Synthesis of 19-substituted steroids of the 16α,17α-cyclohexanopregnane series and study of their interactions with rat uterine cytosol and blood serum proteins

I. S. Levina,[a]★ L. E. Kulikova,[a] A. V. Kamernitsky,[a] E. V. Pokrovskaya,[b] and A. N. Smirnov[b]

[a]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (495) 135 5328. E-mail: li@ioc.ac.ru*
[b]*Department of Biology, M. V. Lomonosov Moscow State University, 1 Leninskie gory, 119992 Moscow, Russian Federation.*

New 16α,17α-cyclohexanopregnanes (pregna-D′$_6$-pentaranes) containing alkyl or heteroatomic substituents in position 19, namely, 19-methylidene-, 19-methyl-, 19-methoxyimino-, and 19-(3-methoxycarbonylpropoxyimino)-16α,17α-cyclohexanopregn-4-ene-3,20-diones were synthesized, and their interactions with the progesterone receptor and pentarane-binding proteins of rat uterus cytosol and rat blood serum was studied. The same substituent in position 19 of the steroid molecule can affect in opposite direction its affinity to these proteins. These results suggest differences in the structures of the ligand-binding pockets of the three proteins in the region surrounding the C(10) atom of the steroid nucleus.

Key words: Wittig reaction, pentaranes, progesterone, binding, receptor.

Using the series of progesterone derivatives fused at positions 16α and 17α to an additional three- to six-membered carbocycle D′ (pregna-D′-pentaranes) synthesized previously,[1] we demonstrated the possibility of separating the biological functions of the natural hormone. Compounds of this series containing six-membered ring D′ were found to possess full agonistic progesterone activity, whereas the other may behave as selective progesterone agonists/antagonists as regards the effect of particular biological functions. Analysis of the interaction kinetics of pregna-D′-pentaranes with the cytosolic progesterone receptor (PR) did not reveal any relationship between the affinity of these ligands and the type of biological activity.[2] In addition, it was found that the affinity of the ligand—receptor complex to the DNA containing a hormone-responsive element depends on the ligand nature. Indeed, an opposite correlation between the DNA affinity of the complex and the agonistic activity *in vivo* has been found for pentaranes with six-membered ring D′. However, no such regularity can be found in the case of pentaranes with three-membered ring D′, which is equally active *in vivo*. Presumably, structural features of pentaranes, first of all, the differences in the conformation of ring D and 17β-side chain cause the conformational differences in the tertiary structure of PR complexes with the ligand, which in turn dictate the differences of action at the transcription level.[3] Yet another factor affecting the interaction of the steroid molecule with the PR and, hence, the PR transcription activity may be the presence or absence of the Me group in position 19 or another β-substituent at the C(10) or C(11) atoms,[4] *i.e.*, the degree of "filling" of the region of the hormone-binding PR pocket. Elucidation of this effect is a necessary condition for the design of new selective hormonal agents. The interaction of hormonal ligands with non-receptor transporting or cellular proteins may serve as an additional factor modulating the efficiency of their action.[5] Therefore, in this work we studied the effect of modification at C(10) on steroid binding to not only the PR but also to previously identified uterine and serum proteins (UP and SP) (pentaranophylins), which specifically interact with pentaranes.[6]

The purpose of this work, as a stage dealing with the effect of the structure of the pentarane molecule on its binding to receptors, was to synthesize new 19-substituted 16α,17α-cyclohexanopregnanes and to study the effect of "filling" of the space above rings A and C in the pentarane molecule by alkyl and heteroatomic substituents in position 10 of the steroid skeleton on the PR, UP, and SP affinities of these compounds.

Results and Discussion

5α-Bromo-6β,19-epoxypentarane **1** (see Ref. 7) was chosen as the starting compound for the preparation of 19-substituted 16α,17α-cyclohexanopregnanes

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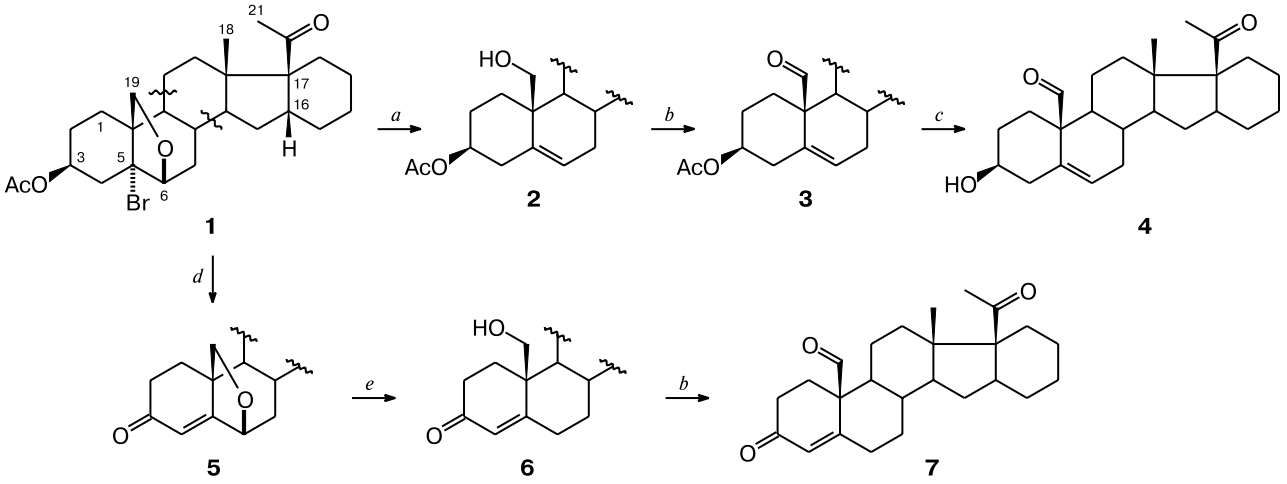
Scheme 1



