Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIX, No. 4

A GENERAL METHOD FOR LABELING OLIGODEOXYNUCLEOTIDES WITH ^{18}F FOR *IN VIVO* PET IMAGING

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Summary

This paper describes an original general method for the synthesis of oligodeoxynucleotides (ODNs) carrying the positron emitter ^{18}F. The labeling strategy developed is simple, reliable, independent of backbone modifications and base sequence. It should be of general applicability for the labeling of natural or modified ODN, provided that the latter bear a phosphorothioate group at their 3' end, and it should be easily transposable to other radiohalogenes such as ^{76}Br or ^{123}I. The methodology we developed uses recently preparative-scale commercially available 3'-phosphorothioate oligodeoxynucleotides and is based on the efficient coupling reaction of oligodeoxynucleotides, containing a single phosphorothioate monoester, and *N*-(4-radiohalobenzyl)-2-bromoacetamide. Yields of 40-45% (decay corrected) of pure [^{18}F]*N*-(4-fluorobenzyl)-2-(ACCGATCCG$^{3'}$-p$_S$)-acetamide [^{18}F]-(**1**) (around 30 mCi or 1.1 GBq) were obtained for the whole synthetic procedure (220 minutes) with respect to [^{18}F]fluoride ion, with specific radioactivities as high as 3 Ci/μmole (111 TBq/μmol) calculated for End of Bombardment (or 750 mCi/μmole (28 TBq/μmol) at End of Synthesis) for a 15 μA, 60 min (54000 μC) irradiation of a 50% [^{18}O]-enriched water target with a 16 MeV proton beam [^{18}O(p,n)^{18}F].

Key Words : fluorine-18, labeled oligodeoxynucleotide, antisense, hybridization, mRNA, positron emission tomography

Introduction

Positron Emission Tomography (PET) uses probes labeled with short-lived positron-emitting isotopes to measure *in vivo* a wide range of physiological parameters, including blood flow, glucose metabolism and the distribution and concentration of receptors, enzymes and neuromediators[1]. One field that PET has yet to explore is the imaging of mRNAs *in vivo* in the manner that *in situ* hybridization realizes on post mortem tissues. This would open the technique of PET to biological parameters for which there are no adequate ligands of the proteic actor, such as

CCC 0362–4803/97/040319–12$17.50
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Received 7 October 1996
Revised 18 November 1996

structural and regulatory elements of the cell, or exogenous molecules such as viral RNA. Furthermore, the high degree of specificity of Watson-Crick base pairing should allow exquisite discrimination between closely related target sequences.

Oligodeoxynucleotides (ODNs) of relatively short length have been known for a long time to hybridize specifically with their RNA of complementary sequence on tissue sections, and more recently to block their target mRNA's expression when administered *in vivo*[2].

For this reason, we sought to develop a general technique for ODNs labeling with positron-emitting isotopes, independent of backbone modifications and base sequence. In principle, the manner in which the ODN is labeled may be dictated by the specific study involved, but in general the principles of simplicity and versatility are best employed to guide the choice of labeling procedure.

Some chemical strategies have already been developed at the monomer level in the hope that they could be applied later on at the oligomer level. For example, thymidine[3] and a thymidine derivative[4] have been labeled with the positron-emitting ^{11}C, but the too short half-life of this isotope ($t_{1/2}$ of 20.4 minutes), prohibits the synthesis of small oligodeoxynucleotides using the well-established phosphotriester methodology. [^{18}F]Fluoro derivatives ($t_{1/2}$ of 110 minutes) of cytidine[5], thymidine[6] and mainly uridine[7-18] were also synthesized (unfortunately often carrier-added). However, even with this longer half-life isotope, ODNs of defined sequence have not been labeled yet using these types of approach.

We have developed an original methodology, using recently preparative-scale commercially available 3'-phosphorothioate oligodeoxynucleotides and based on the efficient coupling reaction of oligodeoxynucleotides containing a single phosphorothioate monoester with an electrophilic moiety[19,20,21]. Terminal labeling of the oligodeoxynucleotide may be advantageous not only for minimal structural perturbation (minor modification of a terminal phosphomonoester is not likely to alter the structure or the stability of a double-stranded or even triple-stranded complex) but also for protection of the ODN toward terminal enzymatic degradation due to the various exonucleases present *in vivo*. Another advantage consist in the fact that terminal labeling should be completely independent of the ODN sequence and therefore also applicable to any sequence. Site-specific earmarking of the ODN phosphate backbone has already been described and a variety of compounds, bearing chemical functions dedicated to thioselective reactions, have been covalently bound to oligodeoxynucleotides containing a phosphorothioate monoester[19,20,21]. Among them, we reasoned that 2-bromo- or 2-iodo-*N*-benzyl-substituted acetamides were, from a synthetic point of view, suitable to act as the carrier of a positron-emitter and conceivable in short-lived isotope chemistry. The benzyl group was chosen as substituent especially for its versatile and easy transposition to the synthesis of *N*-(4-radiohalobenzyl)-2-bromoacetamide using not only ^{18}F as the positron-emitter, but also other radiohalogenes such as ^{76}Br ($t_{1/2}$ of 16 hours) and ^{123}I ($t_{1/2}$ of 13 hours).

