Here we describe the 1.95 Å structure of the clinically used antiprogestin RU486 (mifepristone) in complex with the progesterone receptor (PR). The structure was obtained by taking a crystal of the PR ligand binding domain (LBD) containing the agonist norethindrone, and soaking it in a solution containing the antagonist RU486 for extended times. Clear ligand exchange could be observed in one copy of the PR LBD dimer in the crystal. RU486 binds while PR is in an agonistic conformation, without displacing helix 12. Although this is probably due to the constraints of the crystal lattice, it demonstrates that helix 12 displacement is not a prerequisite for RU486 binding. Interestingly, B-factor analysis clearly shows that helix 12 becomes more flexible after RU486 binding, suggesting that RU486, being a model antagonist, does not induce one fixed conformation of helix 12, but changes its positional equilibrium. This conclusion is confirmed by comparing the structures of RU486 bound to PR and RU486 bound to the glucocorticoid receptor.

The drug RU486, also known as mifepristone, is the only clinically approved antiprogestin (trade name Mifegyne® or Mifeprrox®). It is applied to terminate pregnancy, and has been clinically tested in many more indications (1, 2). Recently, it was shown that RU486 can prevent mammary tumorigenesis in Brca1/p53-deficient mice, implying a use for RU486 in breast cancer therapy (3).

RU486 exerts its clinical effect by binding to the ligand binding domain of the progesterone receptor, although RU486 can also bind to the glucocorticoid receptor (GR) and weakly to the androgen receptor (AR) (4). All these nuclear receptors are close sequence homologs (5). Because the anti-GR activity of RU486 might be problematic in chronic administration (1), past research has focused on finding RU486 variants with more selectivity (4, 6-10) (Table I).

Despite the clinical importance of RU486, there is currently no three-dimensional structure of it bound to PR, its principal target. However, other complexes have been informative, such as that between the PR ligand binding domain and asoprisnil, which is biochemically a full antagonist and is chemically related to RU486 (Table I, ref. 11). Also informative is the crystal structure of RU486 bound to the GR LBD (12).

In all these structures the antagonists bind to a receptor conformation in which the C-terminal helix (called helix 12) is displaced compared to structures of bound agonists. This so-called helix 12 displacement was first seen in the structure of raloxifene bound to the estrogen receptor alpha, and it is commonly thought to be a general nuclear receptor mechanism (5, 13).

The indications from X-ray structures that, in PR, RU486 can induce displacement of helix 12, are supported by biochemical data. For instance the truncation of the PR C-terminus induces RU486 to act as agonist (14). Also, the C-terminus of PR becomes prone to proteolysis when RU486 binds (15). Finally, from modelling RU486 into the structure of bound progesterone it has been concluded that the 11β-substitution of RU486 (Table I) is sterically incompatible with the agonistic conformation of helix 12 (16).

Despite the evidence that RU486 can induce displacement of helix 12, it is still unclear if RU486 obligately dissociates helix 12 through steric repulsion, or if RU486 allows multiple
positions of helix 12, but changes their dynamic equilibrium. In this latter theory, called the dynamic model, RU486 would also be able to bind when helix 12 is in an agonist conformation. Indeed, under rare conditions RU486 can function as an agonist (7), and the compound asoprisnil, in-vivo, is known as a partial agonist (11). The use of corepressor peptides in crystal complexes, such as in the PR-asoprisnil complex (11), might lock helix 12 into its final antagonist position, whereas the compound alone would have more subtle effects. The dynamic model controversy has also arisen with other nuclear receptors, indicating its wider scope (13, 17).

To further elucidate the mechanism of RU486, we have determined the three-dimensional structure of RU486 bound to PR. For this, we developed a novel protocol in which ligands are exchanged in an existing PR crystal in which norethindrone is bound. The crystal lattice restricts the position of helix 12 to the agonist position, but RU486 is still able to bind, proving that it is sterically compatible with an agonist position of helix 12, and suggesting that RU486 works through changing the dynamic equilibrium of helix 12. Comparing our structure with the asoprisnil complex gives insight into the mechanism of helix 12 destabilization, which is confirmed by comparing our structure to that of RU486 bound to GR.

**EXPERIMENTAL PROCEDURES**

**Expression and purification of PR-LBD** – The PR LBD, comprising residues 678-933 was cloned in pET15b (Novagen). Expression was performed in *E. coli* BL21(DE3) star (Invitrogen), by overnight induction at 20°C in presence of 10µM norethindrone. Bacteria were lysed in buffer A (50 mM Tris pH 7.8, 250 mM NaCl, 10% glycerol) with 0.4 mM pefabloc (Roche) and 50µM norethindrone, and purified on NiNTA. Fractions were eluted with buffer A to which we added 10µM β-mercapto-ethanol, 10µM norethindrone and 100 µM imidazole. Elution fractions of the first three column volumes were discarded, the others were collected and treated with 2.5 % thrombin (Kordia) overnight at 4°C to remove the N-terminal his tag. Thrombin was removed by adding benzamidin sepharose (GE healthcare) and centrifugation for 10 min. at 5,000g and harvesting the supernatant. To make the final crystallization sample, the protein was dialyzed to buffer A to which we added 1 mM EDTA, 1 mM DTT and 10 µM norethindrone, and subsequently concentrated in a stirring cell to about 2 mg/ml as measured by its absorbion at 280 nm. The sample was stored at 4°C.

