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Syntheses and Biological Evaluation of Novel 2α-Substituted 1α,25-Dihydroxyvitamin D_3 Analogues

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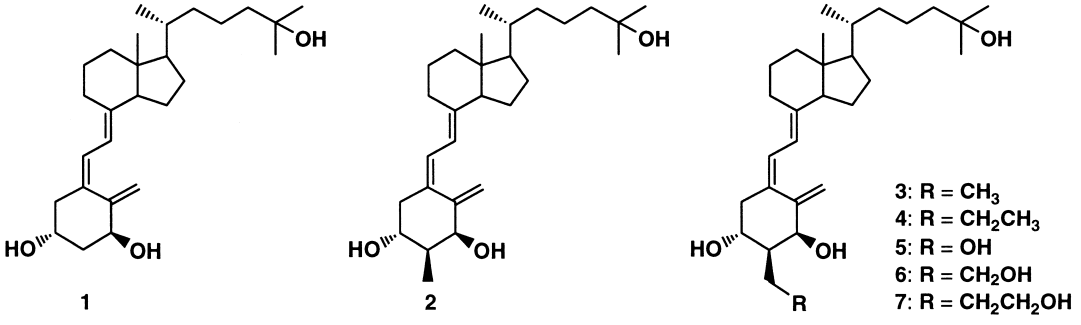
Abstract—Novel 2α-substituted 1α,25-dihydroxyvitamin D_3 analogues were efficiently synthesized and their biological activities were evaluated. 2α-Methyl-1α,25-dihydroxyvitamin D_3 (**2**), whose unique biological activities were previously reported, was modified to 2α-alkyl (ethyl and propyl) and 2α-hydroxyalkyl (hydroxymethyl, hydroxyethyl, and hydroxypropyl) analogues **3**–**7** by elongation of the alkyl chain and/or introduction of a terminal hydroxyl group. 2α-Hydroxypropyl-1α,25-dihydroxyvitamin D_3 (**7**) exhibited an exceptionally potent calcium-regulating effect and a unique activity profile.

The growing interest in 1α,25-dihydroxyvitamin D_3 (**1**), the hormonally active form of vitamin D_3, has prompted numerous efforts to synthesize vitamin D analogues as potential therapeutic agents.[1] A majority of them are modified in the side chain or in the A-ring.[2] A number of non-steroidal vitamin D mimics have been synthesized quite recently.[3]

In order to investigate the A-ring conformation–activity relationships, we synthesized all the possible A-ring diastereomers of 2-methyl-1,25-dihydroxyvitamin D_3 and found that the 2α-methyl isomer (**2**) showed higher potency than **1** in terms of VDR binding affinity, elevation of rat serum Ca concentration, and induction of HL-60 cell differentiation.[4] Furthermore, the combination of this 2α-methyl substitution with 20-epimerization, i.e., double modification, produced a much more potent analogue.[5] These results prompted us to design new analogues with further modification of the 2α-methyl group. We anticipated that if the 2α-methyl group were replaced by a longer 2α-alkyl or the 2α-hydroxyalkyl group, the resulting analogues would provide insight into the biological significance of the 2α-methyl substitution. We report here the synthesis of several new analogues, **3**–**7**, and their biological evaluation.

The analogues were synthesized according to the method developed by Trost and co-workers using palladium-catalyzed coupling of the A-ring enyne synthon with the CD-ring portion.[6] To introduce 2α-substituents into **1**,



