Tetrahedron Vol. 47, No. 23, pp 3753-3772, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00
Pergamon Press plc

A FACILE ROUTE TO 20-HYDROXYECDYSONE AND SIDE CHAIN HOMOLOGUES FROM POSTSTERON[1]

UDO HEDTMANN, RALF KLINTZ, KURT HOBERT,
JADWIGA FRELEK[2], IONTSCHO VLAHOV[3], and PETER WELZEL*

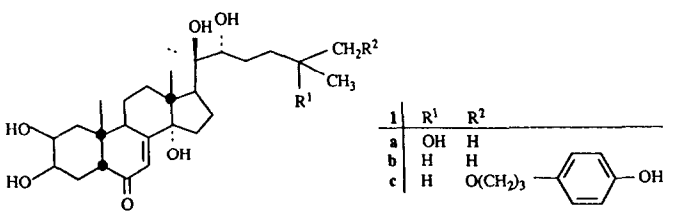
Fakultät für Chemie der Ruhr-Universität, Postfach 102148, D-4630 Bochum (FRG)

(*Received in Germany* 8 *January* 1991)

Abstract - A flexible approach to ecdysteroids, chain elongated at C-26 and C-27, is reported. Key features are the addition of 5-lithio 2,3-dihydrofurans (**3**) to poststeron (**10**) and a stereoselective reduction of the 22-CO group.

Background

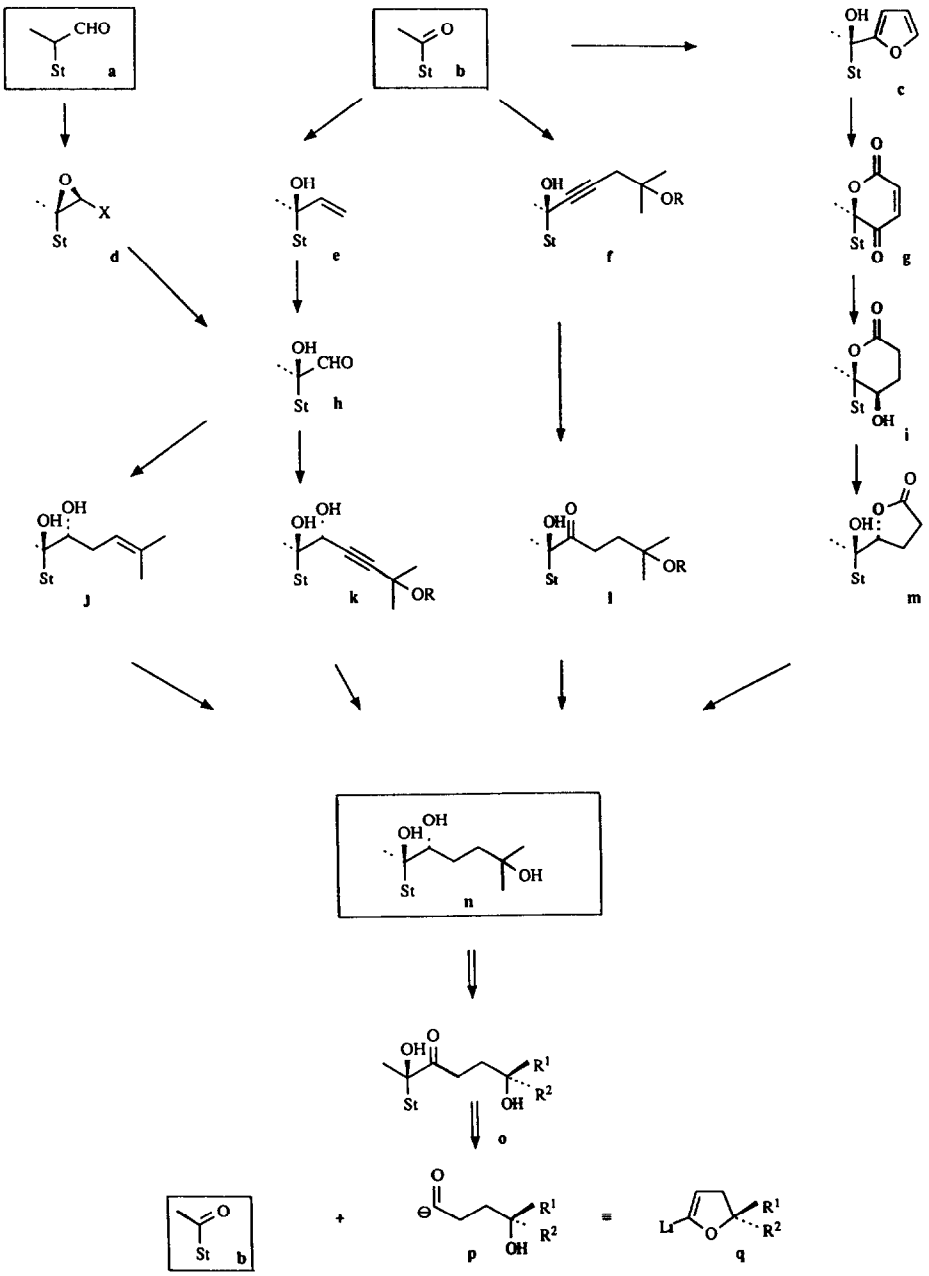
The steroid hormone 20-hydroxyecdysone (**1**) plays a key role in the development of Drosophila melanogaster (and of insects, in general).[4] Drosophila development is divisible into embryogenesis, three larval instars, and the prepupal and pupal stages. Each of these stages is marked by a pulse of **1**. The late third instar pulse triggers the larval-to-adult metamorphosis. In larval salivary glands this puls induces the formation of a small set of puffs. It is assumed that these "early puffs" encode regulatory proteins that repress their own expression and induce the formation of a great number of "late puffs". 1 inducible products are believed to play a key role in initiating metamorphosis.[5] Ashburner et al.[6] have proposed some fifteen years ago that transcription of the early genes is directly induced by an ecdysone-receptor complex. In contrast to vertebrate steroid hormone receptors,[7] the ecdysteroid receptor was until recently virtually uncharacterized (probably caused by the low concentration of the receptor in the target cells and the limited stability of the hormone-receptor complex).[4]



1	R^1	R^2
a	OH	H
b	H	H
c	H	$O(CH_2)_3$ — OH

Scheme 1

We decided some time ago to prepare a biologically active derivative of **1** that (i) contains a (latent) chemically reactive group, and (ii) a radioactive label. Such a compound could be (i) covalently attached to the receptor via the reactive group, thus permitting its identification[8] (affinity labeling[7b]), (ii) bound to a matrix in order to concentrate or even isolate the receptor through affinity chromatography,[9] and (iii), after isolation of the receptor protein, be used in determining the hormone binding site. From structure-activity studies it is known that essentially all the functional groups in **1** are required[10,11]


CHO
St a
O
St b
OH
O
St c
O
X
St d
OH
St e
OH
OR
St f
O
O
St O g
OH
CHO
St h
O
O
St OH i
OH
OH
St j
OH
OH
St OR k
O
OH
OR
St l
O
OH
O
St m
OH
OH
St OH n
O
OH
R1
R2
St OH o
O
R1
R2
OH p
O
St b
Li
O
R1
R2
q

Scheme 2

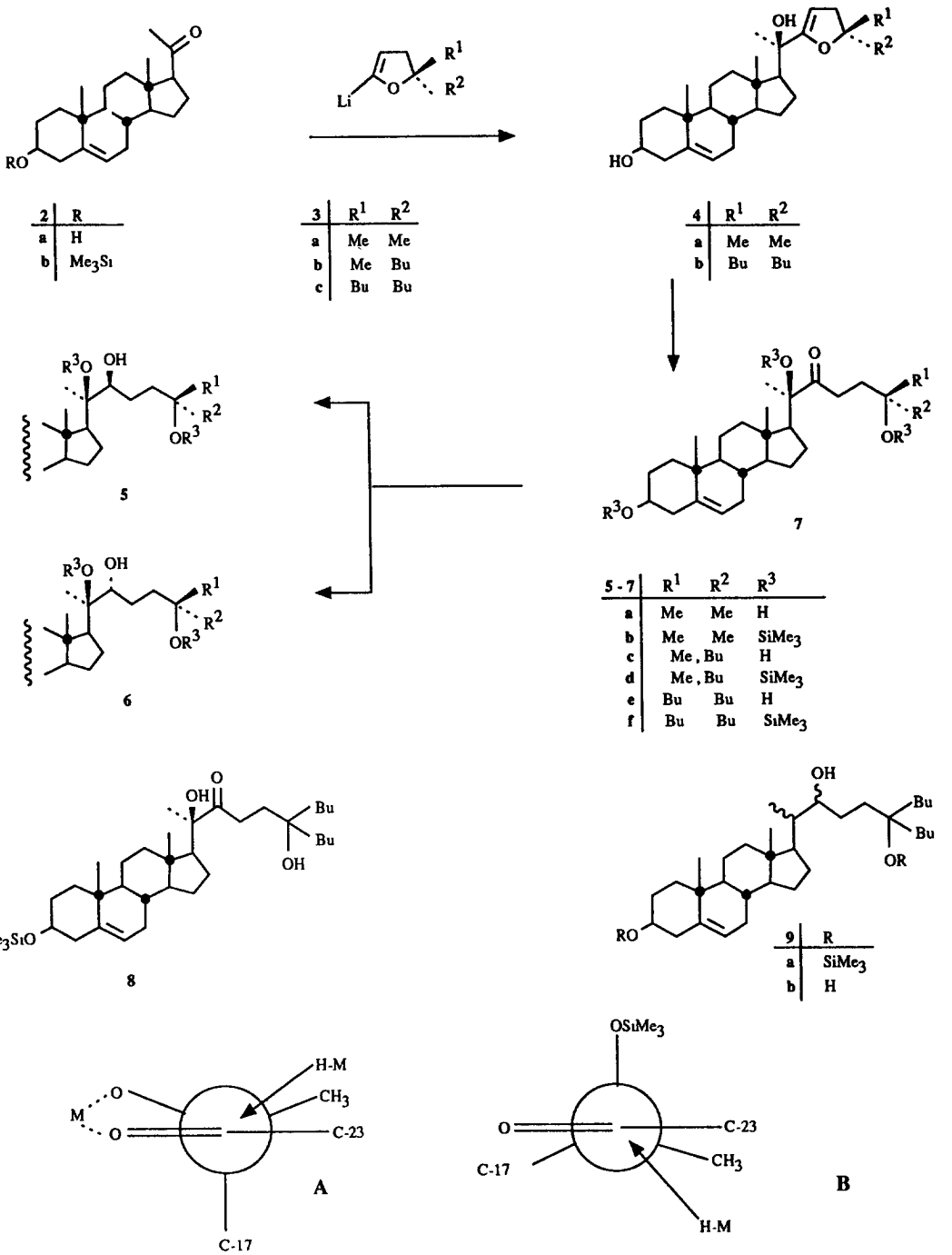
to ensure full biological activity and cannot be used to attach a potential coupling group. However, structural changes at the end of the side-chain appear not to cause a loss of activity as can be judged from the high biological activity of ponasterone A (**1b**) and 26-iodoponasterone A,[8c] and the moderate activity of compound **1c**.[8a]
We report here a flexible approach to the synthesis of ecdysteroids with substituents at C-26 and C-27. These compounds have been shown to be biologically active and one of them was successfully used for affinity labeling of a partly purified ecdysterid receptor (vide infra).

Synthesis Design

Chemical synthesis of ecdysteroids is a longstanding problem.[12] The methods that have been developed for side-chain construction are summarized in Scheme 2. The main problem is, of course, to control the configuration at C-20 and C-22. Starting materials were C-22 or C-20 carbonyl compounds of type **a** and **b**, respectively, which were converted in many synthetic schemes to the central intermediate **h**.[13] Conversion of **b** to **e** follows the Cram rule.[14] Addition of an organometallic reagent to **h** yields an addition product of type **j** or **k** with the correct configuration at C-22 (cyclic Cram model). As can be seen from Scheme 2, formation of the 20-hydroxy-ecdysone side chain as in **n** using this type of approach is rather lengthy. Recently, Kametani et al.[15] introduced the sequence **b** --> **c** --> **g** --> **i** --> **m** --> **n**, in which the configuration at C-20 is controlled by the Cram rule and that at C-22 in the reduction step **g** --> **i**. The most straightforward approach from **b** to **n** was reported by the Schering and Hoffmann - LaRoche groups.[16] It consists of acetylide addition to **b** (**b** --> **f**), hydration of the triple bond (**f** --> **l**) and hydride reduction (**l** --> **n**). The only shortcoming of this process is the poor stereocontrol (**b** --> **f**: d.e. 60%; **l** --> **n**: (22R):(22S) = 1:19[17]). We decided to follow the retrosynthetic sequence depicted in the lower part of Scheme 2. It was anticipated to introduce the complete side chain via acyl anion synthon **p**, for which lithiated dihydrofurans of type **q** were selected as synthetic equivalents. This approach demands a method for the stereoselective synthesis of the dihydrofuran precursors of **q** as well as directing the stereochemical outcome of the 22-keto group reduction in the desired sense. This paper reports the addition of **q** to **b** as well as the reduction of **o** to **n**. The synthesis of compounds **q** was already reported in a preliminary form,[1] a full account of this work will be the subject of a forthcoming publication.