**Ligand replacement** - Crystals of the PR LBD in complex with norethindrone were grown at room temperature from 2µl drops hanging over a mother liquor (ML) of 12-25% PEG4000, 0.1M Hepes pH 6.5 and 100mM Li2SO4. Drops consisted of 1µl protein sample and 1µl ML. Crystals usually appeared after about three days and were kite-shaped.

For reference, one crystal was transferred to a cryoprotectant solution of 80% ML and 20% glycerol, dipped in liquid nitrogen and shipped for data collection to 1.6 Å at 100K. Another crystal was transferred to a ML to which 10µM RU486 was added. The crystal was stored in a sitting drop at room temperature. The solution surrounding the crystal was replaced by fresh solution 10 times over a period of 10 days. After this period the crystal was frozen as described above, and shipped for data collection to 1.95 Å at 100K.

All data were collected by the MXpress service of the ESRF, Grenoble, France. Structures were solved and refined using the ccp4i interface of the CCP4 software suite (18). PDB-entry 1A28, stripped of solvent, ligands and cofactors, was used for molecular replacement. The structure was refined with refmac5 (19) using 2 TLS domains, one for each protein chain and ligand. Corina (20) was used to generate geometry-restraints for the ligands. For final data statistics, see Table II. For electron density, see Fig. 1 and supplemental data. The structure and structure factor of the RU486 complex were submitted to the protein data bank (identifier 2W8Y).

**Activity measurements** – Agonistic and antagonistic profiles of compounds on PR, GR and AR were determined in cellular assays
using protocols similar to those published earlier for PR (21-23). For agonistic profiles we measured nuclear-receptor induced activation of a MMTV-luciferase reporter in CHO cells (22). These CHO cells were stably transfected with the reporter gene and the receptor of interest. For antagonistic profiles, we measured the decrease of activation, induced by a reference compound, by competition with a compound of interest (21). Reference compounds were for AR: dihydrotestosterone, PR: Org 2058 (23), GR: dexamethasone. Assays were run in a standardized fashion. The results are the average of a duplicate test in two different experiments (Table I).

**Binding data** - were taken from the literature (Tables III and IV). Usually RU486 data are reported as EC50s or relative affinities in radio-active competition assays. Because different reference ligands are used, these data are hard to compare. To alleviate this problem we converted EC50_{RU486} to K_{i,RU486} with the Cheng-Prusoff equation (24) as follows: 

$$K_{i,RU486} = \frac{EC50_{RU486}}{(1+\text{[ref]})K_{d,ref}}.$$  

Here [ref] is the reference ligand concentration, and K_{d,ref} the reference ligand dissociation constant. Wherever possible, all input values were taken from the original assay data source (Table IV).

**Conformer calculation** – 16 RU486 conformers were generated with the package ‘Catalyst’ (Accelrys©) These were minimized at the Hartree-Fock level with basis set 3-21G with the program Gaussian03 (Gaussian Inc., 2004). The resulting 5 different conformations were subjected to a rigid body fit into the electron density of the GR and PR RU486 complexes.

**RESULTS AND DISCUSSION**

**Structure of the norethindrone-bound complex** – To compare structures properly, it is very important that they are determined using the same crystallographic protocols. Therefore, we first redetermined the structure of the PR LBD coexpressed with the agonist norethindrone, to 1.55 Å. This structure is essentially identical to the published PR-norethindrone complex 1SQN (25), with an rmsd (C_{n}) of 0.118 Å. The asymmetric unit contains two copies of the PR-LBD, and in both copies norethindrone is present. However, the protein conformation of both copies is not identical (25), as differences occur in the regions comprising residues 703-712, 785-808 and 893-923, which are located N-terminally from helices 3, 6 and 12 respectively (for their location in the LBD, see Fig. 2A).

The PR ligand exchange protocol allows visualization of new compound classes – Using RU486 in the same manner as norethindrone in coexpression and purification did not generate crystals, but led to precipitation of the receptor. Therefore, we resorted to ligand replacement by soaking norethindrone-containing crystals.

In general, soaking protocols are a good way to obtain atomic information on compounds that are scarce or destabilize the protein, such as RU486. However, they have not been widely applied to nuclear receptors, because presence of high-affinity ligands is generally required for LBD stability during purification (26), and such ligands exchange only slowly. Promising experiments for mutant estrogen receptor (ERα) crystals have recently been described (27), but the most advanced protocol with PR thus far involves exchange of ligands during purification (26), which has not worked for RU486 so far.

To circumvent problems with slow exchange, we soaked our crystals in RU486 for a long time (10 days). This resulted in clear replacement of norethindrone by RU486 in one copy of the crystal dimer. This is evidenced by the appearance of electron density for the 11β-substitution (Fig. 1).

Subsequent refinement of the ligand with full occupancy and loose B-factor restraints results in an average B-factor for the 11β-substituent of only 3.5 Å² (2.5 st.dev.) above that of the average of the 17-atom steroid scaffold. This is comparable to the B-factor of the ketone at the 3-position, and therefore this refinement test indicates a high level of exchange. Thus, ligands can be replaced in PR crystals if long soaking times are used.

