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

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Abstract—The synthesis and biological properties of *seco*-C-9,11-bisnor-17-methyl-1α,25-dihydroxyvitamin D_3 novel D-ring analogues are described.

The observation that 1α,25-dihydroxyvitamin 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 (Fig. 1).[1–3]

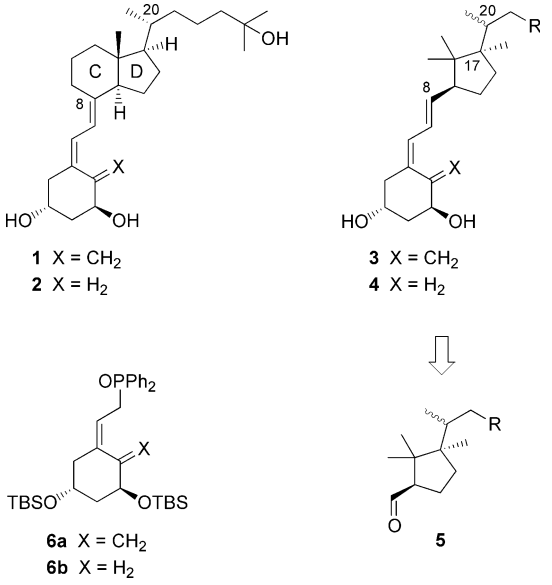

Figure 1.

In the context of a long-standing study[4] of analogues modified in the central part (C-8–C-20) we presently describe the synthesis of new 17-methyl 'D-ring' analogues, lacking the six-membered C-ring, **3** (natural A-ring) and **4** (19-*nor* compounds). The geminal dimethyl group at C-13 (steroid numbering) mimics C-12 and C-18, in **1** and **2** which are known to influence side chain orientations.[3] As this also holds for the C-20 configuration we plan to produce both the *R* (natural) and *S* epimers. It is indeed 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]

Analogues **3** and **4** will be constructed via the Lythgoe coupling[6] of an appropriate 8-formyl-D-ring fragment **5** (from 1*S*,3*R*-camphoric acid **7**) with respectively phosphine oxides **6a**[8] and **6b**.[7a]

In the preceding paper,[9] we described the synthesis of the related 21-*nor*-17-methyl D-ring analogues via the unsaturated ester **8** which was found to be also a valuable precursor for the present target molecules (Scheme 1). Several approaches for the introduction of the required 21-methyl group, for example conjugate addition to **8** or substitution of tosylate **23b** (Scheme 2), failed due to the highly hindered position of C-20.

A viable route was then found, involving a copper mediated SN_2' reaction[10] on allylic bromide **10** obtained from **8** (Scheme 1).