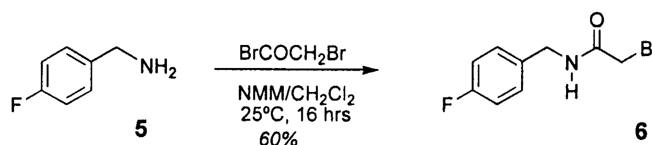
This paper fully describes the radiosynthesis of non-carrier-added [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide and its condensation with the ACCGATCCG 9-mer, 3'phosphorothioate oligodeoxynucleotide chosen as model for this reaction.

Results and Discussion

Chemistry

The synthesis of fluorine-18 labeling precursor **3** is illustrated in the first part of scheme 3. 4-Cyano-*N,N,N*-trimethylanilinium trifluoromethanesulfonate (**3**) was prepared from commercial 4-dimethylaminobenzonitrile (**2**) by reacting with 1.5 equivalent of methyl trifluoromethanesulfonate for 5 hours at reflux in benzene (82% yield). Its choice as precursor for labeling is discussed in the radiochemistry part below.

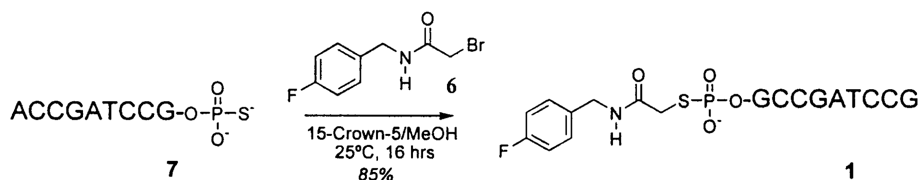
The synthetic route to prepare unlabeled *N*-(4-fluorobenzyl)-2-bromoacetamide (**6**) is shown in scheme 1.



Scheme 1 : Synthesis of *N*-(4-fluorobenzyl)-2-bromoacetamide (**6**)

4-Fluorobenzylamine (**5**) was reacted with 2-bromoacetyl bromide in the presence of *N*-methylmorpholine to give **6** in 60% non-optimized yield. Other fluorine-18 synthesis reference products such as 4-fluorobenzonitrile (**4**) or 4-fluorobenzylamine (**5**) were commercially available.

The reference unlabeled 3'substituted oligodeoxynucleotide was prepared as follows (scheme 2). ACCGATCCG was chosen as a model ODN, for its manageable size, its well-balanced A/C/G/T ratio and the absence of palindromic sequences. The starting lyophilized oligodeoxynucleotide 3'-phosphorothioate (7, sequence : ACCGATCCGp_S) was dissolved in MeOH containing 5% of 15-Crown-5 and stirred overnight at room temperature with 10 equivalents of *N*-(4-fluorobenzyl)-2-bromoacetamide (**6**). Semi-preparative HPLC gave **1** as the highly predominant product (85% yield).



Scheme 2 : Synthesis of unlabeled 3'-substituted oligodeoxynucleotide (**1**)

Its oligodeoxynucleotide nature was in a first time confirmed by RP HPLC (higher retention time than **7**, due to an increase in lipophilicity (following the addition of the fluorobenzyl substituent)), by UV spectrometry (superimposable UV spectra of **7** and **1**) and by polyacrylamide gel electrophoresis (higher migration on the 20%-gel than starting nucleotide **7**, due again to its

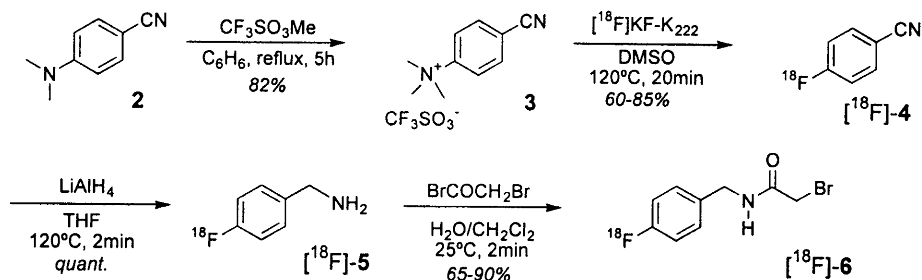
increased lipophilicity but also by the loss of a charge on the substituted phosphate group). In a second time, oligodeoxynucleotidic integrity of the sequence was confirmed after a DNAse/phosphatase enzymatic digestion : standard HPLC assay confirmed all four nucleoside chemical identities and the expected A/C/G/T ratio of 2/4/2/1 for the sequence used. The addition of only one 4-fluorobenzylaminocarbonylmethyl substituent to the oligodeoxynucleotide under the conditions used for the coupling reaction (even with 10 equivalents of reactant **6**) was confirmed by mass spectroscopy (electrospray, negative mode) : a molecular weight of 2944.2 was found for structure **1** (2944.01 calculated). The regioselectivity of the condensation was in accord with the published literature. Only the phosphorothioate function is reactive under the conditions used as verified by ^{31}P NMR : a chemical shift of +15.23 ppm (D_2O, TMP as internal standard) was recorded for the terminal phosphorothioate function in the modified oligodeoxynucleotide (**1**) whereas it was +42.00 ppm in starting oligodeoxynucleotide (**7**); in both oligodeoxynucleotides chemical shifts for the inter-nucleosidic phosphate were between -3.50 and -4.00 ppm. These values are fully in accord with published ^{31}P NMR literature[22,23]

Radiochemistry

[^{18}F]*N*-(4-Fluorobenzyl)-2-bromoacetamide [^{18}F]-(**6**) was prepared in 3 steps from the anilinium sulfonate **3** (scheme 3).

