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14. ABSTRACT

A class of breast tumors, known as ER+, contains significant concentrations of ER which functions to regulate cell growth, and mediate the action of estrogen antagonists. There is a need for the development non-invasive and reliable methods for the determination of tumor ER concentration in the identification of patients predicted to respond to hormone therapy. It has been shown that tumor ER concentration can be determined by imaging, using 18F-labeled ER selective ligands, and that the ER concentrations determined by imaging correlate well with those determined by immunoassay methods on surgical biopsies. Because of the short half-life of fluorine-18, this method is costly, with low availability. Thus, the development of an effective ER imaging agent that is of low cost and widespread availability might eliminate the need for tumor biopsy in the treatment selection for breast cancer patients. We propose the development of radiopharmaceutical imaging agents labeled with 99mTc, which is available at most hospitals at a relatively low cost, as a 99Mo/99mTc generator. Studies conducted in this laboratory suggest that an integrated organometallic design in which technetium bonded to carbon forms a part of the core structure will display the stability, as well the requisite affinity to ER.

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Breast Cancer

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Cyclopentadienyl Rhenium (Technetium) Tricarbonyl Complexes Integrated in Estrogen Receptor (ER) Ligands for ER+ Tumor Imaging

Nathan C. Ackroyd, University of Illinois, Urbana-Champaign, 600 S. Mathews Ave, Urbana, IL, 61801.

Introduction

It is known that many breast tumors contain significant concentrations of ER. In these tumors, known as ER+ tumors, the role of ER is to regulate cell growth, but it can also function to mediate the anti-proliferative effects of estrogen antagonists, such as tamoxifen. It has been shown that ER concentration correlates well with the efficacy of anti-estrogen use in hormone therapy. Tumors with low ER concentration (i.e., ER- tumors) do not respond well to hormone therapy. As a result, chemotherapy is often used instead of hormone therapy, in spite of the high morbidity associated with its use, because chemotherapy is known to be effective in both ER+ and ER- tumors. Unfortunately, roughly half of patients that are successfully treated with chemotherapy could have been treated equally well with hormone therapy and thereby avoided the deleterious side effects of chemotherapy, provided that a reliable means could be used to identify those patients that would respond to hormone therapy. Thus, there is a great need for the development of a non-invasive and reliable method for the determination of ER concentration in tumors that would allow identification of breast cancer patients having ER+ tumors that are likely to respond well to hormone therapy, so that these patients could be spared the side effects of chemotherapy. It has been shown that the ER concentration in breast tumors can be determined by imaging, using ER selective radiopharmaceutical imaging agents, and that the ER concentrations determined by imaging correlate well with those determined by binding or immunoassay methods on surgical biopsies. Currently, the most effective ER imaging agent is a fluorine-18 labeled estrogen. However, because of the short half-life of this radionuclide, this agent is very expensive and is not widely available. Thus, the development of an effective ER imaging agent that is of low cost and widespread availability might eliminate the need for tumor biopsy to determine whether a patient is a good candidate for hormone therapy. We have proposed the development of a radiopharmaceutical imaging agent labeled with ^{99m}Tc, which exhibits a high binding affinity to ER, has high *in vivo* stability, and functions effectively *in vivo* for imaging ER levels in breast tumors. Imaging agents labeled with ^{99m}Tc would be available at most hospitals and at a relatively low cost, because ^{99m}Tc is widely available from a ^{99}Mo/^{99m}Tc generator. Previous studies of technetium–99m labeled ER ligands for use as imaging agents have suffered from several problems. Inorganic chelates of ^{99m}Tc demonstrated molecular instability under biological conditions; also, the large size of many Tc complexes interferes with cellular uptake. Studies conducted in this laboratory suggest that an integrated organometallic design in which technetium bonded to carbon forms a part of the core structure will display the needed stability, as well the potential for high binding affinity to ER. While significant advances have been made, major improvements in radiolabeling techniques and structural design are still needed before an imaging agent using ^{99m}Tc will be effective as a diagnostic tool to identify tumors that will respond to hormone therapy. The structural design motif under investigation is based upon previous work in our laboratory, as well as molecular modeling with comparison to the morphology of the native ER ligand, estradiol.

Body of Report

I. Training

I have attended various organic-chemistry, organometallic-chemistry, and chemical-biology seminars presented by visiting professors and UIUC students.

II. Research

The overall objective of this proposal is to develop a compound bearing a ^{99m}Tc label that exhibits both a high relative binding affinity to ER, has good *in vivo* stability, and functions effectively as an imaging agent for ER in breast tumors. Ultimately, we hope that this compound could be used to image ER+ tumors in a manner that would provide information useful for the selection of the optimal therapy for a breast cancer patient. This aim has been divided into four tasks, which make up the approved Statement of Work.

Task A: (Months 1-18, July 1, 2003 – December 31, 2004)

- Begin model studies for synthesis of compound **PyCR (II)**.
- Begin synthesis of **ACR (IV)**.

Task B: (Months 13-30, August 1, 2004 – December 31, 2005)

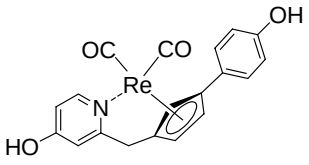
- Execute synthesis of **PyCR (II)**, and determine relative binding affinity.
- Execute synthesis of **PIRB (VI)** analogs and determine relative binding affinity.

Task C: (Months 25-36, July 1, 2005 – June 30, 2006)

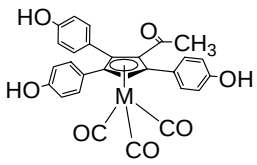
- Execute synthesis of analogs of **PyCR (II)**, with various combinations of alkyl and aryl substitution on the central cyclopentadienyl ring.
- Execute synthesis of **PIRM (VII)** analogs and determine relative binding affinity.
- Execute synthesis of the **ACR** (**IV)**, and determine relative binding affinity.
- Develop synthesis of **CBRN (VIII)**, and determine relative binding affinity.

