DOE/ER/60401--T2

DE92 008729

C. PROGRESS REPORT

C.1. Period

This progress report covers work performed on this project since February 1, 1989, a period of ca. two and a half years.

C.2. Personnel

The following personnel have participated in this project.

Name	Appointment	Period
John A. Katzenellenbogen	Principal Investigator	2/1/89-present
Susan Pochapsky	Graduate Research Assistant	2/1/86-2/1/90
Martin G. Pomper	Graduate Research Assistant	2/1/89-6/1/90
James P. DiZio	Graduate Research Assistant	2/1/89-7/1/91
Aijun Liu	Graduate Research Assistant	2/1/89-present
Rita Fiaschi	Postdoctoral Fellow	9/1/89-9/1/90
Yearn Choe	Postdoctoral Fellow	7/1/91-present
Kathryn E. Carlson	Technician	2/1/89-present

C.3. Summary of Previous Specific Aims and Past Progress

The specific aims of the previous grant application can be summarized as follows:

Previous Aim 1: Synthesize fluorine-substituted progestins from the following high affinity classes: R5020 (promegestone), norgestrel, RU486, and retroprogestins.

Previous Aim 2: Synthesize fluorine-substituted androgens from the following high affinity classes: mibolerone, R1881 (metribolone) and 2-oxometribolone.

Previous Aim 3: Evaluate the receptor binding and non-specific binding of these fluorosteroids by in vitro binding assays.

Previous Aim 4: Develop and optimize fluoride ion substitution reactions suitable for the rapid, efficient and convenient preparation of these fluorosteroids in high specific activity, F-18 labeled form.

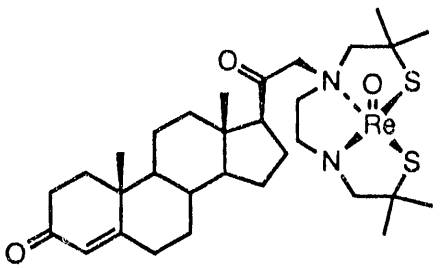
Previous Aim 5: Evaluate the target tissue uptake of the F-18 labeled androgens and progestins in experimental animals.

As is described in detail below, we have made excellent progress on these aims. In addition, we have prepared a progestin labeled with technetium-99m or rhenium-186 that shows high affinity for the progesterone receptor and documents that technetium labeled ligands for steroid receptors can be made. Reference is made to publications that have arisen from this project as "Publication No. ____". These publications are listed in Section C.5. and are collected in the Appendix.

C.4. Progress Under Previous Aim 1: Synthesis of Progestin Imaging Agents

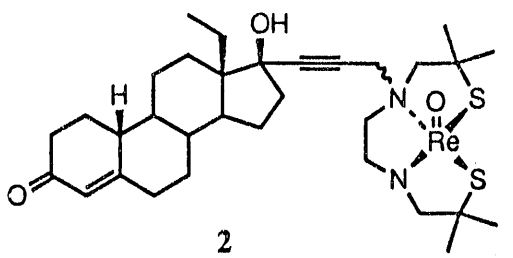
C.4.a.(1) Technetium-99m and Rhenium-186 Labeled Progestins: Synthesis, Binding and In Vivo Tissue Distribution (Publication Nos. 8, 9 and 18)

The most exciting finding during the past three years has come from our investigations of metal conjugates of progestins. We were aware that ligands for the progesterone receptor could tolerate substituents of considerable bulk when placed at the 11β, 16α, 17α or 21 positions; therefore, we made a systematic investigation of conjugates of a basic N_2S_2-metal chelate system with ligands for the progesterone receptor, having attachment at these sites. These systems were then complexed with rhenium. The structures of the compounds we prepared are shown below, and the full details of their synthesis are given in Publication No. 8. The synthesis of conjugate **4** is shown in Scheme 1.



1

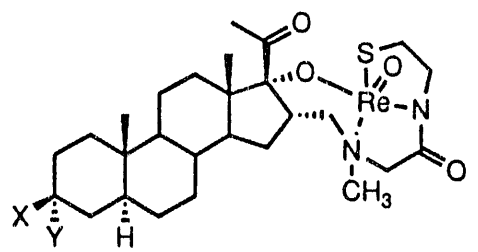
(1:1 mixture of Syn1 and Syn2)



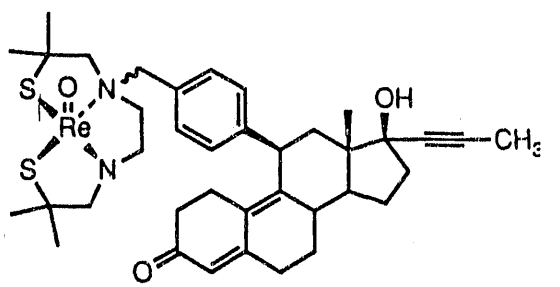
2

syn1,2 : anti1,2

(4 : 1)



3a (X,Y= O)
3b (X= OH, Y=H)



4

	Ratio
Syn1,2	30
anti1	1
anti2	1

we were able to separate some of the anti-diastereomers, the syn isomers were for the most part inseparable.

In competitive binding assays (see Table 1), three of the four progestin metal conjugates showed very low binding affinity for the progesterone receptor, but the diastereomers of the 11β-linked system, a compound based on the antiprogestin RU468, have binding affinities that exceed that of progesterone!

