Synthesis of Optically Pure *o*-Formylcyclopentadienyl Metal Complexes of 17α-Ethynylestradiol. Recognition of the Planar Chirality by the Estrogen Receptor

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A formyl group has been introduced onto the cyclopentadienyl ring of 17α-ethynylestradiol derivatives bearing a ferrocenyl, a cymantrenyl, or a cyrhetrenyl group at the ortho position with respect to the ethynyl group. The presence of this group on the cyclopentadienyl ring induces a planar chirality that provokes the formation of two diastereomers, the *Sp* and *Rp* derivatives. These compounds were prepared from *Sp* and *Rp* 1-formyl-2-iodoferrocenes, -cymantrenes, and -cyrhetrenes, which were separately prepared by using a combination of suitable ortho directing substituents, such as methoxymethyldioxane and *p*-tolylsulfoxide, as well as trimethylsilyl as a temporary blocking group. A cross-coupling reaction between these precursors and 17α-ethynylestradiol led to the formation of the hormone complexes. The ferrocenyl hormone compounds were only obtained from a Sonogashira reaction, the cyrhetrenyl compounds were only formed by using a Stille reaction, and the cymantrenyl compounds were obtained from both reactions. A tentative explanation for this interesting behavior is proposed. The affinity of the *R* diastereomers for the α receptor of estradiol is almost twice that of the *S* diastereomers. This is the first example of estradiol receptor discrimination between organometallic diastereomers possessing planar chirality. However, in contrast to 1,2-disubstituted derivatives, when the formyl group is at the 1,3-position, the receptor does not differentiate between the two planar chiral diastereomers.

Introduction

The incorporation of organometallic fragments into biological molecules is a current strategy for modifying or providing new biological activities to these molecules. This field of research, which is known as bioorganometallic chemistry, is a strongly emerging area.[1] Some organometallic complexes have proven to be of interest either as antitumor agents, as in the case of Fe and Ti metallocenes,[2] or as radiopharmaceuticals, for example the use of radioisotopes of Tc and Re.[3] Over the past few years we have been studying 17β-estradiol, a natural estrogen, in order to determine how one might add organometallic moieties to its skeletal structure and still retain good affinity for its specific receptor. The first hormone complex was obtained by complexation of the aromatic A-ring of 17β-estradiol with the chromium tricarbonyl group; the resulting compound is the first and only example where the organometallic group discriminates between the α and β faces of the steroid.[4] It was found later that the 17α position can accept a large number of organometallic fragments, especially at the terminal carbon of 17α-ethynylestradiol, while retaining a good affinity for the α estrogen receptor (ERα).[5] The 11β position is another possible position for this type of incorporation, but it has been less studied because of synthetic difficulties.[6] In terms of the organometallic

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(1) (a) Jaouen, G. *Bioorganometallics*; Wiley-VCH: Weinheim, Germany, 2005. (b) Vessières, A.; Top, S.; Beck, W.; Hillard, E.; Jaouen, G. *Dalton Trans.* **2005**, 1−12. (c) Jaouen, G.; Top, S.; Vessières, A.; Leclercq, G.; McGlinchey, M. J. *Curr. Med. Chem.* **2004**, *11*, 2505−2517. (d) Van Staveren, D. R.; Metzler-Nolte, N. *Chem. Rev.* **2004**, *104*, 5931−5985. (e) Beck, W.; Severin, K. *Chem. Unserer Zeit* **2002**, *36*, 356−365.

(2) (a) Köpf-Maier, P. *Eur. J. Clin. Pharmacol.* **1994**, *47*, 1−16. (b) Köpf-Maier, P. In *Metal Complexes in Cancer Chemotherapy*; Keppler, B. K., Ed.; VCH: Weinheim, Germany, 1993; pp 259−296. (c) Top, S.; Vessières, A.; Leclercq, G.; Quivy, J.; Tang, J.; Vaissermann, J.; Huché, M.; Jaouen, G. *Chem. Eur. J.* **2003**, *9*, 5223−5236. (d) Top, S.; Kaloun, E. B.; Vessières, A.; Laïos, I.; Leclercq, G.; Jaouen, G. *J. Orgamomet. Chem.* **2002**, *643*−*644*, 350−356.

(3) (a) Nicolini, M.; Bandoli, G.; Mazzi, U. *Technetium and Rhenium in Chemistry and Nuclear Medicine 4*; SG Editoriali: Padova, Italy, 1995. (b) Alberto, R. In *Bioorganometallics*; Jaouen, G., Ed.; Wiley-VCH: Weinheim, Germany, 2005; pp 97−124. (c) Top, S.; Vessières, A.; Pigeon, P.; Rager, M.-N.; Huché, M.; Salomon, E.; Cabestaing, C.; Vaissermann, J.; Jaouen, G. *ChemBioChem* **2004**, *5*, 1104−1113. (d) Bigott, H. M.; Parent, E.; Luyt, L. G.; Katzenellenbogen, J. A.; Welch, M. J. *Bioconjugate Chem.* **2005**, *16*, 255−264.

(4) (a) Top, S.; Jaouen, G.; Vessières, A.; Abjean, J.-P.; Davoust, D.; Rodger, C. A.; Sayer, B. G.; McGlinchey, M. J. *Organometallics* **1985**, *4*, 2143−2150. (b) Vessières, A.; Top, S.; Ismail, A. A.; Butler, I. S.; Louer, M.; Jaouen, G. *Biochemistry* **1988**, *27*, 6659−6666.

(5) (a) Vessières, A.; Jaouen, G.; Gruselle, M.; Rossignol, J. L.; Savignac, M.; Top, S.; Greenfield, S. *J. Steroid Biochem.* **1988**, *30*, 301−305. (b) El Amouri, H.; Vessières, A.; Vichard, D.; Top, S.; Gruselle, M.; Jaouen, G. *J. Med. Chem.* **1992**, *35*, 3130−3135. (c) Vessières, A.; Top, S.; Vaillant, C.; Osella, D.; Mornon, J.-P.; Jaouen, G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 753−755. (d) Top, S.; El Hafa, H.; Vessières, A.; Quivy, J.; Vaissermann, J.; Hughes, D. W.; McGlinchey, M. J.; Mornon, J.-P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc.* **1995**, *117*, 8372−8380. (e) Top, S.; El Hafa, H.; Vessières, A.; Huché, M.; Vaissermann, J.; Jaouen, G. *Chem. Eur. J.* **2002**, *8*, 5241−5249. (f) Stockland, R. A., Jr.; Kohler, M. C.; Guzei, I. A.; Kastner, M. E.; Bawiec, J. A.; Labaree, D. C.; Hochberg, R. B. *Organometallics* **2006**, *25*, 2475−2485. (g) Arterburn, J. B.; Corona, C.; Venkateswara Rao, K.; Carlson, K. E.; Katzenellenbogen, J. A. *J. Org. Chem.* **2003**, *68*, 7063−7070. (h) Osella, D.; Ravera, M.; Nervi, C.; Cavigiolio, G.; Vincenti, M.; Vessières, A.; Jaouen, G. *Eur. J. Inorg. Chem*, **2000**, 491−497. (i) Jackson, A.; Davis, J.; Pither, R. J.; Rodger, A.; Hannon, M. J. *Inorg. Chem.* **2001**, *40*, 3964−3973.

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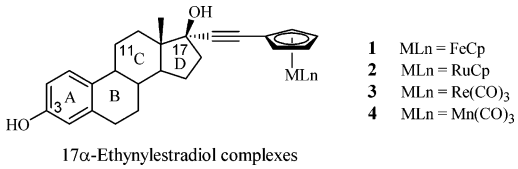
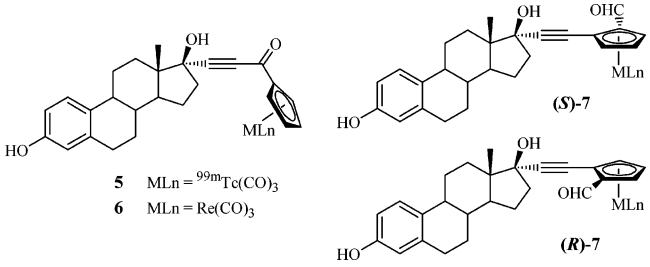
Chart 1



1 MLn = FeCp
2 MLn = RuCp
3 MLn = $Re(CO)_3$
4 MLn = $Mn(CO)_3$

17α-Ethynylestradiol complexes

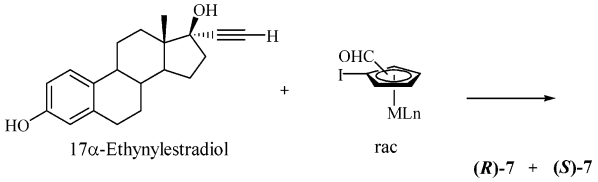
Chart 2



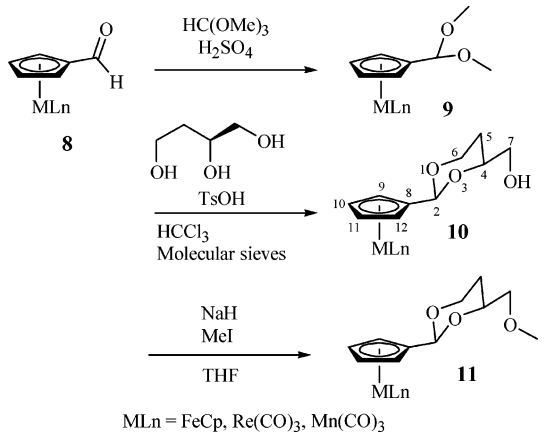
5 MLn = $^{99m}Tc(CO)_3$
6 MLn = $Re(CO)_3$

moiety, ferrocene derivatives seem especially promising; we have found that ferrocifen, an analogue of tamoxifen, has antiproliferative activity against breast cancer for both hormone-dependent and -independent cell lines at very low concentrations (IC_{50} around 0.5 μM).[2c] Additionally, suitable properties of radioisotopes Re-186 and Re-188 for nuclear medicine make the cyclopentadienyltricarbonylrhenium moiety an interesting fragment for the synthesis of radiopharmaceuticals.[7] For these purposes, complexes **1**−**4** had been previously synthesized (Chart 1).[2] Until now, only metal groups with unsubstituted cyclopentadienyl rings have been used. Because the ER affinity of hormones is sensitive to the presence of substituents, an additional functional group on the cyclopentadienyl ring of the complexes would be expected to modify their recognition by the ER. For example, in the course of our search for suitable radiopharmaceuticals for breast cancer diagnosis and therapy, we have prepared the ^{99m}Tc complex **5** and Re complex **6** (Chart 2).[7a] The presence of a CO group between the alkyne functionality and the cyclopentadienyl ring decreases the affinity for the ER. The relative binding affinity (RBA) for **6** is 1.6%, limiting its perspectives for applications as a radiopharmaceutical. In order to improve affinity, we thought it would be interesting to move the CO functionality, necessary for the metal exchange reaction (e.g., Fe for ^{99m}Tc), to another position of the cyclopentadienyl ring: for instance, to make complex **7**. The presence of a formyl substituent in the ortho position induces the formation of two diastereomers, **(*R*)-7** and **(*S*)-7**, by generation of planar chirality on the organometallic fragment. Compound **7** could be prepared from the coupling reaction between 17α-ethynylestradiol and 1-formyl-2-iodocyclopentadienyl metal compounds (Scheme 1), but the resulting two diastereomers are difficult to separate from one another. This problem can be solved by using enantiopure 1-formyl-2-iodocyclopentadienyl metal compounds. The most useful strategy to obtain these types of compounds in their enantiopure forms is the use of diastereoselective ortho lithiation and subsequent reaction with an appropriate coupling reagent. This asymmetric synthesis was first developed in the ferrocene series by Ugi et al. by using a suitable stereogenic ortho-directing

