F-18 LABELLED FLUOROMETHYL-NORBORNYL-WAY. A NEW RADIOPHARMACEUTICAL TO VISUALIZE THE 5-HT1A RECEPTOR

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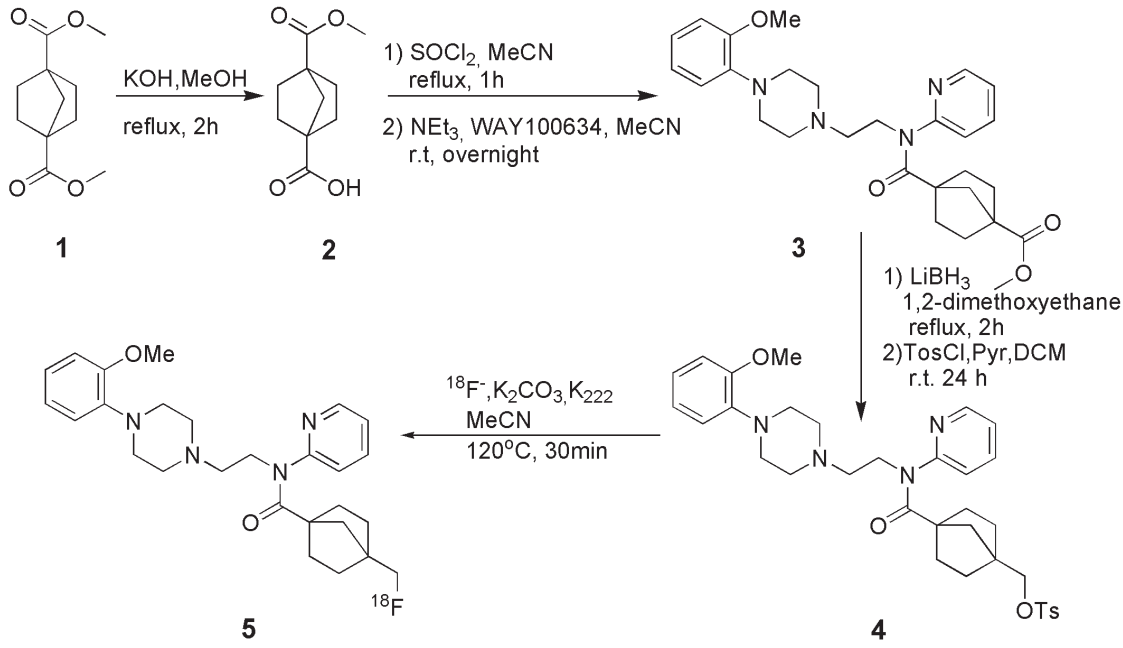
Objectives: In the last few years, we suggested that problems such as defluorination of radiopharmaceuticals can be solved by having the radiolabel on a bridgehead position of e.g. the cubyl moiety, to prevent E2-elimination of HF. In vivo investigations showed that [^{18}F]CH_2F-cubyl-WAY binds to the HT_{1A} receptor in rat brain. Unfortunately, the same studies also indicated some defluorination, presumably as a result of the pseudo-aromatic character of cubane. Furthermore, the precursor of this compound was instable and has to be stored in a refrigerator. For these reasons, we approach to synthesize another WAY derivative; [^{18}F] CH_2F-norbornyl-WAY. Interestingly, initial in vitro screening to investigate the binding affinity showed that CH_2F- cubyl-WAY and CH_2F-norbonyl-WAY were having almost the same Kd-value. Unlike the precursor of CH_2F-cubyl-WAY, the precursor of this novel compound seems to be stable at room-temperature.

Methods: Saponification of (1) with KOH in methanol gave (2) in 60% yield. Treatment with $SOCl_2$ in dry acetonitril to give the acid chloride and subsequently adding WAY100634 and NEt_3 gave (3) in 90% yield. Reduction of (3) with $LiBH_4$ in 1,2-dimethoxyethane followed by a reaction with tosylchloride in dichloromethane gave (4) in 45% overall yield. Finally, a straightforward radiofluorination of (4) was done under standard fluorination conditions in dry acetonitrile for 30 minutes. Separation from the precursor was easily preformed using prep HPLC yielding a (radio)chemically pure product in a radiochemical yield of over 22%.



