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SYNTHESIS OF 1α,25-DIHYDROXY-22-THIAVITAMIN D_3 AND RELATED ANALOGS[1]

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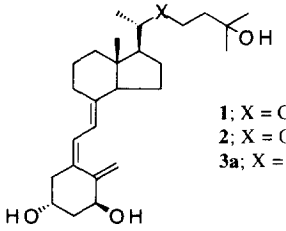
Abstract: The 22-thiolation reaction of the steroidal side chain and the synthesis of 1α,25-dihydroxy-22-thiavitamin D_3 and related analogs bearing side chains of different sizes in combination with 20-epi configuration are described.

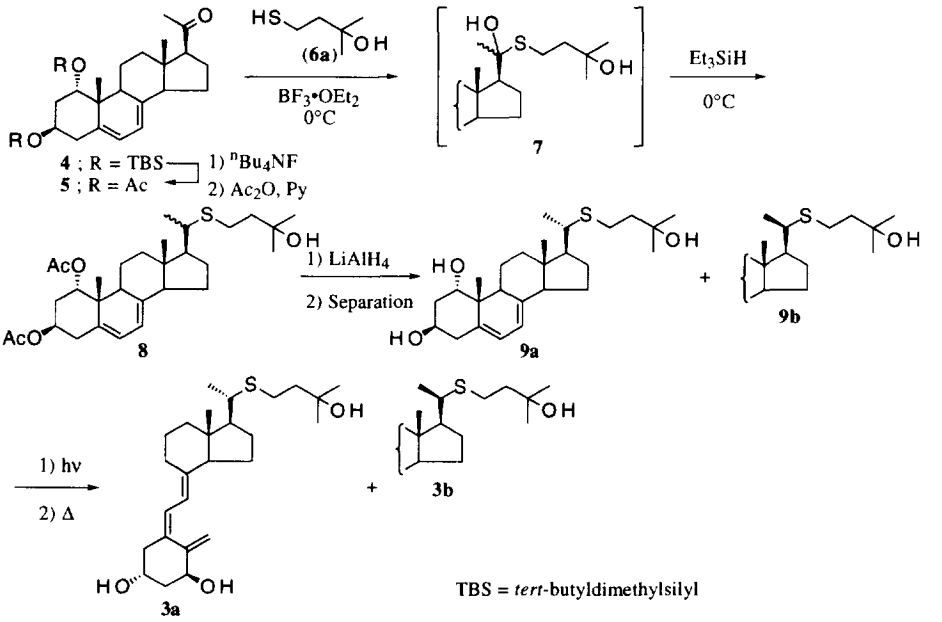
The active vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$, **1**), is now well recognized as one of the potential regulators of cell proliferation and differentiation processes in addition to the regulatory effect of calcium and phosphorous metabolism.[2] With the aim of dissociating these biological activities, new analogs of 1α,25$(OH)_2D_3$ (**1**) have been synthesized.[3] Since we initially synthesized 1α,25-dihydroxy-22-oxavitamin D_3 (22-oxacalcitriol, OCT, **2**)[4] which shows an enhanced *in vitro* differentiation-inducing activity with low *in vivo* calcemic liability, 1α,25$(OH)_2D_3$ analogs possessing heteroatoms in the side chain have been attractive targets during the last decade.[5] As a continuation of our studies of modification on the side chain of **1**, we took a great interest in 1α,25-dihydroxy-22-thiavitamin D_3 (22-thiacalcitriol, TCT, **3a**), a bioisoster of OCT (**2**), from the point of view of bioisosterism.[6] Accordingly, in this paper, we wish to describe the 22-thiolation reaction of the steroidal side chain and the synthesis of TCT (**3a**) and related analogs[7] bearing side chains of different sizes in combination with the unnatural 20-epi forms, in which potent biological properties may be expected as shown in 20(R)-1α,25-dihydroxy-24-homo-26,27-dimethyl-22-oxavitamin D_3.[8]



1; X = CH_2; 1α,25$(OH)_2$ D_3
2; X = O; OCT
3a; X = S; TCT

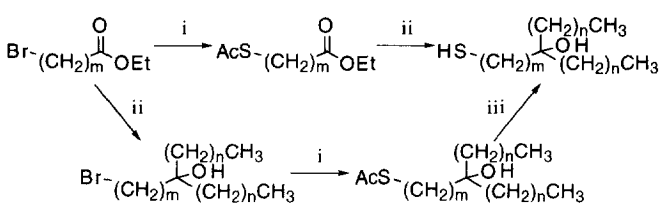
Scheme 1

Initially, we unsuccessfully attempted the 22-thiolation reaction with i) thiocarbonylation with Lawesson's reagent,[9] ii) the Mitsunobu reaction with thioacetic acid,[10] iii) nucleophilic substitution by thiolate anion,[11] etc. All of these efforts led only to the recovery or decomposition of the reactants. After many fruitless trials, we focused on the reductive thiolation of the steroidal ketone that utilized modified methodology of Olah's sulfide