Scheme 1. Cross-Coupling Reaction



Scheme 2. Synthesis of Precursors



group.[8] Kagan et al. have extensively studied this strategy and found that the chiral methoxymethyl-1,3-dioxane group is very efficient for the ortho functionalization of formylferrocene.[9] Thus, enantiopure (*S*)-1-formyl-2-iodo and (*R*)-1-formyl-2-iodoferrocenes have been synthesized. This ortho-directing group was also used in the cymantrene series.[10] In addition, we have found that these two enantiomers can also be prepared from the same precursor by using a trimethylsilyl group to block one of the ortho positions. We now describe here the full synthetic pathway to organometallic hormone complexes and the results of the recognition of planar chirality by the ER. Preliminary results in the cymantrene series have been recently published.[11]

Results and Discussion

(*S*)-1-Formyl-2-iodocyclopentadienyl Compounds 13-Fe, 13-Mn, and 13-Re. Scheme 2 shows the synthetic pathway to the methoxymethyl-1,3-dioxane compounds **11** from formylferrocene, formylcymantrene, and formylcyrhetrene. Following Riant and Kagan's procedure,[9] aldehydes **8** were transformed to ketals **9** by reaction with trimethyl orthoformate in the presence of sulfuric acid. A trans-ketal formation with (*S*)-1,2,4-butanetriol in the presence of TsOH at room temperature gave the hydroxymethyl-1,3-dioxane species **10**. Addition of NaH to the solution of **10** in THF followed by addition of iodomethane produced the methoxymethyl-1,3-dioxanes **11**.

Ortho Electrophile Addition. The attachment of a second substituent at the ortho position was achieved by adding *t*BuLi at −78 °C, followed by the reaction of the electrophile with

(6) Morel, P.; Top, S.; Vessières, A.; Stéphan, E.; Laïos, I.; Leclercq, G.; Jaouen, G. *C. R. Acad. Sci., Ser. IIc: Chim.* **2001**, *4*, 201−205.

(7) (a) Masi, S.; Top, S.; Boubekeur, L.; Jaouen, G.; Mundwiler, S.; Spingler, B.; Alberto, R. *Eur. J. Inorg. Chem.* **2004**, 2013−2017. (b) Spradau, T. W.; Katzenellenbogen, J. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3235−3240. (c) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 3135−3136.

(8) (a) Marquarding, D.; Klusacek, H.; Gokel, G.; Hoffmann, P.; Ugi, I. *J. Am. Chem. Soc.* **1970**, *92*, 5389. (b) Marquarding, D.; Klusacek, H.; Gokel, G.; Hoffmann, P.; Ugi, I. *Angew. Chem.* **1970**, *82*, 360; *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 371.

(9) (a) Riant, O.; Samuel, O.; Kagan, H. B. *J. Am. Chem. Soc.* **1993**, *115*, 5835. (b) Riant, O.; Samuel, O.; Flessner, T.; Taudien, S.; Kagan, H. B. *J. Org. Chem.* **1997**, *62*, 6733−6745.

(10) Son, S. U.; Park, K. H.; Lee, S. J.; Chung, Y. K.; Sweigart, D. A. *Chem. Commun.* **2001**, 1290−1291.

(11) Ferber, B.; Top, S.; Jaouen, G. *J. Organomet. Chem.* **2004**, *689*, 4872−4876.

Scheme 3. Ortho-Electrophilic Addition

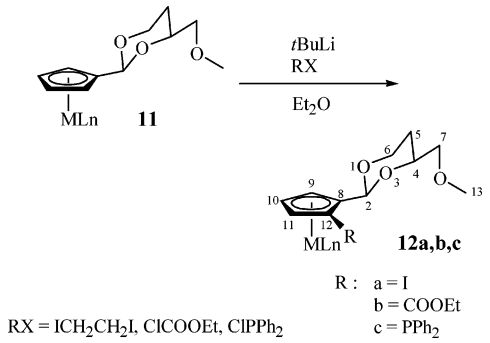


Table 1. Yields of 12

entry	molecule	R	yield, %
1	**12a-Fe**	I	83
2	**12a-Re**	I	63
3	**12b-Re**	COOEt	50
4	**12c-Re**	PPh_2	76
5	**12a-Mn**	I	60

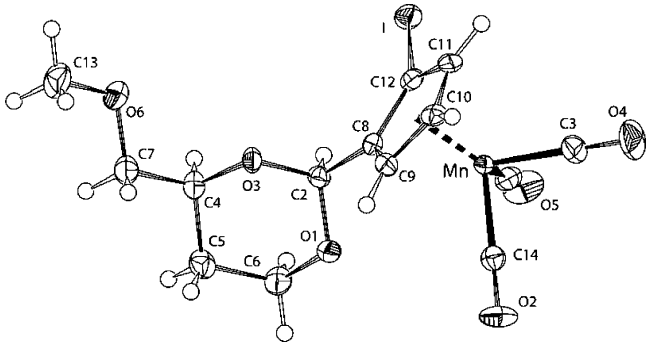


Figure 1. ORTEP view of the complex **12a-Mn**. Selected bond distances (Å) and angles (deg): Mn−C3 = 1.790(4), C3−O4 = 1.156(6), C8−C9 = 1.415(5), C12−I = 2.090(4), C2−C8 = 1.497(5), C2−O1 = 1.415(6); C2−O3−C4 = 112.2(3), C2−O1−C6 = 109.2(3).

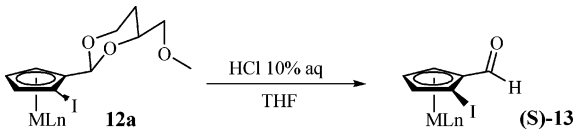
the lithium intermediate (Scheme 3). Addition of 1,2-diiodoethane led to the formation of **(*S*)-12a** in good yield. Under these conditions, the diastereoselectivity was greater than 95:5; only the diastereomer **(*S*)-12a** was obtained, while the second diastereomers were not observed by NMR. The compound **12a-Fe** had already been synthesized by Kagan et al.[9]

Table 1 summarizes the results obtained. We noticed that the lithio intermediates of the Mn and Re series were less stable than the lithio ferrocene derivative. This may explain the relatively low yields for **12a-Re** and **12a-Mn**, 63% and 60%, respectively, compared to that of **12a-Fe**, 83%. In order to verify the diastereoselectivity, two other electrophiles, ethyl chloroformate and chlorodiphenylphosphine, were also used in the case of the cyrhetrene series. **12b-Re** and **12c-Re** were obtained in their enantiopure forms, proving the effectiveness of the ortho-directing group.

The configuration of **12** was confirmed by the single-crystal X-ray structural analysis of **12a-Mn**. The asymmetric unit of the crystal structure of the complex **12a-Mn** (ORTEP view), presented in Figure 1, clearly shows the *S* configuration. We notice that the dioxane ring is almost perpendicular to the cyclopentadienyl ring and the methoxymethyl group stands at the farthest position from the cymantrenyl group. Some selected bond distances and angles are given in the caption of Figure 1.

The removal of the chiral auxiliary was achieved by a mixture of 10% aqueous HCl solution and THF in a 1:1 (v:v) proportion

Scheme 4. Deprotection Reaction



Scheme 5. Synthesis of (*R*)-1-Formyl-2-iodocyclopentadienyl Compounds

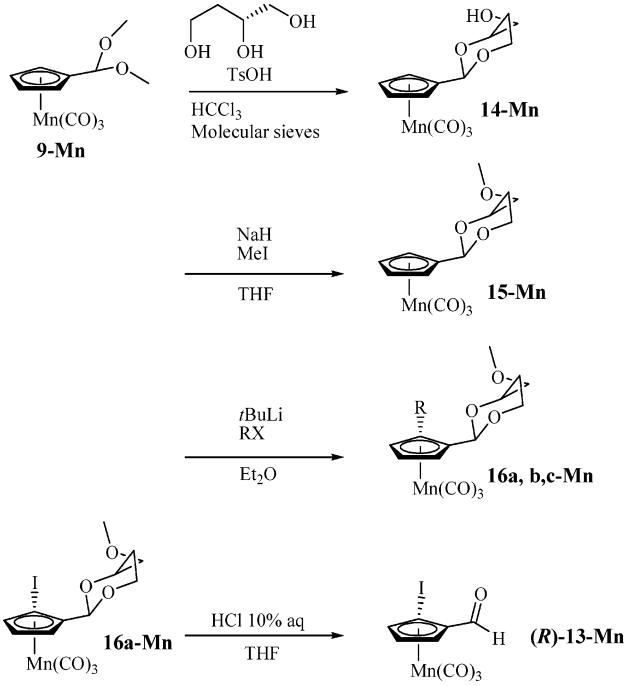


Table 2. Yields of 16

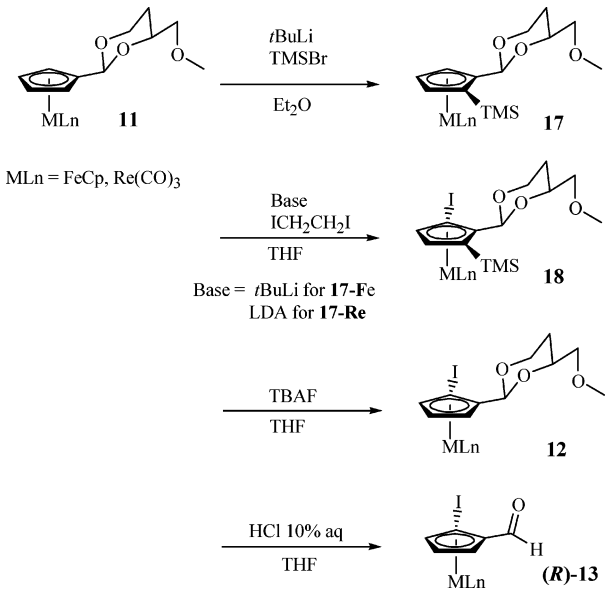
entry	molecule	R	yield, %
1	**16a-Mn**	I	62
2	**16b-Mn**	COOEt	43
3	**16c-Mn**	$P(Ph)_2$	72

(Scheme 4). Under these conditions, the deprotection reaction is very fast for ferrocene (10 min) and slower for cyrhetrene (3 h), while the cymantrene compound needs at least 48 h to achieve a quantitative deprotection.

