Design and Synthesis of Novel 20-*epi* Analogues of Calcitriol with Restricted Side Chain Conformation

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Abstract: The design and efficient preparation of two 20-*epi* analogues of calcitriol with restricted side chain conformation is described. The formation of the tetrahydropyran ring was achieved via zinc chloride mediated etherification of alcohols. Docking experiments show that these novel analogues could be interesting synthetic targets.

Key words: synthesis, vitamin D, analogues, nitriles, diols, drugs

1α,25-Dihydroxyvitamin D_3 [**1**, 1α,25-$(OH)_2$-D_3, calcitriol], the hormonally active form of vitamin D_3[1] (**2**, cholecalciferol), exerts control over important physiological processes, including calcium and phosphorus metabolism, cellular differentiation and immune reactions.[2] However, the clinical utility of this hormone for treatment of cancers and skin disorders is limited by its hypercalcaemic effects. There is accordingly much interest in the design and synthesis of analogues of **1** with more selective (or even different) biological effects (Figure 1).[2]

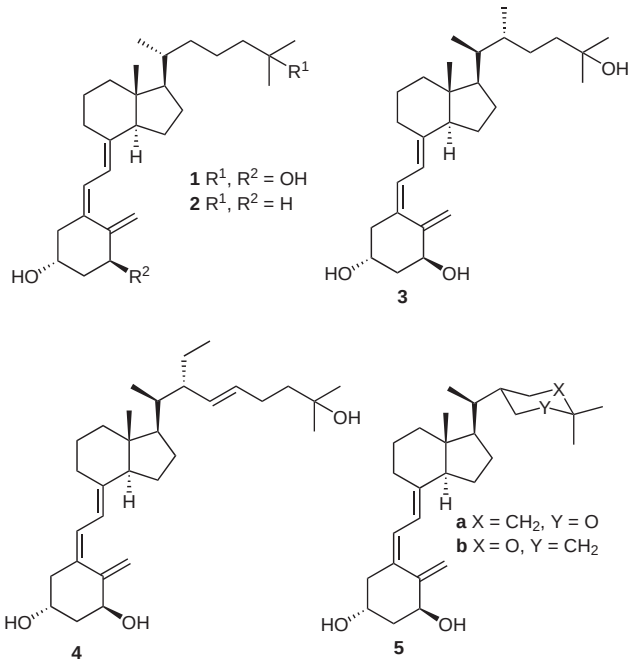

Figure 1 Structure of calcitriol and some of its analogues with restricted side chain conformation.

Most of the analogues of **1** synthesized so far show modifications at the side chain but only a limited number of these analogues are in use as drugs.[2f] The structural modifications capable of substantially enhancing the antiproliferative potency of **1** include: incorporation of an oxygen atom at C-22, methyl homologation at C-26 and C-27, homologation at C-24, epimerization at C-20 and incorporation of rigid, conformationally restricting structural units.[3] The natural 1α,25-$(OH)_2$-D_3 and several synthetic analogues have been recently co-crystallized within the active site of the ligand binding domain (LBD) of the human vitamin D receptor (VDR).[4] This structural information is crucial for the rational design of more potent and selective analogues of the hormone. Pre-orientation of the side chain in a productive conformation is expected to facilitate receptor–ligand binding by lowering the entropic barrier and, hence, a number of semi-rigid analogues of calcitriol with restricted flexibility of the side chain have been reported. Yamada and co-workers showed that homologation at C-22 provided analogues with restricted side chain conformation and in some cases enhanced biological properties.[5] For example, analogue **4** is 100 times more efficient than 1,25-dihydroxyvitamin D_3 (**1**) in cell differentiation, although its affinity for the vitamin D receptor (VDR) is one-seventh of that of **1**, whereas analogue **3** shows the highest VDR binding activity so far known.

As part of our research program on the synthesis of vitamin D analogues with restricted side chain conformation, we previously reported the synthesis of analogues of **1** modified at C-22.[6] We now report our preliminary results on the docking and synthesis of 20-epi analogues of calcitriol with cyclic side chain.

Inspection of the available crystal structures suggests that bulky side chains can be effectively hosted within the active site of the receptor. Therefore, we decided to study the effect of carbocyclic side chains on the binding and selectivity of the vitamin D analogues. Our docking calculations indicate that analogues with hexacyclic side chains should have an enhanced affinity for the receptor as such side chains contribute to improve the receptor–ligand complementarity. For instance, our calculations suggest that compounds **5a** and **5b** should exhibit a similar binding energy to that of the natural ligand. The decrease in the

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binding energy due to the less efficient H-bonds between C25–O and the histidines H305 and H397 is, according to our *in silico* studies, compensated by more efficient van der Waals interactions of the new side chains with the lipophilic residues of the active site. In our experiments, the automated docking program Autodock[17] reproduced with a high degree of accuracy the reported competent conformation of 1α,25-$(OH)_2$-D_3 in complex with the VDR-LBD. The docking energy computed by Autodock for the natural substrate, –15.5 kcal/mol, is similar to the docking energies computed for **5b** and **5a**: –15.5 and –15.4 kcal/mol, respectively (Figure 2).

