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# Synthesis and Biological Activity of 2-Methylene Analogues of Calcitriol and Related Compounds

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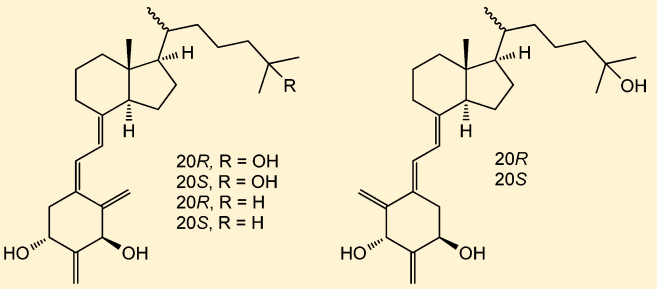
Supporting Information

**ABSTRACT:** In an attempt to prepare vitamin D analogues that are superagonists, (20*R*)- and (20*S*)-isomers of 1$\alpha$-hydroxy-2-methylenevitamin $D_3$ and 1$\alpha$,25-dihydroxy-2-methylenevitamin $D_3$ have been synthesized. To prepare the desired A-ring dienyne fragment, two different approaches were used, both starting from the (−)-quinic acid. The obtained derivative was subsequently coupled with the C,D-ring enol triflates derived from the corresponding Grundmann ketones, using the Sonogashira reaction. Moreover, (20*R*)- and (20*S*)-1$\alpha$,25-dihydroxy-2-methylenevitamin $D_3$ compounds with an (5*E*)-configuration were prepared by iodine catalyzed isomerization. All four 2-methylene analogues of the native hormone were characterized by high in vitro activity. As expected, the 25-desoxy analogues were much less potent. Among the synthesized compounds, two of them, 1$\alpha$,25-dihydroxy-2-methylenevitamin $D_3$ and its C-20 epimer, were found to be almost as active as 2-methylene-19-nor-(20*S*)-1$\alpha$,25-dihydroxyvitamin $D_3$ (2MD) on bone but more active in intestine.




## INTRODUCTION

The most active metabolite of vitamin $D_3$, 1$\alpha$,25-dihydroxyvitamin $D_3$ [calcitriol, 1$\alpha$,25-$(OH)_2D_3$, **1**, Figure 1) plays a crucial role in calcium and phosphate homeostasis.[1] In addition, the physiological role of calcitriol in living organisms is much broader than previously thought and includes the regulation of cellular growth, cell differentiation, and immunomodulation.[2] This has resulted in a search for analogues that target these roles with reduced calcemic activity.[3]

By moving the exomethylene substituent of calcitriol from C-10 to C-2 (compound **2**) there is a marked increase in bone calcium mobilization activity.[4] This effect was even more pronounced when the configuration was changed from 20*R* to 20*S*. Further, this analogue also greatly increased bone formation in vitro[5] as well as in the ovariectomized (OVX) rat model.[6] Moreover in a clinical trial, compound **3** increased bone turnover in postmenopausal women.[7] These findings stimulated synthesis of several other 2-methylene-19-norvitamin D analogues acting as possible agents for the treatment of osteoporosis.[8] To explore the surprising high biological activity of 2MD, 1-desoxy[9] and 3-desoxy analogues of 2MD were synthesized.[10] We also prepared the (20*R*)- and (20*S*)-25-hydroxy-2-methylenevitamin $D_3$ compounds **4** and **5**[11] and the (20*R*) and (20*S*) isomers of 3-desoxy-1$\alpha$,25-dihydroxy-2-methylenevitamin $D_3$ (**6** and **7**).[12] Biological activities of these analogues were compared with previously obtained 2-methylene-substituted vitamin D compounds. Compounds **4**−**7**, possessing both exomethylene moieties at C-2 and C-10, were characterized by pronounced in vivo calcemic activity. However, the lack of the 3$\beta$- and especially the 1$\alpha$-hydroxyl groups caused a marked decrease in the ability to bind the vitamin D receptor (VDR). The 1$\alpha$-hydroxylation of 2,10-dimethylene compounds **4** and **5** occurs at a rate much slower than that of 25-hydroxyvitamin $D_3$. Thus, it is suggested that the next targets in our structure−activity studies should include compounds that possess both the A-ring hydroxyls (1$\alpha$ and 3$\beta$) and both exomethylene substituents at C-2 and C-10. This paper reports the preparation and testing of these compounds, **8**−**13**.[13]

## RESULTS AND DISCUSSION

**Chemistry.** Since the bicyclic vinyl triflates **15**−**17** were known compounds and **18** could be easily prepared from the respective Grundmann ketone by the same method, we concentrated our efforts on the synthesis of the required A-ring dienyne **14**. Two alternative synthetic routes have been elaborated, both starting from the commercially available (−)-(1*S*,3*R*,4*S*,5*R*)-quinic acid (**19**).

First synthetic approach began with the silylation of the secondary hydroxyl in the ester **20** (Scheme 1) easily accessible from **19**.[14] Treatment of the obtained hydroxy ester **21** with sulfuryl chloride in pyridine provided a single dehydration product **22**. The structure of the **22** assignment was based on previous experience in similar systems.[15] Introduction of the methyl group into $\beta$-position of unsaturated ester **22** was

