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Inhibition of Ecdysone Biosynthesis : Preparation of Acetylenic Intermediates

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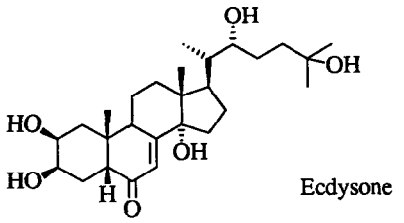
Abstract : Several acetylenic derivatives of ecdysone were synthesized from pregnenolone. These compounds carry an acetylenic function at C-22 and were devised with the aim to inhibit the C-22 hydroxylation of ecdysone biosynthesis by a suicide-substrate mechanism. The five compounds synthesized inhibit the synthesis of ecdysone in the prothoracic glands of *Locusta migratoria in vitro.*

Introduction



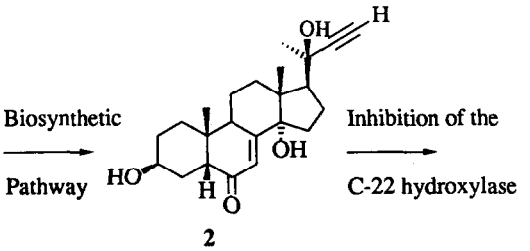
Ecdysone

1

Scheme 1

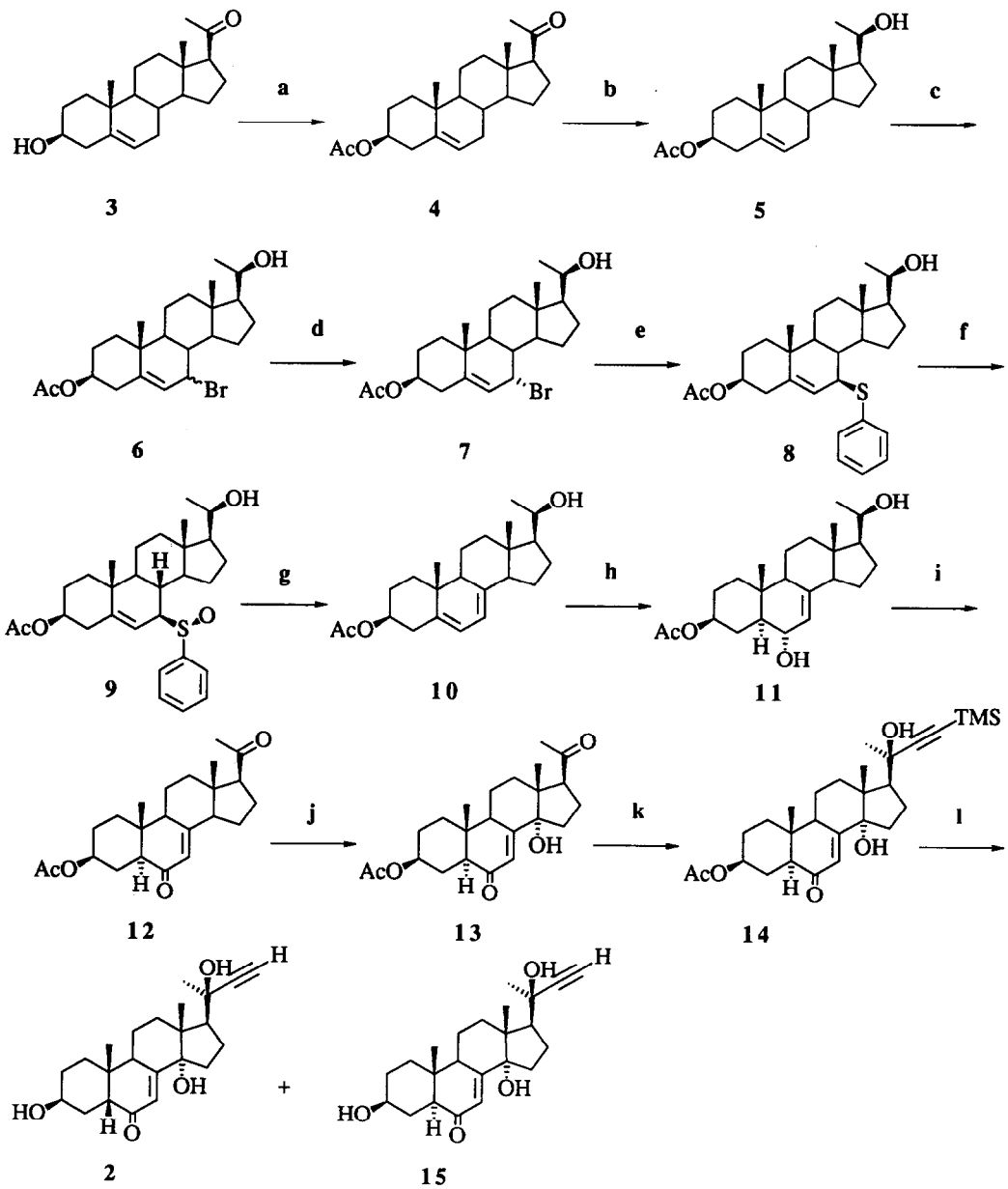
Ecdysone, (**1**, 2β,3β,14α,22*R*,25-pentahydroxy-5β-cholest-7-en-6-one, Scheme 1) the insect moulting hormone, is synthesized during larval and nymphe development in the moulting glands (prothoracic glands in *Locusta migratoria*). It is also produced in the follicular cells of the ovaries in reproductively competent adult females. Though our knowledge of the biosynthetic pathway of ecdysone is still imperfect, we have clearly established the sequence of the three last steps, consisting in a sequence of hydroxylations from 2,22,25-trideoxyecdysone in the following order : at C-25, C-22 and C-2.