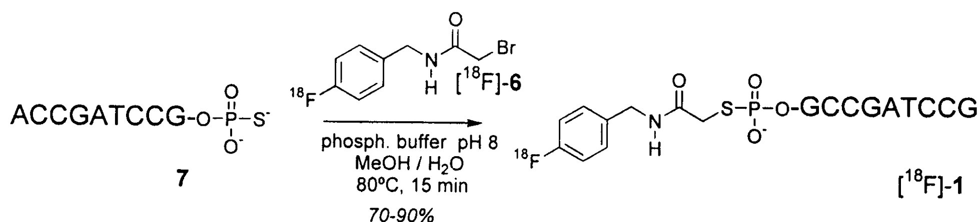
The first step corresponded to the introduction of the fluorine-18 using a nucleophilic aromatic substitution, performed at the para position of the cyano-activated ring of a benzonitrile. *N,N,N*-Trimethylammonium was chosen as para substituent for this substitution, not only for its high potential as leaving group in comparison with a corresponding halo or better nitro substituent, but also for the expected superior precursor separation from the reaction product ([^{18}F]-4-fluorobenzonitrile) due to the differences in the physico-chemical properties[24]. The reaction was performed in hot DMSO (120°C) for 20 minutes using the activated [^{18}F]KF-K_{222} complex[25] as the fluorinating reactant and 4-cyano-*N,N,N*-trimethylanilinium trifluoromethanesulfonate (**3**) as the substrate. The reaction mixture was diluted with water and passed through a Sep-pak C18. 4-[^{18}F]Fluorobenzonitrile was then eluted with THF. The yield of substitution varied from 60% to 85% with respect to [^{18}F]fluoride ion (yields were determined after the Sep-pak elution by the THF over DMSO/H_2O radioactivity counting ratio followed by radiochromatography). Chemical identity was confirmed by co-elution with commercial reference compound **4**. The second step was the reduction of the cyano function of **4**. Best results were obtained when the reduction was performed with $LiAlH_4$ in refluxing THF (120-130°C) for 2 minutes. In order to minimize the amount of $LiAlH_4$, the THF solution of **4** was dried for 5 minutes by standing over 4Å molecular sieve powder prior to reduction. Volatility of the product forbade evaporation till dryness followed by taken up in dry THF. Quantitative yields (determined by radiochromatography) of 4-[^{18}F]fluorobenzylamine (**5**) were usually obtained with an optimized minimum quantity of $LiAlH_4$. The amine-aluminium complex as well as the excess of $LiAlH_4$ were destroyed by adding water and chemical identity was confirmed by co-elution with commercial reference compound **5**. The utilization of DIBAL-H as

well as BH_3.DMS gave only poor yield and were found less convenient in use. The last step is the condensation with 2-bromoacetyl bromide. The reaction occurred cleanly in 2 minutes at room temperature in a 10/1 (v/v) mixture of CH_2Cl_2/H_2O (65 to 90% yield, determined by radiochromatography). Semi-preparative HPLC gave pure [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide [^{18}F]-(**6**). (Co-elution with authentic synthesized unlabeled reference compound **6**).



Scheme 3 : Synthesis of [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide [^{18}F]- (**6**)

Condensation of [^{18}F]-(**6**) with the oligodeoxynucleotidic sequence **7** occurred in less than 15 minutes at 80°C in a mixture of 0.1M aqueous phosphate buffer (pH 8) and methanol (scheme 4). Semi-preparative RP HPLC gave pure expected oligodeoxynucleotide [^{18}F]-(**1**) (Co-elution with authentic synthesized unlabeled reference compound **1**) with condensation yields from 70 to 90%.



Scheme 4 : Condensation of [^{18}F]-(**6**) with ACCGATCCG$^{3'}$-p$_S$ - Synthesis of labeled 3' substituted oligodeoxynucleotide (**1**)

Typically, a 40-45% yield (decay corrected) of pure [^{18}F]*N*-(4-fluorobenzyl)-2-(ACCGATCCG$^{3'}$-p$_S$)-acetamide [^{18}F]-(**1**) (around 30 mCi or 1.1 GBq) were obtained for the whole synthetic procedure (220 minutes) with respect to [^{18}F]fluoride ion, with specific radioactivities as high as 3 Ci/μmole (111 TBq/μmol) calculated for End of Bombardment (or 750 mCi/μmole (28 TBq/μmol) at End of Synthesis) for a 15 μA, 60 min (54000 μC) irradiation of a 50% [^{18}O]-enriched water target with a 16 MeV proton beam [^{18}O(p,n)^{18}F]. The synthesis (included the HPLC purification) of labeled [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide (**6**) is now fully automated on a computer assisted Zymate robot system.