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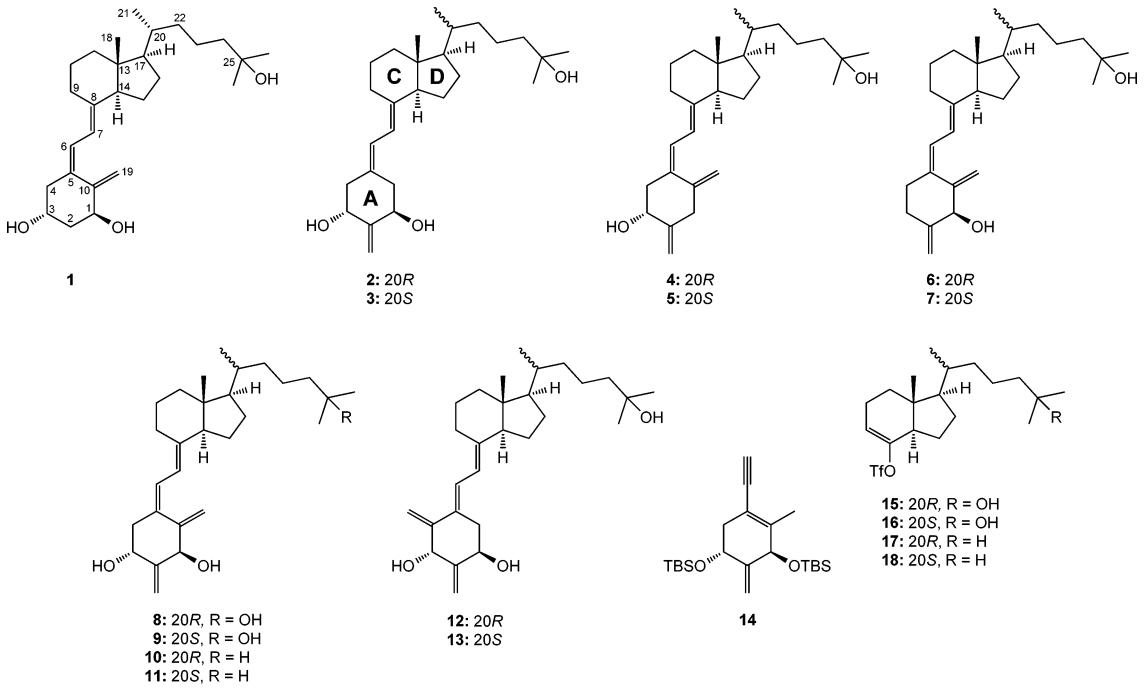
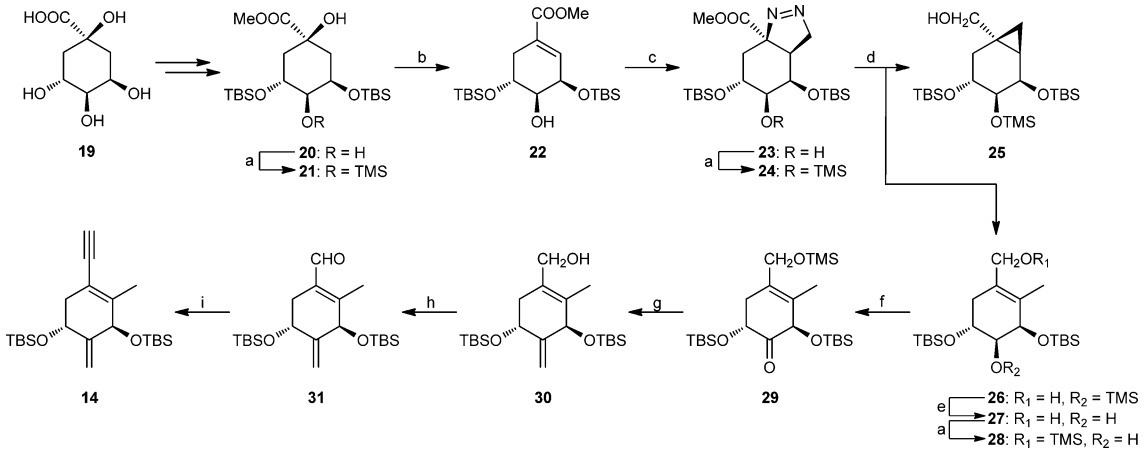



**Figure 1.** Chemical structure of 1$\alpha$,25-dihydroxyvitamin $D_3$ (calcitriol, **1**), the previously synthesized 2-methylene compounds **2**–**7**, 2-methylene analogues described in this work (**8**–**13**), and the building blocks for their synthesis.

**Scheme 1**[a]




[a](a) TMS-Cl, TEA, $CH_2Cl_2$ (**21**: 97%, **24**: 88%, **28**: 88%); (b) $SO_2Cl_2$, pyridine; then MeOH, $CH_2Cl_2$, 68%; (c) $CH_2N_2$, $Et_2O$, 95%; (d) (i) DMF, 125 °C; (ii) DIBALH, $CH_2Cl_2$/toluene, chrom. separation (**25**, 32%; **26**, 32%; two steps); (e) citric acid, MeOH, 89%; (f) Dess–Martin periodinane, $CH_2Cl_2$, 82%; (g) (i) $Ph_3P^+CH_3Br^-$, *n*-BuLi, THF; (ii) 5% HCl, 76% (two steps); (h) PDC, $CH_2Cl_2$, 79%; (i) *n*-BuLi, $TMSCHN_2$, THF, 82%.

achieved by the method described by Desmaele and Tanier.[16] Treatment of **22** with diazomethane resulted in formation of bicyclic adduct **23** as a product of 1,3-dipolar cycloaddition. The observed regioselectivity of the reaction was expected;[17] however, high stereoselectivity of this process was somewhat surprising. Inspection of $^1$H NMR spectrum of the product and comparison of its vicinal coupling constants with those calculated by molecular modeling (Figure 2a) allowed us to propose adduct structure resulting from an attack of the diazomethane from the side of the allylic OTBS group. The hydroxyl group in **23** was then protected as a TMS ether and the silylated adduct **24** subjected to thermolysis in DMF. Unfortunately, the outcome of this process was rather disappointing because two products, resulting from nitrogen extrusion, were formed in comparable quantities, and their separation was possible only after the following reduction step. In addition to the desired allylic alcohol **26**, the bicyclic product **25** was also isolated and its structure was established by $^1$H NMR (Figure 2b). After manipulation of protective groups, **26** was converted to the isomeric alcohol **28**, which was oxidized with Dess–Martin periodinane. The obtained ketone **29** was then subjected to Wittig methylenation and hydrolysis. Oxidation of the formed allylic alcohol **30** with PDC afforded the aldehyde **31** that reacted with the anion of trimethylsilyldiazomethane leading to the target dienyne **14**.

