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Synthesis and crystallographic study of 1,25-dihydroxyergocalciferol analogs

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ABSTRACT

The hybrid analogs of 1,25-dihydroxyergocalciferol (PRI-5201 and PRI-5202) were synthesized as potential anticancer agents using a convergent strategy. The analogs were designed by combining a 19-*nor* modification of the A-ring with the homologated and rigidified ergocalciferol-like side-chain of the previously obtained analogs PRI-1906 and PRI-1907. The strategy also allowed the novel efficient synthesis of 19-*nor*-1,25-dihydroxyergocalciferol (paricalcitol, PRI-5100) and its (24*R*)-diastereomer (PRI-5101). The single crystal X-ray structures of the 19-*nor* analogs (PRI-5100 and PRI-5101) were solved and refined. The A-ring of both analogs adopts exclusively chair β-conformation in the solid state. The side-chain of these analogs is coplanar with the CD-ring plane, while it is perpendicular in 1,25-dihydroxycholecalciferol.

1. Introduction

Chronic renal disease [1] is a pathological state that results in secondary hyperparathyroidism. In this disease kidneys fail to hydroxylate enough of 25-hydroxycholecalciferol (25-hydroxyvitamin D_3) to its active form, 1,25-dihydroxycholecalciferol [calcitriol, 1,25-dihydroxyvitamin D_3, 1,25-$(OH)_2D_3$] and do not adequately excrete phosphate. This leads to hypocalcaemia, and the subsequent increase in parathyroid hormone secretion, in order to increase the serum calcium level. High levels of the parathyroid hormone can be selectively regulated by paricalcitol [2], a vitamin D receptor (VDR) activator. The use of this synthetic vitamin D analog [3], compared to the natural 1,25-$(OH)_2D_3$, might minimize the effects on the calcium and phosphate levels by increasing the activation of VDR in the parathyroid glands, without increasing the concentration of VDR in the intestines or bones. However, the molecular mechanism of action of paricalcitol still remains to be largely unknown. Chronic renal failure has been, until very recently, treated exclusively with the active forms of vitamin D, such as alfacalcidol (1α-hydroxyvitamin D_3, 1α-OH-D_3) and calcitriol. Calcimimetics, like cinacalcet [4], have been recently used in patients on dialysis, but not in the chronic kidney disease predialysis patients, because this therapeutics might increase the phosphate level.

Paricalcitol is also being currently investigated for its anticancer activity. It was found recently that a pre-treatment with paricalcitol might ameliorate the cisplatin-induced renal injury in rats by suppressing the fibrotic, apoptotic and proliferative factors [5]. Moreover, there is a phase II clinical study underway, at the Comprehensive Cancer Center of Wake Forest University, in collaboration with National Cancer Institute, on the influence of paricalcitol on bone remodeling in advanced androgen-insensitive prostate cancer [6]. A phase I clinical trial [7] is being conducted at Roswell Park Cancer Institute with the escalating doses of paricalcitol, in combination with gemcitabine, in patients with advanced malignancies. There is also a feasibility trial on paricalcitol in women with metastatic breast cancer in combination with taxanes or ixabepilone [8].

In our laboratory we designed and synthesized a homologated analog of 1,25-dihydroxyergocalciferol [1,25-dihydroxyvitamin D_2, 1,25-$(OH)_2D_2$] with the conjugated diene moiety in the extended side-chain, as well as a series of its 26,27-congeners [9]. In this series, the analog PRI-1907, with a mono-homologated vitamin D_2 side-chain and branched side-chains at C-25, extended by

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one carbon unit, showed the strongest antiproliferative activity *in vitro*, against the cells of human breast cancer lines T47D and MCF7, as well as human and mouse leukemia lines, HL-60 and WEHI-3, respectively. The activity was 3–150 times stronger, depending on the cell line, than that of 1,25-$(OH)_2D_3$. I this regard, it should be stressed that these cell lines express VDR. However, the PRI-1907 showed higher sub-acute toxicity [10] than the parent analog of the series (PRI-1906) in BDF_1 male mice. The elevation of the serum calcium level [10] as an adverse effect was also higher for the PRI-1907 (63%) than that of the PRI-1906 (23%), compared to the 40% increase for natural 1,25-$(OH)_2D_3$. Therefore, it was the PRI-1906 that was used as the first vitamin D analog to show anticancer potential in the combined treatment of cells with a tyrosine kinase inhibitor (imatinib mesylate) [11]. One of the most rapid effects [12] of the PRI-1906 and other vitamin D analogs was the nuclear accumulation of the vitamin D receptor (VDR), which was found proportional to the differentiation-inducing potential of the analog. The PRI-1906 was as effective as 1,25-$(OH)_2D_3$ in the panel of blast cells isolated from the patients with acute myeloid leukemia (AML) [13]. In this *ex vivo* model [14] the analogs PRI-1906 and PRI-1907 displayed a cell-differentiating activity comparable to that of 1,25-$(OH)_2D_3$. It was also found very recently [15] that the differentiation of blast cells in this model, induced by the PRI-1906, was enhanced by a potent pan-caspase inhibitor QVD to a varying degree, depending on the subtype of leukemia.

In this paper we describe the synthesis of hybrid vitamin D_2 analogs, designed by introducing a 19-*nor* modification into the A-ring of our analogs PRI-1906 and PRI-1907. Moreover, we present here for the first time the solid state X-ray diffraction data for 19-*nor* vitamin D analogs, as well as for their key synthetic intermediate. The new analogs are to be tested for their anticancer potential, compared to paricalcitol (PRI-5100) and its (24*R*)-diastereomer (PRI-5101), as the reference compounds.

2. Experimental

2.1. Synthesis

Samples were dried in a vacuum drier Salvis Lab VC-20 (Donserv). Melting points were recorded on a MEL-TEMP II apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian GEMINI-200, Varian S 500 and Varian S 600 spectrometers. UV spectra were taken in ethanolic solutions on a Shimadzu UV-160A spectrophotometer. Infrared (IR) spectra were taken on a Perkin-Elmer Model 1725X FT-IR spectrophotometer as films of oily substances or $CHCl_3$ solutions. Mass spectra (MS) and high-resolution MS (HRMS) were recorded on a Maldi Spektrometr SYNAPT G2-S HDMS (Waters Corporation Milford, Massachusetts). High-performance liquid chromatography (HPLC) separations were performed using a Knauer Instrument and Eurospher 100 C18 column, 10 μm, 5 cm × 25 cm. X-ray powder diffraction were recorded with the Rigaku *MiniFlex* series with copper radiation Cu Kα, λ = 1.54056 Å. Diffractograms were worked out and interpreted with the aid of DIFFRAC.EVA© software [16]. Column chromatography was carried out on Kiesegel 60 (Merck Art. 9025).

2.1.1. (7aR)-7a-methyl-1-((S)-1′-(phenylsulfonyl)propane-2′-yl)hexahydro-1H-inden-4(2H)-on (**8**)

The solution of (7a*R*)-1-[(*S*)-1-hydroxypropan-2-yl]-7a-methyl-octahydro-1*H*-inden-4-ol (**5**, 5.0 g, 23.6 mmol) in dichloromethane (25 mL) was added to the solution of triphenylphosphine (6.2 g, 23.6 mmol), imidazole (3.2 g, 47.1 mmol) and iodine (6.0 g, 23.6 mmol) in dichloromethane (75 mL). The mixture was stirred at room temperature for 20 h to give the iodide solution **6**. The solution of the crude iodide **6** (6.0 g, 18.6 mmol) in dichloromethane (30 mL) was added to the mixture of pyridinium chlorochromate (15.7 g, 73.0 mmol) and Celite (15 g) in dichloromethane (300 mL). The mixture was stirred at room temperature for 1.5 h and filtered to give the iodoketone **7** (4.7 g, 14.7 mmol). The solution of the iodoketone **7** (4.7 g) and sodium benzenesulfinate (11.0 g, 67.0 mmol) in DMF (30 mL) was stirred at 80 °C for 1.5 h. The mixture was extracted with ethyl acetate and the residue was purified by silica gel chromatography to give the sulfone **8** (4.0 g, 12.0 mmol, 82%, from **7**). The product was crystallized from ethyl acetate (25 mL) to yield the crystalline sulfone **8** (3.4 g, 10.2 mmol, 70%, from **7**); mp 153.5–154.5 °C; UV λ_{max} 218.6, 258.8, 264.8, 271.6 nm; IR (KBr, ν (cm^{-1})): 3385, 3070, 2987, 2965, 2951, 2903, 2881, 2854, 1698, 1469, 1448, 1410, 1381, 1303, 1290, 1241, 1146, 1089, 1057; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.61 (s, 3H, 8-CH_3), 1.25 (d, ^3J = 6,6 Hz, 3H, 3′-CH_3), 3.00 and 3.02 (m, 2H, 1′-CH_2), 7.52–7.93 (m, 5H, Ar–H); ^{13}C NMR (200 MHz, $CDCl_3$) δ: 12.24, 18.87, 20.04, 23.72, 27.13, 31.96, 38.54, 40.69, 49.62, 55.59, 61.59, 61.61, 127.72, 129.26, 133.59, 140.08, 211.10. The analytical sample of **8** was crystallized from acetone. The crystals of the sulfone **8** were dried in the vacuum drier (10 mbar, 40 °C). XRPD (2θ), [°]: 9.08, 11.77, 14.44, 15.15, 16.03, 16.29, 17.21, 18.94, 19.62, 23.54, 25.73, 26.72, 27.03, 28.07; DSC: endothermic peak 154.61 °C (onset 153.72 °C); MS (relative intensity) *m*/*z*: 357 ([M + Na]$^+$/100), 358/16, 691 ([2 M + Na]$^+$/13), 692/5; HRMS calcd for $C_{19}H_{26}O_3NaS$ [M + Na]$^+$: 357.1500, found 357.1512.

