

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

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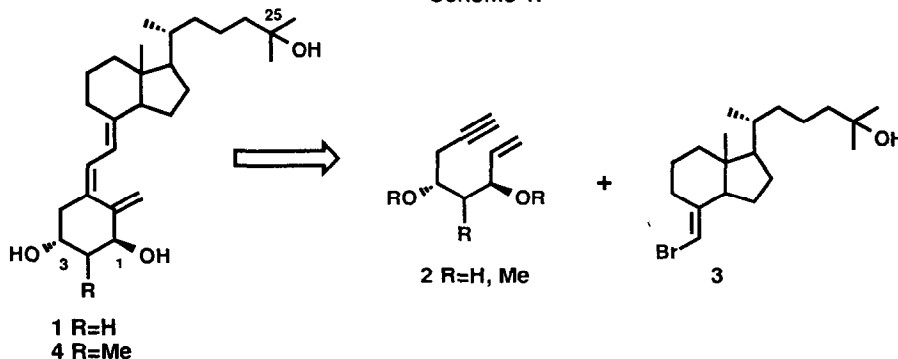
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Abstract: A novel and practical route to the A-ring enyne synthon (2), which can be versatile for a variety of A-ring analogs of 1 α ,25-dihydroxyvitamin D₃ (1), was developed. This novel method led to an improved synthesis of the A-ring diastereomers 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 $\alpha\beta$ -isomer to be more potent than 1. © 1998 Elsevier Science Ltd. All rights reserved.

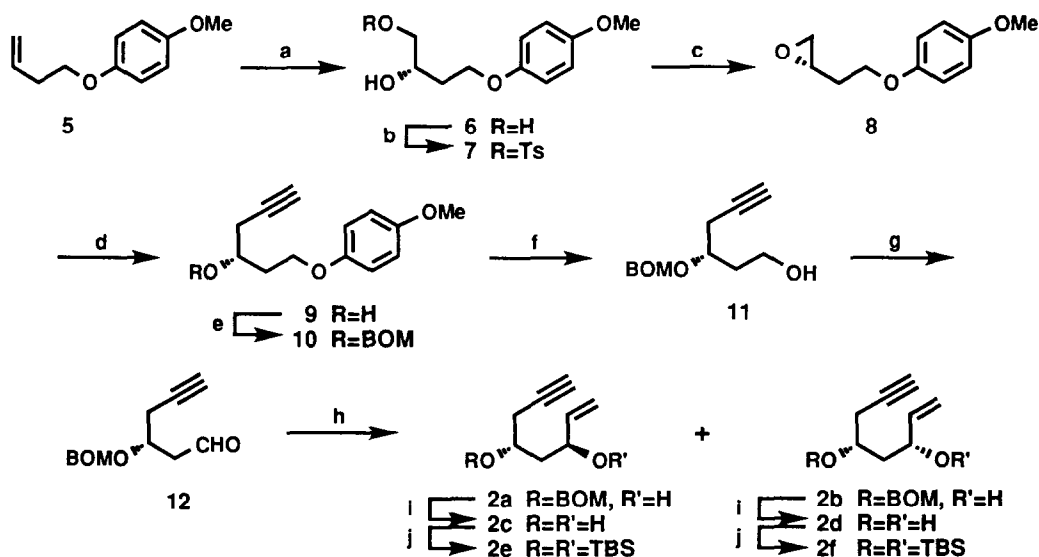
A large number of analogs of 1 α ,25-dihydroxyvitamin D₃ [1 α ,25-(OH)₂VD₃ (1)], the hormonally active metabolite of vitamin D₃, have been synthesized and biologically evaluated to investigate the structure-activity relationships and to develop potential therapeutic agents.¹ A majority of those are altered 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, synthetic 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.