Task D: (Months 12-36, July 1, 2004 – June 30, 2006)

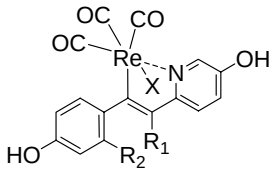
- We will develop methods of radiolabeling using ^{94m}Tc in place of Re and evaluate the *in vivo* tissue distribution of all labeled compounds with promising *in vitro* properties. (To be done through our long-standing collaboration with Professor Michael Welch of the Mallinckrodt Institute of Radiology at Washington University Medical School)



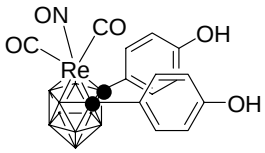
PyCR (II)



ACR (IV) M = Re
ACT (V) M = Tc



PIRB (VI) X = Br
PIRM (VII) X = OCH_3



CBRN (VIII)

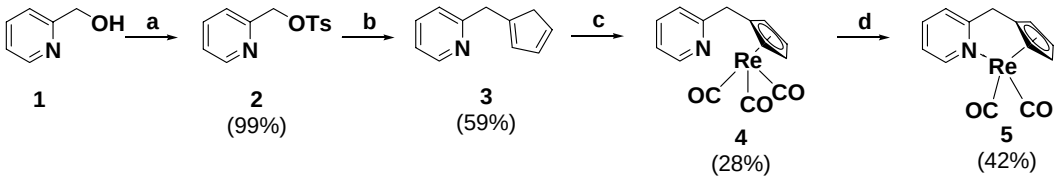
Figure 1. ER Ligand Targets

This final report describes progress made through all thirty-six months of award coverage. A number of unsuccessful synthetic approaches described in annual reports I and II have been omitted, in favor of new approaches to the described statement of work.

Task A Step 1

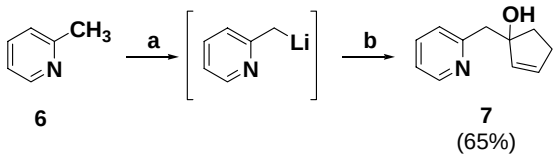
The model study for the synthesis of $\eta^1\eta^5$-complex PyCR **II** has been completed with the synthesis of η^1,η^5-pyridylmethylcyclopentadienyl rhenium(I) dicarbonyl (**5**), which forms the core of the phenol substituted PyCR **II**. Production of **5** proceeds in four steps from commercially available 2-pyridylmethanol **(1)**, as shown in Scheme 1. The essential pyridine to rhenium cyclization occurs via photo-irradiation under inert atmosphere to provide the desired rhenium dicarbonyl complex.[1] Alternatively, the production of the

(pyridylmethyl)cyclopentadiene (**3**) can be accomplished in a one-pot procedure using the lithium anion of picoline (**6**) and cyclopentenone, as shown in Scheme 2, followed by dehydration.[2]



(a) TsCl, KOH, THF, RT, overnight. (b) NaCp, THF, -78 °C. (c) n-BuLi, [ReBr(THF)2(CO)3]2, RT, 20 min. (d) hn, 300 nm. 90 min.

Scheme 1 Synthesis of Pyridyl-Cyclopentadienyl Core

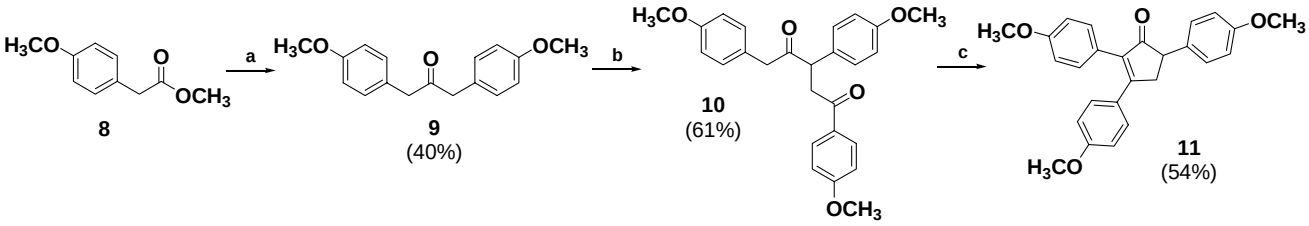


(a) *n*-BuLi, THF, -78 °C, 20 min. (b) 2-cyclopentenone, 20 min.

Scheme 2 Anionic Synthesis of Core

Task A Step 2

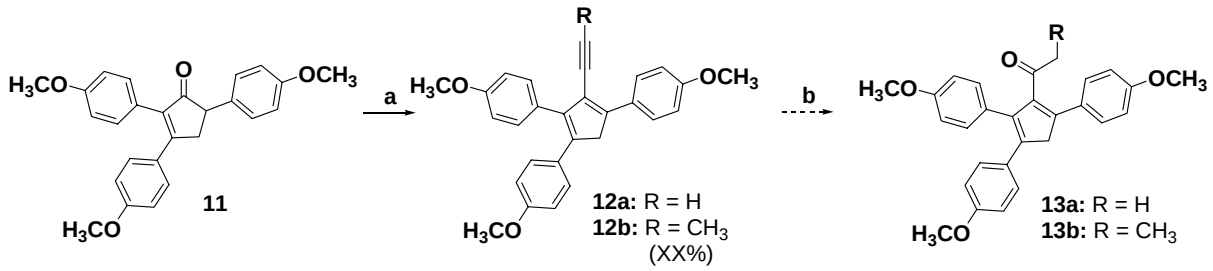
The synthesis of 2,3,5-tris-(4-methoxyphenyl)cyclopentenone (**11**), as shown in Scheme 3, has been completed in three steps, starting from commercially available methyl 4-methoxyphenylacetate (**8**). Several methods have been investigated for the addition of a fourth substituent to the central pentacycle, including nucleophilic addition of organometallic reagents, addition of electrophiles to the cyclopentadiene derived from **11**, Wittig and titanium mediated olefination, followed by hydride transfer, and zirconocene-mediated cyclopentenone formation.



(a) i. LDA, ether, -78 °C, 20 h. ii. AcOH, HCl, reflux, 5h. (b) i. LDA,THF, -78 °C, 1 h. ii. 2-bromo-4'-methoxyacetophenone, 1.5 h. c. methanolic KOH, RT, 20 min.

Scheme 3 Synthesis of Triarylcyclopentenone

Ultimately, the nucleophilic addition of 1-propynylmagnesium bromide to the carbonyl of enone **11** provided the tetra-substituted 2,3,5-tris-(4-methoxyphenyl)-1-propynylcyclopentadiene (**12b**), as shown in Scheme 4. Initial attempts to hydrate the triple bond of alkyne **12b** using transition metal catalysis have thus far failed to provide the desired ketone **13b**. A model study, elucidating potentially successful reaction conditions, is described below as part of Task C.

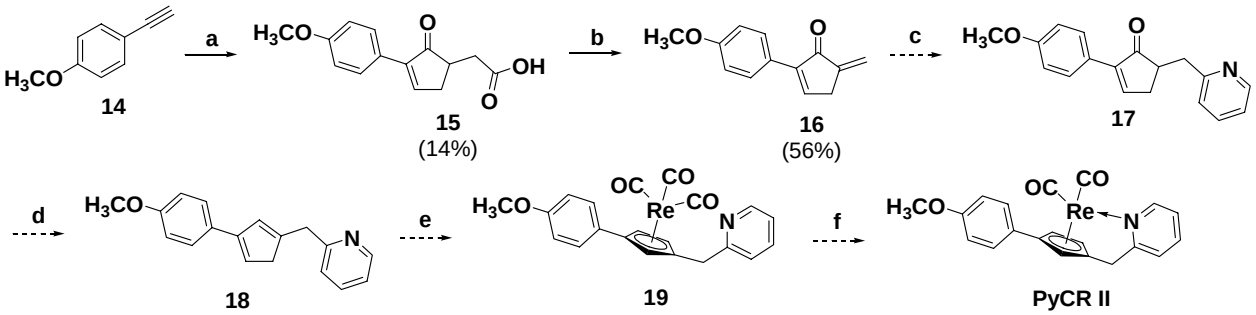


(a) i. TMS-acetylene, *i*-Pr-MgBr, 30 min, 0°C, THF. ii. ethanol. iii. Repeat cycle 6.

