A Radiosynthesis of 6-[C-11]-α-D-Glucose from [C-11]Methyl Iodide.

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D-Glucose has been labeled randomly with C-11 using a photosynthetic route and there are well established methods for preparing 1-[C-11]-D-glucose [1], but a label on C-6 would allow one to follow glucose metabolism longer. We have succeeded in developing a practical and efficient radiosynthesis of 6-[C-11]-D-glucose. One of our earlier studies examined the sequence involving the highly stereo-selective bis-hydroxylation of 5,6-dideoxy-1,2-*O*-isopropylidene-3-*O*-(4-methoxybenzyl)-α-D-*xylo*-hex-5-eno-furanose[2]. With OsO_4, in the presence of an equivalent of an alkaloid ligand[3], this alkene could be converted within minutes into a quantitative yield of diols (D-*gluco*- and L-*ido*-, 87:13), and the diastereomeric diols could be separated by HPLC (SiO_2: EtOAc). This attractive route could only be applied if the pre-requisite alkene could be labeled in the terminal position with C-11. Attempts to label the alkene from [C-11]MeI using the Wittig reaction (BuLi or $KOBu^t$ bases) were disappointing (<14%, decay corr) due to base sensitivity of the labeling aldehyde substrate. This former obstacle has been overcome by using a Wittig type olefination method with C-11 and adapting the recently reported results of Kihlberg et al.[4].

Heating a mixture of 1,2-*O*-isopropylidene-3-*O*-(*tert*-butyldimethylsilyl)-α-D-*xylo*-pentodialdo-1,4-furanose, [C-11]methyltriphenylphosphonium iodide, and epichlorohydrin in 1,2-dibromobenzene afforded 80-60% yields (EOB, 40 min overall) of the labeled alkene after chromatographic clean-up. An equivalent result was obtained by using *n*-butyl ether as trapping solvent for [C-11]MeI and the Wittig reaction (160°C, 7 min, 35 min EOB). Bis-hydroxylation of the labeled alkene (10-15 min reaction) followed by HPLC separation (SiO_2, 70% EtOAc/Hex) of the diol isomers, de-silylation (TBAF/THF, 1 min rt), and liberation of the 1,2-isopropylidene (0.5 N H_2SO_4, reflux 5 min) afforded 6-[C-11]-α-D-glucose (31% overall yield (decay corr) from [C-11]$MePh_3P/I$, 70 min syn time). The identity of labeled glucose was confirmed by HPLC.

The overall reaction sequence cannot be carried out as a one-pot procedure, but in principle is amenable to automation. The success and rate of the OsO_4-diol reaction requires that the labeled alkene be isolated pure before reaction. Ph_3P is a potent poison to this reaction, while other impurities slow the reaction rate.

Figure 1 illustrates HPLC results used for the alkene and diol separations. Based on these results and our observations we anticipate that these processes can be streamlined and scaled to afford large quantities (100mCi, EOS) of 6-[C-11]-D-glucose.

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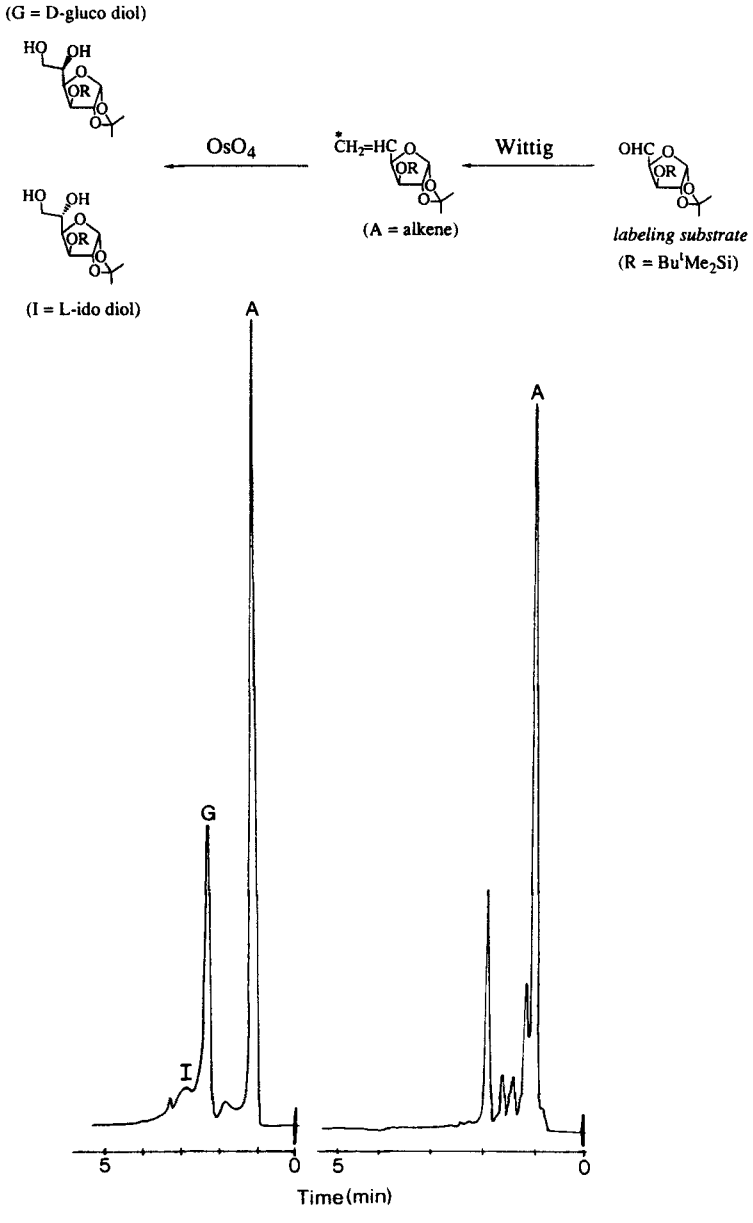

Figure 1: HPLC chromatograms of the Wittig reaction products (right, SiO_2, 10% EtOAc/Hex) and OsO_4 reaction products (left, SiO_2, 70% EtOAc).

1-[^{11}C] Labeled Polyhomoallylic Fatty Acids: Phospholipid Metabolic Tracers for the Brain

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Ex-vivo experiments in rats have demonstrated that the 1-[^{14}C] labeled polyhomoallylic fatty acids, arachidonic (**1a**) and docosahexaenoic (**1b**) acid, label the sn2 positions (>95 %) of different brain phospholipids with little distribution into non-lipid compartments via β oxidation (≤15 %). In contrast, 9,10-[^{3}H]palmitic acid labels the sn1 position (85 %) of brain phospholipids and is equally distributed between lipid and non-lipid compartments (50 % undergoing β oxidation)[1-3]. Most importantly, the turnover rates of these labeled homoallylic fatty acids in brain tissue after iv injection are sensitive to stimulation of second messenger systems whereas the rate of palmitic acid turnover remains unaffected.[3]. In an effort to apply these rat data to the *in vivo* measurement of brain phospholipid metabolism using PET, the corresponding 1-[^{11}C] polyhomoallylic fatty acids were prepared. A facile retro-synthesis involving the radical chain decarboxylation of N-hydroxypyridine-2-thione arachidonate[4] and docosahexaenoate has been developed to synthesize (all Z)-1-bromononadeca-4,7,10,13-tetraene (**2a**), and (all Z)-1-bromoheneicosa-3,6,9,12,15,18-hexaene (**2b**) in approximately 60% overall yield. The Grignard was carbonated with [^{11}C]CO_2 to afford the 1-[^{11}C]polyhomoallylic labeled fatty acid in 50% yield (decay corrected to end of bombardment) in less than 35 minutes (Fig 1). The final radiochemical purities were found to be in excess of 95% by radio-HPLC and TLC.

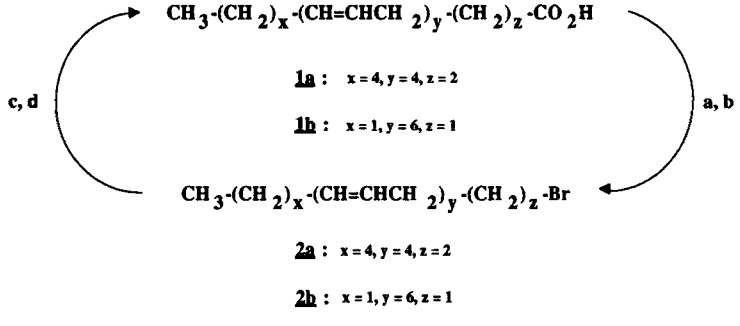

Fig 1: a) TMAOH / $(CO)_2Cl_2$ / toluene; b) N-Hydroxpyridine-2-thione / $BrCCl_3$ / Δ;
c) Mg^0 / Et_2O / >>>rt; d) [^{11}C]CO_2 / Et_2O

PET studies in rhesus monkeys revealed that approximately 0.5 % of the total injected dose of 1-[^{11}C]arachidonic acid was present in brain tissue at 20 minutes and remained constant for nearly 2 hours. The kinetics of the early phase of uptake appears to be consistent with rat data indicating unidirectional pulse-labeling of brain phospholipids. The rate of uptake into brain tissue for 1-[^{11}C]arachidonic acid was found to be approximately twice that of 1-[^{11}C]palmitic acid (1.0 % / min and 0.6 % / min respectively). These initial results suggest that these 1-[^{11}C] fatty acids will be useful radiotracers for the *in vivo* measurement of brain phospholipid turnover.

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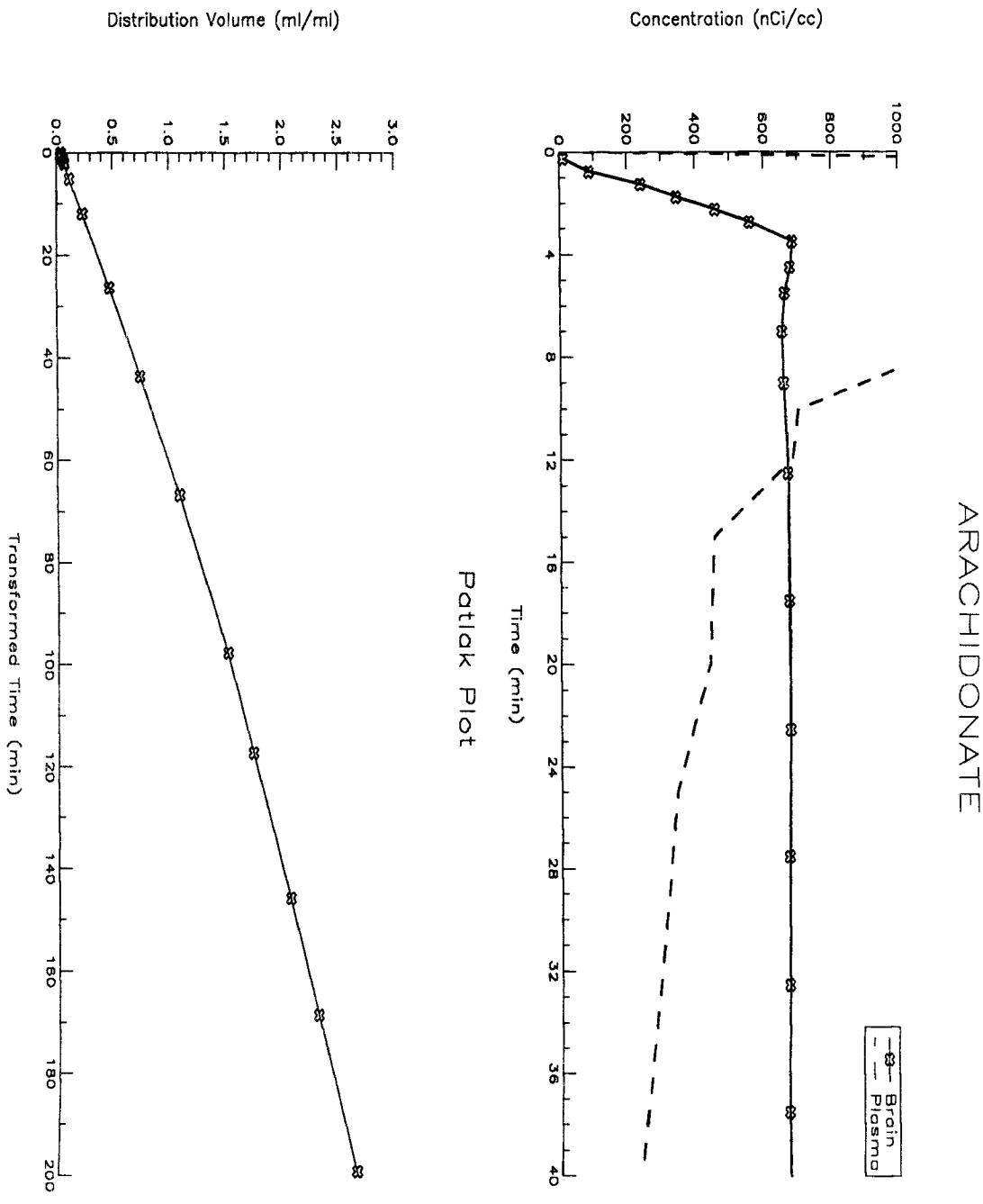

Fig 2. Uptake of 1-[^{11}C]arachidonic acid in brain and clearance from plasma after infusion. (16 mCi in a 9 Kg rhesus monkey / single slice, whole brain region of interest (ROI) / Plasma curve corrected for metabolites /200 min transformed time = 40 min real time / Scanitronics PC2048-15B (6 x 6 x 6 mm resolution)).

Paper L3

Efficient Radiosynthesis of [^{18}F]-Fluoromisonidazole Suitable For Routine PET

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[^{18}F]-Fluoromisonidazole ([^{18}F]-Fmiso,1) represents a promising approach for the detection of hypoxic tissue in ischemic heart and brain, and tumors using PET.[1] It has been shown to metabolically accumulate in hypoxic tissues according to the tissue's degree of hypoxia. Recently, this radiopharmaceutical has been prepared by others[2] and used for detection of hypoxia in human tumors. According to these initial results,[3] [^{18}F]-Fmiso PET imaging provides a convenient, non-invasive means to select patients for conventional radiotherapy and assess tumor reoxygenation during treatment. It is unfortunate that the reported syntheses use a low-yield two-step labeling procedure. The published method would be difficult for the daily routine preparation of large amounts necessary for routine PET use.

We propose a new synthetic approach for preparing [^{18}F]-Fmiso via a rapid and high-yield labeling step. This incorporates the synthesis of an appropriately protected sulfonate precursor to introduce labeled fluoride directly into the final product. Rapid cleavage of the protecting group is then carried out. Thus, [^{18}F]-Fmiso is obtained in an efficient process suitable for routine use for PET imaging.

Initially we considered the use of a cyclic sulfate[4] across two adjacent hydroxyl groups to simultaneously protect and activate the appropriate adjacent hydroxyl groups. Unfortunately, this approach did not allow us to cleanly add the imidazole fragment, and may have suffered from intramolecular attack of the nitroimidazole on the cyclic sulfate. It was, therefore, necessary to explore a more traditional stepwise approach.

