Rhenium Carbonyl Complexes of β-Estradiol Derivatives with High Affinity for the Estradiol Receptor: An Approach to Selective Organometallic Radiopharmaceuticals

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Abstract: The complexes 17α-[(C≡CC_5H_4)$M(CO)_3$]-estradiol, M = Re (**17**) and Mn (**18**), were prepared either by reaction of (LiC≡CC_5H_4)$M(CO)_3$ with estrone or by the Pd-catalyzed coupling reaction of 17α-ethynylestradiol with (C_5H_4I)$Re(CO)_3$. Analogously, 11β-(chloromethyl)-17α-[(C≡CC_5H_4)$Re(CO)_3$]-estradiol (**19**) was prepared from (LiC≡CC_5H_4)$Re(CO)_3$ and 11β-(chloromethyl)estrone. **19** was characterized by X-ray crystallography: space group $P2_12_12_1$ (orthorhombic), a = 8.189(2) Å, b = 15.247(6) Å, c = 20.549(9) Å, V = 2566(2) Å^3, Z = 4. The complexes 17α-[($CH_2C_5H_4$)$M(CO)_3$]-estradiol, M = Re (**22**) and Mn (**23**), were prepared by reaction of (C_5H_4Li)$M(CO)_3$ with spiro[oxirane-2,17β′-estra-1,3,5(10)-trien-3-ol]. The relative binding affinities (RBA's) of these complexes for the estradiol-specific receptor are compared to that of the natural hormone. The low RBA values for **22** (0.8%) and **23** (2.5%) are rationalized on the basis of the flexible character of their bulky 17α substituents; in contrast, the alkynyl derivatives **17** and **18** adopt conformations in which the organometallic fragments lie beneath the steroidal D ring, as in the X-ray structure of **19**, and their relative binding affinities are reasonably good (16% and 15%, respectively). It is proposed that the extraordinarily high RBA for **19** (172% at 25 °C) can be accounted for in terms of an interaction of the 11β-chloromethyl substituent with a Lewis acid proximate to the receptor binding site. Some modeling studies of the dimeric receptor are in agreement with this hypothesis. The lipophilicities of these complexes have been estimated by measuring their partition coefficients between octanol and water, and these $P_{o/w}$ coefficients are related to the degree of nonspecific binding to low affinity sites. The potential utility of isotopically labeled steroidal rhenium complexes both as imaging agents and in radiotherapy is discussed.

Introduction

Thanks to the contributions of molecular biology, we are currently seeing renewed interest in steroid hormones in relation to their specific receptors.[1] This field is particularly important for understanding the phenomena of regulation within the organism, as well as for diagnosis and therapy of certain fairly common cancers. Despite considerable progress in recent years, the mechanism of action of steroidal hormones still remains unexplained at the molecular level. The solution to this problem could well lie in solving the structure of a receptor protein. To date, although improved access to truncated and full length estrogen receptor (ER) expressed in bacteria, in yeast, and in mammalian cells has recently become possible,[2] no complete 3D structure of this family of macromolecules has been published.[3] To begin answering the question of molecular recognition between a hormone and its specific receptor, the only options are either to modify the ligand or to alter the protein by point mutation. In this context, organometallic chemistry can play a role in the alteration of the two association partners.[1,4] It should be noted however that, for reasons of practicality, modification of the steroid by organometallics has been the option most studied.

In particular, it has been shown that complexation of the A ring of estradiol by the $Cr(CO)_3$ group is compatible with good receptor recognition, providing that this moiety is attached on the α face of the bioligand, suggesting that the β face is oriented toward a well-fitted recognition site. It has also been shown that the receptor bonded to the metal carbonyl hormone can be detected by analysis of the M−CO vibrations by Fourier transform infrared spectroscopy.[5]

In addition, modification of the D ring of estradiol by incorporation of organometallic moieties at the 17α position produces a family of affinity markers, **2**−**6**, with the same basic skeleton as the natural hormone.[4] This is compatible with a binding site close to an acid entity (probably a Zn^{2+}) and a

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nucleophilic center (probably cysteine 530). Moreover, it has been shown that estradiol receptors are capable of recognizing metal-complexed steroidal hormones, even when the organometallic moiety possesses considerable steric bulk.[6]

We introduce here a new potential application of organometallic chemistry in the domain of receptology, specifically by synthesis of very stable rhenium derivatives of estradiol with particularly high affinity for the receptor protein. A preliminary communication has already appeared.[7]

Why Rhenium? The use of ^{99m}Tc in nuclear medicine is already well established, and among elements of the same series, the isotopes ^{186}Re and ^{188}Re show promise of increasing use in the development of therapeutic strategies.[8] For a β^--emitting radioelement to be therapeutically useful, a half-life of between 12 h and 5 days is preferred; moreover, for a 1 MeV β^- particle, the depth of penetration into tissue is approximately 5 mm. Furthermore, if some of the disintegrations are accompanied by a 100−300 keV γ photon, the behavior of the radioelement can be conveniently followed by using a γ camera. The nuclear properties of ^{186}Re and ^{188}Re are optimal for these purposes.[9]

In order for the selected molecule to be biomedically useful (in particular for the detection and treatment of hormone-dependent cancers), the rhenium-containing moiety must be firmly attached to the steroid which is used to deliver the radioelement to the estradiol receptor in the nucleus of the target cells. The other crucial requirement is that the hormonal vehicle must retain a high affinity for the specific receptor. Our first attempt to incorporate an organorhenium functionality into the estradiol framework involved the complexation of a series of $Re_2(CO)_7L$ groups, where L = CO, MeCN, pyridine, and PPh_3, onto the alkyne linkage of 17α-ethynylestradiol to give **7**.[10] However, these complexes appeared to be somewhat unstable in the cytosol medium and were not recognized by the estradiol receptor.

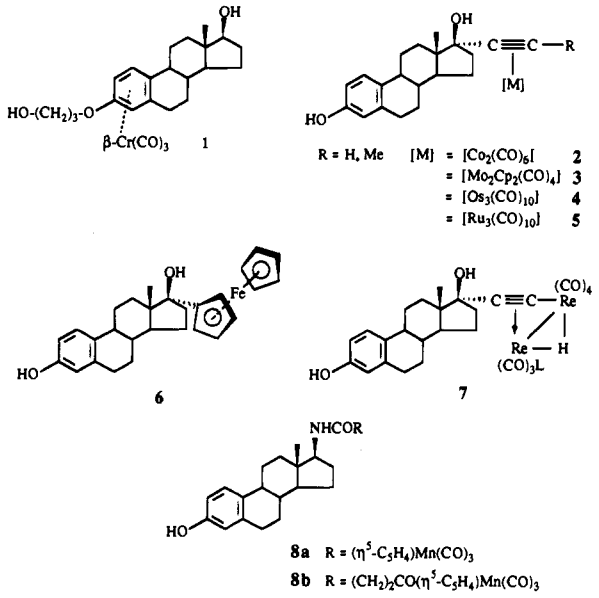
Another interesting development in the area of steroidal−metal complexes has been the search for appropriate technetium-based systems as potential diagnostic imaging agents. Recently, a number of N- and S-coordinated oxo−rhenium chelate complexes have been prepared as models for their Tc analogues; several of these molecules exhibit remarkably high affinity for the progesterone receptor; however, they decompose very rapidly in the biological medium (in some cases, more than 6% per hour).[11]

With the aim of satisfying the above-mentioned criteria, we have prepared a series of Re−carbonyl complexes of estradiol. In these molecules, the organorhenium fragment is firmly bonded at the 17α position, and we now report their syntheses, characterizations, and biochemical activity.

Results and Discussion

Synthetic Aspects. We have previously introduced a variety of organometallic groups into β-estradiol and its derivatives; the aromatic A ring of estrogens proved an excellent site for the attachment of such moieties as $Cr(CO)_3$, $Cr(CO)_2CS$, or $(C_5H_5)Ru^+$, and a number of these molecules have been prepared and characterized.[5,6,12,13] Interestingly, the α- and β-$Cr(CO)_3$ complexes exhibit markedly different relative binding affinities (RBA's) to the estradiol receptor (α, 28%; β, 2%); however, the sensitivity of chromium complexes to sunlight makes them difficult to use in routine biological studies.

