APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organomet. Chem. 2006; **20**: 168–174
Bioorganometallic Chemistry AOC
DOI:10.1002/aoc.1034

Synthesis of cyclopentadienyltricarbonylrhenium substituted benzhydryl species and oestrogen receptor binding properties

D. Plażuk[1,2], F. Le Bideau[1], A. Pérez-Luna[1], E. Stéphan[1], A. Vessières[1], J. Zakrzewski[2] and G. Jaouen[1]*

[1]UMR CNRS 7576, Ecole Nationale Supérieure de Chimie de Paris, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France
[2]Department of Organic Chemistry, University of Łódź, Narutowicza 68, 90-136 Łódź Poland

Received 27 October 2005; Accepted 23 November 2005

6-(4-Methoxyphenyl)fulvene has been efficiently engaged in a process implying nucleophilic attack of methyllithium followed by a transmetallation reaction in the presence of different organorhenium sources {$XRe(CO)_5$: X = Cl, Br, OTf; $BrRe(CH_3CN)_2(CO)_3$, $[BrRe(CO)_3THF]_2$} to afford the corresponding cyclopentadienyltricarbonylrhenium-substituted compound. This study allowed us to determine the best complex for that transformation, and similar reaction conditions were used with success to provide new potential radiopharmaceuticals in the cold series. One of these compounds [the (4,4′-dihydroxybenzhydryl)cyclopentadienyltricarbonylrhenium] displays a good recognition for the α form of the oestrogen receptor.

KEYWORDS: bioorganometallic chemistry; cyclopentadienyl ligands; rhenium; fulvene

INTRODUCTION

Benzhydryl compounds containing hydroxyl groups in the phenyl rings are interesting in the search for new anticancer drugs since they are expected to display high affinity for the oestrogen receptors.[1–3] Such an affinity combined with the presence of a $CpRe(CO)_3$ unit in these molecules, **1a–d** (Scheme 1), could provide promising candidates as potential radiopharmaceuticals.[4]

Because of a potential development in the radioactive series, we needed short and efficient routes to these species with the aim of introducing the metal as late as possible in the synthesis. We previously reported the synthesis of a large number of organometallic-substituted steroids.[5–7] Among them, a non-radioactive $CpRe(CO)_3$-substituted estradiol has been shown to have an excellent binding affinity for the oestrogen receptor (ER) when the organorhenium moiety was fixed at the 17α position.[8] We have also previously described[9] the access to a diastereomeric mixture of $CpRe(CO)_3$-substituted steroids, **3**, from a fulvene, **2**, involving a nucleophilic attack/transmetallation process (Scheme 2).

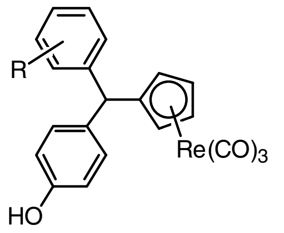
The poor yield (39%) obtained in this two-step procedure led us to look at whether modification of the reaction conditions and the nature of the reactants will result in a more efficient method of synthesis of the substituted $CpRe(CO)_3$ complexes bearing biologically relevant moities. We report herein an improvement of this reaction obtained with the modification of the metallic source, and the application of this procedure to the synthesis of type **1** molecules.

SYNTHESIS OF THE SUBSTITUTED CYCLOPENTADIENYLTRICARBONYLRHENIUM SPECIES

The fulvene, **4**, which is not prone to basic transformation, was picked as a model substrate for the optimization process (Scheme 3). Methyllithium was added to **4** at room temperature and reacted 10 min before the addition of the metallic source in different solvents and at different temperatures within 3 h (Table 1).

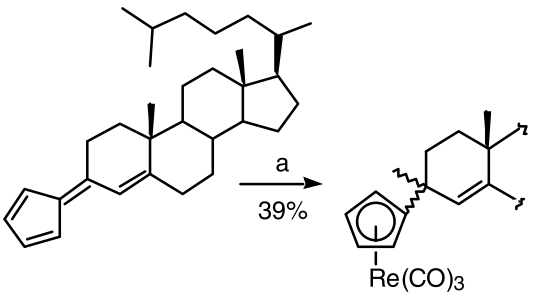
---

*Correspondence to: G. Jaouen, UMR CNRS 7576, Ecole Nationale Supérieure de Chimie de Paris, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France.
E-mail: gerard-jaouen@enscp.fr
Contract/grant sponsor: EC Marie Curie Fellowship; Contract/grant number: HMPT-CT-2000-00186.



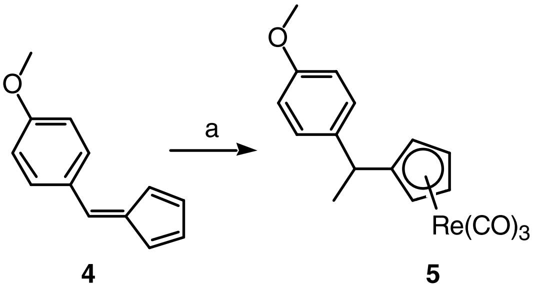
1a R = *p*-OH, **1b** R = *m*-OH
1c R = *o*-OH, **1d** R = *p*-CF$_3$

Scheme 1.



2 **3**

Scheme 2. (a) 1, MeLi, toluene, −78 °C to r.t.; 2, BrRe(CO)$_5$, toluene Δ, 3 h.



4 **5**

Scheme 3. (a) 1, MeLi, r.t. 10 min; 2, metallic source, 3 h (see Table 1).

