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13. ABSTRACT ***(Maximum 200 Words)***

We are developing methods to image her-2-neu oncogene over-expression in breast cancer using positron emission tomography (PET). Small oligodeoxynucleotides (ODNs) that are complementary to the Her-2-neu messenger RNA (mRNA) are being investigated as potential imaging probes. Fluorine-18 (2 hour half-life positron emitter) has been used to label 15-18 mer ODN probes. The labeling of an ODN to Fluorine-18 has been particularly troublesome because of the limited half-life and the complicated chemistry. We have explored multiple strategies and are trying to maximize yield and specific activity of our probes. With adequate synthesis of the ODN probes we will begin further cell testing and small animal imaging with microPET. We expect that the techniques developed will lead to methods to detect breast cancer in living subjects in the case that her-2-neu is over-expressed.

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PI - Signature 10/24/02 Date

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Introduction

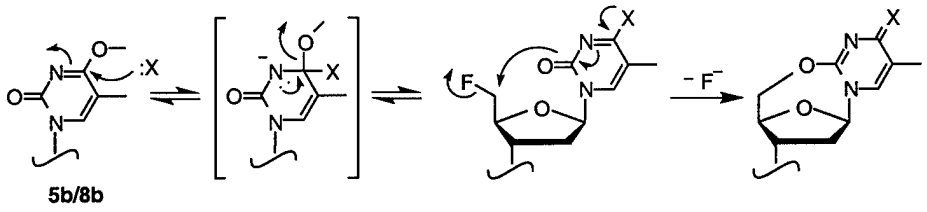
Improved methods to image breast cancer are critically needed in order to lead to earlier initial detection, earlier detection for recurrence, and better management of patients undergoing treatment. Most approaches to date have focused on anatomical changes due to tumor growth (e.g., mammography, computerized tomography, magnetic resonance imaging) or metabolic changes in the tumor (e.g., FDG Positron emission tomography). As molecular oncology continues to shed insight into the molecular basis for breast cancer, methods are needed to directly image molecular abberations in breast cancer cells. We are developing methods using radiolabeled antisense oligodoeoxynucleotides (RASONs) which can be injected via the blood-stream and then accumulate in cells that have sufficient levels of a particular target messenger RNA (mRNA). Normal cells (breast and other tissues) which don't have high level of target mRNA would not lead to intracellular trapping of the RASONs. One known molecular abnormality in about 25% of breast cancer patients is the over-expression of the Her-2-neu (c-erb-B2) oncogene. We have selected this gene as our first target using RASONs labeled with fluorine-18 (a positron emitter). We seek to develop RASONs that can be validated using nude mice carrying human breast cancer tumor xenografts imaged using microPET technology. With pre-clinical proof of their ability to home to breast cancer tumors over-expressing Her-2-neu we hope to have sufficient proof to eventually transition to human applications. It is hoped that this approach will lead to more specific and sensitive detection of breast cancer with over-expression of Her-2-neu and set the foundation for a new antisense based imaging approach which could potentially be applied to many different oncogenes.

Body

Aim 1: The development of ^{18}F-labeled oligodeoxynucleotides (ODNs).

After several attempts to improve the radiochemical yield of an ^{18}F-labeled oligodeoxynucleotide (ODN), we identified critical problems, including low coupling efficiency of the reversed-activation method, partial de[^{18}F]fluorination under the standard $MeNH_2$-NH_4OH condition (Scheme 1) and difficult separation of the ^{18}F-labeled ODN from unreacted and de[^{18}F]fluorinated ODNs. These problems contribute to the poor radiochemical yield and low specific radioactivity (presented by Dr. Joseph C Walsh at the 47th Annual Meeting of the Society of Nuclear Medicine in June 2000: *J. Nucl. Med.* **2000**, *41*, 245P). Consequently, we have been investigating alternative approaches based on [^{18}F]fluorobenzaldehyde as an ^{18}F-labeling agent.

Scheme 1. A possible mechanism of de[^{18}F]fluorination

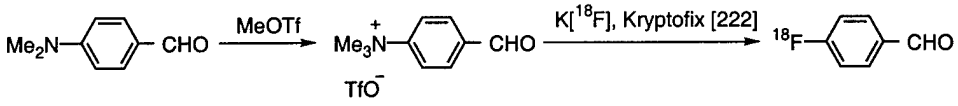


5b/8b

X = $MeNH_2$, NH_3 or OH

1. Synthesis of [^{18}F]fluorobenzaldehyde. According to the reported procedure[1], we have synthesized [^{18}F]fluorobenzaldehyde in ~40% radiochemical yield (decay corrected) within 50 min (Scheme 2).

