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The Preparation of (1α,3β)-3-Hydroxycholestane-4,6-diene-1,25-diol Diacetate from a 5,7-Diene Precursor: A New Method for the Synthesis of Heteroannular Dienes

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Received August 18, 2004

Abstract: Starting from the 7α-bromide **5a**, a regioselective synthesis of (1α,3β)-3-hydroxycholestane-4,6-diene-1,25-diol diacetate (**2**) is described. The preparative removal of contaminating 5,7-diene **9** was accomplished by the formation of the corresponding Diels−Alder adduct **11**. Acetylation of the diacetate **2** followed by acid-catalyzed elimination and rearrangement yielded styrene **13**.

Kinetic control of chemical reactions on a preparative scale can be problematic as the heat and mass control effects can be significantly different than those observed in the laboratory. Oftentimes, routes are selected so that the thermodynamic product will be the desired product; however, in these situations, impurities may be the result of kinetic control.[1] During the course of process development studies on the synthesis of 7-dehydrocholesterol-1α,25-diacetate (**1**),[2] a synthesis of the contaminating 4,6-diene isomer **2** was required for analytical method development and validation. Analytical separation of unsaturated sterols can be problematic,[3] and the well-known protection strategy of 5,7-dienes by the formation of a Diels−Alder adduct[4,5] was to be used to preparatively separate the homoannular diene from the heteroannular diene. A report has recently appeared which has used this technique as a way to isolate and purify steroids containing a 5,7-diene moiety from a fermentation.[6] We report here on the results from this investigation.

The heteroannular diene moiety is also found in many important pharmaceuticals such as compactin and mevastatin.[7] For the bromination, dehydrohalogenation route for the commercial synthesis of triacetate **10**, from cholesterol 1α,3β,25-triacetate (**4**), the heteroannular diene **8** is typically observed as a byproduct.[2] On a manufacturing scale, the success of this approach for the synthesis of 7-dehydrocholesterol 1α,3β,25-triacetate (**10**) resides on the fact that the rate of elimination of the 7α-bromide **6a** to form the 5,7-diene **10** is faster than the rate of equilibration of the 7α-bromide **6a** to the 7β-bromide **6b** and that the 7α-bromide **6a** is the major isomer from the allylic bromination of triacetate **4**. However, because of the potential for a preequilibrium between bromides **6a** and **6b**, it should be possible to influence these reaction rates in such a way that the 4,6-diene **8** is the predominate product. In particular, if the elimination was conducted under conditions in which the 7α and 7β-bromides **6a** and **6b** would equilibrate, then if the rate of elimination to the form the heteroannular diene **8** was faster than the rate to form the homoannular diene **10**, then the 4,6-diene **8** should predominate (Scheme 1).[8]

The substrate chosen for this study was the 7α bromide **5a** in which the 3β-alcohol was protected as a TBDMS ether and the 1α- and 25-hydroxyl groups were protected as acetates. Ultimately, this protecting group strategy would allow for a facile synthesis of (1α,3β)-3-hydroxycholestane-5,7-diene-1,25-diol diacetate (**1**).[2] Our hypothesis was originally shown to be valid when a mixture of crude bromination mixture of halides 7α- and 7β-bromides **5a** and **5b**, which contained an approximate 10:1 ratio of 7α- and 7β-bromides **5a** and **5b** by ^{1}H NMR analysis was treated with tetrabutylammonium fluoride in tetrahydrofuran at room temperature resulted in the formation of ∼11% of the 4,6-diene diacetate **2** as indicated by HPLC analysis. The approximate agreement of the ratio of the dienes **2** and **1** to that of the starting bromides **5a** and **5b** suggested that no equilibration occurred under these conditions and the ratio of dienes **1** and **2** was reflective of the ratio of the starting halides **5a** and **5b**.

