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ACETYLENIC CHOLESTERYL DERIVATIVES AS IRREVERSIBLE INHIBITORS OF ECDYSONE BIOSYNTHESIS.

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Abstract. - Two series of acetylenic derivatives of cholesterol were synthesized from stigmasterol and 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. Two of these compounds (15a, 15f) inhibit the synthesis of ecdysone in follicular cells under in vitro conditions. The inhibition is selective of the C-22 hydroxylase system.

Arthropods occupy most ecological niches in the environment. They are believed to represent more than 90% of the species of the animal kingdom. A major characteristic of their development is the process of regular shedding of the old cuticle and synthesis of a new cuticle. This process, known as molt, is controlled by the steroid hormone ecdysone (4) (Gk. ecdysis = molt). Ecdysone is synthesized during postembryonic development (and possibly during the late stages of embryogenesis) in endocrine glands referred to as "prothoracic glands" in insects and "Y organs" in crustaceans. Reproductively competent female insects also synthesize ecdysone in their ovaries [1,2].

In addition to triggering the cycles of cuticulogenesis, ecdysone is involved in the control of a variety of developmental and reproductive processes, although our information is still fragmentary in this respect [3]. In order to get a better understanding of the functions of ecdysone, and keeping in mind possible practical applications, we have set up a program for the syntheses of selective inhibitors of ecdysone biosynthesis. Suicide substrate inhibitors seemed to be suitable for these studies because of their specific and irreversible action. A suicide substrate is a molecule homologous to the natural substrate of an enzyme and capable of binding irreversibly to this enzyme as a consequence of the enzymatic changes [4-6]; whereby the catalytic activity of the enzyme is destroyed.

Cholesterol is known to be the first C_{27} precursor in the biosynthesis of ecdysone, but until now only the last steps of the pathway of this biosynthesis have been well established. They consist of a sequence of hydroxylations at C-25, C-22 and finally at C-2 [7,8].

As a first approach, we have chosen to synthesize compounds which are able to inhibit irreversibly the hydroxylation at C-22. Recent results have indicated that the enzyme responsible for the C-22 hydroxylation is a cytochrome P-450 dependent monooxygenase[9]. The acetylenic function is known to induce inactivation of this type of enzymes [10,11].

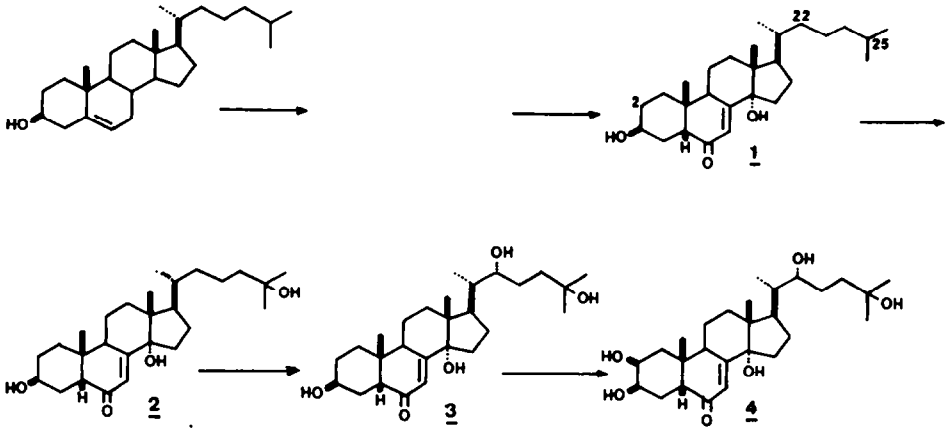


Figure 1 : Ecdysone biosynthesis.

From the chemical standpoint, excellent information is available on the side-chain of steroids [12,13]. We have undertaken the syntheses of sterols having the nucleus of cholesterol and bearing a side chain with a triple bond at C-22. We expected molecules structurally similar to cholesterol to enter more easily the biosynthetic tissue.

In this paper, we describe the syntheses of two series of cholesteryl derivatives and we present several biological results.

CHEMICAL RESULTS.

The syntheses of compounds 8 and 11 were achieved as shown in scheme A. The dichlorovinylderivative 5 was prepared from stigmasterol, without epimerization at C-20, according to Salmond et al.[14]. Treatment with butyllithium converted 5 to the lithium acetylide 6 , the common intermediate.

7(a-d) were obtained quantitatively from 6 by hydrolysis or by alkylations (table A). Alkylation of 6, except in the case of 7b with methyl iodide, was too sluggish in pure THF. This problem was solved by using hexamethylphosphotriamide [15,16].

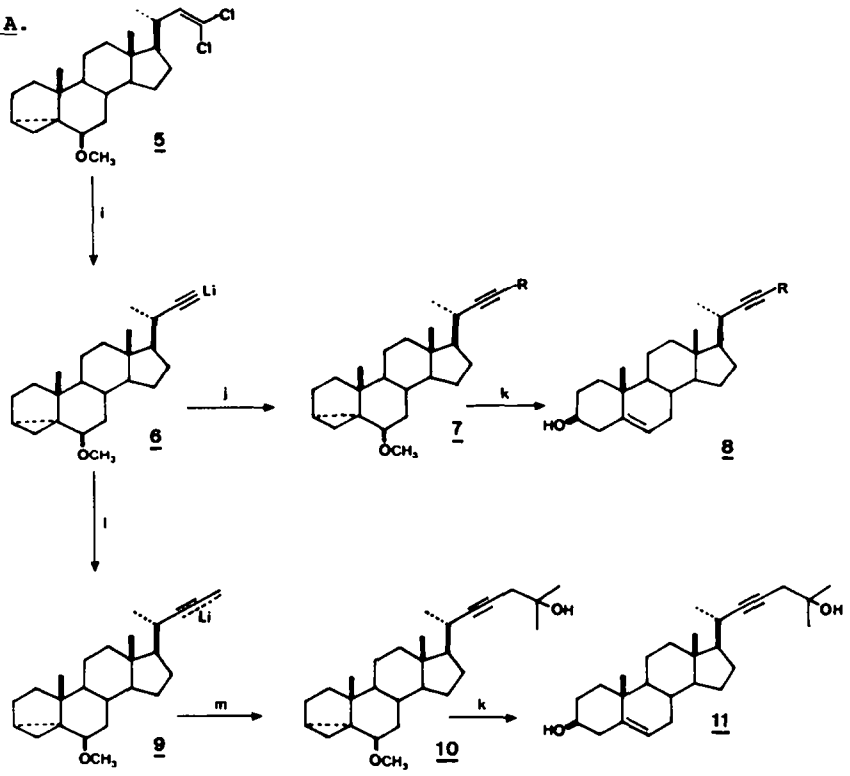
Reaction of 6 with i-butyl bromide gave 2 products. The expected 7e was obtained only in 33% yield, the major reaction, with elimination of HBr in the electrophile [17], furnished 7a (67%). However, the yield of 7e has been improved up to 58% by using the following sequence of operations: after the initial alkylation, 6 was regenerated in situ with another equivalent of BuLi and subsequently alkylated with i-butyl bromide to yield 7e .

