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Synthesis and biological activity of 22-oxa CD-ring modified analogues of 1α,25-dihydroxyvitamin D_3: spiro[5.5]undecane CF-ring analogues

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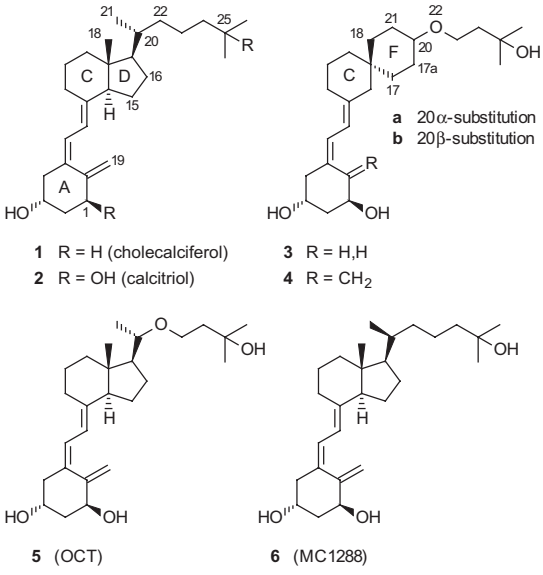
Abstract—The synthesis and biological activity of novel CD-ring modified analogues of 22-oxa-1α,25-dihydroxyvitamin D_3, lacking the D-ring and featuring a connection between C-18 and C-21 (spiro[5.5]undecane CF-ring analogues), is described. The central ring system is conveniently synthesised from an achiral intermediate. The analogues have marginal binding affinity for the nVDR and possess low to moderate genomic activity.

Over the last decade there has been a continuous interest in the development of analogues of $1\alpha,25(OH)_2D_3$ (**2**; calcitriol), the hormonally active metabolite of vitamin D_3 (**1**; cholecalciferol), with the purpose of separating calcemic and prodifferentiating and/or antiproliferative activities.[1] In this context we wish to report here on the synthesis and biological activity of spirocyclic analogues such as **3** and **4**. Among the various structural modifications that have been introduced in the parent compound,[2] a few are of special interest in view of the present work. The 22-oxa modification in the side chain led to the first analogue (**5**, OCT) that showed dissociation in activities.[3] Epimerisation at C-20 (cf. **6**, MC1288) often generates derivatives with enhanced biological activity.[4] And removal of C-19 is usually accompanied by a reduction in calcemic activity.[5] Whereas the above modifications are located in the flexible parts of the molecule, that is the side chain or A-ring, our laboratories have rather focused on structural changes within the central CD-ring skeleton of the molecule,[6] the part that is, from a synthetic point of view, the least accessible one.[7] In this context several series of analogues have been developed that show the desired separation of activities, including ones characterised by ring deletions.[8]



In the accompanying paper bicyclic CE-ring analogues of $1\alpha,25(OH)_2D_3$ (**2**) are described. In this work the central ring system consists of a spiro[5.5]undecane

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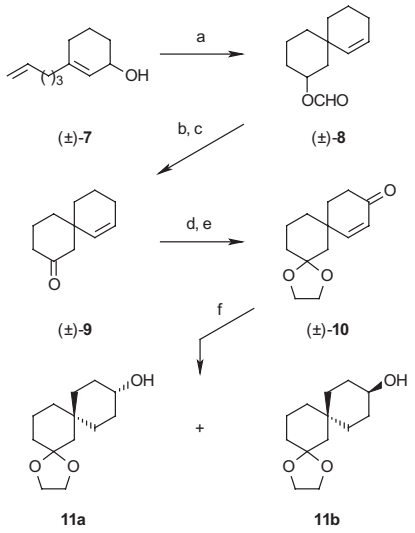
bicyclic moiety, featuring the deletion of C-15 and of C-16, and a connection between C-18 and C-21 (CF-ring system).[9] The choice of a six-membered F-ring with a 22-oxa side chain at C-20 allows for a synthetic scheme with the epimeric alcohols **11a** and **11b** as achiral intermediates, so that the enantioselectivity of the synthesis of the CF-ring system is not an issue.

