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Two-step regio- and stereoselective syntheses of [^{19}F]- and [^{18}F]-2-deoxy-2-(*R*)-fluoro-β-D-allose

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Abstract—Replacement of specific hydroxyl groups by fluorine in carbohydrates is an ongoing challenge from chemical, biological, and pharmaceutical points of view. A rapid and efficient two-step, regio- and stereoselective synthesis of 2-deoxy-2-(*R*)-fluoro-β-D-allose (2-(*R*)-fluoro-2-deoxy-β-D-allose; 2-FDβA), a fluorinated analogue of the rare sugar, D-allose, is described. TAG (3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-arabino-hex-1-enitol or 3,4,6-tri-*O*-acetyl-D-glucal), was fluorinated in anhydrous HF with dilute F_2 in a Ne/He mixture or with CH_3COOF at −60 °C. The fluorinated intermediate was hydrolyzed in 1 N HCl and the hydrolysis product was purified by liquid chromatography and characterized by 1D ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy as well as 2D NMR spectroscopy and mass spectrometry. In addition, ^{18}F-labeled 2-deoxy-2-(*R*)-fluoro-β-D-allose (2-[^{18}F]FDβA) was synthesized for the first time, with an overall decay-corrected radiochemical yield of 33 ± 3% with respect to [^{18}F]F_2, the highest radiochemical yield achieved to date for electrophilic fluorination of TAG. The rapid and high radiochemical yield synthesis of 2-[^{18}F]FDβA has potential as a probe for the bioactivity of D-allose.

Keywords: 2-Deoxy-2-(*R*)-fluoro-D-allose; PET tracer; Regio- and stereoselective fluorination; Elemental fluorine; Fluorine-18

1. Introduction

Substitution of a hydroxyl group by fluorine usually results in molecules that are metabolized without C–F bond attack. Deoxyfluorinated sugars therefore allow studies of carbohydrate transport and metabolism and are key to the isolation of specific biochemical reaction sequences from general metabolic pathways, especially in the functional imaging sciences.[1] Furthermore, ^{18}F-labeled ($t_{1/2} = 110$ min) sugars play an important role in medical applications, including the diagnoses of pathophysiological processes.[2] For example, the elevated glucose metabolism in tumor cells has been used to advantage for the diagnosis and management of cancer by use of the ^{18}F-labeled analogue of 2-deoxy-D-glucose, 2-[^{18}F]fluoro-2-deoxy-D-glucose (2-[^{18}F]FDG), as a glucose tracer in conjunction with positron emission tomography (PET).

There have been several recent publications related to the biological functions of D-allose. For example, D-allose provides very effective protection against neutrophil-related postischemic injury of liver tissue[3] and inhibits segmented neutrophil production and lowers platelet count without detrimental side effects.[4] Such characteristics make D-allose a candidate for the treatment of diseases such as chronic myelogenous leukemia. By analogy with glucose and its fluorinated derivative, 2-FDG, it appeared desirable to synthesize 2-deoxy-2-fluoro-D-allose, which, when labeled with ^{18}F, could serve as a probe of the bioactivity of D-allose.

Johansson and Lindberg[5] have reported the only synthesis of 2-deoxy-2-fluoro-D-allose prior to the present

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work. Their lengthy, multi-step synthesis was carried out by the reaction of an anhydrous chloroform (ethanol-free) solution of the sugar epoxide, methyl 4,6-di-*O*-acetyl-2,3-anhydro-α-D-allopyranoside, with BF_3 in HF at −70 °C. Hydrolysis and deprotection of the reaction intermediate resulted in 2-deoxy-2-fluoro-D-altrose, 68% of which was epimerized to 2-deoxy-2-fluoro-D-allose in 33% aqueous trimethylamine at 60 °C over a period of 5 h. The final reaction product was a mixture of the α- and β-anomers. Although the authors referred to unpublished ^{1}H and ^{19}F NMR data to support their structural characterization, no NMR parameters were provided.

One of the most prevalent synthetic procedures used to introduce fluorine at C2 in carbohydrate molecules is electrophilic addition of fluorine to glycals. Such compounds often show high regio- and stereoselectivity in addition reactions.[2] Adamson et al.[6,7] first reported the syntheses of 2-deoxy-2-fluoro sugars by addition of CF_3OF to 3,4,6-tri-*O*-acetyl-D-glucal (TAG) dissolved in $CFCl_3$ at −80 °C followed by acid catalyzed hydrolysis of the reaction intermediates. The synthesis produced 2-fluoro-2-deoxy-D-glucose (2-FDG) and 2-fluoro-2-deoxy-D-mannose (2-FDM) in good yields. Since then, TAG has been one of the most widely used precursors for the syntheses of 2-deoxy-2-fluoro-sugars.

The introduction of a fluorine atom at C2 by addition to glycals is effected by so-called 'electrophilic fluorinating reagents, $R^{\delta-}$–$F^{\delta+}$'. Because C2 of a glycal is more electronegative than C1 as a result of resonance contributions –O–C(1)=C(2) ↔ –O^+=C(1)–C^-(2), the fluorine of R–F always adds to C2. Reagents in this category include CF_3OF, F_2 diluted with an inert gas, CH_3COOF, and XeF_2.[8] Because almost all electrophilic fluorinating agents are the products of direct fluorination by F_2, it is desirable to use F_2 directly as the electrophilic fluorinating agent, thereby eliminating synthetic steps.