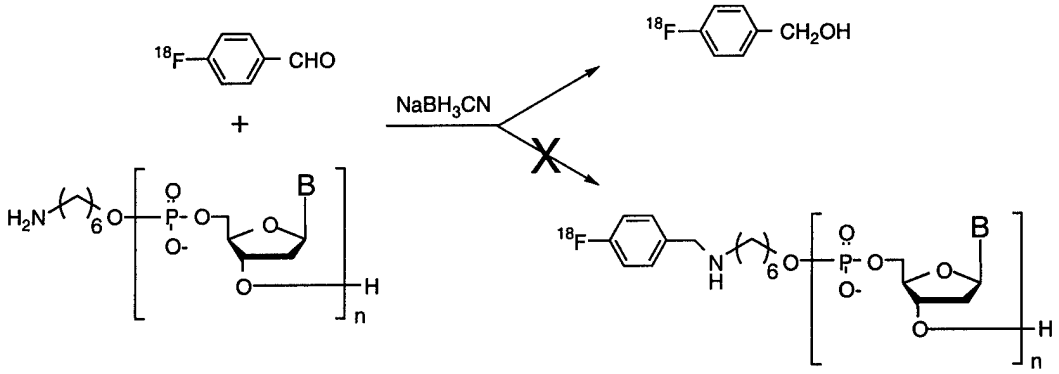
Scheme 2. Synthesis of [^{18}F]fluorobenzaldehyde



2. Attempt to synthesize an ^{18}F-labeled ODN using reductive alkylation of a 5'-amino modified ODN with [^{18}F]fluorobenzaldehyde. The reductive alkylation of amines with aldehyde derivatives has been used frequently to modify biomolecules including ODNs and proteins.[2] We have therefore investigated the reductive alkylation method for ^{18}F-labeling of ODNs. The 5'-aminohexyl modified ODN was synthesized and subjected to reductive alkylation with [^{18}F]fluorobenzaldehyde.

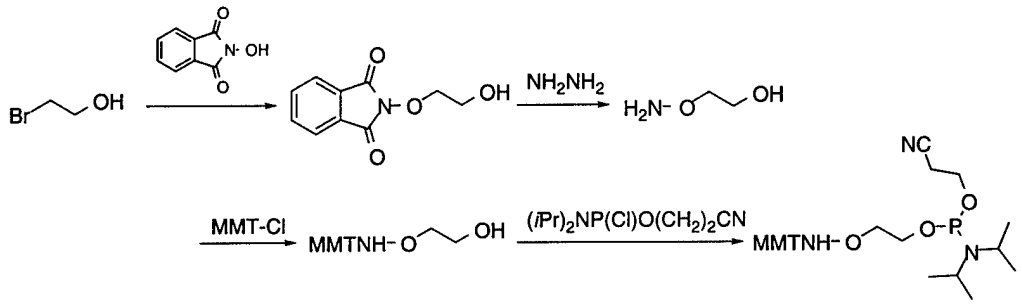
However, examination of the reaction mixture by analytical radio-HPLC indicated the formation of [^{18}F]fluorobenzyl alcohol instead of the expected conjugate (Scheme 3). It seemed that formation of the imine intermediate was not efficient under the low concentration of both substrates, a typical condition of radiochemical synthesis with ^{18}F.

Scheme 3. Unsuccessful reductive alkylation approach



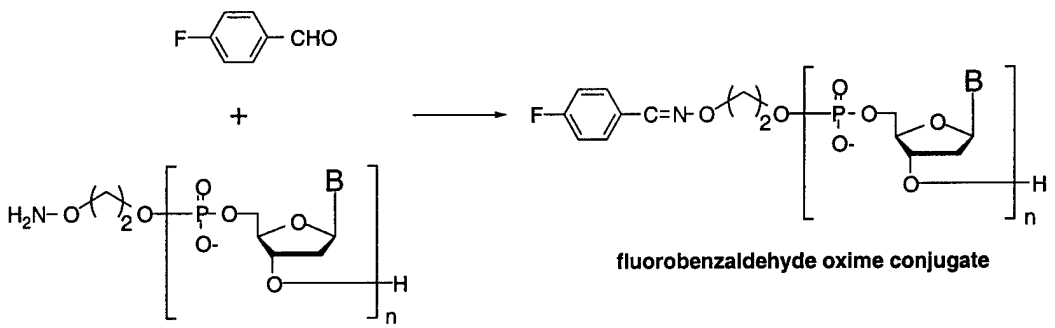
3. Application of the aminooxy–aldehyde coupling reaction for ^{18}F-labeling of ODNs. The aminooxy group is more nucleophilic than the primary amino group due to the so-called α-effect.[3] It is also known that the *O*-alkyl oximes formed upon the reaction of *O*-alkylhydroxylamines with aldehyde compounds are much more stable than the imines derived from primary amines, thus eliminating the use of reducing agents.[3] We have synthesized an aminooxy linker that is ready for incorporation into an ODN synthesizer (Scheme 4). We are currently synthesizing non-radioactive standards of the aminooxy modified ODN as well as the fluorobenzaldehyde oxime conjugate