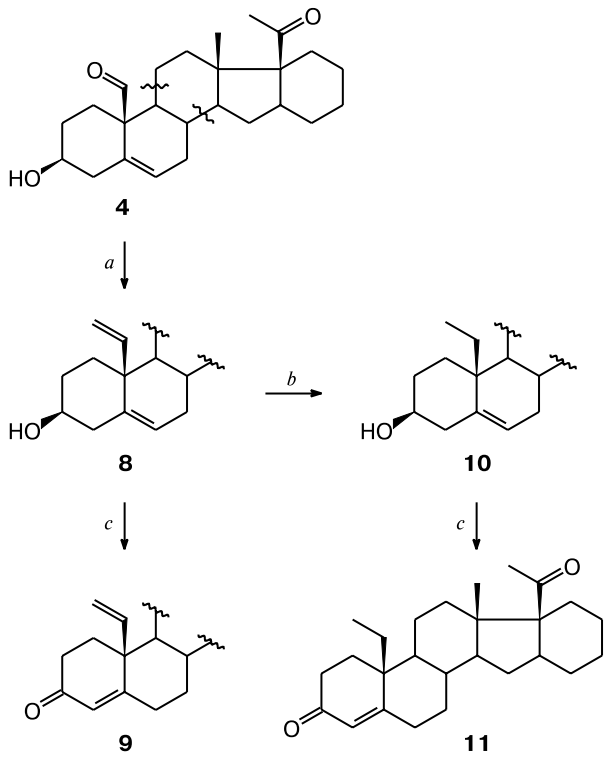
Reagents and conditions: *a*. Zn—AcOH, Pr^iOH, refluxing for 5 h; *b*. PCC—CH_2Cl_2; *c*. KOH—MeOH; *d*. 1) K_2CO_3—MeOH, 2) CrO_3—H_2SO_4, 3) KOAc—MeOH; *e*. Zn—AcOH

(Scheme 1). Epoxide ring cleavage in compound **2**, the subsequent oxidation of 19-hydroxy group with pyridinium chlorochromate (PCC) to give aldehyde **3**, and saponification of the 3-acetoxy group resulted in the key compound, 19-aldehyde **4**. Since the subsequent transformation of aldehyde **4** into Δ^4-3-keto analog **7** proved difficult, we synthesized this compound starting from the known[7] hydroxy enone **6**, which was prepared by successive saponification of the acetoxy group in bromo epoxide **1**, oxidation of the 3-hydroxy group in the alcohol by the Jones reagent, elimination of HBr to give conjugated ketone **5**, and epoxide ring opening in the ketone on treatment with Zn dust in AcOH. Oxidation of 19-hydroxy-Δ^4-3-ketopentarane **6** with PCC yielded aldehyde **7** (see Scheme 1).

19-Methylidenepentarane **8** was synthesized by the Wittig reaction. Refluxing of aldehyde **4** with the ylide, obtained from methyltriphenylphosphonium iodide and Bu^nLi, in THF for 15 h afforded compound **8** in 67% yield (Scheme 2). The attempts to obtain substituted pentarane with a five-carbon chain in position 10 by the reaction of aldehyde **4** with the Wittig reagent generated from 4-carboxybutyltriphenylphosphonium bromide on treatment with dimsyl sodium in DMSO did not lead to the desired outcome. The Oppenauer oxidation of compound **8** yielded Δ^4-3-ketopentarane **9**. The selective catalytic reduction of the 10β-vinyl group of compound **8** yields 19-methylpentarane **10**, which was also transformed into the conjugated ketone **11**.