Retrosynthetic analysis of the desired precursor leads to functionalization equivalent to glycerol, which is readily available. One of the two primary hydroxyl groups is replaced by the nucleophilic imidazole. The other primary hydroxyl group must also be converted to an effective leaving group for fluorine-18 labeling, while the secondary hydroxyl group needs to be protected throughout the process and deprotected after labeling.

Two different groups for alcohol protection are used for the two classes of alcohol present in glycerol. Condensation of glycerol with benzaldehyde gave benzylidene 2 in 25% yield (mp 60-65°C). Treatment of 2 with dihydropyran in the presence of pyridinium *p*-toluenesulfonate gave THP-benzylidene 3 in 75% yield (mp 50-52°C). Selective removal of the benzylidene of 3 with Na/NH_3 gave the diol 4 in 80% yield as an oil, which was converted to the di-toluenesulfonate 5 in 90% yield (mp 107-110°C). Displacement of one of the toluenesulfonates by 2-nitroimidazole in DMF with Cs_2CO_3 provided the target labeling precursor 6. Precursor 6 was purified by alumina column chromatography (50% EtOAc/pet ether, 40% yield). Although the overall yield of the five steps seems low (6%), the majority of the starting materials are readily available and inexpensive. It is worth noting that the limiting reagent in this sythesis with regard to availability and expense , 2-nitroimidazole, is not introduced until the final synthetic step.

The radiosynthesis procedure for 1 is similar to the well-known preparation of [^{18}F]-FDG[5]. The overall radiochemical yield is 45% at 50 min EOB (72% chemical yield based on fluoride). [^{18}F]-Fluoride displacement of 6 proceeded with 80% chemical yield (TLC, HPLC) using 10 mg of 6 in anhydrous AcN in the presence of 13 mg of kryptofix® and 1.8 mg of K_2CO_3 as co-catalysts, and heating at 100°C for 10 min. Rapid removal of the THP ether in 1 N HCl (100°C for 10 min) proceeded quantitatively (TLC, HPLC) to give [^{18}F]-Fmiso. Neutralization, filtration through a short alumina column, C-18 Sep-Pak® and sterilizing filter gave a sterile, isotonic solution of [^{18}F]-Fmiso.

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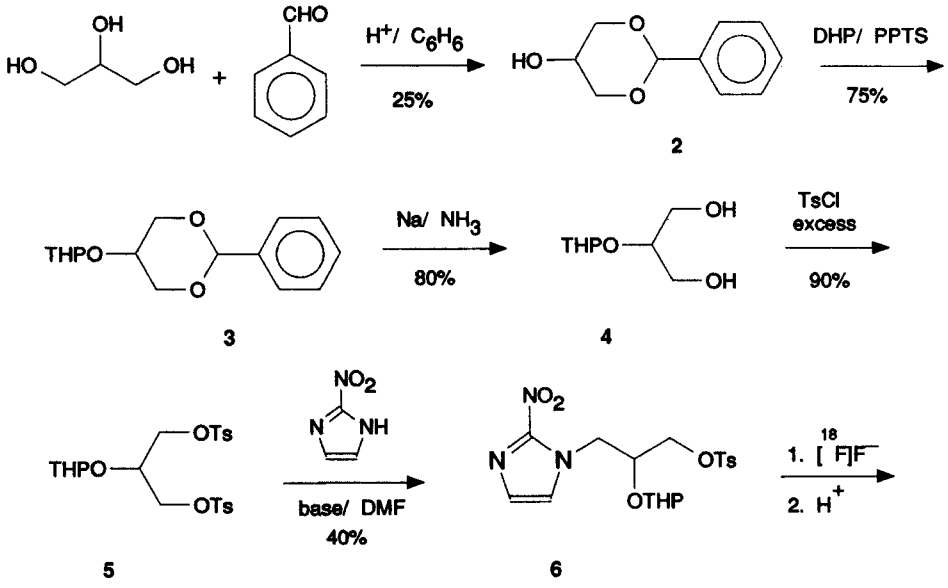

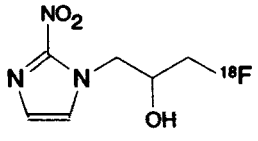

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Fluoromisonidazole

FLUORINE-18 LABELED ANALOGS OF META-IODOBENZYLGUANIDINE.

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Meta-iodobenzylguanidine (MIBG) labeled with ^{131}I or ^{123}I has been used clinically for the diagnosis and therapy of a variety of tumors and has been investigated as a potential tracer of neuronal injury in the heart. If an ^{18}F-labeled analog of MIBG could be synthesized with similar norepinephrine-like properties, it might be possible to pursue the diagnostic applications of MIBG in oncology and cardiology using PET. Since the para-iodo analog of MIBG also exhibited significant adrenal medulla uptake in the dog (1), and since fluorodenitration with the activating group para has been shown to proceed in higher yield than with meta substitution (2), the first compound synthesized was 4-[^{18}F]fluorobenzylguanidine (PFBG).

The synthetic scheme is outlined in Figure 1. 4-[^{18}F]fluorobenzylamine was synthesized from tetrabutylammonium [^{18}F]fluoride as described (3). Reaction with 2-methyl-2-thiopsudourea sulfate at 150°C for 30 min followed by HPLC purification gave the required PFBG. Radiochemical yields were 55-70% in a total synthesis time of 70-75 min (EOB) with a radiochemical purity of 95-99%. The meta isomer MFBG was synthesized using the same method by using 3-nitrobenzonitrile in the first step of the synthesis. Radiochemical yields for MFBG were only 10-15%, much lower than obtained for PFBG. As expected, the fluoro for nitro exchange reaction was the difference (radiochemical yields: para, 65-80%; meta, 12-18%).

The in vitro binding properties of PFBG and MFBG were determined in a live-cell assay using SK-N-SH human neuroblastoma cells. The SK-N-MC line served as a control to correct for nonspecific binding. Incubations were performed in triplicate for various times at 37°C with 4 x 10^5 cells of each line. As shown in Figure 2, the specific binding of MFBG to SK-N-SH cells was 2- 3-fold higher than that of PFBG. For example, at 2 hr, the specific binding of MFBG was 34.5 ± 3.5% compared to 11.2 ± 0.6% for PFBG. Parallel measurements with MIBG yielded a specific binding value of 53.1 ± 1.6%. Thus, although the binding of MFBG was better than PFBG, substitution of fluorine for iodine at the meta position resulted in decreased specific binding in vitro.

The tissue distribution of PFBG and MFBG was measured in normal mice 1, 2, and 4 hr after injection. With both isomers, maximum concentration of ^{18}F was observed in heart and adrenals at all time points (Tables 1 and 2). Adrenal uptake was similar for both isomers and comparable to that observed in paired experiments using MIBG labeled by isotopic exchange. For example, at 1 hr, the mean % injected dose/g in adrenals was 14.6, 15.6, and 14.8% ID/g for PFBG, MFBG and MIBG, respectively. Myocardial uptake of MFBG was higher than that of PFBG, with the differences becoming significant by 2 hr. At 1 hr, myocardial uptake of MFBG was 16.2 ± 3.1% ID/g, a value higher than that for conventionally labeled MIBG (11.5 ± 1.9% ID/g) and essentially identical to that for MIBG labeled using a no-carrier-added synthesis (15.8 ± 1.8% ID/g). We conclude that these ^{18}F-labeled analogs of MIBG, particularly, MFBG, warrant further evaluation as new tracers for PET.

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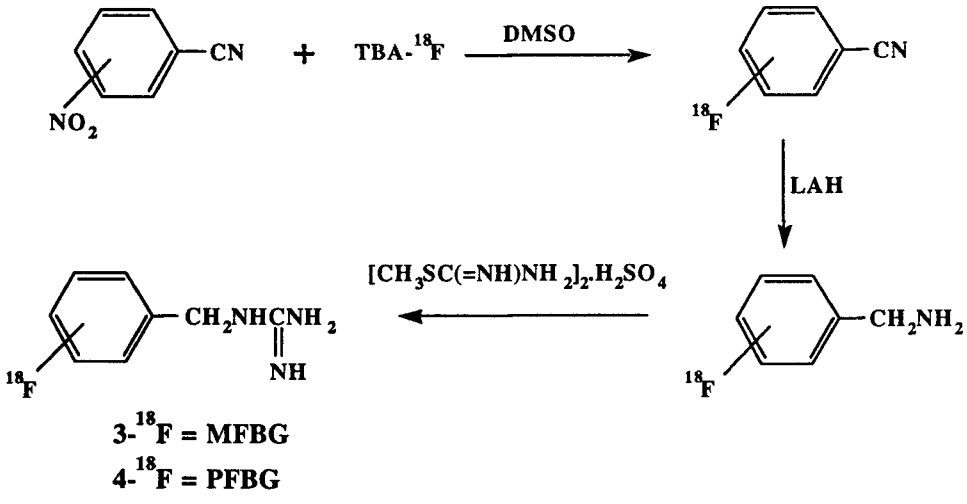

FIGURE 1: SYNTHETIC SCHEME FOR MFBG AND PFBG

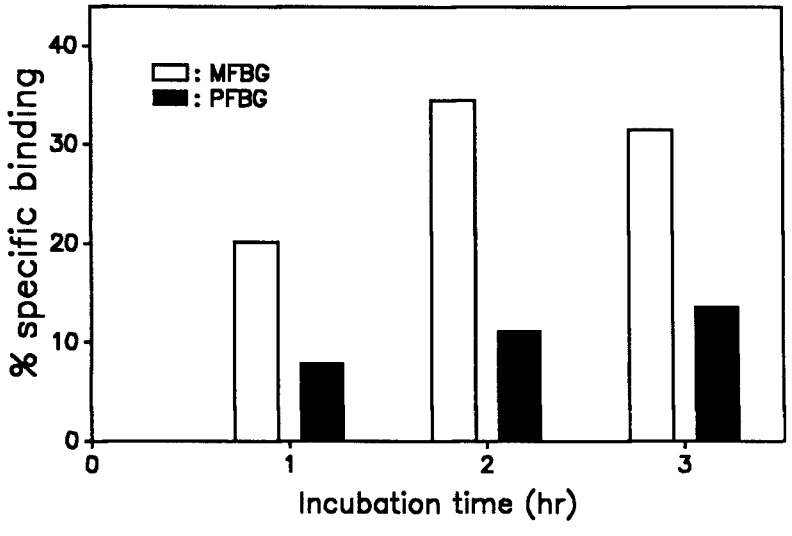

Figure 2: Binding of MFBG and PFBG to SK–N–SH neuroblastoma cells

Table 1. Tissue distribution of [^{18}F]PFBG in normal mice.

	percent injected dose per gram		
Tissue	1hr	2hr	4hr
Liver	7.10 ± 1.07	4.83 ± 0.06	3.15 ± 0.80
Spleen	4.78 ± 0.60	4.24 ± 0.60	3.57 ± 0.40
Lung	4.53 ± 0.90	3.13 ± 0.40	1.98 ± 0.70
Heart	13.35 ± 1.00	9.23 ± 0.90	5.62 ± 0.90
Kidneys	2.55 ± 0.20	2.00 ± 0.31	1.70 ± 0.20
Blood	0.75 ± 0.50	0.50 ± 0.03	0.29 ± 0.06
Brain	0.20 ± 0.04	0.11 ± 0.01	0.06 ± 0.01
Adrenals	14.58 ± 2.01	13.42 ± 3.40	10.17 ± 2.20

Table 2. Tissue distribution of [^{18}F]MFBG in normal mice.

	percent injected dose per gram		
Tissue	1hr	2hr	4hr
Liver	8.39 ± 1.47	4.94 ± 0.65	3.83 ± 0.50
Spleen	4.53 ± 0.30	4.09 ± 0.50	3.70 ± 0.40
Lung	5.55 ± 1.60	3.75 ± 1.00	3.05 ± 0.60
Heart	16.22 ± 3.10	11.82 ± 1.00	8.74 ± 1.10
Kidneys	1.45 ± 0.26	2.71 ± 0.51	1.94 ± 0.30
Blood	0.79 ± 0.09	0.54 ± 0.10	0.46 ± 0.14
Brain	0.55 ± 0.05	0.18 ± 0.01	0.07 ± 0.02
Adrenals	15.58 ± 2.20	16.07 ± 7.50	12.51 ± 3.60

[^{11}C]-2-METHOXY-PHENYL-METYRAPONE FOR PET

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Enzyme inhibition of the 11ß-hydroxylation of deoxycorticosterone (DOC) has been studied using various metyrapone derivatives (1,2). The 2-substituted phenylmetyrapone analogues 2-methoxy-phenyl-metyrapone (X_{11}) and 2-hydroxy-phenylmetyrapone (X_{12}) showed the highest inhibitory effect when compared with metyrapone (X_3), phenylmetyrapone (X_4), and the 4'-bromo-metyrapone-derivative (X_8).

Fig. 1 shows IC_{50}-values (µM) for the inhibition of P-$450_{11ß}$ enzyme activity. Ring A derivatization considerably increased the inhibitory effect when compared with the ring B substituted product (X_8). Binding of ^{3}H - metyrapone to rat adrenal tissue slices indicated exclusively cortical binding (Fig. 2).

2-hydroxy-phenylmetyrapone has been labelled by ^{11}C-methylation within 5 minutes with high incorporation (> 95%). After reversed-phase semi-preparative HPLC the radiochemical purity was > 99%. Reaction conditions for labelling and optimal semi-preparative HPLC-purification will be discussed as well as in-vivo stability.

[^{11}C] - 2-methoxy-phenylmetyrapone may gain considerable importance as a PET ligand for evaluating adrenocortical function, especially with hyperfunctioning adrenals.

