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Note

Synthesis of *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-fucopyranosyl-L-threonine

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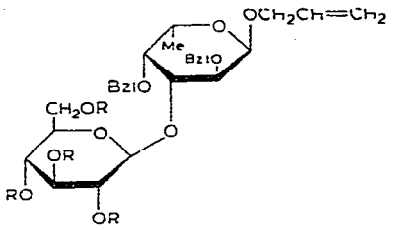
In 1974, Hallgren and his co-workers isolated a disaccharide amino acid from normal, human urine. On the basis of methylation analysis, optical rotation, and other work, the structure *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-fucopyranosyl-L-threonine (**13**) was proposed[1]. Fucose linked to threonine had not previously been found in Nature.

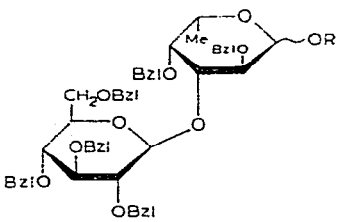
We now report on a confirmation of the assigned structure by an unambiguous synthesis of **13**. L-Fucose (**1**) was converted into allyl α-L-fucopyranoside[2] (**2**) in 48% yield by a modification of the Fischer glycosidation procedure. Partial benzylation of **2** with benzyl chloride and potassium hydroxide in *p*-dioxane–toluene afforded, in addition to the 2,3,4-tribenzyl ether, the 2,4-dibenzyl ether (**3**) and the 3,4-dibenzyl ether (**4**) in 30% and 20% yield, respectively. Partial benzylation of methyl α-L-fucopyranoside affords similar results[3]. The structures of **3** and **4** were demonstrated by methylation analysis. The two dibenzyl ethers were methylated, the benzyl groups were removed by hydrogenolysis, and the resulting mono-*O*-methylfucosides were converted into the corresponding mono-*O*-methylalditol acetates, which were identified by g.l.c.–m.s. as peracetylated 3-*O*-methylfucitol (from **3**) and 2-*O*-methylfucitol (from **4**), respectively.

Allyl 2,4-di-*O*-benzyl-α-L-fucopyranoside (**3**) was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in nitromethane using mercury(II) cyanide as promotor[3], giving the disaccharide glycoside **5** in a 70% yield. Deacetylation of **5** followed by benzylation of the product **6** with benzyl bromide and sodium hydride in *N*,*N*-dimethylformamide yielded **7** (87% yield from **5**). Isomerization of the allyl group in **7** with tris(triphenylphosphine)rhodium(I) chloride[4] and removal of the resulting prop-1-enyl group with mercury(II) chloride and mercury(II) oxide[5] produced the disaccharide derivative **8** (65% from **7**).

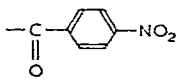
Treatment of **8** with *p*-nitrobenzoyl chloride and pyridine gave an anomeric mixture of 1-benzoates **9** and **10**, which was then treated with hydrogen bromide in dichloromethane at 0°. The resulting glucosyl-fucosyl bromide **11** decomposed immediately on contact with silica gel, and t.l.c. analysis thus gave spots correspond-

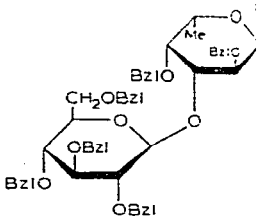


5 R = Ac
6 R = H
7 R = Bzl

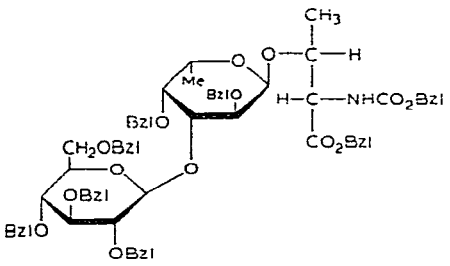


8 R = H

9, 10 R = 



11



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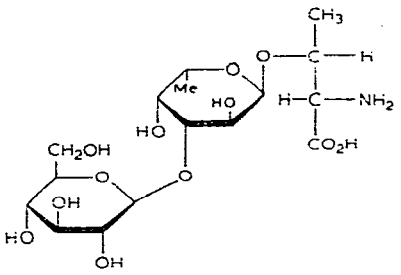

13

ing to the hydrolysis product **8** only. However, a 100-MHz p.m.r. spectrum indicated that **11** was sufficiently pure (>90%) to be used directly in the next step. Condensation of **11** with *N*-benzyloxycarbonyl-L-threonine benzyl ester[6] in the presence of tetraethylammonium bromide and molecular sieve[7] afforded, after chromatography, the protected glycosylfucosylthreonine **12** in a 38% yield. Catalytic hydrogenolysis of **12** yielded the title compound **13** in a 96% yield. Analysis of the product **13** showed the presence of threonine, fucose, and glucose in equimolar proportions. The p.m.r. spectrum of **13** was compatible with the assigned structure.

