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Abstracts

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Metabolism of Octreotide Conjugates of ^{64}Cu-TETA, ^{64}Cu-CPTA and ^{111}In-DTPA

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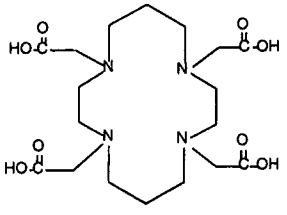
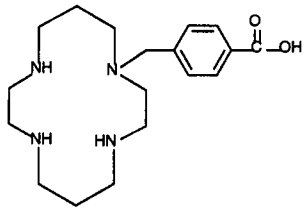
The somatostatin analogue octreotide (Sandostatin®) has been labeled with ^{123}I ($T_{1/2}$ = 13 h)(1) and ^{111}In ($T_{1/2}$ = 67 h)(2) for SPECT imaging, as well as ^{68}Ga ($T_{1/2}$ =1.1 h)(3) and ^{18}F ($T_{1/2}$ =1.8 h)(4) for PET imaging of somatostatin receptor positive tumors. Due to high background activity in the body, optimal SPECT images of tumors in patients of ^{111}In-DTPA-D-Phe^1-octreotide were obtained at 24 hours post-injection. For PET imaging, the short half-lives of ^{68}Ga and ^{18}F may not allow for optimal imaging times when labeled to octreotide, therefore we have focused on labeling octreotide with ^{64}Cu, a positron-emitting radionuclide with a half-life of 12.8 h. Octreotide has been labeled with ^{64}Cu using the macrocyclic chelates; 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA) and 4-[1,4,8,11-tetraazacyclotetradec-1-yl)-methyl]benzoic acid (CPTA) shown below.



TETA



CPTA

The ^{64}Cu-octreotide conjugates of these chelates have been evaluated both *in vitro*, to determine receptor binding affinity and *in vivo*, to determine biodistribution in target and nontarget tissues and have been been compared to the extensively studied ^{111}In-DTPA-octreotide.(5) We have observed a relationship between the biodistribution of octreotide conjugates and the lipophilicity of the chelate and/or overall charge of the metal-chelate complex.(5) There is not, however, a clear interpretation of this relationship. To accurately design second-generation octreotide conjugates the metabolic fate of octreotide complexes needs to be determined. There has been a lack of studies pertaining to the metabolic fate of radiolabeled octreotide complexes even for the commonly researched ^{111}In-DTPA-octreotide. We believe that by determining the site of the isotope in the cell/organ and the nature of the metabolite we can more

thoroughly understand the relationship between the lipophilicity and charge of the metal-chelate octreotide conjugate and its biodistribution.

The metabolites of ^{64}Cu-TETA-octreotide and ^{64}Cu-CPTA-octreotide were analyzed in the blood and urine. Metabolites of ^{64}Cu-TETA-octreotide and ^{111}In-DTPA-octreotide were analyzed in the pancreas, adrenals, liver and kidneys. Metabolic studies were performed using mature female Sprague Dawley rats. Radiolabeled octreotide conjugates were prepared as previously described[5] and were injected into rats via the tail vein. The rats were sacrificed at 1 and 24 hours post-injection and the liver, kidneys, adrenals, pancreas, blood and urine were removed. Tissue samples were homogenized in ethanol/TEA buffer (10mM Tris, 1.5mM EDTA, 3mM sodium azide, pH 7.4) at room temperature. The tissue homogenates were treated with ethanol; the precipitated protein was separated by centrifugation and the supernatant was removed, counted and analyzed by reversed phase HPLC and/or TLC. Organ blanks, where the ^{64}Cu-TETA-octreotide or ^{111}In-DTPA-octreotide injectate was added directly to tissues prior to work-up, were performed as controls.

The fraction of activity *in vivo* that is present as unmetabolized octreotide-labeled conjugate in blood and urine was determined from integration of the HPLC chromatograms. (see Table 1) The percent authentic activity that is unmetabolized was determined using the %intact conjugate obtained from the chromatogram, the %purity of the injectate, the tissue extraction efficiency of sample and the tissue extraction efficiency of the blank.

Table 1. Percent intact ^{64}Cu labeled chelate octreotide in blood and urine. (Chelate = TETA or CPTA)

Time (h)	TETA (blood)	CPTA (blood)	TETA (urine)	CPTA (urine)
0.03	79	38	--	--
0.5	78	38	--	--
1	75	34	100	0
2	42	42	100	39
4	9	15	85	36
6	15	0	--	21
12	0	0	5	0
24	0	0	0	0

As exhibited above, metabolism of ^{64}Cu-TETA-octreotide in the blood occurred rather slowly until 1 h post-injection. The percent of intact ^{64}Cu-TETA-octreotide decreased rapidly from 1 h to 12 h at which time it was completely metabolized. In contrast, the more lipophilic, positively charged ^{64}Cu-CPTA-octreotide was 62% metabolized by 2 min post injection and 100% metabolized in the blood by 6 h post injection.

Urine data has proven quite interesting in that ^{64}Cu-CPTA-octreotide and ^{64}Cu-TETA-octreotide show markedly different results. At 2 h post injection, ^{64}Cu-TETA-octreotide remained 100% intact whereas ^{64}Cu-CPTA-octreotide was only 39% intact. It may be concluded from this data that ^{64}Cu-TETA-octreotide is excreted from the kidneys initially intact whereas ^{64}Cu-CPTA-octreotide is initially excreted mostly as metabolites. It should be noted that metabolism is relatively complete by 12 h post injection for both conjugates.

In organ metabolism studies at 1 h post injection, ^{64}Cu-TETA-octreotide and ^{111}In-DTPA-octreotide were >65% intact in the somatostatin receptor containing organs (adrenal and pancreas). In both clearance organs (kidneys and liver) ^{111}In-DTPA-octreotide was >70% metabolized at 1 h, whereas ^{64}Cu-TETA-octreotide was approximately 50% metabolized. At 24 h, both radiolabeled conjugates were completely metabolized in all organs. Both compounds showed the presence of a more hydrophilic, relatively small molecular weight metabolite. Using reversed phase TLC this metabolite had an R_f of ~0.7 as compared to an R_f of ~0.3 for intact radiolabeled octreotide-chelate.

In conclusion, our studies have shown that metabolism of the three octreotide conjugates studied occur rather rapidly, especially in the clearance organs. Further studies are in progress to more accurately correlate the metabolism and biodistribution of ^{64}Cu- and ^{111}In- octreotide conjugates.

Acknowledgements: This work was supported by NIH CA64475 (C.J.A.), Mallinckrodt Medical, Inc. (C.J.A.), and DOE DE-FG02-87-ER60512 (M.J.W.).

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^{18}F- and ^{131}I-Labeling of the Octadecapeptide Apamin: A Selective Blocker of the Ca^{2+} Dependent K^+ -Channel. Syntheses and In-Vivo Evaluation in NMRI Mice.

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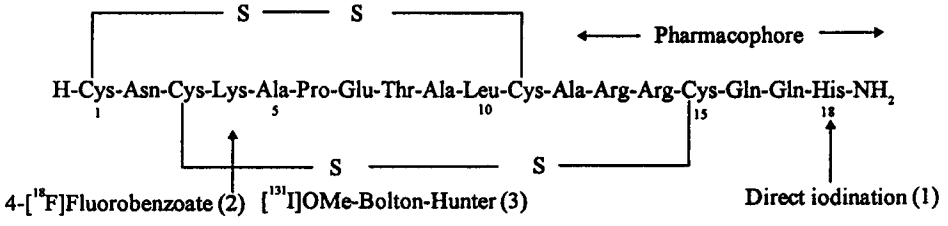
Apamin (scheme 1), an octadecapeptide found in the venom of the honey bee (1), is known to be a selective blocker of Ca^{2+}-dependent K^+ channels (K^+_{Ca}-ch.) (2) and to exhibit high affinity to its binding sites (K_D in the range of 15 to 60 pM (3)). As recently shown by post mortem autoradiography, an anatomically discrete loss of K^+_{Ca}-channels within the hippocampus occurs in Alzheimer's disease (4). Since apamin is different from most peptide toxins in its unusual ability to cross the blood brain barrier (5), radiolabeled apamins are of potential interest for in-vivo receptor mapping of K^+_{Ca}-channels with SPET and PET. For this purpose, ^{131}I- and ^{18}F- labeled apamin was synthesized. The biodistribution and in-vivo stabilities of the different tracers were investigated in NMRI mice and the brain uptake compared.

Initial attempts concerning direct iodination of apamin at His^{18} (1) were carried out via the chloramine-T method according to a published procedure (2). Due to extensive degradation of educt and product observed under these reaction conditions, further experiments were performed using the Iodogen method. However, no significant reduction of fragmentation was found. Moreover, in-vivo studies on NMRI mice using SP-Sephadex C-25 purified product (1, CAT-method) revealed fast deiodination as indicated by values of about 110% iD/g thyroid (60min p.i.). Thus, an alternative labeling procedure via acylation at Lys^4 was performed which allows labeling of apamin under mild conditions.

Encouraged by the recently published results of our group concerning improved in-vivo stability towards deiodination of iodinated tyrosine derivatives such as O-methylated 3-iodotyrosine (OIMT) and O-methylated 3-iodo-α-methyltyrosine (OMIMT) (6), we have O-methylated the Bolton-Hunter reagent and applied N-succinimidyl desamino-(O-methyl)tyrosine (^{131}OMeBH) to the labeling of apamin (2) (scheme 1). Initially, as shown in scheme 2, we prepared ^{131}I-OMeBH by iodination of the corresponding acid (Iodogen, 90% trifluoroacetic acid (TFA), 60°C, 45min, RCY 68±4%) and subsequent activation using O-(N-succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TSTU) (MeCN, 60°C, 2 min, >95%). Direct iodination of OMeBH using TFA was also possible and led to a RCY of about 60% in 15 min (scheme 1, Fig. 1). At longer reaction times the acid-catalyzed ester hydrolysis led to a decrease of the overall radiochemical yield.



Scheme 1: Apamin (amino acid sequence, pharmacophore and labeling sites)

^{18}F-labelling at Lys^{4} (3) was achieved using N-succinimidyl 4-[^{18}F]fluorobenzoate (7,8) which was prepared via an improved synthesis (scheme 3). This procedure involved ^{18}F-fluorination of ethyl 4-(trimethylammonium triflate)benzoate followed by hydrolysis. Educt separation was performed by fixation on a polystyrene cartridge and elution through a cation exchange cartridge. In the last step, activation using N,N,N',N'-tetramethyluronium tetrafluoroborate yielded [^{18}F]SFB in nearly quantitative yield. Subsequent product separation was done by fixation on a poylstyrene cartridge and selective elution. The synthesis was completed in about 35min with a RCY of 55±5%.
To determine the extent of competing acylation at Cys^{1} and His^{18} (as well as active ester hydrolysis catalyzed by His^{18}) and to check the assumption that no protection is necessary at Arg^{13,14}, initial acylations were carried out on a S-Benzyl-CysOMe/ N(α)Ac-HisOMe/ N(α)Ac-ArgOMe amino acid mixture (molar ratio 1:1:2) as a function of pH. The RCY found for the [^{18}F]SFB conjugate of N(α)Ac-LysOMe was >85% at pH >8.5, whereas acylation of N(α)Ac-HisOMe and N(α)Ac-ArgOMe did not exceed 5 and 1% (pH>8.5) respectively. Thus, preparation of (2) and (3) was carried out at pH 8.7 with 0.05 to 1 mg of apamin which giving a RCY of up to 70% (Fig. 2).
In-vivo studies on NMRI mice revealed fast deiodination of (1) (111±36% iD/g thyroid, 60 min p.i.) and a much higher stability for (2) (<10% iD/g thyroid, 60 min). Accumulation of F-18 in bones using (3) did not exceed 2.5% iD/g after 60 min. In accordance with literature data on the distribution of binding sites for potassium channel ligands in rat brain (9), the activity accumulation for both (2) and (3) showed a significantly higher uptake in the hippocampal area (Figs.3 and 4) compared with in the remaining brain tissue.
To demonstrate the preservation of binding properties, the determination of the IC_{50}-values of (2) and (3) by competition experiments on mouse brain synaptosomes are presently being carried out.
From these results we conclude that despite the relatively low absolute activity accumulation in the brain, further studies on the suitability of radiolabeled apamin as a suitable tracer for the imaging of pathological differences of the receptor status of $K^{+}Ca$-channels in-vivo is worthwhile.

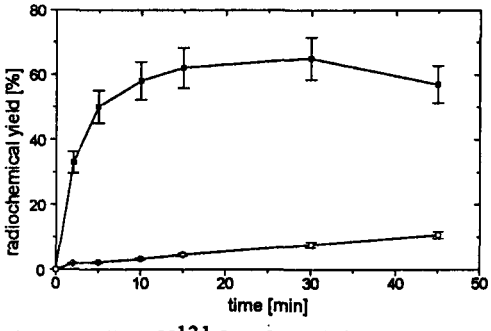


Fig. 1: RCY of [^{131}I]MeOBH (■) and the corresponding acid (□) as function of time (90%TFA, Iodogen, 60°C, 32 mM OMeBH)

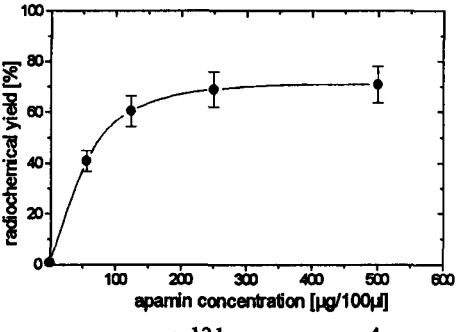
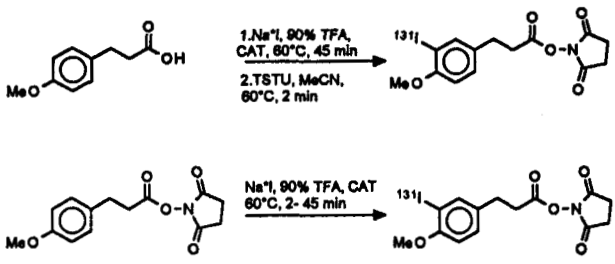
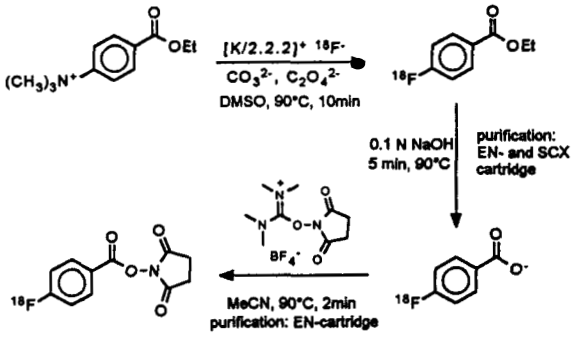


Fig.2: RCY of [^{131}I]OMeBH-Lys^{4}-apamin as function of apamin concentration



Scheme 2: Synthesis of [^{131}I]OMeBH



Scheme 3: Improved synthesis of [^{18}F]SFB

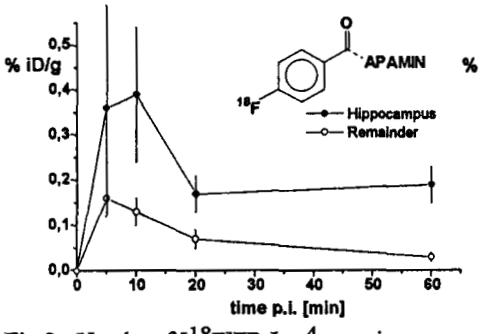


Fig.3: Uptake of [^{18}F]FB-Lys^4-apamin in mouse brain

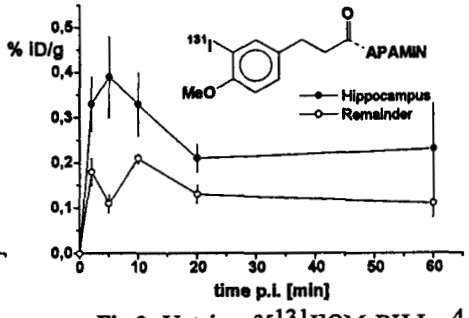


Fig.3: Uptake of [^{131}I]OMeBH-Lys^4-apamin in mouse brain

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Labeling of small peptides with yttrium and gallium via a new DOTA-derivative or DFO using different disuccinimidylic esters as linker

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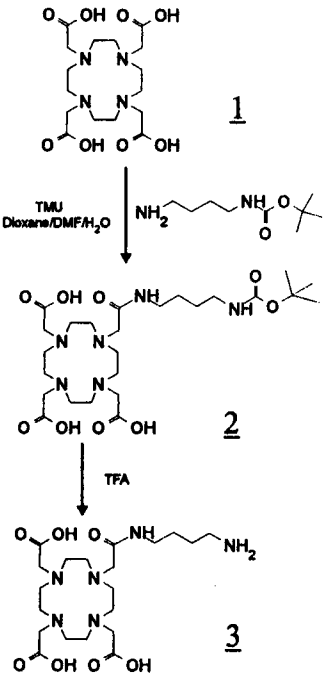
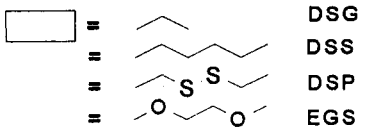
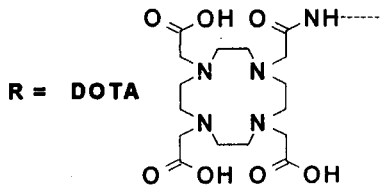
Small peptides such as the somatostatin analogue are of increasing interest in tumor diagnosis. Many procedures have been developed to label these peptides either via halogenation or via the linking of radio-metals like gallium, indium etc. Due to the useful nuclear properties of ^{90}Y ($T_{½}$ = 64.2 h, 100% β-decay) for endo-therapeutic applications, chelating agents are also required for yttrium. Many chelators were synthezised on the basis of diethylenetriamine pentaacetic acid. Due to the low in vivo stability of these complexes, the siderophoric ligand desferrioxamine B (DFO) for gallium as well as derivatives of 1,4,7,10-tetraaza cyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) for yttrium were suggested to yield complexes of high stability, respectively.

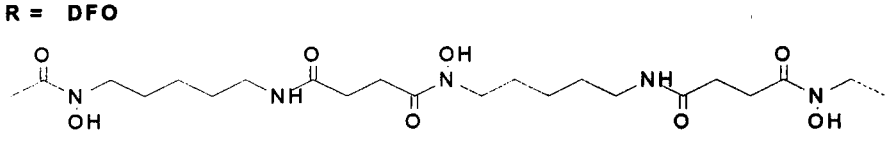
We have synthezised an analogue of DOTA by modifying one of its carboxylic groups forming an butylamine residue; scheme 1. DOTA was prepared by the method of Desreux et al. (1) with 74% yield. In the second step mono-boc-1,4-diaminobutane was coupled to one carboxylic group of the macrocycle using O-(N-succinimidyl)-tetramethyluronium tetrafluoroborat as activating agent. The reaction was carried out in a dioxane/ DMF/H_2O solvent-mixture using diisopropyl-ethylamine as base. The product 2 was purified by anion-exchange chromatography and lyophilized giving a white powder wich was analysed by ^1H-NMR. It was deprotected with trifluoracetic acid, subsequently evaporated and purified by anion exchange chromatography. Overall yields of 3 are > 50%.



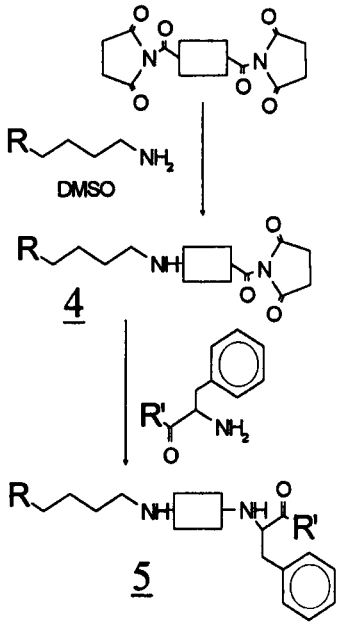
Scheme 1: Derivatization of DOTA







In order to modify the linkage between the chelator (DOTA-derivatives or DFO) and the peptide, several disuccinimidylic esters were used systematically (scheme 2). Initial studies were done using various small petides with an N-terminal phenyalanin group. The parameters of the reaction between the alkyl-amine-residue and the N-terminal amino acid of the NHS-esters were studied in detail. Due to the characteristics of the homobifunctional NHS-esters, crosslinking was observed especially at low peptide to NHS-ester ratios, Fig. 1. At least a five fold excess of the chelator to NHS-ester is needed to obtain yields higher than 80%, Fig. 2. The second step can be carried out without any prior purification. For most of the peptides the overall yield in the linking procedure is higher than 90% except for peptides with histidine residues, known to build unstable side products (Table 1).



Scheme 2: Coupling of DOTA- and DFO to peptides through Disuccinimidylesters

For the labelling of these products with yttrium and gallium the effects of buffer, pH and the complexation kinetics were investigated. Furthermore, their long term stability in aqueous solution was measured. These results were applied to synthesize a set of octreotide analogues of the ^{68}Ga-DFO-succ.-octreotide (2), substituting glutaryl and suberyl by the succinyl-linkage. Lipophilicity, stability and ease of synthesis were evaluated systematically.

In conclusion, the various comercially available NHS esters can be effectively used as variable linkers for the coupling of peptides and chelators.

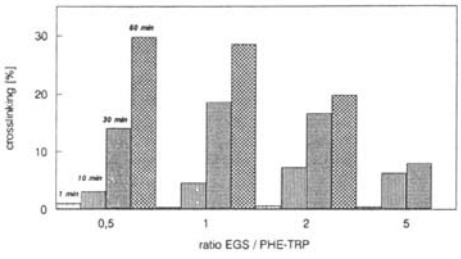


Fig. 1: Crosslinking for different EGS / PHE-TRP-ratios

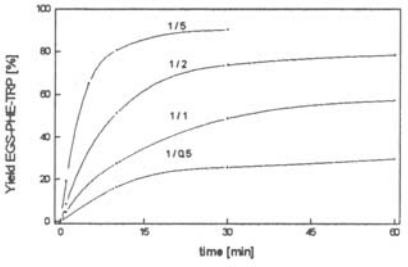


Fig. 2: Kinetics of the reaction EGS + PHE-TRP as a function of educt ratios.

Table 1: Coupling of small peptides to DFO through disuccinimidyl suberate (DSS)

Peptide	educt	product 4 (yield in %)	product 5 (overall yield in %)	cross-linking (in %)
Phe-Trp	2.0	91.9	83.0	6.1
Phe-Tyr	0	96.4	89.8	3.6
Phe-His	3.1	64.4	61.8	32.5 (side products)
Phe-Gly	0	99.3	98.8	0.7
Phe-Gly-Phe-Gly	0	94.8	94.3	5.2
Phe-Ser-Val	0	99.5	92.5	0.5

($c_{peptide}$= 1.5 10^{-3} M; $c_{peptide}$ / c_{DSS}= 1 / 5; c_{DSS} / c_{DFO} = 1 / 2; DMSO (Diisopropylethylamine)

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In vivo uptake kinetics and dosimetric calculations of ^{86}Y-DTPA-octreotide with PET as a model for potential endotherapeutic octreotides labelled with ^{90}Y

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Various radio-labelled somatostatin-analogues such as ^{111}In-DTPA-octreotide (Octreoscan®) or ^{123}I-Tyr^3-Octreotide for example are broadly applied for the diagnosis of different SRIF tumors. The high affinity of the this peptide to somatostatin receptors has stimulated the development of radio-labelled analogues, in view of potential endotherapeutic applications. Yttrium-90 is one of the most promissing candidates amoung the radiotherapeutic isotopes because of its nuclear decay parameter ($T_{1/2}$ = 64 h, 100% β^-) and its convenient availability in n.c.a. state from the ^{90}Sr/^{90}Y generator system. Furthermore, it forms stable chemical bonds to small peptides via appropriate chelators. There is, however, one serious problem to be solved preporatory to the endotherapeutic application of potential ^{90}Y-labelled octreotides, namely the quantitative determination of the in vivo uptake kinetics and the calculation of local radiation doses of the individual radiotherapeutics in patients.

It was the aim of this study to substitute ^{90}Y by the positron emitting yttrium isotope ^{86}Y ($T_{1/2}$ = 14.7 hr, 32% β^+) in the radioyttrium-labelled octreotide in order to measure uptake kinetics of the tracer in tumor bearing rats using a PET scanner and to calculate dosimetric data for organs of interest (1). ^{86}Y-DTPA-octreotide was used as a model analogue. Male Lewis rats (250±50 g) bearing an intrascapular exocrine pancreatic islet cell tumor (≈ 1 g) were used. They were injected i.v. with about 100 MBq of ^{86}Y-DTPA-octreotide of 17 GBq / µmol specific activity. Simultaneously, dynamic PET scans were started for up to 120 min with intervals of 30 sec at the beginning and 10 min at the end.
Uptake kinetics of ^{86}Y-DTPA-octreotide in the tumor are shown for two animals in Fig. 1.

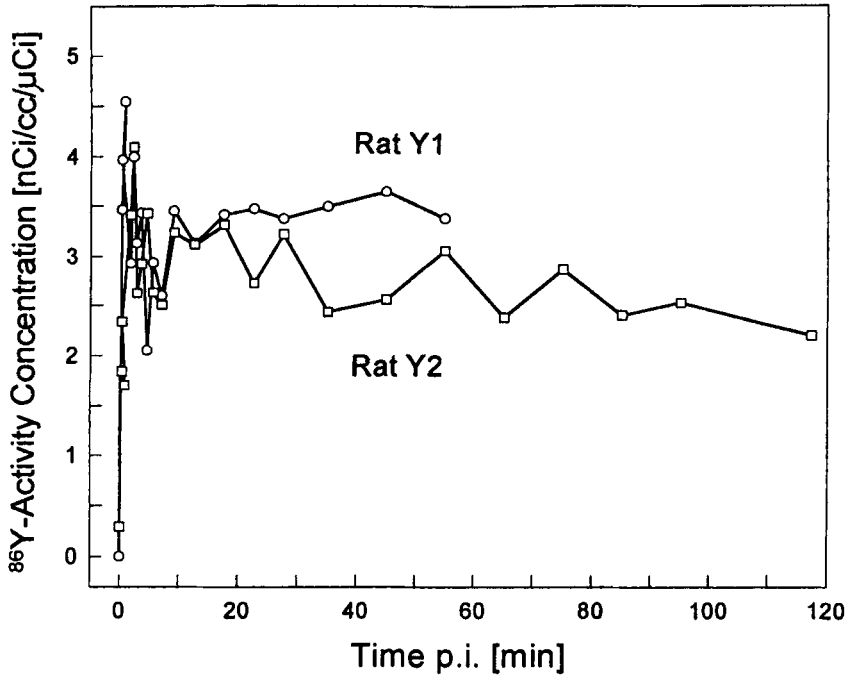

Fig. 1 Tumor uptake kinetics of ^{86}Y-DTPA-octreotide of two male Lewis rats as measured by PET. Data are normalized per one µCi ^{86}Y-DTPA-octreotide injected.

The primary measured absolute ^{86}Y activity concentrations per cm^3 were normalized to one µCi ^{86}Y-DTPA-octreotide injected. For both rats a rapid uptake is observed with maxima of 3.5±0.5 nCi / cm^3 / µCi injected (= 0.35±0.05 % ID / g) at 3.5±0.5 min after injection followed by a slow release of ^{86}Y activity from the tumor with a k_{off} rate of 4.4±0.4 and 2.1±0.2 10^{-5} s^{-1} for the two PET rats.

The uptake kinetics of ^{86}Y-DTPA-octreotide in the tumor, the liver, and the bladder is shown in Fig. 2. There is an increase in ^{86}Y activity concentrations for the liver and the bladder with values being much higher han those for the tumor. The PET data agree with the results of the biodistribution measurements, which are (in % ID / g at 120 after injection, n = 3) 0.39±0.05 for the tumor, 0.76±0.01 for the liver, 0.98±0.16 for the kidneys, and 1.59±0.14 for the bone, for example.

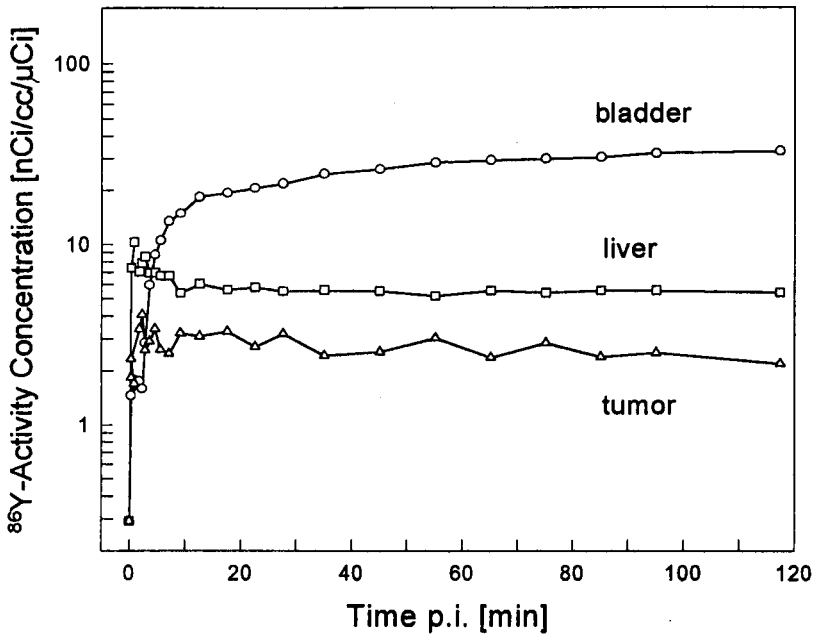

Fig. 2 Uptake kinetics of ^{86}Y-DTPA-octreotide in tumor, liver and bladder of rat Y2 as measured simultaneously by PET. Note logarithmic scale.

Using the uptake kinetics of ^{86}Y-DTPA-octreotide as measured by PET, radiation doses for ^{90}Y-DTPA-octreotide were calculated according to the MIRD recommendations (2). To simplify the dose calculation, only the contribution of the β^--radiation of ^{90}Y (mean energy of 0.934 MeV) and a specific absorbed dose fraction of 1 for the two organs was considered. The results, given as mGy per MBq ^{90}Y-DTPA-octreotide injected, for the tumor and the liver are 6.8 and 11.3 for rat Y1 and 3.2 and 13.5 for rat Y2.

In conclusion, the approach to use ^{86}Y-labelled octreotides and PET to determine individual radiation doses in vivo seems to be a useful tool to evaluate the radiation dosimetry of potential ^{90}Y-labelled octreotide endotherapeutics in patients. Furthermore, it can be used to evaluate chemically different ^{90}Y-chelator/linker-octreotides and finally, to estimate the dose of a given ^{90}Y-labelled octreotide per individual patient.