The reaction of aldehyde **4** with *O*-methylhydroxylamine gave 19-methoxyimine **12** in high yield (Scheme 3). The subsequent Oppenauer oxidation of 3β-hydroxy group gave 19-methoxyimino-Δ^4-3-ketopentarane **14**. Accord-

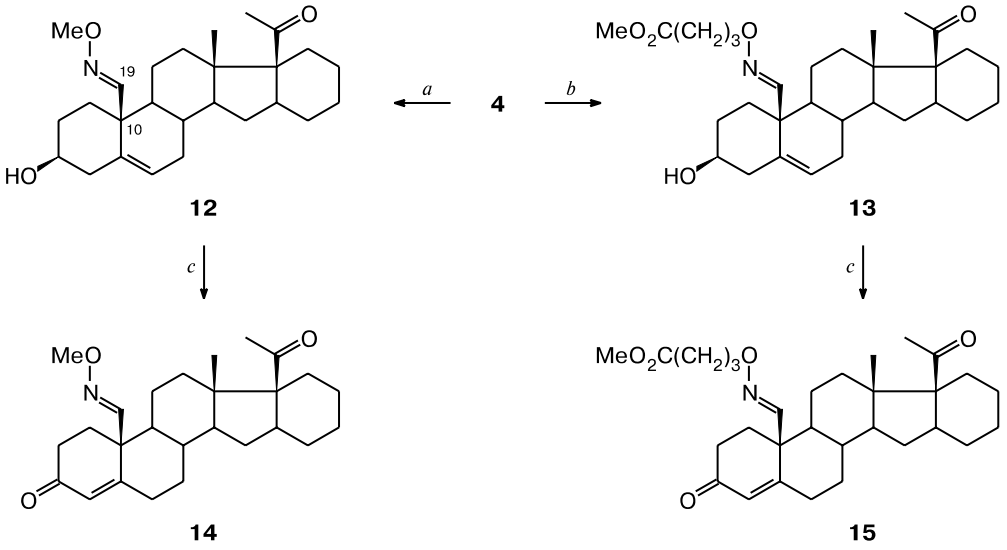
Scheme 2



Reagents and conditions: *a*. $MePPh_3I$—Bu^nLi, THF—ether, refluxing for 15 h; *b*. H_2/Pd—$CaCO_3$, EtOH; *c*. $Al(OPr^i)_3$, cyclohexanone, PhMe, refluxing for 2.5 h.

ing to the same strategy, the reaction of aldehyde **4** with *O*-(3-carboxypropyl)hydroxylamine followed by methylation with diazomethane gave 19-(3-methoxycarbonyl-

Scheme 3



Reagents and conditions: *a*. $MeONH_2 \cdot HCl$—Py, EtOH, 60 °C, 7 h; *b*. $HO_2C(CH_2)_3ONH_2 \cdot HCl$—Py, 60 °C, 5 h; CH_2N_2, MeOH, ether; *c*. $Al(OPr^i)_3$, cyclohexanone, PhMe, refluxing 2.5 h.

propoxyimino) derivative **13**, which was also converted into conjugated ketone **15** in 50% yield based on the starting aldehyde **4** (see Scheme 3). The *E*-configuration was assigned to the 19-oxyimino group in the resulting oximes **12** and **13**, **14** and **15** on the basis of H(19) chemical shifts (δ 7.28, 7.5).[8,9] The structures of all of the obtained compounds follow from the data from ^1H NMR spectra and mass spectra.

To estimate the effect of the substituent in position 19 of the pentarane molecule on its interaction with PR, UP, and SP, we used competitive analysis (displacement of [^{3}H]progesterone **P** (for PR) and 6α-methyl[^{3}H]-16α,17α-cyclohexanoprogesterone **16** (for UP and SP) from complexes with proteins by the synthesized derivatives). Examples of the displacement curves of the [^{3}H]ligands from the complexes with PR, UP, and SP are shown in Fig. 1, *a*—*c*. The relative binding affinities (RBA) of the synthesized compounds presented in Table 1 with respect to the affinity of 16α,17α-cyclohexanoprogesterone **17** (see Ref. 6), taken to be unity, which can be considered as the basic structure for the 19-substituted derivatives obtained, demonstrate the effect of the substituent at C(10) on the affinity to PR, UP, and SP. It can be seen that the introduction of a methylene or methyl substituent into position 19 of the 16α,17α-cyclohexanoprogesterone molecule **17** (pentaranes **9** and **11**, respectively) decreases the PR affinity 30—50-fold. The introduction of oxygen-containing substituents affects PR binding in different ways. For 19-hydroxy compound **6** or 6β,19-oxide **5**, the interaction with PR is much weaker, while 19-aldehyde **7** retains more than 10% of the affinity of the basic pentarane structure **17**. Compounds **5** and **6** also showed a sharp decrease in the affinity for the UP. 19-Methoxyiminopentarane **14** demonstrated a high affinity for the PR, equal to, or higher than, the affinity of the basic pentarane, perhaps, due to the additional interaction with the corresponding amide region of the hormone-binding pocket, which implies the presence of free space for such a substituent. However, the elongation of 19-aldimine substituent together with the ester group at the end of the chain in compound **15** brings about a sharp decrease in binding. A high affinity to the SP (6.5 times as

Table 1. RBA values for 19-substituted 16α,17α-cyclohexanoprogesterones with respect to 16α,17α-cyclohexanoprogesterone **17**