Condensation of 6 with an epoxide, 2-methyl-propan-1,2-oxide, as described in Salmond's procedure [14], gave a mixture of products. An alternative route was explored (scheme A): methyl iodide was added to 6 to form 7b, which was directly converted to the propargylanion 9 by BuLi [18,19]. The ambident anion 9 was treated with acetone to form regioselectively 10 (88%). This one-pot procedure furnished 10 with a better yield than that reported earlier [14].

Acid catalysed hydrolyses of the i-methyl ethers 7 and 10 completed subsequently the syntheses of 8 and 11 (84 to 95%).

The acetylenic derivatives of type 15 were synthesized stereospecifically as shown in scheme B. Their precursors 14 were obtained by reacting the

SCHEME A.



a, R=H; b, R=Me; c, R=Et, d, R=Pr
e, R=i-Bu.

i) 2 BuLi, j) R-X, k) p-TsOH cat., l) MeI, m) Acetone

Table A.

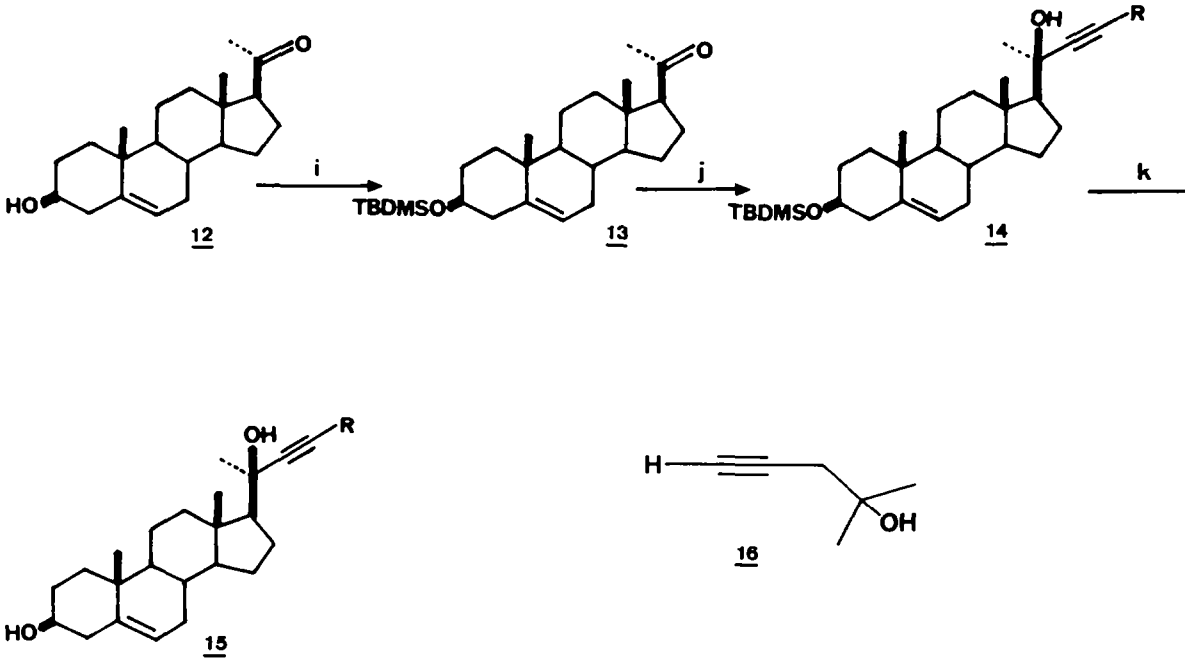
Compounds	Electrophiles	Conversion in %
7a	H_2O	quant.
7b	MeI	quant.
7c	EtBr	quant.
7d	PrBr	quant.
7e	i-BuBr	33
10	acetone	88

corresponding lithium acetylide (3 eq) with the protected pregnenolone 13 [20,21] (Table B). Addition of 13 to the lithiated dianion [22] of carbinol 16 [23] furnished 14f (53%) and the starting material 13 (37%). In situ generation of the trimethylsilyl ether of 16, followed by treatment with methyllithium and 13 gave 14f in 92% yield after acid hydrolysis and purification.

The nucleophilic attack of the lithium acetylide on 13 was stereospecific [12], giving the (20R) stereoisomer with only a trace of the more polar isomer (TLC, NMR). The (20R) and (20S) isomers have quite distinct NMR spectra: C-18 methyl protons appear at about 0.98 ppm in the case of (20R) and at about 0.89 ppm in the case of (20S) isomer.

Fluoride ions (Bu_4NF) removed quantitatively the t-butyl- dimethylsilyl ether moiety in 14, to yield 15 (96 to 98%).

SCHEME B.



i) TBDMSCl, $(i\text{-}Pr)_2NEt$; j) LiC≡CR, -78°C; k) Bu_4NF

Table B.

Compounds 14	R	Isolated yield *in* %
a	$SiMe_3$	93
b	Me	92
c	Et	94
d	Pr	92
e	i-Bu	96
f	$CH_2C(CH_3)_2OH$	92

(15 has respectively the same R as 14 except 15a, R=H)

BIOLOGICAL RESULTS.

All the biological tests were performed on the migratory locust, *Locusta migratoria*. In this insect, ecdysone is produced in the prothoracic glands during postembryonic development and in the follicular cells of the ovaries of adult vitellogenic females [24].

The molecules, which we have synthesized (8, 11, 15), have been tested *in vitro* on the prothoracic glands of the larvae. Three of these compounds (8a, 15a, 15f) induce a decrease in the synthesis of ecdysone. The depressory effect on the biosynthesis was 30% in the presence of 8a and of 60% in the presence of 15a and 15f, at a concentration of 10^{-4}M, and it was dose-dependent. The inhibition of synthesis of ecdysone by these three inhibitors was not suppressed by rinsing the glands, followed by re-incubation in a fresh medium in the absence of inhibitor; the effect was therefore most probably irreversible. Concomitant addition of each of 15a and 15f and tritiated ecdysone precursors

with prothoracic glands indicated that these compounds inhibited selectively the C-22 hydroxylase system. The details of these biological results are described in another publication [25].

