Novel Estradiol Derivatives Labeled with Ru, W, and Co Complexes. Influence on Hormone-Receptor Affinity of Several Organometallic Groups at the 17α Position

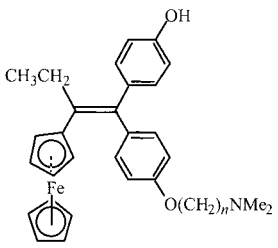
Siden Top,*[a] Hassane El Hafa,[a] Anne Vessières,[a] Michel Huché,[a] Jacqueline Vaissermann,[b] and Gérard Jaouen*[a]

Abstract: In order to elucidate the extent to which recognition of the estrogen receptor is influenced by addition of an organometallic substituent at the 17α position, modification of 17β-estradiol at this position was carried out by using the organometallic groups -C≡C-(η^5-C_5H_4)RuCp, CH_2-(η^5-C_5H_4)RuCp, -C≡C-(η^5-C_5H_4)-$W(CO)_3$(Me), -(C≡CCHO)$Co_2(CO)_6$, and -(C≡CCH_2OH)$Co_2(CO)_6$. The relative binding affinity (*RBA*) values for estradiol receptor alpha showed that recognition was good (*RBA* between 20 and 13.5%) when the organometallic moiety was attached at the end of a rigid alkyne spacer. However, the affinity of the modified hormone for the receptor was severely reduced (*RBA* = 1%) for a substituent such as -CH_2-(η^5-C_5H_4)-RuCp, in which the spacer is reduced to a single flexible sp^3 carbon atom, allowing the organometallic moiety greater freedom of movement around the attachment point. The *RBA* values found were in agreement with results obtained from a molecular-modeling study in which **5**, an organometallic hormone with a rigid spacer, or **7**, a molecule with a flexible spacer, was inserted into the cavity of the recently characterized Ligand-Binding Domain of estrogen receptor alpha.

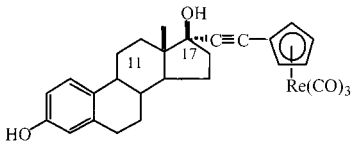
Keywords: bioorganometallic chemistry · cobalt · molecular modeling · ruthenium · steroids · tungsten

Introduction

Bioorganometallic chemistry is a strongly emerging field, whose promise was underlined by a recent special issue of the *Journal of Organometallic Chemistry* devoted to the subject.[1] At the basis of bioorganometallic chemistry is the addition of an organometallic functional group onto a vector or a biological target—a biomolecule, a protein, DNA—in order to modify its properties.[2] Over the past few years this approach has given rise to new types of radiopharmaceuticals,[3] various cytotoxic entities,[4] innovative analytical concepts,[5] and novel bioconversions.[6] The principle of attaching an organometallic functional group to a tamoxifen-type antiestrogen delivery system may well lead to new therapeutic approaches, since such molecules, as for example hydroxyferrocifen, are simultaneously active on both hormone-dependent (MCF7) and hormone-independent (MDAMB231) breast-cancer cell lines.[7]



Hydroxyferrocifen (n=3)



(17α-ethynyl cyrhetrene)estradiol

Over the last few years we have been studying the natural estrogen, estradiol, and searching for organometallic modifications to its skeletal structure that would retain good affinity for its specific receptor.[8] In preference to the chemically more difficult modification at position 11,[9] we chose to attach an alkyne-type rigid spacer at position 17α of the steroid.[10] Molecules such as (17α-ethynylcyrhetrene)estradiol in fact prove to be very well recognized by estrogen receptor α.[10] It remains to be seen whether this (estradiol)–(17α)-C≡C-OM combination (OM = organometallic) can be generalized synthetically to organometallic systems other than rhenium and ferrocene,[11] whether these also show good binding affinities for the receptor, and, if so, why this is the case. We thus

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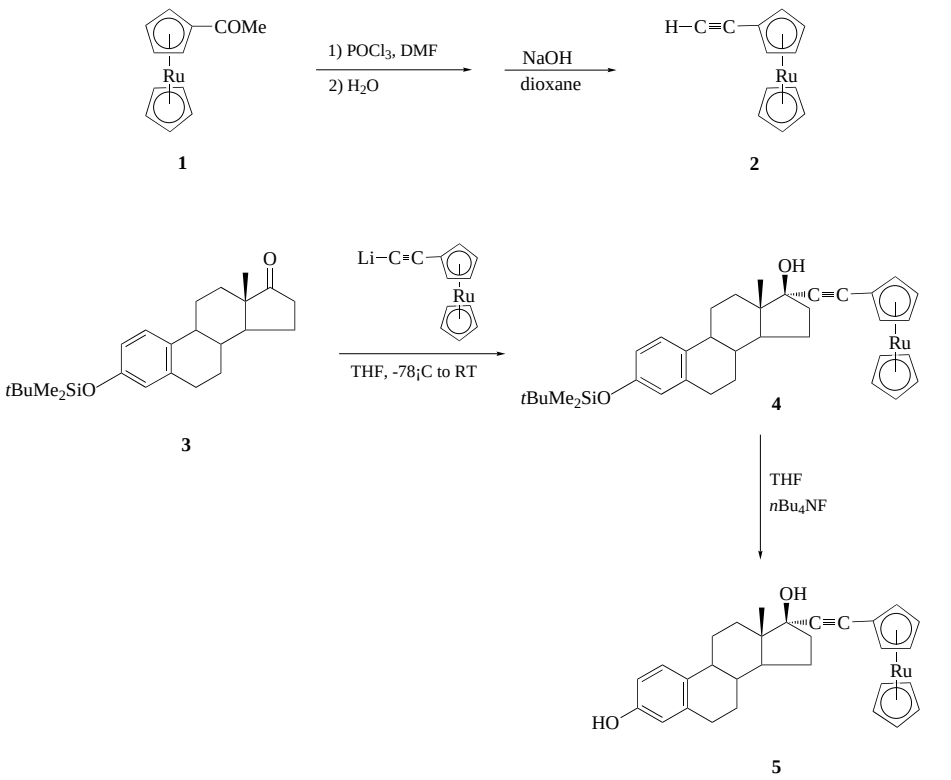
prepared a number of acetylene estradiols modified at position 17α with new complexes of Ru, W, and Co, evaluated their relative binding affinity for estrogen receptor α, and used molecular-modeling techniques to analyze the factors involved in the recognition of the modified hormone by the receptor. The potential applications of these organometallic moieties are related to new cytotoxic effects,[4e] new radiopharmaceuticals,[12] and heavy metals for X-ray structural determination.[13]

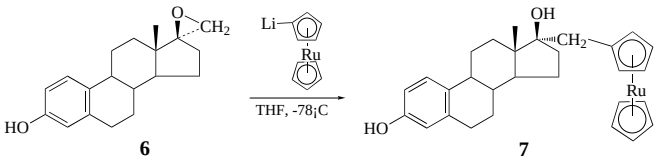


Scheme 1. Synthesis of 17α-(ruthenocenylethynyl)estra-1,3,5(10)-trien-3,17β-diol (**5**).