The substitution of a bromide by a chloride atom on the metallic source does not influence the transmetallation process since these reagents bearing Cl or Br led to the same yield of product **5** (entries 1 and 2) in refluxing toluene. The use of a complex bearing the more labile triflate group, on the other hand, gave a 10% increase in yield under the same conditions (entry 3). We next turned our attention towards BrRe(CH$_3$CN)$_2$(CO)$_3$ and [BrRe(CO)$_3$THF]$_2$, which can be easily synthesized via carbon monoxide displacement from BrRe(CO)$_5$ in refluxing acetonitrile[10] and THF respectively.[11] With the acetonitrile complex, at toluene reflux, the yield of **5** increased dramatically (71%) in comparison with the use of bromorheniumpentacarbonyl (entry 4 vs 1). Using the acetonitrile- and THF-substituted rhenium complexes, compound **5** was obtained in moderate yields (52 and 45% respectively) in toluene at only 25 °C, and despite the lack of solubility encountered for these species at that temperature (entries 5 and 6). This problem was solved working in THF (entry 7) at room temperature, thus allowing access to **5** in good yield (71%), as good as that obtained at toluene reflux with the acetonitrile derivative. That kind of transmetallation was reported from a cyclopentadienyl thalium intermediate using slightly different metallic sources ([ClRe(CO)$_3$THF]$_2$, ClReL$_2$(CO)$_3$ L = CH$_3$CN, pyridine, DME).[12] The best yield (80%) was obtained with the THF-substituted complex at 0 °C in THF. More recently, Mull *et al.* reported the synthesis of substituted cyclopentadienyltricarbonylrhenium species from the corresponding cyclopentadienyllithium compounds at room temperature using [BrRe(CO)$_3$THF]$_2$ with yields ranging from 37 to 83%.[13] THF is the solvent of choice for this reaction because it can also act as a labile ligand after replacement of two carbonyls on the rhenium complex before transmetallation. Nevertheless, the lowest reflux temperature of that solvent in comparison with toluene's one is not in favour of such a substitution within 3 h (the formation of [BrRe(CO)$_3$THF]$_2$ from BrRe(CO)$_5$ needs 22 h to be complete) and could explain the difference in yields in these two solvents (Table 1, entries 1 and 8).

Table 1. Reaction of compound **4** with MeLi followed by transmetallation with different metallic sources under selected conditions

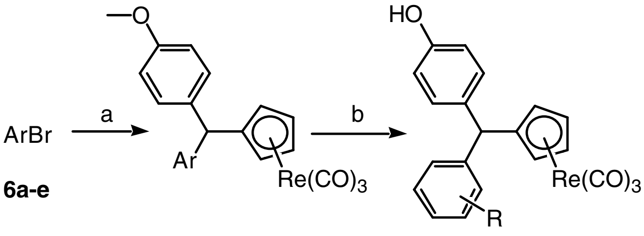
Entry	Metallic source	Solvent	*T* (°C)	Yield (%)[a]
1	BrRe(CO)$_5$	Toluene	110	36
2	ClRe(CO)$_5$	Toluene	110	36
3	CF$_3$SO$_3$Re(CO)$_5$	Toluene	110	45
4	BrRe(CH$_3$CN)$_2$(CO)$_3$	Toluene	110	71
5	BrRe(CH$_3$CN)$_2$(CO)$_3$	Toluene	25	52
6	[BrRe(CO)$_3$THF]$_2$	Toluene	25	45
7	[BrRe(CO)$_3$THF]$_2$	THF	25	71
8	BrRe(CO)$_5$	THF	67	21

[a] Yields of isolated products are based on fulvene.

These results and those reported in the literature show that the most difficult event in such a transmetallation is the loss of two carbonyl ligands in the rhenium complex while the nature of X (Br, Cl, OTf) imposes little if any influence on the reaction.

On the basis of these results, we chose [BrRe(CO)$_3$THF]$_2$ as the metallic source and we synthesized some new cyclopentadienyltricarbonylrhenium derivatives **7a–e** (Scheme 4, Table 2) with slightly modified reaction conditions (see Experimental part).

Five different substituted aryl bromide compounds **6a–e** were transformed into the corresponding aryllithium species through a classical halogen metal exchange. These



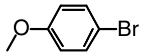
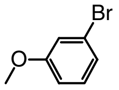
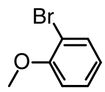
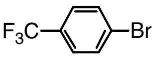
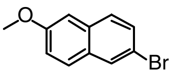
6a-e

7a-e

1a (94%); **1b** (75%)
1c (61%); **1d** (67%)
1e (83%)

Scheme 4. (a) 1, *n*-BuLi, −78 °C, 1 h., THF; 2, **4**, 10 min, r.t.; 3, $[BrRe(CO)_3THF]_2$, THF, r.t. (b) BBr_3, CH_2Cl_2, r.t., 40 min.

Table 2. Reaction of compound **4** with different aryllithiated species followed by a transmetallation with $[BrRe(CO)_3THF]_2$

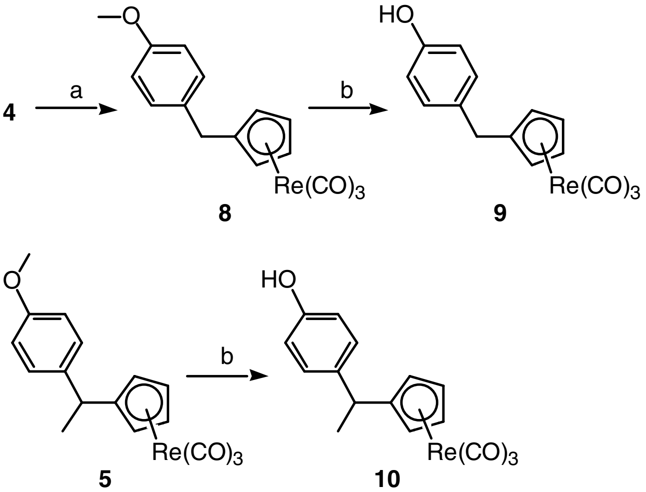
Entry	ArBr	Time	Yield[a]	Compound
1		1 h.	48%	**7a**
2		1 h.	46%	**7b**
3		1 h.	47%	**7c**
4		2 h.	29%	**7d**
5		1 h.	40%	**7e**

[a] Yields of isolated products are based on rhenium.