The synthetic *O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-fucopyranosyl-L-threonine (**13**) and the natural product[1] had the same chromatographic mobilities in ethyl acetate–acetic acid–water (3:1:1), propan-1-ol–ethyl acetate–water (6:1:3), and ethyl acetate–pyridine–water (10:4:3), as well as on Bio-Gel P-2 (200–400 mesh)[1]. The two compounds were indistinguishable in high-voltage electrophoresis[1]. Ninhydrin-degraded, permethylated **13** gave a mass spectrum identical to that given by the natural product treated similarly[1]. The optical rotation of **13** was −146° (water), whereas the value reported[1] for the natural product was −111°. The discrepancy is most probably due to the small amounts of the natural product available[1].

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed at reduced pressure and a bath temperature below 40°. Optical rotations were recorded at room temperature (22–24°) with a Perkin–Elmer 141 instrument. P.m.r. spectra were recorded with Varian A-60 A or XL-100 instruments. Analytical t.l.c. was performed on precoated plates (silica gel F_{254}, Merck). Sulfuric acid was used as spray reagent. Preparative separations were performed on prepacked columns of silica gel (Merck) or on manually packed columns of silica gel (Merck Kieselgel 60, particle size 0.040–0.063 mm). Paper chromatography was performed with Whatman No. 4 paper. Silver nitrate or ninhydrin was used for detection. G.l.c.–m.s. was performed with Perkin–Elmer 900 and 270 or Varian MAT 311 instruments, equipped with the appropriate columns for separation of components prior to recording mass spectra (ionization potential, 70 eV, ion-source temperature, 120°). Acid hydrolysis and subsequent determinations of sugars and amino acid were performed by the methods previously described for the natural product[1].

Allyl α-L-fucopyranoside (**2**). — L-Fucose (25 g) was suspended in dry allyl alcohol (80 ml). Methanol-washed Dowex-50(H^+) ion-exchange resin (12.5 g) was added. After being stirred at 70° for 4 h, the mixture was filtered while hot. On cooling, crystalline **2** (10.3 g) separated. Two repetitions of this procedure with the concentrated mother liquor and fresh allyl alcohol gave more material (8.1 g). One recrystallization from ethyl acetate containing a little methanol yielded **2** (15 g), m.p. 154–158°, $[\alpha]_D$ −190° (*c* 0.5, methanol); lit.[2] m.p. 158–160°, $[\alpha]_D$ −216° (water).

Allyl 2,4-di-O-benzyl-α-L-fucopyranoside (**3**) *and allyl 3,4-di-O-benzyl-α-L-fucopyranoside* (**4**). — Allyl α-L-fucopyranoside (**2**) (9 g) was dissolved in 1:1 *p*-dioxane–toluene (90 ml). Powdered potassium hydroxide (27 g) and benzyl chloride (72 ml) were added, and the mixture was stirred at 65–70° for 6 h. After dilution with toluene (500 ml), the mixture was washed repeatedly with water and then concentrated. After co-distilling several times with water, the material weighed 21 g. Chromatography on a column of silica gel (700 g), packed in toluene and eluted with a toluene–ethyl acetate gradient (up to 30%), gave first allyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranoside as a syrup (9.9 g), contaminated with non-carbohydrate material. The second fraction was **3** (5.10 g) which, when recrystallized from hexane, gave material with m.p. 86–87°, $[\alpha]_D$ −92° (*c* 0.5, chloroform).

Anal. Calc. for $C_{23}H_{28}O_5$: C, 71.9; H, 7.34. Found: C, 72.0; H, 7.23.

The third fraction (1.75 g) was a mixture of **3** and **4**. The fourth fraction (3.45 g) contained pure **4**; recrystallization from hexane gave material with m.p. 85–86°, $[\alpha]_D$ −126° (*c* 0.5, chloroform).

Anal. Calc. for $C_{23}H_{28}O_5$: C, 71.9; H, 7.34. Found: C, 72.0; H, 7.42.

For characterization, the two dibenzyl ethers **3** and **4** were methylated with methyl iodide–sodium methylsulfinylmethanide in methyl sulfoxide[8]. Debenzylation by catalytic hydrogenolysis gave two different monomethylated glycosides. Mass

spectrometry on the derived alditol acetates[9] showed them to be the 3- and 2-*O*-methyl derivatives, respectively.