Scheme 4 Synthesis of Acylcyclopentadiene

Task B Step 1

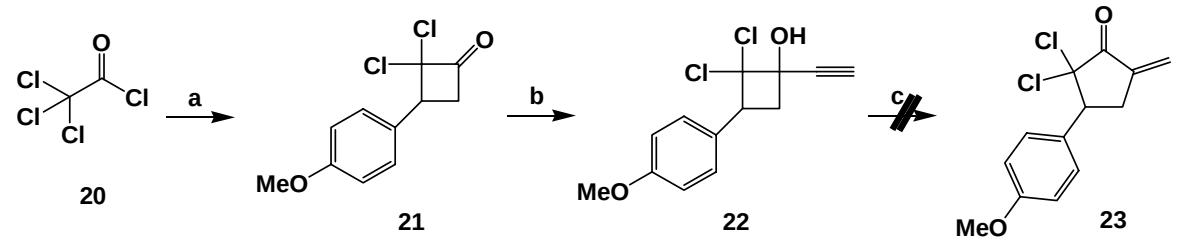
The completion of the model study in Step 1 of Task A led to attempts to synthesize the phenol substituted **PyCR**. Early routes to a hydroxyphenyl substituted pyridylmethyl cyclopentadiene, described in Annual Report I, shared the reactivity limitations of enone **4**, described above. The Pauson-Khand type reaction, described in Annual Report II (Scheme 5) provided small amounts of the desired acid-enone **15**. Ultimately, when attempted on preparative scale the triphasic cyclization reaction to provide the enone carboxylic acid **15** did not provide sufficient material to move beyond the decarboxylative elimination to form dienone **16**.



(a) $NiBr_2$, NaI, $AlBr_3$, Fe, CO, acetone, RT, 5h. (b) 2-Mercaptopyridine N-oxide, DCC, $CBrCl_3$, reflux, 5h. (c) Pyridyl cuprate reagent, BF_3•OEt_2. (d) DIBALH, THF, -78° C, 4h. (e) i. n-BuLi, THF, -78° C, 15 min. ii. $[ReBr(THF)_2(CO)_3]_2$, RT, 20 min. (f) hn, 300nm, THF, 90 min.

Scheme 5 Pauson-Khand Type Synthesis of PyCR II

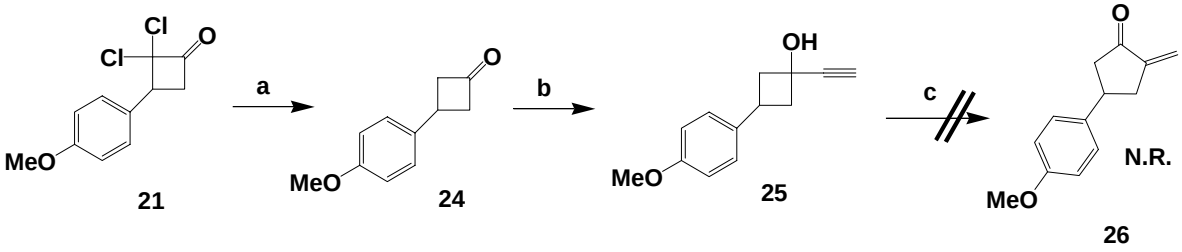
Dienone **16** shows impressive structural similarity to the exomethylene enone product of gold catalyzed ring expansion of cyclic propargylic alcohols.[3] With this in mind, exo enone **23** was synthesized as shown in scheme 6. The production of dichloroketene from trichloroacetyl chloride (**20**), followed by 2+2 cyclization with methoxy styrene provided dichlorocyclobutanone **21**. Addition of acetylide grignard yielded propargylic alcohol **22**, a potential substrate for ring expansion. Upon treatment with the appropriate gold(I) catalyst however, the acetylide moiety was eliminated to again provide cyclobutanone **21** rather than the desired enone **23**.



a) i. $POCl_3$, Zn-Cu, THF. ii. 4-methoxystyrene. b) ethynylmagnesium bromide, THF. c) PPh_3AuCl, $AgSbF_6$.

Scheme 6 Ring Expansion

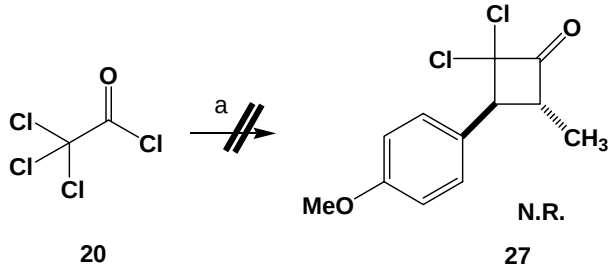
Because literature examples did not contain the α,α-dichloro moiety[3] it was decided that reduction to the methylene, as shown in scheme 7, would provide a substrate more suitable for expansion. Zinc reduction of dichloride **21** provided cyclobutanone **24**. Addition of acetylide yielded propargylic alcohol **25,** which decomposed under ring-expansion conditions rather than the eliminating acetylide as previously-observed.



a) i. Zn, AcOH. b) ethynylmagnesium bromide, THF. c) PPh_3AuCl, $AgSbF_6$.

