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2′-FLUOROMALTOSE: SYNTHESIS AND PROPERTIES OF 4-*O*-(2-DEOXY-2-FLUORO-α-D-GLUCOPYRANOSYL)-D-GLUCOPYRANOSE, AND THE CRYSTAL STRUCTURE OF 2,3-DI-*O*-ACETYL-1,6-ANHYDRO-4-*O*-(3,4-TRI-*O*-ACETYL-2-DEOXY-2-FLUORO-α-D-GLUCOPYRANOSYL)-β-D-GLUCOPYRANOSE

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ABSTRACT

Coupling of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide and 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl bromide with 2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucose, under Koenigs–Knorr reaction conditions gave, in both instances, only the corresponding α-(1→4)-linked disaccharides. In the first case, the linkage was verified by ^{1}H- and ^{19}F-n.m.r., and by X-ray crystallographic analysis of 2,3-di-*O*-acetyl-1,6-anhydro-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl)-β-D-glucopyranose. Deprotection of this disaccharide gave 2′-deoxy-2′-fluoromaltose. Maltose and its fluorinated analogue displayed similar ^{1}H- and ^{13}C-n.m.r. spectra and optical rotations, indicating that these two sugars are isomorphous in solution and suggesting that 2′-deoxy-2′-fluoromaltose might be a suitable substrate for studies of α-glycosidases.

INTRODUCTION

It is widely assumed[1] that distortion of the pyranose ring, prior to carbonium ion formation, is a general feature of glycosidase action. While there is little experimental support for this assumption, it seems reasonable that Nature may use the same general mechanism established for lysozyme. The crystallographic studies by Phillips[2] on lysozyme supported earlier observations[3] that binding of a pyranose unit at subsite D of the enzyme required distortion of the chair conformation. Indeed, a transition-state analogue having a half-chair conformation has been shown to bind tightly to lysozyme and to act as a competitive inhibitor[4].

From an n.m.r. viewpoint, it would be expected that the change in dihedral

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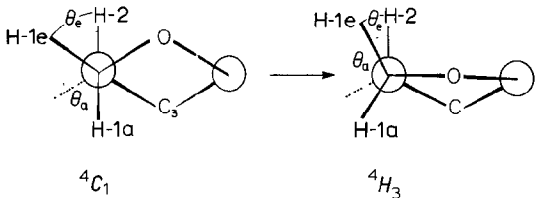
angle between H-1 and H-2, upon distortion of a pyranose ring, should alter the magnitude of $J_{1,2}$. The anomeric proton in the normal chair conformation [$^4C_1$(D)] will subtend a different dihedral angle with an axial proton at C-2 upon distortion to the half-chair [$^4H_3$(D)] conformation (Scheme 1). This approach has been employed in previous studies[5–7], but the experiments were complicated by either rapid hydrolysis of the substrate analogue, or by the inability to observe any changes in the relevant, but small, coupling constants.



$^4C_1$ $^4H_3$

Scheme 1

We have synthesized two 2′-deoxy-2-′-fluorodisaccharides as substrates suitable for n.m.r. studies of the type described here. The powerfully electron-withdrawing fluorine atom should significantly destabilize any carbonium-ion character in the transition state such that, while binding and distortion should occur, hydrolysis would not. This might allow direct observation of the substrate conformation upon binding to an enzyme.

EXPERIMENTAL

General methods. — N.m.r. spectra were recorded with a Varian XL-100 instrument, a 270 MHz spectrometer constructed at UBC with a Bruker console and an Oxford Instruments magnet, and at 400 MHz (100 MHz for ^{13}C) with a Bruker WH-400 spectrometer. Optical rotations were measured with either a Perkin–Elmer 141 or 249–MC polarimeter. High-resolution mass spectra were obtained with a Kratos/AEI MS-50 spectrometer (direct-probe inlet at 70 eV) and the low-resolution spectra with a Varian/MAT CHYB (direct-inlet probe at 70 eV) instrument. Melting points were measured in capillary tubes with a Thomas–Hoover Unimelt apparatus and are uncorrected. T.l.c. was performed on silica gel GF (Merck, 10 cm) and detection made by charring with H_2SO_4. Elemental analyses of all elements except fluorine were performed at UBC; fluorine analyses were performed by Analytische Laboratorien, Germany. Those products that were oils were evaporated in a rotary evaporator, with distillation of benzene from the residue, and then dried under high vacuum. After recrystallization, solids were dried under high vacuum over P_2O_5.

*Crystal-structure analysis of 2,3-di-*O*-acetyl-1,6-anhydro-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-*D*-glucopyranosyl)-β-*D*-glucopyranose.* — The space group was determined to be $P2_12_12_1$, from symmetry and systematic extinc-

tions observed on Weissenberg and precession photographs. A single crystal (0.35 $\times$0.40 $\times$ 0.25 mm) was aligned on a Picker FACS-I diffractometer with the b axis slightly offset from the diffractometer ϕ axis. Data were collected by using graphite-monochromated MoKα radiation ($\lambda K\alpha_1$ = 0.70926 Å). Least-squares refinement of 11 $2\theta > 30°$ values gave the following unit-cell dimensions: a = 8.839(5), b = 8.814(5), c = 32.721(17) Å. The observed density of 1.39 g cm^{-3}, measured by flotation, is in agreement with the calculated value of 1.398 g cm^{-3} based on four formula units of $C_{22}H_{29}FO_{14}$ per unit cell (temperature = 20°).

Intensity data were collected in two shells at room temperature using the $\theta - 2\theta$ scan technique. Reflections having $2\theta \leq 20°$ were measured with a scan width of 1.4° and an increment to allow for dispersion. Data were collected using stationary-counter, stationary-crystal background counts of 10 s on either side of the scan. Reflections having $20° < 2\theta < 40°$ were measured similarly with a scan width of 1.3° and the background counts of 20 s. In both instances the scan rate was 2°/min. Two standard reflections were monitored every 60 reflections. These intensities varied by only $\pm$1.4% during the course of data collection and no correction was made for crystal decay.

Estimated standard deviations were obtained from counting statistics using $\sigma^2(I) = [S + (t_s/t_b)^2B + (0.03\ I)^2]$ with S being the total scan count, B the total background count, t_s and t_b the scan and total background count-times, respectively, and I the net intensity. The 1295 reflections out of a total of 1419 independent reflections having net intensities $I > 2.3\sigma\ (I)$ were considered observed and used in the structure refinement. Lorentz and polarization corrections were applied to the intensities, but no correction was made for absorption. With a value of 1.31 cm^{-1} for the linear absorption coefficient a *maximum* error in F of $\pm$0.33% was estimated to result from the neglect of absorption effects.

