Efficient Stereocontrolled Access to 15- and 16-Hydroxy Steroids

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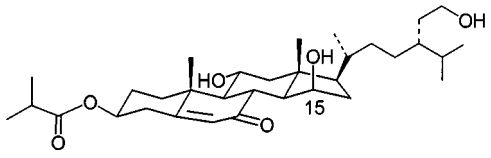
Four epimeric 15- and 16-hydroxy steroids have been stereoselectively synthesized from *epi*-androsterone. The key intermediate is the 3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorchol-16-en-22-ol (**10**), which allows both efficient D-ring functionalization and the possibility of facile side-chain construction. In the course of this synthesis, we have found that the stereochemical outcome of the C-15 carbonyl reduction is strongly dependent on the C-16 and C-17 hybridization.

Introduction

In recent years, the discovery of interesting polyhydroxy steroids, mainly isolated from marine sources,[1] has stimulated the quest for new stereoselective methods capable of introducing hydroxy groups into the steroidal framework.[2] Surprisingly, only a few synthetic efforts have been devoted to C-15 and C-16 D-ring hydroxylation.[3] A literature survey of recent syntheses has revealed that C-15 hydroxylation generally follows stereoselective introduction of the C-17 side chain,[4] while C-16 hydroxylation is efficiently performed starting from 17-oxo steroids[5a] or 16,17-epoxypregnanes.[5b−5d] Unfortunately, none of these methods allows the straightforward construction of various C-17 side chains.

We focused our attention on hydroxylation of the D-ring methylene units as many biologically active steroids possess hydroxy groups linked at these positions.[6] In particular, in the highly cytotoxic steroidal alkaloids cephalostatins,[7] as well as in the oligoglycoside OSW-1,[8] the simultaneous presence of a ketal or a carbonyl group at C-22 and a hydroxy group at C-15 has been hypothesized to be responsible for their potent antitumor activities.[9] Compounds hydroxylated at C-16 also exhibit interesting biological properties. Examples are the oogoniols (e.g. **1**),[10] sex hormones of the water mould *Achlya*, the shark repellent pavonins (e.g. **2**),[11] and the spermatostatic glycoside **3**,[12] isolated from the coelenterate *Sinularia crispa*.

Starfish undoubtedly appear to be the richest source of polyhydroxy steroids.[1][13] Such compounds have been found in almost all the species examined and more than one hundred of them have been reported to date. The vast majority of them shows hydroxylation mainly in positions 3β, 6α (or 6β), 8β, 15α (or 15β), and 16β[13] (e.g. **4**,[14a] **5**,[14b] **6**[14c]). Although largely uninvestigated, the biological activities of these compounds could well be important and useful in physiology and medicine.[6][15]



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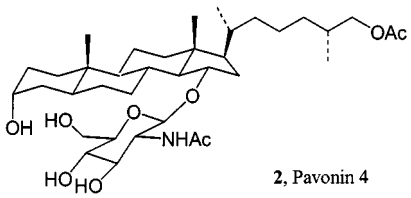
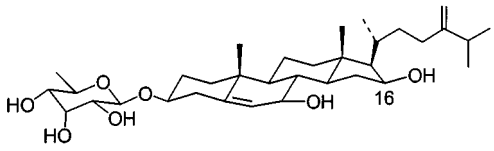


2, Pavonin 4



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With the aim of producing simple model compounds for use in biological studies, and as part of our program devoted to the synthesis of marine steroids and their analogues,[2d,14c,16] we have endeavoured to develop a simple method for D-ring oxy functionalization. In this paper, we report an efficient procedure allowing stereoselective C-15 and C-16 hydroxylation of a simplified steroidal model. The chosen substrate can easily be transformed, in a two-step sequence, into a C-22 aldehyde, which represents a versatile intermediate for the subsequent construction of a wide range of side chains.[17]

Results and Discussion

C-16 Hydroxylation

Commercially available *epi*-androsterone (**7**) was first protected as its *tert*-butyldimethylsilyl (TBS) ether. Silylated **8** was obtained in 96% yield (Scheme 1).[18]

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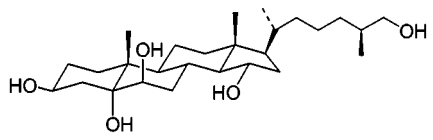

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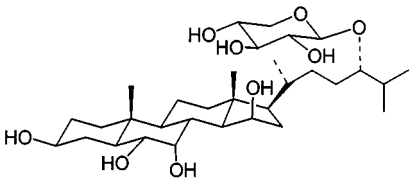
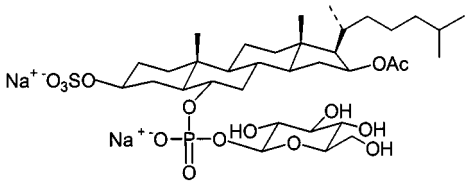


5, Amurensoside D



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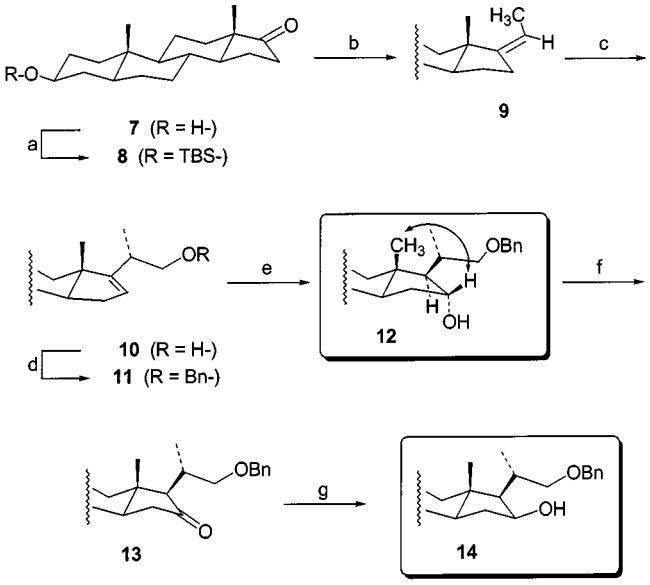