Because the rate of elimination of an allylic iodide would be significantly faster than the rate of elimination of an allylic bromide, the introduction of a soluble iodide source such as tetrabutylammonium iodide should allow

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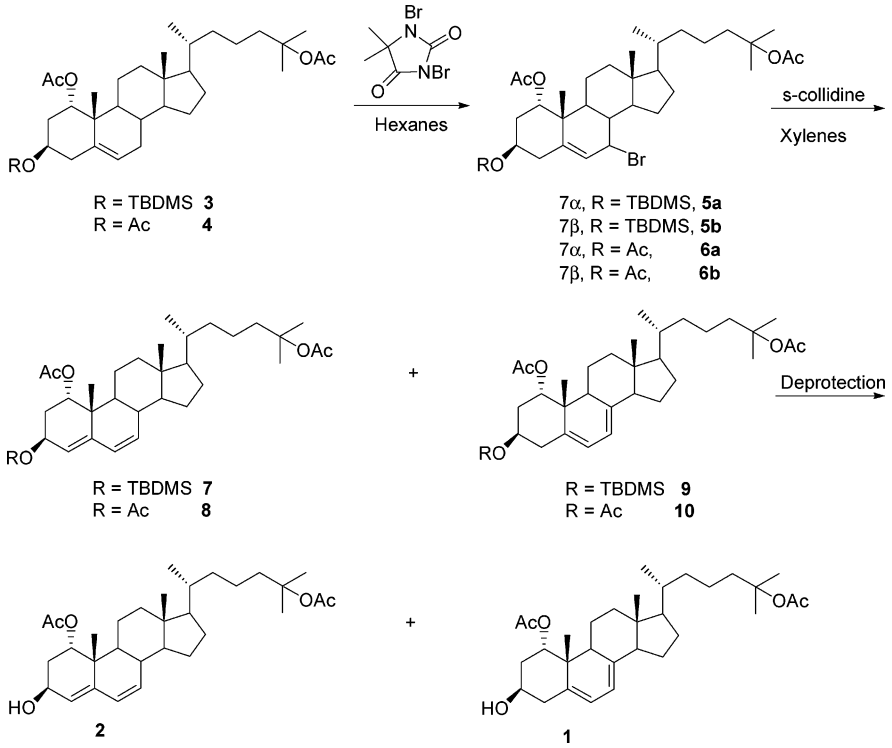
10.1021/jo048556i CCC: $27.50
Published on Web 11/02/2004

SCHEME 1. Synthesis of the Heteroannular Diene 2



R = TBDMS **3**
R = Ac **4**

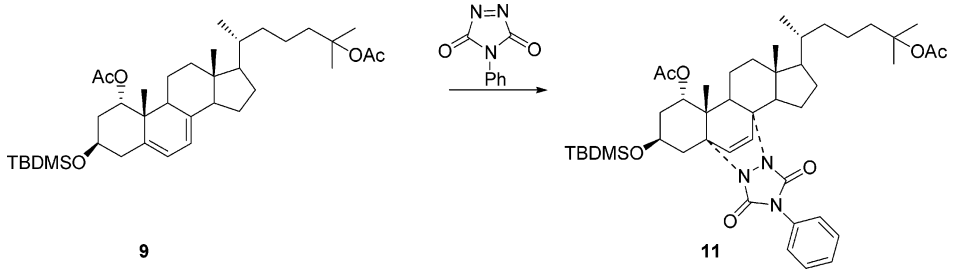
7α, R = TBDMS, **5a**
7β, R = TBDMS, **5b**
7α, R = Ac, **6a**
7β, R = Ac, **6b**

R = TBDMS **7**
R = Ac **8**

R = TBDMS **9**
R = Ac **10**

2 **1**

SCHEME 2. Diels–Alder Reaction of Diene 9



9 **11**

for the in situ preparation of a constant concentration of this reactive species. Because bromide ion would also be solubilized by the presence of the phase-transfer catalyst, equilibration of halides **5a** and **5b** would also occur during the course of the elimination. When the 7α-bromide **5a** was treated with tetrabutylammonium iodide in xylenes in the presence of *sym*-collidine at 60 °C, broadening of the HPLC peak for the 7α-bromide **5a** was seen, and this observation is due to either the presence of significant amounts of the 7*β*-bromide **5b** or the presence of the corresponding 7α- and 7*β*-iodides. After this epimerization at C-7 to yield an unknown mixture of 7α- and 7*β*-halides **5a** and **5b**, the mixture was heated to 100 °C. HPLC analysis revealed it to contain an approximate 6:1 mixture of the TBDMS-protected 4,6-diene diacetate **7** and TBDMS-protected 5,7-diene diacetate **9**. Under these conditions, the relative rates of equilibration of the halides versus elimination have been influenced in such a way that the equilibration of the halides is now competitive with the rate of elimination.