The structure was solved by direct methods, using the 215 E values >1.35, in the computer program MULTAN. The set of phases having the highest combined figure of merit led to solution of the crystal structure. The initial E map gave the positions of 33 out of 37 non-hydrogen atoms; the remaining four atoms were located from a three-dimensional difference synthesis. The structure was refined by full-matrix, least-squares methods with isotropic temperature-parameters to a discrepancy factor $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ of 0.09. A three-dimensional difference map indicated the position of all hydrogen atoms. These were included in the structure-factor part of the calculations with isotropic temperature parameters 10% greater than the atoms to which they were bonded, but were not included in the refinement. Refinement with anisotropic temperature-factors reducted R to 0.042 and gave a weighted residual $R = [\Sigma w(|F_o| - |F_c|^2/\Sigma w|F_o|)^2]^{0.5}$ of 0.054. On the final cycle of least-squared refinement, no parameter shifted by more than 0.01.

A three-dimensional difference Fourier map revealed no peaks greater than 0.15 eÅ^{-3}. The least-square refinement was based on minimization of the function $\Sigma w(|F_o| - |F_c|)^2$. A final weighting scheme of the type $w = 1/\sigma(F_o)^2$ was used in the refinement and an analysis of average values of $w(|F_o| - |F_c|)^2$ showed no unusual

variation in terms of either F_o or sin θ/λ. Atomic scattering-factors for all non-hydrogen atoms were taken from ref. 8, while those for the hydrogen atoms were from ref. 9. No corrections were made for anomalous dispersion. The computer programs used in the structure determination and refinement have been noted elsewhere[10].

Final positional parameters are listed in Table I and interatomic distances and angles are given in Table II. Observed and calculated structure amplitudes and thermal parameters are listed in supplementary material*.

TABLE I

FRACTIONAL ATOMIC COORDINATES ($\times 10^4$) WITH ESTIMATED STANDARD DEVIATIONS IN PARENTHESES

Atom	*x*	*y*	*z*
F-2'	470(4)	−3688(5)	4147(1)
O-5'	2602(5)	−6133(5)	3490(1)
O-6'	4746(5)	−8431(6)	3650(1)
O-11'	6401(7)	−9559(7)	3273(2)
O-3'	2796(5)	−4689(5)	4675(1)
O-7'	3553(9)	−2309(8)	4769(2)
O-4'	5530(5)	−5432(5)	4245(1)
O-9'	5524(7)	−7500(7)	4645(2)
O-1'	1956(5)	−3610(5)	3421(1)
O-5	2759(5)	−1012(5)	3003(1)
O-1	948(5)	−53(5)	2593(1)
O-2	982(5)	−332(5)	3697(1)
O-7	−285(7)	+1754(7)	3842(2)
O-3	−1372(4)	−2004(5)	2939(1)
O-9	−2555(7)	−3918(8)	3250(2)
C-1'	1525(7)	−5010(7)	3593(2)
C-2'	1460(7)	−4873(7)	4053(2)
C-3'	2978(7)	−4561(7)	4243(2)
C-4'	4064(7)	−5789(7)	4105(2)
C-5'	4115(7)	−5801(7)	3638(2)
C-6'	5159(8)	−7022(7)	3468(2)
C-11'	5516(9)	−9621(10)	3547(3)
C-12'	5069(10)	−10993(8)	3764(2)
C-7'	3041(9)	−3452(10)	4903(2)
C-8'	2635(11)	−3736(10)	5337(2)
C-9'	6170(8)	−6438(11)	4503(3)
C-10'	7759(9)	−6012(11)	4600(2)
C-1	1533(7)	+10(7)	2994(2)
C-2	313(7)	−435(7)	3294(2)
C-3	−196(6)	−2057(7)	3235(2)
C-4	1025(7)	−3108(6)	3086(2)
C-5	2115(7)	−2285(8)	2796(2)
C-6	1319(8)	−1533(9)	2437(2)
C-7	555(10)	+773(9)	3941(2)
C-8	1299(9)	+686(9)	4340(2)
C-9	−2507(8)	−2990(11)	2985(3)
C-10	−3735(8)	−2734(11)	2683(3)

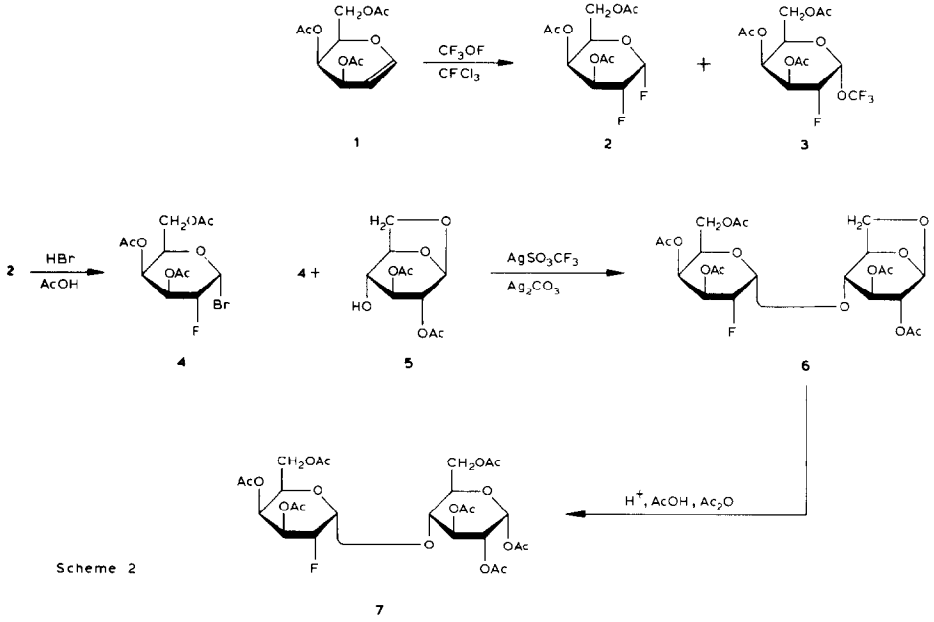
*The table of anisotropic thermal parameters may be obtained from Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to BBA/DD/285/*Carbohydr. Res.*, 132 (1984) 241–259.

