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RAPID SYNTHESIS OF A 5′-FLUORINATED OLIGODEOXY-NUCLEOTIDE: A MODEL ANTISENSE PROBE FOR USE IN IMAGING WITH POSITRON EMISSION TOMOGRAPHY (PET)[1]

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Abstract: 5′-Deoxy-5′-fluoro-O^4-methylthymidine was synthesized by the reaction of the corresponding 5′-*O*-tosylate with KF in the presence of Kryptofix [222] and coupled to a 5′-phosphoramidite-activated CPG-bound oligodeoxynucleotide. The sequence of reactions and purifications were accomplished within 4 h, a necessary condition of the development of radiofluorinated antisense oligodeoxynucleotide probe for use with PET.

Recent years have seen dramatic developments in the application of synthetic antisense oligodeoxynucleotides (ODNs) as inhibitors of specific disease-related gene expression.[2,3] The antisense approach has also preliminarily been explored to develop new biological probes for in vivo imaging of specific gene expression. Gamma-emitting ^{111}In- and ^{99}mTc-labeled antisense ODNs have been recently synthesized for use with single photon emission computed tomography (SPECT) imaging.[4] However, labeling with these radioactive metals requires a sterically bulky chelating group that might alter the binding affinity as well as cellular transport and distribution of the parent ODNs. In addition, SPECT has a lower resolution than positron emission tomography (PET) (8–12 mm vs. 2–6 mm, respectively). Furthermore, as compared with SPECT, PET allows for greater quantitative accuracy that is essential for developing a quantitative in vivo imaging assay.[5] Therefore, we[1] and others[6] have been exploring the development of the antisense ODN probes labeled with positron emitting fluorine-18 to image the biodistribution of ODNs and specific gene expression using PET. Fluorine-18 (96.9% β^+ emission), due to its close isosteric relationship with hydrogen,[7] offers a suitable alternative to mimic the biological behavior of the parent ODN. In this communication we report a rapid synthesis of 5′-fluoro-ODN that should be applicable for use with radiolabeled fluorine. The target antisense ODN is a 10-mer, d[C CGC CAG CTC], complementary to the 5′ translation start region of the her-2-neu proto-oncogene mRNA.[8] A high affinity is essential for the detection of an amplified oncogene mRNA that is present with a Bmax in the range of 1–1000 pM.[9] It has been reported that a deca-ribonucleotide binds to a single-stranded region of its complementary mRNA with affinity constants in the range of 0.01–0.1 pM.[10] In addition, a stretch of 10 nucleotide bases and high order structure requirements of hybridization should be enough to provide a high binding selectivity.[11] It is therefore conceivable that the antisense probe may detect even the lower level of target mRNA with a signal to noise ratio of ~10:1 (based on the ratio of Bmax to Kd at equilibrium). We have decided to use [^{18}F]fluoride and introduce it to the 5′-end of the above ODNs for the following reasons: (1) a compound with high specific activity (~10^3–10^4 Ci/mmol) can be attained with [^{18}F]fluoride,[12] which is neccesary for detecting relatively low levels of target mRNA; (2) the 5′-deoxy-5′-fluoro analogue of nucleoside has been shown to be stable under physiological condition;[13] (3) a fluorine-18 labeled nucleoside is introduced in the last step avoiding an extra radiation-exposure time and dilution of radioactivity; and (4) the half-life of ^{18}F is likely sufficient for kinetic determination of transport and specific binding as well as clearance of the unbound ODN.[14]

