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A NOVEL AND PRACTICAL ROUTE TO A-RING ENYNE SYNTHON FOR 1α,25-DIHYDROXYVITAMIN D3 ANALOGS: SYNTHESIS OF A-RING DIASTEREOMERS OF 1α,25-DIHYDROXY-VITAMIN D3 AND 2-METHYL-1,25-DIHYDROXYVITAMIN D3

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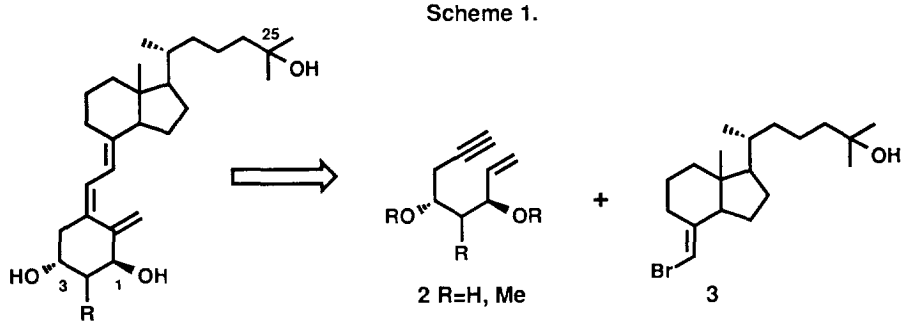
Abstract: A novel and practical route to the A-ring enyne synthon (**2**), which can be versatile for a variety of A-ring analogs of 1α,25-dihydroxyvitamin D3 (**1**), was developed. This novel method led to an improved synthesis of the A-ring diastereomers of **1**, the compounds **13-15**, and synthesis of the new analogs, 2-methyl-1,25-dihydroxyvitamin D3 (**4**) with its all possible diastereomers. The biological evaluation of the 2-methyl analogs showed the ααβ-isomer to be more potent than **1**.

A large number of analogs of 1α,25-dihydroxyvitamin D3 [1α,25-(OH)2VD3 (**1**)], the hormonally active metabolite of vitamin D3, have been synthesized and biologically evaluated to investigate the structure-activity relationships and to develop potential therapeutic agents.[1] A majority of those are altered in the side-chain, providing many useful analogs with high potency or selective activity, while modification of the A-ring has attracted attention in recent years because it can afford useful analogs exhibiting unique activity profiles as well.[2]

For synthesis of A-ring analogs, the convergent method is much more advantageous over the classical steroidal approach because it can be more effective and flexible.[3] In particular, the procedure reported by Trost and co-workers using palladium-catalyzed coupling of the A-ring enyne synthon (**2**) with the CD-ring portion (**3**) would be most useful (Scheme 1).[4] However, syntheitc route to **2** (R=H), which can be versatile and applicable to a wide variety of A-ring analogs, have not been fully established.[5] In this paper, we describe a novel and practical route to all four possible diastereomers of **2** (R=H), and its application to the synthesis of the A-ring diastereomers of **1** and the new analogs, 2-methyl-1,25-dihydroxyvitamin D3 (**4**) with its all possible diastereomers. The biological evaluation of the 2-methyl analogs is also reported.

Scheme 1.