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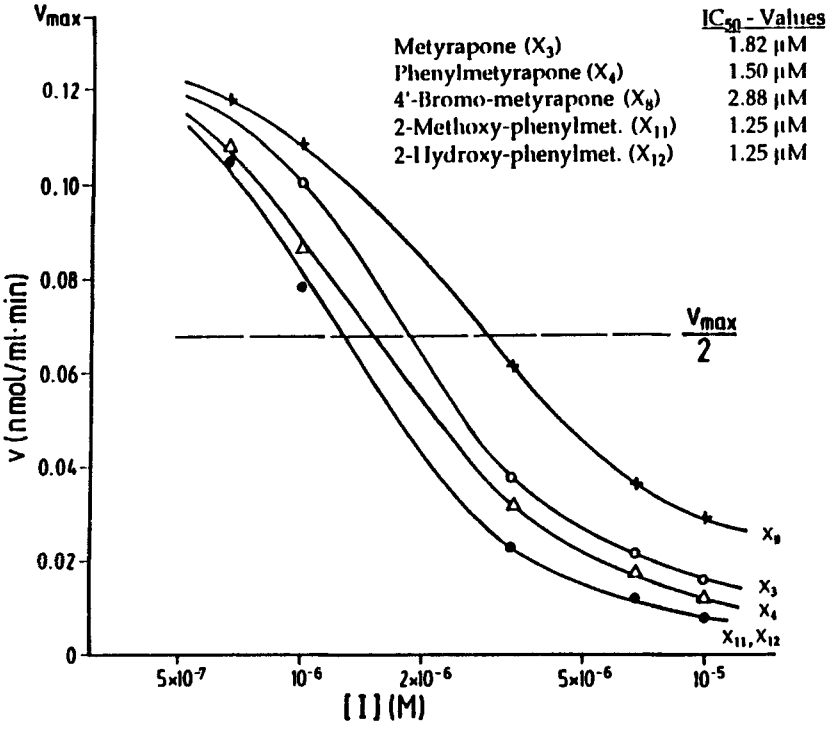

Fig. 1 Inhibitory effect of metyrapone-derivatives on the 11ß-hydroxylation of deoxycorticosterone (^{14}C-DOC) expressed as the reaction rate vs the log of inhibitor concentration

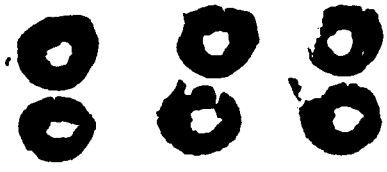

Fig. 2 Autoradiography of rat adrenal tissue slices (16 µm) after incubation with ^{3}H-metyrapol (50 nM) for 1 hr. The tissue slices were rinsed with saline medium prior to a 5 day-exposure to X-ray film

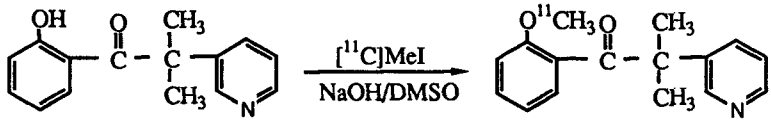

Fig. 3 Incorporation of [^{11}C] methyl-iodide to [^{11}C]-2-methoxyphenyl-metyrapone

Synthesis and evaluation of ^{11}C- and ^{18}F-labeled purine nucleosides as a tracer for adenosine transport and metabolism

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In mammals, adenosine is transported across cell membranes by carriers called nucleoside transport systems and inside the cells is metabolized in two ways depending on its concentration; at low (uM-range) concentration, it is preferentially metabolized into AMP by adenosine kinase, while at high (mM-range) concentration, it is deaminated into inosine by adenosine deaminase. It is therefore expected that adenosine or its analogs labeled with positron-emitting radionuclides such as ^{13}N, ^{11}C, and ^{18}F in a NCA state would become metabolically-trapped agents, if they are properly designed as for enzymatic properties. In this study, we have investigated the potentials of two positron-labeled purine nucleosides, ^{11}C-6-methylmercaptopurine ribose (^{11}C-MMPR) and ^{18}F-6-fluoropurine ribose (^{18}F-FPR), (Fig.1).

NCA ^{18}F-FPR was prepared by the previously reported method (1). NCA ^{11}C-MMPR was obtained as follows; 6-mercaptopurine ribose (1mg) was reacted with ^{11}C-methyl iodide in DMF (0.5ml) at 90°C for 3 min and the reaction solution was injected into the HPLC (MegapaQ SILICA, 1x25cm, elution with EtOAc:iso-PrOH=99:1, flow rate 7ml/min, UV 300 nm) and the fraction corresponding to ^{11}C-MMPR was collected. The time required for synthesis and purification was 15-20 min and the yield was 36-73mCi (3 runs). Specific activity was 3500-5200 mCi/mmol. The radiochemical purity was more than 98% as determined by HPLC (Finepak C18, analytical column, gradient elution for 5 min from (A)=AcCN:H2O/0.1%TEF/0.1%TFA=2:98 to (B)=20:80, flow rate 3ml/min).

Mice (C3H strain, male) were used for biodistribution study. In the case of ^{11}C-MMPR (Fig.2), at 1 min after injection, such organs as blood (red blood cells), lung, heart and kidneys showed high uptakes. All the organs showed the retention curves of radioactivity (This compound is known to be a good substrate of adenosine kinase). In contrast, the radioactivities from ^{18}F-FPR were rapidly eliminated from all the organs examined except bone, which suggested in vivo defluorination of this compound (Fig.3). This was confirmed by in vitro incubation of ^{18}F-FPR with calf intestinal adenosine deaminase.

Of the two nucleosides and one base, ^{18}F-6-fluoropurine, the transport mechanisms in the lung were studied. A mixture of the tracer and a nucleoside transport inhibitor (dypiridamole or para-nitrobenzylthioinosine) was injected in mice and the lung uptake was measured at 15-60 seconds after injection. As compared with control mice, about 60% of decreases in the lung uptake were observed in the case of ^{11}C-MMPR and ^{18}F-FPR, but no significant change for the base (Fig.4).

In summary, ^{11}C-MMPR and ^{18}F-FPR behave similarly in the transport but very differently in the metabolism; while ^{11}C-MMPR showed the retention curve, ^{18}F-FPR showed the washout curve of the radioactivity.

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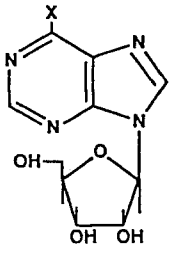
Fig.1 Structure of 11C-MMPR and 18F-FPR



MMPR: X=$^{11}CH^{3}S$
FPR: X=^{18}F

Fig.4 The effect of nucleoside transport inhibitor on the lung uptake of purine derivatives

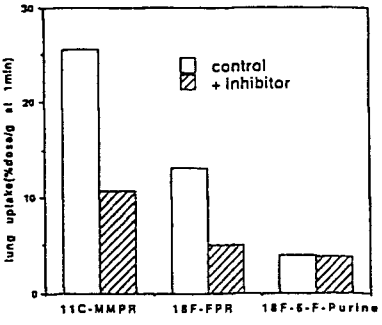


Fig.2 Organ distribution of ^{11}C-MMPR in mice

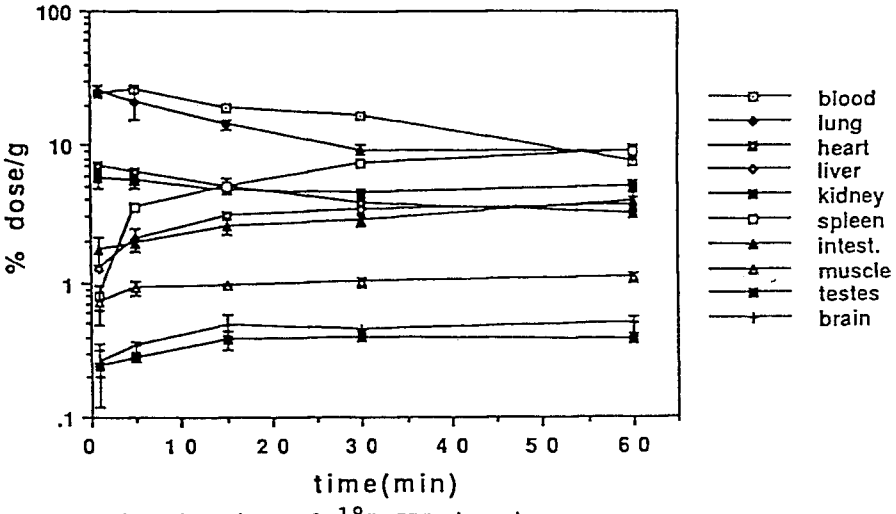
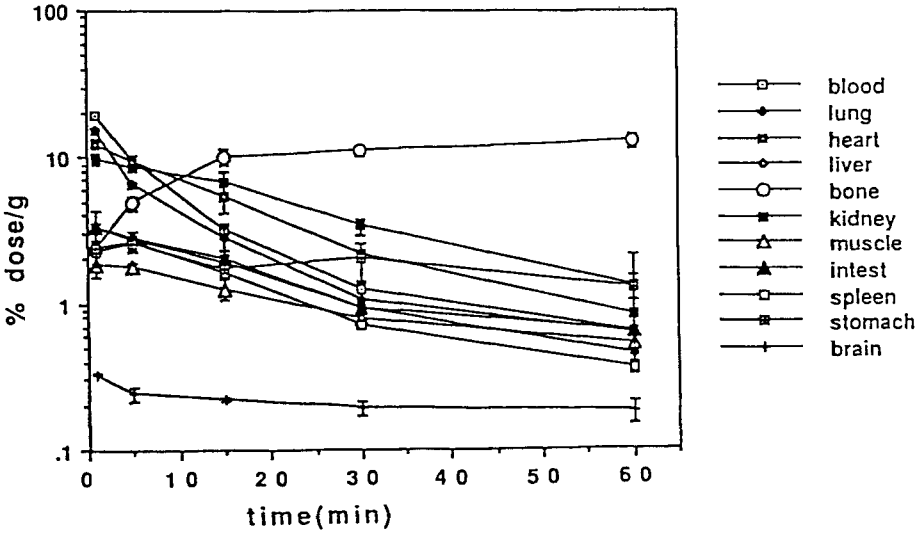


Fig.3 Organ distribution of ^{18}F-FPR in mice



Paper L7

SYNTHESIS AND *IN VIVO* BEHAVIOR OF F-18 LABELED ANALOG OF D-TALOSE: 2-DEOXY-2-[^{18}F]FLUORO-D-TALOSE

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Fluorine substituted analog of D-talose, 2-deoxy-2-fluoro-D-talose (FDT), is C2-epimer of 2-deoxy-2-fluoro-D-galactose (FDGal), F-18 labeled analog of which has used as a radiotracer for the study of liver function by PET[1,2]. FDGal enters a D-galactose metabolic pathway in mammalian tissues and initially is phosphorylated by galactokinase. Studies on the substrate specificity of galactokinase, showing that D-talose can be accepted as a substrate for galactokinase[3], have led us to consider that FDT could behave similarly as a substrate for galactokinase. We became interested to compare the *in vivo* behavior of F-18 labeled FDT ([^{18}F]FDT) in tumor-bearing animals with those reported for the [^{18}F]FDGal. We have now developed a high-yield synthesis of [^{18}F]FDT using [^{18}F]fluoride ion and elucidated its *in vivo* behavior by both ^{18}F-radiotracer and ^{19}F-NMR techniques.

[^{18}F]FDT was prepared by a two-step procedure (Fig. 1). Aminopolyether supported potassium [^{18}F]fluoride ($K^{18}F$/Kry222) was used as a [^{18}F]fluorinating agent for the fluorination of the precursor (1). The obtained intermediate (2) was purified by Silica Sep-Pak filtration. The deprotection of 2 was achieved by the short treatment with 2M-BBr_3 in CH_2Cl_2. After neutralization of the acidic solution of [^{18}F]FDT with AG11X8 resin column and subsequent purification with both C18 and Alumina Sep-Pak columns, an aqueous [^{18}F]FDT was obtained in 33% radiochemical yield. Nonradioactive FDT was also prepared in 53% yield by the fluorination of 1 with Me_4NF followed by deprotection with 2M-BBr_3. ^{19}F-NMR spectrum showed that FDT was a mixture of both the anomers of the pyranoses (87%) and the furanoses (13%) (Fig. 2A).

Biodistribution of [^{18}F]FDT in mice bearing fibrosarcoma (Table 1), showing the highest uptake of radioactivity in the liver followed by the kidney, small intestine, and tumor, had a marked resemblance to those of [^{18}F]FDGal. It was reported that [^{18}F]FDGal is highly taken up in the liver and is trapped in the forms of [^{18}F]FDGal-1-P and UDP-[^{18}F]fluorohexoses[4-6]. In our metabolic studies of FDT in mice by ^{19}F-NMR spectroscopy, however, only one metabolite with negligible amount of FDT was detected in the water extractable fraction from the mouse liver (Fig. 2B) and this metabolite was determined to be FDT-1-P by the following findings. First, the metabolite of FDT had the same ^{19}F-chemical shift (-37.50 ppm) with that of the product obtained by the enzymatic reaction of FDT with yeast galactokinase (Fig. 2C). Second, the metabolite was converted to FDT by the action of alkaline phosphatase (Fig. 2D). Furthermore, the high liver uptake of [^{18}F]FDT in normal rats was strongly inhibited by co-injection of D-galactose. These results strongly suggest

that [^{18}F]FDT enters the D-galactose metabolic pathway and accumulates as [^{18}F]FDT-1-P following phosphorylation by galactokinase in some organs such as liver and tumor. Thus [^{18}F]FDT would be expected to be a new radiotracer for the measurement of galactokinase activity by PET.

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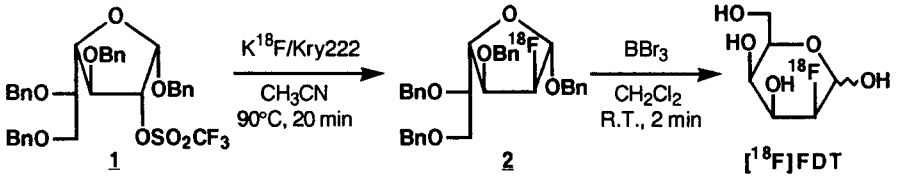

[Radiochemical yield: 33% (decay uncorrected), Radiochemical purity: >98%, Synthesis time: 80 min from start of the radiofluorination.]

Fig.1 Radiosynthesis of 2-Deoxy-2-[^{18}F]fluoro-D-talose ([^{18}F]FDT).

Table 1. Tissue Distribution of Radioactivity in Mice Bearing Fibrosarcoma Following Intravenous Administration of [^{18}F]FDT.

Tissue	Uptake (%dose/g) *			
	10 min	30 min	60 min	120 min
Blood	2.96 ± 0.75	2.55 ± 0.46	2.15 ± 0.34	2.43 ± 0.30
Liver	28.9 ± 5.30	34.9 ± 2.51	38.4 ± 4.43	39.4 ± 1.74
Small intestine	12.1 ± 0.81	12.9 ± 1.75	9.95 ± 0.75	8.75 ± 0.83
Kidney	16.1 ± 2.57	15.9 ± 2.16	10.5 ± 1.45	9.35 ± 0.59
Tumor †	5.23 ± 1.05	5.69 ± 1.20	5.96 ± 0.66	6.08 ± 0.17
Brain	2.36 ± 0.49	2.69 ± 0.31	2.78 ± 0.25	2.63 ± 0.16
Bone	0.98 ± 0.26	0.57 ± 0.04	0.39 ± 0.07	0.53 ± 0.09

* Mean value ± SD of 3-4 female C3H mice

† 3-Methylcholanthrene-induced fibrosarcoma was inoculated subctaneously into right hind leg muscle of female C3H mice. These mice were used for biodistribution at 9-10 days after inoculation.

