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SYNTHESIS OF 3ß-ACETOXY-1ß,2ß-EPOXY-25-HYDROXY- CHOLESTA- 5,7-DIENE AND 2ß,25-DIHYDROXYVITAMIN D_3

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This paper is dedicated to Professor Dr. R. Wiechert on the occasion of his 65th birthday.

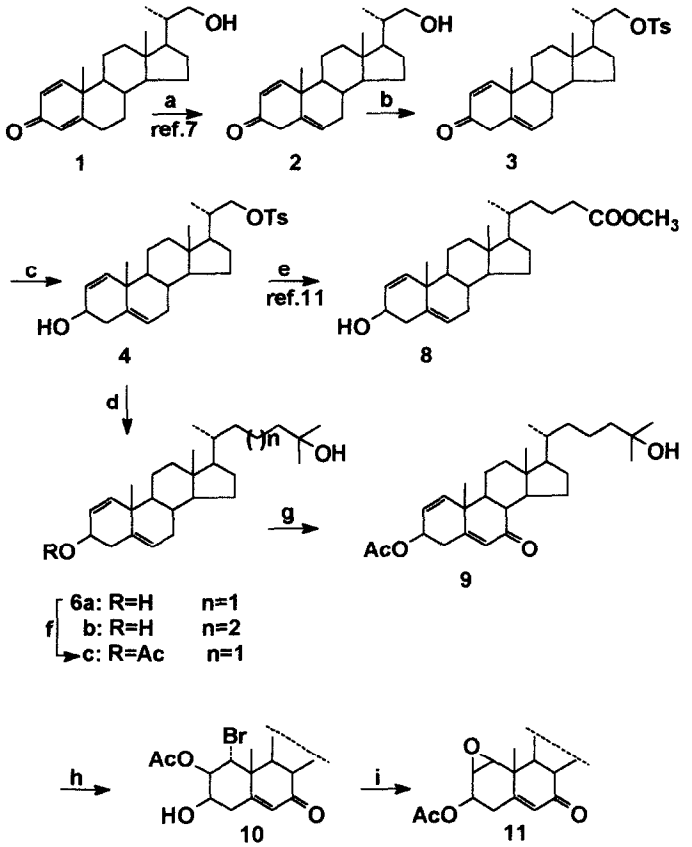
Abstract: The new key compounds **4** and **9** and the 25-hydroxy provitamin **13**, possessing an 1ß,2ß-epoxy group, have been synthesized from **1**. **13** has been converted to 2ß,25-dihydroxyvitamin D_3(**18**), using $LiAlH_4$ reduction and an efficient new photochemical process.

Besides its classical role in calcium homeostasis, 1α,25-$(OH)_2$-D_3 inhibits cell proliferation, induces cell differentiation and shows immunoregulatory effects.[1] Therefore a possible use in the treatment of cancer and psoriasis is suggested. Much effort is directed to the development of analogues possessing high cell differentiating activity and low calcitropic activity. By now a lot of compounds exhibiting promising therapeutic properties are known, especially side chain analogues,[2] but also some A-ring derivatives.[3] In spite of this progress we feel that much more effort is necessary to get an insight into structure activity relationship.[4] Therefore we decided to synthesize key compounds suitable for the synthesis of side chain and A-ring analogues. The present paper describes in a short form our route of synthesizing such key compounds and the 1ß,2ß-epoxy provitamin **13**, the reduction to the 2ß-hydroxy compound **14** and the photochemical conversion to 2ß,25-dihydroxyvitamin D_3 (**18**), using a simultaneous irradiation with UV light at two wavelengths, which enabled us to get a high yield of previtamin. Such a sequence of reactions, combined with an effective separation of the photoproducts, is competitive to well-established convergent syntheses of vitamin D analogs which avoid photochemical step.[5] An advantage of syntheses proceeding via the provitamins is the use of rigid steroid skeleton for control regio- and stereochemistry.

