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Investigation on the synthesis of 25-hydroxycholesterol

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ABSTRACT

A very efficient and environmentally benign method has been developed for the synthesis of 25-hydroxycholesterol. The reaction was performed in THF–water (4:1, v/v) using NBS as the brominating agent, followed by the easy reduction of C–Br with lithium aluminum hydride in THF, to yield the final product corresponding to a Markovnikov's rule. Excellent yields and regioselectivity have been obtained.

1. Introduction

Vitamin D_3 is an important biological regulator of calcium and phosphorus metabolism [1]. It is now established that the parent vitamin D_3 is sequentially metabolized in various tissues to the steroid hormone 1,25-$(OH)_2$-D_3 which exerts the highest biological activity of all vitamin D_3 metabolites. This hormonal derivative stimulates the intestinal absorption of calcium and phosphorus, and the mobilization of bone calcium through a target organ receptor mediated mechanism [2]. A common characteristic feature of these metabolites is the C-25 hydroxy group. Thus, the introduction of 25-hydroxy group into an appropriate substrate would be a key step in the synthesis of these compounds.

Using *in situ* generated ethyl(trifluoromethyl)dioxirane (ETDO), a facile synthesis was developed by Ogawa et al. [3] for 25-hydroxycholesterol, as well as its 3-sulfate (overall yield of the sulfate, 24%) and 24-oxocholesterol (16%), starting from cholesterol. However, long linear synthetic route and low yields are major hitches. Unlike cholesterol, the conventional starting material for preparing certain steroids (for example 25-hydroxycholesterol), desmosterol already contains a reactive side chain (Δ^{24}). Desmosterol plays an important role, as a labile intermediate, in the biosynthesis of cholesterol in animals. It was included in a filtrate of recrystallization of crude lanolin which was made from lanolin alcohol obtained by saponification of wool grease, a washing waste of wool, and the content of desmosterol reached 10–25% [4,5].

The reaction of mercuric acetate with desmosterol leads to the addition on the double bond of the groups –OH on one side, and –HgOAc on the other. It can be followed by the easy reduction of the C–Hg bond with sodium borohydride in sodium hydroxide/water, to yield the 25-hydroxycholesterol corresponding to a Markovnikov addition of water on the double bond. The nuclear Δ^5 double bond, which is quite reactive towards most electrophilic reagents, was left untouched. This remarkable selectivity has been confirmed in 1992, but this study has not been extended. Mercuric acetate is an environmental problem, obviously because of the poisonous nature of the reagent and of the products of the reaction. Care must be taken, even when working with small amounts, during the reaction and for the disposal of the residues [6].

The vicinal functionalization of carbon–carbon double bond is a powerful synthetic tool for organic chemists. In particular, selective introduction of two different functional groups, such as hydroxyl and halogen, has attracted sustained attention in organic synthesis [7]. Halohydrins are usually prepared *via* the ring opening of epoxides using hydrogen halides or metal halides. These procedures are associated with the formation of byproducts such as *vic*-dihalides and 1,2-diols. Meanwhile, these procedures require prior synthesis of epoxide. Apart from this, there are two general approaches for heterolytic addition of water and halogen to an olefinic bond. One involves the usage of molecular halogen, $TsNBr_2$, [8] N-halosaccharin [9] or N-halosuccinimide [10–23] for halogenation, and the other employs metal halide along with an oxidizing agent [24,25]. Cheap and available N-halosuccinimide, in particular N-bromosuccinimide (NBS) and N-chlorosuccinimide (NCS), are the better choice of halogen sources over other hazardous reagents for such transformations.

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From these points of view we have undertaken the syntheses of the 25-hydroxycholesterol using inexpensive reagents and available steroid starting compound. We were able to develop a facile synthesis of naturally occurring oxysterols, 25-hydroxycholesterol (**1**), from desmosterol (**2**) by using N-halosuccinimide *via* halohydrin reaction. Then, the reductive of halides is achieved by lithium aluminum hydride ($LiAlH_4$) in THF (Scheme 1). To the best of our knowledge, there are no examples describing the formation of 25-hydroxycholesterol *via* halohydrin reaction.

2. Experimental

Melting points were determined using WRR melting point apparatus. ^1H and ^{13}C NMR spectra were recorded on Bruker AV-400 spectrometer (Bruker Corporation, America) at working frequencies 400 and 100 MHz. respectively in $CDCl_3$ And with TMS as the internal standard. Chemical shifts are expressed in ppm downfield from TMS and observed coupling constants (*J*) are given in Hertz (Hz). Starting materials and reagents were commercially purchased and used without further purification. The progress of the reactions was monitored by thin-layer chromatography (TLC) Analytical thin-layer chromatography (TLC) was conducted using silica gel plates (200 μm) containing a fluorescent indicator (silica gel 60 F_{254}). Detection was performed by spraying with molybdophosphoric acid (5%) at 120 °C Column chromatography was performed using silica gel, 200–300 mesh, and elution was performed with *n*-hexane/ethyl acetate.

