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Bioorganic & Medicinal Chemistry Letters 16 (2006) 6139–6142
Bioorganic & Medicinal Chemistry Letters

Rapid microwave-assisted fluorination yielding novel 5′-deoxy-5′-fluorouridine derivatives

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Received 16 July 2006; revised 20 August 2006; accepted 23 August 2006
Available online 7 September 2006

Abstract—The preparation of ^{18}F-labeled ligands for positron emission tomography (PET) and the subsequent imaging have to be completed within a half-life of the neutron-deficient isotope (^{18}F = 110 min). In this paper, we report a rapid fluorination approach to obtain 5′-deoxy-5′-fluoro-substituted uracil nucleoside analogues. Nucleophilic substitution at the 5′-position of the nucleosides was achieved within 45 min providing excellent yields of 75–92% by application of microwaves.

Fluorine-18-labeled bioorganic molecules such as nucleosides have been utilized as useful tracers for positron emission tomography (PET), which has recently become a powerful diagnostic method.[1] PET diagnostics are used in research as well as in hospitals for diagnosis, for example, of heart diseases, seizure disorders, and in oncology.[2] Furthermore, the monitoring of (patho)physiological processes in vivo by PET is very useful for the prediction of the performance of a therapy.[1]

PET is based on the labeling of physiological compounds or pharmacological agents with neutron-deficient nuclides, such as carbon-11, fluorine-18, nitrogen-13, oxygen-15, or gallium-68. Fluorine-18 is the most commonly used nuclide for PET studies because of its half-life of 110 min, which is considerably longer than that of other positron-emitting nuclides (e.g., ^{11}C: 20 min, ^{15}O: 2 min). In the synthesis of PET ligands, the radiolabel (e.g., ^{18}F) should be introduced in the last or one of the last reaction steps, and its introduction has to be performed fast and efficiently due to the short half-life.[1]

Apart from their use as PET ligands in ^{18}F-labeled form, non-radioactive fluorinated nucleosides have become an important subject of research[3–5] in medicinal chemistry due to their antiviral and anticancer activities. Many bioorganic structures containing fluorine have been synthesized and investigated. Specially, 5-fluorouracil (5FU, Fig. 1) is widely used as an antimetabolite in anticancer therapy. However, the cytotoxic properties of 5FU are a significant problem and limit the clinical efficacy of such agents.[6] To improve its pharmacological and pharmacokinetic properties, 5-FU nucleosides and nucleoside analogues have been prepared. Currently, 5′-deoxy-5-fluorouridine (5′-DFUR) is under investigation as well-tolerated drug, showing promising effects/results for long-term chemotherapy in treatment of patients with recurrent breast cancer.[7]

In addition, the existence of a specific membrane receptor for uridine has recently been postulated.[8,9] *N*(3)-Phenacyluridine was identified as a potent hypnotic compound[10] and found to be the most potent known ligand of the uridine binding site in rat and bovine brain

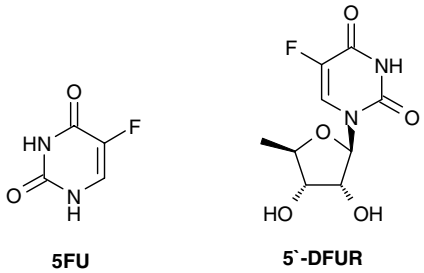

Figure 1. Fluorinated pyrimidine and its derivative.

Keywords: 5′-Deoxy-5′-fluorouridine derivatives; Microwave; Fluorinated nucleosides.
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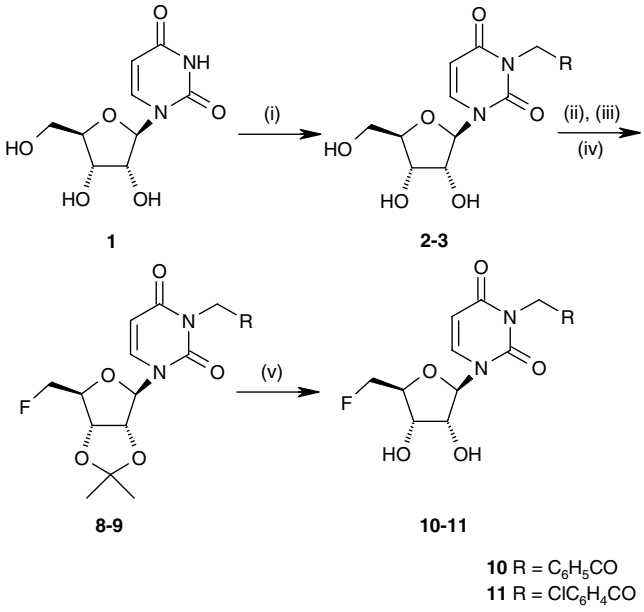
doi:10.1016/j.bmcl.2006.08.093

membrane preparations, for example, of striatum, thalamus, and cortex.[8]

