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Synthesis of Novel Hapten Derivatives of 1α,25-Dihydroxyvitamin D_3 and Its 20-Epi Analogue[1]

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Hapten derivatives of 1α,25-dihydroxyvitamin D_3 and its 20-epimer were synthesized and conjugated to a carrier protein for raising polyclonal antibodies. The haptens were linked through spacers at C-16, thereby exposing both the A-ring and the side chain of the molecules, to maximize antibody specificity. The spacers were introduced via stereoselective hydroboration of 16-ene intermediates as the key step. In immunoassays, the antibodies raised toward the natural hormone were selective to this compound over derivatives with modifications in the A-ring or the side chain. The antibodies toward the 20-epimer, however, were unable to recognize modifications in the side chain.

Introduction

Vitamin D has been recognized as a pluripotent hormone involved in a variety of biological processes, including calcium homeostasis, cell differentiation and proliferation, and immunology.[2] The metabolite 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$; Figure 1) has been identified as the hormonally most active form of vitamin D. The detailed mechanism of action of vitamin D is yet unclear, but it has been established that it involves interaction with a specific vitamin D receptor (VDR) which undergoes dimerization with other nuclear receptors, in particular the retinoid X receptor. The receptors then bind to response elements on DNA, causing stimulation/inhibition of protein synthesis.[3] For therapeutic use, analogues of 1,25$(OH)_2D_3$ have been synthesized with enhanced antiproliferative activity and with reduced calcemic activity. Vitamin D analogues have been developed as antipsoriatic drugs and show promise in the treatment of cancer and other proliferative disorders.[4] Most analogues synthesized to date have been compounds with modified C-20 side chains.[5]

One group of analogues that has attracted particular attention is characterized by an inverted stereochemistry at the C-20 position relative to that of the natural hormone (Figure 1). These so-called "20-epi" analogues are, in many cases, more potent in regulating cell growth and differentiation than the corresponding compounds with normal C-20 stereochemistry. In particular, 20-epi analogues exhibit immunosuppressive properties.[6]

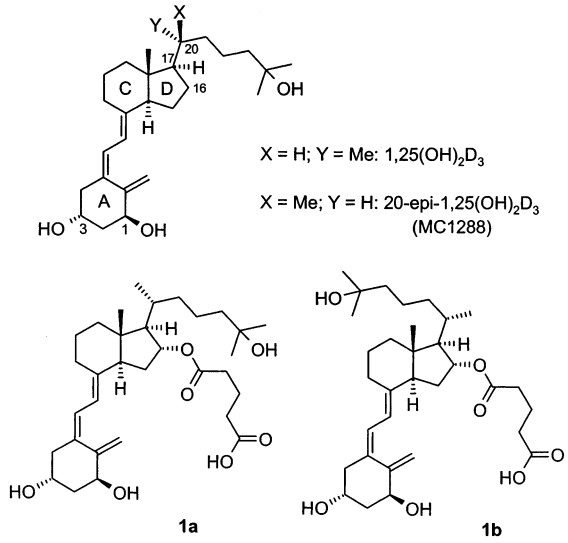

FIGURE 1. The natural hormone 1,25$(OH)_2D_3$, its 20-epimer (MC1288), and corresponding haptens **1a** and **1b** used for raising antibodies.

The purpose of the present study was to produce and compare specific antibodies to 1,25$(OH)_2D_3$ and 20-epi-1,25$(OH)_2D_3$. We were interested to see if the antibodies could distinguish between the normal and C-20 epimeric side chains. If this is the case, it would be possible to raise specific antibodies to new 20-epi analogues selected

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(1) This work was taken in part from the doctoral thesis of Lars Blæhr (Royal Danish School of Pharmacy, Copenhagen) and was partially presented at the 10th Workshop on Vitamin D: *Vitamin D. Chemistry, Biology and Clinical Applications of the Steroid Hormone*; Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Printing and Reprographics, University of California: Riverside, CA, 1997; p 91.

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as drug candidates. Such antibodies would be useful in clinical settings for analyzing serum concentrations of the drugs in patients. In this study, we designed and synthesized hapten derivatives of 1,25$(OH)_2D_3$ and 20-epi-1,25$(OH)_2D_3$ (**1a** and **1b**; Figure 1) for raising antibodies. The haptens have a spacer for carrier conjugation incorporated at the C-16α position to expose the A-ring and the side chain, two structural elements that together distinguish 1,25$(OH)_2D_3$ from a host of other natural vitamin D metabolites. It has previously been shown that exposing these elements in the hapten leads to high antibody specificity.[7] Thus, cross-reaction with related metabolites, especially 25-hydroxyvitamin D_3 (lacking the 1α-OH) present in high concentrations in serum, is minimized.

We report herein the syntheses of the two haptens and the preliminary examination of the properties of polyclonal antibodies raised toward bovine serum albumin (BSA) conjugates of the haptens.

Results and Discussion

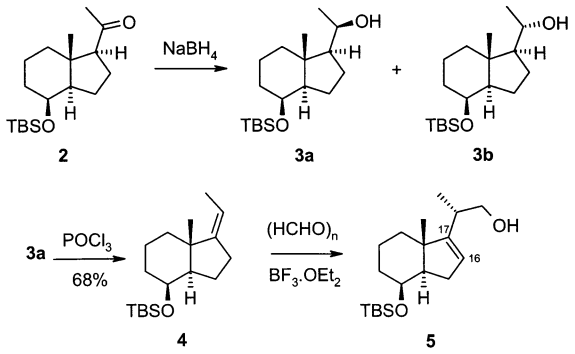
There have been several reports in the literature on the synthesis of haptens for raising polyclonal antibodies to 1,25$(OH)_2D_3$. The first haptens reported were derivatives of 1,25$(OH)_2D_3$ conjugated to a carrier protein through spacers attached to one of the inherent hydroxyl groups in the molecule.[8] However, polyclonal antibodies resulting from immunization with these conjugates were not specific to 1,25$(OH)_2D_3$, probably because it is essential to expose all three hydroxyl groups of 1,25$(OH)_2D_3$ in the hapten to obtain a high specificity. Later on it was shown that the introduction of a fourth hydroxyl group for conjugation improved the antibody specificity considerably.[7a] Thus, we decided to follow this strategy for our study and introduce a hydroxyl group at the C-16 position.

Synthesis of 16-Glutaryloxy-1,25$(OH)_2D_3$. We decided to introduce a 16,17 double bond as a starting point for functionalizing the C-16 position. Subsequent hydroboration/oxidation of the double bond would be expected to regiospecifically introduce a hydroxyl group at this position, providing a chemical handle for attaching the spacer.

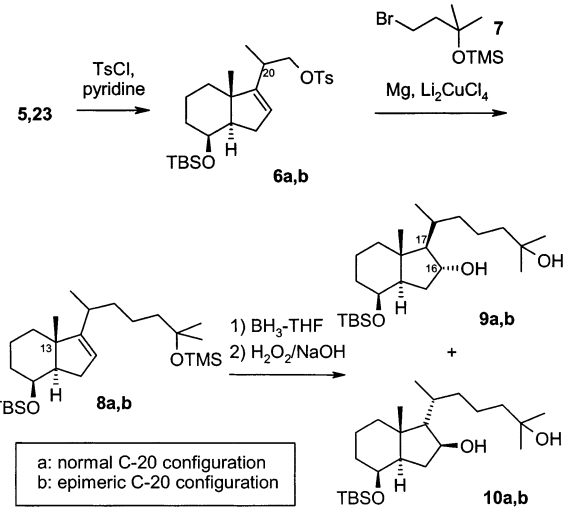
To obviate the problem of chemoselective hydroboration of a 16-ene in the presence of the triene system of vitamin D, we chose to work with CD-ring intermediates, deferring assembly of the triene unit until the final steps of the synthesis in a convergent approach.[5]

Introduction of the 16,17 double bond[9] involved ene reaction of formaldehyde with the ethylidene compound **4**, which was obtained from the known ketone **2**[10] (Scheme 1). Reduction of the ketone with $NaBH_4$ gave two epimeric alcohols[11] in the ratio (*R:S*) 85:15 which were easily separated by chromatography. The *R*-isomer (**3a**) was converted to the (*Z*)-ethylidene compound **4** by E2 elimination of water with $POCl_3$.[12] Ene reaction with paraformaldehyde under BF_3•OEt_2 catalysis resulted in highly stereoselective conversion to the homoallylic alcohol **5**, the angular methyl group directing attack of aldehyde solely from the α-face of the molecule.[13]

SCHEME 1



SCHEME 2



The 25-hydroxylated vitamin D_3 side chain was assembled by a modification of a previously described procedure used to make 1,25$(OH)_2D_3$ (Scheme 2).[14] Thus, tosylation of the primary alcohol **5** to **6a** followed by coupling of the Grignard reagent of **7**[15] in the presence of Li_2CuCl_4[16] gave **8a**.

