Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 1827–1835

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of Novel Progestin–Rhenium Conjugates as Potential Ligands for the Progesterone Receptor

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Received 9 November 1998; accepted 1 March 1999

Abstract—To assist in the development of technetium-based radiopharmaceuticals that are useful for the diagnostic imaging of steroid receptor-positive breast tumors, we have synthesized a series of small-sized metal chelates according to '$n+1$' mixed-ligand, thioether-carbonyl and organometallic designs. In these preliminary investigations, rhenium was used as a model for the radioactive technetium. The metal chelates contain the rhenium metal in several oxidation states, being $+5$, $+3$, and $+1$, and they were attached to 21-substituted progesterone derivatives. A competitive receptor-binding assay (rat uterine cytosol, 0°C) was used to determine the binding affinity of these conjugates for the progesterone receptor. The highest affinity of 9% (RU5020 = 100%) was obtained with a '$3+1$' mixed-ligand complex, containing a NMe group as the central donor atom in the tridentate ligand part. This value reflects a relative binding affinity of 75% compared with the parent steroid progesterone.

Introduction

Progestins play important roles in the preparation of the uterus for pregnancy, in the maintenance of pregnancy, and in the maintenance of the female reproductive organs. The physiological effects of progestins are mediated by their interaction with the progesterone receptor (PgR).[1] In combination with the estrogen receptor (ER), the PgR plays an essential role as a biochemical marker for breast cancer and for responsiveness to endocrine therapy in the treatment of breast cancer patients.[2,3]

Compared to the high affinity of 3,17β-estradiol for the ER ($K_D = 0.2$ nM), the affinity of progesterone for the PgR is relatively poor ($K_D = 3$ nM). Nevertheless, several synthetic progestins have exhibited much higher affinity for the PgR, and a higher biological activity than progesterone itself.[4–8] These high affinity ligands include representatives from both the pregnane and androstane series, and they even include compounds bearing large substituents, such as those shown in Scheme 1.

In a series of reports Katzenellenbogen and co-workers explored the tolerance of the progesterone receptor to ligands containing bulky rhenium and technetium complexes attached to various positions of the steroid molecule through tetradentate N_2S_2-chelates.[6–8] Of the compounds studied, the 11β-substituted derivatives showed especially high affinity for the PgR. The corresponding technetium-99m conjugates were studied in rats, and they showed specific receptor mediated uptake in the rat uterus. However, these compounds were not suitable for imaging because their high lipophilicity also resulted in a high uptake in non-target tissue.[6–8]

Our ongoing interest focused on technetium-based radiotracers for the steroid receptor system, and we have exploited some alternative chelate systems, such as the '$n+1$' mixed-ligand design,[9,10,12] the thioether-carbonyl design,[11,13] and a modified organometallic design.[14]

Herein, we report on the influence that the structure of novel rhenium–progesterone conjugates has in terms of their binding to the progesterone receptor. The easy access to 21-substituted progesterones as well as the reported substantial receptor affinity of some 21-substituted progesterone derivatives,[6] prompted us to work out a synthesis of various rhenium chelates according to

Key words: 21-Substituted progesterone; progesterone receptor; relative binding affinities; rhenium.
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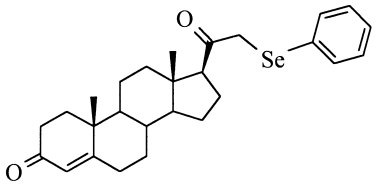
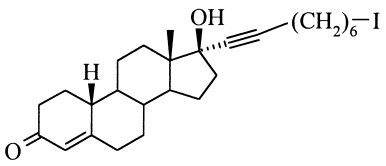
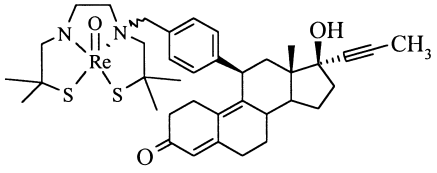
0968-0896/99/$ - see front matter
PII: S0968-0896(99)00119-4



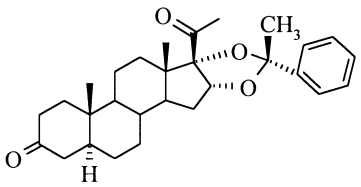
21-phenylseleno progestin
RBA = 59



17α-substituted iodo progestin
RBA = 138



11β-substituted Re-BAT-progestin
RBA = 283 (anti-isomer)



16α,17α-substituted progestin
RBA = 129

Scheme 1. High affinity ligands for the progesterone receptor.

'$n+1$', dithioether-carbonyl and organometallic designs attached to the 21-position of progesterone. At the initial stage of this investigations, we used rhenium as a non-radioactive model for the technetium because the chemistry and biochemistry of rhenium and technetium are known to be closely related.[15] By varying the structure of the chelate unit, we have synthesized a novel set of rhenium–progesterone conjugates, which were evaluated in a competitive radiometric binding assay to determine their binding affinities towards the PgR. In all cases the introduction of the metal chelates lowers the receptor binding affinity.

Results and Discussion

Chemistry

Synthesis of '3+1' mixed-ligand complexes of 21-substituted progesterone 7 and 8. The principle underlying the '3+1' mixed-ligand design[9,10,12] consists of the saturation of three coordination sites of the oxometal(V) core by a small-sized tridentate 'SXS' ligand and filling the remaining fourth position with a monodentate coligand. For technetium and rhenium in the oxidation state +5, a mercaptide sulfur is the preferred donor group for the monodentate ligand as it provides stable binding of the metal via a single donor atom. Thus, chemical modification was aimed at the introduction of a thiol group into the steroid. The synthesis of 21-mercapto-progesterone **3**, capable of forming '3+1' mixed-ligand complexes, is shown in Scheme 2.

Starting from commercially available deoxycorticosterone **1**, we converted the hydroxy group into the corresponding thiol group following the Mitsunobu procedure[16] with thiobenzoic acid, PPh_3 and DIAD. The thiobenzoate **2** was then treated with 1 N NaOMe in MeOH, and the thiol **3** was obtained as a solid in a 66% yield, based on alcohol **1**.

21-Mercaptoprogesterone **3** was subjected to typical conditions for the formation of the corresponding '3+1' mixed-ligand complexes **7** and **8**.