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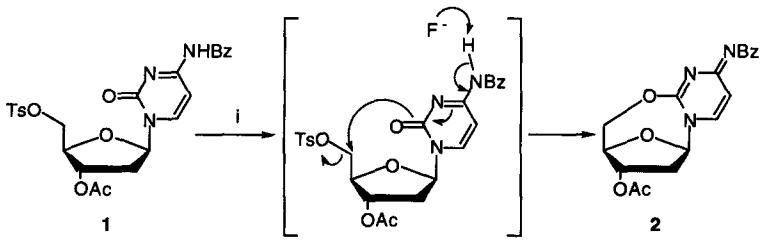
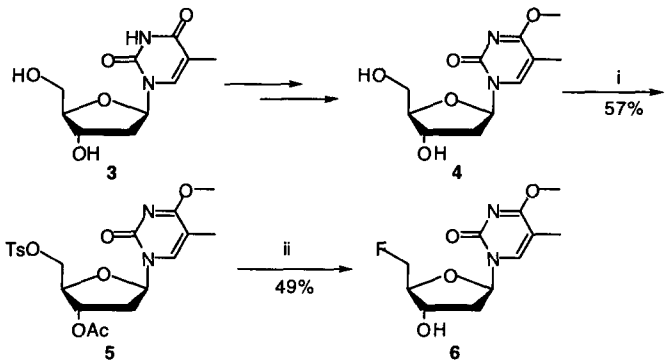
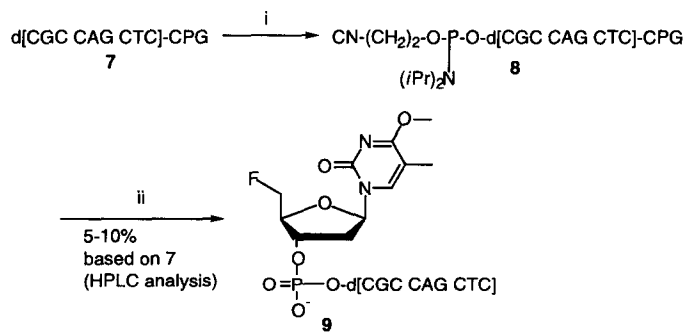


Scheme 1. *Reagents*: (i) KF/Kryptofix-[222], MeCN, 120 °C, 15.



Scheme 2. *Reagents*: (i) (1) TsCl, Py; (2) Ac_2O, Py; (ii) (1) KF/Kryptofix-[222], MeCN, 100 °C, 15 min; (2) conc NH_4OH, 100 °C, 15 min; (3) C_{18}-HPLC, MeOH:H_2O (40:60).

A number of elegant approaches to synthesize fluorinated nucleosides and nucleotides have been described.[15] In addition, a wide variety of reagents for fluorination are currently available.[16] Among these methods and reagents, only a few can be adapted to the specific constraints of ^{18}F-chemistry. These included the need to complete a series of reactions within 2–3 half-lives, after cyclotron production of the radionuclide, and the use of a large amount of radioactivity (~1 Ci) to compensate for radioactive decay and synthetic yields.[17] Our synthetic strategy is comprised of two key steps: synthesis of a 5′-deoxy-5′-fluoro-nucleoside followed by its incorporation into a CPG-bound ODN by the reverse-activation method introduced by Tan et al.[18]



Scheme 3. *Reagents*: (i) $(iPr)_2NP(Cl)O(CH_2)_2CN$, $(iPr)_2EtN$, 1-methylimidazole, Py, MeCN, rt, 1 h; (ii) (1) **6**, 1*H*-tetrazole, MeCN, rt, 30 min; (2) I_2, H_2O; (3) $MeNH_2$:NH_4OH (1:1), 50 °C, 10 min; (4) ion-exchange HPLC (POROS 20 HQ), buffer A: 23 mM Tris-HCl, 1 mM EDTA, pH 8.0 with H_2O:acetonitrile (90:10), buffer B: A containing 1.0 M NaCl, 10–60% B in 30 min.

First, the known 5′-*O*-tosyl derivative of cytidine[19] **1** was subjected to nucleophilic fluorination using KF and an azocrown ether, Kryptofix [222][20] (Scheme 1). The reaction, however, yielded only the 2,5′-anhydride **2** formed via nucleophilic attack by the 2-carbonyl oxygen initiated by proton abstraction from N^4 by fluoride.[21]

In order to avoid the intramolecular cyclization, we then chose the O^4-methylthymidine derivative **5**. O^4-Methylthymidine **4** acts as pseudo-cytidine by paring with guanosine.[22] According to a literature procedure,[23] thymidine **3** was converted to **4** in 37% yield (Scheme 2). Selective tosylation of **4** by the method of Reist et al.[24] followed by acetylation gave the precursor **5** in 57% yield. Fluorination was performed using two equivalents of KF and Kryptofix [222] in anhydrous MeCN at 100 °C in a sealed tube for 15 min.[25] The reaction mixture was subsequently treated with concentrated NH_4OH at 100 °C in a sealed tube for another 15 min. Purification by reverse-phase HPLC[26] afforded 5′-deoxy-5′-fluoro-O^4-methylthymidine **6** as a powder in 49% yield.[27] The structure was confirmed by ^{19}F NMR and HRMS.[26] Fluorination and purification were completed within 2 h.

