[^{11}C]-FLUMAZENIL METABOLITES : MEASUREMENTS OF UNCHANGED LIGAND IN PLASMA USING THIN LAYER CHROMATOGRAPHY AND RAPID LIQUID CHROMATOGRAPHY.

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To study in vivo benzodiazepine (BZ) receptors using PET, Flumazenil, an imidazobenzodiazepine with selective antagonistic actions, has been labeled with ^{11}C on its methyl group (1). The accurate determination of in vivo binding parameters using biomathematical models requires the knowledge of radioligand metabolism and the measurement of the plasmatic concentration of unchanged radioligand is mandatory. The present report describes and compares rapid and simple analytical procedures to measure unchanged [^{11}C]-Flumazenil in plasma.

After IV injection of 10 mCi of [^{11}C]-Flumazenil in baboon, blood samples are taken from 0.5 to 60 min. For radio-TLC analysis 0.2 mL plasma sample is deproteinized by precipitation using 2 mL methanol, the precipitate is washed with 1 mL CH_3OH (radioactivity recovery yield >95%) and the supernatants are mixed, then concentrated under vacuum. Separation of unchanged radioligand from its metabolites is performed on silicagel plates (Si60 Merck) by TLC with CH_2Cl_2-CH_3OH (95-5) and by OPTLC with CH_2Cl_2-C_2H_5OH (90-10). Whatever the system used, the radioactive distributions, measured on the chromatograms with a high sensitive analyser, show that [^{11}C]-Flumazenil (rf=0.76) is mostly metabolized in two radioactive compounds : a polar metabolite I (rf=0.05) and a lipophilic metabolite II (rf=0.53) which correspond respectively to a free acidic and an hydroxy-ethyl ester metabolite of the radioligand (2).

Unchanged [^{11}C]-Flumazenil has also been analysed by rapid chromatography on C18 cartridges. Plasma samples, diluted 10 fold with a H_2O-CH_3OH (90-10) mixture are poured on the column, then washed with 1M NaOH. The ratio of column to plasma radioactivities can be considered as an index of the fraction of unchanged ligand in plasma. However polar metabolite II remain on the column with unchanged [^{11}C]-Flumazenil. The concentrations of unchanged radioligand found using these two analytical procedures are well correlated : r = 0.86.

In baboon, the variation of the percentage of unchanged radioligand in plasma, according to time after injection (min), follows a biexponential law:

$$\% = 41\ e^{-0.37t} + 59\ e^{-0.00423t}$$

(half-lives = 1.9 and 165 min)

1 h post injection, 45% of the radioactivity of the plasma is related to unchanged [^{11}C]-Flumazenil. The concentration of the acid metabolite (I) is always 9 times higher than that of the lipophilic metabolite (II).
These results allow to correct the input function of the kinetic model used to quantify [^{11}C]-Flumazenil binding parameters on central BZ receptors.

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INVESTIGATIONS ON THE IN VIVO STABILITY AND THE BIODISTRIBUTION OF ALPRAZOLAM; A BENZODIAZEPINE RECEPTOR AGONIST. W.R. Banks, H. Yamakita, G.A. Digenis, College of Pharmacy, University of Kentucky Lexington, KY 40536-0082.

Agents labelled with the positron emitter carbon-11 for in vivo studies of the benzodiazepine (BZ) receptor have typically suffered from the placement of the radiolabel into a metabolically unstable position. The ease of labelling using a carbon-11 alkylating agent gives rise to the in vivo loss of radioactivity resulting in extraneous sources of cerebral radioactivity chemically unrelated to the parent ligand, and to the distribution of labelled metabolites into the target organ (1,2).

Clearly, the alternative strategy was to label a high affinity BZ possessing a stable labelling site, whose metabolites were polar in plasma and rapidly excreted; hence their cerebral uptake negligible (3). We have reported the high specific activity synthesis of carbon-11 labelled alprazolam [^{11}C]-APZ (a potent (Ki 3.4 nM) triazolobenzodiazepine) whereby the label was situated into the heteroaromatic ring (4).

The present report will illustrate the application of an analytical method developed for determination of metabolite contribution to radioactivity levels in the brain and plasma of naive and pretreated rats, as well as biodistribution data, using identically labelled carbon-14 alprazolam (5).

Male Sprague-Dawley rats received carbon-14 alprazolam (11μCi, 29.7 mCi/mmol, iv) and were serially sacrificed. Pretreated rats received Xanax$^{(R)}$ (0.2 mg/kg, iv) 30 min prior to radioinjection. Upon sacrificing, organs were taken, weighed, aliquots solubilized, and analyzed by LSC. The results are shown in Table 1 and are expressed as % id/g tissue. Distribution was extensive and rapid with a moderate to fast blood clearance. Brain uptake was high and was the only organ affected by the pretreating dose (33% of control animals).

For metabolite studies 30% brain homogenates and plasma (100μl) were subjected to an extraction (91% and 84% efficient for brain and plasma respectively) and a tlc system designed for the separation of APZ and its two major metabolites (α-hydroxy and 4-hydroxy-APZ). The results expressed as percent isolated radioactivity (Table 2) were used to correct the % id/g values. In the worst case greater than 94% (mean of means) of the radioactivity, in the rat brain (60 min post-dosing), but only 60% of plasma radioactivity was attributable to alprazolam.

In light of the metabolic profile, high specific activity synthesis with carbon-11, and high receptor affinity, [^{11}C]-APZ should find applicability to imaging studies of the BZ receptor.

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TABLE 1 TISSUE DISTRIBUTION OF CARBON-14 LABELLED ALPRAZOLAM[a]

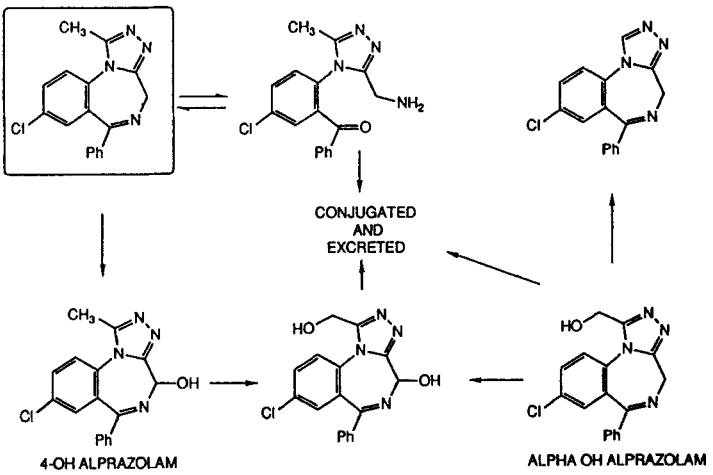
	CONTROL			PRETREATMENT		
	Time after Injection (min)					
Tissue	10	30	60	10	30	60
	Average relative Retention ± sd[b]					
Brain	0.840±0.081	0.269±0.052	0.118±0.034	0.604±0.024	0.267±0.030	0.157±0.014
Heart	2.236±0.058	1.112±0.165	0.563±0.154	1.959±0.063	1.207±0.072	0.732±0.035
Lung	1.757±0.048	0.949±0.195	0.516±0.117	1.497±0.073	0.927±0.073	0.608±0.041
Liver	5.571±0.517	4.954±0.363	2.75 ±0.317	6.841±0.387	4.665±0.454	3.087±0.241
Kidney	3.920±0.181	2.662±0.433	1.592±0.344	3.697±0.207	2.96 ±0.023	2.312±0.211
Spleen	1.510±0.045	0.849±0.097	0.438±0.069	1.554±0.250	1.048±0.202	0.550±0.008
Intestine	2.100±0.212	2.295±0.953	3.804±2.213	1.936±0.124	4.151±4.021	4.310±3.517
Stomach	0.474±0.213	1.282±1.490	0.997±0.609	0.934±0.201	1.311±0.677	0.806±0.192
Eye	0.456±0.086	0.276±6.048	0.147±0.018	0.394±0.052	0.236±0.023	0.153±0.023
Testes	0.493±0.083	0.351±0.054	0.222±0.040	0.453±0.019	0.375±0.027	0.286±0.041
Plasma	0.688±0.009	0.436±0.071	0.253±0.054	0.636±0.049	0.486±0.027	0.291±0.006
Brain/ Plasma	1.22	0.617	0.466	0.949	0.534	0.539

[a]Rats (250-300 g male Sprague-Dawley) received approximately 11 µCi [^{14}C]-ĀPZ(Sp Act 95.94 µCi/mg) via femoral vein in 0.5 mL propylene glycol/H_2O (50/50 v/v).
[b]All time points represent averages of four rats with the exception of the 10 min pretreatment time point which represents three rats.

TABLE 2 PERCENT OF ISOLATED RADIOACTIVITY FROM PLASMA (P) AND BRAIN (B) HOMOGENATES[a,d]

	Control						Pretreated					
	PERCENT OF ISOLATED RADIOACTIVITY[b]											
Time	10		30		60		10		30		60	
	P	B	P	B	P	B	P	B	P	B	P	B
4-OH[c]	21.54 (2.41)	0.18 (0.17)	32.48 (3.80)	1.37 (1.25)	31.74 (7.1)	2.31 (2.30)	15.08 (0.81)	0.35 (0.25)	20.94 (4.82)	2.10 (0.99)	37.15 (3.60)	3.82 (3.46)
α-OH[c]	2.96 (0.47)	0.51 (0.36)	4.83 (1.35)	1.19 (1.25)	5.37 (1.20)	1.20 (1.82)	4.85 (1.09)	0.62 (0.94)	3.69 (1.09)	0.77 (0.68)	2.05 (1.48)	1.69 (2.67)
APZ	75.20 (2.33)	99.31 (3.92)	60.45 (4.06)	97.03 (2.00)	59.38 (5.63)	95.39 (3.91)	79.06 (1.74)	98.80 (1.21)	74.91 (4.81)	95.25 (1.33)	60.70 (4.81)	94.11 (2.71)
Sum	99.7	100	97.8	99.6	96.5	98.9	98.9	99.8	94.5	98.1	99.9	99.6

[a]All values represent the average ± sd of 4 rats, sampled in duplicate except for the pretreated 10 min time point n=3.
[b]Percents calculated from the sum of all radioactivities isolated from tlc plate as described in the text.
[c]4-OH; 4-hydroxy-alprazolam: α-OH; α hydroxyalprazolam: alprazolam.
[d]Values in parenthesis represent standard deviations.



Partial metabolism of alprazolam (boxed).

NEW RADIOIODINATED LIGAND FOR SPECT STUDIES OF BENZODIAZEPINE RECEPTOR.

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In the search for the in vivo benzodiazepine receptor imaging studies, the recent availability of ^{123}I and the good spatial resolution reached by SPECT call for the development of radioiodinated compounds with high affinity for benzodiazepine receptor. On this search, based on the studies on structure-pharmacological activity relationship of the 1,4-benzodiazepine series, the 2' position in the C-5-phenyl ring of diazepam was selected as the best exploitable site for the iodination and the effect of the iodination at 2' position was comparatively studied with diazepam derivatives iodinated at other position (3' and 4').

In the synthesis of iodinated diazepam derivatives, since the application of common methodology through the amino benzophenone was difficult, a new synthetic methodology through the iodobenzyl chloride reaction with N-(4-chlorophenyl)-4-methyl-ethylenediamine was estimated: after cyclization and oxidation, the iododiazepam (IDZ) was made available. The synthesized diazepam derivatives were confirmed by IR, NMR and mass spectra.

The receptor affinity was measured from the ability to inhibit specific ^{3}H-diazepam binding to rat cortical membranes. Among the tested derivatives, only the 2'-iododiazepam (2'-IDZ) showed high affinity, reaching approximately 3 times higher value than the corresponding parent compound, diazepam.

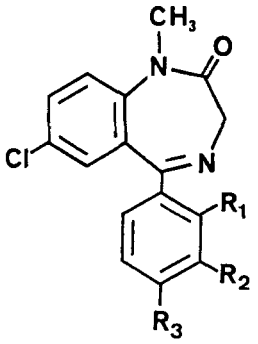
Then, as for its utilization in the in vitro saturation binding study and in the in vivo biodistribution, the radioiodination of 2'-IDZ was carried out by bromine-iodine exchange reaction of brominated derivative with $Na^{125}I$ (specific activity: 200 - 300 Ci/mmol).

Binding of ^{125}I-2'-IDZ to rat cortical membranes was saturable and Scatchard analysis revealed an homogeneous population of binding sites with an apparent dissociation constant (Kd) of 0.33 nM and maximum number of binding sites (Bmax) of 1.33 pmol/mg protein.

In mice distribution studies, ^{125}I-2'-IDZ showed a rapid and high uptake into the brain. The brain uptake and brain-to-blood ratio of this compound were higher than those of N-(^{11}C-methyl)-diazepam. Furthermore, among the various cerebral regions analyzed, this compound displayed highest accumulation in the cortex, a region of high benzodiazepine receptor density. This cortical binding was saturable and displaced by the administration of diazepam, fludiazepam and Ro-15-1788,

compounds bound to benzodiazepine receptors in vivo. These in vivo rersults were also assessed by macroautoradiographic studies.

These data indicated that the newly synthesized 2'-IDZ holds great potential for the SPECT study of benzodiazepine receptor.



	R_1	R_2	R_3
2'-IDZ :	I	H	H
3'-IDZ :	H	I	H
4'-IDZ :	H	H	I

SYNTHESIS OF NCA 11,17β-DIHYDROXY-6-METHYL-17α-(3-[^{18}F]FLUOROPROP-1-YNYL)ANDROSTA-1,4,6-TRIEN-3-ONE AS A POTENTIAL GLUCOCORTICOID RECEPTOR LIGAND FOR NEURO-PET STUDIES.

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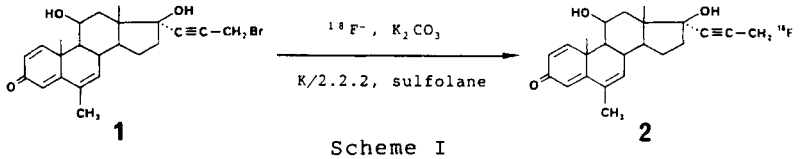
Glucocorticoids appear to exert physiologic, biochemical and behavioral effects on the central nervous system (1). The presence of glucocorticoid binding sites have been demonstrated in human brain by autoradiographic studies (2). In order to visualize the brain glucocorticoid binding sites by PET, we have synthesized n.c.a. 11,17β-dihydroxy-6-methyl-17α-(3-[^{18}F]fluoroprop-1-ynyl)androsta-1,4,6-trien-3-one (2), a fluorine-18 analog of the selective type II glucocorticoid receptor agonist RU 28362 (3).

The radiochemical synthesis of 2 is outlined in Scheme I. The n.c.a. fluoride-18 was produced by the $^{18}O(p,n)^{18}F$ reaction on 50% enriched [^{18}O]H_2O with 16 MeV proton. The activity thus obtained was delivered into a siliconized tube containing 8.8 μmol of Kryptofix 2.2.2. and 4.3 μmol of K_2CO_3. After evaporation of the water under a stream of N_2 at 120 °C for 30 min, approximately 70-90% of the activity was solubilized in 600 μl tetramethylene sulfone (sulfolane) and transferred to a reacti-vial (4) containing 3.7 μmol of 1 (RU 52444). The compound 2 ([^{18}F]RU 52461) was prepared by fluoride substitution of the bromide in 1 at 100 °C for 10 min. The unreacted $^{18}F^-$ was then passed through a C-18 Sep-pak with water (2x20 ml) and the desired fluoro compound 2 was eluted with CH_2Cl_2 (2x20 ml). The final product 2 was purified by two successive HPLC (on a silica gel and PRP-1 column), with a radiochemical yield of 12-25% (corrected for decay) and a specific activity of 900-1500 mCi/μmol (33-56 GBq/μmol, end of synthesis). The overall synthesis time was nearly 140 min. The chemical manipulations, up to the HPLC separation, were executed by a Zymark robot. The identity of the radiopharmaceutical was confirmed by its HPLC retention time which was identical to that of coinjected unlabeled 2 (RU 52461).

Biodistribution studies in mature male rats and in vivo distribution of 2 by PET on a baboon are in progress.

We wish to thank Roussel Uclaf for the generous gift of RU 52444 and RU 52461. This work was supported in part by the Commissariat à l'Energie Atomique and Fonds de la Recherche en Santé du Québec.



1 2

Scheme I

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IMAGING AGENTS FOR BRAIN CORTICOSTEROID RECEPTORS: SYNTHESIS AND TISSUE DISTRIBUTION OF 21-[^{18}F]FLUORO-DEACYLCORTIVAZOL.

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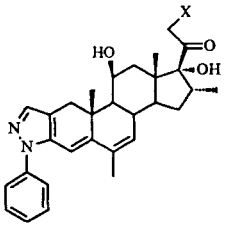
The characteristic degeneration of neurons in the hippocampus seen in Alzheimer's disease may be exacerbated by the action of corticosteroids, as prolonged elevated levels of cortisol may place the hippocampus in a state of vulnerability to additional metabolic insults. Since cortisol levels are, in part, regulated by the stress response system through negative feedback on corticosteroid receptors in the hippocampus, a temporary or permanent decrease in corticosteroid receptors (i.e. down-regulation or receptor loss) can result in prolonged elevation of cortisol. Therefore, a method for in vivo assay of hippocampal corticosteroid receptors might provide a means for assessing susceptibility of the hippocampus to neuronal damage.

We have previously reported the synthesis and tissue distribution of fluorine-18 labeled analogues of the synthetic corticosteroids 3'-(3-oxo-7α-propyl-17β-hydroxyandrost-4-ene-17α-yl)-propanoic acid lactone (RU26752) and 11β,17β-dihydroxy-6-methyl-androsta-1,4,6-trien-3-one (RU28362), which possess high affinity and specificity for the type I (MR or mineralocorticoid preferring) and type II (GR or glucocorticoid preferring) corticosteroid receptors, respectively.[1] 21-[^{18}F]fluorinated analogues of the naturally occurring corticosteroids deoxycorticosterone and corticosterone and the synthetic steroid triamcinolone acetonide have also been studied. In spite of their high affinity for the target receptors, these compounds showed little target tissue (hippocampal) uptake. Furthermore, moderate to severe metabolic defluorination was indicated by high activity levels in bone.

Recently, we have prepared the 21-fluoro analogue (II) of the unique, high affinity type II selective ligand deacylcortivazol (I). The fluorine-substituted analogue retains forty percent of the binding affinity of the parent ligand. This compound has been prepared in fluorine-18 labeled form at an effective specific activity of 370 Ci/mmol by fluoride ion (Bu_4NF) displacement on the triflate precursor.



I X = OH
II X = F

In vivo biodistribution in mature adrenalectomized male rats (ca. 200 g) showed higher brain uptake at 15 min than any of the corticosteroids previously studied, with only a 33% decrease in activity bound in target tissue after 8 h. Brain to blood ratios at 2 h and 8 h post injection were 6.71 ± 0.26 and 5.43 ± 0.64. The extent of in vivo defluorination was less than for previous ligands. Target tissue uptake, however, could not be blocked by pretreatment of animals with excess corticosterone or cortivazol. Further studies into the binding and metabolism of this compound are planned.

This work was supported by grants from the American Health Assistance Foundation (A88267), the Department of Energy (DE FG02 84ER60218), and the National Institutes of Health (HL13851).

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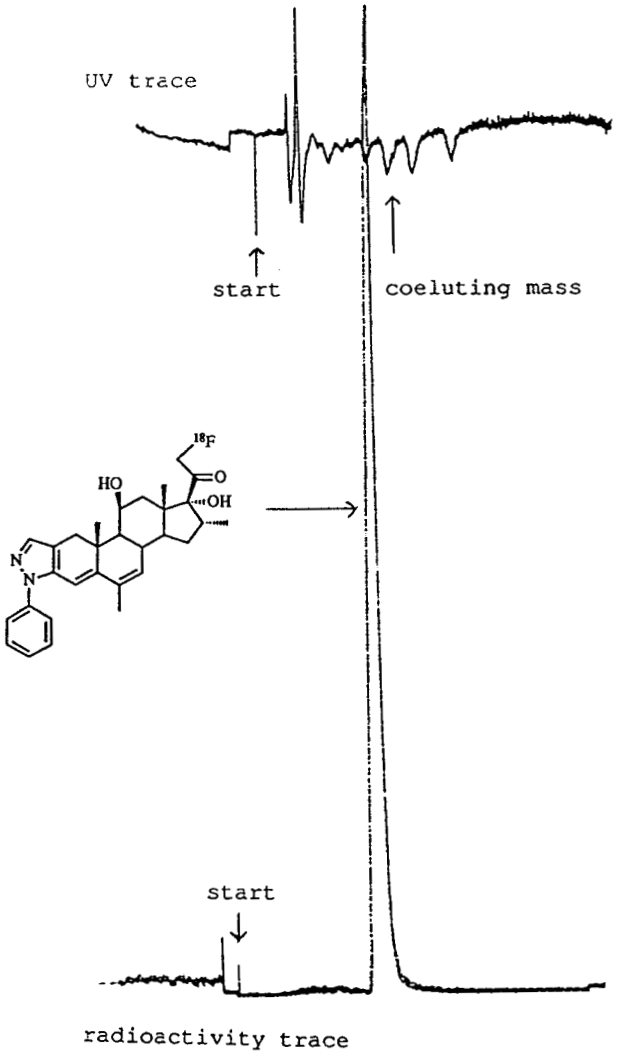
Table I. In vivo biodistribution of 21-[^{18}F]fluorodeacylcortivazol (II) in mature male adrenalectomized rats (n = 5).

% ID/g	15 min	2 h	2 h blocked (corticosterone)
blood	.076 ± .010	.048 ± .003	.053 ± .006
cortex	.387 ± .033	.295 ± .022	.332 ± .037
hippocampus	.281 ± .030	.289 ± .031	.305 ± .036
pituitary	.957 ± .145	.918 ± .113	.742 ± .082
rest of brain	.340 ± .038	.298 ± .020	.332 ± .036
bone	.261 ± .040	.484 ± .037	.447 ± .038

% ID/g	2 min	2 h	2 h low dose
blood	.200 ± .073	.044 ± .005	.052 ± .015
liver	3.23 ± 1.05	2.10 ± .83	2.36 ± 1.03
kidney	1.86 ± .49	1.02 ± .10	.893 ± .250
muscle	.101 ± .052	.302 ± .045	.301 ± .102
fat	.156 ± .068	1.49 ± .22	1.20 ± .41
cortex	.282 ± .071	.305 ± .031	.322 ± .144
hippocampus	.189 ± .046	.293 ± .038	.220 ± .100
pituitary	1.24 ± .298	.946 ± .130	1.35 ± .79
rest of brain	.400 ± .417	.298 ± .033	.249 ± .080
bone	.172 ± .053	.488 ± .054	.618 ± .111
thymus	.219 ± .108	.577 ± .075	.555 ± .209

% ID/g	4 h	4 h blocked (cortivazol)	8 h
blood	.039 ± .003	.050 ± .005	.034 ± .006
liver	2.14 ± .13	1.79 ± .34	1.60 ± .37
kidney	.920 ± .090	.700 ± .102	.736 ± .136
muscle	.346 ± .045	.315 ± .080	.265 ± .036
fat	1.22 ± .32	2.01 ± .29	1.47 ± .21
cortex	.268 ± .027	.221 ± .037	.214 ± .058
hippocampus	.278 ± .050	.230 ± .048	.200 ± .032
pituitary	.973 ± .085	.554 ± .124	.702 ± .210
rest of brain	.261 ± .016	.257 ± .047	.186 ± .031
bone	.757 ± .050	.510 ± .114	1.16 ± .24
thymus	.622 ± .060	.424 ± .075	.560 ± .117



Quality control analysis of 21-[^{18}F]fluorodeacylcortivazol (**II**).

Reversed phase (C-18) analytical HPLC.
Solvent system: 75% CH_3CN/25% H_2O.

DEVELOPMENT OF A F-18 LABELED ANTAGONIST FOR THE A_1 ADENOSINE RECEPTOR.

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Recently we reported a prosthetic group methodology for the preparation of [^{18}F] labeled insulin.[1] The radiosynthesis was based on coupling an [^{18}F]-fluoromethylbenzoyl alkyldiamine to a preactivated N-hydroxysuccinimidoyl insulin derivative. As an extension of this general methodology we have applied this to the ^{18}F labeling of an antagonist for the A_1 adenosine receptor.

Derivatives of A_1 antagonists bearing the 8-(4-carboxymethyloxyphenyl)-1,3-dipropylxanthine (XCC) pharmacophore have been well characterized.[2] These high affinity synthetic analogs of theophylline are easily modified via derivatization of the carboxymethyloxy group.

The first step in this two step radiosynthesis involves preparation of the ^{18}F labeled prosthetic group by S_N2 displacement of bromide for [^{18}F]-fluoride. To a solution already containing tetramethylammonium hydroxide (1.8μmol) and [^{18}F]-fluoride in acetonitrile (500μL), N-(4-bromomethylbenzoyl)-N'-tert-butyloxycarbonyl ethylenediamine (800μmol) was added. After 15 min at 100 C the resulting mixture was purified by HPLC. N-([^{18}F]-4-fluoromethylbenzoyl)-N'-tert-butyloxycarbonyl ethylenediamine was extracted from HPLC eluent with a C-18 BOND ELUT and the t-Boc protecting group removed by treatment with trifluoroacetic acid (200μL) at room temperature for 5 min. The first step was completed in 70 min in 59% yield (corrected for decay).

After removal of the TFA the activity was dissolved in DMSO (25μL) containing triethylamine (1μL). To this was then added the N-hydroxysuccinimidoyl ester of XCC (443μg;0.9μmol) and the mixture allowed to react at room temperature for 5 min. The reaction mixture was again purified by HPLC. The total synthesis required approximately 2 hrs to complete in an overall yield of 45% (corrected for decay). N-([^{18}F]-4-fluoromethylbenzoyl)ethylenediamine, [^{18}F]-FMB reacts almost quantitatively (TLC) with N-hydroxysuccinimdoyl esters at room temperature in less than 5 min. The final radiochemical and chemical purity for [^{18}F]-FMB-XCC was in excess of 95% with specific activities greater than 15 Ci/μmol at the end of synthesis.

This prosthetic group methodology has proven to be a good general method for labeling functionalized drugs. The [^{18}F]-FMB-XCC congener is presently being evaluated as a possible PET ligand.

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RADIOSYNTHESIS OF N.C.A. [O-METHYL-^{11}C]NIMODIPINE

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Nimodipine is a calcium antagonistic compound with a preferential effect on cerebral vessels [1] and a high affinity to the L-channel of neurons [2]. Its regional pharmacokinetics in brain and heart can be studied with PET using the ^{11}C-labeled compound. The pharmaceutical was previously labeled with ^{14}C in the 2-position of the dihydropyridine ring for metabolic studies [3] and with ^{11}C in the isopropyl estergroup for PET studies [4]. Since O-demethylation and methoxyethylester cleavage are known to be the first steps of catabolism in addition to dehydrogenation [5], [^{11}C-isopropyl]nimodipine will lead to labeled metabolites (for structure see Scheme 1). We have therefore labeled nimodipine in the O-methyl group. Direct methylation of the corresponding alcohol with [^{11}C]methyliodide is not possible due to alkylation in the N-position of the dihydropyridine ring in presence of strong bases. Alternatively [^{11}C]methoxyethanol was at first prepared from glycol and [^{11}C]methyliodide and subsequently condensed with the imidazolide of the corresponding carboxylic acid precursor (cf. Scheme 1).

At first [^{11}C]methoxyethanol was prepared in glycol as solvent since sodium and potassium glycolate are only poorly soluble in dipolar aprotic solvents. In addition, the reactivity of the glycolate anion for nucleophilic substitution of iodide in CH_3I was very low under these conditions. Satisfying yields were only obtained after 10 minutes at temperatures > 90°C (see Fig. 1). At 130°C the reaction time could be shortened to 2 minutes with a radiochemical yield of 70 % as determined by gas chromatography.

However, preparative isolation of [^{11}C]methoxyethanol from glycol or further esterification therein was not successful. Solubility tests of potassium glycolate in polar solvents with addition of the phase-transfer-catalyst 18-crown-6 showed the sequence of solubility dioxane > butanol > acetonitrile ≈ acetone. However, methylation succeeded in acetonitrile. With 10 µmol of $KOCH_2CH_2OH$ in 1 ml CH_3CN 85±6 % radiochemical yield (based on [^{11}C]CH_3I) of [^{11}C]$H_3COCH_2CH_2OH$ was obtained at 110°C within 5 minutes.

Optimization studies with equimolar amounts of methoxyethanol and the carboxyl-imidazolide lead at 130°C to a yield of 80 % of nimodipine with acetonitrile as solvent. Thus, the two step synthesis could be performed in a one-pot reaction. Presence of 18-crown-6 made it necessary to use a threefold excess of the base NaH. Application of the optimized conditions (130°C, 5 min, 24 µmol imidazolide, 2 ml CH_3CN) to the n.c.a. radiosynthesis allowed the preparation of [^{11}C-methyl]nimodipine with a radiochemical yield of 22±3 % (based on [^{11}C]CH_3I). Reverse phase HPLC was used to isolate the product from the carboxylic acid and the 2'-hydroxyethylester.

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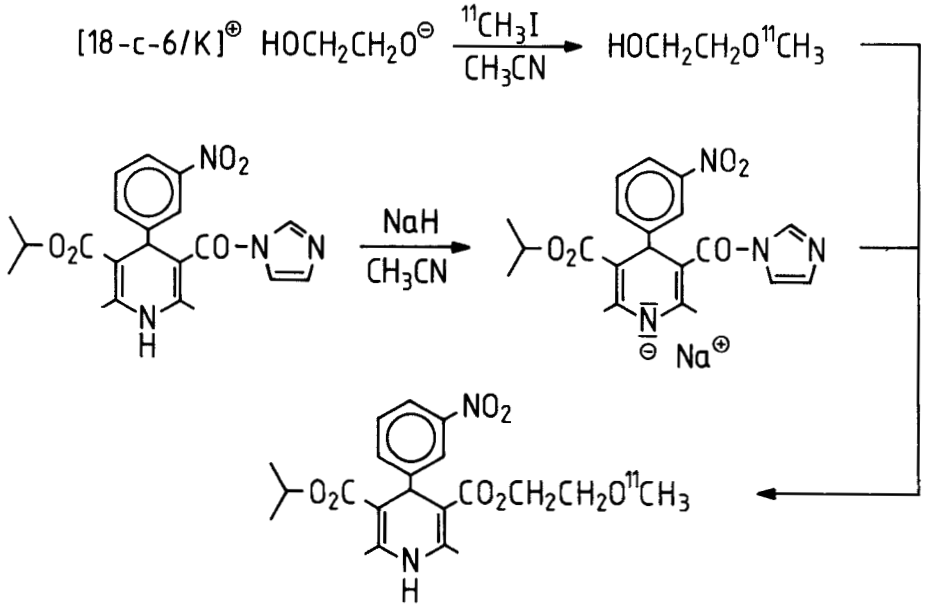

Scheme 1: Radiosynthesis of n.c.a. [^{11}C-methyl]-nimodipine via [^{11}C]methoxyethanol

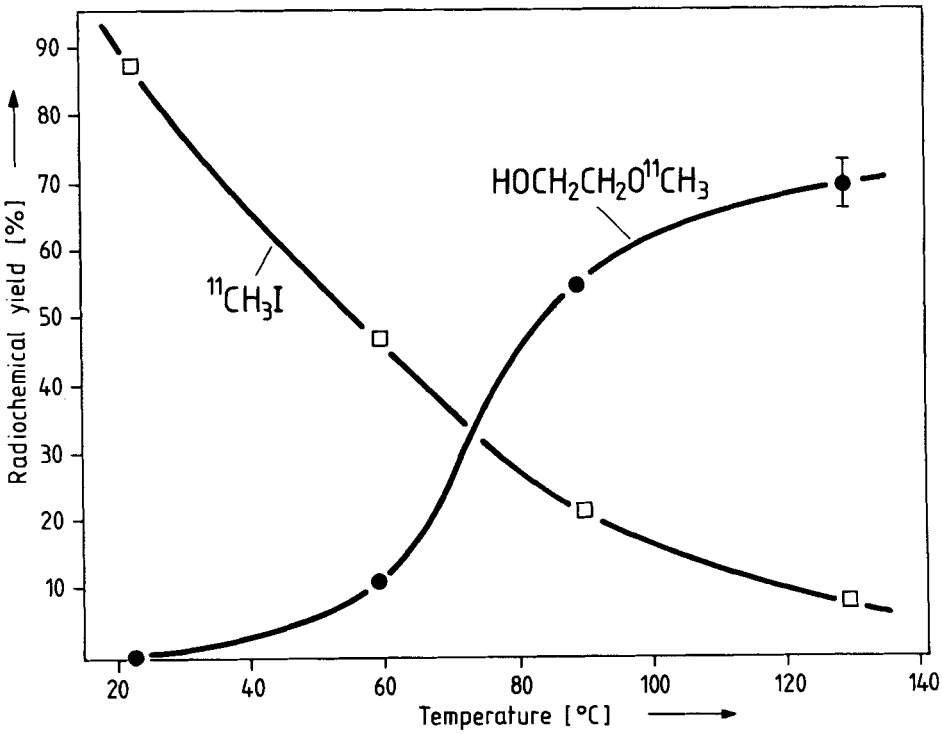

Fig. 1: Temperature dependence of sodium glycolate ^{11}C-methylation with n.c.a. [^{11}C]CH_3I in glycol as solvent (10 min reaction time)

EVALUATION OF NEUTRAL AND CHARGED mAChR LIGANDS AS POTENTIAL PET AGENTS FOR MYOCARDIAL IMAGING

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Evaluation of neutral muscarinic receptor (mAChR) ligands using a combination of *in vitro* and *ex vivo* techniques permitted selection of a potential PET brain imaging agent from a number of candidates (1). TRB, a tropanyl benzilate selected on this basis, was radiolabeled using C-11 and has demonstrated utility for detection of mAChR in human brain (2). Although TRB has a high affinity for the M_2 mAChR in the myocardium, it is unacceptable as a myocardial imaging agent due to significant non-specific localization in the lung. Reports indicating the usefulness of quaternized QNB (MQNB) as a myocardial imaging agent (3) lead us to explore the *in vitro* binding affinity of a series of quaternized mAChR antagonists. The longer half life of ^{18}F also prompted an investigation into the binding of some neutral and quaternized fluoro derivatives.

The first series of ligands evaluated were N-methyl quaternized analogs of the neutral mAChR ligands TRB, NMPB and QNB. The data compiled in Table 1 show that quaternization using methyl iodide does not alter the IC50 value significantly. The IC50 values were obtained using competitive binding assays in mouse heart homogenates using ^{3}H-QNB as the radioactive ligand. Assays were done in triplicate and repeated at least twice. Analysis of the data using Lundon-2 software indicates that binding is to a single site for all ligands.

The second series of ligands used various benzyl halides as quaternizing agents in an effort to determine the steric tolerance at the receptor for quaternized ligands. In the quaternized QNB series two fluorinated benzyl halides were evaluated: 2- and 4-fluorobenzyl halides. In both cases, the IC50 increased by an order of magnitude relative to the methyl quaternized analog. The 4-fluoro derivative showed higher affinity for the receptor (IC50 was 14nM) compared to the 2-fluoro derivative (IC50 was 35nM). In the NMPB series, use of benzyl halide increased the IC50 by about 6-fold relative to DMPB (11.1nM vs 1.8nM). Comparable benzylated TRB derivatives have not yet been evaluated. Analysis of the data also indicated the presence of only a single binding site.