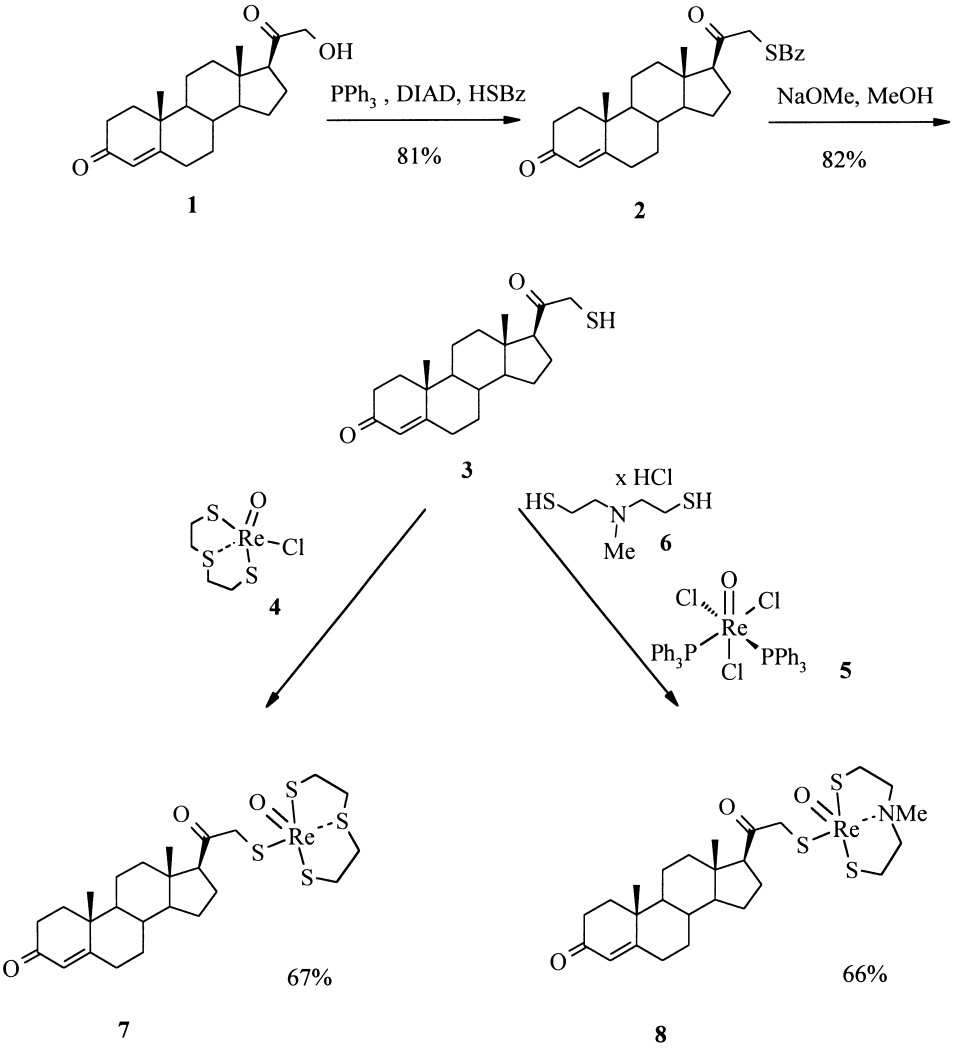
The reaction of the chlorine-containing oxorhenium(V) precursor **4**[17] with thiol **3** in refluxing acetonitrile gave the 'SSS' mixed-ligand complex **7** as brown crystals in satisfactory yield of 67%. The reaction could be monitored by the color change of the dark-blue rhenium-precursor **4** into the red mixed-ligand complex **7**.

An alternate route was employed for the synthesis of complex **8**. The reaction of equimolar amounts of oxotrichlorobis(triphenylphosphine)rhenium(V) **5** and tridentate 'SNMeS' ligand **6** with a slight excess of monodentate thiol ligand **3** gave the green complex **8** in 66% yield after flash chromatography. Due to the low reactivity of oxorhenium(V) precursor **5**, formation of the desired '3+1' mixed-ligand complex required refluxing in 1 N methanolic NaOAc for at least 2 h.

The '3+1' mixed-ligand complexes obtained by this process differ with respect to the neutral donor atom in the tridentate ligand part 'SXS', which is X = S for **7** and X = NMe for **8**.

The IR spectra of both complexes show intense Re=O stretching bands at 960 cm^{-1} (for **7**) and 954 cm^{-1} (for **8**). In the ^1H NMR spectrum, the 'SSS' complex **7** exhibits a characteristic AB-quartet (J_{AB} = 14.2 Hz) at 4.63 ppm for the protons adjacent to the 20-keto group, in addition to the complex coupling pattern of a ABCD-system for the ethylene protons of the tridentate 'SSS' ligand. The α-keto-CH_2 protons of the analogous 'SNMeS' complex **8**, on the other hand, display only broad signals.

Crystals of the 'SSS' complex **7** suitable for X-ray single crystal analysis were obtained by slow crystallization of

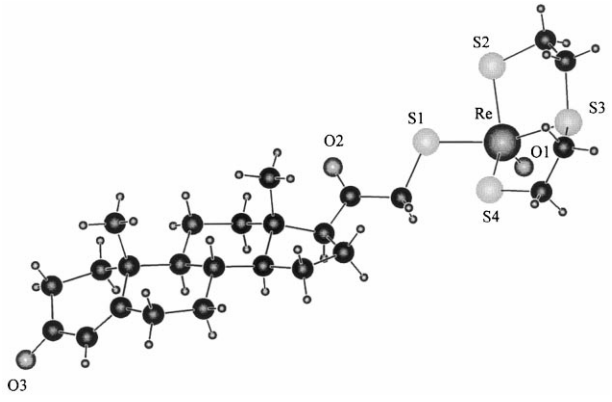


Scheme 2. Synthesis of '3 + 1' mixed ligand complexes **7** and **8**.

7 from an acetone:*n*-hexane solution at room temperature. The X-ray structure of complex **7** is shown in Scheme 3 (Table 1).

The four sulfur donor atoms in this complex are arranged in a square-pyramidal geometry around the oxorhenium(V) core, as is typical of 'SSS' coordinated '3 + 1' mixed-ligand complexes. The metal lies above the equatorial plane of the four basal sulfur donor atoms, and the oxo group is at the apex. The metal oxo portion of the square-pyramidal chelate moiety exhibits an *anti*-like orientation with respect to the β-orientated methyl groups at position 10 and 13 of the progesterone molecule.