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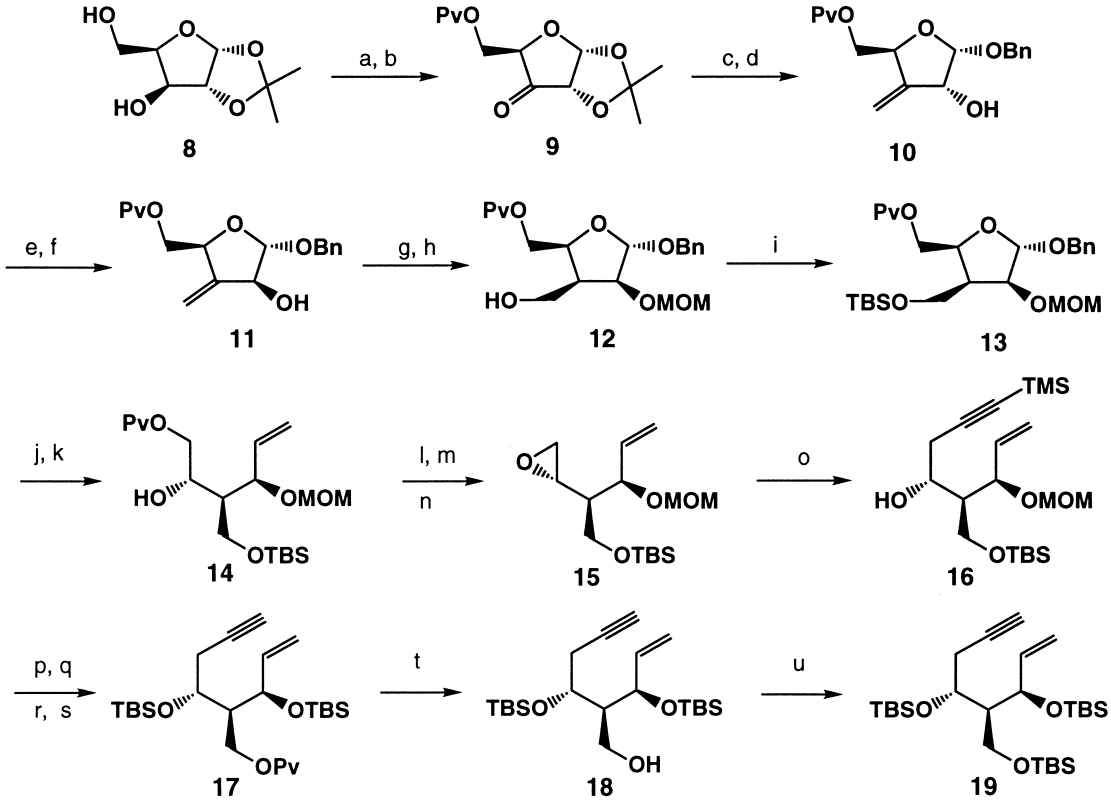
the synthesis of the A-ring portion was started with a D-xylose derivative (**8**), as shown in Scheme 1. Selective protection of the C-5 primary alcohol of **8** with a pivaloyl group, followed by oxidation with PCC gave the ketone **9** in 98% yield. Conversion of the ketone to an exomethylene group by means of the Wittig reaction, and subsequent treatment with 1 N HCl/BnOH afforded the alcohol **10** in 58% yield along with the β-isomer (32% yield). The C-2 hydroxyl group of **10** was inverted via the Mitsunobu reaction to give the secondary alcohol **11**, corresponding to the 1α-hydroxyl group of vitamin D analogues, in 84% yield. After protection of the alcohol moiety of **11** with a methoxymethyl (MOM) group, the exomethylene group was selectively converted to the 3β-hydroxymethyl compound **12** in high yield by hydroboration with 9-BBN. Protection of the primary hydroxyl group with a *tert*-butyldimethylsilyl (TBS) group afforded the TBS ether **13** in 99% yield. Thus, modification of the C-2 and C-3 hydroxyl groups of the D-xylose derivative, which correspond to the C-1 hydroxyl group and C-2 alkyl or hydroxyalkyl group of the vitamin D analogues, respectively, was accomplished.