Although the IC50 values for the quaternized benzyl derivatives suggests that there is some steric tolerance at the mAChR, a quaternized ligand containing fluorine with IC50 values comparable to the N-methyl derivatives would be more desirable. Our approach to this target was to begin with evaluation of neutral antagonists containing fluorine. Thus, N-fluoroethyl nor-TRB (FENTRB) and N-fluoroethyl piperidyl benzilate (FEPB) were synthesized and evaluated. Replacing N-methyl with N-fluoroethyl lead to small increases in IC50 values: FENTRB had a

value of 3.3nM compared to 1.6nM for TRB and FEPB had a value of 7.4nM compared to 1.0 for NMPB. These small changes in IC50 values suggest that quaternization of these ligands would lead to derivatives with excellent affinity for the mAChR receptor. Synthesis of quaternized derivatives is underway.

One other quaternized derivative was evaluated: DAMP. Comparison between DMPB and DAMP permits evaluation of the effect of replacing a 2-hydroxy group with hydrogen in the benzilic acid half of the molecule. The IC50 value for DAMP was 10.7, about six times greater than for DMPB. This suggests that the 2-hydroxy increases receptor affinity.

In conclusion, quaternization with methyl iodide yields mAChR ligands with potential as myocardial imaging agents. Further evaluation of these ligands using the ex vivo assay will be conducted.

Table 1. Comparison of neutral and N-methyl quaternized mAChR ligands in mouse heart homogenates.

Neutral	IC50	N-methyl	IC50
TRB	1.6nM	MTRB	1.5nM
QNB	0.8nM	MQNB	1.1nM
NMPB	1.0nM	DMPB	1.8nM

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SYNTHESIS OF LABELLED DIPHENTLETHYLENETROPANE WITH F-18 OR C-11

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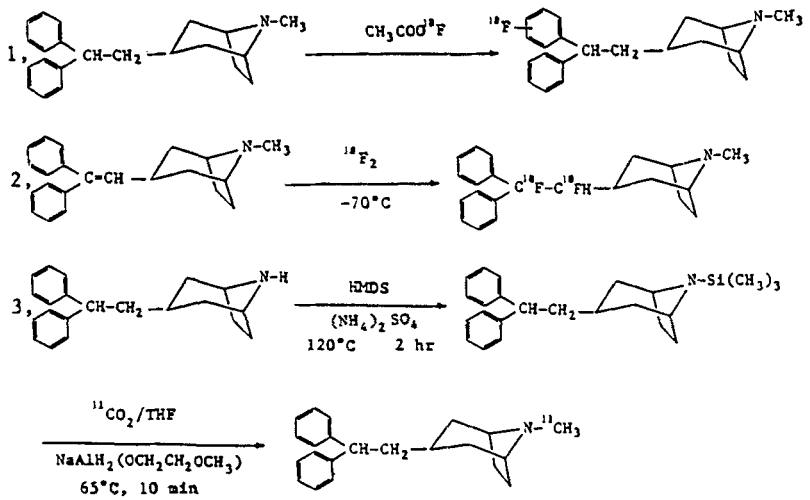
Diphenylethylenetropane (DPET) is a long-acting anticholinergic compound whose action duration for more than 12 hours. In the current studies DPET was labelled with F-18 or C-11 suggested their potential as a muscarinic receptor imaging agents using the positron emission tomography. F-18 and C-11 was introduced into the different positions in the DPET molecule by the following schemes:



In the scheme 1, F-18 was labelled at the benzene ring forming F-18 fluoro-DPET, specific activity 325 mCi/mmol, radiochemical purity 95% by HPLC, radiochemical yield 6%, the procedures took 100 min. In the scheme 2, F-18 was introduced into the ethylene giving F-18 difluoro-DPET, specific activity 416 mCi/mmol, radiochemical purity 95% by HPLC, radiochemical yield 7%, the procedures took 120 min. In the scheme 3, C-11 was connected with the nitrogen atom in the tropane ring, C-11 DPET specific activity 21 Ci/mmol, radiochemical purity 98% by HPLC, radiochemical yield 30%, the procedures took 45 min.

The results of preliminary in vivo imaging studies indicate that the labelled compounds easily pass through the blood-brain barrie. Both compounds bind to cholinergic receptors in the brain and the heart. The selectivity of in vivo binding characteristics of these labelled tropane compounds remains to be elucidated.

TOWARDS A CHIRAL PRECURSOR FOR THE AUTOMATED RADIOSYNTHESIS OF CARBON-11 LABELLED *S*-CGP 12177

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CGP 12177 [(4-(3-*t*-butylamino-2-hydroxypropoxy)-benzimidazol-2-one] binds with high affinity to β–adrenergic receptors.[1] Racemic CGP 12177 can be labelled with carbon-11 by treating racemic 1-(3-*t*-butylamino-2-hydroxypropoxy)-2,3-diaminobenzene (I) with nca [^{11}C]phosgene[2] and has been reported as a promising radioligand for PET studies of β receptors in heart.[3]

In vitro studies with *S*- and *R*-[^{3}H]CGP 12177 have shown that the *S*-enantiomer has approximately 80-fold greater affinity than the *R*-enantiomer for β-adrenergic receptors.[4] Also our studies of *S*- and *R,S*-[^{3}H]CGP 12177 injected intravenously into rats have shown that the *S*-enantiomer gives an approximately twofold greater specific signal, as represented by the ratio of β-receptor bound radioliogand to non-specifically bound radioligand, both in heart and in lung. Therefore if possible it is clearly desirable to use the carbon-11 labelled *S*-enantiomer rather than the racemate for PET studies of β-adrenergic receptors. We therefore set out to prepare the *S*-enantiomer of the precursor I (VIII) for this purpose.

We were unable to separate the enantiomers of I or its progenitors by HPLC using any of a number of different chiral columns, including Chiralcel OD and Cyclobond I columns. (These columns also fail to resolve racemic CGP 12177). We therefore sought a convenient synthetic route to the *S*-enantiomer of the precursor (VIII). A twelve step synthesis of cold *S*-CGP 12177 starting from 2,3-dinitroaminophenol is known, but does not proceed through the diamine VIII.[4] Here we describe our approach (Scheme 1) to the synthesis of VIII, based on the known ability of enantiomerically pure *R*- or *S*-glycidyl-3-nitrobenzenesulphonate to react with phenoxide ions rapidly and efficiently,[5,6] and of the epoxide ring of the resultant epoxy ethers to open by reaction with amines giving products in which the chirality of the original glycidyl enantiomer is retained.[5]

The starting material 2,3-dinitrophenol (II) was reduced to 2,3-diaminophenol (III) and the latter was acetylated to give 2,3-diacetylaminophenol (IV).[3] Reaction of (IV) with sodium hydride in DMF under nitrogen, followed by addition of *S*-glycidyl-3-nitrobenzenesulphonate (V) gave *S*-[1-(2,3-diacetylaminophenoxy)]-2,3-epoxypropane (VI). Refluxing VI with *t*-butylamine gave *S*-[1-(2,3-diacetylaminophenoxy)]-3-(*N*-*t*-butylamino)-propane-2-ol (VII). Compounds II, III, VI and VII were characterised by mass spectrometry and by ^{1}H and ^{13}C-NMR spectroscopy. On the basis that *S*-[1- (2.3,-diacetylaminophenoxy)]-3-(*N*-benzyl-*N*-*t*-butylamino)proprane-2-ol deacetylates without racemisation upon treatment with potassium hydroxide, we expect to obtain the *S*-enantiomer (VIII) by treating VII similarly. Work is in progresss to isolate and characterise this product.

The radioynthesis of carbon-11 labelled *S*-CGP 12177 from VIII will be carried out in fully automated apparatus now used to prepare racemic [^{11}C]CGP 12177 from nca [^{11}C]phosgene, itself prepared according to Landais & Crouzel[7] (Scheme 2). This apparatus delivers up to 5 GBq of radiochemically and chemically pure ^{11}C-labelled CGP 12177 with a specific activity up to 10 GBq/μmol at 40 min after EOB.

Acknowledgements The authors are grateful to Glaxo Ltd for financial support, to Ciba-Geigy AG for CGP 12177 and related compounds and to Professor K.B. Sharplesss for useful discussions.

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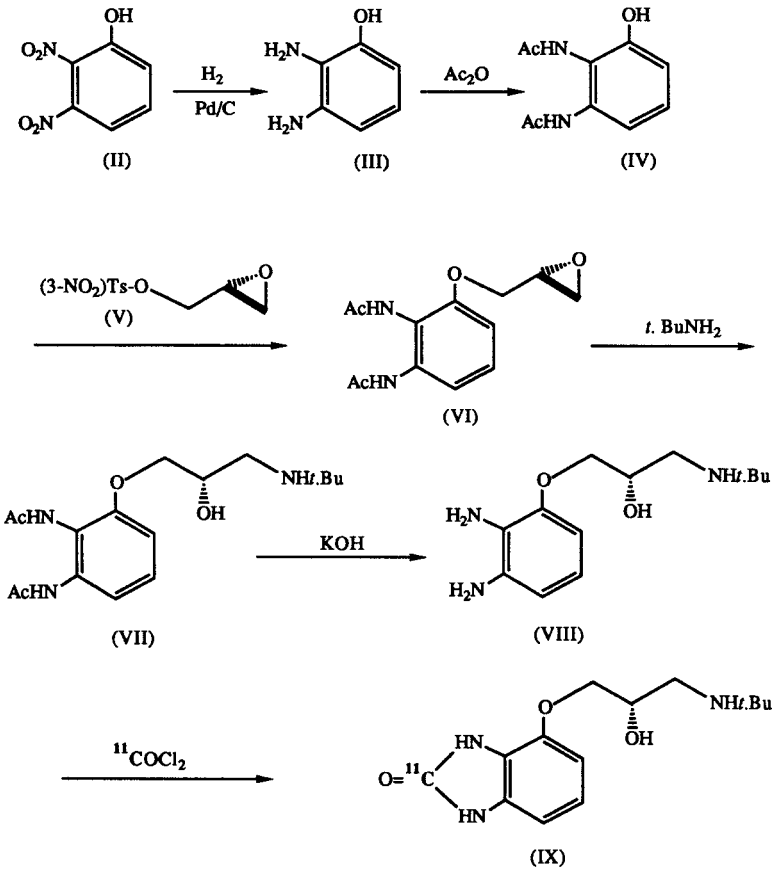
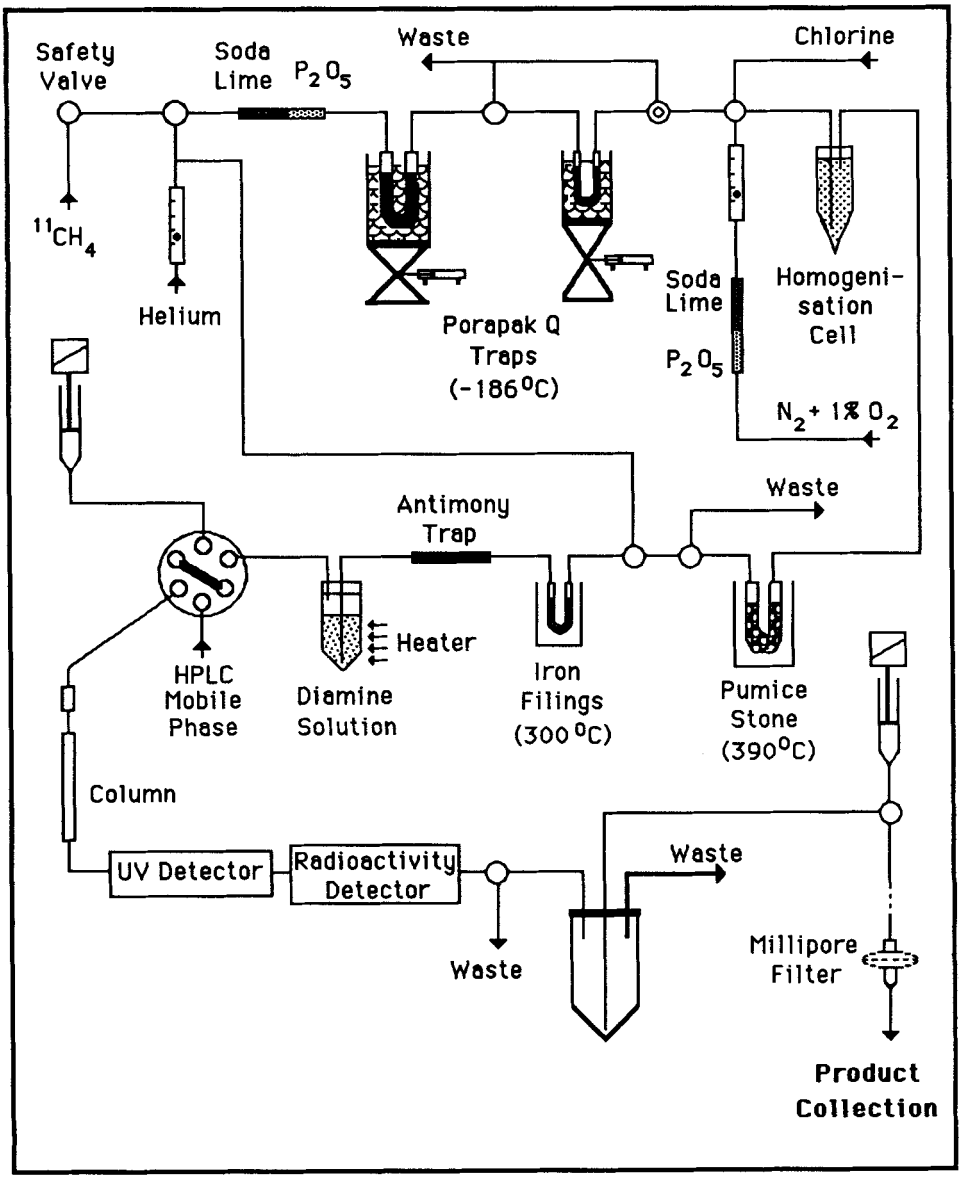


Scheme 1. Proposed synthesis of *S*-[^{11}C]CGP 12177



Scheme 2: Apparatus for the automated preparation of [^{11}C]CGP 12177. All components are controlled external to the closed hot cell.

^{11}C-LABELLING OF OHMEFENTANYL : AN AGONIST FOR μ-OPIATE RECEPTOR.

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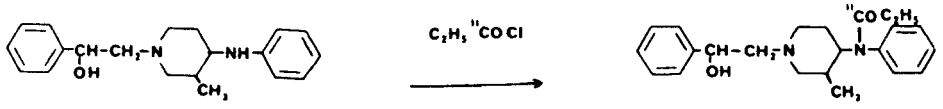
Ohmefentanyl : cis-N-[1-(βhydroxy-βPhenylethyl)-3-methyl-4-piperydyl]-N-Phenyl propionamide, is a synthetic narcotic analgesic agent (1). The analgesic activity of this compound is 28 times more potent than that of fentanyl and 6300 times more than that of morphine. By using [^{3}H] ohmefentanyl, it has been shown that it has a high affinity and specificity for opiate receptors of mice brain. The high affinity binding sites (k_D = 0.32 nM) correlates with the μ receptors (2). These results encouraged us to label this compound with carbon-11 to visualize "in vivo" the μ receptors by Positron Emission Tomography.

The carbon-11 is produced by the (p, α) reaction on pure nitrogen (99,999 % Purity). By irradiating with 20 MeV protons, 30 μA, 30 minutes, 1.5 Ci $^{11}CO_2$ is obtained. From $^{11}CO_2$, the [^{11}C]propyl chloride is initially produced by the method described by S. Luthra (3).

$$C_2H_5MgBr \xrightarrow[\text{Di ethyl ether}]{^{11}CO_2} C_2H_5{}^{11}COMgBr \xrightarrow[\text{2,6 di-ter butyl pyridine}]{\text{Phtaloyl Dichloride}} C_2H_5{}^{11}COCl$$

The [^{11}C]acid chloride released by heating (bp : 79°C) is carried by a slow stream of nitrogen into a solution of amine (I) in dioxane. After 5 minutes at 60°C, the solution is injected on a HPLC column : Partisil 10 SCX, Magnum 9/25 Whatman, eluant = water/EtOH 65/35, pH 2.3 Phosphate buffer 10^{-2}M flow rate 6 ml/min, T_R (II) = 10 minutes, T_R (I) = 25 minutes. Starting with 1.5 Ci $^{11}CO_2$, several tens mci of [^{11}C]Ohmefentanyl with high specific activity are obtained. The product ready for injection is obtained 40 minutes after the end of bombardement.



(I) (II)

Scheme of [^{11}C]ohmefentanyl radiosynthesis

Acknowledgements
We wish to thank Dr. Zhu, Shangai Institute of Materia medica, Academia Sinica for graciously providing sample of ohmefentanyl and amine precursor.

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Differences in the hepatic metabolism of ^{99m}Tc and ^{125}I labelled galactosyl neoglycoalbumin

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Galactosyl neoglycoalbumin(NGA) is a synthetic ligand for the asialoglycoprotein receptor(hepatic binding protein) which resides exclusively on hepatocytes. Uptake of ^{99m}Tc allows assessment of liver function based on receptor concentration as well as dynamic liver imaging. However, most biochemical studies on the uptake and hepatic mechanism of asiaglycoproteins have used ^{125}I labelled ligands. We have undertaken, therefore, a comparative study of NGA uptake and hepatic metabolism in the isolated perfused rat liver using both ^{99m}Tc and ^{125}I radiolabelled NGA. First pass hepatic uptake of a pulse(150pmol) of ^{125}I and ^{99m}Tc labelled NGA was similar($37\pm3\%$ vs $44\pm5\%$) showing that both labelling methods did not alter receptor binding. Of the ^{125}I-NGA taken up, 82% of the ^{125}I was released at the sinusoidal pole of the hepatocyte and reappeared in the perfusate(mainly as small MW metabolites) whilst 8% was excreted unchanged in the bile and 7% remained in the liver 1h after the pulse. Of the ^{99m}Tc-NGA taken up, only 4% of the ^{99m}Tc was returned to the sinusoidal pole of the hepatocyte whilst 40% was excreted in bile and 55% remained in the liver 1h after the pulse.

These differences in the hepatic metabolism of ^{125}I and ^{99m}Tc NGA can be related to the different methods of attachment of the radiolabel to the ligand and the implications of this finding will be discussed in detail.

SYNTHESIS OF [11C]RANITIDINE: A POTENTIAL PET IMAGING AGENT FOR H2 RECEPTORS IN HEART.

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Ranitidine hydrochloride is a highly potent H2 receptor antagonist of value in treatement of peptic ulceration.
In order to visualize the H_2-receptors in heart by PET, we have undertaken the synthesis and labelling with [11]carbon (β^+, t = 20,4mn) of ranitidine (fig.1).
In a first place, the precursor of ranitidine was synthesized.
The furfuryl alcohol 1 is aminomethylated by treatement with formaldehyde and dimethylamine as a case of the Mannich reaction.
When allowed to react with cysteamine hydrochloride in concentrated hydrochloride acid at 80°C, 2 is converted into the amine 3.
Condensation of 3 with methylisocyanate leads to the urea 4.
The urea 4 was converted in the carbodiimide 5 using triphenylphosphine and methylamine in methylene chloride (fig.1).

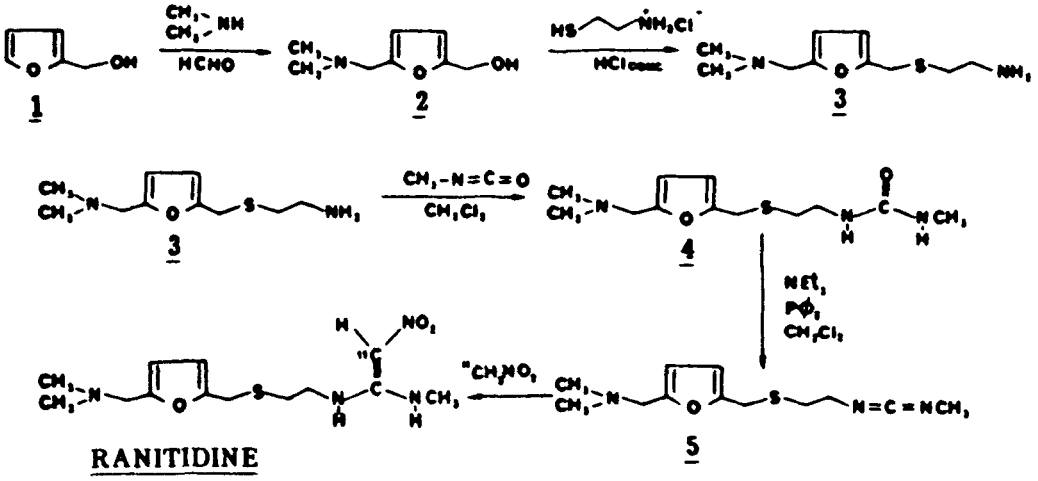

figure 1

The ^{11}C-labelled precursor is ^{11}C-nitromethane, which could be prepared in two ways (fig.2).
The first method is the one from K.O. SCHOEPS (1), in which the nitromethane is synthesized on-line from ^{11}C-labelled methyl iodide obtained from ^{11}C-carbon dioxide, by use of a heated silver nitrite column (fig.2), the radiochemical yield based on carbon dioxide is 40%.
In the second method ^{11}C-nitromethane is synthesized from ^{11}C-labelled methyliodide with sodium nitrite in dimethylformamide under sonication.The radiochemical yield based on carbon dioxide is 55% (fig.2).

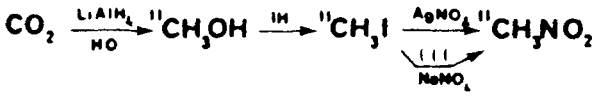

figure 2

The condensation of ^{11}C-nitromethane with the carbodiimide 4 occurs when the nitromethane carbanion is prepared under anhydrous conditions(2). We used three differents methods for the preparation of the carbanion:
1) 11C-Nitromethane reacts with sodium hydride in DMF at -30°C for 3mn, then carbodiimide 5 is added, the reaction is continued at 60°C for 10mn. The radiochemical yield based on ^{11}C-nitromethane is 5%.
2) ^{11}C-Nitromethane reacts with butyl lithium and diisopropylamine at -78°C for 3mn, then carbodiimide is added, the reaction is continued under the same conditions as before. The radiochemical yield based on ^{11}C-nitromethane is 10%.
3) ^{11}C-Ranitidine is one-pot prepared using potassium hydroxide in dimethylformamide with ^{11}C-nitromethane and the carbodiimide 5 under sonication for 15mn.Radiochemical yield based on ^{11}C-nitromethane is 15%.

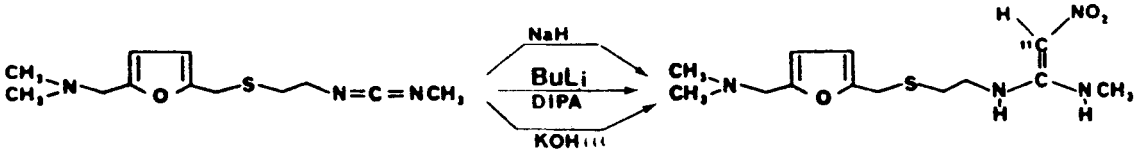

figure 3

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IMPROVED AND AUTOMATED "ONE-POT" RADIOSYNTHESES OF [^{11}C]DIPRENORPHINE AND [^{11}C]BUPRENORPHINE

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We originally reported radiosyntheses of the opiate receptor radioligands, [^{11}C]diprenorphine[1] and [^{11}C]buprenorphine,[2] using nca [1-^{11}C]cyclopropanecarbonyl chloride to label the C-20 methylene position in two steps. Subsequently, Lever *et al.*[3] reported a simpler procedure for labelling diprenorphine in the 6-methoxy position in a single step using the more easily prepared nca [^{11}C]iodomethane as labelling agent. However we have found that the precursor used in this radiosynthesis (6-*desmethyl*-3-*t*-butyldimethylsilyl-diprenorphine - reference sample and synthetic details kindly given by Dr J.R. Lever) to be unstable under the basic conditions required for [^{11}C]methylation and to give preferentially the by-product labelled at the C-3 position by *O*-alkylation. This accounts to some extent for the modest radiochemical yield of [^{11}C]diprenorphine from the procedure of Lever *et al.*[3]

Consequently, we have examined alternative protecting groups for the 3-hydroxy position. We have found that a 3-*O*-trityl group has excellent stability to the basic conditions (sodium hydride/DMF) necessary for [^{11}C]methylation at the 6-position and that it can be removed rapidly by mild acid hydrolysis. Further the 3-*O*-trityl protected precursor gave much higher radiochemical yields of 6-*O*-[^{11}C]methylated product than the 3-*O*-*t*-butyl-dimethylsilyl protected precursor in reactions with [^{11}C[iodomethane under the same conditions. We have therefore developed an improved "one-pot" radiosynthesis of [^{11}C]diprenorphine from [^{11}C]iodomethane based on the use of the 3-*O*-trityl protected precursor (Scheme 1).

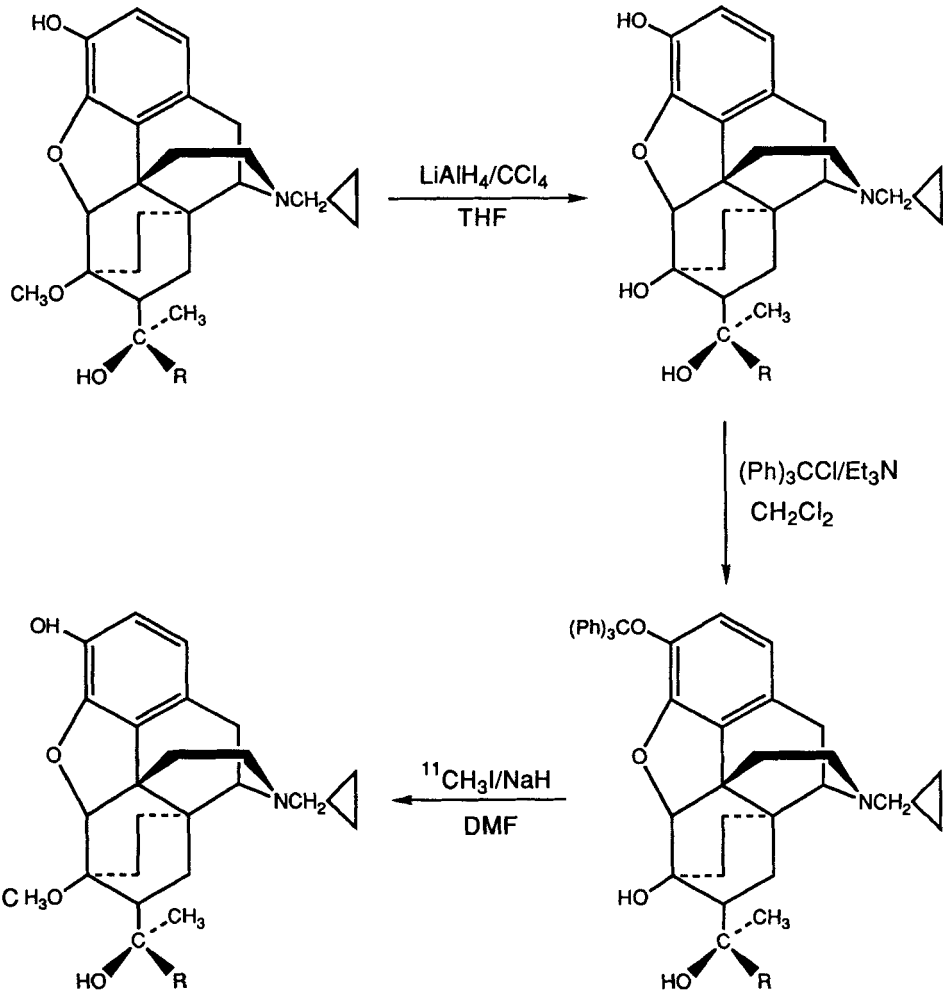
Thus [^{11}C]iodomethane is trapped in DMF (0.3 mL) containing the 3-*O*-trityl-protected precursor (*ca* 2 mg) and sodium hydride (4-5 mg) at ambient temperature. The reaction vessel is heated at 90 ^{0}C for 5 min. Then the *O*-trityl group is removed by treatment with aqueous hydrochloric acid (2 M, 0.25 mL) for 2 min. Radiochemically and chemically pure [6-*O*-*methyl*-^{11}C]diprenorphine is obtained by work-up on C_{18} Sep-pak followed by reverse phase HPLC. The radiosynthesis is fully automated using a programmable controller and reliably delivers 2.5-3.5 GBq of [6-*O*-*methyl*-^{11}C]diprenorphine with a specific activity of 8-11 GBq/μmol at EOS (*ca* 47 min from EOB).

[^{11}C]Buprenorphine has been prepared analogously in similar radiochemical yield and with equivalent specific activity, radiochemical purity and chemical purity.

The *O*-trityl precursor to [^{11}C]diprenorphine is easily prepared from diprenorphine itself by 6-*O*-demethylation according to Lever *et al.*[3] and by subsequent reaction with trityl chloride. The *O*-trityl precursor to [^{11}C]buprenorphine is similarly prepared from buprenorphine (Scheme 1).

References:

1. Luthra S.K., Pike V.W. and Brady F. *J. Chem. Soc. Chem.Commun.*, 1423 (1985).
2. Luthra S.K., Pike V.W., Brady F., Horlock P.L., Prenant C. and Crouzel C. *Appl. Radiat. Isot.*, **38**, 65 (1987).
3. Lever J.R., Dannals R.F., Wilson A.A., Ravert H.T. and Wagner H.N. Jr. *Tetrahedron Lett.*, **28**, 4015 (1987).



R = Me; [^{11}C]diprenorphine
R = t.Bu; [^{11}C]buprenorphine

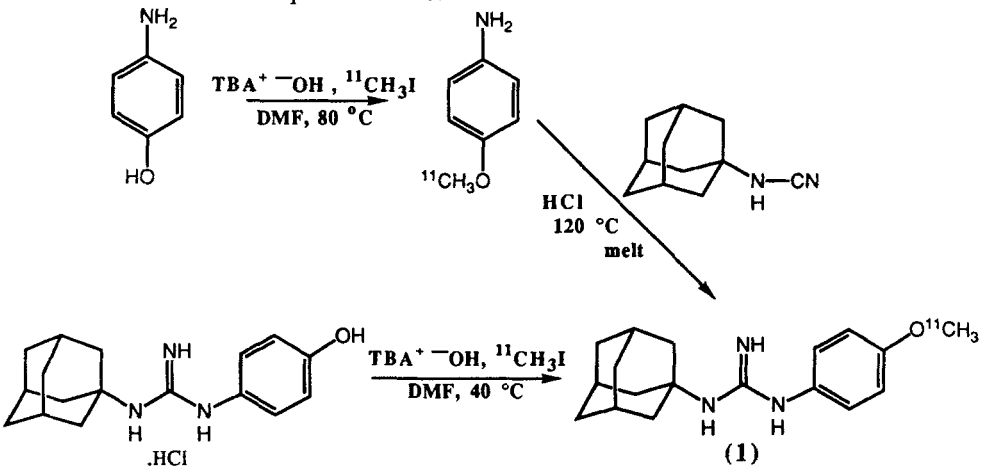
Scheme 1. Radiosyntheses of [^{11}C]diprenorphine and [^{11}C]buprenorphine.

1 - (4 - [^{11}C] - METHOXYPHENYL) - 3 - (1 - ADAMANTYL) GUANIDINE: A POTENTIAL RADIOTRACER FOR PET IMAGING OF SIGMA RECEPTORS.

A.A. Wilson, R.F. Dannals, H.T. Ravert and H.N. Wagner, Jr. Divisions of Nuclear Medicine and Radiation Health Sciences, The Johns Hopkins Medical Institutions, Baltimore, MD 21205-2179

Neuroleptics of diverse chemical classes have been found to bind to sigma receptors and it has been suggested that psychotomimetic benzomorphans such as SKF 10,047 act through the sigma receptor. Additionally, a relationship between the binding ability of cocaine analogues to sigma receptors and their ability to induce psychosis has been observed. This has led to speculation that certain types of psychosis may be treated with sigma selective antagonists. Localization and quantification of sigma receptors in normals, psychotic patients, and experienced drug users, using PET or SPECT, could help to explain the role of the sigma receptor in normals, the mechanism of drug induced psychosis, and the relationship between sigma receptors and psychotic individuals.

1-Adamantyl congeners of the selective sigma receptor ligand 1,3-ditolylguanidine (DTG) are potent inhibitors of [^{3}H]DTG binding and their IC$_{50}$s have been correlated with their potency in an in vitro bioassay of sigma receptor induced response. We report here the radiosynthesis of 1-(4-[^{11}C]-methoxyphenyl)-3-(1-adamantyl)guanidine (**1**) by two different methods; a two step method which may be applicable to the preparation of other labelled guanidines, and a one-step method which is most suitable for this particular radiotracer.



Our initial two-step route involves the methylation of the anion of 4-hydroxyaniline with [^{11}C]iodomethane followed by the acid catalysed reaction of the resultant labelled aniline with 1-adamantyl cyanamide. Time considerations required that this reaction be carried out as a melt at 120 °C. This method seems suitable for the synthesis of other labelled guanidines via labelled anilines (e.g [^{18}F]4-fluoroaniline). The one-step radiosynthesis of (**1**) is more efficient however, involving direct methylation of a phenolate precursor generated in situ.

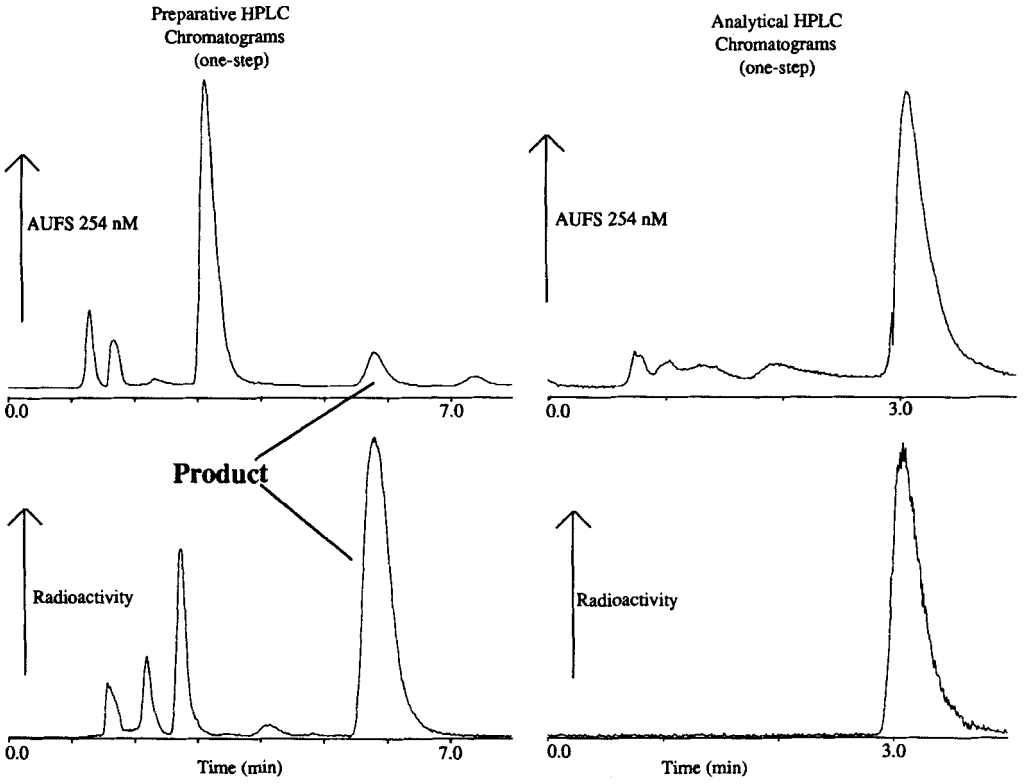
After HPLC purification and formulation both methods gave radiochemically pure (**1**). Average preparation times (from EOB), radiochemical yields (from [^{11}C]iodomethane), and specific activities (at EOS) for the two-step method were 20 min, 17 %, and 1240 mCi / μmole respectively, and for the one-step were 32 min, 12.4 %, and 1160 mCi / μmole.