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Evaluation of Some Direct Labeling Technologies of Tc-99m Monoclonal Antibody in Effiency and Targeting

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This study was aimed to evaluate some different methodologies (1) (2) (3) for Tc-99m labelling of monoclonal antibody can affect labelling efficiency and scintigraphic characteristics. The effects of antibody reduction and transition weak ligand used on labelling efficiency and the availibility of using Tc-99m-antibody labelled without 2-mercapto-ethanol pretreatment in scintigraphy were tested. The ior-ceal, hig and home-developed monoclonal antibody 1A5 were used. 2-Mercapto-ethanol in 0.05 M PBS solution (pH 7.4) was added to antibody PBS solution with 2-mercaptoethanol:antibody ratio of 1000:1, swirled and incubated for 30 min at room temperature (20° C) in nitrogen atmosphere. The reduced antibody was purified on Sephadex G-25 column (1.5x7 cm), previously equilibrated with 0.1% BSA, PBS rinsed, and eluted with 0.05 M PBS containing 1 mM 2-mercaptoethanol monitoring by UV detector and with nitrogen purged. The protein containing fractions were purged with nitrogen and after being sampled to measure its concentration divided into aliquots frozen immediately at -70° C stored ready for use. A domestic MDP kit vial (containing 5 mg $SnCl_2$ and 5 mg MDP) was reconstituted with 0.05 ml nitrogen purged saline and 20 μl was added to every 0.2 mg reduced (unreduced) antibody (in 0.05 M PBS). Tc-99m-pertechnetate solution of 74 MBq(2 mCi) each was added, made equal volume with saline, swirled and after 30 min at room temperature (20° C) purified over Sephadex G-50 using saline as eluant. Labelling efficiency was analyzed by paper chromatography using saline as mobile phase. The labelling efficiencies obtained from reduced antibody were evidently higher than that from unreduced antibody ($P < 0.01$). The tartrate solution reconstituted from a kit vial (containing 2 mg $SnCl_2$ and 20 mg tartaric acid) with 0.2 ml purged saline solution made by dissolving 5 mg $SnCl_2$ and 50 mg citric acid in 5 ml purged saline and $SnCl_2$ solution without ligand (as blank) used in parallel with the above MDP solution. Each solution containing same amount of stannous ion (20 μg) was added to 0.15 mg of above reduced antibody. 74 MBq(2 mCi) Tc-99m-pertechnetate was added and made equal volume with saline. After 30 min at room temperature (20° C) samples were analyzed. The results showed that the weak ligand had very significant effect over blank on labelling efficiency and MDP was the best among the three ligands. The domestic-developed

anti-lung cancer monoclonal antibody LC-1 fragment Fab and anti-melanoma monoclonal antibody Ng76(used as control) were used to evaluate scintigraphic application of Tc-99m-MoAb labelled without 2-mercaptoethanol pretreatment. 200 μ l of MDP solution containing 100 μ g stannous ion was added separately to 2 mg of LC-1 Fab and Ng76 in 0.05 M saline solution. About 740 MBq(20 mCi) of Tc-99m-pertechnetate was added and after 30 min at room temperature (20° C) the labelling efficiency was ~ 85%. The labelled antibodies were i.v. administrated separately to lung cancer bearing BALB/C nude mice with dose of 15 μ l(containing 80 MBq)/each. Biodistributioin and scintigraphic imaging of animals were performed at 24 hr post injection. The distribution of LC-1 fragment had high tumour uptake over other tissues except kidney and bone. The target/nontarget ratios were 14± 7.0; 13± 6.7; 9.8± 8.6; 4.2± 2.3; 2.5± 1.1; 1.1± 0.9; 1.3± 1.2 for muscle, blood, heart, lung, spleen, liver and stomach. The animal imaging also exhibit good tumour concentration. While as control monoclonal antibody Ng76 had different distributioin and no significant tumour concentration. The T/N ratios for greater part of organs were less than 1.

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Electrochemical reduction of ^{188}Re for protein labelling

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As the reduction of ^{188}Re(VII) for antibody labelling employs a vast excess of stannous chloride which often results in dimininshed biological activity, electrochemical reduction was investigated as alternative method for ^{188}Re(VII) reduction.

The "H" type electrochemical cell with tungsten cathode and platinum anode was constructed, and 7 M HCl was used as supporting electrolyte. Reduction of $^{188}ReO_4^-$ was conducted at 25 - 35 V (I = 0.05 - 0.5 A, current density on cathode $4x10^{-3}$ - $4x10^{-2}$ A/cm2) for 15 min. Ascending paper chromatography on Whatman DE81 paper in 7 M HCl at 4°C confirmed that 75% of ^{188}Re was reduced to 5+ oxidation state [1].

Three methods of recovery of ^{188}Re(V) from 7 M HCl media in the form suitable for direct labelling of proteins were investigated: I - evaporation of HCl followed by addition of 0.5 - 1.0 M sodium gluconate or citrate solution at pH = 5 - 7; II - extraction of ^{188}Re into the acetylacetone phase followed by re-extraction into sodium gluconate or acetate solution; III - formation of ^{188}Re(V)-KCNS complex, extraction of the resultant complex into acetylacetone–chloroform phase, evaporation of chloroform under nitrogen and suspension of residue into sodium gluconate or acetate solutions. In each case the percentage of ^{188}Re(V) complexed was determined by ITLC - SG with acetone and saline as mobile phases.

A typical procedure of radiolabelling the DD 3B6/22 murine antifibrin antibody involved exposing Fab' fragments (0.9 mg/ml) in 0.5 - 1.0 M sodium acetate or gluconate solution at pH = 5 - 7 to the equal volume of ^{188}Re(V) complexes described above. Reactions were conducted at various temperatures (4-37°C) and time intervals (15 - 90 min). Size exclusion chromatography (P6-DG column; 1.0 x 2.5 cm; eluent 0.5 M sodium acetate) was used to separate the radiolabelled protein from unreacted ^{188}Re.

^{188}Re(V) recovered from 7 M HCl for each method I - III and complexation are summarised in

Table 1. Methods I - III provide good extraction of electrochemically reduced ^{188}Re(V) from 7 M HCl media into aqueous solutions of chelating agents. As direct labelling of Fab' fragments involves transchelation of ^{188}Re(V), high complexation of ^{188}Re(V) with gluconate or citrate is mandatory for labelling. Methods II and III provide relatively high complexation of ^{188}Re(V). Lower percentage of complex formation for method I may be explained by re-oxidation of ^{188}Re(V) to ^{188}Re(VII) during evaporation procedure.

Table 1 Extraction into aqueous phase and complexation of electrochemically reduced ^{188}Re(V)

Method	^{188}Re in aqueous solution, %	^{188}Re(V) complexed, %
I	80	15
II	45	80
III	70	50

The labelling of the reduced Fab' fragments of DD 3B6/22 monoclonal antibody with electrochemically reduced ^{188}Re have not been successful due to instability of ^{188}Re(V) and its complexes in the absence of reducing agent. However, we believe that electrochemically reduced ^{188}Re may play an important role in protein labelling with bifunctional chelating agents, and the work with developed *in house* bifunctional chelating agent is currently ongoing.

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Labelling of proteins with ^{166}Ho

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The physical properties of ^{166}Ho make it ideal for radiotherapeutic applications - high energy beta rays (E_{av} = 666 keV); low intensity and low energy suitable for imaging γ-rays (80.5 keV, 6%); 26.4 h half-life and stable daughter ^{166}Er. Non-carrier free ^{166}Ho may be produced in a nuclear reactor by ^{165}Ho [n,γ] reaction. Recently it has been shown that ^{166}Ho [1] can be obtained in carrier-free form by separation from its parent ^{166}Dy employing reverse phase ion exchange chromatography on Aminex-A5 HPLC column and α-HIBA (α-hydroxyisobutyric acid) as eluent. The availability of carrier-free ^{166}Ho make it an attractive nuclide for labelling of proteins.

Production of ^{166}Ho

$^{164}Dy_2O_3$ (2.8 mg) was irradiated in the ANSTO reactor (5.0 x 10^{13} $n.s^{-1}.cm^{-2}$) for 120 h and "cooled" for 2 days allowing short-lived ^{165}Dy to decay to ^{165}Ho. The target was digested in 9 M HCl (1 ml), evaporated to dryness and taken up in of 0.01 M HNO_3 (500 µl). This was followed by separation of ^{166}Ho from ^{166}Dy on commercially available Alltech HEMA-IEC BIO 1000 sulphobutyl column (4.6 x 250 mm, p=1600 psi) and 0.085 M α-HIBA as eluent. In a similar manner to that described elsewhere [1], the amount of ^{165}Ho in this preparation was determined by titrating with Arsenazo-III (2,2'-[1,8-Dihydroxy-3,6-disulfo-2,7-naphthalene-bis(azo)dibenzenearsonic acid), and monitoring UV absorbance of the Ho - Arsenazo-III complex formed. Results indicated that ~2.8 x 10^{-2} mg (1.7 x 10^{-7} M) of ^{165}Ho was present per 13-15 mCi of ^{166}Ho (preparation A, ~0.54 mCi/µg of ^{165}Ho). The remaining ^{166}Dy was eluted from the column, and after 2.5 days the ^{166}Ho /^{166}Dy mixture was reloaded on the column for separation. This separation provided ~ 4.5 - 5 mCi of the desired carrier-free ^{166}Ho (preparation B). As no measurable amount (>0.6 µg) of ^{165}Ho was detected in preparation B, specific activity

was calculated to be ~8 mCi/μg of ^{165}Ho. Purified from α-HIBA, carrier-free ^{166}Ho was resuspended in 0.1 M sodium acetate buffer (pH=4.0 - 5.5; 200 - 300μl). Non-carrier free ^{166}Ho was produced by irradiation of $^{165}Ho_2O_3$ (2 mg) in the reactor for 1 h to yield $6.7x10^{-3}$ mCi/μg of ^{165}Ho (preparation C).

Labelling of human serum albumin (HSA) with ^{166}Ho

Diethylenetriaminepentaacetic acid (DTPA) anhydride in dimethylsulfoxide (DMSO) (10-40 μl) was added to HSA (1 ml, 10 mg/ml) in $NaHCO_3$ buffer (pH=8.5, 0.05 M), so the molar excess of DTPA anhydride to HSA varied from 1 to 300. The mixture was shaken and allowed to react for 10 min, concentrated by Centricon-30 microconcentrator followed by washing with 0.1 M sodium acetate buffer (pH=4.0, 4 x 1 ml). Final concentration of DTPA-HSA conjugate was ~50 mg/ml. Varied volumes of ^{166}Ho solution (15 - 200 μl) in sodium acetate buffer (pH=4.0, 0.1 M) were added to the DTPA-HSA conjugate and left to react for 10 min. The ^{166}Ho-DTPA-HSA product was purified on Sephadex G-25 column (15 cm x 0.7 cm; eluent PBS). Non-specific binding was determined by addition of ^{166}Ho (15 - 200 μl) to HSA in the absence of DTPA anhydride. As shown in Fig.1, the ratio of specific activity (Sp Act) of ^{166}Ho-DTPA-HSA to specific activity of ^{166}Ho-HSA is a function of the protein to anhydride molar ratio.

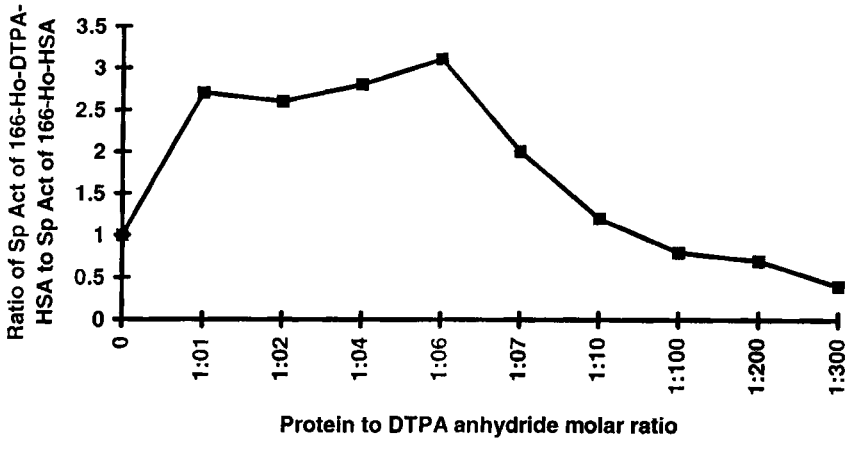

Fig. 1 Normalised specific activity of ^{166}Ho -DTPA-HSA versus protein to anhydride molar ratio

The same dependence of relative coupling efficiency at a fixed protein concentration upon the protein to anhydride molar ratio has been observed elsewhere [2]. Due to rapid hydrolysis of Ho

at pH > 6, eluents at pH=4.5 - 5.5 (e.g. 0.1 M sodium acetate) were found to be more suitable than PBS for purification of ^{166}Ho-DTPA-HSA from unconjugated Ho using size exclusion chromatography.

Labelling of monoclonal antibodies with ^{166}Ho

The same method to that described above was employed to label the murine antifibrin DD-3B6/22 monoclonal antibody (Fab'_2 fragments, supplied by Dr.P.Bundesen, AGEN Biomedical Ltd, Brisbane, Qld, Australia). Only 1:1 molar ratio of Fab'_2 to DTPA anhydride was used for conjugation. Table 1 presents labelling yields using different preparations of ^{166}Ho.

Table 1. Labelling efficiency of DTPA-Fab'_2 conjugate* with various preparations of ^{166}Ho

^{166}Ho preparation	[^{165}Ho], M	Radiolabelling yield, %
A	1.7×10^{-7}	1
B	no ^{165}Ho 3.3×10^{-11} M ^{166}Ho	12
C	5.5×10^{-6}	< 1

Using carrier-free ^{166}Ho in 0.1 M sodium acetate buffer at pH=4.5, 12% labelling efficiency of DTPA-Fab'_2 fragments was achieved in 30 min at room temperature (non-specific binding 3%). Specific activity of final product ^{166}Ho-DTPA-Fab'_2 was 2.4 mCi/mg. Radioimmunoassay was performed using Immulon-4 Dividastrips (Dynatech Laboratories) coated with human D-Dimer. Immunoreactive fraction of labelled species was 64%.

This study clearly demonstrates the advantage of using carrier-free ^{166}Ho for protein labeling in comparison with non-carrier free. Considerably higher labelling efficiency and specific activity are achieved using relatively lower amounts of activity, emphasising important role of carrier-free ^{166}Ho in radioimmunotherapy.

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*Starting amount of Fab'_2 1.84×10^{-9} M

BIOKINETICS OF MONOCLONAL ANTIBODIES LABELLED WITH RADIO-LANTHANIDES AND 225-AC IN XENOGRAFTED NUDE MICE:

PRELIMINARY RESULTS

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INTRODUCTION Monoclonal antibodies have found wide nuclear-medical application as vehicles to carry radionuclides to tumor cells. Unfortunately the number of available radionuclides, and thus the diversity of types and energy of radiation, are limited. Further, commercial available radiotracers often contain significant amounts of inactive components impairing the labelling procedure. Here we demonstrate that lanthanide radioisotopes can be introduced into appropriate chelator-antibody conjugates under very mild conditions. The biokinetic behavior of radiolabelled immunoconjugates was compared with that of the corresponding ^{111}In derivative. In-labelled bioconjugates have a well understood biokinetic behavior and are commonly used as radio-pharmaceutical (Abdel-Nabi and Doerr 1993).

EXPERIMENTAL: Radio isotopes: Carrier free preparations of ^{153}Gd, ^{169}Yb, ^{167}Tm, ^{141}Ce, ^{225}Ac were produced using the on-line isotope separator facility ISOLDE at CERN (E.Kugler et al. 1992). In unspecific nuclear interaction induced by the 1 GeV pulsed proton beam of the CERN PS Booster (spallation, fission and fragmentation) a very complex mixture of nuclear reaction products is formed. After the mass separation process in most cases an additional radiochemical purification step (isobaric separation) is required. Cation-exchange chromatography was applied using α-HIB (α-HIB = α-hydroxyisobutyric acid) as eluting agent. For the production of ^{225}Ac and ^{141}Ce a 55 g/cm^2 thick U-carbide target was used, the other rare earth isotopes were produced from a 220 g/cm^2 Ta-foil target. The hot surface ionisation ion source provides selective ionisation of radioisotopes of group I, II and IIIb elements. From the primary obtained ^{225}Ra a small radionuclide generator based on a cation-exchange column (Aminex A5) was build. From the Aminex-A5 column pure ^{225}Ac can be eluted using 1 M α-HIB, while the ^{225}Ra remains on the column. The α-HIB used in all radiochemical purification steps was removed by evaporation and the remaining radioisotopes were redissolved in 10 - 50 µL of 0.05 M HCl. The obtained preparations were carrierfree and isotopically clean. ^{111}In was purchased from Amersham.

Preparation and labelling of the chelator-antibody conjugate: Aminobenzyl-DTPA, in which the DTPA is derivatized on the carbon backbone so as to retain the highest metal-ion affinity (Brechbiel et al., 1986), was converted to its aminooxyacetyl derivative. It was then site specifically conjugated to the murine anti-CEA IgG1 monoclonal antibody MAB 35 by oxime formation with aldehyde groups introduced on the antibody oligosacharides by mild periodate oxidation (Pochon et al. 1989). The chelator-antibody conjugate was then labelled by 2 h incubation with the radio nuclides in acetate solution. The labelled protein was isolated by gel filtration.

Biokinetic studies: Antibody-chelator conjugates labelled with different radionuclides were injected simultaneously into the tail vein of nude mice bearing xenografted colorectal tumors. At various times, mice were sacrified and dissected. The radionuclide content of the organs (expressed in % i.d./g tissue) was analyzed by gamma ray spectroscopy.

RESULTS: Labelling yields: All rare earth elements as well as Ac could clearly be introduced into the protein fraction with labelling yields between 75 and 95 %.

Biokinetic studies: The comparison of the biokinetics of some lanthanides and indium is summerized in Fig.1 - 3. Fig.1 shows the biokinetics for ^{153}Gd labelled bioconjugates. The simultanously measured biokinetics for ^{111}In labelled antibodies (not presented in the figure) showed

exactly the same behaviour except for the liver, were the uptake was slightly smaller compared to Gd. Fig. 2 demonstrates that the blood clearance for the antibodies labelled with different lanthanides and In is practically identical, while in case of Ac a significant faster clearance is observed indicating a low in-vivo stability. The simultanously measured tumor uptake (Fig.3) confirms this findings, similar uptakes for the rare earth and In labelled bioconjugates and satisfactory in-vivo stability and a fast tumor clearance in case of a Ac-labelled antibodies. Only small differences in the uptake of lanthanides and Indium in other organs (liver, kidney, femur) have been measured.

An increase of DTPA groups bound per antibody up to 10 did not affect the biodistribution.

CONCLUSION: Radio-lanthanides can form radioimmunoconjugates showing an in vivo behaviour comparable to clinically used ^{111}In immunoconjugates. Consequently the complete range of lanthanide radio-isotopes can be used in radioimmunoconjugates. Thus we have access to all types of radiations with a wide range of energy and half life, including β^+-emmitters which can be used for in-vivo dosimetry with PET and β^-- and α-emitters for radio-immuno therapy.

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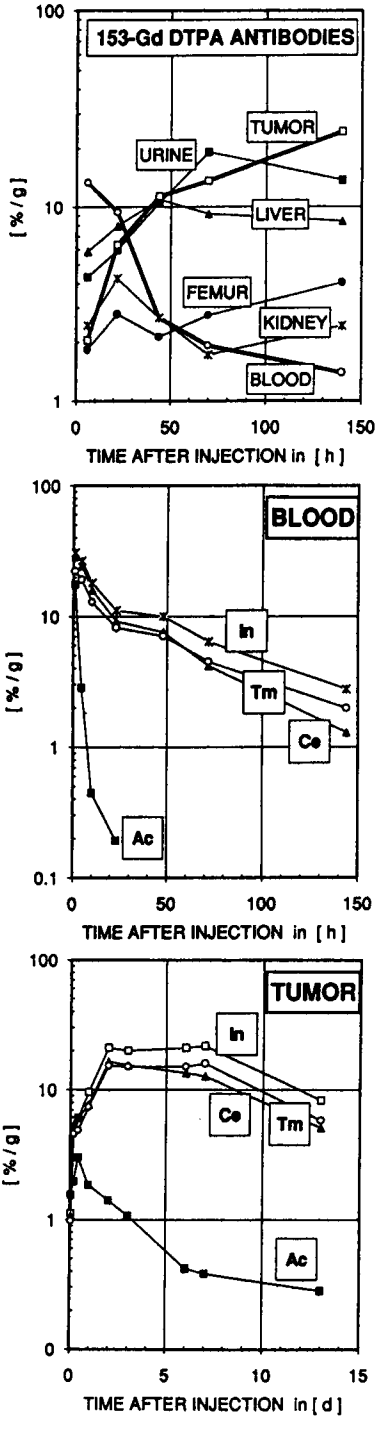
LEGENDS

Fig.1 Biokinetics for monoclonal antibodies labelled with ^{153}Gd. The simultanously measured ^{111}In bio-distribution (not shown in the fig.) is practically identically.

Fig.2 Blood clearance of monoclonal antibodies labelled with radio-lanthanides and ^{225}Ac (produced at CERN ISOLDE) and ^{111}In (purchased from Amersham) in tumor bearing nude mice

Fig.3 Tumor uptake of monoclonal antibodies labeled with ^{153}Gd, ^{169}Yb, ^{167}Tm, ^{141}Ce and ^{225}Ac (carrier free, produced at CERN ISOLDE) and ^{111}In (purchased from Amersham) in tumor bearing nude mice.



THE DIRECT LABELLING OF IgG ANTIBODY WITH Tc-99m USING FSA AS A REDUCING AGENT FOR PRETREATING

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As an alternative to dithiothreitol, 2-mercaptoethanol, etc. reductants(1),We have recently reported a modification of the direct Tc-99m labelling method of IgG antibody(2). In this paper, we report an improved method using FSA as a reducing agent for pretreating and using $SnCl_2$ as a reducing agent for the labelling of IgG antibody with Tc-99m. We have study on the effect of pH, temperature, buffer, FSA amount etc. on the labelling yieled of IgG antibodies using FSA for pretreating.

The antibody was first changed from buffer into saline and concentrated to about 10 mg/ml by ultra-filtration. The IgG antibody (2mg)in saline was pretreated by reaction with a appropriated buffer solution and FSA as a reducing agents at 39℃ for 2hr and then adding appropriated $SnCl_2$. $^{99m}TcO^-_4$ for labelling at room temperature. The results are sumarized in Tables 1-2.

Radiochemical purity and in vitro stability were measured by a combination of ITLC. TCA and stable labeling methods. Two develop systems in acetone and in methanol: ammonia acetate (10% in water)=1:1(v/v)were used for the ITLS. 0.003M EDTA. phosphate buffered saline and the fresh cysteine solution of a different concentration were prepared for Transchelation Challenge Test.

Table 3 is the results on frozen test of IgG kit. The results for the assay of immunoreactivity of the reduced and labeled antibody(MAb170) are shown in Table 4.

In conclusion, FSA is a mild reducing agent for IgG antibodies pretreating which may be of value in keeping immunoreactivity of IgG antibodies(3). It could be split for disulfide bone in IgG antibodies and it is not necessary to remove from the reduced IgG antibodies before the IgG antibodies are reacted with Tc-99m. The results suggest that the reported method provides a direct and efficient means of labeling IgG antibody with Tc-99m and offers the possibility of performing labeling by a kit.

TABLE 1. IgG LABELING WITH Tc-99m USING FSA+$SnCl_2$ METHOD

No	Labeling	TLC-A % bound	TLC-M.N % bound	TCA % bound
1	Tc-99m	99.6	97.2	97.4
2	Tc-99m	99.7	97.2	96.8

* A=acetone M.N=methanol: ammonia acetate

TABLE 2. MAb170 LABELING WITH Tc-99m USING FSA+$SnCl_2$ METHOD

No	Labeling	TLC-A % bound	TLC-M.N % bound	TCA % bound
1	Tc-99m	99.6	96.1	96.6
2	Tc-99m	99.7	91.7	90.8

TABLE 3. FROZEN TEST FOR IgG KIT USING FSA+$SnCl_2$ METHOD

Analysis	Fresh	Frozen (-70°) % bound		
	(25℃)% bound	6d	8d	49d
TLC-A	98.9	99.3	99.5	98.9
TLC-M.N	94.5	92.0	97.1	95.8
TCA	93.8	97.5	94.2	97.7

* Fresh--new preparations

Frozen--keep kits in cold storage at-70℃ for several days

TABLE 4. THE ASSAY OF IMMUNOREACT IVITY OF THE REDUCED AND Tc-99m LABELED ANTIBODY (MAb170)

Sample conc.ng	STD 170 % INHIB	#1 % INHIB	#2 % INHIB
40	92.19	94.29	94.07
20	83.39	87.33	87.33
10	65.54	73.34	73.53
5	45.93	55.15	52.22
2.5	24.58	26.39	32.31

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Re-188 labelling of DD-3B6/22 Fab' Antibody for Radioimmunotherapy.

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Rhenium-188 (γ =155 keV (15 %); β^- = E_{max} 2.1 MeV and $t_{1/2}$ 17 hrs) can be obtained in a carrier-free and salt-free form from the $^{188}W/^{188}Re$ generator [1]. Positive results in a pilot clinical trial of ^{99m}Tc-DD-3B6/22 Fab' for the diagnosis of ovarian cancer encouraged the development of the ^{188}Re-DD-3B6/22 Fab' - a matched pair of radioimmunoconjugates for diagnosis and therapy. As its coordination chemistry is similar to that of ^{99m}Tc, similar radiolabelling techniques for ^{99m}Tc-DD-3B6/22 Fab' were employed for direct labelling with ^{188}Re. The *in vitro* and *in vivo* stability as well as the biodistribution of the ^{188}Re-DD-3B6/22 Fab' in nude mice are reported.

Rhenium-188 was eluted from a $^{188}W/^{188}Re$ generator with 0.05 M NH_4NO_3 and the eluent evaporated to dryness. The residual ^{188}Re was dissolved in a range of buffers - 0.2 M acetate (pH 4.0 - 5.5), 0.2 M phosphate (pH 5.0-6.0) and 0.2 M sodium carbonate (pH 6.5-8.5). Excess $SnCl_2$ (2 mg/ml) was used to reduce the ReO_4^- - Re(VII) to its Re(V) for complexation at various pH in sodium gluconate (0.5 M) or sodium citrate (0.5 M) in the presence and absence of an inert atmosphere. Percent complexation (confirmed by ITLC-SG mobile phase acetone; ReO_4^- : R_f = 0.95; Re(complex) : Rf = 0.05) in each case is illustrated in Fig. 1., 2. and 3. Comparing the effect of gluconate complexation at various pHs (4.0 - 8.5) in various buffers (Fig. 1.) shows complexation decreases rapidly with increasing pH. An inert atmosphere was essential for this reduction reaction as Re(V) readily oxidises to Re(VII) in air. Optimum complexation of ≈ 75 % was achieved at pH 4.0 - 4.5 in 90 min at 37°C for both citrate and gluconate.

The DD-3B6/22 Fab' was produced by the reduction of $F(ab')_2$ with dithiothreitol (DTT) at 37°C for 30 min. The ^{188}Re(V) complexes of gluconate and citrate formed *in situ* were incubated for 1 h at 37°C with the purified (by size exclusion chromatography) Fab'. Transchelation of the ^{188}Re onto the endogenous thiol groups of the Fab' was found to be 20 times more effective for the ^{188}Re complexes of gluconate than that of citrate. Specific activity of final product was 2.9 mCi/mg with an immunoreactive fraction of 77%. The concentration of $SnCl_2$ was increased (2.0 - 5.0 mg/ml) in an effort to increase ^{188}Re(V) available for transchelation, however immunoreactivity

was severely compromised (IRF = 37% at 5mg/ml). *In vitro* stability of the ^{188}Re-DD-3B6/22 Fab' at 4°C in PBS pH 7.2 was assessed by HPLC over a 48 h time period (see Fig. 4.). Result show upto 30% of the ^{188}Re was lost from the Fab' fragment within 24 h. The rate of ^{188}Re lost from the protein was considerably reduced after 24 h.

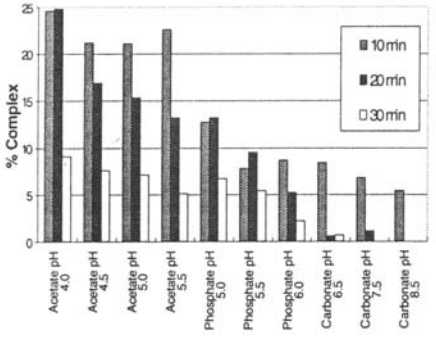

Fig. 1. Effect of pH on gluconate complexation of ^{188}Re in various buffers[a]

[a] at 37°C in the absence of nitrogen.

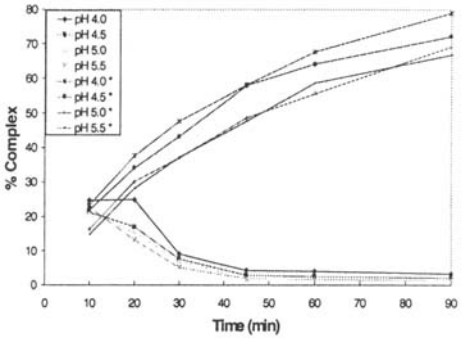

Fig. 2. Effect of pH on gluconate complexation of ^{188}Re[a]

[a] 0.2 M sodium acetate at 37°C. *under nitrogen.

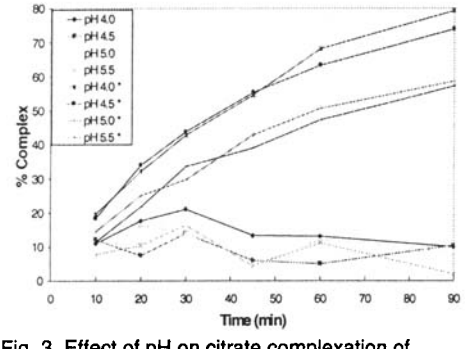

Fig. 3. Effect of pH on citrate complexation of ^{188}Re[a]

[a] 0.2 M sodium acetate at 37°C. *under nitrogen.

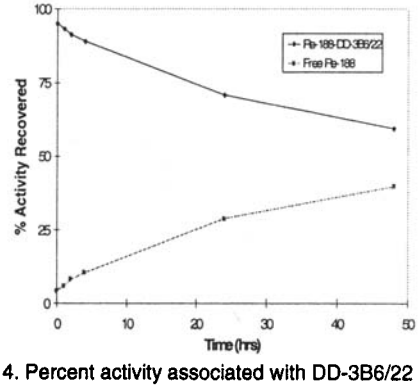

Fig. 4. Percent activity associated with DD-3B6/22 Fab' when store at 4°C.

As the DD-3B6/22 antibody only recognises the cross-linked fibrin of humans and primates, a *pseudo* animal model was established. Pharmacokinetics of the final product was evaluated in nude mice transplanted with both D-dimer (antigen +ve) and glycine (antigen -ve) sepharose beads in the left and right hind flanks, respectively. Results (see Table.1 and Fig. 5. and 6.) show that ^{188}Re-DD-3B6/22 Fab' clears rapidly from the blood (α phase = 1.1 $\pm$ 0.1 h; β phase = 5.7 $\pm$ 0.6 h) and is excreted through the renal system.