2.1.2. ((1R, 3R)-5-(2-iodoethylidene)cyclohexane-1,3-diyl)bis(tert-butyldimethylsilyloxy) (**13**)

Iodine (24.0 g, 94.5 mmol) was added to the solution of triphenylphosphine (24.7 g, 94.3 mmol) and imidazole (12.8 g, 188.2 mmol) in dichloromethane (300 mL) and was stirred at 0 °C. 2-((3*R*,5*R*)-[Bis(*tert*-butyldimethylsilyloxy)-cyclohexylidene)ethanol **12** (20.0 g, 51.8 mmol) was added after 30 min and the mixture was stirred at room temperature for 24 h. The residue was purified by silica gel chromatography to give the iodide **13** as wax (25.2 g, 50.8 mmol, 98%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.0–0.1 (m, 12H, 2xSi$(CH_3)_2$), 0.86–0.88 (m, 18H, 2xSiC$(CH_3)_3$), 1.5–2.5 (m, 6H, 2-CH_2, 4-CH_2, 6-CH_2), 3.8–4.2 (m, 2H, 2′-CH_2), 5.6 (dd, ^3J = 9.5 Hz, ^3J = 9.1 Hz, 1H, 1′-CH); ^{13}C NMR (200 MHz, $CDCl_3$) δ: −4.89, −4.82, −4.77, −4.72, 2.15, 18.05, 18.11, 25.79, 25.82, 25.87, 36.88, 43.39, 44.76, 66.99, 67.85, 76.37, 77.00, 77.64, 123.15, 139.80.

2.1.3. (3R, 5R)-[bis(tert-butyldimethylsilyloxy)]-cyclohexylidenethyldiphenylphosphine oxide (**14**)

The solution of iodide **13** (11.4 g, 23.0 mmol) in tetrahydrofuran (40.0 mL) was added dropwise to the solution of diphenylphosphine (5.6 mL, 32.0 mmol) and n-BuLi (1.6 M in hexane, 20.0 mL, 32.0 mmol) in tetrahydrofuran (20.0 mL) and then stirred at 0 °C. The mixture was stirred for 1 h and then H_2O_2 solution (18%, 100 mL) was added. The stirring at 0 °C was continued for 1 h. The mixture was extracted with dichloromethane and the residue purified by silica gel chromatography to give the phosphine oxide **14** (10.3 g, 17.9 mmol, 78%). UV λ_{max} 210.8, 259.6, 265.8, 272.4 nm; IR (KBr, ν (cm^{-1})): 3056, 2953, 2928, 2886, 2855, 1471, 1462, 1437, 1360, 1252, 1199, 1107, 1077, 1051, 1005, 961, 919, 900, 867, 836, 805, 775; ^{1}H NMR (200 MHz, $CDCl_3$) δ: −0.02–0.01 (m, 12H, 2xSi$(CH_3)_2$), 0.83–0.84 (m, 18H, 2xSiC$(CH_3)_3$), 1.65 (dd, ^3J = 5.5 Hz, ^3J = 5.1 Hz, 2H, 4-CH_2), 1.80–2.29 (m, 4H, 2-CH_2, 6-CH_2), 2.95–3.28 (m, 2H, 8-CH_2), 3.9–4.1 (m, 2H, 3-CH_2, 5-CH_2), 5.20–5.40 (m, 1H, 7-CH), 7.10–7.80 (m, 12H, Ar–H); ^{13}C NMR (200 MHz, $CDCl_3$) δ: −4.93, −4.91, −4.85, −4.82, 17.98, 18.06, 25.71, 25.76, 29.62, 31.01, 37.02, 43.26, 44.89, 44.94, 67.37,

67.40, 67.70, 67.75, 113.56, 113.72, 128.31, 128.35, 128.55, 128.57, 130.90, 131.04, 131.08, 131.22, 131.65, 131.68, 138.86, 139.10. MS (relative intensity) *m/z*: 593 ([M + Na]$^+$/100), 594/39, 571 ([M + H]$^+$/25), 1163([2 M + Na]$^+$/23), 307/20, 1164/18, 595/10, 595/10, 572/7 1142 ([2 M + H]$^+$/7), 1165/8, 1167/5, 308/5; HRMS (ESI): calcd for $C_{32}H_{51}0_3NaSi_2P$ [M + Na]$^+$: 593.3012, found 593.3032.

2.1.4. (7E)-(1R,3R)-24-phenylsulfonyl-1,3-bis(tert-butyldimethylsililoxy)-9,10-seco-19,22,23-trinor-chola-5,7-dien (**15**)

The solution of the sulfone **8** (0.11 g, 0.33 mmol) in THF (0.7 mL) was added drop-wise to the phosphine oxide solution **14** (0.33 g, 0.58 mmol) in THF (0.7 mL) at −60 °C and stirred at −40 °C for 2 h. The resulting mixture was extracted with hexane–ethyl acetate and purified by silica gel chromatography to give the sulfone **15** as foam (0.18 g, 0.26 mmol, 79%); mp 184.3–186.1 °C; UV λ_{max} 243.6, 251.8, 261.6 nm; IR (KBr, ν (cm^{-1})): 2923, 2855, 1732, 1674, 1620, 1587, 1471, 1447, 1379, 1361, 1306, 1252, 1149, 1086, 1051, 1026, 1006, 961, 921, 836, 774; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.05 (s, 6H, 2xSiCH_3), 0.52 (s, 3H, 18-CH_3), 0.86 (m, 18H, 2xSiC(CH_3)$_3$), 1.23 (d, ^3J = 6,6 Hz, 3H, 21-CH_3), 3.95–4.18 (m, 2H, 24-CH_2), 5.79 (d, ^3J = 11 Hz, 1H, 6-CH), 6.18 (d, ^3J = 11 Hz, 1H, 7-CH), 7.50–7.94 (m, 5H, Ar–H); ^{13}C NMR (200 MHz, $CDCl_3$) δ: −4.89, −4.69, −4.78, 11.88, 18.09, 20.26, 22.08, 23.17, 25.84, 27.48, 28.49, 29.69, 32.57, 36.77, 40.29, 43.66, 45.68, 45.99, 55.76, 56.13, 62.04, 67.91, 68.06, 116.54, 121.50, 127.86, 129.24, 133.49, 134.19, 139.77, 140.38; MS (relative intensity) *m/z*: 709 ([M + Na]$^+$/100), 710/47, 711/18, 1397 [2 M + Na]$^+$/13), 1399/8, 712/5. HRMS (ESI): calcd for $C_{39}H_{66}0_4NaSi_2S$ [M + Na]$^+$: 709.4118, found 709.4112. The analytical sample of **15** was crystallized from ethyl acetate. The crystals were dried in the vacuum drier (10 mbar, 40 °C). XRPD (2θ), [°]: 10.42, 15.30, 15.72, 17.16, 18.04, 19.71, 20.79, 21.94, 22.61, 23.97, 24.93, 25.86, 27.59, 29.39.

2.1.5. (1R,3R,7E,22E,24E)-24a-homo-19-nor-9,10-secoergosta-5,7,22,24-tetraen-1,3,25-triol (PRI-5201, **1**)

