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Synthesis of 21-nor-22-oxa-1α,25-dihydroxyvitamin D_3 derivatives in quest of a drug with low calcemic activity

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Abstract—Two 21-nor-22-oxa-1α,25-dihydroxyvitamin D_3 derivatives have been synthesized in quest of a drug with lower calcemic activity than Maxacalcitol, 22-oxa-1α,25-dihydroxyvitamin D_3, being used as antihyperparathyroidism and antipsoriatic drug. Of two 21-nor products obtained, the product carrying one carbon elongated side chain with diethylcarbinol moiety has been found to exhibit comparable differentiation-inducing activity to Maxacalcitol with much lower exhibition of calcemic activity.

Owing to a wide range of activities in addition to its role in calcium homeostasis (calcemic activity), 1α,25-dihydroxyvitamin D_3 **1**, known as Calcitriol, and its derivatives have received a great deal of attention from both biological and synthetic points of view.[1] In our extensive synthetic studies in this field, we prepared a 22-oxa-analogue of Calcitriol **1** named as Maxacalcitol **2** which was found to exhibit higher differentiation-inducing activity than Calcitriol **1** with lower calcemic activity[2] and, on the basis of this finding, we have developed a practical antihyperparathyroidism and antipsoriatic drug with low calcemic activity.[3,4] During the investigation we have also found that calcemic activity is greatly reduced compared with the reduction of differentiation-inducing activity in other Calcitriol analogues, **3a** and **3b**, which are lacking 21-methyl functionality.[5,6] In this regard, the Leo group in Denmark reported an interesting observation that the 21-epimer of 22-oxa-1α,25-dihydroxyvitamin D_3 carrying one carbon elongated side chain with diethylcarbinol moiety named as Lexacalcitol **4** enhances significantly both calcemic and differentiation-inducing activities[7,8] though its calcemic activity seemed to be somewhat higher than for practical purposes.[9] Since it is essential to enforce the differentiation-inducing activity or to reduce the calcemic activity compared with Maxacalcitol **2** for the development of a new drug having better drug profile, we were very interested in the above findings, namely, a greater reduction in the calcemic activity relative to the differentiation-inducing activity observed in the 21-nor compounds, **3a** and **3b**, and a greater amplification on both calcemic and differentiation-inducing activities in Lexacalcitol **4**, the 21-epimer with elongated side chain. In order to know the effect of these two factors on the improvement of the drug profile, we synthesized two 21-nor-22-oxa-1α,25-dihydroxyvitamin D_3 derivatives, 21-nor-Maxacalcitol **5** and 21-nor-Lexacalcitol **6**, for biological evaluation. We wish to report here our preliminary result providing a promising clue to the control of calcemic activity and differentiation-inducing activity for the improvement of the drug profile (Fig. 1).

The synthesis started from the known tetracyclic ketone[3a] **7** which was first transformed into the primary alcohol **9**, regio- and stereoselectively, in excellent overall yield via the *exo*-methylene **8** by Wittig methylenation followed by hydroboration–oxidation reaction. The etherification of the primary alcohol **9** with an appropriate alkyl halide or a sulfonate, such as 2-methyl-2-triethylsiloxybutyl bromide and 2-methyl-2-triethylsiloxybutyl tosylate, under standard Williamson conditions completely failed to give the desired product. The

