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A Convenient Stereoselective Synthesis of Castasterone and its Analogues Using Arsenic Ylides

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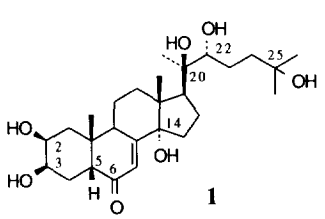
Abstract: (2α,3α,22S)-Trihydroxy-5α-cholestan-6-one **3** and (2α,3α,22R)-trihydroxy-5α-cholestan-6-one **4** have been synthesized with high stereoselectivity. The key step is the coupling of the aldehyde **5** with an arsenic ylide, followed by an *in situ* DIBAH reduction. A second arsenic ylide was used to prepare the key allylic alcohol intermediate **15**, which allows the synthesis of castasterone **2**. Copyright © 1996 Elsevier Science Ltd

Introduction

Brassinosteroids are naturally occurring steroids present in a wide variety of plants and display biological activity on plant growth and development[1]. It has been demonstrated that brassinosteroids counteract the effect of the insect moulting hormone, 20-hydroxyecdysone[2] **1**. The most effective natural compound was castasterone **2**.



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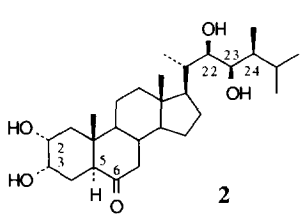

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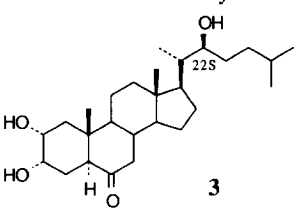
Hence, we are at present exploring the potential of using brassinosteroids as antihormones in the study of the receptor and the mode of action of ecdysteroids[3]. In the first approach, we investigated the influence of the configuration of the C-22 hydroxyl group. A coupling reaction with (3-methyl-2-butenyl) triphenylarsonium tetrafluoroborate and the C-22 aldehyde **5**, allowed the synthesis of molecules **3** and **4** bearing a C-22S and C-22R hydroxyl function respectively and containing the castasterone nucleus. By employing propenyl triphenylarsonium tetrafluoroborate, we have prepared the allylic alcohol **15** a useful intermediate for the synthesis of castasterone **2**. Initial biological studies have been carried out to investigate their antiecdysteroid potentiality.

Results and discussion

The synthesis of C-22 hydroxylated side-chains, via organometallic reagents, has already been reported[4-7] and are non-stereoselective. A synthesis of 22R-hydroxydesmosterol using an arsenic ylide which reacts in a suitable solvent[8] with carbonyl compounds to give an unsaturated epoxide[9], followed by a reduction with the super hydride $LiBHEt_3$, seemed to be more attractive. However, this method suffers from problems of reproducibility.

DIBAH was employed instead of $LiBHEt_3$ for the synthesis of (2α,3α,22S)-trihydroxy-5α-cholestan-6-one **3** and (2α,3α,22R)-trihydroxy-5α-cholestan-6-one **4**. This method gives good yields and affords compound **3** with excellent stereoselectivity.



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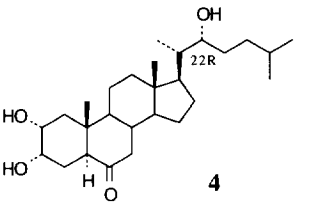

