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SYNTHESIS OF TETRASACCHARIDES RELATED TO THE ANTIGENIC DETERMINANTS FROM THE GLYCOPEPTIDOLIPID ANTIGENS OF SEROVARS 9 AND 25 IN THE *Mycobacterium avium–M. intracellulare–M. scrofulaceum* SEROCOMPLEX*

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ABSTRACT

The synthesis of the tetrasaccharides *O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose (**36**) and *O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose (**41**) is described. The former and the latter structures, respectively, have been proposed as the carbohydrate chains in the polar glycopeptidolipid antigens of serovars 9 and 25 in the *Mycobacterium avium–M. intracellulare–M. scrofulaceum* serocomplex. Glycosylation of allyl 2,3-di-*O*-methyl-α-L-fucopyranoside with 4-*O*-benzoyl-2,3-di-*O*-methyl-α-L-fucopyranosyl chloride gave the α-linked disaccharide derivative, which was *O*-deallylated and converted into the corresponding disaccharide α-chloride. This was coupled with benzyl 3,4-di-*O*-benzyl-6-deoxy-2-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-α-L-talopyranoside (**32**) to give a fully protected tetrasaccharide derivative, which was deprotected to furnish **36**. Likewise, 3-*O*-benzyl-4-*O*-(3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl)-2-*O*-methyl-α-L-fucopyranosyl chloride, prepared by way of condensation of allyl 3-*O*-benzoyl-2-*O*-methyl-α-L-fucopyranoside with 2-*O*-methyl-3,4-di-*O*-(*p*-nitrobenzoyl)-α-L-fucopyranosyl bromide, reacted with **32**, to provide, after removal of blocking groups, **41**.

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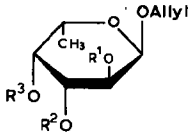
INTRODUCTION

Brennan *et al.*[1–4] have studied the "C-mycosidic" glycopeptidolipid-typing, surface antigens from serovarieties in the *Mycobacterium avium–M. intracellulare–M. scrofulaceum* (MAIS) serocomplex, and proposed[4], from detailed structural studies of the specific oligosaccharides liberated by reductive elimination from the antigens as the oligosaccharide-alditols, that the carbohydrate chains from serovars 9 and 25 are *O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose (**36**) and *O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose (**41**), respectively. In a previous paper[5], we have reported the synthesis of 6-deoxy-2-*O*-(α-L-rhamnopyranosyl)-L-talose, which occurs as the inner unit of the tetrasaccharides **36** and **41** as well as of the carbohydrate chains of several other serovars[2–4] of the MAIS complex, and the preparation of benzyl 3,4-di-*O*-benzyl-6-deoxy-2-*O*-(2,4-di-*O*-benzoyl-α-L-talopyranoside (**32**), which could serve as a common glycosyl acceptor for the synthesis of complete oligosaccharide chains from various serovars. We now report the synthesis of the tetrasaccharides **36** and **41** by unambiguous routes. A comparison of alditol **37**, formed from tetrasaccharide **36**, with the alditol derived[4] from the natural antigen shows discord. Accordingly, revision of the structure of the carbohydrate chain in the glycopeptidolipid from serovar 9 will be necessary before a complete synthesis can be attempted. Similar considerations may apply to the carbohydrate chain from the serovar 25 antigen.

RESULTS AND DISCUSSION

The synthetic methods employed here for the preparation of **36** and **41** are based on (*a*) the synthesis and characterization of the respective outer-disaccharide units, namely, 4-*O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-2,3-di-*O*-methyl-L-fucose (**19**) and 2-*O*-methyl-4-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-L-fucose (**27**), of **36** and **41**; (*b*) synthesis of the suitably protected, disaccharide glycosyl donors corresponding to **19** and **27**; and (*c*) block condensation of **32** with each of the glycosyl donors, followed by removal of protecting groups.

Synthesis of the outer disaccharide **19**. — Acetonation of allyl α-L-fucopyranoside[6] (**1**) with 2,2-dimethoxypropane and acetone in the presence of a trace of *p*-toluenesulfonic acid at room temperature gave a mixture from which the 3,4-isopropylidene acetal **2** directly crystallized in 74% yield. Fractionation of the mother liquor from **2** on a column of silica gel afforded a further 16% yield of **2**. When the reaction was performed without the added acetone, **1** remained unchanged in a considerable proportion, which prevented the direct crystallization of **2** from the reaction mixture. The structure of **2** was confirmed by its conversion into the known[7,8] 2-*O*-methyl-L-fucose (**9**). Methylation[9] of **2** with methyl iodide and sodium hydride in *N,N*-dimethylformamide gave, in 92% yield, the 3,4-*O*-iso-

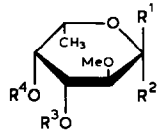


1 $R^1 = R^2 = R^3 = H$
2 $R^1 = H, R^2, R^3 = CMe_2$
3 $R^1 = Me, R^2, R^3 = CMe_2$
4 $R^1 = Me, R^2 = R^3 = H$
5 $R^1 = Me, R^2 = H, R^3 = Bz$
6 $R^1 = R^2 = Me, R^3 = Bz$
7 $R^1 = R^2 = Me, R^3 = H$
8 $R^1 = Me, R^2 = Bzl, R^3 = H$

propylidene-2-*O*-methyl derivative **3**, which was deisopropylidenated with aqueous acetic acid to afford allyl 2-*O*-methyl-α-L-fucopyranoside (**4**) in 93% yield. Mild acid hydrolysis of **4** produced the reducing sugar **9**, in 85% yield.

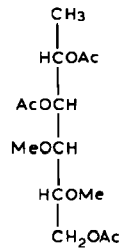
Treatment of **4** with trimethyl orthobenzoate in acetonitrile in the presence of *p*-toluenesulfonic acid, followed by acid-catalyzed rearrangement of the orthobenzoate[10], gave allyl 4-*O*-benzoyl-2-*O*-methyl-α-L-fucopyranoside (**5**) in 79% yield. The location of the benzoyl group in **5** was established in the following way. Methylation of **5** with diazomethane–boron trifluoride etherate[11] gave, in 82% yield, allyl 4-*O*-benzoyl-2,3-di-*O*-methyl-α-L-fucopyranoside (**6**), which was *O*-debenzoylated with sodium methoxide to afford allyl 2,3-di-*O*-methyl-α-L-fucopyranoside (**7**) in 93% yield. The benzoyl group on O-4 in **6** resisted an attempt at conventional *O*-debenzoylation, so that heating of **6** in methanol under reflux, in the presence of sodium methoxide, was necessary to effect the removal of the benzoyl group. Hydrolysis of **7** gave the known[12,13] 2,3-di-*O*-methyl-L-fucose (**10**). As **10** had not been obtained crystalline, it was sequentially reduced with sodium borohydride and acetylated to afford 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-fucitol (**16**), whose substitution pattern was shown by its mass spectrum.

Isomerization of the allyl group in **6** to a 1-propenyl group with tris(triphenylphosphine)rhodium(I) chloride[14,15], in the presence of 1,4-diazabicyclo[2.2.2]-octane[14], followed by hydrolysis of the resulting 1-propenyl group with mercuric chloride and mercuric oxide[16], gave 4-*O*-benzoyl-2,3-di-*O*-methyl-L-fucose (**11**) in 74% yield. The hydroxy derivative **11** was readily transformed by treatment with oxalyl chloride in dichloromethane, in the presence of a catalytic amount of *N*,*N*-dimethylformamide[17], into the corresponding crystalline α-chloride **12**.