Lundt and Pedersen[9–11] extensively studied the reactions of TAG and other 3,4,6-tri-*O*-substituted glycals with and in anhydrous hydrogen fluoride (aHF) solvent and in mixed solvent media containing HF. The reaction of TAG with aHF resulted in a 1,2-unsaturated 3,4-dioxolenium ion, which was stable in aHF at −70 °C for several hours. The dioxolenium ion was hydrolyzed in the presence of a small amount of water and epimerization occurred at C3, with the configuration at C3 changing from *S* in the starting material to *R* in the product.[10]

Drawing on the synthetic approach of Lundt and Pedersen[10] and the established synthetic procedure used to introduce fluorine at C2 in hexapyranoses,[6,7,12–14] a rapid and efficient two-step synthesis of 2-deoxy-2-(*R*)-fluoro-β-D-allose (2-(*R*)-fluoro-2-deoxy-β-D-allose; 2-FDβA), resulting from electrophilic fluorination of TAG, is reported in this paper. A detailed ^{1}H, ^{13}C, and ^{19}F NMR study has provided the first unambiguous structural characterization of 2-FDβA. In addition, the synthesis of ^{18}F-labeled 2-FDβA (2-[^{18}F]FDβA), using [^{18}F]F_2 as the electrophilic fluorinating agent, is reported for the first time.

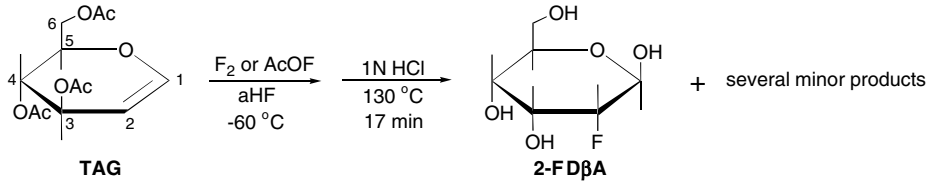
2. Results and discussion

2.1. Synthesis of 2-FDβA

The synthesis of 2-FDβA was accomplished by electrophilic fluorination of commercially available TAG with F_2 or CH_3COOF in aHF at −60 °C, followed by acid catalyzed hydrolysis and purification using liquid chromatography (Scheme 1). The compound was characterized by conventional 1D ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy, selective 1D ^{1}H and 2D ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopy experiments, and by mass spectrometry.

2.2. Structural characterization of 2-FDβA by 1D and 2D NMR spectroscopy

Among the methods for determining the compositions and stereochemistries of sugar mixtures, NMR spectroscopy has emerged as the most reliable.[15] The structures of monosaccharides and fluorinated monosaccharides can be readily analyzed, in most instances, by conventional 1D NMR techniques and by reference to the ^{1}H and ^{13}C NMR parameters of related compounds.[16] Moreover, the conformations of the deoxyfluorinated sugars can be reliably determined by ^{1}H NMR spectroscopy.[17] Whereas resonances of non-anomeric protons of different conformers usually overlap, those of anomeric protons are often well separated from each other.[15] In cases where ^{1}H spectral data are difficult to obtain,



Scheme 1. Synthesis of 2-(*R*)-fluoro-2-deoxy-β-D-allose (2-FDβA).

^{19}F NMR spectroscopy can be used to probe the configuration and/or conformation of fluorocarbohydrates.[18]

The 1D ^{1}H NMR spectrum (Fig. 1) of the products of Scheme 1 was too complex to permit full and unambiguous analysis and extraction of NMR parameters. However, the ^{1}H resonances corresponding to the anomeric proton could be readily assigned. The full NMR structural characterization of 2-FDβA was accomplished by carrying out selective 1D ^{1}H and 2D correlation spectroscopy experiments. The ^{1}H NMR parameters for 2-FDβA are summarized in Table 1.

The ^{1}H NMR spectrum showed only one type of anomeric proton resonance. Thus, it could be immediately concluded that the major carbohydrate product synthesized in Scheme 1 existed in either the α- or β-form. The doublet of doublets (dd), centered at 5.21 ppm, was assigned to the anomeric proton, H1. The smaller coupling (1.4 Hz) was characteristic of an axial–equatorial coupling between H1 and F, and established that the fluorine atom was bonded to C2. Moreover, the larger coupling (8.2 Hz) showed that the monosaccharide possessed a trans-diaxial arrangement of hydrogen atoms at C1 and C2. As a result, the absolute configuration at C1 was assigned as *R*, i.e., the fluorocarbohydrate had the β-form. Furthermore, comparison of the magnitudes of $^{3}J_{H1,H2}$ and $^{3}J_{H1,F}$ measured in the present study with those of the β-anomers of 2-FDG ($^{3}J_{H1,H2} = 7.8$ Hz; $^{3}J_{H1,F} = 2.5$ Hz)[19,20] and 2-fluoro-2-deoxy-D-galactose ($^{3}J_{H1,H2} = 7.3$ Hz; $^{3}J_{H1,F} = 3.0$ Hz)[21–23] indicated that 2-fluoro-2-deoxy-D-allose predominantly existed as the β-form in equilibrated aqueous solutions of the products. This is consistent with the observation that the anomeric equilibrium for the D-*ribo*- and D-*allo*-pyranose series favors the β-D-pyranose form.[24–26] This is also in agreement with the reported compositions of D-allose and D-gulose and their amino sugar derivatives in equilibrium mixtures in which the β-pyranose forms of the sugars dominate (Table 2).

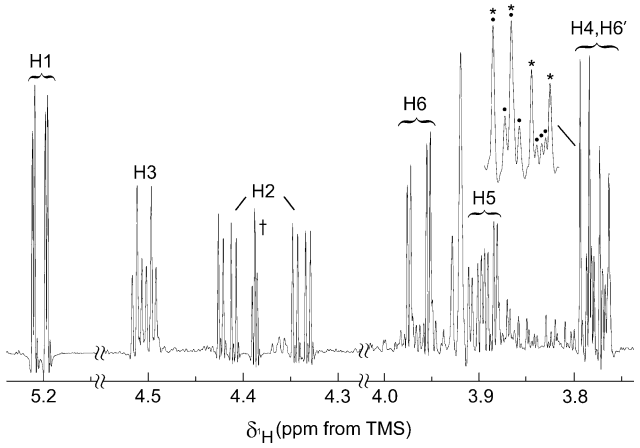


Figure 1. The ^{1}H NMR spectrum (D_2O solvent at 25 °C) of the products resulting from the electrophilic fluorination of TAG in aHF. The spectrum was processed using Gaussian multiplication and presaturation of the HDO solvent resonance at 4.77 ppm. Dots (•) and asterisks (*) denote H4 and H6′ resonances, respectively. The dagger (†) denotes an unassigned 'triplet' (δ(^{1}H) 4.39 ppm; 1.6 Hz splitting).

