Bioorganic & Medicinal Chemistry 21 (2013) 5209–5217

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design and synthesis of novel 1,25-dihydroxyvitamin D_3 analogues having a spiro-oxetane fused at the C2 position in the A-ring

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ARTICLE INFO

Article history:
Received 26 May 2013
Revised 11 June 2013
Accepted 12 June 2013
Available online 20 June 2013

Keywords:
Vitamin
Hormone
Nuclear receptor
Oxetane
Steroid

ABSTRACT

Four structurally novel stereoisomeric analogues of 1,25-dihydroxyvitamin D_3 (**3a–d**) bearing a spiro-oxetane fused at the C2 position of the A-ring have been designed and synthesised in a convergent manner. The requisite A-ring enyne precursors (**13a**,**b**) for the vitamin D analogues (**3a**,**b**) and (**3c**,**d**), respectively, were synthesised from pentaerythritol according to an eleven-step procedure. Preliminary biological evaluation of the analogues using the bovine thymus vitamin D receptor (VDR) suggested that the incorporation of the spiro-oxetane moiety instead of a *gem*-dimethyl group at the C2 position had a beneficial effect on the VDR affinity.

1. Introduction

The hormonally active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (**1**: Fig. 1), exhibits a broad range of biological activities, including cell differentiation-inducing, anti-proliferative and apoptosis-stimulating activities in many normal and malignant cells, in addition to its classical role in calcium-phosphorus homeostasis.[1] The vitamin D receptor (VDR) is generally considered to be the major molecular target of vitamin D_3 and its active metabolites. The VDR itself belongs to the nuclear receptor super-family, and behaves as a ligand-inducible transcriptional factor. Further insights into the structure–function relationships of vitamin D and its metabolites are needed to allow for our understanding of these beneficial effects on cells in disease states to be developed into therapeutic agents against cancer, psoriasis and immune disorders, as well as developing a deeper understanding of the subtype-free VDR and how it could be used to deliver a variety of different actions.

The binding of the *seco*-steroid hormone **1** to the ligand-binding domain (LBD) of VDR triggers a conformational change in the trans-activation domain of the receptor (AF-2) that initiates an entire sequence of reactions resulting in a genomic response.[1] The specific interactions of modified ligands with the LBD have been the subject of considerable levels of attention from chemists, because the interface provided by the VDR for the binding of transcriptional co-activators, as well as the overall stability of the complex and the transcriptional machinery, can be influenced by modifications to the ligands, particularly to the A-ring of **1**.

The binding mode of **1** to the receptor, as well as the binding modes of several other active VDR ligands, have been solved by X-ray crystallography, with the results indicating that all three of the hydroxy groups in **1**, as well as in the active ligands, were recognised by the same two amino acid residues via hydrogen bonding interactions.[2] The X-ray crystal structure of the VDR complexed with **1** also revealed the presence of a hydrophilic region in the vicinity of the A-ring that was capable of accommodating bulky substituents at the C2 position of the A-ring as in the *seco*-steroids.[2c] Several groups, including our own, found a series of important A-ring analogues with elevated affinities for VDR via the introduction of a substituent with hydrogen bonding capability.[3] In addition, it has been suggested that this cavity consists of a sleek hydrophobic area surrounded by lipophilic amino acid residues such as Phe-150, Leu-233 and Tyr-236, in the close vicinity of the C2 position in the A-ring.[3d] In this regard, we generated a series of the C2-methyl analogues (**2a–c**)[3a–c] with unique activity profiles, including 2,2-dimethyl-1α,25-dihydroxyvitamin D_3 (**2c**: Fig. 1),[3e] bearing substituents that could access this hydrophobic area.

Four-membered cyclic ethers, which are otherwise known as oxetanes, can act as surrogates not only for geminal dimethyl groups, but also for 'deeper' carbonyl groups with high hydrogen bonding avidity.[4] The nominal analogy of oxetanes to carbonyl groups has been demonstrated, and the former can be beneficial when a larger volume occupancy and deeper oxygen placement are required in the receptor pocket.[4b] The substitution of a geminal

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http://dx.doi.org/10.1016/j.bmc.2013.06.032

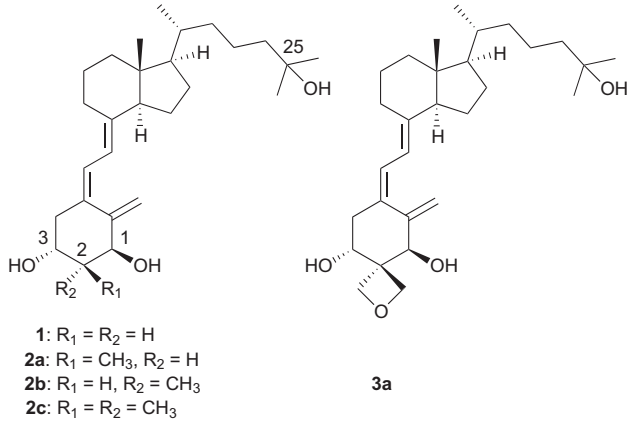


Figure 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (**1**), the C2-methyl-introduced vitamin D_3 (**2a–c**) and novel vitamin D analogue (**3a**) having a spiro-oxetane fused at the C2 position in the A-ring.

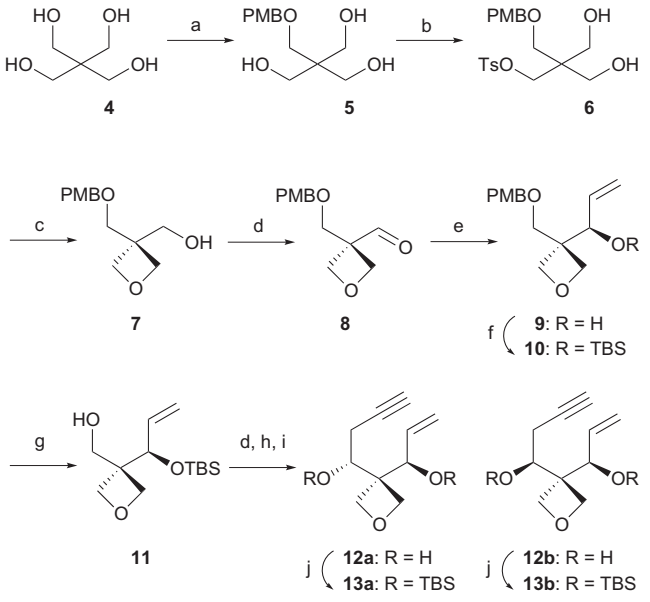
dimethyl group with an oxetane should lead to an increase in aqueous solubility, as well as a reduction in the rate of metabolic degradation in the majority of cases,[4b] and changes of this type could be advantageous for VDR ligands. In view of our results with the C2-methyl analogues, it was envisaged that the incorporation of a spiro-oxetane at the C2 position in the A-ring of **1** would be of interest to generate novel ligands capable of better filling the hydrophilic cavity. Herein, we describe the synthesis of all four possible A-ring stereoisomers incorporating a spiro-oxetane fused at the C2 position (**3a–d**) as novel vitamin D analogues capable of filling the hydrophilic cavity with an oxetane, combined with beneficial effects of the C2-methyl substituent.

2. Results and discussion

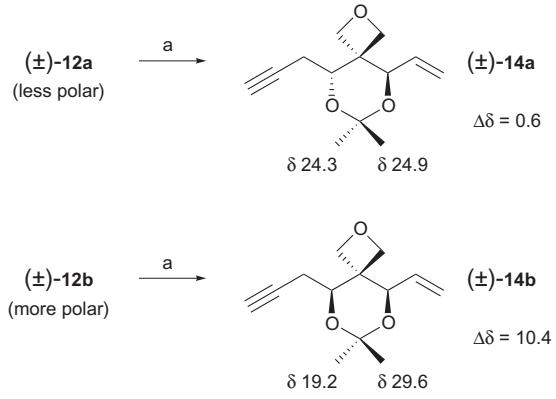
2.1. Synthesis

The syntheses of these novel *seco*-steroid analogues bearing a spiro-cyclic oxetane fused at the C2 position in the A-ring were conducted according to the convergent method pioneered by Trost et al.[5] The A-ring enyne precursors (**13a**,**b**) were synthesised from pentaerythritol (**4**), according to the route depicted in the scheme (Scheme 1). The protection of one of the four hydroxy groups in pentaerythritol (**4**) with a *p*-methoxybenzyl (PMB) group gave the triol **5** in 75% yield.[6] Conversion of **5** to the mono-tosylate **6**, followed by treatment with sodium hydride, provided the key oxetane intermediate **7** in excellent yield.[7] Oxidation of the primary alcohol in **7** using the Swern procedure gave the corresponding aldehyde **8** in 99% yield. Subsequent treatment of the aldehyde with vinylmagnesium bromide afforded an enantiomeric mixture of the allylic alcohols (±)-**9** in 81% yield. The protection of the secondary alcohol in (±)-**9** with a *tert*-butyldimethylsilyl (TBS) ether was achieved under standard conditions using TBS chloride (TBSCl) and imidazole to give (±)-**10** in 96% yield. Subsequent deprotection of the PMB group with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) gave the primary alcohol (±)-**11** in 95% yield without affecting the oxetane structure. The primary alcohol (±)-**11** was then oxidised using the Swern procedure to afford the corresponding aldehyde in 93% yield, which was reacted with allenylmagnesium bromide to give a diastereomeric mixture of homopropargyl alcohols in 95% yield, as an approximately 45:55 mixture of the diastereomers in favour of the 1,3-*syn*-diols. Following the removal of the TBS protective group with tetra-*n*-butylammonium fluoride (TBAF), it became possible to separate the resulting mixture of the requisite A-ring enyne synthons (±)-**12a** and (±)-**12b** by silica gel column chromatography. The relative stereochemistries of the 1,3-diols in the different enantiomeric mixtures were determined by ^{13}C NMR analysis of the corresponding acetonides (Scheme 2).[8] A variety of different conditions were evaluated for the protection of the diols, including TBSCl-imidazole, TBS trifluoromethanesulfonate (triflate)-2,6-lutidine, and *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide.[9] Unfortunately, however, these methods failed to provide the desired products in good yield. The protection of the diols in (±)-**12a** and (±)-**12b** as the corresponding TBS groups was ultimately achieved using *tert*-butyldimethylsilyloxy *N*-phenylbenzimidate (TBS-BEZA)[10] in the presence of catalytic pyridinium trifluoromethanesulfonate (triflate) to afford the requisite A-ring precursors (±)-**13a** and (±)-**13b**, respectively, in 80% yield in both cases.