2.1. General procedure for the synthesis of desmosterol acetate 3

To a solution of the desmosterol (20 g, 0.05 mol) in hexane (150 mL), DMAP (200 mg) and acetic anhydride (10 g, 0.1 mol) were added, after stirring at 50 °C in 3 h (TLC control, TLC solvents: *n*-hexane/EtOAc (8:1, v/v)), the reaction mixture was successively washed with water, HCl solution (5%wt.) and saturated $NaHCO_3$ solution. Desmosterol acetate (18.85 g, 85.0%) was obtained by evaporating in a vacuum and recrystallization in EtOH.

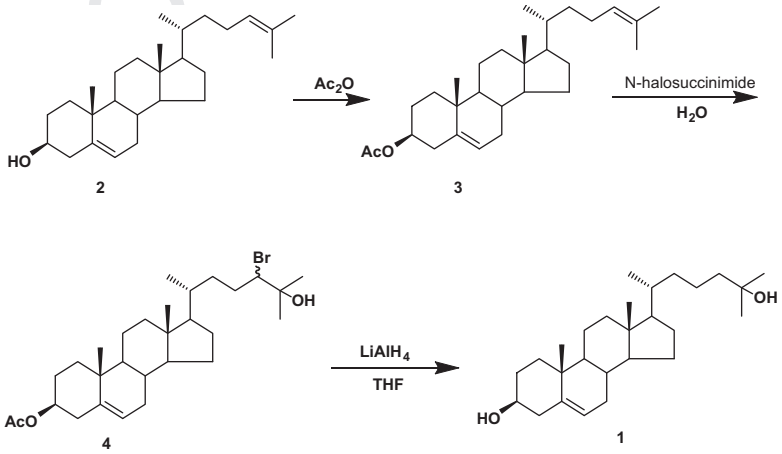
3 [26]: mp: 89.1–90.1 °C (lit. Mp: 91–92 °C) ^1H NMR ($CDCl_3$, 400 MHz): δ 5.38 (*d*, *J* = 4.0 Hz, 1H, 6-CH), 5.10 (*t*, *J* = 6.4 Hz, 1H, 24-CH), 4.60 (*m*, 1H, 3-CH), 1.61 (*s*, 3H, 26-CH_3), 1.53 (*s*, 3H, 27-CH_3), 1.01 (*s*, 3H, 19-CH_3), 0.86 (*d*, *J* = 6.5 Hz, 3H, 21-CH_3), 0.69 (*s*, 3H, 18-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.52 (C-18), 18.31 (C-21), 19.29 (C-19), 19.97 (C-23), 21.68 (C-11), 22.12 (–CO<u>C</u>H_3), 24.95 (C-27 and C-28), 25.37 (C-15), 26.40 (C-16), 28.42 (C-2), 32.52 (C-7 and C-8), 36.27 (C-20), 36.70 (C-22), 37.23 (C-10), 37.64 (C-1), 38.77 (C-12), 40.36 (C-4), 42.98 (C-13), 50.66 (C-9), 56.69 (C-17), 57.31 (C-14), 74.64 (C-3), 123.31 (C-6), 125.88 (C-24), 131.59 (C-25), 140.29 (C-5), 171.23 (–<u>C</u>OCH_3).

2.2. General procedure for the synthesis of bromohydrins 4

To a well-stirred solution of desmosterol acetate **3** (0.427 g, 1 mmol) in THF–water (4:1) (50 mL), NBS (0.213 g, 1.2 mmol) was added, and the reaction mixture was allowed to stir at −10 °C. Progress of the reaction was monitored by TLC (TLC solvents: *n*-hexane/EtOAc (8:1, v/v)). After 2 h, 10% aqueous sodium thiosulfate was added to destroy the excess NBS. The reaction mixture was extracted with dichloromethane (3 × 20 mL) and successively washed with saturated $NaHCO_3$ solution (20 mL × 2) and saturated NaCl solution (20 mL). The extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. Purification of the crude product by column chromatography on silica gel (200–300 mesh) with a mixture of *n*-hexane/EtOAc (8:1, v/v) as an eluent to give bromohydrins **4** (0.44 g, 85%).