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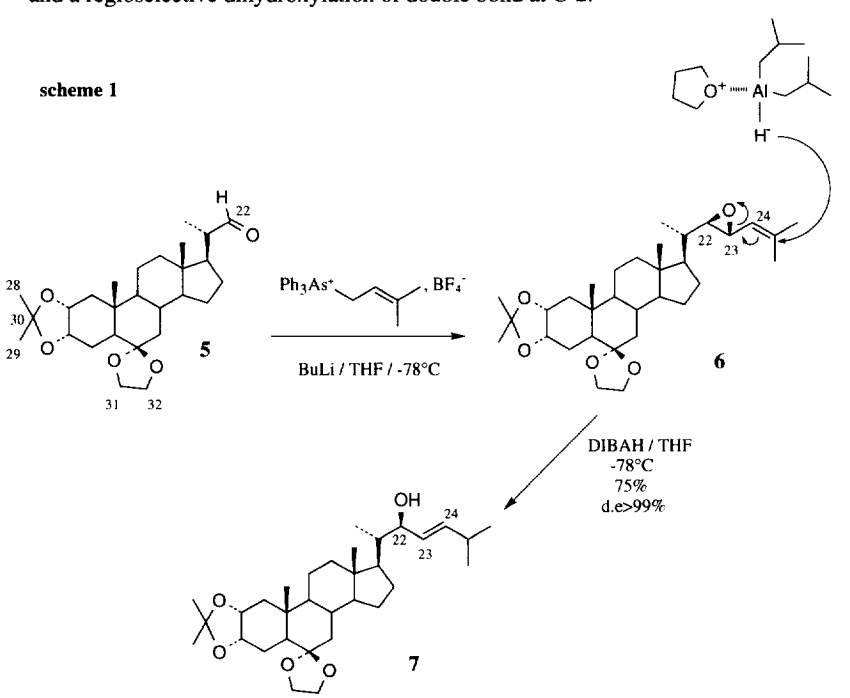
The key step of this synthesis is the condensation of the semistabilized arsenic ylide[10], (3-methyl-2-butenyl)triphenylarsonium tetrafluoroborate[9] with aldehyde **5** to form the α,β-unsaturated epoxide **6** (Scheme 1). Compound **5** was prepared from C-3 protected stigmasterol *via* a regioselective hydroboration of double bond at C-5[11] and a regioselective dihydroxylation of double bond at C-2.

scheme 1



$LiBHEt_3$ allows a specific attack on C-23, but very often does not lead to complete reduction of the epoxide, undoubtedly due to steric reasons. During the $LiBHEt_3$ reduction, the temperature should also be carefully controlled to give an optimum yield. In contrast, DIBAH easily induces complete reduction of the

epoxide **6**. The opening of the epoxide **6** occurs with the participation of the double-bond at C-24 and leads to the unsaturated alcohol **7** in 75% yield and over 99% enantiomeric excess (in less than 1hr and without control of temperature). The (E)-geometry of the newly generated C-23 double bond of **7** was confirmed by the 400MHz ^{1}H-NMR spectum, which showed a characteristic $J_{H\text{-}23,\ H\text{-}24}$= 16Hz.

Reduction of the unsaturated epoxide, 2-ethenyl-2-methyloxirane (Fig. 1), by DIBAH was shown to occur with or without the participation of the double bond according to the solvent[12]. In an apolar solvent the non-complexed aluminium hydride will deliver the necessary hydride ion after formation of donor-acceptor complex **8** with the oxirane moiety through a six membered-ring (Fig. 1).

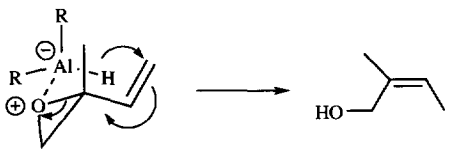


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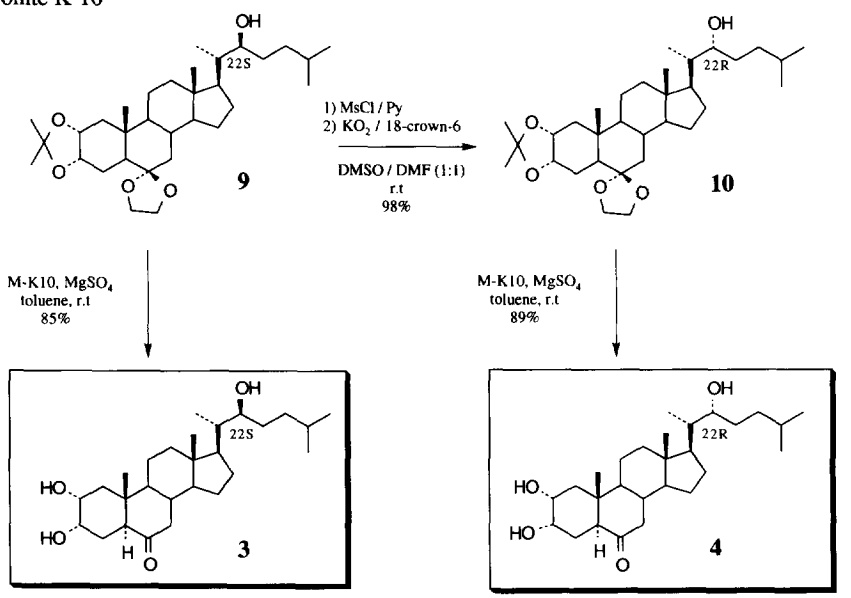
Fig. 1

The coupling reaction with the arsenic ylide and the reduction of epoxide can be carried out in one pot with THF. The bulky complex formed by the DIBAH in THF gives preference to an attack at C-25, due to steric hindrance (Scheme 1). This assumption is supported by the NMR assignment of C-22H and C-23H in the opening of epoxide **12** *(vide infra)*.