A drawback of ligand exchange is that the crystal lattice constrains the conformation of the protein. When a compound lacks affinity for the
restrained receptor conformation, ligand exchange does not take place or the crystals deteriorate due to induced fit. Both are frequent results in our hands (data not shown). However, when we do see exchange, as for RU486, the ligand has affinity for the restrained protein conformation and therefore the observed binding mode has thermodynamical relevance.

The flexible loop 785-808 could be a ligand entry route – Interestingly, compound exchange of RU486 takes place in only 1 copy of the dimeric PR LBD. In the other copy, norethindrone remains bound. This selective affinity is also observed when soaking with other compounds (data not shown). However, sometimes exchange is observed in both copies, indicating that the second PR LBD copy is accessible in the crystal, and that it must have lower steroid affinity, or a slower rate of ligand entry. This difference must arise from conformational differences in the PR LBDs, which are most prominent around the loop 785-808. This loop is also a region of substantial conformational variability (~1.0 Å) when our structure is compared to the PR/norethindrone, PR/asoprisnil, and the GR/RU486 complexes (see below). A Cys798Val mutation in this loop abrogates all progesterone binding activity of PR (28), even though Cys798 is not in direct contact with the ligand. Interestingly, the same region was recently suggested to allow ligand entry in ERα (27). Therefore we propose that also in PR, the flexible loop 785-808 is involved in ligand entry.

The binding mode of RU486 explains antiprogestin resistance – The interactions of the steroidal rings of RU486 are similar to those of other steroids such as norethindrone (ref. 11, Fig. 2), and in line with mutagenesis results (28). The ketone of the A-ring anchors to Arg 766 and Gln725. The 17β-hydroxyl on the D-ring forms a water-mediated hydrogen bond to Asn 719, and is oriented towards Thr 894 at 4.5 Å distance. The 17α-propynyl of RU486 is accommodated in a cavity formed by Leu 715, Leu 718, Phe 794, Leu 797, Met 801 and Tyr 890 (ref. 11, Fig. 2 and 3).

Additional interactions are formed by the 11β-substitution of RU486. This binds into the hydrophobic pocket lined by Gly 722, Trp 755, Met 759, and Met 909 (Fig. 2 and 3), which is also the 11β-substituent pocket in other nuclear receptors (5, 11).

Interestingly, the terminal dimethylamine is not coplanar with the phenyl moiety of the 11β-substitution. This strained conformation is supported by the electron density (Fig. 1). To see if it also occurs in small molecule crystal structures, we analyzed 1347 N,N-dimethylanilines without ortho substituents in the Cambridge Structural Database (CSD, ref. 29). These show a median angle of 8.8° between the plane through the phenyl ring and the plane through the dimethylamino-group, revealing that the skew is a property of dimethylanilines.

The terminal dimethylamine engages in a π–π interaction with the main chain carbonyl group of Gly722 (3.9 Å). This Gly 722 is a close neighbour of Gln 725 that anchors the steroid A-ring, and together they form a loop that clasps one side of the antiprogestin.

In the past, a Gly722Cys variation in the PR of chicken and hamsters was found to cause resistance to RU486 (30). From modelling it was concluded that a Cys 722 side chain could potentially block RU486 binding (16). This is confirmed in our structure. If modelled, the Cβ of Cys 722 would be at 2.0 Å distance from the phenyl moiety in RU486, leading to incompatible steric clashes.

RU486 binds to an agonist-like conformation of the LBD – When the RU486-bound PR LBD is superimposed on a norethindrone-bound PR LBD (we used the equivalent copy in the unsoaked structure), this shows that helix 12 has a similar orientation (Fig. 2). Therefore, RU486 binds to an agonistic conformation of the PR LBD. Clearly, this is caused by the restraints of the crystal contacts, which were formed when the crystal was grown in presence of norethindrone.

It is surprising that RU486 binds to the ‘agonistic’ PR-LBD conformation at all, since in the past, it was predicted that the dimethylaniline would clash with Met 909 in helix 12 and Trp 755 (16). In our structure we only observe a more subtle steric conflict. In comparison to the norethindrone complex, the side chain of Met 909 becomes disordered and the neighbouring Ile 913 assumes a different
rotamer (Fig. 1). There is only incomplete electron density for the side chain of Met909, and any rotamer we model remains sterically uncomfortably distanced from the terminal dimethylamine. In reality we probably observe the average of an ensemble of subtly different dimethylaniline and Met 909 orientations, each sterically allowed.

Other differences are observed, compared to the norethindrone complex, in the two flexible loops 785-808 and 703-712. After RU486 binding these have shifted 0.5 Å and 0.3 Å respectively, inducing a (partially occupied) peptide bond flip of Ser 792. These shifts widen the hormone binding pocket. It appears as if RU486 wrenches itself in the LBD, between Phe 794 in the loop 785-809, and Met 909 in helix 12 (Fig. 2). Thus, although RU486 is bound with helix 12 in the agonist position, the LBD is clearly changed.

**RU486 binding induces higher B-factors in helix 12** – The structures of the PR-LBD bound to norethindrone and RU486 were determined using the same crystallographic protocols, and since they have the same crystal contacts, we can attribute any differences in main-chain B-factors to ligand binding. B-factor differences between the individual structures were calculated as $B_{i,\text{RU486}} - B_{i,\text{norethindrone}}$ for every atom $i$, for the equivalent PR copies. When plotted, these differences indicate substantial effects, particularly in the region of helix 12 (Fig. 2A and supplemental data). Thus, even though helix 12 has an ‘agonist-like’ orientation, RU486 clearly increases its flexibility.

