Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3533–3536

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Efficient Synthesis of New Stable 1α,25-Dihydroxy-19-norvitamin D_3 Analogues Containing Amide Bond

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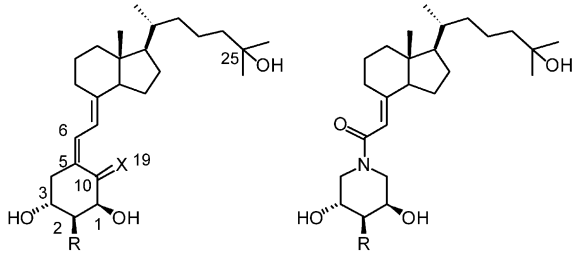
Received 5 August 2002; accepted 17 September 2002

Abstract—The design and synthesis of new 1α,25-dihydroxy-19-norvitamin D_3 analogues **3a–c**, which have an amide bond in the molecule instead of the diene, are described. The A-ring moiety was constructed by a (3*S*,5*S*)-3,5-dihydroxypiperidine derivative (**9**, **11**, or **13**) prepared from D-mannose, and a CD-ring carboxylic acid **16** was synthesized from Grundmann's ketone. Coupling those parts gave desired **3a–c** in good yield. This strategy can be applied in combinatorial chemistry; therefore, those compounds would be applicable as useful tools in the development of new drugs.

1α,25-Dihydroxyvitamin D_3 (**1**) is the major active metabolite of vitamin D_3, which exerts hormonal control over important physiological processes, including calcium and phosphorus metabolism, cellular differentiation and immune reactions.[1] A number of vitamin D analogues have been synthesized in order to investigate the biological roles of vitamin D and to develop potential therapeutic agents.[2] Since effective doses of **1** may cause hypercalcemia, the therapeutic use of **1** in the treatment of certain cancers and skin disorders is limited. Dissociation of the calcemic activity from the other activities of **1** is one of the important issues in searching for novel drugs based on vitamin D analogues. DeLuca et al. synthesized a 19-nor derivative **2**, which is a non-calcemic analogue of **1** that still preserves its cell differentiating activities (Fig. 1).[3]

Previously, we synthesized several 1α,25-dihydroxyvitamin D_3 derivatives, which systematically introduced a 2α-alkyl, 2α-hydroxyalkyl, or 2α-hydroxyalkoxyl group into the parent hormone **1**, in order to investigate the A-ring conformation– and structure–activity relationships.[4–6] Some of these analogues exhibited interesting biological activities, in particular, 2α-methyl (**1a**),[4] 2α-hydroxypropyl (**1b**),[5] and 2α-hydroxypropoxyl (**1c**)[6] analogues showed higher potency than the natural hormone **1** in terms of bovine thymus vitamin D receptor (VDR) binding affinity, elevation of rat serum calcium concentration, and induction of HL-60 cell differentiation (Fig. 1). For example, compound **1b** exhibited 3-fold higher VDR binding affinity and more than double potency in inducing differentiation of HL-60 cells as compared to **1**.[5a]



1: R = H, X = CH_2
1a: R = CH_3, X = CH_2
1b: R = $(CH_2)_3OH$, X = CH_2
1c: R = $O(CH_2)_3OH$, X = CH_2
2: R = H, X = H_2

3a: R = H
3b: R = OH
3c: R = $O(CH_2)_3OH$

Figure 1. Structures of the natural hormone **1**, and its 2α-substituted analogues **1a–c** with higher binding affinity for VDR than that of **1**, DeLuca's 19-nor derivative **2**, and amide derivatives **3a–c**.

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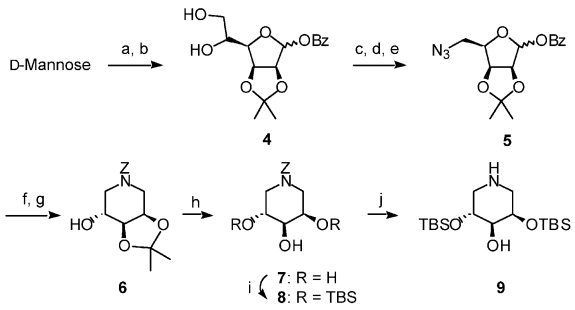
PII: S0960-894X(02)00800-4

Based on these results, we then focused on modification of the diene moiety in the 19-nor skeleton of the B-*seco* steroid compound.[7] We anticipated that if the diene was replaced by an amide bond, the resulting analogues would retain the original biological activities of 19-nor-vitamin D_3 (**2**), since they would possibly take on a similar 'flat' conformation.

