and −3), 4.04 (minor) (1 H, dd, J = 9.3, 4.6 Hz, H-1 or 3), 5.82 (1 H, d, J = 11.2 Hz, H-7), 6.21, 6.27 (3:2) (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 704 (M^+, 24), 642 (12), 585 (74), 73 (100).

*1α-Hydroxy-25-methoxymethoxy-2-spiro[oxirane]-19-norvitamin D_3 (**18**).* A 1.0 M THF solution of TBAF (377 μL, 0.377 mmol) was added to a solution of **17** (88.7 mg, 0.126 mmol) in THF (1 mL), and the mixture was stirred at room temperature for 4 h. The mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (6 g) and eluted with 50% AcOEt/hexane to give 1,3-diol **18** (58.9 mg, 98%) as a mixture of epimers at C2. **18**: ^{1}HNMR ($CDCl_3$) δ 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.62, 2.72 (3:2) (1 H, dd, J = 13.6, 3.9 Hz), 2.85 and 3.08, 2.94, and 2.99 (3:2) (each 1 H, d, J = 4.7 Hz, $-CH_2OC-$), 3.37 (3 H, s, OCH_3), 3.81 (1 H, m, H-1 or −3), 3.91, 3.98 (3:2)(1 H, m, H-1 or −3), 4.71 (2 H, s, OCH_2O), 5.87 (1 H, m, H-7), 6.39 (1 H, m, H-6). MS m/z (%) 476 (M^+, 25), 414 (100).

*1α,2-Dihydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 (**19**).* $LiAlH_4$ (3.3 mg, 0.088 mmol) was added to a solution of diol **18** (20.9 mg, 0.044 mmol) in THF (500 μL), and the mixture was stirred at room temperature for 16 h. A solution of saturated potassium sodium tartrate was added to the reaction, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 70% AcOEt/hexane to give triol **19** (17.2 mg, 82%) as a 3:2 mixture of epimers at C2. **19**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.27, 1.30 (3:2) (3 H, s, H-2Me), 3.37 (3 H, s, OCH_3), 3.74 (2 H, m, H-1, 3), 4.71 (2 H, s, OCH_2O), 5.83 (1 H, m, H-7), 6.32 (1 H, m, H-6). MS m/z (%) 478 (M^+, 45), 416 (100), 398 (13).

*1α,2-Dihydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**20a**) and 1,2-Dimethyl Acetal (**20b**).* To a solution of triol **19** (34.6 mg, 0.072 mmol) in 2,2-dimethoxypropane (1 mL) was added $TsOH·H_2O$ (1.4 mg, 7.23 μmol), and the mixture was stirred at room temperature for 1 h. The mixture was extracted with AcOEt, and the extract was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 20% AcOEt/hexane to give **20** (28.4 mg, 64%) as a mixture of 2,3- and 1,2-diol acetonides (3:2). **20**: ^{1}H NMR ($CDCl_3$) δ 0.54, 0.55 (3:2) (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.30, 1.34 (3:2) (3 H, s, H-2Me), 1.38, 1.45 (major) (each 3 H, s, C$\underline{(CH_3)_2}$), 1.39, 1.46 (minor) (each 3 H, s, C$\underline{(CH_3)_2}$), 3.37 (3 H, s, OCH_3), 3.88, 3.82 (3:2) (1 H, m, H-1 or −3), 4.04 (minor) (1 H, t, J = 4.1 Hz, H-1 or −3), 4.10 (major) (1 H, t, J = 4.4 Hz, H-1 or −3), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.26 (1 H, m, H-6). MS m/z (%) 518 (M^+, 97), 456 (100), 413 (11), 398 (33), 380 (23).

*1α-(2-tert-Butyldimethylsilyloxyethoxy)-2-hydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**22a**) and 1α,2-Dihydroxy-25-(methoxylmethoxy)-2-methyl-19-norvitamin D_3 3-(2-tert-Butyldimethylsilyloxyethyl) Ether 1,2-Dimethyl Acetal (**22a′**).* To a solution of alcohol **20** (11.8 mg, 0.028 mmol) in DMF (400 μL) were added NaH (60% in oil, 33.1 mg, 0.827 mmol) and a solution of tosylate **21a** (62.5 mg, 0.189 mmol) in DMF (500 μL). The mixture was stirred for 4 h at room temperature and then quenched with ice water. The mixture was extracted with 50% AcOEt/hexane, washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 5% AcOEt/hexane to give **22a** and **22a′** (11.8 mg, 63%) as a mixture (3:2) of isomers. **22a** and **22a′**: ^{1}H NMR ($CDCl_3$) δ 0.07, 0.05 (3:2)(6 H, s, Si-Me × 2), 0.54, 0.55 (3:2)(3 H, s, H-18), 0.90, 0.89 (3:2) (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.325, 1.334 (3:2)(3 H, s, H-2Me), 1.37, 1.45 (major) (each 3 H, s, C$\underline{(CH_3)_2}$) 1.38, 1.47 (minor) (each 3 H, s, C$\underline{(CH_3)_2}$), 3.37 (3 H, s, OCH_3), 3.75−4.06 (6 H, m, H-1 and 3, O$\underline{(CH_2)_2}$OTBS), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.23 (1 H, m, H-6). MS m/z (%) 676 (M^+, 2), 614 (4), 599 (3), 556 (2), 438 (100).

*1α-(3-tert-Butyldimethylsilyloxypropoxy)-2-hydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**22b**) and 1α,2-Dihydroxy-25-(methoxylmethoxy)-2-methyl-19-norvitamin D_3 3-(3-tert-Butyldimethylsilyloxypropyl) Ether 1,2-Dimethyl Acetal (**22b′**).* Monohydroxy compound **20** (16.9 mg, 0.033 mmol) dissolved in DMF (900 μL) was treated with NaH (60% in oil, 52.0 mg, 1.30 mmol) and then tosylate **21b** (90.6 mg, 0.263 mmol) as in the synthesis of **22a** and **22a′**. The ethers **22b** and **22b′** (11.1 mg, 49%) were obtained after similar workup as a 3:2 mixture of isomers. **22b** and **22b′**: ^{1}H NMR ($CDCl_3$) δ 0.05, 0.04 (3:2) (6 H, s, Si-Me × 2), 0.54, 0.55 (3:2) (3 H, s, H-18), 0.89, 0.88 (3:2)(9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.32, 1.33 (3:2) (3 H, s, H-2Me), 1.37, 1.44 (major) (each 3 H, s, C$\underline{(CH_3)_2}$) 1.39, 1.47 (minor) (each 3 H, s, C$\underline{(CH_3)_2}$), 3.37 (3 H, s, OCH_3), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.21 (1 H, m, H-6).

*1α-(4-tert-Butyldimethylsilyloxybutoxy)-2-hydroxy-25-(methoxymethoxy)-2-methyl-19-norvitamin D_3 2,3-Dimethyl Acetal (**22c**) and 1α,2-Dihydroxy-25-(methoxylmethoxy)-2-methyl-19-norvitamin D_3 3-(4-tert-Butyldimethylsilyloxybutyl) Ether 1,2-Dimethyl Acetal (**22c′**).* Monohydroxy compound **20** (11.9 mg, 0.023 mmol) in DMF (700 μL) was similarly treated with NaH (60% in oil, 27.5 mg, 0.688 mmol) and tosylate **21c** (49.4 mg, 0.138 mmol). The ethers **22c** and **22c′** (10.6 mg, 65%) were obtained after similar workup as a mixture (3:2) of isomers. **22c** and **22c′**: ^{1}H NMR ($CDCl_3$) δ 0.05, 0.04 (3:2) (6 H, s, Si-Me × 2), 0.54, 0.55 (3:2) (3 H, s, H-18), 0.891, 0.887 (3:2) (9 H, s, Si-*t*Bu), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.32, 1.33 (3:2)(3 H, s, H-2Me), 1.37, 1.45 (major) (each 3 H, s, C$\underline{(CH_3)_2}$), 1.39, 1.47 (minor) (each 3 H, s, C$\underline{(CH_3)_2}$), 3.37 (3 H, s, OCH_3), 3.39−3.70 (5 H, m, H-1 or 3, O$\underline{CH_2}(CH_2)_2\underline{CH_2}$OTBS), 4.00, 4.05 (3:2)(1 H, m, H-1 or 3), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, m, H-7), 6.23 (1 H, m, H-6).