Hydrogenation of **7** over 10% Pd/C in the presence of sodium nitrite gave alcohol **9** quantitatively. Compound **9** is inverted into alcohol **10** in 98% yield according to Corey's procedure[13]: the mesylate of alcohol **9** reacts with potassium superoxide in the presence of 18-crown-6 in dimethyl sulphoxide/dimethyl formamide, 1/1. Compounds **9** and **10** were converted to the corresponding alcohols **3** and **4** by Montmorillonite K-10[14]



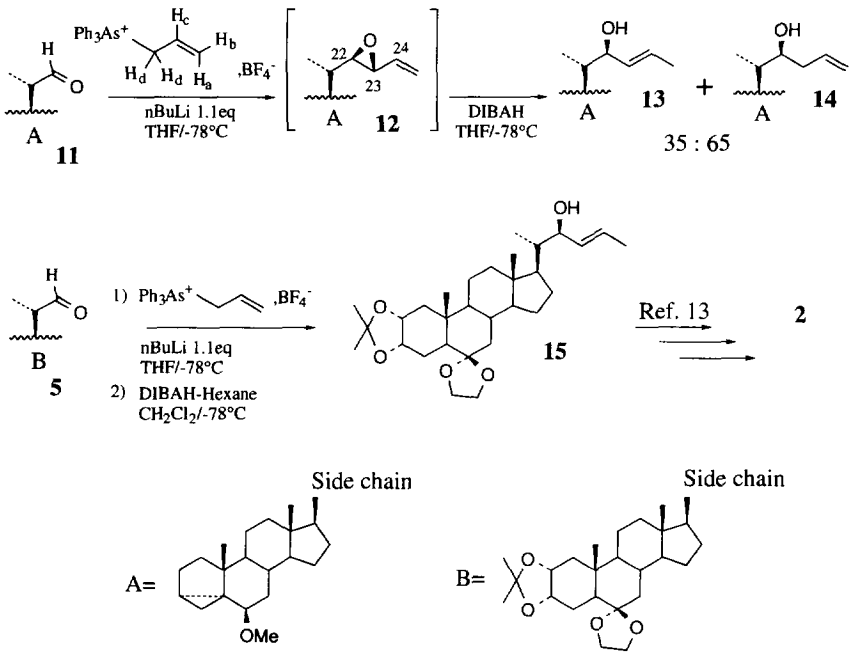
Scheme 2

This arsenic ylide approach was then employed to prepare castasterone **2**, a well known representative of the brassinosteroids.

Aldehyde **11** is reacted with propenyl triphenylarsonium tetrafluoroborate in anhydrous THF and reduced in one pot as described previously. A 35/65 mixture of the unseparable regioisomers **13** and **14**, as determined by ^1H NMR, was obtained (Scheme 3).

The 22-H signal of **14** in the mixture was assigned at 3.7 ppm as a triplet (J= 3.4Hz) while that of the allylic alcohol **13** was assigned at 4.18 ppm as a broad singlet.

The C-23 position of **12** seemed to be more easily accessible by the DIBAH/THF complex than the C-23 position of **6**. So, the complexed aluminium reagent delivers the necessary hydride through a 1,2-oxirane opening as demonstrated by Lenox[10].



Scheme 3

To avoid the 1,2-epoxide opening of **12**, we have evaporated it to dryness and dissolved it in dry dichloromethane. Reduction of **12** in the presence of a 1M DIBAH solution in hexane produced only allylic alcohol **13** in 75% yield and over 99% enantiomeric excess.

The same procedure was used to achieve the synthesis of castasterone **2**. Addition of propenyltriphenylarsonium tetrafluoroborate to the C-22 aldehyde **5** leads to the unsaturated alcohol **15** with a 73% yield and over 99% enantiomeric excess (Scheme 3).

