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# Synthesis of (25R)-Cholest-5-ene-3β, 26-diol and its Radiolabeled Analog

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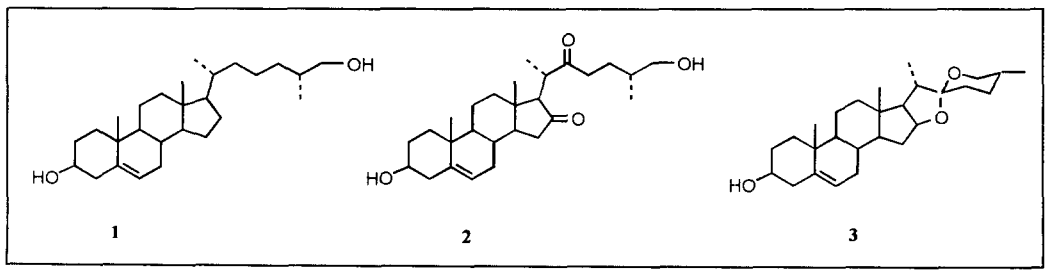
Summary: A new, convenient and stereoselective route to the synthesis of (25R)-cholest-5-ene-3β,26-diol (**1**) and its radiolabeled analog **4** is described. The key step is a Julia condensation of sulfone **6** with aldehyde **12** to furnish compound **13**. Further reduction of the α-hydroxysulfone moiety afforded 22,23-unsaturated i-steroid **14**. The double bond was reduced by hydrogen and by tritium to provide substrates for the preparation of **1** and **4**, respectively. © 1997 Elsevier Science Ltd.

The title compound **1** is an intermediate[1] in the metabolic pathway from cholesterol to bile acids.[2] 27-Hydroxycholesterol (**1**) was found to be an inhibitor of HMG CoA reductase and has been shown to down regulate LDL receptor binding in fibroblasts. The absence of the sterol 27-hydroxylase enzyme is the metabolic basis of a genetically determined disease referred as cerebrotendinous xanthomatosis. In addition inhibition of DNA synthesis[3] and antitumor properties have been reported.[4]