Model Studies using Pregnenolone (**2a**) as Substrate

Treatment of **2** with the lithiated dihydrofurans **3a-3c** led to the formation of the very acid-sensitive addition products of type **4** which were normally directly converted to the corresponding 22-ketones **7**. Only **4b** was isolated and fully characterized. If **2a** with the free 3-OH group was the starting material, an excess (normally 4 equiv.) of **3** had to be employed. For silyl ether **2b** 1 equiv. of **3c** proved to be sufficient. The yield of **7** was in the range of 90% corrected for recovered **2**. In all cases only about 70% of the keto steroid **2**



2	R
a	H
b	Me_3Si

3	R^1	R^2
a	Me	Me
b	Me	Bu
c	Bu	Bu

4	R^1	R^2
a	Me	Me
b	Bu	Bu

5-7	R^1	R^2	R^3
a	Me	Me	H
b	Me	Me	$SiMe_3$
c	Me, Bu		H
d	Me, Bu		$SiMe_3$
e	Bu	Bu	H
f	Bu	Bu	$SiMe_3$

9	R
a	$SiMe_3$
b	H

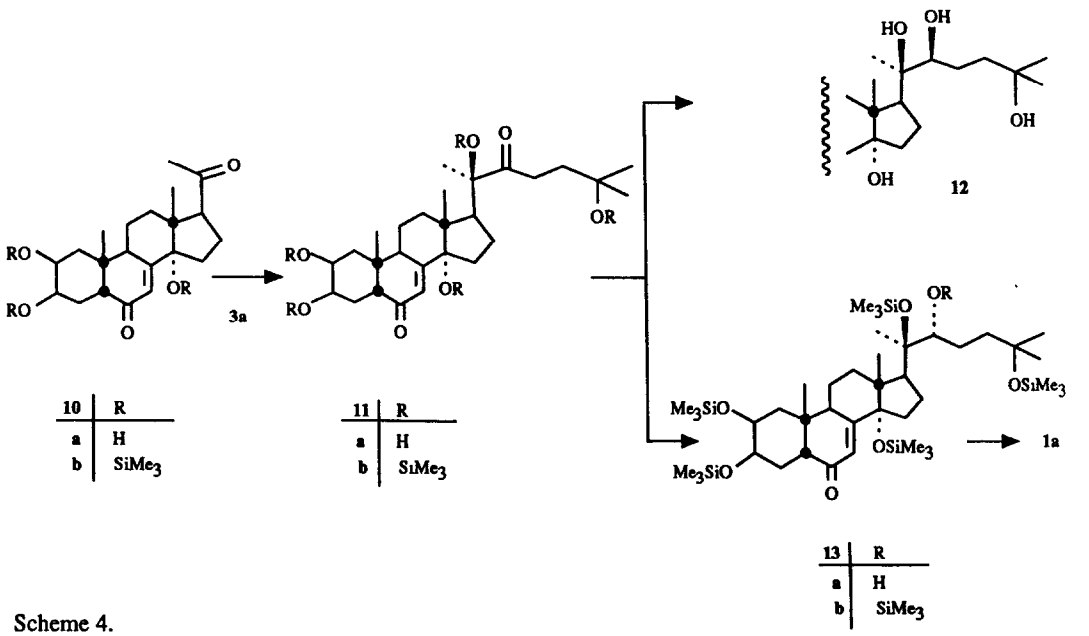
Scheme 3.

were consumed. We assume that part of **2** is converted by **3** into the corresponding enolate from which **2** is restored during the work-up procedure. In preliminary experiments we tried to overcome this difficulty using nucleophiles derived from **3** by transmetallation ($MgBr_2$ x Et_2O, Et_2AlCl, $CeCl_3$) but without success.[18] Addition of DMPU[19] to a reaction mixture of **2b** and **3c** led to the complete recovery of **2b**.[18,20]

In all cases studied a single stereoisomer **7** was formed which is assumed to have the (20R) configuration based on chemical precedent[21] and the chemical shift of the 21-CH_3 group (δ (CH_3-21) for **7a**: 1.68).[22]

Reduction of **7a** with $NaBH_4$ yielded the (22S) and the (22R) compounds **5a** and **6a**, respectively, in a 3:1 ratio. In small-scale test experiments similar results were obtained using DIBAH and $LiAlH(O^tBu)_3$.[23] These results are in agreement with the previous observations mentioned above.[17] The stereochemical outcome of these reductions is tentatively explained on the basis of the cyclic Cram model (see **A** in Scheme **3**).[24,25]

On the other hand, protection of the OH groups in **5a** by trimethylsilyl ether formation with trimethylsilyl triflate,[26,27] followed by DIBAH reduction nicely yielded mainly the desired (22R) compound **6a** (after deprotection with Bu_4NF), probably via transition state geometry as depicted in **B**.[28] Assignment of the configuration at C-22 in **5a** and **6a** is based on the chemical shifts of the CH_3-21 which is known to be larger for the (22S) than for the (22R) isomer (about 0.1 ppm[29], see Table 1).



10	R
a	H
b	$SiMe_3$

11	R
a	H
b	$SiMe_3$

13	R
a	H
b	$SiMe_3$

Scheme 4.

Preparation of 20-Hydroxyecdysone (**1a**)

Poststerone **10a** reacted with an excess of lithium compound **3a** to an addition product the enol ether group of which was cleaved with 0.1. HCl producing **11a** in 79% yield. Reduction of **11a** with $LiAlH(O^tBu)_3$ led to a 16:1 mixture of **12** and **1a**. The keto function at C-6 was stable under these conditions. Unfortunately, after conversion of **11a** to the persilylated **11b** the keto functions turned out to be completely stable toward $LiAlH(O^tBu)_3$.[1b] DIBAH as reducing agent caused reduction of the 22-keto group in the desired stereochemical sense but the 6-oxo group was also reduced (vide infra). After allylic oxidation with MnO_2 and silyl ether removal with TBAF a 39:1 mixture (HPLC) of **1a** and **12** was obtained in 80% yield. **1a** was identical with an authentic sample.

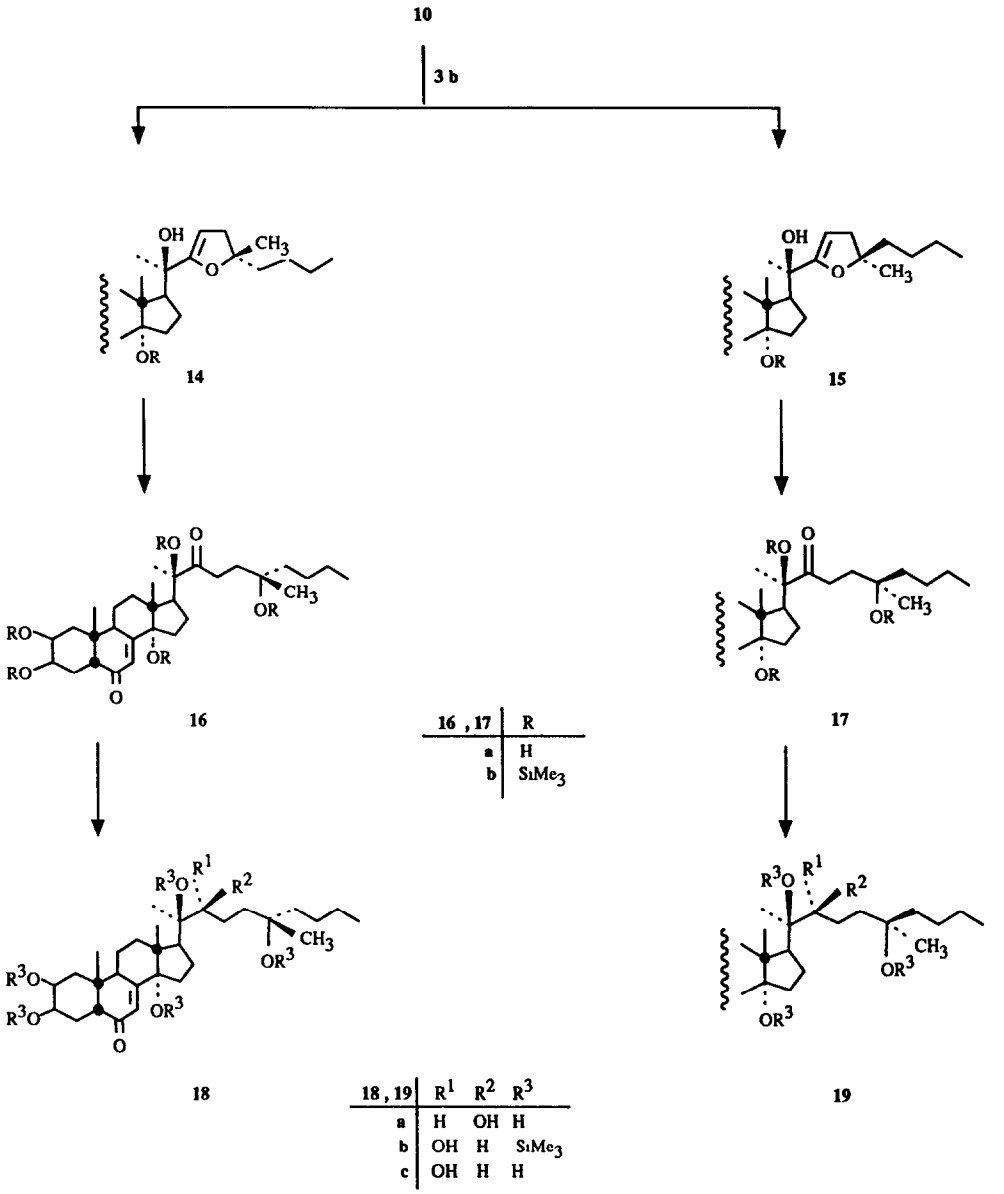
Preparation of Side Chain Homologues **18a, 19a, 18c and 19c**

The 1:1 mixture of diastereoisomers obtained from poststerone (**10a**) and rac.-**3b** could be separated only after silyl ether formation, to provide pure samples of **16b** and **17b**. Reduction of both compounds with DIBAH and subsequent re-oxidation of the 6-OH group with MnO_2 produced **18b** and **19b**, respectively, each contaminated with traces of the corresponding (22S)-isomer. Cleavage of the silyl ether groups (**18b** --> **18c**, **19b** --> **19c**) followed by HPLC showed the d.e. in the DIBAH reduction step to be 94%. Direct reduction of the mixture of **16a** and **17a** with $LiAlH(O^tBu)_3$ provided **18a** and **19a**, which now could be separated by HPLC. In order to interelate these compounds with **18c** and **19c**, respectively, the silyl groups were removed from pure **17b** . Subsequent reduction with $LiAlH(O^tBu)_3$ furnished **19a**.

The configuration at C-22 depicted in formulae **18** and **19** was expected from the results obtained in the reduction of **11a** and **11b**, respectively. These assignments are consistent with those of the ^1H NMR studies, as summarized in Table 1. Thus, as in the case of **12** and **1a**, for the pairs **18a/18c** and **19a/19c** the 21-CH_3 groups in the (22S) isomers (**18a**, **19a**) are more deshielded.

Final proof of the configuration at C-22 was obtained from the 300 nm CD band of the in-situ generated complexes of **1a**, **12**, and **18c** with $Mo_2(OAc)_4$. This band can (inter alia) be used to determine the absolute configuration of optically active 1,2-diols.[30] From Figure 1 it is obvious that the spectra of **1a** and **18c** complexes are almost identical in the 300 nm region . One may conclude, therefore, that **18c** has the same configuration at C-22 as **1a**. One has however to be careful since the CD curves depicted in Figure 1 are most probably sum curves of three Cotton effects: the side-chain and ring A diol complexes, and the enone chromophore. Since the interfering ring A diol and enone Cotton effects are, however, equal in all compounds they are eliminated in the difference spectra. The complete identity of the difference spectra (**1a** - **12**) and (**18c** - **12**) (see Figures 1a and 1b) demonstrates unambiguously that **1a** and **18c** have the same configuration at C-22.

The configuration at C-25 can not be determined by this method. Attempts to obtain suitable crystals for an X-ray analysis were unsuccessful. The problem was finally solved by a stereoselective synthesis. Optically active **3b** on reaction with persilylated poststerone **10b**



16 , 17	R
a	H
b	$SiMe_3$

18 , 19	R^1	R^2	R^3
a	H	OH	H
b	OH	H	$SiMe_3$
c	OH	H	H

Scheme 5.

