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25-Hydroxydihydrotachysterol$_2$ An Innovative Synthesis of a Key Metabolite of Dihydrotachysterol$_2$

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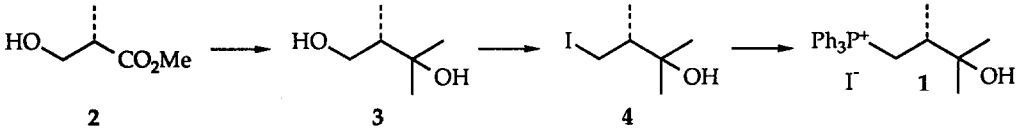
***Abstract**: A new synthesis of 25-hydroxydihydrotachysterol$_2$ is described. The hydroxylated side-chain is constructed stereoselectively using a chiral Wittig reagent. The A-ring synthon is introduced utilising the Wittig-Horner method as developed by Lythgoe et al. The preparation of the metabolite is carried out in 18 steps.*

INTRODUCTION

The D vitamins have received considerable chemical and biochemical attention over the past few decades. Vitamin D_2, its metabolites and analogues contain an additional chiral centre at C^{24} and an *E* C^{22}-C^{23} double bond compared to vitamin D_3 compounds, making their syntheses much more challenging and complex. Whereas the 25-hydroxylated vitamin D_2 metabolite has been synthesised in various ways,[1-4] direct synthesis of the title compound has not been published hitherto. The first synthesis of 25-hydroxydihydrotachysterol$_2$ (25-OH-DHT$_2$) was presented on the vitamin D conference in the U. S. A. in 1988.[5] This rather lenghty preparation is based on an iodine catalysed ultraviolet conversion of the bis-protected 25-hydroxy-D_2 metabolite, followed by the stereoselective reduction of the exocyclic C^{10}-C^{19} double bond.[4,6,7] This approach of the required DHT$_2$ metabolite seemed rather awkward to us, as it involves a low yield conversion of a difficult to prepare D_2 metabolite. We therefore chose for a more direct synthetic approach including the introduction of the DHT$_2$ A-ring according to the method described by Lythgoe *et al.*,[8,9,10] and the construction of the side-chain using the Wittig reaction.

RESULTS and DISCUSSION

a. Starting compound: the Wittig reagent. The required phosphonium salt for the construction of the 25-hydroxylated DHT_2 side-chain is depicted in scheme 1 (compound **1**). The synthesis of **1** is already mentioned (in short) in our recently published report.[11] The preparation was done in four steps, starting from **2** in a 73% overall yield. Synthetic and spectroscopic details of compound **1** (and related compounds) were published recently.[12,13]



2 **3** **4**

Scheme 1.

b. Starting compound: the CD synthon aldehyde.[14] A simple and straightforward synthetic route towards an appropriate CD synthon was chosen. Keto-aldehyde **5** (figure 1) which was prepared from vitamin D_2 was first used as the starting compound for the 25-OH-DHT_2 synthesis. We anticipated that the ylide from phosphonium salt **1** would react regioselectively with the aldehyde moiety, as aldehydes are much more reactive towards ylides than ketones.[15]

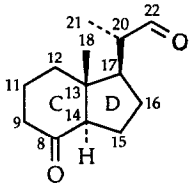
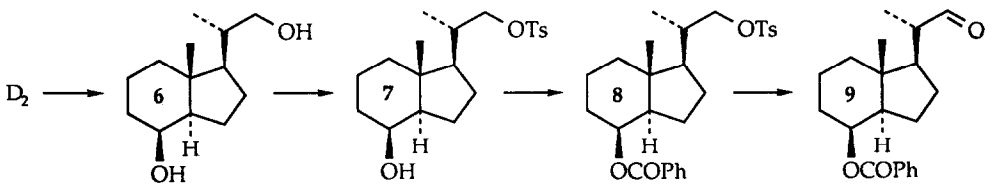


Figure 1. Keto-aldehyde **5**.

Ozonolysis of ergocaliferol in absolute methanol at -78°C, followed by reduction with dimethyl sulfide (Me_2S) resulted in keto-aldehyde **5** (figure 1) in a 65% yield. Reaction of our phosphorus ylide with compound **5** resulted in a mixture of unidentifiable reaction products. This result was rather unexpected. In an attempt to surpress the suspected aldol condensation reactions, we used a deficiency of MeLi. However, a similar disappointing result was obtained, indicating the rather basic behaviour of the ylide from phosphonium salt **1** towards substrate **5**. In our second approach we chose the aldehyde synthesis as described by Sardina *et al.* (see scheme 2).[2]



Scheme 2.

Diol **6** was readily obtained in an 85% yield by ozonolysis of vitamin D_2, followed by reduction of the ozonides with sodium borohydride ($NaBH_4$).[2] The crystal structure of the Inhoffen-Lythgoe diol[16,17] (figure 2) confirms the retention of the vitamin D_2 stereochemical stucture.

