PRACTICAL AND FACILE ROUTE TO A FUNCTIONAL INTERMEDIATE FROM STIGMASTEROL FOR THE SYNTHESIS OF 1α-HYDROXYVITAMIN D₃ AND RELATED COMPOUNDS*₁

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Abstract – As a functional and versatile intermediate for the synthesis of 1α-hydroxyvitamin D₃ 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 D₂ (2) (ergocalciferol) to vitamin D₇ (7) and are distinguished by their differing side chains (Figure 1).² Among them it is well-established that vitamin D₃ (3) (cholecalciferol) ingested into foods or synthesized in the skin is metabolized to 25-hydroxyvitamin D₃ (8) (calcifediol) in the liver, which is further hydroxylated at the 1α-position in the kidney to produce the active form, 1α,25-dihydroxyvitamin D₃ (9) (calcitriol) (Scheme 1).³ 1α,25-Dihydroxyvitamin D₃ (9) is well recognized as a potent regulator of calcium and

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

*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. 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. 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).
13 reported in a literature,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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).

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{structures.png}
\caption{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)}
\end{figure}

**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%).\textsuperscript{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 \textit{i}-stigmasterol methyl ether, in 69.0% total yield, following Mosettig’s procedure.\textsuperscript{22,23} Thus, β-sitosterol (16) was oxidized with aluminium isopropoxide [Al(i-PrO)$_3$] and \textit{N}-methyl-4-piperidone\textsuperscript{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 \textit{t}-butoxide (\textit{t}-BuOK) gave 1,5-dien-3-one (19) in 75.5%
yield, which was reduced with sodium borohydride (NaBH₄) in the presence of cerium chloride (CeCl₃)⁵⁵ 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-chloroperbenzoic 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) CeCl₃·7H₂O/MeOH/NaBH₄/CH₂Cl₂, -5 °C. e) Ac₂O/pyridine, rt. f) NBS/hexane, reflux then γ-collidine/xylene, reflux. g) LiAlH₄/THF, -5 °C. h) PTAD/CH₂Cl₂, rt then TBSCl/imidazole/DMF, rt. i) MCPBA/CH₂Cl₂, rt. j) TBAF/THF, rt. k) DMI, 140 °C.
Based on related chemistry, the preparation of \textbf{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 1\(\alpha\)-hydroxyvitamin D\(\gamma\) (13) and 2\(\beta\)-tritiated 1\(\alpha\)-hydroxyvitamin D\(\gamma\).\(^{27}\) Also the nucleophilic cleavage of epoxide in \textbf{14} with alkoxy or alkyl groups would give the way to 2\(\beta\)-substituted 1\(\alpha\)-hydroxyvitamin D\(\gamma\) derivatives.\(^{28,29}\) Further synthetic studies using \textbf{14} as a key intermediate will be conducted elsewhere.

**CONCLUSION**

Through the practical and facile 17-step sequence from inexpensive stigmasterol (15), 1,2\(\alpha\)-epoxide (14) was obtained in 17\% overall yield. Based on the epoxy ring in \textbf{14}, introduction of 1\(\alpha\)-hydroxy moiety in 13 is possible. Since \textbf{14} could also serve as a key intermediate for the synthesis of related analogs of 13 the development of 1\(\alpha\)-hydroxyvitamin D\(\gamma\) (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.

\(\beta\)-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\(_2\)O, 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). [\(\alpha\)]\(_D\) -47.91 (c 1.274, CHCl\(_3\)) (lit.\(^{22}\) [\(\alpha\)]\(_D\) -49 (c 1, CHCl\(_3\)). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 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 (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 (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, 5.1 Hz).

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 as colorless crystals: mp 120-121 °C (lit. 122 °C). [α]_D^{26} -39.94 (c 1.01, CHCl_3) (lit. 122 °C, [α]_D^{26} -37.5 (c 1, CHCl_3)). IR (film): ν 3035, 2956, 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 120-121 °C (lit. 122 °C, mp 121-122 °C).

