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PRACTICAL AND FACILE ROUTE TO A FUNCTIONAL INTERMEDIATE FROM STIGMASTEROL FOR THE SYNTHESIS OF 1α-HYDROXYVITAMIN D_5 AND RELATED COMPOUNDS*[1]

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Abstract – As a functional and versatile intermediate for the synthesis of 1α-hydroxyvitamin D_5 and related compounds, 1α,2α-epoxy-3β-hydroxystigmasta-5,7-diene was synthesized by a practical and facile 17-step route from stigmasterol in 17% overall yield.

INTRODUCTION

Currently, six forms of vitamin D (**1**) are known. They range from vitamin D_2 (**2**) (ergocalciferol) to vitamin D_7 (**7**) and are distinguished by their differing side chains (Figure 1).[2] Among them it is well-established that vitamin D_3 (**3**) (cholecalciferol) ingested into foods or synthesized in the skin is metabolized to 25-hydroxyvitamin D_3 (**8**) (calcifediol) in the liver, which is further hydroxylated at the 1α-position in the kidney to produce the active form, 1α,25-dihydroxyvitamin D_3 (**9**) (calcitriol) (Scheme 1).[3] 1α,25-Dihydroxyvitamin D_3 (**9**) is well recognized as a potent regulator of calcium and

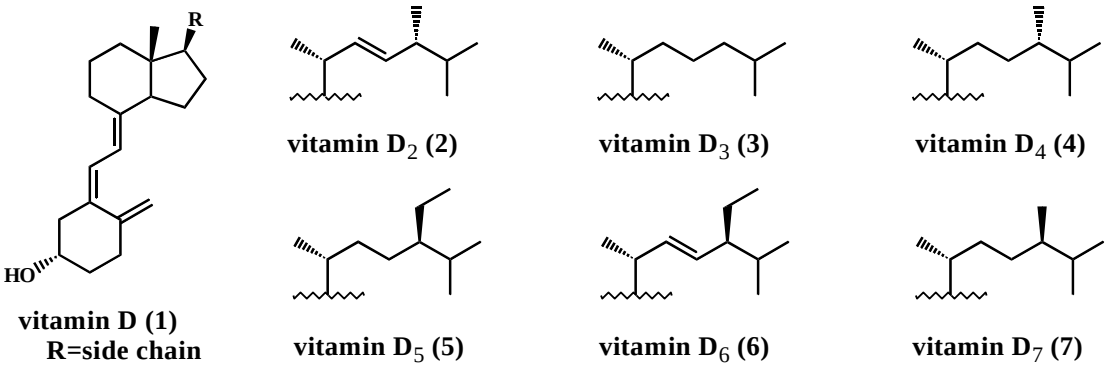

Figure 1. Six forms of vitamin D (**1**) distinguished by differing side chains

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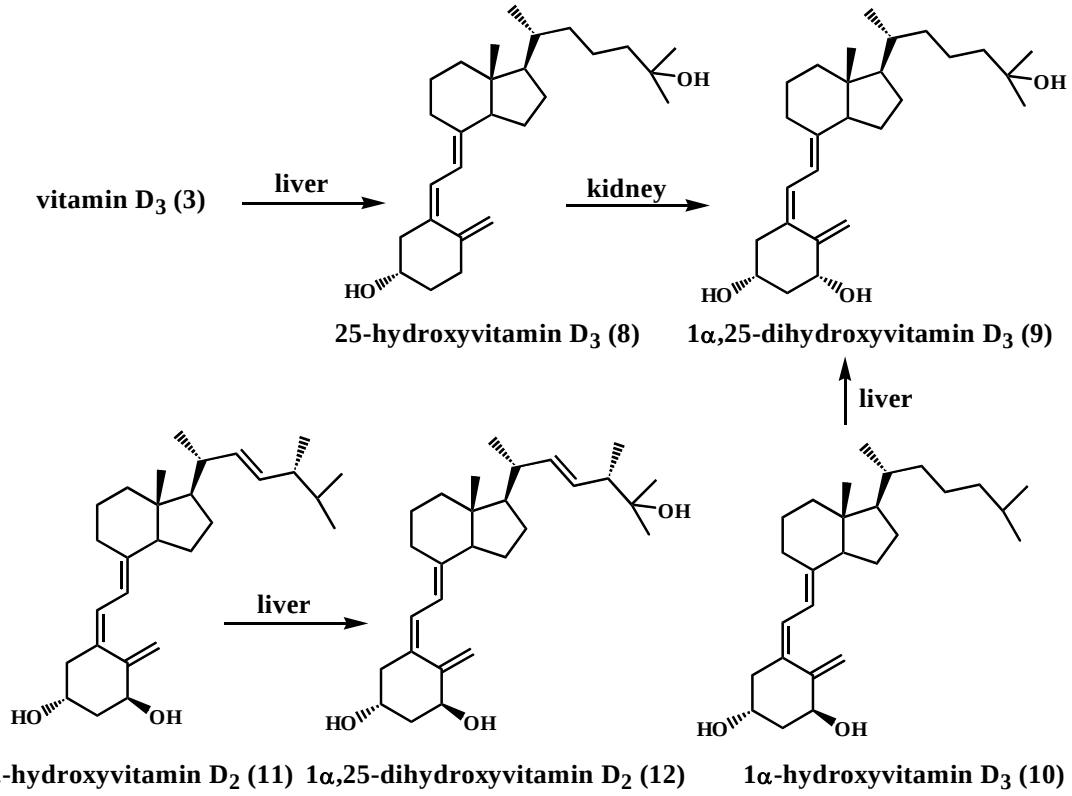
*This paper is dedicated to Professor Dr. Lutz F. Tietze on the occasion of his 75th birthday.

phosphorous metabolism while also possessing regulatory effects on cell proliferation and differentiation processes.[4] In case of vitamin D_2 (**2**), activation pathway and biological activity are almost similar to vitamin D_3 (**3**), although much less information for **2** is available compared to **3**.[2] Concerning the biological properties of vitamin D_4 (**4**), vitamin D_5 (**5**), vitamin D_6 (**6**), and vitamin D_7 (**7**), very little is known about their activation pathways and active metabolites (Figure 1).[5]

