Synthetic Strategy for Organometallic Complexes of Rhenium with Exceptionally High Affinity for the Oestradiol Receptor; their Potential Use as Imaging and Therapeutic Agents

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Compounds such as 11β-chloromethyl-17α-[cyclopentadienyl (rhenium tricarbonyl)-ethynyl]-oestradiol are readily accessible, are stable in biological media and show very high affinity for the oestradiol receptor, offering, because of their potential use as radio-pharmaceuticals, new approaches in the treatment of breast cancer.

The nuclear medicinal potential of chelates of ^{99m}Tc (*e.g.* the N_2S_2, bisamino–bisthiol systems) has been extensively studied. This radio-element can be generated from $^{99m}TcO_4^-$,[1] which is commercially available. Isotopes ^{186}Re and ^{188}Re also show promise in the development of new therapeutic strategies.[2–5]

Hormone-dependent cancers (*e.g.* breast cancer) should be receptive to a radio-pharmaceutical approach using the hormone as vector.[6] The oestradiol receptor localised in target cell nuclei and strongly associates with oestrogen, which then binds strongly in a dimerized form to DNA, regulating gene expression.[7] The hormonal vehicle must also have high affinity for the receptor. The hormones obtained with the chelate method, however, are unstable in biological media,[6c] and because the substituents are bulky and of high molecular mass compared with hormonal ligand only certain positions on the hormonal skeleton are viable without losing recognition.

We here present a new strategy, to overcome these problems using cold (*i.e.* non-radioactive) rhenium as the model metal to form strong, covalent metal–carbon bonds. In particular, we describe the synthesis of 11β-chloromethyl-17α-[cyclopentadienyl(rhenium tricarbonyl)-ethynyl]oestra-1,3,5-(10)-triene-3,17β-diol **3**, which has high affinity for the oestradiol receptor.

To obtain a good association of modified oestradiols with the oestrogen receptor (E_2R_c), it is essential to preserve the hydroxy functions at C-3 and C-17, whereas modification of the 17α position by a rigid and polarisable chain such as in **1** and **2** is sometimes compatible with maintenance of a high level of recognition.[8–12] Furthermore, substituents with p electrons, *e.g.* CH_2Cl or OMe, when they are placed in position 11β of oestradiol, increase the value of the relative binding affinity (RBA).[8–9]

Product **3** was synthesized by generating (η^5-C_5H_4-C≡C-Li)[$Re(CO_3]$] by treating dry Bu^{n}Li in THF at −60 °C with (η^5-C_5H_4-C≡C–H)[$Re(CO)_3$], obtained by the method of Stille *et al.*[13] This complex was then treated with 11β-chloromethyl-oestrone at −60 °C. After hydrolysis and evaporation of the solvent, the resulting product was purified by chromatography on silica gel plates with THF–pentane (1 : 3) as eluent, to give **3**, colourless crystals, 78% yield.

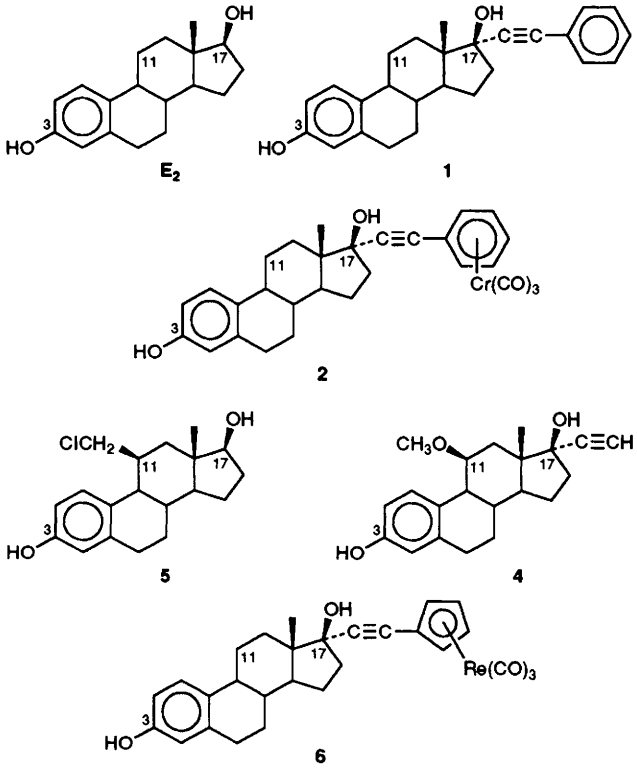
The estimated residence time of natural oestrogens in target cells *in vivo* is *ca.* 6–12 h,[6a] and the number of E_2R_c molecules per cell in hormone-dependent cancers is *ca.* 10 000–30 000. Thus, saturation with vector ligands of very high affinity and specificity is necessary to maximise the residence time for increased efficacy.