Synthesis of '4 + 1' mixed-ligand complex of 21-substituted progesterone 14. A alternative '4 + 1' mixed-ligand design[9,18] employs a rhenium(III) moiety coordinated with the tripodal chelating sulfur ligand 2,2′,2′-nitrilotris-ethanethiol ('NS_3') and a monodentate isocyanide ligand. Convenient access to the '4 + 1' complexes in one step[19] was achieved by the reaction of phosphane-containing tripodal rhenium(III) precursor **13**[16] complex with an isocyanide generated in situ.



Scheme 3. X-ray structure of complex **7**.

For the synthesis of isocyanide precursor **12**, a modified Gabriel reagent[20] was used. First, the α-hydroxy ketone **1** was converted into the corresponding α-bromo ketone **10** in 87% yield for two steps, via the mesylate **9** and substitution with LiBr in acetone. The displacement of the bromide in **10** was accomplished by the reaction of

Table 1. Selected bond lengths and angles for **7**

Bond length (Å)		Bond angles (Å)	
Re-O1	1.668	O1-Re-S1	105.2
Re-S1	2.304	O1-Re-S2	114.9
Re-S2	2.300	O1-Re-S3	101.2
Re-S3	2.380	O1-Re-S4	115.4
Re-S4	2.306	S1-Re-S2	81.3
		S1-Re-S3	153.4
		S1-Re-S4	88.6
		S2-Re-S3	84.6
		S2-Re-S4	129.7
		S3-Re-S4	83.3

1.2 equiv of sodium diformylamide in refluxing acetonitrile to provide the α-bis-formylamino ketone **11** in an excellent yield of 94%. Treatment of **11** in EtOH with a catalytic amount of KOH at room temperature removed one formyl group, providing the formamide **12** in 93% yield, whereas the treatment of **11** with HCl in refluxing EtOH produced the amine hydrochloride.

The one-pot procedure, with formamide **12** and rhenium(III) precursor **13**[18] in presence of the $POCl_3$/diisopropylamine as the dehydrating agent, was used to prepare the desired '4 + 1' complex **14** (Scheme 4). The reaction was started at −78°C and was then allowed to warm to room temperature over 2 h. Complex **14** was obtained in 53% yield as an olive-green solid, after purification by flash chromatography.

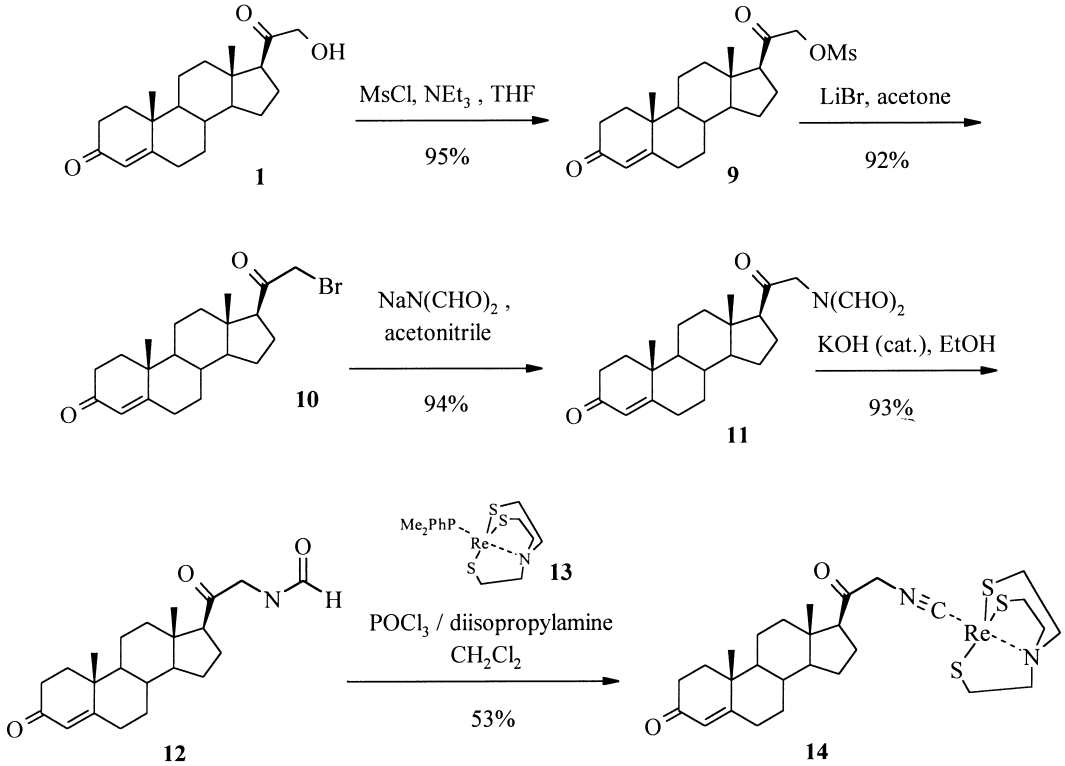
The IR-spectrum of **14** shows a strong vibrational band at 1938 cm^{-1}, indicative of the isocyanide group attached to the rhenium(III) core. In the ^1H NMR, the characteristic AB-quartet at 5.46 ppm ($J = 19.5$ Hz) is indicative of the ketone diastereotopic α-methylene protons. A broad signal at 3.00 ppm represents the 12 protons of the tripodal 'NS_3' ligand.

Synthesis of dithioether–carbonyl complex of 21-substituted progesterone 17. Recently, use of the organometallic aquocomplex $[M(OH_2)_3(CO)_3]^+$ has been proposed to prepare low valency technetium(I) or rhenium(I) complexes.[21,22] Due to its known tendency to bridge Re(I) or Tc(I) centers,[23] a mercapto group appeared to be a poor linker. Therefore, we have chosen a bidentate thioether group as a ligand suitable for forming a monomeric complex.