[α]_D^{27} -39.94 (c 1.01, CHCl_3) (lit. 122 °C, [α]_D^{27} -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 5.37-5.34 (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): 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 MgSO4, 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. mp 88.8-90 °C). $[\alpha]D_28^{28} +84.77$ (c 0.650, CHCl3) (lit. $[\alpha]D_25^{25}$ +82 (c 0.650, CHCl3)). $^1$H-NMR (CDCl3): δ 5.72 (1H, s), 2.46-2.24 (4H, m), 2.06-1.99 (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 (CDCl3): 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), 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. NaHCO3 and saturated aq. NaCl, dried over MgSO4, 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 (CDCl3): 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 (CDCl3): 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 C29H46O 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 H2O (10 mL), extracted with AcOEt, washed with saturated aq. NaHCO3 and saturated aq. NaCl, dried over MgSO4, 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. $[\alpha]D_27^{27} +67.75$ (c 0.996, CHCl3). IR (film): ν 3018, 2959, 2870, 1686, 1462, 1383, 1261, 1216, 1106, 1024, 843, 758 cm$^{-1}$. $^1$H-NMR (CDCl3): δ 6.99 (1H, d, $J$=10.2 Hz), 5.88 (1H, d,
$J=10.1 \text{ Hz}$), 5.44 (1H, m), 3.36 (1H, br dd, $J=6.5 \text{ Hz}$), 0.88-0.80 (9H, m), 0.73 (3H, s). $^{13}$C-NMR (CDCl$_3$): $\delta$ 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.

$\beta$-Hydroxystigmasta-1,5-diene (20): To a stirred solution of CeCl$_3$7H$_2$O (782 mg, 2.1 mmol) in MeOH (20 mL), was added $\mathbf{19}$ (715 mg, 1.74 mmol) in CH$_2$Cl$_2$ (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$_2$O and saturated aq. NaCl, dried over MgSO$_4$, evaporated, and chromatographed on silica gel. Elution with hexane/AcOEt (4:1) gave $\mathbf{20}$ (642 mg, 89.6%) as a colorless solid. Recrystallization from acetone/MeOH gave analytically pure $\mathbf{20}$ as colorless flakes: mp 146-147 °C. $\left[\alpha\right]_D^{27}$-2.72 (c 0.776, CHCl$_3$). IR (CHCl$_3$): $\nu$ 3438, 3032, 2960, 2870, 1643, 1464, 1382, 1219, 1038, 1021 cm$^{-1}$. $^1$H-NMR (CDCl$_3$): $\delta$ 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$_3$): $\delta$ 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$^+$, 100%), 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.

$\beta$-Acetoxystigmasta-1,5-diene (21): A mixture of $\mathbf{20}$ (458 mg, 1.11 mmol) and Ac$_2$O (1 mL) in pyridine (2 mL) was stirred at room temperature for 15 h, poured into H$_2$O, 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 $\mathbf{21}$ (503 mg, 100%) as a colorless solid. Recrystallization from acetone/MeOH gave analytically pure $\mathbf{21}$ as colorless flakes: mp 58.5-60 °C. $\left[\alpha\right]_D^{28}$+21.54 (c 0.886, CHCl$_3$). IR (film): $\nu$ 3028, 2959, 2871, 1739, 1461, 1368, 1237, 1024 cm$^{-1}$. $^1$H-NMR (CDCl$_3$): $\delta$ 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$_3$): $\delta$ 170.84...
3β-Acetoxy stigma – 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 H₂O. The organic layer was washed with saturated aq. NaCl, dried over MgSO₄, 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 (CHCl₃): δ 2960, 2872, 1728, 1464, 1373, 1248, 1220, 1217, 1025, 843 cm⁻¹. ¹H-NMR (CDCl₃): 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=6.5 Hz), 0.88-0.80 (9H, m), 0.63 (3H, s).

