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Synthesis and Biological Evaluation of 1α,24-Dihydroxy-25-nitrovitamin D_3

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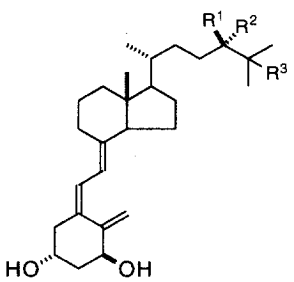
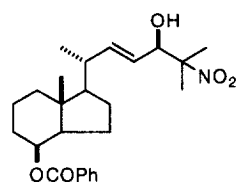
Abstract: 1α,24(*R*)-Dihydroxy-25-nitrovitamin D_3 **1** and 1α,24(*S*)-dihydroxy-25-nitrovitamin D_3 **2** were synthesized using the palladium-catalyzed alkylative enyne cyclization reaction. Their biological properties were studied based on VDR binding affinity and HL-60 cell differentiation activity.

1α,25-Dihydroxyvitamin D_3 **3**, an active metabolite of vitamin D_3, mediates calcium and phosphorous homeostasis,[1] and influences cell proliferation and cell differentiation.[2] For separating the calcemic effect from the differentiation activity, many structural analogues of **3** have been synthesized. Among them, 1α,24(*R*)-dihydroxyvitamin D_3[3] **4** is known to induce keratinocyte differentiation[4] with less hypercalcemic activity, and is used as a therapeutic agent for psoriasis. Although **4** is a potent active Vitamin D_3 analogue, it is also known to be metabolized to 1α,24(*R*),25-trihydroxyvitamin D_3 **6** thus reducing its biological activities.[5]

We previously reported[6] the preparation of the CD-ring synthon **7** having a nitro group in the side chain using the asymmetric nitroaldol reaction, which could be utilized after denitration for the synthesis of **4**. On the other hand, active vitamin D_3 analogues, which focused on the inhibition of hydroxylation at the 25-position, by the introduction of a substituent have been rarely reported.[7] Herein, we wish to describe the synthesis of 1α,24(*R*)-dihydroxy-25-nitrovitamin D_3 **1** and 1α,24(*S*)-dihydroxy-25-nitrovitamin D_3 **2**, which are the first analogues of vitamin D_3 bearing a nitro group in the side chain, and also the results of their biological properties.



1 : R^1 = OH, R^2 = H, R^3 = NO_2
2 : R^1 = H , R^2 = OH, R^3 = NO_2
3 : R^1 = H, R^2 = H, R^3 = OH
4 : R^1 = OH, R^2 = H, R^3 = H
5 : R^1 = H, R^2 = OH, R^3 = H
6 : R^1 = OH, R^2 = H, R^3 = OH