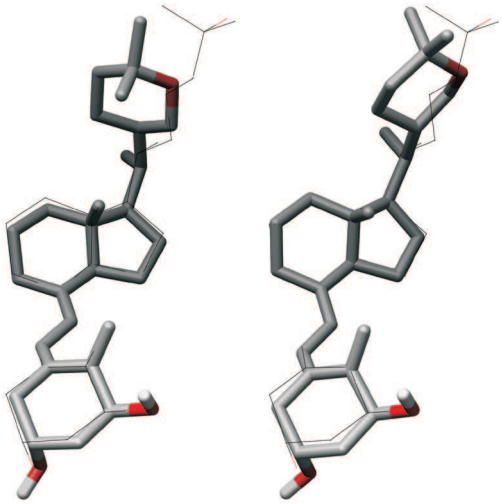
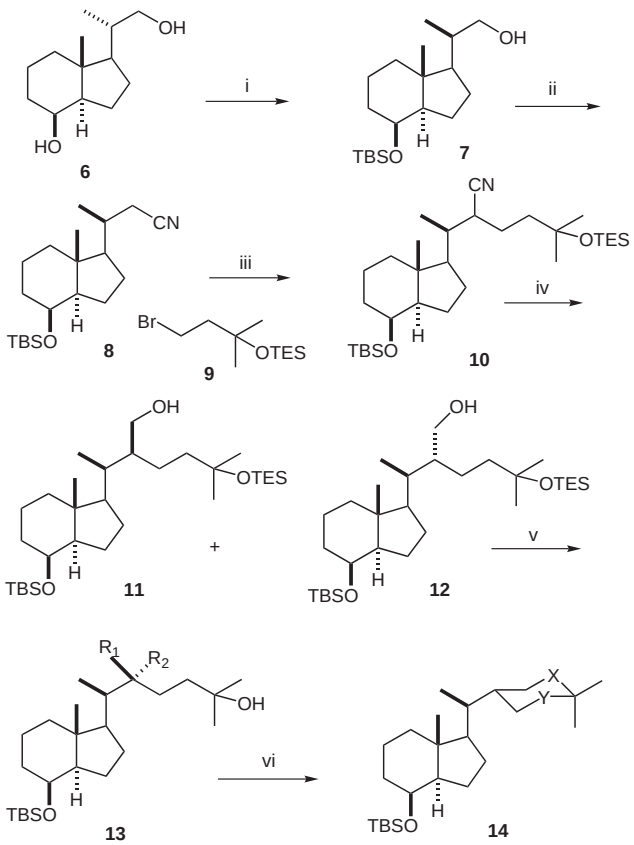


Figure 2 Most stable conformations of **5b** (left) and **5a** (right) superimposed to the active conformation of calcitriol.

The introduction of a hydroxyl group in selected positions of the side chains of these compounds is predicted to improve the docking energies by approximately 2.5 kcal/mol (data not shown). However, the preparation of such compounds would require important synthetic efforts. In order to test the accuracy of our computational predictions, we decided to synthesize and test compounds **5a** and **5b**, readily available by using a modification of a synthetic methodology developed by us for the preparation of other vitamin D analogues.[6] If the preliminary biological tests, currently underway, confirm our binding model, we plan to accomplish the synthesis of more complex structures based upon the scaffold presented here. The synthesis of the cyclic side chain moieties is outlined in Scheme 1.

Alcohol **7** was readily obtained from the Inhoffen–Lythgoe diol **6** using known methodology.[7,8] Tosylation of alcohol **7** and displacement of the tosylate with sodium cyanide afforded nitrile **8**,[9,10] which was deprotonated with two equivalents of LDA in THF at –78 °C and reacted with bromide **9**[11] to afford nitriles **10**[10] as a mixture of unseparable diastereoisomers. Reaction of the nitriles **10** with DIBAL-H in dichloromethane at –10 °C and subsequent acid work up afforded the corresponding aldehydes which were taken up in methanol and reacted with excess of sodium borohydride[12] to afford a mixture of alcohols **11**[10] and **12**,[10] which were cleanly separated by flash chromatography as colorless oils (17 and 44%, respectively). At this stage the stereochemistry of **11** and **12** at C22 was unknown. Both diastereoisomeric alcohols were selectively desilylated to give diols **13**[10] in excellent yields. Diols **13** were subjected to a very efficient zinc chloride mediated etherification[13] to give **14**,[10] which contained the desired cyclic side chain. The silyl protecting group of **14** was removed on refluxing THF affording alcohols **15**.[10] We were delighted to see that **15a** on recrystallization from ethyl acetate–hexane afforded crystals, which were subjected to X-ray crystallographic analysis, establishing its structure to be that shown in Figure 3.