Several inhibitors, based upon the suicide substrate strategy, have been synthesized and proven to be active toward prothoracic glands in *Locusta*[1,2]. However, we have found reasonable to assume that, with these inhibitors, the cholesteryl nucleus was oxidized into an ecdysteroid inhibitor, **2**, by the biosynthetic pathway before reaching its target, the C-22 hydroxylase, which was inactivated by the acetylenic function (Scheme 2).



2

Scheme 2



a Ac_2O, 120°C (quant.) **b** L-Selectride®, THF, -78°C (Quant.) **c** Dibromantine, $NaHCO_3$, refluxing hexane **d** nBu_4NBr, THF, r.t. **e** Thiophenol, Et_3N, r.t. **f** mCPBA, AcOEt **g** 70°C, Toluene (53% starting from compound **3**) **h** BH_3.DMS, CH_2Cl_2, 25°C, sodium perborate, 0°C (77 %) **i** PCC, CH_2Cl_2, r.t. (80 %) **j** SeO_2, dioxanne, 80°C (74 %) **k** TMS-CCLi, THF, -78°C (88 %) **l** K_2CO_3, MeOH, THF, H_2O (95 %).

Scheme 3

In an attempt to check this hypothesis, we decided to synthesize inhibitors possessing an ecdysteroid

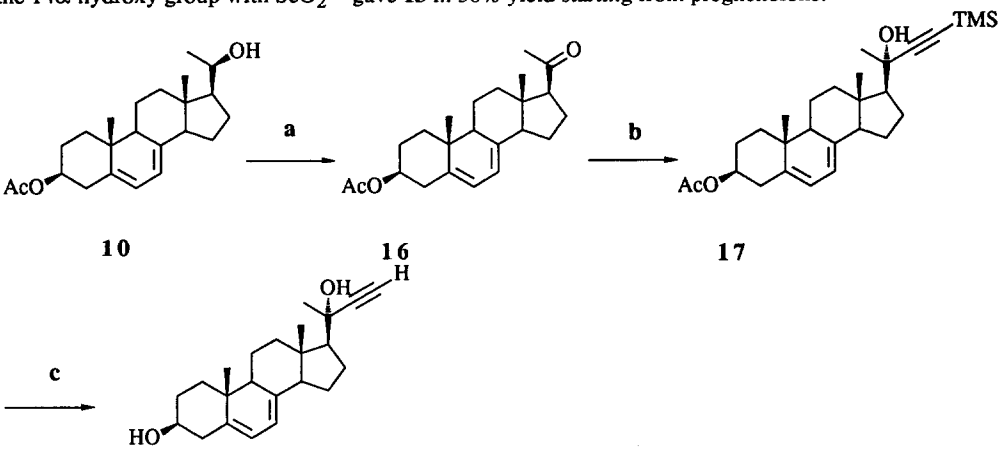
nucleus. Furthermore, we needed to use a divergent versatile synthesis and to obtain large amounts of inhibitors for biological studies. We found existing methods[3-6] not to be satisfactory in that aim and therefore investigated a new synthesis involving the hydroboration of a $\Delta^{5,7}$ diene as a key reaction[7]. In a first approach, we observed that allylic bromination did not give reproducible yields with TBDMS used as a protective group of the hydroxyle at C-3[8], however, we found acetate to be appropriate in that direction.

Chemical results and discussion

Starting from pregnenolone (**3**, Scheme 3), the $\Delta^{5,7}$ diene was selectively introduced by allylic bromination followed by the cis elimination of the corresponding sulfoxides, according to a procedure described by Confalone *et al*[9]. All our attempts to synthesize the $\Delta^{5,7}$ diene by using the Shapiro reaction[10] were unsuccessfull, because of the limited yields obtained in the course of the allylic oxidation at C-7.

However, reduction of the acetate protected pregnenolone (**4**) with L-Selectride® avoided the formation of the anti-Cram adduct and a tedious separation. It selectively gave the alcohol **5** in very good yields. Allylic bromination with 2,3-dibromo-5,5-dimethylhydantoin gave a mixture of 7α and 7β bromides (**6**) in a 1:1 ratio. Upon equilibration with nBu_4NBr, which was more efficient than LiBr in our hands, the 7α isomer predominated. Substitution with thiophenol then gave the corresponding sulfurs, where the 7β isomer was the major isomer. Oxidation with mCPBA gave a mixture of diastereomer (**9**), and a cis elimination of the 7β sulfoxides, in thermic interconversion at 70°C, gave the diene **10**, free of the $\Delta^{4,6}$ diene as shown by U.V. data, in 53 % yield. We would emphasize that no chromatography is required until this step.

