lies somewhat below 0.2 cm^{-1} and a small rhombic component is present.[13] The resonance positions of Figure 2b–d remain unaffected upon cooling the sample to 77 K.

Currently, we are engaged in a search for other examples of low-spin mononuclear and mixed-spin polynuclear manganese(II) complexes. The possible transformation of the latter into reactive mixed-valence species via chemical and/or electrochemical electron transfer is also under scrutiny. The $Mn_3L_6^+$ species observed cyclic voltammetrically is however too unstable to be isolated as salts.

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Registry No. Mn_3L_6, 114201-32-0; K[MnL_3], 114220-78-9; [Ph_4As][MnL_3], 114201-34-2; [Ph_4As][FeL_3], 114201-36-4.

Supplementary Material Available: Figures comparing the IR spectra (KBr disk) of [Ph_4As][FeL_3] and [Ph_4As][MnL_3] (Figure 3) and of $MnFe_2L_6$ and Mn_3L_6 (Figure 4) in the region 350–1600 cm^{-1} (3 pages). Ordering information is given on any current masthead page.

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Transition-Metal Carbonyl Complexes in Progesterone Receptor Assay

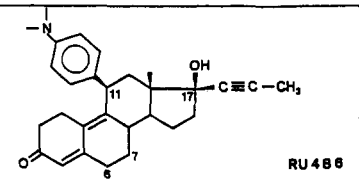
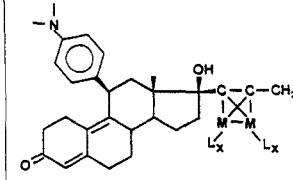
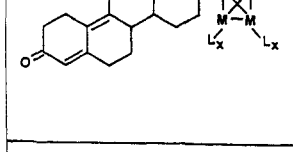
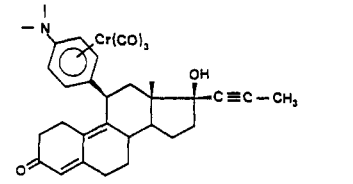
Sir:

Current biochemical steroid hormone receptor assays, particularly assays for estradiol and progesterone receptors, are important in therapy[1,2] but remain expensive, are difficult to perform, and require radiolabeled ligands.[3] An alternative receptor assay would ideally involve: a sensitivity comparable to that of radioisotopic detection methods, together with a lower cost; a marker with an indefinite shelf life exempt from any legal restrictions or licensing requirements.

A receptor assay based on metal carbonyl complexes [$M_x(CO)_y$] as markers and FT-IR spectroscopy as the detection method may fulfill very satisfactorily the above criteria.[4] Metal carbonyl complexes exhibit strong infrared absorption bands in the ν(CO) region between 2200 and 1800 cm^{-1}, a region normally devoid of strong absorption by other species, and thus can be detected by FT-IR spectroscopy in very low concentrations. In this paper, we will demonstrate for the first time the utilization of metal carbonyl moieties as markers in progesterone receptor assay.

While therapeutically useful synthetic antiestrogens, antiandrogens, antiglucocorticoids, and antimineralocorticoids have been available for some time,[5-8] only quite recently has a promising new synthetic antiprogestin called mifepristone (**1**, RU 486 [17β-hydroxy-11β-(4-(dimethylamino)phenyl)-17α-(1-propynyl)estra-4,9-dien-3-one]; see Table I) been developed.[9,10] This compound has high affinity for both the progesterone (PR) and the glucocorticoid (GR) receptor and has potent antiprogestin and antiglucocorticoid activity in vitro and in vivo.[11-13] Furthermore, mifepristone (**1**) appears to be a good candidate for transition-metal carbonyl labeling owing to the existence of several potential sites for complexation. The mapping of PR binding sites suggests close and rigid contact between the receptor and both the steroid A-ring and its "C_4, C_7, C_{14}" surface and a more labile

Table I. Relative Binding Affinities (RBA) of RU 486 and RU 486 Complexes for the Cytosol Progestin (PR) and Glucocorticoid (GR) Receptors[a]

	RBA	
	PR	GR
 **1** RU486	389	251
 **2**	13	17
 **3**	10	8
 **4**	20	29

[a] The RBA values for progestin (PR) and glucocorticoid (GR) receptors were measured in a routine screening assay as previously described.[22,23] Rabbit uterus and [^{3}H]R 5020 were used for PR; rat thymus and [^{3}H]dexamethasone were used for GR. Cytosol was incubated for 24 h at 0 °C. The RBA value of progesterone for PR and that of dexamethasone for GR were taken to be equal to 100.