*Allyl 2,4-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*L*-fucopyranoside* (**5**). — The dibenzyl ether **3** (2.69 g) was dissolved in dry nitromethane–benzene (1:1, 280 ml), and solvent (110 ml) was distilled off to ensure complete dryness. After cooling to room temperature, mercury(II) cyanide (2.52 g) and tetra-*O*-acetyl-α-D-glucopyranosyl bromide (3.77 g) were added, and the mixture was stirred at 40° for 20 h. Dilution with benzene (500 ml), washing with saturated, aqueous sodium hydrogen carbonate, drying, filtering, and evaporation gave a syrup (6.20 g) which crystallized on standing. Chromatography on a column of silica gel (600 g) with toluene–ethyl acetate (6:4) gave a chromatographically homogeneous syrup which crystallized. Recrystallization from ethyl acetate–hexane gave needles (3.50 g), m.p. 109–110°, $[\alpha]_D$ −46° (*c* 0.5, chloroform).

Anal. Calc. for $C_{37}H_{46}O_{14}$: C, 62.2; H, 6.49. Found: C, 62.3; H, 6.38.

P.m.r. data ($CDCl_3$): δ (downfield from tetramethylsilane) 7.36 (m, 10 H, Ph-*H*), 5.65–6.10 (octet, 1 H, OCH_2–C*H*=CH_2), 1.88 and 2.03 (2 s, 3 H and 9 H, O–COCH_3), and 1.12 (d, 3 H, $J_{5,6}$ 6.5 Hz, fucose H-6).

*Allyl 2,4-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-α-*L*-fucopyranoside* (**7**). — To a solution of **5** (2.40 g) in methanol (30 ml) was added methanol saturated with ammonia (18%, 6 ml), and the mixture was left overnight at room temperature. Concentration then gave **6** as a chromatographically homogeneous foam (1.92 g), which was taken up in dry *N*,*N*-dimethylformamide (30 ml). Sodium hydride (80% in oil; 0.61 g) was added, and the mixture was stirred at room temperature for 30 min. After the dropwise addition of freshly distilled benzyl bromide (4.8 ml), the mixture was stirred for 3 h. A little methanol was added to destroy the excess of sodium hydride, whereupon the reaction mixture was partitioned between chloroform (250 ml) and water (250 ml). The organic layer was washed twice with water and concentrated at 1 mmHg and 60°. Chromatography of the residue on a column of silica gel (300 g, packed in toluene) with a toluene–ethyl acetate gradient (up to 20%) gave pure **7** as a colourless syrup (2.67 g), $[\alpha]_D$ −26° (*c* 0.5, chloroform). P.m.r. data ($CDCl_3$): δ 7.15–7.35 (m, 30 H, Ph-*H*), 5.66–6.09 (octet, 1 H, OCH_2–C*H*=CH_2), and 1.05 (d, 3 H, $J_{5,6}$ 6.5 Hz, fucose H-6).

*2,4-Di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*L*-fucose* (**8**). — The perbenzylated allyl disaccharide **7** (2.10 g) in ethanol–benzene–water (40:16:5, 61 ml) was boiled under reflux with tris(triphenylphosphine)rhodium(I) chloride (31 mg) and diazabicyclo[2.2.2]octane (220 mg). After 2.5 h, only a trace of starting material remained (t.l.c.; toluene–ethyl acetate, 8:2). The mixture was diluted with water and extracted with ethyl ether. The organic layer was washed with saturated, aqueous potassium chloride, acidified to pH 2 with hydrochloric acid, washed again with aqueous potassium chloride, dried with anhydrous magnesium sulfate, and evaporated. The residual syrup was taken up in acetone–water (10:1, 30 ml). Mercury(II) oxide (630 mg) was added, and a solution of mercury(II) chloride (630 mg) in acetone–water (10:1, 10 ml) was added dropwise with stirring. The reaction was

instantaneous (t.l.c.). The mixture was filtered through Celite, concentrated, and extracted with ether. The organic layer was washed with half-saturated, aqueous potassium iodide, dried ($MgSO_4$), and evaporated. The residual syrup (1.84 g) was chromatographed on a prepacked column (size C) of silica gel. Toluene–ethyl acetate (6:4) eluted chromatographically pure **8** as a syrup (1.30 g), $[\alpha]_D -9°$ (*c* 0.5, chloroform).