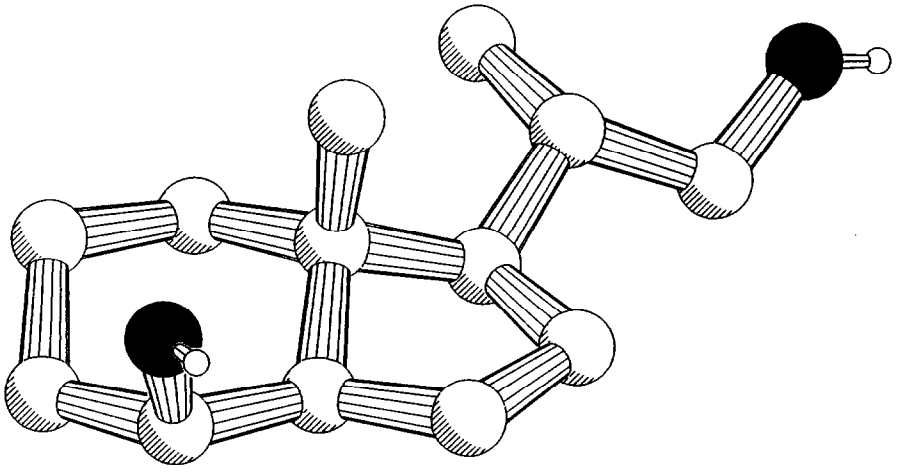


Figure 2.

Selective monotosylation of the primary hydroxy group of diol **6**, followed by benzoylation of the remaining free secondary hydroxy group resulted in tosyl-benzoate **8**. Its crystal structure showed that the stereochemical configuration of the starting material **6** was retained. Sardina *et al.*, however, reported the tosyl-benzoate **8** to be an oil.[2]

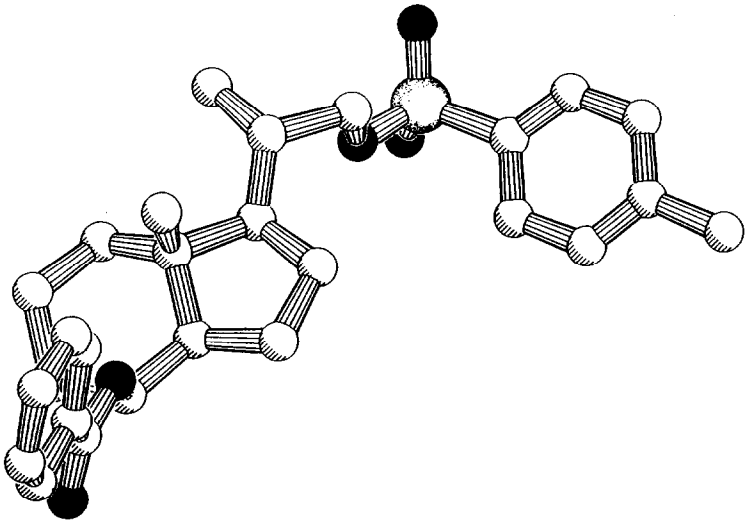
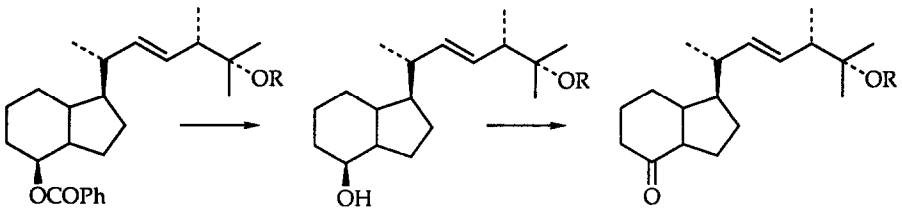


Figure 3.

The Kornblum oxidation[18] of tosyl-benzoate **8**, as described in the report of Sardina,[2] could not be reproduced by us. A complete epimerisation of C^{20} had taken place (this could best be observed on ^{13}C NMR: two diastereoisomers were visible). We repeated the experiment several times with the same result. Therefore, a different synthetic route had to be chosen.

In 1977, Lythgoe *et al.* published the synthesis of aldehyde **9** as a starting compound for the synthesis of the Windaus and Grundmann's ketone.[17] This preparation comprises the complete benzoylation of diol **6** in pyridine with benzoyl chloride, followed by removal of the primary benzoate group with ethanolic 0.55 M potassium hydroxide resulting in the secondary monobenzoate of diol **6**. We reproduced this synthesis with a 95% overall yield. Oxidation of the latter with pyridinium chlorochromate (PCC) rendered **9** in a 97% yield.[19] The overall yield of the synthesis of aldehyde **9**, starting from ergocalciferol, is a high 78%.

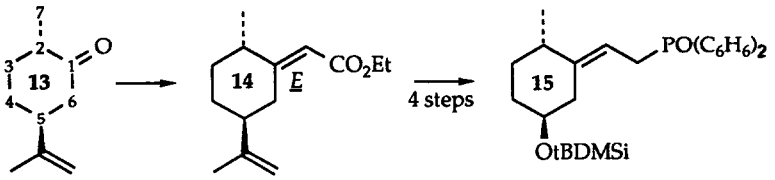
c. Starting compound: the 25-OH-Windaus and Grundmann's ketone.[20] The condensation of the ylide of phosphonium salt **1** and aldehyde **9** was carried out in ether.[11,12] As expected, the reaction proceeded fully stereoselectively.[21,22] The maximum yield of the reaction was 50%, with an 1.5 equivalent amount of ylide made with a deficiency of MeLi. This result suggests a competition between the nucleophilic and basic behaviour of the ylide; the conjecture was supported by the retrieval of epimerised aldehyde **9** from the reaction mixture. The side-reaction could not be eliminated. The introduction of the C^{22}-C^{23} *E* double bond was achieved by adding **9** to the ylide of compound **1** (in ether) at -40°C. The resulting yellow suspension was stirred for 20 h at room temperature, leading to the desired compound in a 45-50% yield. The coupling product was reacted with $ClCH_2OCH_3$ in CH_2Cl_2 (with diisopropylethyl amine as a base) in order to protect the tertiary hydroxy group (scheme 3: R= MOM; methoxy methyl). Subsequent removal of the benzoate group of **10** with $LiAlH_4$ in THF at 0°C resulted in alcohol **11** quantitatively. Oxidation of **11** with pyridinium chlorochromate (PCC) afforded **12** in a quantitative yield.