Scheme 4. Synthesis of aminooxy linker



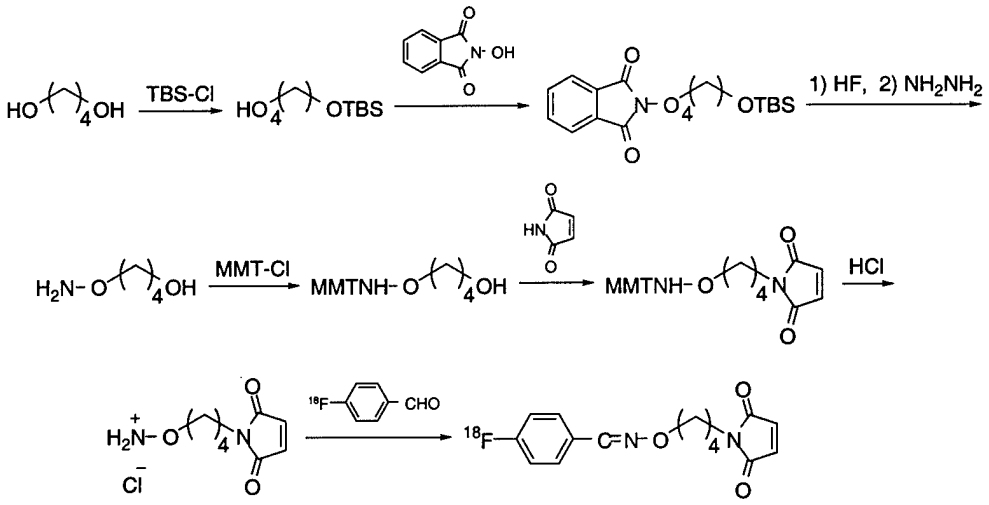
(Scheme 5).

Scheme 5. Synthesis of non-radioactive standard



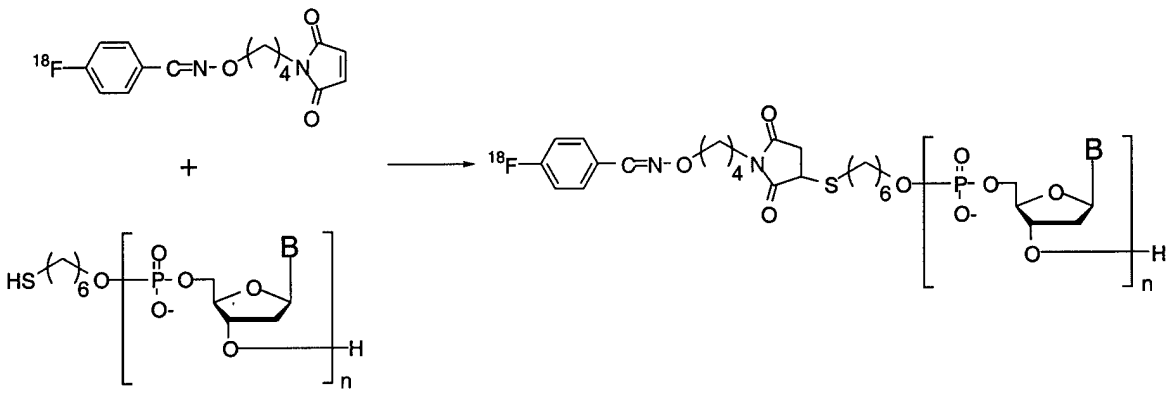
4. Development of an oxime-based thiol-reactive ^{18}F-labeling agent and its use in ^{18}F-labeling of ODNs. We have succeeded in synthesizing a novel thiol-reactive ^{18}F-labeling agent by taking advantage of the selectivity in the aminooxy–aldehyde coupling reaction (Scheme 6). The reaction proceeded smoothly yielding the product in ~16% radiochemical yield (decay corrected) with 100 min. We are currently optimizing the reaction condition. We will soon investigate the conjugation of this thiol-reactive ^{18}F-labeling agent with a 5'-thiol modified ODN as well as a 3'-phosphorothioate ODN (Scheme 7).