A second method of attachment involves attack by a metal-containing nucleophile on a suitable estrone precursor to give, in all cases studied so far, the 17β-OH epimer. This result is obtained whether one first introduces an alkyne (which is subsequently converted into a tetrahedral cluster by treatment with $Co_2(CO)_8$ or $(C_5H_5)_2Mo_2(CO)_4$ to give **2** or **3**)[14] or whether a bulky organometallic nucleophile, such as lithioferrocene, is used.[15]



The (cyclopentadienyl)$M(CO)_3$ (M = Mn, Tc) group has already been incorporated into drugs[16] and into estradiol derivatives;[17] for example, in **8b**, the organomanganese fragment was attached by an alkyl chain to a 17-amido linkage. However, since the absence of the 17β-hydroxyl substituent considerably reduces any effective binding to the receptor site,[18] we chose

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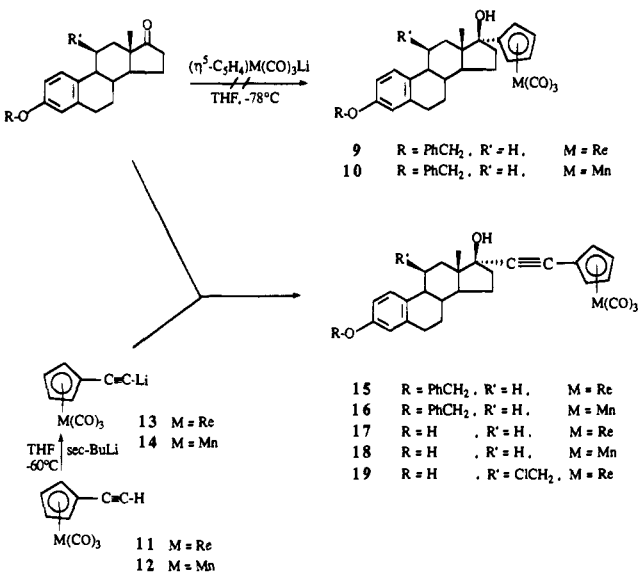
Scheme 1



to retain the 17β-OH linkage and instead bind the organorhenium moiety at the 17α position.

Surprisingly, treatment of 3-(benzyloxy)estrone with $(C_5H_4Li)M(CO)_3$, where M = Mn or Re, did not yield useful quantities of the desired alcohols **9** or **10**. The reasons for this failure are not immediately apparent since the corresponding reaction with $(C_5H_4Li)Fe(C_5H_5)$ proceeded satisfactorily, and the steric requirements for the formation of **6**, **9**, and **10** should not be markedly different. Consequently, we chose to introduce either (a) an ethynyl linkage or (b) a methylene moiety to act as a spacer group between the cyclopentadienyl unit and C(17) of the steroidal D ring. These goals have been accomplished in several ways.

The alkynyl–cyclopentadienyl complexes $(\eta^5\text{-}C_5H_4C{\equiv}CH)M(CO)_3$, where M = Re (**11**) or Mn (**12**), have been previously reported.[19] Lithiation of the ethynyl functionalities gave the corresponding $(\eta^5\text{-}C_5H_4C{\equiv}CLi)M(CO)_3$ reagents, **13** and **14**, which, upon treatment with 3-(benzyloxy)estrone, led to the desired products **15** (Re) and **16** (Mn) in yields of 71% and 65%, respectively. As shown in Scheme 1, removal of the benzyloxy protecting group in **15** (**16**) gave the desired estradiol **17** (**18**) bearing the organometallic fragment. However, it is not mandatory to protect the 3-hydroxy position in the estrone starting material. Instead, one can simply use a 2-fold excess of lithio reagent **13** (or **14**) which first deprotonates the phenol and then undergoes nucleophilic attack at the ketonic site. In order to avoid unnecessary wastage of the $(\eta^5\text{-}C_5H_4C{\equiv}CLi)M(CO)_3$ reagent (**13**) the phenol function can be initially converted into phenolate by treatment with *n*-BuLi. Using this method, **17** and **18** were then obtained directly in 61% and 28% yield, respectively. In the case of 11β-(chloromethyl)estrone, because of the small amount of steroid available, an excess of $(\eta^5\text{-}C_5H_4C{\equiv}CLi)Re(CO)_3$ was used to reduce the estrone; the rhenium complex **19** was obtained in 78% yield. In all cases, only the 17β-hydroxy diastereomer was isolated after chromatographic purification.

Another possible route to the alkynylcyclopentadienyl complexes **15**–**19** involves the cross-coupling reaction of an alkyne with an organic halide in the presence of catalytic amounts of Pd(0) and Cu(I).[20] Although this reaction gives good yields for purely organic compounds, only poor yields (≈20%) have been obtained for the reaction of 17α-ethynylestradiol with $(C_6H_5Cl)Cr(CO)_3$.[21] Indeed, $(C_5H_4I)Re(CO)_3$ or $(C_5H_4I)Mn(CO)_3$ failed to yield coupling products with ethynylestradiol. Another method of surmounting this obstacle involves the use of a stannyl group to activate the alkyne prior to reaction with the halo compound.[19] As shown in Scheme 2, functionalization of the 3-position of 17α-ethynylestradiol with a *tert*-butyldimethylsiloxy protecting group, followed by treatment with Bu_3SnOMe at 90 °C,[22] gave the stannylated ethynylestradiol **20** in 29% yield. In the presence of a catalytic amount of $(MeCN)_2PdCl_2$, the reaction of **20** with $(C_5H_4I)Re(CO)_3$ produces **17** in 33% yield. Overall, the alkynyllithium reagent **13** provides a more efficient route to **17** than does the cross-coupling reaction.

The attachment of a (cyclopentadienyl)$M(CO)_3$ fragment to the estradiol framework via a methylene spacer is readily accomplished via nucleophilic attack on spiro[oxirane-2,17β′-estra-1,3,5(10)-trien-3-ol] (**21**) by using $(C_5H_4Li)M(CO)_3$, where M = Mn or Re, to yield **22** and **23**. The epoxide ring-opening shown in Scheme 3 proceeds in 65–70% yield without the need to protect the phenol function, and only the 17β-OH epimer is isolated after chromatographic separation of the products.[23]

NMR Spectroscopy. The NMR spectra of these steroidal systems were assigned by the usual combination of one- and two-dimensional techniques and are collected in the Experimental Section. There was, however, an unusual observation that the ^{13}C resonances of those carbons at or near the hydroxyl substituents were doubled when the spectrum was recorded in acetone, but not in chloroform, dichloromethane, methanol, toluene, or dimethyl sulfoxide. Thus, in acetone-d_6 solvent, the resonances assignable to C(2), C(3), C(4), C(16), C(17), and C(20) are doubled in **19** and in 17α-ethynyl-3,17β-estradiol; however, when the 3-hydroxy substituent is protected as either a benzyloxy or methoxy group, the C(2), C(3), and C(4) peaks are again singlets but C(16), C(17), and C(20) retain their doublet character. One can perhaps rationalize these observations in terms of hemiketal formation by addition to the solvent. Although the equilibrium for hemiketal formation normally lies heavily in favor of the alcohol and the ketone, the high concentration of acetone (the solvent) perturbs the equilibrium such that the hemiketal is readily detectable. We are unaware of any previous reports of such effects in ketonic solvents and offer the suggestion that it may provide a simple method of measuring the extent of hemiketal formation with alcohols or phenols bearing substituents of differing electronic character; these aspects will be reported elsewhere.

X-ray Crystallography. Since the mutual recognition of a hormone and its receptor site may be crucially dependent upon subtle changes in molecular geometry, we chose to determine the structure of the alkynylcyclopentadienyl rhenium complex **19**, which exhibits an extraordinarily high relative binding affinity for the estradiol receptor site (see below). **19** crystallizes from CH_2Cl_2/pentane in the orthorhombic space group $P2_12_12_1$. Crystallographic data are collected in Table 1, and atom coordinates together with bond lengths and angles are available as supporting information.

