.10, 2.09 (2 s, 6H, 2 OCOC*H*$_3$), 1.68 (s, 3 H, CC*H*$_3$), and 1.33 (d, 3 H, $J_{5,6}$ 5.5 Hz, H-6).

Anal. Calc. for $C_{12}H_{19}N_3O_6$: C, 47.83; H, 6.35; N, 13.94. Found: C, 48.19; H, 6.56; N, 13.82.

3-O-Acetyl-4-azido-4,6-dideoxy-3-C-methyl-2-O-methyl-α,β-L-mannopyranose (**25**). — A solution of **24** (360 mg, 1.19 mmol) and hydrazine acetate (167 mg) in dry

N,N-dimethylformamide (5 mL) was kept for 24 h at room temperature. The solution was diluted with dichloromethane (40 mL), washed with water (3 × 25 mL), dried, and concentrated to a residue, which was chromatographed on silica gel (5:1 light petroleum–ethyl acetate) to afford solid **25** (250 mg, 81%), $[\alpha]_D$ $-28.1° \rightarrow -18.4°$ at 24 h (*c* 0.98); ^{1}H-n.m.r.: δ 5.26, 4.77 (2 s, 1 H, H-1α,1β), 4.18, 4.15 (2 s, 1 H, H-2), 3.57, 3.39 (2 s, 3 H, OCH_3), 2.12, 2.08 (2 s, 3 H, OCOCH_3), 1.71, 1.59 (2 s, 3 H, CCH_3), 1.34, and 1.31 (2 d, 3 H, $J_{5,6}$ 6.0, 6.2 Hz, H-6).

Anal. Calc. for $C_{10}H_{17}N_3O_5$: C, 46.32; H, 6.60; N, 16.20. Found: C, 46.57; H, 6.78; N, 16.63.

Allyl O-*(3-*O*-acetyl-4-azido-4,6-dideoxy-3-*C*-methyl-2-*O*-methyl-α-*L*-mannopyranosyl)-(1→3)-4-*O*-benzoyl-2-*O*-methyl-α-*D*-rhamnopyranoside* (**27**). — Trichloroacetonitrile (0.323 mL) and 1,5-diazabicyclo[5.4.0]undec-5-ene (0.08 mL) were added to **25** (140 mg, 0.54 mmol) in dry dichloromethane (5 mL) and the solution was kept for 20 min at room temperature. The reaction mixture was concentrated and the residue was purified by passage through a short column of silica gel with (3:1) light petroleum–diethyl ether as eluant to give 3-*O*-acetyl-4-azido-4,6-dideoxy-3-*C*-methyl-2-*O*-methyl-α-L-mannopyranosyl trichloroacetimidate (**26**) (211 mg, 97%) as a crystalline solid which was used directly for glycosylation; ^{1}H-n.m.r.: δ 8.65 (s, 1 H, NH), 6.30 (s, 1 H, H-1), 3.46 (s, 3 H, OCH_3), 2.10 (s, 3 H, OCOCH_3), 1.74 (s, 3 H, CCH_3), and 1.35 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6).

A mixture of **26** (118 mg, 0.29 mmol), **6** (70 mg, 0.21 mmol), and 4A molecular sieves (0.5 g) in dry dichloromethane (10 mL) was stirred under argon for 1 h, boron trifluoride etherate (47 μL, 1.25 equiv.) was added, and stirring was continued overnight. The reaction mixture was filtered through Celite, the residue was washed with dichloromethane, the combined filtrate and washings were concentrated, and the syrupy product was chromatographed on silica gel (40:1 chloroform–acetone) to afford **27** (110 mg, 90%) as a foam, $[\alpha]_D$ $-43°$ (*c* 0.5); ^{1}H-n.m.r.: δ 8.02–7.42 (m, 5 H, Ph-H), 4.95 (2 overlapping s, 2 H, H-1,1′), 3.54, 3.35 (2 s, 6 H, OCH_3), 2.01 (s, 3 H, OCOCH_3), 1.41 (s, 3 H, CCH_3), 1.24, and 0.96 (2 d, 6 H, $J_{5,6}$ 7.2, 5.8 Hz, H-6,6′); ^{13}C: δ 96.64 ($^1J_{C,H}$ 168.4 Hz, C-1 or 1′), 93.68 ($^1J_{C,H}$ 165.4 Hz, C-1′ or 1); exact mass: calc. for $C_{27}H_{37}N_3O_{10}$ + Na, 586.2376; found, 586.2411.