Hydroboration/oxidation of the resulting diene **10** furnished the allylic alcohol **11**. However, the classical $NaOH/H_2O_2$ procedure afforded a significant deprotection of the acetate group and had to be replaced by a milder one, employing sodium perborate[11], in order to obtain good yields of the desired allylic alcohol. Oxidation in the C-6 and C-20 (or C-17) positions with PCC gave compound **12** in good yields. Introduction of the 14α-hydroxy group with SeO_2[12] gave **13** in 30% yield starting from pregnenolone.



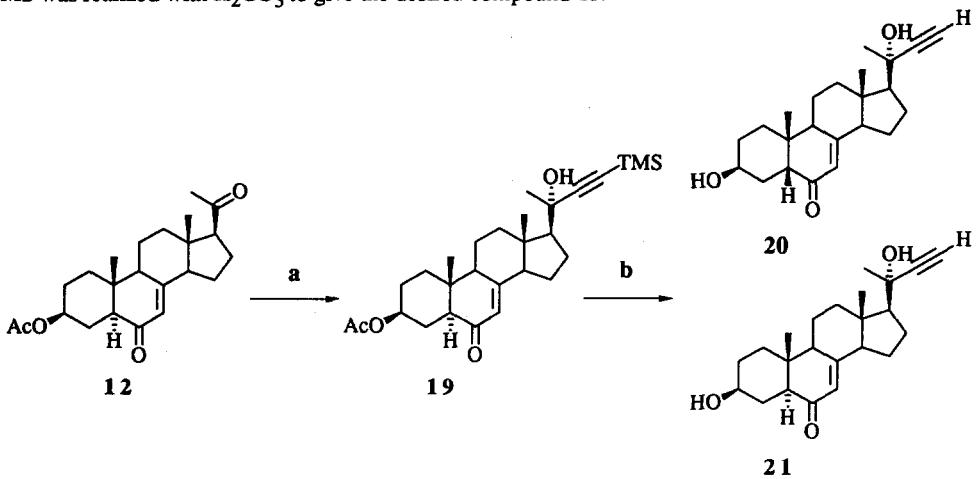
10 **16** **17** **18**

a NMO, TPAP, CH_2Cl_2, 4Å MS (92 %) b TMS-C≡CLi, THF, -78°C (82 %) c K_2CO_3, MeOH/H_2O (80%).

Scheme 4

Surprisingly, we were unable to introduce the acetylenic side chain with the corresponding Grignard, even with a large excess of this reagent. However, we found that treatment of compound **13** with 4.0 eq. of lithium trimethylsilylacetylide gave pure **14** in 88 % yield, without any deprotection of the acetate nor reaction of the conjugated ketone, which we believe to be protected by enolization. Basic treatment with K_2CO_3 in MeOH/THF/H_2O enabled us to deprotect both TMS and acetate groups and to epimerize at C-5 in one pot, to give a mixture of **2** and **15** in 95 % yield starting from pregnenolone, in a 1:4 ratio according to ^1H NMR.

Recently, 7-Dehydrocholesterol has been shown to be a precursor of the ecdysone synthesis[13]. Thus, we were interested in the synthesis of a dienic inhibitor and therefore started from the dienic intermediate **10** (Scheme 4). Oxidation at C-20 was realized with the TPAP/NMO catalytic system from Griffith and Ley[14,15], to furnish compound **16** in very good yields. Condensation of the side chain then gave **17**, deprotection of TMS was realized with K_2CO_3 to give the desired compound **18**.



a TMS-C≡CLi, THF, -78°C (79 %) **b** K_2CO_3, MeOH/H_2O (83 %).

Scheme 5

In the same way, we have synthesized a 14-dehydro inhibitor, starting from compound **12**, as shown on Scheme 5.

Thus in conclusion, we have been able to establish a new route to functionalized inhibitors, by introducing a $\Delta^{5,7}$ diene regiospecifically. The final epimerization at C-7 allowed us to obtain the desired 5β compounds efficiently. We should point out that a stereoselective synthesis would be much longer, since the trans A/B ring junction is favoured in our case.

Unfortunately, these inhibitors did not show any significant improvement in their inhibition effects when compared to the corresponding cholesteryl inhibitor[1], excepted for the dienic compound **18**. Its inhibitory effect has been determined *in vitro* on ecdeysone biosynthesis in larval prothoracic glands (74% depressory effect at a concentration of 10^{-5}M, 34% at 10^{-6}M) of *Locusta migratoria*.