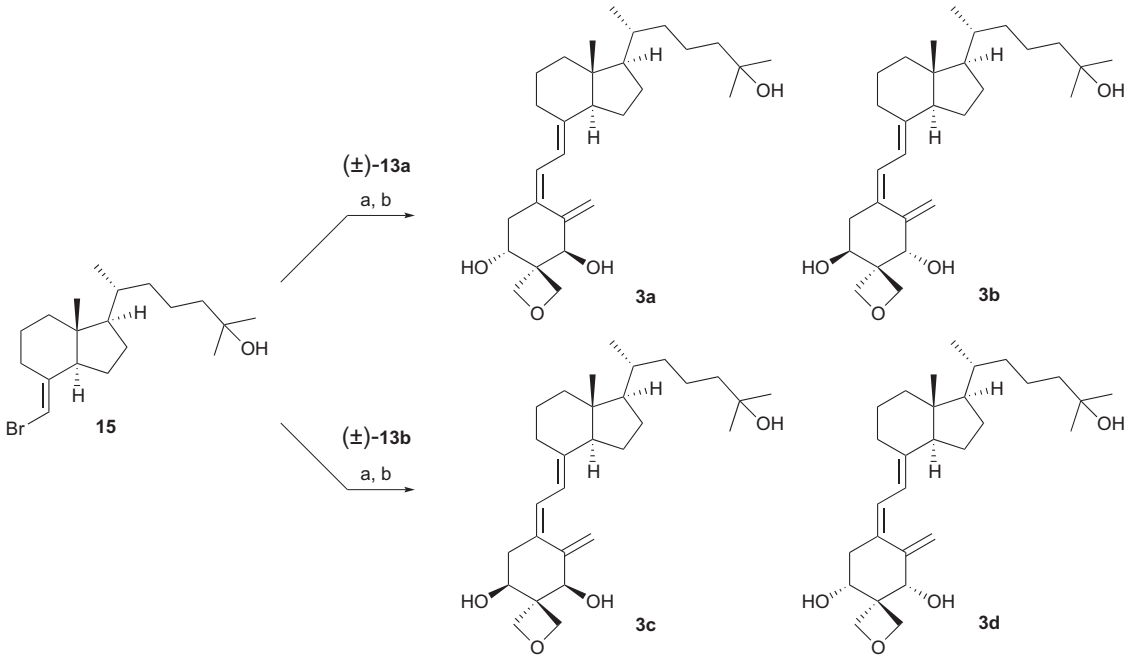
The coupling reactions of the protected A-ring enyne precursors (±)-**13a** and (±)-**13b** with the CD-ring portion **15**[5] in the presence of tetrakis(triphenylphosphine)palladium, followed by deprotection with TBAF, proceeded smoothly to afford the novel *seco*-steroids **3a**,**b** and **3c**,**d**, respectively (Scheme 3). The presence of a spiro-oxetane structure in the four A-ring stereoisomers (**3a–d**) was



Scheme 1. *Reagents*: (a) NaH; PMBCl, TBAI/DMF, 75%; (b) TsCl/pyridine, 63%; (c) NaH/THF, 90%; (d) oxalyl chloride, DMSO, Et_3N/CH_2Cl_2, 99% from **8**, 93% from **11**; (e) vinylmagnesium bromide/toluene, 81%; (f) TBSCl, imidazole/CH_2Cl_2, 96%; (g) DDQ/CH_2Cl_2, 95%; (h) allenylmagnesium bromide/ether, 95%; (i) TBAF/THF, 96%; (j) TBS-BEZA, pyridinium triflate/CH_2Cl_2, 80% from **12a**,**b**.



Scheme 2. Determination of relative stereochemistry of the 1,3-diols by ^{13}C NMR analysis. *Reagents*: (a) 2,2-dimethoxypropane, CSA, 82% from (±)-**12a**, 62% from (±)-**12b**.



Scheme 3. Coupling reactions of the A-ring enynes with the CD-ring portion. *Reagents*: (a) $(Ph_3P)_4Pd$, Et_3N/toluene; (b) TBAF/THF, 16–20% (two steps).

confirmed by ^1H NMR analysis. The NMR spectra of the stereoisomers showed four distinctive doublets with geminal coupling constants in the range of 6.1–6.5 Hz, indicating the presence of a slightly restricted four-membered ring. In the case of **3a**, the vicinal coupling constant of 6.9 Hz between the H(3α and H(4β protons suggested that the introduction of the spiro-oxetane had not had a significant effect on the conformational preference of the A-ring, based on a comparison with the parent compound **1** and its 2,2-dimethyl counterpart **2c**.[11] The absolute stereochemistry of the 1,3-diols in the A-ring was determined using the bis-MTPA methods that we developed previously (Fig. 2).[12] The four stereoisomeric spiro-oxetane analogues were purified using a recycling HPLC method to afford pure specimens for biological evaluation.

Table 1
Vitamin D receptor binding affinities of the four A-ring stereoisomeric spiro-oxetane analogues (**3a**–**d**)[a]

Compounds	VDR affinity[b]
1	100
2a	400[c]
2b	13[c]
2c	3[d]
3a (1*R*, 3*R*)	5
3b (1*S*, 3*S*)	0.001
3c (1*R*, 3*S*)	0.04
3d (1*S*, 3*R*)	0.002

[a] The potency of 1α,25-dihydroxyvitamin D_3 (**1**) was normalised as 100.
[b] Bovine thymus vitamin D receptor.
[c] Ref. 3a.
[d] Ref. 3e.

2.2. Vitamin D receptor (VDR) binding affinity

The affinities of the novel compounds for the VDR were evaluated using bovine thymus VDR. Table 1 provides a summary of the relative VDR binding affinities of the spiro-oxetane analogues (**3a**–**d**) in comparison with the natural hormone **1**, the potency of which was normalised to 100 by definition, as well as the 2α-methyl-(**2a**)[3a] 2β-methyl-(**2b**)[3a] and 2,2-dimethyl analogues (**2c**).[3e] Of the four stereoisomers (**3a**–**d**), isomer **3a**, which possessed the 1α- and 3β-hydroxy groups in the natural configuration, showed the highest level of affinity toward the VDR. Furthermore, the replacement of the C2-*gem*-dimethyl group of **2c** with the oxetane in this case resulted in a slight increase in the affinity toward the VDR. The other stereoisomers (**3b**–**d**) showed a reduced affinity to the VDR relative to the parent A-ring stereoisomers of the natural hormone **1**. It is noteworthy that isomer **3d** bearing

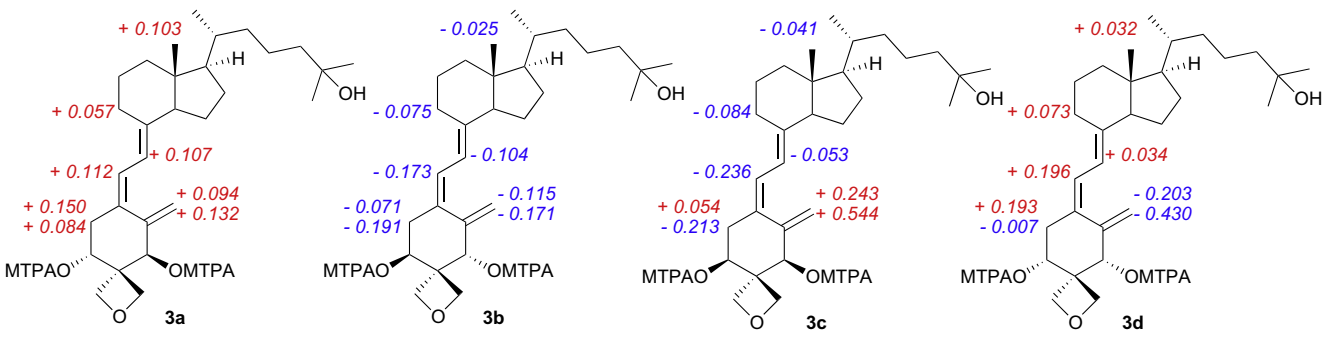


Figure 2. Determination of absolute configuration at the C1 and C3 positions of **3a**–**d** by ^1H NMR analysis of their bis-MTPA esters.[12]

the 1β- and 3β-hydroxy groups, which showed the weakest level of binding to the VDR of the four A-ring stereoisomers,[3b] showed a recognisable level of affinity. The introduction of an oxetane at the C2 position as in **3d**, particularly the oxygen atom, could therefore provide some degree of compensation for the unfavourable interactions provided by the 1β-hydroxy group.