Table 3. RBA and log $P_{o/w}$ for compounds **1a–e, 9** and **10**

	RBA (%) cytosol	RBA (%) on ERβ	log $P_{o/w}$
1a	12.6 ± 1.8	5.9 ± 0.2	4.37
1b	2.2 ± 0.7	0.67 ± 0.08	4.59
1c	0.47 ± 0.08	0.5 ± 0.2	4.57
1d	1.45 ± 0.07	0.37 ± 0.04	6.05
1e	6.5 ± 0.85	7.7 ± 3.1	5.00
9	0.27 ± 0.0	0.015 ± 0.05	4.22
10	2.6 ± 0.46	0.26 ± 0.2	4.33
17β-Estradiol	100	100	3.1

intermediates were reacted *in situ* with compound **4** before the addition of $[BrRe(CO)_3THF]_2$ to afford **7a–e** in moderate to good yields (29–48%, overall yield) (yields are not as good as in the case of methyllithium, which is less hindered than the aryl aryllithium species used here). In the case of compound **6d**, the electron-withdrawing effect of the CF_3 group making the aryllithium species less nucleophilic is probably responsible for the lower yield and longer reaction time (Table 2, entry 4).



8 **9** **5** **10**

Scheme 5. (a) 1, $LiAlH_4$, −78 °C to r.t., 1 h, THF; 2, $[BrRe(CO)_3THF]_2$, THF, r.t. (b) BBr_3, CH_2Cl_2, r.t., 40 min.

We also used $LiAlH_4$ for the synthesis of **8**, the reduced form of **4** (Scheme 5).

Eventually, the corresponding hydroxy compounds **1a–e** (Scheme 4), **9** and **10** (Scheme 5) have been obtained in 61–94% yields by a routine demethylation of **7a–e, 5** and **8** with BBr_3 in dichloromethane at room temperature.[14]

BIOCHEMICAL STUDIES

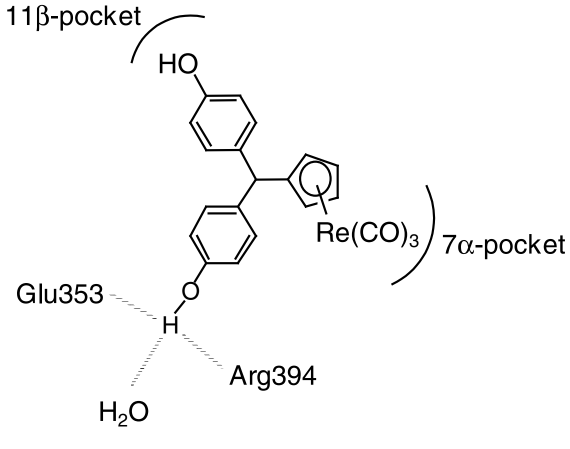
The relative binding affinities (RBA) values of complexes **1a–e, 9** and **10** were measured for both the α and β forms of the oestrogen receptor (ERα or ERβ). For the sake of comparison with previously published values, we used lamb uterine cytosol as the source of ERα and purified ERβ (Table 3).

The diphenol complex **1a** with the two OH groups in the *para* positions shows a remarkable affinity for the α receptor (RBA = 12.6% by comparison with 17β-estradiol taken at 100%). However by changing the position of one OH group, the RBA value decreases (*m*-OH, **1b**, RBA = 2.2%; *o*-OH, **1c**, RBA = 0.47%). A decrease in RBA values is also observed for the β receptor upon changing the position of one OH group from *para* (RBA = 5.9%) to *meta* and *ortho* positions (RBA = 1.2 and 1.4% respectively). The replacement of one *para*-OH group for CF_3 also decreases the affinity. Interestingly, compound **1e** with one phenol substituted for a naphthol shows a relatively good affinity both for the α (RBA = 6.5%) and β receptor (RBA = 7.7%). Finally, replacement of one phenol by H (compound **9**) or CH_3 (compound **10**) clearly decreases the affinity. In this series of complexes, the *para*-diphenol complex **1a** exhibits the best potential for further studies, in terms of ER recognition.

The lipophilicity ($\log P_{o/w}$) of these complexes was determined by HPLC (Table 3). The reference value in this series of measurements is estradiol ($\log P_{o/w} = 3.1$). It is worth noting that, if all organometallic complexes have $\log P_{o/w}$ values higher than those of estradiol, then the penetration of the compounds inside the cells will be made easier. Breast tissues are relatively fat and a too high lipophilicity would hinder selectivity for the target cells containing ERs. It then appears that molecules such as **1a** with a $\log P_{o/w} = 4.37$ would be a good compromise in term of cell selectivity.

Several X-Ray crystal structures of the ligand-binding domains (LBDs) of the ER (α or β) bound to various bioligands have become available.[15–17] In addition, recent papers have used molecular modelling as an aid to understanding the structure–affinity relationships of several 1,1-diaryl-ethylene motifs with the ERs binding site.[18,19] We have also previously used this approach in the OH-ferrocifen series.[20] Based on Katzenellenbogen's work[21] for 1,1-paradiphenol motifs, we suggest that the binding of one of the phenols in **1a** is similar to the association of the A ring of estradiol with Glu 353, H_2O, Arg 394 while the other phenol group is directed towards the 11-β pocket (Scheme 6).

