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ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 9077–9079

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A general approach to the synthesis of enantiopure 19-*nor*-Vitamin D_3 and its C-2 phosphate analogs prepared from cyclohexadienyl sulfone†

Vikas Sikervar, James C. Fleet and Philip L. Fuchs*

Received 27th April 2012, Accepted 28th May 2012
DOI: 10.1039/c2cc33054g

Published on 01 August 2012 on http://pubs.rsc.org | doi:10.1039/C2CC33054G
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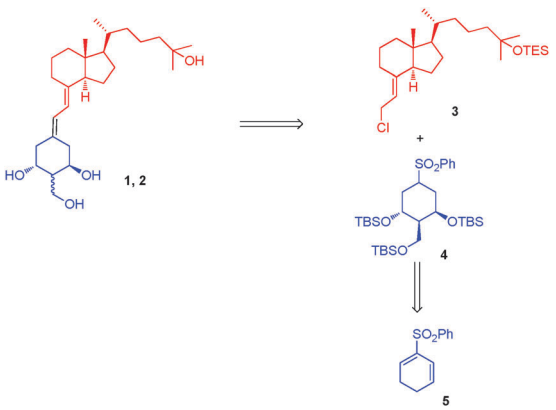
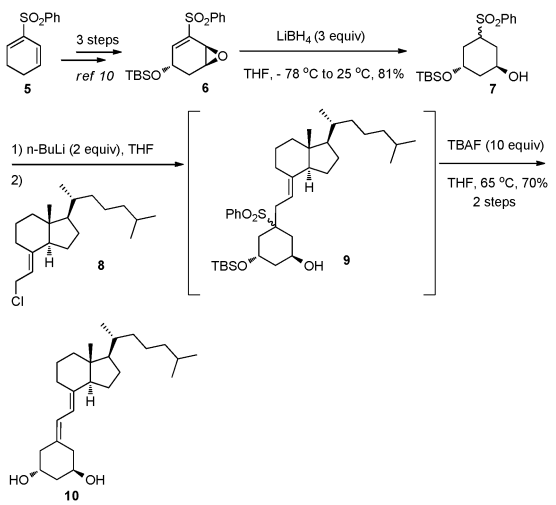
A synthesis of enantiopure 19-*nor*-Vitamin D_3 and its C-2 substituted cyclic phosphate analogs is achieved *via in situ* trapping of an α-sulfonyl anion with a CD-ring allyl chloride and 1,2-eliminative desulfonylation exploiting the basic properties of TBAF. The A-ring is prepared *via anti*-selective dithiane addition to vinyl sulfone and $LiBH_4$ mediated sequential bis reduction of an epoxy vinyl sulfone.

1,25-Dihydroxyvitamin D_3, [1α,25$(OH)_2D_3$] is an active metabolite of Vitamin D_3 and is involved in the regulation of calcium homeostasis and bone metabolism. In addition, it suppresses the growth of numerous human cancer cell lines by inhibiting cell cycle progression and inducing cell death.[1] Therapeutic applications have proven difficult because of severe side effects such as hypercalcemia. Design of Vitamin D_3 analogs with effective antiproliferative activity combined with reduced calcemic effects has been an extensive area of research aimed at overcoming this hurdle.[2] DeLuca *et al.* reported in 1990 that the deletion of the C10,19 exocyclic olefin renders the molecule less calcemic and significantly increases stimulation of differentiation and growth inhibition of tumor cells.[3] This finding has spurred the synthesis of 19-nor-Vitamin D_3 and its analogs, a number of which are currently used as drugs.[4] A number of analogs with C-2 substituted products are found to have higher cell differentiating properties and VDR binding affinity.[5] In addition, introduction of a phosphonate group in the upper side chain also reduces the calcemic effects of these analogs.[6] Phosphorus-bearing A-rings and their effects on VDR binding affinity and hypercalcemic properties are unknown. We now report the synthesis and *in vitro* properties of four A-ring cyclic phosphate analogs. The synthetic strategy as shown in Scheme 1 involves the coupling of A-ring sulfone **4** with CD-ring allyl chloride **3**. The A-ring sulfone **4** is prepared by series of transformations on cyclohexadienyl sulfone **5**.



Scheme 1 Retrosynthetic analysis.



Scheme 2 Synthesis of 19-*nor*-1α$(OH)_2D_3$.

As shown in Scheme 2, a concise route to 19-*nor*-1α$(OH)_2D_3$[7] involves alkylation of an alkoxide-sulfonyl dianion derived from **7** with allyl chloride **8**.[8] Achiral dienyl sulfone **5** is the ultimate starting material, which is prepared on the kg scale.[9] Application of the standard three-step synthesis provides enantiopure epoxide **6**,[10] which suffers sequential double conjugate reduction with $LiBH_4$ to afford sulfone **7** in 2.8 : 1 dr in 81% yield. O,C-bismetallation of **7** using 2.0 equiv of n-BuLi gives the dianion, which is treated with allyl chloride **3**[11] to deliver alkylated product **9**. One-pot TBAF–mediated desulfonylation with concomitant silyl ether deprotection directly provides diene **10** in 70% overall yield from **7**.