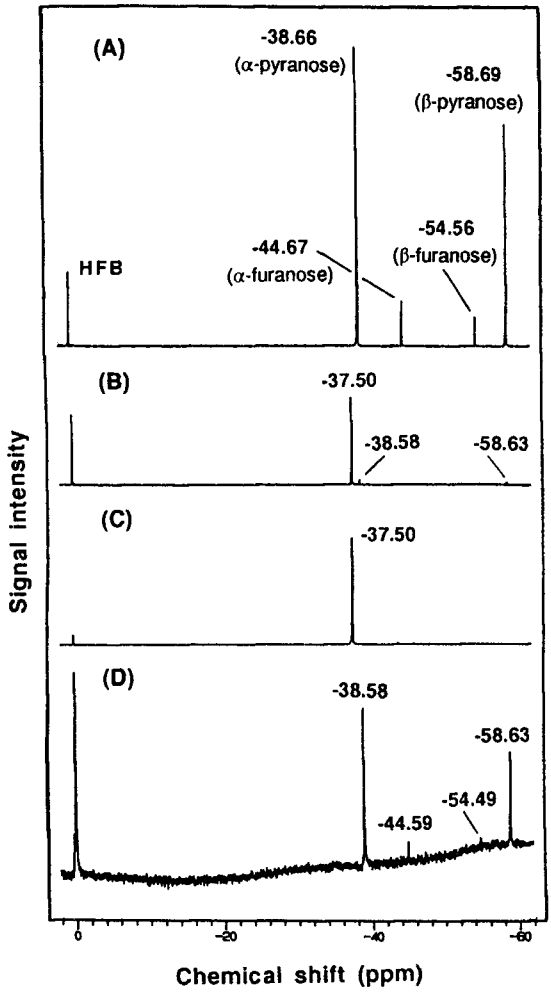

Fig. 2 ^{19}F-NMR (254 MHz) Spectral Analyses of the metabolite of FDT

(A) authentic FDT in water. **(B)** water extractable fraction from C3H mouse liver. FDT was injected (60 mg/kg) intravenously through the tail vein. The liver was removed at 60 min after injection, heated at 100°C for 3 min to deactivate enzymes, homogenized with water, and centrifuged. **(C)** product of the enzymatic reaction of FDT with yeast galactokinase.[4] **(D)** the liver extract after treatment with alkaline phosphatase.[4] **HFB**: hexafluorobenzene as an external standard.

New Results Concerning the Metabolism of ω-(4'-Iodophenyl)pentadecanoic acid (IPPA)

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For several years the radioiodinated fatty acid ω-(4'-Iodophenyl)-pentadecanoic acid (IPPA) is now routinely used as a scintigraphic heart agent. In order to understand the metabolism some experimental effort was undertaken and published in a series of papers. Among all only one contribution tried to elucidate the molecular fate of IPPA using Langendorff-isolated-rat hearts, chromatographic separation techniques and mass spectrometric identification of the separated metabolites. According to GLC-MS measurements the metabolites in the heart perfusate were identified as 4-iodobenzoic acid, 3-(4'-iodophenyl)propenoic acid and 3-(4'-iodophenyl)-propanoic acid. The heart on the other side contained about 80% of IPPA in esterised form[1].

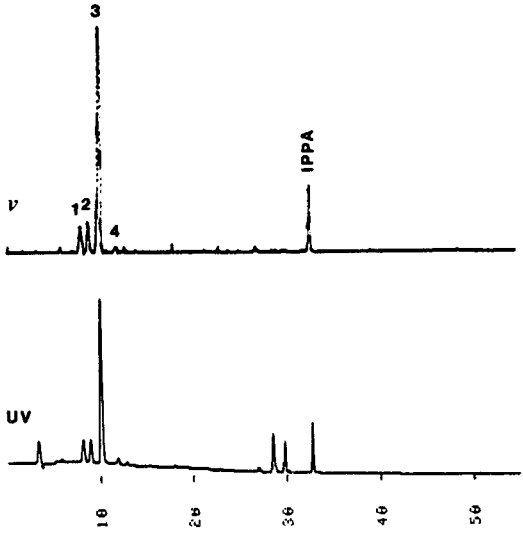


Figure 1. γ- and UV- profiles of HPLC of rat heart perfusate. Numbers indicate analysed metabolites.

While using IPPA as a reference in the analysis of metabolites of modified fatty acids recently synthesised in our laboratory[2], we obtained reproducible IPPA degradation products which were not in accordance with the results cited above[1]. In contrast to the commonly accepted opinion, that the final metabolite of IPPA is 4-iodobenzoic acid, we could not detect measurable amounts of this compound in the rat-heart perfusates. Figure 1 depicts the UV- and γ-traces of the perfusate showing the main metabolites **1** - **4**. These metabolites were separated using HPLC and analysed by neg. FAB-MS. According to these measurements we assigned the molecular masses to the following compounds: 5-(4'-iodophenyl)-3-hydroxy-pentanoic acid **1**, 3-(4'-iodophenyl)propanoic acid **2**, 3-(4'-iodophenyl)propenoic acid **3** and 5-(4'-iodophenyl)-2-pentenoic acid **4**

(Figure 2). In addition, it is noteworthy that metabolite 3 was formed with the highest concentration and that no β-keto-fatty

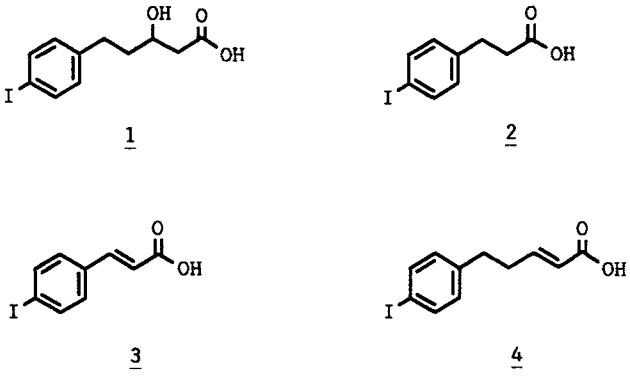


Figure 2. IPPA metabolites 1 - 4 isolated with HPLC and analysed by FAB-MS

acid metabolite was detected. The steric bulkiness and the conjugation of the double bond with the π-electrons of the phenyl group may be the key factors which stop further enzymatic degradation in heart muscle cells. This led to compound 3 as the terminal metabolite. Further metabolism which forms 4-iodophenylbenzoic acid must therefore be attributed to the enzyme function of other organs, e.g. of the liver.

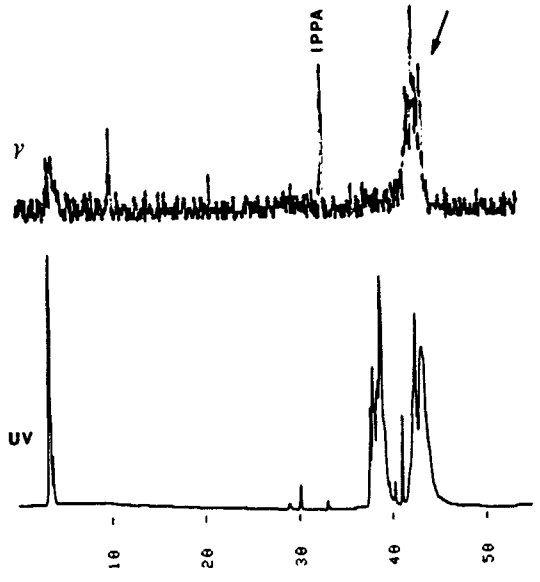
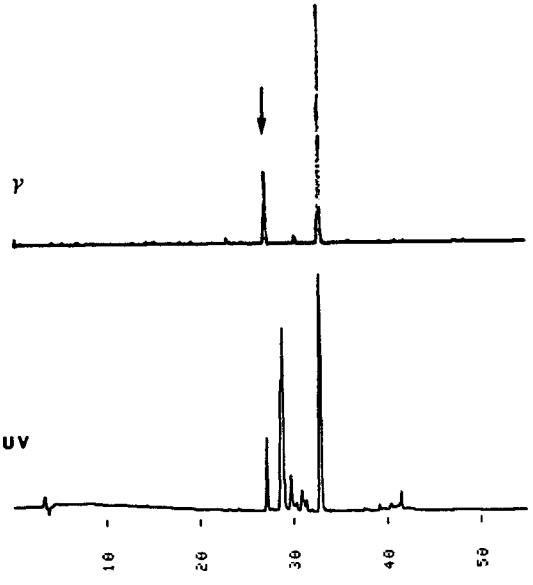
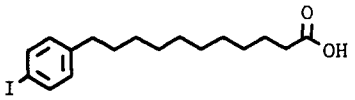


Figure 3. γ- and UV- profiles of HPLC of rat heart extract. Arrow indicates ester fraction.

The HPLC obtained from the lipid extract of the perfused rat heart is shown in Figure 3. The distribution of the IPPA activity into esters and free fatty acid was comparable to earlier results[1]. In order to prove the integrity of IPPA in the ester fraction we isolated the ester fraction coeluting with the activity peak. Hydrolysis of the esters with KOH/EtOH liberated the fatty acids quantitatively. As expected IPPA was identified in the hydrolysate by HPLC and FAB-MS. However, an additional, more hydrophilic and in other experiments even in higher concentrations appearing metabolite (**5**) was detected (Figure 4). According to FAB-MS this metabo-





5

Figure 4. γ- and UV- profiles of HPLC of hydrolysed ester fraction. Arrow indicates metabolite 5.

lite showed a mass which was reduced by 56 a.m.u.'s. as compared with IPPA. Therefore, compound **5** relates to 11-(4'-iodophenyl)undecanoic acid.

The appearance of a single IPPA metabolite in the ester fraction of rat-heart cells may lead to speculations concerning the mechanism of formation. It may either be produced by cytosolic fatty acid synthesis or, to our opinion less favorably, by mitochondrial cleavage with reuptake in the ester fraction. Additionally, the almost exclusive accumulation of just one metabolite in the ester fraction is the result of high enzyme selectivity.

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Paper L9

PREPARATION AND IN VIVO COMPARISON OF ^{62}Cu-LABELED BLOOD-POOL AGENTS. C.J. Anderson, M.J. Welch, C.J. Weinheimer*, S.R. Bergmann*. Mallinckrodt Institute of Radiology and *Cardiovascular Division, Washington University Medical School, St. Louis, MO 63110.

The ^{62}Zn/^{62}Cu generator is a source of positron-emitting radiotracers, and has been used in the production of ^{62}Cu-PTSM, a radiopharmaceutical for studying myocardial perfusion by positron emission tomography (PET)(1). It would be desirable to have a ^{62}Cu-radiopharmaceutical for delineation of blood volume to use with ^{62}Cu-PTSM for correction of radioactivity emanating from the vascular space. One possible vascular agent is ^{62}Cu-labeled human serum albumin (HSA), utilizing the bifunctional chelate 6-*p*-bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid (Br-benzyl-TETA)(2). ^{62}Cu-benzyl-TETA-HSA has been shown to give images that are comparable to ^{15}O labeled carbon monoxide; however, the preparation includes a time-consuming spin column purification step to remove unlabeled copper, and the overall yield is only about 16% (without decay correction). An ideal vascular agent would be a a copper-labeled protein conjugated to a bifunctional chelate which can be labeled in less than ten minutes in high yield and is stable in vivo for at least 30 minutes. Fujibayashi, et. al. have labeled ^{62}Cu to a dithiosemicarbazone-human serum albumin (DTS-HSA) conjugate for measuring blood volume in a dog model (3). The labeling of ^{62}Cu to DTS-HSA was accomplished by mixing the ^{62}Cu in 200 mM glycine with the DTS-HSA solution. The labeling efficiency was consistantly greater than 95%, and no spin column purification was necessary to remove free copper. In this study we have compared blood clearance of ^{67}Cu-labeled DTS-HSA and benzyl-TETA-HSA in adult female Sprague-Dawley rats. In PET experiments with a dog, myocardial blood volume images were obtained using ^{62}Cu-DTS-HSA and ^{62}Cu-benzyl-TETA-HSA. These images were compared to images obtained with C^{15}O.

Bromo-benzyl-TETA was synthesized and conjugated to HSA as described by McCall, et. al. (4). *p*-Carboxyethylphenylglyoxal-*N',N''*-dimethyldithiosemicarbazone (CE-DTS) was prepared by a modified method of the synthesis described by McPherson, et. al. (5). CE-DTS was conjugated to HSA via the coupling agent diphenylphosphoryl azide (DPPA) (3) and purified using centricon membranes. Labeling of ^{67}Cu and ^{62}Cu to benzyl-TETA-HSA was accomplished by adding copper acetate to the conjugated protein, and purifying by spin column chromatography. The labeling efficiencies ranged from 60-75%. Copper-labeled DTS-HSA was prepared similarly; however, spin column chromatography showed a labeling effiency of greater than 95%.

Blood clearance and biodistribution of ^{67}Cu-benzyl-TETA-HSA and ^{67}Cu-DTS-HSA were determined by removing a blood sample by direct cardiac puncture at the appropriate times after i.v. injection. The blood was counted in a NaI well counter, and the % injected dose/gram was determined for each sample. The blood clearance of both agents were comparable out to 30 minutes, however copper-labeled benzyl-TETA-HSA was stable for a longer time period.

Due to consistent labeling effiencies of greater than 95%, in the preparation of ^{62}Cu-DTS-HSA for dog imaging the final spin column purification was excluded. This gave a final yield of ^{62}Cu-DTS-HSA that was over three times that of ^{62}Cu-benzyl-TETA-HSA (without decay correction). To evaluate the imaging characteristics of the ^{62}Cu-agents, 7 mCi of

^{62}Cu-benzyl-TETA-HSA and 20 mCi of ^{62}Cu-DTS-HSA were administered sequentially to an anesthetized dog. Static tomagraphic reconstructions were compared with those obtained after administration by inhalation of 40 mCi of $C^{15}O$, which binds irreversibly to erythrocytes. Images obtained with ^{62}Cu-labeled benzyl-TETA-HSA and DTS-HSA showed identical blood pool distribution compared to those obtained with $C^{15}O$. The simpler method of production and higher yield of ^{62}Cu-DTS-HSA as well as the high quality of the blood pool images suggest that this radiopharmaceutical would be of more practical use than ^{62}Cu-benzyl-TETA-HSA.

Acknowledgements: The authors greatfully acknowledge Dr. Claude Meares for providing Br-benzyl-TETA. This work was sponsored by DOE grant # DE-FG02-87-ER60512.

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TABLE 1

Blood clearance of the copper-labeled blood-pool radiotracers 1-60 minutes after i.v. injection in adult female Sprague-Dawley rats. The data is reported as the mean ± standard deviation.

Time (minutes)	% ID/g HSA-DTS	% ID/g Benzyl-TETA-HSA
2	7.51 ± 0.24	5.67 ± 1.08
5	6.92 ± 0.39	5.59 ± 1.05
10	6.61 ± 0.57	5.30 ± 0.95
20	4.71 ± 0.48	4.93 ± 0.99
60	3.26 ± 0.43	4.62 ± 0.58

Paper L10

RETENTION MECHANISM OF Cu-62-PTSM IN THE BRAIN: IRREVERSIBLE REDUCTION OF Cu-PTSM BY MITOCHONDRIAL ENZYME.