10 **11** **12**

Scheme 3.

d. Starting compound: the DHT_2 A-ring synthon.[23] As we developed a convergent synthesis for the title compound based on the Lythgoe procedure,[9] a DHT_2 A-ring synthesis was devised. S-(+)-Carvone was used as the starting material, thus certifying the correct absolute configuration if the reduction of the double bond could be carried out stereoselectively. Reduction with lithium bronze ($Li(NH_3)_4$) of S-(+)-carvone[24] afforded **13** almost stereoselectively (a small amount of saturated alcohol of **13** and some of the other diastereoisomer was isolated: *cis/trans*: 10/90).



Scheme 4.

The preparation of phosphonate **15** (S,S'), and its enantiomeric counterpart (the R,R' variant) are mentioned in our previous article.[23] The overall yield, starting from ester **14**, is 40%.

e. Synthesis of 25-hydroxydihydrotachysterol$_2$. The Wittig-Horner coupling of ketone **12** and phosphine oxide **15** was carried out in the standard Lythgoe manner.[8-10] We expected the stereoselectivity of this Wittig-Horner reaction to be comparable with the stereoselectivity as found in the condensation carried out with vitamin D A-ring synthons.[2,3] The condensation afforded the bis-protected 25-OH-DHT$_2$ in a 60% yield in a total stereoselective fashion. The methoxymethyl- and *tert*-butyldimethylsilyl protective moieties of the bis-protected 25-OH-DHT$_2$ were simultaneously removed with an AG-50WX4 proton ion-exchange resin, affording the 25-hydroxylated dihydrotachysterol$_2$ **16** in a 75% yield.[2]

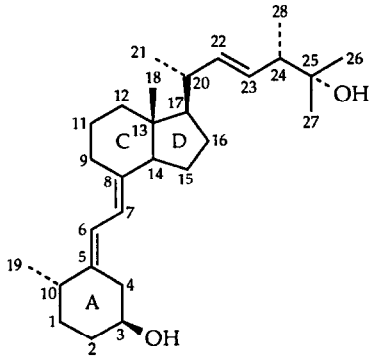


Figure 4. 25-Hydroxydihydrotachysterol$_2$ **16**.

EXPERIMENTAL SECTION

Glassware was dried with the flame while being evacuated. All reactions were carried out under nitrogen with magnetic stirring. Standard Schlenk techniques were used where mentioned. Solvents were freshly distilled before use from the appropiate drying agents. Column chromatography was performed on SiO_2. Proton NMR spectra were recorded at 300 MHz on a Bruker AC300 spectrometer with $CDCl_3$ as solvent and internal standard, chemical shifts being reported as ppm downfield from Me_4Si (the proton NMR spectrum of keto-aldehyde **5** was measured at 90 MHz on a Varian EM 390). Coupling constants *J* are reported in Hz. Carbon-13 NMR spectra were obtained on a Bruker AC300 at 75.4 MHz with $CDCl_3$ as solvent and internal standard. Phosphorus-31 NMR spectra were recorded at 80.9 MHz on a Bruker AC200 spectrometer; chemical shifts

are referenced to 85% H_3PO_4 (external). Mass spectroscopy was performed on a Kratos MS 80 GC-MS combination apparatus. The mass spectrum (Fast Atom Bombardment) of compound **15** was performed on a Jeol-JMS-AX505W mass spectrometer with a Hewlett-Packard 9000 data system.

R-(+)-(2,3-Dimethylbutyl)triphenylphosphonium iodide 1[11,12]

Phosphonium salt **1** was synthesised in 4 steps starting from S-(+)-methyl 2-methyl-3-hydroxy-propanoate **2** in a 73% overall yield.[11,12] Anomalous single crystal X-ray diffraction showed that the stereogenic centre of the starting compound was retained.[13] The formation of the ylide from phosphonium salt **1** is described below.

De-*A*,*B*-8-oxo-23,24-dinorcholan-22-al (5)

Vitamin D_2 (1.5 g, 0.0038 mol) was dissolved in 75 ml absolute methanol, and cooled to -78°C. Ozone was led through the solution until a grey-blue colour appeared (10 min). The ozone flow was discontinued, and the reaction mixture was purged with N_2 at -78°C until all dissolved ozone was removed (KI test). Subsequently, 1.5 ml (1.3 g, 0.021 mol) Me_2S was added. The temperature of the reaction mixture was allowed to rise slowly to room temperature, and the solution was stirred for an additional 2 h. The solvents were removed under reduced pressure. A light yellow syrup remained. The reaction products were seperated on SiO_2 with 15% EtOAc/hexane. Keto-aldehyde **5** was obtained in a 65% yield. ^1H NMR (numbering as in figure 2): δ 9.65 (1H, d, H^{22}, $^3J_{22\text{-}20}$= 2.8), δ 2.7-1.3 (17H, broad m), δ 1.15 (3H, d, CH_3^{21}, $^3J_{21\text{-}20}$= 6.2), δ 0.7 (3H, s, CH_3^{18}); ^{13}C NMR (numbering as in figure 2): δ 210.8 (C^8), δ 203.8 (C^{22}), δ 60.6 (C^{14}), δ 51.1 (C^{20}), δ 49.6 (C^{13}), δ 48.7 (C^{17}), δ 40.5 (C^{12}), δ 38.2 (C^9), δ 26.0 (C^{16}), δ 23.5 (C^{15}), δ 19.2 (C^{11}) δ 13.1 (C^{18} or C^{21}), δ 12.4 (C^{18} or C^{21}); MS, *m /e* 208 (M^+, 8), 193 (M^+-$CH_3{}^{\bullet 21}$, 10), 179 (M^+-CH=O$^\bullet$, 9), 165 (35), 151 (M^+-H_3C-C$^\bullet$H-CHO, 64), 124 (30), 111 (100), 96 (35), 81 (63), 68 (30), 55 (50), 41 (22), 28 (20).