3. Conclusions

In conclusion, we have designed and synthesised four novel A-ring analogues of active vitamin D_3 bearing an oxetane fused at the C2 position. A convergent synthetic method using palladium catalysis allowed us to reach the novel *seco*-steroids bearing a unique spiro-oxetane structure (**3a**–**d**). The enyne precursors (**13a**,**b**) required for the construction of the A-ring were prepared from pentaerythritol according to an eleven-step procedure in excellent overall yield. The introduction of a spiro-oxetane into the A-ring resulted in an increase of the polarity and solubility properties of the products compared with their geminal dimethyl counterparts. Our concise synthetic route from pentaerythritol to the enyne precursors (**13a**,**b**) provided an effective demonstration that the oxetane structure is considerably robust and tolerant of a variety of synthetic conditions, which may be of additional importance to any future work conducted in this area. Preliminary biological evaluation of the novel analogues using bovine thymus VDR suggested that the incorporation of a spiro-oxetane at the C2 position instead of a *gem*-dimethyl group had a beneficial effect on the VDR affinity.

4. Experimental section

4.1. General information

NMR spectra were recorded on a Bruker AVANCE-400, a Bruker AVANCE-700, or a JEOL ECX-400 spectrometer. The chemical shifts have been expressed in ppm relative to tetramethylsilane (TMS). Mass spectra and electrosprey ionisation high-resolution mass spectra (ESI-HRMS) were recorded on a Bruker micrOTOF. Ultraviolet spectra were recorded with a Jasco V-660 spectrophotometer. Recycling preparative HPLC was performed on a Shimadzu CBM-20A equipped with an LC-6AD pump and an SPD-M20A diode array detector.

4.2. 2-Hydroxymethyl-2-(4-methoxybenzyloxy)methyl-1,3-propanediol (5)

To a solution of pentaerythritol **4** (3.40 g, 25.0 mmol) and tetra-*n*-buthylammonium iodide (TBAI) (250 mg, 0.68 mmol) in dry *N*,*N*-dimethylformamide (DMF) (37.5 mL) was added sodium hydride (405 mg, 60% in mineral oil, 28.1 mmol) in a potion-wise manner with stirring under an atmosphere of argon at 0 °C. Upon completion of the addition, the stirring was continued for 1 h at room temperature. A solution of *p*-methoxybenzyl chloride (PMBCl) (1.06 g, 6.76 mmol) in dry DMF (1.25 mL) was then added to the reaction mixture with stirring at 0 °C, and the resulting mixture was stirred overnight at room temperature. After the addition of methanol (3.0 mL) to the reaction mixture at 0 °C, the solvent was removed under the reduced pressure to give a residue, from which **5** (1.30 g) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane/methanol = 10:5:1) as a colourless solid in 75% yield.

Compound ***5***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 2.53 (3H, t, J = 5.6 Hz), 3.48 (2H, s), 3.71 (6H, d, J = 5.6 Hz), 3.80 (3H, s), 4.44 (2H, s), 6.88 (2H, d, J = 8.8 Hz), 7.22 (2H, d, J = 8.8 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 44.9, 55.3, 65.0, 72.0, 73.5, 113.9, 129.3, 129.6, 159.4; HRMS (ESI^+) m/z calcd for $C_{13}H_{20}O_5Na$ $[M+Na]^+$ 279.1203, found 279.1217.

4.3. 3-Hydroxy-2-hydroxymethyl-2-[(4-methoxybenzyloxy)methyl]propyl 4-methylbenzenesulfonate (6)

To a stirred solution of **5** (6.67 g, 26.0 mmol) in dry pyridine (70 mL) was added *p*-toluenesulfonyl chloride (5.45 g, 28.6 mmol) under an atmosphere of argon at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. After the addition of water (25 mL) to the reaction mixture at 0 °C, the solvent was removed under the reduced pressure to give a residue, which was dissolved with CH_2Cl_2 (400 mL) before being washed with brine (70 mL). The organic layer was dried over sodium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **6** (6.72 g) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane/methanol = 10:5:1) as a colourless oil in 63% yield.

Compound ***6***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 2.30 (2H, t, J = 6.0 Hz), 2.45 (3H, s), 3.42 (2H, s), 3.63 (4H, d, J = 6.0 Hz), 3.81 (3H, s), 4.13 (2H, s), 4.37 (2H, s), 6.86 (2H, d, J = 8.4 Hz), 7.16 (2H, d, J = 8.4 Hz), 7.34 (2H, d, J = 8.4 Hz), 7.78 (2H, d, J = 8.4 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 21.5, 44.9, 55.1, 62.9, 69.1, 69.7, 73.2, 113.7, 127.8, 129.0, 129.5, 129.8, 132.3, 144.9, 159.2, 145.1, 159.4; HRMS (ESI^+) m/z calcd for $C_{20}H_{26}NaO_7S$ $[M+Na]^+$ 433.1291, found 433.1242.

4.4. 3-[(4-Methoxybenzyloxy)methyl]-oxetan-3-ylmethanol (7)

To a solution of **6** (4.50 g, 11.0 mmol) in dry THF (45 mL) was added sodium hydride (660 mg, 60% in mineral oil, 16.5 mmol) in a potion-wise manner with stirring under an atmosphere of argon at 0 °C, and the resulting mixture was stirred for 16 h at room temperature. After the addition of water (30 mL) to the reaction mixture at 0 °C, the whole was extracted with ethyl acetate (200 mL × 3). The combined organic layer was washed with brine, dried over sodium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **7** (2.36 g) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 2:1) as a colourless oil in 90% yield.

Compound ***7***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 2.30 (1H, t, J = 5.6 Hz), 3.78 (2H, s), 3.81 (3H, s), 3.93 (2H, d, J = 5.6 Hz), 4.41 (2H, d, J = 6.0 Hz), 4.48 (2H, d, J = 6.0 Hz), 4.49 (2H, s), 6.90 (2H, d, J = 8.8 Hz), 7.22 (2H, d, J = 8.8 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 43.9, 55.2, 66.3, 73.3, 73.4, 76.5, 113.8, 129.3, 129.6, 159.4; HRMS (ESI^+) m/z calcd for $C_{13}H_{18}O_4Na$ $[M+Na]^+$, 261.1097, found 261.1080.

4.5. 3-[(4-Methoxybenzyloxy)methyl]-oxetane-3-carbaldehyde (8)

A solution of oxalyl chloride (0.42 mL, 0.61 g, 4.8 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of dimethylsulfoxide (DMSO) (0.68 mL, 9.7 mmol) in dry CH_2Cl_2 (5 mL) under an atmosphere of argon at −78 °C, and the mixture was stirred at the same temperature for 30 min. The resulting mixture was transferred to a solution of **7** (500 mg, 2.1 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture was stirred at −78 °C for 40 min. Subsequently, neat triethylamine (2.6 mL, 18.5 mmol) was added to the reaction mixture followed by stirring for 50 min, while the temperature was elevated from −78 to 0 °C. The reaction was quenched by the addition of water (5 mL) and the whole was extracted with ether (100 mL × 3). The combined organic layer was washed with brine (5 mL), dried over sodium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **8** (490 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:2) as a colourless oil in 99% yield.

Compound ***8***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 3.80 (3H, s), 3.89 (2H, s), 4.50 (2H, d, J = 8.0 Hz), 4.52 (2H, s), 4.79 (2H, d, J = 8.0 Hz),

6.87 (2H, d, J = 8.8 Hz), 7.22 (2H, d, J = 8.8 Hz), 9.85 (1H, s); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 53.1, 53.3, 69.6, 73.26, 73.31, 113.9, 129.4, 159.4, 199.9; HRMS (ESI^+) m/z calcd for $C_{13}H_{16}O_4Na$ $[M+Na]^+$ 259.0940, found 259.0946.

4.6. 1-{3-(4-Methoxybenzyloxy)methyl-oxetan-3-yl}prop-2-en-1-ol (9)

A solution of vinylmagnesium bromide (14% in THF, 0.29 mL, 0.29 mmol) was added to a stirred solution of **8** (64 mg, 0.27 mmol) in dry toluene (5 mL) at −78 °C in a drop-wise manner under an atmosphere of argon. After having been stirred for 35 min at the same temperature, the reaction was quenched by the addition of saturated aqueous NH_4Cl. The resultant mixture was extracted with ethyl acetate (30 mL × 3), and the combined organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which **9** (58 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:2) as a colourless oil in 81% yield.