This molecule does not open the 11β-pocket but, in the closed oestrogenic conformation, there are two polar residues near the N-terminus of helix-3. This second OH group might be well positioned to engage in hydrogen bonding with Thr 347 and Asp 351. It is worth noticing that in ERβ only Thr 299 is within hydrogen bonding distance for a *para* phenol. Upon modifying the position of the phenolic OH group (*para* OH for *meta* OH and *ortho* OH) the establishment of a hydrogen bond is less favourable for these racemic mixtures. This tentative explanation for the different binding affinities will be sustained or not in the future by our own modelling studies. It remains that **1a** shows a good potential in terms of its recognition by the oestrogen receptor.



Scheme 6.

CONCLUSION

The results presented above describe an easy access to non-radioactive cyclopentadienyltricarbonylrhenium-substituted species as new potential radiopharmaceuticals. This access is based on a one-pot process involving a nucleophilic attack on a fulvene followed by a transmetallation. The nature of the metallic source, for this second reaction was studied in detail and we showed that the best results were obtained using a THF derivative of the bromorheniumpentacarbonyl at room temperature in THF. This strategy was applied with success to the synthesis of several benzhydryl compounds containing at least one hydroxyl group in the phenyl ring. The study of their binding affinities for the oestrogen receptors has shown that compound **1a** recognizes the two isoforms of ER with an affinity sufficiently high for further study.

EXPERIMENTAL

Chemical part

^1H NMR and ^{13}C NMR spectra were taken on a 200 MHz Bruker AC 200 spectrometer. Chemical shifts are reported in ppm and referenced to the residual proton resonances of the solvents. Infrared (IR) spectra were recorded using a BOMEN MB spectrometer. Mass spectra were obtained on NERMAG R1010C apparatus. HRMS were measured at the Service de Spectrométrie de Masse of the Ecole Normale Supérieure (Paris, France). Melting points were measured on a Büchi B-510 apparatus and were uncorrected. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F 254. Silica gel Merck Gerudan SI (40–63 μm) was used for column chromatography. Elemental analyses were measured at the microanalysis laboratory of the Pierre et Marie Curie University (Paris, France) or of the CNRS (Gif sur Yvette, France). Anhydrous methanol (99.8%) was purchased from Aldrich. Reactions were carried out in flame-dried Schlenk glassware under an inert atmosphere (argon). $BrRe(CO)_3(CH_3CN)_2$,[10] $[BrRe(CO)_3THF]_2$,[11] $ClRe(CO)_5$[22] and $CF_3SO_3Re(CO)_5$[23] were synthesized according to the literature. Compounds 4[24] and 5[25] were identified by comparison with the published data.

Reaction of 6-(4-methoxyphenyl)fulvene with MeLi and $XRe(CO)_3L_2$

General procedure

MeLi ($c = 1.16$ M in diethyl ether, 0.660 mL, 0.8 mmol) was added dropwise at room temperature to a solution of 6-(4-methoxyphenyl)fulvene **4** (94 mg, 0.5 mmol) in 1.2 ml solvent. After 10 min, the metallic source (0.8 mmol) was added and the mixture was stirred for 3 h at different temperatures (see Table 1). The reaction mixture was poured into water, extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated. Purification by flash chromatography (petroleum ether–diethyl ether = 9 : 1) afforded the desired product **5** as a pale yellow solid.

General procedure for the reaction of 6-(4-methoxyphenyl)fulvene 4 with different aryllithium followed by transmetallation with $[BrRe(CO)_3(THF)]_2$

To a solution of arylbromide **6ae** (1 mmol) in 5 mL THF was added *n*-BuLi ($c = 1.6$, 0.614 mL, 1 mmol) at −78 °C. The reaction mixture was stirred at that temperature for 1 h. A solution of 6-(4-methoxyphenyl)fulvene (246 mg, 1.3 mmol) in 5 ml THF was then added at −78 °C and the cold bath removed. After 15 min, a solution of $[BrRe(CO)_3(THF)]_2$ (282 mg, 0.3 mmol) in 5 ml THF was added in one portion and the reaction mixture was stirred for 1 h at room temperature, quenched with water, extracted by CH_2Cl_2, dried over $MgSO_4$ and concentrated. Products **7a–e** were isolated by flash chromatography (petroleum ether–ether = 9 : 1).

(4,4′-dimethoxybenzhydryl) cyclopentadienyltricarbonylrhenium, 7a

Pale yellow solid. ^1H NMR (200 MHz, $CDCl_3$) δ 3.78 (s, 6H, OCH_3); 5.09 (s, 1H, C*H*); 5.21 (t, $J = 2.2$ Hz, 2H, Cp); 5.26 (t, $J = 2.2$ Hz, 2H, Cp); 6.83 (d, J = 8.8 Hz, 2H, C*H*$COCH_3$); 7.26 (d, J = 8.7 Hz, 2H, C*H*CHCOCH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 48.5 (CH); 55.3 (OMe); 82.9 (Cp); 86.7 (Cp); 113.3 (Cp, Q); 113.8 (C-3); 129.5 (C-2); 135.3 (Ar); 158.4 (Ar); 194.5 (CO); IR (KBr) 2023; 1934; 1914 cm^{-1}; MS: 562 $(M)^{+\bullet}$; 532 $(M\text{-}CH_2O)^{+\bullet}$; 478 $(M - 3\,CO)^{+\bullet}$; m.p.: 93 °C.

