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SYNTHESIS OF OLIGOSACCHARIDES OF L-FUCOSE CONTAINING α- AND β-ANOMERIC CONFIGURATIONS IN THE SAME MOLECULE

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ABSTRACT

Some L-fucopyranosyl di-, tri-, and tetra-saccharides containing D-glucose and D-galactose have been synthesised. The use of mercuric cyanide and 2-*O*-benzyl-3,4-di-*O*-*p*-nitrobenzoyl-α-L-fucopyranosyl bromide gave α-L-fucopyranosides stereospecifically, but 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide gave mixtures with selectivity favouring the β anomer. A tetrasaccharide was prepared containing both α- and β-L-fucopyranosyl residues in the same molecule, as part of a structure occurring in some extracellular bacterial polysaccharides. The configuration and positions of substitution of fucopyranosyl residues were clearly shown by ^{1}H- and ^{13}C-n.m.r. data.

INTRODUCTION

Complex extracellular polysaccharides are produced by many Gram-negative bacteria[1]. Colanic acid produced by some *Escherichia coli* and *Salmonella* species contains a hexasaccharide repeating-unit with both α- and β-fucopyranosyl residues[2]. The β-L-fucopyranosyl linkage is extremely rare in Nature and raises interesting biochemical questions[3].

Earlier studies of the stereochemistry of the Koenigs–Knorr reaction led to methods for preparing both α- and β-L-fucopyranosides, so that controlled synthesis of such repeating structures should be feasible[4,5]. Various oligosaccharides containing both of these residues have now been prepared.

RESULTS AND DISCUSSION

Reaction of 2-*O*-benzyl-3,4-di-*O*-*p*-nitrobenzoyl-α-L-fucopyranosyl bromide[6] (**1**) with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**3**) in the presence of mercuric cyanide, followed by catalytic deacylation, gave 3-*O*-(2-*O*-benzyl-α-L-fucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**5**). The yield of **5** (from **1**) was 60% if excess of **3** was used. Since 40% of **3** was recovered and could be recycled, this approach involved the most economical use of the expensive bromide **1**.

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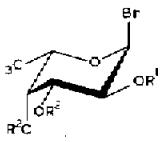
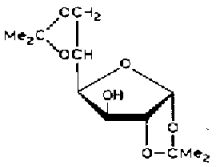


1 R = Bzl, R^2 = pNO$_2$Bz
2 R = R^2 = Ac



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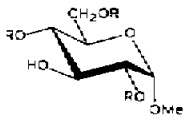
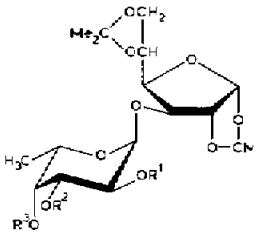
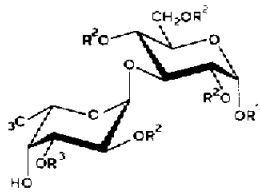


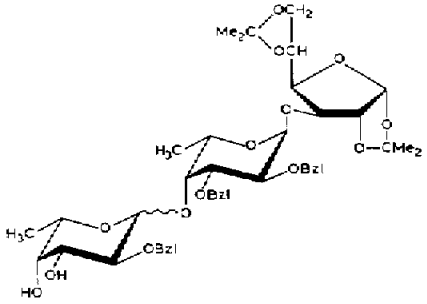
4 R = Bzl



5 R = Bzl, R^2 = R^3 = H
6 R = R^2 = Bzl, R^3 = H
7 R = R^3 = Bzl, R^2 = Ac



8 R^1 = Me, R^2 = Bzl, R^3 = H
9 R^1 = Me, R^2 = R^3 = Bzl
10 R^1 = Me, R^2 = R^3 = H
11 R^1 = R^2 = R^3 = H



12 ~ = α
13 ~ = β

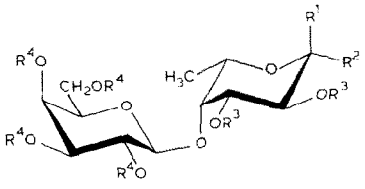
Treatment of **5** with approximately equimolar proportions of benzyl bromide and sodium hydride in *N*,*N*-dimethylformamide, followed by acetylation, gave the 3-acetate (**7**) and 4-acetate (**6**, R^3 = Ac) in the ratio of 3:2, which were characterised on the basis of their n.m.r. data.

Regiospecific benzylation of **5** was achieved by using the stannylidene complex[7], to give 58.5% of syrupy 3-*O*-(2,3-di-*O*-benzyl-α-L-fucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**) after purification *via* the 4-acetate. Catalytic hydrogenolysis of **5**, followed by selective acid hydrolysis, gave 3-*O*-α-L-fucopyranosyl-D-glucose (**11**).