An alternative route to the A-ring fragment **14** started with the keto lactone **32** (Scheme 2) prepared from the quinic acid **19** by the described method.[18] Wittig reaction introduced the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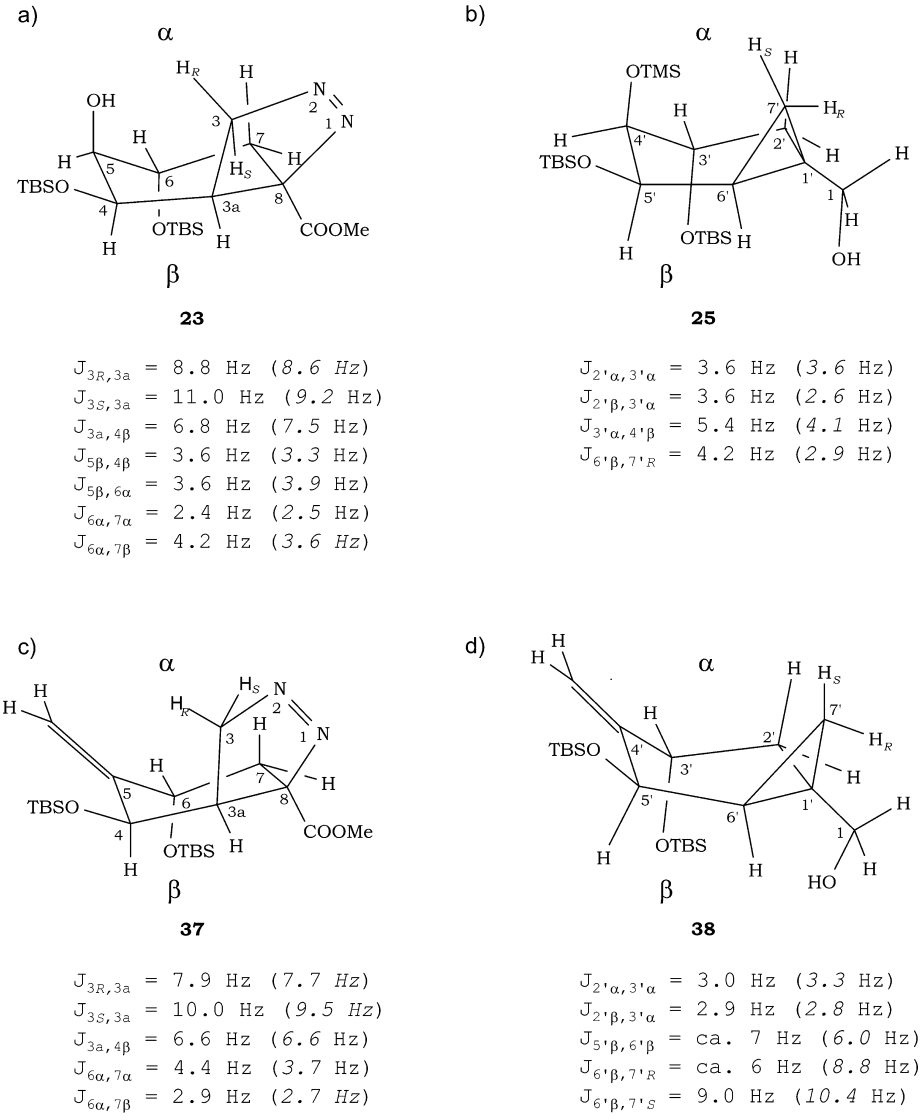


**Figure 2.** Preferred, energy-minimized (HyperChem) conformations of bicyclic diazomethane adducts **23** (a) and **37** (c) and bicyclic products **25** (b) and **38** (d) resulting from the pyrolysis of the adducts and the following reduction. The most informative $^1$H−$^1$H constants are listed; values of calculated couplings (PC MODEL) are enclosed in parentheses.

exomethylene substituent at the early stage of synthesis, and methanolysis of **33** gave the dihydroxy ester **34**. As a result of the symmetrical substitution of its cyclohexane ring, the subsequent dehydration process with Martin sulfurane furnished a single elimination product **36** from **35**. 1,3-Dipolar cycloaddition of diazomethane to the unsaturated ester **36** proved to be highly regio- and stereoselective, occurring solely from the side of an allylic OTBS substituent. Inspection of the $^1$H NMR spectra of the formed adduct **37**, supported by molecular mechanics calculations, allowed us to establish its structure and preferred conformation (Figure 2c). The subsequent thermolysis process of **37** followed by DIBALH reduction provided two isomeric compounds. However, the yield of the desired allylic alcohol **30**, a direct precursor of the A-ring fragment **14**, was significantly higher (60%) than that of the minor bicyclic product **38** (34%; Figure 3d). A comparison of the two synthetic paths described above (Schemes 1 and 2) indicates that the overall yield of **30** (from quinic acid) is almost twice as high in the latter method (5.5% versus 10%).

