Synthesis of tritiated 1α,25-dihydroxy-22-oxavitamin D3[1]

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SUMMARY

Synthesis of two tritiated 1α,25-dihydroxy-22-oxavitamin D_3 (OCT), [26-$^3H_3$]OCT (**3**) and [2β-^{3}H]OCT (**4**), is described. [26-$^3H_3$]OCT (**3**) was prepared by tritiation at the side chain with tritiated methylmagnesium iodide and [2β-^{3}H]OCT (**4**) was labeled at the A-ring by tritiation with sodium borotritiide.

KEYWORDS: 1α,25-dihydroxy-22-oxavitamin D_3 (OCT), [26-$^3H_3$]OCT, [2β-^{3}H]OCT, sodium borotritiide, 1α,25-dihydroxyvitamin D_3

INTRODUCTION

Considerable attention has been focused on the synthesis of analogues of 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$] (**1**) aiming to separate the differentiation-inducing activity of human myeloid leukemia cells from the regulatory effect on calcium and phosphorous metabolism.[2] We have already reported that 1α,25-dihydroxy-22-oxavitamin D_3 (OCT) (**2**) shows potent *in vitro* differentiation-inducing activity with low *in vivo* calcemic liability.[3] OCT (**2**) is now being clinically investigated as a candidate for the treatment of secondary hyperparathyroidism[4] and psoriasis.[5]

During the course of our development of OCT (**2**), the synthesis of tritiated OCT was needed for pharmacokinetic and metabolic studies. In this paper we wish to describe the synthesis of two tritiated OCT at different positions, namely [26-$^3H_3$]OCT (**3**) tritiated at the

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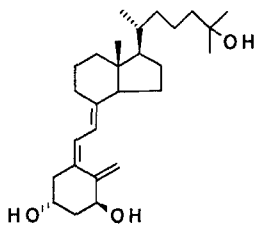
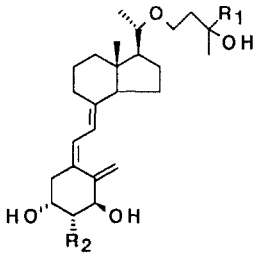
side chain of 2 with tritiated methylmagnesium iodide and [2β-^{3}H]OCT (4) labeled at the A-ring of 2 by tritiation with sodium borotritiide (NaB[$^3H_4$]) (Scheme 1).



(1); 1α,25(OH)$_2$D$_3$

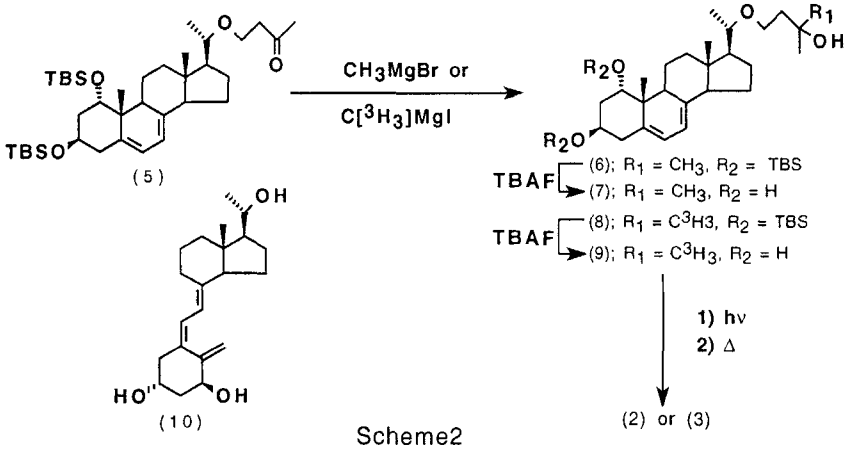


(2); R_1 = CH_3, R_2 = H: OCT
(3); R_1 = C^3H_3, R_2 = H: [26-^{3}H$_3$]OCT
(4); R_1 = CH_3, R_2 = ^{3}H: [2β-^{3}H]OCT

Scheme 1

SYNTHESIS

First, we undertook the synthesis of [26-^{3}H$_3$]OCT (3). Introduction of the C-26 methyl group was performed by alkylation of the known ketone (5).[3a] Thus, treatment of 5 with methylmagnesium bromide (CH_3MgBr) at -15°C gave the methylated derivative (6) in 79% yield, which was then desilylated to proOCT (7) in 85% yield. Subsequent irradiation of 7 with a high pressure mercury lamp through a Vycor filter, followed by thermal isomerization provided OCT (2) in 17% yield, as described before.[3b] A similar reaction of 5 with [^{3}H$_3$]methylmagnesium iodide (C[^{3}H$_3$]MgI) prepared from [^{3}H$_3$]methyl iodide (50Ci, at 85Ci/mmol) furnished the tritiated product (8) (5.4Ci, 80-81% purity). A similar desilylation