*1α-(2-Hydroxyethoxy)-2β,25-dihydroxy-2α-methyl-19-norvitamin D_3 (**2c**).* CSA (24.3 mg, 0.105 mmol) was added to a solution of **22a** and **22a′** (11.8 mg, 0.017 mmol) in MeOH (300 μL), and the mixture was stirred for 1.5 h at room temperature. Then, 5% $NaHCO_3$ was added to the reaction, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (3 g) and eluted with 70% AcOEt/hexane to give a 3:2 mixture of regioisomers **2c** and **2c′** (7.2 mg, 87%). The mixture was separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min] to give **2c** (2.59 mg, RT 21.08) and **2c′** (1.67 mg, RT 23.75). **2c**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.29 (3 H, s, H-2Me), 2.37, 2.49 (each 1 H, m, H-4), 2.79 (1 H, dd, J = 12.6, 4.1 Hz, H-9), 2.91 (1 H, dd, J = 13.7, 4.4 Hz, H-10), 3.48−3.81 (6 H, m), 5.83 (1 H, d, J = 11.2 Hz, H-7), 6.26 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 478 (M^+, 100), 460 (58), 442 (18). UV λ_{max} (EtOH): 244, 252, and 261 nm. **2c′** ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.94 (3 H, d, J = 6.5 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.30 (3 H, s, H-2Me), 2.60 (1 H, dd, J = 13.7, 4.2 Hz), 2.73 (1 H, dd, J = 14.7, 5.5 Hz),2.79 (1 H, m), 3.55−3.74 (6 H, m), 5.81 (1 H, d, J = 11.1 Hz, H-7), 6.32 (1 H, d, J = 11.1 Hz, H-6). MS m/z (%) 478 (M^+, 100), 460 (55), 442 (24). UV λ_{max} (EtOH): 244 nm, 252 nm, 261 nm.

*1α-(3-Hydroxypropoxy)-2β,25-dihydroxy-2α-methyl-19-norvitamin D_3 (**2d**).* Deprotection of **22b** and **22b′** (11.1 mg, 0.016 mmol) by the procedure described above gave a 3:2 mixture of **2d** and **2d′** (4.3 mg, 54%). The mixture was separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min to give **2d** (2.37 mg, RT: 20.18) and **2d′** (1.37 mg, RT: 21.60). **2d** ^{1}H NMR ($CDCl_3$) δ: 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.27 (3 H, s, H-2Me), 2.09 (1 H, dd, J = 13.1, 9.8 Hz, H-10), 2.36 (1 H, dd, J = 14.0, 5.2 Hz, H-4), 2.47 (1 H, m, H-4), 2.80 (1 H, dd, J = 12.3, 4.1 Hz, H-9), 2.89 (1H, dd, J = 13.7, 4.2 Hz, H-10), 3.44−3.86 (6 H, m), 5.83 (1 H, d, J = 11.2 Hz, H-7), 6.27 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 492 (M^+, 100), 474 (65), 456 (26). UV λ_{max} (EtOH): 244, 252, and 261 nm. **2d′** ^{1}H NMR ($CDCl_3$) δ 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.5 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.25 (3 H, s, H-2Me), 2.60−2.81 (3H, m), 3.50−3.88 (6 H, m), 5.81 (1 H, d, J = 11.2 Hz, H-7), 6.32 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%): 492 (M^+, 38), 474 (45), 456 (33), 135 (100). UV λ_{max} (EtOH): 244 nm, 252 nm, 262 nm.

*1α-(4-Hydroxybutoxy)-2β,25-dihydroxy-2α-methyl-19-norvitamin D_3 (**2e**).* Deprotection of **22c** and **22c′** (10.6 mg, 0.015 mmol) was similarly carried out by treatment with CSA (20.9 mg, 0.090

mmol) in THF/MeOH (1:1 400 μL) to give a 3:2 mixture of **2e** and **2e′** (4.6 mg, 60%). The isomers were separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min] to give **2e** (3.68 mg, RT: 28.66) and **2e′** (0.90 mg, RT: 32.68). **2e** ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.28 (3 H, s, H-2Me), 2.09 (1H, dd, J = 13.3, 9.7 Hz, H-10), 3.43−3.64 (2 H, OCH_2), 3.65−3.74 (4 H, m), 5.82 (1 H, d, J = 11.2 Hz, H-7), 6.26 (1 H, d, J = 11.2 Hz, H-6). Mass m/z (%) 507 (M^+, 6), 489 (4), 471 (1), 75 (100). UV λ_{max} (EtOH): 244 nm, 252 nm, 261 nm. **2e′** ^{1}H NMR ($CDCl_3$) δ 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 1.27 (3 H, s, H-2Me), 2.60−2.81 (3 H, m), 3.49 (2 H, OCH_2), 3.67 (3 H, m), 3.76 (1 H, m), 5.82 (1 H, d, J = 11.2 Hz, H-7), 6.31 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%) 507 (M^+, 2), 489 (1), 75 (100). UV λ_{max} (EtOH): 244, 252, and 262 nm.

*5α-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-one (**24a**).* To a solution of iPr_2NH (706 μL, 5.04 mmol) in THF (10 mL) at −20 °C was added *n*-BuLi (2.8 mL, 4.37 mmol, 1.58 M hexane), and the mixture was stirred for 15 min. Grundman's ketone (1.09 g, 3.36 mmol) in THF (5 mL) was added to the LDA solution at −78 °C, the mixture was stirred for 1 h at that temperature, and then TMSCl (436 μL, 5.04 mmol) and Et_3N (702 μL, 5.04 mmol) were added. The temperature of the reaction was raised to −20 °C for 1.5 h. The solvent was evaporated, the residue was dissolved in hexane, and the mixture was passed through Celite to give **23** (1.34 g). To a solution of **23** (500.5 mg, 1.262 mmol) in THF (3 mL) at 0 °C was added MeLi (1.2 M Et_2O solution, 1.16 mL, 1.388 mmol) and stirred for 1 h. This solution of enolate was added to a solution of iodobutane (718 μL, 6.31 mmol) and HMPA (439 μL, 2.524 mmol) in THF (2 mL) at 0 °C, and the mixture was stirred at 0 °C for 3 h. A saturated NH_4Cl solution was added to the reaction, the solution was extracted with AcOEt, and the organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (20 g) and eluted with 5% AcOEt/hexane to give **24a** (249.2 mg, 52%). **24a**: ^{1}H NMR ($CDCl_3$) δ 0.64 (3 H, s, H-18), 0.87 (3 H, t, J = 7.1 Hz, $(CH_2)_3\underline{CH_3}$), 0.95 (3 H, d, J = 6.1 Hz, H-21), 1.21 (6 H, s, H-26,27), 2.02 (1 H, m, H-9), 2.60 (1 H, dd, J = 11.6, 7.4 Hz, H-14), 3.37 (3 H, OMe), 4.70 (2 H, s, $O\underline{CH_2}O$). MS m/z (%) 380(M^+, 2), 365 (7), 318 (45), 227 (24), 262 (100), 219 (209, 103 (41).

*5-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-4-vinyl-octahydro-inden-4-ol (**25a**).* A solution of **24a** (303 mg, 0.80 mmol) in THF (3 mL) at 0 °C was treated with vinyl magnesium bromide (1.66 mL, 1.662 mmol THF solution), and the mixture was stirred for 3 h. The reaction was quenched by adding a 1 N HCl solution and extracted with AcOEt, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 10% AcOEt/hexane to give **25a** (297.5 mg, 88%). **25a**: ^{1}H NMR ($CDCl_3$) δ 0.88 (3 H, t, J = 7.1 Hz, $(CH_2)_3\underline{CH_3}$), 0.91 (3 H, d, J = 6.5 Hz, H-21), 0.96(3 H, s, H-189, 1.21 (6 H, s, H-26,27), 2.03 (1 H, m, H-14), 3.37 (3 H, s, OMe), 4.70 (2 H, s, OCH_2O), 5.02 (1 H, dd, J = 10.8, 1.6 Hz, H-6), 5.23 (1 H, dd, J = 17.2, 1.6 Hz, H-6), 5.94 (1 H, dd, J = 17.2, 10.8 Hz, H-7).

*[5-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-acetaldehyde (**26a**).* To a solution of **25a** (268.7 mg, 0.657 mmol) in CH_2Cl_2 (15 mL) was added PCC (723 mg, 3.288 mmol) and Celite (0.8 g), and the mixture was stirred at room temperature for 23 h. The reaction mixture was directly chromatographed on silica gel (10 g) and eluted with 5% AcOEt/hexane to give aldehyde **26a** (172.8 mg, 86%). **26a**: ^{1}H NMR ($CDCl_3$) δ 0.61 (3 H, s, H-18), 0.88 (3H, t, J = 7.2 Hz, $(CH_2)_3\underline{CH_3}$), 0.94 (3 H, d, J = 5.7 Hz,H-21), 1.21 (6 H, s, H-26,27), 2.36 (1 H, m, H-14), 3.37 (3 H, s, OMe), 3.39 (1 H, m, H-9), 4.71 (2 H, s, OCH_2O), 5.78 (1 H, d, J = 8.3 Hz, H-7), 10.06 (1 H, d, J = 8.3 Hz, CHO).