The A-ring building block **14** was than coupled with the C,D-ring vinyl triflates **15**−**18**, obtained from the corresponding Grundmann ketones.[4,19] Three former hydrindane compounds were described in literature,[12,20,21] and **18** was prepared in an analogous fashion (Supporting Information). Thus, Sonogashira reaction of dienyne **14** with the vinyl triflate **15** carried out using reactions described by Mourino[21] furnished the expected trienyne **39** (Scheme 3) in which the triple bond was selectively hydrogenated by a Lindlar catalyst poisoned with quinoline. Thermal rearrangement of the obtained previtamin D analogue **43** afforded the protected vitamin D compound **47** in quantitative yield. Removal of the three silyl protecting groups was less efficient but provided the target 2-methylene-1$\alpha$,25-$(OH)_2D_3$ (**8**). The Sonogashira protocol was also applied to other coupling reactions between dienyne **14** and the vinyl triflates **16**−**18**. The obtained trienynes **40**−**42** were converted to the respective final vitamin D analogues **9**−**11** as described above. For the preparation of the 5*E*-compounds **12** and **13**, the well-known iodine-catalyzed isomerization[22] was used (Scheme 4).


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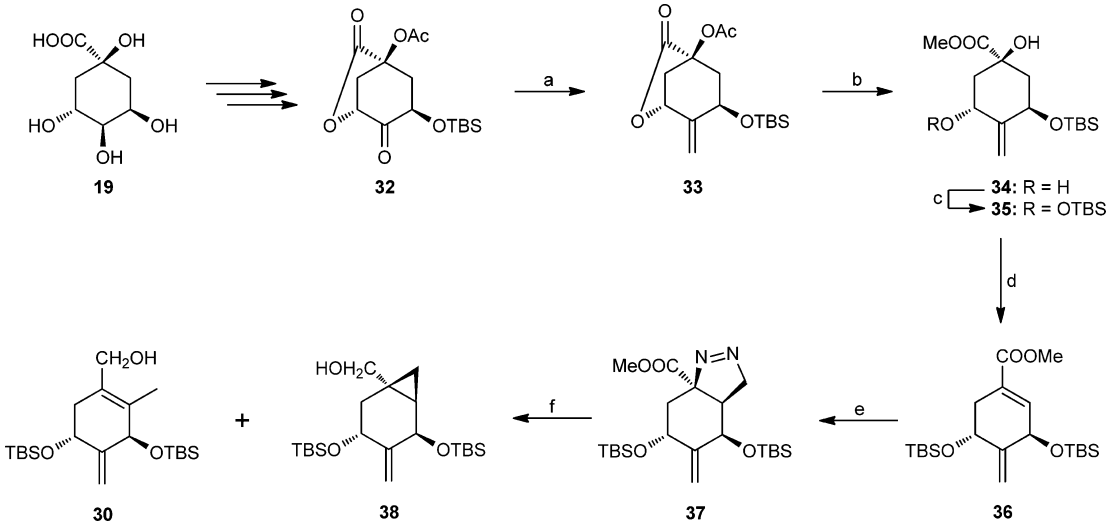
Scheme 2[a]




[a](a) $Ph_3P^+CH_3Br^-$, *t*-BuOK, THF, 73%; (b) $CH_3OMe$, MeOH, 79%; (c) TBSOTf, 2,6-lutidine, $CH_2Cl_2$, 95%; (d) Martin sulfurane, $CCl_4$, 90%; (e) $CH_2N_2$, $Et_2O$, 99%; (f) (i) DMF, 125 °C; (ii) DIBALH, $CH_2Cl_2$/toluene, chrom separation (**30**, 60%; **38**, 34%; two steps).

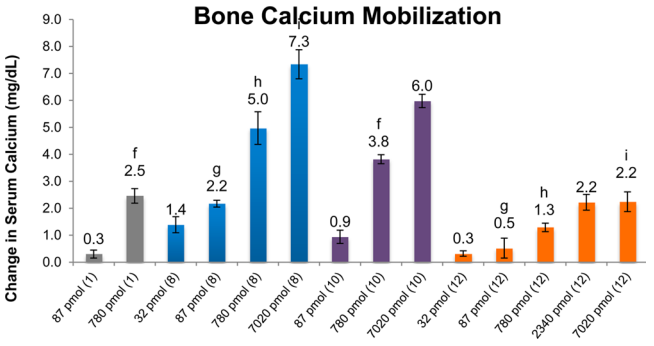



**Figure 3.** Bone calcium mobilization of 1$\alpha$,25-$(OH)_2D_3$ (**1**), its synthesized 2-methylene analogues **8** and **12**, and 2-methylene analogue of 1$\alpha$-OH-$D_3$ (**10**).

**Biological Evaluation.** The affinity of the analogues for the full-length recombinant rat receptor was compared to that of 1$\alpha$,25-$(OH)_2D_3$ (**1**) and 2MD (**3**). It was established (Table 1) that 1$\alpha$,25-dihydroxy-2-methylenevitamin $D_3$ (**8**) as well as its (20*S*)- and (5*E*,20*S*)-isomers bound the VDR slightly more effectively as the natural hormone **1**, whereas (5*E*)-compound **12** had 2.5-fold higher affinity for the receptor. Not unexpectedly, the lack of 25-hydroxyl in the analogues **10** and **11** resulted in much lower binding ability, decreased by 2 orders of magnitude from **1**.

With the exception of **10**, the obtained compounds were strongly antiproliferative. Analogue **9** and its (5*E*)-counterpart **13** were either as effective or more effective than 2MD in inducing HL-60 cells to differentiate into monocytes.