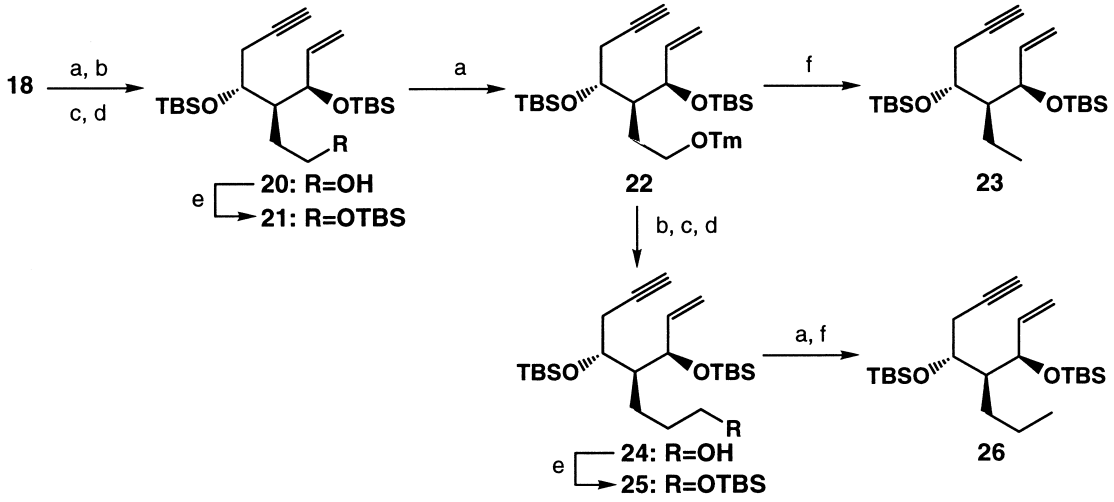
The requisite A-ring synthons were obtained from the D-xylose derivative **13**. Hydrogenolysis of the benzyl group of **13**, followed by ring opening by means of the Wittig reaction afforded **14** in good yield. This was converted to the epoxide **15** by sequential treatment with DIBAL-H, 2,4,6-trimethylbenzenesulfonyl chloride (TmCl) and lithium hexamethyldisilazide (LiHMDS) in high yield. The acetylene unit was introduced by the reaction of **15** with (trimethylsilyl)acetylene/*n*-BuLi-BF_3·OEt_2 in THF to give the alcohol **16** in 80% yield. Removal of both silyl protecting groups (TMS and TBS) by tetrabutylammonium fluoride (TBAF), and subsequent manipulation of the protecting groups by selective pivaloylation, removal of MOM group and silylation afforded the fully protected triol **17** in good yield. Finally, further protecting group manipulation through **18** gave the desired A-ring synthon **19** in high yield.

Elongation of the 2α-substituent of the A-ring synthon was carried out in a conventional manner as shown in Scheme 2. Sequential sulfonylation, cyanide addition and reduction of the alcohol **18** furnished the single-carbon-elongated alcohol **20** in good overall yield, and this was protected to give the A-ring synthon **21**. The alcohol **20** was further converted to the 2α-ethyl derivative through the tosylate **22**. In the same manner, the double-carbon-elongated synthons **25** and **26** were prepared from **22**–**24**.

Finally, palladium-catalyzed coupling of the A-ring synthons **19**, **21**, **23**, **25** and **26** with the CD-ring portion **27**, followed by deprotection with camphorsulfonic acid (CSA) in MeOH gave the 2α-alkyl and 2α-hydroxyalkyl analogues **3**–**7**[7] as shown in Scheme 3. Thus, we have



Scheme 1. (a) PvCl, Py, 86%; (b) PCC, MS 4A, CH_2Cl_2, 98%; (c) $Ph_3P^+CH_3Br^-$, KHMDS, THF, 85%; (d) 1 N HCl, BnOH, 1,4-dioxane/toluene, 58%; (e) *p*-nitrobenzoic acid, Ph_3P, DEAD, THF; (f) 10 mM NaOH, H_2O/MeOH, 84% (2 steps); (g) MOMCl, DPIEA, TBAI, CH_2Cl_2, 88%; (h) 9-BBN, THF, then 3 M NaOH, 30% H_2O_2, 85%; (i) TBSCl, imidazole, DMF, 99%; (j) H_2, $Pd(OH)_2$, EtOH; (k) $Ph_3P^+CH_{33}Br^-$, LiHMDS, THF, 79% (2 steps); (l) DIBAL-H CH_2Cl_2, 84%; (m) TmCl, DMAP, CH_2Cl_2; (n) LiHMDS, THF, 86% (2 steps); (o) TMSC≡CH, *n*-BuLi, BF_3·OEt_2, THF, 80%; (p) TBAF, THF, 99%; (q) PvCl, Py/CH_2Cl_2, 85%; (r) PPTS, *t*-BuOH, 74%; (s) TBSOTf, 2,6,-lutidine, CH_2Cl_2, 99%; (t) DIBAL-H, CH_2Cl_2, 96%; (u) TBSCL, imidazole, DMF, 88%.