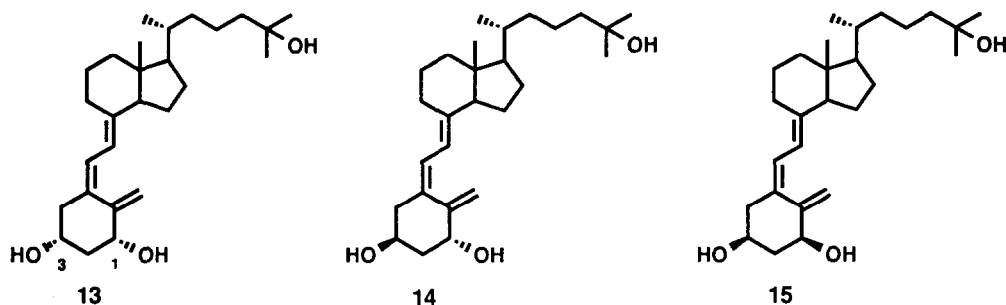
Scheme 2.



(a) AD-mix- α , t BuOH-H₂O, 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, CH₂Cl₂, rt, 90% (f) CAN, MeCN-H₂O, 0 °C, 99% (g) PDC, CH₂Cl₂, rt, 96% (h) VinylMgBr, toluene, -78 °C, 62% (i) PhSH-BF₃·Et₂O, CH₂Cl₂, 0 °C, 62% (j) TBSOTf, 2,6-Lutidine, CH₂Cl₂, 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., $[\alpha]_D^{26}$ -4.2° (c =0.38, CHCl₃).⁶ This was recrystallized from CH₂Cl₂-hexane to yield the enantiomerically pure specimen, mp. 64 °C, $[\alpha]_D^{26}$ -5.2° (c =1.35, CHCl₃). The diol **6** was converted to the epoxide **8**, $[\alpha]_D^{25}$ -13.6° (c =1.65, CHCl₃), by sequential treatment with TsCl and lithium hexamethyldisilazide through the mono-tosylate **7**, $[\alpha]_D^{26}$ +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**, $[\alpha]_D^{26}$ -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**, $[\alpha]_D^{28}$ -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**, $[\alpha]_D^{27}$ -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**, $[\alpha]_D^{26}$ -33.2° (c =1.01, CHCl₃), and **2b**, $[\alpha]_D^{27}$ -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 ¹H NMR analysis of their MTPA ester.⁸ Finally, the BOM protecting group was removed by PhSH-BF₃·OEt₂ to afford the desired enynes **2c**, $[\alpha]_D^{25}$ -1.2° (c =1.25, CHCl₃), and **2d**, $[\alpha]_D^{28}$ -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 $1\alpha,25\text{-(OH)}_2\text{VD}_3$, 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 $1\alpha,25\text{-(OH)}_2\text{VD}_3$.¹⁰ Therefore, these diastereomers have become important as well as $1\alpha,25\text{-(OH)}_2\text{VD}_3$ 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 $\text{Pd}(\text{dba})_3\text{-PPh}_3\text{-Et}_3\text{N}$ 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