Table 1. ^{1}H NMR signals of CH_3-21 of compounds in [d_5] pyridine

Config.	Compound: δ value				
22S	**5a**: 1.66	**12**: 1.71	**18a**, **19a**: 1.73		
22R	**6a**: 1.55	**13a**: 1.60	**18c**, **19c**: 1.61	**21a**, **21b**: 1.59	**6c**: 1.52

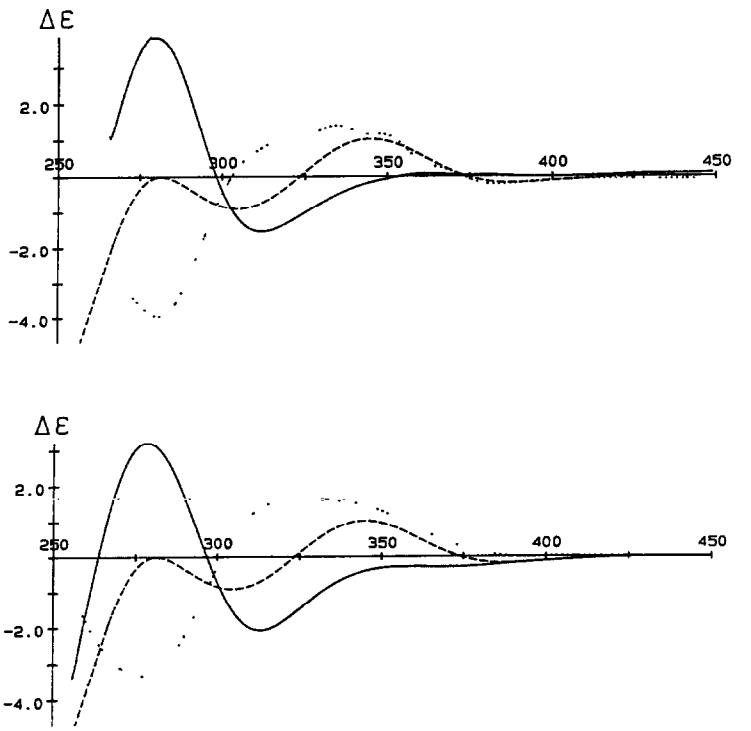


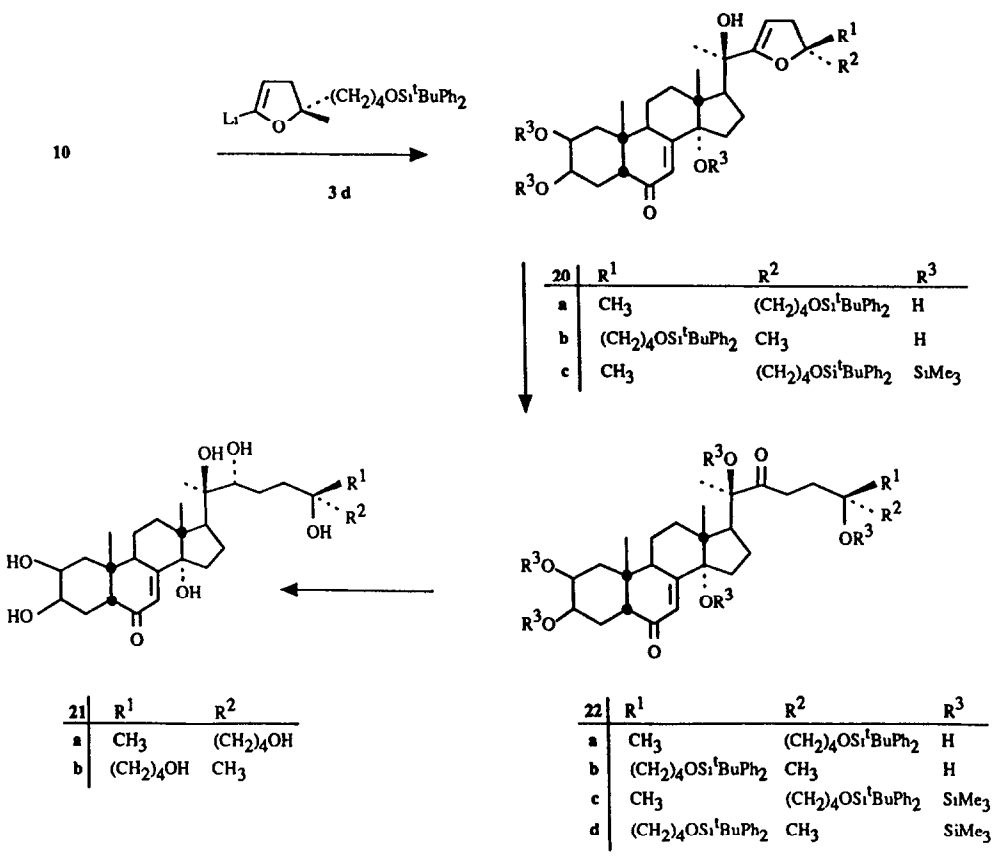
Figure 1. Assignment of configuration at C-22 in **18c**:
a: CD spectra of **12** (---) and **1a** (···) in the presence of [$Mo_2(OAc)_4$] in DMSO solution and difference curve of the spectra of **12** and **1a** (——).
b: CD spectra of **12** (---) and **18c** (···) in the presence of [$Mo_2(OAc)_4$] in DMSO solution and difference curve of the spectra of **12** and **18c** (——).

followed by acid hydrolysis and resilylation provided pure **16b** identical with the specimen obtained after separation of the **16b/17b** mixture described above.

Preparation of **21a** and **21b**

In analogy to the experiments described above, compounds **21a** and **21b** were prepared from **10a** and rac.-**3d** via **20a/20b** and the ketones **22a** and **22b**. In contrast to the case described above HPLC separation of **22a** and **22b** did not pose any problems. In this series, it turned out to be necessary to study the DIBAH reduction (after silylation) more in detail. In model

experiments it was found that persilylated 20-hydroxyecdysone (**13b**) was readily reduced with 2.2 equiv. DIBAH in THF (4h at 20°C). Reoxidation with MnO_2 and F^--mediated desilylation provided **1a** in 81% overall yield. On the other hand, the 22-oxo group in **7f** was practically inert under these conditions even after 10 hours. Only the formation of decomposition products was observed. Under forced conditions (10 equiv. of DIBAH, 4h) the keto group was reduced. Besides the desired reduction product **6f** the over-reduced compound **9a** was obtained. The structure was determined after desilylation (**9a**—>**9b**), the configurations at C-20 and C-22 are unknown. One may conclude from these experiments that in the reduction of the silylated ecdysteroids the carbonyl group at C-6 reacts faster than that at C-22. Furthermore, the rather slow reduction of the 22-oxo group with DIBAH in THF may be accompanied by side reactions. This difficulty could be circumvented when the DIBAH reduction of **7f** was performed in toluene rather than in THF.



20	R^1	R^2	R^3
a	CH_3	$(CH_2)_4OSi^tBuPh_2$	H
b	$(CH_2)_4OSi^tBuPh_2$	CH_3	H
c	CH_3	$(CH_2)_4OSi^tBuPh_2$	$SiMe_3$

21	R^1	R^2
a	CH_3	$(CH_2)_4OH$
b	$(CH_2)_4OH$	CH_3

22	R^1	R^2	R^3
a	CH_3	$(CH_2)_4OSi^tBuPh_2$	H
b	$(CH_2)_4OSi^tBuPh_2$	CH_3	H
c	CH_3	$(CH_2)_4OSi^tBuPh_2$	$SiMe_3$
d	$(CH_2)_4OSi^tBuPh_2$	CH_3	$SiMe_3$

Scheme 6.

6f was readily formed without any side products being observed. Application of these reaction conditions to the reduction of **22c**, followed by MnO_2 oxidation and deprotection furnished **21a** in 82% overall yield.

The (R) configuration at C-22 follows from the reduction procedure and is supported by the chemical shift of the CH_3-21 protons (see Table 1).

The configuration at C-25 was established by a stereochemically unambiguous synthesis. The reaction of **10a** with an excess of optically active **3d** provided almost exclusively **22a**, identical with a sample obtained by separation of **22a/22b** as described above. **10b** was treated with 1.0 equiv. of **3d** to yield **20c**. Enol ether cleavage followed by resilylation gave **22c** from which **21a** was prepared using the already described procedure.

Epilogue

The affinity of ecdysteroids **1a**, **18c**, **19c**, **21a**, and **21b** for receptor proteins has been measured by their ability to displace bound [$^3H_2$]ponasterone A. Receptor preparations were obtained from nuclei of Drosophila melanogaster embryos and from the cytoplasm of Drosophila pupae. In addition, the ecdysteroid concentration required for 50% puff induction at 74EF and 75B in salivary gland chromosomes of larval Drosophila (third instar) was determined. A comparison of these values showed no significant difference between the activity of these ecdysteroids to bind *in vitro* to the receptor and to induce *in vivo* puffs. As for **1a**, the relative receptor binding affinity and relative puff induction of the side chain homologues were about 1% of the corresponding values of ponasterone A.[1b]

Furthermore, a radioligand was obtained from the ecdysteroid homologue **21a** by selective bromoacetylation of the primary hydroxyl group in the side chain with $BrCH_2{}^{14}COOH$. This compound was shown by the group of Professor Pongs to react rapidly, quantitatively, and irreversibly with the ecdysteroid receptor. Analysis of the labeled receptor protein has identified two different peptides.[5b]

EXPERIMENTAL

General

All O_2- or moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of argon. Sensitive liquids and solutions were transferred by syringe and were introduced into the reaction flasks through rubber septa. Usual work-up means partitioning the reaction mixture between water and an organic solvent (given in paranthesis), drying the combined organic solutions over Na_2SO_4 and removal of the solvent by distillation in vacuo at 40°C, using a rotatory evaporator. The instrumentation used was: ^1H NMR: WP-80 (Bruker); AM-400 (Bruker); ^{13}C NMR: AM-400 (Bruker); IR: Perkin Elmer 257; MS: MAT-731 and MAT-CH-5 (Finnigan); HPLC: High-pressure chromatography using pump system model 6000 (Waters Associates Inc.), UV-detector LC-3 (Pye Unicam), stainless steel columns 25.0 cm x 0.4 cm (analytical) or 25.0 cm x 2 cm (preparative), stationary phase and eluent are given in parenthesis; MPLC: Medium-pressure chromatography using 31.0 cm x 2.5 cm glass tubes, silica gel Grace (50 µm), Duramat pump (CFG); Thomachrom UV detector (Reichelt). The FAB mass spectra were obtained using a Finnigan MAT-731 instrument. Samples were dissolved in DMSO, and the matrix (given in parenthesis) was added. The solutions were placed on a stainless steel probe tip and bombarded with 6 KeV Xenon from a modified Saddle Field Ion Source.

General procedure for the preparation of 2-lithio derivatives of 2,3-dihydrofurans (3).
Procedure a: To a solution of the respective 2,3-dihydrofuran 1 equiv. of TMEDA in THF 1.0 equiv. of n-BuLi (1.5 M solution in hexane) was added at -78° C. The solution was allowed to warm to 20°C (1-2.5 h) and was stirred at 20°C for 15 min.
Procedure b: To a solution of the 2,3-dihydrofuran in THF t-BuLi (1.5 M solution in pentane) was added at -78°C. The solution was allowed to warm to ambient temperature (1-2.5 h) and was then stirred at 20°C for 15 min.

(20R)-3β,20,25-Trihydroxy-cholest-5-en-22-one (7a).
To a solution of **3a** (7 mmol, procedure b) a solution of pregnenolone (**2a**, 600 mg, 1.89 mmol) in dry THF (20 ml) was added dropwise at -78°C . The mixture was stirred for 1 h at -78°C and then for 1 h at 20°C. Usual work-up (Et_2O) and MPLC (hexanes-ethyl acetate 2:1 -> 1:1) gave a mixture of two compounds (**7a** and probably **4a**, 430 mg) along with pure **7a** (140 mg) and **2a** (93 mg, 15 %). Total yield of **4a** and **7a**: 90 % (based on **2a**). To a solution of the above mixture of **4a** and **7a** (150 mg) in THF-water (20:1, 10.5 ml) was added at 20°C HCl (0.1 mol/l, 0.5 ml). After being stirred for 30 min at 20°C the mixture was neutralized with solid K_2CO_3. Filtration, solvent evaporation and LC (hexanes-ethyl acetate 2:1) gave **7a** (105 mg, 70%).- M.p. 186-189°C (from acetone).- ^1H NMR (80 MHz, $CDCl_3$): δ = 0.91 (s, CH_3-18), 1.01 (s, CH_3-19), 1.23 (s, CH_3-26, CH_3-27), 1.46 (s, CH_3-21), 2.50-2.80 (CH_2-23), 3.30-3.75 (3-H), 3.90 (s, OH), 5.32 ($W_{1/2}$=8 Hz, 6-H).- IR (KBr): 1690 cm^{-1} (CO).- MS: m/z (%) = 414 ([M-H_2O]$^+$, 0.8), 399 (2), 381 (3), 317 (92), 299 (63), 281 (17), 255 (17), 229 (8), 199 (9), 159 (37), 113 (55), 43 (100).- (Found: C, 74.81; H, 10.16. $C_{27}H_{44}O_4$ (432.6) requires C, 74.96 H, 10.25%).

Reduction of 7a.
To a solution of $NaBH_4$ (110 mg, 2.8 mmol) in water (0.35 ml) and NaOH (5%, 0.4 ml) was added dropwise at 20°C a solution of **7a** (80 mg, 0.18 mmol) in THF (5 ml), and the mixture was stirred at 20°C for 16 h. Usual work-up (Et_2O) and MPLC (hexanes-ethyl acetate-ethanol 30:30:1) gave **6a** (16 mg, 20%) and **5a** (48 mg, 59%).

(20R,22R)-Cholest-5-ene-3β,20,22,25-tetraol (6a).
M.p. 231-234°C (from acetone-methanol).- ^1H NMR (80 MHz, C_5D_5N): δ = 1.05 and 1.18 (2 s's, CH_3-18, CH_3-19), 1.47 (s, CH_3-26, CH_3-27), 1.56 (s, CH_3-21), 3.60-4.10 (3-H, 22-H), 5.43 ($W_{1/2}$=8 Hz, 6-H).- MS: m/z (%) = 434 ([M]$^+$, 0.2), 417 (0.3), 401 (3), 384 (2), 360 (2), 330 (5), 317 (100), 299 (63), 281 (15), 255 (14), 229 (9), 159 (41), 43 (99).- (Found: C, 74.39; H, 10.74. $C_{27}H_{46}O_4$ (434.6) requires C, 74.61; H, 10.66%).