Results and Discussion

Synthesis of ruthenium complexes derived from estradiol: Scheme 1 shows the synthesis of 17α-(ruthenocenylethynyl)-estradiol (**5**). This synthesis involves the use of ethynylruthenocene (**2**) obtained from acetylruthenocene (**1**) following the method reported by Rausch et al.[14]

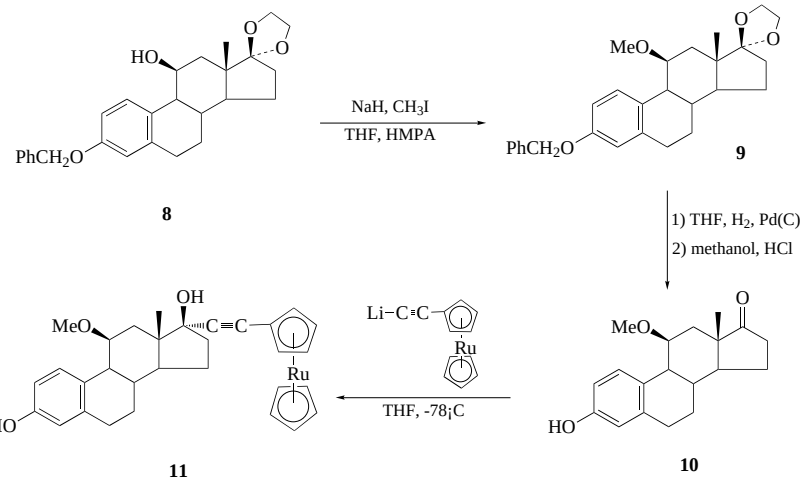
Ethynylruthenocenyl lithium is obtained by addition of butyllithium to **2**. The organolithium thus formed is then allowed to react with the protected estrone **3**. The protected hormone complex **4** is formed initially in 55% yield. This complex is converted into 17α-(ruthenocenylethynyl)-estradiol **5** simply by stirring **4** with the deprotection agent, nBu$_4$NF.



Scheme 2. Synthesis of 17α-(ruthenocenylmethyl)estra-1,3,5(10)-trien-3,17β-diol (**7**).

In order to be able to compare the results with those for a complex containing a shorter and less rigid spacer, 17α-(ruthenocenylmethyl)-estradiol (**7**) was prepared from 17β-spiro-oxiranyl estradiol (**6**) (Scheme 2). Lithium ruthenocenyl, obtained by addition of nBuLi to ruthenocene, was allowed to react with compound **6** at $-78\,^{\circ}$C. Complex **7** was obtained in 65% yield.

Attachment of an organic group in the 11β position often affects recognition.[10] The synthesis of 17α-(ruthenocenylethynyl)-11β-methoxyestradiol (**11**) (Scheme 3) utilizes diprotected 11β-hydroxy-estrone **8**. Compound **8**, obtained by the method described by Zeicher and Quivy,[15] is allowed to react with NaH and then with an excess of MeI to give, in the first instance, the methoxy derivative **9**. Successive deprotection of the benzyl and the glycoxy ethylene by hydrogenation and by action of HCl gives 11β-methoxy estrone **10**. This is finally allowed to react with ethynylruthenocenyl lithium to give **11**, with a 60% yield for this final step.



Scheme 3. Synthesis of 17α-(ruthenocenylethynyl)-11β-methoxyestra-1,3,5(10)-trien-3,17β-diol (**11**).

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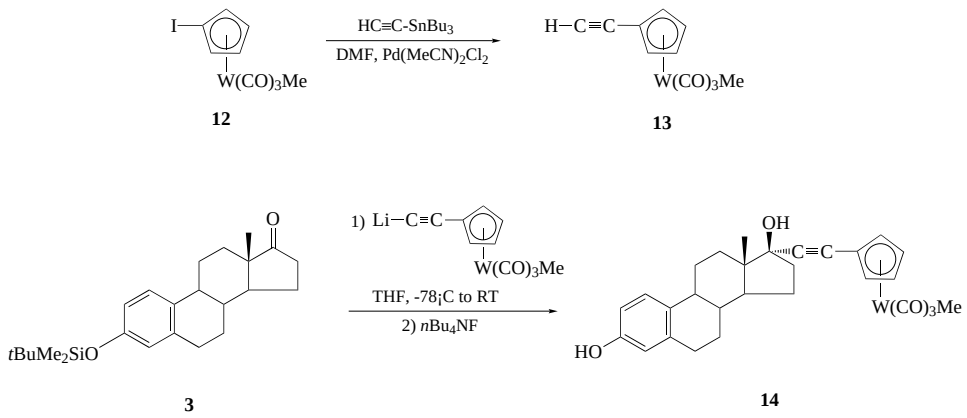
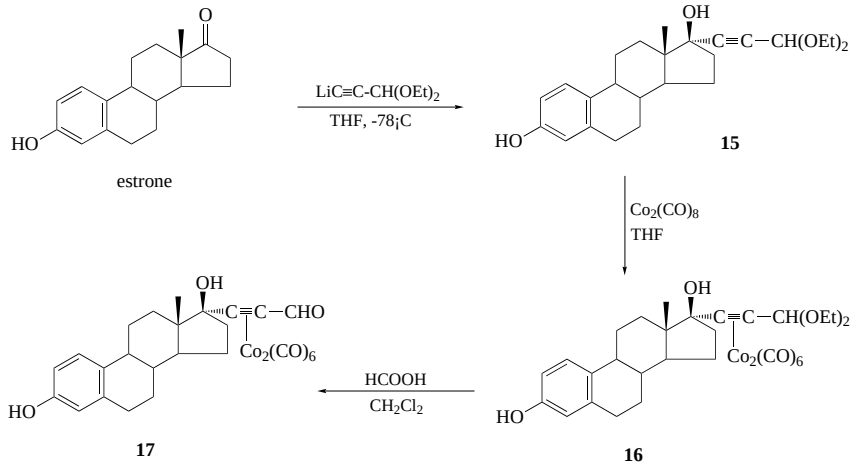
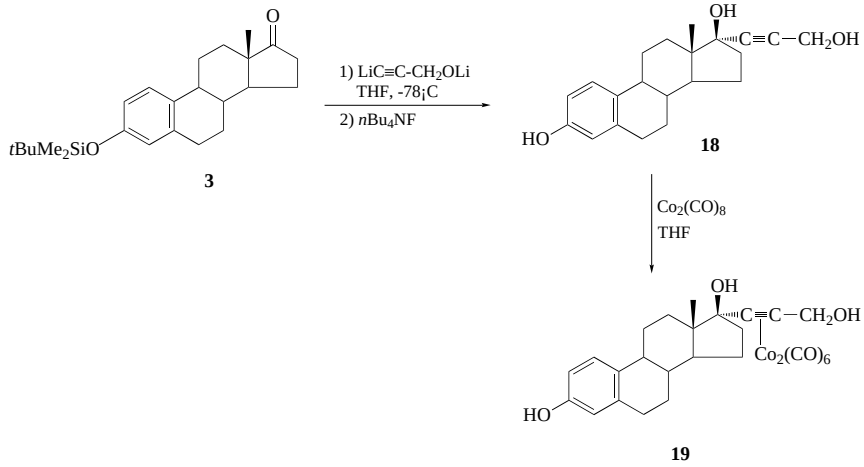


Scheme 4. Synthesis of tungsten complex **14**.



Scheme 5. Synthesis of cobalt complex **17**.



Scheme 6. Synthesis of cobalt complex **19**.

Synthesis of estradiol derivatives of tungsten complexes: Scheme 4 shows the synthetic route for the tungsten complex **14**. (Iodocyclopentadienyl)(methyl)tungsten tricarbonyl, **12**, was first prepared by addition of an excess of iodine to lithium (cyclopentadienyl)(methyl)tungsten tricarbonyl. Compound **12** was then allowed to undergo a Stille coupling reaction with tributylstannyl acetylene to give **13**.[16] Lithiation of **13** followed by addition of protected estrone **3** gave the complex **14** in 60% yield.

Synthesis of estradiol derivatives of cobalt complexes: The Co complex **17** was prepared from estrone in three steps (Scheme 5). Addition of $LiC{\equiv}CCH(OEt)_2$ to estrone yielded **15**. Stirring **15** with $Co_2(CO)_8$ yielded the complex **16**. Deprotection of **16** with HCOOH gave the final product **17**. This compound is unusual in that an aldehyde function remains at the end of the chain; this offers further applicability.