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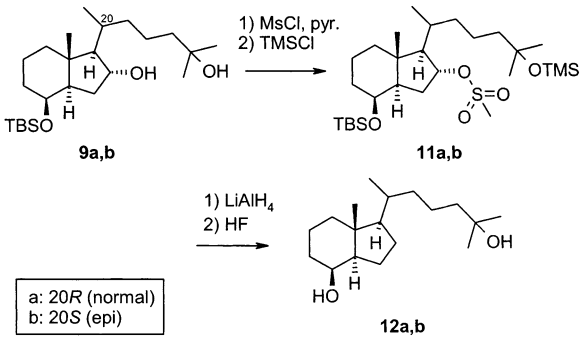
SCHEME 3



It is known that hydrogenation of 16-ene steroids takes place exclusively from the α-face due to steric hindrance afforded by the angular C-13 methyl group.[13] We anticipated that hydroboration of the double bond in **8a** would exhibit the same facial selectivity. Treating **8a** with diborane followed by oxidation with H_2O_2 resulted in two isomeric alcohols (**9a** and **10a**) in the ratio 5:1. The predominant isomer showed a signal in ^{1}H NMR at 4.0 ppm (H-16), and this chemical shift corresponded with spectra of 16α-hydroxy steroids found in the literature.[17] The minor isomer displayed a signal at 4.3 ppm which was in accordance with the literature data of 16*β*-hydroxy steroids. Consequently, the major and minor isomers were assigned the structures of **9a** and **10a**, respectively. Since hydroboration is a *syn*-addition, the isomers should also be 17-epimers. However, neither comparison of chemical shifts nor 2D NOE experiments supplied conclusive evidence for assigning the absolute configuration at C-17.

Therefore, we decided to confirm our assignments by converting **9a** to its corresponding 16-deoxy compound, which is known.[18] Thus, the alcohol was converted to the mesylate, subsequently reduced with LAH, and deprotected (Scheme 3) to give **12a**, which was identical (^{1}H, mp) with literature data. Evidently, hydroboration of a 16,17 double bond does preferentially take place from the least hindered α-face. Bulkier borane reagents such as 9-BBN, which might have given improved selectivity, were not effective at hydroborating the double bond.

The hydroboration/oxidation step resulted in adventitious cleavage of the TMS group in the side chain. (In some runs, the TMS intermediates were the isolated products, from which the diols **9a** and **10a** could be recovered after brief treatment with a catalytic amount of PPTS in ethanol/THF.[19]) Fortunately, the unprotected tertiary alcohol did not interfere in the following steps. The secondary alcohol **9a** was protected as an acetate to give compound **13a** (Scheme 4), and the TBS group was removed with hydrofluoric acid, affording **14a**. Oxidation of the secondary alcohol with PDC and reprotection of the tertiary alcohol afforded **16a**, which was coupled with A-ring synthon **17** under standard conditions.[20] The acetate group was replaced with a hemiglutaroyl group, and deprotection with TBAF generated the final hapten **1a**.

Synthesis of 20-Epi-16-Glutaryloxy-1,25(OH)$_2$D$_3$. The synthesis of the corresponding 1,25(OH)$_2$D$_3$ hapten with 20-epi (*S*) configuration (**1b**) followed a similar synthetic route. However, because the altered configuration at C-20 had some unexpected influence on the outcome of some reactions, a few modifications proved to be necessary.

To establish the *S*-configuration at C-20, the *E*-isomer of alkene **4** was required for the ene reaction with formaldehyde (cf. Scheme 1). The *E*-isomer could be formed by stereospecific dehydration of the alcohol **3b** obtained from reduction of ketone **2**. However, since the yield of this alcohol was poor, we looked at ways to isomerize alkene **4** from the previous sequence, which was available in larger amounts, to the corresponding *E*-isomer.

Although a procedure has been described for the interconversion of 17-ethylidene isomers (standard sequence of epoxidation, ring-opening with lithium diphenylphosphide, and methylation),[21] we decided on another approach, involving hydroboration of the double bond followed by E2 elimination of water (Scheme 5). The hydroboration of (*Z*)-17-ethylidene steroids has been reported to occur predominantly by attack from the α-face of the molecule, affording the (20*S*)-alcohol upon oxidation.[22] Thus, hydroboration/oxidation of the (*Z*)-ethylidene compound **4** afforded the (*S*)-alcohol **3b** in good yield, and subsequent dehydration gave rise to the (*E*)-ethylidene compound **21**, which now could be prepared in adequate amounts.

We then proceeded with ene reaction of **21** with formaldehyde to produce the homoallylic alcohol **23**. Attack from the less hindered α-face would establish the desired 20-epi configuration (cf. ref 12). Surprisingly, when the (*E*)-alkene **21** was subjected to the same reaction conditions used with the (*Z*)-alkene **4** in the previous synthesis (Scheme 1), a compound was isolated as the major product with the correct molecular mass (determined by MS), but without the expected vinylic signals in its ^{1}H NMR and ^{13}C NMR spectra. The compound was identified as the oxetane **22**, apparently as a single diastereomer, which presumably is the product of α-attack as shown (Scheme 6). TLC showed that **23** was initially formed to some extent in the reaction as a transient intermediate.

The generation of oxetanes in ene reactions is known. It has been postulated that the mechanism of the ene reaction in certain cases involves the formation of a 2 + 2 transition state complex (an oxetane) before the rate-determining step to the ene product.[23] Alternatively, the oxetane could be formed via an intermediate characteristic of the Prins reaction.[24] Thus, instead of the concerted

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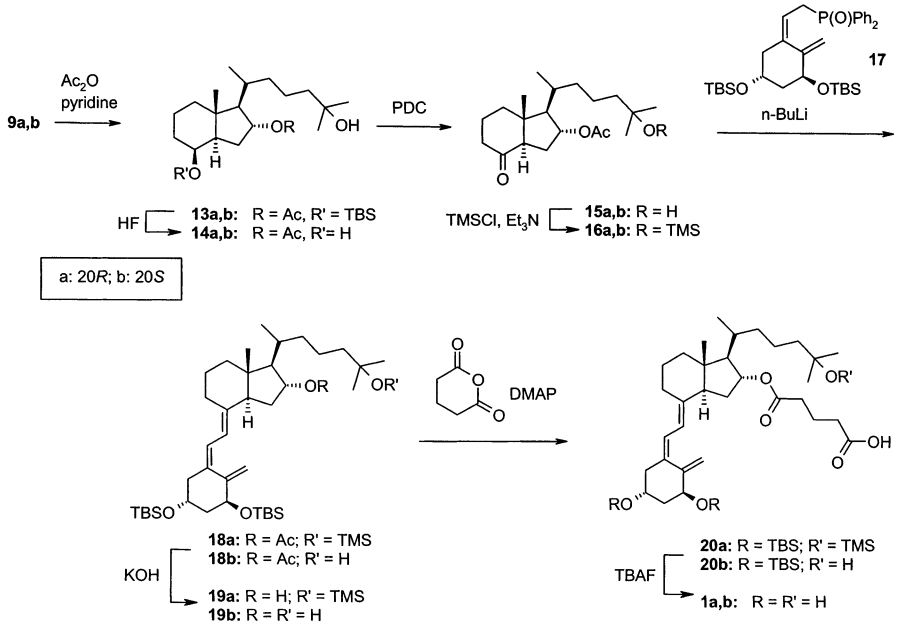
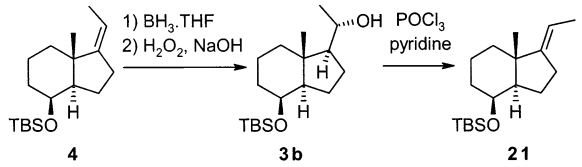
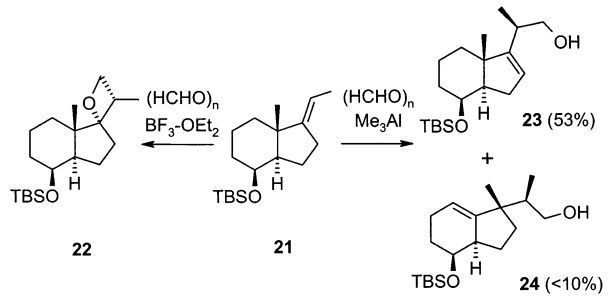
SCHEME 4



SCHEME 5



SCHEME 6



addition process typical of the ene reaction, the alkene attacks the formaldehyde, thereby generating a tertiary carbenium ion that collapses to the oxetane **22**.

On the basis of this mechanism, the formation of the oxetane could presumably be minimized by using another Lewis acid to alter the reactivity of the oxy anion. A number of other Lewis acids were evaluated for the reaction above, and it was found that substituting $BF_3 \cdot OEt_2$ with trimethylaluminum led to a good yield of the desired homoallylic alcohol **23** (with the recovery of 17% starting material). While only trace amounts of oxetane were formed, a new byproduct (**24**) was produced, resulting from a Wagner−Meerwein rearrangement.[25]

The alcohol **23** was then converted to the hapten **1b** using a sequence similar to that described for the preparation of hapten **1a** (Schemes 2 and 4). As before, hydroboration of the 16-ene intermediate **8b** with $BH_3 \cdot THF$ and subsequent oxidation with H_2O_2 afforded two isomeric alcohols (**9b** and **10b**) in the ratio 8.5:1. Again, the hydrogen at the C-17 position of the major isomer **9b** was established to be α-oriented (natural configuration) by converting the isomer to the known 16-deoxy compound **12b** (Scheme 3) and correlating with literature data.[26] Due to the *syn*-addition mechanism of hydroboration, it then follows that the 16-OH of **9b** is also α-oriented. This configuration was also confirmed by an NOE between 16-H and the angular C-13 methyl group. In the case of the isomer **10b**, no NOE was observed between 16-H and the C-13 methyl, and this points to the configuration as drawn. The spectrum of **10b** also revealed an NOE between 16-H and 17-H, which is consistent with the *trans*-configuration of the protons in a *gauche*-orientation.