In the present paper, we shall focus on the activity of 15a and 15f on ecdysone biosynthesis in the follicular cells of the adult female. To appreciate the inhibitory capacity of these molecules, we have studied their influence on the conversion of a labelled precursor of ecdysone, 2,22-dideoxyecdysone 2 [26], in the follicular cells.

Ovaries were excised from adult vitellogenic females and the oocytes (60-100) were divided into three equivalent batches which were separately incubated with equal quantities of labelled 2,22-dideoxyecdysone. In addition, to one batch we added 15a, and to a second 15f, the third serving as a control. The labelled metabolites of the three incubations were extracted, separated by HPLC and identified by co-elution with reference substances as ecdysone (4), 2-deoxyecdysone (3) and 22-deoxyecdysone.

Table C presents the conversion rates observed during the incubations. In comparison to the control, there is a 30% decrease in the conversion of precursor 2 to ecdysone in the presence of 15a and of 66% in the presence of 15f. Hence these two molecules have an inhibitory effect on the biosynthesis of ecdysone in the follicular cells *in vitro*. The level of conversion of 2 to 22-deoxyecdysone is similar in all three cases. The C-2 hydroxylation is therefore not affected. In comparison to the control, there is a 42% decrease in the conversion of 2 to 2-deoxyecdysone in the presence of 15a and of 78% in the presence of 15f. The C-22 hydroxylation system is strongly depressed. These results indicate that 15a and 15f inhibit the C-22 hydroxylation during ecdysone biosynthesis in the follicular cells.

Conversion products %	Unconverted 2,22-dideoxy-ecdysone (2)	22-Deoxy-ecdysone	2-Deoxy-ecdysone (3)	Ecdysone (4)
Control	54	7.5	5.9	3.6
Inhibitor 15a	61	7.6	3.4	2.5
Inhibitor 15f	67	7.8	1.3	1.2

Table C.

The oocytes of five vitellogenic females (when the ecdysteroids synthesis is maximum) were dissected and divided equally in three tubes. Each tube contained 1 ml of Landureau's medium and 270 kBq of tritiated 2,22-dideoxyecdysone (2) (specific activity 22 TBq/mmole). The first tube was the control. In the two others, 15a and 15f were added to react at a final concentration of 10^{-4}M. After 16 h of incubation, the labelled ecdysteroids were extracted and separated by reversed phase HPLC on a C-18 column and identified by co-elution with reference substances. The radioactivity of each labelled metabolite was measured by scintillation counting. The rate of conversion in each metabolite was calculated in comparison with the total of labelling and expressed in percentage. In this table we have not taken into account the minor unidentified conversion products.

CONCLUSION.

The reduction of the concentration of circulating ecdysteroids in insects is a challenge which has been taken up by many authors [27]. The techniques adopted aim at suppressing the first precursor of ecdysone biosynthesis, i.e. cholesterol. Insects are unable to synthesize de novo cholesterol which they take up from the diet, except for phytophageous insects, which rely on dealkylation of phytosterols for their cholesterol supply. Prestwich and coll. [28,29] and Ikekawa and coll. [30] have developed inhibitors of the dealkylation process of phytosterols. An indirect approach has been used by Costet et al. [31]. Plants treated with a systemic fungicide of the morpholin family replace their Δ^5sterols to a large extent by cyclopropylsterols and Δ^8sterols as a result of the selective inhibition of two enzymes of the biosynthetic pathway of plant sterols. When reared on experimental plants, insects were shown to be unable to produce normal levels of ecdysone, as a direct result of the deficiences of Δ^5sterols in their diet.

These approaches used to lower the normal levels of cholesterol have some limitations when the biological roles of ecdysone are to be investigated. Indeed, as cholesterol is an essential contituent of eukaryotic membranes, its depletion can affect non-specific processes unrelated to any hormonal control by ecdysone. These drawbacks could be overcome by the synthesis of specific inhibitors of the last stages of ecdysone biosynthesis. Several of the molecules which we have now synthesized are of potential interest as they selectively and irreversibly inhibit C-22 hydroxylation. We are developping our efforts to synthesize related compounds effective on this and other enzymes of ecdysone biosynthesis, at lower concentrations. In particular we will synthesize compounds having the ecdysone ring system (instead of the bare cholesterol ring system as in the present paper), and bearing an acetylenic group at C-22.

EXPERIMENTAL.

Melting points were measured on a Reichert microscope and are uncorrected. (α)D were measured on a Perkin-Elmer 141 polarimeter in $CHCl_3$. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer and a Pye Unicam SP3-300S infrared spectrophotometer Philips. Absorptions are given in cm-1. NMR spectra were recorded on a Brucker SY (200MHz) apparatus with $CHCl_3$ (7.27 ppm) or CH_2Cl_2 (5.35 ppm) as internal standard for ^1H NMR and $CDCl_3$ (76.9 ppm) or CD_2Cl_2 (53.6 ppm) as internal standard for ^{13}C NMR. The chemical shifts are reported in ppm downfield from TMS. MS were measured on a Thomson THN 208 and a LKB 9000S apparatus by direct introduction using an ionization potential of 70 ev.. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyses of the CNRS. GLC analysis were carried out on a Girdel Chromatograph 300 fitted with a flame ionization detector. A packed glass column (OV1, 3%) (210 cm x 0.2 cm) was used. TLC were run on pre-coated plates of silica gel 60F254 (Merck) and silica gel (40 - 63 µm, Merck) was used for column chromatography.

Conversions given in the table A were determined employing the following conditions of GLC: injector and detector temperatures were respectively 200 and 300°C, GLC was run from 200 to 290°C (5°C/min) with a flow of 25 ml/min (He).

General procedure for the preparation of lithium acetylide (6).

BuLi in hexane (2.2 eq, 820 µl, 1.2 mmol.) was added dropwise to a stirred solution (-78°C) of the dichlorovinylpregnane [14] (5) (230 mg, 0.56 mmol.) in dry THF (20 ml) under argon. Stirring was continued for 1h at -78°C and another 2h at R.T. This solution was used for alkylation.

6β-Methoxy-3α,5-cyclo-24-nor-5α-chol-22-yne (7a).