Our designed analogues are shown in Figure 1. The A-ring moiety was substituted with a piperidine ring having two hydroxyls at C1α and C3β (*seco*-steroidal numbering) with the appropriate stereochemistry, which play a crucial role in VDR binding and biological actions.[8] The diene part was converted to an amide bond to connect with the normal CD-ring moiety. We also planned modification at the C2 position with the substituent of the 3-hydroxypropoxyl group, because this motif can strengthen binding affinity to VDR.[5,9] The amide structures **3a–c** would allow the N–C6 bond to rotate, whose original structure of the C5–C6 bond is fixed in the natural hormone **1**. The relative positions of the two hydroxyls at C1α and C3β toward the VDR ligand binding domain (LBD) are retained with the rotation, and the artificial C2 substituent may settle down in an α or a β-orientation in preference when **3b** or **3c** complexes with LBD. In Figure 2, the X-ray crystal structure of **1** (red) docking in LBD of VDR[10] and the modeled structure of the amide D_3 **3a** (blue) in the same cavity of the VDR are shown. The latter structure constructed by molecular dynamics calculations (AMBER* force field) using MacroModel ver. 6.5, and **3a** could interact with the VDR through six hydrogen bonds (dotted lines) from the C1α, C3β, and C25-hydroxyls as in **1**. This modeling also encouraged us to synthesize the amide D_3 analogues.

Scheme 1 shows the synthesis of the A-ring portions **8** (an intermediate) and **9**. Treatment of D-mannose with anhydrous $CuSO_4$ and a catalytic amount of concd H_2SO_4 in acetone followed by benzoylation of the anomeric hydroxyl group gave a D-mannofuranose derivative in 86% yield. Selective deprotection of the O4–O6 acetonide using 70% aqueous acetic acid produced D-mannofuranoside **4**. Oxidative diol cleavage of **4** with sodium periodate, and reduction of the resultant aldehyde afforded the primary alcohol. Azide **5** was prepared from the alcohol through tosylate in good yield. After removal of the benzoyl group of **5**, reductive amination with H_2/cat. $Pd(OH)_2$ in methanol followed by protection of the amino group with Cbz gave the piperidine derivative **6** in 87% yield. Deprotection of the acetonide of **6**, then introduction of the TBS group to the C1 and C3 hydroxyl groups afforded **8** in 92 and 87% yields, respectively. Finally, the Cbz group was hydrogenated with cat. Pd/C to give the desired A-ring part **9** for **3b** in 71% yield.



Scheme 1. Reagents and conditions: (a) (i) anhydrous $CuSO_4$, acetone, concd H_2SO_4, (ii) BzCl, pyridine (86% in two steps); (b) 70% AcOH aq (quant); (c) (i) $NaIO_4$, Et_2O, H_2O, (ii) $NaBH_4$, MeOH (64% in two steps); (d) TsCl, pyridine (75%); (e) NaN_3, DMF (72%); (f) 1 N NaOH aq, MeOH (97%); (g) (i) H_2, cat. $Pd(OH)_2$, MeOH, (ii) CbzCl, Et_2O, sat. $NaHCO_3$ aq (87% in two steps); (h) 80% AcOH aq, 90 °C (92%); (i) TBSCl, Et_3N, DMF (87%); (j) H_2, cat. Pd/C, MeOH (71%).

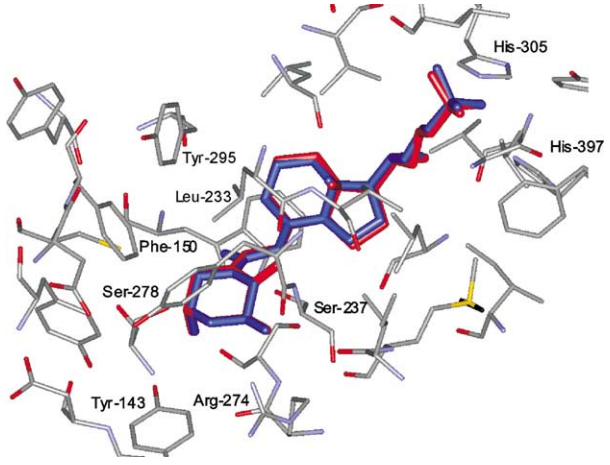
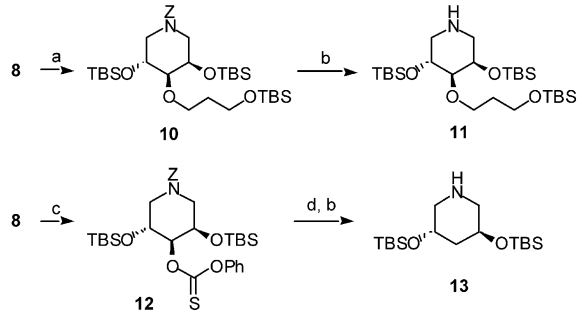