A renaissance of interest in the field of antimetabolites of nucleic acid biosynthesis reports that specific binding sites or receptors for uridine on cell membranes may exist and particularly the requirement for procedures that will allow the preparation of ^{18}F-labeled PET ligands prompted us to develop an efficient synthetic strategy for 5′-deoxy-5′-fluorinated uridine and base-modified derivatives. The preparation of ^{18}F-labeled PET ligands may also be a useful tool for the investigation of uridine receptors.

The described rapid microwave-assisted fluorination in the 5′-position of nucleosides and nucleoside analogues is superior to previously described synthetic strategies,[11–15] some of which suffer from poor yields, require numerous synthetic steps, or long reaction times. For the preparation of fluorine-18-labeled bioorganic molecules, the application of microwave irradiation is advantageous and would lead to high yield with very short reaction time.[21,22] For aliphatic nucleophilic radiofluorination, a reactive leaving group like tosylate is required.[21] In contrast, in aromatic nucleophilic substitutions halogen atoms are preferred as efficient leaving groups.[22]

Commercially available uridine **1** was alkylated according to patent literature[16] with commercially available phenacyl bromide or 4-chlorophenacyl bromide to generate the intermediates **2** and **3** (Scheme 1).



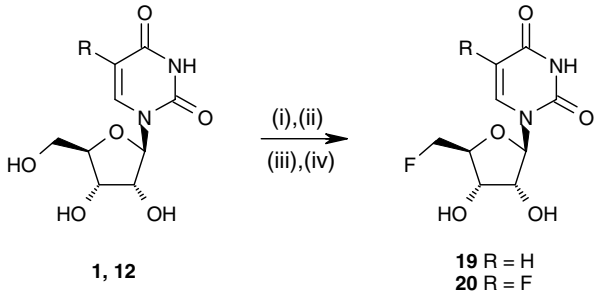
Scheme 1. Reagents and conditions: (i) acetone, DMSO, potassium carbonate, phenacyl bromide or 4-chlorophenacyl bromide, 10 h, 70 °C; (ii) acetone, *p*-toluenesulfonic acid monohydrate, 4 Å molecular sieves, 2.5 h, pyridine; (iii) dry pyridine, *p*-toluenesulfonic acid chloride, 2 h, rt; (iv) dioxane, TBAF·HF, pyridine, sealed tube, 15.0 bar, 160 W, 130 °C, 45 min; (v) hydrogen chloride (10%) in methanol and water, 10 min, reflux.

The vicinal 2′- and 3′-hydroxyl groups of the nucleoside intermediates were protected with an isopropylidene group to afford the 2′,3′-*O*-isopropylidene nucleosides **4** and **5**. Then the free primary alcohol function at the 5′-position was tosylated[17,18] yielding compounds **6** and **7**, and subsequently replaced by fluoride.[19] The procedure for the fluorination described in the literature generally takes more than 20 h, which is far too long for the synthesis of PET tracers.[15] However, the application of microwaves considerably reduces the reaction time. By the application of microwave-assisted fluorination, compounds **8** and **9** were obtained in very good yields (75–83%) within a short reaction time of only 45 min.

The removal of the isopropylidene protecting group by standard methods[11] delivered high yields but took more than 30 min. Thus a compromise between yield and reaction time had to be found in order to meet the needs of the synthesis of PET ligands. The intermediate products **8** and **9** were treated with a solution of hydrogen chloride (10%) in methanol and water, and refluxed for 10 min. These reaction conditions[17,18] led to the formation of the desired fluorinated nucleosides **10** and **11** without side products in acceptable yields of 33–35%.[23] The deprotection in acidic milieu gave poor results due to the instability of the nucleosidic bond at lower pH values and the high temperature. By applying an analogous synthetic strategy the fluorinated nucleosides **19** and **20** (Scheme 2) were successfully prepared with similarly high yields for the fluorination step (92% for **19**, 78% for **20**).