Substituent in position 10	Compound	RBA		
		Progesterone receptor	Pentaranophylin	
			uterus	serum
CH_2—O—C(6)	**5**	0.045	0.0085	1.08
CH_2OH	**6**	0.015	0.0146	—
HCO	**7**	0.134	0.944	—
CH_2=CH	**9**	0.021	0.174	0.54
$MeCH_2$	**11**	0.029	0.176	1.31
MeON=CH	**14**	1.33	0.0035	6.48
$MeO_2C(CH_2)_3ON$=CH	**15**	0.0023	0.0058	2.02
Me	**17***	1	1	1

* The affinities of 16α,17α-cyclohexanoprogesterone **17** to these three proteins were determined previously.[6]

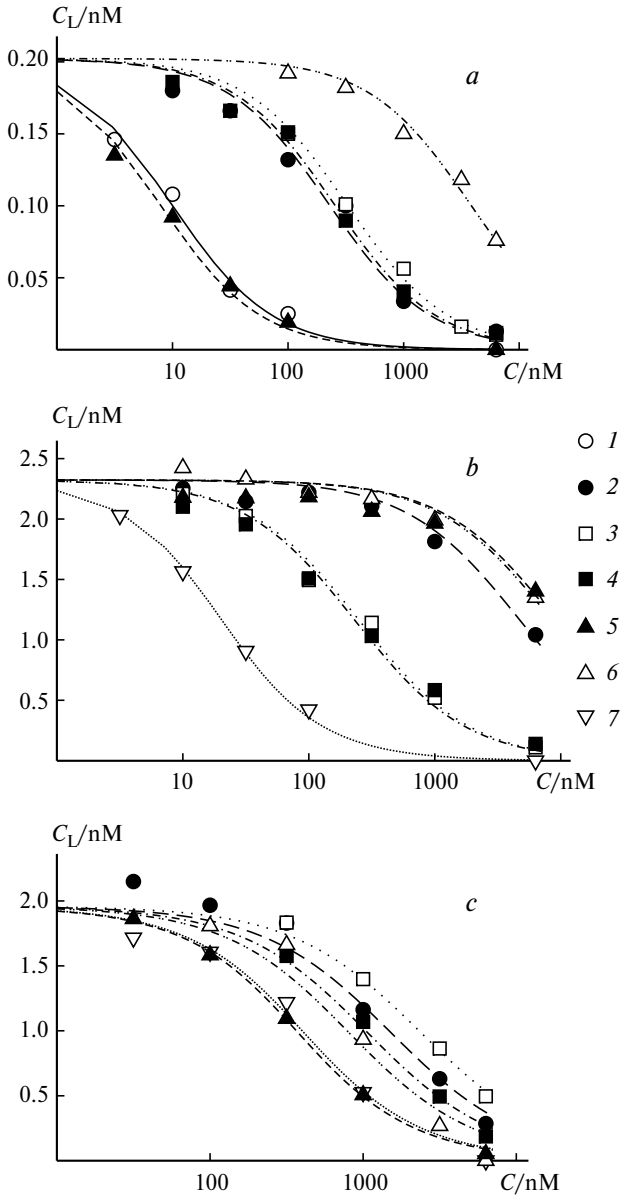


Fig. 1. Displacement curves of the [^{3}H]ligands from complexes with rat proteins by the steroids: (*a*) [^{3}H]progesterone **P** complexes with the PR; (*b*) 6α-methyl[^{3}H]16α,17α-cyclohexanoprogesterone **16** complexes with the UP; (*c*) complexes of **16** with the rat blood SP: progesterone **P** (*1*), compounds **5** (*2*), **9** (*3*), **11** (*4*), **14** (*5*), **15** (*6*), and **16** (*7*); *C* is the competitor concentration, C_L is the concentration of the bound [^{3}H]-ligand.

high as that of 6α-methyl-16α,17α-cyclohexanoprogesterone (**16**)) and a very low affinity to the UP were found for 19-*O*-methyloxime **14**. The other 19-substituents in the pentarane molecule exerted a rather weak influence on the affinity to the SP. Presumably, the ligand-binding pocket of the SP has a rather large cavity in the region adjacent to the steroid C(10) atom, which allows incorporation of diverse substituents of the steroid molecule. The aldehyde substituent in compound **7** did not exert a noticeable effect on the pentarane affinity to the UP, whereas larger substituents inhibited binding, indicating that the ligand-binding pocket of this protein has a limited free space above C(10) and can accommodate only small substituents (Me and HCO). The binding data obtained suggest that the ligand-binding pocket of the PR contains a cavity adjacent to the C(19) atom of the steroids. This cavity in the PR may incorporate amino acid residues able to serve as hydrogen bond donors. However, judging by the results, this cavity in pentaranophylins is different in principle. Probably, modification of the steroid molecule in position 19 may contribute to discrimination of the pentarane interaction with various proteins, and, hence, to the selectivity of their biological action.

Thus, the results obtained allow one to estimate preliminarily the size of the ligand-binding protein pockets at C(10) and, in the future, to obtain compounds possessing high affinity to the PR and to use 19-substituted steroids with an appropriate spacer as affinity ligands (see Ref. 10).

