FINAL REPORT

DOE Project No. **DE FG02 86ER60401**

Project PI: **John A. Katzenellenbogen**

Institution: **University of Illinois, Urbana-Champaign**

Title: "Novel Chemical Strategies for Labeling Small Molecule Ligands for Androgen, Progestin, and Peroxisome Proliferator-Activated Receptors for Imaging Prostate and Breast Cancer and the Heart."

Period

This final report covers work performed on this project from February 1, 1998 – May 31, 2006

Summary of Progress

The specific aims of this project can be summarized as follows:

- ***Aim 1:*** Prepare and evaluate radiolabeled ligands for the peroxisome proliferator-activated receptor γ (PPARγ), a new nuclear hormone receptor target for tumor imaging and hormone therapy.
- ***Aim 2:*** Prepare steroids labeled with a cyclopentadienyl tricarbonyl technetium or rhenium unit.
- ***Aim 3:*** Prepare and evaluate other organometallic systems of novel design as ligand mimics and halogenated ligands for nuclear hormone receptor-based tumor imaging.

As is described in detail below, we made excellent progress on all three of these aims; the highlights of our progress are the following:

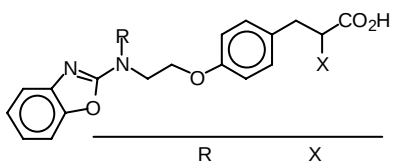
- we have prepared the first fluorine-18 labeled analogs of ligands for the PPARγ receptor and used these in tissue distribution studies in rats
- we have developed three new methods for the synthesis of cyclopentadienyltricarbonyl rhenium and technetium ($CpRe(CO)_3$ and $CpTc(CO)_3$) systems and we have adapted these to the synthesis of steroids labeled with these metals, as well as ligands for other receptor systems
- we have prepared a number of fluorine-18 labeled steroidal and non-steroidal androgens and measured their tissue distribution in rats
- we have prepared iodine and bromine-labeled progestins with high progesterone receptor binding affinity
- we have prepared inorganic metal tricarbonyl complexes and steroid receptor ligands in which the metal tricarbonyl unit is an integral part off the ligand core.

Throughout the detailed description of our past progress, reference is made to publications that have arisen from each section as ["**Pub. No. (#)**"]. Reference is made only to the refereed papers [**1–36**], which are listed in the end of this report.

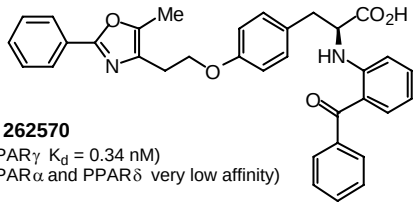
Detailed Final Report

1. Progress Under Previous Aim 1: Prepare and evaluate radiolabeled ligands for the peroxisome proliferator-activated receptor γ (PPARγ), a new nuclear hormone receptor target for tumor imaging and hormone therapy. [Pub. No. 18, 33, 34].

Because it is believed that the PPARγ receptor may be a good target for imaging small metastatic tumors, we first undertook the synthesis of two representative, high affinity PPARγ ligand in fluorine-18 labeled form (**9** and **22**, Scheme 1, left). The parent compound is known to have high affinity ligand for PPARγ, and the synthesis of precursors suitable for labeling these two analogs with fluorine-18 proceeded uneventfully, although it was a challenging, multistep synthesis.



	R	X
9	$–CH_2CH_2F$	–O-Ph
22	$–CH_3$	$–OCH_2CH_2F$



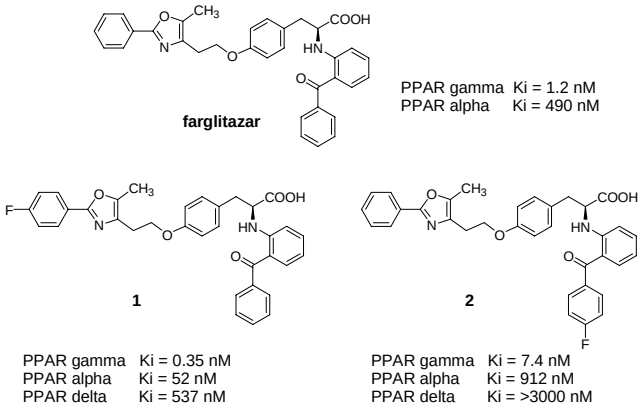
GI 262570
(PPARγ K_d = 0.34 nM)
(PPARα and PPARδ very low affinity)

Scheme 1. Fluorine-Substituted PPARγ Ligands Synthesized (left) and for Possible Further Consideration (right).

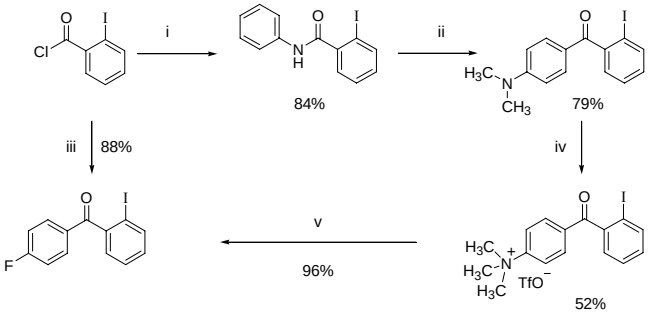
One of the fluorine-substituted products (**22**) was found to have relatively high affinity for PPARγ, at least in the low nanomolar range (7 nM), comparable to that of the parent ligand, and we prepared this analog in fluorine-18 labeled form in reasonable yield and at sufficiently high specific activity to conduct tissue distribution studies in rats and in mice bearing human breast tumor xenografts. We looked for selective accumulation in normal tissues and tumors that are known to contain PPARγ, but we did not find significant selective uptake in these tissues, and no tissue showed uptake that could be blocked by coadministration of a blocking dose of unlabeled ligand.

In evaluating our experience with these PPARγ ligands, we recognize that this target system presents two special challenges for imaging, low receptor density and unusual ligand pharmacology. First, the concentration of this receptor, even in the richest tissues, is considerably lower than that of the steroid receptors. Thus, in order to image this receptor effectively, one would need a ligand with exceedingly high affinity and high specific activity. Since we initiated this project, higher affinity PPARγ ligands such as GI 262570 (Scheme 1, right; K_d = 0.34 nM) have been reported.[70] This compound forms the basis of a new series of radiohalogenated ligands that we plan to investigate for the PPARγ target. The second challenge is pharmacokinetic: most of the potent PPARγ ligands are lipophilic acids that are highly bound to serum proteins and have long clearance times.

We prepared several new PPARγ ligands based on the GI262570 structure; the structures of two of these are shown in Scheme 2. Our selection of the site of halogen substitution (**X**) is based a site known to be tolerant of substitution in this ligand series. Our synthesis of compound **2** was patterned after that described in the literature by the Glaxo-Wellcome group and is shown in Schemes 3 and 4.

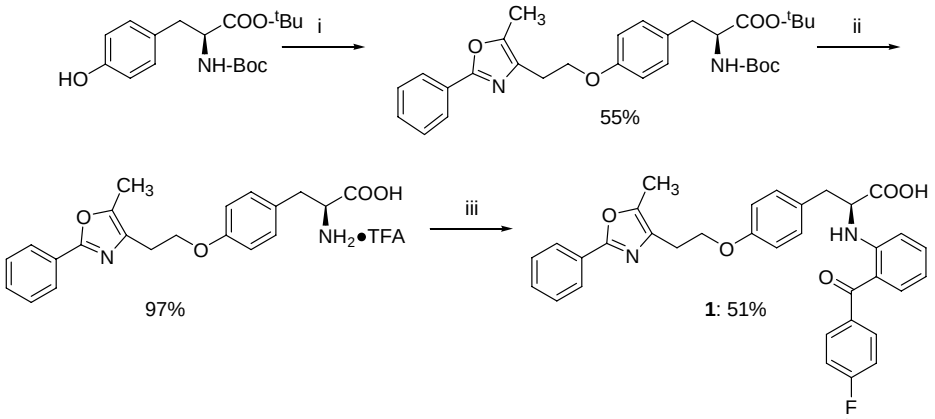


Scheme 2. Two new Radiohalogenated PPARγ Ligands



[a]Reaction conditions: (i) aniline, pyridine, rt, 12 h; (ii) 1) $POCl_3$, N,N-dimethylaniline, 150 °C, 4 h, 2) 50% aq. HCl, 100 °C, 2 h; (iii) fluorobenzene, $AlCl_3$, $FeCl_3$, rt, 20 h; (iv) $CF_3SO_2OCH_3$, CH_2Cl_2, rt, 12 h; (v) TBAF, CH_3CN, 90 °C 1 h.

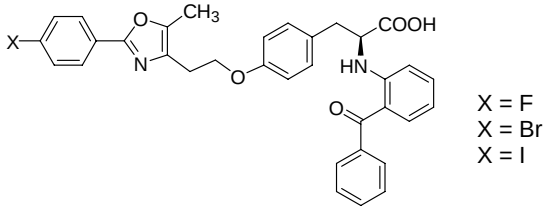
Scheme 3. Synthesis of the benzophenone portion of PPARγ ligand **1**.



[a]Reaction conditions: (i) 2-(5-methyl-2-phenyloxazol-4-yl)ethanol, PPh_3, DEAD, THF, 0 °C ~ rt, 12 h; (ii) TFA/CH_2Cl_2, rt, 6 h; (iii) **13**, K_2CO_3, CuI, DMF, 90 °C, 4 h.

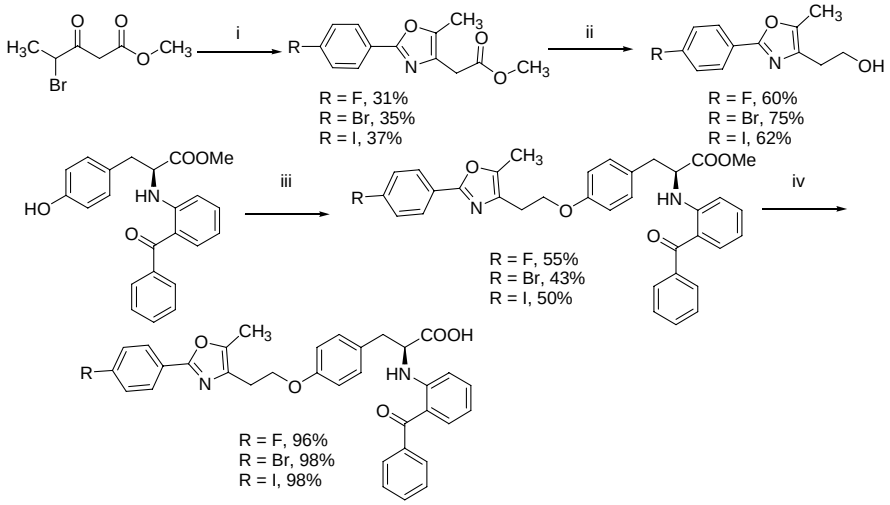
Scheme 4. Completion of the synthesis of the PPARγ ligand **1**.

The other PPARγ ligand based on GI262570 are shown in Scheme 5 below; in addition to the fluoro analog, there are bromine and iodine analogs.