The RBA values were measured for **3** and **6** at two temperatures, using the cytosol of lamb uterus as the receptor

Table 1 Binding affinity of some oestradiol derivatives for the oestrogen receptor and their log $P_{o/w}$ values

	RBA[a]		
	$T = 0$ °C	$T = 25$ °C	log $P_{o/w}$[b]
Oestradiol, E_2	100[c]	100[c]	3.30
17α-Ethynyl-11β-methoxy-oestradiol, **4**	14	185	3.01[d]
11β-Chloromethyloestradiol, **5**	100	260	3.55
6	16	15	5.31
3	29	172	5.56

[a] The RBAs of the compounds for the oestrogen receptor are determined as described in ref. 14. [b] Log of octanol–water partition coefficient was measured by the method of Minick *et al.*[18] using a reverse phase HPLC method. [c] Value by definition. [d] Value from ref. 15.



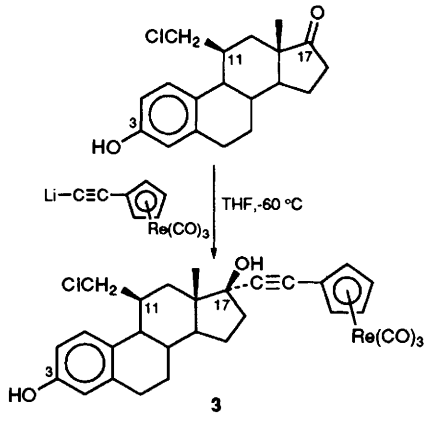


Fig. 1

source and tritiated oestradiol as tracer, according to the previously described method.[14] An increase in the RBA value of **3** was noted depending on the incubation temperature, going from 29% at 0 °C (kinetic control ratio) to 172% at 25 °C (thermodynamic control ratio). On the other hand, for **6**, which lacks an 11β substituent, an increase in the temperature had no influence on the RBA value (Table 1). The positive effect of temperature on affinity is fairly rare but not unknown. In fact, this situation exists for the 17α-ethynyl-oestradiol-11β-methoxy **4** (RBA at 25 °C = 185%)[15] and for the 11β-chloromethyl oestradiol **5** (RBA at 25 °C = 260%) with natural oestradiol defined as 100%. This has been explained by the fact that these oestrogens disassociate themselves from the receptor more slowly than natural oestradiol.[8] In fact the residence time of **5** on the receptor ($t_{1/2}$) exceeds 50 h,[16] because the equilibrium created by **5** with E_2R_c is further displaced in favour of the complex. The extraordinary character of this interaction means that compounds of this type, that possess heteroelements at 11β, are very useful in the study of the mode of interaction of oestrogens with their association site. An interesting hypothesis lies in the presence of a Zn^{2+} cation near the binding site which effects an extra complexation with the modified bioligand.[17]

For imaging agents, the radio-pharmaceutical must show limited uptake in non-target tissues such as fat and bone. The relative importance of these factors is influenced by the lipophilicity and metabolic stability of the species under study. In a preliminary *in vitro* conservation test over 3 months in an alcohol–water mixture at 4 °C, compound **3** displayed very high stability, in contrast to its arene homologue $Cr(CO)_3$ **2** which undergoes rapid decomplexation by photochemical oxidation in air. We therefore used FTIR spectroscopy to ascertain that organometallic complex **3** has not decomposed after 3 h incubation at 25 °C in the presence of lamb uterus cytosol and precipitation.

Octanol–water partitions coefficients (log $P_{o/w}$) as a measure of lipophilicity, have been shown to correlate well with the non-specific binding of steroids[15] (Table 1).[18] Recently, the liquid chromatographic method of Minick,[18] has been found to accurately reproduce octanol–water partition values measured with the shake flask method.

It is clear that the modifications performed on the hormone, including the addition of $-CH_2Cl$, $-C\equiv C-$, $(\eta\text{-}C_5H_5)Re(CO)_3$ groups, all increase the lipophilicity of molecule **3**. It is nonetheless true that the values obtained for the rhenium chelates in the progesterone series are higher (log *k ca.* 6.3) than those observed for the organometallics. If necessary, it would be straightforward to lower the lipophilicity of the compounds, *e.g.* in **3** by replacing the 11β-CH_2Cl group with a substituent such as 11β-OMe. Access to organometallic molecules with non-specific binding levels of less than 10% at receptor saturation is thus clearly an attainable goal.

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