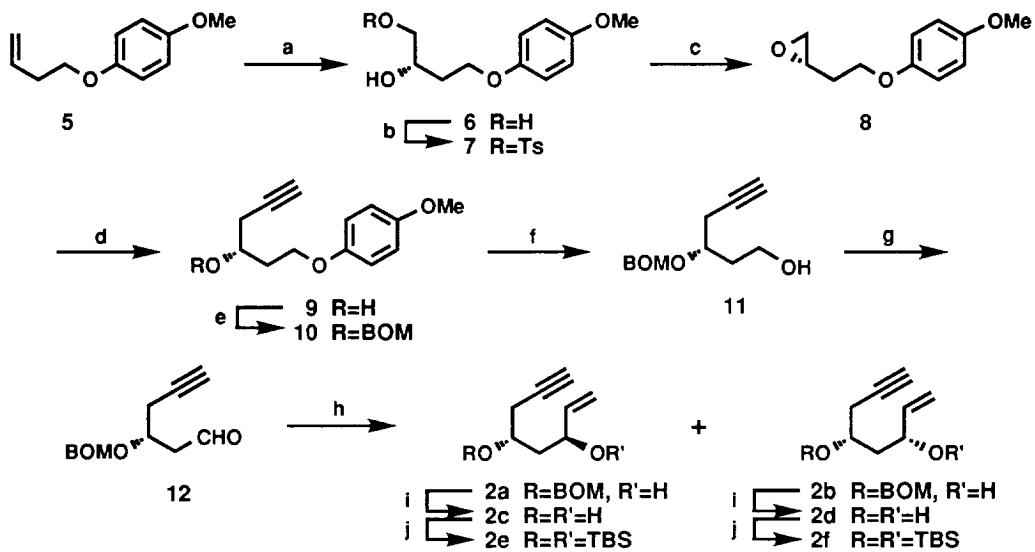
1 R=H
4 R=Me

2 R=H, Me

3

PII: S0960-894X(97)10204-9

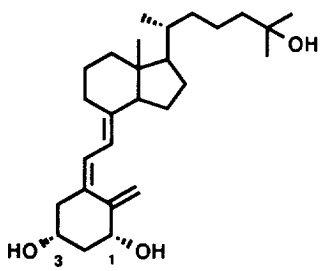
Scheme 2.



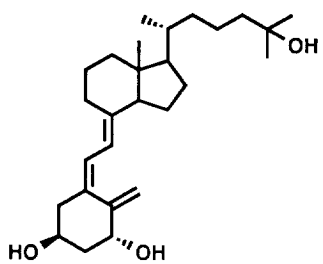
(a) AD-mix-α, ^{t}BuOH-H_2O, 0 °C, 95% (b) TsCl, pyridine, 0 °C, 94% (c) Lithium hexamethyldisilazide, THF, 0 °C, 84% (d) Lithium acetylide-EDA, DMSO, rt, 86% (e) BOMCl, EtN(Pr^i)$_2$, CH_2Cl_2, rt, 90% (f) CAN, MeCN-H_2O, 0 °C, 99% (g) PDC, CH_2Cl_2, rt, 96% (h) VinylMgBr, toluene, -78 °C, 62% (i) PhSH-BF_3·Et_2O, CH_2Cl_2, 0 °C, 62% (j) TBSOTf, 2,6-Lutidine, CH_2Cl_2, 0 °C, 99%