The following steps: Sharpless epoxidation, epoxide opening with i-$Pr_2CuCNLi_2$ and deprotection, have been described previously[15] and are used to afford castasterone **2** whose spectral data are identical with those reported elsewhere (m.p, $[\alpha]_D$, I.R, NMR)[16].

We have evaluated the biological activity of castasterone **2** and brassinosteroid-like compounds **3** and **4** in an *in vitro* assay[17] based on the ecdysteroid-specific responses of the B_{II}(l[2]mbn) tumorous blood cell line of *Drosophila melanogaster*. Compounds **3** and **4** were cytotoxic at 10^{-4} M. Compound **2** did not show any agonistic or antagonistic activities at 10^{-4} M but was toxic at 10^{-3} M.

However, the situation *in vivo* can be very different from that of *in vitro*. Thus, studies are underway to investigate these possibilities.

Experimental

Melting points were measured on a Reichert hot stage microscope and are uncorrected. Optical rotation ($[\alpha]_D$) were measured on a Perkin-Elmer 241 polarimeter in $CHCl_3$. IR spectra were recorded in KBr on a Perkin-Elmer 881 infrared spectrophotometer. NMR spectra were recorded on a Bruker SY(200MHz) and AM (400MHz) spectometer using $CDCl_3$ (δ= 7.26 ppm) as internal standard for ^{1}H-NMR, $CDCl_3$ (δ= 77.0 ppm) as internal standard for ^{13}C-NMR. The chemical shifts are reported in ppm downfield from TMS (*,°,$^+$ = interchangeable assignment). The attribution of the different carbons (C, CH, CH_2 or CH_3) was determined by ^{13}C to ^{1}H polarisation transfer (DEPT). MS were measured on a TRIO 2000 apparatus by direct introduction, or coupled to a GC DB5 column (J. W); an ionization potential of 70 eV was used. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyse of CNRS. TLC were run on pre-coated plates of silica gel 60F254 (Merck), dipped in a solution of vanillin (1g) in EtOH/H_2SO_4 (95/5, 1l) and heated on a hot plate to reveal the compounds. Medium pressure chromatography (P= 0.5-1.1 bar) was conducted on silica gel (40-63 mm, Merck) columns. All solvents were freshly distilled before use; Pyridine was dried by reflux over CaH_2 for several hours; THF was purified by distillation from a dark purple solution of sodium benzophenone dianion. Air- or moisture- sensitive reactions were conducted in flame-dried glassware and under an inert atmosphere. All the commercial reagents were purchased from Aldrich, Janssen, or Lancaster.

(2α,3α,22S)-2,3-Isopropylidene-dioxy-6,6-ethylenedioxy-5α-cholest-23-en-22-ol (7)

(3-Methyl-2-butenyl) triphenylarsonium tetrafluoroborate (6.2 g; 13.4 mmol) was stirred at -78°C in THF (100 ml). To this suspension, a solution of 1.5M BuLi in THF was added dropwise until a slight pale red colour persisted and then BuLi (8.4 ml; 12.7 mmol) was added at once. After 30 min stirring, aldehyde **5** (4.6 g; 10.3 mmol) in THF (20 ml) was added dropwise to the red solution. After 30 min the solution was cooled from room temperature to -78°C and a 1M solution of DIBAH in THF (30 ml; 30 mmol) was added slowly and the mixture was stirred 30 min. AcOEt (50 ml) and a saturated solution of potassium, sodium tartrate were added. The solution was extracted with Et_2O (5x100 ml). The organic layer was washed with brine (100 ml), dried over $MgSO_4$ and concentrated under vacuum. The colourless oil chromatographed on silica gel using hexane/ethyl acetate 95:5 - 9:1 as eluent, and afforded pure compound **7**. Yield: 3.9 g (75%).

