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Selective Crystallization of an Allylic 7α-Bromide: A Facile Synthesis of (1α,3β)-3-Hydroxycholeste-5,7-diene-1,25-diol Diacetate

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Abstract:

An efficient synthesis of (3β)-7-dehydro-1α,25-diacetoxycholesterol (1) is described. When acetonitrile was used as the crystallizing solvent, only the 7α-bromide 6a was isolated and bromide 6a was prepared in a 69% yield starting from 3β-TBDMS-1α,25-diacetoxycholesterol (5). Dehydrobromination with *sym*-collidine led to the regioselective formation of only the 5,7-diene 7. After deprotection with concentrated hydrochloric acid, the diene diacetate 1 was obtained in an overall yield of 44% without chromatographic separations. When tetrabutylammonium fluoride trihydrate was used as the base, the 7β-fluoride 10 was isolated.

Introduction

Control of impurity formation in intermediates and in the final product is a fundamental issue in process development. In exploratory chemistry, chromatographic separations are often employed as a facile means of purification, and if a compound matures to the process development stage, other purification scenarios are considered as a means to minimize the accumulation of process impurities. In chemical development, one approach to address this issue is to understand the experimental factors which are responsible for the formation of byproducts, and we have recently elaborated on a study in which knowledge of potential competing pathways was coupled with a statistical design of experiments to predict the process control limits.[1] A more empirical method would be to develop a process in which appropriate purification conditions can be identified for key intermediates; in doing so, the control of impurity formation resides in the selectivity and in the robustness of the crystallization or the purification process. Structural modification of intermediates has been one approach in which desirable crystallization characteristics can be imparted to intermediates, and in a recent report, the substitution of a methyl ester for an ethyl ester eliminated the need for a chromatographic purification as the methyl ester was a well-defined crystalline solid, whereas the ethyl ester was a relatively low-melting solid.[2]

During the course of chemical development studies for the synthesis of (1α,3β)-3-hydroxycholeste-5,7-diene-1,25-diol diacetate (**1**), a process was required in which there was control over the formation of the undesired 4,6-diene impurity[3] and in which only the diacetate (**1**) would be formed (Scheme 1). In the original work by Uskokovíc, these requirements were met by an acid-catalyzed conversion of the 4,6-diene triacetate to less polar molecules, which could be readily removed by chromatography, and by a selective saponification of the 5,7-diene triacetate. The overall yield in this process from Ro 21-3245 (**2**) to Ro 21-5536 (**1**) was 42%, and two chromatographic separations were used for the purification of the 5,7-diene triacetate and the final diacetate (**1**).[4] Prior experience had shown that, when the 3β and 1α positions of 1α,3β-dihydroxy-preg-5-ene derivatives were protected as a TBDMS ether, this functional group imparted desirable crystallization characteristics to these intermediates.[1] For the synthesis of diacetate **1**, if a similar trend was observed, then the preparation of 3β-TBMDS derivatives may allow for selective crystallization of the 7α-bromide **6a** as well as exclusive formation of the diacetate **1** during the removal of the TBDMS protecting group. We wish to report on the results from this investigation.

Results and Discussion

As expected, when 1α,25-dihydroxycholesterol (Ro 21-3245) (**2**)[1] was reacted with *tert*-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole at room temperature, the only product was the 3β-TBDMS ether **3**, and ether **3** was isolated in a quantitative yield. Acetylation of ether **3** with acetic anhydride in triethylamine (TEA) as the solvent and a catalytic amount of (dimethylamino)-pyridine (DMAP) afforded the diacetate **5**. The overall yield for this two-step conversion was 98%. Surprisingly, attempts to telescope these two steps to eliminate the extractive workup and isolation procedure for the TBDMS ether **3** were not successful. If the acetylation was conducted at room temperature, the monoacetate **4** could be isolated.[5]

Despite the fact that other approaches for the exclusive formation of homoannular dienes have been reported,[6] from a process development perspective, the precedent of the success of the dehydrobromination route on a commercial scale for the synthesis of 7-dehydrocholesterol warranted a

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(1) Van Arnum, S. D.; Moffet, H.; Carpenter, B. K. *Org. Process Res. Dev.* **2004**, *8*, 769−776 and references therein.

(2) Daniewski, A. R.; Liu, W.; Okabe, M. *Org. Process Res. Dev.* **2004**, *8*, 411−414.