Biodistribution: four male wister rats received an injection of 15 MBq (300μL) of this [^{18}F] fluoromethylbicyclo[2.2.1]heptyl-WAY, in the tail vein. Rats were sacrificed 45 minutes post injection. Several tissues and distinct brain regions were dissected, weighed and counted for radioactivity.

Results: In vivo biodistribution studies revealed that this [^{18}F]CH_2F-norbonyl-WAY has a relatively high uptake in the regions of interest of the rat brain (hippocampus and cortex). The ratio's of tissue of interest/cerebellum at 45 minutes are shown in the table. We also found that [^{18}F]CH_2F-norbonyl-WAY has a higher brain uptake and less liver and kidney uptake compared to [^{18}F] CH_2F-cubyl-WAY. Furthermore, no abnormal bone uptake was detected.

	Ratio's
Striatum/ Cerebellum	1.31
OccCortex/ Cerebellum	1.80
Hippocampus/ Cerebellum	5.56

Conclusions: Radiofluorinated norbornyl-WAY is easily accessible and has a stable carbon-radioisotope bond. Further studies using PET-imaging are planned to confirm our findings. Finally, this study showed that [^{18}F]CH_2F-norbonyl-WAY is a promising radiopharmaceutical to visualize the 5-HT_{1A} receptor.

Research Support: This research has been made possible by financial support of the Dutch Technology Foundation (STW).

SYNTHESIS AND FLUORINE-18 LABELING OF CANDIDATE METABOLICALLY-RESISTANT MGLUR5 LIGANDS

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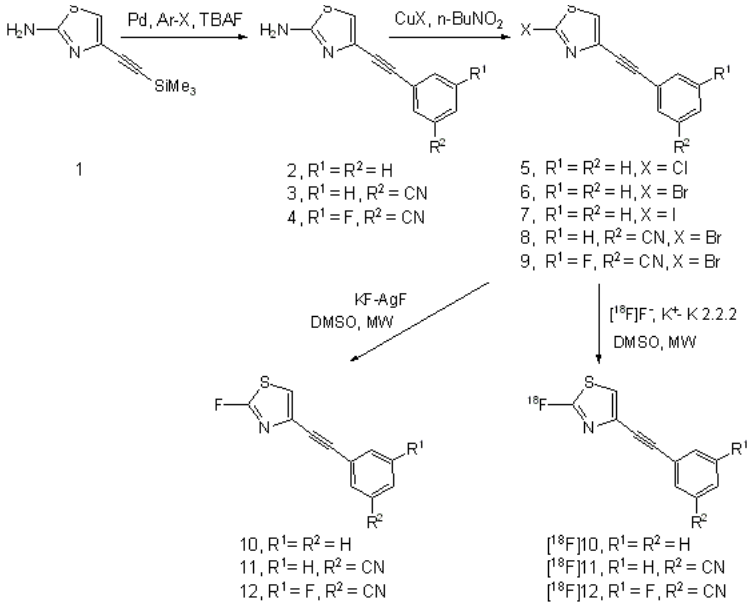
Objectives: The effective mGluR5 radioligand, [^{18}F]SP203 (3-fluoro-5-[[2-([^{18}F]fluoromethyl)thiazol-4-yl]ethynyl]benzonitrile), produces a single radiometabolite by defluorination in monkey blood [Siméon et al., J. Med. Chem., 2007, 50, 3256] and rat brain but fortuitously not in human subjects [Brown et al., J. Nucl. Med., 2008, 49, 2042]. In monkey, radiodefluorination hampers quantification of brain mGluR5 density since [^{18}F]fluoride ion accumulates in nearby skull leading to spill-over of radioactivity through the partial volume effect. As a strategy to produce an ^{18}F-labeled PET mGluR5 radioligand devoid of radiodefluorination in monkey, we set out to synthesize and label a new class of ligands related to SP203 in which the 2-fluoromethyl group on the 1,3-thiazole ring is replaced with fluorine (Scheme).