Experimental

Melting points were measured on a Reichert hot stage microscope and are uncorrected. $[\alpha]_D$ were measured on a Perkin-Elmer 141 polarimeter in $CHCl_3$. IR spectra were recorded in KBr on a Perkin-Elmer infrared spectrophotometer. UV spectra were

measured on a Kontron-Uvikon 810 UV-vis spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Brucker SY (200 MHz) aparatus with $CHCl_3$ (δ=7.26 ppm)as internal standard for NMR ^1H, $CDCL_3$ (δ=77.02 ppm) as internal standard for ^{13}C NMR. The chemical shifts are reported in ppm downfield from TMS (=interchangeable assignement). MS were measured on a LKB 9000 S apparatus by direct introduction, or coupled to a GC (OV-1 column); an ionization potential of 70 eV was used. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyse of CNRS. TLC plates were run on pre-coated plates of silica gel 60 F 254 (Merck), dipped in a solution of vanillin (1 g) in $EtOH/H_2SO_4$ (95/5, 1 l) and heated on a hot plate to reveal the compounds. Medium pressure chromatography (P=0.4-1.0 bar) was conducted on silica gel (40-63 mm, Merck) columns. All solvents were freshly distilled before use. Air or moisture sensitive reactions were conducted in flame-dried glasware.

3β-Acetoxypregn-5-en-20-one (4)

Pregnenolone (3) (10g, 31.6 mmol.) was acetylated by treatment with freshly distilled acetic anhydride (15ml) at 120°C for 45 mn. The mixture was cooled to r. t., filtered and washed with cold methanol. The residue, dried over KOH under high vacuum, gave the acetate 2 quantitatively (11.3 g), which cristallized in CH_2Cl_2.

4 : Mp : 148-150°C; **^1H-NMR** δ :.0.63 (s, 3H, H-18); 1.02 (s, 3H, H-19); 2.02 (s, 3H, H-COOMe); 2.12 (s, 3H, H-21); 4.60 (m, 1H, $w_{1/2}$=25 Hz, H-3); 5.37 (d, 1H, J=5.2 Hz, H-6); **Microanalysis** : calc. for $C_{23}H_{34}O_3$ (358.53) : C, 77.05, H, 9.56. Found : C, 77.08, H, 9.56.

(20*R*)-3β-Acetoxy-20-hydroxypregn-5-ene (5)

Compound 4 (10 g, 27.9 mmol.) was dissolved in dry THF (200 ml) under Ar, and the resulting solution was cooled to 0°C. L-Selectride® 1M in THF (1.2 eq., 27.9 ml, 27.9 mmol.) was added dropwise and the resulting mixture was stirred for 30 mn. Water (30 ml) was cautiously added, followed by sodium perborate. The mixture was extracted with Et_2O, washed with water. The organic layer was dried over Na_2SO_4 and evaporated to dryness, to give compound 3 (9.65 g, 96%), which cristallized in CH_2Cl_2.

5 : R_f=0.42 (hex/AcOEt 8/2); **Mp** 146-150°C; $[\alpha]_D^{24}$=-73 (c=1, $CHCl_3$); **IR** ν (cm^{-1}) : 3564s, 2954s, 2912s, 2890s, 2865s, 1720s, 1450s, 1374s, 1258s, 1110s, 1032s; **^1H-NMR** δ : 0.77 (s, 3H, H-18); 2.02 (s, 3H, H-Ac); 3.73 (qd, 1H, J_1=6.0 Hz, J_2=3.5 Hz, H-20); 4.59 (m, 1H; $w_{1/2}$=25 Hz, H-3); 5.36 (d, 1H, J=5.2 Hz, H-6); **^{13}C-NMR** δ : 12.4 (18), 19.4 (19), 21.0 (11), 21.5 (Me-Ac), 23.7 (21), 24.6 (15), 25.7 (16), 27.8 (2), 31.8 (8), 32.0 (7), 36.9 (10), 37.0 (1), 38.2 (12), 39.9 (4), 42.8 (13), 50.1 (9), 56.2 (17), 58.5 (14), 70.6 (20), 74.0 (3), 122.5 (6), 140.0 (5), 170.7 (CO-Ac); **MS** m/z : 300 (100%), 285 (16), 282 (5), 267 (10), 258 (2), 255 (7), 253 (4), 241 (5); **Microanalysis** : calc. for $C_{23}H_{36}O_3$ (360.54) C, 76.62, H, 10.06. Found : C, 76.45, H, 10.00.

