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DOE/ER/60460-T3

DOE/ER/60460--T3

DE92 013797

Harvard-MIT Research Program in Short-Lived Radiopharmaceuticals

Technical Progress Report

1991

S. James Adelstein, M.D., Ph.D.
Program Director

Project 1: Studies in Technetium Chemistry (9/1/90-9/1/91)

A. Personnel

Name	Title	Dates of Service	Percent Effort
Alun G. Jones	Associate Professor	09/01/90-present	25
Alan Davison	Professor	09/01/90-present	10
James F. Kronauge	Instructor	09/01/90-present	40
Ashfaq Mahmood	Instructor	10/01/90-present	50
Joel Wolff	Graduate Student	09/01/90-present	100

B. Publications

The following publications represent work partially supported by this funding.

1. Leon A, Verdera S, Leon E, Mut F, Kronauge JF, Jones AG. $^{99m}Tc(CPI)_6^+$: Desarrollo de un juego de reactivos liofilizados y estudios radiofarmacologicos. Rev Esp Med Nuclear 1990; 9: 5-22.

2. Delmon-Moingeon LI, Mahmood A, Davison A, Jones AG. Strategies for labeling monoclonal antibodies and antibody-like molecules with technetium-99m. J Nucl Biol Med. 1991; 35: 47-59.

3. Kronauge JF, Davison A, Roseberry A, Costello CE, Jones AG. Synthesis and identification of the monocation technetium(I) hexakis(2-carbomethoxyisopropylisocyanide) and its hydrolysis products. Inorg Chem (in press).

4. Nicholson T, Davison A, Jones AG. The synthesis of a technetium(V) phenylimido complex from pertechnetate. The single crystal x-ray structure of $[TcCl_3(NPh)(PPh_3)_2].CH_2Cl_2$. Inorg Chim Acta (in press).

5. Kronauge JF, Chiu ML, Cone JS, Davison A, Holman BL, Jones AG, Piwnica-Worms D. Comparison of neutral and cationic myocardial perfusion agents: characteristics of accumulation in cultured cells. Nuc Med Biol. (in press).

6. Verdera ES, Leon AS, Leon ET, Souto B, Oliver P, Rodriguez A, Tamosiunas G, Esponda A, Jones AG, Kronauge JF. Radiopharmacological studies of ^{99m}Tc-CPI: Experience with isolated rat atrial tissue. Nuc Med Biol. (in press).

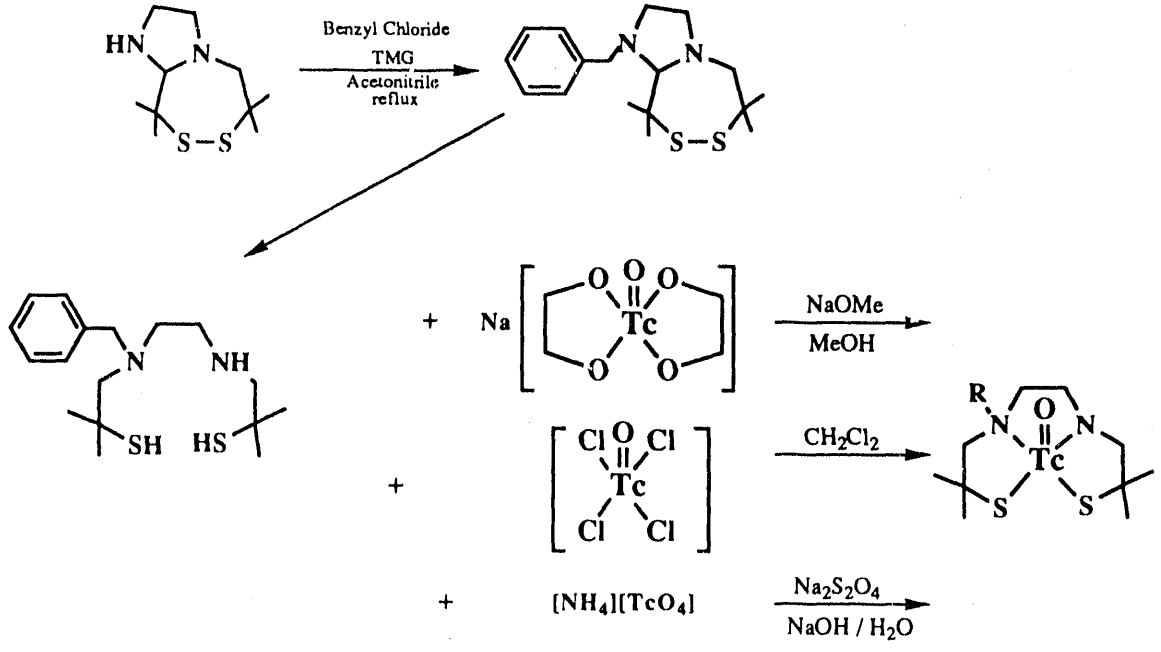
7. Kronauge JF, Leon AS, Verdera S, Balter HS, Leon ET, Mut F, Oliviera MC, Garcia FA, Holman BL, Davison A, Jones AG. Distribution kinetics and radiopharmacological studies of the myocardial perfusion agent {technetium(2-carbomethoxy-2-methyl)ethylisocyanide}$_6^+$, [^{99m}Tc-CPI]. J Nuc Med. (submitted).

8. Dizio JP, Fiaschi R, Davison A, Jones AG, Katzenellenbogen JA. Progestin-rhenium complexes: metal-labeled steroids with high receptor binding affinity, potential receptor-directed agents for diagnostic imaging or therapy. Bioconj Chem. (submitted).

C. Report

In this past year, the aim of developing a chelating system for technetium and rhenium that will allow labeling of peptides and other small biologically active molecules has moved forward. Given our experience with such systems for technetium we believe that the concept of a Tc or Re complex preformed prior to its being linked to a biomolecule is a valid and achievable goal using a functionalized tetradentate N_2S_2 donor. This approach, using C-substituted ligands, has been studied but suffers from two disadvantages. First, there are roughly equal amounts of both *syn* and *anti* isomers formed upon chelation with the metal. Second, the resultant complexes are anions (3). To achieve a neutral complex when bonded to a TcO^{3+} core, a mono N(amine)-substituted diamine dithiol (DADT) or an N-substituted amine amide dithiol (AADT) is required.

The known chemistry for the synthesis of DADT ligands is rather complex. Of particular interest, however, is the mild reduction of 1,2-dithia-5,8-diazacyclodeca-4,8-dienes by sodium borohydride. Reduction of only one of the two imine bonds occurs, leaving the second imine and the disulfide bond intact (4), and yielding a bicyclic compound: an imidazolinol[1,2-d]dithiazepine. Subsequent reduction with lithium aluminum hydride gives a DADT ligand (5).

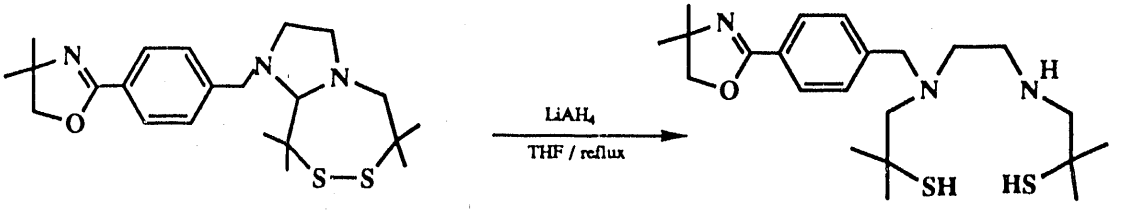


The dithiazepine can be readily mono-alkylated, followed by $LiAlH_4$ reduction and complexation to the oxo(Tc) or oxo(Re) cores, to give the expected neutral N-substituted compounds. For the N-methyl derivative and for the N-benzyl derivative we have found the *syn* isomer predominates. The results for the N-methyl derivative are essentially in agreement with those subsequently reported by Lever (6). In the case of the N-benzyl, no *anti* isomers could be detected by HPLC or by NMR spectroscopy. Our X-ray structures of the Tc and Re N-benzyl

compounds confirmed the *syn* configuration.

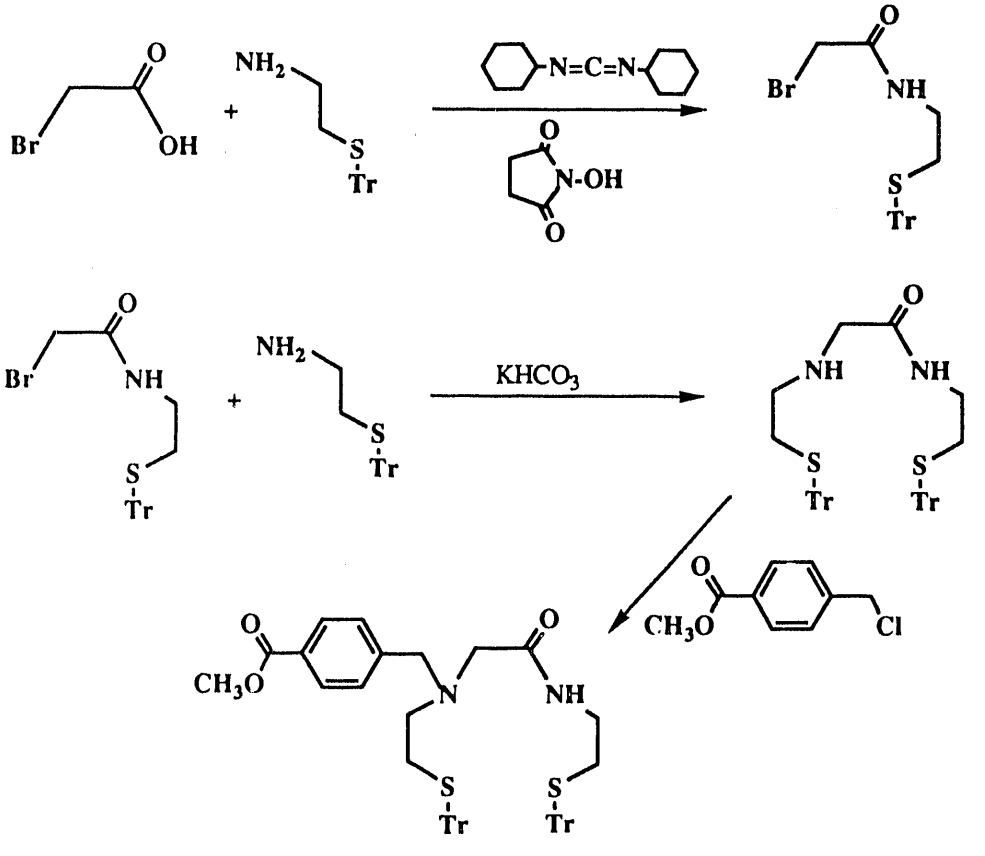
In order to provide a linker for binding to a peptide we intitially chose to explore the use of a carboxylic acid moiety at the 4- position of an N-benzyl DADT ligand. The choice was made to prevent attack of the pendant carboxylate at the sixth coordination site *trans* to the metal oxo group and to minimize potential enzymatic cleavage of the complex to peptide bond (since benzamides do not occur in peptides).