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA. E-mail: pfuchs@purdue.edu; Fax: +1765-494-0239; Tel: +1765-494-5242

† Electronic supplementary information (ESI) available. CCDC 879401. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc33054g

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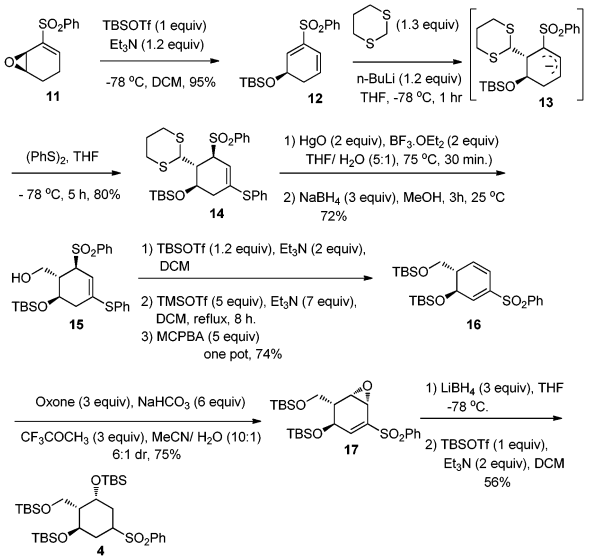
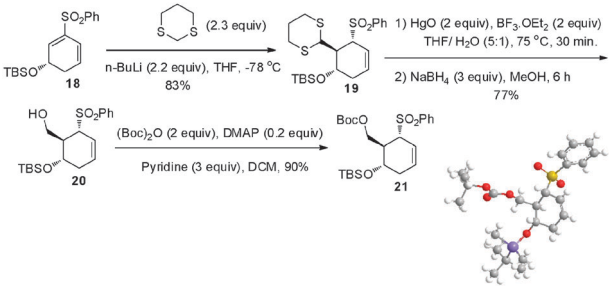


Scheme 3 Synthesis of sulfone **4**.



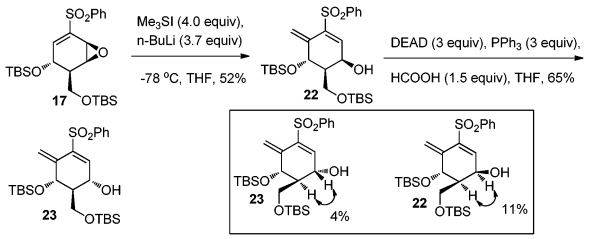
Scheme 4 Synthesis of allyl sulfone **21**.

To access the C3-hydroxymethyl A-ring needed for preparation of the cyclic phosphate targets, dithiane is used as a hydroxymethyl synthon. Significantly, dienyl sulfone **12** (Scheme 3) suffers conjugate addition[12] of lithiated 1,3-dithiane giving allyl sulfonyl anion **13**, which was quenched with diphenyldisulfide providing the thermodynamic vinyl sulfide **14** as a single regio- and stereoisomer.[13,17] Dienyl sulfone **12** is prepared *via* TBSOTf catalyzed opening of the epoxide **11** which in turn is prepared from dienyl sulfone **5** in 70% yield and 99% ee using Jacobsen's catalyst.[14] HgO mediated deprotection of thioacetal **14** to the aldehyde[15] and subsequent reduction with $NaBH_4$ gave alcohol **15**. One-pot TBS protection, 1,4-elimination and mCPBA oxidation provided dienyl sulfone **16**.[16] Epoxidation using *in situ* generated TFDO proceeds with 6 : 1 dr. Double conjugate-reduction of epoxy vinyl sulfone **17** with $LiBH_4$ gave the secondary alcohol, which provided sulfone **4** in 56% yield with > 90% purity upon silyl ether protection (Scheme 3).

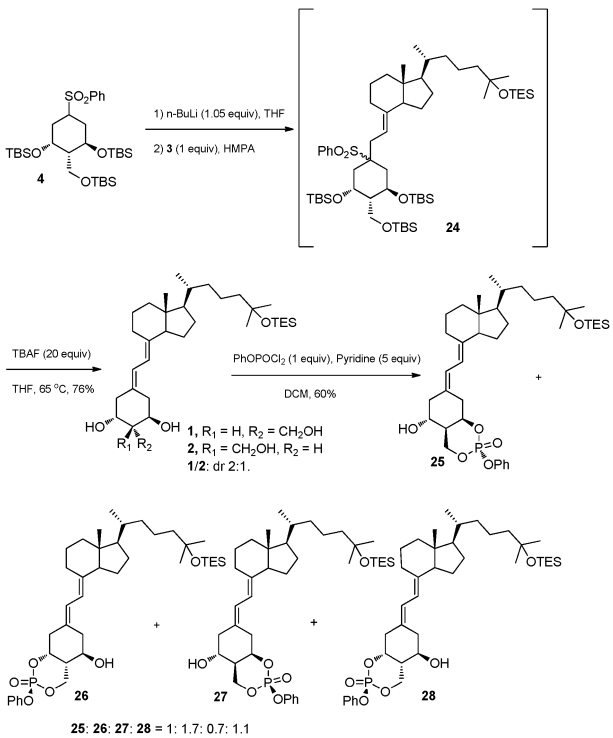
The stereochemistry of the dithiane addition to the dienyl sufone **18** is verified by X-ray crystallography of allyl sulfone **21** (Scheme 4).

