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PREPARATION AND CHARACTERIZATION OF 2-DEOXY-2-[^{18}F]FLUORO-D-GALACTOSE.

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SUMMARY

2-Deoxy-2-[^{18}F]fluoro-D-galactose was prepared using gaseous [^{18}F]acetyl hypofluorite or [^{18}F]F_2 and tri-O-acetyl-D-galactal as substrate. The product was obtained in a surprisingly high epimeric purity (>90%), even with [^{18}F]F_2 as fluorinating reagent, as demonstrated by HPLC and high-resolution ^{1}H- and ^{19}F-NMR spectroscopy. This is the first report of a highly stereoselective reaction using molecular fluorine in a polar protic solvent. Epimerization at C-3 of galactal led to α-2-deoxy-2-fluoro-D-idose and α-2-deoxy-2-fluoro-D-gulose as minor products. A simple apparatus for a partially remote-controlled preparation of 2-deoxy-2-[^{18}F]fluoro-D-galactose is also described.

Key Words: 2-Deoxy-2-[^{18}F]fluoro-D-galactose, 500 MHz ^{1}H-NMR, ^{19}F-NMR, Fluorohexoses, PET.

INTRODUCTION

As a part of our studies of fluorinated hexose analogs [1] 2-deoxy-2-fluoro-D-galactose **1** has been prepared in high yield and high epimeric purity by direct fluorination of tri-O-acetyl-D-galactal **2a** or galactal **2b** using molecular fluorine diluted in neon. Compound **1** was labelled with ^{18}F (half life = 109.7 min) using [^{18}F]F_2 or [^{18}F]acetyl hypofluorite and substrate **2a** via standard preparation procedures. An almost pure product was obtained in both cases.

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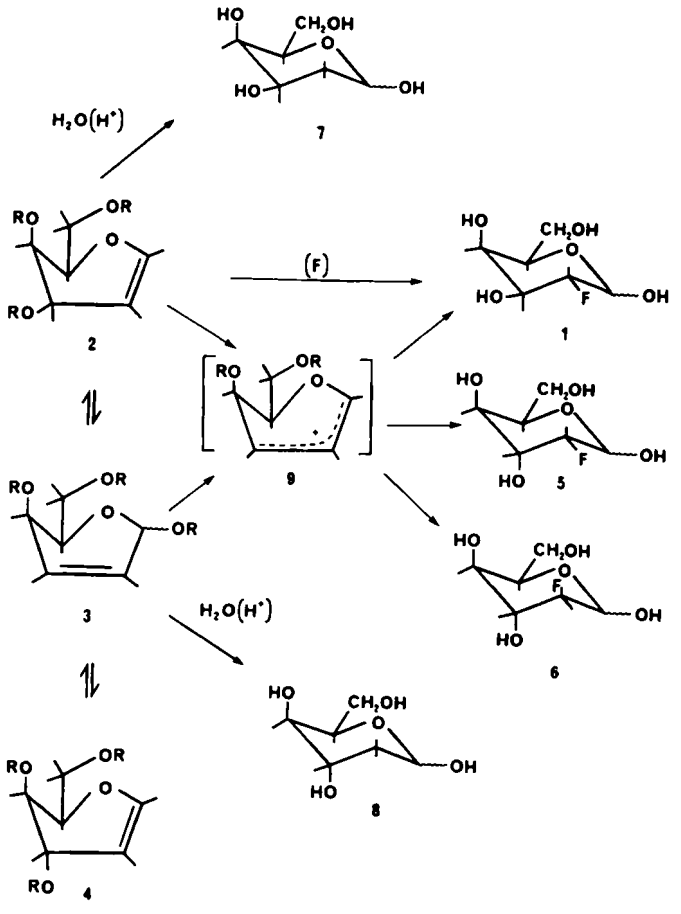

[F] = Exp. A,C: F_2 (acetic acid)
B: CH_3COOF (acetic acid)
D: F_2 (water)

2a-4a,9a: R = acetyl

2b-4b,9b: R = H

Fig. 1. Preparation of 2-deoxy-2-fluoro-D-galactose 1 and [^{18}F]-1 in acetic acid or water. Ring protons have been omitted for clarity. Compound 1 will be formed predominantly from 2, assuming that the addition of fluorine to the 1,2 double bond with anomerization is faster than the allylic rearrangement, or in part from 3, with strong solvent interaction involving the carbocation 9; 5 and 6 (with inversion at C-3) will most likely be obtained from 9 via the rearrangement pathway or possibly from 4 if epimerization is rapid. Analogously, 7 is obtainable predominantly from 2; 8 can arise via migration of the 1,2 double bond followed by addition of water during our work-up procedure but is not expected to be derived from 4; however, the presence of 4 in our reaction mixture cannot be excluded (see [22-34] and text).