To prevent reduction of the aromatic carboxylic acid or its ester by $LiAlH_4$ in the dithiazepine reduction to the DADT ligand, the carboxylate was protected as a 4,4-dimethyl-2-



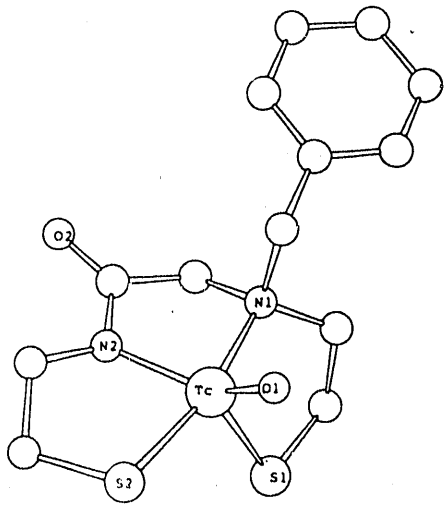
oxazoline. Unfortunately the reduction step gave several products, including the required DADT ligand in only 10% yield. The DADT ligand readily formed the desired technetium complex, but removal of the oxazoline protection did not occur in any appreciable yield.

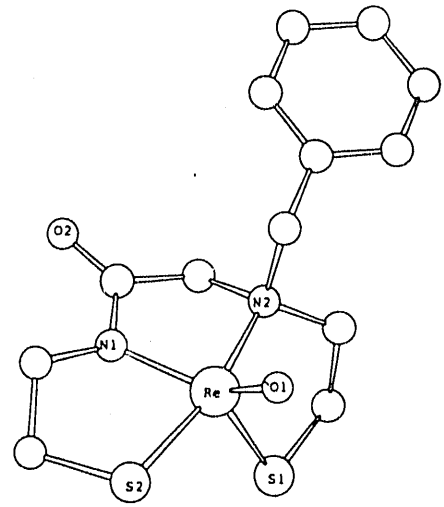
At this stage it was decided that a new synthetic strategy should be attempted, i.e., to preserve an N_2S_2 backbone that could be uniquely N-functionalized and provide a tri-negative ligand with a *p*-carboxylate benzyl substitutent in order to retain the features found in the DADT ligands. Such a ligand would be an amine amide dithiol (AADT). Shortly after we had begun



our synthesis, another example of such an amine amide dithiol was reported at the 8th International Symposium on Radiopharmaceutical Chemistry at Princeton last year, when Fritzberg presented the isomeric ligand backbone (MAMA), though without any details of the synthetic procedure (7).

The N-benzyl and the *p*-carbomethoxy-N-benzyl ligands have been synthesized. The Tc and Re complexes of the N-benzyl ligand have been characterized by single crystal X-ray determinations and shown to have the *syn* configuration. These results are shown below, together with some representative bond distances and angles. As in the case of the benzyl DADT derivatives, there is no evidence for the production of the *anti* isomer in these complexes.





d(Tc=O)	1.663(8)
d(Tc-S)	2.263(4)
	2.263(4)
d(Tc-N)	2.19(1) (amine)
	1.96(1) (amide)
∠ (S-Tc-S)	86.9(2)
∠ (N-Tc-N)	79.1(4)
∠ (O-Tc-S)	115.7(3)
	107.4(3)
∠ (O-Tc-N)	118.3(5)
	102.4(4)

Tc 0.74A above N_2S_2 plane

d(Re=O)	1.668(9)
d(Re-S)	2.269(4)
	2.266(4)
d(Tc-N)	2.18(1) (amine)
	1.97(1) (amide)
∠ (S-Re-S)	88.2(2)
∠ (N-Re-N)	80.0(4)
∠ (O-Re-S)	115.1(3)
	107.5(3)
∠ (O-Re-N)	118.2(4)
	100.9(4)

Re 0.74A above N_2S_2 plane

Recently, in collaboration with Dr. Katzenellenbogen of the University of Illinois, a DADT ligand has been attached to a steroid and subsequently radiolabeled with both ^{99m}Tc and ^{186}Re for in vitro and in vivo evaluation (8). It should be noted that in this case, unlike the situation with proteins and peptides, the metal bonding site is well defined.

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8. Dizio JP, Fiaschi R, Davison A, Jones AG, Katzenellenbogen JA. Bioconj Chem. (submitted).

D. Goals for the balance of this cycle

The goals for the remaining period of this funding remain the same as stated in original renewal submission.

Project 2: **Evaluation of Technetium Acetylacetonates as Potential Cerebral Blood Flow Agents** (August 1, 1990 - August 1, 1991)

A. Personnel

Name	Title	Dates of Service	Percent Effort
Alan B. Packard	Assistant Professor	08/01/90-present	50
S. Ted Treves	Professor	08/01/90-present	5

B. Publications

1991

1. Packard AB. Synthesis and characterization of a ^{99}Tc-porphyrin complex. J Labelled Compd Radiopharm 1991; 30:14-15.

In Press

1. Packard AB. The synthesis and biodistribution of technetium-99m complexes of ß-diketonato ligands. II. 1,1,1-Trifluoro-2,4-pentanedione and 1,1,1,5,5,5-hexafluoro-2,4-pentanedione. Nucl Med Biol.

C. Report

The objective of this project has been the exploration of the chemical and biological properties of technetium complexes of ß-diketone ligands (e.g. acac). Over the last two years this effort has evolved into an evaluation of $Tc(acac)_3$ as a starting material for the synthesis of technetium-porphyrin complexes. This project has provided promising preliminary results which have been used in support of applications to DOE and NIH for more extensive support. Our previous studies with Tc ß-diketones led to the evaluation of the use of the ß-diketone complexes as starting materials for the synthesis of Tc-porphyrins. Complexes of the type $M(acac)_n$ (acac = 2,4-pentanedione, n = 2,3) are commonly used as starting materials for the synthesis of metalloporphyrins (1), and the investigation of this reaction with $^{99}Tc(acac)_3$ is a natural extension of the project. Radiolabeled metalloporphyrins have attracted considerable interest as possible tumor-avid radiopharmaceuticals because of the use of porphyrins as tumor sensitizers in photodynamic therapy (PDT) (2,3). There are, however, only two examples of ^{99}Tc-porphyrin complexes in the literature (4,5) and no examples of well-characterized ^{99m}Tc-porphyrin complexes. This is quite remarkable considering the interest that has been generated by PDT and the extensive use of ^{99m}Tc in nuclear medicine. The synthesis and characterization of ^{99}Tc complexes of TPP (H_2TPP = tetraphenylporphyrin) have, therefore, been undertaken as the first step towards the long-term goal of preparing ^{99m}Tc complexes of water-soluble porphyrins for evaluation as tumor-avid radiopharmaceuticals.

I. Synthesis of Technetium-Porphyrin Complexes

As described previously, the focus of our attention has been the reaction of $^{99}Tc(acac)_3$ with H_2TPP in molten phenol. This leads to the formation of a Tc-TPP complex of the type Tc(O)(X)(TPP) where X is the second axial ligand (6).

$$Tc(acac)_3 + H_2TPP \xrightarrow[200\ °C]{PhOH} Tc(O)(X)(TPP)$$

The excess phenol is removed by sublimation or by extraction of the reaction mixture with 1 N NaOH, and the crude product is dissolved in methylene chloride and chromatographed on silica gel using methylene chloride and methylene chloride/methanol mixtures as eluents. Unreacted H_2TPP and $Tc(acac)_3$ elute first followed by the Tc-TPP complex. The product is isolated by reprecipitation from methylene chloride with n-hexane or methanol.

The uv-visible spectrum of this material shows a pattern typical of metalloporphyrins and similar to that of ReCl(O)(TTP) (7) and $Os(O)_2(TTP)$ (8). The infrared spectrum includes bands typical of Tc=O (940 cm^{-1}) (9) as well as those due to the TPP ligand and phenolate (OPH^-) (10). The mass spectrum, obtained in the FAB^+ mode, includes peaks at m/z=727 ($Tc(O)TPP^+$), 712 ($TcTPP+H^+$), and 651 ($Tc(O)TPP-C_6H_4^+$). No peak is observed near m/z=820 ($Tc(O)(OPh)(TPP)^+$). This pattern is similar to that of Re(O)(OPh)(OEP) (11). These results are not sufficient to identify the second axial ligand (X) as it is easily lost in the mass spectrometer ionization process and it has also proven difficult to remove the excess phenol completely from the reaction mixture.

One effort to drive the reaction to a single product has been derived from the work of Che et al. with osmium porphyrins (12). These investigators have reported that heating a mixture of $Os(O)_2(P)$ ($H_2P=H_2TPP$ or H_2OEP, H_2OEP=octaethylporphyrin) and ascorbic acid in molten phenol for 5 min at 100 °C produced $Os(OPh)_2(P)$.

A similar method has been applied to the mixture of Tc-OEP products. The combined ^{99}Tc containing fractions from the silica gel chromatography of a batch of Tc-OEP prepared from $Tc(acac)_3$ and H_2OEP in phenol is evaporated to dryness, phenol (5 g) and ascorbic acid (100 mg) are added, and the mixture is heated at 100 °C for 5 min. The mixture is allowed to cool to room temperature and washed with a large volume of water to remove the excess phenol and ascorbic acid. The TLC of this product is identical to that of the starting material and still contains three bands indicating that the product has not been driven to a single chemical form.

The problems that have been encountered with eliminating excess phenol from the reaction mixtures have led to the consideration of alternative solvents that might be more easily removed. Glacial acetic acid, which has used successfully by Lawrence et al. in the synthesis of Tc(O)(OAc)(OEP) (5), has been evaluated as a possible solvent for a sealed-tube reaction. It is anticipated that the sealed tube will permit a faster reaction because of the higher reaction temperatures that can be achieved. A similar approach has been used successfully in our earlier studies of technetium complexes of low-boiling ß-diketones.