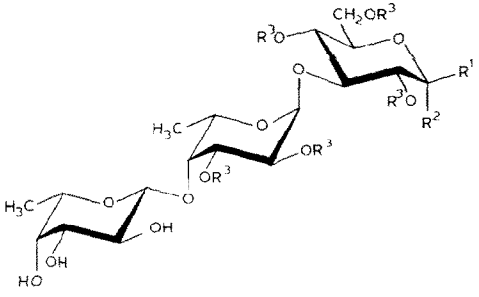
Reaction of methyl 2,4,6-tri-*O*-benzyl-α-D-glycopyranoside[8] (**4**) with **1** under Koenigs–Knorr conditions, followed by catalytic deacylation, afforded a 70% yield of methyl 2,4,6-tri-*O*-benzyl-3-*O*-(2-*O*-benzyl-α-L-fucopyranosyl)-α-D-glucopyranoside (**8**). The n.m.r. spectrum and optical rotation of **8** were consistent with the α-L-fucopyranosyl structure and, as in the synthesis of **5**, there was no evidence for the formation of the β anomer.

Benzylation of the stannylidene complex of **8** led to regiospecific substitution at HO-3 (equatorial), and 82% of the pentabenzyl ether **9** was obtained. The structure of **9** was established by comparison of its n.m.r. spectrum with that of its acetate, and confirmed by the fact that partial hydrolysis of **9** with acid gave **4** and 2,3-di-*O*-benzyl-L-fucose.

Condensation of **6** with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[9] (**2**) in the presence of mercuric cyanide did not proceed stereospecifically. In 1:1 nitromethane–benzene, the terminal α,β-L-Fuc*p* ratio (**12**:**13**) was 1:2, whereas, in acetonitrile, the reaction was much more stereoselective (α,β ratio, 1:9), although the total yield was rather lower. The trisaccharide derivatives **12** and **13** were isolated by column chromatography. Likewise, condensation of **9** with **2** in nitromethane–benzene, followed by deacylation and column chromatography, gave 46.5% of the β-L-Fuc*p*-substituted disaccharide derivative **19** and 11% of the α anomer. The 80-MHz n.m.r. spectrum of **19** was sufficiently well resolved to enable identification of both the α- and β-L-Fuc*p* residues present. Catalytic hydrogenolysis of **19** afforded crystalline methyl *O*-β-L-fucopyranosyl-(1→4)-*O*-α-L-fucopyranosyl-(1→3)-α-D-glucopyranoside (**20**).



14 R^1 = OMe, R^2 = R^4 = H, R^3 = Bzl
15 R^1 = OMe, R^2 = R^3 = R^4 = H
16 R^1 = OAc, R^2 = H, R^3 = R^4 = Ac
17 R^1 = Br, R^2 = H, R^3 = R^4 = OAc
18 R^1 = R^3 = R^4 = H, R^2 = OH



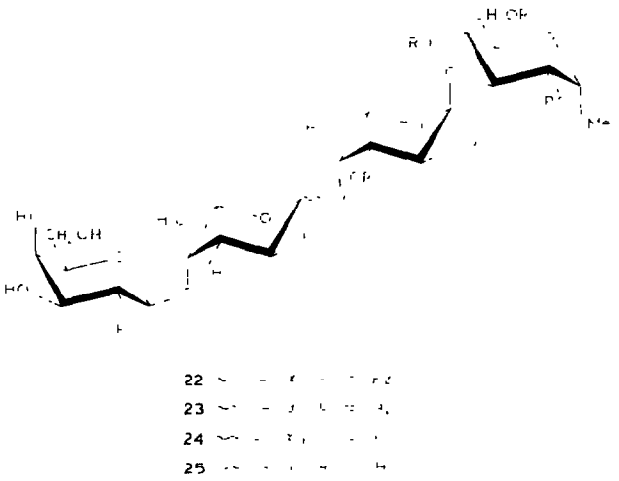
19 R^1 = H, R^2 = OMe, R^3 = Bzl
20 R^1 = R^3 = H, R^2 = OMe
21 R^1, R^2 = H,OH, R^3 = H

Methyl 2,3-di-*O*-benzyl-4-*O*-β-D-galactopyranosyl-α-L-fucopyranoside (**14**) was synthesised in >80% yield from methyl 2,3-di-*O*-benzyl-α-L-fucopyranoside and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide followed by catalytic deacetylation. The crystalline product was anomerically pure, and had the expected n.m.r. spectrum and sign of optical rotation. Acetylation of **14** followed by acetolysis gave 4-*O*-β-D-galactopyranosyl-L-fucose hepta-acetate (**16**) which contained ~90% of the α anomer, as indicated by the p.m.r. spectrum.