Compound ***9***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 3.78 (1H, d, J = 8.8 Hz), 3.81 (3H, s), 3.82 (1H, d, J = 8.8 Hz), 4.25 (1H, d, J = 6.4 Hz), 4.47 (2H, s), 4.50 (3H, complex), 4.68 (1H, d, J = 6.2 Hz), 5.25 (1H, dt, J = 10.5, 1.5 Hz), 5.37 (1H, dt, J = 17.1, 1.5 Hz), 5.87 (1H, ddd, J = 17.1, 10.5, 6.0 Hz), 6.89 (2H, m), 7.23 (2H, m); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 45.8, 55.3, 73.2, 73.5, 75.2, 76.3, 77.4, 113.9, 117.1, 129.3, 129.5, 136.3, 159.5; HRMS (ESI^+) m/z calcd for $C_{15}H_{20}O_4Na$ [M + Na]$^+$ 287.1254, found 287.1254.

4.7. 3-{[1-(*tert*-Butyldimethylsilyl)oxy]prop-2-enyl}-3-[(4-methoxybenzyl)oxy]methyl-oxetane (10)

To a stirred solution of **9** (1.60 g, 6.1 mmol) and imidazole (1.24 g, 18.2 mmol) in dry CH_2Cl_2 (60 mL) was added *tert*-butyldimethylsilyl chloride (TBSCl) (1.82 g, 12.1 mmol) at 0 °C under an atmosphere of argon, and the resulting mixture was stirred at room temperature for 9 h. Additional charges of imidazole (1.24 g, 18.2 mmol) and TBSCl (1.82 g, 12.1 mmol) were then made to the reaction, and the resulting mixture was stirred at room temperature for 13 h. The reaction mixture was then diluted with ethyl acetate (300 mL) and was washed successively with water (30 mL), and brine (30 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:2) to give **10** (2.20 g) as a colourless oil in 96% yield.

Compound ***10***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.03 (3H, s), 0.06 (3H, s), 0.88 (9H, s), 3.55 (1H, d, J = 9.2 Hz), 3.71 (1H, d, J = 9.2 Hz), 3.81 (3H, s), 4.32 (1H, d, J = 5.8 Hz), 4.35 (1H, br d, J = 7.4 Hz), 4.42 (1H, d, J = 5.9 Hz), 4.44 (1H, d, J = 11.7 Hz), 4.50 (1H, d, J = 11.7 Hz), 4.54 (1H, d, J = 5.8 Hz), 4.59 (1H, d, J = 5.9 Hz), 5.17 (1H, ddd, J = 10.3, 1.7, 0.9 Hz), 5.24 (1H, ddd, J = 17.2, 1.7, 1.2 Hz), 5.82 (1H, ddd, J = 17.2, 10.3, 7.4 Hz), 6.88 (2H, m), 7.26 (2H, m); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ −5.0, −3.9, 18.1, 25.8, 47.6, 55.3, 71.2, 73.1, 74.4, 75.1, 75.3, 113.8, 117.1, 129.3, 130.3, 137.2, 159.2; HRMS (ESI^+) m/z calcd for $C_{21}H_{34}NaO_4Si$ $[M+Na]^+$ 401.2119, found 401.2074.

4.8. 3-{[1-(*tert*-Butyldimethylsilyl)oxy]prop-2-enyl}-3-hydroxymethyl-oxetane (11)

To a stirred solution of **10** (160 mg, 0.42 mmol) in a mixture of CH_2Cl_2 (5 mL) and water (0.5 mL) was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (143 mg, 0.63 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 50 min. The reaction mixture was then diluted with ether (50 mL), and was washed successively with saturated aqueous $NaHCO_3$ (5 mL × 2), and then with brine (5 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated to afford a residue, from which **11** (102 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:3) as a colourless oil in 95% yield.

Compound ***11***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.07 (3H, s), 0.12 (3H, s), 2.79 (1H, dd, J = 8.0, 2.8 Hz), 3.93 (1H, dd, J = 11.1, 8.0 Hz), 4.02 (1H, ddd, J = 11.1, 2.7, 1.0 Hz), 4.19 (1H, d, J = 6.5 Hz), 4.37 (1H, d, J = 6.5 Hz), 4.57 (1H, dd, J = 6.1, 1.0 Hz), 4.64 (1H, d, J = 6.2 Hz), 4.66 (1H, br d, J = 7.0 Hz), 5.26 (1H, ddd, J = 10.4, 1.4, 1.2 Hz), 5.32 (1H, dt, J = 17.2, 1.4 Hz), 5.84 (1H, ddd, J = 17.2, 10.4, 7.0 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ −5.1, −4.1, 18.0, 25.7, 46.9, 65.5, 74.9, 77.6, 79.2, 117.7, 136.1; HRMS (ESI^+) m/z calcd for $C_{13}H_{26}NaO_3Si$ $[M+Na]^+$ 281.1543, found 281.1543.

4.9. (1*RS*)-{3-[1-(*RS*)-Hydroxyprop-2-en-1-yl]oxetan-3-yl}but-3-yn-1-ol (12a:1,3-*anti*-diol) and (1*SR*)-{3-[1-(*RS*)-hydroxyprop-2-en-1-yl]oxetan-3-yl}but-3-yn-1-ol (12b:1,3-*syn*-diol)

A solution of oxalyl chloride (0.84 mL, 1.24 g, 9.80 mmol) in CH_2Cl_2 (15 mL) was added to a solution of DMSO (1.39 mL, 19.6 mmol) in dry CH_2Cl_2 (15 mL) under an atmosphere of argon at −78 °C, and the mixture was stirred at the same temperature for 30 min. The resulting mixture was transferred to a solution of **11** (1.10 g, 4.26 mmol) in dry CH_2Cl_2 (30 mL), and the reaction mixture was stirred at −78 °C for 40 min. Subsequently, neat triethylamine (5.2 mL, 37.5 mmol) was added to the reaction mixture followed by stirring for 1 h, while the temperature was elevated from −78–0 °C. The reaction was quenched by the addition of water (15 mL) and the whole was extracted with ether (80 mL × 3). The combined organic layer was washed with brine, dried over sodium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which the desired aldehyde (1.03 g) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:9) as a colourless oil in 93% yield.

3-[1-(*tert*-Butyldimethylsilyloxy)prop-2-en-1-yl]oxetane-3-carbaldehydee ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.07 (3H, s), 0.10 (3H, s), 0.88 (9H, s), 4.51 (1H, d, J = 6.5 Hz), 4.58 (1H, d, J = 6.3 Hz), 4.67 (1H, br d, J = 7.0 Hz), 4.731 (1H, d, J = 6.3 Hz), 4.734 (1H, d, J = 6.5 Hz), 5.28 (1H, ddd, J = 10.4, 1.2, 1.1 Hz), 5.35 (1H, dt, J = 17.1, 1.3 Hz), 5.84 (1H, ddd, J = 17.1, 10.4, 7.0 Hz), 9.88 (1H, s); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ −5.1, −4.0, 18.0, 25.6, 57.0, 72.1, 73.7, 75.3, 118.4, 135.6, 200.6; HRMS (ESI^+) m/z calcd for $C_{13}H_{26}NaO_3Si$ $[M+Na]^+$ 281.1543, found 281.1552.

To a stirred solution of the aldehyde (1.03 g, 4.02 mmol) dissolved in dry ether (40 mL) was added a solution of allenylmagnesium bromide (*ca.* 2 M in ether, 12 mL, 24 mmol) at −78 °C under an atmosphere of argon. After having been stirred for 30 min at the same temperature, the reaction was quenched by the addition of saturated aqueous NH_4Cl (20 mL). The resultant mixture was extracted with ethyl acetate (50 mL × 3), and the combined organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the filtrate afforded a residue, from which a diastereomeric mixture of homopropargyl alchohols (1.13 g) was obtained by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:5) as a colourless oil in 95% yield. The ratio between the minor and major isomers was deduced to be 45:55 by ^{1}H NMR analysis using the protons at 4.18 ppm and 3.98 ppm, respectively.

A mixture of alcohols: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) (minor isomer) δ 0.10 (3H, s), 0.14 (3H, s), 0.88 (9H, s), 2.06 (1H, t, J = 2.6 Hz), 2.68 (2H, dd, J = 6.7, 2.7 Hz), 3.73 (1H, d, J = 3.3 Hz), 4.18 (1H, dt, J = 6.7, 3.3 Hz), 4.42 (1H, d, J = 6.7 Hz), 4.48 (1H, d, J = 6.4 Hz), 4.67 (1H, d, J = 6.4 Hz), 4.68 (1H, d, J = 6.7 Hz), 4.69 (1H, br d, J = 7.4 Hz), 5.33 (1H, ddd, J = 10.4, 1.2, 1.0 Hz), 5.36 (1H, dt, J = 17.2, 1.2 Hz), 5.94 (1H, ddd, J = 17.2, 10.4, 7.4 Hz); (major isomer) δ 0.07 (3H, s), 0.11 (3H, s), 0.90 (9H, s), 2.07 (1H, t, J = 2.6 Hz), 2.50 (1H, ddd, J = 16.9, 8.7, 2.7 Hz), 2.64 (1H, d, J = 4.7 Hz), 2.70 (1H, ddd, J = 16.9, 4.1, 2.7 Hz), 3.98 (1H, dt, J = 8.8, 4.4 Hz), 4.40 (1H, d, J = 7.0 Hz), 4.46 (1H, d, J = 6.4 Hz), 4.53 (1H, d, J = 7.0 Hz), 4.56

(1H, br d, J = 7.2 Hz), 4.63 (1H, d, J = 6.3 Hz), 5.26 (1H, ddd, J = 10.4, 1.4, 1.2 Hz), 5.33 (1H, dt, J = 17.1, 1.4 Hz), 5.89 (1H, ddd, J = 17.1, 10.4, 7.2 Hz).

