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Stereospecific fluorination of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-sulfonate esters: preparation of a versatile intermediate for synthesis of 2′-[^{18}F]-fluoro-arabinonucleosides

Mian M. Alauddin[a], Peter S. Conti[a,*,1], Thomas Mathew[b], John D. Fissekis[a], G.K. Surya Prakash[b], Kyoichi A. Watanabe[c,2]

[a]*PET Imaging Science Center, University of Southern California, Los Angles, CA 90033, USA*
[b]*Loker Hydrocarbon Research Institute, University of Southern California, Los Angeles, CA 90033, USA*
[c]*Laboratory of Organic Chemistry, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*

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Abstract

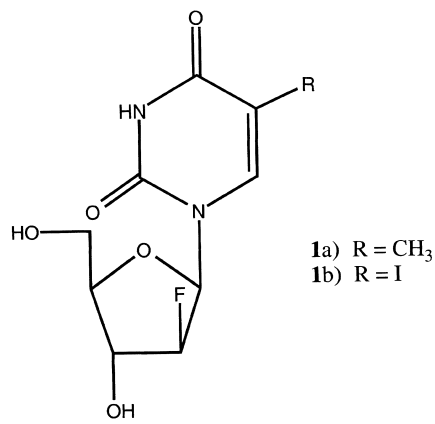
A detailed investigation on fluorination of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-sulphonate esters is reported. Various combinations of sulfonate esters, fluorinating agents and solvents were evaluated in this study. Organic ammonium fluoride, in particular *n*-Bu_4NF, was found to be better fluorinating agent than inorganic fluoride, and 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-trifluoromethylsulphonate ester appeared to be the best substrate. The developed method is suitable for stereospecific (*arabino*) incorporation of radiofluorine (^{18}F) into the sugar moiety.

Keywords: Fluorination; *n*-Bu_4NF; Nucleophilic radiofluorine

1. Introduction

Pyrimidine-1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-nucleosides and their radiolabeled derivatives are potentially important compounds [1–5]. 2′-Fluoro-5-[^{11}*C*-methyl]-1-β-D-arabinofuranosyluracil (^{11}C-FMAU) **1a** (Fig. 1) is being studied as a marker for cell proliferation by positron emission tomography (PET) [3,4]. In addition, radiolabeled 2′-fluoro-5-iodo-1-β-D-arabino-furanosyluracil (FIAU) **1b** is considered to be a useful agent for imaging the expression of transfected genes in vivo [5]. ^{11}C-FMAU **1a** was first synthesized in our laboratory [3], in quantities suitable for animal and patient studies. However, the short half-life of C-11 ($t_{1/2}$=20 min) limits the clinical application of that compound. Therefore, labeling with the longer-lived PET isotope fluorine-18 ($t_{1/2}$=110 min) in the glycone would be more advantageous.

The direct, stereospecific (*arabino*) introduction of fluorine by an S_N2 substitution at the 2′-position of the furanosyl moiety in a pyrimidine nucleoside has not been possible [1,6–8]. Alternatively, the incorporation of fluorine in the *arabino* configuration at C-2 of the sugar followed by coupling with the pyrimidine base has been successful [1,9–12]. The common methods for the stereospecific (*arabino*) incorporation of fluorine involve use of KHF_2 or $Et_3N{\cdot}3HF$ as fluorinating agent in excess (6 eq) [11,13], and require long reaction times (6 h). These methods are not, however, satisfactory for incorporation of radiofluorine.



1a) R = CH_3
1b) R = I

Fig. 1. Structure of FMAU (**1a**) and FIAU (**1b**).

* Corresponding author. Tel.: +1-323-442-5940; fax: +1-323-442-5778.
E-mail address: pconti@hsc.usu.edu (P.S. Conti).
[1] Present address: Suite 101, 2250 Alcazar st. Los Angeles, CA 90033, USA.
[2] Present address: Pharmasset, Inc., 1860 Montreal Road, Tucker, GA 30084, USA.

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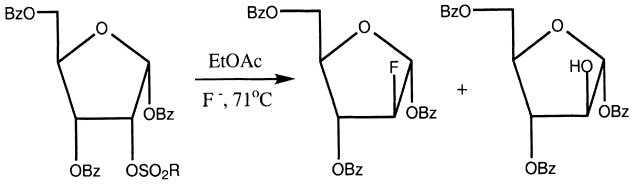

2a) R = F
2b) R = CH_3
2c) R = CF_3
2d) R = $PhCH_3$

Fig. 2. General methodology of fluorination reactions.