The stability of the ^{18}F-labeled probe and the detection limit were evaluated by polyacrylamide-urea gel electrophoresis of serial dilutions of the [^{18}F]ODN. The probe in solution was found to be remarkably stable, since there was no apparent degradation at room temperature up to 3 hours after the radiosynthesis and the detection limit with the phosphorimager technique was estimated to be 20 nCi. However, the stability of the probe *in vivo* may be less satisfactorily, since only about 50% of the radioactivity was recovered as unchanged [^{18}F]ODN after 2 hours incubation in human plasma at 37°C.

Experimental

General

Chemicals were purchased from Aldrich, Fluka or Sigma France and were used without further purification. Lyophilized, HPLC-RP purified oligodeoxynucleotides 3'-phosphorothioate were purchased from Genset, France. TLC were run on pre-coated plates of silicagel 60F254 (Merck). The compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by iodine staining and/or (3) by dipping the TLC-plates in a 1% ninhydrin solution in ethanol (or 1% aqueous $KMnO_4$) and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyzer. Flash chromatography was conducted on silicagel 63-200 μm (Merck) at 0.3 bars (Ar). HPLC were run on Waters systems equipped with a 510 pump (isocratic elution) or a 600 pump with a 600 controller (gradient elution), 490E UV-multiwavelength detectors and Geiger-Müller radioactivity detectors. UV spectra were recorded on a Kontron-Uvikon 880 UV-Vis spectrophotometer. IR spectra were recorded in $CHCl_3$ on a Perkin-Elmer IR/TF Paragon 1000 spectrophotometer (frequencies are reported in cm^{-1}). NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated solvents (DMSO-d_6, δ = 2.50 ppm) and/or TMS as internal standards for ^1H NMR as well as the deuteriated solvents (DMSO-d_6, δ = 39.5 ppm) and/or TMS as internal standards for ^{13}C NMR. ^{31}P NMR were recorded on the same apparatus using TMP (δ = 0.0 ppm) as internal standard. The chemical shifts are reported in ppm, downfield either from TMS (^1H NMR and ^{13}C NMR) or from TMP (^{31}P NMR) (s, d, t, dd, b for singlet, doublet, triplet, doublet of doublet and broad respectively). The mass spectra (MS), DCI/NH_4^+ were measured on a Nermag R10-10 apparatus (ionization potential of 70 eV was used) and on a Quattro VG (Fison, Manchester, UK) for electrospray ionization (negative mode). Air- or moisture sensitive reactions were conducted in heat gun-dried glassware, under an inert atmosphere and with freshly distilled solvents.

Chemistry

4-Cyano-*N,N,N*-trimethylanilinium trifluoromethanesulfonate (**3**)

A solution of 21.1 g of 4-(dimethylamino)benzonitrile (144 mmol, MW : 146.19) and 22.6 mL of methyl trifluoromethanesulfonate (200 mmol, 1.4 eq, MW : 164.10, d : 1.450) in 300 mL of

dry benzene was refluxed for 5 h under an argon atmosphere. The solid residue was filtered off and dissolved in 300 mL of water. This aqueous solution was washed once with 200 mL of CH_2Cl_2, once with 200 mL of $CHCl_3$ and concentrated to dryness. The residue was recrystallized twice from CH_2Cl_2 containing a few drops of MeOH to give 36.6 g of pure 4-cyano-*N,N,N*-trimethylanilinium trifluoromethanesulfonate (**3**) as pale yellow needles (82% yield).
Rf (MeOH/AcOH : 50/50) : 0.4. ^1H NMR (DMSO-d_6, 338.0K) : δ : 8.30-8.00 (2b, 4H) ; 3.70 (s, 9H). ^{13}C NMR (DMSO-d_6, 338.0K) : δ : 150.0 [C] ; 133.9 [CH] ; 121.8 [CH] ; 120.6 [q, J : 325 Hz, CF_3] ; 116.9 [C] ; 113.2 [C] ; 56.4 [CH_3].

4-Fluorobenzonitrile (**4**) and 4-fluorobenzylamine (**5**) are commercially available.

N-(4-Fluorobenzyl)-2-bromoacetamide (**6**)