Experimental

Melting points were determined on a Boetius hot stage. ^{1}H NMR spectra were recorded on a Bruker WM-250 instrument in $CDCl_3$. The residual $CHCl_3$ signal (δ 7.27) was used as the internal standard. Mass spectra were run on a Kratos MS 30 instrument with direct injection into the ion source (EI, 70 eV) at temperatures of 150—200 °C. Analytical TLC was performed on Silica gel 60 F_{254} plates (Merck) in hexane—acetone and hexane—ether mixtures. The spots were visualized with a 1% solution of $CeSO_4$ in 10% aqueous H_2SO_4 with subsequent heating. Preparative separation was carried out by column chromatography on silica gel Kieselgel 60 (0.063—0.100 mm, Merck) at a compound : sorbent ratio of 1 : 40. Specific rotation was measured on a JASCO DIP-360 polarimeter in $CHCl_3$ at 22 °C.

Usual workup of organic extracts implies washing to a neutral reaction of wash water, drying with $MgSO_4$, and evaporation of the solvent *in vacuo*.

3β-Acetoxy-19-hydroxy-16α,17α-cyclohexanopregn-5-en-20-one (2). A mixture of bromo epoxide acetate **1** (5.4 g, 10.7 mmol),[7] Zn dust (10 g), and glacial AcOH (10 mL) in Pr^{i}OH (200 mL) was refluxed under argon for 5 h. The residue was filtered off and washed on the filter with Pr^{i}OH (3×15 mL), and the filtrate was concentrated *in vacuo*. The residue was dissolved in EtOAc, worked up, and chromatographed in an acetone—heptane system (2 : 8) to give 3.75 g (82%) of 19-hydroxypentarane **2**, m.p. 188—190 °C (from an acetone—hexane mixture), $[\alpha]_D$ −28 (*c* 1.0). ^{1}H NMR, δ: 0.74, 2.05, 2.12 (all s, 3 H each, 18-Me, 3-OAc, 21-Me); 2.96 (m, 1 H, H(16)); 3.63 (d, 1 H, H(19), J = 13.6 Hz); 3.86 (d, 1 H, H(19), J = 13.6 Hz); 4.65 (m, 1 H, H(3)); 5.76 (m, 1 H, H(6)). MS, m/z (I_{rel} (%)): 368 [M − AcOH]$^+$ (21), 337 [M − AcOH − CH_2OH]$^+$ (100). $C_{27}H_{40}O_4$. Calculated: M = 428.

3β-Acetoxy-20-oxo-16α,17α-cyclohexanopregn-5-en-19-al (3). PCC (2.06 g, 9.56 mmol) was added in portions under argon to a stirred ice-cooled solution of 19-hydroxypentarane **2** (1.92 g, 4.48 mmol) in anhydrous CH_2Cl_2 (40 mL), and the mixture was stirred for 3 h. The reaction mixture was filtered through a column with silica gel (40 g) and washed with CH_2Cl_2 (200 mL). The filtrate was concentrated *in vacuo* and the chromatographically pure oxidation product **3** (1.79 g, 94%) was used in the next stage. The analytical specimen had m.p. 149—151 °C (from an acetone—hexane mixture). ^{1}H NMR, δ: 0.63, 2.01, 2.14 (all s, 3 H each, H(18), 3-OAc, H(21)); 2.96 (m, 1 H, H(16)); 4.60 (m, 1 H, H(3)); 5.88 (m, 1 H, H(6)); 9.65 (s, 1 H, H(19)). MS, *m/z* (I_{rel} (%)): 426 $[M]^+$ (1), 366 $[M - AcOH]^+$ (16), 337 $[M - AcOH - CHO]^+$ (100). $C_{27}H_{38}O_4$. Calculated: M = 426.

3β-Hydroxy-20-oxo-16α,17α-cyclohexanopregn-5-en-19-al (4). Potassium hydroxide (0.73 g, 12.9 mmol) in H_2O (5 mL) was added to a suspension of compound **3** (1.79 g, 4.19 mmol) in MeOH (130 mL), and the mixture was stirred for 3 h at 20 °C under argon. The reaction mixture was neutralized with 10% HCl with ice cooling and poured into water (1 L), and the white precipitate formed was filtered off, and dried to give 1.53 g (95%) of aldehyde **4**. The analytical sample had m.p. 191—194 °C (from aqueous MeOH), $[\alpha]_D$ −117 (*c* 1.0). ^{1}H NMR, δ: 0.63, 2.14 (both s, 3 H each, H(18), H(21)); 2.96 (m, 1 H, H(16)); 3.50 (m, 1 H, H(3)); 5.88 (m, 1 H, H(6)); 9.65 (s, 1 H, H(19)). MS, *m/z* (I_{rel} (%)): 355 $[M - CHO]^+$ (24). $C_{25}H_{36}O_3$. Calculated: M = 384.