Methods: Halo precursors (5–9) for radiolabeling were prepared by Sonogashira cross-coupling of (1) with the appropriate haloarenes followed by regioselective halogenation with CuX (X = Cl, Br, I for compounds 5–7, respectively; X = Br for compounds 8 and 9) in the presence of n-butyl nitrite [Siméon et al., J. Org. Chem., In press]. The corresponding fluoro analogs (10–12) were obtained in 25–40% yield by treating the bromides (6, 8 or 9) with KF in DMSO under microwave irradiation at 150 W (10 min, 150 °C). Use of a KF-AgF mixture improved yields to 75%. An aqueous solution (100 μL) of potassium carbonate (0.5 mg) and kryptofix 2.2.2 (5 mg) was added to aqueous cyclotron-produced [^{18}F]fluoride ion (150–250 μL), and dried by three cycles of acetonitrile addition-evaporation under a nitrogen stream. Each halo precursor (5–9) (~ 1 mg) was heated with the generated [^{18}F]F^--K^+-Kryptofix 2.2.2 complex for different times and temperatures in MeCN or DMSO (750 μL) under microwave irradiation at 90 W (80 to 150 °C). Radioactive products were purified on a semi-preparative size Luna C18 column being eluted with acetonitrile: aq. 10 mM ammonium formate (60: 40, v/v) at 4.5 mL/min, and then analyzed by co-injection with reference ligand and LC-MS analysis of carrier.



Scheme. Syntheses and radiolabeling of ligands 10-12.

Results: Treatment of 5, 6 or 7 with [^{18}F]fluoride ion in DMSO under microwave irradiation (5–10 min, 150 °C, 90 W) produced [^{18}F]10 in 19, 16 or 3% decay-corrected radiochemical yield (RCY), respectively. Radiofluorination of 6 at 130 °C in DMSO under argon for 10 min gave [^{18}F]10 in much higher RCY (52%). The radiofluorination of 9 under mild conditions (MeCN, 80 °C, 30 min) gave [^{18}F]12 in only 4% RCY, but under harsher conditions (DMSO, 130 °C, 10 min) gave [^{18}F]12 in high RCY (86%). Under the latter conditions [^{18}F]11 was produced from 8 in 37% RCY.

Conclusions: The reaction of 2-halo substituted 1,3-thiazoles with [^{18}F]fluoride ion gave the [^{18}F]2-fluoro analogs in high RCYs. This reaction was applied successfully to the labeling of new candidate radioligands for imaging mGluR5 receptors; these may prove to be resistant to radiodefluorination in vivo. Further work is in progress to assess the pharmacology of these new ligands, preceding possible in vivo evaluation of the radioligands and their metabolic fates in vivo.

SYNTHESIS OF THE KAPPA OPIOID AGONIST LIGAND [11C]GR103545 WITH HIGH SPECIFIC ACTIVITY: A FACILE, ONE-POT PROCEDURE THROUGH TRANSCARBOXYLATION

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Objectives: [^{11}C]GR103545 is a potent selective kappa opioid receptor (k-OR) agonist[1]. Previous studies demonstrated favorable properties of [^{11}C]GR103545 as a PET tracer for in vivo imaging of cerebralk -OR.[2,3] But use of [^{11}C]GR103545 in human imaging studies has been hampered by difficulties of its multiple-step radiosynthesis (Method A), leading generally to low and variable radiochemical yield with low specific activity (SA). We sought to develop a facile high-yielding method for preparing high SA [^{11}C]GR103545.

Methods: GR103545 contains a methylcarbamate group. Recent literature has described synthesis of N-alkyl carbamates from amines via transcarboxylation reaction with DBU-CO_2 carbamic complex followed by alkylation.[4] We adapted the transcarboxylation process for radiosynthesis of [^{11}C]GR103545 (Method B). Briefly, the amine precursor 1 and DBU-CO_2 (1.2 to 2.5 equivalents) was dissolved in DMF, either with or without the presence of 1-3 equivalents each of Cs_2CO_3 and tetrabutylammonium triflate (TBATf), to generate the intermediate carbamic ion 2. [^{11}C]Methyl triflate was then bubbled into the solution at ambient temp, and allowed to react for 5 min to afford [^{11}C]GR103545. Radiosynthesis was carried out in the FxC automated module.