(3) Van Arnum, S. D.; Carpenter, B. K.; Parrish, D. R.; MacIntrye, A. *J. Org. Chem.* **2004**, *69*, 8529−8532.

(4) Uskokovíc, M. R.; Barwid, T. A.; Iacobelli, J. A.; Baggiolini, E. (Hoffmann-La Roche, Inc.). U.S. Patent 3,993,675, 1976; *Chem. Abstr.* **1977**, *86*, 16846.

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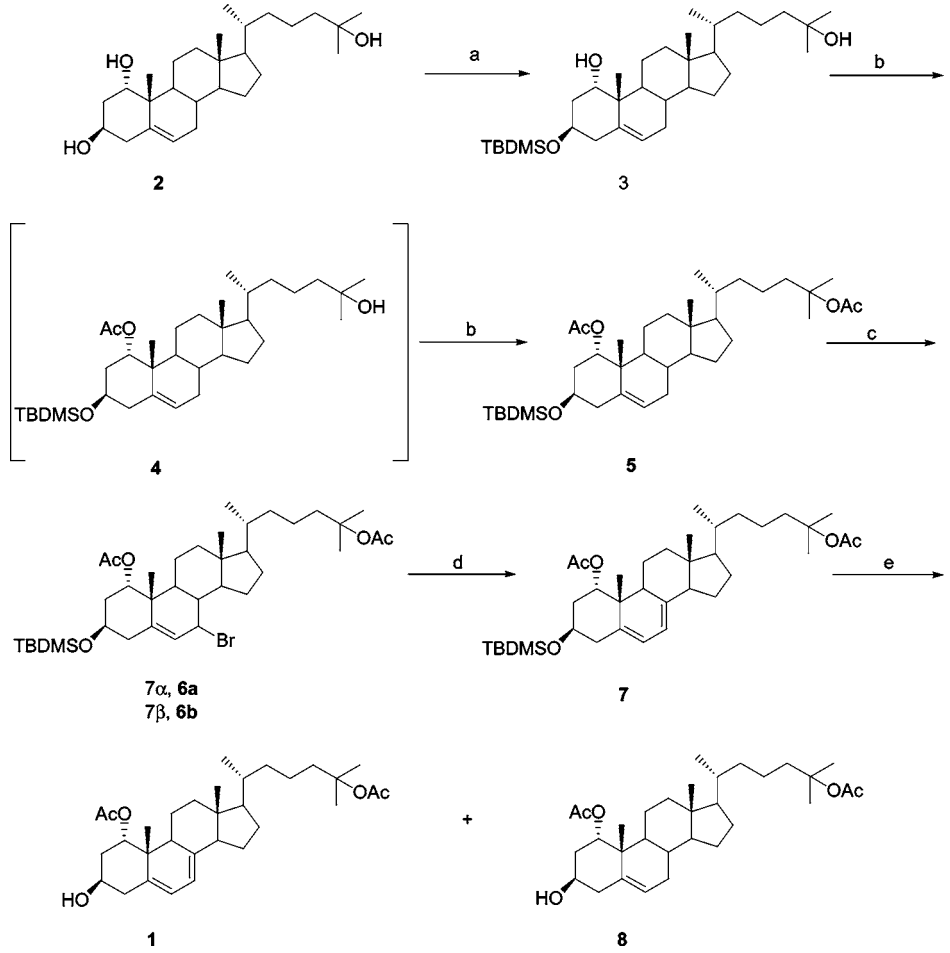
Scheme 1. **Synthesis of the diacetate 1**[a]



[a] Conditions: (a) TBDMSCl, imidazole, DMF, rt, 100%. (b) Ac_2O, DMAP, TEA, reflux, 98%. (c) 1,3-Dibromo-5,5-dimethylhydantoin, *sym*-collidine, TBABr, hexanes, 69%. (d) *sym*-Collidine, diglyme, 100 °C. (e) Aqueous hydrochloric acid, overall de 58%.

