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Synthesis of Potential Cytochrome P45011β -Generated Intermediates

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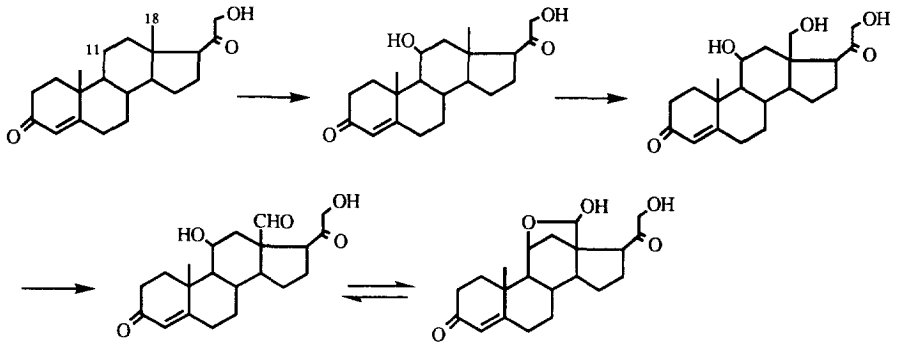
ABSTRACT : Cytochrome $P\text{-}450_{11\beta}$ is a key enzyme in the biosynthesis of aldosterone which catalyzes the transformation of 11-deoxycorticosterone (DOC) to aldosterone *via* three successive hydroxylations (at C-11 and at C-18). This enzyme is irreversibly inactivated by 18-VP **1** and 18-EP **4**. In order to determine the mechanism of the inactivation, we have synthesized three potential reactive enzyme-generated intermediates **2**, **3** and **5**.

INTRODUCTION

Mammalian cytochrome $P450_{11\beta}$ catalyzes the biosynthesis of aldosterone from 11-deoxycorticosterone by oxidation at C-11 and C-18 in three O_2-NADPH dependent steps. The first hydroxylation occurs at C-11, followed by two hydroxylations at C-18 leading to aldosterone (Scheme 1). Selective inhibition of the oxidation at C-18 might offer a way to control aldosterone activity, responsible for electrolytic balance and blood pressure, without interference with the corticosterone pathway.



Scheme 1. Biosynthesis of aldosterone

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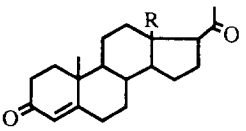
We have designed and synthesized several potential mechanism-based inactivators of the last two steps of the catalysed reaction. The most active compounds 18VP **1** and 18EP **4** are both progesterone analogues characterized by an unsaturation at C-18.[1] It has now been established in our laboratory that they were irreversible mechanism-based inhibitors[2] but the mechanism is not yet solved. As for other cyt P450 oxygen dependent enzymes, inactivation could involve reaction of the double or triple bond with the Fe^{V}=O species, generated in the catalytic cycle, leading to the heme N-alkylation product[3]. Alternatively, it could involve the α,β-unsaturated ketone **3** (or **6**), produced from **1** (or from **4**) by two enzymatic oxidations *via* an allylic alcohol **2** (or a propargylic alcohol **5**) respectively. Oxidation of allylic and propargylic alcohols has already been proposed to lead to enzyme inactivation, by a Michael addition of an enzyme nucleophile to the newly formed α,β-unsaturated compounds4.

In order to provide insight about the mechanism of cyt $P450_{11\beta}$ inactivation by 18VP **1** and 18EP **4**, we have synthesized three postulated reactive enzyme-generated intermediates **2, 3** and **5** (Figure 1).



Potential mechanism-based inhibitors	Postulated reactive intermediates
	2 R = -CHOH-CH=CH_2 (18R or 18S)
1 R = -CH_2-CH=CH_2	3 R = -CO-CH=CH_2
4 R = -CH_2-C≡CH	5 R = -CHOH-C≡CH (18R or 18S)
	6 R = -CO-C≡CH

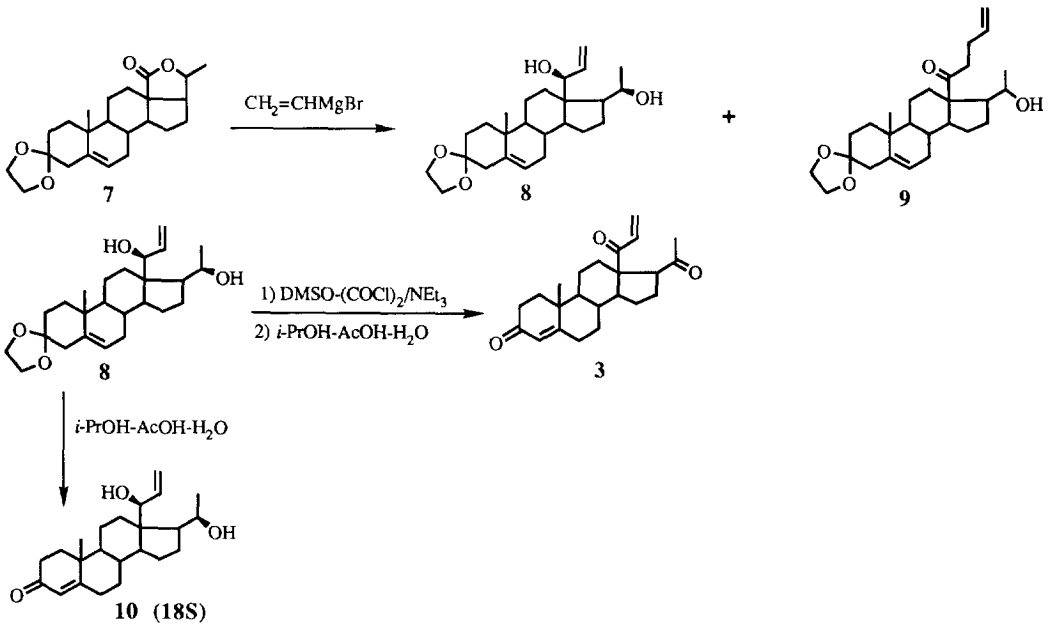
Figure 1. Structures of potential cytochrome $P450_{11\beta}$-generated intermediates

RESULTS AND DISCUSSION

First approach to enone 3 (Scheme 2)

The most logical approach to synthesize the α,β-unsaturated ketone was the addition of a vinylmagnesium halide on the carbonyl group of the known lactone **7**, synthesized by a well described photochemical reaction from pregnenolone5.