In a large scale, to a well-stirred solution of desmosterol acetate **3** (4.27 g, 10 mmol) in THF–water (4:1) (300 mL), NBS (2.13 g, 12 mmol) was added, and the reaction mixture was allowed to stir at −10 °C. Progress of the reaction was monitored by TLC (TLC solvents: *n*-hexane/EtOAc (8:1, v/v)). After 4 h, 10% aqueous sodium thiosulfate was added to destroy the excess NBS. The reaction mixture was extracted with dichloromethane (3 × 300 mL) and successively washed with saturated $NaHCO_3$ solution (200 mL × 2) and saturated NaCl solution (200 mL). The extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. Purification of the crude product by column chromatography on silica gel (200–300 mesh) with a mixture of *n*-hexane/EtOAc (8:1, v/v) as an eluent to give bromohydrins **4** (4.2 g, 80.8%).

4: mp: 148.7–149.9 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.38 (*d*, *J* = 4.0 Hz, 1H, 6-CH), 4.60 (*m*, 1H, 25-OH), 2.68 (*m*. 1H, 24-CH), 1.31 (*s*, 3H, 26-CH_3), 1.27 (*s*, 3H, 27-CH_3), 1.01 (*s*, 3H, 19-CH_3), 0.94 (*d*, *J* = 6.5 Hz, 3H, 21-CH_3), 0.69 (*s*, 3H, 18-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.76 (C-18), 18.56 (C-21), 19.20 (C-19), 20.90 (C-11), 21.34 (–CO<u>C</u>H_3), 25.30 (C-27 and C-28), 25.58 (C-15), 27.65 (C-16), 28.12 (C-2), 31.73 (C-23), 32.25 (C-7 and C-8), 35.55 (C-20), 36.47 (C-22), 36.87 (C-10), 38.00 (C-1), 39.60 (C-12), 42.23 (C-4), 49.87 (C-13), 55.80 (C-9), 56.56 (C-17), 58.05 (C-14), 64.71 (C-24), 64.84 (C-25), 73.87 (C-3), 122.50 (C-6), 139.53 (C-5), 171.23 (–<u>C</u>OCH_3).



Scheme 1. Synthesis of 25-hydroxycholesterol (**1**) with desmosterol (**2**) as starting compound.

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2.3. General procedure for the synthesis of 25-hydroxycholesterol **1**

A solution of bromohydrins 4 (0.523 g, 1 mmol) in THF (30 mL) under a nitrogen atmosphere was treated with $LiAlH_4$ (0.15 g, 4 mmol) at 5 °C. Then the reaction mixture was stirred at 70 °C for 3 h (TLC control, TLC solvents: *n*-hexane/EtOAc (2:1, v/v)). Again cooled, the reaction mixture was added dropwise to a HCl solution (5%wt.). Then the mixture extracted with dichloromethane (3 × 20 mL) and successively washed with saturated $NaHCO_3$ solution (20 mL × 2) and saturated NaCl solution (20 mL). The extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. 25-hydroxycholesterol (0.38 g, 95%) was thus obtained after recrystallization in toluene.

In a large scale, A solution of bromohydrins 4 (5.23 g, 10 mmol) in THF (100 mL) under a nitrogen atmosphere was treated with $LiAlH_4$ (1.5 g, 40 mmol) at 5 °C. Then the reaction mixture was stirred at 70 °C for 3 h (TLC control, TLC solvents: *n*-hexane/EtOAc (2:1, v/v)). Again cooled, the reaction mixture was added dropwise to a HCl solution (5%wt.). Then the mixture extracted with dichloromethane (3 × 300 mL) and successively washed with saturated $NaHCO_3$ solution (200 mL × 2) and saturated NaCl solution (200 mL). The extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. 25-hydroxycholesterol (3.9 g, 97%) was thus obtained after recrystallization in toluene.

1 [3]: mp: 176.4–177.1 °C (lit. Mp: 178–180 °C). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.37 (*d*, *J* = 3.4 Hz, 1H, 6-CH), 3.50 (*m*, 1H, 3-CH), 1.23 (*s*, 6H, 26- and 27-CH_3), 1.03 (*s*, 3H, 19-CH_3), 0.94 (*d*, *J* = 6.5 Hz, 3H, 21-CH_3), 0.70 (*s*, 3H, 18-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.76 (C-18), 18.58 (C-21), 19.30 (C-19), 20.66 (C-23), 20.97 (C-11), 24.18 (C-15), 28.15 (C-16), 29.09 (C-26), 29.25 (C-27), 31.54 (C-2), 31.79 (C-7 and C-8), 35.64 (C-20), 36.36 (C-22 and C-10), 37.15 (C-1), 39.67 (C-12), 42.21 (C-4 and C-13), 44.31 (C-24), 50.01 (C-9), 55.96 (C-17), 56.65 (C-14), 71.03 (C-25), 71.67 (C-3), 121.58 (C-6), 140.67 (C-5).