The Co complex **19** was prepared in a similar way (Scheme 6). We found that the addition of $LiC{\equiv}CCH_2OLi$, generated from propargylic alcohol, to estrone produced **18** in low yield. An acceptable yield of **18** (47%) was obtained by adding $LiC{\equiv}CCH_2OLi$ to protected estrone **3**, followed by deprotection with nBu_4NF. Stirring **18** with $Co_2(CO)_8$ yielded the complex **19**.

The addition reaction of an organolithium to estrone to give compounds **5**, **11**, **14**, **15**, and **18** is theoretically a diastereogenic reaction. However due to the particular structure of estrone, and especially to the presence of the methyl group in position 13β, this reaction is stereospecific and yields almost exclusively the 17α isomer. This behavior is well known in this series.[10, 17] NMR studies show the presence of only one diastereoisomer; this is consistent with the X-ray crystallographic analysis of compound **5**.

X-ray crystal structure of 5: Determination of the structure of the modified hormone is very useful in the later analysis of receptor binding. Compound **5** gave crystals suitable for an X-ray crystallographic-structural determination, and the data are given in Table 1. The ORTEP diagram of **5** is shown in Figure 1.

Table 1. Summary of crystallographic data for **5**.

Formula	$C_{30}H_{32}O_2Ru$
M_w	525.6
a [Å]	7.587(2)
b [Å]	16.540(3)
c [Å]	19.344(6)
V [Å3]	2427(1)
Z	4
crystal system	orthorhombic
space group	$P2_12_12_1$
μ [cm^{-1}]	6.56
ρ [g cm^{-3}]	1.44
diffractometer	Enraf-Nonius CAD4
radiation	$Mo_{K\alpha}$ ($\lambda = 0.71069$ Å)
scan type	$\omega/2\theta$
scan range [°]	$0.8 + 0.345\ \mathrm{tg}\theta$
θ limits [°]	1–24
T	RT
Octants collected	0.8, 0.18, 0.22
No of data collected	2212
No of unique data collected	2188
No of unique data used for refinement	959 $(F_o)2 > 3\sigma(F_o)^2$
$R = \Sigma \vert \vert F_o \vert - \vert F_c \vert \vert / S \vert F_o \vert$	0.0539
R_w[a] $= \{\Sigma w(\vert F_o \vert - \vert F_c \vert)^2 / \Sigma w F_o^2\}^{1/2}$	0.0624
absorption correction	DIFABS (min = 0.87, max = 1)
extinction parameter	none
goodness of fit (s)	1.17
No. of variables	149
$\Delta\rho_{min}$ [e Å^{-3}]	−0.47
$\Delta\rho_{max}$ [e Å^{-3}]	0.62

[a] $w = w'[1-((\vert \vert F_o \vert - \vert F_c \vert \vert)/6\sigma(F_o))^2]^2$ with $w' = 1/\Sigma\rho \mathrm{ArTr}(X)$ with three coefficients 3.79, −0.810, and 2.74 for a Chebyshev Series, for which X is F_c/F_c(max).

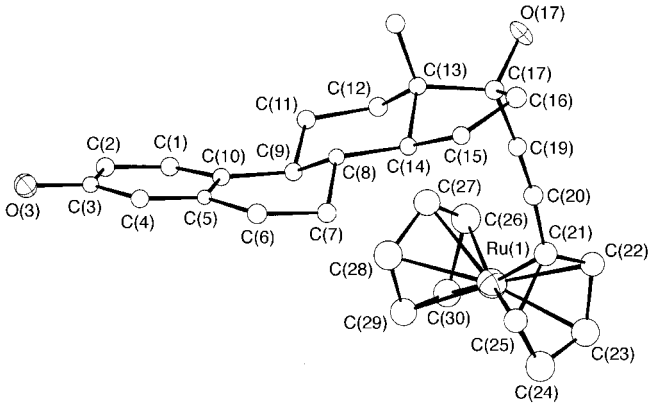
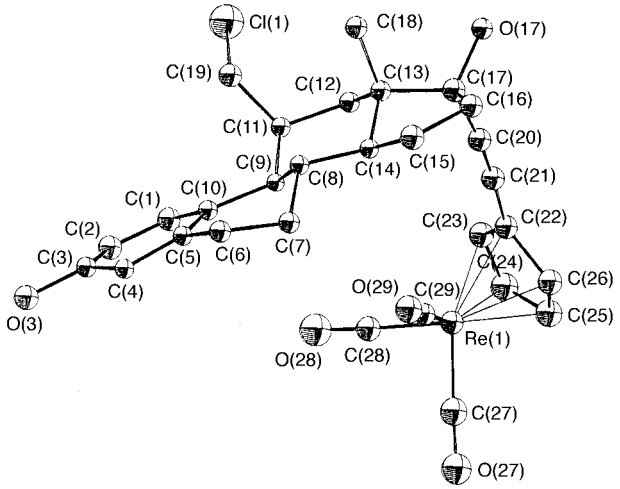
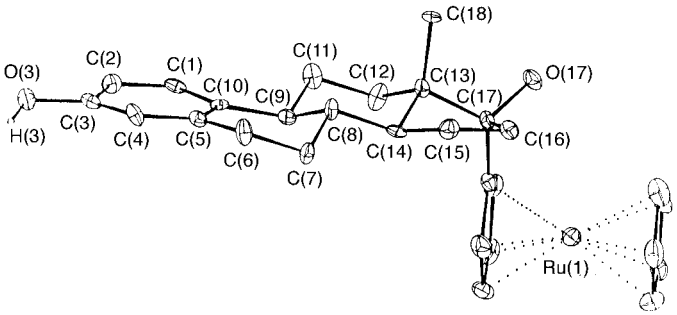


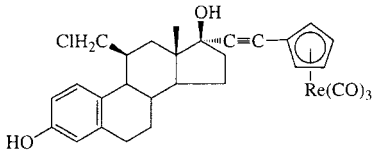
Figure 1. X-ray structure of **5**.

The structure of **5** shows that the steroid's skeletal arrangement is not noticeably different from that of 17β-estradiol.[18] Structural analysis confirmed the position of the ruthenocenyl group at 17α. It is interesting to compare the structure of **5** with those of **20**[10] and **21**[19] determined previously by us (Figure 2). In the case of **21**, in which the organometallic moiety (η^5-C_5H_4)RuCp is attached without a spacer in position 17α, the ruthenocenyl group is twisted towards the opposing face of the steroidal skeleton, probably owing to the steric effect of the neighboring D ring. In compound **5**, on the other hand, the existence of the ethynyl spacer eliminates this problem. The ruthenocenyl group can turn freely and prefers a position almost directly below the D ring, probably for reasons of compactness. In contrast, there is a great structural similarity between **5** and **20**. As in the case of **20**, the ethynyl linkage is not perfectly linear, and shows a slight deformation. In fact the C(17)-C(19)-C(20) angle proves to be 177.0° (20) and the C(19)-C(20)-C(21) angle is 174.7° (20).

Biochemical studies: At this point it is important to establish the affinity of the modified hormones for the estradiol







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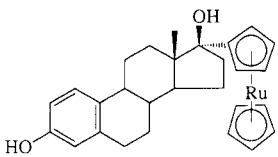

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Figure 2. X-ray structures of **20** and **21**.

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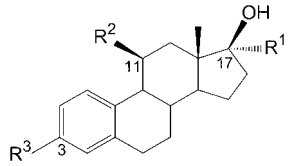
receptor (ER). Measurement of Relative Binding Affinities (*RBA*) was performed by using the previously published method[8d] based on competition between the modified hormone and tritium-labeled 17β-estradiol. The values obtained are summarized in Table 2. By definition, the *RBA* value of 17β-estradiol is 100%.