(*R*)-1-Formyl-2-iodocyclopentadienyl Compounds 13-Fe, 13-Mn, and 13-Re. Following the same procedure as that for the synthesis of the (*S*)-1-formyl-2-iodocyclopentadienyl compounds **13**, the corresponding *R* enantiomers ((*R*)-1-formyl-2-iodocyclopentadienyl compounds **13**) can be prepared by using (*R*)-1,2,4-butanetriol instead of (*S*)-1,2,4-butanetriol. Scheme 5 shows the synthetic pathway for the Mn compounds.

The results of the electrophilic addition reaction are depicted in Table 2. When using diiodoethane as reagent, the yield of **16a-Mn** is similar to that of its diastereomer **12a-Mn**, 62%. This yield increases to 80% when I_2 is used instead of diiodoethane. It is worth noting the difference in reactivity between the cymantrene and ferrocene series; in contrast to the cymantrene compound, the ferrocene compound is not formed in significant yield when I_2 is used. Ethyl chloroformate and chlorodiphenylphosphine gave **16b-Mn** and **16c-Mn** in 43% and 72% yields, respectively. The removal of the chiral auxiliary was achieved by a mixture of 10% aqueous HCl and THF in a proportion of 1:1 (v:v), giving **(*R*)-13-Mn**. All compounds were isolated with an enantioselectivity greater than 95%. Therefore, it is clear that the methoxymethyl-1,3-dioxane group is an excellent ortho-directing group.

Scheme 6. Synthesis of (*R*)-1-Formyl-2-iodocyclopentadienyl Compounds



The synthesis of **16-Mn** requires the repetition of the whole synthetic pathway starting from **9-Mn**. In order to avoid this tedious procedure, we have designed a different and straightforward method for the ferrocene and cyrhetrene series (Scheme 6).[11]

Following the Overman et al. and Manoury et al. procedure,[12,13] we first introduced the trimethylsilyl group at the favored ortho position in order to temporarily block this position. Ortho lithiation was accomplished by the addition of *t*BuLi to a solution of **11** in diethyl ether at −78 °C. The mixture was stirred for 10 min at low temperature and then at room temperature for 45 min in the case of cyrhetrene and for 1 h for the ferrocene series. The mixture was cooled again to −78 °C before addition of $ClSiMe_3$. After this mixture was stirred at −78 °C for 30 min and at room temperature for 1 h, the solvent and unreacted silane were removed under vacuum. The crude product, **17**, was dissolved again in diethyl ether (Fe) or in THF (Mn, Re) and cooled to −78 °C. *t*BuLi (Fe) or LDA (Re) was added, followed by addition of diiodoethane. The compound **18-Fe** was isolated in 43% yield with respect to **11-Fe**, and **18-Re** was isolated in 50% yield from **11-Re**. In the case of the ferrocene series, a second deprotonation by *t*BuLi occurs on the unsubstituted Cp ring, forming 1-acetal-2-(trimethylsilyl)-1′-iodoferrocene as a byproduct after the reaction with chlorotrimethylsilane. We found that a slow warming from −78 °C to room temperature minimizes the formation of this byproduct. In the case of cyrhetrene, the yield is limited by the degradation of the lithium intermediate. The trimethylsilyl group was eliminated by using TBAF, leading to the formation of **(*R*)-13-Fe** and **(*R*)-13-Re**. When *t*BuLi was used in the cyrhetrene and cymantrene series during the second deprotonation, a total degradation was observed and no expected products (**17-Re** and **17-Mn**) were obtained. This degradation may be due to the formation of a Fisher carbene as a result of the reaction between the base and the carbonyl groups.[14] The use of LDA instead of *t*BuLi, which is less nucleophilic, permitted deprotonation without degradation.

Cross-Coupling Reactions. The synthesis of the (*S*)-1-formyl-2-iodo- and (*R*)-1-formyl-2-iodocyclopentadienyl compounds **13** in their pure enantiomeric forms allows the synthesis of optically pure hormone complexes. This can be done by using a cross-coupling reaction with ethynylestradiol (Scheme 7). We have used two coupling reactions, the Stille and the Sonogashira reactions. We found that the reactivity of the iodo compounds is highly variable from one series to another. The conditions for the Sonogashira[15] reaction are 5% $PdCl_2(PPh_3)_2$, 5% $Cu(OAc)_2{\cdot}H_2O$, and diisopropylamine as solvent; the conditions for the Stille[16] reaction are 10% $PdCl_2(MeCN)_2$, tributyltin−ethynylestradiol, and DMF as solvent.

Table 3 gives the results obtained. Neither the (*S*)- nor the (*R*)-1-formyl-2-iodoferrocenes coupled with tributyltin−ethynylestradiol under the Stille reaction conditions, while the Sonogashira reaction gave very good yields of hormone complexes: 70% for **(*R*)-7-Fe** and 77% for **(*S*)-7-Fe**. In contrast, (*S*)- and (*R*)-1-formyl-2-iodocyclopentadienyltricarbonylrhenium did not react under the Sonogashira conditions, while the Stille reaction gave acceptable yields (30−50%). In the case of the cymantrene series, we found that (*S*)- and (*R*)-1-formyl-2-iodocyclopentadienyltricarbonylmanganese undergo coupling under both reaction conditions. A better yield of **(*R*)-7-Mn** was obtained with the Stille reaction (68% versus 45%), while **(*S*)-7-Mn**, in contrast, was obtained in similar yields for both reactions. It is commonly accepted that these palladium-catalyzed reactions are mainly under the control of two reaction steps: the fast oxidative addition of aryl halide on Pd(0) and slow transmetalation. The efficiency of the reaction is improved by decelerating the first step and accelerating the second step in order to reach the stationary regime of the catalytic cycle.[19] In addition, it has been found that the oxidative addition of aryl chlorides to $Pd(PPh_3)_4$ is accelerated by the presence of an electron-withdrawing group on the ring[20] or by complexation of the aryl ring with the electron-withdrawing group $Cr(CO)_3$.[21] Denisovich and Gubin have found that the acidity of the C−H bond of ferrocene, cymantrene, and cyrhetrene increases from ferrocene to cyrhetrene (pK_a = 39, 31, and 30, respectively).[22] As the ferrocenyl group is a stronger electron donor in comparison to the phenyl group, we presume that the ease of oxidative addition follows the order iodoformylcyrhetrene > iodoformylcymantrene > iodoformylferrocene. On the other hand, it has been shown that nucleophiles possessing alkene or alkyne functionalities decelerate the oxidative addition reaction by sequestering the reactive Pd(0) under the less reactive Pd^0(alkene) or Pd^0(alkyne) complex.[19] It is likely that, under Stille reaction conditions, the fast oxidative addition of iodocyrhetrene is efficiently decelerated by sequestering Pd(0) in the [(alkynyl-

(12) Donde, Y.; Overman, L. E. *J. Am. Chem. Soc.*, **1999**, *121*, 2933.
(13) Chiffre, J.; Coppel, Y.; Balavoine, G. G. A.; Daran, J. C.; Manoury, E. *Organometallics* **2002**, *21*, 4552.
(14) For example: Maas, G.; Mayer, D. *J. Organomet. Chem.* **2001**, *617*−618, 339−345.
(15) (a) Pudelski, J. K.; Callstrom, M. R. *Organometallics* **1994**, *13*, 3095−3109. (b) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467. (c) Osella, D.; Nervi, C.; Galeotti, F.; Cavigiolio, G.; Vessières, A.; Jaouen, G. *Helv. Chim. Acta* **2001**, *84*, 3289.
(16) (a) Lo Sterzo, C.; Miller, M. M.; Stille, J. K. *Organometallics* **1989**, *8*, 2331−2337. (b) Lo Sterzo, C.; Stille, J. K. *Organometallics* **1990**, *9*, 687−694. (c) Top, S.; El Hafa, H.; Vessières, A.; Quivy, J.; Vaissermann, J.; Hughes, D. W.; McGlinchey, M. J.; Mornon, J. P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc.* **1995**, *117*, 8372.
(17) Ferber, B.; Top, S.; Welter, R.; Jaouen, G. *Chem. Eur. J.* **2006**, *12*, 2081−2086.
(18) Pike, A. C.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; Engstrom, O.; Ljunggren, J.; Gustafsson, J.-A.; Carlquist, M. *EMBO J.* **1999**, *18*, 4608−4618.
(19) Jutand, A. *Pure Appl. Chem.* **2004**, *76*, 565−576.
(20) Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508−524.
(21) Dufaud, V.; Thivole-Cazat, J.; Basset, J.-M.; Mathieu, R.; Jaud, J.; Waissermann, J. *Organometallics* **1991**, *10*, 4005−4015.
(22) Denisovich, L. I.; Gubin, S. P. *J. Organomet. Chem.* **1973**, *57*, 109−119.