Scheme 2. (a) TmCL, DMAP, Ch_2Cl_2; (b) NaCN, DMSO, 61–81% (2 steps); (c) DIBAL-H, CH_2Cl_2, 85–89%; (d) $NaBH_4$, MeOH, 82–98%; (e) TBSCl, imidazole, DMF, 78–83%; (f) LAH, Et_2O, 80% (2 steps).

synthesized five 2α-substituted 1α,25-dihydroxyvitamin D_3 analogues.

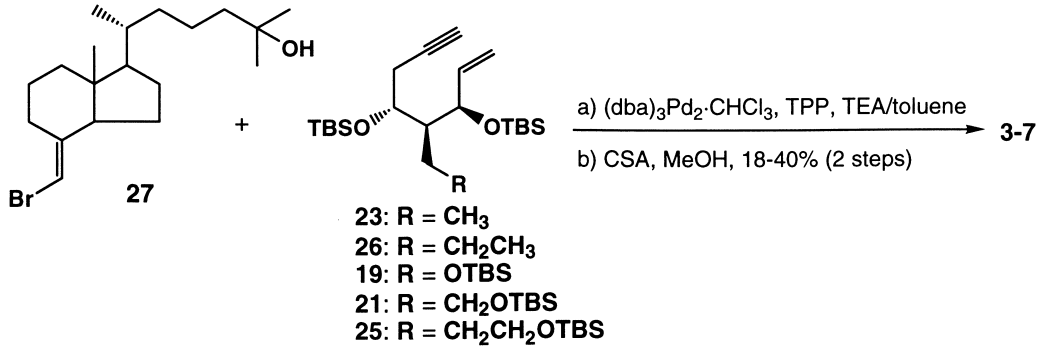
The results of biological evaluation are summarized in Table 1, in comparison with those of 1α,25-dihydroxyvitamin D_3 (**1**) and 2α-methyl-1α,25-dihydroxyvitamin D_3 (**2**). First of all, we examined the receptor binding in an assay using bovine thymus VDR.[8] The 2α-alkyl analogues **3** and **4** showed much lower binding affinity than the 2α-methyl analogue **2**; in other words, the shortest-chain analogue has the highest affinity among the 2α-alkyl analogues. In contrast, the 2α-hydroxypropyl analogue **7**, bearing the most elongated chain, showed the highest binding affinity, 3-fold higher than that of **1**, among the 2α-hydroxyalkyl analogues.[9] Therefore, the mode of binding of the 2α-hydroxyalkyl analogues may be different from that of the 2α-alkyl analogues. Steric hindrance of the 2α-alkyl group and hydrogen bonding ability of the 2α-hydroxyalkyl group are presumably involved in the difference of the mode of binding. Okano et al. previously reported the VDR binding affinity of 2β-alkyl (methyl, ethyl, propyl, butyl, pentyl, and hexyl) and 2β-hydroxyalkyl (hydroxypropyl, hydroxybutyl, hydroxypentyl, and hydroxyhexyl) analogues.[10] In contrast with those compounds, some of the 2α-substituted analogues showed significantly higher binding affinity than 1α,25-dihydroxyvitamin D_3.