Our starting material, (20S)-20-hydroxymethyl-pregna-1,4-diene-3-one (**1**), available by microbial degradation of sterols,[6] has already been used for the synthesis of the vitamin D metabolites 1α,25-$(OH)_2$-D_3

and 24,25-$(OH)_2$-D_3 via the corresponding intermediates 1α,25-dihydroxy-cholesterol[7] and (20S)-3ß-acetoxy-20-phenylsulfonylmethyl-5,7-pregnadiene.[8]

Scheme 1

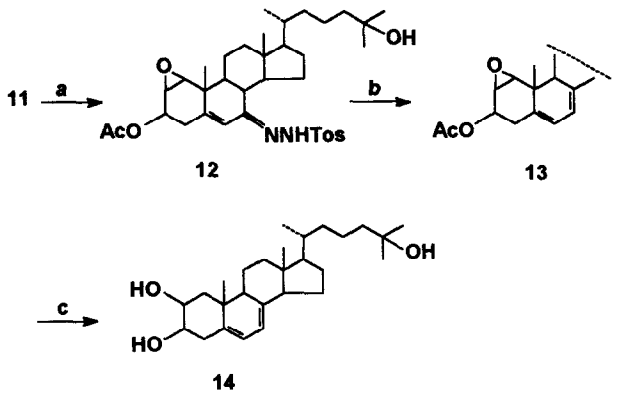


a: tert-BuOK/DMSO, r.t. b: p-TosCl/Py, -10 °C to 0 °C, 4h. c: i $CaCl_2$/EtOH/$NaBH_4$, -20 °C, 3/EtOH, -20 °C, 3h; ii acetone,H_2O, dil. HCl, 0 °C. d: i $ClMgCH_2CH_2C(CH_3)_2OSiMe_3$ (5a)/THF or $ClMgCH_2CH_2CH_2C(CH_3)_2OSiMe_3$ (5b)/THF, CuI, 0 °C/ 2h, r.t./ 14h; ii NH_4Cl/H_2O, HCl, CH_2Cl_2. e: i NaI/DMF, Δ, ii (dipy)Ni(CH_2CH_2COO) (7)/MnI_2/DMF, 48h, r.t.; iii dil.HCl/ether, H_2O; iv CH_2N_2 /ether. f: Ac_2O/Py, r.t. g: $Na_2Cr_2O_7$ (3.3 Mol% related to steroid), silica gel (Merck Co.), benzene, tert-BuOOH (80%), 50 °C, 5h. h: NBS/THF/H_2O, r.t., 3h. i: CH_3COONa/EtOH, 80 °C, 1h.

Deconjugation of 1 as previously described[7] and tosylation of the 22-hydroxy group (see Scheme 1) provided compound 3 possessing a 22-tosyloxy group suitable for C-C coupling reactions. Reduction of 3 with $Ca(BH_4)$[9] gave the 3ß-hydroxy compound 4 in a smooth reaction. The total yield from 1 to 4 is nearly 60 %. Compound 4 represents a key intermediate for the synthesis of compounds with different side chains: reaction