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In our preliminary survay studies, radiocopper labeled bisthiosemicarbazone (Cu-DTS) complexes showed high brain accumulation (1). Among the Cu-DTS complexes, Cu-pyruvaldehyde-bis(N4-methylthiosemicarbazone) (Cu-PTSM, Fig. 1) showed a most potential chracteristics for brain imaging (2). The high brain extraction of Cu-PTSM is considered to be a result of its appropriate lipophilicity as well as its small molecular size. However, its retention mechanism was still unknown. In this study, chemical structure and valents states of Cu-PTSM in the brain was evaluated using electron spin resonance spectrometry (ESR) and retention mechanism of Cu-PTSM in the brain was discussed.

In mouse brain homogenate, Cu-PTSM was quickly changed to ESR-inactive form, namely reduced monovalent Cu. This reduction was largely inhibited at 4 °C incubation with brain homogenate. In addition, pre-heat treatment (60 °C, 1.5 min) of the brain homogenate also deminished its reduction ability. These results indicated the contribution of some enzyme(s) to the reduction of Cu-PTSM.

To identify the reduction site of the brain cell, sub-cellular fractionation was performed. ESR signal of Cu-PTSM was reduced only in the mitochondrial fraction, but not in the nucleus, microsome or soluble fractions (Fig. 2). Gathering these results, it was concluded that Cu-PTSM was reduced by mitochondrial enzyme system.

It was considered that Cu-PTSM might be evaluated as a blood flow tracer, as far as mitochondrial function is normal. However, if there is mitochondria-selective damages in some diseased states, Cu-PTSM accumulation might be modified.

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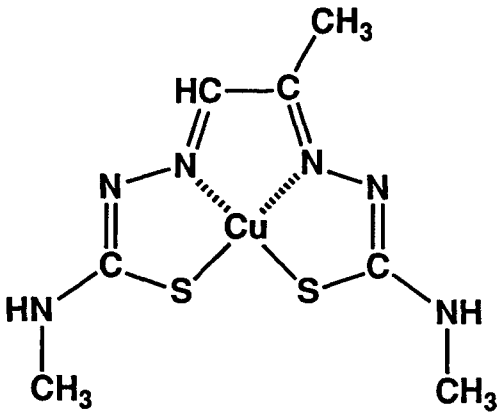

Figure 1. Structure of Cu-PTSM

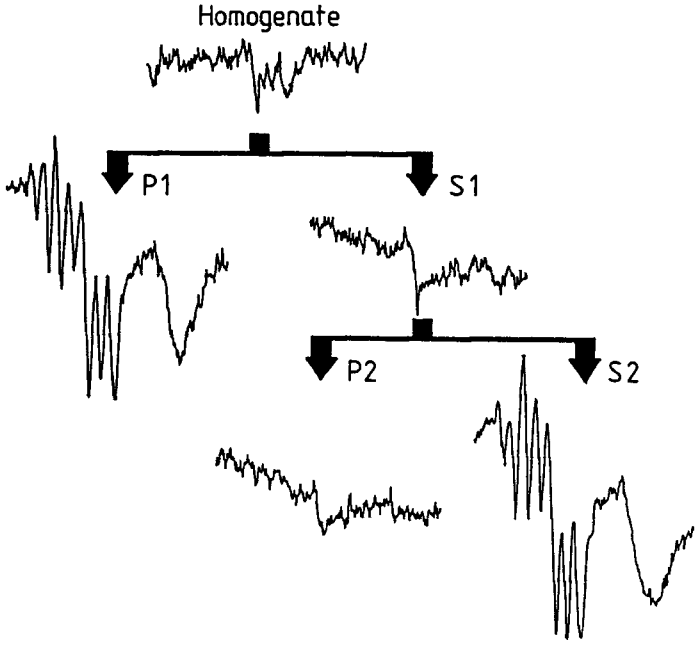

Figure 2. ESR spectra of Cu-PTSM in sub-cellular fractions.

Synthesis of 2-[^{18}F]FDG Using Tetraalkylammonium Bicarbonates.

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Kryptofix 222-K_2CO_3 (K222-K_2CO_3) is the most widely used phase transfer reagent (PTR) for the preparation of 2-[^{18}F]fluoro-2-deoxyglucose (2-[^{18}F]FDG) from 1,3,4,6,-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose (triflate).[1] A major concern with K222 is its toxicity in laboratory animals and the limited knowledge of its pharmacological response in humans.[2] Although methods for the removal of K222 have been described,[1,3] the potential of K222 in the patient formulation is a risk, especially for automated systems. Tetraalkylammonium hydroxides have been effectively used as PTRs for the preparation of 2-[^{18}F]FDG although yields are inferior to K222-K_2CO_3.[4] We have reinvestigated tetraalkylammonium salts in an effort to improve the yield of 2-[^{18}F]FDG, because they have an extensive documented history of therapeutic use in humans.[5]

We have found that tetraethylammonium bicarbonate ($TEAHCO_3$) and tetra-*n*-butylammonium bicarbonate ($TBAHCO_3$) provide yields of 2FDG that are significantly improved relative to tetraethylammonium hydroxide (TEAOH) and comparable to K222-K_2CO_3 (in our hands). We have confirmed these yields in syntheses that employ NCA ^{18}F and 2 umol ^{19}F.

The tetraalkylammonium bicarbonates ($TAAHCO_3$) were prepared by bubbling CO_2 through an aqueous solution of the tetraalkylammonium hydroxide (pH > 12) until the pH had stabilized at 7-8. The flask was evacuated to remove excess CO_2, concentrated to remove water and taken up in CH_3CN. The concentration of the $TAAHCO_3$ was confirmed by treating 1 ml of the solution with excess acetic acid and measuring the volume of CO_2 released.

In a typical synthesis with ^{19}F, 50 umol $TEAHCO_3$ (in CH_3CN) and 2 umol HF (in 0.2 ml 18 megohm-cm H_2O) was added to 4 ml of CH_3CN (4A molecular sieves) in a 25ml borosilicate glass flask. The mixture was concentrated under reduced pressure with a helium bleed and vigorous stirring in a 65°C oil bath. An additional 5 ml of CH_3CN was added and the reaction mixture concentrated. To the reaction vessel was added 40 mg (83.3 umol) of triflate in 4 ml of CH_3CN. The mixture was stirred for 8 min under helium in a 100°C oil bath. The reaction mixture was concentrated, treated with 2 ml of 2N HCl and heated for 20 min in a 125°C oil bath under helium. The reaction mixture was passed through an ion retardation column (BioRad AG11A8) to effect neutralization, a C18 sep pak (Waters) to effect decolorization and

a neutral alumina sep pak (Waters) to remove fluoride. The products of the cold synthesis were analyzed and quantitated by anion exchange HPLC coupled to pulsed amperometric detection. The typical synthesis with NCA ^{18}F was carried out in a similar fashion. After EOB, the enriched water ($H_2{}^{18}O$) was recovered by distillation and the ^{18}F was taken up in sterile water. Syntheses were run with 10-20 mCi of activity. Nitrogen, instead of helium was used as the inert gas. The decay corrected yield of 2-[^{18}F]FDG was determined by measurement of the activity in the final product mixture coupled with analysis of the final product mixture with radio-TLC. The radiochemical purity of the 2-[^{18}F]FDG was >95%.

Table 1 contains results of syntheses with 2 umol of $H^{19}F$ and 80 umol of triflate; yields are relative to starting ^{19}F. The amount of PTR used is indicated in the table and gave the best and most reproducible yield for that PTR. Table 2 contains results with 10-20 mCi of ^{18}F, NCA and 80 umol of triflate; yields are decay corrected.

Table 1 **Yields of 2-[^{19}F]FDG**

Phase Transfer Reagent	PTR (umol)	Yield	Std. Dev.	n
TEAOH	68	5%	3%	4
TEA_2CO_3	68	20%	3%	3
$TEAHCO_3$	51	41%	2%	2
$TBAHCO_3$	43	63%	1%	2
Kryptofix 222-K_2CO_3	85	44%	3%	2

Table 2 **Yields of 2-[^{18}F]FDG**

Phase Transfer Reagent	PTR (umol)	Yield (EOB)	Std. Dev.	n
TEAOH	68	4%		1
$TEAHCO_3$	51	48%	9%	3
$TBAHCO_3$	43	63%	9%	3
Kryptofix 222-K_2CO_3	67	57%	14%	4

Both $TEAHCO_3$ and $TBAHCO_3$ provide higher yields of 2-[^{18}F]FDG or 2-[^{19}F]FDG than does TEAOH. Even though the yield for 2-[^{18}F]FDG using TEAOH in this study is lower than a literature value (12-17% using TBAOH[4]), $TEAHCO_3$ and $TBAHCO_3$ represent a substantial improvement and give yields that are comparable to yields that we have obtained with K222-K_2CO_3. A possible explanation for the improved performance of the bicarbonate versus hydroxide salts is destruction of the starting mannose triflate tetraacetate via elimination or acetate hydrolysis by hydroxide. Replacement of hydroxide by the less nucleophilic and less basic bicarbonate reduces this nonproductive consumption of the mannose triflate tetraacetate. This explanation is consistent with the observation that TEA_2CO_3 provides a yield of 2FDG that is midway between the yields seen with TEAOH and $TEAHCO_3$.

It is important to point out that the syntheses described are carried out without an intermediate purification step to remove the PTR (either K222 or tetraalkylammonium) prior to hydrolysis.[1,3] K222 was removed via a sulfonic acid functionalized polystyrene resin (Dowex 50W) after HCl hydrolysis.[6] We have found that TAA salts may also be effectively removed using the same resin or via sulfonic acid derivatized silica gel (Bakerbond SPE). Using TLC and an iodoplatinate stain, we are unable to detect TEA, TBA or K222 using this clean-up method in the final aqueous solution The presence of the PTR during hydrolysis does not adversely affect yield and eliminating the intermediate purification step greatly simplifies the synthesis protocol, an important goal for automation and a reduced synthesis time.

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Paper L12

DEVELOPMENT AND VALIDATION OF A SOLVENT EXTRACTION TECHNIQUE FOR DETERMINATION OF Cu-PTSM IN BLOOD.

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Copper-62 labeled pyruvaldehyde *bis*(N^4-methylthiosemicarbazonato)copper(II), Cu(PTSM), is a potential generator-based PET radiopharmaceutical for evaluation of cerebral and myocardial blood flow (1). Quantitation of tissue perfusion with this tracer will require determination of the fraction of copper radioactivity in arterial blood that chemically remains in the form of Cu(PTSM) at various times following intravenous injection. We report here an investigation of the partitioning of Cu(PTSM) between blood plasma and red cells and the development of a solvent extraction technique for determination of the Cu(PTSM) content of blood. For these studies longer-lived ^{67}Cu has been employed as a convenient substitute for ^{62}Cu.

The partitioning of [^{67}Cu]-Cu(PTSM) between plasma and red blood cells was evaluated *in vitro* using both human and animal blood. One minute after mixing [^{67}Cu]-Cu(PTSM) with human blood (hematocrit = 44%) approximately 75% of the ^{67}Cu radioactivity is found in the plasma phase. This distribution of activity remains constant when the blood is kept at 4°C; however, at room temperature and 37°C the fraction of ^{67}Cu radioactivity associated with red cells is found to increase with time (Table 1). The partitioning of [^{67}Cu]-Cu(PTSM) between blood plasma and red cells appears to be species dependent. For example, one minute after mixing [^{67}Cu]-Cu(PTSM) with rat blood (hematocrit = 45%) only 55% of the ^{67}Cu-radioactivity is found in the plasma phase.

Using a variety of alcohols, solvent extraction was investigated as a technique to recover the lipophilic [^{67}Cu]-Cu(PTSM) complex from blood. One minute after mixing [^{67}Cu]-Cu(PTSM) with human blood, a 10 μL blood sample was added to 1.0 mL of the various alcohols and vortex mixed. The blood-alcohol suspension was then centrifuged to precipitate the denatured proteins and cell debris and the fraction of radioactivity remaining in the alcohol solution measured. Octanol consistently provided higher and more reproducible extraction of ^{67}Cu-radioactivity than the other alcohols examined; as the alcohol was varied the efficiency of ^{67}Cu extraction was found to progressively decrease (from >80% to 35%) in the order: *n*-octanol > *iso*-amyl alcohol >

Table 1. Distribution of [^{67}Cu]-Cu(PTSM) Between the Plasma and Red Cell Fractions of Human Blood *In Vitro*.

Time Post-Mixing (minutes)	Percentage of ^{67}Cu Radioactivity in Plasma Fraction		
	4°C	20°C	37°C
1	78.0 ± 1.1%	75.1 ± 1.2%	76.1 ± 0.6%
5	72.8 ± 0.7%	70.5 ± 2.9%	70.6 ± 0.7%
10	71.5 ± 1.2%	69.6 ± 0.9%	64.2 ± 1.7%
20	71.3 ± 1.1%	66.6 ± 1.2%	57.0 ± 3.1%

Values shown represent the mean of three measurements and their standard deviation.

isopropanol > ethanol > *n*-butanol > *n*-propanol. Consequently, *n*-octanol was employed as the alcohol of choice in all subsequent studies.

The stability of [^{67}Cu]-Cu(PTSM) in either whole blood, plasma, or washed red blood cells was determined using this octanol extraction procedure to analyze ^{67}Cu-blood (or ^{67}Cu-blood component) mixtures at various times post-mixing *in vitro*. The data presented in Table 2 shows that the recovery of [^{67}Cu]-Cu(PTSM) from whole blood or saline-suspended red blood cells is high (*ca* 90%) immediately after mixing, but declines slowly with time. Incubation of the [^{67}Cu]-Cu(PTSM)-blood mixture at 37°C instead of room temperature results in a more rapid decomposition of the Cu(PTSM) radiopharmaceutical, as evidenced by a more rapid decline in the fraction of octanol extractable radioactivity. However, following mixing of [^{67}Cu]-Cu(PTSM) with plasma *ca* 97% of the ^{67}Cu-radioactivity can be consistently recovered by octanol extraction, regardless of the time post-mixing (Table 2). Analysis of the octanol extract by thin layer chromatography in all cases shows that >95% of the extracted radioactivity is present as Cu(PTSM) (silica gel TLC plates eluted with ethanol).

Table 2. Recovery of ^{67}Cu-(PTSM) from Human Blood and Blood Components by Octanol Extraction*.

Time Post-Mixing (minutes**)	Percentage Total ^{67}Cu-Activity Extracted into Octanol		
	Whole Blood	Plasma	Red Cells
0.25	88.9 ± 1.0	97.2 ± 0.4	89.9 ± 0.9
0.50	88.5 ± 2.0	96.9 ± 0.5	88.9 ± 0.7
1.0	88.3 ± 1.2	97.8 ± 0.0	87.9 ± 0.4
5.0	85.5 ± 0.9	96.8 ± 1.0	74.4 ± 3.1
10.0	78.7 ± 0.4	93.1 ± 3.7	66.1 ± 0.7
20.0	67.6 ± 3.3	96.6 ± 0.9	53.0 ± 0.4

*Values shown represent the mean ± standard deviation of 3 samples; **All blood samples were incubated at 20 °C.