n-BuLi (1.6 M in hexane, 2.2 mL, 3.5 mmol) was added to the solution of diisopropylamine (0.7 mL, 5.0 mmol) in tetrahydrofuran (2 mL) at −60 °C. The solution of the sulfone **15** (2.0 g, 2.9 mmol) in tetrahydrofuran (8 mL) was added to the mixture. The stirring continued for 15 min and the freshly destilled ethyl 3-methyl-4-oxobut-2-enoate **16** (0.6 g, 4.2 mmol) was added. The mixture was stirred at −60 °C for 1 h and extracted with tetrahydrofuran. The solvent was replaced with ethyl ether (2.5 mL) and methyl magnesium bromide (3 M, 1.8 mL, 5.4 mmol) was added to the resulting crude ester **17**. The mixture was stirred for 30 min and extracted with *t*-butyl methyl ether to give the crude alcohol **18**. The saturated methanolic solution of Na_2HPO_4 (15 mL) and sodium amalgam (10%, 10 g) were added and the mixture was stirred at room temperature for 2 h. The mixture was extracted with *t*-butyl methyl ether to give the crude silylated alcohol **20**. This was dissolved in tetrahydrofuran (3 mL) and tetrabutylammonium fluoride (1 M, 3.8 ml) was added. The solution was stirred at 74 °C for 2 h and extracted with tetrahydrofuran. The product was purified by silica gel chromatography and crystallized from ethyl acetate to give the analog **1** (0.55 g, 1.3 mmol, 45% from **15**); mp 93.2–96.4 °C UV λ_{max} 242.6, 251.2, 261.6 nm; IR (KBr, ν (cm^{-1})): 3320, 2970, 2941, 2869, 1614, 1454, 1444, 1376, 1363, 1350, 1217, 1185, 1086, 1056; ^{1}H NMR (600 MHz, $CDCl_3$); δ 0.57 (s, 3H, 18-CH_3), 1,05 (d, ^3J = 6.6 Hz, 3H, 21-CH_3), 1,40 (s, 6H, 26-CH_3, 27-CH_3), 1,94 (s, 3H, 28-CH_3), 4,01 (m, 1H, 1-CH), 4,09 (m, 1H, 3-CH), 5,50 (s, 1H, 24a-CH), 5,52 (m, 1H, 22-CH), 5,85 (d, ^3J = 11.2 Hz, 1H, 7-CH), 5,93 (d, ^3J = 15.6 Hz, 1H, 23-CH), 6,28 (d, ^3J = 11.2 Hz, 1H, 6-CH); ^{3}C NMR (600 MHz, $CDCl_3$) δ: 12.17, 13.07, 20.61, 22.08, 23.33, 27.66, 28.75, 30.94, 31.05, 36.70, 40.25, 40.31, 41.72, 44.27, 45.64, 56.20, 56.37, 66.70, 66.99, 70.87, 115.38, 123.18, 131.73, 132.85, 135.07, 135.17, 136.59, 142.35; MS (relative intensity) *m/z*: 451 ([M + Na]$^+$/100), 411/36, 519/34, 452/32, 495/23, 520/12, 880/9, 587/8, 881/6, 581/6. HRMS (ESI): calcd for $C_{28}H_{44}0_3Na$ [M + Na]$^+$: 451.3188, found 451.3185; XRPD (2θ), [°]: 3.35, 3.67, 3.84, 4.09, 4.37, 4.64, 4.82, 4.89, 5.12, 5.33, 5.57, 6.32, 6.69, 8.35, 9 64, 16.68.

2.1.6. (1R,3R,7E,22E,24E)-24a,26,27-trihomo-19-nor-9,10-secoergosta-5,7,22.24-tetraen-1,3,25-triol (PRI-5202, **2**)

n-BuLi (1.6 M in hexane, 2.4 mL, 3.8 mmol) was added to the solution of diisopropylamine (0.8 mL, 5.5 mmol) in tetrahydrofuran (2 mL) at −60 °C. The solution of sulfone **15** (2.2 g, 3.2 mmol) in tetrahydrofuran (10 mL) was added to this mixture. The stirring continued for 15 min and the freshly destilled ethyl 3-methyl-4-oxobut-2-enoate **16** (0.7 g, 4.9 mmol) was added. The mixture was stirred at −60 °C for 1 h and extracted with tetrahydrofuran. The solvent was replaced with ethyl ether (3.0 mL) and ethyl magnesium bromide (3 M, 2.0 mL, 6.0 mmol) was added to the resulting crude ester **17**. The mixture was stirred for 30 min and extracted with *t*-butyl methyl ether to give the crude alcohol **19**. The saturated methanolic solution of Na_2HPO_4 (15 mL) and sodium amalgam (10%, 12 g) were added and the mixture was stirred at room temperature for 2 h. The mixture was extracted with *t*-butyl methyl ether to give the crude silylated alcohol **21**. This was dissolved in tetrahydrofuran (3 mL) and tetrabutylammonium fluoride (1 M, 4.0 ml, 4.0 mmol) was added. The solution was stirred at 76 °C for 2 h and extracted with tetrahydrofuran. The product was purified by silica gel chromatography and crystallized from ethyl acetate to give the analog **2** (0.64 g, 1.4 mmol, 44% from **15**); mp 71.6–74.7 °C; UV λ_{max} 242.8, 251.6, 261.6 nm; IR (KBr, ν (cm^{-1})): 3384, 2930, 2874, 1725, 1618, 1457, 1374, 1257, 1135, 1047, 964, 965; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.57 (s, 3H, 18-CH_3), 0.89 (m, 6H, 28-CH_3, 29-CH_3), 1.06 (d, ^3J = 6.6 Hz, 3H, 21-CH_3), 1.63 (q, ^3J = 7.2 Hz, 4H, 26-CH_2, 27-CH_2), 1.96 (d, ^3J = 1.2 Hz, 3H, 30-CH_3), 4.04 (m, 1H, 1-CH), 4.11 (m, 1H, 3-CH), 5.31 (s, 1H, 24a-CH), 5.50 (dd, ^3J = 8.6 Hz, ^3J = 8.6 Hz, 1H, 22-CH), 5.85 (d, ^3J = 11.2 Hz, 1H, 7-CH), 5.96 (d, ^3J = 15.6 Hz, 1H, 23-CH), 6.30 (d, ^3J = 11.2 Hz, 1H, 6-CH); ^{13}C NMR (600 MHz, $CDCl_3$) δ: 8.16, 8.18, 13.35, 20.71, 22.21, 23.45, 27.73, 28.89, 33.91, 33.95, 37.13, 40.32, 40.33, 42.12, 44.59, 45.76, 56.31, 56.52, 67.13, 67.36, 76.38, 115.33, 123.71, 131.28, 133.28, 134.48, 134.54, 136.05, 142.83; MS (relative intensity) *m/z*: 479 ([M + Na]$^+$]/100), 480/33, 936 [2 M + Na]$^+$/22, 937/19, 137/19, 1393/13, 697/10, 1394/5; HRMS (ESI): calcd for $C_{30}H_{48}0_3Na$ [M + Na]$^+$: 479.3501, found 479.3499.

2.1.7. (R)-3-hydroxy-2,3-dimethylbutanal (***24a***)

The solution of methyl (*S*)-(+)-3-hydroxy-2-methylpropionate **22a** (15.0 g, 127.1 mmol) in tetrahydrofuran (40 mL) was added to the solution of methyl magnesium bromide (3 M, 160 mL, 480.0 mmol) cooled to −10 °C. The mixture was stirred for 3 h under argon and extracted with *t*-butyl methyl ether. The residue was distilled under reduced pressure (1 mmHg/73–75 °C) to give the crude diol **23a** as oil (11.9 g, 100.8 mmol, 79%). Dess-Martin periodinane (40.0 g, 94.3 mmol) was added to the solution of the diol **23a** (6.8 g, 57.6 mmol) in dichloromethane (150 mL) which was then stirred at room temperature for 1 h. The mixture was extracted with dichloromethane and distilled under reduced pressure (1 mmHg/43–45 °C) to give the aldehyde **24a** as oil (4.8 g, 41.4 mmol, 72% from **23a**). IR (film, ν (cm^{-1})): 3427, 2975, 2942, 2882, 2736, 1712, 1460, 1371, 1272, 1158, 954; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.14 (d, ^3J = 7.2 Hz, 3H, 2-CH_3), 1.25 (s, 3H, 3-CH_3), 1.30 (s, 3H, 4-CH_3), 2.46 (dq, ^3J = 7.3 Hz, ^3J = 1.8 Hz, 1H, 2-H), 2.74 (bs, 1H, 3-OH), 9.83 (d, ^3J = 1.8 Hz, 1H, 1-CHO); ^{13}C NMR (200 MHz, $CDCl_3$) δ: 9.28, 26.21, 28.42, 55.64, 71.75, 205.98; HRMS calcd for $C_6H_{12}0_2Na$ [M + Na]$^+$: 139.0735, found 139.0733.

*2.1.8. (S)-3-hydroxy-2,3-dimethylbutanal (**24b**)*

The diol **23b** was obtained as described for the diol **23a** starting from the methyl (*R*)-(+)-3-hydroxy-2-methylpropionate **22b** (15.1 g, 127.6 mmol). The crude diol **23b** was distilled under reduced pressure (1 mmHg/73–75 °C) to give the diol **23b** as oil (11.6 g, 98.3 mmol 77%). The aldehyde **24b** was obtained as described for the aldehyde **24a** starting from the diol **23b**. The crude product was distilled under reduced pressure (1 mmHg/47–75 °C) to give the aldehyde **24b** (6.3 g, 54.3 mmol, 55% from **23b**) as oil. IR (film, ν (cm^{-1})): 3427, 2975, 2942, 2882, 2736, 1712, 1460, 1371, 1272, 1158, 954; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.15 (d, ^3J = 7.2 Hz, 3H, 2-CH_3), 1.25 (s, 3H, 3-CH_3), 1.30 (s, 3H, 4-CH_3), 2.45 (dq, ^3J = 7.3 Hz, ^3J = 1.8 Hz, 1H, 2-H), 2.71 (bs, 1H, 3-OH), 9.82 (d, ^3J = 1.8 Hz, 1H, 1-CHO); ^{13}C NMR (200 MHz, $CDCl_3$) δ: 9.50, 26.39, 28.61, 55.79, 71.99, 206.12; HRMS calcd for $C_6H_{12}0_2Na$ $[M + Na]^+$: 139.0735, found 139.0736.