Scheme 1.Reagents and conditions: (a) 1.4 equiv. DBU, 1.2 equiv. TBSCl, CH_2Cl_2, room temp., overnight, 96%; (b) 2.7 equiv. *t*BuOK, 3.0 equiv. $EtPPh_3Br$, THF, 3 h, reflux, 85%; (c) 5.7 equiv. paraformaldehyde, 0.1 equiv. $BF_3{\cdot}OEt_2$, CH_2Cl_2, room temp., 0.1 h. 75%; (d) 3.0 equiv. NaH, 4.0 equiv. BnBr, 0.1 equiv. TBAI, THF, reflux, overnight, 68%; (e) 1) 3.8 equiv. $BH_3{\cdot}SMe_2$, THF, 0°C → room temp., overnight, 2) HOO^-, reflux, 1 h, 60%; (f) 2.0 equiv. PDC, molecular sieves, 1 h, 100%; (g) 2.0 equiv. $LiAlH_4$, THF, 80%

The C-17 side chain was then attached to the protected alcohol following well-established vitamin D chemistry.[17b] The 17-oxo steroid was thus converted into the (*Z*)-17(20)-ethylidene steroid **9** in 85% yield by means of a Wittig reaction.[19] An ene reaction of **9** with paraformaldehyde[19] in the presence of a catalytic amount of boron trifluoride−diethyl ether afforded stereospecifically the alcohol **10** in 75% yield. The 3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorchol-16-en-22-ol (**10**) was benzylated in the conventional way, affording the ether **11** in 68% yield.

The required hydroxy functionality at C-16 was introduced by treating **11** with borane−dimethyl sulfide, followed by oxidation with alkaline hydrogen peroxide, giving **12** in 60% yield (see Experimental Section). The 16α configuration of the hydroxy group was confirmed by comparing the ^{1}H-NMR resonances of 16-H and 18-H_3 with those of other 16α-hydroxy steroids.[20] Moreover, a ROESY[21] experiment on compound **12** (Scheme 1) showed a cross-peak between the signals at δ = 4.09 (16-H) and 0.68 (18-H_3), confirming the 22-(benzyloxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorcholan-16α-ol structure.

The C-16 epimer **14** was easily obtained by a two-step inversion. Treatment of alcohol **12** with PDC[22] conveniently afforded the ketone **13** in quantitative yield, which was stereoselectively reduced with $LiAlH_4$[23] to provide the required 16β-OH sterol **14** in 80% yield (*de* > 97%, ^{1}H-NMR analysis).

C-15 Hydroxylation

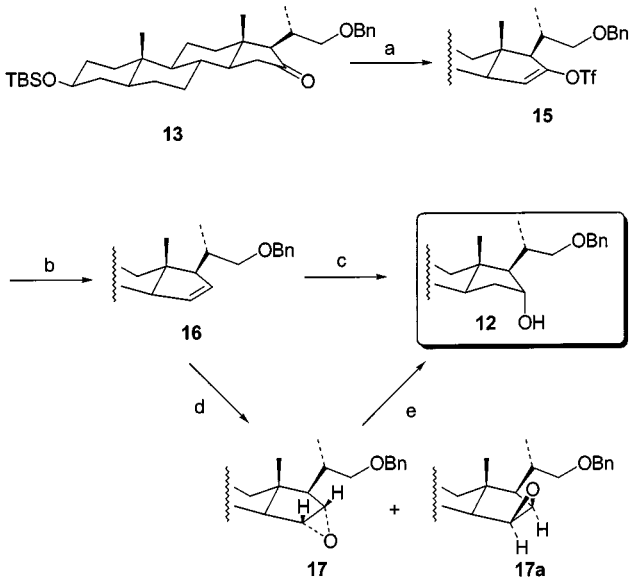
A number of different synthetic methods for C-15 oxygenation have been reported for 17-alkylated steroids.[24] The standard procedure involves Δ^{14} hydroboration to give a 15α alcohol. The synthesis of Δ^{14} sterols is currently achieved mainly through the conversion of $\Delta^{5,7}$-sterols to $\Delta^{7,14}$-sterols under acidic conditions.[25]

In view of the limited scope of the existing methods, we considered various routes for the stereoselective C-15 functionalization of a synthetically flexible steroidal precursor. In our first approach (Scheme 2), we explored the possibility of converting ketone **13** into the alkene **16** and then introducing the 15-OH group regio- and stereoselectively.

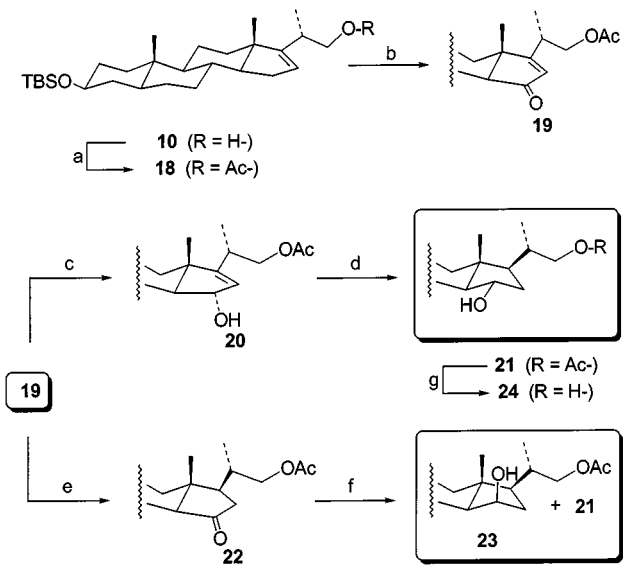
The key Δ^{15} steroid **16** was easily synthesized in two steps using palladium(0)-mediated deoxygenation methodology. Thus, kinetic enolization of ketone **13** with lithium bis(trimethylsilyl)amide [$LiN(TMS)_2$] and quenching of the enolate with *N*-phenyltrifluoromethanesulfonimide[26] [$PhN(SO_2CF_3)_2$] gave the stable enol triflate **15**. Deoxygenation of the latter with tributyltin hydride (Bu_3SnH) and tetrakis(triphenylphosphane)palladium(0)[27] afforded 22-(benzyloxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorchol-15-ene (**16**) in 58% yield (two steps).

