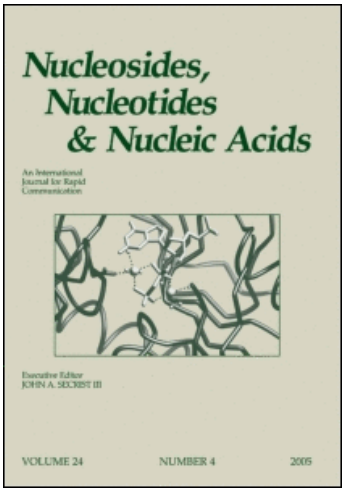


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Labeling of an Antisense Oligonucleotide with [^{18}F]FPy5yne

James A. H. Inkster[a]; Michael J. Adam[b]; Tim Storr[a]; Thomas J. Ruth[ab]
[a] Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada [b] TRIUMF, Vancouver, British Columbia, Canada

Online publication date: 24 November 2009

To cite this Article Inkster, James A. H. , Adam, Michael J. , Storr, Tim and Ruth, Thomas J.(2009) 'Labeling of an Antisense Oligonucleotide with [^{18}F]FPy5yne', Nucleosides, Nucleotides and Nucleic Acids, 28: 11, 1131 — 1143
To link to this Article: DOI: 10.1080/15257770903400691
URL: http://dx.doi.org/10.1080/15257770903400691

Nucleosides, Nucleotides and Nucleic Acids, 28:1131–1143, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770903400691

Taylor & Francis
Taylor & Francis Group

LABELING OF AN ANTISENSE OLIGONUCLEOTIDE WITH [^{18}F]FPy5yne

James A. H. Inkster,[1,2] Michael J. Adam,[2] Tim Storr,[1] and Thomas J. Ruth[1,2]

[1]*Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada*
[2]*TRIUMF, Vancouver, British Columbia, Canada*

□ *Functional imaging of gene expression in vivo with short-lived positron emitter ^{18}F remains an unrealized goal, in part because the long reaction times and challenging protocols typically required to label nucleic acid-based molecular probes with this radionuclide ($t_{1/2}$ = 109.8 minutes). To this end, we synthesized prosthetic group 2-[^{18}F]fluoro-3-(hex-5-ynyloxy)pyridine ([^{18}F]FPy5yne), used previously to label peptides, and coupled it to an oligodeoxyribonucleotide with ^{18}F by way of a Cu^{I}-mediated azide/alkyne cycloaddition reaction. HPLC-purified [^{18}F]FPy5yne was ligated to a 5′-azide-modified DNA sequence antisense to* mdr1 *mRNA in the presence of Cu^{I}-stabilizing ligand tris(benzyltriazolylmethyl)amine and 2,6-lutidine. Non-decay corrected, collected yields of the ^{18}F-labeled oligonucleotide from end-of-bombardment were 3.9% ± 0.5% (*n = *3; 24.6% ± 0.5% decay corrected). Shortest preparation time was 276 minutes from start of synthesis.*

Keywords Click chemistry; antisense; heteroaromatic substitution; ^{18}F; PET chemistry

INTRODUCTION

Despite only modest successes since its inception, antisense imaging remains a promising tool for the identification and treatment of genetic diseases, particularly cancer.[1] In vivo molecular imaging of radiolabeled DNA analogues prepared complementary to disease-upregulated or disease-specific mRNA could allow for the determination of early cellular response to treatment, the assessment of antisense therapeutics, and the

Received 7 August 2009; accepted 9 October 2009.

The authors thank Ken Buckley, Cornelia Höhr, and the rest of TRIUMF Nuclear Medicine Division and TR13 Cyclotron Operations for providing them with [^{18}F]fluoride. They also thank Morgan Hughes (SAMS Centre, University of Calgary) for performing the MALDI-TOF cited herein, as well as the University of British Columbia Mass Spectroscopy and NMR facilities for their contributions. This work was supported by a Canadian Institutes of Health Research Operating Grant and a TRIUMF Life Sciences Grant.

Address correspondence to James A. H. Inkster, TRIUMF, 4004 Wesbrook Mall, Vancouver, British Columbia, Canada V6T 2A3. E-mail: jamesi@triumf.ca

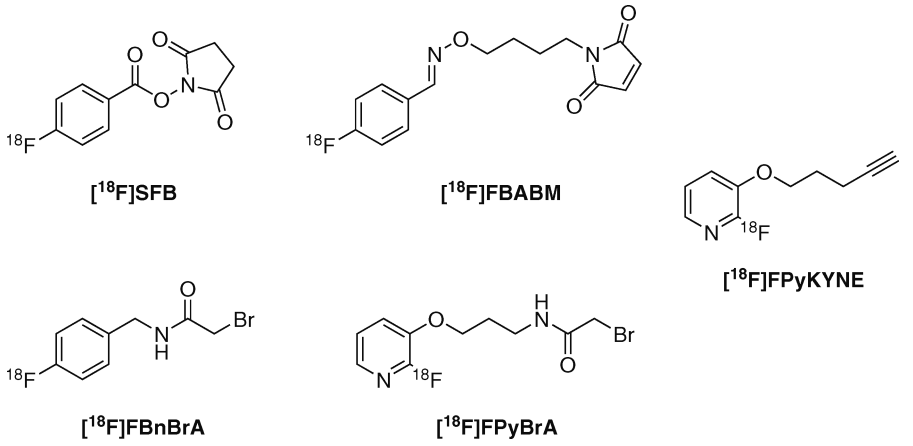


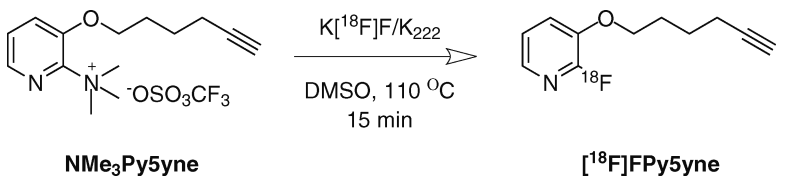
FIGURE 1 Selected prosthetic groups for the ^{18}F-labeling of biological molecules for PET imaging.