*2.1.9. (1R,3R,7E,22E)-19-nor-9,10-secoergosta-5,7,22-trieno-1,3,25-triol (paricalcitol, PRI-5100, **3**)*

The solution of the sulfone **15** (2.6 g, 3.8 mmol) in tetrahydrofuran (15 mL) was added to the solution of n-BuLi (1.6 M in hexane, 5.9 mL) and diisopropylamine (1.4 mL, 9.9 mmol) in tetrahydrofuran (8 mL) was added at −60 °C. The mixture was stirred for 15 min and the aldehyde **24a** (1.8 g, 15.5 mmol) was added. The mixture was stirred at −60 °C for 1 h and the crude hydroxysulfone **25a** was extracted with tetrahydrofuran. The saturated methanolic solution of Na_2HPO_4 (20 mL) and sodium amalgam (3 g) were added to the resulting crude sulfones **25a** (2.4 g, 3.0 mmol, 80%) and the suspension was stirred at room temperature for 2 h. The silylated alcohol **26a** (1.4 g, 2.1 mmol, 70%) was extracted with *t*-butyl methyl ether and the solvent replaced with the mixture of methanol – chloroform (1:1, 16 mL). Camphorsulfonic acid (900 mg) was added and the solution was stirred at room temperature for 1.5 h. The product was purified by silica gel chromatography and crystallized from ethyl acetate to give the paricalcitol **3** (0.5 g, 1.2 mmol, 57%). UV λ_{max} 243.6, 251.8, 261.4 nm; IR (KBr, ν (cm^{-1}): 3286, 2949, 2883, 1615, 1457, 1378, 1368, 1284, 1193, 1133, 1124, 1049, 975; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.56 (s, 3H, 13-CH_3), 1.01 (d, ^3J = 6.9 Hz, 3H, 21-CH_3), 1.04 (d, ^3J = 6.9 Hz, 3H, 28-CH_3), 1.14 (s, 3H, 27-CH_3), 1.17 (s, 3H, 26-CH_3), 4.05 (m, 1H, 1-CH), 4.11 (m, 1H, 3-CH), 5.34 (m, 2H, 22-CH, 23-CH), 5.85 (d, ^3J = 11.4 Hz, 1H, 6-CH), 6.31 (d, ^3J = 11.4 Hz, 1H, 7-CH); ^{13}C NMR (600 MHz, $CDCl_3$) δ: 12.11, 15.26, 20.77, 22.08, 23.31, 26.12, 26.61, 27.66, 28.71, 36.62, 40.25, 40.32, 41.60, 44.18, 45.53, 47.87, 56.13, 56.19, 66.53, 66.84, 72.45, 115.43, 122.99, 129.20, 131.87, 138.43, 142.07; MS (relative intensity) *m*/*z*: 439 ($[M + Na]^+$)/100), 440/25, 461/11, 399/8; HRMS (ESI): calcd for $C_{27}H_{44}0_3Na$ $[M + Na]^+$: 439.3188, found 439.3178.

*2.1.10. (1R,3R,24R,7E,22E)-19-nor-9,10-secoergosta-5,7,22-trien-1,3,25-triol (PRI – 5101, **4**)*

The analog **4** was obtained as described for the analog **3,** starting from the aldehyde **24b** (0.84 g, 7.24 mmol) and the sulfone **15** (1.43 g, 2.08 mmol), through the intermediate hydroxysulfone **25b** (1.29 g, 1.61 mmol, 77%) and the silylated alcohol **26b** (0.72 g, 1.11 mmol, 69%). The product was crystallized from ethyl acetate to give the analog **4** (0.25 g, 0.60 mmol, 54%). UV λ_{max} 243.6, 251.8, 261.4 nm; IR (KBr, ν (cm^{-1})): 3400, 2978, 2945, 2869, 1741, 1616, 1376, 1284, 1220, 1165, 1088, 1045, 974; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.56 (s, 3H, 13-CH_3), 1.00 (d, ^3J = 7.0 Hz, 3H, 21-CH_3), 1.04 (d, ^3J = 6.4 Hz, 3H, 28-CH_3), 1.13 (s, 3H, 27-CH_3), 1.18 (s, 3H, 26-CH_3), 4.04 (m, 1H, 1-CH), 4.11 (m, 1H, 3-CH), 5.27 (m, 1H, 22-CH), 5.35 (m, 1H, 23-CH), 5.84 (d, ^3J = 11.4 Hz, 1H, 6-CH), 6.30 (d, ^3J = 11.4 Hz, 1H, 7-CH); ^{13}C NMR (600 MHz, $CDCl_3$) δ: 12.28, 15.82, 20.87, 22.30, 23.43, 26.23, 26.92, 28.23, 28.87, 37.15, 40.32, 40.62, 42.15, 44.65, 45.68, 48.33, 56.06, 56.31, 67.16, 67.38, 72.40, 115.34, 123.75, 129.36, 131.25, 139.30, 142.78; MS (relative intensity) *m*/*z*: 439 ($[M + Na]^+$)/100), 399/38, 440/28, 507/17, 501/13, 400/11, 381/8, 251/7, 305/6; HRMS (ESI): calcd for $C_{27}H_{44}0_3Na$ $[M + Na]^+$: 439.3188, found 439.3179.

2.2. Crystallography

The data for PRI-5100 (**3**), PRI-5101 (**4**) and CD-ring sulfone **8** were collected using the BRUKER KAPPA APEXII ULTRA diffractometer controlled by APEXII software [17], equipped with the MoKα rotating anode X-ray source (λ = 0.71073 A, 50.0 kV, 22.0 mA) monochromatized by multi-layer optics and the APEX-II CCD detector. The experiments were carried out at 100 K using the Oxford Cryostream cooling device. The crystal was mounted on a thin cactus needle with a droplet of Pantone-N oil and immediately cooled. Indexing, integration and initial scaling were performed with *SAINT* [18] and *SADABS* software [19]. The data collection and processing statistics are reported in the tables for respective structures.

The crystal was positioned at 40 mm from the CCD camera. 2103, 1853 and 1818 frames were measured at 0.5° intervals with a counting time of 30–45 s for PRI-5100, PRI-5101 and synthon CD, respectively. The structures were solved by direct methods approach using the SHELXS-97 program and refined with the SHELXL-97 [20]. Multi-scan absorption correction has been applied in the scaling procedure.

The refinement was based on F^2 for all reflections except those with negative intensities. Weighted *R* factors wR and all goodness-of-fit *S* values were based on F^2, whereas conventional *R* factors were based on the amplitudes, with *F* set to zero for negative F^2. The $F_0^2 > 2\sigma(F_0^2)$ criterion was applied only for R factors calculation and was not relevant to the choice of reflections for the refinement. The *R* factors based on F^2 are for all structures about twice as large as those based on *F*. Scattering factors were taken from Tables 4.2.6.8 and 6.1.1.4 from the International Crystallographic Tables Vol. C [21]. All details regarding X-ray experiment have been summarized in Table 1.

3. Results and discussion

3.1. Syntheses

The analogs **1** (PRI-5201) and **2** (PRI-5202) (Fig. 1) were conceived by combining modifications in both parts of the vitamin D molecule involved in receptor binding, *i.e.* in the A-ring and in the side-chain. We anticipated that these hybrid analogs might show amplified anticancer activity due to a stronger interaction with VDR. To test this hypothesis we have now formally deleted the C-19 methylene group from our analogs PRI-1906 and PRI-1907, like in the structure of paricalcitol (**3**, PRI-5100) and its (24*R*)-diastereomer (**4**, PRI-5101). The other vitamin D hybrid analogs with the removed C-19 methylene and modified (shortened) side-chain [22] have already exhibited a highly advantageous pattern of biological activity.

19-*Nor*-1,25-dihydroxyergocalciferol (paricalcitol, **3**, Fig. 1) was first obtained [3] by the removal of 19-methylene from 25-hydroxyvitamin D_2, followed by diastereoselective hydroxylation at carbon C-1, in a multistep linear synthesis. Classical methods, well established in vitamin D chemistry, were also used for this purpose [23]. Thus, the intermediate 3,5-cyclovitamin, conceived by Sheves and Mazur [24], was hydroxylated at C-1 by the method of Paaren and DeLuca [25] using Sharpless allylic hydroxylation [26]. The key step was the oxidative cleavage of the C10–C19 diol. A number of modifications of this method and the syntheses of intermediates were also developed [27], [28] using various activating groups at

Table 1
Experimental data of X-ray studies of the analogs **3** (PRI-5100), **4** (PRI-5101) and CD-ring sulfone **8**.