Results: [11C]GR103545 was produced in 9.4 ± 1.2% radiochemical yield (from [11C]MeOTf, decay-uncorrected) and SA of 8.18 ± 3.47 Ci/mmol at end of synthesis (EOS, n = 9). Total synthesis time was 43 min from end of bombardment (EOB). Starting from the same amount of [11C]CO2 (~2.5 Ci), this new process gave 68.0 ± 13.6 mCi (n = 9) of [11C]GR103545 ready for administration, whereas the literature method provided 18.2 ± 12.0 mCi with SA of 0.87 ± 0.43 Ci/mmol at the end of a 50 min synthesis (n = 15). Compared to literature method2,3, our new method gives, on average, 3.7 times more of formulated product and 9.4 times higher SA. Furthermore, since this is a one-pot, two-step process akin to any radiosynthesis using N-/O-[C-11]methylation, it is simple, reliable and fully amenable to automation.

Conclusions: A reproducible and high-yielding process was developed to produce the kappa opioid tracer [11C]GR103545 in high SA. Schoultz et al. reported a similar process where CO2 gas was bubbled into the precursor solution for one hour before methylation to effect the radiosynthesis5. Compared to the method of Schoultz et al., our new process is much simpler and more convenient, in that the DBU-CO2 complex is a solid, thus can be handled easily and weighed out accurately. Radiosynthesis can be carried out in a completely automated manner in a commercially available chemistry module. The availability of a facile process to produce [11C]GR103545 with high SA should help pave the way for moving this radiotracer to imaging applications in humans.

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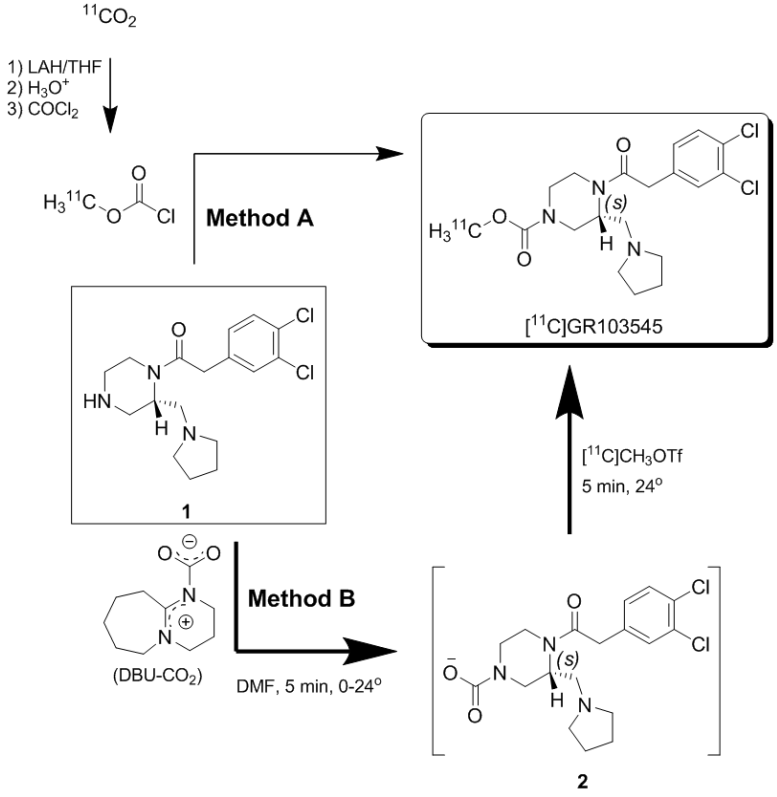

SYNTHESIS AND IN VIVO EVALUATION OF 2-{3-[1-CARBOXY-2-(4-[125I]IODO-BENZYLSULFANYL)-ETHYL]-UREIDO}-PENTANEDIOIC ACID ([125I]DCIBC)

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Objectives: Prostate Specific Membrane Antigen (PSMA) is a cell surface protein overexpressed in prostate cancer and neovasculature of most solid tumors. It is an attractive target for imaging and therapy of prostate and potentially other cancers. Previously, we demonstrated several successful PET and SPECT imaging of PSMA-expressing xenografts using radiohalogenated urea-based PSMA inhibitors, which include 125I-labeled tyrosine-glutamate-urea and lysine-glutamate-urea conjugates [1-2] using direct radioiodination methods, and an 18F-labeled cysteine-glutamate-urea (DCFBC) utilizing a radiofluorinated prosthetic group [3]. Herein we report the first radioiodinated cysteine-glutamate-urea PSMA inhibitor: 2-{3-[1-carboxy-2-(4-[125I]iodo-benzylsulfanyl)-ethyl]-ureido}-pentanedioic acid ([125I]DCIBC).