The mechanism of helix 12 destabilization – It is interesting how the single steric clash between Met 909 and RU486 can lead to destabilization of the entire helix 12 as measured by B-factors. This can be explained because Met 909 on the helix interface protrudes deeply into the LBD core and represents a hydrophobic hook that can be dissociated by RU486. In addition, the main chain N atom of Met 909 is located at the amino-terminal side of helix 12, where it is capped by Glu 723 (Fig. 2B), which functions as the residue that stabilizes the helix 12 dipole (31). A small shift of Met 909 will therefore lead to disruption of this dipole system, leading to destabilization of the entire helix.

**Differences in the binding mode to PR of RU486 and asoprisnil** – Recently, the three-dimensional structure of the PR LBD was reported, bound to the antagonist asoprisnil and a SMRT peptide (PDB code 2OVH, ref. 11) (Fig. 3). For unknown reasons, asoprisnil and SMRT stabilize the complex sufficiently to allow cocrystallization experiments, whereas in our hands RU486, even in combination with peptides, yields an unstable complex. Nevertheless, RU486 and asoprisnil bind to PR with similar affinities (Table III).

In the asoprisnil structure helix 12 is rearranged (ref. 11, Fig 3), to make room for binding of the SMRT peptide. Thereby, the asoprisnil complex represents a PR structure in full antagonistic mode (5, 11).

Apart from the helix 12 shift, our PR/RU486 and the PR/asoprisnil structures overlap well, though again the loops 703-712 and 785-808 show conformational flexibility. RU486 and asoprisnil have a similar binding orientation, but asoprisnil is positioned 0.8 Å ‘upwards’ towards Met 909 in helix 12, which is now out of the way. At the bottom of the pocket, Phe 794 (1.1 Å) and Leu 797 (0.8 Å) are upwardly shifted, to match the 17α-substituent of asoprisnil. The Phe 794 and Leu 797 sidechains of the asoprisnil structure would need to shift downward to accommodate the 17α-substituent of RU486. Therefore, compared to RU486, asoprisnil appears squeezed out of the LBD, towards helix 12 (Fig. 3). This demonstrates that the loop 785-808 and the 17α-substituent also contribute to helix 12 displacement and thereby to antiprogestin activity (Fig. 4).

Our RU486 complex is an intermediate in antagonist action – Using the above analyses, we can place our structure in the route of antiprogestin action (Fig. 4). Normally, before treatment, progesterone is bound to the PR-LBD. This is represented by the structure with PDB code 1A28. Upon treatment, progesterone is replaced by RU486, and helix 12 is destabilized. Its new position is a dynamic equilibrium that includes the agonist position (as in our structure) and the antagonist position...
RU486 binds with similar affinity to PR and GR – To compare the binding affinities of RU486 for PR and GR we converted historic binding EC50s to Ki (Table IV). This indicates that RU486 binds to native PR with an average Ki of 1.9 nM, and that the Ki for GR is similar at about 2 nM. The Ki for the separate LBDs (15-40 nM) is higher than for full length receptors, but still substantial (Table IV).

Next we superimposed our PR/RU486 complex on the published GR/RU486 complex (PDB code 1NHZ, ref. 12). This latter structure again shows a shifted helix 12 conformation, although in the GR/RU486 structure, the space freed up by the helix 12 shift is filled up by helix 12 from another GR monomer, and thus care should be taken in interpreting this structure as a full antagonistic conformation (12). Nevertheless, the comparison still yields meaningful insights.

RU486 binds with a different conformation to PR and GR – Interestingly, the steroid A ring of RU486 has a different puckering conformation in both complexes (Fig. 5). To confirm this, we calculated the experimental electron densities belonging to both complexes and show that the RU486 conformation in the 1NHZ structure does not fit our density and vice versa (Fig. 5).

To check the physical reality of the different conformations we calculated RU486 conformers ab initio. The lowest energy conformation is similar to that observed in the GR electron density (12). A higher energy conformation, which matches CSD entry FAFGOI (32) (+1.7 kcal/mol in vacuo), fits in the electron density of our PR structure (Fig. 5). Similar disorder in the A ring of 3-keto-Δ^4 steroids has been observed before in the X-ray structures of 3-keto-desogestrel (33) and nandrolone (34). We conclude that in PR, RU486 binds in a somewhat strained conformation. Since this conformation is also seen in the PR/asoprisnil complex, it is probably not induced by the agonistic helix 12 position, but by differences in the binding pockets of GR and PR.

RU486 has different binding interactions in PR and GR – The PR/RU486 and the GR/RU486 complexes differ in their conformation of helix 12 and to a lesser extent in the conformations of the loops of residues 785-808 and 703-712 (548-557 and 630-653 in GR) (Fig. 6). Moreover, in GR, the 11β-dimethyl-aniline of RU486 has shifted 1.4 Å and occupies space freed up by helix 12 rearrangement (Fig. 6). In this ‘GR’ orientation, the phenyl group would stereochemically clash at 2.4 Å with Asn 719 in the PR conformation. However, in GR, the equivalent Asn 564 has assumed a different rotamer (made possible by the helix-12 shift), which still binds the ligand’s 17β-hydroxyl group, but now via two water molecules instead of one.(Fig. 6).

