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Complexes Integrated in Estrogen Receptor Ligands for ER+
Tumor Imaging

PRINCIPAL INVESTIGATOR: Nathan C. Ackroyd
John A. Katzenellenbogen, Ph.D.

CONTRACTING ORGANIZATION: University of Illinois
Champaign, Illinois 61820-6242

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13. ABSTRACT (Maximum 200 Words) 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 ¹⁸ F-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 ^{99m} Tc, which is available at most hospitals at a relatively low cost, as a ⁹⁹ Mo/ ^{99m} Tc 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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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 $^{99}\text{Mo}/^{99m}\text{Tc}$ 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

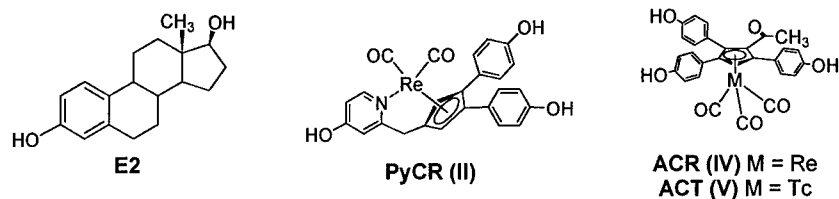
In the past year I have attended the American Chemical Society National Organic Symposium in Bloomington Indiana, June 8-12, 2003, and the Jensen Symposium on Nuclear Receptors and Endocrine Disorders in Cincinnati, Ohio, December 5-7, 2003. I have also attended various organic-chemistry, organometallic-chemistry, and chemical-biology seminars presented by visiting professors and UIUC students. I assisted Dr Katzenellenbogen in teaching his class "Isotopically Labeled Compounds in Chemistry and Biology".

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.

Figure 1



Task A: (Months 1-18)

- Execute synthesis of ACR (IV), and determine relative binding affinity.
- Begin model studies for synthesis of PyCR (II).

Task B: (Months 13-30)

- Develop methods of radiolabeling ACT (V), using $[^{99m}\text{Tc}(\text{H}_2\text{O})_3(\text{CO})_3]^+$ under aqueous conditions.
- Execute synthesis of PyCR (II), and determine relative binding affinity.

Task C: (Months 25-36)

- Execute synthesis of analogs of PyCR, with various combinations of alkyl and aryl substitution on the central cyclopentadienyl ring.

Task D: (Months 12-36)

- We will evaluate the *in vivo* tissue distribution of all Tc 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)

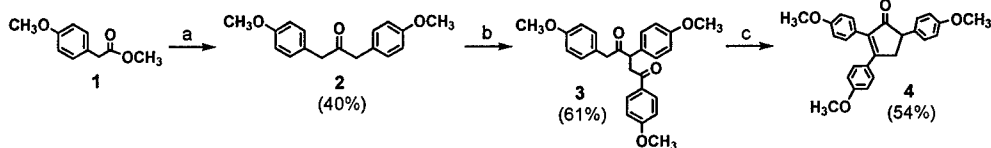
The first twelve months of award coverage described in the approved Statement of Work includes two steps listed as Task A: First, execute synthesis of 1-acetyl-2,3,5-tris-(4-methoxyphenyl)-cyclopentadienylrhenium(I) tricarbonyl (ACR, IV) and determine relative binding affinity; second, begin model studies for synthesis of η^1, η^5 -1,2-(4-hydroxyphenyl)-4-pyridylmethylcyclopentadienyl rhenium(I) dicarbonyl (PyCR, II). Progress has been made towards completion of Step 1, the synthesis of ACR; however, certain synthetic difficulties were encountered in the preparation of the tetra-substituted cyclopentadiene precursor **6a**, and this has prevented the completion of this step to date; work on this step will be continued in the future. Step 2, model studies for the synthesis of the η^1, η^5 PyCR (II) has been completed. As a result, work has begun on a portion of task B initially intended for the second year of award support. The problems encountered in the synthesis of the complexes ACR and PyCR has led to a re-evaluation of targets, and some proposed modification being made of the specific targets the approved statement of work; these changes are still consistent with the overall goal of developing technetium-labeled ligand for the estrogen receptor for *in vivo* imaging of ER+ breast tumors.