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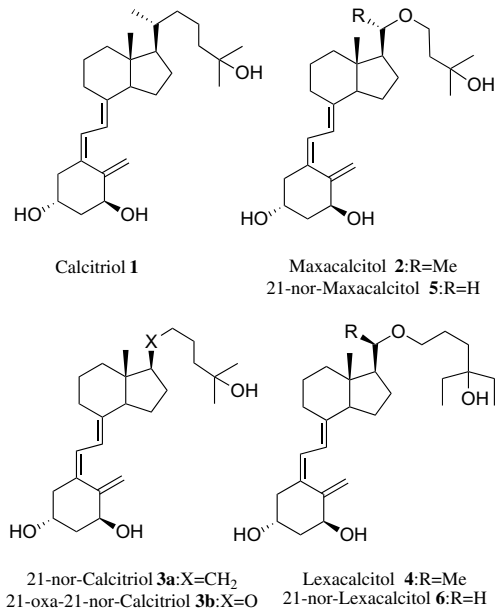
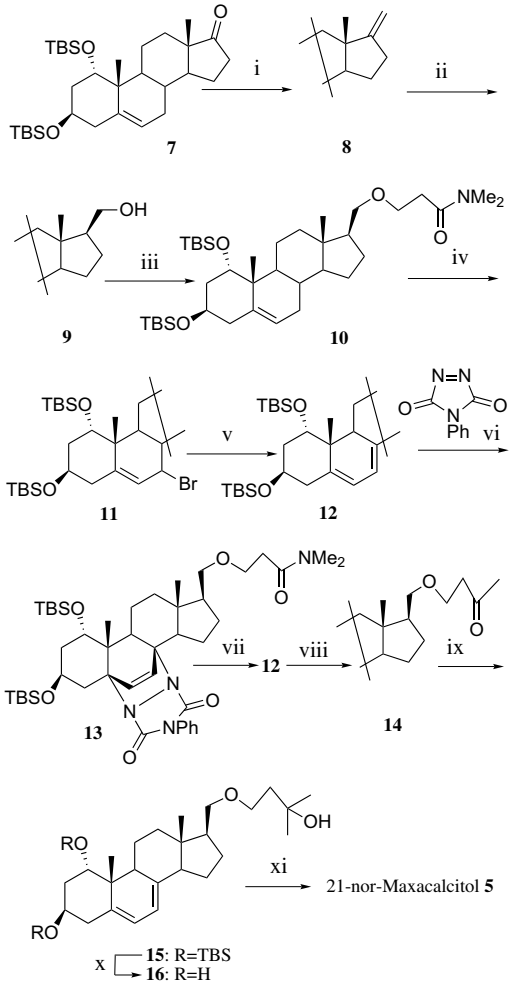


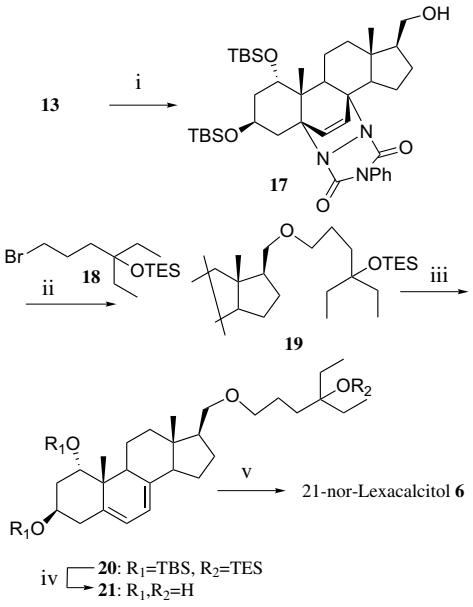
Figure 1.

observed difficulty in the Williamson synthesis was apparently due to the steric effect of the methyl and the *O*-protecting groups on the tertiary center of the alkylating agents, which shields the nucleophilic attack of the steroidal primary alcohol **9** in the S_N2 pathway.[10] We had, therefore, to take a rather circuitous route. Thus, the alcohol **9** was reacted with *N*,*N*-dimethylacrylamide in THF in the presence of sodium hydride to initiate a Michael 1,4-addition[3c] to give rise to the ether **10**. As expected, the reaction of the alcohol **9** with the less hindered electrophile proceeded without difficulty to afford the ether **10** in a satisfactory yield. When an acrylate ester or methyl vinyl ketone was used in place of the amide as a Michael acceptor, an undesired secondary reaction took place under the conditions to give a complex mixture. By following the standard conditions established in the synthesis of vitamin D_3 derivatives,[1d,6a] the ether **10** obtained was treated with NBS in hexane to give the allyl bromide **11**, which was treated with γ-collidine in toluene to generate the 1,3-diene **12** accompanied by inseparable by-products. The crude diene **12** was first treated with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD)[11] to give the adduct **13** for purification. The adduct **13** after purification was then heated in DMI at 140 °C to regenerate the diene **12** by retro-Diels–Alder reaction. Overall yield of the diene **12** from the ether **10** through a four-step sequence was 13%. At this point, transformation of the tertiary amide **12** into the tertiary alcohol **15** was carried out though it was found to be not an easy task. It required two steps and the cerium reagent[12] was essential in each of the steps so as to have the nucleophilic reaction proceed.[3c] Thus, the reaction of the reagent, prepared in situ from methylmagnesium bromide and cerium chloride in THF, was treated with the amide **12** and gave the methyl ketone **14**, which was again treated with the same cerium reagent to afford the tertiary alcohol **15**. Deprotection of **15** under standard conditions[3] afforded the triol **16**, which on the photolysis under the conditions established for the 21-methyl analogues[3] furnished 21-nor-Maxacalcitol **5** through concomitant photo-cycloreversion and thermal hydrogen migration.[1] Yield of the final step was 22%[13] (Scheme 1).