9 $R^1 = R^3 = R^4 = H, R^2 = OH$
10 $R^1, R^2 = H, OH, R^3 = Me, R^4 = H$
11 $R^1, R^2 = H, OH, R^3 = Me, R^4 = Bz$
12 $R^1 = Cl, R^2 = H, R^3 = Me, R^4 = Bz$
13 $R^1 = OH, R^2 = R^3 = H, R^4 = Me$
14 $R^1 = H, R^2 = ONBz, R^3 = R^4 = NBz$
15 $R^1 = Br, R^2 = H, R^3 = R^4 = NBz$

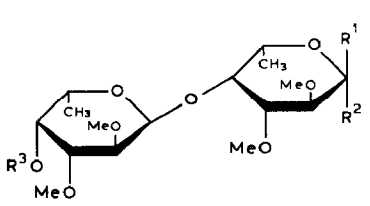
NBz = $COC_6H_4NO_2(p)$

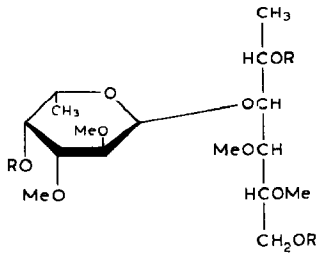


16

Condensation of **7** with 1.9 mol. equiv. of **12** in 1:1 benzene–nitromethane at 45°, in the presence of mercuric cyanide, gave, as the major product, allyl 4-*O*-(4-*O*-benzoyl-2,3-di-*O*-methyl-α-L-fucopyranosyl)-2,3-di-*O*-methyl-α-L-fucopyranoside (**17**), which was directly isolated crystalline in 43% yield from the reaction mixture, and fractionation of the mother liquor on a column of silica gel afforded another 30% of **17**. The α-L configuration at the newly introduced, interglycosidic linkage in **17** was indicated[18] by the ^{13}C-n.m.r. spectrum, which showed two signals for the anomeric carbon atoms at δ 99.65 ($^1J_{CH}$ 166.1 Hz) and 95.21 ($^1J_{CH}$ 167.8 Hz).

Compound **17** was *O*-debenzoylated by boiling with sodium methoxide in methanol to give the crystalline 2,3,2′,3′-tetra-*O*-methyl derivative **18**. Isomerization of the allyl group in **18** with the rhodium complex[14,15] in the presence of the base[14], followed by hydrolysis of the resulting 1-propenyl group with mercuric chloride and mercuric oxide[16], furnished crystalline **19**. Reduction of **19** with sodium borohydride provided crystalline 4-*O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-2,3-di-*O*-methyl-L-fucitol (**22**). In the ^{1}H-n.m.r. spectrum of **22**, the H-1′ resonance appeared at δ 5.02 as a doublet with $J_{1',2'}$ 2.6 Hz, consistent with the α-L configuration at C-1′. Acetylation of **22** gave crystalline **23**.





17 R^1 = Allyl, R^2 = H, R^3 = Bz
18 R^1 = Allyl, R^2 = R^3 = H
19 R^1, R^2 = H,OH, R^3 = H
20 R^1, R^2 = H,OH, R^3 = Bz
21 R^1 = Cl, R^2 = H, R^3 = Bz

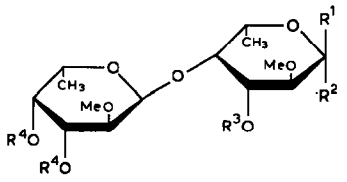
22 R = H
23 R = Ac

Synthesis of the outer disaccharide **27**. — Regioselective benzylation of the dibutylstannylene derivative of **4** in benzene with benzyl bromide, in the presence of tetrabutylammonium bromide[5,19], gave allyl 3-*O*-benzyl-2-*O*-methyl-α-L-fucopyranoside (**8**) in 81% yield. The structure of **8** was confirmed by methylation[9], followed by *O*-deallylation and hydrolysis, to give the known[12] 2,4-di-*O*-methyl-α-L-fucose (**13**).

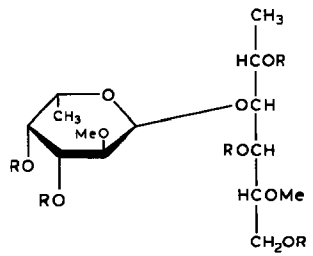
Esterification of **9** with *p*-nitrobenzoyl chloride in pyridine afforded, in 79% yield, crystalline 2-*O*-methyl-1,3,4-tri-*O*-(*p*-nitrobenzoyl)-β-L-fucopyranose (**14**), the anomeric configuration of the product being assigned on the basis of its ^{1}H-n.m.r. spectrum. Treatment[20] of **14** with hydrogen bromide–dichloromethane at room temperature converted it into the corresponding α-bromide **15**, which was isolated crystalline in 88% yield.

Reaction of **8** with 2 mol. equiv. of **15** in dichloromethane and *N,N*-dimethylformamide, in the presence of tetraethylammonium bromide and molecular sieve[21], at room temperature for 5 days gave a mixture shown by t.l.c. to contain, in addition to a considerable amount of unreacted **8** and **15**, a major product, accompanied by traces of a marginally faster-migrating, unidentified product that could not be removed by chromatography. Therefore, the mixture was *O*-deacetylated and the product was chromatographed on a column of silica gel to give, in 49% yield, allyl 3-*O*-benzyl-2-*O*-methyl-4-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-α-L-fucopyranoside (**24**), which on acetylation afforded the crystalline 3′,4′-di-*O*-acetyl-3-*O*-benzyl-2,2′-di-*O*-methyl derivative **25**. The ^{13}C-n.m.r. spectrum of **25** showed two signals for two anomeric carbon atoms having the α-L configuration[18], at δ 99.11 ($^1J_{CH}$ 168.6 Hz) and 95.56 ($^1J_{CH}$ 166.5 Hz).

In a more practical approach to **25**, condensation of **8** with 2 mol. equiv. of **15** in 1:1 benzene–nitromethane at 0°, in the presence of mercuric cyanide, followed by *O*-deacylation, isolation of the major product by chromatography on a column of silica gel, and acetylation, gave **25** in 75% yield. When **8** was treated with **15** under the foregoing conditions, but at room or elevated temperatures, the yield of **25** greatly decreased and side-products were formed.



24 R^1 = OAllyl, R^2 = R^4 = H, R^3 = Bzl
25 R^1 = OAllyl, R^2 = H, R^3 = Bzl, R^4 = Ac
26 R^1, R^2 = H,OH, R^3 = Bzl, R^4 = H
27 R^1 = R^3 = R^4 = H, R^2 = OH
28 R^1, R^2 = H,OH, R^3 = Bzl, R^4 = Ac
29 R^1 = Cl, R^2 = H, R^3 = Bzl, R^4 = Ac



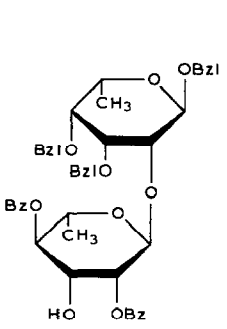
30 R = H
31 R = Ac

O-Deallylation of **24** gave the 3-*O*-benzyl-2,2′-di-*O*-methyl derivative **26**, which was hydrogenolyzed in acetic acid in the presence of palladium-on-charcoal, to give crystalline **27**. Reduction of **27** with sodium borohydride afforded 2-*O*-methyl-4-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-L-fucitol (**30**), whose ^{1}H-n.m.r. spectrum showed the H-1′ resonance at δ 5.29 as a doublet with $J_{1',2'}$ 3.75 Hz, consistent with the α-L configuration at C-1′. Acetylation of **30** produced crystalline **31**.

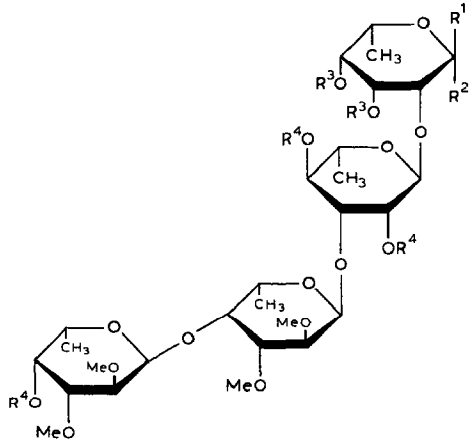
Synthesis of the tetrasaccharides **36** *and* **41**. — Compounds **17** and **25** were *O*-deallylated to give the hydroxy derivatives **20** and **28**, respectively. With oxalyl chloride–*N,N*-dimethylformamide in dichloromethane[17], **20** and **28** were trans-

formed into 4-*O*-(4-*O*-benzoyl-2,3-di-*O*-methyl-α-L-fucopyranosyl)-2,3-di-*O*-methyl-α-L-fucopyranosyl chloride (**21**) and 3-*O*-benzyl-4-*O*-(3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl)-2-*O*-methyl-α-L-fucopyranosyl chloride (**29**), respectively.