*2-[5-Butyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-ethanol (**26b**).* $NaBH_4$ (13.9 mg, 0.367 mmol) in EtOH (2 mL) was added to a solution of aldehyde **26a** (200.5 mg, 0.493 mg) in EtOH (1.5 mL) at 0 °C, and the mixture was stirred for 1 h. Water was added to the reaction, and the mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g) and eluted with 20−25% AcOEt/hexane to give **26b** (172.8 mg, 86%). **26b**: ^{1}HNMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.88 (3 H, t, J = 7.3 Hz, $(CH_2)_3\underline{CH_3}$), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.21 (6 H, s, H-26,27), 2.12 (1 H, m, H-14), 2.62 (1 H, m, H-9), 3.37 (3 H, s, OMe), 4.15, 4.25 (each 1 H, m, H-6), 4.71 (2 H, s, OCH_2O), 5.23 (1 H, m, H-7). MS e/z (%) 408 (M^+, 0), 346 (11), 328 (58), 313 (23), 271 (19), 243 (33), 217 (100).

*5-Butyl-4-[2-(diphenyl-phosphinoyl)-ethylidene]-1-(5-methoxy-methoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-indene (**27**).* To a solution **26b** (156 mg, 0.382 mmol) in THF (2 mL) at 0 °C were added a solution of *n*-BuLi (1.58 M hexane, 266 μL, 0.420 mmol) and then a solution of TsCl (87.4 mg, 0.458 mmol) in THF (0.2 mL), and the mixture was stirred for 5 min. In another flask, to a solution of diphenylphosphine (133 μL, 0.764 mmol) in THF (1 mL) at 0 °C was added a solution of *n*-BuLi (1.58 M hexane, 484 μL, 0.764 mmol) to yield a red solution. This red solution was slowly added via a double headed needle to the above solution of tosylate until the red color did not disappear in the solution, and the mixture was stirred for 30 min. Water (20 μL) was added to the reaction, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C, 10% H_2O_2 (3 mL) was added to this solution, and the mixture was stirred at 0 °C for 1 h. Sodium thiosulfate (2 N) was added to the reaction, the mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (8 g) and eluted with 40% AcOEt/hexane to give Wittig−Horner reagent **27** (173.7 mg, 77%). **27**: ^{1}H NMR ($CDCl_3$) δ 0.23 (3 H, s, H-18), 0.86 (3 H, t, J = 7.4 Hz, $(CH_2)_3\underline{CH_3}$ overlapped with H-21), 1.20 (6 H, s, H-26,27), 2.02 (1 H, m, H-14), 2.49 (1 H, m, H-9), 2.98, 3.34 (each 1 H, m, H-6), 3.36 (3 H, s, OMe), 4.70 (2 H, s, OCH_2O), 5.04 (1 H, m, H-7), 7.43−7.78 (10 H, m, aromatic H). MS m/z (%) 592 (M^+, 2), 530 (100), 473 (33), 419 (4), 216 (74), 202 (91).

*9α-Butyl-1α-(tert-butyldimethylsilyloxy)-25-methoxymethoxy-19-norvitamin D_3 3-(tert-Butyldimethylsilyl) Ether (**29**).* To a solution of phosphine oxide **27** (124.1 mg, 0.209 mmol) in THF (2 mL) at −78 °C were added HMPA (36 μL, 0.209 mmol) and *n*-BuLi (1.58 M hexane solution, 132 μL, 0.209 mmol), and the mixture was stirred for 15 min. To this solution was slowly added ketone **28** (37.5 mg, 0.105 mmol) in THF (1 mL), the mixture was stirred for 1 h at −78 °C, and then the temperature of the reaction was slowly raised to room temperature, and the mixture was stirred at room temperature for 3 h. The reaction was quenched by adding saturated NH_4Cl and extracted with AcOEt, and the organic layer was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (8 g) and eluted with 2% AcOEt/hexane to yield **29** (15.7 mg, 21%, 81% on the basis of recovered **27**) and then eluted with 40% AcOEt/hexane to give starting phosphine oxide **27** (91.3 mg, 74%). **29**: ^{1}H NMR ($CDCl_3$) δ 0.048, 0.053 (each 6H, s, SiMe), 0.54 (3 H, s, H-18), 0.865, 0.87 (each 9H, s, $\underline{t\text{-Bu}}$Si overlapped with $(CH)_2\underline{CH_3}$), 0.92 (3 H, d, J = 6.3 Hz, H-21), 1.21 (6 H, s, H-26,27), 2.83 (1 H, m),3.37 (3 H, s, OMe), 4.71 (2 H, s, OCH_2O), 4.07 (2 H, m, H-1,3), 5.83 (1 H, d, J = 11.2 Hz, H-7), 6.14 (1 H, d, J = 11.2 Hz, H-6).

*9α-Butyl-1α,25-dihydroxy-19-norvitamin D_3 (**4a**).* To a solution of **29** (28.5 mg, 0.039 mmol) in MeOH (1 mL) was added CSA (54.2 mg, 0.233 mmol), and the mixture was stirred for 1.5 h at room temperature. A 5% $NaHCO_3$ solution was added to the reaction, the solution was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (4 g) and eluted with 70% AcOEt/hexane to give **4a** (17.2 mg, 96%). Since **4a** contains a minor product, it was purified by HPLC (YMC-Pack ODS-AM SH-342-5, 15% H_2O/MeOH, 8 mL/min) to yield **4a** (11.8 mg) and its geometrical isomer **4a′** (1.0 mg) at the 7-position. **4a**: ^{1}H NMR ($CDCl_3$) δ 0.54 (3 H, s, H-18), 0.87 (3 H, t, 7.2 Hz, $(CH_2)_3\underline{CH_3}$), 0.93 (3 H, d, J = 6.4 Hz, H-21),1.22 (6 H, s, H-26,27), 2.82 (1 H, m, H-9), 4.07 (2 H, m, H-1,3), 5.87 (1 H, d, J = 11.2 Hz, H-7), 6.31 (1 H, d, J = 11.2 Hz, H-6). UV (EtOH) λ_{max} (ε) 244 (27,000), 252 (31,000) and 261 nm (21,000). MS z/e (%) 460 (M^+, 21), 442 (100), 424 (36), 406 (15), 331 (31), 313 (32), 295 (21). **4a′** [(7Z)-9α-Butyl-1α,25-dihydroxy-19-norvita-

dx.doi.org/10.1021/jm400537h | J. Med. Chem. 2013, 56, 6745−6760

min D_3]: ^{1}HNMR ($CDCl_3$) δ 0.64 (3 H, s, H-18), 0.87 (3 H, t, J = 7.2 Hz, $(CH_2)_3\underline{CH_3}$), 0.94 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26,27), 4.07 (2 H, m, H-1,3), 6.12 (1 H, d, J = 11.5 Hz, H-7), 6.46 (1 H, d, J = 11.5 Hz, H-6). UV (EtOH) λ_{max} 244, 252, and 261 nm. MS z/e (%) 460 (M^+, 18), 442 (100), 424 (33), 406 (17), 331 (36), 313 (33), 295 (22).

*5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-one (**24b**).* To a solution of iPr$_2$NH (706 μL, 5.04 mmol) in THF (10 mL) at −20 °C was added n-BuLi (2.8 mL, 4.37 mmol, 1.58 M hexane), and the mixture was stirred for 15 min. Ketone **5** (1.09 g, 3.36 mmol) in THF (5 mL) was added to the LDA solution at −78 °C, the mixture was stirred for 1 h at that temperature, and then TMSCl (436 μL, 5.04 mmol) and Et_3N (702 μL, 5.04 mmol) were added. The temperature of the reaction was raised to −20 °C for 1.5 h. The solvent was evaporated, the residue was dissolved in hexane, and the mixture was passed through Celite to give **23** (1.34 g). To a solution of silyl enol ether **23** (1.3 g, 3.36 mmol) in THF (5 mL) at 0 °C was added a solution of MeLi (1.2 M Et_2O, 3.4 mL, 4.06 mmol), and the mixture was stirred for 1 h. This mixture was added to a solution of allyl iodide (631 μL, 6.77 mmol) in THF (2 mL) at −78 °C. The mixture was stirred for 1 h while raising the temperature to −45 °C. NH_4Cl solution was added to the reaction, and the mixture was extracted with AcOEt, the extract was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was chromatographed on silica gel (30 g) with 8−10% AcOEt/hexane to give **24b** (887.7 mg, 73%, 2 steps). **24b** ^{1}H NMR ($CDCl_3$) δ 0.66 (3 H, s, H-18), 0.96 (3 H, d, J = 6.2 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.57 (1 H, dd, J = 11.5, 7.4 Hz, H-14), 3.37 (3 H, s, OCH_3), 4.70 (2 H, s, OCH_2O), 5.09 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.68 (1 H, m, $CH_2\underline{CH}{=}CH_2$). MS m/z (%) 364 (M^+, 6), 323 (8), 302 (76), 261 (17), 219 (68), 191 (25), 55 (100).