Treatment of the lactone **7** with a large excess (16 equiv.) of vinylmagnesium bromide in refluxing toluene overnight [6] gave two unexpected products with 70% yield in the ratio 45 / 55. The first product **8** exhibited an ABXY system at 4.26, 5.20 and 6.30 ppm in the ^{1}H NMR spectrum. The absence of carbonyl absorption and the presence of bands at 3600 (sharp) and 3450 (broad) cm^{-1} in IR spectrum confirmed the structural features of an allylic alcohol. The formation of this product could be explained by reduction of the carbonyl group at C-18 with the metal hydride eliminated in the reaction mixture from the metal alkoxyde at C-20[7]. It was hydrolysed under acidic conditions to give compound **10** identical to the product obtained as described in Scheme 3. The second product which was attributed structure **9** according to the spectral data, results from the 1,4 addition of a second vinyl moiety on the intermediate enone.



Scheme 2. Synthesis of 18-oxo-18-vinylprogesterone **3** from lactone **7**

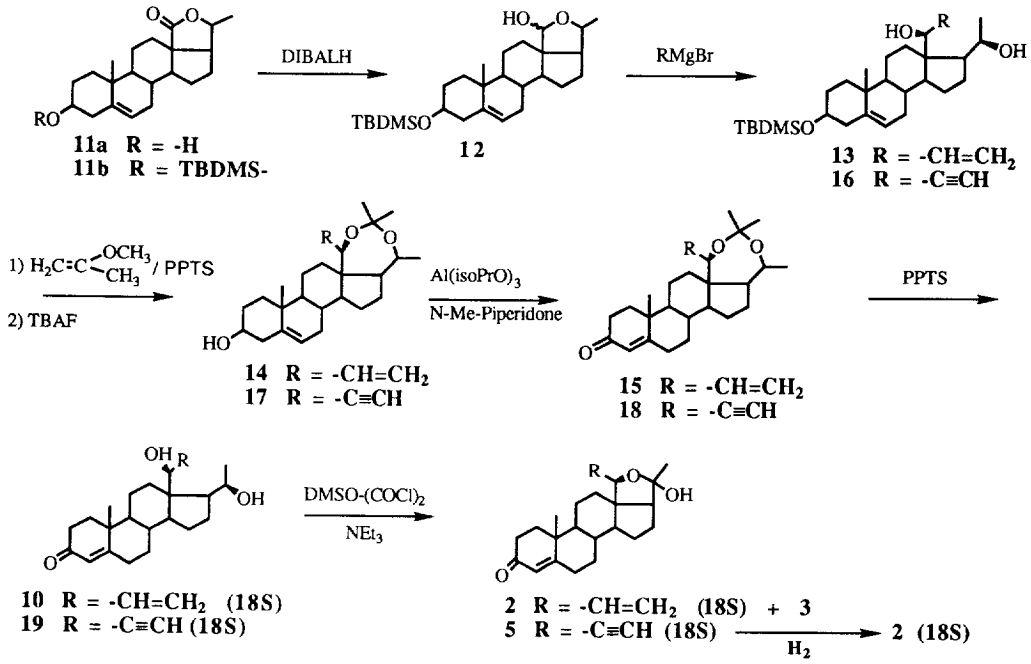
Several attempts to increase the yield of the 1,2-addition product were carried out. We first limited the Grignard reagent excess (8 and 12 equivalents). But the 1,4 product **9** remained the main product and the reaction course was much longer to get to completion. In order to prevent the 1,4-addition we attempted an alternative route involving a competitive reduction of the intermediate enone in a one-step conversion of lactone **7** to allylic alcohol **8** with $RMgX\text{-}LiBH_4$[8]. Unfortunately, the yield of the allylic alcohol **8** in the complex mixture was not increased. The use of a silylvinyl magnesium reagent[9] for addition on the lactone **7** was expected to decrease the undesirable 1,4 addition by steric hindrance of the trimethylsilyl group. Indeed, nucleophilic attack of this bulky reagent at the hindered acyl moiety of the lactone **7** was in that case disfavoured and occurred at C-20 leading to the carboxylic acid at C-18, as main product[10].

The diol **8** was then oxidized by the Swern procedure and the desired enone **3** was obtained by removal of the ethylenedioxy-protecting group at C-3 with a poor yield because of an intervening Michael addition on the double bond.

The synthesis of the corresponding α,β-acetylenic ketone **6** was attempted by treatment of the lactone (**7** or **11a**) either with ethynyl magnesium halide (Br, Cl) or with lithium acetylide[11], in the presence of boron trifluoride etherate[12]. We also examined the reactivity of organocerium (III) reagents, generated by transmetallation of organolithium with Cerium (III) halide, towards the lactone[13]. In each case, the starting material was recovered from the reaction mixture in high yield.

Alternate approaches to the enone 3

As an alternate approach to improve the yield of **3**, we investigated two other routes, *via* an allylic alcohol, obtained by addition at C-18 of the same Grignard reagent either on the lactol **12** (Scheme 3), or on the free aldehyde group at C-18 of the 3,20-bis-protected 18-oxoprogesterone **20** (Scheme 4)[14].



Scheme 3. Synthesis of 18-hydroxy-18-vinylprogesterone **2** and 18-hydroxy-18-ethynylprogesterone **5** from lactol **12**

The diastereoisomeric mixture of the 3-O-silyl-protected lactols **12** was obtained from the lactone **11b** by reduction with DIBALH (Scheme 3). The cyclic 3-ketal lactone **7** was resistant to reduction with DIBALH into lactol. We have already observed the same long range effects for the synthesis of **1**[1a]. Treatment of **12** with vinylmagnesium bromide in toluene-THF at room temperature rapidly afforded the allylic alcohol **13** as a single diastereoisomer[15]. The diol **13** was protected as acetonide[16] and the silyl ether group at C-3 was then removed by TBAF in THF to give **14**. The ketone **15** was obtained by oxidation of **14** at C-3 by the Oppenauer procedure, and the deprotection led to the keto-diol **10**. An X-ray analysis of **10** allowed us to ascertain the S-configuration of alcohol at C-18 as shown on Figure 2. Oxidation under Swern conditions afforded mainly 18-hydroxy-18-vinylprogesterone **2** (18S), obtained as hemiketal, and the enone **3**.