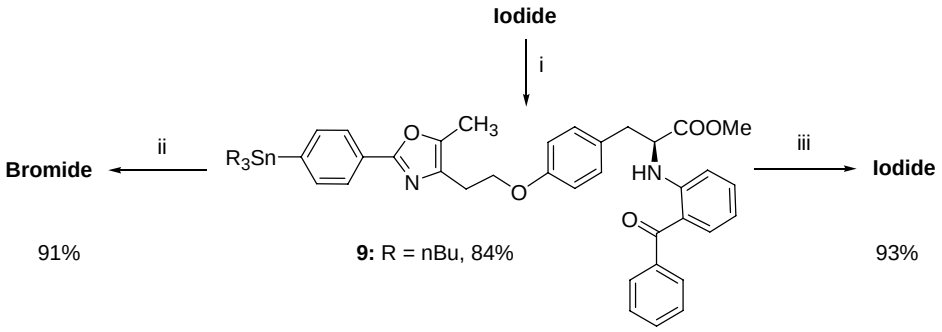
Scheme 5. Other new Radiohalogenated PPARγ Ligands

Synthesis of these three ligands in unlabeled form is given in Scheme 6 below. The synthesis designed for radiobromination or radioiodination proceeds through the aryl tin intermediate, shown in Scheme 7, and the route suitable for radiofluorination proceeds through a diaryliodonium salt, as shown in Schemes 8 and 9, below.



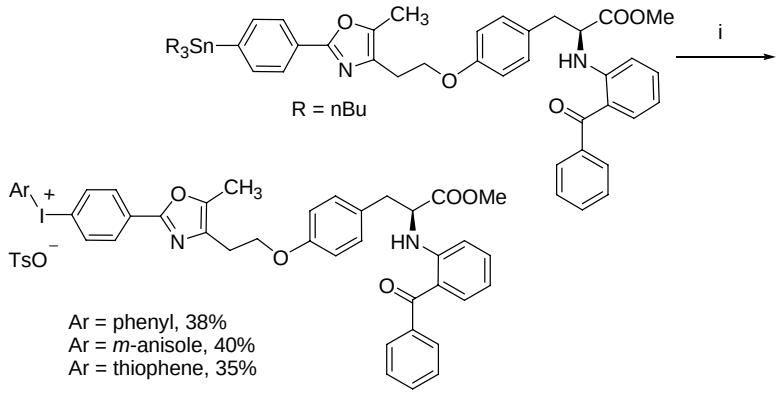
[a]Reaction conditions: (i) 4-halobenzamide, 1,2-dichlorobenzene, 130 °C, 24 h; (ii) LAH, THF, 0 °C, 1 h, or $NaBH_4$, MeOH, rt, 6 h; (iii) PPh_3, DEAD, THF, 0 °C ~ rt, 12 h; (iv) LiOH, THF/MeOH, rt, 6 h.

Scheme 6. Synthesis of three new halogenated PPARγ Ligands in unlabeled form



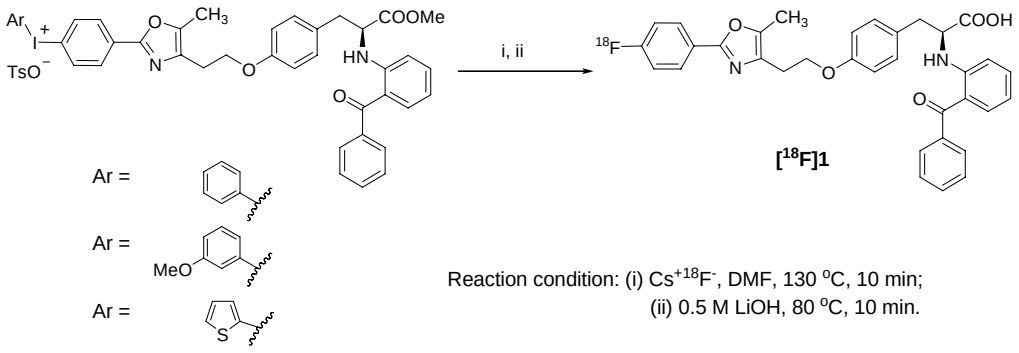
[a]Reaction conditions: (i) $(PPh_3)_4Pd$, Sn_2Bu_6, Toluene, 120 °C, 12 h; (ii) (a) NH_4Br, CH_3CO_3H, CH_3COOH, rt, 30 min, (b) LiOH, THF/MeOH, rt, 1 h.; (iii) (a) NaI, CH_3CO_3H, CH_3COOH, rt, 30 min, (b) LiOH, THF/MeOH, rt, 1 h.

Scheme 7. Synthetic sequence suitable for radiobromination and radioiodination of PPAR γ Ligands .



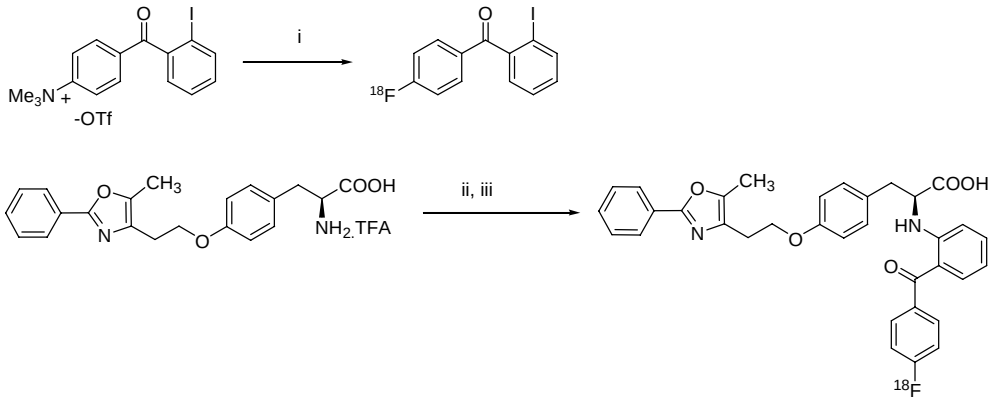
[a]Reaction conditions: (i) Various Koser reagents, CH_2Cl_2, rt, 12 h.

Scheme 8. Synthesis of diaryliodonium salt precursor for F-18 labeling of PPAR γ Ligands .



Scheme 9. Reaction of diaryliodonium salt precursor for F-18 labeling of PPAR γ Ligands .

One of these new PPARγ ligands was labeled with F-18, as shown in Scheme 10. Tissue distributions done in Sprague-Dawley rats (Tabl 1) showed pronounced uptake in brown fat, the tissue in non-tumor bearing animals that is highest in PPARγ. Uptake in brown fat also could be blocked by coadministration of a high dose of unlabeled compound, further suggesting that the uptake in this tissue is due to PPARγ.



Reaction condition: (i) $K^{+18}F^-$, Kryptofix, MeCN, 110 °C, 10 min; (ii) tetrabutylammonium hydroxide (40% aqueous solution); (iii) **[^{18}F]benzophenone**, CuI, DMF, N_2, 130 °C, 20 min.

Scheme 10. F-18 labeling of a PPAR γ Ligands.

TABLE 1. Biodistribution of **[^{18}F]1** in Normal Mautre Female Sprague-Dawkey Rats

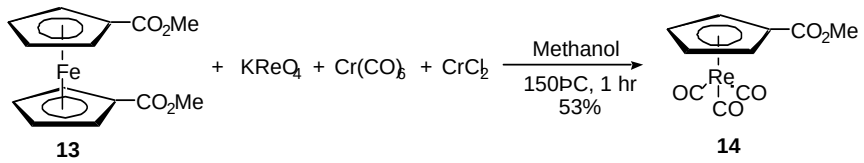
	Mean percentage injected dose per gram ± SD (n = 5)			
Tissue/organ	1 h	1 h (blocked)	2 h	2 h (blocked)
Blood	0.07 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
Lung	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
Liver	11.52 ± 3.80	8.36 ± 2.21	6.28 ± 1.89	5.51 ± 1.91
Spleen	0.13 ± 0.03	0.13 ± 0.03	0.07 ± 0.03	0.10 ± 0.04
Kidney	0.17 ± 0.05	0.14 ± 0.03	0.09 ± 0.02	0.08 ± 0.02
Muscle	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
White Fat	0.05 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Brown Fat	0.13 ± 0.01	0.07 ± 0.01	0.12 ± 0.03	0.08 ± 0.01
Heart	0.07 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
Bone	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.01

2. Progress Under Previous Aim 2: **Develop methods for labeling small molecules with a cyclopentadienyl tricarbonyl technetium or rhenium unit. [Pub. No. 1, 2, 4, 5, 7-9, 11-15, 20, 25, 26]**

In our previous application, we reported that certain steroids containing a cyclopentadienyl tricarbonyl rhenium group ($CpRe(CO)_3$) appeared to be promising ligands for receptors. This organometallic function provides a novel means of incorporating rhenium ($CpRe(CO)_3$) and technetium ($CpTc(CO)_3$) into small organic molecules via chemical linkages that are small, non-polar and very stable. Despite the unique structural features of the $CpRe(CO)_3$ and $CpTc(CO)_3$ groups and their promise for radiolabeling, what had been missing was an effective method for preparing these systems efficiently and conveniently at the tracer level and at high specific activity. Most known preparations of these systems utilized slow reactions that proceeded under awkward conditions—involving autoclaves to contain high pressures of carbon monoxide—to reduce pertechnetate or perrhenate to the dimetal decacarbonyl state, from which the pentacarbonyl bromide is prepared and then reacted with the Cp anion. During the past project period, we have developed three new methods for preparing the $CpMet(CO)_3$ system conveniently and efficiently.

2.a. Double Ligand Transfer (DLT) Reaction – We have successfully adapted an unusual ligand transfer process termed the "central atom exchange" reaction or the "double ligand transfer" (DLT) reaction for the preparation of $CpRe(CO)_3$ or Tc-99m labeled $CpTc(CO)_3$ at high specific activity. During this exchange reaction, shown in Scheme 11, pertechnetate or perrhenate is reduced by Cr(III) in the presence of a

source of CO ($Cr(CO)_6$) and a Cp donor (*bis*-methoxycarbonylferrocene, **13**). Although the reaction requires high temperatures, it can be performed conveniently in a sealed tube, and good yields are obtained; in fact, higher yields are obtained with Tc-99m at the tracer level than with larger quantities of Re and Tc-99. The modifications of this central atom exchange reaction that we have made avoid the use of manganese carbonyls, which was an essential component of the original description of this reaction. The use of manganese carbonyls resulted in the production of $CpMn(CO)_3$, which proved difficult to separate from the $CpRe(CO)_3$ and $CpTc(CO)_3$ products, greatly reducing the effective specific activity of the Tc-99m compound. The only limitation on this reaction is that exchange occurs readily only when the ferrocene system is substituted with electron withdrawing groups. These groups appear to provide the needed labilization of the Cp system required for transfer from the Fe to the Tc or Re centers.

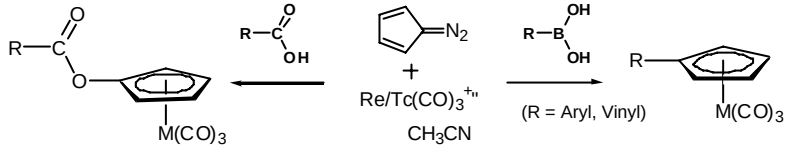


13 **14**

Scheme 11. Modified Double Ligand Transfer Reaction that Gives High Effective Specific Activity

With support from this project, we have demonstrated the utility of this reaction for labeling various receptor ligands with Tc-99m; these are described below. Initially, we did this through a $CpTc(CO)_3$ active ester, which could readily be prepared using the DLT reaction; we also labeled molecules with ferrocene precursors having ketone linkages. However, despite the success we had with the DLT in a number of systems, we recognized the need to expand methods for the preparation of $CpTc(CO)_3$ and $CpRe(CO)_3$ systems. We explored a number of alternative approaches and have found two new methods that provide a great expansion of methods for the preparation of these $CpMet(CO)_3$ systems that can be used for radiolabeling with short-lived isotopes: the three-component condensation and the Cp-tin transfer reactions. These two new methods are discussed in the sections below.