Scheme 6. Synthesis of oxime-based thiol-reactive ^{18}F-labeling agent



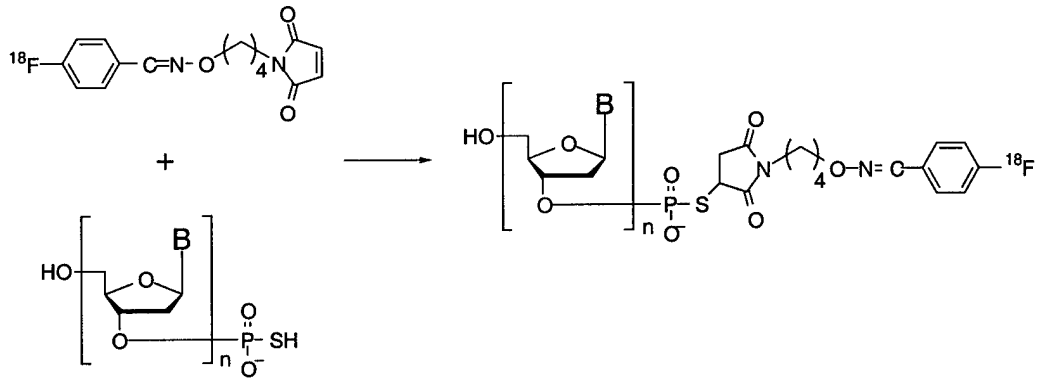
Scheme 7. Future plans

Plan 1



5'-thiol modified ODN

Plan 2



3'-phosphorothioate ODN

Also see References:

1. Haka MS, Kilbourn MR, Watkins GL, Toorongian SA. "Aryltrimethylammonium trifluoromethanesulfonates as precursors to aryl [^{18}F]fluorides: improved synthesis of [^{18}F]GBR-13119." *J. Labelled Cpd. Radiopharm.* **1988**, *27*, 823–833.
2. Brinkley M. "A brief survey of methods for preparing protein conjugates with dyes, haptens, and cross-linking reagents." *Bioconjugate Chem.* **1992**, *3*, 2–13.
3. Muir, TW. "A chemical approach to the construction of multimeric protein assemblies." *Structure* **1995**, *3*, 649–652.

<u>Aim 2:</u> The development of (15-20)-mer oligodeoxynucleotides for targeting the Her-2-neu (c-erbB-2) proto-oncogene mRNA. We have studied several candidate sequences for targeting the Her-2-neu mRNA. Through structural analysis we have previoiusly defined several optimal sequence that we feel should be accessible by our RASON probes. We have completed synthesis of modified-backbone ODNs in order

to improve their plasma stability. We find that 2' O-methyl modified ODNs may be optimal for eventual use *in vivo*. We are also currently exploring 2' methoxy-ethoxy modified ODNs as potential probes. We are getting some help from scientists at ISIS Pharmaceuticals (Carlsbad, CA) which is a company that specializes in ODN therapeutics.

Aim 3: Tissue culture testing of the developed probes to determine the specificity and kinetics of the probe for the c-erbB-2 mRNA. We have studied 4 cell lines for their levels of Her-2-neu expression. These include a MCF-7 control cell line, a MCF-7 over expressing Her-2-neu, SK-BR-3, and SK-OV-3. We now await the labeling of this ODN sequence with Fluorine-18 after Aim 1 leads to improved yields for our RASON probes. Then cell culture uptake and efflux studies will be performed with the RASON probes (antisense and control probes).

Aim 4: To study the targeting properties of ^{18}F-labeled antisense-oligodeoxynucleotides *in vivo* in a mouse animal model using PET.

There has been no progress towards this aim as compared to the previous report. We still await further progress of Aim 1 to go further in Aim 4. As stated last year we have performed some very preliminary studies in two control nude mice in order to understand the limitations of injecting our 18F-ODN probes into mice and imaging with a microPET. Because the yields of 18F-ODN are still very low (see also Aim 1), we have not been able to get satisfactory images of biodistribution of the tracer. We will be able to better characterize the biodistribution when more tracer is routinely available. We have also been able to grow xenografted tumors in mice (e.g., MCF-7) in order to eventually use these tumor models to image with microPET and our 18F-ODN probes.

Key Research Accomplishments

- Synthesis of 18F-oligodeoxynucleotide (ODN) probes in low yields
- Multiple strategies for synthesis thoroughly explored this last year
- Purification of 18F-ODN probes for cell culture testing and *in vivo* testing
- Assessment of hybridization potential of 18F-ODN with target mRNA through T_m measurements
- Synthesis of 2'-o-methyl modified ODNs for improved plasma stability
- Specific Activity measurements of 18F-ODN probes
- Isolation of an 18-mer antisense sequence that should have optimal targeting properties for Her-2-neu
- Study of cell lines for levels of Her-2-neu over-expression
- Preliminary biodistribution studies of 18F-ODN probes in control mice using microPET

Reportable Outcomes

Presentation of 18F radiolabeling approach utilizing newly developed chemistry. Presented in poster form by Dr. Joe Walsh at Annual meeting of the Society of Nuclear Medicine in June, 2000.