(20R,22S)-Cholest-5-ene-3β,20,22,25-tetraol (5a).
5a: M.p. 196-199°C (from methanol).- ^1H NMR (80 MHz, C_5D_5N): δ = 1.08 and 1.20 (2 s's, CH_3-18 and CH_3-19), 1.42 (s, CH_3-26, CH_3-27), 1.66 (s, CH_3-21), 3.60-4.10 (3-H, 22-H); 5.42 ($W_{1/2}$= 8 Hz, 6-H).- IR (KBr): 1650 cm^{-1} (C=C).- MS: m/z (%) = 434 ([M]$^+$, 0.3), 416 (0.2), 401 (3), 383 (2), 360 (4), 317 (100), 299 (58), 281 (17), 255 (14), 229 (8), 159 (31), 43 (70).- (Found: C, 71.88; H, 10.14. $C_{27}H_{46}O4\cdot H_2O$ (452.6) requires C, 71.96; H, 10.29%).

(20R)-3β,20,25-Tris-(trimethyl-silanyloxy)-cholest-5-en-22-one (7b).
To a solution of **7a** (30.4 mg, 69 μmol) in dry THF (3 ml) and 2,6-lutidine (63 μl, 55 μmol) was added dropwise at 20°C trimethylsilyl triflate (47 μl, 0.241 mmol) and the mixture was stirred at 20°C for 30 min. Usual work-up (Et_2O) and LC (hexanes-ethyl acetate-NEt_3 60:1:0.3) gave **7b** (38 mg, 86%).- ^1H NMR (80 MHz, C_6D_6): δ = 0.17, 0.18, and 0.30 (3 s's, $Si(CH_3)_3$), 0.89 and 0.98 (2 s's, CH_3-18, CH_3-19), 1.26 (s, CH_3-26, CH_3-27), 1.48 (s, CH_3-21), 2.61-2.93 (CH_2-23), 3.43-3.90 (3-H), 5.37 ($W_{1/2}$=7 Hz, 6-H).- IR (CCl_4): 1715 cm^{-1} (C=O).- $C_{36}H_{68}O_4Si_3$ (649.2), MS: m/z (%) = 648 ([M]$^+$, 0.1), 633 (3), 543 (4), 461 (100), 371 (5), 281 (22), 143 (51), 117 (70), 73 (53).

(20R,22R)-3β,20,25-Tris-(trimethyl-silanyloxy)-cholest-5-en-22-ol (6b).
To a solution of **7b** (prepared from **7a**, 29.9 mg, 68 μmol, as described above) in dry THF (2.0 ml) was added dropwise at -78°C DIBAH (1.2M in toluene, 0.28 ml, 0.34 mmol). The mixture was stirred for 3 h being allowed to warm to 20°C. Usual work-up (Et_2O) and LC (hexanes-ethyl acetate-NEt_3 50:1:0.25) gave **6b** (31 mg, 72%) along with a mixture (4 mg) of **6b** and another compound (probably the (22S) isomer).- ^1H NMR (C_6D_6): δ = 0.18, 0.34 (2s's, $Si(CH_3)_3$-

signals), 0.95 and 1.00 (2 s's, CH_3-18 and CH_3-19), 1.17 (s, CH_3-26, CH_3-27), 1.35 (s, CH_3-21), 3.32-3.90 (3-H, 22-H), 5.39 ($W_{1/2}$=7 Hz, 6-H).- IR (CCl_4): 3630, 3500-3300 cm^{-1} (OH).- $C_{36}H_{70}O_4Si_3$ (651.2) MS: m/z (%) = 650 ([M]$^+$, 0.1), 635 (0.7), 619 (0.6), 545 (5), 461 (100), 371 (5), 281 (24), 143 (46), 117 (67), 73 (47).

General procedure for the silyl ether cleavage.
To a solution of the silyl ether in dry THF was added at 20°C TBAF (0.1M solution in THF, 1.3 equiv per silyl group). The mixture was stirred at 20°C for 1 h. After solvent evaporation the crude reaction product was purified by LC.

6a from 6b.
After silyl ether cleavage in **6b** (general procedure) and LC (hexanes-ethyl acetate-ethanol 10:10:1) **6a** (76%) was obtained, identical with the product of $NaBH_4$ reduction of **7a**.

(20R,25RS)-3β,20,25-Trihydroxy-27-propyl-cholest-5-en-22-one (7c).
7c was prepared from **2a** and rac.-**3b** (procedure b) as described for **7a**. LC (hexanes-ethyl acetate 2:1) gave 85% of a mixture of two C-25 diastereoisomeres.- ^{1}H NMR (80 MHz, $CDCl_3$): δ = 0.89 (s, CH_3-18), 1.02 (s, CH_3-19), 1.17 (s, CH_3-26), 1.45 (s, CH_3-21), 2.50-2.75 (CH_2-23); 3.30-3.75 (3-H), 3.91 (s, OH), 5.32 ($W_{1/2}$ = 7 Hz, 6-H).- IR ($CHCl_3$): 3595-3450 (OH), 1695 cm^{-1} (C=O).- $C_{30}H_{50}O_4$ (474.4), MS: m/z (%) = 456.3591 ([M-H_2O]$^+$, 4, Calc for $C_{30}H_{48}O_3$: 456.3603), 438 (2), 399 (2), 381 (2), 358 (6), 317 (19), 299 (17), 184 (42), 183 (40), 43 (100).

Reduction of 7c.
7c was reduced with DIBAH as described for **7a**. MPLC (hexanes-acetone 2:1) gave **5c** (47%) and a mixture of **5c** and **6c** (34%).

(20R,22S,25RS)-27-Propyl-cholest-5-ene-3β,20,22,25-tetraol (5c).
^{1}H NMR (80 MHz, $CDCl_3$): δ = 0.91 (s, CH_3-18), 1.03 (s, CH_3-19), 1.21 (s, CH_3-26), 1.29 (s, CH_3-21), 3.20-3.80 (3-H, 22-H), 5.36 ($W_{1/2}$ = 7 Hz, 6-H).- IR ($CHCl_3$): 3660-3300 (OH), 1600 cm^{-1} (C=C).- $C_{30}H_{52}O_4$ (476.7), MS: m/z (%) = 443 ([M-H_2O-CH_3]$^+$, 1.2), 425 (1), 401.3036 ([M-C_4H_9-H_2O]$^+$, Calc for $C_{24}H_{41}O_3$: 401.3055), 383 (2), 360 (3), 317 (86), 299 (54), 281 (16), 271 (8), 255 (12), 43 (100).

(20R,25RS)-27-Propyl-3β-20,25-tris-(trimethylsilanyloxy)-cholest-5-en-22-one (7d).
7d was obtained from **7c** as described for **7b**. LC (hexanes-ethyl acetate-NEt_3 50:1:0.25), yield 90%.- ^{1}H NMR (80 MHz, C_6D_6): δ = 0.19, 0.20, 0.31 (3s's, $Si(CH_3)_3$), 0.88 (s, CH_3-18), 0.98 (s, CH_3-19), 1.16 (s, CH_3-26), 1.49 (s, CH_3-21), 2.65-2.95 (CH_2-23), 3.45-3.80 (3-H), 5.38 ($W_{1/2}$ = 7 Hz, 6-H).- IR (CCl_4): 1710 cm^{-1} (C=O).- $C_{39}H_{74}O_4Si_3$ (691.2), MS: m/z (%) = 675.4665 ([M-CH_3]$^+$, 1, Calc for $C_{36}H_{71}O_4Si_3$: 675.4660), 633 (2), 585 (2), 543 (1), 461 (41), 281 (13), 143 (50), 117 (95), 73 (100).

(20R,22R,25RS)-27-Propyl-3β,20,25-tris-(trimethylsilanyloxy)-cholest-5-en-22-ol (6d).
6d was obtained from **7d** by DIBAH reduction as described for **6b** (yield: 76%).- ^{1}H NMR (80 MHz, C_6D_6): δ = 0.18, 0.20, 0.31 (3 s's, $Si(CH_3)_3$), 0.92 (s, CH_3-18), 1.00 (s, CH_3-19), 1.28 (s, CH_3-26), 1.35 (s, CH_3-21), 3.30-3.80 (3-H and 22-H), 5.38 ($W_{1/2}$ = 7 Hz, 6-H).- IR (CCl_4): 3630, 3520 cm^{-1} (OH).- $C_{39}H_{76}O_4Si_3$ (693.2), MS: m/z (%) = 587.4317 ([M-CH_3-$HOSi(CH_3)_3$]$^+$, 0.4, Calc for $C_{35}H_{63}O_3Si_2$: 587.4316), 545 (1), 461 (6), 445 (4), 370 (5), 117 (42), 73 (100).

(20R,22R,25RS)-26-Propyl-cholest-5-ene-3β,20,22,25-tetraol (6c).
6d was converted to **6c** using the general silyl ether cleavage procedure. LC (hexanes-acetone 2:1) provided **6d** (73%) and a mixture of **6c** and **5c** (9%).- ^{1}H NMR (80 MHz, $CDCl_3$): δ = 0.89 (s, CH_3-18), 1.02 (s, CH_3-19), 1.19 (s, CH_3-27), 1.26 (s, CH_3-21, for $\delta_{pyridine}$, see Table 1), 3.30-3.80 (3-H and 22-H), 5.36 ($W_{1/2}$ = 7 Hz, 6-H).- IR ($CHCl_3$): 3660-3300 (OH), 1600 cm^{-1} (C=C).- $C_{30}H_{52}O_4$ (476.7), MS: m/z (%) = 440 ([M-H_2O]$^+$, 2), 425 (2), 401.3044 ([M-C_4H_9-H_2O]$^+$, 8, Calc for $C_{26}H_{41}O_3$: 401.3055), 383 (3), 365 (2), 360 (3), 317 (100), 299 (56).

Addition of **3c** to **2a**.
To a solution of **3c** (7.0 mmol, procedure b) in THF (10 ml) a solution of **2a** (579.9 mg, 1.83 mmol) in dry THF (20 ml) was added at -78°C within 25 min. The mixture was then allowed to warm to 20°C (4.75 h). Usual work up (Et_2O) followed by MPLC (hexanes-ethyl acetate-NEt_3 5:1:0.1) gave **4b** (506.1 mg, 56%), **7e** (109.6 mg, 12%) and **2a** (166.7 mg, 26%).

(20R)-26,27-Dipropyl-22,25-epoxy-cholest-5,22-diene-3β,20-diol (**4b**).
^1H NMR (80 MHz, $CDCl_3$): δ = 0.82 (s, CH_3-18), 0.99 (s, CH_3-19), 0.80-1.00 (CH_3-30, CH_3-33), 1.40 (s, CH_3-21), 2.35 (d, J = 2.4 Hz, CH_2-24), 3.50 ($w_{1/2}$ = 18 Hz, 3-H), 4.48 (t, J = 2.4 Hz, 23-H), 5.34 (d, J = 4.2 Hz, 6-H).- IR (CCl_4): 3600-3200 (OH), 1705 cm^{-1} (C=C-O).- $C_{33}H_{52}O_3$ (498.8), MS: m/z (%) = 498.4071 ([M]$^+$, 7, Calc 498.4073), 480 (12), 465 (2), 423 (8), 358 (17), 299 (28), 226 (100), 225 (69), 197 (59), 55 (96).

(20R)-26,27-Dipropyl-3β,20,25-trihydroxy-cholest-5-en-22-one (**7e**).
mp.: 101-103°C (from Et_2O-hexane).- ^1H NMR (80 MHz, $CDCl_3$) δ = 0.89 (s, CH_3-18), 0.80-1.05 (CH_3-30, CH_3-33), 1.02 (s, CH_3-19), 1.45 (s, CH_3-21), 2.48-2.77 (CH_2-23), 3.46 ($w_{1/2}$ = 21 Hz, 3-H), 3.95 (broad s, OH), 5.32 ("d", J = 4.0 Hz, 6-H).- IR (CCl_4) = 3600-3100 (OH), 1700 cm^{-1} (C=O).- MS: m/z (%) = 480 ([M-$2H_2O$]$^+$, 24), 465 ([M-$2H_2O$-CH_3]$^+$, 3), 423 (41), 317 (45), 299 (42), 197 (64), 151 (63), 55 (100).-(Found C, 76.69, H, 11.00, $C_{33}H_{56}O_4$ (516.8) requires C, 76.70, H, 10.92%).

(20R)-20,25-Dihydroxy-26,27-dipropyl-3β-(trimethylsilanyloxy)-cholest-5-en-22-one (**8**).
a.-To a solution of **3c** (98 µmol, procedure b) in dry THF (0.5 ml) a solution of **2b** (38.1 mg, 98 µmol) in THF (1.0 ml) was added at -78°C and the mixture was allowed to warm up 20°C within 4.75 h. Usual work up (Et_2O) followed by LC led to **8** (22%), 63% of **2b** were recovered.
b.-To a solution of **3c** (106 µmol, procedure a) in dry THF (1.0 ml) a solution of **2b** (37.4 mg, 96.4 µmol) in THF (0.5 ml) was added at -78°C and the solution was allowed to warm to 20°C within 4h. Usual work-up (Et_2O) followed by treatment with 0.1 N HCl (1.0 ml) in THF (5.0 ml, stirring at 20°C for 2h), usual work up (Et_2O) and LC (hexanes-ethyl acetate 5:1) gave **8** (8%), **7e** (42%), **2b** (1%), and **2a** (38%).- ^1H NMR (80 MHz, $CDCl_3$): 0.14 (s, $Si(CH_3)_3$ signals), 0.88 (s, CH_3-18), 0.80-1.03 (CH_3-30, CH_3-33), 0.96 (s, CH_3-19), 1.48 (s, CH_3-21), 2.48-2.73 (CH_2-23), 3.50 ($w_{1/2}$ = 16 Hz, 3-H), 3.95 (broad s, OH), 5.33 ("d", J = 4.4 Hz, 6-H).- IR (CCl_4): 3610-3400 (OH), 1705 cm^{-1} (C=O).- $C_{36}H_{64}O_4Si$ (589.0), MS: m/z (%) = 570.4454 ([M-H_2O]$^+$, 1.3, Calc for $C_{36}H_{62}O_3Si$: 570.4468), 552 (4), 527 (1), 430 (5), 389 (5), 299 (24), 226 (74), 225 (62), 197 (100).