Supporting data
for
"1 - (4 - [^{11}C] - METHOXYPHENYL) - 3 - (1 - ADAMANTYL) GUANIDINE: A POTENTIAL RADIOTRACER FOR PET IMAGING OF SIGMA RECEPTORS". A.A. Wilson et al

Compound (**1**) has an IC_{50} of 10 nM versus [^{3}H]DTG. In the same assay DTG has an IC_{50} of 28 nM.

The product from both reactions co-chromatographed with each other and with authentic cold (**1**) using three HPLC columns of varying chemistries:
a) a C18 reverse-phase column which is fully end-capped;
b) a non end-capped C18 reverse-phase column;
c) a silica column.

Shown on the next page are copies of the preparative and analytical HPLC chromatograms of the reaction mixtures and the final products respectively from the one-step radiosynthesis of (**1**).



SYNTHESIS OF CARBON-11 LABELLED CYCLOPENTYLTHEOPHYLLINE : A RADIOLIGAND FOR PET STUDIES OF ADENOSINE RECEPTORS

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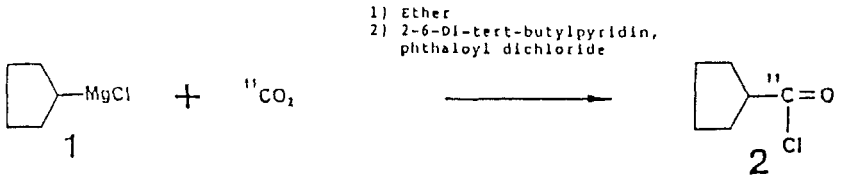
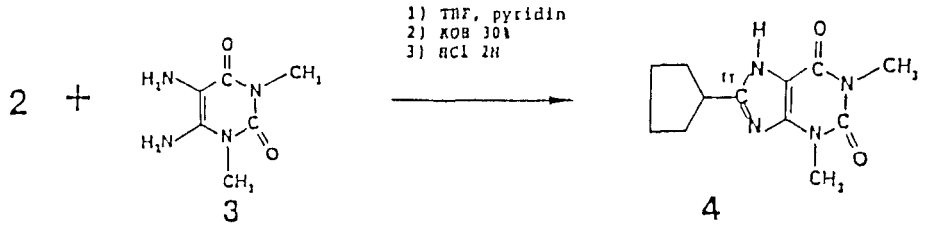
Adenosine is presently considered as a neuromodulator (1). An adenosine system has been described including secretory neurons (2) with a diffused distribution, specific receptors (3) and a reuptake system (4) distributed heterogeneously in different anatomic areas. Only, in man, the receptors have been localized post-mortem. In order to localize the adenosine receptors in vivo by PET, we chose to label with carbon-11 8-cyclopentyltheophylline 4 (CPT), a known adenosine antagonist A_1 receptors. [^{11}C]CPT is prepared by condensation of [^{11}C]cyclopentane carbonyl chloride 2 with 5,6 diamino 1,3 dimethyl uracil hydrate 3.

The carbon-11 is produced by the (p, α) reaction on pure nitrogen (99,9999 % Purity). By irradiating with 20 MeV protons (30 µA) for 30 minutes, 1.5 Ci (55 Gbq) $^{11}CO_2$ is obtained. From $^{11}CO_2$, the [^{11}C]cyclopentane carbonyl chloride is at first produced by the method described by S. Luthra (5).



1 2

The [^{11}C]acid chloride released by heating at 160° is carried by a light stream of nitrogen into 100 µl of THF/pyridine 80/20 containing 10 µMol. of 3.



3 4

The reaction mixture was heated at 70°C for 2 minutes, then 120 µl of KOH (30%) was added and heated at 120°C for 10 minutes. The solution was neutralized with 500 µl HCl (2N) then injected onto a HPLC column (µ Bondapak C_{18}, ϕ 7.8, L 300, H_2O/EtOH-70/30, buffered with NaH_2PO_4, 10^{-2}M, 5 ml/min) to give [^{11}C]CPT 4 (radiochemical yield 56 % based on acid chloride, decay corrected) with a retention time of 13 minutes. Few tens mCi of 4 were obtained 40-45 minutes after EOB.

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4. Bisserbe J.C., Deckert J. and Marangos P.J. Neurosci. Letters, 66, 341-345 (1986).
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ROUTINE PLASMA ANALYSIS FOR 2-[^{18}F]FLUOROTYROSINE BY SAX-CARTRIDGES

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There is a great necessity for simple, reliable, and fast methods of plasma analysis. The determination of the actual plasma-content of a radiopharmaceutical as a function of time after injection, i.e. its input function, is one of the prerequisites for tracer kinetic quantitation in vivo. While HPLC is generally the most effective method, it is rather time consuming and expensive in a routine setting with numerous plasma samples. The other problem encountered is the low level of radioactivity in extended (pharmacokinetic) studies. Pretreatment of plasma to make HPLC injectable solutions or to concentrate the activity is complicated and susceptible to error. This is also true for TLC-methods which are simple and cheap but where samples mostly must be extracted and concentrated.

For the routine determination of the amino acid analogue 2-[^{18}F]-fluorotyrosine (2-^{18}FTyr) in patient's plasma samples a cartridge-filtration procedure was developed. It was known that ^{18}F-activity in plasma appears in three forms: the fluoroamino acid, labeled proteins and fluoride [1]. Attempts using small alumina, silica gel or RP material filled columns were unsatisfactory. Separation of $^{18}F^-$ and 2-^{18}FTyr was simply achieved on small SAX columns (Adsorbex^R SAX, 400 mg, Merck) by elution with phosphate buffer even in presence of cold plasma. In case of labeled plasma, however, some breakthrough of small proteins or peptides was noticed which necessitates precipitation of proteins before SAX cartridge filtration.

The analytical procedure is as follows: 100 μl of plasma is diluted with 100 μl of phosphate buffer (pH 9), then 100 μl of methanol is added and the proteins separated by centrifugation. The supernatant and an equal solution used for washing the precipitate are transferred to the SAX-columns, which are conditioned by each 1 ml of methanol and buffer. Up to 10 columns are then extracted at the same time by four portions of 1 ml buffer solution by means of a dedicated vacuum chamber. The activity content in the eluate is related to the radioactivity in an authentic plasma sample to calculate the fraction of 2-^{18}FTyr.

The results obtained agree within 5% with those determined by reverse phase HPLC [1,2] as is shown for the plasma curve of one patient in Fig. 1. Although the procedure was especially developed for 2-^{18}FTyr and still needs optimization to avoid previous precipitation of proteins, it will also be useful for other amino acids and radiopharmaceuticals if there are no metabolites with similar elutropic properties as the parent compound. In the latter case HPLC methods seem mandatory.

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[2] Coenen H.H., Franken K., Kling P., Stöcklin G., Appl. Radiat. Isot. 39, 1243 (1988)

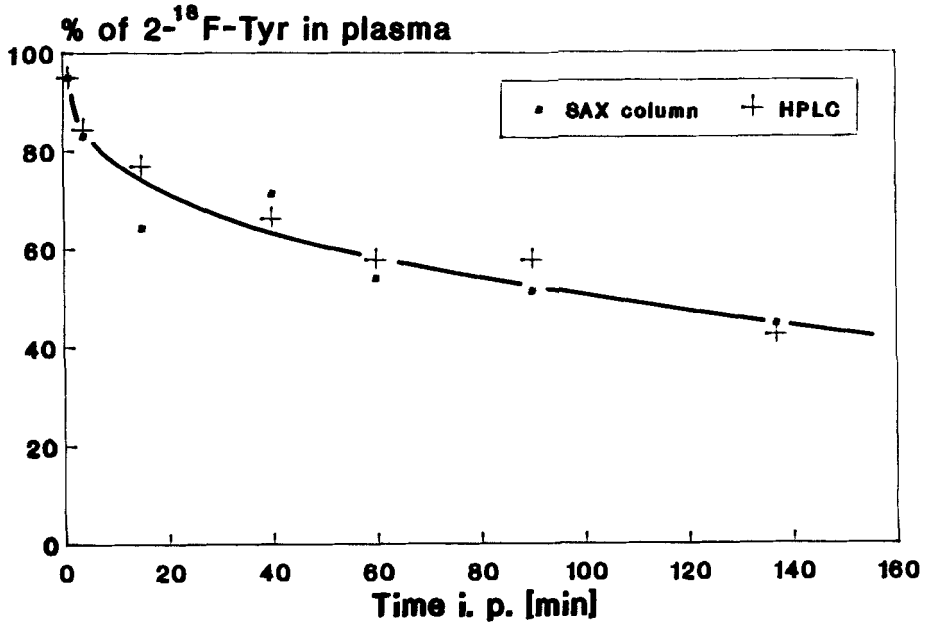

Fig. 1 Results of analysis of patient plasma by HPLC and SAX columns for content of 2-[^{18}F]fluorotyrosine (% of plasma radioactivity)

[F-18]Fluoro-meta-L-tyrosine is a better PET tracer than [F-18] fluoro-L-dopa for the deliniation of dopaminergic structures in the human brain

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F-Dopa was originally designed und developed to measure presynaptic dopaminergic function with PET (1). Kinetic evaluation of brain PET studies with F-dopa is made difficult because of a metabolite of F-dopa, the peripherally formed 3-O-methyl-[F-18] fluoro-L-dopa (OMeFdopa). Like F-dopa, it also enters the brain and thus contributes unspecifically to the F-18 accumulation in the brain. To circumvent this problem the trace aminoacid meta-L-tyrosine labelled with positron emitter has been suggested by deJesus (2). Meta-tyrosine, like dopa, is an substrate for the brain enzyme dopa decarboxylase (DDC).
We set out to compare the utility of [F-18]fluoro-meta-L-tyrosine (FmLtyr) for PET investigations of dopaminergic brain regions with that of F-dopa.
Syntheses: Our general electrophilic radiofluorination process with [F-18]fluorine gas and hydrogen fluoride as solvent was used to prepare FmLtyr, [F-18]fluoro-meta-tyramine (F-mTA) and 3-hydroxy-[F-18]fluoro-phenylacetic acid (FHPAA) (3) as well as F-dopa and OMeFdopa (4). The compounds were isolated by HPLC. The new fluorinated compounds were identified by mass spectrometry and by their H-1, C-13 and F19 NMR spectra.
Distribution of FmLtyr in the brain: When FmLtyr was injected into rhesus monkeys, the distribution of F-18 within the brain at autopsy reflects the known intracerebral distribution of dopa decarboxylase and that of meta-tyramine in man (Table 1).
When FmLtyr was injected into man the same preferential striatal accumulation of F-18 was demonstrated with PET, very much like that with F-dopa. Further, in patients with unilateral Parkinsons disease the characteristic contralateral reduction in the putamenal DDC was also demonstrated by FmLtyr.
Although F-dopa and FmLtyr each produce comparable PET images in controls and in parkinsonian patients, the F-18 associated with these tracers follow a different time-course in cerebral tissue (Fig.1 and 2). The ratio striatum to occiput at 120 min was 4.4 to 1 in the case of FmLtyr and only 1.7 to 1 with F-dopa. Faster clearance of F-18 from the non striatal tissue is responsible for the improved deliniation of the striatal tissue.
Metabolism of FmLtyr was investigated in plasma, putamen, caudate nucleus and occipital cortex of rhesus monkeys. After the injection of FmLtyr the tissues were were homogenized in 7% perchloric acid, then centrifuged and the supernatant was analyzed by calibrated reversed-phase HPLC. In all tissues only 3 metabolites were found; F-m-TA, FHPAA and a unidentified conjugate of a F-18-containing metabolite (Fig.3). At early times there was some F-m-TA in the DDC-rich brain regions, caudate nucleus and putamen. The major metabolite at later times was FHPAA as the result of the subsequent action of DDC and monoamine oxidase.
Conclusion: The better deliniation of striatum with FmLtyr is not the result of improved striatal retention but it is the

consequence of much lower retention of F-18 in non-dopaminergic tissue. Kinetic analysis of the accumulation time course can provide a quantitave measure of the activity of dopa decarboxylase.

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Garnett et al Nature 305:137 (1983)
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4.Chirakal et al J.Nucl.Med. 27:417 (1986)

Distribution of ^{18}F in the Monkey Brain

after an i.v. injection of [^{18}F]Fluoro-L-m-tyrosine

Brain Region	20 minutes	90 minutes
Caudate Nucleus	127	105
Putamen	106	88
Thalamus	83	44
Hypothalamus	73	45
Substantia nigra	63	58
Occipital cortex	67	19
Parietal cortex	66	22
Cingulate cortex	68	16

Regional concentration of ^{18}F is given as percent of dose of ^{18}F per gram tissue per gram body weight.

Table 1

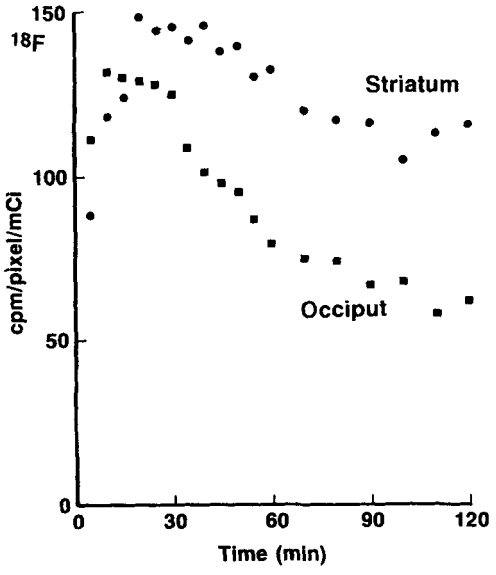

Figure 1

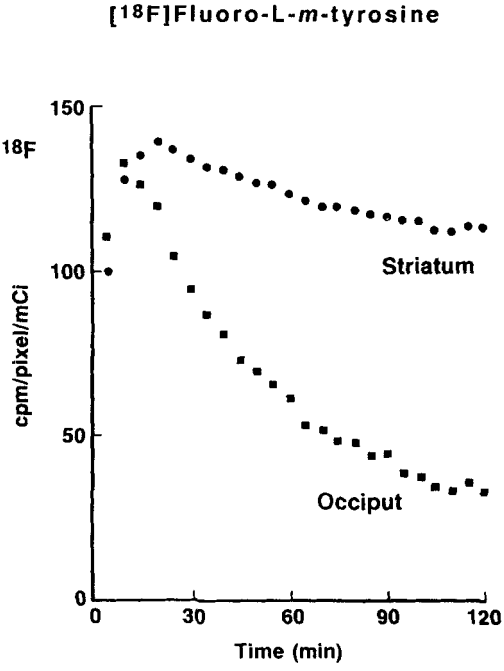

Figure 2

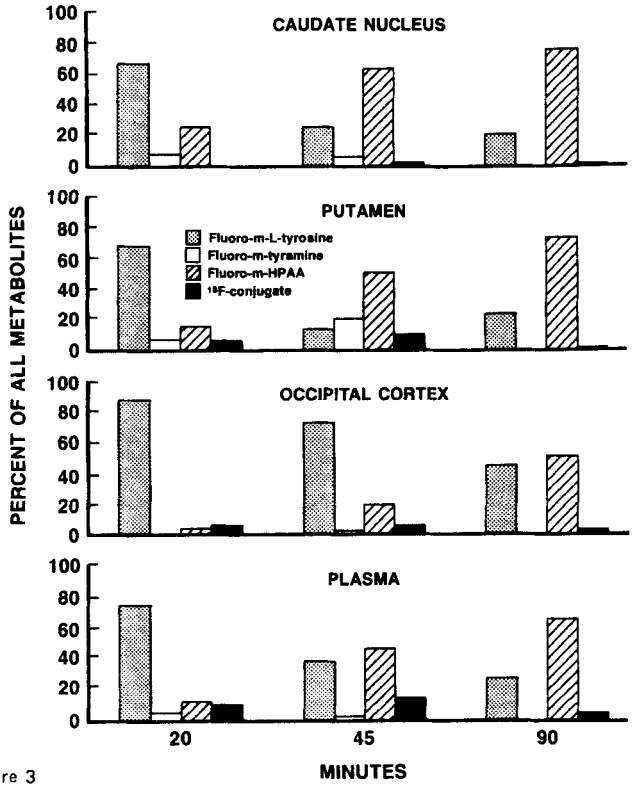
CAUDATE NUCLEUS
PUTAMEN
OCCIPITAL CORTEX
PLASMA
Fluoro-m-L-tyrosine
Fluoro-m-tyramine
Fluoro-m-HPAA
18F-conjugate
PERCENT OF ALL METABOLITES
100
80
60
40
20
0
20
45
90
MINUTES

Figure 3

NCA ASYMMETRIC SYNTHESIS OF 6-[^{18}F]-FLUORO-L-DOPA

Lemaire C, Guillaume M, Cantineau R, Plenevaux A, Christiaens L.
Cyclotron Research Center- Liège University- Belgium

6-[^{18}F]-fluoro-L-dopa has found wide application as a tracer for presynaptic dopamine function in cerebral studies with PET in humans. The main synthetic routes so far reported for the preparation of this radiopharmaceutical are based on the electrophilic radiofluorination of L-dopa or its protected derivatives using reagents such as [^{18}F-F_2], XeF_2[^{18}F] or acetyl hypofluorite-[^{18}F](1). These methods involve the addition of carrier. As an alternative we have developed two different no-carrier-added nucleophilic syntheses of this radiotracer based on the nucleophilic displacement of nitro groups by ^{18}F- of two commercially available compounds, 6-nitroveratraldehyde and 6-nitropiperonal, followed by multi-step syntheses (2).

The first method involves condensation of the labeled benzaldehyde derivative with phenyloxazolone followed by hydrolysis (2). This yields a racemic mixture of the amino acid and consequently a chiral HPLC separation and removal of copper from the final product are necessary. An asymmetric synthesis has been therefore investigated using a chiral agent (3). Although stereoselectivity was observed using this second method, a chiral HPLC separation with its accompanying disadvantages was still required.

For routine production of 6-[^{18}F]-fluoro-L-dopa via this nucleophilic pathway the stereoselectivity of the alkylation reaction would have to be increased and the HPLC separation avoided. Accordingly, a major research objective of our group has therefore been to investigate various inductors of chirality, which could lead from the same intermediate, the previously described [^{18}F]fluorobenzyl bromide (3), to an enantiomeric excess as close as possible to 100 % in the final product. The results obtained with two new chiral inductors are presented here.

The chiral starting compounds selected were first the 1-(S)-(-)camphor imine of tert-butyl glycinate (A) easily synthesized from commercially available 1-(S)-(-)camphor (4) and secondly (S)-(-)-1-Boc-2-tert-Butyl-3-methyl-4-imidazolidinon [(S)- Boc-BMI (B)] (5) now commercially available from Merck (Scheme 1).

These two asymmetric syntheses involve the alkylation of these inductors of chirality in THF in presence of LDA(TMPLi) at minus 78°C with the 6-fluoro-3,4-dimethoxybenzyl bromide which is easily prepared from 6-fluoroveratrole. The alkylated products C and D (Aryl = F-C_6H_4-$(OCH_3)_2$ were separated by chromatography on silica gel column (ether/hexane) and fully characterized by ^{1}H NMR and mass spectroscopy.

Compound C : Mp: 85-86°C. ^{1}H NMR(400 MHz)($CDCl_3$): δ 6.62-6.47(2H, m, aromatic H's), 3.99(1H, m), 3.74(3H, s), 3.72(3H, s), 3.18(1H, s), 2.86(1H, s), 2.18(1H, m), 1.7-1.35 (5H, m), 1.33(9H, s), 1.24(1H, m), 0.81(3H, s), 0.75(3H, s), 0.66(3H, s). MS, m/e: (relative intensity) 433(3.2, M+), 332(88.2), 264(9.7), 208(100), 169(67.7).
Compound D : Mp: 125°C. ^{1}H NMR(400 MHz)($CDCl_3$): δ 6.53(1H,d), 6.48(1H,d), 4.75(1H,s), 4.24(1H, s), 3.75(3H, s), 3.74(3H, s), 3.19(1H, d), 2.78(3H, s), 1.55 (1H, s), 1.38(9H, s), 0.89(9H, s). MS , m/e: (relative intensity) 425 (17, M+), 369(62.1), 367(32), 311(45.6), 268(27), 267(100), 169(81).

The diastereoisomeric excesses were determined by NMR and HPLC and found to be 83% and ≥ 95% respectively.

Extension of this procedure to the n.c.a. radiofluorination with [^{18}F]6-fluoro-3,4-dimethoxybenzyl bromide gave 6-[^{18}F]-fluoro-L-dopa in variable yield dependant on the purity of the starting fluorinated compound. In comparison with the camphor derivative, the Boc-BMI method seems more promising since the alkylation reaction proceeds without production of a racemic mixture, the diastereoselectivity being not affected by electronic factors from the alkylating agent. Moreover, the imidazolidone seems to be less sensitive to racemisation during hydrolysis than the camphor derivative and leads to higher enantiomeric excess of the free amino-acids.

Starting from other fluoro aldehydes such as fluorobenzaldehyde, 4-methoxy-2-fluorobenzaldehyde and 4-fluoro-3-methoxybenzaldehyde it was possible to extend this general method to the preparation of other potentially interesting L-[^{18}F]-labelled amino acids (4-fluorophenylalanine, 2-fluorotyrosine, 4-fluoro-m-tyrosine).

Since it yields an enantiomeric excess close to 100 % this general NCA fluorination route should lead, after optimization, to the preparation of various [^{18}F]labeled aromatic L- amino acids in sufficiently high activity (EOS) for cerebral studies with PET. Work in this area is currently in progress.

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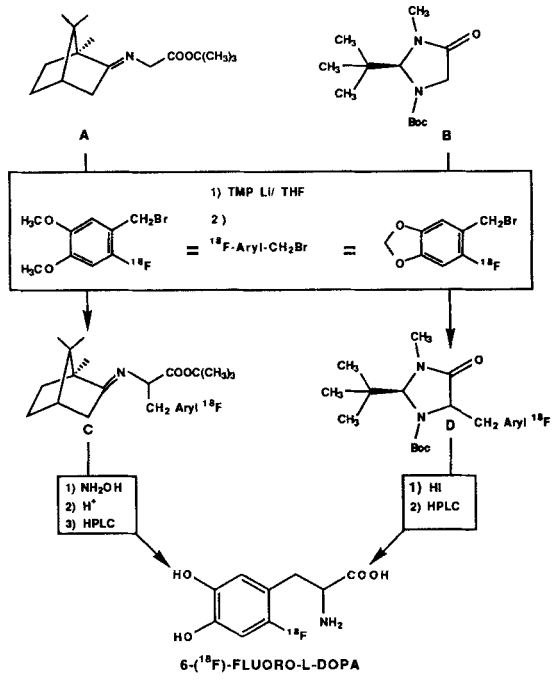

Scheme 1. Asymmetric synthesis of n.c.a. 6-(^{18}F)fluoro-L-dopa starting from two different chiral compounds

RADIOIODINATED PRESYNAPTIC MARKERS FOR GABA AND DOPAMINERGIC NEURONS.

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Division of Nuclear Medicine, University of Michigan, Ann Arbor, MI 48109

The dopaminergic and GABA neuronal systems play a prominent role in the etiology of certain psychiatric and neurological diseases. For example abnormalities in the dopamine system have been implicated in the development of Parkinsons disease and schizophrenia whereas GABA dysfunctions have been implicated in the manifestations of epilepsy and other seizure disorders.

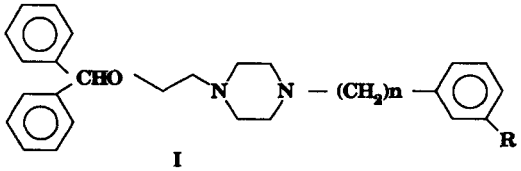
Radioligands for PET studies of dopaminergic systems have focused on dopamine receptors (eg., [C-11]-and [F-18]N-methylspiperone and [I-123]iodobenzamide), precursors to dopamine synthesis (6-[^{18}F]fluorodopa) and presynatic dopamine uptake inhibitors ([F-18]GBR 13119). In contrast radioligands developed for the GABA system have been limited entirely to the benzodiazepine subset of the GABA-benzodiazepine receptor complex.

Successful PET studies with [F-18]GBR 13119, a presynaptic dopamine uptake inhibitor, have led us to develop radioiodinated presynaptic markers for dopaminergic and GABA neurons for possible SPECT application. Compounds structurally related to I (R=H, n=2,3) and II (R=Cl) are potent uptake inhibitors of GABA and dopamine, respectively. The iodinated analogs of I and II (R=I) have been synthesized and labeled with I-125 in high specific activity by the solid-state ammonium sulfate technique. Following isotopic exchange removal of free I-125 from [I-125]I (n=2,3) was accomplished using an IRA-400 anion exchange resin. Further purification by reversed phase preparative HPLC [C-18; 5-μm particle, 150 x 4.6 mm; 0.1 M NH_4OAc:95% ETOH (1:4); 1 mL min^{-1}] afforded the radioligands in 99% chemical and radiochemical purity in a directly injectable form after dilution with normal saline. The chemistry, radiochemistry and preliminary bioevaluation of these radioligands will be discussed.

RADIOSYNTHETIC DATA

LIGAND	RADIOCHEMICAL YIELD	SPECIFIC ACT. (Ci/mmol)	RAD. PURITY
[I-125]I (n=2)	65%	135	99%
[I-125]I (n=3)	68%	152	99%
[I-125]II	60%	128	98%

PRESYNAPTIC UPTAKE INHIBITORS



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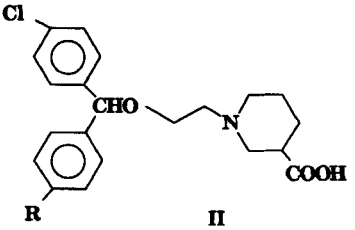

II

F-18-LABELLED PET TRACERS FOR CNS PRESYNAPTIC DOPAMINE NEURONS.

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The CNS dopamine (DA) system is involved in several neuropsychiatric processes and has been the target of radiotracer development since the early 1970s (1). In order to have selectivity, a tracer for dopamine must share some, if not all, of the important biochemical characteristics of this neurotransmitter. 6-Fluoro-L-dopa (6-FD), developed and pioneered by Firnau et al., has been shown by several laboratories to behave analogously to L-dopa in the following respects; (a) transport into the brain, (b) metabolism involving all the enzymes involved in DA metabolism, (c) vesicular storage of 6-fluorodopamine (6F-DA) and (d) action of 6F-DA as false DA neurotransmitter. Human PET studies using [F-18]-6-FD has provided important insights into Parkinson's and related disorders, dystonia, and MPTP intoxication. Although 6-FD is a poor substrate of the enzyme catechol-O-methyltransferase (COMT), PET studies with this radiotracer have been complicated by the formation of the O-methyl-6-FD. This suggests that 6-FD may be sub-optimal as a PET DA tracer.

We have previously proposed m-tyrosine analogs as PET tracers of CNS DA since these analogs are not COMT substrates but retain other important L-dopa properties (2). PET studies performed in monkeys using [F-18]-4-fluoro-m-tyrosine (FMT) (3) and in man using [F-18]-6-fluoro-m-tyrosine (4) have shown improved image contrast compared to [F-18]-6-FD scans due possibly to the reduced number of metabolites formed by FMT. Although the major [F-18]-labelled metabolite found by both groups within an hour of injection into rats and monkeys was [F-18]-fluoro-m-hydroxyphenylacetic acid, the time course of the clearance of this radio-metabolite appear to be long in the time scale of the PET study resulting into exceptionally good PET images of DA-rich brain regions. However, the relevance of this finding to the utility of PET scans with[F-18]-FMT in the evaluation of presynaptic DA nerve terminals needs to be clarified by further biochemical studies.

A novel m-tyrosine analog, also a dopa decarboxylase (DC) substrate, β-fluoro-methylene-m-tyrosine (FMMT) (MDL 72394), was recently developed by Palfreyman et al. (5) as a selective monoamine oxidase (MAO) inhibitor. FMMT is decarboxylated to form β-fluoro-methylene-m-tyramine, a potent and irreversible MAO inhibitor. This unique property of FMMT and the fact that in humans DC enzymes are highly concentrated in CNS areas innervated with DA terminals suggested that, because of the regionally selective decarboxylation of [F-18]-labelled FMMT analog leading to a product irreversibly bound to mitochondrial MAO found in DA neurons, PET studies of presynaptic DA neurons with this tracer may be simplified.

We have synthesized [F-18]-fluoro-FMMT by the direct reaction of FMMT in 1:1 trifluoroacetic acid-acetic acid with [F-18]-AcOF. Under unoptimized conditions, the total decay-corrected HPLC-isolated radiochemical yield was 61.8% ± 12.6% (n=5) with about one hour of synthesis time from EOB. Analysis of the product by semipreparative HPLC showed three positional isomers. These products were identified, using NMR (H-1, C-13, F-19) analysis of HPLC-pure samples to be 2-, 6-fluoro-FMMT and 2,6-difluoro-FMMT with distribution shown in Scheme 1.

Studies using mice (n=3-4) pretreated with 1 μmol/kg benserazide, a DC inhibitor, showed that after i.p. injection of [F-18]-6-F-FMMT, striatum to cerebellum (S/C) uptake ratios were 1.04, 1.62, and 1.72 at 30, 60, and 120 minutes postinjection. With 200 μg/kg benserazide, S/C ratio was 3.85 at 2 hours. Biodistribution and pharmacokinetics of [F-18]-6-F-FMMT show that elimination of the radioactivity involved the kidneys more than the liver. We have also performed preliminary studies *in vitro* using rat synaptosomal preparations which show uptake of 6-F-FMMT to be similar to that of dopamine.

These results demonstrate the potential of 6-F-FMMT as PET imaging agent for dopamine neurons. With the possible advantage of "metabolic trapping", the mathematical modelling of PET scans using [F-18]-F-FMMT may be simpler than either 6-FD or FMT scans since a model similar to the deoxyglucose method may be applicable. More biochemical and pharmacological studies both *in vivo* and *in vitro* are needed to validate the use of this novel PET tracer.

The evolution of presynaptic DA radiopharmaceuticals from 6-FD to FMT to F-FMMT was directed at simplifying PET studies of CNS DA sytem by minimizing metabolite formation. However, this is at the expense of tracing correspondingly fewer steps in DA metabolism and turnover. Whether this approach will become useful in the clinic needs to be determined.

We thank Dr. M. Palfreyman of Merrell Dow Research Institute, Cinncinnati, OH for providing MDL 72394 for this study. Research supported in part by NIH Grant NS-26621 and a Wisconsin Alumni Research Foundation Grant-in-Aid.

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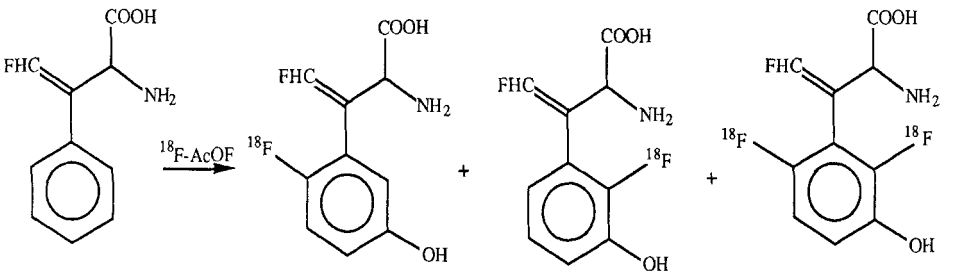
SCHEME 1



β-Fluoromethylene-m-tyrosine (FMMT)	6-F-FMMT	2-F-FMMT	2,6-difluoro-FMMT
Total Radiochemical Yield (n=5)	25.3% ± 5.9%	22.1% ± 3.8%	14.4% ± 4.2%
Range	19% - 33%	18% - 28%	8% - 18%

QUALITY ASSURANCE METHODS FOR SOME AGENTS USED IN PET STUDIES OF THE DOPAMINERGIC SYSTEM

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Though L-6-[^{18}F]fluoro-DOPA, *S*-[*N-methyl*-^{11}C]nomifensine and [*O-methyl*-^{11}C]raclopride now find increasing application, little has been reported on their quality assurance. The application of rigorous quality assurance, giving unequivocal characterisation of the product, is rendered difficult by the short half-lives of the incorporated radioisotopes. Nonetheless high standards of safety and efficacy must apply to these products as to any other pharmaceuticals and where possible quality control should be performed on each production before administration to man. In our laboratory quality assurance rests on establishing *good manufacturing practice* supported by rapid *routine quality control.*

In establishing good manufacturing practice indirect techniques (*e.g.* NMR spectroscopy) and direct techniques (*e.g.* radiochromatography) are extensively applied to characterise the product from a developing procedure, and to investigate any contamination. Table1 details the type of information that has been gleaned from the techniques that we initially applied to the aforementioned radiopharmaceuticals. The importance of applying thorough analysis to the radioactive products from even well described radiosyntheses cannot be over-emphasised; in our experience the type and level of likely contaminants can depend critically on minute details of the synthetic protocol, details that can vary between production centres. Examples are the presence or absence of L-6-hydroxy-DOPA and *O*-ethyl*desmethyl*raclopride in L-6-[^{18}F]fluoro-DOPA and [*O-methyl*-^{11}C]raclopride respectively. For this reason it is imperative that synthetic protocols are tightly defined and adhered to.

In order to establish radiochemical purity, chemical purity and specific activity before product administration we have developed rapid routine quality control procedures based on analytical HPLC methods for L-6-[^{18}F]fluoro-DOPA, *S*-[*N-methyl*-^{11}C]nomifensine and [*O-methyl*-^{11}C]raclopride (Table 2). We regard the performance of such independent quality control before product administration to be mandatory for safety and efficacy. In our experience analytical data taken from production separations (*e.g.* preparative chromatograms) cannot be relied upon, even qualitatively, especially when chromatographic columns give incomplete separations of product or are prone to deterioration in efficiency (for example the reverse phase columns commonly used to separate the positional isomers of L-[^{18}F]fluoro-DOPA prepared by non-regioselective fluorination). Further these methods can be applied to *formulated materials* close to the time of administration. This is an important consideration for radipharmaceuticals that may show poor stability in formulated form (*e.g.* *S*-[*N-methyl*-^{11}C]nomifensine and L-6-[^{18}F]fluoro-DOPA).

