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Vitamin D_3: Synthesis of *seco* C-9,11,21-*trisnor*-17-Methyl-1α, 25-dihydroxyvitamin D_3 Analogues

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Abstract—The synthesis and biological activities of *seco* C-9,11,21-*trisnor*-17-methyl-1α,25-dihydroxyvitamin D_3 analogues (D-ring analogues) are described.

The observation that 1α,25-dihydroxy vitamin D_3 (**1**; calcitriol) is active in the regulation of cell proliferation and differentiation, next to the classical role in calcium-bone homeostasis, has led in recent years to the development of analogues capable of dissociating cell differentiating effects from calcemic effects.[1,2] Among the three fragments of the vitamin D skeleton especially structural modifications of the side chain and of the A-ring have been studied in the past.[3]

Some years ago, we embarked on an extensive study of the structure–function relationship with the focus on the least studied part of the molecule, that is the central CD-ring regio.[4] In this respect we decided stripping the molecule to its five-carbon backbone (C-8–C-20) and resubstituting it again in various ways. Presently we want to describe 21-*nor*, 17-methyl D-ring analogues lacking the six-membered C-ring, that is with general formula **3** and **4**. We decided to select a 'D-ring' carrying a *gem*-dimethyl group at C-13 (steroid numbering) as these substituents mimic respectively the angular C-18 methyl group and C-12 in the parent steroid **1**, which are known to have an influence on restricting the side chain orientations.[3,5] It is generally assumed that the relative position in space of the 1α- and 25-hydroxy groups is important for the biological activity and that the side chain occupies a very restricted topology at the binding site of the vitamin D receptor (VDR).[3,5,6] This is also influenced by the 21-methyl group; it is of interest to see if its absence can be compensated by a 17-methyl substituent. Also some 19-*nor* analogues **4** are described, as this structural feature lowers the toxic calcemic effect (**1** vs **2**).[7]

(1*S*,3*R*)-Camphoric acid **5** is an ideal template for the central fragment (D-ring). Construction of analogues will involve (i) chemoselective manipulation of the carboxylic functions, (ii) formation of side chains and (iii) producing the C-8 aldehyde function needed for coupling with A-ring precursors **6a**,[8] and **6b** (Fig. 1).[7a]

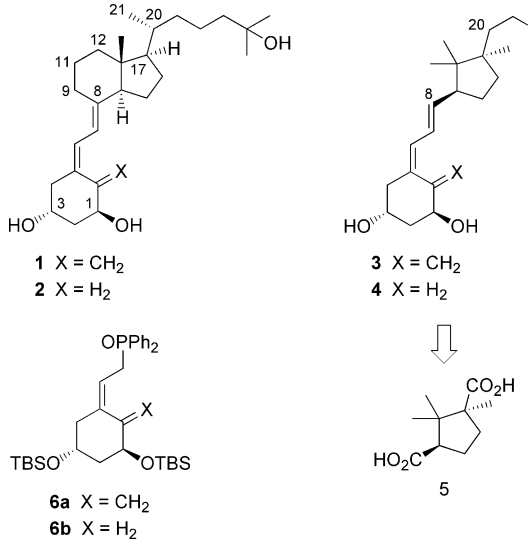

Figure 1.