The molecular structure (Figure 1) shows clearly that the organorhenium moiety is on the α face of the hormone and

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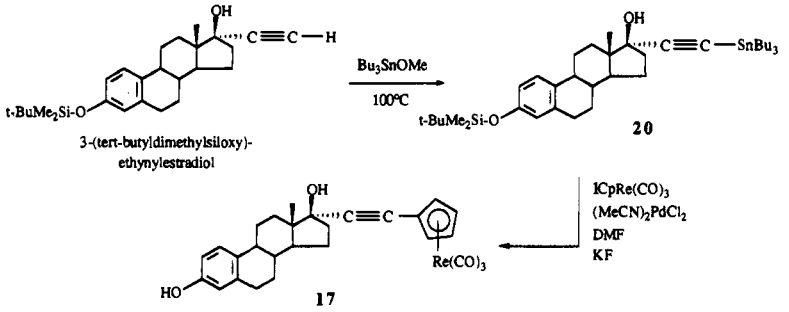
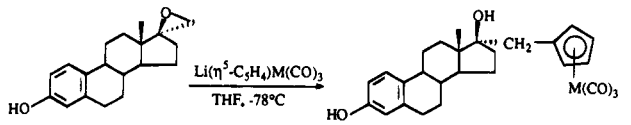
Scheme 2



Scheme 3



21 **22** M = Re; **23** M = Mn

Table 1. Summary of Crystallographic Data for **19**

chem form	$C_{29}H_{28}O_5ClRe$
fw	678.19
cryst syst	orthorhombic
space group	$P2_12_12_1$
Z	4
a, Å	8.189(2)
b, Å	15.247(6)
c, Å	20.549(9)
V, Å^3	2566(2)
F(000)	1336
ϱ(calcd), g cm^{-3}	1.76
μ (Mo Kα), cm^{-1}	49.4
diffractometer	CAD4
monochromator	graphite
radiation	Mo Kα (0.71070)
temp, °C	20
scan type	$\omega/2\theta$
scan range θ, deg	0.8 + 0.34 tan θ
2θ range, deg	3–46
no. of refln collected	2058
no. of refln used (criteria)	1331 ($I > 3\sigma(I)$)
R	0.044
R_w[a]	0.050
absorpn corrn[b]	min 0.86, max 1.34
weighting scheme	unit weights
rms (shift/esd) (last ref)	0.08
l.s. parameters	145

[a] $R_w = [\sum_i W_i(F_o - F_c)^2/\sum_i W_i F_o^2]^{1/2}$. [b] Difabs: Walker, N.; Stuart, D. *Acta Crystallogr.* **1983**, *A39*, 159.

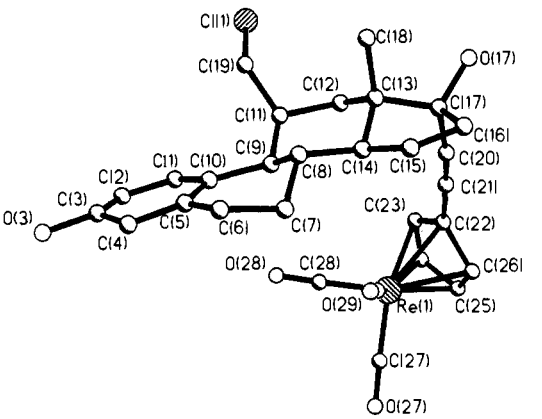


Figure 1. View of the molecular structure of 17α-[(cyclopentadienyl-tricarbonyl rhenium)ethynyl]-11β-(chloromethyl)estra-1,3,5-(10)-triene-3,17β-diol (**19**), showing the atom numbering. Selected bond lengths (Å) and angles (deg): C(17)–C(20), 1.48(3); C(20)–C(21), 1.17(3); C(21)–C(22), 1.44(3); C(22)–C(23), 1.39(3); C(22)–Re(1), 2.30(2); Re(1)–C(27), 1.87(2); C(27)–O(27), 1.18(2); C(11)–C(19), 1.54(3); C(19)–Cl(1), 1.79(2); C(17)–C(20)–C(21), 171.7°(27); C(20)–C(21)–C(22), 176.8°(27); C(11)–C(12)–C(13), 116.0°(19); C(9)–C(11)–C(12), 109.9°(17).

also confirms the presence of the chloromethyl group at the 11β position. With respect to the structure of β-estradiol,[24] the steroidal skeleton of **19** shows some slight deformations. The bulky chloromethyl substituent at the 11β position and the methyl group at C(13) are disposed in a 1,3-diaxial orientation and this results in an opening up of the C(11)–C(12)–C(13) angle (116° versus 110° in β-estradiol). A similar effect has previously been observed in 11β-methoxy-β-estradiol, for which the C(11)–C(12)–C(13) angle is 113°.[25] The widening of this angle is complemented by a narrowing of the C(9)–C(11)–C(12) angle by 3.8° (109.9° versus 113.7° in β-estradiol). The C(13)–C(17)–C(16) angle is almost unchanged (103.5° in **19**; 105.2° in β-estradiol; 104° in 11β-methoxy-β-estradiol); overall, substitution at the 17α position has a rather minimal effect on the conformation of the D ring. It is especially noteworthy that the Re(CO)$_3$ moiety in **19**, at least in the solid state, is positioned proximally, *i.e.*, directly beneath the steroidal C and D rings. This contrasts with the situation for 17α-ferrocenyl-β-estradiol (**6**), in which the organometallic fragment adopts a distal orientation relative to the steroidal framework.[26] Indeed in **19**, molecular modeling studies indicate no steric constraint to rotation of the CpRe(CO)$_3$ unit; in **6**, where there is no alkyne spacer between the steroid and the ferrocenyl group, the proximal rotamer would engender severe steric problems. We observe a clear deformation of the linearity of the alkynyl bonds in **19**; the C(17)–C(20)–C(21) angle is 171.7°, but the C(20)–C(21)–C(22) angle is still almost linear (176.8°).

It is the conventional wisdom that the association of the steroidal hormone with the receptor is mediated by hydrogen-bonding interactions with the hydroxyl substituents at C(3) and C(17). In the solid state, many sterols are held together by a network of intermolecular hydrogen bonds, and this phenomenon is beautifully exemplified by the X-ray crystal structure of β-estradiol hemihydrate.[24] Likewise, as depicted in Figure 2, an intricate pattern of intermolecular hydrogen bonds is evident in the packing diagram for **19**. In the case shown, a 3-hydroxy substituent is hydrogen bonded to two other molecules via their 17-hydroxy functionalities. At the other end of the same molecule, the situation is reversed whereby the 17-OH group interacts with the 3-hydroxy substituents in two other molecules. The O···O distances for these two types of bond are about 2.80 Å.

We have previously reported the X-ray crystal structure of [(estradiol)Ru(C$_5$Me$_5$)]$^+$[CF$_3$SO$_3$]$^-$ in which the 3-OH substitu-

(24) Busetta, B.; Hospital, M. *Acta Crystallogr.* **1972**, *B28*, 560–567.
(25) Courselle, C.; Busetta, B.; Precigoux, G.; Hospital, M. *Acta Crystallogr.* **1973**, *B29*, 2462–2465.
(26) Vichard, D.; Gruselle, M.; Jaouen, G.; Nefedova, M. N.; Mamedyarova, I. A.; Sokolov, V. I.; Vaissermann, J. *J. Organomet. Chem.* **1994**, *484*, 1–8.

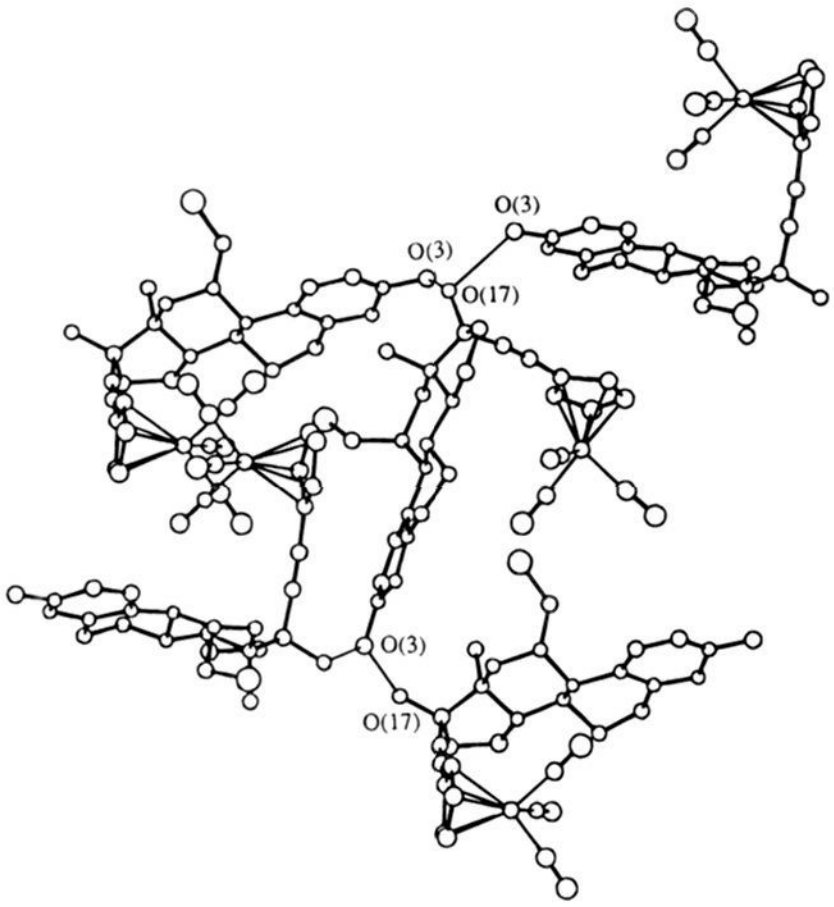


Figure 2. View of the packing of **19** in the solid state showing the intermolecular O—H···O hydrogen bonds which link the monomers.

ent of one cation is hydrogen-bonded to O(17) of its nearest neighbor. Furthermore, this 17-OH group forms another hydrogen bond with an oxygen of the triflate counteranion. Similarly, in our studies on $Cr(CO)_3$ derivatives of hexestrol, the X-ray crystallographic data revealed head-to-tail hydrogen-bonded interactions between the hydroxyl substituents.[27]

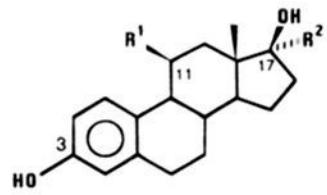
Binding Affinities of Metal-Complexed Hormonal Steroids. A crucial requirement for the potential analytical or therapeutic utility of any of these hormonal organometallics is that the incorporation of the metal carbonyl moiety does not unduly reduce the affinity of the modified hormone for its natural receptor. In Table 2 we list the relative binding affinities measured for a series of organometallic derivatives of estradiol. (RBA values are quoted relative to 3,17β-estradiol, which is taken as 100%.)