Scheme 7. Synthesis of Complexes of 17α-Ethynylestradiol

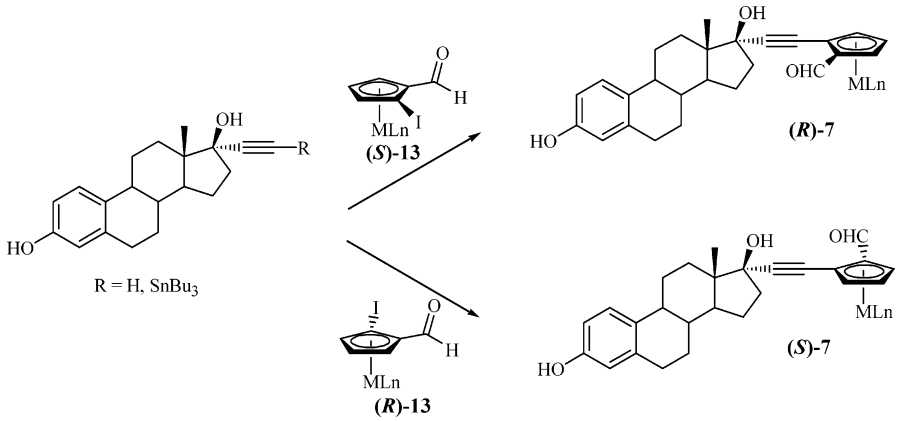


Table 3. Yields of Hormone Complexes

entry	molecule	reacn type	yield, %
1	**(*R*)-7-Fe**	Sonogashira	70
2	**(*R*)-7-Fe**	Stille	0
3	**(*S*)-7-Fe**	Sonogashira	77
4	**(*S*)-7-Fe**	Stille	0
5	**(*R*)-7-Re**	Sonogashira	
6	**(*R*)-7-Re**	Stille	30
7	**(*S*)-7-Re**	Sonogashira	0
8	**(*S*)-7-Re**	Stille	50
9	**(*R*)-7-Mn**	Sonogashira	45
10	**(*R*)-7-Mn**	Stille	68
11	**(*S*)-7-Mn**	Sonogashira	45
12	**(*S*)-7-Mn**	Stille	43

stannane)(Pd)] form, making the oxidative addition rate compatible with that of the transmetalation. Under Sonogashira reaction conditions, the rate of oxidative addition does not match that of transmetalation because the deceleration effect of [(alkynylcopper)(Pd)] or [(alkyne)(Pd)] is less efficient or the rate of transmetalation is too slow. Inversely, in the case of iodoformylferrocene, the reaction conditions are idealy reached in the Songashira reaction, while under Stille reaction conditions, the oxidative addtion step may be too slow compared to the transmetalation. Only the oxidative addition rate of iodoformylcymantrene can match both reations. This hypothesis needs to be verified by experiments, as other factors could also be involved in the mechanism: for example, the reductive elimination step or the nature of the solvent.

The *R* configuration of **(*R*)-7-Fe** was confirmed by single-crystal X-ray analysis. The asymmetric unit of the crystal structure is illustrated in Figure 2. The molecule in the solid state is characterized by the position of the ferrocenyl group, which orients itself almost directly below the D ring of the steroid skeleton. This compact conformation has also been observed for cyrhetrenyl and ruthenocenyl complexes of 17α-ethynylestradiol.[5d,e] Once again the ethynyl linkage is not perfectly linear and shows a slight deformation. The C17−C19−C20 and C19−C20−C21 angles are 173.9(3) and 175.5(2) Å, respectively. Moreover, a bifurcated hydrogen bond between O1/O3 and O2 occurs in this molecular structure (O2−H2O- - -O1 = 2.770(3) Å, 1.97 Å, 158°; O3−H3O- - -O2 = 2.755(3) Å, 2.01 Å, 147°) (Figure 3).

Biochemical Results. The relative binding affinity values (RBAs) for the two forms of the estrogen receptors (ERα and ER*β*) and the lipophilicity (log Po/w) of the ferrocenyl and cymantrenyl complexes were measured and are shown in Table 4.

All the complexes are clearly recognized by the α form of the estrogen receptor (ERα), and the RBA values of the ferrocenyl complexes **(*R*)-7-Fe** and **(*R*)-** and **(*S*)-21-Fe**[17] are above 10%: i.e., significantly higher than the value found for **19** (RBA = 3.1%).[7a] Interestingly, the values found for the *R* and *S* 1,2-disubstituted ferrocenyl complexes are markedly different (10.7 and 5.2, respectively), while similar high values (14.45 and 13.5%) are found for the *R* and *S* 1,3-complexes **(*R*)-** and **(*S*)-21-Fe**, respectively. This is the first time, to our knowledge, that a difference in recognition by the ER has been

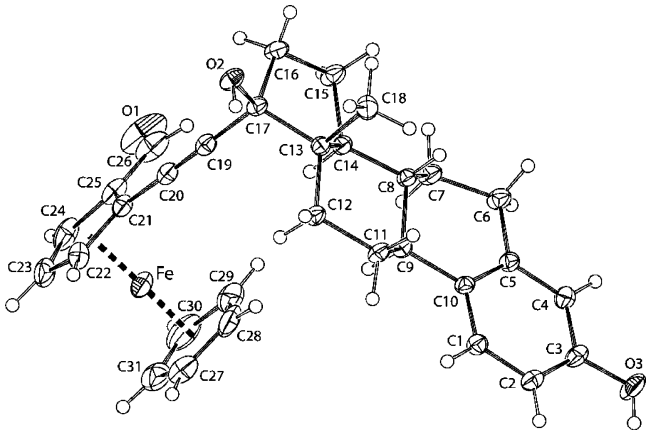


Figure 2. ORTEP view of the complex **(*R*)-7-Fe**. Selected bond distances (Å) and angles (deg): Fe-C29 = 2.032(3), Fe−C21 = 2.041(3), C27−C31 = 1.391(5), C24−C23 = 1.400(4), C20−C21 = 1.441(4); C8−C14−C13 = 112.4(2).

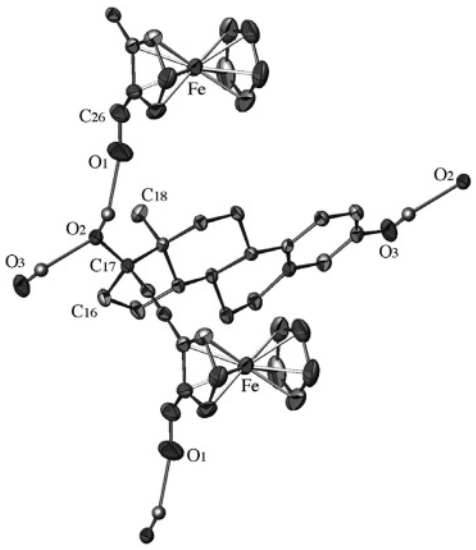
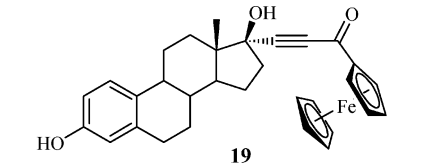


Figure 3. View of the complex **(*R*)-7-Fe** with bifurcated hydrogen bonds between O1/O3 and O2.

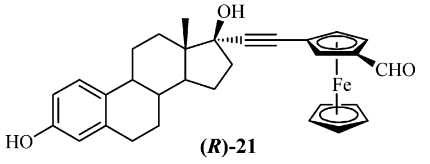
Table 4. Relative Binding Affinities (RBAs) for ERα (Cytosol) and ER*β* (Purified) and Lipophilicity (log Po/w) of the Ferrocenyl− and Cymantrenyl−Estradiol Derivatives

		RBA, %[a]	
compd	log Po/w	on ERα	on ER*β*
(*R*)-7-Fe	4.3	10.7 ± 0.8	0.98 ± 0.02
(*S*)-7-Fe	4.6	5.2 ± 0.9	0.4 ± 0.1
(*R*)-7-Mn	4.7	5.4 ± 0.1	0.6 ± 0.05
(*S*)-7-Mn	4.9	3.1 ± 0.2	0
(*R*)-21-Fe	4.3	14.45 ± 0.05	1.0 ± 0.1
(*S*)-21-Fe	4.3	13.5 ± 0.9	1.6 ± 0.4
19	nd	3.0 ± 0.1	0.9 ± 0.1

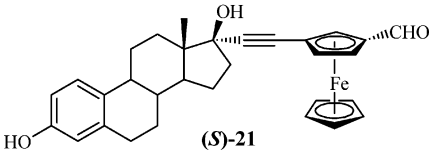
[a] Mean of two experiments ± range.



19



(*R*)-21



(*S*)-21

shown for two diasteromers possessing organometallic planar chirality. On the other hand, the RBA values found for the *β* form of the estrogen receptor are low for all the ferrocenyl complexes (between 1.6 and 0.4). This can be explained by the fact that the hydrophobic pocket of the active site for the *β* receptor is smaller than that of the α receptor (390 and 490 Å^3, respectively).[18] Finally the RBA values found for the cymantrenyl complexes are lower than those of the corresponding ferrocenyl complexes, but the difference between the *R* and *S* 1,2-disubstituted cymantrenyl complexes still exists. The lipophilicity values of the complexes are, as expected, superior to those of estradiol (log Po/w = 3.3) and 17α-ethynylestradiol (3.76), due to the hydrophobic character of the organometallic moieties, which has been well documented.[5,6]

The effect of the iron complexes **(*R*)-** and **(*S*)-7** and **(*R*)-** and **(*S*)-21**, on the proliferation of hormone-dependent MCF-7 breast cancer cells has been studied and compared to the effect of estradiol, the reference estrogen (Figure 4).

At a molarity of 1 *μ*M, all of the complexes display a strong estrogenic effect, equivalent to that found for estradiol and apparently independent of the difference in affinities found for the ER. These results are comparable to those previously described for 17α-ferrocenylethynylestradiol (**1**).[23] At this concentration, there is no cytotoxic expression of the ferrocene moiety, as was found in the series of ferrocenyl−hydroxytamoxifen or ferrocenyl−diphenol compounds; in the latter cases, cytotoxicity seems to be related to a particular 1-(*p*-hydroxyphenyl)-2-ferrocenylethene motif.[3c,24]

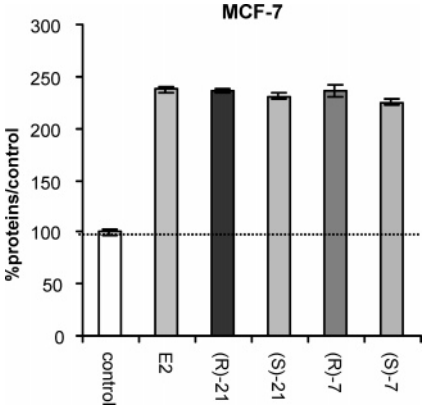


Figure 4. Effect of estradiol (E2 at 1 nM) and of the estradiol−ferrocenyl complexes (1 *μ*M) on the proliferation of MCF-7 cells (hormone-dependent breast cancer cells) after 6 days of culture: representative data of one experiment performed twice with similar results (eight measurements ± limits of confidence; $P = 0.1$, $t = 1.895$).