The Wittig reaction with the ylide from **1** was carried out as described below. GC-MS showed a large number of reaction products with a mass ranging between ±208-±656.

De-*A*,*B*-23,24-dinor-22-hydroxycholan-8β-ol (Inhoffen-Lythgoe Diol 6)[2]

Vitamin D_2 (16 g, 0.04 mol) was dissolved in 1000 ml absolute methanol to which was added 14 ml of dry pyridine. At -78°C, a stream of ozone was passed through the reaction mixture until a grey-blue colour appeared. After 3.5 h, the ozone flow was discontinued, and the reaction mixture (still at -78°C) was purged with N_2 to remove the remaining dissolved ozone (KI test). $NaBH_4$ (4.0 g, 0.11 mol) was added in one portion (a considerable amount of gas evolved, and the temperature rose to -70°C), and the resulting solution was stirred at -78°C for 20 min while a gentle stream of nitrogen was maintained. This operation was repeated twice before the reaction mixture was allowed to reach room temperature overnight. A final amount of $NaBH_4$ was added (2 g, 0.055 mol) and the reaction mixture was stirred for an additional 1 h. The solvents were removed under reduced pressure, and the remaining brown slurry was continuously extracted (Soxhlet) for 24 h with refluxing diethylether. The etheral extracts were washed with a 5% HCl aqueous solution and H_2O, and subsequently dried over $MgSO_4$. Filtration and removal of the

solvents *in vacuo* resulted in a residue which was dissolved in ether. Slow addition of pentane resulted in the crystallisation of the diol. The Inhoffen-Lythgoe diol was obtained in a 85% yield (7.2 g, 0.034 mol). ^{1}H NMR (conventional steroid numbering is used): δ 4.08 (1H, broad s, H^{8}), δ 3.60 (1H, dd, H^{22a} or H^{22b}, $^2J_{22a\text{-}22b}$= 12.3, $^3J_{22\text{-}20}$= 4.6), δ 3.37 (1H, dd, H^{22a} or H^{22b}, $^2J_{22a\text{-}22b}$= 12.3, $^2J_{22\text{-}20}$= 7.8), δ 2.08-1.68 (4H, broad m), δ 1.68-1.08 (11H, broad m), δ 1.05 (3H, d, CH_3^{21}, $^3J_{21\text{-}20}$= 7.2), δ 0.94 (3H, s, CH_3^{18}); ^{13}C NMR (conventional steroid numbering is used): δ 69.2 (C^{8}), δ 67.6 (C^{22}), δ 52.7, (C^{14}), δ 52.3 (C^{17}),δ 41.6 (C^{13}), δ 40.2 (C^{12}), δ 38.2 (C^{20}), δ 33.5 (C^{9}), δ 26.6 (C^{16}), δ 22.6 (C^{15}), δ 17.4 (C^{11}), δ 16.6 (C^{18}), δ 13.6 (C^{21}). Single crystals of the Inhoffen-Lythgoe diol were grown by vapour diffusion of pentane in a solution of the diol in CH_2Cl_2.[30]

De-*A*,*B*-(benzoyloxy)-23,24-dinor-22-(benzoyloxy)cholane (the Dibenzoate of Diol 6)

Inhoffen-Lythgoe diol **6** (7.2 g, 0.034 mol) was dissolved in pyridine (100 ml), and 0.15 g DMAP (dimethylamino pyridine) was added. The resulting solution was cooled to 0°C, after which benzoyl chloride (14.3 g, 0.1 mol) was added. The reaction mixture was kept in the refrigerator for 48 h. Ice was added, and the resulting suspension was thoroughly extracted with ether. The combined organic extracts were washed with an aqueous 5% HCl solution, water, an aqueous 5% H_2NOH solution, and water, after which the organic extracts were dried over $MgSO_4$. Removal of the solvents *in vacuo* afforded the dibenzoate of diol **6** in a 98% yield (13.9 g, 0.033 mol).
^{13}C NMR (conventional steroid numbering is used): δ 166.7 (Ph-(C=O)-OR), δ 166.4 (Ph-(C=O)-OR), δ 132.9 (C$^{ar}_{para}$), δ 132.8 (C$^{ar}_{para}$), δ 130.9 (C$^{ar}_{ipso}$-COOR), δ 130.5 (C$^{ar}_{ipso}$-COOR), δ 129.6 (C$^{ar}_{ortho}$-COOR), δ 129.5 (C$^{ar}_{ortho}$-COOR), δ 128.4 (C$^{ar}_{meta}$-COOR), δ 128.3 (C$^{ar}_{meta}$-COOR), δ 72.0 (C^{22}), δ 69.8 (C^{8}), δ 53.3 (C^{14}), δ 51.4 (C^{17}), δ 42.1 (C^{13}), δ 39.8 (C^{20}), δ 35.7 (C^{12}), δ 30.5 (C^{9}), δ 26.6 (C^{16}), δ 22.7 (C^{15}), δ 18.0 (C^{11}), δ 17.3 (C^{18}), δ 13.6 (C^{21}).