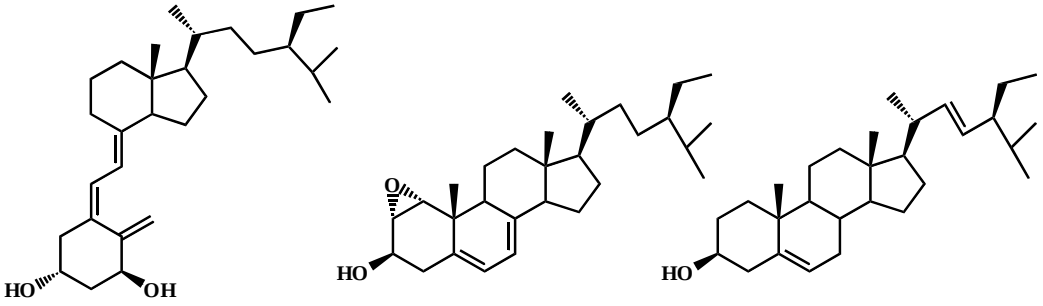


Scheme 1. Activation of vitamin D_3 (**3**) and synthetic prodrugs, 1α-hydroxyvitamin D_3 (**10**) and 1α-hydroxyvitamin D_2 (**11**), to biologically active forms (**9**) and (**12**)

1α-Hydroxyvitamin D_3 (**10**) (alfacalcidol) has been widely used as a synthetic prodrug for 1α,25-dihydroxvitamin D_3 (**9**) in the treatment of hypocalcemia, chronic renal failure, hypoparathyroidism and osteoporosis.[6,7] 1α-Hydroxyvitamin D_2 (**11**) (doxercalciferol), a synthetic prodrug of 1α,25-dihydroxyvitamin D_2 (**12**), has been clinically used for the treatment of secondary hyperparathyroidism (Scheme 1).[7-9] 1α-Hydroxyvitamin D_5 (**13**) is a synthetic analog of vitamin D_5 (**5**) and shows prevention and therapy of breast, prostate, and colon cancers.[10-12] Although it has been announced that phase I clinical trial with 1α-hydroxyvitamin D_5 (**13**) is expected to be initiated for breast cancer patients soon,[13] activation pathway and metabolites of **13** are not, however, delineated. This is in part due to the lack of labelled compounds such as tritiated **13**, which is an indispensable material for pharmacokinetic and metabolic studies of **13**.[14,15] Because of ambiguous synthetic route to

13 reported in a literature,[16,17] structure activity relationships between 1α-hydroxyvitamin D_5 (**13**) and its derivatives against tumor cells have not been delineated. We believe that reliable and efficient synthetic method available for **13** and related compounds, including labelled compounds and putative metabolites, would accelerate the development of **13** as a promising drug candidate for the treatment of cancer (Figure 2).

In the steroid-vitamin D chemistry, photoreaction of steroidal 5,7-diene system called as provitamin D to generate previtamin D, which is further converted to vitamin D by subsequent thermal isomerization, is one of the most important and well-known reactions.[18,19] The steroidal 5,7-diene structure having the requisite side chain to each vitamin D, therefore, should be a useful key intermediate as provitamin D.[20] In this paper we describe the practical and facile route to a functional and versatile steroidal intermediate, 1α,2α-epoxy-3β-hydroxystigmasta-5,7-diene (1,2α-epoxide) (**14**) with 5,7-diene structure, from inexpensive stigmasterol (**15**) for the synthesis of 1α-hydroxyvitamin D_5 (**13**) and related compounds (Figure 2).



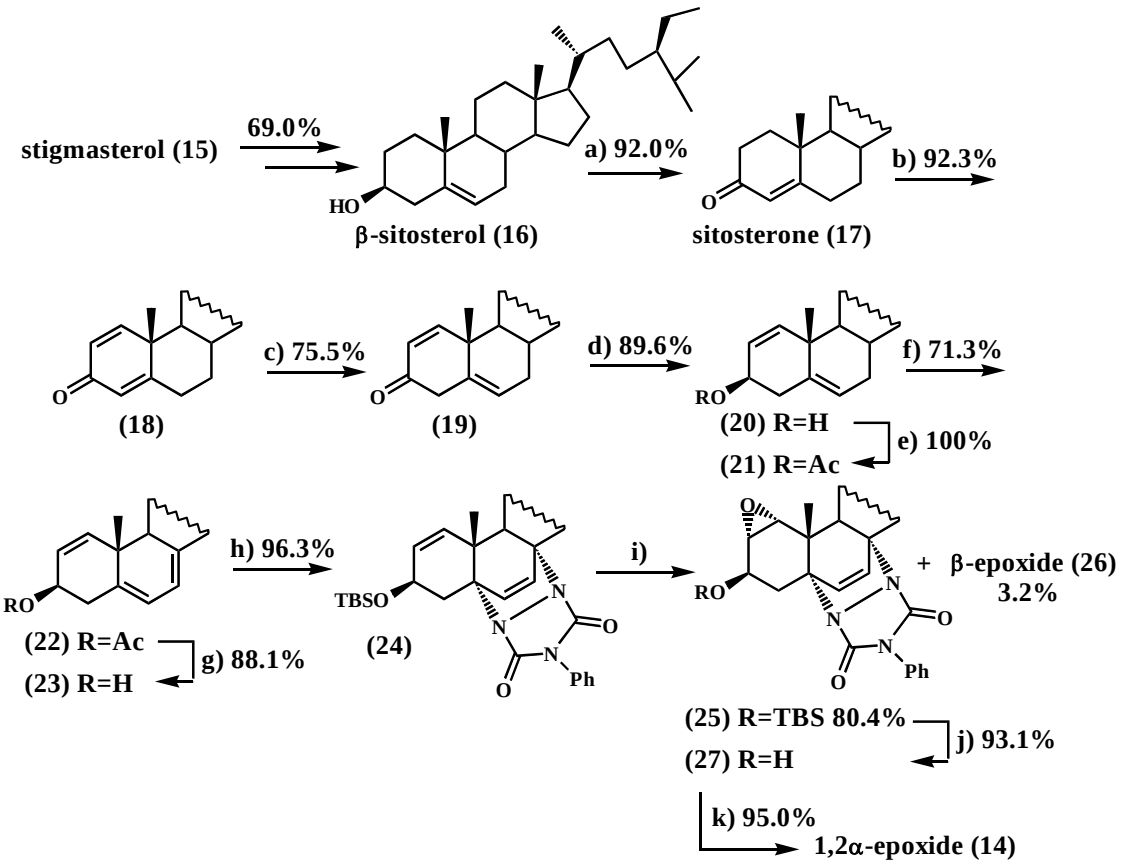
1α-hydroxyvitamin D_5 (13) **1,2α-epoxide (14)** **stigmasterol (15)**