differentiation between cancer phenotypes. Antisense oligonucleotides have been labeled with the positron-emitting radionuclide ^{18}F for use in positron emission tomography (PET) imaging by way of ^{18}F-bearing prosthetic groups. Bioconjugations have been performed *via* isothiocyante formation,[2] photoconjugation,[3] acylation reactions with **[^{18}F]SFB**,[4–6] and chemospecific alkylations with **[^{18}F]FBABM**,[7] **[^{18}F]FBnBrA**,[8] and **[^{18}F]FPyBrA**[9] (Figure 1). [^{18}F]FPyBrA was prepared by way of an efficient nucleophilic *hetero*aromatic [^{18}F]fluorination[10] from the corresponding 2-nitro and 2-trimethylammonium triflate pyridines. Unfortunately, an additional two radiochemical steps (Boc deprotection, followed by condensation with bromoacetyl bromide) are required to complete the synthesis of this bifunctional molecule. Originally designed to chemospecifically radiolabel phosphorothioate DNA, [^{18}F]FPyBrA has been subsequently used to label a variety of potential antisense drugs and imaging agents with ^{18}F, including Speigelmers,[11] short interfering RNA (siRNA),[12] and polyamide nucleic acids (PNAs).[13]

The Huisgen 1,3-dipolar cycloaddition reaction[14] has found utility in a number of peptide- and protein-based bioconjugations, as researchers exploit the rate enhancement and product diastereospecificity afforded this reaction by the presence of Cu^{I} catalyst.[15,16] In this fashion, a number of radioactive prosthetic ligands have been recently developed for the labeling of targeting peptides with the short-lived positron-emitting radionuclides ^{11}C[17] ($t_{1/2}$ = 20.4 minutes) and ^{18}F[18–21] ($t_{1/2}$ = 109.8 minutes). In addition, a few ^{18}F analogues of small biological molecules (sugars, amino acids and nucleosides) have been prepared by way of what is now commonly referred to as the Cu^{I}-catalyzed azide/alkyne cycloaddition (CuAAC) reaction.[22,23] However, use of this coupling strategy in postsynthetic nucleic acid-based bioconjugations—radiochemical or otherwise—is not as

widespread. Presumably, this fact stems in part from the destructive effects of nucleobase oxidation arising from the in situ Cu^{I}-catalyzed production of hydroxyl radical, a phenomenon that has been previously reviewed.[24] Thus, the first modification of an oligonucleotide using the Huisgen 1,3-dipolar cycloaddition reaction was performed in the absence of Cu^{I} catalyst, furnishing fluorescent DNA in good yields but as a mixture of 1,4- and 1,5-triazole diasteromers.[25] However, the discovery of Cu^{I}-stabilizing catalyst tris-(benzyltriazolylmethyl)amine (TBTA) has largely overcome the problem of copper-mediated DNA damage.[26] It has been hypothesized that the [1,2,3]-triazole moieties of this tetradentate ligand protect the metal centre from unwanted oxidation and subsequent destructive radical formation, dissociating only to allow the formation of the catalytic Cu^{I}-acetylide/ligand complex.[27] Resin-bound[28] and water-soluble[29] versions of TBTA have been reported. Thus, a small but growing number of DNA analogues have been prepared using TBTA-mediated CuAAC chemistry, including large duplex MTase substrates,[30] densely modified PCR fragments,[31] and conjugates from arylacetylene-modified sequences,[32] as well as end-sealed DNA duplexes.[33] In addition, a microwave-assisted coupling of galactosyl azide derivatives with alkyne-functionalized T_{12} on solid support has been reported.[34] Two excellent reviews describing the growing use of CuAAC in nucleoside, nucleotide, and oligonucleotide chemistry have recently been published.[26,35] Despite these successes however, this 'click' reaction has until now not been exploited in the radiolabeling of oligonucleotide-based molecular probes.

Recently, Kuhnast et al. reported the fully automated radiosynthesis of alkyne-bearing prosthetic group 2-[^{18}F]fluoro-3-pentyne-4-yn-1-yloxypyridine (**[^{18}F]FPyKYNE**, Figure 1).[36] Shortly after, yet independently from, we published the synthesis of a closely related analog, 2-[^{18}F]fluoro-3-(hex-5-ynyloxy)pyridine (**[^{18}F]FPy5yne**), and used it to ^{18}F-label a short azide-bearing model peptide.[37] Both [^{18}F]FPyKYNE and [^{18}F]FPy5yne were prepared by way of an efficient nucleophilic heteroaromatic substitution reaction using Kryptofix 2.2.2-bound K[^{18}F]F in a single radiochemical step (Scheme 1). Herein, we expand the utility of [^{18}F]FPy5yne to include molecular probes for antisense imaging by describing the conjugation of [^{18}F]FPy5yne to a 5′-azide-modified antisense oligodeoxyribonucleotide (**N_3-ODN**), which was first prepared from a 5′-aminohexyl-modified DNA 20mer (**ODN**; Scheme 2). The chosen sequence is complementary to mRNA transcribed from *mdr1*, which encodes the multidrug resistance protein P-glycoprotein (Pgp). Transcriptional suppression of *mdr1* by antisense phosphorothioate oligonucleotides has shown to effectively inhibit expression of Pgp in human tumour xenografts.[38] Moreover, ^{99m}Tc-labeled phosphorothioate DNA antisense to *mdr1* was shown to accumulate in KB-G2 epidermoid tumours in mice



SCHEME 1 Radiosynthesis of ^{18}F-bearing prosthetic group [^{18}F]FPy5yne.

at statistically higher levels relative to a labeled sense strand control.[39] This model system has been used extensively to test the efficacy of novel modifications and delivery agents intended to improve the tumour-targeting capabilities of radiolabeled nucleic acid-based imaging agents.[39–43]