The activity of the synthesized compounds in inducing transcription of a vitamin D target gene was examined using the 1,25$(OH)_2D_3$ hydroxylase (*CYP24A1*) promoter. Analogues **8** and **12**, with the natural configuration at C-20, had transcriptional activity equal to that of 1$\alpha$,25-$(OH)_2D_3$ (**1**). 25-Desoxy compounds **10** and **11** also induced a dose-dependent activation of the *CYP24A1* gene but were less active than **1** by an order of magnitude. As in the previous assay of all tested compounds, the most pronounced activity was exhibited by (20*S*)-analogues **9** and **13**.

The results of in vivo testing of these analogues clearly indicate that 1$\alpha$,25-dihydroxy-2-methylenevitamin $D_3$ (**8**) and its isomer **9** with an "unnatural" (20*S*)-configuration were the most active in raising of serum calcium at the expense of bone (Figures 3 and 4). Their 25-desoxy counterparts **10** and **11** were less active but nevertheless more active than calcitriol. Interestingly, the (5*E*)-configuration found in analogues **12** and **13** resulted in decreased activity on bone calcium mobilization (serum calcium). The same pattern of activity was observed for intestinal calcium transport (Figures 5 and 6).

## CONCLUSIONS

Two alternative synthetic paths to prepare 2-methylene-substituted vitamin D compounds were used, both providing the desired target compounds which were then tested biologically (Table 1, Figures 3–6). Introduction of an additional A-ring exomethylene group at C-2 in 1$\alpha$,25-$(OH)_2D_3$ (**1**) increased both bone calcium mobilization and intestinal calcium transport in vivo without any change in in vitro tests. The (20*S*)-analogues with both a 2- and 10-methylene group (compound **9**) or pseudo-4-methylene group (compound **13**) have VDR binding and HL-60 activity similar to 2MD, whereas their transcriptional activity was decreased 3 and 6 times, respectively, as compared to **3**. The bone calcium mobilization activity of (20*S*)-1$\alpha$,25-dihydroxy-2-methylene-vitamin $D_3$ (**9**) was found to be slightly lower than its 19-nor analogue **3**, similar to its 20*R*-epimer **8** and approximately an order of magnitude higher than calcitriol. However, in the intestine, both compounds **8** and **9** were either equal or slightly more potent than **1** and **3**. Strong calcemic activity of analogues **10** and **11** lacking the 25-hydroxyl suggests their efficient enzymatic hydroxylation in vivo. Because of their considerable in vivo activity, these compounds might be considered potential prodrugs.

## EXPERIMENTAL SECTION

**Chemistry.** Optical rotations were measured in chloroform using PerkinElmer models 241 and 343 polarimeters at 22 °C. Ultraviolet (UV) absorption spectra were obtained on a Shimadzu UV-1800 UV spectrophotometer in 100% EtOH. All nuclear magnetic resonance



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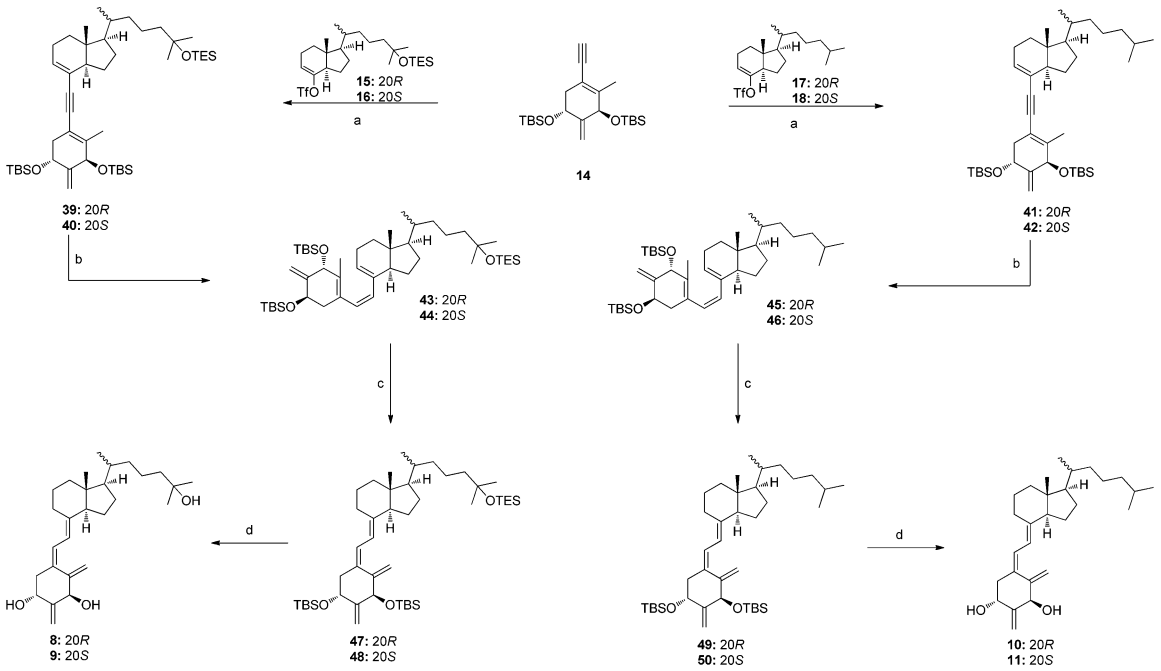
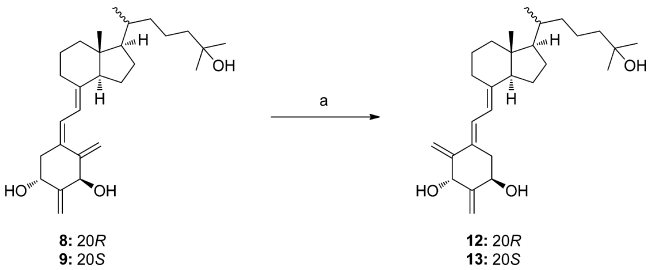
**Scheme 3**[a]




[a](a) $(PPh_3)_2Pd(OAc)_2$, CuI, $Et_2NH$, DMF (**39**, 92%; **40**, 54%; **41**, 84%; **42**, 98%); (b) Lindlar cat., $H_2$, hexane (**43**, 70%; **44**, 84%; **45**, 83%; **46**, 85%); (c) 65 °C, hexane (**47**, 100%; **48**, 70%; **49**, 70%; **50**, 91%); (d) TBAF, THF (**8**, 40%; **9**, 16%; **10**, 40%; **11**, 46%).

