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SUBSTRATES TO STUDY THE MECHANISM OF VITAMIN D HYDROXYLATION: SYNTHESIS OF [24R-^{2}H]-25-HYDROXYPROVITAMIN D_3

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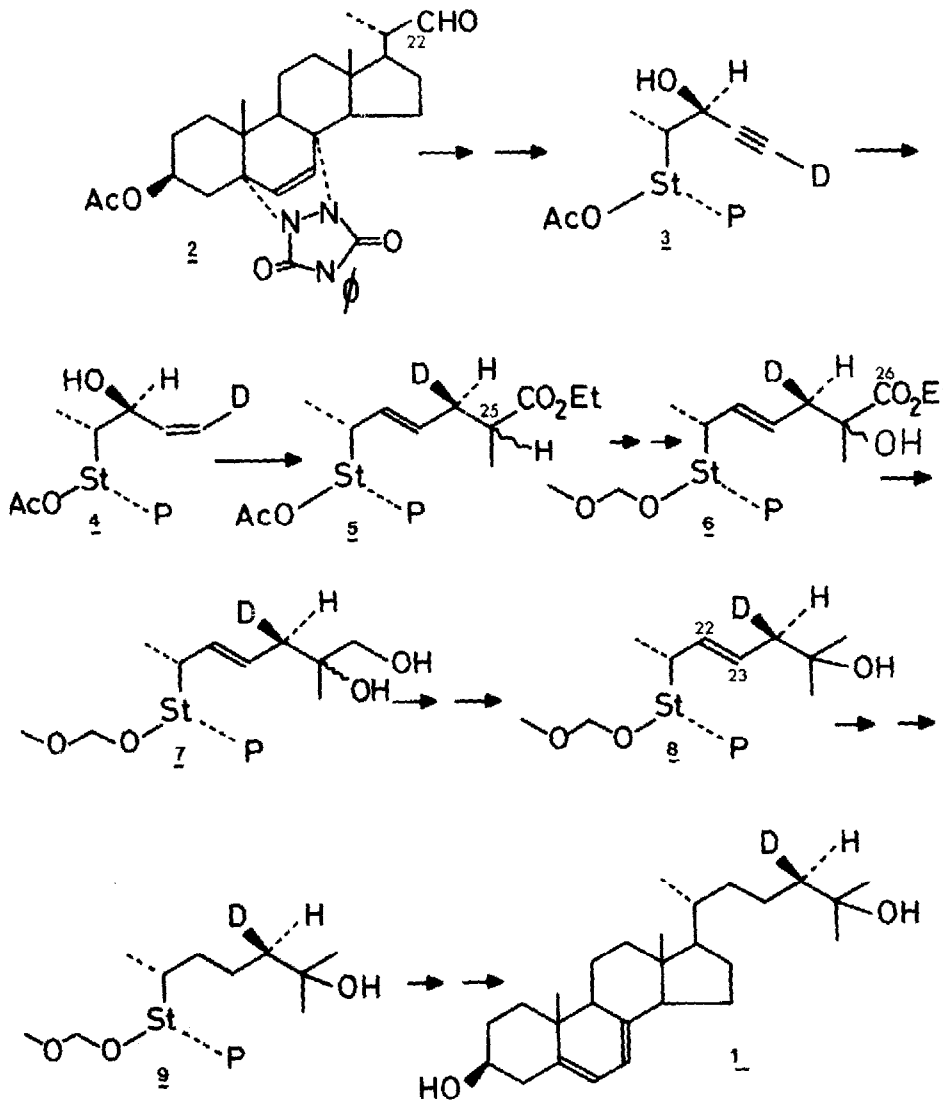
Summary: [24R-^{2}H]-25-Hydroxyprovitamin D_3 has been synthesised, in order that the corresponding vitamin may be used to study the stereochemistry of hydroxylation in production of the metabolite 24R,25-dihydroxycholecalciferol. The stereospecific introduction of deuterium at C-24 is effected by means of a Claisen rearrangement.

