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LIGANDS FOR THE ESTROGEN RECEPTOR, CONTAINING CYCLOPENTADIENYLTRICARBONYLRHENIUM UNITS

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Abstract: As model systems for estrogens labeled with technetium-99m that might be used as in vivo imaging agents for estrogen receptor (ER)-positive breast tumors, we have prepared and determined the ER binding affinity of a series of nonsteroidal and steroidal estrogens substituted with a cyclopentadienyltricarbonylrhenium unit. While this organometallic unit interfered with ER binding when it was tethered close to the ligand, those analogs in which it was attached through a 17α-ethynyl link (provided that the link was not polar) showed high ER affinity.

Many breast tumors contain estrogen receptors (ER) and can be imaged in vivo on the basis of their selective uptake and retention of estrogens labeled with suitable radionuclides.[1] Such ER-based diagnostic imaging can be used to identify both primary and metastatic tumor sites,[1–4] but more importantly, it can be used to predict the liklihood of a favorable response to hormone therapy.[5,6]

Up to now, ER-based diagnostic imaging agents have been estrogens labeled with halogen radioisotopes, such as I-123, Br-77, or F-18.[1] Despite the success of these radiopharmaceuticals, the short half-lives or limited availability of these radioisotopes have constrained the application of ER-based imaging. The popularity of the radionuclide technetium-99m for diagnostic imaging arises from both its excellent gamma emission characteristics and the great convenience and cost-effectiveness of its availability through the Mo-99/Tc-99m generator system.[7–9] Therefore, a premium has been placed on the development of diagnostic imaging agents based on this radionuclide.

The challenge of labeling small molecule ligands for receptor systems with technetium, a metal, has generated much interest, and this field has been reviewed recently.[10] Two general approaches have been taken to the labeling of receptor ligands with technetium: One is an *inorganic* approach, in which a multidentate heteroatom chelate is used to bind a technetium cation, generally, as a Tc(V) oxo system. A number of receptor ligands have been labeled in this inorganic fashion with technetium (or more frequently with the group VII congener rhenium) and investigated as imaging agents.[10] Some of the agents for membrane receptors proved effective, but none of the steroids gave appropriate tissue distribution.

The other approach for labeling receptor ligands is an *organometallic* one, in which a lower valency technetium is tethered through metal carbon bonds. A particularly attractive system for the organometallic tethering is the cyclopentadienyltricarbonyltechnetium ($CpTc(CO)_3$) unit[11] or the corresponding rhenium system ($CpRe(CO)_3$).[11] This unit is compact, nonpolar, and very stable. There have been a limited number of reports of

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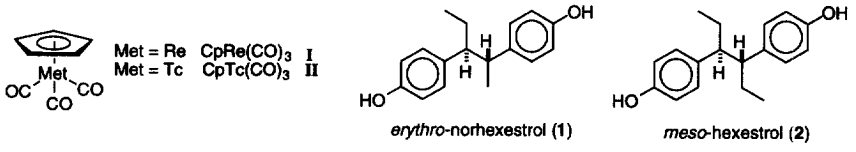
steroids labeled with $CpRe(CO)_3$ systems,[12] but these have proved difficult to prepare in Tc-99m labeled form, so, they have not been studied in vivo.

In this investigation, we describe the preparation of several non-steroidal and steroidal estrogens bearing a $CpRe(CO)_3$ unit as model systems for ER-based imaging agents that might eventually be labeled with Tc-99m. For example, some conjugates are prepared from organometallic precursors that can be synthesized using a novel double ligand transfer reaction on which we have reported recently.[13–15] All of the nonsteroidal ligands we have prepared show low affinity for ER, but some of the steroidal analogs bind to ER with high affinity.