(20R)-26,27-Dipropyl-3β,20,25-tris-(trimethylsilanyloxy)-cholest-5-en-22-one (**7f**).
a) **7e** was silylated as described for **7a** (82 % yield).- b) **4b** was opened with HCl and then silylated (74% yield over two steps).- ^1H NMR (400 MHz, C_6D_6): δ = 0.175, 0.233, 0.314 (3s's, $Si(CH_3)_3$ signals), 0.88 (s, CH_3-18), 0.91 and 0.92 (2 t's, J = 6.6 Hz, CH_3-30 and CH_3-33), 0.98 (s, CH_3-19), 1.49 (s, CH_3-21), 2.51-2.59 (17-H), 2.72 and 2.89 (2 ddd's, J = 4.7, 11.0, 18.2 Hz, CH_2-23), 3.66 ($w_{1/2}$ = 18 Hz, 3-H), 5.37 (d, J = 6.0 Hz, 6-H).- IR (CCl_4) : 1720 cm^{-1} (C=O).- $C_{42}H_{80}O_4Si_3$ (733.3), MS: m/z (%) = 717 ([M-CH_3]$^+$, 16), 675.4568 ([M-C_4H_9]$^+$, 5, Calc for $C_{38}H_{71}O_4Si_3$: 675.4661), 627 (4), 585 (3), 461 (100).

Reduction of **7f**.
a) in THF: To a solution of **7f** (4.1 mg, 5.6 µmol) in dry THF (1.0 ml) DIBAH (1.5 M in toluene, 41.1 µl, 61.6 µl) was added dropwise . The solution was allowed to warm to 20°C within 4h. Usual work-up (CH_2Cl_2) followed by SC (hexanes-ethyl acetate-NEt_3 100:1:0.4) gave **6f** (2.7 mg, 65 %) and **9a** (0.5 mg, 12 %).- b) The same procedure with toluene as solvent (1.5 equiv. of DIBAH) gave **6f** in 50% yield, 26% of **7f** were recovered.

(20R,22R)-26,27-Dipropyl-3β,20,25-tris-(trimethylsilanyloxy)-cholest-5-en-22-ol (**6f**).
^1H NMR (400 MHz, C_6D_6): δ = 0.181, 0.250, 0.313 (3 s's, $Si(CH_3)_3$ signals), 0.922 (s, CH_3-18), 0.929 (t, J = 6.1 Hz, CH_3-30 and CH_3-33), 0.963 (s, CH_3-19), 1.347 (s, CH_3-21), 2.57 (broad t, J = 10.5 Hz, 17-H), 3.48 ($w_{1/2}$ = 12 Hz, 22-H), 3.66 ($w_{1/2}$ = 20 Hz, 3-H), 5.39 (d, J = 4.6 Hz, 6-H).- IR (CCl_4): 3610 - 3400 cm^{-1} (OH).- $C_{42}H_{82}O_4Si_3$ (735.4), MS: m/z (%) = 735 ([M]$^+$, 0.15), 661 (0.3), 644 (0.2), 642 (0.7), 629 (2), 587.4288 ([M-HOSiMe_3-C_4H_9]$^+$, 22, Calc for $C_{35}H_{63}O_3Si_2$: 587.4316), 401 (32), 215 (43), 117 (45), 73 (100).

(20Ξ,22Ξ)-26,27-Dipropyl-3β,25-bis-(trimethylsilanyloxy)-cholest-5-en-22-ol (**9a**).
^{1}H NMR (400 MHz, C_6D_6): δ = 0.179, 0.263 (2 s's, $Si(CH_3)_3$ signals), 0.650 (s, CH_3-18), 0.937 and 0.940 (2 t's, J = 7.1 Hz, CH_3-30 and CH_3-33), 0.964 (s, CH_3-19), 2.43 (ddd, J = 13, 6, 2 Hz, 1H), 2.56 (broad t, J = 12 Hz, 17-H), 3.65 ($w_{1/2}$ = 20 Hz, 3-H), 3.91 ($w_{1/2}$ = 10 Hz, 22-H), 5.40 (d, J = 5 Hz, 6-H).- $C_{39}H_{74}O_3Si_2$ (647.2), MS: m/z (%) = 589.4476 ([M-C_4H_9]$^+$, 4, Calc for $C_{35}H_{65}O_3Si_2$: 589.4473), 571 (3), 499 (14), 255 (24), 215 (59), 183 (78), 73 (100).

(20R,22R)-26,27-Dipropyl-cholest-5-ene-3β,20,22,25-tetraol (**6e**).
6f was desilyated (see general procedure) to give **6e**, after LC (hexanes-ethyl acetate 1:1), in 90% yield.- ^{1}H NMR (400 MHz, C_5D_5N): δ = 0.891 and 0.896 (2t's, J = 7.0 Hz, CH_3-30 and CH_3-33), 1.053 (s, CH_3-19), 1.172 (s, CH_3-18), 1.570 (s, CH_3-21), 3.85 ($w_{1/2}$ = 20 Hz, 3-H and 22-H), 4.69, 5.32, 6.07 and 6.17 (4 broad s's, OH), 5.43 ("d", J = 4.0 Hz, 6-H).- IR ($CHCl_3$): 3670-3400 cm^{-1} (OH).- $C_{33}H_{58}O_4$ (518.8), MS: m/z (%) = 482.4125 ([M-2H_2O]$^+$, 13, Calc for $C_{33}H_{54}O_2$: 482.4124), 464 (6), 443 (20), 425 (14), 317 (100), 299 (57).

(20Ξ,22Ξ)-26,27-Dipropyl-cholest-5-ene-3β,22,25-triol (**9b**).
9a was desilylated (see general procedure) to give, after LC (hexanes-ethyl acetate 1:1) **9b** in 72% yield.- ^{1}H NMR (400 MHz, C_5D_5N): δ = 0.739 (s, CH_3-18), 0.897 and 0.901 (2t's, J = 7.4 Hz, CH_3-30 and CH_3-33), 1.056 (s, CH_3-19), 1.13 (d, J = 8.4 Hz, CH_3-21), 3.85 ($w_{1/2}$ = 20 Hz, 3-H), 4.21 ($w_{1/2}$ = 5 Hz, 22-H), 5.25 and 6.16 (2 broad s's, OH), 5.27 (d, J = 5.3 Hz, OH), 5.42 ("d", J = 4.7 Hz, 6-H).- $C_{33}H_{60}O_3$ (502.8), MS: m/z (%) = 445 ([M-C_4H_9]$^+$, 1), 427.3574 ([M-C_4H_9-H_2O]$^+$, 9, Calc for $C_{29}H_{47}O_2$: 427.3576), 409 (4), 183 (100).

(20R)-2β,3β,14,20,25-Pentahydroxy-5β-cholest-7-ene-6,22-dione (**11a**).
To a solution of **3a** (1.61 mmol, procedure b) in THF (2.0 ml) a solution of **10a** (35 mg, 97 µmol) in THF (8.0 ml) was added dropwise at -78°C . The reaction mixture was stirred for 3 h, being allowed to warm to 20°C. After addition of saturated aq.NaCl (5 ml) the organic layer was separated. The aqueous layer was extracted with n-butanol (3x5 ml) and the combined organic solutions were washed with water (5 ml). After solvent removal, addition of water (20 ml), and lyophilisation the residue was dissolved in THF (5 ml) and treated with 0.1N HCl (1.5 ml) for 1 h at 20°C. Neutralisation with solid K_2CO_3, phase separation, addition of water to the organic phase, lyophilisation, and finally LC (CH_2Cl_2-methanol 12:1) gave **11a** (30 mg, 65%) and recovered **10a** (5 mg, 17%).- M.p. 207-209°C (from methanol, lit.[16]: 209-210°C).- IR (KBr): 1700 (CO), 1650 cm^{-1} (unsat. CO).- ^{1}H NMR[31] (400 MHz, C_5D_5N): δ = 1.06 (s, CH_3-19), 1.12 (s, CH_3-18), 1.35 and 1.37 (2 s's, CH_3-26, CH_3-27), 1.68 (s, CH_3-21), 2.64 (m, 1H), 3.02 (dd, J=4 Hz, 13 Hz, 5-H), 3.21-3.32 (17-H, CH_2-23), 3.55-3.63 (9-H), 4.13-4.20 (2-H), 4.24 ($W_{1/2}$=8 Hz, 3-H), 6.22 (d, J=2.5 Hz, 7-H).- $C_{27}H_{42}O_7$ (478.7), FAB-MS (glycerol): m/z (%) = 501 ([M+Na]$^+$, 20), 479 ([M+H]$^+$, 31), 461 (100), 443 (35), 425 (28), 363 (5), 348 (39),329 (35), 303 (45).

Reduction of **11a** with LiAlH(O^{t}Bu)$_3$.
To a solution of **11a** (25 mg, 52 µmol) in dry THF (8 ml) LiAlH(O^{t}Bu)$_3$ (0.5 M solution in THF, 1.2 ml, 0.6 mmol) was added dropwise at 0°C . The mixture was stirred for 3 h at -78°C and for 1 h at 20°C. Usual work-up (1-butanol), addition of water to the combined organic solutions, lyophilisation, and LC (CH_2Cl_2-methanol 8:1) gave **12** (17.2 mg 68%) and **1a** (1.5 mg, 6%). In the reaction mixture the **12a**:**1a** ratio as determined by HPLC (5µm RP-18, methanol-water 2:3) was 16:1.

(20R,22S)-2β,3β,14,20,22,25-Hexahydroxy-5β-cholest-7-en-6-one (**12**).
M.p. 258-260°C (from methanol, lit.[16]: 259-260°C).- ^{1}H NMR (80 MHz, C_5D_5N): δ = 1.08 (s, CH_3-19, 1.22 (s, CH_3-18), 1.40 (s, CH_3-26, CH_3-27), 1.71 (s, CH_3-21), 2.85-3.15 (5-H), 3.40-3.75 (9-H, 17-H), 4.00-4.35 (2-H, 3-H), 6.22 (d, J = 2 Hz, 7-H).- IR (KBr): 1650 cm^{-1} (unsat. CO).- $C_{27}H_{44}O_7$ (480.7), FAB MS (glycerol): m/z (%) = 481 ([M+H]$^+$, 30), 463 (78), 445 (100), 427.6 (36), 411.5 (23), 393 (20), 371 (42), 347 (40), 331 (57), 329 (72), 303 (70), 301 (93).

(20R,22R)-2β,3β,14,20,25-Pentakis-(trimethyl-silanyloxy)-5β-cholest-7-ene-6,22-dione (**11b**).
To a solution of **11a** (20 mg, 0.041 mmol) and 2,6-lutidine (76 µl, 0.066 mmol) in dry THF (4.0 ml) trimethylsilyl triflate (64 µl, 0.33 mmol) was added dropwise at 20°C . The mixture was stirred at 20°C for 1 h. Usual work-up (Et_2O) and LC (hexanes-ethyl acetate-NEt_3

40:1:0.2) gave **11b** (28 mg, 74%).- IR (CCl_4): 1715 (CO), 1665 cm^{-1} (unsat. CO).- ^1H NMR (400 MHz, C_6D_6): δ = 0.07, 0.15, 0.16, 0.22, and 0.33 (5 s's, $Si(CH_3)_3$ signals), 0.69 (s, CH_3-19), 0.91 (s, CH_3-18), 1.17 (2 s's, CH_3-26, CH_3-27), 1.39 (s, CH_3-21), 2.50 (t, J = 9.5 Hz, 17-H), 2.57-2.67 and 2.78-2.88 (CH_2-23), 2.95 (dd, J = 3.5 Hz, 13.0 Hz, 5-H), 3.06 ($W_{1/2}$=23 Hz, 9-H),3.91 ($W_{1/2}$ = 21 Hz, 2-H), 4.00 ($W_{1/2}$ = 7 Hz, 3-H), 5.93 (d, J = 2.2 Hz, 7-H).- $C_{42}H_{82}O_7Si_5$ (838.9), MS: m/z (%) = 823.4664 ([M-CH_3]$^+$, 1.3, Calc for $C_{41}H_{79}O_7Si_5$: 823.4672), 733 (3), 662 (2), 651 (2), 643 (2), 635 (1), 561 (100).

(20R,22R)-2β,3β,14,20,22,25-Hexakis-(trimethylsilanyloxy)-5β-cholest-7-en-6-one (13b).
1a was silylated as described for **7a** (86% yield).- ^1H NMR (400 MHz, C_6D_6): δ = 0.138, 0.141, 0.173, 0.224, 0.236 and 0.237 (6s's, $Si(CH_3)_3$ signals), 0.73 (s, CH_3-19), 0.94 (s, CH_3-18), 1.29 (s, 6H, CH_3-26 and CH_3-27), 2.70 (t, J =9.4 Hz, 17-H), 2.95 (dd, J = 13.8 Hz, 4.0 Hz, 5-H), 3.09 ($W_{1/2}$ = 12 Hz, 22-H), 3.35 (d, J = 8.3 Hz, 9-H), 3.93 ($W_{1/2}$ = 12 Hz, 2-H), 3.99 (broad s, 3-H), 6.02 (d, J = 2.1 Hz, 7-H).- IR (CCl_4): 1675 cm^{-1} (unsat. C=O).- $C_{45}H_{92}O_7Si_6$ (913.7), MS: m/z (%) = 897 ([M-CH_3]$^+$, 0.65), 894 ([M-H_2O], 0.7), 807(0.7), 561.3252 ([M-$C_{15}H_{42}O_3Si_3$]$^+$, 88, Calc for $C_{30}H_{53}O_4Si_3$: 561.3208), 171 (21), 147(22), 73 (100).