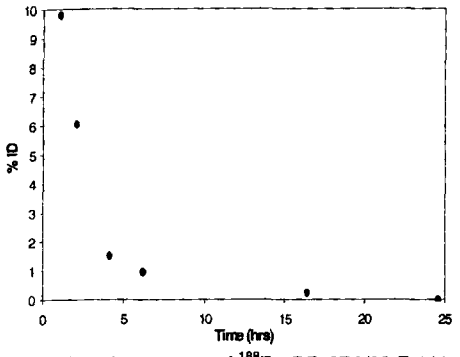

Fig. 5. Blood clearance of ^{188}Re-DD-3B6/22 Fab' in nude mice.

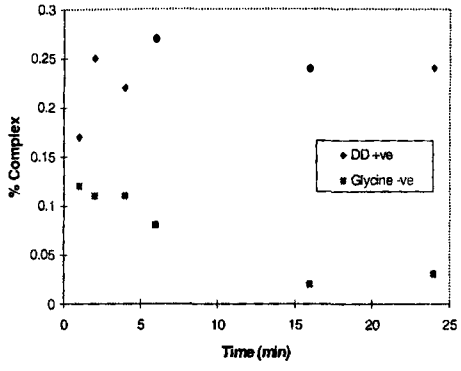

Fig. 6. Localisation to D-dimer (antigen +ve) and glycine (antigen -ve) sepharose beads in nude mice.

Table 1. Biodistribution of ^{188}Re-DD-3B6/22 Fab' in nude mice

% ID/G												
TIME	1 h		2 h		4 h		6 h		16 h		24 h	
ORGAN	MEAN	S.D.	MEAN	S.D	MEAN	S.D	MEAN	S.D	MEAN	S.D	MEAN	S.D
LIVER	2.36	0.82	2.46	0.25	1.06	0.14	0.73	0.04	0.22	0.02	0.11	0.01
SPLEEN	1.52	0.57	1.49	0.22	0.74	0.15	0.52	0.13	0.28	0.05	0.15	0.04
KIDNEY	108.03	37.32	124.32	14.54	73.67	16.72	42.87	5.87	8.86	0.9	5.54	1.09
BONE	1.84	0.76	1.65	0.31	0.58	0.17	0.51	0.09	0.23	0.06	0.14	0.04
LUNGS	3.81	1.15	8.69	4.54	12.84	16.99	1.24	0.62	3.02	1.71	0.2	0.12
HEART	2.91	0.76	1.97	0.28	0.61	0.21	0.39	0.06	0.18	0.09	0.06	0.07
BLOOD	8.12	2.91	5.66	2.37	1.56	0.31	0.9	0.13	0.3	0.04	0.11	0.01
BLADDER	43.14	65.65	19.01	21.14	9.26	11.23	6.44	6.29	1.13	0.43	0.39	0.18
STOMACH	3.67	1.56	9.05	4.6	1.31	0.71	0.74	0.1	0.25	0.11	0.13	0.05
SM INT	2.21	0.83	3.25	1.15	0.67	0.16	0.42	0.11	0.11	0.03	0.05	0.01
CAECUM	3.22	2.05	3.04	0.84	0.74	0.16	0.81	0.4	0.16	0.09	0.17	0.04
TARGET												
+ve *	0.17	0.12	0.25	0.14	0.22	0.11	0.27	0.07	0.24	0.04	0.24	0.13
- ve *	0.09	0.11	0.11	0.05	0.11	0.04	0.08	0.02	0.02	0.01	0.03	0.04

* Data represented as %ID.

Localisation to the target was specific, with a ratio +ve : -ve controls of 5:1 ($P < 0.001$). Specificity was achieved within 6 h and maintained for upto 24 h post-injection. The retention of ^{188}Re-DD-3B6/22 Fab' at the target site tends to indicate that the product is sufficiently stable in *in vivo* for the localised ^{188}Re to decay and potentially administer the required dose. Preliminary work to date indicates that therapeutic levels (upto 30 mCi) of ^{188}Re-DD-3B6/22 Fab' are achievable with this antibody confirming its potential use in therapy of ovarian cancer.

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Labeling of Monoclonal Antibodies (MAbs) with *N*-Succinimidyl 3-hydroxy-4-[^{131}I]iodobenzoate (*m*SHIB).

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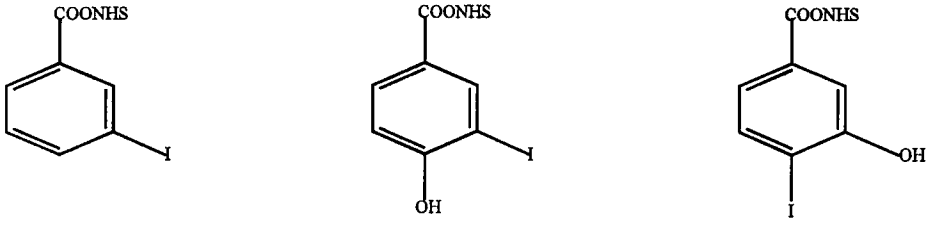
The extensive *in vivo* deiodination observed with directly radioiodinated MAbs generally has been attributed to the presence of an hydroxy group *ortho* to iodine. However, we have shown that the *ortho* hydroxy group does not always result in significant deiodination.[1] This was further substantiated by comparing the biodistribution of a MAb radioiodinated using our prototypical agent, *N*-succinimidyl 3-[^{131}I]iodobenzoate (SIB)[2] with the same MAb radioiodinated with an agent, *N*-succinimidyl 4-hydroxy-3[^{131}I]iodobenzoate, *p*SHIB[3] in which a hydroxy group was introduced *ortho* to the iodine in SIB. The SHIB agent is of particular interest because of its potential adaptability to simple kit formulation. With respect to *in vivo* deiodination, MAb labeled with this agent was only slightly inferior to that labeled with SIB and several-fold better than the directly iodinated MAb. Unfortunately, the coupling yields for *p*SHIB with MAbs was unacceptably low. It was reasoned that this may be due to the presence of a hydroxy group at the *para* position of the carboxylic ester group. To investigate this, an isomeric compound (*m*SHIB) wherein the hydroxy group was moved to the *meta* position was prepared and its potential for radioiodinating MAbs was evaluated.



SIB *p*SHIB *m*SHIB

NHS = *N*-succinimidyl

Chart 1. Chemical structures of *N*-succinimidyl 3-iodobenzoate (SIB), *N*-succinimidyl 4-hydroxy-3-iodobenzoate (*p*SHIB) and *N*-succinimidyl 3-hydroxy-4-iodobenzoate (*m*SHIB).

Meta-hydroxybenzoic acid (MHBA) was radioiodinated using chloramine-T in a 10 min reaction. A yield versus concentration of MHBA showed that a 2 mM concentration of MHBA is necessary to obtain the optimum yield of 70-75% (Figure 1A). The 3-hydroxy-4-[^{131}I]iodobenzoic acid (HIBA) was isolated by reverse-phase HPLC and converted to *m*SHIB in more than 80% radiochemical yield (determined by normal-phase HPLC) by treating it with *N*-hydroxysuccinimide and DCC for 5-10 min. No advantage in yield was obtained by increasing the reaction time up to 1 hr (Figure 1B). MAb 81C6 could be conjugated with HPLC-purified *m*SHIB in 40-60% yield in 20 min; increasing the incubation time upto 1 hr did not result in any significant increase in

conjugation efficiency. In comparison, only 10-15% coupling yield was obtained when *p*SHIB was used.[3] The specific binding of 81C6 labeled with ^{131}I using *m*SHIB to D 54 MG human glioma homogenate was 70 ± 9%. In comparison, the specific binding was 80 ± 7% when 81C6 was labeled with [^{125}I]SIB. The immunoreactivity did not change appreciably when the nuclides were switched. A paired-label biodistribution of 81C6 labeled with [^{131}I]*m*SHIB and with [^{125}I]SIB was determined in normal mice. Generally the uptake of ^{131}I was higher in almost all tissues especially at later time points. Although such a difference in uptake was seen between 81C6 labeled with [^{131}I]*p*SHIB and that with [^{125}I]SIB[3], the magnitude of the difference was much higher in the present case. The thyroid uptake was similar to that observed earlier with *p*SHIB (Figure 1). Initially, the difference in uptake of ^{131}I and ^{125}I was small but statistically significant ($p < 0.005$). With time the difference increased; on the seventh day the ^{131}I uptake was 2.5-fold higher than that of ^{125}I.

In conclusion, *m*SHIB could be prepared in good yields and, as predicted, the yield of coupling to protein was higher with this isomer. The inertness of 81C6 labeled with *m*SHIB to *in vivo* deiodination was similar to the one labeled with *p*SHIB . The usefulness of this labeling agent will be investigated in future tissue distribution experiments in nude mice bearing tumor xenografts. We are currently developing a kit-formulation for the preparation of *m*SHIB.

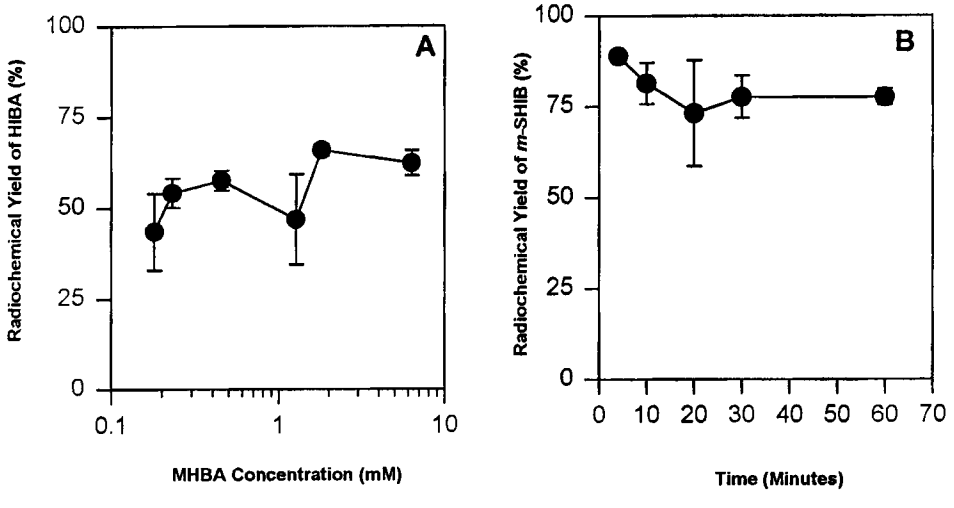


Figure 1. A) Dependence of radiochemical yield of [^{131}I]HIBA production on the concentration of *meta*-hydroxybenzoic acid. B) Yield as a function of time in the formation of *m*[^{131}I]SHIB.

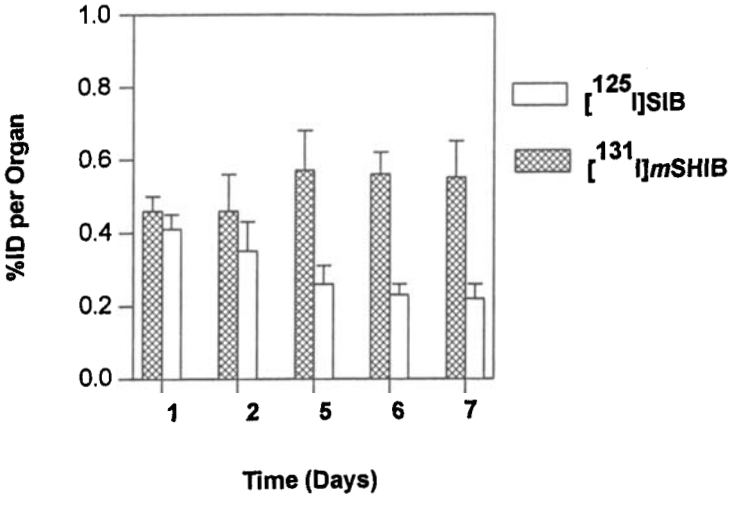


Figure 2. Thyroid uptake in mice following the co-administration of 81C6 labeled with [^{125}I]SIB and with *m*[^{131}I]SHIB.

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Catabolism of Label from an F(ab')$_2$ Fragment Radioiodinated using *N*-Succinimidyl 3-iodobenzoate and Iodogen Methods.

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Understanding of the nature of labeled species generated *in vivo* following administration of labeled monoclonal antibodies (MAbs) could facilitate the design of next-generation radiolabeling methods. In spite of the less than ideal nuclear decay characteristics of radioiodine, the more rapid normal tissue clearance of radioiodine compared to radiometals has resulted in its continued preference for clinical MAb therapy trials. When MAbs are labeled via the direct iodination of their tyrosine residues, high thyroid uptake is observed, providing indirect evidence for *in vivo* dehalogenation (1). This has led to the development of alternative radioiodination approaches using *N*-succinimidyl 3- or 4-iodobenzoate which have resulted in decreased thyroid uptake (2,3) and improved tumor localization (4) in mice. The current study was undertaken to determine the labeled catabolites created following the exposure to tissue *in vitro* and *in vivo* of an F(ab')$_2$ fragment radioiodinated using *N*-succinimidyl 3-iodobenzoate (SIB) and the Iodogen method.

The F(ab')$_2$ of Mel-14, a MAb reactive with a tumor-associated chondroitin sulfate proteoglycan, was labeled with ^{125}I using SIB (4) or the Iodogen method. Balb/c mice were injected with 10 μCi of ^{125}I-labeled F(ab')$_2$ MAb fragment and killed 3 hr later. Tissues of interest were removed, washed with saline and homogenized. After centrifugation, the supernatant was separated and analyzed using size exclusion HPLC performed using a Bio-Sil SEC 250 gel filtration column (Bio-Rad, 600 x 7.5 mm) eluted with PBS at a flow rate of 1 mL/min. Fractions of 0.5 mL were collected and counted using an automated γ-counter. Molecular weight for different peaks was ascertained by running gel filtration molecular weight standards using identical conditions. In addition, liver, spleen, kidneys, blood and urine were collected from another group of Balb/c mice. Tissues were homogenized and incubated *ex vivo* with 10 μCi of ^{125}I-labeled Mel-14 F(ab')$_2$. After incubation @ 37^{0}C for 3 h, homogenates were centrifuged and supernatants were analyzed using size exclusion HPLC as described above. Compounds with molecular weights of less than 10 kD were separated from the supernatants (*in vivo* and *ex vivo* samples) using Centricon-10 cartridges and were analyzed by HPLC using a reverse phase column (C-18, 10μ, 250 x 4.6 mm) eluted in isocratic mode with methanol:water:acetic acid (45:55:0.2) at a flow rate of 1 mL/min. One mL fractions were collected and counted for radioactivity in an automated γ-counter. Cold compounds including iodobenzoic acid (IBA), the glycine conjugate of IBA (IBA-Gly), and the lysine conjugate of IBA (IBA-Lys) were run on HPLC to determine the retention time of these potential catabolic products.

Based on size exclusion HPLC profiles, the distribution of radioactivity in tissues was grouped into four molecular weight (MW) categories: >150 kD, ~100 kD, ~30-70 kD, and <30 kD. As would be anticipated, the largest differences observed between the two labeling methods were in the lowest molecular weight category (Table 1). Two- and seven-fold higher levels of <30 kD species were present in liver and kidney, respectively, in the iodogen samples compared with those obtained from animals injected with F(ab')$_2$ labeled using SIB. In serum samples from SIB group, 87% of supernatant activity was present as the 100kD species (reflecting intact MAb fragment) while for Iodogen group only 33% was present as intact F(ab')$_2$ with 31% present as the ~50kD species (Figure 1).

Reverse-phase HPLC analysis showed a distribution of radioactivity in four major species: iodide, IBA, IBA-Gly, and IBA-Lys. *Ex vivo* incubation of urine with MAb fragment labeled using SIB yielded two major peaks corresponding to iodide and IBA, while *in vivo* urine sample showed the presence of IBA-Lys and IBA-Gly as major peaks, with <3% radioactivity present as iodide (Figure 2). Urine samples from the Iodogen group contained iodide as the predominant peak (>75%) and iodotyrosine as a minor peak. *In vivo* liver samples from the SIB group exhibited a distribution of radioactivity between IBA (30%), IBA-Lys (55%), and IBA-Gly (5%), whereas those from the Iodogen group showed >80% of the radioactivity as iodide with the remainder as the monoiodotyrosine. Unlike other tissues, kidney supernatant from the SIB group included IBA as the major catabolite (Figure 3).

In conclusion, these studies provide direct evidence for the generation of iodide as the predominating catabolic product from a MAb fragment radioiodinated using the Iodogen method. This is consistent with previous speculations that *in vivo* dehalogenation may be the major event in the catabolism of radioiodinated proteins. For MAbs radioiodinated using SIB method, except for the kidneys, IBA-Lys predominates as the catabolic product with minimal radioactivity present as iodide.

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Table 1. Size exclusion HPLC analysis of tissue supernatants from SIB and iodogen groups

Sample		Supernatant Activity (%)			
		>150 kD	~100 kD	~50 kD	<30 kD
Liver					
	SIB	10	13	63	10
	Iodogen	9	9	57	20
Spleen					
	SIB	6	31	29	13
	Iodogen	5	31	32	16
Lungs					
	SIB	2	70	19	1
	Iodogen	3	65	12	13
Kidney					
	SIB	7	15	65	3
	Iodogen	11	11	54	21
Serum					
	SIB	7	87	4	1
	Iodogen	8	33	31	20

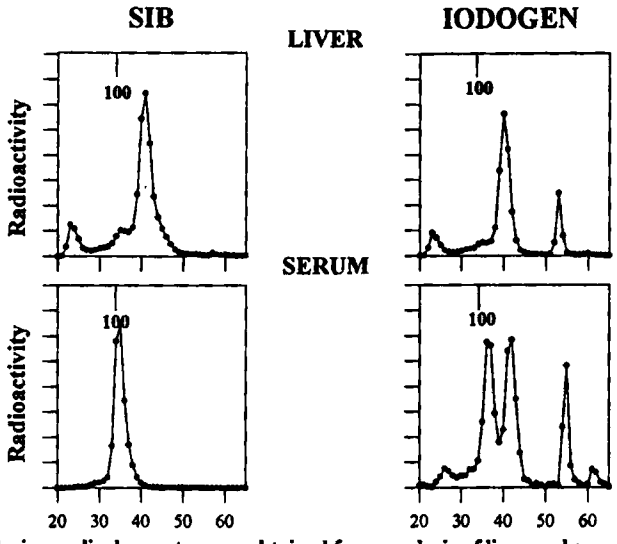

Figure 1. Size exclusion radiochromatogram obtained from analysis of liver and serum. Samples were obtained after injection of Mel-14 F(ab')$_2$ labeled using SIB (left) and iodogen (right).

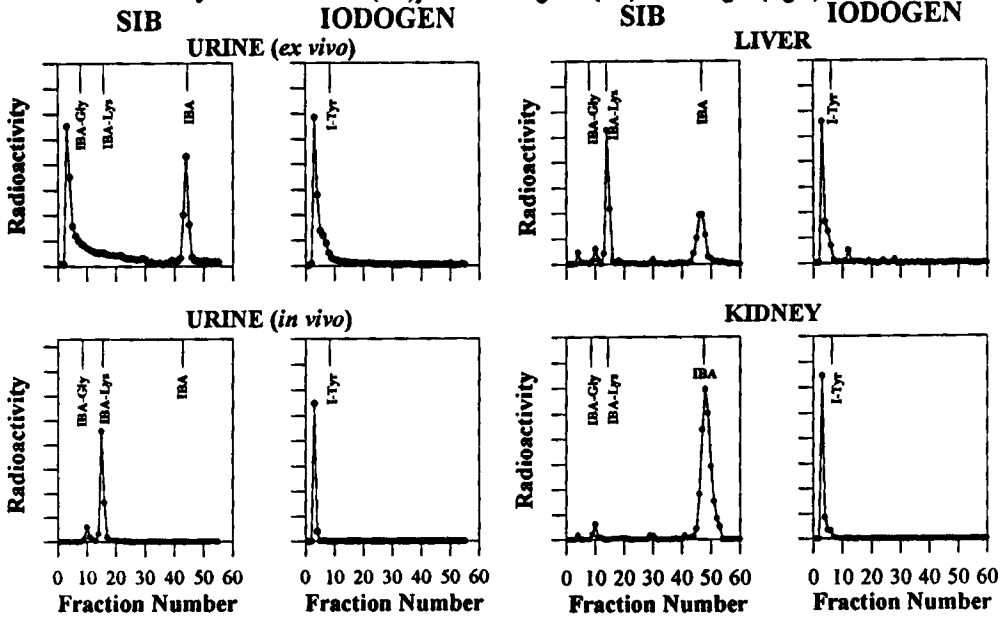

Figure 2. Reverse phase HPLC chromatogram from analysis of urine obtained 3 h after injection of radioiodinated Mel-14 F(ab')$_2$ in normal mice (in vivo) or after 3 h incubation with urine (top).

Figure 3. Reverse phase HPLC chromatogram from analysis of supernatants from liver (top) and kidneys (bottom). Tissues obtained after injection of radioiodinated Mel-14 F(ab')$_2$.

PREPARATION AND *IN VIVO* EVALUATION OF RADIOLABELED LDTPA PROTEIN CONJUGATES.

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The site specific delivery of radioactive metal ions using chelate-biomolecule conjugates has potential medicinal applications in radiodiagnostics, radiotherapy (1), and magnetic resonance imaging (2). The preparation of functionalized chelate derivatives for conjugation, like DTPA (3) and DOTA (4), are generally multi-step procedures with extremely low yields. The notable exception is the DTPA dianhydride which can react with primary amines to form protein conjugates. The major draw back to DTPA dianhydride is substantial cross-linking between the chelate and protein, greatly reducing the yield of protein conjugate. Recently, the high yield synthesis of N,N-bis[2[N',N'-bis(carboxymethyl)amino]ethyl]-4-amino-L-phenylalanine (LDTPA), a bifunctional chelate with one site for protein attachment, was reported (5), but biological data was not given.

We have prepared gram quantities of LDTPA, and converted the aromatic amino group to an isothiocyanate for protein conjugation (Figure 1). This was

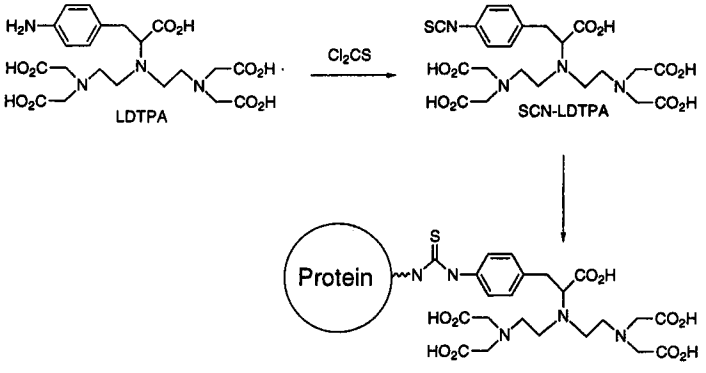

Figure 1

readily achieved by treating LDTPA with thiophosgene (6) and precipitating the product from methanol with dry acetone. The isothiocyanate derivative (SCN-LDTPA) was conjugated to BSA and a series of glycoproteins to evaluate the uptake and clearance of the radiolabeled conjugate. Glycoproteins with

mannose and galactose residues were used, and they are targeted to mannose and asialoglycoproteins receptors, respectively.

The LDTPA BSA and glycoprotein conjugates were radiolabeled with either $^{111}In^{3+}$ or $^{153}Gd^{3+}$, and the biodistributions assayed. The biodistributions of ^{111}In-LDTPA-BSA indicated that the compound was initially circulating in the blood, but then cleared rapidly through the kidneys. ^{111}In-LDTPA-mannose BSA and ^{111}In-LDTPA-galactose BSA showed rapid accumulation in the liver with slow clearance over 24 hours. The ^{111}In-LDTPA-mannose BSA cleared primarily through the kidneys, while ^{111}In-LDTPA-galacose BSA cleared primarily through the lower large intestine.

The biodistributions of ^{153}Gd-LDTPA-BSA was similar to the 111-indium compound, with clearance primarily through the kidneys. The release of $^{153}Gd^{3+}$ from the protein conjugate *in vivo* can be inferred by monitoring the accumulation of radioactivity in the bone. Significantly less bone accumulation of ^{153}Gd was observed with ^{153}Gd-LDTPA-BSA at all time points compared to $^{153}Gd^{3+}$ labeled DTPA dianhydride BSA protein conjugate (7). ^{153}Gd-LDTPA-mannose BSA and ^{153}Gd-LDTPA-galactose BSA both exhibited rapid uptake in the liver followed by slow clearance. Both glycoproteins showed significant accumulation of radioactivity in the bone matrix with time.

The bifunctional chelate LDTPA has been prepared in large quantities and modified for protein conjugation. The conjugation reaction between SCN-LDTPA and the protein showed no cross-linking, offering an advantage for the preparation of $^{111}In^{3+}$ radiopharmaceuticals currently employing DTPA dianhydride. LDTPA-BSA demonstrated a higher affinity for $^{153}Gd^{3+}$ *in vivo* compared to the DTPA dianhydride protein conjugate.

Acknowledgments: This work was supported by Mallinckrodt Medical, Inc.

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Biodistribution and Metabolism of the MAb 1A3 and 1A3-F(ab')$_2$ when Labeled with $^{64/67}$Cu Through Four Different Bifunctional Chelates

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Bifunctional chelating agents (BCAs) are required to complex radiometals to biomolecules such as proteins and peptides. Copper-64 (β^+, $T_{1/2}$ = 12.8 h) has shown potential as a therapeutic and diagnostic radiometal and macrocyclic BCAs are necessary for *in vivo* stability.(1) 1A3 and 1A3-F(ab')$_2$ are anti-colorectal MAbs that have been labeled with ^{64}Cu using the bifunctional chelate, 6-bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (BAT), and evaluated in rat and hamster models.(2) ^{64}Cu-BAT-1A3 and ^{64}Cu-BAT-1A3-F(ab')$_2$ had superior tumor uptake in a hamster model than either the ^{111}In- or ^{125}I-labeled antibodies; however, the kidney uptake of ^{64}Cu-BAT-1A3-F(ab')$_2$ was high. The bifunctional chelate, 4-[(1,4,8,11-tetraazacyclotetradec-1-yl)-methyl]benzoic acid (CPTA), has been previously conjugated to 1A3 and 1A3-F(ab')$_2$, labeled with copper, and compared to the BAT conjugates in both rats and hamsters.(3) In this study, we have synthesized BAT, CPTA, 6-isothiocyanatobenzyl-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (SCN-TETA) and 4-[(1,4,7,10,13-pentaazacyclopentadec-1-yl)-methyl]benzoic acid (PCBA) for conjugation to 1A3 and 1A3-F(ab')$_2$ and radiolabeling with copper isotopes. The four bifunctional chelates (Figure 1), when conjugated to 1A3 and 1A3-F(ab')$_2$ and labeled with ^{64}Cu, are compared with respect to their biodistribution in rats and hamsters. The metabolism of the SCN-TETA, CPTA, and PCBA conjugates in Sprague-Dawley rats will also be discussed.

The four bifunctional chelates were conjugated to 1A3 and 1A3-F(ab')$_2$, labeled with ^{64}Cu, and evaluated in normal Sprague-Dawley rats and Golden Syrian hamsters implanted with GW39 human colon cancer tumors. The rat and

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hamster biodistribution data for all of the ^{64}Cu labeled 1A3 conjugates were comparable, except for a significantly lower uptake in the liver and kidneys of the BAT conjugate (data not shown). The biodistribution data of the ^{64}Cu labeled 1A3-F(ab')$_2$ conjugates at 24 hours post-injection are presented in Tables 1 and 2. The tumor uptake for all of the conjugates are similar; however, both rats and hamsters show a large kidney uptake. The kidney uptake for the CPTA and PCBA conjugates are significantly greater than the kidney uptake for the BAT and SCN-TETA conjugates in rats and hamsters. Also, the SCN-TETA conjugate has a significantly lower kidney uptake in the hamster kidneys than the BAT conjugate.

Figure 1

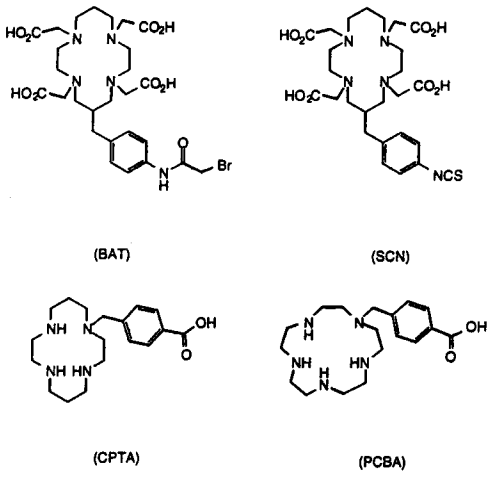

Table 1: Biodistribution in Golden Syrian Hamsters (%ID/g)

organ	^{64}Cu-BAT-1A3-F(ab')$_2$	^{64}Cu-SCN-TETA-1A3-F(ab')$_2$	^{64}Cu-CPTA-1A3-F(ab')$_2$	^{64}Cu-PCBA-1A3-F(ab')$_2$
Blood	1.44 ± 0.24	1.19 ± 0.06	0.92 ± 0.20	1.05 ± 0.15
Liver	1.68 ± 0.31	1.26 ± 0.12	2.71 ± 0.46	2.96 ± 0.70
Tumor	11.71 ±1.52	8.17 ± 0.51	9.00 ± 1.14	13.71 ± 2.59
Kidney	7.99 ± 0.84	3.35 ± 0.69	23.57 ± 2.41	17.56 ± 2.71
Muscle	0.22 ± 0.04	0.40 ± 0.07	0.27 ± 0.04	0.34 ± 0.04

The metabolism of ^{67}Cu labeled SCN-TETA-1A3, CPTA-1A3, and PCBA-1A3 was examined in rat livers and the metabolism of ^{67}Cu labeled SCN-TETA-1A3-F(ab')$_2$, CPTA-1A3-F(ab')$_2$, and PCBA-1A3-F(ab')$_2$ was studied in rat kidneys.

Liver and kidney homogenates were analyzed by size exclusion chromatography. All of the kidney conjugates were converted to a small molecular weight species by 24 hours. The liver conjugates showed the appearance of a peak at ~35 kDa, with the majority of the remaining activity associated with the intact antibody. Superoxide dismutase (SOD) is a copper containing enzyme (MW = 32 kDa) that has a concentration in the rat liver that is twice as great as any other organ. Our data suggest that ^{67}Cu is transchelating to SOD in the liver, causing the peak at ~35 kDa. Comparison of the ^{67}Cu-CPTA-1A3-F(ab')$_2$ metabolite to known standards by TLC show that the main small molecular weight metabolite is ^{67}Cu-CPTA-ε-lysine. Our results indicate that the ^{67}Cu labeled 1A3-F(ab')$_2$ conjugates were rapidly degraded to the ^{67}Cu-chelate-ε-lysine and the kidney activity may be dependent on the exportation of these lysine metabolites.