The α-anomeric tetraacetate of 1 has been described recently and was determined to be the product of a selective *cis*-addition of acetyl hypofluorite to 2a with an 84% yield in epimerically pure form [2,3]. We corroborated this result, and, in addition, clearly confirmed by ^{1}H- and ^{19}F-NMR measurements that almost the same high epimeric purity is obtained using F_2 diluted in neon (1% F_2). This suggests, that under certain conditions (e.g. in polar protic solvents) molecular fluorine can add stereoselectively across a double bond, supporting a similar observation and hypothesis suggested by Bida and Satyamurthy [4,5] in a reexamination of the synthesis of 2-deoxy-2[^{18}F]fluoro-D-glucose. Thus, a high chemical yield of an isomerically pure product together with an easy and clean synthesis suggested 1 as an attractive tracer for biochemical studies *in vivo*, either by means of ^{19}F-NMR spectroscopy, or by positron emission tomography (PET) when 1 has been labelled with ^{18}F, as shown in a recent report [6].

The route by which the galactopyranose analog 1 enters the galactose metabolic pathway involves first phosphorylation at C-1 by galactokinase to give the 1-1-phosphate. Then the UDP-2-deoxy-2-fluoro-D-pyranose analog may be formed by non-specific UDP-glucose uridylyltransferase and UDP-galactose-4'-epimerase or directly by a minor pathway from the 1-1-phosphate and UTP followed by isomerization at C-4. It has been shown that UDP-2-substituted hexose analogs such as 1 do not act as uridylate donors in uridylylation reactions; thus, the unusual substrate 2-deoxy-2-[^{18}F]fluoro-D-galactose finally accumulates as a UDP-hexose analog with trapping by uridylate [7]. Likewise, 2-deoxy-2-fluoro-D-glucose diverts uridylate to the UDP-2-deoxy-glucopyranose analog, but a very much higher concentration of the derivative is required due to competition for cellular metabolism and uptake.[8,9]

These features of galactopyranose metabolism suggest strongly that ^{18}F-1 may be sufficiently selective for examining the biochemistry of tumors and diseased tissue in which enzymes of the galactose pathway are involved. The concept has the virtue of being immediately testable, preferentially in hepatomas using common PET procedures as well as in hepatocytes using ^{19}F-NMR techniques with the unlabelled analogue.

Therefore, we describe here a facile and practical synthesis and purification of 1 and ^{18}F-1 applying different preparation methods with substrate 2a. The purity of ^{18}F-1 has been demonstrated by ^{1}H- and ^{19}F-NMR spectroscopy and by HPLC. Compound

1 has been previously prepared and isolated in moderate yield by Adamson and Marcus using CF_3OF [10,11] and also by Haradira *et al.* applying the nucleophilic displacement of the triflyl-residue by fluoride at the C-2 position of a protected talo-pyranoside [12]. ^{18}F-1 for PET experiments has been produced by Tada *et al.* using $[^{18}F]F_2$ and triacetyl-D-galactal [13] by a method similar to that presented here. However, the chemical identity of ^{18}F-1 was checked preliminarily by a crude HPLC method only, without the presentation of authentic analytical data. Additional but fragmentary information may be found in the work of Diksic and Jolly [14,15], who applied ^{1}H- and ^{19}F-NMR techniques for the analysis of crude reaction mixtures, obtained from the fluorination of D-galactal in aqueous solution. Unfortunately they did not separate their products. Thus, a complete and detailed characterization of ^{18}F-1 is still lacking. Therefore, we wish to augment the previous reports by presenting an unambiguous characterization of 2-deoxy-2-[^{18}F]fluoro-D-galactose. All compounds presented in Fig. 1 were obtained by independent synthesis or repeated semi-preparative HPLC runs of crude reaction mixtures.

MATERIALS AND METHODS

Tri-O-acetyl-D-galactal (3,4,6,-tri-O-acetyl-1,5-anhydro-D-*lyxo*-hex-1-enitol) 2a was obtained from E.Merck, Darmstadt as a stringy, slightly yellow liquid which may be used without further purification. Very pure crystalline 2a may be obtained by passing a $CHCl_3$ solution of the crude substance through a short column of anhydrous $MgSO_4$. Evaporation of the solvent (room temperature) and careful distillation (without water cooling) of the sirupy residue in a high vacuum ($<10^{-3}$ torr) and at a bath temperature of not more than 150 °C gave a product which crystallizes on standing at 6-8 °C (mp = 28 °C). De-O-acetylation by sodium in methanol [16] afforded 2b, which was recrystallized twice from ethylacetate (white material, mp ≈ 103 °C). The 500 MHz ^{1}H-NMR spectrum of 2b indicated that it was >99.5% pure.

$[^{18}F]F_2$ was produced in a common ^{20}Ne-target system [17] with 0.1% F_2 in the target gas. A 1 hr irradiation with ca. 9 MEV deuterons at 12-15 μA delivered an average ^{18}F-radioactivity of 2 x 10^9 Bq. A typical synthesis was started using 30–35 μmoles of elemental fluorine labelled with ^{18}F. Random experiments without radioactivity were carried out using up to 3 mmoles of elemental fluorine diluted to 1% in Ne. Labelled and non-labelled acetyl hypofluorite were prepared as described in [18] and [19], respectively.