The selectively protected, disaccharide glycosyl acceptor **32** was treated with 2 mol. equiv. of **21** in toluene, in the presence of silver trifluoromethanesulfonate[22–24], 2,4,6-trimethylpyridine[23,24], and molecular sieve, and afforded (78% yield after chromatography) benzyl *O*-(4-*O*-benzoyl-2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-6-deoxy-L-talopyranoside (**33**). *O*-Debenzoylation of **33** afforded **34**, which on hydrogenolysis furnished **36** as an amorphous solid. Acetylation of **34** gave **35**. Reduction of **36** with sodium borohydride provided the tetrasaccharide-alditol **37**.



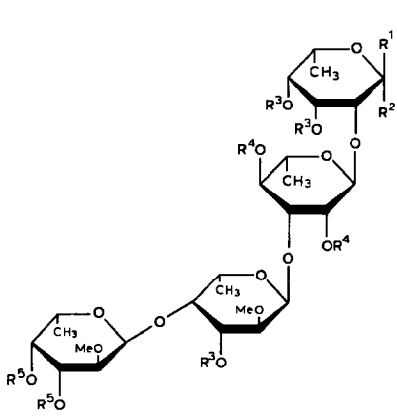
32



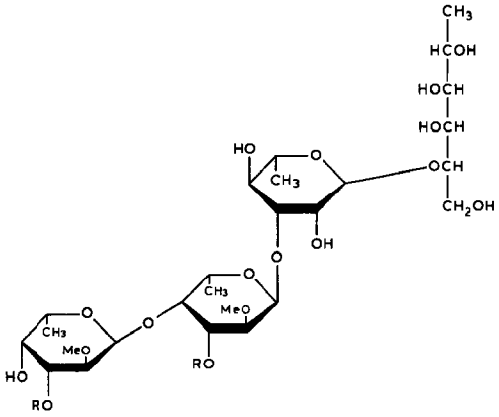
33 R^1 = OBzl, R^2 = H, R^3 = Bzl, R^4 = Bz
34 R^1 = OBzl, R^2 = H, R^3 = Bzl, R^4 = H
35 R^1 = OBzl, R^2 = H, R^3 = Bzl, R^4 = Ac
36 R^1, R^2 = H, OH, R^3 = R^4 = H

Glycosylation of **32** with **29** under conditions similar to those employed for the coupling of **32** with **21** gave (82% yield after chromatography) benzyl *O*-(3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-6-deoxy-α-L-talopyranoside (**38**), which was *O*-deacetylated to afford **39**. Acetylation of **39** gave crystalline **40**. Hydrogenolysis of **39** furnished **41** as an amorphous solid. Reduction of **41** produced the tetrasaccharide-alditol **42**.

The structures of the tetrasaccharide-alditols **37** and **42**, which were implied by their methods of synthesis, were fully confirmed in respect of anomeric configurations by their n.m.r. spectra and in respect of inter-sugar linkage types by methylation analysis, thus ruling out any possibility of benzoyl migration in the



38 R^1 = OBzl, R^2 = H, R^3 = Bzl, R^4 = Bz, R^5 = Ac
39 R^1 = OBzl, R^2 = R^4 = R^5 = H, R^3 = Bzl
40 R^1 = OBzl, R^2 = H, R^3 = Bzl, R^4 = R^5 = Ac
41 R^1, R^2 = H,OH, R^3 = R^4 = R^5 = H



37 R = Me
42 R = H

rhamnopyranosyl residue of the inner-disaccharide derivative **32** during the coupling with the outer-disaccharide derivative **21** or **29**. However, a direct comparison, by t.l.c. and ^{1}H-n.m.r. spectroscopy, of the tetrasaccharide-alditol **37** with the oligosaccharide-alditol (*r*-9) derived from the glycopeptidolipid antigen of serovar 9 showed that the two compounds were not identical. The structure proposed[4] for *r*-9 was based largely on internally consistent compositional and mass-spectral data for the pertrideuteriomethylated derivative, and a signal at δ 4.53 in the ^{1}H-n.m.r. spectrum of the parent oligosaccharide, indicative of a possible fourth anomeric proton, was not assigned significance. A re-examination[25] of the *r*-9 oligosaccharide-alditol has now indicated that the additional residue is that of a uronic acid, probably in a terminal position. Since the compositional data for the alkylated derivative of the synthetic tetrasaccharide-alditol **37** and of that derived from *r*-9 are indistinguishable, it is probable that these compounds are identical, but that formation of the latter under the strongly basic conditions required for trideuteriomethylation of *r*-9 resulted from base-catalyzed degradation[26] with complete loss of the uronic acid residue and alkylation of an exposed hydroxyl group in a non-terminal residue. Experiments to verify this suggestion and to establish the identity and site of attachment of the putative uronic acid residue are in progress. A comparison of the ^{1}H-n.m.r. spectrum of the tetrasaccharide-alditol **42** with that obtained previously for a sample of *r*-25 suggests that this latter oligosaccharide-alditol may also contain an unrecognized sugar constituent. Full details of the re-examinations of *r*-9 and *r*-25 will be reported elsewhere.

The synthetic procedures described here will undoubtedly form the basis for more extended syntheses, leading to the assembly of the complete oligosaccharide chains in confirmation of the revised structures and for the provision of materials for immunological studies.

EXPERIMENTAL

Unless stated otherwise, the general experimental conditions were the same as those described previously[5]. Column chromatography was performed on silica gel (Merck, 9385). ^{1}H-N.m.r. spectra were recorded with Varian A-60A and JEOL-FX 270 spectrometers (Kyoto), and ^{13}C- and some ^{1}H-n.m.r. spectra with a Nicolet NT-360 spectrometer (Colorado State University Regional NMR Center) on solutions in $CDCl_3$ unless stated otherwise. G.l.c.–m.s. was performed on glass-capillary or S.C.O.T. columns of silicone gum OV-225 connected to a VG Micromass 16F mass spectrometer (Toronto and Fort Collins). Microanalyses were performed at the Faculty of Pharmaceutical Sciences, Kyoto University. The following solvent systems were used for chromatography: *1*, 4:1; *2*, 2:1; *3*, 1:1; and *4*, 1:2 hexane–ethyl acetate; *5*, 4:1 and *6*, 9:1 benzene–ethanol; *7*, 2:1; *8*, 1:3; and *9*, 1:4 benzene–ethyl acetate; *10*, 4:1 and *11*, 9:1 chloroform–methanol; and *12*, 65:15:2 chloroform–methanol–water.

*Allyl 3,4-*O*-isopropylidene-α-*L*-fucopyranoside* (**2**). — A mixture of **1** (11.50 g), 2,2-dimethoxypropane (46 mL), dry acetone (20 mL), and *p*-toluenesulfonic acid (0.25 g) was stirred for 1 h at room temperature and then neutralized with Amberlite IR-45 (HO^-) resin. The resin was collected and washed with methanol, and the combined filtrate and washings were concentrated. Crystallization and recrystallization of the residue from pentane at 0° gave **2** (10.20 g, 74%), m.p. 35–36°, $[\alpha]_D^{20}$ −153° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 4.87 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 2.62 (d, 1 H, $J_{2,HO\text{-}2}$ 6.5 Hz, exchangeable with D_2O, HO-2), 1.50 and 1.35 (2 s, each 3 H, CMe_2), and 1.30 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me).