	3, PRI-5100	**4**, PRI-5101	CD-ring sulfone **8**
Crystal data			
Chemical formula	$C_{27}H_{44}O_3$	$C_{27}H_{44}O_3$	$C_{19}H_{26}O_3S$
M_r	416.62	416.62	334.46
Crystal system, space group	Orthorhombic, $P2_12_12$	Monoclinic, C2	Orthorhombic, $P2_12_12_1$
Temperature (K)	100	100	100
a, b, c (Å), β (°)	12.2484 (4), 32.5866 (10), 6.1390 (2)	32.880 (2), 6.1351 (4), 11.8711 (9), 99.503(4)	7.8331 (3), 10.9261 (4), 20.2743 (9)
V (Å^3)	2450.28 (14)	2361.8 (3)	1735.18 (12)
Z	4	4	4
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
m (mm^{-1})	0.07	0.07	0.2
Crystal size (mm)	0.30 × 0.10 × 0.10	0.10 × 0.10 × 0.05	0.25 × 0.15 × 0.15
Data collection			
T_{min}, T_{max}	0.979, 0.993	0.993, 0.996	0.952, 0.971
No. of measured, independent and observed [$I > 2s(I)$] reflections	63008, 4294, 3660	20998, 2850, 2435	39858, 5511, 5045
R_{int}	0.062	0.047	0.036
(sin $\Theta/\lambda)_{max}$ (Å^{-1})	0.716	0.641	0.723
Refinement			
$R[F^2 > 2s(F^2)]$, $wR(F^2)$, S	0.039, 0.102, 1.06	0.041, 0.114, 1.08	0.033, 0.086, 1.06
No. of reflections	4294	2850	5511
No. of parameters	315	279	210
No. of restraints	0	1	0
H-atom treatment	H atoms treated with the mixture of independent and constrained refinement	H-atom parameters constrained	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.30, −0.19	0.24, −0.23	0.40, −0.19
Absolute structure	–	–	Flack H D (1983), Acta Cryst. A39, 876–881
Flack parameter	–	–	−0.01 (5)
CCDC Id No.*	CCDC 917878	CCDC 917879	CCDC 917880

* CCDC 917878–917880 entries contain supplementary crystallographic data for the studied crystals. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via <www.ccdc.cam.ac.uk/data_request/cif>.

C-22 (aldehyde or diphenylphosphonium salt) and at the "C-23" in the side-chain (diphenylphosphonium salt or sulfone). The convergent syntheses [29] of paricalcitol were also described using the phosphine oxide of the A-ring and CD-ring fragment with a masked aldehyde or a diphenylphosphonium moiety at C-22.

However, none of these methods allowed for the convenient preparation of both side-chain modified analogs of 19-*nor*-1,25-$(OH)_2D_2$ and paricalcitol from the same advanced intermediates. Our retrosynthetic analysis (Scheme 1) of this class of compounds indicated that all the compounds of our interest might be conveniently obtained in a convergent manner from the new synthon ACD and the respective side-chain fragment (synthon S). The phenylsulfonyl moiety at C-22 should give, in a Julia olefination with a side-chain aldehyde (synthon S), the highest preference for the *trans* geometry of $C_{22,23}$ double bond. It should be feasible to construct the synthon ACD from the synthon CD and the synthon A using the methods well established in vitamin D chemistry [30]. The new synthon CD should be accessible by the degradative oxidation of commercial vitamin D_2. The synthesis of the synthon A was designed from an achiral precursor, 1,3,5-trihydroxybenzene with the use of a known enzymatic kinetic resolution. The synthon A has been previously used for the construction of 19-*nor*-vitamin D analogs, however it was coupled with the CD fragment already containing an alkyl side-chain, thus limiting the synthesis to a single selected analog [31].

Our synthesis of a new synthon CD (8, Scheme 2) started from the Inhoffen-Lythgoe diol 5 [32]. The diol was obtained by the exhaustive ozonation of commercial vitamin D_2 followed by the reductive workup. The Appel reaction [33] of the diol 5 with triphenylphosphine and iodine resulted in the iodide 6. The PCC oxidation [34] of the crude iodide 6, followed by the reaction of the resulting crude iodoketone 7 with sodium benzenesulfonate gave the synthon CD 8. As we anticipated, this synthon, bearing a phenylsulphonyl moiety, showed a very strong tendency for crystallization. The compound easily formed very large crystals of high purity from a number of solvents including ethyl acetate, acetone, *t*-butylmethyl ether, acetonitrile, 1,2-dimethoxyethane, toluene, methanol and from the mixtures of thereof. This allowed the efficient purification of this key intermediate and effective removal of the impurities from all its former synthetic steps.

For the synthesis of the synthon A (**14,** Scheme 3) we selected an enzymatic approach starting from the achiral precursor, 1,3,5-trihydroxybenzene (phloroglucinol) [35], [36] which is available in industrial quantity as a widely used source of pharmaceuticals. The catalytic reduction [37] of this substrate resulted in a mixture of diastereomeric trihydroxycyclohexanes. The peracetyletion of this mixture, followed by an enzymatic hydrolysis and chromatographic separation yielded (3*R*,5*R*)-3,5-diacetoxy-cyclohexan-1-ol (not shown). The Dess-Martin oxidation and Peterson olefination of this alcohol gave the respective diacetoxyester of high enantiomeric purity. The synthesis of this ester was also described [31] starting from the natural chiral precursor [(1*S*,3*R*,4*S*,5*R*)-1,3,4,5-tetrahydroxy-cyclohexane-carboxylic acid, quinic acid]. However, in this method, the Barton–McCombie radical deoxygenation [38] of the intermediate thiocarbonylimidazolide at C-4 with tributhylthin hydride was associated with the unbearable smell of thio-compounds which, from our point of view, made any further up-scaling of this method impractical. The replacement of both acetoxy groups with silyl protecting groups was followed by the reduction of the resulting ester with sodium bis(2-methoxyethoxy)aluminum dihydride (Red-Al^R) to give the alcohol **12**. The Appel reaction [33] of the alcohol **12** with triphenylphosphine and iodine [39] resulted in the iodide **13.** The alkylation of deprotonated diphenylphosphine with this iodide, followed by the oxidation with hydrogen peroxide yielded the phosphine oxide **14** (synthon A). In our hands the total yield of **14** from the alcohol **12** through the intermediate iodide **13** was substantially higher than that of **14** obtained through the respective chloride intermediate (not shown).

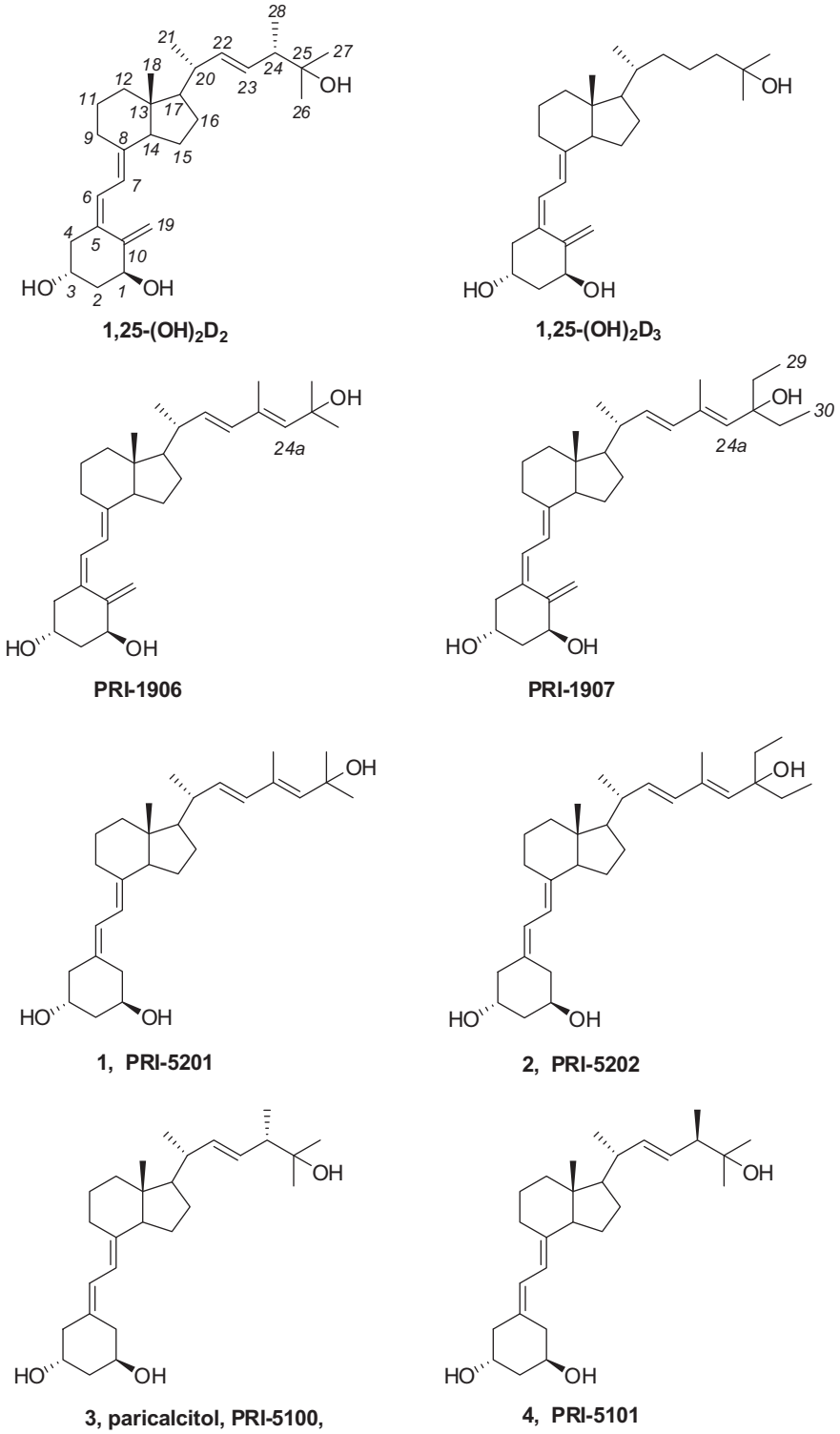
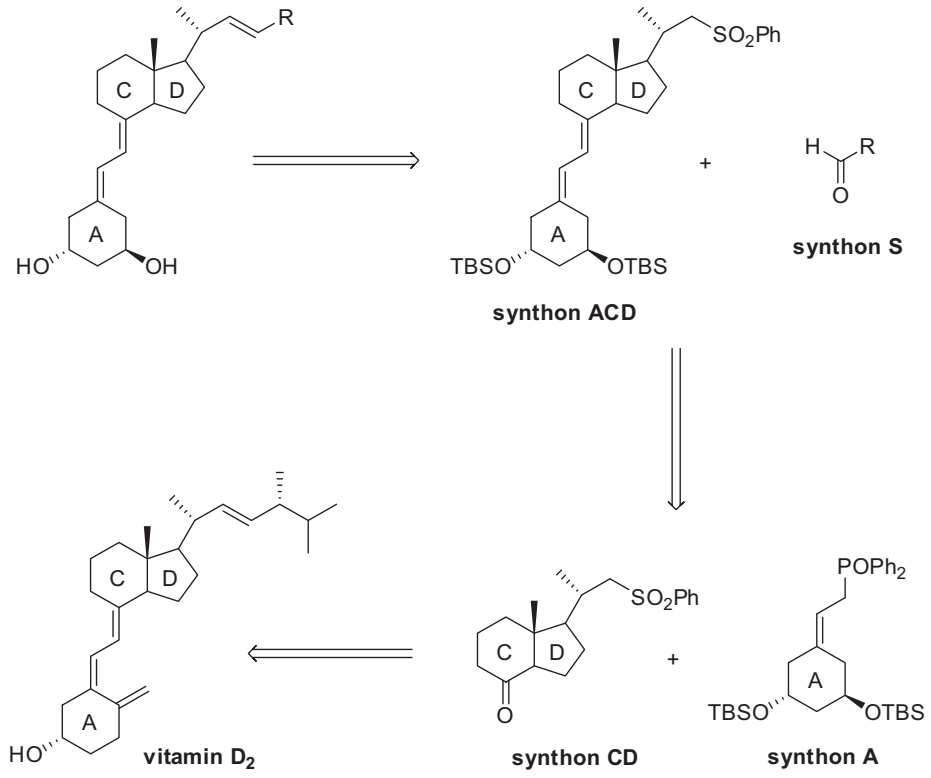


