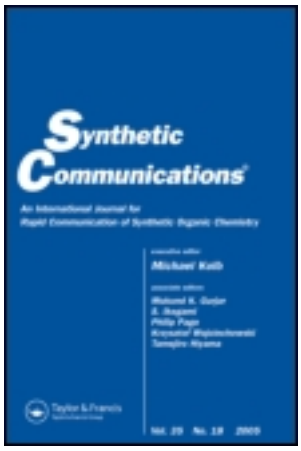

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SYNTHESIS OF 2′-DEOXY-2′-FLUORO-1-β-D-ARABINOFURANOSYL URACIL DERIVATIVES: A METHOD SUITABLE FOR PREPARATION OF [^{18}F]-LABELED NUCLEOSIDES

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ABSTRACT

N-glycosylation of 2,4-*bis*-*O*-(trimethylsilyl)-pyrimidine bases with 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-1-(Br, OBz)-α-D-arabinose derivatives are reported. 1-Bromo-arabinose provides high yield and a favorable anomeric ratio (β/α) of pyrimidine nucleoside in either MeCN or CH_2Cl–CH_2Cl. This method should be suitable for the synthesis of 2′-deoxy-2′-[^{18}F]fluoro-1-β-D-arabinofuranosyluracil derivatives.

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2′-Deoxy-2′-fluoro-5-[^{11}C-methyl]-1-β-D-arabinofuranosyluracil (^{11}C-FMAU), first synthesized in our laboratory, is being studied as a marker for cell proliferation by positron emission tomography (PET).[1–3] However, the short half-life of ^{11}C ($t_{1/2} = 20$ min) limits the clinical application of the compound. The corresponding analogue labeled with fluorine-18 ($t_{1/2} = 110$ min) would be more advantageous.

Attempted syntheses of 2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyluracils by the direct introduction of fluorine have hitherto failed.[4,5] However, the incorporation of fluorine in the *arabino* configuration at C-2 of the sugar followed by coupling with the pyrimidine base has been successful toward the synthesis of these nucleosides.[6–8] The most common method involves conversion of the protected sugar to its 1-bromo-derivative, which is then treated with a protected pyrimidine base over a lengthy period of time ranging from 16 h to 7 d.[7,8] These conditions are not suitable for radiochemical synthesis with short half-life isotopes such as ^{18}F. Another method of coupling the glycosyl moiety to the pyrimidine base involves the use of a Friedel-Crafts catalyst and 2-substituted ribofuranose, which produced, exclusively, the β-anomer of the nucleoside.[9]

Very limited work has been done on pyrimidine glycosylation with a protected 2-deoxy sugar.[9,10] In our effort toward the synthesis of ^{18}F-FMAU, we have investigated reactions of 2,4-*bis*-*O*-(trimethylsilyl)uracil derivatives **1a** and **1b** with 2-deoxy-2-fluoro-1,3,5-*tri*-*O*-benzoyl-α-D-arabinofuranose **2a** using several catalysts under a variety of experimental conditions. In addition, we have investigated the preparation of 1-bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-arabinofuranose **2b** in short time, and its subsequent coupling with a protected pyrimidine.

2,4-*Bis*-*O*-(trimethylsilyl)uracil derivatives **1a** and **1b** were prepared in high yields following a literature method.[10] Compound **2a** was prepared either by a literature method[11] or by one developed in our laboratory.[12] Through careful optimization of solvent and reaction conditions we have developed the synthesis of **2b** in high yield with a reduction of time from 16 to 0.5 h. Compound **2b** was exclusively the α-anomer as observed by ^{1}H NMR spectroscopy ($J_{1,2} = 0$).

Figure 1 represents the coupling reactions of protected pyrimidine base and protected sugar.

Tables 1 and 2 represent the coupling of **1a** and **2b**, and **1a** and **2a** in various conditions, respectively.

The reactions of **1a** with **2b** in MeCN at 80°C resulted in 80–86% chemical yield of the coupled product in the ratio of 65 : 35 (β : α). In CH_2Cl–CH_2Cl, these reactions produced a higher yield, and the anomeric ratio was further enhanced to 10 : 1 (Table 1, Entry 5). The required reaction time for the coupling of **1a** with **2b** at 80°C was longer in CH_2Cl–CH_2Cl

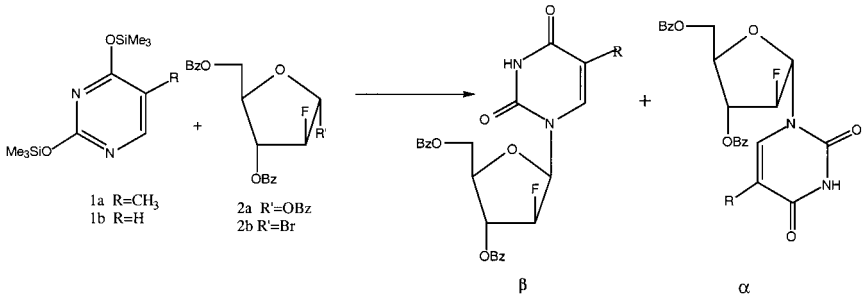

Figure 1.