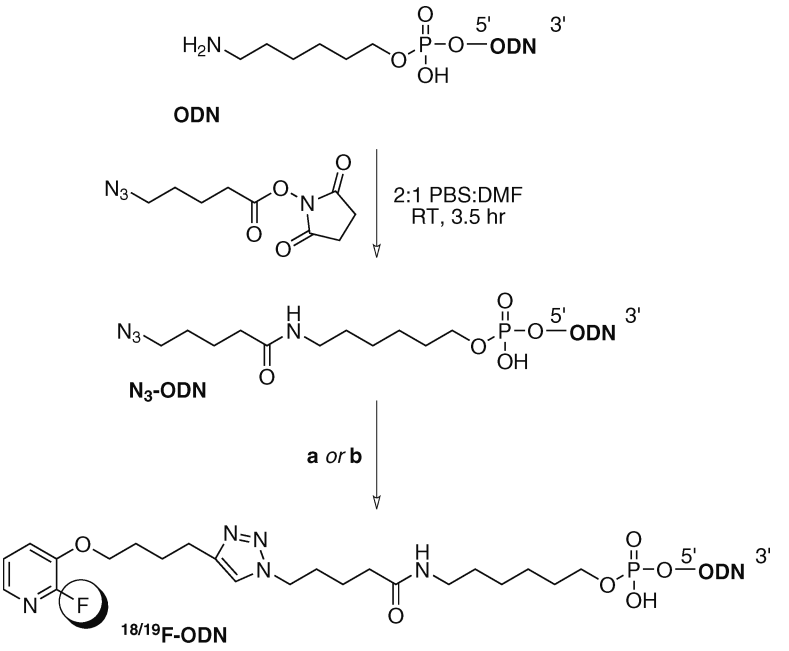
RESULTS

Chemistry

For the purposes of labeling **ODN** by way of Cu^{I}-catalyzed azide/alkyne cycloaddition, the 5′- aminohexyl oligonucleotide was first converted to 5′-azide modified **N_3-ODN** *via* acylation with succinimidyl 5-azidovalerate[25] (Scheme 2). Analytical standard **^{19}F-ODN** was obtained *via* bioconjugation of **N_3-ODN** and [^{19}F]FPy5yne in the presence of TBTA, tetrakis(acetonitrilo)copper(I) hexfluorophosphate and *N,N*-diisopropylethamine (DIEA). The expected MALDI-TOF mass spectra of modified oligonucleotides **N_3-ODN** and **^{19}F-ODN** were obtained. Our choice of copper was based on its solubility in DMF. Later, during radiochemical preparations, we found that 99.9999% CuBr afforded products of higher radiochemical purity. This is consistent with a previous report describing CuAAC ligations to an azide-modified cell surface protein of *E. coli*, where the purity of the copper catalyst had a marked effect on reaction yield.[44] Also observed previously was a large amount of oligonucleotide strand scission when Cu^{I} salt was used alone, *sans* TBTA chelate.[31] As for the addition of DIEA during cold coupling reactions, this nitrogen base has been shown to inhibit side product formation in peptide-based CuAAC radiobioconjugations.[18] Later, during radiochemical preparations, we found 2,6-lutidine to be equally efficient.

Radiochemistry

[^{18}F]FPy5yne was synthesized from its 2-trimethylammonium triflate pyridinyl precursor (**NMe_3Py5yne**) under standard K[^{18}F]F-K_{222}/K_2CO_3 conditions (Scheme 1), with two minor modifications to our described protocol.[37] First, the reaction volume was reduced from 0.7 mL to



a) [^{19}F]FPy5yne, $Cu(CH_3CN)_4PF_6$, TBTA, DIEA in 4:1 PBS:DMF, RT, 48 hr.
b) [^{18}F]FPy5yne, CuBr, TBTA, 2,6-lutidine in ~2:1 PBS:DMF, 75°C, 20 min.

SCHEME 2 Preparation of 5′-azide modified oligonucleotide precursor (**N$_3$-ODN**), radioactive ^{18}F-labeled oligonucleotide (**^{18}F-ODN**) and ^{19}F-labeled synthetic standard (**^{19}F-ODN**).

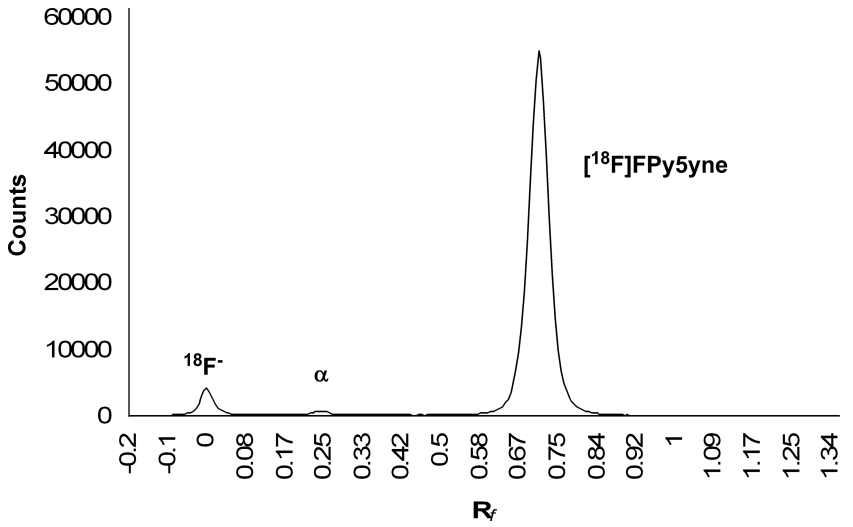


FIGURE 2 Silica gel radioTLC of crude [^{18}F]FPy5yne reaction mixture. Eluent: Ethyl acetate. R_f = 0: Free [^{18}F]fluoride. R_f = 0.24: Undetermined radioimpurity α. R_f = 0.71: [^{18}F]FPy5yne. Reaction yields determined in this fashion represent the percentage of the radioactive peak area corresponding to ^{18}F-labeled product relative to the area of total radioactivity.