Anal. Calc. for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25. Found: C, 59.08; H, 8.30.

The mother liquors were concentrated to a syrup, column chromatography (solvent *3*) of which gave more **2** (2.21 g, 16%).

*Allyl 3,4-*O*-isopropylidene-2-*O*-methyl-α-*L*-fucopyranoside* (**3**). — A solution of **2** (23.11 g) in anhydrous *N,N*-dimethylformamide (250 mL) was stirred for 1 h with sodium hydride (9.0 g; 50% in mineral oil) at room temperature and then cooled to 0°. Methyl iodide (25 mL) was added dropwise during 30 min, and the mixture was stirred for 6 h at room temperature. Methanol was added to decompose the excess of hydride, and most of the solvent was evaporated. A solution of the residue in chloroform was washed with water, dried, and concentrated. Column chromatography (solvent *1*) of the residue gave **3** as a syrup (22.64 g, 92%), $[\alpha]_D^{19}$ −165° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 4.93 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.52 (s, 3 H, OMe), 1.55 and 1.36 (2 s, each 3 H, CMe_2), and 1.33 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me).

*Allyl 2-*O*-methyl-α-*L*-fucopyranoside* (**4**). — To a solution of **3** (25.82 g) in acetic acid (120 mL) at 90° was added water (80 mL) in small portions, and the mixture was stirred for 15 min, then cooled, and concentrated. The last traces of the solvents were removed by repeated evaporation of toluene from the residue, column chromatography (solvent *5*) of which then gave **4** as a syrup (20.33 g, 93%),

$[\alpha]_D^{20}$ −195° (*c* 1.6, chloroform). ^{1}H-N.m.r. data: δ 5.05 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.46 (s, 3 H, OMe), 3.17 and 3.07 (2 d, each 1 H, $J_{3,HO-3} = J_{4,HO-4} = 3.0$ Hz, exchangeable with D_2O, HO-3,4), and 1.28 (d, 3 H, $J_{5,6}$ 6.0 Hz, Me).

2-O-Methyl-β-L-fucose (**9**). — A solution of **4** (15.06 g) in M sulfuric acid (380 mL) was stirred for 6 h at 100°, then neutralized with barium carbonate, and filtered. The insoluble material was washed with water, and the combined filtrate and washings were concentrated. The residue was recrystallized from ethanol to give **9** (10.45 g, 85%), m.p. 151–152°, $[\alpha]_D^{20}$ −52° (3 min) → −89° (*c* 1.2, water; 2 h, constant); lit. m.p. 150–152°, $[\alpha]_D^{18}$ −87° (*c* 1, water)[7]; m.p. 153.5–155°, $[\alpha]_D^{21}$ −91° (*c* 1, water)[8].

Allyl 4-O-benzoyl-2-O-methyl-α-L-fucopyranoside (**5**). — A mixture of **4** (20.05 g), trimethyl orthobenzoate (30 mL), and *p*-toluenesulfonic acid (0.3 g) in dry acetonitrile (160 mL) was stirred for 2 h at room temperature. Trimethylamine (2 mL) was added and the mixture was concentrated to dryness. A solution of the residue in aqueous 80% acetic acid (80 mL) was stirred for 10 min at room temperature and then concentrated, and toluene was evaporated from the residue. Column chromatography (solvent *7*) of the residue gave **5** as a syrup (22.39 g, 79%), $[\alpha]_D^{20}$ −139° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 7.38–7.28 (m, 5 H, Ph), 3.47 (s, 3 H, OMe), 2.75 (d, 1 H, $J_{3,HO-3}$ 3.0 Hz, exchangeable with D_2O, HO-3), and 1.18 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me).

Allyl 4-O-benzoyl-2,3-di-O-methyl-α-L-fucopyranoside (**6**). — Diazomethane in dichloromethane was gradually added to a stirred solution of **5** (16.44 g) in dichloromethane (50 mL) containing boron trifluoride etherate (0.5 mL) at −10° until a faint yellow color persisted, and the mixture was then kept for 1 h at room temperature. Polymethylene was removed, and the filtrate was washed successively with aqueous sodium hydrogencarbonate and water, dried, and concentrated. Column chromatography (solvent *2*) of the residue gave **6** as a syrup (14.15 g, 82%), $[\alpha]_D^{20}$ −152° (*c* 1.4, chloroform). ^{1}H-N.m.r. data: δ 8.20–7.23 (m, 5 H, Ph), 3.51 and 3.44 (2 s, each 3 H, 2 OMe), and 1.18 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me).

Allyl 2,3-di-O-methyl-α-L-fucopyranoside (**7**). — A solution of **6** (7.54 g) in methanol (100 mL) containing M sodium methoxide (5 mL) was boiled for 1 h under reflux, then cooled, neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. Column chromatography (solvent *4*) of the residue gave **7** as a syrup (4.85 g, 93%), $[\alpha]_D^{20}$ −175° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 3.50 and 3.47 (2 s, each 3 H, 2 OMe), 2.53 (bs, 1 H, exchangeable with D_2O, HO-4), and 1.30 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me).

Hydrolysis of **7** (0.47 g) in M sulfuric acid (15 mL) for 5 h at 100°, followed by processing of the mixture (as described for the preparation of **9**) and column chromatography (solvent *12*) of the product, gave 2,3-di-*O*-methyl-L-fucose (**10**) as an amorphous solid (0.33 g, 85%), $[\alpha]_D^{20}$ −81 → −108° (*c* 1.7, water; 3 h, constant); lit. $[\alpha]_D^{18}$ −97° (*c* 3, water)[12]; m.p. 75–76°, $[\alpha]_D^{30}$ −101° (*c* 1.05, water)[13].

Reduction of **10** with sodium borohydride, followed by acetylation, afforded 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-fucitol (**16**), which was homogeneous in g.l.c.

and gave a mass spectrum containing fragment ions at *m/z* 117, 143, and 203.

4-O-Benzoyl-2,3-di-O-methyl-L-fucose (**11**). — A mixture of **6** (11.09 g), tris(triphenylphosphine)rhodium(I) chloride (0.8 g), and 1,4-diazabicyclo[2.2.2]-octane (4.0 g) in 7:3:1 ethanol–toluene–water (280 mL) was boiled for 8 h under reflux and then concentrated to dryness, and the residue was extracted with dichloromethane. The extract was washed successively with water, cold M hydrochloric acid, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. To a solution of the residue in 1:1 acetone–water (200 mL) was added mercuric oxide (3.5 g) followed by a solution of mercuric chloride (3.5 g) in 9:1 acetone–water (60 mL). After the suspension had been stirred for 30 min at room temperature, the solids were removed, the filtrate was concentrated, and a solution of the syrupy residue in ether was washed successively with water, aqueous potassium iodide, and water, then dried, and concentrated. Column chromatography (solvent *4*) of the residue gave **11** as an amorphous powder (7.23 g, 74%), $[\alpha]_D^{27}$ −120.5° (*c* 1.3, chloroform).

Anal. Calc. for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 60.91; H, 6.73.

4-O-Benzoyl-2,3-di-O-methyl-α-L-fucopyranosyl chloride (**12**). — A solution of oxalyl chloride (15 mL) in dichloromethane (45 mL) was added dropwise at 0° to a solution of **11** (9.20 g) in dichloromethane (60 mL) containing *N,N*-dimethylformamide (1.5 mL). The mixture was kept for 1 h at room temperature and then concentrated, a solution of the residue in 1:1 hexane–ethyl acetate (80 mL) was filtered through a layer of silica gel (10 g) and concentrated, and the residue was recrystallized from ether–light petroleum to afford **12** (8.31 g, 85%), m.p. 122.5–123.5°, $[\alpha]_D^{23}$ −261° (*c* 1.2, dichloromethane). ^{1}H-N.m.r. data: δ 6.38 (bs, 1 H, H-1), 3.53 and 3.47 (2 s, each 3 H, 2 OMe), and 1.23 (d, 3 H, $J_{5,6}$ Hz, Me).