Mp: 126-128°C; $[\alpha]_D^{23}$= +25 ($CHCl_3$, c= 1.12); **IR** ν_{max} cm^{-1}: 3489(m), 2945(s), 2874(s), 1461(m), 1377(m), 1051(s); **^{1}H-NMR** 200MHz ($CDCl_3$) δ: 0.66(s, 3H, CH_3-18); 0.81(s, 3H, CH_3-19); 0.86(d, 3H, J= 5.9Hz, CH_3-21); 0.97(d, 6H, J= 2Hz, CH_3-26+CH_3-27); 1.30(s, 3H, acetonide); 1.46(s, 3H, acetonide); 2.27(m, 1H, H-25); 3.7-4(m, 4H, OCH_2CH_2O); 4.02-4.12(m, 1H, H-2); 4.17(d, 1H, J= 4.8Hz, H-22); 4.24(d, 1H, J= 3.7Hz, H-3); 5.53(2 dd, 2H, J= 16Hz and J= 4.28Hz, H-23, H-24); **GCMS** (TMS) m/z: 573(0.3)(M$^+$TMS-15); 171(100.0); 81(18.9); 73(26.1); **Microanalysis**: calcd for $C_{32}H_{52}O_5$ (516.4) C: 74.36; H: 10.15; found C: 74.2; H: 10.0; **^{13}C-NMR** in Table 1

(2α,3α,22S)-2,3-Isopropylidene-dioxy-6,6-ethylenedioxy-5α-cholestan-22-ol (9)

Compounds **7** (90 mg; 0.18 mmol) in 20 ml MeOH:AcOEt (1:1), 40 mg Pd/C 10% and 30 mg $NaNO_2$ was stirred 4 hr in an H_2 atmosphere. Filtration of the catalyst through Celite and evaporation of the solvents affords a colourless solid. Water and CH_2Cl_2 are added. The solution was extracted twice with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated to give alcohol **9** quantitatively.

Mp: 146-148°C; $[\alpha]_D^{23}$= +30 ($CHCl_3$, c= 1.72); **IR** ν_{max} cm^{-1}: 3500(m), 2937(s), 2823(s), 1285(s), 1220(s), 1150(s), 1085(s); **^{1}H-NMR** 200MHz ($CDCl_3$) δ: 0.66(s, 3H, CH_3-18); 0.78(s, 3H, CH_3-19); 0.88(d, 9H, J= 6.5Hz, CH_3-21+CH_3-26+CH_3-27); 1.31(s, 3H, acetonide); 1.47(s, 3H, acetonide); 3.6(dd, 1H, J= 4.4Hz and J= 7.5Hz, H-22); 3.7-4(m, 4H, OCH_2CH_2O); 4.02-4.12(m, 1H, H-2); 4.24(d, 1H, J= 3.7Hz, H-3); **GCMS** (TMS) m/z: 575(2.3)(M$^+$TMS-15); 450(7.7); 355(6.9); 280(9.8); 170(100.0); 75(66.3); 68(78.2); **Microanalysis**: calcd for $C_{32}H_{54}O_5$ (518.4) C: 74.07; H: 10.49; found C: 74.2; H: 10.3; **^{13}C-NMR** in Table 1

(2α,3α,22R)-2,3-Isopropylidene-dioxy-6,6-ethylenedioxy-5α-cholestan-22-ol (10)

To the alcohol **9** (100 mg, 0.193 mmol) dissolved in pyridine (5 ml), mesyl chloride (372 ml, 4.8 mmol) was added at 0°C under stirring. After 5hr, ice and water (20 ml) are added and the solution was extracted with Et_2O (3x10 ml). The ether phase was washed with water (3x5 ml) and saturated brine (5 ml), and evaporated to dryness. The slightly yellow mesylate crystallizes; yield: 110 mg (96%). The mesylate formed was checked by ^{1}H-NMR; the H-22 shifts to 4.8-4.9 ppm and the methyl of CH_3SO_3R appears at 2.99 ppm. The crude mesylate (100 mg, 0.185 mmol) and 18-crown-6 (487 mg, 1.84 mmol) are dissolved in a 1:1 mixture of DMSO and DMF (8 ml). Potassium superoxide (39.2 mg, 0.55 mmol) was added and the solution

was stirred under argon. After 5 hr, the superoxide has almost completely dissolved and the reaction was over. Water (20 ml) and a few drops of 1M solution of HCl are added and the solution was extracted with Et_2O (3x20 ml). The ether phase was washed with water (3x20 ml) and saturated brine (10 ml), dried ($MgSO_4$) and evaporated to dryness. The crude product was chromatographed on silica gel using hexane/ethyl acetate 95:5 - 9:1 as eluent; yield: 98 mg (98%).