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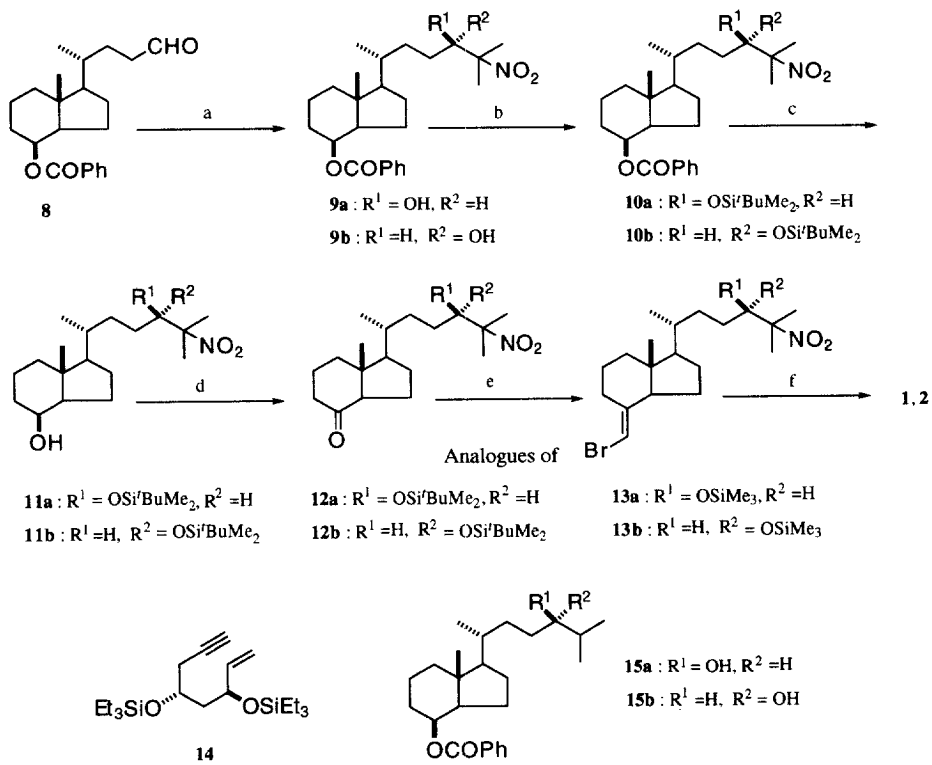
PII: S0960-894X(99)00013-X

Synthesis

The key synthons **13a** and **13b** for the palladium-catalyzed alkylative enyne cyclization reaction,[8a] which is considered one of the most useful methods for constructing the Vitamin D triene system,[8] were prepared from the known CD-ring aldehyde[9] **8** (Scheme 1).

The aldehyde **8** was subjected to the non-stereospecific nitroaldol reaction[10] with 2-nitropropane using $^{t}BuMe_2SiCl$, tetrabutylammonium fluoride, and triethylamine to afford diastereomeric nitroaldol products **9a** (41%) and **9b** (32%) after separation by column chromatography. Each absolute configuration of **9a** and **9b** was determined by HPLC analysis by comparing the retention time of each denitration product **15a** and **15b** using Bu_3SnH in the presence of 2,2'-azobisisobutyronitrile (AIBN)[6] with that of authentic samples after the denitration. The silylation of the nitroaldol adducts **9a** and **9b** led to the respective silylated alcohols **10a** (99%) and **10b** (96%). The deprotection of benzoates **10a** and **10b** was carried out by reduction with $^{i}Bu_2AlH$ to give alcohols **11a** (94%) and **11b** (99%). The oxidation of the resulting alcohols **11a** and **11b** with pyridinium chlorochromate (PCC) yielded ketones **12a** (75%) and **12b** (91%) according to the cited literature.[9] The bromomethylation of the ketones followed by exchange of the protecting group from TBDMS to TMS furnished the key CD-ring synthons **13a** (44%) and **13b** (46%). Each CD-ring synthon was



Scheme 1. a) $^{i}PrNO_2$, NEt_3, Bu_4NF, $^{t}BuMe_2SiCl$; b) $^{t}BuMe_2SiOTf$, 2,6-lutidine; c) $^{i}Bu_2AlH$; d) PCC; e) (1)$Ph_3P^{+}CH_2Br\ Br^{-}$, $NaN(TMS)_2$, (2)$LiBF_4$, H_2SO_4, (3)Me_3Si-imidazole; f) (1)**13**, $Pd_2(dba)_3{\cdot}CHCl_3$, PPh_3, NEt_3, (2) pyridinium *p*-toluenesulfonate.

coupled with the A-ring enyne[11] **14** using $Pd_2(dba)_3 \cdot CHCl_3$, triethylamine and triphenylphosphine, and subsequently deprotected with pyridinium *p*-toluensulfonate to yield 1α,24(*R*)-dihydroxy-25-nitrovitamin D_3 **1** (41%) and 1α,24(*S*)-dihydroxy-25-nitrovitamin D_3 **2** (42%), respectively.[12] These obtained compounds showed satisfactory spectral data (NMR, MS, UV, etc).