When $Tc(acac)_3$, H_2TPP, and glacial acetic acid are heated in a sealed tube for 24 h, there is no evidence for the formation of a Tc-TPP complex (TLC). With DMF as solvent, there is also no evidence for the formation of a Tc-TPP complex. Another solvent that has been evaluated is N-methyl-2-pyrrolidinone (NMP, bp 202 °C). In this case, the TLC of the reaction mixture reveals the presence of a new material at the origin of the TLC plate which increases in quantity with longer reaction times. Further investigation, however, has suggested that the predominant component of this material is TcO_2.

The NMP reaction has been repeated with the addition of Sn^0 (powder). In this case, the TLC of the reaction mixture over time reveals that the peak at the origin forms more quickly than in the

absence of Sn^0. Subsequent investigation suggests that this material is not TcO_2, and more extensive examination of this reaction is planned. It must be noted that the possiblity exists that a Sn^{IV}-TPP complex is being formed either in lieu of or in addition to the desired Tc-TPP complex.

The recent publication of an article by Buchler and Kruppa has provided several useful ways in which to deal with the problem of axial ligand exchange (13). In addition, these investigators have confirmed our earlier hypothesis that an exchange reaction was occuring on the chromatographic column between the original complex, the -OH groups of the adsorbant, and methanol in the eluent.

As adapted from the report of Buchler and Kruppa, the exchange reaction proceeds according to the following series of reactions:

Tc(O)(TPP)(X) + HO-Si/adsorbant ---> Tc(O)(TPP)O-Si/adsorbant + HX

Tc(O)(TPP)O-Si/adsorbant + MeOH ---> Tc(O)(TPP)OMe + HO-Si/adsorbant

Buchler and Kruppa have also noted that the species Re(O)(P)(X) are subject to hydrolysis and condensation to form oxo-bridged dimers (e.g. $[Re(O)(P)]_2O$). We have recently begun an examination of the range of substitution products and have obtained crystals of complexes that we believe to be Tc(O)(OMe)(TPP) and $[Tc(O)(TPP)]_2O$.

As methanol is used (with methylene chloride) to elute the complex from the silica gel column, the first effort has been to isolate the methoxy complex. Using the procedure reported by Buchler and Kruppa, a mixture of several different Tc(O)(TPP)(X) complexes has been taken to dryness, dissolved in 20 ml of $CHCl_3$, and 5 ml of MeOH and 100 mg of NaOAc added. The suspension is then heated at reflux for 12 h. The reaction mixture is taken to dryness (rotary evaporator) and washed with water to remove HOAc and NaOAc. The residue is then dissolved in $CHCl_3$, dried with $MgSO_4$, and recrystallized from $CHCl_3$/MeOH, 10:1. The TLC of the solution (silica gel, 5% MeOH/CH_2Cl_2) shows a single spot with R_f slightly less than 1.0. The electronic spectrum of the methoxy complex is similar to that of Re(O)(OMe)(TPP) (13) except for the position of the Soret band which is displaced by approximately 60 nm relative to that of the rhenium complex (398 nm for the technetium complex versus 460 nm for the rhenium complex). A sample of this material has been provided to the mass spectrometry laboratory at MIT but results are not yet available.

A similar reaction has been carried out to prepare the oxo-bridged dimer. A solution of the mixed products of the original phenol reaction has been taken to dryness, redissolved in toluene (20 ml) and then heated with 2 ml of 2 N NaOH for 6 h. The solvent is removed (rotary evaporator), and a large volume of water is added to the reaction flask to precipitate the product. This solid is washed with water until the pH of the wash is <8. The precipitate is then recrystallized from CH_2Cl_2/toluene. The TLC of this material shows the bulk of the material to be present at a single spot with R_f = 0.84, but also indicates two very small components with R_f = 1.0 and 0.41. Again, this is very different from the TLC of the starting material. None of the previous reactions has revealed a product with R_f = 0.84, suggesting that the dimer is not a major component of the original reaction mixture. Comparison of the spectrum of this product to that of the corresponding rhenium complex reveals a pattern similar to that observed with the methoxy complex. Crystals of this material have also been provided to the MIT mass spectrometry laboratory, but the results are not yet available. Efforts are currently underway to obtain larger crystals of these complexes that can be used for x-ray crystallography.

Another observation by Buchler and Kruppa that is relevant to the "axial ligand problem" is the report that $ReCl_3(P)$ is formed when rhenium porphyrins are prepared from $ReCl_5$ in trichlorobenzene. This complex is analogous to $Tc(CO)_3(P)$ which is formed by the reaction of $Tc_2(CO)_{10}$ with porphyrins (4). This suggests the possibility that one of the early products of the reaction of $Tc(acac)_3$ and $H_2(P)$ in phenol may be Tc(acac)(P) in which the acac ligand is bound to the technetium atom out of the plane of the porphyrin ligand in a manner similar to that observed for $Tc(CO)_3(P)$ and $ReCl_3(P)$. No effort has

yet been made to examine the possiblity, but this may be one of the (many) components present in the reaction mixture.

As part of our continuing collaboration with Davison and Jones, studies of ^{99}Tc-porphyrins have also been undertaken at MIT. The emphasis of the MIT studies has been on the evaluation of the new intermediates that have been developed in Dr. Davison's laboratory as starting materials for a more facile synthesis of ^{99}Tc porphyrins that might be more easily extended to the no-carrier-added ^{99m}Tc level.

The first starting material investigated is $TcCl_3(PPh_3)_2MeCN$ (A. Davison, J. Cook, personal communication), which has proved to be a useful starting material for the synthesis of other new technetium complexes (14). Like $Tc(acac)_3$, this complex is neutral and soluble in many of the same solvents that can be used to dissolve the porphyrin ligand. Octaethylporphyrin and $TcCl_3(PPh_3)_2MeCN$ are heated at 160 °C with excess phenol for 13 h. The resulting brown-green solution is chromatographed on silica gel using methylene chloride as the eluent. The third (green) band is collected, flash chromatographed on alumina, and the product precipitated by addition of pentane. The electronic spectrum of this material includes a Soret band at 372 nm with a small shoulder at 420 nm as well as a peak at 230 nm (phenol). The spectrum is consistent with that expected for a metal-OEP complex (1) and also reveals the presence of phenol, either as a contaminant or as one of the axial ligands. The mass spectrum, recorded in the FAB$^+$ mode, indicates the major peaks listed in Table 1.

Table 1. FAB$^+$ mass spectrum of ^{99}Tc-OEP product

m/z	rel. int.	assignment
647	100	$[Tc(O)(OEP)]^+$
419	25	$[Tc(O)(P)]^+$ (i.e., loss of all 8 ethyl groups)
739	5	$[Tc(O)(OPh)(OEP)-H]^+$
663	<5	$[Tc(O)_2(OEP)]^+$

Peaks have also been observed that correspond to the sequential loss of the ethyl groups from the ligand (e.g. m/z=632, 619, etc.).

These results are consistent with formulation of the product as [Tc(O)(X)(OEP)] but, as with the $Tc(acac)_3$/TPP system, are not adequate to identify the second axial ligand: the second axial ligand is frequently lost from metalloporphyrin complexes during the ionization process (1), and the product is known to contain small amounts of phenol. The peaks at m/z = 663 and 739 may, therefore, be observed if [Tc(O)(OPh)(OEP)] is the major product from which phenol is lost either as OPh or Ph during mass spectroscopy, if the major product is [Tc(O)(X)(OEP)] contaminated with a small amount of [Tc(O)(OPh)(OEP)] or if [Tc(O)(OPh)(OEP)] is formed <u>during</u> mass spectroscopy by the reaction of [Tc(O)(X)(OEP)] with the phenol contaminant. In any case, the results do confirm that a complex of the type [Tc(O)(X)(OEP)] has been prepared.

The infrared spectrum also confirms the presence of the OEP ligand but does not include peaks typical of either Tc=O or O=Tc=O (9). The presence of phenol is also confirmed by the infrared spectrum. One explanation for the "missing" Tc=O peak is substitution of the oxo ligand by Br^- during the preparation of the sample as a KBr pellet (15).

A proton NMR has been obtained using $CDCl_3$ as a solvent. Peaks have been observed at 2.05 ppm, $C\underline{H}_3$-CH_2-P; 4.32 ppm, CH_3-$C\underline{H}_2$-P; and 10.7 ppm, methine H. These values are similar to those observed for $[Os(O)_2(OEP)]$ (16). Again, the spectrum confirms the presence of phenol in the sample.

As the complex has proven quite difficult to separate from phenol, a derivative has been prepared in the hope that it will be less difficult to purify. The synthesis of the <u>trans</u>-triphenylphosphine complex has been attempted as described by Collman et al. for the synthesis of $[Re(PPH_3)_2(TPP)]$ (7). The

[Tc(O)(X)(OEP)] product is dissolved in toluene and treated with excess PPh_3. When the PPh_3 is added, the color of the solution turns orange. The orange product is precipitated with ether and the mass spectrum obtained. This spectrum contains a peak consistent with the formation of $[Tc(PPh_3)_2(OEP)]$. The infrared spectrum contains no peak due to Tc=O, although the caution noted above must be considered, and does contain peaks characteristic of the OEP ligand. This product, however, is also contaminated with phenol. These results confirm that a Tc-OEP complex has been prepared, but that this product remains contaminated with phenol, as was observed with the Tc-TPP complexes prepared at Children's Hospital. Other solvents have been used in an attempt to circumvent this problem, but there is no evidence for the formation of a Tc-OEP complex in these reaction mixtures.

A second complex recently synthesized at MIT has also been evaluated as a starting material for the synthesis of Tc-porphyrins (A. Davison, T. Nicholson, personal communication). A xylene solution of the imido complex $Tc(NPh)Cl_3(PPh_3)_2$ is heated at 180 °C for 12 to 18 h with excess H_2TPP. The reaction mixture is chromatographed on silica gel using methylene chloride as the eluent, and a green band is collected. The electronic spectrum of this fraction is consistent with the presence of a metal-OEP complex, and the material has been found to be insoluble in methanol. More complete characterization is underway at this time. In the absence of the problems associated with the use of phenol as a solvent, it is anticipated that this synthesis can be extended to the no-carrier-added level. If this proves possible, it will be the first example of a chemically characterized ^{99m}Tc-porphyrin.