Mp: 189-190°C; $[\alpha]_D^{23}$= +39 ($CHCl_3$, c= 1.02); **IR**: ν_{max} cm^{-1} 3440(s), 2957(s), 2880(s), 1383(m), 1715(w), 1056(s), 1040(s), 958(w); **^{1}H-NMR** 200MHz ($CDCl_3$) δ: 0.68(s, 3H, CH_3-18); 0.78(s, 3H, CH_3-19); 0.9(d, 3H, J= 2Hz, CH_3-26); 0.93(d, 3H, J= 2Hz, CH_3-27); 0.95(d, 3H, J= 6.5Hz, CH_3-21); 1.31(s, 3H, acetonide); 1.46(s, 3H, acetonide); 3.62(d, 1H, J= 9.7Hz, H-22); 3.7-4(m, 4H, OCH_2CH_2O); 4.02-4.12(m, 1H, H-2); 4.24(d, 1H, J= 3.7Hz, H-3); The mass spectrum of **10** is identical with that of **9**; **^{13}C-NMR** in Table 1

(2α,3α,22R)-Trihydroxy-5α-cholestan-6-one (4)

To a solution of alcohol **10** (60 mg; 0.116 mmol) dissolved in toluene (10 ml) was added $MgSO_4$ (150 mg) and a few drops of water. To the stirred mixture, Montmorillonit ("clay 10", 220 mg) was added quickly and in one portion. The suspension was stirred vigorously at room temperature for 1 hr. Addition of AcOEt, filtration through Celite and evaporation of the solvents afforded compound **4**. Alcohol **4** was purified by chromatography on silica gel using hexane/ethyl acetate 6:4 - 5:5 - 4:6 as eluent. Yield: 45 mg (89%).

Mp: 207-209°C; $[\alpha]_D^{23}$= +2.3 ($CHCl_3$, c= 2.84); **IR** ν_{max} cm^{-1}: 3399(m), 2940(s), 2862(m), 1707(s), 1462(w), 1383(w); **^{1}H-NMR** 400 MHz ($CDCl_3$) δ: 0.67(s, 3H, CH_3-18); 0.74(s, 3H, CH_3-19); 0.88(d, 3H, J= 6.4Hz, CH_3-26); 0.89(d, 3H, J= 6.4Hz, CH_3-27); 0.91(d, 3H, J= 6.7Hz, CH_3-21); 2.28(dd, 1H, J= 4.3Hz and J= 13.1Hz, H-5); 2.66(dd, 1H, J= 2.7Hz and J= 12.6Hz, H-7); 3.6(d, 1H, J= 9.7Hz, H-22); 3.7-3.76(m, 1H, H-2); 4.02(d, 1H, J= 2.4Hz, H-3); **GCMS** (TMS) m/z: 635(3.6)(M^+3TMS-15); 550(14.8); 460(22.2); 445(9.2); 357(5.4); 331(8.4); 173(100.0); 147(17.3); 83(53.2); 73(55.2); **Microanalysis**: calcd for $C_{27}H_{46}O_4$ (434.4) C: 74.59; H: 10.67; found C: 74.7; H: 10.6; **^{13}C-NMR** in Table 1

(2α,3α,22S)-Trihydroxy-5α-cholestan-6-one (3)

Preparation from **9** according to the method described for the preparation of **4**; yield: 85%.

Mp: 195-197°C, $[\alpha]_D^{23}$= -6 ($CHCl_3$, c= 0.77); **IR** ν_{max} cm^{-1}: 3500(m), 2953(s), 2854(m), 1710(s), 1468(w), 1395(w); **^{1}H-NMR** 400 MHz ($CDCl_3$) δ: 0.67(s, 3H, CH_3-18); 0.75(s, 3H, CH_3-19); 0.9(d, 9H, J= 6.5Hz, CH_3-26+CH_3-27+CH_3-21); 2.28(dd, 1H, J= 4.3Hz and J= 13.1Hz, H-5); 2.66(dd, 1H, J= 2.7Hz and J= 12.6Hz, H-7); 3.61(dd, 1H, J= 4.4Hz and J= 7.5Hz, H-22); 3.7-3.78(m, 1H, H-2); 4.02(d, 1H, J= 2.4Hz, H-3); **MS** (FAB$^+$) m/z: 435(2.4)(MH^+, $C_{27}H_{47}O_4$); 391(13.4); 307(9.5); 289(7.8); 167(24.5); 154(100.0); **Microanalysis**: calcd for $C_{27}H_{46}O_4$ (434.4) C: 74.59; H: 10.67; found C: 74.8; H: 10.9; **^{13}C-NMR** in Table 1