Anal. Calc. for $C_{15}H_{19}ClO_5$: C, 57.23; H, 6.08. Found: C, 57.30; H, 6.12.

Allyl 4-O-(4-O-benzoyl-2,3-di-O-methyl-α-L-fucopyranosyl)-2,3-di-O-methyl-α-L-fucopyranoside (**17**). — A solution of **7** (2.65 g, 11.4 mmol) in 1:1 benzene–nitromethane (110 mL) was concentrated to ~80 mL and then cooled to 45°. Mercuric cyanide (5.50 g, 21.8 mmol) and **12** (6.85 g, 21.8 mmol) were added, and the mixture was stirred for 16 h at 45°, then diluted with benzene, washed successively with water, aqueous potassium iodide, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. Crystallization of the residue from light petroleum gave **17** (2.51 g, 43%), m.p. 125–127°, $[\alpha]_D^{20}$ −167° (*c* 1.2, chloroform). N.m.r. data: ^{1}H, δ 8.03–7.43 (m, 5 H, Ph), 3.56 (s, 3 H, OMe), 3.50 (s, 6 H, 2 OMe), 3.46 (s, 3 H, OMe), and 1.39 and 1.34 (2 d, each 3 H, $J_{5,6} = J_{5',6'}$ = 6.5 Hz, 2 Me); ^{13}C, 99.65 ($^1J_{CH}$ 166.1 Hz, C-1 or C-1′) and 95.21 ($^1J_{CH}$ 167.8 Hz, C-1 or 1′).

Anal. Calc. for $C_{26}H_{38}O_{10}$: C, 61.16; H, 7.50. Found: C, 61.17; H, 7.57.

The mother liquors were concentrated and column chromatography (solvent *3*) of the residue gave more **17** (1.75 g, 30%).

Allyl 4-O-(2,3-di-O-methyl-α-L-fucopyranosyl)-2,3-di-O-methyl-α-L-fucopyranoside (**18**). — A solution of **17** (1.44 g) in methanol (25 mL) containing M

sodium methoxide (2 mL) was boiled for 70 min under reflux, and then processed as described for the preparation of **7**. Column chromatography (solvent *9*) of the product gave **18** (1.04 g, 90%), m.p. 86–87° and 104–105° (from ether–light petroleum), $[\alpha]_D^{20}$ −234° (*c* 1.4, chloroform). ^{1}H-N.m.r. data: δ 3.53 (s, 3 H, OMe), 3.51 (s, 6 H, 2 OMe), 3.48 (s, 3 H, OMe), 2.47 (bs, 1 H, exchangeable with D_2O, HO-4′), and 1.37 and 1.30 (2 d, each 3 H, $J_{5,6} = J_{5',6'} = 6.5$ Hz, 2 Me).

Anal. Calc. for $C_{19}H_{34}O_9$: C, 56.14; H, 8.43. Found: C, 56.28; H, 8.49.

4-O-(2,3-Di-O-methyl-α-L-fucopyranosyl)-2,3-di-O-methyl-L-fucose (**19**). — A mixture of **18** (0.81 g), tris(triphenylphosphine)rhodium(I) chloride (0.2 g), and 1,4-diazabicyclo[2.2.2]octane (0.9 g) in 7:3:1 ethanol–toluene–water (30 mL) was boiled for 8 h under reflux, then processed as described for the preparation **11**, and concentrated to dryness. To a solution of the residue in 9:1 acetone–water (10 mL) was added mercuric oxide (0.3 g) followed, dropwise with stirring, by a solution of mercuric chloride (0.3 g) in 9:1 acetone–water (10 mL), and the mixture was processed as described for the preparation of **11**. Column chromatography (solvent *6*) of the residue gave **19** (0.54 g, 73%), m.p. 143.5–144.5° (from ether–light petroleum), $[\alpha]_D^{26}$ −188° (*c* 1, chloroform).

Anal. Calc. for $C_{16}H_{30}O_9$: C, 52.45; H, 8.25. Found: C, 52.56; H, 8.35.

4-O-(2,3-Di-O-methyl-α-L-fucopyranosyl)-2,3-di-O-methyl-L-fucitol (**22**). — Compound **19** (0.38 g) was reduced with sodium borohydride (30 mg) in water (10 mL) overnight at room temperature. The solution was treated with Amberlite IR-120 (H^+) resin to decompose the excess of hydride, the resin was collected and washed with water, and the combined filtrate and washings were concentrated. Methanol was evaporated several times from the residue which crystallized from ether–ethanol to afford **22** (0.33 g, 87%), m.p. 121.5–122.5°, $[\alpha]_D^{26}$ −106° (*c* 0.7, methanol). ^{1}H-N.m.r. data (270 MHz, acetone-d_6): δ 5.02 (d, 1 H, $J_{1',2'}$ 2.6 Hz, H-1), 3.49, 3.44, 3.43, and 3.40 (4 s, each 3 H, 4 OMe), and 1.21 (d, 3 H, $J_{5,6}$ or $J_{5',6'}$ 6.6 Hz, Me), and 1.31 (d, 3 H, $J_{5,6}$ or $J_{5',6'}$ 6.3 Hz, Me).

Anal. Calc. for $C_{16}H_{32}O_9$: C, 52.16; H, 8.75. Found: C, 52.24; H, 8.69.

1,5-Di-O-acetyl-4-O-(4-O-acetyl-2,3-di-O-methyl-α-L-fucopyranosyl)-2,3-di-O-methyl-L-fucitol (**23**). — Conventional acetylation of **22** (0.16 g) with 1:1 acetic anhydride–pyridine (3 mL) overnight at room temperature gave **23** (0.14 g, 88%), m.p. 112–113° (from ether–light petroleum), $[\alpha]_D^{26}$ −102° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 3.50 (s, 3 H, OMe), 3.47 (s, 6 H, 2 OMe), 3.42 (s, 3 H, OMe), 2.16, 2.08, and 2.05 (3 s, each 3 H, 3 OAc), 1.30 (d, 3 H, $J_{5,6}$ or $J_{5',6'}$ 5.5 Hz, Me), and 1.14 (d, 3 H, $J_{5,6}$ or $J_{5',6'}$ 6.5 Hz, Me).

Anal. Calc. for $C_{22}H_{38}O_{12}$: C, 53.43; H, 7.75. Found: C, 53.58; H, 7.88.

Allyl 3-O-benzyl-2-O-methyl-α-L-fucopyranoside (**8**). — A solution of **4** (8.02 g) in benzene (400 mL) containing dibutyltin oxide (10 g) was boiled under reflux for 2 h with continuous removal of water. After concentration of the solution to ~300 mL, tetrabutylammonium bromide (11.8 g) and benzyl bromide (8.7 mL) were added, and the mixture was stirred overnight at 100° and then concentrated. The residue was subjected to column chromatography. Elution with hexane

removed the excess of benzyl bromide. Subsequent elution with solvent *3* gave **8** as a syrup (9.18 g, 81%), $[\alpha]_D^{20}$ −114° (*c* 1.4, chloroform). ^{1}H-N.m.r. data: δ 7.32 (s, 5 H, Ph), 4.72 (ABq, 2 H, *J* 12.0 Hz, PhCH_2), 3.50 (s, 3 H, OMe), 2.50 (bs, 1 H, exchangeable with D_2O, HO-4), and 1.27 (s, 3 H, $J_{5,6}$ 6.5 Hz, Me).