As anticipated, typical separation techniques such as chromatography or crystallization failed for this mixture of dienes. Because of this, the required separation was based on the fact that the 5,7-diene **9** is a reactive diene in a Diels–Alder sense whereas the 4,6-diene **7** would not react with dienophiles.[6] 4-Phenyl-1,2,4-triazoline-3,5-dione readily reacted with the 5,7-diene **9** to form the Diels–Alder adduct **11**, whereas the 4,6-diene **7** remained unchanged.[4,5] Because of the significant differences in the polarity of these compounds, they were readily separated by crystallization (Scheme 2).

The kinetics and aromatization of the acid-catalyzed elimination of anthranyl derivatives has recently been described,[9] and the in the Roche 1α,25-dihydroxycholesterol-based synthesis of calcitriol,[10] the separation of 1α,3*β*,25-triacetoxycholesta-5,7-diene (**10**) from the 4,6-diene triacetate **8** was achieved by the acid-catalyzed elimination of triacetate **8** to yield either triene **12** or the aromatized monoacetate **13**.[2] In the presence of *p*-toluenesulfonic acid in dioxane at 70 °C, the triacetate **8** was consumed very rapidly to yield a component which was assigned as the 2,4,6-triene diacetate **12**. Upon further reaction, the triene diacetate **12** disappeared and the aromatized monoacetate **13** appeared. The structure

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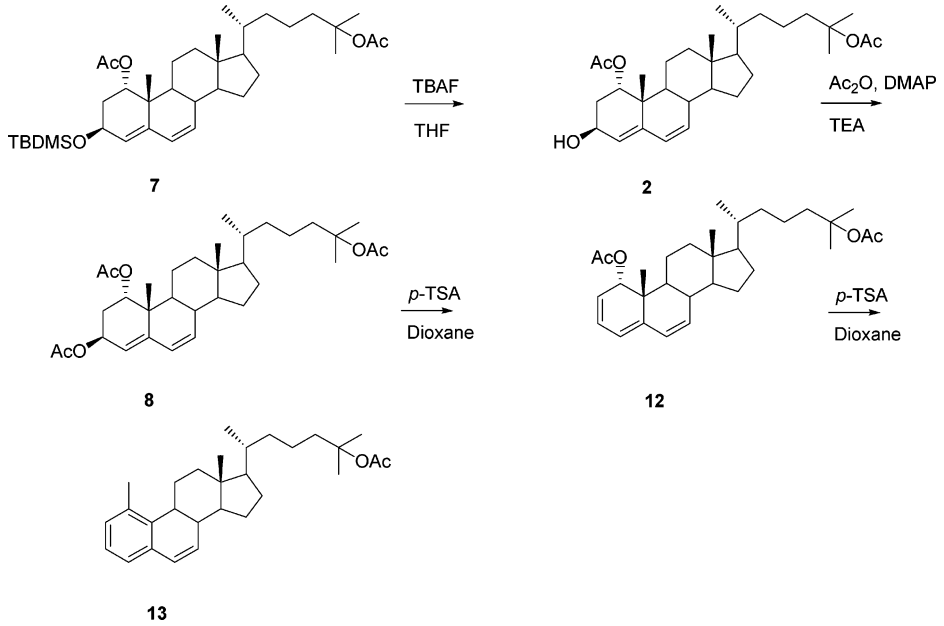
SCHEME 3. Synthesis and Acid-Catalyzed Dehydration of 4,6-Diene Triacetate 8



of styrene **13** was confirmed by ^{1}H NMR, ^{13}C NMR, and IR analysis (Scheme 3).[11]

The 19-norcholestatriene[12] and the 19-norcholestatetrane[12b,13] substructures are found in many natural products, and a possible synthesis of these compounds would be from appropriately substituted heteroannular dienes. As the separation of the mixture of isomeric dienes was accomplished by a Diels−Alder reaction, reaction of a mixture of dienes with a polymer-bound dienophile should allow for the separation of these isomers and the preparation of combinatorial libraries of heteroannular dienes.[14] Subsequent elaboration would yield either 19-norcholestatetrane or 19-norcholestatriene derivatives. Hydrolysis of the [4 + 2] adducts when, for example, maleic anhydride is used as a dienophile and subsequent removal by basic extraction may be an alternative way to separate libraries of homoannular and heteroannular dienes.