Table 2. Relative binding affinity (RBA) of the compounds for the estrogen receptor. $R^2 = H$, except for **11** ($R^2 = MeO$); $R^3 = OH$, except for **4** ($R^3 = t$BuMe$_2$SiO).



Compound	R^1	RBA [0 °C, 3 h]
17β-estradiol	H	100
4	$R^1 = C\equiv C$-(η^5-C_5H_4)RuCp $R^3 = t$BuMe$_2$SiO	0
5	$C\equiv C$-(η^5-C_5H_4)RuCp	13.5
7	CH_2-(η^5-C_5H_4)RuCp	1.0
11	$R^1 = C\equiv C$-(η^5-C_5H_4)RuCp, $R^2 = MeO$	19.5
14	$C\equiv C$-(η^5-C_5H_4)W(CO)$_3$(Me)	17
17	$C\equiv C$-CHO $Co_2(CO)_6$	2.9
18	$C\equiv CCH_2OH$	19
19	$C\equiv C$-CH_2OH $Co_2(CO)_6$	3.3
22	$C\equiv C$-(η^5-C_5H_4)Re(CO)$_3$	16[a]
23	CH_2-(η^5-C_5H_4)Re(CO)$_3$	0.8[a]

[a] Value from ref. [10].

It is known that the hydroxyl in position 3 forms a hydrogen bond with residues Glu353 and Arg394 of the receptor, and that elimination of this phenol function lowers recognition considerably. This observation is confirmed again here by the affinity of **4**, in which the hydroxyl in position 3 has been exchanged for a silylated ether. This compound elicits no recognition by the receptor. In **5**, the regeneration of the hydroxyl in position 3 increased the *RBA* to 13.5%. This value can be compared to that of the tungsten compound **14**, which differs from **4** only in the organometallic group. The affinity of **14** for the receptor is slightly higher (17%) but closely comparable to that of **5**. The presence of a methoxy in the 11β position, as in compound **11**, increases the *RBA* from 13.5% for **5** to 19.5% for **11**. Conversely, replacing the rigid ethynyl spacer by methylene in **7** strongly decreases recognition, giving an *RBA* value of only 1%. This result confirms those we obtained with the rhenium complexes **22** and **23**.[10] These complexes gave *RBA* values of 16% and 0.8%, respectively. In light of these results it can be seen that a neutral organometallic moiety attached to the terminal carbon of the 17α-ethynyl group of estradiol is well tolerated by the receptor. Within certain limits, the particular metal group used (Ru, W, Re) does not seem to be an important factor, since the *RBA* values of the compounds all fall within a narrow range (13.5–20%). This observation was also confirmed for compound **18**, in which the organic group CH_2OH is attached to the terminal carbon. An *RBA* of 19% was found in this case. On the other hand, when steric hindrance is introduced close to the D ring by complexation of the triple bond by a $Co_2(CO)_6$ group, as in compound **19**, the *RBA* is again reduced, to 3.3%. This matches the result for **17** (*RBA* = 2.9%).

We also measured the lipophilic value ($\log P_{o/w}$) of these complexes, since this value is an indication of the facility with which compounds can cross the cellular membrane. The values found for representative complexes are listed in Table 3. As observed previously,[10, 19] it can be seen that all the organometallic hormones are more lipophilic than estradiol. There is no noticeable variation between the three metals Ru, W, and Re. These values appear to be a good compromise for a potential pharmaceutical product. They are high enough to allow the product to enter the cell, and, being under 6, they are also not too high—higher values could cause them to be retained preferentially by fatty tissues in vivo, thus making it more difficult for them to reach their target tissues.

Table 3. Partition coefficients ($\log P_{o/w}$) of some 17α-estradiol derivatives.[a]

Compound	$\log P_{o/w}$
estradiol	3.3
5	5.3
14	4.9
22	5.3[b]

[a] Octanol/water partition coefficient ($\log P_{o/w}$) were determined by the HPLC method as described in ref. [10]. [b] Value from ref. [10].

Molecular modeling: The X-ray crystallographic-structural determination of the Ligand Binding Domain (LBD) of estrogen receptor α with estrogenic and antiestrogenic ligands attached at the binding site has recently become available.[20] Since the crystal structure of the organometallic bioligand **5** has been obtained here, we were able to perform molecular modeling studies to attempt to visualize the differences in receptor binding between **5** and **7**, and thus perhaps account for their great difference in affinity.

The choice of the human ER (hERα) site, initially occupied by hydroxytamoxifen,[20b] was dictated by the fact that it is the only cavity described as being large enough to allow calculation of the correlations. Since deformation of the corresponding protein only impacts the 11β position of estradiol, this has little effect on the amino acid residues of the protein chain at 17α of estradiol, and it is the latter that is the subject of this study.

Mac Spartan Pro software was used for the molecular modeling.[21] Only the amino acids forming the wall of the cavity were retained. Hydroxytamoxifen was removed and replaced successively by the bioligands under study. The position of the bioligand was energetically optimized, with all the heavy atoms (i.e., all except hydrogen) in the cavity immobilized. Then the side chain of the amino acid His524 was released. This was justified in view of the fact that this

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part of the cavity has been shown to be flexible.[22] Energy minimization was then carried out, with all the heavy atoms immobilized except for those of the mediator and of the side chain of His524, by using the Merck molecular force field (MMFF).[21] This established the ideal position for the bioligand. We then established the affinity of the hormone for its cavity by semiempirical PM3 quantum-mechanical methods. This involved calculation of the overall bioligand-cavity energy as well as the individual bioligand and cavity energies, the last two conserving the conformation they had in the complete molecule. This allows calculation of the $\Delta_r H^\circ$ enthalpy variation of the reaction:

bioligand + protein binding site → supramolecular complex

For 17α-ruthenocenylethynylestradiol **5**, the enthalpy variation is 1.6 kcal mol^{-1}, a low endothermic value that favors association. The molecular model obtained with the Molview program[23] (Figure 3) shows that there is a constricted area in the cavity at the ethynyl level, but the narrow structure of the alkyne is a good fit and allows the organometallic complex to pass comfortably through the bottleneck.

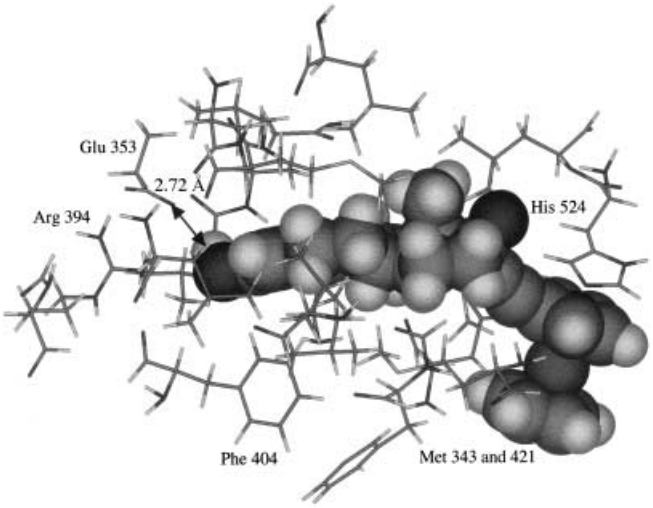

Figure 3. 17α-ruthenocenylethynylestradiol (**5**) in the hERα cavity. The bioligand **5** is represented as a space-filling model and the amino acids as rods. The His524 amino acid is clearly visible on the right of the cavity. The ethynylruthenocenyl group is also bordered in its lower side by two hydrophobic amino acid residues Met343 and Met421. A shrinkage, which is well adapted to accommodate the rigid ethynyl group, can be clearly seen in front of the 17α-position of **5**; this allows the ruthenocenyl group to avoid steric constraints inside the cavity. This model must be similar for related structures.