The synthesized fluorine-substituted nucleoside derivatives were characterized by ^{1}H, ^{13}C-NMR, and CHN elemental analysis. The typical coupling constants between carbon and fluorine atoms were observed for the products **10**, **11**, **19**, and **20** (^{1}J (C-5′, F) = 167–171 Hz, ^{2}J (C-4′, F) = 20.0 Hz and ^{3}J (C-3′, F) = 5.0 Hz).

The results obtained in this study underline the efficiency of the microwave-assisted, rapid fluorination of nucleosides delivering high yields in a very short time. No significant formation of side products was observed. The chromatographic purification of the intermediate as well



Scheme 2. Reagents and conditions: (i) acetone, *p*-toluenesulfonic acid monohydrate, 4 Å molecular sieves, 2.5 h, pyridine; (ii) dry pyridine, *p*-toluenesulfonic acid chloride, 2 h, rt; (iii) dioxane, TBAF·HF, pyridine, sealed tube, 15.0 bar, 130 °C, 160 W, 45 min; (iv) hydrogen chloride (10%) in methanol and water, 10 min, reflux.

as the final products was quick and convenient. Final yields may be further optimized, for example, by introduction of different protecting groups, and the duration of the fluorination reaction may be further shortened by optimizing the reaction conditions. The synthesized novel uracil nucleoside derivatives **10**, **11**, as well as the rapid preparation of the chemotherapeutically highly active nucleosides **19** and **20**[20] (Scheme 1) will be investigated for their biological properties, including their interaction with the uridine binding site, and with enzymes of the uracil nucleoside metabolic pathway. It is expected that the 5′-flourine atom, which can be envisaged as a bioisosterical replacement of the 5′-hydroxy group, can imitate the corresponding ribonucleosides in many aspects, however phosphorylation at the 5′-position will no longer be possible.

Acknowledgments

H. Phuoc Le dedicates this article to Maria Diem Loc Le, his deceased sister. Thanks are due to Prof. Dr. Hans Suschitzky, The Ramage Laboratories, Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT, UK, for critical reading of the manuscript.