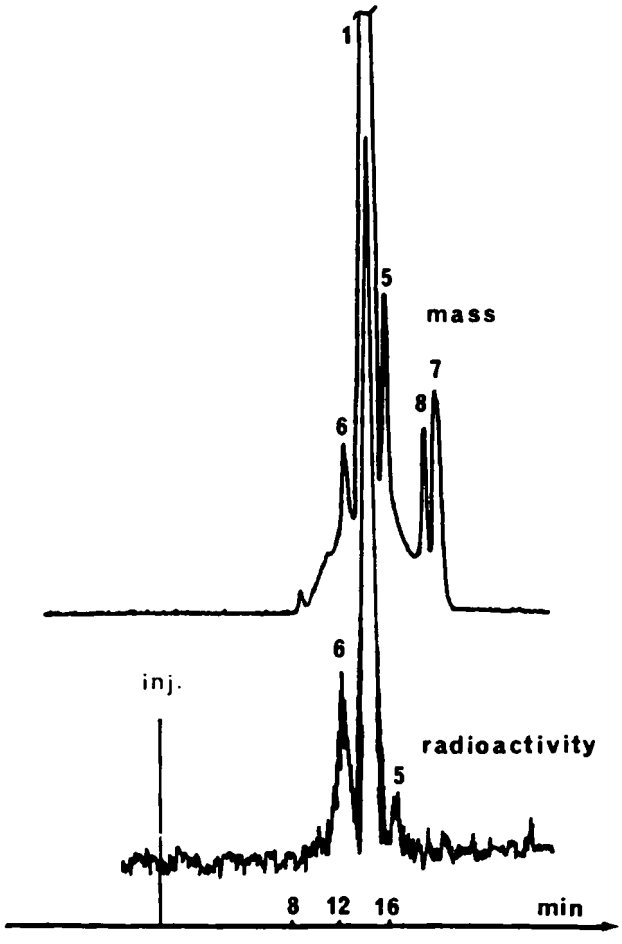

Fig. 2. HPLC purification of ^{18}F-1 with peaks numbered according to Fig. 1. Pump: Beckman Model 110A, 0.5 ml/min; detector: Altex, RI; Waters U6K 2 ml injection loop; Eldex column heater regulated at 85 °C; recorded at 2.5 mm/min. Analysis for lead in the effluent showed 90 ppm Pb^{2+}. The retention time of ^{18}F-1 was t_R = 13.6 min. Minor radioactive impurities were detected at t_R = 12.8 min and 16.4 min (see text). The product peaks are labelled as in Fig. 1. Radioactivity peaks of ^{18}F-5 and ^{18}F-6 appear with vertical scale increased two-fold.

Analysis of 1 was carried out by HPLC in degassed water on a lead(II)-loaded monosaccharide analysis column (Bio-Rad, Aminex HPX-87P, 300 x 7.8 mm). Sample purification was achieved on a Ca^{2+}-loaded pre-column (Bio-Rad, Aminex HPX-87C, 30 x 4.6 mm). The separation was run with the column temperature controlled at 85 °C and a flow rate of 0.5 ml/min. A typical chromatogram is shown in Fig. 2 with details given in the legend. The purity of the product could also be confirmed at the extreme column operating conditions

of 0.05 ml/min at room temperature (see [1] for selective column operation).

Preparation of ^{18}F-1 from tri-O-acetyl-D-galactal.

Experiment A: Approximately 55 mg (200 µmoles) of 2a were dissolved in 15 ml of acetic acid. [^{18}F]F_2 (30-35 µmoles, 2 x 10^9 Bq) was released from the target system at a flow of 80-100 ml/min and bubbled through the solution of 2a at room temperature. Evaporation of acetic acid at a bath temperature of ≤ 60 °C and subsequent hydrolysis of the fluorinated pyranose acetate (5 ml 1 M HCl, 120 °C, 15 min) gave the desired ^{18}F-1. Hydrogen chloride was removed in vacuum to near dryness, and the residue was taken up with 2 ml of wet CH_3CN (containing 0.3% water by volume) and evaporated again. Then 5 ml of the same solvent were applied to dissolve the crude product, which was passed through a silica column (70 x 10 mm, silica 60, Merck No. 9385) topped with 15 mg of active charcoal, using 50 ml of CH_3CN (0.3% water). The first 5 ml were discarded; the rest was pumped into a heated sample vial (85 °C) using compressed air with continuous evaporation of the solvent by a stream of nitrogen. The slightly yellow residue was taken up with water (0.5 ml) and loaded onto the Aminex HPX-87P column. The fraction at 13.6 min (see Fig. 2) was collected with about 1 ml of water. It delivered ca. 4 x 10^8 Bq of pure ^{18}F-1 with 20% yield 60 min after the end of bombardment (relative to the originally trapped [^{18}F]F_2 radioactivity without correction for decay).

Experiment B: Alternatively, gaseous [^{18}F]acetyl hypofluorite was used in the preparation of ^{18}F-1. It was obtained from damp solid potassium acetate ($CH_3CO_2K/CH_3CO_2H/H_2O$ = 1/1.6/0.2) and introduced into a solution of 2a at a flow of ca. 100 ml/min at ambient temperature. Approximately 8 x 10^8 Bq of radioactivity were trapped in the substrate solution. Work up as above gave ^{18}F-1 (5 x 10^8 Bq) in 1 ml of water as virtually the only labelled product (2.8% total radioactive impurities were observed; see Results and Discussion). The yield was 62.5% (80.2% when corrected for decay) based on the originally trapped [^{18}F]acetyl hypofluorite.