As a test of our ability to quantitate "available" [^{67}Cu]-Cu(PTSM) in blood, the biodistribution of [^{67}Cu]-Cu(PTSM) was determined in ketamine anesthetized rats following i.v. injection of tracer that had been pre-mixed with rat blood for 2 to 25 min. Octanol extraction was performed on each ^{67}Cu(PTSM)-blood mixture at the time of injection to quantitate the fraction of injected ^{67}Cu radioactivity that remained as Cu(PTSM). In control rats receiving [^{67}Cu]-Cu(PTSM) in saline 1.60 ± 0.22% of the injected dose was found in the brain at 5 min. post-injection (n = 6); while only 0.90 ± 0.41% of the injected dose of ^{67}Cu-radioactivity was found in the brains of animals (n = 22) that received [^{67}Cu]-Cu(PTSM) pre-mixed with blood. However, when this latter data was individually corrected by the octanol extraction efficiency of the injectate, the mean percent of injected ^{67}Cu-PTSM dose in the brain became identical to the control animals (1.68 ± 0.32; n = 22). These results indicate that octanol extraction provides a suitable means to quantitate [^{67}Cu]-Cu(PTSM) levels in blood.

Acknowledgement

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Paper L13

SYNTHESIS OF FLUORINE-18 LABELED 4-FLUORO-4-DEOXY-D-GLUCOSE AS A POTENTIAL BRAIN, HEART AND TUMOR IMAGING AGENT

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Fluorinated carbohydrate analogues in which fluorine-18 replaced a hydroxyl group at carbon position-2 of D-glucose (2-fluoro-2-deoxy-D-glucose, 2-FDG), carbon position-2 of D-mannose (2-fluoro-2-deoxy-D-mannose, 2-FDM) and carbon position-3 of D-glucose (3-fluoro-3-deoxy-D-glucose, 3-FDG) show significant brain, heart and tumor uptake and pronounced retention. Another potential positron emitting D-glucose analog is fluorine-18 4-fluoro-4-deoxy-D-glucose, 4-FDG. 4-fluoro-4-deoxy-D-glucose behaves similar (Km=84mM, Vmax=0.1) to 3-FDG (Km=70mM, Vmax=0.1) as a substrate for hexokinase (1). Kinetic studies of the transport of 4-FDG across the blood-brain barrier and into the myocyte are not available. However, 4-FDG affinity for the carrier responsible for D-glucose transport into these cells (2) should be similar to 3-FDG (Ki=6.9mM) and D-glucose (Ki=6.3mM) due to presence of hydrogen bonding between the fluorine atom at C-4 in the D-gluco configuration and the carrier which is a structural requirement for transport (2).

Because of our interest in providing the regional distribution of radiotracers which show brain, heart and tumor specificity, we report the development of the synthesis of fluorine-18 labeled 4-fluoro-4-deoxy-D-glucose. The synthetic approach chosen for 4-FDG (Figure 1) involved displacement of a 4-triflate group. The triflate substrate 3 was prepared by treating methyl-B-D-galactose with benzoyl chloride followed trifluoromethane sulfonic anhydride. Treatment of 3 with $K^{18}F$/K 2.2.2 in acetonitrile at 85 C for 5 min followed by hydrolysis gave F-18 4-FDG (5) in 50% (EOB). Evaluation of F-18 labeled 8 will be presented.

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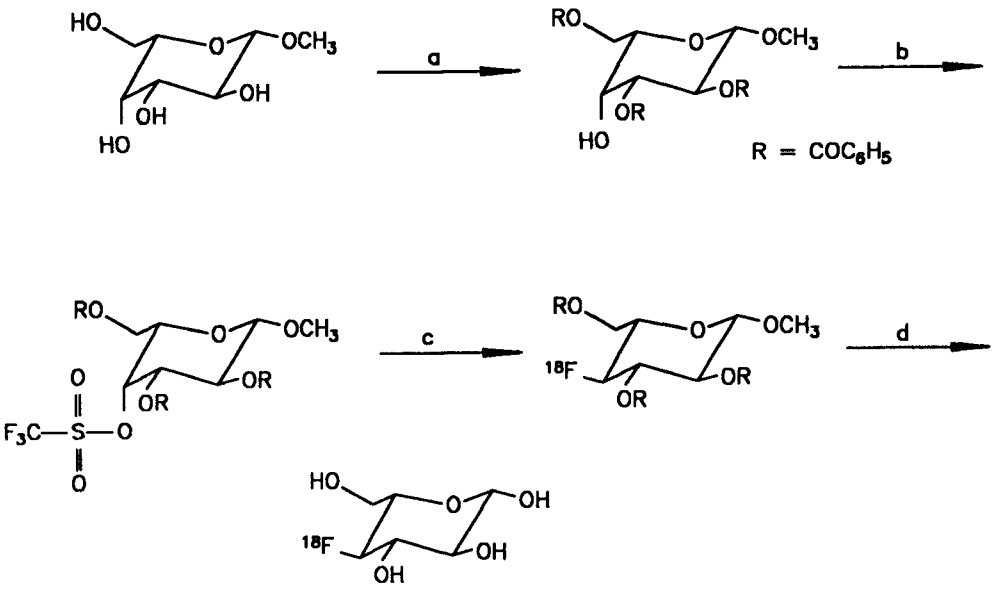

Reagents: a = C_6H_5COCl, pyridine; b = $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2;

c = $K^{18}F$, K222, CH_3CN; d = 1NHCl

Paper L14

FLUORINATION VIA CYCLIC SULFAMIDATES: HIGH YIELD STEREOSPECIFIC SYNTHESIS OF β-[F-18]FLUOROMETHAMPHETAMINES. M.E. Van Dort, Y-W Jung, M.R. Kilbourn, D.M. Wieland. University of Michigan Medical Center, Division of Nuclear Medicine, Ann Arbor, MI 48109-0552

Aliphatic nucleophillic substitution of suitable leaving groups with [F-18]fluoride is a commonly utilized radiofluorination method for the synthesis of many radiopharmaceuticals. In general, sulfonate esters are the most often utilized leaving groups of which triflates(trifluoromethanesulfonates) offer the best radiochemical yields.[1]

Recently cyclic sulfamidates have been reported to undergo nucleophillic substitution at oxygen with a variety of nucleophiles to provide enantiomerically pure products in good to excellent yields.[2] Lyle and coworkers have also reported the synthesis of two isomeric enantiomerically pure β-fluoromethamphetamines from chiral acyclic sulfamate precursors.[3] In order to test the synthetic utility of chiral cyclic sulfamidates for the stereospecific synthesis of β-fluorophenethylamines, two model compounds were synthesized and their reactivity and stereospecificity of [F-18]fluoride substitution examined.

The cyclic sulfamidate precursor 2 was synthesized in two steps by treatment of commercially available (1R)(2S)(-)ephedrine with $SOCl_2/Et_3N$ in CH_2Cl_2 at -78° C and oxidation of the resulting sulfamidite with $NaIO_4/RuCl_3$. Treatment of 2 with KF/CaF_2 and kryptofix 222 in refluxing CH_3CN followed by acid hydrolysis with 3N HCl afforded enantiomerically pure (1S)(2S)-β-fluoromethamphetamine 4 identified by the characteristic 1H NMR splitting pattern of the C-1 methine proton. A similarly sequence of reactions on (1S)(2S)(+)pseudoephedrine afforded (1R)(2S)-β-fluoromethamphetamine.

Reaction of 2 with no-carrier-added [F-18]fluoride ion provided the corresponding F-18 labeled N-sulfonic acid derivative 3 which was hydrolysed *in situ* to provide (1S)(2S)-β-[F-18]fluoromethamphetamine 4 in 99% radiochemical purity. The isolated radiochemical yield was 50-55% (EOS) in a 50 min synthesis time. Similarly (1R)(2S)-β-[F-18]fluoromethamphetamine was prepared in comparable radiochemical yield and purity from its corresponding cyclic sulfamidate.

In conclusion this route allows for the stereospecific introduction of [F-18]fluoride in high radiochemical yields at a carbon atom β to a secondary amine. The tracers display excellent in vivo stability towards defluorination in preliminary rat biodistribution studies. The application of this novel method towards the radiosynthesis of other biogenic amines is currently in progress.

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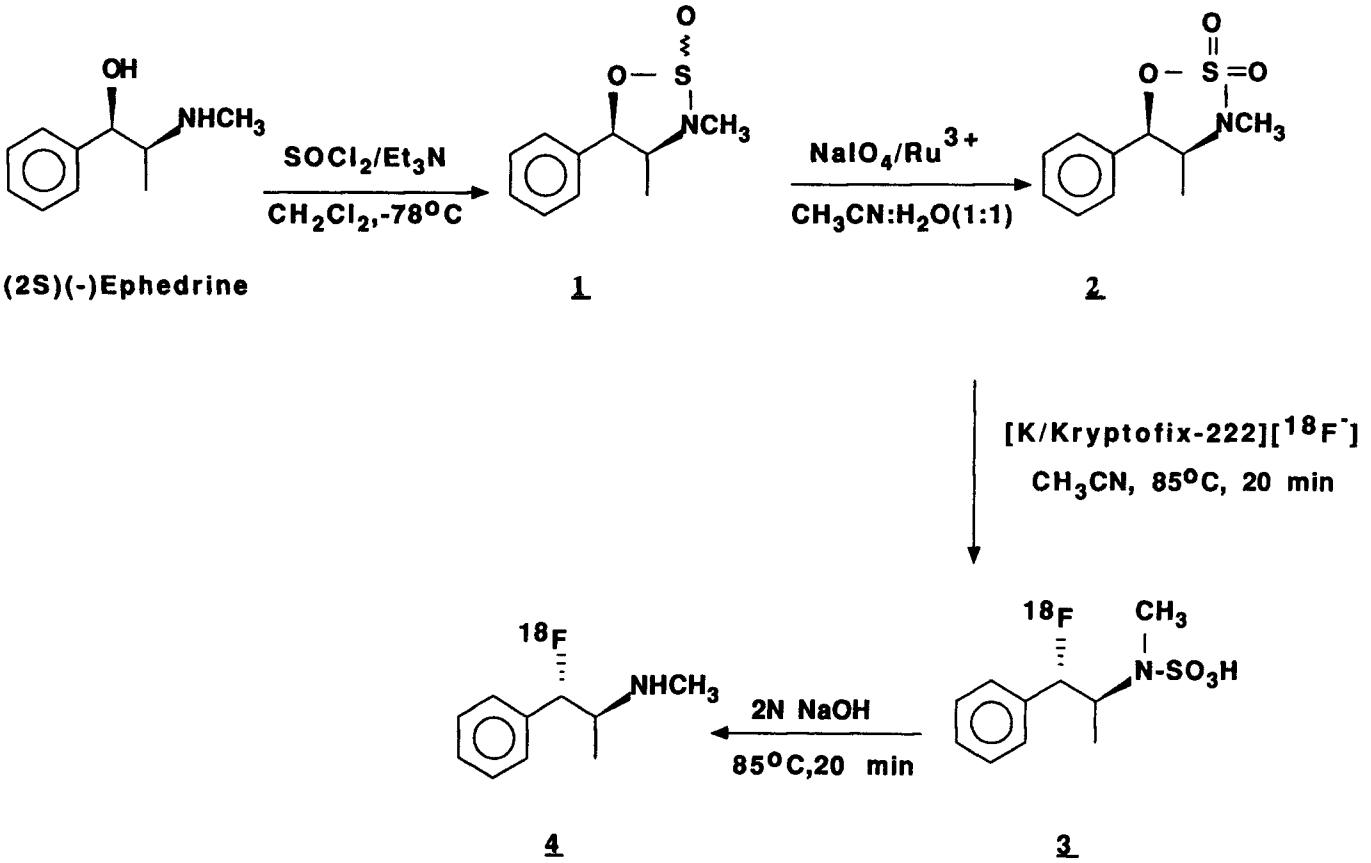
SYNTHESIS OF (1S,2S)-ß-[^{18}F]FLUOROMETHAMPHETAMINE



(1R)(2S)(-)Ephedrine

1

2

3

4

A NEW METHOD FOR THE SYNTHESIS AND FLUORINE-18 LABELLING OF 2- DEOXY-2-FLUORO-D-GALACTOSE. W.Barry Edwards, Carmen S. Dence, Michael J. Welch, Mallinckrodt Insitute of Radiology, Washington University Medical School, St. Louis, MO 63110.

2-Deoxy-2-[^{18}F]-fluoro-D-galactose (^{18}FDGal) has been proven to be a useful tracer to measure galactose metabolism in the liver with positron emission tomography[1]. ^{18}FDGal has recently been synthesized via ^{18}F ion displacement of a triflate[2]. Following this strategy, we have improved the synthesis of both ^{18}FDGal and the unlabelled galactose 4 (FDGal).

Preparation of the fluorinated galactose began with the commercially available methyl-β-D-galactose. Modification of the established conditions[3] gave the protected carbohydrate 1. A subsequent alteration of the published oxidation[2] of 1 followed by lithium aluminum hydride reduction yielded the talose derivative 2. Reaction of 2 with triflic anhydride and pyridine furnished the key triflate intermediate 3 in good yield. Fluoride displacement followed by acid hydrolysis gave FDGal. The structures of the the unlabelled compounds were determined with ^{1}H,^{13}C, and ^{19}F-NMR and mass spectrometry.

The synthesis of ^{18}FDGal required the [^{18}F]-fluoride displacement of the triflate function of 3. Deprotection by acid hydrolysis of the [^{18}F]-labelled intermediate afforded a single radioactive product as shown by radio-HPLC and -TLC. The retention time of this product corresponded to that of FDGal. The radiochemical synthesis required about 90 minutes and purity of ^{18}FDGal was at least 98%, with a radiochemical yield of about 30% (non-decay corrected).

This work was supported by NIH cyclotron grant HL 13851.

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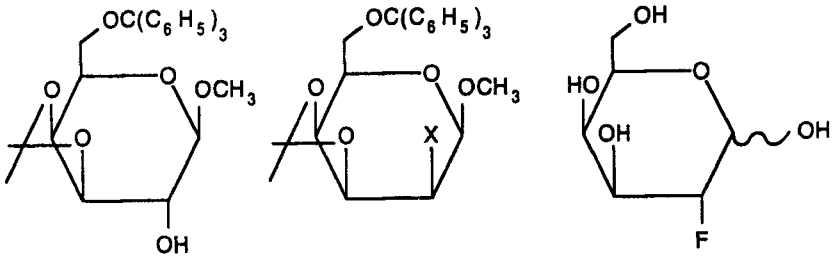
Figure 1



1

2 X=OH

3 X=OSO_2CF_3

FDGal

4

AN IMPROVED SYNTHESIS OF 1-[^{11}C]-D-GLUCOSE

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The preparation of 1-[^{11}C]-D-glucose using the well known Kiliani synthesis has been accomplished by Shiue (1), and was later automated by two groups (2,3). A modified synthesis using diborane as the reducing agent for the intermediate aldontriles has also been published (4). More recently, the availability [^{11}C]-nitromethane has now made feasible a synthesis of the desired sugar by the Nef reaction (5). In all these approaches the final product, after purification by ion exchange open-column chromatography, is obtained as a mixture of the epimers 1-[^{11}C]-D-glucose and 1-[^{11}C]-D-mannose. The reported ratio of glucose to mannose ranges from 0.25 to 0.5.