Hydroboration−oxidation of the alkene **16** proved to be poorly regio- and stereoselective. From the complex mixture of isomers produced, the previously synthesized 16α alcohol **12** was isolated only in low yield. In view of this, an alternative strategy was considered. **16** was epoxidized with *m*-chloroperbenzoic acid to give two diastereomeric oxiranes **17** and **17a** in an 82:18 ratio as a separable mixture.

The stereochemical assignment of **17** was accomplished through a combination of COSY-45, HETCOR, and ROESY[21] techniques. In particular, the ROESY spectrum showed two key cross-peaks (Figure 1): a correlation between the signals at δ = 3.21 (15-H) and 0.92 (18-H_3) and



Scheme 2. Reagents and conditions; (a) 3.0 equiv. $LiN(TMS)_2$, 2.4 equiv. $PhN(SO_2CF_3)_2$, THF, −78°C → room temp., 75%; (b) 4.4 equiv. LiCl, 0.1 equiv. $Pd(PPh_3)_4$, 2.8 equiv. Bu_3SnH, THF, reflux, 4 h, 77%; (c) 1) 3.8 equiv. $BH_3{\cdot}SMe_2$, 0°C → room temperature, overnight, 2) HOO^-, reflux, 1 h, 6% of **12** plus other unidentified isomers; (d) 2.0 equiv. *m*CPBA, CH_2Cl_2, 0°C → room temp., 3 h (**7**: 46%, **7a**: 10%); (e) 3.0 equiv. $LiAlH_4$, reflux, 25% of **12** plus other unidentified isomers



Scheme 3. Reagents and conditions: (a) equiv. pyridine, 3.8 equiv. $(CH_3CO)_2O$, 0.04 equiv. DMAP, CH_2Cl_2, room temp., 3 h, 100%; (b) 19 equiv. $CrCO_3$, 19 equiv. dimethylpyrazole, CH_2Cl_2, −40°C → room temp., 6 h, 42%; (c) 1.0 equiv. $CeCl_3$, 1.0 equiv. $NaBH_4$, THF/MeOH, 2 h, 100%; (d) H_2, Pt/C, AcOEt, overnight, 98% (e) H_2, Pt/C, EtOAc, overnight, 72%; (f) 2.8 equiv. $NaBH_4$, EtOH/THF, 3 h (**23**: 68%, **21**: 11%); (g) 0.8 equiv. K_2CO_3, MeOH, 2h, 72%

another between the signals at δ = 3.21 (15-H) and 1.96

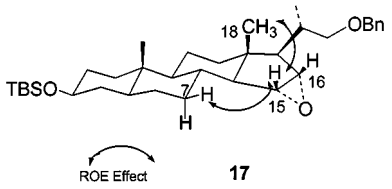


Figure 1

(7β-H_{eq}), supporting the α configuration of the epoxide **17**.

Subsequent reduction of **17** with $LiAlH_4$[28] gave the unwanted 16α-hydroxy derivative **12**. In the hope of reversing the regioselectivity of the reductive step we explored the use of alternative reducing reagents (Superhydride®, DIBAL-H), but no improvements were observed.

Attention was then turned to the Δ^{15} steroid **10** once more. In order to introduce the oxygen function at C-15, we planned a short three-step strategy involving chromium-mediated allylic oxidation followed by two reductive steps (C-15 carbonyl reduction and Δ^{16} hydrogenation, Scheme 3).

The free OH group in **10** was thus quantitatively protected as acetate (**10** → **18**). CrO_3/dimethylpyrazole[29] allylic oxidation then afforded the Δ^{16}-15-oxo compound **19** in 42% yield.[30] Reduction of the enone under Luche conditions[31] and subsequent Δ^{16} hydrogenation produced isomerically pure 15α alcohol **21** in 98% yield (two steps, *de* > 97%, ^{1}H-NMR analysis). Surprisingly, the inverse reaction sequence (platinum-mediated hydrogenation of **19** followed by $NaBH_4$ reduction) furnished the 15β alcohol **23** accompanied by its epimer **21** in an 88:12 separable mixture (other isomers were not detected).

The configuration at C-15 in **21** and **23** was assigned on the basis of a ROESY experiment on **21**. This showed a key cross-peak between the 18-CH_3 group (δ = 0.70) and the proton at C-15 (δ = 3.96), establishing the α configuration of the 15-hydroxy group. Moreover, according to literature data,[11b] a signal at δ = 3.96 is consistent with a 15β proton, for which a chemical shift of δ ≈ 3.90 can be expected. A 15α proton resonates further downfield at δ ≈ 4.20.[4c] Such a value was found for the C-15 proton of **23** (δ = 4.20).

Attention was then turned to determination of the configuration at C-17 in **21** and **23**. It is known that the direction of hydrogen attack on a Δ^{16} double bond is dependent on the configuration at C-14:[32] A 14α configuration leads to α-face hydrogenation, whereas the 14β isomer is saturated from the β side. To confirm the β configuration of the C-17 side chain, we deacetylated compound **21** and found a good agreement between the ^{1}H-NMR shifts of the protons at C-19, C-20, and C-21 and the corresponding values found for the known 1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorchol-5-en-22-ol.[19] This evidence also confirmed the 17β configuration of the alcohol **23**. In fact, as indicated in Scheme 2, $NaBH_4$ reduction of the stereoisomerically pure ketone **22** produced some **21**.

The stereochemical outcome of the $NaBH_4$ reduction indicates an unexpected dependence on the C-15 and C-16 hybridization. A rationalization of the stereo-dependent reduction was attempted by considering the minimized conformations of **21** and **23** (not shown) obtained by molecular

dynamics simulation and energy minimization calculations using the MM2 force field.[33] However, in neither case could we find any obvious explanation for the observed steric outcome of the hydride transfer.