Elimination of the Primary Benzoate Group[17]

The dibenzoate of diol **6** (13.9 g, 0.033 mol) was added at room temperature to a KOH solution (5.1 g, 0.09 mol) in ethanol (150 ml). The resulting reaction mixture was stirred for 3 h, after which ice and an aqueous 5% HCl solution were added until pH4. The solution was thoroughly extracted with ether, and the combined organic extracts were dried with $MgSO_4$. The solvents were removed under reduced pressure, and the residue was chromatographed on SiO_2 with a 25% EtOAc/ hexane eluens. The monobenzoate was isolated in a 90% yield (9.5 g, 0.030 mol). ^{13}C NMR (conventional steroid numbering is used): δ 166.1 (Ph-(C=O)-OR), δ 132.5 (C$^{ar}_{para}$-COOR), δ 130.5 (C$^{ar}_{ipso}$-COOR), δ 129.3 (C$^{ar}_{ortho}$-COOR), δ 128.1 (C$^{ar}_{meta}$-COOR), δ 71.9 (C^{22}), δ 67.1 (C^{8}), δ 52.5 (C^{14}), δ 51.1 (C^{17}), δ 41.7 (C^{20}), δ 39.5 (C^{13}), δ 38.2 (C^{12}), δ 30.3 (C^{9}), δ 26.3, (C^{16}), δ 22.5 (C^{15}), δ 17.7 (C^{11}), δ 16.5 (C^{18}), δ 13.4 (C^{21}).

De-*A*,*B*-8β-(benzoyloxy)-23,24-dinorcholan-22-al (9)

The monobenzoate (9.5 g, 0.03 mol) was dissolved in 50 ml methylene chloride. To the resulting solution was added pyridinium chlorochromate (12.9 g, 0.06 mol) at 0°C. The subsequent reaction mixture was stirred for two hours at room temperature. The resulting black suspension was

subsequently filtered through a florisil packed column. Diethyl ether was used as eluens. The reaction vessel was thoroughly rinsed with ether, and the resulting etheral solution was also filtered through the florisil. The solvents of the obtained clear colourless solution were evaporated *in vacuo*, and aldehyde **9** was obtained in a 95% yield (9.1 g, 0.029 mol) which was of good purity, and the aldehyde was used directly in the next step. ^{13}C NMR (conventional steroid numbering is used): δ 203.1 (C^{22}), δ 166.0 (Ph-(C=O)-OR), δ 132.9 (C^{ar}_{para}-COOR), δ 131.5 (C^{ar}_{ipso}-COOR), δ 129.9 (C^{ar}_{ortho}-COOR), δ 128.7 (C^{ar}_{meta}-COOR), δ 71.7 (C^{8}), δ 51.4 (C^{14} or C^{17}), δ 50.9 (C^{14} or C^{17}), δ 49.2 (C^{20}), δ 42.3 (C^{13}), δ 39.7, (C^{12}), δ 30.7 (C^{9}), δ 26.1 (C^{16}), δ 23.2 (C^{15}), δ 18.2 (C^{11}), δ 13.9 (C^{18} or C^{21}), δ 13.3 (C^{18} or C^{21}).

(22*E*,24*S*)-De-*A*,*B*-25-[(methoxymethyl)oxy]ergost-22-en-8-one (12)

Phosphonium salt **1** (0.98 g, 0.002 mol) was suspended in 5 ml of diethylether in a 25 ml Schlenk vessel, after which 2 equivalents of MeLi (1.6 M in ether, containing 0.4% LiCl) were added at -20°C. The temperature of the reaction mixture was allowed to rise to 20°C, and was stirred for an additional 2 h. The aldehyde **9** (2.4 g, 0.0075 mol), dissolved in ether, was added to the ylide at -40°C, causing an instantaneous dissipation of the red ylide colour, after which the reaction mixture was stirred at -40°C for 2 h. The resulting yellow suspension was stirred for an additional 20 h at room temperature. Water, and an aqueous HCl solution (2.5%) were added until pH4. The resulting mixture was thoroughly extracted with ether, and the combined ether layers were dried over $MgSO_4$. The solvents were removed under reduced pressure affording a yellow coloured residue, which was chromatographed on SiO_2 with 20% EtOAc/hexane. The desired compound was isolated in a 45% yield (1.3 g, 0.0034 mol). ^1H NMR (conventional steroid numbering is used): δ 8.04 (2H, m, H^{ar}), δ 7.45 (3H, m, H^{ar}), δ 5.39 (1H, broad s , H^{8}), δ 5.31 (1H, m, H^{22} and H^{23}), δ 2.1 (4H, m), δ 1.9-1.4 (11H, m), δ 1.14 (3H, s, CH_3^{26} or CH_3^{27}), δ 1.12 (3H, s, CH_3^{26} or CH_3^{27}), δ 1.05 (3H, s, CH_3^{18}), δ 1.03 (3H, d, CH_3^{21} or CH_3^{28}, ^3J= 6.6), δ 0.98 (3H, d, CH_3^{21} or CH_3^{28}, ^3J= 6.9); ^{13}C NMR (conventional steroid numbering is used): δ 166.3 (Ph-(C=O)-OR), δ 138.4 (C^{23}), δ 132.6 (C^{ar}_{para}-COOR), δ 130.7 (C^{ar}_{ipso}-COOR), δ 129.4 (C^{ar}_{ortho}-COOR), δ 129.3 (C^{22}), δ 128.2 (C^{ar}_{meta}-COOR), δ 72.1 (C^{25}), δ 72.0 (C^{8}), δ 56.0 (C^{17}), δ 51.5 (C^{14}), δ 47.9 (C^{24}), δ 41.7 (C^{13}), δ 39.8 (C^{12} or C^{20}), δ 39.7 (C^{12} or C^{20}), δ 30.4 (C^{9}), δ 27.4 (C^{16}), δ 26.7 (C^{26} or C^{27}), δ 26.5 (C^{26} or C^{27}), δ 22.5 (C^{15}), δ 20.5 (C^{21}), δ 17.9 (C^{11}), δ 15.4 (C^{28}), δ 13.6 (C^{18}).