Scheme2

of **8** afforded **9** in 99% yield, which was transformed to [26-^{3}H$_3$]OCT (**3**) by irradiation and thermal isomerization in 29% yield, the specific activity of which was found to be 86.5Ci/mmol (Scheme 2).

In the preliminary *in vitro* metabolic studies of OCT (**2**), the major metabolite of OCT appeared to be pentanorOCT (**10**)[6] which lost the radioactivity of [26-^{3}H$_3$]OCT (**3**), however, [26-^{3}H$_3$]OCT (**3**) has been used as a tracer in a radioimmunoassay experiment of OCT (**2**)[7] due to its high specific activity .

Next, taking the metabolic loss of the tritiated methyl group into consideration, we turned our attention to the synthesis of OCT labeled at the A-ring. We chose [2β-^{3}H]OCT (**4**) as a target compound. To synthesize **4**, the α-epoxide (**27**) was needed for the introduction of tritium into the A-ring. The α-epoxide (**27**) was prepared from the alcohol (**11**) as follows;

a) Alkylation of the known alcohol (**11**)[8] with *N,N*-dimethylacrylamide[9] giving **12** in 86% yield

b) Methylation of **12** with MeMgBr/cerium(III) chloride ($CeCl_3$)[9] giving **13**

c) Methylation of **13** with MeMgBr/$CeCl_3$[9] giving **14** in 75% yield from **11**

d) Desilylation of **14** with tetrabutylammonium fluoride (TBAF) giving **15** quantitatively

e) Oppenauer oxidation of **15** giving **16** in 88% yield

f) Oxidation of **16** with dichlorodicyanoquinone (DDQ)[10] giving **17** in 63% yield

g) Deconjugation of **17** with sodium ethoxide (NaOEt)[11] giving **18** in 64% yield based upon the recovery of **17**

h) Reduction of **18** with sodium borohydride ($NaBH_4$)[11] giving **19** in 74% yield

i) Acetylation of **19** giving **20** in 88% yield

j) Bromination of **20** with N-bromosuccinimide (NBS)[11] and dehydrobromination with TBAF giving **21**

k) Deacetylation of **21** with lithium aluminum hydride ($LiAlH_4$) giving **22**

l) 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) addition to **22** giving **23** in 11% yield from **20**

m) Silylation of **23** giving **24** quantitatively

n) Epoxidation of **24** with *m*-chloroperbenzoic acid (MCPBA)[11] giving **25** in 71% yield