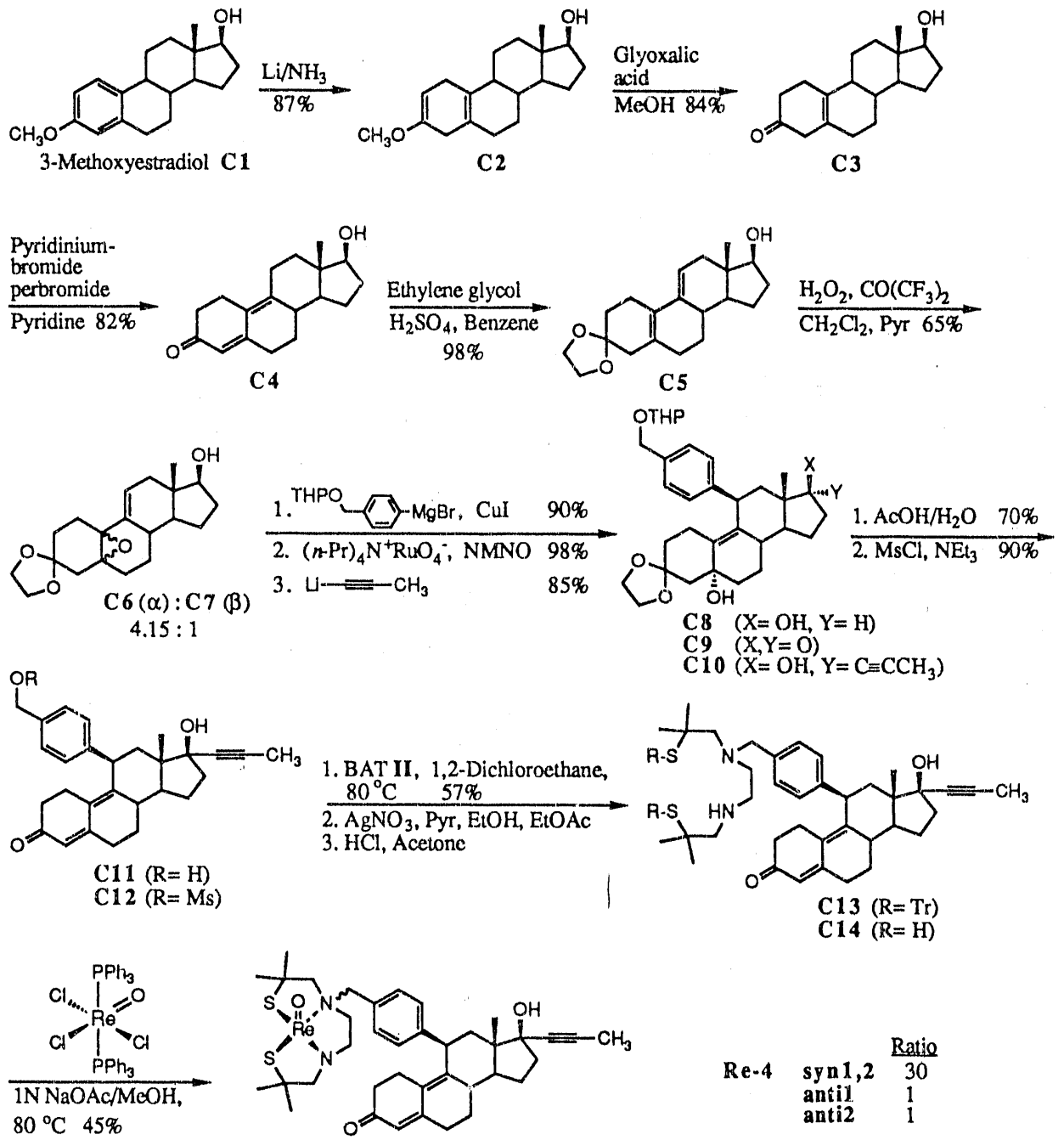
Table 1. Binding Affinity of Progesterone Systems for the Progesterone Receptor.[a]

Compound	RBA[b] (%)
R5020	645
C-21 Series	
Progesterone	100
1(syn,1,2) (Re Chelate)	0.03
17α-Series	
Norgestrel	308 ± 110
2(syn1,2) (Re Chelate)	**0.06**
2(anti1,2) (Re Chelate)	**1.8**
16α,17α Series	
Precursor to **3a**	0.16 ± 0.04
Precursor to **3b**	0.05 ± 0.02
3a (3-keto) **(Re Chelate)**	**0.10 ± 0.01**
3b (3-hydroxy) **(Re Chelate)**	**0.07 ± 0.03**
11β-Series	
C11 (benzyl alcohol precursor)	97
C12 (benzyl mesylate precursor)	107
C14 (11β-BAT I estradien precursor)	68
4 (syn1,2) (Re chelate)	**178**
4 (syn1,2) (Tc Chelate)	**162 ± 24**
4 (anti1) (Re Chelate)	**283 ± 109**
4 (anti1) (Tc Chelate)	**303 ± 9**
4 (anti2) (Re Chelate)	**62 ± 27**
4 (anti2) (Tc Chelate)	**40 ± 8**

[a]Relative binding affinities were determined by a competitive radiometric binding assay, using uterine cytosol from estrogen primed immature rats as a source of receptor, tritium-labeled R5020 as tracer, and charcoal dextran as adsorbant of free tracer

[b]RBA = relative binding affinity. Association constant as a percent of the affinity of progesterone. Although R5020 was used as the tracer, binding affinities are expressed relative to the affinity of progesterone.

We then prepared the 11β-linked progestin in technetium-99m (**Tc-99m-4**) and rhenium-186 (**Re-186-4**) labeled form. With these radiolabeled ligands, we were able to demonstrate directly their high affinity binding to the progesterone receptor and the selectivity of this binding. Binding curves and Scatchard plots are shown in Figure 1. In addition, we have performed tissue



Scheme 1. Synthesis of Progestin-Chelate Conjugate **4** and its Oxorhenium Complex **Re-4.**

There are four possible stereoisomers of each of these systems, since the metal complex has two stereogenic centers, namely the metal and the tertiary nitrogen. Only one isomer of compound **3** was found, but the other three systems gave all four, with the syn diastereomers (those in which the bond linking the N_2S_2 chelator to the steroid is syn to the metal oxo bond) being produced in larger quantities, which is consistent with reports in the literature [39]. While

distribution of these compounds in estrogen-primed female rats; selected results are shown in Figures 2 and 3. A complete account of all of these experiments is found in Publication No. 9.

The binding curves (Fig. 1) show that both complexes **Tc-99m-4** and **Re-186-4** show high affinity binding to the progesterone receptor. The K_d values of 4.95 nM and 4.76 nM, respectively, (Fig. 1, Panels C), are consistent with the competitive binding affinities (RBA values, Table 1). The binding selectivity of these compounds (Fig. 1, Panels A), however, is only moderate, with the Tc-99m complex showing more specific binding than the Re-186 complex.

The uptake of these compounds is not highly selective for the target tissue, the uterus, because they are so lipophilic (log P measured by reversed phase HPLC is ca. 6.3, vs ca. 4.5 for most progestins) (see below). Nevertheless, as seen in Panels A of Figs. 2 and 3 at 6 h, the