In summary, the synthesis of (1α,3*β*)-3-hydroxycholestane-4,6-diene-1,25-diol diacetate (**2**) from a 5,7-diene precursor **5a** was achieved by kinetic control of the equilibration and the elimination. In a laboratory flask, the kinetics for the desired and undesired reactions were sufficiently different that this apparatus was suitable for the synthesis of diacetate **7**. One approach to reduce or eliminate the formation of the undesired 5,7-diene **9** could involve the evaluation of this chemistry in a two stage microreactor[1] in which precise control of the temperature and the residence time should allow for yield improvements of the kinetic product, the 4,6-diene diacetate **7**.

Experimental Section

Preparation of (1α,3*β*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]cholestane-4,6-diene-1,25-diol Diacetate (Ro 25-1342) (7). The 7α-bromide, Ro 25-0650 (**5a**) (27.80 g, 40.0 mmol), 10.6 mL (80.2 mmol) of *sym*-collidine, 0.78 mL (2.1 mmol) of tetrabutylammonium iodide, and 640 mL of xylenes were combined and heated to 60 °C for 2 h. The reaction was heated to 100 °C and held overnight at this temperature. By area % HPLC, the reaction contained 68% of the 4,6-TBDMS-diene diacetate **7**, 10.5% of the 5,7-TBDMS-diene diacetate **9,** and 4.5% of the starting alkene (1α,3*β*)-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]cholestan-5-ene-1,25-diol (Ro 25-0649) (**3**) in a ratio of 15:2.3:1 of **7**:**9**:**3** respectively. After an aqueous workup with ethyl acetate as the extracting solvent, acetonitrile was used as a fractional crystallization solvent. A white solid (2.53 g) was obtained which contained by HPLC, 80% of the 4,6-diene **7**, and 20% of the 5,7-diene **9**.

Acetone (20 mL) was added, and the batch was cooled to 0 °C. 4-Phenyl-1,2,4-triazoline-3,5-dione was added until, by HPLC, there was none of the 5,7-diene **10** present. Acetone was removed under vacuum, and 20 mL of acetonitrile was added. The slurry was cooled to less than 0 °C. The batch was filtered, and the solid was air-dried. There was obtained 0.51 g of the 4,6-diene diacetate **7** as a white solid. By HPLC, the purity was greater than 99.5%. The 4,6-diene diacetate **7** had: mp 90−92 °C; $[\alpha]^{RT} = -26.67$ (1.0, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max} = 250$ nm ($\epsilon = 24\,404$); ^{1}H NMR ($CDCl_3$) δ 0.023 (s, 3H) 0.034 (s, 3H), 0.68 (s, 3H), 0.85 (s, 9H), 1.01 (s, 3H), 1.37 (s, 6H), 1.92 (s, 3H), 1.98 (s, 3H), 2.24−

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2.26 (m, 1H), 4.34 (m, 1H), 4.95 (m, 1H), 5.35 (m, 1H), H-4), 5.59 (m, 1H, J_{H6}-J_{H7} = 9.97 Hz), 5.92 (m, 1H, $J_{H6}-J_{H7}$ = 9.97 Hz); ^{13}C NMR ($CDCl_3$) δ −4.5, 12.0, 18.3, 18.5, 19.1, 20.4, 20.5, 21.0, 22.5, 23.8, 25.9, 26.1, 28.2, 32.7, 35.7, 36.2, 37.2, 38.2, 39.8, 41.1, 43.5, 44.6, 54.2, 56.1, 65.7, 75.0, 82.5, 126.5, 128.2, 131.1, 140.2, 170.4, 170.7; IR (KBr) (cm^{-1}) 2949 (m), 1738 (s), 1368 (m), 1243 (s). Anal. Calcd for $C_{37}H_{62}O_5Si$: C, 72.26; H, 10.16. Found: C, 72.27; H, 10.47.