The synthesis of **11a** and **11b** is shown in Scheme 1. Their spirocyclic skeleton results from the known cationic π cyclisation of allylic alcohol *rac*-**7** in formic acid leading to formate *rac*-**8** in 92% yield.[10] The precursor alcohol is readily obtained from 3-ethoxy-2-cyclohexen-1-one via a sequence involving: (i) addition of the Grignard derivative of 5-bromo-1-pentene; (ii) acid hydrolysis to 3-(4-pentenyl)-2-cyclohexen-1-one (85% yield); (iii) reduction with lithium aluminum hydride (99% yield).[10] The further conversion of formate *rac*-**8** to enone *rac*-**10** involves the reductive removal of the formate ester group with lithium aluminum hydride (95% yield), followed by Swern oxidation of the resulting alcohols to *rac*-**9** (93% yield). After protection of the carbonyl group as ethylene ketal (96% yield), allylic oxidation with pyridinium dichromate and *tert*-butylhydroperoxide leads to *rac*-**10** (64% yield). The catalytic hydrogenation of *rac*-**10** (Pd/C in MeOH) affords a mixture of the achiral alcohols **11a** and **11b**, which is separated by chromatography (52% and 45% isolated yield, respectively). The stereochemical assignment of both isomers follows from ^{1}H NMR COSY and NOE measurements that were performed on the corresponding ketones **12a** and **12b**, obtained by acid hydrolysis (Fig. 1). The final transformation of **11a** and **11b** into the corresponding stereoisomeric analogues **3a**, **4a** and **3b**, **4b** involves the consecutive introduction of the flexible parts of the molecule, that is the side chain and the *seco*-B,A-ring portion (Scheme 2). Alkylation of the



Scheme 1. Reagents and conditions: (a) HCOOH, rt (92%); (b) $LiAlH_4$, THF, 0 °C (95%); (c) $(COCl)_2$, Et_3N, DMSO, CH_2Cl_2, −60 °C (93%); (d) $HO(CH_2)_2OH$, TsOH, toluene, reflux (96%); (e) PDC, *t*-BuOOH, Celite, PhH, rt (64%); (f) H_2, Pd/C, MeOH, rt (**a**: 52%; **b**: 45%).

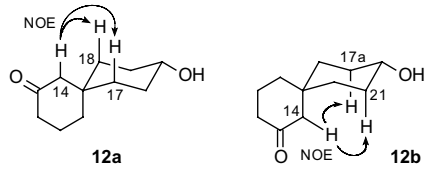
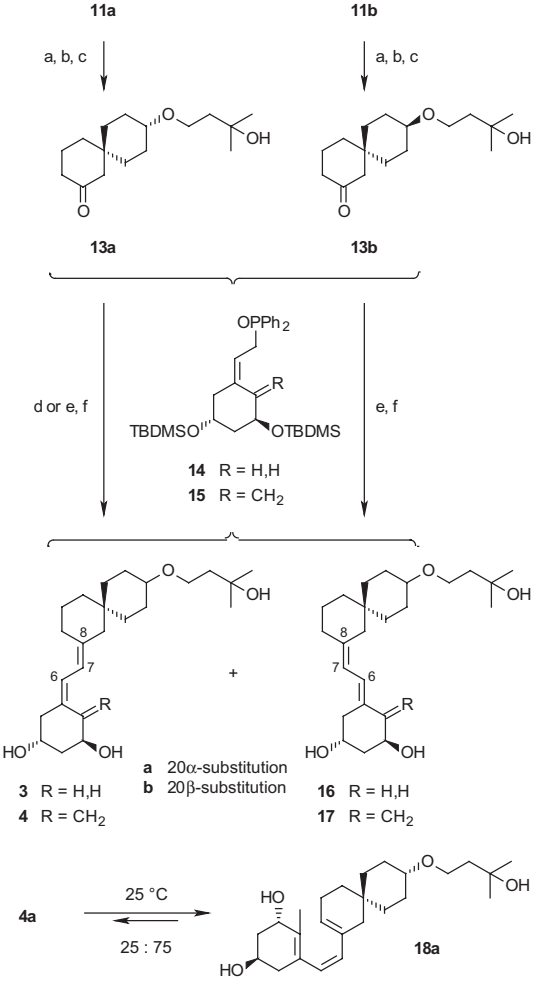


Figure 1. ^{1}H NMR NOE measurements of **12a** and **12b**.

alkoxides derived from **11** (NaH) with 4-bromo-2-methyl-2-butene in the presence of tetrabutylammonium iodide (DMF, 25 °C), followed by mercuric acetate assisted water addition and treatment with sodium borohydride (NaOH), led after acid hydrolysis to ketones **13** (overall yield: **a**: 61%; **b**: 53%).