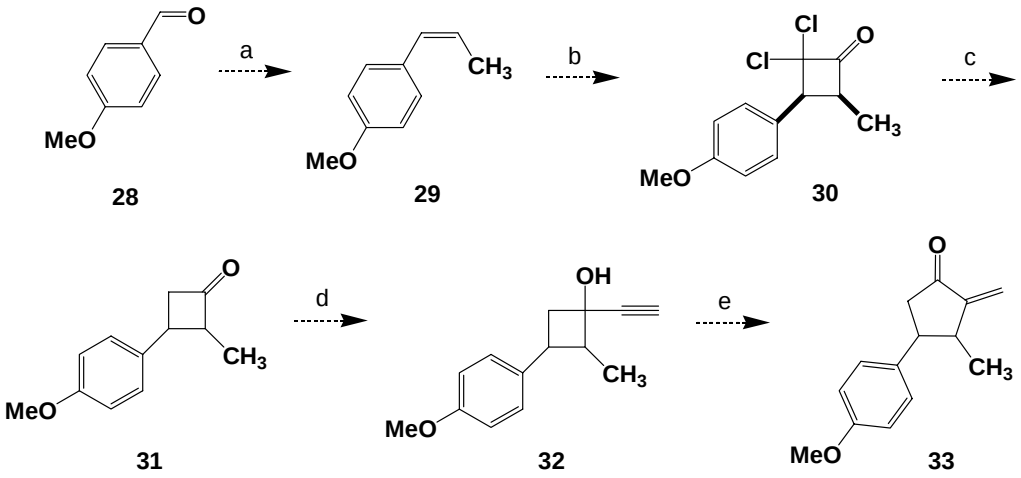
Scheme 7 De-chlorinated Ring Expansion

It was thought that α-substitution would improve the migratory aptitude of the cyclobutane ring carbon; consequently, production of the *trans* α-methyl analog (**27**) of dichloro cyclobutanone **21**was attempted (scheme 8). Reaction of dichloroketene with commercially available *trans*-anethole failed, likely due to the steric requirements of the 2+2 cyclization. Synthesis of *cis*-anethole (**29**) for the production of *cis*-aryl methyl cyclobutanone **30** (scheme 9) is ongoing.



a) i. $POCl_3$, Zn-Cu, THF. ii. *trans*-anethole.

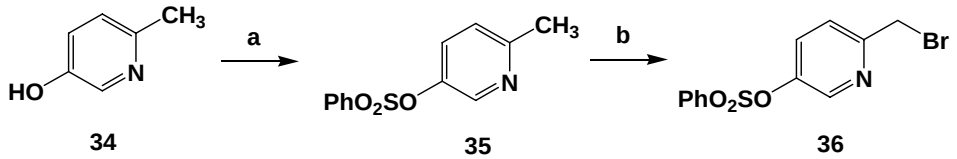
Scheme 8 Methyl Substitution Substrate



a) ethyltriphenylphosphonium bromide, NaH, THF. b) $POCl_3$, An-Cu, THF. c) i. Zn, AcOH.
d) ethynylmagnesium bromide, THF. e) PPh_3AuCl, $AgSbF_6$.

Scheme 9 Putative Synthesis of Exo Enone

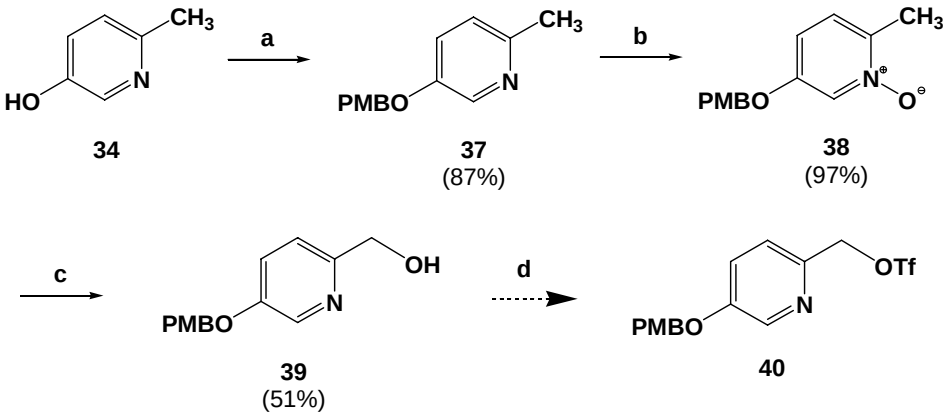
An alternative approach currently under investigation is the S_N2 displacement of a leaving group from a protected hydroxy picoline, using an aryl-substituted cyclopentadienide. The synthesis of bromide **36** is shown in scheme 10. The protection of hydroxypicoline **34** as the radical-stable benzene sulfonate yields picoline **35**, which undergoes radical bromination to provide bromide **36**.



a) $PhSO_2Cl$, NEt_3, THF. b) NBS, AIBN, CCl_4.

Scheme 10 Synthesis of Pyridylmethyl Bromide

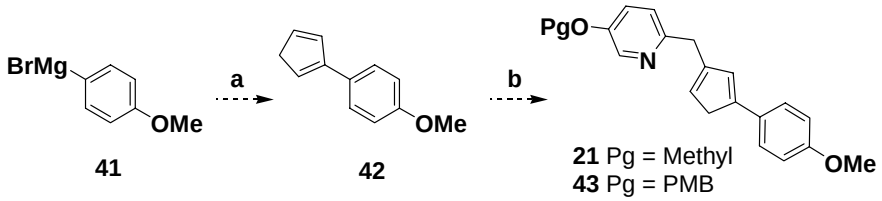
Alternatively, the hydroxymethyl pyridine 34 can be activated as a trifluoromethane sulfonate **40**, as shown in scheme 11. Synthesis of the dihydroxy complexes begins with the protection of the hydroxypicoline **34**, as the *p*-methoxybenzyl ether **37**. Oxidation to the N-oxide **38** with peroxy acid, with subsequent rearrangement provides the alcohol **39**. Treatment of **39** with triflic anhydride in the presence of base produces triflate **40**.



(a) PMBCl, NaH, THF. (b) mCPBA, $CHCl_3$. (c) i. $(CF_3CO)_2O$, CH_2Cl_2. ii. CH_3OH. (c) MnO_2, CH_2CL_2. (d) $(TfO)_2O$, NEt_3, THF.

Scheme 11 Synthesis of Pyridylmethyl Triflate

The aryl-substituted cyclopentadiene **42** required as the nucleophilic component for S_N2 displacement is potentially synthesized via addition of Grignard reagent **41** to cyclopentenone, followed by dehydration to form **42**. Initial trials of this reaction have demonstrated both its utility in forming product and the instability of the mono-substituted Cp **42** under ambient conditions. Deprotonation using strong acid, followed by S_N2 displacement using either bromide **36** or triflate **40** is expected to provide the pyridyl substituted Cp **21** or **43**, suitable for labeling.

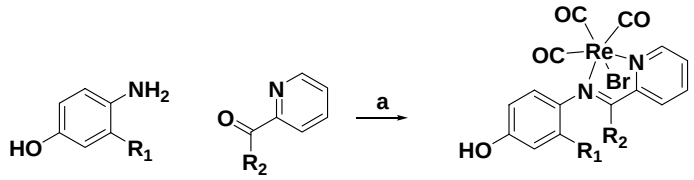


(a) 2-cyclopeten-1-one, THF. (b) i. nBuLi, THF. ii. **36** or **40**.