Partially remote-controlled apparatus.

The apparatus of Fig. 3 has been assembled to prepare ^{18}F-labelled deoxy sugars under remote control. The heart of the

system is a remote-operated multiway rotary valve with a 20 ml motor-driven syringe **A** connected to the mainport **M** of the valve. The flask containing the radioactive fluorinated tetraacetate of the desired deoxyhexose was first connected to the micro-evaporator **B** (Büchi) and then introduced into the heating bath at 60 °C. A controlled vacuum was switched on and smoothly regulated to the endpoint of 50 torr by operating the pump valve **V** outside of the hot cell. During the evaporation (5 min) the motor-driven syringe was filled with 5 ml of 1 M HCl from a storage bottle **S**. After most of the acetic acid had been distilled off (3 min) the temperature was adjusted to 120 °C with a heating rate of 15 °C/min. Then the evaporator was flushed by opening line **a** and HCl was introduced through port 1 of the rotary valve. Line **a** was closed and a mild vacuum was applied during the first 10 min of hydrolysis; then increasing amounts of HCl were distilled off with the distillation rate regulated by the pump valve **V**. The motor-driven syringe was washed with water and filled with 7 ml of CH_3CN

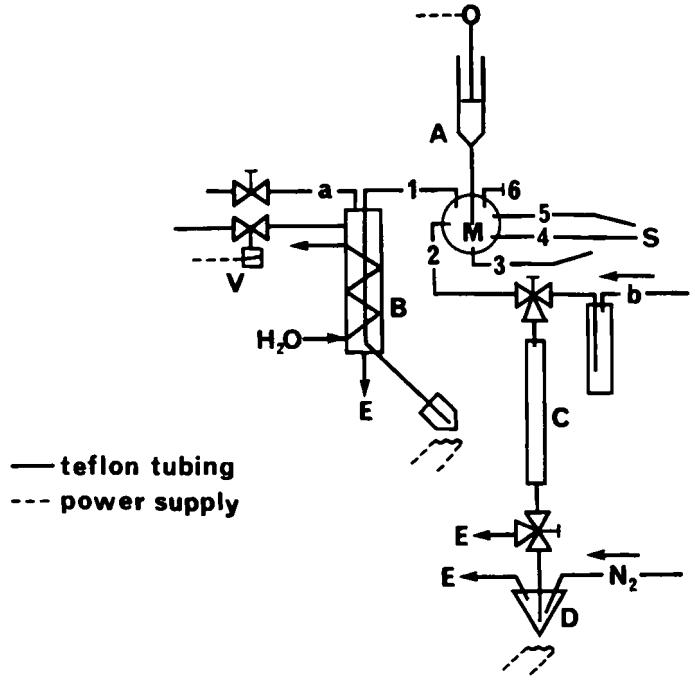

Fig. 3. Apparatus for the preparation of ^{18}F-labelled deoxy sugars. **A**: motor-driven syringe connected to a remote-operated multiway rotary valve (Latek, Heidelberg, F.R.G.) through the mainport **M**; **B**: micro-evaporator with silicon oil heating bath; **S**: solvent storage bottles connected to ports 3, 4, and 5 of the rotary valve; **a**: vent; **V**: pump valve operated by a vacuum controller (Brand EVC 1, Wertheim, F.R.G.); **C**: silica column and solvent supply; **b**: compressed air; **D**: sample vial; **E**: exit for waste.

(0.3% water) during hydrolysis. After the evaporation of HCl was nearly complete, line a was opened again and 2 ml of CH_3CN were introduced and evaporated. Before this operation the heating bath had been moved down and switched off, and the rotary valve was positioned to the deadpoint 6 during evaporation of the first fraction of the CH_3CN/H_2O mixture. The remaining CH_3CN was then used to dissolve the residual crude product (rotary valve at point 1 and line a opened). It was transferred (line 2) to the silica column C (230-400 mesh) topped with active charcoal and chromatographed with the aid of compressed air using 50 ml of CH_3CN (0.3% H_2O). After the first 5 ml of the eluate were discarded, the product was collected in the heated vessel D from which the solvent was evaporated through a slow stream of N_2. The product was redissolved in 0.5 ml water and injected into the HPLC system.