In view of the expected problems related to vitamin–previtamin equilibration (see accompanying paper), it was decided to first synthesise the 19-nor analogues **3a** and **3b**. The appendage of the A-ring was achieved using the reliable Wittig–Horner based procedure developed



Scheme 2. Reagents and conditions: (a) (i) NaH, DMF, rt; (ii) $Me_2C{=}CHCH_2Br$, *n*-Bu_4NI, rt (**a**: 83%; **b**: 71%); (b) (i) $Hg(OAc)_2$, THF–H_2O, rt; (ii) $NaBH_4$, NaOH, rt (**a**: 86%; **b**: 90%); (c) TsOH, Me_2CO, H_2O, rt (**a**: 85%; **b**: 83%); (d) **14**, *n*-BuLi, THF, −78 °C (**a**: 47%; **b**: 72%); (e) **15**, *n*-BuLi, THF, −78 °C (59%); (f) TBAF, THF, rt (80–86%).

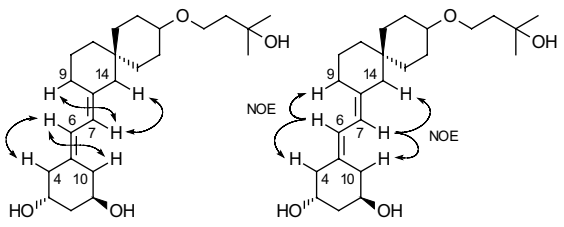


Figure 2. ^{1}H NMR COSY (left) and NOE studies (right) of **3a** and **3b**.

by Lythgoe and co-workers.[11] Attachment of the 19-nor A-ring to ketone **13a** was realised using the known derivative **14**.[12] After fluoride-induced silyl ether deprotection, a mixture of dienes **3a** and **16a** (ratio 8:2) was obtained, which could not be separated by chromatography. The identification of the major isomer as the (*E*)-derivative **3a** rests on ^{1}H NMR COSY and NOE measurements of the mixture (Fig. 2). When the same sequence was applied to the epimeric ketone **13b**, again a mixture of dienes **3b** and **16b** (ratio 8:2) was obtained from which now, however, analogue **3b** could be isolated in pure form by HPLC. The (*E*)-stereochemistry of **3b** was also confirmed by ^{1}H NMR analysis (Fig. 2).

Given the *Z*-contaminant in the 19-nor **a** series, the Wittig–Horner reaction was as yet carried out using the natural A-ring in order to obtain analogue **4a**. The sequence was performed on ketone **13a** using the known phosphine oxide **15**,[13] which after removal of the TBDMS protective groups (tetrabutylammonium fluoride) led to a separable mixture of isomeric (*E*)- and (*Z*)-trienes **4a** and **17a**, in favour of the desired (*E*)-derivative **4a** (ratio 85:15). Somewhat unexpected (see accompanying paper) **4a** was found to be rather stable with regard to the equilibration towards the previtamin form **18a** (Scheme 2).[14] The stereochemical assignment of the (*E*)-7,8-double bond in **4a** follows from ^{1}H NMR NOE difference experiments that were performed on the mixture of the TBDMS protected derivatives (Fig. 3). Analytical and spectral data consistent with the depicted structures were obtained for all compounds.[15]

The results of the biological evaluation of **3a** (contaminated with **16a**), **3b**, **16b** and **4a** are given in Table 1. Inspection of the table learns that: (i) the binding affinity for the VDR of all derivatives is very low, (ii) *Z*-isomer **16b** is inactive, so that the moderate activity of impure **3a** probably originates from the major *E*-isomer, (iii) all derivatives possess low calcemic activity, (iv) impure **3a** shows the aimed at separation of calcemic and genomic

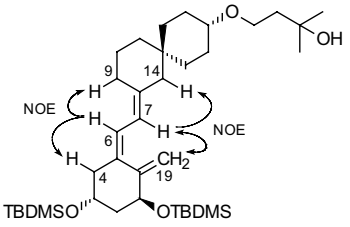


Figure 3. ^{1}H NMR NOE measurements of TBDMS protected **4a**.

Table 1. Biological activity of CF-ring 22-oxa-1α,25(OH)$_2$D$_3$ analogues[a]

	VDR[b] (pig)	HL-60[c]	MCF-7[d]	Ca serum[e] (mice)
2	100	100	100	100
3a[f]	<0.1	8	60	<0.25
3b	<0.1	6	7	<0.25
16b	<0.1	<1	0	<0.25
4a	0.5	—	10	<0.25

[a] The activities are presented as relative values, the reference value of 1α,25(OH)$_2$D$_3$ (**2**) being defined as 100.
[b] Binding affinity for the nuclear vitamin D receptor.
[c] Prodifferentiating activity on human leukemia HL-60 cells.
[d] Antiproliferative activity on human breast cancer MCF-7 cells.
[e] Effect on Ca level in blood serum.
[f] Contaminated with 20% of **16a**.

effects. The lack of affinity for the VDR is presumably due to the rigidity of the central hydrophobic part of the molecule. Future work will be directed towards establishing more flexible spirocyclic systems in which at least one of the central rings will consist of a five-membered ring.