study of this chemistry for the synthesis of diacetate **1**.[7] Allylic bromination of a hexanes solution of diacetate **5** with 1,3-dibromo-5,5-dimethylhydantoin in the presence of *sym*-collidine and a catalytic amount of tetrabutylammonium bromide (TBABr) yielded the 7-brominated product as a mixture of 7α- and 7*β*-epimers, **6a** and **6b**, respectively. This mixture of epimers typically contained 90% of the 7α-bromide **6a** and 10% of the 7*β*-bromide **6b** as indicated by ^{1}H NMR analysis; the CH resonance of the 7α-bromide **6a** was observed at 4.66 ppm, whereas the CH resonance of the 7*β*-bromide **6b** was observed at 4.82 ppm in deuteriochloroform. This particular reaction proved to be somewhat erratic, and typically 1−5% of starting material **5** by HPLC analysis was present after the bromination.[8] After the bromination was complete, the reacting solvent was replaced with acetonitrile. Tetrabutylammonium bromide was present during the reaction so that, during the crystallization of the 7α-bromide **6a**, equilibration of the mixture of the 7α- and 7*β*-bromides **6a** and **6b** in the supernatant would occur during the crystallization and this could yield to an increased recovery of the 7α-bromide **6a** (Scheme 2). The presence of tetrabutylammonium bromide may have had a negative impact on the bromination; however, this effect was not studied in detail, and for our reported preparation, there was an incomplete consumption of starting material **5**. The addition of a relatively small amount of tetabutylammonium bromide to a reactor in which the filtrate from the bromination reaction would be charged was an operation which was to be avoided, and further experimental work would be necessary to better identify the particular requirement for tetrabutylammonium bromide and the potential of this reagent

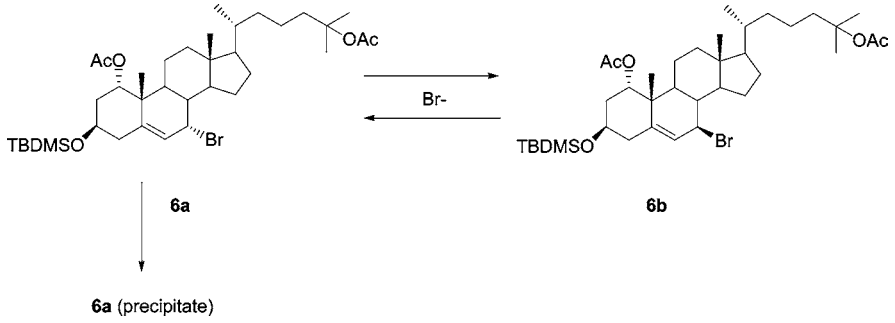
(5) The monoacetate, Ro 25-2495 (**4**) had the following properties: Anal. Calcd for $C_{35}H_{62}O_4Si$: C, 73.12; H: 10.87. Found: C, 73.05; H, 10.91; $[\alpha]^{22} = -5.81$ (0.88, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.043 (s, 6H), 0.66 (s, 3H), 0.87 (s, 9H), 0.91 (d, 3H), 1.06 (s, 3H), 1.21 (s, 6H), 2.03 (s, 3H), 2.28 (m, 2H), 3.82 (m, 1H), 5.00 (m, 1H), 5.25 (m, 1H); ^{13}C NMR ($CDCl_3$) δ −4.6, 11.9, 18.3, 18.7, 19.5, 20.4, 20.7, 21.1, 24.3, 25.9, 28.2, 29.3, 31.7, 31.8, 35.7, 36.2, 36.5, 39.7, 40.3, 42.0, 42.2, 42.4, 44.7, 56.0, 56.8, 67.4, 71.1, 75.6, 123.9, 137.9, 170.3. IR (KBr) (cm^{-1}) 3455 (b), 2950 (s), 1740 (s), 1371 (s), 1239 (s).

(6) (a) Confalone, P. N.; Kulesha, I. D.; Usokovíc, M. R. *J. Org. Chem.* **1981**, *46*, 1030−1032. (b) Takahashi, T.; Nakagawa, N.; Minoshima, T.; Yamada, H.; Tsuji, J. *Tetrahedron Lett.* **1990**, *31*, 4333−4336.

(7) For a review, see: Hirsch, A. L. Vitamin D. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed.; John Wiley & Sons: New York, 1998; Vol. 25, pp 217−256.

(8) The following HPLC conditions were used. A Zorbax ODS column with dimensions of 25.0 cm × 4.6 mm was used in conjunction with a mobile phase that consisted of a mixture of 65% acetonitrile and 35% methylene chloride. The flow rate was 0.8 mL/min. A Varex evaporative light-scattering detector (ELSD II) (operating at an exhaust temperature of 40 °C, a heater temperature of 49 °C, and a gas flow of 60 mm, 21 psig) was used as a detector. The following retention times were obtained: Ro 21-3245 (**2**), 3.5 min; Ro 21-5536 (**1**), 3.8 min, Ro 25-0648 (**3**) 6.1 min; Ro 25-0650 (**6a**) 6.6 min; Ro 25-1342 (**9**) 6.9 min; Ro 24-2495 (**4**) 7.0 min; Ro 25-0953 (**7**) 7.4 min; Ro 25-0649 (**5**) 7.9 min.

