January 1997 Notes Chem. Pharm. Bull. 45(1) 185—188 (1997) 185

# Synthesis and Biological Activity of a 1α,25-Dihydroxyvitamin $D_2$ Analog Bearing an Amide Group in the Side-Chain

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Received June 3, 1996; accepted September 7, 1996

**Synthesis of a 1α,25-dihydroxyvitamin $D_2$ analog (3), in which the double bond in the side-chain is replaced by an amide group, is described. Condensation of a carboxylic acid (8) with an amine (6) gave an amide (9), which in turn led to 3 *via* several steps. The analog (3) could not bind to the chick cytosol vitamin D receptor, which indicated the importance of the hydrophobic interaction of the C(22)–C(23) double bond in 1α,25-dihydroxyvitamin $D_2$ (2) with the vitamin D receptor.**

**Key words** 1α,25-dihydroxyvitamin $D_2$ analog; isosteric replacement; synthesis; vitamin D receptor binding

1α,25-Dihydroxyvitamin $D_3$ (**1**), the hormonally active form of vitamin $D_3$, mediates calcium and phosphorus homeostasis, and bone resorption and mineralization. Moreover, **1** has a variety of biological roles in many tissues and cells.1) Accordingly, a number of vitamin $D_3$ analogs have been prepared to investigate the functional roles of vitamin D and to develop therapeutic agents.2) Of the analogs synthesized, those altered in the side-chain are of particular interest because some of them show higher potency than the parent compound3) and others show preferential activity on cell differentiation and proliferation over calcium absorption,4) which could be therapeutically important.

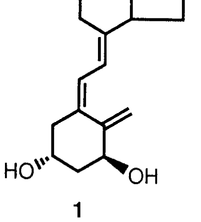
1α,25-Dihydroxyvitamin $D_2$ (**2**), the counterpart of **1** for vitamin $D_2$, acts in a similar way to **1**,5) in spite of having somewhat different structure in the side-chain, but less is known about its metabolism and activities.6) Furthermore, in contrast to the case of **1**, there have been only a few studies on syntheses and pharmacological activities of 1α,25-dihydroxyvitamin $D_2$ analogs.7−10)

We report here the synthesis and preliminary results on the biological activities of a new 1α,25-dihydroxyvitamin $D_2$ analog **3**, in which the double bond in the side-chain is replaced by an amide bond.11) Amide and *trans*-olefin bonds are isosteric to each other12) and this isosteric replacement has been successfully applied in peptide13) and retinoid14) chemistry. Since an amide bond adopts planar *s-trans* conformation because of resonance, it is geometrically similar to a *trans*-olefin, but electrostatically these two bonds are considerably different from each other owing to the presence of the polar heteroatoms in the amide group. Calculation of the geometry and the electrostatic potential of **2** and **3** using MOPAC (ver. 5) based on the X-ray crystallographic data of **2**15) showed that the most stable conformation of **3** is virtually identical to that of **2** and there exists a negative charge at oxygen and a small positive charge at nitrogen in the amide group of **3**, which is markedly different from the electronic potential of **2**.

The synthesis of the analog **3** is shown in Charts 1 and 2. Firstly, the optically active amine moiety was prepared from D-alanine. The Grignard reaction of *N*-$^t$Boc-D-alanine methylester (**4**) with methylmagnesium bromide gave the alcohol **5** in 86% yield. The Boc protective group of **5** was removed by treatment with trifluoroacetic acid (TFA) and the resulting TFA salt was converted to the crystalline hydrochloride **6** in 89% overall yield.

The steroidal carboxylic acid **8** was prepared from the corresponding alcohol **7**, which we have used as an intermediate for the synthesis of other vitamin D analogs.8) Unexpectedly, however, the direct oxidation from **7** to **8** was unsuccessful with conventional oxidants (PDC, $MnO_2$, $Ag_2O$, $SeO_2$ and $KMnO_4$), so that this was accomplished in a stepwise manner, that is, Swern oxidation followed by $KMnO_4$, in 60% yield.