*5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-4-vinyl-octahydro-inden-4-ol (**25b**).* To a solution of **24b** (887.7 mg, 2.4 mmol) in THF (7 mL) at 0 °C was added a solution of vinyl magnesium bromide (1 M THF solution, 4.87 mL, 4.87 mmol), and the mixture was stirred for 2 h. The reaction mixture was treated as described above for the synthesis of **25a**. The residue was chromatographed on silica gel (10 g) and eluted with 10% AcOEt/hexane to give **25b** (759.1 mg, 79%). **25b**: ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, d, J = 6.5 Hz, H-21), 0.97 (3 H, s, H-18), 1.21(6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 4.71 (2 H, s, OCH_2O), 4.99 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.05 (1 H, dd, J = 10.8, 1.5 Hz, H-6), 5.25 (1 H, dd, J = 17.2, 1.5 Hz, H-6), 5.69 (1 H, m, $CH_2\underline{CH}{=}CH_2$), 5.91 (1 H, dd, J = 17.2, 10.8 Hz, H-7). MS m/z (%) 392 (no M^+), 330 (53), 312 (50), 297 (14), 271 (20), 247 (87), 219 (20), 201 (49), 55 (100).

*[5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-acetaldehyde (**26c**).* To a solution of **25b** (759.1 mg, 1.93 mmol) in CH_2Cl_2 (30 mL) were added PCC (1.67 g, 7.73 mmol) and Celite (1.7 g), and the mixture was stirred at room temperature for 23 h. The reaction mixture was chromatographed on silica gel (20 g) and eluted with 2% AcOEt/hexane to give aldehyde **26c** (669.3 mg, 89%). **26c**: ^{1}H NMR ($CDCl_3$) δ 0.62 (3 H, s, H-18), 0.95 (3 H, d, J = 5.7 Hz, H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 3.47 (1 H, m, H-9), 4.71 (2 H, s, OCH_2O), 5.03 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.68 (1 H, m, $CH_2\underline{CH}{=}CH_2$), 5.76 (1 H, dd, J = 8.3, 1.5 Hz, H-7), 10.01 (1 H, d, J = 8.2 Hz, CHO). MS m/z (%) 390 (M^+, 6), 328 (71), 287 (52), 217 (28), 215 (100).

*2-[5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-ethanol (**26d**).* $NaBH_4$ (63.9 mg, 1.69 mmol) was added to a solution of aldehyde **26c** (660.1 mg, 1.69 mmol) in EtOH (10 mL) at 0 °C, and the mixture was stirred for 1 h. The mixture was treated as described above for the synthesis of **26b**. Chromatography of the product on silica gel (20 g) and elution with 20−25% AcOEt/hexane gave alcohol **26d** (400.6 mg, 61%). **26d**: ^{1}H NMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.93 (3 H, d, J = 6.4 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.73 (1 H, dd, J = 13.9, 7.1 Hz, H-9), 3.37 (3 H, s, OCH_3), 4.14 (2 H, d, J = 6.8 Hz, H-6), 4.71 (2 H, s, OCH_2O), 4.97 (2 H, m, $CH_2CH{=}\underline{CH_2}$), 5.28 (1 H, dd, J = 7.1, 1.6 Hz, H-7), 5.70 (1 H, m, $CH_2\underline{CH}{=}CH_2$). MS m/z (%) 392 (no M^+), 330 (100), 312 (53), 299 (64), 289 (19), 271 (41), 245 (47), 219 (32), 217 (49), 201 (72).

*2-{2-[5-Allyl-1-(5-methoxymethoxy-1,5-dimethyl-hexyl)-7a-methyl-octahydro-inden-4-ylidene]-ethanesulfonyl}-benzothiazole (**30**).* To a solution of alcohol **26d** (108.8 mg, 0.277 mmol) in CH_2Cl_2 (2 mL) were added Ph_3P (109 mg, 0.416 mmol), 2-mercaptobenzothiazole (69.5 mg, 0.416 mmol), and DIAD (57.4 μL, 0.277 mmol), and the mixture was stirred at 1 h. The solvent was evaporated, and the residue was dissolved in EtOH (2.5 mL) and cooled to 0 °C. To this solution were added 30% H_2O_2 (300 μL) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (68.5 mg, 0.055 mmol), and the mixture was stirred for 1 h at room temperature. The reaction was quenched with 2 N Na_2SO_3 solution, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 7% AcOEt/hexane to give **30** (158 mg, 97%). **30**: ^{1}H NMR ($CDCl_3$) δ: 0.17 (3 H, s, H-18), 0.84 (3 H, d, J = 6.1 Hz, H-21), 1.20 (6 H, s, H-26, 27), 2.70 (1 H, m, H-9), 3.36 (3 H, s, OCH_3), 4.06 (1 H, ddd, J = 14.4, 5.9, 2.0 Hz, H-6), 4.54 (1 H, dd, J = 14.4, 9.8 Hz, H-6), 4.69 (2 H, s, OCH_2O), 4.94 (2 H, m, $CH{=}\underline{CH_2}$), 5.06 (1 H, m, H-7), 5.64 (1 H, m, $\underline{CH}{=}CH_2$), 7.61, 8.00, 8.21 (2 H, 1 H, 1 H, m, arom-H).

*9α-Allyl-1α-(tert-butyldimethylsilyloxy)-2-[2-(tert-butyldimethyl-silyloxy)-ethylidene]-25-methoxymethoxy-19-norvitamin D_3 3-(tert-Butyldimethylsily) Ether (**32**).* A solution of LHMDS (1.0 M THF, 230 μL, 0.23 mmol) was added to a solution of sulphone **30** (158.0 mg, 0.269 mmol) in THF (1.5 mL) at −78 °C, the solution was stirred for 30 min, and to this solution, a solution of A-ring ketone **31** (69.3 mg, 0.155 mmol) in THF (1 mL) was added. The mixture was stirred for 1 h at −78 °C, the temperature was raised to 0 °C, and the mixture was stirred at that temperature for 1 h. Saturated NH_4Cl solution was added to the reaction, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g) and eluted with 3% AcOEt/hexane to give **32** (112.4 mg, 83% on the basis of recovered **30**) as a mixture of E- and Z-isomers (5:3) and with 10% AcOEt/hexane sulphone **30** (64.8 mg, 41%). **32a** (E isomer): ^{1}H NMR ($CDCl_3$) δ 0.05−0.09 (18 H, Si-Me × 6), 0.56 (3 H, s, H-18), 0.82−0.94 (30 H, Si-tBu × 3, H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 4.25−4.45 (3 H, m, H-1, $\underline{CH_2}$OTBS), 4.71 (2 H, s, OCH_2O), 4.78 (1 H, m, H-3), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.61 (1 H, m, C=CH), 5.72 (1 H, m, $\underline{CH}{=}CH_2$), 5.89 (1 H, d, J = 11.1 Hz, H-7), 6.12 (1 H, d, J = 11.1 Hz, H-6). **32b** (Z isomer): ^{1}H NMR ($CDCl_3$) δ 0.05−0.09 (18 H, Si-Me × 6), 0.54 (3 H, s, H-18), 0.82−0.94 (30 H, Si-tBu × 3, H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 4.25−4.45 (3 H, m, H-3, $\underline{CH_2}$OTBS), 4.71 (2 H, s, OCH_2O), 4.78 (1 H, m, H-1), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.61 (1 H, m, C=CH), 5.72 (1 H, m, $\underline{CH}{=}CH_2$), 5.83 (1 H, d, J = 10.4 Hz, H-7), 6.22 (1 H, d, J = 10.4 Hz, H-6). MS m/z (%) 872 (M^+, 3), 740 (68), 678 (100), 621 (8).

*9α-Allyl-1α,25-dihydroxy-2-(2-hydroxyethylidene)-19-norvitamin D_3 (**4b and 4c**).* CSA (77 mg, 0.332 mmol) was added to a solution of **32** (48.0 mg, 55 μmol) in MeOH (1 mL), and the mixture was stirred for 1.5 h at room temperature. A 5% $NaHCO_3$ solution was added to the reaction, the mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 2% MeOH/AcOEt to give a mixture of **4b** and **4c** (E- and Z-isomers at the position 2) (24.2 mg, 90%). The mixture was separated by HPLC [YMC-Pack ODS-AM SH-342-5, 20% H_2O/MeOH, 8 mL/min] to give **4b** (E-isomer) (14.15 mg, RT 34.80) and **4c** (Z-isomer) (8.49 mg, RT 37.10). **4b**: ^{1}H NMR ($CDCl_3$) δ: 0.55 (3 H, s, H-18), 0.94 (3 H, d, J = 6.2 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.42 (2 H, m, H-4), 2.92 (1 H, m, H-9), 3.13 (1 H, dd, J = 13.0, 4.8 Hz, H-10), 4.15 (1 H, dd, J = 12.5, 5.8 Hz, $\underline{CH_2}$OH), 4.37 (1 H, dd, J = 12.5, 8.1 Hz, $\underline{CH_2}$OH), 4.40 (1 H, m, H-1), 4.82 (1 H, m, H-3), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.73 (1 H, m, $\underline{CH}{=}CH_2$), 5.80 (1 H, m, C=CH), 5.90 (1 H, d, J = 11.1 Hz, H-7), 6.26 (1 H, d, J = 11.1 Hz, H-6). UV λ_{max} (EtOH) 247, 255, and 264 nm. **4c**: ^{1}H NMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.94 (3 H, d, J = 6.2 Hz, H-21), 1.22 (6 H, s, H-26, 27), 4.19 (1 H, dd, J = 12.5, 6.0 Hz, $\underline{CH_2}$OH), 4.37 (1 H, dd, J = 12.5, 7.9 Hz, $\underline{CH_2}$OH), 4.45 (1 H, m, H-3), 4.87 (1 H, m, H-1), 4.97 (2 H, m, $CH{=}\underline{CH_2}$), 5.72 (1 H, m,

dx.doi.org/10.1021/jm400537h | J. Med. Chem. 2013, 56, 6745−6760

CH=CH_2), 5.81 (1 H, m, C=CH), 5.87 (1 H, d, J = 11.2 Hz, H-7), 6.37 (1 H, d, J = 11.2 Hz, H-6). MS m/z (%): 486 (M^+, 17), 468 (17), 450 (30), 432 (61), 391 (100). UV λ_{max} (EtOH) 247, 255, and 264 nm.