Preparation of (1α,3*β*)-3-Hydroxycholestan-4,6-diene-1,25-diol Diacetate (Ro 25-1343) (2). To a solution of 0.66 g (1.07 mmol) of the TBDMS 4,6-diene diacetate 7 in 10 mL of tetrahydrofuran was added 1.01 g (3.9 mmol) of tetrabutylammonium fluoride trihydrate. After the mixture was stirred for 1 h at room temperature, the reaction was complete. After an extractive aqueous workup with ethyl acetate, the organic layer was treated with Florosil and the solvents were removed under vacuum. There was obtained 0.61 g of a yellow oil. The material was chromatographed on silica gel using a mixture of 50% toluene and 50% ethyl acetate as the eluent. There was obtained 0.46 g (85.9%) of the 4,6-diene diacetate **2** as a colorless oil. A sample of the oil was treated with hexanes and Ro 25-1343 (**2**) solidified as a white solid. Ro 25-1343 (**2**) had: mp (94) 96−98 °C; $[\alpha]^{RT}$ = −34.27 (0.29, $CHCl_3$); UV (CH_3CN) λ_{max} = 239.6 (ϵ = 23,043); ^{1}H NMR ($CDCl_3$) δ 0.72 (s, 3H), 0.90 (d, 3H,), 1.06 (s, 3H), 1.42 (s, 6H), 1.97 (s, 3H), 2.02 (s, 3H), 2.40 (m, 1H), 4.40 (m, 1H), 5.00 (d, 1H), 5.48 (d, 1H), 5.66 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 12.0, 18.5, 19.2, 20.4, 21.0, 22.5, 23.8, 26.1, 28.2, 32.5, 35.7, 36.2, 37.2, 38.3, 39.8, 41.1, 43.5, 44.5, 54.1, 56.1, 65.1, 74.7, 82.6, 125.3, 128.0, 131.8, 141.4, 170.5, 170.7; IR (KBr) (cm^{-1}) 3446 (b), 2944 (s), 1736 (s), 1668 (w), 1617 (w), 1368 (m), 1245 (s). Anal. Calcd for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.29; H, 9.61.

Preparation of 1-Methyl-25-(acetyloxy)-19-norcholesta-6-tetraene (Ro 25-2410) (13). To 6.69 g (6.2 mmol) of (1α,3*β*)-cholesta-5,7-diene-1,3,25-triol triacetate (**10**) which contained approximately 10% of the 4,6-diene triacetate **8** by HPLC in 52.9 mL of anhydrous dioxane was added 0.613 g of *p*-toluenesulfonic acid monohydrate.[2] The batch was heated to 70 °C for 30 min. When the 4,6-diene triacetate **8** was the starting material, the reaction after 4 h and 25 min at 70 °C contained by HPLC approximately 72% of tetraene **13**. Ethyl acetate was added and was followed by an extractive workup. A total of 6.88 g of a residue was successively chromatographed using mixtures of 90% toluene and ethyl acetate and 95% toluene and 5% ethyl acetate as the eluents. There was obtained 0.10 g of tetraene **13**. The spectral properties of the tetraene **13** were in agreement with those obtained from a pure sample of the 4,6-diene triacetate **8**. The tetraene **13** had: ^{1}H NMR ($CDCl_3$) δ 0.72 (s, 3H), 0.95 (d, 3H), 1.43 (s, 6H), 1.98 (s, 3H), 2.55 (s, 3H), 2.49 (m, 1H), 5.93 (d, 1H), 6.42 (d, 1H), 6.86−7.04 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 11.8, 18.5, 20.5, 22.5, 24.4, 25.0, 26.1, 26.9, 28.7, 29.8, 35.8, 36.2, 39.0, 40.4, 41.2, 46.0, 54.1, 56.4, 82.5, 125.2, 126.0, 128.8, 132.3, 132.7, 135.2, 136.0, 136.8, 170.5; IR (neat) (cm^{-1}) 2947 (s), 1721 (s), 1269 (s), 909 (s), 734 (s).

Acknowledgment. We thank the Analytical Development department of Pharmaceutical Process Development for their assistance and, in particular, James Johnson and Carolyn Cicio for the spectral assignments and we, also, thank the Physical Chemistry Department of Hoffmann-La Roche.

Supporting Information Available: HPLC conditions, characterization of bromide **5a**, and the preparation of Ro 25-1558 (**8**) and Ro 25-1040 (**11**). This material is available free of charge via the Internet at http://pubs.acs.org.

JO048556I