TABLE II

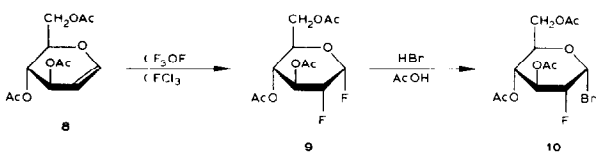
INTERATOMIC DISTANCES (Å) AND ANGLES (deg.) (STANDARD ERRORS IN PARENTHESES)

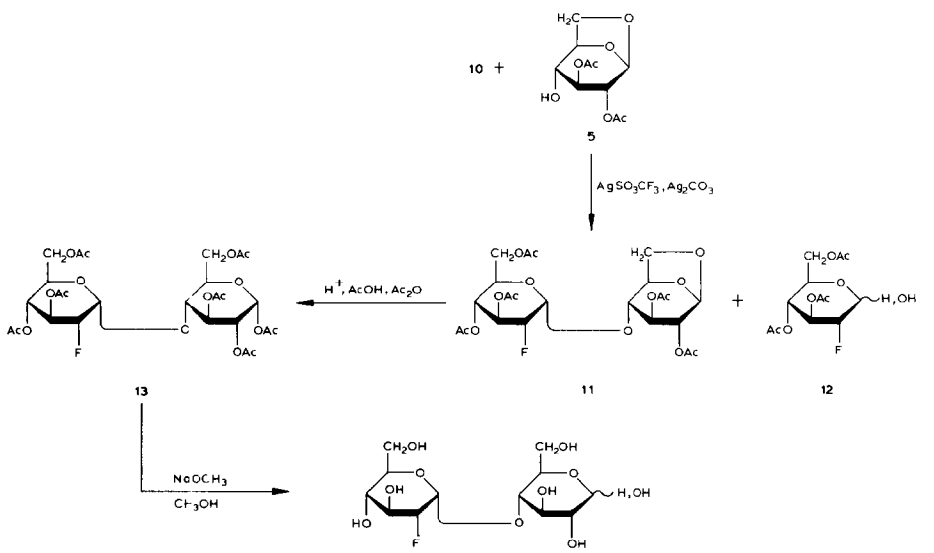
Bond	*Distance*	*Bond*	*Distance*
C-1′–C-2′	1.511(8)	C-11′–O-11′	1.191(9)
C-1′–O-5′	1.414(7)	O-1′–C-4	1.440(7)
C-1′–O-1′	1.409(7)	C-1–C-2	1.508(9)
C-2′–C-3′	1.504(9)	C-1–O-5	1.409(7)
C-2′–F-2′	1.397(7)	C-1–O-1	1.413(7)
C-3′–C-4′	1.516(8)	C-2–C-3	1.511(8)
C-3′–O-3′	1.427(7)	C-2–O-2	1.448(7)
O-3′–C-7′	1.338(9)	O-2–C-7	1.310(8)
C-7′–C-8′	1.487(10)	C-7–C-8	1.463(10)
C-7′–O-7′	1.187(8)	C-7–O-7	1.191(8)
C-4′–C-5′	1.532(8)	C-3–C-4	1.504(8)
C-4′–O-4′	1.410(7)	C-3–O-3	1.423(7)
O-4′–C-9′	1.348(9)	O-3–C-9	1.336(9)
C-9′–C-10′	1.487(11)	C-9–C-10	1.485(11)
C-9′–O-9′	1.191(9)	C-9–O-9	1.194(9)
C-5′–O-5′	1.452(7)	C-4–C-5	1.536(9)
C-5′–C-6′	1.523(8)	C-5–O-5	1.429(7)
C-6′–O-6′	1.425(8)	C-5–C-6	1.521(9)
O-6′–C-11′	1.295(8)	C-6–O-1	1.439(8)

Bond	*Angle*	*Bond*	*Angle*
O-5′–C-1′–C-2′	108.6(5)	C-1′–O-5′-C-5′	113.5(4)
O-5′–C-1′–O-1′	109.6(5)	C-1′–O-1′-C-4	114.7(5)
C-2′–C-1′–O-1′	109.8(5)	O-5–C-1–C-2	111.7(5)
C-1′–C-2′–C-3′	113.1(5)	O-5–C-1–O-1	105.9(5)
C-1′–C-2′–F-2′	107.6(5)	C-2–C-1–O-1	109.4(5)
C-3′–C-2′–F-2′	109.3(5)	C-1–C-2–C-3	112.2(5)
C-2′–C-3′–C-4′	108.2(5)	C-1–C-2–O-2	106.5(5)
C-2′–C-3′–O-3′	107.1(5)	C-3–C-2–O-2	107.2(5)
C-4′–C-3′–O-3′	108.0(5)	C-2–O-2–C-7	119.0(5)
C-3′–O-3′–C-7′	118.0(5)	O-2–C-7–C-8	112.4(7)
O-3′–C-7′–C-8′	110.9(7)	O-2–C-7–O-7	123.5(7)
O-3′–C-7′–O-7′	123.2(7)	O-7–C-7–C-8	124.0(7)
C-8′–C-7′–O-7′	125.9(8)	C-2–C-3–C-4	114.2(5)
C-3′–C-4′–C-5′	108.7(5)	C-2–C-3–O-3	105.8(5)
C-3′–C-4′–O-4′	109.1(5)	C-4–C-3–O-3	108.8(4)
C-5′–C-4′–O-4′	107.4(5)	C-3–O-3–C-9	116.8(6)
C-4′–O-4′–C-9′	116.2(5)	O-3–C-9–C-10	112.0(8)
O-4′–C-9′–C-10′	111.3(8)	O-3–C-9–O-9	123.7(7)
O-4′–C-9′–O-9′	124.0(6)	C-10–C-9–O-9	124.2(8)
C-10′–C-9′–O-9′	124.6(9)	C-3–C-4–O-1′	110.7(5)
C-4′–C-5′–O-5′	107.9(5)	C-3–C-4–C-5	111.1(5)
C-4′–C-5′–C-6′	112.8(5)	O-1–C-4–C-5	104.9(5)
O-5′–C-5′–C-6′	107.1(5)	C-4–C-5–C-6	113.2(5)
C-5′–C-6′–O-6′	108.0(5)	C-4–C-5–O-5	109.1(5)
C-6′–O-6′–C-11′	117.5(5)	C-6–C-5–O-5	102.0(5)
O-6′–C-11′–C-12′	113.7(7)	C-5–O-5–C-1	100.8(4)
O-6′–C-11′–O-11′	120.2(9)	C-5–C-6–O-1	103.1(5)
C-12′–C-11′–O-11′	125.7(8)	C-6–O-1–C-1	106.4(5)



Scheme 2





Scheme 3

3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl bromide (**4**). — Dry 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl fluoride (**2**, 200.2 mg, 0.645 mmol)[11,12] was dissolved in 30–32% HBr in AcOH (Eastman Kodak Co., 4 mL) and acetic anhydride (20 drops) was added. The solution was stirred overnight at room temperature in the dark. Argon was bubbled through the mixture for 2 h to purge HBr, and then toluene (5 mL) was added and the solution evaporated to dryness at 30°. Toluene (3 × 10 mL) was then evaporated from the resulting oil at the same temperature; 1:1 dichloromethane–benzene (10 mL) and decolorizing carbon (~500 mg) were added, and the mixture was stirred for 0.5 h. The mixture was filtered and the residue washed well with ethanol and dichloromethane to give a light-yellow filtrate. Drying the filtrate gave 236.7 mg (99.3%) of an oil that crystallized from ether–hexane, m.p. 79°, $[\alpha]_D^{21.9}$ +64.2° (*c* 2.6, $CHCl_3$). High-resolution m.s.; calc. for $C_{12}H_{16}BrFO_7$: 371.013; found: 371.011). This compound decomposed on keeping and was stored at 0°.