The synthetic route to **2** (R=H) and its diastereomers is outlined in Scheme 2. The Sharpless asymmetric dihydroxylation (AD) of the olefin **5** with AD-mix-α afforded the diol **6** in 95% yield with the natural 3(*S*)-configuration and 93% e.e., $[\alpha]^{26}_D$ -4.2° (*c*=0.38, CHCl3).[6] This was recrystallized from CH_2Cl_2-hexane to yield the enantiomerically pure specimen, mp. 64 °C, $[\alpha]^{26}_D$ -5.2° (*c*=1.35, CHCl3). The diol **6** was converted to the epoxide **8**, $[\alpha]^{25}_D$ -13.6° (*c*=1.65, CHCl3), by sequential treatment with TsCl and lithium hexamethyldisilazide through the mono-tosylate **7**, $[\alpha]^{26}_D$ +2.4° (*c*=1.00, CHCl3), in high yield. The acetylene unit was introduced by the reaction of **8** with lithium acetylide-ethylenediamine complex in DMSO to give the alcohol **9**, $[\alpha]^{26}_D$ -9.8° (*c*=1.18, CHCl3), in 86% yield. Protection of the resulting secondary alcohol of **9** by benzyloxymethyl (BOM) group yielded the BOM ether **10**, $[\alpha]^{28}_D$ -19.4° (*c*=1.04, CHCl3), in 90% yield, followed by deprotection of the *p*-anisyl group with cerium (IV) diammonium nitrate (CAN)[7] furnished the primary alcohol **11**, $[\alpha]^{27}_D$ -50.3° (*c*=1.74, CHCl3), in quantitative yield. Introduction of the vinyl group to the aldehyde **12**, obtained from **11** with PDC in 96% yield, proceeded smoothly to give the allyl alcohol **2a**, $[\alpha]^{26}_D$ -33.2° (*c*=1.01, CHCl3), and **2b**, $[\alpha]^{27}_D$ -31.5° (*c*=1.09, CHCl3), in 62% yield as a 1: 1 mixture of the diastereomers. These isomers were readily separable by silica gel column chromatography and the C-1 configuration of each isomer was determined by ^{1}H NMR analysis of their MTPA ester.[8] Finally, the BOM protecting group was removed by PhSH-BF_3·OEt_2 to afford the desired enynes **2c**, $[\alpha]^{25}_D$ -1.2° (*c*=1.25, CHCl3), and **2d**, $[\alpha]^{28}_D$ -10.3° (*c*=0.70, CHCl3), from **2a** and **2b** , respectively, in 62% yield. Using AD-mix-β instead of AD-mix-α for the AD reaction with **5** led to the enantiomers corresponding to **2c** and **2d** in the same way as above. Thus, we have synthesized all four possible diastereomers of A-ring enyne synthon **2** (R=H) in good overall yield, 11% for 8 steps, starting from **5**.

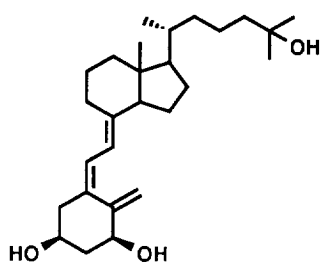
This novel method was applied to an improved synthesis of the A-ring diastereomers of 1α,25-$(OH)_2VD_3$, the compounds **13**, **14** and **15**, which were previously synthesized by Okamura and co-workers.[9a] The recent studies demonstrated that the 1β-isomer (**13**) is an antagonist of the nongenomic but not genomic biological response,[9b] and the 3-epimer (**14**) is a target tissue specific metabolite of 1α,25-$(OH)_2VD_3$.[10] Therefore, these diastereomers have become important as well as 1α,25-$(OH)_2VD_3$ itself in vitamin D research. The method described above should be useful for synthesis of these diastereomers. In fact, coupling of the disilyl ether **2f**, obtained from the diol **2d** with TBSOTf in 96% yield, with the CD-ring portion (**3**) catalyzed by $Pd_2(dba)_3$-PPh_3-Et_3N in toluene at 120 °C for 6h, followed by deprotection with camphorsulfonic acid (CSA) in MeOH gave the 1β-isomer (**13**)[11] in 52% yield. The same treatment of the enantiomers of **2e** and **2f** afforded the rest of the diastereomers **14**[11] and **15**.[11]



13



14



15

We further applied this novel method to synthesiz the new analogs, 2-methyl-1,25-dihydroxyvitamin D_3 (**4**) with its all possible diasteromers. The unique biological profile of 2β-(hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 (ED-71) prompted us to synthesize these structually simpler analogs.[12] In addition, they may be suitable to investigate the A-ring conformation-activity relationships.[13]