Fig. 1. Structures of the natural hormonal form of vitamin D_2 [1,25-$(OH)_2D_2$] and vitamin D_3, [1,25-$(OH)_2D_3$], structures of the previously described [9] analogs PRI-1906 and PRI-1907, structures of hybrid analogs of 1,25-dihydroxyergocalciferol (**1**, PRI-5201 and **2**, PRI-5202) and structures of paricalcitol (**3**, PRI-5100) and of its (24*R*)-diastereomer (**4**, PRI-5101).

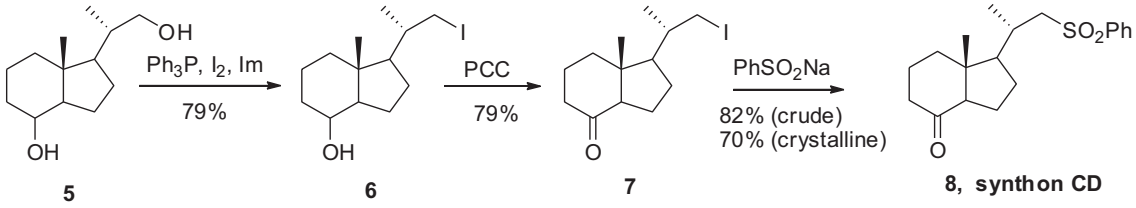
The Wittig-Horner condensation of a phospinoxy anion, generated from the phosphine oxide **14** (synthon A, Scheme 4), with the ketone **8** gave the new sulfone **15** (synthon ACD) as the key intermediate in the synthesis of the title compounds.

The Julia olefination of the deprotonated sulfone **15** with the aldehyde **16** (Scheme 5) gave the mixture [40] of diastereomeric hydroxysulfones **17**. The mixture was reacted with a Grignard reagent to give the respective tertiary alcohols **18** and **19**. The radical dehydroxy-desulfonylation of **18** and **19** with sodium amalgam in buffered methanol, followed by the tetrabutylammonium fluoride catalyzed desilylation gave the analogs **1** and **2**, respectively.

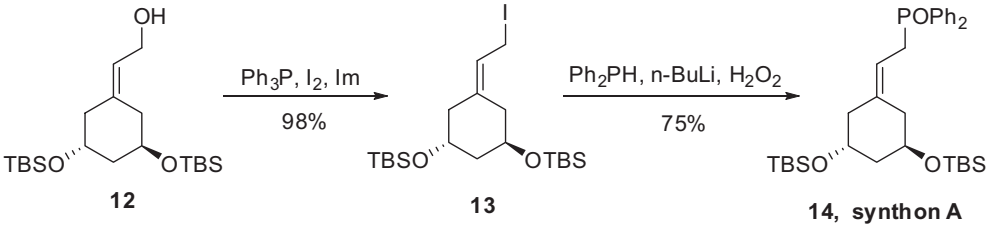
The synthesis of the side-chain fragments of the paricalcitol (**3**) and its (24*R*)-diastereomer **4** started from the homochiral methyl (*S*)- and (*R*)-3-hydroxy-2-methylpropionate **22a** and **22b** (Scheme 6), respectively. The Grignard reaction of these esters, followed by the Dess-Martin oxidation of the resulting crude diols



Scheme 1. Retrosynthetic analysis of the hybrid analogs of 1,25-dihydroxyergocalciferol.



Scheme 2. Synthesis of the synthon CD (**8**).



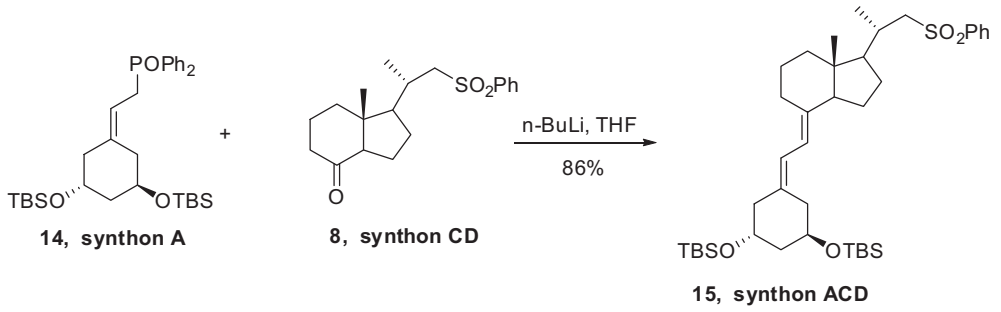
Scheme 3. Synthesis of the synthon A (**14**).

23a and **23b**, respectively, yielded the aldehydes **24a** and **24b**, respectively (see Scheme 7).

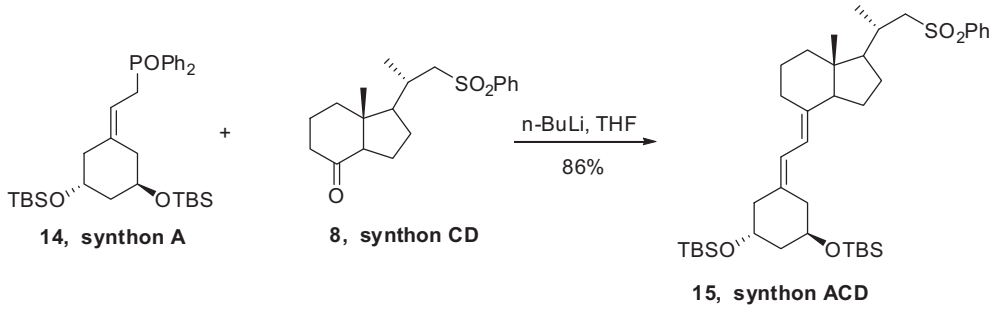
According to our design, the paricalcitol (**3**) and its (24*R*)-diastereomer **4** were obtained by the method similar to the one developed for the analogs **1** and **2** (Scheme 5). Thus the deprotonated sulfone **15** was coupled with the side-chain aldehyde **24a** and **24b** to give, following the dehydroxy-desulfonylation and desilylation, the paricalcitol (**3**) and its (24*R*)-diastereomer **4**, respectively. The paricalcitol (**3**) thus obtained was found identical in all respects with the one obtained from the synthon A (**14**, Scheme 4) synthesized from quinic acid.