Methods: Synthesis of [125I]DCIBC is shown in Figure 1. Cysteine-glutamate-urea tri-p-methoxybenzyl ester 1 was prepared by a multi-step synthesis and reacted with p-tributylstannylbenzyl methanesulfonate to give radioiodination precursor 2. Precursor 2 was radiolabeled using [125I]NaI and N-chlorosuccinimide (NCS) in methanol (room temp, 20 min) followed by deprotection (TFA, anisole, 5 min) and RP-HPLC to give [125I]DCIBC in radiochemical yield of 53-59% (SA > 1,700 Ci/mmol). SCID mice bearing a PSMA+ PC-3 PIP tumor and a PSMA- PC-3 flu tumor on either shoulder were injected via tail vein with 2 μCi of [125I]DCIBC for biodistribution and with 1 mCi for SPECT imaging. Mice were sacrificed at various time points; tumor, blood, and major organs were harvested, weighed, and radioactivity counted.

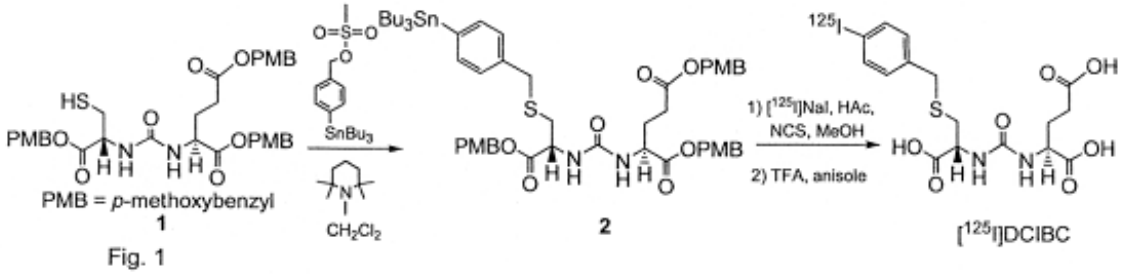

Fig. 1

Results: The biodistribution and imaging showed significant uptake of [125I]DCIBC in the PSMA+ PC-3 PIP tumors with low uptake in PSMA- PC-3 flu tumors. Uptake in PSMA+ PIP tumors ranged from 8.0±1.7 %ID/g at 1 h to 5.5±0.6 at 4 h; uptake in PSMA- flu tumors ranged from 1.3±0.2 at 1 h to 1.0±0.3 %ID/g at 4 h. The PIP/flu ratio was 6:1 at 1 h pi and 5:1 at 4 h pi. By 24 h, radioactivity cleared from normal organs except liver and kidneys.

Conclusions: [125I]DCIBC can be quickly and easily prepared. It localizes in PSMA expressing tumors. This tracer is an attractive candidate for imaging prostate cancer.

Research Support: This work was supported by the following grants: CA92871, EB005324, CA1114111, MH080580

References: [1] Foss, C. A.; Mease, R. C.; Fan, H.; Wang, Y.; Ravert, H. T.; Dannals, R. F.; Olszewski, R.; Heston, W. D.; Kozikowski, A. P.; Pomper, M. G. Clin. Cancer Res. 2005, 11, 40224028. [2] Chen, Y.; Foss, C. A.; Byun, Y.; Nimmagadda, S.; Pullambhatla, M.; Fox, J. J.; Castanares, M.; Lupold, S. E.; Babich, J. W.; Mease R.C.; Pomper, M. G. J. Med. Chem., 2008, 51, 79337943. [3] Mease, R. C.; Dusich, C. L.; Foss, C. A.; Ravert, H. T.; Dannals, R. F.; Seidel, J.; Prideaux, A.; Fox, J. J.; Sgouros, G.; Kozikowski, A. P.; Pomper, M. G. Clin. Cancer Res. 2008, 14, 3036-3043.