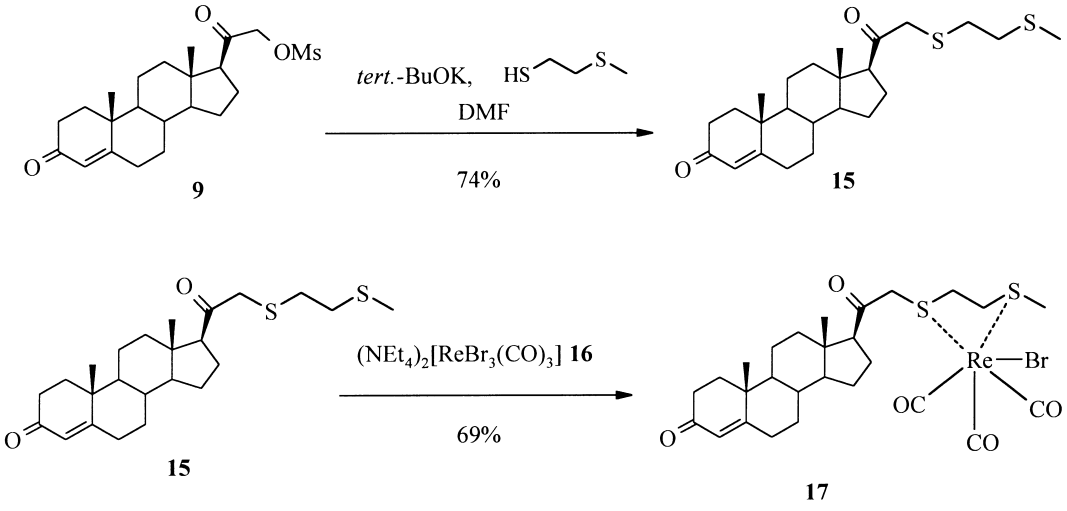
The dithioether **15** was easily obtained in a 74% yield by the displacement of mesylate **9** with 2-(methylmercapto)-ethanethiol and *tert*-BuOK as the base in DMF at room temperature. The synthesis of complex **17** is shown in Scheme 5.

The coordination with the π-acceptor dithioether ligand **15** displaces two of the three halogen atoms of the low valency metal(I) carbonyl precursor $[ReBr_3(CO)_3]^{2-}$ **16**,[21] providing neutral complex **17**. In this complex, rhenium is in the +1 oxidation state, which suggests that this coordination compound will have high kinetic stability.

^1H NMR analysis of complex **17** reveals two singlets for the 18-methyl group, which indicate that complex **17** exists as 1:1 mixture of diastereomers.



Scheme 4. Synthesis of '4 + 1' mixed-ligand complex **14** with $POCl_3$/diisopropylamine as the dehydrating agent.



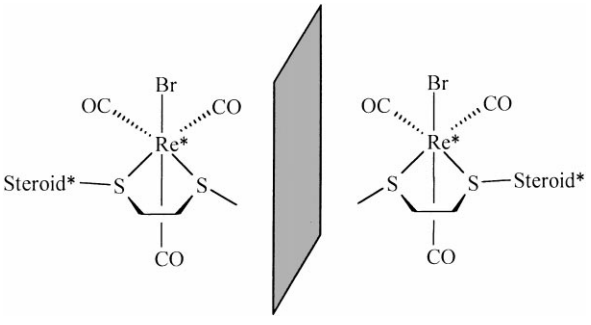
Scheme 5. Synthesis of complex **17**.

The attachment of the tricarbonyl–bromo–rhenium(I) core through the dithioether sulfur leads to a rhenium(I) chelate in which the rhenium metal is a stereogenic center (Scheme 6). Furthermore, coordination of the prochiral thioether sulfur also results in the formation of stereogenic sulfur donor atoms. The formation of stereoisomers during the complexation procedure reflects a general feature of the dithioether–carbonyl design when a non-symmetrical dithioether is used.

Synthesis of organometallic complex of 21-substituted progesterone 19. An organometallic cyclopentadienyltricarbonylrhenium(I) complex **19** was synthesized in excellent yield (96%) by the coupling of 21-hydroxyprogesterone **1** with cyclopenta-dienytricarbonylrhenium(I) carboxylic acid (CpTR-COOH) **18**[24] using 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC) as the dehydrating agent and DMAP as the catalytic base (Scheme 7). Rhenium(I) precursor **18** was easily obtained by a central metal exchange reaction starting from ferrocene dimethyl ester, by a previously described method.[24]

Receptor binding affinity

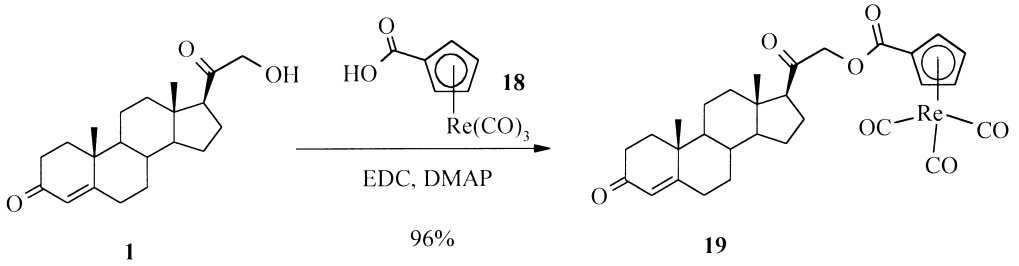
Progesterone receptor binding affinity of 21-substituted progesterone rhenium complexes. The PgR binding affinities of the rhenium–progesterone conjugates were measured in vitro by a competitive binding assay involving the tritium-labeled synthetic progestin RU5020 as tracer, estrogen-primed rat uterine cytosol as a source of PgR, and dextran-coated charcoal as an adsorbant for removing free ligand. The binding assay was performed by incubation of PgR with increasing concentrations of steroid competitor and [^{3}H]RU5020 at 0°C. The low temperature was used because of the poor stability of PgR compared with ER. Relative to RU5020, progesterone has an affinity of 12%.[25] The binding affinities of all of the rhenium–progesterone complexes prepared in this study are listed in Table 2.



Scheme 6. Diastereomers of complex **17**.

The highest affinity of 9% (RU5020 = 100%) was observed with '3 + 1' mixed-ligand complex **7**, containing a NMe group as the central donor atom in the tridentate ligand part. This value reflects a relative binding affinity of 75% compared to the natural progestin progesterone. Replacement of the NMe central donor atom in the tridentate ligand part of complex **8** with sulfur, as in the corresponding 'SSS' complex **7**, results in a 50% reduction in binding affinity, to 4.7% relative to RU5020. The sterically more bulky '4 + 1' mixed-ligand complex **14** shows only a low affinity (1.5% relative to RU5020 or 12% relative to progesterone). Binding affinity drops very markedly for the dithioether–carbonyl complex **17**. This complex displays a negligible affinity of 0.02% compared to RU5020. On the other hand, the related organometallic complex **19** still exhibits a substantial binding affinity of 2.2% relative to RU5020, which is still 18% of the binding affinity of progesterone. The very low affinity of dithioether complex **17** may be attributed, in part, to the diastereomeric nature of the complex. Furthermore, the presence of an additional bulky bromine atom in complex **17** might also have a detrimental effect on its binding to the receptor.