Table 1.

Entry	Solvent	Catalyst	Temp/Time	Yield	Product ($\beta:\alpha$)
1	MeCN		80°C/50 min	86–90%	65 : 35
2	MeCN	$SnCl_4$	70°C/50 min	58%	40 : 60
3	CH_2Cl–CH_2Cl		97°C/1 h	90–95%	85 : 15
4	CH_2Cl–CH_2Cl		97°C/1 h	70–75%*	85 : 15
5	CH_2Cl–CH_2Cl		80°C/3.25 h	95%	91 : 9

*Started from 1-benzoate ester, yield in two steps.

(3.25 h) than in MeCN (50 min). However, the temperature of the reaction in CH_2Cl–CH_2Cl could be increased up to 97°C to achieve high yield in a relatively shorter time maintaining the high anomeric selectivity. Thus in 1h at 97°C the chemical yield was similar as those obtained in 3.25 h at 80°C, while the anomeric ratio ($\beta:\alpha$) was only slightly lower at the higher temperature (Table 1, Entry 3). The two step process, i.e., preparation of **2b** and its and subsequent coupling, leads to an overall yield of 70–75% with an anomeric ratio of 85 : 15 ($\beta:\alpha$) (Table 1, Entry 4).

We have also carried out the coupling of **1a** with **2a** using Friedel-Crafts catalysts. In these reactions, solvent plays a very important role in determining the anomeric selectivity. In the highly polar solvent MeCN the formation of the α anomer predominates. Thus reactions of **2a** with **1a** in MeCN/$SnCl_4$ produced the two anomers in the ratio of 28 : 72 ($\beta:\alpha$) in 70–76% combined chemical yield. In CH_2Cl–CH_2Cl/$SnCl_4$ the anomeric ratio was reversed to 70 : 30 ($\beta:\alpha$) (Table 2, Entry 2); however, the combined yield was reduced to 30–32%. The addition of catalyst to the reactions of 1-bromo-sugar (**2b**) with **1a** in MeCN/$SnCl_4$ resulted in a lower chemical

Table 2.

Entry	Solvent	Catalyst	Temp./Time	Yield	Product ($\beta:\alpha$)
1	MeCN	$SnCl_4$	80°C/45 min	70–76%	28 : 72
2	CH_2Cl–CH_2Cl	$SnCl_4$	80°C/1 h	30–32%	70 : 30
3	CCl_4	$SnCl_4$	80°C/1 h	20%	67 : 33
4	MeCN	BF_3	80°C/1 h	14%	29 : 71
5	MeCN	$TiCl_4$	80°C/1 h	5%	29 : 71
6	MeCN	$ZnCl_2$	80°C/1 h	No reaction	

yield (58%) and a ratio of 40 : 60 ($\beta:\alpha$) (Table 1 Entry 2). It appears that in both instances (**2a** and **2b**), the catalyst ($SnCl_4$) influences the course of reaction through a S_N1 mechanism by the formation of a carbocation as suggested earlier.[8] Tin tetrachloride ($SnCl_4$) was found to be the best catalyst compared to others ($ZnCl_2$, BF_3-etherate and $TiCl_4$) used in this study. In comparison to the direct, one step coupling of **2a** (Method 2) the two step procedure (Method 1) that involves the conversion of **2a** to **2b** prior to coupling, lengthens the overall reaction time by 40 min. Although Method 2 requires 40 min additional time the overall yield of the desired anomer is much higher, and therefore has an advantage over the other method. Experiments with **1b** produced similar results as in the case of **1a**.

The protected coupled product could be hydrolyzed in 5 min using sodium methoxide at 80°C. Analysis by HPLC of this free nucleoside showed a mixture of two products, α and β, co-eluted with authentic samples (Figure 2), and consistent with the literature.[7] The ratio of the anomers was consistent with that estimated by ^{1}H NMR spectra. Preparation and subsequent coupling of **2b** with a protected pyrimidine base in CH_2Cl–CH_2Cl should be suitable to the synthesis of 2′-deoxy-2′-[^{18}F]fluoro-1-β-D-arabinofuranosyluracil derivatives for PET imaging.

EXPERIMENTAL

Proton NMR spectra were recorded on a Brucker 360 or 500 MHz spectrometer using tetramethylsilane as an internal reference at the University of Southern California. Mass spectra were obtained on a Finnigan 400 mass spectrometer at the University of Minnesota using an ammonia chemical ionization technique. High performance Liquid Chromatography (HPLC) was performed on a system using a 510 pump (Waters Associates), a UV detector (Isco) operated at 254 nm and a C_{18}

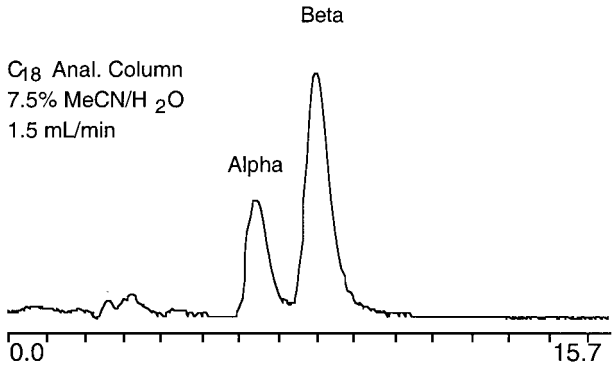

Figure 2.

reverse phase analytical column (Alltech). A MeCN/H_2O solvent system (7.5% MeCN) was used as eluent.