Propenyl triphenyl arsonium tetrafluoroborate

Triphenylarsine (4 g, 13 mmol) was dissoved in CH_3CN (50 ml) and 3-bromopropene(1.65ml, ~1,5 eq) was added under argon. The mixture was protected from light with an aluminium foil. After 3 days, the solution was evaporated to dryness. To the crude bromide in CH_2Cl_2 (80 ml) was added a solution of 150 g $NaBF_4$ in water (200 ml) and the mixture was stirred vigorously for 20 min. The solution was extracted with CH_2Cl_2/hexane, 2:1 (3x80 ml). The organic phase was dried ($MgSO_4$) and evaporated to dryness. The crude salt was triturated in Et_2O to give white crystals. Yield: 4.12 g (73%).

Mp: 138-140°C; **IR** $\nu_{max}$$cm^{-1}$: 2896(w), 1487(v), 1439(v), 1084(s), 746(w), 689(m); **^{1}H-NMR** 200MHz ($CDCl_3$) δ: 4.25(d, 2H, J_{dc}= 7.6Hz); 5.37(d, 1H, J_{bc}= 10Hz); 5.49(d, 1H, J_{ac}= 16.8Hz); 5.81(ddt, 1H, J_{ca}= 16.8Hz, J_{cb}= 10Hz, J_{cd}= 7.6Hz); 7.8(m, 15Harom); **MS** (FAB$^+$) m/z: 781(2); 347(100); 229(14); **Microanalysis**: calcd for $C_{21}H_{20}AsBF_4$ (433.8) C: 58.09; H: 4.65; found C: 58.1; H: 4.5; **^{13}C-NMR** 50.3MHz ($CDCl_3$) δ: 29.7(CH_2); 120.8(C); 124(=CH-); 126(=CH_2); 131(2CH arom); 132(2CH arom); 134(CH arom).

(3β,5α,6β,22S,23E)-6-Methoxy-3,5-cyclo-26,27-dinorcholest-23-en-22-ol (13)

Propenyl triphenylarsonium tetrafluoroborate (220 mg, 0.51 mmol) was stirred at -78°C in dry THF (4 ml). A solution of 1.55M BuLi (0.32 ml, 0.49 mmol) in hexane was added dropwise until a slight colour persists. After 30 min stirring, aldehyde **5** (150 mg, 0.43 mmol) in dry THF (2 ml) was added dropwise to the red solution. The solution remains slightly coloured. The reaction was monitored by TLC (deactivated with a Et_2O/Et_3N: 96/4 mixture). After 35 min stirring at -78°C the solvent was removed under reduced pressure without heating. The slightly coloured oil was then dried under high vacuum and dissolved in CH_2Cl_2 (5 ml). The solution was cooled again to -78°C and a 1M solution of DIBAH in hexane (0.78 ml; 0.7 mmol) was added slowly. The solution was allowed to slowly warm to room temperature. AcOEt and a saturated potassium, sodium tartrate solution was added. The solution was extracted with Et_2O (3x20 ml). The organic layer was washed with brine, drived over $MgSO_4$ and concentrated under vacuum. Chromatography using hexane/ethyl acetate 97:3 - 90:10 as eluent afforded the pure compound **13**; yield: 126 mg (75%).

Mp: 101-103°C; $[\alpha]_D^{23}$= +26 ($CHCl_3$, c= 0.9); **IR** $\nu_{max}cm^{-1}$: 3450(s), 2963(s), 2047(s), 1458(m), 1373(m), 1140(m), 1092(s), 976(w); **^{1}H-NMR** 200 MHz ($CDCl_3$) δ: 0,42(dd, 1H, J= 8Hz, J= 5Hz, H-4α); 0,63(t, 1H, J= 5Hz, H-4β); 0,72(s, 3H, CH_3-18); 0.89(3H, d, J= 6.2Hz, CH_3-21); 1.01(s, 3H, CH_3-19); 1.7(d, 3H, J= 5.14Hz, CH_3-25); 2.76(t, 1H, J= 2.8Hz, H-6); 3.31(s, 3H, OMe); 4.18(s, 1H, $w^{1/2}$= 10Hz, H-22); 5.45-5.71 (complex m, 2H, $w^{1/2}$= 10Hz, H-23+H-24); **MS** (EI^+) m/z: 386 (5.8); 371(8.11); 331(13.08); 316(17.3); 283(100); 213(24.7); 121(48.6); 71(87.1); **Microanalysis**: calcd for $C_{26}H_{42}O_2$ (386,3) C: 80.76; H: 10.95; found C: 80.9; H:10.8; **^{13}C-NMR** in Table 1