Compound **8** (1.15 g) was methylated[9] in *N,N*-dimethylformamide (10 mL) with methyl iodide (1 mL) and sodium hydride (0.4 g; 50% in mineral oil). The product was boiled with tris(triphenylphosphine)rhodium(I) chloride (50 mg) and 1,4-diazabicyclo[2.2.2]octane (0.3 g) in 8:3:1 ethanol–toluene–water (30 mL) under reflux overnight. The mixture was processed and treated with mercuric oxide (0.3 g) and mercuric chloride in 9:1 acetone–water (10 mL), as described for the preparation of **11**. A solution of the product in acetic acid (10 mL) was hydrogenated in the presence of 10% Pd/C (0.8 g) at atmospheric pressure for 1 day, and then filtered through a Celite pad which was washed with methanol. The combined filtrate and washings were concentrated. Column chromatography (solvent *6*) of the residue gave 2,4-di-*O*-methyl-α-L-fucose (**13**; 0.51 g, 71%), m.p. 131–133° (from chloroform–light petroleum), $[\alpha]_D^{20}$ −129 (3 min) → −89° (*c* 1.1, water; 2 h, constant); lit.[12] m.p. 131–132°, $[\alpha]_D^{18}$ −85° (*c* 0.85, water).

2-O-Methyl-1,3,4-tri-O-(p-nitrobenzoyl)-β-L-fucopyranose (**14**). — *p*-Nitrobenzoyl chloride (70 g) was added portionwise at 0° to a solution of **9** (14.80 g) in anhydrous pyridine (200 mL), and the mixture was kept overnight at room temperature. The precipitate was collected and washed extensively with aqueous sodium hydrogencarbonate, and a solution in chloroform was washed with aqueous sodium hydrogencarbonate and water, dried, and concentrated. Recrystallization of the resulting white solid twice from ethyl acetate gave **14** (41.1 g, 79%), m.p. 197.5–198.5°, $[\alpha]_D^{20}$ −149° (*c* 1.8, chloroform). ^{1}H-N.m.r. data: δ 6.03 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.58 (s, 3 H, OMe), and 1.38 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me).

Anal. Calc. for $C_{28}H_{23}N_3O_{14}$: C, 53.77; H, 3.71; N, 6.72. Found: C, 53.78; H, 3.58; N, 6.67.

2-O-Methyl-3,4-di-O-(p-nitrobenzoyl)-α-L-fucopyranosyl bromide (**15**). — A saturated solution of hydrogen bromide in dichloromethane (190 mL) was added to a solution of **14** (12.52 g) in dichloromethane (110 mL). After 2 h at room temperature, the precipitated *p*-nitrobenzoic acid was removed, and the filtrate was washed successively with cold aqueous sodium hydrogencarbonate and cold water, dried, and concentrated. Crystallization of the residue from ethyl acetate–hexane gave **15** (9.51 g, 88%), m.p. 168–169° (dec.), $[\alpha]_D^{23}$ −299° (*c* 1.6, dichloromethane). ^{1}H-N.m.r. data: δ 6.83 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.52 (s, 3 H, OMe), and 1.34 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me).

Anal. Calc. for $C_{21}H_{19}BrN_2O_{10}$: C, 46.77; H, 3.55. Found: C, 46.90; H, 3.44.

Allyl 3-O-benzyl-2-O-methyl-4-O-(2-O-methyl-α-L-fucopyranosyl)-α-L-fucopyranoside (**24**). — A solution of **8** (2.70 g, 8.8 mmol) in dry dichloromethane (30 mL) was stirred for 2 h at room temperature in the presence of tetraethylammonium bromide (3.68 g, 17.5 mmol) and molecular sieve Type 4A (10 g). A solution of **15** (9.44 g, 17.5 mmol) in dichloromethane (15 mL) and dry *N,N*-di-

methylformamide (15 mL) was then added, and the mixture was stirred for 5 days at room temperature. Methanol (20 mL) was added, and the mixture was stirred for 3 h at room temperature, then filtered, and concentrated to dryness. A solution of the residue in chloroform was washed with water, dried, and concentrated. The residue was dissolved in methanol (80 mL) and dichloromethane (30 mL), and treated with methanolic M sodium methoxide (5 mL) overnight at room temperature. The solution was neutralized with acetic acid and concentrated. Column chromatography (solvent *6*) of the residue gave **24** as a syrup (2.01 g, 49%), $[\alpha]_D^{20}$ −215° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.47–7.23 (m, 5 H, Ph), 3.52 and 3.49 (2 s, each 3 H, 2 OMe), and 1.28 and 0.98 (2 d, each 3 H, $J_{5,6} = J_{5',6'} = 6.5$ Hz, 2 Me).

Allyl 3-O-benzyl-4-O-(3,4-di-O-acetyl-2-O-methyl-α-L-fucopyranosyl)-2-O-methyl-α-L-fucopyranoside (**25**). — (*a*) Acetylation of **24** (0.66 g) in 1:1 acetic anhydride–pyridine (8 mL) gave **25** (0.72 g, 92%), m.p. 107–108° (from ether–hexane), $[\alpha]_D^{20}$ −239.5° (*c* 1.1, chloroform). N.m.r. data: ^{1}H, δ 7.45–7.23 (m, 5 H, Ph), 3.54 and 3.47 (2 s, each 3 H, 2 OMe), 2.12 and 2.00 (2 s, each 3 H, 2 OAc), and 1.29 and 0.75 (2 s, each 3 H, $J_{5,6} = J_{5',6'} = 6.5$ Hz, 2 Me); ^{13}C, 99.11 ($^1J_{CH}$ 168.6 Hz, C-1 or C-1′) and 95.56 ($^1J_{CH}$ 166.5 Hz, C-1 or C-1′).

Anal. Calc. for $C_{28}H_{40}O_{11}$: C, 60.86; H, 7.30. Found: C, 60.60; H, 7.30.

(*b*) A solution of **8** (8.05 g, 26 mmol) in 1:1 benzene–nitromethane (350 mL) containing powdered mercuric cyanide (13.10 g, 52 mmol) was concentrated to ~280 mL, and then cooled to 0°. A solution of **15** (28.16 g, 52 mmol) in 1:1 benzene–nitromethane (100 mL) was added dropwise during 1 h with stirring. After being stirred for 1 h at 0°, the mixture was allowed to reach room temperature, then stirred for 1 h, and processed as described for the preparation of **17**. The product was treated with methanolic M sodium methoxide (10 mL) overnight at room temperature and then processed as described for the preparation of **24**. Column chromatography (solvent *5*) of the product gave crude **24** (11.0 g) as a syrup, which was acetylated with 1:1 acetic anhydride–pyridine (140 mL). Recrystallization of the product twice from ether–hexane afforded **25** (10.82 g, 75%), m.p. and mixture m.p. 107–108°, $[\alpha]_D^{23}$ −238° (*c* 1, chloroform).

2-O-Methyl-4-O-(2-O-methyl-α-L-fucopyranosyl)-L-fucose (**27**). — Compound **24** (1.65 g) was boiled with tris(triphenylphosphine)rhodium(I) chloride (0.2 g) and 1,4-diazabicyclo[2.2.2]octane (1.0 g) in 8:3:1 ethanol–toluene–water (60 mL) under reflux for 16 h. The mixture was processed and the product was treated with mercuric oxide (0.2 g) and mercuric chloride (0.2 g) in 9:1 acetone–water (30 mL), as described above. Column chromatography (solvent *5*) of the product gave 3-*O*-benzyl-2-*O*-methyl-4-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-L-fucose (**26**) as a syrup (1.51 g, 74%), $[\alpha]_D^{20}$ −156° (*c* 1.4, methanol).

A solution of **26** (1.31 g) in acetic acid (15 mL) was hydrogenolyzed in the presence of 10% Pd/C (1.2 g) for 1 day. Processing of the mixture, as described for the preparation of **13**, and column chromatography (solvent *11*) of the product gave **27** (0.92 g, 89%), m.p. 93.5–94.5° (from ethanol–ether), $[\alpha]_D^{20}$ −164 (3 min) → 184° (*c* 0.9, water; 3 h, constant).

Anal. Calc. for $C_{14}H_{26}O_9$: C, 49.70; H, 7.75. Found: C, 49.84; H, 7.83.