Aqueous NaCl was added to the solution of lithium acetylide 6 in THF (0.56 mmol., 20 ml). The mixture was extracted with ether, washed with 6N HCl, water,

then dried on Na_2SO_4, filtered over silica gel and evaporated to give 7a (190mg,quant.).

mp= 74-76°C (lit. [14], 74-76°C). (α)D = +54° (c = 0.8). IR: 3320 (m), 3080 (w), 2110 (w), 1110 (s). ^1H NMR ($CDCl_3$): 0.43 (1H, dd : J1 = 8 Hz and J2 = 5 Hz, H-4), 0.65 (1H, t : J = 5 Hz, H-4), 0.74 (3H, s, H-18), 1.02 (3H, s, H-19), 1.22 (3H, d : J = 7 Hz, H-21), 2.01 (1H, d: J = 2.5 Hz, H-23), 2.43 (1H, m : w1/2 = 20 Hz, H-20), 2.77 (1H, m : w1/2 = 6.5Hz, H-6), 3.32 (3H, s, MeO). ^{13}C NMR in table 1. MS m/e: 340 (M^+, 100), 325 (44), 308 (50), 285 (54), 255 (31).

General Procedure for the deprotection of the i-ether group.

A solution of 7 (56 mmol.) and p-TsOH acid (catalytic amount) in dioxan/water (20 ml, 7/3) was stirred at 80°C for 3h, and then poured into an aqueous solution of NaCl. Extraction with ether, and chromatography over silica gel (toluen-AcOEt, 5%) gave 8.

24-Nor chol-5-en-22-yn-3β-ol (8a).

7a (190 mg) was hydrolysed according to the general procedure to give 8a (163mg, 89%).

mp= 145-146°C (EtOH). (α)D = -50° (c = 3.5). IR: 3620 (m), 3490 (bm), 3280 (s), 3260 (s), 3040 (w), 1670 (w), 1070 (m). ^1H NMR ($CDCl_3$): 0.71 (3H, s, H-18), 1.01(3H, s, H-19), 1.23 (3H, d : J = 7 Hz, H-21), 2.03 (1H, d : J = 2 Hz, H-23), 2.48 (1H, m : w1/2 = 18 Hz, H-20), 3.54 (1H, m : w1/2 = 25 Hz, H-3), 5.35 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 326(M^+, 100), 311 (13), 308 (29), 293 (12), 273 (15), 255 (11), 241 (19), 215 (12). Found: C, 84.65; H, 10.58. Calc for $C_{23}H_{34}O$ (326.5) : C, 84.60; H, 10.50.

Chol-5-en-22-yn-3β-ol (8b)from 6.

MeI (75 µl, 12 mmol.) was added dropwise to a stirred and cooled solution (-10°C) of lithium acetylide 6 in dry THF (20 ml, 0.56 mmol.) under argon. After stirring for 3h at R.T. the usual work-up gave the crude product 7b and after hydrolysis, according to the general procedure, 8b was obtained (172 mg, 90%).

mp= 104-105°C (methanol). (α)D = -46° (c = 2.8). IR: 3540 (bs), 3040 (w), 1670 (w), 1070 (m). ^1H NMR ($CDCl_3$): 0.69 (3H, s, H-18), 1.01 (3H, s, H-19), 1.17 (3H, d : J = 7 Hz, H-21), 1.77 (3H, d : J = 2 Hz, H-24), 2.39 (1H, m : w1/2 = 20 Hz,H-20), 3.55 (1H, m : w1/2 = 25 Hz, H-3), 5.36 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 340 (M+, 100), 325 (14), 322 (17), 307 (14), 273 (20), 255 (35), 229 (17). Found :C, 82.28; H, 10.79. Calc. for $C_{24}H_{36}O$, 0.5 H_2O (349.54) : C, 82.46; H, 10.67.

26,27-Dinor cholest-5-en-22-yn-3β-ol (8c) from 6.

EtBr (126 µl, 1.7 mmol.) and dry HMPA (2 ml) were added dropwise to a stirred and cooled solution (-10°C) of lithium acetylide 6 in dry THF (0.56 mmol., 20 ml). After stirring for 1.5 h at R.T., 8c was isolated in the similar way as described for 8b (178 mg, 90%).

mp= 112-113°C (acetone). (α)D = -44° (c = 0.9). IR: 3440 (bm), 3040 (w), 1670 (w), 1060 (s). ^1H NMR ($CDCl_3$): 0.70 (3H, s, H-18), 1.01 (3H, s, H-19), 1.10 (3H, t : J = 7.5 Hz, H-25), 1.17 (3H, d : J = 7 Hz, H-21), 2.34 (2H, qd : J1 = 7.5 Hz and J2 = 2 Hz, H-24), 2.42 (1H, m : w1/2 = 20 Hz, H-20), 3.55 (1H, m : w1/2 = 25 Hz, H-3), 5.35 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 354 (M+, 100), 339 (16), 336 (18), 321 (12), 273 (21), 269 (14), 255 (32), 243 (21). Found: C, 84.80; H, 11.08. Calc. for $C_{25}H_{38}O$ (354.6) : C, 84.69; H, 10.80.

27-Nor cholest-5-en-22-yn-3β-ol (8d) from 6.

8d (173 mg, 84%) was prepared in the same manner as described for the synthesis of 8b, using PrBr (155 µl, 1.7 mmol.), dry HMPA (2 ml) and lithium acetylide 6 in THF (0.56 mmoles, 20 ml).

mp= 107-108°C and 114-115°C (MeOH). $(\alpha)_D$ = -43° (c = 1.3). IR: 3440 (bm), 3040 (w), 1670 (w), 1070 (s). ^1H NMR ($CDCl_3$): 0.69 (3H, s, H-18), 0.96 (3H, t : J = 7.5 Hz, H-26), 1.00 (3H, s, H-19), 1.17 (3H, d : J = 7 Hz, H-21), 2.11 (2H, td : J_1 = 7 Hz and J_2 = 2 Hz, H-24), 2.43 (1H, m : $w_{1/2}$ = 20 Hz, H-20), 3.52 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.35 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e:368 (M+, 100), 353 (12), 350 (18), 335 (13), 283 (15), 273 (19), 257 (23), 255 (28). Found: C, 84.83; H, 11.10. Calc. for $C_{26}H_{40}O$ (368.58) : C, 84.71; H, 10.94.