Earlier, we developed a method suitable for stereospecific (*arabino*) incorporation of radioactive fluorine in the 2-position of the protected sugar [14,15]. The chemical yield was variable, but could be as high as 30%. However, the radiochemical yield was quite low (2–6%). In our continuing effort towards the synthesis of ^{18}F-FMAU, we have investigated reactions of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-sulphonate esters **2a–c** with several fluorinating agents, under a variety of experimental conditions (Fig. 2). Here, we report a procedure for stereospecific (*arabino*) incorporation of radiofluorine into the protected sugar, much superior to the currently available method in terms of simplicity and radiochemical yield.

2. Experimental

2.1. Reagents and instrumentation

All reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and used without further purification. 2-*O*-(Fluorosulfonyl)-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose **2a**, 2-*O*-(methylsulfonyl)-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose **2b** and 2-*O*-(trifluoromethylsulfonyl)-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose **2c** were prepared following literature methods [11,13].

Proton and ^{19}F NMR spectra were recorded on a Brucker 360 or 500 MHz spectrometer in chloroform-D_3 using tetramethylsilane as an internal reference, and trichlorofluoromethane as an external reference, respectively. Mass spectra were obtained on a Finnigan 400 mass spectrometer at the University of Minnesota using ammonia chemical ionization technique. High performance liquid chromatography (HPLC) was performed on a Waters Associates system using a 510 pump, UV detector (Isco) operated at 254 nm, and a radioactivity detector with single-channel analyzer (Technical Associates, Canoga Park, CA) using semi-preparative or analytical C_{18} reverse phase columns (Alltech). A MeCN/H_2O solvent system (70% MeCN) was used.

2.2. Fluorinating reagents

Tetraethylammonium fluoride (Et_4NF), tetrapropylammonium fluoride (n-Pr_4NF) and tetrabutylammonium fluoride (n-Bu_4NF) were prepared from their hydroxy-compounds by neutralization with aqueous hydrofluoric acid (48% HF) to pH 7.00. The aqueous solution was dried by azeotropic evaporation of water with acetonitrile before the fluorination reaction. Triethylamine tri-(hydrogen fluoride) ($Et_3N{\cdot}3HF$) was prepared by adding cold, anhydrous HF into Et_3N at low temperature (dry ice acetone).

2.3. 2-Deoxy-2-fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose 3

All fluorination reactions were performed in a similar manner; a representative procedure is described here. Each precursor (fluorosulfonate, methylsulfonate or trifluoromethylsulfonate, 10–12 mg) was dissolved in either EtOAc or MeCN (0.5 ml), and the solution was added to the dry fluorination reagent under argon in a V-vial. The vial was heated in a heating block at 71–72°C for 20 min, when TLC (12% acetone in hexane) showed no starting material remained. The reaction mixture was cooled to room temperature, solvent evaporated, and purified by HPLC (C_{18} semi-prep. column, 70% MeCN/H_2O, flow 4.5 ml/min). The appropriate fraction was collected and evaporated to dryness to provide the pure product. ^{1}H NMR: δ=8.05–8.15 (m, 6H, aromatic), 7.55–7.64 (m, 3H, aromatic), 7.39–7.48 (m, 6H, aromatic), 6.74 (d, 1H, C_1H, J=8.6 Hz), 5.61 (dd, 1H, C_3H, J =19.4 and 2.9 Hz), 5.37 (d, 1H, C_2H, J=48.6 Hz), 4.68–4.79 (m, 3H, C_4H and C_5H). ^{19}F NMR: δ=−190.8. MS: 465 (M+1, 10), 391 (35), 343 (75), 242 (100).

2.4. 1,3,5-Tri-O-benzoyl-α-D-arabinofuranose 4

This compound was essentially the major product in most of the fluorination reactions. It was isolated during HPLC purification of the crude material in 44–60% yield, and characterized by ^{1}H NMR spectroscopy and mass spectrometry. ^{1}H NMR: δ=8.04–8.14 (m, 6H, aromatic), 7.60–7.66 (m, 3H, aromatic), 7.41–7.49 (m, 6H, aromatic), 6.75 (s, 1H, C_1H), 5.82 (s, 1H, C_2H), 5.68 (d, 1H, C_3H, J =3.5 Hz), 4.81–4.84 (m, 1H, C_4H), 4.71–4.75 (m, 2H, C_5H). MS: 461 (M−1, 5), 445 (100), 242 (85), 201 (70).