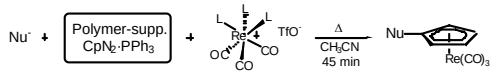
2.b. Three-Component Condensation – Hermann had shown that $CpTc(CO)_3$ systems could be prepared by reaction of diazocyclopentadiene with bromotechnetium pentacarbonyl. This was a sluggish reaction that had the additional disadvantage that it gave only the product having a bromine substituent on the Cp ring, that is, the halogen nucleophile that was prebound by the metal became incorporated in the carbon skeleton. We reasoned that if we removed this halogen from the metal precursor, then we might be able to introduce a variety of exogenous nucleophiles and have them become incorporated in the Cp ring. Indeed, we found that by replacing $BrTc(CO)_5$ with the $Re(CO)_3^+$ or $Tc(CO)_3^+$, cations in which the halogen had been removed using silver triflate, we could introduce other nucleophiles (Scheme 12). The nucleophiles that worked best were carboxylates, which gave as products acyloxyCpMet$(CO)_3$ (Scheme 3, left), and boronic acids, which gave aryl and vinyl CpMet $(CO)_3$ systems (Scheme 3, right).

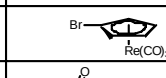
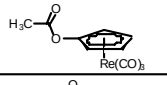
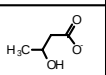
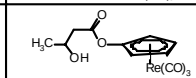
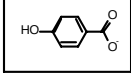
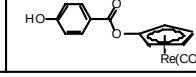


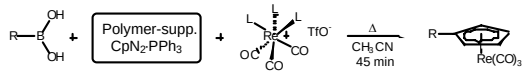
Scheme 12. Three-Component Condensation to Prepare Acyloxy and Aryl/Vinyl $CpMet(CO)_3$ Systems

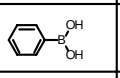
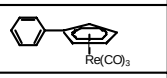
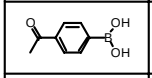
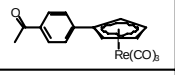
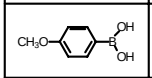
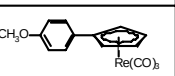
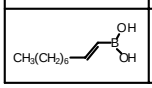
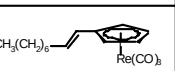
The three-component condensation works under very mild conditions and proceeds in good yields. Examples of both types of this reaction are given in Table 2 below, and the technetium and rhenium organometallic structures that we can produce by this method are more complex than any hithertofore prepared. Additional examples are given below. The acyloxy-$CpMet(CO)_3$ systems can be considered the "carboxy-inversion analogs" of the $CpMet(CO)_3$ carboxylates that come from the DLT reaction. The ester link to the Cp phenol is rather stable. The vinyl and aryl-substituted systems are novel.

Table 2. Structures and Isolated Yields in the Three-Component Condensation with Various Cyclopentadienes

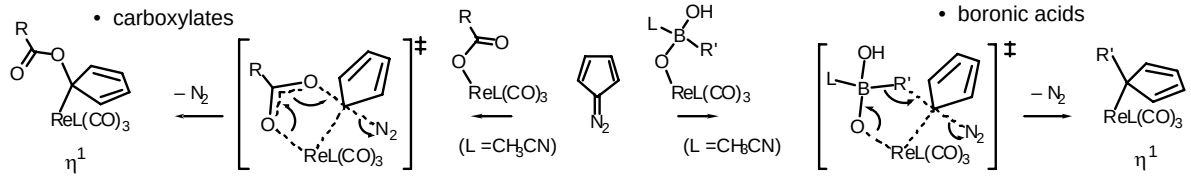


Nu⁻	complex	"free" CpN_2	polymer-supp. reagent
Br^-		67	71
H_3C–C(=O)–O⁻		69	67
		59	60
		72	71



boronic acid	complex	"free" CpN_2	polymer-supp. reagent
		64	53
		34	41
		74	56
		51	45

Because this is an unusual transformation, we examined its mechanism in some detail. Through a combination of spectroscopic examinations of reaction intermediates, using FT-IR and NMR with C-13 labeled precursors, as well as a linear free energy study of various para-substituted carboxylates, we were able to conclude that the reaction proceeds in the following fashion: As illustrated in Scheme 13, there is an initial interaction between the metal and the nucleophile (i.e., carboxylate oxygen or boronic acid oxygen); the diazo-substituted carbon then interacts with the metal center; there is then a synchronous transfer through a multi-center transition state of the metal-bound nucleophile (i.e., oxygen in the case of carboxylate nucleophiles or aryl or vinyl group in the case of the boronic acids) to this center with loss of nitrogen, and finally, the initial η^1-Cp product isomerizes to the η^5-Cp product (the latter isomerization not shown).



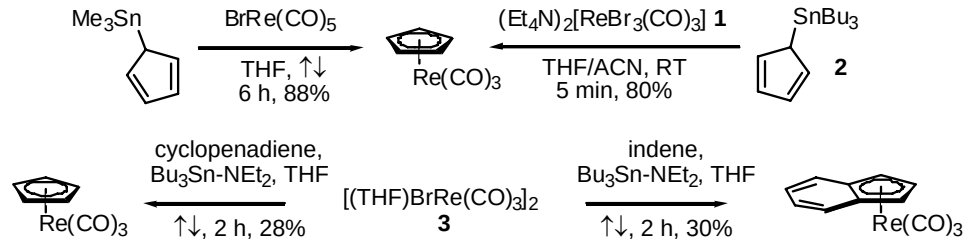
Scheme 13. Transition State for the Three-Component Condensation with Carboxylates and Boronic Acids.

The critical component of this reaction is diazocyclopentadiene, a molecule that is easy to prepare but is unstable on storage and potentially explosive, clearly undesirable characteristics for general synthesis and particularly for routine radiochemical preparations that might be done by medical technicians. We found, however, that the stable, crystalline adduct of diazocyclopentadiene and triphenylphosphine works just as well in the three-component condenstation as does diazocyclopentadiene itself. Furthermore, this adduct can be prepared from triphenylphosphine tethered on a solid support. This provides the added convenience of removing from the reaction product any phosphorous-containing material.

The three-component condensation greatly expands the scope of $CpMet(CO)_3$ systems that can be prepared, but it is still limited to molecules with a single substituent on the Cp ring. Therefore, we sought yet another method that would be applicable to the preparation of multiply substituted systems.

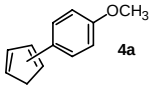
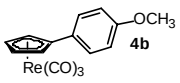
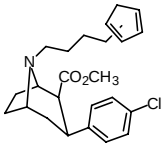
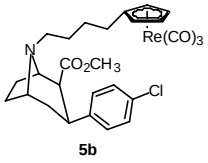
2.c. Cp-Tin Transfer Reaction – We were aware that it is possible to prepare $CpTc(CO)_3$ and $CpRe(CO)_3$ systems directly by the reaction of various Cp donors and the technetium and rhenium carbonyl cations. Typically, the Cp thallium salts are used. We were, however, more intrigued by the use of Cp tin systems as donors, because these seemed to proceed under mild conditions. In fact, as shown in Scheme 14, we found that a variety of Cp-tin systems, which can be prepared simply by the reaction of cyclopentadiene with a tributyltin amide, worked as excellent Cp transfer agents, reacting readily with metal carbonyl precursors. The reaction with the rhenium tricarbonyl cation, in particular, was very fast. These reactions do not require that halogen be removed from the tricarbonylmetal cation precursor, and they proceed even more rapidly and under milder conditions than the three-component condensation. In addition, we found that formation of the Cp-tin intermediate and the Cp transfer to the tricarbonyl metal center could be done in one

pot, under aprotic conditions in THF as solvent and the THF-soluble precursor **3**. The yields in the one-pot protocol are not high, but the conversion is very convenient. Thus, we have developed a very general method for the synthesis of both singly and multiply substituted CpMet(CO)$_3$ systems. Examples of this are shown in Table 3. It is of note that, though a collaboration with Radiotracer, Inc., we have used this method to prepare some CpMet(CO)$_3$ tropane systems as ligands for the dopamine transporter.



Scheme 14. Cp-Tin Transfer Reaction According to the Two-Step Protocol (top) and One-Pot Protocol (bottom).

Table 3. Structures and Isolated Yields in the Reaction of **3** with Various Cyclopentadienes and Indene

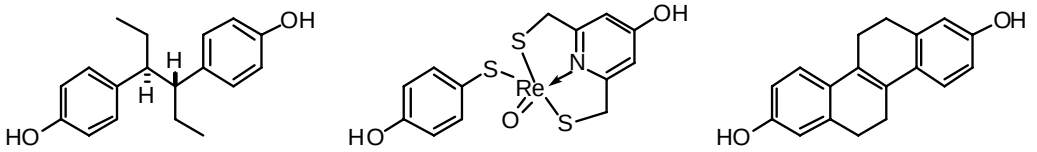
Entry	Reactant	Yield (%)	Product
1		28	
2		30	
3		65	
4		26	

5a **5b**

3. Progress Under Previous Aim 3: Prepare and evaluate metal complexes and organometallic systems of novel design as ligand mimics and analogs for nuclear hormone receptor-based tumor imaging [Pub. No. 3, 6, 10-12, 15, 16]

3.a. Synthesis of <u>Integrated</u> Oxotechnetium Amino-Thiol Complexes that Mimic Steroids. – Prior to the last period of funding, it appeared that it might be possible to prepare ***integrated*** inorganic oxotechnetium amino-thiol complexes that would effectively mimic not only the geometry of steroids but the receptor binding affinity properties of these hormones as well. The syntheses of these systems proved to be a formidable challenge at which we ultimately succeeded. However, despite our considerable efforts, we were unable to obtain any *integrated N_2S_2 bis-bidentate* or related tetradentate chelate system that demonstrated the proper combination of sufficient stability and significant receptor binding affinity. Nevertheless, despite our lack of biological success in this endeavor, through this work we did expand our chemical facility for the preparation of these metal complexes.

Because of the success that others had achieved in the synthesis of stable 3+1 oxotechnetium amino-thiol complexes,[77] we undertook the preparation of a series of 3+1 complexes whose shape mimicked that of non-steroidal ligands for the estrogen receptor. (Estrogens were prepared for simplicity, to evaluate feasiblity of preparing the synthetically more complex androgens and progestins.) The complexes were patterned after the shape and size of the non-steroidal receptor ligands, hexestrol and tetrahydrochrysenediol. The structure of the best of these complexes is shown in Scheme 15.