Synthesis of 21-nor-Lexacalcitol **6** utilized the cycloadduct **13** formed in the synthesis of 21-nor-Maxacalcitol **5** shown above. Thus, treatment of the adduct **13** with potassium *tert*-butoxide induced retro-Michael cleavage giving rise to an excellent yield of the primary alcohol **17**. In contrast to the above mentioned etherification reaction between the primary alcohol **9** and the γ,γ,γ-trisubstituted primary electrophiles, the Williamson etherification between the alcohol **17** and the δ,δ,δ-trisubstituted primary bromide **18** proceeded without difficulty to generate the desired ether **19** in good yield. This was apparently due to the one-carbon elongation in the electrophile, which lessened the steric congestion to allow the standard S_N2 pathway giving rise to the ether



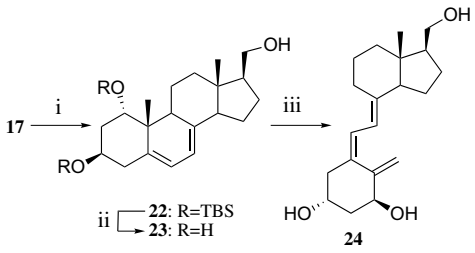
Scheme 1. Reagents and conditions: (i) PPh_3CH_3Br, *t*-BuOK, THF, 90%; (ii) 9-BBN, THF, then 30% H_2O_2, 3 M aq NaOH, quant; (iii) *N*,*N*-dimethylacrylamide, NaH, THF, 72%; (iv) NBS, AIBN, hexane; (v) γ-collidine, toluene; (vi) 4-phenyl-1,2,4-triazoline-3,5-dione, CH_2Cl_2, 19% (three steps); (vii) DMI, 140 °C, 69%; (viii) MeMgBr, $CeCl_3$, THF; (ix) MeMgBr, $CeCl_3$, THF, 68% (two steps); (x) TBAF, THF, 70%; (xi) *hν*, THF, 22%.



Scheme 2. Reagents and conditions: (i) *t*-BuOK, THF, 83%; (ii) *t*-BuOK, 18-crown-6, THF, 73%; (iii) DMI, 140 °C, 71%; (iv) TBAF, THF, 70%; (v) *hv*, THF, 8%.

19. Thermolysis of **19** in DMI at 140 °C induced a retro-Diels–Alder reaction to leave the tetracyclic diene **20**, which was deprotected to give the triol **21**. On photolysis, the triol **21** furnished 21-nor-Lexacalcitol[13] **6**. Yield of the final step was 8% (Scheme 2).

Moreover, we also synthesized the primary alcohol **24**, which is presumed to be the common metabolite of both 21-nor-Maxacalcitol **5** and 21-nor-Lexacalcitol **6** for a comparison in the biological evaluation. The synthesis utilized the intermediate **17** in the synthesis of 21-nor-Lexacalcitol **6**. Thus, the primary alcohol **17** was heated in DMI under the same conditions as above to induce a retro-Diels–Alder reaction to give the diene **22**, which



Scheme 3. Reagents and conditions: (i) DMI, 140 °C, 73%; (ii) TBAF, THF, 68%; (iii) *hv*, THF, 8%.

gave the triol **23** on deprotection. Photolysis of **23** under the same conditions above furnished the target primary alcohol **24** in 8% yield[13] (Scheme 3).