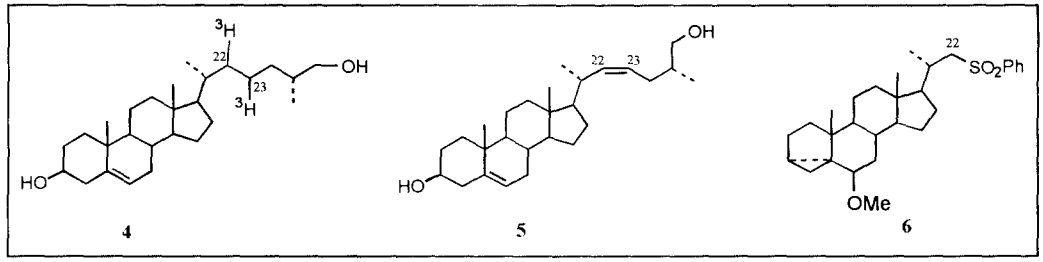



**1** **2** **3**

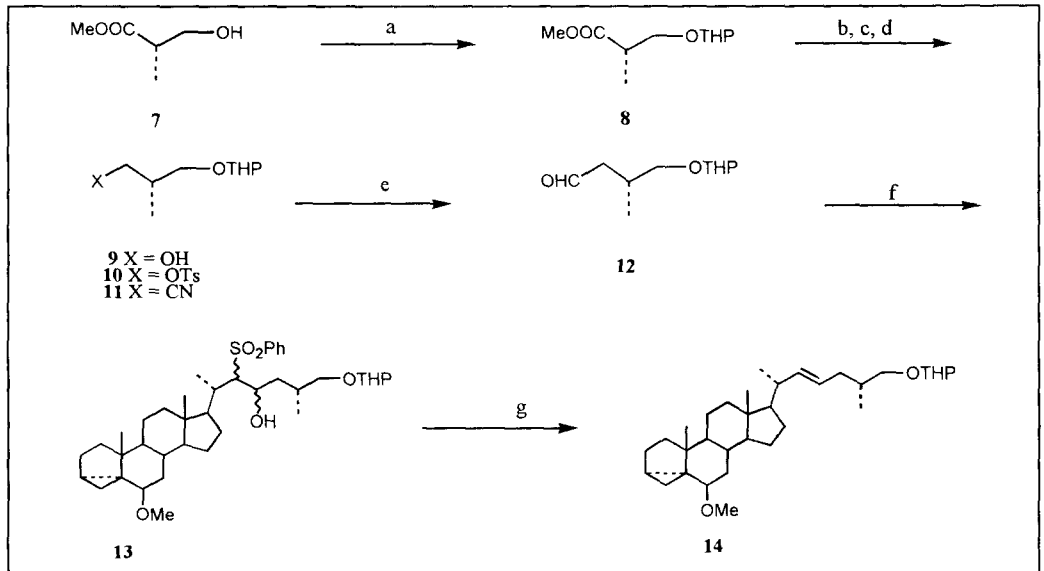
For our study of cholesterol metabolism, chemically and diastereomerically pure **1** and its radiolabeled (tritiated) analog were required. Since the side chain in **1** is degraded in the metabolic process to the shorter C-24 metabolite, tritium should be introduced at positions before C-24. The metabolically unstable C-2, C-3, C-5, C-6 and C-7 positions should also be excluded. Classical routes to the synthesis of **1** utilized two readily available natural isoprenoids, kryptogenin[5] (**2**) and diosgenin[6] (**3**). In principle these are efficient approaches.

The syntheses involved two-stage reduction of the 16 and 22 oxo functions accompanied by the elaboration of the 3 and 27-hydroxyl groups. Unfortunately these methods suffer from several faults. One problem is the potential for epimerization at the C-25 stereocenter.[7] In addition to the use of **2** and **3** as starting points, other routes to optically active **1** have been described.[8] However none of these methods were deemed suitable for the synthesis of radiolabeled **1** under the requirements described above.[9] Recently Schroepfer's group described[6f,9] the preparation of (25R) 22,23 di-[$^3$H]-26-hydroxycholesterol (**4**) from compound **5**. The olefin **5** was obtained in several steps, in low yield from a by-product which was formed during preparation of **1** from **3**.

Recently methodology was developed, which provides synthesis of **1** by alkylation of the readily available sulfone **6**[10] with an optically pure alkylating agent.[11]

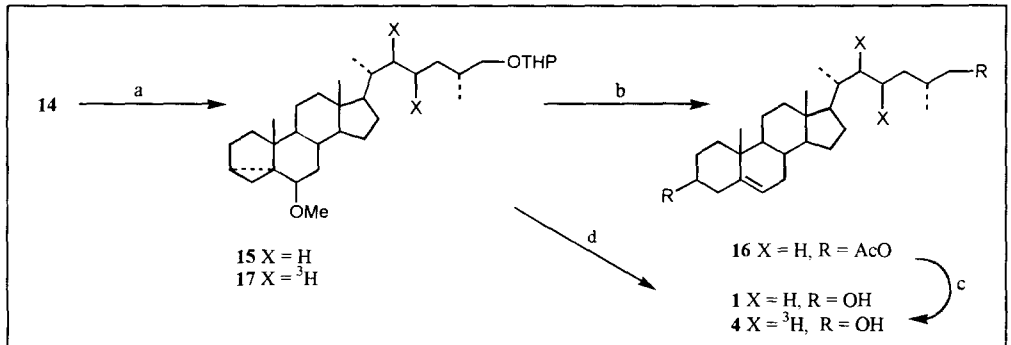



It was envisaged that introduction of the 22,23-unsaturation could be accomplished from the sulfone **6** and an appropriate $C_{23}$-$C_{27}$ fragment *via* Julia coupling.[12]




**Scheme 1**. *Reagents and conditions:* a). DHP, $CH_2Cl_2$, CSA; b). $LiAlH_4$, THF, 82%; c). TsCl, py, DMAP, 95%; d). KCN, MeOH, DMF, HMPA, 80 °C, 12h, 81%; e). DIBAL-H, toluene, -70°C, 89%; f). **6** + BuLi, THF, -70°C, then **12**, 65%; g). Na-Hg, MeOH, $Na_2HPO_4$ aq., 0 °C, 51%.