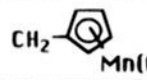
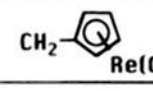
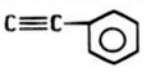
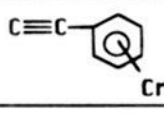
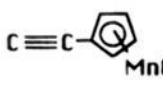
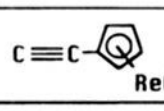
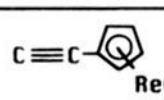
There is a marked difference in behavior between the $CpM(CO)_3$ complexes, M = Re or Mn, depending on the identity of the spacer linking the organometallic fragment to C(17). When the intervening group is ethynyl, as in **17** and **18**, the RBA values are reasonably good, but for the methylene bridged systems **22** and **23**, recognition is very poor. RBA values have been reported for many purely organic 17α-substituted estradiols, including those bearing alkyl, alkenyl, and alkynyl groups of chain lengths varying from 3 to 11 carbons.[23a,b] These data were interpreted in terms of (a) the hydrophobic versus hydrophilic character of the 17α substituent and (b) the steric bulk and conformational flexibility of the 17α chain. Certainly, for molecules **22** and **23**, the freedom to rotate about the C(17)—CH_2 and CH_2—Cp bonds allows the very bulky (cyclopentadienyl)$M(CO)_3$ moiety to sweep out an enormous cone angle. Figure 3 demonstrates clearly the flexible character of these methylene-bridged systems which were modeled by taking the X-ray crystal structure of the alkynyl-bridged complex **19** and replacing the spacer by a CH_2 group.

In Figure 3a the organometallic fragment is oriented away from the steroidal D ring and severely restricts access to the 17-OH functionality. Successive rotations about the CH_2—Cp and C(17)—CH_2 bonds generate the conformations depicted in

(27) Jaouen, G.; Top, S.; Vessières, A.; Sayer, B. G.; Frampton, S. C.; McGlinchey, M. J. *Organometallics* **1992**, *11*, 4061—4068.

Table 2. Relative Binding Affinities of Some Estradiol Derivatives[a]



Compound	R^1	R^2	RBA
estradiol	H	H	100
23	H		2.5
22	H		0.8
24	H		25
25	H		11
26	H		25
27	H		24
18	H		15
17	H		16
19	$ClCH_2$		29

[a] Estrogen receptor binding affinity (RBA) is determined in a competitive radioreceptor binding assay, using lamb uterine cytosol as a source of receptor and [^{3}H]estradiol as a tracer. Bound fractions were measured by protamine sulfate assay as described in ref 6b.

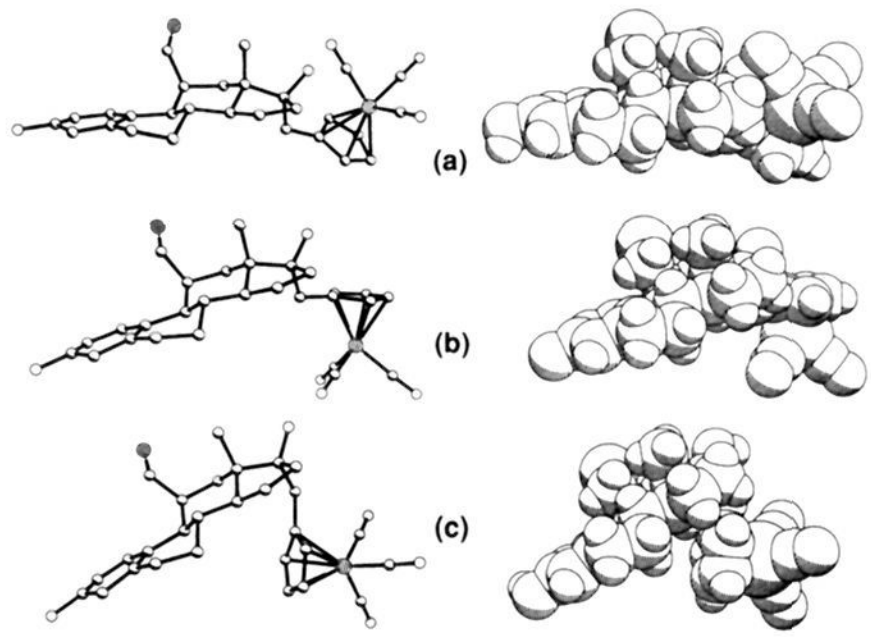


Figure 3. Some conformations of **23** generated in a molecular modeling program by successive rotations about the CH_2—Cp and C(17)—CH_2 bonds.

Figure 3b,c. The steric hindrance attributable to the voluminous (cyclopentadienyl)$Re(CO)_3$ group is evident from the accompanying space-filling models. These molecular modeling studies illustrate that the organometallic units in the methylene complexes **22** and **23** cannot be conveniently "tucked away" below the steroidal D ring. This contrasts markedly with the X-ray crystal structure of the 17α-($C{\equiv}CC_5H_4$)$Re(CO)_3$ substi-

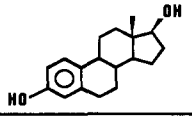
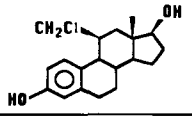
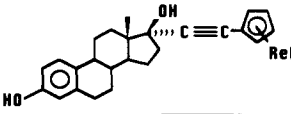
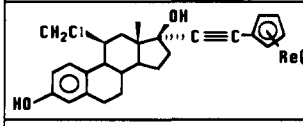
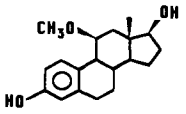
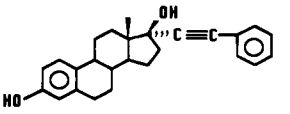
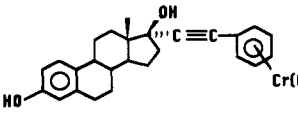
tuted molecule **19**, in which the pendant organometallic fragment is preferentially site directed beneath the steroidal C and D rings, at least in the solid state.

In accord with these data, we note that attachment of a $Cr(CO)_3$ unit onto a 17α-phenyl substituent reduces the RBA from 25% in **24** to 11% in **25**. In contrast, incorporation of an ethynyl spacer between C(17) and the phenyl ring, **26**, leaves the RBA value unchanged after complexation with $Cr(CO)_3$, **27**. Apparently, it is important to restrict the degrees of freedom available to the 17α group.

We turn now to the systems possessing an 11β-chloromethyl substituent: at 0 °C, the free ligand, **28**, has an RBA equal to that of estradiol, but at 25 °C, this increases very dramatically to 280%. Gratifyingly, the same behavior is found for 11β-(chloromethyl)-17α-[(C≡CC_5H_4)$Re(CO)_3$]-estradiol (**19**), where the RBA value increases from 29% at 0 °C, to 172% at 25 °C. *This is the first example of an organometallic derivative of estradiol which possesses a better relative binding affinity for the estradiol receptor site than does the natural hormone.* Katzenellenbogen has pointed out that measurements at the higher temperature more likely reflect the true relative binding affinities, as an equilibrium between the receptor and the ligand has been established more completely.[28] Indeed, the crucial parameter here appears to be the residence time at the receptor site which is on the order of 6−12 h for estradiol itself but which is more than 2 days for 11β-(chloromethyl)estradiol (**28**). Animal studies demonstrated the accumulation of a radioiodinated 11β-(chloromethyl)estradiol derivative up to 48 h postinjection.[29] Similar temperature-dependent behavior has been reported for 11β-methoxyestradiol,[30] even though its RBA value is much lower than 11β-(chloromethyl)estradiol.