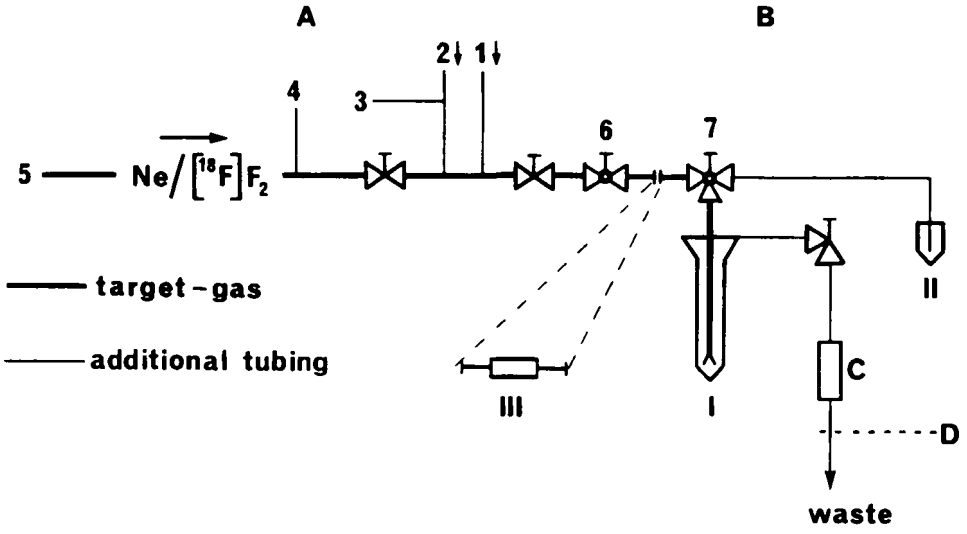

Fig. 4. Gas supply for the target system and trapping of ^{18}F radioactivity. A: shielded gas supply (Ni-tubing) with hand-operated bellow valves (stainless steel), 1: Ne, 2: Ne/F_2 mixture (2% F_2), 3: vacuum, 4: pressure gauge, 5: target, 6: Monel-needle valve (flow control); B: trapping system (teflon tubing), 7: three-way valve (Kel-F), I: trap with shielding, II: evacuated vial, III: CH_3COOK/CH_3COOH tube; C: soda lime/active charcoal, D: flow-meter.

It is important that after the hydrolysis the solvents *should not* be removed to complete dryness. All storage solvents, gas supply valves and the vacuum pump are placed outside the hot cell. This apparatus is now in use for the preparation of 2-deoxy-2[^{18}F]fluoro-D-glucose, 2-deoxy-2[^{18}F]fluoro-D-galactose, and with

a slight modification for the preparation of 5-[^{18}F]fluorouracil. Trapping of [^{18}F]F_2 or [^{18}F]acetyl hypofluorite was done in the cyclotron vault in a shielded hand-operated apparatus near the ^{18}F-target system (Fig.4).

Preparation of unlabelled 2-deoxy-2-fluoro-D-galactose 1.

Experiment C: from tri-O-acetyl-D-galactal **2a**. Compound **2a** (1.36 g = ca. 5 mmole) was dissolved in 40 ml of acetic acid. F_2, diluted to 1% in Ne was bubbled through this solution at a flow rate of 100 ml/min for 2 h in an apparatus described in detail by Vyplel [20]. The resulting clear, but green solution was evaporated to dryness, and the residue was suspended in 30 ml of 1 M HCl and refluxed for 1 h. Then the mixture was neutralized with saturated $NaHCO_3$ solution, evaporated and finally extracted several times with 10 ml portions of hot ethanol. The ethanol extract was decolorized with active charcoal and filtered off. 2-deoxy-2-fluoro-D-galactose was precipitated from the filtrate by ethyl acetate to give 645 mg (71%) of a pure white powder which, after recrystallization from ethanol/ethyl acetate, showed a melting point of 131 °C (lit. 133-136 °C [11]). Analysis calculated for $C_6H_{11}O_5F$: C 39.56%, H 6.04%; found C 39.55%, H 6.21%; $[\alpha]_D$ = 91.5° (c = 2.1, water).

Experiment D: from D-galactal **2b**. Compound **2b** (146 mg, 1 mmole) was dissolved in 20 ml of water containing 82 mg of sodium acetate and was treated as above with diluted F_2 for 1 h. The solution was evaporated and the oily, slightly yellow residue was dissolved in water and chromatographed on Aminex HPX-87P in three successive runs yielding 115 mg (63%) of **1** (mp. 133 °C, analysis found: C 39.48%, H 6.13%).

TABLE 1. Synthesis of 2-deoxy-2-[^{18}F]fluoro-D-galactose and 2-deoxy-2-fluoro-D-galactose.

Experiment[a]	Yield(%)		Spec. Activity		Purity(%)[b]	
	^{18}F-**1**	**1**	μg/mCi[c]	ε[d]	RC	C
A	20[e]		130	14.02	90	86
B	62.5[f]		118	13.92	97.5	81
C		71				>98
D		63				>98

(a) Experiments A, B and C in acetic acid; D in water.
(b) from HPLC peak areas; RC = radiochemical purity, C = chemical purity.
(c) determined by HPLC; RI detector thermostatted at 15 °C; radioactivity measured separately using a Capintec ionization chamber.
(d) $\varepsilon = A_i/A^*$ shows how far the radiolabelled product differs from the perfect carrier free state A_i ($A_i = \lambda N_L$, λ = decay constant and N_L Avogadro constant, A^* = actual specific activity measured for the labelled compound) on a logarithmic scale, as defined in [21].
(e) yield at the end of synthesis and purification; based on $[^{18}F]F_2$ initially trapped in the substrate solution.
(f) yield at the end of synthesis and purification; based on the radioactivity of reagent that could be transferred to the substrate solution.