Figure 2. Structures of 1α-hydroxyvitamin D_5 (**13**), a synthetic analog of vitamin D_5 (**5**), 1,2α-epoxide (**14**), a functional intermediate for the synthesis of **13** and related compounds, and stigmasterol (**15**)

SYNTHESIS OF 1,2α-EPOXIDE FROM STIGMASTEROL

Since commercially available β-sitosterol (**16**) (22,23-dihydrostigmasterol), extracted from wheat germ oil, contains inseparable impurities such as campesterol (20-30%) and dihydrobrassicasterol (10-30%), the purity of **16** is quite low (~50%).[21] We, therefore, prepared pure **16** from commercial 95% stigmasterol (**15**) by 5-step sequence (tosylation, methanolysis, hydrogenation, acetolysis, and hydrolysis as described the details in experimental section) *via* *i*-stigmasterol methyl ether, in 69.0% total yield, following Mosettig's procedure.[22,23] Thus, β-sitosterol (**16**) was oxidized with aluminium isopropoxide [Al(*i*-PrO)$_3$] and *N*-methyl-4-piperidone[24] in 92.0% yield to sitosterone (**17**), which was further oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to 1,4-dien-3-one (**18**) in 92.3% yield. Treatment of **18** with potassium *t*-butoxide (*t*-BuOK) gave 1,5-dien-3-one (**19**) in 75.5%

yield, which was reduced with sodium borohydride ($NaBH_4$) in the presence of cerium chloride ($CeCl_3$)[25] yielding 3β-hydroxy-1,5-diene (**20**) in 89.6% yield. After quantitative protection of the hydroxy moiety in **20** as the acetate **21**, the 5,7-diene system in **22** (provitamin D framework) was obtained by bromination with *N*-bromosuccinimide (NBS) and dehydrobromination with γ-collidine in 71.3% yield. Deacetylation of **22** afforded 3β-hydroxy-1,5,7-triene (**23**) in 88.1% yield. The 5,7-diene moiety in **23** was protected by forming an adduct with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) to give the PTAD adduct (**24**) after protection of hydroxy part as the *t*-butyldimethylsilyl (TBS) ether in 96.3% yield. Epoxidation reaction of **24** with *m*-chroloperbenzoic acid (MCPBA) occurred predominantly from the less congested α-face to afford α-epoxide (**25**) in 80.4% yield accompanied with β-epoxide (**26**) (3.2% yield), which were readily separated by silica gel column chromatography. After deprotection of TBS group in **25** (93.1% yield), the retro-cycloaddition reaction of the PTAD adduct (**27**) to regenerate the 5,7-diene system was carried out by heating (140 °C) **27** in 1,3-dimethyl-2-imidazolidinone (DMI)[26] giving rise to 1,2α-epoxide (**14**) in 95.0% yield (Scheme 2).



Scheme 2. Synthesis of 1,2α-epoxide (**14**) from stigmasterol (**15**) Reagents and conditions: a) *N*-methyl-4-piperidone/toluene, Al(*i*-PrO)$_3$, reflux. b) DDQ/dioxane, rt. c) *t*-BuOK/THF, rt. d) $CeCl_3 \cdot 7H_2O$/MeOH/$NaBH_4$/CH_2Cl_2, -5 °C. e) Ac_2O/pyridine, rt. f) NBS/hexane, reflux then γ-collidine/xylene, reflux. g) $LiAlH_4$/THF, -5 °C. h) PTAD/CH_2Cl_2, rt then TBSCl/imidazole/DMF, rt. i) MCPBA/CH_2Cl_2, rt. j) TBAF/THF, rt. k) DMI, 140 °C.

Based on related chemistry, the preparation of **14** as a key intermediate allows for a potential reductive epoxy ring opening reaction with sodium borohydride or sodium borotritide, photoirradiation and subsequent thermal isomerization reaction provide 1α-hydroxyvitamin D_5 (**13**) and 2β-tritiated 1α-hydroxyvitamin D_5.[27] Also the nucleophilic cleavage of epoxide in **14** with alkoxy or alkyl groups would give the way to 2β-substituted 1α-hydroxyvitamin D_5 derivatives.[28,29] Further synthetic studies using **14** as a key intermediate will be conducted elsewhere.

CONCLUSION

Through the practical and facile 17-step sequence from inexpensive stigmasterol (**15**), 1,2α-epoxide (**14**) was obtained in 17% overall yield. Based on the epoxy ring in **14**, introduction of 1α-hydroxy moiety in **13** is possible. Since **14** could also serve as a key intermediate for the synthesis of related analogs of **13** the development of 1α-hydroxyvitamin D_5 (**13**) as a promising drug candidate for the treatment of cancer is now possible.

EXPERIMENTAL

All reactions with exception of hydrogenation were carried out under an atmosphere of dry nitrogen. Melting points (mp) were determined on a Yanako micro melting point apparatus and uncorrected. Infrared (IR) spectra were obtained using JASCO IR-700 and JASCO FT/IR-410 spectrometers. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on JEOL AL-400, Bruker Avance III HD400, and JEOL-JNM-ECA 600 spectrometers in $CDCl_3$ with tetramethylsilane as an internal reference. Mass (MS) spectra were measured with JEOL JMS-DX 303, JMS-700, and JMS-T 100 GC instruments. Optical rotation values were obtained with JASCO DIP-4 and P-2200 polarimeters.