1α-(tert-Butyldimethylsilyloxy)-2-[2-(tert-butyldimethylsilyloxy)-ethylidene]-9α-(3-hydroxypropyl)-25-methoxymethoxy-19-norvitamin D_3 3-(tert-Butyldimethylsily) Ether (33). To a solution of **32** (57.7 mg, 0.0661 mmol, *E*:*Z*, 5:3) in THF (300 μL) was added 9-borabicyclo[3.3.1]nonane (9-BBN) (1.32 mL, 0.661 mmol, 10.0 equiv) at room temperature, and the mixture was stirred for 1.5 h. MeOH (220 μL) was added to the reaction, and the mixture was stirred for 15 min. The mixture was cooled to 0 °C, and 6 M NaOH (220.2 μL, 1.32 mmol, 20.0 equiv) and 30% H_2O_2 (220 μL) were added. The mixture was stirred at room temperature for 1 h. To the reaction, 2 N HCl was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (10 g) and eluted with 8% EtOAc/hexane to give **33a** (2*E*-isomer, 13.1 mg, 22%) and with 10% AcOEt/hexane to give **33b** (2*Z*-isomer, 11.1 mg, 19%). **33a**: ^{1}H NMR ($CDCl_3$) δ 0.05–0.08 (18 H, Si-Me × 6), 0.55 (3 H, s, H-18), 0.83, 0.89, 0.93 (each 9 H, s, Si-*t*Bu × 3, overlapped with H-21), 1.22 (6 H, s, H-26, 27), 2.86 (1 H, m, H-9), 3.00 (1 H, dd, J = 12.7, 4.7 Hz, H-10), 3.37 (3 H, s, OCH_3), 3.61 (2 H, m, CH_2OH), 4.25–4.38 (3 H, m, H-1, CH_2OTBS), 4.71 (2 H, s, OCH_2O), 4.78 (1 H, m, H-3), 5.61 (1 H, m, C=CH), 5.93 (1 H, d, J = 11.1 Hz, H-7), 6.11 (1 H, d, J = 11.1 Hz, H-6). MS m/z (%) 890 (M^+, 2), 828 (5), 758 (52), 696 (100), 626 (10), 75 (93). **33b**: ^{1}H NMR ($CDCl_3$) δ 0.06–0.09 (18 H, Si-Me × 6), 0.54 (3 H, s, H-18), 0.82, 0.90, 0.93 (each 9 H, s, Si-*t*Bu × 3, overlapped with H-21), 1.22 (6 H, s, H-26, 27), 3.37 (3 H, s, OCH_3), 3.61 (2 H, m, CH_2OH), 4.30 (2 H, m, CH_2OTBS), 4.44 (1 H, m, H-3), 4.71 (2 H, s, OCH_2O), 4.83 (1 H, m, H-1), 5.61 (1 H, m, C=CH), 5.87 (1 H, d, J = 10.4 Hz, H-7), 6.22 (1 H, d, J = 10.4 Hz, H-6). MS m/z (%) 890 (M^+, 1), 828 (1), 758 (20), 696 (22), 626 (4), 75 (100).

(2E)-1α,25-Dihydroxy-2-(2-hydroxyethylidene)-9α-(3-hydroxypropyl)-19-norvitamin D_3 (4d). To a solution of **33a** (13.1 mg, 0.0147 mmol) in MeOH (300 μL) was added CSA (34.1 mg, 0.147 mmol, 10.0 equiv), and the mixture was stirred for 2 h. Then, 5% $NaHCO_3$ was added to the reaction, the mixture was extracted with AcOEt, and the organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (5 g) and eluted with 5% MeOH/AcOH to give **4d** (5.7 mg, 77%). **4d**: ^{1}H NMR ($CDCl_3$) δ: 0.56 (3 H, s, H-18), 0.94 (3 H, d, J = 6.0 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.17 (1 H, t, J = 8.7 Hz), 2.29, 2.41 (each 1 H, m, H-4), 2.89 (1 H, m, H-9), 3.16 (1 H, m, H-10), 3.48, 3.60 (each 1 H, m, CH_2CH_2OH), 4.09 (1 H, m, CH_2OH), 4.31 (3 H, m, H-1, CH_2OH, OH), 4.40 (1 H, br.s, OH), 4.80 (1 H, m, H-3), 5.74 (1 H, m, C=CH), 5.97 (1 H, d, J = 10.6 Hz, H-7), 6.11 (1 H, d, J = 10.6 Hz, H-6). MS m/z (%): 504 (M^+, 1), 486 (10), 468 (34), 450 (57), 432 (24), 386 (100), 339 (14). UV λ_{max} (EtOH): 246, 254, and 264 nm.

(2Z)-1α,25-Dihydroxy-2-(2-hydroxyethylidene)-9α-(3-hydroxypropyl)-19-norvitamin D_3 (4e). **33b** (2*Z*-isomer, 11.1 mg, 0.0124 mmol) was deprotected similarly to give **4e** (3.4 mg, 54%). **4e**: ^{1}H NMR ($CDCl_3$) δ 0.56 (3 H, s, H-18), 0.93 (3 H, d, J = 6.3 Hz, H-21), 1.22 (6 H, s, H-26, 27), 2.38 (1 H, m, H-10), 2.66 (1 H, dd, J = 13.0, 4.6 Hz, H-4), 2.76 (1 H, dd, J = 14.0, 5.7 Hz, H-10), 2.89 (1 H, m, H-9), 3.61 (2 H, m, CH_2CH_2OH), 4.26 (1 H, dd, J = 12.6, 6.4 Hz, CH_2OH), 4.36 (1 H, dd, J = 12.6, 7.1 Hz, CH_2OH), 4.43 (1 H, m, H-3), 4.84 (1 H, m, H-1), 5.84 (1 H, m, C=CH), 5.91 (1 H, d, J = 11.0 Hz, H-7), 6.37 (1 H, d, J = 11.0 Hz, H-6). MS m/z (%) 504 (M^+, 1), 486 (17), 468 (58), 450 (100), 432 (41), 339 (23). UV λ_{max} (EtOH): 246, 254, and 264 nm.

Graphical Manipulations and Ligand Docking. Graphical manipulations were performed using SYBYL (Tripos, St. Louis).

■ ASSOCIATED CONTENT

Supporting Information

Figures S1–S6 and Tables S1–S3. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

PDB ID codes: 3VT3, 3VT4, 3VT5, 3VT6, 3VT7, 3VT8, and 3VT9.

■ AUTHOR INFORMATION

Corresponding Authors

*(M.N.) Phone: +81 3 3972 8111 ext 2363. E-mail: makoto-n.str@tmd.ac.jp.

*(S.Y.) Phone: +81 3 3972 8111 ext 2363. E-mail: yamada.vd@image.ocn.ne.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank professor Hector F. DeLuca at Department of Biochemistry, University of Wisconsin—Madison, for kindly providing an expression plasmid of the rVDR-LBD. We thank Dr. Harumi Fukada at Graduate School of Life and Environmental Sciences, Osaka Prefecture University, for guiding the CD spectral study. We thank the beamline staff of the KEK-PF's Structural Biology Research Center for helpful suggestions for acquiring X-ray diffraction images.