Condensation of **9** and the glycosyl bromide **17** (prepared from **16**), followed by catalytic deacetylation and column chromatography, gave methyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(α- and β-L-fucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**22**, 22%; and **23**, 52.5%). Both compounds were crystalline solids, and the p.m.r. data and optical rotations accorded with the structures assigned.

Catalytic debenzylation of **22** and **23** produced the methyl tetrasaccharides **24** and **25**, respectively, which could not be crystallised, and catalytic deacetylation of **16** afforded 4-*O*-β-D-galactopyranosyl-β-L-fucopyranose (**18**).

The reaction of the glycosyl bromide **1** (benzylated at position 2) with two different nucleophiles (**3** and **4**) proceeded stereospecifically and gave α-glycosides,



but the glycosyl bromides **2** and **17** (acetylated at position 2) gave β-glycosides. High-resolution p.m.r. spectra of some of these oligosaccharide pairs (**20** and its α anomer, **22** and **23**, **24** and **25**) showed the marked effect of anomeric differences upon the CMe signals, which can serve as markers. Anomeric configurations could generally be established on the basis of the H-1 signals of the fucopyranosyl residues. ^{13}C-N.m.r. spectra of some of the products, including the two tetrasaccharides (**24** and **25**), confirmed the structures ascribed, and showed quite clearly the anomeric configurations and positions of substitution of the glycopyranosyl residues (Table I).

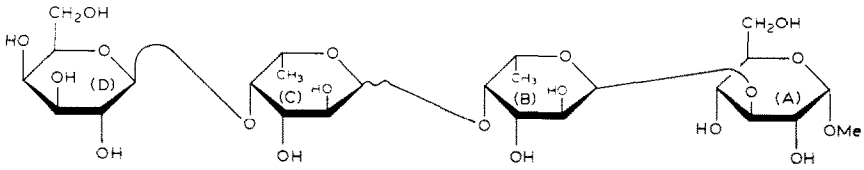
EXPERIMENTAL

For general methods, see ref. 10. ^{1}H-N.m.r. spectra were recorded with a Bruker HFX-10 (90 MHz) or WH-270 (270 MHz) spectrometer. ^{13}C-N.m.r. spectra were recorded for solutions in D_2O with a Bruker WH-270 spectrometer. Chemical shifts for ^{13}C-n.m.r. spectra are given relative to that of Me_4Si by using internal 1,4-dioxane (δ 67.4). When the solvent is not specified for n.m.r. spectra, $CDCl_3$ is implied. All optical rotations were determined at 24–25°.

3-O-(2-O-Benzyl-α-L-fucopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**5**). — To a stirred solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**3**; 4.5 g, 17.3 mmol) in 1:1 nitromethane–benzene (40 mL), dried by partial evaporation of the solvent, were added mercuric cyanide (3 g, 12 mmol) and 2-*O*-benzyl-3,4-di-*O*-*p*-nitrobenzoyl-α-L-fucopyranosyl bromide (**1**, 7.0 g, 11.4 mmol) in three portions during 18 h. After an additional 12 h at ambient temperature, the mixture was diluted with benzene, and the organic layer was washed successively with saturated, aqueous sodium hydrogencarbonate and water, dried, and concentrated. A solution of the residue in 1:2 chloroform–methanol was conventionally deacylated catalytically with sodium methoxide, concentrated to a small

TABLE I

^{13}C-N M R DATA[a] FOR L-FUCOPYRANOSIDES[b]



	10	15	20	24	25
Ring D					
C-1		104.3		104.2	104.1
C-2		72.1		72.1	72.4
C-3		73 4		73.3	73.1
C-4		69 5		69 4	68.8 or 69.5
C-5		76.0		75.9	75 9
C-6		61 8		61.8	61.9
Ring C					
C-1	100.3	100.4	105.2	101.3	105.2
C-2	68.9	69.5	71.1	68.9 or 69.2	72.2
C-3	70.4	69.8	74 0	69.6 or 69.8	73.2
C-4	72.4	81.3	72.4	81 1	79.8
C-5	67.6	67.5	71 7	68.1	71.9
C-6	16.0	16.1	16.4	15.9 or 16.0	16.4
Ring B					
C-1			100 2	100.3	100 2
C-2			68.9	68.9 or 69.2	68.8 or 69.5
C-3			69.7	69.6 or 69.8	69.7
C-4			82.5	81.5	82.7
C-5			67.3	68.0	67.2
C-6			16.1	15 9 or 16 0	16.0
Ring A					
C-1	100.0		100.1	100.1	100.0
C-2	72.4		72.2	72.4	72 5
C-3	81.0		81.0	81 1	81.0
C-4	69 2		69.7	70.2	70.2
C-5	72.7		72.4	72.4	72.9
C-6	61.4		61.4	61.4	61 4
OMe	55.8	56.0	55.8	55.8	55.8