The results that have been obtained in the last year reflect a growing knowledge of the problems associated with the synthesis and an increasing ability to address them, particularly the issue of the labile axial ligands. The ability to more precisely control the axial ligands of these complexes has, in turn, facilitated their characterization. More complete understanding of the behavior of the axial ligands will also allow us to concentrate on the synthesis of the complexes from $Tc(acac)_3$ in phenol rather than having to find a better solvent or to develop a completely new synthetic method.

II. References

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Project 3: **Labeling Antibodies for the Radioimmunodiagnosis and Radioimmunotherapy of Cancer** (August 1, 1990 - August 1, 1991)

A. Personnel

Name	Title	Dates of Service	Percent Effort
Amin I. Kassis	Associate Professor	08/01/90-present	10
S. James Adelstein	Professor	08/01/90-present	10
Janina Baranowska-Kortylewicz	Lecturer	08/01/90-present	20
Zbigniew P. Kortylewicz	Research Fellow	08/01/90-present	50
Rebekah A. Taube	Research Associate	08/01/90-present	60
Annick D. Van den Abbeele	Assistant Professor	08/01/90-present	65

B. Publications

1990

Papers

1. Mariani G, Kassis AI, Adelstein SJ. Antibody internalization by tumor cells: Implications for tumor diagnosis and therapy. J Nucl Med Allied Sci 1990; 34:51-55.
2. Van den Abbeele AD, Aaronson RA, Taube RA, Adelstein SJ, Kassis AI. Preadsorption of radiolabeled monoclonal antibodies to liver and spleen tissues leads to nigher tumor-to-normal-tissue ratios. J Nucl Med Allied Sci 1990; 34:94-102.
3. Adelstein SJ, Kassis AI, Baranowska-Kortylewicz J, Van den Abbeele AD, Mariani G, Ito S. Potential for tumor therapy with iodine-125 labeled immunoglobulins. Nucl Med Biol 1990; 18:43-44.

Abstracts

1. Mariani G, Kassis AI, Adelstein SJ. An experimental model to explore the potentials of antibody internalization for tumor diagnosis and therapy. In: Sagripanti A, Carpi A, eds. Abstract Book of the Second International Congress on Advances in Management of Malignancies, Ascoli Piceno, Italy, May 28-June 1, 1990, p. 113.

1991

Papers

1. Van den Abbeele AD, Aaronson RA, Daher S, Taube RA, Adelstein SJ, Kassis AI. Antigen-binding site protection during radiolabeling leads to a higher immunoreactive fraction. J Nucl Med 1991; 32:116-122.

2. Tumeh SS, Carvalho PA, Van den Abbeele AD, Maguire JH, Rauh DA, Neptune M, Kassis AI. Detection of focal infection by ^{111}In-human polyclonal IgG. J Nucl Biol Med 1991; 35:4-9.

Abstracts

1. Baranowska-Kortylewicz J Adelstein SJ, Kassis AI. Sulfhydryl-selective, photoaffinity, radioiodinating and fluorescent reagent for proteins. Presented at the Thirty-second National Organic Chemistry Symposium, Minneapolis, MN, June 16-20, 1991.

In Press

1. Khawli LA, El-Shourbagy T, Baranowska-Kortylewicz J, Kassis AI. m-[^{125}I]Iodoaniline - A useful reagent for the radiolabeling of biotin. Nucl Med Biol.
2. Khawli LA, Van den Abbeele AD, Kassis AI. N-(m-[^{125}I]iodophenyl)maleimide: A new and useful agent for high yield radiolabeling of antibodies. Nucl Med Biol.

C. Report

The goal of this project is to establish reproducible methods for the production of radiolabeled monoclonal antibodies whose original immunocompetence and biodistribution characteristics are intact.

I. Chemistry Studies

a. Assessment of Alterations in the Primary Structure of Antibodies. Our preliminary evaluation of the damage suffered by the primary structure of antibodies during routine radioiodination indicates that the involved amino acids include tryptophan and cystine residues. In fact, substantial oxidative damage of the indole portion of tryptophan as measured by uv spectroscopy has been observed. These results suggest that the extent of the oxidation depends on the oxidant used as well as on the molar ratio of that reagent to protein (**Fig. 1**). For example, the destruction of tryptophan by Iodobeads is about 10%, which is significantly less than with other oxidants, and the process is accelerated in the presence of sodium iodide. On the other hand, about 50% of the available tryptophan residues are oxidized in the presence of Iodogen at the typically used molar ratio of 30:1 (Iodogen:IgG). Some oxidation of the disulfide bonds also takes place in the presence of chloramine T (CT), and our studies indicate that even with a molar ratio of CT to IgG as low as 10:1 (typically used >300:1), up to 50% of the easily accessible disulfides are damaged (Fig. 2).

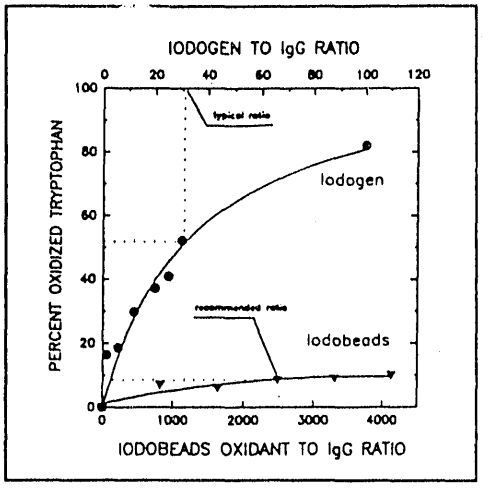


Figure 1. Kinetics of tryptophan oxidation as function of oxidant concentration.

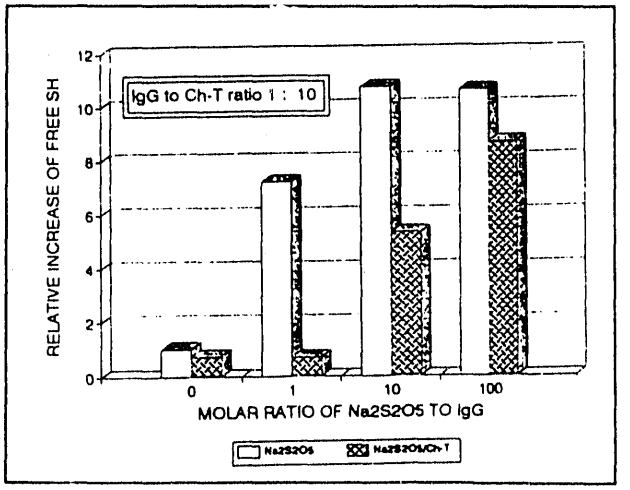
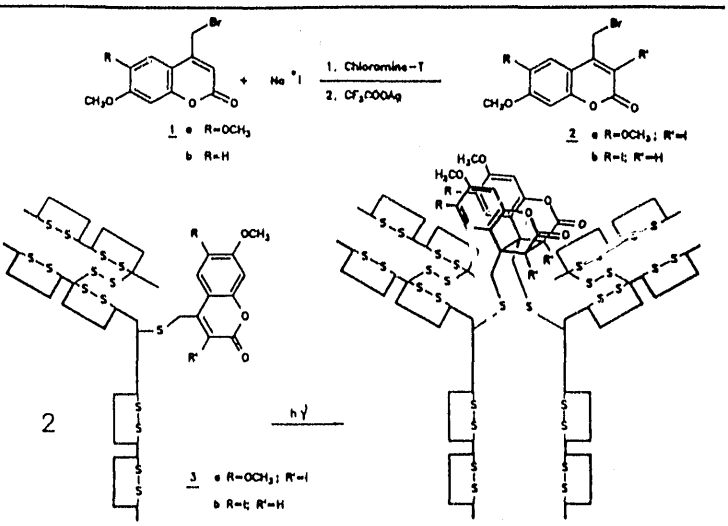


Figure 2. Thiol group production in IgG molecules exposed to chloramine T.

b. Indirect Labeling of Antibody HL Molecules with ^{125}I Labeled Derivatives. To radio-

iodinate (and eventually astatinate) proteins at a site that is distant from the antigen-binding sites, we have synthesized two iodocoumarin (*IC) derivatives (**Scheme 1**), 3-[^{125}I/^{127}I]iodo-4-bromomethyl-6,7-dimethoxy-2-oxo-2H-benzopyran and [^{125}I/^{127}I]iodo-4-bromomethyl-7-methoxy-2-oxo-2H-benzopyran, that will photochemically dimerize the two halves of the antibody molecule and form radiolabeled bivalent antibody molecules (HL-*IC-*IC-HL/ Fab'-*IC-*IC-Fab'). The 3-iodo analog (2a) produces dimers with the iodine atoms on the cyclobutane portion of the dimer. Since aliphatic iodides are less stable in vivo than aromatic ones, the 6-iodo derivative of coumarin (2b) has also been prepared (i.e. the iodine atom is located on the aromatic ring).



Scheme 1

4-Bromomethyl-6,7-dimethoxy-2-oxo-2H-benzopyran (1a) and 4-bromomethyl-7-methoxy-2-oxo-2H-benzopyran (1b) are fluorescent reagents that react exclusively with the free sulfhydryl groups of proteins. The radioiodination of these reagents in the presence of silver trifluoroacetate with CT or N-chlorosuccinimide as the oxidant gives the 3-iodo-2a and 6-iodo-2b derivatives, respectively. Both compounds also react selectively with thiol groups generated during the reduction of disulfide bonds of antibody molecules with dithiothreitol (**Scheme 1**). Unsensitized photodimerization of antibodies conjugated to 1a, 1b, 2a, or 2b regenerates immunoglobulins selectively labeled at the sulfhydryl groups in their hinge region. Polyacrylamide-sodium dodecyl sulfate electrophoresis of uv-irradiated immunoglobulins conjugated to 1a, 1b, 2a, or 2b and treated with mercaptoethanol has confirmed the location and covalent character of this conjugate.

c. Radiolabeled Biotin Derivatives. The exceptional affinity of biotin for avidin has been explored for use in tumor diagnosis (^{123}I) and therapy (^{211}At) with avidin-biotin-antibody conjugates in conjunction with radiolabeled biotin. There are however several problems associated with this approach. One difficulty is the preparation of a no-carrier-added radiolabeled biotin derivative in high yield while preserving its high affinity for avidin.