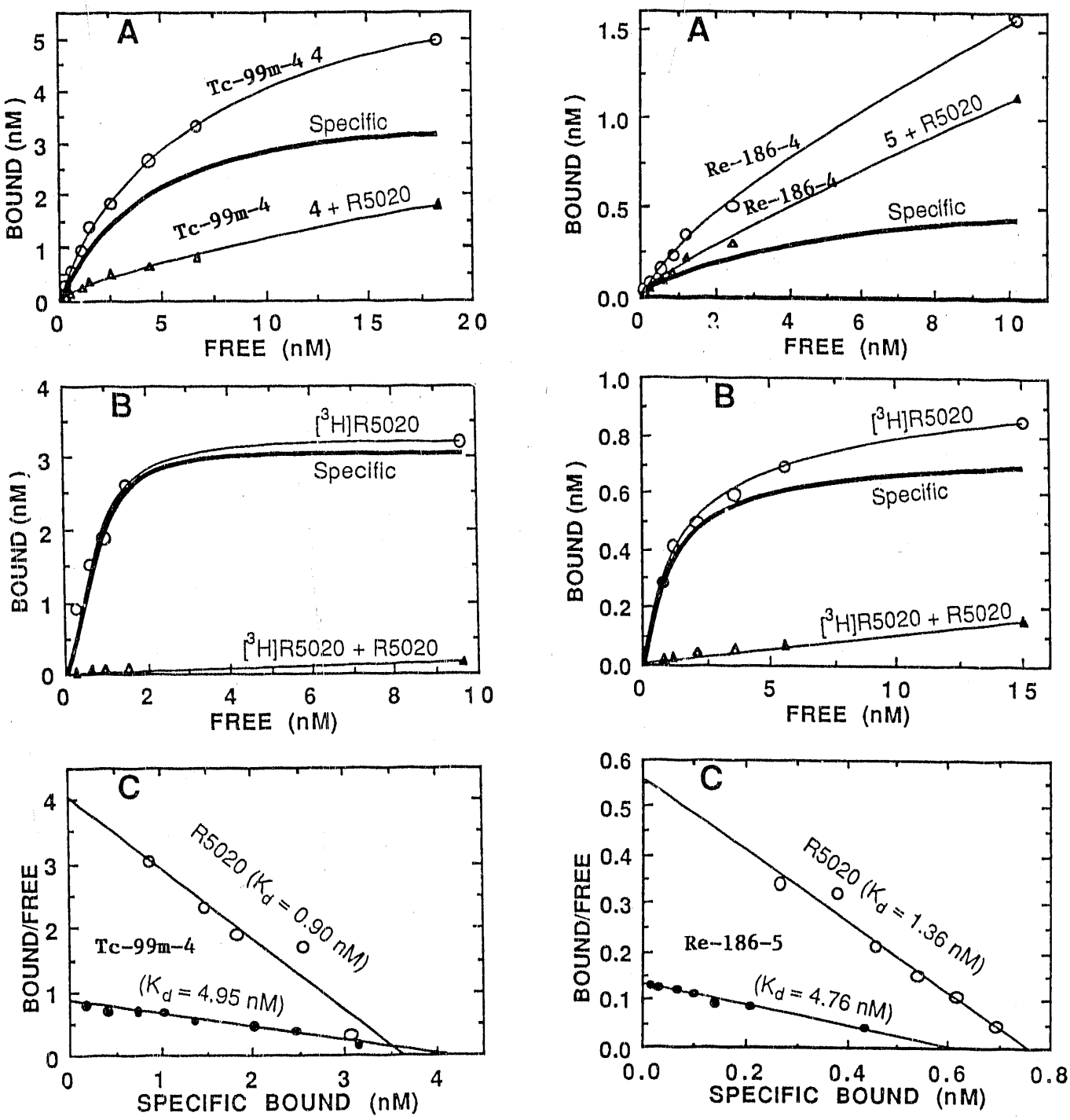


Figure 1. Left: Multipoint binding assay data for **Tc-99m-4** (Panel A) and R5020 (Panel B). Panel C describes the data converted to Scatchard plots. Right: Multipoint binding assay data for **Re-186-4** (Panel A) and R5020 (Panel B). Panel C describes the data converted to Scatchard plots.

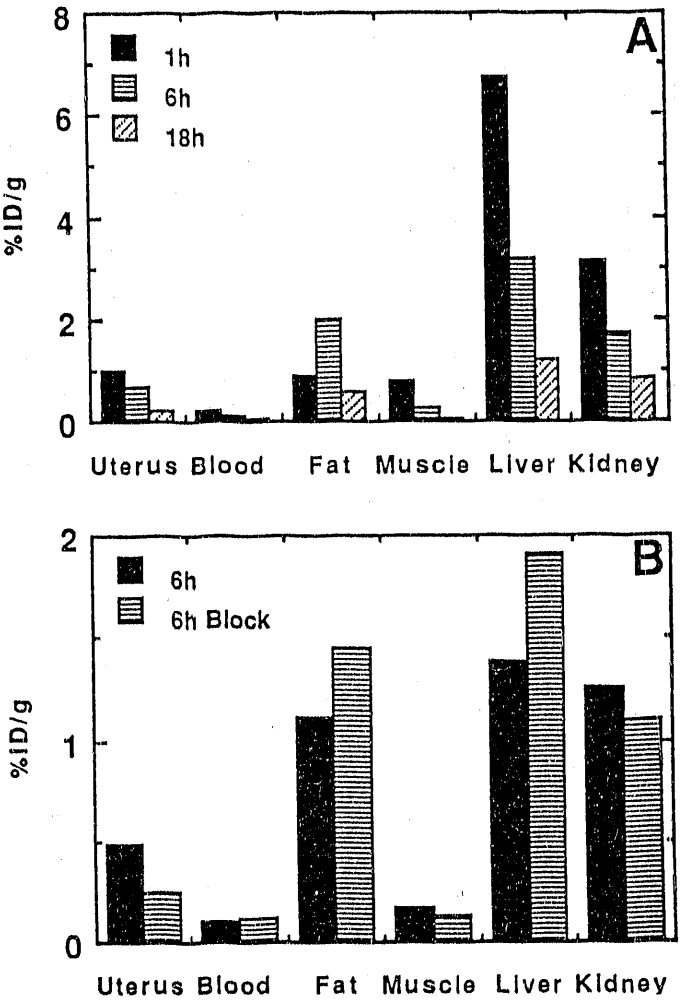


Figure 2. Comparison of rat tissue uptake for **Tc-99m-4**. Panel A shows the uptake in various tissues at 1, 6, and 18 hours. Panel B shows the uptake after 6 hours for rats coinjected with 18 μg ORG2058 (blocked) and rats not coinjected.

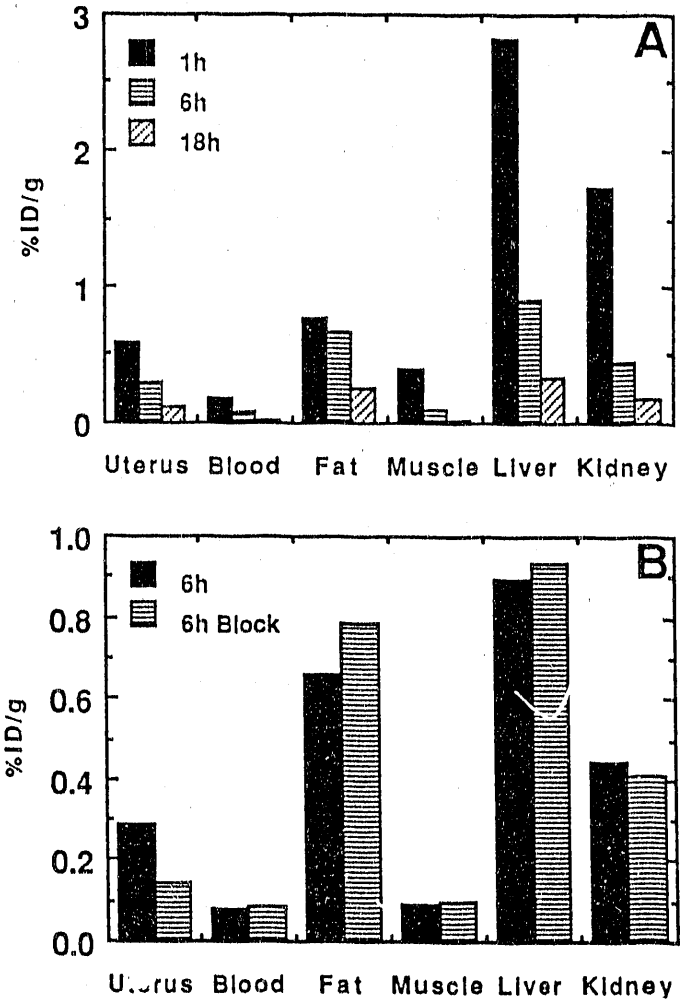


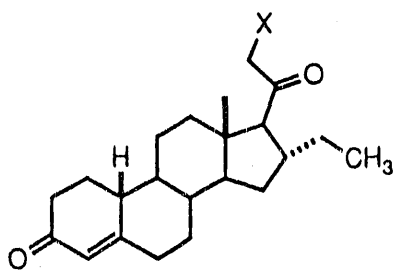
Figure 3. Comparison of rat tissue uptake for **Re-186-4**. Panel A shows the uptake in various tissues at 1, 6, and 18 hours. Panel B shows the uptake after 6 hours for rats coinjected with 18 μg ORG2058 (blocked) and rats not coinjected.