To a solution containing 13.0 mL of 4-fluorobenzylamine (**5**, 114 mmol, MW : 125.15, d : 1.095) and 10.2 mL of *N*-methylmorpholine (114 mmol, 1 eq, MW : 101.15, d : 1.13) in 100 mL of CH_2Cl_2 was added dropwise 10.0 mL of bromoacetyl bromide (114 mmol, 1 eq, MW : 201.86, d : 2.3) while maintaining the temperature under 30°C. After addition, the reaction mixture was stirred at room temperature overnight. The mixture was then filtered, the filtrate was concentrated to dryness and the residue was chromatographed on silica gel. Elution with heptane/EtOAc (90/10 to 60/40) gave 16.8 g of pure *N*-(4-fluorobenzyl)-2-bromoacetamide (**6**) as a white solid (60% yield).
Rf (heptane/EtOAc : 50/50) : 0.35. IR ν : 3272 ; 3084 ; 1644 ; 1604 ; 1565 ; 1508 ; 1432 ; 1314 ; 1216 ; 1131 ; 820. ^1H NMR (DMSO-d_6, 300.0K) : δ : 8.80 (bt, 1H) ; 7.32 (dd, J : 8.1 Hz and 5.70 Hz, 2H) ; 7.15 (t, J : 8.1 Hz, 2H) ; 4.31 (d, J : 6 Hz, 2H) ; 3.92 (s, 2H). ^{13}C NMR (DMSO-d_6, 300.0K) : δ : 166.1 [C] ; 161.3 [C, d, J : 249 Hz] ; 135.0 [C, d, J : 2 Hz] ; 129.2 [CH, d, J : 8 Hz] ; 115.1 [CH, d, J : 23 Hz] ; 41.9 [CH_2] ; 29.4 [CH_2]. MS : 265 [M + NH_4^+] ; 263 [M + NH_4^+] ; 248 [M + H^+] ; 246 [M + H^+].

N-(4-Fluorobenzyl)-2-(ACCGATCCG$^{3'}$-p_S)-acetamide (**1**)

95 OD of the 3'p_S-oligomer (**7**, ACCGATCCG, ~ 1.0 μmol, MW : 2778.85) were dissolved in 2 mL of MeOH containing 100 μL of 15-Crown-5 (0.5 mmol, MW : 220.27, d : 1.109). To this solution was added 2.5 mg of *N*-(4-fluorobenzyl)-2-bromoacetamide (10.0 μmol, MW : 246.08). The mixture was stirred at room temperature overnight, concentrated to dryness and the white residue taken up in 1 mL of water. A 100 μL aliquot was purified on a C18 semipreparative HPLC column (μBondapak™, Waters, 7.8 x 300 mm, 125 Å, 10 μm). Gradient elution (linear in 5 min from 95/5 to 90/10, then linear in 10 min from 90/10 to 85/15, then wash-out for 10 min at 50/50; flow rate : 6 mL/min and UV detection at 254 nm) using 50 mM aq. TEAA/CH_3CN gave 8 OD of pure *N*-(4-fluorobenzyl)-2-(ACCGATCCG$^{3'}$-p_S)-acetamide (**1**, 85% yield).
Rt : 12.0-12.5 min (C18 HPLC, see above for elution conditions). UV : λ_{max} : 260 nm (ε_{estim} : 93500). ^{31}P NMR (D_2O, 298.0K) : δ : + 15.23 (-OP(O)(OH)(S-acetamide)) ; - 4.00 (-OP(O)$(OH)_2$). MS (electrospray) : 2944.2 (theor. 2944.01).

Standard polyacrylamide gel electrophoresis analysis, as well as UV and DNAse/phosphatase assay were run by Genset, France (see acknowledgment part).

for reference : ACCGATCCG$^{3'}$-p$_S$ (7) :

Rt : 10.0-10.5 min (C18 HPLC, see above for elution conditions). UV : λ_{max} : 260 nm (ε_{mes} : 92000 ; ε_{calc} : 92500). ^{31}P NMR (D_2O, 298.0K) : δ : + 42.00 (-OP(O)(OH)(SH)) ; - 4.00 (-OP(O)$(OH)_2$). MS (electrospray) : 2778.8 (theor. 2778.85).

Radiochemistry

Production of aqueous [^{18}F]F$^-$

[^{18}F]F$^-$ was produced on a CGR-MeV 520 cyclotron by irradiation of a water target (water-cooled stainless steel target-holder equipped with an 12 µm titanium window He-cooled) either using a 30 MeV helium-3 beam on Millipore-grade water [^{16}O(^{3}He,p)^{18}F] or a 16 MeV proton beam on 50 to 95% [^{18}O]-enriched water [^{18}O(p,n)^{18}F].

Preparation of [^{18}F]N-(4-fluorobenzyl)-2-bromoacetamide ([^{18}F]-6)