Scheme 2. **Crystallization process for the 7α-bromide 6a**



to alter the ratio of bromides **6a** and **6b** during the crystallization of the 7α-bromide **6a**.

Within the Process Research and Development department at Bristol-Meyers Squibb, Kiau and others have recently reported on the experimental development of an efficient crystallization procedure which utilized crystallization-induced dynamic resolution to isolate a chiral α-bromo carboxylic acid, and in this work as well, tetrabutylammonium bromide was the soluble halide source.[9] We have shown that the use of tetrabutylammonium iodide is an effective way to alter the ratios of halides.[3] The requirement for this soluble halide source is that it can alter the ratio of halides, and removal of the 7α-bromide **6a** by crystallization will perturb the equilibrium. The use of a soluble halide source has the potential to improve the recovery in the crystallization; however, our work does not show that this occurs during the isolation of the 7α-bromide **6a**.

When acetonitrile was used as the recrystallization solvent, epimerically pure 7α-bromide **6a** was isolated in a 69% yield from Ro 25-0649 (**5**). Because the 3*β*-TBDMS ether group causes compounds such as **6a** to be soluble in nonpolar solvents, solvents such as hexanes are not suitable for recrystallization. The allylic bromide **6a** is labile towards nucleophiles, and consequently, polar solvents such as methanol were not evaluated. Acetonitrile as a polar, nonnucleophilic solvent was the solvent of choice for the recrystallization. The instability of allylic bromide **6a** is evident in the fact that, although it could be dried and handled in air, storage at room temperature for several weeks under atmospheric conditions led to a deterioration of bromide **6a**, and 7-hydroxy compounds were tentatively assigned as degradants. This hydrolysis produces hydrobromic acid, which also caused cleavage of the 3*β*-TBDMS ether in the solid state. On scale-up of this route, a likely scenario would be that the dehydrobromination reaction would be conducted soon after bromide **6a** was isolated.

Because the project was discontinued before a mass balance study of the crystallization could be completed, the rationale for the lost yield of 31% could not be ascertained experimentally. The presence of *sym*-collidine could solubilize bromide **6a**, and other less lipophilic bases were not evaluated. A base was present as a precaution against any deprotection of the 3*β*-silyl ether The solubility of the **6a** bromide in acteonitrile at −10 °C was also not determined.

For the Roche patented process in which the three hydroxy groups were protected as acetates, the yield of the crude bromide from the triacetate is quantitative, and the overall yield from the crude bromide to the 5,7-diene triacetate is 64%.[4] In addition to the dehydrobromination step, this process includes an acid-catalyzed elimination to convert the 4,6-diene triacetate to less polar products.[3,4] Because of this transformation, a chromatographic purification of the 5,7-diene triacetate is possible.[4] For comparative purposes, the TBDMS process yields a 5,7-diene precursor **6a** at essentially the same yield without a chromatographic separation.

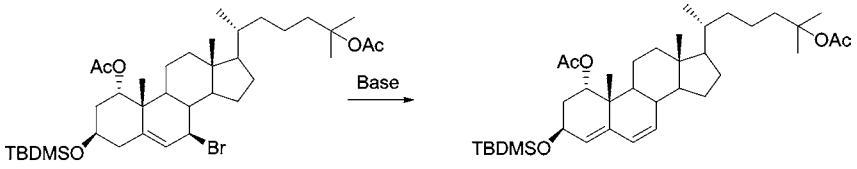
Elimination of hydrogen bromide from the 7α-bromide **6a** by *sym*-collidine in either xylenes, glyme, or diglyme yielded only the TBDMS 5,7-diene **7**.[10] No epimerization of the 7α-bromide **6a** occurred under these conditions as evidenced by the fact that there was no evidence by HPLC[8] for the formation of the related TBDMS 4,6-diene **9**. This observation substantiated our framework for a suitable process for the 5,7-diene diacetate **1** and validated our recently reported results on the formation of the 4,6-diene **9**. An authentic sample of the 4,6-diene diacetate **9** was prepared by conducting the elimination under conditions in which the 7α-bromide **6a** would equilibrate to the 7*β*-bromide **6b** (Scheme 3).[3] To avoid an isolation step for the TBDMS 5,7-diene **7**, digylme was used as the solvent for the dehydrobromination because it would be compatible with the reaction conditions for the removal of the TBDMS protecting group in the 5,7-diene **7**.