Radiopharmaceutical	*Technique*	*Information obtainable*	*Findings*
L-6-[^{18}F]Fluoro-DOPA[a]	^{19}F-NMR	Isomeric purity Contamination by F-compounds	94 % 2-F-DOPA
	M.S.	Mol. wt. of product and contaminants	215 233 (di-fluoro-DOPA)
	Chiral TLC	Enantiomeric purity of product	> 99 %
	HPLC	Product stability **Radiochem. purity** **Specific activity** **Contaminants**	Stable to 60 min 93.8 % (n =30) 4 - 8 MBq/μmol L-DOPA 2-F-DOPA 6-OH-DOPA Trace unknowns
S-[^{11}C]Nomifensine[b]	Chiral HPLC	**Chiral purity of precursor**	100 %
	^{13}C-NMR	Position of label	*N*-methyl
	M.S.	Molecular weight	238
	HPLC	Product stability **Radiochem. purity** **Specific activity** **Contaminants**	Unstable 89.2 % (n =30) 12 -50 GBq/μmol Trace unknowns
[^{11}C]Raclopride[c]	^{13}C-NMR	Position of label	*O*-methyl
	M.S.	Molecular weight of product and contaminants	346 360 (ethyl analogue)
	HPLC	Product stability **Radiochem. purity** **Specific activity** **Contaminants**	Stable up to 74 min 98.4 % (n =30) 12 -40 GBq/μmol Trace precursor Trace unknowns

[a] Prepared essentially according to Adam *et al., J. Nucl. Med.* (1986) 27, 1462.
b Prepared by a modification of the method of Ulin *et al., Appl. Radiat. Isot.* (1989) **40**,171.
c Prepared by the method of Ehrin *et al., J. Label. Compd. Radiopharm.* (1987) **24**, 931.

Table 1. Techniques applied to establishing good manufacturing procedure for some agents for PET studies of the dopaminergic system. The mentioned techniques are also supplemented by general tests for pH, tonicity, sterility and apyrogenicity for each material. Parameters in bold type are determined on each batch of material by the methods detailed in Table 2

Radiopharmaceutical	*Method*	*Compounds determined*	*Retention times (min)*
L-6-[^{18}F]Fluoro-DOPA	HPLC on Nucleosil 5 C18 (250 X 4.6 mm) 0.07 M KH_2PO_4 1 ml/min	6-F-DOPA 2-F-DOPA L-DOPA 6-OH-DOPA Difluoro-DOPA Trace unknowns	9.60 9.00 6.85 6.13 13 8.5
S-[^{11}C]Nomifensine[b]	Chiral HPLC on Cyclobond I (250 X 4.6 mm) MeOH/TEA (O.2 M) O.5 mL/min	*S-nor*nomifensine *R-nor*nomifensine	9.6 12.4
	HPLC on μ-Bondapak C-18 (300 X 3.9mm) MeCN/H_3PO_4 (0.01 M) 20/80 by vol. 2 mL/min	Nomifensine *Nor*nomifensine Trace unknown Trace unknown	4.33 3.4 6.3 7.0
[^{11}C]Raclopride[c]	HPLC on μ-Bondapak C-18 (300 X 3.9mm) MeCN/H_3PO_4 (0.01 M) 27/73 by vol 2 mL/min	Raclopride Desmethylraclopride *O*-Ethyl-analogue Unknown Unknown	6.0 4.5 8.5 3.2 4.5

Table 2. Techniques routinely applied to the quality assuarance of some agents used for PET studies of the dopaminergic system. Each HPLC method uses radioactivity and U.V. absorbance detection. The mentioned techniques are also routinely supplemented by general tests for pH and periodically for sterility and apyrogenicity.

Synthesis and Evaluation of [^{123}I]-labelled Compounds to Study the Dopamine System of the Brain.

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As part of our ongoing study to synthesize ^{123}I-labelled SPECT brain imaging agents we have recently prepared three compounds to study the dopamine system of the brain; L-6-[^{123}I]iododopa (6-ID) (1), L-6-[^{123}I]iodo-*m*-tyrosine (6-IMT) and N,N-dimethyl[β-(3,4-diacetoxy-6-[^{123}I]iodophenyl)]ethylamine (IDDE).

6-ID was prepared as an analog to 6-fluorodopa for potential use as an agent to study dopamine metabolism with SPECT. 6-ID was synthesized by the exchange of $^{123}I^-$ for bromine on L-6-bromodopa in buffer (pH 4) for 35 min at 97°C. The synthesis was complete in approximately 1 hr with a radiochemical yield of 50%. It was shown that 6-ID accumulated in the rat striatum with a striatum:whole brain ratio of 1.36 at 1 hr and 1.11 at 2 hr. Further animal experiments are under way and will be discussed.

Recently, Barrio and co-workers (2) have prepared 4-[^{18}F]fluoro-L-*m*-tyrosine to study striatal dopaminergic function. They found that this compound accumulates in the striatum of monkeys with a striatum/cerebellum ratio of 4 at 180 min. Based on this report we prepared the iodinated analog (6-IMT) with the aim to study the same function using SPECT. 6-IMT was synthesized by the direct iodination of L-*m*-tyrosine with [^{123}I]NaI and Chloramine-T. The crude product was purified by reverse phase HPLC to give the final product in 57% yield. The preliminary biodistribution in rats showed a higher concentration in the cerebellum but no selective uptake in the striatum at 1 or 2 hr after injection.

[^{123}I]-IDDE was prepared as a possible SPECT brain imaging agent for the dopamine system and as a potential radiotracer for monoamine oxidase (MAO) activity in the brain. It has been shown that the non iodinated version of this compound crosses the blood brain barrier and activates dopamine receptors in the CNS (3). It has also been show that N,N-dimethylphenethylamine derivatives enter the mouse brain and are deaminated by MAO to dimethylamine and phenylacetaldehyde (4). IDDE may also be deaminated by MAO to produce labelled dihydroxy-phenylacetaldehyde which may be trapped in the brain. IDDE was synthesized by the iodination of a mercury derivative of N,N-dimethyl-3,4-dimethoxy-phenethylamine using [^{123}I]NaI and Chloramine-T. The O-methyl groups were removed with BBr_3 and the molecule acetylated to afford IDDE in 43% radiochemical yield. Initial animal experiments are currently under way.

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SYNTHESIS OF IODINE-123 LABELED 3-O-(E)-3-IODOPROPEN-2-YL-D-GLUCOSE: A POTENTIAL NEW AGENT FOR THE ASSESSMENT OF GLUCOSE TRANSPORT INTO THE BRAIN AND HEART USING SPECT

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The glucose analogues F-18 labeled 2-fluoro-2-deoxy-D-glucose (2-FDG) and C-11 labeled 3-O-methyl-D-glucose (CMG) have been used noninvasively to evaluate the regional cerebral glucose utilization in patients with ischemic brain disease. These glucose analogues are transported across the blood-brain-barrier by the same carrier system as glucose. Clinical studies using F-18 labeled 2-FDG (1) and carbon-11 labeled CMG (2) in stroke patients have shown accumulation defects frequently not detectable by CT. These findings suggest a reduction in the transport of glucose in the ischemic region occurs prior to anatomical changes detectable with CT.

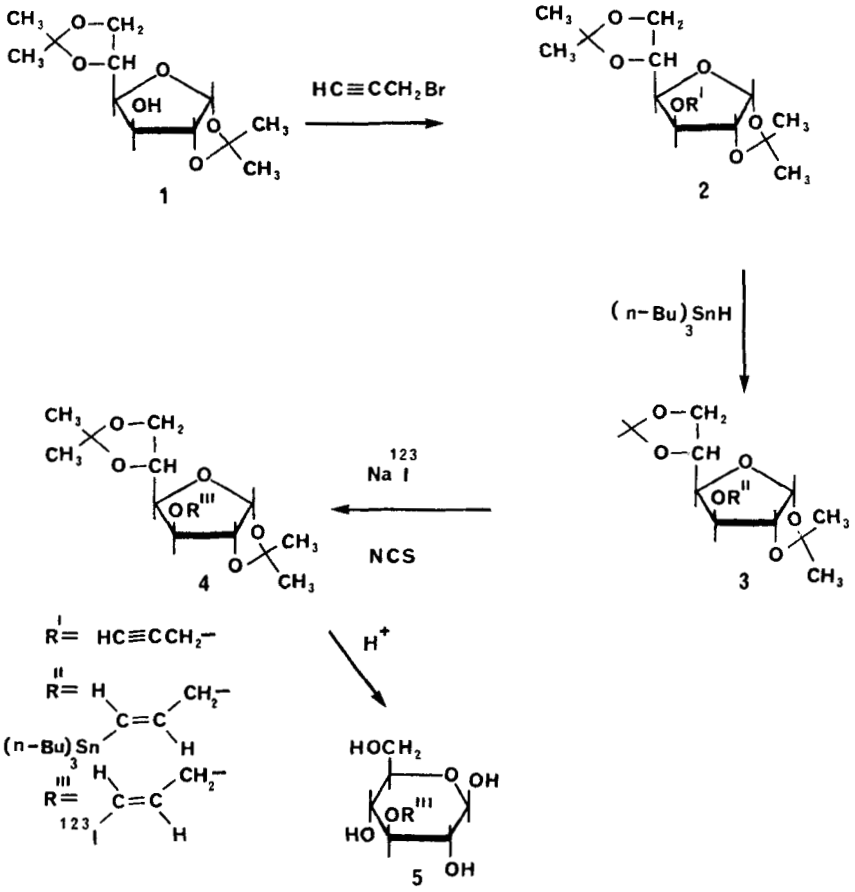
The carbohydrate analogues 2-FDG and CMG exhibit different biochemical behavior following transport into the brain. Once in the brain 2-FDG enters the glycolytic pathway and is phosphorylated by the enzyme hexokinase to 2-FDG-6-PO_4. Following the hexokinase reaction 2-FDG-6-PO_4 is not metabolized further nor returns to the blood resulting in prolonged cerebral retention. On the other hand, CMG does not enter the glycolytic pathway once in the brain but returns to the circulating blood. CMG concentrates and is retained in the brain for a time long enough to allow the measurement of CMG concentration in different brain regions.

The glucose derivative 3-O-propen-2-yl-D-glucose has an affinity Ki(10 mM)=25 for the protein carrier responsible for transporting sugars across cell membranes (3) similar to that of CMG Ki(10mM)=12. Because of the attractive radionuclidic properties of iodine-123, a single photon emitter (159 keV) with a 13.3 h half-life, radioiodinated analogues of 3-O-propen-2-yl-D-glucose may enable the study of perfusion and transport of glucose in patients with ischemic brain disease by SPECT. The goal of this study is to develop a synthesis of iodine-123 labeled 3-O-(E)-3-iodopropen-2-yl-D-glucose (5) as an analogue of CMG with the objective of achieving high brain and heart uptake.

Iodine-123 labeled 3-O-(E)-3-iodopropen-2-yl-D-glucose was synthesized in a 4 step sequence of reactions outlined in figure 1. The alkynyl sugar 3-O-propyn-2-yl 1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (2) was prepared in 80% yield from commercially available 1,2:5,6-di-isopropylidene-α-D-glucofuranoside by treatment with n-BuLi in THF followed by propargyl bromide. Hydrostannylation of 2 with tri-n-butyltin hydride/AIBN gave the key intermediate 3-O-(E)-(tributyltin)-propen-2-yl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (3) in 95% yield. Treatment of 3 with sodium iodide and NCS or I_2 in methylene dichloride gave 3-O-(E)-iodopropen-

2-yl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (4) in 67% and 90% yield respectively. Acid hydrolysis of 4 gave 3-O-(E)-3-iodopropen-2-yl-D-glucose (5). Iodine-123 labeled 5 was prepared in 60% yield from 3 by treatment with Na[I-123]iodide and NCS followed by hydrolysis. Tissue distribution of I-123 5 in rats will be presented.

Figure 1. Synthesis of Iodine-123 Labeled 3-O-(E)-3-Iodopropen-2-yl-D-Glucose



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SYNTHESIS OF IODINE-123 LABELED 4-O-(E)-3-IODOPROPEN-2-YL-2-DEOXY-D-GLUCOSE AND 4-O-(E)-3-IODOPROPEN-2-YL-D-GLUCOSE AS POTENTIAL BRAIN AND HEART IMAGING AGENTS

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Glucose is the primary energy source of the brain and also an important metabolic substrate for the heart under hypoxic conditions and for cell proliferation in tumors. Studies at a number of clinical institutions with in-house cyclotrons and positron emission tomographic (PET) devices have demonstrated that accurate diagnosis and management of disease can be achieved in patients with brain disorders, heart disorders and cancer using the positron emitting tracer fluorine-18 labeled 2-fluoro-2-deoxy-D-glucose (2-FDG). However, only a few clinical centers worldwide have in-house cyclotrons and PET devices. Thus, only a limited patient population can presently utilize diagnostic techniques available from 2-FDG.

Iodine 123 labeled glucose analogues which exhibit behavior similar to that of 2-FDG are attractive candidates for widespread clinical use since the single photon emitting radionuclide iodine-123 is commercially available and SPECT systems are routinely used at most clinical institutions.

Because of interest in providing the widespread availability of radiotracers which show behavior similar to 2-FDG, we report the development of the synthesis of iodine-123 labeled 4-O-(E)-3-iodopropen-2-yl-D-glucose and 4-O-(E)-3-iodopropen-2-yl-2-deoxy-D-glucose. The design of these glucose analogues as SPECT radiotracers was suggested by the work of Barnett and coworkers (1) who demonstrated in a general model for binding of sugars to the transport system which can traverse mammalian membranes that glucose derivatives substituted with 4-O-alkyl groups have an affinity for the carrier comparable to that of D-glucose.

The synthetic approach chosen for the preparation of 4-O-(E)-3-iodopropen-2-yl-D-glucose (8) (figure 1) involved the scission of a 3,4-anhydro sugar with propargyl alcohol. The propargyl substrate 4-O-propyn-2-yl-2-O-para-toluenesulfonyl-1,6-anhydro-ß-D-glucopyranoside (2) was prepared by treating 2-O-para-tolueneulfonyl-1,6-3,4-dianhydro-ß-D-glucopyranoside (1) with propargyl alcohol. Treatment of 2 with sodium methoxide gave 4-O-propyn-2-yl-1,6-2,3-dianhydro-ß-D-glucopyranoside (3). Acid hydrolysis of 3 followed by Ac_2O/pyridine gave the key intermediate 4-O-propyn-2-yl-1,2,3,4-O-tetraacetyl-ß-D-glucopyranoside (5). Hydrostannylation of 4 with $(n\text{-}Bu)_3SnH$ gave 4-O-(E)-3-(tributyltin)propen-2-yl-1,2,3,4-O-tetraacetyl-ß-D-glucopyranoside (6). Treatment of 6 with Na[I-123]I and NCS followed by acid hydrolysis gave I-123 labeled 8.

The synthetic approach for iodine-123 labeled 4-O-(E)-3-iodopropen-2-yl-2-deoxy-D-glucose (14) involved treatment of 3 with superhydride (figure 2) followed by the 5-step reaction sequence described above for 8. Evaluation of I-123 labeled 8 and 14 will be presented.

Research sponsored by NIH under contract GM-39081.

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Figure 1. Synthesis of Iodine-123 Labeled 4-O-(E)-3-Iodopropen-2-yl-D-Glucose

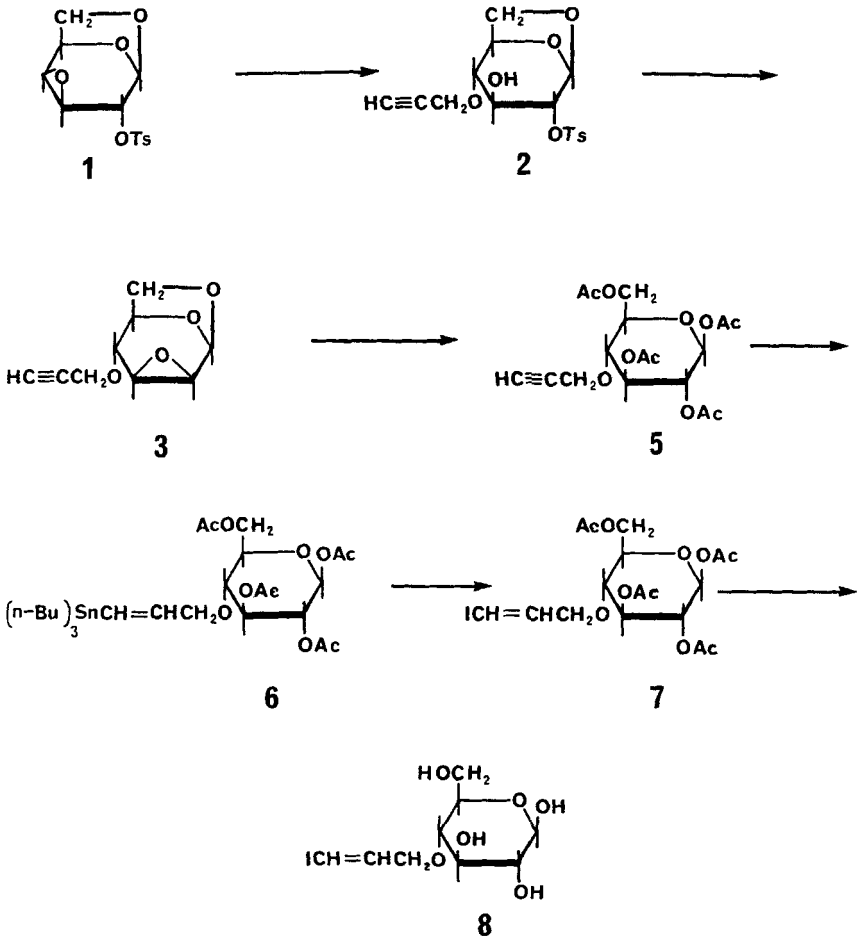
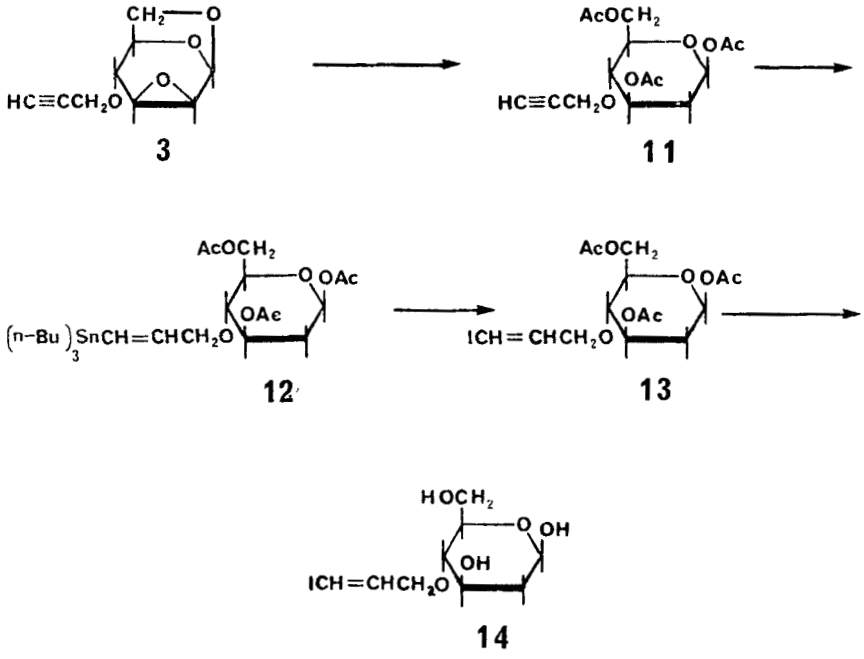


Figure 2. Synthesis of Iodine-123 Labeled 4-O-(E)-3-Iodopropen-2-yl-2-deoxy-D-Glucose



SEARCH FOR A NON-METABOLIZABLE PET TRACER FOR HEART NEURONAL IMAGING.

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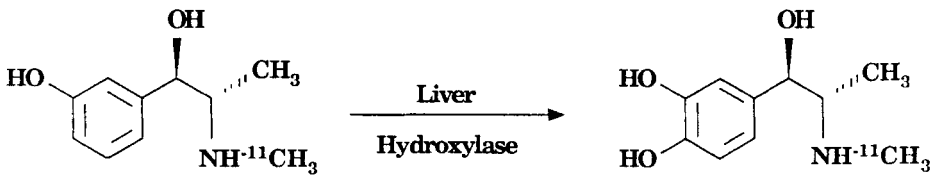
The tracer (1R,2S)-(-)-[^{11}C]-meta-hydroxyephedrine (MHED) is used successfully at our institution to study neuronal heart diseases and neuroendocrine tumors (1). MHED is a false neurotransmitter which is a substrate for neither MAO nor COMT. However, MHED is rapidly metabolized in humans (n=17), most likely to α-methylepinephrine and its 3-O-methyl ether by the initial action of liver microsomal hydroxylase (2,3). Fortunately, based on studies in guinea pigs, which is the animal model most predictive of primate metabolism, no metabolites of MHED appear in the heart. However, to optimize the accuracy of kinetic measurements in heart tissue, the temporal pattern of blood metabolites must be determined for each patient study. Our clinic operates two whole-body PET scanners so two patients are often scanned with MHED at the same time. Metabolic workup presently requires manual Sep-Pak chromatography of each blood sample. Because of the 20 min half-life of carbon-11, metabolic studies require rapid work by 2-3 diligent technicians in a scene not unlike Santa's workshop the week before Christmas. This presentation will describe our efforts to develop a [^{11}C]-labeled neuronal tracer that is completely resistant to metabolism on the PET-imaging time scale.



MHED
(1R,2S)-(-)-[^{11}C]meta-Hydroxyephedrine

α-Methylnorepinephrine

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DIRECT CHROMATOGRAPHIC ANALYSIS OF METABOLITES OF LIPOPHILIC TRACERS IN WHOLE BLOOD BY ISRP CHROMATOGRAPHY.

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High Performance Liquid Chromatography (HPLC) is frequently used in studies of metabolism of radioactive and non-radioactive compounds in whole blood and other biological samples. Because of the small particle size (< 10μ) of the majority of HPLC column packing materials, analyses of drugs and metabolites in whole blood must utilize a pretreatment process, which involves either separation of cellular material from plasma, or solvent extraction, or both. These pretreatment steps are undesirable. They restrict rapid analysis in time dependent studies; they may alter delicate equilibria; results may be perturbed due to different solvent extraction efficiencies of the hydrophobic drugs and their metabolites.

HPLC using columns packed with 5μ Internal Surface Reverse Phase (ISRP) resin have shown utility in the analysis of lipophilic drugs and their metabolites by direct injection of serum or plasma samples (1,2). We have developed a method for rapid on-line separation of small hydrophobic components from cellular whole blood components. This is achieved through the use of 75μ GFF (glycine-phenylalanine-phenylalanine) glass bead ISRP chromatographic material packed into a small HPLC column, and used in conjunction with an HPLC system containing a switching valve and a second analytical column (figure 1). When heparinized whole blood is applied to a 75μ GFF ISRP column, and the column eluted with an isotonic eluent, lipophilic compounds free in plasma are retained by the column, while plasma proteins, blood cells, and compounds bound to these blood components pass through the ISRP column. Following the elution of these components, the lipophilic compounds retained on the ISRP column are eluted by increasing the percentage of organic solvent in the eluent, and are further resolved (if required) by the analytical column.

We have applied this analytical method to the study of metabolism of the ^{99m}Tc-BATO (Boronic acid Adducts of Technetium diOxime) cerebral and myocardial perfusion tracers, previously described by Treher et al (3). The ^{99m}Tc-BATO complex was mixed with heparinized whole blood. At set periods, a 20-40 μL aliquot of the whole blood was injected onto the 50 x 4.6 mm glass bead GFF ISRP column, which was eluted with 0.1M ammonium citrate at pH = 5.0 at a flow rate of 1 mL/min. The column eluate was directed to the radiometric detector to quantitate the amount of radioactivity which is associated with the whole blood components. After 3.5 minutes, eluate from the column was redirected through a C_8 Nucleosil analytical column and to the radiometric detector. At this time, the solvent composition was changed through a linear gradient to 72:28 CH_3CN:0.1M ammonium citrate. This caused the removal of the free hydrophobic components from the ISRP resin, which were resolved on the Nucleosil column.

To compare results from the ISRP procedure with a conventional method of analysis, ^{99m}TcCl(DMG)$_3$2MP and ^{99m}TcCl(CDO)$_3$MeB were incubated in whole rat blood at

37°C, and the percentage bound to red blood cells and protein over time was determined both by the ISRP method (without the analytical column) and by centrifugation. Results are shown in figure 2. In both cases, the centrifugation method provides an essentially constant value at all time points, whereas the ISRP method demonstrates the uptake of tracer on red blood cells and protein at early time points. This difference is attributed to the long sample processing time (20-25 minutes) required by the centrifugation method compared to the rapid separation achieved by the ISRP method. For ^{99m}TcCl(CDO)$_3$MeB, the graphs demonstrate that the two methods provide similar values for equilibrium binding. However, in the study with ^{99m}TcCl(DMG)$_3$2MP, the centrifugation method demonstrates a lower equilibrium value than that obtained with the ISRP method, perhaps indicating that the processing required in the centrifugation method disturbs the equilibrium more than is the case with the ISRP method.

The following results were from ISRP/Nucleosil HPLC analysis of a sample of ^{99m}TcCl(DMG)$_3$2MP in whole human blood at 37°C.

Time (min) *	% bound to RBCs+protein	% chloro **	% metabolites		
			unidentified	% hydroxy***	unidentified
1	2.5	93.7	0.4	1.1	1.5
5	22.4	53.6	4.8	11.3	5.6
20	38.3	29.3	4.5	13.1	10.2
25	41.9	28.9	5.2	12.3	10.5
40	48.6	9.2	6.3	17.2	14.4

* (Duration of incubation of ^{99m}TcCl(DMG)$_3$2MP in whole blood)

** % Non-bound, unmodified ^{99m}TcCl(DMG)$_3$2MP.

*** % Non-bound, ^{99m}TcOH(DMG)$_3$2MP.

In whole human blood, the $t_{1/2}$ for loss of free ^{99m}TcCl(DMG)$_3$2MP is 10.4 minutes, with the generation of protein/RBC bound ^{99m}Tc, ^{99m}TcOH(DMG)$_3$2MP (by exchange of the axial chloro ligand by hydroxyl ion(4)), and at least two unidentified metabolites.

This novel HPLC method provides a rapid, convenient, and reliable method for the analysis of radioactive and non-radioactive lipophilic components in whole blood. Variation in the secondary analytical column, solvents, and detection system should allow this system to be used in many drug metabolism studies.

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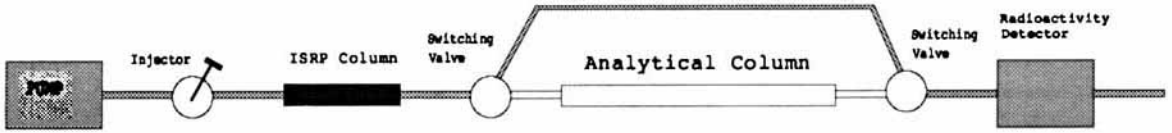
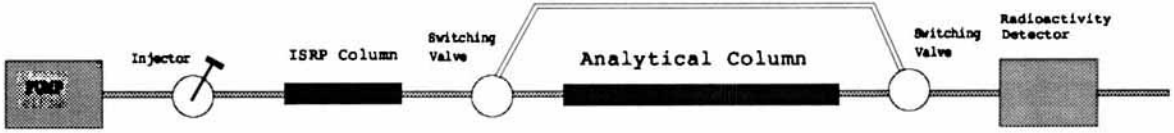
Figure 1. ISRP chromatography in the analysis of lipophilic components in whole blood.

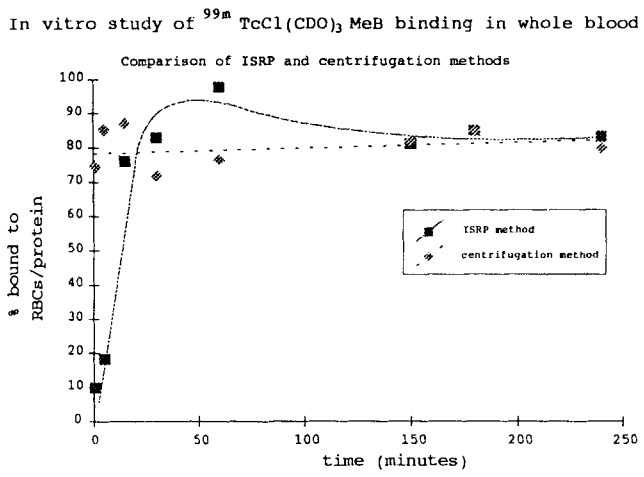


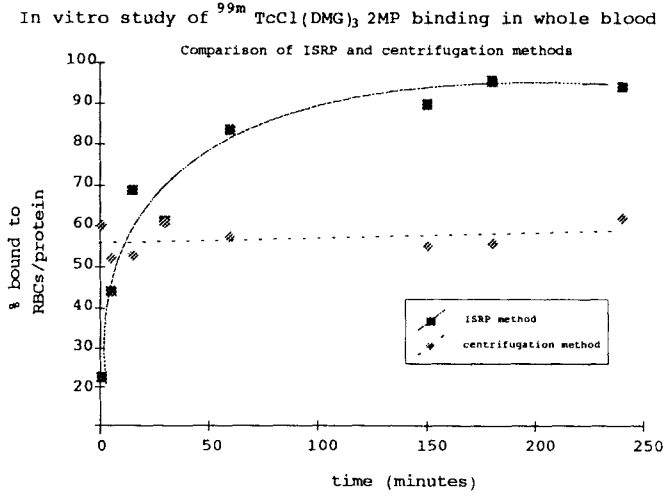
a. Initially, eluent flow proceeds directly from the ISRP column to the radioactivity detector. Free analyte is retained by the column, while analyte bound to blood cells and plasma proteins is eluted.



b. After all blood cells and proteins have been eluted, flow is redirected through the analytical column, increasing the organic solvent content to remove lipophilic components from the ISRP column, with subsequent separation of these components by the analytical column.

Figure 2. Comparison of the ISRP method with centrifugation





The Sequential Production of [^{18}F]-6-FDOPA and [^{18}F]-F-m-TYROSINE (or [^{18}F]-2-FDG) from the Same Dilute [^{18}F]F_2 Target Gas

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The recovery of [^{18}F] from our target is 65 ± 8% when 0.4% F_2 in neon is the target gas[1]. The fraction of [^{18}F] trapped in the reaction vessel during direct fluorination of aromatic compounds is dependent upon the solvent used for the reaction. For the fluorination of L-DOPA in HF/BF_3, the [^{18}F] trapped in the reaction vessel is 36 ± 4% whereas in other solvents it is 60 ± 5%[2]. The percent recovery of [^{18}F] is even lower for less activated aromatic rings such as ortho, meta and para-tyrosine in HF/BF_3 (12-16%). The aim of this work is to demonstrate how to utilize this unreacted, readily available, [^{18}F]F_2 for developmental work.

The experimental approach is to bubble the unreacted gas from the reaction vessel through a second vessel containing a different substrate in a suitable solvent. This way we are able to utilize almost 100% of the total radioactivity recovered from the target. This simple procedure is practical because, [^{18}F]F_2 does not exchange with the fluorine in HF or BF_4^-. We have used this method to produce, in sequence, mCi quantities of [^{18}F]-6-FDOPA and [^{18}F]F-m-TYROSINE (Scheme 1) and [^{18}F]-6-FDOPA and [^{18}F]-2-FDG (Scheme 2). The practical applications of this method are: 1. the synthesis and characterisation of new [^{18}F]-labelled compounds such as the metabolites of fluorodopa and fluoro-m-tyrosine. 2. the study of reactivity and orientation of direct fluorination of aromatic compounds in different solvents.

The reported [^{18}F]F_2 yield from the reaction ^{18}O (p,n) ^{18}F is 500-700 mCi after 30 min. irradiation with 11 Mev protons at a beam current of 30 uA[3]. We envisage that our sequential production scheme would provide us with large amounts of [^{18}F]F_2 for radiopharmaceutical production and for developmental work from a single irradiation of 0-18 target gas. By making use of this routinely produced, yet normally wasted, [^{18}F]F_2, we can save up to $30,000 per year based on the cost of target gas alone.

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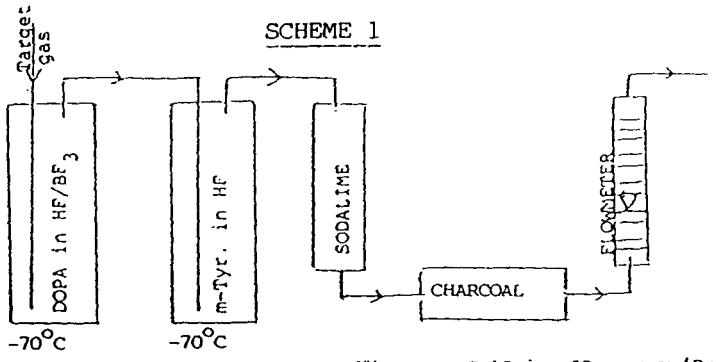
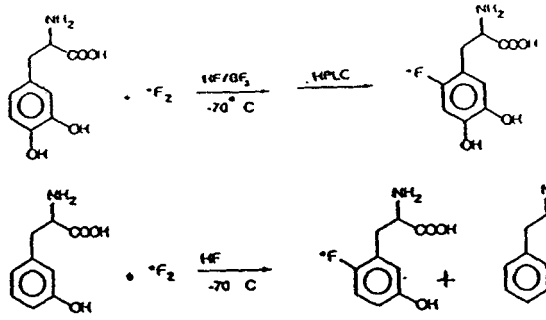
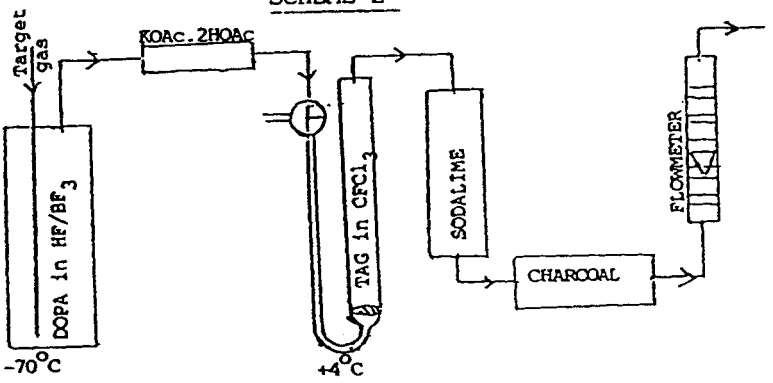
SCHEME 1

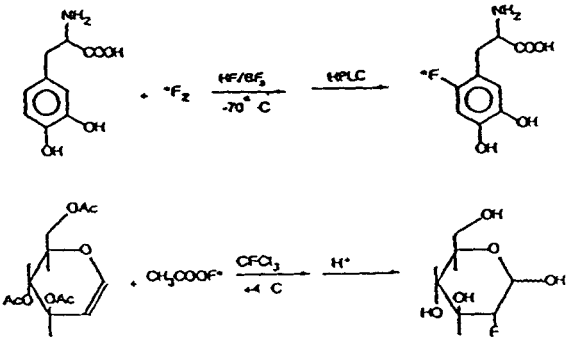




F-18 in Reaction Vessel	%Recovery From the Target	Product (mCi)	Synthesis Time(min)
55mCi	27	4.5	125
67mCi	33	4.1	165

SCHEME 2





F-18 in Reaction Vessel	%Recovery From the Target	Product (mCi)	Synthesis Time(min)
80mCi	39	6.7	130
17.4	8	6.1	90

F-18 in KOAc.2HOAc = 28.6 mCi (14% recovery from target

EVALUATION OF F-18-LABELLED DOPAMINE TRACERS USING *IN VIVO* MICRODIALYSIS.