The higher binding affinity of the '3 + 1' mixed-ligand complexes **7** and **8** in comparison to all of the other complexes suggests that the chelate unit may be engaged in direct interaction with the binding site of the receptor. The oxygen of the rhenium–oxo core could serve as



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Scheme 7. Synthesis of cyclopentadienyltricarbonylrhenium(I) complex **19**.

Table 2. Relative binding affinities (RBAs) of 21-substituted progesterone–rhenium complexes for the progesterone receptor ($RBA_{RU5020} = 100$)

Compound	Substituents	RBA (0°C) (%)
RU5020		100
Progesterone		12
7	21-S-ReO 'SSS'	4.7
8	21-S-ReO 'SNMeS'	9.0
14	21-NC-Re(NS_3)	1.5
17	21-'SS'-ReBr($CO)_3$	0.02
19	21-Cp$Re(CO)_3$	2.2

a potential H-acceptor for hydrogen bonding with H-donors from the receptor protein, such as residues containing free carboxylic acid groups.

The central donor atom in the tridentate ligand part (S or NMe) affects the rhenium–oxygen bond length and, therefore, the binding character of the rhenium–oxo core. It is known from the literature, that with NMe as the central donor atom, the rhenium–oxygen bond is lengthened compared with that in the corresponding sulfur containing complexes.[26] The longer bond lengths found for the rhenium–oxo core in 'SNMe' complexes are in the range which is characteristic of a rhenium–oxygen double bond, whereas the shorter rhenium–oxygen bond lengths found in the corresponding 'SSS' complexes indicate a contribution from a triple bond.

Hence, the oxygen of the rhenium–oxygen bond is more electron-rich in 'SNMeS' complex **8** compared to that of 'SSS' complex **7**, and this would improve its capability to act as a H-acceptor, which might account for its higher binding affinity.

Conclusions

We have described the preparation of several novel PgR binding ligands labeled with rhenium by four different means, three inorganic complexes and one organometallic complex. In all cases the introduction of the metal chelates lowers the binding to the receptor. Nevertheless, we could show that some of these complexes still have substantial binding affinity for the progesterone receptor. Our studies reveal interesting effects of variation in the structure of the chelate unit, in terms of receptor binding: the capacity of the rhenium–oxo core to act as a H-acceptor in hydrogen binding with potential H-donors on the receptor protein appears to favour the '3 + 1' mixed-ligand design compared to the other approaches presented herein, and complexes containing a NMe group as the cental donor atom exhibit especially promising receptor binding properties. Further investigations concerning the in vivo stability of all complexes are planned using the corresponding technetium-99m complexes.

Experimental

General

Melting points were determined on a Boëtius melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254 plastic plates, with visualization under UV (254 nm) and/or polyphosphomolybdic acid (PMA). Flash chromatography was performed as described by Still et al.[27] using Merck silica gel (0.040–0.063 mm). Proton ^1H NMR spectra were recorded on a Varian Inova-400 at 400 MHz with $CDCl_3$. ^{13}C NMR spectra were obtained on a Varian Inova-400 at 100 MHz with $CDCl_3$. The number of the carbon atoms is given in parentheses. Infrared spectra (IR) were obtained on a Perkin–Elmer FTIR Specord 2000 spectrometer in the indicated phase. High-resolution electron-impact mass spectra were obtained on a Finnigan MAT 90. High resolution fast atom bombardment (FAB) spectra were obtained on a Varian MAT 731, employing a dithiothreitol matrix. Elemental analyses were obtained on a LECO CHNS 932 elemental analyser.

Solvents and reagents were purchased from Sigma, Fluka, or Aldrich. Compounds **4**,[17] **13**,[18] **16**,[21] and **18**[24] were prepared according to literature procedures.

The standard procedure for product isolation involves quenching of the reaction mixture in an aqueous solution, followed by a thorough extraction with $CHCl_3$, washing of the extract with water and brine, drying over $MgSO_4$, filtration and evaporation of the solvent under reduced pressure.

Chemical synthesis

21-[(*S*)-Benzoylthio]-progesterone (2). DIAD (1.3 mL, 6 mmol) was added dropwise to 1.57 g (6 mol) PPh_3 in

20 mL dry THF at 0°C. After strirring for 0.5 h at 0°C deoxycorticosterone **1** (1 g, 3 mmol) and thiobenzoic acid (740 μL, 6 mmol) in 10 mL THF were added within 10 min. A clear orange solution developed and strirring was continued for 1 h at 0°C and 1 h at room temperature. Standard work up (saturated $NaHCO_3$-solution, $CHCl_3$, 1 N HCl, $NaHCO_3$-solution, water, brine, $MgSO_4$) and evaporation of the solvent under reduced pressure provided a yellow residue, which was purified by flash chromatography (1. *n*-hexane:EtOAc, 25:1; 2. *n*-hexane:EtOAc, 10:1; 3. *n*-hexane:EtOAc, 2:1) to give 1.1 g (81%) of **2** as colorless crystals. Mp 181–182°C; IR (KBr) $\tilde{\upsilon} = 1714$, 1672, 1664 cm^{-1} (s, C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.72 (s, 3H; 18-CH_3), 1.17 (s, 3H; 19-CH_3), 2.77 (t, 1H, $J = 8.8$ Hz, 17α-H), 3.92 (s, 2H; $-CH_2$-SBz), 5.72 (s, 1H; 4-H), 7.42–7.98 (m, 5H; Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.77 (20), 199.38 (3), 190.54 (SC=O), 170.82 (5), 136.20, 133.67, 128.62, 127.36, 123.88 (4), 61.98 (17), 55.97 (14), 53.50 (9), 44.53 (13), 40.52 (21), 38.51 (12), 38.45 (10), 35.64 (1), 35.51 (8), 33.88 (2), 32.69 (6), 31.80 (7), 24.38 (15), 23.45 (16), 21.01 (11), 17.31 (19), 13.35 (18). Anal. calcd for $C_{28}H_{34}O_3S$: C, 74.63; H, 7.60; S, 7.11; found: C, 74.45; H, 7.55; S, 6.99.