2.5. 2-Acetyl-1,3,5-tri-O-benzoyl-α-D-arabinofuranose 5

The precursor (**2a**, 7.7 mg) was dissolved in EtOAc (0.5 ml) in a V-vial under argon. Acetic acid (1.2 eq) and n-Bu_4NF (1.2 eq) were added and the reaction mixture was heated in a heating block at 71–72°C for 95 min. The reaction mixture was cooled to room temperature, solvent evaporated, and purified by HPLC. The desired product was isolated in 75% yield. ^{1}H NMR: δ=8.07–8.12 (m, 6H, aromatic), 7.55–7.64 (m, 3H, aromatic), 7.41–7.47 (m, 6H, aromatic), 6.58 (s, 1H, C_1H), 5.58 (s, 1H, C_2H), 5.52 (d, 1H, C_3H, J=3.4 Hz), 4.71–4.77 (m, 3H, C_4H and C_5H), 2.09 (s, 3H, acetate). MS: 522, M+NH_4 (42), 383 (100), 343

(28), 104 (65). Exact mass calculated for $C_{28}H_{28}NO_9$ ($M+NH_4$) 522.1766, found 522.1766.

2.6. 2-Deoxy-2-[^{18}F]-fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose **3**

[^{18}F]-Fluoride was reacted with *n*-Bu_4NHCO_3 and evaporated to dryness azeotropicaly with MeCN (1.2 ml). To the dry residue, *n*-$Bu_4N^{18}F$, a solution of **2a** or **2c** (5–7 mg) in ethyl acetate (0.5 ml) was added, and the reaction mixture was heated at 71–72°C for 20 min. The crude reaction mixture was passed through a sep-pack cartridge (silica gel), and eluted with EtOAc. After evaporation of the solvent, the residue was dissolved in the HPLC solvent (70% MeCN/H_2O), and chromatographed using a C_{18} semi-prep column. The appropriate fraction containing the radioactive species was isolated and evaporated to dryness. The radiochemical yields were 32–41% for **2c** and 3–6% for **2a**, respectively, both in >99% radiochemical purity.

3. Results and discussion

The precursors, fluorosulfonate **2a**, mesylate **2b** and triflate **2c** were prepared following literature methods in high yields [11,13]. Like other investigators, we were not successful in preparing the tosylate **2d** by the reaction of tosyl chloride on 1,3,5-tri-*O*-benzoyl ribofuranose [11]. We carried out extensive studies on the preparation of 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose **3** by fluorination of the fluorosulfonate **2a**. Most of these experiments were performed in EtOAc, which has been reported to be the best solvent for this reaction [13]. In addition, MeCN and DMF were also used in some experiments for comparison. Fig. 2 represents the general methodology of such fluorination reactions.

Table 1 represents the results of fluorination with various substrates, fluorinating agents, solvents and time at 71–72°C. We have investigated each of three substrates against various fluorinating agents. When reacted with *n*-Bu_4NF, the triflate **2c** and fluorosulfonate **2a** produced **3** in 30–40 and 12–15% yield, respectively. In contrast, the mesylate **2b** did not produce any product. *n*-Bu_4NF in either EtOAc or MeCN led to successful fluorination of **2a** and **2c**, although MeCN was optimal for isolation of the pure product. Fluorinations with either tetra-alkyl ammonium fluorides resulted in relatively higher yield (12–16%) in EtOAc. In MeCN, the yields were slightly lower (10%) in the reactions of Et_4NF and *n*-Pr_4NF, but this difference was not significant in the case of *n*-Bu_4NF. The small difference of yields (Table 1) in the reactions of tetra-alkyl ammonium fluorides with these solvents, may be due to the relative solubility of the reagents, and thus the availability of the fluoride ion in solution. It has been suggested, that the solvent plays an important role in reactions of the fluoride ion [16]. Our results also suggest that choice of solvent is important, with EtOAc appearing to be a better solvent for organic ammonium fluorides in these fluorination reactions in terms of yields. EtOAc was also

Table 1
Fluorination reactions of substrates with various fluorinating agents and solvents at 71–72°C