■ ABBREVIATIONS USED

VDR, vitamin D receptor; HVDRR, hereditary vitamin D-resistant rickets; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; RXR, 9-*cis*-retinoic acid receptor; VDRE, vitamin D-responsive elements; AF-2, activation function 2; DBD, DNA binding domain; LBD, ligand binding domain; LBP, ligand-binding pocket; PPARγ, peroxisome proliferator-activating receptor γ; CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5

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JOURNAL OF LIPID RESEARCH

Crystal structures of complexes of vitamin D receptor ligand-binding domain with lithocholic acid derivatives

Hiroyuki Masuno,[1,*] Teikichi Ikura,[1,†] Daisuke Morizono,[2,*] Isamu Orita,[*] Sachiko Yamada,[§] Masato Shimizu,[*] and Nobutoshi Ito[3,†]

Institute of Biomaterials and Bioengineering,[*] Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan; Medical Research Institute,[†] Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8510, Japan; and School of Medicine,[§] Nihon University, Itabashi-ku, Tokyo 173-8610, Japan

Abstract The secondary bile acid lithocholic acid (LCA) and its derivatives act as selective modulators of the vitamin D receptor (VDR), although their structures fundamentally differ from that of the natural hormone 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. Here, we have determined the crystal structures of the ligand-binding domain of rat VDR (VDR-LBD) in ternary complexes with a synthetic partial peptide of the coactivator MED1 (mediator of RNA polymerase II transcription subunit 1) and four ligands, LCA, 3-keto LCA, LCA acetate, and LCA propionate, with the goal of elucidating their agonistic mechanism. LCA and its derivatives bind to the same ligand-binding pocket (LBP) of VDR-LBD that 1,25$(OH)_2D_3$ binds to, but in the opposite orientation; their A-ring is positioned at the top of the LBP, whereas their acyclic tail is located at the bottom of the LBP. However, most of the hydrophobic and hydrophilic interactions observed in the complex with 1,25$(OH)_2D_3$ are reproduced in the complexes with LCA and its derivatives. Additional interactions between VDR-LBD and the C-3 substituents of the A-ring are also observed in the complexes with LCA and its derivatives. These may result in the observed difference in the potency among the LCA-type ligands.—Masuno, H., T. Ikura, D. Morizono, I. Orita, S. Yamada, M. Shimizu, and N. Ito. **Crystal structures of complexes of vitamin D receptor ligand-binding domain with lithocholic acid derivatives.** *J. Lipid Res.* **2013.** 54: **2206–2213.**

Supplementary key words nuclear receptor • structure-function relationship • bile acid • hypercalcemia

The active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], regulates calcium homeostasis (1). It also promotes cellular differentiation, inhibits cellular proliferation, and suppresses the immune system (2–7). It has been used clinically to treat renal osteodystrophy, vitamin D-dependent rickets type I, and X-linked hypophosphatemic rickets, among other conditions (8–14). Most of its effects are mediated by its specific binding to the vitamin D receptor (VDR), which is a member of nuclear receptor (NR) super family (15). When 1,25$(OH)_2D_3$ is bound to VDR, it activates it by inducing conformational changes. The activated complex, VDR/1,25$(OH)_2D_3$, binds as a heterodimer with the retinoid X receptor (RXR) to vitamin D response elements located in the promoter region of the target genes. Recruitment of coactivator proteins to this heterodimer is also essential to the transactivation. However, clinical use of 1,25$(OH)_2D_3$ is limited because therapeutic doses can give rise to significant hypercalciuria and hypercalcemia (16). A number of synthetic ligands to VDR have been developed for medical use; however, most of them can also cause similar problems because they are derived from 1,25$(OH)_2D_3$.

Several synthetic compounds without the vitamin D_3 scaffold have been reported to bind to VDR and have VDR-modulating activities, including growth inhibition of cancer cells and keratinocytes and induction of leukemic cell differentiation, with less calcium mobilization side effects than 1,25$(OH)_2D_3$ (17). Therefore, these synthetic compounds are expected to be therapeutics for cancer, leukemia, and psoriasis. Subsequently, Makishima et al. discovered that secondary bile acids, including lithocholic acid (LCA) and its derivatives, also behaved as VDR agonists (18–20). LCA acts as a detergent to stabilize fats for absorption, and it has been implicated in human and experimental animal carcinogenesis. However, the agonistic behavior of LCA as a ligand recognized by VDR was not common knowledge because the structure of LCA is completely different from that of vitamin D_3. Additional studies showed that VDR had dual functions as a metabolic sensor of bile acids and as an endocrine receptor for 1,25$(OH)_2D_3$.

Abbreviations: AcOEt, ethyl acetate; AF2, activation function 2; LBD, ligand-binding domain; LBP, ligand-binding pocket; LCA, lithocholic acid; MED1, mediator of RNA polymerase II transcription subunit 1; NR, nuclear receptor; 1,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; RMSD, root-mean-square deviation; RXR, retinoid X receptor; VDR, vitamin D receptor.

[1] H. Masuno and T. Ikura contributed equally to this work.

[2] Present address of D. Morizono: Teva API Japan LTD, Minato-ku, Tokyo 105-0001, Japan.

[3] To whom correspondence should be addressed.
e-mail: ito.str@tmd.ac.jp

Manuscript received 31 March 2013 and in revised form 9 May 2013.

Published, JLR Papers in Press, May 17, 2013
DOI 10.1194/jlr.M038307

Both functions are closely related to colon cancer suppression (21). Although theoretical studies were performed to elucidate how LCA binds to $1,25(OH)_2D_3$, the agonistic mechanism of VDR by LCA was still unclear (18, 22).

Here we determined the crystal structures of the ligand binding domain (LBD) of rat VDR in ternary complexes with a synthetic peptide containing the target sequence of the coactivator MED1 (mediator of RNA polymerase II transcription subunit 1, also known as ARC205 and DRIP205) and the ligands LCA, 3-keto LCA, LCA acetate, and LCA propionate (**Fig. 1**) to investigate how LCA and its derivatives bind to VDR and how they act as the agonists. The structures reveal that LCA and its derivatives bind to the same ligand-binding pocket (LBP) of VDR that $1,25(OH)_2D_3$ binds to (23–31), but in the opposite orientation. Comparison of these structures also show how LCA and its derivatives mimic $1,25(OH)_2D_3$ and give insight into how the C-3 substituents on the A-ring affect the activity of each ligand. The structures also provide a sound basis for designing new compounds using the scaffold of LCA.

MATERIALS AND METHODS

Preparation of LCA ligands

LCA, 3-keto LCA, and LCA acetate were commercially obtained. LCA propionate was synthesized as follows. Boron trifluoride diethyl ether complex (44 μl, 0.33 mmol) was added to a stirred solution of LCA (504.5 mg, 1.34 mmol) in a mixture of propionic anhydride (1.67 ml) and tetrahydrofuran (5 ml) at 0°C, and the resulting solution was stirred at room temperature for 14.5 h. Aqueous sodium hydrogen carbonate was added to this solution, and the reaction mixture was stirred at room temperature for 1 h. Crude LCA propionate was extracted from the reaction mixture with ethyl acetate (AcOEt). The organic layer containing LCA propionate was washed with brine and dried over anhydrous magnesium sulfate, and the solvents were evaporated under house vacuum. The residue was purified by chromatography on silica gel (15 g) with 10% AcOEt/hexane to yield 460 mg (79%) of LCA propionate. The product was recrystallized on AcOEt/hexane.

Protein expression, purification, and crystallization

Rat VDR-LBD (residues 116–423, Δ165–211) was expressed, purified, and crystallized as described by Vanhooke et al. and Nakabayashi et al. (31, 32). The purity and homogeneity of the protein was assessed by SDS-PAGE. The concentration of the protein was determined by UV absorption at 280 nm with molar extinction coefficients estimated using the method developed by Pace et al. (33). The 13mer synthetic oligopeptide (KNHPMLMNLLKDN), which corresponds to residues 625–637 of rat MED1, was purchased from World Gene Co., Ltd. (Tokyo, Japan). Each of the four ligands, LCA, 3-keto LCA, LCA acetate, and LCA propionate, was used to prepare a ternary complex of VDR-LBD/peptide/ligand in 10 mM Tris·HCl (pH 7.0), 10 mM dithiothreitol, and 0.02% sodium azide. All the ternary complexes were crystallized at 20°C in a series of precipitant solutions containing 0.1–0.4 M sodium formate, 12–22% (w/v) polyethylene glycol 4000, and 0–10% (v/v) ethylene glycol.

X-ray diffraction data collection and structural analysis

The crystals were flash-frozen using mother liquor supplemented with 10% ethylene glycol. Diffraction data for the ternary complexes were collected at 95 K at beamline BL-6A at the Photon Factory of the High Energy Accelerator Research Organization and were integrated and scaled with HKL2000 (HKL Research, Inc.). The space group for each complex is *C*2; the unit cell dimensions are listed in **Table 1** with one complex per asymmetrical unit. The structures were solved by molecular replacement by using the crystal structure of the ternary complex reported by Vanhooke et al. (PDB code: 1RK3) as the search model in CNS (31, 34). Refinement was performed with CNS and XtalView (35).