Stereochemistry of epoxide **17** is assigned by synthesis of cross-conjugated dienyl alcohols **22** and **23** and comparing the NOE difference between the two protons as shown in Scheme 4.

Addition of dimethylsulfonium methylide[17] to epoxyvinyl sulfone **17** gave alcohol **22**, which on Mitsunobu inversion



Scheme 5 NOE comparison of alcohols **22** and **23**.



Scheme 6 Synthesis of cyclic phosphates.

with formic acid provided diastereomer **23** after formate hydrolysis (Scheme 5).[18]

Sulfone **4** is metallated with 1.05 equiv of n-BuLi and the sulfonyl anion thus created is quenched with 1 equiv of allyl chloride **3** to generate the coupled product **24** (Scheme 6). The intermediate **24** is treated with 20 equiv of TBAF to form the dienes **1** and **2** in a 2 : 1 ratio in 78% yield. The mixture of dienes **1** and **2** is subjected to reaction with 1.0 equiv of phenyl dichlorophosphoridate to give the required targets **25**, **26** **27**, **28** in 1 : 1.7 : 0.7 : 1.1 dr ratio and 60% yield. The four compounds were separated on HPLC and stereochemistry assigned based on NOESY, 31P and COSY spectra.†

Impact of the vitamin D analogs on the expression of vitamin D-responsive genes in two human colon cancer cell lines was examined: Caco-2 cells and SW480-ADH (Fig. 1)[19]. These cells respond to 1,25 dihydroxyvitamin D_3 treatment by inducing the expression of various target genes. The most responsive vitamin D regulated gene is for the enzyme CYP24 (a cytochrome P450 family member called 25 hydroxyvitamin D, 24 hydroxylase) – this is expressed in both cell lines. In SW480-ADH cells, $1\alpha,25(OH)_2D_3$ treatment also induces expression of E-cadherin, a tight junction protein that is a marker of cell

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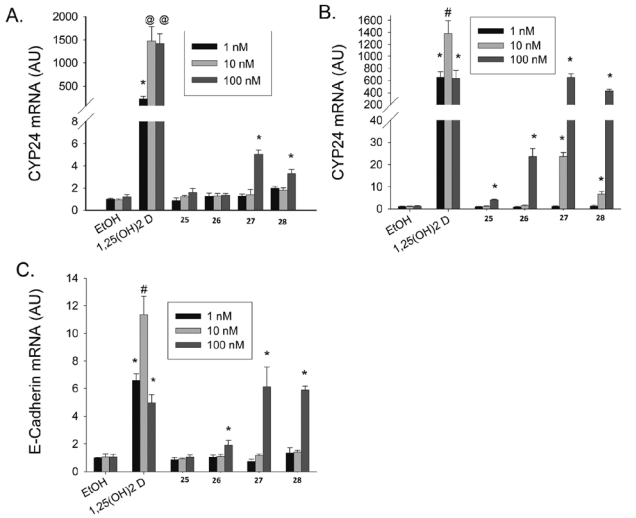

Fig. 1 Effect of phosphate analogs in Caco-2 and SW480-ADH cell lines. The effect of phosphate modified A-ring analogs on expression of two vitamin D responsive genes in the human colonic carcinoma cell lines Caco-2 and SW-480ADH. (A) CYP24 mRNA in Caco-2, (B) CYP24 mRNA in SW480-ADH, (C) E-cadherin mRNA in SW480-ADH. Cells were treated for 6 h with varying doses of vitamin D compounds or with concentration matched ethanol vehicle (EtOH). RNA was isolated and analyzed by real time-PCR. Data are normalized to the expression of the control gene RPLPO. Bars represent the mean ± SEM (n = 4). * significantly different from the ethanol control group, # significantly different from all other groups, @ significantly different from all but one group.

differentiation. Phosphate analogs **25**, **26**, **27** and **28** were found to be less active than 1α,25(OH)$_2$D$_3$ in Caco-2 cell lines. While in SW480-ADH cell lines, phosphate analogs **25** and **26** showed less activity but **27** and **28** were found to have slightly higher activity at 100 nM.

In summary, we have reported a highly convergent synthesis of 19-nor-Vitamin D$_3$ and its four cyclic phosphates from dienyl sulfone **5**.

We thank Arlene Rothwell and Karl Wood for providing MS data. We thank John Harwood for providing some of the NMR data. We thank Purdue Cancer center for providing the Ross fellowship.

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