Scheme 12 Synthesis of Aryl Pyridyl Cp

Task B Step 2

As discussed in Annual Report I, the synthetic ease of imine formation, as well as the strong coordination of the nitrogen of pyridine makes a pyridyl-imine based scaffold attractive for the bidentate portion of a 2+1 ligand system in the production of radio-labeled ER ligands. The anionic monodentate ligand is bromide for the pyridyl-imine rhenium tricarbonyl bromide (**PIRB VI**) system. A nomenclature of PIRB ligands is based on whether a hydroxyl appears on the aniline moiety (A-ring mimic), the pyridine (D-ring mimic), and the identity of substituents R_1, and R_2. As shown in Scheme 6, a variety of commercially available hydroxyaniline derivatives **44-46** were combined with either pyridinecarboxaldehyde **47** or acetylpyridine **48** in refluxing methanol**,** followed by addition of a rhenium tricarbonyl salt formed the monohydroxy rhenium complexes **49a-d**, with the aniline moiety forming the A-ring mimic.



44: R_1 = H
45: R_1 = CH_3
46: R_1 = Cl

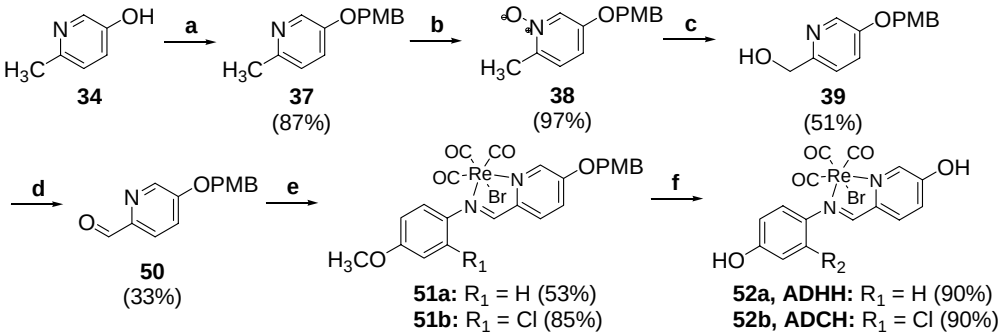
47: R_2 = H
48: R_2 = CH_3

49a, AHH: R_1 = H R_2 = H (XX)%
49b, AMH: R_1 = CH_3 R_2 = H (54)%
49c, ACH: R_1 = Cl R_2 = H (XX)%
49d, AHM: R_1 = H R_2 = CH_3 (75)%

(a) i. methanol, reflux, 2h. ii. $[NEt_3]_2[ReBr_3(CO)_3]$, 5 min.

Scheme 13 Synthesis of Monohydroxy Imines

Synthesis of the analogous dihydroxy complexes begins with the protection of the hydroxypicoline **34**, as the *p*-methoxybenzyl ether **37**. Oxidation to the N-oxide **38** with peroxy acid, with subsequent rearrangement provides the alcohol **39**. Benzylic oxidation yields the aldehyde **50** needed for imine formation. Condensation with anisidine derivatives, followed by addition of the rhenium salt causes the protected rhenium complexes **51a-b** to precipitate from solution. Deprotection with Lewis acid boron tribromide reveals the dihydroxy complexes **52a-b**.

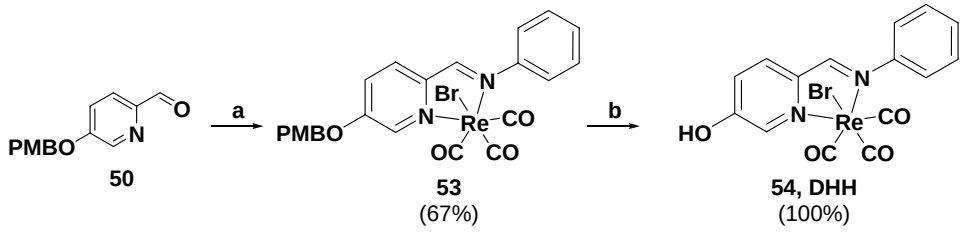


34
37 (87%)
38 (97%)
39 (51%)
50 (33%)
51a: R_1 = H (53%)
51b: R_1 = Cl (85%)
52a, ADHH: R_1 = H (90%)
52b, ADCH: R_1 = Cl (90%)

(a) PMBCl, NaH, THF. (b) mCPBA, $CHCl_3$. (c) i. $(CF_3CO)_2O$, CH_2Cl_2. ii. CH_3OH. (c) MnO_2, CH_2CL_2. (d) i. Anisidine, CH_3OH, reflux. ii. $[NEt_4]_2[ReBr_3(CO)_3]$. (e) BBr_3, CH_2Cl_2, -78° C - RT.

Scheme 14 Synthesis of Bis-Hydroxy Imines

The corollary to monohydroxy **49a**, in which the pyridine moiety forms the A-ring mimic, is formed using the above procedure, starting with protected hydroxy aldehyde **50**, which undergoes condensation with unsubstituted aniline to form rhenium complex **53**. Deprotection reveals the monohydroxy complex **54.**



50
53 (67%)
54, DHH (100%)

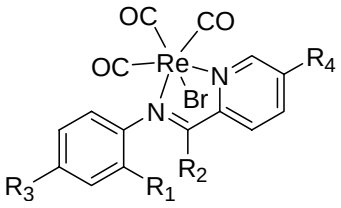
(a) i. Aniline, CH_3OH, reflux, 2h. ii. [NEt4]2[ReBr3(CO)3]. (b) BBr_3, CH_2CL_2, -78° C - RT.

Scheme 15 Synthesis of Isomeric Monohydroxy Imine

For all **PIRB** complexes synthesized, binding affinities relative to estradiol (100%) are listed in Table 1 for both ERα and β. The highest affinity compound thus far is **52a**, **ADHH**. Among mono-hydroxy compounds **49a-d, 54**, substitution in the R_2 position increases binding affinity. This is supported by molecular modeling (Figure 2), which for lead compound **ADHH (52a)**, chloro mono-hydroxy **ACH** (**49c)**, and bis-hydroxy **ADCH**

(52b), show free space in the receptor opposite the imine carbon. This suggests that a number of bis-hydroxy keto-imines be synthesized in order to improve binding affinity. Substitution in the R_1 position has mixed effects, raising binding affinity for **ACH (49c)**, but lowering binding affinity for **AMH (49b)**, relative to H-substituted **AHH (49a)**. This effect cannot be explained by the difference in halogen versus alkyl, as the substitution of a chloride, which has previously improved binding affinity, lowered it for **ADCH (52b)** versus **ADHH (52a)**.