Cholest-5-en-22-yn-3β-ol (8e) from 6.

i-BuBr (130 µl, 1.2 mmol.) and dry HMPA (2 ml) were added dropwise to a stirred and cooled solution (-10°C) of lithium acetylide 6 in dry THF (0.56 mmol., 20 ml) under Ar. After 5h at R.T., the solution was cooled again at -78°C and BuLi in hexane (1.2 ml, 1.2 mmol.) was introduced dropwise. The reaction temperature was raised to -10°C during 25 min and another quantity of i-BuBr (130 µl, 1.2 mmol.) was introduced dropwise. The stirring was continued for further 3h at R.T. Usual work-up and chromatography over silica gel

(hexane-Et_2O 2%) gave 7e, which was hydrolysed to yield 8e (94 mg, 44%).
mp= 110-111°C (methanol). $(\alpha)_D$ = -40° (c = 0.8). IR: 3420 (bm), 3040 (w), 1670 (w), 1070 (s). ^1H NMR ($CDCl_3$): 0.70 (3H, s, H-18), 0.95 (6H, d : J = 6.5 Hz, H-26 and H-27), 1.01 (3H, s, H-19), 1.18 (3H, d : J = 7 Hz, H-21), 2.03 (2H, dd : J_1 = 6.5 Hz and J_2 = 2 Hz H-24), 2.44 (1H, m : $w_{1/2}$ = 20 Hz, H-20), 3.55 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.35 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 382 (M+, 100), 367 (11), 364 (14), 349 (7), 297 (10), 273 (18), 271 (14), 255 (17). Found: C, 82.91; H, 11.33. Calc. for $C_{27}H_{42}O$, 0.5 H_2O (391.61) : C, 82.80; H, 11.07.

Cholest-5-en-22-yn-3β,25-diol (11) from 6.

MeI (30 µl, 0.48 mmol.) was added dropwise to a stirred and cooled solution (-10°C) of lithium acetylide 6 in dry THF (0.37 mmol, 10 ml). After stirring for 3h at R.T., the solution was cooled to 0°C and BuLi in hexane (650 µl, 0.91 mmol.) was introduced dropwise. The stirring was continued for 2 h at 0°C, and then the solution was further cooled to -78°C and anhydrous acetone (110 µl, 1.48 mmol.) was added dropwise. The reaction mixture was stirred at -78°C for 1h. Usual work-up and chromatography over silica gel (hexane-Et_2O, 7%) yielded compound 10. Hydrolysis of i-ether group and chromatography over silica gel (hexane-EtOH, 2%) furnished 11 (114 mg, 78%).
mp= 167-168°C (AcOEt). $(\alpha)_D$ = -37.5° (c = 0.5). IR: 3350 (bs), 3040 (w), 1670 (w), 1170 (m), 1070 (s). ^1H NMR ($CDCl_3$): 0.72 (3H, s, H-18), 1.02 (3H, s, H-19), 1.21 (3H, d : J = 7 Hz, H-21), 1.29 (6H, s, H-26 and H-27), 2.34 (2H, d : J = 2 Hz, H-24), 2.48 (1H, m : $w_{1/2}$ = 20 Hz, H-20), 3.54 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.36 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS (50 e.V) m/e: 398 (M+, 10), 383 (5), 380 (4), 340 (92), 325 (11), 322 (24), 307 (14), 272 (100), 255 (44). Found: C, 81.59; H, 10.53. Calc. for $C_{27}H_{42}O_2$ (398.61) : C, 81.35; H, 10.62.

3β-t-Butyldimethylsilyloxy-pregn-5-en-20-one (13).

A solution of TBDMSCl (1.2 eq., 10.5 g, 68 mmol.) in dry CH_2Cl_2 (45 ml) was added to a stirred mixture of pregnenolone (12) (18 g, 57 mmol.) in dry $(i\text{-}Pr)_2EtN$ (15 ml, 86 mmol.) and CH_2Cl_2/DMF (90 ml, 65/25) under Ar. The solution became homogeneous after 20 min. and a white precipitate was formed. The stirring was continued for 2.5h and then 1M aqueous K_2CO_3 (50 ml) was poured into the solution. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined extracts were dried (K_2CO_3) and evaporated to dryness. The crude product (24 g) was crytallyzed in CH_2Cl_2-EtOH to give pure 13 (22.2 g, 90%).
mp= 163-165°C (lit [20] 162-164°C). $(\alpha)_D$ = +21° (c = 6). IR: 3040 (w), 1705 (s), 1250 (s), 1085 (s). ^1H NMR ($CDCl_3$): 0.07 (6H, s, $Si(Me)_2$), 0.64 (3H, s, H-18), 0.90 (9H, s, $SiC(CH_3)_3$), 1.01 (3H, s, H-19), 2.13 (3H, s, H-21), 3.49 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.34 (1H, d : J = 5 Hz, H-6). MS m/e: 430 (M^+, 1), 415 (2), 373 (100), 355 (2), 297 (5), 281 (6). Found: C, 75.19; H, 10.79. Calc. for $C_{27}H_{46}O_2Si$ (430.73) : C, 75.28; H, 10.77.

General procedure for the reaction of protected pregnenolone (13) with 1-Alkynyllithium.

BuLi in hexane (1.3 ml, 3 mmol.) was added dropwise to a stirred and cooled solution (-78°C) of alkyne (3 mmol.) in dry THF (10 ml) under argon. After 1h, a solution of 13 (430 mg, 1 mmol.) in THF (10 ml) was introduced dropwise. The stirring was continued for further 1h at -78°C, then the dry ice bath was removed and Na_2SO_4, 10 H_2O was added. The mixture was filtered over SiO_2 and evaporated to dryness. Medium pressure chromatography over silica gel (P= 0.5 bar, Hexane-Et_2O 7.5%) gave compound 14.

3β-t-Butyldimethylsilyloxy-23-trimethylsilyl-24-nor-chol-5-en-22-yn-20(R)-ol (14a).

14a (492 mg, 93%) was prepared according to the general procedure as described above, using trimethylsilylacetylene (412 µl, 3 mmol.).
mp= 124-125°C (ethanol). $(\alpha)_D$ = -7° (c = 1.5). IR: 3610 (m), 3460 (bm), 3040 (w), 2160 (w), 1260 (s), 1130 (m), 1090 (s). ^1H NMR (CD_2Cl_2): 0.07 (6H, s, $Si(Me)_2$), 0.17 (9H, s, $Si(Me)_3$), 0.90 (9H, s, $SiC(Me)_3$), 0.98 (3H, s, H-18), 1.03 (3H, s, H-19), 1.45 (3H, s, H-21), 3.50 (1H, m : $w_{1/2}$ = 25 Hz, H-3). ^{13}C NMR in table 1. MS (C.I., NH_3), m/e: 546 ($(M+NH_4)^+$, 9), 528 (25), 511 (75), 488 (100), 471 (87). Found: C, 72.85; H, 10.63. Calc. for $C_{32}H_{56}O_2Si_2$ (528.95): C, 72.66, H, 10.67.