3β-Hydroxy stigma – 1,5,7-triene (23): To a stirred mixture of LiAlH₄ (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 NH₄OH at -5 °C, extracted with AcOEt, washed with saturated aq. NaCl, dried over MgSO₄, 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. [α]D²⁶ -110.23 (c 0.584, CHCl₃). IR (film): ν 3347, 3023, 2928, 1643, 1455, 1383, 1319, 1216, 1153, 1064, 1041, 833 cm⁻¹. ¹H-NMR (CDCl₃): 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). ¹³C-NMR (CDCl₃): 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 C₂⁹H₄₆O: 410.3549, found 410.3532.

PTAD adduct of 3β-tert-butyldimethylsilyloxy stigma – 1,5,7-triene (24): To a stirred solution of 23 (1.168 g, 2.85 mmol) in CH₂Cl₂ (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 H₂O, extracted with AcOEt, washed with saturated aq. NaCl, dried over MgSO₄, 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. 

\[ \alpha \] D₂₆ +6.41 (c 0.560, CHCl₃). IR (CHCl₃): \( \nu \) 3012, 2958, 2932, 2858, 1748, 1690, 1504, 1470, 1308, 1256, 1067, 1050, 908, 837 cm⁻¹. ¹H-NMR (CDCl₃): \( \delta \) 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). ¹³C-NMR (CDCl₃): \( \delta \) 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), 35.53 (d), 35.00 (d), 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 C₄₃H₆₅N₃O₃Si: 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-butyldimethylsilyloystigmasta-5,7-diene (25):** A mixture of 24 (2.754 g, 3.94 mmol) and MCPBA (1.700 g, 9.85 mmol) in CH₂Cl₂ (300 mL) was stirred at room temperature for 168 h, washed with 3% aq. Na₂S₂O₃, saturated aq. NaHCO₃, and saturated aq. NaCl, dried over MgSO₄, 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. \[ \alpha \] D₂₅ -55.42 (c 0.566, CHCl₃). IR (film): \( \nu \) 2956, 1752, 1698, 1502, 1463, 1402, 1251, 1085, 838, 753 cm⁻¹. ¹H-NMR (CDCl₃): \( \delta \) 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). ¹³C-NMR (CDCl₃): \( \delta \) 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), 32.76 (s), 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 C₄₃H₆₅N₃O₃Si: C, 73.77; H, 9.36; N, 6.00. Found: C, 73.68; H, 9.37; N, 5.98.
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_{3}O_{4}Si: C, 72.12; H, 9.15; N, 5.87. Found: C, 72.00; H, 9.16; N, 5.81.

26: IR (film): \( \nu \) 2960, 2930, 1757, 1702, 1504, 1408, 1102, 1070, 838 cm\(^{-1}\).

1H-NMR (CDCl\(_3\)): \( \delta \) 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): \( \nu \) 3279, 2958, 1748, 1694, 1601, 1456, 1403, 1308, 1240, 1218, 1139, 1079, 1035, 829, 790, 757 cm\(^{-1}\).

1H-NMR (CDCl\(_3\)): \( \delta \) 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\(_2\)O), 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). 13C-NMR (CDCl\(_3\)): \( \delta \) 146.80 (s), 144.93 (s), 136.51 (s), 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_{3}O_{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\(_2\)O 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): \( \nu \) 3377, 3002, 1609, 1464, 1377, 1216, 1060, 1044, 925, 840, 751 cm\(^{-1}\). 1H-NMR (CDCl\(_3\)): \( \delta \) 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$_2$O), 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$_3$): $\delta$ 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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REFERENCES AND NOTES
13. Information presented at the symposium organized by Vitamin D Workshop INC., ‘VITAMIN
D/CANCER MEETING. Cancer Chemoprevention & Cancer Treatment: Is there a role for vitamin D, 1α,25(OH)2-vitamin D3, or new analogs (deltanoids)?’ which was held November 17-19, 2004, in NIH Campus Bethesda, Maryland USA (Abstract p. 61).