Scheme 3 shows the synthetic route as exemplified by the ααβ-isomer (the Greek letters denote the configuration at C-1, C-2, and C-3, respectively, in the vitamin D numbering system). Methyl (*R*)-(-)-3-hydroxy-2-methylpropionate (**16**) was converted to the olefin (**17**), $[\alpha]^{26}_D$ -2.4° (c=1.01, $CHCl_3$), by conventional manner in high yield, which on subsequent treatment with mCPBA to afford the epoxide (**18**), $[\alpha]^{26}_D$ -0.76° (c=1.18, $CHCl_3$), in 97% yield. The acetylene unit was introduced by the reaction of **18** with ethynyltrimethylsilane/BuLi-BF_3·OEt_2 in THF, giving a 1: 1 mixture of the alcohols **19a** , $[\alpha]^{26}_D$ +3.3° (c=1.19, $CHCl_3$), and **19b**, $[\alpha]^{26}_D$ -3.0° (c=1.01, $CHCl_3$), in 85% yield. These isomers were readily separable by silica gel column chromatography, and the absolute configuration at C-3 of each isomer was determined by the ^1H NMR analysis of its MTPA esters.[8] Protection of the 3-OH in the 3(*R*) isomer **19a** with THP, followed by treatment with TBAF furnished the primary alcohol **20**, which was then oxidized by the Swern procedure to give the aldehyde **21** in excellent yield. The reaction of **21** with vinylmagnesium bromide in the presence of $CeCl_3$ afforded a 1: 1 mixture of the diasteromeric allyl alcohols **22a** and **22b** in 92% yield. After removal of the THP protecting group, the resulting mixture of the diols **23a** and **23b** was readily separable by silica gel column chromatography, and the relative stereochemistry of the 1,3-diol in each isomer was determined by ^{13}C NMR analysis of its acetonide.[14] Finally, palladium-catalyzed coupling of the disilyl ether **24a**, $[\alpha]^{26}_D$ +10.3° (c=1.15, $CHCl_3$), obtained from **23a** with t-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) in quantitative yield,

with the CD-ring portion **3**, followed by deprotection with camphorsulfonic acid (CSA) in MeOH gave the ααβ-isomer of the analog **2**(ααβ)[15] in good yield. The same treatment of the isomer **23b** produced the βαβ-isomer. The rest of the 2α-methyl analogs, the ααα- and βαα-isomers, were synthesized from the alcohol **19b** by the same sequence of the reactions as from **19a** to **4**. Using methyl (*S*)-(+)-3-hydroxy-2-methylpropionate, the enantiomer of **16**, as the starting material afforded the other four isomers of the 2β-methyl analogs. Thus, we have synthesized all eight posibble diastereomers of the 2-methyl-1α,25-dihydroxyvitamin D3 (**4**).

Table 1. The relative potency of the 2-methyl analogs to 1α,25-$(OH)_2VD_3$ (**1**).[a]