[a]For solutions in D_2O; δ in p.p.m from the signal for Me_4Si (internal 1,4-dioxane, δ 67.4). [b]A, α-D-Glc*p*; B, α-L-Fuc*p*, C, α- or β-L-Fuc*p*; D, β-D-Gal*p*

volume, and diluted with chloroform, and the organic layer was washed twice with water. The aqueous washings were back-extracted four times with chloroform, and the combined organic layers were dried (Na_2SO_4) and concentrated. The residue was eluted from silica gel with benzene–ether (1:1), to give methyl *p*-nitrobenzoate and **3** (1.8 g, 40%). Elution with benzene–ether–methanol (14:14:1) then gave **5** (3.5 g; 60%, based on **1**), m.p. 169–171° (from ethanol), $[\alpha]_D$ −123.5° (*c* 0.77,

chloroform). N.m.r. data: δ 1.24 (3 H, *J* 7 Hz, H-6′), 1.27, 1.39, 1.47 (6 H), 4.92 (*J* 3 Hz, H-1 of α-L-Fuc*p*), 5.76 (*J* 3.6 Hz, H-1 of α-D-Glc*f*), and 7.34 (5 H).

Anal. Calc. for $C_{25}H_{36}O_{10}$: C, 60.47; H, 7.31. Found: C, 60.60; H, 7.18.

3-O-(2,3-Di-O-benzyl-α-L-fucopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**6**). — Sodium hydride (0.067 g, 1.4 mmol) was added to a stirred solution of **5** (0.72 g, 1.4 mmol) in *N*,*N*-dimethylformamide (20 mL) followed, after 2 h at ambient temperature, by benzyl bromide (0.17 mL, 1.4 mmol), and the mixture was stirred for a further 1 h. After the addition of methanol (1 mL) and water in excess, the mixture was extracted with toluene, and the organic layer was washed with water and concentrated. The residue was conventionally acetylated with acetic anhydride–pyridine, and the product was eluted from a column of silica gel with benzene–ether (9:1) to give, first and presumably, the 4-acetate of **6** (0.22 g, 24.3%), $[\alpha]_D$ −73°. N.m.r. data: δ 2.16 (*a*-OAc). Eluted second was, presumably, 3-*O*-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-fucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**7**, 0.30 g, 33.2%), $[\alpha]_D$ −80° (*c* 2.3, chloroform). N.m.r. data: δ 1.97 (*e*-OAc).

The former compound was obtained in better yield by regiospecific benzylation of the stannylidene complex of **5**. A solution of **5** (2.9 g, 5.65 mmol) was boiled under reflux with dibutyltin oxide (1.5 g, 5.9 mmol) in benzene (60 mL) for 4 h with azeotropic removal of water. After concentration of the solution to 35 mL and reduction of the temperature to 80°, tetrabutylammonium iodide (0.5 g) and benzyl bromide (1.4 mL, 11.5 mmol) were added, and the mixture was stirred overnight at 80°. T.l.c. (4:1 benzene–ether) then showed essentially one spot. After addition of aqueous sodium hydrogencarbonate, the organic layer was washed with water, dried, and concentrated. The residue was conventionally acetylated and the product was found to be the 4-acetate of **6**. Coloured contaminants were removed by column chromatography, and the product (2.3 g) was catalytically deacetylated to give amorphous **6** (2.0 g, 58.5%), $[\alpha]_D$ −70° (*c* 1, chloroform).

Anal. Calc. for $C_{35}H_{42}O_{10}$: C, 65.51; H, 7.22. Found: C, 65.35; H, 7.04.

3-O-α-L-Fucopyranosyl-D-glucose (**11**). — A solution of **5** (0.17 g) in aqueous 90% ethanol (50 mL) containing 10% Pd/C (0.2 g) was catalytically hydrogenolysed at 3 atm. overnight and the filtered solution, which showed one spot in t.l.c. (9:1 chloroform–methanol), was concentrated. The residue was dissolved in ethanol (5 mL), aqueous 0.5% sulfuric acid (5 mL) was added, and the solution was kept overnight at ambient temperature, neutralised ($BaCO_3$), filtered, and concentrated. A solution of the residue in chloroform–methanol–water (60:25:4) was added to a column of silica gel. Elution with 2-propanol–water (7:3) gave **11** (74 mg, 69%), $[\alpha]_D$ −47° (*c* 1.47, methanol).