(20*R*)-3β-Acetoxy-20-hydroxypregna-5,7-diene (10)

A mixture of compound 5 (5g, 13.9 mmol.), dibromantin (1.0 eq., 4.0 g, 13.9 mmol.) and sodium hydrogenocarbonate (5.4 eq., 6.3 g, 75.3 mmol.) in dry hexane (240 ml) was heated under reflux for 1.5 h. The reaction mixture was cooled to 0°C, filtered to remove 5,5-dimethylhydantoin and inorganic salts and washed with cold Et_2O, the filtrate was evaporated to dryness. The residue was taken up in dry THF at 0°C (150 ml) and treated with nBu_4NBr (0.1 eq., 448 mg, 1.4 mmol.) under Ar for 30 mn. The mixture was treated with distilled Et_3N (1.3 eq., 2.5 ml, 18.1 mmol.) and thiophenol (1.3 eq., 1.9 ml, 18.1 mmol.). After beeing stirred for 1.5 h at r.t., the reaction mixture was extracted with Et_2O, washed with 1M HCl and twice with water. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in AcOEt (90 ml), cooled to 0°C and treated with 70% mCPBA (1.1 eq., 3.8 g, 15.3 mmol.) for 20 mn. The mixture was washed with 10% $NaHCO_3$ and water. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in toluene (120 ml), treated with dry Et_3N (2.2 eq., 4.3 ml, 30.7 mmol.), heated at 70°C for 20 h, cooled, and washed twice with water. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed through silica, eluting with hexane/AcOEt 9/1, allowing us to obtain pure 8 (2.6 g, 53%).

10 : R_f=0.72 (hex/AcOEt 5/5); **Mp** 148-152°C; $[\alpha]_D^{24}$=-95 (c=1, $CHCl_3$); **IR** ν (cm^{-1}) : 3450w, 2940s, 2932s, 2880s, 1716s, 1702s, 1448s, 1378s, 1252s, 1040s; **^1H-NMR** δ : 0.70 (s, 3H, H-18); 0.96 (s, 3H, H-19); 1.16 (d, 3H, J=6.1 Hz, H-21); 2.04 (s, 3H, H-Ac), 3.73 (qd, 1H, J_1=5.9 Hz, J_2=4.2 Hz, H-20); 4.71 (m, 1H, $w_{1/2}$=25 Hz, H-3); 5.39 (m, 1H, $w_{1/2}$=12.5 Hz, H-

7); 5.57 (m, 1H, $w_{1/2}$=10 Hz, H-6); **^{13}C-NMR** δ : 12.3 (18), 16.2 (19), 21.0 (11), 21.4 (Me-Ac), 23.3 (15), 23.7 (21), 25.5 (16), 28.2 (2), 36.8 (1), 37.2 (10), 38.0 (12), 39.3 (4), 43.0 (13), 54.0 (9), 58.3 (14), 70.7 (20), 72.8 (3), 116.5 (7), 120.2 (6), 136.9 (5), 141.0 (8), 170.5 (CO-Ac); **MS** m/z : 300 (100%), 288 (2), 285 (15), 282 (6), 267 (10), 258 (2), 255 (8), 253 (3), 241 (4); **UV** λ_{max} (ε) :268 (6080), 279 (6280), 290 (3380); **Microanalysis** : calc for $C_{23}H_{34}O_3$ (358.53) : C, 77.05, H, 9.56. Found : C, 76.50, H, 9.81.

(20*R*)-3β-Acetoxy-6α,20-dihydroxypregn-7-ene (11)

To a solution of **10** (1g, 2.79 mmol.) in dry THF (20 ml) at 0°C and under Ar was added BH_3.DMS complex 1M in THF (4.0 eq., 11.2 ml, 11.2 mmol.) dropwise, over a period of 5 mn. After 10 to 15 mn, water was introduced in such a way that the temperature was maintained at 0°C. A stoichiometric amount of $NaBO_3.4H_2O$ (1.0 eq, 429 mg, 2.79 mmol.) was added, the mixture was allowed to warm up to room temperature and was stirred for 2h while vigorous stirring. The two phases were separated, and the aqueous phase extracted with Et_2O. The combined organic phases were washed with brine, dried with $MgSO_4$, and the solvent was evaporated, the residue was chromatographed through silica, eluting with hexane/AcOEt 7/3, yielding pure **9** (809 mg, 77 %).

11 : R_f=0.47 (hex/AcOEt 5/5); **Mp** 195-196°C; $[\alpha]_D^{22}$=+3 (c=1, $CHCl_3$); **IR** ν (cm^{-1}) : 3420w, 2956s, 2878s, 1730s, 1448s, 1376s, 1361s, 1244m, 1032s; **^{1}H-NMR** δ : 0.63 (s, 3H, H-18), 0.87 (s, 3H, H-19), 1.17 (s, 3H, H-21), 2.03 (s, 3H, H-Ac), 3.72 (qd, 1H, J_1=6.3 Hz, J_2=3.5 Hz, H-20), 4.70 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.19 (m, 1H, $w_{1/2}$=10 Hz, H-6), 6.52 (bs, 1H, H-7); **^{13}C-NMR** δ : 12.4 (18), 13.8 (19), 21.2 (11), 21.3 (Me-Ac), 23.1 (15), 23.7 (21), 25.3 (16), 27.4 (2), 29.9 (4), 35.4 (10), 37.0 (1), 39.5 (12), 43.6 (13), 49.0 (9)*, 49.2 (5)*, 54.3 (14), 58.5 (17), 70.0 (6), 70.7 (20), 73.2 (3), 122.1 (7), 141.2 (8), 170.5 (CO-Ac); **MS** m/z : 376 (17%, M^+), 358 (17), 343 (6), 316 (54), 314 (7), 301 (16), 298 (100), 283 (78), 280 (8); **Microanalysis** : calc for $C_{23}H_{36}O_4$ (376.54) : C, 73.37, H, 9.64. Found : C, 73.25, H, 9.71.