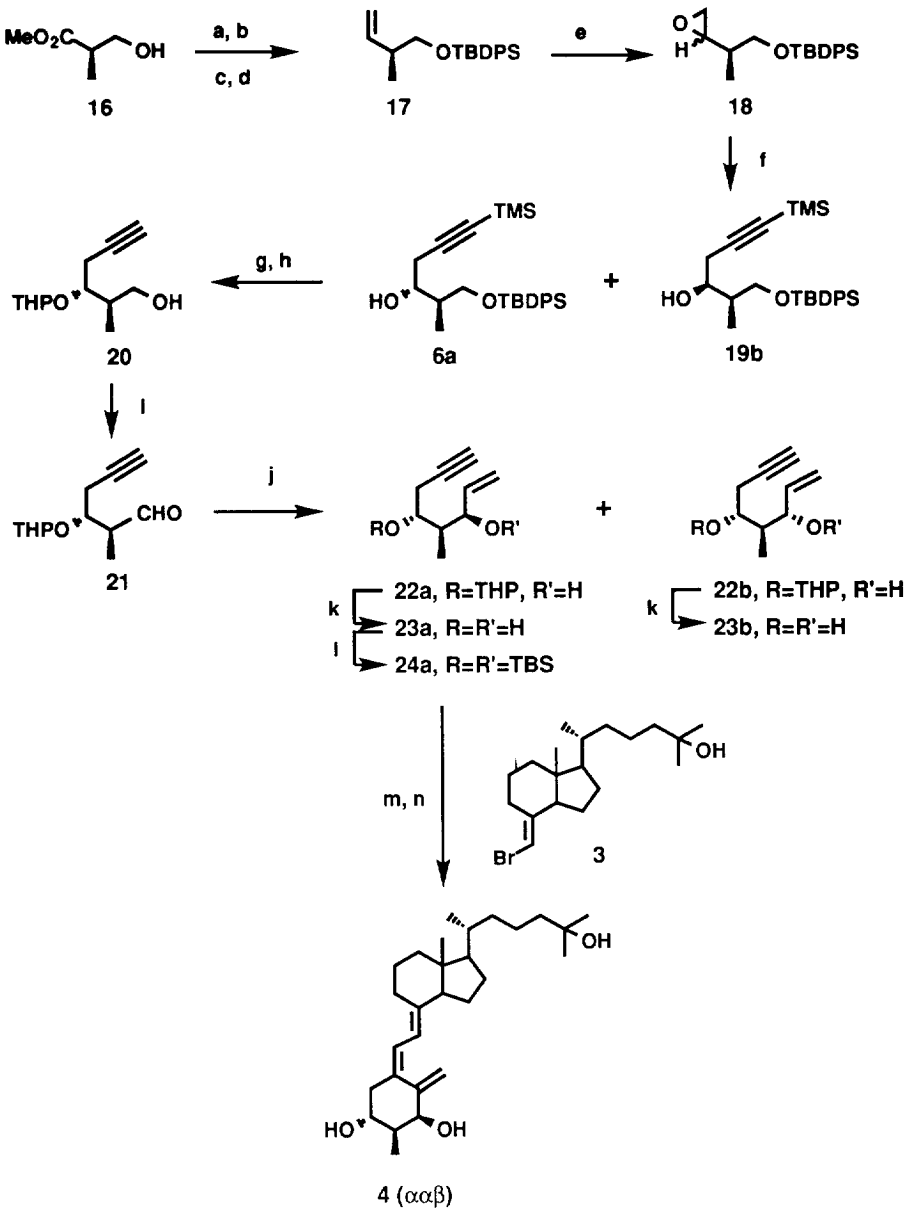
compounds[b]	VDR[c]	Ca[d]	HL-60	DBP[e]
1α,25-$(OH)_2VD_3$	100	100	100	100
ααβ	400	400	200	68
αββ	13	2	10	79
ααα	4	NT[f]	13	45
αβα	0.3	NT	1.5	21
βαβ	<0.1	NT	1.0	200
βββ	<0.1	NT	1.5	1000
βαα	<0.1	NT	0.5	1200
ββα	0.8	NT	3.0	1300

(a) The potency of 1α,25-$(OH)_2VD_3$ is normalized to 100. (b) The Greek letters denote the configuration at C-1, C-2, and C-3, respectively.(c) Bovine thymus (d) Rat serum Ca level (e) Calf serum (f) Not tested.

The results of the biological evaluation of the 2-methyl analogs are summarized in Table 1. The potency was highly dependent on each isomer, that is, the stereochemistry of the A-ring substituents. In vitamin D receptor (VDR) binding using bovine thymus,[16] the 1α-compounds exhibited significant to high affinity whereas the 1β-compounds had virtually no affinity, which was consistent with those previously reported.[9b] Furthermore, of the 1α-compounds, the 2α-methyl isomers (ααβ- and ααα-isomers) showed much higher potency than the corresponding 2β-methyl isomers (αββ- and αβα-isomers). Thus, the ααβ-isomer showed 4-fold higher affinity than 1α,25-$(OH)_2VD_3$ (**1**). This isomer also exhibited 4-fold higher potency in elevation of rat serum Ca concentration,[17] while its 2-epimer, the αββ-isomer, had quite low activity. The rank order of potency in HL-60 cell differentiation[18] was almost parallel to that of the VDR binding, and hence, the ααβ-isomer was twice as potent as the parent compound. In binding to the vitamin D binding protein (DBP) using calf serum,[19] the 1β-isomers showed high affinity, while the 1α-isomers had only poor affinity, which was again consistent with those previously reported.[20]