Lipophilicities of Metal-Complexed Hormones. It has recently been shown by Katzenellenbogen that there is a good correlation between the in vivo uptake rate of a molecule in fatty tissue and its lipophilicity. Moreover, by altering the lipophilicity of the steroid through synthetic modification, one can change its tissue permeability by allowing more or less of the ligand to enter cells in ER-rich target tissues or nontarget tissues.[31] The lipophilicity is measured by its octanol/water partition coefficient, $P_{o/w}$. Thanks to the method described by Pomper et al.,[32] it is now possible to carry out the measurement by HPLC, thus avoiding the tedious "shake-flask" method of measurement. Tables permitting the calculation of log $P_{o/w}$ values for a given organic molecule do exist, but these tables do not take all parameters into account, and moreover, there are no data available, to our knowledge, for organometallic complexes. We have therefore measured the log $P_{o/w}$ values of a number of hormones, both complexed and noncomplexed, by HPLC (Table 3). All the hormones described in this study are more lipophilic than estradiol itself. We can therefore note here that the addition of a CH_2Cl fragment increases the log $P_{o/w}$ value by 0.25 and that the attachment of an aromatic substituent in position 17α produces an increase of 1.4. In the case of organometallic hormones, the addition of a $Cr(CO)_3$ moiety on an aromatic ring in the 17α position increases the value by 0.32. The value for the $Re(CO)_3$ entity alone cannot be obtained because the corresponding organic hormone bearing a noncomplexed cyclopentadienyl cannot exist. It should be noted however that the log $P_{o/w}$ values of the two rhenium complexes are quite high (5.31 and 5.56, respectively); however, they are noticeably lower than those obtained by Katzenellenbogen for the rhenium chelates of progesterone.[11] The log $P_{o/w}$ values for the latter are all above 6.25, corresponding to an increase of 2.4−2.6 with respect to progesterone, while for organometallic complexes the increase is only 2.1. The attachment of pertinent substituents at the 11β position should decrease the partition coefficient, if needed.

Table 3. Relative Binding Affinities and Partition Coefficients (log $P_{o/w}$) of Some Estradiol Derivatives

Compound	RBA[a]		log$P_{o/w}$[b]
	0°C	25°C	
	100	100	3.30
	100	260	3.55
	16	15	5.31
	29	172	5.56
	9.7	86	2.55
	25		4.65
	24	14	5.03

[a] RBA values were measured as described in Table 2. [b] Octanol−water partition coefficients (log $P_{o/w}$) were determined by HPLC method as described by Pomper et al.[32]

Modeling Studies: Location of Compound 19 in the Postulated Binding Site of the Receptor Dimer. While X-ray crystallographic and NMR studies have provided a reasonable understanding of the DNA binding domain and of the dimerization regions of the glucocorticoids and of the estrogens, the hormone binding domain (HBD) is less reliably characterized.[33] Several molecular modeling studies of the hormone binding domain of ER have appeared, but all yield different descriptions of the site of association.[34] However, it is known that, upon attachment of the hormone, a *dimeric* form of the receptor

(28) (a) Bindal, R. D.; Carlson, K. E.; Reiner, G. C.; Katzenellenbogen, J. A. J. *J. Steroid Biochem.* **1987**, *28*, 361−370. (b) VanBrocklin, H. F.; Carlson, K. E.; Katzenellenbogen, J. A.; Welch, M. J. *J. Med. Chem.* **1993**, *36*, 1619−1629.

(29) Zeicher, M.; Delcorde, A.; Quivy, J.; Dupuis, Y.; Verbist, A.; Frühling, J. *Nucl. Med. Biol.* **1995**, in press.

(30) Ojasoo, T.; Raynaud, J. P. *Cancer. Res.* **1978**, *38*, 4186−4198.

(31) VanBrocklin, H. F.; Liu, A.; Welch, M. J.; O'Neil, J. P.; Katzenellenbogen, J. A. *Steroids* **1994**, *59*, 34−45.

(32) Pomper, M. G.; VanBrocklin, H.; Thieme, A. M.; Thomas, R. D.; Kiesewetter, D. O.; Carlson, K. E.; Mathias, C. J.; Welch, M. J.; Katzenellenbogen, J. A. *J. Med. Chem.* **1990**, *33*, 3143−3155.

(33) (a) Lee, M. S.; Kliewer, S. A.; Provencal, J.; Wright, P. E.; Evans, R. M. *Science* **1993**, *260*, 1117−1120. (b) Luisi, B. F.; Xu, W. X.; Otwinowski, Z.; Freedman, L. P.; Yamamoto, K. R.; Sigler, P. B. *Nature* **1991**, *352*, 497−505. (c) Schwabe, J. W. R.; Chapman, L.; Finch, J. T.; Rhodes, D. *Cell* **1993**, *75*, 567−578.

(34) (a) Lewis, D. F. V.; Lake, B. G. *Xenobiotica* **1993**, *23*, 79−96. (b) Goldstein, R. A.; Katzenellenbogen, J. A.; Luthey-Schulten, Z. A.; Seielstad, D. A.; Wolynes, P. G. *Proc. Natl. Acad. Sci. U.S.A.*, **1993**, *90*, 9949−9953. (c) Gaboriaud, C.; Bissery, V.; Benchetrit, T.; Mornon, J. P. *FEBS Lett.* **1987**, *224*, 149−155. (d) Lemesle-Varloot, L.; Henrissat, B.; Gaboriaud, C.; Bissery, V.; Morgat, A.; Mornon, J. P. *Biochimie* **1990**, *72*, 555−574. (e) Höltje, H. D.; Dall, N. *Pharmazie* **1993**, *48*, 243−249.

Figure 4. Stereoview of the binding site of the estradiol ligand in the dimeric form of the estradiol receptor model (E_2R) such as deduced from the SERPIN hypothesis.[3] The hypothetical zinc atom (not shown) would be localized near Cys 530 and Cys 381 of the lower monomer on the left hand side and near His 488 of the upper monomer on the right hand side of the diagram.

becomes associated with DNA and triggers the transcriptional machinery. Currently, only a single model of this dimer—the true active complex—is available,[3] and we offer here a preliminary study of the behavior of **19** with respect to this dimer model.

The 3D structure proposed by Mornon and Thoreau for the hormone binding domain of the nuclear hormone receptors (NHR) is based on homology modeling starting from the serine protease inhibitors (SERPINS). In particular, for the hormone binding domain of human ER, this model takes account of the following experimental data: firstly, the affinity labeling of Cys 530 and of Cys 381 by the aziridines of 4-hydroxytamoxifen and of ketononestrol when docked in the estradiol binding site,[35] secondly, the dimerization related to ligand binding,[36] and thirdly, a possible Zn binding site.[4]

Figure 4 depicts the environment of the postulated hormone binding site with 11β-(chloromethyl)-17α-(C≡CC_6H_5)-estradiol docked in it. The 17α-(C≡CC_6H_5) derivative was used instead of the 17α-(C≡CC_5H_4)Re(CO)$_3$ complex to allow molecular mechanics calculations; this is justifiable in terms of the X-ray crystal structure of the rhenium complex **19**, which shows the Re(CO)$_3$ moiety to be tucked under the steroidal ring in an *endo* fashion.

Figure 4 shows only one of the two steroids located in the model dimer; the 17α-(C≡CC_6H_5) substituent is directed toward the interface between the monomers, and this interfacial localization of the bulky side chain may explain how ER can accommodate such a long molecular fragment. Most interestingly, the 11β-chloromethyl moiety is directed toward Cys 530 and Cys 381, suggesting that they could all participate in coordination to a metal, probably zinc.

The distances shown in Figure 4 are slightly too long relative to those anticipated for such a metal binding site, and we emphasize the qualitative nature of the model. It is noteworthy that no Zn^{2+} was introduced into the model, so the structure obtained takes no account of the coordination capacity of such an atom which could bring about the necessary small local adjustments. We note that the presence of Zn^{2+} in the hormone binding domain of the estradiol receptor has already been demonstrated by use of ^{65}Zn; however, its precise location has not yet been established.[37]

It has been suggested that the interaction between the dimer of the 90 kDa heat shock protein (hsp 90) and the monomer of ER involves the simultaneous occurrence of leucine zipper-like pairing and the formation of cysteine- and histidine-mediated metallic bonds between these species.[38] Moreover, cysteine 530, which has lysine neighbors at positions 529 and 531, may, like cysteine 381, be implicated in the metal-mediated dimerization process. The ability of proximal cysteines and lysines to create N—H···S hydrogen bonds has been shown to be an important factor in the structure and stability of metal-bonded sites in proteins.[39] The hydrogen bonding to sulfur is thought to decrease the metal—sulfur pπ—dπ antibonding interaction, thereby stabilizing the metal—thiolate complex.