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23. To a solution of 2′,3′-*O*-isopropylidene-3-(2-oxo-2-phenylethyl)-5′-*O*-tosyluridine **6** (0.7 g, 1.26 mmol) in 2 mL of dry dioxane, 0.51 mL (2.52 mmol) of 50% of *tetra*-butylammonium hydrogen difluoride solution in acetonitrile and 0.2 g (2.52 mmol) of dry pyridine were added. The reaction was performed in a pressure tube upon microwave irradiation (15.0 bar, 160 W, 130 °C, 45 min). Subsequently the solvent was removed under reduced pressure and the residue was poured onto a silica gel column and eluted with diethyl ether. 5′-Deoxy-5′-fluoro-2′,3′-*O*-isopropylidene-3-(2-oxo-2-phenylethyl)uridine **8** (0.425 g, 1.0 mmol, 83% yield) was obtained as a slightly yellow oil; ^{1}H NMR in CD_3OD, δ (ppm): 8.1 (d, 2H, H-10′, H-14′, J = 8.0 Hz), 7.8 (d, 1H, H-6, J = 7.4 Hz), 7.7 (m, 1H, H-12′), 7.6 (m, 2H, H-11′, H-13′), 5.9 (d, 1H, H-1′, J = 2.2 Hz), 5.8 (d, 1H, H-5, J = 7.4 Hz), 5.45 (s, 2H, H-7′), 5.1 (dd, 1H, H-3′, J = 2.1 Hz, J = 3.2 Hz), 4.9 (dd, 1H, H-2′, J = 2.2 Hz, J = 3.2 Hz), 4.65 (m, 2H, H-5′, J = 5.6 Hz, $J_{F\text{-}5'\text{–}C\text{-}5'}$ = 46 Hz), 4.4 (m, 1H, H-4′, J = 2.1 Hz, J = 5.6 Hz, $J_{F\text{-}5'\text{–}H\text{-}4}$ = 25.5 Hz), 1.6 (s, 3H), 1.40 (s, 3H); ^{13}C NMR in CD_3OD, δ (ppm): 194.1 (C8′), 165.0 (C4), 152.4 (C2), 142.7 (C6), 136.4 (C9′), 135.4 (C12′), 130.3 (C10′, C14′), 129.5 (C11′, C13′), 115.6 (C6′), 102.2 (C5), 96.3 (C1′), 88.0 (d, C4′, $J_{F\text{-}5'\text{–}C\text{-}4'}$ = 21.0 Hz), 86.3 (C2′), 85.0 (d, C5′, $J_{F\text{-}5'\text{–}C\text{-}5'}$ = 169.0 Hz), 82.2 (d, C3′, $J_{F\text{-}5'\text{–}C\text{-}3'}$ = 7.0 Hz), 48.0 (C7′),27.5 (CH_3), 25.7 (CH_3). Applying the described procedure for **8**, 2′,3′-*O*-isopropylidene-3-[2-oxo-2-(4-chloro)phenylethyl)]-5′-*O*-tosyluridine **7** (0.4 g, 0.68 mmol) in 2 mL of dry dioxane, 0.28 mL (1.36 mmol) of 50% of *tetra*-butylammonium hydrogen difluoride solution in acetonitrile, and 0.11 g (1.36 mmol) of dry pyridine were added, 5′-deoxy-5′-fluoro-2′,3′-*O*-isopropylidene-3-[2-oxo-2-(4-chloro)phenylethyl]-uridine **9** (0.3 g, 0.51 mmol, 75% yield) was obtained as a slightly yellow oil; ^{1}H NMR in CD_3OD, δ (ppm): 8.0 (d, 2H, H-10′, H-14′, J = 8.5 Hz), 7.75 (d, 1H, H-6, J = 7.4 Hz), 7.6 (d, 2H, H-11′, H-13′, J = 8.5 Hz), 5.9 (d, 1H, H-1′, J = 2.2 Hz), 5.88 (d, 1H, H-5, J = 7.4 Hz), 5.4 (s, 2H, H-7′), 5.0 (m, 1H, H-4′, J = 2.1 Hz, J = 4.4 Hz, $J_{F\text{-}5'\text{–}H\text{-}4'}$ = 26.0 Hz), 4.9 (dd, 1H, H-2′, J = 2.2 Hz, J = 3.3 Hz), 4.65 (m, 2H, H-5′, J = 4.4 Hz, $J_{F\text{-}5'\text{–}H\text{-}5'}$ = 47.0 Hz), 4.4 (d, 1H, H-3′, J = 2.2 Hz, J = 3.3 Hz), 1.6 (s, 3H), 1.4 (s, 3H); ^{13}C NMR in CD_3OD, δ (ppm): 193.0 (C8′), 164.6 (C4), 152.3 (C2), 142.7 (C6), 142.6 (C12′), 135.0 (C9′), 131.1 (C10′, C14′), 130.6 (C11′, C13′), 115.6 (C6′), 102.3 (C5), 96.2 (C1′), 88.0 (d, C4′, $J_{F\text{-}5'\text{–}C\text{-}4'}$ = 20.5 Hz), 86.0 (C2′), 85.0 (d, C5′, $J_{F\text{-}5'\text{–}C\text{-}5'}$ = 169.0 Hz), 82.0 (d, C3′, $J_{F\text{-}5'\text{–}C\text{-}3'}$ = 7.0 Hz), 48.0 (C7′), 27.7 (CH_3), 25.7 (CH_3). The obtained intermediate **8** (0.4 g, 1.0 mmol) was dissolved in 5 mL of MeOH containing aq. HCl (1.35 mL of 37% aq HCl: 3.65 mL of MeOH) and refluxed for 10 min. After cooling at rt and neutralization with sodium hydrogen carbonate, the reaction mixture was extracted with ethyl acetate (2× 30 mL). The organic phases were combined and dried over Na_2SO_4. After filtration and removal of the solvent under reduced pressure, the product was recrystallized from 7 mL of diethyl ether. Pure product of **10** (0.128 g, 0.35 mmol, 35% yield) was obtained as an off-white solid; ^{1}H NMR in CD_3OD, δ (ppm): 8.1 (m, 2H, H-10′, H-14′),