Coupling of **6** to the CPG-bound 9-base ODN[28] **7** was carried out by the reverse-activation protocol[18] (Scheme 3). Phosphitylation of **7** was successful by treatment with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite and *N,N*-diisopropylethylamine in the presence of 1-methylimidazole and pyridine in anhydrous MeCN at room temperature for 1 h. The resulting phosphoramidite[29] **8** was then reacted with **6** in MeCN containing 1*H*-tetrazole at rt for 30 min. After oxidation with aqueous iodine, the product ODN was simultaneously deprotected and cleaved from the CPG following the standard $MeNH_2$-NH_4OH treatment at 50 °C for 10 min. The crude mixture was purified by ion-exchange HPLC (POROS 20 HQ) to yield the desired 5′-fluorinated ODN **9** in 5–10% yield based on **7** analyzed by HPLC.[30] The structure of **9** was confirmed by MALDI-TOF MS.[30] The total time required for coupling and purification was 2 h.

The present work demonstrates that the synthesis of 5′-fluorinated antisense ODN can be accomplished within 4 h, a neccesary condition for F-18 labeling. Since the fluorination of the nucleoside and the activation of CPG-bound ODN can be performed concurrently, the total reaction time could be reduced further. Synthesis of [^{18}F]fluorinated antisense ODN as well as its in vitro and in vivo applications will be reported elsewhere.

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References and Notes

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25 The condition has not been optimized.
26. Compound **6**: HPLC purification: column: Econosil C18 10U, 250 × 10 mm; gradient: methanol/H_2O (40/60); flow rate: 4.7 mL/min.; retention time: 10.8 min. ^{19}F NMR (Bruker AM360, $CDCl_3$, $CFCl_3$), δ -233.5. ^{1}H NMR spectra ($CDCl_3$, TMS) δ 1.95 (s, 3, OCH_3), 2.11 (br ddd, 1, $J_{H\text{-}2'a,H\text{-}2'b}$ = 13.5 Hz, $J_{H\text{-}2'a,H\text{-}3'}$ = 6.5 Hz, $J_{H\text{-}2'a,H\text{-}1'}$ = 4.0 Hz, H-2′a), 2.59 (ddd, 1, $J_{H\text{-}2'b,H\text{-}2'a}$ = 13.6 Hz, $J_{H\text{-}2'b,H\text{-}3'}$ = 5.6 Hz, $J_{H\text{-}2'b,H\text{-}1'}$ = 3.7 Hz, H-2′b), 3.57 (br s, 1, O*H*), 3.98 (s, 3, CH_3), 4.14 (dddd, 1, $J_{H\text{-}4',F\text{-}5'}$ = 33.0 Hz, $J_{H\text{-}4',H\text{-}3'}$ = 3.3 Hz, $J_{H\text{-}4',H\text{-}5'a}$ = 2.0 Hz, $J_{H\text{-}4',H\text{-}5'b}$ = 2.0 Hz, H-4′), 4.57 (m, 1, H-3′), 4.64 (ddd, 1, $J_{H\text{-}5'a,F\text{-}5'}$ = 48.5 Hz, $J_{H\text{-}5'a,H\text{-}5'b}$ = 10.7 Hz, $J_{H\text{-}5'a,H\text{-}4'}$ = 2.0 Hz, H-5′a), 4.72 (ddd, 1, $J_{H\text{-}5'b,F\text{-}5'}$ = 46.5 Hz, $J_{H\text{-}5'b,H\text{-}5'a}$ = 10.7 Hz, $J_{H\text{-}5'b,H\text{-}4'}$ = 2.0 Hz, H-5′b), 6.40 (dd, 1, $J_{H\text{-}1',H\text{-}2'a}$ = 4.0 Hz, $J_{H\text{-}1',H\text{-}2'b}$ = 3.7 Hz, H-1′), 7.59 (s, 1, H-6). HRMS (electrospray) $[M+H]^+$, obsd: 259.1092, calcd: 259.1094.
27 The yield was calculated basing on 4-*O*-methyl-3′-*O*-acetyl-5′-*O*-tosyl thymidine **5**.
28. The CPG-bound ODN **7** was prepared on a Beckman 1000M DNA synthesizer, following standard phosphoramidite chemistry.
29. The quality of the CPG-bound phosphoramidite **8** can be evaluated as described in ref 11.
30. 5′-Fluorinated ODN **9**. HPLC purification: column: POROS 20 HQ, 100 × 4.6 mm; eluent A: H_2O, 25 mM Tris-HCl, 1 mM EDTA, pH 8.0 with H_2O/acetonitrile (90/10); eluent B: A plus 1 M NaCl; gradient: 20–50% B in 15 min; flow rate 4 mL/min; retention time: 7.3 min. MS (MALDI–TOF)$[M + H]^+$, obd: 2979, calcd: 2980.