Table 1. PIRB Relative Binding Affinity (E2 = 100%)



	R_1	**R_2**	**R_3**	**R_4**	**ERα**	**ERβ**
49a, AHH	H	H	OH	H	0.015%	0.011%
49b, AMH	CH_3	H	OH	H	<0.005%	<0.005%
49c, ACH	Cl	H	OH	H	0.327%	0.0185%
49d, AHM	H	CH_3	OH	H	0.071%	0.055%
52a, ADHH	H	H	OH	OH	0.271%	2.29%
52b, ADCH	Cl	H	OH	OH	<0.01%	0.01%
54, DHH	H	H	H	OH	<0.01%	0.015%

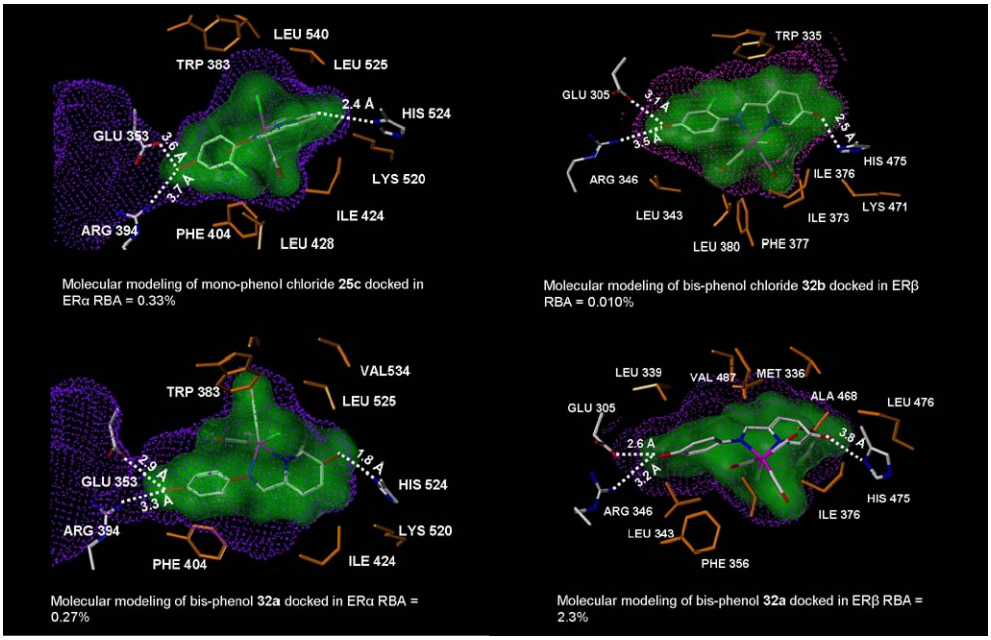
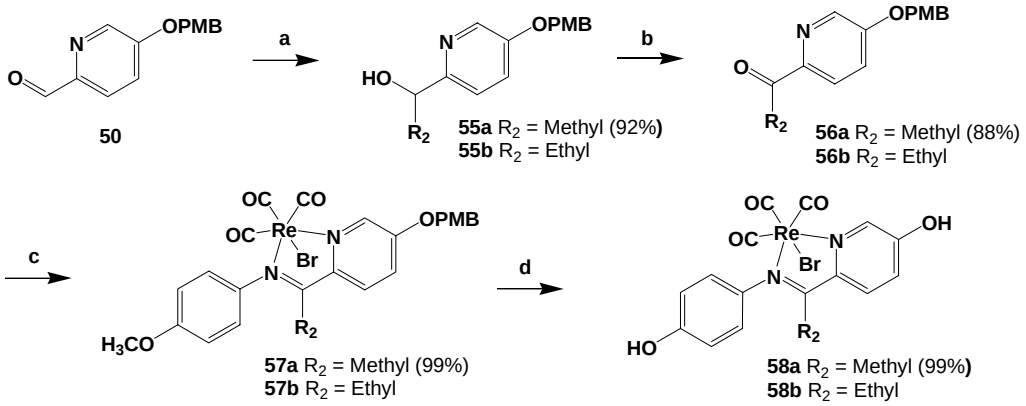


Figure 2. Molecular Modeling of Selected PIRB Ligands

Synthesis of the keto-imine analog to **49c**, ADHM (**61a**) is shown in scheme 16. Addition of methyl grignard reagent to aldehyde **50** provides secondary alcohol **55a**, which is reoxidized using manganese dioxide to ketone **56a**. Imine formation, followed by rhenium addition provides ether-protected ADHM (**60a**), which is deprotected with boron tribromide to form **61a**. Binding affinity of ADHM (**61a**) to the estrogen receptor has yet to be determined.

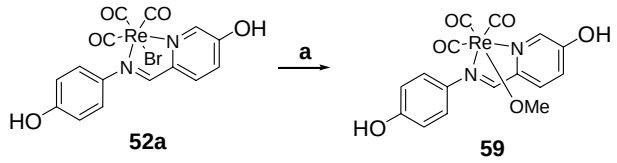


a)R_2MgBr, THF. b) MnO_2, CH_2Cl_2. c) i. Anisidine, MeOH. ii. $[NEt_4]_2[ReBr_3(CO)_3]$ d)BBr_3,CH_2Cl_2.

Scheme 16 Synthesis of Keto-imine Complexes

Task C Step 2

The conversion of bromide complexes PIRB to the methoxy complex PIRM has been initiated. Conversion of **ADHH** (**52a)** to the methoxy compound proceeds successfully, according to initial NMR spectroscopy experiments. To date, the high polarity of the resulting methoxy complex has complicated final purification of methoxy complex **59**. Future plans include the use of reverse phase HPLC, followed by characterization and binding affinity studies.

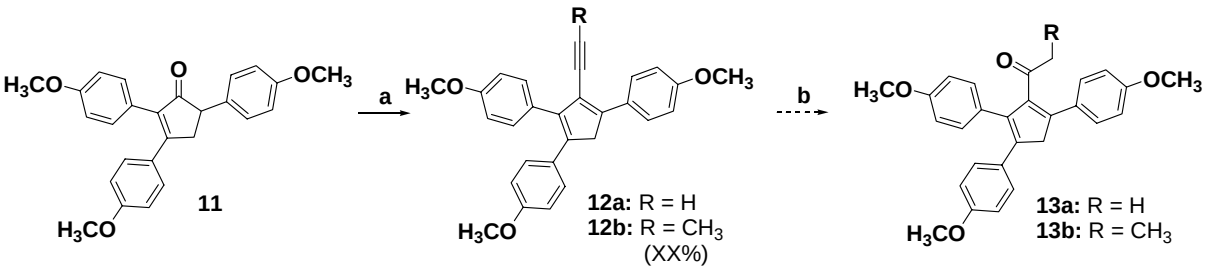


(a) MeOH, NaOH, RT, 1 h.