We began synthesis[13] of the side chain building block from the commercially available and optically active hydroxyester 7 (Scheme 1). The alcohol function in 7 was protected as THP acetal (equimolar mixture of diastereomers), then the resulting crude mixture[14] **8** was subjected to $LiAlH_4$ reduction to afford a mixture of the two diastereomeric alcohols **9** in high overall yield (82%). Further reaction of **9** with TsCl in pyridine provided the mixture of diastereomeric tosylates **10** in excellent yield (95%). Heating of **10** with KCN in MeOH-DMF-HMPA solution gave a mixture of cyanides **11** in 81% yield. The IR spectrum of **11** showed an absorption corresponding to a CN group at 2245 $cm^{-1}$. Reduction of the cyano group in **11** by DIBAL-H gave a mixture of the two aldehydes **12**. The $^1H$ NMR spectrum showed a double triplet at 9.8 ppm corresponding to the formyl group. The aldehydes **12** were condensed with sulfone **6** to give the mixture of steroidal hydroxysulfones **13** in 65% yield. Mass spectral analysis of **13** showed a signal at m/z = 657, corresponding to the $[M+H]^+$ ion. Reductive elimination of the α-hydroxysulfone moiety in **13** by sodium amalgam afforded a mixture of olefins **14** in moderate yield (51%); the absorption corresponding to the formed double bond was observed as multiplets at 5.17-5.24 ppm.




**Scheme 2.** *Reagents and conditions:* a). $H_2$, Pd-C, AcOEt, $NEt_3$, 98%, ($^3H_2$, 1,4-dioxane, Pd-C); b). $ZnCl_2$, $Ac_2O$, 81% ; c). MeOH, Amberlyst, 89%; d). 1,4-Dioxane, $H_2O$, PTSOH, Δ.

The synthesis of **1** from **14** was accomplished in three steps (Scheme 2). The double bond in **14** was efficiently (98%) reduced by hydrogen with Pd-C as a catalyst, in the presence of triethylamine, to afford i-steroid **15**, as a mixture of the THP acetals. Compounds **15** was subjected to acid catalyzed ($ZnCl_2$) ring opening to furnish diacetate **16** in good yield (81%). The absence of OMe group signals arise from i-steroid, and the presence of acetyl singlets at 2.03 and 2.05 ppm provided proof for the formation of **16**. Base catalyzed methanolysis of **16** afforded **1** in 89% yield. Spectrochemical data for (25R)-cholest-5-ene-3β, 26-diol (**1**) were identical in all respects with those reported previously.[5,6,11]

Substitution of tritium for the reduction of **14**, followed by hydrolysis of the resultant cyclosteroid **17** afforded tritiated analog **4**,[15] which exhibited high specific activity (55 Ci/mmol). The distribution of tritium determined by $^3H$ NMR is consistent with data for deuterated 27-hydroxycholesterol reported by Ni et al.[9]

In conclusion, we presented a new method for the synthesis of the diastereomerically pure (25R)-cholest-5-ene-3β, 26-diol (**1**) and its radiolabeled analog **4**, with the tritium in metabolically and chemically stable positions. The described procedure can be considered as a general method for the synthesis of the 22,23 deuterium or tritium labeled steroids.

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**Notes and references:**

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15). Olefin **14** has two prochiral centers, C-22 and C-23. Tritiation yielded the 22, 23 diastereomeric mixture.

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