**Scheme 4**[a]




[a](a) $I_2$, $h\nu$, $Et_2O$ (**12**, 65%; **13**, 64%).

spectra were recorded in deuteriochloroform using Varian Unity Plus (200 MHz), Bruker DMX-400 (400 MHz), Bruker DMX-500 (500 MHz). COSY spectra, spin decoupling as well as NOE, DEPT 90, and DEPT 135 experiments were used to assign particular signals in the $^1H$ and $^{13}C$ NMR spectra. Chemical shifts ($\delta$) are reported in parts per million relative to $(CH_3)_4Si$ ($\delta$ 0.00) as an internal standard. Abbreviations used are singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). High resolution mass spectra were registered on LCT (TOF) or Mass Quattro LC spectrometer. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a model 6000A solvent delivery system, model U6K Universal injector, and model 486 tunable absorbance detectors. Solvents were dried and distilled following standard procedures.

The purity of final compounds was determined by HPLC, indicating at least 99% purity. Two HPLC columns (9.4 mm × 25 cm Zorbax-Sil and 9.4 mm × 25 cm Zorbax Eclipse XDB-C18) were used (Table 2 in Supporting Information). The purity and identity of the synthesized vitamins were confirmed by inspection of their $^1H$ NMR and high-resolution mass spectra.

The known vinyl triflates **15**,[20] **16**,[12] and **17**[21] were obtained according to the procedure of De Clercq et al.;[23] an analogous method was used for the preparation of the (20*S*)-trilate **18** (Supporting Information) from the corresponding Grundmann ketone. The starting hydroxy ester **20**[14] and lactone **32**[18] were synthesized from (−)-quinic acid (**19**).

**(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-4-[(trimethylsilyl)oxy]cyclohexanecarboxylic Acid Methyl Ester (21).** To a stirred solution of the dihydroxy ester **20** (1.03 g, 2.4 mmol) in anhydrous methylene chloride (5 mL) and triethylamine (600 $\mu$L) was added chlorotrimethylsilane (490 $\mu$L, 3.9 mmol) at 0 °C. After 2 h the reaction was quenched with saturated $NaHCO_3$ and extracted with methylene chloride. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography on silica using hexane/ethyl acetate (9:1) to give protected compound **21** (1.17 g, 97%) as a colorless oil.

**(3*R*,4*S*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-hydroxycyclohex-1-enecarboxylic Acid Methyl Ester (22).** To a stirred solution of hydroxy ester **21** (11.23 g, 22.2 mmol) in anhydrous methylene chloride (100 mL) and pyridine (23 mL) was dropwise added sulfuryl chloride (6.8 mL, 84 mmol) at −78 °C under argon. The white suspension was stirred for 5 h at this temperature, and then methanol (12 mL) was dropwise added. The mixture was allowed to warm up to room temperature, and stirring was continued for 17 h. The reaction was quenched with brine and extracted with diethyl ether. The organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica using hexane/ethyl acetate (95:5) to afford an oily unsaturated ester **22** (1.17 g, 97%).

**(3a*R*,4*R*,5*S*,6*R*,7a*R*)-4,6-Bis[(*tert*-butyldimethylsilyl)oxy]-5-hydroxy-3,3a,4,5,6,7-hexahydroindazole-7a-carboxylic Acid Methyl Ester (23).** To a solution of unsaturated ester **22** (1.94 g, 4.66 mmol) in diethyl ether (10 mL) was added an ether solution of diazomethane [20 mL (prepared according to the procedure of Arndt)].[24] The mixture was stirred at room temperature for 24 h in the darkness. The solvent was evaporated and the crude product was purified by column chromatography on silica using hexane/ethyl acetate (92:8) to give a pale yellow, oily adduct **23** (2.03 g, 95%).

**(3a*R*,4*R*,5*S*,6*R*,7a*R*)-4,6-Bis[(*tert*-butyldimethylsilyl)oxy]-5-[(trimethylsilyl)oxy]-3,3a,4,5,6,7-hexahydroindazole-7a-carboxylic Acid Methyl Ester (24).** Silylation of bicyclic compound **23** was performed according to procedure described for compound **20**; the reaction was carried out overnight at room temperature. Purification of the crude product by column chromatography on silica


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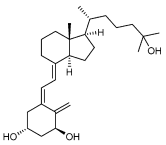
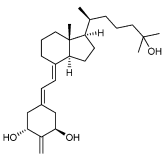
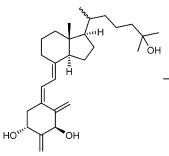
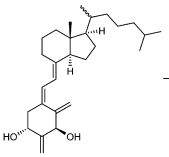
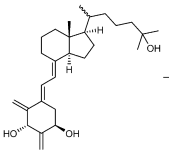
Table 1. Relative VDR Binding Activities,[a] HL-60 Differentiating Activities,[b] and Transcriptional Activities[c] of the Vitamin D Hormone (1), 2MD (3), and the Vitamin D Analogues 8−13

| Compd. Structure | Comp. No. | VDR Binding[a] | HL-60[b] differentiation | 24OHase[c] transcription |
|---|---|---|---|---|
| | | $K_i$ Ratio | $ED_{50}$ Ratio | $ED_{50}$ Ratio |
|  | **1** | 1 | 1 | 1 |
|  | **3** | 1 | 25 | 29 |
|  | **8** | 1.4 | 0.7 | 1 |
| | **9** | 1.1 | 29 | 10 |
|  | **10** | 0.01 | 0.07 | 0.07 |
| | **11** | 0.01 | 0.7 | 0.07 |
|  | **12** | 2.5 | 0.4 | 1 |
| | **13** | 1.1 | 20 | 5 |