As envisioned in this scenario, the receptor dimerization process would cause *both* hydrophobic van der Waals-type interactions with leucine zipper-like heptads *and* interactions of zinc cations with cysteine and histidine residues in the two monomers *at the same time*. Cysteines 381 and 530, which lie close to the hormone binding site at the interface of the two monomers, are good candidates for the establishment of a link to the metal, although they are insufficient in themselves to control dimerization. The study of the coordination chemistry of receptors will undoubtedly lead to further clarification of these

(35) (a) Katzenellenbogen, B. S.; Fang, F.; Ince, B. A.; Pakdel, F.; Wooge, C. H.; Wrenn, C. K. *Breast Cancer Res. Treat.* **1993**, *27*, 17–26. (b) Reese, J. C.; Katzenellenbogen, B. S. *J. Biol. Chem.* **1991**, *266*, 10880–10887. (c) Reese, J. C.; Wooge, C. H.; Katzenellenbogen, B. S. *Mol. Endocrinol.* **1992**, *6*, 2160–2166. (d) Harlow, K. W.; Smith, D. N.; Katzenellenbogen, J. A.; Greene, G. L.; Katzenellenbogen, B. S. *J. Biol. Chem.* **1989**, *264*, 17476–17485.

(36) Green, S. *Nature* **1993**, *361*, 590–591.

(37) Humeny, A.; Thole, H. H. *Biochem. Biophys. Res. Commun.* **1993**, *194*, 1248–1255.

(38) Schwartz, J. A.; Mizukami, H. *Med. Hypotheses* **1991**, *35*, 140–145.

(39) Chung, W. P.; Dewan, J. C.; Walters, M. A. *J. Am. Chem. Soc.* **1991**, *113*, 525–530.

processes in the future; this may involve replacement of Zn^{2+} by Cu^{2+}, mutation of the coordination potential in the protein (such as at His 488), or modification of the ligand.

Concluding Remarks

These RBA and $P_{o/w}$ data for organometallic complexes of estradiol should be placed in the perspective of recent reports concerning the mode of interaction between estradiol and its specific receptor site. While this remains one of the important unsolved problems in molecular biology, there are intimations of conceptual breakthroughs in the near future. Evidence is emerging that there are nucleophilic sulfur residues of a coordination unit involving an acidic metal (presumably Zn^{2+}) and presumably cysteines 530 and 381 (human estrogen receptor numbering)[3,35] in close vicinity to the estradiol binding site. In this context, it is particularly noteworthy that those estradiol derivatives which exhibit enhanced RBA values at 25 °C normally bear an 11β functionality which includes an atom possessing one or more nonbonding electron pairs. These include CH_2Cl, OMe, or $C_6H_4NMe_2$ (which is the 11β substituent in RU 486, the controversial abortifaciant now available in several countries.) One is tempted to associate this enhanced activity with the ability to coordinate an electron pair to the Lewis acid, thus adding an extra point of attachment between the hormone and its specific receptor. However, one must note that 11β-ethyl-16β-fluoroestradiol has also been reported to have a remarkably high RBA value,[28b] so clearly other factors can come into play. Interactions with hydrophobic aromatic amino acids have also been proposed as an alternative model.[40]

In terms of the practical development of 11β-(chloromethyl)-17α-[($C{\equiv}CC_5H_4$)$Re(CO)_3$]-estradiol (**19**), which has an excellent RBA value but also a slightly high $P_{o/w}$ coefficient, one must seek ways to lower the lipophilicity of the organometallic fragment without reducing the RBA and the specific binding index. Finally, it will be necessary to develop a synthetic route which allows the rapid incorporation of a radioisotope of rhenium. We note that $(C_5H_5)Re(CO)_3$ can be obtained easily from $Re_2(CO)_{10}$, which is itself readily prepared from $^{186}ReO_4^-$ [41] or Re_2O_7.[42] It would appear that the cross-coupling route to **19** (or some suitably modified analogue) will need to be improved so as to avoid the relatively time-consuming preparation of (η^5-$C_5H_4C{\equiv}CH$)$Re(CO)_3$.[43] It is also evident that these rhenium complexes are excellent models for their technetium analogues.[44] Future manuscripts will describe our progress toward these objectives. Indeed, Wenzel and Klinge have very recently reported the technetium analogue of our rhenium complex **17**.[45]

Experimental Section

General Procedures. All reactions were performed under a dry argon atmosphere using standard Schlenk techniques. $Re_2(CO)_{10}$ was purchased from Strem Co.; other reagents and solvents were obtained from Aldrich Chemical Co. and Janssen Chemical Co. Solvents were purified by conventional distillation techniques under argon. IR spectra were recorded on a Bomem Michelson 100 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AM-250 and AM-200 spectrometers. Mass spectra were obtained by the Service de Spectrometrie de Masse of the E.N.S.C.P., Paris, and C.N.R.S., Vernaison, France. 11β-(Chloromethyl)estrone and spiro[oxirane-2,-17β'-estra-1,3,5(10)-trien-3-ol] were provided by Medgenix S. A. (η^5-C_5H_4I)$Re(CO)_3$, (η^5-C_5H_4I)$Mn(CO)_3$, (η^5-$C_5H_4C{\equiv}CH$)$Re(CO)_3$, and (η^5-$C_5H_4C{\equiv}CH$)$Mn(CO)_3$ were prepared according to the literature method.[19]

17α-((Tributylstannyl)ethynyl)estra-1,3,5(10)-triene-3,17β-diol (20). A mixture of 3-(*tert*-butyldimethylsiloxy)-17α-ethynylestradiol (0.410 g, 1.00 mmol) and Bu_3SnOMe (0.480 g, 1.50 mmol) was heated for 15 h at 90 °C with concomitant elimination of methanol. The crude product obtained was purified by chromatography on silica gel plates using ether/pentane (1:5) as the eluent to give **20** (0.230 g, 0.39 mmol; 39%). ^1H NMR (200 MHz, $CDCl_3$): δ 7.15 (d, 1H, J = 8.4 Hz, H-1), 6.63 (dd, 2H, J = 8.4 Hz, 2.6 Hz, H-2), 6.56 (d, 1H, J = 2.6 Hz, H-4), 2.81 (m, 2H, H-6α, H-6β), 0.99 (s, 9H, *t*-Bu), 0.91 (s, 3H, Me-13), 0.20 (s, 6H, Me_2).

17α-[(Cyclopentadienyltricarbonylrhenio)ethynyl]-3-benzyloxy-17β-hydroxyestra-1,3,5(10)-triene (15). *sec*-BuLi (0.61 mL of a 1.3 M solution in hexane; 0.79 mmol) was added to a solution of (η^5-$C_5H_4C{\equiv}CH$)$Re(CO)_3$ (0.145 g, 0.40 mmol) in THF (3 mL) at −70 °C. After stirring for 30 min, a solution of 3-(benzyloxy)estrone (0.252 g, 0.70 mmol) in THF (4 mL) was slowly added to the solution of the phenolate anion which was maintained at −70 °C. The stirring was continued overnight, during which time the temperature was allowed to rise slowly to room temperature. After hydrolysis with ice water, ether extraction, and solvent removal, the residue was chromatographed on silica gel plates using ether/pentane (1:2) as the eluent. The first fraction to elute was unreacted (η^5-$C_5H_4C{\equiv}CH$)$Re(CO)_3$ (0.010 g). The second fraction yielded **15** (0.205 g, 0.28 mmol; 74%), mp 80 °C, as a beige solid. The mass spectrum (EI, 70 eV, Re = 187) showed peaks at *m/z* 720 $[M]^+$, 692 $[M - CO]^+$, 636 $[M - 3CO]^+$. IR (CH_2Cl_2): ν_{CO} at 2025 (s), 1930 cm^{-1} (s). ^1H NMR (250 MHz, $CDCl_3$): δ 7.39 (m, 5 H, Ph), 7.23 (d, 1 H, J = 8.5 Hz, H-1), 6.80 (dd, 1 H, J = 8.5 Hz, 2.7 Hz, H-2), 6.73 (d, 1 H, J = 2.7 Hz, H-4), 5.61 (t, 2 H, J = 2.2 Hz, (η^5-C_5H_4)), 5.30 (t, 2H, J = 2.2 Hz, (η^5-C_5H_4)), 5.04 (s, 2 H, CH_2-Ph), 2.85 (m, 2 H, H-6), 0.91 (s, 3 H, Me-13). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 193.00 (s, 3 CO), 156.47 (C-3), 137.74 (C-5), 137.07 (C_{ip}, Ph), 132.56 (C-10), 128.27 (C_m, Ph), 127.17 (C_p, Ph), 127.56 (C_o, Ph), 126.11 (C-1), 114.56 (C-4), 112.04 (C-2), 79.92 (C-17), 87.25, 87.20, 83.86, 83.81 (4C, η^5-C_5H_4), 85.57 (C-1' of (η^5-C_5H_4)), 92.88, 76.68 (C≡C), 69.69 (CH_2Ph), 49.45 (C-14), 47.55 (C-13), 43.15 (C-9), 39.14 (C-8), 38.64 (C-16), 32.72 (C-12), 29.52 (C-6), 26.86 (C-7), 26.11 (C-11), 22.60 (C-15), 12.54 (Me-13). Anal. Calcd for $C_{35}H_{33}O_5Re$: C, 58.39; H, 4.62. Found: C, 58.65; H, 4.65.