Table 1. Relative potency of the synthesized analogues[a]

Compound	VDR[b] binding affinity	DBP[c] binding affinity	HL-60 cell[d] differentiation	Ca mobilization[e]
1 (1α,25-$(OH)_2VD_3$)	100	100	100	100
2 (2α-methyl)	400	44	444	658
3 (2α-ethyl)	40	48	106	68
4 (2α-propyl)	20	21	44	636
5 (2α-hydroxymethyl)	20	95	10	124
6 (2α-hydroxyethyl)	70	74	86	372
7 (2α-hydroxypropyl)	300	362	240	50,866

[a]The potency of 1α,25-$(OH)_2VD_3$ is normalized to 100 in each case.
[b]Bovine thymus.
[c]Rat serum.
[d]Cell differentiation was assessed in terms of expression of antigen CD11b.
[e]Rat serum Ca level.

The affinity for vitamin D binding protein (DBP)[11] was tested using rat serum DBP. The binding affinity was decreased in the 2α-alkyl analogues including **2**, whereas it was largely retained or even increased in the 2α-hydroxyalkyl analogues. In particular, the analogue **7** showed very high affinity for DBP, being similar in that respect to the β-isomer (2β-hydroxypropoxy-1α,25-dihydroxyvitamin D_3: ED-71).[12] This result implies that



Scheme 3.

there is no discrimination between the α- and β-isomers of the 2-hydroxypropyl analogues in DBP binding.

The rank order of potency for inducing differentiation in HL-60 cells[13] was parallel to that of VDR binding; that is, as the chain length became longer, the potency decreased in 2α-alkyl analogues, whereas it increased in the 2α-hydroxyalkyl analogues. Only compounds **2** and **7** showed much higher activity than 1α,25-dihydroxyvitamin D_3 in this experiment.

Most of the new analogues exhibited higher calcium-mobilizing activity[14] than **1**; in particular, the analogue **7** showed approximately 500 times higher potency, and this remarkably high activity is unique among vitamin D analogues reported to date. Therefore, this 2α-hydroxypropyl analogue **7** may provide clues to achieving separation of the biological functions of vitamin D. In the case of the hydroxyalkyl series, the longer the chain, the higher the activity. This rank order of potency is parallel to that of VDR binding affinity, implying that the calcium-regulating effect of the 2α-hydroxyalkyl analogues may involve interaction with VDR. In the alkyl series, in which VDR binding affinity decreased with increasing chain length, the 2α-propyl analogue **4** showed anomalously high calcium-mobilizing activity, comparable to that of **2**. This result is not easy to rationalize in terms of a VDR-dependent mechanism, and therefore, the analogue **4** may exert its calcium-regulating effect through a distinct mechanism from the other analogues.

Thus, the activity profiles of the synthesized analogues are high structure-sensitive, in that even a single-carbon chain difference greatly alters the profile. Consequently, these analogues should be useful for studies on the action mechanism of vitamin D, and also as lead compounds for developing therapeutic agents. Further studies, however, are needed to elucidate fully the activity profiles and modes of action of these analogues.

In this study, we have developed a synthetic route to A-ring enyne synthon, which can be useful for 2α-substituted vitamin D analogues, starting from D-xylose, and utilized it for the synthesis of five novel 2α-substituted vitamin D analogues **3**–**7** by palladium-catalyzed convergent method. In the light of the importance of 2-substituted vitamin D analogues, as exemplified by ED-71,[10,12] this novel procedure has a great advantage, because it should be applicable to a variety of 2α-substituted vitamin D analogues. Biological evaluation of these analogues demonstrated that they have unique activity profiles, and in particular, the 2α-hydroxypropyl analogue **7** exhibited exceptionally potent calcium-regulating activity. Further investigation along this line should provide insight into the biological significance of 2-substituted vitamin D analogues.