Table 1. ^{1}H NMR parameters for 2-FDβA

	^{1}H Chemical shift (ppm)	Multiplicity[a]	Coupling constant (Hz)	
H1	5.21	dd	$^{3}J_{H1,F} = 1.4$	$^{3}J_{H1,H2} = 8.2$
H2	4.38	ddd	$^{2}J_{H2,F} = 47.0$	$^{3}J_{H2,H3} = 3.1$
H3	4.51	ddd[b]	$^{3}J_{H3,F} = 8.9$	$^{3}J_{H3,H4} = 3.0$
H4	3.78	ddd	$^{4}J_{H4,F} = 1.6$	$^{3}J_{H4,H5} = 10.0$
H5	3.90	ddd	$^{3}J_{H5,H6} = 2.3$	$^{3}J_{H5,H6'} = 5.8$
H6	3.97	dd	$^{2}J_{H6,H6'} = 12.3$	
H6′	3.78	dd		

[a] The abbreviations denote doublet of doublets (dd) and doublet of doublets of doublets (ddd).

[b] This multiplet appears as a doublet of 'triplets' because $^{3}J_{H3,H4} \approx {}^{3}J_{H2,H3}$.

A ^{1}H–^{1}H gradient COSY spectrum (Fig. 2) showed a strong correlation between the H1 resonance and a doublet of doublets of doublets centered at 4.38 ppm. Selective 1D TOCSY experiments and coupling constants were used to assign the multiplets to H2. The magnitudes of the $^{2}J_{H2,F}$, $^{3}J_{H2,H1}$, and $^{3}J_{H2,H3}$ couplings unequivocally established the absolute configuration at C2 as *R*. The ^{1}H–^{1}H COSY and selective 1D TOCSY experiments were also used to assign the H3 resonance to the doublet of pseudo-triplets at 4.51 ppm. The larger (8.9 Hz) coupling is characteristic of equatorial–equatorial coupling between H3 and fluorine bonded to C2. Each pseudo-triplet resulted from the overlap of two sets of doublets having an average *J*-value of 3.0 Hz and resulted from the three-bond equatorial–axial couplings, $^{3}J_{H2,H3}$ and $^{3}J_{H3,H4}$. The magnitudes of $^{3}J_{H3,F}$, $^{3}J_{H3,H4}$, and $^{3}J_{H3,H2}$ confirmed the *R*-configuration at C3.

In addition to correlation between H2 and H3, the ^{1}H–^{1}H COSY spectrum showed correlation between H3 and the proton(s) responsible for the complex multiplet centered at 3.78 ppm. Although it was certain that this multiplet included the H4 resonance, it was apparent from peak integration that the multiplet corresponded to two hydrogen atoms. A selective 1D COSY experiment showed that the multiplet's complexity and integrated intensity resulted from overlap of the H4 and H6′ resonances. The magnitudes of the trans-diaxial coupling between H4 and H5 (10.0 Hz) and the three-bond axial–equatorial coupling between H4 and H3 (3.0 Hz) confirmed that the absolute configuration at C4 was *R*. A long-range coupling (1.4 Hz) between H4 and the fluorine bonded to C2 was also observed.

The H5 and the diastereotopic H6 and H6′ resonances were assigned by the use of a ^{1}H–^{1}H COSY experiment as well as by the magnitude of the coupling between H4

Table 2. The compositions (%) of D-allose and D-gulose and their amino sugars in D_2O

Compound	Temp (°C)	Pyranose (%)		Furanose (%)		References
		α	β	α	β	
D-Allose	31	14	77.5	3.5	5	27
D-Gulose	22	16	81	—	3	28
2-Acetamido-2-deoxy-D-allose	23–25	14	72	9	5	26
2-Acetamido-2-deoxy-D-gulose	23–25	17	74	3	6	26

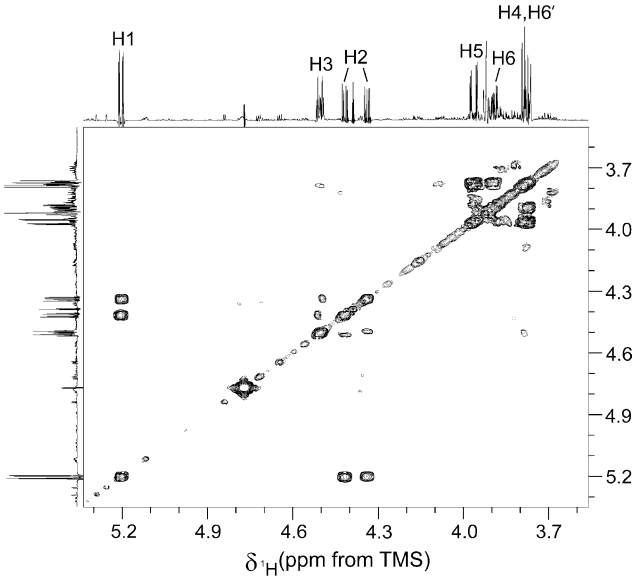


Figure 2. The ^1H–^1H gradient COSY spectrum (D_2O solvent at 25 °C) of the products resulting from the electrophilic fluorination of TAG in aHF.