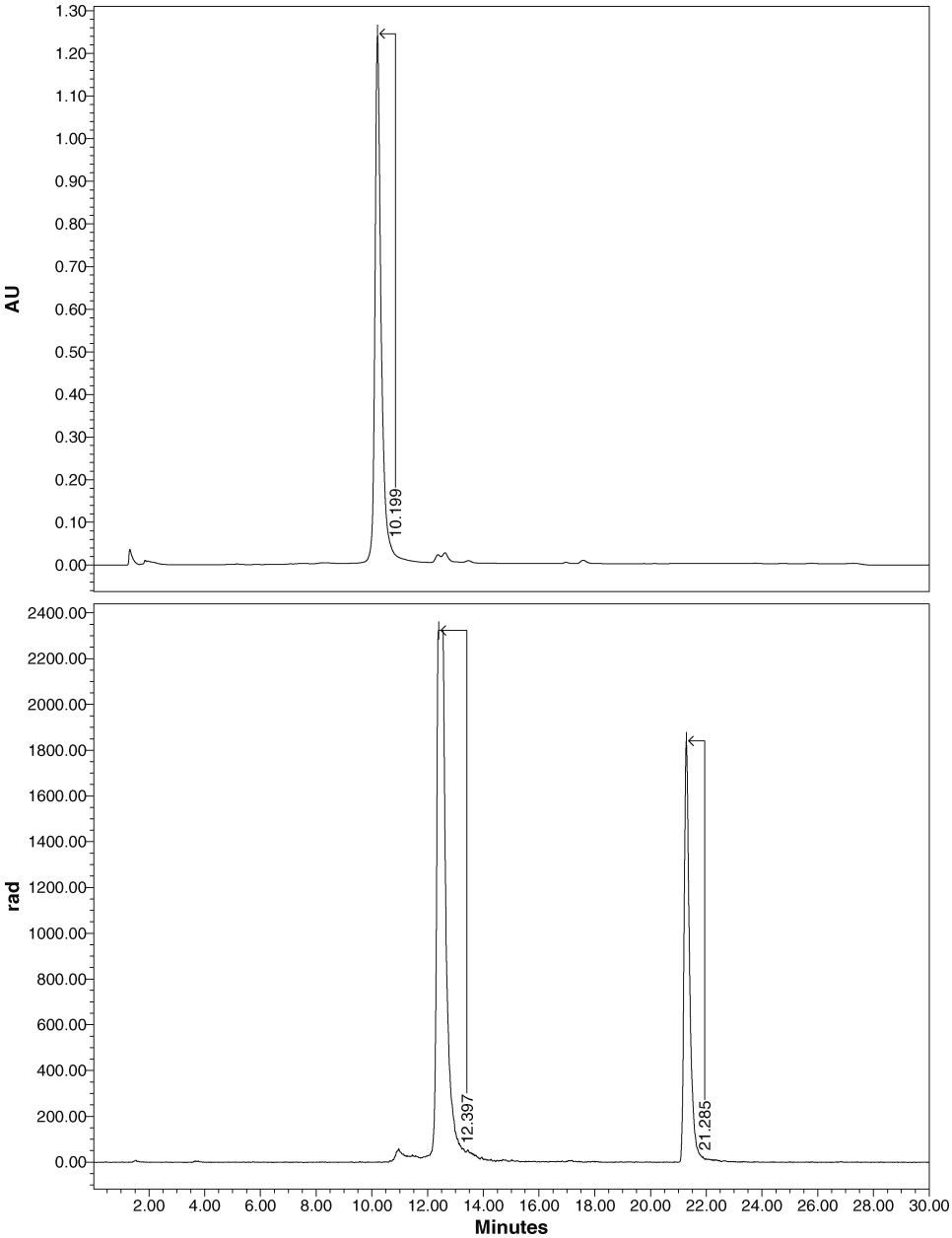


FIGURE 3 Preparative radioHPLC of the **^{18}F-ODN** reaction mixture. Top: UV trace (AU, absorbance units), 260 nm. Bottom: Radioactive trace (rad, arbitrary voltage deflection). R_t = 10.2 minutes: **N_3-ODN**. R_t = 12.4 minutes: **^{18}F-ODN**. R_t = 21.3 minutes: [^{18}F]FPy5yne.

0.5 mL DMSO. Second, in order to minimize product loss of [^{18}F]FPy5yne due to evaporation, concentration of the purified prosthetic group from methylene chloride was performed at room temperature (instead of 50°C) over 10 minutes. As such, the eluent was concentrated to ~100–150 μL,

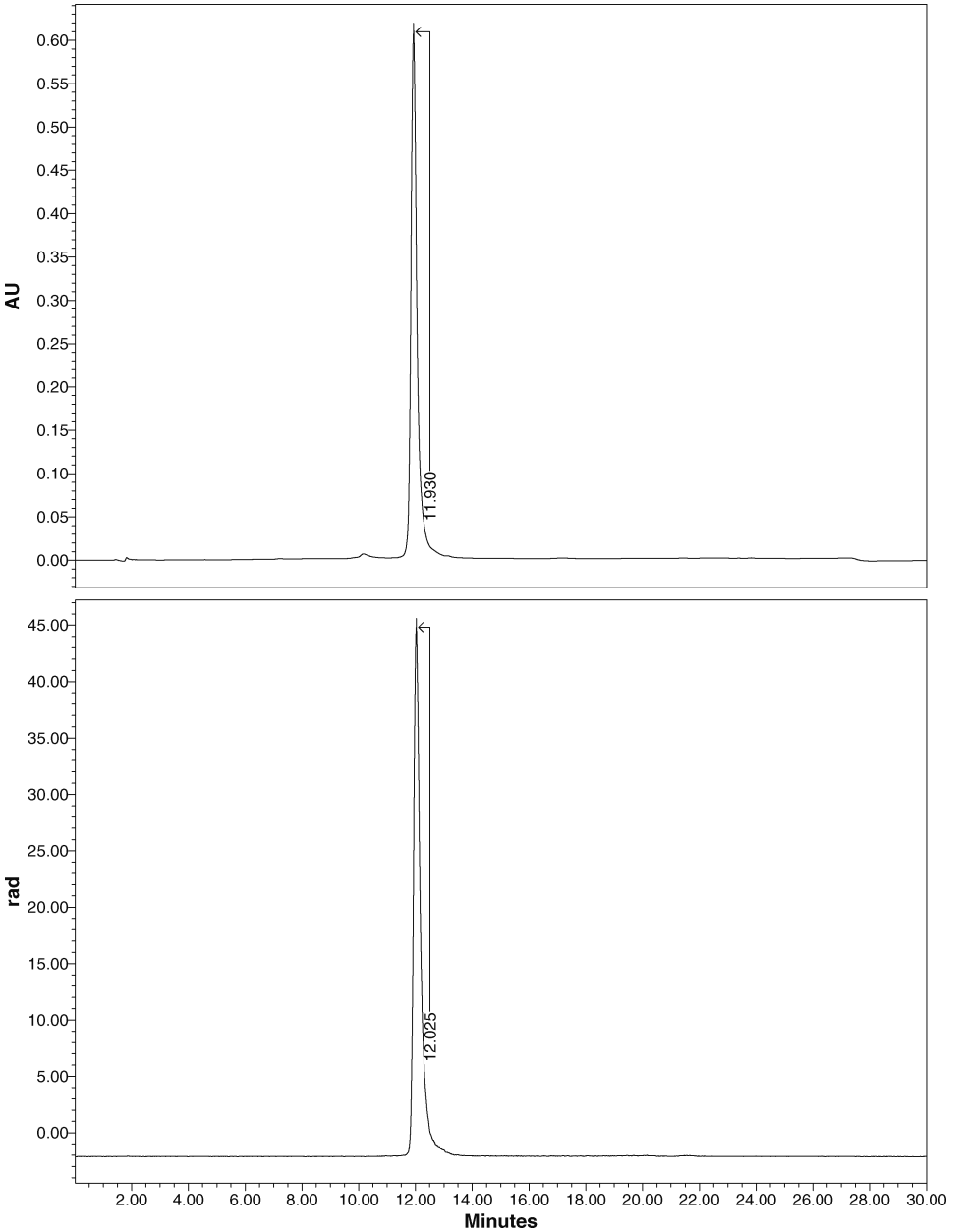


FIGURE 4 RadioHPLC co-injection of **^{18}F-ODN** final formulation admixed with ^{19}F synthetic standard (**^{19}F-ODN**). Top: UV trace (AU, absorbance units), 260 nm. Bottom: Radioactive trace (rad, arbitrary voltage deflection).