(3,4′-dimethoxybenzhydryl) cyclopentadienyltricarbonylrhenium, 7b

Pale yellow oil ^1H NMR (200 MHz, $CDCl_3$) δ 3.78 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 5.10 (s, 1H, C*H*); 5.3–5.2 (m, 4H, Cp); 6.71 (t, $J = 2.2$ Hz, 1H, H-2); 6.73 (d, $J = 8, 0$ Hz, 1H, H-4); 6.77 (ddd, $J = 8.2$, 2.5, 0.8 Hz, H-6); 6.84 (d, $J = 8.8$ Hz, 2H, H-3′); 7.08 (d, $J = 8.6$ Hz, 2H, H-2′); 7.21 (t, $J = 7.9$ Hz, 1H, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.3 (CH); 55.2 (OCH_3); 55.3 (OCH_3); 82.8; 83.2; 86.7; 87.0 (Cp-H); 111.9 (C-6); 112.6 (Cp Q); 113.9 (C-3′); 114.7 (C-2); 121.0 (C-4); 129.4 (C-5); 129.6 (C-2′); 134.8; 144.8; 158.5; 159.6; 194.2 (CO); IR (KBr): 2020; 1918 cm^{-1}; MS: 562 $(M)^{+\bullet}$; 534 $(M\text{-}CO)^{+\bullet}$; 478 $(M - 3\,CO)^{+\bullet}$. Anal. Calcd for $C_{23}H_{19}O_5Re$: C, 49.19; H, 3.41. Found: C, 50.01; H, 3.55.

(2,4′-dimethoxybenzhydryl) cyclopentadienyl-tricarbonylrhenium, 7c

Pale yellow solid; ^1H NMR (400 MHz, $CDCl_3$) δ 3.77 (s, 6H, OCH_3); 5.20 (t, $J = 1.7$ Hz, 1H, Cp); 5.24 (t, $J = 1.5$ Hz, 1H, Cp); 5.28 (t, $J = 1.7$ Hz, 1H, Cp); 5.31 (t, $J = 1.4$ Hz, 1H, Cp); 5.55 (s, 1H, C*H*); 6.82 (d, $J = 8.7$ Hz, 2H, H-3′); 6.86 (dd, $J = 8.2$, 0.7 Hz, 1H, H-3); 6.91 (dt, $J = 7.6$, 1.0 Hz, 1H, H-5); 7.01 (dd, $J = 7.6$, 1.7 Hz, 1H, H-6); 7.09 (d, $J = 8.6$ Hz, 2H, H-2′); 7.22 (dt, $J = 7.6$, 1.7 Hz, 1H, H-4)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.0 (CH); 55.2 (OCH_3); 81.6 (Cp-H); 83.4 (Cp-H); 86.8 (Cp-H); 88.3 (Cp-H); 110.7 (C-3); 111.4 (Cp-CH); 113.6 (C-3′); 120.1 (C-5); 128.1 (C-4); 129.0 (C-6); 129.6 (C-2′); 132.0; 134.8; 156.2; 158.2; 194.3 (CO); IR (KBr): 2020; 1921; MS: 562 $(M)^{+\bullet}$; 532 $(M\text{-}CO)^{+\bullet}$; 478 $(M - 3\,CO)^{+\bullet}$. Anal. Calcd for $C_{23}H_{19}O_5Re$: C, 49.19; H, 3.41. Found: C, 49.36; H.3.45; m.p.: 100 °C.

(4-methoxy-4′-trifluoromethyl) benzhydrylcyclopentadienyltricarbonylrhenium, 7d

Pale yellow oil; ^1H NMR (200 MHz, $CDCl_3$) δ 3.79 (s, 3H, OCH_3); 5.1–5.3 (m, 4H, Cp); 6.86 (d, $J = 11.6$ Hz, 2H, Ar); 7.05 (d, $J = 11.6$ Hz, 2H, Ar); 7.28 (d, $J = 8.0$ Hz, 2H, Ar); 7.57 (d, $J = 8.2$ Hz, 2H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 49.3; 55.4; 83.2; 83.5; 56.7; 87.2; 111.5; 114.2; 124.2 (q, $J = 271.6$ Hz, CF_3); 129.0; 129.5; 134.1; 147.3; 158.8; 194.0; IR (KBr) 2023; 1924 cm^{-1}; MS: 600 $(M)^{+\bullet}$; 581 $(M\text{-}F)^+$; 570 $(M\text{-}CH_2O)^{+\bullet}$; 516 $(M - 3\,CO)^{+\bullet}$; m.p.: 62 °C.

[4-methoxy-α-(6-methoxy-2-naphthyl)-benzyl] cyclopentadienyl-tricarbonylrhenium, 7e

Pale yellow solid; ^1H NMR (400 MHz, $CDCl_3$) δ 3.80 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3); 5.3–5.2 (m, 5H, Cp and CH); 6.86 (d, $J = 8.7$ Hz, 2H, H_{Ph}-3); 7.1–7.2 (m, 3H, H_{Ph}-2 and $H_{Napht.}$-7); 7.17 (d, $J = 2, 5$ Hz, $H_{Napht.}$-5); 7.26 (dd, $J = 8.5$, 1.8 Hz, 1H, $H_{Napht.}$-3); 7.50 (s, 1H, $H_{Napht.}$-1); 7.68 (d, $J = 8.5$ Hz, 1H, $H_{Napht.}$-4); 7.69 (d, $J = 8.9$ Hz, 1H, $H_{Napht.}$-8)); ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.2 (CH); 55.3 (OCH_3); 82.6; 83.5; 86.7; 87.1 (*Cp*-H); 105.6 ($C_{Napht.}$-7); 112.9 (*Cp*-C); 113.9 ($C_{Ph.}$-3); 119.1 ($C_{Napht.}$-5); 126.8 ($C_{Napht.}$-1); 127.0 ($C_{Napht.}$-4); 127.5 ($C_{Napht.}$-3); 128.7; 129.4 ($C_{Napht.}$-8); 129.8 (C_{Ph}-2); 133.5; 134.9; 138.5; 157.8; 158.5; 194.3 (CO); IR (KBr) 2019; 1919 cm^{-1}; MS 612 $(M)^{+\bullet}$; 528 $(M - 3\,CO)^{+\bullet}$; Anal Calc. For $C_{27}H_{21}O_5Re$: C, 53.02; H, 3.46. Found: C, 53.01; H, 3.59; m.p.: 62 °C.