Conclusions

We have developed a simple and general method for the stereocontrolled introduction of hydroxy groups at C-15 and C-16 of steroidal substrates having a C-22 truncated side chain, through straightforward functional group transformations. The present synthetic strategy shows a high degree of stereoselectivity in most of the critical stereodifferentiating steps. Extension of this approach to precursors other than *epi*-androsterone and possible application to the synthesis of biologically important polyhydroxy steroids is currently in progress.

Experimental Section

General Remarks: All reactions were carried out under dry argon using freshly distilled solvents unless otherwise noted. Tetrahydrofuran was distilled from sodium/benzophenone. Toluene and dichloromethane were distilled from calcium hydride. Glassware was flame-dried prior to use. Where appropriate, compounds were dried by azeotropic removal of water with toluene under reduced pressure. Commercial reagents were purchased from Aldrich or Fluka and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel plates (0.25 mm); spots were visualized using UV light or by spraying with $H_2SO_4/Ce(SO_4)_2$ solution and drying. Reaction temperatures were measured externally. Flash chromatography was performed on Merck silica gel 60 (particle size 0.040−0.063 mm). Yields refer to chromatographically and spectroscopically (^{1}H-NMR) pure materials. − NMR spectra were recorded in $CDCl_3$ solutions on Bruker AM-250 and DRX 400 spectrometers at ambient temperatures. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: δ_H = 7.26, $^{13}CDCl_3$: δ_C = 77.0). − Optical rotations were measured in $CHCl_3$ solutions with a JASCO DIP-1000 polarimeter. − Mass spectra (EI, 70 eV) were recorded with a VG TRIO 2000 mass spectrometer. − Melting points were measured with an Electrothermal 9100 digital apparatus.

22-(Benzyloxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorcholan-16α-ol (12): To a solution of **11** (0.75 g, 1.40 mmol) in THF (10 mL) at 0°C was slowly added $BH_3{\cdot}SMe_2$ (2.7 mL, 2.0 M in THF, 5.4 mmol). After 0.1 h, the mixture was allowed to warm to room temperature and stirred for a further 20 h. The solution was then cooled at 0°C once more, whereupon absolute ethanol (2.5 mL), 3.0 M NaOH solution (3.5 mL), and aqueous H_2O_2 (0.7 mL, 30%) were successively added. The reaction mixture was refluxed for 1 h, concentrated in vacuo to remove the excess THF, and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue, containing a mixture of unidentified sterols, was flash-chromatographed (0−20% diethyl ether in petroleum ether) to give stereoisomerically pure **12** as a glassy solid (0.46 g, 60%). − R_f = 0.57 (silica gel, 30% diethyl ether in petroleum ether). − $[\alpha]_D^{20}$ = −1.6 (*c* = 1.0, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.04 [s, 6 H, $(CH_3)_2Si$], 0.68 (s, 3 H, 18-Me), 0.78 (s, 3 H, 19-Me), 0.88 [s, 9 H, $(CH_3)_3C$], 1.03 (d, 3 H, *J* = 7.0 Hz, 21-Me), 3.47 (dd, 1 H, *J* = 9.5 Hz, *J* = 4.8 Hz, 22-H), 3.54 (m, 1 H, 3-H), 3.65 (dd, 1 H, *J* = 9.5 Hz, *J* = 3.9 Hz, 22-H′), 4.09 (m, 1 H, 16-H), 4.47 (d, 1 H, *J* = 11.7 Hz, C*H*Ph), 4.55 (d, 1 H, *J* = 11.7 Hz, C*H*′Ph), 7.32 (m, 5 H, C_6H_5). − ^{13}C NMR (100 MHz): δ = −4.5 (× 2), 12.3, 13.8, 18.2 (× 2), 20.9, 25.9 (× 3), 28.7, 32.0 (× 2), 34.2, 35.1, 35.2, 35.5, 37.1, 38.7, 40.0, 44.7, 45.0, 53.4, 54.3, 64.8, 72.2, 73.5, 76.1, 76.9, 127.8 (× 3), 128.5 (× 2), 137.5. − EI MS; *m*/*z* (%): 554 (2) [M^+·], 497 (100) [M^+ − *t*Bu]. − $C_{35}H_{58}O_3Si$ (554.92): calcd. C 75.75, H 10.53; found C 75.95, H 10.17.

22-(Benzyloxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorcholan-16-one (13): To a solution of **12** (0.42 g, 0.76 mmol) in CH_2Cl_2 (10 mL) were added 4-Å molecular sieves (m.s., 0.6 g) and PDC (0.55 g, 1.46 mmol). After 1 h, the reaction mixture was diluted with diethyl ether (10 mL). Filtration through a short pad of Celite and $CaSO_4$ (10% in weight) afforded a clear solution, which was concentrated in vacuo to give **13** as a white solid (0.42 g, 100%); m.p. 75−77°C. − R_f = 0.82 (silica gel, 30% diethyl ether in petroleum ether). − $[\alpha]_D^{20}$ = −74.7 (*c* = 0.3, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.05 [s, 6 H, $(CH_3)_2Si$], 0.80 (s, 3 H, 19-Me), 0.82 (s, 3 H, 18-Me), 0.89 [s, 9 H, $(CH_3)_3C$], 1.08 (d, 3 H, *J* = 7.0 Hz, 21-Me), 3.58 (m, 2 H, 3-H and 22-H), 3.69 (dd, 1 H, *J* = 9.0 Hz, *J* = 3.9 Hz, 22-H′), 4.50 (s, 2 H, CH_2Ph), 7.32 (m, 5 H, C_6H_5). − ^{13}C NMR (100 MHz): δ = −4.6 (× 2), 12.3, 13.5, 17.0, 18.2, 20.7, 25.9 (× 3), 28.4, 31.8, 32.0, 32.1, 34.3, 35.5, 36.8, 38.5, 38.7, 38.9, 43.0, 44.9, 50.7, 54.1, 64.1, 71.9, 72.8, 74.1, 127.2, 127.4 (× 2), 128.1 (× 2), 138.9, 218.6. − EI MS; *m*/*z* (%): 552 (0.5) [M^+·], 495 (100) [M^+ − *t*Bu].