(20R,22R)-22-Hydroxy-2β,3β,14,20,25-pentakis-(trimethyl-silanyloxy)-5β-cholest-7-en-6-one (13a).
To a solution of **11b** (10 mg, 0.012 mmol) in dry THF (2.0 ml) DIBAH (1.2 M solution in toluene, 75 µl, 0.09 mmol) was added dropwise at -78°C. The mixture was stirred for 4 h, being allowed to warm to 20°C. Usual work-up (Et_2O) gave a crude product which was carefully dried (16 h at 100-200 Pa), dissolved in dry CH_2Cl_2 (2.0 ml), and then treated with MnO_2 (70 mg, freshly prepared[32]) for 16 h at 20°C. Filtration though SiO_2, solvent evaporation and LC (hexanes-ethyl acetate-NEt_3 40:1:0.2) gave **13a** along with traces of its (22S) isomer (8.3 mg, 83%). The d.e. was determined after silyl group removal (vide infra).- IR (CCl_4): 3640, 3500-3350 (OH), 1665 cm^{-1} (unsat. CO).- ^1H NMR (400 MHz, C_6D_6): δ = 0.09, 0.18, 0.23, and 0.36 (5 s's, $Si(CH_3)_3$ signals), 0.78 (s, CH_3-19), 0.93 (s, CH_3-18), 1.17 and 1.19 (2 s's, CH_3-26, CH_3-27), 1.29 (s, CH_3-21), 2.25 (t, J=9 Hz, 17-H), 2.54 (presumably OH), 2.97 (dd, J=3.5 Hz, 13.0 Hz, 5-H), 3.07 ($W_{1/2}$=24 Hz, 9-H), 3.45 ($W_{1/2}$=16 Hz, 22-H), 3.93 ($W_{1/2}$=20 Hz, 2-H), 4.01 ($W_{1/2}$=7 Hz, 3-H), 6.00 (d, J = 2.3 Hz, 7-H).- $C_{42}H_{84}O_7Si_5$ (840.9), MS: m/z (%) = 825.4858 ([M-CH_3]$^+$, 0.7, Calc for $C_{41}H_{81}O_7Si_5$: 825.4828), 735 (4), 664 (1), 651 (4), 645 (1), 635 (1), 561 (100).

Reduction of **13b**.
DIBAH reduction (reaction time 4h) of **13b** as described for **13a** gave a polar product, which was converted to **1a** by MnO_2 oxidation and silyl group cleavage in 81% overall yield.

(20R,22R)-2β,3β,14,20,22,25-Hexahydroxy-5β-cholest-7-en-6-one (1a).
1a (with traces of **12**) was prepared from the sample of **13a** desribed above by silyl ether cleavage (general procedure). Yield after LC (CH_2Cl_2-methanol 5:1) 80%, d.e. = 95% (determined by HPLC (5µm RP-18, methanol-water 3:4)). Formation of **1a** and **12** was secured by TLC and HPLC comparison.-^1H NMR (400 MHz, C_5D_5N): 1.07 (s, CH_3-19), 1.22 (s, CH_3-18), 1.37 (s, CH_3-26, CH_3-27), 1.60 (s, CH_3-21), 2.97 - 3.03 (m, 5-H, 17-H), 3.59 ($W_{1/2}$ = 20 Hz, 9-H), 3.88 ($W_{1/2}$ = 10 Hz, 22-H), 4.14 - 4.25 (2-H, 3-H), 4.72 (s, OH), 5.24 (s, OH), 6.03 (d, J = 5.9 Hz, OH), 6.10 (d, J = 1.8 Hz, OH), 6.18 (d, OH), 6.27 (d, J = 2.2 Hz, 7-H), 6.32 (s, OH).- ^{13}C NMR (100 MHz, C_5D_5N): δ = 17.95 (C-18), 21.16 (C-21), 21.54, 21.76, 24.53 (C-19), 27.52, 30.06, 30.18, 31.83, 32.05, 32.51, 34.46, 38.03, 38.72, 42.71, 48.15 (C-5), 50.14 (C-17), 51.45 (C-13), 68.11 (C-3), 68.20 (C-2), 69.61 (C-25), 76.89 (C-22), 77.59 (C-20), 84.22 (C-14), 121.71 (C-7), 166.60 (C-8), 203.58 (C-6).

2β,3β,14-Tris-(trimethylsilanyloxy)-5β-pregn-7-ene-6,20-dione (10b).
10a was silylated with 3 equiv. of 2,6-lutidine and trimethylsilyl triflate as described for **7a** (81% yield after LC (hexanes-ethyl acetate-NEt_3 10:1:0.1)).- ^1H NMR (400 MHz, $CDCl_3$): δ = 0.08, 0.10, 0.12 (3s's, $Si(CH_3)_3$ signals), 0.56 (s, CH_3-18), 0.92 (s, CH_3-19), 2.13 (s, CH_3-21), 2.50 (dd, J=4.1 Hz, 13.2 Hz, 5-H), 2.96 ($W_{1/2}$ = 8 Hz, 9-H), 3.73 ($W_{1/2}$ = 12 Hz, 2-H), 3.89 (broad s, 3-H), 5.81 (d, J = 2.3 Hz, 7-H).- IR (CCl_4): 1705 (C=O), 1670 cm^{-1} (unsat. C=O).- MS: m/z (%) = 578.3279 ([M]$^+$, Calc for $C_{30}H_{54}O_5Si_3$: 578.3279, 4), 550 (4), 488(4), 466 (34), 185 (91), 73 (100).

(20R,25RS)-22,25-Epoxy-20-hydroxy-27-propyl-2β,3β,14-tris(trimethyl-silanyloxy)-7,22-dien-6-one (**14b/15b**).
To a solution of rac.-**3b** (17.3 µmol, procedure a) in THF (1.0 ml) was added at -78°C under argon a solution of **10b** (10.0 mg, 17.3 µmol) in dry THF (1.0 ml) and the mixture was stirred for 5h being allowed to warm to 20°C. Usual work-up (CH_2Cl_2), followed by MPLC (hexanes-ethyl acetate-NEt_3 10:1:0.1) gave **14b/15b** (5.2 mg, 42%, not completly pure) and **10b** (4.7 mg, 47%).- ^1H NMR (400 MHz, C_6D_6): δ = 0.088, 0.173, 0.221 (3s's, $Si(CH_3)_3$ signals), 0.79 (s, CH_3-18), 0.81 (s, CH_3-18), 0.896 (t, J = 10.0 Hz, CH_3-30 and CH_3-33), 1.21 and 1.24 (2s's, CH_3-19, CH_3-26), 1.46 (s, CH_3-21), 2.93 (dd, J = 1.7, 5.2 Hz, 5-H), 3.05 ($w_{1/2}$ = 16 Hz, 17-H), 3.91 (d, J = 10 Hz, 2-H), 4.00 (broad s, 3-H), 4.55 (broad s, 2*1H, 23-H), 6.00 (broad s, 7-H).- IR (CCl_4): 3580 (OH), 1660 cm^{-1} (C=O).- $C_{39}H_{70}O_6Si_3$ (719.2), MS: m/z (%): 718 ($[M]^+$, 1.2), 628 (1), 579.3303 ($[M-C_9H_{15}O]^+$, 2, Calc for $C_{30}H_{55}O_5Si_3$: 579.3358), 536 (5), 466 (10), 376 (4.8), 185 (29), 184 (27), 147 (33), 73 (100).

(20R,25RS)-2β,3β,14,20,25-Pentahydroxy-27-propyl-5β-cholest-7-ene-6,22-dione (**16a/17a**).
16a/17a were prepared from **10a** and rac.-**3b** as described for **11a**. MPLC (5µm RP-18, CH_3OH-H_2O-CH_3CN 6:3:1) and LC (CH_2Cl_2-CH_3OH 10:1) gave **16a/17a** (68%), which could not be separated.- ^1H NMR (80 MHz, C_5D_5N): δ = 0.89 (t, J = 6.0 Hz, CH_3-30), 1.04 (s, CH_3-19), 1.10 (s, CH_3-18), 1.30 (s, CH_3-26), 1.70 (s, CH_3-21), 2.80-3.70 (5-H, 9-H, 17-H and CH_2-23), 4.00-4.30(2-H and 3-H), 6.19 (d, J = 2.0 Hz, 7-H).

Reduction of **16a/17a**.
16a/17a was reduced with $Li(^tBuO)_3AlH$ as described for **11a**. HPLC (5 µm RP-18, CH_3OH-H_2O 1:1) showed 2 large and 2 small peaks corresponding to the diastereoisomers isomeric at C-22 and C-25. Separation of the C-22 from the C-25 isomers was unsufficient for d.e. determination but allowed the isolation of pure **18a** (34%) and **19a** (34%). Structural assignment and d.e. determination rest on the reduction of pure **17a** (see below).

(20R,22S,25R)-2β,3β,14,20,22,25-Hexahydroxy-26-propyl-5β-cholest-7-en-6-one (**19a**).
mp: 219-221°C (from CH_3OH-H_2O).- ^1H NMR (400 MHz, C_5D_5N): δ = 0.83 (t, J = 7.0 Hz, CH_3-30), 1.07 (s, CH_3-19), 1.23 (s, CH_3-18), 1.39 (s, CH_3-26), 1.73 (s, CH_3-21), 3.01 (dd, J = 3.5 Hz, 13 Hz, 5-H), 3.51-3.65 (9-H and 17-H), 3.94 (broad d, J = 9.5 Hz, 22-H), 4.27 ($w_{1/2}$ = 21 Hz, 2-H), 4.35 ($w_{1/2}$ = 7 Hz, 3-H), 6.24 (d, J = 2.2 Hz, 7-H).- IR (KBr): 1670-1640 cm^{-1} (unsat. C=O).- $C_{30}H_{50}O_7$ (522.7), FAB MS (glycerol): m/z (%) = 523 ($[M+H]^+$, 54), 505 (69), 487 (100), 469 (43), 453 (12).

(20R,22S,25S)-2β,3β,14,20,22,25-Hexahydroxy-27-propyl-5β-cholest-7-en-6-one (**18a**).
^1H NMR (400 MHz, C_5D_5N): identical with the spectrum of **19a**.- IR (KBr): 1640 cm^{-1} (unsat. C=O).- $C_{30}H_{50}O_7$ (522.7), FAB MS (glycerol) m/z (%) = 523 ($[M+H]^+$, 35), 505 (60), 487 (100), 469 (43), 451 (12).

Silylation of **16a/17a**.
The **16a/17a** mixture was silylated as described for **11a** to give 76% of a mixture of C-25 diastereoisomers. Prep. HPLC (5µm Si100, i-octane-dioxane-i-propanol 200:0.20:0.25) gave **17b** (35%) and **16b** (36%), and 6% of fraction containing both of them. Configurational assignment at C-25 is based on HPLC comparison with the sample of **16b** that is described in the following paragraph.- HPLC (5 µm Si100 (Merck), i-octane-i-propanol-dioxane 200:0.25:0.20), retention times: **17b**: 17.6 min, and **16b**: 18.1 min.

Formation of **16b** from **10b** and (S)-**3b**.
Reaction of **10b** with (S)-**3b** (as described above for **10b** and rac.-**3b**) gave **14b** (36%). 33% of **10b** were recovered. Enol ether cleavage and subsequent silylation (**14b** -> **16b**) were performed as described in the **16b/17b** series (see above). Yield: 37% (based on **14b**).

(20R,25S)-27-Propyl-2β,3β,14,20,25-pentakis-(trimethylsilanyloxy)-5β-cholest-7-ene-6,22-dione (**16b**).
^1H NMR (400 MHz, C_6D_6): δ = 0.07, 0.17, 0.19, 0.22 and 0.33 (5s's, $Si(CH_3)_3$ signals), 0.70 (s, CH_3-19), 0.92 (s, CH_3-18), 1.17 (s, CH_3-26), 1.41 (s, CH_3-21), 2.51 ($W_{1/2}$ = 20 Hz, 17-H), 2.57-2.67 and 2.77-2.87 (CH_2-23), 2.94 (dd, J = 3.5 Hz, 13 Hz, 5-H), 3.05 ($w_{1/2}$ =24 Hz,

9-H), 3.91 ($W_{1/2}$ = 20 Hz, 2-H), 4.00 ($W_{1/2}$ = 8 Hz, 3-H), 5.94 (d, J =2.2 Hz, 7-H) and unidentified signals at δ = 0.90 and 1.36.- IR (CCl_4): 1715 (C=O), 1665 cm^{-1} (unsat. C=O).- $C_{45}H_{88}O_7Si_5$ (881.6), MS: m/z (%) = 865.5147 ($[M-CH_3]^+$, 1.0, Calc for $C_{44}H_{85}O_7Si_5$: 865.5142), 823 (2), 775 (3), 753 (2), 685 (3), 651 (2), 561 (100).

(20R,25R)-27-Propyl-2β,3β,14,20,25-pentakis-(trimethylsilanyloxy)-5β-cholest-7-ene-6,22-dione (17b).
^1H NMR (400 MHz, C_6D_6): identical with the spectrum of **16b**, exception: δ = 2.56-2.66 and 2.81-2.91 (CH_2-23), 3.06 ($W_{1/2}$ = 23 Hz, 9-H).- IR (CCl_4): 1715 (C=O), 1665 cm^{-1} (unsat. C=O).- $C_{45}H_{88}O_7Si_5$ (881.6), MS: m/z (%) = 865.5140 ($[M-CH_3]^+$, 0.8, Calc for $C_{44}H_{85}O_7Si_5$: 865.5142), 823 (2), 775 (2), 753 (1), 685 (2), 651 (1), 561 (100).