Anal. Calc. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 44.04; H, 6.95.

Methyl 2,4,6-tri-O-benzyl-3-O-(2-O-benzyl-α-L-fucopyranosyl)-α-D-glucopyranoside (**8**). — Methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 4.4 g, 9.5 mmol), mercuric cyanide (2.0 g, 8 mmol), and **1** (5.0 g, 8.1 mmol) in nitromethane–benzene (30 mL) were allowed to react, as described above for **5**, for a total of 18

h, followed by work-up and catalytic deacylation. Column chromatography of the product gave **4** (1.6 g, 36.4%) and **8** (4.0 g, 70% calculated on **1**), which was eluted by benzene–ether (1:1). The product did not crystallise and had $[\alpha]_D$ −25° (*c* 1.4, chloroform). N.m.r. data: δ 0.85 (3 H, *J* 8 Hz, H-6′), 3.30 (3 H), 4.71 (*J* 4 Hz, H-1 of α-L-Fuc*p*), 5.75 (*J* 4 Hz, H-1 of α-Glc*p*), and 7.25–7.34 (20 H).

Anal. Calc. for $C_{41}H_{48}O_{10}$: C, 70.26; H, 6.90. Found: C, 70.52; H, 7.05.

Methyl 2,4,6-tri-O-benzyl-3-O-(2,3-di-O-benzyl-α-L-fucopyranosyl)-α-D-glucopyranoside (**9**). — Benzylation of **8** (6.6 g) in benzene *via* the stannylidene complex gave essentially a single product which, after purification by elution from silica gel with benzene–ether (4:1), was a syrup (6.1 g, 82%), $[\alpha]_D$ −28° (*c* 1.3, chloroform). N.m.r. data: δ 0.83 (*J* 8 Hz, H-6′), 3.30 (3 H), 5.70 (*J* 3.5 Hz), and 7.21–7.30 (25 H).

Anal. Calc. for $C_{48}H_{54}O_{10}$: C, 72.89; H, 6.88. Found: C, 72.60; H, 6.75.

Conventional acetylation of **9** gave a product with an n.m.r. signal at δ 2.14, presumably due to AcO-4(*a*).

3-O-[2,3-Di-O-benzyl-4-O-(α- and β-L-fucopyranosyl)-α-L-fucopyranosyl]-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**12** *and* **13**). — Compound **6** (1.0 g, 1.70 mmol), 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide (**2**; 0.60 g, 1.94 mmol, added in portions), and mercuric cyanide (0.50 g, 2 mmol) were allowed to react in 1:1 nitromethane–benzene (20 mL), as described for **5**, for 24 h. After work-up and catalytic deacetylation, extraction of the product with chloroform gave a three-component mixture (t.l.c.). Column chromatography (9:1 chloroform–methanol) gave **6** (0.3 g, 30%), followed by **13** (0.55 g, 44%) and **12** (0.25 g, 20%).

Compound **12** had $[\alpha]_D$ −106.5° (*c* 0.6, chloroform). N.m.r. data: δ 1.19–1.46 (18 H), 5.75 (H, *J* 3.5 Hz), and 7.25–7.32 (10 H).

Anal. Calc. for $C_{38}H_{52}O_{14}$: C, 62.28; H, 7.15. Found: C, 62.35; H, 7.40.

Compound **13** had $[\alpha]_D$ −30.5° (*c* 1.6, chloroform). N.m.r. data: δ 1.13–1.48 (18 H), 5.75 (H, *J* 3.5 Hz), and 7.26–7.29 (10 H).

Anal. Found: C, 62.28; H, 7.02.

Similar reaction of **6** (1.7 g) in acetonitrile gave **12** (0.10 g, 4.4%) and **13** (0.80 g, 35.6%), together with **6** (0.65 g, 38.2%).

Methyl 2,3-di-O-benzyl-4-O-β-D-galactopyranosyl-α-L-fucopyranoside (**14**). — Reaction of methyl 2,3-di-*O*-benzyl-α-L-fucopyranoside (13 g, 34.2 mmol), mercuric cyanide (13 g, 50 mmol), and tetra-*O*-acetyl-α-D-galactopyranosyl bromide (21.0 g, 51 mmol) in nitromethane–benzene (1:1) for 24 h, followed by catalytic deacetylation and column chromatography, gave a crystalline solid (15.5 g, 81%) eluted by chloroform–methanol (9:1). Recrystallisation from ethyl acetate–acetone gave **14**, m.p. 184–186°, $[\alpha]_D$ −86° (*c* 1.1, 95% ethanol). N.m.r. data [$(CD_3)_2SO$]: δ 1.27 (*J* 8 Hz, H-6), 3.70 (OMe), and 7.31 (10 H).