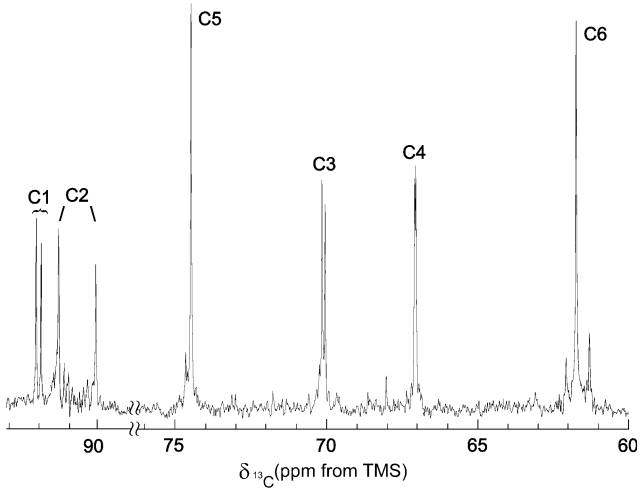


Figure 3. The ^{13}C NMR spectrum (D_2O solvent at 25 °C) of the products resulting from the electrophilic fluorination of TAG in aHF.

Table 3. ^{13}C NMR parameters for 2-FDβA

^{13}C	Chemical shift[a] (ppm)		Multiplicity[b]	Coupling constant (Hz)
C1	91.93	(96.4)	d	$^2J_{C1,F} = 24.5$
C2	90.67	(74.2)	d	$^1J_{C2,F} = 185.5$
C3	70.09	(74.3)	d	$^2J_{C3,F} = 15.9$
C4	67.05	(69.5)	d	$^3J_{C4,F} = 5.5$
C5	74.47	(76.5)	s	
C6	61.75	(64.1)	s	

[a] Chemical shifts in parentheses are those of β-D-allose.[29]
[b] The abbreviations denote doublet (d) and singlet (s).

and H5. The doublet of doublets centered at 3.90 ppm was assigned to H5. As noted above, the 10.0 Hz coupling arose from $^3J_{H5,H4}$. The two smaller couplings, 5.8 and 2.3 Hz, corresponded to $^3J_{H5,H6}$ and $^3J_{H5,H6'}$, respectively, which arose from coupling of H5 to the diastereotopic H6 (3.97 ppm) and H6′ (3.78 ppm) protons.

Assignments of the ^{13}C resonances (Fig. 3 and Table 3) were aided by the ^{13}C–^{19}F coupling constants and by a ^{13}C–^1H heteronuclear single quantum correlation (HSQC) experiment. The ^{13}C NMR spectrum provided further evidence that Scheme 1 produced 2-FDβA as the major carbohydrate product. The ^{13}C resonance of the anomeric carbon, C1, appeared as a doublet (d) at the highest frequency, 91.93 ppm, with $^2J_{C1,F} = 24.5$ Hz. The chemical shift of C2, bearing the fluorine ligand and being adjacent to the anomeric carbon atom, occurred at 90.67 ppm and gave rise to a one-bond ^{13}C–^{19}F coupling (185.5 Hz) that was consistent with fluorine bonded to an sp^3 hybridized carbon. Carbon-3 resonance appeared at 70.09 ppm and the magnitude of the $^2J_{C3,F}$ coupling was 15.9 Hz. The three-bond coupling between C4, which appeared at 67.05 ppm, and the fluorine environment was 5.5 Hz. The chemical shift of C5 (74.47 ppm) appeared at higher frequency than those of C3 and C4, because C5 is bonded to the ring oxygen. The C6 resonance (61.75 ppm) appeared at the lowest frequency.

The 1D ^{19}F NMR spectrum of the products (Fig. 4) showed an intense doublet of doublets of pseudo-triplets centered at −199.99 ppm, corresponding to 2-FDβA, along with a weak, unassigned doublet of doublets centered at −199.44 ppm. The largest coupling (47.0 Hz) was assigned to the two-bond coupling between fluorine and H2. The three-bond coupling between fluorine and H3 was 8.2 Hz. Finally, the pseudo-triplet resulted from overlap of two doublets (average J-value, ca. 1.4 Hz) corresponding to $^3J_{F,H1}$ and $^4J_{F,H4}$. The 1D ^{19}F NMR spectrum confirmed that 2-FDβA was the major product of the electrophilic fluorination of TAG in aHF. It

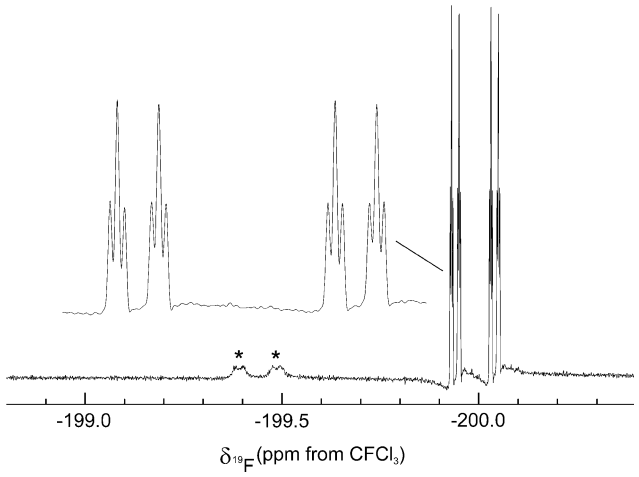


Figure 4. The ^{19}F NMR spectrum (D_2O solvent at 30 °C) of the products resulting from the electrophilic fluorination of TAG in aHF. The spectrum was processed using Gaussian multiplication. Asterisks (*) denotes an unassigned resonance ('doublet of doublets') arising from a minor component ($\delta(^{19}F)$, 199.44 ppm; $^2J_{F,H}$, 44.35 Hz and $^3J_{F,H}$, 7.67 Hz) characteristic of a fluorocarbohydrate.

is significant that neither 2-FDG nor 2-FDM was produced in this reaction, whereas electrophilic fluorinations of TAG with F_2 and AcOF in any other solvent medium result in both 2-FDG and 2-FDM[13,30–34] (also see synthesis of 2-[^{18}F]FDβA).