The acetylenic analogue **19** was obtained from lactol **12** by reaction with ethynyl magnesium bromide, followed by the same steps of protection, deprotection and oxidation according to Scheme 3. Oxidation under Swern conditions did not produce any triketone but exclusively the product of monooxidation at C-20, the 18-ethynyl-18-hydroxyprogesterone **5** (18S), as hemiketal. The configuration of alcohol at C-18 in **5** was correlated by hydrogenation of hemiketal **5** leading to hemiketal **2**.

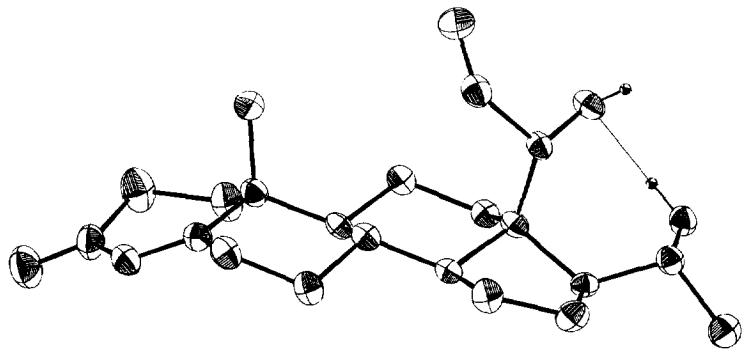
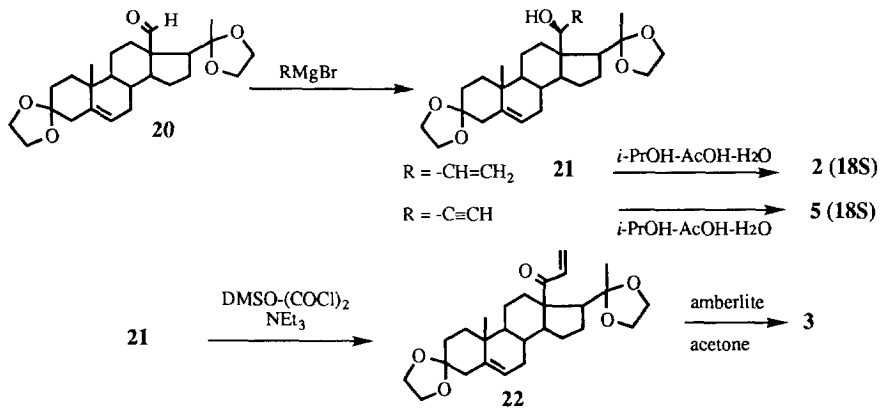


Figure 2. ORTEP drawing of the structure of compound **10**

An alternate preparation of enone **3** with an improved yield was achieved by addition of the Grignard reagent on the free aldehyde group of the 3,20-bis-ethylene ketal **20** (Scheme 4). Treatment of the aldehyde **20**[14] with vinyl magnesium bromide in THF at 0°C afforded the allylic alcohol **21** in 84% yield. The absolute configuration (18S) of the alcohol group at C-18 in **21** was correlated with that of **10** since the same compound **2** was obtained by deprotection of **21** and oxidation of **10** at C-20. A Swern oxidation of **21** gave the 3,20-bisketal **22** which after deprotection, afforded the desired enone **3**.



Scheme 4. Synthesis of **2**, **3** and **5** from aldehyde **20**

We attempted to obtain the allylic alcohol **2** (18R) by a 1,2 reduction of enone **22** with methoxyalumino hydride in the presence of cerium chloride[17] and with DIBALH. The formation of only the 1,4 -product in each case revealed the important hindrance of the carbonyl group at C-18. Taking into account the above results, we did not try the Mitsunobu reaction to invert the configuration of the alcohol function in **21**. This reaction would very likely lead to the SN2' substitution product. Therefore, we decided to try the Dittmer procedure that uses epoxidation, mesylation and reduction by telluride of the allylic alcohol[18]. But selective epoxidation of the exo double bond at C-18 failed.

In summary, we have synthesized the three postulated cyt $P450_{11\beta}$-generated intermediates **2** (18S) and **3** for 18VP **1**, and **5** (18S) for 18EP **4**. Inhibition of the aldosterone biosynthesis induced by these compounds

was evaluated both on rat adrenal homogenate[1b,1c] and bovine purified enzyme[2]. The biochemical results will be presented elsewhere.

EXPERIMENTAL SECTION

General

Melting points (mp) were determined on a Kofler apparatus and were uncorrected. ^{13}C and ^{1}H NMR spectra were recorded in $CDCl_3$, either on a JEOL 400 or on a Brucker AC 200 spectrometer. Chemical shifts are expressed in ppm relative to TMS and coupling constants in Hz. The chemical shifts and assignments for the ^{13}C spectra of steroids **2-22** are given in Table I. IR spectra were recorded in $CHCl_3$ on a Perkin Elmer 1420 spectrometer. Optical rotations were measured in $CHCl_3$ with a Perkin Elmer 241 polarimeter. Mass spectra were carried out by the "Centre de Spectrochimie de l'Université Paris VI" and by the "Service de spectrométrie de masse de l'ENSCP". IC (NH_3) Mass spectra were obtained with a RIBER MAG R 10.10 and high resolution mass spectra on a KRATOS MS 50 spectrometer. Whenever possible, purification of products and intermediates was achieved by crystallization in isopropyl ether - CH_2Cl_2 and followed by microanalysis (carried out by the "Centre de Microanalyse de l'Université Paris VI"). Some of them (**10**, **14**, and **16**) co-crystallized with water. Otherwise, hemiketals[19] **2**, **5** and intermediate alcohols, **8**, **9**, **12**, **15**, **17**, **19** were purified by column chromatography and analyzed by (HR)MS. Analytical TLC was carried out on 0.20-mm E. Merck precoated silica gel plates (60F-254) with detection by UV light or sulfuric acid (30 %) spray followed by heating.