The resulting acid **8** was condensed with the amine **6** by the use of dicyclohexylcarbodiimide (DCC) as a coupling agent to give the amide **9** in 25% yield. Attempts to improve the yield by the other methods for amide bond formation using $SOCl_2$/Py or carbonyldiimidazole (CDI)



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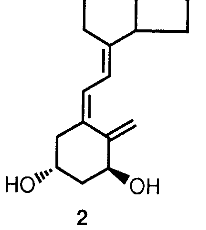

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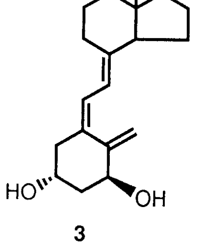

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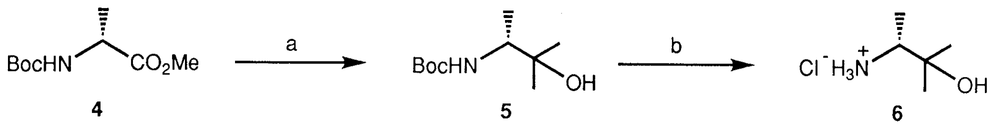
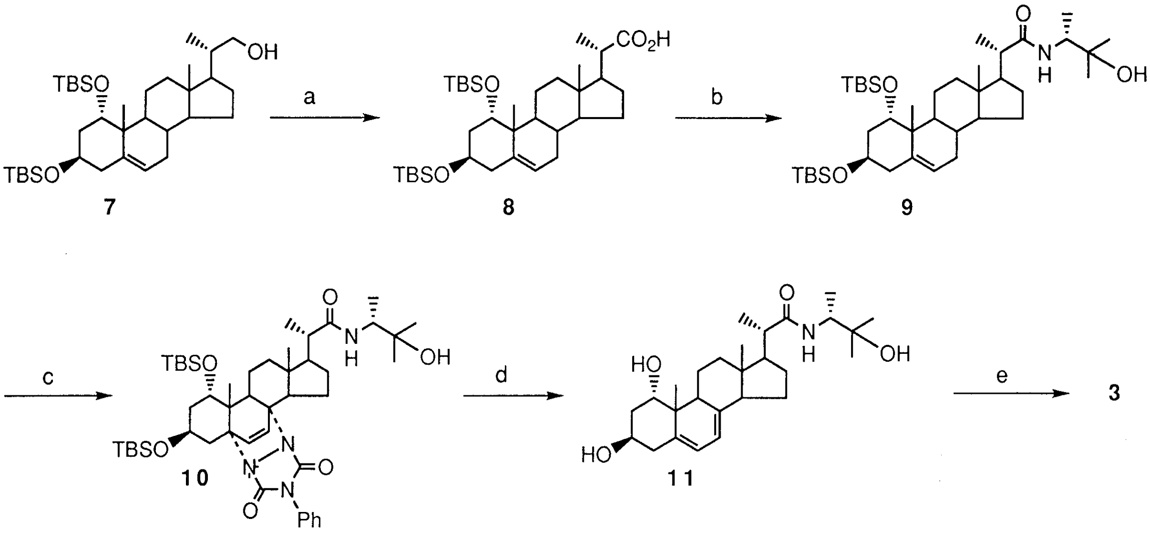


a, MeMgBr; b, 1) TFA 2) HCl

Chart 1



a, 1) DMSO-$(COCl)_2/Et_3N$ 2) $KMnO_4$; b, 1) DCC/DMAP 2) **6**/DBU; c, 1) NBS 2) n-$Bu_4NBr$ 3) n-$Bu_4NF$ 4) PTAD; d, 1) n-$Bu_4NF$ 2) $K_2CO_3$/DMSO; e, 1) hν 2) reflux/EtOH

Chart 2

resulted in failure. The amide **9** was led to the analog **3** *via* usual vitamin D chemistry. Thus, preparation of the 5,7-diene followed by the reaction with 4-phenyl-1,2,4-triazolidine-3,5-dione (PTAD) gave **10** in 10% yield, which was converted to the provitamin **11** by sequential deprotection of the silyl groups and PTAD in 51% overall yield. Photochemical and thermal isomerization of the provitamin **11** finally afforded the analog **3** in 10% yield after HPLC purification.