In GR, the 17α-propynyl group of RU486 delves 1.6 Å deeper into its subpocket compared to PR. This is due to presence of the flexible Met 639 in GR instead of the bulky Phe 794 as in PR. This orientation is stabilized in GR by Gln 642 forming a 2.7 Å hydrogen bond to the 17β-hydroxyl of RU486. This hydrogen bond is strengthened by the burial between Met 560, Met 639 and Tyr 735. In PR, Gln 642 is replaced by Leu 797, which is unable to form hydrogen bonds to RU486 (Fig. 6). Therefore, although RU486 binds to both PR and GR with similar affinity, there are clear differences in its binding interactions.

Specificity differences in RU486-like compounds – To compare the activities of RU486 and related compounds, we determined their activities in our own set of cellular assays (Table I). Binding data for many of these compounds were reported earlier (ref. 4, Table IV), but here our cellular assays allow discrimination between agonistic and antagonistic activities.

Our results confirm that RU486 has full antagonistic activity in PR and AR, but interestingly in GR, RU486 is a partial antagonist (with 10% agonistic activity). On the atomic level, this difference might be caused by RU486 binding more deeply into the
17α-propynyl-binding pocket in GR. This might ensure less clashes between the 11β-substitution of RU486 and helix 12, at the other end of the LBD, thereby facilitating binding of helix 12 in its agonist conformation, leading to a more partial compound profile on GR.

Structures help to understand different profiles of RU486-related compounds – Our activity data show that asoprisnil and onapristone are more specific for PR than RU486, conforming earlier results (ref. 7, Table I). Underlying this increased specificity are the chemical differences between the compounds, which are mostly located around the 11 and 17 positions of the steroid ring (Table I).

As for the 11β-substitution, our structure, and biological data (6, 14), suggests that it does not solely disrupt helix 12, but also provides binding energy. Replacing the 11β-terminal dimethylamine by an oxime leads to threefold stronger binding to PR (6). Probably, through its increased coplanarity with the phenyl group, the oxime can stack better to Gly 722. Alternatively, the oxime might fit better in the solvent exposed environment after helix 12 displacement. However, as binding interactions are essentially similar in PR and GR, it is unlikely that the 11β-substituents in Table I confer selectivity.

At the 17-position, introduction of an alkyl ether in asoprisnil or alcohol in onapristone reduces GR activity (refs 6, 7, Table I). Both the 17β-methoxy of asoprisnil and the 17β-hydroxypropyl of onapristone can be accommodated in PR, but clash with Gln 642 in GR. In this way, differences at the 17-position may play a role in the selectivity of these antiprogestins.

Conclusion – Our PR/RU486 structure resembles an intermediate in antiprogestin action and gives new insight into how RU486 acts on PR. Our structure suggests that RU486 does not induce one particular receptor conformation, but changes the equilibrium of the helix 12 position. Importantly, the whole LBD cooperates in helix 12 displacement through concerted action of various loops and residues. Apart from RU486, many more drugs are 11β-substituted steroids or related antihormones (5, 13). Through this structure, the molecular mechanism of this whole drug class can be better understood.