Conclusion

In summary, a formyl group has been introduced to the cyclopentadienyl ring of 17α-ethynylestradiol derivatives bearing a ferrocenyl, a cymantrenyl, or a cyrhetrenyl group. The presence of this group on the cyclopentadienyl ring generates planar chirality that provokes the formation of two diastereomers, the *Sp* and *Rp* derivatives. These compounds were prepared from the *Sp* and *Rp* 1-formyl-2-iodoferrocenes, -cymantrenes, and -cyrhetrenes, which were separately prepared by using a combination of suitable ortho-directing substituents, such as methoxymethyldioxane and *p*-tolylsulfoxide, as well as trimethylsilyl as a temporary blocking group. A cross-coupling reaction between these precursors and 17α-ethynylestradiol led to the formation of the hormone complexes. Interestingly, the ferrocenyl compounds could only be obtained from a Sonogashira reaction, while the cyrhetrenyl compounds were only formed under Stille conditions, and the cymantrenyl compounds were obtained from both reactions. In comparison to **19**, which is characterized by the presence of a carbonyl functionality between the ethynyl group and the cyclopentadienyl ring, compounds **(*R*)-7-Fe** and **(*S*)-7-Fe** have a better affinity for ERα. It is worth noting that the affinity of the *R* diastereomers **(*R*)-7-Fe** and **(*R*)-7-Mn** is almost twice that of the *S* diastereomers. This is the first time that the estradiol receptor has been shown to recognize the planar chirality of organometallic compounds. In contrast to the case for 1,2-disubstituted derivatives, when the formyl group is at the 1,3-position, as in the case of **(*R*)-21** and **(*S*)-21**, the receptor does not differentiate between the two diastereomers. These molecules **7** and **21** show a proliferative effect with MCF-7 breast cancer cell lines, indicating estrogenic behavior.

Experimental Section

Anhydrous THF and diethyl ether were distilled from sodium/benzophenone. Flash chromatography was performed on silica gel Si 60 (40−63 *μ*M). FT-IR spectra were recorded on a BOMEM Michelson-100 spectrometer. ^{1}H and ^{13}C NMR spectra were acquired on Bruker 300 and 400 spectrometers. Mass spectrometry was performed on a Nermag R 10-10C spectrometer. High-

(23) Vessières, A.; Spera, D.; Top, S.; Misterkiewicz, B.; Heldt, J. M.; Hillard, E. A.; Huché, M.; Plamont, M.-A.; Napolitano, E.; Fiaschi, R.; Jaouen, G. *ChemMedChem* **2006**, DOI: 10.1002/cmdc.200600176.

(24) (a) Vessières, A.; Top, S.; Pigeon, P.; Hillard, E. A.; Boubekeur, L.; Spera, D.; Jaouen, G. *J. Med. Chem.* **2005**, *48*, 3937−3940. (b) Hillard, E. A.; Vessières, A.; Thouin, L.; Jaouen, G.; Amatore, C. *Angew. Chem.* **2005**, *118*, 291−296; *Angew. Chem., Int. Ed.* **2005**, *45*, 285−290.

resolution mass spectrometry (HRMS) was performed on a JEOL MS 700 instrument. Melting points were measured with a Kofler device. The compounds **9-Fe**, **10-Fe**, **11-Fe**, **12a-Fe**, and **(*S*)-13-Fe** were prepared according to literature procedures.[9] The syntheses of compounds **9-Mn**, **10-Mn**, **11-Mn**, **12a-Mn**, **(*S*)-13-Mn, (*R*)-13-Mn**, **14-Mn**, **15-Mn**, **16a-Mn**, **(*S*)-7-Mn**, and **(*R*)-7-Mn** have been reported.[11]

(2*S*,4*S*)-4-(Hydroxymethyl)-2-cyrhetrenyl-[1,3]-dioxane (10-Re). Formylcyrhetrene (1.40 g, 3.7 mmol) was dissolved in trimethyl orthoformate (10 mL) under argon. One drop of H_2SO_4 was added. After the mixture was stirred for 3 h at room temperature, dry K_2CO_3 was added in small portions. The solution was filtered through Celite, and the solvent was removed under reduced pressure. The crude oil obtained was dissolved in dry chloroform (7 mL), and *S*-(−)-1,2,4-butanetriol was added, followed by *p*-toluenesulfonic acid (5%). After the mixture was stirred overnight, K_2CO_3 was added and the solution was filtered through Celite. The filtrate was diluted with diethyl ether (20 mL) and washed three times with water (10 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified on a silica gel column using 1:1 pentane−ethyl acetate as eluent. **10-Re** was obtained as a colorless solid in 60% yield (1.00 g). Mp: 60 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.38 (m, 1H, eq-H5), 1.86 (dq, 1H, J = 5.2 Hz, J = 12.1 Hz, ax-H5), 2.15 (s, 1H, OH), 3.64 (m, 2H, H7), 3.84 (td, 1H, J = 2.7 Hz, J = 11.9 Hz, eq-H6), 3.93 (dd, 1H, J = 2.7 Hz, J = 5.8 Hz, H4), 4.18 (dd, 1H, J = 5.0 Hz, J = 11.4 Hz, ax-H6), 5.27 (m, 3H, H10, H11, and H2), 5.50 (m, 1H, H9 or H10), 5.54 (m, 1H, H9 or H10). ^{13}C{^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 26.4 (C5), 65.5 (C6), 66.4 (C7), 77.5 (C4), 83.4 and 83.5 (C10 and C11), 83.7 and 84.1 (C9 and C12), 95.7 (C2), 107.3 (C8), 194.1 (CO). Anal. Calcd for $C_{13}H_{13}O_6Re$: C, 34.59; H, 2.90. Found: C, 34.57; H, 2.94.

(2*S*,4*S*)-4-(Methoxymethyl)-2-cyrhetrenyl-[1,3]-dioxane (11-Re). In a Schlenk tube, **10-Re** (1.44 g, 2.47 mmol) was dissolved in THF (6 mL) at room temperature. A suspension of sodium hydride (60% in mineral oil, 0.148 g, 3.7 mmol) in THF (2 mL) was added in small portions to the solution of **10-Re**. After 1 h of stirring, iodomethane (230 μL, 3.7 mmol) was added and the stirring was maintained overnight. The mixture was then hydrolyzed with water. The crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude oil obtained was diluted in dichloromethane and was filtered through a silica gel pad. The solvent was evaporated, giving quantitatively **11-Re** as a colorless solid. Mp: 60 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.44 (m, 1H, ax-H5), 1.76 (dq, 1H, J = 5.0 Hz, J = 12.3 Hz, eq-H5), 3.35 (dd, 1H, J = 4.4 Hz, J = 8.9 Hz, H7), 3.38 (s, 3H, H13), 3.48 (dd, 1H, J = 6.1 Hz, J = 10.5 Hz, H7), 3.83 (td, 1H, J = 2.5 Hz, J = 11.9 Hz, eq-H6), 3.95 (m, 1H, H4), 4.16 (ddd, 1H, J = 1.0 Hz, J = 4.9 Hz, J = 11.5 Hz, ax-H6), 5.23 (m, 2H, H10 and H11), 5.25 (s, 1H, H2), 5.50 (t, 2H, J = 2.2 Hz, H9 and H12). ^{13}C{^{1}H} NMR (75.5 MHz, $CDCl_3$): δ 27.8 (C5), 59.8 (C13), 66.8 (C6), 75.5 (C7), 76.4 (C4), 83.3, 83.5, 83.6, and 83.9 (C9, C10, C11, and C12), 95.8 (C2), 108.0 (C8), 194.2 (CO). Anal. Calcd for $C_{14}H_{15}O_6Re$: C, 36.12; H, 3.25. Found: C, 36.69; H, 3.29. $[\alpha]_D^{25}$ = +6° (c = 9.0 g/L, $CHCl_3$). IR ($HCCl_3$): ν 2026, 1934 cm^{-1} (CO).

(2*S*,4*S*,*S*$_{Cyr}$)-4-(Methoxymethyl)-2α-(*o*-iodocyrhetrenyl)-[1,3]-dioxane (12a-Re). 11-Re (200 mg, 0.43 mmol) was dissolved in anhydrous diethyl ether (3 mL) under argon. The solution was cooled to −78 °C, and ^{t}BuLi (0.37 mL, 0.56 mmol) was added dropwise. After 10 min at low temperature, the cold bath was removed and the mixture was stirred at room temperature for 45 min. A pale yellow precipitate was formed. The mixture was then cooled again to −78 °C, and 1,2-diiodoethane (158 mg, 0.56 mmol) dissolved in dry THF (1.5 mL) was added dropwise. The mixture was stirred at −78 °C for 10 min and for 2 h at room temperature. The mixture was quenched with saturated $Na_2S_2O_3$ solution. The crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude oil obtained was purified by flash chromatography on silica gel using 8:2 pentane−diethyl ether as eluent. The **12a-Re** obtained (180 mg, 63% yield) was crystallized in pentane. Mp: 60 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.48 (m, 1H, ax-H5), 1.83 (qd, 1H, J = 5.2 Hz, J = 12.4 Hz, eq-H5), 3.40 (s, 3H, H13), 3.42 (dd, 1H, J = 2.2 Hz, J = 7.3 Hz, H7), 3.52 (dd, 1H, J = 5.9 Hz, J = 10.5 Hz, H7′), 3.91 (td, 1H, J = 2.6 Hz, J = 11.8 Hz, eq-H6), 4.20 (m, 1H, H4), 4.23 (ddd, 1H, J = 1.1 Hz, J = 5.1 Hz, J = 11.5 Hz, ax-H6), 5.19 (s, 1H, H2), 5.30 (t, 1H, J = 2.9 Hz, H11), 5.50 (dd, 1H, J = 2.0 Hz, J = 2.7 Hz, H10), 5.55 (dd, 1H, J = 2.0 Hz, J = 3.0 Hz, H9). ^{13}C{^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 27.5 (C5), 43.4 (C12), 59.5 (C13), 66.9 (C6), 75.1 (C7), 76.6 (C4), 84.2, 86.5, and 90.1 (C9, C10, and C11), 97.3 (C2), 108.2 (C8), 193.6 (CO). Anal. Calcd for $C_{14}H_{14}IO_6Re$: C, 28.43; H, 2.39. Found: C, 28.55; H, 2.21. $[\alpha]_D^{25}$ = −4° (c = 2.0 g/L, $CHCl_3$). IR ($HCCl_3$): ν 2029, 1941 cm^{-1} (CO).