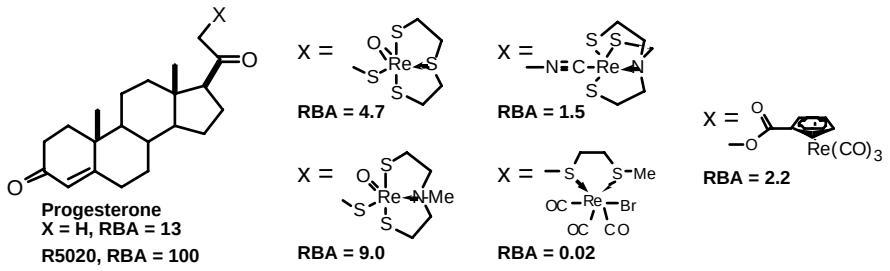


Scheme 15. 3 + 1 Integrated NS_3 Complex (center) and its Parent Ligands (left and right)

The 3 + 1 complex was quite stable, and by NMR analysis, we found that the unusual tridentate 2,6-bis(mercaptomethyl)pyridine ligand adopts a non-symmetrical twisted geometry that makes the methylene groups non-equivalent, but becomes fluxional at higher temperatures. However, despite their interesting structure, these 3+1 complexes did not have significant affinity for the estrogen receptor, so the further effort required to prepare analogs that might bind to androgen and progestin receptors was not deemed worthwhile.

3.b.. Synthesis of Steroids Labeled with Technetium and Rhenium in a <u>Pendant</u> Fashion. – With support of this project and through a collaboration with the radiopharmaceutical group at Rossingdorf, Germany, we have prepared and examined the stability and receptor affinity of a number of steroids onto which are appended a variety of rhenium and technetium complexes, notably tetradentate 3+1 oxotechnetium/rhenium(V) complexes, and also derivatives of technetium/rhenium in other oxidation states, such as pentadentate technetium/rhenium (III) isonitrile complexes, dithioether-halide complexes of tricarbonyltechnetium/rhenium (I), and cyclopentadienyltricarbonyltechnetium/rhenium (I). The structures of these "pendant" complexes and the routes used for their preparation are summarized below in Schemes 7-9.

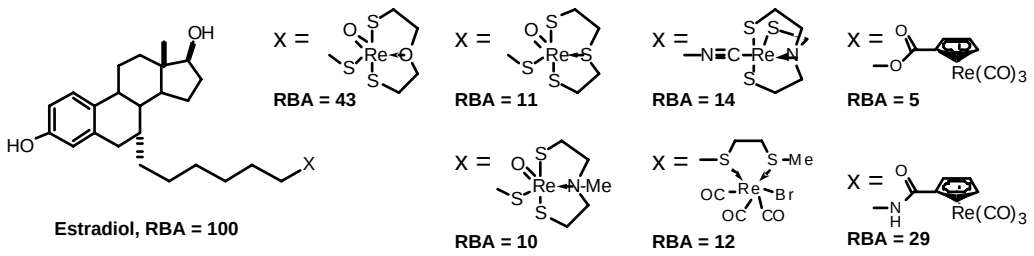
The first series we prepared were progestins complexes labeled at the C-21 position with a set of rhenium complexes that were attached through a thiol (for oxo-Re(V) 3+1 complexes), an isonitrile (for a pentadentate Re(III)-isonitrile complex), a dithioether (for a tricarbonylrhenium(I) bromide complex), and an oxygen (for a carbonyl-linked $CpRe(CO)_3$ complex) (Scheme 16). We could obtain these complexes in good yield and in pure form for binding affinity measurements, and, in fact, some of them showed good affinity for the progesterone receptor. However, the highest affinity analogs were the 3+1 complexes in which the steroid was attached to the monodentate component. Considering the results of ligand challenge assays recently developed by the Rossindorf group, it is likely that under the conditions of the binding assay the metal center dissociated from this thiolate ligand, so what was being measured was the relatively high affinity of the steroid free thiol, not the complex itself. However, the more stable isonitrile and $CpRe(CO)_3$ complexes did show significant affinity. The low overall affinity of these complexes might be ascribed to the C-21 not being the most favorable site for substitution on progestins.



Scheme 16. C-21 Conjugates of Progesterone and Rhenium Chelates and Organometallics

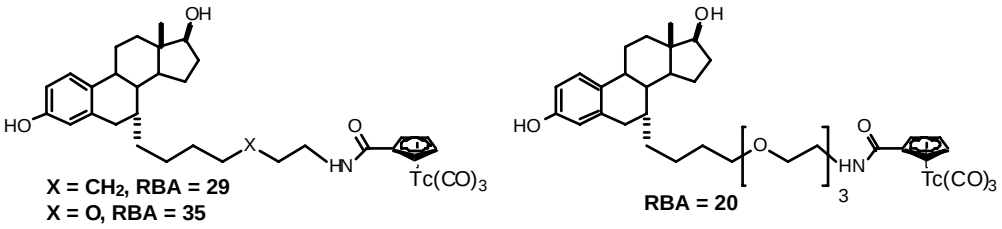
The second series of steroids labeled with rhenium/technetium in a pendant fashion were estrogens (again for chemical simplicity) labeled at the 7α position with the same set of complexes (Scheme 17). The 7α position was selected because it is known that estrogens can be substituted with long chains (up to 15 atoms long) at this position with preservation of binding affinity.[78] As before, we tend to discount the high affinity of the 3+1 complexes because of the tendency of these complexes to dissociate under the assay conditions. On the other hand, the other three types of complexes, the isonitrile, the bisthioether and the $CpMet(CO)_3$ complex all showed very favorable affinities. We were particularly encouraged by the high affinity shown by the rhenium $CpMet(CO)_3$ complex with the amide link, which showed an affinity that was

ca. 30% that of the parent ligand! These results certainly validated our selection of the 7α position as a preferred site for tethering a bulky substituent to this steroid ligand.



Scheme 17. 7α Conjugates of Estradiol and Rhenium Chelates and Organometallics

Based on the intriguingly high affinity of the stable $CpRe(CO)_3$ complex, we prepared this derivative labeled with Tc-99m (Scheme 18). In addition, because we had found the octanol-water partition coefficients (log $P^{o/w}$) of these complexes to be very high, we prepared as well, two analogs in which we had introduced ether linkages in the chain tethering the $CpMet(CO)_3$ complex to the C-7α position, in an attempt to lower the log $P^{o/w}$ values. In one case, we kept the chain length the same (six atoms to the amine) but introduced a single ether, and in the second case, we extended the chain to include three ether functions. Although each substitution of a methylene group by an ether oxygen is predicted to reduce the log $P^{o/w}$ of a compound by 1.5,[16] we found, surprisingly, that the lipophilicity of these new analogs was only marginally reduced from that of the parent compound.



Scheme 18. 7α Conjugates of Estradiol and $CpTc(CO)_3$.

Nevertheless, we continued in our efforts to prepare these compounds in Tc-99m labeled form, and we were successful in this endeavor, so that we were able to conduct tissue distribution studies in immature rats. Unfortunately, despite the favorable binding affinity of these analogs for the receptor, we found that all three of these compound showed only very modest uptake in the uterus, the primary target tissue for estrogens. Very little of this uptake was blocked by the coadministration of unlabeled estradiol.

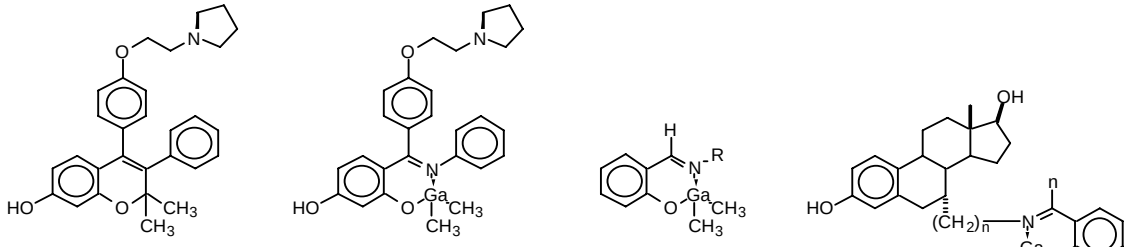
Our interpretation of these results is that despite our efforts to increase their polarity, the $CpMet(CO)_3$-estrogen derivatives are too lipophilic to show favorable receptor-directed tissue distribution properties in vivo. Their high affinity for the receptor can be demonstrated in in vitro assays, where the concentration of other proteins that would compete for their binding in vivo is low, but in vivo, extensive protein binding hinders their distribution and the cell membrane penetration that is required for their entry into target tissues. By reaching this conclusion, we have redirected our efforts towards the design of a technetium-labeled steroid receptor ligand to use the *organometallic* CpT-Met unit as an integral part of the ligand core.

4. Progress Under Previous Aims 4: Prepare and evaluate other novel radiolabeled androgens and progestins and their analogs [Pub. No. 1, 10, 15-19, 22-24, 27, 29-32, 35, 36]

4.a. Synthesis of gallium-substituted analogs of nuclear hormone receptor ligands – Gallium radioisotopes hold considerable promise for the development of diagnostic imaging agents. Rather than using the gallium(III) ion, which requires hexacoordinate chelation to form stable complexes, we have worked with the dimethylgallium cation that forms tetravalent complexes that are stable in water. The tetrahedral geometry of these complexes makes them close structural analogs of a tetrahedral carbon. This affords interesting

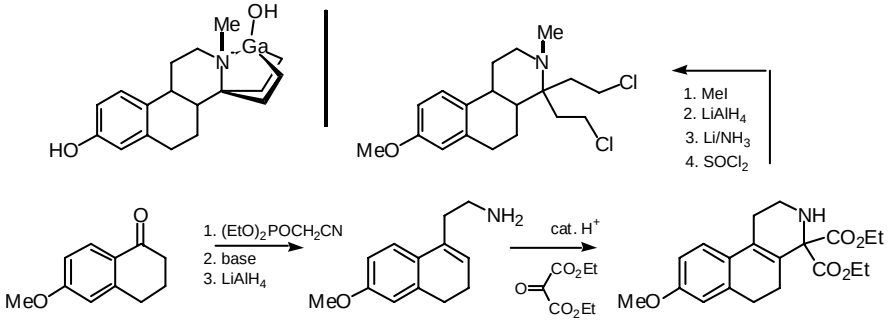
structural opportunities for the preparation of metal-containing ligands that mimic the structure of ligands for nuclear hormone receptors.

Previously, we prepared two types of dimethylgallium compounds (Scheme 19). The first was an integrated complex in which the dimethyl gallium unit substituted for an isopropyl group (**20**), and in the second, a dimethylgallium salicylaldimine system was a pendant substituent on a non-steroidal estrogen (**22**). However, we found that neither system was sufficiently stable in water.



Scheme 19. Dimethylgallium Complexes with an Inorganic Tether.

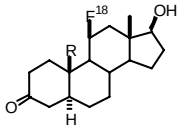
In an alternative approach, we have designed a receptor ligand in which the gallium is tethered to the ligand through an organometallic bond, rather than an inorganic chelate. We have made good progress on the synthesis of this system (Scheme 20), preparing the key dihalide intermediate from which the gallium species is to be prepared through a di-Grignard reagent and gallium trichloride. Work on this system will continue under the support of this project but will not be described further in this proposal.



Scheme 20. Synthetic Progress Towards a Novel Organometallic Gallium Steroid Mimic

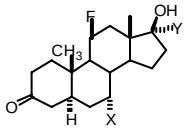
4.b. Prepare selected fluorine-18 labeled androgens and progestins for imaging prostate and breast cancer] –

F-18 Labeled Androgens with for Improved AR Imaging. We have made two analogs of 11β-fluoro-DHT (**17a**), a compound that has very high affinity for AR, but appeared to be undergoing rapid metabolism in vivo. These analogs have methyl substitutents at the 7α or the 17α position (**18ab**), sites at which substitution typically increases potency in vivo, presumably by slowing androgen metabolism. We have completed the syntheses of these analogs, and have worked out conditions for their F-18 labeling by a bromofluorination-reduction sequence that we have previously reported (not shown).[81] They have good affinity for the androgen receptor, and their tissue distribution studies will be completed soon.