In preliminary evaluation of biological activities, the analog **3** was found not to bind to the chick cytosol vitamin D receptor, showing almost no affinity even at much higher concentrations ($>10^4$ fold) than those effective for the parent compound **2**. This could be a consequence of repulsion between the polar amide moiety and the corresponding hydrophobic site in the receptor. Therefore, this study indicates the importance of the hydrophobic interaction of the C(22)–C(23) double bond in 1α,25-dihydroxyvitamin $D_2$ (**2**) with the vitamin D receptor. Further studies on biological activities are in progress.

## Experimental

Melting points are uncorrected. Spectral data were recorded on the following instruments: $^1$H-NMR, JEOL GSX-400; MS, JEOL JMS-D 300; IR, JASCO FT/IR-8000; optical rotations, JASCO DIP-370. Tetramethylsilane (TMS) was used as an internal standard for $^1$H-NMR. Wakogel C-300 (Wako Pure Chemical Industries Ltd.) was used for silica gel flash chromatography. HPLC separations were performed on a Waters LC equipped with a 510 HPLC pump and 484 tunable absorbance detector (Waters Associates).

**(3*R*)-3-[*N*-*tert*-Butyloxycarbonyl)amino]-2-methylbutan-2-ol (5)** To a stirred solution of *N*-Boc-D-alanine methylester (8.12 g, 40 mmol) in THF (50 ml) was added 3.0 M MeMgBr/ether (55 ml, 164 mmol, 4.1 eq) at 0 °C under argon, and the mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of a small amount of brine, and the mixture was diluted with AcOEt, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (100 g, acetone–$CHCl_3$, 1:25) to give **5** (7.52 g, 86%) as a colorless oil: $[\alpha]_D$ +1.6° ($c$=1.6, $CHCl_3$). $^1$H-NMR ($CDCl_3$) δ: 1.13 (3H, d, $J$=7.0), 1.18 (3H, s), 1.23 (3H, s), 1.45 (9H, s), 2.16 (1H, br s), 3.57—3.64 (1H, m), 4.68 (1H, br s). IR (neat): 3441, 1694, 1506, 1250, 1173 cm$^{-1}$. MS $m/z$: 203 ($M^+$), 130 ($M-{}^t$BuO), 112 ($M-{}^t$BuO$-H_2O$). HR-MS $m/z$: 203.1518 ($M^+$) Calcd for $C_{10}H_{21}NO_3$ 203.1521.

**(3*R*)-3-Amino-2-methylbutan-2-ol Hydrochloride (6)** To a stirred solution of the alcohol **5** (1.05 g, 5.2 mmol) in $CHCl_3$ (10 ml) was added $CF_3CO_2H$ (4 ml), and the mixture was stirred at room temperature for 10 min. After evaporation of the solvent, the residue was dissolved in MeOH (1 ml). Concentrated HCl (0.6 ml) was added, and the mixture was stirred at room temperature for 1 min. The mixture was concentrated and the crystalline crude product was recrystallized from ether–EtOH to give **6** (0.64 g, 89%) as colorless needles: mp 136.5—137.5 °C; $[\alpha]_D$ −8.0° ($c$=0.89, MeOH). $^1$H-NMR ($CD_3OD$) δ: 1.19 (3H, s), 1.26 (3H, d, $J$=7.0), 1.28 (3H, s), 3.16 (1H, q, $J$=7.0). IR (Nujol): 3162, 1510, 1157 cm$^{-1}$. *Anal.* Calcd for $C_5H_{13}NO\cdot HCl$: C, 43.01; H, 10.11; N, 10.03. Found: C, 43.01; H, 10.23, N, 10.12.