Scheme 17 Synthesis of Methoxy Complex

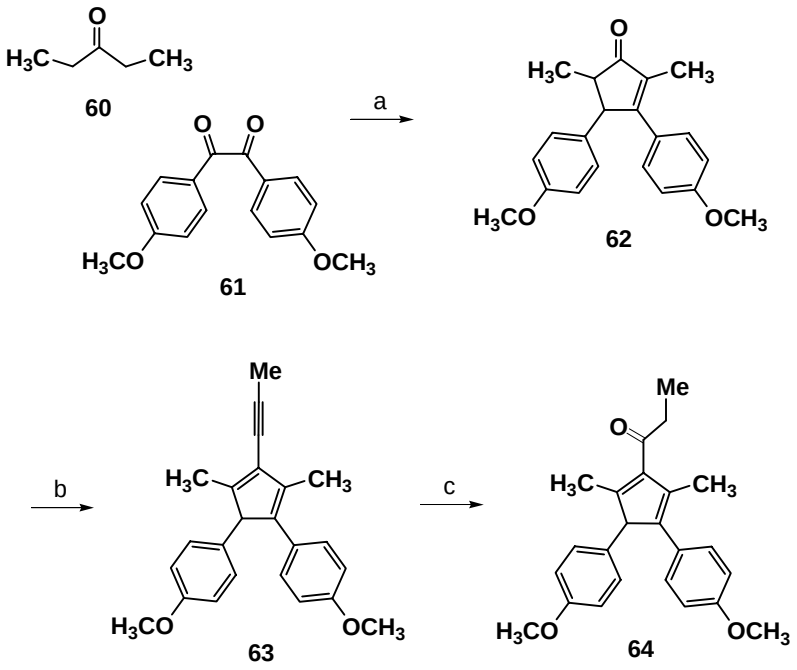
Task C Step 3

As discussed above for Task A, the successful synthesis of ketone **13** depends upon the hydration of alkyne **12** (See Scheme 4). In order to determine the requisite conditions, model compound **63** was synthesized as shown in scheme 18. Enone **62** is synthesized via the dehydrative cyclization of 3-pentanone (**60**) with 4,4'-dimethoxybenzil (**61**) to form enone **62**, which is converted to alkyne **63** by the addition of propynyl grignard. An investigation of transition metal catalysis revealed that substoichiometric amounts of HgO, in the presence of acetic acid, resulted in clean conversion to acylketone **64**. Resynthesis of alkyne **12**, the intended substrate for this method, is ongoing.



(a) i. TMS-acetylene, *i*-Pr-MgBr, 30 min, 0°C, THF. ii. ethanol. iii. Repeat cycle 6.

Scheme 18 Synthesis of Acylcyclopentadiene

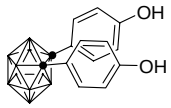


a) i. EtOH, KOH. ii. HI, AcOH. iii. Na_2SO_3. b) i. Propynylmagnesium bromide, THF. ii. EtOH quench. iii. repeat 2x c) HgO, acetone, H_2O, AcOH

Scheme 19 Model Hydration System

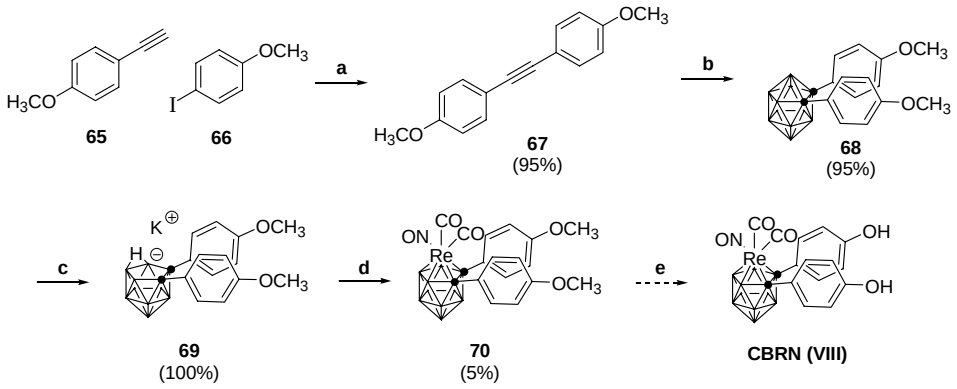
Task C Step 4

The lipophilic character of the estrogen receptor, coupled with the lipophilic nature of *closo*-carboranes make them attractive scaffolds upon which to build ER ligands. Indeed, work is this field by Endo[4-6] has produced a number of carborane-based ER ligands with good binding affinity (See Figure 3). Radiolabeling under aqueous conditions produces an anionic tricarbonyl species (See Scheme 19).[7] Use of a di-cationic rhenium-nitroso-dicarbonyl to balance the charge[8-10] provides the potential for a neutral metallocarborane, capable of binding to the estrogen receptor. To test this hypothesis, Sonogashira coupling of methoxyphenyl acetylene **65** with iodoanisole **66** provides the bis-methoxyphenyl acetylene **(67)**, which is refluxed with decaborane to form the *ortho*-*closo*-carborane **68**. The *closo*-carborane is degraded to the *nido*-carborane potassium salt **69**, using potassium hydroxide. Literature methods for the metallation of nido-carbanions such as **69** have only provided metallo-carborane **70** in minimal yields. Investigations of alternate counter-ions, bases, solvents and sources of rhenium (I) have in my hands failed to provide increased metallation efficiency.