2,3-Di-O-acetyl-1,6-anhydro-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl)-β-D-glucopyranose (**6**). — 2,3-Di-*O*-acetyl-1,6-anhydro-β-D-glucose[13,14] (**5**, 492 mg, 2.0 mmol) in abs. ether (80 mL) was treated with silver carbonate (450 mg, prepared according to the method of Wolfrom *et al.*[15]), silver trifluoromethanesulfonate (140 mg), and Drierite (2.4 g). The mixture was stirred in a stoppered flask for 30 min at room temperature. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl bromide (**4**, 742 mg, 2.0 mmol) was added in one portion and the mixture stirred for 1 h. An additional 250 mg of **4** was then added and the stoppered mixture was stirred overnight at room temperature and filtered through Celite. The residue was washed with ether and dichloromethane, and the combined filtrates were evaporated and the residue was chromatographed on silica gel (140 g, Woelm, activity 2) with 7:4 (v/v) benzene–ethyl acetate to give 421 mg (39.2%) of **6**. T.l.c. of the mixture, before chromatography, on silica gel (1:1 benzene–ethyl acetate) showed four components: **4** (R_F 0.73), a side product (later identified as 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-galactose by the same procedure that was used for **12**); (R_F 0.53), **6** (R_F 0.43), and **5** (R_F 0.26). Column chromatography gave the same relative elution-pattern. Recrystallization from ethanol gave an analytical sample, m.p. 175–176°, $[\alpha]_D^{23.5}$ +53.7° (*c* 0.9, $CHCl_3$); $[\alpha]_D^{23}$ +54.5° (*c* 1.1, $CHCl_3$) for the 2′-OAc analogue[16].

Anal. Calc. for $C_{22}H_{29}FO_{14}$: C, 49.25; H, 5.45; F, 3.54. Found: C, 49.05; H, 5.26; F, 3.36.

1,2,3,6-Tetra-O-acetyl-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl)-α-D-glucopyranose (**7**). — Compound **6** (30 mg, 0.06 mmol) was stirred for 1 h at room temperature in an acetolysis solution (1.1 mL) composed of acetic anhydride (45 mL), acetic acid (15 mL), and sulfuric acid (1 mL). Fused anhydrous sodium acetate (300 mg, Fisher Scientific) was added and the solution was stirred for 10 min. Ice (20 g) was then added and the mixture stirred for another 30 min. The resulting solution was extracted with dichloromethane (3 × 100 mL) and the combined extracts were washed successively with water (100 mL), 2M $KHCO_3$ (100

mL), water (100 mL), and then dried over $MgSO_4$. The mixture was filtered and the filtrate taken to dryness to give an oil. Addition of abs. ethanol (1 mL) followed by cooling to 0° gave white crystals that were filtered off and washed with petroleum ether (b.p. 30–60°) and then dried to give 28 mg (73.0%) of the product **7** as the α anomer; m.p. 158–159°, $[\alpha]_D^{23.5}$ +101° (*c* 0.95, $CHCl_3$), lit.[16] $[\alpha]_D^{27}$ +117° (*c* 1.14, $CHCl_3$) for 2′-OAc analogue.

Anal. Calc. for $C_{26}H_{35}FO_{17}$: C, 48.00; H, 5.53; F, 2.98. Found: C, 48.71; H, 5.50; F, 2.81.

*3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-*D*-glucopyranosyl bromide*[17] (**10**). — Compound **9** (1.99 g, 6.42 mmol) was dissolved in 25 mL of 30–32% HBr in AcOH and acetic anhydride (1 mL) was added. The solution was stirred for 2 h at room temperature in the dark. Argon was bubbled through for 2 h to purge HBr and the solution was evaporated at 30°. Evaporation of toluene (4 × 20 mL) from the residue at the same temperature and further drying under high vacuum gave a tan-coloured oil. This product was dissolved in the minimum of ether, an equal volume of light petroleum ether was added, and the solution was allowed to crystallize at 0°. The resulting white crystals were filtered off, washed with light petroleum ether, and dried to give 2.17 g (91.0%) of pure product; m.p. 75–76° (lit.[17], 79–80°), $[\alpha]_D^{23.5}$ +209° (*c* 1.1, $CHCl_3$), (lit.[17] $[\alpha]_D^{25}$ +229° in 1–2% $CHCl_3$).

Anal. Calc. for $C_{12}H_{16}BrFO_7$: C, 38.83; H, 4.35; Br, 21.53; F, 5.12. Found: C, 38.84; H, 4.44; Br, 21.80; F, 5.26.

*2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-*D*-glucopyranosyl)-α-*D*-glucopyranose* (**11**). — Into a 35-mL dry round-bottom flask were introduced **5** (62.6 mg, 0.254 mmol), silver carbonate[15] (67.9 mg, 0.246 mmol), $AgSO_3CF_3$ (8.7 mg, 0.073 mmol), Drierite (309.6 mg, 2.28 mmol) (all dried for two days over P_2O_5 under high vacuum in the dark) and abs. ether (10 mL). The mixture was stirred at room temperature in a stoppered flask in the absence of light for 0.5 h and then 92.7 mg of **10** was added under the same conditions. After 20 min, an additional 29.9 mg was added (0.330 mmol total). The mixture was stirred overnight, filtered through a fine sintered-glass funnel, and the residue washed well with dichloromethane. T.l.c. of the filtrate showed three spots, as indicated next. The dried filtrate (272.5 mg) was dissolved in the minimum of dichloromethane and separated on silica gel (100 g, Woelm, activity 1.5) by elution with 7:4 benzene–ethyl acetate to yield 34.9 mg of a side product **12** (R_F 0.507), 82.8 mg of the disaccharide **11** (R_F 0.422), and starting material (R_F 0.273). The crude, oily **11** crystallized from ether. Recrystallization from dry ethanol gave 74 mg (54.4%) of product; m.p. 150–151°, $[\alpha]_D^{22.1}$ +49.4° (*c* 0.78, $CHCl_3$); $[\alpha]_D^{20}$ +48.9 (*c* 5.44, $CHCl_3$) for the 2′-OAc analogue[18].

Anal. Calc. for $C_{22}H_{29}FO_{14}$: C, 49.25; H, 5.45; F, 3.54. Found: C, 49.01; H, 5.43; F, 3.68.