Immunoassays

The haptens **1a** and **1b** were converted to their corresponding *N*-hydroxysuccinimide esters and coupled to BSA.[27] Rabbits were immunized biweekly with the resulting conjugates for 3 months. The polyclonal antisera thus produced were examined for cross-reactivity with a series of vitamin D metabolites in a standard radioimmunoassay using radiolabeled $1,25(OH)_2D_3$ as ligand. The results are presented in Table 1.

The antibodies to **1a** (normal side chain configuration) are clearly selective toward $1,25(OH)_2D_3$ over its metabolites and over 20-epi-$1,25(OH)_2D_3$. Removal of either the 25-hydroxy group (1-hydroxyvitamin D_3) or the 1-hydroxy group (25-hydroxyvitamin D_3) resulted in a 100-fold decrease in binding. Removal of both hydroxyl groups (vitamin D_3) resulted in a binding of less than 0.01%

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TABLE 1. Specificity of Antibodies Derived from Immunization in Rabbits with BSA Conjugates of 1a and 1b

metabolite	percent cross-reaction[a] antibody to **1a**	antibody to **1b**
1,25(OH)$_2$D$_3$	100	100
20-epi-1,25(OH)$_2$D$_3$	8	50
25-hydroxyvitamin D$_3$	1.3	<0.01
1-hydroxyvitamin D$_3$	1.3	1.5
vitamin D$_3$	<0.01	<0.01

[a] The percent cross-reaction of a metabolite is defined as (dose of 1,25(OH)$_2$D$_3$ required to displace 50% of the bound radioligand)/(dose of metabolite required to displace 50% of the bound radioligand) × 100.

compared to that of 1,25(OH)$_2$D$_3$. These results indicate that both the side chain and the A-ring are important for binding, and an alteration in either moiety leads to a reduction in binding affinity.

In contrast, the antibodies toward **1b** (epimeric side chain configuration) did not discriminate between the natural hormone and its 20-epimer, but we detected a large difference in affinity for 1-hydroxyvitamin D$_3$ and 25-hydroxyvitamin D$_3$. This result indicates that the antibody has a stronger binding preference for the A-ring moiety than for the side chain. Alterations in the side chain do not seem to influence the binding affinity to a large degree, while the antibody is much more sensitive to alterations in the A-ring.

In conclusion, we have successfully prepared the pure 16α-glutaryloxy derivatives of 1α,25(OH)$_2$D$_3$ and 20-epi-1α,25(OH)$_2$D$_3$. These derivatives were used as haptens for the generation of antibodies toward the two vitamin D compounds. The haptens were designed to present the most characteristic parts of the vitamin D molecule, the A-ring and the side chain, to maximize the specificity of the antibodies. This was achieved to a satisfactory level with the derivative of the natural hormone, but less so for the corresponding epi compound. An explanation could be that the side chain in the epi antigen interacts with the carrier protein and thereby becomes less accessible to the antibody-producing B-cells. Further work concerning antibodies against epi compounds should concentrate on other linker positions to obtain more specific antibodies. Thus, this study has given us further information on how to make useful haptens of specific vitamin D derivatives.

Experimental Section

General Procedures. THF was dried by distillation under argon from sodium benzophenone ketyl immediately prior to use. DMSO was dried by distillation under argon from CaH_2 and then stored over 4 Å molecular sieves. All other solvents (CH_2Cl_2, Et_2O, DMF, pyridine) were dried by storage over 4 Å molecular sieves. Other commercial reagents were used as received. Water-sensitive reactions were performed in oven-dried (130 °C) or flame-dried glassware under argon. Thin-layer chromatography was carried out on precoated silica gel 60 F_{254} glass plates. Plates were visualized using UV radiation (254 nm) or with 4 M H_2SO_4 containing 1.5% cerium sulfate and 1.0% molybdic acid followed by heating to 150 °C. Standard workup means extracting 2−3 times with an organic solvent (ethyl acetate unless otherwise stated), washing with water and brine, drying over $MgSO_4$ and removing the solvent under reduced pressure. Flash chromatography was performed as described previously[28] using silica gel 60, 40−63 μm. NMR spectra were obtained in $CDCl_3$ unless otherwise stated. NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are reported in parts per million downfield from that of internal trimethylsilane. For compounds containing silicon, $CHCl_3$ was used as reference (7.25 ppm in ^{1}H NMR, 76.8 ppm in ^{13}C NMR). All spectra were recorded at the Department of Spectroscopy at LEO Pharma. Melting points are uncorrected.

(5*Z*,7*E*)-(1*R*,3*S*)-9,10-Secocholesta-5,7,10(19)-triene-1,3,25-triol-16α-yl Hydrogen Glutarate (1a). To a solution of silyl ether **20a** (32 mg, 0.04 mmol) in THF (4 mL) was added TBAF (119 mg, 0.4 mmol), and the resulting solution was heated to 60 °C for 5 h and then cooled to room temperature. After standard workup, the residue was purified by flash chromatography (CH_2Cl_2/methanol), affording 7 mg (33%) of hemiglutarate **1a** as a colorless oil. ^{1}H NMR (500 MHz, CD_3OD): δ 0.63 (3H, s), 0.99 (3H, d, $J \approx 6.3$ Hz), 1.16 (6H, s), 1.0−2.1 (series of m), 2.24 (2H, t), 2.33 (2H, t), 2.54 (2H, m), 2.88 (1H, br d), 4.12 (1H, m), 4.35 (1H, m), 4.89 (2H, m), 5.29 (1H, m), 6.01 (1H, d, $J \approx 11.2$ Hz), 6.31 (1H, d, $J \approx 11.1$ Hz). MS: *m*/*z* 396 (M − glutaryl − H_2O), 378 (M − glutaryl − $2H_2O$), 360 (M − glutaryl − $3H_2O$), 249. HRMS (EI): *m*/*z* calcd for $C_{27}H_{36}$ ([M − HOOC$(CH_2)_3$COOH − $3H_2O]^+$) 360.2817, found 360.2820.

(5*Z*,7*E*)-(1*R*,3*S*,20*S*)-9,10-Secocholesta-5,7,10(19)-triene-1,3,25-triol-16α-yl Hydrogen Glutarate (1b). Following the procedure described for the preparation of **1a**, 26 mg of **20b** was converted to 11 mg (59%) of **1b** as a colorless oil. ^{1}H NMR (CD_3OD): δ 0.62 (3H, s), 0.88 (3H, d, $J \approx 6.2$ Hz), 1.17 (6H, s), 1.2−2.2 (series of m), 2.31 (1H, m), 2.33 (4H, m), 2.51 (1H, dd), 2.90 (1H, m), 4.11 (1H, m), 4.34 (1H, m), 4.88 (1H, m), 4.96 (1H, m), 5.29 (1H, m), 6.02 (1H, d, $J \approx 11.1$ Hz), 6.31 (1H, d, $J \approx 11.1$ Hz). ^{13}C NMR (CD_3OD): δ 14.1, 18.8, 21.5, 22.0, 24.0, 29.3, 29.7, 33.7, 34.1, 34.4, 34.7, 37.9, 41.5, 43.7, 45.1, 46.2, 47.5, 48.2, 54.8, 62.7, 67.4, 71.5, 71.6, 79.7, 112.4, 119.5, 124.6, 136.5, 140.9, 149.8,174.7, 176.9. HRMS (EI): *m*/*z* calcd for $C_{32}H_{46}O_5$ [M − $2H_2O]^+$ 510.3345, found 510.3358.

(20*S*)-Des-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]pregnan-20-ol (3b).[11a] To a solution of (*Z*)-ethylidene **4** (2.40 g, 8.40 mmol) in dry THF (80 mL) was added BH_3·THF (1.0 M in THF, 15 mL). The resulting mixture was stirred at room temperature for 2.5 h, and then water (60 mL) was slowly added. After the mixture was cooled in an ice bath, H_2O_2 (30% aqueous, 40 mL) was added from a dropping funnel over a 10−15 min period. After complete addition, the suspension was stirred at 0 °C for 2 h and extracted twice with EtOAc. The combined organic layers were washed with 10% $NaHSO_4$, water, and brine, dried, and evaporated. Flash chromatography (15% EtOAc/pentanes) afforded 2.0 g (77%) of alcohol **3b**. ^{1}H NMR: δ −0.01 (3H, s), 0.00 (3H, s), 0.87 (9H, s), 0.90 (3H, s), 1.19 (3H, d, $J \approx 6.3$ Hz), 1.1−2.0 (series of m), 3.67 (1H, m), 4.01 (1H, m). ^{13}C NMR: δ −5.4, −5.0, 14.2, 17.2, 17.8, 22.8, 23.2, 24.8, 25.6, 34.1, 39.6, 41.2, 52.7, 58.8, 69.0, 70.0. HRMS (EI): *m*/*z* calcd for $C_{18}H_{34}OSi$ [M − $H_2O]^+$ 294.2379, found 294.2389.