The 1.0-1.5 mL of aqueous [^{18}F]fluoride from the target were combined with 14.0-15.0 mg of Kryptofix® K_{222} and 2.0-4.5 mg of K_2CO_3 in a silicon coated glass tube. The resulting solution was gently concentrated to dryness at 110-120°C under a nitrogen stream for 15-20 min. The residue was then dissolved in 200 µL of freshly distilled DMSO and transferred to a 2 mL reaction vial containing 4.0 to 6.0 mg of the triflate salt of 4-trimethylammoniumbenzonitrile (**3**). The evaporation tube was rinsed twice with 200 µL of DMSO which was then added to the previous reaction mixture. Resolubilization yields were about 60-80% of the original [^{18}F]fluoride ion. The reaction vial was then tightly closed and heated without stirring at 120-130°C for 20 min. The resulting yellow-brown reaction mixture was cooled using an ice/water bath, diluted with 3 mL of water and passed through a C18 Sep-pak cartridge (Waters). The cartridge was washed with 0.5 mL of water and partially dried for 5 min by applying a nitrogen stream. 4-[^{18}F]Fluorobenzonitrile [^{18}F]-(**4**) was eluted with 4 mL of THF (Less than 5% of the total radioactivity amount was left on the cartridge). The yield of substitution varied from 60% to 80% with respect to [^{18}F]fluoride ion (yields were determined after the Sep-pak elution by the THF over DMSO/H_2O radioactivity counting ratio followed by radiochromatography (SiO_2-TLC, eluant : heptane/EtOAc : 95/5, Rf : 4-[^{18}F]fluorobenzonitrile : 0.35 and Rf : [^{18}F]fluoride ion : 0.0 or eluant : heptane/EtOAc : 50/50, Rf : 4-[^{18}F]fluorobenzonitrile : 0.80 and Rf : [^{18}F]fluoride ion : 0.0). The mentioned THF solution was allowed to stand for 5 min on 1.0 g of oven-dried 4 Å grounded molecular sieve. Another 3 mL of THF was used to wash the above powder and to transfer the 4-[^{18}F]fluorobenzonitrile directly into a 8 mL reaction vial containing 20 mg of dry powdered $LiALH_4$. The vessel was then tightly closed and heated for 2 min at 120-130°C (strong reflux). The yield of reduction was usually quantitative as determined by radiochromatography (SiO_2-TLC, eluant : heptane/EtOAc : 50/50, Rf : 4-[^{18}F]fluorobenzonitrile : 0.80 and Rf : [^{18}F]fluorobenzylamine-aluminium complex : 0.0). The

resulting gray-white suspension was cooled using an ice/water bath and concentrated to dryness. The amine-aluminium complex as well as the excess of $LiALH_4$ were destroyed by adding 300 μL of H_2O. The white aqueous residue was then diluted with 3 mL of CH_2Cl_2 (SiO_2-TLC, eluant : CH_2Cl_2/MeOH/TEA : 90/10/3, Rf : [^{18}F] fluorobenzylamine : 0.25). After addition of 30 μL of a 1.0 M solution of bromoacetyl bromide in CH_2Cl_2, the white milky suspension was allowed to react 2-3 min at room temperature with a smooth air-bubbling agitation. The yield of condensation with $BrCOCH_2Br$ varied from 65% to 90% as determined by radiochromatography (SiO_2-TLC, eluant : heptane/EtOAc : 50/50, Rf [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide : 0.35 and Rf : [^{18}F]fluorobenzylamine : 0.0). The reaction mixture was then filtered on cotton and the precipitate washed twice with 1.0 mL of CH_2Cl_2. The filtrate was concentrated to dryness, the residue dissolved in 1-2 mL of CH_2Cl_2 (or the HPLC solvent used for purification) and the crude was injected onto a SiO_2 semipreparative HPLC column (Prep Nova-Pak® HR Silica, Waters, 7.8 x 300 mm, 60 Å, 6 μm). Isocratic elution (flow rate : 3 mL/min and UV detection at 254 nm) using CH_2Cl_2/EtOAc (95/5) gave pure labeled [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide ([^{18}F]-(**6**), Rt : 8.0-9.0 min). The synthesis (included the HPLC purification) of labeled [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide [^{18}F]-(**6**) is now fully automated on a computer assisted Zymate robot system.

Condensation of labeled [^{18}F]N-(4-fluorobenzyl)-2-bromoacetamide [^{18}F]-(6) with ACCGATCCG 3'-terminal phosphorothioate oligodeoxynucleotide (7)

The previous HPLC collected [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide [^{18}F]-(**6**) was concentrated to dryness and the residue was dissolved in 500 μL of MeOH. To this solution was then added 500 μL of H_2O followed by 50 μL of 1.0 M phosphate buffer pH 8.0 and finally 0.1-0.2 μmol of the ACCGATCCG 3'-phosphorothioate oligodeoxynucleotide (**7**) in 30-70 μL of H_2O. The resulting mixture was then heated for 10-15 min at 70-80°C. The yield of coupling varied from 70 to 90% as determined by radiochromatography (SiO_2-TLC, eluant : heptane/EtOAc : 50/50, Rf [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide : 0.35 and Rf : [^{18}F]-oligodeoxynucleotide : 0.0). The mixture was then concentrated to dryness, the residue was dissolved in 1.0 mL of H_2O and injected onto a C18 semipreparative HPLC column (μBondapak™, Waters, 7.8 x 300 mm, 125 Å, 10 μm). Gradient elution (linear in 5 min from 95/5 to 90/10, then linear in 10 min from 90/10 to 85/15, then wash-out for 10 min at 50/50; flow rate : 6 mL/min and UV detection at 254 nm) using 50 mM aq. TEAA/CH_3CN gave pure labeled [^{18}F]*N*-(4-fluorobenzyl)-2-(ACCGATCCG$^{3'}$-p_S)-acetamide ([^{18}F]-(**1**), Rt : 12.0-12.5 min). The same yield as previously estimated by radio-TLC was observed after HPLC. Final conditioning as a TEAA-free injectable pharmaceutical form was performed by elution with physiological serum on a NAP-10 (Pharmacia) Sephadex® G-25 DNA grade column.