The side product was purified on a column of silica gel (30 g, Woelm, activity 1.5) with 1:1 benzene–ethyl acetate and then dried. The resulting oil had $[\alpha]_D^{21.7}$ +100° (*c* 0.8, $CHCl_3$). High-resolution m.s.; calc. for $C_{12}H_{17}FO_8$: 308.090; found:

308.087. Synthesis of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucose[19] (**12**) (see text) gave a product having $[\alpha]_D^{21.9}$ +99.80° (*c* 0.9, $CHCl_3$), which behaved similarly to the side product in t.l.c. T.l.c. of the side product in the presence of a trace of acetic acid gave two spots, whose relative intensities in order of their elution rate was 2:1. Three columns were used in an attempt to separate these two components, without success (see Discussion). These columns and the relevant R_F values of the two components were: (*1*) 1:1:0.1 ether–petroleum ether (b.p. 30–60°)–AcOH at ambient temperature; R_F 0.455, 0.344; (*2*) as in (*1*) but at 4°; R_F 0.276, 0.191; and (*3*) 2:1 ether–petroleum ether (b.p. 30–60°)-trace of AcOH; R_F 0.612, 0.510.

*1,2,3,6-Tetra-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-*D*-glucopyranosyl)-α-*D*-glucopyranose* (**13**). — Dry **11** (70 mg, 0.130 mmol) was treated in a manner identical to that for the production of **7** from **6** to give 50 mg (60%) of **13** as the α anomer; m.p. 148.5–149.5°, $[\alpha]_D^{21.7}$ +107° (*c* 0.8, $CHCl_3$); lit.[20] $[\alpha]_D^{20}$ +122.8° (*c* 5.01, $CHCl_3$) for the 2′-OAc analogue.

Anal. Calc. for $C_{26}H_{35}FO_{17}$: C, 48.90; H, 5.53; F, 2.98. Found: C, 48.65; H, 5.33; F, 3.14.

4-O-*(2-Deoxy-2-fluoro-α-D-glucopyranosyl)-D-glucopyranose* (**14**) (*2′-deoxy-2′-fluoromaltose).* — Dry **13** (34.4 mg, 0.054 mmol) was dissolved in abs. methanol (4.9 mL) under a stream of N_2. The solution was then syringed into a flask under a positive pressure of N_2. Methanolic $NaOCH_3$ (M, 0.1 mL, freshly prepared) was then added similarly and the solution stirred for 3 h at room temperature. A small amount of charcoal (500 mg) was added with stirring and then Amberlite IR-120 (H, 300 mg) resin was added to remove Na^+ ions. When the mixture reached neutrality (as indicated by pH paper), it was filtered through a fine-glass filter funnel and the residue washed well with methanol. Evaporation of the filtrate gave 18.42 mg of the product (99.5%) as a transparent glass that did not crystallize; $[\alpha]_D^{23.5}$ +109° (*c* 0.9, pyridine, 20 h). The $[\alpha]_D^{21}$ value for D(+)-maltose (Nutritional Biochemicals Corporation) was +106° (*c* 1.12, pyridine, 20 h).

RESULTS

Lactose (4-*O*-β-galactopyranosyl-D-glucopyranose) is the substrate for the widely studied enzyme β-D-galactosidase[21] and we chose 2′-deoxy-2′-fluorolactose as our first synthetic target. Treatment of 3,4,6-tri-*O*-acetyl-D-galactal (**1**) with trifluoromethyl hypofluorite (CF_3OF) gave a mixture of the corresponding epimeric 2-fluoro sugars[11,12], two of which are shown in Scheme 2. Chromatography on dry kieselgel gave 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl fluoride (**2**) and the corresponding trifluoromethyl galactoside **3**. These fluoro sugars were subsequently converted into the galactosyl bromide **4**, which was in turn treated under Koenigs–Knorr conditions with 2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose[13,14] (**5**). The only disaccharide isolated from this reaction under the conditions employed was the α-(1→4)-linked disaccharide **6**. The nature of the α link-

TABLE III

COUPLING CONSTANTS (Hz) AND ^{19}F CHEMICAL SHIFTS FOR DISACCHARIDES AS DETERMINED FROM $^{1}H^{a}$ AND $^{19}F^{b}$ SPECTRA

Compound[c]	*F-2'*	*H-1',H-2'*	*H-1',F-2'*	*H-2',F-2'*	*H-2',H-3'*	*H-3',H-4'*	*H-3',F-2'*	*H-4',F-2'*	*H-4',H-5'*	*Solvent*
6	(208.0)	4.0	0.0 (0.0)	49.5 (48.8)	10.5[d]	3.4	11.8 (9.3)	3.5 (2.8)	1.13[e]	$CDCl_3$
6	(206.1)		(0.0)	(47.5)			(11.8)	2.4		Me_2SO-d_6
7	(207.1)	4.0	0.0 (0.0)	48.5 (49.8)	9.5	3.5	11.8 (9.9)	3.5 (2.4)	1.20	$CDCl_3$
7 (198.0, 198.1)[f]	(198.2, 198.4)		(0.0)	(49.2)			(13.1)			Me_2SO-d_6
11	(200.9)	3.9	0.0 (0.0)	49.0 (48.8)	9.5	9.5	11.8 (11.5)	0.0	9.8	$CDCl_3$
11	(200.2)		(0.0)	(48.9)			(12.0)			Me_2SO-d_6
13	(195.5)	3.8	0.0 (0.0)	49.5 (48.8)	9.6	9.5	11.5 (11.4)	0.0	9.5	$CDCl_3$
13	(199.5)		(0.0)	(48.4)			(11.6)			Me_2SO-d_6
14 (197.2, 197.3)[f]	(197.0, 197.1)	4.0	(0.0)	49.0 (49.0)	9.6		[i] (13.1)			D_2O[g]
14 (198.0, 198.1)[f]	(198.2, 198.4)	3.8	0.0 (0.0)	49.1 (49.0)	9.5	9.5	13.2 (13.4)	0.0	9.5	Me_2SO-d_6[g]
14		4.0		49.6	9.6		[j]			D_2O[h]

[a]Recorded at 100 MHz. [b]Recorded at 94.1 MHz. [c]Chemical shifts in p.p.m. upfield from $CFCl_3$. [d]Average from measurements at H-2' and H-3'. [e]Average from measurements at H-4' and H-5'. [f]Chemical shifts measured from undecoupled spectra. [g]Protons recorded at 270 MHz. [h]Protons recorded at 400 MHz. [i]Unresolved. [j]Unassigned.

age was indicated by the ^{1}H- and ^{19}F-n.m.r. spectra (Table III) on the basis of the $J_{1',2'}$ values. In addition, the specific rotation of **6** is close to that of the corresponding non-fluorinated 2′-OAc analogue (see Experimental). Opening of the anhydride of **6** gave **7**, which again exhibited a similar rotation to that of the corresponding nonfluorinated 2′-OAc disaccharide.