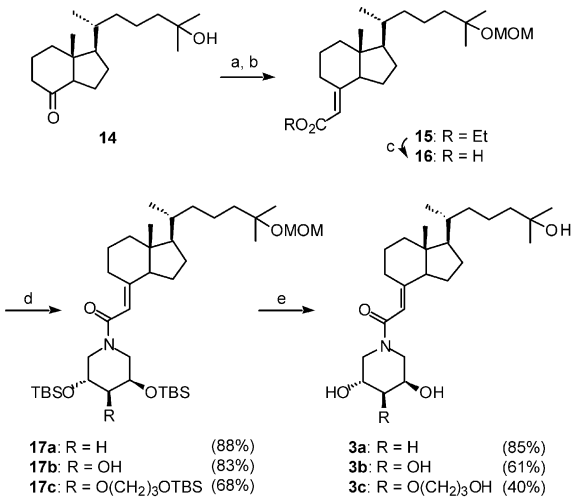
Figure 2. Crystal structure of VDR bound to **1** (red) by Moras et al.[10] and computer modeling of **3a** (blue) in the VDR ligand binding domain.

We synthesized two additional piperidine derivatives **11** and **13** from **8** as shown in Scheme 2. The A-ring portion **11** was synthesized by Williamson ether synthesis using (3-bromopropoxy)-*tert*-butyldimethylsilane and sodium hydride, followed by hydrogenolysis of the Cbz group. On the other hand, the natural type of A-ring portion **13** was obtained by radical reduction of thiocarbonate **12** with tributyltin hydride and AIBN in good yield.

The CD-ring portion was prepared from Grundmann's ketone **14**[11] as shown in Scheme 3. After protecting the C25 hydroxyl group with MOM, Horner–Wadsworth–Emmons reaction using triethyl phosphonoacetate and sodium hydride in THF gave the ester **15** in an excellent



Scheme 2. Reagents and conditions: (a) $TBSO(CH_2)_3Br$, NaH, DMF (20%); (b) H_2, Pd/C, MeOH (quant); (c) PhOC(S)Cl, DMAP, CH_3CN (36%); (d) Bu_3SnH, AIBN, benzene (95%).



Scheme 3. Reagents and conditions: (a) MOMCl, DIEA, CH_2Cl_2 (quant); (b) triethyl phosphonoacetate, NaH, THF (98%); (c) 1 N NaOH, MeOH (86%); (d) **9**, **11**, or **13**, BOP, DIEA, DMF (68–88%); (e) *p*-TsOH, MeOH (40–85%).

yield.[12] The ethyl ester of **15** was subjected to hydrolysis by 1 N aqueous NaOH in a MeOH solution to afford the desired carboxylic acid **16** in 86% yield. Subsequent condensation of **16** with the piperidine derivatives **9**, **11**, and **13** using a BOP reagent[13] and DIEA provided the amides **17a–c** in good yields, respectively. Finally, deprotection under acidic conditions furnished the target amide analogues **3a–c**[14] in considerable yield.

We tested VDR binding affinity of the amide analogues **3a–c** using bovine thymus VDR[15] though, unfortunately, we could not recognize the affinity for the VDR (3×10^{-6}, 2×10^{-6}, and 3×10^{-6} times that of **1** for **3a**, **3b**, and **3c**, respectively).[16]

Two reasons were considered. One would be the electrostatic difference between the amide moiety and the diene part. The polar amide bond in **3a–c** could not be well suited for the hydrophobic region surrounded by amino acid residues Phe-150, Leu-233, Tyr-295, and Trp-286 of VDR, which would originally accept the diene part of **2**. The other would be the difference in steric energy (9.8 kcal/mol; ab initio MO method, HF/6–31G*) between the active structure in the VDR and the energy minimized structure. Figure 3a shows the energy minimized structure of **3a** (green) lacking the side chain, as calculated by Monte Carlo conformational search and superimposed on the modeled structure of **3a** (yellow) binding to the VDR. Conformation of the A-ring is quite different from each other. However, the X-ray structure of **1** (yellow) in the VDR and the energy minimized structure of **1** (green) are shown in Figure 3b, in which the latter modeled structure is very similar to the X-ray structure in VDR. It may be difficult for VDR to recognize the amide analogue **3a** as a ligand in a solution.