(*Z*)-Des-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]pregn-17(20)-ene (4). A solution of alcohol **3a**[11a] (2.0 g, 6.4 mmol) in dry pyridine (50 mL) was cooled to 0−5 °C, and $POCl_3$ (6 mL) was added slowly over 20 min. The mixture was stirred overnight in the slowly melting ice bath and then poured into 175 mL of ice-cooled ethyl acetate. Cold water (75 mL) was added and the mixture acidified with HCl (aqueous, 4 M) to pH 3.0 (approximately 130 mL). The water phase was separated and extracted with ethyl acetate. The combined organic layers were washed with 1 M HCl (40 mL), water, $NaHCO_3$, water, and brine, then dried, and concentrated to 1.76 g. The oil was chromatographed (2% ethyl acetate/pentanes) to yield 1.7 g (68%) of **4** as a colorless oil. ^{1}H NMR: δ 0.00 (6H, s), 0.88 (9H, s), 1.10 (3H, s), 1.3−2.4 (series of m), 1.63 (3H, m), 4.06 (1H, m), 5.02 (1H, m). ^{13}C NMR: δ −5.3, −5.0, 12.8, 17.7, 17.8,

(28) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

19.3, 23.6, 25.6, 30.2, 34.2, 37.9, 44.0, 52.5, 69.5, 112.1, 150.6. Anal. Calcd for $C_{18}H_{34}OSi$: C, 73.40; H, 11.64. Found: C, 73.36; H, 11.56.

Des-A,B-23,24-dinor-8*β*-[(*tert*-butyldimethylsilyl)oxy]-chol-16-en-22-ol (5). A suspension of alkene **4** (760 mg, 2.58 mmol) and paraformaldehyde (387 mg, 12.9 mmol) in 80 mL of dry CH_2Cl_2 was treated with 4 drops of $BF_3{\cdot}OEt_2$ and stirred for 8 min at room temperature. The purple suspension was poured into water, and after standard workup with CH_2Cl_2, the residue (a yellow oil) was purified by flash chromatography to yield 497 mg (59%) of **5** as a colorless oil. ^{1}H NMR: δ 0.02 (6H, s), 0.88 (9H, s), 1.11 (3H, d, $J \approx 6.9$ Hz), 1.03 (3H, s), 1.2−2.0 (series of m), 2.23 (1H, m), 2.38 (1H, m), 3.46 (2H, m), 4.09 (1H, m), 5.44 (1H, m). ^{13}C NMR: δ −5.4, −5.0, 17.8, 18.2, 18.9, 25.6, 30.7, 34.4, 34.5, 35.3, 46.5, 54.8, 66.4, 68.7, 121.6, 157.4. HRMS (EI): *m*/*z* calcd for $C_{19}H_{36}O_2Si$ (M^+) 324.2484, found 324.2498.

Des-A,B-23,24-dinor-8*β*-[(*tert*-butyldimethylsilyl)oxy]-22-[(*p*-tolylsulfonyl)oxy]chol-16-ene (6a). Alcohol **5** (497 mg, 1.53 mmol) and pyridine (1.82 g, 23.0 mmol) were dissolved in dry CH_2Cl_2, and the solution was cooled to 0 °C. *p*-Toluenesulfonyl chloride (1.46 g, 7.66 mmol) was added, and the reaction mixture was stirred at 0 °C for 30 min and then at room temperature overnight. Methanol (5 mL) was added and the mixture stirred for an additional 45 min and then poured into water (50 mL). The mixture was extracted twice with ether, and the combined ether layers were washed with water (twice), 1 M HCl, water, and brine. The organic phase was dried and concentrated. Purification by flash chromatography afforded **6a** (699 mg, 95%) as a colorless oil. ^{1}H NMR: δ 0.00 (6H, s), 0.86 (9H, s), 0.91 (3H, s), 0.99 (3H, d, $J \approx 6.9$ Hz), 1.2−1.9 (series of m), 2.13 (1H, dd), 2.3−2.5 (1H, m), 2.43 (3H, s), 3.85 (1H, dd, $J \approx 8.8$ Hz), 4.00 (1H, dd, $J \approx 9.4$, 5.6 Hz), 4.05 (1H, m), 5.21 (1H, m), 7.32 (2H, d, $J \approx 8.1$ Hz), 7.77 (2H, dd, $J \approx 8.1$, 1.8 Hz). ^{13}C NMR: δ −5.4, −5.1, 17.7, 17.8, 18.3, 18.7, 21.4, 25.5, 30.7, 31.0, 34.3, 35.1, 46.6, 54.4, 68.6, 74.0, 122.3, 127.7, 129.5, 133.1, 144.3, 155.2. Anal. Calcd for $C_{26}H_{42}O_4SSi$: C, 65.23; H, 8.84; S, 6.70. Found: C, 65.09; H, 8.86; S, 6.92.

(20*R*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]-22-[(*p*-tolylsulfonyl)oxymethyl]pregn-16-ene (6b). Following the procedure described for the preparation of **6a**, alcohol **23** (1.75 g, 5.39 mmol) was converted to 2.14 g of tosylate **6b** (83%) as a white solid. Mp: 59.0−60.5 °C (recrystallized from *n*-hexane). ^{1}H NMR: δ −0.01 (3H, s), 0.00 (3H, s), 0.86 (9H, s), 0.95 (3H, s), 1.06 (3H, d, $J \approx 7.0$ Hz), 1.06 (1H, m), 1.3−1.9 (series of m), 2.17 (1H, ddd, $J \approx 11.7$, 1.5 Hz), 2.4 (1H, m), 2.43 (3H, s), 3.63 (1H, dd, $J \approx 9.6$, 8.5 Hz), 3.97 (1H, $J \approx 9.6$, 4.4 Hz), 4.03 (1H, m), 5.35 (1H, br t, $J \approx 1.5$ Hz), 7.33 (2H, d, $J \approx 7.7$ Hz), 7.76 (2H, dt, $J \approx 8.2$, 1.8 Hz). ^{13}C NMR: δ −5.4, −5.1, 17.7, 17.8, 18.8, 21.4, 25.5, 30.7, 30.9, 34.3, 35.1, 46.6, 54.2, 68.6, 74.1, 124.0, 127.8, 129.5, 133.1, 144.3, 154.5. HRMS (EI): *m*/*z* calcd for $C_{25}H_{39}O_4SSi$ [M − CH_3]$^+$ 463.2338, found 463.2309. Anal. Calcd for $C_{26}H_{42}O_4SSi$: C, 65.23; H, 8.84; S, 6.70. Found: C, 65.40; H, 8.77; S, 6.58.

Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)oxy]cholest-16-ene (8a). Magnesium turnings (910 mg, 37.4 mmol) and freshly distilled THF (20 mL) were placed in a dry two-necked flask equipped with a condenser and an addition funnel. 4-Bromo-2-methyl-2-(trimethylsilyloxy)-butane[15] (**7**) (8.21 g, 34.3 mmol) was placed in the funnel, and approximately 1/10 was added to the Mg suspension. By heating to reflux, the reaction started, and the remaining bromide was added so that a steady reflux was maintained. After complete addition, the reaction mixture was cooled to room temperature. Meanwhile, LiCl (132 mg, 3.12 mmol) and $CuCl_2$ (210 mg, 1.56 mmol) were dissolved in dry THF (10 mL) by sonication and added to the reaction mixture. After being stirred for 10 min, the mixture was cooled in an ice bath, and a solution of tosylate **6a** (1.49 mg, 3.12 mmol) in THF (10 mL) was added. Stirring was continued for 60 min at 0 °C, after which ether (50 mL) was added and the mixture was then poured into ice−water. After standard workup with ether, flash chromatography (ether/pentanes, 0.5% → 1.0%) afforded 956 mg (66%) of crude **8a** as an oil. ^{1}H NMR: δ 0.02 (6H, s), 0.08 (9H, s), 0.88 (9H, s), 0.97 (3H, d, $J \approx 6.6$ Hz), 1.01 (3H, s), 1.18 (6H, 2 s), 1.2−2.1 (series of m), 2.23 (1H, m), 4.08 (1H, m), 5.25 (1H, m). ^{13}C NMR: δ −5.4, −5.1, 2.4, 17.8, 17.9, 18.8, 22.1, 22.2, 25.6, 29.6, 29.9, 30.6, 31.5, 34.6, 35.6, 37.0, 44.8, 46.5, 54.9, 69.0, 73.9, 119.2, 160.5. HRMS (EI): *m*/*z* calcd for $C_{26}H_{51}O_2Si_2$ [M − CH_3]$^+$ 451.3428, found 451.3430. Anal. Calcd for $C_{27}H_{54}O_2Si_2$: C, 69.46; H, 11.66. Found: C, 69.58; H, 11.69.

(20*S*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)oxy]cholest-16-ene (8b). Following the procedure described for the preparation of **8a**, 1.9 g of tosylate **6b** (4.0 mmol) was converted to 1.67 g (90%) of **8b** (colorless oil). ^{1}H NMR: δ 0.01 (6H, s), 0.08 (9H, s), 0.88 (9H, s), 1.02 (3H, s), 1.04 (3H, d, $J \approx 6.6$ Hz), 1.18 (6H, s), 1.2−2.1 (series of m), 1.86 (1H, ddd, $J \approx 14.4$, 6.2, 3.2 Hz), 2.21 (1H, ddt, m), 4.08 (1H, m), 5.28 (1H, m). ^{13}C NMR: δ −5.4, −5.0, 2.4, 17.8, 17.9, 19.2, 21.1, 22.1, 25.6, 29.7, 30.6, 30.9, 34.6, 35.5, 38.0, 44.7, 46.8, 54.6, 69.0, 73.9, 119.8, 161.0. HRMS (EI): *m*/*z* calcd for $C_{27}H_{54}O_2Si_2$ (M^+) 466.3662, found 466.3648.

Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]cholestane-16α,25-diol (9a) and (17*S*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]cholestane-16*β*,25-diol (10a). A solution of **8a** (420 mg, 0.90 mmol) in dry THF (10 mL) was treated with $BH_3{\cdot}THF$ (7.7 mL, 1.0 M in hexane) at 0 °C and stirred in the melting ice bath overnight. The reaction mixture was cooled in the ice bath, and water (3 mL), aqueous NaOH (3 N, 10 mL), and aqueous H_2O_2 (30%, 5 mL) were slowly added while the temperature was maintained below 6 °C. After being stirred at 0 °C for 2 h, the mixture was acidified to pH 7.5 with HCl (4 M). Standard workup with ether afforded 817 mg of crude product as an oil, which was purified by flash chromatography (ethyl acetate/petroleum ether) to give 254 mg of deprotected alcohol **9a** (68%) and 53 mg of the isomer **10a** (14%), both as colorless oils.

Data for Isomer 9a. ^{1}H NMR: δ −0.03 (3H, s), −0.02 (3H, s), 0.85 (9H, s), 0.91 (3H, d), 0.92 (3H, s), 0.98 (1H, dd, $J \approx 10.2$, 5.4 Hz), 1.18 (6H, s), 1.2−2.0 (series of m), 2.05 (1H, dt), 3.99 (1H, m), 4.02 (1H, m). ^{13}C NMR: δ −5.4, −5.0, 15.1, 17.1, 17.8, 18.7, 20.7, 25.6, 28.8, 29.3, 33.3, 34.1, 35.9, 36.0, 40.5, 43.8, 43.9, 49.8, 67.0, 68.8, 70.9, 75.9. HRMS (EI): *m*/*z* calcd for $C_{24}H_{46}O_2Si$ [M − H_2O]$^+$ 394.3267, found 394.3255. Anal. Calcd for $C_{27}H_{56}O_3Si_2$ (as 25-*O*-TMS ether): C, 66.88; H, 11.64. Found: C, 66.97; H, 11.59.

Data for Isomer 10a (as 25-*O*-TMS Ether). ^{1}H NMR: δ 0.01 (6H, s), 0.10 (9H, s), 0.8−2.1 (series of m), 3.98 (1H, m), 4.31 (1H, m). ^{13}C NMR: δ −5.3, −5.0, 2.4, 14.9, 17.2, 17.8, 17.9, 20.9, 25.6, 29.4, 29.6, 29.9, 34.2, 35.5, 36.1, 40.5, 41.8, 44.8, 50.7, 61.8, 68.7, 71.6, 73.9. HRMS (ES): *m*/*z* calcd for $C_{27}H_{57}O_3Si_2$ [M + H]$^+$ 485.3846, found 485.3834.

(20*S*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]cholestane-16α,25-diol (9b) and (17*S*,20*S*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]cholestane-16*β*,25-diol (10b). Using the procedure described for the preparation of **9a**, 1.5 g of alkene **8b** (3.2 mmol) was converted to 840 mg of deprotected alcohol **9b** (64%) as a white solid, mp 104−106 °C (recrystallized from *n*-hexane), and 98 mg (7.4%) of isomer **10b** as a colorless oil.

Data for Isomer 9b. ^{1}H NMR: δ −0.01 (3H, s), 0.00 (3H, s), 0.87 (9H, s), 0.91 (3H, s), 1.01 (3H, d, $J \approx 6.9$ Hz), 1.01 (1H, dd), 1.1−1.8 (series of m), 1.20 (6H, s), 1.86 (1H, m), 2.06 (1H, dt, $J \approx 13.5$, 8.3 Hz), 4.02 (1H, m), 4.04 (1H, m). ^{13}C NMR (125 MHz): δ −5.4, −5.0, 15.5, 17.2, 17.8, 18.5, 20.6, 25.6, 29.0, 32.3, 34.1, 35.5, 36.2, 40.3, 43.9, 44.0, 49.8, 66.3, 68.8, 70.9, 75.6. MS: *m*/*z* 412 (M^+), 394 (M − H_2O), 379 (M − H_2O − Me), 337 (M − H_2O − *t*-Bu), 279 (M − H_2O − TBS), 263 (M − H_2O − OTBS), 95. HRMS (EI): *m*/*z* calcd for $C_{24}H_{46}O_2Si$ [M − H_2O]$^+$ 394.3267, found 394.3264. Anal. Calcd for $C_{24}H_{48}O_3Si$: C, 69.84; H, 11.72. Found: C, 69.85; H, 11.63.

Data for Isomer **10b**. ^{1}H NMR: δ 0.01 (6H, s), 0.88 (9H, s), 0.97 (1H, m), 0.97 (3H, d, $J \approx 6.5$ Hz), 1.08 (3H, s), 1.20 (6H,

s), 1.1−2.1 (series of m), 3.97 (1H, m), 4.30 (1H, m). ^{13}C NMR: δ −5.3, −5.0, 15.1, 17.3, 17.8, 18.4, 20.6, 25.6, 29.0, 29.2, 34.1, 34.6, 35.6, 40.6, 41.9, 44.0, 50.9, 61.7, 68.7, 70.9, 72.0. HRMS (ES): *m/z* calcd for $C_{24}H_{47}O_2Si$ [$MH^+ - H_2O$] 395.3345, found 395.3449.

Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]-16α-[(methylsulfonyl)oxy]-25-[(trimethylsily)oxy]cholestane (11a). To a solution of 125 mg (0.30 mmol) of **9a** in pyridine (5 mL) was added methanesulfonyl chloride (50 μL) at 0 °C. The solution was stirred at 0−5 °C for 3 h, then TMSCl (50 μL) was added, and stirring was continued for another 45 min. After addition of water (0.5 mL), the mixture was poured into ice/water (25 mL) and extracted with ether. The combined ether layers were washed with water, aqueous $CuSO_4$ (0.2 M), and brine, dried, and evaported. Flash chromatography afforded 146 mg (87%) of **11a** as a colorless oil. ^{1}H NMR: δ − 0.02 (3H, s), −0.00 (3H, s), 0.08 (9H, s), 0.86 (9H, s), 0.92 (3H, s), 0.95 (3H, overlapping d), 1.1−1.8 (series of m), 1.19 (6H, s), 1.94 (1H, br d), 2.09 (1H, dt, $J \approx$ 14.1, 6.4 Hz), 2.97 (3H, s), 4.03 (1H, m), 4.95 (1H, m).

(20*S*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]-16α-[(methylsulfonyl)oxy]-25-[(trimethylsily)oxy]cholestane (11b). Using the procedure described for the preparation of **11a**, 60 mg of alcohol **9b** (0.15 mmol) was converted to 69 mg of mesylate **11b** (85%) as a colorless oil. ^{1}H NMR: δ −0.01 (3H, s), 0.00 (3H, s), 0.09 (9H, s), 0.86 (9H, s), 0.91 (3H, s), 0.98 (3H, d, $J \approx$ 6.5 Hz), 1.19 (6H, s), 1.1−2.0 (series of m), 2.11 (1H, dt, $J \approx$ 14.1, 7.9 Hz), 2.98 (3H, s), 4.03 (1H, m), 5.00 (1H, t, $J \approx$ 7.1 Hz). ^{13}C NMR: δ −5.4, −5.0, 2.4, 15.5, 17.0, 17.8, 18.1, 20.7, 25.6, 29.6, 29.7, 31.6, 32.7, 34.0, 36.5, 39.0, 39.7, 42.8, 44.8, 49.8, 62.2, 68.2, 73.8, 85.9. HRMS (ES): *m/z* calcd for $C_{28}H_{59}O_5SSi_2$ [M + H]$^+$ 563.3622, found 563.3635.

Des-A,B-cholestane-8*β*,25-diol (12a). $LiAlH_4$ (118 mg, 3.11 mmol) was added to a solution of mesylate **11a** in ether (10 mL). The mixture was heated to reflux for 3.5 h, at which point the reaction was complete. Ethyl acetate was added until gas evolution ceased. After standard workup with ether, the residue was dissolved in THF and acetonitrile (each 1 mL), treated with aqueous HF (48%, 0.5 mL), and stirred overnight at room temperature. Water (1 mL) was added, and after being stirred for 45 min, the mixture was extracted three times with CH_2Cl_2. The combined extracts were then washed with 2 M $KHCO_3$, water, and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography to afford 21 mg (68%) of **12a** as a white solid, which was recrystallized in hexane/dichloromethane for analysis. Mp: 91−91.5 °C (lit.[18] mp: 91−92 °C). ^{1}H NMR: δ 0.91 (3H, s, $J \approx$ 6.9 Hz), 0.93 (3H, s), 1.21 (6H, s), 1.0−1.9 (series of m), 1.95−2.05 (1H, m), 4.07 (1H, m). ^{13}C NMR: δ 13.5, 17.5, 18.5, 20.8, 22.5, 27.2, 29.2, 29.4, 33.6, 35.3, 36.3, 40.4, 41.9, 44.4, 52.6, 56.7, 69.5, 71.1.