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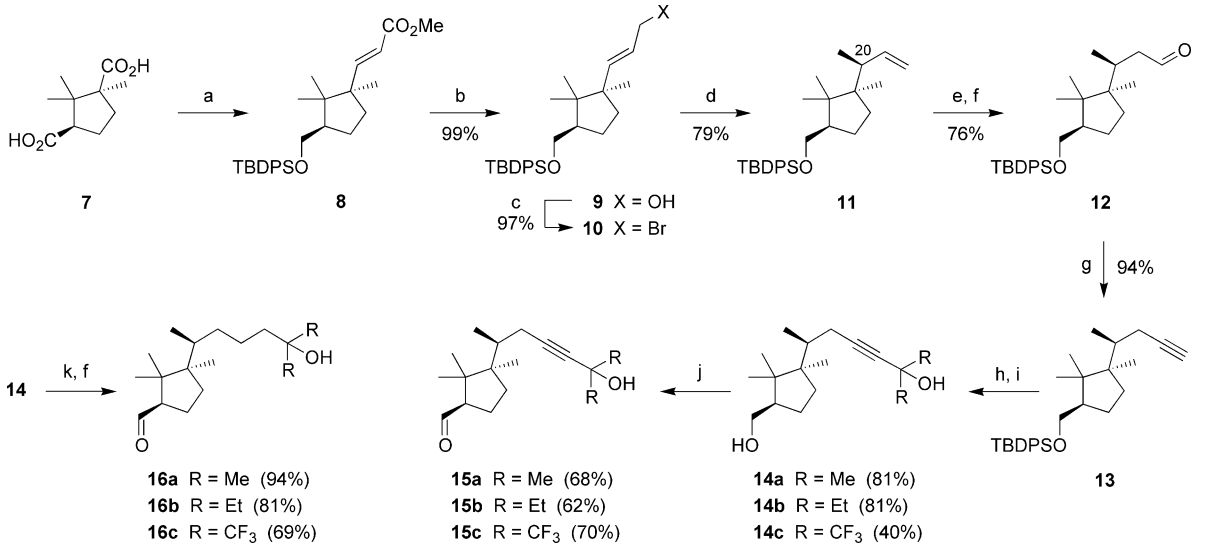
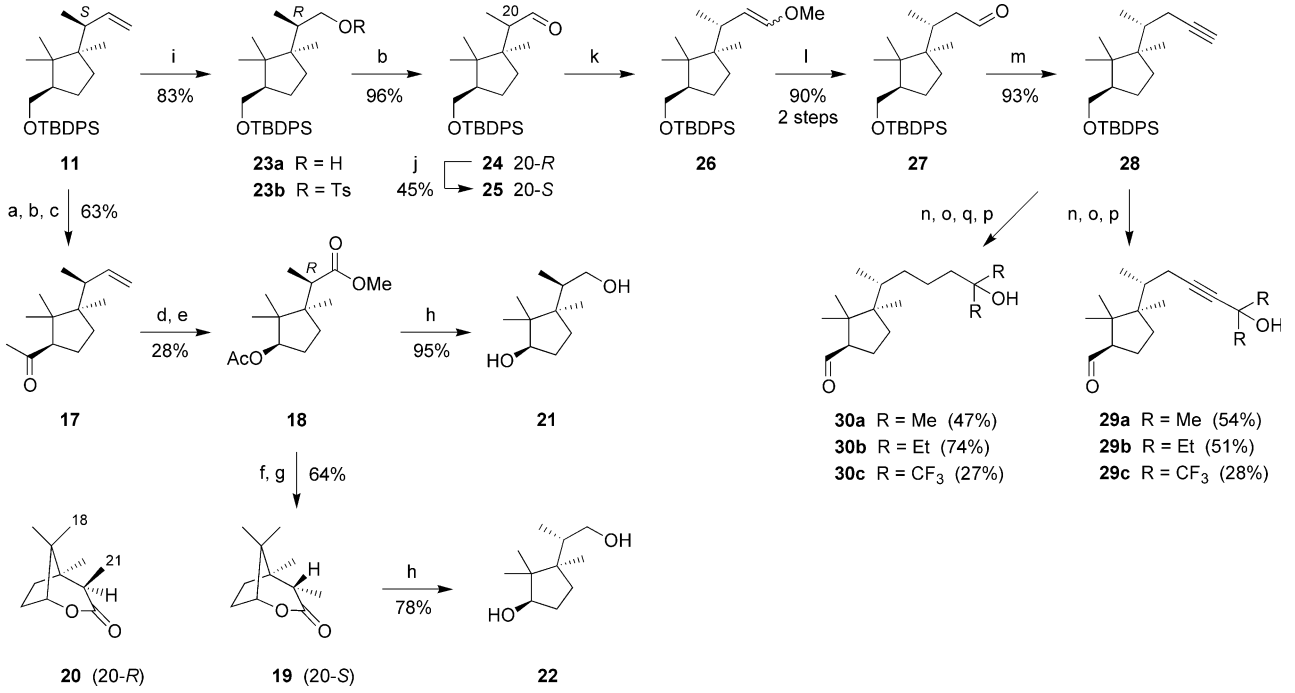
The reaction was completely diastereoselective and led to **11** with unnatural 20(*S*) configuration. This is in accordance with the Felkin rule[11] and with Corey's model[12] of d–π* complexation in SN_2' reactions.

Chemical proof followed from routine transformation of **11** into the rigid lactone **19** (Scheme 2). Lactonisation of intermediate **18** led to **19**(*S*) instead of the — on the basis of Felkin's model — expected **20**(*R*). Structure **19** was established by NOE, COSY, ^1H and ^{13}C NMR experiments. Lactone **19** is the thermodynamically most stable epimer as in **20** there is severe sterical hindrance due to the 1,3-diaxial interaction between C-18 and C-21. That, indeed acid catalyzed epimerization had occurred during the lactonisation was shown by reduction of **18** and **19** which respectively led to the two epimeric diols **21** and **22**.