J.C. Walsh, D. Pan, N. Satyamurthy, J.R. Barrio, M.E. Phelps, T. Toyokuni, S.S. Gambhir. Use of 5'Deoxy-[18F] Fluoro-4-0-Methylthymidine in the Synthesis of 18F-Labeled Antisense Oligodeoxynucleotide Propbes for Imaging Gene Expression with PET. J. Nucl. Med, 41(5):245P, 2000.

J.C. Walsh, L.M. Fleming, N. Satyamurthy, J.R. Barrio, M.E. Phelps, S.S. Gambhir, T. Toyokuni. Application of Silicon-Fluoride Chemistry for the Development of Amine-Reactive 18F-Labeling Agents for Biomolecules. J. Nucl. Med, 41(5):249P, 2000.

Conclusions

The results to date demonstrate that it is possible to label oligodexoynucleotide molecules with Fluorine-18 (a positron emitter). We still continue to optimize the chemistry in order to achieve significant yields at a high specific activity. Many of the other Aims are ready to proceed once we have sufficient F-18 labeled ODNs. These include study of cell culture models and *in vivo* animal tumor models using microPET imaging technology. The groundwork has also been set for further study in cell culture models, and *in vivo* animal models. With continued funding of this work it should be possible to understand the feasibility of using RASON probes to image Her-2-neu over-expression in breast cancer with PET imaging.

References

J.C. Walsh, D. Pan, N. Satyamurthy, J.R. Barrio, M.E. Phelps, T. Toyokuni, S.S. Gambhir. Use of 5'Deoxy-[18F] Fluoro-4-0-Methylthymidine in the Synthesis of 18F-Labeled Antisense Oligodeoxynucleotide Propbes for Imaging Gene Expression with PET. J. Nucl. Med, 41(5):245P, 2000.

J.C. Walsh, L.M. Fleming, N. Satyamurthy, J.R. Barrio, M.E. Phelps, S.S. Gambhir, T. Toyokuni. Application of Silicon-Fluoride Chemistry for the Development of Amine-Reactive 18F-Labeling Agents for Biomolecules. J. Nucl. Med, 41(5):249P, 2000.

Appendices

H. T. Ravert, O. Vaterlein, L. Kerenyi, R. E. Gibson, C. Ryan, T. Hamill, H. D. Burns, and R. F. Dannals, The Johns Hopkins Medical Institutions, Baltimore, MD; Department of Radiopharmacology, Merck Research Laboratories, West Point, PA. (500367)

Objectives: Endothelin (ET), a potent vasoconstrictive peptide, acts by binding to two major receptor subtypes, ET-A and ET-B. These receptors have not yet been imaged in vivo by PET. This study was undertaken to determine if [^{11}C]L-753, 037, [(+)-(5S, 6R, 7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-methoxyphenyl]-5-(3, 4-methylenedioxyphenyl) cyclopenteno [1, 2-b]pyridine-6-carboxylate], a new mixed ET-A/ET-B receptor antagonist, could be used to label endothelin receptors in vivo. **Methods:** [^{11}C]-L-753, 037 was synthesized by [^{11}C]-methylation of a phenolic precursor, L-843, 974. Its in vivo kinetics, biodistribution, and binding characteristics were evaluated in mice. The specificity of receptor binding was assessed using the selective ET-A antagonist, L-753, 164. **Results:** Kinetic studies in mice showed the highest tracer uptake at 5 min post-injection (p.i.) in the liver (25.0 % injected dose; ID/g), followed by kidneys (18.7 %ID/g), lungs (15.2 %ID/g) and heart (5.6 %ID/g). Initial uptake in liver, kidneys and lungs was followed by a rapid wash-out during the next 10 min and then by a very slow clearance up to 2 hours p.i. By contrast, the activity in the heart remained almost unchanged over 2 hours. Administration of 1 mg/kg of both L-753, 164 (ET-A selective antagonist) and L-753, 037 (mixed ET-A/ET-B antagonist), resulted in significant inhibition of [^{11}C]L-753, 037 binding in mouse heart, lungs, kidneys and adrenal glands. Inhibition by L-753, 164 in the heart was dose-dependant (16, 72 and 96% at 0.1, 1.0, and 10 mg/kg, respectively). In the dog, a dynamic PET study of the heart showed high tracer accumulation at 55-95 minutes p.i. Pre-injection of L-753, 164 (1.0 mg/kg) 30 min before [^{11}C]L-753, 037 administration, led to a 58% reduction in tracer binding at 85 min. **Conclusion:** The results suggest that [^{11}C]L-753, 037 binds to endothelin receptors in vivo and is a promising candidate for investigation of these receptors by PET.

No. 1081

USE OF 5′-DEOXY-5′-[^{18}F]FLUORO-4-O-METHYLTHYMIDINE IN THE SYNTHESIS OF ^{18}F-LABELED ANTISENSE OLIGODEOXYNUCLEOTIDE PROPBES FOR IMAGING GENE EXPRESSION WITH PET.