(20*S*)-Des-A,B-cholestane-8*β*,25-diol (12b).[26] Using the procedure described for the preparation of **12a**, 66 mg of mesylate **11b** (0.12 mmol) was converted to 15 mg of diol **12b** (44%) as a white solid. Mp: 79.5−81 °C. ^{1}H NMR: δ 0.84 (3H, d, $J \approx$ 6.5 Hz), 0.93 (3H, s), 1.21 (6H, s), 1.0−2.1 (series of m), 4.07 (1H, m). ^{13}C NMR: δ 13.8, 17.5, 18.5, 20.9, 22.4, 27.1, 29.2, 29.3, 33.6, 34.7, 35.7, 40.4, 41.9, 44.3, 52.7, 56.3, 69.4, 71.1. HRMS (EI): *m/z* calcd for $C_{18}H_{32}O$ [M − H_2O]$^+$ 264.2453, found 264.2466.

Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]cholestan-25-ol-16α-yl Acetate (13a). Alcohol **9a** (293 mg, 0.710 mmol) dissolved in pyridine (5 mL) was treated with acetic anhydride (1.10 mL) at 0 °C under argon. After being stirred at room temperature for 3 h, the reaction mixture was poured into water (20 mL), cooled in an ice bath, and acidified to pH 2.5 with aqueous HCl (4 M). After standard workup using ether, the residue was purified by flash chromatography to afford 240 mg of **13a** (74%) as an oil. ^{1}H NMR: δ −0.04 (3H, s), −0.02 (3H, s), 0.85 (9H, s), 0.91 (3H, d, $J \approx$ 6.6 Hz), 0.93 (3H, s), 1.18 (6H, s), 1.0−2.0 (series of m), 1.99 (3H, s), 2.12 (1H, dt, $J \approx$ 13.9, 8.2 Hz), 3.98 (1H, m), 4.88 (1H, m). ^{13}C NMR: δ −5.4, −5.0, 15.0, 17.1, 17.8, 18.6, 20.3, 21.3, 25.6, 29.0, 29.2, 33.0, 33.3, 34.0, 35.4, 40.4, 43.0, 44.1, 50.1, 61.8, 68.5, 70.8, 78.8, 170.7. HRMS (EI): *m/z* calcd for $C_{26}H_{50}O_4Si$ (M^+) 454.3478, found 454.3486. Anal. Calcd for $C_{26}H_{50}O_4Si$: C, 68.67; H, 11.08. Found: C, 68.16; H, 11.07.

(20*S*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]cholestan-25-ol-16α-yl Acetate (13b). Following the procedure described for the preparation of **13a**, 395 mg (0.96 mmol) of **9b** was converted quantitatively to **13b** (colorless oil). ^{1}H NMR: δ −0.03 (3H, s), −0.01 (3H, s), 0.82 (3H, d, $J \approx$ 6.9 Hz), 0.86 (9H, s), 0.93 (3H, s), 1.20 (6H, s), 1.1−1.8 (series of m), 1.88 (1H, m), 2.00 (3H, s), 2.13 (1H, dt, $J \approx$ 13.7, 8.4 Hz), 3.99 (1H, m), 4.95 (1H, br t, $J \approx$ 6.9 Hz). ^{13}C NMR: δ −5.4, −5.0, 15.4, 17.1, 17.8, 17.9, 20.5, 21.3, 25.6, 29.0, 29.1, 32.1, 33.0, 34.1, 36.1, 40.1, 43.0, 44.0, 50.1, 61.2, 68.4, 70.8, 78.3, 170.7. HRMS (ES): *m/z* calcd for $C_{26}H_{50}O_4SiNa$ [M + Na]$^+$ 477.3376, found 477.3374.

Des-A,B-cholestane-8*β*,25-diol-16α-yl Acetate (14a). To a solution of **13a** (210 mg, 0.462 mmol) in CH_3CN (7 mL) was added aqueous HF (40%, 230 μL), and the resulting mixture was stirred at room temperature for 8 h. The reaction mixture was neutralized with saturated $NaHCO_3$ and concentrated by evaporation. After standard workup, the residual oil was purified by flash chromatography (ethyl acetate/pentanes), affording 101 mg of **14a** (64%) as a colorless oil. ^{1}H NMR: δ 0.92 (3H, d, $J \approx$ 6.6 Hz), 0.95 (3H, s), 1.18 (6H, s),1.0−2.0 (series of m), 2.00 (3H, s), 2.18 (1H, dt, $J \approx$ 13.9, 8.2 Hz), 4.07 (1H, m), 4.90 (1H, t, $J \approx$ 6.9 Hz). ^{13}C NMR: δ 14.7, 16.9, 18.6, 20.3, 21.3, 29.0, 29.2, 32.9, 33.1, 33.4, 35.3, 40.2, 42.7, 44.1, 49.6, 61.6, 68.2, 70.8, 78.5, 170.6. HRMS (EI): *m/z* calcd for $C_{20}H_{34}O_3$ [M − H_2O]$^+$ 322.2509, found 322.2482.

(20*S*)-Des-A,B-cholestane-8*β*,25-diol-16α-yl Acetate (14b). Following the procedure described for the preparation of **14a**, 444 mg (0.96 mmol) of **13b** was converted to 257 mg of **14b** (79%) as a colorless oil. ^{1}H NMR: δ 0.85 (3H, d, $J \approx$ 6.7 Hz), 0.97 (3H, s), 1.22 (6H, s), 1.1−2.0 (series of m), 2.02 (3H, s), 2.19 (1H, dt, $J \approx$ 13.9, 8.4 Hz), 4.09 (1H, m), 4.98 (1H, br t, $J \approx$ 7.1 Hz). ^{13}C NMR: δ 15.3, 17.2, 18.1, 20.8, 21.5, 29.27, 29.34, 32.4, 32.8, 33.7, 36.3, 40.1, 43.0, 44.2, 49.9, 61.3, 68.4, 71.0, 78.2, 170.9. MS: *m/z* 322 (M − H_2O), 280 (M − HOAc), 262 (M − H_2O − HOAc), 247 (M − H_2O − CH_3 − HOAc). HRMS (EI): *m/z* calcd for $C_{20}H_{34}O_3$ [M − H_2O]$^+$ 322.2509, found 322.2509.

Des-A,B-25-hydroxycholestan-8-on-16α-yl Acetate (15a). PDC (219 mg, 0.583 mmol) was added to an ice/water-cooled solution of **14a** (131 mg, 0.385 mmol) in dry CH_2Cl_2 (20 mL). The resulting suspension was then stirred at room temperature for 6 h, filtered through Celite, and evaporated. The residue was redissolved in ethyl acetate, washed twice with saturated $NaHCO_3$, dried, and evaporated. The resulting crude material was purified by flash chromatography (ethyl ether/petroleum ether, 3:1), affording 108 mg (83%) of **15a** as an oil. ^{1}H NMR: δ 0.68 (3H, s), 0.99 (3H, d, $J \approx$ 6.4 Hz), 1.21 (6H, s), 1.0−3.4 (series of m), 2.01 (3H, s), 2.72 (1H, dd, $J \approx$ 12.6, 6.8 Hz), 4.95 (1H, dd, $J \approx$ 7.2, 6.1 Hz). ^{13}C NMR: δ 14.0, 18.9, 20.5, 21.4, 23.5, 29.3, 29.5, 30.0, 33.6, 35.5, 38.9, 40.8, 44.2, 50.1, 59.3, 61.7, 70.9, 78.3, 170.5, 210.4. HRMS (EI): *m/z* calcd for $C_{20}H_{34}O_4$ (M^+) 338.2457, found 338.2480.

(20*S*)-Des-A,B-25-hydroxycholestan-8-on-16α-yl Acetate (15b). Following the procedure described for the preparation of **15a**, 228 mg of **14b** was converted to 216 mg of **15b** (95%, colorless oil). ^{1}H NMR: δ 0.67 (3H, s), 0.89 (3H, d, $J \approx$ 6.5 Hz), 1.23 (6H, s), 1.1−2.1 (series of m), 2.01 (3H, s), 2.29 (3H, m), 2.73 (1H, dd, $J \approx$ 12.2, 6.9), 5.00 (1H, m). ^{13}C NMR: δ 14.2, 18.0, 20.8, 21.4, 23.5, 29.3, 29.4, 29.7, 32.7, 36.4, 38.6, 40.8, 44.1, 50.1, 59.2, 61.1, 70.9, 77.5, 170.5, 210.3. HRMS (ES): *m/z* calcd for $C_{20}H_{34}O_4Na$ [M + Na]$^+$ 361.2355, found 361.2337.