Anal. Calc. for $C_{27}H_{36}O_{10}$: C, 62.05; H, 6.95. Found: C, 62.00; H, 7.07.

Methyl 4-O-β-D-galactopyranosyl-α-L-fucopyranoside (**15**). — Catalytic hydrogenolysis of **14** (4.5 g), followed by the usual work-up, gave a solid that was precipitated in amorphous form (2.6 g, 89%) on cooling a hot solution in methanol–

ethyl acetate and had m.p. ~150°, $[\alpha]_D$ −103° (*c* 1.5, methanol). N.m.r. data: (270 MHz, D_2O): δ 1.21 (3 H, *J* 6.5 Hz, H-6), 3.29 (3 H), and 4.33 (H, *J* 6.5 Hz, H-1 of β-Gal*p*).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.11. Found: C, 45.62; H, 7.42.

*4-O-β-*D*-Galactopyranosyl-*L*-fucose 1,2,3,2',3',4',6'-hepta-acetate* (**16**). — Treatment of **15** (3.2 g) with acetic anhydride–pyridine overnight at ambient temperature gave a syrupy product, $[\alpha]_D$ −46.5° (*c* 1.7, chloroform). To a stirred solution of this product in 5:1 acetic anhydride–acetic acid (60 mL) at 5° was added 1:9 sulfuric acid–acetic anhydride (4 mL). The mixture was kept overnight at 5°, stirred with excess of cold aqueous sodium hydrogencarbonate for 2 h, and extracted with chloroform. The extract contained a product with R_{15} 0.8 (1:1 benzene–ether), and traces of **15**. Column chromatography (1:1 benzene–ether) gave **16** as a syrup (4.65 g, 80%), $[\alpha]_D$ −84°(*c* 2, dichloromethane). N.m.r. data: δ 1.26 (*J* 8 Hz, H-6), 1.98–2.17 (21 H, including 2.7 H at 2.10 presumably due to AcO-1*a* of Fuc*p*), and 6.8 (0.9 H, *J* 3 Hz, H-1 of α-acetate).

Anal. Calc. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.51; H, 5.76.

*Methyl 3-O-(4-O-β-*L*-fucopyranosyl-α-*L*-fucopyranosyl)-α-*D*-glucopyranoside* (**20**). — Reaction of **9** (1.9 g, 2.4 mmol), mercuric cyanide (0.8 g, 3.2 mmol), and **2** (1.5 g, 5 mmol) in 1:1 nitromethane–benzene (25 mL), as described above for **5**, followed by catalytic deacetylation and column chromatography, gave **9** (0.65 g, 34%), eluted with benzene–ether–methanol (14:14:1), chloroform–methanol (19:1) eluted a major product (1.05 g, 46.5%), $[\alpha]_D$ −36° (*c* 1, chloroform), followed by a minor product (0.25 g, 11%), $[\alpha]_D$ −86° (*c* 1.5, chloroform). N.m.r. data: major product, δ 0.78 (3 H, *J* 8 Hz), 1.22 (3 H, *J* 8 Hz), 3.30 (3 H), 4.05 (*J* 8 Hz, H-1 of β-L-Fuc*p*), 4.78 (*J* 3 Hz, H-1 of α-L-Fuc*p*), 5.65 (*J* 3 Hz, H-1 of α-D-Glc*p*), and 7.21–7.30 (25 H); minor product, δ 0.78 (3 H, *J* 8 Hz), 1.00 (3 H, *J* 8 Hz), 3.29 (3 H), and 7.21–7.30 (25 H).

The major fraction was hydrogenolysed in 95% ethanol over 10% Pd/C, and the product was purified by column chromatography. Chloroform–methanol–water (60:25:4) eluted a white solid (0.35 g, 80%) that crystallised from ethanol, to give **20**, m.p. 223–226°, $[\alpha]_D$ −24° (*c* 1, methanol). N.m.r. data: (270 MHz, D_2O): δ 1.14 (3 H, *J* 7 Hz), 1.195 (3 H, *J* 6.9 Hz), 3.33 (3 H), 4.47 (1 H, *J* 7.6, β-L-Fuc*p*), and 5.13 (1 H, *J* 3.8, α-L-Fuc*p*).

Anal. Calc. for $C_{19}H_{34}O_{14}$: C, 46.91; H, 7.04. Found: C, 46.68; H, 7.02.