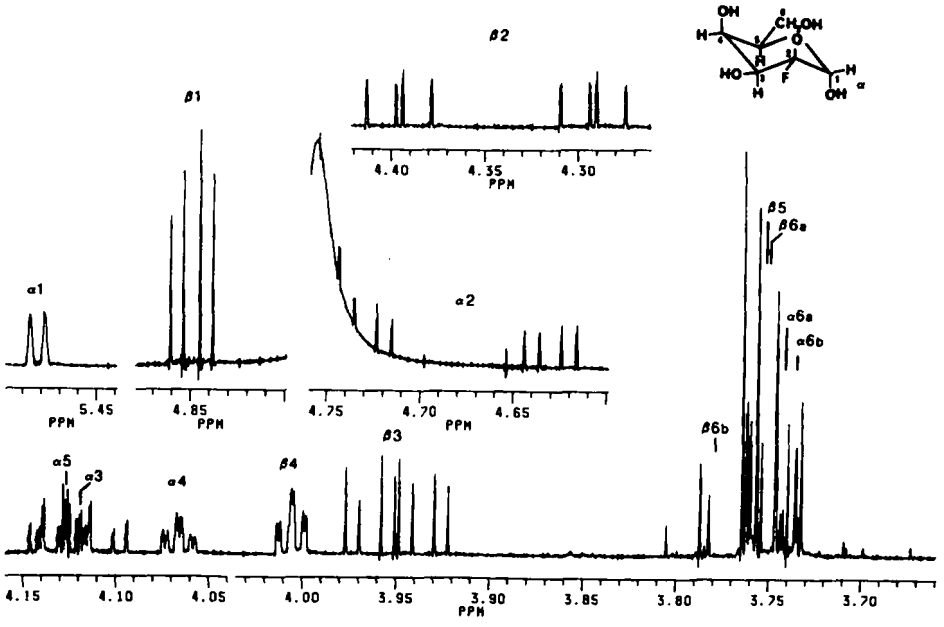

Fig. 5. 500 MHz ^{1}H-NMR spectrum of 2-deoxy-2-fluoro-D-galactose in D_2O at 28 °C. Resolution enhancement has been performed using the Lorentz-Gauss lineshape transformation routine of the Bruker software. Individual protons for the α- and β-anomers are labelled with marks at their chemical shift positions.

NMR spectroscopy.

The identity and purity of 1 was checked by means of conventional high-resolution ^{1}H- and ^{19}F-NMR at 500 and 470 MHz, respectively, using a Bruker AM-500 FT NMR spectrometer (for details see [1]). Samples were dissolved in D_2O in 5 mm sample tubes with 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionic acid

(TSP) as chemical shift reference. ^{19}F chemical shifts are relative to C_6F_6 (-87.279 ppm) in C_6D_6 contained in a 2 mm coaxial capillary, whereby 1 mM trifluoroacetic acid in D_2O = 0 ppm. The resolution-enhanced ^{1}H-spectrum of 1 is shown in Fig. 5. The spectrum could be completely assigned, and the ^{1}H- and ^{19}F-NMR parameters are summarized in Tables 2 and 3. Integration of the ^{1}H- and ^{19}F-spectra gave anomer populations of 40.8% α and 59.2% β (±0.2%). Much of the spectrum could be analyzed by simple inspection; however, the protons α-6a,6b and β-5,6a,6b overlap and are strongly second-order so that a simulation with iterative fit to the measured spectrum was performed (PANIC program in the Bruker software package) to determine shifts and couplings.

RESULTS AND DISCUSSION

The results obtained from fluorination of tri-O-acetyl-D-galactal (2a) and D-galactal (2b) are summarized in Table 1. The ^{19}F-NMR spectrum of the product mixture of Experiment B showed, in addition to the α- and β-anomers of 1, two impurities (5 and 6 in Fig. 1) for which a number of ^{1}H-signals could also be assigned unequivocally. Since the galactal starting material was found to be >99% pure by ^{1}H-NMR, the minor products observed must arise via additional complex reaction pathways. These occur even after refluxing 2a in acetic acid for only 10 min, probably by the well-known route which involves allylic migration of the 1,2 double bond in a Ferrier-type rearrangement reaction leading to compound 3 [22-25]. Our NMR data for the 2-fluoro analogs of glucose, mannose [1] and galactose (Tables 2 and 3) provide a sufficient basis for analyzing the data of Table 4 and arriving at the structural assignments presented therein. First, considering ^{19}F chemical shifts, it is observed that for equatorial-F at C-2 the configurational change β to α (equatorial -OH to axial -OH) causes a $\delta\delta_F$ of only -0.17 ppm for both glucose [1] and galactose analogs. In contrast changing the OH at C-4 from equatorial to axial gives $\delta\delta_F$ = -8.14 ppm. For axial -F at C-2 the anomeric effect is quite large ($\delta\delta_F$ = +18.4 ppm for mannose β to α). These observations suggest that equatorial -F will be shifted only slightly by a configuration change at C-3, whereas axial -F should show a strong effect. In this sense the structures 5 (gulose) and 6 (idose) are consistent with the δ_F assignments. Galactose is converted to gulose by inversion at C-3 and the value of $\delta\delta_F$ = +1.25 ppm. On the other hand the α-idose analog has axial -F and

two neighboring axial -OH and the shift difference compared to α-mannose is $\delta\delta_F$ = +13.3 ppm, which includes a large positive contribution for the change equatorial -OH to axial -OH at C-3 and a smaller (probably negative) contribution for equatorial -OH to axial -OH at C-4.