The radiohalogenation procedures based on electrophilic substitution of N-biotinyl-3-tri(n-butyl)stannylaniline require strong oxidants of iodine, and the presence of these leads to extensive oxidation of the sulfur atom within the biotin ring. Therefore, the yield of the desired ^{125}I-anilide of biotin is very low (≤5%) and its purification difficult. Nevertheless, this iododerivative exhibits a K_a of ~3.5 x 10^{14} M^{-1} (**Fig. 3**), although it has been reported that the formation of sulfoxide and sulfone reduces the association constant at least 10^4 times. Our early attempts to synthesize radioiodinated biotin from no-carrier-added radioiodoaniline have also failed because of the low reactivity of the biotin derivatives employed and the side reactions taking place in preference to anilide formation. Furthermore the overall reaction times are far too long for the preparation of biotin labeled with short-lived isotopes such as ^{123}I or ^{211}At.

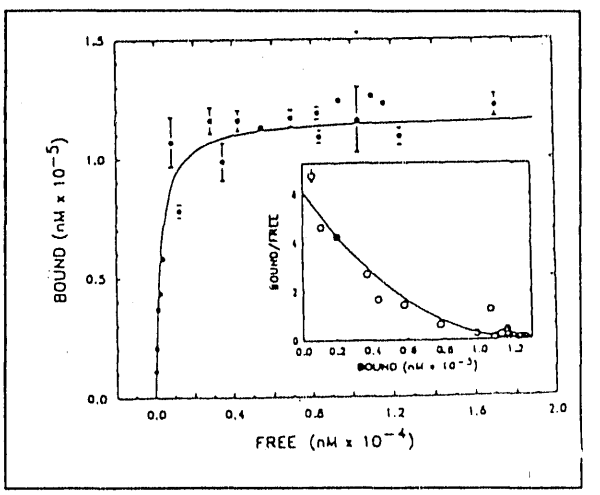
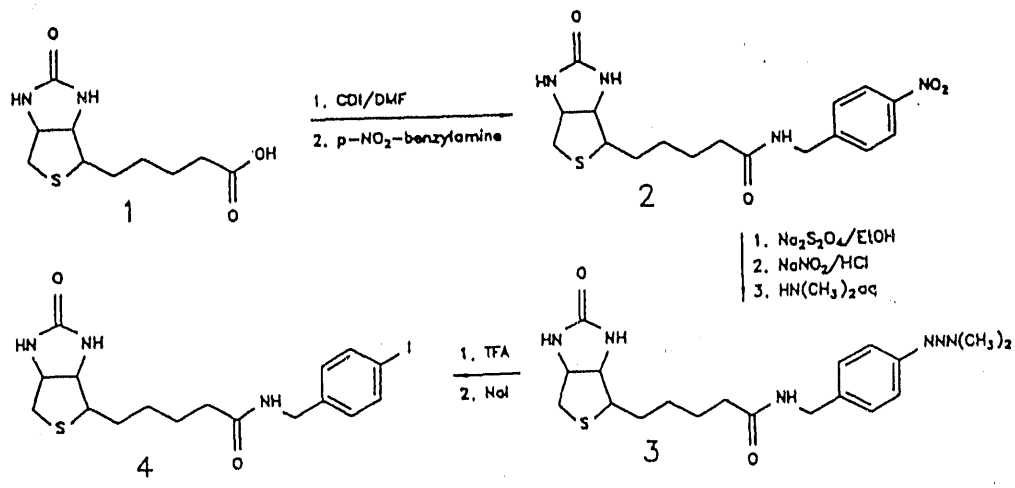


Figure 3. Binding kinetics of radioiodinated anilide of biotin.



Scheme 2

We have explored another synthetic path to radiolabeled biotin based on triazine derivatives. This allows for rapid introduction of $^{125}I/^{127}I$ without oxidants or addition of carrier during the final iodination step (**Scheme 2**). According to our method, the p-nitrobenzylamide **2** of biotin, obtained in high yield after activation of biotin with N,N-carbonyl diimidazole, is reduced to p-aminobenzylamide and then diazotized. The diazonium salt is reacted in situ with an excess of dimethylamine to give the corresponding triazine **3**. This compound serves as a convenient precursor of p-iodobenzylamidobiotin. Radioiodination requires only the acidification of the acetonitrile solution of **3** prior to the addition of sodium iodide. The reaction is instantaneous, giving only gaseous dimethyl amine as a by-product. An average isolated yield of **4** is 40% (n=5) following silica gel column purification.

The binding of **4** to avidin has been studied under equilibrium dialysis conditions in phosphate buffered saline (PBS), pH 7.2, at a constant avidin concentration of 30 nM and variable concentrations of $^{125}I/^{127}I$-benzylamidobiotin. The data have been analyzed using nonlinear regression and the association constant, determined from the Scatchard plot (**Fig. 4**), is 2.54×10^{14} M^{-1} with a correlation coefficient of 0.84 and the number of binding sites 4.7 ± 0.9.

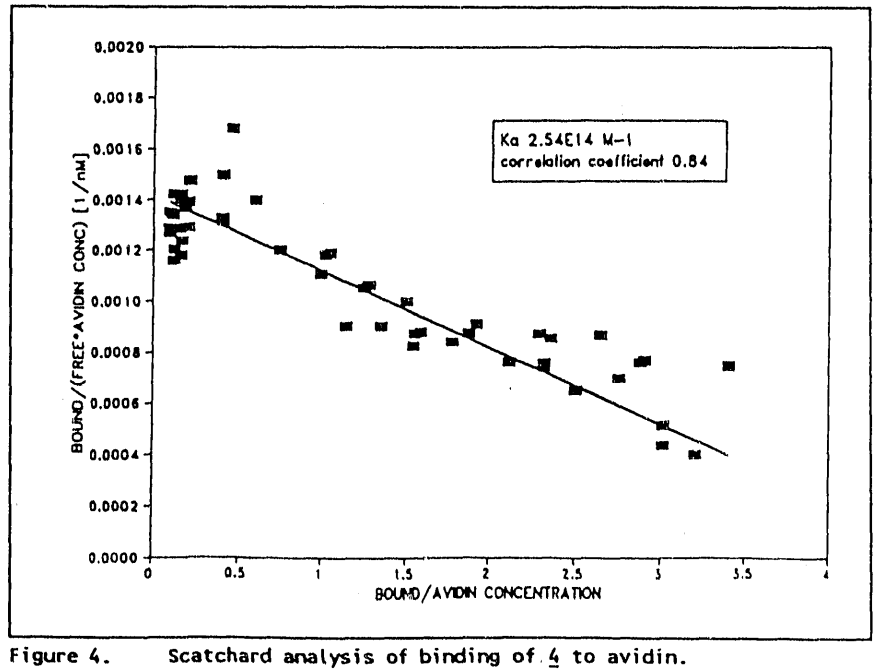


Figure 4. Scatchard analysis of binding of **4** to avidin.

II. In Vitro Studies

Semi-Preparative Isoelectrophoresis of Antibodies. The electrophoretic mobility (EM) of

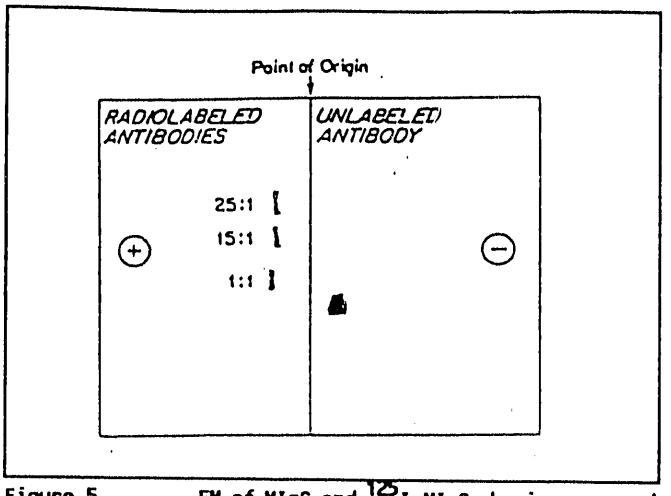


Figure 5. EM of MIgG and ^{125}I-MIgG showing reversal of Ab charge following iodination.

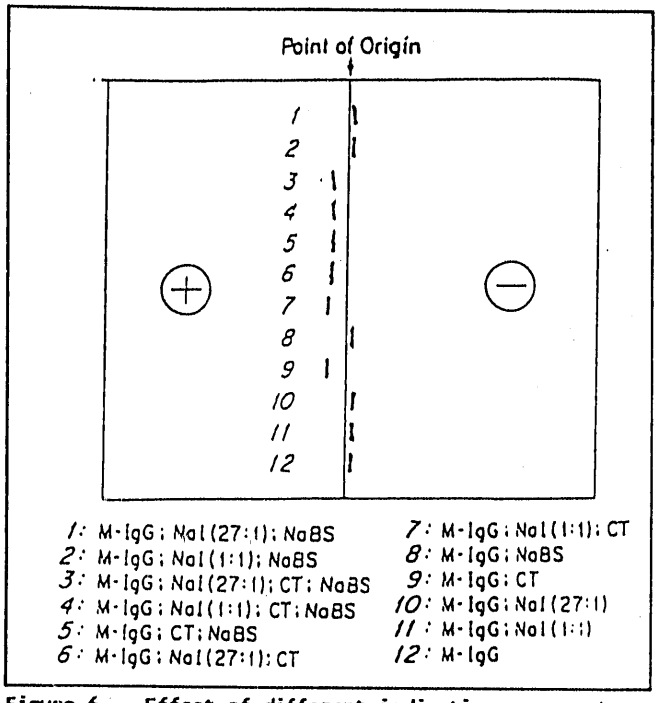


Figure 6. Effect of different iodination components on EM of MIgG.