Acknowledgements

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14. The vitamin–previtamin equilibrium **4a**–**18a** (Scheme 2) was investigated ($CDCl_3$, rt). Starting from the vitamin as well as the previtamin the same equilibrium composition was reached and was found to be in favour of previtamin **18a** (25:75), but the equilibration was very slow (about 90 days starting from **4a**).
15. Spectral data, for *rac*-**9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.64 (dt, $J = 10.1$, 3.7 Hz, 1H), 5.42 (br d, $J = 10.1$ Hz, 1H), 2.32–2.26 (m, 2H), 2.21 (AB, $J = 13.9$ Hz, 2H), 1.95–1.82 (m, 4H), 1.72 (m, 1H), 1.63–1.52 (m, 3H), 1.51–1.40 (m, 2H) ppm. For *rac*-**10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (d, $J = 10.3$ Hz, 1H), 5.86 (d, $J = 10.3$ Hz, 1H), 3.95–3.87 (m, 4H), 2.42 (t, $J = 6.8$ Hz, 2H), 2.01 (dt, $J = 13.4$, 6.6 Hz, 1H), 1.89 (dt, $J = 13.5$, 6.9 Hz, 1H), 1.77–1.57 (m, 7H), 1.44 (m, 1H) ppm. For **11a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.88 (s, 4H), 3.59 (m, 1H), 1.72–1.67 (m, 5H), 1.56–1.55 (m, 4H), 1.43–1.37 (m, 6H), 1.19 (m, 2H) ppm. For **11b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.95–3.89 (m, 4H), 3.63 (m, 1H), 1.80–1.73 (m, 4H), 1.63–1.56 (m, 6H), 1.47–1.40 (m, 3H), 1.26 (m, 2H), 1.14 (m, 2H) ppm. For **13a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.69 (t, $J = 5.8$ Hz, 2H), 3.62 (s, 1H), 3.33 (tt, $J = 7.9$, 3.9 Hz, 1H), 2.29 (t, $J = 6.8$ Hz, 2H), 2.17 (s, 2H), 1.84 (m, 2H), 1.75 (t, $J = 5.6$ Hz, 2H), 1.74 (m, 2H), 1.68 (dd, $J = 6.1$, 6.1 Hz, 2H), 1.58 (s, 1H), 1.58–1.49 (m, 4H), 1.24 (s, 6H), 1.22 (m, 2H) ppm. For **13b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.68 (t, $J = 5.8$ Hz, 2H), 3.30 (tt, $J = 8.2$, 4.0 Hz, 1H), 3.60 (s, 1H), 2.29 (t, $J = 6.8$ Hz, 2H), 2.26 (s, 2H), 1.86 (m, 2H), 1.76 (m, 2H), 1.75 (t, $J = 5.8$ Hz, 2H), 1.61 (dd, $J = 6.1$, 6.1 Hz, 2H), 1.55 (m, 2H), 1.49 (m, 2H), 1.24 (m, 2H), 1.23 (s, 6H) ppm. For *E*/*Z*-mixture **3a**/**16a** (ratio 8:2): ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.22 (*A*B, $J = 11.3$ Hz, 1H of *E*-isomer), 6.20 (*A*B, $J = 11.3$ Hz, 1H of *Z*-isomer), 6.10 (A*B*, $J = 11.3$ Hz, 1H of *Z*-isomer), 5.95 (A*B*, $J = 11.3$ Hz, 1H of *E*-isomer), 4.03 (br s, 2H), 3.68 (t, $J = 5.8$ Hz, 2H), 3.54 (s, 1H), 3.30 (tt, $J = 8.3$, 4.1 Hz, 1H), 2.60 (dd, $J = 13.3$, 3.8 Hz, 1H), 2.45 (dd, $J = 13.3$, 3.6 Hz, 1H), 2.29–2.21 (m, 2H), 2.17–2.12 (m, 2H), 1.96 (s, 2H), 1.87–1.70 (m, 4H), 1.71 (t, $J = 5.8$ Hz, 2H), 1.61 (br s, 2H), 1.55–1.40 (m, 8H), 1.19 (s, 6H), 1.15–1.10 (m, 2H) ppm. For **3b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.24 (*A*B, $J = 11.3$ Hz, 1H), 5.94 (A*B*, $J = 11.3$ Hz, 1H), 4.12–4.07 (m, 2H), 3.79 (br s, 1H), 3.72 (t, $J = 5.7$ Hz, 2H), 3.27 (tt, $J = 8.9$, 3.9 Hz, 1H), 2.63 (dd, $J = 13.4$, 3.6 Hz, 1H), 2.49 (dd, $J = 13.2$, 3.6 Hz, 1H), 2.32–2.16 (m, 4H), 2.06 (*A*B, $J = 13.3$ Hz, 1H), 2.02 (A*B*, $J = 13.3$ Hz, 1H), 1.92–1.82 (m, 2H), 1.76 (t, $J = 5.7$ Hz, 2H), 1.75–1.72 (m, 10H), 1.58–1.42 (m, 2H), 1.25 (s, 6H), 1.11–1.07 (m, 2H) ppm. For **16b**: ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.23 (*A*B, $J = 11.2$ Hz, 1H), 6.11 (A*B*, $J = 11.2$ Hz, 1H), 4.05–4.01 (m, 2H), 3.70 (t, $J = 5.8$ Hz, 2H), 3.60 (br s, 1H), 3.26 (tt, $J = 9.1$, 4.1 Hz, 1H), 2.59 (dd, $J = 13.3$, 3.8 Hz, 1H), 2.46 (dd, $J = 13.1$, 3.6 Hz, 1H), 2.26 (dd, $J = 13.3$, 7.4 Hz, 1H), 2.23 (*A*B, $J = 13.2$ Hz, 1H), 2.18 (A*B*, $J = 13.2$ Hz, 1H), 2.16 (dd, $J = 13.2$, 7.1 Hz, 1H), 2.14–2.11 (m, 2H), 1.86–1.73 (m, 4H), 1.73 (t, $J = 5.8$ Hz, 2H), 1.58–1.54 (m, 5H), 1.50–1.42 (m, 3H), 1.40–1.38 (m, 2H), 1.21 (s, 6H), 1.16–1.09 (m, 2H) ppm. For **4a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.31 (*A*B, $J = 11.2$ Hz, 1H), 6.05 (A*B*, $J = 11.2$ Hz, 1H), 5.32 (s, 1H), 4.98 (s, 1H), 4.43 (br s, 1H), 4.23–4.19 (br m, 1H), 3.80 (br s, 1H), 3.69 (t, $J = 5.7$ Hz, 2H), 3.29 (tt, $J = 7.9$, 3.9 Hz, 1H), 2.60 (dd, $J = 13.1$, 3.6 Hz, 1H), 2.31–2.22 (m, 3H), 1.96 (t, $J = 5.6$ Hz, 2H), 1.91 (s, 2H), 1.74 (t, $J = 5.7$ Hz, 2H), 1.71–1.69 (m, 2H), 1.59 (br s, 2H), 1.54–1.43 (m, 8H), 1.24 (s, 6H), 1.13–1.08 (m, 2H) ppm. For **17a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.27 (*A*B, $J = 11.3$ Hz, 1H), 6.19 (A*B*, $J = 11.3$ Hz, 1H), 5.32 (s, 1H), 5.00 (s, 1H), 4.42 (br s, 1H), 4.21 (br m, 1H), 3.77 (br s, 1H), 3.70 (t, $J = 5.8$ Hz, 2H), 3.31 (tt, $J = 8.6$, 3.6 Hz, 1H), 2.59 (dd, $J = 13.1$, 3.7 Hz, 1H), 2.28 (dd, $J = 13.0$, 7.4 Hz, 1H), 2.09 (s, 2H), 2.08 (t, $J = 6.2$ Hz, 2H), 1.98–1.94 (m, 2H), 1.75 (t, $J = 5.8$ Hz, 2H), 1.76–1.74 (m, 2H), 1.59–1.43 (m, 10H), 1.24 (s, 6H), 1.15–1.11 (m, 2H) ppm. For **18a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (*A*B, $J = 12.4$ Hz, 1H), 5.70 (A*B*, $J = 12.4$ Hz, 1H), 5.64 (br s, 1H), 4.19 (br s, 1H), 4.14–4.09 (m, 1H), 3.79 (br s, 1H), 3.70 (t, $J = 5.7$ Hz, 2H), 3.32 (tt, $J = 8.0$, 4.0 Hz, 1H), 2.44 (dd, $J = 16.7$, 4.7 Hz, 1H), 2.08–2.03 (m, 3H), 1.86 (*A*B, $J = 16.9$ Hz, 1H), 1.82 (A*B*, $J = 16.9$ Hz, 1H), 1.72 (s, 3H), 1.76–1.41 (m, 14H), 1.24 (s, 6H), 1.14–0.90 (m, 2H) ppm.