β-Sitosterol (16)[22,23,30,31]: A mixture of **15** (5.0 g, 12.1 mmol) and *p*-TsCl (6.0 g, 31.5 mmol) in pyridine (70 mL) was stirred at room temperature for 24 h. The mixture was poured into cold saturated $NaHCO_3$. The resulting solid was collected by filtration, washed with H_2O, and dried in a vacuum oven at 60 °C. Recrystallization from acetone gave tosylate (6.267 g, 91.2%) as colorless crystals: mp 137-140 °C (lit.[22] mp 147-148 °C). $[\alpha]_D^{29}$ -47.91 (*c* 1.274, $CHCl_3$) (lit.[22] $[\alpha]_D$ -49 (*c* 1, $CHCl_3$)). ^{1}H NMR ($CDCl_3$): δ 7.79 (2H, d, *J*=8.3 Hz), 7.32 (2H, d, *J*=8.0 Hz), 5.32-5.28 (1H, m), 5.14 (1H, dd, *J*=15.2, 8.5 Hz), 5.01 (1H, dd, *J*=15.2, 8.6 Hz), 4.38-4.28 (1H, m), 2.48-2.38 (1H, m), 2.44 (3H, s), 2.27 (1H, ddd, *J*=13.2, 5.1, 1.8 Hz), 2.08-1.90 (3H, m), 1.85-1.78 (2H, m), 1.75-1.64 (2H, m), 1.01 (3H, d, *J*=6.6 Hz), 0.97 (3H, s), 0.85-0.78 (9H, m), 0.68 (3H, s). A mixture of KOAc (4.50 g, 45.9 mmol) and

the above-mentioned tosylate (4.45 g, 7.86 mmol) in dry MeOH (200 mL) was refluxed for 2 h. After removal of the solvent under reduced pressure, the residue was extracted with AcOEt, washed with H_2O, saturated aq. $NaHCO_3$, and saturated aq. NaCl, dried over K_2CO_3, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (16:1) gave *i*-stigmasteryl methyl ether[22,23] (3.011 g, 89.9%). ^{1}H-NMR ($CDCl_3$): δ 5.15 (1H. dd, *J*=15.2, 8.6 Hz), 5.01 (1H, dd, *J*=15.2, 8.6 Hz), 3.32 (3H, s), 2.77 (1H, br t, *J*=2.8 Hz), 2.10-2.01 (1H, m), 1.97 (1H, dt, *J*=12.5, 3.3 Hz), 1.89 (1H, dt, *J*=13.4, 3.0 Hz), 1.81-1.65 (3H, m), 1.03 (3H, s), 0.91-0.78 (9H, m), 0.85 (3H, d, J=6.4 Hz), 0.73 (3H, s), 0.65 (1H, br t, *J*=4.7 Hz), 0.43 (1H, dd, *J*=8.0, 5.1 Hz). The above-mentioned *i*-stigmasteryl methyl ether (3.011 g, 7.07 mmol) in 95% EtOH (75 mL) was hydrogenated in the presence of 10% Pd/C (500 mg) at room temperature. After the absorption of equimolar H_2, the insoluble material was filtered off. The filtrate was concentrated under reduced pressure to give practically pure 22,23-dihydrostigmasteryl methyl ether[22] (2.959 g, 97.8%) as a colorless foam which was used without further purification. IR (film): ν 3058, 2956, 2868, 1464, 1382, 1201, 1183, 1098, 1015 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.32 (3H, s), 2.77 (1H, t, *J*=2.6 Hz), 1.99 (1H, dt, *J*=12.5, 3.2 Hz), 1.90 (1H, dt, *J*=13.5, 2.9 Hz), 1.02 (3H, s), 0.96-0.79 (13H, m), 0.91 (3H, d, *J*=6.9 Hz), 0.72 (3H, s), 0.65 (1H, br t, *J*=4.5 Hz), 0.43 (1H, dd, *J*=8.0, 5.1Hz). A mixture of the above-mentioned 22,23-dihydrostigmasteryl methyl ether (4.496 g, 10.5 mmol) and freshly fused $Zn(OAc)_2$ (9.25 g, 50.4 mmol) in AcOH (200 mL) was refluxed for 2 h, diluted with H_2O, cooled to 0 °C, and filtered to give practically pure 22,23-dihydrostigmasteryl acetate (4.440 g, 91.9%) as colorless crystals. Recrystallization from acetone/MeOH gave analytically pure 22,23-dihydrostigmasteryl acetate[22,32] as colorless crystals: mp 120-121 °C (lit.[22,32] mp 121-122 °C). $[\alpha]_D^{26}$ -39.94 (*c* 1.01, $CHCl_3$) (lit.[22] $[\alpha]_D$ -37.5 (*c* 1, $CHCl_3$)). IR (film): ν 2938, 2868, 1733, 1464, 1373, 1244, 1033, 796, 736 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.37 (1H, br d, *J*=4.8 Hz), 4.65-4.55 (1H, m), 2.34-2.29 (2H, m), 2.05-1.93 (2H, m), 2.03 (3H, s), 1.90-1.78 (3H, m), 1.02 (3H, s), 0.92 (3H, d, *J*=6.5 Hz), 0.88-0.80 (9H, m), 0.68 (3H, s). A mixture of the above-mentioned 22,23-dihydrostigmasteryl acetate (2.943 g, 6.45 mmol) and KOH (1.848 g, 33 mmol) in MeOH (60 mL) and Et_2O (60 mL) was stirred at room temperature for 15 h. After removal of the solvent under reduced pressure, the residue was extracted with AcOEt, washed with H_2O and saturated aq. NaCl, dried over $MgSO_4$ and evaporated to give **16** (2.499 g, 93.6%) as colorless crystals. Recrystallization from acetone/MeOH gave analytically pure **16** as colorless crystals: mp 136-138 °C (lit.[22] mp 137.5-138 °C, lit.[23] mp 135.5-136 °C, lit.[30] mp 136.5-138 °C, lit.[31] mp 139-140 °C). $[\alpha]_D^{27}$ -33.19 (*c* 1.002, $CHCl_3$) (lit.[22] $[\alpha]_D$ -33 (*c* 1, $CHCl_3$), lit.[23] $[\alpha]_D^{24}$ -34.3 (*c* 1, $CHCl_3$)). ^{1}H-NMR ($CDCl_3$): δ 5.37-5.34 (1H, m), 3.57-3.48 (1H, m), 2.33-2.19 (2H, m), 2.04-1.94 (2H, m), 1.89-1.79 (3H, m), 1.01 (3H, s), 0.92 (3H, d, *J*=7.6 Hz), 0.88-0.80 (9H, m), 0.68 (3H, s).