A goal of the present work was to find reaction conditions which would maximize the ratio of glucose to mannose in the Kiliani synthesis. Among the best understood mechanisms for the separation of carbohydrate isomers is their complexation with borate ions (6-8). We decided to test the use of a borate buffer to form a D-arabinose-borate complex prior to the attack by CN^- in order to direct the stereochemistry.

With the use of a 0.033M borate buffer, we successfully raised the ratio from 0.52 ± 0.25 to 1.80 ± 0.57 in favor of glucose. The use of a higher molarity buffer, 0.3M borate-formate at pH 8.2, was also found to improve the ratio to 1.52 ± 0.18 which is less than that with the more dilute buffer. The overall radiochemical yield for the mixture of sugars was also higher with the dilute borate buffer, 30%, vs 15-20% obtained with the borate-formate buffer.

Another goal of the project was to construct a reliable system for the synthesis of $NH_4{}^{11}CN$ starting with $^{11}CO_2$ (9). The $NH_4{}^{11}CN$ is delivered into a versatile, easy-to-operate remote gantry system for the synthesis of 1-[^{11}C]-D-glucose among other compounds.

We are currently producing 1-[^{11}C]-D-glucose in a ready-to-inject form in amounts from 5

to 20 mCi. The radiochemical purity is 98%-99.5% and the synthesis time, including HPLC preparative separation is 50 to 55 minutes.

This work was supported by NIH grants HL13851 and NS28700.

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FLEROXACIN, A QUINOLONE ANTIBACTERIAL AGENT, LABELING WITH FLUORINE -18 FOR PHARMACOKINETIC STUDIES.

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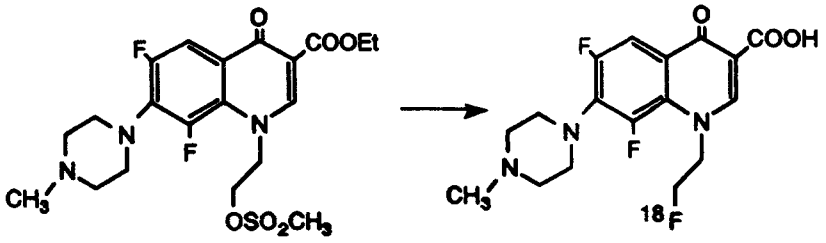
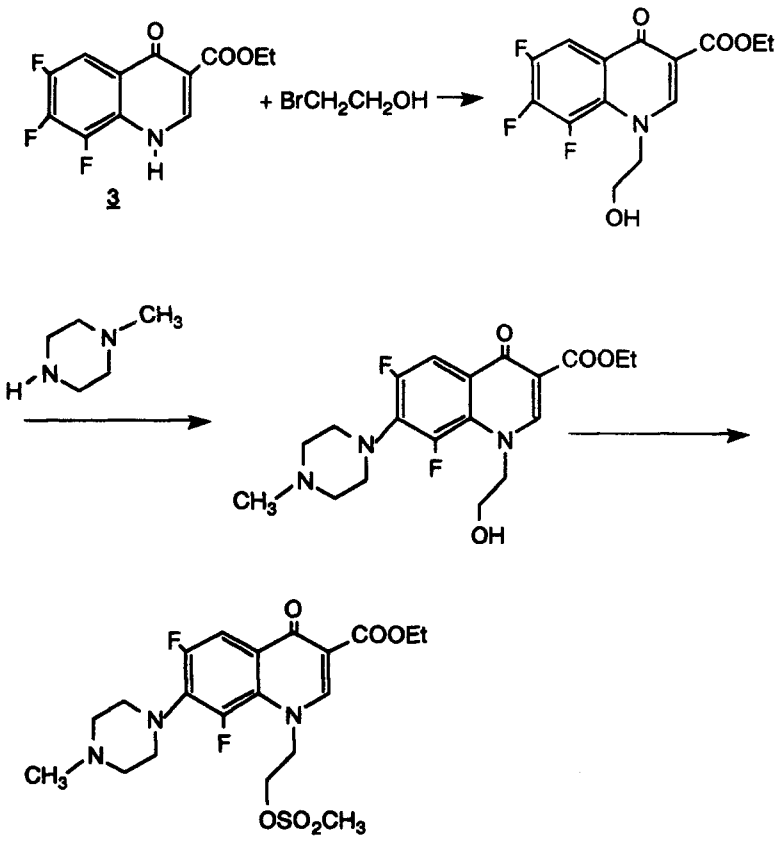
Fleroxacin (RO 23-6240) is a new broad spectrum trifluoro quinolone antibacterial. To study the in vivo pharmcokinetics of fleroxacin the drug was labeled with ^{18}F by nucleophilic substitution of the mesylate precursor 1 (Scheme 1). The synthesis of 1 is shown in Scheme 2.



Scheme 1

^{18}F in $H_2{}^{18}O$ (1 ml) was added to a 5 ml reacti-vial containing kryptofix (13.75 umol), K_2CO_3 (6.25 umol), KF (0.5 umol) and 25 ul phosphate buffer pH 7. The solution was dried by evaporation under nitrogen at 110°C followed by addition of acetonitrile. A solution of 1 (6.25 umol) in 0.5 ml CH_2Cl_2 was added to the reacti-vial and the solution heated at 80°C for 12 min. Purification of the ethyl ester of 2 was done on silica gel Sep-Pak column with CH_2Cl_2: EtOH: NH_4OH, 90:10:1 as eluate. The solution was evaporated and the ester hydrolyzed with 0.5N NaOH (1 ml) at 110°C for 12 min . Lactic acid (4 ml) was added and the solution filtered through 0.22 u membrane filter. The time required for synthesis was 90 min and the yield was 5%-8% (EOS). The radiochemical purity of 2 (>93%) was ascertained by HPLC on Waters Novapak C18 column (10 cm, 4 u) eluting with 0.01M phosphate buffer pH 3: MeOH, 4:1 at 1.3 ml/min (retention time 4.9 min). No detectable amounts of kryptofix were found by TLC analysis.

Based on the small scale synthesis of 2 a robotic synthesis was developed that yields a high purity product. Adequate amounts of 2 (50-80 mci) for both animal and human studies can be easily prepared.



Scheme 2

Compound 3 was synthesized according to the literature procedure (1).

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Paper L18

A Modified and Improved System For Synthesis of ^{18}F-2-Deoxy-2-Fluoro-D-Glucose (2-FDG) based on the resin exchanged method.

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ABSTRACT

2-FDG is the most widely used radiopharmaceutical for positron emission tomography (PET). Many different synthetic methods have been described to produce this important radiotracer since it's introduction. The most recent method involves the use of nucleophilic exchange on a quaternary 4-aminopyridinium resin(1). Although this method is successful and may have some advantages compared to other reported methods, nevertheless it suffers from unreliability (due to blockade of the resin column) and low production yields. We have developed a modified system using minimum amounts of the resin (0.3g) as reactant in a "U"-type filter vessel. This system would allow an efficient entrapment of ^{18}F-fluoride ion (the entrapment for more than seventy runs ranged between %93 to %98 and with a mean of %96) in the resin. The resin is then allowed to react with 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-D-mannopyranose in acetonitrile (1-3mL) with stirring in an oil bath at 120C for 5 minutes. This has increased the yield of the reaction and also eliminate the possibility of the column blockade. The fluorinated material is then hydrolysed and purified to give 2-FDG reliably with yields ranging from %25 to %40, and with a mean of %30 (N=71). This means that we routinely produce about 150mCi of 2-FDG at the end of synthesis starting with about 500mCi of ^{18}F fluoride ion at the end of bombardment.

EXPERIMENTAL

^{18}F-Fluoride was produced by ^{18}O(p,n)^{18}F reaction on ^{18}O-Water (95-97% enrichment, Isotec) in all silver target. The "Merrifield resin" (Aldrich Chem. Co.) was modified as reported in the literature[1] by reacting 4-(4-Methyl-1-piperidino)pyridine (Reilly and Tar Chem. Co., Indianapolis, Indiana) in acetonitrile. Ion retardation resin was obtained from Bio-Rad. C-18, and Silica cartridge were obtained from Waters Ass. All other chemicals were obtained from Aldrich, and used without further purification.

^{18}F-FDG Synthesis

The schematic diagram of our system is shown in figure 1. below.

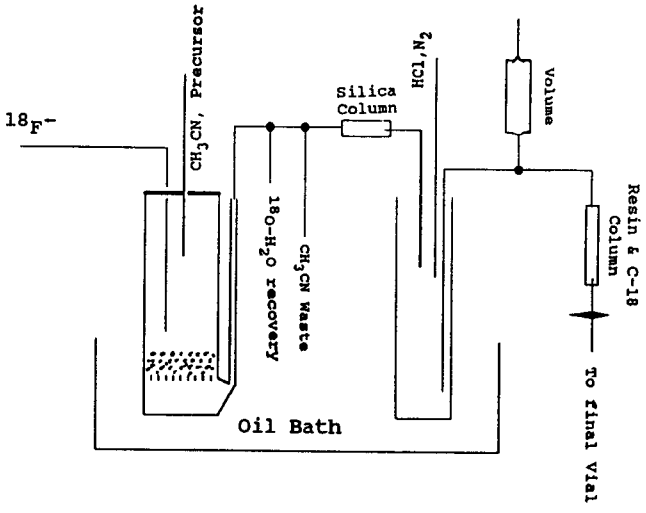

Figure 1. Schematic diagram of the system for 2-FDG synthesis using slurry resin method.

The ^{18}O-water carrying ^{18}F-fluoride ion is pushed through a Teflon tubing from the target to the synthesis apparatus by means of slow helium gas. This solution is directly passed through the resin

where ^{18}F-fluoride is trapped, and the ^{18}O-water is transferred to a collecting vial for future use. The resin is then washed twice with cold acetonitrile, and twice with hot acetonitrile before it is dried by a stream of nitrogen. A solution of 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-D-mannopyrannose (20mg) in acetonitrile (2mL) is then added to the resin and heated with stirring for 5 minutes. The solution is then passed through a silica gel cartridge into the hydrolysis tube. The resin is then washed with hot acetonitrile (2 X 2mL), and the washes are added to the hydrolysis tube. The acetonitrile is then evaporated by heating and slow stream of nitrogen to yield the fluorinated precursor. A solution of 1/N hydrochloric acid is then added to this material and heated for 15 minutes for hydrolysis. The acidic solution is then combined with sterile saline (8mL), which then pulled into the volume, and pushed through a C-18 column, ion retardation resin column, and a 0.22 micron filter into a sterile multi-dose vial.

RESULTS AND DISCUSSION

We have used this system for 71 successful synthesis. When we first started to use this system before fine tuning the yields were low, but we now produce a reliable respectable yields of over %25(decay uncorrected). However we still feel that the yields can be improved since a major portion of ^{18}F seems to be untouched bound to the resin. We have attempted to solve this problem by using functionalized glass beads unsuccessfully.

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Paper L19

SYNTHESIS OF LABELLED THYMIDINE USING ENZYME BASED HOLLOW FIBER MEMBRANE BIOREACTORS. Jeffrey Hughes and Michael Jay, College of Pharmacy, University of Kentucky, Lexington KY, 40536-0082, USA

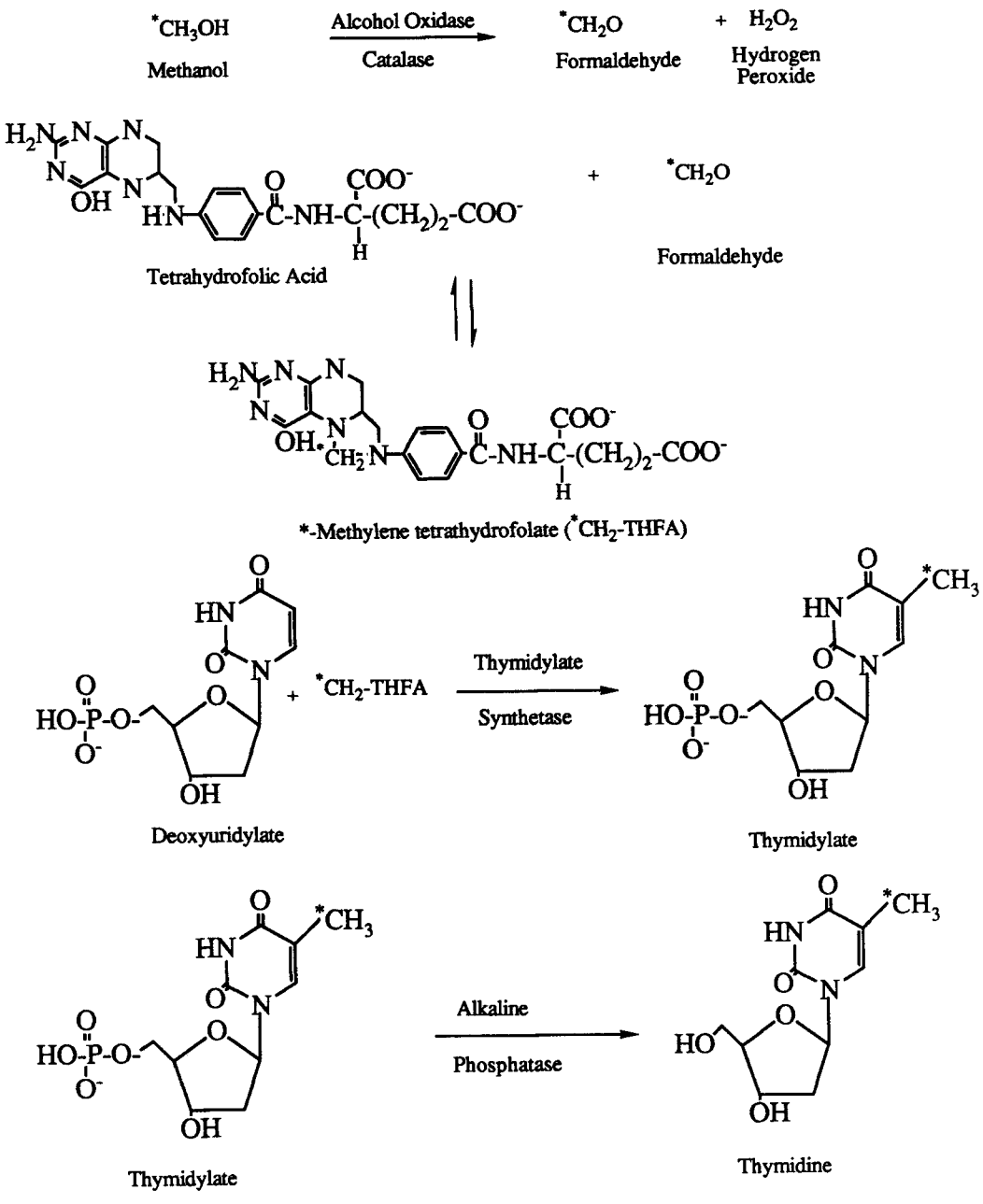
C-11 labelled thymidine and its analogs have potential uses in the detection and monitoring of therapy for a number of diseases states including viral infections and malignancies. Several different approaches have been utilized for the introduction of the C-11 label into the target thymidine molecule. The techniques used include both synthetic and biosynthetic methods. The major limitation to the simpler biosynthetic methods are in the time consuming purification steps. Improvement of the yield and purification of the final product can be accomplished by the immobilization of enzyme onto a hollow fiber membrane support (HFM). The membrane support would serve two roles: 1) simplify purification and 2) increase yield due to decreased resident times which are inherent with HFM. In this vein an enzyme bioreactor system has been developed which has the potential for rapid and efficient synthesis of C-11-thymidine from C-11-methanol.(Scheme 1) The system consists of three separate enzyme immobilized hollow fiber reactors. The first reactor is an immobilized alcohol oxidase/catalase system for the conversion of methanol to formaldehyde. The second reactor composed of immobilized thymidylate synthetase is used for the conversion of deoxyuridylate (dUMP), in the presence of labelled methylene tetrahydrofolate (methylene-THFA), to thymidylate. The final component is an immobilized alkaline phosphatase system for the dephosphorylation of labelled thymidylate to a labelled thymidine.