(4-methoxybenzyl) cyclopentadienyltricarbonylrhenium, 8

Pale yellow solid; ^1H NMR (200 MHz, $CDCl_3$) δ 3.69 (s, 2H, CH_2); 3.80 (s, 3H, OCH_3); 5.24 (s, 4H, Cp); 6.86 (d, $J = 8.6$ Hz, 2H, H-3); 7.13 (d, $J = 8.6$ Hz, 2H, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 33.5 (CH); 55.3 (OMe); 83.7 (Cp); 83.8 (Cp); 100.5 (Cp-CH_2); 114.1 (C-3); 129.5 (C-2); 131.3 (Ar); 188.5 (Ar); 194.4 (CO); IR (KBr) 2011; 1919 (CO) cm^{-1}; MS: 456 (M^+); 428 $(M\text{-}CO)^+$; 372 $(M - 3\,CO)^+$. Anal. Calcd for $C_{16}H_{13}O_4Re$: C, 42.19; H, 2.88. Found: C, 42.18; H, 2.94; m.p.: 65 °C.

General procedure for demethylation of phenols methyl ethers with BBr_3

To a solution of **7a–e, 5** or **8** (1 mmol) in 5 ml of CH_2Cl_2 was added at room temperature a solution of BBr_3 in CH_2Cl_2 (1 M, 5 mmol of BBr_3 for each OCH_3 group). The resulting mixture was stirred at room temperature as long as starting material remained (control by TLC). The reaction mixture was hydrolysed with a saturated solution of $NaHCO_3$ (20 ml), extracted with CH_2Cl_2 (3×15 ml). Purification by flash chromatography (CH_2Cl_2–ethyl acetate = 97 : 3) afforded product **1a–e, 9** or **10** which were crystalized from a mixture of CH_2Cl_2 and pentane.

(4,4′-dihydroxybenzhydryl) cyclopentadienyltricarbonylrhenium, 1a

White powder. ^1H NMR (200 MHz, $CDCl_3$) δ 4.72 (s, 1H, CH); 5.21 (t, $J = 2.2$ Hz, 1H, Cp); 5.26 (t, $J = 2.2$ Hz, 1H, Cp); 5.31 (s, 2H, Cp); 6.76 (d, $J = 8.8$ Hz, 2H, H-3); 7.01 (d, $J = 8.4$ Hz, 2H, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 48.5 (CH); 82.9 (Cp); 86.8 (Cp); 113.0 (Cp-CH); 115.3 (C-3); 129.7 (C-2); 135.5; 154.3; 194.2 (CO); IR (KBr) 2020; 1925 (CO) cm^{-1}; MS: 534 $(M)^{+\bullet}$; 450 $(M - 3\,CO)^{+\bullet}$. Anal. calcd for $C_{21}H_{15}O_5Re$: C, 47.27; H, 2.83. Found: C, 47.09; H, 2.93; m.p.: 75 °C.

3,4′-dihydroxybenzhydrylcyclopentadienyltricarbonylrhenium, ***1b***

Pale yellow powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.04 (s, 1H, CH); 6.61 (t, $J = 1.8$ Hz, 1H, H-2); 6.7–6.8 (m, 4H, H-3′, H-4, H-6); 7.01 (d, $J = 8.4$ Hz, 2H, H-2′); 7.16 (t, $J = 7.8$ Hz, 1H, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.1 (CH); 82.6 (Cp); 83.3 (Cp); 86.8 (Cp); 87.2 (Cp); 112.1 (C-6); 113.9 (Cp-CH); 115.4 (C-3′); 115.6 (C-2); 121.2 (C-4); 129.7 (C-5); 129.8 (C-2′); 134.9; 145.0; 154.3; 155.5; 194.2 (CO); IR (KBr) 2016; 1916 (CO)

(2,4′-dihydroxybenzhydryl) cyclopentadienyltricarbonylrhenium, ***1c***

White solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.83 (s, 1H, OH); 4.94 (s, 1H, OH); 5.20 (t, $J = 1.8$ Hz; 2H, Cp); 5.3–5.4 (m, 3H, Cp + CH); 6.7–7.2 (m, 8H, Ar); ^{13}C NMR (50 MHz, $CDCl_3$) δ 43.2 (CH); 81.1 (Cp); 84.5 (Cp); 86.5 (Cp); 88.5 (Cp); 111.1 (C-3); 115.5 (Cp-CH); 116.3 (C-3′); 120.9 (C-5); 128.4 (C-4); 129.2 (C-6); 129.9 (C-2′); 130.4; 133.6; 152.6; 154.5; 194.1 (CO); IR (KBr) 2019; 1922 (CO); MS: 534 $(M)^{+\bullet}$; 506 $(M\text{-}CO)^{+\bullet}$; 450 $(M - 3\,CO)^{+\bullet}$; 441 $(M - HOC_6H_4)^+$. Anal. calcd for $C_{21}H_{15}O_5Re$: C, 47.27; H, 2.83. Found: C, 47.08; H, 2.99; m.p.: 80 °C.