Table 1: Biodistribution in Sprague-Dawley Rats (%ID/g)

organ	^{64}Cu-BAT-1A3-F(ab')$_2$	^{64}Cu-SCN-TETA-1A3-F(ab')$_2$	^{64}Cu-CPTA-1A3-F(ab')$_2$	^{64}Cu-PCBA-1A3-F(ab')$_2$
Blood	0.59 ± 0.09	0.50 ± 0.05	0.21 ± 0.01	0.29 ± 0.02
Liver	1.24 ± 0.16	1.27 ± 0.06	2.30 ± 0.40	1.81 ± 0.27
Kidney	13.57 ± 2.56	15.35 ± 2.55	27.58 ± 2.25	23.83 ± 1.89
Muscle	0.17 ± 0.03	0.19 ± 0.03	0.11 ± 0.04	0.12 ± 0.02

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TECHNETIUM-99m-LABELED HYDRAZINO NICOTINAMIDE DERIVATIZED INTACT AND F(ab')$_2$ 1A3, AN ANTICOLORECTAL MONOCLONAL ANTIBODY (MAb). S.W. Schwarz, J.M. Connett*, C.J. Anderson, M.J.Welch, G.W. Philpott* Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110, *Department of Surgery, Jewish Hospital, St. Louis, MO 63110.

We are interested in labeling 1A3, an anticolorectal carcinoma MAb, with ^{99m}Tc to increase the sensitivity of the antibody for γ-camera imaging due to the favorable decay characteristics of ^{99m}Tc. We are currently involved in Phase I-II clinical trials of intact 1A3 and F(ab')$_2$ labeled with the positron emitting isotope ^{64}Cu. These radiopharmaceuticals have exhibited significant tumor uptake and blood clearance by 24 hours post injection (1) (2).

In this study we have investigated the attachment of ^{99m}Tc via the bifunctional chelate succinimidyl 6-hydrazino nicotinate hydrochloride (SHNH) to intact 1A3 and F(ab')$_2$ fragments. Conjugation was performed as previously described with some modifications (1). Briefly, a 5 molar excess of freshly dissolved SHNH (30 mM in DMF) was added dropwise to a stirred solution of MAb in O.l M sodium phosphate pH 7.8. The solution was stirred for 5 h at RT and protected from light. Purification of the intact SHNH-1A3 was accomplished by dialysis against 10 mM sodium citrate pH 5.2 at 4° C for 24 h. Purification of the SHNH-1A3-F(ab')$_2$ recquired fast protein liquid chromatography (FPLC) separation of the conjugate and excess SHNH. This was accomplished using an HR 16/50 Superose column eluted at 0.4 mL/min with 0.1 M sodium phosphate pH 7.8. The eluate was monitored for u.v. absorption at 280 nm. The conjugate eluted with a retention of ~32 minutes, corresponding to a MW of ~110,000 Da. The protein peak was concentrated using Centricon-30 filtration units, and diluted to a final concentration of 3 mg/mL using 20 mM sodium citrate pH 5.2.

The nicotinyl hydrazine modified MAbs were radiolabeled with ^{99m}Tc using ^{99m}Tc-tricine [tris(hydroxymethyl)methylglycine] (2) to provide the recquired Tc(V) oxo species. Tricine kits were prepared (72 mg/mL tricine, 100 μg/mL stannous chloride, pH 6.0) and stored frozen at -80° C in 1 mL aliquots. ^{99m}Tc-tricine was freshly prepard by mixing equal

volumes of the tricine kit and $^{99m}TcO_4^-$ and immediately adding the ^{99m}Tc-tricine to the SHNH-MAb. The mixture was incubated for 10 minutes at room temperature. Radiochemical purity was determined using ITLC-SG strips in 0.1 M sodium citrate pH 5.5, and FPLC analysis on a Superose-12 column eluted with 20 mM Hepes and 150 mM NaCl. All preparations had >98% of the radioactivity bound to the MAb. No purification of the ^{99m}Tc-SHNH-MAbs was recquired. Immunoreactivity (IR) was determined using a direct binding assay under conditions of antigen excess. IR values for ^{99m}Tc-SHNH-1A3 ranged from 85-89%, and for ^{99m}Tc-SHNH-1A3-F(ab')$_2$ the range was 85-92%.

To determine the *in-vivo* behavior of the ^{99m}Tc-labeled intact 1A3 and 1A3-F(ab')$_2$, biodistribution studies were performed in Golden Syrian hamsters implanted with GW39 human colon carcinoma. The %ID/g tumor were obtained for 5, 19 and 24 hours post injection. Uptake was maximized for ^{99m}Tc-SHNH-1A3 at 24 hours, and for ^{99m}Tc-SHNH-1A3-F(ab')$_2$ at 19 hours post injection (13.55±1.18 and 11.27±3.36 respectively). These results were compared to the biodistribution of ^{64}Cu-benzyl-TETA-1A3 intact and F(ab')$_2$, and ^{125}I-1A3 intact and ^{123}I-F(ab')$_2$ at 6 and 24 hours in the same animal model (Table 1 and 2). The uptake in the tumor for both ^{99m}Tc-labeled intact 1A3 and F(ab')$_2$ is greater at 24 h than the iodinated MAbs and in the same range as the ^{64}Cu-labeled MAbs. These exciting results with ^{99m}Tc-labeled-1A3 indicate *in-vivo* stability, tumor uptake comparable to clinically effective ^{64}Cu-TETA-1A3 intact and F(ab')$_2$, and TNT ratios at 24 h (Table 3) which would allow visualization of primary and metastatic disease.

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Tissue	%ID/g 5 h ^{99m}Tc-SHNH-1A3	%ID/g 24 h ^{99m}Tc-SHNH-1A3	%ID/g 6 h ^{64}Cu-TETA-1A3	%ID/g 24 h ^{64}Cu-TETA-1A3	%ID/g 6 h ^{125}I-1A3	%ID/g 24 h ^{125}I-1A3
Blood	5.82±1.30	3.49±0.15	5.52±1.97	4.11±0.25	5.47±0.38	4.04±0.44
Liver	3.58±1.34	2.37±0.42	1.96±0.88	1.56±0.07	1.68±0.21	1.26±0.10
Kidney	1.71±0.53	1.14±0.12	1.66±0.56	1.17±0.06	3.51±0.35	3.51±0.21
Tumor	3.20±1.76	13.55±1.18	2.79±1.80	14.4±4.8	3.58±1.07	8.99±1.67
Muscle	0.14±0.03	0.21±0.05	0.09±0.03	0.21±0.03	0.22±0.05	0.29±0.04

TABLE 1 Biodistribution in Golden Syrian hamsters carrying GW39 human colon carcinoma of intact ^{99m}Tc-SHNH-1A3, ^{64}Cu-TETA-1A3, and ^{125}I-1A3 at 5 or 6 and 24 hours post injection (n = 4-5 for each group). Values are the mean %ID/g tissue ± S.D.

Tissue	%ID/g 5 h ^{99m}Tc-SHNH-1A3-F(ab')$_2$	%ID/g 24 h ^{99m}Tc-SHNH-1A3-F(ab')$_2$	%ID/g 6 h ^{64}Cu-TETA-1A3-F(ab')$_2$	%ID/g 24 h ^{64}Cu-TETA-1A3-F(ab')$_2$	%ID/g 6 h ^{123}I-1A3-(Fab')$_2$	%ID/g 24 h ^{123}I-1A3-(Fab')$_2$
Blood	5.15±2.61	1.90±0.13	5.44±0.58	1.33±0.18	3.96±1.21	1.12±0.26
Liver	2.32±0.53	1.99±0.21	1.73±0.22	1.56±0.18	0.74±0.29	0.31±0.04
Kidney	12.90±3.61	17.01±2.89	10.72±0.85	7.66±0.97	2.42±0.89	0.98±0.20
Tumor	7.18±1.10	10.31±1.70	10.47±2.16	11.77±1.47	4.68±2.63	8.99±0.59
Muscle	0.33±0.07	0.37±0.07			0.19±0.07	0.18±0.02

TABLE 2. Biodistribution in Golden Syrian hamsters carrying GW39 human colon carcinoma of ^{99m}Tc-SHNH-1A3-F(ab')$_2$, ^{64}Cu-TETA-1A3-F(ab')$_2$, and ^{125}I-1A3-F(ab')$_2$ at 5 or 6 and 24 hours post injection (n = 4-5 for each group). Values are the mean %ID/g tissue ± S.D.

MAb	Time (hours)	Tumor/Blood	Tumor/Liver	Tumor/Kidney
^{99m}Tc-SHNH-1A3	5	0.53±0.25	0.89±0.37	1.83±0.88
	24	3.87±0.37	5.78±0.55	11.93±1.56
^{99m}Tc-SHNH-1A3-F(ab')$_2$	5	2.97±0.09	3.17±0.62	0.66±0.03
	24	5.47±1.06	5.20±0.87	0.59±0.15

TABLE 3.Tumor to non-tumor ratios for blood, liver and kidney obtained in Golden Syrian Hamsters at 4-5 and 24 hours post injection for intact ^{99m}Tc-SHNH-1A3 and ^{99m}Tc-SHNH-1A3-F(ab')$_2$. n = 4 or 5 for each group. Values are the mean ± S.D. of the indicated tumor/non-tumor ratio of each animal.

IMPROVED ONE STEP SYNTHESIS OF N-SUCCINIMIDYL 4-[^{18}F](FLUOROMETHYL) BENZOATE FOR PROTEIN LABELING

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We previously reported a method of one step synthesis of fluorine-18 labeled N-succinimidyl 4-[^{18}F](fluoromethyl)benzoate for protein labeling (1). We showed that by carefully controlling the reaction conditions and increasing the reactivity of the leaving group the N-succinimidyl 4-[^{18}F](fluoromethyl)-benzoate can be prepared in one step in 15% (± 4%) labeling yield (EOS) after normal phase HPLC purification. We also showed that by using reversed phase HPLC the specific activity of N-succinimidyl 4-[^{18}F](fluoromethyl)benzoate can be greatly enhanced (2). The radiochemical yield obtained from reversed phase HPLC is comparable to that of obtained from normal phase HPLC. However, one drawback of the purification of [^{18}F]-N-succinimidyl 4-[^{18}F]-(fluoromethyl)benzoate with reversed phased HPLC is the post HPLC purification handling of radioactivity mainly due to removal of relatively large volumes of aqueous solution using a C-18 Sep-Pak cartridge trapping technique. Evaporation of the aqueous solution is not practical due to the instability of N-succinimidyl 4-[^{18}F](fluoromethyl)benzoate in aqueous solution and the short half life of F-18. The C-18 Sep-Pak cartridge trapping requires washing and drying which lead to substantial recovery and decay loses. At the end of this process, only about 70% of activity from HPLC is available for protein labeling. The objective of our current investigation is to increase the radiochemical yield and to generalize the condition and structural requirement for one step synthesis of a F-18 labeled active ester suitable for protein labeling. Different active esters, leaving groups, and carboxylic acids were studied in order to obtained optimal results.

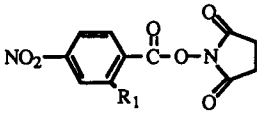
Ligands used in the study

The esters used in this study can be divided into five groups according to the structure of the acids: **a**) benzoic acid esters with the leaving on the benzene ring (1-3) ; **b**) substituted 4-methyl benzoic acid esters (4-19); **c**) substituted 4-ethyl benzoic acid esters (20-21); **d**) substituted 4-methyl phenyl acetic acid esters (22-23); **e**) substituted aliphatic acid esters (24-25). These compounds are listed in Figure 1.

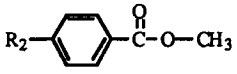
Radiolabeling of esters

The radiofluorination of esters were carried out using potassium carbonate/Kryptofix and F-18 fluoride with acetone or acetonitrile as the solvent at varing temperatures. The anhydrous [^{18}F]fluoride ion was prepared by removing water with argon flow at 105 °C using 3x0.3 ml dry acetonitrile. The products were analyzed using silica gel radio TLC along with fluorinated standards which can be visualized by UV. TLC radiochromatograms were obtained using a Bioscan radioactive scanner.

Figure 1. Compounds used in the study



R_1 = H (1), NO_2 (2)



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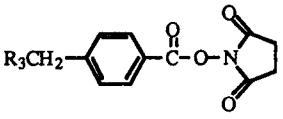
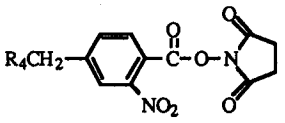


R_3 = Br (5), TsO (6), NsO (7), NO_2-NsO (8), PF (9)



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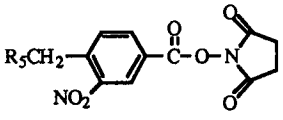
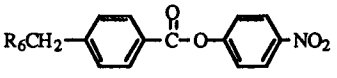
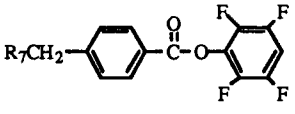


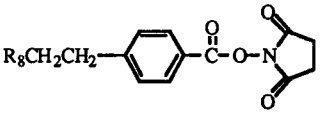
R_5 = Br (13), TsO (14), NsO (15)



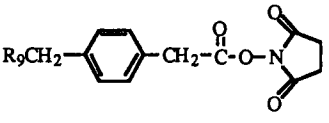
R_6 = Br (16), TsO (17)



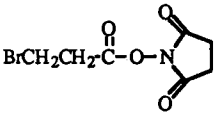
R_7 = Br (18), TsO (19)



R_8 = Br (20), NsO (21)



R_9 = Br (22), NsO (23)



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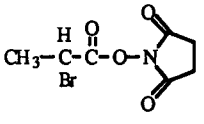

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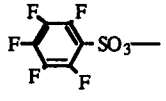
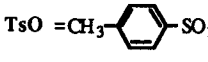
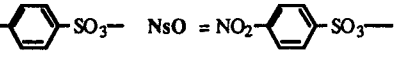
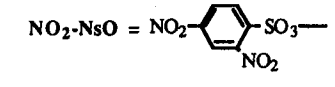
PF = 

TsO = 

NsO = 

NO_2-NsO = 

Results.

When compounds **1-3** were used for labeling, only compound **3** can be labeled at high temperature. Radiofluorination of compounds **1** and **2** resulted in decomposition (1). Results indicate that radiofluorinated active esters of this group can not be made in one step and can only be made in multi-steps as reported by other group (3). When compounds **4-19** were used for labeling, only methyl ester and N-succinimyl esters can be radiolabeled in one step with different labeling yields depending on the leaving group and ring substitution (compounds **4-15** in Table 1). For active esters the best results were obtained at room temperature using acetone as the solvent. Labeling yields increase with the better leaving groups (NO_2-NsO > NsO > PF > TsO > Br) and decrease with eletron withdrawing ring substitution (Table 1) with the 3-nitro compound having the lowest reactivity (compouds **13, 14,** and **15**).

Table 1. Labeling yields of active esters in group b using acetone as the solvent at the room temperature

Compound	5	6	7	8	9	10	11	12	13	14	15
Yield	5%	10%	15%	25%	12%	1%	5%	10%	<1%	~1%	<5%

When 4-nitrophenol and tetrafluorophenol were used as the active ester (compounds **16-19**), the acid fluoride was the only radioactive product. These types of F-18 labeled esters have been reported for labeling proteins, but can be only made in multi-steps (4). Compounds **20** and **21** can also be labeled in one step with somewhat lower reactivity compared to compounds **5** and **6**, whereas the compounds **22** and **23** can not be radiolabeled in one step at all. The major difference here is that compounds **22** and **23** have α hydrogens which can be deprotonated during the fluorination leading to the decomposition. By the same argument, the compounds **24** and **25** can not be made in one step either. In conclusion, by using a better leaving group NO_2-NsO instead of NsO we achived a 65% increase in labeling yields (from 15% ± 4% to 24% ± 4%). The results indicated that only N-succinimidyl esters without an α hydrogen can be radiolabeled with F-18 in one step. The NO_2-NsO leaving group represents a significant improvement in labeling yield compared to the previous method (1).

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IDENTIFICATION OF METABOLITES OF F-18 LABELED ANTI-TAC DISULFIDE STABILIZED MONOCLONAL ANTIBODY VARIABLE-REGION FRAGMENTS IN MICE.

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Radiolabeled monoclonal antibodies and monoclonal antibody fragments are being evaluated as diagnostic and therapeutic agents for tumors. The advantage of using monoclonal antibody fragments is that the localization of monoclonal antibody fragments is much faster than whole antibody and the distribution is more uniform due to smaller size of the fragments. This would allow the use of short lived radioisotopes for radiolabeling. We report here the labeling of anti-Tac disulfide stabilized Fv fragment (dsFv) with fluorine-18 and the identification of metabolites of radiolabeled dsFv in nude mice. This dsFv is genetically-engineered from a murine monoclonal antibody that recognizes the IL-2α receptor (1). IL-2α is expressed on activated T-cells, various leukemias and lymphomas and has been proposed as a target for immunotherapy (2). When the variable-region fragments (Fv fragments) are linked by a covalent peptide bond they are know as single chain Fv (scFv), whereas when they are linked by an interchain disulfide bond they are known as disulfide-stabilized Fv (dsFv). Anti-Tac dsFv has been shown to be more stable than its Fv or scFv counterpart and has been evaluated as an immunotoxin conjugate (3) and as an I-125 Bolton-Hunter reagent labeled fragment (4). The biodistribution study of I-125 Bolton-Hunter reagent labeled fragments showed fast blood clearance and high kidney uptake. The study with F-18 labeled dsFv showed similar results. The blood clearance of F-18 dsFv was rapid with less than 10% retained in the blood by 15 minutes and 70% of F-18 activity was absorbed by kidney. The tumor uptake reached a maximum between 30-40 minutes. The size of the anti-Tac dsFv is a main factor in determining glomerular filtration. Wochner et al have shown that glomerular filtration is important in the clearance of IgG fragments, but not important in the clearance of intact antibody (5). Arend et al reported that the main mechanism for catabolism of Fab fragments is removal from circulation by glomerular filtration and subsequent re-absorption and degradation in the proximal tubule (6). In order to better understand the retention of F-18 activity by kidney and to improve the target to non-target radios, it is an important step to identify the metabolites of F-18 labeled dsFv. It has been reported that the major metabolite of ^{111}In-DTPA-glycoprotein is the ^{111}In-DTPA-ε-lysine and the amide bonds between radiometal chelate complex and lysine are stable in vivo (7). Another group has also reported the identification of the N-acetylated-lysine adduct of Tc-99m-N_2S_2 chelate in the urine of rats and mice after the administration of Tc-99m-N_2S_2 preformed chelate labeled monoclonal antibody Fab fragment (8).

Preparation of F-18 Labeled dsFv. Anti-Tac dsFv was labeled with F-18 using N-succinimidyl 4-[^{18}F](fluoromethyl)benzoate prepared according to previously described method (9). The F-18 labeled Anti-Tac dsFv was purified with a Beckman SEC 2000 size exclusion

HPLC column using PBS buffer (pH 6.9). Specific activity of F-18 anti-Tac dsFv at the end of synthesis was about 13 mCi/mg and showed more than 98% of radiochemical purity as determined by size exclusion HPLC. The immunoreactivity of the radiolabeled anti-Tac dsFv was > 82%.

Preparation of possible radioactive metabolites of F-18 dsFv. In order to analyze the metabolites from F-18 dsFv, several likely metabolites were prepared to serve as standards: 4-[^{18}F]- (fluoromethyl)benzoic acid (FMBz), N-ε–4-[^{18}F] fluoro-methylbenzoyl lysine (FBL), N-ε-4-[^{18}F]fluoromethylbenzoyl(α-N-acetyl) lysine (FBNAL), N-ε–4-[^{18}F](fluoromethylbenzoyl) lysinyl-alanine (F-LYS-ALA), and ananyl-N-ε-4-[^{18}F](fluoromethylbenzol) lysine (ALA-F-LYS). The latter two were chosen based on the sequence of anti-Tac dsFv. FMBz was prepared by hydrolyzing N-succinimidyl 4-[^{18}F](fluoromethyl)benzoate with 0.5 M sodium carbonate. FBL, FBNAL, F-LYS-ALA, and ALA-F-LYS were prepared by reacting lysine, N-α-acetyl lysine, alanyl lysine, and lysinyl alanine with N-succinimidyl 4-[^{18}F](fluoromethyl)benzoate in 50% acetonitrile. TLC radiochromatogram showed 100% incorporation of F-18 activity to the amino acids and dipeptides.

Analysis of Metabolites with TLC. Serum and urine samples obtained at 15, 45, and 90 min were analyzed using silica gel TLC. Approximately 10 μl of urine, serum and radiolabeled standards were spotted on the plates and developed using three different mobile phases: 15:15:1 ethyl acetate:hexane:acetic acid (A); 15:15:1 ethyl acetate:methanol:acetic acid (B); 15:15:1 ethyl acetate:methanol: ammonium hydroxide(C). TLC radiochromatograms were obtained using a Bioscan radioactive scanner.

Analysis of Metabolites with HPLC. Standards and selected urine samples were analyzed by HPLC equipped with a reversed phase radial-pak cartridge with 3% acetonitrile in 5 mM sodium phosphate (pH 6.54) with a flow rate of 1 ml/min. The radiolabeled anti-Tac dsFv and selected serum and urine samples were analyzed using size exclusion HPLC equipped with a on-line NaI gamma detector using a TSK 2000 column. The column was eluted with 0.067 M PBS, 0.1 mM KCl buffer (pH 6.8) with a flow rate of 0.5 ml/min.

RESULTS.

Evaluation of the circulating radioactivity showed that anti-Tac dsFv cleared rapidly from the blood with an increasing fraction of the circulating radioactivity appearing as metabolites. The fraction of radioactivity appearing as metabolites in serum and urine increased over time whereas the fraction present as the anti-Tac dsFv parent decreased (Table 1). The analysis of urine and serum samples along with the known F-18 standards on silica gel TLC plates showed that the main metabolite was N-ε-4-[^{18}F]fluoromethylbenzoyl(α-N-acetyl) lysine and the minor metabolite was N-ε–4-[^{18}F] fluoromethylbenzoyl lysine in urine. The main metabolite in serum was N-ε–4-[^{18}F] fluoromethylbenzoyl lysine and the minor metabolite was N-ε-4-[^{18}F]fluoromethylbenzoyl(α-N-acetyl) lysine (Table 2).

Table 1. % Radioactivity in anti-Tac fraction (size exclusion HPLC)

Time	In serum	In urine	
15 min	74% ± 8% (4*)	56% ± 36% (4)	* number of study
45 min	39% ± 10% (4)	2% ± 3% (3)	
90 min	32% (1)	7% ± 11% (3)	

The R_f values of metabolites obtained with different solvent system gave us a hint of the identity of the metabolites. N-ε-4-[^{18}F]fluoromethylbenzoyl(α-N-acetyl) lysine does not have a free amine and added base in the solvent did not affect its R_f value. On the other hand, N-ε–4-[^{18}F] fluoromethylbenzoyl lysine and the two dipeptides have free amine group and added base greatly changed their R_f values. The results of reversed phased HPLC of standards and urine samples also confirm the identity of the metabolites. The reversed phased HPLC of urine samples showed that the major peak had a R_t (20.3 min) identical to that of N-ε-4-[^{18}F]fluoromethylbenzoyl(α-N-acetyl) lysine and the minor peak had a R_t (14.8 min) identical to N-ε–4-[^{18}F] fluoro-methylbenzoyl lysine.

Table 1. R_f value of reference F-18 compounds using silica gel TLC

Solvent	FMBz	FBL	FBNAL	ALA-F-LYS	F-LYS-ALA
A	0.18	0	0	0	0
B	-	0.11	0.44	0.14	0.14
C	-	0.36	0.56	0.51	0.51

No 4-[^{18}F] (fluoromethyl)benzoic acid was detected in all urine and serum samples. This indicates that the amide bond between lysine and benzoic acid is stable in vivo. The result also rule out the presence of dipeptides with the F-18 attached to them. This study is the first to identify the N-ε–4-[^{18}F]fluoro-methylbenzoyl lysine and the N-acetylated N-ε–4-[^{18}F] fluoro-methylbenzoyl lysine as the metabolites of radiolabeled antibody fragments in both urine and serum.

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Synthesis and Evaluation of *para*-Iodohippuric Acid Tetrafluorophenyl Ester for Radioiodination of Antibodies.

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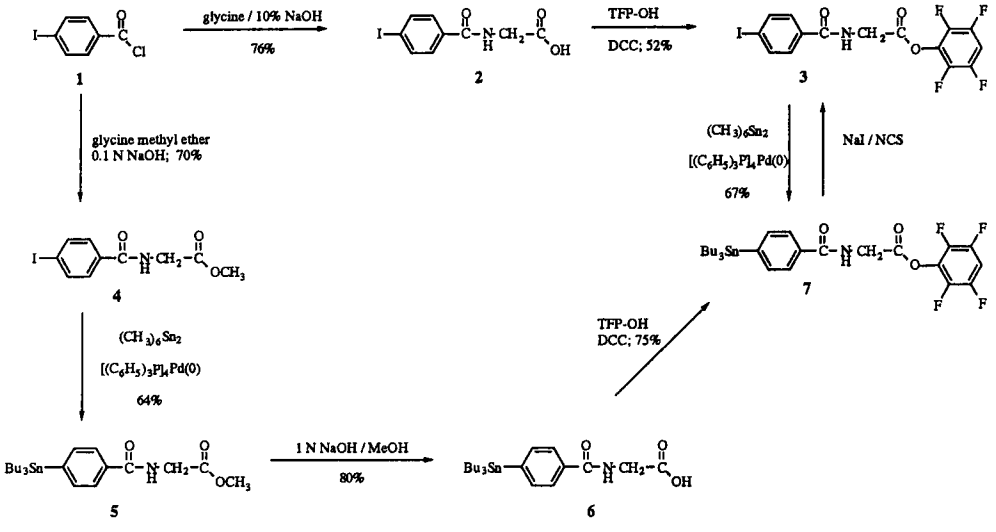
Conjugates for radiohalogenation of antibodies have been extensively studied and several have proven to be useful for that application [1]. Of the many different conjugates, *para*- and *meta*-iodobenzoyl derivatives [2,3] have been studied the most. While these two reagents have similar characteristics (radiochemical yields, protein modification results, and iodine stability), one notable difference has been that the kidney retention of radioactivity for antibody fragments labeled with the *para*-iodobenzoate is considerably higher than that found with the *meta*-iodobenzoate [4] or chloramine-T labeled antibodies [5]. The difference in kidney retention has been attributed to the fact that the metabolite found in urine for the *para*-iodobenzoyl conjugate is primarily the lysine adduct [6], whereas free *m*-benzoic acid is thought to be released on metabolism of *meta*-iodobenzoyl conjugate [7]. This difference prompted us to evaluate iodohippuryl conjugated antibody fragments since iodohippuric acid, **2**, might be released as a metabolite from its conjugation with lysine moieties, and **2** would not be expected to be retained in the kidney.

We have previously prepared the N-hydroxysuccinimide (NHS) ester of *p*-tri-*n*-butylstannylhippuric acid for antibody conjugation, however, antibody conjugations were not conducted with that compound [1]. In our present studies, *p*-tri-*n*-butylstannylhippurate tetrafluorophenyl (TFP) ester, **7**, and *p*-iodohippurate tetrafluorophenyl ester, **3**, were targeted as the TFP ester is more stable to hydrolysis. Initial studies to prepare **7** involved reaction of *p*-tri-*n*-butylstannylbenzoic acid NHS ester (PIB reagent [2]) with glycine. It was surprising that, under the conditions studied, no product was obtained. Indeed, no reaction was obtained with a number of different amine containing compounds. Thus, a different synthetic route had to be designed. Ultimately, two different successful approaches to prepare the stannyl derivative **7** were studied, as depicted in Figure 1. From those studies, we conclude that synthesis of **7** is best accomplished by conversion from **3**. The conditions and reaction yields are listed in Figure 1.

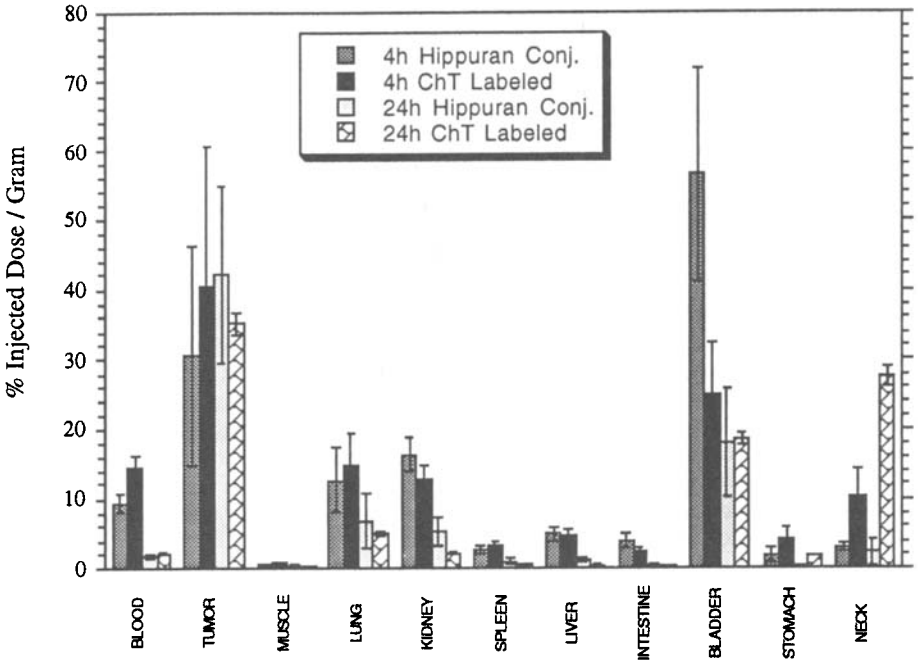
Radioiodination of **7** was conducted by its reaction with N-chlorosuccinimide (NCS) / Na[^{125}I]I in MeOH containing 1% HOAc. Radiochemical yields of 44% and 45% of **3** were obtained in two reactions. Radiolabeled **3** was isolated from the HPLC effluent so that **7** would not be present to conjugate with the antibody. Our previous labeling method using PIB had not required HPLC separation prior to antibody conjugation as we had found no tin associated with the antibody (Plasma Emission Spectrometry). Although we had previously believed that this was due solely to insolubility of the arylstannane, it is likely that the non-reactivity of the NHS ester with amines was a significant factor.

Figure 1: Synthesis of *p*-Tri-n-Butylstannylhippurate and *p*-Iodohippurate Tetrafluorophenyl Esters.