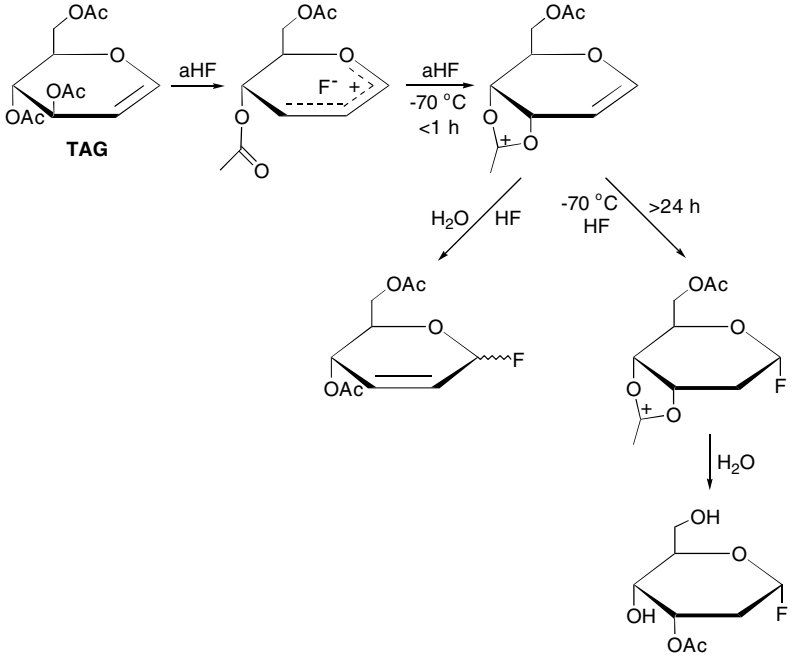
2.3. Mass spectrometric results

Negative ion electrospray ionization mass spectrometry of the reaction products resulting from Scheme 1 also confirmed that 2-FDβA was the major product. The spectrum showed the deprotonated molecular ion, $[M-H]^-$, corresponding to 2-FDβA at *m*/*z* 181. However, the base peak in the spectrum appeared at *m*/*z* 217, which corresponds to the chloride adduct of neutral 2-FDβA.

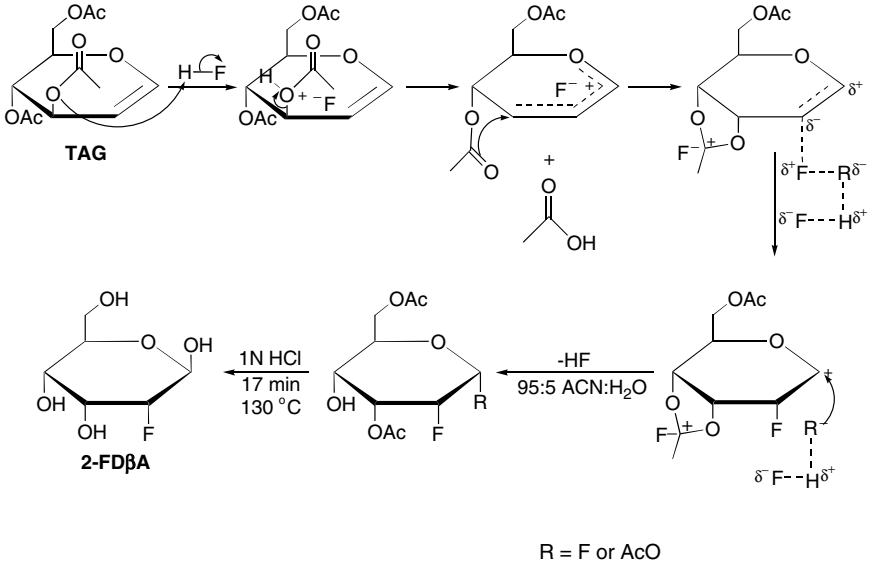
2.4. Proposed mechanistic route to 2-FDβA

Fluorination of glycals by F_2 and AcOF in polar solvents and aqueous solutions usually results in difluoro and 2-deoxy-2-fluoro intermediates, respectively, in which the electrophilic fluorinating agent is added across the double bond to give a *syn*-arrangement.[12–14] The absence of an *anti*-isomer is attributed to the fact that, unlike other halogens, fluorine cannot form a halonium ion. Therefore, the initial fluorocarbonium ion intermediate resulting from electrophilic attack of C2 by fluorine is not bridged.[35] The carbonium ion rapidly combines with the counter ion, F^- or AcO^-. Both the fluorine ligand and acetoxy group on C1 are susceptible to hydrolysis in dilute acid media.

Lundt and Pedersen[10] proposed that the reaction of TAG with aHF (Scheme 2) proceeded by protonation of the oxygen atom bonded to C3 and subsequent cleavage of the *O*-acetyl group was likely assisted by attack from the acyloxy group at C4. These workers showed, from a freshly prepared aHF solution of TAG that the double bond of the corresponding 1,2-unsaturated 3,4-dioxolenium ion was initially intact at −70 °C. After a period of 24 h, the ^1H NMR spectrum showed that aHF had added across the double bond of the



Scheme 2. Reaction of TAG with aHF.[10]



Scheme 3. A proposed partial mechanism for the synthesis of 2-FDβA by electrophilic fluorination of TAG in aHF.

dioxolenium ion in a Markovnikov fashion. The presence of a small amount of water hydrolyzed the dioxolenium ion and resulted in epimerization at C3.[10]

In the present study, fluorinations were routinely carried out at −60 °C within 30 min following addition of aHF to TAG, thus avoiding significant HF addition to the double bond of the 1,2-unsaturated dioxolenium ion prior to fluorination. A proposed partial mechanism for the synthesis of 2-FDβA in the present work is provided in Scheme 3, which incorporates the existing synthetic route to 2-deoxy-2-fluoro sugars and the proposed synthetic route[10] that gives rise to the 1,2-unsaturated 3,4-dioxolenium ion and its epimerization at C3.

The exclusive existence of the β-anomer of 2-deoxy-2-(*R*)-fluoro-D-allose, as indicated in the ^{1}H and ^{13}C NMR spectra, is consistent with the data presented in Table 2. The high stereoselectivity of TAG toward fluorination in aHF to give 2-deoxy-2-(*R*)-fluoro-D-allose, however, has yet to be accounted for.