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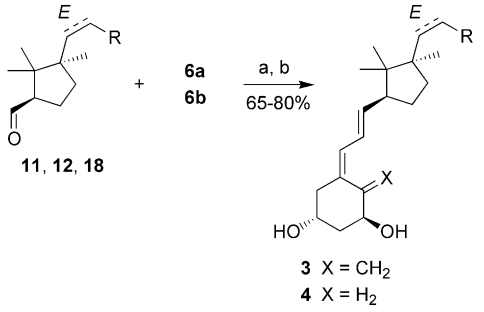
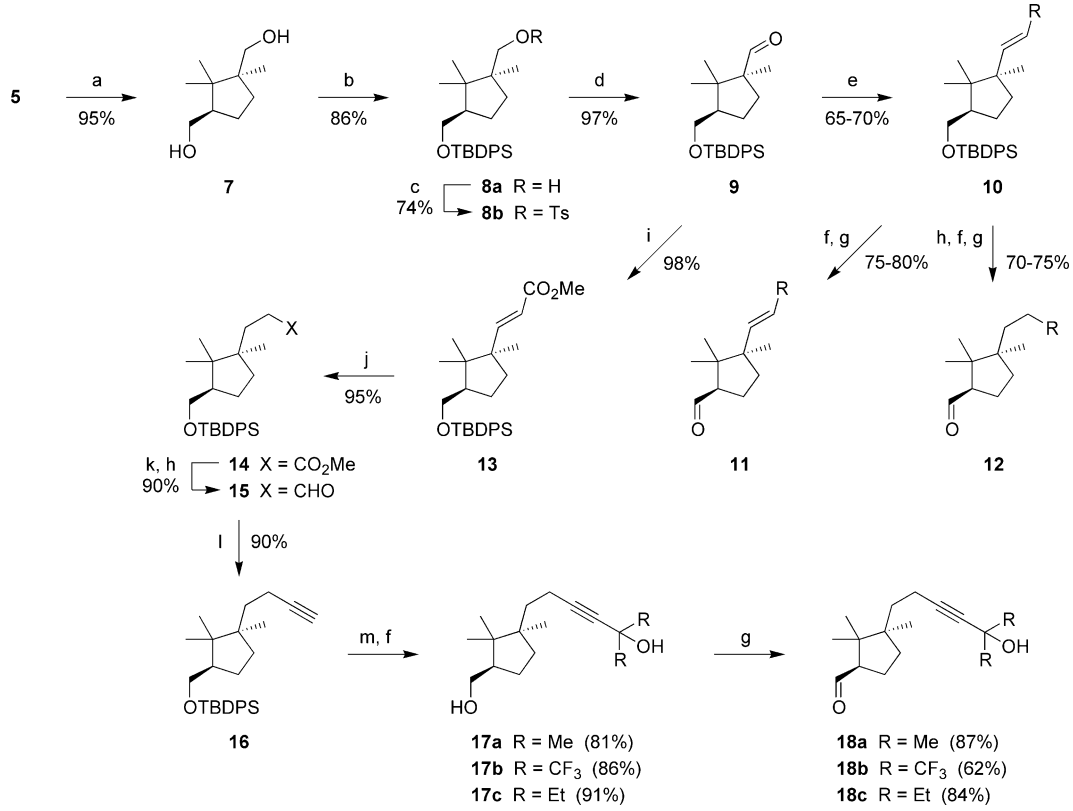
A highly efficient two-step differentiation of the carboxylic functions in **5** was attained via silylation of the corresponding diol **7** affording **8a** (87%) next to the di-TBDPS ether (5%) (Scheme 1). This method was far superior to an approach involving the C-8 mono-methyl ester (97%; MeOH, concd H_2SO_4, 30 °C) of **5** and selective reduction of both carboxylic functions. Introduction of the side chain via tosylate **8b** was impossible because all attempted substitutions at the sterically hindered C-20, failed. We therefore turned our attention to addition reactions on aldehyde precursor **9**. Julia coupling[9] with the appropriate phenylsulfone ($PhSO_2CH_2R$) and reductive elimination[10] led in good yields to the E-double bond isomers **10** (for respective side chain moieties R, see Scheme 2). The 25-hydroxy group in R is protected as a TES ether. Subsequent deprotection and oxidation gave the required C-8-formyl precursors **11** (for analogues WY 621, WY 624 and WY 903). For the synthesis of aldehyde **12** (synthesis of saturated side chain analogues KS 699, KS 703, WY 619, WY 625, WY 838 and WY 836) **10** was first hydrogenated.

Of special interest are 23-yne vitamin D_3 analogues with a conformationally restricted side chain; they frequently display enhanced biological properties.[3] In the present context their synthesis was performed via an initial Horner–Emmons reaction[11] on **9**. After catalytic hydrogenation of resulting **13**, the ester function in **14** was transformed into an aldehyde function in a two-step sequence (reduction to the corresponding alcohol and Swern oxidation[12]). On large scale the direct Dibal-H reduction was troublesome as it invariably led to mixtures of **14**, **15** and the primary alcohol. Then,



4 (X = H_2)	**3** (X = CH_2)	C_{20}-C_{22}	R
WY 838	KS 699	single	$(CH_2)_2C(Me)_2OH$
WY 836	KS 703	single	$(CH_2)_2C(Et)_2OH$
-	WY 619	single	$(CH_2)_2C(CF_3)_2OH$
-	WY 625	single	CH_2-$CF_2C(Me)_2OH$
-	WY 621	double (*E*)	$(CH_2)_2C(CF_3)_2OH$
WY 903	WY 624	double (*E*)	CH_2-$CF_2C(Me)_2OH$
WY 906	WY 722	single	≡C-$(Me)_2OH$
CD 578	WY 718		≡C-$(CF_3)_2OH$
-	WY 727	single	≡C-$(Et)_2OH$

Scheme 2. (a) *n*-BuLi, THF, −78→−20 °C, 3 h; (b) TBAF, THF, rt, 12 h.