*2-O-Methyl-4-O-(2-O-methyl-α-L-fucopyranosyl)-L-fucitol (***30***).* — Reduction of **27** (0.35 g) with sodium borohydride (30 mg) in water (10 mL), as described for the preparation of **22**, gave **30** as a syrup (0.31 g, 89%), $[\alpha]_D^{20}$ −128° (*c* 0.8, water). ^{1}H-N.m.r. data (D_2O): δ 5.29 (d, 1 H, $J_{1,2}$ 3.75 Hz, H-1′), 6.88 and 6.83 (2 s, each 3 H, 2 OMe), and 1.30 and 1.23 (2 d, each 3 H, $J_{5,6} = J_{5',6'} = 6.5$ Hz, 2 Me).

1,3,5-Tri-O-acetyl-4-O-(3,4-di-O-acetyl-2-O-methyl-α-L-fucopyranosyl)-2-O-methyl-L-fucitol (**31**). — Acetylation of **30** (0.17 g) gave **31** (0.24 g, 89%), m.p. 103–104° (from ether–light petroleum), $[\alpha]_D^{20}$ −98° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 3.51 and 3.47 (2 s, each 3 H, 2 OMe), 2.17 (s, 3 H, OAc), 2.7 (s, 6 H, 2 OAc), 2.02 (s, 6 H, 2 OAc), and 1.47 and 1.11 (2 d, each 3 H, $J_{5,6} = J_{5',6'} = 6.5$ Hz, 2 Me).

Anal. Calc. for $C_{24}H_{38}O_{14}$: C, 52.36; H, 6.96. Found: C, 52.56; H, 7.08.

4-O-(4-O-Benzoyl-2,3-di-O-methyl-α-L-fucopyranosyl)-2,3-di-O-methyl-L-fucose (**20**). — A mixture of **17** (5.03 g), tris(triphenylphosphine)rhodium(I) chloride (0.3 g), and 1,4-diazabicyclo[2.2.2]octane (1.5 g) in 8:3:1 ethanol–toluene–water (150 mL) was boiled for 7 h under reflux. The mixture was processed and the product was treated with mercuric oxide (2 g) and mercuric chloride (2 g) in 9:1 acetone–water (100 mL) as described above. Column chromatography (solvent *8*) of the resulting syrup gave **20** as an amorphous powder (3.85 g, 83%), $[\alpha]_D^{20}$ −167° (*c* 1.2, chloroform).

Anal. Calc. for $C_{23}H_{34}O_{10}$: C, 58.71; H, 7.28. Found: C, 58.79; H, 7.40.

3-O-Benzyl-4-O-(3,4-di-O-acetyl-2-O-methyl-α-L-fucopyranosyl)-2-O-methyl-L-fucose (**28**). — Compound **25** (10.05 g) was boiled with tris(triphenylphosphine)-rhodium(I) chloride (0.5 g) and 1,4-diazabicyclo[2.2.2]octane (2.4 g) in 8:3:1 ethanol–toluene–water (300 mL) under reflux for 6 h. The mixture was processed and the product was treated with mercuric oxide (3 g) and mercuric chloride (3 g) in 9:1 acetone–water (200 mL) as described above. Chromatography (solvent *8*) of the resulting syrup gave **28** as an amorphous powder (7.65 g, 82%), $[\alpha]_D^{20}$ −145° (*c* 1, chloroform).

Anal. Calc. for $C_{25}H_{36}O_{11}$: C, 58.58; H, 7.08. Found: C, 58.70; H, 7.16.

Benzyl O-(4-O-benzoyl-2,3-di-O-methyl-α-L-fucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-fucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-O-benzyl-6-deoxy-α-L-talopyranoside (**33**). — Treatment of **20** (3.40 g) in dichloromethane (25 mL) containing *N,N*-dimethylformamide (0.18 mL) with a solution of oxalyl chloride (1.85 mL) in dichloromethane (10 mL), as described for the preparation of **12**, gave 4-*O*-(4-*O*-benzoyl-2,3-di-*O*-methyl-α-L-fucopyranosyl)-2,3-di-*O*-methyl-α-L-fucopyranosyl chloride (**21**) as an amorphous powder (3.36 g, 95%), $[\alpha]_D^{26}$ −276° (*c* 3.5, dichloromethane), which was used in the glycosylation step without purification. ^{1}H-N.m.r. data: δ 8.18–7.42 (m, 5 H, Ph), 6.37 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 3.55, 3.53, 3.49, and 3.44 (4 s, each 3 H, 4 OMe), and 1.42 and 1.19 (2 d, each 3 H, $J_{5,6} = J_{5',6'} = 6.5$ Hz, 2 Me).

A solution of **21** (3.08 g, 6.3 mmol) in dry toluene (20 mL) was added drop-

wise to a stirred mixture of **32** (2.48 g, 3.1 mmol), silver triflate (1.94 g, 7.6 mmol), 2,4,6-trimethylpyridine (1 mL, 7.6 mmol), and molecular sieve Type 4A (5 g) in toluene (30 mL) at −30°. After being stirred for 1 h at −20°, the mixture was allowed to warm gradually to room temperature, then stirred for 1 h, and filtered, and the solids were washed with ether. The combined filtrate and washings were washed successively with water, cold M hydrochloric acid, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. Column chromatography (solvent *2*), of the residue gave **33** as an amorphous powder (3.05 g, 78%), $[\alpha]_D^{26}$ −75° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 8.25–7.23 (m, 30 H, 6 Ph), 3.46, 3.43, 3.24, and 2.75 (4 s, each 3 H, 4 OMe), and 1.43–1.00 (m, 12 H, 4 Me).

Anal. Calc. for $C_{70}H_{80}O_{20}$: C, 67.73; H, 6.50. Found: C, 68.01; H, 6.54.

O-(2,3-Di-O-methyl-α-L-fucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-fucopyranosyl)-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose (**36**). — A solution of **33** (2.50 g) in methanol (30 mL) and dichloromethane (10 mL) was boiled with methanolic M sodium methoxide (3 mL) for 2 h under reflux. Processing of the mixture, as described for the preparation of **7**, gave a syrup, which, on column chromatography (solvent *11*), gave benzyl *O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-6-deoxy-α-L-talopyranoside (**34**) as an amorphous powder (1.72 g, 92%), $[\alpha]_D^{27}$ −176° (*c* 0.8, chloroform).

Hydrogenolysis of **34** (1.02 g), using the procedure described above, and column chromatography (solvent *10*) of the product, afforded **36** as an amorphous solid (0.62 g, 86%), $[\alpha]_D^{26}$ −177° (*c* 1.4, water).

Anal. Calc. for $C_{28}H_{50}O_{17}$: C, 51.06; H, 7.65. Found: C, 50.89; H, 7.78.

Benzyl O-(4-O-acetyl-2,3-di-O-methyl-α-L-fucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-fucopyranosyl)-(1→3)-O-(2,4-di-O-acetyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-O-benzyl-6-deoxy-α-L-talopyranoside (**35**). — Acetylation of **34** (0.43 g) gave **35** as an amorphous powder (0.46 g, 92%), $[\alpha]_D^{27}$ −125° (*c* 1.9, chloroform). ^{1}H-N.m.r. data: δ 7.47–7.18 (m, 15 H, 3 Ph), 3.47 (s, 3 H, OMe), 3.40 (s, 6 H, 2 OMe), 3.25 (s, 3 H, OMe), 2.13, 2.11, and 2.03 (3 s, each 3 H, 3 OAc), 1.32 (d, 3 H, $J_{5,6}$ 6.5 Hz, Me), 1.18 (d, $J_{5,6}$ 6.5 Hz, Me), and 1.09 (d, 6 H, each $J_{5,6}$ 6.5 Hz, 2 Me).

Anal. Calc. for $C_{55}H_{74}O_{20}$: C, 62.61; H, 7.07. Found: C, 62.82; H, 7.21.