[a]Competitive binding of 1$\alpha$,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues to the full-length recombinant rat vitamin D receptor. The experiments were carried out in duplicate on two different occasions. The $K_i$ values are derived from the dose−response curves and represent the inhibition constant when radiolabeled 1$\alpha$,25-$(OH)_2D_3$ is present at 1 nM and a $K_d$ of 0.2 nM is used. The numbers shown in the table are expressed as the average ratio of the 1$\alpha$,25-$(OH)_2D_3$ $K_i$ to the $K_i$ for the analogue. [b]Induction of differentiation of HL-60 promyelocytes to monocytes by 1$\alpha$,25-$(OH)_2D_3$ (**1**) and the synthesized vitamin D analogues. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated in duplicate two times. The $ED_{50}$ values are derived from the dose−response curves and represent the analogue concentration capable of inducing 50% maturation. The numbers shown in the table are expressed as the average ratio of the 1$\alpha$,25-$(OH)_2D_3$ $ED_{50}$ to the $ED_{50}$ for the analogue. [c]Transcriptional assay in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. The $ED_{50}$ values are derived from dose−response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. The numbers shown in the table are expressed as the average ratio of the 1$\alpha$,25-$(OH)_2D_3$ $ED_{50}$ to the $ED_{50}$ for the analogue.

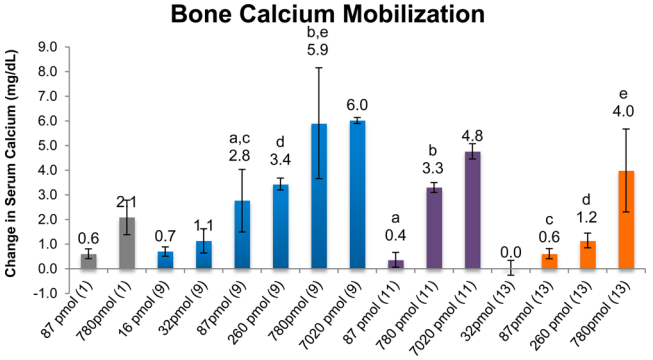



**Figure 4.** Bone calcium mobilization of 1$\alpha$,25-$(OH)_2D_3$ (**1**), its synthesized 2-methylene analogues **9** and **13**, and 2-methylene analogue of (20*S*)-1$\alpha$-OH-$D_3$ (**11**).

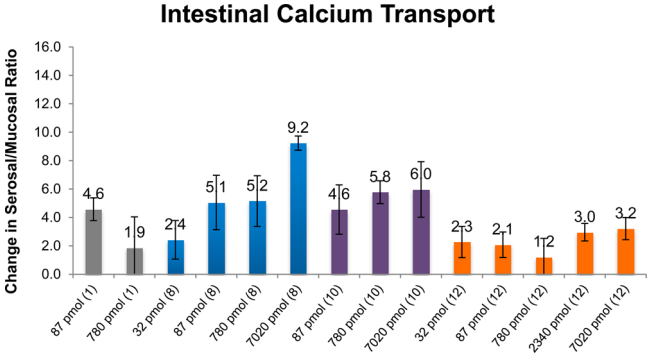



**Figure 5.** Intestinal calcium transport of 1$\alpha$,25-$(OH)_2D_3$ (**1**), its synthesized 2-methylene analogues **8** and **12**, and 2-methylene analogue of 1$\alpha$-OH-$D_3$ (**10**).

using hexane/ethyl acetate (95:5) gave silylated product **24** (102 mg, 88%) as a pale yellow oil.

**[(1′*S*,3′*R*,4′*S*,′5*R*,6′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[(trimethylsilyl)oxy]bicyclo[4.1.0]hept-1-yl]methanol (25)**



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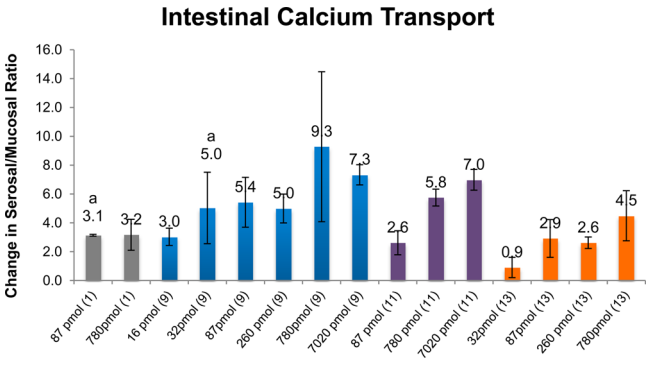


**Figure 6.** Intestinal calcium transport of 1$\alpha$,25-$(OH)_2D_3$ (**1**), its synthesized 2-methylene analogues **9** and **13**, and 2-methylene analogue of (20*S*)-1$\alpha$-OH-$D_3$ (**11**).