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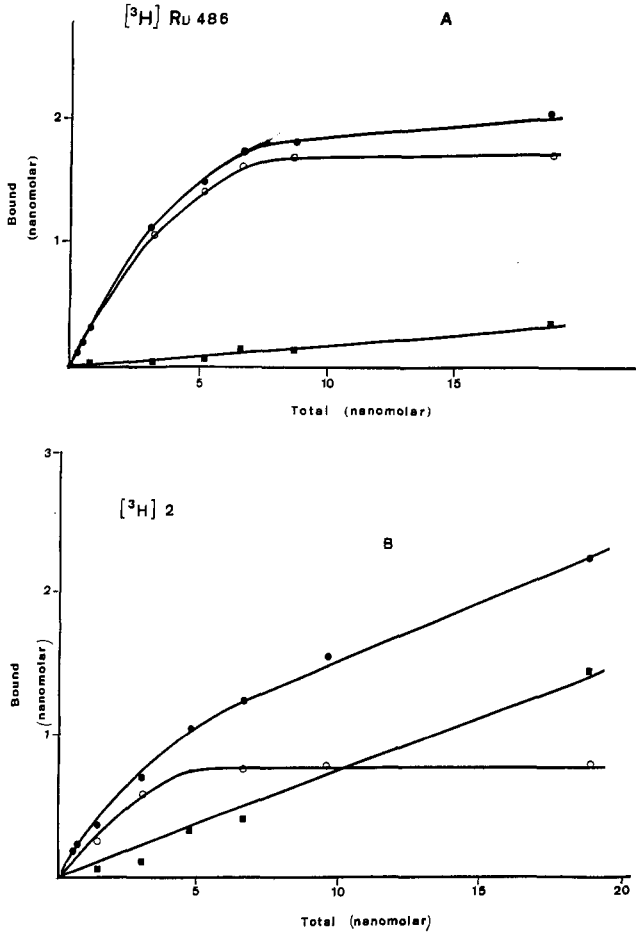

Figure 1. Interaction of [^{3}H]RU 486 and [^{3}H]**2** with uterine cytosol progestin receptor. Uteri from estrogen-primed female immature rabbits (25 μg of estradiol sc 96 h before sacrifice) were homogenized in 25 volumes (*w*/*v*) of buffer (Tris-HCl (10 mM Tris-HCl, 0.25 M, sucrose pH 7.4). Cytosol was prepared and incubated for 3 h at 0 °C with increasing concentrations of [^{3}H]RU 486 (A) or [^{3}H]**2** (B). Cytosol concentration was 0.95 mg of protein/mL. The amount of [^{3}H]RU 486 or of [^{3}H]**2** bound was determined by precipitation from solution with protamine sulfate.[24] Nonspecific binding (■) was determined in parallel incubations containing a 500-fold excess of cold RU 486. Key: (○) specific binding; (●) total binding.

association at "C_{11}, C_{12}, C_{17}".[14,15] Therefore, it may be postulated that with the complexation of a suitable organometallic moiety either to the arene at the 11β-position (compound **4**) or to the alkyne at the 17α position (compounds **2** and **3**), the recognition properties of the receptor for the modified hormone would be preserved. As shown in Table I, this expectation is confirmed by the values of relative binding affinities (RBA) for **2**–**4**.[16] As

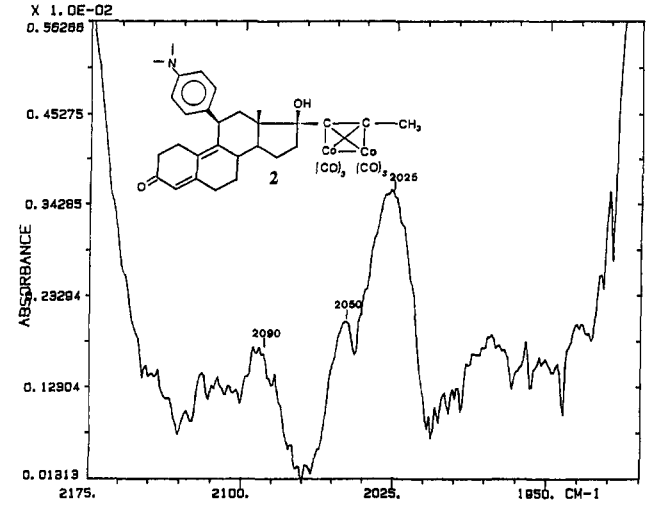

Figure 2. Difference spectrum obtained by the subtraction of the FT-IR spectrum of the precipitated receptor protein subsequent to in vitro incubation of cytosol with **2** (10^{-8} M in the presence of 500-fold unlabeled RU 486) from that of the spectrum of the precipitated protein after in vitro incubation of cytosol with **2** (10^{-8} M).

noticed for organometallic derivatives of estradiol,[4] complexation brings about a decrease of the RBA values. This tendency appears more striking for the 17α-complexed products **2** and **3** (for which the decrease is proportional to the steric hindrance of the label) than for complex **4** in which the $Cr(CO)_3$ label is on the 11β-arene ring. This result is consistent with the existence of a bulky hydrophobic pocket in the corresponding part of the receptor.[20] However, all these complexes show RBA values equal or superior to 10%, indicating that they should be capable of selective labeling of the specific receptor.[21]

In order to prove that the organometallic hormone is specifically bound at the progesterone receptor site, we prepared compound **2**, the easiest to obtain, tritium labeled at the 6,7-positions[19] and performed in vitro experiments with rabbit uterus receptor.