3β-t-Butyldimethylsilyloxy-chol-5-en-22-yn-20(R)-ol (14b).

A solution of dichloro-1,1-propene (300 µl, 3.15 mmol.) in dry THF (2 ml) was added dropwise to a stirred an cooled solution (-78°C) of BuLi (2.5 ml, 6 mmol.) in THF (10 ml) under Ar. After 1h at -78°C and another 1h at R.T., the reaction mixture was further cooled (-78°C) and a solution of 13 in THF (10 ml, 1 mmol.) was added dropwise. The stirring was continued for 1h at -78°C. Usual

work-up and chromatography, as described above, gave 14b (432 mg, 92%).
mp= 142-146°C. $(\alpha)_D$ = -21.5° (c = 1.5). IR: 3620 (m), 3440 (bw), 3040 (w), 2260 (w), 1260 (s), 1130 (m), 1090 (s). ^{1}H NMR ($CDCl_3$): 0.06 (6H, s, $Si(Me)_2$), 0.90 (9H, s, $SiC(Me)_3$), 0.96 (3H, s, H-18), 1.01 (3H, s, H-19), 1.46 (3H, s, H-21), 1.83 (3H, s, H-24), 3.50 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.35 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 470 (M^+, 2), 455 (3), 452 (2), 413 (100), 395 (19), 373 (17), 331 (17).

3β-t-Butyldimethylsilyloxy-26,27-dinor-cholest-5-en-22-yn-20(R)-ol (14c).

14c (455mg, 94%) was prepared according to the general procedure, using a solution of 1-butynyllithium in THF (10 ml, 3 mmol.).
mp= 125-129°C. $(\alpha)_D$ = -17° (c = 1.8). IR: 3610 (w), 3490 (bm), 3040 (w), 2240 (w), 1255 (s), 1135 (s), 1100 (s). ^{1}H NMR ($CDCl_3$): 0.06 (6H, s, $Si(Me)_2$), 0.90 (9H, s, $SiC(Me)_3$), 0.97 (3H, s, H-18), 1.01 (3H, s, H-19), 1.12 (3H, t : J = 7.5 Hz, H-25), 1.45 (3H, s, H-21), 2.20 (2H, q : J = 7.5 Hz, H-24), 3.49 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.35 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 484 (M^+, 1), 469 (3), 466 (1), 427 (100), 409 (15), 399 (3), 373 (29), 331 (15).

3β-t-Butyldimethylsilyloxy-27-nor-cholest-5-en-22-yn-20(R)-ol (14d).

14d (460mg, 92%) was prepared according to the general procedure, using 1-pentyne (300 µl, 3 mmol.).
mp= 112-114°C (ethanol). $(\alpha)_D$ = -16° (c = 1.2). IR: 3620 (w), 3500 (bm), 3040 (w), 2235 (w), 1260 (m), 1140 (m), 1100 (s). ^{1}H NMR ($CDCl_3$): 0.06 (6H, s, $Si(Me)_2$), 0.89 (9H, s, $SiC(Me)_3$), 0.96 (3H, t : J = 7 Hz, H-26), 0.97 (3H, s, H-18), 1.01 (3H, s, H-19), 1.46 (3H, s, H-21), 2.17 (2H, t : J = 7 Hz, H-26), 3.51 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.31 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 498 (M^+, 2), 483 (3), 480 (4), 441 (100), 423 (19), 373 (8). Found: C, 77.23; H, 11.07. Calc. for $C_{32}H_{54}O_2Si$ (498.85): C, 77.04; H, 10.91.

3β-t-Butyldimethylsilyloxy-cholest-5-en-22-yn-20(R)-ol (14e).

14e (493 mg, 96%) was prepared according to the general procedure, using 4-methyl-1-pentyne [32] (350 µl, 3 mmol.).
mp= 114-117°C. $(\alpha)_D$ = -15° (c = 2). IR: 3610 (w), 3520 (m), 3040 (w), 2230 (w), 1250 (m), 1130 (m), 1090 (s). ^{1}H NMR ($CDCl_3$): 0.06 (6H, s, $Si(Me)_2$), 0.89 (9H, s, $SiC(Me)_3$), 0.97 (6H, d : J = 6.5 Hz, H-26 and H-27), 0.98 (3H, s, H-18), 1.02 (3H, s, H-19), 1.47 (3H, s, H-21), 2.09 (2H, d : J = 6.5 Hz, H-24), 3.49 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.32 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 512 (M^+, 1), 497 (2), 494 (1), 455 (100), 437 (10), 373 (8), 331 (10).

3β-t-Butyldimethylsilyloxy-cholest-5-en-22-yn-20(R),25-diol (14f).

The carbinol 16 [23] (450 µl, 4 mmol.) was added dropwise to a stirred and cooled mixture (-78°C) of MeLi (2.35 ml, 4 mmol, in ether) in dry THF (10 ml) under Ar. The reaction temperature was raised to -30°C during a 30 min period afterwards trimethylchlorosilane (508 µl, 4 mmol) was introduced dropwise. The stirring was continued for 1h at R.T., and the reaction was further cooled to -78°C. Another quantity of MeLi (2.30 ml, 3.91 mmol) was added dropwise, followed , after stirring for 1h, by a solution of 13 in THF (1 mmol, 10 ml). After 1h, the dry-ice bath was removed and the reaction temperature was raised to 0°C. An aqueous NH_4Cl (20 ml, 6N) was poured into the solution. The mixture was acidified to pH = 1 with HCl (1N) and then was stirred for 4h at R.T. The organic phase was separated and the aqueous phase was extracted with ether. The combined extracts were dried (Na_2SO_4) and evaporated to dryness. Medium pressure chromatography over silica gel (P= 0.2 bar, hexane-Et_2O-EtOH: 700-296-4) yielded compound 14f (487 mg, 92%).
mp= 157-158°C (ethanol). $(\alpha)_D$ = -13° (c = 1.7). IR: 3380 (bs), 3040 (w), 2240 (w), 1250 (s), 1130 (s), 1100 (s). ^{1}H NMR ($CDCl_3$): 0.06 (6H, s, $Si(Me)_2$), 0.89 (9H, s, $SiC(Me)_3$), 0.98 (3H, s, H-18), 1.01 (3H, s, H-19), 1.31 (6H, s, H-26 and H-27), 1.50 (3H, s, H-21), 2.41 (2H, s, H-24), 3.49 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.32 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 528 (M^+, 1), 513 (3), 510 (2), 495 (2), 471 (100), 453 (18), 435 (3), 413 (2), 395 (5), 373 (15), 331 (14). Found: C, 74.78; H, 10.92. Calc. for $C_{33}H_{56}O_3Si$ (528.87): C, 74.94; H, 10.67.