RESULTS

Overall structures of the ternary complexes

The crystal structures of the ternary complexes of VDR-LBD with LCA and its three derivatives were determined at 1.9–2.2 Å resolution by X-ray crystallography (Table 1 and **Fig. 2A**). Most of the residues in the complexes were unambiguously determined; however, the N-terminal region (Ala116–Gln122), the middle region (Asp160–Gly164 and Ser212–Leu217), and the C-terminal end (Ser423) of

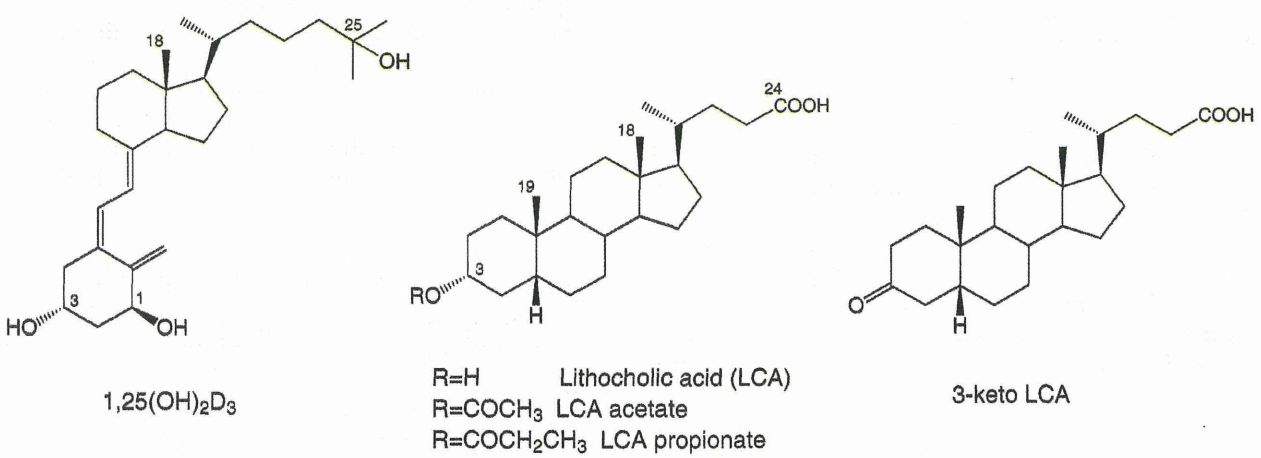

Fig. 1. Chemical structures of $1,25(OH)_2D_3$, LCA, and LCA derivatives.

TABLE 1. Data collection and refinement statistics

Ligands	LCA	LCA Acetate	LCA Propionate	3-keto LCA
Data collection				
Unit cell dimensions				
a (Å)	154.5	154.4	154.5	153.8
b (Å)	42.4	42.0	42.8	42.3
c (Å)	41.6	41.5	41.6	41.5
β (degree)	96.8	96.2	96.3	96.4
Resolution (Å)	50–1.9 (1.96–1.90)	50–2.2 (2.28–2.20)	50–2.2 (2.28–2.20)	50–1.9 (1.96–1.90)
Completeness (%)	99.2 (99.9)	99.3 (95.9)	99.6 (96.8)	99.2 (99.8)
Redundancy	3.6 (3.6)	4.3 (4.0)	3.6 (3.4)	3.6 (3.5)
$I/\sigma(I)$	43.1 (6.2)	26.2 (4.4)	30.3 (3.9)	41.3 (4.7)
R_{sym} (%)	3.2 (26.4)	5.8 (29.5)	4.5 (35.7)	4.4 (32.9)
Refinement				
R (%)	23.3	22.0	21.1	23.4
R-free (%)	26.3	27.6	26.5	27.9
RMSD bond lengths (Å)	0.0058	0.0068	0.0069	0.0065
RMSD bond angles (degree)	1.25	1.19	1.18	1.22
Number of atoms				
Protein	1925	1942	1942	1926
Peptide	92	92	92	92
Ligand	27	30	31	27
Water	108	49	56	110
Average B factor ($Å^2$)	41.0	50.1	49.9	43.8

VDR-LBD, and Asp636 and Asn637 of the MED1 peptide were not detected, probably due to fluctuation in these regions. Two more residues at the C-terminal end (Glu421 and Ile422) were also undetectable in the complexes with LCA and 3-keto LCA. Most of these missing residues were previously reported as invisible in studies of other ternary complexes of VDR and are likely a characteristic common to crystals of VDR complexes (23–31).

The overall structures of VDR-LBD in the four complexes are nearly identical (Fig. 2B). The root-mean-square deviations (RMSD) between the proteins in the LCA complex and each of the 3-keto LCA, LCA acetate, and LCA propionate complexes are 0.34, 0.52, and 0.52 Å, respectively, using the Cα atoms of Lys123–Met159 and Ser218–Asn420. Furthermore, the RMSD between the proteins in the LCA and $1,25(OH)_2D_3$ complexes is 0.49 Å for the overall structure (Fig. 2C). Therefore, no significant structural differences were found among the proteins in the ternary complexes with LCA, its derivatives, or $1,25(OH)_2D_3$ (28, 31).

Structures of the ligands and their interactions with VDR-LDB

Proteins in the NR super family have a common ligand-binding pocket (LBP). Residues in helices 1, 3, 5, 11, and 12, all β-turns, and loops 6–7 and 11–12 form the framework for the LBP of VDR. The natural hormone $1,25(OH)_2D_3$ is accommodated in the LBP. In the present study, we observed clear electron density in the LBP, as was previously reported in the complex with $1,25(OH)_2D_3$ (**Fig. 3A**) (28), and crystallographic refinement allowed us unambiguous determination of the structure of LCA and its derivatives in the complex (Figs. 2A and 3A).

Except for their respective substituents, LCA and its three derivatives are accommodated in the LBP of VDR-LBD with almost identical structures. However, their orientation

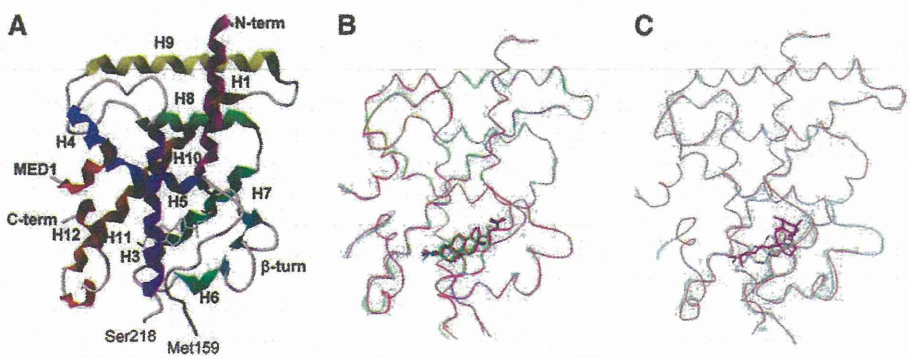

Fig. 2. Overall structures of ternary complexes of VDR with LCA derivatives. A: Complex structure of VDR with LCA. VDR and MED1 are represented by ribbons, and LCA is represented by a stick. The helices of VDR are numbered after that of the human RXR. B: Superposition of Cα traces of the four ternary complexes of VDR with LCA derivatives. Complexes with LCA, 3-keto LCA, LCA acetate, and LCA propionate are represented in red, green, cyan, and magenta, respectively. C: Superposition of Cα trace of ternary complexes of VDR with LCA (cyan) and $1,25(OH)_2D_3$ (red) complex.

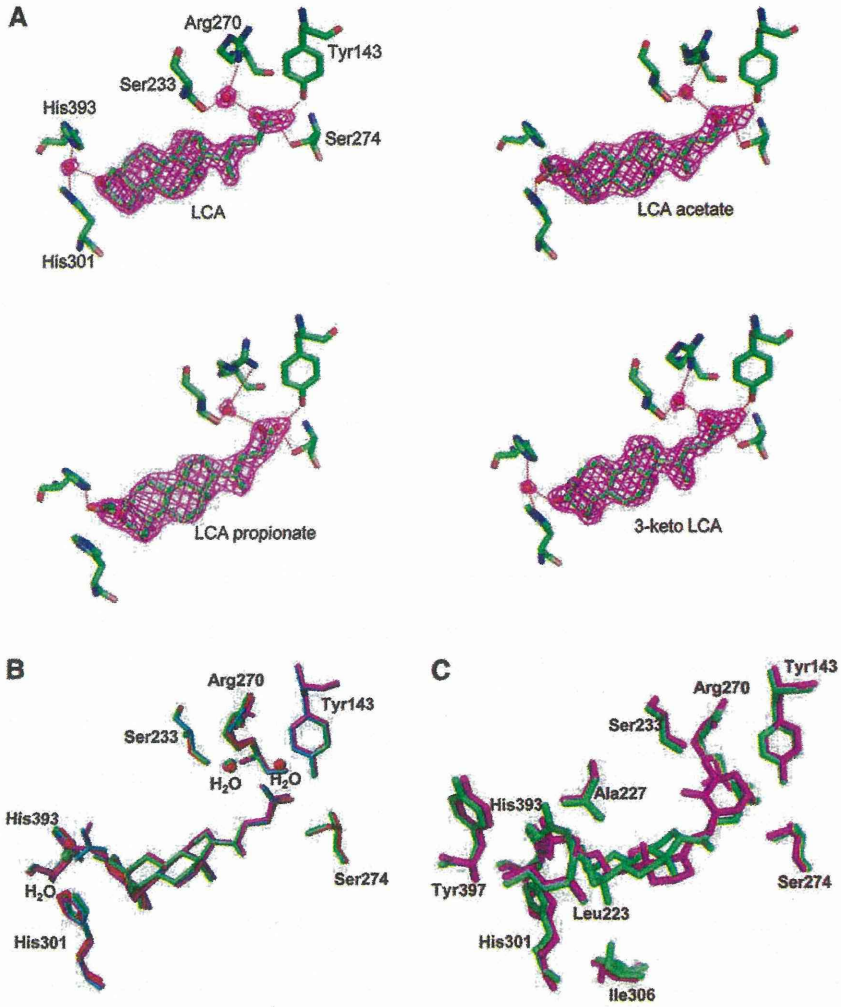

Fig. 3. A: Hydrogen-bonding network between VDR and LCA derivatives, LCA, 3-keto LCA, LCA acetate, and LCA propionate. The purple fishnet is the F_o-F_c omit-annealed difference Fourier electron-density map contoured at 3σ level from the average of the map. The oxygen atoms of water are represented by red spheres. B: Superposition of the four LCA derivatives in VDR complexes. LCA, 3-keto LCA, LCA acetate, and LCA propionate are represented in red, green, cyan, and magenta, respectively. C: Superposition of LCA acetate (green) and $1,25(OH)_2D_3$ (magenta) in VDR complexes.