Sitosterone (17)[33]: A mixture of **16** (2.142 g, 5.18 mmol) and *N*-methyl-4-piperidone (10.8 mL, 88.1 mmol) in toluene (96 mL) was refluxed for 1 h. To the stirred mixture, was added Al(*i*-PrO)$_3$ (3.17 g, 15.5 mmol). The resulting mixture was refluxed for 6 h, cooled, diluted with AcOEt, washed with 5% HCl and saturated NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (4:1) gave **17** (1.964 g, 92.0%) as colorless crystals. Recrystallization from acetone gave analytically pure **17** as colorless crystals: mp 88-89 °C (lit.[33] mp 88.8-90 °C). $[\alpha]_D^{28}$ +84.77 (*c* 2.008, $CHCl_3$) (lit.[33] $[\alpha]_D^{25}$ +82 (*c* 0.650, $CHCl_3$)). ^{1}H-NMR ($CDCl_3$): δ 5.72 (1H, s), 2.46-2.24 (4H, m), 2.06-1.99 (2H, m), 1.90-1.82 (2H, m), 1.18 (3H, s), 0.92 (3H, d, *J*=6.4 Hz), 0.85 (3H, t, *J*=7.3 Hz), 0.84 (3H, d, *J*=6.9 Hz), 0.81 (3H, d, *J*=6.9 Hz), 0.71 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 199.65 (s), 171.71 (s), 123.82 (d), 56.09 (d), 55.97 (d), 54.00 (d), 45.91 (d), 42.47 (s), 39.71 (t), 38.67 (s), 36.19 (d), 35.78 (t), 35.71 (t), 34.06 (t), 33.96 (t), 33.02 (t), 32.13 (t), 29.23 (d), 28.27 (t), 26.16 (d), 24.26 (t), 23.15 (t), 21.11 (t), 19.90 (q), 19.11 (q), 18.79 (q), 17.47 (q), 12.03 (q).

Stigmasta-1,4-dien-3-one (18): A mixture of **17** (2.509 g, 6.09 mmol) and DDQ (2.074 g, 9.13 mmol) in dioxane (60 mL) was refluxed for 19 h and cooled to room temperature. The insoluble material was filtered off. The filtrate was diluted with AcOEt, washed with saturated aq. $NaHCO_3$ and saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (8:1) gave **18** (2.305 g, 92.3%) as a pale yellow solid. IR (film): ν 2957, 1716, 1660, 1602, 1463, 1383, 1293, 1215, 1103, 889, 808, 756 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.07 (1H, d, *J*=10.1 Hz), 6.23 (1H, dd, *J*=10.1, 1.8 Hz), 6.08 (1H, s), 2.53-2.42 (1H, m), 2.39-2.32 (1H, m), 2.04 (1H, dt, *J*=12.8, 3.4 Hz), 1.98-1.92 (1H, m), 1.91-1.80 (2H, m), 1.23 (3H, s), 0.91 (3H, d, *J*=6.5 Hz), 0.87-0.79 (9H, m), 0.74 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 186.51 (s), 169.54 (s), 156.10 (d), 127.51 (d), 123.84 (d), 56.07 (d), 55.55 (d), 52.49 (d), 45.90 (d), 43.73 (s), 42.74 (s), 39.58 (t), 36.17 (d), 35.62 (d), 33.92 (t), 33.78 (t), 33.02 (t), 29.23 (d), 28.22 (t), 26.17 (t), 24.47 (t), 23.14 (t), 22.94 (t), 19.90 (q), 19.10 (q), 18.78 (q), 18.74 (q), 12.12 (q), 12.05 (q). MS *m/z*: 410 (M^+), 275, 269, 227, 173, 147, 122 (100%). HRMS calcd for $C_{29}H_{46}O$ 410.3549, found 410.3530.

Stigmasta-1,5-dien-3-one (19): To a stirred solution of **18** (381 mg, 0.93 mmol) in dry THF (15 mL), was added *t*-BuOK (521 mg, 4.65 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 h, poured into an ice-cold mixture of AcOH (2.5 mL) and H_2O (10 mL), extracted with AcOEt, washed with saturated aq. $NaHCO_3$ and saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (8:1) gave **19** (288 mg, 75.5%) as a solid. Recrystallization from acetone/MeOH gave analytically pure **19** as colorless flakes: mp 142-144 °C. $[\alpha]_D^{27}$ +67.75 (*c* 0.996, $CHCl_3$). IR (film): ν 3018, 2959, 2870, 1686, 1462, 1383, 1261, 1216, 1106, 1024, 843, 758 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 6.99 (1H, d, *J*=10.2 Hz), 5.88 (1H, d,

J=10.1 Hz), 5.44 (1H, m), 3.36 (1H, br dd, *J*=17.4, 2.6 Hz), 2.91 (1H, d, *J*=17.3 Hz), 2.12-2.02 (2H, m), 1.22 (3H, s), 0.94 (3H, d, *J*=6.5 Hz), 0.88-0.80 (9H, m), 0.73 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 198.59 (s), 156.43 (d), 135.80 (s), 126.47 (d), 123.69 (d), 56.76 (d), 55.98 (d), 45.84 (d), 45.58 (d), 45.36 (t), 42.37 (s), 39.98 (s), 39.53 (t), 36.18 (d), 33.92 (t), 31.92 (d), 31.18 (t), 29.15 (d), 28.27 (t), 26.04 (t), 24.21 (t), 23.09 (t), 21.01 (t), 19.90 (q), 19.28 (q), 19.08 (q), 18.82 (q), 12.04 (q), 12.01 (q). MS *m/z*: 410 (M^+, 100%), 395, 382, 275, 269, 227, 173, 147, 135, 122, 95. *Anal*. Calcd for $C_{29}H_{46}O$: C, 84.81; H, 11.29. Found: C, 84.35; H, 11.14.