REFERENCES
1. Cadepond, F., Ulmann, A., and Beaulieu, E-E. (1997) *Annu. Rev. Med.*, 48, 129-156
2. Leonhardt, S.A., and Edwards, D.P. (2002) *Exp. Biol. Med.*, 227, 969-980
3. Poole, A.J., Li, Y., Kim, Y., Lin, S-C.J., Lee, W.H., and Lee, E.Y.H.P. (2006) *Science*, 314, 1467-1470
4. Kloosterboer, H.J., Deckers, G.H., de Gooyer, M.E., Dijkema, R., Orlemans, E.O.M., and Schoonen, W.G.E.J. (1995) *Ann. NY Acad. Sci.*, 761, 192-201
5. Greschik, H., and Moras, D. (2003) *Curr. Top. Med. Chem.*, 3, 1573-1599
6. Winneker, R.C., Fensome, A., Zhang, P., Yudt, M.R., McComas, C., and Unwalla, R.J. (2008) *Steroids*, 73, 689-701
7. Wehle, H., Moll, J., and Cato, A.C.B. (1995) *Steroids*, 60, 368-374
8. Pullen, M.A., Laping, N., Edwards, R., and Bray, J. (2006) *Steroids*, 71, 792-798
9. Attardi, B.J., Burgenson, J., Hild, S.A., and Reel, J.R. (2004) *J. Steroid Biochem. Molec. Biol.*, 88, 277-288
10. Palmer, S., Campen, C.A., Allan, G.F., Rybczynski, P., Haynes-Johnson, D., Hutchins, A., Kraft, P., Kiddoe, M., Lai, M-T., Lombardi, E., Pedersen, P., Hodgen, G., and Combs, D.W. (2000) *J. Steroid Biochem. Molec. Biol.*, 75, 33-42
11. Madauss, K.P., Grygielko, E.T., Deng, S-J, Sulpizio, A.C., Stanley, T.B., Wu, C., Short, S.A.,
    Thompson, S.K., Steward, E.L., Lapin, N.J., Williams, S.P., and Bray, J.D. (2007) *Molecular
    Endocrinology*, 21, 1066-1081
12. Kauppi, B., Jakob, C., Färnegårdh, M., Yang, J., Ahola, H., Alarcon, M., Calles, K., Engström, O.,
    Harlan, J., Muchmore, S., Ramqvist, A-K., Thorell, S., Öhman, L., Greer, J., Gustafsson, J-Å.,
    Carlstedt-Duke, J., and Carlquist, M. (2003) *J. Biol. Chem.*, 278, 22748-22754
13. Kong, E.H., Pike, A.C.W., and Hubbard, R.E. (2003) *Biochem. Soc. Trans.*, 31, 56-59
14. Vegeto, E., Allan, G.F., Schrader, W.T., Tsai, M-J., McDonnell, D.P., and O’Malley, B.W. (1992)
    *Cell*, 69, 703-713
15. Allan, G.F., Leng, X., Tsai, S.Y., Weigel, N.L., Edwards, D.P., Tsai, M-J., and O’Malley, B.W.
    (1992) *J. Biol. Chem.*, 267, 19513-19520
16. Williams, S.P., and Sigler, P.B. (1998) *Nature*, 393, 392-395
17. Nakabayashi, M., Yamada, S., Yoshimoto, N., Tanaka, T., Igarashi, M., Ikura, T., Ito, N.,
    Makishima, M., Tokiwa, H., DeLuca, H.F., and Shimizu, M. (2008) *J. Med. Chem.*, 51, 5320-5329
18. Potterton, E., Briggs, P., Turkenburg, M., and Dodson, E.J. (2003) *Acta Cryst.*, D59, 1131-1137
19. Murshudov, G.N., Vagin, A.A., and Dodson, E.J. (1997) *Acta Cryst.*, D53, 240-255
20. Copeland, R.A. (2000) *Enzymes*, Wiley, New York
21. Madauss, K.P., Deng, S-J., Austin, R.J.H., Lambert, M.H., McLay, I., Pritchard, J., Short, S.A.,
    Stewart, E.L., Uings, I.J., and Williams, S.P. (2004) *J. Med. Chem.*, 47, 3381-3387
22. Hassell, A.M., An, G., Bledsoe, R.K., Carter III, H.L., Deng, S-J. J., Gampe, R.T., Grisard, T.E.,
    Madauss, K.P., Nolte, R.T., Rocque, W.J., Wang, L., Weaver, K.L., Williams, S.P., Wisely, G.B.,
    Xu, R., and Shewchuk, L.M. (2007) *Acta Cryst.*, D63, 72-79
23. Nettles, K.W., Bruning, J.B., Gil G., Nowak J., Sharma, S.K., Hahm, J.B., Kulp, K., Hochberg,
    R.B., Zhou, H., Katzenellenbogen, J.A., Katzenellenbogen, B.S., Kim, Y., Joachimiak, A., and
    Greene, G.L. (2008) *Nature Chemical Biology*, 4, 241-247
24. Letz, M., Bringmann, P., Mann, M., Mueller-Fahnow, A., Reipert, D., Scholz, P., Wurtz, J-M.,
    and Egner, U. (1999) *Biochim. Biophys. Acta*, 1429, 391-400
25. Allen, F.H. (2002) *Acta Cryst.*, B58, 380-388
26. Benhamou, B., Garcia, T., Lerouge, T., Vergezac, A., Gofflo, D., Bigigne, C., Chambon, P., and
    Gronemeyer, H. (1992) *Science*, 255, 206-209
27. Branden, C., and Tooze, J. (1991) *Introduction to protein structure*, Garland, New York
28. Reisch, J., Zappel, J., Rao, A.A.R., and Henkel, G. (1994) *Arch. Pharm.*, 327, 809-811
29. van Geerestein V.J., Kanters, J.A., and Kroon, J. (1987) *Acta Cryst.*, C43, 2398-2401
30. Precigoux, G., Busetta, B., Courseille, C., and Hospital, M. (1975) *Acta Cryst.*, B31, 1527-1532
31. Vellieux, F.M.D., and Dijkstra, B.W. (1997) *J. Appl. Cryst.*, 30, 396-399
36. Jones, D.G., Liang, X., Stewart, E.L., Noe, R.A., Kallander, L.S., Madauss, K.P., Williams, S.P., Thompson, S.K., Gray, D.W., and Hoekstra, W.J. (2005) *Bioorganic Med. Chem. Letters*, **15**, 3203-3206

37. Honer, C., Nam, K., Fink, C., Marshall, P., Ksander, G., Chatelain, R.E., Cornell, W., Steele, R., Schweitzer, R., and Schumacher, C. (2003) *Molecular Pharmacology*, **63**, 1012-1020

**FOOTNOTES**

The authors acknowledge Elspeth Gordon and Stephanie Monaco of the ESRF, Grenoble, for data collection. They thank Hans Hamersma, Cor Kuil, Scott Lusher, Sabine Mulders, Martin-Jan Smit and Arie Visser for comments on the manuscript, Tsang Lam and Maria van Rosmalen for measuring activity data, and Diep Vu for supporting crystallization work.

**FIGURE LEGENDS**

Table I
ago: agonistic activity, ant: antagonistic activity, efficacy is measured by curve height at maximal effect and expressed as percentage of reference compound effect (see experimental section), nr: no response, nd: not determined. The indicated error margins are standard deviations calculated over all measurements (minimum of four data points). PR activity was measured on the B isoform. The top left panel shows the nomenclature of the steroid ring for clarity.