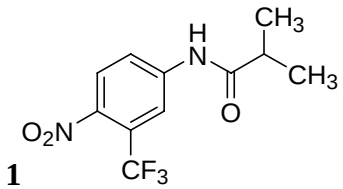
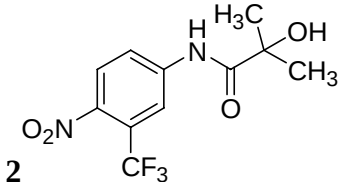
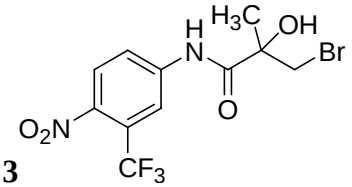
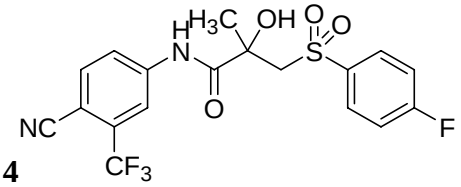
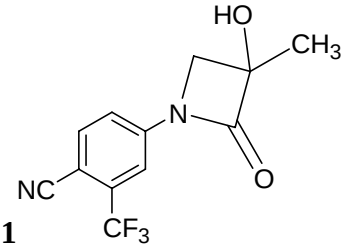
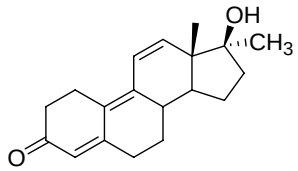
		AR (RBA)	SHBG (RBA)
17a	(R=CH_3)	53	1740
17b	(R=H)	75	134
		(R1881=100)	(E_2=100)

	X	Y	AR (RBA)	SHBG (RBA)
18a	H	Me	36	604
18b	Me	H	32	332



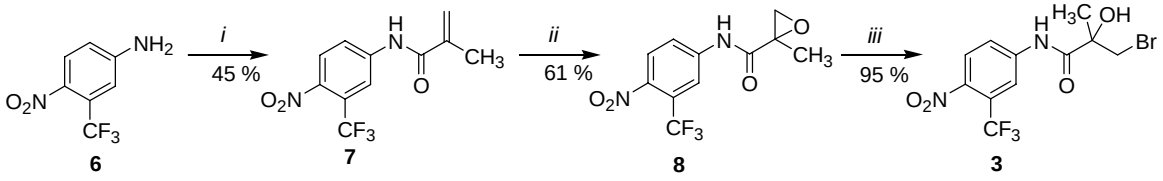
We have prepared a number of non-steroidal androgens with halogen labels, some of which we have prepared in radiolabeled form and evaluated for their tissue distribution in rats. The structures and binding affinities of some of these compounds are shown in Table 3.

Table 3: RBA data of non-steroidal AR ligands and standard (R1881).

Compound	RBA[a,b]
 **1**	0.0014
 **2**	0.045 ± 0.033
 **3**	1.90 ± 0.18
 **4**	0.044 ± 0.020
 **11**	0.014
 **R1881**	100

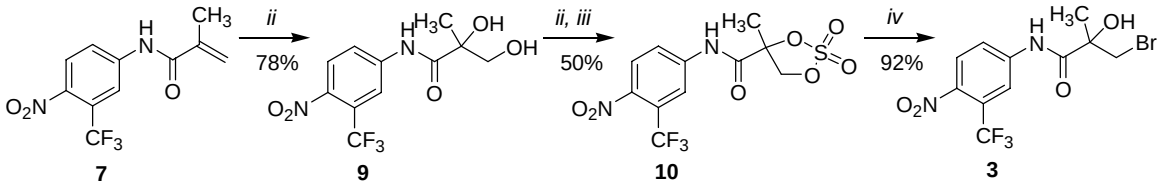
[a] Relative Binding Affinity (RBA) where R1881 is 100%. The K_d value of R1881 is 0.6 nM [32].
[b] Competitive Radiometric Binding Assays were done with purified ligand binding domain of rat AR (Panvera/Invitrogen), using [^{3}H]R1881 as tracer, as previously described [33].

The synthesis of several of these by different approaches are shown in the schemes below. Two approaches to bromoflutamide, one of the highest affinity non-steroidal androgens, are shown in Schemes 21 and 22.



i) Methacryloyl chloride, TEA, CH_2Cl_2. ii) Formic acid, H_2O_2. THF. iii) Ammonium Bromide, acetone.

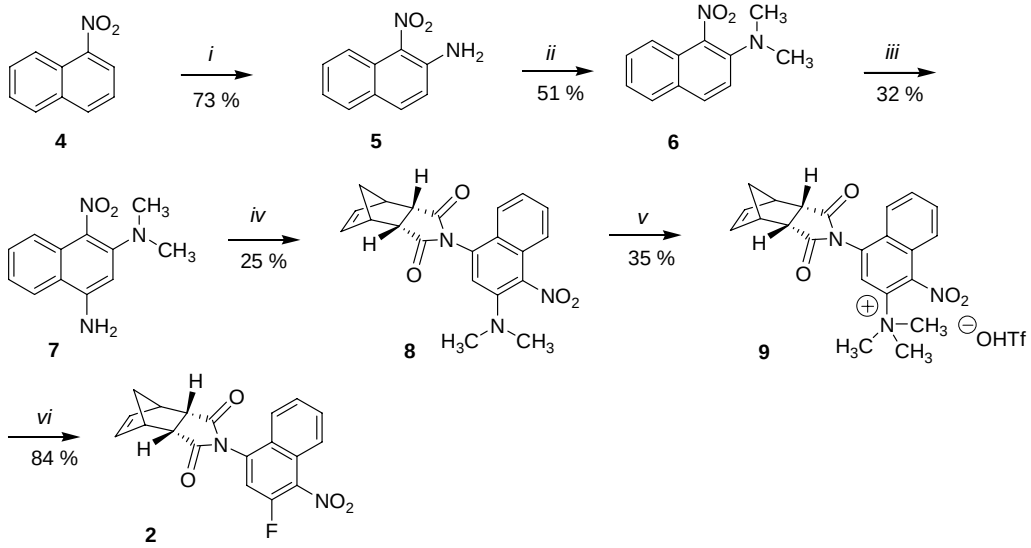
Scheme 21: Synthesis of 3-bromo-hydroxyflutamide from epoxide intermediate (8).



i) N-methylmorpholine N-oxide, osmium tetroxide, THF. ii) Thionyl chloride, THF. iii) $NaIO_4$, $RuCl_3$•3 H_2O, CH_3CN. iv) NH_4Br, Acetone.

Scheme 22: Synthesis of 3-Bromo-hydroxyflutamide from epoxide intermediate

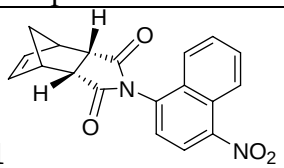
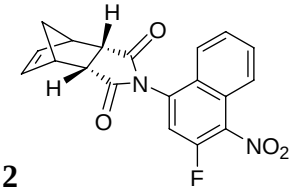
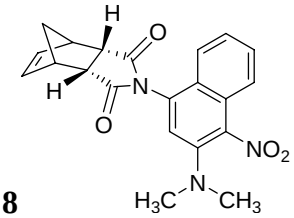
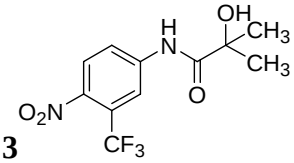
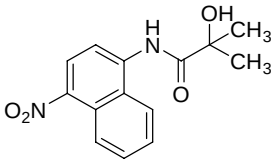
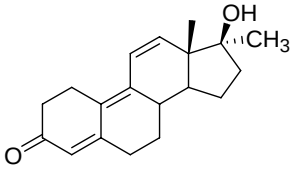
We also explored a novel non-steroidal antiandrogen and developed an approach to label it with fluorine-18. The synthesis is shown in Scheme 23. The androgen receptor binding affinities of members of this series are given in Table 4.



i) Benzothiazole-2-sulfenamide, t-BuOK, DMF. ii) CH_3I, NaH, HMPA, THF. iii) N-Tetramethylenethiocarbamoylsulfenamide, t-BuOK, DMF. iv) cis-5-Norborene-endo-2,3-dicarboxylic anhydride, HOAc. v) MeOTf, 1,2 Dichloroethane. vi) TBAF, CH_3CN

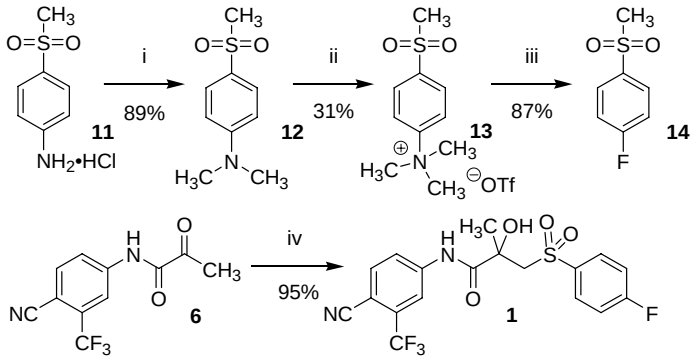
Scheme 23: Synthesis of 3-F-NNDI, a non-steroidal antiandrogen.

Table 4: Androgen Receptor Binding Affinities of Non-Steroidal AR Ligands and the Standard, R1881 (Expressed as Relative Binding Affinity [RBA] values).

Compound	RBA[a,b]
 **1**	0.54 ± 0.48
 **2**	0.20 ± 0.098
 **8**	<0.004 ± 0.001
 **3**	0.045 ± 0.033
 **10**	0.016 ± 0.0021
 **R1881**	100

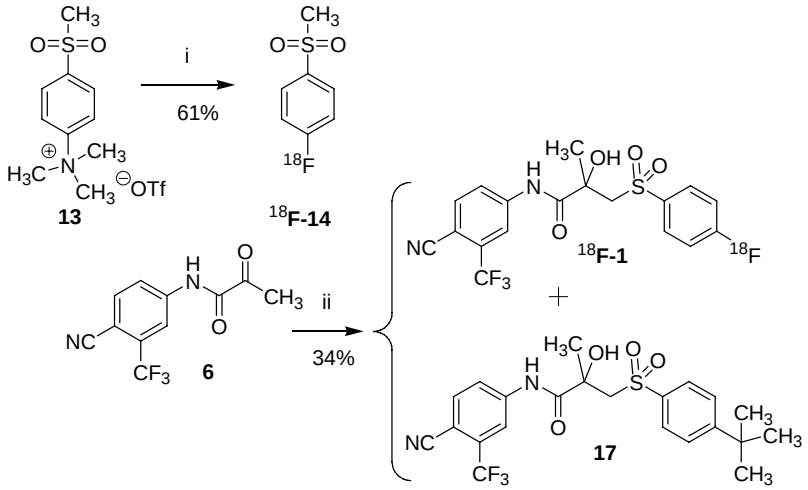
[a] Relative Binding Affinity (RBA) values are IC_{50}[R1881]/IC_{50}[compound] X 100. By definition, R1881 has an RBA value of 100. The K_d value of R1881 is 0.6 nM [5].
[b] Competitive Radiometric Binding Assays were done with purified ligand binding domain of rat AR (Panvera/Invitrogen), using [^{3}H]R1881 as tracer, as previously described [31].