Figure 1 shows the saturation curves obtained after incubation of rabbit uterus receptor in the presence of increasing amounts of [^{3}H]**2** and [^{3}H]**1**. This experiment proves that both [^{3}H]**1** and the organometallic-labeled hormone [^{3}H]**2** bind to the progestin receptor. This binding is saturable and reversible. After 3 h of incubation at 0 °C, the level of nonspecific binding was found to be higher for [^{3}H]**2** than for [^{3}H]**1**. However, at saturation concentration (4×10^{-9} M), the level of specific binding for [^{3}H]**2** was twice that of the nonspecific binding. Thus, complex **2** was found to be suited for progesterone receptor assay.

Since the behavior of compound **2** toward PR has been fully established, this molecule was selected for the Fourier transform infrared (FT-IR) measurements. While, as shown in Figure 1, the level of non-specific-binding is relatively high in the presence of **2**, we are interested in detecting the amount of hormone *specifically* bound to the receptor. For this purpose the following samples have been prepared:

Experiment 1. The FT-IR spectrum of the precipitated receptor protein obtained from in vitro incubation of compound **2** (1×10^{-8}

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(16) **1** was obtained from Roussel-Uclaf (Romainville, France). **2** was prepared by reaction of RU 486 (**1**) with $Co_2(CO)_8$ in THF at room temperature. After purification by TLC, **2** was obtained in 82% yield. Decomposition occurs at about 160 °C. ^{1}H NMR (acetone-d_6, δ): 13-CH_3, 0.8 s; $N(CH_3)_2$, 2.9 s; $\equiv CCH_3$, 2.9 s; 11-H, 4.5 dd; 4-H, 5.6 s; aromatic, 6.61–7.1 [α ^{20}D]: −274° (*c* 2.77 × 10^{-3} g/mL, $CHCl_3$). **3** was obtained from a mixture of RU 486 (**1**) and $Mo_2Cp_2(CO)_4$ prepared according to Curtis[17] in THF that was heated for 4 h at 50 °C. After purification by TLC, **3** was obtained in 41% yield. Decomposition occurs at about 160 °C. ^{1}H NMR (C_6D_6, δ): 13-CH_3, 0.79 s; $\equiv CCH_3$, 2.61 s; Cp, 4.94–5.19. [α^{22} D]: +170° (*c* 2.23 × 10^{-3} g/mL, CH_2Cl_2). **4** could not be prepared by direct complexation with $Cr(CO)_6$, but was obtained by heating RU 486 (**1**) with $(NH_3)_3Cr(CO)_3$ in THF according to Vebrel[18] (72% yield). Decomposition occurs at about 170 °C. ^{1}H NMR (acetone-d_6, δ): 13-CH_3, 0.7 s; $\equiv CCH_3$, 1.8 s; aromatic, 4.9–6.1. [α^{19} D]: −41° (*c* 2.46 × 10^{-3} g/mL, acetone).
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(19) [6,7-^{3}H]**2** with a specific activity of 4.8 Ci/mmol was prepared by direct complexation with $Co_2(CO)_8$ of 1 mCi of [6,7-^{3}H]RU 486 (AS = 50.6 Ci/mmol diluted 1/10 by addition of cold RU 486 in order to get a sufficient amount for the synthesis) as indicated for the synthesis of the cold complex **2**.[16] Yield: 68%. For the calculation of the specific activity, the concentration of the radioactive solution was determined by UV spectroscopy (using the characteristic absorption band of the complex at 392 nm).
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M) with rabbit uterine cytosol (containing 560 fmol/mg of proteins of PR) was recorded for the sample pressed into a 3-mm wafer after protamine sulfate precipitation,[24] with no prior dilution in an alkali-metal halide matrix.

Experiment 2. A 3-mm wafer was prepared under the same conditions as in experiment 1 except a 500-fold excess of the unlabeled steroid (RU 486) was added to the incubation medium (to saturate the receptor specific binding sites).