Our studies employed the anti-renal cell carcinoma antibody A6H. Its Fab' fragment was used since previous studies with Fab and Fab' fragments had demonstrated a large difference in kidney radioactivity (>2x) [2,5] at 4 hours post (co)injection when radioiodinated with *p*-iodobenzoyl (PIB) or by chloramine-T labeling. The sulfhydryl groups of the A6H Fab' fragments were capped with N-ethylmaleimide prior to labeling so that thioesters would not be formed. To conduct the comparison in tumor bearing athymic mice, A6H Fab' was radioiodinated with [^{125}I]**3** and separately with Na[^{131}I]I / chloramine-T. The A6H Fab' radioiodinated with [^{125}I]**3** had an immunoreactivity of 44% and A6H Fab' radioiodinated with Na[^{131}I]I / chloramine-T had an immunoreactivity of 42%. While these values are low, they are normal for radiolabeled A6H Fab'. A biodistribution of the co-injected preparations is shown graphically in Figure 2. It is important to note that the percent injected dose of radioactivity in the kidney at 4 h is approximately equivalent for both radioiodination methods. This is quite different from the results obtained with PIB labeled A6H Fab' [5], suggesting that the kidney retention is different with the hippurate conjugate. However, some caution must be taken in that interpretation as the blood levels of the two radiolabeled preparations were significantly different at the 4 h time. As expected, the levels of radioiodine in the stomach and neck for the [^{125}I]**3**-labeled A6H Fab' indicate that it was stable to *in vivo* dehalogenation.

Figure 2: Biodistribution of Coinjected *p*-[^{125}I]Iodohippuryl- and Na[^{131}I]I / Chloramine-T labeled A6H Fab' in Athymic Mice (n = 8) bearing a Human Renal Cell Carcinoma Xenograft (TK-82).



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Production of Zirconium-labelled Antibodies and their Biodistribution in Tumour-bearing Mice

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For planning radioimmunotherapy, it is important to know the biodistribution of the radiolabelled antibody. Positron Emission Tomography can provide these data by non-invasive measurements of an antibody labelled with a positron emitter. The relatively long half-life of ^{89}Zr ($T_{1/2}$=78 h) makes this positron emitter suitable for this purpose. Furthermore, ^{89}Zr decays for 23% by positron emission and it can be produced in high yield with a high radionuclidic purity (1). The aim of this study was to investigate whether antibodies can be labelled with Zr-isotopes, without altering their pharmaco-kinetical behaviour.

The antibodies 323A3 and E48 (IgG) have been modified with desferal, a chelating agent with a high complexation affinity for Zr (2). Modification was performed in a two-step procedure (Scheme 1). First maleimide groups were incorporated into the antibodies by reaction of the lysine-groups with SMCC. The thioester of SATA-Df was converted into the free thiol with hydroxylamine in order to react with the malemide groups of the antibodies. The excess of SATA-Df was succesfully removed by gelfiltration. The labelling was performed easily by adding the premodified protein to isolated Zr. After incubation for one hour the yield of labelling was found to be more than 90% and the antibodies retained their immunoreactivity (>80%).

The biodistribution of the ^{88}Zr-labelled antibodies in mice bearing non-specific and specific xenografts was determined and compared with the biodistribution of the same antibodies labelled with ^{99m}Tc and ^{123}I. From the results of these studies, which are depicted in Figure 1, it can be concluded that the developed procedure is a good method of general applicability to label proteins with Zr-isotopes, that the tumour accumulation must be due to specific binding of the monoclonal antibodies to the antigens on the tumour cells and that it seems justified to assume that the Zr-labelled antibodies are stable *in vivo*. The usefulness of the ^{89}Zr-labelled Mab's as PET-conjugate was confirmed by PET-imaging studies. The images, obtained without interference of the 900 keV gamma-radiation, showed an obvious accumulation of ^{89}Zr in the tumours. Even a small tumour (50 mg) was clearly visualized (see Figure 2 as an example).

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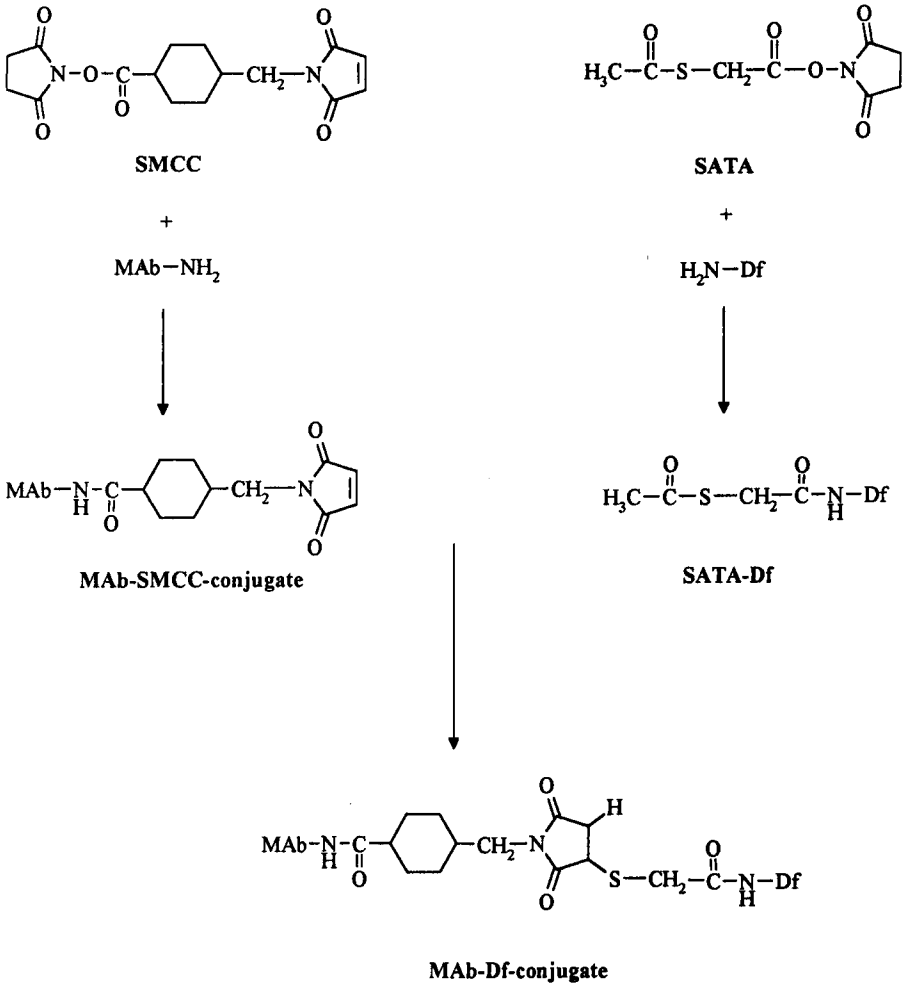
SMCC
SATA
+
MAb–NH2
H2N–Df
MAb-SMCC-conjugate
SATA-Df
MAb-Df-conjugate

Scheme 1

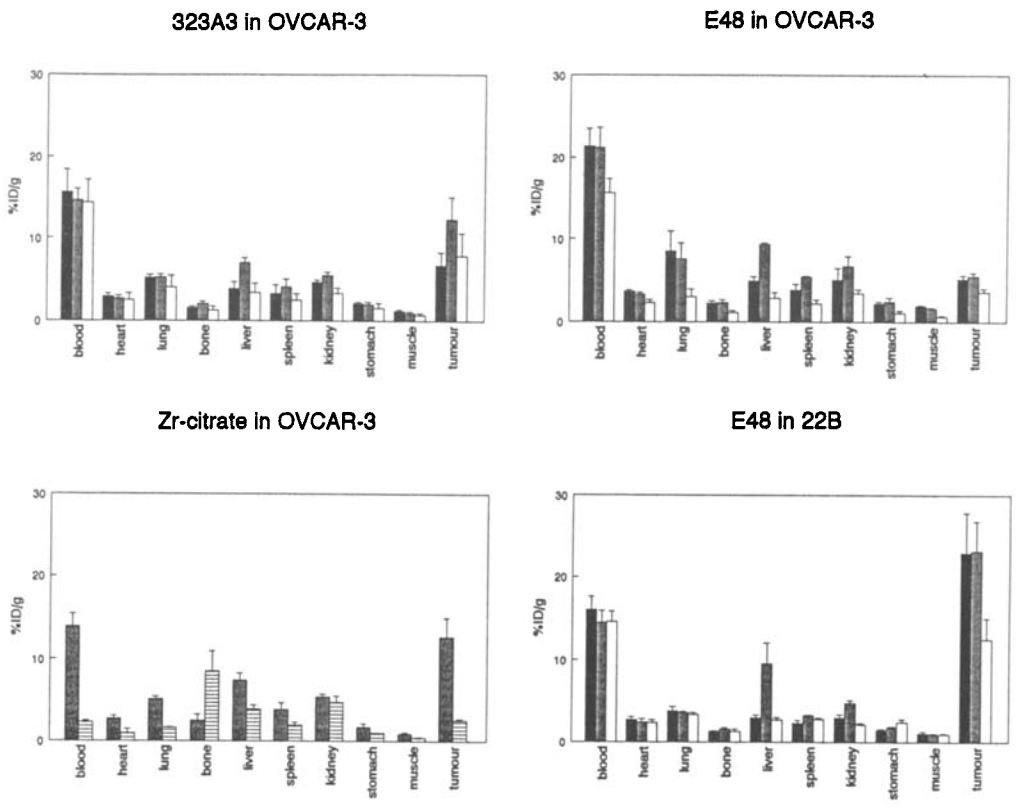

Figure 1. Biodistribution of ■ ^{99m}Tc-MAb ■ ^{89}Zr-MAb □ ^{125}I-MAb ▤ ^{89}Zr-citrate in tumour-bearing nude mice 24 hours post injection.

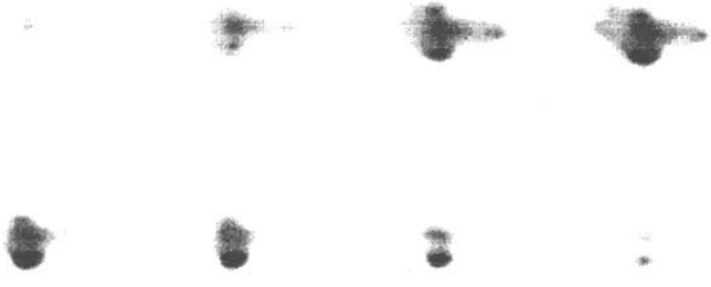

Figure 2. PET-image of a mouse injected with ^{89}Zr-323A3

SYNTHESIS OF RADIOIODINATED BIOTIN DERIVATIVES. Catherine F. Foulon, S. James Adelstein and Amin I. Kassis. Harvard Medical School, Department of Radiology, 50 Binney Street, Boston, MA 02115.

The localization and therapy of tumors using radiolabeled antibodies is impaired by the extremely low uptake within the tumor and poor target/non-target ratios (1,2). This limitation may be overcome by an approach (3,4) that utilizes the high avidity of avidin or streptavidin for biotin, a 244 Da vitamin found in low concentration in blood and tissues (5). Because the noncovalently bound avidin/streptavidin--biotin complex has an extremely low dissociation constant (Kd of about 10^{-15} M), investigators have used these two molecules as binding pairs to bridge molecules that have no affinity for each other and to target various radionuclides. So far, two distinct strategies tested in animals and humans have been designed: the "two-step" and the "three-step" approaches. The former consists in injecting an avidin-antibody or biotin-antibody conjugate, followed several days later by radiolabeled biotin (6) or avidin (7,8), respectively. The latter approach relies on injecting a biotinylated antibody, followed by an excess of avidin, and several days later, radiolabeled biotin (9). Each of these protocols shows enhancement of the tumor/background ratio and tumors are vizualized 20 min post injection of the tracer with an average tumor uptake of 0.012% ID/g, 3 hr post injection (9). These encouraging results motivated us to pursue the development of a series of radioiodinated biotin derivatives for use in cancer radioimmunoscintigraphy or radioimmunotherapy (10,11). By modifying the molecular structure of such derivatives, we hope to identify a molecule that exhibits a high and selective affinity for avidin/streptavidin and that displays more suitable in vivo pharmacokinetic properties. The structural modifications are based on the length of the alkyl arm (presence of not of the caproyl chain) and nature of the prosthetic group carrying the iodine. As depicted in the synthetic scheme, the metallic precursors (**1a**, **2a**, **3a** and **4a**) were obtained by the action of either 3-tributylstannylaniline or 3-tributylstannylbenzylamine with each of the succinimido derivatives of biotin, whereas the iodinated biotin derivatives (**1b**, **2b**, **3b** and **4b**) were obtained by the action of either 3-iodoaniline or 3-iodobenzylamine with each of the biotin derivatives activated via isobutyl-chloroformate. The four tributylstannyl derivatives as well as the cold iodinated analogs have been characterized by NMR and elemental analysis. The synthesis of biotinyl-3-[^{125}I]iodoanilide (**1c**), biotinyl-3-[^{125}I]iodobenzylamide (**2c**), biotinyl-γ-

caproyl-3-[^{125}I]iodoanilide (**3c**) and biotinyl-γ-caproyl-3-[^{125}I]iodbenzylamide (**4c**) was achieved by destannylation of their respective tributylstannyl analog (**1a**, **2a**, **3a** and **4a**) using Na^{125}I, N-chlorosuccinimide as the oxidant and followed by treatment with sodium metabisulfite. The radioiodinated compounds were purified by elution through reverse-phase cartridge with MeOH/Water (65/35). The solvent was evaporated under a stream of nitrogen and the sample solubilized in water for in-vitro assays. Radiochemical yields ranged between 90-95%. As a quality control, each radiolabeled biotin derivative was authenticated by coinjecting the respective cold iodinated analog (**1b**, **2b**, **3b** and **4b**) onto a C18 HPLC column (MeOH/Water: 65/35, 1 mL/min). In-vitro binding assays and in-vivo stability studies of (**1c**), (**2c**), (**3c**) and (**4c**) are currently under way and the results will be presented at the meeting.

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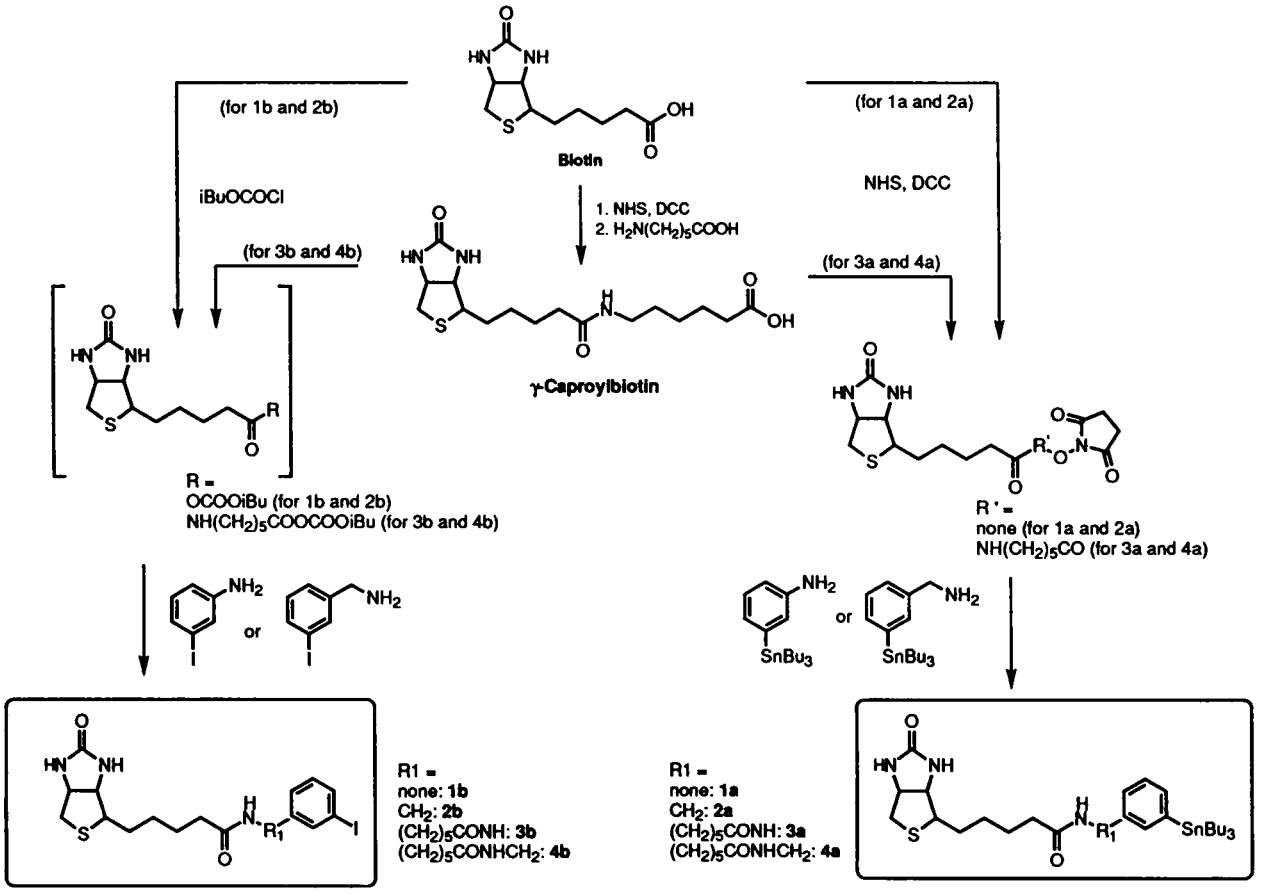
Biotin
(for 1b and 2b)
iBuOCOCl
(for 3b and 4b)
1. NHS, DCC
2. H2N(CH2)5COOH
γ-Caproylbiotin
(for 1a and 2a)
NHS, DCC
(for 3a and 4a)
R =
OCOOiBu (for 1b and 2b)
NH(CH2)5COOCOOiBu (for 3b and 4b)
R' =
none (for 1a and 2a)
NH(CH2)5CO (for 3a and 4a)
or
or
R1 =
none: 1b
CH2: 2b
(CH2)5CONH: 3b
(CH2)5CONHCH2: 4b
R1 =
none: 1a
CH2: 2a
(CH2)5CONH: 3a
(CH2)5CONHCH2: 4a

In-111 Avidin and In-111 Streptavidin Localization in Tumor as a Function of Dose Administered. WANG, T.S.T.; DAS, S.; GREENSPAN, R.; VAN HEERTUM, R.L.; AND FAWWAZ, R.A. College of Physicians and Surgeons, Columbia University, New York, NY 10032. U.S.A.

Biotin-containing intranuclear inclusion bodies have been demonstrated in various tumor types (1-4). Carbohydrate utilization has been shown to be elevated in many tumor tissues (5) and biotin plays an important role in carbohydrate metabolism (6). Based on these observations, we investigated the role of radiolabeled avidin and streptavidin, which have a high affinity for biotin, as potenital tumor localizing agents. In experiments performed in nude mice bearing human breast carcinoma and injected with In-111-Avidin or In-111-Streptavidin, the overall tumor to normal tissue ratios were superior to those achieved following the administration of Fluorine-18-fluorodeoxyglucose (F-18-FDG), Tc-99m-Sestamibi, In-111-IgM-MAb, In-111-IgG-72-MAb, and In-111-Octreotide (7).

Since the mass of normal tissues is much greater than that of tumor, and since biotin is utilized by all normal tissues, one would predict that following the administration of In-111 avidin or In-111-streptavidin in tumor bearing animals, the vast majority of the label would localize in normal tissues.

This study was designed to determine whether increasing the amount of avidin or streptavidin administered would result in improved tumor to normal tissue ratios by saturating normal binding sites.

In-111-Avidin and In-111 Streptavidin labeling was achieved via bifunctional chelation using the bicyclic anhydride of DTPA method (8). Human breast carcinoma (MCF-7) xenografts were implanted subcutaneously into the back of nude mice weighing about 20 g. When tumor size reached approximately 0.5 cm., 20 uCi of In-111-Avidin or In-111-Streptavidin was injected intravenously

through the tail vein. The following dose levels were used: 1, 10, and 100 ug per mouse (Four animals were used per dose level). The animals were sacrificed 3 days post injection and tissues obtained for determination of percent of injected dose per gram of tissue. As demonstrated in Table 1 and 2, tumor to normal tissue ratios were directly proportional to the dose of Avidin or Steptavidin adminstered.

Since Avidin and Streptavidin are relatively non-toxic agents (9), these results warrant further studies to determine whether further dose increments results in even greater improvement in tumor to normal tissue ratios.

Supported by a Grant from the Irving A. Hansen Memorial Foundation.

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Table 1. BIODISTRIBUTION RATIO OF TUMOR TO NORMAL TISSUE

Tissue	F-18-FDG	Tc-99m Sestamibi	In-111-IgM MAb	In-111-IgG MAb-B-72	In-111 Octreotide	In-111-SAV	In-111-AV
Blood	6.6	10.1	6.0	0.9	8.0	18.2	17.1
Lung	1.3	1.4	1.0	1.3	0.3	2.3	2.1
Heart	0.1	0.1	2.9	1.0	4.0	3.4	2..5
Liver	5.4	0.2	0.7	0.6	0.5	0.6	0.3
Spleen	1.8	0.8	0.2	1.3	1.2	0.9	0.5
Kidney	1.5	0.03	0.1	0.9	0.06	0.4	0.3
Muscle	0.9	0.39	5.4	4.9	4.1	6.8	7.9
Bone	1.3	1.0	0.2	3.4	1.1	2.7	1.6
Small Int.	1.3	0.1	0.9	3.8	0.5	3.6	2.7
Large Int.	1.3	0.2	1.1	4.8	0.06	2.2	1.5

SAV=Streptavidin, AV=Avidin

Table 2. RATIO OF TUMOR TO NORMAL TISSUES OF UPTAKE OF In-111-AVIDIN

Tissue	1 ug	10 ug	100 ug
Blood	3.4	7.3	17.1
Lung	1.3	1.6	2.1
Heart	1.2	1.4	2.5
Liver	0.01	0.02	0.3
Spleen	0.04	0.05	0.5
Kidney	0.1	0.2	0.3
Muscle	1.3	4.4	7.9
Bone	0.8	0.6	1.6
Small Int.	2.3	2.9	2.7
Large Int.	2.7	1.9	1.5

Binding of radioyttrium to human serum albumin: Relevance to endotherapeutic applications of ^{90}Y-labelled pharmaceutics

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The endotherapeutic application of ^{90}Y-labelled monoclonal antibodies, peptides, complexes or particles has increased over the last decade in parallel to an improvement of radiochemical syntheses, in vitro stability and in vivo behaviour of these radiotherapeutics. In contrast, only little and contradictory information is available on the physiological behaviour of the radioyttrium cation in the blood itself, i.e. its ability to bind to serum proteins [1-5]. The binding of trivalent metals to proteins is actually a topic of basic bioinorganic chemistry, but it is also relevant to radiopharmaceutical chemistry. It is mandatory to study in particular those ^{90}Y-labelled radiotherapeutics, from which Y(III) is released in vivo and subsequently transfered to serum proteins, which in turn develop their own radiotherapeutic action.

In order to investigate the radioyttrium binding to the serum proteins, human serum albumin (HSA) was used as a model protein and ^{86}Y ($T_{1/2}$ = 14.7 h, 32% β^+) or ^{88}Y ($T_{1/2}$ = 106 d, EC) as radioyttrium isotope. The separation of protein-bound from non-protein-bound radioyttrium was carried out on a gel filtration column filled with Sephadex G-25 using TRIS-acetate as eluent. For comparison ultrafiltration studies with Amicon microconcentrators microcon-30 were performed. The activity distribution was measured via γ-counting. Standard incubation time of radioyttrium in solutions of 10 mg/mL HSA (ionic strength: 0.15 M) was 23 h, and the following experimental parameters were varied systematically: (a) type of the endogenous anion present in the solutions including chloride, fluoride (labelled with [^{18}F]F$^-$), sulfate (labelled with [^{35}S]SO_4^{2-}), citrate and phosphate (labelled with [^{32}P]PO_4^{3-}), (b) concentration of Y(III) carrier, (c) pH and (d) competing citrate concentration.

While the binding of Y(III) to HSA was low (< 2%; determined by gel filtration) in the absence of any anion or in the presence of chloride, fluoride, sulfate and citrate, a significant enhancement of Y(III) bound to HSA (ca. 80%) was observed in the presence of the phosphate anion. Phosphate, in turn, was effectively bound to HSA (about 80% in the presence of Y(III)), but almost neglegibly (< 2%) in the absence of Y(III), indicating that Y(III) and phosphate are bound synergistically to HSA; Table 1.

Table 1 Percent binding of ^{88}Y(III) and endogenous anions to HSA

Anion	Concentration in serum	^{88}Y-HSA-binding	Anion-HSA-binding
Chloride	100 mM	2%	-
Bicarbonate	27 mM	3%	-
Sulfate	1 mM	2.5%	1.5%
Fluoride	0.02 mM	1%	1%
Citrate	0.1 mM	2%	-
Phosphate	**2 mM**	**82%**	**81%**

The evidence for the simultaneous binding of Y (III) and phosphate to HSA could be confirmed in human blood serum. Maximum binding was observed at concentration ratios [Y(III)] : [phosphate] of about 1 : 1 for both HSA and human serum. Results are illustrated for HSA in Fig. 1. The number of binding sites for the yttrium-phosphate-species on HSA was determined to be about 14.

Increasing the citrate concentration lead to a destruction of the supposed complex between yttrium, phosphate and HSA. The latter process may be described by following complex formation equilibrium: $Y(PO_4)HSA + cit^{3-} \leftrightarrow [Y(cit)_n]^{3-n} + PO_4^{3-} + HSA$.

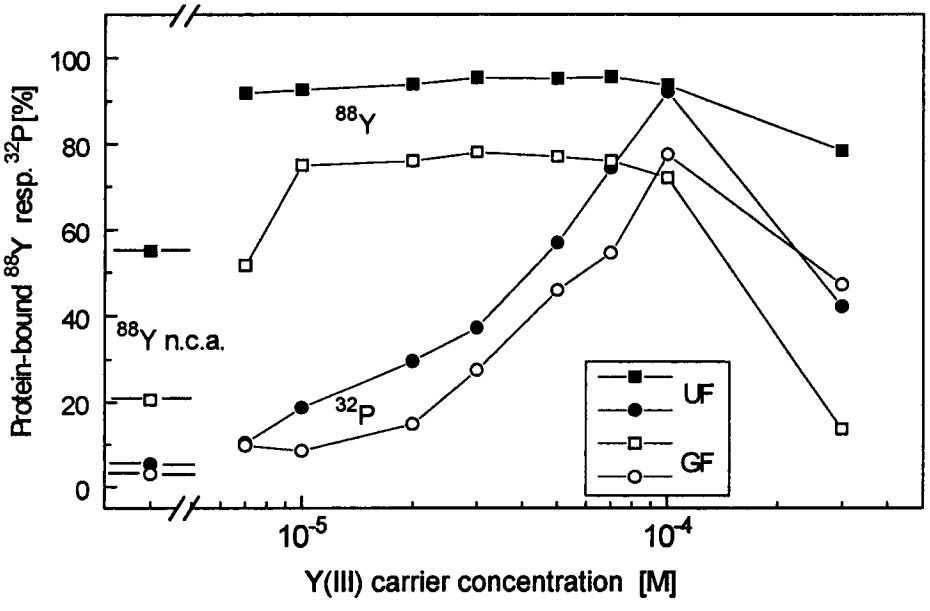

Fig. 1 Percentage of [^{88}Y]Y(III) and [^{32}P]phosphate bound to HSA vs. Y(III) carrier concentration (GF = gel filtration, UF = ultrafiltration)

The results indicate that Y(III) is effectively bound to HSA, almost exclusively due to the interaction of the endogenous phosphate anion. As compared to binary models discussed in the literature, this process may be described in terms of a ternary complex formation between yttrium(III), phosphate and HSA. Ligands L^{a-} with high $[YL_n]^{3-an}$ complex formation constants (e.g. citrate, EDTMP, EDTA etc.) are able to competitively destruct this ternary complex. It seems possible to quantify the number of binding sites and the apparent association constant of this complex. However, an HSA stabilized yttrium phosphate colloid formation cannot be completely excluded. On the other hand, the effect of Y(III) bound synergistically with phosphate to HSA could be detected also for other trivalent metals. Phosphate, for example, is bound to HSA to a small degree only in the presence of Sc(III), Ga(III) and In(III), but in higher amounts in the presence of the lanthanides Ln(III). There is even evidence that the tendency to form a ternary complex is dependent on the ionic radius of the trivalent metal. cf. Fig. 2.

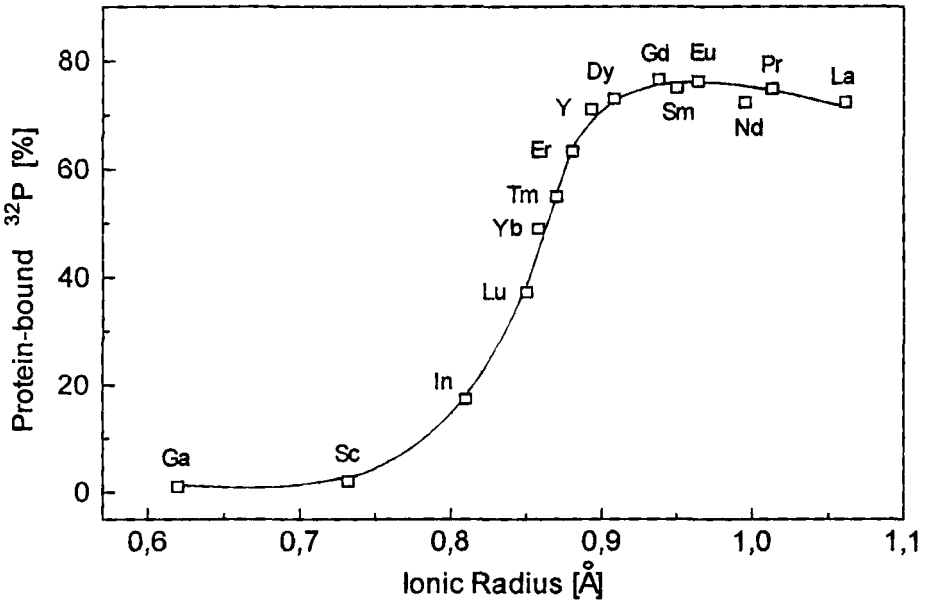

Fig. 2 Binding of [^{32}P]phosphate to HSA vs. the ionic radius of various Me(III)

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PALLADIUM MEDIATED SYNTHESIS OF [^{18}F]FLUOROPHENYL ALKENES OR ARENES

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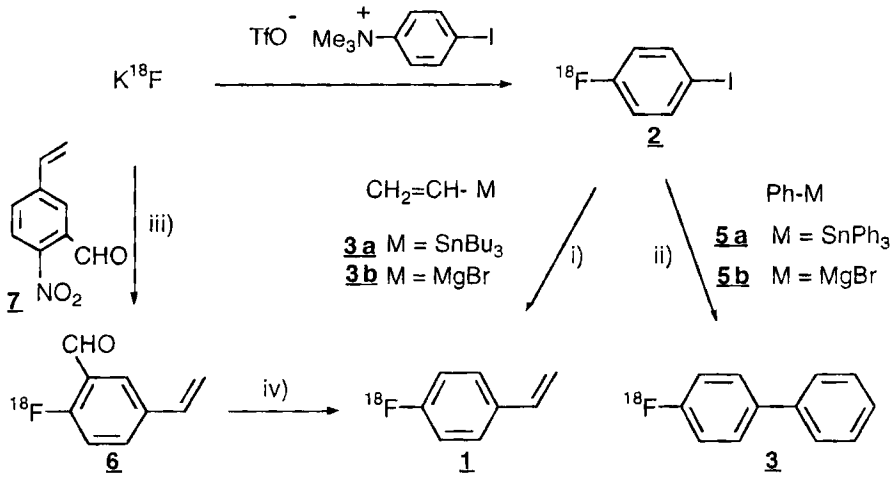
The 4-fluorophenyl group is frequently encountered in molecules of biological interest (GBR 12936[1], WIN 35,428[2]) and we have previously developped a rapid synthesis of [^{18}F]fluorophenylpiperazine[3]. We have also shown that [^{18}F]fluorophenylalkenes can be obtained in a two step procedure from [^{18}F] fluoride using a Wittig reaction on [^{18}F] fluorobenzaldehyde[1]. The major drawback of this approach is the formation of a mixture of both Z and E diastereoisomers (ratio 40/60). Alkene arylations can be successfully carried out by reaction of an organometallic and an aryl halide using a palladium catalyst. This reaction is often quite regio and stereospecific[4]. A particulary attractive feature of the use of stannane derivatives is the toleration of a wide variety of substituents such as unprotected carbonyl or acetate functions[5].This methodology has been recently used in carbon-11 chemistry for the [^{11}C]cyanation of halogenoaromatics[6] and in the synthesis of [^{11}C]ketones[7]. With the aim of synthezising alkenes or (hetero)aromatic compounds bearing a [^{18}F]fluorophenyl group on an sp^2 carbon and [^{18}F]fluorophenyl(cyclo)alkanes, we have explored the usefulness of some palladium mediated reactions of organometallics on 4-[^{18}F]fluoroiodobenzene and we report here our preliminary results.