17α-[(Cyclopentadienyltricarbonylmanganio)ethynyl]-3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene (16). *sec*-BuLi (0.61 mL of a 1.3 M solution in hexane; 0.80 mmol) was added to a solution of (η^5-$C_5H_4C{\equiv}CH$)$Mn(CO)_3$ (0.182 g, 0.79 mmol) in THF (3 mL) at −70 °C. After stirring for 30 min, a solution of 3-(benzyloxy)estrone (0.180 g, 0.50 mmol) in THF (4 mL) was slowly added to the solution of the phenolate anion which was maintained at −70 °C. The stirring was continued overnight, during which time the temperature was allowed to rise slowly to room temperature. After hydrolysis with ice water, ether extraction, and solvent removal, the residue was chromatographed on silica gel plates using ether/pentane (1:4) as the eluent to give **16** (0.183 g, 0.32 mmol; 65%), mp 70 °C, as a beige solid. The mass spectrum (DCI, 70 eV) showed peaks at *m/z* 606 $[M + NH_4]^+$, 588 $[M + NH_4 - H_2O]^+$, 571 $[M + H - H_2O]^+$. IR (CH_2Cl_2): ν_{CO} at 2024 (s), 1940 cm^{-1} (s). ^1H NMR (250 MHz, $CDCl_3$): δ 7.39 (m, 5 H, Ph), 7.23 (d, 1 H, J = 8.4 Hz, H-1), 6.80 (dd, 1 H, J = 8.4 Hz, 2.6 Hz, H-2), 6.73 (d, 1 H, J = 2.6 Hz, H-4), 5.00 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 4.70 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 5.04 (s, 2 H, CH_2Ph), 2.85 (m, 2 H, H-6), 0.91 (s, 3 H, Me-13). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ224.22 (s, 3 CO), 156.70 (C-3), 137.98 (C-5), 137.31 (C_{ip}, Ph), 132.82 (C-10), 128.50 (C_m, Ph), 127.80 (C_p, Ph), 127.41 (C_o, Ph), 126.34 (C-1), 114.80 (C-4), 112.26 (C-2), 80.22 (C-17), 86.23, 86.16, 82.12, 82.02 (4C, η^5-C_5H_4), 82.36 (C-1' of η^5-C_5H_4), 92.63, 78.20 (C≡C), 69.92 (CH_2Ph), 49.63 (C-14), 47.66 (C-13), 43.41 (C-9), 39.38 (C-8), 38.91 (C-16), 32.93 (C-12), 29.76 (C-6), 27.14 (C-7), 26.35 (C-11), 22.82 (C-15), 12.76 (Me-13). Anal. Calcd for $C_{35}H_{33}O_5Mn$: C, 71.42; H, 5.65. Found: C, 71.77; H, 5.91.

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17α-[(Cyclopentadienyltricarbonylrhenio)ethynyl]estra-1,3,5(10)-triene-3,17β-diol (17). (a) From Estrone and an Alkynyllithium. A solution of (η^5-$C_5H_4C{\equiv}CLi$)$Re(CO)_3$ was prepared as described above from (η^5-$C_5H_4C{\equiv}CH$)$Re(CO)_3$ (0.216 g, 0.60 mmol) and *sec*-BuLi (1 mmol) at −50 °C. In a second Schlenk tube, estrone (0.270 g, 1.00 mmol) was dissolved in THF (10 mL), cooled to −50 °C, and treated with *sec*-BuLi (1.00 mmol). The phenolate solution then slowly added to the alkynyllithium solution which was maintained at −60 °C. After hydrolysis with ice water, ether extraction, and solvent removal, the residue was purified by thin layer chromatography on silica gel plates using ether/pentane (1:1) as the eluent to give **17** (0.225 g, 0.36 mmol; 61%), mp 161 °C, as a colorless solid. The mass spectrum (EI, 70 eV) showed peaks at *m/z* 630 $[M]^+$, 612 $[M - H_2O]^+$, 602 $[M - CO]^+$, 546 $[M - 3CO]^+$. IR (CH_2Cl_2): ν_{CO} at 2025 (s), 1930 cm^{-1} (s). ^1H NMR (250 MHz, CD_2Cl_2): δ 7.14 (d, 1 H, J = 8.4 Hz, H-1), 6.61 (dd, 1 H, J = 8.4 Hz, 2.1 Hz, H-2), 6.55 (d, 1 H, J = 2.1 Hz, H-4), 5.71 (s, 1 H, OH-3), 5.63 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 5.32 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 2.78 (m, 2 H, H-6), 0.89 (s, 3 H, Me-13). ^{13}C NMR (62.9 MHz, CD_2Cl_2): δ 193.29 (CO), 153.23 (C-3), 137.92 (C-5), 132.07 (C-10), 126.06 (C-1), 114.80 (C-4), 112.27 (C-2), 79.84 (C-17), 87.52, 87.45, 84.04, 83.95 (4 C, η^5-C_5H_4), 85.49 (C-1′ of η^5-C_5H_4), 92.71, 76.54 (C≡C), 49.31 (C-14), 47.46 (C-13), 43.07 (C-9), 39.12 (C-8), 38.52 (C-16), 32.64 (C-12), 29.24 (C-6), 26.75 (C-7), 26.11 (C-11), 22.42 (C-15), 12.31 (Me-13). Anal. Calcd for $C_{28}H_{27}O_5Re$: C, 53.40; H, 4.32. Found: C, 54.17; H, 4.32.

(b) By Palladium-Catalyzed Coupling. 20 (0.171 g, 0.39 mmol), (η^5-C_5H_4I)$Re(CO)_3$ (0.139 g, 0.30 mmol), and $(MeCN)_2PdCl_2$ (0.016 g, 0.06 mmol) were dissolved in dimethylformamide (6 mL), and the solution was stirred overnight at room temperature. Diethyl ether (10 mL) and a aqueous solution of KF 50% (5 mL) were added, and the stirring was maintained for 2 h. After hydrolysis with water, ether extraction, and solvent removal, the residue was chromatographed on silica gel plates using ether/pentane (1:1) as the eluent to give **17** (0.070 g, 0.11 mmol; 33%) as a colorless solid.

17α-[(Cyclopentadienyltricarbonylmanganio)ethynyl]estra-1,3,5(10)-triene-3,17β-diol (18). By following the same procedure as for **17**, the manganese complex **18** was prepared from (η^5-$C_5H_4C{\equiv}CH$)$Mn(CO)_3$ (0.230 g, 1.00 mmol), *sec*-BuLi (1.00 mmol, 0.77 mL), and estrone (0.270 g, 1.00 mmol). After the workup, the residue was chromatographed on silica gel plates using acetone/pentane (1:3) as the eluent to yield **18** (0.140 g, 0.28 mmol; 28% yield), mp 218 °C as a beige solid. The mass spectrum (DCI, 70 eV) showed peaks at *m/z* 516 $[M + NH_4]^+$, 498 $[M + NH_4 - H_2O]^+$, 481 $[M + H - H_2O]^+$. IR (CH_2Cl_2): ν_{CO} at 2025 (s), 1940 cm^{-1} (s). ^1H NMR (250 MHz, CD_2Cl_2): δ 7.14 (d, 1 H, J = 8.2 Hz, H-1), 6.59 (dd, 1 H, J = 8.2 Hz, 1.9 Hz, H-2), 6.54 (d, 1 H, J = 1.9 Hz, H-4), 5.03 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 4.87 (s, 1 H, OH-3), 4.73 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 2.80 (m, 2 H, H-6), 0.88 (s, 3 H, Me-13). ^{13}C NMR (62.9 MHz, CD_2Cl_2): δ 224.17 (CO), 153.07 (C-3), 138.02 (C-5), 132.31 (C-10), 126.11 (C-1), 114.72 (C-4), 112.31 (C-2), 79.85 (C-17), 86.20, 86.11, 82.05, 81.92 (4 C, η^5-C_5H_4), 82.00 (C-1′ of η^5-C_5H_4), 92.32, 77.76 (C≡C), 49.31 (C-14), 47.32 (C-13), 43.12 (C-9), 39.13 (C-8), 38.62 (C-16), 32.64 (C-12), 29.25 (C-6), 26.80 (C-7), 26.14 (C-11), 22.43 (C-15), 12.26 (Me-13). Anal. Calcd for $C_{28}H_{27}O_5Mn$: C, 67.47; H, 5.46. Found: C, 68.11; H, 5.66.