3,20-Dioxo-16α,17α-cyclohexanopregn-4-en-19-al (7). PCC (0.44 g, 2.02 mmol) was added in portions under argon to an ice-cooled stirred solution of 19-hydroxy-16α,17α-cyclohexanopregn-4-ene-3,20-dione **6** (0.43 g, 1.12 mmol) (see Ref. 7) in anhydrous CH_2Cl_2 (10 mL), and the mixture was kept for 2 h. The reaction mixture was filtered through a column with silica gel and washed with CH_2Cl_2 (50 mL). The filtrate was concentrated *in vacuo* to 3—5 mL, hexane (10 mL) was added, and the precipitated white crystalline powder was recrystallized from an acetone—hexane mixture to give 0.095 g (22%) of aldehyde **7**, m.p. 187—190 °C. ^{1}H NMR, δ: 0.71, 2.14 (both s, 3 H each, H(18), H(21)); 2.96 (m, 1 H, H(16)); 5.99 (s, 1 H, H(4)); 9.94 (s, 1 H, H(19)). MS, *m/z* (I_{rel} (%)): 382 $[M]^+$ (15), 353 $[M - CHO]^+$ (9), 339 $[M - Ac]^+$ (43). $C_{25}H_{34}O_3$. Calculated: M = 382.

3β-Hydroxy-19-methylidene-16α,17α-cyclohexanopregn-5-en-20-one (8). A 1.35 *M* solution of Bu^nLi in hexane (11 mL) was added under argon to a suspension of methyltriphenylphosphonium iodide (5.86 g, 14.5 mmol) in anhydrous ether (50 mL), and the mixture was stirred for 1.5 h at 20 °C. Aldehyde **4** (0.9 g, 2.34 mmol) in anhydrous THF (40 mL) was added to the resulting ylide, and the mixture was refluxed for 16 h. The solvents were removed *in vacuo*, the residue was dissolved in EtOAc, and the oily residue obtained after usual workup was chromatographed in the acetone—petroleum ether system (10 : 90 → 14 : 86) to give 0.6 g (67%) of product **8**, m.p. 237—239 °C (from an acetone—hexane mixture), $[\alpha]_D$ −98 (*c* 1.0). ^{1}H NMR, δ: 0.61, 2.14 (both s, 3 H each, H(18), H(21)); 2.96 (m, 1 H, H(16)); 3.54 (m, 1 H, H(3)); 4.98 (dd, 1 H, H(19′b), $J_{19'a,19'b}$ = 1.0 Hz); 5.29 (dd, 1 H, H(19′a)); 5.58 (br.s, 1 H, H(6)); 5.65 (dd, 1 H, H(19), $J_{19,19'a}$ = 11.0 Hz, $J_{19,19'b}$ = 18.0 Hz). MS, *m/z* (I_{rel} (%)): 382 $[M]^+$ (12), 339 $[M - Ac]^+$ (11), 321 $[M - Ac - H_2O]^+$ (40). $C_{26}H_{38}O_2$. Calculated: M = 382.

19-Methylidene-16α,17α-cyclohexanopregn-4-ene-3,20-dione (9). 19-Methylidenepentarane **8** (0.3 g, 0.78 mmol) was subjected to Oppenauer oxidation (0.33 g of $Al(Pr^iO)_3$, 3 mL of cyclohexanone, 30 mL of toluene, refluxing for 2.5 h). The reaction mixture was acidified with dilute AcOH, and the organic layer was separated. The oily residue obtained after the usual workup was diluted with hexane, the precipitated crystalline powder was filtered off, and the mother liquor was chromatographed on a column using an acetone—petroleum ether mixture as an eluent (8 : 92). The yield of the conjugated ketone **9** was 0.17 g (57%), m.p. 223—225 °C (from an acetone—hexane mixture), $[\alpha]_D$ +112 (*c* 1.0). ^{1}H NMR, δ: 0.70, 2.14 (both s, 3 H each, H(18), H(21)); 2.96 (m, 1 H, H(16)); 4.98 (dd, 1 H, H(19′b), $J_{19'a,19'b}$ = 1.0 Hz); 5.29 (dd, 1 H, H(19′a)); 5.91 (br.s, 1 H, H(4)); 5.95 (dd, 1 H, H(19), $J_{19,19'a}$ = 11.0 Hz, $J_{19,19'b}$ = 18.0 Hz). MS, *m/z* (I_{rel} (%)): 380 $[M]^+$ (20), 337 $[M - Ac]^+$ (26). $C_{26}H_{36}O_2$. Calculated: M = 380.

3β-Hydroxy-19-methyl-16α,17α-cyclohexanopregn-5-en-20-one (10). A solution of 19-methylidenepentarane **8** (0.3 g, 0.78 mmol) in dioxane (25 mL) was hydrogenated over 5% $Pd/CaCO_3$ (0.07 g) at room temperature and atmospheric pressure until hydrogen absorption ceased and the starting compound disappeared (TLC). The catalyst was filtered off, the filtrate was concentrated *in vacuo*, and the resulting crystalline residue was recrystallized from acetone to give 0.25 g (83%) of compound **10**, m.p. 236—241 °C, $[\alpha]_D$ −10 (*c* 1.0). ^{1}H NMR, δ: 0.74, 2.15 (both s, 3 H each, H(18), H(21)); 0.86 (t, 3 H, H(19′)); 2.96 (m, 1 H, H(16)); 3.55 (m, 1 H, H(3)); 5.54 (m, 1 H, H(6)). MS, *m/z* (I_{rel} (%)): 384 $[M]^+$ (9), 355 $[M - Et]^+$ (100), 337 $[M - Et - H_2O]^+$ (58). $C_{26}H_{40}O_2$. Calculated: M = 384.