2.5. Synthesis of 2-FDβA in weakly acidic media

The reactivities of F_2 toward TAG in weakly acidic media, namely trifluoroacetic acid (TFA) and formic acid (FA), were also studied. These reactions, however, provided significantly lower stereoselectivities for 2-FDβA than those carried out in aHF. In both TFA and FA, electrophilic fluorination of TAG produced 2-FDG, 2-FDM and 2-FDβA in 1.3:1.0:1.6 ratios, based on their integrated ^{19}F NMR spectra. This demonstrates that TAG does not completely convert to the 1,2-unsaturated 3,4-dioxolenium ion in weakly acidic media and that a significant amount of the precursor is intact at the time of fluorination.

2.6. Synthesis of ^{18}F-labeled 2-FDβA

To determine an overall product yield with respect to F_2, the electrophilic fluorination of TAG in aHF was carried out using $[^{18}F]F_2$ as the electrophilic fluorinator. The overall decay-corrected radiochemical yield (RCY) of the resulting 2-[^{18}F]FDβA was 33 ± 3% with respect to $[^{18}F]F_2$, which is the highest RCY achieved to date for electrophilic fluorination of TAG using $[^{18}F]F_2$ in any solvent system. The radiochemical purity of 2-[^{18}F]FDβA was 96.3 ± 3 and 85 ± 7%, as determined by radio-HPLC and radio-TLC, respectively. Furthermore, ^{19}F NMR studies showed that neither 2-[^{18}F]FDG nor 2-[^{18}F]FDM were produced. The former is widely used for the study of local glucose metabolism in humans and the latter is a significant contaminant when 2-[^{18}F]FDG is produced by electrophilic fluorination methods. The absence of 2-[^{18}F]FDG and 2-[^{18}F]FDM in 2-[^{18}F]FDβA is a significant factor when used for PET imaging purposes, because the presence of either contaminant would contribute to background noise, reducing the signal-to-noise ratio in the resulting image, and would unnecessarily increase the radiation dose to the patient.

3. Conclusion

A reliable and rapid two-step highly regio- and stereoselective synthesis of 2-FDβA by electrophilic fluorination of TAG in aHF has been achieved. The high regioselectivity results from exclusive fluorination at C2. Stereoselectivity resulted in an *R* configuration at C2 of the product, whereas all other known electrophilic fluorinations of TAG result in products having both *R* and *S*

configurations at C2. Such high regio- and stereoselectivities are often hard to achieve from electrophilic fluorinations. The total synthesis time was approximately 45 min, which is more rapid and efficient than the existing synthesis of 2-deoxy-2-fluoro-D-allose reported by Johansson and Lindberg.[5] In addition, 2-[^{18}F]FDβA has been synthesized for the first time with an overall decay-corrected RCY of 33 ± 3% with respect to [^{18}F]F_2. Work is underway to determine the potential application of 2-[^{18}F]FDβA in PET as a tracer for D-allose metabolism.

4. Experimental

Caution: It is recommended that proper first-aid treatment procedures[36–38] be known and available to all laboratory personnel prior to repeating portions of this study that deal with the use of aHF and F_2. Skin contact with even small amounts of aHF or F_2 may result in painful burns if immediate and proper treatment is not given. Any incident involving direct contact with liquid aHF, HF vapor, F_2 gas, and aqueous solutions of HF must be aggressively treated and brought to the attention of qualified medical personnel for appropriate follow-up treatment.

4.1. Standard techniques

The use of radioactive isotopes was carried out in a safe and effective manner in compliance with all requirements of the Canadian Nuclear Safety Commission (CNSC) and Radioisotope Protection Committee (RPC).

4.2. Materials

Enriched [^{18}O]O_2 (^{18}O, 99 at. %, Isotec), neon (99.999%, Air Products), 1% F_2 in neon (Canadian Liquid Air), helium (99.9999%, Matheson), anhydrous hydrogen fluoride (Air Products, 99.9%), trifluoroacetic acid (Caledon, 99%), formic acid (Sigma–Aldrich; 98–100%), 3,4,6-tri-*O*-acetyl-D-glucal (Aldrich, 98%), anhydrous ethyl ether (Aldrich, 99.0%), and HPLC grade acetonitrile (Caledon, 99.8%) were used without further purification and/or drying. Sterile, deionized water was used in all aqueous procedures.

4.3. Production of [^{18}F]F_2

Fluorine-18 labeled F_2 was produced by the ^{18}O(p,n)^{18}F nuclear reaction using a Siemens RDS 112 proton cyclotron operating at 11 MeV by the use of 'double shoot' method[39] in the Nuclear Medicine Department, Hamilton Health Sciences as previously described.[40] An aluminum target (11 mL) was pressurized to 15–17 atm with enriched [^{18}O]O_2 and irradiated for 30 min using a 30 μA proton beam (*production shoot*). After irradiation, the [^{18}O]O_2 was recovered from the target by condensing it at −196 °C onto molecular sieves (Varian VacSorb) contained in a 316 stainless steel Whitey® cylinder (75 mL). The target was then evacuated to remove residual ^{18}O, flushed with neon (ca. 7 atm), and re-evacuated. The target was then filled with 1% F_2 (30–50 μmol) in neon, pressurized to 20 atm with neon, and irradiated for 15 min in a 15 μA proton beam (*recovery shoot*). The [^{18}F]F_2 was sporadically released from the target into a continuous stream of helium (10 mL/min) until the target pressure dropped to 2 atm. Helium was used as the sweep gas to transfer [^{18}F]F_2 from the target into the hot cell and to help dissipate the heat evolved during the fluorination reaction.