Having obtained the desired 21-demethylated compounds, 21-nor-Maxacalcitol **5** and 21-nor-Lexacalcitol **6**, in hand, we next examined their biological activities with respect to calcemic and differentiation-inducing activities in comparison to their corresponding 21-methyl derivatives, Maxacalcitol **2** and Lexacalcitol **4**, using Calcitriol **1** as standard. Calcemic activity was evaluated on the basis of in vivo calcium ion level in mice, while intrinsic activity as a vitamin D was evaluated on the basis of the affinities to chick vitamin D receptor (VDR) and to rat vitamin D binding protein (DBP) and differentiation-inducing activity in HL-60 cell, which are compiled in Table 1. For brevity, the relationship between calcemic activity and differentiation-inducing activity (HL-60) in two compounds, in addition to the 21-methyl compounds and the presumed metabolite, was also depicted in Figure 2 as a relative diagram based on Calcitriol **1** as standard. As seen in the figure, both 21-nor-Maxacalcitol **5** and 21-nor-Lexacalcitol **6** exhibited no significant calcemic activities which were almost the same as the primary alcohol **24**, while 21-methyl compounds **2** and **4**, in particular the latter, exhibited a considerable extent of activity higher

Table 1. Biological properties of Calcitriol **1**, Maxacalcitol **2**, Lexacalcitol **4**, 21-nor-Maxacalcitol **5**, 21-nor-Lexacalcitol **6**, and the primary alcohol **24**

	VDR[a] (%)	DBP[b] (%)	HL-60[c] EC_{50} [M]	In vivo Ca (i.v.)[d] [mmol/L]
				24 h/48 h (Mean ± SE, $n = 5$)
Vehicle				1.34 ± 0.02/1.32 ± 0.04
Calcitriol **1**	100	100	7.73×10^{-9}	1.80 ± 0.15[#]/1.63 ± 0.09[#]
Maxacalcitol **2**	12.28	0.07	1.82×10^{-9}	1.42 ± 0.03[e#]/1.39 ± 0.03[e]
Lexacalcitol **4**	66.89	n.d.[f]	1.19×10^{-11}[g]	1.77 ± 0.14[h#]/2.27 ± 0.16[h#]
5	0.65	<0.20	1.59×10^{-8}	1.35 ± 0.04/1.30 ± 0.04
6	6.91	<0.20	1.75×10^{-9}	1.36 ± 0.05/1.34 ± 0.02
24	0.01	<0.20	n.a.[i]	1.37 ± 0.03/1.33 ± 0.03

[a] Affinity for chick vitamin D receptor.
[b] Affinity for rat vitamin D binding protein.
[c] Differentiation-inducing effect on HL-60 cells.
[d] Ionized calcium levels after 30 μg/kg of analogues administration to ddY mice. The data were analyzed by unpaired Student's *t*-test compared to vehicle. Differences $^{\#}p < 0.05$ were considered significantly different.
[e] Vehicle: 1.33 ± 0.05 ($n = 5$, 24 h), 1.35 ± 0.05 ($n = 5$, 48 h), Calcitriol **1**: 1.84 ± 0.22 ($n = 5$, 24 h), 1.77 ± 0.13 ($n = 5$, 48 h).
[f] Not determined.
[g] Calcitriol **1**: 8.46×10^{-9}.
[h] Vehicle: 1.39 ± 0.02 ($n = 5$, 24 h), 1.41 ± 0.03 ($n = 5$, 48 h), Calcitriol **1**: 1.69 ± 0.06 ($n = 5$, 24 h), 1.63 ± 0.04 ($n = 5$, 48 h).
[i] No activity.

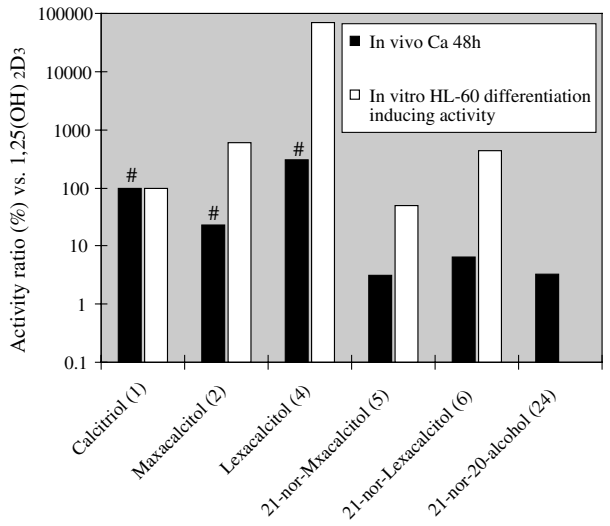


Figure 2. Relative diagram between in vivo calcemic and differentiation-inducing (HL-60) activities. HL-60 differentiation-inducing activity relative potency (%) = (Calcitriol EC_{50}/analogue EC_{50}) × 100. In vivo calcemic activity (48 h) relative potency (%) = ({analogue's [Ca mmol/L] – Vehicle's [Ca mmol/L]}/{Calcitriol's [Ca mmol/L] – Vehicle's [Ca mmol/L]}) × 100. $^{\#}p < 0.05$ compared to vehicle by unpaired Student's *t*-test.