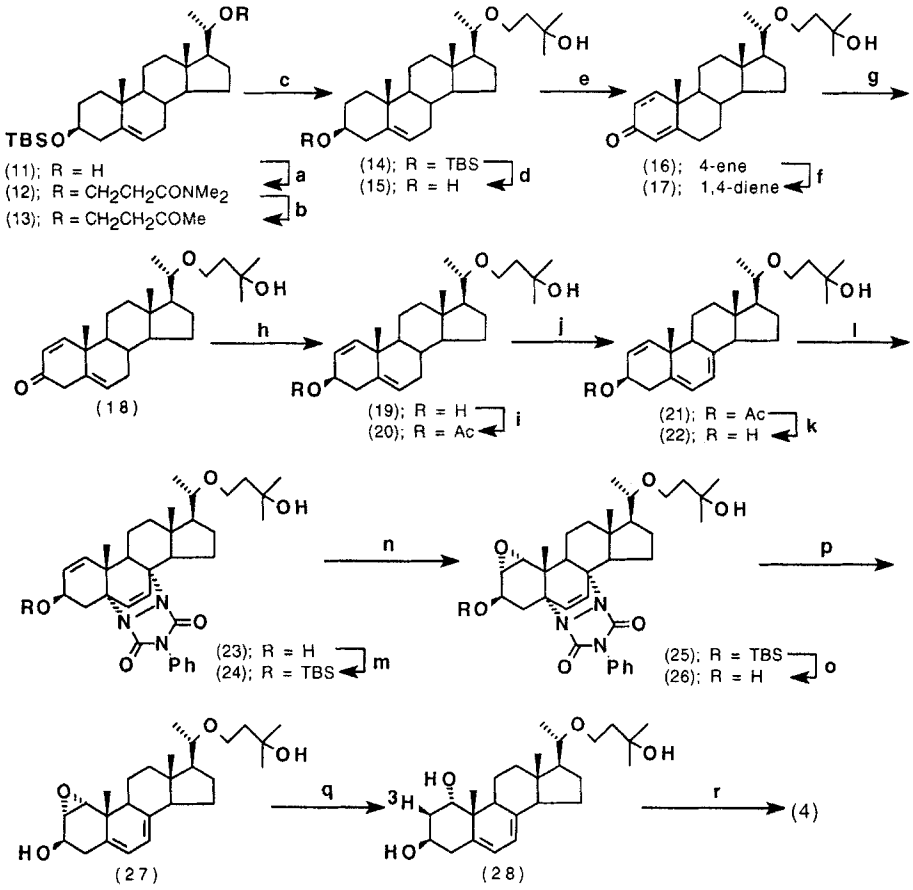
o) Desilylation of **25** with TBAF giving **26** in 93% yield

p) Retrocycloaddition[12] of **26** giving **27** in 84% yield.

As described before[13], α-epoxide (**27**) was successfully reduced with NaB[^{3}H$_4$] (2.0Ci, 50Ci/mmol) to tritiated proOCT (**28**) (103.5mCi) in 29% chemical yield and 21%

radiochemical yield. Irradiation of **28**, followed by thermal isomerization provided [2β-^{3}H]OCT (**4**) in 29% yield, whose specific activity was found to be 15.0Ci/mmol (Scheme 3).

Since the tritium label of [2β-^{3}H]OCT (**4**) is retained after *in vivo* administration, [2β-^{3}H]OCT (**4**) has been used in pharmacokinetic and metabolic studies although its specific activity is low compared to [26-$^3H_3$]OCT (**3**). The detailed results of pharmacokinetic and metabolic studies of OCT (2) will be reported elsewhere.



a) NaH, CH_2=CHCONMe$_2$; b) MeMgBr/$CeCl_3$; c) MeMgBr/$CeCl_3$; d) ^{n}Bu$_4$NF; e) Al(O^{i}Pr)$_3$, cyclohexanone; f) DDQ; g) NaOEt; h) $NaBH_4$; i) Ac_2O, pyridine; j) i: NBS, ii: ^{n}Bu$_4$NBr, iii: ^{n}Bu$_4$NF; k) $LiAlH_4$; l) PTAD; m) TBSCl, imidazole; n) MCPBA; o) ^{n}Bu$_4$NF; p) 140°C; q) NaB[$^3H_4$]; r) hv then heating

Scheme 3

EXPERIMENTAL

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded with a Hitachi 270-30 spectrometer, proton nuclear magnetic resonance (NMR) spectra with a JEOL FX-200, mass spectra (MS) with a Shimadzu GCMS QP-1000, high-resolution mass spectra (HR-MS) with a VG Auto Spec Q, and ultra violet (UV) spectra with a Shimadzu UV-240. The apparatus used for high-performance liquid chromatography (HPLC) was a Tosoh CCP with UV detector UV-8010 and RI detector RS-8000. HPLC was carried out on a YMC A-312 at a flow rate of 1ml/min with MeOH/H_2O (80:20). Radioactivity was measured with an Aloka LSC-900.

All reactions were carried out under an atmosphere of dry argon or nitrogen. Flash column chromatography was carried out with Merck Kieselgel 60, 230-400 mesh, and preparative thin layer chromatography (TLC) was performed on 20 × 20cm plates coated with a 0.25mm thickness of Merck Kieselgel 60 containing F_{254} indicator. NaB[$^3H_4$] was purchased from Amersham Japan (code No. TRK 838).