**(20*S*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-5-pregnene-20-carboxylic Acid (8)** A mixture of DMSO (0.52 ml, 7.14 mmol, 14.0 eq) and oxalyl chloride (0.32 ml, 3.57 mmol, 7.0 eq) in dry $CH_2Cl_2$ (6 ml) was stirred at −78 °C for 30 min under argon. Then the alcohol **7** (300 mg, 0.51 mmol) in dry $CH_2Cl_2$ (6 ml) was added, and the mixture was stirred at −78 °C. After 30 min, $Et_3N$ (0.91 ml, 6.34 mmol, 12.5 eq) was added to the reaction mixture at −78 °C and the whole was stirred at −78 °C for 15 min, then poured into saturated $CuSO_4$ solution and extracted with $CH_2Cl_2$. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated to give an aldehyde as colorless needles. $^1$H-NMR ($CDCl_3$) $\delta$: 0.02 (3H, s), 0.03 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.72 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 0.96 (3H, s), 1.12 (3H, d, $J=6.7$), 3.77 (1H, dd, $J=3.7$, 1.5), 3.98 (1H, tt, $J=4.7$, 10.9), 5.45 (1H, d, $J=5.6$), 9.57 (1H, d, $J=3.1$). MS $m/z$: 574 ($M^+$), 559 (M−Me), 517 ($\mathrm{M}-{}^t\mathrm{Bu}$). HR-MS $m/z$: 517.3520 ($\mathrm{M}-{}^t\mathrm{Bu}$) Calcd for $C_{30}H_{53}O_3Si_2$ 517.3531.

The resulting aldehyde was dissolved in acetone (30 ml), and $KMnO_4$ was added until a purple color developed. The reaction was quenched by the addition of saturated $Na_2S_2O_3$ solution and the mixture was filtered through Celite. The filtrate was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (40 g, AcOEt–hexane, 1 : 20) to give **8** (180 mg, 60%) as colorless needles: mp 246.5—248 °C (MeOH); $[\alpha]_D$ +1.3° ($c=0.55$, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.03 (3H, s), 0.04 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.70 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 0.96 (3H, s), 1.24 (3H, d, $J=7.0$), 2.43 (1H, dq, $J=10.7$, 7.0 ), 3.76 (1H, d, $J=1.6$), 3.99 (1H, tt, $J=5.9$, 10.5), 5.45 (1H, d, $J=5.9$). IR ($CHCl_3$): 1715 cm$^{-1}$. MS $m/z$: 590 ($M^+$), 575 (M−Me), 572 ($M-H_2O$), 533 ($\mathrm{M}-{}^t\mathrm{Bu}$). HR-MS $m/z$: 590.4172 ($M^+$) Calcd for $C_{34}H_{62}O_4Si_2$ 590.4186. *Anal.* Calcd for $C_{34}H_{62}O_4Si_2$: C, 69.10; H, 10.57. Found: C, 68.88; H, 10.72.

**(24*R*)-23-Aza-1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]-22-oxo-5-ergosten-25-ol (9)** A solution of the carboxylic acid **8** (487 mg, 0.83 mmol) and 4-dimethylaminopyridine (DMAP) (152 mg, 1.13 mmol, 1.5 eq) in dry $CH_2Cl_2$ (20 ml) was treated with DCC (257 mg, 1.13 mmol, 1.5 eq) and the mixture was stirred at room temperature under argon. After 1.5 h, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.62 ml, 4.15 mmol, 5.0 eq) and the amine hydrochloride **6** (584 mg, 4.15 mmol, 5.0 eq) were successively added, and the mixture was stirred at room temperature for 2 h under argon, poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (15 g, AcOEt–hexane, 1 : 20, 1 : 10, 1 : 5) to give **9** (140 mg, 25%) as a colorless, viscous oil: $[\alpha]_D$ +2.6° ($c=0.52$, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.02 (3H, s), 0.03 (3H, s), 0.04 (3H, s), 0.06 (3H, s), 0.70 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 0.95 (3H, s), 1.13 (3H, d, $J=6.7$), 1.19 (3H, d, $J=6.7$), 1.20 (3H, s), 1.22 (3H, s), 3.76 (1H, d, $J=1.6$), 3.88 (1H, dq, $J=6.7$, 8.9), 3.98 (1H, tt, $J=5.2$, 10.5), 5.44 (1H, d, $J=5.6$), 5.59 (1H, d, $J=8.9$). IR ($CHCl_3$): 1651 cm$^{-1}$. MS $m/z$: 675 ($M^+$), 657 ($M-H_2O$), 642 ($M-H_2O-Me$), 618 ($\mathrm{M}-{}^t\mathrm{Bu}$). HR-MS $m/z$: 600.4268 ($\mathrm{M}-{}^t\mathrm{Bu}-\mathrm{H_2O}$) Calcd for $C_{35}H_{62}NO_3Si_2$ 600.4265.