Conversely, for 17α-ruthenocenylmethylestradiol, **7**, the enthalpy variation 12.3 kcal mol^{-1}, an endothermic value that is unfavorable for association. The molecular model (Figure 4) shows that the bottleneck mentioned earlier is a bad fit for the methylene, which is not linear. This is aggravated by the closeness of the bulky ruthenocenyl group, which sterically hinders this part of the receptor.

Finally, Figure 5 shows 17α-ethynylestradiol in the cavity. It can be seen that there are minimal steric constraints, as suggested by the *RBA* value of 107 %.[24]

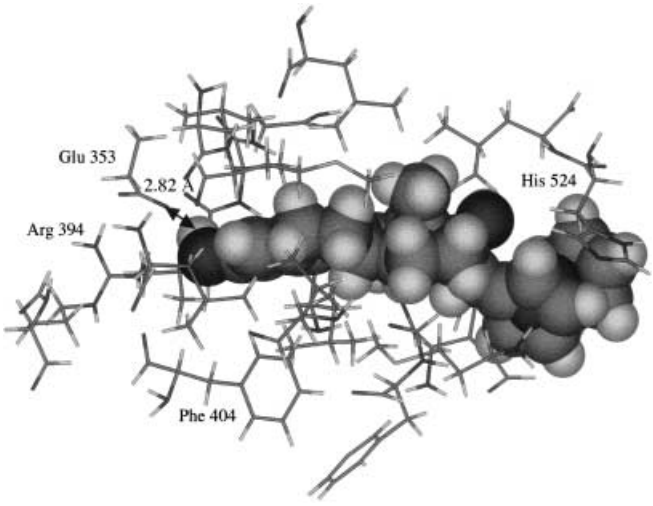

Figure 4. 17α-ruthenocenylmethylestradiol (**7**) in the hERα cavity. The hormone **7** is represented as a space-filling model and the amino acids as rods. The amino acid His524 is clearly visible on the right of the cavity. Note the steric hindrance between the -$CH_2(\eta^5$-$C_5H_4)$RuCp group and the receptor residues neighboring His524.

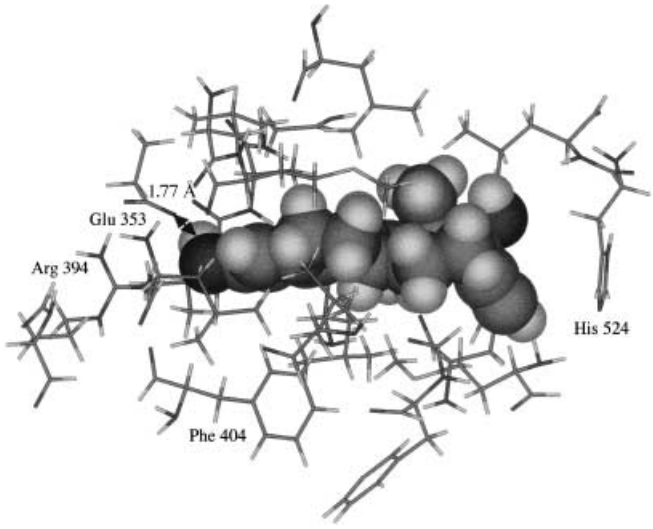

Figure 5. 17α-ethynyl-estradiol in the hERα cavity. The hormone is represented as a space-filling model and the amino acid as rods. The amino acid His524 is clearly visible on the right hand of the cavity. It can be seen here that the ethynyl group at the 17α position does not present any steric constraint at this level, near to the narrow hydrophobic channel.

The software used does not permit calculation of the free enthalpy variation, $\Delta_r G^\circ$, or of the entropic variation $\Delta_r S^\circ$. However, the two $\Delta_r H^\circ$ energy values found for **5** and **7** are so different that it is possible to conclude that the difference in affinities correlates well with the *RBA* values, and thus to have confidence in the observed experimental results. In addition, several authors have stressed the importance of the hydrogen bond between the carboxylate function of Glu353 and the phenol group at position 3 of estradiol.[25] According to our modeling studies, this length for compounds **5** and **7** is, respectively, 2.72 and 2.82 Å. This lengthening also reflects the steric hindrance at the other end of the organometallic steroid. For ethynyl estradiol, which exhibits no steric constraint, this distance is 1.77 Å.

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Conclusion

In summary, the affinity measurements suggest three trends:

a) If the organometallic, or an organic group, is attached at the end of a rigid -C≡C- ethynyl chain at the 17α position of the steroid, recognition of estrogen receptor α is good for any type of organometallic group, in this case neutral or lipophilic.
b) If the spacer is shortened to a single flexible sp^3 carbon atom, creating greater mobility for the organometallic entity at 17α and a greater space-filling volume, affinity is reduced.
c) If the bulky organometallic moiety is moved towards the D ring of the steroid, even on a rigid spacer, affinity is again decreased.

The results observed are in agreement with the molecular-modeling studies performed with **5** and **7**. On the His524 side, facing a possible substituent at position 17α, the estrogen receptor possesses a narrow region that can be turned to advantage, since it accommodates rigid, narrow substituents such as an alkyne group. In addition, if a bulky organometallic moiety is attached to the end of this rigid spacer, the affinity remains acceptable since, in this case, the organometallic group lies outside the zone of steric constraint with this part of the protein. This general pattern should hold true for a whole series of molecules with this type of structure. To our knowledge, this is the first molecular-level explanation for this kind of behavior; it may prove useful in the future in evaluating factors that influence recognition when designing customized estrogen vectors for well-defined targets, for example organometallic radiopharmaceutical products attached to bioligands of this type.[3d, 2f]

Experimental Section

General procedures: All reactions were performed under a dry argon atmosphere by 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. ^{1}H 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-Chloromethylestrone and 17β-oxiranylestra-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.[16]

Acetylruthenocene 1: Acetyl ruthenocene was prepared following the procedure described by Rausch et al.[14] Ruthenocene (0.600 g, 2.59 mmol) was dissolved in acetic anhydride (1 mL) and 1,2-dichloroethane (4 mL). Phosphoric acid (0.2 mL, 85 %) was added to this solution. The mixture was heated at reflux for 15 min. The solution was then hydrolyzed with ice water, and the product was extracted with dichloromethane. After drying over magnesium sulfate, the solution was filtered and concentrated with a rotary evaporator. The crude product was purified by TLC chromatography with dichloromethane as eluent to yield acetyl ruthenocene as a yellow solid (0.400 g, 56 % yield). M.p. 118 °C; ^{1}H NMR (200 MHz, $CDCl_3$) $\delta = 5.10$ and 4.78 (t, t, 2H; 2H; C_5H_4), 4.59 (s, 5H; C_5H_5), 1.58 (s, 3H; Me); elemental analysis calcd (%) for $C_{12}H_{12}ORu$: C 52.74, H 4.43; found: C 52.56, H 4.55. Unreacted ruthenocene (100 mg) was also isolated.

Ethynylruthenocene 2: $POCl_3$ (0.918 g; 6 mmol) was dissolved in DMF (4 mL) at 0 °C. A solution of acetyl ruthenocene (0.546 g, 2 mmol) in DMF (8 mL) was added dropwise to this solution. The mixture was stirred at 0 °C for 15 min and then at room temperature for 2 h. The resulting orange solution was poured into a MeCOONa solution (20 %, 100 mL). After 1 h of stirring, the solution had turned yellow. The product was extracted with dichloromethane. After drying over magnesium sulfate, the solution was filtered and concentrated with a rotary evaporator. The crude product obtained was dissolved in dioxane (40 mL). The solution was heated at reflux, then NaOH solution (0.5 N, 50 mL) was added. The reflux was maintained for 20 min. After hydrolysis with ice water, neutralization with 1/10 HCl solution, ether extraction, and solvent removal, the residue was chromatographed on silica gel plates with dichloromethane/pentane (1:6) as eluent. Ethynyl ruthenocene was finally isolated as a yellow solid (0.100 g, 20 % yield). ^{1}H NMR (200 MHz, $CDCl_3$) $\delta = 4.87$ and 4.56 (t, t, 2H; 2H; C_5H_4), 4.61 (s, 5H; C_5H_5), 2.66 (s, 1H; CH).