Acknowledgements

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7. **3**: ^{1}H NMR (400 MHz, $CDCl_3$–D_2O/TMS) δ 0.53 (3H, s), 0.94 (d, 3H, J=6.6 Hz), 0.95 (3H, t, J=7.4 Hz), 1.21 (6H, s), 2.24 (1H, dd, J=8.0, 12.8 Hz), 2.66 (1H, dd, J=4.4, 12.8 Hz), 2.83 (1H, m), 3.89 (1H, dt, J=4.4, 8.0 Hz), 4.39 (1H, d, J=2.2 Hz), 4.99 (1H, d, J=2.2 Hz), 5.27 (1H, d, J=1.5 Hz), 6.00 (1H, d, J=11.4 Hz), 6.40 (1H, d, J=11.4 Hz) ; HREIMS 444.3604 calcd for $C_{29}H_{48}O_3$ (M^+) 444.3603. **4**: ^{1}H NMR (400 MHz, $CDCl_3$–D_2O/TMS) δ 0.53 (3H, s), 0.94 (3H, d, J=6.4 Hz), 1.01 (3H, t, J=6.8 Hz), 1.21 (6H, s), 2.24 (1H, dd, J=8.4, 13.2 Hz), 2.66 (1H, dd, J=4.0, 13.2 Hz), 2.83 (1H, m), 3.88 (1H, dt, J=4.4, 8.4 Hz), 4.36 (1H, d, J=3.3 Hz), 4.99 (1H, d, J=1.8 Hz), 5.26 (1H, d, J=1.8 Hz), 6.00 (1H, d, J=11.4 Hz), 6.40 (1H, d, J=11.4 Hz); HREIMS 458.3755 calcd for $C_{30}H_{50}O_3$ (M^+) 458.3760. **5**: ^{1}H NMR (400 MHz, $CDCl_3$–D_2O/TMS) δ 0.53 (3H, s), 0.94 (3H, d, J=6.8 Hz), 1.21 (6H, s), 2.31 (2H, dd, J=9.9, 13.0 Hz), 2.67 (1H, dd, J=4.6, 13.0 Hz), 2.84 (1H, m), 3.96 (1H, dd, J=4.6, 11.2 Hz), 4.05 (1H, dd, J=4.6, 11.2 Hz), 4.24 (1H, dt, J=4.6, 9.9 Hz), 4.46 (1H, d, J=2.9 Hz), 5.02 (1H, d, J=1.8 Hz), 5.29 (1H, d, J=1.8 Hz), 5.98 (1H, d, J=11.0 Hz), 6.45 (1H, d, J=11.0 Hz); HREIMS m/z 428.3287 calcd for $C_{28}H_{44}O_3$ (M^+-H_2O) 428.3290. **6**: ^{1}H NMR (400 MHz, $CDCl_3$–D_2O/TMS) δ 0.53 (3H, s), 0.94 (3H, d, J=6.4 Hz), 1.21 (6H,s), 2.26 (1H, dd, J=8.0, 13.0 Hz), 2.66 (1H, dd, J=4.0, 13.0 Hz), 2.83 (1H, m), 3.79 (2H, m), 3.94 (1H, dt, J=4.0, 8.0 Hz), 4.37 (1H, d, J=2.2 Hz), 5.02 (1H, d, J=1.8 Hz), 5.30 (1H, bs), 6.01 (1H, d, J=11.2 Hz), 6.40 (1H, d, J=11.2 Hz); HREIMS m/z 460.3557 calcd for $C_{29}H_{48}O_4$ (M^+) 460.3553. **7**: ^{1}H NMR (400 MHz, $CDCl_3$-D_2O/TMS) δ 0.53 (3H, s), 0.93 (3H, d, J=6.6 Hz), 1.21 (6H, s), 2.25 (1H, dd, J=8.0, 13.2 Hz), 2.66 (1H, dd, J=4.0, 13.2 Hz), 2.83 (1H, m), 3.71 (2H, t, J=5.6 Hz), 3.90 (dt, 1H, J=4.0, 8.0 Hz), 4.38 (d, 1H, J=2.9 Hz), 5.00 (1H, d, J=1.8 Hz), 5.28 (1H, bs), 6.00 (1H, d, J=11.2 Hz), 6.40 (1H, d, J=11.2 Hz); HREIMS 474.3709 calcd for $C_{30}H_{50}O_4$ (M^+) 474.3709.
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