2,4-Di-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α,β-L-fucopyranosyl p-nitrobenzoate (**9α**, **10β**). — The disaccharide **8** (1.22 g) was dissolved in dry, ethanol-free dichloromethane (25 ml) containing pyridine (0.79 ml). *p*-Nitrobenzoyl chloride (703 mg) was added with stirring at 0°, and the reaction mixture was then left overnight at room temperature. T.l.c. (toluene–ethyl acetate, 8:2) revealed two spots corresponding to the α- and β-*p*-nitrobenzoates. The reaction mixture was stirred with crushed ice for 5 min, diluted with dichloromethane, and washed with M hydrochloric acid, saturated, aqueous sodium hydrogen carbonate, and water. Drying ($MgSO_4$) and concentration gave a syrup (1.92 g), which was purified by chromatography on a prepacked column (size C) of silica gel. Toluene–ethyl acetate (85:15) first eluted **9** as a syrup (440 mg), $[\alpha]_D -42°$ (*c* 0.45, chloroform); then a fraction containing a mixture of **9** and **10** (247 mg); and finally pure **10** as a syrup (396 mg), $[\alpha]_D +27°$ (*c* 0.5, chloroform). P.m.r. data ($CDCl_3$): for **9**, δ 6.55 (d, 1 H, $J_{1,2}$ 4 Hz, fucose H-1); for **10**; δ 5.84 (d, 1 H, $J_{1,2}$ 7 Hz, fucose H-1).

N-Benzyloxycarbonyl-3-O-[2,4-di-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-L-fucopyranosyl]-L-threonine benzyl ester (**12**). — A mixture of the *p*-nitrobenzoates **9** and **10** (778 mg) in dry, ethanol-free dichloromethane (15 ml) was cooled to 0°. Dichloromethane (3 ml), saturated at room temperature with hydrogen bromide, was added and the reaction mixture was stirred at 0°. When no starting material remained (t.l.c., ~15 min), the reaction was interrupted by the addition of 2 g of molecular sieve (4Å), and the mixture was filtered. Concentration gave the bromo sugar **11** as a syrup, contaminated with solid *p*-nitrobenzoic acid. The 100-MHz p.m.r. spectrum in toluene showed a doublet (*J* 3.5 Hz) at δ 6.48, indicative of an α configuration. The crude, syrupy **11** was immediately dissolved in dry ethanol-free dichloromethane (2 ml), and the solution was filtered and added to a solution of *N*-benzyloxycarbonyl-L-threonine benzyl ester[6] (343 mg) and tetraethylammonium bromide (210 mg) in dichloromethane (8 ml) containing molecular sieve (1000 mg). The mixture was stirred at room temperature for 24 h, filtered through Celite, washed with water, saturated, aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and concentrated to give a syrup (750 mg), which was chromatographed on a column of silica gel. Elution with toluene–ethyl acetate (85:15) gave pure **12** as a colourless syrup (350 mg), $[\alpha]_D -28°$ (*c* 0.45, chloroform).

Anal. Calc. for $C_{73}H_{77}NO_{14}$: C, 73.5; H, 6.51; N, 1.17. Found: C, 73.5; H, 6.44; N, 1.17.

P.m.r. data ($CDCl_3$): δ 7.0–7.4 (m, 40 H, Ph-*H*), 6.51 (d, N*H*), 1.19 and 0.90 (2 d, each 3 H, each *J* 6 Hz, fucose and threonine CH_3).

O-β-D-Glucopyranosyl-(1→3)-O-α-L-fucopyranosyl-L-threonine (**13**). — The protected disaccharide amino acid **12** (125 mg) was dissolved in ethanol–water–acetic

acid (4:1:1, 36 ml) and hydrogenolysed with 10% palladium-on-carbon (300 mg) at 60 p.s.i. and room temperature for 5 h. The filtered solution was then concentrated, the residue was taken up in a little water, and the solution was filtered and lyophilized. The residue (43 mg), according to a 100-MHz p.m.r. spectrum, contained minor impurities only. Gel filtration on a column of Sephadex G-25 yielded pure material, $[\alpha]_D$ −146° (*c* 0.5, water). P.m.r. data (D_2O): δ [downfield from sodium 3-(trimethylsilyl)propanesulfonate] 5.06 (d, 1 H, $J_{1,2}$ 3 Hz, fucose H-1), 4.36–4.66 [m, 2 H, glucose H-1 ($J_{1,2}$ 7.5 Hz) and threonine O-C*H*(CH_3)-], 1.33 (d, 3 H, *J* 6.4 Hz, threonine CH_3), and 1.20 (d, 3 H, *J* 6.2 Hz, fucose CH_3). The latter two signals were differentiated by irradiating at the threonine O-C*H*(CH_3) signal.

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