17α-(Ruthenocenylethynyl)estra-1,3,5(10)-trien-3,17β-diol (5): *n*BuLi (0.50 mL of a 1.6 M solution in hexane, 0.8 mmol) was added to a solution of ethynylruthenocene (0.200 g, 0.78 mmol) in THF (5 mL) cooled to −78 °C. After the mixture had been stirring for 1 h, a solution of 3-*tert*-butyldimethylsiloxyestrone **3** (0.200 g, 0.78 mmol) in THF (3 mL) was slowly added over 30 min. The stirring was continued for 3 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 with ether/pentane (1:2) as eluent. The first fraction to elute was unreacted ethynylruthenocene (0.010 g). The second fraction yielded 17α-(ruthenocenylethynyl)estra-1,3,5(10)-trien-3-*tert*-butyldimethylsiloxy-17β-ol (0.170 g, 55 % yield). The latter was dissolved in THF (5 mL), and a solution of nBu$_4$NF (2 mL 1 M) in THF was added. After 10 min of stirring and a work-up, the crude product obtained was purified by TLC chromatography with diethyl ether/pentane (1:1) as eluent. Finally, 17α-(ruthenocenylethynyl)estra-1,3,5(10)-trien-3,17β-diol was isolated in 60 % yield. M.p. 230 °C; ^{1}H NMR (250 MHz, CD_3COCD_3): $\delta = 7.94$ (s, 1H; OH), 7.13 (d, $J = 8.3$ Hz, 1H; H(1)), 6.59 (dd, $J = 8.3$ and 2.6 Hz, 1H; H(2)), 6.52 (d, $J = 2.6$ Hz, 1H; H(4)), 4.80 (t, $J = 1.7$ Hz, 2H; C_5H_4), 4.54 (t, $J = 1.7$ Hz, 2H; C_5H_4), 4.57 (s, 5H; Cp), 2.78 (m, 2H; H(6)), 0.89 (s, 3H; Me-13); ^{13}C NMR (62.89 MHz, CD_3COCD_3) $\delta =$ 155.9 (C(3)), 138.4 (C(5)), 131.9 (C(10)), 127.1 (C(1)), 115.9 (C(4)), 113.6 (C(2)), 90.8 (C_5H_4), 80.0 (≡C), 82.5 (C(17)), 74.2–74.1, 71.2, 71.1 (C_5H_4), 72.1 (Cp), 69.7 (C≡, 50.4 (C(14)), 48.5 (C(13)), 44.9 (C(9)), 40.6 (C(8)), 39.8–33.8 (C(12), C(16)), 30.3 (C(6)), 28.3–27.4 (C(7), C(11)), 23.5 (C(15)), 13.4 (Me-13); MS (70 eV, EI): *m*/*z*: 526 (100) [M^+], 508 (21) [$M^+ - H_2O$], 298 (26), 270 (78), 256 (56): elemental analysis calcd (%) for $C_{30}H_{32}O_2Ru$: C 68.55, H 6.13; found: C 68.43, H 6.06.

17α-Ruthenocenylmethylestra-1,3,5(10)-trien-3,17β-diol (7): Ruthenocene (0.181 g, 0.78 mmol) in THF (5 mL) was cooled to −78 °C and treated with *n*BuLi (0.50 mL of 1.6 M solution in hexane, 0.80 mmol). After stirring for 1 h, a solution of spiro-17β-oxiranylestra-1,3,5(10)-trien-3-ol, (0.100 g, 0.35 mmol) in THF (2 mL) was slowly added to the organolithium solution maintained at −78 °C. The stirring was continued for 2 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 with diethyl ether/pentane (4:6) as eluent to give 17α-ruthenocenylmethylestra-1,3,5(10)-trien-3,17β-diol (0.117 g, 65 % yield. M.p. 130 °C, $R_f = 0.22$; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 7.15$ (d, $J = 8.4$ Hz, 1H; H(1)), 6.63 (dd, $J = 8.4$ and 2.5 Hz, 1H; H(2)), 6.56 (d, $J = 2.5$ Hz, 1H; H(4)), 4.65 (m, 2H; C_5H_4), 4.55 (s, 5H; Cp) 4.52 (m, 2H; C_5H_4), 2.81 (m, 2H; H(6)), 2.53 and 2.47 (d,d, $J = 14.0$ Hz, 1H, 1H; CH_2), 0.94 (s, 3H; Me-13); ^{13}C NMR (62.89 MHz, $CDCl_3$) $\delta = 153.5$ (C(3)), 138.4 (C(5)), 132.8 (C(10)), 126.6 (C(1)), 115.3 (C(4)), 112.7 (C(2)), 87.6 (C_{ip} of C_5H_4), 82.6 (C(17)), 73.4–72.7, 70.4, 70.0 (C_5H_4), 71.1 (5C, Cp), 49.4 (C(14)), 46.5 (C(13)), 43.9 (C(9)), 39.6 (C(8)), 36.6–34.7–31.7 (C(12), $CH_2C_5H_4$, C(16)), 29.8 (C(6)), 27.5–26.4 (C(7), C(11)), 23.6 (C(15)), 14.5 (Me-13); MS (70 eV, EI): *m*/*z*: 516 (21) [M^+], 245 (100), 167 (17).

Synthesis of 9: In a flask equipped with a dropping funnel, compound **8** (1.500 g, 3.57 mmol) was dissolved in THF (50 mL) and HMPA (3.7 mL). A suspension of NaH (0.70 g, 17.8 mmol) in THF (10 mL) was added dropwise to the solution. After 2 h of stirring at room temperature, an excess of MeI (15 mL) was added to the mixture. The mixture was then heated at reflux for 12 h. The solvent was evaporated, and the crude product was dissolved in dichloromethane. The solution obtained was

washed with water, dried with $MgSO_4$, and evaporated. The yellow oil obtained was chromatographed on silica gel plates with diethyl ether/pentane (1:3) as eluent. Finally, **9** was obtained as a white solid (1.06 g, 71 % yield). M.p. 120 °C; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 7.2$ (d, $J = 8.5$ Hz, 1H; H(1)), 6.8 (dd, $J = 8.5$ and 2.6 Hz, 1H; H(2)), 6.77 (d, $J = 2.6$ Hz, 1H; H(4)), 5.04 (s, 2H; $PhCH_2$), 4.2 (m, 1H; H(11)), 3.31 (s, 3H; OCH_3), 3.89 (m, 4H; $O(CH_2)O$), 1.10 (s, 3H; Me-18); MS (70 eV, EI): m/z: 434 (3) [M^+], 372 (1), 287 (1); elemental analysis calcd (%) for $C_{28}H_{34}O_4Ru$: C 77.39, H 7.89; found: C 76.54, H 8.10.