Scheme 1. (a) See ref 8; (b) DIBALH, CH_2Cl_2, −78→50 °C, 2.5 h; (c) CBr_4, Ph_3P, CH_2Cl_2, −60→35 °C, 2 h; (d) $CuBrMe_2S$, MeLi, $ZnCl_2$, THF, −78→−50 °C, 4.5 h, then **9**, −78→rt, 5.5 h; (e) 9-BBN, THF, 50 °C, 3.5 h, then 30% H_2O_2, 50 °C, 1 h; (f) Py·SO_3, DMSO–CH_2Cl_2, −20→15 °C, 1.5 h, (g) $(MeO)_2P(O)CHN_2$, *t*-BuOK, THF, −78 °C, 40 min, **11**, −78→rt; (h) LDA, HMPA, THF, −20 °C, then R_2CO, −30→5 °C, 2–3 h; (i) TBAF, THF, rt; (j) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78→−10 °C, 2–3 h; (k) 5%, Rh/Al_2O_3, 1 atm H_2, EtOAc, rt 8 h.

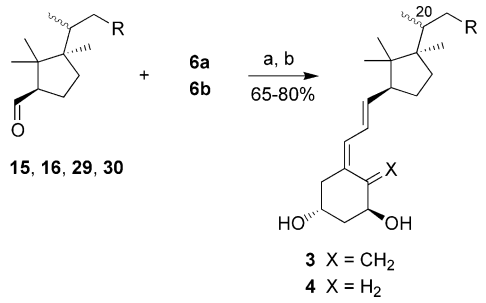


Scheme 2. (a) TBAF, THF, rt, 4 h; (b) $(COCl_2)$, DMSO, −78 °C, then Et_3N, −78→−10 °C, 3 h; (c) (i) MeMgBr, THF, 20 °C, 2 h, (ii) PDC, CH_2Cl_2, rt, 12 h; (d) O_3, NaOH, MeOH–CH_2Cl_2, −78 °C, 3 h; (e) *m*-CPBA, $CHCl_3$, rt 3 days; (f) LiOH, THF–MeOH, rt, overnight; (g) TsOH, CH_2Cl_2, rt, 12 h, (h): DIBALH, CH_2Cl_2, −78→−40 °C, 3 h; (i) O_3, hexane–CH_2Cl_2–MeOH, py, −78 °C, 2 h, then, $NaBH_4$, −78→20 °C, 4 h; (j) DBU, CH_2Cl_2, rt, 12 h, 45–49% conversion; (k) (i) $MeOCH_2PPh_3$·Cl, LDA, THF–HMPA (5:2), −20→0 °C, 1 h, then **25**, −40→rt, 2.5 h; (l) $Hg(OAc)_2$, THF, rt, 1 h, then 7% KI, (m) $(MeO)_2P(O)CHN_2$ *t*-BuOK, THF, −78 °C, 1 h, **27**, −75 °C, →rt, 16 h; (n) LDA, HMPA, THF, −20 °C, then R_2CO, −30→5 °C, 2→3 h; (o) TBAF, THF, rt; (p) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78→−10 °C, 2–3 h; (q) 5%, Rh/Al_2O_3, 1 atm H_2, EtOAc, rt 8 h.

Transformation of **11** into the C-8 formyl precursors **15** and **16** is straightforward; hydroboration and oxidation gave **12**, which upon Seyferth's[13] reaction led to alkyne **13**. Reaction of lithiated **13** with a series of ketones gave **14a**,**b**,**c** which were then transformed into **14a**,**b**,**c** (two steps) and **16a**,**b**,**c** (three steps).