Anal. Calc. for $C_{27}H_{37}N_3O_{10}$: C, 57.54; H, 6.61; N, 7.45. Found: C, 57.35; H, 6.71; N, 7.44.

Allyl O-*(4,6-dideoxy-4-formamido-3-*C*-methyl-2-*O*-methyl-α-*L*-mannopyranosyl)-(1→3)-2-*O*-methyl-α-*D*-rhamnopyranoside* (**29**). — A solution of **27** (80 mg, 0.14 mmol) in methanolic 0.85M sodium methoxide (0.4 mL) was heated under reflux for 12 h. The solution was neutralized by treatment with Amberlite IR-120 (H$^+$) resin, filtered, and concentrated, and the residue was purified by chromatography on silica gel (15:1 chloroform–acetone) to yield **28** (56 mg) as syrup, $[\alpha]_D$ $-2.0°$ (*c* 3.1); ^{1}H-n.m.r.: δ 5.03 (s, 1 H, H-1 or 1′), 4.86 (d, 1 H, $J_{1,2}$ 1.45 Hz, H-1′ or 1), 3.52, 3.45 (2 s, 6 H, OCH_3), 1.41 (s, 3 H, C-3′-CH_3), 1.33, and 1.32 (2 d, 6 H, $J_{5,6}$ 6.0 and 6.14 Hz, H-6, 6′).

A solution of **28** (46 mg, 0.11 mmol) in 2:1 pyridine–water (7.5 mL) was saturated with hydrogen sulfide for 45 min at 40°. Stirring was continued for 24 h at 40°, the solution was filtered and concentrated, and the residue in ethyl formate (10 mL) was heated under reflux for 54 h. The solution was concentrated and the residue was chromatographed on silica gel (2:1 acetone–chloroform) to give **29** (26 mg, 56%) as a solid, m.p. 65–67°, $[\alpha]_D$ +19° (*c* 0.66); ^{1}H-n.m.r.: δ 8.31, 8.02 (s and d, 1 H, $J_{CH,NH}$ 11.4 Hz, *E* and *Z* *H*CONH), 5.06–4.87 (4 s, 2 H, H-1,1′ for *E* and *Z* isomers), 3.53, 3.46 (2 s, 6 H, OC*H*$_3$), and 1.36–1.26 (m, 9 H, H-6,6′, C-3′-C*H*$_3$); exact mass: calc. for $C_{19}H_{33}NO_9$ + H, 420.223; found, 420.222.