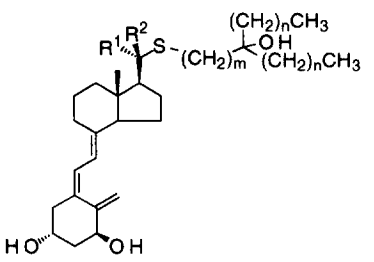
Scheme 2

formation.[12] Thus, 20-keto-compound (**5**), obtained from the known ketone (**4**),[13] was treated with thiol (**6a**) in the presence of boron trifluoride etherate at 0°C in CH_2Cl_2 to form the *in situ* hemithioacetal (**7**). Treatment of **7**, without isolation, with triethylsilane at 0°C afforded the sulfide (**8**) as inseparable diastereomers in 45% yield with 35% recovery of the starting ketone (**5**). This application of reductive thiolation to the steroidal side chain provides useful methodology to construct analogs of $1\alpha,25(OH)_2D_3$ (**1**) and related compounds, although the reaction conditions have not yet been optimized. Deacetylation of the sulfide (**8**) with $LiAlH_4$ gave the 20(S)-sulfide (**9a**) and 20(R)-sulfide (**9b**) in 22% and 42% yield, respectively, after separation by preparative TLC. The stereochemistry at C-20 for **9a** and **9b** was tentatively assigned by ^1H NMR chemical shift of C-21 methyl substituent, in which C-21 methyl resonance for 20(S)-isomer (**9a**: 1.41ppm) was downfielded by 0.10ppm relative to the 20(R)-isomer (**9b**: 1.31ppm) as shown in the general pattern.[14] Subsequent irradiation of **9a** and **9b** in ethanol at 0°C using a high-pressure mercury lamp through a Vycor filter followed by thermal isomerization under reflux in ethanol gave rise to TCT (**3a**)[15] and its 20-epimer (**3b**)[16] (Scheme 2).



Compound No.	m	n
6a	2	0
6b	2	1
6c	2	2
6d	3	0
6e	3	1
6f	4	0
6g	5	0

Scheme 3



$R^1 = CH_3$, $R^2 = H$; 20(S) configuration
$R^1 = H$, $R^2 = CH_3$; 20(R) configuration

Table 1

Compound No.	Configuration at 20-position	m	n
3a	S	2	0
3b	R	2	0
3c	S	2	1
3d	R	2	1
3e	S	2	2
3f	R	2	2
3g	S	3	0
3h	R	3	0
3i	S	3	1
3j	R	3	1
3k	S	4	0
3l	R	4	0
3m	S	5	0
3n	R	5	0

A similar reaction of the ketone (**5**) with various thiols (**6b-6g**), obtained from the corresponding bromoesters as shown in Scheme 3, gave sulfides in similar yields and ratios to **9a** and **9b**. The sulfides were converted to the 22-thia-derivatives of 1α,25(OH)$_2$D$_3$ (**1**) bearing different-sized side chains with six pairs of 20(S) and 20(R)-isomers as shown in Table 1.

In a preliminary *in vitro* study of the differentiation-inducing activity of human myeloid leukemia cells (HL-60) into macrophages estimated by superoxide anion production,[17] every 20(R)-isomer showed more potent activities than the corresponding 20(S)-isomer. Among these synthesized analogs, the most potent analog, 20(R)-1α,25-dihydroxy-26,27-dimethyl-22-thiavitamin D_3 (**3d**) (ED_{50} : 3.52×10^{-13}M), was about 100,000 times as active as 1α,25(OH)$_2$D$_3$ (**1**). Further biological properties including the regulatory effect of calcium and phosphorous metabolism are now under investigation and will be reported elsewhere.

Acknowledgment

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References and Notes

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15. **3a**; ^{1}H-NMR ($CDCl_3$, 200MHz) δ: 6.37 (d, *J* = 11.1Hz, 1H), 6.01 (d, *J* = 11.1Hz, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.48-4.35 (br, 1H), 4.30-4.17 (br, 1H), 2.63 (t, *J* = 7.8Hz, 2H), 1.39 (d, *J* = 6.6Hz, 3H), 1.25 (s, 6H), 0.58 (s, 3H); IR(neat) 3380, 2920, 1440, 1370, 1050cm^{-1}; UV (EtOH) λ_{max} 264nm, λ_{min} 227nm; HRMS m/z calcd for $C_{26}H_{42}O_3S$ 434.2855, found 434.2898.
16. **3b**; ^{1}H-NMR ($CDCl_3$, 200MHz) δ: 6.37 (d, *J* = 11.1Hz, 1H), 6.02 (d, *J* = 11.1Hz, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.48-4.36 (br, 1H), 4.30-4.13 (br, 1H), 2.61 (t, *J* = 8.3Hz, 2H), 1.30 (d, *J* = 6.6Hz, 3H), 1.25 (s, 6H), 0.62 (s, 3H); IR(neat) 3390, 2920, 1450, 1380, 1060cm^{-1}; UV (EtOH) λ_{max} 264nm, λ_{min} 227nm; HRMS m/z calcd for $C_{26}H_{42}O_3S$ 434.2855, found 434.2837.
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