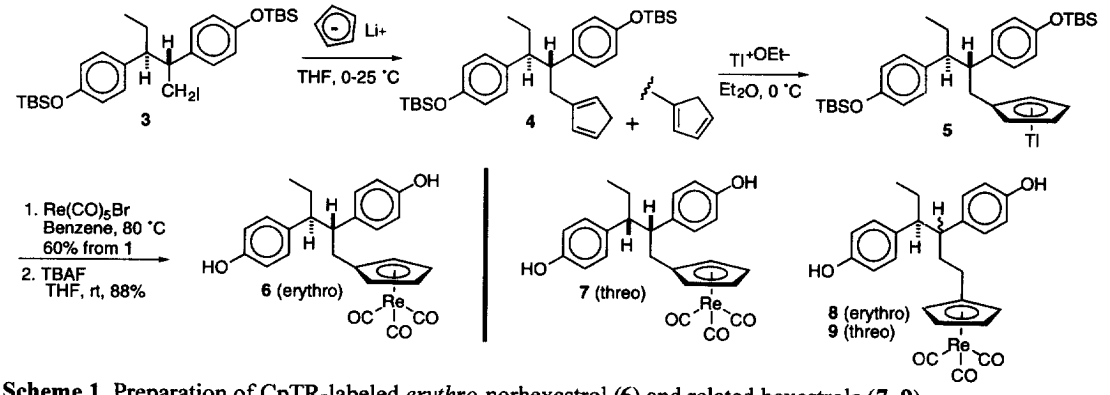
Results and Discussion

Synthesis of Non-Steroidal Estrogens Labeled with $CpRe(CO)_3$

The nonsteroidal estrogens that we have prepared with $CpRe(CO)_3$ substituents are all analogs of the high affinity ER ligands *erythro*-norhexestrol (**1**) or *meso*-hexestrol (**2**). These simple molecules, as the erythro or meso diastereomers, bind to ER with an affinity that is greater than that of estradiol (E_2); the *threo* or *dl* diastereomers, by contrast, have low affinity (cf. Table 1).



To label the norhexestrol and hexestrol systems, an indirect approach was used: $Re(CO)_5Br$ served as a $Re(CO)_3$ donor to introduce the metal into cyclopentadiene-derivatized precursor estrogens. The *erythro*-norhexestrol labeling sequence is shown in Scheme 1. A cyclopentadienyl group was introduced into the iodo derivative **3** (readily prepared from the previously described methyl ether[16,17]) via a displacement reaction using cyclopentadienyllithium (Cp-Li), to provide **4** as a mixture of diene isomers in high yield. The reaction with



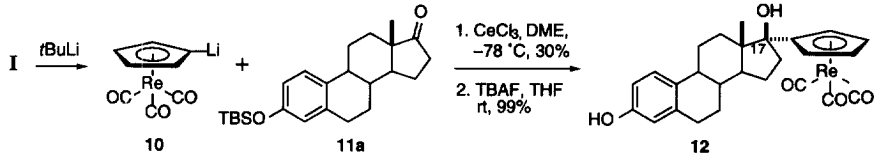
Scheme 1. Preparation of CpTR-labeled *erythro*-norhexestrol (**6**) and related hexestrols (**7–9**).

$Re(CO)_5Br$ was performed using the Cp-thallium intermediate **5**. The TBS protecting groups were removed with tetrabutylammonium fluoride (TBAF) to provide *erythro*-norhexestrol derivative **6**. The corresponding *threo*-norhexestrol derivative **7**, as well as the *erythro*- and *threo*-hexestrol analogs **8** and **9**, were prepared in an analogous fashion.

Steroidal Estrogens Labeled with $CpRe(CO)_3$

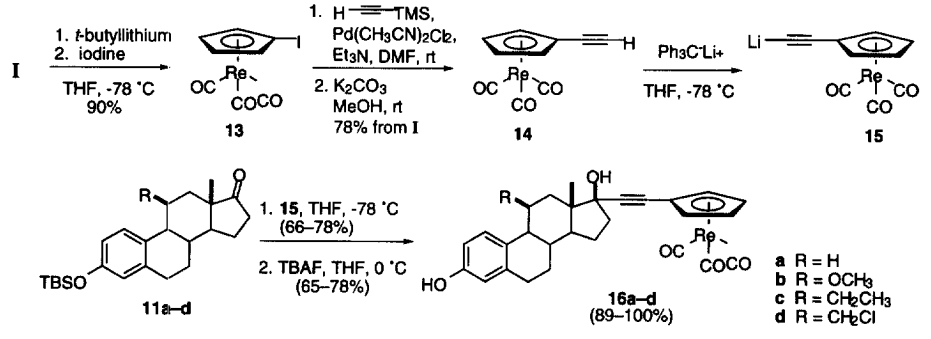
All of the steroidal estrogens were substituted with the $CpRe(CO)_3$ unit at the 17α position, although through various linkages. The first linkage was a direct bond between the steroid and the $CpRe(CO)_3$ group. Brief treatment of $CpRe(CO)_3$ (I) with *t*-butyllithium produces the known anion **10**. Although the reaction of the lithium salt **10** with TBS-estrone **11a** gave mostly deprotonation and aldol condensation products, the corresponding Ce(III) salt[18] gave satisfactory yields of the 17α-$CpRe(CO)_3$ adduct which was deprotected to provide the free estradiol derivative **12** (Scheme 2).