ERa RBA = 48%

Figure 3 BE361

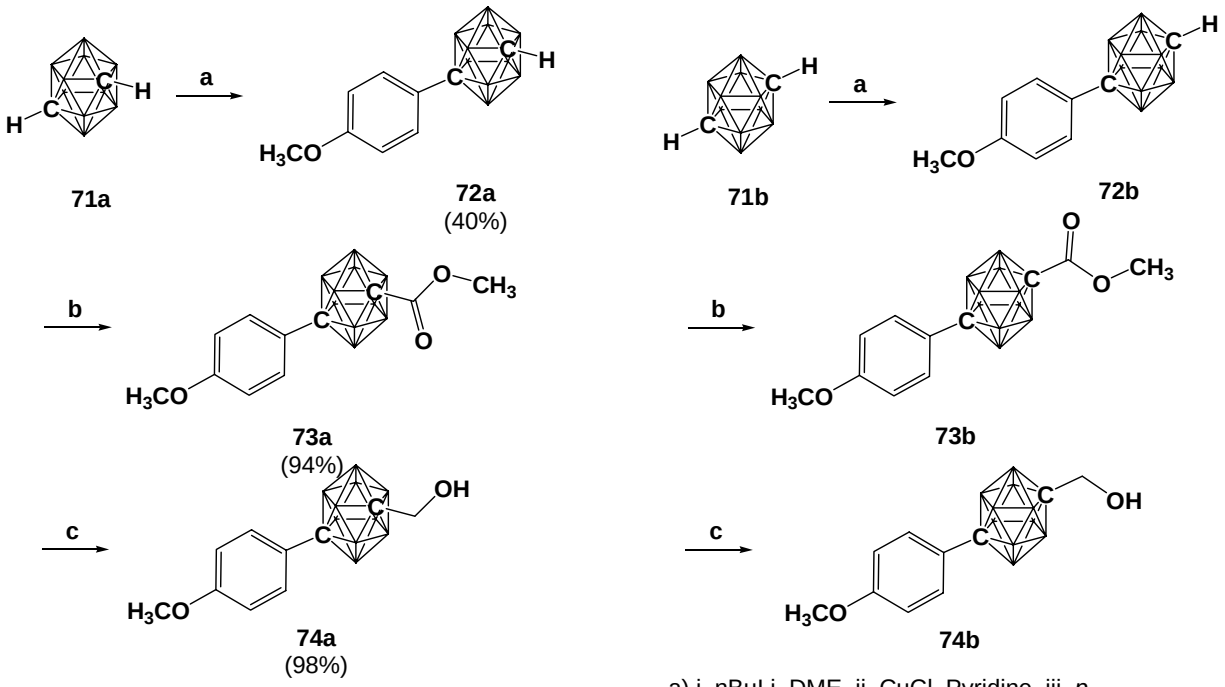


(a) $Pd(PPH_3)_4$, CUI, TEA, ACN, RT, 3h. (b) $B_{10}H_{14}$, Et_2S, Pr_2O, reflux, 12h. (c) ethanolic KOH, RT, 18h. (d) $KF_{(aq)}$, MeOH, 85° C, 1 h. ii. $[NEt_4]_2[Re(CO)_3Br_3]$. 85° C, 12h. iii. $NOBF_4$. (e) BBr_3, CH_2Cl_2, -78° - RT.

Scheme 20 Synthesis of CBRN

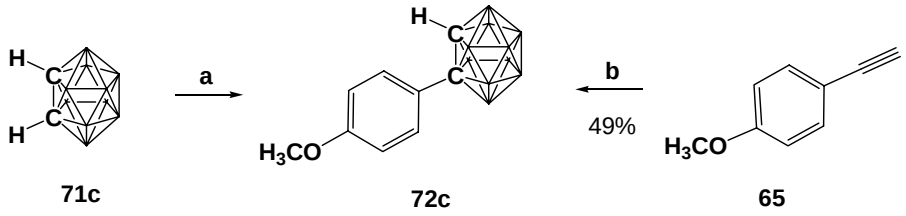
The electron density of *nido*-carborane **69** is high, due to the presence of two electron donating aryl substituents. It was thought that replacing one of these rings with a less donating substituent would increase the acidity of the bridging hydrogen and increase the metallation efficiency. In addition, reducing the steric demand of the carborane substituents could act to improve the facility of approach by the metal complex during metallation. A number of carboranes successfully metallated by Valiant, *et. al*[7] contain primary alkyl chains, terminated by a Lewis base, which could act as a tether to assist the metal's approach. With these design features in mind, it was decided to synthesize *meta* and *para* arylhydroxymethyl carboranes **74a** and **74b** for metallation.[4]

The syntheses of the *meta*- and *para*- aryl and hydroxymethyl substituted carboranes are shown in scheme 21. Copper catalyzed arylation of the commercially-available carborane **71a-c** provides mono-aryl carborane **72a-c**, which is converted to ester **73a,b** via deprotonation and attack on methyl chloroformate. Reduction with lithium aluminum hydride provides hydroxymethyl carborane **74a,b**.



Scheme 21 Synthesis of *meta*- and *para*-Carborane Ligands

Mono Aryl *ortho*-carborane **72c** was synthesized via copper catalyzed coupling of methoxyphenyl iodide to the commercially available *ortho* carborane **71c**, as well as by the conversion of methoxyphenyl acetylene to the carborane using decaborane (Scheme 22). Degradation of the *closo*- carboranes **74a** and **72c** to the uncapped *nido*- form, and subsequent attempts at metallation did not provide workable quantities of metallated product.



a) i. nBuLi, DME. ii. CuCl, Pyridine. iii. *p*-methoxyphenyl iodide. b) $B_{10}H_{14}$, Et_2S, nPr,O

Scheme 22 Synthesis of Mono Aryl Carborane

Key Research Accomplishments

- Task A Step 1, Model Study for compounds PyCR completed.
- Task A Step 2, Synthesis of ACR two steps from completion.
- Task B Step 1, Synthesis of monoaryl analog to PyCR ongoing using a number of parallel schemes.
- Task B, Step 2, Synthesis of a small library of PIRB ligands accomplished. Lead compound is ADHH with RBA to ERα of 2.3%.
- Task C Step 2, Synthesis of crude ADHH methoxy analog is awaiting purification.
- Task C Step 3, Model study for Hydration has revealed appropriate conditions for the completion of synthesis of ACR.
- Task C Step 4, A variety of known carborane-based ligands for ER have been synthesized. Metallation has been attempted, yielding minimal amounts of product.

Reportable Outcomes

- Poster Presentation **Cyclopentadienyl Rhenium (Technetium) Tricarbonyl Complexes Integrated in Estrogen Receptor (ER) Ligands for ER+ Tumor Imaging**, at the 9th Congress of the World Federation of Nuclear Medicine & Biology at COEX Seoul, Korea, October 22~27, 2006.

Conclusions

A number of ligands for the Estrogen Receptor have been synthesized, with promising binding affinity among the PIRB family of ligands while the PIRM family of ligands shows increased *in vitro* stability. Ongoing studies directed towards the further development of this pyridyl-imine class of ligands promise to lead to ligands with both good binding affinity and suitable stability for labeling and *in vivo* testing. The ACR and PyCR ligand classes are both near completion, with two steps remaining for ACR and multiple synthetic schemes leading to PyCR underway. The CBRN class of ligands has shown low potential for radio-metallation in my hands. As ligands with appropriate binding affinity and stability are completed, radio-labeling and *in vivo* animal studies will be undertaken with our collaborator, Michael Welch, of the Mallinckrodt Institute of Radiology at Washington University Medical School.

Abbreviations

ACR	1-acetyl-2,3,5-tris-(4-methoxyphenyl)-cyclopentadienylrhenium(I) tricarbonyl **(IV)**
ACT	1-acetyl-2,3,5-tris-(4-methoxyphenyl)-cyclopentadienyltechnetium(I) tricarbonyl **(V)**
ER	Estrogen Receptor
ER+	Estrogen Receptor Positive
ER-	Estrogen Receptor Negative
CBRN	(3)-1,2-bis-(4-hydroxyphenyl)-1,2-dicarbadodecahydroundecaborate (-2) nitroso rhenium dicarbonyl
PyCR	η^1,η^5-1 -(4-hydroxyphenyl)-3-pyridylmethylcyclopentadienyl rhenium(I) dicarbonyl **(II)**
PIRB	{Bromo[*N*-(2-pyridinylmethylene)-4-hydroxyphenylamine]rhenium(I) tricarbonyl}
PIRM	{Methoxy[*N*-(2-pyridinylmethylene)-4-hydroxyphenylamine]rhenium(I) tricarbonyl}
RBA	Relative Binding Affinity

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