(20R,25R)-2β,3β,14,20,25-Pentahydroxy-27-propyl-5β-cholest-7-ene-6,22-dione (17a).
17b was desilylated (see general procedure) to give after LC (CH_2Cl_2-MeOH 12:1) **17a** in quantitative yield.- ^1H NMR (80 MHz, C_5D_5N): δ = 0.89 (t, J = 6 Hz, CH_3-30), 1.04, 1.10 (2s's, CH_3-18, CH_3-19), 1.30 (s, CH_3-26), 1.70 (s, CH_3-21), 2.80-3.70 (5-H, 9-H, 17-H, CH_2-23), 4.00-4.30 (2-H, 3-H), 6.19 (d, J = 2.0 Hz, 7-H).- $C_{30}H_{48}O_7$ (520.7), FAB MS (glycerol): m/z (%) = 521 ($[M+H]^+$, 26), 503 (100), 485 (30), 467 (17).

Reduction of 17a.
17a was reduced with $Li(^tBuO)_3AlH$ in THF as described for **11a**. Analytical HPLC (5 µm Si 100, $CHCl_3$-CH_3OH 7:1) showed the presence of 2 peaks in a 16:1 ratio (corresponding to a d.e. = 88% for the reduction of the 22-CO group). The main peak correlated with **19a**, obtained from the reduction of the **16a** /**17a** mixture as described above.

(20R,22R,25S)-22-Hydroxy-26-propyl-2β,3β,14,20,25-pentakis-(trimethyl-silanyloxy)-5β-cholest-7-en-6-one (18b).[33]
Conversion of **16b** to **18b** by DIBAH reduction and subsequent MnO_2 oxidation was performed as described for **11b**-->**13a** (see there). Yield: 77%.- ^1H NMR (400 MHz, C_6D_6): δ = 0.09, 0.18, 0.21, 0.22 and 0.32 (5s's, $Si(CH_3)_3$ signals), 0.78 (s, CH_3-19), 0.92 (s, CH_3-18), 1.21 (s, CH_3-26), 1.31 (s, CH_3-21), 2.28 (t, J = 9.5 Hz, 17-H), 2.36 ("d", OH), 2.96 (dd, J = 3.5 Hz, 13 Hz, 5-H), 3.06 ($W_{1/2}$ =24 Hz, 9-H), 3.45 (dd, J = 3 Hz, 10 Hz, 22-H), 3.92 ($W_{1/2}$ = 21 Hz, 2-H), 4.02 ($W_{1/2}$ = 8 Hz, 3-H), 6.00 (d, J = 2.2 Hz, 7-H).- IR (CCl_4): 3630-3300 (OH), 1665 cm^{-1} (unsat. C=O).- $C_{45}H_{90}O_7Si_5$ (883.6), MS: m/z (%) = 867 ($[M-CH_3]^+$, 0.3), 795 (1), 777 (1), 735 (2), 707 (1), 687 (1), 651 (10), 561 (100).

(20R,22R,25R)-22-Hydroxy-27-propyl-2β,3β,14,20,25-pentakis-(trimethyl-silanyloxy)-5β-cholest-7-en-6-one (19b).[33]
Conversion of **17b** to **19b** by DIBAH reduction and subsequent MnO_2 oxidation was performed as described for **11b**-->**13a** (see there). Yield: 71%.- ^1H NMR (400 MHz, C_6D_6): δ = 0.10, 0.17, 0.22, 0.23 and 0.34 (5s's, $Si(CH_3)_3$ signals), 0.78 (s, CH_3-19), 0.92 (s, CH_3-18), 1.19 (s, CH_3-26), 1.30 (s, CH_3-21), 2.25 (t, J = 9.5 Hz, 17-H), 2.33 ("d", OH), 2.95 (dd, J = 3.5 Hz, 13 Hz, 5-H), 3.06 ($W_{1/2}$ = 24 Hz, 9-H), 3.44 ($W_{1/2}$ = 18 Hz, 22-H), 3.92 ($W_{1/2}$ = 20 Hz, 2-H), 4.00 ($W_{1/2}$ = 8 Hz, 3-H), 6.00 (d, J = 2.2 Hz, 7-H).- IR (CCl_4): 3630-3300 (OH), 1665 cm^{-1} (unsat. C=O).- $C_{45}H_{90}O_7Si_5$ (883.6), MS: m/z (%) = 867 ($[M-CH_3]^+$, 1), 825 (1), 777 (1), 708 (2), 687 (1), 651 (2), 634 (4), 631 (5), 561 (36), 509 (44), 493 (40), 440 (32), 421 (100).

(20R,22R,25S)-2β,3β,14,20,22,25-Hexahydroxy-27-propyl-5β-cholest-7-en-6-one (18c).
18b was desilylated (general procedure) to give **18c** (83% yield). The d.e. (94%) of the DIBAH reduction[33] was determined by HPLC (5 µm RP-18, MeOH-H_2O 3:4).- ^1H NMR (400 MHz, C_5D_5N): δ = 0.85 (t, J = 7.5 Hz, CH_3-30), 1.08 (s, CH_3-19), 1.22 (s, CH_3-18), 1.36 (s, CH_3-26), 1.61 (s, CH_3-21), 3.02 ($W_{1/2}$ = 20 Hz, 5-H and 17-H), 3.57 ($W_{1/2}$ = 23 Hz, 9-H), 3.92 ($W_{1/2}$ = 16 Hz, 22-H), 4.20 ($W_{1/2}$= 21 Hz, 2-H), 4.27 ($W_{1/2}$ = 7 Hz, 3-H), 6.25 (d, J = 2.0 Hz, 7-H), signals at δ = 0.89 (t), 1.38 (q) and 3.57(m), probably traces of Bu_4NX.- IR(KBr): 1665-1635 cm^{-1} (unsat. C=O).- $C_{30}H_{50}O_7$ (522.7), FAB-MS (glycerol): m/z (%) = 523 ($[M+H]^+$, 57), 505 (87), 487 (100), 469 (55).

(20R,22R,25R)-2β,3β,14,20,22,25-Hexahydroxy-27-propyl-5β-cholest-7-en-6-one (19c).
19b was desilylated (general procedure) to give **19c** (83% yield). The d.e. (94%) of the DIBAH reduction[33] was determined by HPLC (5 µm RP-18, MeOH-H_2O 3:4).- ^1H NMR (400 MHz, C_5D_5N): δ =

0.84 (t, J = 7.0 Hz, CH_3-30), 1.07 (s, CH_3-19), 1.22 (s, CH_3-18), 1.35 (s, CH_3-26), 1.61 (s, CH_3-21), 3.01 ($w_{1/2}$ = 22 Hz, 2H, 5-H and 17-H), 3.52 ($w_{1/2}$ = 23 Hz, 9-H), 3.90 ($w_{1/2}$ = 17 Hz, 22-H), 4.19 ($w_{1/2}$ = 23 Hz, 2-H), 4.25 ($w_{1/2}$ = 8 Hz, 3-H), 6.26 (d, J = 2.0 Hz, 7-H), signals at 0.89 (t), 1.38 (q) and 3.57 (m), traces of Bu_4NX.- IR(KBr): 1645 cm^{-1} (unsat. C=O).- $C_{30}H_{50}O_7$ (522.7), FAB MS (glycerol): 523 ($[M+H]^+$, 42), 505 (72), 487 (100), 469 (43).

(20R,25S)-27-(3-(tert.-Butyl-diphenyl-silanyloxy)-propyl)-22,25-epoxy-20-hydroxy-2β,3β,14-tris-(trimethylsilanyloxy)-5β-cholest-7,22-dien-6-one (**20c**).

To a solution of **3d** (96.1 µmol, procedure b) in dry THF (2.0 ml) a solution of **10b** (50.5 mg, 87.4 µmol) in THF (1.0 ml) was added at -78°C, and the mixture was stirred for 4.2 h while warming up to 20°C. Usual work up (Et_2O) followed by LC (hexanes-ethyl acetate-NEt_3 12:1:0.1) gave **3d** (23.3 mg, 56%), **10a** (17.3 mg, 34%), **20c** (36.8 mg, 43 %).- ^1H NMR (400 MHz, $CDCl_3$): δ = 0.04, 0.06 and 0.10 (3s's, $Si(CH_3)_3$ signals), 0.78 (s, CH_3-19), 0.92 (s, CH_3-18), 1.02 (s, $C(CH_3)_3$), 1.22 (s, 6H, CH_3-21 and CH_3-26), 2.93 ($W_{1/2}$ = 12 Hz, 5-H), 3.37 ($W_{1/2}$ = 12 Hz, 9-H), 3.63 (t, J = 6.1 Hz, CH_2-30), 3.71 (m, $w_{1/2}$ = 16 Hz, 2-H), 3.88 (broad s, 3-H), 4.52 (t, J = 2.2 Hz, 23-H), 5.78 (d, J = 2.1 Hz, 7-H), 7.33-7.42 and 7.62-7.66 (Ar-H).- IR (CCl_4): 3600-3200 (OH), 1715 (enol ether), 1670 (unsat. C=O), 1640, 1590 cm^{-1} (C=C).-$C_{55}H_{88}O_7Si_4$ (973.6), MS: m/z (%) = 873 ($[M-C_4H_9-H_2O-CH_3]^+$, 0.3), 799 (0.3), 725 (0.4), 605 (0.9), 577 (3), 503 (10), 466 (15), 429(19), 355 (25), 337 (78), 199(100), 73 (53).

Addition of (±)-**3d** to **10a**.

Reaction of (±)-**3d** with **10a**, as described for the synthesis of **11a**, followed by LC (ethyl acetate-ethanol 25:1) gave a mixture of **22a** and **22b** (75%, based on **10a**). Prep. HPLC (i-octane-$CHCl_3$-ethanol 15:15:1) gave **22b** (35%), **22a** (33%), and a mixture of both diastereomeres (7%).

Addition of (S)-**3d** to **10a**.

Reaction of (S)-**3d** with **10a**, as described for the synthesis of **11a**, followed by LC (ethyl acetate-ethanol 30:1) gave **22a** (50%, based on **10a**). The specimen of **22a** obtained in this experiment was identical (HPLC) with **22a** obtained in the preceding experiment.- HPLC (5 µm Si 100, i-octane-$CHCl_3$-ethanol 5:5:1), retention times: **22b**: 16 min and **22a**: 18 min.

(20R,25S)-27-[3-(tert.-Butyl-diphenyl-silanyloxy)-propyl]-2β,3β,14,20,25-5β-pentahydroxy-cholest-7-ene-6,22-dione (**22a**).

^1H-NMR (400 MHz, $CDCl_3$): δ = 0.85 (s, CH_3-19), 0.98 (s, CH_3-18), 1.03 (s, 9H, Si^tBu), 1.15 (s, CH_3-26), 1.42 (s, CH_3-21), 2.41 (dd, J = 3.5 Hz, 13 Hz, 5-H), 2.54-2.70 (17-H, CH_2-23), 3.00 ($w_{1/2}$ = 23Hz, 9-H), 3.65 (t, J = 6.0 Hz, CH_2-30), 3.86 ($w_{1/2}$ = 23 Hz, 2-H), 4.01 ($w_{1/2}$ = 9 Hz, 3-H), 4.09 (s, OH, exchanges with D_2O), 5.78 (d, J = 2.2 Hz, 7-H), 7.38 and 7.66 (Ar-H), unidentified small signals at δ = 0.88 and 1.24.- IR ($CHCl_3$): 3650-3200 (OH), 1695 (C=O), 1650 cm^{-1} (unsat. C=O).- Neither EI nor FAB MS could be obtained.

(20R,25R)-27-[3-(tert.-Butyl-diphenylsilanyloxy)-propyl]-2β,3β,14,20,25-penta-hydroxy-5β-cholest-7-ene-6,22-dione (**22b**) .

^1H-NMR (400 MHz, $CDCl_3$): δ = 0.87 (s, CH_3-19), 0.99 (s, CH_3-18), 1.03 (s, 9H, Si^tBu), 1.13 (s, CH_3-26), 1.43 (s, CH_3-21), 2.42 (dd, J= 3.5 Hz, 13 Hz, 5-H), 2.55-2.68 (17-H, CH_2-23), 3.00 ($w_{1/2}$ = 23 Hz, 9-H), 3.65 (t, J = 6.0Hz, CH_2-30), 3.86 ($W_{1/2}$ = 22 Hz, 2-H), 4.02 ($w_{1/2}$ = 9 Hz, 3-H), 4.09 (s, OH, exchanges with D_2O), 5.79 (d, J = 2.2 Hz, 7-H), 7.38 and 7.66 (Ar-H), unidentified small signals at δ = 0.88 and 1.25.- IR ($CHCl_3$): 3650-3200 (OH), 1700 (C=O), 1655 cm^{-1} (unsat. C=O).-Neither EI nor FAB MS could be obtained.

(20R,25R)-27-[3-(tert.-Butyl-diphenyl-silanyloxy)-propyl]-2β,3β,14,20,25-pentakis-(trimethyl-silanyloxy)-5β-cholest-7-ene-6,22-dione (**22d**).