That the α-linked disaccharide **6** was the only product obtained posed two problems. The first was that the α-linkage gave a compound that is not a derivative of lactose (which is β-linked). Secondly, the mechanism whereby the α linkage became the sole product was not apparent. Our objectives were subsequently pursued by coupling 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (**10**) with the anhydroglucose derivative **5**, as the formation of an α-linked disaccharide would give a derivative of maltose, whereas the β-linked disaccharide would be a derivative of cellobiose, and either of these disaccharides would be suitable for enzymic studies of the types already described.

Treatment of 3,4,6-tri-*O*-acetyl-D-glucal (**8**) with CF_3OF gave products containing 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl fluoride[22] (**9**) which was converted into the bromide **10**, as indicated in Scheme 3. Coupling of **10** with the anhydro sugar **5** gave, as the only isolable disaccharide, compound **11**. Analysis of the $J_{1',2'}$ values of **11** (Table III) indicated that once again an α-(1→4) glycosidic

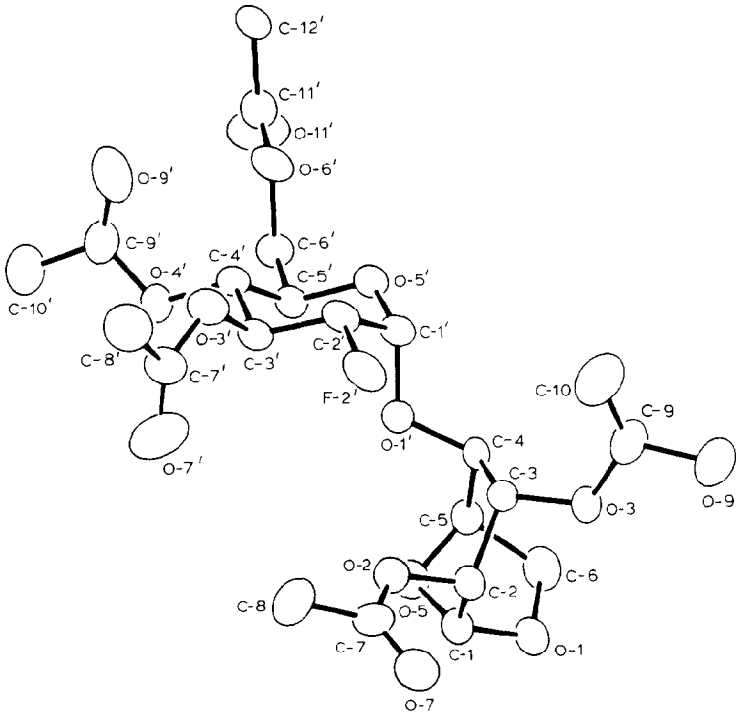


Fig. 1. X-Ray crystal structure of 2,3-di-*O*-acetyl-1,6-anhydro-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl)-β-D-glucopyranose (**11**).

linkage had been formed. As the apparently exclusive formation of an α linkage posed some mechanistic questions, we subjected **11** to X-ray crystallographic analysis to ensure that an α linkage had indeed been formed.

The crystal structure and atom labelling for 2,3-di-*O*-acetyl-1,6-anhydro-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl)-β-D-glucopyranose (**11**) are shown in Fig. 1 and outlined in Tables I and II. The bond lengths (Table II) are in good agreement with those observed for α-D-glucose[23] and 1,6-anhydro-β-D-glucopyranose[24]. The glucopyranose ring has the acetoxyl substitutents equatorially disposed at C-2′, C-3′, C-4′, and C-5′, and the anhydro moiety has the pyranose ring in the $^1C_4$(D) conformation with the acetoxyl substituents at C-2 and C-3 in axial dispositions. Of special note here, and confirming the results already discussed, the disaccharide linkage has the α configuration.

The anhydro bridge across the pyranose ring forms a seven-membered ring having a boat conformation, as observed in the structure of 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose[25] and 1,6-anhydro-β-D-glucopyranose[24]. In the latter case, energy calculations indicated that this conformation is ~1 kcal. mol^{-1} more favorable than an alternative conformer having the pyranose ring in the boat form and the seven-membered ring in the chair form. The bridge distorts the pyranose ring, resulting in compression of the C-1–O-5–C-5 angle to 100.8(5)° and extension

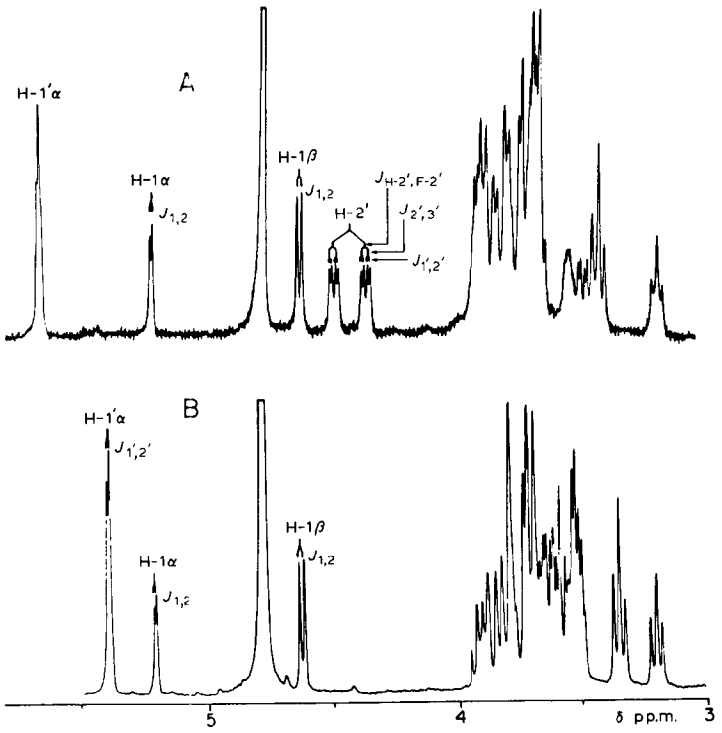


Fig. 2. 400-MHz ^{1}H-N.m.r. spectra in D_2O. A, 2′-deoxy-2′-fluoromaltose (**14**); B, maltose.