(2*S*,4*S*,*S*$_{Cyr}$)-4-(Methoxymethyl)-2α-(*o*-ethylformatocyrhetrenyl)-[1,3]-dioxane (12b-Re). 11-Re (50 mg, 0.12 mmol) was dissolved in anhydrous diethyl ether (4 mL) under argon. The solution was cooled to −78 °C, and ^{t}BuLi (0.26 mL, 0.39 mmol) was added dropwise. After 10 min at low temperature, the cold bath was removed and the mixture was stirred at room temperature for 45 min. A pale yellow precipitate was formed. The mixture was then cooled again to −78 °C, and ethyl chloroformate (15 μL, 0.153 mmol) was then added dropwise. The mixture was stirred at −78 °C for 10 min and 2 h at room temperature. The mixture was quenched with water. The crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product obtained was purified by flash chromatography on silica gel using 1:1 pentane−diethyl ether as eluent. **12b-Re** (34 mg) was obtained as a colorless oil in 46% yield. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.27 (t, 3H, J = 5.4 Hz, CH_2CH_3), 1.39 (d, 1H, J = 13.5 Hz, eq-H5), 1.82 (qd, 1H, J = 5.4 Hz, J = 13.2 Hz, ax-H5), 3.30 (s, 3H, H13), 3.34 (m, 1H, H7), 3.42 (dd, 1H, J = 4.2 Hz, J = 8.7 Hz, H7), 3.84 (td, 1H, J = 12.0 Hz, J = 21.6 Hz, eq-H6), 3.95 (m, 1H, H4), 4.25 (m, 2H, ax-H6 and CH_2CH_3), 5.22 (t, 1H, J = 2.4 Hz, H10), 5.58 (t, 1H, J = 2.4 Hz, H9), 5.79 (s, 1H, H2), 5.76 (dd, 1H, J = 2.1 Hz, J = 2.7 Hz, H11). ^{13}C{^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 14.1 (CH_2CH_3), 27.4 (C5), 59.3 (C13), 61.3 (C6), 66.8 (C7), 67.2 (CH_2CH_3), 74.9, 83.6 (C2), 84.1 (C10), 86.5 (C12), 88.3 (C9), 95.4 (C11), 110.1 (C8), 163.6 (CO), 192.3 (CO). Anal. Calcd for $C_{17}H_{19}O_8Re$: C, 37.98; H, 3.56. Found: C, 38.19; H, 3.88. $[\alpha]_D^{25}$ = −6° (c = 2.0 g/L, $CHCl_3$). IR ($HCCl_3$): ν 2026, 1937 cm^{-1} (CO).

(2*S*,4*S*,*S*$_{Cyr}$)-4-(Methoxymethyl)-2α-(*o*-(diphenylphosphino)-cyrhetrenyl)-[1,3]-dioxane (12c-Re). 11-Re (50 mg, 0.12 mmol) was dissolved in anhydrous diethyl ether (4 mL) under argon. The solution was cooled to −78 °C, and ^{t}BuLi (0.107 mL, 0.153 mmol) was added dropwise. After 10 min at low temperature, the cold bath was removed and the mixture was stirred at room temperature for 45 min. A pale yellow precipitate was formed. The mixture was then cooled again to −78 °C, and chlorodiphenylphosphine (27.5 μL, 0.153 mmol) was added dropwise. The mixture was stirred at −78 °C for 10 min and for 2 h at room temperature. The mixture was quenched with water. The crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude oil obtained was purified by flash chromatography on silica gel using 1:1 pentane−diethyl ether as eluent. **12c-Re** was obtained as a white solid in 76% yield (66 mg). Mp: 73 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.38 (m, 1H, eq-H5), 1.77 (qd,

1H, J = 5.1 Hz, J = 12.4 Hz, ax-H5), 2.94 (dd, 1H, J = 4.9 Hz, J = 10.4 Hz, H7), 3.02 (dd, 1H, J = 4.7 Hz, J = 10.2 Hz, H7), 3.12 (s, 3H, H13), 3.73 (m, 2H, eq-H6 and H4), 4.14 (dd, 1H, J = 4.0 Hz, J = 10.3 Hz, ax-H6), 4.94 (ddd, 1H, J = 0.6 Hz, J = 1.7 Hz, J = 3.4 Hz, H10), 5.20 (td, 1H, J = 0.8 Hz, J = 2.6 Hz, H9), 5.41 (d, 1H, J = 1.9 Hz, H2), 5.78 (m, 1H, H12), 7.35 (m, 6H), 7.43 (m, 4H). $^{13}C\{^1H\}$ NMR (62.5 MHz, $CDCl_3$): δ 27.5 (C5), 59.1 (C13) 66.7 (C6), 74.5 (C7), 75.9 (C4), 81.8 (C9), 86.0 (C2), 91.8 (C12), 91.8 (C11), 95.9 (C8), 96.0 (C10), 128.1, 128.1, 128.4, 128.5, 128.6, 129.5, 132.6, 132.9, 134.5, 134.8, 193.3 (CO). Anal. Calcd for $C_{26}H_{24}O_6PRe\cdot1.5CH_2Cl_2$: C, 42.51; H, 3.50. Found: C, 42.76; H, 3.48. $[\alpha]_D^{25} = -27°$ (c = 2.0 g/L, $CHCl_3$). IR ($HCCl_3$): ν 2029, 1937 cm^{-1} (CO).

(*S*)-*o*-Iodoformylcyrhetrene ((*S*)-13-Re). 12a-Re (407 mg, 0.68 mmol) was dissolved in THF (10 mL). A 10 mL portion of 10% aqueous HCl was added, and the mixture was stirred for 3 h. The acid was neutralized with solid K_2CO_3 and diluted with diethyl ether (40 mL). The organic layer was washed three times with water, dried over $MgSO_4$, and filtered through silica gel. The solvent was evaporated under reduced pressure, and the **(*S*)-13-Re** obtained was directly used in the cross-coupling reaction (300 mg, 90% yield). ^1H NMR (250 MHz, $CDCl_3$): δ 5.54 (*t*, 1H, J = 3.0 Hz, H5), 5.70 (dd, 1H, J = 1.8 Hz, J = 2.6 Hz, H3), 5.95 (dd, 1H, J = 1.8 Hz, J = 3.2 Hz, H4), 9.58 (s, 1H, CHO).

(S_{Cyr})-17α-[(*o*-formylcyrhetrenyl)ethynyl]estradiol ((*S*)-7-Re). 17α-[(Tributyltin)ethynyl]estradiol (360.2 mg, 0.61 mmol) and (*R*)-*o*-iodoformylcyrhetrene (**(*R*)-13-Re**; 300 mg, 0.61 mmol) were dissolved in DMF (8 mL). Dichlorobis(acetonitrile)palladium(II) (15.8 mg, 0.06 mmol) was added under argon, and the mixture was stirred overnight at room temperature. The mixture was quenched with water, and the crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product obtained was purified by preparative TLC using 2:8 pentane−diethyl ether as eluent, giving **(*S*)-7-Re** in 50% yield as a white solid (201 mg). ^1H NMR (250 MHz, $CDCl_3$): δ 0.91 (s, 3H, H18), 2.81 (m, 2H, H6), 4.91 (s, br, 1H, OH-3), 5.44 (t, 1H, J = 2.7 Hz, H3′), 5.60 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, H4′), 5.96 (dd, 1H, J = 1.8 Hz, J = 3.0 Hz, H2′), 6.56 (d, 1H, J = 2.7 Hz, H4), 6.63 (dd, 1H, J = 2.7 Hz, J = 8.4 Hz, H2), 7.16 (d, 1H, J = 8.4 Hz, H1), 9.81 (s, 1H, CHO). $^{13}C\{^1H\}$ NMR (62.5 MHz, $CDCl_3$): δ 12.8 (C18), 15.2, 22.9, 26.3, 27.1, 29.6 (C6), 33.1, 39.2, 39.4, 43.4, 47.8, 50.0, 60.5, 73.8, 80.5, 85.3 (C3′), 85.7 (C4′), 85.8 (C2′), 87.5, 92.2 (C1′), 98.7 (C5′), 95.8, 112.7, 115.3, 126.5, 132.3, 138.2, 153.4, 183.5 (CHO), 190.8 (Re*CO*). HRMS for $C_{29}H_{31}O_6NRe$: calcd for ^{187}Re, 676.1710; found, 676.1719. IR ($HCCl_3$): ν 2029, 1940 cm^{-1} (CO).

(2*R*,4*R*,S_{Cyr})-4-(Methoxymethyl)-2α-[5′-(trimethylsilyl)-2′-iodocyrhetrenyl]-[1,3]-dioxane (18-Re). 11-Re (358 mg, 0.77 mmol) was dissolved in diethyl ether (5 mL) under argon. The solution was cooled to −78 °C, and ^{t}BuLi (0.56 mL, 0.85 mmol) was added dropwise. After 10 min at low temperature, the cold bath was removed and the mixture was stirred at room temperature for 45 min. A pale yellow precipitate was formed. The mixture was then cooled again to −78 °C, and bromotrimethylsilane (117 μL, 0.92 mmol) was added dropwise. The mixture was stirred at −78 °C for 10 min and for 2 h at room temperature. Solvent and unreacted bromotrimethylsilane were evaporated under reduced pressure. The mixture was dissolved in THF (5 mL) and then cooled to −78 °C. LDA (0.51 mL, 0.92 mmol) was added dropwise. After 10 min at −78 °C, the mixture was stirred at room temperature for 1 h and and cooled again to −78 °C. Diiodoethane (325 mg, 1.15 mmol) in THF (2 mL) was added. The cooling bath was removed, and the mixture was stirred at room temperature for 3 h. The mixture was diluted with diethyl ether and washed three times with water. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude oil obtained was purified by flash chromatography on silica gel using 8:2 pentane−diethyl ether as eluent. **18-Re** was obtained as a pale yellow oil in 50% yield (255 mg). ^1H NMR (250 MHz, $CDCl_3$): δ 0.29 (s, 9H, $SiMe_3$), 1.57 (d, br, 1H, J = 14.4 Hz, eq-H5), 1.91 (dq, 1H, J = 5.1 Hz, J = 12.4 Hz, ax-H5), 3.37 (s, 3H, H13), 3.43 (dd, 1H, J = 5.4 Hz, J = 9.9 Hz, H7), 3.57 (dd, 1H, J = 5.1 Hz, J = 9.9 Hz, H7), 3.93 (dt, 1H, J = 2.7 Hz, J = 14.7 Hz, eq-H6), 4.04 (m, 1H, H4), 4.26 (dd, 1H, J = 4.2 Hz, J = 11.4 Hz, ax-H6), 5.20 (s, 1H, H2), 5.36 (d, 1H, J = 2.7 Hz, H10), 5.52 (d, 1H, J = 2.7 Hz, H11). $^{13}C\{^1H\}$ NMR (62.5 MHz, $CDCl_3$): δ 1.0 ($SiMe_3$), 29.7 (C5), 46.9 (C9), 59.1 (C13), 66.5 (C6), 74.5 (C7), 76.8 (C4), 91.3 (C10), 95.6 (C11), 98.7 (C2), 115.9 (C8), 193.9 (Re*CO*). Anal. Calcd for $C_{17}H_{22}IReO_6Si$: C, 38.36; H, 4.17. Found: C, 38.12; H, 4.01. $[\alpha]_D^{25} = -17°$ (c = 8.0 g/L, $CHCl_3$). IR ($HCCl_3$): ν 2029, 1940 cm^{-1} (CO).