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The preclinical pharmacokinetic evaluation of brain radiopharmaceuticals has involved the sacrifice of small animals at time intervals after injection followed by dissection and analysis of cerebral regions of interest (1). In larger animals such as monkeys and man, cerebral time-activity curves are obtained by PET while plasma samples are analyzed for metabolites and results are assumed to reflect cerebral metabolism. In some cases, dissecting and analyzing brains of monkeys sacrificed at different times has provided unequivocal information on the pharmacokinetics and metabolism of brain radiopharmaceuticals (2). With the recent development of *in vivo* microdialysis (3), a more efficient and easier method of evaluating brain radiopharmaceuticals is now available.

In vivo microdialysis involves the stereotaxic placement of small dialysis probes in one or more areas in a living brain which allows sampling the extracellular space. These microdialysis probes are continously perfused with artificial cerebrospinal fluid which permits small molecules to pass into or out of the semipermeable dialysis membrane as driven by concentration gradients. The perfusates are collected and analyzed using sensitive methods such as GC/MS or HPLC with electrochemical detector. For the radiochemical analysis required in this study, a unique β-γ-γ three-fold coincidence flow-through HPLC detector with subnanocurie sensitivity was recently developed in our laboratory.

We have used *in vivo* microdialysis in the evaluation of presynatic dopamine tracers in rat brains. The apparatus used in this study consisted of (a) a microinjection pump ($10^{-6} \leq$ flowrate $\leq$ 1ml/min), (b) a Kopf stereotaxic apparatus to place and hold the microdialysis probe (20 x 0.65 mm cannula, 3 x 0.52 mm membrane with 20 kilodalton cutoff) in proper position in brain, and (c) a mini-fraction collector driven by the preset volume of the pump. Ion pair HPLC (C18 150 x 4.6 mm, 3μ, 9:1 octylsulfonate, NaOAc, EDTA, n-BuNH_2:MeOH, 1.5ml/min) was used to analyze each fraction for the different [F-18]-labelled species.

[F-18]-6-fluoro-L-dopa was prepared using the method of Luxen et al. (4). About 700 μCi 6-FD was injected i.p. into an anesthetized rat pretreated with 0.75 mg/kg benserazide, a dopa decarboxylase inhibitor. The microdialysis probe was inserted, 90 minutes before 6-FD injection, into the right striatum (coordinates: AP= -0.26 mm, L= -3.2 mm, and V= -6.4 mm, with respect to bregma and dura surface based on Paxinos and Watson's "Rat Brain Atlas"). Perfusion rate was 80μl/min and 20-minute fractions were collected and analyzed by radioHPLC with β-γ-γ detector. The time course of the radioactivity in the perfusate (Figure 1) is consistent with results reported for L-dopa under similar experimental conditions (5). Each perfusion fraction contained less than a nanocurie of [F-18]. The radiochromatogram of fraction 6 (2 hr postinjection) shows 2 major peaks which elute close to the dopamine metabolites, DOPAC and HVA. After 2.5 hr postinjection, the animal was sacrificed and blood collected. The radioHPLC analysis of the plasma (ca. 50 nCi) show peaks we identifiy as 6-FD, 3-O-Me-6-FD, F-DOPAC, F-DA, F-HVA and a small amount of F-3-O-methyl-tyramine. These results show that metabolite distribution seen in the plasma is different from that in the striatal extracellular space. Further, this study shows the utility of *in vivo* microdialysis coupled with HPLC and β-γ-γ detection in the assessment of cerebral metabolism and pharmacokinetics of 6-FD in the rat. Studies involving uptake, storage, release and metabolism of presynaptic tracers can be performed using the appropriate pharmacological agents such as blockers and inhibitors of each metabolic step which can be introduced via the microdialysis probe. For post synaptic tracers, on the other hand, in addition to being able to assess cerebral metabolism and pharmacokinetics using fewer animals, the effect of the competing endogenous ligand can be simultaneously evaluated. We believe that the ultimate use of this technique may be in the final validation of any new brain radiopharmaceuticals wherein microdialysis is done while a non-human primate is being imaged with PET or SPECT.

We thank Drs. Weiler and Rudy of the UW School of Pharmacy for their assistance in setting up these microdialysis experiments. This research is supported in part by NIH Grant NS-26621 and the Wisconsin Alumni Research Foundation.

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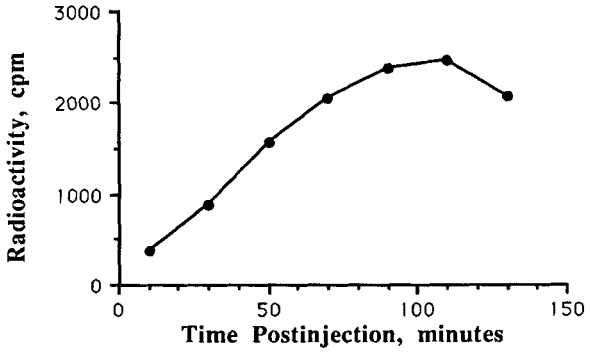

Figure 1. Time Course of F-18 radioactivity in microdialysis fractions after the injection i.p. of [F-18]-6-FD into a rat

A SINGLE COLUMN, RAPID QUALITY CONTROL PROCEDURE FOR 6-[^{18}F]FLUORO-L-DOPA AND 6-[^{18}F]FLUORODOPAMINE PET IMAGING AGENTS.

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6-[^{18}F]Fluoro-L-dopa and 6-[^{18}F]fluorodopamine are promising imaging agents for visualizing cerebral dopaminergic centers and cardiac sympathetic innervation and function. Administration of these radiochemicals to humans requires a means to determine the purity of these agents prior to injection. The quality control testing must also be rapid, because the half-life of ^{18}F is 109.8 min. Here we describe such a method using high performance liquid chromatography (HPLC) with UV and radioactivity detection using a single column. The procedure can resolve these fluorinated catechols, their isomers, and the amino acid precursor, dihydroxyphenylalanine (L-dopa) within 10 minutes. This allows the chemical and radiochemical purity, as well as specific activity, to be determined prior to the administration of the drug to humans.

The chromatographic separation is performed using a high speed C-18 column (4.6 x 50 mm, 3μm). The mobile phase buffer was prepared according to Eisenhofer et al. (1) and pumped using an acetonitrile gradient, as follows: phase A: 20% acetonitrile in buffer, mobile phase B: 0.5% acetonitrile in buffer. Prior to each analysis, the column was equilibrated at 100% B for a minimum of 10 min @ 2 mL/min. The flow was reduced to 1 mL/min for 5 min and the sample injected. One minute after injection a linear gradient from 100% B to 0% B over 2.5 min was commenced. Dopa, 2-fluorodopa, and 6-fluorodopa eluted at 2.00, 2.40, and 2.68 min. Then, elution at 100% A was maintained for seven minutes to effect the elution of the 2 and 6-fluorodopamine, which eluted at 5.53 and 5.77 min. Equilibration at 2 mL/min 100% B for 10 min resulted in reproducible retention times in subsequent analyses. The entire elution was complete in less than 10 min. Monitoring the UV absorbance at 220 nm provided chromatography with equivalent response factors while still maintaining high sensitivity for these analyses. The method reproducibly detected 6 ng on column. Using 10 μL of final product with a specific activity of 200 mCi/mmol, the mass on column would be three orders of magnitude greater than the limit of detection for either of the radiopharmaceutical products.

This method offers the advantages of speed, resolution and efficiency. The short analysis time and high resolution of the fluorinated catechols makes it an excellent method for rapid quality control analysis.

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DO THE METABOLITES OF 6-[F-18]FLUORO-L-DOPA AND OF [F-18]FLUORO-META-L-TYROSINE CONTRIBUTE TO THE F-18 ACCUMULATION IN THE HUMAN BRAIN?

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In man, F-dopa is metabolized peripherally by dopa decarboxylase to fluoro-dopamine and by catechol-O-transferase to 3-O-methyl-6[F-18]fluoro-L-dopa (OMeFdopa) (1,2). OMeFdopa, like F-dopa itself, is thought to enter the brain from the blood. Therefore, the total F-18 accumulation in the brain may not only reflect the dopaminergic function but also the unspecific retention of OMeFdopa. Kinetic analysis of F-18 accumulation-time curves is complicated because of the additional feed.

FmLtyr is not expected to be a substrate of catechol-O-methyl transferase. In the periphery, it is quickly metabolized by the subsequent action of dopadecarboxylase and monoamine oxidase to 3-hydroxy-[F-18]fluoro-phenylacetic acid (FHPAA) which is the major metabolite in the blood.

We set out to determine to what extent these metabolites contribute to the accumulation of F-18 in the human brain.

Syntheses: Our general electrophilic radiofluorination process with [F-18]fluorine gas in hydrogen fluoride as solvent was used to prepare F-dopa, FmLtyr, OMeFdopa and FHPAA. The compounds were isolated by HPLC. The new fluorinated compounds were identified by mass spectrometry and by their H-1, C-13 and F-19 NMR spectra (3,4).

PET Studies: On separate occasions 3 mCi doses of FmLtyr, F-dopa, OMeFdopa and FHPAA was given i.v. to a healthy member of the laboratory staff. The F-18 accumulation was measured up to 2 hours with a single ring positron tomograph with a spatial resolution of 8 mm. The amount of F-18 in each region of interest was expressed as the average cpm/pixel within the region normalized to the number of mCi of F-18 injected. The time accumulation-curves are given in Fig. 1 and 2. The metabolite of F-dopa, OMeFdopa, enters all regions of the brain; on a per-mCi-basis, to about 1/2 that of F-dopa. The metabolite of FmLtyr, FHPAA, accumulates only to 1/20 of that of FmLtyr.

Conclusion: F-18 accumulation-time data in the human brain, created by the application of FmLtyr, are not "contaminated" by its F-18-carrying major peripheral metabolite. This is in contrast to F-dopa.

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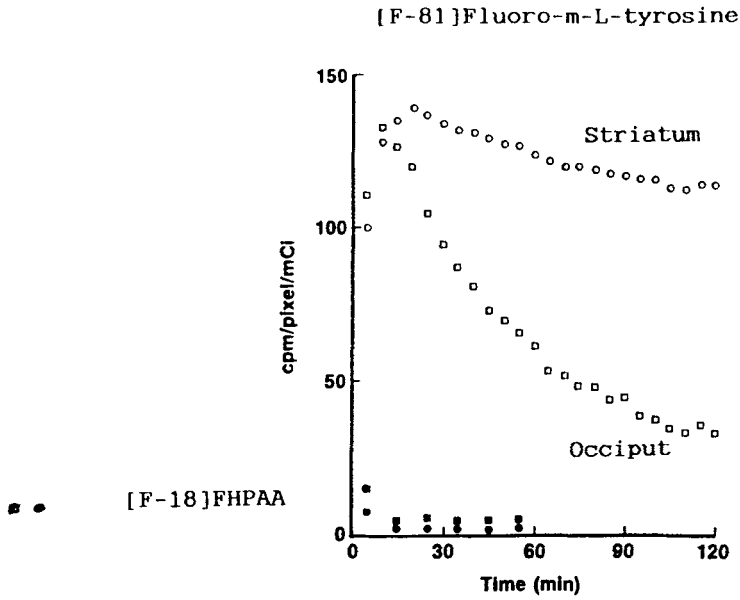

Figure 1

Time course of ^{18}F in the human striatum (o) and occiput (□) after i.v. injection of FmLtyr (open symbols) and FHPAA (closed symbols).

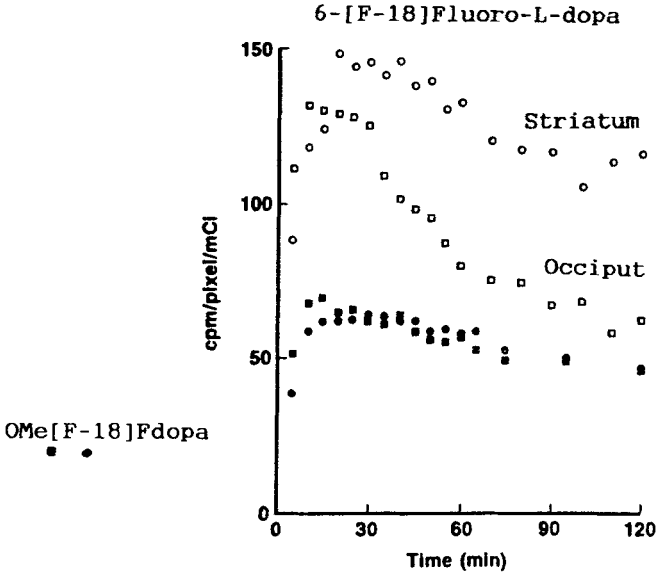

Figure 2

Time course of ^{18}F in the human striatum (o) and occiput (□) after i.v. injection of Fdopa (open symbols) and OMeFdopa (closed symbols).

SYNTHESIS AND EVALUATION OF FLUORINE-18 LABELED 4-O-3-FLUOROPROPYL-D-GLUCOSE AND 4-O-3-FLUOROPROPYL-2-DEOXY-D-GLUCOSE: POTENTIAL AGENTS FOR THE ASSESSMENT OF LOCAL GLUCOSE TRANSPORT AND PERFUSION IN THE BRAIN AND HEART

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The accepted mechanism for the transport of sugars across mammalian membranes involves the formation of a sugar-protein "carrier" complex which can traverse the membrane. The sugars are believed to bind to specific sites within the protein by hydrogen bonds. The structural and binding requirements for transport of sugars across mammalian membranes are strict. In general all of the hydroxyl groups must be in the trans-glucose configuration. More specifically groups (O and F) which can form a hydrogen bond from the protein "carrier" to the sugar must be present at sugar positions 1,3,4 and 6.

Carbon-11 labeled 3-O-methyl-D-glucose (CMG) is a glucose analogue with a high affinity Ki(10mM)=12 for the protein "carrier" which transports glucose Ki(10mM)=6.3 across the blood brain barrier but which does not enter the glycolytic pathway (1). CMG shows rapid brain and heart uptake (2) and has been shown to be a valuable agent for the study of ischemic brain disease (3). 4-O-propyl-D-glucose is an analogue which has an affinity Ki(5mM)=10.2 for the protein 'carrier' comparable with that of glucose, but has not been investigated as an agent to evaluate glucose utilization in the brain.

This study was undertaken to synthesize and evaluate in laboratory animals both by tissue distribution and imaging studies two model agents, fluorine-18 labeled 4-O-3-fluoropropyl-D-glucose and 4-O-3-fluoropropyl-2-deoxy-D-glucose. These agents may prove to be more advantageous than CMG for clinical applications, since unlike carbon-11 t1/2=20 min, the relatively long t1/2=110 min of F-18 will allow a single batch production to serve numerous patients.

The synthetic approach for preparation of 4-O-3-fluoropropyl-D-glucose (8) entailed a six step sequence of reactions delineated in figure 1. The starting material 1,6-2,3-dianhydro-2-O-para-toluenesulfonyl-ß-D-glucopyranoside (1) was treated with 3-chloropropan-1-ol to afford 1,6-anhydro-4-O-3-chloropropyl-2-O-para-toluenesulfonyl-ß-D-glucopyranoside (2). The key iodo substrate 4-O-3-iodopropyl-1,2,3,6-tetraacetyl-ß-D-glucopyranoside (5) was prepared by treatment of 2 with sodium methoxide followed by reflux with a H+ resin, Ac_2O/pyridine and NaI/CH_3CN. Fluorine-18 labeled 8 was prepared in an automated synthesis unit (CPCU, CTI Cyclotron Systems, Berkley Calif.) in 50% yield (EOB) by a F-18(NCA) fluoride ion/CH_3CN/K2.2.2. for I substitution of 5 followed by acid hydrolysis.

Fluorine-18 labeled 4-O-3-fluoropropyl-2-deoxy-D-glucose (14) was prepared in a similar manner (figure 2) in 50% yield (EOB) by treatment of 1,6-2,3-dianhydro-4-O-3-chloropropyl-ß-D-glucopyranoside (3) with lithium aluminum hydride followed by the four step sequence

of reactions described above for 8. Tissue distribution and imaging studies of F-18 labeled 8 and 14 will be presented.

Research sponsored by NIH under contract GM39081.

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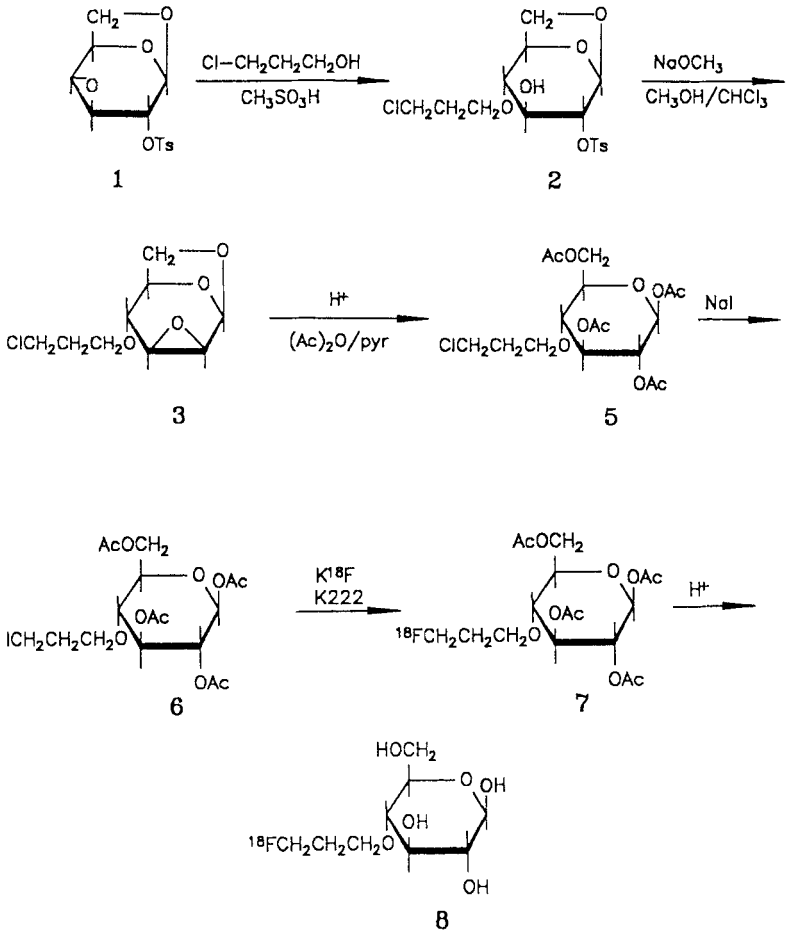

Figure 1. Synthesis of Fluorine-18 Labeled 4-O-3-Fluoropropyl-D-Glucose

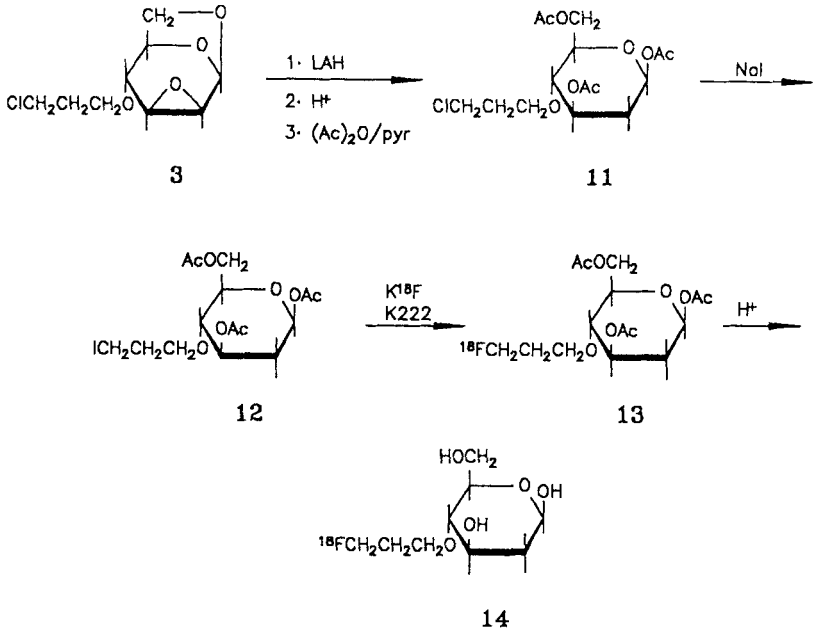

Figure 2. Synthesis of Fluorine-18 Labeled 4-O-3-Fluoropropyl-2-deoxy-D-Glucose

SYNTHETIC METHODS DIRECTED TOWARD A PRACTICAL RADIOSYNTHESIS OF 6-[C-11]-D-GLUCOSE.

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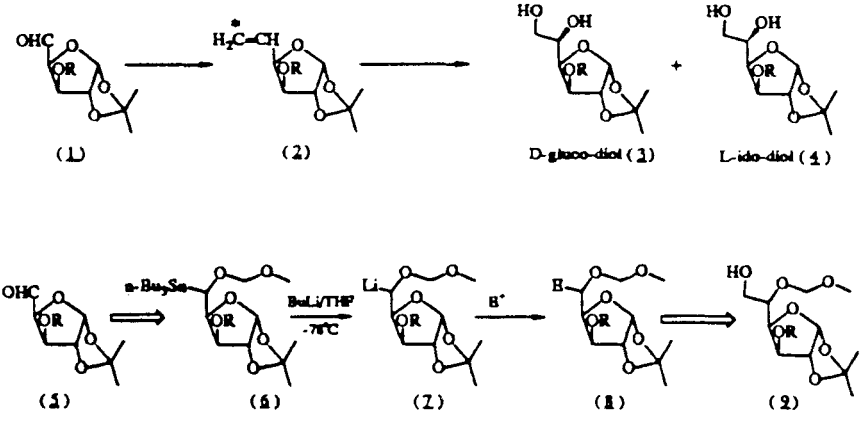
As part of our interest to in the differential utilization of 1-[C-11]- and 6-[C-11]-D-glucose, to study the pentose shunt in normal and tumor tissues, we sought a practical radiosynthesis of the 6-[C-11] compound. Previously, we presented the development of a rapid method for the highly selective synthesis of 6-[C-11]-D-glucose over the compound with opposing absolute stereochemistry at C-5, L-idose[1]. A practical chromatographic method for the separation of these two diastereomers at an early stage of the synthesis was also established. The synthetic studies involved the radiosynthesis of the alkene (2), via a labeled Wittig reagent. Attempts to improve the poor radiochemical yield of the alkene (15%, EOB) have been unsucessful to date. A concurrent effort to overcome these shortcomings has investigated the use of configurationally stable α-alkoxy-organolithium reagents[2] with labeled CO_2 and CH_2O or their equivalents. We have prepared a mixture of α-alkoxy-stannanes (6) from which the requisite stereo-defined compound for glucose synthesis can be isolated. Treatment of these stannanes with 1 equiv. of n-BuLi in THF at low temperature produces α-alkoxy-organolithium reagents (7). The mixture of the resultant carbanionc reagents reacts efficiently with chlorotrimethylsilane to afford a mixture of the corresponding α-alkoxy-silanes. In a completely analogous fashion, treatment of the stannanes (6) with n-BuLi, followed by carbonation with CO_2 and [C-11] CO_2 and subsequent reduction (LAH) of the putative (carboxylate) intermediate has not afforded the desired alcohols (9). The course of events during these carbonations is under investigation to circumvent this unexpected result. Results of a similar nature will be presented concerning the alternate use of formaldehyde and equivalent reagents.



This work was supported in part by USPHS grant CA42045.

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Supporting Data: SYNTHESIS OF IODOVINYLMISONIDAZOLE FOR IMAGING HYPOXIA JE Biskupiak

Cell line	FMISO	IVM
RIF-1	27.3	25.6
EMT-6	12.6	10.4
V-79	27.6	13.7
CaOs-1	21.1	9.1
Rat Myocytes	20.3	22.0

ANOXIC:OXIC UPTAKE RATIOS

Uptake ratios in four tumor cell lines and rat myocytes (a nonmalignant tissue) were determined. The cells were rinsed and covered with a thin layer of medium containing either 50 μM of [H-3] FMISO or [I-131] IVM. The cells were exposed to the desired oxygen concentrations (oxic: 95% air/5% CO_2 or anoxic: 95% N_2/5% CO_2) using the thin film culture techniques of Koch.(1) Uptake of radiolabeled drug was accomplished by incubation for 3 hrs at 37°C. The medium was removed and the cells were rinsed twice with cold medium to remove unbound labeled drug. The tumor cells were harvested by trypsinization and a proportion of the total sample counted. The plates containing the rat myocytes were scraped and the cell material counted.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF ESTERIFIED RADIOIODINATED GLUCOSE DERIVATIVE.

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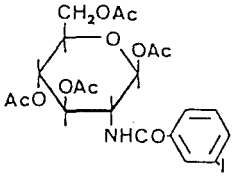
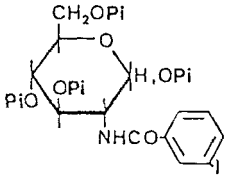
Recently, we have developed the N-(m-iodobenzoyl)-D-glucosamine (BGA), a radioiodinated glucose derivative with ability to inhibit the phosphorylation reaction of hexokinase(1). In the present work, to improve the permeability of BGA through the blood-brain barrier, two BGA prodrugs, the 1,3,4,6-tetra-O-acetyl-BGA (ABGA), and 1,3,4,6-tetra-O-pivaloyl-BGA (PBGA)(Fig.1) were synthesized by esterification with acetyl group and pivaloyl group, respectively. Their biological functionality were investigated by in vitro studies (lipophilicity and enzymatic cleavage) followed by in vivo experiments.

Fig. 1 Chemical structure of esterified glucose derivative



Ac ; CH_3CO-

1,3,4,6-tetra-O-acetyl-N-(m-iodobenzoyl)-glucosamine (ABGA)



Pi ; $(CH_3)_3CCO$-

1,3,4,6-tetra-O-pivaloyl-N-(m-iodobenzoyl)-glucosamine (PBGA)

Esterification of BGA was carried out by acetyl chloride (ABGA) and pivaloyl chloride (PBGA), respectively, in a mixture of THF and pyridine; chemical characterization performed by elemental analysis and NMR. Radioiodination (I-125) carried out by isotopic exchange reaction in the presence of divalent Cu as catalyzer with radiochemical yield of more than 80 %, followed by the purification on silicagel column chromatography (solvent, chloroform:ether=4:1). The radiochemical purity of both labelled compounds was more than 97 % as determined by TLC (solvent, chloroform:methanol=7:3).

The lipophilicity of both compounds were assayed by octanol-phosphate buffer extraction. The partition ratio of ABGA and PBGA was 400-900 times higher than that of BGA (Table 1); these were clearly reflected on an increase of the Brain Uptake Index(2) value, compared with that of BGA. The enzymatic cleavage of both

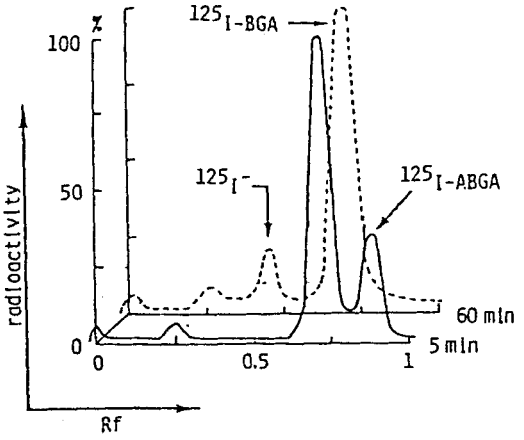
Table 1 Partition ratio of ^{125}I-ABGA and ^{125}I-PBGA between octanol and phosphate buffer (pH 7.4).

^{125}I-BGA	^{125}I-ABGA	^{125}I-PBGA
0.47(0.05)*	193.8(5.1)	481.3(153.2)

* Each value represents mean(s.d.) for 3 trials.

esterified compounds was assayed with pig liver esterase; ABGA was converted into BGA in only 5 minutes incubation, while PBGA remained unchanged for 3 hours. In the biodistribution studies in normal mice, the radioactivity of both compounds in brain showed higher uptake and longer retention than that of BGA. Then, the analysis of brain homogenate was carried out by TLC(Fig.2). As also observed in vitro, at 5 min post injection, only I-125-ABGA released free I-125-BGA, while no changes were detected with I-125-PBGA.

Fig. 2 Brain homogenate analysis of ^{125}I-ABGA in normal mice.



* Analyzed by TLC (silicagel plate; $CHCl_3$:MeOH = 7:3).

In conclusion, permeability of BGA was enhanced by the esterification and above all, ABGA function as a drug delivery of BGA into the brain was demonstrated.

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6-[^{18}F]FLUORO-L-DOPA KINETICS WITH PET: CORRELATION WITH ITS BIOCHEMICAL METABOLISM IN NORMAL AND MPTP-TREATED MONKEYS.

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6-[^{18}F]Fluoro-L-DOPA(FDOPA) is a functional analog of L-DOPA that is used to assess the integrity of the dopaminergic system in vivo with PET (1). Characterization of FDOPA metabolites generated from its peripheral and brain metabolism is necessary to develop tracer kinetic models for valid interpretation of FDOPA/PET studies. This biochemical information is essential for comparison of normal with diseased states to detect metabolic changes that could affect the validity of the tracer model. Accordingly, we have examined FDOPA biochemistry in both normal and MPTP-treated monkeys in conjunction with FDOPA/PET studies.

After initial FDOPA/PET scans, two monkeys received intracarotid injections of MPTP to effect ipsilateral striatal lesions. Approximately 8 months later, the monkeys received a final FDOPA/PET scan and were sacrificed the next day, 60 min after another FDOPA injection. Control monkeys received a similar FDOPA/PET study and FDOPA injection prior to sacrifice. Brains were removed within 30 min; putamen, cerebellum and cortex were dissected on ice and analyzed by HPLC for both endogenous catecholamines and FDOPA metabolites (2). Time-total activity curves were generated from PET data. Comparison of pre- versus post-MPTP PET scans for the same monkey revealed a 40-50% reduction in total activity for the lesioned striatum of MPTP treated monkeys at 60 min. This was comparable to the approximately 50% reduction in total counts observed in biochemical analysis of the lesioned putamen. Total radioactivity in the putamen of both normal and non-lesioned striatum/MPTP treated monkeys was distributed as follows:3-O-methyl-6-[^{18}F]fluoro-L-DOPA(3-OMFD), 35%; 6-[^{18}F]fluorodopamine (FDA), 35%; [^{18}F]fluorohomovanillic acid (FHVA), 15%; and the remaining 5-10% was FDA sulfate, 3,4-dihydroxy-6-[^{18}F]fluorophenylacetic (FDOPAC), and FDOPA. In contrast, for MPTP treated monkeys the 3-OMFD metabolite accounted for 75%, and FHVA for 10% of the total putamen activity; FDA and FDOPAC were not present in significant amounts. The (FHVA + FDOPAC)/FDA ratio, indicative of FDA turnover, for controls was 1:2; however, for MPTP-treated monkeys, significant levels of FHVA plus the very low levels of FDA resulted in a ratio of > 6:1, suggestive of increased turnover of FDA.

Thus, from biochemical data it can be established that FDA accumulation in MPTP-lesioned putamen was less than 5% that of control animals when the 3-OMFD contribution is subtracted. Non-invasive evaluation of turnover changes observed from biochemical analyses are now being studied with tracer kinetic models sensitive to clearance changes detectable with PET.

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[^{18}F]-2-FDG AS A TOOL FOR STUDYING HEXOKINASE KINETICS.

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In the basic research related to the development of radiolabelled glucose analogues or to sugar metabolism, the measurement of hexokinase kinetics is very important. Until now those kinetics are carried out by means of spectrophotometric methods based on a two step enzymatic reaction : 1° phosphorylation by the enzym hexokinase, 2° reaction of the phosphate with NADP catalysed by glucose-6-phosphate dehydrogenase to yield NADPH.

In the spectrophotometric methods the formation of NADPH as a function of time is directly or indirectly measured and is supposed to reflect the hexokinase kinetics occuring in 1°. When it is the purpose to study the interaction of compounds with hexokinase by means of the spectrophotometric method, a simultaneous interaction with glucose-6-phosphate dehydrogenase can not be ruled out without applying differential kinetic measurements.

The article of S.J. Gatley et al (1) about the quality control of [^{18}F]-2-FDG preparations using the hexokinase reaction in vitro was the basic idea of the method proposed in this paper dealing with the direct measurement of hexokinase kinetics by the measurement of the activity related to [^{18}F]-2-FDG-6-phosphate. [^{18}F]-2-FDG was prepared using the method of Hamacher et al (2).

For each essay the compounds of interest were incubated together with [^{18}F]-2-FDG in appropriate reaction conditions. At different times a 100 µl aliquot was transferred to a mini anion exchange column (400 mg Dowex 1X8, Cl^- form in a 4 mm Ø column), which was immediately rinsed with 4 ml of H_2O. The activity on the resin (suspended in 4 ml) and eluate was measured. The % of [^{18}F]-2-FDG -6-phosphate is expressed as the ratio of the activity on the resin to the initial activity in the aliquot (brought to 4 ml).

Hexoses that are greedy substrates for hexokinase can be present in the radiopharmaceutical preparation. Their amount was estimated by spectrometry using a Lineweaver-Burk plot as a standard curve.

Each [^{18}F]-2-FDG essay contained about 10^{-7} moles of glucose. The ion exchange column retained up to 2.10^{-5} moles of glucose-6-P quantitatively and the ion exchange process was shown not to be disturbed by the presence of molecular hexoses and by the organic compounds studied.

When plotting ln(100-%) as a function of time, linear curves were obtained for the reference glucose as well as in the presence of competitive and non competitive inhibiting compounds (decreased slopes) showing that this method is appropriate for hexokinase studies.

The authors thank FGWO (grant 3.0099.89) and Mallinchrodt Diagnostica Holland BV for financial support.

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An Automated System for the Synthesis of L-6-[^{18}F]Fluorodopa

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TRIUMF - University of British Columbia, Vancouver, B.C., Canada V6T 2A3

The PET chemistry group at TRIUMF has completely automated the synthesis of L-6-[^{18}F]Fluorodopa. This was done to reduce the exposure of lab technicians to radioactive and corrosive chemicals used in the process. It also improves reliability and reproducibility of the synthesis.

The chemical synthesis is performed using the fluorodemercuration reaction as previously described (1).

The automation is controlled by an IBM Personal Computer AT running the MS-DOS Operating System. The software for the synthesis was written using the Microsoft QuickBASIC Compiler. Graphic display features were created using the Connell Scientific Graphics EGA Toolkit.

Hardware for the synthesis is composed of standard Teflon tubing and fittings, glass vessels, and other chemistry apparatus installed in a fume hood. Gases and fluids are transferred between vessels through electromechanical valves using vacuum and air pressure.

A customized rotary evaporator is used to heat and evaporate solutions. The resulting product is removed from the rotary evaporator by rotating the entire assembly and transferring the fluid to an HPLC injector using air pressure (2).

Purification of the product is accomplished by reverse phase HPLC. A radioactivity detector monitors the eluant activity and automatically routes the product to a sterile multi-injection vial.