O-(2,3-Di-O-methyl-α-L-fucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-fucopyranosyl)-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talitol (**37**). — Reduction of **36** (0.39 g) with sodium borohydride, as described above, and column chromatography (solvent *10*) of the product gave **37** as a hygroscopic, amorphous powder (0.34 g, 87%), $[\alpha]_D^{26}$ −179° (*c* 0.9, water). ^{1}H-N.m.r. data (270 MHz, D_2O): δ 5.45 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1 of α-L-Fuc*p*), 5.17 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1 of α-L-Fuc*p*), 4.94 (s, 1 H, H-1 of α-L-Rha*p*), 3.51 (s, 3 H, OMe), 3.47 (s, 6 H, 2 OMe), 3.43 (s, 3 H, OMe), 1.34 (d, 3 H, $J_{5,6}$ 6.9 Hz, Me), 1.31 (d, 3 H, $J_{5,6}$ 6.3 Hz, Me), and 1.24 (d, 6 H, each $J_{5,6}$ 6.3 Hz, 2 Me).

Benzyl O-(3,4-di-O-acetyl-2-O-methyl-α-L-fucopyranosyl)-(1→4)-O-(3-O-

benzyl-2-O-methyl-α-L-fucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-O-benzyl-6-deoxy-α-L-talopyranoside (**38**). — A solution of **28** (4.59 g) in dichloromethane (35 mL) and *N,N*-dimethylformamide (0.23 mL) was treated with a solution of oxalyl chloride (2.22 mL) in dichloromethane (10 mL), and processed as described previously, to give 3-*O*-benzyl-4-*O*-(3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl)-2-*O*-methyl-α-L-fucopyranosyl chloride (**29**) as an amorphous powder (4.42 g, 93%), $[\alpha]_D^{26}$ −260° (*c* 1.8, dichloromethane), which was used in the coupling reaction without purification. ^{1}H-N.m.r. data: δ 7.53–7.25 (m, 5 H, Ph), 6.31 (bs, 1 H, H-1), 3.55 and 3.48 (2 s, each 3 H, 2 OMe), 2.13 and 1.99 (2 s, each 3 H, 2 OAc), and 1.32 and 0.78 (2 d, each 3 H, $J_{5,6} = J_{5',6'}$ = 6.5 Hz, 2 Me).

A solution of **29** (4.31 g, 8.1 mmol) in toluene (30 mL) was added at −30° to a stirred mixture of **32** (3.20 g, 4.05 mmol), silver triflate (2.50 g, 9.7 mmol), and 2,4,6-trimethylpyridine (1.28 mL, 9.7 mmol) in toluene (40 mL). The mixture was processed as described for the preparation of **33**. Column chromatography (solvent *2*) of the product gave **38** as an amorphous powder (4.28 g, 82%), $[\alpha]_D^{26}$ −79° (*c* 1.9, chloroform). ^{1}H-N.m.r. data: δ 8.18–7.18 (m, 30 H, 6 Ph), 3.37, 2.74, 2.07, and 1.96 (4 s, each 3 H, 4 OMe), and 1.37, 1.18, 0.94, and 0.63 (4 d, each 3 H, each $J_{5,6}$ 6.5 Hz, 4 Me).

Anal. Calc. for $C_{72}H_{82}O_{21}$: C, 67.37; H, 6.44. Found: C, 67.55; H, 6.59.

O-*(2-O-Methyl-α-L-fucopyranosyl)-(1→4)-O-(2-O-methyl-α-L-fucopyranosyl)-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talose* (**41**). — A solution of **38** (3.97 g) in methanol (40 mL) and dichloromethane (20 mL) was treated with methanolic M sodium methoxide (4 mL) overnight at room temperature, and then processed as described for the preparation of **7**. Column chromatography (solvent *11*) of the product gave benzyl *O*-(2-*O*-methyl-α-L-fucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-methyl-α-L-fucopyranosyl)-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-6-deoxy-α-L-talopyranoside (**39**) as an amorphous powder (2.86 g, 93%), $[\alpha]_D^{20}$ −131° (*c* 0.8, chloroform).

Hydrogenolysis of **39** (1.50 g), followed by column chromatography (solvent *10*) of the product, gave **41** as an amorphous solid (0.84 g, 88%), $[\alpha]_D^{26}$ −176° (*c* 1.2, water).

Anal. Calc. for $C_{26}H_{46}O_{17}$: C, 49.52; H, 7.35. Found: C, 49.41; H, 7.50.

Benzyl O-*(3,4-di-O-acetyl-2-O-methyl-α-L-fucopyranosyl)-(1→4)-O-(3-O-benzyl-2-O-methyl-α-L-fucopyranosyl)-(1→3)-O-(2,4-di-O-acetyl-3-O-α-L-rhamno*pyranosyl)-(1→*2)-3,4-di-O-benzyl-6-deoxy-α-L-talopyranoside* (**40**). — Acetylation of **39** (0.52 g) gave **40** (0.56 g, 92%), m.p. 164–165° (from hexane–ether), $[\alpha]_D^{20}$ −131° (*c* 1.7, chloroform). ^{1}H-N.m.r. data: δ 7.43–7.17 (m, 20 H, 4 Ph), 3.43 and 3.32 (2 s, each 3 H, 2 OMe), 2.11, 2.05, 2.02, and 2.0 (4 s, each 3 H, 4 OAc), and 1.32, 1.13, 1.07, and 0.73 (4 d, each 3 H, each $J_{5,6}$ 6.5 Hz, 4 Me).

Anal. Calc. for $C_{62}H_{78}O_{21}$: C, 64.24; H, 6.78. Found: C, 64.33; H, 6.78.

O-*(2-O-Methyl-α-L-fucopyranosyl)-(1→4)-O-(2-O-methyl-α-L-fucopyranosyl)-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talitol* (**42**). — Reduction of

41 (0.35 g) with sodium borohydride and column chromatography (solvent *10*) of the product gave **42** as a hygroscopic, amorphous powder (0.32 g, 91%), $[\alpha]_D^{26}$ −174° (*c* 1, water). ^{1}H-N.m.r. data (270 MHz, D_2O): δ 5.44 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1 of α-L-Fuc*p*), 5.16 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1 of α-L-Fuc*p*), 4.94 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1 of α-L-Rha*p*), 3.53 and 3.49 (2 s, each 3 H, 2 OMe), 1.33 (d, 3 H, $J_{5,6}$ 6.9 Hz, Me), and 1.30, 1.24, and 1.19 (3 d, each 3 H, $J_{5,6}$ 6.6 Hz, 3 Me).

Trideuteriomethylation of tetrasaccharide-alditols **37** *and* **42**. — Each alditol (10 mg) was kept in 0.37M sodium methylsulfinylmethanide in methyl sulfoxide (0.8 mL) for 4 h, and trideuteriomethyl iodide (0.4 mL) was added dropwise to the cooled solution. After 4 h, the solution was partitioned between chloroform and water, and the organic layer was dried and concentrated. The resulting syrup was subjected to column chromatography (chloroform) on Sephadex LH-20 and silica gel. The resulting pertrideuteriomethylated oligosaccharide was successively hydrolyzed (0.5M sulfuric acid, 4 h, at 100°), reduced with sodium borohydride, and acetylated. Thus, **37** gave the acetates of 6-deoxy-1,3,4,5-tetra-*O*-trideuteriomethyltalitol, 2,3-di-*O*-methyl-4-*O*-trideuteriomethylfucitol, 2,3-di-*O*-methylfucitol, and 2,4-di-*O*-trideuteriomethylrhamnitol. In addition, a component of high retention time gave fragment ions consistent with those arising from reducing (*m/z* 223, 188, 163) and non-reducing units (*m/z* 217, 182, 146, 109, 62) of the disaccharide-alditol, *O*-(3-*O*-acetyl-2,4-di-*O*-trideuteriomethylrhamnopyranosyl)-(1→2)-6-deoxy-1,3,4,5-tetra-*O*-trideuteriomethyltalitol, formed on incomplete hydrolysis.

Likewise, **42** afforded the above products, with the exception that both fucitol derivatives carried a trideuteriomethyl group at O-3.

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