(*R*)-*o*-Iodoformylcyrhetrene ((*R*)-13-Re). 18-Re (126 mg, 0.19 mmol) was dissolved in THF (10 mL), and TBAF (1M in THF, 0.38 mL) was added dropwise. The mixture was stirred at room temperature for 2 h and then diluted with diethyl ether (50 mL). The organic layer was washed three times with water and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2, and the solution was filtered through silica gel. The solvent was removed under reduced pressure and then dissolved in THF (10 mL). A 10 mL portion of 10% aqueous HCl was added, and the mixture was stirred for 3 h. The acid was neutralized with solid K_2CO_3 and diluted with diethyl ether (40 mL). The organic layer was washed three times with water, dried over $MgSO_4$, and filtered through silica gel. The solvent was evaporated under reduced pressure, and the **(*R*)-13-Re** obtained was directly used in the cross-coupling reaction (73 mg, 80% yield).

(R_{Cyr})-17α-[(*o*-formylcyrhetrenyl)ethynyl]estradiol ((*R*)-7-Re). 17α-[(tributyltin)ethynyl]estradiol (360.2 mg, 0.61 mmol) and (*S*)-*o*-iodoformylcyrhetrene (**(*S*)-13-Re**) (300 mg, 0.61 mmol) were dissolved in DMF (8 mL). Dichlorobis(acetonitrile)palladium(II) (15.8 mg, 0.06 mmol) was added under argon, and the mixture was stirred overnight at room temperature. The mixture was quenched with water, and the crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product obtained was purified by preparative TLC using 2:8 pentane−diethyl ether as eluent, giving **(*R*)-7-Re** in 30% yield as a white solid (120 mg). ^1H NMR (250 MHz, $CDCl_3$): δ 0.91 (s, 3H, H18), 2.81 (s, 2H, H6), 4.81 (s, br, 1H, OH-3), 5.44 (t, 1H, J = 0.9 Hz, H3′), 5.61 (dd, 1H, J = 1.8 Hz, J = 2.7 Hz, H4′), 5.97 (dd, 1H, J = 1.8 Hz, J = 3.3 Hz, H2′), 6.56 (d, 1H, J = 2.7 Hz, H4), 6.63 (dd, 1H, J = 3.0 Hz, J = 8.4 Hz, H2), 7.16 (d, 1H, J = 8.4 Hz, H1), 9.82 (s, 1H, CHO). $^{13}C\{^1H\}$ NMR (62.5 MHz, $CDCl_3$): δ 12.8 (C18), 15.2, 22.9, 26.3, 27.1, 29.5 (C6), 33.1, 33.2, 39.2, 39.4, 43.4, 47.8, 49.9, 60.5, 73.8, 80.5, 85.3 (C3′), 85.7 (C4′), 85.7 (C2′), 87.5, 92.2 (C1′), 95.7 (C5′), 98.7, 112.7, 115.2, 126.5, 132.3, 138.2, 153.4, 183.4 (CHO), 190.8 (Re*CO*). HRMS for $C_{29}H_{31}O_6NRe$: calcd for ^{187}Re, 676.1710; found, 676.1710. IR ($HCCl_3$): ν 2029, 1940 cm^{-1} (CO).

(2*S*,4*S*,R_{Fc})-4-(Methoxymethyl)-2α-[5′-(trimethylsilyl)-2′-iodoferrocenyl]-[1,3]-dioxane (18-Fe). 11-Fe (1100 mg, 3.48 mmol) was dissolved in anhydrous diethyl ether (16 mL) under argon. The solution was cooled to −78 °C, and ^{t}BuLi (2.8 mL, 4.17 mmol) was added dropwise. After 10 min at low temperature, the cold bath was removed and the mixture was stirred at room temperature for 1 h. An orange precipitate was formed. The mixture was then cooled again to −78 °C, and bromotrimethylsilane (643 μL, 4.5 mmol) was added dropwise. The mixture was stirred at −78 °C for 10 min and for 2 h at room temperature. The solvent was removed under reduced pressure. The crude oil obtained was dissolved again in diethyl ether (16 mL), and the solution was cooled to −78 °C. ^{t}BuLi (2.8 mL, 4.17 mmol) was added dropwise, and the mixture was stirred at this temperature for 10 min and at room temperature for 1 h, giving a red solution. The mixture was

then cooled again to −78 °C, and a solution of diiodoethane (1470 mg, 5.22 mmol) in THF (6 mL) was added dropwise. The cooling bath was removed, and the mixture was stirred at room temperature for 3 h. The mixture was quenched with water. The crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude oil obtained was purified by flash chromatography on silica gel using 1:9 pentane−diethyl ether as eluent, giving **18-Fe** as an orange oil in 43% yield (770 mg). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.29 (s, 9H, $SiMe_3$), 1.50 (dd, 1H, J = 1.3 Hz, J = 13.2 Hz, eq-H5), 1.82 (qd, 1H, J = 5.3 Hz, J = 12.4 Hz, ax-H5), 3.43 (s, 3H, H13), 3.46 (dd, 1H, J = 6.6 Hz, J = 9.6 Hz, H7), 3.63 (dd, 1H, J = 6.6 Hz, J = 10.0 Hz, H7′), 3.93 (td, 1H, J = 2.6 Hz, J = 14.1 Hz, eq-H6), 4.11 (m, 2H, H4 and ax-H6), 4.18 (d, 1H, J = 2.5 Hz, H10), 4.20 (s, 5H, Cp), 4.53 (d, 1H, J = 2.5 Hz, H11), 5.41 (s, 1H, H2). ^{13}C{^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 1.2 ($SiMe_3$), 27.9 (C5), 46.1 (C9), 59.9 (C13), 67.0 (C6), 72.8 (Cp), 75.6 (C7), 75.8 (C4), 76.3 (10), 76.8 (C11), 89.5 (C8), 102.1 (C2). Anal. Calcd for $C_{19}H_{27}FeIO_3Si$: C, 44.38; H, 5.29. Found: C, 44.19; H 5.44. $[\alpha]_D^{25}$ = −53° (c = 5.0 g/L, $CHCl_3$).

(*R*)-*o*-Iodoformylferrocene ((*R*)-13-Fe). 18-Fe (410 mg, 0.8 mmol) was dissolved in THF (10 mL). TBAF (1 M in THF, 1.59 mL, 1.6 mmol) was added, and the mixture was refluxed overnight. The mixture was diluted with diethyl ether, and the organic layer was washed three times with water. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude oil obtained was purified by flash chromatography on silica gel using 8:1 pentane−diethyl ether as eluent, giving **(*R*)-12-Fe** quantitatively as an orange oil (350 mg). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.48 (m, 1H, eq-H5), 1.79 (m, 1H, ax-H5), 3.47 (s, 3H, H13), 3.49 (dd, 1H, J = 3.0 Hz, J = 10.3 Hz, H7), 3.59 (dd, 1H, J = 6.2 Hz, J = 10.6 Hz, H7′), 3.94 (td, 1H, J = 2.5 Hz, J = 11.7 Hz, eq-H6), 4.23 (m, 1H, H4), 4.17 (m, 1H, ax-H6), 4.18 (s, 5H, Cp), 4.21 (t, 1H, J = 2.5 Hz, H11), 4.39 (dd, 1H, J = 1.5 Hz, J = 2.4 Hz, H12), 4.46 (dd, 1H, J = 1.5 Hz, J = 2.6 Hz, H10), 5.37 (s, 1H, H2). ^{13}C{^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 29.0 (C5), 59.5 (C13), 41.6 (C4), 67.0 (C6), 68.8 (C11), 72.0 (Cp), 74.8 (C12), 75.7 (C7), 76.4 (C10), 86.4 (C8), 100.7 (C2). Anal. Calcd for $C_{16}H_{19}FeIO_3$: C, 43.47; H, 4.33. Found: C, 43.20; H, 4.37. **(*R*)-12-Fe** was dissolved in THF (10 mL). A 10 mL portion of aqueous HCl (10%) was added, and the mixture was stirred for 3 h. The acid was neutralized with solid K_2CO_3 and diluted with diethyl ether (40 mL). The organic layer was washed three times with water, dried over $MgSO_4$, and filtered through silica gel. The solvent was evaporated under reduced pressure, giving **(*R*)-13-Fe**, which was directly used in the cross-coupling reaction.

(R_{Cyr})-17α-[(*o*-Formylferrocenyl)ethynyl]estradiol ((*R*)-7-Fe). 17α-Ethynylestradiol (156 mg, 0.5 mmol) and (*S*)-*o*-iodoformylferrocene (**(*S*)-13-Fe**; 203.9 mg, 0.8 mmol) were dissolved in degassed diisopropylamine (15 mL). Then copper(II) acetate hydrate (5 mg, 0.025 mmol) and dichlorobis(triphenylphosphine)palladium-(II) (17.5 mg, 0.025 mmol) were added into the solution. The mixture was refluxed for 2 h. The mixture was quenched with water, and the crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product obtained was purified by preparative TLC using 2:8 pentane−diethyl ether as eluent, giving **(*R*)-7-Fe** (313 mg) in 77% yield. ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.94 (s, 3H, H18), 1.23 (m, 1H), 1.46 (m, 3H), 1.86 (m, 4H), 2.39 (m, 2H), 2.82 (m, 2H, H6), 4.31 (s, 5H), 4.65 (t, 1H, J = 2.7 Hz, H3′), 4.82 (dd, 1H, J = 2.6 Hz, J = 4.0 Hz, H4′), 4.92 (dd, 1H, J = 1.4 Hz, J = 2.7 Hz, H2′), 6.58 (d, 1H, J = 2.5 Hz, H4), 6.64 (dd, 1H, J = 8.4 Hz, J = 2.5 Hz, H2), 7.17 (d, 1H, J = 8.3 Hz, H1), 10.19 (s, 1H, CHO). ^{13}C{^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 13.0 (C18), 23.0, 26.5, 27.5, 29.7, 33.3, 39.3, 39.5, 43.9, 47.7, 50.6, 53.5, 68.6, 68.7, 71.6 (C6′), 72.9 (C4′), 79.3 (C19), 80.6 (C2′), 81.1 (C20), 93.5 (C1′), 112.8 (C2), 115.4 (C4), 126.6 (C1), 132.3 (C10), 138.2 (C5), 153.6 (C3), 193.2 (C7′). HRMS for $C_{31}H_{33}O_3Fe$: calcd, 509.1780; found, 509.1777. IR ($HCCl_3$): ν 1672 cm^{-1} (CO).