Scheme 1 *Reagents and conditions*: (i) see ref. 7, 8; (ii) (a) *p*-TsCl, pyridine, r.t. (72%); (b) NaCN, DMSO, 90 °C (94%); (iii) LDA, THF, –78 °C, **9** (93%); (iv) DIBAL-H, CH_2Cl_2, –10 °C, HCl, $NaBH_4$, MeOH (**11**: 17%; **12**: 44%); (v) *n*-Bu_4NF, THF, r.t. (**13a**: 97%; **13b**: 94%); (vi) $ZnCl_2$, $ClCH_2CH_2Cl$, r.t. (**14a**: 76%; **14b**: 93%).

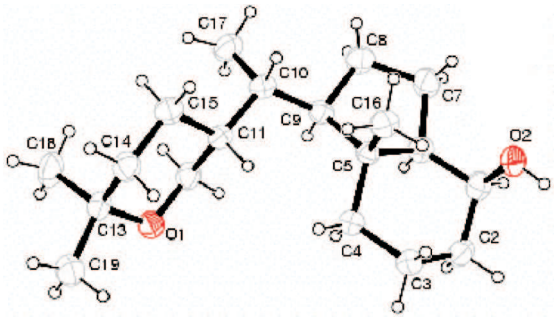
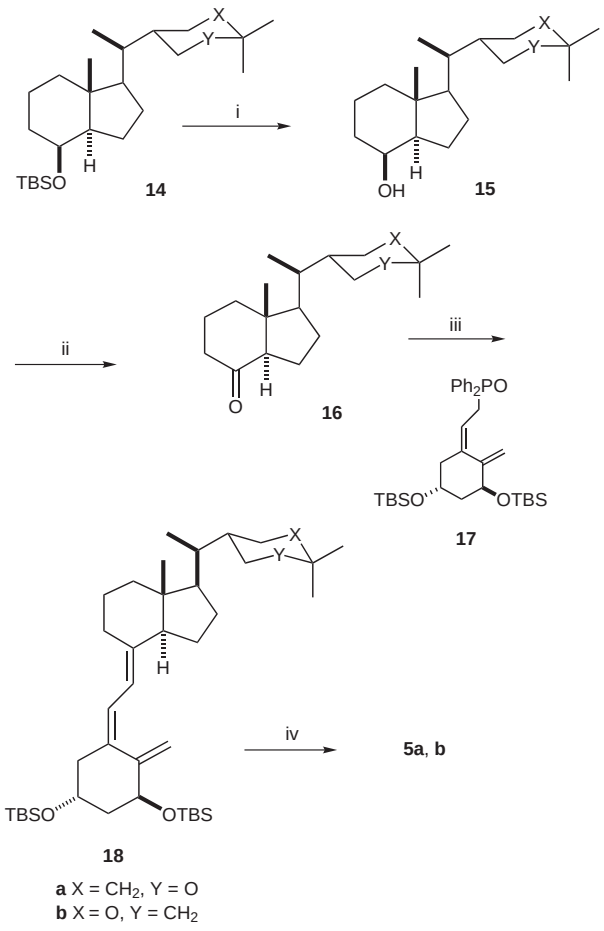


Figure 3 X ray structure of **15a**.

Oxidation of alcohols **15** with pyridinium dichromate afforded ketone **16**,[10] so setting the stage for the Wittig–Horner reaction with phosphine oxide **17**[14] leading to the desired analogues **5a**[15] and **5b**[16] [**5a** (54%); **5b** (52%); two steps (Scheme 2)].



Scheme 2 *Reagents and conditions*: (i) *n*-Bu_4NF, THF, reflux (**15a**: 77%; **15b**: 77%); (ii) PDC, CH_2Cl_2, r.t. (**16a**: 94%; **16b**: 99%); (iii) **17**; *n*-BuLi, THF, –78 °C [**18a** (70%); **18b** (69%)]; (iv) *n*-Bu_4NF, THF, r.t. [**5a** (77%); **5b** (76%)].