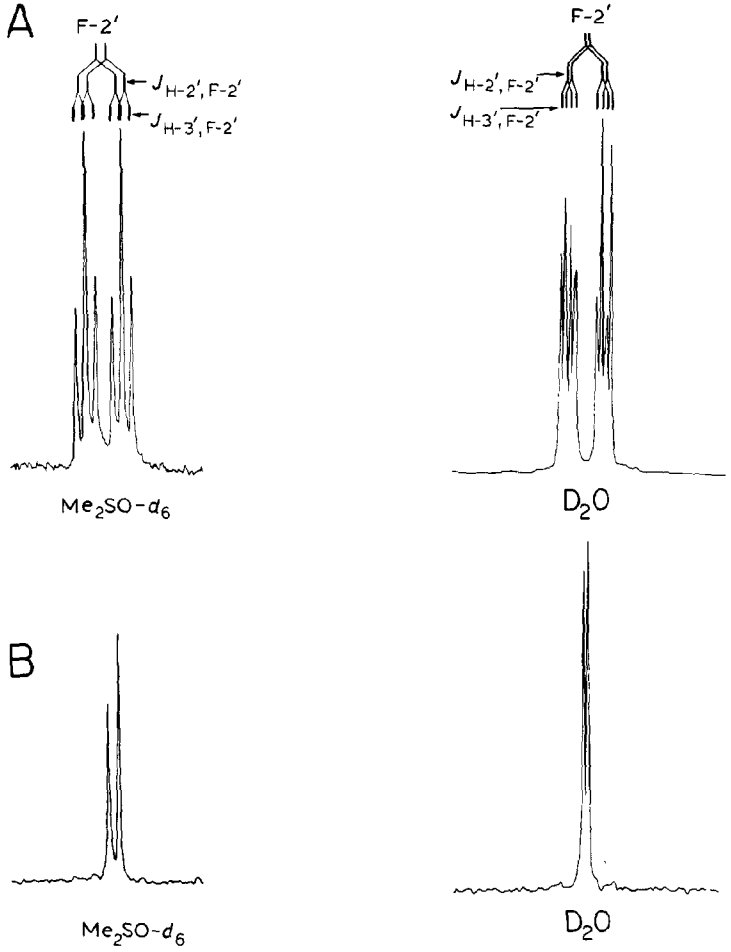


Fig. 3. 94.1-MHz ^{19}F-N.m.r. spectra of **14** (signals are upfield of $CFCl_3$); A, ^{1}H-coupled spectra; B, ^{1}H-decoupled spectra.

of the C-2–C-3–C-4 angle to 114.2(5)° as compared with the corresponding values of 113.5(4)° and 108.2(5)° for the unbridged pyranose ring. The extent of the distortion may also be observed by comparison of the dihedral angles for the six-membered rings. These dihedral angles are given in Table II, together with other selected dihedral angles.

The five-membered ring formed by the anhydro bridge has an envelope conformation; the phase angle of pseudorotation, Δ, of 176° being very close to the envelope value of 180°, as compared with a value of 144° for a half-chair conformation. Atom O-5 is 0.64 Å from the least-squares plane defined by the other four atoms of the ring and the flap angle is 44.7°.

Having confirmed that an α linkage had been generated, the anhydro sugar

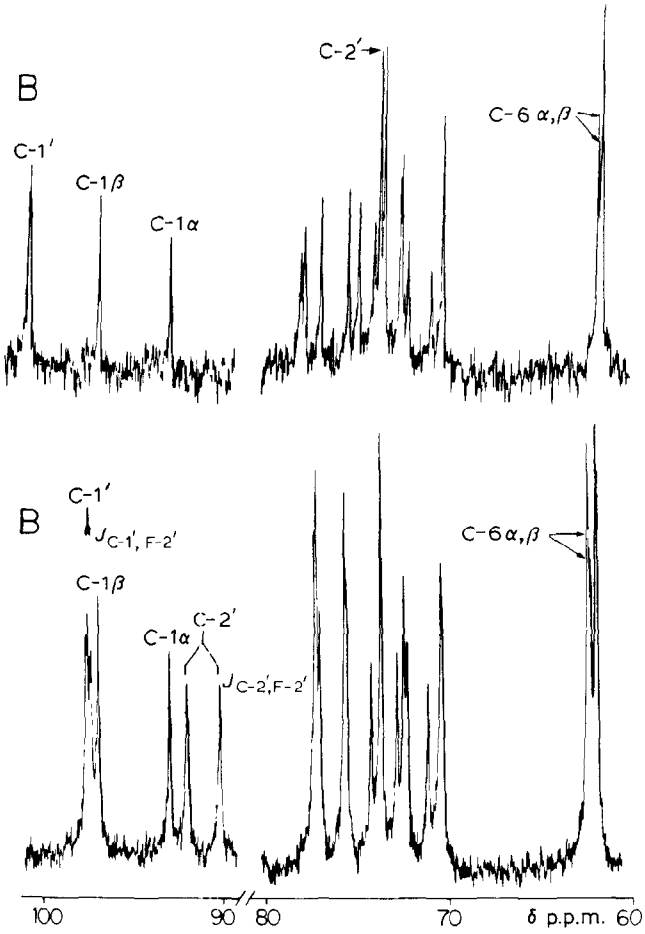


Fig. 4. 100.62-MHz ^{13}C-N.m.r. spectra in D_2O; A, maltose; B, 2'-deoxy-2'-fluoromaltose (**14**).

was opened to give the heptaacetate (**13**) of 2'-deoxy-2'-fluoromaltose, which was deacetylated to give the syrupy 2'-deoxy-2'-fluoromaltose (**14**) (Scheme 3). The ^{1}H-n.m.r. spectrum of **14** (Table III, Fig. 2) is consistent with the assigned structure. The ^{19}F-n.m.r. spectrum of **14** (Fig. 3) showed two closely spaced resonances (at 198.2 and 198.4 p.p.m.) indicating two species in solution. Acetylation of **14** in acetic anhydride and pyridine gave a mixture of two compounds identical on t.l.c. with the α and β anomers of the peracetate **13** from which **14** had originally been prepared, indicating that no decomposition had occurred in the original deprotection step; compound **14** thus displayed inherently two different fluorine resonances. In addition, acetyl resonances were not detected in either the ^{1}H or ^{13}C spectra of **14**, indicating that complete deprotection had occurred (Fig. 2, 4; tables IV and V).