*Methyl O-β-*D*-galactopyranosyl-(1→4)-O-(α- and β-*L*-fucopyranosyl)-(1→4)-O-(2,3-di-O-benzyl-α-*L*-fucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-α-*D*-glucopyranoside* (**22** *and* **23**). — A solution of **16** (4.0 g) in acetic acid (20 mL) and HBr–HOAc (45%, 20 mL) was kept at 5° for 30 min, and t.l.c. then showed that reaction was complete. The mixture was extracted with dichloromethane, and the extract was washed with aqueous sodium hydrogencarbonate and ice–water, and then concentrated. T.l.c. (1:1 benzene–ether) showed almost homogeneous **17** (4.3 g, 100%), $[\alpha]_D$ −141° (*c* 1, dichloromethane). Reaction of **17** (4.0 g, 6.2 mmol) with **9** (3.0 g, 3.8 mmol) and mercuric cyanide (1.5 g, 6 mmol) in 1:1 nitromethane–ben-

zene (35 mL) for 2 days, as described for **5**, followed by catalytic deacetylation and column chromatography (9:1 chloroform–methanol), gave **23** (2.7 g, 52.5%) followed by **22** (1.2 g, 22%). Both **22** and **23** crystallised from ethanol–ether.

Compound **22** had m.p. 178–180°, $[\alpha]_D$ −80° (*c* 0.9, methanol). N.m.r. data (270 MHz): δ 0.76 (3 H, *J* 7 Hz), 0.92 (3 H, *J* 6.5 Hz), 3.25 (3 H), 4.75 (H, *J* 3.5 Hz), 5.65 (H, *J* 3.5 Hz), and 7.10–7.40 (25 H).

Anal. Calc. for $C_{60}H_{79}O_{19}$: C, 65.26; H, 7.21. Found: C, 65.31; H, 7.00.

Compound **23** had m.p. 143–145°, $[\alpha]_D$ −40° (*c* 0.9 methanol). N.m.r. data (270 MHz): δ 0.75 (3 H, *J* 7 Hz), 1.26 (3 H, *J* 6.5 Hz), 3.25 (3 H), 4.10 (H, *J* 7.5 Hz), 5.65 (H, *J* 3.5 Hz), and 7.10–7.40 (25 H).

Anal. Found: C, 65.13; H, 6.92.

Methyl O-β-D-*galactopyranosyl*-(*1*→*4*)-O-α-L-*fucopyranosyl*-(*1*→*4*)-O-α-L-*fucopyranosyl*-(*1*→*3*)-α-D-*glucopyranoside* (**24**). — Hydrogenolysis of **22** (0.38 g) in 95% ethanol, as described above, gave essentially one product (0.15 g, 67%) which did not crystallise even after column chromatography (elution with 60:35:8 chloroform–methanol–water). It had $[\alpha]_D$ −75° (*c* 1.2, methanol). N.m.r. data (270 MHz, D_2O): δ 1.18 (6 H, *J* 6.5 Hz, H-6′,6″), 3.34 (3 H), 4.33 (H, *J* 7.1 Hz, H-1 of β-D-Gal*p*), 4.90 (H, *J* 3.5 Hz, H-1 of α-L-Fuc*p*), and 5.17 (H, *J* 3.8 Hz, H-1 of α-L-Fuc*p*).

Anal. Calc. for $C_{25}H_{44}O_{19}$: C, 46.29; H, 6.84. Found: C, 46.01; H, 6.95.

Methyl O-β-D-*galactopyranosyl*-(*1*→*4*)-O-β-L-*fucopyranosyl*-(*1*→*4*)-O-α-L-*fucopyranosyl*-(*1*→*3*)-α-D-*glucopyranoside* (**25**). — Similar treatment of **23** (1.0 g) afforded, after chromatography, a glass (0.50 g, 85%), R_{19} ~1.4 (60:35:8 chloroform–methanol–water), $[\alpha]_D$ −23° (*c* 1.1, methanol). N.m.r. data (270 MHz, D_2O): δ 1.14 (3 H, *J* 6.5 Hz), 1.25 (3 H, *J* 6.5 Hz), 3.33 (3 H), 4.13 (H, *J* 7, H-1 of β-L-Fuc*p*), 4.38 (H, *J* 7 Hz, H-1 of β-D-Gal*p*), and 5.14 (*J* 3.5 Hz, H-1 of α-L-Fuc*p*).

Anal. Calc. for $C_{25}H_{44}O_{19}$: C, 46.29; H, 6.84. Found: C, 45.95; H, 6.70.