The deprotection of diene **7** was effected by concentrated hydrochloric acid at 0 °C. The addition of water precipitated the product, and hexanes were added as a nonmiscible solvent to separate the byproduct of the deprotection, *tert*-butyldimethylsilanol from diacetate **1**. From the 7α-bromide **6a**, the diene diacetate **1** was prepared in an overall yield of 58%. The product was contaminated with levels of the monoene diacetate **8** which resulted from an incomplete consumption of starting material in the bromination step.[11]

(9) Kiau, S.; Discordia, R. P.; Madding, G.; Okuniewicz, F. J.; Rosso, V.; Venit, J. *J. Org. Chem.* **2004**, *69*, 4256−4261.

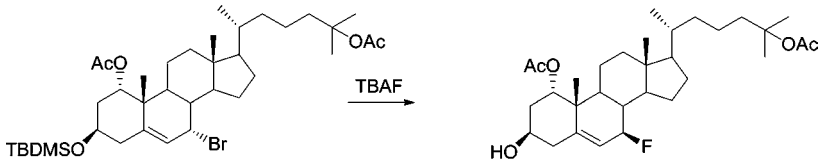
(10) The TBDMS 5,7-diene diacetate, Ro 25-0953 (**7**), had the following properties: mp 96−97 °C; Anal. Calcd for $C_{37}H_{62}O_5Si$: C, 72.26; H, 10.16. Found: C, 72.20, H: 10.26; $[\alpha]^{22} = -7.38$ (1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.61 (s, 3H), 0.88 (s, 9H), 0.93 (d, 3H), 0.94 (s, 3H), 1.42 (s, 6H), 1.97 (s, 3H), 2.06 (s, 3H), 2.42 (b, 3H), 3.93 (m, 1H), 4.97 (m, 1H), 5.39 (m, 1H), 5.67 (m, 1H); ^{13}C NMR ($CDCl_3$) δ −4.8, 11.9, 16.1, 18.2, 18.7, 20.3, 20.4, 21.1, 22.5, 23.0, 25.8, 26.0, 28.0, 36.1, 36.2, 38.1, 39.1, 40.4, 40.9, 41.1, 42.9, 54.6, 55.9, 66.2, 75.3, 82.5, 115.4, 120.8, 136.9, 141.0, 170.1, 170.4; IR (KBr) (cm^{-1}) 2953 (s), 1730 (s), 1237 (s), 1093 (s).

Scheme 3. **Formation of the 4,6-diene diacetate 9**



6b **9**

Scheme 4. **Formation of the 7*β* fluoride 10**



6a **10**

A patented process has claimed that, when freeze-dried tetrabutylammonium fluoride is used as a base, the homoannular diene is the only product.[12] When tetrabutylammonium fluoride trihydrate was used as a base, the elimination of bromide **6a** was also accompanied by deprotection of the silyl ether protecting group. Despite the presence of soluble bromide, there was no evidence for the formation of the 4,6-diene. However, a new impurity was observed and was identified as the 7*β*-fluoride **10**.[13] The 7*β*-fluoride **10** results from a S_N2 addition of fluoride ion to the 7α-bromide **6a** (Scheme 4). Because the 5,7-diene **7** readily forms a Diels adduct with dienophiles such as 4-phenyl-1,2,4-triazoline-3,5-dione,[3] separation of the 7*β*-fluoride impurity **10** was achieved by the formation of the Diels−Alder adduct of **1**.

For the Roche patented procedure, a selective saponification at the 3*β* position was followed by a chromatographic purification. The yield in this conversion was 65%, and the overall yield in the process from Ro 21-3245 (**2**) to Ro21-5536 (**1**) was 42%. The chromatographic separation was presumably necessary to separate the diacetate (**1**) from any other isomeric diacetates or monoacetates.[4] Because for the TBDMS route only the diacetate (**1**) is formed, a purification scheme to separate these kinds of impurities is not required.