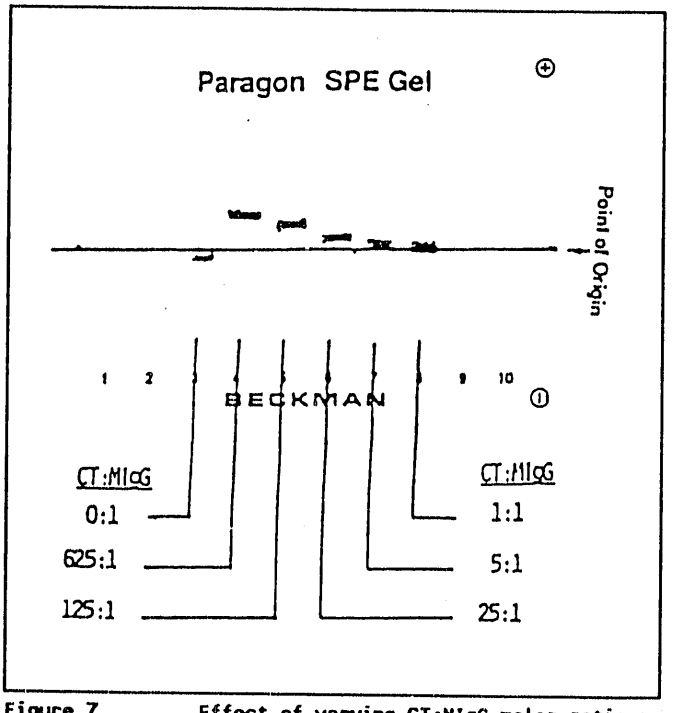


Figure 7. Effect of varying CT:MIgG molar ratio on EM of MIgG.

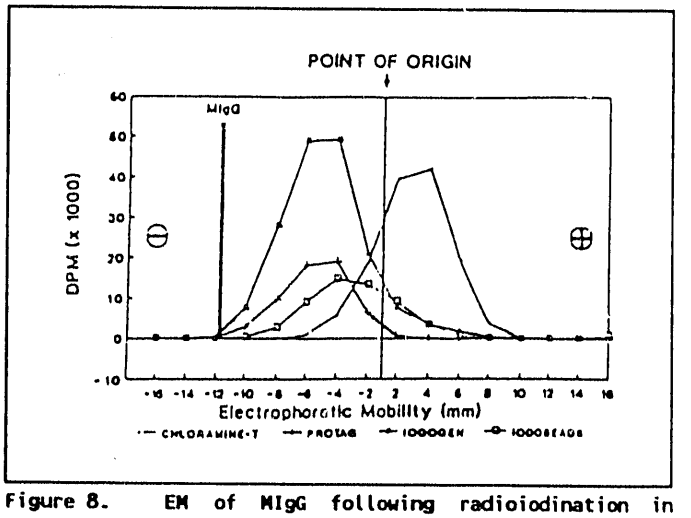


Figure 8. EM of MIgG following radioiodination in presence of various oxidants.

antibodies (Ab) is altered following various manipulations. Figure 5 demonstrates the EM of native mouse IgG (MIgG) and ^{125}I-MIgG (I:Ab ratios of 1:1, 15:1 and 25:1). The results indicate that, as expected, native MIgG moves toward the anode, i.e. is positively charged. Direct iodination, however, induces a reversal of the charge on the protein that is independent of the number of iodine atoms per antibody molecule. Analysis, singly and in combination, of the effect of the reagents used during the iodination procedure has shown that the reversal of the charge is due to the oxidizing agent CT and is independent of the presence of NaI or the reducing agent sodium bisulfite (Fig. 6). The alterations in the electrophoretic mobilities of MIgG are directly proportional to the concentration of CT present during the iodination. Decreasing the molar ratio of CT to MIgG (from 625:1 to 1:1) results in a corresponding decrease in the charge reversal (Fig. 7). Unlike CT, Protag, Iodobeads and Iodogen do not lead to

not lead to charge reversal even though all three agents cause an alteration in the electrophoretic mobilities of MIgG (**Fig. 8**). We have also compared the EM of goat anti-mouse IgG (GAM-IgG) radioiodinated (^{125}I) at three I:Ab ratios (1:1, 15:1, 35:1) either directly or following protection of the antigen-binding sites (Ab radioiodinated as an antigen-antibody complex) and have observed that the iodination of these antibodies changes their electrophoretic mobilities. When the antibody is labeled in the protected state, however, the degree of change is less (**Fig. 9**). These results therefore demonstrate that the binding of an antibody to its antigen prior to radiolabeling prevents major conformational changes as reflected by electrophoresis.

We have begun a collaborative effort with Dr. Calvin Saravis (New England Deaconess Hospital, Boston) in which a recently developed apparatus that separates proteins based on their isoelectric points (pI) is being examined as a means of determining whether the pI of an antibody following radiolabeling could affect its kinetics of biodistribution and tumor targeting. Rabbit anti-human serum albumin IgG has been subjected to isoelectric focusing (IEF) using the Semi-Preparative IEF apparatus. One ml of antibody (7.5 mg) is diluted in 147 ml of distilled water and 1.5 ml (40% solution) of pH 4.0-to-7.0 and 0.5 ml of pH 3.5-to-10.0 ampholytes are added. The antibody is focused at room temperature for 2.5 h. The pH of each of the 20 fractions collected is determined. When the pH values are plotted against fraction number, the pH gradient formed is linear over the range 4.5 to 9.0 (**Fig. 10**). The fractions are then concentrated from the 7.5 ml initial volume to 500 µl and their protein content determined (over 98% of the protein has been recovered). The concentrated fractions are then subjected to isoelectric focusing in an EC isoelectric focusing chamber equipped with a solid-state thermal control unit using a pre-cast 1% agarose IEF gel, pH 3.0 to 10.0. Our results indicate that this antibody is composed of several isoforms of variable pI (**Fig. 11**) that can be separated in milligram quantities and isolated using this novel apparatus. Preliminary findings with the MoAb A1D2 are similar.

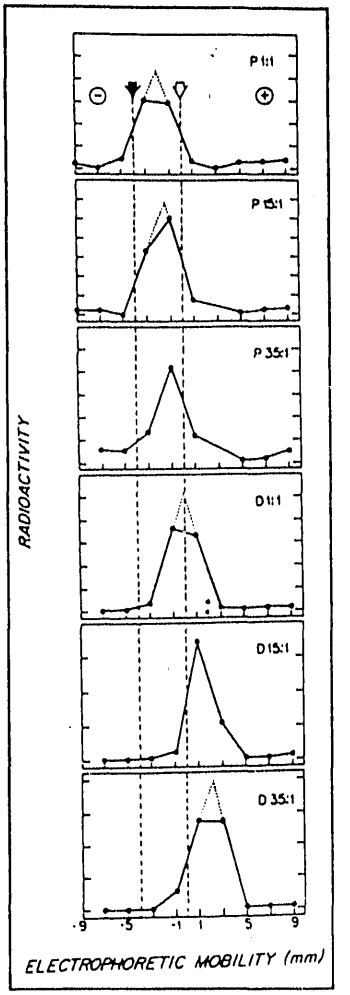


Figure 9. EM of GAM radioiodinated at 3 I:Ab ratios (1:1, 15:1, 35:1) either directly (D) or following protection (P). Closed arrow: EM of unlabeled GAM; open arrow: sample loading zone.

III. In Vivo Studies

a. Biodistribution of Radiolabeled MOv18 in Ovarian Cancer Xenografts and Normal Nude Mice. MOv18 is an IgG1 monoclonal antibody (MoAb) that is specific to epithelial ovarian cancer. To determine clinical applicability of the MoAb for radioimmunodiagnosis and radioimmunotherapy in ovarian cancer patients, we have examined the localization of ^{125}I labeled MOv18 and its tumor uptake in an animal model and have compared it with the anti-ovarian cancer MoAb OC 125 (whole IgG1).

Immunohistochemical staining using immunoperoxidase techniques has shown that MOv18 binds to OVCAR:3 (NIH) cells from ascitic fluid and solid tumors. Nude mice are each inoculated intraperitoneally (i.p.) with 10^7 OVCAR-3 cells. When solid tumors as well as ascites have developed, the animals are injected i.p. with the radiolabeled MoAb (5 µCi, 75 µg; molar ratio 1:1). Biodistribution studies are performed for 5 days. Tumor-bearing and nontumor-bearing (control) mice have been studied.

The MOv18 target/nontarget ratio is about 2 in most organs even at 4 h post injection. The binding to normal organs decreases much faster than to the tumor, thus increasing tumor/nontumor ratios. Blood clearance in normal mice is similar for both immunoglobulins. In tumor-bearing animals, however, blood clearance of OC 125 is much faster than that of MOv18. Tumor targeting with MOv18

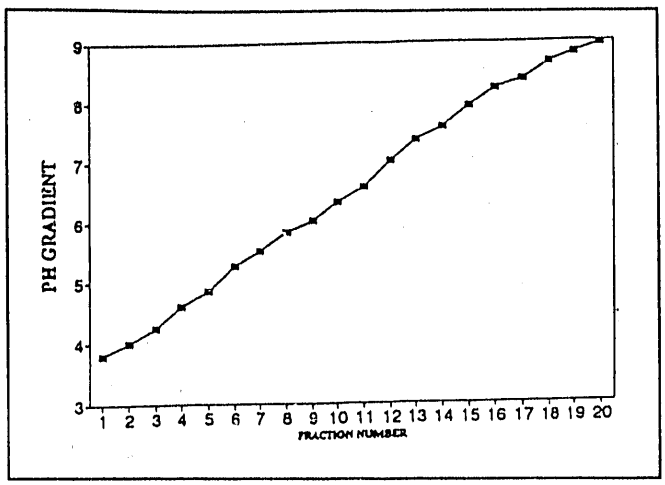


Figure 10. pH gradient obtained by semi prep EF of mouse MoAb.