We further applied this novel method to synthesize the new analogs, 2-methyl-1,25-dihydroxyvitamin D₃ (**4**) with its all possible diastereomers. The unique biological profile of $2\beta\text{-(hydroxypropoxy)-}1\alpha,25\text{-dihydroxyvitamin D}_3$ (ED-71) prompted us to synthesize these structurally 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 $\alpha\alpha\beta$ -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**), $[\alpha]^{26}_{\text{D}} -2.4^\circ$ ($c=1.01$, CHCl_3), by conventional manner in high yield, which on subsequent treatment with mCPBA to afford the epoxide (**18**), $[\alpha]^{26}_{\text{D}} -0.76^\circ$ ($c=1.18$, CHCl_3), in 97% yield. The acetylene unit was introduced by the reaction of **18** with ethynyltrimethylsilane/ $\text{BuLi-BF}_3\text{-OEt}_2$ in THF, giving a 1:1 mixture of the alcohols **19a**, $[\alpha]^{26}_{\text{D}} +3.3^\circ$ ($c=1.19$, CHCl_3), and **19b**, $[\alpha]^{26}_{\text{D}} -3.0^\circ$ ($c=1.01$, 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 diastereomeric 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 acetone.¹⁴ Finally, palladium-catalyzed coupling of the disilyl ether **24a**, $[\alpha]^{26}_{\text{D}} +10.3^\circ$ ($c=1.15$, 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 $\alpha\alpha\beta$ -isomer of the analog **2**($\alpha\alpha\beta$)¹⁵ in good yield. The same treatment of the isomer **23b** produced the $\beta\alpha\beta$ -isomer. The rest of the 2 α -methyl analogs, the $\alpha\alpha\alpha$ - and $\beta\alpha\alpha$ -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 2 β -methyl analogs. Thus, we have synthesized all eight possible diastereomers of the 2-methyl-1 α ,25-dihydroxyvitamin D₃ (**4**).

Table 1. The relative potency of the 2-methyl analogs to 1 α ,25-(OH)₂VD₃ (**1**).^a

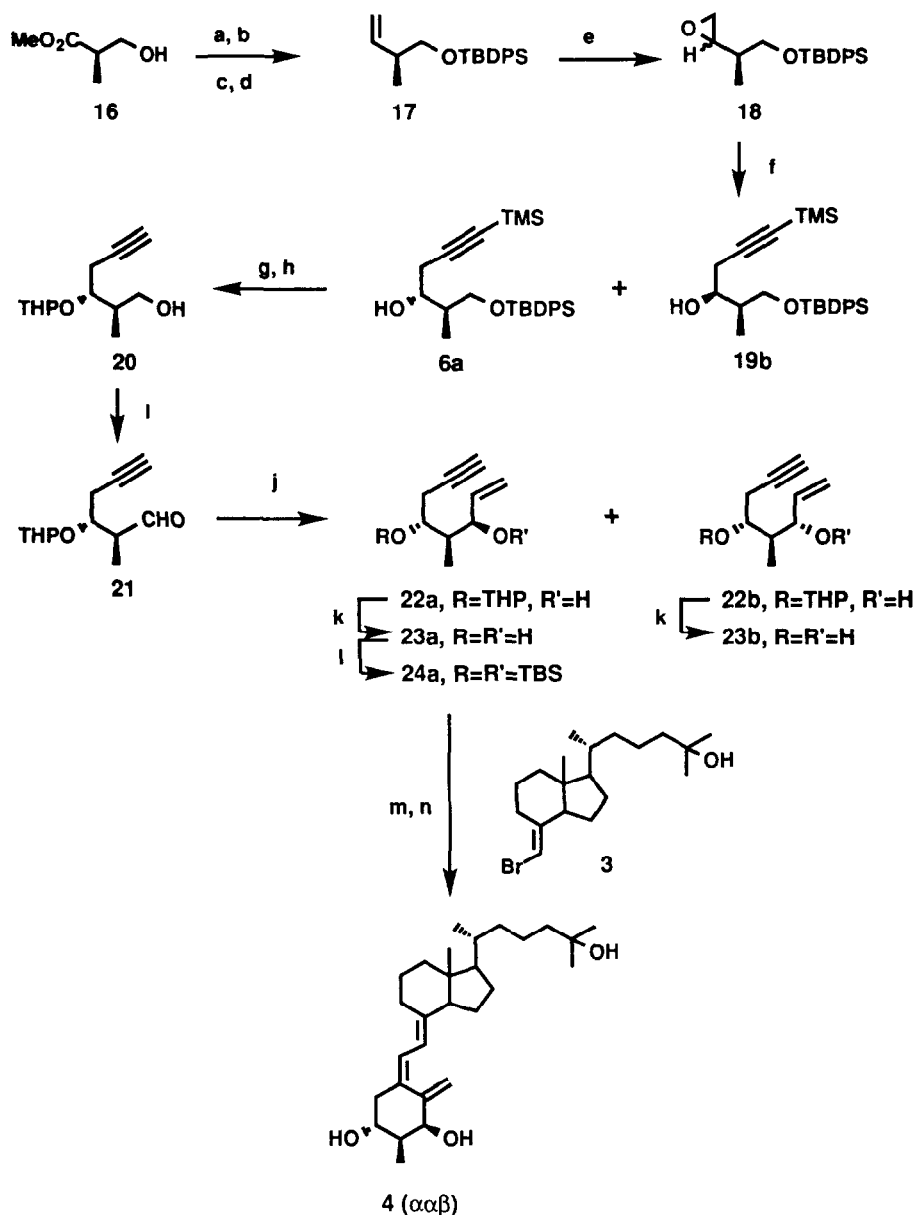
compounds ^b	VDR ^c	Ca ^d	HL-60	DBP ^e
1 α ,25-(OH) ₂ VD ₃	100	100	100	100
$\alpha\alpha\beta$	400	400	200	68
$\alpha\beta\beta$	13	2	10	79
$\alpha\alpha\alpha$	4	NT ^f	13	45
$\alpha\beta\alpha$	0.3	NT	1.5	21
$\beta\alpha\beta$	<0.1	NT	1.0	200
$\beta\beta\beta$	<0.1	NT	1.5	1000
$\beta\alpha\alpha$	<0.1	NT	0.5	1200
$\beta\beta\alpha$	0.8	NT	3.0	1300