uptake by the uterus is ca. 3-fold greater than that of a non-target organ such as muscle and 3.6-5.4-fold greater than that of the blood. Furthermore, when progesterone receptor sites are blocked by co-administration of an excess of unlabeled progestin (Panels B, Figs. 2 and 3), the only significant decrease in uptake is seen in the uterus (ca. 50%). Thus, although they are too lipophilic to show uptake that is highly selective for target tissues, there is a significant component of receptor-mediated uptake of these two complexes. It is also clear that further studies are needed in order to see whether the in vivo distribution properties of these compounds can be optimized; these studies are described under New Specific Aim 1, in Section D.1.

It is of note that these technetium and rhenium progesterone complexes are the first receptor ligands labeled with a radioactive metal that retain binding affinity for receptor that is comparable to that of the natural receptor ligand. This demonstration opens the possibility of developing other receptor ligands labeled with these readily available radioactive metal ions, and would extend greatly the applicability of these imaging agents.

C.4.a.(2) Human Breast Cancer Imaging Studies with FENP (Publication Nos. 1, 7, and 16)

At the end of the previous grant period, we reported the synthesis of 21-fluoro-16α-ethyl-19-norprogesterone (**5**, FENP), a fluorine-substituted analog of ORG2058 (**6**), a potent synthetic progestin from the Organon company ORG2058. As was reported in a publication that appeared during this grant period [34] (Publ. No. 1), the rat and human progesterone receptor binding affinity of FENP and its tissue distribution in rats were excellent, comparable to or better than that of ORG2058.



	5 FENP (X = F)	**6** ORG 2058 (X = OH)
RBA[a]	697	204
% ID/g uterus at 1 h	6.4	4.8
uterus/muscle at 1 h	16	15
uterus/blood at 1 h	26	3.4

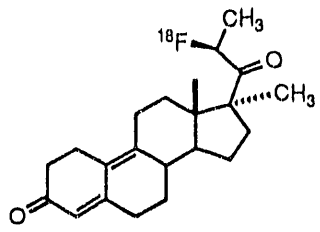
[a]These RBA values are relative to R5020 = 100; on this scale the RBA of progesterone is 16.

On the basis of these excellent binding and uptake characteristics, we worked with our collaborators in St. Louis to have FENP tested in human breast cancer patients [36] (Publ. No. 7). Although [^{18}F] FENP gave positive images of breast tumors in ca. 50% of the cases, the level of uptake was low and the target to background contrast was poor, and both were unrelated to tumor progesterone receptor content measured on tumor biopsy samples *in vitro*. These findings were very different from those we had experienced previously with a fluorine-18 labeled estrogen in imaging human breast tumors [14].

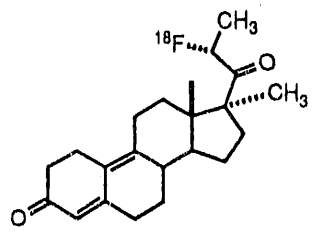
We believe that the failure of FENP to show high quality images in humans results from two factors,: (1) the high lipophilicity of this compound that derives from the replacement of the 21-hydroxyl group with a fluorine, and (2) the high metabolic lability of FENP in humans. We have found high metabolic defluorination rates with other steroids labeled with fluorine-18 at C-21 [35,38]. Also, preliminary comparative metabolism studies using rat and human hepatocyte systems showed that metabolite generation from FENP in the human liver cell system, particularly the generation of hydrophobic metabolites, was more rapid than in the rat system [36] (Publ. Nos. 7, 16). Thus, a part of New Specific Aim 4 (Section D.4.) involves the design and synthesis of new, less lipophilic analogs of FENP that have the C-21 hydroxy group intact and have fluorine placed at a position that we anticipate will be less metabolically vulnerable.

C.4.a.(3) Three New Fluorine-18 Labeled Progestins: Synthesis, Binding and In Vivo Tissue Distribution Studies (Publication Nos. 2 and 12)

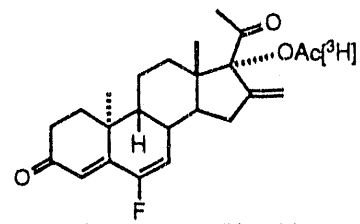
We have synthesized three fluorine-substituted ligands that have high affinity for the progesterone receptor, and we have studied their receptor binding and their tissue distribution. Their structures are shown below.



7 (21S)



8 (21 R)



9 DU41165

	7 (21S)	8 (21 R)	9 DU41165
RBA[a]	45	11	145
% ID/g uterus at 1 h	2.1	0.45	7.1
uterus/muscle at 1 h	4.4	1.2	15.6
uterus/blood at 1 h	4.3	0.8	32

[a]These RBA values are relative to R5020 = 100; on this scale the RBA of progesterone is 16.

The first two **7** and **8** are the epimeric 21-fluoro derivatives of R5020. We have shown that R5020 is taken up by the uterus with high selectivity [33], and it is known that R5020 undergoes hydroxylation to the epimeric C21 hydroxy derivatives [39]. Thus, we prepared the fluoro analogs from the corresponding epimeric hydroxy compounds, one of which (21S) is a particularly high affinity ligand for the progesterone receptor [39]. In each case, fluoride ion displacement on the triflate derivatives proceeded with inversion, so that the low binding hydroxy epimer (21R) gave the high binding fluoro compound (21S), and vice versa. The relative binding affinity (RBA) values of these compounds for the progesterone receptor are shown beneath their structures, together with a summary of their tissue distribution properties in estrogen-primed immature rats. The high affinity analog (**7**) shows good uptake, but less than that of FENP. The low affinity analog (**8**) gave very poor uptake.

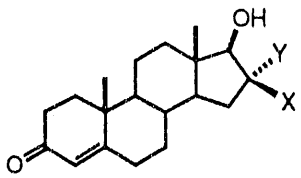
The third compound is DU41165 (**9**), a retroprogestin, that is, a compound in which the configurations of centers C-9 and 10 are inverted. This compound was reported to have an exceedingly high binding affinity for the progesterone receptor [40]. In our hands, we found that its affinity was high, though lower than the original report. Since it is difficult to prepare this compound in fluorine-18 labeled form, we synthesized DU41165 in tritium-labeled form for tissue distribution studies. Indeed, we find that its uptake behavior is better than any progestin that we

have studied so far both in terms of uptake efficiency and selectivity. Further studies on fluorine-18 labeled progestins that involve an investigation of strategies for labeling DU41165 with fluorine-18 form part of New Specific Aim 4 (Section D.4.).