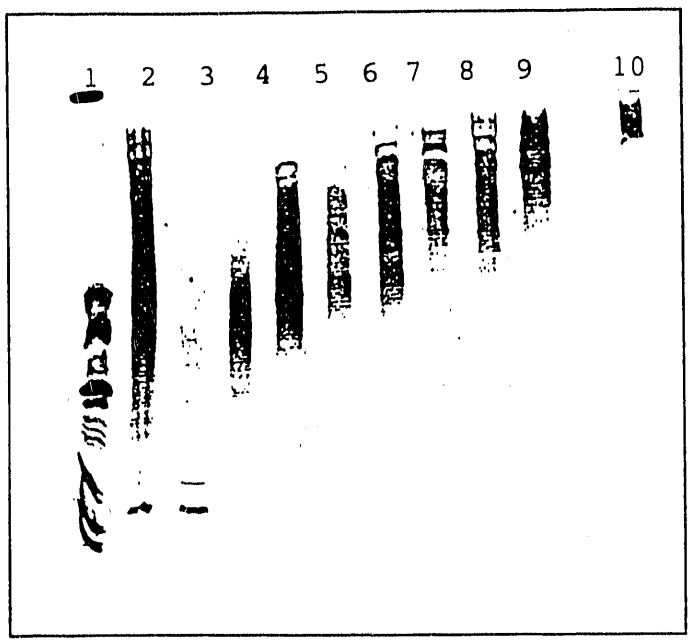


Figure 11. Gel IEF of representative fractions (3-10) obtained following semi prep IEF fractionation of RIgG (anti-HSA). 1: standards; 2: native RIgG.

is 3.5% of the injected dose at 24 h in contrast to 0.48% for OC 125. Although an acceptable tumor/liver ratio is obtained within the first 4 h (1.8), the ratio continues to increase and reaches a maximum of 2.3 by 48 h. Comparable results have been obtained with other tissues. Similarly, radioiodinated OC 125 has a better tumor/liver ratio at 48 h. Based on these results, we conclude that this MoAb is promising for radioimmunodetection and applicable to radioimmunotherapy.

b. Use of ^{111}In Labeled Nonspecific Polyclonal IgG in Patients with Acute Inflammatory/Infectious Processes [J Nucl Biol Med 1991; 35:4-9]. Eleven patients with suspected foci of inflammation and/or infection (7 with abdominal source, 3 with musculoskeletal source, 1 with thoracic aorta source) have been scanned with ^{111}In labeled polyclonal human IgG. The test has scored truly positive in 7 patients, truly negative in 3 patients, and falsely negative in 1 patient. All the true positive cases have shown abnormally increased radiopharmaceutical uptake at the site of infection by 6 h, suggesting the diagnosis, although the intensity of uptake has increased progressively up to 24 h later. There have been no untoward effects noted in this series. This examination is potentially useful in the early depiction of focal sources of infection/inflammation.

c. Use of ^{131}I Labeled Monoclonal Antibody OC 125 for Intraperitoneal Radioimmunotherapy of Refractory Ovarian Carcinoma. We have concluded a phase I therapeutic trial that has examined the feasibility of i.p. radioimmunotherapy utilizing escalating doses of ^{131}I labeled OC 125 F(ab')$_2$. Twenty-nine patients have been treated with a single dose of radiolabeled antibody. Twenty-eight patients have been evaluable for dose related toxicity. The toxicities most frequently observed are hematologic and gastrointestinal. Hematologic toxicity has been noted in 5/14 (36%) patients receiving 18 to 87 mCi and in 12/14 patients (71%) receiving 100 to 144 mCi ($p=0.018$). The median white blood cell nadir of 2 to 3 k/μl (range 1.4-3.5 k/μl) has occurred at a median of 4.5 weeks and the median platelet nadir of 41 k/μl (range 20-78 k/μl) at a median of 6.5 weeks. Mild gastrointestinal toxicity has been observed in 4/14 (28%) patients at doses $\leq$100 mCi whereas at doses $\geq$100 mCi, 11/14 (79%) patients have developed nausea, vomiting, or chronic ileus ($p=0.021$). This toxicity has been present most frequently in patients with protracted urinary ^{131}I excretion. We conclude that ^{131}I labeled OC 125 can be safely administered i.p. Hematologic and gastrointestinal toxicity is predictable and related to the dose of isotope and rate of isotope clearance.

Project 4: **Antibody Modification for Radioimmune Imaging** (August 1, 1990 - August 1, 1991)

A. Personnel

Name	Title	Dates of Service	Percent Effort
Alan J. Fischman	Associate Professor	08/01/90-present	as needed
H. William Strauss	Professor	08/01/90-present	as needed

B. Publications

1990

Papers

1. Abrams MJ, Juweid M, tenKate CI, Schwartz DA, Hauser MM, Gaul FE, Fuccello AJ, Rubin RH, Strauss HW, Fischman AJ. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. J Nucl Med 1990; 31:2022-2028.

1991

Papers

1. tenKate CI, Fischman AJ, Rubin RH, Fuccello AJ, Rexinger D, Wilkinson RA, Du L, Khaw BA Strauss HW. Effect of isoelectric point on biodistribution and inflammation-imaging properties of indium-111 labeled, nonspecific, polyclonal human IgG. Eur J Nucl Med 1991; 17:305-309.
2. Fishman JA, Strauss HW, Fischman AJ, Nedelman M, Callahan R, Khaw BA, Rubin RH. Imaging of pneumocystis carinii pneumonia with ^{111}In-labeled nonspecific IgG: An experimental study. Nucl Med Commun 1991; 12:175-187.
3. Fischman AJ, Pike MC, Kroon D, Fuccello AJ, Rexinger D, tenKate C, Wilkinson R, Rubin RH, Strauss HW. Imaging of focal sites of bacterial infection in rats with indium-111-labeled chemotactic peptide analogs. J Nucl Med 1991; 32:483-491.
4. Strauss HW, Fischman AJ, Khaw BA. Non-tumor applications of radioimmune imaging. Nucl Med Biol 1991; 18:127-134.

In Press

1. Tompkins RG, Fischman AJ, Yarmush ML. Imaging infection with antibodies: A method to localize occult infections. Chest.
2. Juweid M, Fischman AJ, Rubin RH, Baum R, Strauss HW. Comparison of ^{99m}Tc-labeled monoclonal anti-granulocyte antibody and ^{111}In-labeled IgG for the detection of focal sites of infection in rats. Nucl Med Commun.

C. Report

I. Synthesis and Biological Testing of ^{99m}Tc-IgG

During the past year, due to limitations in funding, our work was concentrated on the application of novel ligands for protein labeling, specifically on the development and preliminary testing of a nicotinyl hydrazine derivative for labeling IgG.

The nicotinyl hydrazine modified IgG was readily labeled with ^{99m}Tc by reaction with ^{99m}Tc-glucoheptonate. Radiochemical purity was routinely greater than 90%.

The biological behavior of the ^{99m}Tc labeled IgG was examined in rats with focal sites of inflammation due to E. coli and in normal controls. The imaging results were compared with those obtained using ^{111}In labeled IgG. The ^{99m}Tc and ^{111}In labeled IgG had virtually identical biodistribution properties. Both the concentration achieved at sites of infection and the distribution in normal organs were comparable (1).

II. References

1. Abrams MJ, Juweid M, tenKate CI, Schwartz DA, Hauser MM, Gaul FE, Fuccello AJ, Rubin RH, Strauss HW, Fischman AJ. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. J Nucl Med 1990; 31:2022-2028.

Project 5: **Antibody Labeling with Positron-Emitting Radionuclides** (August 1, 1990 - August 1, 1991)

A. Personnel

Name	Title	Dates of Service	Percent Effort
David R. Elmaleh	Associate Professor	08/01/90-present	60
Eli Livni	Assistant Professor	08/01/90-present	35
Robert N. Hanson	Professor	08/01/90-present	as needed

B. Publications

1990

Papers

1. Carter EA, Barlai-Kovach M, Elmaleh DR, Livni E. Acute alcohol ingestion reduces fatty acid extraction of the heart, liver and small intestine. Alcohol Clin Exp Res 1990; 14:781-784.
2. Fink GD, Montgomery JA, David F, Garneau M, Livni E, Elmaleh DR, Strauss HW, Brunengraber H. Metabolism of β-methyl-heptadecanoic acid in the perfused rat heart and liver. J Nucl Med 1990; 31:1823-1830.

Abstracts

1. Herman LW, Elmaleh DR, Fischman AJ, Hanson RN, Strauss HW. The use of pentafluorophenyl derivatives for the ^{18}F labeling of proteins. In: Abstract Book of the Eighth International Symposium on Radiopharmaceutical Chemistry, Princeton, NJ, June 24-29, 1990, pp. 212-213.
2. Shen X, Hanson RN, Elmaleh DR. Synthesis and evaluation of radioiodinated tetrafluorophenyl m-iodobenzoate and tetrafluorophenyl-5-iodopentenoates as conjugating agents for proteins and antibodies. In: Abstract Book of the Eighth International Symposium on Radiopharmaceutical Chemistry, Princeton, NJ, June 24-29, 1990, pp. 229-230.
3. Livni E, Fischman AJ, Elmaleh DR, Du L, Strauss HW, Rubin RH, Dahl RJ, Robson W, Margouleff D, Liss R, Webb D, Ray S, Sinclair I. [^{18}F]-Fluconazole: Synthesis, biodistribution in rats, and imaging of rabbits. In: Abstract Book of the Eighth International Symposium on Radiopharmaceutical Chemistry, Princeton, NJ, June 24-29, 1990, pp. 420-421.

1991

Papers

1. Nishizawa K, Okunieff P, Elmaleh D, McKusick KA, Strauss HW, Suit HD. Blood flow of human soft tissue sarcomas measured by thallium-201 scanning: Prediction of tumor response to radiation. Int J Radiat Oncol Biol Phys 1991; 20:593-597.

Abstracts

1. Brownell A-L, Isacson O, Schumacher J, Elmaleh DR, Madras BK, Brownell GL. Glucose utilization and N-[C-11]-methyl-2-carbomethoxy-3-phenyl tropane ([C-11]PT) studies of the primate model of Huntington's disease. J Nucl Med 1991; 32:981.
2. Elmaleh DR, Brownell A-L, Isacson O, Schumacher J, Livni E, Madras BK, Brownell GL. Preparation and in vivo imaging of N-[C-11]-methyl-2-carbomethoxy-3-phenyl tropane ([C-11]PT) in monkey brain. J Nucl Med 1991; 32:1009.
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In Press

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C. Report

The main objective of the project is to create better methods for labeling peptides and proteins with positron-emitting radionuclides of the halogen series. During the past year our goal has been to develop syntheses for radiofluorination and radioiodination precursors that can label proteins in high specific activity.

Pentafluorophenyl derivatives have been shown to be susceptible to nucleophilic attack, primarily at the position para to the nonfluorine group, the rate being proportional to the electron withdrawing strength of the fluorine moiety. We have found efficient ^{18}F for F exchange in pentafluorophenyl (PFP) derivatives bearing a wide variety of electron withdrawing groups. We have also had some success in labeling human serum albumin (HSA) with pentafluorobenzaldehyde with ^{18}F incorporated into the phenyl ring using the tetrabutylammonium salt in DMSO. Since activated esters are also useful for the covalent attachment of ligands to amino acids, we have examined a series of pentafluorophenyl activated esters. Pentafluorophenyl esters decomposed under the exchange conditions. However, the 2,3,5,6-tetrafluorophenyl-pentafluorobenzoate readily incorporates ^{18}F and reacts quickly with HSA to give an absolute yield of 15% labeled HSA in 72 min. The tetrafluorophenoxy moiety does not contain sufficient electron withdrawing groups to undergo ^{18}F for F exchange, thereby reducing the overall incorporation of ^{18}F into the protein. This has been verified by hydrolysis of the ester followed by radiographic TLC of the products; no activity is associated with the recovered tetrafluorophenol.