Task A Step 1

The synthesis of 2,3,5-tris-(4-methoxyphenyl)cyclopentenone (**4**), as shown in Scheme 1, has been completed in three steps, starting from commercially available methyl 4-methoxyphenylacetate (**1**). Several methods have been investigated for the addition of a fourth substituent to the central cyclopentenone system, including nucleophilic addition of organometallic reagents, addition of electrophiles to the cyclopentadiene derived from **4**, Wittig and titanium mediated olefination, followed by hydride transfer, and zirconocene-mediated cyclopentenone formation. Ultimately, the nucleophilic addition of 1-propynylmagnesium bromide to the carbonyl of enone **4** provided the tetra-substituted 2,3,5-tris-(4-methoxyphenyl)-1-propynylcyclopentadiene (**5b**),

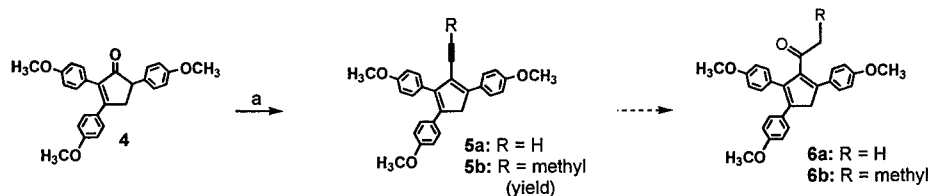
as shown in Scheme 2. Conditions to hydrate the triple bond of alkyne **5b** using transition metal catalysis to provide the desired ketone **6b**, are being investigated.

Scheme 1



(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 2

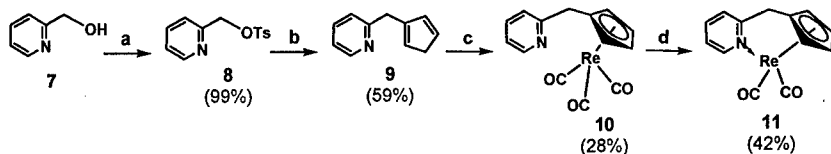


(a) i. TMS-acetylene, *i*-Pr-MgBr, 30 min, 0 °C, THF. ii. ethanol. iii. Repeat cycle 6.

Task A Step 2

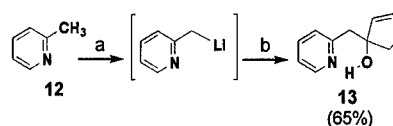
The model study for the synthesis of $\eta^1\eta^5$ -complex PyCR **II** has been completed with the synthesis of $\eta^1\eta^5$ -pyridylmethylcyclopentadienyl rhenium(I) dicarbonyl (**11**), which forms the core of the phenol substituted PyCR **II**. Production of **11** proceeds in four steps from commercially available 2-pyridylmethanol (**8**), as shown in Scheme 3. The essential pyridine to rhenium cyclization occurs via photo-irradiation under inert atmosphere to provide the desired rhenium dicarbonyl complex. Alternatively, the production of the (pyridylmethyl)cyclopentadiene (**9**) can be accomplished in a one-pot procedure using the lithium anion of picoline (**12**) and cyclopentenone, as shown in Scheme 4.

Scheme 3



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

Scheme 4

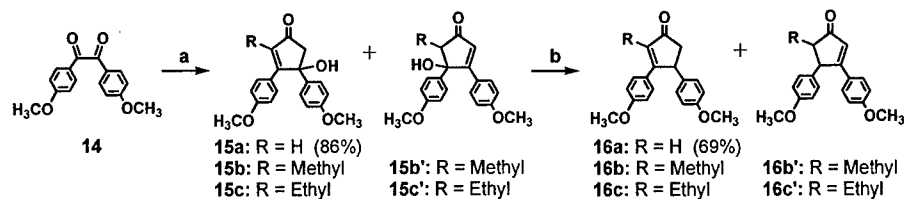


(a) *n*-BuLi, THF, -78 °C, 20 min. (b) 2-cyclopentenone, 20 min.