Bicalutamide is an effective non-steroidal androgen used for endocrine therapy of prostate cancer. We have developed a method for isotopic labeling of bicalutamide with F-18. The synthesis of unlabeled material is shown in Scheme 24. The radiochemical synthesis, shown in Scheme 25, used a pseudocarrier approach.



(i) NaH, CH_3I, HMPA, THF. (ii) MeOTf, CH_2Cl_2.
(iii) TBAF, DMSO, 90 °C. (iv) **14**, *n*-BuLi, THF, -78 °C.

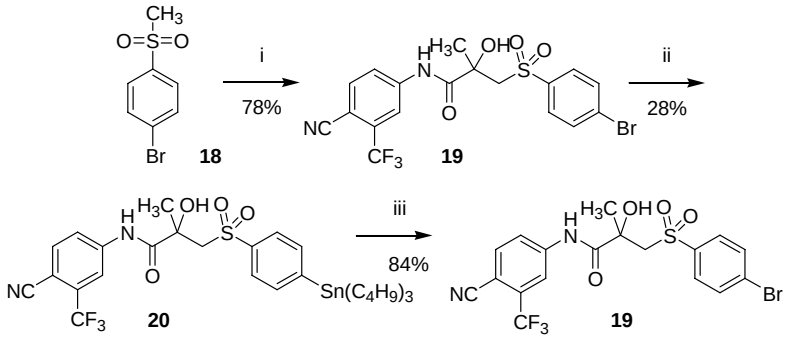
Scheme 24: Convergent synthesis of bicalutamide



(i) TBA[^{18}F]F, DMSO, 90 °C. (iv) ^{18}**F-14**,**16** *n*-BuLi, THF, -78 °C.

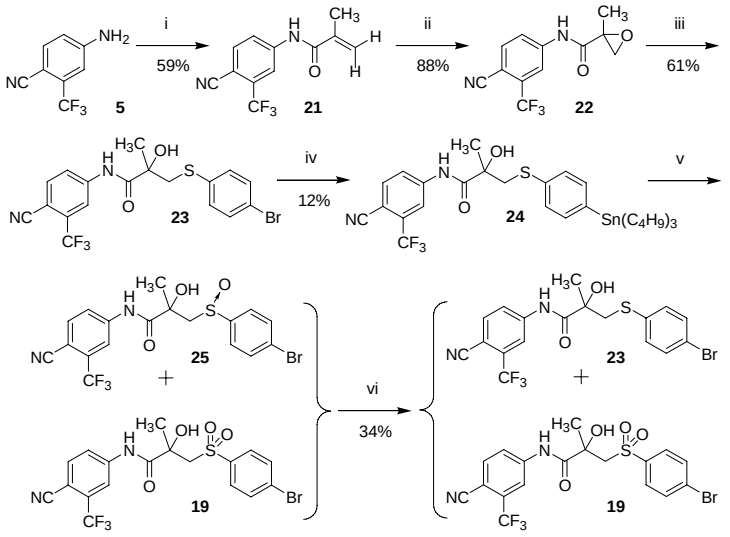
Scheme 25: Radiosynthesis of ^{18}F-Bicalutamide

Another interesting analog of bicalutamide is the compounds with a bromine, rather than a fluorine, substituent, called, colloquially, bromo-bicalutamide. A synthesis of this material is shown in Scheme 26; the synthesis of a thioether analog, reported to have higher binding affinity, is shown in Scheme 27.



(i) nBuLi, **6**, THF. (ii) Hexabutyl ditin, $Pd(PPh_3)_4$, TEA, tol. (iii) NH_4Br, 10% HOOAc, HOAc.

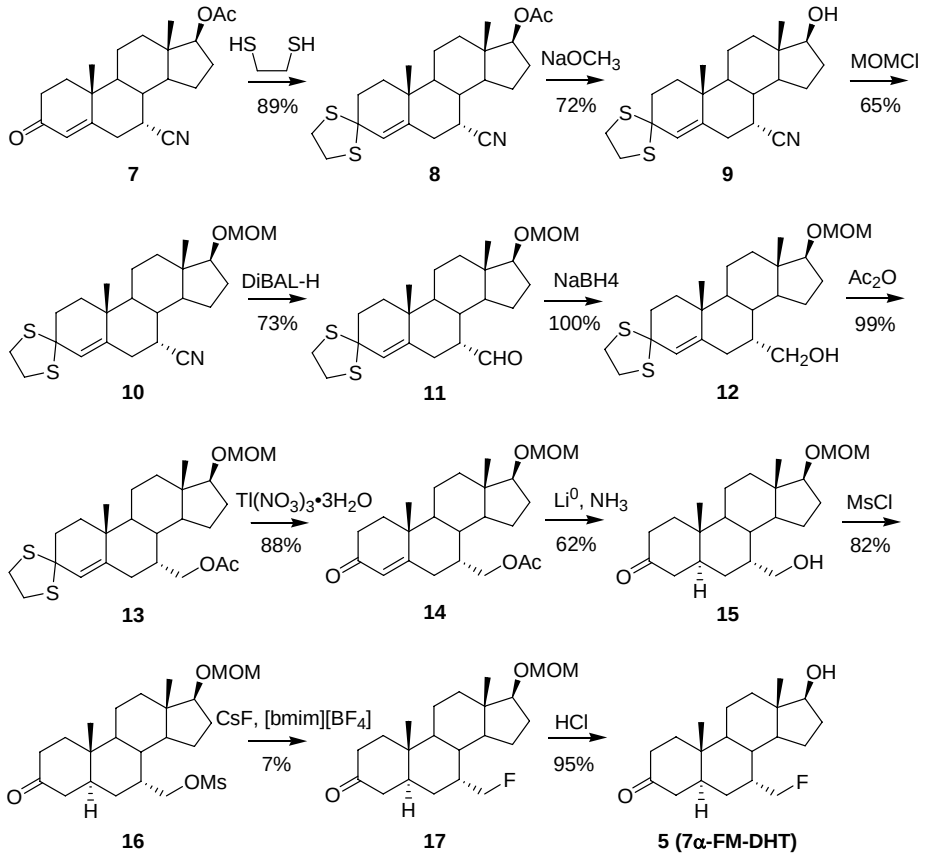
Scheme 26: Synthesis of 4-Bromobicalutamide



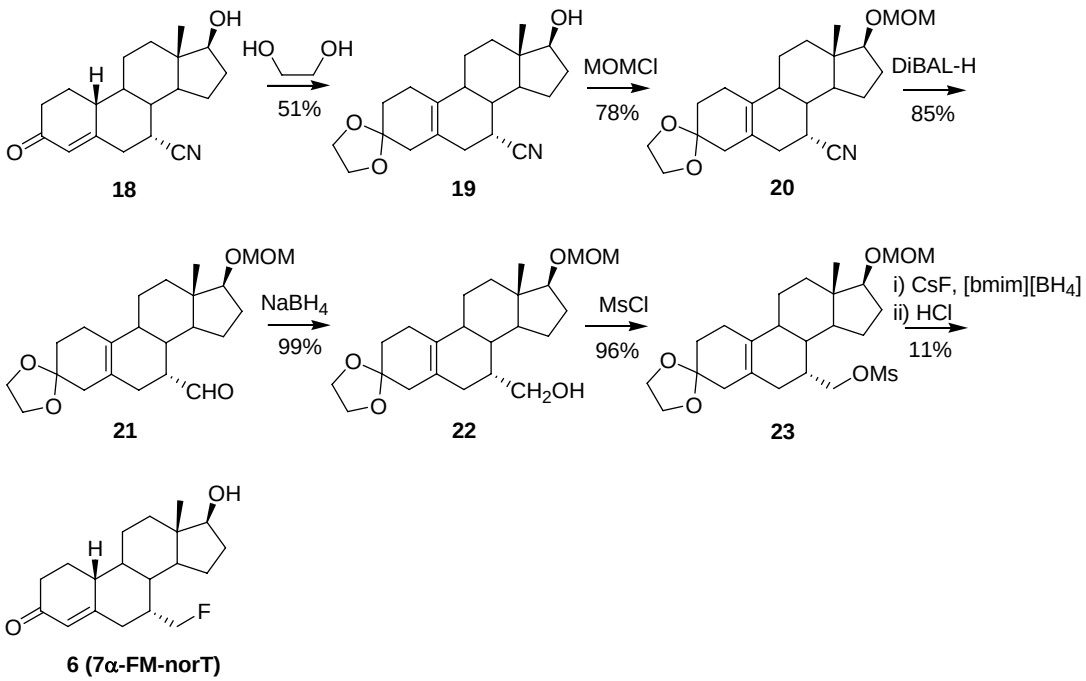
(i) Methacroyl chloride, TEA, CH_2Cl_2. (ii) H_2O_2, HOCHO.
(iii) 4-bromo-thiophenol, NaH, THF. (iv) Hexabutyl ditin, $Pd(PPh_3)_4$, tol.
(v) 1.6% HOOAc / HOAc, NH_4Br. (vi) Diethyl chlorophosphite, CH_2Cl_2.

Scheme 27: Synthesis of 4-Bromo-thiobicalutamide

We have prepared two F-18 labeled steroidal androgens, 7α-fluoromethyl-5α-dihydrotestosterone and 7α-fluoromethyl-19-nortestosterone. The syntheses of these two targets are shown in Schemes 28 and 19. The androgen receptor binding affinities of members of this series is given in Table 5.

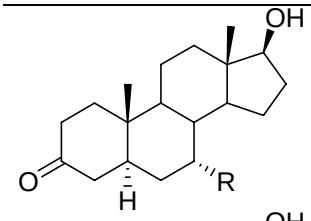
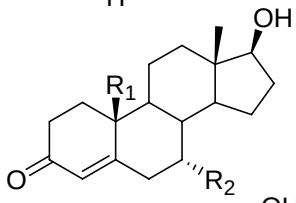
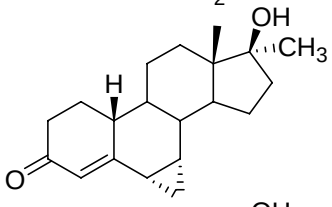
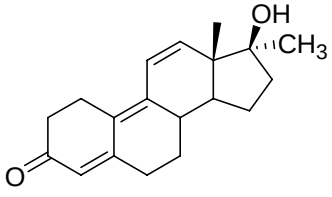


Scheme 28: Synthesis of 7α-fluoromethyl-5α-dihydrotestosterone.



Scheme 29: Synthesis of 7α-fluoromethyl-19-nortestosterone

Table 5: Binding affinities of various steroidal ligands for the AR and SHBG proteins

compound	Relative binding affinity (RBA)[a] AR	SHBG
 R = H (DHT)	60 ± 16	2125 ± 999
27 R = CH_2OH	0.17 ± 0.03	2.0 ± 0.17
6 R = CH_2F	39 ± 7.1	170 ± 7.1
 R_1 = H, R_2 = H (nor-T)	31 ± 1.5	30 ± 6.8
R_1 = CH_3, R_2 = H (T)	5.9 ± 1.3	417 ± 88
5 R_1 = H, R_2 = CH_2F	33 ± 1.6	7.8 ± 1.3
18 R_1 = H, R_2 = CN	1.4 ± 0.30	0.032 ± .006
 **25**	2.5 ± 1.7	1.3 ± 1.1
 **26** R1881	100	4.0 ± 0.9

[a]RBA values are the average of 2-3 determinations ± standard deviation.

