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Synthesis of 2′-*C*-β-Fluoromethyluridine

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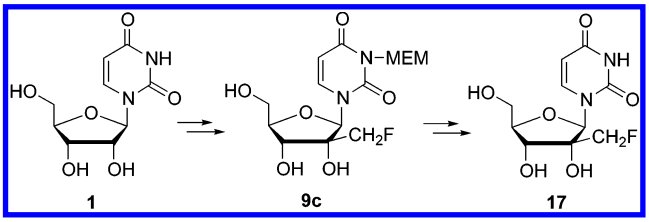
ABSTRACT



2′-*C*-β-Fluoromethyluridine (17) represents both a potentially important biological agent and a tool for biochemical analysis. Here we describe the first synthesis of this compound starting from uridine. The key steps include protection of the uracil base with methoxyethoxymethyl (MEM) chloride, conversion to the corresponding 2′-*C*-α-epoxide, and regioselective opening of the oxirane ring with potassium fluoride/hydrogen fluoride. Subsequent acetylation of the 3′- and 5′-hydroxyl groups enables MEM removal using *B*-bromocatecholborane. Deacetylation generates the parent nucleoside, 2′-*C*-β-flurormethyluridine.

2′-Fluoromethylnucleosides represent important targets for synthesis due to their potential value as clinically useful medicinal agents and as biochemical probes. As medicinal agents, 2′-*C*-β-methylnucleosides possess anticancer and antiviral properties and function as inhibitors of enzymes.[1] Additionally, fluorine substitution within a nucleoside may enhance clinical efficacy by altering drug metabolism, lipophilicity, and reactivity.[2] As biochemical probes, 2′-*C*-β-fluoromethyl nucleosides may provide important tools for functional analysis of biologically significant RNA molecules. A series of ribonucleoside analogues containing 2′-*C*-β-methyl groups of increasing fluorine substitution (CH_3, CH_2F, CHF_2, or CF_3) might allow systematic variation of the pK_a of the 2′-hydroxyl group over a broad range while maintaining a similar structural context. Such a series of nucleosides could unleash the power of physical organic approaches to study the critical biological role played by the 2′-hydroxyl group of RNA. In previous work, we reported the syntheses of 2′-*C*-β-methyl and 2′-*C*-β-trifluoromethyl ribonucleosides.[3] Here we describe the synthesis of the 2′-*C*-β-monofluoromethyl nucleoside, 2′-*C*-β-fluoromethyl-uridine.

Yoshimura et al. successfully prepared a derivative of 1-(2′-*C*-α-monofluoromethyl-β-D-arabinofuranosyl)uridine from the corresponding 2′-β-spiroepoxy-uridine by regioselective opening of the oxirane ring with potassium fluoride/hydrogen fluoride.[4] We envisioned that the analogous reaction with an appropriate 2′-α-spiroepoxy derivative could

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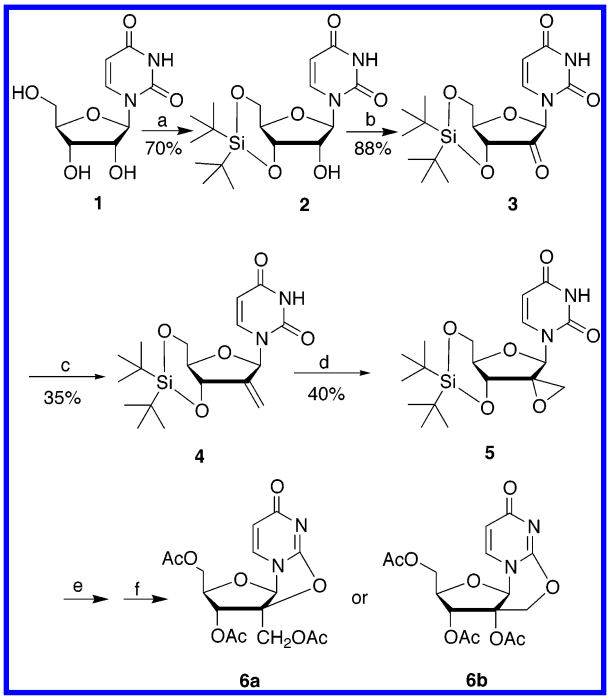
generate the 2′-fluoromethyl group on the β-face of the ribose ring to give 1-(2′-*C*-β-monofluoromethyl-β-D-ribofuranosyl)-uridine. Scheme 1 shows our strategy for the synthesis of