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-(3-hydroxy-3-methylbutyloxy)-pregna-5,7-diene (6)

To a stirred solution of the ketone (5) (73mg, 0.12mmol) in THF (6ml), was added CH_3MgBr (3M solution in Et_2O, 0.3ml, 0.9mmol) dropwise at -15°C. The mixture was then stirred at the same temperature for 1h and quenched by dropwise addition of aqueous NH_4Cl. The mixture was poured into aqueous NH_4Cl and extracted with $CHCl_3$. The extract was washed with saturated NaCl. The combined aqueous layer was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by preparative TLC developed with *n*-hexane/AcOEt (85:15) to give the alcohol (6) (59mg, 79%) which was identical (TLC, IR, NMR, MS and UV) with the authentic sample of 6.[3b]

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-20(*S*)-([4-$^3H_3$]-3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (8)

Mg powder (20mg) was taken in a 50ml two-necked round-bottomed flask fitted with a rubber-septum and tap-adapter and pumped to a hard vacuum in a manifold. Et_2O (10ml) was introduced into the flask and the mixture was frozen. [$^3H_3$]Methyl iodide (50Ci, 85Ci/mmol) was distilled into the flask and the mixture was warmed to 30°C for 0.5h. The mixture was then cooled to -15°C and the ketone (5) (100mg, 0.16mmol) in Et_2O was added. The reaction mixture was stirred at the same temperature for 2h, and quenched by dropwise addition of aqueous NH_4Cl. The mixture was poured into aqueous NH_4Cl and extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and evaporated to give crude 8 (8.3Ci, 58-68% purity). The crude product was purified by preparative TLC developed with *n*-hexane/AcOEt (85:15) to give practically pure 8 (5.4Ci, 80-81% purity) which was used without further purification. Analytically pure 8 was obtained by HPLC purification on an Ultrasphere ODS column eluted with EtOH/ H_2O (9:1) in 94-98% radiochemical purity.

1α,3β-Dihydroxy-20(*S*)-([4-$^3H_3$]-3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (9)

A solution of 8 (600mCi) and TBAF (1M solution in THF, 300μl, 300μmol) in THF (2.5ml) was refluxed mildly for 15h. The mixture was then diluted with AcOEt, washed with H_2O, 10% HCl, saturated $NaHCO_3$ and saturated NaCl, and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude 9, which was purified by preparative TLC developed with CH_2Cl_2/EtOH (10:1) to give 9 (594mCi, 99%).

1α,3β-Dihydroxy-20(*S*)-([4-$^3H_3$]-3-hydroxy-3-methylbutyloxy)-9,10-secopregna-5,7,10(19)-triene; [26-$^3H_3$]OCT (3)

A solution of 9 (594mCi) in EtOH (200ml) was irradiated using a 400W high pressure mercury lamp with a Vycor filter at 0°C for 1.5min. The mixture was then refluxed for 2h, and

concentrated *in vacuo*. The residue was purified by preparative TLC developed with CH_2Cl_2/EtOH (10:1) to give **3** (175mCi, 29%).

3β-(*tert*-Butyldimethylsilyloxy)-20(*S*)-(2-dimethylaminocarbonylethyloxy)-pregn-5-ene (12)

To a stirred mixture of NaH (10.0g, 250mmol) in THF (30ml) was added dropwise a solution of the alcohol (**11**) (94.6g, 250mmol) and *N,N*-dimethylacrylamide (51.6ml, 501mmol) in THF (250ml) at 0°C during 0.5h. The resulting mixture was then stirred at room temperature for 3h, poured into saturated NH_4Cl, extracted with AcOEt. The extract was washed with saturated NH_4Cl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with AcOEt as the eluant to give **12** (91.5g, 86%) as a colorless powder; mp. 141 - 142°C; IR (neat): 2955, 2930, 2890, 2855, 1645, 1630, 1250, 1085, 835cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.06 (6H, s), 0.64 (3H, s), 0.89 (9H, s), 0.99 (3H, s), 1.16 (3H, d, J = 6.3Hz), 2.42 - 2.69 (2H, m), 2.94 and 3.02 (total 6H, each s), 3.19 - 3.33 (1H, m), 3.38 - 3.59 (2H, m), 3.75 - 3.93 (1H, m), 5.31 (1H, brd, J = 4.4Hz); EI-MS (m/z): 531 (M^+), 358 (100%).