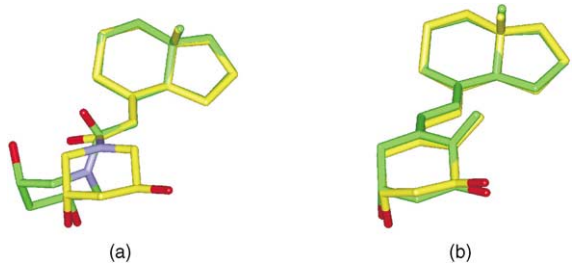


Figure 3. (a) The energy minimized structure of **3a** (green) and the modeled structure of **3a** (yellow) docking in VDR; (b) the X-ray structure of **1** (yellow) in VDR[10] and the energy minimized structure of **1** (green).

In summary, we have developed an efficient systematic route for synthesizing new stable amide analogues **3a–c** utilizing D-mannose. Takahashi et al. recently reported the synthesis of 1α,25-dihydroxyvitamin D_3 derivatives by the combinatorial procedure.[17] Our amide analogues can also be applied to this synthetic method and would easily provide many kinds of this class of D_3 analogues. We consider that these syntheses and analogues would contribute to the elucidation of vitamin D_3 action mechanisms with medicinal actions. Further biological studies of the amide analogues are currently in progress in our laboratory.

Acknowledgements

We are grateful to Miss Junko Shimode and Miss Maroka Kitsukawa for the spectroscopic measurements. This work was supported in part by Kanagawa Academy of Science and Technology Research Grants, which we gratefully acknowledge.

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14. Data for **3a**: ^{1}H NMR (600 MHz, CD_3OD) δ 0.66 (3H, s), 0.97 (1H, d, J = 6.6 Hz), 1.05–1.10 (1H, m), 1.16–1.18 (7H, m), 1.23–1.29 (2H, m), 1.31–1.48 (9H, m), 1.54–1.59 (2H, m), 1.62–1.67 (2H, m), 1.73–1.80 (2H, m), 1.85–1.89 (1H, m), 1.92–1.98 (1H, m), 2.04 (1H, dt, J = 3.0, 12.9 Hz), 2.04–2.10 (1H, m), 2.72 (1H, dt, J = 2.9, 13.6 Hz), 3.27 (1H, dd, J = 6.9, 12.9 Hz), 3.38 (1H, dd, J = 6.0, 13.2 Hz), 3.53 (1H, dd, J = 3.0, 13.2 Hz), 3.84 (1H, dd, J = 3.3, 12.9 Hz), 3.95–4.00 (2H, m), 5.61 (1H, s). HRMS (EI) *m*/*z*: calcd for $C_{25}H_{43}O_4N$ 421.3192. Found 421.3181 (M^+). Data for **3b**: ^{1}H NMR (600 MHz, CD_2Cl_2, selected) δ 0.61 (3H, s), 0.95 (1H, d, J = 6.6 Hz), 1.07 (1H, t, J = 8.1 Hz), 1.92 (1H, m), 2.03–2.06 (2H, m), 2.74–2.83 (2H, m), 2.95–3.02 (1H, m), 3.23 (1H, br d, J = 13.2 Hz), 3.50 (1H, br d, J = 6.0 Hz), 3.57 (1H, br d, J = 6.0 Hz), 3.78 (1H, br s), 3.85 (0.6H, br d, J = 13.2 Hz), 3.94–3.97 (1H, m), 4.04 (0.4H, br s), 4.34 (1H, br d, J = 10.8 Hz), 5.58 (1H, s). HRMS (EI) *m*/*z*: calcd for $C_{25}H_{43}O_5N$ 437.3144. Found 437.3148 (M^+). Data for **3c**: ^{1}H NMR (600 MHz, CD_2Cl_2, selected) δ 0.61 (3H, s), 0.95 (1H, d, J = 6.6 Hz), 3.63–3.67 (4H, m,-OC**H$_2$**C**H$_2$**C**H$_2$**OH), 5.56 (1H, s). HRMS (EI) *m*/*z*: calcd for $C_{28}H_{49}O_6N$ 495.3560. Found 495.3558 (M^+).
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