In this study, we have developed a novel and practical route to the A-ring enyne synthon (**2**) for 1α,25-$(OH)_2VD_3$ analogs. It is noteworthy that this method produces all possible diastereomers in the same way, which is marked contrast to the known procedures. This feature resulted in the improved synthesis of the A-ring diastereomers of 1α,25-$(OH)_2VD_3$, the compounds **13**-**15**. Furthermore, this method can be versatile for synthesis of a wide variety of A-ring analogs with substituent(s) at any position. We have indeed synthesized all eight possible diastereomers of 2-methyl-1,25-dihydroxyvitamin D3 (**4**) according to this method. Of the isomers thus synthesized, the ααβ-isomer showed particularly high potency in VDR binding and in elevation of serum Ca concentration. This implies that such a simple modification can lead to a potent analog and the potency is highly dependent on the configuration not only of the C-1 and C-3 hydroxy groups but also of the 2-methyl group.

Scheme 3



(a) TBDPSCl, imidazole, CH_2Cl_2, rt, quant. (b) DIBAL-H, toluene, -78 °C-rt, 90% (c) Swern, 97% (d) $Ph_3P{=}CH_2$, THF, 0 °C-rt, 89% (e) mCPBA, CH_2Cl_2, 0 °C-rt, 97% (f) Ethynyltrimethylsilane/BuLi-$BF_3{\cdot}OEt_2$, THF, -78 °C-rt, 85% (g) DHP, pTsOH, CH_2Cl_2, 0 ° C, 95% (h) TBAF, THF, 96% (i) Swern, 98% (j) VinylMgBr/$CeCl_3$, THF, -78 °C, 92% (k) pTsOH, MeOH, rt, 71% (l) TBSOTf, 2,6-Lutidine, CH_2Cl_2, rt, 99% (m) $Pd_2(dba)_3$-PPh_3-Et_3N, toluene, 120 °C (n) CSA/MeOH, 67%