3β-(*tert*-Butyldimethylsilyloxy)-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregn-5-ene (14)

$CeCl_3 \cdot 7H_2O$ (212g, 568mmol) was heated at 250°C in dry oven for 1h, then at 140°C *in vacuo* (0.5mmHg) with stirring for 1h, and cooled to room temperature. THF (605ml) was added and stirring was continued for 0.5h. CH_3MgBr (3M solution in Et_2O, 172ml, 516mmol) was added at -20°C, and stirring was continued at the same temperature for 0.5h. To a resulting suspension, **12** (91.5g, 172mmol) was added during 20min at -20°C. The mixture was stirred at the same temperature for 30min, then poured into saturated NH_4Cl. The separated organic layer was dried over $MgSO_4$ and evaporated to give crude **13** (72.4g) as a colorless powder which was used without further purification.

Treatment of crude **13** (72.4g) with $CH_3MgBr/CeCl_3$ in THF prepared in the same scale and manner as described above, gave crude **14** (85.9g), which was washed with cold MeOH (400ml) to give practically pure **14** (64.4g, 72%) as a colorless powder. The analytically pure **14** was obtained as a colorless powder by flash column chromatography with *n*-hexane/AcOEt (1:1) as the eluant; mp. 171 - 172°C; IR (KBr): 3520, 2960, 2930, 1380, 1250, 1090, 835, 780cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.05 (6H, s), 0.66 (3H, s), 0.89 (9H, s), 0.99 (3H, s), 1.18 (3H, d, J = 6.1Hz), 1.23 (6H, s), 3.17 - 3.32 (1H, m), 3.40 - 3.55 (2H, m), 3.76 - 3.89 (1H, m), 5.27 - 5.34 (1H, m); EI-MS (m/z): 518 (M^+), 69 (100%); Anal. Calcd for $C_{32}H_{58}O_3Si$: C, 74.07; H, 11.27. Found: C 74.18; H, 11.52.

3β-Hydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregn-5-ene (15)

A mixture of **14** (67.0g, 129mmol) and TBAF (1M solution in THF, 645ml, 645mmol) was stirred at room temperature for 72h, and concentrated *in vacuo*. The residue was taken up with AcOEt, washed with 10% HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with CH_2Cl_2/EtOH (50:3) as the eluant to give **15** (52.9g, quantitatively) as a colorless powder; mp. 152.5 - 153.5°C; IR (KBr): 3350, 1465, 1440, 1370, 1150, 1085, 1050cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.67 (3H, s), 1.00 (3H, s), 1.19 (3H, d, J = 6.1Hz), 1.23 (6H, s), 3.18 - 3.32 (1H, m), 3.41 - 3.60 (2H, m), 3.71 - 3.90 (1H, m), 5.30 - 5.38 (1H, m); EI-MS (m/z): 404 (M^+), 69 (100%); HR-MS Calcd for $C_{26}H_{44}O_3$: 404.3290. Found: 404.3283.

20(*S*)-(3-Hydroxy-3-methylbutyloxy)pregn-4-ene-3-one (16)

A solution of **15** (52.9g, 131mmol) and cyclohexanone (150g, 1.53mol) in toluene (1.12l) was azeotropically refluxed for 3h. To the resulting reflux solution, was added aluminum isopropoxide (13.3g, 65.3mmol) dropwise during 0.5h. The mixture was refluxed for 2.5h, and concentrated *in vacuo*. The residue was taken up with AcOEt, washed with aqueous Rochelle Salt and saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (1:1) as the eluant to give **16** (46.0g, 88%) as a pale yellow powder; mp. 72 - 74°C; IR (neat): 3430, 1670, 1615, 1445, 1370,

1225, 1070cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.70 (3H, s), 1.18 (3H, d, J = 5.8Hz), 1.18(3H, s), 1.23 (6H, s), 3.23 - 3.31 (1H, m), 3.41 - 3.55 (1H, m), 3.74 - 3.91 (1H, m), 5.73 (1H, s); EI-MS (m/z): 402 (M^+), 69 (100%); HR-MS Calcd for $C_{26}H_{42}O_3$: 402.3134. Found: 402.3137.