22b was silylated as described for **11a**. LC (hexanes-ethyl acetate-NEt_3 30:1:0.15) gave **22d** in 79% yield.- ^1H NMR (400 MHz, C_6D_6), δ = 0.06, 0.17, 0.18, 0.24 and 0.34 (5s's, $Si(CH_3)_3$ signals), 0.70 (s, CH_3-19), 0.92 (s, CH_3 -18), 1.16 (s, CH_3-26), 1.20 (s, Si^tBu), 1.41 (s, CH_3-21), 2.51 ($w_{1/2}$ = 19Hz, 17-H), 2.56-2.67 and 2.75-2.86 (CH_2-23), 2.96 (dd, J = 3.5 Hz, 13 Hz, 5-H), 3.07 ($w_{1/2}$ = 23 Hz, 9-H), 3.69 (t, J = 6.0 Hz, CH_2-30), 3.92 ($w_{1/2}$ = 20 Hz, 2-H), 4.00 ($w_{1/2}$ = 8 Hz, 3-H), 5.94 (d, J = 2.2 Hz, 7-H), 7.26 and 7.79 (Ar-H).- IR (CCl_4): 1715 (C=O), 1665 cm^{-1} (unsat. CO).- $C_{61}H_{106}O_8Si_6$ (1135.9), MS: m/z (%) = 1119 ($[M-CH_3]^+$, 2),

1077.5817 ([M-C_4H_9]$^+$, 13, Calc for $C_{57}H_{97}O_8Si_6$: 1077.5808), 987.5 (18), 897.5 (13), 807.5 (7), 561 (100).

(20R,25S)-27-[3-(tert.-Butyl-diphenyl-silanyloxy)-propyl]-2β,3β,14,20,25-pentakis-(trimethyl-silanyloxy)-5β-cholest-7-ene-6,22-dione (**22c**).

a) **22a** was silylated as described for **11a** to give, after LC (see preceding experiment) **22c** in 71% yield. b) **22c** was also obtained from **20a** by (i) opening with HCl (see formation of **11a**), (ii) silylation, (iii) LC (see above) in 78% overall yield.- ^{1}H NMR (400 MHz, C_6D_6), δ = 0.07, 0.18, 0.19, 0.25 and 0.35 (5s's, $Si(CH_3)_3$ signals), 0.70 (s, CH_3-19), 0.92 (s, CH_3-18), 1.17 (s, CH_3-26), 1.21 (s, Si^{t}Bu), 1.42 (s, CH_3-21), 2.51 ($W_{1/2}$ = 21 Hz, 17-H), 2.55-2.64 and 2.80-2.91 (CH_2-23), 2.95 (dd, J = 3.5 Hz, 13 Hz, 5-H), 3.07 ($W_{1/2}$ = 22 Hz, 9-H), 3.69 (t, J = 6.0 Hz, CH_2-30), 3.92 ($W_{1/2}$ = 20Hz, 2-H), 4.00 ($W_{1/2}$ = 7 Hz, 3-H), 5.94 (d, J = 2.2 Hz, 7-H), 7.27 and 7.80 (Ar-H).- IR (CCl_4): 1715 (C=O), 1665 cm^{-1} (unsat. C=O).- $C_{81}H_{106}O_8Si_6$ (1135.9), MS: m/z (%) = 1119 ([M-CH_3]$^+$, 2), 1077.5798 ([M-C_4H_9]$^+$, 17, Calc for $C_{57}H_{97}O_8Si_6$: 1077.5808), 987.5 (10), 897.5 (17), 807.5 (9), 561 (100).

(20R,22R,25R)-2β,3β,14,20,22,25-Hexahydroxy-27-(3-hydroxy-propyl)-5β-cholest-7-en-6-one (**21b**).

21b was obtained from **22d** (as described for **1a**) by (i) DIBAH reduction in THF, (ii) oxidation with MnO_2 in CH_2Cl_2, (iii) desilylation, (iv) LC (CH_2Cl_2-MeOH 5:1) in 43% yield.- ^{1}H NMR (400 MHz, C_5D_5N), δ = 1.08 (s, CH_3-19), 1.22 (s, CH_3-18), 1.37 (s, CH_3-26), 1.59 (s, CH_3-21), 2.98 - 3.07 (5-H, 17-H), 3.59 ($W_{1/2}$ = 20 Hz, 9-H), 3.89 ($W_{1/2}$ = 14 Hz, CH_2-30, 22-H), 4.15 - 4.26 (2-H, 3-H), 6.26 (d, J = 2.2 Hz, 7-H).- IR (KBr):1635 cm^{-1} (unsat. C=O).- $C_{30}H_{50}O_8$ (538.7), FAB MS (glycerol): m/z (%) = 539 ([M+H]$^+$, 17), 521 (32), 503 (42), 487 (54), 485 (100), 469 (22), 467 (23).

(20R,22R,25S)-2β,3β,14,20,22,25-Hexahydroxy-27-(3-hydroxy-propyl)-5β-cholest-7-en-6-one (**21a**).

21a was obtained from **22c** by (i) DIBAH reduction in toluene (cf.**7f**, procedure 2), (ii) oxidation with MnO_2 in CH_2Cl_2, (iii) desilylation, (iv) LC (CH_2Cl_2-MeOH 8:1) in 83% overall yield.- ^{1}H NMR spectrum (400 MHz, C_5D_5N) was identical with that of **21b** with the exception of : δ = 3.87 ($W_{1/2}$ = 11 Hz, CH_2-30, 22-H).- IR (KBr): 1635 cm^{-1} (unsat. C=O).- $C_{30}H_{50}O_8$ (538.7), FAB MS (DMSO-glycerol): m/z (%) = 539 ([M+H]$^+$, 14), 521 (33), 503 (45), 487 (53), 485 (100), 469 (24), 467 (22).

Acknowledgements - It is a pleasure to thank Dr.D.Müller, Dr.W.Dietrich, and their colleagues for the MS and NMR spectra. Financial support by the Deutsche Forschungsgemeinschaft, the Minister für Wissenschaft und Forschung des Landes Nordrhein Westfalen (PhD grant to R.K.), and the Fonds der Chemischen Industrie is gratefully acknowledged.

References and Notes

[1] Preliminary Communications: a) U.Hedtmann, P.Welzel, *Tetrahedron Lett.* **1985**, *26*, 2773-2774; b) U.Hedtmann, K.Hobert, R.Klintz, P.Welzel, J.Frelek, M.Strangmann-Diekmann, A.Klone, O.Pongs, *Angew.Chem.* **1989**, *101*, 1543-1545, *Angew.Chem.Int.Ed.Engl.* **1989**, *28*, 1515-1518.

[2] Permanent Address: Department of Chemistry, Warszawa University, ul.Pasteura 1, PL-02-093 Warszawa (Poland)

[3] Permanent Address: Institute of Organic Chemistry and Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia (Bulgaria)

[4] J.Koolman (ed.): *Ecdysone - From Chemistry to Mode of Action*, Georg Thieme Verlag, Stuttgart, 1989.

[5] For most recent work, see a) G.Feigl, M.Gram, O.Pongs, *Nucleic Acids Res.* **1989**, *17*, 7167-7177; b) M.Strangmann-Diekmann, A.Klone, A.Ozyhar, F.Kreklau, H.-H.Kiltz, U.Hedtmann, P.Welzel, O.Pongs, *Eur.J.Biochem.* **1990**, *189*, 137-143; c) K.C.Burtis, C.S.Thummel, C.W.Jones, F.D.Karim, D.S.Hogness, *Cell* **1990**, *61*, 85-99; d) C.S.Thummel, K.C.Burtis, D.S.Hogness, *Cell* **1990**, *61*, 101-111; e) W.A.Segraves, D.S.Hogness, *Genes Devel.* **1990**, *4*, 204-219.

[6] M.Ashburner, *Dev.Biol.* **1974**, *39*, 141-157; M.Ashburner, C.Chihara, P.Meltzer, G.Richards, *Cold Spring Harbor Symp.Quant.Biol.* **1974**, *38*, 655-662.
[7] Reviews: a) S.Green, P.Chambon, *Nature (London)*, **1986** , *324*, 209-252; b) H.Gronemeyer (ed.): *Affinity Labelling and Cloning of Steroid and Thyroid Hormone receptors*, Ellis Horwood, VCH, Chichester/Weinheim, 1988.
[8] For recent experiments of this type, see a) D.Guédin-Vuong, Y.Nakatani, B.Luu, G.Ourisson, *Tetrahedron Lett.* **1985**, *26*, 5959-5962 and *Croat.Chem.Acta* **1985**, *58*, 547-557, b) cf. B.A.Sage, D.H.S.Horn, T.M.Landon, J.D.O'Connor, *Arch.Ins.Biochem.Physiol.* **1986**, 25-33, c) P.Cherbas, L.Cherbas, S.-S.Lee, K.Nakanishi, *Proc.Natl.Acad.Sci.USA* **1988**, *85*, 2096-2100.
[9] M.Lombest, M.Claire, P.Lustenberger, A.Michaud, M.-E.Rafestin-Oblin, *J.Biol.Chem.* **1987**, *262*, 8121-8127.
[10] P.Cherbas, D.A.Trainor, R.J.Stonard, K.Nakanishi, *J.Chem.Soc., Chem.Commun.* **1982**, 1307-1308.
[11] Review: R.Bergamasco, D.H.S.Horn in J.A.Hoffmann (ed.): *Progress in Ecdysone Research*, Elsevier/North Holland Biomedical Press, Amsterdam 1980, p. 299-324.
[12] Review: T.Kametani, M.Tsubuki, lc.cit.[4], p.74-96.
[13] a) G.Hüppi, J.B.Siddall, *J.Am.Chem.Soc.* **1967**, *89*, 6790-6792 ; b) H.Hikino, K.Mohri, Y.Hikino, S.Arihara, T.Takemoto, H.Mori, K.Shibata, *Tetrahedron* **1976**, *32*, 3015-3021, and previous work cited therein; c)M.Adamczyk, E.K.Dolence, D.S.Watt, M.R.Christy, J.H.Reibenspies, O.P.Anderson, *J.Org.Chem.* **1984**, *49*, 1378-1382; d) G.M.Segal, S.Zh.Sydykov, I.V.Torgov, *Izv.Akad.Nauk.SSR Ser.Khim.* **1982**, 317-325.
[14] D.J.Cram, F.A.Abd Elhafez, *J.Am.Chem.Soc.* **1962**, *74*, 5828-5835; J.Mulzer, *Nachr.Chem.Tech.Lab.* **1984**, *32*, 16-18.
[15] T.Kametani, M.Tsubuki, K.Higurashi, T.Honda, *J.Org.Chem.* **1986**, *51*, 2932-2939.
[16] U.Kerb, R.Wiechert, A.Furlenmeier, A.Fürst, *Tetrahedron Lett.* **1968**, 4277-4280.
[17] Personal communication of Prof.Wiechert, see also N.K.Chaudhuri, R.Nickolson, H.Kimball, M.Gut, *Steroids* **1970**, *15*, 525-539.
[18] R.Klintz, Dissertation, Ruhr-Universität Bochum, 1990.
[19] 1,3-Dimethyltetrahydro-2(1H)-pyrimidinon, T.Mukhopadhyay, D.Seebach, *Helv.Chim.Acta* **1982**, *65*, 385-391.
[20] see E.Juraisti, E.L.Eliel, *Tetrahedron Lett.* **1977**, 543-546; K.S.Kyler, D.S.Watt, *J.Org.Chem.* **1981**, *46*, 5182-5188.
[21] J.Redpath, F.J.Zeelen, *Chem.Soc.Rev.* **1983**, *12*, 75-98.
[22] D.M.Piatak, J.Wicha, *Chem.Rev.* **1978**, *78*, 199-241.
[23] U.Hedtmann, Dissertation, Ruhr-Universität Bochum, 1987.
[24] Less clear in this respect is the observation that even on reduction of **7a** with $LiAlH(O^tBu)_3$ in the presence of TMEDA or HMPA and with Bu_4NBH_4 **5a** was the main product;[23] cf. J.A.Katzenellenbogen, S.B.Bowlus, *J.Org.Chem* **1972**, *38*, 627-632; M.T.Reetz, *Angew.Chem.* **1984**, *96*, 542-555, *Angew.Chem.Int.Ed.Engl.* **1984**, *23*, 556; E.K.Dolence, M.Adamczyk, D.S.Watt, G.B.Russell, D.H.S.Horn, *Tetrahedron Lett.* **1985**, *26*, 1189-1192; M.Bhupathy, T.Cohen, *Tetrahedron Lett.* **1985**, *26*, 2619-2622; Y.Yamamoto, K.Maruyama, *J.Am.Chem.Soc.* **1985**, *107*, 6411-6413.
[25] cf. X.Chen, E.R.Hortelano, E.L.Eliel, S.V.Frye, *J.Am.Chem.Soc.* **1990**, *112*, 6130-6131.
[26] E.J.Corey, H.Cho, C.Rücker, D.H.Hua, *Tetrahedron Lett.* **1981**, *22*, 3455-3458.
[27] This silylation method gave more reliable results than the previously published with N,O-bis(trimethylsilyl)acetamide method.[18a]
[28] N.T.Anh, *Top.Curr.Chem.* **1980**, *88*, 145-162; M.Cherest, H.Felkin, N.Prudent, *Tetrahedron Lett.* **1968**, 2199-2204.
[29] see ref.[16]
[30] J.Frelek, Z.Majer, A.Perkowska, G.Snatzke, I.Vlahov, U.Wagner, *Pure Appl.Chem.* **1985**, *57*, 441-451.
[31] Assignment by comparison with the spectrum of **1a**.
[32] J.Attenburrow, A.F.B.Cameron, J.H.Chapman, R.M.Evans, B.A.Hems, A.B.A.Jansen, T.Walker, *J.Chem.Soc.* **1952**, 1094-1111.
[33] **18b** and **19b**, respectively, contained small amounts of the respective (22S) isomer formed in the DIBAH reduction step. The d.e. was determined after silyl group removal.