J. C. Walsh*, D. Pan, N. Satyamurthy, J. R. Barrio, M. E. Phelps, T. Toyokuni, and S. S. Gambhir, University of California at Los Angeles School of Medicine, Los Angeles, CA. (500601)

We have been involved in the development of ^{18}F-labeled oligodeoxynucleotides (ODNs) for the use as PET antisense probes to image mRNA expression in living subjects. Our approach is to synthesize 5′-deoxy-5′-[^{18}F]fluorinated nucleoside which is then coupled to a pre-assembled ODN on a solid-support using the reverse-activation protocol. We have previously reported preliminary results on the synthesis of an ^{18}F-labeled ODN (16-mer) using this approach. We now present detailed examination of the synthesis identifying some problems associated with our approach. Synthesis of 5′-deoxy-5′-[^{18}F]fluoro-4-O-methylthymidine occurs efficiently yielding ~25% radiochemical yield (decay corrected) with >99% radiochemical purity. However, subsequent coupling of 1 to a pre-assembled ODN (15 mer), followed by deprotection and purification of the ^{18}F-ODN (16 mer), is found problematic. First, the coupling is inefficient due to competing hydrolysis of the phosphoramidite by trace quantities of water. The radiochemical yield is generally in the range of 0.01–0.1% (decay corrected). Second, deprotection of the ^{18}F-ODN using conventional AMA reagent (NH4OH–MeNH2) at 65 °C for 7–10 min is accompanied by partial de[^{18}F]fluorination. It seems that amine bases attack the O4-methylthymine portion to form the 5-methylcytosine derivatives, which then cyclize to the 2, 5′-anhydride releasing [^{18}F]fluoride. On the basis of HPLC analysis, 10-min exposure of the protected ^{18}F-ODN to the AMA reagent gives rise to radioactivity corresponding to [^{18}F]fluoride, partially deprotected ^{18}F-ODN and the desired ^{18}F-ODN in a ratio of 1.4 : 1.8 : 1.0. The ratio can be improved to 1.0 : 6.6 : 2.3 by 7-min exposure. Third, it is difficult to separate the ^{18}F-ODN from the unreacted 15-mer resulting in the low specific activity of <500 Ci/mmol (decay corrected). We are currently addressing these problems and the progress will be presented.

University, Atlanta, GA. (100232)

Objectives: To develop a ^{99m}Tc renal imaging agent with the clearance equivalent to ^{131}I OIH by using new N_3S ligands. **Methods:** The tetradentate chelates N-(2-(pyridylamido)ethyl)-L-(or D-)cysteine (PAEC) were synthesized in high yields by condensation of N-(2-aminoethyl)-L-(or D-)cysteine with succinimidylpicolinate and characterized. PAEC ^{99m}Tc labeling by the Glucoscan kit method (pH 8-11) afforded *syn* and *anti* isomers. Biodistribution, renal clearance (Cl) and renal extraction fraction (EF) studies of the *syn*-TcO(L- and D-PAEC) were performed in rats (n = 6) using OIH as an internal control. The Re derivative of PAEC was prepared by ligand exchange reaction with $ReO_2I(PPh_3)_2$. **Results:** Four TcO(PAEC) stereoisomers are possible because the new ligand is chiral and the complex forms *syn* and *anti* isomers. Two radiochemical products (3:1 ratio) were obtained at the optimal labeling conditions (pH 8). This ratio can be increased by using higher pH, but the combined yield (*syn* + *anti*) is lower. The *anti* isomers were unstable (~ 80% decomposed at 3 h) and thus were not included in the animal studies. *syn*-TcO(L-PAEC) was efficiently extracted by the kidney (65 ± 4% of the dose was found in the kidneys and bladder 30 minutes post injection; the clearance was 75 ± 6% and the extraction fraction was 91 ± 13% that of OIH). In comparison, *syn*-TcO(D-PAEC) had a clearance and EF only 33 ± 3% and 52 ± 13% of OIH, respectively. ReO(L-PAEC) obtained in 23% yield was a mixture of *syn* and *anti* isomers (5:1 ratio) as revealed by ^{1}H NMR and HPLC analysis. **Conclusion:** PAEC ligands form ^{99m}Tc and Re complexes as a mixture of diastereomers (*syn* and *anti*) at pH 8. The *syn*-TcO(L-PAEC) isomer shows good renal clearance characteristics but the need for HPLC purification limits its use as a clinical renal imaging agent.

No. 1083

INDIUM AND COPPER FORCEFIELDS SUITABLE FOR THE MOLECULAR MODELLING OF LABELLED BIFUNCTIONAL CHELATE PEPTIDE CONJUGATES.