**(24*R*)-23-Aza-1α,3β-bis[(*tert*-butyldimethylsilyl)oxy]-22-oxo-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidine-1,2-diyl)-6-ergosten-25-ol (10)** A mixture of the amide **9** (120 mg, 0.18 mmol), *N*-bromosuccinimide (NBS) (60 mg, 0.324 mmol, 1.8 eq), $NaHCO_3$ (102 mg, 1.22 mmol, 6.8 eq) and benzoyl peroxide (catalytic amount) in hexane (15 ml) was refluxed for 1 h under argon. After having cooled, the mixture was filtered through Celite and the filtrate was evaporated. The residue was dissolved in THF (6 ml), then $Et_3N$ (0.25 ml, 1.8 mmol, 10 eq) and $n$-$Bu_4NBr$ (5.7 mg, 0.018 mmol, 0.1 eq) were successively added. The mixture was stirred at 0 °C for 15 min, then 1.0 M $n$-$Bu_4NF$/THF (1.25 ml, 1.25 mol, 7 eq) was added and the whole was further stirred at 0 °C for 4 h under argon, then poured into saturated $NH_4Cl$ solution and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in AcOEt (6 ml) and PTAD was added to the solution until a red color developed. The solvent was evaporated and the crude product was purified by silica gel flash chromatography (1 g, AcOEt–hexane, 1 : 3, 1 : 1) to give **10** (17 mg, 12%) as a colorless, viscous oil: $[\alpha]_D$ −14.0° ($c=0.62$, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.07 (3H, s), 0.08 (3H, s), 0.10 (3H, s), 0.12 (3H, s), 0.82 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 0.92 (3H, s), 1.13 (3H, d, $J=6.7$), 1.19 (3H, s), 1.22 (3H, s), 1.23 (3H, d, $J=6.0$), 3.25 (1H, dd, $J=5.1$, 14.5), 3.84 (1H, d, $J=2.4$), 3.90 (1H, tq, $J=6.0$, 8.5), 4.77 (1H, tt, $J=4.8$, 9.7), 5.62 (1H, d, $J=8.5$), 6.23 (1H, d, $J=7.3$), 6.36 (1H, d, $J=7.3$), 7.2—7.5 (5H, m). IR ($CHCl_3$): 1740, 1689, 1649 cm$^{-1}$. UV $\lambda_{max}$ ($CHCl_3$): 240 ($\varepsilon$ 2960), 262 (2680) nm. MS $m/z$: 673 (M−PTAD), 655 ($M-PTAD-H_2O$), 616 ($\mathrm{M}-\mathrm{PTAD}-{}^t\mathrm{Bu}$), 598 ($\mathrm{M}-\mathrm{PTAD}-{}^t\mathrm{Bu}-\mathrm{H_2O}$), 541 ($\mathrm{M}-\mathrm{PTAD}-2{}^t\mathrm{Bu}-\mathrm{H_2O}$). HR-MS $m/z$: 674.5001 (M−PTAD+H) Calcd for $C_{39}H_{72}NO_4Si_2$ 674.5000.

**(24*R*)-23-Aza-22-oxo-5,7-ergostadiene-1α,3β,25-triol (11)** A solution of the PTAD adduct **10** (15 mg, 0.02 mmol) in THF (2 ml) was treated with 1.0 M $n$-$Bu_4NF$/THF (0.36 ml, 0.36 mmol, 18 eq) and the mixture was heated at 75 °C for 2 h under argon. After having cooled, the reaction mixture was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (1 g, 2.5—4% MeOH–$CHCl_3$) to give a triol (8.5 mg, 70%) as an amorphous solid: $[\alpha]_D$ −28.8° ($c=0.44$, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.77 (3H, s), 0.87 (3H, s), 1.05 (3H, d, $J=7.0$), 1.12 (3H, s), 1.14 (3H, d, $J=6.5$), 1.15 (3H, s), 2.63 (2H, dt, $J=13.0$, 6.5), 3.12 (1H, dd, $J=5.2$, 14.5), 3.79 (1H, d, $J=2.6$), 3.83 (1H, dq, $J=8.8$, 6.5), 4.85 (1H, tt, $J=5.2$, 11.0), 5.53 (1H, d, $J=8.8$), 6.20 (1H, d, $J=8.2$), 6.33 (1H, d, $J=8.2$), 7.2—7.5 (5H, m). IR ($CHCl_3$): 3433, 1747, 1684, 1655 cm$^{-1}$. MS $m/z$: 445 (M−PTAD), 427 ($M-PTAD-H_2O$), 409 ($M-PTAD-2H_2O$), 391 ($M-PTAD-3H_2O$). HR-MS $m/z$: 445.3193 (M−PTAD) Calcd for $C_{27}H_{43}NO_4$ 445.3190.