A solution of tetra-*n*-buthylammonium fluoride (TBAF) (1.0 M in THF, 1.4 mL, 1.4 mmol) was added to a stirred solution of the diastereomeric mixture as described above (340 mg, 1.2 mmol) in THF (5 mL) at 0 °C in a drop-wise manner, and the resulting mixture was stirred for 1 h at room temperature. Brine was then added to the reaction mixture and the whole was extracted with ethyl acetate (30 mL × 3). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate, and filtered. Evaporation of the filtrate afforded a residue, from which **12a** (89 mg, 42%, less polar) and **12b** (124 mg, 57%, more polar) were obtained by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:3) both as colourless oils in a combined yield of 99%.

Compound ***12a***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 2.07 (1H, t, J = 2.8 Hz), 2.60 (1H, ddd, J = 16.8, 6.1, 2.7 Hz), 2.70 (1H, ddd, J = 16.8, 7.9, 2.8 Hz), 2.71 (1H, complex), 3.12 (1H, br d, J = 6.0 Hz), 4.29 (1H, dt, J = 7.8, 6.1 Hz), 4.42 (1H, d, J = 6.9 Hz), 4.59 (2H, m), 4.61 (1H, d, J = 7.0 Hz), 4.63 (1H, m), 5.42 (1H, dt, J = 10.4, 1.3 Hz), 5.48 (1H, dt, J = 17.1, 1.3 Hz), 6.20 (1H, ddd, J = 17.1, 10.4, 6.4 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 23.1, 48.3, 71.2, 73.6, 74.8, 75.3, 75.7, 80.1, 118.6, 135.9; HRMS (ESI^+) m/z calcd for $C_{10}H_{14}NaO_3$ $[M+Na]^+$ 205.0835, found 205.0816.

Compound ***12b***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 2.09 (1H, t, J = 2.6 Hz), 2.15 (1H, d, J = 3.5 Hz), 2.61 (1H, ddd, J = 16.9, 8.2, 2.6 Hz), 2.62 (1H, d, J = 4.1 Hz), 2.69 (1H, ddd, J = 16.9, 4.3, 2.6 Hz), 4.10 (1H, dt, J = 8.4, 4.3 Hz), 4.49 (1H, d, J = 6.6 Hz), 4.53 (1H, m), 4.57 (1H, d, J = 6.6 Hz), 4.63 (1H, d, J = 6.6 Hz), 4.66 (1H, d, J = 6.6 Hz), 5.37 (1H, dt, J = 10.4, 1.3 Hz), 5.45 (1H, dt, J = 17.1, 1.3 Hz), 6.17 (1H, ddd, J = 17.1, 10.4, 6.6 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 23.6, 49.4, 71.3, 71.5, 73.2, 74.6, 75.1, 80.8, 118.2, 136.6; HRMS (ESI^+) m/z calcd for $C_{10}H_{14}NaO_3$ $[M+Na]^+$ 205.0835, found 205.0821.

4.10. 3-{[1-(*RS*)-(*tert*-Butyldimethylsilyl)oxy]but-3-ynyl}-3-{[1-(*RS*)-(*tert*-butyldimethylsilyl)oxy]prop-2-enyl}oxetane (13a)

To a stirred solution of **12a** (66 mg, 0.36 mmol) and pyridinium trifluoromethanesulfonate (67 mg, 0.29 mmol) in dry THF (5 mL) was added *tert*-butyldimethylsilyloxy *N*-phenylbenzimidate (TBS-BEZA) (677 mg, 2.17 mmol) at −20 °C under an atmosphere of argon, and the resulting mixture was stirred at the same temperature for 2 h. Water (1 mL) was added to the reaction mixture, and the whole was extracted with ether (10 mL × 3). The combined organic layer was washed with brine (5 mL), dried over sodium sulfate, and filtered. The filtrate was concentrated under the reduced pressure to give a residue, from which **13a** (119 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:99) as a colourless oil in 80% yield.

Compound ***13a***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.04 (3H, s), 0.06 (3H, s), 0.070 (3H, s), 0.072 (3H, s), 0.90 (9H, s), 0.92 (9H, s), 1.99 (1H, t, J = 2.7 Hz), 2.59 (2H, dd, J = 7.2, 2.7 Hz), 3.73 (1H, d, J = 10.1 Hz), 4.00 (1H, d, J = 10.1 Hz), 4.27 (1H, br d, J = 7.8 Hz), 4.30 (1H, d, J = 5.7 Hz), 4.56 (1H, d, J = 5.8 Hz), 4.88 (1H, t, J = 7.2 Hz), 5.17 (1H, ddd, J = 10.3, 1.7, 0.7 Hz), 5.22 (1H, ddd, J = 17.2, 1.7, 1.0 Hz), 5.83 (1H, ddd, J = 17.2, 10.3, 7.7 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ −5.6, −4.9, −3.9, 18.1, 22.1, 25.8, 50.5, 61.6, 70.3, 70.6, 72.2, 74.1, 80.2, 81.7, 117.2, 137.7; HRMS (ESI^+) m/z calcd for $C_{22}H_{42}NaO_3Si_2$ $[M+Na]^+$ 433.2565, found 433.2524.

4.11. 3-{[1-(*SR*)-(*tert*-Butyldimethylsilyl)oxy]but-3-ynyl}-3-{[1-(*RS*)-(*tert*-butyldimethylsilyl)oxy]prop-2-enyl}oxetane (13b)

To a stirred solution of **12b** (114 mg, 0.63 mmol) and pyridinium trifluoromethanesulfonate (143 mg, 0.63 mmol) in dry THF (7 mL) was added TBS-BEZA (1.56 g, 5.00 mmol) at 0 °C under an atmosphere of argon, and the resulting mixture was stirred at the same temperature for 1.5 h. Water (1.5 mL) was then added to the reaction mixture, and the whole was extracted with ether (10 mL × 3). The combined organic layer was washed with brine (5 mL), dried over sodium sulfate, and filtered. The filtrate was concentrated under the reduced pressure to give a residue, from which **13b** (204 mg) was separated by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:49) as a colourless oil in 80% yield.

Compound ***13b***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.06 (3H, s), 0.09 (3H, s), 0.10 (3H, s), 0.14 (3H, s), 0.90 (9H, s), 0.93 (9H, s), 1.93 (1H, t, J = 2.7 Hz), 2.45 (1H, ddd, J = 17.2, 4.8, 2.7 Hz), 2.52 (1H, ddd, J = 17.2, 5.1, 2.8 Hz), 4.06 (1H, t, J = 4.9 Hz), 4.33 (1H, d, J = 6.1 Hz), 4.44 (1H, br d, J = 7.1 Hz), 4.46 (1H, d, J = 5.9 Hz), 4.62 (1H, d, J = 6.0 Hz), 4.80 (1H, d, J = 6.1 Hz), 5.26 (1H, br d, J = 10.3 Hz), 5.31 (1H, br d, J = 17.1 Hz), 6.03 (1H, ddd, J = 17.1, 10.3, 7.5 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ −4.8, −4.0, −3.6, 18.2, 23.8, 25.8, 52.0, 70.6, 70.8, 73.6, 74.2, 74.9, 81.3, 117.4, 137.5; HRMS (ESI^+) m/z calcd for $C_{22}H_{42}NaO_3Si_2$ $[M+Na]^+$ 433.2565, found 433.2521.

4.12. General procedure for synocedure for synthesis of acetonides

To a stirred solution of the 1,3-diols (**12a**,**b**:10–28 mg) in dimethoxypropane (1–1.5 mL) was added camphorsulfonic acid (CSA) (0.2 equiv) at room temperature under an atmosphere of argon, and the resulting mixture was stirred for 0.5–1.5 h. Evaporation of the solvent, followed by purification by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:4) gave the corresponding acetonides (**14a**,**b**) as colourless oils in 62–82% yields. The *syn*-1,3-diol required an extended reaction time to complete the reaction with a slightly reduced yield.

Compound ***14a***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 1.36 (3H, s), 1.37 (3H, s), 2.08 (1H, t, J = 2.6 Hz), 2.64 (1H, ddd, J = 16.9, 7.9, 2.6 Hz), 2.81 (1H, ddd, J = 16.9, 4.7, 2.6 Hz), 3.91 (1H, dd, J = 7.9, 4.7 Hz), 4.25 (1H, br d, J = 5.9 Hz), 4.29 (1H, d, J = 7.3 Hz), 4.34 (1H, d, J = 6.8 Hz), 4.63 (1H, d, J = 6.8 Hz), 4.73 (1H, d, J = 7.2 Hz), 5.43 (1H, ddd, J = 10.6, 1.7, 1.4 Hz), 5.48 (1H, dt, J = 17.2, 1.6 Hz), 6.17 (1H, ddd, J = 17.2, 10.6, 6.0 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 21.2, 24.3, 24.9, 47.6, 70.3, 71.0, 73.3, 74.1, 75.2, 80.5, 101.2, 118.6, 133.1.