Ga-68- AND In-111-LABELED SMALL MOLECULE INHIBITORS OF PSMA FOR PROSTATE CANCER IMAGING

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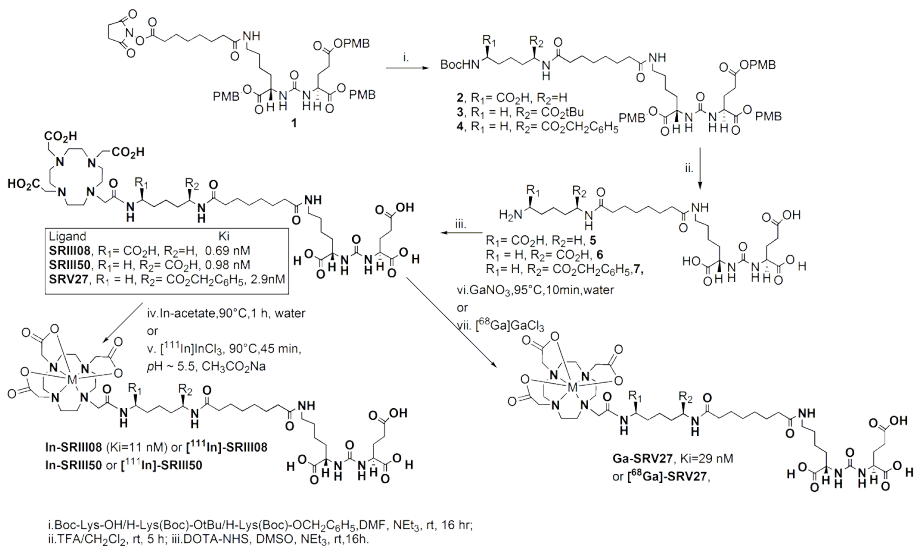
Objectives: The integral membrane protein prostate-specific membrane antigen (PSMA) is becoming increasingly recognized as a viable target for imaging and therapy of cancer. We have previously demonstrated the ability to image PSMA-expressing prostate tumor xenografts with radiohalogenated, urea-based, low molecular weight inhibitors of PSMA.1,2 Recently we have extended this work to include the radiometal Tc-99m.3 To retain the binding affinity of the urea inhibitors, a linker moiety was introduced between the amino functionalized PSMA urea and the metal chelator (Figure 1). We have now extended this work further to include other radiometals such as Ga-68 for PET and In-111 for SPECT. Here we report the tumor targeting potential of the Ga-68-labeled DOTA urea inhibitor [68Ga]SRV27 and the In-111-labeled
DOTA urea inhibitors [111In]SRIII08 and [111In]SRIII50 (Figure 1).

Figure 1



i.Boc-Lys-OH/H-Lys(Boc)-OtBu/H-Lys(Boc)-$OCH_2C_6H_5$,DMF, NEt_3, rt, 16 hr;
ii.TFA/CH_2Cl_2, rt, 5 h; iii.DOTA-NHS, DMSO, NEt_3, rt,16h.

Methods: Compounds were synthesized as outlined in Figure 1 and were characterized using standard spectroscopic techniques. SPECT-CT imaging was performed on an XSPECT scanner using either a SCID mouse bearing a PSMA+ LNCaP tumor or using a SCID mouse bearing PSMA+ PC-3 PIP and PSMA- PC-3 flu tumors, one on either shoulder, and ~1 mCi (37 MBq) of each agent via tail vein injection. Compound [111In]SRIII08 was tested in an ex vivo biodistribution study. Mice (4 per time point) were sacrificed at various time points. Tumor, blood, and major organs were harvested, weighed, and radioactivity was counted.