4-[^{18}F]fluorophenylethene **1**

4-[^{18}F]fluoroiodobenzene **2** prepared according to the literature[8] was allowed to react with tributylvinylstannane **3a** (14 µmol) under different conditions [solvent (DMF or toluene), temperature (60, 110 or 150°C), catalysts : tetrakistriphenylphosphine palladium[9] **4a**, palladium acetate - tri-*o*-tolylphosphine[10] **4b**, tris(dibenzylideneacetone)dipalladium-triphenyl arsine[11] **4c**, copper (I) iodide[11] **4d**]. The highest yield in [^{18}F]fluorophenyl ethene **1** [53% from [^{18}F]fluoroiodobenzene **2**, 90 min total synthesis time from [^{18}F]fluoride including HPLC (µ-bondapack C_{18}, MeCN:H_2O 50:50 v/v flow : 2.4 mLmin^{-1}, λ : 254 nm)] was obtained when the iodide **2** and the stannane **3a** were heated for 5 min in DMF in the presence of **4c** and **4d**. The co-catalytic effect of the cuprous iodide **4d** appeared strongly solvent dependent. If no significant influence was observed in DMF, the isolated radiochemical yield was largely improved (from 26 to 42 %) by adding cuprous iodide to **4c** when the reaction was carried out in toluene. The usefulness of the stannane derivative was shown by the failure to isolate any coupling adduct from the reaction of vinylmagnesium bromide **3b** and the aryl iodide **2** under palladium catalysis.



i) **3a** (14 µmol), DMF (1 mL), 60°C, 5 min, 2 % Pd_2dBa_3, 16 % $AsPh_3$, 8 % CuI ; **3b** (960 µmol), THF (1 mL), 5 min, reflux, 0.9 % $Pd(PPh_3)_4$ ii) **5a** (10 µmol), HMPA (1 mL), 120°C, 5 min, 0.7 % $Pd(PPh_3)_4$; **5b** (960 µmol), THF (1 mL), 5 min, reflux, 0.9 % $Pd(PPh_3)_4$ iii) **7** (85 µmol), DMSO (1 mL), 150°C, 15 min iv) $RhCl(PPh_3)_3$ (13.5 µmol), benzonitrile (1 mL), 2 min (microwave oven).

*[^{18}F]Biphenyl **3***

The reactions of [^{18}F]fluorophenyliodide **2** with either phenylmagnesium bromide **5b** or triphenylstannane **5a** in the presence of tetrakistriphenylphosphine palladium **4a**[12] or benzylchlorobis(triphenylphosphine)dipalladium[13] **4e** have been compared. The results presented in table 1 showed that the highest yields are obtained when HMPA and **4a** were used as solvent and catalyst respectively. [^{18}F]Biphenyl **3** was identified by HPLC [µ-porasil, heptane, flow : 2 $mLmn^{-1}$, λ : 254 nm].

Table 1 : Cross-coupling reactions of **2** with **5a** or **5b**

Ph-M	Solvent	Catalyst (%µmol) (a)	Isolated Radiochemical Yield (%) (b)
Ph-MgBr	THF	**4a** (2.2)	0
	THF	**4a** (1.9)	20
	THF	**4a** (0.9)	53
Ph-$SnPh_3$	THF	**4a** (0.7)	0
	THF	**4e** (0.7)	15
	HMPA	**4a** (0.7)	86
	HMPA	**4e** (0.7)	80

(a) in % versus **5a** or **5b**

(b) from 4-[^{18}F]fluoroiodobenzene **2**

*4-[^{18}F]fluorophenylethene **1** via decarbonylation of 2-[^{18}F]fluoro-5-vinylbenzaldehyde **6***

A second approach to the synthesis of 4-[^{18}F]fluorophenylalkenes based on the easy [^{18}F]fluorination of an 2-nitrobenzaldehyde[14] and the possibility of removing the aldehyde group by decarbonylation[15,16] was envisaged. 2-[^{18}F]fluoro-5-vinylbenzaldehyde **6** (Rf = 0.46, heptane:AcOEt 50:50 v/v) prepared in 33% yield (not optimized) from its nitro precursor **7**[17] [K^{18}F/K$_{222}$, DMSO (1 mL), 150°C, 15 min],was treated with tris(triphenylphosphine)rhodium chloride in benzonitrile for 2 min in a microwave oven. The [^{18}F]fluorophenylethene **1** was obtained in 70% yield from the aldehyde **6** (23% overall yield from [^{18}F]fluoride, and 80 min total synthesis time).

Work is in progress to improve both the yields and specific radioactivities and to synthesize polyfunctionnal compounds.

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A study of α,α,α-Trifluorotoluenes Mediated [^{18}F]Fluoro-for-Nitro Exchange. A Useful Intermediate in the Synthesis of Highly Fluorinated Radiopharmaceuticals.

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The high lipophilicity and the strong electron-withdrawing effect of the trifluoromethyl group make aryltrifluoromethyl derivatives a desirable moiety in the development of novel pharmaceuticals.[1] Three recent examples are the serotonin[2] agonist 1 and the nonpeptidal antagonists[3] 2 and 3 of substance P (Figure 1). It has been demonstrated in some cases that biological activity does not significantly change upon replacing one hydrogen atom with a fluorine atom.[4] Therefore, development of an efficient way of labeling drugs containing both a trifluoromethyl group and a fluorine-18 directly substituted on an aromatic ring may be an interesting approach to radiopharmaceutical design. [^{18}F]fluoro-for-nitro exchange is an important method for the labeling of various radiopharmaceuticals. In order for this reaction to be effective, the aromatic ring must be activated to nucleophilic attack. This has been well demonstrated[5] by successful radiolabeling of nitroacetophenone and nitrobenzonitrile. To extend this approach we have evaluated ^{18}F radiolabeling of aromatic nitro compounds that contain a trifluoromethyl substituent.

Reaction of resolubilized [^{18}F]fluoride ion with 2-nitro-α,α,α-trifluorotoluene (4a) (microwave, 2 minutes) gave 2-[^{18}F]fluoro-α,α,α-trifluorotoluene (5a) in 80% radiochemical yield (Figure 2). No isotopic exchange[6] was observed by HPLC. The yield was less impressive for the para position, and in this case because of the lower reactivity of the nitro group we also observed 1-4% isotopic exchange. As with many other electron-withdrawing groups, the nitro at the meta position did not react at all. Addition of another electron-withdrawing group at the meta position as in compound 4d activated the nitro group and the yield increased to 80%, while no isotopic exchange was observed. Product 5d is a possible synthon for the potent serotonin agonist 1.

Encouraged by these results, we turned our attention toward the synthesis of compound 9 , a model for the antagonist 2. Reaction of 2-chloro-5-nitrobenzaldehyde (6) with dibromodifluoromethane[7] in the presence of copper gave the 5-nitro-2-trifluoromethyl benzaldehyde (7) in 70% yield (Figure 3). The latter was reacted with K^{18}F/kryptofix in an conventional microwave for 2.5 min to give the desired 5-[^{18}F]fluoro-2-trifluoromethyl benzaldehyde (8) in 75% radiochemical yield, and the compound was readily purified on a C18 Sep Pak. Reductive alkylation with sec-butylamine and $NaCNBH_3$ produced

compound 9 in 65% radiochemical yield. We attempted to incorporate the ^{18}F at the final step by radiolabeling the imine 10. However, this gave a low yield of 11(20%), an observation similar to those obtained for the simple toluenes. In summary, we have evaluated the ability of the aryltrifluoromethyl derivatives to mediate [^{18}F]fluoro-for-nitro exchange in simple trifluorotoluenes and have synthesized a model compound that has potential as a novel radiopharmaceutical .

This research was supported by the National Institutes of Health (PO1-HL-13851) and the Fulbright Commission (Scholarship to E.M.).

Figure1

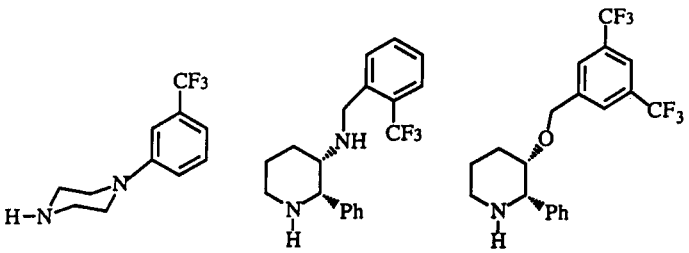
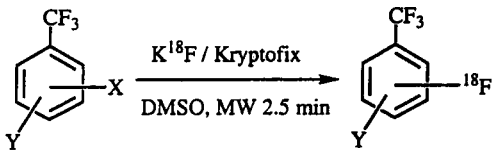
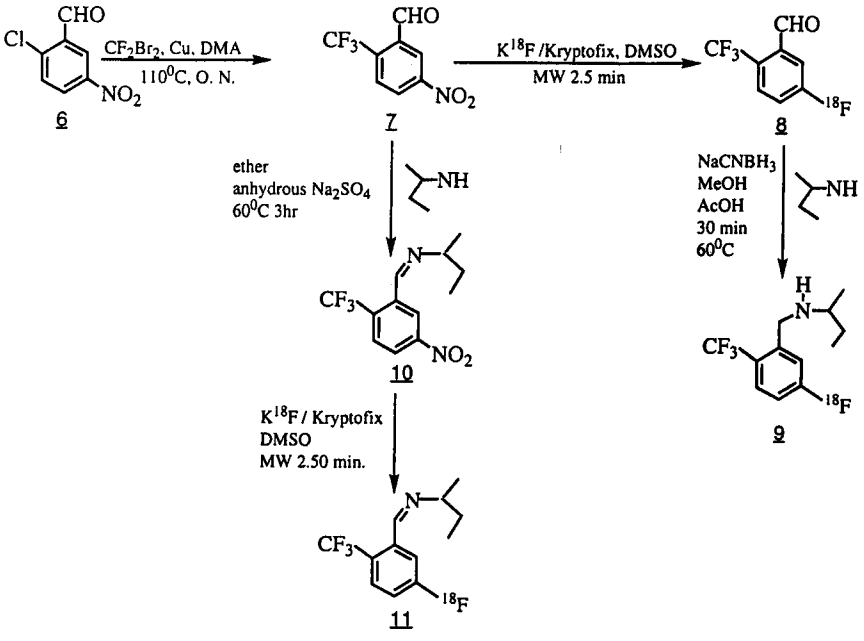


Figure 2



4a : X = o-NO_2, Y = H	5a 80% yield 97% radiochemical purity
4b : X = p-NO_2, Y = H	5b 15% yield 80% radiochemical purity
4c : X = m-NO_2, Y = H	no reaction
4d : X = m-NO_2, Y = m-NO_2	5d 80% yield 97% radiochemical purity

Figure 3



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Labelling of the CFC-alternative, 2*H*-Heptafluoropropane (HFC 227ea), with Fluorine-18

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2*H*-Heptafluoropropane (HFC 227ea) is proposed as an alternative to ozone-depleting bromofluorocarbons (Halons) and chlorofluorocarbons (CFCs) in several applications, including their use as fire extinguishants and aerosol propellants. This compound is also being considered as a propellant in metered-dose inhalers for the administration of drugs. This raises questions about the biological fate and distribution of HFC 227ea when inhaled by humans. Our recent study (1) on the CFC-replacement, 1,1,1,2-tetrafluoroethane, suggests that the disposition of HFC 227ea *in vivo* could be investigated by whole-body γ-counting after labelling the fluorocarbon with positron-emitting, fluorine-18 ($t_{1/2}$ = 109.7 min). Thus, it was of interest to develop a method for labelling HFC 227ea in high radiochemical yield, radiochemical purity and chemical purity with fluorine-18.

The proton-irradiation of ^{18}O-enriched water is an efficient route to high radioactivities of [^{18}F]fluoride at high specific radioactivity (2). We have investigated several approaches to label HFC 227ea with fluorine-18, based on reactions of [^{18}F]fluoride in the presence of potassium cation-aminopolyether 2.2.2 (K^+-APE 2.2.2), a powerful radiofluoridation reagent, with various fluorocarbon substrates.

Reactions were performed with or without acetonitrile as solvent in a sealed glassy carbon vessel under nitrogen (3). Volatile products were analyzed by radio-GC on either, *i*) a 60/80 Carbopack B/5% Fluorcol SP alloy column (10 ft. x 1/8 in. o.d.; Supelco Ltd.) operated with helium (30 ml/min; 30 p.s.i.) and the oven programme: hold 15 °C for 10 min; raise 5 °C/min to 100 °C; hold 30 min (retention times: hexafluoropropene, 7.7 min; HFC 227ea, 10.9 min; perfluoro-isohex-2-ene, 29.6 min; 1-iodo-2*H*-hexafluoropropane, 35 min) or *ii*) a Carboxen-1000 stainless-steel column (3.05 m x 1/8 in. o.d.; Supelco Ltd.) operated isothermally at 225 °C with helium (60 ml/min; 60 p.s.i.) (retention times: hexafluoropropene, 14.3 min; HFC 227ea, 24.8 min). GC-MS, equipped with a B.P. 1 column (5 mm thick dimethyl siloxane, 25 mm X 0.33 mm i.d.; SGE Ltd.) and quadrupole spectrometer, was also used for analysis.

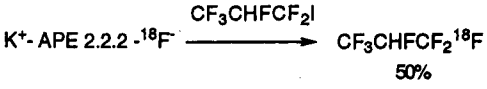
Nucleophilic substitution reactions were considered first. Reaction of 2*H*-hexafluoroisopropyl tosylate (30 mg,) with K^+-APE 2.2.2-[^{18}F]fluoride in acetonitrile (400 μL) (30 min, 100 °C; N_2, 30 p.s.i.) gave [^{18}F]HFC 227ea in less than 2% radiochemical yield (decay-corrected) and three other unidentified volatile radioactive products (12%) (Scheme 1).

$$K^+\text{-APE 2.2.2-}{}^{18}F^- \xrightarrow{(CF_3)_2CHOTs} \underset{2\%}{(CF_3)_2CH{}^{18}F} + \underset{12\%}{\text{other radioactive products}}$$

Scheme 1: Reaction of K^+-APE 2.2.2-[^{18}F]fluoride with 2*H*-hexafluoroisopropyl tosylate.

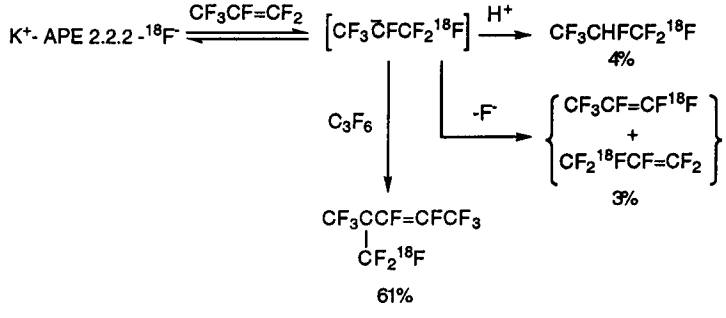
By contrast, the reaction of 1-iodo-2*H*-hexafluoropropane with K^+-APE

2.2.2-[^{18}F]fluoride in acetonitrile (400 μL) (30 min, 50°C; N_2, 50 p.s.i.) gave [^{18}F]HFC 227ea in high radiochemical yield (50%, decay-corrected). No other volatile radioactive product was observed (Scheme 2).



Scheme 2: Reaction of K$^+$-APE 2.2.2-[^{18}F]fluoride with 1-iodo-2*H*-hexafluoropropane.

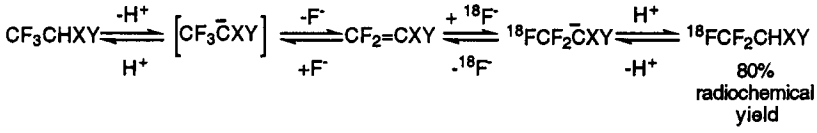
The reaction of [^{18}F]fluoride with trifluoroethylene gives [1-^{18}F]1,1,1,2-tetrafluoroethane in high radiochemical yield; the reaction is tantamount to 'hydrogen [^{18}F]fluoride addition' (4). However, in our hands the analogous reaction of hexafluoropropene with K$^+$-APE 2.2.2-[^{18}F]fluoride in acetonitrile (400 μL) at 95 °C for 30 min gave a mixture of volatile radioactive products (80.6%; decay-corrected), consisting of [^{18}F]HFC 227ea (10%), [^{18}F]hexafluoropropene (28%), [^{18}F]perfluoro-isohex-2-ene (76%) and an unknown (6%). These observations are consistent with an equilibrium operating between hexafluoropropene and the carbanion formed by addition of [^{18}F]fluoride (Scheme 3). This carbanion is presumed to react further with hexafluoropropene to give the major radioactive product, [^{18}F]perfluoro-isohex-2-ene, or to eliminate fluoride to give [^{18}F]HFC 227ea as a minor product. Fluoride-induced dimerisations of hexafluoropropene are well-known (5). However, Finch *et al.* (6) recently report that the reaction of [^{18}F]fluoride with hexafluoropropene, using a high temperature (160°C) and short reaction time (5 min), gives [^{18}F]HFC 227ea in high radiochemical yield and purity,



Scheme 3: The reaction of K$^+$-APE 2.2.2-[^{18}F]fluoride with hexafluoropropene.

Several 1,1,1-trifluoroalkanes can incorporate fluorine-18 by exchange with K$^+$-APE 2.2.2-[^{18}F]fluoride (3,7,8). The mechanism almost certainly involves deprotonation of the hydrofluorocarbon followed by fluoride loss from the trifluoromethyl group of the generated carbanion, with the reverse sequence resulting in the incorporation of [^{18}F]fluoride (7,8) (Scheme 4). Exchange is favoured when the intermediate carbanion is stabilised (7) and able to extract a

proton from the reaction milieu. The trifluoromethyl group is highly electron-withdrawing and can stabilise the intermediate carbanion (9). Stabilisation increases with the number of trifluoromethyl or other electron-withdrawing substituents bonded to the carbon bearing the formal negative charge (7–9) Exceptionally, the strongly electronegative fluoro group is weakly effective at stabilising an intermediate carbanion because of its opposing mesomeric effect (5,7). Thus, it was of interest to test whether HFC 227ea, which has two trifluoromethyl and one fluoro group bonded to a single carbon atom, could be labelled with fluorine-18 by exchange.



Scheme 4: Reaction of K⁺-APE-2.2.2-[^{18}F]fluoride with 1,1,1-trifluoroalkanes.

The reaction of HFC 227ea (40 p.s.i.; ca. 110 mg) with K⁺-APE 2.2.2-[^{18}F]fluoride in acetonitrile (500 μL) at 100 °C for 30 min gave [^{18}F]HFC 227ea (81% radiochemical yield, decay-corrected) as the major (> 99%) volatile radioactive product. The above reaction was repeated without acetonitrile and incorporated 80% of the radioactivity (decay-corrected) into [^{18}F]HFC 227ea. The supposed mechanism implies that labelling is in the 1-position (3,4,10).

The exchange reaction without solvent was chosen for its simplicity and high radiochemical yield for the synthesis of high radioactivities of pure [1-^{18}F]HFC 227ea. A lead-shielded automated apparatus including preparative GC, previously developed for the synthesis of [1-^{18}F]1,1,1,2-tetrafluoroethane [3], was modified with respect to GC conditions for the production of high radioactivities of [1-^{18}F]HFC 227ea. [1-^{18}F]HFC 227ea was produced in 80 min with a radiochemical purity of greater than 99% and a chemical purity of 98.4%. This product is suitable for the study of the disposition of HFA 227ea *in vivo* by whole-body γ-counting. The same apparatus could be used to produce pure no-carrier-added product by the reaction of [^{18}F]fluoride with 1-iodo-2*H*-hexafluoropropane.

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Halogen Activation of the Aromatic Ring. A new Approach for the Synthesis of 2- and 3-[^{18}F]Fluoroalkylarenes ?

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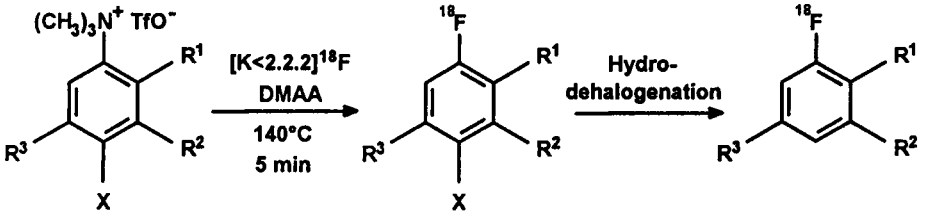
The N,N,N-trimethylammonium moiety is often used as a potent and useful leaving group in the synthesis of fluorine-18 labeled aromatic compounds. Based on earlier results (1) a ^{18}F-for-N$^+$(CH$_3$)$_3$ substitution on 4-iodo-2- or 4-iodo-2-alkyl-N,N,N-trimethylanilinium triflates followed by a hydro-dehalogenation might be a promising synthetic pathway for the synthesis of 2- and 3-[^{18}F]fluoroalkylarenes (Scheme 1). However, recent studies of the ^{18}F-for-N$^+$(CH$_3$)$_3$ substitution on halogen activated alkylarene precursors were unable to confirm this assumption.



X = Cl, Br, I R^2 = H, CH$_3$, C$_2$H$_5$, CH$_2$COOEt,
R^1 = H, CH$_3$ R^3 = H, OCH$_3$

Scheme 1: Synthesis of 2- and 3-[^{18}F]fluoroalkylarenes via halogen activation of the aromatic ring

The fluorination reaction was performed in dimethylacetamide (DMAA) at 140°C within 5 min and in the presence of [K⊂2.2.2]$_2$CO$_3$ cryptate as [^{18}F]fluoride activator. Under these conditions the ^{18}F-for-N$^+$(CH$_3$)$_3$ substitution on 4-iodo-3-methylphenyl-N,N,N-trimethylanilinium triflate led to 3-[^{18}F]fluoro-6-iodotoluene in apparent radiochemical yields (RCY) of about 45%, based on the solubilized activity (EOS). The separation of the labeled compound by solid phase extraction on a SEP-PAK C-18 cartridge led to a real preparative RCY of only 9%. This remarkable difference is mainly due to the formation of volatile [^{18}F]CH$_3$F which is a well known byproduct in the case of a ^{18}F-for-N$^+$(CH$_3$)$_3$ substitution on N,N,N-trimethylanilinium salts (2, 3)

and which escapes total activity measurement, unless special precautions are taken. Additional evidence is given by recent gaschromatographic studies of the preparation of 4-[^{18}F]fluorophenyllithium via 4-[^{18}F]fluoro-iodobenzene (cf. (4)) where 15 to 23% and 70 to 80% were found for 4-[^{18}F]fluoro-iodobenzene and methyl [^{18}F]fluoride, respectively. Thus, the previously reported yields (1, 4) must be revised downward by a factor of 3 to 5.

Variation in the type, size and position of the alkyl substituent led to a further reduction of the radiochemical yields, corresponding to the increasing electron density in the aromatic ring. For example, with 2-[^{18}F]fluoro-5-iodotoluene and 3-[^{18}F]fluoro-2-iodo-4-methylanisole radiochemical yields of about 7% were obtained, whereas for 2-ethyl-4-[^{18}F]fluoro-iodobenzene a radiochemical yields of 5% was found. With respect to functionalized aliphatic side chains, the synthesis of 3-[^{18}F]fluoro-2-iodophenylacetic acid ethyl ester was investigated. However, in all cases the radiochemical yields were below 1%.

In order to study the possibility of hydro-dehalogenation, the extracted organic compounds were eluted from the SEP-PAK C-18 cartridge with 1 mL acetonitrile or 1,4-dioxane. The dehalogenation step was performed with sodium borohydride (5), sodium cyanoborohydride (6) or tri-n-butyltinhydride (7) because of their different strenght of reduction. The variation of the reductive potential was necessary in order to prevent the reduction of functionalized aliphatic side chains (cf. Scheme 1).

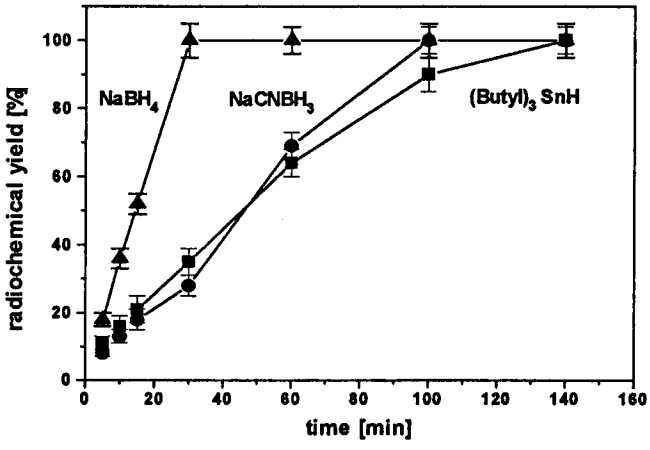


Fig. 1: Influence of the reduction agent on the radiochemical yield of n.c.a. 3-[^{18}F]fluorotoluene (based on 3-[^{18}F]fluoro-2-iodotoluene). Reaction conditions : [hydro-deiodination agent] 35 mmol/L, uv-activation (λ = 254nm), room temperature, 1 mL acetonitrile.

A fast and quantitative hydro-dehalogenation of 3-[^{18}F]fluoro-2-iodotoluene was found in the case of sodium borohydride (100% transformation within 30 min). Because $NaBH_4$ is known to reduce aldehydes or keto groups to primary and secondary alcohols, comparative studies were done using sodium cyanoborohydride and tri-n-butyltinhydride in order to determine mild reaction conditions (cf. Fig. 1). Sodium cyanoborohydride as well as tri-n-butyltinhydride led to a quantitative hydro-dehalogenation of 3-[^{18}F]fluoro-2-iodo-toluene within 140 min. The variation of the hydride concentration in the reaction mixture showed that a tenfold excess of the hydride was necessary for quantitative dehalogenation within 15 min (concentration based on the amount of N,N-dimethylaniline derivative as macroscopic side product in the solution).

The influence of thermal vs photolytic activation for reduction of 3-[^{18}F]fluoro-2-iodo-toluene on the radiochemical yield of 3-[^{18}F]fluorotoluene was studied. Using thermal activation a quantitative hydro-deiodination was obtained within 15 min, whereas photolytic activation yielded about 80% based on the starting activity of n.c.a. 3-[^{18}F]fluoro-2-iodotoluene.

Although, the reductive deiodination can be performed quantitatively, the overall radiochemical yield (< 10 %) appeares too low for radiolabeling purposes. Preparation of n.c.a. 2- and 3-[^{18}F]fluoroalkylarenes via Scheme 1. depends on improvement of the first reaction step in order to be comparative to multi step synthetic methods.

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RADIOSYNTHESIS OF 4-[^{18}F] FLUORONAPHTHOL DERIVATIVE

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We recently reported a Baeyer-Villiger reaction with m-chloroperbenzoic acid (MCPBA) and trifluoroacetic acid (TFAA) as catalyst to obtain [^{18}F]fluorophenol from a series of [^{18}F] non-symmetrical ketones[1]. The success of this method was extended to more complex structures such as [^{18}F]naphthol derivative. Compound **1** was prepared in a two step sequence that began with a nucleophilic substitution with [^{18}F]fluoride followed by the oxidation reaction (scheme 1).

With an interest to maximize the synthetic potential of this reaction, we have investigated the effect of different activating groups (nitro or trialkylammonium), on the aromatic ring, towards the nucleophilic reaction on the yield of the [^{18}F] fluoride displacement. Various conditions were explored with n.c.a [^{18}F] fluoride and the optimal reaction conditions for the fluoration were found to be: 160°C over 20min with 15mg of **3a** (**3b** : 140°C)as precursor and DMSO as solvent. These conditions gave 25% and 45% as radiochemical yields of crude product **2** from **3a** and **3b** respectively, assessed by radioTLC. The work up of these compounds on a C18 Sep-Pak followed by a Si Sep-Pak purification gave radiochemically pure yields of **2** in 5-7% and 20-25% respectively. Compound **3a** was prepared in three steps from 5,6,7,8 tetrahydro-1-naphthol **4** (nitration, alkylation and Heck reaction) and **3b** was obtained from **3a** by reduction and N-alkylation of the nitro group.

The oxidation reaction of **2** was carried out in a sealed reactor at 100°C for 15min with MCPBA (100mg) and CF_3COOH (200µl) in dichloromethane. After

cooling, the solution was washed with water. KOH (1M) was added, the mixture heated at 40-50°C for 10min , neutralized to pH 7-8 and extracted with dichloromethane. Compound **1** was isolated in 25-40% radiochemical yield from **2**, analyzed by radioTLC, coeluted with authentic sample and counted. Work is now in progress to apply this procedure to a biologically active compound which possesses this structure and presents a great interest in TEP.