Des-A,B-25-[(trimethylsilyl)oxy]cholestan-8-on-16α-yl Acetate (16a). To a solution of **15a** (108 mg, 0.319 mmol) in dry CH_2Cl_2 (10 mL) were added DMAP (1 mg, 0.010 mmol), Et_3N (0.16 mL, 1.16 mmol), and TMSCl (0.16 mL, 1.28 mmol)

at 0 °C under argon. The resulting mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was quenched with water and extracted twice with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were washed with water and concentrated under reduced pressure. The residue was taken up in ether, washed twice with water and brine, dried, and evaporated to dryness. Flash chromatography in ethyl acetate/pentanes afforded 118 mg (90%) of **16a** as an oil. ^{1}H NMR: δ 0.07 (9H, s), 0.66 (3H, s), 0.96 (3H, d, $J \approx 6.4$ Hz), 1.17 (6H, s), 1.0−2.4 (series of m), 1.98 (3H, s), 2.70 (1H, dd, $J \approx 12.6$, 6.8 Hz), 4.90 (1H, dd, $J \approx 7.2$, 6.2 Hz), ^{13}C NMR: δ 2.4, 13.7, 18.6, 20.3, 21.1, 23.3, 29.6, 29.7, 29.8, 33.4, 35.2, 38.7, 40.6, 45.0, 49.9, 59.1, 61.6, 73.7, 78.0, 170.2, 210.1. MS: *m*/*z* 322 (M − H_2O), 262 (M − H_2O − CH_3COOH). HRMS (EI): *m*/*z* calcd for $C_{22}H_{39}O_4Si$ [M − CH_3]$^+$ 395.2618, found 395.2600.

(20*S*)-Des-A,B-25-[(trimethylsilyl)oxy]cholestan-8-on-16α-yl Acetate (16b). Following the procedure described for the preparation of **16a**, **15b** was converted to **16b** (79% from **14b)** as a colorless oil. ^{1}H NMR: δ 0.09 (9H, s), 0.65 (3H, s), 0.86 (3H, d, $J \approx 6.5$), 1.20 (6H, s), 1.1−2.1 (series of m), 2.00 (3H, s), 2.27 (3H, m), 2.71 (1H, dd, $J \approx 6.9$, 12.2 Hz), 4.98 (1H, m). ^{13}C NMR: δ 2.4, 14.0, 17.8, 20.5, 21.2, 23.3, 29.5, 29.6, 29.7, 32.5, 36.2, 38.4, 40.6, 44.8, 49.9, 59.0, 61.0, 73.7, 77.4, 170.3, 210.1. MS: *m*/*z* 395 (M − CH_3), 335 (M − HOAc − CH_3), 131. HRMS (EI): *m*/*z* calcd for $C_{22}H_{39}O_4Si$ [M − CH_3]$^+$ 395.2618, found 395.2614.

(5*Z*,7*E*)-(1*R*,3*S*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)oxy]-9,10-secocholesta-5,7,10(19)-trien-16α-yl Acetate (18a). Ketone **16a** and phosphine oxide **17**[20] were dried thoroughly with an oil pump. A solution of **17** (248 mg, 0.425 mmol) in dry THF (10 mL) was treated with *n*-BuLi (1.56 M, 275 μL) at −78 °C under argon. The resulting orange solution was stirred for 25 min at −78 °C, upon which a solution of **16a** (95 mg, 0.231 mmol) in THF (2 mL) was added over 5 min. The reaction mixture was stirred at −78 °C for 2 h, warmed slowly to room temperature, where stirring was continued for 3 h, and finally quenched with a few drops of water. The solvent was removed in vacuo. The residue was dissolved in a 1:1 mixture of ethyl acetate and petroleum ether, washed with saturated $NaHCO_3$ and brine, and dried. Filtration and evaporation afforded 381 mg of residue that was chromatographed on silica gel (2.5% ethyl acetate/petroleum ether) to give 160 mg (89%) of **18a** as a colorless oil. ^{1}H NMR: δ 0.05 (12H, s), 0.08 (9H, s), 0.57 (3H, s), 0.86 (18H, s), 0.94 (3H, d, $J \approx 5.9$ Hz), 1.18 (6H, s), 1.0−2.1 (series of m), 2.00 (3H, s), 2.23 (2H, m), 2.42 (1H, dd, $J \approx 13.1$, 3.4 Hz), 2.83 (1H, m), 4.18 (1H, m), 4.35 (1H, m), 4.81 (1H, d, $J \approx 2.3$ Hz), 4.90 (1H, m), 5.15 (1H, $J \approx 1.6$ Hz), 5.93 (1H, d, $J \approx 11.2$ Hz), 6.20 (1H, d, $J \approx 11.2$ Hz). ^{13}C NMR: δ −5.2−5.0, −4.9, −4.7, 2.4, 13.1, 17.9, 18.0, 18.7, 20.4, 21.3, 22.8, 25.6, 25.7, 28.5, 29.6, 29.8, 32.4, 33.9, 35.4, 40.4, 44.6, 45.1, 45.9, 46.1, 53.5, 61.9, 67.3, 71.9, 73.8, 78.9, 111.3, 118.1, 122.6, 135.4, 139.1, 147.9, 170.6. HRMS (EI): *m*/*z* calcd for $C_{44}H_{82}O_5Si_3$ (M^+) 774.5470, found 774.5467.

(5*Z*,7*E*)-(1*R*,3*S*,20*S*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-9,10-secocholesta- 5,7,10(19)-trien-25-ol-16α-yl Acetate (18b). Following the procedure described for the preparation of **18a**, 98 mg of **16b** was converted to 103 mg (61%) of **18b** (TMS deprotected) and 60 mg (32%) of TMS-protected product, both as colorless oils. Spectral data of the TMS-protected product were recorded. ^{1}H NMR: δ 0.04 (6H, s), 0.05 (6H, s), 0.09 (9H, s), 0.56 (3H, s), 0.86 (18H, s), 1.19 (6H, s), 1.1−2.1 (series of m), 2.01 (3H, s), 2.23 (2H, m), 2.43 (1H, dd, $J \approx 13.4$, 3.8 Hz), 2.84 (1H, br d, $J \approx 12.6$ Hz), 4.18 (1H, septet, $J \approx 3.4$ Hz), 4.35 (1H, dd, $J \approx 6.5$, 3.8 Hz), 4.81 (1H, br d, $J \approx 2.3$ Hz), 4.96 (1H, dd, $J \approx 7.2$, 5.7 Hz), 5.16 (1H, br d, $J \approx 1.5$ Hz), 5.93 (1H, d, $J \approx 11.4$ Hz), 6.20 (1H, d, $J \approx 11.4$ Hz). ^{13}C NMR: δ −5.3, −5.0, −4.9, −4.7, 2.4, 13.3, 17.90, 17.93, 18,0, 20.5, 21.3, 22.8, 25.59, 25.65, 28.5, 29.6, 29.7, 32.2, 33.0, 36.0, 40.2, 44.5, 44.9, 45.9, 46.1, 53.4, 61.3, 67.3, 71.9, 73.8, 78.4, 111.3, 118.0, 122.6, 135.4, 139.1, 147.9, 170.7. MS: *m*/*z* 774 (M), 714 (M − HOAc), 642 (70, M − HOTBS), 582 (M − HOAc − TBSOH), 513 (M − 2TBSOH), 131. HRMS (EI): *m*/*z* calcd for $C_{44}H_{82}O_5Si_3$ (M^+) 774.5470, found 774.5467.

(5*Z*,7*E*)-(1*R*,3*S*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)oxy]-9,10-secocholesta-5,7,10(19)-trien-16α-ol (19a). Acetate **18a** (135 mg, 0.174 mmol) was added to a solution of KOH in methanol (0.4 M, 20 mL), and the resulting mixture was stirred at 40 °C for 6 h. The mixture was concentrated to approximately half the initial volume and diluted with ethyl acetate. After standard workup, the residue was purified by flash chromatography (ethyl acetate/pentanes) to give 70 mg (55%) of **19a** as a colorless oil. ^{1}H NMR: δ 0.05 (12H, s), 0.10 (9H, s), 0.56 (3H, s), 0.86 (18H, s), 0.95 (1H, dd, $J \approx 7.4$, 3.8 Hz), 1.20 (6H, s), 1.1−2.5 (series of m), 2.82 (1H, m), 4.00 (1H, m), 4.18 (1H, m), 4.36 (1H, m), 4.85 (1H, m), 5.17 (1H, m), 5.95 (1H, d, $J \approx 11.2$ Hz), 6.21 (1H, d, $J \approx 11.1$ Hz). ^{13}C NMR: δ −5.2, −5.0, −4.9, −4.8, 2.5, 13.3, 17.95, 18.0, 18.7, 21.3, 22.8, 25.6, 25.7, 28.5, 29.6, 29.7, 30.1, 34.2, 34.9, 36.0, 40.6, 44.6, 44.9, 45.8, 46.9, 53.3, 67.3, 67.6, 71.9, 73.9, 111.1, 118.0, 122.8, 135.2, 139.7, 148.1. MS: *m*/*z* 732 (M), 600 (M − TBSOH), 248. HRMS (EI): *m*/*z* calcd for $C_{42}H_{80}O_4Si_3$ (M^+) 732.5364, found 732.5331.