3.2. Single crystal X-ray diffraction

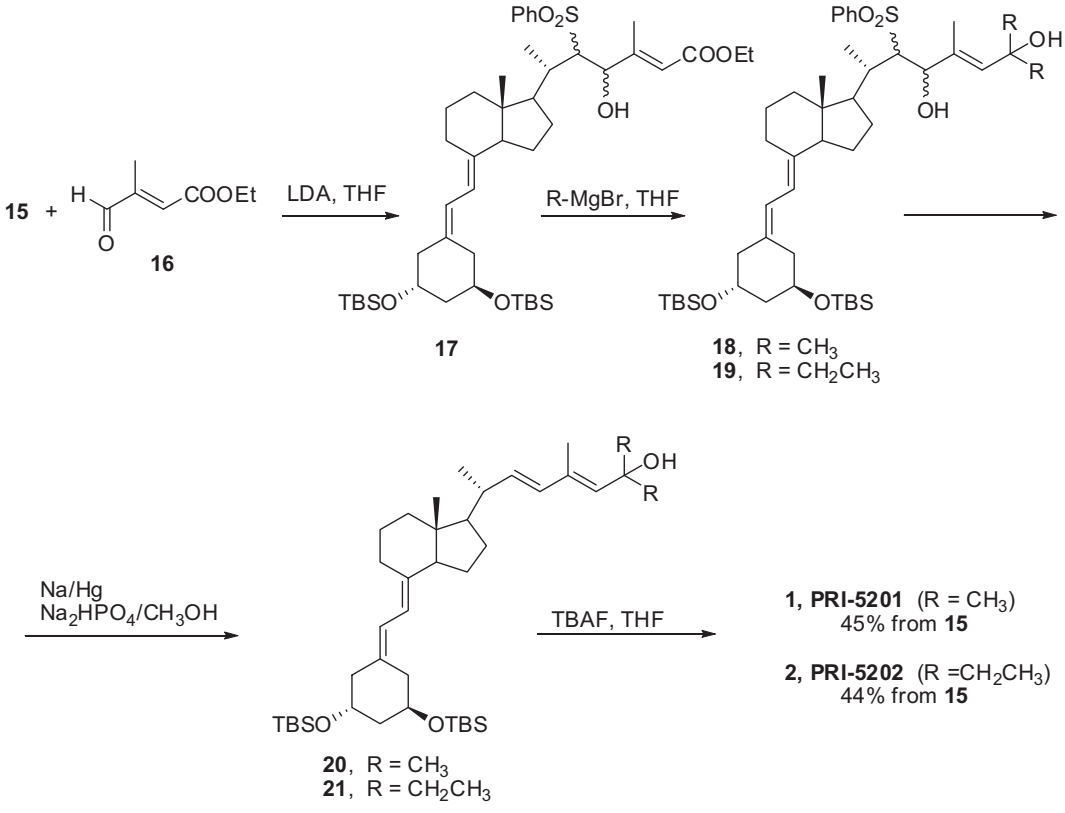
The single crystal X-ray studies of 1α-hydroxylated vitamin D analogs have shown that in most of these molecules the cyclohexane A-ring adopts a slightly distorted chair β-conformation (3-OH is axial) in the solid state. This conformation was found in the solid state initially for calcipotriol monohydrate [41] and later for 1,25-$(OH)_2D_3$ [42] trihydrate, as well as for anhydrous 1α-OH-D_2 and 1α-OH-D_3 [43]. The only exception is (24*R*)-1,24-dihydroxyvitamin D_3 monohydrate (tacalcitol) with the α-conformation [44] of the A-ring in the solid state. In the organic solutions of vitamins D



Scheme 4. Synthesis of the synthon ACD (**15**).

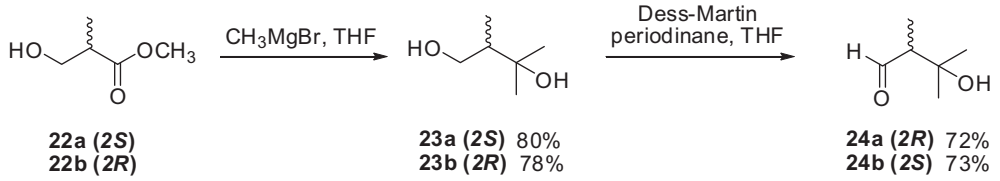


Scheme 5. Synthesis of the hybrid analogs of 1,25-dihydroxyergocalciferol (**1**, PRI-5201 and **2**, PRI-5202).



Scheme 6. Synthesis of the side-chain fragment **24a** of paricalcitol (PRI-5100) and **24b** of its (24*R*)-diastereomer (PRI-5101).

both conformers are in the equilibrium of α– and β–form [45,46] as determined by ^{1}H NMR. However, the crystal structures of the ligand binding domain of VDR liganded with vitamin D confirm that it is the β-conformation [47,48] of the A-ring of vitamin D when bound to the receptor. It was therefore of interest to learn the crystal structure of 19-*nor* vitamins D lacking the exocyclic C10-C19 double bond that might distort the conformation of the A-ring. None of the solid state crystal structures of numerous



Scheme 7. Synthesis of the paricalcitol **3** (PRI-5100) and (*24R*)-diastereomer **4** (PRI-5101).

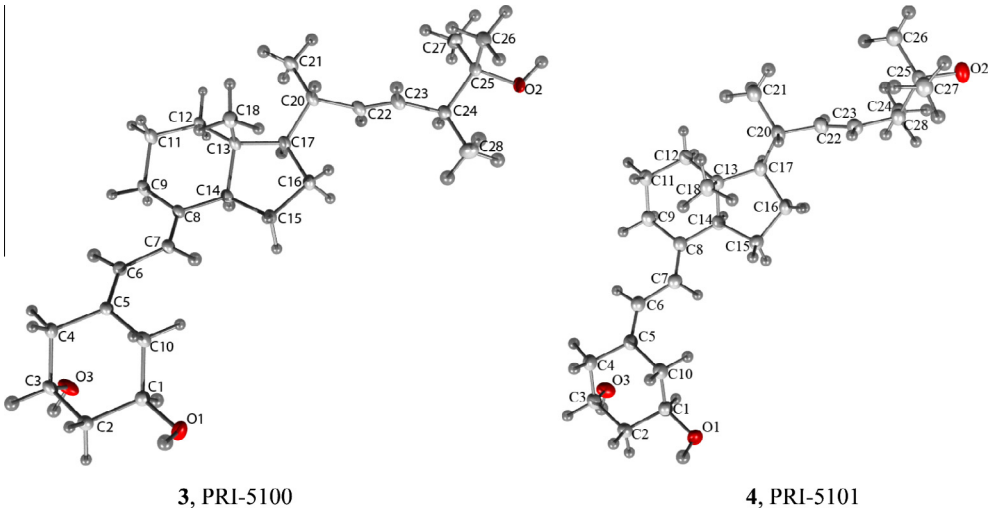


Fig. 2. Molecular structure of **3** (PRI-5100) and **4** (PRI-5101) with atomic displacement parameters (ADPs) at the 50% probability level.

19-*nor* vitamins D synthesized up to the present has been solved and refined so far. Therefore we present some first examples below.

The analogs **3** and **4** crystallize in the monoclinic $P2_12_12$ and C2 space groups, respectively, each of them with one molecule in the independent part of the unit cell. These are chiral space groups. However, due to the bad quality of the crystals and weak diffraction form single crystals of **3** and **4,** we were unable to establish the absolute structures for them, although the data collection was carried out at low temperature. The numbering schemes of atoms with atomic displacement parameters calculated at the 50% probability level are shown in Fig. 2. There are no solvent molecules in the crystal lattices of these compounds, whereas in 1,25-$(OH)_2D_3$ two water molecules are present.

The conformation of the A-ring in both structures is in the β-form, with the OH group at C3 in the axial position. There is a small distortion from the ideal cyclohexagonal form. The 5,7-diene fragment is symmetric in **3** [1.343(2), 1.450(2), 1.340(2) Å for the C5C6, C6C7 and C7C8 bonds lengths, respectively] and in **4** [1.335(3), 1.452(3), 1.335(3) Å for the equivalent bonds]. The C5C6C7C8 torsion angle is equal to 177.7(2) and -175.8(3) for **3** and **4**, respectively.

The mean plains for the A-ring and the CD-ring fragments have been calculated to compare their mutual orientation. The angles between those planes are equal to 13.7° and 6.6° for **3** (PRI-5100) and **4** (PRI-5101), correspondingly. Previous crystallographic studies of 1,25-$(OH)_2D_3$ [42] revealed that this angle was 6.6°. The aliphatic side-chain C17-C26 adopts different conformations for the new 19-*nor* vitamin D derivatives than for 1,25-$(OH)_2D_3$ [42]. The C17C20C22C23 and C22C23C24C25 torsion angles are: −121.2(3)°, 118.6(3)° and −116.9(2)°, 118.7(2)° for PRI-5100 and PRI-5101, respectively, whereas the equivalent torsion angles for the 1,25-$(OH)_2D_3$ [42] are close to 180° (−176.5(6)° and 169.2(7)°).