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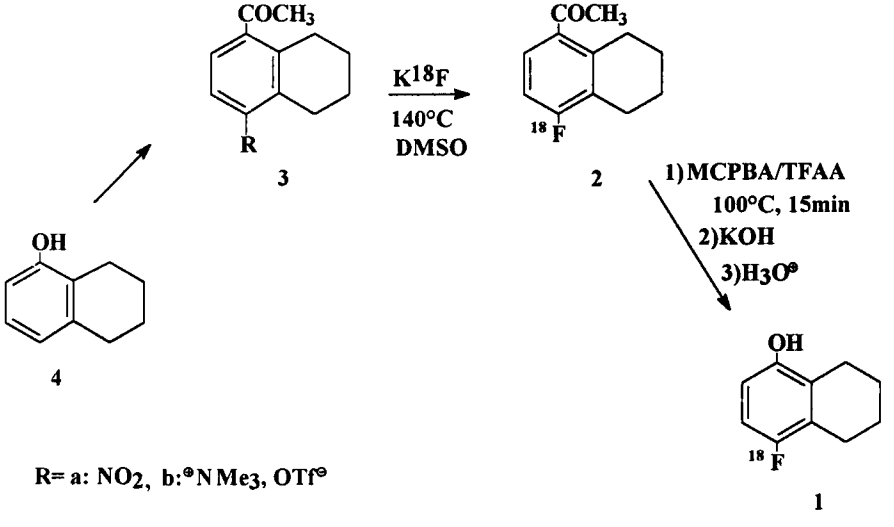

SCHEME 1

Synthesis of α-[^{18}F]Fluoro-ß-alanine via a nucleophilic Route.

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Study of the pharmacokinetics of the anti-cancer drug 5-Fluorouracil (5FU) is an area of current interest especially in view of the use of this agent in combination with modulators such as Folinic acid , PALA and Interferron in order to increase it's efficacy[1,2]. Positron Emission Tomography (PET)[3,4] has been used for such studies with the aim of optimising the use of this agent. However 5-FU is rapidly catabolised *in vivo* to α-fluoro-β-alanine (FBAL)[3,6] and "contamination" of the PET signal by this metabolite currently restrains data interpretation[4]. We have previously reported on the kinetics of [^{18}F]FBAL in rats, using [^{18}F]FBAL recovered from the urine of other rats which had been injected with 5[^{18}F]FU.[6] Here we report a synthesis of [^{18}F]FBAL, the availability of which will allow us to extend these studies to man to assist interpretation and modelling of data from PET studies with 5[^{18}F]FU.

The cold synthesis of α-fluoro-ß-alanine by fluorination of *N,N*-dibenzyl-isoserine benzyl ester using DAST to give *N,N*-dibenzyl-α-fluoro-ß-alanine benzyl ester followed by debenzylation using hydrogenolysis has previously been reported[7]. This route is tedious and unsuitable for synthesis of α-[^{18}F]fluoro-ß-alanine. Although [^{18}F]DAST has been labelled with fluorine-18, the synthesis is difficult and uses carrier added [^{18}F]fluorine[8]. Consequently we sought an alternative synthesis of α-[^{18}F]fluoro-ß-alanine via a nucleophilic route using no-carrier-added [^{18}F]fluoride.

Here we report a novel and rapid synthesis of α-[^{18}F]fluoro-ß-alanine by displacement of bromine from *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester, by cryptand activated [^{18}F]fluoride anion , to give *N,N*-dibenzyl-α-[^{18}F]fluoro-ß-alanine benzyl ester followed by a novel one step catalytic debenzylation to give α-[^{18}F]fluoro-ß-alanine.

The carboxyl and amino functions of isoserine (I) were simultaneously benzylated in a novel one step procedure as shown in Figure 1,

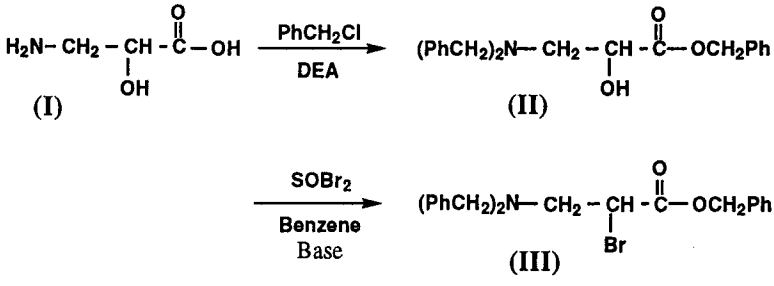
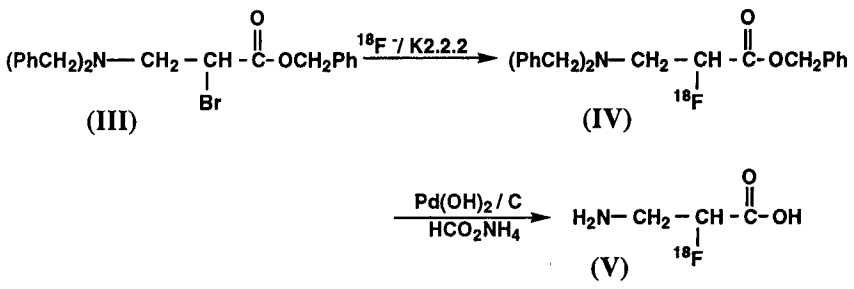


Figure 1. Synthesis of *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester (III) from isoserine (I).

The *N,N*-dibenzyl-isoserine benzyl ester (II) was then converted to *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester (III) by bromination with thionyl bromide as shown in Figure 1.



(III) (IV) (V)

Figure 2. Synthesis of α-[^{18}F]fluoro-ß-alanine.

Nucleophilic displacement of bromine from *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester (III) to give *N,N*-dibenzyl-α-[18F]fluoro-ß-alanine benzyl ester (IV) was achieved using [^{18}F]fluoride anion activated by Kryptofix 222 as shown in Figure 2. Incorporation of fluoride was 75 - 90%. The benzyl protecting groups were rapidly removed using a suspension of palladium hydroxide and ammonium formate in methanol. In preliminary experiments radiochemical yields of [^{18}F]FBAL of 18 - 24% were obtained. In the cold reaction yields of FBAL were in excess of 60% and work is in progress to optimize the radiochemical yield by incorporating a preliminary clean-up before debenzylation.

N,N-dibenzyl-isoserine benzyl ester (II)

Benzyl chloride (28 ml, 242 mmol) was added slowly over 25 minutes dropwise to a boiling solution of (*R,S*)-isoserine (1.25g,11.9mmol) in ethanol (85ml), containing diisopropylethylamine (16ml, 92mmol) and distilled water (40ml), with stirring. The mixture was refluxed and was found by mass spectrometry and tlc to go to completion within 1 hour.. Extraction with chloroform and vacuum distillation (9.5mmHg) at 130°-135°C to remove benzyl chloride and base left a clear, viscous liquid which was found to be *N,N*-dibenzyl-isoserine benzyl ester in good yield (75-80%). Mass spectrometry: CI+ve mode, *m/z* = (210) $[(PhCH_2)_2NCH_2]^+$ and (376) $[M+H]^+$. EI mode, *m/z* = (210) $[(PhCH_2)_2NCH_2]^+$. ^{13}CNMR ($CDCl_3$) δ 56.18($\underline{C}H_2CH$), 58.60($(Ph\underline{C}H_2)_2$), 66.96($Ph\underline{C}H_2O$), 69.18($\underline{C}HOH$), 127.24, 128.34, 128.38, 128.55, 128.81, 129.05, 135.25 and 138.48, (Aromatic) and 173.39ppm ($\underline{C}O$).

N,N-dibenzyl-α-bromo-ß-alanine benzyl ester (III)

Thionyl bromide (220 µL, 2.8 mmol) was added to a stirred solution of *N,N*-dibenzyl-isoserine benzyl ester (980mg, 2.6mmol), in dry benzene (20 mL) containing pentamethylpiperidine (510 µL; 2.8 mmol) and pyridinium hydrobromide (12 mg, 0.07 mmol), at 7 °C. After removal of a precipitate of pentamethylpiperidinium hydrobromide, N,N-dibenzyl-α-bromo-ß-alanine benzyl ester was extracted into ethyl acetate (40 mL) as the hydrobromide, converted to the free base by addition of triethylamine (150 µL). Extraction with hexane (30 mL) followed by removal of solvent gave *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester as a clear viscous liquid (746 mg, 65% yield).

Mass spectometry: CI +ve mode, *m/z* = (210) $[(PhCH_2)_2NCH_2]^+$ and (438, 440) $[M+H]^+$. EI mode, *m/z* = (210) $[(PhCH_2)_2NCH_2]^+$. ^{13}C NMR ($CDCl_3$) δ 42.57($\underline{C}HBr$), 58.40($\underline{C}H_2CH$), 58.92($(Ph\underline{C}H_2)_2$), 67.50($Ph\underline{C}H_2O$), 127.4, 128.4, 128.4, 128.5, 128.67 129.1, 135.39; and 138.6 (Aromatic) and 169.35ppm ($\underline{C}O$).

N,N-dibenzyl-α-[^{18}F]fluoro-ß-alanine benzyl ester (IV)

Cyclotron-produced nca [^{18}F]fluoride in ^{18}O-enriched (20%) water was adsorbed onto an anion exchange resin (Biorad AG1X8, carbonate form) and eluted with potassium chloride solution (0.03mol dm^{-3}). This solution was added to a platinum crucible containing Kryptofix 2.2.2 (26mg). The water was azeotropically removed by adding and evaporating off dry

acetonitrile (3 x 2mL). A solution of *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester (6mg) in acetonitrile (2mL) was then added to the crucible, which was heated at 120°C for 15min. The progress of the reaction was monitored by autoradiography and tlc using authentic *N,N*-dibenzyl-α-fluoro-ß-alanine benzyl ester as standard and was found to go to completion within 15min to form *N,N*-dibenzyl-α-[^{18}F]fluoro-ß-alanine benzyl ester in radiochemical yields of 75 - 90% as determined by tlc (Silica ; methanol:triethylamine; 99:1; Rf 0.7).

α-[^{18}F]fluoro-ß-alanine (V)

After removal of acetonitrile from the reaction mixture a suspension of palladium hydroxide (20%) on carbon (3mg) and ammonium formate (60mg) in methanol (2mL) was added to the crucible containing *N,N*-dibenzyl-α-[^{18}F]fluoro-ß-alanine benzyl ester. The mixture was covered and heated at 120°C for 15min. α-[^{18}F]fluoro-ß-alanine (V) was produced in radiochemical yields of 18-24% as determined by tlc (Silica plate; methanol : triethylamine 99:1; R_F=0.4) and autoradiography with authentic α-fluoro-ß-alanine.

The work described was carried out starting from racemic isoserine. This produces racemic *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester and consequently racemic α-[^{18}F]fluoro-ß-alanine. An HPLC method has been successfully developed for chiral separation of the enantiomers of *N,N*-dibenzyl-α-bromo-ß-alanine benzyl ester using a Chiralcel OD semi-prep column eluted with hexane:isopropanol (99.7 : 0.3)). Work is in progress to synthesise (+) α-[^{18}F]fluoro-ß-alanine using the appropriate enantiomerically pure benzylated bromide which is required for human PET studies.

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THE ROUTINE SYNTHESIS OF FLUORINE-18 16α-FLUOROESTRADIOL: THE USE OF 3-METHOXYMETHYL-EPIESTRIOL-16β,17β-CYCLIC SULFATE AS THE STARTING SUBSTRATE.

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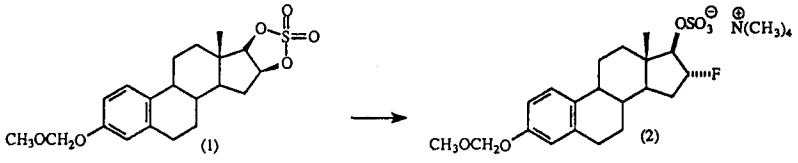
The use of 16β,17β-cyclic sulfate derivatives as substrates for the preparation of fluorine-18 labelled16α-fluoroestradiol has been reported before[1,2,3], with variations on the protecting group used at the phenolic three position being the primary variable discussed. The methoxymethyl derivative (1) at three has proved to be the most convenient protecting group and we would like to describe the progression of the use of this compound, from a developmental compound to the substrate of choice for the routine synthesis of fluorine-18 16α-fluoroestradiol for clinical use.



The synthesis of the methoxymethyl cyclic sulfate will be described elsewhere. The compound is a stable crystalline derivative. A sample has been kept at room temperature for more than a year without any change in properties. It reacts with tetramethyl ammonium fluoride in a quantitative fashion to give a stable crystalline tetramethyl ammonium salt (2). There is no sign of the alternative product of ring opening, the 17α-fluoro-16β-sulfate either by NMR or HPLC. Refluxing the salt in methanol with strong cation exchange resin for 90 minutes removes both 17-sulfate and the 3-methoxymethyl group to give 16α-fluoroestradiol identical to the literature compound[4].

Fluorine-18 is prepared by the $^{18}O(p,n)^{18}F$[5] reaction, using oxygen-18 water as target material and the fluoride is trapped on a anion exchange resin in the hydroxide form[6], eluted with an appropriate base and dried by azeotropic distillation with acetonitrile. Details of the target and isolation system will be described elsewhere. Fluorination was then performed in refluxing acetonitrile. The fluorination of the cyclic sulfate was examined using either elution of the column with 20 μmoles of potassium carbonate and subsequently complexing the potassium with [2,2,2]-Kryptofix[7] or elution with 20 μmoles of tetrabutyl ammonium hydroxide. The potassium carbonate typically elutes >95% of the fluoride from the column but consistent fluorination yields could only be obtained using amounts of the cyclic sulfate equivalent to the quantity of base, that is 20 μmoles. 20 μmoles of tetrabutyl ammonium hydroxide typically elute about 80% of the fluorine-18 from the ion exchange column. The amount of fluoride eluted is somewhat variable, but consistently high fluorination yields could be obtained using as little as 3 μmoles of the cyclic sulfate. The fluorination reaction is heated under argon for ten minutes at a bath temperature of 95°C, such that the acetonitrile will evaporate. When the solvent has evaporated the residue is dissolved in 0.75 mls of a solution of 1:1ethanol and 0.1 M ammonium acetate (the mobile phase for the HPLC column). This solution is loaded onto the HPLC column, a semi-prep Phenomenex C-18 column with 7 micron particles running at 5 ml min. The fluoride is eluted at 2 minutes and the product (2) comes off at 6 minutes. Unreacted cyclic sulfate is not eluted from the column under these conditions. Starting from a 10 minute

bombardment there are 2-5 mCi's in the fluoride fraction and 70-75 mCi's in the product fraction. Analytical HPLC shows the fluorosulfate salt (2) as single peak with <0.1% fluoride. There is no detectable UV absorption at 284 nm's, the UV max for the estradiol MOM ethers.

Removal of the protecting groups did not follow the macroscopic scale reaction. Refluxing with cation exchange resin quickly and cleanly removed the MOM group but did not remove the 17 sulfate, even after 90 minutes reflux in water. The probable difference between the two reactions is that the macroscopic hydrolysis of the sulfate may be autocatalytic in that a strong acid is produced by the hydrolysis. On the no carrier added scale this will not be a factor. Both groups are removed by refluxing for ten minutes in 20% HCl. However this reaction is not completely clean. The percentage fluoride rises from <0.1% to 2-3% and two additional organic radioactive peaks appear on the analytical HPLC. They comprise <5% of the total radioactivity. The solution is then neutralized with sodium hydroxide and sodium bicarbonate, put on a Sep-Pak, washed with water to remove the salts and the produced fluoride during the hydrolysis. The 16α-fluoroestradiol is washed off with 25% ethanol solution. This removes all but a trace of the 2 products produced by the hydrolysis.

This sequence produces 50-55 mCi's of the final product from a 10 minute bombardment. There are no detectable UV absorption peaks in the product and so we do not have a measured specific activity at this point. The minimum detectable amount would put the specific activity at >1 Curie per μmole. At this point we have not performed an estrogen bioassay on the produced solution so that we cannot give the total effective estrogen content of the solution.

Acknowledgments: This work was supported in part by Grant CA 42045 from the National Cancer Institute.

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Radiosynthesis of [^{18}F]Fluticasone Propionate for Lung Deposition Studies with PET

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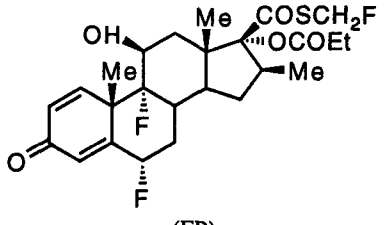
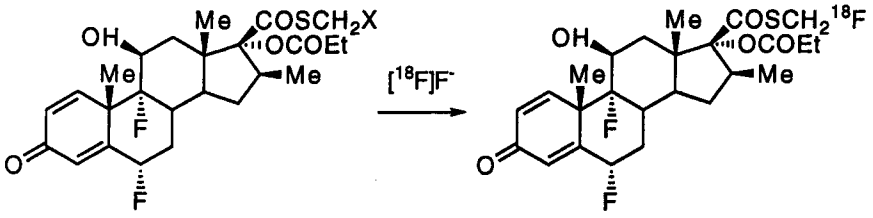
Fluticasone propionate [(*S*)-fluoromethyl 6α,9α-difluoro-11α-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)androsta-1,4-diene-17β-carbothioate, (FP)] is a potent (1) anti-inflammatory steroid with several therapeutic indications, including use as an anti-asthmatic when administered as sized particles by inhalation from a metered-dose inhaler (MDI) (2,3). Further information on the deposition of FP within the human lung and its subsequent pharmacokinetics is of interest (3). Positron emission tomography (PET) is a potentially powerful technique for the study of the deposition of FP in human lung, provided that the drug can be labelled isotopically with a positron-emitter and used to label FP particles uniformly within an MDI.



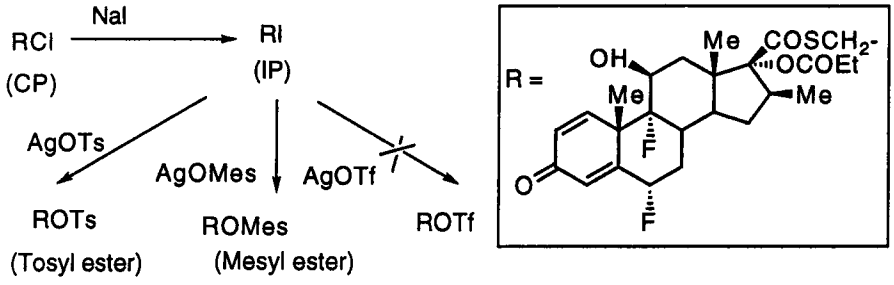
(FP)

FP contains three fluorine atoms. We chose to develop a method for labelling FP with positron-emitting fluorine-18 ($t_{1/2}$ = 109.6 min) rather than shorter-lived carbon-11 ($t_{1/2}$ = 20.4 min), since this allows more time for radiochemistry, formulation and data acquisition in PET studies. We sought to develop a labelling procedure from [^{18}F]fluoride, which can be produced in high radioactivity at a no carrier-added level of specific radioactivity by the proton irradiation of ^{18}O-enriched water (4). Labelling by nucleophilic substitution in the 6-position would require the difficult synthesis of an activated precursor and probably lack stereochemical control. Since the opening of an epoxide with anhydrous hydrogen fluoride is a standard procedure for the introduction of the *trans* 9α-fluoro-11β-hydroxy system into steroids, we originally explored the opening of the corresponding 9β,11β-epoxide with [^{18}F]fluoride under a variety of conditions, but without success. De Groot (5) has attempted similar reactions in progestins, also without success. We therefore considered labelling in the fluoromethyl group. In the preparation of FP, this fluorine is introduced by treating the 17β-carbothioic acid with bromofluoromethane or fluoroiodomethane (6). This approach could be considered for labelling with fluorine-18 since [^{18}F]bromofluoromethane can be prepared from [^{18}F]fluoride (7,8). However, we considered that a single step labelling procedure, based on nucleophilic displacement of a suitable leaving group (Scheme 1) would be preferred.



Scheme 1: Proposed radiosynthesis of [^{18}F]FP by nucleophilic substitution from [^{18}F]fluoride.

The known chloro analogue of FP, chloroticasone propionate (CP) (6), was used to prepare precursors with leaving groups suitable for nucleophilic displacement by [^{18}F]fluoride. The iodo analogue (iodoticasone propionate; IP) was first obtained by treating CP with sodium iodide in acetone (Scheme 2). Other precursors were then obtained by exchanging the iodo group for mesylate or tosylate by treatment with their silver salts in acetonitrile. However, all attempts to prepare the triflate from the iodo analogue by treatment with silver triflate failed. The synthesised precursors were fully characterised by ^{1}H- and ^{13}C-NMR spectroscopy, infrared spectroscopy, MS, TLC and elemental analysis.



Scheme 2: Synthesis of precursors for the preparation of [^{18}F]FP.

Radiochemistry was performed using 'no-carried-added' [^{18}F]fluoride produced by proton irradiation of ^{18}O-enriched water (*ca* 20% enrichment) (4). [^{18}F]Fluoride was recovered by adsorption onto an anion exchange resin (9). The resin was eluted with potassium iodide solution (0.095M) in preference to potassium carbonate solution, which due to its higher basicity, reacted with the FP precursors to form dimeric byproducts. The [^{18}F]fluoride was then converted into the powerfully nucleophilic radiofluorinating agent, K+-APE 2.2.2-[^{18}F]fluoride, in a glassy-carbon reaction vessel (9). This reagent was then reacted with the FP precursors (*ca* 10 mg) in acetonitrile (600 µL) at 100 ^{0}C under a nitrogen pressure of 20 psi for 30 min.

Products were analyzed by HPLC (Lichrosphere 100 RP-18, 5 µ column, 150 x 4.6 mm i.d.; eluted with acetonitrile-water (60:40 v/v) at 1.5 ml/min with eluate monitored for radioactivity and absorbance at 254 nm). [^{18}F]FP was identified by co-elution with a sample of authentic FP and decay-corrected radiochemical yields from [^{18}F]fluoride were measured (Table 1).

Table 1. Results from nucleophilic substitution reactions for the preparation of [^{18}F]FP.

FP precursor	Radiochemical yield of [^{18}F]FP (%)
Iodo	4– 5 (n = 4)
Mesyl ester	15–18 (n = 5)
Tosyl ester	18–25 (n = 8)

The highest radiochemical yield of [^{18}F]FP was obtained by substitution in the tosylate precursor. This method has been chosen for the routine production of [^{18}F]FP for which an automated radiosynthesis has been devised. The total preparation takes 110 min and gives [^{18}F]FP in 18–25% radiochemical yield, decay-corrected, with a specific radioactivity of 40 mCi/μmol at the end of synthesis (from an irradiation producing 12 mCi of [^{18}F]fluoride). The amount of carrier is 20 μg. The low specific radioactivity may be due to the production of fluoride carrier from the decomposition of the FP precursor during the reaction. A preparative HPLC method has been devised, based on the use of a Primesphere C18-HC, column (250 x 10 mm; 5 μ) eluted with acetonitrile-water (52: 48 v/v) at a flow rate of 9 mL/min. This has been found to give a chemical purity of > 95%.

A reaction of one equivalent of potassium fluoride with tosylate precursor in the presence of APE 2.2.2 gave fluticasone propionate after HPLC purification under the same conditions used for the preparation of [^{18}F]FP. This product was chemically pure as assessed by LC with diode array detector analysis of the UV spectrum and by LC-MS and LC-NMR.

Thus, an effective method has been developed for labelling FP with fluorine-18 in high activity, chemical purity and radiochemical purity. The radiosynthesis is now being automated to produce high radioactivities of [^{18}F]FP for introduction into FP particles within an MDI.

Acknowledgement. The authors are grateful to Professor D.S. Davies (RPMS) for his interest in this work, to Mr M.J. Daniel (GRD)for useful discussions and to Mr Colin J. Steel (MRC) for the production of [^{18}F]fluoride for these studies.

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PALLADIUM-MEDIATED COUPLING REACTIONS OF ^{18}F-LABELLED ARYLHALIDES WITH ORGANOTIN COMPOUNDS

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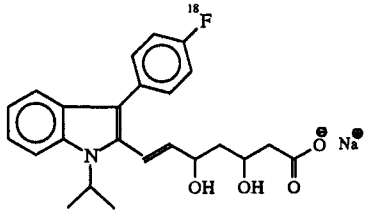
Palladium-promoted coupling reactions of organic halides with organotin compounds has been extensively used in carbon-carbon bond forming reactions.[1,2] These type of coupling reactions has previously been utilised in rapid labelling synthesis involving the short-lived radionuclide ^{11}C.[3] In development of the ^{18}F-labelled cholesterole inhibitor fluvastatine **1** for metabolic studies using the PET-technique, a mild incorporation of the label was required due to the presence of sensitive functional groups attached to the indole skeleton.



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The palladium-promoted cross-coupling of p-[^{18}F]fluorobromobenzene **2** with an indole trialkyltin derivative was considered as a possible route to prepare ^{18}F-labelled fluvastatine. With the intention to evaluate this metal-mediated approach, a number of model reactions were investigated, as examplified in Scheme 1.

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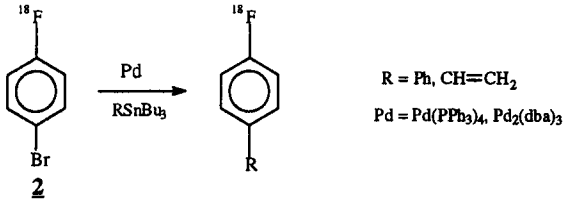

Scheme 1

Production of p-[^{18}F]fluorobromobenzene has previously been reported in the literature[4]. In the present study another approach to obtain **2** was investigated. The p-[^{18}F]fluorobromobenzene was produced from 3-bromo-6-nitrobensaldehyde via nucleophilic aromatic substitution with ^{18}F$^-$ and subsequent decarbonylation using Wilkinson's catalyst. In the decarbonylation reaction, a slight modification of the previously described procedure was used.[5] The p-[^{18}F]fluorobromobenzene was obtained in approximately 70% radiochemical yield as determined by analytical HPLC. Purification of p-[^{18}F]fluorobromobenzene was performed either by solid phase extraction or by distillation, which appears to be a promising purification procedure. The coupling reaction of the purified **2** with various organotin model compounds was investigated using different solvents such as DMF, dioxane, DMSO and toluene in temperatures between 60-130°C. The cross-coupled products were obtained in radiochemical yields between 40-90 % within 20 min reaction time. Work is now in progress to optimise the reaction conditions in the cross-coupling of p-[^{18}F]fluorobromobenzene with indole trialkyltin derivatives.

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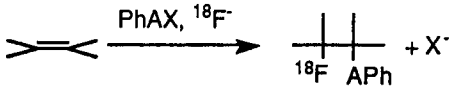
COMPARISON OF [^{18}F]FLUOROSULFENYLATION TO [^{18}F]FLUORO-SELENENYLATION. Hatano, Kentaro and Yanagisawa, Touru, Cyclotron Research Center, Iwate Medical University, Takizawa, Iwate Japan 020-01

In the last symposium we reported a new radiofluorinating reaction, [^{18}F]fluoroselenenylation, from no-carrier-added (NCA) [^{18}F]fluoride (1). This reaction consisted of *in situ* preparation of benzeneselenenyl [^{18}F]fluoride followed by addition reaction across double bond of alkene (Figure 1). [^{18}F]Fluoroselenide thus afforded was found to be converted to [^{18}F]fluoroalkene by oxidative desenenylation by ozone, however, radiochemical yield and reproducibility was not sufficiently high. In this study we examined analogous [^{18}F]fluorosulfenylation (Figure 1) as another novel labeling procedure. Fluorosulfenylation was found earlier than fluoroselenenylation (2) but the application was also limited (3, 4). It might, in part, be attributed to that products, fluorothioethers, could also be obtained *via* fluoro-Pummerer reaction of sulfoxides by diethylaminosulfur trifluoride (5).

Figure 1



A=S, Se X=Br, Cl

Benzenesulfenyl chloride was added dropwise to ice cooled suspension of different kind of [^{18}F]fluoride and alkene (4-allylanisole) in CH_2Cl_2. Reaction mixture was stirred for a subsequent hour at room temperature. Formation of [^{18}F]fluorothioether *via* addition reaction of *in situ* generalized benzenesulfenyl [^{18}F]fluoride across double bond was observed by radio-TLC. Regioselectivity of the reaction was also inspected by normal phase HPLC analysis. About 80% of product was Markovnikov adduct.

Table 1 Radiochemical Yields of [^{18}F]Fluorosulfenylation From Various [^{18}F]Fluorides

[^{18}F]Fluoride (Additive)	Radiochemical Yield (%)	
	[^{18}F]Fluorothioether	Byproduct
CA Ag[^{18}F] (AgF)	29.2	12.6
NCA Ag[^{18}F] ($AgOCOCH_3$)	1.4	3.0
NCA Ag[^{18}F] ($AgCO_3$)	No Reaction	
NCA [K/APE][^{18}F] (K_2CO_3, APE)	11.2	7.9
NCA [K/APE][^{18}F] ($KOCOCH_3$, APE)	2.9	-
NCA [K/APE][^{18}F] ($(KOCO)_2$, APE)	4.7	-

CA=Carrier-Added, NCA=No-Carrier-Added, APE=Aminopolyether (Kryptofix 222 (Merck, Germany)

Significant amount of polar byproduct was obtained together with desired product (Table 1). When carrier-added Ag[^{18}F] was used as labeling precursor radiochemical yield of [^{18}F]fluorosulfenylation was lower than that of [^{18}F]fluoroselenenylation. (Up to 61% of

[^{18}F]fluoroselenide was yielded under same reaction procedure.) But with NCA K[^{18}F] activated by aminopolyether (APE) yield of [^{18}F]fluorothioether was as good as corresponding selenide. More [^{18}F]fluorothioether would be obtained, provided the byproduction were reduced

Generally, selenides are easily converted to corresponding alkene *via* oxidation. Selenoxide fragmentation proceeds readily even at room temperature. In contrast desulfurization of sulfoxide could only be achieved under pyrolytic condition (6). The problem of [^{18}F]fluoroselenenylation was instability of product especially in high specific activity that led to unsuccessful yield of oxidative desenenylation. Therefore rather stable sulfur intermediate was considered advantageous. In addition labeled sulfoxides could also be further converted by Pummerer or other rearrangement reactions. Radiochemical yield of [^{18}F]fluorosulfenylation was comparable to that of [^{18}F]fluoroselenenylation with NCA [K/APE]$^{+}$[^{18}F]$^{-}$. From this data and forecasted stability and synthetic possibility of [^{18}F]fluorothioether it can be concluded that [^{18}F]fluorosulfenylation is superior to [^{18}F]fluoroselenenylation.

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4-[^{10}B]BORONO-2-[^{18}F]FLUORO-L-PHENYLALANINE-FRUCTOSE COMPLEX FOR USE IN TIMING BORON NEUTRON CAPTURE THERAPY (BNCT)

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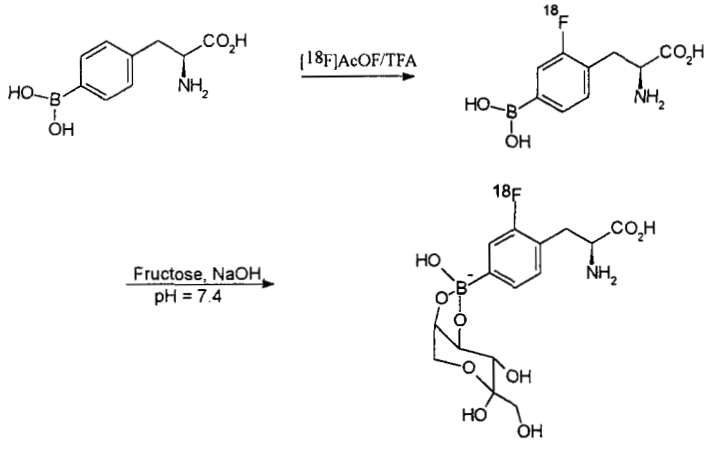
BNCT is a form of radiotherapy that relies on the selective concentration of boron-10 in the targeted tumors. 4-Boronophenylanine (BPA) has been successfully utilized to treat patients with glioblastoma multiforme and occular melanoma in Japan,[1] Australia[2] and, more recently, in the U.S. The complexation of BPA with fructose increases its solubility in solution,[3] at pH 7.4, from 1.8 mg/mL to about 100 mg/mL. In addition, the BPA-fructose complex improves both the tumor-to-blood and tumor-to-normal brain concentration ratios from 3:1 to 3.6:1.