Task B

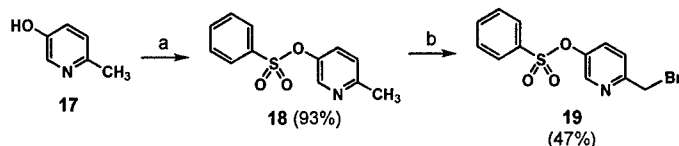
The completion of the model study in Step 2 of Task A led to attempts to synthesize the phenol substituted PyCR. Requisite to completion of Task B is the synthesis of 3,4-bis-(4-methoxyphenyl)cyclopentenone (**16a**), which was synthesized in two steps from dimethoxybenzil (**14**), as shown in Scheme 5. Several methods were used to produce a protected-hydroxy substituted picoline anion, including the production of 2-bromomethylpyridine-5-benzenesulfonate (**19**), as shown in Scheme 6. Deprotonation of protected 5-hydroxypicoline benzenesulfonate (**18**) using strong base, generated anion density at an aromatic carbon rather than the desired methyl group. Generation of the Grignard reagent of **19** using magnesium turnings or anthracene-magnesium complex **20** provided only starting material bromide. Attempts to add the lithiated picoline **12** to **16a**, a reaction similar to that shown in Scheme 4, has thus far provided only starting materials.

Scheme 5



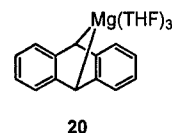
(a) ketone, KOH, ethanol, 80 °C, 2h. (b) i. HI, AcOH, reflux, 10 min. ii. Na₂SO_{3(aq)}.

Scheme 6



(a) benzenesulfonyl chloride, NEt₃, CH₂Cl₂, 0 °C, 90 min. (b) NBS, AIBN, CCl₄, benzene, 4 h, reflux.

Figure 2



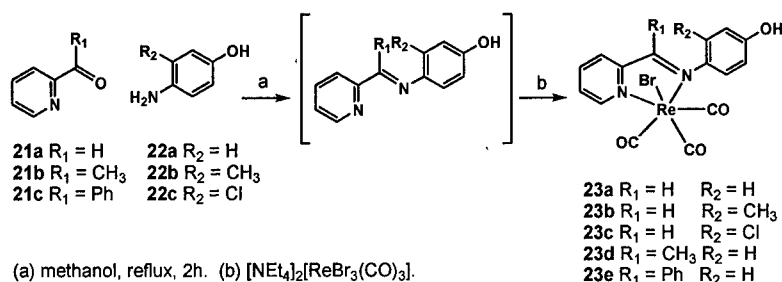
Modification of Approved Statement of Work

The problems we have been having in adding a fourth substituent to 2,3,5-tris-(4-methoxyphenyl)cyclopentenone (**4**) and a substituted picoline to 3,4-bis-(4-methoxyphenyl)cyclopentenone (**16a**), to produce ACR and PyCR respectively, is presumably being caused by the electron donation of the β -methoxyphenyl substituent on both compounds, because model compounds without a methoxy group add nucleophiles readily. The reactivity of these enones is predicated upon the electron-poor character of the carbonyl carbon. The electron-rich oxygen of the β -methoxyphenyl is in electronic conjugation with the carbonyl, due to the presence of the alkene portion of the enone. Because of this, the electron-poor character of the carbonyl is reduced, which has led to a lack of reactivity towards electron rich reaction partners. In the majority of cases, unreacted starting material was re-isolated in impure form.