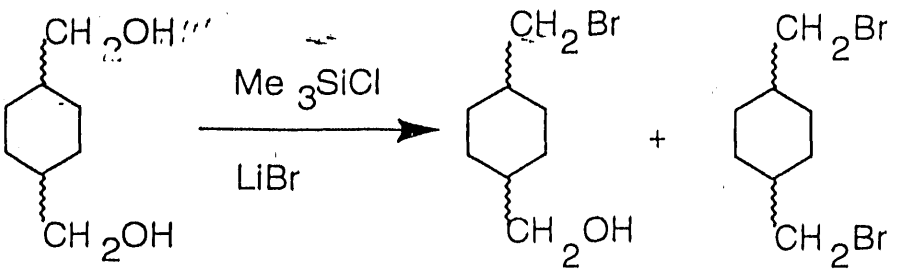
The radiolabeling methods that we use must label peptides/proteins in high specific activity. The molecular weight of proteins/antibodies is high, necessitating high concentrations of these substances to achieve reasonable labeling efficiency. Chemotactic peptides generally act as ligands for the receptor site and their pharmacological effects begin at very low doses. To eliminate toxicity and saturability effects, these radiolabeled peptides must be used in low concentrations. The development of methods that allow high specific activity labeling alleviates these problems. In the past year two precursors that will be suitable for labeling with ^{18}F in high specific activity have been developed.

I. Synthesis of Cyclohexanedimethanol Ditosylate

Paratoluenesulfonyl chloride is added to a solution of cyclohexanedimethanol in dry pyridine and the mixture is stirred overnight at room temperature. The mixture is diluted with water and filtered. The

crystals are washed with water and dried. ^{1}H NMR spectra show peaks at 2.460 ppm (s, SO_2-⬡-CH_3), 3.45 and 3.83 ppm (m, -CH_2-⬡-CH_2-), 7.34 and 7.78 ppm (m, aromatic protons).

Chlorotrimethylsilane is added to a solution of LiBr in dry CH_3CN with stirring under a N_2 atmosphere. Cyclohexanedimethanol is added and the mixture is refluxed overnight (**Scheme 1**). The reaction mixture is take up in ether, washed successively with water, $NaHCO_3$ solution, and brine. After drying, evaporation of solvent yields a mixture of product and starting material. The product is isolated on preparatory TLC with EtOAc:CH_2Cl_2:hexane, 5:15:80, as solvent. A very small amount of dibromide is formed. ^{1}H NMR spectra of product show peaks at 3.305 ppm (d, 6 Hz, $BrCH_2$-⬡-) and 3.481 ppm (d, 6 Hz, $HOCH_2$-⬡-). The dibromide shows a peak at 3.3 ppm (d, 6 Hz, $BrCH_2$-⬡-CH_2Br).



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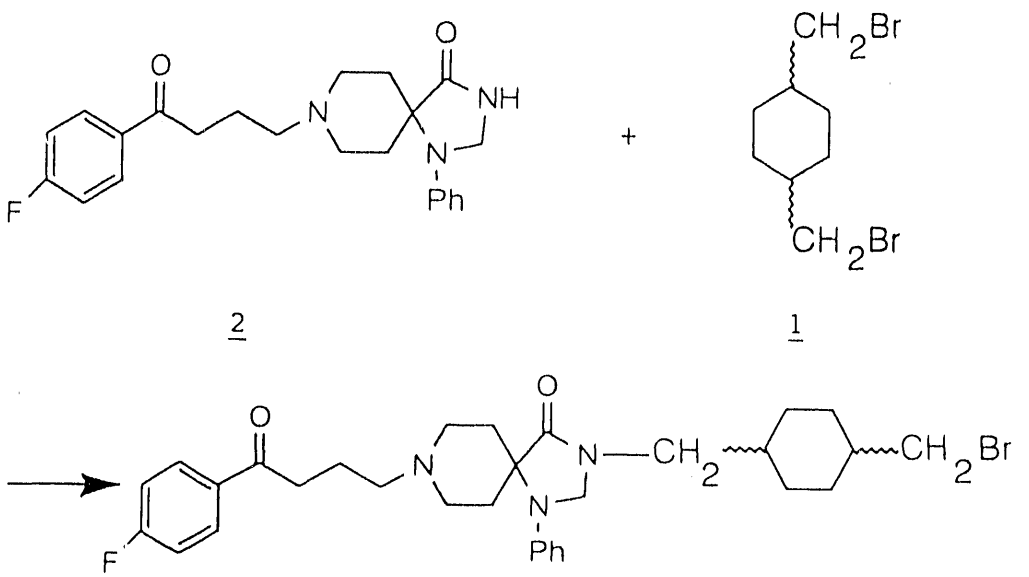
Scheme 1. Synthesis of cyclohexyldimethylbromide.

Spiperone (2) has been refluxed with cyclohexyldimethylbromide in benzene together with sodium hydroxide solution and tetrabutylammonium hydrogen sulfate (Scheme 2) to form 4-[^{18}F]fluoromethyl-cyclohexylmethylspiperone.

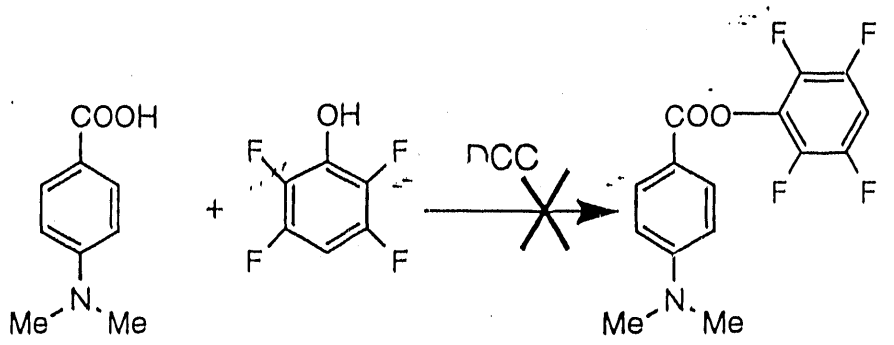


2 1

Scheme 2. Synthesis of 4-[^{18}F]fluoromethylcyclohexylmethylspiperone.

II. Synthesis of a Trimethylammoniumbenzoate Activated Ester

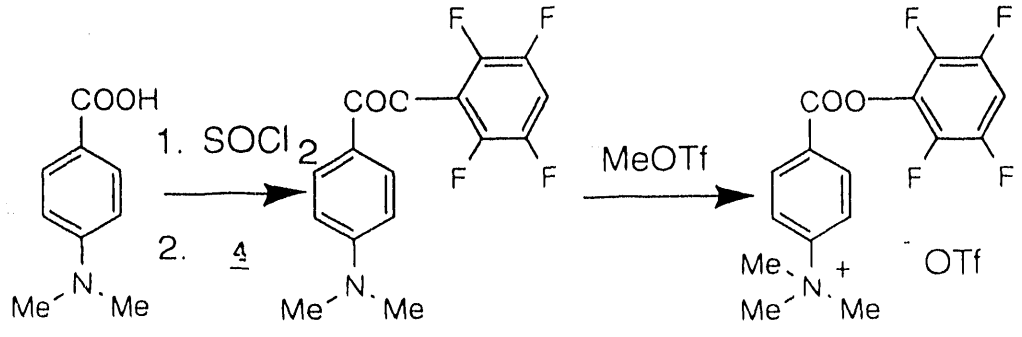
Several attempts have been made to synthesize tetrafluorophenyl-p-dimethylaminobenzoate (5) by condensation of p-dimethylaminobenzoic acid (3) and tetrafluorophenol (4) with 1,3-dicyclohexylcarbodiimide (DCC) without success. Changing the reaction conditions has not improved the yield (**Scheme 3**).



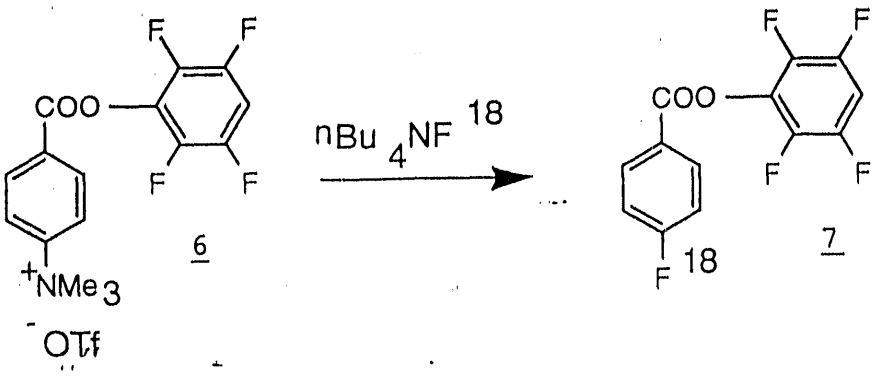
3 4 5

Scheme 3. Attempted condensation of tetrafluorophenyl-p-dimethylaminobenzoate with 1,3-dicyclohexylcarbodiimide.

Because of this failure a new approach has been devised (**Scheme 4**). Since acid chloride is generally a very reactive species, the acid (3) is converted to p-dimethylaminobenzoyl chloride by thionyl chloride. This acid chloride is reacted with tetrafluorophenol (4) to give the ester (5). This ester has been purified on a silica gel column. ^{1}H NMR spectra of product show peaks at 3.11 ppm (s, -N(CH_3)$_2$), 6.73 and 8.08 ppm (d, -OOC-C$_6$H$_4$-N<) and 7.01 ppm (m, -C$_6$HF$_4$). Then the dimethylamino group of 5 is reacted with methyltrifluoromethane sulfonate (MeOTf) to yield solid tetrafluorophenyl-p-trimethylammonium triflic benzoate (6). The ammonium salt 6 will be purified and its structure identified. If the needed compound has been made, it will be labeled with ^{18}F via [^{18}F]tetrabutylammonium fluoride to yield 7 and conjugated with some natural products to establish its general application.



3 5 6



$$R\text{-}NH_2 + \underline{7} \dashrightarrow R\text{-}NH\text{-}CO\text{-}C_6H_4\text{-}{}^{18}F$$

Scheme 4. Successful synthesis of tetrafluorophenyl-p-trimethylammonium triflic benzoate and its proposed use to label protein.

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