The valves, rotary evaporator, heater, and HPLC system are interfaced to the IBM PC using the OPTO-22 OPTOMUX control system. This system uses optically isolated assemblies to control and monitor various analog and digital signals. It uses a serial communication protocol to link all the assemblies to the IBM PC.

The software is composed of a series of routines to sequence through the required steps of the synthesis. The length of time for each step is adjustable and the terminating conditions can be changed by the operator. A graphic display mimics the process to give the operator a good understanding of the state of the process. The operator does not have to approach the fume hood to monitor the process. The radioactivity monitored in the HPLC eluant is plotted on the screen, and the total radioactivity is calculated.

For setup and testing the system, an interactive control version of the program was developed. A mouse is used to select a valve and change the state of the valve. The other controllable devices, such as the heater and HPLC, can also be activated using the mouse.

Data is logged by the system to record the results of each step in the process. The radiochromatogram is recorded for later processing, display and record keeping.

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SUB-NANOCURIE HPLC ANALYSIS OF LABELLED METABOLITES WITH A FLOW-THROUGH β^+–DETECTOR.

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The need for a low background level, flow-through detector is crucial for HPLC analysis of nCi samples that arise in clinical work (eg. blood borne metabolites of 6-(18-F)-fluoro-L-DOPA) and in basic research involving microdialysis of animal preparations. The figure of merit to optimize is given by $\varepsilon/B^{1/2}$, setting the balance point between sacrificing efficiency ε in order to reduce the bacground B. We have invoked the three-fold coincidence (the positron and its annihilation gammas, β–γ–γ) with fast (16 ns FWHM) electronics and efficient detectors in order to reduce the background rate to $10^{-2}>B>10^{-6}$ cps. The corresponding efficiency , $11\% > \varepsilon > 2\%$ is then determined by the lower level thresholds on the β and γ channels, shown in Figure 1.

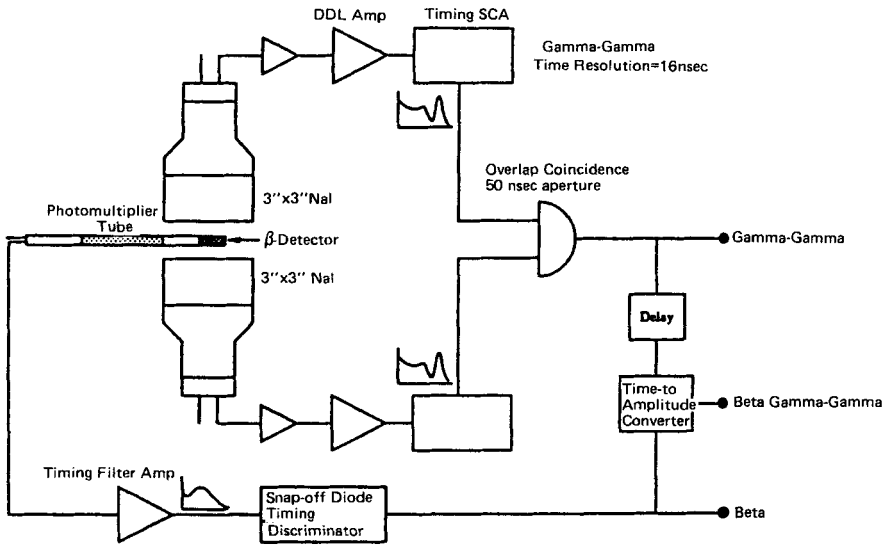

Fig 1

The β-detector is a spiral-wound tube of BC-400 plastic scintillator (60 cm long, 1/16" OD, 1/32" ID, internal volume 300 μL) potted into a flat disc (14 mm thick) and optically coupled to a RCA 4516 PM tube. This disc is sandwiched between two heavily shielded (400 kg Pb) 3"x3" NaI detectors , with the overall efficiency limited by the solid angle achievable. For the beta detector the integration of the energy distribution above the lower threshold intends to exclude the background noise, namely the single and multiple electron emission from the photocathode, influenced by the photomultiplier temperature. The extent

to which this exponentially decreasing bacground intrudes into the actual beta spectra depends on the light yield , the effectivness of the optical coupling , the photofluorescent decay constant of the organic scintillator and the integration time of the timing electronics processing the beta detector's signal . Experimental beta efficiencies were 50% for F-18 and 70% for N-13.

The performance of the system was excercised over a 7 decade dynamic range by measuring the decay of aqueous F-18 in the detector over a period of 4 days, shown in Figure 2. The lower level discriminators for the NaI detectors were set at 50 keV, and for the beta detector at 30 keV.

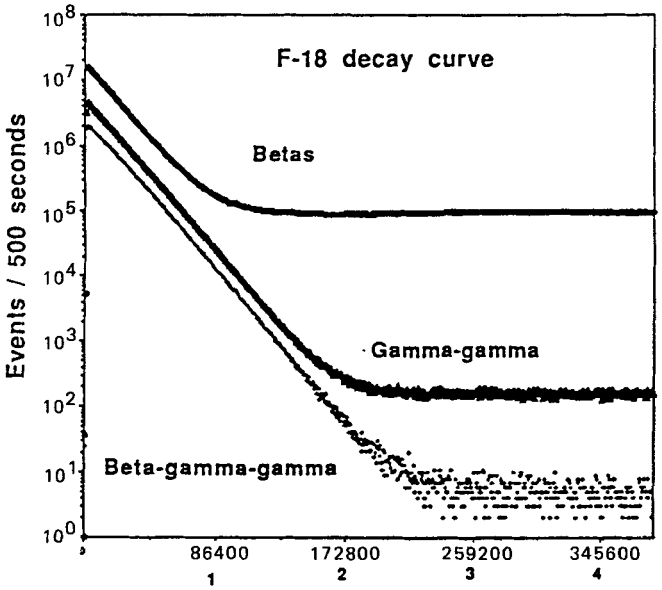

Fig 2

The chromatographic resolution demands careful attention to the flow paths , minimizing dead volumes and matching the detector volume to the sampling volume and column resolution. In Figure 3 an actual chromatogram is shown of microdialysis eluate from rat brain (0.8 nCi F-18 labelled metabolites) injected on a HPLC column . The effect of the successive coincidence criteria on the improvement in $\varepsilon/B^{1/2}$ can be seen in Fig 3, as the background recedes to reveal the metabolite signal previously buried in the noise. Future developments will center on the use of larger gamma detectors to improve the solid angle and subsequent system efficiency.

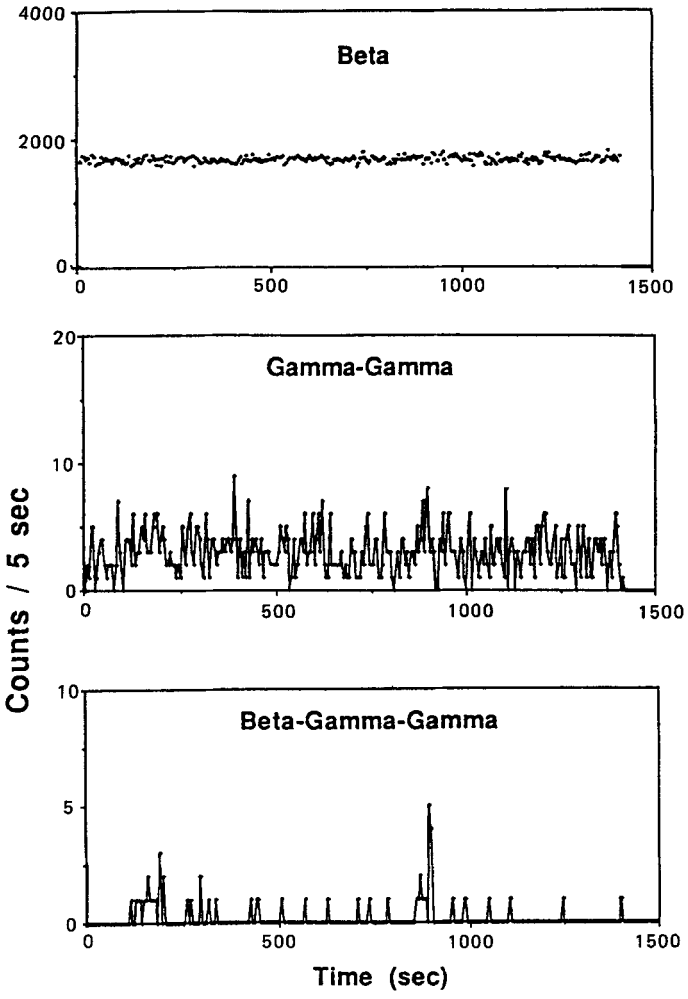
Beta
4000
2000
0
0
500
1000
1500
Gamma-Gamma
20
10
0
Beta-Gamma-Gamma
10
5
0
Counts / 5 sec
Time (sec)

Fig. 3

COMPARISON OF Tc-99m LABELED ANTIBODY Fab' CHELATOR CONJUGATES HAVING MONO, DI, AND TRIESTER LINKAGES.

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Centocor, Inc. Radiopharmaceutical Research and Development,
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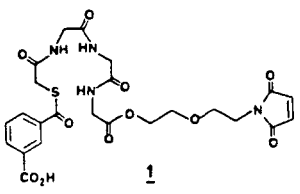
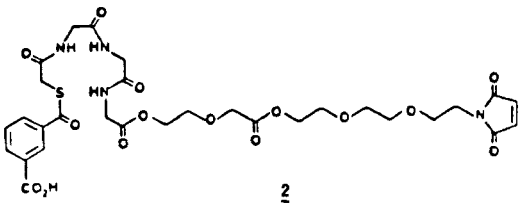
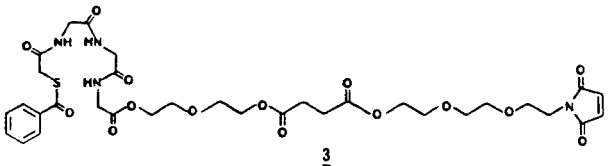
We have developed a bifunctional chelator for labeling antibody Fab' fragments based on the N_3S core of mercaptoacetyltriglycine in which the last glycine is linked through an ester group to an N-alkylated maleimide (1). The presence of the ester group resulted in a labeled conjugate having enhanced clearance through the renal system. In order to assess whether the presence of additional ester functions in the linking group would further enhance clearance of the radiopharmaceutical, bifunctional chelators having di (2) and tri (3) ester functions were synthesized. The chelators were site-specifically attached to the sulfhydryls of Fab' fragments through the maleimides. After deblocking of the ligand sulfhydryl, the conjugates were labeled with Tc-99m by ligand exchange. The radiolabeling was quantitative after 30 minutes, purification was not necessary, and the labeled conjugates remained immunoreactive. Upon incubation in fresh human serum at 37°C for 18 hours, 12% of the radioactivity was no longer protein bound to the monoester, 75% was lost from the diester, and 43% was lost from the triester, presumably due to hydrolysis. The differences observed in serum stability were not apparent in mouse biodistribution studies. All three of the Tc-99m labeled ester linked conjugates exhibited more rapid kidney clearance than an amide linked Tc-99m Fab' conjugate; however, the differences between the ester linked conjugates were not statistically significant. The similarities between the different ester linked Tc-99m conjugates held for the other major organs as well. Thus, the presence of additional ester groups in the linking moiety of the Tc-99m labeled Fab' chelator conjugates had no significant effect over that of a single ester on the in vivo clearance of the radioactivity in mice.



1



2



3

THE EFFECT OF CONJUGATION OF MONOCLONAL ANTIBODY WITH ELECTRONEGATIVELY CHARGED POLYMERS ON IMMUNOREACTIVITY. TST Wang, RA Fawwaz and PO Alderson, Columbia University, New York, New York 10032

Tumor cells in culture will take protein molecules such as albumin and ferritin and the intracellular transport of these proteins can be enhanced by basic polyamino acids and amino-dextrans (1,2). A similar enhancement may occur with the anti-tumor monoclonal antibodies (MAbs). The purpose of this study is to synthesize In-111 labeled MAb conjugated with electronegatively charged polymers and evaluate whether these polymers retain their immunoreactivity.

Poly-L-lysine (PL) (3.8 kd) was reacted with MADTPA in PBS, pH 8.6 to form PL-DTPAs and was purified by exhaustive dialysis. The free NH2- groups of PL were measured by trinitrobenzene sulfonic acid titration (3). PL-DTPAs were treated with sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate in PBS, pH 8.2 to form the p-maleimido derivative of PL-DTPAs (II). Anti-human breast carcinoma MAb -BC-75 was reacted with 2-iminothiolane in PBS pH 8.6, to yield its mercapto derivative (III), (II) and (III) were combined to form MAb-BC-75-PL-DTPAs (IV), which finally chelated with In-111. The product (V) was purified from a Bio Rad P-30 column, and eluted with PBS, pH 7.4. Under similar conditions, In-111-MAb-BC-75-dextran-DTPAs (VI) was also prepared using amino-dextran (DE) (40 kd) was the starting material. In-111-MAb-BC-75-DTPA (VIII) (mixed anhydride of DTPA method), which was prepared in a similar way but does not contain electronegative polymers, and evaluate as a control.

Direct binding of the MAb to human breast carcinoma cells and control cells were performed. The results of cell binding experiments (N=3 per agent) in the region of antigen excess (X ±SD) were as follows: 71.6 ± 4.52% for (V) and 82.4 ± 5.31% for (VI) vs. 35.7 ± 1.17% for (VII) ($p < 0.01$). Binding of all agents to the control lymphoid cell line was less than 3%.

These results suggest that the conjugation of electronegatively charged polymers, PL-DTPAs and the DE-DTPAs to MAb do not cause significant loss of MAb immunoreactivity. Moreover, in the MAb-BC-75 conjugated with PL-DTPAs and DE-DTPAs proved superior to labeling with DTPA alone. The findings suggest that further study of electronegative polymer MAbs as tumor seeking agents is warranted.

IMMUNOREACTIVITY

Compound	Cell Binding Assay Percent of Binding (N=4)
MAb-BC-75	100
In-111-MAb-BC-75 poly-L-lysine-DTPAs (V)	71.6 ± 4.52
In-111-MAb-BC-75-dextran-DTPAs (VI)	82.4 ± 5.31
In-111-MAb-BC-75-DTPA (VII)	35.7 ± 1.17

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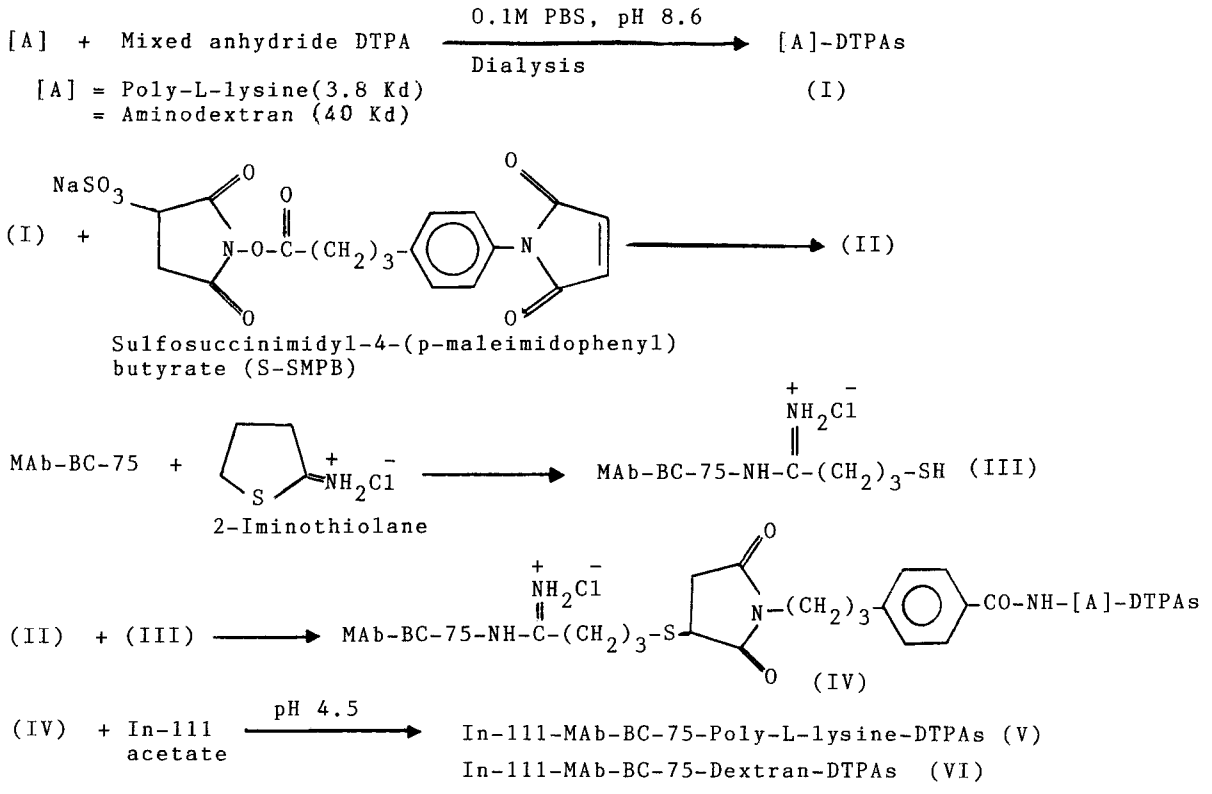
SYNTHESES OF In-111-MAb-BC-75-POLY-L-LYSINE-DTPAs and In-111-MAb-BC-75-DEXTRAN-DTPAs



EFFECT OF ANTIBODY CONCENTRATION ON POLYMERIC ANTIBODY FORMATION BY CYCLIC DTPA DIANHYDRIDE REACTION AND RELATIONSHIP BETWEEN POLYMER FORMATION AND IMMUNOREACTIVITY OF ANTIBODY.

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The conjugation of DTPA to antibody using cyclic DTPA dianhydride (cDTPAA) produces cross linked antibody-DTPA polymers in addition to monomeric antibody-DTPA dependent upon the concentrations of antibody and cDTPAA. The purpose of this research was to investigate the effect of antibody concentration on the polymer formation and the relationship between the polymer formation and the immunoreactivity of antibody.

Anti-human serum albumin antibody IgG (Ab) was used as a model antibody for this study. cDTPAA (3.6×10^{-4} M) was reacted with Ab at 18 mg/ml (1.2×10^{-4} M) and 2.0 mg/ml (1.3×10^{-5} M) in 0.1 M Na bicarbonate buffer at pH 8.4 at room temperature for 1 hr. This reaction mixture was then reacted with In-111Cl_3 in a buffer mixture containing 0.1 M Na acetate and 0.01 M Na citrate at pH 5.0. The labeling reaction was continued until there was no free In-111 ion based on silica gel TLC (Macherey-Nagel, Germany) developed with 2:2:1 10 % ammonium formate:methanol:0.2 M citric acid. R_f values are 0, 5.6 and 8.3 for Ab-DTPA-In-111, DTPA-In-111 and unreacted In-111 ion. Aliquots of the reaction mixture containing 400 ug Ab and 5 uCi of In-111 activity was analyzed with size exclusion HPLC (Bio-Sill TSK 250 column 7.8 x 600 mm, 1 ml/min flow rate, 0.1 M Na phosphate/0.1 M Na sulfate 1:1, pH 6.6) and affinity chromatography with a combination column containing Sepharose 4B-HSA (1 cm) on the top of Sephadex G-50 (45 cm). The combination column was first eluted with 0.02 M Na phosphate (pH 7.4)/0.5 M NaCl to separate deactivated Ab from DTPA and immunoreactive Ab bound to the affinity column was dissociated from the antigen by elution with 0.2 M glycine.HCl (pH 2.2)/0.5 M NaCl. The HPLC retention time of polymeric Ab, monomeric Ab and DTPA-In-111 was 13, 18, and 27 min, respectively.

The conjugation yield based on the concentration of cDTPAA was 73 % for the reaction with Ab at 18 mg/ml and 18 % for the 2 mg/ml Ab reaction as assessed by In-111 activity distributed between Ab-DTPA and free DTPA. The number of DTPA molecules conjugated per Ab estimated based on the conjugation yield was 2 for the higher Ab concentration and 5 for the the lower antibody concentration. The HPLC analysis showed that the conjugation reaction with the lower Ab concentration did not produce polymer apppreciably whereas the reaction with the higher Ab concentration produced 10 % polymer as assessed by uv peak intensity. The affinity chromatographic analysis indicates that the conjugation reaction with the lower Ab concentration and the higher Ab concentration decreased the immunoreactivity to 77 and 84 %, respectively. The immunoreactivity of the unconjugated Ab was 89 % based on affinity chromatography.

These results indicate that the conjugation reaction with a higher Ab concentration produces a higher percentage of Ab polymer for the conjugation reactions producing a similar number of DTPA molecules conjugated per Ab. The deactivation of Ab is, however, influenced more by the number of DTPA molecules conjugated per Ab than by the polymerization of Ab.

SEARCH FOR RADIOLABELED ANTIBODIES WITH HIGH TARGET TO NON-TARGET RATIO. Y. Arano, H. Matsushima, M. Tagawa, M. Koizumi, Y. Watanabe, K. Endo, J. Konishi and A. Yokoyama. Faculty of Pharmaceutical Sciences and School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

In radioimmunoimaging and therapy, localization of radioactivity in non-target organs and its slow clearance constitute major problems when metallic radionuclides are used as labels for antibodies. For the non-specific radioactivity localization, the in-vivo behavior of antibodies themselves seems to be one of the most critical factors responsible (1, 2). In order to reduce the radioactivity from non-target organs or accelerate the clearance, the radioactivity removal as an intact chelate enhancing their urine excretion might constitute an attractive method for achieving a high target to non-target ratio.

To pursue the above task, an ester containing heterogeous bifunctional reagent, a Maleimidoethyl Succinylsuccinate (MESS) (Fig.1) was designed and synthesized. MESS possesses a site for binding Ga-67 deferoxamine and that for binding antibodies. Under physiological condition, hydrolysis of the ester bonding might occur by the action of carboxyesterase in non-target organs, and Ga-67 succinyldeferoxamine (Ga-SDF) chelate can be released from the radiolabeled antibodies. Ga-SDF has been reported to be a potential alternative for ortho-iodo hippuran (3); on the other hand, high stability of Ga-deferoxamine chelate under physiological environment has been well demonstrated (4, 5). In this study, Ga-67 labeled monoclonal IgG_1 and its $F(ab')_2$ fragment against Osteogenic Sarcoma (Ga-MESS-IgG and Ga-MESS-$F(ab')_2$) were prepared by the reaction of MESS with antibodies and deferoxamine, followed by the Ga-67 labeling with 67-$GaCl_3$ in PBS (pH 6.0). For the evaluation of this drug-design, in-vitro release of Ga-SDF from the Ga-67 labeled antibodies and in-vivo studies in nude mice were carried out.

HPLC analyses of the two Ga-67 labeled conjugates showed more than 95 % radiochemical yields without intermolecular cross-linking. Preserved immunoreactivity was also confirmed by cell binding assay. Both labeled preparations released Ga-SDF upon incubation in sera or in the presence of carboxyesterase; Ga-MESS-$F(ab')_2$ generated more Ga-SDF than Ga-MESS-IgG. When injected into nude mice bearing Osteogenic Sarcoma, Ga-MESS-IgG showed relatively rapid blood clearance with low non-specific radioactivity localization in the liver, spleen and kidney, but tumor to blood reached a ratio of 6 after 24 hours post-injection (Table 1). Ga-MESS-$F(ab')_2$ showed faster blood clearance with low radioactivity localization in the liver and the spleen (Table 1). As a result, in only 6 hours post-injection, tumor to blood ratio reached the value of 3. These results suggest the validity of presently designed MESS for the target imaging using Ga-67 labeled antibodies, due to an efficient release of Ga-SDF upon metabolism in non-target organs. Using this versatile reagent, MESS or its derivatives, replacement of Ga-SDF by another chelates for more appropriate radionuclides are now underway.

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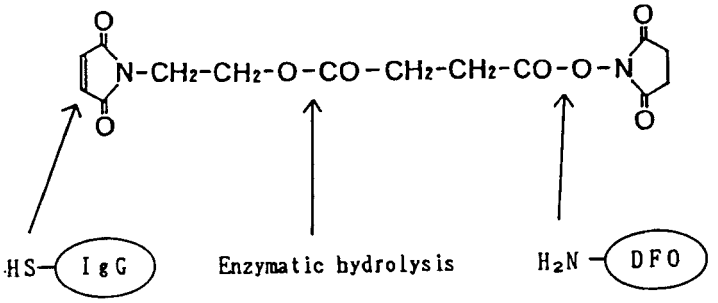

Fig. 1. Chemical structure of MESS.

Table 1. Biodistribution of Ga-MESS-IgG and Ga-MESS-F(ab')$_2$ in nude mice bearing Osteogenic Sarcoma*.

	Ga-MESS-IgG	Ga-MESS-F(ab')$_2$
Organs	24 hr.	6 hr.
Blood	6.66 (2.04)	4.27 (0.23)
LIver	4.88 (1.19)	2.15 (0.26)
Kidney	5.08 (0.74)	29.92 (2.92)
Spleen	2.02 (0.51)	0.98 (0.31)
Tumor	33.19 (8.47)	13.08 (4.45)

* Each value represents mean % dose/gram (1 s.d.) for 3 animals.

EVALUATION OF A NEW BIFUNCTIONAL CHELATE FOR RADIOLABELING ANTIBODIES WITH INDIUM-111. S.W. Schwarz, C.J. Mathias, Y. Sun*, W.G. Dilley, S.A. Wells, Jr., A.E. Martell*, M.J. Welch. Departments of Radiology and Surgery, Washington University School of Medicine, St. Louis, MO 63110; *Texas A&M University, College Station, TX 77840.

We have recently synthesized two new bifunctional chelates, N-(2-hydroxy-3,5-dimethylbenzyl)-N-(2-hydroxy-5-bromoacetamidobenzyl)-ethylenediamine-N,N'-diacetic acid ($BrMe_2$HBED) and N,N'-bis(2-hydroxybenzyl)-1-(4-bromoactamidobenzyl)1,2-ethylenediamine-N,N'-diacetic acid (BrΦHBED), with potential for binding trivalent metals to antibodies. (Scheme 1) Previous work has shown that these bifunctional chelates will bind to indium antibodies (Ab) with a high degree of stability.(1-2) In this study an antiparathyroid, IgGl type, monoclonal antibody (BB5) was conjugated with these new ligands and radiolabeled with In-111. We have compared these In-111-labeled-Abs to conventionally radiolabeled-BB5 (iodinated and DTPA-conjugated), in a nude mouse model. In this mouse model human parathyroid human parathyroid tissue, obtained from patients with primary hyperparathyroidism, was minced into small pieces (1-3mm^3) and implanted into either Swiss nude mice or Balb/c athymic nude mice in the rear flank musculature as descrived previously.(4)

The conjugation with $BrMe_2$HBED was carried out by dissolving $BrMe_2$HBED in absolute ethanol (1.0mg/ml), evaporating the solvent, and redissolving the ligand (L) in the buffered Ab (>3mg/mL) solution, pH 7-8. The molar ratio of L to Ab used was 10:1. After a 24h incubation at RT, the $BrMe_2$HBED-BB5 conjugate was isolated from the L on a 1 mL Sephadex G-50 spin column equilibrated with 0.01M sodium citrate, pH 8. Radiolabeling was accomplished by adding ^{111}In-citrate, pH 7-8, to the purified $BrMe_2$HBED-BB5. (^{111}In-citrate was prepared by adding $^{111}InCl_3$ (~300mCi/mL) to 0.1 M sodium citrate, pH 8.) The reaction was incubated at room temperature for 24h, then purified on a 1 mL Sephadex G-50 spin column equilibrated with 0.01M sodium citrate, pH 8. The recovered radiolabeled-BB5 was assayed for protein concentration (~90% recovery) and the labeling efficiency was determined.

BrΦHBED was conjugated to BB5 by mixing a 10:1 (L:Ab) mole ratio of L (2mg/mL) in 0.01 M sodium citrate (pH 8) and BB5 (>3mg/ml) in 0.01M sodium citrate (pH 8) and incubating at RT for 24h. The BrΦHBED-BB5 complex was isolated from free L on a 1mL Sephadex G-50 spin column equilibrated with 0.01M sodium citrate, pH 8. Radiolabeling was achieved by adding ^{111}In-citrate to the purified BrΦHBED-BB5 and incubating at room temperature for 1h. The reaction was purified on a 1 mL Sephadex G-50 spin column equilibrated with 0.01M sodium citrate (pH 8).

Diethylenetriaminepentaacetic acid (DTPA) cyclic anhydride (Sigma Chemical Co.) was purchased and used without further purification. Conjugation of DTPA cyclic anhydride with BB5 was accomplished by evaporating the appropriate volume (to achieve 10:1 mole ratio, L:Ab) of dissolved L solution (1mg/2-5ml chloroform) with a stream of nitrogen and reconstituting the L in the buffered Ab solution (>3mg/ml, pH 5). Unbound DTPA was separated from the L-conjugated-BB5 with a 1mL Sephadex G-50 spin column equilibrated with either 0.04M sodium acetate, pH 5, or 0.01M sodium citrate, pH 6. Radiolabeling was accomplished by the addition of ^{111}In-citrate or ^{111}In-acetate, pH 5.5. The reaction was incubated at RT for 1h, and purified on a 1 mL Sephadex G-50 spin column equilibrated in the corresponding buffer.

The iodogen method was used to radioiodinate the intact antibody.(4) Prior to use, the radiolabeled antibody was purified from the unbound radioiodine using a Sephadex G-50 spin column equilibrated with 0.01M sodium phosphate pH 7.2.

The biodistribution studies were performed by co-injecting each mouse via tail vein with 25μg of ^{111}In-labeled-BB5 (conjugated with DTPA cyclic anhydride, BrMe$_2$HBED, or BrΦHBED) and 25μg ^{125}I-BB5. Animals were sacrificed at 24, 72, 96 and/or 120 hours after the radiolabeled MAb injection. Samples of blood, liver, kidney, muscle, and parathyroid were removed and weighed. These data were expressed as %ID/gm and target to nontarget ratios calculated (Table 1). It is seen that higher target to nontarget ratios are obtained with BrΦHBED due to more rapid clearance from the blood and muscle. These data show BrΦHBED to be the bifunctional chelate of choice for labeling BB5.

This work was supported in part by NIH grants CA42925 and CA42632.

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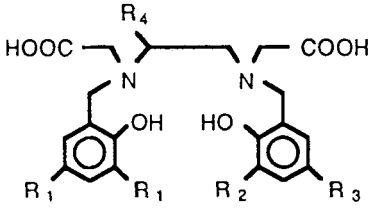
Scheme 1.



	R_1	R_2	R_3	R_4
BrMe$_2$HBED	CH_3	H	$NHCOCH_2Br$	H
BrΦHBED	H	H	H	$CH_2\Phi NHCOCH_2Br$
BrMe$_4$ΦHBED	CH_3	CH_3	CH_3	$CH_2\Phi NHCOCH_2Br$

Table 1. Target to nontarget ratios 24h and 96h after injection of radiolabeled-BB5.

	^{111}In-BrΦHBED	^{111}In-BrMe$_2$HBED	^{111}In-DTPA Cyclic Anhydride	^{125}I
24h				
pT/blood	1.94 ± 0.21	1.13 ± 0.20	1.19 ± 0.28	2.01 ± 0.62
pT/muscle	21.65 ± 4.72	6.99 ± 0.16	11.73 ± 12.08	10.82 ± 3.47
96h				
pT/blood	6.88*	4.01 ± 1.61	2.44 ± .85	1.26 ± .44
pT/muscle	61.13*	19.79 ± 9.01	19.99 ± 8.42	20.92 ± 10.82

* Extrapolated from 72 and 120h data

PHENOLIC POLYAMINOCARBOXYLIC ACIDS FOR LABELING OF MONOCLONAL ANTIBODIES WITH RADIONUCLIDES OF GALLIUM.

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Gallium (Ga) chelates with diethylenetriaminepentaacetic acid (DTPA) are of low in vitro stability (log K_1 25.5) and thus are unsuiteable as a label for immunoscintigraphy (1). N,N'-ethylene-bis(2-hydroxyphenylglycine) (EHPG) and N,N'-bis (2-hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid (HBED) are much stronger Ga chelators with stability constants of log K_1 33.6 and 39.6 respectively (2), which should prevent invivo transchelation of Ga by transferrin. The introduction of additional functional groups at the phenolic rings for covalent attachment of the ligands to proteins results in bifunctional ligands which should be more preferable for labeling monoclonal antibodies (MAbs) whith Ga than DTPA. We have synthetized a propionic acid substituted EHPG in a one step reaction according to the method of Dexter (3) (Scheme 1). This ligand was first labeled with Ga-67 and subsequently the chelate was coupled to MAbs via carbodiimid. While labeling of the ligand was quantitative, coupling of the chelate to MAbs, whitout an appreciable loss in immunoreactivity, ammounted to 20%.

A bifunctional HBED-ligand with aliphatic isothiocyanate groups for protein coupling was prepared by a Mannich reaction starting with triflouracetyltyramine (Scheme 2). Coupling of the ligand resulted in a thiourea linkage with the MAbs. Labeling of these MAb-HBED conjugates was carried out either with In-111 in a citrate buffer pH 6.0 or with Ga-67 in a MES-buffer pH 4.8 . Labeling efficiency for both nuclides was >85%. Biodistribution experiments were carried out with the murine MAb A2.6 (IgG_1) directed against the 14-ASML-1 tumor (rat sarcoma) grewing in nude mice. The chelate to MAb molar ratio for all MAb conjugates was adjusted to be 1:1. Uptake of Ga-67 EHPG and Ga-67 or In-111 HBED labeled MAbs in blood, tumor and liver was compared with that of In-111 DTPA or I-131 labeled MAbs. For coupling of DTPA the bicyclic anhydride was used, I-131 labeling was carried out using IODO-GEN as the oxidant (Table 1).

Compared to In-DTPA, In-111 HBED showed a faster activity decrease in blood and a much higher liver uptake, indicating a lower invivo stability than In DTPA. Ga-67 activity after administration of Ga-67 EHPG or HPED labeled MAbs accumulated to a lesser extent in the liver but had a similar uptake in tumors than In-DTPA. These results indicate the potential of EPHG and HBED for Ga-labeling of MAbs. Ga-labeling of MAbs might be of interest for immunoscitigraphy with the positronemitter Ga-68 and bispecific MAbs as proposed and outlined by Goodwin et al (4).