(a) The potency of 1 α ,25-(OH)₂VD₃ 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 1 α -compounds exhibited significant to high affinity whereas the 1 β -compounds had virtually no affinity, which was consistent with those previously reported.^{9b} Furthermore, of the 1 α -compounds, the 2 α -methyl isomers ($\alpha\alpha\beta$ - and $\alpha\alpha\alpha$ -isomers) showed much higher potency than the corresponding 2 β -methyl isomers ($\alpha\beta\beta$ - and $\alpha\beta\alpha$ -isomers). Thus, the $\alpha\alpha\beta$ -isomer showed 4-fold higher affinity than 1 α ,25-(OH)₂VD₃ (**1**). This isomer also exhibited 4-fold higher potency in elevation of rat serum Ca concentration,¹⁷ while its 2-epimer, the $\alpha\beta\beta$ -isomer, had quite low activity. The rank order of potency in HL-60 cell differentiation¹⁸ was almost parallel to that of the VDR binding, and hence, the $\alpha\alpha\beta$ -isomer was twice as potent as the parent compound. In binding to the vitamin D binding protein (DBP) using calf serum,¹⁹ the 1 β -isomers showed high affinity, while the 1 α -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 1 α ,25-(OH)₂VD₃ 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 1 α ,25-(OH)₂VD₃, 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 $\alpha\alpha\beta$ -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) $\text{Ph}_3\text{P}=\text{CH}_2$, THF, 0°C -rt, 89% (e) mCPBA, CH_2Cl_2 , 0°C -rt, 97% (f) Ethynyltrimethylsilane/BuLi- $\text{BF}_3\cdot\text{OEt}_2$, 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) $\text{Pd}_2(\text{dba})_3\text{-PPh}_3\text{-Et}_3\text{N}$, toluene, 120°C (n) CSA/MeOH, 67%

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- 2**($\alpha\alpha\beta$): ^1H 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 ($\text{M}^+-\text{H}_2\text{O}$), 394 ($\text{M}^+-2\text{H}_2\text{O}$), 376 ($\text{M}^+-3\text{H}_2\text{O}$), 361 ($\text{M}^+-3\text{H}_2\text{O}-\text{Me}$); HRMS m/z 430.3447 calcd for $\text{C}_{28}\text{H}_{46}\text{O}_3$ 430.3447.
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