19-Methyl-16α,17α-cyclohexanopregn-4-en-3,20-dione (11). A similar procedure starting from compound **10** (0.15 g) gave conjugated ketone **11** (0.09 g, 60%), m.p. 193—195 °C, $[\alpha]_D$ +31 (*c* 1.0). ^{1}H NMR, δ: 0.74, 2.15 (both s, 3 H each, H(18), H(21)); 0.90 (t, 3 H, H(19′)); 2.96 (m, 1 H, H(16)); 5.92 (br.s, 1 H, H(4)). MS, *m/z* (I_{rel} (%)): 382 $[M]^+$ (9), 339 $[M - Ac]^+$ (35). $C_{26}H_{38}O_2$. Calculated: M = 382.

3β-Hydroxy-19(*E*)-methoxyimino-16α,17α-cyclohexanopregn-5-ene-20-one (12). A mixture (0.3 g, 0.78 mmol) of aldehyde **4**, $MeONH_2 \cdot HCl$ (0.13 g, 1.56 mmol), and Py (0.1 mL) in EtOH (15 mL) was kept for 8 h at 60 °C and poured into ice water. The crystalline precipitate was filtered off, washed with water, dried in air, and recrystallized from an acetone—hexane mixture to give 0.19 g (59%) of methoxy oxime **12**, m.p. 164—166 °C, $[\alpha]_D$ −118 (*c* 1.0). ^{1}H NMR, δ: 0.66, 2.14 (both s, 3 H each, H(18), H(21)); 2.96 (m, 1 H, H(16)); 3.55 (m, 1 H, H(3)); 3.86 (s, 3 H, OMe); 5.62 (m, 1 H, H(6)); 7.28 (s, 1 H, H(19)). MS, *m/z* (I_{rel} (%)): 395 $[M - H_2O]^+$ (6), 382 $[M - MeO]^+$ (11), 364 $[M - MeO - H_2O]^+$ (51). $C_{26}H_{39}NO_3$. Calculated: M = 413.

3β-Hydroxy-19(*E*)-(3-methoxycarbonylpropoxyimino)-16α,17α-cyclohexanopregn-5-en-20-one (13). A mixture of aldehyde **4** (0.21 g, 0.55 mmol) and *O*-(3-carboxypropyl)hydroxylamine hydrochloride (0.15 g, 1 mmol)[11] in Py (3 mL) was kept for 5 h at 50 °C, and subjected to azeotropic distillation with heptane (2×30 mL). Water (10 mL) and EtOAc (50 mL) were added to the residue. The organic layer was washed with dilute HCl, $NaHCO_3$, and water and dried. The residue obtained after solvent evaporation was dissolved in MeOH (10 mL) and kept for 2 h with an ethereal solution (20 mL) of diazomethane (from

2 g of nitrosomethylurea and 3.6 mL of 45% KOH) at 20 °C. The solvents were evaporated *in vacuo* and the residue was chromatographed in an acetone—petroleum ether—MeOH system (10 : 89 : 1) to give 0.22 g (80%) of aldoxime **13**, m.p. 160—162 °C (from ether), $[\alpha]_D$ −107 (*c* 1.0). ^{1}H NMR, δ: 0.66, 2.15 (both s, 3 H each, H(18), H(21)); 2.40 (m, 4 H, $(CH_2)_2$); 2.96 (m, 1 H, H(16)); 3.55 (m, 1 H, H(3)); 3.69 (s, 3 H, COOMe); 4.00 (m, 2 H, (CH_2)ON=); 5.60 (m, 1 H, H(6)); 7.28 (s, 1 H, H(19)).

19(*E*)-Methoxyimino-16α,17α-cyclohexanopregn-4-ene-3,20-dione (14). 19-Pentaran **12** (0.19 g, 0.46 mmol) was subjected to Oppenauer oxidation ($Al(Pr^iO)_3$ (0.22 g), cyclohexanone (4 mL), toluene (20 mL), refluxing for 2.5 h). The reaction mixture was acidified with dilute AcOH and the toluene layer was separated. The usual workup and chromatography of the residue (elution with petroleum ether containing 1 to 7% acetone) gave 0.11 g (58%) of conjugated ketone **14**, m.p. 154—156 °C (from an ether—hexane mixture), $[\alpha]_D$ +157 (*c* 1.0). ^{1}H NMR, δ: 0.71, 2.15 (both s, 3 H each, H(18), H(21)); 2.96 (m, 1 H, H(16)); 3.86 (s, 3 H, OMe); 5.99 (br.s, 1 H, H(4)); 7.52 (s, 1 H, H(19)). MS, *m/z* (I_{rel} (%)): 411 $[M]^+$ (3.5), 380 $[M - MeO]^+$ (42). $C_{26}H_{37}NO_3$. Calculated: M = 411.