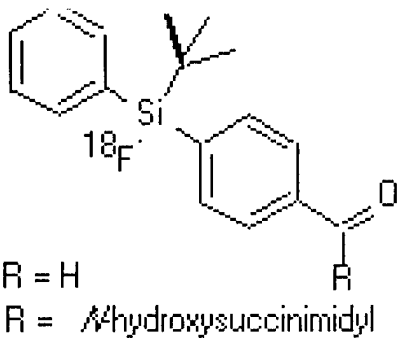
D. E. Reichert*, P. O. Norrby, and M. J. Welch, Washington University School of Medicine, St. Louis, MO; Royal Danish School of Pharmacy, Copenhagen, Denmark. (100184)

Molecular mechanics parameters for In(III) and Cu(II) have been developed for the AMBER force field, as implemented within the commercial package MacroModel, based on crystallographic and *ab initio* data. These parameters were then utilized in a study of the conformational preferences of several small peptides and their metal bound bifunctional chelate (BFC) conjugates. Octreotide is a synthetic octapeptide analog of somatostatin, which when conjugated to various BFC's such as DTPA, DOTA, and TETA and radiolabelled with various radionuclides such as ^{111}In, ^{90}Y, and ^{64}Cu has found use in imaging and radiotherapy of somatostatin receptor positive tumors. This cyclic peptide and several analogs of octreotide, such as octreotate with the terminal threonol replaced by threonine, and Tyr^3-octreotide with the Phe^3 replaced by tyrosine were modelled with this force field. These studies were performed using the GB/SA aqueous solvation model, in order to examine the conformational preferences of the parent peptides in a more realistic environment than the usual vacuum. The studies were then repeated with BFC conjugates (DTPA and DOTA) of these peptides labelled with both In(III) and Cu(II). The results from these studies indicate that the choice of radiometal and bifunctional chelate can have significant effects on the conformational preferences of the peptide and therefore on binding to the targeted receptor.

No. 1097

DIFFERENT CHELATORS (HYNIC, DTPA, MAG3) INFLUENCE THE BEHAVIOR OF ^{99m}Tc IN CELL CULTURE WHEN USED TO RADIOLABEL ANTISENSE DNA. Y. M. Zhang*, N. Liu, and D. J. Hnatowich, University of Massachusetts Medical School, Worcester, MA. (500221)

We have shown recently that cell accumulation in culture of antisense DNA is strongly influenced by the presence of a ^{99m}Tc-MAG3 group for radiolabeling. **Objectives:** In this investigation, we have compared the in vitro behavior of ^{99m}Tc when radiolabeled to one antisense uniform phosphorothioate DNA (i.e. 5′-GCGTGCCTCCTCACTGGC) by three different methods. **Methods:** An 18-mer antisense DNA against the RIa subunit of PKA was obtained with a primary amine on the 5′ end via a 6-member alkyl linker. The amine was conjugated with the NHS esters of HYNIC and MAG3 and by the cyclic anhydride of DTPA. **Results:** By surface plasmon resonance, the association rate constants for hybridization to the uniformly phosphorothiolated sense DNA was unchanged by the conjugation from that of unconjugated antisense DNA in the case of HYNIC and MAG3, but was significantly reduced by conjugation with DTPA, possibly because of anhydride attack on the nitrogenous bases. Labeling efficiencies and specific activities for ^{99m}Tc were highest for HYNIC (tricine) and MAG3 compared to DTPA while stability to cysteine transchelation was in the order HYNIC>DTPA>MAG3. Incubation of labeled DNA in 37°C serum and cellular media showed protein binding by size exclusion HPLC in the order HYNIC>MAG3>DTPA with the phosphorothioate backbone presumably contributing. In each case, radiolabeled and intact DNA was still detectable after 24 hrs. To test cellular uptake, ACHN tumor cells were used after RT-PCR showed that the RIa mRNA is expressed in this cell line. The order of cellular accumulation of ^{99m}Tc was DTPA>hynic>MAG3 with the differences becoming more significant with time between about 4-24 hrs. The rate of ^{99m}Tc egress from cells was found to be MAG3>HYNIC>DTPA which partially explains the order of cellular accumulation. **Conclusion:** Although these results were obtained for one antisense DNA in one cell type, we conclude that the success of antisense imaging may depend, in part, on the method of radiolabeling. This investigation was conducted with financial support from the US Department of Energy.