*Allyl O-(3-acetyl-4-azido-4,6-dideoxy-3-C-methyl-2-O-methyl-α-*L*-mannopyranosyl)-(1→3)-O-(4-O-benzoyl-2-O-methyl-α-*D*-rhamnopyranosyl)-(1→3)-4-O-benzoyl-2-O-methyl-α-*L*-fucopyranoside* (**31**). — A mixture of **26** (110 mg, 0.27 mmol), **11** (150 mg, 0.25 mmol), and 4A molecular sieves (0.75 g) was stirred under argon for 30 min, boron trifluoride etherate (0.44 mL) was added, and stirring was continued overnight. Sodium hydrogencarbonate (0.1 g) was added and after 10 min the mixture was filtered through Celite, the residue was washed with dichloromethane, and the combined filtrate and washings were washed with water (2 × 20 mL), dried, and concentrated. The residue was chromatographed on silica gel (3:2 chloroform–acetone) to furnish **31** (211 mg, 89%) as a solid foam, m.p. 75–77°, $[\alpha]_D$ −150° (*c* 0.73); ^{1}H-n.m.r.: δ 8.18–7.41 (m, 10 H, Ph-H), 5.51–5.16 (m, 6 H, H-1,1′,4,4′, =C*H*$_2$), 4.75 (s, 1 H, H-1″), 3.53, 3.44, 3.24 (3 s, 9 H, OC*H*$_3$), 1.97 (s, 3 H, OCOC*H*$_3$), 1.30 (s, 3 H, C-3″-C*H*$_3$), 1.23, 1.19, and 0.65 (3 d, 9 H, $J_{5,6}$ 5.8, 6.3, 5.7 Hz, H-6,6′,6″); ^{13}C: δ 169.3, 165.9, 165.0 (3 C=O), 98.63, 94.78, 92.72 ($^1J_{C,H}$ 172.0, 168.1, 166.0 Hz, C-1,1′,1″).

Anal. Calc. for $C_{41}H_{53}N_3O_{15}$: C, 59.48; H, 6.45; N, 5.07. Found: C, 59.85; H, 6.43; N, 4.73.

*Allyl O-(4,6-dideoxy-4-formamido-3-C-methyl-2-O-methyl-α-*L*-mannopyranosyl)-(1→3)-O-(2-O-methyl-α-*D*-rhamnopyranosyl)-(1→3)-2-O-methyl-α-*L*-fucopyranoside* (**33**). — A solution of **31** (150 mg, 0.18 mmol) in methanolic 0.1M sodium methoxide (8.5 mL) was heated for 30 h at 60°, neutralized by treatment with Amberlite IR-120 (H$^+$) resin, and concentrated. The residue was chromatographed on silica gel (2:1 chloroform–acetone) to give **32** (84 mg, 80%) as a solid, m.p. 184–186°, $[\alpha]_D$ −73° (*c* 1.34); ^{1}H-n.m.r.: δ 5.17, 5.05 (2 s, 2 H, H-1′,1″), 5.02 (d, 1 H, $J_{1,2}$ 3.56 Hz, H-1), 3.53, 3.47, 3.41 (3 s, 9 H, OC*H*$_3$), 1.40 (s, 3 H, C-3″-C*H*$_3$), 1.31, and 1.27 (2 d, 6 H and 3 H, $J_{5,6}$ 6.1, 6.6 Hz, H-6,6′,6″); ^{13}C: δ 98.89, 96.05, 95.10 ($^1J_{CH}$ 170.9, 167.8, 167.3 Hz, C-1,1′,1″).

A solution of **32** (35 mg, 0.06 mmol) in 2:1 pyridine–water (6 mL) was saturated with hydrogen sulfide for 45 min at 40° and stirring was continued for 24 h at the same temperature. The solution was concentrated to dryness, the residue in ethyl formate (10 mL) was heated under reflux for 45 h, and the solution was concentrated. The residue was chromatographed on silica gel (1:2 chloroform–acetone) to furnish **33** (21 mg, 60%) as a solid, m.p. 104–106°, $[\alpha]_D$ −60° (*c* 1.7); ^{1}H-n.m.r.: δ 8.3, 8.0 (s and d, 1 H, $J_{CH,NH}$ 11.4 Hz, *E* and *Z* *H*CONH), 5.18, 5.05 (2 s, 2 H, H-1′,1″), 5.02 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 3.54, 3.47, 3.42 (3 s, 9 H, OC*H*$_3$), 1.34, 1.29, 1.26 (3 d, 9 H, $J_{5,6}$ 6.9, 7.6, 6.9 Hz, H-6,6′,6″), and 1.25 (s, 3 H, C-3″-C*H*$_3$); exact mass: calc. for $C_{26}H_{45}NO_{13}$ + H, 580.296; found: 580.301.

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