4.4. Electrophilic fluorinations of TAG in aHF using F_2 and [^{18}F]F_2

TAG (20 mg; 73.5 μmol) was loaded into a 5/16 in. o.d. × 5/32 in. i.d. FEP (perfluoroethylene/perfluoropropylene co-polymer) reaction vessel and attached to a Kel-F (trifluorochloropolyethylene polymer) Y-piece with 1/4 in. o.d. × 1/8 in. i.d. ends. One arm of the Y-piece was connected through a Kel-F valve to a vacuum line used to dispense aHF. A 1/16 in. o.d. × 1/32 in. i.d. length of Teflon tubing was fed through a 1/4 in. o.d. × 1/16 in. Teflon® Swagelok® reducing union and connected to the remaining arm of the Y-piece. The Teflon® tube was fed into and to the bottom of the FEP reaction vessel and sealed at the 1/16 in. Teflon® union connection. Anhydrous HF was condensed into the reaction vessel at −196 °C. The reaction vessel and contents were equilibrated at −60 °C and the reaction vessel was disconnected from the vacuum line. The Teflon® tube was connected to a stainless steel vacuum manifold (28.5 mL) used to dispense F_2. The initial manifold pressure of 8 atm of 1% F_2 (ca. 95 μmol) in neon was adjusted to 27 atm with helium. The remaining arm of the Y-piece was connected to a separate 1/16 in. o.d. Teflon® tube, which was led into a 1 N NaOH solution that served to trap the HF and unreacted F_2 gases as they emerged from the reaction vessel. Approximately 20 atm of the resulting F_2/Ne/He mixture (ca. 70 μmol of F_2) was passed through the substrate solution.

When electrophilic fluorinations were carried out using [^{18}F]F_2, the reactor configuration remained unchanged except that the Teflon® tube was connected to the [^{18}F]F_2 target line. A helium sweep gas line was connected to the [^{18}F]F_2 target line (between the target and the reaction vessel) and helium was slowly passed through the line. Aliquots of [^{18}F]F_2 gas were released from the target by periodically opening and closing the target valve. Fluorine-18 labeled F_2 gas and the helium sweep gas were passed through the reaction vessel

containing the substrate solution. The effluent gas from the reaction vessel was trapped in the NaOH solution before it was vented into the hot cell. The amount of $[^{18}F]F_2$ that had reacted was determined by counting the amount of radioactivity present in the reaction mixture.

Once the desired amount of F_2 or $[^{18}F]F_2$ gas had passed through the substrate solution, the reaction vessel was disconnected from the F_2 line and the NaOH trap and the HF solvent was removed under dynamic vacuum and trapped in a FEP U-tube cooled to −196 °C.

4.5. Gas–solid phase generation of CH_3COOF and $[^{18}F]CH_3COOF$ from $KOAc(HOAc)_{1.5}$

The complex, $KOAc(HOAc)_{1.5}$, was prepared as previously described.[41] A 2 in. long 1/4 in. o.d. × 1/8 in. i.d. FEP column, fitted with a 1/4 in. o.d. × 1/16 in. Teflon® Swagelok® reducing union on one end and a 1/4 in. to 1/16 in. stainless steel Swagelok® reducing union on the other end, was packed to a depth of 1 in. with $KOAc(HOAc)_{1.5}$. The Teflon® Swagelok® union was connected to a 1/16 in. o.d. stainless steel F_2 line and 8 atm of 1% F_2 in neon was diluted to 27 atm with helium inside a stainless steel vacuum manifold (28.5 mL). Approximately 20 atm of the resulting F_2/Ne/He mixture was passed through the column containing the $KOAc(HOAc)_{1.5}$ complex, which resulted in the formation of CH_3COOF gas.

In the case of $[^{18}F]CH_3COOF$, the $KOAc(HOAc)_{1.5}$ column was connected, through the Teflon® Swagelok® union, to a 1/16 in. stainless steel tube leading from the $[^{18}F]F_2$ target cell. After irradiation, the target gas was slowly metered through the $KOAc(HOAc)_{1.5}$ complex, forming $[^{18}F]CH_3COOF$ gas.

4.6. Electrophilic fluorinations of TAG in aHF using CH_3COOF and $[^{18}F]CH_3COOF$

The reaction conditions were identical to those described above. The $KOAc(HOAc)_{1.5}$ column used to generate CH_3COOF and $[^{18}F]CH_3COOF$ gas was connected to a 1/16 in. o.d. × 1/32 in. i.d. Teflon® tube, which was fed through one of the arms of a Kel-F Y-piece into a 5/16 in. o.d. × 5/32 in. i.d. FEP reaction vessel containing TAG (20 mg; 73.5 μmol) in aHF (ca. 1 mL) and connected as described above. The fluorination was carried out using the procedure described above. Once the desired amount of CH_3COOF or $[^{18}F]CH_3COOF$ had passed through the substrate solution, the reaction vessel was disconnected from the CH_3COOF or $[^{18}F]CH_3COOF$ line and the NaOH trap. Anhydrous HF solvent was removed under vacuum by pumping through an FEP U-tube cooled to −196 °C.

4.7. Hydrolysis of the reaction intermediate resulting from electrophilic fluorination of TAG

The reaction intermediate was recovered from the reaction vessel by dissolution in a 5% water/CH_3CN, followed by two rinsings with the same solvent mixture. The combined rinsings were then passed through a silica Sep-Pak® (Waters Corporation, Milford, Massachusetts, USA) and collected in a 10 mL Reacti-Vial® equipped with a magnetic stirring bar. The solvent was evaporated to dryness under a slow stream of nitrogen gas in a Reacti-Therm® (Pierce) set at 130 °C. A 2.0 mL aliquot of 1 N HCl was added to the Reacti-Vial® and the residue was hydrolyzed at 130 °C for 17 min.

4.8. Separation and purification of the hydrolysis product by liquid chromatography (LC)

A 1 cm o.d. × 17 cm long column (Bio-Rad) was packed with ion retardation resin (Bio-Rad AG® 11 A8, 50–100 mesh) and the column was washed with water. An alumina Sep-Pak® (Waters Corporation, Milford, Massachusetts, USA) was conditioned with 10 mL of water and attached to the end of the ion exchange column. A C_{18} Sep-Pak® (Waters Corporation, Milford, Massachusetts, USA) was conditioned with 4 mL of absolute ethanol followed by 10 mL of water and attached to the end of the alumina Sep-Pak®. The end of the C_{18} Sep-Pak® was attached to a 30 mL multi-dose vial through a 0.2 μM filter.