Table II
Data between brackets indicate the last resolution shell. a$R_{	ext{pim}} = \frac{\sum_i \left| \frac{1}{N-1} \right| \left[ \sum_i I_i(h) - \langle I(h) \rangle \right]}{\sum_i I_i(h)}$, where $I_i$ is the observed intensity and $\langle I \rangle$ is the average intensity of multiple observations from symmetry-related reflections. It is an indicator of the precision of the final merged and averaged data-set. b$R_{\text{free}}$ was determined using 5% of the data.

Table III
ruPR= rabbit uterine PR. In the LBD, rabbit and human PR have identical sequences.

Table IV
Data were taken from literature. Unless otherwise indicated, all data in a row originate from the reference in the first column. ruPR: rabbit uterine PR, hPR: human PR, rtGR: rabbit thymic GR, hGR: human GR. PR-A and PR-B are isoforms, prog:progesterone, dex: dexamethasone, nr: not reported. ano errors reported. bas reported by literature source, or calculated from EC50 of reference ligand (ref) in the same assay through $K_{d,\text{ref}} = \frac{EC50_{\text{ref}} \cdot [\text{ref}]}{24}$ c$K_{d,\text{ref}}$ taken from Pullen et al. (8) as Palmer et al. (10) report 0.05nM which seems very low. d$K_{d,\text{ref}}$ taken from Attardi et al. (9). econverted from EC50 using $K_i = \frac{EC50_{\text{RU486}}}{(1+[\text{ref}]/K_{d,\text{ref}})}$. The average $K_i$ of RU486 for full length PR (not LBD only) is 1.9 nM ±1.4.
**Fig. 1.** $F_o-F_c$ OMIT electron density around the ligands in the PR LBD after soaking. The map is contoured at 3 standard deviations (3σ): A: the PR copy in the asymmetric unit which shows norethindrone bound. Residue Met 909 assumes a double conformation. B: the other PR copy, which shows electron density for RU486 and displacement of Met 909. The map was calculated after taking out the ligands from the final PDB file followed by additional refinement with refmac (19) to remove remaining phase bias. The structure shown is the final PDB file. In the supplemental data, a $2F_o-F_c$ OMIT map, calculated according to Bhat’s protocol (35) is shown.

**Fig. 2.** Binding of RU486 (purple) in the PR LBD (blue/red). A: Ribbon view of the PR LBD copy in the dimer that binds RU486. The colouring represents B-factor changes compared to the equiconformational LBD copy in the PR norethindrone complex. Changes range from -4 (blue) to +44 (red) Å$^2$, and are predominantly restricted to the loop 785-808 and helix 12. The conformation of helix 12 is agonistic, closely packed against the LBD core B: Superposition of the two PR LBD copies in the dimer, one containing RU486 (colouring as in panel A), one containing norethindrone (grey). Important residues and loops are marked.

**Fig. 3.** Superposition of the PR LBDs bound to asoprisnil and to RU486. The asoprisnil complex (copper) was obtained from the PDB (code 2OVH, ref. 11). The RU486 complex is shown in blue. The corepressor SMRT in this asoprisnil complex is coloured green. A: overview of the total LBD, B & C: detailed views of the ligand binding pocket.

**Fig. 4.** The mechanism of antagonistic action in PR. The circle outlines the PR LBD pocket, capped by helix 12 (rectangle). Top left: when progesterone is bound, the LBD is in a tightened agonist conformation (model after PDB structure 1A28). Top right: when an antagonist such as RU486 displaces progesterone, the LBD pocket widens by pressure on Phe 794 and Met 909 (model after our structure). Low right: this pressure can be released by helix 12 displacement, followed by return of Phe 794 and the loop 785-808 to a more relaxed conformation. This conformation is further stabilized by corepressor binding (model after the PDB structure 2OVH).

**Fig. 5:** $2F_o-F_c$ electron densities (1.2 σ contouring) for our PR/RU486 complex (2W8Y, panel A) and the GR/RU486 complex (1NHZ, panel B, diffraction data obtained from the PDB). Ligands modelled in the original X-ray structures are in grey. The cyan conformation is the low-energy inverted half-chair conformation (1β, 2α) that fits the GR density. The dark blue conformation shows the normal half-chair conformation (1α, 2β) that fits the PR density and which has somewhat higher energy in vacuo. The *ab initio* ligands (cyan/blue) were rigid body fitted into the density using the program Coot.