Substrate	Fluorinating agent	Solvent	Time	Yield (%)
2a	$(Et)_4NF$ (1.2 eq)	EtOAc	20 min	13
2a	$(Et)_4NF$ (1.2 eq)	EtOAc/THF	20 min	9.0
2a	$(Et)_4NF$ (1.2 eq)	MeCN	20 min	10.5
2a	$(nPr)_4NF$ (1.2 eq)	EtOAc	20 min	12.5
2a	$(nPr)_4NF$ (1.2 eq)	MeCN	20 min	10.5
2a	$(n Bu)_4NF$ (1.2 eq)	EtOAc	20 min	15.7
2a	$(n Bu)_4NF$ (1.2 eq)	MeCN	20 min	14.5
2a	CsF (10.0 eq)	EtOAc	45 min	5.0
2a	CsF (10.0 eq)	EtOAc	96 min	10.5
2a	CsF (3.4 eq)	MeCN	45 min	12.3
2a	CsF (3.4 eq)	MeCN	75 min	17.5
2a	CsF (3.3 eq)	DMF	75 min	5.3
2a	CsF (8.7 eq)	DMF	45 min	14.0
2a	$(Et)_3N{\cdot}3HF/(Et)_3N$ (6 eq)	EtOAc	2.5 h	56.0
2a	$(Et)_3N{\cdot}3HF/(Et)_3N$ (6 eq)	EtOAc	4 h	60.5
2a	$(Et)_3N{\cdot}3HF/(Et)_3N$ (6 eq)	MeCN	4 h	35.5
2a	$(n Bu)_4NF+Et_3N{\cdot}3HF$	EtOAc	30 min	30
2a	$(n Bu)_4N$(F-18)$F+Et_3N{\cdot}3HF$	EtOAc	30 min	6[a]
2b	$(n Bu)_4NF$ (1.2 eq)	EtOAc	20 min	no product
2c	$(n Bu)_4NF$ (1.2 eq)	EtOAc	20 min	31
2c	$(n Bu)_4NF$ (1.2 eq)	EtOAc	20 min	34
2c	$(n Bu)_4NF$ (1.2 eq)	EtOAc	20 min	30
2c	$(n Bu)_4N^{18}F$ (trace)	EtOAc	20 min	32[a]
2c	$(n Bu)_4N^{18}F$ (trace)	EtOAc	20 min	41[a]
2c	$(n Bu)_4N^{18}F$ (trace)	EtOAc	20 min	35[a]

[a] Radiochemical yield.

found to be much better solvent in the reactions with the other organic fluoride $Et_3N{\cdot}3HF/Et_3N$, but the reaction time was much longer. Cesium fluoride (CsF), in the presence of a crown ether, produced higher yields in MeCN than in EtOAc. A highly polar aprotic solvent, DMF, however, did not produce a high yield of product with CsF unless the amount of CsF was increased to more than two-fold compared to that used in MeCN.

All reactions using the fluorosulfonate ester **2a** produced low yield of the desired product **3** within 20–30 min except in one instance when two reagents were used. Combination of *n*-Bu_4NF and $Et_3N{\cdot}3HF$ (3:2) appeared to produce the higher yield within shorter reaction time (30 min). The chemical yield could be improved from 15 to 30% by manipulation of the reagent composition, with the radiochemical yield improved proportionally from 3 to 6%. This persistent low radiochemical yield was due to an undesirable competitive exchange, between radioactive fluoride and the covalently bound fluorine on the leaving group. The nucleophilic radiofluorine (fluoride) is consumed by displacing the isotopic fluorine on the sulfonate ester leaving group (Fig. 3) and thus becomes unavailable for the desired substitution reaction. The net result of this facile exchange is a significant reduction in radiochemical yield. Although, **2a** is known to be stable [13], the sulfur fluorine bond have been reported to be very labile [17]. Therefore, it is likely that the radioactive fluoride in solution is equilibrated fast with the non-radioactive fluoride before the apparently slower displacement of the sulfonate ester is completed.

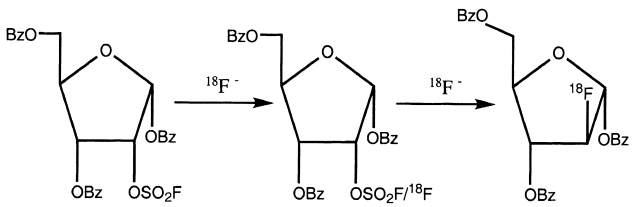

Fig. 3. Exchange reaction between radiofluorine and isotopic stable fluorine.