22-(Benzyloxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorcholan-16β-ol (14): To a solution of **13** (0.025 g, 0.045 mmol) in THF (1 mL) was added $LiAlH_4$ (0.09 mL, 1.0 M in THF, 0.09 mmol). The reaction mixture was stirred for 1 h and then quenched with ethyl acetate (0.5 mL) and NH_4OH (0.5 mL, 10% aqueous solution). Filtration of the resulting mixture through a short pad of Celite and concentration in vacuo gave **14** as a white solid (0.020 g, 80%); m.p. 104−106°C. − R_f = 0.58 (silica gel, 30% diethyl ether in petroleum ether). − $[\alpha]_D^{20}$ = −14.4 (*c* = 1.0, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.05 [s, 6 H, $(CH_3)_2Si$], 0.80 (s, 3 H, 19-Me), 0.88 [s, 12 H, 18-Me and $(CH_3)_3C$], 0.96 (d, 3 H, *J* = 7.0 Hz, 21-Me), 3.37 (dd, 1 H, *J* = 9.0 Hz, *J* = 8.9 Hz, 22-H), 3.42 (dd, 1 H, *J* = 9.0 Hz, *J* = 3.1 Hz, 22-H′), 3.54 (m, 1 H, 3-H), 4.31 (m, 1 H, 16-H), 4.51 (s, 2 H, CH_2Ph), 7.32 (m, 5 H, C_6H_5). − ^{13}C NMR (100 MHz): δ = −4.6 (× 2), 12.3, 13.2, 17.1, 18.2, 20.9, 25.9 (× 3), 28.7, 30.8, 31.9, 32.1, 35.1 (× 2), 35.5, 37.1, 38.6, 40.4, 43.0, 45.0, 54.2, 54.5, 63.0, 72.2, 73.4, 73.7, 79.2, 127.7 (× 2), 127.8, 128.5 (× 2), 137.5. − EI MS; *m*/*z* (%): 554 (0.5) [M^+·], 497 (100) [M^+ − *t*Bu]. − $C_{35}H_{58}O_3Si$ (554.92): calcd. C 75.75, H 10.53; found C 75.07, H 10.91

22-(Acetoxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorchol-16-en-15-one (19): To a suspension of chromium trioxide (0.395 g, 3.95 mmol) in CH_2Cl_2 (3.5 mL) at −20°C was added 2,5-dimethylpyrazole (0.379 g, 3.95 mmol). The reaction mixture was stirred at −20°C for 0.5 h. To the dark-red solution thus obtained, **18** (0.100 g, 0.205 mmol) was added in one portion and the resulting mixture was stirred at −20°C for 5 h. Then, 5 N NaOH solution (2 mL) was added and the mixture was stirred at 0°C for 0.5 h. It was then extracted with CH_2Cl_2 and the combined extracts were washed with 1 N HCl solution, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was flash-chromatographed (20−40% diethyl ether in petroleum ether) to give **19** as a white solid (0.043 g, 42%); m.p. 178−179°C. − R_f = 0.17 (silica gel, 20% diethyl ether in petroleum ether). − $[\alpha]_D^{20}$ = −2.6 (*c* = 2.0, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.04 [s, 6 H, $(CH_3)_2Si$], 0.85

(s, 3 H, 19-Me), 0.87 [s, 9 H, (CH_3)$_3$C], 1.02 (s, 3 H, 18-Me), 1.14 (d, 3 H, J = 7.0 Hz, 21-Me), 2.02 (s, 3 H, CH_3CO), 2.67 (dq, 1 H, J = 13.0 Hz, J = 3.0 Hz, 7-Hβ), 2.79 (m, 1 H, 20-H), 3.53 (m, 1 H, 3-H), 4.07 (dd, 1 H, J = 9.0 Hz, J = 6.6 Hz, 22-H), 4.14 (dd, 1 H, J = 9.0 Hz, J = 7.3 Hz, 22-H′), 5.68 (s, 1 H, 16-H). − ^{13}C NMR (100 MHz): δ = −4.6 (× 2), 12.4, 17.8, 18.2, 20.5, 20.8, 23.4, 25.9 (× 3), 28.3, 30.4, 31.9, 32.3, 32.4, 32.5, 35.9, 37.0, 38.5, 45.2, 47.0, 55.1, 63.8, 67.0, 71.9, 125.6, 170.8, 183.6, 207.3. − EI MS; *m*/*z* (%): 502 (1) [M$^+$·], 445 (100) [M$^+$ − *t*Bu].

22-(Acetoxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorchol-16-en-15α-ol (20): To a solution of **19** (0.100 g, 0.199 mmol) in THF/MeOH (2:1) (1.5 mL) were added cerium trichloride (0.071 g, 0.192 mmol) and $NaBH_4$ (0.008 g, 0.21 mmol). The reaction mixture was stirred at room temp. for 2 h and then quenched with water (1 mL), concentrated in vacuo to remove the excess THF and MeOH, and extracted three times with CH_2Cl_2. The organic layer was washed with 0.1 N HCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was flash-chromatographed (30% diethyl ether in petroleum ether) to give **20** as a white solid (0.100 g, 100%); m.p. 75−76°C. − R_f = 0.61 (silica gel, 30% diethyl ether in petroleum ether). − [α]$_D^{20}$ = +46.4 (c = 1.0, $CHCl_3$). − ^{1}H NMR (250 MHz): δ = 0.04 [s, 6 H, (CH_3)$_2$Si], 0.81 (s, 3 H, 18-Me), 0.83 (s, 3 H, 19-Me), 0.87 [s, 9 H, (CH_3)$_3$C], 1.06 (d, 3 H, J = 7.0 Hz, 21-Me), 2.03 (s, 3 H, CH_3CO), 2.43 (m, 1 H, 20-H), 3.54 (m, 1 H, 3-H), 3.91 (dd, 1 H, J = 10.7 Hz, J = 7.9 Hz, 22-H), 4.09 (dd, 1 H, J = 10.7 Hz, J = 6.2 Hz, 22-H′), 4.50 (d, 1 H, J = 8.5 Hz, 15-H), 5.35 (m, 1 H, 16-H). − ^{13}C NMR (62.5 MHz): δ = −4.6 (× 2), 12.4, 18.2, 18.5, 19.3, 20.9 (× 2), 25.9 (× 3), 28.6, 31.1, 31.9, 32.6, 34.2, 34.9, 35.7, 37.0, 38.6, 45.0, 47.8, 54.8, 65.2, 68.2, 72.0, 77.7, 127.5, 157.6, 171.1. − EI MS; *m*/*z* (%): 504 (1) [M$^+$·], 447 (100) [M$^+$ − *t*Bu].