Scheme 1[a]

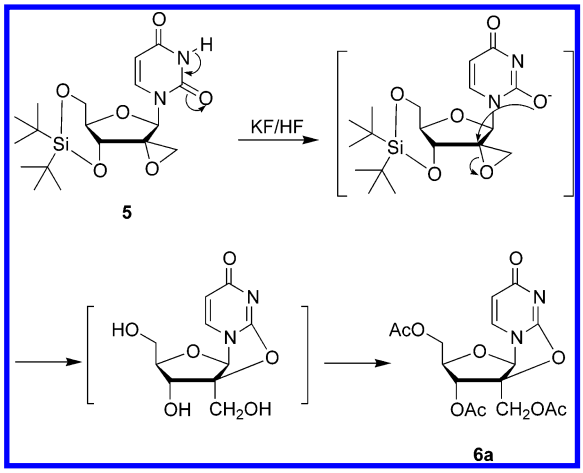


[a] Reaction conditions: (a) dichloro-di-*tert*-butylsilane, $AgNO_3$; (b) Dess−Martin periodinane, CH_2Cl_2, 24 h; (c) methyltriphenylphosphonium bromide, potassium *tert*-pentoxide, ether, 48 h; (d) MCPBA, CH_2Cl_2, 48 h; (e) KHF_2, 2-methoxyethanol; (f) Ac_2O, pyridine.

the 2′-α-spiro epoxide **5**. Reaction of uridine with di-*tert*-butylsilyl chloride and silver nitrate in DMF at room-temperature protected the 3′- and 5′-hydroxyl groups as the silyl ether **2** in 70% yield.[5] Dess−Martin periodinane in dichloromethane at room-temperature oxidized **2** in 88% yield to the corresponding 2′-keto nucleoside **3**,[6] which was dehydrated by azeotropic distillation with toluene before further use. Treatment of **3** with methylenetriphenylphosphine, produced in situ from methyltriphenylphosphonium bromide and potassium *tert*-pentoxide in ether, installed the 2′-methylene group to give **4**.[7] Epoxidation of the 2′-methylene group with MCPBA in methylene chloride generated the diastereomerically pure α-spiro-epoxide **5** in 40% yield. NOE experiments supported the stereochemical assignment at C-2′: irradiation at one of the oxirane protons enhanced only the H-3′ resonance; irradiation at the other oxirane proton weakly enhanced H-1′ only.

Exposure of the α-epoxide **5** with KF/HF in 2-methoxyethanol at 130 °C generated a highly polar product due to removal of the di-*tert*-butylsilyl group. To facilitate purification, we peracylated the crude product by treatment with acetic anhydride in pyridine. Analytical characterization (^{1}H NMR, ^{13}C NMR, MS, and elemental analysis) of this product showed no fluoromethyl group and was consistent with two possible products: 3′,5′-di-*O*-acetyl-2′-*C*-α-acetyloxymethyl-2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil **6a** or 2′,3′,5′-*O*-acetyl-2′-*C*-β-hydroxylmethyl-2,2′-anhydro-1-(β-D-ribofuranosyl)uracil **6b** (Scheme 1). To distinguish between these two possibilities, we carried out single-crystal X-ray diffraction analysis. The structural data established the product identity as 3′,5′-di-*O*-acetyl-2′-*C*-α-acetyloxymethyl-2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil **6a**,[8] suggesting that under the reaction conditions, the 2-keto oxygen of the uracil heterocycle attacks the α-epoxide at C-2′ (Scheme 2). The

Scheme 2



formation of **6a** supports our assignment of **5** as the α-epoxide. Yoshimura et al. reported no such reaction in the case of the β-epoxide,[4] presumably because the cis relationship between the epoxide and the nucleophile prohibits backside attack at C-2′.