General procedure for the deprotection of silylether compound.

Tetrabutylammonium fluoride (Bu_4NF), (1.5 eq, 1M in THF) was added to a stirred solution of silylether 14 in dry THF (0.5 M). After 24h at R.T., Na_2SO_4, 10 H_2O was added into the solution. The mixture was filtered and evaporated to dryness. The crude compound was chromatographed over silica gel (P= 0.5 bar, hexane-Et_2O 30%) to give compound 15.

24-Nor chol-5-en-22-yn-3β,20(R)-diol (15a).

15a (96%) was prepared according to the general procedure as described

N°C	7a(1)	8a(1)	8b(1)	8c(1)	8d(1)	8e(1)	11(2)	15a(1)	15b(2)	15c(2)	15d(2)	15e(2)	15f(2)	13(1)	14a(1)	14b(1)	14c(1)	14d(1)	14e(2)	14f(2)	N°C
1	33.2	37.3	37.4	37.2	37.2	37.3	37.4	36.9	37.7	37.7	37.6	37.6	37.6	37.4	37.3	37.7	37.3	37.7	37.8	37.6	1
2	24.0'	31.7*	31.7*	31.6*	31.6*	31.6*	31.8*	30.7'	31.9'	32.1'	32.0'	32.0'	32.1'	31.8*	31.8*	32.2*	31.8*	32.2*	32.3*	32.2*	2
3	21.4°	71.8	71.8	71.7	71.7	71.7	71.8	70.8	71.9	72.0	71.9	71.9	71.9	72.5	72.5	72.9	72.5	73.0	73.0	72.9	3
4	13.0	42.7	42.4	42.3	42.2	42.2	42.5	41.4	42.5	42.7	43.2	42.6	42.7	42.8	42.7	43.1	42.8	43.2	43.2	43.1	4
5	35.2	140.8	140.9	140.7	140.7	140.7	141.1	140.6	141.6	141.4	141.3	141.3	141.4	141.5	141.6	142.0	141.6	142.3	142.0	141.9	5
6	82.2	121.6	121.6	121.6	121.6	121.5	121.5	120.9	121.7	121.6	121.6	121.5	121.6	120.7	120.9	121.2	120.9	121.5	121.3	121.2	6
7	34.9	31.9*	31.9*	31.8*	31.8*	31.9*	32.0*	31.4'	32.3'	32.2'	32.2'	32.1'	32.2'	32.0*	32.0*	32.4*	32.0*	32.4*	32.5*	32.4*	7
8	30.3	31.9	31.9	31.7	31.7	31.8	32.0	31.8	31.9	31.8	31.7	31.7	31.8	31.9	31.3	31.7	31.3	31.7	31.8	31.7	8
9	48.0	50.3	50.3	50.1	50.1	50.3	50.4	49.9	50.7	50.6	50.5	50.5	50.6	50.1	50.0	50.5	50.1	50.7	50.7	50.6	9
10	43.3$	36.6	36.6	36.4	36.4	36.6	36.7	36.1	37.0	36.9	36.8	36.8	36.9	36.6	36.5	36.9	36.6	36.9	37.0	36.9	10
11	22.5	21.0	21.0	20.8	20.8	20.9	21.1	20.4	21.3	21.2	21.1°	21.1	21.2	21.0	20.7	21.1	20.8	20.9'	21.2	21.1	11
12	39.4	39.1	39.2	39.0	38.9	39.1	39.2	39.6	40.7	40.7	40.6	40.6	40.6	38.9	40.2	40.6	40.2	40.7	40.7	40.6	12
13	42.6$	42.4	42.3	42.2	42.2	42.2	42.5	42.9	43.6	43.5	43.5	43.4	43.5	43.9	43.2	43.5	43.1	43.5	43.6	43.5	13
14	56.1	56.5	56.6	56.4	56.4	56.5	56.6	56.0	56.9	56.8	56.7	56.7	56.7	56.9	56.2	56.7	56.3	56.8	56.9	56.7	14
15	24.8'	24.2	24.3	24.1	24.1	24.3	24.3	24.6*	24.6*	24.5*	24.5*	24.5*	24.5*	22.9	24.1	24.5	24.1	24.4	24.6	24.5	15
16	27.3	27.2	27.4	27.2	27.2	27.4	27.7	23.7*	25.5*	25.5*	25.5*	25.5*	25.6*	24.4	25.2	25.5	25.2	25.5	25.7	25.6	16
17	55.4	55.4	56.0	55.9	55.9	56.1	56.0	59.8	61.0	60.9	60.8	60.7	60.7	63.7	60.5	60.4	60.4	60.9	60.8	60.6	17
18	12.4	12.2	12.2	12.1	12.1	12.2	12.2	12.6	13.4	13.5	13.5	13.5	13.7	13.1	13.4	13.6	13.4°	13.4°	13.6	13.7	18
19	19.2	19.4	19.4	19.3	19.3	19.4	19.4	18.7	19.6	19.5	19.4	19.4	19.5	19.3	19.3	19.7	19.3	19.4	19.6	19.5	19
20	27.5	27.5	27.7	27.5	27.5	27.7	27.9	70.1	71.3	71.6	71.5	71.5	71.6	209.2	71.4	71.6	71.2	71.6	71.6	71.5	20
21	21.2°	21.4	21.7	21.7	21.7	21.8	21.7	31.0	32.8	33.0	33.1	33.2	33.3	31.3	32.5	33.0	32.8	33.1	33.3	33.2	21
22	89.3	89.3	84.1	84.3	85.1	85.9	88.4	87.3	83.6	87.1	86.0	85.3	87.4		109.7	83.4	87.0	86.0	85.5°	87.3	22
23	68.2	68.5	75.6	81.8	80.2	79.4	76.8	72.9	81.3	83.9	84.8	84.8	82.5		89.7	81.5	83.3	84.8	84.9°	82.5	23
24			3.47	12.4	20.7	28.1	34.6		3.4	12.6	20.9°	28.1	34.7			3.8	12.4	21.1'	28.2	34.7	24
25				14.4	22.5	28.4	69.9			13.9	22.4	28.3	70.2				13.7°	22.3	28.4	70.2	25
26					13.4	21.9	28.5				13.5	22.0	28.8					13.4°	22.1	28.8	26
27						21.9	28.5					22.0	28.8						22.1	28.8	27
1'	56.4													-4.6	-4.7		-4.7	-4.7	-4.5	-4.6	1'
														18.1	18.1	18.1	18.1		18.4	18.3	2'
														25.8	25.9	26.2	25.9	25.8	26.0	25.9	3'
															-0.3						1"

Table 1.