Both of these compounds show promise as agents for imaging androgen receptors in prostate cancer.

Publications from Previous Funding Period

Refereed Publications

1. T. W. Spradau and J. A. Katzenellenbogen, Preparation of Cyclopentadienyltricarbonylrhenium Complexes Using a Double Ligand Transfer Reaction. *Organometallics,* **1998**, *17*, 2009-2017.
2. F. Minutolo and J. A. Katzenellenbogen, A Convenient Three-Component Synthesis of Substituted Cyclopentadienyl Tricarbonyl Rhenium Complexes. *J. Am. Chem. Soc.*, **1998**, *120*, 4514-4515.
3. R. W. Chesnut and J. A. Katzenellenbogen, Four-Coordinate Dimethylgallium Compounds Vary in Stability toward Hydrolysis. *Organometallics,* **1998**, *17*, 4889-4896.
4. T. W. Spradau and J. A. Katzenellenbogen, Protein and Peptide Labeling with (cyclopentadienyl)tricarbonyl Rhenium and Technetium. *Bioconjugate Chem.,* **1998**, *9,* 765-772.
5. F. Minutolo and J. A. Katzenellenbogen, Boronic Acids in the Three-Component Synthesis of Carbon-Substituted Cyclopentadienyl Tricarbonyl Rhenium Complexes. *J. Am. Chem. Soc.,* **1998**, *120*, 13264-13265.
6. M. B. Skaddan, K. E. Carlson, and J. A. Katzenellenbogen. Integrated "3+1" Oxorhenium(V) Complexes as Estrogen Mimics. *Bioconjugate Chem.*, **1999**, *10*, 119-129.
7. J. A. Katzenellenbogen, F. Minutolo. A Polymer-Supported Phosphazine as a Stable and Practical Reagent in the Three-Component Synthesis of Substituted (Cyclopentadienyl)-tricarbonylrhenium Complexes, *Angew. Chem. Int. Ed.*, **1999**, *38*, 1617-1620.
8. F. Minutolo, J. A. Katzenellenbogen. Three-Component Synthesis of Substituted n^5-Cyclopentadienyltricarbonylrhenium Complexes: Scope, Limitations, and Mechanistic Interpretations, *Organometallics*, **1999**, *18*, 2519-2530.
9. F. Minutolo, S.R. Wilson, and J. A. Katzenellenbogen. Crystallographic Evidence for the Electronic Distribution in (2,4-Cyclopentadien-1-ylidenehydrazanon)triphenylphosphorane. *Acta. Cryst.,* **1999**, *C55*, 1016-1019.
10. F. Wüst, M. B. Skaddan, P. Leibnitz, H. Spies, J. A. Katzenellenbogen, and B. Johanssen. Synthesis of Novel Progestin-Rhenium Conjugates as Potential Ligands for the Progesterone Receptor. *Bioorg. Med. Chem.*, **1999**, *7*, 1827-1835.
11. M. B. Skaddan, F. R. Wüst, J. A. Katzenellenbogen. Synthesis and Binding Affinities of Novel Re-Containing 7α-Substituted Estradiol Complexes: Models for Breast Cancer Imaging Agents. *J. Org. Chem.*, **1999**, *64*, 8108-8121.
12. M. B. Skaddan, F. R Wüst, S. Jonson, R. Syhre, M. J. Welch, H. Spies, J. A. Katzenellenbogen. Radiochemical Synthesis and Tissue Distrubution of Tc-99m-Labeled 7α- Substituted Estradiol Complexes. *Nuc. Med. Bio.*, **2000**, *27*, 269-278.
13. F. Minutolo and J. A. Katzenellenbogen, A Convenient Three-Component Synthesis of Substituted Cyclopentadienyl Tricarbonyl Re(I) and Tc(I) Complexes and its Potential for Radiolabeling. In *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine* (M. Nicolini and U. Mazzi, Ed.), **1999**, *5*, 109-116.
14. J. A. Katzenellenbogen, R. Minutolo, T. W. Spradau, M. B. Skaddan, Preserving Bioactivity of Small Molecules Labeled with Technetium and Rhenium: An Organometallic Approch. In *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine* (M. Nicolini and U. Mazzi, Ed.), **1999**, *5*, 363-372.
15. F. Wüst, M. B. Skaddan, K. E. Carlson, P. Leibnitz, J. A. Katzenellenbogen, H. Spies, and B. Johannson, Synthesis and Receptor Binding of Novel Progestin-Rhenium Complexes. In *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine* (M. Nicolini and U. Mazzi, Ed.), **1999**, *5*, 491-495.
16. R. R. Cesati III and J. A. Katzenellenbogen. Preparation of Hexahydro-benzo[f]isoquinolines Using a Vinylogous Pictect-Spengler Cyclization. *Org. Lett.*, **2000**, *2,* 3635-3638.
17. R. R. Cesati III, J. A. Katzenellenbogen. One-Pot Formation of Substituted Cyclopentadienyl and Indenyltricarbonyl Rhenium Complexes Through In Situ Generation of Cyclopentadienyl- and Indenyltributylstannanes. *J. Am. Chem. Soc.*, **2001**, *123*, 4093-4094.

18. S. H. Kim, S. D. Jonson, M. J. Welch, J. A. Katzenellenbogen. Fluorine-Substituted Ligands for the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ): Potential Imaging Agents for Metastatic Tumors. *Bioconjugate Chem.*, **2001**, *12*, 439-450.
19. J. B. Downer, L. A. Jones, J. Engelbach, Lich, L. L., W. Mao, K. E. Carlson, J. A. Katzenellenbogen, M. J. Welch. Comparison of Animal Models for the Evaluation of Radiolabeled Androgens. *Nucl. Med. Biol.*, **2001**, *28*, 613-626.
20. R. R. Cesati III, G. Tamagnan, R. M. Baldwin, S. S. Zoghbi, R. B. Innes, R. J. Baldessarini, and J. A. Katzenellenbogen. Synthesis of Cyclopentadienyl Tricarbonyl Rhenium Phenyl-Tropanes by Direct Double Ligand Transfer with Ferrocene Precursors: Development of Imaging Agents for the Dopamine Transporter, *Bioconj. Chem.*, **2002**, *13*, 29-39.
21. E. S. Mull, V. J. Sattigeri, J. A. Katzenellenbogen. Aryl Cyclopentidienyl Tricarbonyl Rhenium Complexes: Novel Ligands for the Estrogen Receptor with Potential Uses as Radiopharmaceuticals. *Bioorg. Med. Chem. Lett.*, **2002**, *10,* 1381-1398.
22. D. Vijaykumar, W. Mao, K. S. Kirschbaum, J. A. Katzenellenbogen. An Efficient Route for the Preparation of a 21-Fluoro Progestin-16α,17α-Dioxolane, a High Affinity Ligand for PET Imaging of the Progesterone Receptor. *J. Org. Chem.*, **2002**, *67*, 4904-4910.
23. L. Luyt, J. A. Katzenellenbogen. A Trithiolate Tripodal Bifunctional Ligand for the Radiolabeling of Peptides with Gallium (III). *Bioconjugate Chem.*, **2002**, *13*, 1140-1145.
24. F. Wüst, K. E. Carlson, J. A. Katzenellenbogen. Synthesis of Novel Arylpyrazolo Corticosteroids as Potential Ligands for Imaging Brain Glucocorticoid Receptors, *Steroids*, **2003**, *68*, 177-191.
25. L. G. Luyt, H. M. Bigott, M. J. Welch, J. A. Katzenellenbogen. 7α and 17α-Substituted Estrogens Containing Tridentate Tricarbonyl Rhenium/Technetium Complexes: Synthesis of Estrogen Receptor Imaging Agents and Evaluation Using MicroPET with Technetium-94m, *Bioorg. Med. Chem.*, **2003**, *11*, 4977-4989.
26. H. M. Bigott, E. Parent, L. G. Luyt, J. A. Katzenellenbogen, M. J. Welch. Design and Synthesis of Functionalized Cyclopentadienyl Tircarbonyl Metal Complexes for Technetium-94m PET Imaging of Estrogen Receptors. *Bioconj. Chem.*, **2005**, *16*, 255-264.
27. F. Dehdashti, J. Picus, J. M. Michalski, C, S. Dence, B. A. Siegel, J. A. Katzenellenbogen, M. J. Welch. Positron Tomographic Assessment of Androgen Receptors in Prostatic Carcinoma. *Eur. J. Nucl. Med. Molec. Imaging*, **2005**, *32*, 344-350.
28. C-K Chu, J-H Kim, D. W. Kim, K-H Chung, J. A. Katzenellenbogen, D. Y. Chi. Aromatic Fluorination by Decomposition of Triazenes in Ionic Liquids. *Bull. Korean Chem. Soc.* **2005**, *26*, 599-602.
29. D. Zhou, K. Carlson, J. A. Katzenellenbogen, M. J. Welch. Bromine- and Iodine-substituted 16 Alpha, 17 Alpha-dioxolane Progestins for Breast Tumor Imaging and Radiotherapy: Sythesis and Receptor Binding Affinity. *J. Med. Chem.*, **2006**, *49*, 4737-4744.
30. E. E. Parent, C. S. Dence, T. L. Sharp, M. J. Welch, J. A. Katzenellenbogen. Synthesis and Biological Evaluation of a Fluorine-18-Labeled Nonsteroidal Androgen Receptor Antagonist, n-(3-[^{18}F]Fluoro-4-Nitronaphthyl)-cis-5-Norbornene-Endo-2,3-Dicarboxylic Imide. *Nuc. Med. Bio.*, **2006**, *33*, 615-624.
31. Jacobson, D. Laky, K. E. Carlson, S. Elgavish, M. Gozin, E. Even-Sapir, I. Leibovitc, M. Gutman, R. Chisin, J. A. Katzenellenbogen, and E. Mishani. Chiral Dimethylaimine Flutamide Derivatives – Modeling, Synthesis, Androgen Receptor Affinities, and Carbon-11 Labeling. *Nuc. Med. Bio.*, **2006**, *33*, 695-704.
32. E. E. Parent, C. Jenks, T. Sharp, M. J. Welch, J. A. Katzenellenbogen. Synthesis and Biological Evaluation of a Non-Steroidal Bromine-76 Labeled Androgen Receptor Ligand: 3-[^{76}Br]Bromo-hydroxyflutamide. *Nuc. Med. Bio.*, **2006**, *33*, 705-713.
33. B. C. Lee, K. C. Lee, H. Lee, R. H. Mach, and J. A. Katzenellenbogen. Synthesis and Binding Affinity of a Fluorine-Substituted Peroxisome Proliferator-Activated Gamma (PPARγ) Ligand as a Potential Positron Emission Tomography (PET) Imaging Agent. *Bioconj. Chem.*, **2007**, *18*, 507-13.