24R,25-Dihydroxycholecalciferol is an important metabolite of vitamin D_3,[1,2] which may be concerned with calcium action in bone.[3,4] The site of 24-hydroxylation is kidney,[5] but whether hydroxylation occurs with retention or inversion of configuration is unknown. The expectation is that hydroxylation would occur with retention of configuration (insertion of oxygen into the C-H bond).[6] We report here the synthesis of [24R-^{2}H]-25-hydroxyprovitamin D_3 (1), suitable for conversion into the corresponding vitamin, and hence for testing the mechanism of hydroxylation.

The C-22 aldehyde 2,[7] in which the ring B diene has been protected by reaction with phenyltriazolinedione, was prepared from ergosterol acetate. The aldehyde underwent selective attack when treated with 2 equivalents of lithium trimethylsilyacetylide in tetrahydrofuran (THF) at -78°. The reaction was quenched (aqueous NH_4Cl) after 3h, and the resulting mixture of diastereoisomers at C-22 (3:1 ratio) separated by short column chromatography.[8] The major diastereoisomer (mp 200-201, 50% yield) was shown to possess the 22R stereochemistry by X-ray crystallography of its p-bromobenzoate (mp 198-199).[9] The synthesis was continued with this diastereoisomer, and the trimethylsilyl group removed, by treatment with 10 equivalents of dry benzyltrimethylammonium



St = steroid nucleus
P = ring-B protecting group

fluoride in THF containing 100 molar equivalents of 99.8% D_2O, to give 3. This acetylene was converted to the Z-allylic alcohol 4 by catalytic hydrogenation over Lindlar's catalyst in THF. Upon reaction of 4 with triethylorthopropionate, and propionic acid as catalyst, in refluxing dry benzene for 16h, the Claisen rearrangement product 5 was obtained in 80% yield as a mixture of diastereoisomers at C-25.[10] In order to obtain the quoted yield, it was necessary to titrate the crude product with a small amount of phenyltriazolinedione, to replace a portion of the ring B protecting group which is lost .

At a later stage in the synthesis, a base-stable protecting group is required for the 3β-OH group.[11] Reaction of 5 with potassium carbonate in dry ethanol gave the corresponding 3β-alcohol, which afforded the 3β-methoxymethyl ether upon treatment with chloromethylmethyl ether and N,N-diisopropylethylamine in CH_2Cl_2. This product was converted[12] to the α-hydroxy ester 6 in 75% yield upon reaction of the C-25 anion (generated by use of 1.2 equivalents of lithium isopropylcyclohexylamide at -78° in THF) with oxygen at -78°, and reduction of the resulting peroxide with triethyl phosphite. The C-26 ester group was then removed by (i) reduction of 6 to the C-26 alcohol with $LiBH_4$ in THF to give 7 (95% yield) and (ii) reduction of the C-26 tosylate (tosyl chloride/pyridine) with $LiBH_4$ in refluxing THF to give 8 (70% yield).

Reaction of 8 with BH_3 (1 equivalent) in THF for 1 h at room temperature gave a C(22)-C(23) adduct which, unfortunately, was not cleaved by acetic acid. The adduct was therefore worked-up oxidatively (H_2O_2 in aqueous sodium carbonate) to give a 23,25-diol, which was converted to the 23-tosylate (tosyl chloride/pyridine), and this in turn was reduced with $LiBH_4$ in THF for 12h to give 9 (20% overall yield from 8). Removal of the protecting groups (TsOH in MeOH under reflux for 2h, followed by reaction with $LiAlH_4$ in THF at 50° for 12h) gave [24R-^{2}H]-25-hydroxyprovitamin D_3 (1) in 70% overall yield from 9.

The problems associated with removal of the C(22)-C(23) double bond detract from this route to introduce chirality at C-24 in a saturated sidechain. However, it is noteworthy that an analogue of 3, in which CH_3 replaced deuterium, would upon $LiAlH_4$ reduction,[13] Claisen rearrangement, C-25

hydroxylation, and reduction furnish 25,26-dihydroxyvitamin D_2 in a relatively efficient synthesis. We are currently exploring this route.

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