3β-Hydroxystigmasta-1,5-diene (20): To a stirred solution of $CeCl_3 7H_2O$ (782 mg, 2.1 mmol) in MeOH (20 mL), was added **19** (715 mg, 1.74 mmol) in CH_2Cl_2 (15 mL) at -5 °C. To the mixture, was added $NaBH_4$ (79 mg, 2.1 mmol) portionwise at the same temperature. The resulting mixture was stirred at the same temperature for 1.5 h, quenched by addition with acetone, concentrated under reduced pressure, extracted with AcOEt, washed with H_2O and saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (4:1) gave **20** (642 mg, 89.6%) as a colorless solid. Recrystallization from acetone/MeOH gave analytically pure **20** as colorless flakes: mp 146-147 °C. $[\alpha]_D^{27}$ -2.72 (*c* 0.776, $CHCl_3$). IR ($CHCl_3$): ν 3438, 3032, 2960, 2870, 1643, 1464, 1382, 1219, 1038, 1021 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.79 (1H, dd, *J*=10.1, 1.9 Hz), 5.54 (1H, br d, *J*=10.0 Hz), 5.43-5.40 (1H, m), 4.21 (1H, br), 2.47 (1H, ddd, *J*=12.0, 6.0, 1.3 Hz), 2.35-2.26 (1H, m), 2.07-1.99 (2H, m), 1.90-1.80 (1H, m), 1.09 (3H, s), 0.92 (3H, d, *J*=6.5 Hz), 0.84 (3H, t, *J*=7.0 Hz), 0.83 (3H, d, *J*=7.0 Hz), 0.81 (3H, d, *J*=6.9 Hz), 0.70 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 138.87 (s), 136.64 (d), 129.27 (d), 121.99 (d), 69.90 (d), 56.90 (d), 56.06 (d), 46.80 (d), 45.86 (d), 42.38 (s), 40.51 (t), 39.73 (t), 38.57 (s), 36.22 (d), 33.97 (t), 31.81 (d), 31.36 (t), 29.17 (d), 28.30 (t), 26.08 (t), 24.28 (t), 23.11 (t), 21.93 (q), 21.09 (t), 19.92 (q), 19.09 (q), 18.83 (q), 12.05 (q), 12.02 (q). MS *m/z*: 412 (M^+), 397, 394, 383 (100%), 379, 342, 275, 253, 211, 143, 135, 118, 95. *Anal*. Calcd for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found: C, 84.12; H, 11.73.

3β-Acetoxystigmasta-1,5-diene (21): A mixture of **20** (458 mg, 1.11 mmol) and Ac_2O (1 mL) in pyridine (2 mL) was stirred at room temperature for 15 h, poured into H_2O, extracted with AcOEt, washed with 10% HCl, saturated aq. $NaHCO_3$, and saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (7:1) gave **21** (503 mg, 100%) as a colorless solid. Recrystallization from acetone/MeOH gave analytically pure **21** as colorless flakes: mp 58.5-60 °C. $[\alpha]_D^{28}$ +21.54 (*c* 0.886, $CHCl_3$). IR (film): ν 3028, 2959, 2871, 1739, 1461, 1368, 1237, 1024 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.87 (1H, dd, *J*=10.2, 1.9 Hz), 5.48-5.44 (2H, m), 5.28-5.22 (1H, m), 2.51 (1H, ddd, *J*=12.0, 6.2, 1.3 Hz), 2.42-2.34 (1H, m), 2.07 (3H, s), 2.06-1.98 (2H, m), 1.88-1.79 (1H, m), 1.10 (3H, s), 0.92 (3H, d, *J*=6.6 Hz), 0.88-0.79 (9H, m), 0.70 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 170.84

(s), 138.14 (d), 137.79 (s), 125.17 (d), 123.09 (d), 72.17 (d), 56.85 (d), 56.04 (d), 46.63 (d), 45.87 (d), 42.38 (s), 39.69 (t), 38.61 (s), 36.23 (d), 35.97 (t), 33.97 (t), 31.74 (d), 31.38 (t), 29.18 (d), 28.30 (t), 26.08 (t), 24.27 (t), 23.11 (t), 21.70 (q), 21.48 (q), 21.07 (t), 19.92 (q), 19.10 (q), 18.83 (q), 12.06 (q), 12.02 (q). MS *m/z*: 454 (M^+), 439, 394, 379, 275, 211, 143, 135, 118 (100%). *Anal*. Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.83; H, 11.10.

3β-Acetoxystigmasta-1,5,7-triene (22): A mixture of **21** (4.585 g, 10.1 mmol) and NBS (2.51 g, 14.1 mmol) in hexane (100 mL) was refluxed for 1.25 h. After cooling to room temperature, the insoluble material was filtered off. The filtrate was concentrated under reduced pressure. The residue and γ-collidine (13 mL) were dissolved in xylene (90 mL). The resulting mixture was refluxed for 1.5 h, cooled to room temperature, and diluted with toluene and H_2O. The organic layer was washed with saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (10:1) gave **22** (3.253 g, 71.3%) as a pale yellow semi-solid, which was used without further purification. IR ($CHCl_3$): ν 2960, 2872, 1728, 1464, 1373, 1248, 1220, 1217, 1025, 843 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.80 (1H, dd, *J*=10.2, 1.8 Hz), 5.70 (1H, br d, *J*=4.0 Hz), 5.55 (1H, d, *J*=10.2 Hz), 5.48-5.44 (1H, m), 5.37-5.29 (1H, m), 2.62 (1H, ddd, *J*=12.6, 5.6, 1.0 Hz), 2.36 (1H, br t, *J*=11.2 Hz), 2.08 (3H, s), 1.01 (3H, s), 0.95 (3H, d, *J*=6.4 Hz), 0.88-0.80 (9H, m), 0.63 (3H, s).

3β-Hydroxystigmasta-1,5,7-triene (23): To a stirred mixture of $LiAlH_4$ (118 mg, 3.11 mmol) in THF (20 mL), was added **22** (1.125 g, 2.49 mmol) in THF (10 mL) dropwise at -5 °C. The resulting mixture was stirred at the same temperature for 1 h and at room temperature for 1 h, quenched with NH_4OH at -5 °C, extracted with AcOEt, washed with saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (4:1) gave **23** (899 mg, 88.1%) as a colorless solid. Recrystallization from acetone/MeOH gave analytically pure **23** as colorless crystals: mp 142-144 °C. $[\alpha]_D^{26}$ -110.23 (*c* 0.584, $CHCl_3$). IR (film): ν 3347, 3023, 2928, 1643, 1455, 1383, 1319, 1216, 1153, 1064, 1041, 833 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.73 (1H, dd, *J*=10.2, 1.9 Hz), 5.69 (1H, dd, *J*=5.4, 1.9 Hz), 5.63 (1H, d, *J*=10.1 Hz), 5.48-5.44 (1H, m), 4.35-4.27 (1H, br), 2.59 (1H, ddd, *J*=12.6, 5.6, 1.3 Hz), 2.27 (1H, br t, *J*=10.8 Hz), 2.14-2.08 (1H, m), 1.01 (3H, s), 0.95 (3H, d, *J*=6.5 Hz), 0.88-0.80 (9H, m), 0.63 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 143.06 (s), 137.94 (s), 136.42 (d), 130.16 (d), 119.58 (d), 116.85 (d), 68.98 (d), 55.84 (d), 54.83 (d), 45.91 (d), 43.27 (d), 42.87 (s), 39.59 (t), 39.05 (t), 38.59 (s), 36.58 (d), 33.95 (t), 29.22 (d), 28.15 (t), 26.18 (t), 23.17 (t), 23.14 (t), 21.00 (t), 19.93 (q), 19.11 (q), 18.99 (q), 18.22 (q), 12.07 (q), 11.92 (q). MS *m/z*: 410 (M^+), 392, 377, 367, 251, 209, 181, 141 (100%). HRMS calcd for $C_{29}H_{46}O$ 410.3549, found 410.3532.