(2α,3α,5α,22S,23E)-6,6-Ethylenedioxy-2,3-isopropylidenedioxy-26,27-dinorcholest-23-en-22-ol (15)

Preparation from **5** according to the procedure described for the preparation of **13** using propenyl triphenyl arsonium tetrafluoroborate yield: 73% colourless oil.

IR $\nu_{max}cm^{-1}$: 3483(s), 1664(s), 1210(m), 948(v), 728(w); **^{1}H-NMR** 200MHz ($CDCl_3$) δ: 0.66(s, 3H, CH_3-18); 0.84(s, 3H, CH_3-19); 0.89(d, 3H, J= 6.2Hz, CH_3-21); 1.32(s, 3H, acetonide); 1.47 (s, 3H, acetonide); 1.7(d, 3H, J= 5.14Hz, CH_3-25), 3.7-3.97(m, 4H, OCH_2CH_2O); 4.04-4.15(m, 1H, H-2); 4.18(br, 1H, H-22); 4.27(d, 1H, J= 3.7Hz, H-3); 5.46-5.72 (complex m, 2H, $w^{1/2}$= 9.8 Hz, H-23+H-24); **MS** (EI^+) m/z: 488(16); 473(42); 417(41); 387(67); 259(68); 178(78); 81(100); **Microanalysis**: calcd for $C_{30}H_{48}O_5$ (488,3) C: 73.72; H: 9.91; found C: 74.1; H: 10.1; **^{13}C-NMR** in Table 1

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	3	4	7	9	10	13	15
1	40,3	40,2	39,6	39,9	39,9	33,4	39,6
2	68,3*	68,3*	72,9*	73,1*	73*	24,2	72,9*
3	68,4*	68,4*	73*	73,2*	73,2*	21,5	73*
4	26,3	26,3	41	41,3	41	13,1	41,1
5	53,7	53,8	45	45,7	45,2	35,3	45,6
6	211,9	212,1	107,6	107,8	107,6	82,4	107,6
7	46,8	46,7	42,7	42,9	42,7	35,1	42,8
8	37,7	37,7	33	33,2	33,1	30,6	32,9
9	50,7	50,8	52,6	52,8	52,6	48	53,1
10	42,6	42,6	42,5	42,7	42,7	42,7	42,5
11	21,2	21,2	20,8	21	21	21,5	20,8
12	39,5	39,4	27,7	27,9	27,9	40,2	28,6
13	42,9	43,3	38,1	38,2	38,6	43,4	38,1
14	56,6	56,3	55,8	56	55,7	56,4	55,9
15	23,9	24	22	22,2	22,2	25	22
16	27,6	27,7	24,2	24,4	24,2	27,9	24,3
17	52,5	53,1	53	53,2	53,8	52,6	52,6
18	11,9+	12,4+	11,9+	11,7+	12,2+	12,2+	12,1+
19	13,5	13,6	13,4	13,6	13,4	19,3	13,5
20	40,1	42,5	41,5	40,4	42,8	41,5	41,5
21	11,5+	12+	11,9+	12,2+	12,7+	12,1+	12,2+
22	73,8	74	74	74	74,1	74,2	74,2
23	33,3	27,6	129,6	33,4	27,9	125,4	125,3
24	35,7	36,1	137,6	35,9	35,8	133,9	133,9
25	28,2	28,2	30,8	28,4	28,2	17,8	17,8
26	22,5°	22,9°	22,5°	22,8°	22,8°		
27	22,7°	22,5°	22,5°	22,9°	22,9°		
28			26,6	26,8	26,8		
29			28,7	28,4	28,7		
30			109,7	109,9	109,7		
31			64,2	64,4	64,2		
32			65,4	65,7	65,6		
-OMe						56,6	

Table 1: ^{13}C NMR chemical shifts

δ_c (100 MHz; standard Me_4Si). *(or °, +): interchangeable assignment.
The assignments are based upon: (1) shielding data; (2) by comparison with the spectra of closely related steroids[16] and brassinosteroids[17]

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