Compound ***14b***: ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 1.37 (3H, s), 1.49 (3H, s), 2.05 (1H, t, J = 2.7 Hz), 2.64 (1H, ddd, J = 17.2, 7.7, 2.7 Hz), 3.01 (1H, ddd, J = 17.2, 4.1, 2.7 Hz), 4.00 (1H, dd, J = 7.7, 4.2 Hz), 4.23 (1H, br d, J = 6.4 Hz), 4.32 (1H, d, J = 7.0 Hz), 4.46 (1H, J = 6.9 Hz), 4.49 (1H, d, J = 7.2 Hz), 4.58 (1H, d, J = 7.2 Hz), 5.489 (1H, dt, J = 10.1, 1.5 Hz), 5.490 (1H, dt, J = 17.5, 1.5 Hz), 6.22 (1H, ddd, J = 17.5, 10.1, 6.3 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 19.2, 21.2, 29.6, 43.9, 69.8, 70.9, 72.1, 72.5, 74.7, 81.4, 99.4, 120.0, 133.3.

4.13. (5*Z*,7*E*)-(1*R*,3*R*)-2,2-(Methyleneoxy)methano-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3a) and (5*Z*,7*E*)-(1*S*,3*S*)-2,2-(methyleneoxy)methano-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3b)

To a stirred solution of the A-ring enyne precursor **13a** (50 mg, 0.12 mmol) and the CD-ring portion **15** (52 mg, 0.15 mmol) in a mixture of toluene (6.3 mL) and triethylamine (2.2 mL) was added tetrakis(triphenylphosphine)palladium (70 mg, 0.06 mmol) at room temperature under an atmosphere of argon. After having been heated at reflux for 40 min, the reaction mixture was diluted with ether and filtered through a small pad of silica gel. Evaporation of the filtrate gave a residue, which was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:19, then 1:1) to give a mixture of protected vitamins (82 mg). The crude vitamins dissolved in THF (2 mL) were treated with TBAF (1.0 M

in THF, 0.85 mL, 0.85 mmol) at room temperature for 16 h. Brine was added to the mixture and the whole was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:2, then 6:1), followed by silica gel preparative TLC (ethyl acetate/*n*-hexane = 6:1) to give **3a**,**b** (9.0 mg) in 16% yield (two steps). Separation and further purification of **3a** (less polar) and **3b** (more polar) were conducted on a recycling HPLC (COSMOSIL® Cholester column, 10 × 250 mm, 5 mL min^{-1}, methanol/water = 75:25).

Compound ***3a***: UV (EtOH) λ_{max} 266 nm, λ_{min} 227 nm; ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.53 (3H, s), 0.93 (3H, d, *J* = 6.4 Hz), 1.21 (6H, s), 1.98 (1H, m), 2.19 (1H, dd, *J* = 14.0, 6.9 Hz), 2.46 (1H, dd, *J* = 13.9, 3.5 Hz), 2.80 (1H, dd, *J* = 11.6, 3.5 Hz), 4.31 (1H, dd, *J* = 6.7, 3.9 Hz), 4.46 (1H, d, *J* = 6.3 Hz), 4.49 (1H, br s), 4.52 (1H, d, *J* = 6.1 Hz), 4.60 (1H, d, *J* = 6.3 Hz), 4.67 (1H, d, *J* = 6.1 Hz), 5.05 (1H, s), 5.44 (1H, s), 5.98 (1H, d, *J* = 11.3 Hz), 6.36 (1H, d, *J* = 11.2 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 12.0, 18.8, 20.8, 22.2, 23.6, 27.6, 29.1, 29.2, 29.3, 36.1, 36.4, 40.4, 41.2, 44.4, 45.9, 50.0, 56.3, 56.5, 70.3, 71.1, 74.1, 74.2, 74.8, 114.0, 116.7, 125.2, 131.8, 143.8, 144.6; HRMS (ESI$^+$) *m*/*z* calcd for $C_{29}H_{46}NaO_4$ $[M+Na]^+$ 481.3288, found 481.3277.

Compound ***3b***: UV (EtOH) λ_{max} 266 nm, λ_{min} 227 nm; ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.54 (3H, s), 0.94 (1H, d, *J* = 6.4 Hz), 1.22 (6H, s), 1.99 (1H, m), 2.17 (1H, dd, *J* = 13.4, 7.9 Hz), 2.48 (1H, dd, *J* = 13.9, 4.0 Hz), 2.81 (1H, dd, *J* = 12.4, 4.3 Hz), 4.27 (1H, m), 4.46 (1H, d, *J* = 6.2 Hz), 4.54 (1H, d, *J* = 6.4 Hz), 4.56 (1H, br s), 4.60 (1H, d, *J* = 6.3 Hz), 4.70 (1H, d, *J* = 6.2 Hz), 5.07 (1H, s), 5.44 (1H, s), 5.98 (1H, d, *J* = 11.3 Hz), 6.38 (1H, d, *J* = 11.3 Hz); ^{13}C NMR (175 MHz, TMS, $CDCl_3$) δ 12.0, 18.8, 20.8, 22.3, 23.6, 27.6, 29.1, 29.2, 29.4, 36.1, 36.4, 40.4, 41.4, 44.4, 46.0, 49.9, 56.3, 56.5, 69.7, 71.1, 74.4, 75.3, 114.8, 116.6, 125.4, 131.5, 144.0, 144.2; HRMS (ESI$^+$) *m*/*z* calcd for $C_{29}H_{46}NaO_4$ $[M+Na]^+$ 481.3288, found 481.3279.

4.14. (5*Z*,7*E*)-(1*R*,3*S*)-2,2-(Methyleneoxy)methano-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3c) and (5*Z*,7*E*)-(1*S*,3*R*)-2,2-(methyleneoxy)methano-9,10-*seco*-5,7,10(19)-cholestatriene-1,3,25-triol (3d)

To a stirred solution of the A-ring enyne precursor **13b** (55 mg, 0.13 mmol) and the CD-ring portion **15** (57 mg, 0.16 mmol) in a mixture of toluene (6.8 mL) and triethylamine (2.4 mL) was added tetrakis(triphenylphosphine)palladium (46 mg, 0.04 mmol) at room temperature under an atmosphere of argon. After having been heated at reflux for 40 min, the reaction mixture was diluted with ether, and filtered through a small pad of silica gel. Evaporation of the filtrate gave a residue, which was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:10), followed by silica gel preparative TLC (ethyl acetate/*n*-hexane = 1:2), to give a mixture of protected vitamins (56 mg). The vitamins were then dissolved in THF (0.8 mL) before being treated with TBAF (1.0 M in THF, 0.24 mL, 0.24 mmol) at room temperature for 1.5 h. Brine was added to the mixture and the whole was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:4, then 2:1), followed by silica gel preparative TLC (ethyl acetate/*n*-hexane = 5:1) to give **3c**,**d** (12 mg) in 20% yield (two steps). Separation and further purification of **3c** (more polar) and **3d** (less polar) were conducted on a recycling HPLC (COSMOSIL® Cholester column, 10 × 250 mm, 5 mL min^{-1}, methanol/water = 75:25).

Compound ***3c***: UV (EtOH) λ_{max} 264 nm, λ_{min} 228 nm; ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.53 (3H, s), 0.93 (3H, d, *J* = 6.4 Hz), 1.21 (6H, s), 1.99 (1H, m), 2.39 (1H, m), 2.44 (1H, dd, *J* = 14.5, 4.0 Hz), 2.83 (1H, dd, *J* = 12.4, 4.0 Hz), 4.30 (1H, m), 4.31 (1H, d, *J* = 6.3 Hz), 4.35 (1H, d, *J* = 6.4 Hz), 4.58 (1H, br s), 4.71 (1H, d, *J* = 6.4 Hz), 4.76 (1H, d, *J* = 6.5 Hz), 5.05 (1H, d, *J* = 1.5 Hz), 5.37 (1H, d, *J* = 1.5 Hz), 5.99 (1H, d, *J* = 11.3 Hz), 6.47 (1H, d, *J* = 11.3 Hz); ^{13}C NMR (175 MHz, TMS, $CDCl_3$) δ 12.1, 18.8, 20.8, 22.2, 23.4, 27.6, 29.1, 29.2, 29.3, 36.1, 36.4, 40.5, 41.3, 44.4, 45.9, 47.4, 56.3, 56.5, 71.1, 73.4, 76.1, 79.5, 116.1, 116.7, 126.6, 129.4, 143.9; HRMS (ESI$^+$) *m*/*z* calcd for $C_{29}H_{46}NaO_4$ $[M+Na]^+$ 481.3288, found 481.3286.

Compound ***3d***: UV (EtOH) λ_{max} 264 nm, λ_{min} 227 nm; ^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.55 (3H, s), 0.94 (3H, d, *J* = 6.4 Hz), 1.21 (6H, s), 2.39 (1H, m), 2.45 (1H, dd, *J* = 14.4, 3.6 Hz), 2.83 (1H, dd, *J* = 11.9, 4.0 Hz), 4.29 (1H, d, *J* = 6.4 Hz), 4.33 (1H, d, *J* = 6.5 Hz), 4.35 (1H, m), 4.62 (1H, br s), 4.72 (1H, d, *J* = 6.5 Hz), 4.76 (1H, d, *J* = 6.5 Hz), 5.08 (1H, d, *J* = 1.9 Hz), 5.39 (1H, d, *J* = 1.8 Hz), 6.04 (1H, d, *J* = 11.3 Hz), 6.48 (1H, d, *J* = 11.5 Hz); ^{13}C NMR (100 MHz, TMS, $CDCl_3$) δ 11.9, 18.8, 20.8, 22.3, 23.7, 27.6, 29.15, 29.23, 29.4, 36.1, 36.4, 40.4, 41.3, 44.4, 46.0, 47.3, 56.3, 56.5, 71.1, 73.6, 76.3, 79.9, 116.4, 116.6, 126.7, 129.5, 143.98, 144.03; HRMS (ESI$^+$) *m*/*z* calcd for $C_{29}H_{46}NaO_4$ $[M+Na]^+$ 481.3288, found 481.3269.