The subtraction of the FT-IR spectrum of the latter sample, in which the organometallic-labeled steroid present is nonspecifically bound, from that of the first sample yields a difference spectrum in which the carbonyl intensity is proportional to the extent of specific binding in the first sample. In order to compensate for the difference in thickness between the two sample wafers, the absorbances of the ν(OH) overtone of the protein at 6700 cm^{-1} in the two spectra were measured and a normalization factor N was calculated from the ratio of these intensities (eq 1) (A is the absorbance of a ν(CO) band.

$$A(\text{sp}) = A(\text{expt 1}) - N(A(\text{expt 2})) \quad (1)$$

The difference spectrum represented by eq 1 (Figure 2) shows clearly two of the bands characteristic of the carbonyl moiety of the cluster at 2025 and 2050 cm^{-1}, while the third and least intense signal at 2090 cm^{-1} is not clearly discerned above the background noise. The amount of organometallic cluster detected here is in the range of 1 pmol. In order to achieve the necessary sensitivity to observe these relatively weak signals, we employed an InSb detector, which is the most sensitive detector in the 2100-1900-cm^{-1} region available commercially. This set of experiments shows the feasibility of this new sensitive receptor assay of wide applicability in principle, together with a burgeoning area of application for transition-metal cluster complexes.

Acknowledgment. We wish to thank Roussel-Uclaf, the ANVAR, the NSERC, and Bomem for facilities.

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Examination of an Alternate Structure for $Ba_2YCu_3O_{6+x}$ Using Single-Crystal X-ray Diffraction

Sir:

With the discovery that $Ba_2YCu_3O_{6+x}$ exhibits superconductivity at approximately 90 K, a great deal of work has been done to elucidate the crystal structure of this material. Neutron powder diffraction[1,2] and X-ray single-crystal diffraction[3,4] on both the orthorhombic and tetragonal forms of this material have proposed a layered variant of perovskite, with oxygen vacancies both in the Y layer and in the Cu layer between the Ba layers. In all this work, the cations have been ordered, with possibly some small interchange between the Y and Ba sites.[5] However, measurements using X-ray absorption near-edge structure (XANES)[6,7] have led to the proposal of an alternate structure, where Cu has substituted into the Y site at a level of 0.2-0.3 mole fraction. We have examined the single-crystal X-ray data taken on a number of tetragonal and orthorhombic crystals to determine if this model might be correct. Since the X-ray scattering factors for Cu and Y are very different (unlike the neutron scattering factors), the single-crystal X-ray structure should be sensitive to this substitution.

Table I. R Values Obtained for Cu Substitution into the Y Site

	mole fraction of Cu			
preparation	0.0	0.05	0.10	0.20
orthorhombic	0.0521	0.0529	0.0541	0.0548
tetragonal (700 °C, N_2)	0.0427	0.0467	0.0620	0.1198
tetragonal (melt grown)	0.0317	0.0318	0.0322	0.0321

Experimental Section. The crystals analyzed were prepared by standard techniques, and the analysis of their structures has appeared previously.[3,4] Briefly, single-crystal X-ray data were taken by using an automated Nicolet P3F diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71069 Å). The materials were assumed to be modified perovskites, and therefore, atomic positions were assigned on the basis of this structure and refined by using the SHELXTL program package.[8] The major difference between the orthorhombic and tetragonal structures is that the Cu layer between the Ba layers contains partially occupied O sites in the orthorhombic material, while these sites are vacant in the tetragonal form.

There is nothing unusual about the Y sites in any of these crystals that would make one suspect substitution. Both the positional and thermal parameters are well-behaved. In order to test the model proposed from the XANES data, the structures of an orthorhombic crystal,[3] a tetragonal crystal prepared by annealing at 700 °C under nitrogen,[4] and a tetragonal crystal grown from a melt[9] were refined with the occupancy of the Y site at ($^1/_2$, $^1/_2$, $^1/_2$) fixed at various Y/Cu ratios. The site was always assumed to be fully occupied.

Results and Discussion. The R values obtained from each of the refinements are presented in Table I. The differences between the tetragonal and orthorhombic material are caused not by differences in the cation positions but by differences only in the oxygen positions. Therefore, one would assume that any disorder in the cation positions would manifest itself in both orthorhombic and tetragonal material. In all three cases examined, the R value increased as Cu was allowed to substitute into the Y site, even though the number of parameters being refined also increased. In addition, the thermal parameters for the Cu atom became very large. Therefore, if any substitution for Y by Cu is taking place, it is at a level much lower than the 0.2-0.3 mole fraction determined from the XANES data. We feel that the perovskite structure with the ordered cations proposed from both neutron and X-ray diffraction measurements is, therefore, correct and that there must be another explanation for the XANES data.

One should note that the reason for the proposed substitution of Cu in the Y site is the observation of a 2.5 Å Cu-O distance. We wish to point out that, in the accepted structure of the tetragonal material, we determined a Cu-O distance of 2.466 (13) Å between the Cu in the layers near Y and the O in the Ba layers.

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