((1) = in $CDCl_3$, (2) = in CD_2Cl_2, ppm from TMS,
', °, $ or * = interchangeable assignement).

Side chain carbons have chemical shifts attributed according to the incrementation method [33,34,35].

above, using nBu_4NF (3 eq) and a solution of 14a in THF.
mp= 215°-217°C (Ethanol). $(\alpha)_D$ = -41° (c = 1). IR: 3640 (w), 3440 (s), 3280 (m), 3040 (w), 2120 (w), 1130 (m), 1045 (m). ^1H NMR ($CDCl_3$): 0.99 (3H, s, H-18), 1.03 (3H, s, H-19), 1.52 (3H, s, H-21), 2.53 (1H, s, H-23), 3.53 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.36 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 342 (M^+, 100), 327 (10), 324 (30), 273 (26), 255 (42), 241 (13). Found: C, 80.43; H, 10.27. Calc. for $C_{23}H_{34}O_2$ (342.50) : C, 80.65; H, 10.01.

Chol-5-en-22-yn-3β,20(R)-diol (15b).

15b (98%) was prepared according to the general procedure.
mp= 188-190°C (methanol). $(\alpha)_D$ = -33° (c = 1.5). IR: 3400 (s), 3040 (w), 2260 (w), 1135 (m), 1055 (s). ^1H NMR ($CDCl_3$): 0.96 (3H, s, H-18), 1.02 (3H, s, H-19), 1.46 (3H, s, H-21), 1.83 (3H, s, H-24), 3.53 (1H, m : $w_{1/2}$ = 25 Hz, H-3), 5.36 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 356 (M^+, 72), 341 (5), 338 (14), 323 (5), 274 (33), 256 (100), 241 (22). Found: C, 80.86; H, 10.44. Calc for $C_{24}H_{36}O_2$ (356.53) : C, 80.85; H, 10.18.

26,27-Dinor-cholest-5-en-22-yn-3β,20(R)-diol (15c).

15c (96%) was prepared according to the general procedure.
mp= 135-136°C (acetone). $(\alpha)_D$ = -30° (c = 0.8). IR: 3440 (s), 3040 (w), 2240 (w), 1140 (m), 1045 (m). ^1H NMR ($CDCl_3$): 0.98 (3H, s, H-18), 1.03 (3H, s, H-19), 1.12 (3H, t : J = 7.5 Hz, H-25), 1.45 (3H, s, H-21) 2.20 (2H, q : J = 7.5 Hz, H-24) 3.53 (1H, m : w1/2 = 25 Hz, H-3), 5.36 (1H, d : J = 5 Hz, H-6). ^{13}C NMR in table 1. MS m/e: 370 (M^+, 29), 355 (4), 352 (7), 274 (79), 256 (100), 241 (21). Found: C, 79.11; H, 10.31. Calc for $C_{25}H_{38}O_2$, 0.5 H_2O (379.56) : C, 79.10;H, 10.36.

27-Nor-cholest-5-en-22-yn-3β,20(R)-diol (15d).

15d (98%) was prepared according to the general procedure.
mp= 130-131°C (acetone). $(\alpha)_D$ = -27° (c = 1). IR: 3330 (s), 3040 (w), 2260 (w), 1140 (m), 1050 (m). ^1H NMR (CD_2Cl_2): 0.97 (3H, s, H-18), 0.97 (3H, t : J = 7 Hz, H-26), 1.02 (3H, s, H-19), 1.42 (3H, s, H-21), 2.17 (2H, t : J = 7 Hz, H-24), 3.50 (1H, m : $w_{1/2}$ = 25 Hz, H-3). ^{13}C NMR in table 1. MS m/e: 384 (M^+, 24), 369 (4), 366 (9), 274 (67), 256 (100), 241 (25). Found: C, 79.39; H, 10.81. Calc. for $C_{26}H_{40}O_2$, 0.5 H_2O (393.59) : C, 79.34; H, 10.50.

Cholest-5-en-22-yn-3β,20(R)-diol (15e).

15e (98%) was prepared according to the general procedure.
mp= 139-140°C (acetone). $(\alpha)_D$ = -22° (c = 1.3). IR: 3430 (s), 3040 (w), 2240 (w), 1135 (m), 1045 (m). ^1H NMR (CD_2Cl_2): 0.98 (6H, d : J = 6.5 Hz, H-26 and H-27), 0.99 (3H, s, H-18), 1.04 (3H, s, H-19), 1.45 (3H, s, H-21), 2.11 (2H, d : J = 6.5 Hz, H-24), 3.50 (1H, m : $w_{1/2}$ = 25 Hz, H-3). ^{13}C NMR in table 1. MS m/e: 398 (M^+, 15), 383 (s), 380 (6), 274 (57), 256 (100), 241 (16). Found: C, 79.65; H, 10.83. Calc. for $C_{27}H_{42}O_2$, 0.5 H_2O (407.61) : C, 79.55; H, 10.63.

Cholest-5-en-22-yn-3β,20(R),25-triol (15f).

15f (96%) was prepared according to the general procedure. Chromatography (P= 0.15 bar; hexane-ether-ethanol : 50-47-3) gave the pure compound.
mp= 220-221°C (AcOEt). $(\alpha)_D$ = -23° (c = 0.8) IR: 3420 (s), 3040 (w), 2260 (w), 1135 (m), 1060 (m). ^1H NMR (CD_2Cl_2): 1.00 (3H, s, H-18), 1.04 (3H, s, H-19), 1.29 (6H, s, H-26 and H-27), 1.49 (3H, s, H-21), 2.40 (2H, s, H-24), 3.49 (1H, m : $w_{1/2}$ = 25 Hz, H-3). ^{13}C NMR in table 1. MS m/e: 414 (M^+, 13), 399 (1), 396 (6), 381 (5), 338 (13), 274 (45), 256 (100), 241 (14). Found:C, 78.23; H, 10.36. Calc. for $C_{27}H_{42}O_3$ (414.61) : C, 78.21; H, 10.21.

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