4.15. General procedure for synthesis of bis-MTPA esters

A solution of the vitamins (from 0.3 to 0.5 mg) described above dissolved in dry CH_2Cl_2 was treated with DMAP (26 equiv) and (*R*)- or (*S*)-MTPACl (16 equiv) at room temperature under an atmosphere of argon. The reaction mixture was purified by preparative TLC (ethyl acetate/*n*-hexane = 1:2) without pretreatment to afford the corresponding bis-(*S*)-MTPA or bis-(*R*)-MTPA ester.

4.15.1. Bis-(*S*)-MTPA ester of 3a

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.441 (3H, s), 0.931 (3H, d, *J* = 6.6 Hz), 1.216 (6H, s), 2.243 (1H, m), 2.737 (1H, dd, *J* = 13.2, 4.7 Hz), 2.802 (1H, m), 3.472 (3H, s), 3.542 (3H, s), 3.985 (1H, d, *J* = 6.7 Hz), 4.073 (1H, d, *J* = 6.7 Hz), 4.199 (1H, d, *J* = 6.4 Hz), 4.608 (1H, d, *J* = 6.5 Hz), 5.181 (1H, dd, *J* = 10.3, 4.5 Hz), 5.298 (1H, s), 5.679 (1H,s), 5.892 (1H, d, *J* = 11.8 Hz), 5.916 (1H, s), 6.470 (1H, d, *J* = 11.4 Hz), 7.345–7.716 (10H, m); HRMS (ESI$^+$) *m*/*z* calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4079.

4.15.2. Bis-(*R*)-MTPA ester of 3a

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.338 (3H, s), 0.931 (1H, d, *J* = 6.4 Hz), 1.225 (6H, s), 2.159 (1H, m), 2.587 (1H, m), 2.745 (1H, m), 3.434 (3H, s), 3.598 (3H, s), 4.268 (1H, d, *J* = 6.6 Hz), 4.331 (1H, d, *J* = 6.7 Hz), 4.391 (1H, d, *J* = 6.7 Hz), 4.617 (1H, d, *J* = 6.9 Hz) 5.204 (1H, s), 5.338 (1H, dd, *J* = 10.8, 5.8 Hz), 5.547 (1H, s), 5.785 (1H, d, *J* = 11.2 Hz), 5.971 (1H, s), 6.358 (1H, d, *J* = 10.9 Hz), 7.344–7.591 (10H, m); HRMS (ESI$^+$) *m*/*z* calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4045.

4.15.3. Bis-(*S*)-MTPA ester of 3b

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.511 (3H, s), 0.934 (3H, d, *J* = 6.4 Hz), 1.218 (6H, s), 2.163 (1H, m), 2.555 (1H, dd, *J* = 13.5, 4.4 Hz), 2.730 (1H, m), 3.458 (3H, s), 3.589 (3H, s), 4.265 (1H, d, *J* = 6.7 Hz), 4.365 (1H, d, *J* = 6.7 Hz), 4.406 (1H, d, *J* = 6.7 Hz), 4.617 (1H, d, *J* = 6.7 Hz), 5.226 (1H, s), 5.339 (1H, dd, *J* = 10.5, 5.0 Hz), 5.547 (1H, s), 5.847 (1H, d, *J* = 11.6 Hz), 5.907 (1H, s), 6.316 (1H, d, *J* = 10.8 Hz), 7.331–7.569 (10H, m); HRMS (ESI$^+$) *m*/*z* calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4039.

4.15.4. Bis-(*R*)-MTPA ester of 3b

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.536 (3H, s), 0.940 (3H, d, *J* = 6.4 Hz), 1.216 (6H, s), 2.234 (1H, m), 2.746 (1H, dd, *J* = 13.1, 4.5 Hz), 2.805 (1H, m), 3.461 (3H, s), 3.555 (3H, s), 4.082 (1H, d, *J* = 6.8 Hz), 4.172 (1H, d, *J* = 6.7 Hz), 4.215 (1H, d, *J* = 6.5 Hz), 4.632 (1H, d, *J* = 6.4 Hz), 5.216 (1H, dd, *J* = 10.5, 4.6 Hz), 5.347 (1H, s), 5.718 (1H, s), 5.951 (1H, d, *J* = 11.6 Hz), 5.966 (1H, s), 6.489 (1H, d, *J* = 11.6 Hz), 7.295–7.562 (10H, m); HRMS (ESI$^+$) *m*/*z* calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4042.

4.15.5. Bis-(*S*)-MTPA ester of 3c

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.430 (3H, s), 0.942 (3H, d, J = 6.4 Hz), 1.218 (6H, s), 1.85 (1H, m), 1.93–2.03 (2H, m), 2.302 (1H, dd, J = 14.4, 7.0 Hz), 2.480 (1H, dd, J = 14.4, 4.0 Hz), 2.667 (1H, m), 3.216 (3H, s), 3.307 (3H, s), 4.203 (1H, d, J = 6.6 Hz), 4.321 (1H, d, J = 6.8 Hz), 4.342 (1H, d, J = 6.8 Hz), 4.393 (1H, d, J = 6.7 Hz), 5.138 (1H, s), 5.430 (1H, dd, J = 6.8, 4.2 Hz), 5.477 (1H, s), 5.694 (1H, s), 5.767 (1H, d, J = 11.4 Hz), 6.150 (1H, d, J = 11.4 Hz), 7.387–7.568 (10H, m); HRMS (ESI$^+$) m/z calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4090.

4.15.6. Bis-(*R*)-MTPA ester of 3c

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.471 (3H, s), 0.939 (3H, d, J = 6.3 Hz), 1.228 (6H, s), 2.248 (1H, m), 2.693 (1H, dd, J = 13.2, 4.9 Hz), 2.752 (1H, dd, J = 12.2, 3.5 Hz), 3.552 (3H, s), 3.647 (3H, s), 4.232 (1H, d, J = 6.9 Hz), 4.276 (1H, d, J = 6.8 Hz), 4.479 (1H, d, J = 6.4 Hz), 4.523 (1H, d, J = 6.4 Hz), 4.895 (1H, s), 4.933 (1H, s), 5.122 (1H, dd, J = 10.6, 4.8 Hz), 5.524 (1H, s), 5.820 (1H, d, J = 11.2 Hz), 6.390 (1H, d, J = 10.9 Hz), 7.382–7.716 (10H, m); HRMS (ESI$^+$) m/z calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4083.

4.15.7. Bis-(*S*)-MTPA ester of 3d

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.552 (3H, s), 0.944 (3H, d, J = 6.4 Hz), 1.223 (6H, s), 2.271 (1H, dd, J = 11.6, 9.9 Hz), 2.682 (1H, dd, J = 13.5, 4.8 Hz), 2.758 (1H, m), 3.481 (3H, s), 3.603 (3H, s), 4.242 (1H, d, J = 6.9 Hz), 4.280 (1H, d, J = 6.7 Hz), 4.462 (1H, d, J = 6.4 Hz), 4.492 (1H, d, J = 6.3 Hz), 4.901 (1H, s), 4.962 (1H, s), 5.174 (1H, dd, J = 9.9, 4.5 Hz), 5.558 (1H, s), 5.812 (1H, d, J = 11.2 Hz), 6.386 (1H, d, J = 11.1 Hz), 7.379–7.421 (10H, m); HRMS (ESI$^+$) m/z calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4085.

4.15.8. Bis-(*R*)-MTPA ester of 3d

^{1}H NMR (400 MHz, TMS, $CDCl_3$) δ 0.520 (3H, s), 0.935 (3H, d, J = 6.3 Hz), 1.218 (6H, s), 2.278 (1H, dd, J = 14.0, 7.9 Hz), 2.489 (1H, dd, J = 13,9, 4.4 Hz), 2.685 (1H, m), 3.292 (3H, s), 3.361 (3H, s), 4.265 (1H, d, J = 6.6 Hz), 4.324 (1H, d, J = 6.7 Hz), 4.376 (1H, d, J = 6.6 Hz), 4.391 (1H, d, J = 6.3 Hz), 5.104 (1H, s), 5.388 (1H, dd, J = 7.4, 4.1 Hz), 5.405 (1H, s), 5.682 (1H, s), 5.778 (1H, d, J = 10.9 Hz), 6.190 (1H, d, J = 11.5 Hz), 7.348–7.571 (10H, m); HRMS (ESI$^+$) m/z calcd for $C_{49}H_{60}F_6NaO_8$ $[M+Na]^+$ 913.4085, found 913.4122.