(4-hydroxy-4′-trifluoromethylbenzhydryl) cyclopentadienyltricarbonylrhenium, ***1d***

Green powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.0–5.2 (m, 5H, Cp + CH); 6.69 (d, $J = 9.4$ Hz, 2H, H_{Ph}); 6.92 (d, $J = 9.4$ Hz, 2H, H_{Ph}); 7.18 (d, $J = 7.9$ Hz, 2H, H_{Ph}); 7.48 (d, $J = 7.9$ Hz, 2H, H_{Ph}); ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.1 (CH); 82.9 (Cp); 83.5 (Cp); 86.6 (Cp); 87.1 (Cp); 111.2 (Cp Q); 115.5 (CH_{Ph}); 125.4 (C_{Ph} Q); 125.5 (C_{Ph} Q); 128.9 (CH_{Ph}); 129.8 (CH_{Ph}); 134.2 (C_{Ph} Q); 147.1 (C_{Ph} Q); 154.7 (C_{Ph} Q); 193.9 (CO); IR (KBr) 2021; 1916 (CO); MS: 586 $(M)^{+\bullet}$; 502 $(M - 3\,CO)^{+\bullet}$. Anal. calcd for $C_{22}H_{14}F_3O_4Re$: C, 45.13; H, 2.41. Found: C, 44.95; H, 2.60.

[4-hydroxy-α-(6-hydroxy-2-naphthyl)-benzyl] cyclopentadienyltricarbonylrhenium, ***1e***

Pink powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.03 (s, OH); 5.25 (s, 5H, Cp + CH); 6.77 (d, $J = 8.4$ Hz, 2H, H_{Ph}-3); 7.0–7.2 (m, 4H, $H_{Napht.}$-5, $H_{Napht.}$-7, H_{Ph}-2); 7.2–7.3 (m, 1H, $H_{Napht.}$-3); 7.47 (s, 1H, $H_{Napht.}$-1); 7.59 (d, $J = 8.6$ Hz, 1H, $H_{Napht.}$-4); 7.68 (d, $J = 9.4$ Hz, 1H, $H_{Napht.}$-8); ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.2 (CH); 82.5 (Cp); 83.5 (Cp); 86.7 (Cp); 87.2 (Cp); 109.4 ($C_{Napht.}$-7); 112.6 (Cp-CH); 115.3 (C_{Ph}-3); 118.1 ($C_{Napht.}$-5); 126.7 ($C_{Napht.}$-1); 126.8 ($C_{Napht.}$-4); 127.7 ($C_{Napht.}$-3); 128.6; 129.8 ($C_{Napht.}$-8); 130.0 (C_{Ph}-2); 133.4; 135.0; 138.5; 153.5; 154.4; 194.2 (CO); MS: 584 $(M)^{+\bullet}$; 500 $(M - 3\,CO)^{+\bullet}$. Anal. calcd for $C_{25}H_{17}O_5Re$: C, 51.45; H, 2.94. Found: C, 51.23; H, 3.16.

(4-hydroxybenzyl) cyclopentadienyltricarbonylrhenium, ***9***

White solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.78 (s, 2H, CH_2); 5.1–5.3 (m, 4H, Cp); 5.30 (s, OH); 6.80 (d, $J = 8.4$ Hz, 2H, H-3); 7.08 (d, $J = 8.6$ Hz, 2H, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 33.5 (CH_2); 83.6 (Cp); 83.9 (Cp); 110.3 (Cp-CH_2); 115.5 (C-3); 129.8 (C-2); 131.5; 154.4; 194.4 (CO); IR; MS:442 (M^+); 414 $(M\text{-}CO)^+$; 358$(M - 3\,CO)^+$. Anal. calcd for $C_{15}H_{11}O_4Re$: C, 40.81; H, 2.51. Found: C, 40.57; H, 2.69; m.p.: 54 °C.

1,1-(4-hydroxyphenyl) cyclopentadienyltricarbonylrhenium ethane, ***10***

White solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48 (d, $J = 7.0$ Hz, 3H, CH_3); 3.80 (q, $J = 7.2$ Hz, 1H, CH); 5.20 (t, $J = 2.2$ Hz, 2H, Cp); 5.23 (t, $J = 2.2$ Hz, 1H, Cp); 5.31 (t, $J = 2.0$ Hz, 1H, Cp); 6.80 (d, $J = 8.6$ Hz, 2H, H-3); 7.09 (d, $J = 8.6$ Hz, 2H, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.8 (CH_3); 37.5 (CH); 53.4 (Cp); 81.8 (Cp); 83.7 (Cp); 84.1 (Cp); 115.4 (Cp-CH); 116.0 (C-3); 128.2 (C-2); 137.0; 154.3; 194.5 (CO); IR (KBr) 2016; 1930 (CO) cm^{-1}; MS: 456 (M^+); 428 $(M\text{-}CO)^+$; 372 $(M - 3\,CO)^+$. Anal. calcd for $C_{16}H_{13}O_4Re$: C, 42.19; H, 2.88. Found: C, 42.20; H, 3.06; m.p.: 68 °C.

Biochemical part

Materials

17β-oestradiol, 4-OH-Tam (*Z*), glutamine, protamine sulfate were obtained from Sigma-Aldrich (France). Stock solutions (1×10^{-3} M) of the compounds to be tested were prepared in DMSO and were kept at 4 °C in the dark; under these conditions they are stable for at least 2 months. Serial dilutions were prepared in phosphate buffer just prior to use.

Oestrogen receptor sources

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. Purified EPβ were from Invitrogen corporation.