More important, of course, are the vicinal coupling constants in assigning configurations. The values of J_{2F} and J_{22} definitely rule out any axial-axial (aa) vicinal interactions, and the observed couplings are consistent with the complete set of

TABLE 2. 500 MHz ^{1}H-NMR data for 2-deoxy-2-fluoro-D-galactose in D_2O at 28 °C.

		Chemical Shifts in ppm[a]						
anomer	%	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
α	40.7	5.4814	4.6794	4.1197	4.065	4.1275	3.7435*	3.7358*
β	59.3	4.8491	4.3438	3.9491	4.005	3.7526*	3.7506*	3.7769*

	Coupling constants J_{HH} in Hz[b]										
	$^2J_{12}$	$^3J_{23}$	$^3J_{34}$	$^3J_{45}$	$^3J_{56a}$	$^3J_{56b}$	$^2J_{6a6b}$	$^4J_{13}$	$^4J_{15}$	$^4J_{24}$	$^5J_{14}$
α	4.03 ea	9.98 aa	3.56 ae	1.26 ea	4.23*	8.16*	-11.67*	0.41 ea	0.6 ea	0.25 ae	0.4 ee
β	7.77 aa	9.52 aa	3.64 ae	1.11*ea	4.41*	7.88*	-11.71*	<0.1 aa	<0.2 aa	0.24 ae	<0.2 ae

a) relative to TSP; values marked with * were obtained by iterative spectrum simulation; other values are the midpoints of the nearly first-order multiplets.

b) e = equatorial, a = axial are codes for the orientation of the two protons involved in a given coupling; values marked with * were obtained from the iterative simulation, other couplings (without sign) were obtained from the peak frequency listing.

TABLE 3. 470 MHz ^{19}F-NMR data for 2-deoxy-2-fluoro-D-galactose in D_2O at 28 °C.

anomer	%	δ_F (ppm)[a]	Coupling constants J_{HF} in Hz[b]				
			$^2J_{2F}$	$^3J_{3F}$	$^3J_{1F}$	$^4J_{4F}$	$^5J_{6F}$
α	41	-131.957	49.73	12.52 ae	<0.2 ee	3.85 ee	<0.1 ae
β	59	-131.785	51.92	14.25 ae	3.37 ae	3.3 ee	<0.2 ae

a) chemical shifts relative to 1 mM trifluoroacetic acid in D_2O = 0 ppm.

b) a = axial, e = equatorial are codes used to denote the orientations of H and F.

TABLE 4. NMR parameters for minor products in the synthesis of 2-deoxy-2-fluoro-D-galactose.

Product	Yield(%)	δ_F (ppm)	$^2J_{2F}$	$^3J_{3F}$	$^3J_{1F}$	$^3J_{12}$	$^2J_{22}$	$^3J_{34}$	$^4J_{13}$	$^3J_{56a}$	$^3J_{56b}$
5	0.9	-130.713	52.7	18.9 ee	3.8 ee	4.2 ea	5.9 ae		1.0 ee		
6	2.1	-115.808	50.7	23.6 ea	11.5 ea	1.1 ee	2.4 ee	6.1 ee	0.4 ee	4.6	7.4

	^{1}H Chemical shifts	H-1	H-2	H-3	H-6a	H-6b	
5		5.425	4.957				
6		5.512	4.897	4.359	3.714	3.653	($^2J_{6a6b}$ = -11.7)

5 = α-2-deoxy-2-fluoro-D-gulose, 6 = α-2-deoxy-2-fluoro-D-idose

interactions defined by structures 5 and 6. For example, for the gulose analog 5 $^3J_{1,2}$ = 4.2 compares well with values of 3.9 and 4.0 for the (ea) configuration in glucose and galactose analogs, respectively, ie. H-1 is equatorial and H-2 is axial, consistent also with $^3J_{1,F}$ = 3.8 (ee). $^3J_{2,3}$ = 5.9 implies H-3 is equatorial, consistent with $^3J_{3,F}$ = 18.9 (ee). Finally, the configuration at C-4 is expected to be the same as that for the galactose analogue based on the above arguments concerning δ_F.

For the idose analog 6 the small values of $^3J_{1,2}$ and $^3J_{2,3}$ clearly place protons H-1,2,3 equatorial. The configuration at C-4 must be that of the galactose analogue, based on $^3J_{3,4}$ and the fact that the couplings between H-5 and H-6a,6b agree well with those for the galactose analog (Table 2) but not with those for glucose or mannose (2.3, 5.6 [1]). The corresponding β-anomers of 5 and 6 were not detected.

Mechanistically, minor products 5 and 6 could arise unambiguously from the epimer 4 (C-3 inverted), assuming that in acetic acid the initial 1,2 to 2,3 migration of the double bond is reversible, giving 4 via 3. An attempt to demonstrate this pathway failed; however, the involvement of possible S_N1' or S_N2' mechanisms cannot be ruled out under the conditions of the syntheses. The fluorination of 3 as a source of 5 and 6 appears to be more likely and is supported by well-investigated analogous reactions [25-27], which were also performed in acetic acid. In any case, the products presented in Fig. 1 represent the result of competing attack of fluorine or acetyl hypofluorite on 2 or on the resonance-stabilized carbocation 9, probably kinetically controlled. Acetic acid or water as solvent would promote epimerization at C-3 and, more importantly, anomerization of the products, thus leading to enhanced stereoselectivity at the anomeric carbon. Anomeric and allylic effects both favor the formation of α-anomers [28-31]. Thus, in our case of the formation of 5 and 6 via the carbocation intermediate, one would expect almost exclusively the α-anomers of these compounds, as indeed was observed.