The hydrolysis product was added to the top of the LC column and eluted with 10 mL of water into the 30 mL multi-dose vial. Ionic species and unhydrolyzed organic compounds remained on the column while ~10 mL of the hydrolyzed product solution was collected in the multi-dose vial.

4.9. NMR spectroscopy

NMR samples were prepared by drying an aqueous solution of the final products on a rotary evaporator and redissolving the resulting thin film in D_2O. Samples were allowed to equilibrate for in excess of 36 h before recording their NMR spectra. Samples were locked to deuterium in D_2O and spectra were acquired without spinning the samples. Spectra were externally referenced with respect to neat TMS (^1H and ^{13}C, 25 °C) and neat $CFCl_3$ (^{19}F, 30 °C).

Proton and ^{13}C NMR spectra were recorded at 600.130 and 150.903 MHz, respectively, on a Bruker Avance 600 MHz NMR spectrometer using a 5-mm triple broad band inverse probe. The sample temperature was maintained at 25 °C by means of a BVT 3000 digital temperature controller.

Proton NMR spectra were obtained in 48 scans in 64 K data points over a 5.00 kHz spectral width corresponding to an acquisition time of 6.55 s and a digital resolution of 0.08 Hz/point. The residual HDO solvent peak was suppressed by presaturation during a 1.5 s relaxation delay between acquisitions. The data were zero-filled to 128 K before Fourier transformation. The free induction decays (FIDs) were processed using Gaussian multiplication with a Gaussian broadening of 0.1 and −2.00 Hz line broadening.

Proton correlation spectroscopy (COSY) spectra were recorded in the absolute value mode using the pulse sequence 90°-t_1-45°-ACQ and included pulse field gradients for coherence selection. The data were acquired in 16 scans for each of the 256 FIDs that contained 2 K data points in the F_2 dimension over a 5.00 kHz spectral width. The ^{1}H 90° pulse width was 8.0 μs. A 1.0 s relaxation delay was used between acquisitions. Zero-filling in the F_1 dimension produced a 1 K × 1 K data matrix with a digital resolution of 4.88 Hz/point in both dimensions. During the 2D Fourier transformation, a sine-bell squared window function was applied to both dimensions. The transformed data were then symmetrized.

Selective 1D total correlation spectroscopy (TOCSY) ^{1}H spectra with pulsed field gradients were recorded over an 8.09 kHz spectral width in 64 K data points corresponding to a 4.05 s acquisition time. Selective excitation was provided by Gaussian-shaped pulses with a 180° pulse width corresponding to 83.4 ms. This pulse was followed by the standard TOCSY MLEV-17 spin-lock pulse sequence. The 90° spin-lock pulse width was 63.0 μs. A 1.0 s relaxation delay was used. The spin-lock period was 100 ms, which was followed by a z-filter that contained 10 variable delay times ranging from 4 to 18 ms. The transmitter offset was adjusted to the frequency of the ^{1}H resonance being selectively excited. The spectra were acquired in 200 scans and the FIDs were processed using exponential multiplication with a 0.20 Hz line broadening and were zero-filled to 128 K of memory before Fourier transformation.

A selective 1D COSY ^{1}H spectrum was obtained in 320 scans over an 8.09 kHz spectral width in 64 K data points corresponding to a 4.05 s acquisition time. The data were processed in the same manner as for the selective 1D TOCSY ^{1}H spectra discussed above.

The ^{13}C NMR spectrum was acquired over a 36.231 kHz spectral width in 53,100 scans in 32 K data points with an acquisition time of 0.45 s and a data point resolution of 1.11 Hz/point. The ^{13}C pulse width was 4 μs (30° pulse angle) and a relaxation delay of 0.5 s was employed. The FIDs were zero-filled to 64 K prior to Fourier transformation and were processed using exponential multiplication with a line broadening of 4.0 Hz and linear back-prediction.

An inverse-detected ^{1}H–^{13}C 2D chemical shift correlation spectrum was acquired in the phase-sensitive mode using the pulsed field gradient version of the heteronuclear single quantum coherence (HSQC) pulse sequence. The FIDs in the F_2 (^{1}H) dimension were recorded over a 5.00 kHz spectral width in 2 K data points. The 256 FIDs in the F_1 (^{13}C) dimension were obtained over a 27.10 kHz spectral width. Each FID was acquired in 32 scans. The fixed delays during the pulse sequence were a 1.0 s relaxation delay and a delay for polarization transfer of 0.001786 s. The 90° ^{1}H and ^{13}C pulses were 8.0 and 14.0 μs, respectively. The data were processed using a sine-bell squared window function shifted by π/2 in both dimensions and a linear prediction to 256 data points in the F_1 dimension followed by zero-filling to 1 K.

Fluorine-19 NMR spectra were recorded at 470.592 MHz on a Bruker Avance DRX-500 NMR spectrometer using a 5-mm broad band inverse probe with the ^{1}H coil tuned to the ^{19}F frequency. The spectra were obtained over a 6.89 kHz spectral width in 64 K data points corresponding to an acquisition time of 4.76 s and a data point resolution of 0.11 Hz/point. Adequate signal-to-noise was achieved in 60 scans. The ^{19}F pulse width was 2.5 μs (30° pulse angle) and a relaxation delay of 1.0 s was used. The FIDs were processed using Gaussian multiplication with a Gaussian broadening of 0.2 and −1.50 Hz line broadening. The data were zero-filled to 128 K before Fourier transformation. The sample temperature was maintained at 30 °C by means of a Bruker BVT 3000 digital variable temperature unit.

4.10. Mass spectrometry

The mass spectrometric analyses were carried out on a Waters Micromass Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray ionization interface. Samples were prepared in water, which was also used as the blank. The blank and analytes were injected by use of a pneumatically assisted Rheodyne 7010 injector equipped with a 10 μL injection loop at a flow rate of 10 μL/min.

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