References and Notes

1. Ettinger, R. A.; DeLuca, H. F. *Adv. Drug Res.* **1996**, *28*, 269.
2. Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocri. Rev.* **1995**, *16*, 200.
3. (a) Dai, H.; Posner, G. H.*Synthesis* **1994**, 1383; (b) Zhu, G.-D.; Okamura, W. H.*Chem. Rev.* **1995**, *95*, 1877.
4. Trost, B. M.; Dumas, J.; Villa, M. *J. Am. Chem. Soc.* **1992**, *114*, 9836.
5. For the synthesis of **2** and its derivatives, see: (a) Tazumi, K.; Ogasawara, K. *J. Chem. Soc. Chem. Commun.* 1994, 1903; (b) Trost, B. M.; Hanson, P. R. *Tetrahedron Lett.* **1994**, *35*, 8119; (c) Moriarty, R. M.; Kim., J.; Brumer III, H. *Tetrahedron Lett.* **1995**, *36*, 51; (d) Vrielynck, S.; Vandewalle, M.; Garía, A. M.; Mascareñas, J. L.; Mouriño, A. *Tetrahedron Lett.* **1995**, *36*, 9023; (e) Moriarty, R. M.; Kim., J.; Brumer III, H. *Tetrahedron Lett.* **1995**, *36*, 9265.
6. (a) Corey, E. J.; Guzman-Perez, A.; Noe, M. C. *Tetrahedron Lett.* **1995**, *36*, 3481; (b) Corey, E. J.; Guzman-Perez, A.; Noe, M. C.*J. Am. Chem. Soc.* **1995**, *117*, 10805.
7. Fukuyama, T.; Laird, A. A.; Hotchkiss, L. M. *Tetrahedron Lett.* **1985**, *26*, 6291.
8. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
9. (a) Muralidharan, K. R.; de Lera, A. R.; Isaeff, S. D.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1993**, *58*, 1895; (b) Norman, A. W.; Bouillon, R.; Farach-Carson, M. C.; Bishop, J. E.; Zhou, L.-X.; Nemere, L.; Zhao, J.; Muralidharan, K. R.; Okamura, W. H. *J. Biol. Chem.* **1993**, *268*, 20022.
10. (a) Reddy, G. S. In *Vitamin D: Chemistry, Biology and Clinical Applications of the Steroid Hormone*; Norman, A. W.; Bouillon, R.; Thomasset, M., Ed.; University of California, Printing and Reprogrphics: Riverside, 1997; pp. 139-146. (b) Masuda, S.; Okano, T.; Kamao, M.; Kobayashi, T *ibid*; pp. 159-160.
11. All the spectral data of these compounds were consistent with those previously reported in Ref. 10a.
12. Ono, Y.; Watanabe, H.; Shiraishi, A.; Takeda, S.; Higuchi, Y.; Sato, K.; Tsugawa, N.; Okano, T.; Kobayashi, T.; Kubodera, N. *Chem. Pharm. Bull.* **1997**, *45*, 1626, and the references cited therein.
13. Okamura and co-workers reported that the A-ring of **1** is equilibrated between two chair comformers, the α- and the β-form, in 1: 1 ratio by the ^{1}H NMR analysis, and proposed that the β-form, in which the 1α-hydroxyl is equatorially oriented, may be responsible for biological activity. Synthesis and biological evaluation of the 2-methyl analogs **4** could settle out this issue because introduction of 2-methyl group would resulted in quilibrium shift to either one way in certain extent. The conformational analysis of the A-ring of **4** and its relationships to the biological activities are in progress and will be reported in due course; see: (a) Okamura, W. H.; Norman, A. W.; Wing, R. M. *Pro. Nat. Acad. Sci. USA* **1974**, *71*, 4194; (b) Wing, R. M.; Okamura, W. H.; Rego, A.; Pirio, M. R.; Norman, A. W. *J. Am. Chem. Soc.* **1975**, *97*, 4980.
14. Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511.
15. **2**(ααβ): ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 0.55 (3H, s), 0.94 (3H, d, J=6.4), 1.02 (3H, d, J=7.0), 1.22 (6H, s), 2.36 (1H, dd, J=5.5, 14.1), 2.65 (1H, dd, J=2.8, 14.1), 2.83 (1H, dd, J=4.3, 12.5), 3.72 (1H, bs), 3.97 (1H, d, J=3.0), 5.07 (1H, d, J=1.8), 5.30 (1H, d, J=1.8), 6.05 (1H, d, J=11.3), 6.43 (1H, d, J=11.3); UV (EtOH) λ_{max} 262 nm; MS *m/z* 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$), 376 (M^+-$3H_2O$), 361 (M^+-$3H_2O$-Me); HRMS *m/z* 430.3447 calcd for $C_{28}H_{46}O_3$ 430.3447.
16. Imae, Y.; Manaka, A.; Yoshida, N.; Ishimi, Y.; Shinki, T.; Abe, E.; Suda, T.; Konno, K.; Takayama, H.; Yamada, S. *Biochim. Biophys. Acta* **1994**, *1213*, 302.
17. Ishizuka, S.; Norman, A. W. *J. Steroid Biochem.* **1986**, *25*, 505.
18. Collins, S. J.; Ruscetti, F. W.; Gallagher, R. E.; Gallo, R. C. *J. Exp. Med.* **1979**, *149*, 969.
19. Honda, A.; Mori, Y.; Otomo, S.; Ishizuka, S.; Ikekawa, N. *Steroids* **1991**, *56*, 142.
20. Bishop, J. E.; Collins, E. D.; Okamura, W. H.; Norman, A. W. *J. Bone Min. Res.* **1994**, *9*, 1277.