21-Mercaptoprogesterone (3). Thiobenzoate **2** (200 mg, 0.44 mmol) was dissolved in 20 mL MeOH. Then, 880 μL (0.88 mmol) 1 N NaOMe solution was added and the mixture was stirred for 2 h at ambient temperature under an argon atmosphere. Standard work up (1 N HCl, $CHCl_3$, $NaHCO_3$-solution, water, brine, $MgSO_4$) and evaporation of the solvent under reduced pressure provided a yellow oil, which was purified by flash chromatography (*n*-hexane:EtOAc, 4:1) to give 125 mg (82%) of **3** as colorless crystals. Mp 172–175°C; IR (KBr) $\tilde{\upsilon} = 2569$ cm^{-1} (w, S-H), 1705, 1662 cm^{-1} (s, C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.68 (s, 3H; 18-CH_3), 1.18 (s, 3H; 19-CH_3), 1.99 (t, 1H, $J = 7.0$ Hz, S-H), 2.72 (t, 1H, $J = 8.8$ Hz, 17α-H), 3.35 (d-AB, 2H, $J_{AB} = 15.8$ Hz, $J = 7.0$ Hz, -CH_2-SH), 5.73 (s, 1H; 4-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.51 (20), 199.39 (3), 170.71 (5), 123.97 (4), 61.47 (17), 55.95 (14), 53.55 (9), 44.53 (13), 38.57 (12), 38.53 (10), 35.70 (1), 35.56 (21), 35.53 (8), 33.91 (2), 32.71 (6), 31.83 (7), 24.42 (15), 23.59 (16), 20.97 (11), 17.34 (19), 13.62 (18). Anal. calcd for $C_{21}H_{30}O_2S$: C, 72.79; H, 8.73; S, 9.25; found: C, 72.44; H, 8.25; S, 8.81.

[3-Thiapentane-1,5-dithiolato][4-pregnen-3,20 dione-21-thiolato]oxorhenium(V) (7). Chloro(3-thiapentane-1,5-dithiolato)oxorhenium(V) **4** (187 mg, 480 μmol) was dissolved in 10 mL hot acetonitrile while stirring. Thiol **3** (110 mg, 320 μmol) in 10 mL acetonitrile was added. The mixture was refluxed for 2 h. Flash chromatography (1. *n*-hexane:EtOAc, 2:1; 2. $CHCl_3$:MeOH, 50:1) gave 150 mg (67%) of **7** as red crystals. Mp 189–191°C. IR (KBr) $\tilde{\upsilon} = 961$ cm^{-1} (s, Re=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 3H; 18-CH_3), 1.18 (s, 3H; 19-CH_3), 2.01 (m, 2H; A-part ABCD-system 'SSS'), 2.72 (t, 1H, $J = 8.9$ Hz, 17α-H), 3.15 (td, 2H, $J = 13.9$ Hz, $J = 4.1$ Hz, B-part ABCD-system 'SSS'), 3.95 (dtd, 2H, $J = 10.2$ Hz, $J = 3.9$ Hz, $J = 1.3$ Hz, C-part ABCD-system 'SSS'), 4.30 (dd, 2H, $J = 13.3$ Hz, $J = 4.8$ Hz, D-part ABCD-system 'SSS'), 4.63 (AB, 2H, $J = 14.3$ Hz, -CH_2-S-ReO 'SSS'), 5.73 (s, 1H; 4-H). LRMS (FAB negative) 697 (50.5), 698 (20.0), 699 (100) M-H, 700 (37.1), 701 (19.0). HRMS calcd for $C_{25}H_{38}O_3S_4Re$ [M + H]: 701.1261; found: 701.1254. Anal. calcd for $C_{25}H_{37}O_3S_4Re$: C, 42.90; H, 5.33; S, 18.32; found: C, 42.22; H, 5.30; S, 18.34.

[3-(*N*-Methyl)azapentane-1,5-dithiolato][4-pregnen-3,20-dione-21-thiolato]oxo-rhenium(V) (8). Thiol **3** (54 mg, 156 μmol), 26.6 mg (142 μmol) tridentate ligand **6** and 118 mg (142 μmol) oxorhenium(V) precursor **5** in 4 mL MeOH were combined in a flask. Then, 1.5 mL of 1 N NaOAc in MeOH was added, and the solution was refluxed for 1 h, during which time the heterogeneous light green reaction mixture turned to a clear dark green solution. Flash chromatography (1. *n*-hexane:EtOAc, 4:1; 2. CH_2Cl_2; 3. acetone:CH_2Cl_2, 1:39; 4. acetone:CH_2Cl_2, 1:19) gave 65 mg (66%) of **8** as a green foam. IR (KBr) $\tilde{\upsilon} = 954$ cm^{-1} (s, Re=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.71 (s, 3H; 18-CH_3), 1.17 (s, 3H; 19-CH_3), 2.68 (m, 2H; A-part ABCD-system 'SNS'), 2.89 (t, 1H, $J = 8.9$ Hz, 17α-H), 3.14 (m, 4H; B- and C-part ABCD-system 'SNS'), 3.35 (s, 3H; N-CH_3), 3.58 (m, 2H; D-part ABCD-system 'SNS'), 4.30–4.60 (m, 2H; -CH_2-S-ReO 'SNS'), 5.71 (s, 1H; 4-H). HRMS (FAB positive) calcd for $C_{25}H_{38}NO_3S_4Re$ [M + H]: 698.1806; found: 698.1820.

21-(Methylsulfonyl)oxy-progesterone (9). Deoxycorticosterone **1** (750 mg, 2.27 mmol) was dissolved in 40 mL THF. After the addition of 700 μL Et_3N and cooling to 0°C, 220 μL (2.8 mmol) MsCl in 10 mL THF was slowly added. The mixture was stirred for 2 h at 0°C and 4 h at room temperature. Standard work up (water, $CHCl_3$) and flushing through a short silica gel plug provided 882 mg (95%) of the mesylate **9** as colorless crystals after the evaporation of the solvent under reduced pressure. Mp 152–154°C; IR (KBr) $\tilde{\upsilon} = 1716$, 1661 cm^{-1} (s, C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.69 (s, 3H; 18-CH_3), 1.16 (s, 3H; 19-CH_3), 2.51 (t, 1H, $J = 8.8$ Hz, 17α-H), 3.21 (s, 3H; SO_2-CH_3), 4.76 (AB, 2H; $J_{AB} = 17.2$ Hz, CH_2-OMs), 5.71 (s, 1H; 4-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.86 (20), 199.31 (3), 170.53 (5), 123.94 (4), 72.69 (17), 58.65 (SO_3CH_3), 56.05 (14), 53.38 (9), 44.84 (13), 39.17 (21), 38.46 (12), 38.28 (10), 35.61 (1), 35.46 (8), 33.89 (2), 32.62 (6), 31.74 (7), 24.37 (15), 22.79 (16), 20.90 (11), 17.28 (19), 13.35 (18). Anal. calcd for $C_{22}H_{32}O_5S$: C, 64.68; H, 7.89; S, 7.85; found: C, 64.55; H, 7.98; S, 7.58.