Determination of the RBA of the compounds for ERα (from cytosol), and ERβ purified

Sheep uterine cytosol prepared in buffer A (0.05 M Tris–HCl, 0.25 M sucrose, 0.1% ß-mercaptoethanol, pH 7.4 at 25 °C) as previously described[26] was used as a source of cytosolic ERα. For purified ERβ, 10 µl of the solution containing 3500 pmol/mL were 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 silanized 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-^{3}H]- estradiol (2×10^{-9} M, specific activity 1.62 TBq/mmol, NEN Life Science, Boston, MA, USA) in the presence of nine concentrations of 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 [^{3}H]-estradiol (Y) was calculated using the logit transformation of Y [logit$Y$: $\ln(y/1 - Y)$] vs the log of the mass of the competing steroid. The concentration of unlabeled steroid required to displace 50% of the bound [^{3}H]-estradiol was calculated for each steroid tested, and the results expressed as RBA. The RBA value of estradiol is by definition equal to 100%.

Measurement of octanol/water partition coefficient ($\log P_{o/w}$) of the compounds

The $\log P_{o/w}$ values of the compounds were determined by reversed-phase HPLC on a C-8 column (nucleosil 5.C8, from Macherey Nagel, France) according to the method previously described by Minick[27] and Pomper.[28] Measurement of the chromatographic capacity factors (k') for each compounds 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-morpholino propanesulfonic acid) buffer pH 7.4 (prepared in 1-octanol-saturated water). These capacity factors are extrapolated to 100% of the aqueous component given the value of k'_w. $\log P_{o/w}$ (y) is then obtained by the formula: $\log P_{o/w} = 0.13418 + 0.98452 \log k'_w$.

Acknowledgements

D.P.'s stay in Paris was supported through a European Community Marie Curie Fellowship (HMPT-CT-2000-00186).

REFERENCES

1. Matthews JB, Twomey K, Zacharewski TR. *Chem. Res. Toxicol.* 2001; **14**: 149.
2. Rivas A, Lacroix M, Olea-Serrano F, Laíos I, Leclercq G, Olea N. *J. Steroid Biochem. Mol. Biol.* 2002; **82**: 45.
3. Bindal RD, Golab JT, Katzenellenbogen J. *J. Am. Chem. Soc.* 1990; **112**: 7861.
4. Le Bideau F, Salmain M, Top S, Jaouen G. *Chem. Eur. J.* 2001; **7**: 2289.
5. Masi S, Top S, Boubeker L, Jaouen G, Mundwiler S, Spingler B, Alberto R. *Eur. J. Inorg. Chem.* 2004; 2013.
6. Osella D, Galeotti F, Cavigiolio G, Nervi C, Hardcastle KI, Vessières A, Jaouen G. *Helvet. Chim. Acta* 2002; **85**: 2918.
7. Ferber B, Top S, Jaouen G. *J. Organomet. Chem.* 2004; **689**: 4872.
8. Top S, El Hafa H, Vessières A, Quivy J, Vaissermann J, Hughes DW, McGlinchey MJ, Mornon J-P, Thoreau E, Jaouen G. *J. Am. Chem. Soc.* 1995; **117**: 8372.
9. Le Bideau F, Pérez-Luna A, Marrot J, Rager M-N, Stephan E, Top S, Jaouen G. *Tetrahedron* 2001; **57**: 3939.
10. Farona MF, Kraus KF. *Inorg. Chem.* 1970; **9**: 1700.
11. Vitali D, Calderazzo F. *Gazz. Chim. Ital.* 1972; **102**: 587.
12. Bosch WH, Englert U, Pfister B, Stauber R, Salzer A. *J. Organomet.Chem.* 1996; **506**: 273.
13. Mull ES, Sattigeri VJ, Rodriguez AL, Katzenellenbogen JA. *Bioorg. Med. Chem.* 2002; **10**: 1381.
14. Vickery E, Pahler L, Eisenbraun E. *J. Org. Chem.* 1979; **24**: 4444.
15. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL. *Cell* 1998; **95**: 927.
16. Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, Ljunggren J, Gustafsson J, Carlquist M. *EMBO J.* 1999; **18**: 4608.
17. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson J-A, Carlquist M. *Nature* 1997; **389**: 753.
18. Kim S-H, Katzenellenbogen JA. *Bioorg. Med. Chem.* 2000; **8**: 785.
19. Mull ES, Sattigeri VJ, Rodriguez AL, Katzenellenbogen JA. *Bioorg. Med. Chem.* 2002; **10**: 1381.
20. Top S, Vessières A, Leclercq G, Quivy J, Tang J, Vaissermann J, Huché M, Jaouen G. *Chem. Eur. J.* 2003; **9**: 5223.
21. Muthyala RS, Sheng S, Carlson KE, Katzenellenbogen BS, Katzenellenbogen JA. *J. Med. Chem.* 2003; **46**: 1589.
22. Davis R, Duvrant JLA, Rowland CC. *J. Organomet. Chem.* 1986; **315**: 119.
23. Nitschke J, Schmidt SP, Trogler VC. *Inorg. Chem.* 1985; **24**: 1972.
24. Lee GCM, Tobias B, Holmes JM, Harcourt DA, Garst ME. *J. Am.Chem. Soc.* 1990; **112**: 9330.
25. Le Bideau F, Hénique J, Pigeon P, Joerger JM, Top S, Jaouen G. *J. Organomet. Chem.* 2003; **668**: 140.
26. Vessières A, Top S, Ismail AA, Butler IS, Loüer M, Jaouen G. *Biochemistry* 1988; **27**: 6659.
27. Minick DJ, Frenz JH, Patrick MA, Brent DA. *J. Med. Chem.* 1988; **31**: 1923.
28. Pomper MG, VanBrocklin H, Thieme AM, Thomas RD, Kiesewetter DO, Carlson KE, Mathias CJ, Welch MJ, Katzenellenbogen JA. *J. Med. Chem.* 1990; **33**: 3143.