3β-Acetoxypregn-7-en-6,20-dione (12)

To a stirred suspension of PCC (3 eq., 1.2 g, 5.78 mmol.) in dry CH_2Cl_2 (10 ml) with 4Å molecular sieve was added compound **11** (700 mg, 1.86 mmol.) in CH_2Cl_2 (2 ml). After 30 mn at room temperature, dry Et_2O (10 ml) was added and the supernatant liquid was decanted from the black gum. The insoluble residue was washed with dry Et_2O (3x) and became a black granular solid. The combined organic solution was passed through a short pad of Florisil® and the solvent was removed by distillation. Compound **12** was separated through silica eluted with Tol/Hex/EtOH 10/83/7 yielding pure **12** (554 mg, 80 %).

12 : R_f=0.29 (hex/tol/EtOH 7.5/1/1.5); **Mp** 190-193°C; $[\alpha]_D^{24}$=+26 (c=1, $CHCl_3$); **IR** ν (cm^{-1}) : 3432w, 2958s, 2873s, 1728s, 1702s, 1666s, 1364s, 1246s, 1036s; **^{1}H-NMR** δ : 0.58 (s, 3H, H-18), 0.88 (s, 3H, H-19), 2.68 (dd, 1H, J_1=8.5 Hz, J_2=4.0 Hz, H-5), 4.73 (m, 1H, $w_{1/2}$= 25 Hz, H-3), 5.75 (d, 1H, J=2.7 Hz, H-7); **^{13}C-NMR** δ : 13.2 (18), 13.7 (19), 21.3 (Me-Ac), 21.8 (11), 22.8 (15)*, 22.9 (16)*, 26.4 (1)◊, 26.8 (2)◊, 31.3 (21), 36.7 (4), 37.9 (12), 38.4 (10), 45.3 (13), 50.0 (9), 53.3 (5), 55.4 (14), 63.2 (17), 72.8 (3), 123.6 (7), 161.6 (8), 170.5 (CO-Ac), 199.6 (6), 208.3 (20); **MS** m/z : 372 (75%, M^+), 357 (4), 354 (5), 329 (25), 312 (100), 301 (5), 297 (48), 284 (9); **UV** λ_{max} (ε) : 237 (11790); **Microanalysis** : calc for $C_{23}H_{32}O_4$ (372.51) : C, 74.16, H, 8.66. Found : C, 73.98, H, 8.68.

3β-Acetoxy-14α-hydroxypregn-7-en-6,20-dione (13)

A solution of compound **12** (200 mg, 0.54 mmol.) in dioxane (12 ml) was treated with SeO_2 (10 eq., 596 mg, 5.37 mmol.) at 80°C for 5 mn. The reaction mixture was allowed to cool down to room temperature and filtered through celite. After evaporation to dryness, the crude mixture was chromatographed through silica eluting with hex/AcOEt 7/3, yielding pure **13** (154 mg, 74 %).

13 : R_f=0.24 (hex/tol/EtOH 7.5/1/1.5); **Mp** 218-220°C; $[\alpha]_D^{24}$=+47 (c=1, $CHCl_3$); **IR** ν (cm^{-1}) : 3474w, 2977s, 2958s, 2950s, 2876s, 1732s, 1686s, 1658s, 1377s, 1364s, 1240s, 1208s, 1042s; **^{1}H-NMR** δ : 0.64 (s, 3H, H-18), 0.87 (s, 3H, H-21), 2.72 (dd, 1H, J_1=8.7 Hz, J_2=4.0 Hz, H-5), 3.29 (bt, 1H, J=8.7 Hz, H-9), 4.72 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.91 (d, 1H, J=2.7 Hz, H-7); **^{13}C-NMR** δ : 12.9 (18), 17.1 (19), 20.5 (11), 21.1 (16), 21.3 (Me-Ac), 26.2 (1)*, 26.7 (2)*, 29.7 (15), 31.4 (21), 32.0 (12), 36.5 (4), 38.5 (10), 45.9 (9), 47.4 (13), 53.3 (5), 58.8 (17), 72.7 (3), 84.4 (14), 123.2 (7), 162.3 (8), 170.6 (CO-Ac), 199.6 (6), 209.4 (20); **MS** m/z : 388 (1%, M^+), 372 (4), 370 (9), 360 (5), 338 (32), 310 (4), 300 (7), 295 (6), 289 (9), 285 (11), 276

(100), 274 (57), 267 (18); UV λ_{max} (ε) : 234 (10070) **Microanalysis** : calc for $C_{23}H_{32}O_5$ (388.51) : C, 71.11, H, 8.30. Found : C, 71.00, H, 8.39.