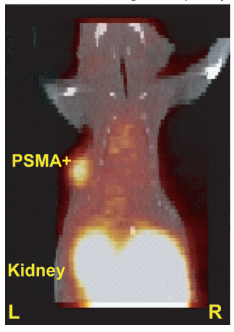
Results: The PSMA inhibitory constants (Ki values) of compounds tested are in shown Figure 1. SRV27 was radiolabeled with Ga-68 in good radiochemical yield (~ 90%) and in radiochemical high purity (> 98 %). PET imaging revealed that [68Ga] SRV27 demonstrated site-specific uptake in PSMA+ tumor on summed images obtained from 0.5 – 2 h postinjection. No significant nontarget uptake was identified. Further ex vivo biodistribution will be presented. [111In]SRIII08 and [111In]SRIII50 were prepared in good yield (~ 60%) and in high radiochemical purity (> 98 %). SPECT imaging demonstrated specific uptake in PSMA+ PIP tumor. [111In]SRIII08 showed PSMA+ PIP-tumor uptake values of 3.17 ± 1, 1.14 ± 0.6, 1.08 ± 0.3 and 0.24 ± 0.01% ID/g at 0.5 h, 1 h, 2 h and 5 h respectively. Radioactivity cleared rapidly from tumor and other normal organs. No uptake was identified in PSMA- flu tumor.

Conclusions: All three radiolabeled DOTA-chelated urea analogs localized in PSMA+ tumor xenografts. However, [111In] SRIII08 cleared rapidly from tumor and normal organs due to its hydrophilic nature compared to our previous Tc-99m-labeled compounds. This preliminary study indicates that radiometal-labeled DOTA-linker ureas are promising candidates for PET/ SPECT imaging of prostate cancer.

Research Support: This work was supported by the following grants: NIH R24CA92871, NIH R21 CA114111, and DoD PC050999

References: 1. 1. 1.J Med Chem 2008; 51:7933-7943. 2.Clin Cancer Res; 2008 14:3036. 3.J Med Chem 2008; 51:4504-4517.

PET-CT image of a PSMA+ LnCaP Tumor Xenograft Model
for [68Ga]-SRV27 during 0.5h-2h post injection



THE FIRST TECHNETIUM-LABELED SUBSTRATES FOR HUMAN THYMIDINE KINASE 1

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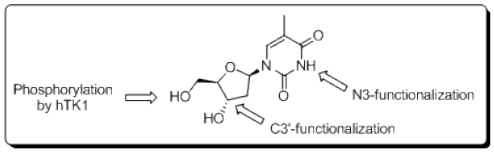
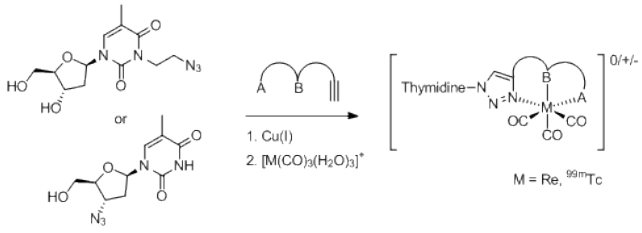
Objectives: Human thymidine kinase 1 (hTK1) is expressed in all neoplastic cells, but is virtually absent in non-proliferating cells, making it an ideal target for proliferation markers. Although hTK1 has proven to be a suitable target for non-invasive imaging of cancer cell proliferation using radiolabeled substrates such as 18F-fluorothymidine, up to now no radiometal-labeled thymidine derivatives suitable for single photon emission tomography (SPECT), and which are substrates for hTK1, have been reported. We aimed, therefore, to synthesize thymidine derivatives based on the inexpensive and readily available nuclide technetium-99m, and to establish the structural requirements necessary to maintain activity towards the enzyme.

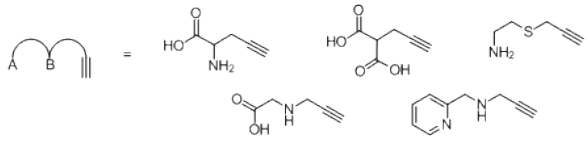
Methods: Two series of thymidine analogues with efficient metal chelating systems were prepared in parallel from either 3'-azido-3'-deoxythymidine (AZT) or an N3-functionalized azido-thymidine derivative and a set of suitable alkynes using the Cu(I) catalyzed azide-alkyne cycloaddition. The reaction mixtures were labeled directly with Tc-99m before the radiolabeled products were purified by HPLC. Phosphorylation of the Tc-labeled thymidine analogues in the presence of hTK1 was followed by HPLC. Rhenium analogues were also prepared and fully characterized, and their phosphorylation was followed by HPLC and mass spectroscopy. The relative rates of phosphorylation of the novel hTK1 substrates were measured by UV spectroscopy using a coupled pyruvate kinase-lactate dehydrogenase assay.