17α-[(Cyclopentadienyltricarbonylrhenio)ethynyl]-11β-(chloromethyl)estra-1,3,5(10)-triene-3,17β-diol (19). As for **17**, a solution of (η^5-$C_5H_4C{\equiv}CLi$)$Re(CO)_3$ was prepared from (η^5-$C_5H_4C{\equiv}CH$)$Re(CO)_3$ (0.216 g, 0.60 mmol) and *sec*-BuLi (0.70 mmol, 0.54 mL of 1.3 M solution in hexane) at −60 °C. After being stirred for 20 min, a solution of 11β-(chloromethyl)estrone (0.064 g, 0.20 mmol) in THF (10 mL) was slowly added to the alkynyllithium solution maintained at −60 °C. The stirring was continued overnight, during which time the temperature was allowed to rise slowly to room temperature. After addition of THF (20 mL) followed by water (0.5 mL), the solution was filtered over silica gel and evaporated to dryness. The residue was purified by thin layer chromatography on silica gel plates using THF/pentane (1:3) as the eluent to give **19** (0.105 g, 0.15 mmol; 78%), mp 219 °C, as a colorless solid. (η^5-$C_5H_4C{\equiv}CH$)$Re(CO)_3$ (0.145 g) and 11β-(chloromethyl)estrone (0.007 g) were also recovered. The mass spectrum of **19** (EI, 70 eV) showed peaks at *m/z* 678 $[M]^+$ and 650 $[M - CO]^+$. IR (CH_2Cl_2): ν_{CO} at 2025 (s), 1930 cm^{-1} (s). ^1H NMR (250 MHz, CD_2Cl_2): δ 7.09 (d, 1 H, J = 8.5 Hz, H-1), 6.65 (dd, 1 H, J = 8.5 Hz, 2.7 Hz, H-2), 6.54 (d, 1 H, J = 2.7 Hz, H-4), 5.66 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 5.34 (t, 2 H, J = 2.2 Hz, η^5-C_5H_4), 4.97 (s, 1 H, OH-3), 3.53 (m, 2 H, CH_2Cl), 2.72 (m, 2 H, H-6), 1.04 (s, 3 H, Me-13). ^{13}C NMR (62.9 MHz, CD_2Cl_2): δ 193.98 (CO), 153.86 (C-3), 139.70 (C-5), 128.76 (C-10), 127.73 (C-1), 115.84 (C-4), 113.79 (C-2), 77.88 (C-17), 88.39, 88.32, 84.76 (4 C, η^5-C_5H_4), 85.84 (C-1′ of η^5-C_5H_4), 93.33, 75.69 (C≡C), 51.77 ($ClCH_2$), 48.55 (C-13), 48.18 (C-14), 46.48 (C-9), 39.32 (C-11 and C-16), 35.75 (C-8), 33.40 (C-12), 30.35 (C-6), 27.17 (C-7), 23.05 (C-15), 16.16 (Me-13). Anal. Calcd for $C_{29}H_{28}O_5ClRe$: C, 51.35; H, 4.16. Found: C, 51.45; H, 4.23.

17α-[(Cyclopentadienyltricarbonylrhenio)methyl]estra-1,3,5(10)-triene-3,17β-diol (22). $CpRe(CO)_3$ (0.177 g, 0.53 mmol) in THF (5 mL) was cooled to −78 °C and treated with *n*-BuLi (0.33 mL of 1.6 M solution in hexane; 0.53 mmol). After being stirred for 1 h, a solution of spiro[oxirane-2,17β′-estra-1,3,5(10)-trien-3-ol] (**21**) (0.050 g, 0.18 mmol) in THF (2 mL) was slowly added to the organo-lithium solution maintained at −78 °C. The stirring was continued for 4 h, during which time the temperature was allowed to rise slowly to room temperature. After hydrolysis with ice water, ether extraction, and solvent removal, the residue was chromatographed on silica gel plates using ether/pentane (4:6) as the eluent to give **22** (0.081 g, 0.13 mmol; 65%), mp 120 °C, as a colorless solid. The mass spectrum (DCI, 70 eV) showed peaks at *m/z* 638 $[M + NH_4]^+$, 621 $[M + NH_4 - H_2O]^+$ and 603 $[M + H - H_2O]^+$. IR (CH_2Cl_2): ν_{CO} at 2020 (s), 1921 cm^{-1} (s). ^1H NMR (250 MHz, CD_3CN): δ 7.10 (d, 1 H, J = 8.6 Hz, H-1), 6.53 (dd, 1 H, J = 8.6 Hz, 2.5 Hz, H-2), 6.47 (d, 1 H, J = 2.5 Hz, H-4), 5.56 (m, 2 H, (η^5-C_5H_4)), 5.45 (m, 2 H, (η^5-C_5H_4)), 2.77 (m, 2 H, H-6), 2.63 and 2.48 (dd, J = 13.8 Hz, CH_2Ph), 0.94 (s, 3 H, Me-13). ^{13}C NMR (62.9 MHz, CD_3CN): δ 196.54 (CO), 155.54 (C-3), 139.04 (C-5), 132.73 (C-10), 127.32 (C-1), 115.99 (C-4), 113.55 (C-2), 109.16 (C-17), 87.62, 87.54, 86.10, 84.32 (4 C, η^5-C_5H_4), 84.16 (C-1′ of η^5-C_5H_4), 50.19 (C-14), 47.74 (C-13), 44.58 (C-9), 40.79 (C-8), 37.06, 33.76, 32.16, (C-12, C-16, $CH_2(\eta^5$-$C_5H_4)$), 30.29 (C-6), 28.27 (C-7), 27.21 (C-11), 23.88 (C-15), 15.16 (Me-13). Anal. Calcd for $C_{27}H_{29}O_5Re$: C, 52.56; H, 4.69. Found: C, 51.79; H, 4.83.

17α-[(Cyclopentadienyltricarbonylmanganio)methyl]estra-1,3,5(10)-triene-3,17β-diol (23). As for **22**, $CpMn(CO)_3$, *n*-BuLi and spiro[oxirane-2,17β′-estra-1,3,5(10)-trien-3-ol] (**21**) gave **23** in 70% yield, mp 160 °C. The mass spectrum (CI, 70 eV) showed peaks at *m/z* 506 $[M + NH_4]^+$ and 472 $[M + H - H_2O]^+$. IR (CH_2Cl_2): ν_{CO} at 2018 (s), 1930 cm^{-1} (s). ^1H NMR (250 MHz, CD_2Cl_2): δ 7.16 (d, 1 H, J = 8.4 Hz, H-1), 6.64 (dd, 1 H, J = 8.4 Hz, 2.6 Hz, H-2), 6.57 (d, 1 H, J = 2.6 Hz, H-4), 4.87 (s, 1 H, OH-3), 4.71 (m, 4 H, η^5-C_5H_4), 2.83 (m, 2 H, H-6), 2.53 and 2.31 (d and d, J = 13.8 Hz, CH_2Ph), 0.93 (s, 3 H, Me-13). ^{13}C NMR (62.9 MHz, CD_2Cl_2): δ 225.24 (CO), 153.39 (C-3), 138.20 (C-5), 132.53 (C-10), 126.52 (C-1), 115.25 (C-4), 112.72 (C-2), 102.80 (C-17), 84.71, 84.53, 82.79, 81.24 (4 C, η^5-C_5H_4), 83.31 (C-1′ of η^5-C_5H_4), 49.50 (C-14), 46.82 (C-13), 43.86 (C-9), 39.69 (C-8), 36.16, 34.32, 31.34 (C-12, C-16, $CH_2(\eta^5$-$C_5H_4)$), 29.61 (C-6), 27.39 (C-7), 26.24 (C-11), 23.26 (C-15), 14.35 (Me-13). Anal. Calcd for $C_{27}H_{29}O_5Mn$: C, 66.40; H, 5.98. Found: C, 65.90; H, 6.26.

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Supporting Information Available: Tables giving crystallographic data, fractional coordinates, interatomic distances, and bond angles (4 pages) listing of structure factors for **19** (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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