Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 381–384

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

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

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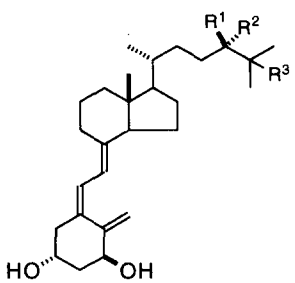
Received 2 November 1998; accepted 16 December 1998

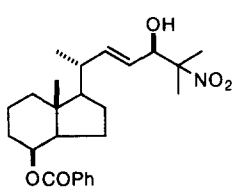
Abstract: 1α,24(*R*)-Dihydroxy-25-nitrovitamin D_3 **1** and 1α,24(*S*)-dihydroxy-25-nitrovitamin D_3 **2** were synthesized using the palladium-catalyzed alkylative enyne cyclization reaction. Their biological properties were studied based on VDR binding affinity and HL-60 cell differentiation activity.

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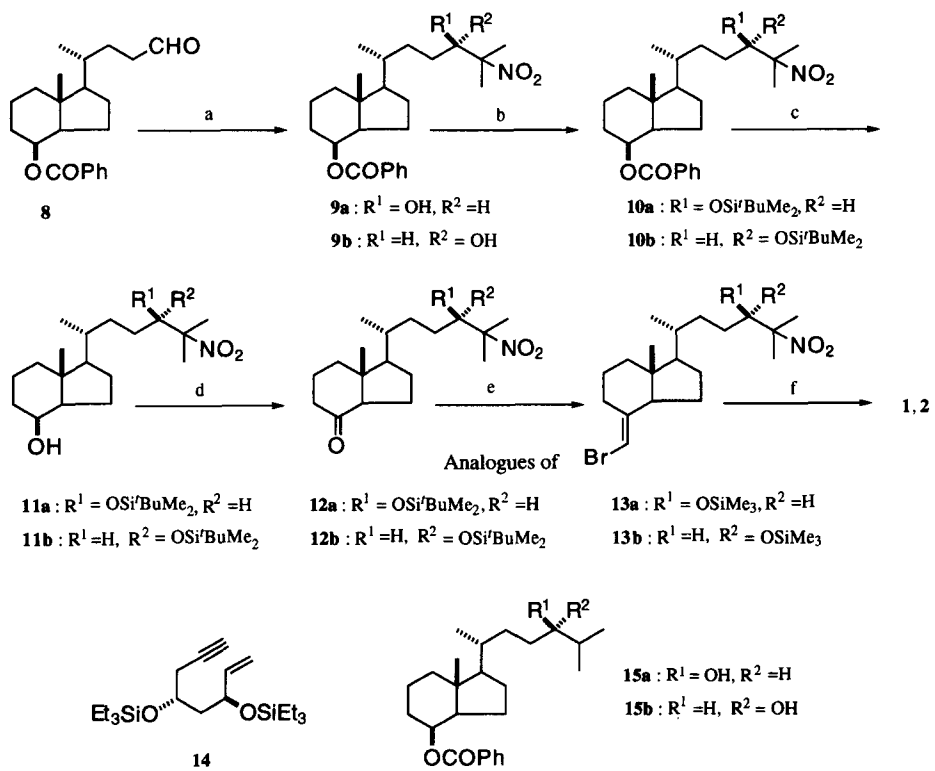
PII: S0960-894X(99)00013-X

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$_2$SiCl, 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$_2$AlH 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$_4$NF, ^{t}BuMe$_2$SiCl; b) ^{t}BuMe$_2$SiOTf, 2,6-lutidine; c) ^{i}Bu$_2$AlH; d) PCC; e) (1)Ph$_3$P$^+$CH$_2$Br Br$^-$, NaN(TMS)$_2$, (2)LiBF$_4$, H_2SO_4, (3)Me$_3$Si-imidazole; f) (1)**13**, Pd$_2$(dba)$_3$·CHCl$_3$, PPh$_3$, NEt$_3$, (2) pyridinium *p*-toluenesulfonate.

coupled with the A-ring enyne[11] **14** using $Pd_2(dba)_3$·$CHCl_3$, 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.

Acknowledgment

The authors gratefully acknowledge S. Sugihara for his technical assistance. We also thank Dr. T. Tanaka for his suggestions.

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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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