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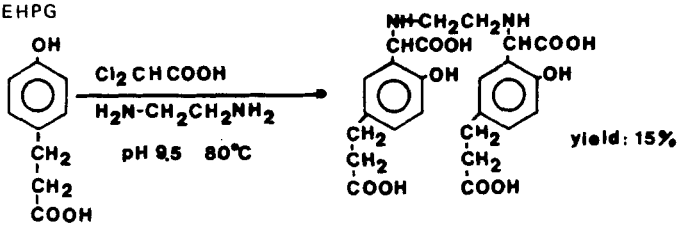
Table 1

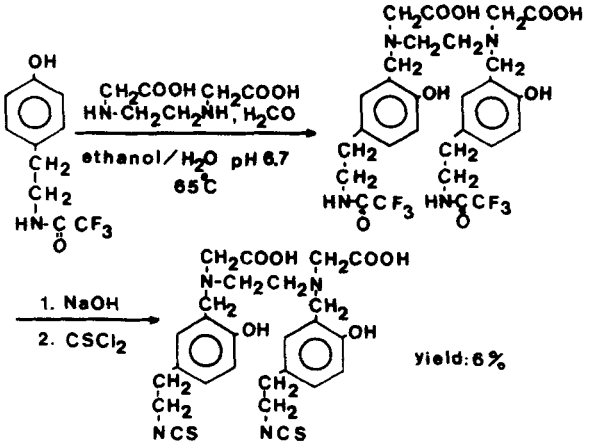
Substance	Tumor	Blood	Liver
Ga-67 HBED	14.7±4.6*	8.4±1.3	3.3±0.4
Ga-67 EDDHA	18.7±3.6	5.3±1.2	3.2±0.6
In-111 HBED	20.8±2.0	3.1±1.0	9.8±1.0
In-111 DTPA	19.2±3.3	5.5±2.5	5.0±0.8
I-131	5.7±1.8	4.3±1.5	0.8±0.2

*% of inj.dose per gramm of tissue ±1SD; 120h p.i.; n=6

Scheme 1 EHPG



Scheme 2 HBED



NEW BIFUNCTIONAL CHELATORS FOR ANTIBODY LABELING WITH METALLIC RADIONUCLIDES.

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The use of monoclonal antibodies (MAb) in clinical oncology is becoming a new method in cancer diagnosis and treatment. Many parameters have to be optimized before this new modality can contribute substantially to the management of the disease. Our research goals are devoted towards the labeling procedure. This has to be easy to perform, cost effective, reliable and available on the time of request. The best way to do this is by use of metallic radionuclides and bifunctional chelators. This method has the special advantage that a lyophilised antibody ligand conjugate can be prepared and stored in advance and a tumorseeking radiopharmaceutical can be prepared by simply adding a solution of a metallic radionuclide prior to application to it.

We report on the successful labeling of a MAb with the diagnostic radionuclides indium-111 and technetium-99m and the beta-emitting therapeutic radionuclides Cu-67 and Y-90. Two new bifunctional chelators based on tetraazamacrocycles have been synthesized for immobilization of In-111. The bifunctional ligands loose only 0,1% and 0,4% of the label within 24 hrs under physiological conditions to serum transferrin. One of these ligands also appears to stabilize Y-90 in vivo well enough for an application. A bifunctional ligand based on a propylenediaminedioxime was synthesized for antibody labeling with Tc-99m. It forms stable Tc (V) complexes which survive physiological conditions. Moreover Cu-67 was bound to MAb b-12 which is directed against a mucin-like molecule on breast tumor cells. Results on tumor bearing mice showed good tumor uptake but high concentrations of MAb b-12 in the liver. A comparison of a biodistribution of MAb b-12 labeled with the four radionuclides will be made and discussed.

EFFECT OF CHELATE RIGIDITY ON IN VIVO RADIOIMMUNOCONJUGATE DISTRIBUTION.
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This study was carried out to evaluate the effect of backbone rigidity in a series of polyaminocarboxylates (PACs) on the biodistribution of corresponding radioimmunoconjugates. Rigidity was introduced by substitution of trans-1,2-diaminocyclohexane for an ethylenediamine portion of EDTA, DTPA, and TTHA. The cyclohexane ring locks two chelating nitrogens into a favorable position and prevents them from rotating away from the metal upon dissociation. In addition, the steric bulk of the ring may inhibit the approach of competing ligands in-vivo. The radiometals used in this study (^{111}In, ^{57}Co, ^{203}Pb, ^{67}Cu) display a wide range of serum stability as DTPA immunoconjugates (^{111}In high, ^{57}Co moderate, ^{203}Pb and ^{67}Cu low). This range provides a baseline from which to assess the effects of ligand rigidity.

The cyclohexyl derivatives of DTPA and TTHA (CDTPA and CTTHA) were synthesized as outlined in Scheme 1. Trans-1,2-diaminocyclohexane was converted to its mono-t-Boc derivative 1 prior to reaction with isobutylchloroformate (IBC) and t-Boc-glycine to give 2 or was reacted directly with IBC and t-Boc-glycine to give 3. Hydrolysis of t-Boc groups and reduction of the amides produced 4 and 5. Alkylation with bromoacetic acid gave CDTPA and CTTHA. Cyclic anhydrides and/or N-hydroxysuccinimide (NHS) esters were used to prepare anticolon ca 17-1A immunoconjugates. Room temperature acetic anhydride/pyridine dehydration of EDTA yielded a mixture of mono- and dianhydride products (NMR) while under similar conditions the monoanhydride of cyclohexyl EDTA (CDTAMA) precipitated selectively. NHS esters of CDTA and DTPA were made from CDTAMA and DTPADA[1], while NHS esters of TTHA, CDTPA, and CTTHA were prepared directly using dicyclohexyl carbodiimide and excess NHS in DMSO[2], because acetic anhydride/pyridine dehydration failed to produce the desired anhydrides. All derivatives were isolated and characterized by NMR.

Radiometal immunoconjugates were purified by HPLC (Zorbax GF-250) and monomeric fractions were injected into either normal BNL mice or human colon ca (SW948) xenografted nude mice. Only the EDTAMA/EDTADA mixture gave significant high molecular weight material (~ 30%); other preparations contained ≤3% of such material. Biodistribution data are summarized in Tables 1 and 2. With ^{111}In, CDTA and CTTHA gave higher tumor uptake than with EDTA and TTHA; this uptake was comparable to CDTPA and DTPA. In addition, CDTA gave the lowest kidney and bone retention. Tumor uptake at 24h greatly increased for ^{203}Pb labeled CDTA-17-1A compared to DTPA-17-1A (Table 1); both preparations, however, gave high kidney and bone retention. The greatest effect of the cyclohexyl ring was seen with ^{57}Co (Table 2). The order of tumor uptake at 96h was CDTA>CDTPA>CTTHA>DTPA≈EDTA. The distribution (data not shown) of ^{67}Cu-CDTA-17-1A in normal mice was indistinguishable from that of $^{67}CuCl_2$.

In conclusion, the introduction of backbone rigidity into PACs can improve biodistribution of radioimmunoconjugates when labeled with metals whose DTPA complexes are of high or moderate stability in-vivo. The effect is most pronounced with CDTA, possibly because the highest percentage of its binding sites (2 of 5, 40%) are constrained by the cyclohexane ring. The rigidity in this system also helps counter the shortage of donor atoms available for coordination. CDTA immunoconjugates attached through the cyclohexane ring are expected to improve these results.

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This research was supported by the United States Department of Energy, Office of Health and Environmental Research, under Contract No. DE-AC02-76CH00016.

Table 1. Biodistribution (% dose/g) of ^{111}In and ^{203}Pb Labeled 17-1A Conjugates in Nude Tumor Mice

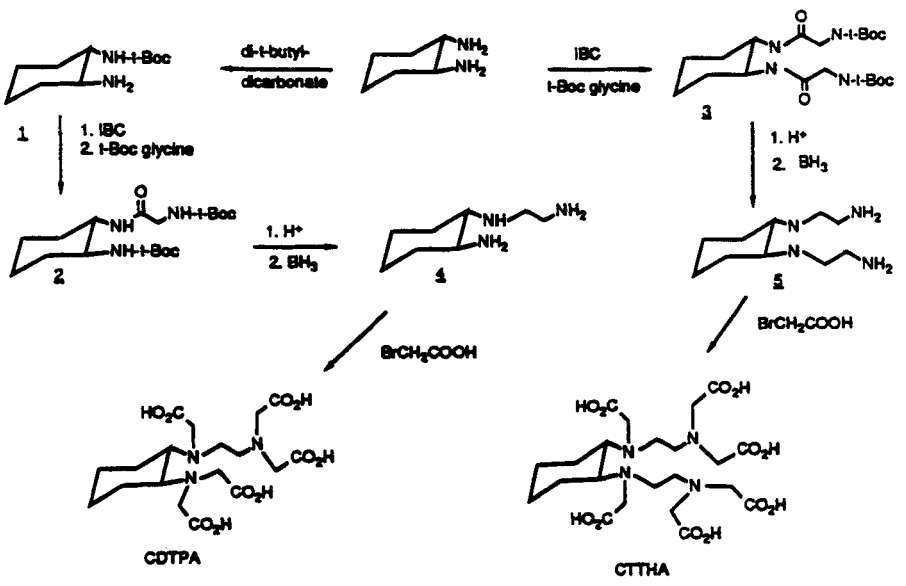
Ligand	Nuclide	Time (h)	n	Blood	Liver	Kidney	Bone	Tumor	%Recov.
EDTAMA &	^{111}In	24	2	3.2	7.3	24.4	7.2	6.2	90
EDTADA		96	6	0.5±0.2	7.4±2.8	12.7±2.5	4.8±1.4	2.8±1.2	60
CDTAMA	^{111}In	24	4	4.2±2.6	9.0±1.5	4.2±0.8	6.0±1.6	14.0±3.0	96
		96	4	1.0±1.0	7.1±2.2	4.3±1.9	2.5±1.0	6.0±3.3	63
CDTA-1NHS	^{111}In	24	4	6.8±3.5	7.5±2.1	4.7±0.3	2.6±0.9	12.3±1.2	85
		96	5	0.3±0.2	7.7±0.6	3.7±0.6	2.2±0.4	3.0±0.4	50
DTPADA	^{111}In	24	6	5.6±2.8	8.8±2.5	8.8±1.6	5.2±2.0	11.2±1.7	96
		96	6	2.6±2.0	6.5±0.8	11.6±2.2	3.6±1.1	9.8±4.1	71
DTPA-2NHS	^{111}In	24	3	11.6±1.2	6.8±0.1	12.0±1.6	4.6±0.4	14.1±2.0	94
		96	3	0.4±0.02	6.6±0.9	11.5±0.5	6.3±0.6	6.0±0.6	64
CDTPA-2NHS	^{111}In	24	3	9.8±4.7	7.2±1.2	7.3±1.2	5.5±0.9	16.8±2.0	100
		96	3	0.4±0.1	8.7±1.4	5.3±0.7	7.1±1.3	5.9±1.2	68
TTHA-1NHS	^{111}In	24	3	2.0±0.2	5.4±0.3	26.3±2.5	7.8±1.4	5.4±1.5	80
		96	3	0.3±0.1	6.0±0.8	14.8±3.0	6.7±1.3	2.9±0.3	61
CTTHA-2NHS	^{111}In	24	3	6.7±3.2	5.9±0.8	13.8±1.3	6.4±2.0	11.8±2.0	78
		96	3	2.9±2.6	5.6±0.7	9.4±1.2	5.1±0.5	9.5±3.0	71
CDTAMA	^{203}Pb	24	3	8.6±0.9	6.4±0.7	16.5±0.7	12.1±0.4	6.1±0.2	76
		96	3	1.3±0.3	2.2±0.3	6.5±0.2	14.1±1.5	1.1±1.1	48
DTPADA	^{203}Pb	24	3	3.6±0.1	5.3±0.2	18.6±4.3	13.0±1.6	1.1±0.2	48
		96	3	0.9±0.1	2.7±0.4	6.3±1.5	8.1±0.3	0.4±0.1	35

Table 2. Biodistribution (% dose/g) of ^{57}Co Labeled 17-1A Conjugates in Nude Tumor Mice

Ligand	Time (h)	n	Blood	Liver	Kidney	Bone	Tumor	%Recov.
EDTAMA &	24	3	2.2±0.1	4.1±0.6	3.0±0.02	1.0±0.16	3.1±0.2	35
EDTADA	96	3	0.2±0.01	2.0±0.2	1.2±0.04	0.5±0.03	0.5±0.02	10
CDTAMA	24	5	12.9±5.2	5.7±1.8	3.5±1.0	2.5±0.7	12.8±3.5	79
	96	5	1.5±1.6	2.7±0.4	1.9±0.2	1.4±0.9	4.1±2.7	31
CDTA-1NHS	24	5	6.0±2.2	7.2±2.5	3.3±0.7	4.4±1.8	14.2±3.8	78
	96	4	5.5±3.5	3.2±0.6	2.5±0.4	1.3±0.3	11.2±4.8	41
DTPADA	24	6	2.2±0.7	5.8±0.7	3.6±0.3	1.9±0.3	5.6±1.1	41
	96	7	0.4±0.2	2.2±0.5	1.6±0.4	0.6±0.2	0.9±0.4	10
DTPA-1NHS	24	3	5.2±0.8	6.1±0.5	3.8±0.4	0.8±0.2	6.2±2.1	41
	96	3	0.3±0.1	1.8±0.2	1.7±0.2	0.5±0.1	0.8±0.4	11
CDTPA-2NHS	24	3	3.7±0.7	4.8±1.2	3.0±0.2	2.0±0.1	6.2±1.5	44
	96	3	1.0±0.8	1.8±0.3	1.5±0.1	0.7±0.2	2.4±1.2	16
CTTHA-2NHS	24	2	1.9	5.9	3.9	1.7	5.9	35
	96	2	0.6	2.3	2.0	0.4	1.4	13

Scheme 1

Synthesis of Cyclohexyl -DTPA and -TTHA



USING TECHNETIUM-99m MH-1 MONOCLONAL ANTIBODY TO IMAGE THROMBI IN A RABBIT JUGULAR VEIN. K.M. Miller, J.H. Wible, Jr., J.R. Coveney, L.R. Lyle, Mallinckrodt Medical, Inc., St. Louis, MO; P.E. Gargan, V.A. Ploplis, American Biogenetic Sciences, Inc., Notre Dame, IN; A.R. Fritzberg, NeoRx Corporation, Seattle, WA.

The monoclonal antibody MH-1 developed at American Biogenetic Sciences recognizes an epitope on cross-linked fibrin and binds with high affinity ($K_D = 6.7 \times 10^{-10}$M). This IgG_1k MAb does not cross react with fibrinogen. It also does not react with oligopeptides in fibrin degradation products, in contrast to some other reported antifibrin MAb's. These properties make this MAb potentially useful as an *in vivo* diagnostic agent to image thrombi. The MH-1 was labeled with a Tc-99m triamide thiolate (N_3S) bifunctional chelate using a modified version of the preformed chelate approach developed at NeoRx. Purified Tc-99m MH-1 was characterized by HPLC, SDS-PAGE, and IEF. Tc-99m MH-1 (120 μCi/μg) was injected into rabbits (200-400 μCi/kg) with experimentally-induced thrombi. Serial scintigrams and arterial blood samples were acquired for 6 hours, then thrombi and tissues were assayed for Tc-99m concentration. The average weight of thrombi was 44.2 mg (range: 36 - 62 mg). Clot-to-blood ratios were determined as were half-lives of tracer elimination from the blood using a two-compartment model. Results were compared to those for a Tc-99m labeled antibody not specific for fibrin, Tc-99m NR-Lu-10, a MAb that is specific for several carcinomas. The Tc-99m MH-1 reliably visualized thrombi within 2.5 hours post-injection; the Tc-99m NR-Lu-10 did not image thrombi at any time. Analysis of the Tc-99m concentrations produced these results:

	Tc-99m MH-1	Tc-99m NR-Lu-10
n	4	2
clot-to-blood	16.3 (range: 8.7 - 28.3)	0.19 (range: 0.18 - 0.21)
$t_{1/2}$ (hours)	3.7 (range: 3.1 - 4.0)	11.3 (range: 9.6 - 13.5)

The clot-to-blood ratios for Tc-99m MH-1 at 6 hours post-injection are comparable to or higher than values obtained after much longer periods (18-48 hours) for other reported radiolabeled antifibrin MAb's. The $t_{1/2}$ for Tc-99m MH-1 is considerably shorter than is normally found for whole MAb's and might relate to the acidic isoelectric point (pI = 5.8) of MH-1. These results indicate that Tc-99m MH-1 may be efficacious as an *in vivo* diagnostic agent for the detection of thrombi.

Tumor targeting with low molecular weight radiopharmaceuticals versus monoclonal antibodies

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It is commonly accepted that the major limitation of tissue targeting with monoclonal antibodies (MAbs) resides in the blood-tissue barrier which is operative on high molecular weight (Mw) carrier molecules. To overcome this problem, multi-step approaches have recently been introduced which include bispecific antibodies or antibody conjugates together with low Mw radiolabeled species, the combination of both elements leading to a process of labeling in vivo.

Test systems. To evaluate the respective targeting potential of high and low Mw radiopharmaceuticals we have chosen two tumor model systems wherein selective accumulation of both types is taking place. In human B-lymphoma BJAB xenografts a MAb against a lymphocyte differentiation antigen was compared to ^{67}Ga, while a MAb with irrelevant specificity and ^{111}In were used for control purposes. In human neuroblastoma SK-N-SH xenografts, MAb BW575/9 (Behringwerke, Marburg, FRG) was compared to ^{131}I-MIBG. Biokinetic data were obtained by (quantitative) autoradiography and by conventional biodistribution analysis using the paired label assay. The intra-tumoral radioactivity distribution pattern at several time points was analyzed by computer-aided densitometry of macro-autoradiographs.
Results. In both systems the low Mw radiopharmaceutical showed rapid uptake in tumor tissue. Labeled antibodies on the other hand took 24-48 h to achieve peak uptake values (Tables 1 and 2). Blood clearance on the other hand was moderate to rapid with ^{67}Ga and ^{131}I-MIBG, respectively, but it was slow with the MAbs. This resulted in high contrast with both low Mw radiopharmaceuticals at early time points. The absolute amount of radioactivity uptake was low with MIGB in neuroblastoma tissue and with both MAbs as well as with ^{111}In in BJAB lymphoma tissue. Uptake was high with MAb 575/9 in neuroblastoma and with ^{67}Ga in lymphoma.
The accumulation patterns produced by the individual pharmaceuticals were documented and semi-quantitatively evaluated by computer-aided densitometry of autoradiographic images (not shown in the abstract). In neuroblastoma SK-N-SH, the distribution was remarkably homogeneous with both MAb BW575/9 and MIBG. In lymphoma BJAB, ^{67}Ga produced a homogeneous distribution pattern, while uptake of MAb HD37 was strictly confined to the periphery of lymphoma BJAB. Non-specific MAb and ^{111}In showed weak accumulation and diffuse distribution with a slight preference for peripheral uptake in the case of In. Paired label experiment and autoradiographic data, taken together, yielded an interpretation of the strikingly different accumulation behaviour of high and low Mw radiopharmaceuticals in both systems.

Discussion. The two models studied with a pair of "specifically" accumulating radiopharmaceuticals of high and low Mw showed divergent results which highlight most of the phenomena which are relevant to transport and binding in tumor tissue. The outstanding characteristic of the neuroblastoma model is a very loose texture of tumor tissue. Consequently, targeting molecules get access to virtually every part of the nodule, although the rate of transport is clearly slower with the high Mw species. Since blood clearance of the low Mw MIBG is so rapid, the overall input, and, hence, the absolute amount of uptake of the low Mw species is low. The tumor resembles the renal cell carcinoma described by Sands et al. (1). The lymphoma situation is more complicated because of two incoherent elements. 1) MAb access is restricted to the outermost cell layers (2). To our knowledge, this xenograft presents the most stringent accessibility limitations for macromolecules which have ever been encountered in vivo. 2) The low Mw radiopharmaceuticals, i.e. ^{67}Ga and ^{111}In, are both transported by high Mw transferrin, but the association constant for Ga under physiological conditions is so low that a considerable portion of the nuclide coexists in a low Mw form. This is reflected by a moderately rapid clearance from the blood and a marked uptake of Ga in bone tissue. In view of both factors it is important to note that ^{67}Ga, due to its predominant presences as a low Mw species, does not meet the severe accessibility limitations imposed on macromolecules. Hence, it is accumulated rapidly and homogeneously in BJAB tissue. ^{111}In, on the other hand, which is almost exclusively presented in a high Mw state, shows uptake values hardly above non-specific antibody, and uptake is confined to the periphery of nodules. In fact, the uptake of ^{67}Ga in BJAB xenografts models a multi-step approach: Ga-transferrin in the blood represents a low affinity buffer from which Ga is released and transported into solid tumor tissue. Within the lymphoma tissue, Ga is again bound to pre-existing apotransferrin and the Ga-transferrin complex is specifically taken up via the transferrin receptor of lymphoma cells.
In summary, the results obtained with high and low Mw radiopharmaceuticals in the BJAB system illustrate the potential benefit from complex targeting approaches in situations of severely limited accessibility.

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COMPARISON OF ACCUMULATION RATES OF HIGH AND LOW MW RADIOPHARMACEUTICALS IN HUMAN TUMOR XENOGRAFTS

Table 1. B-lymphoma BJAB: Specific MAb (HD37) and control MAb (B40) versus ^{67}Ga and ^{111}In .
n=4-6 animals per group. 70-80 kBq of iodinated MAbs, 300-400 kBq of either In or Ga per animal.

	% injected dose per gram				
Time	Tumor	Blood	Liver	Muscle	Bone
^{125}I-B40					
24h	1.76	14.06	3.60	1.42	1.61
48h	1.93	10.56	2.28	0.92	1.17
^{125}I-HD37					
24h	3.73	21.95	4.86	1.27	1.55
48h	2.08	7.20	1.85	0.80	0.46
^{111}In					
24h	2.22	1.78	8.60	1.08	5.67
48h	2.69	0.66	7.56	0.87	3.41
^{67}Ga					
24h	7.30	2.34	6.35	0.45	8.41
48h	6.21	1.04	4.13	0.41	7.05

Table 2. Neuroblastoma SK-N-SH: Specific MAb 575/9 versus MIBG
n=4 animals per group. 75 kBq of ^{125}I-575/9 and 370 kBq of ^{131}I-MIBG per animal.

	% injected dose per gram				
Time	Tumor	Blood	Liver	Musc	Bone
^{125}I-BW575/9					
4h	8.34	19.34	5.26	1.43	1.79
24h	16.35	11.19	2.63	1.39	1.40
48h	23.72	7.28	2.12	1.00	1.40
^{131}I-MIBG					
4h	2.91	.57	4.01	1.04	.85
24h	1.91	.05	.51	.13	.14
48h	.61	.01	.18	.04	.07

Radiochemical Synthesis of ^{89}Zr-Porphyrin for Positron Label of Monoclonal Antibodies.
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Current research efforts in radiolabelling MAbs commonly use bifunctional chelators as agents to complex radiometals. However, the resulting metal chelates are not sufficiently stable *in vivo* towards transcomplexation with serum protein (1). Thus, the stability of the bifunctional metal chelator to loss of the radiometal is of critical importance.

^{89}Zr is a positron emitting radionuclide ($t_{1/2}$ = 78 hrs, 22.3% positron emission and 76.6% electron capture with a 909 keV gamma emission (2)) which we propose to use as a label for MAbs to quantitate the time-dependent deposition of MAbs in normal and tumor tissues using PET. The greater resolution of PET compared with SPECT or planar imaging can provide more accurate data as input functions for dosimetry calculations.

We chose porphyrins as chelates for zirconium for the following favorable characteristics: 1) They form stable zirconium complexes which do not demetalate in 100% sulfuric acid (3). 2) They can be synthesized with a variety of peripheral functional groups for covalent attachment to MAbs (4). 3) They are not toxic at clinically required concentrations. We report here the synthesis and characterization of zirconium porphyrins using water soluble porphyrins, such as H_2TPPS_4 and H_2TPPS_3COOH (Figure 1). Characterization of these compounds was based on ^{1}H NMR, UV/visible, and mass spectroscopies.

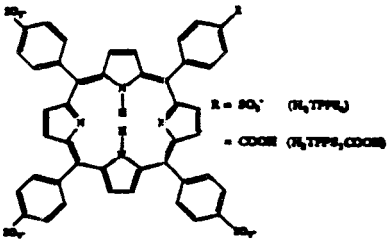

Figure 1

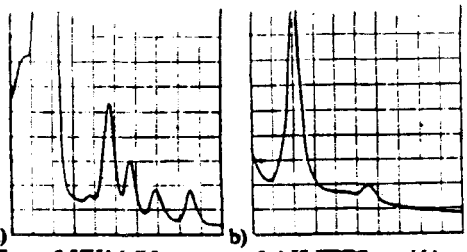

Figure 2 UV/visible spectra of a) H_2TPPS_4 and b) $ZrTPPS_4$ (λ = 350-700 nm).

^{89}Zr was produced by ^{89}Y(p,n)^{89}Zr nuclear reaction and separated from yttrium by extraction with 0.5 M 2-thenoyltrifluoroacetone (TTA) (5,6). ^{89}Zr$TPPS_4$ and ^{89}Zr$TPPS_3COOH$ were synthesized by the reaction of porphyrin in phenol with $Zr(TTA)_4$ carrier added. A mixture of $^{89}Zr(TTA)_4$ carrier added and porphyrin (2:1) in phenol was refluxed for 2 hours. Completion of reaction was judged by UV/visible spectroscopy (Figure 2) before vacuum removal of phenol. ^{89}Zr$TPPS_4$ and ^{89}Zr$TPPS_3COOH$ were separated from free base porphyrins by preparative TLC on reversed phase C_2 plates developed with 0.1 M NH_4OAc pH 7.0 and methanol (60:40) to determine a (20%) radiosynthetic yield counting ^{89}Zr radioactivity in a well gamma counter.

Experiments to couple ^{89}Zr-porphyrins with MAbs are underway.

This work was supported in part by DOE grant DE-FG06-89ER60870.

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RADIOCHEMISTRY OF LEAD-203 FOR RADIOLLABELING ANTIBODY CONJUGATES
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Lead-203 [52 h, 279 (80.1%) KeV] was recognized (1,2) as a potentially useful tracer for tumour specific radiopharmaceuticals due to its favourable nuclear and chemical properties. The emission of the intense 279 KeV gamma-ray makes this nuclide especially attractive for use in SPECT of deep organs. There has also been a resurgent interest in this radionuclide as a conjugated label of monoclonal antibodies. For these applications the Pb-203 should be free of carrier Pb and metal ion contamination. Cyclotron production of Pb-203 via the (d,2n) and (p,n) reactions on high chemical purity natural Tl will invariably contain 6-10 ppm Pb from the target. In order to overcome this problem we use enriched Tl-203 (96% obtained from ORNL) as the target for co-production of Pb-203 and Pb-201/Tl-201 generators (3). This target is highly depleted in Pb since it has been recycled over 200 times. In a typical run 0.5-1 GBq of Pb-203 is produced (700 μAh of 26.4 MeV protons on 35-41 mg/cm^2 of 96% enriched Tl-203). After separation of the Tl-201 the Pb solution [containing Pb-203, ca. 5% Pb-201 and trace amounts of Fe (4)] is wet-ashed from concentrated HNO_3. The residue is taken-up in 8 M HCl and passed through a Dowex 1 x 8 column (1.5 cm x 1 cm) to selectively remove Fe(III) and Tl(III). Then the volume of the solution is reduced and the Pb-203 is shipped from Riyadh to the NIH. The small amount of Tl-201 which grows-in during shipment and residual Fe(III) is removed by repeating the ion-exchange step at the NIH prior to use of the Pb-203.

We have labelled the monoclonal antibody B72.3, conjugated with 2-(p-isothiocyanatobenzyl)DOTA, with Pb-203 in 30% yield. We are currently investigating the biodistribution and in vivo stability of this labelled antibody in nude mice. Tumour to normal tissue ratios displayed stable tumour localization at 72 and 144 hr. There was no major accretion in any of the normal organs.

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RADIOIODINATION OF PROTEINS BY A NEW CONJUGATION TECHNIQUE WITH ACTIVATED PARAMETHOXYPHENYLISOTHIOCYANATE.

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Radioiodinated proteins labeled by the N-hydroxyphenylsuccinimide conjugation (1), are found unstable in vivo; on the other hand, thiourea bond formed between activated phenyl isothiocyanate and the unprotonated C-amino group of lysine group of the protein is found stable in vivo.

The para methoxy phenyl isothiocyanate (PMPITC) was radioiodinated by the chloramine-T or Iodogen method (2-4). The labeling efficiency was (40-55)%. The retention times of radioiodinated PMITC (RPMITC) and free iodide eluted with 50% aceto-nitrile from a C-18 reverse phase column were 7.6 and 1.7 minutes respectively. The PMPITC (RPMPITC) was extracted with ether and evaporated to dryness. After dissolving in DMSO, it was conjugated to the model proteins: canine albumin and gamma globulin (Figure 1). The parameters of concentration of PMITC, oxidant, protein, pH and PMPITC/substrate stoichiometry were optimized (Figure 2) and the immunoreactivity was checked by the affinity column. The competing hydrolysis reaction could be minimized by conjugation at the pH range of 6-8. The conjugation efficiency was (35-45)%. A specific activity of 2-10 mCi/mg was obtained. The shelf-life of RPMPITC when stored in ether at 4°C was 60 days. The radioiodinated product was further analyzed by the HPLC system. with a Spherogel column (TSK-3000). The retention times of the radioiodinated dog albumin and radioiodide were 9.2 and 14.4 minutes respectively. The biodistribution was carried out by the intravenous injection in black mice; 35-50% and 15-25% of the injected albumin were present in blood at 24 and 48 hours. This conjugation method provides a simple technique of radioiodination of protein, peptide and oligonucleotide, lacking activated phenol ring. These tracers could be used for the studies of turnover, imaging and hybridization.

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Acknowledgements

The supports of Department of Energy grant DE-FG05-88ER60728, Florida High Technology and Industry Council, Baxter-Bentley Corporation are gratefully acknowledged.

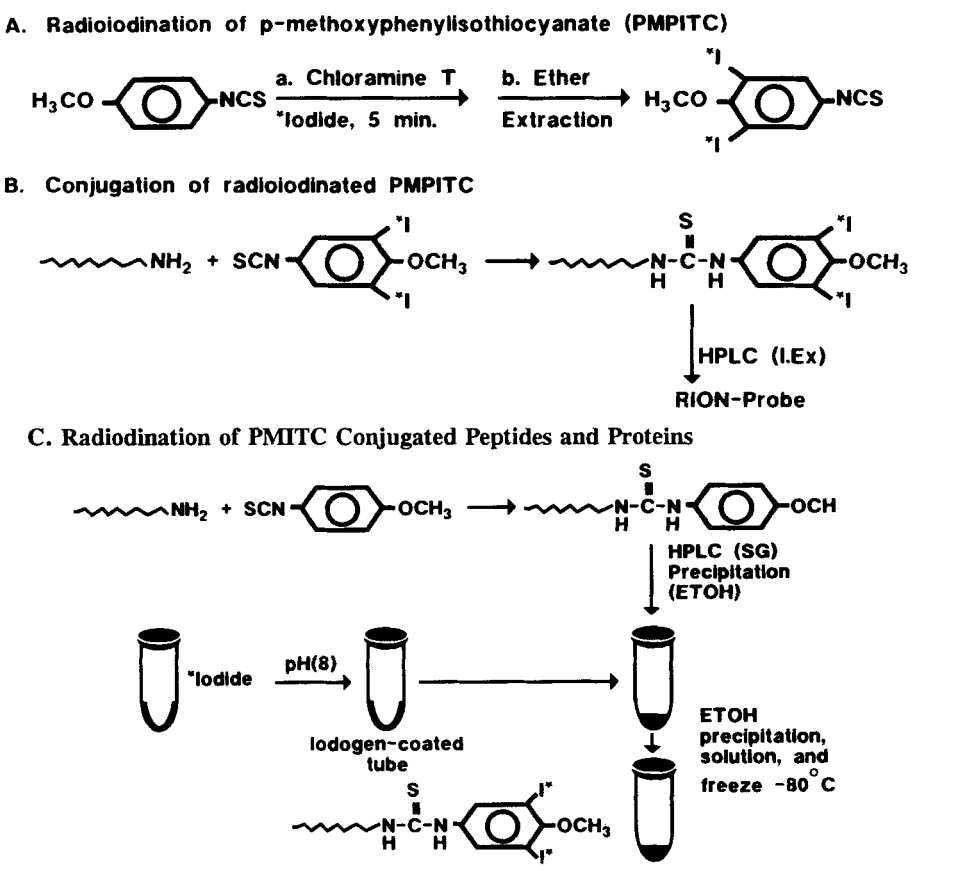

Figure 1. The radioiodination of 4-paramethoxyphenyl isothiocyanate and conjugation of RPMPITC with peptides, proteins and oligonucleotides.

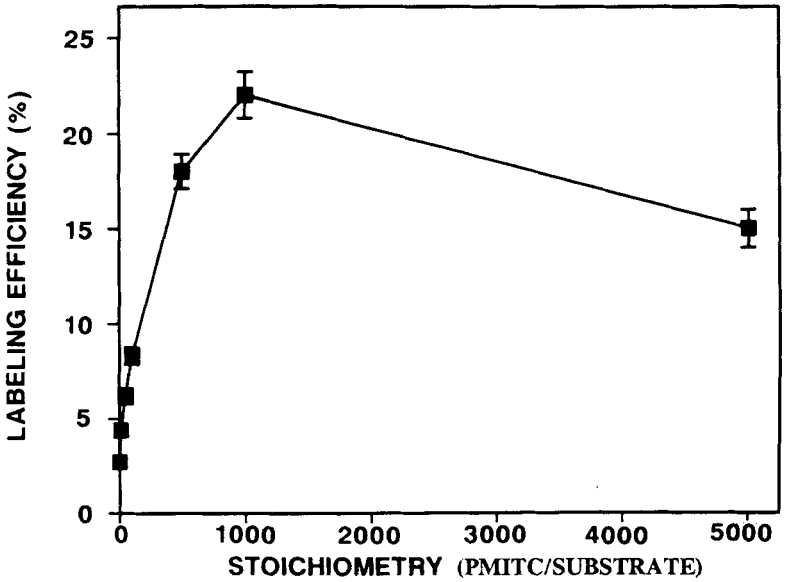

Figure 2. The effect of PMPITC/substrate on the labeling efficiency

A SHORTLIVED METALRADIONUCLIDE LABELED IMMUNOGLOBULIN-G FOR IMMUNORADIOMETRIC ASSAY: AN APPROACH FOR ACHIEVING Ga-LABELING OF IMMUNOGLOBULIN-G WITH HIGH SPECIFIC RADIOACTIVITY.

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Immunoradiometric assay (IRMA) has become an essential analysis methodology in the bio-science field. Recent research, being directed towards the molecular-level, requires highly sensitive analytic systems. However, carrier-free I-125, the most popular labeling nuclide, has sensitivity limits as high as fmole level. To detect molecules of lower concentration range than this, new labeling methodology with higher specific radioactivity is needed.

To satisfy this requirement, radionuclide with shorter half life was of great interest. Under carrier-free conditions, half-life is in inverse proportion to specific radioactivity. In this study, radiogallium (Ga-67: half life = 72 hr, Ga-68: half life = 68 min) was selected as a nuclide for IgG labeling, using deferoxamine (DF) as a bifunctional chelating agent [1-5]. Theoretically, the combination of Ga-67 and DF-IgG should reach 7400-11100 MBq/mg of specific radioactivity. However, it actually reaches only 37 MBq/mg protein, 10 times lower than I-125. This might be caused by the binding of DF-IgG with Fe ions, tracely contaminated in the Ga-source. Thus, purification and concentration of the Ga-source was performed by extraction for the achievement of Ga-labeling of IgG with very high specific radioactivity.