**Fig. 6.** Superposition of RU486 bound to GR (cyan) and PR (blue). The GR structure was obtained from the PDB (code 1NHZ, ref. 12). A: overview of the total LBD. B: detail of the ligand binding pocket. The shifted steroid ring orientation and the different puckering in the steroid A-ring can be clearly observed.
| Structure | Trivial name               | PR-B activity ago (nM) (efficacy) | GR activity ago (nM) (efficacy) | AR activity ago (nM) (efficacy) |
|-----------|---------------------------|----------------------------------|---------------------------------|---------------------------------|
| ![Progesterone structure](image1) | Progesterone               | 0.60±0.01 (89%)                  | >1000                           | > 100                           |
| ![Norethindrone structure](image2) | Norethindrone/ norethisterone | 0.39±0.04 (101%)                | >1000                           | 126±3 (98%)                    |
| ![RU486/ mifepristone structure](image3) | RU486/ mifepristone        | >1000                            | >1000                           | 36±12 (41%)                    |
| ![J867/ Asoprisnil structure](image4) | J867/ Asoprisnil (Jenapharm/ TAP/Bayer-Schering) | 0.14±0.02 (96%)                | 3.5±1.5 (10%)                  | >1000                           |
| ![ZK98299/ Onapristone structure](image5) | ZK98299/ Onapristone (Bayer-Schering) | 3.4±0.4 (98%)                   | >1000                           | >1000                           |
Table II

|                              | Norethindrone | RU486   |
|------------------------------|---------------|---------|
| Space group                  | P2₁           | P2₁     |
| Unit cell (Å³)               | 57.71x64.26   | 58.16x63.90 |
| x70.12                       | x70.05        |         |
| β-angle (°)                  | 95.65         | 95.57   |
| Wavelength (Å)               | 0.939         | 0.934   |
| Resolution (Å)               | 69.84-1.55    | 69.67-1.95 |
| (1.59-1.55)                  | (2.06-1.95)   |         |
| Completeness                 | 98.5%         | 99.8%   |
| (85.9%)                      | (99.3%)       |         |
| R_pim a                      | 0.058 (0.378) | 0.047 (0.284) |
| Mn(I/σ)                      | 11.4 (1.9)    | 13.9 (3.1) |
| R factor / R_free b          | 0.156/0.187   | 0.174/0.208 |
| Protein atoms                | 4111          | 4113    |
| Ligand atoms                 | 44            | 54      |
| Water molecules              | 573           | 199     |
| Other molecules              | 35            | 21      |
| (eg glycerol, SO₄³⁻)         |               |         |
| Rmsd bonds, Å                | 0.014         | 0.008   |
| Rmsd angles (°)              | 1.4           | 1.096   |
| B-factors (ave, Å²)          |               |         |
| Main chain                   | 16.2          | 24.2    |
| Side chain                   | 18.2          | 24.9    |
| Water molecules              | 30.3          | 27.2    |
| Ligand A-chain               | 16.5          | 25.6    |
| Ligand B-chain               | 17.1          | 26.4    |
| Wilson B-factor (Å²)         | 16.4          | 21.2    |
| PDB identifier               | identical to  | 2W8Y    |
|                             | 1SQN          |         |
Table III

| Binding constants of RU486 and asoprisnil for PR data from ref 8 |
|-----------------------------------------------|
| **RU486** (nM) | **Asoprisnil** (nM) |
| ruPR (Kᵢ) | 0.82±0.01 | 0.85±0.01 |

Table IV

| Binding constants of RU 486 for PR and GR |
|------------------------------------------|
| **Nuclear receptor** | **RU486 data** (EC50) | **reference ligand** (identity, [nM]) | **ref. ligand**<sup>b</sup> (Kᵢ, nM) | **RU486**<sup>c</sup> (Kᵢ, nM) |
|----------------------|-----------------------|----------------------------------------|-------------------------------------|---------------------|
| **PR**               |                       |                                        |                                     |                     |
| ruPR (8)             | nr                    | R5020 (5)                              | 2.0                                 | 0.82±0.01           |
| hPR-A (9)            | 10.6 nM±1.3           | prog (6.8)                             | 1.2                                 | 1.6±0.4             |
| hPR-B (9)            | 9.5 nM±0.9            | prog (6.8)                             | 0.9                                 | 1.1±0.2             |
| ruPR (9)             | 11.5 nM±0.9           | prog (6.8)                             | 4.8                                 | 4.8±0.5             |
| ruPR (10)            | 3.0 nM<sup>a</sup>    | R5020 (0.4)                            | 2.0<sup>c</sup>                     | 2.5<sup>a</sup>     |
| hPR (T47D, ref. 10)  | 1.0 nM<sup>a</sup>    | R5020 (0.4)                            | 2.0<sup>c</sup>                     | 0.83<sup>a</sup>    |
| hPR LBD (36)         | nr                    | prog analog (nr)                       | 10                                  | 10.0±0.3            |
| hPR LBD (37)         | 307 nM<sup>a</sup>    | prog analog (nr)                       | 10                                  | 40<sup>a</sup>      |
| **GR**               |                       |                                        |                                     |                     |
| rtGR (9)             | 9.1 nM±0.8            | dex (6)                                | 2.2                                 | 2.4±3.2             |
| hGR (IM9, ref. 10)   | 2.0 nM<sup>a</sup>    | dex (0.4)                              | 2.2<sup>d</sup>                     | 1.7<sup>a</sup>     |
| hGR LBD (37)         | 100 nM<sup>a</sup>    | dex analog (1)                         | 0.18                                | 15<sup>a</sup>      |
Figure 1
Figure 2
Figure 3
The X-ray structure of RU486 bound to the progesterone receptor in a destabilized agonistic conformation
Hans C.A. Raaijmakers, Judith E. Versteegh and Joost C.M. Uitdehaag

J. Biol. Chem. published online April 16, 2009

Access the most updated version of this article at doi: 10.1074/jbc.M109.007872

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2009/04/16/M109.007872.DC1