It should be mentioned that one of the impurities in the synthesis of 2-deoxy-2-fluoro-D-glucose and 2-deoxy-2-fluoro-D-mannose, which we tentatively assigned as 3-deoxy-3-fluoro-D-mannose [1], gives NMR data that we can now analyze to be consistent only with β-2-deoxy-2-fluoro-D-allose { δ_F = -123.07 ppm; $^2J_{2,F}$ = 50.5 Hz, $^3J_{3,F}$ = 15.5 (ee), $^3J_{1,F}$ = 4.0 (ae), δ_{H-2} = 4.491, $^3J_{1,2}$ = 8.8 (aa), $^3J_{2,3}$ = 6.0 (ae) }. Thus, this minor product of the glucose synthesis also has an inverted

configuration at C-3, just as for 5 and 6 in the galactose synthesis. Work is still progress to confirm these interpretations by independent synthesis.

The expected epimer of 1, 2-deoxy-2-fluoro-D-talose (F axial), could not be detected (< 0.1%), contrary to the observation of Diksic and Jolly [14,15]. This remarkably high stereoselectivity of the reaction, in contrast to the reaction with D-glucal as substrate [1], is apparently due to additional steric and/or kinetic restraints, caused by the axial -OR at C-4 and the effect of a dipolar protic solvent. This selectivity is essentially the same whether using acetyl hypofluorite or molecular fluorine diluted in neon as fluorinating reagent. Thus, a nucleophilic attack on fluorine by the sp^2-carbon C-2 of the substrate is a plausible mechanism.

As will be discussed in a future report, we also have evidence that the high electron affinity of fluorine supports attack of double bonds, assisted by the ease of ejection of acetate as leaving group for an S_N2 reaction of acetyl hypofluorite. Furthermore, acetyl hypofluorite, stable for > 24 h when dissolved in acetic acid, shows a very broad ^{19}F-NMR resonance (δ_F = ca. +240 ppm), indicating that fluorine in CH_3COOF is labile and undergoing some form of chemical exchange or protonation equilibrium.

It was easily verified by the reaction of $[^{18}F]F_2$ with substrate 2a (Experiment A) that compounds 6 and 5 were formed as radioactive contaminants. These were found by HPLC at t_R = 12.8 min (6 = 8.5% of crude material) and at t_R = 16.4 min (5 = 0.8%) which also accounts for the above-mentioned course of the fluorination reaction.

Major non-fluorinated side products formed in the crude preparation were compounds 2b, 3b and the 2-deoxygalactose 7, which variably represented up to 12% of the product mixture, as determined from HPLC peak areas. This is apparently the result of the main features of glucal-water interactions [32-35] and of the well-known acid-catalyzed addition of water to the double bond of 2a or 3a during hydrolysis of excess 2a [22-24, 34]. That a small amount of 3a was involved in this reaction follows from the appearance of 2-deoxygulose 8, detected at levels of up to 1.5% of the total product mixture. Most of 2b and 3b could be removed by the silica gel filtration during the preparation of ^{18}F-1, whereas the final purification could be achieved only by HPLC.

CONCLUSIONS

It has been shown that 2-deoxy-2-[^{18}F]fluoro-D-galactose and the unlabelled compound are obtainable with simple chemistry and with high epimeric purity, most efficiently using molecular fluorine in a polar protic solvent such as acetic acid or water. Moreover, it has been demonstrated that ^{1}H- and ^{19}F-NMR spectroscopy represent excellent methods for the analysis of ^{18}F-labelled radiopharmaceuticals obtained by carrier-added synthesis, which, in combination with high-resolution HPLC procedures, significantly improve standard methods of quality control.

The title compound, 2-deoxy-2-[^{18}F]fluoro-D-galactose, has significant potential for the study of metabolic pathways in hepatoma cells as a UMP-trapping sugar analog, especially in combination with synergistically acting inhibitors of the *de novo* UMP-synthesis or with pyrimidine ribonucleoside analogs (e.g. 5-fluorouridine, which may also be labelled with ^{18}F). Thus, it is suggested that this compound be utilized as a tool for control of the selectivity and effectiveness of tumor chemotherapy involving anti-pyrimidines [7].

Furthermore, it must be stressed that *in vivo* PET-studies with ^{18}F-labelled substrate analogs should be supplemented by ^{19}F-NMR spectroscopy of normal and diseased tissue samples or, when possible, *in vivo* [36] to achieve a maximum of qualitative and quantitative diagnostic information concerning uptake and metabolism of these substances.

However, all of these techniques profit from a highly developed and refined chemistry which guaranties serial preparations of selected drugs with very high purity. It is hoped that the present report will contribute to this goal as well as increase our repertoire of chemical strategies for enhancing the tumor specifity of such diagnostic or therapeutic agents.

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