the bulk of which is presumably residual water. Average radiochemical incorporation of [^{18}F]fluoride was 89.6% ± 2.0% ($n = 4$) as determined by radioTLC (Figure 2). Although this nucleophilic substitution proceeds adequately in acetonitrile, reaction yields in this solvent are mitigated

slightly by the enhancement of a radioimpurity (denoted α in Figure 2) relative to reactions in DMSO. Non-decay corrected, collected yields from start-of-synthesis (30.1% ± 1.5%; $n = 4$) were consistent with those reported earlier. Typical preparation time of [^{18}F]FPy5yne was 90 minutes from start-of-synthesis.

^{18}F-labeled antisense oligonucleotide **^{18}F-ODN** was prepared by mixing the azide-modified **N_3-ODN** with [^{18}F]FPy5yne in the presence of copper(I) bromide (10 equiv.) and TBTA (15 equiv.) for 15 minutes at 75°C (Scheme 2). In this case, the efficiency of the bioconjugation reaction could not be estimated with good certainty by HPLC, as the hydrophobic prosthetic group is only partially soluble in PBS, which was used to quench the reaction prior to HPLC purification. Indeed, upon transfer of the mixture to HPLC, 16% of the total radioactivity remained in the microcentrifuge tube. However, the collected non-decay corrected bioconjugate yield, which is ultimately a more meaningful value, was found to be 18.0% ± 3.4% ($n = 3$). Figure 3 shows an aliquot of a preparative bioconjugation reaction mixture. After HPLC purification and desalting by size exclusion chromatography, the non-decay corrected, collected yield of **^{18}F-ODN** was 3.9% ± 0.5% ($n = 3$) from end-of-bombardment (24.6% ± 0.5% decay corrected). The chemical nature of **^{18}F-ODN** was confirmed by way of HPLC co-injection with its ^{19}F standard (Figure 4). Shortest total preparation time was 276 minutes from start-of-synthesis.

CONCLUSION

Current consensus suggests that the future exploitation of DNA analogues for molecular imaging or therapy will likely require a carrier to improve their translocation across cellular barriers. To this end, a wealth of cell-penetrating antisense conjugates and delivery systems are continuously being explored.[45] Radiolabeled analogues of these chimeric macromolecules have been prepared that might eventually allow for the clinical diagnosis and staging of neoplastic growth and other pathological processes that upregulate gene expression.[41,46–48] Additionally, positron-emitting derivatives of potential DNA-based therapeutics can be used to assess their targeting potential and pharmacokinetics in living systems.[11,12,49,50] However, in the case of a short-lived radionuclide such as ^{18}F, highly optimized radiosynthetic procedures are essential to the successful development of these complex, multi-component imaging agents. To this end, we have used the 2-[^{18}F]fluoropyridinyl prosthetic group [^{18}F]FPy5yne to radiolabel an azide-bearing DNA sequence antisense to mRNA transcribed by the *mdr1* gene. This "prosthetic group" approach represents the first synthesis of a ^{18}F-labeled DNA analogue by way of CuAAC chemistry. More importantly, this method should be considered as 'proof-of-principle' for

the eventual radiosynthesis of other nucleic acid-based PET imaging agents, including biologically stable antisense PNAs and morpholinos, aptamers, and siRNAs. We are now directing our efforts towards the ^{18}F-labeling of a cell-penetrating steric-block antisense probe for the PET imaging of breast cancer in a murine model.

EXPERIMENTAL

Chemicals

Oxygen-18-enriched water ([^{18}O]H_2O, >97% pure) was purchased from Rotem Industries (Beer Sheva, Israel). Desalted 5′-amino-C6-modified oligodeoxyribonucleotide bearing the sequence 5′-CCA TCC CGA CCT CGC GCT CC-3′ (**ODN**) was purchased from IDT (Coralville, IA, USA). *N*-Succinimidyl 5-azidovalerate, [25,51,52] precursor NMe_3Py5yne[37] and TBTA[27] were synthesized in-house according to published procedures. Most chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) or Alfa Aesar (Ward Hill, MA, USA) in their standard purities, with the exception of CuBr (Aldrich), which was purchased 99.9999% pure. All organic solvents used in radiochemical synthesis were designated anhydrous.

Analytical Methods

Radio thin layer chromatography (TLC) was performed on 60F_{254} silica gel plates purchased from Sorbent Technologies (Atlanta, GA, USA). RadioTLCs were obtained using a Bioscan System 200 Imaging scanner. All reverse phase HPLC reported herein utilized a Waters 600 Controller in combination with a Waters 2487 Dual λ Absorbance Detector and a Bioscan NaI detector. All HPLC solvents were filtered prior to use.

HPLC A Column: Phenomenex Luna PFP(2) C18 (250 × 10 mm, 5μm); Detector: 214, 260 nm; Program: flow = 4 mL/min, 50:50 MeCN:0.1% TFA. HPLC B Column: Phenomenex Clarity Oligo-RP (100 × 10 mm, 5 μm); Detector: 260 nm; Program: gradient elution, flow = 4.75 ml/min, 10% to 60% MeOH in 50 mM triethylammonium acetate buffer (containing 5% acetonitrile; pH 7.0) over 20 minutes, then hold for 5 minutes. Identification of oligonucleotides **N_3-ODN** and **^{19}F-ODN** by MALDI-TOF was preformed on an Applied Biosystems (Foster City, CA, USA) Voyager-DE STR instrument with 3-hydroxypicolinic acid as matrix.