The immobilization of the various enzymes was accomplished by introducing the enzymes into an aldehyde activated ethylhydroxyl cellulose coated polysulfone membrane. A Schiff base was formed between a terminal amine group from a lysine residue for the enzyme and the aldehyde on the membrane. This was reduced to a stable secondary amine via incubation with sodium cyanoborohydride. (Scheme 2) The substrates were then introduced, via a peristaltic pump, through the lumen of the HFM. Product was collected through the shell side of the HFM system. By varying the residence time (contact time) through the bioreactors optimum yield could be obtained.

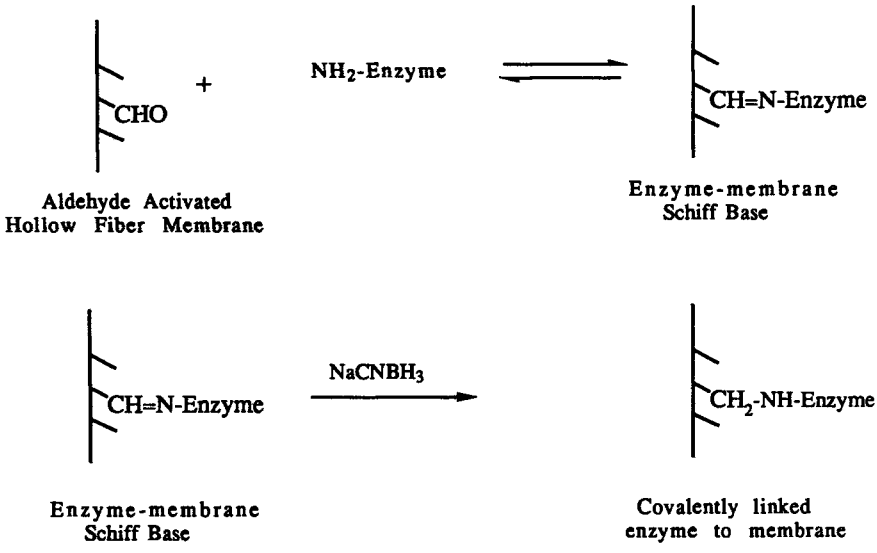
Production of formaldehyde from methanol was accomplished with the alcohol oxidase/catalase bioreactor. Both enzymes were attached to the HFS via secondary amine linkages. Optimum residence times in this system offered value of 0.5 umole of formaldehyde produced per minute. The enzyme catalase is included in the bioreactor to degrade the side production of hydrogen peroxide, potential poison of alcohol oxidase. Pre-incubation of labelled formaldehyde with tetrahydrofolic acid is done to encourage the formation of labelled methylene-THFA over hemithioacetal formation from the mercaptoethanol in the enzymatic buffer solution.

Thymidylate synthetase was covalently attached to the pore wall surfaces of the microporous hollow fiber membrane as described above. When dUMP and labelled methylene-THFA were recirculated through the second bioreactor production yields in the range of 56 nmoles/min were achieved. Compared to the alcohol oxidase system the production rate is lower but manageable. The lower yield is most likely due to the purity of the enzyme. It is hoped that with higher purity enzyme the yield of the of reaction can be increased.

The final component of the enzymatic bioreactor consisted of the enzyme alkaline phosphatase, a non-specific dephosphorylating enzyme. This system was used for the conversion of the nucleotide thymidylate to nucleoside thymidine. The enzyme was again covalently attached to the HFS. Conversion rates of 2 umoles/min could be obtained with this system.


*CH3OH
Methanol
Alcohol Oxidase
Catalase
*CH2O
Formaldehyde
+ H2O2
Hydrogen Peroxide
Tetrahydrofolic Acid
+ *CH2O
Formaldehyde
*-Methylene tetrathydrofolate (*CH2-THFA)
Deoxyuridylate
+ *CH2-THFA
Thymidylate Synthetase
Thymidylate
Thymidylate
Alkaline Phosphatase
Thymidine

Scheme 1



Scheme 2

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Paper L20

SYNTHESIS OF RADIOLABELLED ω–IODOALKYL GLUCOSIDES.

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Laurent Mauclaire [3], Christophe Morin * [1], Michel Vidal [1].

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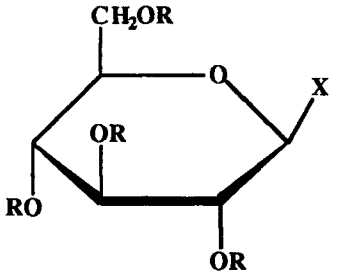
D-Glucose plays a pivotal role as an energy-yielding substrate through glycolysis and as such, it has received particular attention in nuclear medicine for imaging purposes. Despite various efforts to introduce iodine on a glucose backbone, no method, to the best of our knowledge, has addressed functionnalisation of the anomeric position.

Barnett *et al.* have shown during structural variation studies (1) that among other derivatives, n-propyl- β- D-glucopyranoside was a substrate for glucose transporters. As the bulkiness of iodine is similar to that of a methyl group, a CH2-CH2-I structural unit was thus considered, which led us to synthesize 1. Although a preparation of (unlabelled) 1 can be found in the literature (2), the synthesis starts with the unstable peracetylbromoglucose; therefore in the present case, another route has been used.

Commercially available 2 was reacted with 2-bromoethanol in the presence of freshly distilled boron trifluoroetherate (3) to afford 3 in over 80 % isolated yield. A single glucoside was formed (most likely through the anchimeric participation of the neighbouring acetyl group) and these conditions proved to be superior to other methods; good evidence for the beta-glucoside being formed were characteristic nmr absorptions (δ H–1 = 4.5 ppm ; J = 7.8 Hz ; δ C-1 = 100.8 ppm).

Halogen exchange (NaI - acetone) followed by Zemplèn deacetylation in chloroform / methanol at -20°c afforded 4 , whose structure was ascertained by complete ^{13}C and ^{1}H nmr assignments, with particular help of double quantum H/H 2D experiments .

Stability assays of 1 were carried out before any labelling experiments. 1 was stable in aqueous solutions (no degradation being observed after several months) or under " strong" labelling conditions (100 °C - several hours).



1 : R = H, X = $OCH_2CH_2I^*$
2 : R = Ac, X = OAc
3 : R = Ac, X = OCH_2CH_2Br
4 : R = Ac, X = OCH_2CH_2I
5 : R = H, X = $OCH_2CH_2OCH_2CH_2I$
6 : R = Ac, X = $OCH_2CH_2OCH_2CH_2OTs$

Introduction of radio iodine was performed through halogen exchange using an added carrier to minimize the impact of secondary reactions on free iodide (4). This was performed with either Na ^{123}I or Na ^{125}I in 90 - 100 % radiochemical yield. After ion-exchange purification, the desired glycoside is isolated in pure form suitable for bioassay studies.

Another higher analogue, namely 5, has also been prepared using a similar route; thus, glycosidation of 2 with the monotosylate of diethylene glycol (5-6) in the presence of stannic chloride in acetonitrile (8) afforded 6 which was then converted to 5 as described above. In 5 however, the lipophilicity of the now longer "side-chain" is being lessened by the presence of an additional oxygen .

Acknowledgment : M.-C. Salon is thanked for her help in recording double quanta homonuclear experiments.

References and note.

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LABELLING OF D-GLUCOSAMINE IN POSITION 1 WITH [^{11}C]CYANIDE

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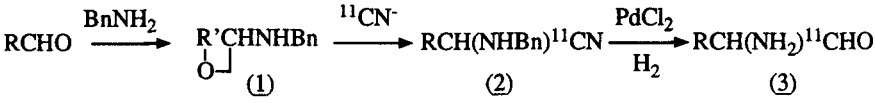
D-Glucosamine (2-amino-2-deoxy-D-glucose), the predominant hexosamine in brain glycoproteins (1), has been labelled with ^{15}N by Hornemann, (2) from cyanide and ^{15}N-labelled N-benzyl-arabinosylamine (1). The N-acetyl derivatives of D-glucosamine have previously been labelled in the acetyl group with ^{11}C (3) and ^{18}F (4). The latter has been used for PET studies of its tissue distribution and particularly its uptake in tumors (5). We present here an adaptation of Hornemanns method for labelling D-glucosamine with carbon-11 in position 1, by using [^{11}C]cyanide and (1) in a two step reaction as shown below.



R=$C_4H_9O_4$, R'=$C_4H_8O_3$

D-Arabinose and benzylamine were refluxed in EtOH to generate (1). Carrier KCN was added and [^{11}C]NH_4CN trapped directly in the precursor solution. At the end-of-trapping the reaction mixture was heated at 60°C for 5 min to produce the α-amino-nitrile (2) in 60-80% yields. The solvent was subsequently evaporated and HCl and $PdCl_2$ added. The reaction mixture was heated at 60°C for another 15 min under a stream of H_2(g). After cooling, the solution was neutralized and the catalyst removed by filtration. [1-^{11}C]-D-Glucosamine (3) was isolated by straight-phase HPLC. As confirmed by analytical HPLC, this procedure produced almost exclusively the D-glucosamine epimer. The radiochemical conversion was 20-30%, based on the trapped [^{11}C]cyanide.

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Paper L22

THE PREPARATION OF AN ^{11}C-LABELLED 5-LIPOXYGENASE PRODUCT, 5(S)-HYDROXY-6(R)-(N-1-[^{11}C]ACETYL)CYSTEINYL-7,9-TRANS-11,14-CIS-EICOSATETRAENOIC ACID.

Franz Oberdorfer, Thilo Siegel, Albrecht Guhlmann, Dietrich Keppler, Wolfgang Maier-Borst, Deutsches Krebsforschungszentrum, Institut für Radiologie und Pathophysiologie, im Neuenheimer Feld 280, D-6900 Heidelberg, FRG.

5(S)-Hydroxy-6(R)-(N-1-[^{11}C]acetyl)cysteinyl-7,9-trans-11,14-cis-eicosatetraenoic acid **1** was prepared through the reaction of leukotriene E_4 and 1-[^{11}C]acetyl chloride (Fig.1).The compound was obtained with 3.5% isolated yield, based on 1-[^{11}C]acetyl chloride. The preparation required 50 min from the end of radioisotope production. The chemical and radiochemical purity of an injectable dose of N-1-[^{11}C]acetyl leukotriene E_4 was 95%. The remaining impurity seemed to be the sulfoxide of 5(S)-hydroxy-6(R,S)-(N-1-[^{11}C]acetyl)cysteinyl-7,9-trans-11-(cis,trans)-14-cis-eicosatetraenoic acid. Considerable amounts of the 11-trans isomer of the target compound were detected in some preparations. These batches were also released for initial animal experiments [1]. The average specific activity of the carrier added preparation was 2 GBq/μmol at the time of application.

Leukotrienes are the principal metabolites of arachidonic acid metabolism, produced through the 5-lipoxygenase pathway in mammalian tissue [2-5]. Various syntheses of these natural compounds and a variety of their synthetic derivatives are described which contributed rapidly to the large progress made in the biological evaluation of the leukotrienes as important mediators in desease states in man [5]. Our interest has been directed towards the *in vivo* pattern of leukotriene transport and elimination by positron-emission-tomography [1] using the most practical ^{11}C-labelled leukotriene metabolite - with regard to its preparation.

Details of the apparatus and of the labelling procedure will be described. Analytical procedures corroborating the chemical identity and *in-vivo* behaviour of the target compound will be given.

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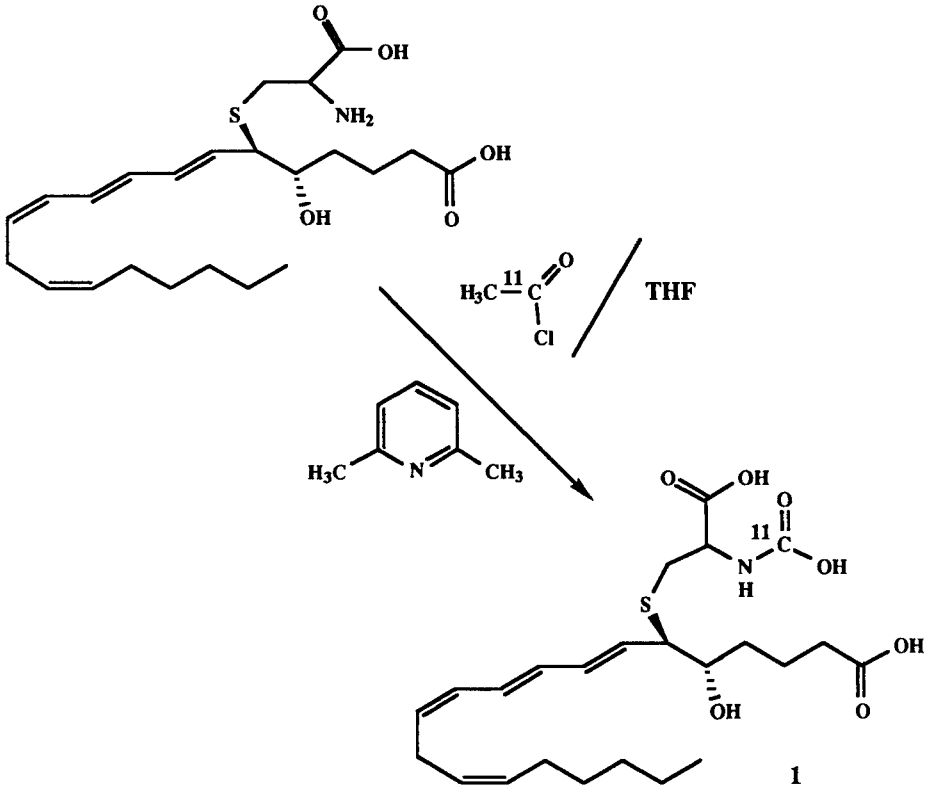

Figure 1: Procedure for the preparation of 5(S)-hydroxy-6(R)-(N-[1-^{11}C]acetyl)cysteinyl-7,9-trans-11,14-cis-eicosatetraenoic acid.