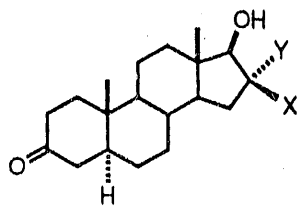
C.4.b. Previous Aim 2: Synthesis of Fluorine-Substituted Androgens

C.4.b.(1) Synthesis of Fluorine-Substituted Analogs (Publication Nos. 5, 10 and 15)

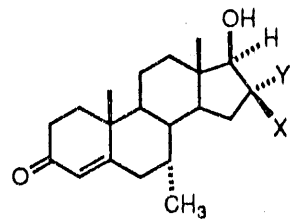
We have completed the synthesis of ten fluorine-substituted androgens whose structures are shown below. The compounds represent members of the testosterone, dihydrotestosterone, mibolerone, methyltrienolone and 7α-methyl-19-nortestosterone (MNT) classes, with fluorine substitution at both C-20 and C-16 (both α and β). A full description of their preparation is given in Publication Nos. 5 and 10.



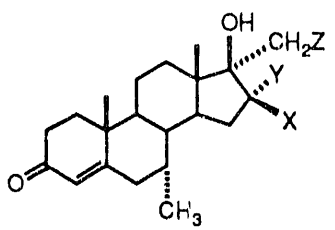
	X	Y	RBA
testosterone	H	H	5.0
10	F	H	2.1
11	H	F	0.3



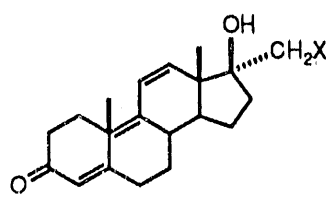
	X	Y	RBA
dihydrotestosterone	H	H	60.2
12	F	H	42.7
13	H	F	3.7



	X	Y	RBA
MNT	H	H	156
14	F	H	36.5
15	H	F	21.9



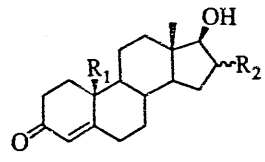
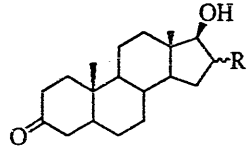
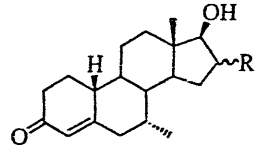
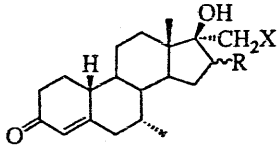
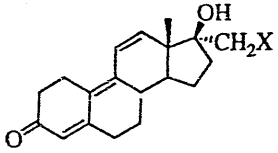
	X	Y	Z	RBA
mibolerone	H	H	H	118
16	F	H	H	30.8
17	H	F	H	37.3
18	H	H	F	53.2



	X	RBA
R1881	H	100
19	F	18.8

We have measured the binding properties of these compounds towards androgen receptor, progesterone receptor, and mineralocorticoid receptor and towards sex steroid binding protein. These binding data are given below the structures as well as in Table 2.

Table 2 RBA Values of Fluorinated Androgens and Their Protio Analogs for AR, PgR, MR and SBP

Compound		Relative Binding Affinity[a]			
		AR	PgR	MR	SBP
	$R_1 = H$ $R_2 = H$ (nor-T)	30.6 ± 1.5	3.10 ± 1.00	2.00 ± 1.20	29.9 ± 6.8
	$R_1 = CH_3$ $R_2 = H$ (T)	5.9 ± 1.3	0.09 ± 0.02	4.19 ± 3.44	417 ± 88
	$R_1 = CH_3$ $R_2 = \beta$-F (**10**)	2.1 ± 1.0	---	---	---
	$R_1 = CH_3$ $R_2 = \alpha$-F (**11**)	0.3 ± 0.1	---	---	---
	R = H (DHT)	60.2 ± 16.0	0.23 ± 0.10	1.17 ± 0.14	2125 ± 999
	R = β-F (**12**)	42.7 ± 2.0	0.12 ± 0.04	0.06 ± 0.01	385 ± 83
	R = α-F (**13**)	3.7 ± 0.8	0.07 ± 0.01	0.05 ± 0.01	228 ± 54
	R = H (MNT)	155.7 ± 14.4	3.7 ± 0.1	0.74 ± 0.32	28.7 ± 9.4
	R = β-F (**14**)	36.5 ± 4.4	1.6 ± 0.3	0.05 ± 0.01	3.75 ± 0.93
	R = α-F (**15**)	21.9 ± 3.3	5.7 ± 3.7	0.21 ± 0.10	4.01 ± 1.50
	R = H X = H (Mib)	118.0 ± 4.0	20.4 ± 4.2	5.70 ± 0.47	19.0 ± 8.3
	R = β-F X = H (**16**)	30.8 ± 5.4	3.0 ± 0.6	0.08 ± 0.01	1.3 ± 0.7
	R = α-F X = H (**17**)	37.3 ± 9.8	14.4 ± 1.5	0.37 ± 0.09	3.1 ± 0.8
	R = H X = F (**18**)	53.2 ± 3.1	10.3 ± 2.0	0.86 ± 0.07	3.0 ± 1.9
	X = H (R1881)	(100)	43.7 ± 9.1	25.4 ± 1.3	4.0 ± 0.9
	X = F (**19**)	18.8 ± 5.6	10.5 ± 1.2	4.5 ± 1.4	2.2 ± 0.3

[a] Relative binding affinities were determined by a competitive radiometric binding assay. Values are the average of two or more determinations ± range (n = 2) or s.d. (n ≥ 3) and are expressed on a percent scale relative to the affinity of the tritium-labeled tracer (AR=R1881, PgR=R5020, MR=Aldosterone, SBP=E_2).

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DE FG02 86ER60401

There are some very interesting trends found in the binding of these compounds. In six cases, the affinity for the androgen receptor relative to the tracer compound R1881 is 20% or greater, a number that we have set as the lower limit for these compounds to be considered for further studies. The highest affinity compounds for the androgen receptor are those from the dihydrotestosterone, mibolerone and 7α-methyl-19-nortestosterone (MNT) classes; those from the testosterone and R1881 classes were lower. The testosterone case is an interesting one. Testosterone, a relative low affinity ligand for the androgen receptor, is the major circulating form of androgens *in vivo*, but in the target tissue it is converted to the higher affinity dihydrotestosterone by the action of 5α-reductase. As will be described below, it appears that the same thing occurs with 16β-fluorotestosterone.

Fluorine substitution reduces androgen receptor binding affinity, but only by a modest factor. Where comparisons can be made, fluorine substitution at 16β is tolerated better than at 16α- in the testosterone and dihydrotestosterone series, but orientation makes less difference in the MNT and mibolerone series. This β-epimeric preference for fluorine at C-16 for androgens is just the reverse that of 16-fluoroestrogens for the estrogen receptor.

The binding affinity of these fluoro androgens for the progesterone and mineralocorticoid receptors was also measured (Table 2). (The affinity of compounds in these series for the glucocorticoid receptor is known to be very low.) Heterologous binding is lower for the natural androgens, testosterone and dihydrotestosterone, than for the synthetic ones. On the other hand, affinity for the serum binder, sex steroid binding protein (SBP), is much greater for the natural than for the synthetic androgens. Fluorine substitution tends to reduce the affinity of all of these compounds for the heterologous receptors and the serum binders, so the affinity ratios of androgen receptor to other binders, in most cases, remains about the same or improves relative to the parent compounds.