34. B. C. Lee, K. C. Lee, H. Lee, R. H. Mach, J. A. Katzenellenbogen. Strategies for the Labeling of Halogen-Substituted PPAR-Gamma (PPARγ) Ligands: Potential Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) Imaging Agents. *Bioconj. Chem.*,**2007**, *18*, 514-23..
35. E. E. Parent, C. S. Dence, C. Jenks, T. L. Sharp, M. J. Welch, J. A. Katzenellenbogen. Synthesis and Biological Evaluation of [^{18}F]Bicalutamide, 4-[^{76}Br]Bromobicalutamide, and 4-[^{76}Br]Bromo-thiobicalutamide as Non-Steroidal Androgens for Prostate Cancer Imaging. *J. Med. Chem.*, *Submitted*.
36. E. E. Parent, K. E. Carlson, and J. A. Katzenellenbogen. Synthesis of 7α-Fluoromethyl-dihydrotestosterone and 7α-Fluoromethyl-nortestosterone, Structurally Paired Androgens Designed to Probe the Role of Sex Hormone Binding Globulin in Imaging Androgen Receptors in Prostate Tumors by Positron Emission Tomography. *J. Org. Chem., Submitted*.

Abstracts

A1. F. Wüst, R. Berger, J. A. Katzenellenbogen, R. Alberto, P. A. Schubiger, H. Spies, and B. Johannsen. Rheniumkomplexe von Steroidalen Estogenen, Androgenen und Progestinen. Deutsche Gesellshaft für Nuclearmedizin e.V. 36. Internatioonale Jahrestagung, Leipzig, Germany, April **1998**.

A2. F. Wüst, J. A. Katzenellenbogen, H. Spies, and B. Johannsen. Rhenium Complexes of 17α-Substituted Estradiol Capable of Binding to the Estrogen Receptor. Joint WFNM&B and EANM Congress, Berlin, Germany, August 30–September 4, **1998**.

A3. M. B. Skaddan, F. R. Wüst, and J. A. Katzenellenbogen. Novel Rhenium-Containing Estrogen Mimics as Potential Imaging Agents for Breast Cancer. 216th National Meeting of the American Chemical Society, Boston, August 23-27, **1998**.

A4. J. A. Katzenellenbogen, F. Minutolo, T. W. Spradau, and M. B. Skaddan. Preserving Bioactivity of Small Molecules labeled with Technetium and Rhenium: an Organometallic Approach. 5th International Symposium on Technetiumn in Chemistry and Nuclear Medicine, Bressanone, Italy, September 6–9, **1998**. *Quart. J. Nucl. Med. 42 (3S) 3,* **1998**.

A5. F. Minutolo, and J. A. Katzenellenbogen. A Convenient Three-Component Synthesis of Substituted Cyclopentadienyl Tricarbonyl Re(I) and Tc(I) Complexes and its Potential for Radiolabeling. 5th International Symposium on Technetiumn in Chemistry and Nuclear Medicine, Bressanone, Italy, September 6–9, **1998**. *Quart. J. Nucl. Med. 42 (3S) 19* **1998**.

A6. F. Wüst, M. B. Skaddan, J. A. Katzenellenbogen, and H. Spies. Synthesis and Receptor Binding of Novel Progestin-Rhenium Complexes. 5th International Symposium on Technetiumn in Chemistry and Nuclear Medicine, Bressanone, Italy, September 6–9, **1998**. *Quart. J. Nucl. Med. 42 (3S) 41* **1998**.

A7. J. A. Katzenellenbogen. Steroid Radiopharmaceuticals Labeled with Organometallic Rhenium and Technetium. 217th ACS National Meeting, Anaheim, CA, March, **1999**.

A8. R. R. Cesati, G. Tamagnan, R. m. Baldwin, S. S. Zoghbi, R. B. Innis, J. A. Katzenellenbogen. Synthesis of Cyclopentadienyl Tricarbonyl Technetium Phenyl-Tropane Derivatives by Direct Double Ligand Transfer with Ferrocene Precursors. 13th International Symposium on Radiopharmaceutical Chemistry, June 27-30, **1999**.

A9. M. B. Skaddan, F. R. Wust, M. J. Welch, J. A. Katzenellenbogen. Synthesis and Biological Evaluation of 7α Re/Tc "3+1" and Cyclopentadienyltricarbonylmetal (CpT-Met) Estrogen Mimics Based on the Conjugated Design. 13th International Symposium on Radiopharmaceutical Chemistry, June 27-30, **1999**.

A10. F. Minutolo, J. A. Katzenellenbogen. New Route to Substituted $CpRe(CO)_3$ and $CpTc(CO)_3$ Complexes using Diazocyclopentadiene: A Mechanistic Analysis. 13th International Symposium on Radiopharmaceutical Chemistry, June 27-30, **1999**.

A11. F. Minutolo, J. A. Katzenellenbogen. A Practical Solid-Phase Diazocyclopentadiene Source for the Synthesis of Substituted $CpRe(CO)_3$ and $CpTc(CO)_3$ Complexes. 13th International Symposium on Radiopharmaceutical Chemistry, June 27-30, **1999**.

A12. G. Tamagnan, R. R. Cesati III, S. S. Zoghbi, A. Varrone, A. Tikriti, J. P. Seibyl, N. Kula, R. J. Baldessarini, J. A. Katzenellenbogen, R. B. Innis, and R. M. Baldwin. Labeling, Dopamine Transporter Affinity, and Brain Uptake of the First Cyclopentadienyltricarbonyl-[^{99m}Technetium]-Tropane Conjugate. 47th Meeting of the Society for Nuclear Medicine, June 3-7, **2000**.

A13. J A Katzenellenbogen, F. Dehdashti, J. E. Mortimer, B A, Siegel , K. Trinkaus , M J. Welch. Imaging Breast and Prostate Cancer for Steroid Receptors and Metabolism: An Approach to Predicting Response to Hormone Therapy. Hormones and Cancer 2000, Port Douglas, Australia, November 3-7, **2000**.

A14. N. I. Gorshkov, J. A Katzenellenbogen, L. G. Luyt, A. A. Lumpov, A. E. Miroslavov, D. N. Suglobov. New Dithiocarbamato-Carboxylate Chelation Unites for Linking M(CO)3+ (M=Tc, Re) Species to Other Molecules. "14th International Symposium on Radiopharmaceutical Chemistry" Journal of Labelled Compound & Radiopharmaceuticals. June **2001**.

A15. S. H. Kim, S. D. Jonson, M. J. Welch, J. A. Katzenellenbogen. Fluorine-Substituted Ligands for the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ): Potential Imaging Agents for Metastatic Tumors. "14th International Symposium on Radiopharmaceutical Chemistry" Journal of Labelled Compound & Radiopharmaceuticals. June **2001**.

A16. L. G. Luyt and J. A. Katzenellenbogen. Tridentate Re(I)/Tc(I) Tricarbonyl Estradiol Complexes. "14th International Symposium on Radiopharmaceutical Chemistry" Journal of Labelled Compound & Radiopharmaceuticals. June **2001**.

A17. L. G. Luyt, J. A. Katzenellenbogen, H. M. Bigott, M. J. Welch. Preparation of Cyclopentadienyltricarbonyl Technetium-94m Complexes. "14th International Symposium on Radiopharmaceutical Chemistry" Journal of Labelled Compound & Radiopharmaceuticals. June **2001**.

A18. L. G. Luyt, J. A. Katzenellenbogen. Synthesis of a Functionalized Gallium(III) Tripodyl Complex. "14th International Symposium on Radiopharmaceutical Chemistry" Journal of Labelled Compound & Radiopharmaceuticals. June **2001**.

A19. A. E. Miroslavov, N. I. Gorshkov, J. A. Katzenellenbogen, L. G. Luyt, A. A. Lumpov, and D. N. Suglobov. Technetium and Rhenium Tricarbonyl Complexes with Thiosemicarbazide Ligands. "14 Radiochemistry Conference." Czech Republic. April 14-19, **2002**.

A20. N. I. Gorshkov, J. A. Katzenellenbogen, L. G. Luyt, A. A. Lumbov, A. E. Miroslavov and D. N. Suglobov, Technetium and Rhenium in Chemistry and Nuclear Medicine, "6th International Symposium on Technetium and Rhenium" Bressanone, Italy, September 4-7, **2002**.

A21. F. Dehdashti, J. Picus, J. M. Michalski, C. Dence, B. A. Siegel, J. A. Katzenellenbogen, M. J. Welch. Positron Tomographic Assessment of Androgen Receptors in Prostate Carcinoma, "SNM 50th Annual Meeting", New Orleans, LA, June 21-25, **2003**.

A22. H. M. Bigott, L. G. Luyt, M. J. Welch, J. A. Katzenellenbogen. Synthesis of Rhenium/Technetium Labeled Estrogen Receptor Imaging Agents: Evaluation Using Micropet with Technetium-94M, "15th International Symposium on Radiopharmaceutical Chemistry", Sydney, Australia, August, 2003.

A23. J. A. Katzenellenbogen. Imaging nuclear hormone receptors in vivo. "227th National ACS Meeting", Anaheim, CA, March **2004**.

A24. J. W. Seo, D. Y. Chi, L. G. Luyt, C. S. Dence, T. L. Sharp, F. Wuest, R. H. Mach, M. J. Welch, J. A. Katzenellenbogen. Sigma-2 Selective Fluorinated Ligands: Synthetic Method and Biodistribution

Study. "International Symposium on Radiopharmaceutical Chemistry 16 ISRC 2005", Iowa City, IA, June **2005**.

A25. J. W. Seo, D. Y. Chi, L. G. Luyt, C. S. Dence, T. L. Sharp, F. Wuest, R. H. Mach, M. J. Welch, J. A. Katzenellenbogen. Novel Fluorine-18 Labeled Estrogen Receptor Ligands: Fluoroalkyl-Cyclofenil Analogues. "International Symposium on Radiopharmaceutical Chemistry 16 ISRC 2005", Iowa City, IA, June **2005**.

A26. D. Zhou, K. E. Carlson, R. H. Mach, J. A. Katzenellenbogen, M. J. Welch. 16α,17α-Dioxolane Bromine- and Iodine-Substituted Progestins for Breast Tumor Imaging and Radiotherapy: Synthesis. "International Symposium on Radiopharmaceutical Chemistry 16 ISRC 2005", Iowa City, IA, June **2005**.

A27. B. Lee, K. Lee, J. A. Katzenellenbogen. Synthesis of Fluorine-Labeled Ligands for PET Imaging of the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ). "International Symposium on Radiopharmaceutical Chemistry 16 ISRC 2005", Iowa City, IA, June **2005**.

A28. N. C. Ackroyd, J. A. Katzenellenbogen. Cyclopentadienyl Rhenium (Technetium) Tricarbonyl Complexes Integrated in Estrogen Receptor (ER) Ligands for ER+ Tumor Imaging. "International Symposium on Radiopharmaceutical Chemistry 16 ISRC 2005", Iowa City, IA, June **2005**.

A29. M. L. Nickels, J. A. Katzenellenbogen. Novel Tacn Complexes as Estrogen Receptor Ligands for Tumor Imaging. "International Symposium on Radiopharmaceutical Chemistry 16 ISRC 2005", Iowa City, IA, June **2005**.

A30. D. W. Kim, D. H. Barnett, J. R. Gunther, B. S. Katzenellenbogen, and J. A. Katzenellenbogen. Inhibitors of Carbonic Anhydrase XII Suitable for F-18 Radiolabeling. "International Symposium on Radiopharmaceutical Chemistry 16 ISRC 2005", Iowa City, IA, June **2005**.

A31. J. A. Katzenellenbogen. Probing Estrogen Receptor Stucture and Function: Ligands, Fluorophores, and In Vivo Imaging. "Great Lakes Nuclear Receptor Meeting", Madison, Wisconsin, October 14-15 **2005**.