Methyl 3-O-α-L-*fucopyranosyl*-α-D-*glucopyranoside* (**10**). — Hydrogenolysis of **8** (0.90 g) in 95% ethanol, as described above, gave a solid product (0.40 g, 92%) which was almost completely homogeneous in t.l.c. (4:1 chloroform–methanol). Recrystallisation from ethanol gave needles of **10**, m.p. 197–199°, $[\alpha]_D$ −20° (*c* 0.7, methanol). N.m.r. data (270 MHz, D_2O): δ 1.07 (3 H, *J* 6.5 Hz, H-6′), 3.32 (3 H), and 5.11 (H, *J* 3 Hz, H-1 of α-L-Fuc*p*).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.11. Found: C, 46.01; H, 7.05.

4-O-β-D-*Galactopyranosyl*-L-*fucose* (**18**). — Compound **16** (0.8 g) was catalytically deacetylated in methanol at 5° overnight. T.l.c. (60:35:8 chloroform–methanol–water) showed a single, slowly-migrating spot. The solution was stirred with excess of Dowex 50 (H^+) resin and, after filtration, concentrated, and the residue was recrystallised from ethanol containing a little methanol, to give needles of **18** (0.35 g, 83%), m.p. 182–184°, $[\alpha]_D$ −39° (5 min) → −48° (18 h) (*c* 1, water).

Anal. Calc. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 43.89; H, 6.85.

O-β-L-*Fucopyranosyl*-(*1*→*4*)-O-α-L-*fucopyranosyl*-(*1*→*3*)-D-*glucose* (**21**). —

Hydrogenolysis of **13** (0.50 g) in ethanol, as described above, followed by deacetalation of the residue in 1:1 1,4-dioxane–1% sulfuric acid (10 mL) at ambient temperature overnight, gave a product that was almost homogeneous in t.l.c. (60:25:4 chloroform–methanol–water). The solution was neutralised with $BaCO_3$, filtered, and concentrated, and the residue was purified by column chromatography, to give **21** (0.25 g, 78%), which did not crystallise and had $[\alpha]_D$ −60° (*c* 1.2, methanol). ^{13}C-N.m.r. data: δ 105.3, 74.1, 72.8, 72.6, 71.2, and 16.5 (assigned to α-L-Fuc*p*); 100.3, 82.6, 69.9, 69.2, 67.5, and 16.3 (assigned to 4-*O*-substituted α-L-Fuc*p*); typical α- and β-D-Glc*p* peaks were also present, including those at δ 93.0 and 96.6 (C-1 of α-D-Glc*p* and β-D-Glc*p*, respectively).

Anal. Calc. for $C_{18}H_{32}O_{14}$: C, 45.76; H, 6.83. Found: C, 45.38; H, 6.49.

Partial, acid hydrolysis of **9**. — A solution of **9** (0.47 g) in 1:1 1,4-dioxane–M H_2SO_4 (40 mL) was boiled under reflux for 3 h, cooled, neutralised ($BaCO_3$), and concentrated. The residue was subjected to chromatography (1:1 benzene–ether) to give, first, **4** (0.22 g, 83%), and then 2,3-di-*O*-benzyl-L-fucose (0.17 g, 83.5%) which was identical (t.l.c., optical rotation, p.m.r. spectrum) with an authentic specimen and clearly differentiated by these criteria from 2,4- and 3,4-di-*O*-benzyl-L-fucose[11].

REFERENCES

1 I. W. SUTHERLAND in I. W. SUTHERLAND (Ed.), *Surface Carbohydrates of the Prokaryotic Cell*, Academic Press, New York, 1977, pp. 27–96.
2 P. J. GAREGG, B. LINDBERG, T. ONN AND T. HOLME, *Acta Chem. Scand.*, 25 (1971) 1185–1194; P. J. GAREGG, B. LINDBERG, T. ONN AND I. W. SUTHERLAND, *ibid.*, 25 (1971) 2103–2108.
3 H. M. FLOWERS, *Adv. Carbohydr. Chem. Biochem.*, 39 (1981) 279–345.
4 H. M. FLOWERS, *Carbohydr. Res.*, 74 (1979) 177–185.
5 H. PAULSEN, *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 156–173.
6 M. DEJTER-JUSZYNSKI AND H. M. FLOWERS, *Carbohydr. Res.*, 23 (1972) 41–45.
7 C. AUGÉ, S. DAVID AND A. VEYRIÈRES, *Chem. Commun.*, (1976) 373–376.
8 S. S. KOTO, Y. TAKESE AND S. ZEN, *Bull. Chem. Soc. Jpn.*, 45 (1972) 291–293.
9 H. M. FLOWERS, A. LEVY AND N. SHARON, *Carbohydr. Res.*, 4 (1967) 189–195.
10 H. M. FLOWERS, *Carbohydr. Res.*, 18 (1971) 211–218.
11 M. DEJTER-JUSZYNSKI AND H. M. FLOWERS, *Carbohydr. Res.*, 28 (1973) 61–74.