(5*Z*,7*E*)-(1*R*,3*S*,20*S*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-9,10-secocholesta-5,7,10(19)-triene-16α,25-diol (19b). Following the procedure described for the preparation of **19a**, **18b** was converted quantitatively to **19b** (colorless oil). ^{1}H NMR: δ 0.04−0.05 (12H, singlets), 0.54 (3H, s), 0.86 (18H, s), 1.03 (3H, d, $J \approx 6.9$ Hz), 1.20 (6H, s), 1.2−2.0 (series of m), 2.20 (1H, dd, $J \approx 13.0$, 7.6 Hz), 2.36 (1H, overlaid m), 2.43 (1H, dd, $J \approx 13.0$, 3.8 Hz), 2.82 (1H, br d, $J \approx 13.40$ Hz), 4.04 (1H, m), 4.17 (1H, m), 4.36 (1H, dd, $J \approx 6.5$, 3.8 Hz), 4.84 (1H, m), 5.16 (1H, m), 5.94 (1H, d, $J \approx 11.1$ Hz), 6.21 (1H, d, $J \approx 11.1$ Hz). ^{13}C NMR: δ −5.3, −5.0, −4.89, −4.86, 13.5, 17.95, 18.0, 18.4, 20.6, 22.7, 25.61, 25.65, 28.4, 29.1, 33.2, 34.6, 36.1, 40.3, 44.0, 44.6, 45.8, 46.8, 53.2, 66.3, 67.3, 70.9, 71.9, 75.9, 111.1, 118.0, 122.8, 135.3, 139.5, 148.1. HRMS (EI): *m*/*z* calcd for $C_{39}H_{72}O_4Si_2$ (M^+) 660.4969, found 660.4952.

(5*Z*,7*E*)-(1*R*,3*S*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-25-[(trimethylsilyl)oxy]-9,10-secocholesta-5,7,10(19)-trien-16α-yl Hydrogen Glutarate (20a). To a solution of **19a** (70 mg, 0.096 mmol) in CH_2Cl_2 (5 mL) were added DMAP (70 mg, 0.573 mmol) and glutaric anhydride (54 mg, 0.477 mmol). The resulting solution was stirred at 4 °C for 3 days. Standard workup with CH_2Cl_2 followed by flash chromatography (30% EtOAc/pentanes) afforded 32 mg of **20a** (39%) as a colorless oil. ^{1}H NMR: δ 0.04 and 0.05 (12H, singlets), 0.08 (9H, s), 0.56 (3H, s), 0.85 (9H, s), 0.87 (9H, s), 0.93 (3H, d, $J \approx 5.7$ Hz), 1.18 (6H, s), 1.2−2.7 (series of m), 2.83 (1H, m), 4.18 (1H, m), 4.35 (1H, m), 4.82 (1H, m), 4.93 (1H, m), 5.14 (1H, m), 5.92 (1H, d, $J \approx 11.1$ Hz), 6.21 (1H, d, $J \approx 11.2$ Hz). ^{13}C NMR: δ −5.2, −5.0, −4.9, −4.7, 2.5, 13.2, 17.9, 18.7, 20.1, 20.8, 22.8, 25.6, 25.7, 28.6, 29.6, 29.7, 32.4, 33.6, 33.8, 35.4, 40.4, 44.6, 44.9, 46.1, 46.2, 53.5, 62.3, 67.2, 72.2, 73.9, 78.9, 111.7, 118.0, 122.7, 135.3, 139.1, 147.6, 172.8. MS: *m*/*z* 846 (M), 582 (M − 2TBSOH), 75. HRMS (EI): *m*/*z* calcd for $C_{47}H_{86}O_7Si_3$ (M^+) 846.5681, found 846.5635.

(5*Z*,7*E*)-(1*R*,3*S*,20*S*)-1,3-Bis[(*tert*-butyldimethylsilyl)oxy]-9,10-secocholesta-5,7,10(19)-trien-25-ol-16α-yl Hydrogen Glutarate (20b). Following the procedure described for the preparation of **20a**, 44 mg of **19b** was converted to 40 mg (77%) of **20b** as a colorless oil. ^{1}H NMR: δ 0.04, 0.05, and 0.05 (12H, 3 s), 0.55 (3H, s), 0.86 (18H, s), 0.85 (3H, overlaid), 1.21 (6H, s), 1.3−2.5 (series of m), 2.33 and 2.38 (4H, overlapping t), 2.84 (1H, m), 4.18 (1H, m), 4.35 (1H, m), 4.82 (1H, m), 5.00 (1H, m), 5.16 (1H, m), 5.93 (1H, d, $J \approx 11.2$ Hz), 6.20 (1H, d, $J \approx 11.1$ Hz). ^{13}C NMR: δ −5.2, −5.0, −4.9, −4.7, 13.5, 17.9, 19.9, 20.6, 22.7, 25.6, 25.7, 28.5, 29.0, 29.1, 32.2, 32.6, 33.0, 33.6, 36.4, 40.0, 43.9, 44.5, 46.0, 46.1, 53.4, 61.1, 67.2, 71.1, 72.0, 78.0, 111.4, 118.0, 122.6, 135.5, 139.0, 147.8, 172.4, 177.9. HRMS: (EI) *m*/*z* calcd for $C_{44}H_{78}O_7Si_2$ (M^+) 774.5286, found 774.5278.

(*E*)-Des-A,B-8*β*-[(*tert*-butyldimethylsilyl)oxy]-17(20)-pregnene (21). Following the procedure described for the

preparation of **7**, 2.36 g of alcohol **3b** (7.55 mmol) was converted to 1.88 g of alkene **21** (85%) as a colorless oil. ^{1}H NMR: δ 0.01 (6H, s), 0.88 (9H, s), 0.97 (3H, s), 1.1−1.9 (series of m), 1.52 (3H, dt, $J \approx 6.6$, 1.5 Hz), 2.10−2.35 (2H, m), 4.07 (1H, q, $J \approx 2.6$ Hz), 4.97 (1H, tq, $J \approx 6.4$, 2.5 Hz). ^{13}C NMR: δ −5.3, −5.0, 13.4, 17.5, 17.8, 21.6, 23.3, 25.3, 25.6, 34.6, 36.7, 43.1, 51.4, 69.2, 108.8, 153.2. HRMS (EI): *m*/*z* calcd for $C_{18}H_{34}OSi$ (M^+) 294.2379, found 294.2391.

Des-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-17,20-epoxymethanopregnane (22). To a solution of (*E*)-alkene **21** (468 mg, 1.59 mmol) in dry CH_2Cl_2 (50 mL) were added paraformaldehyde (239 mg, 7.94 mmol) and $BF_3{\cdot}OEt_2$ (3 drops, ∼23 mg, 0.16 mmol) under argon. The resulting red-brown suspension was stirred for 10 min at room temperature and then poured into water (70 mL). After standard workup, the crude product was purified by flash chromatography (10% EtOAc/pentanes) to afford 354 mg of oxetane **22** (69%) as a colorless oil. ^{1}H NMR (500 MHz, d_6-DMSO): δ 0.02 (3H, s), 0.03 (3H, s), 0.86 (3H, d, $J \approx 6.9$ Hz), 0.87 (9H, s), 0.95 (3H, s), 1.2−1.9 (series of m), 3.14 (1H, dd, $J \approx 11.1$, 8.5 Hz), 3.69 (1H, dd, $J \approx 8.2$, 7.3 Hz), 3.93 (1H, m). ^{13}C NMR: δ −5.0, −4.8, 10.0, 18.0, 18.1, 21.8, 24.9, 25.7, 30.0, 30.0, 32.1, 43.9, 52.3, 52.5, 69.9, 70.8, 93.4. IR ($CHCl_3$): 835, 1035, 1090, 1255, 1460, 2800−3000 (s) cm^{-1}. MS: *m*/*z* 324 (M^+), 267 (M − *t*-Bu), 193 (M − OTBS), 175, 135, 75.

(20*R*)-Des-A,B-8β-[(*tert*-butyldimethylsilyl)oxy]-20-hydroxymethylpregn-16-ene (23). To a solution of alkene **21** (2.1 g, 7.1 mmol) in CH_2Cl_2 (150 mL) was added paraformaldehyde (1.07 g, 35.7 mmol), and the resulting suspension was cooled in an ice bath. Trimethylaluminum (2 M in heptanes, 9 mL) was added using a syringe, and the reaction mixture was stirred for 4.5 h at 0 °C. Then, additional Me_3Al (9 mL) was added. After the mixture was stirred for another 2 h, methanol was carefully added until the reaction subsided. This was followed by the addition of water to fully quench the reaction. While being cooled in an ice bath, the mixture was acidified to pH 3.0 with aqueous HCl. After standard workup with CH_2Cl_2, the residue was purified by flash chromatography (12% EtOAc/pentanes) to afford 1.22 g of alcohol **23** (colorless oil, 53%) and 358 mg (17%) of starting material **21**. In some runs, the byproduct **24** was produced in <10% yield as a colorless oil. ^{1}H NMR: δ 0.01 (6H, 2 s), 0.87 (9H, s), 1.03 (3H, s), 1.10 (3H, d, $J \approx 7.0$ Hz), 1.2−2.0 (series of m), 2.25 (1H, m), 2.37 (1H, m), 3.46 (2H, m), 4.08 (1H, m), 5.44 (1H, br t, $J \approx 1.5$ Hz). ^{13}C NMR: δ −5.4, −5.1, 17.8, 17.9, 18.9, 25.6, 30.8, 33.9, 34.4, 35.4, 46.8, 54.5, 66.4, 68.7, 123.3, 156.7. HRMS (EI): *m*/*z* calcd for $C_{19}H_{36}O_2Si$ (M^+) 324.2485, found 324.2487.

The NMR spectra of the byproduct **24** are available in the Supporting Information.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for new compounds and experimental details of the radioimmunoassay. This material is available free of charge via the Internet at http://pubs.acs.org.

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