Chemistry. Preparation of 5′-azide modified oligonucletotide (N_3-ODN). Desalted 5′-amino-C6-modified oligodeoxyribonucleotide **ODN** (226 nmol) was dissolved in 100 μL phosphate-buffered saline (150 mM, pH 7.2). An aliquot (50 uL) of 53 mg/mL *N*-succinimidyl 5-azidovalerate (11 μmol) in DMF was added. The reaction was shaken vigorously for 3.5 hours, then diluted with 1.5 mL of TEAA buffer (5 mM, pH 7.0), purified

on a Phenomenex Clarity desalting column (3 μm, 200 mg), and concentrated in vacuo to afford **N_3-ODN**. This material was used in a subsequent CuAAC ligation as is; however, an analytically pure sample was prepared by further purification by HPLC (HPLC B, R_t = 10.2 minutes), followed by vacuum concentration and desalting on an NAP-10 size exclusion column (GE Healthcare). MALDI-TOF: Calcd. 6240.2 Found. 6243.3.

Preparation of 5′-^{19}F-modified oligonucleotide (^{19}F-ODN). A sample of **N_3-ODN** (62.5 nmol) in a 2 mL microcentrifuge tube was dissolved in 100 μL PBS (150 mM, pH 7.2) and 240 μg (1.2 μmol) of [^{19}F]FPy5yne in DMF (25 μL) was added, followed by DIEA (2.2 μL, 1.2 μmol). To this mixture was added a freshly prepared solution (25 μL) of $Cu(CH_3CN)_4PF_6$ (19.2 mg/mL) and TBTA (20.8 mg/mL) in DMF. The sample was shaken vigorously for 48 hours, diluted with 200 μL water, passed through a 0.45 μm Millex-HV filter (Millipore) to remove insolubles, then purified by HPLC (HPLC B). The collected portion (R_t = 12.4 minutes) was concentrated by vacuum drying, and desalted on a NAP-10 size exclusion column (GE Healthcare). MALDI-TOF: Calcd. 6433.4. Found. 6439.9.

Radiochemistry

No-carrier-added [^{18}F]fluoride was produced by 13 MeV proton bombardment of [^{18}O] water [^{18}O(p,n)^{18}F reaction].[53] Typical production was 65–100 mCi of [^{18}F]F^- at end of bombardment for a 10 μA, 5 minute irradiation.

2-[^{18}F]Fluoro-3-(hex-5-ynyloxy)pyridine ([^{18}F]FPy5yne). The radiosynthesis of [^{18}F]FPy5yne via nucleopholic heteroaromatic substitution of NMe_3Py5yne has been described previously.[37] To clarify, DMSO (0.5 mL) was employed as reaction solvent. Reaction temperature was 110°C; reaction time was 15 minutes. The ^{18}F-labeled prosthetic group was trapped on a tC18 solid phase extraction column (Waters), eluted with acetonitrile (1 mL), then separated from the precursor molecule and [^{18}F]fluorodemethylation[54] contaminant 2-dimethylamino-3-(hex-5-ynyloxy)pyridine by semi-preparative HPLC (HPLC A). [^{18}F]FPy5yne was extracted from HPLC eluent using another tC18 column, eluted off the column with methylene chloride (2 mL), and *carefully* concentrated to ~100–150 μL (residual water) in a 5 mL conical glass vial under a stream of He at room temperature over 10 minutes.

5′-^{18}F-modified oligonucleotide (^{18}F-ODN). Dimethylformamide (100 μL) was added to an aqueous solution of [^{18}F]FPy5yne (~150 μL; 20.4 mCi) and the prosthetic molecule was transferred (84% efficiency) to a microcentrifuge tube containing **N_3-ODN** (200 nmol) in 100 μL PBS (150 mM, pH 7.2). To this mixture was added a solution of Cu^I-TBTA complex (50 μL), followed by 2,6-lutidine (4 μL, 17.3 μmol). The Cu^I-TBTA slurry was prepared immediately beforehand by addition of

TBTA (15.8 mg, 29.8 μmol) in DMF (250 μL) to CuBr powder (2.7 mg, 18.8 μmol), followed by the addition of water (250 μL). The reaction was heated at 75°C for 20 minutes, then diluted to 1 mL with PBS (150 mM, pH 7.2), centrifuged for 2 minutes (12,000 min^{-1}) and purified by HPLC (HPLC B). The transfer efficiency in this step was 84%. The collected portion (~6 mL) was concentrated to ~100 μL at 110°C under a stream of He, diluted with 400 μL H_2O, and desalted on a NAP-5 column (GE Healthcare, Mississauga, ON, Canada). The final formulation (1 mL H_2O) contained 4.03 mCi of **^{18}F-ODN**.