Table I. ^{13}C NMR Data (50MHz) of the steroids 2 to 22

	C-3	C-4	C-5	C-6	C-18	C-18a	C-18b	19	20	21	acetonide Cq	acetonide 2 CH_3		dioxolane	t-BuSi	diMeSi
2	199.5	123.8	171.1	b	85.0	140.1	117.6	17.2	106.7	24.9						
3 †	199.3	124.0	170.6	b	202.9	134.6	126.6	17.2	208.7	30.2						
5	199.2	123.9	170.6	b	72.0[d]	84.2	75.1[d]	17.4	108.1	b						
8 *†	109.8	b	140.5	122.0	74.0	142.1	114.5	19.0	70.5	23.2				64.2; 64.3		
10	199.5	123.8	171.2	b	74.0	141.5	115.7	17.2	70.7	22.6				64.2; 64.2		
11b	72.6	b	141.8	120.3	178.9			19.3	82.4	22.6					25.9	c
12	72.5	b	141.9 / 141.5	121.0	103.4 / 101.6			19.6	84.2 / 82.3	23.0 / 23.5					25.9	- 4.5
13	72.8	b	141.9	121.0	74.4	141.7	115.9	19.4	71.0	22.8					26.1	- 4.4
14 †	71.7	b	141.0	121.3	73.5	140.6	114.4	19.1	74.2	22.3	101.2	23.3	30.3			
15	199.3	123.8	171.1	b	74.1[d]	140.6	114.6	17.2	73.4 [d]	b	101.2	b	b			
16	72.5	b	141.0	120.7	62.8	86.0	76.2	19.0	70.0	22.2					25.8	-4.7
17 †	71.8	b	141.0	121.1	60.9	85.1	77.2	19.2	74.3	22.2	102.0	30.7	24.7			
18	199.3	123.7	171.3	b	60.8	85.4	77.3	17.4	74.1	22.1	102.1	25.0	30.0			
19	199.3	124.0	178.9	b	62.7	86.1	77.4	17.3	70.2	22.5						
21	109.4	41.7	140.2	122.0	74.3	139.7	114.7	18.7	111.7	24.0				62.9; 64.2; 64.4; 64.5		
22	109.5	b	140.1	122.0	203.1	135.7	124.0	18.8	110.8	22.5				64.5; 64.3; 63.6; 63.2		

†: ^{13}C NMR (100MHz); * in C_6D_6; [b] not assigned; [c] not recorded; [d] can be interchanged.

Starting materials. Compounds **7**, **11a**, **20** were prepared by previously described methods[5, 14].

3,3-Ethylenedioxy-18-20-dihydroxy-18-vinylpregn-5-ene (8) and 3,3-Ethylenedioxy-20-hydroxy-18-oxo[but-4-enyl]-pregn-5-ene (9). The lactone **7** (220 mg, 0.6 mmol) was dissolved in anhydrous toluene (5.4 mL) under argon. The solution was cooled at 0 °C and vinyl magnesium bromide (16 eq, 9.6 mL, 1M in THF) was added. The mixture was refluxed. After 16 h, the reaction was complete and gave two main products. The mixture was cooled and hydrolysed by a saturated solution of NH_4Cl (50 mL), extracted with CH_2Cl_2, washed with HCl 5% and water. The resulted oil was chromatographed on silica gel (cyclohexane-ethyl acetate 75:25) to give **8** (75 mg, 31.5 %, Rf = 0.48 in cyclohexane-ethyl acetate 50:50) and **9** (97 mg, 38.5%, Rf = 0.60 in the same solvent system).

8 : IR 3600, 3450 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.02 (s, 3H, H-19), 1.17 (d, 3H, J = 6.1 Hz, H-21), 3.98 (m, 5H, H-20 and -O-CH_2-CH_2-O-), 4.26 (d, 1H, J = 8.0 Hz, H-18), 5.20 (m, 2H, J = 17.3 Hz and 10.5 Hz,

-CH=C$\underline{H}_2$), 5.35 (m, 1H, H-6), 6.30 (m, 1H, J = 17.3 Hz and 10.5 Hz, -C$\underline{H}$=CH_2); MS (EI) *m/z* 402 (M^+), (CI) *m/z* 403 (MH^+).

9 : IR 3600, 1685 cm^{-1}; ^1H NMR (200 MHz) δ 0.90 (s, 3H, H-19), 1.14 (d, 3H, J = 6.1 Hz, H-21), 3.38 (m, 1H, H-20), 3.94 (m, 4H, -O-CH_2-CH_2-O-), 5.02 (m, 2H, -CH=C$\underline{H}_2$), 5.35 (m, 1H, H-6), 6.83 (m, 1H, -C$\underline{H}$=CH_2); ^{13}C NMR (50 MHz) (C_6D_6) 213.7, 140.0, 138.3, 121.7, 114.8, 109.3, 69.9, 64.3, 24.0, 19.0; MS (EI) *m/z* 428 (M^+). With time, this product was in equilibrium with its hemiketal form as detected by ^1H NMR spectrum.

A sample of product **9** was acetylated by acetic anhydride in pyridine to give the 20- acetate, IR 1720, 1685 cm^{-1}; ^1H NMR δ 0.85 (s, 3H, H-19), 1.14 (d, 3H, J = 6.1 Hz, H-21), 2.10 (s, 3H, -$COCH_3$), 3.94 (m, 4H, -O-CH_2-CH_2-O-), 4.48 (m, 1H, H-20), 5.00 (m, 2H, -CH=C$\underline{H}_2$), 5.35 (m, 1H, H-6), 6.78 (m, 1H, -C$\underline{H}$=CH_2); MS (CI) *m/z* 471 (MH^+).

18,20-Dihydroxy-18-vinylpregn-4-ene-3-one (10).

According to Scheme 2 (from lactone **7***).* The bis protected diol **8** (70 mg, 0.17 mmol) was refluxed in solution P (5 mL isopropanol - 5 mL H_2O - 1 mL acetic acid) (4.7 mL) during 4 h. After usual work-up, a purification by silica gel chromatography gave pure **10** (21 mg, 33%).