1-Bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranose 2b

The fluorosugar **2a** (50 mg, 0.11 mmol) was dissolved in 1,2-dichloroethane (3 ml) under argon. Hydrogen bromide (HBr) in acetic acid (30%, 0.5 ml) was added, and the reaction mixture was heated at 80°C for 30 min. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2 (15 ml), washed with saturated $NaHCO_3$ soln (15 ml), dried and evaporated to dryness. After flash chromatography on silica gel using 12% acetone in hexane as eluent, 40 mg of pure product **2b** was obtained (88% yield). ^{1}H NMR: (δ): 8.1–8.2 (m, 4H, aromatic), 7.5–7.7 (m, 2H, aromatic), 7.4–7.5 (m, 4H, aromatic), 6.65 (d, 1H, 1′H, $J = 12$ Hz), 5.55 (dm, 1H, 3′H, $J = 20$ Hz), 5.61 (d, 1H, 2′H, $J = 50$ Hz), 4.6–4.9 (m, 3H, 4′H & 5′H). High resolution MS: M + H, Calculated 423.0223; found 423.0223.

2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-1-β-D-arabinofuranosyluracil Derivative

Method 1: Compound **2b** (25–30 mg) was dissolved in either acetonitrile or 1,2-dichloroethane (0.5 ml) under argon in a V-vial. Freshly prepared 2,4-*bis*-*O*-(trimethylsilyl)uracil derivative **1a** (8–9 equiv.) was dissolved in either MeCN or CH_2Cl–CH_2Cl (1.5 ml) and added to the reaction vial and the

mixture heated at 80–81°C for 50 min, when TLC (30% acetone in hexane) showed no remaining starting material. The reaction mixture was cooled to room temperature and the solvent was evaporated. The residue was dissolved in CH_2Cl_2, the solution washed with water, dried and evaporated to dryness. The crude product was purified on silica gel using 25% acetone in hexane as eluent. Pure product (by TLC) was obtained in 80–86% yield, as a mixture of α and β anomers. ^{1}H NMR: (α): (δ): 9.26 (s, 1H, NH), 8.0–8.2 (m, 4H, aromatic), 7.5–7.7 (m, 2H, aromatic), 7.2–7.5 (m, 4H, aromatic), 6.16 (d, 1H, 1′H, $J = 15.5$ Hz), 5.69 (d, 1H, 3′H, $J = 15$ Hz), 5.49 (d, 1H, 2′H, $J = 48$ Hz), 4.2–5.0 (m, 3H, 4′H and 5′H), 1.86 (s, 3H, methyl). ^{1}H NMR: (β): (δ): 9.10 (s, 1H, NH), 8.0–8.2 (m, 4H, aromatic), 7.5–7.7 (m, 2H, aromatic), 7.2–7.5 (m, 4H, aromatic), 6.34 (dd, 1H, 1′H, $J = 22.5$ Hz and 2.8 Hz), 5.62 (dt, 1H, 3′H, $J = 17.5$ Hz and 2.8 Hz), 5.30 (dd, 1H, 2′H, $J = 50$ Hz), 4.7–4.8 (m, 1H, 4′H), 4.5–4.6 (m, 2H, 5′H), 1.73 (s, 3H, methyl).

Method 2: Compound **2a** (25–30 mg) was dissolved in either MeCN or CH_2Cl–CH_2Cl (0.5 ml) under argon in a V-vial. The protected uracil derivative **1a** (8–9 equiv.) was dissolved in appropriate solvents (1.5 ml) and the solution added to the reaction vial followed by addition of $SnCl_4$ (10 equiv.). The vial was heated at 80–81°C for 45 min, when TLC showed absence of starting material. The reaction mixture was cooled to room temperature, worked up and purified as described in Method 1. Pure product (by TLC) was obtained in 70–76% yield. ^{1}H NMR spectrum showed a mixture of α and β anomers.

2-Deoxy-2-fluoro-1-β-D-arabinofuranosyluracil Nucleoside

The protected-coupled product (5 mg) was dissolved in methanol (2 ml). Sodium methoxide (1M, 0.1 ml) was added, and the mixture was heated for 5 min at 80°C when TLC showed that no starting material remained. The reaction mixture was neutralized with dil. HCl, filtered and analyzed by HPLC using a C_{18} reverse phase analytical column and 7.5% MeCN in water as eluting solvent at a flow of 1.5 ml/min. HPLC chromatogram (Figure 2) showed to be a mixture of two products α and β in the ratio as observed by ^{1}H NMR spectra, and co-eluted with the known product.

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