Selective Ga-67 extraction was rapidly completed with butyl acetate from Ga-67 aqueous solution containing 5 N HBr. Under this condition, Fe extraction could be lowered to 0.6 %, when 0.1 M ascorbic acid was present as a Fe-reducing agent. After extraction, the butyl acetate layer was collected, evaporated and Ga-67 dissolved in 20-30 ul of 0.05 N Fe-free HCl solution to make Ga-Labeling Solution (Ga-LS). In the Ga-LS, Fe was not detectable when Fe-free HCl was used as a reference. After the purification, high Ga-67 concentration of 9287 MBq/ml was obtained. Using the Ga-LS and DF-IgG with DF/IgG conjugation level of 0.7-0.8, very high labeling efficiency (> 95 %) was obtained even under low protein concentration condition (200 ug/ml, Fig. 1). Under the same condition, commercial Ga-67 showed rather low labeling efficiency. As for the specific radioactivity, 872 MBq/mg IgG could be achieved (Table 1).

For the application of Ga-DF-IgG to the IRMA system, two further conditions must be satisfied; high labeling stability and preserved immunoreactivity of Ga-DF-IgG. As for the former, 24 hours standing under 10000 times dilution showed no alteration, an indication of high stability. With respect to the latter, the labeling conditions had no effect on the immunoreactivity of IgG (Fig. 2). In addition, in our previous paper, the effect of DF conjugation with IgG on immunoreactivity has been negligible [5]. Thus, this labeling methodology was totally inert to the immunoreactivity of the IgG.

In conclusion, IgG could be labeled with Ga-67 with higher specific radioactivity than that of I-125. Unlike the case of I-125, labeling was completed by simple mixing of the Ga-LS and DF-IgG solution, without following purification. Moreover, the use of shorter half-lived radionuclide will contribute to the reduction of radioactive waste. The present method will provide a new type of IRMA system having higher sensivity and easier handling.

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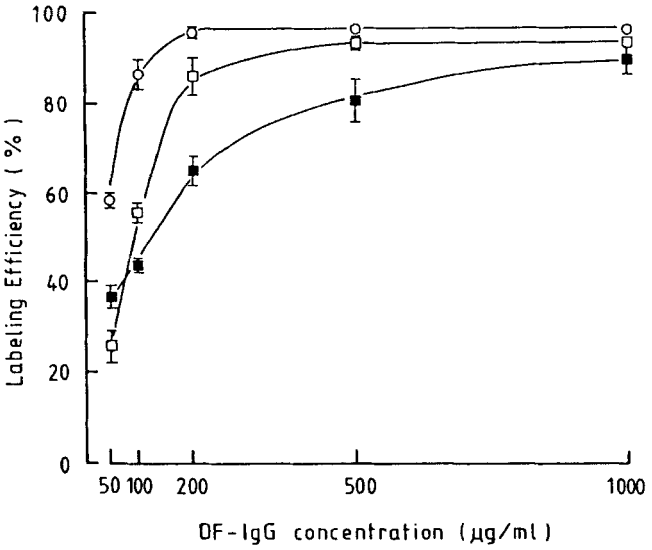

Fig. 1. The labeling efficiency of DF-IgG conjugates(50, 100, 200, 500 and 1000 ug/ml) when they were labeled with the prepared ^{67}Ga-LS (○ , n=5), $^{67}GaCl_3$ (■ , n=4), or $^{67}GaCl_3$ with ascorbic acid (□ , n=4). Each point represents the mean and each bar represents the s.d.

Table 1. Labeling efficiency and specific activity of ^{67}Ga-DF-IgG labeled with high concentration ^{67}Ga-LS.

						mean±s.d.
^{67}Ga-LS (MBq/ml)	10175	9731	9361	8510	8658	9287±705
Labeling efficiency (%)	93.9	91.9	92.2	95.8	96.3	94.0±2.01
Specific activity (MBq/mg)	955	895	862	814	833	872±55.7

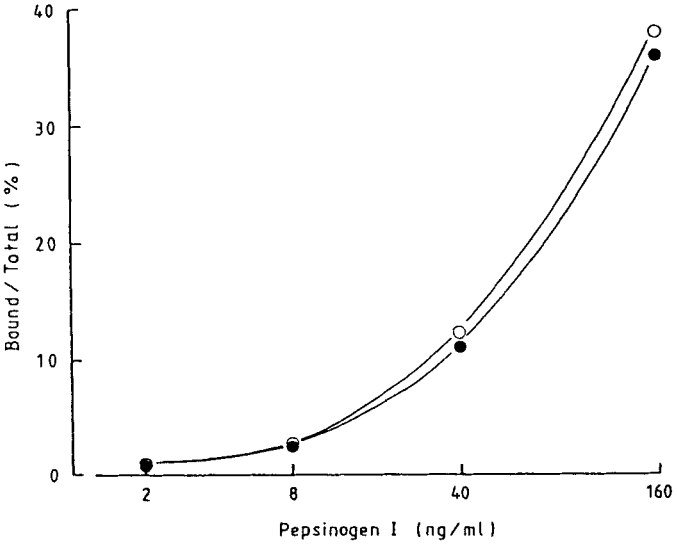

Fig. 2. Standard curves of IRMA, Pepsinogen I RIABEAD, using the beads treated for 1 hr under the labeling condition(O) and the control(●). Each point is the mean of triplicated study.

LIPOSOMES, A NEW VEHICLE FOR THERAPEUTIC APPLICATIONS OF RE-186 AND RE-188

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The increasing interest in the application of liposomes in pharmaceutical preparations, parallelled by the search for safe application of radionuclides for therapy, led to the in vitro investigation of Re-liposomes. The properties of this new preparation are allowing us to decide whether in vivo applications are usable and if so which ones may be considered.

Liposomes consist of a phospholipidbilayer that forms a vesicle, containing an aqueous inner volume. These biodegradable and non irritating liposomes are produced by gelfiltration and have a diameter of 70-80 nm.

Three possibilities have been tested to load the liposomes with radioactive Re (fig. 1):

1) Entrapping of a watersoluble complex (e.g. ReO_4^-) in the aqueous inner compartment of the liposomes.
2) Incorporation of a very lipophilic complex (e.g. Rephos, fig. 2) in the membrane of the liposomes.
3) Derivatisation of a ligand (e.g. DTPA) with stearylamine and anchoring this ligand into the liposome bilayer during the liposome formation. Complex formation is followed by using perrhenate and a reducing agent.

The yield of the first and third type of loading was very low, only about 0.6 % and 1.5 % of radioactive Re was bound. The best result was obtained with the second type, where up to 54 % incorporation and a good stability were observed. The Rephos complex was prepared with the natural isotope mixture of Re-185 (37.4 %) and Re-187 (62.6 %), and was irradiated with neutrons to produce Re-186 and Re-188. A high yield of radioactive Rephos complex was observed, only 10-15 % ReO_4^- have been detected after irradiation and no other byproducts.

The procedure was usually as follows: 1.4 mg Rephos were irradiated in a quarz ampoule with a neutron-flux of $5.84 \cdot 10^{13}$n $cm^{-2}sec^{-1}$ during 44.5 h. At EOB we found 2276 MBq Re-188, 707 MBq Re-186, 11.7 MBq Cl-38, 1.8 MBq P-32.

We suggest Re-liposomes for therapy especially for the radiosynoviorthesis of the knee.

fig. 1

The three principal possibilities of loading liposomes with Rhenium.

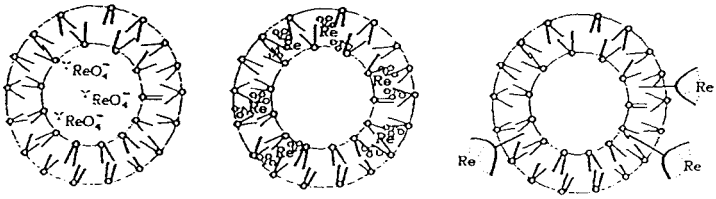
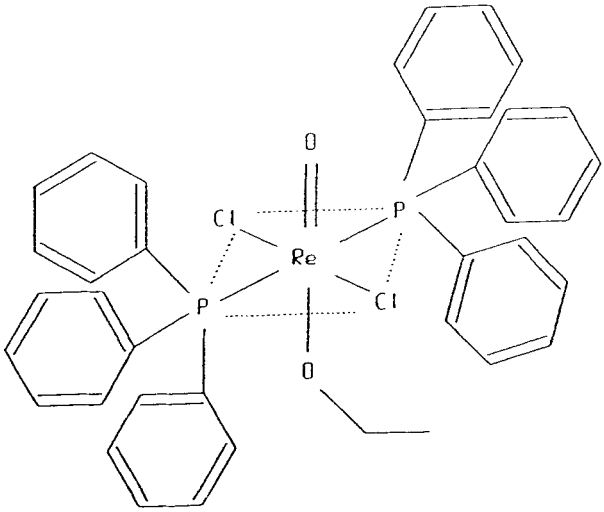


fig. 2

The structure of Oxodichloroethoxy-bis (triphenylphosphine)-rhenium(V), i.e. the Rephos-complex.



Evaluation of monoclonal antibody uptake in small target volumes by quantitative autoradiography.

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There are targeting situations which can only be adequately dealt with when it becomes possible to determine antibody uptake in **small target volumes**: 1) In many systems intra-tumoral radioactivity deposition is patchy. Thus it is important to know the uptake ratio of hot foci versus cold background areas. 2) With respect to small spontaneous metastases it is essential to evaluate uptake both in terms of the absolute amount and the relation to macroscopic lesions. In both situations, relevant data can only be gathered by autoradiographic techniques. In our work emphasis was put on control and correction procedures designed to verify that the finally obtained computer read-out was reflecting the actual radioactivity level in the volume of interest.
To obtain quantitative data from autoradiographic images a transformation of the film density signal into Bq per unit volume is needed. The inherent calibration problem could partly be solved by using tissue-eqivalent standard preparations. However, when aiming at a quantification of radioactivity in small areas (<2mm diameter), special criteria had to be elaborated for the reconstruction of the area in the dose matrix and for the correct integration of the radioactivity content.

The method was applied to the murine lymphoma ESb-Mp because this lymphoma variant reproducibly yields a high number of spontaneous liver metastases and because a selectively binding monoclonal antibody was available. We were able to measure radioactivity concentrations in metastases with diameters down to 200 µm, to define the size-dependence of uptake, and to compare the ranges of uptake recorded in small metastases as opposed to macroscopic tumor nodules. Alternatively, a semi-quantitative version of the method resulting in radioactivity profiles through selected regions was applied to xenograft nodules. These showed non-uniform radioactivity distribution in the form of either intra-tumoral hot foci or preferential accumulation in the periphery. The profiles yielded data pertaining to relative accumulation in "hot" and "cold" areas as well as to shifts in the radioactivity distribution occurring with increasing time intervals after injection of labeled antibody.
On the basis of data gathered in model experiments, we discuss the experimental conditions that are suitable for either a truly quantitative determination of radioactivity concentrations per voxel or only a semi-quantitative evaluation. Further aspects of concern are the spatial resolution and the minimal size of an area to be analyzed, as well as the problem of a three-dimensional evaluation to be achieved by compiling consecutive tissue slices. We think that the basic concepts of our approach will be readily applicable to similar problems in other systems.

This work was supported by the Tumorzentrum Heidelberg-Mannheim.

Radiometal Labeling of Immunoproteins: A Study of the Covalent Attachment of SCN-Bzl-DTPA Ligands to Immunoglobulin.

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Recently, we have reported a series of biodistribution studies of radiolabeled MoAb employing SCN-Bzl-EDTA, SCN-Bzl-DTPA, and various alkyl derivatives of SCN-Bzl-DTPA (1-3). We have now conducted a systematic study of the chemical factors which influence binding of the SCN-Bzl-DTPA family of ligands [specifically, ^{14}C-labeled 2-(p-isothiocyanatobenzyl)-4-methyl-diethylenetriaminepentaacetic acid (1M3B-DTPA)] to proteins. Variables explored include: pH, reaction period, temperature, and ligand and protein concentrations. With the exception of protein concentration, these variables have significant effects on the rate of protein conjugation. In addition, the effect of the concentration of chelate-ligand-conjugated-IgG (conjugated-IgG) on the ^{111}In-radiolabeling yield was also examined. Human IgG, a polyclonal protein with an average molecular weight of 150 kDa, was used as the protein model for these investigations. This choice was primarily based on the ready availability of this protein in high purity and high concentration. Finally, the direct applicability of the results and methodology presented in this work was demonstrated by results from conjugations of three clinically used MoAb's (B72.3, Anti-Tac, and UPC10) with the 1M3B-DTPA ligand. All measurements of ligand binding are presented in terms of a final chelate ligand to protein molar ratio [$(CL/P)_f$; moles of ligands bound per mole of protein] as determined by use of ^{14}C labeled ligands.

The effect of pH was demonstrated by a 7 fold increase in the labeling efficiency when pH was increased from 6.7 to 9.0. Above pH 9.0 the reaction rate was rather independent of pH (Figure 1). The kinetics of the reaction were studied at 5, 15, 25, and 35°C. For reaction periods up to 17 hours an exponential dependence of yield on temperature was obtained which was expected for a bimolecular reaction. This exponential dependence was diminished over longer reaction periods and at higher temperatures (Figure 2). First order dependence of the reaction rate on the ligand concentration was obtained over ligand concentration range of $3.0x10^{-5}$ to $5.5x10^{-4}$ M (Table 1). A 10-fold increase in the concentration of IgG, from 2.4 to 23.4 mg/mL resulted in only a 1.4-fold increase in the $(CL/P)_f$, from 1.12 to 1.58. When IgG and three other monoclonal antibodies were simultaneously conjugated under identical experimental conditions, no significant variation was observed in conjugation efficiencies. The results of the effect of the concentration of 1M3B-DTPA-IgG on the radiolabeling yield at constant ^{111}In activity are summarized in Table II. The radiolabeling yield remains about 90% when the concentration of conjugated IgG [with $(CL/P)_f=0.23$] in the reaction mixture decreased over an order of magnitude from $1.3x10^{-5}$M to $1.7x10^{-6}$M. Further decrease in concentration of conjugated IgG resulted in a decrease in radiolabeling yield. When the concentration of conjugated IgG was $6.7x10^{-6}$M, the yield dropped to 69%. The ratio of activity (μCi) incorporated per microgram of protein also decreased from 4.8 to 0.32 as concentration of conjugated protein increased from $6.7x10^{-7}$M to $1.3x10^{-5}$M (Table II, column 4). It was not possible to attain a 100% radiolabeling yield under any conditions. These results indicate that it is possible to define a set of standard conditions for the reaction of SCN-ligand with MoAbs.

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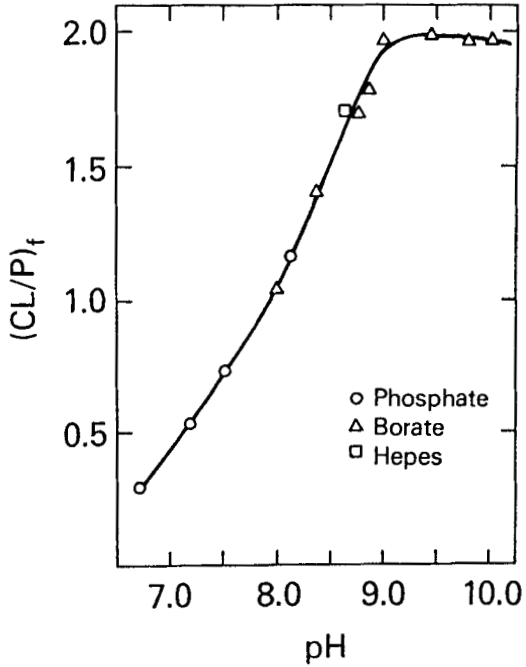

Figure 1. Variation of $(CL/P)_f$ as a function of pH. $(CL/P)_i$=3.9±0.4, $[IgG]_{rxn}$=6.1x10^{-5} M, Reaction Period = 16.6 h, pH=8.62.

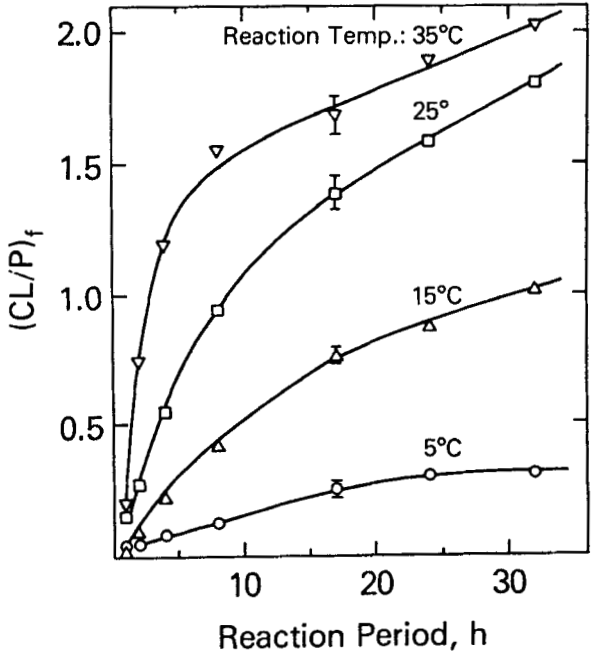

Figure 2. Effect of reaction period on $(CL/P)_f$ for various temperatures. $(CL/P)_i$=3.7, $[IgG]_{rxn}$=5.7x10^{-5} M, pH=8.42.

Table I. Effect of Concentration of Chelate-Ligand[a]

[ligand], M	$(CL/P)_f$	$(CL/P)_i$[b]	$(CL/P)_{0i}$
$3.88x10^{-5}$	0.73	0.33	0.45
$6.25x10^{-5}$	1.17	0.55	0.47
$1.17x10^{-4}$	2.19	0.93	0.43
$1.82x10^{-4}$	3.41	1.42	0.42
$2.42x10^{-4}$	4.54	1.84	0.41
$5.50x10^{-4}$	10.3	3.71	0.36

a) [IgG] = $(4.6\pm0.2)x10^{-5}$; (6.9±0.3 mg/ml), T = 27°C, Rxn period = 17.3 h, pH = 8.62, Vol. of rxn = 460 μl. b) $(CL/P)_i$ = initial chelate ligand to protein molar ratio.

Table II. In-111 Labeling of Conjugated IgG; Effect of the Concentration of Conjugated-IgG on Radiolabeling Yield at Constant ^{111}In Activity[a]

[Conj-IgG], M	$(CL/P)_f$	Radiolabeling[b] Yield, %	Ratio of ^{111}In to Conj-IgG, μCi/μg	Fraction of[c] Available Sites Occupied by ^{111}In, %
0.0[b]	0.0	0.0	0.0	0.0
$6.7x10^{-7}$	0.23	69	4.8	31
$1.3x10^{-6}$	"	72	2.5	16
$1.7x10^{-6}$	"	89	2.5	16
$3.3x10^{-6}$	"	92	1.3	8.2
$6.7x10^{-6}$	"	93	0.65	4.1
$1.3x10^{-5}$	"	90	0.32	2.0
$1.3x10^{-5}$	0.35	92	0.32	1.4

a) Vol. of rxn mixture = 150 ul, T = 27 °C, pH = 4.0-4.2, Rxn period = 30 min, Activity of ^{111}In = 350 μCi. Concentration of protein in the reaction mixture was adjusted to $1.3x10^{-5}$ M by addition of unconjugated IgG. b) 1.0 mg of unconjugated IgG. c) Based on the specific activity of commercialy available ^{111}In.

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TC-99M LABELED MONOCLONAL ANTIBODIES: EVALUATION OF AGENTS FOR DIRECT LABELING.

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The major thrust of current investigations into the use of radiolabeled monoclonal antibodies (MAbs) is focused on the development of their applications for diagnostic imaging. The suitability of it's physical characteristics and availability throughout the world make Tc-99m attractive for this purpose. Among the several methods reported, the procedures in which MAbs are labeled directly with Tc-99m via the controlled reduction of protein disulfide groups to sulfhydryls are most appealing because of their simplicity, possible preservation of immunoreactivity, and adaptability to a "kit" procedure (1-5). The objective of this work was to evaluate agents that will reduce protein disulfides to sulfhydryls, determine the optimal chemical conditions for maximum Tc-99m binding, assess the stability of the tracer and examine immunoreactivity of the protein following the labeling procedure. The agents $SnCl_2$, 2-mercaptoethanol (2-ME), the threo (DTT) and erythreo (DTE) isomers of 2-3 dihydroxy-1,4-dithiobutane or ascorbic acid (AA) were incubated in predetermined molar ratios at 22°C for 60 min. with IgG or IgM isotypes. Excess of agent was eliminated and MAbs treated with Tc-99m, incubated with no more than 0.8 ug/ul $Na_2S_2O_4$. The quantity of radioactivity associated with protein was determined by HPLC and ITLC. The optimal protein to reducing agent molar ratios and the % Tc-99m associated with protein are given in Table-1. Using each agent several Tc-99m preparations of one antibody (IgM, MCA-480) specific for human neutrophil membrane antigen lacto-N-fucopentoase, were examined for immunospecificity (Table-2). Binding to cells not bearing the specific antigen was only 16 +0.5%. These data indicate that the better results are obtained with AA as reducing agent. An MAb (IgG) labeled with Tc-99m using AA was also evaluated with immobilized specific antigen assay. Results are given in Table-3. The tracer was stable when challenged with 500 molar excess of DTPA and HSA, transchelating <1% of the radioactivity. Iodoacetate, ($CH_2S^- + ICH_2COO^- \longrightarrow CH_2SCH_2COO^- + I^-$) added to reduced protein decreased Tc-99m binding as did cysteine in 1:1 (81%) and 100:1 (36%) molar ratio to protein. These indicated sulfhydryls as binding sites for Tc-99m. No greater than 2.7% of the available disulfides, as determined spectrophotometrically, were reduced and no fragmentation of the MAb was observed.

We conclude that i) controlled reduction of MAb disulfides is an efficient approach for labeling MAb with Tc-99m, ii) AA is the best agent among the five evaluated, and iii) the method may also be useful for labeling MAbs with Re-186 for therapy.

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Table-1

PROTEIN TO REDUCING AGENT MOLAR RATIOS FOR THE OPTIMAL QUANTITY OF TC-99M INCORPORATED.

Reducing Agent	2-ME	$SnCl_2$	DTT	DTE	AA	AA
Type of MAb	IgM	IgM	IgM	IgM	IgM	IgG
Molar Ratio	1000	0.5mM*	1500	1000	3500	3500
%Tc99m	55±3	65±3	55±3	55±3	98±5	96±3

*Taken directly from Ref(1).

Table-2

IMMUNOREACTIVITY OF HUMAN NEUTROPHIL SPECIFIC MCA-480 (IGM) FOLLOWING RADIOLABELING WITH IN-111 AND TC-99M

Radionuclide	Intermediate Agent	% Cell bound
In-111 (control)	C-DTPA	75±2
Tc-99m	AA	84±1
Tc-99m	$SnCl_2$	66±1
Tc-99m	2-ME	63±4
Tc-99m	DTT	62±3
Tc-99m	DTE	62±2

Table-3

INCORPORATION OF TC-99M IN PROTEIN AND % BOUND TO SP. ANTIGEN IMMOBILIZED ON SEPHAROSE BEADS.

Prep. No.	%Tc-99m bound to IgG		Sepharose Column	
	ITLC	HPLC	% bound to Column	%Total Recovery
I	95	89	84	99
II	98	91	82	91
III	96	93	82	98
	96.3±1.5	91±2	82.6±1.1	96 ± 4.3

RADIOPHARMACEUTICAL FOR HEPATOCELLULAR CARCINOMA (I) IODINE-125 LABELING ANTIFERRITIN: AN IMPROVED METHOD

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Iodine-131 antiferritin, a new treatment modality in hepatoma: a radiation therapy oncology group study by S.E. Order et al.. (1)Liver Cancer Institute of Shanghai Medical University (2) has applied I-131-antiferritin to treatment of hepatoma and has successfully treated hepatoma with comprehensive therapy of radiolabelled antiferritin and surgical excision. At present, usually in clinic radioiodined antiferritin used lactoperoxidase or chloramin T method. With this method, dozens millicuries iodine-131 were used. After reaction, I-131-antiferritin and free I-131 were necessarily separated and consuming-time and high radiation dose. The labeling efficiency of this procedure is only 75%.

In order to search for simple and rapid radioiodinated method and increase labeling efficiency, we used I-125 to label antiferritin with Iodogen method and studied labeling condition of this method and factors of influencing labeling efficiency. So as to apply to I-131 labeling antiferritin.

Labeling procedure: 0.020ml (16.9mg/ml) antiferritin was added to Iodogen tube 0.100mg and 0.05ml NaI-125 saline solution (1.4mCi/ml) was added the reaction for 10 minutes. The purity was characterized by ITLC silica gel plate and developed solvent system of chloroform: formic acid: acetic acid (3:1:1) was used. Chromatograms were scanned in PANAX ratemeter radioactivity thin chromatography scanner or the chromatograms were sectioned into 0.5 cm strips counted for radioactivity with Compu Gamma (LKB-1282). Labeling product is at original. Rf of free iodine is 0.9-1.0. See figure 1.

Influence of Iodogen to antiferritin weight ratio for labeling efficiency: Different Iodogen to antiferritin weight ratio (Iodogen/antiferritin (w/w): 1.2, 1.5, 2.3, 3) were reacted at 25°C in saline. Different labeling efficiencies were obtained. See figure 2. Fugure 2 shows that the highest labeling efficiency of 95% can be obtained when Iodogen to antiferritin weight ratio was 1.5. In the case, purification by gel filtration column can be omitted.

Influence of solution pH for labeling efficiency: Constanting Iodogen to antiferritin weight ratio at 1.5 reaction time (10 min.)and reaction temp. (25°C), solution pH: 5.1, 6.1, 7.0, 8.6, 9.5 respectively in bicarbonate buffer. The results were seen in figure 3. Figure 3 shows that the labeling efficiency was highest when pH of solution was 6-7. It was more than 90%. The optimal pH value of Iodogen method demanded: the acidic or neutral condition. It is approximate to the solution pH.

Influence of reaction time to labeling efficiency: Constanting Iodogen to antiferritin weight ratio at 1.5, pH at 6-7, temp. at 25°C and observing influence of different reaction times to labeling efficiency, the results were seen in figure 4. Figure 4 shows that the labeling efficiency is increased with increase of reaction time. The labeling efficiency was kept stable and the value was more than 90% when time is longer than 10 minutes.

Influence of reaction temperature to labeling efficiency: Constanting other reaction condition, the temperature of reaction system were changed between 0-25°C (0°C, 5°C, 10°C, 20°C, 25°C). The results can be seen in figure 5. The figure 5 shows that the labeling efficiency was more than 90% when temperature was changed between 20-25°C. Even though the reaction temp. was 0°C, the labeling efficiency can also be attained 70%. It indicated that influence of the temperature to labeling efficiency is not obvious. It is very favorable in clinical appliance.

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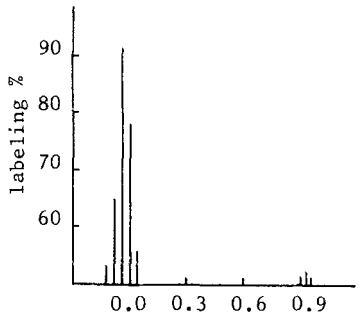

Fig. 1 Rf I-125 and I-125 antiferitin values

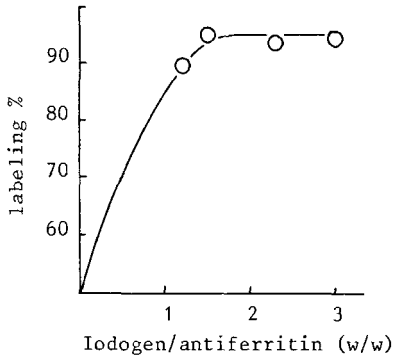

Fig 2. Relations between the labeling % & w/w ratio

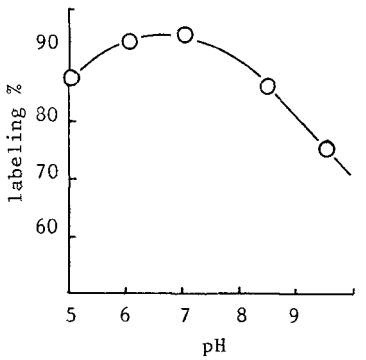

Fig. 3. Relations between the labeling % & the pH values

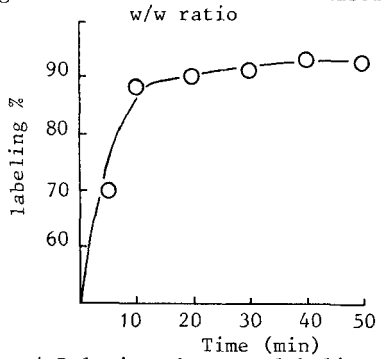

Fig. 4 Relations between labeling % & time

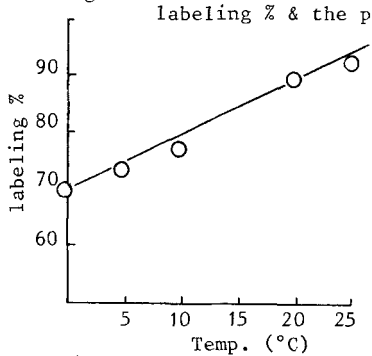

Fig. 5 Relations between labeling % & temp.

RADIOPHARMACEUTICAL FOR HEPATOCELLULAR CARCINOMA (II) DTPA CONJUGATION WITH ANTIFERRITIN AND RADIOLABELED WITH INDIUM

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The radiolabeling antibodies have been trialed in Clinical. Antibodies were labeledwith radioiodine. But radioiodinated antibodies have been proven to be unstable in vivo, e.g. dehalogenation (1). It is apparently reason for the low level of radioiodine in tumor. An alternative approach is radiometallabelled antibody which was covalently modified with bifunctional agent.

Radioiodinated antiferritin has been used in therapy of hepatoma (2,3). In our university, radioiodinated antiferritin has been used in patients of hepatoma. As above reason, we have labeled antiferritin with radioindium. At first we used In-113m for basic studies which purpose is imaging (In-111) and therapy (Y-90) in clinical. For the effective atomic radius of yttrium approaches indium (In 0.81A Y 0.93A), it is possible that the labeling conditions of radioindium can be used to radioyttrium.

The antiferritin was purified from antiserum with sulfate ammonium method, the DTPA cyclic anhydride was synthesized according to the method of C.H. Paik et al (4). Preparing antiferritin-DTPA conjugates in 0.05M bicarbonate buffer solution (pH 8) at molar ratio 1:2, the conjugates was purified by gel filtration chromatograph and determined immunoactivity of the conjugates by Ouchterlony. The results show that immunoactivity of DTPA-antiferritin was same as natural antiferritin. The pure conjugates was labeled with In-113m acetate. The radiolabeled antiferritin was analyzed by ITLC with two solvent systems.

TABLE 1. Rf Values of Radiolabeled Antiferritin and The Radioactive Impurities

Solvent	Ab-DTPA-In113m	DTPA-In-113m	In-113m
pH 6.2, 0.1M actate buffer	0	0.6	0
$CHCl_3$:MeOH:HAc 3:1:1	0	0	0.9

Labeling efficiency attained 98%

The conjugation conditions of DTPA-antiferritin were studied at different molar ratios, pH and antiferritin concentrations. The coupling efficiency of DTPA was measured and the C/P was calculated. (C/P-average number of DTPA molecules attached per antibody molecule).

TABLE 2. Coupling Efficiency and C/P at Different Antiferritin:cDTPA Molar Ratio (Antiferritin Concentration 18.3mg/ml)

Antiferritin:cDTPA	Coupling Efficiency	C/P
1:1	24.0%	0.24
1:2	53.0%	1.07
1:4	61.5%	2.5
1:6	44.1%	2.7

TABLE 3. Coupling Efficiency and C/P at Different pH Values

pH	Coupling Efficiency	C/P
5.09	8.04%	0.32
6.17	31.84%	1.27
7.27	75.6%	3.01
8.00	64.7%	2.53
9.00	44.2%	1.82

TABLE 4. Coupling Efficiency and C/P at Different Antiferritin Concentration (Antiferritin: cDTPA=1:4)

antiferritin concentration	coupling efficiency	C/P
9.7mg/ml	28.6%	1.14
15.2mg/ml	61.7%	2.47
19.4mg/ml	75.2%	3.01
30.4mg/ml	61.0%	2.44

The optimal coupling conditions are pH 7-7.5, antiferritin:cDTPA=1:4, antiferritin concentration 18-20mg/ml. We tried to label antiferritin with Y-90 (contain carrier). For the radioactivity of Y-90 was very low, the labeling efficiency was 30%. We measured immunoactivity of antiferritin-DTPA-Y-90 with RIA method. Its immunoactivity is little change. It is probably that the labeling efficiency can be greatly increase when NCA Y-90 was used. Thus, this basic research provides a method for high labeling efficiency of antiferritin-DTPA-In-113m. The labeling conditions of In-113m can be used to In-111 and Y-90 for the tumor positive imaging and targeting therapy for hepatocellular carcinoma.

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SYNTHESIS OF [^{11}C] MDL 72222 AS A POTENTIAL RADIOPHARMACEUTICAL FOR THE STUDY OF THE 5-HYDROXYTRYPTAMINE 3 ($5HT_3$) RECEPTOR

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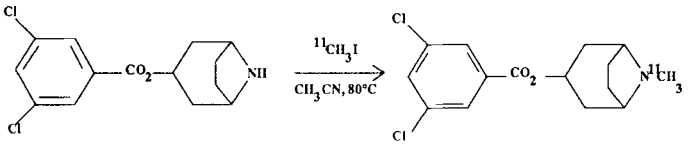
The eventual goal of this work is to determine the distribution of the $5HT_3$ serotonin receptor subtype in the human brain using positron emission tomography (PET). 5-Hydroxytryptamine (5-HT) receptors have been divided into three major classes. *In vitro* radioligand binding techniques have been developed to detect and analyze $5\text{-}HT_3$ binding sites in both the CNS (1-6) and in neuroblastoma cell lines (7a, 7b). Hence, the $5\text{-}HT_3$ receptors have been evidenced *in vitro*, but not yet *in vivo*. Accordingly, we have prepared a positron emitting analogue of MDL 72222 to assess its potential as an imaging radioligand for PET.

Labelling of [^{11}C] MDL 72222 8-[^{11}C]methyl-8-azabicyclo [3.2.1] octan-3-yl-3,5 dichlorobenzoate, endo], was accomplished by N-[^{11}C]alkylation of the desmethyl compound, as is shown in scheme 1.



Scheme 1

Eight min of reaction at 80°C result in 70-75% radiochemical yield based on [^{11}C] CH_3 I. [^{11}C] MDL 72222 and the nor precursor were separated on a µ Porasil (WATERS) HPLC column (78 x 300 mm) by elution with an isocratic mixture: chloroform with 8 % of a 2 % ethylamine - 2 % water in ethanol. Detection takes place at 254 nm, with a flow rate of 3 mL/min. Under these conditions the retention times for [^{11}C] MDL 72222 and the nor precursor were 8 min and 11 min respectively. After evaporation of solvents, the product was formulated for intravenous injection in physiological saline. HPLC gave a product which was found by mass spectrometry to have an identical fragmentation pattern as the authentic sample. The total synthesis time was 40-45 min and the average specific activity obtained (EOS) was on the order of 12.6 - 14.8 GBq/µmole (340 - 400 mCi/µmole) .The radiochemical purity was superior to 99 %.

Investigations using this radiotracer for the *in vivo* study of the $5HT_3$ receptors are presently under way.

Acknowledgements : the authors wish to thank Dr. R. JONES of MERRELL DOW RESEARCH INSTITUTE and for generously providing samples of MDL 72222 and nor precursor for these studies. They thank also F. GOURAND for her technical assistance in this work.

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