Protection of uracil at N-3 might eliminate the formation of **6a** and consequently enhance fluoride attack on the epoxide. We tested the feasibility of four different uracil protecting groups (Scheme 3): benzoyl (Bz), triphenylmethane-sulfenyl (TPMS),[9] 2-methoxyethoxyl-methyl (MEM),[10] and *p*-methoxybenzyl (PMB).[11] The MEM derivative proved to be the most effective. Treatment of **7c** with MCPBA in dichloromethane for 2 days produced the corresponding α-epoxide **8c** in 30% yield (50% of the starting material was also recovered). Longer reaction times resulted in significantly lower yields. NOE experiments analogous to those for **5** confirmed the stereochemistry at C-2′.

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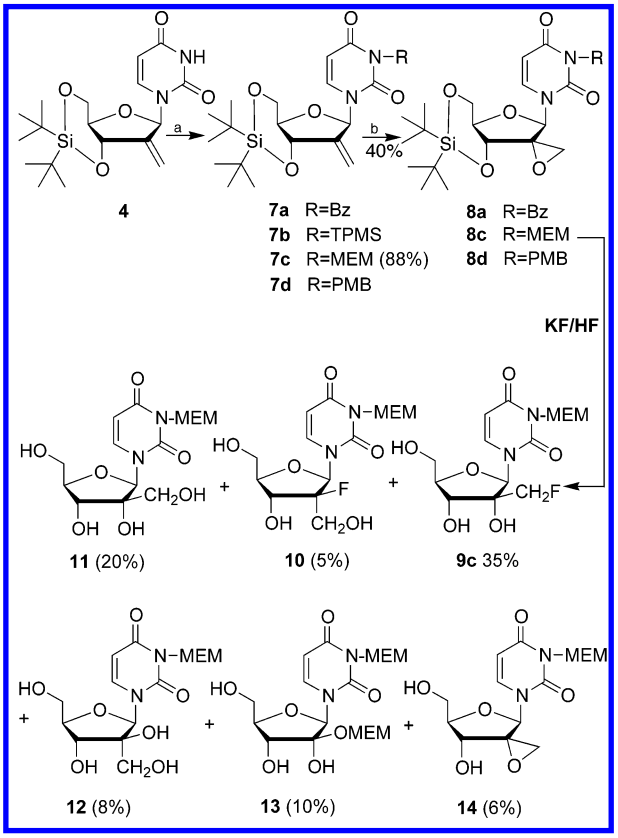
Scheme 3[a]

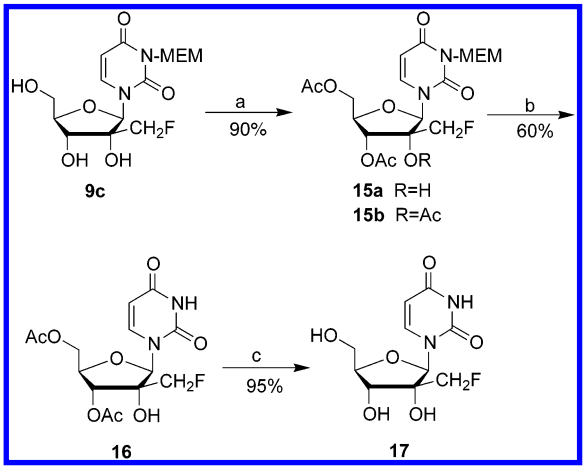


[a] Reaction conditions: (a) diisopropylethylamine, CH_2Cl_2, benzoyl chloride (or MEM-Cl or triphenylmethane sulfenyl chloride); (b) CH_2Cl_2, MCPBA.