Biological Evaluation

Vitamin D receptor (VDR) binding affinity was evaluated using chick intestinal VDR.[13] 1α,24(*R*)-Dihydroxy-25-nitrovitamin D_3 **1** showed a high affinity to VDR comparable to that of 1α,25-dihydroxyvitamin D_3 **3** and 1α,24(*R*)-Dihydroxyvitamin D_3 **4**. Whereas, 1α,24(*S*)-dihydroxy-25-nitrovitamin D_3 **2** showed about one-tenth the affinity of **1** as almost similar affinity to 1α,24(*S*)-dihydroxyvitamin D_3 **5**.

Concerning the cell differentiation activity toward HL-60 cells,[14] **1** exhibited almost a 2-fold higher activity than **3** similar to **4**. On the other hand, the activity of **2** was about 10 times lower than those of the three derivatives (**1**, **3**, **4**) similar to **5**.

These results showed that the nitro group at the 25-position seemed to have little effect on both the vitamin D receptor (VDR) binding affinity and cell differentiation activity toward HL-60 cells.

Table1. Biological Activity of 1α,25-Dihydroxyvitamin D_3 Analogues[1)]

Analogue	VDR binding[2)]	HL-60 cell differentiation[3)]
1α,24(*R*)-dihydroxy-25-nitrovitamin D_3 **1**	93	182
1α,24(*S*)-dihydroxy-25-nitrovitamin D_3 **2**	10	12
1α,25-dihydroxyvitamin D_3 **3**	100	100
1α,24(*R*)-dihydroxyvitamin D_3 **4**	131	182
1α,24(*S*)-dihydroxyvitamin D_3 **5**	10	18

1) The activity of all analogues are compared with that of 1α,25-Dihydroxyvitamin D_3 **3**.

2) Binding was assessed by relative affinity for chick intestinal vitamin D receptor.

3) Cell differentiation was assessed in terms of 4-nitro-blue tetrazolium (NBT) reductivity.

Conclusion

We have synthesized two novel analogues of active vitamin D_3 having a nitro group at the 25-position. The 24R-isomer (1α,24(*R*)-dihydroxy-25-nitorovitamin D_3 **1**) showed comparable biological activities to 1α,25-dihydroxyvitamin D_3 **3** in VDR binding affinity and cell differentiation activity and is considered promising candidate for further evaluation.

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12. **1**: ^{1}H NMR (200 MHz, $CDCl_3$, ppm) δ 0.56 (3H, s), 0.92 (3H, d, *J* = 6 Hz), 1.05 - 2.90 (20H, m), 1.57 (3H, s), 1.58 (3H, s), 3.90 - 4.05 (1H, m), 4.15 - 4.30 (1H, m), 4.40 - 4.50 (1H, m), 4.95 - 5.05 (1H, m), 5.30 - 5.40 (1H, m), 6.02 (1H, d, *J* = 12 Hz), 6.38 (1H, d, *J* = 12 Hz); UV (EtOH) λ_{max} 264 nm; MS *m/z* 461 (M^+); HRMS *m/z* 461.3121, calcd. for $C_{27}H_{43}NO_5$: 461.3141.

    2: ^{1}H NMR (200 MHz, $CDCl_3$, ppm) δ 0.56 (3H, s), 0.92 (3H, d, *J* = 6 Hz), 1.05 - 2.90 (20H, m), 1.57 (3H, s), 1.58 (3H, s), 3.90 - 4.05 (1H, m), 4.15 - 4.30 (1H, m), 4.40 - 4.50 (1H, m), 4.95 - 5.05 (1H, m), 5.30 - 5.40 (1H, m), 6.02 (1H, d, *J* = 12 Hz), 6.38 (1H, d, *J* = 12 Hz); UV (EtOH) λ_{max} 264 nm; MS *m/z* 461 (M^+); HRMS *m/z* 461.3132 , calcd. for $C_{27}H_{43}NO_5$: 461.3141.
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