(11) The monoene diacetate, Ro 25-1280 (**8**) had the following properties: mp 92−95 °C.; Anal. Calcd for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found C, 73.66; H, 10.33; $[\alpha]^{22} = -10.22$ (1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.66 (s, 3H), 0.87 (d, 3H), 1.07 (s, 3H), 1.41 (s, 6H), 1.97 (s, 3H), 2.03 (s, 3H), 2.38 (m, 2H), 3.86 (m, 1H), 5.05 (m, 1H), 5.51 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 11.9, 18.6, 19.5, 20.4, 20.5, 21.1, 22.5, 24.3, 26.1, 28.2, 31.7, 31.8, 35.7, 36.3, 39.7, 40.4, 41.2, 41.5, 42.2, 42.4, 56.2, 56.8, 66.6, 75.2, 82.6, 124.2, 137.2, 170.3, 170.6; IR (KBr) (cm^{-1}) 3440 (br), 2943 (s), 1737 (s), 1249 (s).

(12) Rappoldt, M. P.; Pauli, L. F.; Hoogendoorn, J. (Duphar International Research B.V.). U.S. Patent 4,464,298, 1984; *Chem. Abstr*. **1984**, *100*, 6955.

(13) The 7*β*-flouride, Ro 25-1729 (**10**) had the following properties: mp 71−74 °C.; Anal. Calcd for $C_{31}H_{49}FO_5$: C, 71.50; H, 9.49. Found: C, 71.78; H, 9.59; $[\alpha]^{rt} = +2.61$ (1.0, dioxane); ^{19}F NMR (referenced to $CFCl_3$) ($CDCl_3$) δ 64.4 (d, 7-CF, $J = 50.8$ Hz); ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3H), 0.91 (s, 3H), 1.19 (s, 3H), 1.41 (s, 6H), 1.96 (s, 3H), 2.03 (s, 3H), 2.42 (m, 2H), 3.88 (m, 1H), 4.67 (dd, 1H), 5.02 (m, 1H), 5.53 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 11.7, 18.6, 18.9, 20.4, 22.5, 25.6, 25.98 26.0, 27.3, 34.2, 55.5, 55.6, 66.1, 74.8, 83.8, 93.6 (C7, $J = 163$ Hz), 124.0, 124.4, 142.4, 142.8, 170.0, 170.5; IR (KBr) (cm^{-1}) 3481 (b), 2950 (s), 1737 (s), 1245 (s), 611 (w).

Conclusions

An efficient method for the synthesis of (1α,3*β*)-3-hydroxycholeste-5,7-diene-1,25-diol diacetate (**1**) has been described. Central to this route was the identification of appropriate crystallization conditions for the labile allylic 7α-bromide **6a**, and a more detailed study of the epimerization and crystallization conditions should afford enhanced yields of bromide **6a**. Dehydrobromination of bromide **6a** yielded only the desired 5,7-diene **7**. Without chromatographic separations, the overall yield of diacetate **1** in this four-step sequence was 44%. The selection of the TBDMS group for the protection of the 3*β*-alcohol eliminated two chromatographic separations and afforded the diacetate (**1**) in a comparable yield.

Experimental Section

The NMR spectra were obtained using either a Varian Gemini 200 MHz spectrophotometer or a Varian Unity 400 MHz spectrophotometer. All NMR spectra are referenced to tetramethylsilane unless otherwise noted. IR spectra were obtained on an Analect FX 6260 FTIR spectrophotometer.

Preparation of (1α,3*β*)-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]cholestan-5-ene-1,25-diol (Ro 25-0648) (3). 1α,-25-Dihydroxycholesterol (Ro 21-3245) (**2**)[1] (192.5 g, 0.46 mol), 83.4 g (1.21 mol) of imidazole, 91.3 g (0.605 mol) of *tert*-butyldimethylsilyl chloride, and 1463 mL of *N*,*N*-dimethylformamide were combined. The reaction was stirred at 24−26 °C for 2.5 h. After this time period, the reaction was complete as indicated by HPLC.[8] Over a 20-min period, 210 mL of methanol was added, and the batch was stirred for 1 h at 23−24 °C. Ethyl acetate (1.9 L) and water (1.9 L) were added, and the batch was stirred for 1.25 h at room temperature. The bottom aqueous layer was separated, and it was back-extracted with 2 × 450 mL of ethyl acetate. The combined organic layers were washed with a solution containing 82 g of sodium bicarbonate and 918 mL of water. The layers were separated, and the organic layer was washed with 1.0 L of saturated sodium chloride solution. After a solvent exchange with methanol to a final volume of 2.1 L, 1.5 L of water was added, and the batch was cooled to 0−5 °C. The batch was filtered, and the product was washed with a mixture of 50% water and 50% methanol. The solid was