(20*R*)-3β-Acetoxy-23-trimethylsilyl-14α,20-dihydroxy-24-norchol-7-en-22-yn-6-one (14)

Lithium trimethylsilylacetylide (4.0 eq., 1.03 mmol., prepared from trimethylsilylacetylene and 1.6M BuLi in THF) was added dropwise to a solution of **13** (100 mg, 0.26 mmol.) in dry THF (5 ml) at -78°C under inert atmosphere. After 5 mn, the dry ice bath was removed and $Na_2SO_4.10H_2O$ was added. The mixture was filtered over SiO_2 and evaporated to dryness. The residue was chromatographed through silica eluting with hex/AcOEt 9/1 to 7/3 to yield pure **14** (110 mg, 88 %).

14 : R_f=0.47 (hex/AcOEt 4/6); **Mp** 179-182°C; $[\alpha]_D^{23}$=+13 (c=0.5, $CHCl_3$); **IR** ν (cm^{-1}) : 3480w, 3310s, 2942s, 2168s, 2876s, 1732s, 1722s, 1670s, 1248s, 844s; **^{1}H-NMR** δ : 0.14 (s, 9H, H-$SiMe_3$), 0.88 (s, 3H, H-18), 0.97 (s, 3H, H-19), 1.51 (s, 3H, H-21), 2.09 (s, 3H, H-Ac), 3.64 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.91 (d, 1H, J=2.8 Hz, H-7); **^{13}C-NMR** δ : -0.2 ($SiMe_3$), 12.9 (18), 17.2 (19), 20.4 (11), 21.3 (Me-Ac), 23.6 (16), 26.3 (15)*, 26.7 (2)*, 30.7 (1), 32.0 (12), 33.2 (21), 36.5 (4), 38.6 (10), 45.9 (9), 47.3 (13), 53.4 (5), 54.4 (17), 71.2 (20), 72.8 (3), 84.4 (14), 90.5 (22), 109.6 (23), 122.8 (7), 163.2 (8), 170.6 (CO-Ac), 199.8 (6); **MS** m/z : 486 (1, M^+), 471 (1), 458 (2), 453 (2), 443 (9), 426 (100), 411 (3), 386 (4), 354 (5), 346 (4), 328 (6), 317 (4), 313 (4), 303 (6), 286 (5), 276 (69); **Microanalysis** : calc for $C_{28}H_{42}O_5Si_1$ (486.73) : C, 69.10, H, 8.70. Found : C, 68.92, H, 8.76.

(20*R*)-3β,5β,14α,20-trihydroxy-24-norchol-7-en-22-yn-6-one (2) and (20*R*)-3β,5α,14α,20-trihydroxy-24-norchol-7-en-22-yn-6-one (15)

Compound **14** was added to a mixture of K_2CO_3 (10 eq., 142 mg, 1.02 mmol.) in MeOH/H_2O 1/1 (2 ml). The reaction was refluxed for 10 mn and then cooled down to 0°C, to be extracted with AcOEt, thus giving a mixture of the 5α and 5β isomers **15** and **2** (36 mg, 95 %).

15 : R_f=0.53 (AcOEt/MeOH 9/1); **Mp** 251-253°C; **IR** ν (cm^{-1}) : 3444w, 2968s, 2941s, 1652s, 1628s, 1390s, 1252s, 1128s, 1066s; **^{1}H-NMR** (MeOD) δ : 0.85 (s, 3H, H-18); 0.97 (s, 3H, H-19); 1.48 (s, 3H, H-21); 2.74 (ddd, 1H, J_1=7.5 Hz, J_2=3.0 Hz, J_3=2.6 Hz, H-9), 2.83 (s, 1H, H-23), 3.54 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.82 (d, 1H, J=2.7 Hz, H-7); **MS** m/z : 372 (100%, M^+), 357 (11), 354 (41), 346 (13), 344 (22), 339 (37), 328 (12), 321 (25); **UV** λ_{max} (ε) : 235 (5530); **Microanalysis** : calc for $C_{23}H_{32}O_4$ (372.51) : C, 74.16, H, 8.66. Found : C, 74.10, H, 8.72.

3β-Acetoxypregna-5,7-dien-20-one (16)

Dry NMO (1.5 eq., 98 mg, 0.84 mmol., which was dried prior to use under vacuum at 90°C for 4h) was dissolved in dry CH_2Cl_2 (5 ml) with 4Å molecular sieve and was stirred at r. t. for 10 mn. Compound **10** (200 mg, 0.56 mmol.) was then added, followed by a catalytical amount of TPAP (0.5 mol %, 1 mg, 3 μmol.). After 30 mn, the reaction mixture was diluted with CH_2Cl_2 (25 ml), washed with saturated aqueous sodium sulfite, with brine and with saturated aqueous $CuSO_4$. The organic layer was dried over $MgSO_4$ and evaporated to dryness. After chromatography through silica eluted with hex/AcOEt 9/1, compound **16** was isolated with 92% yield.