A mixture of the triol (8.0 mg, 0.013 mmol) and $K_2CO_3$ (21 mg, 0.156 mmol, 12 eq) in DMSO (1 ml) was heated at 110 °C for 18 h under argon. After having cooled, the mixture was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel flash chromatography (1 g, 4% MeOH−$CHCl_3$) to give **11** (4.2 mg, 73%) as a colorless foam: $[\alpha]_D$ −8.5° ($c=0.17$, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.64 (3H, s), 0.95 (3H, s), 1.14 (3H, d, $J=7.0$), 1.20 (3H, s), 1.22 (3H, d, $J=6.9$), 1.23 (3H, s), 3.77 (1H, br s), 3.89 (1H, dq, $J=8.4$, 6.9), 4.07 (1H, m), 5.39 (1H, dt, $J=5.5$, 2.0), 5.62 (1H, d, $J=8.4$), 5.73 (1H, dd, $J=2.7$, 5.5). IR ($CHCl_3$): 3441, 1651 cm$^{-1}$. UV $\lambda_{max}$ (EtOH): 264 ($\varepsilon$ 7270), 273 (9820), 283 (10650), 295 (6600) nm. MS $m/z$: 445 ($M^+$), 427 ($M-H_2O$), 409 ($M-2H_2O$), 391 ($M-3H_2O$). HR-MS $m/z$: 445.3192 ($M^+$) Calcd for $C_{27}H_{43}NO_4$ 445.3190.

**(24*R*)-23-Aza-22-oxo-9,10-seco-5,7,10(19)-ergostatriene-1α,3β,25-triol (3)** A solution of the provitamin **11** (10.5 mg, 0.02 mmol) in 1: 10 THF–ether (100 ml) was cooled to 0 °C and deoxygenated by bubbling argon through the solution for 40 min. The solution was irradiated with a high-pressure mercury lamp fitted with a Vycor filter at 0 °C for 6 min. The solvent was evaporated at below 25 °C and the residue was dissolved in EtOH (50 ml). The solution was refluxed for 1 h under argon, then evaporated. The crude product was purified by repeated HPLC [(1) Lichrosorb Si-60, 10 × 250 mm, iso-PrOH–$CH_2Cl_2$ (13 : 87) (2) Zorbax-SIL, 4.6 × 250 mm × 2, hexane–$CH_2Cl_2$–MeOH–iso-PrOH (60 : 30 : 5 : 5)] to give **3** (1.1 mg, 10%) as a white foam: $[\alpha]_D$ +1.5° ($c=0.0023$, EtOH). $^1$H-NMR ($CDCl_3$) $\delta$: 0.56 (3H, s), 1.14 (3H, d, $J=7.0$), 1.20 (3H, s), 1.21 (3H, d, $J=6.3$), 1.23 (3H, s), 3.89 (1H, dq, $J=8.2$, 6.3), 4.23 (1H, m), 4.43 (1H, m), 4.99 (1H, s), 5.32 (1H, t, $J=1.8$), 5.59 (1H, d, $J=8.2$), 6.02 (1H, d, $J=11.3$), 6.38 (1H, d, $J=11.3$). IR ($CHCl_3$): 3441, 1651 cm$^{-1}$. UV $\lambda_{max}$ (EtOH): 265 ($\varepsilon$ 18300) nm. MS $m/z$: 445 ($M^+$), 427 ($M^+-H_2O$), 409 ($M-2H_2O$). HR-MS $m/z$: 445.3189 ($M^+$) Calcd for $C_{27}H_{43}NO_4$ 445.3192.

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