The favorable β−conformation of the A-ring in the previous [42] and present studies of the vitamin D analogues suggests the stabilizing influence of the hydrogen bonds. Both structures show the same motif – R_6^6 (hydrogen bond ring with 6 donors and 6 acceptors) – in the hydrogen bond pattern. All geometric parameters for both molecules studied have been summarized in Table 2. Hydrogen bond rings are built around the 2-fold axis. The molecules are packed into layers where the hydrophobic and hydrophilic forms are stack alternately (Fig. 3 (a) and (b)). The most significant layer motif is mutual for the paricalcitol (**3**, PRI-5100) and (24*R*)-diastereomer (**4**, PRI-5101), and vitamin D_3. However, the hydrogen bonds form the R_4^4 motif with three molecules of vitamin D and one molecule of water. The size of molecule of water and the lack of strong dispersive interaction

Table 2
The geometric parameters for intermolecular hydrogen bonds for **3** (PRI-5100, upper line) and **4** (PRI-5101, bottom line).

D–H···A	Symmetry code for the acceptor	d(D–H)	d(H···A)	d(D···A)	<(DHA)
O(1)–H(1O)···O(3)	x, y, z − 1	0.83	1.95	2.746(2)	161
	x, y−1, z	*0.84*	*1.97*	*2.770(3)*	*159.2*
O(2)–H(2O)···O(1)	x + 1/2, −y + 1/2, −z	0.90	1.86	2.759(2)	173
	−x + 3/2, y + 1/2, −z + 1	*0.84*	*2.00*	*2.800(2)*	*160.0*
O(3)–H(3O)···O(2)	−x + 1/2, y − 1/2, −z + 1	0.83	1.90	2.706(2)	163
	x − 1/2, y + 1/2, z − 1	*0.84*	*2.06*	*2.894(3)*	*171.8*

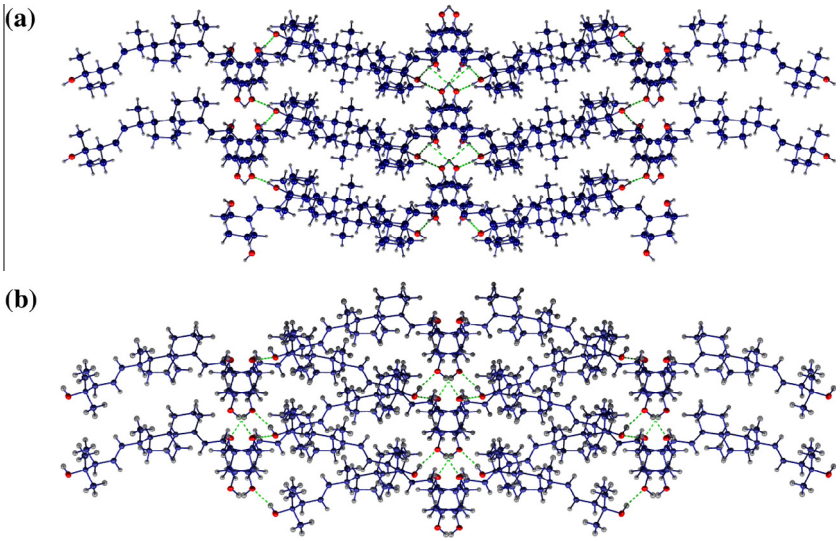


Fig. 3. Packing diagram for (a) **3**, PRI-5100 and (b) **4**, PRI-5101. Hydrogen bonds drown in green dashed lines.

with it allowed to form a smaller hydrogen-bonded ring (see Fig. 4).

Additionally, the crystals of the CD-ring sulfone **8** (see figure below) have been crystallized and the diffractional data for such a single crystal have been collected. The CD-ring sulfone **8** forms large colorless crystals in the orthorhombic $P2_12_12_1$ space group with one molecule in the asymmetric unit. Similarly to the previous compounds, the CD-ring sulfone **8** forms a layered structure, where identical layers can be found. Only very weak H···H and H···π interactions and weak C–H···O interaction occur in this structure.

4. Conclusions

The hybrid analogs of 19-*nor*-1,25-$(OH)_2D_2$, **1** and **2**, combining both the A-ring and side-chain modifications, were synthesized by

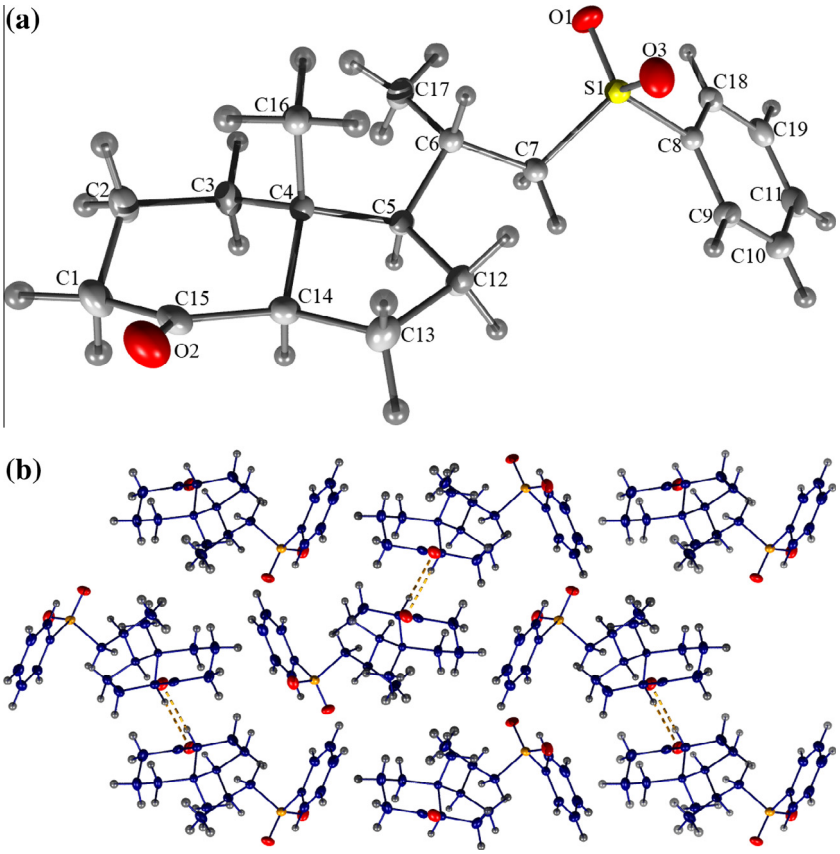


Fig. 4. (a) Molecular structure of the CD-ring sulfone **8** with atomic displacement parameters (ADPs) at the the 50% probability level. (b) Molecular packing diagram for the CD-ring sulfone (**8**). Weak C–H. . .O interaction illustrated by some orange dashed lines.

a convergent strategy using the new advanced intermediates **8** and **15**. This semi-total synthesis was already designed in a way that can be upscaled for potential pharmaceutical purposes. The synthesis starts from the commercial and easily available starting materials and proceeds through convenient bench-stable intermediates. As expected, the CD-ring synthon **8**, containing a rigid skeleton and phenylsulfonyl moiety, crystallized easily and allowed very convenient removal of all the impurities resulting from its multistep synthesis from natural vitamin D_2. Thus the compound might represent a convenient intermediate in pharmaceutical syntheses of various vitamin D analogs of therapeutic importance. Despite numerous attempts, the new hybrid analogs **1** and **2** resisted crystallization in a number of organic solvents. However, the paricalcitol (**3**) and its C-24 diastereomer **4**, obtained by the new synthesis as the reference compounds for a biological activity study, gave the crystals suitable for a crystallographic study. This way we have got the opportunity to solve and refine the solid phase structure of 19-*nor* analogs of 1,25-$(OH)_2D_2$ for the first time.

The crystallographic studies confirmed that the A-ring in both 1α-hydroxylated vitamin D analogs **3** and **4**, even without C10C19 methylene, exists in the solid state in the β-form, with the OH group at C3 in the axial position. This might suggest the need for the re-evaluation of the X-ray solid state structure of tacalcitol, as the exclusive example of the α-form of the A-ring [44].

Due to the flexibility around single bonds the aliphatic side-chain C17-C26 adopts similar conformation for both 19-*nor* C-24 diastereomers **3** and **4**, as measured by the torsion angles C17C20C22C23 and C22C23C24C25. As expected, these torsion angles are both very much different than those for 1,25-$(OH)_2D_3$ [42], containing neither a double bond nor C-24 methyl in the side-chain. The side-chain of analogs **3** and **4** is coplanar with the CD-ring fragment, whereas in the 1,25-$(OH)_2D_3$ it is located in the perpendicular manner.

Very unexpectedly, the crystallographic studies revealed that the additional methyl at C-24 in the side-chain of both **3** and **4** strongly influences the mutual orientation of the mean planes for the A-ring and the CD-ring fragment. This angle was identical for **4** and 1,25-$(OH)_2D_3$ [42] but it was twice higher for **3**. Further syntheses of hybrid analogs of both vitamin D_2 and D_3 is under way in this laboratory. The evaluation of the activity profile of the analogs 1 and 2 is under way in the collaborating laboratory and it will constitute the subject of the future publication.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2013.06.001.

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