Radiopharmaceutical Chemistry Track: Radiopharmacy Posters

Hall 2

No. 1098

APPLICATION OF SILICON-FLUORIDE CHEMISTRY FOR THE DEVELOPMENT OF AMINE-REACTIVE ^{18}F-LABELING AGENTS FOR BIOMOLECULES. J. C. Walsh*, L. M. Fleming, N. Satyamurthy, J. R. Barrio, M. E. Phelps, S. S. Gambhir, and T. Toyokuni, University of California at Los Angeles School of Medicine, Los Angeles, CA; University of California at Los Angeles, Los Angeles, CA. (500296)

Target-specific imaging agents are becoming increasingly important for the non-invasive, in vivo diagnostics of various diseases. Targeting molecules include receptor-specific peptide ligands, oligonucleotides and antibody fragments. Although PET provides better image quality and resolution than SPECT, most target-specific imaging agents under development are those labeled with ^{99m}Tc for SPECT imaging. This is partially due to the ease of ^{99m}Tc labeling that involves a mixing of a targeting molecule modified by a bifunctional chelator with a ^{99m}Tc precursor in aqueous environment. We are developing such a practical method for ^{18}F-labeling of targeting molecules based on silicon-fluoride chemistry. We have previously demonstrated the feasibility of this approach synthesizing a prototype of thiol-reactive ^{18}F-labeling agents (J. Labell. Comp. Radiopharm. 1999, 42, S1-S3). Thus, the maleimide-derivatised silanol (Si-OH) was converted into the corresponding Si-^{18}F by mixing Si-OH with cyclotron-produced aqueous [^{18}F]fluoride in the presence of HI. We now expand this approach to include amine-reactive ^{18}F-labeling agents, namely the Si-^{18}F derivatives (1 and 2) containing an aromatic aldehyde or N-hydroxysuccinimidyl ester group, respectively. Optimization of [^{18}F]fluorination as well as in vivo stability of the Si-^{18}F bond, using a normal mouse with microPET, are currently under investigation.

No. 1099

HPLC PROTOCOLS FOR ROUTINE TC RADIOPHARMACEUTICAL QUALITY CONTROL. J. B. Slater*, J. W. Gunn, and P. S. Assaad, Loma Linda University, Loma Linda, CA. (101383)

Objectives: We have developed a cost-efficient method to ensure quality control of radiopharmaceuticals on a daily basis. Typically the integrity of Tc-labeled drugs is affirmed using ITLC. However; due to its accuracy and reproducibility, HPLC is favored over paper chromatography. Solvent protocols adequate for assessing the purity of five Tc radiopharmaceuticals were found using HPLC. **Methods:** A Waters HPLC system was used with their Millenium^{32} software. The system included the Waters 600 pump, 996 UV array dectector, 717 injector system and two bioscan radiation dectectors. The four solvents used were: (A) 0.05M NH_4SO4:MeOH (35:65) (B) 0.01M PO_4 buffer (pH 6.5):ethanol (95:5) (C) C^3HCN (D) 0.01M PO_4 buffer (pH 7.0):C^3HCN:MeOH (48:50:2) The solvents were used in the following manner: Cardiolite® (100%A), MAG_3® (100%B), Ceretec® (100%D), Choletec® (65%B & 35%C) HDP® (40%B & 60%C gradient to 100%B). Each run on HPLC consisted of two injections, each with a duration of six minutes. The first injection was used to equilibrate the system, while the second run was used to evaluate the pharmacological purity of each kit. **Results:** The technetium bound to the kit was separated from the unbound technetium by an average of three minutes. The unbound technetium was found to have a retention time of approximately 1-2 minutes, while the bound technetium appeared at 4-5 minutes. Using techniques provided by the software each of these two peaks can be integrated to precisely analyze the quality of the labeled drug. **Conclusion:** Using HPLC, we were able to determine solvent systems to analyze the purity of five commonly used Tc radiopharmaceuticals. This method is user friendly since it allows one to test these radiopharmaceuticals in any combination or order. This makes HPLC a practical approach for routine quality control of Tc radiopharmaceuticals.

No. 1100

EFFECT OF CHEMOTHERAPEUTIC DRUGS ON THE BIODISTRIBUTION OF A RADIOPHARMACEUTICAL USED FOR RENAL EVALUATIONS IN BALB/C FEMALE MICE. M. Bernardo-Filho*, D. M. Mattos, M. L. Gomes, R. S. Freitas, E. F. Paula, E. M. Boasquevisque, and V. N. Cardoso, Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, Brazil; Instituto do Câncer (INCA), Rio de Janeiro, Brazil; Universidade Federal de Minas Gerias (UFMG), Belo Horizonte, Brazil. (100080)

The biodistribution of radiopharmaceuticals can be altered by drugs. Knowledge of such altered biodistribution is important both in making diagnostic inferences and in dosimetric considerations. Vincristine and mitomycin-C are used in chemotherapeutic regimens. The biological activities of vincristine can be explained by its ability to bind to tubulin and to block the capability of the protein to polymerize into microtubules. The inability to segregate chromosomes correctly during mitosis presumably leads to cell death. Mitomycin-C becomes a bifunctional or trifunctional alkylating agent. This drug inhibits deoxyribonucleic acid