All fluorinating agents, except $Et_3N{\cdot}3HF/Et_3N$, produced a common by-product (45–60%), which was characterized as 1,3,5-tri-*O*-benzoyl-1-α-D-arabinofuranose **4**. This product was produced by the competitive nucleophilic substitution of the sulfonate esters with a hydroxide ion from either an excess of tetra-alkyl ammonium hydroxide or traces of water present in the reaction mixture. In the reactions with $Et_3N{\cdot}3HF/Et_3N$, this by-product was not formed, since the overall reaction was under acidic conditions.

A second by-product was formed in small amounts when the reactions were performed in EtOAc. It was identified as 2-acetyl-1,3,5,-tri-*O*-benzoyl-1-α-D-arabinofuranose **5**. The formation of **5** may be explained by the nucleophilic substitution of the sulfonate ester by trace of acetate ion present in EtOAc. Fluoride ion is known to be a strong base, a potent hydrogen bond electron donor, and a weak nucleophile [18]. As an electron donor it forms strong hydrogen bonded complex with acetic acid producing acetate nucleophile, which competes with fluoride as a nucleophile and produces the acetoxy compound. In order to verify the formation of the compound **5**, separate experiments were performed with *n*-Bu_4NF (1.2 eq) in the presence of same equivalent acetic acid. The major product in these experiments was **5** in 70–75% yields.

Of the three substrates studied **2a**, **2b**, and **2c**, only **2a** and **2c** produced the desired product. The failure of the mesylate **2b** to yield **3** may be due to an electronic effect; i.e. the mesylate may not be a good leaving group in this system. When fluorination of the triflate **2c** was performed using radioactive fluoride the radiochemical yield was 32–41%. In this reaction, the radioactive fluoride was consumed in the substitution reaction producing high yield of the desired compound. An HPLC chromatogram of radiolabeled fluorosugar **3** is represented in Fig. 4.

Earlier studies demonstrated that the reaction of sulfonate esters **2b** and **2c** with *n*-Bu_4NF resulted, either in an elimination product or in the recovery of the starting material [11]. These authors explained their results on the basis of an earlier suggestion indicating that nucleophilic displacement at C_2 creates an unfavorable alignment of dipoles in the transition state, particularly when the substituent at C_1 is the α-D-anomer [19]. Our results, however, showed the absence of any elimination product or starting material, and instead two products, 2-fluoro-and 2-hydroxy-arabinose derivatives produced by nucleophilic substitution (Fig. 2). These results, therefore, suggest that the fluorosulfonate and trifluormethylsulfonate esters of 1,3,5-tri-*O*-benzoyl-2-ribose do undergo nucleophilic substitution with fluoride, acetate and hydroxide ions, while mesylate is inactive in this system. Furthermore, nucleophilic substitution of trifluoromethanesulfonate ester at the C_2 position of a xylo sugar has also been reported [20]. Since chemical yield in fluorination reaction of sulfonate esters with *n*-But_4NF in EtOAc and in MeCN are comparable, use of MeCN might be advantageous to avoid the formation of the acetate **5** which

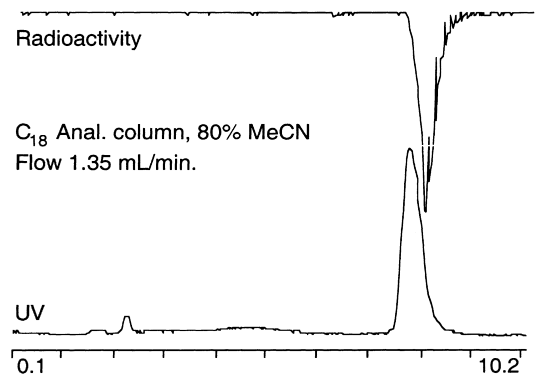

Fig. 4. HPLC chromatogram of [F-18]-labeled-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose.

is, rather, difficult to separate from the desired product during HPLC purification.

4. Conclusion

We have investigated, fluorination of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-sulfonyl esters with several fluorides in various solvents, and developed a suitable method for incorporation of radiofluorine into the protected sugar. The 2-*O*-trifluoromethyl-sulfonyl-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose was found to be the best substrate. Tetrabutylammonium fluoride in either EtOAc or MeCN led to successful fluorination, although MeCN is optimal. For radiolabeling with ^{18}F, the triflate is the optimal substrate, which consistently produces the higher radiochemical yield in the series studied. The 2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose **3**, successfully labeled with ^{18}F, will be a potential intermediate for synthesis of ^{18}F labeled nucleoside analogues for PET imaging.

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