Methods

The 1.8 Å resolution crystal structure of an engineered ligand binding domain of human VDR[4] (Protein Data Bank code 1DB1) was used for the docking experiments. The ligands and the protein were prepared for docking with MOE (Molecular Operating Environment, Chemical Computing Group Inc., Montreal) and Autodock Tools. Autogrid[17] was used to generate the grid maps for an approximately $23 \times 23 \times 23$ Å^3 cell box centered on the ligand. Docking experiments were carried out using the Lamarckian genetic algorithm implemented in the automated docking program Autodock[17], version 3.0. Default parameters were employed except for: number of GA runs, 256; maximum number of energy evaluations, 2.5×10^6; and maximum number of generations, 2.7×10^5.

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(15) Compound **5a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.37 (1 H, d, *J* = 11.3 Hz, =CH-6 or =CH-7), 6.01 (1 H, d, *J* = 11.30 Hz, =CH-6 or =CH-7), 5.31 (1 H, s, CH_2-19), 4.99 (1 H, s, CH_2-19), 4.42 (1 H, dd, *J* = 7.69, 4.27 Hz, CHOH), 4.22 (1 H, m, CHOH), 3.66 (1 H, d, *J* = 11.52 Hz, CH_2O), 3.47 (1 H, t, *J* = 11.38 Hz, CH_2O), 2.82 (1 H, m), 2.59 (1 H, m), 2.31 (2 H, m), 1.18 (3 H, s, CH_3-26 or CH_3-27), 1.16 (3 H, s, CH_3-26 or CH_3-27), 0.81 (3 H, d, *J* = 6.80 Hz, CH_3-21), 0.52 (3 H, s, CH_3-18). ^{13}C NMR ($CDCl_3$): δ = 147.67 (C=), 143.08 (C=), 132.91 (C=), 125.15 (CH=), 117.20 (CH=), 111.90 (CH_2=), 71.01 (C-25), 70.87 (CH), 66.87 (CH), 62.76 (CH_2), 56.35 (CH), 53.50 (CH), 46.16 (C-13), 45.30 (CH_2), 42.89 (CH_2), 39.85 (CH_2), 39.13 (CH), 37.89 (CH), 37.16 (CH_2), 31.35 (CH_3), 29.12 (CH_2), 26.96 (CH_2), 24.86 (CH_2), 23.45 (CH_2), 22.20 (CH_2), 21.57 (CH_3), 14.30 (CH_3), 12.10 (CH_3). HRMS: *m/z* calcd for $C_{28}H_{45}O_3$: 429.3369; found: 429.3390.
(16) Compound **5b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.36 (1 H, d, *J* = 11.3 Hz, =CH-6 or =CH-7), 6.01 (1 H, d, *J* = 11.3 Hz, =CH-6 or =CH-7), 5.31 (1 H, s, CH2-19), 4.99 (1 H, s, CH2-19), 4.42 (1 H, dd, *J* = 8.0, 4.3 Hz, CHOH), 4.23 (1 H, m, CHOH), 3.53 (1 H, t, *J* = 11.3, CH_2O), 3.43 (1 H, dd, *J* = 11.1, 3.0 Hz, CH_2O), 2.82 (1 H, dd, *J* = 10.5, 3.7 Hz), 2.58 (1 H, dd, *J* = 9.9, 3.3 Hz), 2.30 (1 H, dd, *J* = 13.5, 6.4 Hz), 1.19 (3 H, s, CH_3-26 or CH_3-27), 1.17 (3 H, s, CH_3-26 or CH_3-27), 0.77 (3 H, d, *J* = 6.8 Hz, CH_3-21), 0.54 (3 H, s, CH_3-18). ^{13}C NMR ($CDCl_3$): δ = 147.67 (C=), 142.93 (C=), 132.99 (C=), 124.96 (CH=), 117.14 (CH=), 111.74 (CH_2=), 71.35 (C-25), 70.82 (CH), 66.87 (CH), 65.85 (CH_2), 56.38 (CH), 53.08 (CH), 46.02 (C-13), 45.27 (CH_2), 42.89 (CH_2), 39.80 (CH_2), 38.70 (CH), 36.55 (CH_2), 36.27 (CH), 31.34 (CH_3), 29.69 (CH_2), 27.25 (CH_2), 23.52 (CH_2), 22.12 (CH_2), 21.51 (CH_3), 19.73 (CH_2), 14.39 (CH_3), 11.89 (CH_3). HRMS: *m/z* calcd for $C_{28}H_{45}O_3$: 429.3369; found: 429.3377.
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