7.8 (d, 1H, H-6, J = 7.6 Hz), 7.7 (m, 1H, H-12′), 7.6 (m, 2H, H-11′, H-13′), 5.9 (d, 1H, H-1′, J = 3.3 Hz), 5.8 (d, 1H, H-5, J = 7.6 Hz), 5.5 (s, 2H, H-7′), 5.4 4.85 (m, 1H, H-4′), 4.8 (m, 1H, H-3′, J = 4.3 Hz, J = 5.2 Hz, $J_{\text{F-5′–H-4′}}$ = 19.5 Hz), 4.65 (dd, 1H, H-2′, J = 3.3 Hz, J = 5.2 Hz), 4.25 (m, 2H, H-5′, J = 4.3 Hz, $J_{\text{F-5′–H-5′}}$ = 47.0 Hz); ^{13}C NMR in CD_3OD, δ (ppm): 194.2 (C8′), 165.0 (C4), 152.6 (C2), 140.6 (C6), 136.5 (C9′), 135.4 (C12′), 130.3 (C10′, C14′), 129.4 (C11′, C13′), 102.4 (C5), 92.5 (C1′), 84.5 (d, C4′, $J_{\text{F-5′–C-4′}}$ = 20.5 Hz), 84.0 (d, C5′, $J_{\text{F-5′–C-5′}}$ = 169.0 Hz), 76.0 (C2′), 70.5 (d, C3′, $J_{\text{F-5′–C-3′}}$ = 5.0 Hz), 48.0 (C7′); IR (KBr) $\tilde{V}$ (cm^{-1}) 3508-3289 (2× O–H), 3106 (C–H_{arom}), 2950 (C–H_2), 1707 (OCNCO), 1695 (CO), 1662 (CON), 1597 (C═C), 1229(CH_2–F); MS (EI, 75 °C): m/z (%) 364 (21) [M^+], 346 (50), 329 (55), 289 (12), 259 (7), 170 (8), 105 (100), 77 (44), 59 (7); HRMS: Calcd for $C_{17}H_{17}FN_2O_6$: 364.1071. Found: 364.107. Anal. Calcd for $C_{17}H_{17}FN_2O_6$ (364.34): C, 53.40; H, 5.01; N, 7.33. Found: C, 53.20; H, 5.21; N, 7.53. Applying the described procedure described above for **10** to the starting material **9** (0.35 g, 0.8 mmol), **11** (0.108 g, 0.27 mmol, 33% yield) was obtained as a white solid; ^{1}H NMR in CD_3OD, δ (ppm): 8.1 (d, 2H, H-10′, H-14′, J = 8.2 Hz), 7.8 (d, 1H, H-6, J = 7.6 Hz), 7.6 (d, 2H, H-11′, H-13′, J = 8.2 Hz), 5.9 (d, 1H, H-1′, J = 3.3 Hz), 5.8 (d, 1H, H-5, J = 7.6 Hz), 5.45 (s, 2H, H-7′), 4.85 (m, 1H, H-4′, J = 4.4 Hz, J = 5.1 Hz, $J_{\text{F-5′–H-4′}}$ = 20.5 Hz), 4.75 (dd, 1H, H-3′, J = 4.4 Hz, J = 5.1 Hz), 4.65 (dd, 1H, H-2′, J = 3.3 Hz, J = 5.1 Hz), 4.2 (m, 2H, H-5′, J = 4.4 Hz, $J_{\text{F-5′–H-5′}}$ = 46.9 Hz); ^{13}C NMR in CD_3OD, δ (ppm): 193.2 (C8′), 165.0 (C4), 153.0 (C2), 142.0 (C6), 140.5 (C12′), 135.0 (C9′), 131.2 (C10′, C14′), 130.6 (C11′, C13′), 102.3 (C5), 92.5 (C1′), 84.5 (d, C4′, $J_{\text{F-5′–C-4}}$ = 20.0 Hz), 83.4 (d, C5′, $J_{\text{F-5′–C-5′}}$ = 169.0 Hz), 76.0 (C2′), 70.5 (d, C3′, $J_{\text{F-5′–C-3′}}$ = 5.0 Hz), 48.4 (C7′); IR (KBr) $\tilde{V}$ (cm^{-1}) 3598–3359 (2× O–H), 3104 (C–H_{arom}), 2951(C–H_2), 1710 (OCNCO), 1698 (CO), 1661 (CON), 1589 (C═C), 1229 (CH_2–F); MS (EI, 75 °C): m/z (%) 398 (41) [M^+], 382 (25), 380 (75), 363 (32), 323 (57), 304 (35), 293 (20), 137 (100), 96 (7), 73 (4), 57 (5); HRMS: Calcd for $C_{17}H_{16}ClFN_2O_6$: 398.0681. Found: 398.0682. Anal. Calcd for $C_{17}H_{16}ClFN_2O_6{\cdot}H_2O$ (416.79): C, 48.99; H, 4.35; N, 6.72. Found: C, 48.50; H, 4.34; N, 6.70.