Recently, 4-borono-2-[^{18}F]fluoro-L-phenylalanine (L-[^{18}F]FBPA) was prepared for evaluation as a tumor imaging agent.[4] It was discovered that the [^{18}F]FBPA uptake in melanoma was competitively reduced by BPA loading. It has been postulated that complexing [^{18}F]FBPA with fructose might enhance its tumor uptake. We wish to report the synthesis of the [^{18}F]FBPA-fructose complex (Scheme 1).

Scheme 1



[^{18}F]AcOF was bubbled into a solution of BPA•HCl (1) (100 μmol) in TFA (5 mL) at room temperature. After removal of the TFA, the residue was treated with fructose (45 mg), sodium hydroxide (0.25 mL, 0.24 *N*) and sodium bicarbonate buffer (pH = 7.4, 1.0 mL). The mixture was charged on to a C-18 Sep-Pak cartridge, eluted with water and 1.0 mL fractions were collected. The fractions containing the [^{18}F]FBPA-fructose complex were identified by reverse-phase chiral TLC (acetonitrile-methanol-water, 4:1:1) and by HPLC utilizing a reverse-phase column and an eluant mixture of methanol and 0.8% acetic acid (15:85) which contained EDTA (1.0 *mM*), sodium octylsulphate (1.0 *mM*) and sufficient sodium hydroxide to maintain pH = 4.0. The radiochemical yield of [^{18}F]FBPA was 45-55% corrected to EOB based on the [^{18}F]AcOF. The selectivity of [^{18}F]FBPA-fructose complex for tumor imaging with PET is currently being evaluated.

Acknowledgements: Research sponsored by The Department of Energy and The Robert H. Cole Foundation.

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3-[^{18}F]FLUOROBENZIDINE : A PRECURSOR TO FLUORINE-18 LABELED CONGO RED, A POTENTIAL IMAGING AGENT FOR ALZHEIMER'S DISEASE

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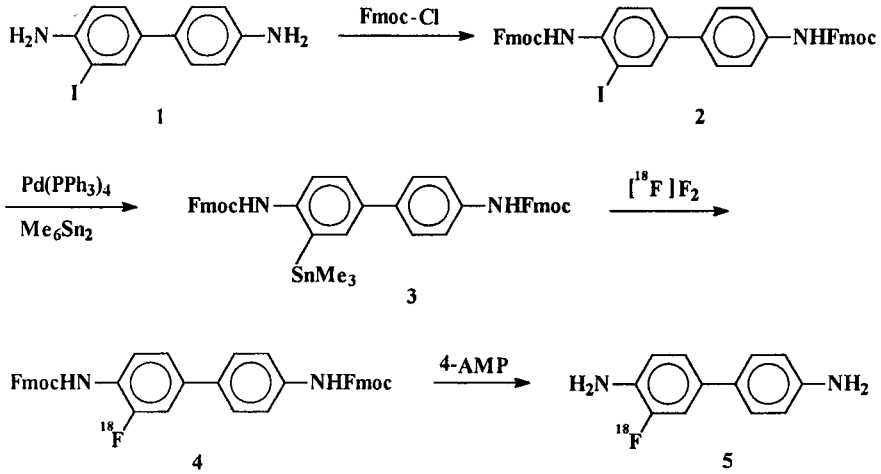
Alzheimer's disease (AD) is a neurodegenerative disorder in humans in which there is extensive brain damage caused by β-amyloid deposition. AD can be definitively diagnosed through the examination of amyloid in brain tissue using Congo Red (CR) staining.[1] The inability to assess amyloid deposition in AD until after death precludes the detailed study of this debilitating illness. It would be advantageous to develop a diagnostic marker to probe amyloid deposits using PET imaging. Based on the X-ray crystal structure of the CR-amyloid protein complex, Turnell and Finch have demonstrated that one molecule of CR intercalates between two anti-parallel β-strands of protein.[2] This suggests that CR derivatives may be effective agents for imaging amyloid deposits *in vivo*. Indeed, a radioiodinated CR derivative was synthesized and was found to localize in amyloid tissue.[3] We have synthesized fluorine-19 labeled CR and have demonstrated specific binding to amyloid protein, *in vitro*.[4] We now wish to report the synthesis of 3-[^{18}F]fluorobenzidine, a precursor to fluorine-18 labeled CR.

The synthesis is based on the electrophilic radiofluorination of a protected stannylated benzidine **3.** 3-Iodobenzidine, **1**, was used as the starting material. The amino groups of **1** were protected as Fmoc derivatives. [When formyl, acetyl or t-BOC functionalities were used as protecting groups, the resultant materials exhibited poor solubility in organic solvents.] Reaction of the iodo derivative, **2**, with hexamethylditin gave the stannylated benzidine, **3**. A chloroform solution of **3** was treated with [^{18}F]F_2 obtained from proton irradiation of gaseous [^{18}O]O_2. The resulting fluorine-18 labeled compound, **4**, was treated with 4-(aminomethyl)piperidine (4-AMP) and washed with acidic phosphate buffer. After purification by column chromatography, 3-[^{18}F]fluoro-benzidine, **5**, was obtained in 20% radiochemical yield. The synthesis, including fluori-nation and deprotection, required sixty minutes.

The conversion of **5**, to fluorine-18 labeled CR involves a diazotization followed by a coupling reaction using the sodium salt of 4-aminonaphthalenesulfonic acid. Preliminary studies indicate that this can be accomplished in less than thirty minutes.

Acknowledgement: Research sponsored by The Department of Energy and The Robert H. Cole Foundation.



1 2 3 4 5

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EVALUATION OF NEW PRECURSORS FOR THE ELECTROPHILIC SYNTHESIS OF 6-[^{18}F]FLUORODOPA

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Introduction

A number of methods for the electrophilic synthesis of 6-[^{18}F]Fluorodopa (FDOPA) have been reported in the literature (1-5). Among these, the regioselective fluorination of 3,4-dimethoxy-6-trifluoroacetoxy-mercurio-L-phenyl alanine ethyl ester **4a** with AcO^{18}F is reported to produce the highest yields (up to 11% decay corrected to EOB) (4). Despite the use of vigourous hydrolysis conditions (57% HI, 135 °C, 20 minutes), a common cause of yield losses using this method results from the incomplete hydrolysis of the methoxy protecting groups and subsequent preparative HPLC purification of the crude product. The use of more labile protecting groups and alternative hydrolysis conditions were therefore investigated with the aim of increasing the overall yield of this electophilic synthesis.

Methods

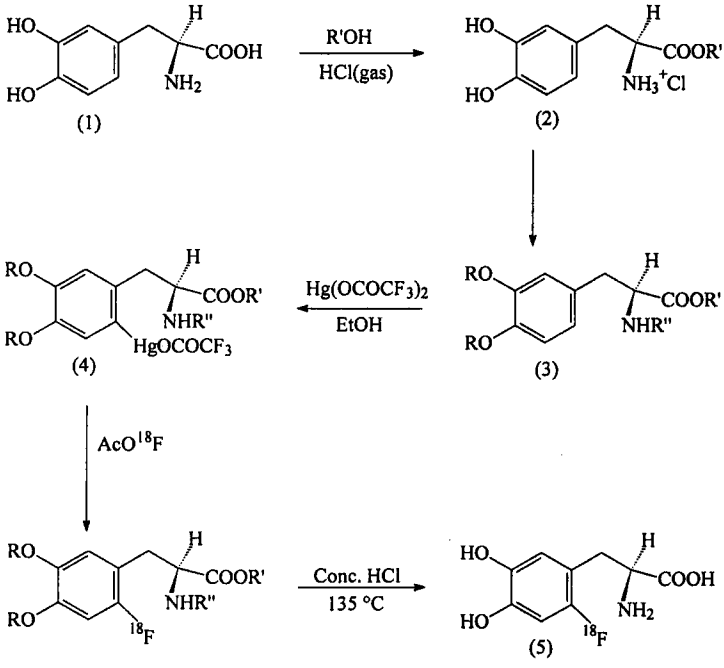
Precursors **4b-e** (see Fig 1) were prepared from L-DOPA (**1**) using the respective alcohols and hydrochloric acid gas to yield the ester **2b-e**. The aromatic hydroxy groups and the amine group were simultaneously protected by either reaction with di-t-butyl dicarbonate or with acetic anhydride to produce **3b-e**. Subsequent treatment of **3b-e** with mercuric trifluoroacetate in ethanol gave the respective mercurio derivatives **4b-e**. **4b-e** were labelled using [^{18}F]acetyl hypofluorite in $CHCl_3$ and subsequently passed over a Si SepPak to a hydrolysis vessel. After evaporation of the organic solvent, concentrated hydrochloric acid was added to the residue and heated at 135 °C for 20 minutes to produce ^{18}F-DOPA (**5**).

Figure 1

Cmpd.	R	R'	R''
a	Me	Et	$COCF_3$
b	^{t}Boc	Me	^{t}Boc
c	^{t}Boc	$CH(CH_3)_2$	^{t}Boc
d	Ac	Et	Ac
e	^{t}Boc	Et	Ac

Results

Preliminary studies indicate that by using substrates **3b,c**, radiochemically pure [^{18}F]FDOPA can be produced with a synthesis time of ca. 45 min (from EOB). Determination of radiochemical yields and the labelling of subtrates **4d,e** are currently under investigation. These initial results indicate that the yields of [^{18}F]FDOPA may be increased, and the synthetic procedure simplified by the careful choice of protecting groups in the labelling precursor. Work is also in progress to analyse the content of mercury, organics and other amino acids in the final product to determine wether the final HPLC purification step may be omitted.



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A strategy for the labeling of [F-18]-3'-deoxy-3'-fluorothymidine: [F-18]FLT.

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Key words: fluorine-18, nucleoside, neoplastic, DNA synthesis.

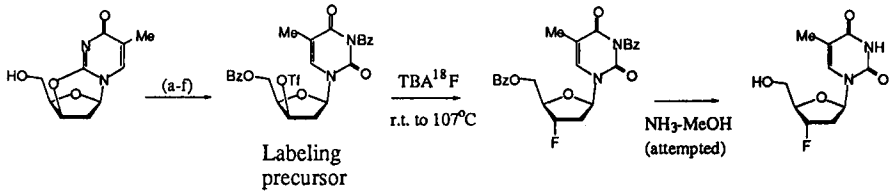
Introduction: The thymidine (TdR) analogs: 3'-deoxy-3'-fluorothymidine (FLT) and 3'-azido-3'-deoxythymidine (AZT) display some unique biochemical characteristics in vivo. While FLT and AZT are both 5'-phosphorylated by thymidine kinase (TK) and trapped in cells, they are also resistant to rapid nucleoside-glycosyl-bond cleavage by thymidine phosphorylase (TdR-Pase). FLT and AZT resistance to TdR-Pase sharply contrast to the behavior of injected thymidine--which is aggressively degraded by TdR-Pase in blood and some other tissues. This situation demands that imaging protocols using [C-11]TdR account for gross degradation of the tracer in competition with its bioactivation, and eventual incorporation into DNA. These considerations suggested to us that [F-18]FLT and [C-11]AZT could be useful [C-11]thymidine substitutes for imaging characteristics of DNA synthesis in vivo. The longer lived [F-18] label on FLT will allow us to image for longer periods than with [C-11] labeled analogs.

Labeling strategy: Our no-carrier-added radiosynthesis of [F-18]FLT was designed to overcome some pitfalls in the low yield (7%) method previously reported by Wilson et al.[1]. Our labeling precursor is a reactive triflate, with both the 5'-OH and the pyrimidine moiety of the nucleoside protected as benzoyl derivatives (see Fig 1). This contrasts with the earlier work which used the less reactive mesylate on C-3' and a pyrimidine ring unprotected, which is susceptible to ionization with carbonate ion. As well, a 5'-O-trityl-group was previously used and it required removal under strictly controlled acidic conditions to avoid competitive hydrolysis (glycosyl bond) of liberated FLT. Our strategy was to use benzoyl protecting groups that could be cleaved under mild basic conditions, thereby avoiding the acid sensitivity of FLT. As well, a benzoyl group on the pyrimidine ring precludes ionization of the N_3-H or its complexation with TBAF, in a similar way that TBAF can form strong H-bonded complexes with β-dicarbonyl structures [2] and promote N-3 alkylations of pyrimidines [3]. The potential for this type of fluoride complexation was suggested by our recent report of a crystalline TBAF complex with β-pseudothymidine [4].

Unlabeled studies: Our labeling precursor was prepared by a six step synthesis from 2-O,3'-anhydrothymidine (Fig. 1). Fluorination of the labeling precursor with TBAF at r.t. in d^3-MeCN and ^{19}F NMR observation showed a clean reaction to produce a fluorine product with a 16-line resonance (non-overlapping, d,d,d,d) centered at δ -177 ppm. These values are consistent with those reported by Wilson et al.[1] for FLT. This result led us to pursue our radiolabeling.

Radiolabeling method: The "triflate" labeling precursor (1.6 μmol/25 μL dry MeCN) was treated at r.t. with no-carrier-added [F-18]fluoride (s.a. 2-3Ci/μmol) in TBA-OH (1.25 μmol/50 μL dry MeCN) as a stirred reaction within a closed 1 mL glass V-vial. The progress of the reaction was monitored by radioTLC (SiO_2, A: 50% EtOAc/Hex and B: 20% MeOH/$CHCl_3$) (see Fig. 2). After 5 min at r.t. a non-polar product (15% total activity) had cleanly formed (Fig 2:A-tlc system-A), and we assign this to labeled and protected FLT. After 15 min at r.t. the activity distribution had not significantly changed. However, heating the reaction at 107°C for 2 min improved the product yield to 84% of the total activity (Fig 2:B-tlc system A; Fig 2:C-tlc system B). We next attempted a base promoted hydrolysis of the benzoyl protecting groups using 2M anhydrous ammonia in methanol (200 μL) for 15 min at rt then heating at 107°C for 5 min. Essentially complete conversion of the non-polar product to material with R_f=0 (same R_f as fluoride) had occurred (Fig 2:D-tlc system-B). Also, HPLC (C-18) analysis showed that no labeled FLT was present and that activity eluted in the void volume, suggestive of [F-18]fluoride was the labeled material. We believe that the hydrolysis

conditions were simply too harsh and that fluoride was eliminated from the first formed product. Hydrolysis with less concentrated ammonia at rt, or slightly above, should effect the benzoyl group cleavage without elimination of fluoride. We are encouraged by our preliminary results and are studying this radiosynthesis in more detail.



Reaction conditions (a-f): (a) LiOH, water, r.t, quant. (b) TBDMSiCl (2 eq), pyr/DMF, 79%; (c) BzCl, EtNPr$^i_2$, MeCN, reflux, 88% (d) TBAF/THF, r.t., 85%; (e) BzCl, pyr, 78%; (f) Tf_2O, 4-Me-2,6-diBu^t-pyr, CH_2Cl_2, -78°C to r.t., 56%.

FIGURE 1: Precursor synthesis and radiolabeling reactions for [F-18]FLT.

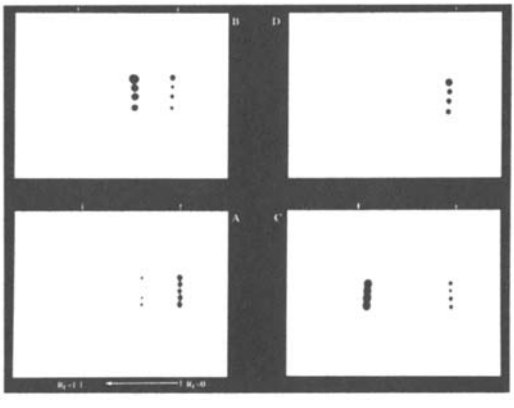


FIGURE 2: TLC-radiochromatograms of reaction mixtures during the radiosynthesis of [F-18]FLT.

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Radiosynthesis of (C-11)*methyl*-labeled AZT from (C-11)methyl iodide. John R. Grierson, Anthony F. Shields, Jeanne M. Link. Department of Radiology, School of Medicine, University of Washington, RC-05, Seattle, WA, 98195, Ph/FAX: (206) 548-6247; 543-3495.

Key words: C-11, methyl iodide, organometallic labeling, neoplastic, nucleoside, DNA synthesis.

Introduction: Carbon-11 labeled thymidine is a useful radiopharmaceutical for imaging DNA synthetic activity in vivo. Thymidine is specifically incorporated into DNA and its intermediate anabolites (dTMP, dTDP and dTTP) are retained in cells. However, a problem with using thymidine for PET imaging is that it enters into processes biochemical at a crossroads, where the larger share of the injected dose is shuttled off towards its irreversible degradation, whereas the balance of the dose, perhaps as little as 10-20%, is exclusively used to make DNA. This situation limits contrast between proliferating and non-proliferating tissues in images and complicates modeling. Other practical problems with thymidine use are its technically demanding labeling (e.g., 2-(C-11)-TdR) and detailing thymidine's metabolic breakdown within minutes of injection.

The pharmaceutical AZT (3'-azido-3'-deoxy-thymidine) is a structural mimic of thymidine, that is known to form 5'-phosphorylated nucleotides (AZTMP, AZTDP and AZTTP) within cells and can partially chain-terminate replicating DNA. As well, AZT is resistant to degradation by thymidine phosphorylase. The in vivo biochemistry of AZT, and the fact that it is a prescription drug, attracted us to label it with carbon-11.

Research: Our radiolabeling of AZT (Figure 1) adapts the method of (C-11)-methyl iodide alkylation of pyrimidyl dianions, developed for the (C-11)methyl labeling of thymidine[1,2]. Prior to this report the method had been exclusively reserved for thymidine syntheses. It was not known if the azido moiety would be tolerated during reactions with butyllithium. The labeling precursor for our radiosynthesis is 3'-azido-5-bromo-5'-(tert-butyldiphenylsilyl)-2',3'-dideoxy-1-β-D-*erythro*-pentofuranosyluracil (5-Br-5'-TBDPSi-dAZU).

Methods and Results: The protected bromo nucleoside was lithiated in dry THF (16 μmol/3 mL) with 2.0 equiv. of butyllithium at -78°C for 20 seconds then treated with carrier-added (C-11)-methyl iodide in THF (8 μmol/0.75 mL). The cold reaction was continued for 2 minutes then warmed to 0°C for 2 minutes. Addition of sat. aq. NH_4Cl (150 μL) to the reaction, then decantation and rapid filtration of the organic solution through a pipette column of silica gel afforded a crude product solution. Radio-TLC (SiO_2: 60% EtOAc/Hex) analysis of the product showed a major component with an R_f identical to a TBDP-silylated AZT standard. The crude product was desilylated with TBAF-THF (40 μmol, rt, 5 min) then the solvent was removed. The residue was extracted with 20% 0.1N NH_4OAc/MeOH (5 mL) and the mixture was then filtered through a 0.2 μm filter. The filtrate was analyzed by HPLC (C-18: see Figure 2). From the injected solution, 83% of the activity was recovered and 40% of the eluted activity was (C-11)AZT. Overall, a 7% decay-corrected yield of isolated (C-11)-AZT was calculated, with a synthesis time of 50 min.

(C-11)Methyl iodide was prepared by a method described in these proceedings (J. Link et al.), then trapped in cold (-78°C) THF containing carrier MeI. The labeling precursor was prepared by silylation of AZU with TBDPSiCl (pyr, reflux) and the silyl compound was brominated with LiBr/ceric ammonium nitrate in acetonitrile. Proof of structure for (C-11)-AZT was based on its HPLC identity with authentic unlabeled AZT and the results of a carbon-13 labeling reaction that used the above reaction conditions with 1.1 eq of (C-13)-MeI. Carbon-13 labeling yielded 5-(C-13)*methyl*-5'-R_3SiAZT as the near exclusive labeled product (^{13}C NMR). Its structure was assigned by ^{13}C NMR (δ:12 ppm, q, J $^{13}C,Me$= 129 Hz) and ^{1}H NMR (δ: 1.63 ppm, d, J=129 Hz). All ^{1}H NMR resonances were identical to 5'-R_3SiAZT except the ^{13}C-labeled methyl.

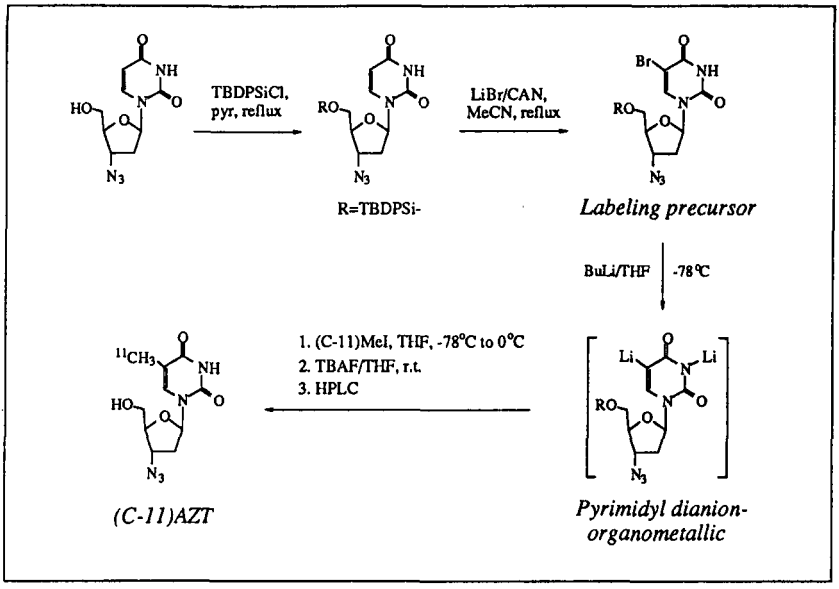

Figure 1: Precursor synthesis and labeling chemistry for (C-11)AZT.

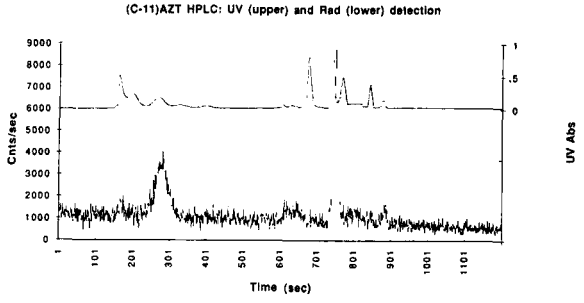

Figure 2: HPLC purification of (C-11)AZT.

Conditions: Phenomenex Ultracarb 5 ODS-30, (250 x 4.6 mm) column, solvent A (MeOH), solvent B (0.1M NH_4OAc): 0-3 min, A/B (1:4); 3-10 min, linear gradient to A/B (4:1); 10-15 min, A/B (4:1). Radioactive peak at approximately 750 sec is (C-11)AZT.

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ALTERNATIVES IN THE SYNTHESIS OF 2-[^{11}C]-THYMIDINE.

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Our laboratory uses [^{11}C]-thymidine labeled in the ring-2 position to image cellular proliferation (1). Its synthesis from [^{11}C]-urea was pioneered by VanderBorght (2) and Labar (3) and has been adapted to the Zymark robot (4). The synthesis has proved difficult to reproduce, especially with robotics, and so we undertook some experiments to develop a more robust approach to this useful radiopharmaceutical. Specifically, we have developed the synthesis of [^{11}C]-urea from cyanide as developed by Emran (5) to utilize robotics, reduce the number of steps, and maximize the yield. We have also evaluated alternative precursors as sources of the 4-carbon skeleton that is cyclocondensed with labeled urea to form the desired [^{11}C]-thymine. Lastly, we have evaluated the requirements for drying urea and other reaction conditions for the cyclocondensation step. With these improvements we produce ~40 mCi (1480 MBq) batches of [^{11}C]-thymidine, HPLC purified and in injectable form, 60 min after a 30 min (40 μA) cyclotron bombardment.

Urea production begins with [^{11}C]-NH_4CN as described earlier (4). The HCN is bubbled through a 22 gauge spinal needle into a 5 mL conical vial containing 0.20 mL of $KMnO_4$ (0.032 M) and 0.05 mL KOH (2 M). We trap 65500±2900 MBq [^{11}C]-NH_4CN 5 min after a 30 min irradiation in a system that has given a consistent cyanide yield for over 600 uses in the past six years. The CN^-/MnO_4^- mixture is warmed to 100±3°C for 1 min and then 0.2 mL $(NH_4)_2SO_4$ (0.75 M) and 0.1 mL EtOH are added. The vial is capped and heated at 170°C for 3 min. Addition of H_2O_2 as recommended in the literature (5) is not required or advantageous in producing the insoluble MnO_2 to remove permanganate from the chemistry. It is known that H_2O_2 and urea form a complex, which presumably decomposes to hydroxyurea (6). The separation of MnO_2 is currently accomplished by filtration through a BioRad disposable column followed by a 0.45 μm nylon mesh. Addition of ~0.3 mL EtOH with 10 μg carrier urea, and a rinse of the reaction vessel with another 0.2 mL EtOH, increases the fraction of [^{11}C]-urea that can be collected and does not prolong the time required for drying the urea. Our procedure for drying is similar to that described (7) and takes about 10-12 min for azeotropic drying using MeCN and under control of the Zymate II. The literature suggests that urea needs to be exceptionally dry for cyclocondensation to occur (2, 3). Our experience, however, is that as much as 10 μL of residual liquid in the urea vessel can be tolerated and still achieve >35% cyclocondensation yields. The amount of urea available for cyclocondensation decreases rapidly if it is dried at >130°C; we typically dry at 100-110°C. Also, if the oleum and organic precursor are added when the urea drying vessel is still hot, the reaction yield is reduced, presumably because of the volatility of the organic precursor. During the cyclocondensation reaction radioactivity can also be lost as $^{11}CO_2$ during sulfamic acid formation or urea can be oxidized by excess SO_3 (8). We therefore monitor the

radioactivity and temperature in the cyclocondensation vessel; the temperature of this heat block never exceeds 130°C.

We have prepared the diethylester of β-methylmalate ("malate") as well as several alternative molecules as the source of carbon atoms for the pyrimidine ring. Details of these precursors are given in Figure 1 and the associated figure legend. Each precursor has been reacted with [^{11}C]-urea to give the results shown in Figure 2. Our choices for alternatives to the "malate" precursor were based on their ease of synthesis and the work by Davidson and Baudish (9) for cyclocondensation of urea with malic acid in oleum. Their hypothesis was that during reaction, malic acid generated malonic semialdehyde ($OHC\text{-}CH_2\text{-}CO_2H$) and this species, or its enol, condensed with urea to form uracil. We reasoned that the "acid, acrylate, and acetal" could be better precursors to malonic semialdehyde, based on their resemblance to its enol, or in the case of the acetal, a reactive hemiacetal. Unlike the malate, which must decarbonylate to form thymine, our alternative precursors do not have to undego this additional, or perhaps even prerequisite decarbonylation.

Each of these precursors have been used for synthesis on the same day, with all of the other reagents and procedures being held constant. Either 9 μL of liquid or 8-14 mg of solid were used. The reactions were only completed through [^{11}C]-thymine. Conversion of thymine to thymidine is done with an enzyme in nearly quantitative yield and works well in our hands. While there were overlapping yields for the different precursors (Figure 1), the variation in results was greatest for the most volatile reagents; the most reproducible yield was for the acid.

In summary, the robotic synthesis of [^{11}C]-thymidine from the malate precursor has not been sufficiently robust in our hands. The acid and the acetal described above have better yields. Our synthesis for patients has used the acrylate, but these results suggest using the acid would give the greatest yield. The technical inconvenience of adding a solid precursor must also be considered.

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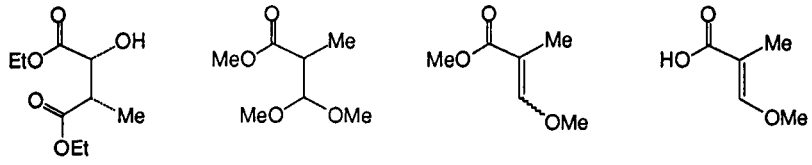

"Malate" "Acetal" "Acrylate" "Acid"

Figure 1. Precursors used for the synthesis of thymine: "Malate" CAS: [74213-61-9], (+)-(2R,3S)-2-hydroxy-3-methyl-butanedioic acid diethyl ester, b.p. 120°C/0.3 mm (Kugelrohr air bath temp.), pure by NMR ($^{1}H/^{13}C$), prepared from (L)-(+)-tartaric acid diethyl ester. "Acetal" CAS: [113681-49-5], 3,3-dimethoxy-2-methylpropanoic acid methyl ester, b.p. 94-98°C/27 mm, pure by NMR ($^{1}H/^{13}C$). "Acrylate" CAS: [35588-82-0] (E-isomer); [14310-92-1] (Z-isomer), (~ 10:1 E/Z)-2-methyl-3-methoxy-2-propenoic acid methyl ester, b.p. 90-93°C/17 mm, >95% pure by NMR ($^{1}H/^{13}C$). "Acid" CAS: [n.a.], (E)-2-methyl-3-methoxy-2-propenoic acid, m.p. 103-104°C, pure by NMR ($^{1}H/^{13}C$).

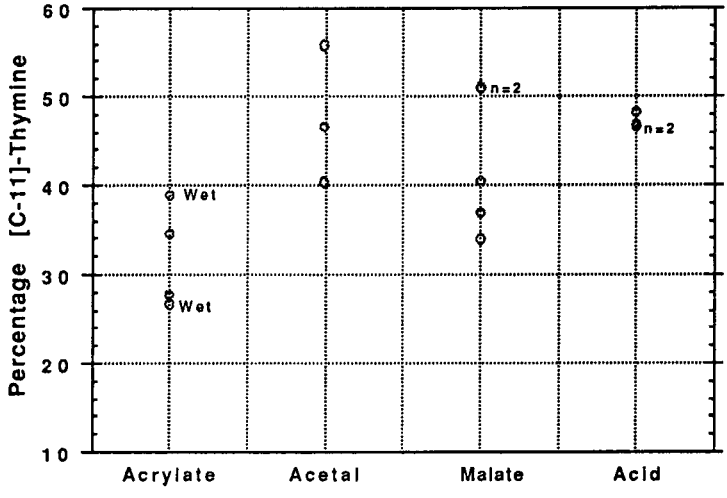

Figure 2. Cyclocondensation yields given as the percentage of [^{11}C]-urea produced that was subsequently incorporated into [^{11}C]-thymine and collected after the AG11A8 resin neutralization step. Data is included only for matched runs where each precursor was evaluated at least once on that date.