19(*E*)-(3-Methoxycarbonylpropoxyimino)-16α,17α-cyclohexanopregn-4-ene-3,20-dione (15). A similar procedure starting from compound **13** (0.18 g) gave 0.06 g (33%) of conjugated ketone **15** as a glassy residue, $[\alpha]_D$ +96 (*c* 1.0). ^{1}H NMR, δ: 0.70, 2.15 (both s, 3 H each, H(18), H(21)); 2.40 (m, 4 H, $(CH_2)_2$); 2.96 (m, 1 H, H(16)); 3.55 (m, 1 H, H(3)); 3.68 (s, 3 H, COOMe); 4.07 (m, 2 H, (CH_2)ON=); 5.88 (m, 1 H, H(4)); 7.50 (s, 1 H, H(19)). MS, *m/z* (I_{rel} (%)): 380 $[M - MeO_2C(CH_2)_3O]^+$ (31), 353 $[M - MeO_2C(CH_2)_3ONCH]^+$ (38), 337 $[M - MeO_2C(CH_2)_3O - Ac]^+$ (22). $C_{30}H_{43}NO_5$. Calculated: M = 497.

Biochemical experiments. Protein sources. Sexually mature virgin female rats of mixed population weighing 200—250 g were used. The animals received intramuscular injections of estradiol, 10 μg in 200 μL of propylene glycol daily for four days, and on the fifth day, they were decapitated. The collected blood was incubated for 1 h at room temperature and for 30 min at 4—6 °C and centrifuged at 3000g for 10 min. The resulting serum was stored at −20 °C. The uteri were ground and homogenized in a glass homogenizer at 0—4° C for 5 min in a buffer solution containing 10 m*M* Tris-HCl (pH 7.5), 10 m*M* KCl, 1 m*M* EDTA, 0.5 m*M* phenylmethylsulfonyl fluoride (PMSF), 1 m*M* dithiothreitol, and 10% glycerol at a tissue : buffer ratio of 1 : 6. The homogenate was centrifuged at 4 °C for 1 h at 50000g. The supernanant fraction with a protein concentration of 4—6 mg mL^{-1} was used immediately. **Steroids.** Commercial progesterone and hydrocortisone (Sigma, USA) and [1,2,6,7-^{3}H]progesterone with a specific radioactivity of 86 Ci $mmol^{-1}$ (Izotop, St. Petersburg) were used; 6α-methyl-[1,2-^{3}H]16α,17α-cyclohexanoprogesterone **16** (43 Ci $mmol^{-1}$) was synthesized as described previously.[12]

The relative binding affinities (RBA) of steroids to the PR were measured using [^{3}H]progesterone in the presence of 3 μ*M* hydrocortisone, and uterus cytosol. The relative binding affinities of the steroids for the UP were measured using 6α-methyl-[^{3}H]16α,17α-cyclohexanoprogesterone **16**, 127 n*M* non-labeled progesterone, and uterine cytosol. The steroid RBA to the SP were determined with 6α-methyl-[^{3}H]16α,17α-cyclohexanoprogesterone **16** and the serum diluted 8—16-fold with the homogenization buffer (see above) without PMSF and dithiothreitol. The cytosol or the dilute serum (100 μL) was incubated at 0—4 °C in the buffer with 100 μL of a steroid mixture for 20 h; the mixture comprised (60—80) · 10^3 pulse min^{-1} (final concentration 3—6 *nM*) of the [^{3}H]ligand and the non-labeled competitor (final concentrations from 0 to 10 μ*M*). The protein-bound and free ligand were separated by incubation with 100 μL of a 2% suspension of the activated charcoal Norit A (Serva, Germany) coated with Dextran-70 (Fluka, Switzerland) for 5 min at 0—4 °C. After centrifugation for 5 min at 3000g, aliquots (250 μL) of the supernatant fraction were taken for radioactivity measurement. The incubation was carried out in BSA-coated quartz tubes.[13] The radioactivity was measured using a dioxane scintillator[14] with a counting efficiency of 20%. The protein content was determined using the Coumassie blue dye.[15] All measurements were carried out in duplicate. Each experiment was reproduced 3—4 times. The equilibrium dissociation constants (K_D) were determined by selecting the parameters K_D and B_{max}, which ensured the least deviation of the experimental data from the "one protein—two ligands" kinetic model.[16] The dimensionless RBA values were calculated from the ratio of the K_d values of progesterone (for the PR) or 6α-methyl-16α,17α-cyclohexanoprogesterone (for the UP and SP) to those of the ligand under comparison determined in separate experiments; the calculated RBA values were then averaged. To unify the analysis of the effect of substituents at the C(10) atom of the pentarane molecule on the affinity to three proteins, the RBA values found for the synthesized compounds were considered as relative values with respect to the affinity of the basic compound, 16α,17α-cyclohexanoprogesterone **17**, to the same proteins.

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