20(*S*)-(3-Hydroxy-3-methylbutyloxy)pregna-1,4-diene-3-one (17)

To a stirred solution of **16** (46.0g, 114mmol) in dioxane (400ml), was added DDQ (37.9g, 160mmol). The mixture was refluxed for 3h, extracted with AcOEt, washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (1:1:9) as the eluant to give **17** (28.8g, 63%) as a yellow powder; mp. 96 - 98°C; IR (neat): 3450, 1670, 1620, 1605, 1450, 1375, 1155, 1085cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.73 (3H, s), 1.19 (3H, s), 1.20 (3H, d, J = 6.4Hz), 1.23 (6H, s), 3.23 - 3.35 (1H, m), 3.44 - 3.56 (1H, m), 3.78 - 3.92 (1H, m), 6.07 (1H, s), 6.23 (1H, d, J = 10.0Hz), 7.04 (1H, d, J = 10.0Hz); EI-MS (m/z): 400 (M^+), 69 (100%); HR-MS Calcd for $C_{26}H_{40}O_3$: 400.2977. Found: 400.2957.

20(*S*)-(3-Hydroxy-3-methylbutyloxy)pregna-1,5-diene-3-one (18)

Na (9.00g, 392mmol) was added in portions to EtOH (120ml). The mixture was stirred at room temperature for 1h, and concentrated *in vacuo*. The residue was taken up with DMSO (370ml) and stirred at room temperature for 0.5h. To this mixture, **17** (28.8g, 72.0mmol) in DMSO (400ml) was added and the stirring was continued at room temperature for 2h. The mixture was then poured into a mixture of AcOH (100ml) and H_2O (400ml), extracted with AcOEt, washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (3:2) as the eluant to give **18** (13.5g, 47%, 64% based on the recovery of **17**) as a pale yellow powder and recovered **17** (7.37g, 26%). **18**; IR (KBr): 3450, 1690, 1620, 1450, 1375, 1265, 1160, 1090cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.72 (3H, s), 1.18 (3H, s), 1.20 (3H, d, J = 6.6Hz), 1.23 (6H, s), 2.91 (1H, d, J = 17.2Hz), 3.20 - 3.32 (1H, m), 3.36 - 3.54 (1H, m), 3.76 - 3.90 (1H, m), 3.79 (1H, d, J = 17.2Hz), 5.44 (1H, brs), 5.88 (1H, d, J = 10.2Hz), 6.97 (1H, d, J = 10.2Hz); EI-MS (m/z): 400 (M^+), 69 (100%).

3β-Hydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-1,5-diene (19)

To a stirred solution of **18** (16.4g, 41.0mmol) in MeOH (445ml), was added $NaBH_4$ (3.89g, 103mmol) at -5°C. The mixture was stirred at the same temperature for 2h, quenched by addition of acetone, and concentrated *in vacuo*. The residue was taken up with CH_2Cl_2 and insoluble material was filtered off. The filtrate was washed with H_2O and saturated NaCl, dried over $MgSO_4$ and evaporated. The crystalline residue was recrystallized from AcOEt to give **19** (12.2g, 74%) as colorless needles; mp. 141 - 143°C; IR (KBr): 3525, 3300, 1665, 1450, 1380, 1370, 1155, 1090, 1050cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.68 (3H, s), 1.09 (3H, s), 1.19 (3H, d, J = 6.2Hz), 1.23 (6H, s), 3.18 - 3.32 (1H, m), 3.40 - 3.54 (1H, m), 3.75 - 3.90 (1H, m), 4.12 - 4.25 (1H, br), 5.38 (1H, brs), 5.52 (1H, d, J = 8.6Hz), 5.76 (1H, d, J = 8.6Hz); EI-MS (m/z): 402 (M^+), 69 (100%); HR-MS Calcd for $C_{26}H_{42}O_3$: 402.3134. Found: 402.3133.

3β-Acetoxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-1,5-diene (20)

To a stirred solution of **19** (3.55g, 8.82mmol) in pyridine (80ml), was added Ac_2O (40ml) at 0°C. The mixture was stirred at the same temperature for 8.5h, poured into H_2O, acidified with diluted HCl and extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (3:1) as the eluant to give **20** (3.46g, 86%) as a pale yellow powder; mp. 72 - 74°C; IR (KBr): 3520, 1730, 1375, 1260, 1240, 1090, 1040cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.69 (3H, s), 1.10 (3H, s), 1.19 (3H, d, J = 6.0Hz), 1.23 (6H, s), 3.20 - 3.35 (1H, m), 3.42 - 3.56 (1H, m), 3.74 - 3.92 (1H, m), 5.16 - 5.31 (1H, m), 5.43 (1H, d, J = 10.0Hz), 5.44 (1H, brs), 5.86 (1H, d, J = 10.0Hz); Anal. Calcd for $C_{28}H_{44}O_4 \cdot H_2O$: C, 72.69; H, 10.02. Found: C 72.76; H, 9.69.