The above mentioned compound (1.3 g, 0.0034 mol) was dissolved in 10 ml CH_2Cl_2 and 10 ml diisopropylethyl amine. At 0°C methoxymethyl chloride (0.32 g, 0.005 mol) was added, and the resulting reaction mixture stirred at room temperature for 24 h. A saturated aqueous NH_4Cl solution was added, and the resulting solution was thoroughly extracted with ether. The combined ether layers were dried over $MgSO_4$. Evaporation of the solvents afforded derivative **10** in a quantitative yield (1.5 g, 0.0034 mol).

$LiAlH_4$ (0.38 g, 0.01 mol) was added to an ice cooled solution of benzoate **10** (1.5 g, 0.0034 mol) in THF (25 ml). The suspension was stirred for 3 h at 0°C. Ice was added, and the resulting mixture was thoroughly extracted with ether. The combined ether layers were dried over $MgSO_4$, and *in vacuo* evaporation of the solvents afforded alcohol **11** in a 80% yield (0.9 g, 0.0027 mol).

To an orange suspension of pyridinium chlorochromate (1.7 g, 0.008 mol) in 25 ml CH_2Cl_2 alcohol **11**, dissolved in methylene chloride, was added at 0°C. The reaction mixture was stirred for 3 h at room temperature. The resulting black suspension was filtered over florisil with ether as the eluens. The reaction vessel was thoroughly rinsed with ether, and the resulting ether solution was also filtered over florisil. The solvents were removed *in vacuo* and the protected 25-OH Windaus and Grundmann's ketone **12** was isolated pure in 90% yield (0.8 g, 0.0024 mol). ^{13}C NMR (conventional steroid numbering is used): δ 211.6 (C^8), δ 136.3 (C^{23}), δ 130.2 (C^{22}), δ 90.8 (OCH_3O), δ 78.0 (C^{25}), δ 61.9 (C^{14}), δ 56.4 (C^{17}), δ 55.0 (OCH_3), δ 49.6 (C^{13}), δ 46.6 (C^{24}), δ 40 8 (C^{12}), δ 39.8 (C^{20}), δ 38.8 (C^9), δ 27.6 (C^{16}), δ 24.6 (C^{26}), δ 24.0 (C^{15}), δ 23.0 (C^{27}), δ 20.7 (C^{21}), δ 19.0 (C^{11}), δ 15.1 (C^{28}), δ 12.6 (C^{18}).

(2S,5S)-Dihydrocarvone (13)

Lithium bronze was made (0.74 g Li, 0.106 mol Li; 7.4 g NH_3 (9.6 ml), 0.44 mol NH_3) according to Mueller *et al.*.[24] S-(+)-Carvon (7.5 g, 0.05 mol) and dry *t*-butyl alcohol, dissolved in ether, were added simultaneously to the lithium bronze (dispersed in ether) at 0°C. The reaction was very vigorous, and occasional cooling was necessary. After completion of the addition, the reaction was stirred for 1/2 h at room temperature. Excess Li was destroyed by the dropwise addition of ethanol dissolved in ether. Water was added, and the solution was thoroughly extracted with ether. The combined organic layers were dried over $MgSO_4$, and *in vacuo* evaporation of the solvents afforded a residue which was chromatographed over SiO_2 with 2% EtOAc/hexane as eluens. The dihydrocarvone **13** was isolated in an 80% yield (6.1 g, 0.04 mol). ^1H NMR (for ring numbering see scheme 4): δ 4.70 (2H, m, $R_2C{=}CH_2$), δ 2.35 (4H, m), δ 2.13 (1H, m, H^5), δ 1.87 (1H, m, H^2), δ 1.68 (3H, broad s, (H_3C-C(R)=CH_2), δ 1.57 (1H, m), δ 1.32 (1H, m), δ 0.98 (3H, d, H^7, $^3J_{7\text{-}2}$= 6.5); ^{13}C NMR (for ring numbering see scheme 4): δ 212.5 (C^1), δ 147.5 (H_3C-C(R)=CH_2), δ 109.5 (H_3C-C(R)=CH_2), δ 46.9 (C^5), δ 46.8 (C^6), δ 44.6 (C^2), δ 34.6 (C^3), δ 30.6 (C^4), δ 20.4 (H_3C-C(R)=CH_2), δ 14.2 (C^7).