with the Grignard compounds **5a**[10] and **5b**[2b] and CuI in tetrahydrofuran provided the 25-hydroxy compound **6a**[9b] and the analogous 24-homo compound **6b**, respectively, in good yields; reaction of the 22-iodide, obtained from **4** with NaI in dimethylformamide and subsequent reaction with the nickelalactone **7** and MnI_2, followed by hydrolysis and esterification with CH_2N_2, gave the ester **8**[11], a suitable starting material for the synthesis of 26,27-homo compounds by Grignard reactions. The further model synthesis is described with compound **6a**, possessing the normal length 25-hydroxy side chain. After acetylation of **6a** with acetic anhydride and pyridine at room temperature the epoxidation of the resulting 3ß-acetoxy compound **6c** with m-chloroperbenzoic acid, in contrast to the literature[12], gave the 5ß,6ß-epoxide and not the desired 1,2-epoxide. For this reason it seemed necessary to deactivate the 5-double bond. One possibility would be the introduction of a 7-oxo group, which would also be useful for the later conversion into the desired 7-double bond.[13] We were able to obtain the hitherto unknown steroid type possessing a protected 3ß-oxygen function and the 1,5-diene-7-oxo system by allylic oxydation, using a catalytic amount of $Na_2Cr_2O_7$ on silica gel and 80% *tert*-butyl hydroperoxide in benzene (50 °C, 4-5 h) in more than 70 %. The epoxidation of the 3ß-acetoxy compound **9** with m-chloroperbenzoic acid in toluene now gave the 1,2-epoxide as expected, but unfortunately as a mixture of α- and ß-epoxide. ^1H NMR analysis revealed the main compound to have the 1ß,2ß-configuration. Therefore we investigated the addition of hypobromous acid (NBS/H_2O, tetrahydrofuran) to the compound **9**.The major product, obtained in good yield, was the 1α-bromo-2ß-acetoxy-3ß-hydroxy compound **10**, which arose from the α-bromonium ion by attack of the neighboring 3ß-acetoxy group. The structure of **10** was determined by ^1H NMR analysis using trichloroacetyl isocyanate. **10** cyclized under mild basic conditions to the 3ß-acetoxy-1ß,2ß-epoxide **11**. Interestingly, a migration of the acetoxy group back to the 3ß-position took place.

Scheme 2



a: p-TosNHNH$_2$/THF, reflux/6h, r.t./12h. b: i LiH/toluene, 100 °C, 1h; ii 0 °C, H$_2$O/MeOH; iii silica gel (Merck 60), benzene/CHCl$_3$ (6/4). c: LiAlH$_4$/ether, r.t.

On this way, the ß-epoxide **11** is conveniently available from **9** in more than 60 %. Investigations to obtain the isomeric α-epoxide with a higher stereoselectivity are now in progress. For the synthesis of the compound **13**, possessing an 1ß,2ß-epoxy group and the 5,7-diene system, **11** were transformed into the tosyl hydrazone[13] **12** (see Scheme 2) without attack at the epoxy group. Treatment of **12** with lithium hydride[13] in toluene gave the desired 1ß,2ß-epoxy provitamin **13** [m.p. 199-201 °C; $[\alpha]_D$ +36 ° ($CHCl_3$); M^+ 456,32000 $C_{29}H_{44}O_4$] in a smooth reaction in yields of 60-70 % from **11**.

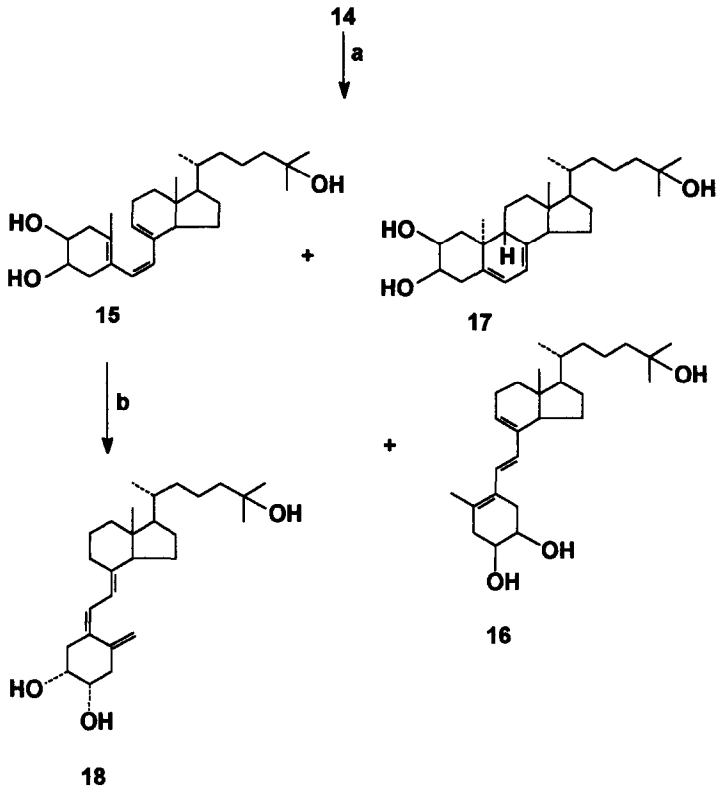
The reduction of **13** with lithium aluminium hydride proceeds with trans-diaxial opening of the epoxide and cleavage of the ester group giving the provitamin **14** with a 2ß-hydroxy group in high yields.