21-Bromoprogesterone (10). Mesylate **9** (1.1 g, 2.7 mmol) was dissolved in 25 mL acetone and 1.54 g (17.7 mmol) LiBr was added in portions while stirring. The mixture became warm and a white precipitate formed. After refluxing for 2 h, the mixture was poured into water and extracted with $CHCl_3$. The organic layer was flushed through a short silica gel plug. After removal of the solvent and recrystallization from acetone 980 mg (92%) of the desired product was obtained. IR (KBr) $\tilde{\upsilon} = 1717$, 1657 cm^{-1} (s, C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.69 (s, 3H; 18-CH_3), 1.18 (s, 3H; 19-CH_3), 2.83 (t, 1H, $J = 8.8$ Hz, 17α-H), 3.90 (AB, 2H; $J_{AB} = 13.2$ Hz, CH_2-

Br), 5.77 (s, 1H; 4-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.83 (20), 199.28 (3), 170.57 (5), 123.96 (4), 60.12 (17), 55.90 (14), 53.50 (9), 44.67 (13), 38.50 (12), 38.46 (10), 35.75 (21), 35.68 (1), 35.53 (8), 33.89 (2), 32.67 (6), 31.80 (7), 24.40 (15), 23.70 (16), 20.95 (11), 17.33 (19), 13.54 (18); HRMS (EI positive, 70 eV) calcd for $C_{21}H_{29}BrO_2$: 392.1351; found: 392.1349. Anal. calcd for $C_{21}H_{29}BrO_2$: C, 64.12; H, 7.43; found: C, 63.55; H, 7.34.

21-(*N*,*N*-Diformylamino)progesterone (11). A mixture of 266 mg (2.80 mmol) $NaN(CHO)_2$ and 900 mg (2.30 mmol) bromide **10** were refluxed in 50 mL acetonitrile for 2 h. Standard work up (water, $CHCl_3$) and flushing through a short silica gel plug gave 830 mg (94%) of **11** after removal of the solvent under reduced pressure. IR (KBr) $\tilde{\upsilon}$ = 1722, 1667 cm^{-1} (s, C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.69 (s, 3H; 18-CH_3), 1.19 (s, 3H; 19-CH_3), 2.59 (t, 1H, J = 9.0 Hz, 17α-H, 4.42 (s, 2H; CH_2-N(CHO)$_2$), 5.73 (s, 1H, 4-H), 8.94 (s, 2H, C*H*O), ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.94 (20), 199.46 (3), 170.77 (5), 163.21 (CHO), 123.98 (4), 60.27 (17), 56.16 (14), 53.52 (9), 48.54 (21), 44.77 (13), 38.53 (12), 38.29 (10), 35.68 (1), 35.51 (8), 33.91 (2), 32.69 (6), 31.82 (7), 24.41 (15), 22.96 (16), 20.02 (11), 17.31 (19), 13.00 (18). Anal. calcd for $C_{23}H_{31}NO_4$: C, 71.66; H, 8.10; N, 3.63; found: C, 70.80; H, 8.11; N, 3.50.

21-(*N*-Formylamino)progesterone (12). Steroid **11** (760 mg, 2.00 mmol) was stirred in 50 mL EtOH with a catalytic amount of KOH at room temperature. The color of the mixture turned to yellow and it was worked up after 0.5 h (removal of EtOH under reduced pressure, water, $CHCl_3$). The organic layer was flushed through a short silica gel plug using $CHCl_3$:MeOH (10:1) to give 665 mg (93%) of **12** after removal of the solvent under reduced pressure. IR (KBr) $\tilde{\upsilon}$ = 1719, 1673 cm^{-1} (s, C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.67 (s, 3H, 18-CH_3), 1.17 (s, 3H, 19-CH_3), 2.52 (t, 1H, J = 9.0 Hz, 17α-H) 4.12 (dAB, 2H, J_{AB} = 20.0 Hz, J = 4.9 Hz, CH_2-NCHO), 5.72 (s, 1H, 4-H), 6.50 (br, 1H, N-H), 8.23 (s, 1H, C*H*O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.99 (20), 199.34 (3), 170.56 (5), 160.83 (CHO), 123.97 (4), 60.69 (17), 55.99 (14), 53.45 (9), 48.99 (21), 44.65 (13), 38.49 (12), 38.43 (10), 35.66 (1), 35.50 (8), 33.87 (2), 32.66 (6), 31.80 (7), 24.38 (15), 22.97 (16), 20.89 (11), 17.31 (19), 13.39 (18); HRMS (EI positive, 70 eV) calcd for $C_{22}H_{31}NO_3$: 357.2304; found: 357.2303.

[Tris(2-thiolatoethyl)amine-21-isocyanidoprogesterone]-rhenium(III) (14). Rhenium(III) precursor **13** (51.9 mg, 100 μmol), 107.3 mg (300 μmol) formamide **12** and 106 μl (750 mmol) diisopropylamine were dissolved in 5 mL CH_2Cl_2 and cooled to −78°C. Then, 28 μL (300 mmol) $POCl_3$ was added, and the mixture was allowed to warm up to room temperature. After stirring for 6 h at room temperature the volume of the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2: acetone, 9:1) to give 30 mg (53% corrected, 11 mg **13** recovered) of the complex **14** as a green powder. IR (KBr) $\tilde{\upsilon}$ = 1938 cm^{-1} (s, NC); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 (s, 3H, 18-CH_3), 1.18 (s, 3H, 19-CH_3), 2.70–3.30 (br, 12H, 'NS_3'), 3.09 (t, 1H, J = 9.0 Hz, 17α-H) 5.46 (AB, 2H, J_{AB} = 19.5 Hz, CH_2-NC-Re 'NS_3'), 5.73 (s, 1H, 4-H). HRMS (FAB positive) calcd for $C_{28}H_{42}N_2O_2S_3Re$ [M + H]: 721.1966; found: 721.1968.