Synthesis of Compound 14

A solution in THF of the anion of triethylphosphonoacetate ($(C_2H_5O)_2P(O)CH_2CO_2C_2H_5$, 35.9 g, 0.16 mol) was made with dry NaH (4.1 g, 0.17 mol; the 80% NaH oil dispersion was washed 5 times with hexane) at -20°C. A vigorous hydrogen evolution was observed at -20°C; after 1 h the reaction mixture (with a light orange colour) was stirred for an additional 2 h at room temperature. At 0°C dihydrocarvone **13** (6.1 g, 0.04 mol) was added, and the resulting reaction mixture was stirred for 24 h at room temperature. Water was added, and the solution was thoroughly extracted with ether, after which the combined organic extracts were dried over $MgSO_4$. The solvents were evaporated under reduced pressure, and the resulting residue was chromatographed over silicium oxide (SiO_2) with 5% EtOAc/hexane as the eluens. Ester **14** was obtained in a 95% yield (8.4 g, 0.038 mol). ^{13}C NMR (for ring numbering see scheme 4): δ 167.0 (R-(C=O)-OR), δ 165.7 (C^1), δ 149.0 (H_3C-C(R)=CH_2), δ 110.7 (C=C(H)-COOR), δ 108.7 (H_3C-C(R)=CH_2), δ 59.4

(H_3C-CH_2-O-CO-R), δ 46.9 (C^5), δ 39.4 (C^2 or C^6), δ 36.8 (C^2 or C^6), δ 35.4 (C^3), δ 31.4 (C^4), δ 20.8 (H_3C-C(R)=CH_2), δ 17.8 (H_3C-CH_2-O-CO-R), δ 14.2 (C^7).

Ozonolysis of the Isopropenyl Group

Ester **14** (2.2 g, 0.01 mol), was dissolved in 100 ml absolute methanol. The solution was cooled to -78°C, and ozone was carefully passed through. The reaction was monitored with TLC. When the reaction was finished, the temperature of the reaction mixture was allowed to come to 20°C, and the methanol was evaporated. Methylene chloride (100 ml), pyridine (2 ml), and DMAP (50 mg) were added, and the solution was cooled to -70°C. 4-Nitrobenzoyl chloride (2.8 g, 0.015 mol) was added, and the temperature of the reaction mixture was allowed to come to room temperature. The reaction mixture was stirred for 16 h. Subsequently, the solution was heated under reflux, after which the solvents were removed *in vacuo*. This afforded a residue which was dissolved in absolute ethanol, followed by the addition of AG-50WX4 ion-exchange resin (15 g, prewashed (3 times) with absolute ethanol). The resulting suspension was stirred at 50°C over-night. The suspension was subsequently filtered, the solvents removed under reduced pressure, and the residue was chromatographed over SiO_2 (from 7.5% EtOAc/hexane to 30% EtOAc/hexane). This afforded the desired alcohol in a 75% yield (1.5 g, 0.0075 mol).

Preparation of Phosphonate 15[3,23]

The hydroxyl group of the alcohol was protected with a *t*BDMSi group (*t*BDMSiCl/imidazol in DMF, overnight stirring, 95% yield), and the ester moiety was subsequently reduced with diisobutylaluminium hydride (DiBAl-H). Thus, the ester (2.0 g, 0.0071 mol) was dissolved in 35 ml toluene, and the resulting solution was cooled to -78°C. Diisobutylaluminium hydride (12 ml 1.5 M in toluene) was added, and the resulting reaction mixture was stirred for 1 h. The solution was subsequently poured in a 2 M disodium tartrate aqueous solution, and the resulting mixture was stirred during 30 min. The extraction was carried out with diethyl ether, and the combined organic layers were dried over $MgSO_4$. The resulting alcohol was isolated in a 97% yield (1.7 g, 0.0069 mol), and was immediately used in the subsequent reaction.

N-chlorosuccinimide (1.9 g, 0.014 mol) was dissolved in 50 ml CH_2Cl_2 and cooled to 0°C. Dimethylsulfide (0.9 g, 0.014 mol) was subsequently added over 15 min. A white voluminous precipitate was formed. After the addition, the resulting suspension was stirred for 15 min. At -20°C the suspension was treated dropwise with a solution of the alcohol in 5 ml of methylene chloride. The reaction mixture was stirred for 30 min at that temperature, after which the temperature of the reaction mixture was allowed to come to room temperature. The mixture was washed with water and brine, and dried with $MgSO_4$. Removal of the solvents resulted in a residue which was dissolved in 50 ml THF. At -70°C, lithium diphenylphosphide, dissolved in THF (0.3 M), was added until the deep orange colour of the phosphide persisted. A few drops of water were added, and the solvents were removed *in vacuo*. The residue was dissolved in 50 ml methylene chloride, and 25 ml of a 10% aqueous hydrogen peroxide solution was added, after which the mixture was vigorously stirred for 14 h. The organic phase was separated, and the