According to Scheme 3 (from lactol **12***).* The crude **15** (305 mg) was dissolved in ethanol (10 mL), and stirred with PPTS. The reaction was complete in 1 h. Aqueous work-up (CH_2Cl_2, $MgSO_4$) and purification by silica gel chromatography (cyclohexane-ethyl acetate 50:50) gave **10** (200 mg, 72%). Two recrystallizations in CH_2Cl_2-isopropyl ether mixture gave pure **10** (138 mg, 50%).

10 : mp 161-162 °C; IR 3600, 3420, 1660 cm^{-1}; $[\alpha]_D^{20}$ (c = 0.4 in $CHCl_3$) + 72°; ^1H RMN (200 MHz) δ 1.17 (s, 3H, H-19), 1.17 (d, 3H, H-21), 3.89 (m, 1H, H-20), 4.29 (d, 1H, J = 7.7 Hz, H-18), 5.25 (m, 2H, AB of ABX, J = 17.1 Hz, 10.1 Hz and 1.5 Hz, -CH=C$\underline{H}_2$) ; 5.71 (s, 1H, H-4), 6.31 (m, 1H, X of ABX, J = 17.1 Hz and 10.1 Hz, -C$\underline{H}$=CH_2); MS (CI) *m/z* 359 (MH^+); HRMS calcd for $C_{23}H_{34}O_3$ 358.2507, found 358.2476. Anal. calcd for $C_{23}H_{34}O_3$: C, 77.04; H, 9.56. Anal. calcd for $C_{23}H_{34}O_3$, 2/10 H_2O: C, 76.27; H, 9.52. Found: C, 76.37; H, 9.42.

3-tert-Butyldimethylsilyloxy-18:20-epoxypregn-5-ene-18-one (11b). TBDMS chloride (250 mg, 1.66 mmol) and DBU (270 µL, 1.8 mmol, dissolved in 12 mL of CH_2Cl_2) were added to 3β-hydroxy-18:20-epoxy-pregn-5-ene-18-one **11a** (500 mg, 1.5 mmol). The mixture was stirred under argon, during 24 h, at room temperature, then diluted with CH_2Cl_2 (100 mL), washed with a saturated solution of NH_4Cl (2 X 50 mL), and water. After concentration and recrystallization, we obtained **11b** (532 mg, 80%), mp 233-234 °C; IR 1745 cm^{-1}; ^1H NMR (200 MHz) δ 0.05 (s,6H, $Si(CH_3)_2$), 0.85 (s, 9H, tBuSi), 1.05 (s, 3H, H-19), 1.35 (d, 3H, J = 6,8 Hz, H-21) ; 3.45 (m, 1H, H-3), 4.35 (q, 1H, J = 6,2 Hz, H-18), 5.32 (m, 1H, H-6). Anal. calcd for $C_{27}H_{44}O_3Si$: C, 72.92; H, 9.98. Found: C, 72.48; H, 9.89.

3-tert-Butyldimethylsilyloxy-18:20-epoxy-18-hydroxypregn-5-ene (12). The lactone **11b** (500 mg, 1.12 mmol) was dissolved under argon, in anhydrous toluene (50 mL) and cooled to -70 °C. DIBALH (6 eq, 4.5 mL, 1.5M in toluene) was added. The mixture was stirred at -70 °C for 1 h 30 and diluted at the same temperature with 2M isopropanol in toluene (20 mL). The agitation was continued for 5 min at -70 °C, then the solution was allowed to warm to 0 °C. H_2O (10 mL) was added and the mixture was stirred for 15 min at room temperature. After filtration, usual work-up (toluene, H_2O and $MgSO_4$) and purification by Flash chromatography (cyclohexane-ethyl acetate 75:25) afforded **12** (380 mg, 76%) in a mixture 18R-18S (ratio 40/60 calculated by NMR); mp 194-195 °C; ^1H NMR (200 MHz) δ 0.05 (s, 6H, $Si(CH_3)_2$), 0.85 (s, 9H, tBuSi), 0.95

and 0.98 (2s, 3H, H-19), 1.21 and 1.27 (2d, 3H, J = 6.8 Hz, H-21), 3.44 (m,1H, H-3), 4.00 (m, 1H, H-20) ; 5.08 (d, 0.4H, $J_{H\text{-}OH}$ = 3.1 Hz, collapsed in D_2O, H-18), 5.25 (m, 1.6H, H-18 and H-6).

3-tert-Butyldimethylsilyloxy-18,20-dihydroxy-18-vinylpregn-5-ene (13). The lactol **12** (310 mg, 0.7 mmol) was dissolved under argon in anhydrous toluene (12 mL) and the solution was cooled to 0 °C. Vinyl magnesim bromide (16 eq, 11.2 mL, 1M in THF) was added. After agitation for 2 h at room temperature, the reaction was complete. A saturated solution of NH_4Cl (25 mL) was added at 0 °C. After usual work-up (ethyl acetate), purification by flash chromatography (cyclohexane-ethyl acetate 75:25), then recrystallization in MeOH, pure **13** (305 mg, 89%) was obtained; mp 190-191 °C; ^1H NMR (200 MHz) δ 0.05 (s, 6H, $Si(CH_3)_2$), 0.85 (s, 9H, tBuSi), 0.98 (s, 3H, H-19), 1.15 (d, 3H, J = 6.2 Hz, H-21), 3.46 (m, 1H, H-3), 3.90 (m, 1H, H-20), 4.26 (d, 1H, J = 7.4 Hz, H-18), 5.16 (m, 2H, J = 17.0 Hz and 9.9 Hz, -CH=C$\underline{H}_2$), 5.30 (m, 1H, H-6), 6.28 (m, 1H, J = 17.0 Hz,9.9 Hz and 7.4 Hz, -C$\underline{H}$=CH_2). Anal. calcd for $C_{29}H_{50}O_3Si$: C, 73. 36; H, 10.62. Found: C, 73.31; H, 10.54.