Scheme 1. (a) $LiAlH_4$, THF–Et_2O, rt, 5 h; (b) TBDPSCI, imidazole, DMF, 0 °C→rt, 5 h; (c) TsCl, Et_3N, DMAP, CH_2Cl_2, Δ, 12 h; (d) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78→0 °C; (e) (i) LDA, THF, −78 °C, 2 h; (ii) Ac_2O, Et_3N, DMAP, −78 °C→rt, 2 h; (iii) Na–Hg, (3.1 or 4.8%), Na_2HPO_4, MeOH, −40→rt, 5 h; (f) TBAF, THF, rt, 4 h; (g) $(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N, −78 °C→−10 °C, 4 h; (h) H_2, 5% Rh/Al_2O_3, H_2, EtOAc, rt, 2–4 h; (i) $(EtO)_2P(O)CH_2CO_2CH_3$, NaH, THF, 0 °C, 2 h; (j) 4 atm. H_2, 10% Pd/C, 3% EtOAc in hexane, rt, 15 h; (k) DIBALH, CH_2Cl_2, −78 °C, 3 h; (l) $(MeO)_2P(O)CHN_2$, *t*-BuOK, THF, −78→0 °C, 15 h; (m) LDA, R_2CO, THF, −40→10 °C, 3 h.

treatment of **15** with dimethyl diazophosphonate afforded alkyne **16**.[13] The remaining carbon atoms of the side chain were introduced upon reaction of lithiated **16** with a ketone (Me_2CO for WY 722 and WY 906, $(CF_3)_2CO$ for WY 718 and CD 578 and Et_2CO for WY 727). Deprotection and Swern oxidation then led to C-8 aldehydes **17**.

Finally, construction of the title compounds **3** and **4** involved Lythgoe coupling of aldehydes **11**, **12** and **17** with A-ring phosphine oxides **6a**[8] and **6b**[7a] followed by deprotection of the hydroxy functions (Scheme 2).

The coupling was performed on D-ring side chain fragments possessing a free 25-hydroxy function therefore an excess of **6a**,**b** (4–5 equiv) was used; the A-ring phosphine oxide could be recuperated. The combined yield of this two-step sequence was higher (65–80%) than for the alternative approach involving TES protection (40–50%).

The affinity to the pig intestinal mucosa vitamin D receptor (VDR) was evaluated as described previously.[14] The relative affinity of the analogues was calculated from their concentration needed to displace 50% of [^{3}H] 1α,25$(OH)_2D_3$ from its receptor compared with the activity of **1** (assigned a value of 100%). The affinity for VDR varied between 40 and 80% compared to **1**.

The in vivo calcemic effects were tested in vitamin D-replete normal NMRI mice by daily intraperitoneal injections of 1α,25$(OH)_2D_3$ **1**, the analogues or the solvent during 7 consecutive days, using serum calcium concentration as parameter. The biological evaluation was determined in vitro on different cell lines (HL 60, MCF-7, MG 63, keratinocytes). All results are the mean of at least 3 experiments and are expressed as percentage activity (at 50% dose response) in comparison with **1** (= 100% activity). All analogues were 10 to more than 100-fold less calcemic than 1α,25$(OH)_2D_3$. The antiproliferative activity was comparable to **1**, except for the more potent fluorinated analogues; especially WY 718 and CD 578 with a 26,27-hexafluoro-23-yne side chain. The latter, a 19-*nor*-analogue, displays high ratios of cell antiproliferation activities versus calcemic effect. Further details of the biological activities and considerations obtained from comparison with other D-ring analogues will be published elsewhere (Table 1).

Table 1. Biological activities

Compd	In vitro studies					In vivo studies
	VDR	HL60	MG-63	MCF-7	Keratinocytes	Ca serum
1	100	100	100	100	100	100
KS 699	30	40	60	80	40	<1
WY 838	40	9	40	30	10	0.25
KS 703	80	100	200	250	90	5
WY 836	75	85	80	100	40	0.25
WY 619	80	150	200	1250	750	10
WY 625	75	80	50	90	1350	3
WY 621	80	50	50	375	300	13
WY 624	40	20	12	70	200	<0.1
WY 903	70	20		10	60	0.5
WY 722	9	100	50	150	400	0.1
WY 906	60	60		70	85	0.25
WY 718	80	215	350	3500	3500	1
CD 578	100	300		2000	4500	1
WY 727	70	90	20	150	90	1

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