Results: Thymidine was functionalized with metal complexes at either the N3 or C3' position. Neutral, cationic and anionic thymidine derivatives were prepared, to assess the influence of both the site of functionalization and of the overall charge on the relative rates of phosphorylation. All of the organometallic complexes retained activity towards the enzyme, however, in the case of the C3'-functionalized compounds, neutral and anionic compounds were the best substrates with phosphorylation rates of 20-28% of the value for the natural substrate thymidine. For the N3-functionalized compounds there was less variation in the phosphorylation rates as a function of overall charge, although neutral and cationic derivatives appeared to be the better substrates with phosphorylation rates <18% of the value for thymidine.

Conclusions: Using the M(CO)3 core we have identified the first metal-labeled substrates for hTK1. Furthermore we have shown that thymidine can be functionalized with a metal complex at either the N3 or C3' position and in both cases maintains activity towards hTK1. The Cu(I) catalyzed azide-alkyne cycloaddition enabled the parallel synthesis of a number of derivatives, all of which were labeled with Tc/Re to produce thymidine analogues with varying physicochemical properties. The synthesis, characterization and relative rates of phosporylation of the metal-labeled thymidine analogues will be presented. In vitro experiments are ongoing to investigate the cell-internalizing ability of the complexes into two human glioblastoma cell lines. The preliminary results of these experiments will also be presented.







SYNTHESIS, RADIOLABELLING AND BIOLOGICAL EVALUATION OF TARGETED METALLACARBORANE (M = 99MTc) DERIVATIVES FOR IMAGING NEURORECEPTORS

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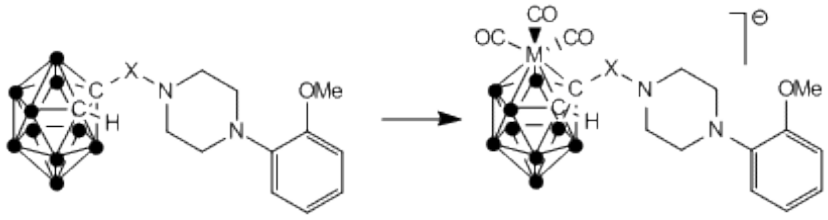
Objectives: Technetium-99m radiopharmaceuticals targeting neuroreceptors have the potential to be used in characterizing neurological disorders such as anxiety and depression. A common problem encountered using ^{99m}Tc as the radionuclide for imaging targets in the brain is the limited amount of agent that gets across the blood-brain barrier (BBB). There is a need to develop new technetium ligands that are compact, stable and easily modified to facilitate uptake and selective binding to the protein of interest. Here, research towards creating a general purpose technology from which technetium compounds that can cross the BBB will be presented.

Methods: Organometallic compounds can be used as compact ligands to develop molecular imaging probes. To create compounds that can cross the BBB, a series of carborane derivatives of WAY-type biomolecules were synthesized in one-pot reactions using an ionic liquid to increase the yields of the closo-carborane compounds. The corresponding rhenium(I) and technetium(I) metallacarborane complexes were prepared in water under mild conditions utilizing microwave heating.

Results: High yields of a new class of WAY derivatives bearing an organometallic core were prepared. The technetium(I) metallacarborane complexes were generated in under 15 minutes by reacting the carborane-WAY ligand with $[^{99m}Tc(CO)_3(H_2O)_3]^+$. High isolated radiochemical yield and specific activity were obtained (25-35 %). The lipophilcity measurements of the derivatives were determined (2.66-2.69) and in vivo imaging were preformed.

Conclusions: Carboranes provide a platform to tailor the imaging agent to have the most appropriate characteristics in order to cross the BBB. The synthesis, radiolabelling and biological evaluation of a series of ^{99m}Tc(I) carborane-WAY derivatives will be presented.



X = $(CH_2)_n$ (n = 1, 3), amido

M = Re, ^{99m}Tc