16 :R_f=0.64 (Hex/AcOEt 5/5); **Mp** 112-114°C; $[\alpha]_D^{23}$=+13.2 (c=0.5, $CHCl_3$); **^{1}H-NMR** δ : 0.68 (s, 3H, H-18), 1.01 (s, 3H, H-19), 2.03 (s, 3H, H-Ac), 2.12 (s, 3H, H-21), 4.69 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.38 (m, 1H, $w_{1/2}$=20 Hz, H-7), 5.56 (m, 1H, $w_{1/2}$=15 Hz, H-6); **Microanalysis** : calc for $C_{23}H_{32}O_3$ (356.51) : C, 77.49, H, 9.05. Found : C, 77.07, H, 9.18.

(20*R*),23-Trimethylsilyl-3β,20-dihydroxy-24-norchol-5,7-dien-22-yn (17)

The same procedure as for **14** was used (105 mg, 82 %)

17 : Mp 135-137°C; $[\alpha]_D^{23}$=-7.4 (c=0.5, $CHCl_3$); **IR** ν (cm^{-1}) : 3484w, 2944s, 2160s, 1712s, 1260s, 840s; **^{1}H-NMR** δ : 0.15 (s, 9H, H-$SiMe_3$), 0.89 (s, 3H, H-18), 0.97 (s, 3H, H-19), 1.47 (s, 3H, H-21), 2.03 (s, 3H, H-Ac), 4.71 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.39 (m, 1H, $w_{1/2}$=12.5 Hz, H-7), 5.57 (m, 1H, $w_{1/2}$=10 Hz, H-6); **MS** m/z : 454 (M^+, 3), 392 (94), 300 (71), 251 (100%), 105 (44); **Microanalysis** : calc for $C_{28}H_{42}O_3Si$ (454.73) : C, 73.96, H, 9.31. Found : C, 73.82, H, 9.42.

(20*R*),3β,20-Dihydroxy-24-norchol-5,7-dien-22-yne (18)

The same procedure as for **15** was used (30 mg, 80 %)

18: R_f=0.49 (Hex/AcOEt 4/6); **Mp** 134-136°C; $[\alpha]_D^{25}$=-36 (c=1, $CHCl_3$); **IR** ν (cm^{-1}) : 3356w, 2942s, 2056s, 1724s, 1448s, 1286s, 1064s; **^{1}H-NMR** δ : 0.90 (s, 3H, H-18), 0.95 (s, 3H, H-19), 1.52 (s, 3H H-21), 2.52 (s, 1H, H-23), 3.64 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.41 (m, 1H, $w_{1/2}$=12.5 Hz, H-7), 5.58 (m, 1H, $w_{1/2}$=10 Hz, H-6); **Microanalysis** : calc for $C_{23}H_{32}O_2$ (340.51) : C, 81.13, H, 9.47. Found : C, 80.92, H, 9.53.

(20*R*),23-trimethylsilyl-3β,20-dihydroxy-24-norchol-7-en-22-yn-6-one (19)

The same procedure as for **14** was used, with 3 eq. acetylide (100 mg, 79 %)

19 : **Mp** 122-125°C; $[\alpha]_D^{23}$=-3 (c=0.5, $CHCl_3$); **IR** ν (cm^{-1}) : 3460w, 2956s, 2162s, 1712s, 1674s, 1250s, 1036s, 840s; **^{1}H-NMR** δ : 0.14 (s, 9H, H-$SiMe_3$), 0.87 (s, 3H, H-18), 0.89 (s, 3H, H-19), 1.49 (s, 3H, H-21), 4.72 (m, 1H, $w_{1/2}$=25 Hz, H-3), 5.73 (bs, 1H, H-7); **MS** m/z : 469 (15), 467 (100 %), 465 (2), 429 (14), 405 (3), 387 (34); **Microanalysis** : calc for $C_{28}H_{42}O_4Si$ (470.73) : C, 71.44, H, 8.99. Found : C, 71.30, H, 9.10.

(20*R*),3β,5β,20-Dihydroxy-24-norchol-7-en-22-yn-6-one (20) and 3β,5α,17β-Dihydroxy-21,24-dinorchol-7-en-22-yn-6-one (21)

The same procedure as for **15** was used (31 mg, 83 %)

21 : R_f=0.21 (Hex/AcOEt 3/7); **Mp** 217-220°C; $[\alpha]_D^{23}$=-3 (c=0.5, $CHCl_3$); **IR** ν (cm^{-1}) : 3424w, 3312s, 1722s, 1664s, 1366s, 1064s; **^{1}H-NMR** δ : 0.89 (s, 3H, H-18), 0.97 (s, 3H, H-19), 1.55 (s, 3H, H-21), 2.68 (s, 1H, H-23), 3.57 (m, 1H, $w_{1/2}$=30 Hz, H-3), 6.18 (s, 1H, H-7); **MS** m/z : 356 (37, M^+), 330 (17), 287 (100), 269 (87), 255 (55); **Microanalysis** : calc for $C_{23}H_{32}O_3$ (356.51) : C, 77.49, H, 9.05. Found : C, 77.09, H, 9.08.

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