dried at 75−80 °C under vacuum. There was obtained 245.5 g of Ro 25-0648 (**3**) as a white powder (100% yield). An analytical sample was prepared by recrystallization from methanol. Ro 25-0648 (**3**) had the following properties: mp 170−171 °C; Anal. Calcd for $C_{33}H_{60}O_3Si$ (532.93): C, 74.38; H, 11.35. Found: C, 73.38; H, 11.42; $[\alpha]^{rt} = -24.97°$ (1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.07 (s, 6H), 0.68 (s, 3H), 0.89 (s, 9H), 0.94 (d, 3H), 1.03 (s, 3H), 1.22 (s, 6H), 1.76 (d, 1H), 1.96 (m, 2H), 2.27 (m, 2H), 3.81 (m, 1H), 4.00 (m, 1H), 5.57 (d, 1H); ^{13}C NMR ($CDCl_3$) δ −4.6, 11.8, 18.2, 18.7, 19.5, 20.3, 20.8, 24.4, 25.9, 28.2, 29.2, 31.8, 31.9, 35.7, 36.5, 38.8, 39.5, 41.6, 41.7, 42.0, 42.3, 44.4, 56.1, 56.6, 67.1, 71.1, 73.0, 125.1, 138.0; IR (KBr) (cm^{-1}) 3440 (br) 2937 (s), 1255 (s), 1095 (s).

Preparation of (1α,3*β*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]cholestan-5-ene-1,25-diol Diacetate (Ro 25-0649) (5). Ro 25-0648 (**3**) (242.5 g, 0.455 mol) was combined with 27.8 g (0.244 mol) of 4-(dimethylamino)-pyridine, 342.7 mL (3.11 mol) of acetic anhydride, and 2.4 L of triethylamine. The batch was heated to reflux and held for 5.5 h. After the reaction was shown to be complete by HPLC analysis,[8] the batch was cooled, and 500 mL of methanol was added. The batch was stirred for 1.25 h at −2 °C. Water (6.0 L) was added slowly, and the slurry was cooled to −4 to −6 °C. After an overnight hold, the batch was filtered, and the cake was washed with 3 × 1.0 L of water (0−5 °C). The solid was dried at 70−80 °C under vacuum. There was obtained 274.0 g of Ro 25-0649 (**5**) as an off-white powder (97.6% yield). Ro 25-0649 (**5**) had the following properties: mp 124−125 °C; Anal. Calcd for $C_{37}H_{64}O_5Si$: C, 72.03; H, 10.46. Found: C, 71.83; H, 10.52; $[\alpha]^{rt} = -1.49°$ (1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.67 (s, 3H), 0.88 (s, 9H), 0.90 (d, 3H), 1.07 (s, 3H), 1.42 (s, 6H), 1.97 (s, 3H), 2.04 (s, 3H), 2.29 (m, 2H), 3.82 (m, 1H), 5.02 (m, 1H), 5.50 (d, 1H); ^{13}C NMR ($CDCl_3$) δ −4.7, 11.9, 18.2, 18.6, 19.5, 20.4, 21.1, 22.5, 24.3, 25.9, 40.3, 41.1, 42.0, 42.1, 42.3, 56.1, 56.8, 67.4, 75.5, 82.5, 123.9, 137.9, 170.2, 170.4; IR (KBr) (cm^{-1}) 2939 (s), 1750 (s), 1250 (s), 1093 (s).