C.4.b.(2) Tissue Distribution Studies of Tritium-Labeled and Fluorine-18 Labeled Androgens In Vivo (Publication Nos. 4, 5, 11 and 15)

As a prelude to our work on fluorine-labeled androgens, we investigated the efficiency and selectivity of uptake of a series of five tritium labeled androgens in the castrate male rat (Publ. No. 5). These uptake data are summarized below in Table 3.

Table 3. Androgen Receptor Binding Affinity and Rat Tissue Distribution of Five Tritium Labeled Androgens in Castrate Male Rats.

		%ID/g prostate			prostate/muscle (prostate/blood)	
Name	RBA	1h	(1h blocked)[a]	4h	1 h	4 h
Testosterone	6	0.44	0.17	0.26	4.5 (3.5)	4.9 (3.9)
Dihydrotestosterone	61	0.39	0.13	0.27	2.8 (2.7)	3.8 (2.4)
19-Nortestosterone	31	0.38	0.10	0.15	3.0 (3.7)	2.9 (5.2)
Mibolerone	118	0.56	0.19	0.50	4.4 (12.3)	6.2 (11.5)
R1881	100	0.69	0.10	0.59	4.4 (10.1)	13.0 (23.4)

[a]36 μg of the corresponding non-radiolabeled androgen was co-injected with the radiotracer.

All five tritium-labeled androgens showed selective uptake by the ventral prostate that at 1 h was 60-85% displaceable by co-injection of an excess of unlabeled androgen. The greatest uptake

was with R1881. The target tissue activity remained high for all compounds up to 4 h after injection, and at 4 h the prostate to blood ratio for Mib and R1881 exceeded 10 and 20, respectively. The uptake efficiency and selectivity of these five androgens appear to be related to their affinity for the androgen receptor and their resistance to metabolism.

Seven of the fluorine-substituted compounds whose preparation was described in the preceding section were prepared in fluorine-18 labeled form. In each case, the radiolabeling step involved F-18 fluoride ion displacement on a reactive triflate or spiro cyclic sulfate derivative. Radiochemical yields were satisfactory, and the effective specific activity after HPLC purification was adequate for tissue distribution studies. The complete details of tissue distribution can be found in Publication Nos. 5 and 11. The key findings are shown in the Figures below.

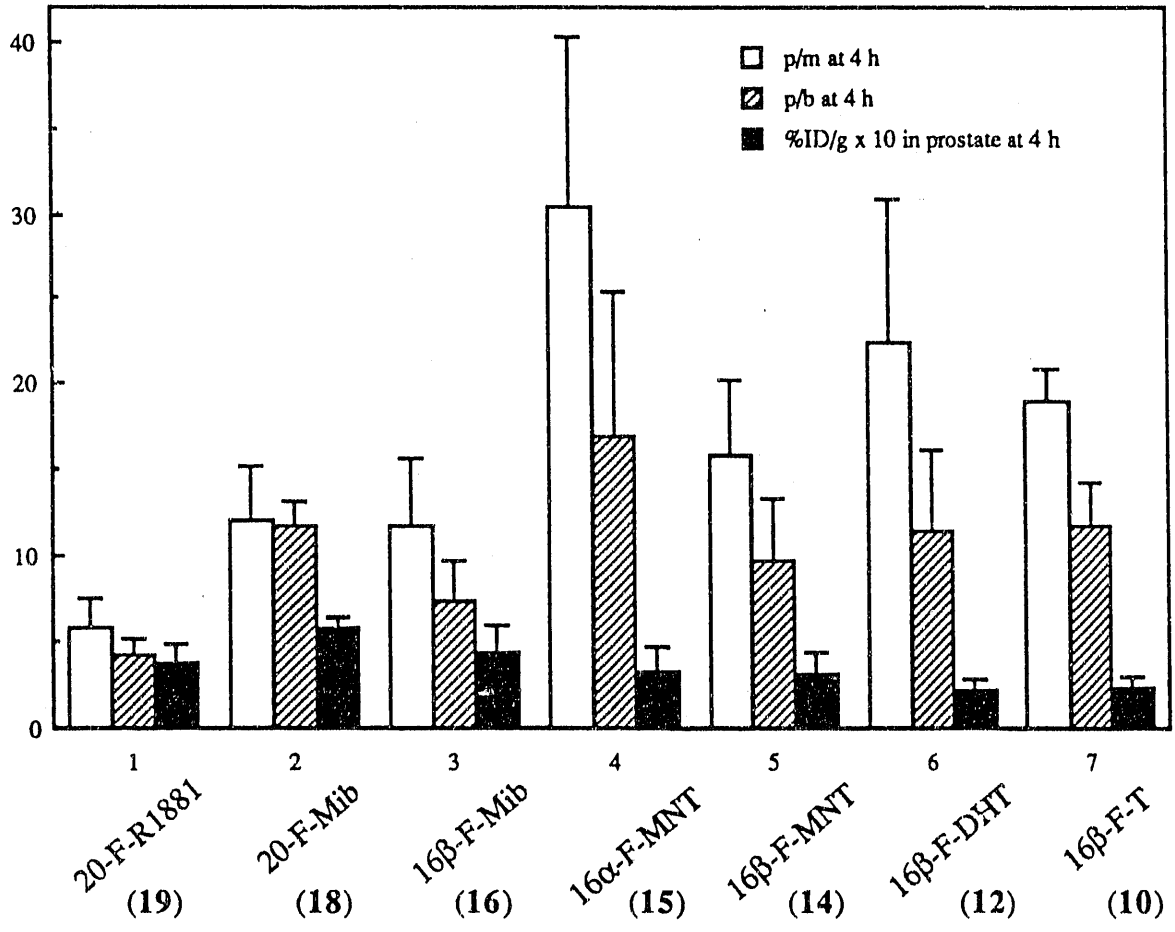


Figure 4 The Highest Uptake Ratios of Prostate to Muscle and to Blood (average values of ventral and dorsal prostate) of Fluorinated Androgens

As seen in Figure 4, all seven compounds showed uptake by the prostate that was both efficient, with %ID/g values ranging from 0.3 to 0.6, and selective, with prostate to muscle and prostate to blood ratios ranging from 6 to 30 and 4 to 16, respectively. Several points are of particular interest. First 16β-F-testosterone, despite its low *in vitro* binding affinity for the androgen receptor, shows efficient and selective uptake *in vivo*, probably because of its conversion to the high affinity 16β-F-dihydrotestosterone analog. This would indicate that the 16β-fluoro substituent does not interfere with the A-ring reduction. For the rest of the compounds there is

generally a reciprocal relationship between uptake efficiency (%ID/g) and uptake selectivity (prostate to muscle or blood ratios). This is consistent with the expected rate of metabolism of these compounds, that is, those that are rapidly metabolized (testosterone, dihydrotestosterone, and MNT) are cleared rapidly, so that prostate uptake is limited, but selectivity is high, since clearance from non-target areas is rapid. On the other hand, the derivatives of mibolerone or R1881, expected to have greater metabolic stability, show greater prostate uptake but lower selectivity, as the extended blood curve would provide more input to the target tissue, but less effective clearance of background activity.