Typically, a 40-45% yield (decay corrected) of pure [^{18}F]*N*-(4-fluorobenzyl)-2-(ACCGATCCG$^{3'}$-p_S)-acetamide [^{18}F]-(**1**) (around 30 mCi or 1.1 GBq) was obtained for the whole synthetic procedure (220 minutes) with respect to [^{18}F]fluoride ion, with specific radioactivities as high as 3 Ci/μmole (111 TBq/μmol) calculated for End of Bombardment (or 750 mCi/μmole (28

TBq/μmol) at End of Synthesis) for a 15 μA, 60 min (54000 μC) irradiation of a 50% [^{18}O]-enriched water target with a 16 MeV proton beam [^{18}O(p,n)^{18}F].

Conclusion

An efficient radiosynthesis of no-carrier-added [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide and its condensation with a 9-mer, 3'phosphorothioate oligodeoxynucleotide are described in this paper. Yields of 40-45% (decay corrected) of pure [^{18}F]*N*-(4-fluorobenzyl)-2-(ACCGATCCG$^{3'}$-p$_S$)-acetamide [^{18}F]-(**1**) (around 30 mCi or 1.1 GBq) were obtained for the whole synthetic procedure (220 minutes) with respect to [^{18}F]fluoride ion, with specific radioactivities as high as 3 Ci/μmole (111 TBq/μmol) calculated for End of Bombardment (or 750 mCi/μmole (28 TBq/μmol) at End of Synthesis) for a 15 μA, 60 min (54000 μC) irradiation of a 50% [^{18}O]-enriched water target with a 16 MeV proton beam [^{18}O(p,n)^{18}F]. The labeling strategy developed is simple, reliable, independent of backbone modifications and base sequence. Therefore, this method should be of general applicability for the labeling of natural or modified ODN, provided that the latter bear a phosphorothioate group at its 3' end. Moreover, the method is easily transposable to other radiohalogenes such as ^{76}Br or ^{123}I.

Given that future studies will bring forward ODNs exhibiting the required characteristics of stability *in vivo*, penetration into the cytoplasm of cells and low non-specific binding, such a method makes envisageable the *in vivo* study of any mRNA of interest to the clinician. Cancers, viral infections, genetic factors associated with the prognosis of diseases, longitudinal studies of the results of grafting, gene therapy and, most of all, antisense therapy could therefore be explored *in vivo*. In this respect, most encouraging is a recent pioneering study showing images of a mammary tumor in mouse after intravenous administration of a γ-emitting antisense probe for c-myc oncogene mRNA[26]. Maximal contrast was reached after one hour, while no imaging was achieved with the corresponding sense probe. This kinetic data suggests that even positron-emitting radioisotopes with short half-lives would be of value for imaging mRNAs with PET.

Acknowledgment

The authors wish to thank cyclotron operators Mr. Daniel Gouel and Mr. Christophe Peronne for performing the irradiations, as well as Miss Isoline Caubere for technical assistance, the Section Analyses Instrumentation Spectroscopiques (Henri Virelizier) for MS recording and Dr Salvatore Terrazino for running the polyacrylamide-urea gels. We express our gratitude to Dr Didier Gasparutto (Genset, Paris, France) for valuable discussions and helpful technical suggestions and support. This study was supported by a grant from the French Ministry of Research (ACC SV12 to B.T.) and BIOMED-2 contract BMH4-CT96-1067.

References

(1) Phelps, M.E., Mazziota, J.C., Schelberg, H.R. In *Positron emission tomography and autoradiography : principles and applications for the brain and heart*; Raven Press : New York, 1986, 690.

(2) Crooke, S.T., Lebleu B. In *Antisense research and applications*, CRC Press : Boca Raton, 1993, 579.

(3) Van der Borght, T., Labar, D., Pauwels, S., Lambotte, L. Production of [2-^{11}C]Thymidine for quantification of cellular proliferation with PET. *Appl. Radiat. Isot.* **1991**, *42*, 103-104.

(4) Goethals, P., Sambre, J., Coene, M., Casteleyn, K., Poupeye, E. A remotely controlled production system for routine preparation of [methyl-^{11}C]thymidine. *Appl. Radiat. Isot.* **1992**, *43*, 952-954.

(5) Visser, G.W.M., Zwaagstra, O., Gorree, G.C.M., Moonen, P., Herscheid, J.D.M., Hoekstra, A. Synthesis and tumor-localizing properties of [^{18}F]-5-fluorocytosine-arabinoside and [^{18}F]-5-fluorocyclocytidine. *Eur. J. Nucl. Med.* **1986**, *12*, 137-140.

(6) Wilson, I.K., Chatterjee, S., Wolf, W. Synthesis of 3'-fluoro-3'-deoxythymidine and studies of its radiolabeling as a tracer for the non-invasive monitoring of drugs against AIDS. *J. Fluorine Chem.* **1991**, *55*, 283-289.

(7) Fowler, J.S., Finn, R.D., Lambrecht, R.M., Wolf, A.P. The synthesis of ^{18}F-5-fluorouracil. *J. Nucl. Med.* **1973**, *14*, 63-64.