PTAD Adduct of 3β-Hydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-1,5,7-triene (23)

A mixture of **20** (3.45g, 7.76mmol) and NBS (1.73g, 9.72mmol) in CCl_4 (100ml) was refluxed for 1.5h. The insoluble material was filtered off and the filtrate was concentrated *in vacuo*. To the stirred residue in THF (80ml), was added tetrabutylammonium bromide (203mg, 0.78mmol) at 0°C and the resulting mixture was stirred at the same temperature for 1h. TBAF (1M solution in THF, 19.4ml, 19.4mmol) was added to the mixture and the stirring was continued at the same temperature for 1h. The mixture was poured into H_2O, extracted with AcOEt, washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (4:1) as the eluant to give crude **21**, which was used without further purification.

To a stirred mixture of $LiAlH_4$ (110mg, 2.90mmol) in THF, was added crude **21** (1.29g, 2.91mmol) in THF (25ml) dropwise at 0°C. The stirring was continued at 0°C for 0.75h, at room temperature for 1h, and with additional $LiAlH_4$ (110mg, 2.90mmol) at room temperature for 1h. The mixture was quenched by addition of 3M NaOH at 0°C, poured into aqueous Rochelle Salt, extracted with AcOEt, washed with saturated NaCl, and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **22**, which was dissolved in CH_2Cl_2 (40ml). PTAD (510mg, 2.91g) in CH_2Cl_2 (10ml) was added and the stirring was continued at room temperature for 1h. Removal of the solvent *in vacuo* left crude **23**, which was purified by flash column chromatography with *n*-hexane/AcOEt (1:1) as the eluant to give **23** (470mg, 11% from **20**) as a yellow powder; ^{1}H-NMR ($CDCl_3$): δ 0.79 (3H, s), 1.05 (3H, s), 1.19 (3H, d, J = 6.1Hz), 1.22 (6H, s), 4.93 - 5.01 (1H, br), 5.70 (2H, brs), 6.24 (1H, d, J = 8.3Hz), 6.40 (1H, d, J = 8.3Hz), 7.24 - 7.40 (5H, m).

PTAD Adduct of 3β-(*tert*-Butyldimethylsilyoxy)-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-1,5,7-triene (24)

A mixture of **23** (545mg, 0.95mmol), TBSCl (428mg, 2.4mmol) and imidazole (386mg, 5.67mmol) in DMF (13ml) was stirred at room temperature for 1.5h and at 40°C for 2h. The mixture was poured into H_2O, extracted with a mixture of *n*-hexane/AcOEt (1:1), washed with H_2O, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (2:1) as the eluant to give **24** (580mg, quantitatively) as a colorless powder; mp. 177-178°C; IR (KBr): 3525, 1760, 1705, 1400, 1095, 1060cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.07 (3H, s), 0.09 (3H, s), 0.79 (3H, s), 0.87 (9H, s), 1.07 (3H, s), 1.19 (3H, d, J = 6.0Hz), 1.21 (6H, s), 3.24 - 3.42 (1H, m), 3.42 - 3.56 (1H, m), 3.76 - 3.88 (1H, br), 4.92 - 5.03 (1H, br), 5.64 (2H, brs), 6.23 (1H, d, J = 8.4Hz), 6.40 (1H, d, J = 8.4Hz), 7.24 - 7.46 (5H, m); EI-MS (m/z): 514 (M^+-PTAD), 141 (100%); Anal. Calcd for $C_{40}H_{59}N_3O_5Si$: C, 69.63; H, 8.62; N, 6.09. Found: C, 69.31; H, 8.93; N, 6.02.