PTAD adduct of 3β-*tert*-butyldimethylsilyloxystigmasta-1,5,7-triene (24): To a stirred solution of **23** (1.168 g, 2.85 mmol) in CH_2Cl_2 (30 mL), was added PTAD (508 mg, 2.90 mmol) at room temperature.

The resulting mixture was stirred at the same temperature for 1 h and concentrated under reduced pressure. A mixture of the resulting residue, TBSCl (1.074 g, 7.13 mmol), and imidazole (1.163 g, 17.1 mmol) in DMF (25 mL) was stirred at room temperature for 15 h, poured into H_2O, extracted with AcOEt, washed with saturated aq. NaCl, dried over $MgSO_4$, and evaporated. The solid residue was purified by recrystallization from acetone/MeOH to give **24** (1.812 g, 91.0%) as colorless crystals: mp 180-182 °C. The mother liquid was further chromatographed on silica gel. Elution with hexane/AcOEt (12:1) gave additional **24** (0.105 g, 5.3%) as a colorless solid. $[\alpha]_D^{26}$ +6.41 (*c* 0.560, $CHCl_3$). IR ($CHCl_3$): ν 3012, 2958, 2932, 2858, 1748, 1690, 1504, 1470, 1408, 1308, 1256, 1067, 1050, 908, 837 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.47-7.37 (4H, m), 7.35-7.28 (1H, m), 6.45 (1H, d, *J*=8.3 Hz), 6.26 (1H, d, *J*=8.2 Hz), 5.71 (1H, d, *J*=10.0 Hz), 5.65 (1H, dd, *J*=9.9, 3.6 Hz), 5.00-4.94 (1H, m), 3.32 (1H, dd, *J*=14.8, 8.0 Hz), 2.50-2.38 (2H, m), 2.31 (1H, dd, *J*=14.8, 6.4 Hz), 2.11-1.97 (2H, m), 1.91 (1H, dd, *J*=12.8, 5.4 Hz), 1.10 (3H, s), 0.94 (3H, d, *J*=6.4 Hz), 0.89 (9H, s), 0.86-0.79 (12H, m), 0.10 (3H, s), 0.08 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 148.56 (s), 146.15 (s), 135.41 (d), 134.42 (d), 131.89 (s), 129.82 (d), 129.27 (d), 129.01 (d), 127.90 (d), 126.50 (d), 65.74 (s), 65.02 (d), 64.45 (s), 55.22 (d), 51.75 (d), 49.19 (d), 45.86 (d), 44.03 (s), 43.04 (s), 38.33 (t), 35.58 (d), 34.50 (t), 33.72 (t), 29.18 (d), 27.56 (t), 26.00 (q), 25.77 (t), 23.22 (t), 23.15 (t), 22.95 (t), 22.09 (d), 19.94 (q), 19.04 (q), 18.08 (s), 12.93 (q), 12.06 (q), -4.38 (q), -4.40 (q). MS *m/z*: 699 (M^+), 524, 509, 467, 392 (100%), 377, 251, 235, 223, 209, 195, 177, 155, 141, 119, 75. *Anal*. Calcd for $C_{43}H_{65}N_3O_3Si$: C, 73.77; H, 9.36; N, 6.00. Found: C, 73.68; H, 9.37; N, 5.98.

PTAD adduct of 1α,2α-epoxy-3β-*tert*-butyldimethylsilyloxystigmasta-5,7-diene (25): A mixture of **24** (2.754 g, 3.94 mmol) and MCPBA (1.700 g, 9.85 mmol) in CH_2Cl_2 (300 mL) was stirred at room temperature for 168 h, washed with 3% aq. $Na_2S_2O_3$, saturated aq. $NaHCO_3$, and saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (6:1) gave 1,2β-epoxide (**26**) (89 mg, 3.2%) and further elution with same solvent gave **25** (2.264 g, 80.4%) as a colorless solid. Recrystallization of **25** from acetone/MeOH gave analytically pure **25** as colorless crystals: mp 109-111 °C. $[\alpha]_D^{25}$ -55.42 (*c* 0.566, $CHCl_3$). IR (film): ν 2956, 1752, 1698, 1502, 1463, 1402, 1251, 1085, 838, 753 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.46-7.38 (4H, m), 7.33-7.28 (1H, m), 6.42 (1H, d, *J*=8.3 Hz), 6.20 (1H, d, *J*=8.2 Hz), 4.92 (1H, dd, *J*=8.4, 5.9 Hz), 3.24 (1H, dd, *J*=15.0, 8.6 Hz), 3.23 (1H, d, *J*=3.6 Hz), 3.14 (1H, d, *J*=3.7 Hz), 2.69 (1H, dd, *J*=12.4, 6.8 Hz), 2.35-2.23 (2H, m), 2.10-2.01 (2H, m), 1.96 (1H, dd, *J*=15.1, 5.8 Hz), 1.09 (3H, s), 0.94 (3H, d, *J*=6.3 Hz), 0.91 (9H, s), 0.86-0.82 (9H, m), 0.81 (3H, d, *J*=6.9 Hz), 0.12 (6H, s). ^{13}C-NMR ($CDCl_3$): δ 146.83 (s), 145.15 (s), 136.70 (d), 132.02 (s), 129.55 (d), 128.95 (d), 127.79 (d), 126.76 (d), 64.18 (s), 63.47 (d), 59.54 (s), 59.14 (d), 56.33 (d), 55.58 (d), 48.95 (d), 48.66 (d), 45.85 (d), 43.88 (s), 40.95 (s), 38.31 (t), 35.53 (d), 33.69 (t), 33.35 (t),