REFERENCES

1. Younes, C.K.; Boisgard, R.; Tavitian, B. Labeled oligonucleotides as radiopharmaceuticals: Pitfalls, problems and perspectives. *Curr. Pharm. Design.* **2002**, 8, 1451–1466.
2. Hedberg, E.; Langstrom, B. Synthesis of 4-([F-18]fluoromethyl)phenyl isothiocyanate and its use in labeling oligonucleotides. *Acta Chem. Scand.* **1997**, 51, 1236–1240.
3. Lange, C.W.; VanBrocklin, H.F.; Taylor, S.E. Photoconjugation of 3-azido-5-nitrobenzyl-[F-18]fluoride to an oligonucleotide aptamer. *J. Labeled Compd. Rad.*. **2002**, 45, 257–268.
4. Hedberg, E.; Langstrom, B. F-18-labeling of oligonucleotides using succinimido 4-[F-18]fluorobenzoate. *Acta Chem. Scand.* **1998**, 52, 1034–1039.
5. Koslowsky, I.; Shahhosseini, S.; Wilson, J.; Mercer, J. Automated radiosynthesis of N-(4-[F-18]flurobenzyl)-2-bromoacetamide: an F-18-labeled reagent for the prosthetic radiolabeling of oligonucleotides. *J. Labeled Compd. Rad.* **2008**, 51, 352–356.
6. Li, J.L.; Trent, J.O.; Bates, P.J.; Ng, C.K. Factors affecting the labeling yield of F-18-labeled AS1411. *J. Labeled Compd. Rad.* **2007**, 50, 1255–1259.
7. Toyokuni, T.; Walsh, J.C.; Dominguez, A.; Phelps, M.E.; Barrio, J.R.; Gambhir, S.S.; Satyamurthy, N. Synthesis of a new heterobifunctional linker, N-[4-(Aminooxy)butyl]maleimide, for facile access to a thiol-reactive F-18-Labeling agent. *Bioconjugate Chem.* **2003**, 14, 1253–1259.
8. Dolle, F.; Hinnen, F.; Vaufrey, F.; Tavitian, B.; Crouzel, C. A general method for labeling oligodeoxynucleotides with F-18 for in vivo PET imaging. *J. Labeled Compd. Rad.* **1997**, 39, 319–330.
9. Kuhnast, B.; de Bruin, A.; Hinnen, F.; Tavitian, B.; Dolle, F. Design and synthesis of a new [F-18]fluoropyridine-based haloacetamide reagent for the labeling of oligonucleotides: 2-bromo-N-[3-(2-[F-18]fluoropyridin-3-yloxy)propyl]acetamide. *Bioconjugate Chem.* **2004**, 15, 617–627.
10. Dolle, F. Fluorine-18-labeled fluoropyridines: Advances in radiopharmaceutical design. *Curr Pharm Design*, **2005**, 11, 3221–3235.
11. Boisgard, R.; Kuhnast, B.; Vonhoff, S.; Younes, C.; Hinnen, F.; Verbavatz, J.M.; Rousseau, B.; Furste, J.; Wlotzka, B.; Dolle, F.; Klussmann, S.; Tavitian, B. In vivo biodistribution and pharmacokinetics of F-18-labeled Spiegelmers: a new class of oligonucleotidic radiopharmaceuticals. *Eur. J. Nucl. Med. Mol. I.* **2005**, 32, 470–477.
12. Viel, T.; Boisgard, R.; Kuhnast, B.; Jego, B.; Siquier-Pernet, K.; Hinnen, F.; Dolle, F.; Tavitian, B. Molecular imaging study on in vivo distribution and pharmacokinetics of modified small interfering (siRNAs). *Oligonucleotides* **2008**, 18, 201–212.
13. Kuhnast, B.; Hinnen, F.; Hamzavi, R.; Boisgard, R.; Tavitian, B.; Nielsen, P.E.; Dolle, F. Fluorine-18 labeling of PNAs functionalized at their pseudo-peptidic backbone for imaging studies with PET. *J. Labeled Compd. Rad.* **2005**, 48, 51–61.
14. Huisgen, R., 1984. 1,3-Dipolarcycloaddition- introduction, survey, mechanism, in *1,3-Dipolarcycloaddition Chemistry*, A. Padwa, Ed.;Wiley, 1984, pp. 1–176.
15. Tornoe, C.W.; Christensen, C.; Meldal, M. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem.* **2002**, 67, 3057–3064.
16. Rostovtsev, V.V.; Green, L.G.; Fokin, V.V.; Sharpless, K.B. A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew Chem. Int. Edit.* **2002**, 41, 2596–2599.

17. Schirrmacher, R.; Lakhrissi, Y.; Jolly, D.; Goodstein, J.; Lucas, P.; Schirrmacher, E. Rapid in situ synthesis of [C-11]methyl azide and its application in C-11 click-chemistry. *Tetrahedron Lett.* **2008**, 49, 4824–4827.
18. Marik, J.; Sutcliffe, J.L. Click for PET: rapid preparation of [F-18]fluoropeptides using Cu-I catalyzed 1,3-dipolar cycloaddition. *Tetrahedron Lett.* **2006**, 47, 6681–6684.
19. Li, Z.B.; Wu, Z.; Chen, K.; Chin, F.T.; Chen, X. Click chemistry for F-18-labeling of RGD peptides and microPET Imaging of tumor integrin alpha(v)beta(3) expression. *Bioconjugate Chem.* **2007**, 18, 1987–1994.
20. Ramenda, T.; Bergmann, R.; Wuest, F. Synthesis of F-18-labeled neurotensin(8–13) via copper-mediated 1,3-dipolar [3+2] cycloaddition reaction. *Letters in Drug Design & Discovery* **2007**, 4, 279–285.
21. Glaser, M.; Arstad, E. "Click labeling" with 2-[F-18]fluoroethylazide for positron emission tomography. *Bioconjugate Chem.* **2007**, 18, 989–993.
22. Sirion, U.; Kim, H.J.; Lee, J.H.; Seo, J.W.; Lee, B.S.; Lee, S.J.; Oh, S.J.; Chi, D.Y. An efficient F-18 labeling method for PET study: Huisgen 1,3-dipolar cycloaddition of bioactive substances and F-18-labeled compounds. *Tetrahedron Lett.* **2007**, 48, 3953–3957.
23. Kim, D.H.; Choe, Y.S.; Jung, K.H.; Lee, K.H.; Choi, J.Y.; Choi, Y.; Kim, B.T. A F-18-labeled glucose analog: Synthesis using a click labeling method and in vitro evaluation. *Arch. Pharm. Res.* **2008**, 31, 587–593.
24. Burrows, C.J.; Muller, J.G. Oxidative nucleobase modifications leading to strand scission. *Chem. Rev.* **1998**, 98, 1109–1151.
25. Seo, T.S.; Li, Z.M.; Ruparel, H.; Ju, J.Y. Click chemistry to construct fluorescent oligonucleotides for DNA sequencing. *J. Org. Chem.* **2003**, 68, 609–612.
26. Gramlich, P.M.E.; Wirges, C.T.; Manetto, A.; Carell, T. Postsynthetic DNA modification through the copper-catalyzed azide-alkyne cycloaddition reaction. *Angew Chem. Int. Edit.* **2008**, 47, 8350–8358.
27. Chan, T.R.; Hilgraf, R.; Sharpless, K.B.; Fokin, V.V. Polytriazoles as copper(I)-stabilizing ligands in catalysis. *Org. Lett.* **2004**, 6, 2853–2855.
28. Chan, T.R.; Fokin, V.V. Polymer-supported copper(I) catalysts for the experimentally simplified azide-alkyne cycloaddition. *Qsar & Combinatorial Science* **2007**, 26, 1274–1279.
29. Hong, V.; Udit, A.K.; Evans, R.A.; Finn, M.G. Electrochemically protected copper(I)-catalyzed azide-alkyne cycloaddition. *ChemBioChem* **2008**, 9, 1481–1486.
30. Weller, R.L.; Rajski, S.R. DNA methyltransferase-moderated click chemistry. *Org. Lett.* **2005**, 7, 2141–2144.
31. Gierlich, J.; Burley, G.A.; Gramlich, P.M.E.; Hammond, D.M.; Carell, T. Click chemistry as a reliable method for the high-density postsynthetic functionalization of alkyne-modified DNA. *Org. Lett.* **2006**, 8, 3639–3642.
32. Ustinov, A.V.; Korshun, V.A. Oligonucleotides containing arylacetylene residues: synthesis and post-synthetic modification via [3+2] cycloaddition. *Russian Chemical Bulletin* **2006**, 55, 1268–1274.
33. El-Sagheer, A.H. Very Stable end-sealed double stranded DNA by click chemistry. *Nucleosides Nucleotides Nucleic Acids* **2009**, 28, 315–323.
34. Bouillon, C.; Meyer, A.; Vidal, S.; Jochum, A.; Chevolot, Y.; Cloarec, J.P.; Praly, J.P.; Vasseur, J.J.; Morvan, F. Microwave assisted "click" chemistry for the synthesis of multiple labeled-carbohydrate oligonucleotides on solid support. *J Org Chem*, **2006**, 71, 4700–4702.
35. Amblard, F.; Cho, J.H.; Schinazi, R.F. Cu(I)-Catalyzed huisgen azide-alkyne 1,3-Dipolar cycloaddition reaction in nucleoside, nucleotide, and oligonucleotide chemistry. *Chem Rev.* **2009**, 109, 4207–4220.
36. Kuhnast, B.; Hinnen, F.; Tavitian, B.; Dolle, F. [F-18]FPyKYNE, a fluoropyridine-based alkyne reagent designed for the fluorine-18 labeling of macromolecules using click chemistry. *J. Labeled Compd. Rad.* **2008**, 51, 336–342.
37. Inkster, J.A.H.; Guérin, B.; Ruth, T.J.; Adam, M.J. Radiosynthesis and bioconjugation of [18F]FPy5yne, a prosthetic group for the 18F labeling of bioactive peptides. *J. Labeled Compd. Rad.* **2008**, 51, 444–452.
38. Ramachandran, C.; Wellham, L.L. Effect of MDR1 phosphorothioate antisense oligodeoxynucleotides in multidrug-resistant human tumor cell lines and xenografts. *Anticancer Res.* **2003**, 23, 2681–2690.
39. Nakamura, K.; Fan, C.; Liu, G.; Gupta, S.; He, J.; Dou, S.; Kubo, A.; Rusckowski, M.; Hnatowich, D.J. Evidence of antisense tumor targeting in mice. *Bioconjugate Chem.* **2004**, 15, 1475–1480.