All of the preceeding routes involve a number of manipulations of the $CpRe(CO)_3$ system that would be awkward for the preparation of the corresponding Tc-99m analogs. We have recently described a facile synthesis of *carbonyl*-substituted $CpRe(CO)_3$ systems[15] that is readily adapted to the synthesis of the corresponding technetium-99m labeled system II.[13,14] Therefore, the last set of analogs we investigated were those in which the $CpRe(CO)_3$ system was attached to the 17α position through a *carbonyl* unit.



Scheme 2. Preparation of 17α-$CpRe(CO)_3$-estradiol (**13**).

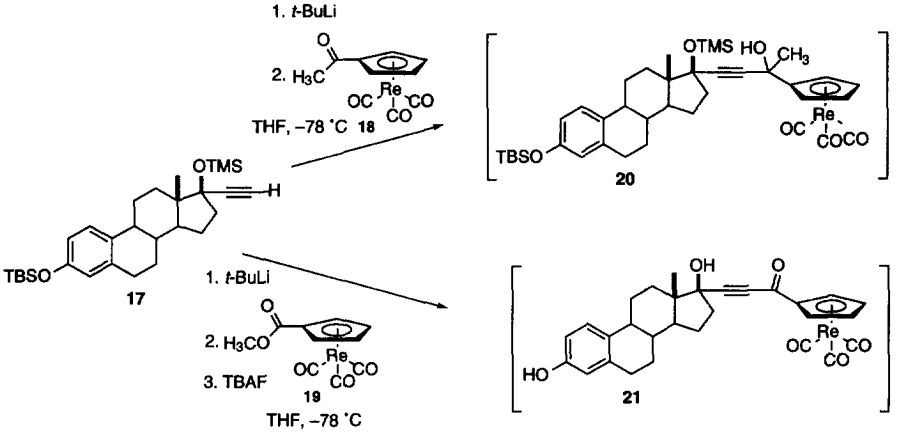
In the next system, the $CpRe(CO)_3$ unit is linked to the 17α position through an ethynyl group. This system had already been investigated to some extent by Jaouen,[12] and one analog (**16d**) was shown to be a high affinity ER ligand.[12] An expeditious route to four of these derivatives is shown in Scheme 3. The TBS-protected



Scheme 3. Preparation of 17α-ethynyl-$Re(CO)_3$ estradiol derivatives.

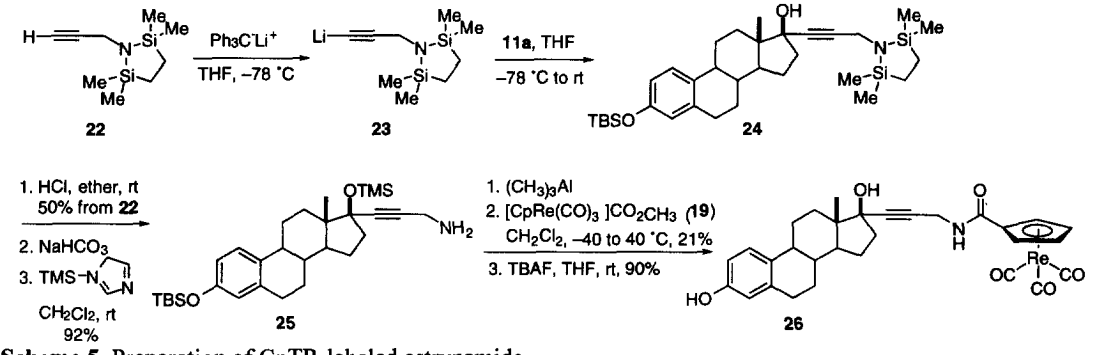
estrone derivatives **11a–d** underwent addition of $CpRe(CO)_3$ ethynyllithium **15**, which was readily prepared from $CpRe(CO)_3$ itself. Subsequent deprotection gave the desired adducts **16a–d**.