29.18 (d), 27.56 (t), 25.87 (q), 25.73 (d), 23.14 (t), 23.03 (t), 23.00 (t), 19.92 (q), 19.02 (q), 18.93 (q), 17.96 (s), 17.40 (q), 12.78 (q), 12.05 (q), -4.62 (q), -4.69 (q). MS *m/z*: 716 (M^+), 584, 539, 407 (100%), 353, 285, 265, 178, 171, 155, 143, 73. *Anal*. Calcd for $C_{43}H_{65}N_3O_4Si$: C, 72.12; H, 9.15; N, 5.87. Found: C, 72.00; H, 9.16; N, 5.81. **26**: IR (film): ν 2960, 2930, 1757, 1702, 1504, 1408, 1102, 1070, 838 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.45-7.37 (4H, m), 7.35-7.30 (1H, m), 6.38 (1H, d, *J*=8.4 Hz), 6.21 (1H, d, *J*=8.2 Hz), 5.00 (1H, ddd, *J*=9.5, 6.6, 2.9 Hz), 3.38 (1H, t, *J*=3.3 Hz), 3.12 (1H, d, *J*=3.8 Hz), 2.92 (1H, dd, *J*=13.9, 6.5 Hz), 2.59-2.49 (1H, m), 2.34 (1H, dd, *J*=12.3, 6.5 Hz), 1.99 (1H, dd, *J*=13.9, 9.5 Hz), 1.02 (3H, s), 0.96 (3H, d, *J*=6.4 Hz), 0.93 (9H, s), 0.86-0.79 (9H, m), 0.78 (3H, s), 0.16 (3H, s), 0.14 (3H, s).

PTAD adduct of 1α,2α-epoxy-3β-hydroxystigmasta-5,7-diene (27): A mixture of **25** (2.195 g, 3.07 mmol) and TBAF (1M solution in THF 15.4 mL, 15.4 mmol) in THF (15 mL) was stirred at room temperature for 2.5 h, diluted with AcOEt, washed with saturated aq. $NaHCO_3$ and saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (1:1) gave **27** (1.718 g, 93.1%) as a colorless solid. Recrystallization from AcOEt/hexane gave analytically pure **27** as a colorless powder: mp 191-193 °C. $[\alpha]_D^{25}$ -80.07 (*c* 0.747, $CHCl_3$). IR (film): ν 3279, 2958, 1748, 1694, 1601, 1502, 1456, 1403, 1308, 1240, 1218, 1139, 1079, 1035, 829, 790, 757 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.46-7.38 (4H, m), 7.33-7.28 (1H, m), 6.43 (1H, d, *J*=8.3 Hz), 6.21 (1H, d, *J*=8.3 Hz), 5.04 (1H, br dd, *J*=12.4, 6.8 Hz), 3.29-3.20 (3H, m), 2.68 (1H, dd, *J*=12.3, 6.8 Hz), 2.48-2.39 (1H, m, disappeared with D_2O), 2.38-2.24 (2H, m), 2.12-1.98 (3H, m), 1.09 (3H, s), 0.94 (3H, d, *J*=6.3 Hz), 0.87-0.79 (12H, m). ^{13}C-NMR ($CDCl_3$): δ 146.80 (s), 144.93 (s), 136.51 (d), 131.87 (s), 129.57 (d), 128.90 (d), 127.88 (d), 126.66 (d), 64.25 (s), 62.53 (d), 59.82 (s), 59.40 (d), 56.25 (d), 55.55 (d), 48.79 (d), 48.62 (d), 45.84 (d), 43.86 (s), 41.04 (s), 38.26 (t), 35.53 (d), 33.68 (t), 31.84 (t), 29.18 (d), 27.54 (t), 25.76 (t), 23.14 (t), 23.00 (t), 22.97 (t), 19.91 (q), 19.02 (q), 18.92 (q), 17.57 (q), 12.74 (q), 12.04 (q). MS *m/z*: 601 (M^+), 426, 407 (100%), 379, 283, 267, 178, 143. *Anal*. Calcd for $C_{37}H_{51}N_3O_4$: C, 73.84; H, 8.54; N, 6.98. Found: C, 73.78; H, 8.59; N, 6.95.

1α,2α-Epoxy-3β-hydroxystigmasta-5,7-diene (14): A solution of **27** (1.418 g, 2.36 mmol) in DMI (140 mL) was stirred at 140 °C for 2 h, concentrated under reduced pressure, diluted with AcOEt, washed with H_2O and saturated aq. NaCl, dried over $MgSO_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (2:1) gave **14** (955 mg, 95.0%) as a colorless solid. Recrystallization from acetone/MeOH gave analytically pure **14** as a colorless flakes: mp 132-133 °C. $[\alpha]_D^{24}$ -52.06 (*c* 0.832, $CHCl_3$). IR (film): ν 3377, 3002, 1609, 1464, 1377, 1216, 1060, 1044, 925, 840, 751 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.72 (br d, *J*=4.3 Hz), 5.42-5.35 (1H, m), 3.91 (1H, quint, *J*=5.1 Hz), 3.33 (1H, dd, *J*=3.6, 0.9 Hz), 3.05 (1H, d, *J*=3.6 Hz), 2.51-2.40 (2H, m), 2.28-2.20 (1H, m), 2.12 (1H, br dt, *J*=12.6, 3.7 Hz),

1.99-1.88 (3H, m, 1H disappeared with D_2O), 1.87-1.77 (2H, m), 1.74-1.62 (2H, m), 1.05 (3H, s), 0.96 (3H, d, *J*=6.5 Hz), 0.88-0.80 (9H, m), 0.64 (3H, s). ^{13}C-NMR ($CDCl_3$): δ 141.61 (s), 133.77 (s), 122.08 (d), 115.98 (d), 67.24 (d), 61.11 (d), 60.31 (d), 55.77 (d), 54.56 (d), 45.89 (d), 42.77 (s), 39.80 (d), 38.86 (t), 38.45 (s), 36.95 (t), 36.58 (d), 33.93 (t), 29.21 (d), 28.11 (t), 26.17 (t), 23.13 (t), 23.08 (t), 20.72 (t), 19.91 (q), 19.10 (q), 18.98 (q), 15.28 (q), 12.05 (q), 11.96 (q). MS *m/z*: 426 (M^+, 100%), 408, 393, 366, 267, 243, 231, 225, 213, 172, 157, 143. HRMS calcd for $C_{29}H_{46}O_2$ 426.3497, found 426.3511.

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