We also noted some interesting trends in bone activity (Figure 5). Since bone scavenges free fluoride ion avidly, bone activity is a good index of metabolic defluorination. The high bone activity seen with the 16β-fluoro compounds suggests that an important route of metabolism is 16α-hydroxylation (cf. Scheme 2). This would give a fluorohydrin that would eliminate to release free fluoride ion. This metabolism is retarded by a 17α-methyl group, is blocked by a 16α-substituent and is of no consequence when fluorine substitution is at C-20.

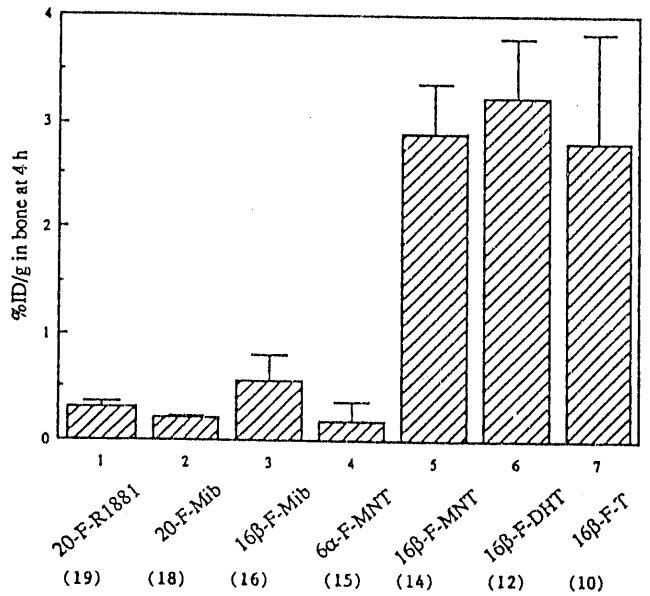
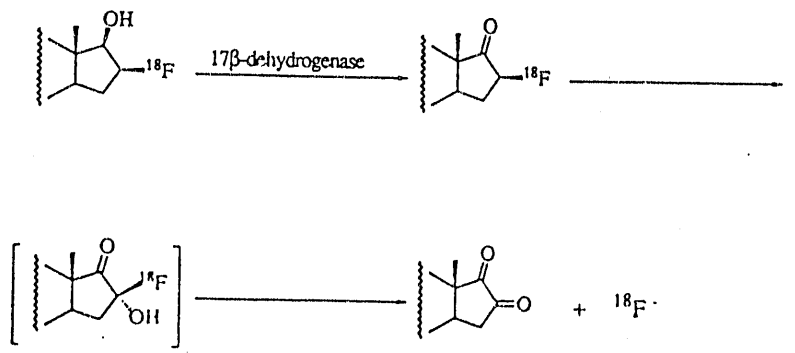


Figure 5. Bone Radioactivity Comparison of Fluorinated Androgens



Scheme 2. Possible route of metabolic defluorination of 16β-F-androgens.

We will be continuing studies on the best of these fluorine substituted androgens, 16α-F-MNT, 20-F-mibolerone, and 16β-F-dihydrotestosterone. Additional studies on compounds in this series are part of New Specific Aim 5 (Section D.5.).

C.4.c. Previous Aim 3: Evaluate the Receptor Binding and Non-Specific Binding of the Fluorosteroids by In Vitro Binding Assays

The results under this aim are imbedded in the progress described under previous aims 1 and 2. In brief, however, we utilize radiometric competitive binding assays to measure the relative affinity of the new androgens and progestins towards both the homologous receptor and other receptors. High affinity, specific tritium-labeled radiotracers are used, with dextran-coated charcoal as the adsorbant of free ligand. In toto, we assay binding to androgen, progesterone, mineralocorticoid and glucorticoid steroid hormone receptors. (The members of these steroidal classes do not bind to the estrogen receptor). We also measure the binding of these compounds to serum steroid transport proteins, sex steroid binding protein for the androgens, and corticosteroid binding globulin for the progestins.

Non-specific binding involves interaction of these steroids with abundant low affinity hydrophobic pockets in proteins and lipid phases, and is generally simply proportional to the lipophilicity of the compound. We measure this lipophilicity by octanol-water partition coefficients, which are estimated by using the reversed phase HPLC method of Minick [13]. This method uses the elution volume measured in a series of carefully selected methanol/0.1% n-decylamine-aqueous MOPS buffer concentrations and provides highly reproducible numbers.

C.4.d. Previous Specific Aim 4: Develop and Optimize Fluoride-Ion Substitution for Labeling with Fluorine-18 (Publication Nos. 1-3, 5 and 11).

This specific aim is embodied in the results described in the previous sections. We have utilized in many cases the displacement of reactive sulfonate esters, especially triflates, by fluoride ion. This proceeds rapidly and efficiently in most cases. We have prepared two fluorine substituted androgens by displacement on a spiro cyclic sulfate. While we reported this method earlier in connection with the work during the previous grant period [27], in the two examples described here, we had to modify the synthesis of the cyclic sulfate because of oxidation sensitive functions elsewhere in the molecule. The use of tetrabutylammonium persulfate (OXONE) in the presence of base worked very well for this sensitive oxidation and gave the desired cyclic sulfate in good yield (Publ. Nos. 5, 11).

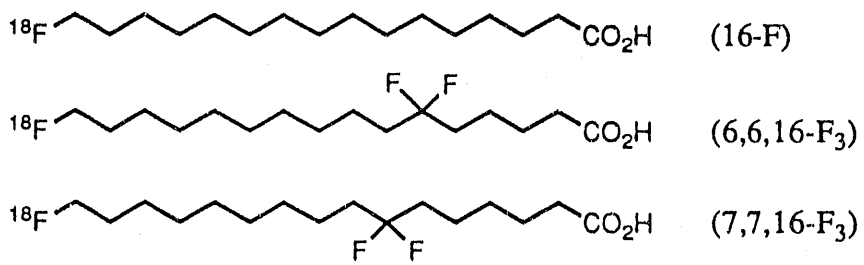
C.4.e. Previous Aim 5: Evaluate the Target Tissue Uptake of the Androgens and Progestins in Experimental Animals (Publication Nos. 1, 2, 4, 5, 9 and 11)

The progress on this aim is imbedded in the progress reported under previous aims 1 and 2. This work is done in St. Louis and involves the use of appropriate animal model systems. For androgens we use estrogen-treated male rats; estrogen treatment has the same effect as orchidectomy, reducing androgen production and increasing the fraction of androgen receptor that is unoccupied by endogenous androgens, but is more convenient and is less stressful to the animals. For progestins, we use estrogen-primed immature female rats. The estrogen treatment in females increases the concentration of progestone receptor. Distribution times generally range from 1-6 h, and at some time points a set of animals is treated with an excess of unlabeled androgen or progestin (for the male and female rats, respectively), to block receptor-mediated uptake. These animal model systems appear to be adequate for characterizing the in vivo uptake properties of these ligands for their intended targets and assessing the selectivity of this distribution.