Synthesis of 10: Compound **9** (0.500 g, 1.15 mmol) was dissolved in THF (18 mL). Pd/C (10 %, 0.25 g) was added, and the flask was filled with hydrogen at atmospheric pressure. The mixture was stirred overnight. After filtration and solvent removal, the crude compound **10** obtained was dissolved in methanol (40 mL), and concentrated aqueous HCl (9 mL) solution was added. The mixture was heated at reflux for 1 h. The solution was concentrated, and then ethyl acetate (100 mL) was added. The solution was washed with water, dried over $MgSO_4$, and evaporated. The crude product obtained was recrystallized from dichloromethane/heptane to give **10** as white crystals (0.32 g, 92 % yield). M.p. 260 °C; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 7.00$ (d, $J = 8.5$ Hz, 1H; H(1)), 6.60 (dd, $J = 8.5$ and 2.6 Hz, 1H; H(2)), 6.57 (d, $J = 2.6$ Hz, 1H; H(4)), 4.20 (m, 1H; H(11)), 3.32 (s, 3H; OCH_3), 1.11 (s, 3H; Me-18); MS (70 eV, EI) m/z: 300 (13) [M^+], 241 (8), 197 (8), 170 (21), 157 (31), 146 (76); elemental analysis calcd (%) for $C_{19}H_{24}O_3$: C 75.97, H 8.05; found: C 75.01, H 8.26.

Synthesis of 11: The synthetic procedure is similar to that for 17α-(ruthenocenylethynyl)estra-1,3,5(10)-trien-3,17β-diol. Compound **11** was obtained in 60 % yield. M.p. 160 °C; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 7.03$ (d, $J = 8.6$ Hz, 1H; H(1)), 6.64 (dd, $J = 8.6$ and 2.7 Hz, 1H; H(2)), 6.54 (d, $J = 2.7$ Hz, 1H; H(4)), 4.80 (t, $J = 1.5$ Hz, 2H; C_5H_4), 4.53 (t, $J = 1.5$ Hz, 2H; C_5H_4), 4.55 (s, 5H; Cp), 3.31 (s, 3H; CH_3O), 2.80 (m, 2H; H(6)), 1.08 (s, 3H; Me-13); ^{13}C NMR (62.89 MHz, $CDCl_3$) $\delta = 153.26$ (C(3)), 138.9 (C5), 129.0 (C(10), 126.9 C(1)), 115.7 (C(4)), 113.7 (C(2)), 88.8 (C_5H_4), 83.6 (C≡), 80.5 (C(17)), 76.8 (C(11), 73.8 – 73.7, 70.7 (3C of C_5H_4), 68.1 (C≡), 71.72 (Cp), 56.4 (OCH_3), 50.6 (C(14)), 49.2 (C(9)), 47.7 C(13)), 39.0 (C(8)), 34.7 (C(16)), 33.0 (C(12)), 29.7 (C(6)), 27.5 (C(7)), 22.8 (C(15)), 14.0 (Me-13); MS (70 eV, EI) m/z: 556 (100) [M^+], 538 (27) [$M^+ - H_2O$], 298 (19), 256 (25).

(Iodocyclopentadienyl)(methyl)tungsten tricarbonyl 12: $CpW(CO)_3Me$ (0.522 g, 1.5 mmol) was dissolved in THF (12 mL). The solution was cooled to − 60 °C, and *n*BuLi (2 mL, 1.3 M, 2.6 mmol) was added. The mixture was stirred at − 60 °C for 1 h. Iodine (0.635 g 2.5 mmol) was then added in one portion. The stirring was maintained for 1 h, during which time the temperature was allowed to rise slowly to room temperature. The solvent was evaporated, and the crude product obtained was chromatographed on silica gel plates with diethyl ether/pentane (1:10) as eluent. (Iodocyclopentantadienyl)(methyl)tungsten tricarbonyl was isolated as a yellow oil (0.300 g 42 % yield). ^{1}H NMR (200 MHz, $CDCl_3$) $\delta = 5.53$ (t, $J =$ 2.3 Hz, 2H; C_5H_4), 5.30 (t, $J = 2.3$ Hz, 2H; C_5H_4), 0.54 (s, 3H; Me); IR (CH_2Cl_2) $\tilde{\nu}_{CO} = 2016$, 1922 cm^{-1}.

Synthesis of HC≡C(η^5-C_5H_4)W(CO)$_3$Me (13): The procedure was similar to that for ethynyl ruthenocene. HC≡C(η^5-C_5H_4)$W(CO)_3Me$ was obtained in 60 % yield. ^{1}H NMR (200 MHz, $CDCl_3$) $\delta = 5.55$ (t, $J = 2.3$ Hz, 2H; C_5H_4), 5.30 (t, $J = 2.3$ Hz, 2H; C_5H_4), 2.96 (s, 1H; CH), 0.54 (s, 3H; Me); IR (CH_2Cl_2) $\tilde{\nu}_{CO} = 2017$, 1922 cm^{-1}.

Synthesis of tungsten complex 14: The procedure was similar to that for 17α-(ruthenocenylethynyl)estra-1,3,5(10)-trien-3,17β-diol. Complex **14** was obtained in 60 % yield. M.p. 212 °C. ^{1}H NMR (250 MHz, $CDCl_3$) $\delta =$ 7.19 (d, $J = 8.4$ Hz, 1H; H(1)), 6.61 (dd, $J = 8.4$ and 2.1 Hz, 1H; H(2)), 6.55 (d, $J = 2.1$ Hz, 1H; H(4)), 5.53 (t, 2H; C_5H_4), 5.31 (t, 2H; C_5H_4), 2.82 (m, 2H; H(6)), 0.91 (s, 3H; Me-13), 0.56 (s, 3H; Me-W); IR (CH_2Cl_2) $\tilde{\nu}_{CO} =$ 2017, 1922 cm^{-1}; MS (70 eV, EI): m/z: 642 (33) [M^+], 558 (69) [$M^+ - 3CO$]; elemental analysis calcd (%) for $C_{29}H_{30}O_5W$: C 54.22, H 4.71; found: C 54.03, H 4.91.

17α-[C≡CH(OEt)$_2$] estradiol (15): *n*BuLi (2.50 mL of a 2.5 M solution in hexane, 6 mmol) was added to a solution of H-C≡$CH(OEt)_2$ (0.769 g, 6 mmol) in THF (30 mL) cooled at − 70 °C. After the mixture had been stirred for 1.5 h, a solution of estrone (0.540 g, 2 mmol) in THF (25 mL) was slowly added over 2 h. The stirring was continued overnight, during which time the temperature was allowed to rise slowly to room temperature. After hydrolysis with ice water, extraction with dichloromethane, and solvent removal, the residue was chromatographed on silica gel plates with diethyl ether/pentane (2:3) as eluent. Compound **15** was isolated as a white solid (0.491 g, 62 % yield). M.p. 80 °C; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 7.09$ (d, $J = 8.5$ Hz, 1H; H(1)), 6.65 (dd, $J = 8.5$ and 2.7 Hz, 1H; H(2)), 6.57 (d, $J = 2.7$ Hz, 1H; H(4)), 5.92 (s, 1H; OH-3), 5.39 (s, 1H; $CH(OEt)_2$), 3.86 – 3.59 (m, 4H; OCH_2CH_3), 2.77 (m, 2H; H(6)), 1.26 (t, $J = 7.1$ Hz, 6H; OCH_2CH_3), 0.86 (s, 3H; Me-13); ^{13}C NMR (62.89 MHz, CD_3CN) $\delta = 153.6$, (C(3)), 138.0 (C(5)), 132.3, (C(10), 126.4 (C(1)), 115.2 (C(4)), 112.7 (C(2)), 91.5 (C(21)), 89.1 or 81.4 (C(19) or C(20)), 79.9 (C(17)), 61.1 (OCH_2CH_3), 49.7 (C(14)), 47.3 (C(13)), 43.3 (C(9)), 39.3 (C(8)), 38.9 (C(16)), 32.9 (C(12)), 29.6 (C(6)), 27.1 (C(7)), 26.3 (C(11)), 22.8 (C(15)), 15.0 (C(18)), 12.7 (OCH_2CH_3); MS (70 eV, EI): m/z: 398 (67) [M^+], 354 (100) [$M^+ -$ EtOH]; elemental analysis calcd (%) for $C_{25}H_{34}O_4$: C 75.34, H 8.60; found: C 75.27, H 7.52.