(S_{Cyr})-17α-[(*o*-Formylferrocenyl)ethynyl]estradiol ((*S*)-7-Fe). 17α-Ethynylestradiol (205.9 mg, 0.66 mmol) and (*R*)-*o*-iodoformylferrocene (**(*R*)-13-Fe**; 268 mg, 0.8 mmol) were dissolved in degassed diisopropylamine (18 mL). Copper(II) acetate hydrate (6.7 mg, 0.033 mmol) and dichlorobis(triphenylphosphine)palladium-(II) (23.4 mg, 0.033 mmol) were added into the solution. The mixture was refluxed for 2 h. The mixture was quenched with water, and the crude product was extracted with dichloromethane. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product obtained was purified by preparative TLC using 2:8 pentane−diethyl ether as eluent, giving 284 mg of **(*S*)-7-Fe** (70% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.94 (s, 3H, H18), 1.23 (m, 1H), 1.46 (m, 3H), 1.86 (m, 4H), 2.39 (m, 2H), 2.82 (m, 2H, H6), 4.31 (s, 5H), 4.64 (t, 1H, J = 2.7 Hz, H3′), 4.82 (dd, 1H, J = 2.6 Hz, J = 4.0 Hz, H4′), 4.92 (dd, 1H, J = 1.4 Hz, J = 2.7 Hz, H2′), 6.58 (d, 1H, J = 2.5 Hz, H4), 6.64 (dd, 1H, J = 8.4 Hz, J = 2.5 Hz, H2), 7.17 (d, 1H, J = 8.3 Hz, H1), 10.21 (s, 1H, CHO). ^{13}C{^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 13.0 (C18), 23.1, 26.5, 27.5, 29.7, 33.3, 39.4, 39.6, 44.0, 47.7, 50.1, 53.5, 66.0, 68.5, 71.6 (Cp), 72.9 (C4′), 79.4 (C19), 80.6 (C2′), 81.1 (C20), 93.5 (C1′), 112.8 (C2), 115.4 (C4), 126.6 (C1), 132.4 (C10), 138.3 (C5), 153.6 (C3), 193.1 (C7′). HRMS for $C_{31}H_{33}O_3Fe$: calcd, 509.1780; found, 509.1774. IR ($HCCl_3$): ν 1672 cm^{-1} (CO).

Crystal Data for the Compounds (*R*)-7-Fe and 12a-Mn. The data collections were performed on a Nonius Kappa-CCD area detector diffractometer (Mo Kα, λ = 0.710 70 Å, ω scan) for the compounds **(*R*)-7-Fe** and **12a-Mn**. The relevant data are summarized in Table 5. The cell parameters were determined from reflections taken from one set of 10 frames (1.0° steps in ϕ angle), each at 20 s exposure. The structures were solved using direct methods (SHELXS97) and refined against F^2 using SHELXL97. The data were not corrected for absorption. All non-hydrogen atoms were refined with anisotropic parameters. The hydrogen atoms were included in their calculated positions and refined with a riding model in SHELXL97. The CCDC reference numbers are 606435 (**(*R*)-7-Fe**) and 60436 (**12a-Mn**).

Biochemical Experiments. (a) Materials. 17β-Estradiol was obtained from Sigma-Aldrich (France). Stock solutions (1×10^{-3} M) of the compounds to be tested were prepared in ethanol and were kept at 4 °C in the dark; under these conditions they are stable for at least 2 weeks. Serial dilutions in ethanol were prepared just prior to use. Dulbecco's modified eagle medium (DMEM) was purchased from Gibco BRL, fetal calf serum was obtained from Dutscher, Brumath, France, and glutamine, estradiol, and protamine sulfate were purchased from Sigma. MCF7 and MDA-MB231 cells were from Human Tumor Cell Bank.

(b) Animal Tissues. Sheep uteri weighing approximately 7 g were obtained from the slaughterhouse at Mantes-la-Jolie, France. They were immediately frozen and kept in liquid nitrogen prior to use.

(c) Determination of the Relative Binding Affinity (RBA) of the Compounds for ERα and ERβ. RBA values were measured on ERα from lamb uterine cytosol and on ERβ purchased from Invitrogen Corp. Sheep uterine cytosol prepared in buffer A (0.05 M Tris-HCl, 0.25 M sucrose, 0.1% β-mercaptoethanol, pH 7.4 at 25 °C) as described in ref 4a was used as a source of ERα. For ERβ, 10 μL of the solution containing 3500 pmol/mL was added to 16 mL of buffer B (10% glycerol, 50 mM Bis-Tris-Propane pH 9, 400 mM KCl, 2 mM DTT, 1 mM EDTA, 0.1% BSA) in a silanised flask. Aliquots (200 μL) of ERα in glass tubes or ERβ in polypropylene tubes were incubated for 3 h at 0 °C with [6,7-^3H]-estradiol (2×10^{-9} M, specific activity 1.62 TBq/mmol, NEN Life Science, Boston, MA) in the presence of nine concentrations of

Table 5. Crystal Data and Refinement Details for the Complexes 12a-Mn and (*R*)-7-Fe

	(*R*)-7-Fe	12a-Mn
	Crystal Data	
formula	$C_{31}H_{32}FeO_3$	$C_{14}H_{14}IMnO_6$
formula wt	508.42	460.09
cryst syst	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_1$
a, Å	9.4090(10)	11.167(2)
b, Å	13.163(3)	6.5790(10)
c, Å	20.138(3)	11.698(2)
α, deg	90.00	90.00
β, deg	90.00	109.232(5)
γ, deg	90.00	90.00
V, Å^3	2494.1(7)	811.5(2)
Z	4	2
density, g cm^{-3}	1.354	1.883
μ(Mo Kα), mm^{-1}	0.636	2.739
F(000)	1072	448
	Data Collection	
temp, K	173(2)	173(2)
radiation, Å	0.710 69 (Mo Kα)	0.710 69 (Mo Kα)
min−max *θ*, deg	1.85−30.03	1.84−27.87
data set (*h*, *k*, *l*)	0−13, 0−18, −28 to +28	-14 to +13, −8 to +8, 0−15
total and unique no. of data, *R*(int)	7287, 4832, 0.0000	3567, 3467, 0.0000
obsd data	$>2\sigma(I)$	$>2\sigma(I)$
	Refinement	
N(rflns), *N*(params)	7287, 316	3567, 199
R2, R1, wR2, wR1, GOF	0.0775, 0.0416, 0.1032, 0.0939, 0.941	0.0295, 0.0275, 0.0935, 0.0839, 1.022
max, av shift/error	0.001, 0.000	0.002, 0.000
Flack *x*	0.000(15)	−0.01(2)
min, max resid dens, e Å^{-3}	−0.456, 0.417	−1.640, 0.635

the hormones to be tested. At the end of the incubation period, the free and bound fractions of the tracer were separated by protamine sulfate precipitation. The percentage reduction in binding of [^3H]-estradiol (Y) was calculated using the logit transformation of *Y* (logit *Y*: $\ln[y/1 - Y]$ versus the log of the mass of the competing steroid. The concentration of unlabeled steroid required to displace 50% of the bound [^3H]-estradiol was calculated for each steroid tested, and the results were expressed as RBA. The RBA value of estradiol is by definition equal to 100%.

(d) Measurement of Octanol/Water Partition Coefficient (log Po/w) of the Compounds. The log Po/w values of the compounds were determined by reverse-phase HPLC on a C-8 column (nucleosil 5.C8, from Macherey Nagel, France) according to the method previously described by Minick[25] and Pomper.[26] Measurement of the chromatographic capacity factors (k') for each compound was done at various concentrations in the range 85−60% methanol (containing 0.25% octanol) and an aqueous phase consisting of 0.15% *n*-decylamine in 0.02 M MOPS (3-morpholinopropanesulfonic acid) buffer pH 7.4 (prepared in 1-octanol-saturated water). These capacity factors (k') are extrapolated to 100% of the aqueous component given the value of k_w'. log Po/w (y) is then obtained by the formula $\log \text{Po/w} = 0.134\,18 + 0.984\,52 \log k_w'$.

(e) Culture Conditions. Cells were maintained in monolayer in DMEM with phenol red (Gibco BRL) supplemented with 8−9% fetal calf serum (Gibco BRL) and glutamine 2 mM (Sigma) at 37 °C in a 5% CO_2 air humidified incubator. For proliferation assays, cells were plated in 1 mL of DMEM medium with phenol red, supplemented with 10% decomplemented and hormone-depleted fetal calf serum and 2 mM glutamine, and incubated. The following day (D0) 1 mL of the same medium containing the compounds to be tested was added to the plates (final volumes of alcohol: 0.1%; four wells for each condition, one plate per day). After 3 days (D3) the incubation medium was removed and fresh medium containing the compounds was added. After 6 days (D6) the total protein content of the plate was analyzed by methylene blue staining as follows. Cell monolayers were fixed for 1 h in methanol, stained for 1 h with methylene blue (1 mg/mL) in PBS, and then washed thoroughly with water. One milliliter of HCl (0.1 M) was then added, and the absorbance of each well was measured at 620 nm with a Biorad spectrophotometer. The results are expressed as the percentage of proteins versus the control.

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Supporting Information Available: Additional details of the crystal data collection and refinement and CIF files giving crystal data for **(*R*)-7-Fe** and **12a-Mn**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Minick, D. J.; Frenz, J. H.; Patrick, M. A.; Brent, D. A. *J. Med. Chem.* **1988**, *31*, 1923−1933.

(26) 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.