3-Hydroxy-18,20-*O*-isopropylidene-18-vinylpregn-5-ene (14). The diol **13** (215 mg, 0.45 mmol) was dissolved in anhydrous DMF (9 mL). Methoxypropene (86 µL, 2eq) and PPTS were added. The mixture was stirred under argon for 15 h at room temperature. Then, the mixture was extracted with ethyl acetate (100ml), washed with water and dried on $MgSO_4$. A mixture of crude 3-tertio-butyldimethylsilyloxy-18:20-*O*-isopropylidene-18-vinylpregn-5-ene and a minor amount of **14** was obtained. The crude was used for the next step. It was dissolved in anhydrous THF (2,5 mL), added with TBAF (1 mL, 1.1M in THF) and stirred for 5 h at room temperature. The solution was diluted with ethyl acetate (100 mL), washed twice with saturated solution NH_4Cl and water. A purification by flash chromatography (CH_2Cl_2-acetone 95:5) gave pure **14** (175 mg, 73% from **13**; 63% from **12** without purification of the intermediate **13**). An analytical sample was obtained by recrystallization in a CH_2Cl_2 / isopropyl ether mixture; mp 210-211 °C; IR 3600 cm^{-1}; ^1H NMR (200 MHz) δ 0.97 (s, 3H, H-19), 1.31 (d, 3H, J = 6.8 Hz, H-21), 1.35 (s, 3H, acetonide); 1.49 (s, 3H, acetonide), 3.50 (m, 1H, H-3), 3.91 (m, 1H, H-20), 4.28 (d, 1H, J = 7.2 Hz, H-18), 5.06 (m, 2H, J = 17.0 Hz and 10.0 Hz, -CH=C$\underline{H}_2$), 5.33 (m, 1H, H-6), 6.10 (m, 1H, J = 17.0 Hz, 10.0 Hz and 7.2 Hz, -C$\underline{H}$=CH_2). Anal. calcd for $C_{26}H_{40}O_3$: C, 77.94; H, 10.07Hz). Anal. calcd for $C_{26}H_{40}O_3$: C, 77.94; H, 10.07. Anal. calcd for $C_{26}H_{40}O_3$, 2/10 H_2O: C, 77.25; H, 10.03. Found: C, 77.18; H, 9.91.

18,20-*O*-isopropyliden-18-vinylpregn-5-ene-3-one (15). The alcohol **14** (310 mg, 0.78 mmol) was dissolved in anhydrous benzene (30 mL) and N-Methyl-4-piperidone (3 mL) was added. The mixture was heated to reflux under argon with a Dean-Stark apparatus. The first 5 mL of distillate were discarded. Aluminium isopropoxyde (400 mg, 2mmol) was added and the mixture refluxed for 5 h. The toluene solution was diluted with 100 mL toluene, washed with 50 mL aqueous 2% sulfuric acid and water, and evaporated. The crude product **15** (305 mg, 98%) was used without purification for the next step; ^1H NMR (200 MHz) δ 1.12 (s, 3H, H-19), 1.38 (d, 3H, H-21), 1.43 (s, 3H, acetonide), 1.50 (s, 3H, acetonide), 3.92 (m, 1H, H-20), 4.32 (d, 1H, J = 7.7 Hz, H-18), 5.10 (m, 2H, -CH=C$\underline{H}_2$), 5.70 (s, 1H, H-4), 6.11 (m, 1H, -C$\underline{H}$=CH_2).

3-tert-Butyldimethylsilyloxy-18-ethynyl-18,20-dihydroxypregn-5-ene (16). The lactol **12** (100mg, 0.22 mmol) was dissolved in anhydrous toluene (5 mL), and cooled to 0 °C, under argon. Ethynyl magnesium bromide (3.65 mmol, 16 eq, 7.3 mL, 0.5 M in THF) was added. The mixture was stirred for 3 h at room temperature. After hydrolysis with a saturated solution of NH_4Cl (25 mL) and extraction with CH_2Cl_2, the crude product (110 mg) was purified by silica gel chromatography (cyclohexane-ethyl acetate 60:40) to give pure **16** (91 mg, 85%). A sample was recrystallized in diethyl ether; mp 224-225 °C; IR 3600, 3480, 3300 cm^{-1}; ^1H NMR (200 MHz) δ 0.05 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, tBuSi), 1.00 (s, 3H, H-19), 1.20 (d, 3H, J =

6.2 Hz, H-21), 2.68 (d, 1H, J = 2.4 Hz, -C≡CH), 3.47 (m, 1H, H-3), 4.07 (m, 1H, H-20), 4.73 (bs, 1H, H-18), 5.32 (m, 1H, H-6); HRMS calcd for $C_{29}H_{48}O_3Si$ 472.3372, found 472.3369. Anal. calcd $C_{29}H_{48}O_3Si$: C, 73.68; H, 10.24. Anal. calcd for $C_{29}H_{48}O_3Si$, 1/2 H_2O: C, 72.30; H, 10.26. Found: C, 72.64; H, 10.03.

18-Ethynyl-3-hydroxy-18,20-*O*-isopropylidene-pregn-5-ene (17). The diol **16** (80 mg, 0.17 mmol) was dissolved in anhydrous DMF (2.5 mL). Methoxypropene (25 µL, 2 eq) and PPTS were added and stirred for 15 h at room temperature. After usual work-up (ethyl acetate), a mixture (74 mg) composed of 3-silyloxy and 3-hydroxy derivatives was obtained. This mixture was dissolved in anhydrous THF (2 mL). TBAF was added (0.3 mL, 1.1M in THF) and stirred for 5 h at room temperature. The solution was diluted with ethyl acetate (50 mL), washed twice with a saturated solution of NH_4Cl and water. A flash chromatography (cyclohexane-ethyl acetate 60:40) gave 59 mg of pure **17** (87%); mp 215-216 °C; IR 3600, 3300 cm^{-1}; ^1H NMR (200 MHz) δ 1.00 (s, 3H, H-19), 1.36 (d, 3H, J = 7.0 Hz, H-21), 1.44 (s, 3H, acetonide), 1.52 (s, 3H, acetonide), 2.59 (d, 1H, J = 2.5 Hz, -C≡CH), 3.51 (m, 1H, H-3), 3.89 (m, 1H, H-20), 4.53 (d, 1H, H-18), 5.34 (m, 1H, H-6).