22-(Acetoxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorcholan-15α-ol (21): To a solution of **20** (0.026 g, 0.051 mmol) in ethyl acetate (1 mL) was added 5% platinum on carbon (0.005 g). The flask was evacuated (40 Torr) and flushed three times with hydrogen. The reaction mixture was then stirred vigorously under hydrogen for 24 h. It was then filtered through a pad of Celite and the filtrate was concentrated. Flash chromatography of the residue (20−40% ethyl acetate in petroleum ether) gave **21** as a colorless oil (0.025 g, 98%). − R_f = 0.62 (silica gel, 40% ethyl acetate in petroleum ether). − [α]$_D^{20}$ = +18.0 (c = 0.6, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.04 [s, 6 H, (CH_3)$_2$Si], 0.70 (s, 3 H, 18-Me), 0.81 (s, 3 H, 19-Me), 0.88 [s, 9 H, (CH_3)$_3$C], 0.99 (d, 3 H, J = 7.0 Hz, 21-Me), 2.05 (s, 3 H, CH_3CO), 3.54 (m, 1 H, 3-H), 3.76 (dd, 1 H, J = 10.8 Hz, J = 7.1 Hz, 22-H), 3.96 (br. t, 1 H, J = 9.0 Hz, 15-H), 4.03 (dd, 1 H, J = 10.8 Hz, J = 3.4 Hz, 22-H′). − ^{13}C NMR (100 MHz): δ = −4.6 (× 2), 12.4, 13.4, 17.0, 18.2, 20.9, 21.1, 25.9 (× 3), 28.6, 31.9, 32.4, 35.1, 35.3, 35.5, 37.2, 38.6, 39.8, 40.0, 44.2, 44.8, 50.5, 54.3, 63.5, 69.3, 72.0, 73.8, 171.2. − EI MS; *m*/*z* (%): 506 (1) [M$^+$·], 450 (100) [M$^+$ − *t*Bu]. − $C_{30}H_{54}O_4Si$ (506.83): calcd. C 71.09, H 10.74; found C 70.43, H 10.86.

22-(Acetoxy)-3β-[(*tert*-*b*utyldimethylsilyl)oxy]-5α-23,24-bisnorcholan-15-one (22): To a solution of **19** (0.100 g, 0.199 mmol) in ethyl acetate (3 mL) was added 5% platinum on carbon (0.020 g). The flask was evacuated (20 Torr) and flushed three times with hydrogen. The reaction mixture was then stirred vigorously under hydrogen for 16 h. It was then filtered through a pad of Celite and the filtrate was concentrated. Flash chromatography of the residue (silica gel, 40% ethyl acetate in petroleum ether) gave **22** as a white solid (0.078 g, 78%); m.p. 178−180°C. − R_f = 0.25 (silica gel, 40% diethyl ether in petroleum ether). − [α]$_D^{20}$ = +33.3 (c = 1.0, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.04 [s, 6 H, (CH_3)$_2$Si], 0.75 (s, 3 H, 18-Me), 0.80 (s, 3 H, 19-Me), 0.87 [s, 9 H, (CH_3)$_3$C], 1.07 (d, 3 H, J = 7.0 Hz, 21-Me), 2.05 (s, 3 H, CH_3CO), 2.41 (dd, 1 H, J = 18.6 Hz, J = 8.7 Hz, 16-H), 2.64 (dq, 1 H, J = 13.2 Hz, J = 3.1 Hz, 7-Hβ), 3.53 (m, 1 H, 3-H), 3.82 (dd, 1 H, J = 10.9 Hz, J = 6.4 Hz, 22-H), 3.99 (dd, 1 H, J = 10.9 Hz, J = 3.7 Hz, 22-H′). − ^{13}C NMR (100 MHz): δ = −4.6 (× 2), 12.2, 13.0, 17.5, 18.2, 20.7, 20.9, 25.9 (× 3), 28.3, 30.6, 31.9, 32.0, 35.3, 35.6, 37.2, 38.5, 39.8, 41.2, 42.3, 45.0, 48.3, 54.0, 65.6, 68.9, 72.0, 171.1, 214.9. − EI MS; *m*/*z* (%): 504 (2) [M$^+$·], 447 (100) [M$^+$ − *t*Bu].