3. Results and discussion

Desmosterol (**2**), obtained from crude lanolin by extraction, was chosen as a suitable starting material. Initially, the desmosterol **2** was transformed into the 3-protected ester **3** by treating with acetic anhydride in the presence of DMAP at 50 °C *via* the usual acetylation to avoid the side reaction of the nuclear Δ^5 double bond.

In the next sequence of reactions, 3-protected ester **3** was transformed into the halohydrins derivative **4** (Scheme 1). As mentioned above, N-halosuccinimide was powerful and versatile. In search for an effective halogenation reagent for the halohydrin reaction, we first studied the reaction of 3-protected ester **3** with NBS or NCS in aqueous THF at different reaction temperature, and the results was presented in Table 1.

Compared with the NCS (Table 1, entry 6), NBS worked much better as the halogenation reagent under the same conditions.

Table 1
Effect of different halogenation reagent.[a]

Entry	Reagent	Temp (°C)	Yield[b] (%)
1	NBS	20	58.1
2	NBS	10	63.7
3	NBS	0	72.3
4	NBS	−10	85.0
5	NBS	−20	84.8
6	NCS	−10	65.6

[a] The substrate **3** was treated with NBS/NCS (1.2 equiv.) in 20% aqueous THF.
[b] Isolated yield.

Table 2
Bromohydrination of **3** with different solvents.[a]

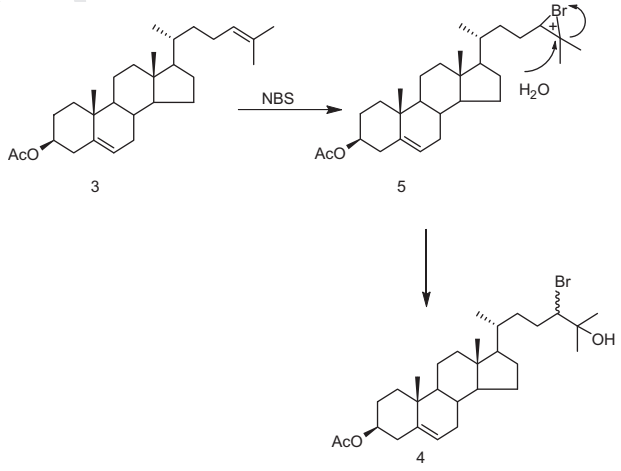
Entry	Solvent	Time (h)	Yield[b] (%)
1	CH_2Cl_2:H_2O(4:1)	24	19.8
2	EtOAc: H_2O(4:1)	24	21.1
3	Toluene:H_2O(4:1)	24	15.0
4	Butanone:H_2O(4:1)	2	68.8
5	*t*-Butanol:H_2O(4:1)	2	60.0
6[c]	Glyme:H_2O(5:1)	2	70.0
7[c]	Acetone:H_2O(10:1)	5	72.3
8	THF:H_2O(4:1)	2	85.0
9	THF:H_2O(10:1)	4	78.8
10	THF:H_2O(30:1)	10	56.6

[a] The substrate **3** was treated with NBS (1.2 equiv.) at −10 °C in different solvents (40 mL).
[b] Isolated yield.
[c] The ratio was determined by solubility of the substrate **3**.

Table 3
Effect of different amount of NBS.[a]

Entry	NBS (eq.)	Yield[b]/%
1	1.0	77.8
2	1.2	85.0
3	1.5	80.3
4	2.0	65.1

[a] The substrate **3** was treated with NBS in 20% aqueous THF at −10 °C.
[b] Isolated yield.

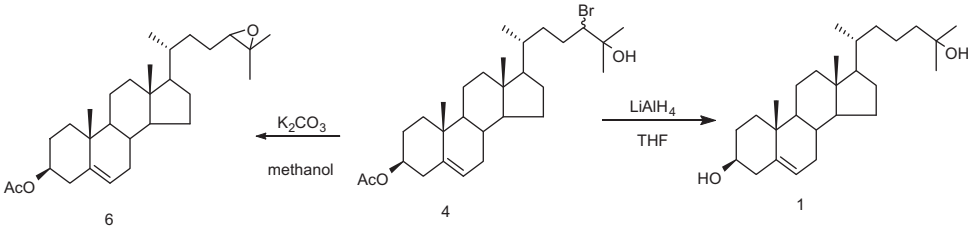


Scheme 2. Probable mechanism of bromination.