PTAD Adduct of 1α,2α-Epoxy-3β-(*tert*-butyldimethylsilyloxy)-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (25)

A mixture of **24** (580mg, 0.84mmol) and MCPBA (363mg, 2.10mmol) in CH_2Cl_2 (91ml) was stirred at room temperature for 168h. The mixture was poured into 3% $Na_2S_2O_3$, washed with saturated $NaHCO_3$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (3:2) as the eluant to give **25** (421mg, 71%) as a colorless foam; IR (neat): 3520, 1750, 1700, 1600, 1500, 1400, 1250, 1150, 1085cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.12 (6H, s), 0.84 (3H, s), 0.91 (9H, s), 1.09 (3H, s), 1.19 (3H, d, J = 6.0Hz), 1.23 (6H, s), 3.08 - 3.28 (2H, m), 3.31 - 3.52 (2H, m), 3.74 - 3.84 (1H, br), 4.92 (1H, br), 6.19 (1H, d, J = 8.2Hz), 6.40 (1H, d, J = 8.2Hz), 7.22 - 7.44 (5H, m); EI-MS (m/z): 514 (M^+-OTBS), 69 (100%); Anal. Calcd for $C_{40}H_{59}N_3O_6Si$: C, 68.05; H, 8.42; N, 5.95. Found: C, 67.73; H, 8.65; N, 5.82.

PTAD Adduct of 1α,2α-Epoxy-3β-hydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (26)

A mixture of **25** (421mg, 0.60mmol) and TBAF (1M in THF, 3ml, 3.00mmol) was stirred at room temperature for 2.5h. The mixture was diluted with AcOEt, washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by

flash column chromatography with CH_2Cl_2/EtOH (50:3) as the eluant to give **26** (327mg, 93%) as a colorless foam; IR (neat): 3450, 1750, 1695, 1600, 1500, 1405, 1150, 1100, 1080cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.83 (3H, s), 1.08 (3H, s), 1.20 (3H, d, J = 6.0Hz), 1.23 (6H, s), 3.16 - 3.56 (3H, m), 3.64 - 3.84 (2H, m), 4.93 - 5.07 (1H, br), 6.19 (1H, d, J = 8.2Hz), 6.40 (1H, d, J = 8.2Hz), 7.24 - 7.46 (5H, m); EI-MS (m/z): 591 (M^+), 69 (100%); Anal. Calcd for $C_{34}H_{45}N_3O_6 \cdot 3/4H_2O$: C, 67.47; H, 7.79; N, 6.94. Found: C, 67.12; H, 7.79; N, 7.43.

1α,2α-Epoxy-3β-hydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)pregna-5,7-diene (27)

A solution of **26** (327mg, 0.55mmol) in 1,3-dimethyl-2-imidazolidinone (DMI, 33ml) was heated at 140°C for 2h. The mixture was diluted with AcOEt and washed three times with H_2O. The combined aqueous layer was extracted with AcOEt, and washed three times with H_2O. The combined organic layer was dried over $MgSO_4$ and evaporated. The crude residue was then purified by flash column chromatography with CH_2Cl_2/EtOH (25:1) as the first eluant, with *n*-hexane/AcOEt (1:1) as the second eluant to give **27** (199mg, 86%) as a colorless powder; IR (KBr): 3350, 2995, 2905, 1395, 1380, 1170, 1110, 1080, 1065, 855cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.61 (3H, s), 1.02 (3H, s), 1.22 (3H, d, J = 7.2Hz), 1.24 (6H, s), 3.00 (1H, d, J = 3.6Hz), 3.20 - 3.28 (1H, br), 3.32 (1H, d, J = 3.6Hz), 3.44 - 3.56 (1H, m), 3.78 - 3.92 (2H, m), 5.32 - 5.40 (1H, m), 5.68 (1H, brd, J = 5.7Hz); EI-MS (m/z): 416 (M^+), 68 (100%); UV (EtOH) λ max: 289, 278, 267nm.

[2β-^{3}H]-1α,3β-Dihydroxy-20(*S*)-(3-hydroxy-3-methylbutyloxy)-9,10-secopregna-5,7,10(19)-triene; [2β-^{3}H]OCT (4)

A solution of **28** (82.9mCi) in EtOH (200ml) was treated in the same manner described in the preparation of **3**. The crude product was purified by preparative TLC developed with CH_2Cl_2/EtOH (10:1) to give **4** (24.1mCi, 29%).

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