22-(Acetoxy)-3β-[(*tert*-butyldimethylsilyl)oxy]-5α-23,24-bisnorcholan-15β-ol (23): To a solution of **22** (0.020 g, 0.039 mmol) in EtOH/THF (2:1) (1.5 mL) was added $NaBH_4$ (0.004 g, 0.108 mmol). The reaction mixture was stirred at room temp. for 3 h and then quenched with water (1 mL), concentrated in vacuo to remove the excess THF and EtOH, and extracted three times with CH_2Cl_2. The organic layer was washed with 0.1 N HCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was flash-chromatographed (30% diethyl ether in petroleum ether) to give **23** (0.014 g, 68%) and **21** (0.002 g, 11%) as colorless oils; **23**. − R_f = 0.35 (silica gel, 50% diethyl ether in petroleum ether). − [α]$_D^{20}$ = −9.6 (c = 1.0, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.04 [s, 6 H, (CH_3)$_2$Si], 0.83 (s, 3 H, 19-Me), 0.88 [s, 9 H, (CH_3)$_3$C], 0.95 (s, 3 H, 18-Me), 1.01 (d, 3 H, J = 7.0 Hz, 21-Me), 2.05 (s, 3 H, CH_3CO), 3.55 (m, 1 H, 3-H), 3.80 (dd, 1 H, J = 10.6 Hz, J = 7.1 Hz, 22-H), 4.05 (dd, 1 H, J = 10.6 Hz, J = 3.4 Hz, 22-H′), 4.20 (br. t, 1 H, J = 6.1 Hz, 15-H). − ^{13}C NMR (100 MHz): δ = −4.6 (× 2), 12.3, 14.7, 17.2, 18.2, 20.9, 21.1, 25.9 (× 3), 28.6, 31.4, 31.5, 31.9, 35.4, 35.6, 37.2, 38.6, 40.4, 41.2, 42.4, 45.1, 53.2, 54.9, 60.7, 69.4, 70.4, 72.1, 171.3. − EI MS; *m*/*z* (%): 506 (1) [M$^+$·], 449 (100) [M$^+$ − *t*Bu]. − $C_{30}H_{54}O_4Si$ (506.83): calcd. C 71.09, H 10.74; found C 69.85, H 10.50.

3β-[(*tert*-Butyldimethylsilyl)oxy]-5α-23,24-bisnorchol-16-ene-15α,22-diol (24): To a solution of **21** (0.009 g, 0.0178 mmol) in methanol (0.2 mL) was added K_2CO_3 (0.002 g, 0.014 mmol). The reaction mixture was stirred vigorously for 2 h, filtered through a pad of Celite, and concentrated in vacuo to give **24** as a glassy solid (0.006 g, 72%). − R_f = 0.11 (silica gel, 40% ethyl acetate in petroleum ether). − [α]$_D^{20}$ = +11.8 (c = 0.67, $CHCl_3$). − ^{1}H NMR (400 MHz): δ = 0.04 [s, 6 H, (CH_3)$_2$Si], 0.70 (s, 3 H, 18-Me), 0.81 (s, 3 H, 19-Me), 0.88 [s, 9 H, (CH_3)$_3$C], 1.02 (d, 3 H, J = 7.0 Hz, 21-Me), 3.34 (dd, 1 H, J = 10.3, 6.0 Hz, 22-H), 3.54 (m, 1 H, 3-H), 3.60 (dd, 1 H, J = 10.3 Hz, J = 2.3 Hz, 22-H′), 3.96 (dt, 1 H, J = 9.1 Hz, J = 2.9 Hz, 15-H). − ^{13}C NMR (100 MHz): δ = −4.6 (× 2), 12.4, 13.4, 16.6, 18.2, 21.1, 25.9 (× 3), 28.6, 31.9, 32.4, 35.1, 35.5, 37.2, 38.2, 38.6, 40.0, 40.1, 44.2, 44.8, 50.2, 54.3, 63.7, 67.8, 72.0, 73.9. − EI MS; *m*/*z* (%): 464 (2) [M$^+$·], 407 (100) [M$^+$ − *t*Bu].

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[1] Reviews: D. J. Faulkner, *Nat. Prod. Rep.* **1998**, *15*, 113 and previous reports in the series; M. V. D′Auria, L. Minale, R. Riccio, *Chem. Rev.* **1993**, *93*, 1839.
[2] Recent examples: [2a] S. Deng, B. Yu, Y. Lou, Y. Hui, *J. Org. Chem.* **1999**, *64*, 202. − [2b] M. Kurosu, L. R. Marcin, T. J. Grinsteiner, Y. Kishi, *J. Am. Chem. Soc.* **1998**, *120*, 6627. −