Preparation of (1α,3*β*,7α)-7-Bromo-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]cholestan-5-ene-1,25-diol (Ro 25-0650) (6a). Ro 25-0649 (**5**) (49.4 g, 0.08 mol) was combined with 0.28 g (0.9 mmol) of tetrabutylammonium bromide and 250 mL of hexanes, which had been previously degassed with nitrogen. The batch was stirred for 5 min to dissolve the Ro 25-0649 (**5**). *sym*-Collidine (29.00 g, 0.24 mol), 17.20 g (0.06 mol) of 1,3-dibromo-5,5-dimethylhydantoin, and 500 mL of hexanes were added. The batch was heated to 55 °C, and the reaction was monitored by HPLC. After 3 h, ∼2.0% of Ro 25-0649 (**5**) was present by HPLC analysis.[8] The batch was cooled, the solids were filtered, and the solids were washed with 2 × 25 mL of hexanes. The combined filtrate was concentrated on a rotary evaporator at a temperature of less than 30 °C to yield 83.35 g of **6a** as a thick, orange oil. Acetonitrile (480 mL) was added, and the batch was stirred at room temperature. The suspension was cooled to −10 °C and was held there for 4 h. The suspension was filtered, and the cake was washed with 2 × 40 mL of −10 °C acetonitrile. The crystals were dried at 20−25 °C under vacuum. There was obtained 38.30 g of Ro 25-0650 (**6a**) as a white powder in a 68.6% yield. In a separate experiment, this preparation afforded an analytical sample. Ro 25-0650 (**6a**)[3] had the following properties: mp 132−137 °C (dec); Anal. Calcd for $C_{37}H_{63}BrO_5Si$: C, 63.86; H, 9.13; Br, 11.48. Found: C, 63.66; H, 9.20; Br, 11.75; $[\alpha]^{rt} = -143.22$ (1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.70 (s, 3H), 0.87 (s, 9H), 0.91 (d, 3H), 1.06 (s, 3H), 1.42 (s, 6H), 1.97 (s, 3H), 2.07 (s, 3H), 2.35 (m, 2H), 3.91 (m, 1H), 4.66 (d, 1H), 5.05 (m, 1H), 5.84 (d, 1H); ^{13}C NMR ($CDCl_3$) δ −4.7, 12.2, 18.1, 18.6, 18.9, 19.9, 20.3, 21.0, 22.5, 23.6, 25.8, 26.0, 27.9, 34.8, 35.6, 36.2, 36.9, 38.7, 41.1, 41.5, 41.6, 42.1, 53.4, 53.8, 58.4, 66.8, 74.7, 82.5, 126.2, 142.2, 170.3, 170.4; IR (KBr) (cm^{-1}) 2939 (s), 1750 (s), 1250 (s), 1100 (s).

Preparation of (1α,3*β*)-3-Hydroxycholeste-5,7-diene-1,25-diol Diacetate (Ro 21-5536) (1). Ro 25-0650 (**6a**) (49.49 g (0.071 mol)) was combined with 12.39 g of *sym*-collidine (0.107 mol) and 250 mL of diglyme. The batch was heated to 100 ± 5 °C for 4 h. The course of the reaction was monitored by HPLC.[8] The reaction was cooled to room temperature and held overnight. The suspension was cooled to 0 °C, and 47.9 mL (0.58 mol) of concentrated hydrochloric acid was added over a 30-min period. After 2 h, the reaction was complete as indicated by HPLC analysis.[8] Ice-cold water (200 mL) was added, and the slurry was cooled to 0 °C and held there for 1.5 h. Hexanes (50 mL) was added, and the batch was stirred for an additional 1.5 h. The batch was filtered, and the cake was washed with 3 × 50 mL of hexanes. The material was air-dried and slurried in 200 mL of hexanes. The slurry was filtered, and the solid was dried under vacuum and at room temperature. There was obtained 20.5 g of 1α,25-diacetoxy-7-dehydrocholesterol**,** Ro 21-5536 (**1**) as a white powder (57.7% yield). The HPLC retention time was in agreement with that of an authentic sample.[4] The diene diacetate **1** had ^1H NMR ($CDCl_3$) δ 0.62 (s, 3H), 0.94 (d, 3H), 1.00 (s, 3H), 1.42 (s, 6H), 1.97 (s, 3H), 2.06 (s, 3H), 2.39 (m, 2H), 2.59 (m, 1H) 3.95 (m, 1H), 5.00 (m, 1H), 5.40 (m, 1H), 5.69 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 11.9, 16.1, 18.7, 20.4, 20.5, 21.2, 22.5, 23.0, 26.1, 28.0, 35.7, 36.0, 36.1, 39.1, 39.9, 40.9, 41.1, 42.9, 54.6, 55.9, 58.1, 65.4, 75.0, 82.6, 115.4, 121.1, 136.3, 141.1, 170.2, 170.5; IR (KBr) (cm^{-1}) 3465 (br), 2950 (s), 1737 (s), 1369 (s), 1244 (s).

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Note Added after ASAP Publication: In the version published on the Internet March 23, 2005, the footnote for Scheme 4 should not appear. The final version, published April 13, 2005, and the print version are correct.

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