TABLE IV

^{1}H-CHEMICAL-SHIFT[a] ASSIGNMENTS FOR THE DISACCHARIDES

Compound	*MHz*	*OAc*[b]	*H-1'*	*H-2'*	*H-3'*	*H-4'*	*H-5'*	*H-6',6''*	*H-1*	*Solvent*
6	100	(6) 1.98 (9) 2.08	5.37	4.72	5.43	5.47	4.45	4.04		$CDCl_3$
7	100	(21) 2.01	5.26	4.73	5.40	5.49			6.27	$CDCl_3$
11	100	(9) 2.00 (6) 2.07	5.32	4.48	5.58	4.98	4.09–4.41			$CDCl_3$
13	100	(21) 2.04–2.30	5.31	4.53	5.51	5.04			6.32	$CDCl_3$
14	270		4.98	4.10	4.56	4.30				Me_2SO-d_6
14	400		5.67	4.43						D_2O
			H-1	*H-2*[c]	*H-3*[c]	*H-4*[c]	*H-5*	*H-6exo*	*H-6'endo*	
6	100		5.46	4.60	4.82	3.58	4.72	3.80	4.02	$CDCl_3$
11	100		5.44	4.56	4.80	3.54	4.68	3.76	3.99	$CDCl_3$

[a]Shifts (δ) in p.p.m. are relative to external Me_4Si. [b]Numbers in parentheses are integrated protons. [c]Tentative assignments.

We conclude that the two resonances in the ^{19}F spectrum (Fig. 3) originate from the α and β anomers of **14**. This conclusion is substantiated by the observation that the α:β ratio measured in the ^{1}H-n.m.r. spectrum was the same as the ratio measured for the proton-decoupled ^{19}F spectrum.

DISCUSSION

The apparently exclusive formation of the α disaccharides **7** and **11** under the conditions employed probably results from the particular properties of the electrophilic glycosyl bromides **4** and **10** and not from any special properties of the nucleophile **5**. Examples of β disaccharide formation from **5** and an α-glycosyl bromide having a participating group at C-2 are well known[13,26]. Specific synthesis of an α-linked disaccharide has been achieved previously by the Koenigs–Knorr reaction[27], regardless of the anomeric configuration of the sugar halide used. In these instances, as in ours, a glycosyl halide having a non-participating group at C-2 was employed, but mercuric cyanide was used in place of silver carbonate; the former is known (but not understood) to result in a high yield of α-linked disaccharides, even with sugars that do have[28] a participating group at C-2. The relationship of those results to our findings thus remains obscure.

The work of Kronzer and Scheurch[29] examined the effects of an excess of alcohol, solvent polarity, and the structure of the C-6 substituent in both direct and silver triflate-assisted methanolysis. Such non-polar solvents as ether tend to increase the proportion of α-linked product; this proportion could be further increased by altering the nature of the C-6 substituent. On the other hand, both of these factors appeared to be overcome when a large excess of methanol was used. We verified this in our work by synthesizing methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranoside[30] using a methanol:**10** ratio of 93:1 under conditions otherwise identical to those used for the disaccharide syntheses. One attempt was made to synthesize **11** by using an excess of alcohol **5** (**5**:**10** = 1.41), but only the α-linked product was obtained, probably because the excess of alcohol was not large enough to permit the formation of a β-linked product.

The apparently exclusive formation of an α-glycosidic linkage during the preparation of **7** and **11** could also be influenced by the fluorine atom at C-2. While the only disaccharide isolated from the condensation of **10** with **5** was **11**, a side product was also isolated and identified as 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucose (**12**), also prepared directly from **10** by treatment with silver carbonate and wet ether[19]. After column chromatography on silica gel, t.l.c. of freshly eluted samples indicated that this product had been separated into two distinct components. However, after a short time, each separated fraction gave a t.l.c. pattern indicative of the starting mixture. The most logical conclusion is that this side product can mutarotate. As acid-catalyzed mutarotation should occur through an intermediate having carbonium-ion character, the latter should be disfavoured by the presence of the fluorine atom at C-2; thus a lack of mutarotation under acidic con-

TABLE V

^{13}C-CHEMICAL-SHIFT[a] ASSIGNMENTS

δ(p.p.m.)	C-1′	C-1β	C-1α	C-2′	$J_{C-2',F-2'}$	$J_{C-1',F-2'}$	α:β[b]	
							C-1	C-6
Maltose	100.50, 100.55	97.1	93.1	73.8			0.77	0.88
2′-Deoxy-2′-fluoromaltose	97.40, 97.20	96.8	92.9	91.0	186.1	18.1	0.79	0.72

[a]Relative to external Me_4Si. [b]Estimated from relative peak-heights.

ditions (namely, on silica gel) is not unlikely. Perhaps the destabilization of carbonium-ion character at C-1 could also be expected to operate during the Koenigs–Knorr condensation. The carbonium ion, while under formation, could interact with one of the acetate protecting groups of **4** and **10**. As the use of either of these glycosyl bromides resulted in only the α glycoside, participation by the acetoxyl group at C-4 is eliminated. Models suggest that acetoxyl substitutents at either C-3 or C-6 could affect the outcome of the reaction, and indeed such effects have been reported[31,32]. Also, once a trace of free bromide ion exists in the mixture, the reaction could then proceed via an even number of displacements. The first reaction would be the attack of the bromide ion to form the β bromide and the second would involve subsequent attack of the alcohol on the β bromide. As the β-glycosyl halide is more reactive than the initial α-glycosyl halide, SN2 displacement of the former with consequent formation of the α-glycosidic linkage could be favored. Therefore, the use of the non-polar solvent (ether) and a minimal quantity of **5**, as well as the presence of acetoxyl groups at C-3 and C-6 and the C-2 fluorine substituent groups may have been determining factors in the present results. It is apparent that a delicate balance of conditions in the Koenigs–Knorr reaction can determine the configurations of the resulting glycosidic linkage.

A comparison of the ^{13}C-n.m.r. spectra of maltose and its fluorinated analogue **14** (Table V, Fig. 4) confirms the structural assignments. In maltose, the C-1′ signals of the anomers appear at 100.50 and 100.55 p.p.m.; the dispersion at 100 MHz (for ^{13}C) allows the two signals to be well resolved. Such observations are common for C-6 of a mutarotated sugar[33–35]. The C-1′ of **14** is similarly resolved into two signals (94.40 and 97.20 p.p.m.) with additional coupling to fluorine. Similarly, C-6 for both sugars appears as two signals under the influence of the anomeric carbon atom.

Finally, the similarity in size and electronegativity of fluorine as compared with a hydroxyl group suggests that the deoxyfluoro sugars described here should show optical rotations similar to those of the parent hydroxyl sugars, provided that the two sugars have the same configurations and conformations, and this was confirmed (see Experimental). The fact that maltose and 2′-deoxy-2′-fluoromaltose (**14**) exhibit the same optical rotations further confirms the n.m.r. structural assign-

ments for **14** and suggests that the two sugars are isomorphous in solution. This behavior, together with the unusual mutarotation of **12**, suggests that a fluorine atom at C-2 of a pyranose ring may destabilize carbonium-ion character at C-1 and also indicates that there is a close structural similarity (even as far as conformation is concerned) between maltose and 2'-fluoromaltose. It is expected that 2'-fluorodisaccharides will prove to be suitable substrates for studies with glycosidases.

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