40. Nakamura, K.; Wang, Y.; Liu, X.R.; Kubo, A.; Hnatowich, D.J. Influence of two transfectors on delivery of Tc-99m antisense DNA in tumor-bearing mice. *Mol. Imaging Biol.* **2006**, 8, 188–192.
41. Nakamura, K.; Wang, Y.; Liu, X.R.; Kubol, A.; Hnatowich, D.J. Cell culture and xenograft-bearing animal studies of radiolabeled antisense DNA-carrier nanoparticles with streptavidin as a linker. *J. Nucl. Med.* **2007**, 48, 1845–1852.
42. Liu, X.; Wang, Y.; Nakamura, K.; Kubo, A.; Hnatowich, D.J. Cell studies of a three-component antisense MORF/tat/Herceptin nanoparticle designed for improved tumor delivery. *Cancer Gene Ther.* **2008**, 15, 126–132.
43. Liu, X.R.; Nakamura, K.; Wang, Y.; Zhang, S.R.; He, J.; Liu, G.Z.; Dou, S.P.; Kubo, A.; Rusckowski, M.; Hnatowich, D. Improved delivery in cell culture of radiolabeled antisense DNAs by duplex formation. *Mol. Imaging Biol.* **2006**, 8, 278–283.
44. Link, A.J.; Vink, M.K.S.; Tirrell, D.A. Presentation and detection of azide functionality in bacterial cell surface proteins. *J. Am. Chem. Soc.* **2004**, 126, 10598–10602.
45. Patil, S.D.; Rhodes, D.G.; Burgess, D.J. DNA-based therapeutics and DNA delivery systems: a comprehensive review. *AAPS J.* **2005**, 7, E61–E77.
46. Tian, X.; Chakrabarti, A.; Amirkhanov, N.; Aruva, M.R.; Zhang, K.; Cardi, C.A.; Lai, S.; Thakur, M.L.; Wickstrom, E. Receptor-mediated internalization of chelator-PNA-peptide hybridization probes for radioimaging or magnetic resonance imaging of oncogene mRNAs in tumours. *Biochem. Soc. Trans.* **2007**, 35, 72–76.
47. Sun, X.K.; Fang, H.F.; Li, X.X.; Rossin, R.; Welch, M.J.; Taylor, J.S. MicroPET imaging of MCF-7 tumors in mice via unr mRNA-targeted peptide nucleic acids. *Bioconjugate Chem.* **2005**, 16, 294–305.
48. Suzuki, T.; Wu, D.; Schlachetzki, F.; Li, J.Y.; Boado, R.J.; Pardridge, W.M. Imaging endogenous gene expression in brain cancer in vivo with 111In-peptide nucleic acid antisense radiopharmaceuticals and brain drug-targeting technology. *J. Nucl. Med.* **2004**, 45, 1766–1775.
49. Ou, X.H.; Tan, T.Z.; He, L.; Li, Y.C.; Li, J.L.; Kuang, A.R. Antitumor effects of radioiodinated antisense oligonuclide mediated by VIP receptor. *Cancer Gene Ther.* **2005**, 12, 313–320.
50. Wang, R.F.; Shen, J.; Qiu, F.; Zhang, C.L. Study on biodistribution and imaging of radioiodinated antisense oligonucleotides in nude mice bearing human lymphoma. *J. Labeled Compd. Rad.* **2007**, 273, 19–23.
51. Khoukhi, N.; Vaultier, M.; Carrie, R. Synthesis and reactivity of methyl gamma-azido butyrates and ethyl delta-azido valerates and of the corresponding acid-chlorides as useful reagents for the aminoalkylation. *Tetrahedron* **1987**, 43, 1811–1822.
52. McGeary, R.P. Facile and chemoselective reduction of carboxylic acids to alcohols using BOP reagent and sodium borohydride. *Tetrahedron Lett.* **1998**, 39, 3319–3322.
53. Ruth, T.J.; Wolf, A.P. Absolute Cross-sections for the production of F-18 Via the O-18 (P, N) F-18 reaction. *Radiochimica Acta* **1979**, 26, 21–24.
54. Sun, H.R.; DiMagno, S.G. Competitive demethylation and substitution in N,N,N-trimethylanilinium fluorides. *J. Fluorine Chem.* **2007**, 128, 806–812.