The bromohydrin reaction of **3** proceeded smoothly at −10 °C within 2 h, it afforded the desired bromohydroxylation product **4** in 85.0% yield (Table 1, entry 4). Then, the reaction temperature was varied from −20 to 20 °C using NBS as the halogenation reagent. Unfortunately, the decrease of the yield was observed as the temperature was increased (Table 1, entries 1–3), which could be ascribed to the selectivity of the nuclear Δ^5 double bond and side chain Δ^{24} double bond. Lower reaction temperature −20 °C has little effect on the yield (Table 1, entry 5). Therefore, −10 °C was chosen for the further experiments.

In search for an appropriate solvent for the bromohydrin reaction, the reactions of **3** and NBS in different solvents (Table 2) were scanned.

Various solvents, such as CH_2Cl_2, EtOAc, toluene, butanone, *t*-butanol, glyme, acetone and THF, in combination with water were studied for this purpose. CH_2Cl_2, EtOAc and toluene gave very



Scheme 3. The synthetic application of bromohydrins.

poor results due to the part conversion of 3-protected ester **3** during the long reaction time in two-phase system (Table 2, entries 1–3), except in the case of butanone (Table 2, entry 4). *t*-Butanol, glyme and acetone gave 60.0–72.3% yield of the desired product (Table 2, entries 5–7). It is interesting to observe that when reaction was carried out in a mixture of *tert*-butyl alcohol and water (4:1 volume ratio) a mixture of bromohydrin and *tert*-butoxybromide was obtained due to the competitive nucleophilicity of *tert*-butyl alcohol and water. A mixture of THF and water in a 4:1 ratio was found to be the best solvent for halohydrin formation (Table 2, entry 8). The reaction takes a relatively longer time in a lower yield when the THF–water ratio was decreased, especially the THF and water in a 30:1 ratio (Table 2, entries 9–10). It provided us the clue that water played an important role to increase the desired product **4** in this process. The nucleophilic solvent water could compete with halide ion leading to incorporation of the latter. An excess of water was employed as nucleophilic reagent to increase the yield of **4**. Therefore, a mixture of THF and water in a 4:1 ratio was selected as solvent because of its solubility of the substrate **3**, highest yield.

Having identified the optimized solvent, we next evaluated the influence of different amount of NBS, as shown in Table 3.

The amount of NBS was varied from 1.0 to 2.0 eq. to study its effect on the bromohydrin of **3** in 20% aqueous THF. The yield of **4** was measured after 2 h of stirring the reaction mixture at −10 °C. It was observed that the yield increases as the amount of NBS was increased (up to 1.2 eq.) and then slowed down (Table 3, entries 1–4). Use of a 2.0 eq. amount of the NBS resulted in a lowest yield. It provided us the clue that the nuclear Δ^5 double bond of compound **4** may react with the NBS further. Therefore, we decided to use 1.2 eq. of NBS for further experiments.

The best result was obtained when substrate **3** was treated with 1.2 equiv. of NBS in 20% aqueous THF at −10 °C within 2 h in the bromohydrin reaction.

A probable mechanistic pathway to explain the regioselectivity of the bromohydrins **4** is depicted in Scheme 2. The mechanism of bromohydrins formation occurs in two steps. A three-membered cyclic bromonium ion intermediate **5** is formed at the initial stage of the reaction due to electrophilic addition of the Br^+ ion (generated from NBS) onto the **3**. Nucleophilic addition of water to intermediate **5** results in bromohydrins formation. The regioselectivity can be explained by Markovnikov's rule, which stated that in the addition of an unsymmetrical reagent to a multiple bond, the positive portion of the reagent is introduced at the less-substituted carbon.

In the next sequence of reaction, the bromohydrins products **4** was transformed into 25-hydroxycholesterol **1** by directly subjecting to the reduction with lithium aluminum hydride in THF, a slight excess of lithium aluminum hydride is generally used. Meanwhile, by action of powdered K_2CO_3 on the bromohydrins products **4** in methanol at room temperature, 24,25-monoepoxide **6** was obtained in good yield (Scheme 3).

4. Conclusion

In conclusion, we have developed an efficient and general method for the synthesis of 25-hydroxycholesterol *via* hydrobromination of desmosterol acetate by using NBS as a bromine source with excellent regioselectivity. The procedure is rapid, easy to perform at −10 °C to give bromohydrins product in good yield. Compared to the method of oxymercuration and hydrodemercuration, the usage of NBS makes it environmentally friendly.

5. Uncited reference

[27]. Q3

Acknowledgments

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