18-Ethynyl-18,20-*O*-isopropylidene-pregn-4-ene-3-one (18). The alcohol **17** (130 mg, 0.32 mmol) was dissolved in toluene (13 mL). N-methylpiperidone (1.3 mL) was added. The mixture was heated to reflux under argon with a Dean-Stark apparatus and the first 2ml were discarded. Aluminium isopropoxyde (165 mg, 0.8 mmol) was added and refluxed for 8 h. The toluene solution was diluted with more solvent. After usual work-up (H_2SO_4 2%), pure **18** (127 mg, 98%) was obtained; ^1H NMR (200 MHz) δ 1.11 (s, 3H, H-19), 1.32 (d, 3H, J = 7.0 Hz, H-21), 1.39 (s, 3H, acetonide), 1.47 (s, 3H, acetonide), 2.62 (d, 1H, J = 2.6 Hz, -C≡CH), 3.85 (m, 1H, H-20), 4.44 (d,1H, J = 2.6 Hz, H-18), 5.66 (s, 1H, H-4).

18,20-Dihydroxy-18-ethynylpregn-4-ene-3-one (19). The acetonide **18** (127 mg) was dissolved in ethanol (5 mL) and stirred 1 h with PPTS. After usual work-up, the diol **19** (105 mg, 92%) was obtained, recrystallized in MeOH; mp 236-237 °C; IR 3600, 3300, 1660 cm^{-1}; ^1H NMR (200 MHz) δ 1.12 (s, 1H, H-19), 1.15 (d, 3H, J = 6.4 Hz, H-21), 2.68 (d, 1H, J = 4 Hz, -C≡CH), 3.96 (m, 1H, H-20), 4.65 (bs, 1H, H-18), 5.67 (s, 1H, H-4); HRMS calcd for $C_{23}H_{32}O_3$ 356.2351, found 356.2351.

3,3,20,20-Bis(ethylenedioxy)-18-hydroxy-18-vinylpregn-5-ene (21). The aldehyde **20** (200 mg, 0.48 mmol) was dissolved in anhydrous THF (2.4 mL), under argon. The solution was cooled to 0 °C and vinyl magnesium bromide (1 mL, 1 M in THF, 1.1 eq) was added dropwise. After 2 h, the reaction was quenched with a saturated NH_4Cl solution (5 mL). The mixture was extracted with CH_2Cl_2, washed with H_2O to give 224 mg of crude product. The same reaction was repeated two times with 350 and 380 mg to give respectively 385 and 389 mg. The whole crude product (998 mg) was purified by crystallization in cyclohexane and silica gel chromatography (cyclohexane-ethylacetate, 3:1, R_f = 0.32) to give 827 mg of pure **21** (84%); mp 144-146 °C; IR 3500 cm^{-1}; $[\alpha]_D^{20}$ (c = 0.5 in $CHCl_3$) -12°; ^1H NMR (200 MHz) δ 0.96 (s, 3H, H-19), 1.32 (s, 3H, H-21), 3.92 (m, 8H, -O-CH_2-CH_2-O-), 4.03 (d, J = 11.4 Hz, supressed in D_2O, -OH), 4.27 (dd, 1H, J = 11.4 and 7.4 Hz, H-18), 5.03 (d, 1H, J = 10.30 Hz, -CH=C<u>H</u>$_2$), 5.15 (d, 1H, J = 17.31 Hz, -CH=C<u>H</u>$_2$), 5.3 (m, 1H, H-6), 6.13 (ddd, 1H, J = 17.31, 10.30 and 7.4 Hz, -C<u>H</u>=CH_2). Anal. calcd for $C_{27}H_{40}O_5$: C, 72.92; H, 9.07. Found: C, 72.93; H, 9.07.

3,3,20,20-Bis(ethylenedioxy)-18-oxo-18-vinylpregn-5-ene (22). Freshly distilled oxalyl chloride (45 µL, 0.5 mmol, 1.1 eq) diluted with anhydrous CH_2Cl_2 (1.12 mL) was cooled to -60 °C, under argon. Anhydrous DMSO (76.5 µL, 1.1 mmol, 2.2 eq) diluted in CH_2Cl_2 (225 µL) was added dropwise. The mixture was stirred to -60 °C for 30 min. Then the alcohol **21** (200 mg, 0.45 mmol) dissolved in CH_2Cl_2

(390 µL) was added and stirred to -60 °C for 30 min. Et_3N (315 µL) was added at -60 °C and the solution was stirred 1 h at room temperature. H_2O (10 mL) was added and after usual work-up (H_2O, CH_2Cl_2), the crude product was purified by chromatography on silica gel (cyclohexane-ethylacetate 3:1, R_f = 0.32) to give 183.5 mg of pure **22** (0.415 mmol, 92%); mp 166-167 °C; IR 1605, 1680 cm^{-1}; $[\alpha]_D^{20}$ (c = 0.5 in $CHCl_3$) + 21.4°; ^1H NMR (200 MHz) δ 0.80 (s, 3H, H-19), 1.19 (s, 3H, H-21), 3.9 (m, 8H, -O-CH_2-CH_2-O-), 5.28 (m, 1H, H-6), 5.41 (dd, 1H, J = 2.2 Hz and 10.26 Hz, -CH-C<u>H</u>$_2$), 6.10 (dd, 1H, J = 2.2 Hz and 17.02 Hz, -CH-C<u>H</u>$_2$), 6.74 (dd, 1H, J = 17.02 Hz and 10.26 Hz, -C<u>H</u>=CH_2). Anal. calcd for $C_{27}H_{38}O_5$: C, 73.26; H, 8.66. Found: C, 73.15; H, 8.62.

18(S)-Hydroxy-3,20-dioxo-18-vinylpregn-4-ene-18,20-hemiketal (18-hydroxy-18-vinylprogesterone) (2).

According to Scheme 3 (from lactol **12***).* Oxalyl chloride (56 µL, 0.65 mmol) was dissolved in anhydrous CH_2Cl_2 (1.5 mL) and cooled to -60 °C under argon. Anhydrous DMSO (0.1mL, 1.4 mmol) dissolved in CH_2Cl_2 (0,3 mL) was added dropwise and the mixture was stirred at -60 °C for 30 min. The alcohol **10** (105 mg, 0.3 mmol) dissolved in CH_2Cl_2 (2,5 mL) was added and the mixture was stirred for 30 min at -60 °C. Et_3N (406 µL) was added and the mixture was allowed to warm to room temperature for 30 min, then poured into H_2O (5 mL). The aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were washed with H_2O, dried on $MgSO_4$ and concentrated to give 120 mg of crude product. A first purification by flash chromatography (cyclohexane-ethyl acetate 50:50) gave 2 products which were separated by silicagel chromatography (toluene-acetonitrile 8.5:1.5) giving 50 mg of **2** (50%) and 25 mg of **3** (25%).