is opposite to that of $1,25(OH)_2D_3$ in both the horizontal and vertical planes (Fig. 3C). The 24-carboxyl group is directed toward the β-turns, and the β-face of the steroid is directed toward helix 7 in the bottom of the LBP, while the A-ring faces helix 12 (Fig. 2A). LCA forms three hydrogen bonds at the carboxyl group. One oxygen atom of the carboxyl group directly forms hydrogen bonds with the hydroxyl groups in the side chains of Tyr143 in helix 1 (the distance between the oxygen atoms of the carboxyl group and the hydroxyl group is 2.46 Å) and Ser274 in helices 4/5 (the distance between the oxygen atoms of the carboxyl group and the hydroxyl group is 2.76 Å). The other oxygen atom of the same carboxyl group interacts via a water molecule (the distance between the oxygen atoms of the water and the carboxyl group is 2.69 Å), with the hydroxyl group in the side chain of Ser233 in helix 3 and the guanidinium group in the side chain of Arg270 in helix 4/5 (the distance between the water molecule and these residues are 2.92 Å and 3.01 Å, respectively) (Fig. 3A). These hydrogen bonds are also observed in the other three complexes. The four rings of the steroid in each of the complexes interact with hydrophobic residues in the LBP through hydrophobic interactions. There are 12 residues (Leu226, Leu229, Val230, Ile264, Ile276, Met268, Trp282, Val296, Ala299, Leu305, Ile306, and Leu309) distributed within 4.3 Å from the rings. Such hydrophobic interactions are also conserved in the other complexes.

Hydrogen bonds between VDR and the ligands are also observed at the other end of the ligands, the C-3 position of the A-ring. The four ligands differ in their substituents at this position. The hydroxyl group of LCA, the carbonyl group of 3-keto LCA, the propionyl group of LCA propionate, and the acetyl group of LCA acetate interact with residues in helix 6, loop 6–7, and helix 11 (Figs. 2A and 3A). In the

complexes with LCA and 3-keto LCA, the oxygen atoms of the respective hydroxyl and carbonyl groups of the substituents interact via a water-mediated hydrogen bond with the nitrogen atoms of imidazole rings of His301 in helix 6 and His393 in helix 11. In contrast, in the complex with LCA acetate, the oxygen atom of the acetyl group directly forms a hydrogen bond with the nitrogen atom of the imidazole ring of His301. In the complex with LCA propionate, the oxygen atom of the propionyl group also directly forms a hydrogen bond with the nitrogen atom of the imidazole ring of His393. Furthermore, the alkyl parts of the two substituents interact with the aromatic rings of Tyr397 in helix 11 and Phe418 in helix 12 and with the side chains of Leu410 and Val414 in helix 12, stabilizing the binding of the two derivatives to the LBP of VDR-LBD (Fig. 3A). From this viewpoint, LCA propionate may be the most effective of the four ligands because it has the longest alkyl part in the substituent.

Structure of the MED1 peptide

NR super family proteins generally include two domains for two types of transactivations, a constitutive activation function (AF-1) and a ligand-dependent activation function (AF-2). The AF-2 domain of the VDR-LBD consists of helices 3, 4, and 12, and loop 3–4, and it interacts with the LXXLL motif known as the NR-box. In the present study, we synthesized a peptide containing the target sequence of the MED1 coactivator and determined the structure of the peptide in the four ternary complexes to investigate whether or not LCA and its derivatives affect coactivator binding. The peptide binds to the AF-2 domain in each of the four complexes similar to the complex with $1,25(OH)_2D_3$. Although two residues of the peptide, Asp636 and Asn637, have no detectable structure as described above, the rest of the peptide forms an α-helix with a kink at Pro628 in each complex (**Fig. 4A**). The structure of the peptide in the four complexes is nearly identical. The RMSDs between the peptides in the LCA complex and the 3-keto LCA, LCA acetate, and LCA propionate complexes are 0.22, 0.58, and 0.69 Å, respectively, using the Cα atoms of Lys625–Lys635. The structure of the peptide in the LCA complex was also compared with that in the $1,25(OH)_2D_3$ complex. The RMSD between the two peptides was calculated at 0.76 Å, indicating no significant structural differences among the peptides in the ternary complexes with LCA, its derivatives, and $1,25(OH)_2D_3$ (Fig. 4B) (28, 31).

The AF-2 domain forms a shallow pit consisting of five hydrophobic residues: Ile238 in helix 3, Ile256 in helix 4/5, Leu259 in helix 4/5, Leu413 in helix 12, and Val417 in helix 12. The peptide binds to this pit through the hydrophobic interactions between the LXXLL motif of the peptide and the complementary pit of the protein. The polar side chains of Lys242 in helix 3 and Glu416 in helix 12 also facilitate the binding of the peptide by clamping it on the both edges of the AF-2 domain (a charge clamping). Two hydrogen bonds are formed between the oxygen atom of the side chain carboxyl group of Glu416 and the amide nitrogen of Met629, and the nitrogen atom of the side chain amino group of Lys242 and the carbonyl oxygen of Leu633. All these interactions observed in the present study are the same as those seen in the complex with $1,25(OH)_2D_3$. Therefore, these results indicate that the interactions between the coactivator MED1 and the AF-2 domain are well conserved in the ternary complexes with LCA and its derivatives.

DISCUSSION

Since the discovery of its function as an agonist of VDR, LCA has been expected to be used a vitamin D alternative, especially because LCA appears to activate VDR without causing hypercalcemia. However, because the functional mechanism of LCA was still unclear, LCA derivatives with higher activities have been found mainly by trial and error. In the present study, we determined the structures of ternary complexes of VDR-LBD with LCA and its derivatives and elucidated how they bind to VRD-LDB.

LCA and its derivatives bind to the same LBP that $1,25(OH)_2D_3$ binds to. However, their orientation is opposite to that of $1,25(OH)_2D_3$ (Fig. 3C). Its A-ring was set on the inlet of the LBP, while its 24-carboxyl group wedged

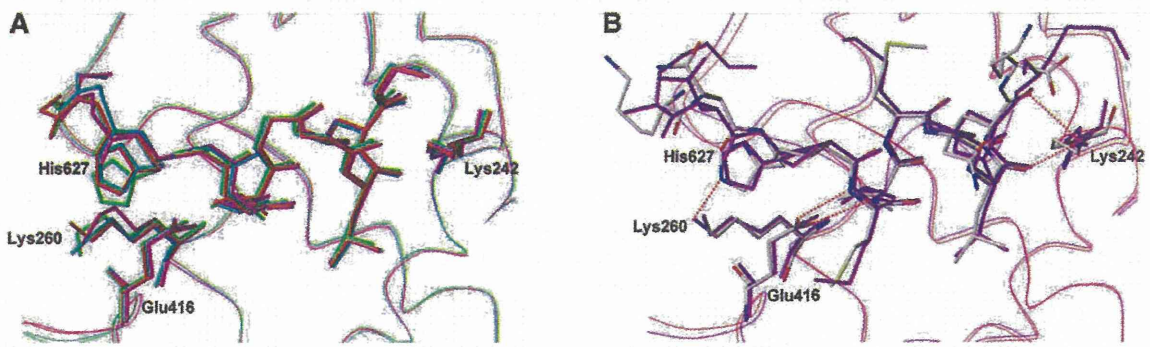

Fig. 4. Structures of MED1 peptides in VDR complexes. MED1 peptide and Lys242 and Glu416 of each VDR complex are represented by sticks; the other parts are represented by ribbons. A: Superposition of MED1 peptides in VDR complexes with LCA (red), 3-keto LCA (green), LCA acetate (cyan), and LCA propionate (magenta). B: Superposition of MED1 peptides in VDR complexes with LCA (magenta) and $1,25(OH)_2D_3$ (white carbons).