Subsequent treatment of the epoxide **8c** with KF/HF in 2-methoxyethanol at 130 °C for 8 h provided **9c** as the major product (35%) together with several byproducts (**10**−**14**; Scheme 3). NMR characterization of **9c** confirmed the presence of the fluoromethyl group $-CH_2F$ ($^2J_{H-F} = 48.0$ Hz, $^1J_{C-F} = 175.3$ Hz; ^{19}F NMR $\delta = -229$ ppm, $CFCl_3$ as a reference).[4,12] To avoid spectral overlap, NOE experiments were conducted on **15b**, the peracetylated form of **9c** (Scheme 4). Irradiation of the fluorine enhanced the resonance at H-3′ strongly but only enhanced the resonance of H-1′ weakly. These results established that the $-CH_2F$ group of **15b** (and by inference **15a** and **9c**) resides on the β-face of the ribose ring.

The formation of major product **9c** indicated that fluoride ion attacked predominantly at the less-hindered carbon of the epoxide, though some attack at the more hindered C-2′ occurred to give a small amount of **10**. The byproducts **11**−**13** arise as a consequence of hydrolysis and solvolysis, respectively. Byproduct **14** arises from initial desilylation of the starting material **8c** but remains only in trace amounts after the reaction. During workup, however, **14** increased at the expense of **9c** until removal of excess KF/HF. Presumably, the 2′-oxygen nucleophilically attacks the fluoromethyl group to displace fluoride and regenerate the epoxide. In contrast to the results for **8c**, exposure of the PMB-protected epoxide **8d** to KF/HF in 2-methoxyethanol under the same

Scheme 4[a]



[a] Reaction conditions: (a) Ac_2O, pyridine; (b) *B*-bromocatecholborane, CH_2Cl_2, rt, 2 h; (c) 1:4 guanidine/guanidine hydrochloride, 1:1 MeOH/CH_2Cl_2.

reaction conditions gave mainly hydrolysis products (corresponding to **11** and **12**).

Reagents known to remove MEM from uracil ($TrBF_4$ or NH_3 in pyridine, or the Lewis acids $TiCl_4$[13] and $ZnBr_2$[14] and bromodimethylborane[15]) failed to remove MEM from **9c** or **15a**. However, *B*-bromocatecholborane[16] in methylene chloride at room-temperature transformed **15a** to **16** in 60% yield. To prepare the parent nucleoside, 2′-*C*-β-fluoromethyluridine, deacetylation of **16** must occur without the loss of fluoride via epoxide formation. A mixture of guanidine and guanidine hydrochloride (1:4) in methanol[17] proved to be effective, yielding **17** in 95% yield (Scheme 4).[18]

In summary, attempts to prepare 2′-*C*-β-monofluoromethyluridine via regioselective oxirane opening of *2′-C*-α-spiroepoxyuridine with fluoride fail because the 2-keto oxygen attacks nucleophilically at C-*2′* to give the corresponding 2,2′-anhydrouridine derivative **6a**. MEM protection of the uracil moiety at N-3 prohibits this reaction such that exposure of the spiroepoxide to KF/HF generates the MEM-protected 2′-*C*-β-fluoromethyl-nucleoside **9c** as the major product. Additional products arise from nucleophilic attack by solvent, water, or fluoride at either carbon of the oxirane. Acetylation of the 3′- and 5′-hydroxyl groups of **9c** followed by treatment with *B*-bromocatecholborane promotes facile removal of MEM group in high yield. Subsequent exposure to guanidine/guanidine hydrochloride removes the acetyl

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(18) Starting from uridine, our nine-step synthesis provided **17** with an overall yield of ∼1.4%.

groups without loss of fluoride to afford 2′-*C*-*β*-fluoromethyluridine. This nucleoside may represent a new class of drugs and/or tools for chemical biology.

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Supporting Information Available: Full experimental and analytical data for compounds **2**−**5**, **6b**, **7c**, **8c**, **9c**, **10**−**14**, **15a**,**b**, **16**, and **17**; $^{19}F-^{1}H$ NOE spectra of **15b**; ^{1}H and ^{13}C NMR spectra of **9c**, **15b**, **16**, and **17**; and further information on MEM resistance to deprotection. This material is available free of charge via the Internet at http://pubs.acs.org.

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