C.4.f. Other Findings

C.4.f.(1) F-18 ω-Fluoro Fatty Acids with Geminal Difluoro Substituents as Potential Metabolically Blocked Myocardial Imaging Agents (Publication No. 3)

We have completed our study of fluorine-18 labeled fatty acids bearing geminal difluoro substituents as potential metabolically blocked myocardial imaging agents. The fluorine-18 labeling of these fatty acids was challenging, but gave satisfactory radiochemical yields and purity. In animal studies, heart uptake by these compounds was modest, and there was relatively little evidence that the geminal difluoro substituent, whether at an even or odd carbon position, had a major suppressive effect on metabolism.



What surprised us was the degree of metabolic defluorination that occurred in these compounds regardless of fluorine substitution at other sites. Defluorination is presumed to occur by ω-hydroxylation followed by fluoride ion elimination from the fluorohydrin.

C.5. Previous Grant Record

C.5.a. Refereed Publication

1. M. G. Pomper, J. A. Katzenellenbogen, M. J. Welch, J. W. Brodack, and C. J. Mathias, 21-[^{18}F]Fluoro-16α-ethyl-19-norprogesterone: Synthesis and Target Tissue Selective Uptake of a Progestin Receptor-Based Radiotracer for Positron Emission Tomography. *J. Med. Chem.*, **1988**, *31*, 1360-1363.

2. M. G. Pomper, K. G. Pinney, K. E. Carlson, H. F. VanBrocklin, C. J. Mathias, M. J. Welch, and J. A. Katzenellenbogen, Target Tissue Uptake Selectivity of Three Fluorine-Substituted Progestins: Potential Imaging Agents for Receptor-Positive Breast Tumors. *Nucl. Med. Biol.*, **1990**, *17*, 309-319.

3. S. S. Pochapsky, H. F. VanBrocklin, M. J. Welch, and J. A. Katzenellenbogen, Synthesis and Tissue Distribution of Fluorine-18 Labeled Trifluorohexadecanoic Acids. Considerations in the Development of Metabolically Blocked Myocardial Imaging Agents. *Bioconjugate Chemistry*, **1990**, 2, 231-244.

4. K. E. Carlson and J. A. Katzenellenbogen, A Comparative Study of the Selectivity and Efficiency of Target Tissue Uptake of Five Tritium Labeled Androgens in the Rat. *J. Steroid Biochem.*, **1990**, *36*, 549-561.

5. A. Liu, J. A. Katzenellenbogen, H. F. VanBrocklin, C. J. Mathias, and M. J. Welch, 20-[^{18}F]Fluoromibolerone, a Positron-Emitting Radiotracer for Androgen Receptors: Synthesis and Tissue Distribution Studies. *J. Nucl. Med.*, **1991**, *32*, 81-88.

6. J. A. Katzenellenbogen, A. Liu, S. J. Brandes, K. E. Carlson, and M. J. Welch, Strategies for the Development of Androgen Receptor-Based Imaging Agents for Prostate Cancer in "Radionuclides in the Prostate Gland" G. S. Limouris and S. K. Shoukla, Eds., Edizioni Associate, Rome, **1991**, p. 91-103.

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8. J. P. DiZio, R. Fiaschi, A. Davison, A. G. Jones and J. A. Katzenellenbogen, Progestin-Rhenium Complexes: Metal-Labeled Steroids with High Receptor Binding Affinity, Potential Receptor-Directed Agents for Diagnostic Imaging or Therapy. *Bioconjugate Chem.*, **1991**, Submitted.

9. J. P. DiZio, C. J. Anderson, A. Davison, G. J. Ehrhardt, K. E. Carlson, M. J. Welch and J. A. Katzenellenbogen, Technetium and Rhenium Labeled Progestins: Synthesis, Receptor Binding, and In Vivo Distribution of an 11β-Substituted Progestin Labeled with Technetium-99m and Rhenium-186. *J. Nucl. Med.*, **1991**, Submitted

10. A. Liu, K. E. Carlson, and J. A. Katzenellenbogen, Synthesis and Receptor Binding Affinity of Fluorine-Substituted Androgens, Potential Imaging Agents for Prostatic Cancer. *J. Med. Chem.*, **1991**, Submitted.

11. A. Liu, C. F. Dence, M. J. Welch, and J. A. Katzenellenbogen, Synthesis and Tissue Distribution Studies of Seven Fluorine-18 Labeled Androgens: Potential Imaging Agents for Prostatic Cancer. *J. Nucl. Med.*, **1991**, Submitted.

C.5.b. **Abstracts**

12. M. G. Pomper, K. G. Pinney, K. E. Carlson, H. F. VanBrocklin, M. J. Welch, J. A. Katzenellenbogen, Uptake Selectivity of Fluorine-substituted Progestins: Analogs of R5020 and a Novel Retroprogestin (The Society of Nuclear Medicine 36th Annual Meetings, St. Louis, MO, June 13-16, 1989), *J. Nucl. Med.*, **30**, 928, 1989.

13. J. A. Katzenellenbogen, M. G. Pomper, A. Liu, M. J. Kochanny, A. M. Thieme, K. E. Carlson, C. J. Mathias, H. VanBrocklin, M. A. Mintun, M. J. Welch, ^{18}F-Labeled Steroids for Positron Emission Tomography of Receptor-Positive Tumors and Brain Receptors, 1989 International Chemical Congress of Pacific Basin Societies (PACIFICHEM), Hawaii, December, 1989.

14. J. A. Katzenellenbogen, M. G. Pomper, A. Liu, M. J. Kochanny, K. E. Carlson, C. J. Mathias, H. VanBrocklin, M. J. Welch, ^{18}F-Labeled Steroids for Positron Emission Tomography of Receptor-Positive Tumors and Brain Receptors, American Chemical Society, 199th National Meeting, Boston, MA, April, 1990.

15. A. Liu, K. E. Carlson, J. A. Katzenellenbogen, H. F. VanBrocklin, C. J. Mathias, M. J. Welch, Androgen Receptor Androgen Receptor-Based Imaging Agents for the Prostate: Synthesis and Tissue Distribution Studies with Tritium and Fluorine-18 Labeled Androgens, 8th International Symposium and Radiopharmaceutical Chemistry, Princeton, NJ, June, 1990.

16. F. Dehdashti, A. H. McGuire, H. F. VanBrocklin, J. W. Brodack, D. P. Anqriole, C. J. Mathias, B. A. Siegel, M. J. Welch, M. G. Pomper, J. A. Katzenellenbogen, Assessment of Progestin Receptors in Breast Carcinoma by Positron Emission Tomography. Society of Nuclear Medicine, Washington, D.C., June, 1990, *J. Nucl. Med.* **1990**, *31*, 746.

17. A. Liu, J. A. Katzenellenbogen, H. F. VanBrocklin, M. J. Welch, Synthesis and Evaluation of Fluorine-18 Labeled Androgens as Imaging Agents for Prostatic Tumors. 201st National ACS Meeting, Atlanta, GA, April 1991.

18. J. P. DiZio, R. Fiaschi, J. A. Katzenellenbogen, C. J. Anderson, G. J. Ehrhardt, and M. J. Welch, Progestin-Rhenium Complexes: Potential Metal-Based Imaging Agents for Steroid Receptors. Society of Nuclear Medicine, 38th Annual Meeting, Cincinnati, OH, June, 1991, *J. Nucl. Med.* **1991**, *32*, 925.

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