An efficient process was developed for the photoisomerization of the non-protected provitamin **14**, which was carried out in a 450 ml photoreactor equipped with a mercury high pressure lamp in the temperature range from -50 to -45° C. Using a filter solution consisting of 2,7-dimethyl-3,6-diaza-cycloheptan-1,6-diene-tetrafluoroborate (DDCHDT) and biphenyl in ethanol, a simultaneous irradiation of **14** in a mixture of *tert*-butyl methyl ether (MTBE) and 10% methanol with light in the range of 285 to 300 nm and >330 nm is possible. Under these conditions the desired previtamin **15** is the main product in the irradiation mixture.[14] The separation of the photoisomers previtamin (**15**), tachysterol (**16**) and lumisterol (**17**) as well as the unconverted provitamin **14** was realized by flash chromatography on silver impregnated silica gel using a modified flash apparature.[15] The isolated previtamin (**15**) gives the desired vitamin (**18**) [m.p. 201-205 °C; M^+ 416,32842 $C_{27}H_{44}O_3$; UV: $\lambda_{max}(CH_3OH)$ 264 nm, ε = 16500] by thermal isomerization. Lumisterol **17** and the separated provitamin **14** were used for recycling, and tachysterol **16** was isomerized to the corresponding previtamin by fluorenone-sensitized irradiation. In this way the vitamin **18**, an isomer of the metabolite 1α,25-dihydroxyvitamin D_3, was obtained in 55-65% yield from the corresponding provitamin. The new vitamin derivative **18** as well as the reversible photoisomers **15**, **16** and **17** have been isolated in a tlc-pure form and have been characterized by UV, mass, and ^1H NMR spectroscopy.

In summary, starting with compound **1** we have obtained the key intermediate **4**, the new steroid type **9** and the 1ß,2ß-epoxy provitamin **13**, which is useful for further synthesis. Furthermore we have developed an efficient photoisomerization of the 2ß-hydroxy provitamin **14** to the previtamin **15**, starting compound for the vitamin **18** in this route.

*Preliminary biological results of compound **18**:* The binding affinity[16] of **18** to the chick intestinal cytosolic receptor was less than 1/1000 compared with 1α,25-$(OH)_2$-D_3. In the induction of HL-60 cell differentiation (ATCC cells)[16] a concentration of 6.0 x 10^{-7} mol/L of **18** gave 50 % of the maximal NBT reduction [4 x 10^{-9} mol/L of 1α,25-$(OH)_2$-D_3; **18** has 1/150 of this activity].

Scheme 3



a: i photoisomerization: MTBE/MeOH (9/1); photoreactor, Hg high pressure lamp, filter solution (DDCHDT/biphenyl), 45 min. ii flash chromatography: Ag^+-doted silica gel 40-63 μm, ethyl acetate/acetone (7/3); fractions: 1. previtamin, 2. tachysterol, 3.provitamin/lumisterol; iii fluorenone-sensitized photoisomerization of fr. 2: MTBE; glass photoreactor, Hg high pressure lamp, filter solution (KNO_2/H_2O), -10 °C, 1h. iv recycling of fr. 3 like i b: i fr. 1 and mixture of [a: iii] in ethyl acetate/n-hexane (3/1), 55 to 60 °C, 6h; ii flash chromatography: silica gel 25-40 μm, ethyl acetate/n-hexane (3/1), fractions: 1. vitamin, 2. previtamin, 3. tachysterol; iii recycling of fr. 3 like [a: iii]; iv recycling thermal isomerization of all previtamin fractions.

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