According to Scheme 3 (from lactol **12***).* The semi-hydrogenation of hemiketal **5** was performed in the presence of ethylacetate (6 mL) as solvent and 5% palladium-on-barium sulfate (125 mg) poisoned with synthetic quinoline (125 µL) as catalyst. The air in the flask was removed by evacuating for 15 min. under agitation and by flushing with hydrogen three times for one hour. The hemiketal **5**, diluted with ethylacetate (2 mL), was then added and the flask was agited by a shaker under hydrogen atmosphere at room temperature for 3 hours. The catalyst was then separated on celite by filtration and the filtrate was distilled. Purification by Al_2O_3 thin layer chromatography (CH_2Cl_2-acetone 95:5) of the crude gave pure hemiketal **2** (22.5 mg, 64 %) as own product.

According to Scheme 4 (from aldehyde **20***).* The aldehyde **20** (200 mg, 0.48 mmol) was dissolved in anhydrous THF (2.3 mL), and cooled to 0 °C under argon. Vinylmagnesium bromide (0.1 mmol, 1 mL, 1M in THF) was added and the mixture was refluxed for 1 h. The usual work-up (CH_2Cl_2) afforded 220 mg of crude product. This crude product was refluxed for 4 h in 15 mL of solution P, neutralized with a solution of K_2HPO_4 (1.5 M), extracted with CH_2Cl_2, washed with water and concentrated. A purification by silica gel chromatography (CH_2Cl_2-acetone 95:5) gave pure **2** (58 mg, 34%) unstable upon recrystallization.

2 : mp 152-153 °C; IR 3580, 1660 cm^{-1}; $[\alpha]_D^{20}$ (c = 0.48 in $CHCl_3$) +102°; ^1H NMR δ 1.01 (s, 3H, H-19), 1.43 (s, 3H, H-21), 4.14 (d, 1H, J = 9.3 Hz, H-18), 5.18 (m, 2H, J = 17.0 Hz and 9.8 Hz, -CH=C<u>H</u>$_2$) 5.69 (s, 1H, H-4), 6.20 (m, 1H, J = 17.0 Hz, 9.8 Hz and 9.3 Hz, -C<u>H</u>=CH_2); HRMS calcd for $C_{23}H_{32}O_3$ 356.2351, found 356.2348.

3,18,20-Trioxo-18-vinylpregn-4-ene(18-oxo-18-vinylprogesterone)(3).

According to Scheme 3 (from lactol **12***).* See 18(*S*)-Hydroxy-3,20-dioxo-18-vinylpregn-4-ene-18,20-hemiketal (18-hydroxy-18-vinylprogesterone) (**2**) .

According to Scheme 4 (from aldehyde **20***).* The enone **22** (53 mg) was dissolved in acetone (13.25 mL) and amberlite resin IRN-77 (1.1 g) was added. The mixture was stirred for 3 days at room temperature in the dark.

After filtration, the crude product was purified on silica gel chromatography (cyclohexane-ethylacetate, 2:1, Rf = 0.25) to give 25 mg of pure **3** (59%).

3 : mp 167-168 °C; IR 1700, 1665, 1660, 1610, 1600 cm^{-1}; $[\alpha]_D^{20}$ (c = 0.5 in $CHCl_3$) + 221°; ^1H NMR (200 MHz) δ 1.03 (s, 3H, H-19), 2.09 (s, 3H, H-21), 5.54 (dd, 1H, J = 10.4 and 2.0 Hz, -CH=C<u>H</u>$_2$), 5.71 (s, 1H, H-4), 6.31 (dd, 1H, J = 17.2 Hz and 2.0 Hz, -CH=C<u>H</u>$_2$), 6.76 (2d, 1H, J = 17.2 Hz and 10.4 Hz, -C<u>H</u>=CH$_2$); HRMS calcd for $C_{23}H_{30}O_3$ 354.2194, found 354.2193. Anal. calcd for $C_{23}H_{30}O_3$: C, 77.92; H, 8.53. Found: C, 77.79; H, 8.92.

18-Ethynyl-18-hydroxy-3,20-dioxopregn-4-ene-18,20-hemiketal (5).

*According to Scheme 3 (from lactol **12**).* Hemiketal **5** was prepared form the diol **19** as described for hemiketal **2** from the diol **10** (quantitative yield).

*According to Scheme 4 (from aldehyde **20**).* The aldehyde **20** (300 mg, 0.72 mmol) was dissolved in anhydrous THF (3.5 mL) under argon, and cooled to 0 °C. Ethynyl magnesium bromide (1.5 mmol, 3 mL, 0.5 M in THF) was added and the mixture was refluxed for 1 h. After cooling, CH_2Cl_2 (200 mL), and a saturated solution of NH_4Cl (20 mL) were added. The organic layer was washed with water and evaporated to give 315 mg of crude product. The crude product was dissolved in 22 mL of solution P (10 mL isopropanol - 10 mL H_2O - 2 mL acetic acid) and refluxed for 4 h. After neutralization with 1,5 M K_2HPO_4 (40 mL), the usual work-up (CH_2Cl_2) afforded crude product (260 mg). A purification by neutral Al_2O_3 chromatography (CH_2Cl_2-acetone 85:15) gave pure **5** (170 mg, 66%), unstable upon recrystallization, mp 160-161 °C; IR 3560, 3300, 1660 cm^{-1}; $[\alpha]_D^{20}$ (c = 0.4 in $CHCl_3$) + 118°; ^1H NMR (200 MHz) δ 1.15 (s, 3H, H-19), 1.48 (s, 3H, H-21), 2.58 (d, 1H, J = 3.1 Hz, -C≡CH), 4.45 (d, 1H, J = 3.1 Hz, H-18), 5.72 (s, 1H, H-4); HRMS calcd for $C_{23}H_{30}O_3$ 354.2194, found 354.2187.

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