4.16. Competitive vitamin D receptor (VDR) binding assay

The bovine thymus VDR receptor was obtained from Yamasa Biochemical (Chiba, Japan) and dissolved in 0.05 M phosphate buffer (pH 7.4) containing 0.3 M KCl and 5 mM dithiothreitol immediately prior to use. The receptor solution (500 μL) was pre-mixed with 50 μL of an ethanol solution of 1α,25-dihydroxyvitamin D_3 or an analogue at various concentrations for 60 min at 25 °C, prior to the before addition of [^{3}H]-1α,25-dihydroxyvitamin D_3 (50 μL). The receptor mixture was then left to stand overnight with 0.1 nM [^{3}H]-1α,25-dihydroxyvitamin D_3 at 4 °C. The bound and free [^{3}H]-1α,25-dihydroxyvitamin D_3 were separated by treatment with a dextran-coated charcoal (Norit SX-II) suspension (200 μL) for 30 min at 4 °C, followed by centrifugation at 3000 rpm for 10 min. The supernatant (500 μL) was mixed with Insta-Gel® Plus (9.5 mL) (PerkinElmer, USA) and the radioactivity was counted. The relative potency of the analogues was calculated from the concentration required to displace 50% of the [^{3}H]-1α,25-dihydroxyvitamin D_3 from the receptor compared with the activity of 1α,25-dihydroxyvitamin D_3, which was assigned as 100 by definition.

Acknowledgements

This work was supported in part by a Grant-in-Aid for scientific research (Grant number 23590142) from the Ministry of Education, Culture, Sports, Science and Technology in Japan.

Supplementary data

Supplementary data (copies of ^{1}H and ^{13}C NMR spectra for the novel compounds **5**–**11**, **12a**,**b**, **13a**,**b**, **14a**,**b** and **3a**–**d**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.06.032.

References and notes

1. (a)*Vitamin D*; Feldman, D., Wesley Pike, J., Adam, J. S., Eds., 3rd ed.; Elsevier Academic Press: Burlington, 2011; (b) Bikle, D. J. *Clin. Endocrinol. Metab.* **2009**, *96*, 26; (c) Haussler, M. R.; Whitfield, G. K.; Kaneko, I.; Haussler, C. A.; Hsieh, D.; Hsieh, J.-C.; Jurutka, P. W. *Calcif. Tissue Int.* **2013**, *92*, 77; (d) Norman, A. W. *Endocrinology* **2006**, *147*, 5542; (e) Plum, L. A.; DeLuca, H. F. *Nat. Rev. Drug Disc.* **2010**, *9*, 941.
2. (a) Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D. *Mol. Cell* **2000**, *5*, 173; (b) Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Mitschler, A.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5491; (c) Hourai, S.; Fujishima, T.; Kittaka, A.; Suhara, Y.; Takayama, H.; Rochel, N.; Moras, D. *J. Med. Chem.* **2006**, *49*, 5199; (d) Eelen, G.; Valle, N.; Sato, Y.; Rochel, N.; Verlinden, L.; De Clercq, P.; Moras, D.; Bouillon, R.; Munõz, A.; Verstuyf, A. *Chem. Biol.* **2008**, *1029*, 15.
3. (a) Konno, K.; Fujishima, T.; Maki, S.; Liu, Z.-P.; Miura, D.; Chokki, M.; Ishizuka, S.; Yamaguchi, K.; Kan, Y.; Kurihara, M.; Miyata, N.; Smith, C.; DeLuca, H. F.; Takayama, H. *J. Med. Chem.* **2000**, *43*, 4247; (b) Fujishima, T.; Konno, K.; Nakagawa, K.; Kurobe, M.; Okano, T.; Takayama, H. *Bioorg. Med. Chem.* **2000**, *8*, 123; (c) Fujishima, T.; Liu, Z.-P.; Konno, K.; Nakagawa, K.; Okano, T.; Yamaguchi, K.; Takayama, H. *Bioorg. Med. Chem.* **2001**, *9*, 525; (d) Fujishima, T.; Konno, K.; Nakagawa, K.; Tanaka, M.; Okano, T.; Kurihara, M.; Miyata, N.; Takayama, H. *Chem. Biol.* **2001**, *1011*, 8; (e) Fujishima, T.; Kittaka, A.; Yamaoka, K.; Takeyama, K.; Kato, S.; Takayama, H. *Org. Biomol. Chem.* **1863**, *2003*, 1; (f) Fujishima, T.; Tsutsumi, R.; Negishi, Y.; Fujii, S.; Takayama, H.; Kittaka, A.; Kurihara, M. *Anticancer Res.* **2006**, *24*, 2633; (g) Kittaka, A.; Suhara, Y.; Takayanagi, H.; Fujishima, T.; Kurihara, M.; Takayama, H. *Org. Lett.* **2000**, *2*, 2619; (h) Suhara, Y.; Nihei, K.; Kurihara, M.; Kittaka, A.; Yamaguchi, K.; Fujishima, T.; Konno, K.; Miyata, N.; Takayama, H. *J. Org. Chem.* **2001**, *66*, 8760; (h) Ono, Y.; Watanabe, H.; Shiraishi, A.; Tanaka, S.; Higuchi, Y.; Sato, K.; Tsugawa, N.; Okano, T.; Kobayashi, T.; Kubodera, N. *Chem. Pharm. Bull.* **1997**, *45*, 1626; (i) Tsugawa, N.; Nakagawa, K.; Kurobe, M.; Ono, Y.; Kubodera, N.; Ozono, K.; Okano, T. *Biol. Pharm. Bull.* **2000**, *23*, 66.
4. (a) Wuitschik, G.; Rogers-Evans, M.; Buckl, A.; Bernasconi, M.; Märki, M.; Godel, T.; Fischer, H.; Wagner, B.; Parrilla, I.; Schuler, F.; Schneider, J.; Alker, A.; Schweizer, W. B.; Müller, K.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4512; (b) Wuitschik, G.; Carreira, E. M.; Wagner, B.; Fischer, H.; Parrilla, I.; Schuler, F.; Rogers-Evans, M.; Müller, K. *J. Med. Chem.* **2010**, *53*, 3227; (c) Burkhard, J. A.; Guérot, C.; Knust, H.; Roger-Evans, M.; Carreira, E. M. *Org. Lett.* **1944**, *2010*, 12.
5. (a) Trost, B. M.; Dumas, J.; Villa, M. *J. Am. Chem. Soc.* **1992**, *114*, 9836; (b) Trost, B. M.; Dumas, J. *J. Am. Chem. Soc.* **1924**, *1992*, 114.
6. For procedures concerning monobenzylation of pentaerythrithol, see: (a) Lehmann, J.; Weitzel, U. P. *Carbohydr. Res.* **1996**, *294*, 65; (b) Brand, M.; Lainé, C.; Réthoré, G.; Laurent, I.; Neven, C.; Lemiègre, L.; Benvegnu, T. *J. Org. Chem.* **2007**, *72*, 8262.
7. For intra-molecular substitution to produce an oxetane, see: (a) Behrendt, J. M.; Bala, K.; Golding, P.; Hailes, H. C. *Tetrahedron Lett.* **2005**, *46*, 643; (b) Jiang, Z.-H.; Budzynski, W. A.; Skeels, L. A.; Kranz, M. J.; Koganty, R. R. *Tetrahedron* **2002**, *58*, 8833.
8. (a) Rychnovsky, S. D.; Skalitzky, J. *Tetrahedron Lett.* **1990**, *31*, 945; (b) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511.
9. For *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide and the successful application of the above reagent, see: (a) Mawhinney, T. P.; Madson, M. A. *J. Org. Chem.* **1982**, *47*, 3336; (b) Boger, D. L.; Kim, S. H.; Mori, Y.; Weng, J.-H.; Rogel, O.; Castle, S. L.; McAtee, J. J. *J. Am. Chem. Soc.* **1862**, *2001*, 123; (c) Pearson, A. J.; Ciurea, D. V.; Velankar, A. *Tetrahedron Lett.* **1922**, *2008*, 49.
10. Misaki, T.; Kurihara, M.; Tanabe, Y. *Chem. Commun.* **2001**, 2478.
11. The A-ring conformational equilibria of **1** and its analogues can be estimated by ^{1}H NMR spectra using the vicinal coupling constants between protons at the C3 and C4 positions, which is confirmed based upon the following references. The above vicinal coupling constant of **3a** ($J_{3\alpha-4\beta}$ = 6.9 Hz) was in good accordance with that of **1** ($J_{3\alpha-4\beta}$ = 6.4 Hz), and with that of **2c** ($J_{3\alpha-4\beta}$ = 6.6 Hz): (a) Eguchi, T.; Ikekawa, N. *Bioorg. Chem.* **1990**, *18*, 19; (b) Anet, F. A. L. *J. Am. Chem. Soc.* **1962**, *84*, 1053.
12. The modified Mosher's method that used a simple derivation of secondary alcohols to their methoxy-trifluoromethyl-phenylacetic acid (MTPA) esters

was shown to be applicable to determine the absolute configuration of 1,3-diols by their bis-MTPA ester products. In the cases of the bis-MTPA esters of 1,3-*syn*-diols (**3c**, **d**), one of the protons at the C4 position that was *syn* to the MTPA ester at the C3 position resulted in an opposite sign value. All the other positions showed no irregular values: Konno, K.; Fujishima, T.; Liu, Z.-P.; Takayama, H. *Chirality***2002**, *14*, 72.