21-(1,4-Dithiapent-1-yl)progesterone (15). 2-Mercaptomethyl-ethanethiol (195 mg, 1.8 mmol) and 202 mg (1.8 mmol) *tert*-BuOK were dissolved in 20 mL DMF under an argon atmosphere and stirred for 0.5 h at 0°C. Afterwards, 700 mg (1.7 mmol) of mesylate **9** in 5 mL DMF was slowly added and the mixture was allowed to warm up to room temperature. After strirring for 4 h at room temperature, standard work up (1 N KH_2PO_4, $CHCl_3$, water, brine, $MgSO_4$), evaporation of the solvent under reduced pressure, and flash chromatography (*n*-hexane:EtOAc, 5:1) provided 530 mg (74%) of the product **15** as colorless crystals. Mp 120–123°C; IR (KBr) $\tilde{\upsilon}$ = 1695, 1670 cm^{-1} (s, C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.68 (s, 3H, 18-CH_3), 1.17 (s, 3H, 19-CH_3), 2.11 (s, 3H, SCH_3), 2.68 (m, 4H,-S-(CH_2)$_2$-SCH_3), 2.89 (t, 1H, J = 8.8 Hz, 17α-H), 3.22 (s, 2H, -CH_2-S-(CH$_2$)$_2$-SCH_3), 5.71 (s, 1H, 4-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.79 (20), 199.33 (3), 170.73 (5), 123.91 (4), 60.04 (17), 55.94 (14), 53.52 (9), 44.54 (13), 41.73 (21), 38.57 (12), 38.50 (10), 35.65 (1), 35.49 (8), 33.88 (2), 33.49 (CH_2), 32.69 (6), 31.83 (7), 31.29 (CH_2), 24.41 (15), 23.68 (16), 20.94 (11), 17.31 (19), 15.40 (SCH_3), 13.63 (18). Anal. calcd for $C_{24}H_{36}O_2S_2$: C, 68.53; H, 8.63; S, 15.24; found: C, 68.36; H, 9.20; S, 15.34.

Bromo-tricarbonyl-{[21-(1,4-dithiapent-1-yl)progesterone]}-rhenium(I) 17. Compound **15** (63 mg, 150 μmol) dissolved in 2 mL acetone was added to a solution of 117 mg (150 μmol) $(NEt_4)_2[ReBr_3(CO)_3]$ **16** in 2 mL methanol. After stirring for 1 h at room temperature, the solvent was evaporated. Dry THF (2 mL) was added, and the precipitate was removed by filtration. The filtrate was concentrated under vacuum, and the oily residue was washed with diethyl ether to give 80 mg (69%) **17**. Mp 221–223°C. IR (KBr) $\tilde{\upsilon}$ = 2033, 1940, 1902 cm^{-1} (s, CO); ^{1}H NMR (400 MHz, $CHCl_3$) δ 0.67, 0.75 (s, 3H, 18-CH_3), 1.18 (m, 3H, 19-CH_3), 5.74 (s, 1H, 4-H); HRMS (FAB positive) calcd for $C_{27}H_{37}BrO_5S_2Re$ [M + H]: 771.0823; found: 771.0817. Anal. calcd for $C_{27}H_{37}BrO_5S_2Re$: C, 42.07; H, 4.71; S, 8.32; found: C, 41.84; H, 4.68; S, 8.25.

Tricarbonyl-[(carbo-4-pregnen-21-oxy)-3,20-dione)cyclopentadienyl]rhenium(I) 19. Progesterone **1** (21.8 mg, 0.066 mmol), CpTR-COOH **18** (25 mg, 0.066 mmol), and DMAP (a few crystals) were combined in 3 mL CH_2Cl_2 at room temperature. It was then cooled to 0°C, and EDC (13.9 mg, 0.073 mmol) was added all at once. It was then slowly warmed to room temperature overnight. The reaction was quenched with $NaHCO_3$ and passed through a silica gel plug, then purified by chromatography (MeOH:CH_2Cl_2, 1:39) to produce a yellow solid (44 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.72 (s, 3H, 18-CH_3), 1.18 (s, 3H, 19-CH_3), 2.53 (t, 1H, J = 8.9 Hz, 17(-H), 4.72 (AB, 2H, J_{AB} = 16.6 Hz, 21-CH_2), 5.39 (m, 2H, Cp-H), 5.73 (s, 1H, 4-H), 6.07 (m, 2H, Cp-H). HRMS (FAB positive) calcd for $C_{30}H_{34}O_7Re$ [M + H]: 693.1862; found: 693.1863.

X-ray crystal structure analysis of complex 7. The X-ray data of **7** were collected at room temperature on a Enraf–Nonius CAD 4 diffractometer, using graphite monochromized Cu-K_α radiation ($\lambda = 0.71069$ Å). The position of the non-hydrogen atoms were determined by the heavy atom technique.

The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 114993.

Crystal data and structure refinement details:

Formula	$C_{25}H_{37}O_3ReS_4$
Formula weight	699.99
Crystal system	monoclinic
Space group	P2 (1)
a(Å)	10.9440 (4)
b(Å)	8.2060 (3)
c(Å)	30.3428 (10)
α(°)	90.00
β(°)	97.2390 (10)
γ(°)	90.00
Volume (Å^3)	2703.3 (2)
Z	4
Temperature (K)	293 (2)
ρ(g/cm^3)	1.72
Absorption coefficient (mm^{-1})	4.829
F(000)	1400
m (MoK_α) (Å)	0.71073
Crystal size (mm^3)	0.45×0.216×0.072
θ-range for data collection	1.35–23.28
Index ranges	$-12 \le h \le 12$ $-8 \le k \le 9$ $-33 \le l \le 31$
Reflections collected	12,021
Independent reflections	7006 [R(int) = 0.0673]
Goodness-of-fit on F^2	1.079
R [I > 2s (I)]	R1 = 0.0499 WR2 = 0.1293
R (all data)	R1 = 0.0561 WR2 = 0.1416
Largest difference peak and hole (e/Å^{-3})	1.491 and −1.362

Determination of the receptor binding affinity. The assays were carried out according to Brandes et al.[25] using a competitive radiometric binding assay with estrogen-primed rat uterine cytosol as a source of the receptor, tritium-labeled RU5020 as tracer, and dextran-coated charcoal as adsorbent for free ligand at 0°C.

Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft and Deutscher Akademischer Austauschdienst (to F.W.) and from the US Department of Energy (to J.A.K. and M.B.S.) is gratefully acknowledged. We are grateful to Kathryn E. Carlson for assistance with receptor binding assays.

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