water layer was thoroughly extracted with CH_2Cl_2. The combined organic layers were washed with an aqueous 10% $Na_2S_2O_8$ solution and dried over $MgSO_4$. Removal of the solvent afforded **15** as a colourless oil in a 40% yield (1.9 g, 0.0041 mol) starting from ester **14**. Chromatography (50% EtOAc/hexane) afforded **15** as a white powder. ^{1}H NMR (for ring number-ing see scheme 4): δ 7.62 (4H, m, $H^{ar}{}_{ortho}$), δ 7.38 (6H, m, $H^{ar}{}_{meta}$ and $H^{ar}{}_{para}$), δ 5.12 (1H, dd, R-CH=CR_2, $^3J_{H\text{-}CHP}$= 7.5, $^3J_{H\text{-}P}$= 7.0), δ 3.20 (1H, m, H^5), δ 3.04 (2H, dd, R_2C=CH-CH_2-P, $^3J_{H\text{-}HC=}$=7.5, $^2J_{H\text{-}P}$= 15.1), δ 2.44 (1H, dd, $H^6{}_{eq}$, $^2J_{6\text{-}6}$= 13.2, $^3J_{6\text{-}5}$= 3.2), δ 1.9-1.1 (6H, m), δ 0.82 (3H, d, H^7, $^3J_{7\text{-}2}$= 7.0), δ 0.78 (9H, s, C_4H_9Si), δ -0.05 (6H, s, $(H_3C)_2$Si); ^{13}C NMR (for ring numbering see scheme 4): δ 145.1 (C^1, $^3J_{C\text{-}P}$= 11.5), δ 132.0 ($C^{ar}{}_{ipso}$, $^1J_{C\text{-}P}$= 90.0), δ 131.0-130.0 ($C^{ar}{}_{meta}$ and $C^{ar}{}_{para}$), δ 128.1 ($C^{ar}{}_{ortho}$, $^2J_{C\text{-}P}$= 11.1), δ 108.5 (C=CH, $^2J_{C\text{-}P}$= 8.0), δ 70.9 (C^5), δ 38.6 (C^3), δ 37.3 (C^2), δ 35.0 (C^4), δ 32.7 (C^6), δ 30.0 (=CH_2P, $^1J_{C\text{-}P}$= 70.0), δ 25.5 ($(H_3C)_3$CSi), δ 17.8 ($(H_3C)_3$CSi), δ 17.4 (C^7), δ -5.0 ($(H_3C)_2$Si); ^{31}P NMR: δ 31.3; MS *m /e* 455 (M^+, 70), 397 (38), 323 (100), 215 (10), 201 (55), 121 (30), 93 (18), 73 (48), 59 (7).

Wittig-Horner Coupling of 12 and 15; Synthesis of 25 Hydroxydihydrotachysterol

A solution of MeLi in ether (0.063 ml, 1.6 M) was slowly added to a -70°C cooled solution of phosphine oxide **15** (0.046 g, 0.1 mmol) in 10 ml THF. A red solution was formed and this was stirred for 1 h at -70°C. Ketone **12** (0.017 g, 0.05 mmol), dissolved in THF, was subsequently added. The resulting reaction mixture was stirred for 2h at -70°C, and became pale orange. The temperature of the solution was allowed to come slowly to 20°C. A few drops of water were added, and the solvents removed under reduced pressure. The residue was dissolved in ether and washed with a saturated aqueous solution of sodium bicarbonate and brine, and dried over magnesium sulfate. Removal of the solvents afforded a residue which was dissolved in deoxygenated methanol (10 ml). Prewashed (3 times with methanol) AG-50WX4 ion exchange resin (1.0 g) was added at room temperature. The resulting suspension was stirred for 24 h. The mixture was subsequently filtered, and the solvents were removed *in vacuo*. Ether was added, and the solution was washed with brine (4 times), and dried with $MgSO_4$. The solvents were evaporated, and the residue was chromatographed over SiO_2 with 30% EtOAc/hexane as eluens. The seco-steroid analogue 25-OH-DHT_2 (10.1 mg, 0.024 mmol) was isolated in a crystalline form from diethyl ether. ^{1}H NMR (conventional steroid numbering is used): δ 6.16 and 5.91 (2H, AB system, H^6 and H^7, $^3J_{6\text{-}7}$= 11.1), 5.35 (2H, double q, H^{22} and H^{23}, $^3J_{22\text{-}23}$= 15.3, $^3J_{22\text{-}20}$= 7.7, $^3J_{23\text{-}24}$= 7.6), δ 3.63 (1H, m, H^3), δ 3.15 (1H, m), δ 2.87 (1H, m), δ 2.20-1.25 (21H, m), δ 1.18 (3H, s, H^{26} or H^{27}), δ 1.14 (3H, s, H^{26} or H^{27}), δ 1.09 (3H, d, H^{28}, $^3J_{28\text{-}24}$= 6.6), δ 1.04 (3H, d, H^{19}, $^3J_{19\text{-}10}$= 6.6), δ 1.01 (3H, d, H^{21}, $^3J_{21\text{-}20}$= 6.9), δ 0.57 (3H, s, H^{18}); ^{13}C NMR (conventional steroid numbering is used):[29] δ 142.0 (C^8), δ 140.0 (C^5), δ 139.1 (C^{23}), δ 129.2 (C^{22}), δ 116.9 (C^6), δ 115.7 (C^7), δ 72.5 (C^{25}), δ 70.9 (C^3), δ 56.4 (C^{14} or C^{17}), δ 56.3 (C^{14} or C^{17}), δ 48.2 (C^{24}), δ 45.8 (C^{13}), δ 40.4 (C^{12}), δ 38.2 (C^4), δ 37.7 (C^{10}), δ 34.9 (C^2), δ 33.2 (C^1), δ 30.9 (C^{20}), δ 28.9 (C^9), δ 27.8 (C^{26} or C^{27}), δ 27.0 (C^{26} or C^{27}), δ 26.4 (C^{16}), δ 23.5 (C^{15}), δ 22.3 (C^{11}), δ 21.0 (C^{21}), δ 17.8 (C^{19}), δ 15.7 (C^{18}), δ 12.3 (C^{28}).

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30. Crystallographic data of diol **6** will be published elsewhere (Kooyman *et al.*).
31. Crystallographic data of tosyl-benzoate **9** will be published elsewhere (Spek *et al.*).