(8) Abrams, D.N., Knaus, E.E., Wiebe, L.I., Helus, F., Maier-Borst, W. 5-^{18}F-5-fluoro-2-deoxyuridine : a potential diagnostic agent for tumor metabolism. *J. Label. Compounds Radiopharm.* **1979**, *16*, 155-156.

(9) Vine, E.N., Young, D., Vine, W.H., Wolf, W. An improved synthesis of ^{18}F-5-fluorouracil. *Int. J. Appl. Radiat. Isot.* **1979**, *30*, 401-405.

(10) Irie, T., Fukushi, K., Ido, T. Synthesis of ^{18}F-6-fluoropurine and ^{18}F-6-fluoro-9-β-D-ribofuranosylpurine. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 445-448.

(11) Irie, T., Fukushi, K. Inoue, O., Yamasaki, T., Ido, T., Nuzaki, T. Preparation of ^{18}F-labeled 6- and 2-fluoro-9-benzylpurine as a potential brain-scanning agent. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 633-636.

(12) Diksic, M., DiRaddo, P. New syntheses of fluoro-compounds by fluorination in water. *Tetrahedron Lett.* **1984**, *25*, 4885-4888.

(13) Diksic, M., Farrokhzad, S., Yamamoto, Y.L., Feindel, W. A simple synthesis of ^{18}F-labeled 5-fluorouracil using acetylhypofluorite. *Int. J. Nucl. Med. Biol.* **1984**, *11*, 141-142.

(14) Shiue, C.-Y., Wolf, A.P., Friedkin, M. Syntheses of 5'-deoxy-5-[^{18}F]fluorouridine and related compounds as probes for measuring tissue proliferation *in vivo*. *J. Label. Compounds Radiopharm.* **1984**, *21*, 865-873.

(15) Visser, G.W.M., Noordhuis, P. Zwaagstra, O., Herscheid, J.D., Hoekstra, A. A simplified synthesis of ^{18}F-labeled cytosine- and uracil-nucleosides. *Appl. Radiat. Isot.* **1986**, *37*, 1074-1076.

(16) Ishiwata, K;, Moma, M., Iwata, R., Ido, T. Automated synthesis of 5-[^{18}F]fluoro-2'-deoxyuridine. *Int. J. Appl. Radiat. Isot.* **1987**, *38*, 467-473.

(17) Visser, G.W.M., Gorree, G.C.M., Dijksman, J.A.R., Bijlma, A.T., Herscheid, J.D.M. Synthesis and evaluation of some new [^{18}F]-5-fluoro-5,6-dihydro-uracil-nucleosides. *J. Label. Compounds Radiopharm.* **1989**, *26*, 227-237.

(18) Oberdorfer, F., Hofmann, E., Maier-Borst, W. Preparation of ^{18}F-labeled 5-fluorouracil of very high purity. *J. Label. Compounds Radiopharm.* **1989**, *27*, 137-146.

(19) Thuong, N.T., Asseline, U. In *Oligonucleotide and analogues, a practical approach : Oligonucleotides attached to intercalors, photoreactive and cleavage agents*, Eckstein, F. Ed., IRL Press : Oxford, 1991, 238-308.

(20) Kurfürst, R., Roig, V., Chassignol, M., Asseline, U., Thuong, N.T. Oligo-α-deoxyribonucleotides with a modified nucleic base and covalently linked to reactive agents. *Tetrahedron* **1993**, *49*, 6975-6990.

(21) Fidanza, J.A., Ozaki, H., McLaughlin, L.W. In *Methods in molecular biology, Vol. 26, Protocols for oligonucleotide conjugates : Functionalization of oligonucleotides by the incorporation of thio-specific reporter groups*, Agrawal, S. Ed., Humana Press Inc : Totowa, NJ, 1994, chapter 4, 121-143.

(22) Gorenstein, D.G. In *Handbook of organophosporus chemistry : advances in phosphorus-31 NMR*, Engel R. Eds, Dekker M. Inc : New York, Basel, Hong Hong, 1992, chapter 9, 435-482.

(23) Gorenstein, D.G. In *Phosphorus-31 NMR : principles and applications*, Gorenstein, D.G. Ed., Academic Press : New York, 1984.

(24) Haka, M.S., Kilbourn, M.R., Watkins, L., Toorongian, S.A. Aryltrimethylammonium trifluoromethanesulfonates as precursors to aryl [^{18}F]fluorides : improved synthesis of [^{18}F]GBR-13119. *J. Label. Compounds Radiopharm.* **1989**, *27*, 823-833.

(25) Hamacher, K., Coenen, H.H., Stöcklin, G. Efficient stereospecific synthesis of NCA 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *J. Nucl. Med.* **1986**, *27*, 235-238.

(26) Dewanjee, M.K., Ghafouripour, A.K., Kapadvanjwala, M., Dewanjee, S., Serafini, A.N., Lopez, D.M., Sfakianakis, G.N. Non-invasive imaging of c-myc oncogene messenger RNA with indium-111-antisense probe in a mammary tumor-bearing mouse model. *J. Nucl. Med.* **1994**, *35*, 1054-1063.