[2c] C. Guo, P. L. Fuchs, *Tetrahedron Lett.* **1998**, *39*, 1099. – [2d] I. Izzo, F. De Riccardis, G. Sodano, *J. Org. Chem.* **1998**, *63*, 4438.
[3] Review of reactions and syntheses of steroids: J. R. Hanson, *Nat. Prod. Rep.* **1998**, *15*, 261 and previous reports in the series.
[4] [4a] T. Takahashi, A. Ootake, J. Tsuji, *Tetrahedron Lett.* **1984**, *25*, 1921. – [4b] T. Takahashi, A. Ootake, J. Tsuji, H. Takibana, *Tetrahedron* **1985**, *41*, 5747. – [4c] D. Liu, L. M. Stuhmiller, T. C. McMorris, *J. Chem. Soc., Perkin Trans. 1* **1988**, 2161. – [4d] S. S. Moon, L. M. Stuhmiller, T. C. McMorris, *J. Org. Chem.* **1989**, *54*, 26. – [4e] M. M. Kabat, *J. Org. Chem.* **1995**, *60*, 1823.
[5] [5a] S. N. Newas, R. K. Tcholakian, *Steroids* **1984**, *43*, 445. – [5b] M. J. S. M. Moreno, M. L. Sa′ e Melo, A. S. Campos Neves, *Tetrahedron Lett.* **1993**, *34*, 353. – [5c] F. J. Brown, C. Djerassi, *J. Am. Chem. Soc.* **1980**, *102*, 807. – [5d] M. Koreeeda, Y. Tanaka, A. Schwartz, *J. Org. Chem.* **1980**, *45*, 1172.
[6] For cytotoxic steroids, see: G. R. Pettit, F. H. Pierson, C. L. Herald in *Anticancer Drugs from Animals, Plants, and Microorganisms*, John Wiley & Sons, New York, **1994**, 74–75, 280–335. For other bioactivities, see: *Dictionary of Steroids* (Eds.: R. A. Hill, D. N. Kirk, H. L. J. Makin, G. M. Murphy), Chapman and Hall, London, **1991**.
[7] G. R. Pettit, M. Inoue, Y. Kamano, D. L. Herald, C. Arm, C. Dufresne, N. D. Christie, J. M. Schmidt, D. L. Doubek, T. S. Krupa, *J. Am. Chem. Soc.* **1988**, *110*, 2006.
[8] S. Kubo, Y. Mimaki, M. Terao, Y. Sashida, T. Nikaida, T. Ohmoto, *Phytochemistry* **1992**, *31*, 3969. Y. Mimaki, M. Kuroda, A. Kameyama, Y. Sashida, T. Hirano, K. Oka, R. Maekawa, T. Wada, K. Sugita, J. A. Butler, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633.
[9] C. Guo, P. L. Fuchs, *Tetrahedron Lett.* **1998**, *39*, 1099.
[10] T. C. McMorris, R. Seshadri, G. R. Weihe, G. P. Arsenault, A. W. Arksdale, *J. Am. Chem. Soc.* **1975**, *97*, 2444.
[11] [11a] K. Tachibana, M. Sakaitani, K. Nakanishi, *Science* **1984**, *226*, 703. – [11b] K. Tachibana, M. Sakaitani, K. Nakanishi, *Tetrahedron* **1985**, *41,* 1027.
[12] L. M. V. Tillekeratne, G. K. Liyanage, W. D. Ratnasooriya, M. B. Ksebati, F. J. Schimitz, *J. Nat. Prod*. **1989**, *52*, 1043.
[13] L. Minale, R. Riccio, F. Zollo, *Prog. Chem. Org. Nat. Prod.* **1993**, *62*, 75–308. L. Minale, R. Riccio, F. Zollo, *Stud. Nat. Prod. Chem., C: Struct. Chem.* **1995**, *15*, 43–110.
[14] [14a] E. Finamore, L. Minale, R. Riccio, G. Rinaldo, F. Zollo, *J. Org. Chem.* **1991**, *56*, 1146. – [14b] R. Riccio, L. Minale, M. Iorizzi, Y. Oshima, T. Yasumoto, *J. Chem. Soc., Perkin Trans. 1* **1988**, 1337. – [14c] F. De Riccardis, M. Iorizzi, L. Minale, R. Riccio, C. Debitus, *Tetrahedron Lett.* **1992**, *33*, 1097.
[15] A. Ganesan, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 611. G. J. Schroepfer, B. C. Sherril, K.-S. Wang, W. K. Wilson, A. Kisic, T. B. Clarkson, *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6861.
[16] F. De Riccardis, G. Sodano, *Recent Res. Dev. Org. Chem.* **1998**, *2*, 419–427.
[17] [17a] D. M. Piatak, J. Wicha, *Chem. Rev.* **1978**, *78*, 199. – [17b] G.-D. Zhu, W. H. Okamura, *Chem. Rev.* **1995**, *95*, 1877.
[18] J. M. Aizpurua, C. Palomo, *Tetrahedron Lett*. **1985**, *26*, 475.
[19] K. Konno, K. Ojima, T. Hayashi, H. Takayama, *Chem. Pharm. Bull.* **1992**, *40*, 1120.
[20] Our assignment is consistent with literature data. For 16α-OH steroids, see: J. Saez, W. Cardona, D. Espinal, S. Blair, J. Mesa, M. Bocar, A. Jossang, *Tetrahedron* **1998**, *54*, 10771 ($\delta_{18\text{-H}}$ = 0.62); G. Piancatelli, A. Scettri, *Gazz. Chim. Ital.* **1976**, *106*, 167 ($\delta_{16\text{-H}}$ = 4.15).
[21] A. Bax, D. G. Davis, *J. Magn. Reson*. **1985**, *63*, 207.
[22] J. Herscovici, K. Antonakis, *J. Chem. Soc., Chem. Commun.* **1980**, 561.
[23] N. R. Schmuff, B. M. Trost, *J. Org. Chem.* **1983**, *48*, 1404.
[24] [24a] Copper-catalysed 1,4-addition to a steroidal unsaturated epoxide: E. J. Parish, M. Tsuda, G. J. Schroepfer, *Chem. Phys. Lipids* **1988**, *49*, 119. – [24b] Remote functionalization: E. Lee, H. H. Lee, H. K. Chang, D. Y. Lim, *Tetrahedron Lett.* **1988**, *29*, 339. – [24c] Δ^{14} hydroboration: E. J. Taylor, C. Djerassi, *J. Org. Chem.* **1977**, *42*, 3571 and references cited therein.
[25] W. K. Wilson, G. J. Schroepfer, *J. Org. Chem.* **1988**, *53*, 1713 and references cited therein.
[26] J. E. McMurry, W. J. Scott, *Tetrahedron Lett*. **1983**, *24*, 979.
[27] W. J. Scott, J. K. Stille *J. Am. Chem. Soc.* **1986**, *108*, 3033.
[28] S. Eguchi, S. Yamaguchi, M. Furuya, M. Morisaki, *Chem. Pharm. Bull.* **1988**, *36*, 2813.
[29] W. G. Salmond, M. A. Barta, J. L. Havens, *J. Org. Chem*. **1978**, *43*, 2057. H.-S. Kim, S. H. Ho, D.-I. Kim, I.-C. Kim, K. H. Cho, Y. B. Park, *BioMed. Chem.* **1995**, *3*, 367.
[30] Alternative methods for the C-15 allylic oxidation (*t*BuOOH, CuI: J. A. R. Salvador, M. L. Sá e Melo, A. S. Campos Neves, *Tetrahedron Lett*. **1997**, *38*, 119; *t*BuOOH, SeO_2: M. de los Angeles Rey, J. A. Martinez-Perez, A. Fernandez-Gacio, K. Halkes, Y. Fall, J. Granja, A. Mouriño, *J. Org. Chem*. **1999**, *64*, 3196) gave lower yields.
[31] C. A. Hoger, A. D. Johnston, W. H. Okamura, *J. Am. Chem. Soc.* **1983**, *105*, 7407.
[32] A. R. Van Horn, C. Djerassi, *J. Am. Chem. Soc.* **1967**, *89*, 651.
[33] U. Burkert, N. Allinger in *Molecular Mechanics*, ACS monograph 177, American Chemical Society, Washington DC, **1982**.
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