Adducts of ethynylestradiol (**17**) and the $CpRe(CO)_3$ ketone (**18**) and ester (**19**) (Scheme 4), namely, compounds **20** and **21**, respectively, proved to be very unstable, and were not judged to be promising candidates for Tc-99m labeling.



Scheme 4. Ethynylestradiol addition to $CpTe(CO)_3$ methyl ketone and methyl ester.

The final $CpRe(CO)_3$ estradiol derivative was the amide **26**. It was prepared by coupling the $CpRe(CO)_3$ ester **19** with an aluminum salt of the 17α-(3-aminopropyn-1-yl)estrogen **25**, which was itself readily prepared from TBS-protected estrone **11a** and the STABASE protected propargyl amine **23** (Scheme 5).



Scheme 5. Preparation of CpTR-labeled estrynamide.

Binding Affinity of $CpRe(CO)_3$-Substituted Estrogens to the Estrogen Receptor

The binding affinity of the $CpRe(CO)_3$-substituted estrogens to the estrogen receptor (ER) was determined in a competitive radiometric binding assay, using [^{3}H]estradiol as tracer and rat uterine cytosol as a source of ER.[19] The binding affinities are expressed as relative binding affinity (RBA) values, where the affinity of estradiol is 100; some assays were performed at both 0 and 25 °C (Table 1).

None of the nonsteroidal $CpRe(CO)_3$ substituted hexestrols or norhexestrols (**6–9**) had substantial affinity for ER. Considering that the $CpRe(CO)_3$ unit is so close to the core of the ligand, this is not surprising. It was of note, however, that threo diastereomers (**7** and **9**) had lower RBA values than the erythro diastereomers (**6** and **8**), reflecting the higher affinity binding of the corresponding diastereomers of the parent ligands.[20]

Table 1. Relative binding affinities (RBA) of estrogen receptor ligands.

	RBA			RBA	
Compound	**0°**	**25°**	**Compound**	**0°**	**25°**
erythro-norhexestrol	229[20]	—	estradiol (E_2)	100	100
6	0.58	—	**12**	0.17	
threo-norhexestrol	2.2[20]	—	**16a**	7.3 (16)[12]	—(15)[12]
7	0.15	—	**26**	0.13	—
			11β-OMe-E_2	9.7[21]	86[21]
meso-hexestrol	300[20]	—	**16b**	53	34
8	0.82	—	11β-Et-E_2	130[21]	1400[21]
dl-hexestrol	3.2[20]	—	**16c**	30	280
9	0.01	—	11β-CH_2Cl-E_2	110*c*	3320[22]
			16d	18 (29)[12]	280 (172)[12]

The steroidal estrogen with the $CpRe(CO)_3$ unit directly bound to the 17α position (**12**) also had very low affinity, but the ethynyl spacer unit, as previously noted by Jaouen, gave estrogen $CpRe(CO)_3$ derivatives with impressively high ER binding affinities (**16a–d**). Again, the highest affinity $CpRe(CO)_3$-substituted analogs (**16c** and **16d**) are derived from the highest affinity parent 11β-ethyl- and chloromethyl-substituted estradiols;[21,22] the high affinity of the 11β-methoxy analog (**16b**) is somewhat unexpected, because the parent steroid has a lower RBA than estradiol.[21] The low affinity of the amide linked $CpRe(CO)_3$ derivative (**26**) was disappointing, but not surprising, as the high polarity of the amide unit is probably introducing polarity in a region of ER where it is poorly tolerated.[23]

The pattern of structure–binding affinity that we have determined for these $CpRe(CO)_3$-substituted estrogens suggests that careful design will be required to obtain analogs that have both high affinity for ER and can be readily prepared as the corresponding [99mTc]-substituted analogs. The high affinity of the ethynyl analogs **16a–d** places a premium on the development of radiosynthetic routes to these compounds.

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