While not insurmountable, as shown by the synthesis of cyclopentadiene **5b**, the challenge of this step has led to the selection of several alternative designs, in keeping with the spirit of the initial statement of work. The central cyclopentadienyl ring of PyCR has been replaced with a monodentate imine moiety for the general class of pyridyl-imine rhenium-tricarbonyl-bromide ligands PyIRB, which maintains the central location of the metal atom. The advantage of the proposed designs over the previously approved work lies in the ease of synthesis, which has allowed a greater number of analogs to be completed in a short amount of time.

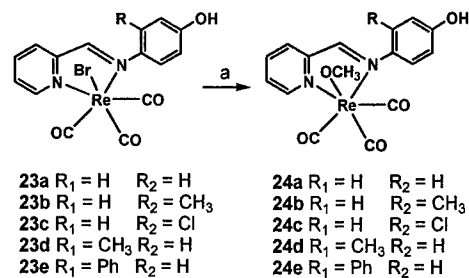
A one-pot procedure, shown in Scheme 7, includes both imine formation and complexation to rhenium tricarbonyl and provides a stable Re(I) complex suitable for RBA testing. Complex **23c** is currently the lead compound with K_i in the high nanomolar concentration range. Addition of silver (I) ion removes the semi-labile Br ligand, forming methoxy complexes **24**, as shown in Scheme 8. Currently, complexes **24a-b** have been submitted for measurement of binding affinity.

Scheme 7



(a) methanol, reflux, 2h. (b) [NEt₄]₂[ReBr₃(CO)₃].

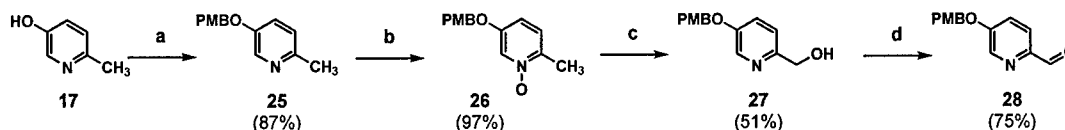
Scheme 8



(a) i. AgBF₄, methanol RT, 30 min. ii. KCN, water, RT, 30 min.

Analogues of complexes **23a-c** bearing a hydroxyl on the pyridine ring requires the synthesis of protected hydroxy-substituted pyridylcarboxaldehyde (**28**). This synthesis, shown in Scheme 9, proceeds in four steps from commercially available 5-hydroxy-2-picoline (**17**).

Scheme 9



(a) methoxybenzyl chloride, NaH, DMF, 1 h, RT. (b) mCPBA, CHCl₃, 18 h, RT. (c) i. triflic anhydride, CH₂Cl₂, 40 min, RT. ii. MeOH. (d) MnO₂, CH₂Cl₂, RT, 3.5 h.

Key Research Accomplishments

- Completion of Task A, Step 2, the model study for synthesis PyCR (**II**).
- Ahead of schedule initiation of Task B, synthesis of phenol-substituted PyCR (**II**), with key intermediates synthesized.
- Design, synthesis and testing of new pyridyl-imine complexes PyIRB with good initial binding affinity to the estrogen receptor

Reportable Outcomes

An abstract has been submitted for the 16th annual International Symposium on Radiopharmaceutical Chemistry, Iowa City, IA, June 24-28, 2005.

Conclusions

Research progress is on schedule with the completion of the model study synthesis, and the early initiation of the synthesis of the phenol substituted PyCR (**II**). Future plans include hydration of the alkynyl substituent on triarylpropynylcyclopentadiene **5b** to provide ketone **6b**, a ligand with potential for aqueous labeling to produce ACR (**IV**). A modification of the accepted statement of work is proposed to include the synthesis of pyridyl-imine complexes which show good initial binding affinity to the estrogen receptor. Future plans include the optimization of this system to maximize the binding affinity into a range with good imaging potential.

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
PyCR	η^1, η^5 -1,2-(4-hydroxyphenyl)-4-pyridylmethylcyclopentadienyl rhenium(I) dicarbonyl (II)
PyIRB	{Bromo[N-(2-pyridinylmethylene)-4-hydroxyphenylamine]rhenium(I) tricarbonyl}
RBA	Relative Binding Affinity