than Calcitriol **1** indicating that the 21-methyl functionality, irrespective of its stereochemistry, plays an important role in the exhibition of calcemic activity. The differentiation-inducing activity, on the other hand, was diminished in the demethyl compounds **5** and **6**, but still remained overwhelmingly in both Calcitriol **1** and Maxacalcitol **2**, indicating the possibility of other factors on side-chain moiety than the 21-methyl functionality in the exhibition of differentiation-inducing activity.

The present study has clearly demonstrated the crucial role of the 21-methyl functionality on the exhibition of calcemic activity irrespective of its stereochemistry. At the same time, the present study suggested the importance of length and bulkiness of the side chain moiety in the 21-nor-derivatives on the exhibition of differentiation-inducing activity.

In conclusion, we have confirmed two structural factors on the side chain of the 22-oxa-1α,25-dihydroxyvitamin D_3 system essential for the control of calcemic activity and differentiation-inducing activity. Further work along the side-chain modification of 21-nor-22-oxa-1α,25-dihydroxyvitamin D_3 derivatives as well as other 21-nor-1α,25-dihydroxyvitamin D_3 derivatives on the basis of the present study is currently in progress.

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13. NMR spectra (270 MHz for ^{1}H and 67.8 MHz for ^{13}C spectra, respectively) of the representative compounds: **5**: ^{1}H NMR: δ 6.37 (d, *J* = 11.2 Hz, 1H), 6.00 (d, *J* = 11.2 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 3.46–3.68 (m, 5H), 3.28–3.34 (m, 1H), 2.81–2.86 (m, 1H), 2.58–2.63 (m, 1H), 2.28–2.35 (m, 1H), 1.50–2.05 (m, 13H), 1.24–1.35 (m, 9H), 0.51 (s, 3H). ^{13}C NMR: δ 147.6, 142.6, 133.1, 124.9, 117.2, 111.8, 73.3, 70.9, 70.6, 68.8, 66.8, 55.8, 50.4, 45.3, 45.0, 42.9, 41.3, 39.1, 29.5, 29.1, 25.2, 23.3, 22.6, 12.6. Compound **6**: ^{1}H NMR: δ 6.38 (d, *J* = 11.2 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.44 (m, 1H), 4.23 (m, 1H), 3.28–3.50 (m, 5H), 2.83–2.87 (m, 1H), 2.57–2.63 (m, 1H), 2.28–2.35 (dd, *J* = 13.4, 6.6 Hz, 1H), 1.26–2.13 (m, 23H), 0.83–0.88 (m, 6H), 0.50 (s, 3H). ^{13}C NMR: δ 147.6, 142.8, 133.0, 124.9, 117.1, 111.8, 73.9,

72.8, 71.8, 70.9, 66.9, 55.8, 50.3, 45.3, 45.1, 42.9, 39.1, 35.5, 31.0, 30.9, 29.1, 25.3, 23.9, 23.3, 22.6, 12.5, 7.9. Compound **24**: ^{1}H NMR: δ 6.38 (d, 11.5 Hz, 1H), 6.02 (d, 11.5 Hz, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.41–4.47 (m, 1H), 4.23–4.24 (m, 1H), 3.71–3.74 (m, 1H), 3.54–3.59 (m, 1H), 2.83–2.89 (dd, J = 13.2, 3.6 Hz, 1H), 2.57–2.63 (dd, J = 13.2, 3.6 Hz, 1H), 2.28–2.36 (dd, J = 13.2, 6.6 Hz, 1H), 1.10–2.08 (m, 16H), 0.53 (s, 3H). ^{13}C NMR: δ 147.7, 142.5, 124.9, 117.3, 111.8, 70.8, 66.9, 64.7, 55.9, 53.3, 45.3, 45.1, 42.9, 39.3, 29.1, 24.9, 23.3, 22.6, 12.5.