For the synthesis of analogues with the natural 20(*R*) configuration (Scheme 2) we planned to equilibrate aldehyde **24** to epimer **25**. Therefore **11** was transformed in two steps to **24**. Base catalyzed equilibration gave a 1:1 ratio of **24** and **25** which could be separated by preparative HPLC. Homologation (1C) of **25** led, via **26**, to **27**, the C-20 epimer of **12**. Subsequent synthesis of precursors **29a**,**b**,**c** and **30a**,**b**,**c** was carried out in essentially the same yields as described for **14a**,**b**,**c** or **15a**,**b**,**c** (see Scheme 1) from **2**.

Finally Lythgoe coupling[6] of the 8-formyl precursors with phosphine oxide **6a** or **6b** and subsequent deprotection afforded the title compounds **3** and **4** (Scheme 3) with six different side chains each with respectively the 20-(*R*) and -(*S*) configuration (see Table 1).



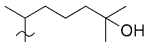
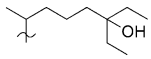
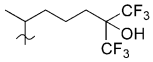
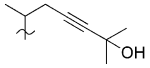
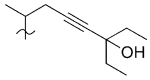
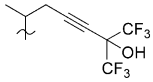
Scheme 3. (a) BuLi, THF, −78 °C→−20 °C, 3 h; (b) TBAF, THF, rt, 12 h.

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)$_2$D$_3$ from its receptor compared with the activity of **1** (assigned a value of 100%). The in vivo calcemic effects were tested in vitamin D-replete normal NMRI mice by daily intraperitoneal injections of 1α,25(OH)$_2$D$_3$, its 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, keratinocytes). All results are the mean of at least three experiments and are expressed as percentage activity (at 50% dose response) in comparison with 1α,25(OH)$_2$D$_3$ (=100% activity). The affinity of the D-ring analogues for VDR varied between 30 and 80% compared to **1** (Table 1).

The antiproliferative (MCF-7, keratinocytes) activities are high, especially for the hexafluoro analogues; as a general observation it appears that, within a pair of 20-epimers, the *S*-epimer is the more potent. Several analogues display high ratios of differentiation between antiproliferative and calcemic effects. The antiproliferative activities of WY 1112 and WY 1113 are comparable with the in vitro activity of known 'top' analogues such as 20-*epi*-22-oxa-24,26–27-trihomo-1α,25(OH)$_2$D$_3$ (KH 1060)[15] or 20-*epi*-22-ethoxy-23-yne-24,26,27-trihomo-1α,25(OH)$_2$D$_3$ (CB 1093).[16]

Table 1. Biological activities

Side chain	Code	C-20	A-ring	VDR	HL-60	MCF-7	Keratinocytes	Calcium serum
	1	*R*	**3**	100	100	100	100	100
	WY 821	*S*	**3**	85	800	2000	1000	8
	WY 1036	*R*	**3**	6	50	60	100	0.25
	WY 1116	*S*	**4**	40	600	400	600	0.5
	WY 1037	*R*	**4**	3	10	8	80	0.12
	WY 826	*S*	**3**	85	1250	15,000	7000	100
	WY 1018	*R*	**3**	50	150	300	400	2
	WY 1117	*S*	**4**	60	500	7500	7000	20
	WY 1019	*R*	**4**	10	80	150	300	0.5
	WY 1112	*S*	**3**	60	400	30,000	20,000	>100
	WY 1038	*R*	**3**	30	150	2500	2400	10
	WY 1113	*S*	**4**	40	400	20,000	16,000	100
	WY 1039	*R*	**4**	9	100	600	1500	50
	WY 9361	*S*	**3**	75	800	1250	3250	3
	WY10061	*R*	**3**	60	80	600	500	3
	WY 1106	*S*	**4**	40	350	1300	2000	4
	WY 1046	*R*	**4**	7	85	70	200	0.12
	WY 9371	*S*	**3**	80	600	750	800	25
	WY 10051	*R*	**3**	80	75	150	80	2
	WY 1107	*S*	**4**	60	400	700	300	2
	WY 1047	*R*	**4**	50	50	85	85	0.25
	WY 939	*S*	**3**	75	1250	8000	15,000	25
	WY 10071	*R*	**3**	60	250	4500	3600	6
	WY 1108	*S*	**4**	50	400	6000	15,000	25
	WY 1048	*R*	**4**	50	300	1500	3000	3

Further details of the biological activities and considerations obtained from comparison with other D-ring analogues will be published elsewhere.

Acknowledgements

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