17α-[(C≡CH(OEt)$_2$)(Co$_2$(CO)$_6$)] estradiol (16): A solution of **15** (0.395 g, 1 mmol) in diethyl ether (5 mL) was added to the solution of $Co_2(CO)_8$ (0.384 g, 1.12 mmol) in diethyl ether (5 mL). After 2 h of stirring at room temperature, the solvent was evaporated. The crude product was chromatographed on silica gel column with diethyl ether/pentane (2:3) as an eluent. Compound **16** was isolated as a red solid (0.507 g, 74 % yield). Decomp. 170 °C; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 7.15$ (d, 1H; H(1)), 6.62 (dd, 1H; H(2)), 6.57 (d, 1H; H(4)), 5.54 (s, 1H; $CH(OEt)_2$), 4.67 (s, 1H; OH-3), 3.86 – 3.59 (m, 4H; OCH_2CH_3), 2.83 (m, 2H; H(6)), 1.32 and 1.31 (t, t, 6H; OCH_2CH_3), 1.05 (s, 3H; Me-13); ^{13}C NMR (62.89 MHz, $CDCl_3$) $\delta =$ 199.5 ($Co_2(CO)_6$), 153.4 (C(3)), 138.2 (C(5)), 132.6 (C(10)), 126.3 (C(1)), 115.2 (C(4)), 112.6 (C(2)), 103.7 (C(21)), 103.2 and 96.1 (C(19) and C(20)), 86.1 (C(17)), 65.8 (OCH_2CH_3), 49.9 (C(14)), 48.5 (C(13)), 42.8 (C(9)), 40.0 (C(8)), 39.5 (C(16)), 32.4 (C(12)), 29.5 (C(6)), 27.4 (C(7)), 26.2 (C(11)), 23.4 (C(15)), 15.5 (C(18)), 15.2 (OCH_2CH_3); IR (KBr) $\tilde{\nu}_{CO} = 2093$, 2055, 2030 cm^{-1}; MS (ElectroSpray): m/z: 707 (92) [M^++Na]; elemental analysis calcd (%) for $C_{31}H_{34}O_{10}Co_2$: C 54.39, H 5.01; found: C 54.57, H 5.13.

17α-[(C≡CHO)(Co$_2$(CO)$_6$)]estradiol 17: Compound **16** (0.300 g, 0.44 mmol) was dissolved in dichloromethane (5 mL). Formic acid (0.312 g, 6.78 mmol) was added, and the mixture was stirred at room temperature for 2 h. After hydrolysis with water, extraction with dichloromethane, and solvent removal, **17** (0.300 g) was isolated. Crystallization from diethyl ether/pentane gave red crystals. M.p. 160 °C; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 10.37$, (s, 1H; CHO), 7.13 (d, 1H; H(1)), 6.62 (dd, 1H; H(2)), 6.57 (d, $J = 2.7$ Hz, 1H; H(4)), 4.70 (s, 1H; OH-3), 2.83 (m, 2H; H(6)), 0.89 (s, 3H; Me-13); ^{13}C NMR (62.89 MHz, $CDCl_3$) $\delta = 198.1$ ($Co_2(CO)_6$), 191.4 (CHO), 153.4, (C(3)), 138.0 (C(5)), 132.2, (C(10)), 126.4 (C(1)), 115.2 (C(4)), 112.7 (C(2)), 105.1 and 87.6 (C(19) and C(20)), 86.3 (C(17)), 50.1 (C(14)), 48.7 (C(13)), 42.8 (C(9)), 42.2 (C(8)), 39.6 (C(16)), 32.5 (C(12)), 29.4 (C(6)), 27.4 (C(7)), 26.0 (C(11)), 23.3 (C(15)), 15.4 (C(18)); IR (KBr) $\tilde{\nu}_{CO} = 2101$, 2064, 2034 cm^{-1}; MS (ElectroSpray): m/z: 633 (76) [M^++Na].

17α-(C≡CH$_2$OH) estradiol 18:[26] *n*BuLi (2.80 mL of a 2.5 M solution in hexane, 7 mmol) was added to a solution of HC≡CH_2OH (0.336 g, 6 mmol) in THF (25 mL) cooled to − 60 °C. After the mixture had been stirred for 30 min, the cooling bath was removed for 10 min, and then the solution was cooled again to − 78 °C. A solution of protected estrone **3** (0.384 g, 1 mmol) in THF (10 mL) was slowly added over 1 h. The stirring was continued overnight, during which time the temperature was allowed to rise slowly to room temperature. A solution of nBu_4NF (1 M, 1 mmol) in THF (1 mL) was then added, and the stirring was maintained for 10 min. After hydrolysis with ice water, neutralization with 10 % HCl solution, extraction with dichloromethane, and solvent removal, the residue was chromatographed on silica gel column with diethyl ether as eluent. Compound **18** was isolated as a colorless solid (0.152 g, 47 %). M.p. 228 °C; ^{1}H NMR (200 MHz, [D_6]acetone) $\delta = 8.01$ (s, 1H; OH), 7.09 (d, $J = 8.4$ Hz, 1H; H(1)), 6.58 (dd, $J = 8.4$ and 2.7 Hz, 1H; H(2)), 6.51 (d, $J = 2.7$ Hz, 1H; H(4)), 4.23 (s, 2H; CH_2OH), 2.77 (m, 2H; H(6)), 0.87 (s, 3H; Me-13); MS (70 eV, EI): m/z: 326 (12) [M^+], 308 (6), 293 (10), 270 (6); elemental analysis calcd (%) for $C_{21}H_{26}O_3.H_2O$: C 73.22, H 8.19; found: C 73.00, H 8.39.

17α-[(C≡CH$_2$OH)(Co$_2$(CO)$_6$)] estradiol 19: Compound **18** (0.152 g, 0.47 mmol) was dissolved in THF (3 mL), and $Co_2(CO)_8$ (0.161 g, 0.47 mmol) was added to the solution. The mixture turned red after it had been stirred for 20 min. The solution was then filtered over a 1 cm-thick silica gel pad. After evaporation of solvent, the crude product was obtained as a red oil (0.266 g). After crystallization in diethyl ether/pentane, **19** was isolated as red crystals (0.065 g, 23 %). Decomp. 170 °C;

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^{1}H NMR (200 MHz, [D$_6$]acetone) $\delta = 7.95$ (s, 1H; OH), 7.09 (d, $J = 8.4$ Hz, 1H; H(1)), 6.58 (dd, $J = 8.4$ and 2.8 Hz, 1H; H(2)), 6.51 (d, $J = 2.8$ Hz, 1H; H(4)), 4.97 (m, 2H; CH_2OH), 2.83 (m, 2H; H(6)), 1.08 (s, 3H; Me-13); MS (ElectroSpray): m/z: 635 (100) [M^++Na].

Determination of the relative binding affinity (*RBA*) of the complexes for the estrogen receptor alpha: Sheep-uterine cytosol prepared as previously described[8d] was used as the source of ERα. Aliquots (200 µL) were incubated for 3 h at 0 °C with 2×10^{-9} M of [6,7-^{3}H]-estradiol (2×10^{-9} M, specific activity 1.96 TBq mmol^{-1}) in the presence of nine concentrations of unlabelled estradiol or of the complex to be tested. The final dilutions of the hormones were made from a 10^{-3} M stock solution in ethanol with a final percentage of ethanol in the incubation medium of 5%. At the end of the incubation period, the free and bound fractions of the tracer were separated by protamine sulfate. The relative binding affinity (*RBA*) of the compounds was the concentration of the unlabelled estradiol/compound required to inhibit half of the specific [^{3}H]-estradiol binding with the affinity of estradiol set by definition at 100%.

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