**and [(1′*S*,3′*R*,4′*S*,′5*R*,6′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)-oxy]-4′-[(trimethylsilyl)oxy]bicyclo[4.1.0]hept-1-yl]methanol (25) and [(3′*R*,4′*S*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-2′-methyl-4′-[(trimethylsilyl)oxy]cyclohex-1′-enyl]methanol (26).** A solution of compound **24** (118 mg, 0.22 mmol) in anhydrous DMF (3 mL) was heated at 125 °C under argon. After 16 h solvent was removed. The crude product was dissolved in methylene chloride (2.5 mL) and cooled to −78 °C. Diisobutylaluminum hydride (1 M in methylene chloride; 1 mL, 1 mmol) was added to the mixture, and stirring was continued for 1 h. The reaction was quenched with saturated $NH_4Cl$ and 1 M potassium−sodium tartrate and extracted with methylene chloride. The solvent was evaporated and the crude product was purified by column chromatography on silica using hexane/diethyl ether/ethyl acetate (95:4:1) to give semicrystalline alcohols **25** (34 mg, 32%) and **26** (34 mg, 32%).

**(1*S*,2*R*,6*R*)-2,6-Bis[(*tert*-butyldimethylsilyl)oxy]-4-hydroxy-methyl-3-methylcyclohex-3-enol (27).** To a stirred solution of alcohol **26** (131 mg, 0.28 mmol) in methanol (12 mL) was added citric acid hydrate (240 mg, 1.11 mmol). The reaction was continued for 3 h, solvent was evaporated, saturated $NaHCO_3$ was added to the residue, and the mixture was extracted with ethyl acetate. The organic phase was dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography on silica using hexane/ethyl acetate (9:1) to afford pure semicrystalline diol **27** (100 mg, 89%).

**(1*S*,2*R*,6*R*)-2,6-Bis[(*tert*-butyldimethylsilyl)oxy]-3-methyl-4-[(trimethylsilyl)oxy]methylcyclohex-3-enol (28).** Silylation of compound **27** (80 mg, 0.20 mmol) was performed according to procedure described for compound **20**; the reaction was carried out at −78 °C for 30 min. Purification of the crude product by column chromatography on silica using hexane/ethyl acetate (95:5) gave silylated product **28** (83 mg, 88%) as a pale yellow oil.

**(1*S*,2*R*,6*R*)-2,6-Bis[(*tert*-butyldimethylsilyl)oxy]-3-methyl-4-[(trimethylsilyl)oxy]methylcyclohex-3-enone (29).** Dess−Martin periodinane (1.58 g, 3.73 mmol) was added to a solution of alcohol **28** (1.178 g, 2.49 mmol) in anhydrous methylene chloride (60 mL). The mixture was stirred for 1 h at room temperature under argon atmosphere. Then reaction was quenched with saturated $Na_2S_2O_3$ and saturated $NaHCO_3$. The mixture was extracted with methylene chloride, and the organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica using hexane/ethyl acetate (95:5) to afford ketone **29** (1.05 g, 89%) as a colorless oil.

**[(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methyl-4-methylene-cyclohex-1-enyl]methanol (30).** *n*-Butyllithium (1.6 M in hexane; 0.6 mL, 0.96 mmol) was added dropwise to methyltriphenylphosphonium bromide (190 mg, 0.53 mmol) in anhydrous THF (3 mL) at −78 °C. After 15 min another portion of phosphonium salt (190 mg, 0.53 mmol) was added, and the solution was stirred at 0 °C for 40 min. The orange-red mixture was then cooled to −78 °C and siphoned to the precooled (−78 °C) solution of the ketone **29** (127 mg, 0.27 mmol) in anhydrous THF (1 mL). The reaction mixture was stirred at −78 °C for 2 h and then at room temperature overnight. The mixture was poured into 5% HCl and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give an orange oily residue which was applied on a silica Sep-Pak cartridge. Elution with hexane/ethyl acetate (95:5) gave pure alcohol **30** (81 mg, 76%) as a colorless oil.

**(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methyl-4-methylenecyclohex-1-enecarbaldehyde (31).** The mixture of alcohol **30** (16 mg, 40.2 $\mu$mol) and pyridinium dichromate (48.5 mg, 225.1 $\mu$mol) in anhydrous methylene chloride (0.7 mL) was stirred vigorously at room temperature for 4 h. The reaction mixture was then filtered through a pad of Celite (washed with methylene chloride), and the solvents were removed under reduced pressure. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane/diethyl ether (98:2) to yield the aldehyde **31** (12.6 mg, 79%) as a colorless oil.

**(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-2-methyl-4-methylenecyclohexene (14).** *n*-Butyllithium (1.6 M in hexanes; 25.5 $\mu$L, 40.8 $\mu$mol) was added to a solution of (trimethylsilyl)diazomethane (2.0 M in hexane, 19.5 $\mu$L, 39 $\mu$mol) in anhydrous THF (50 $\mu$L) at −78 °C under argon, and a solution of aldehyde **31** (12.6 mg, 31.8 $\mu$mol) in dry THF (100 $\mu$L + 50 $\mu$L) was added via cannula. After 1 h the cooling bath was removed and stirring was continued at room temperature overnight. Water was added, and the mixture was extracted with hexane, dried ($Na_2SO_4$), and concentrated. The crude product was applied on a silica Sep-Pak cartridge and eluted with hexane to afford dienyne **14** (10 mg, 82%).

**(1*R*,3*R*,5*R*)-1-Acetoxy-3-[(*tert*-butyldimethylsilyl)oxy]-4-methylene-6-oxabicyclo[3.2.1]octan-7-one (33).** A solution of potassium *tert*-butoxide in THF (1.0 M; 746 $\mu$L, 746 $\mu$mol) was added dropwise to a stirred suspension of methyltriphenylphosphonium bromide (280 mg, 784.6 $\mu$mol) in anhydrous THF (5.5 mL) at 0 °C. The mixture was warmed up to room temperature and stirred for additional 10 min. A solution of ketone **32** (126 mg, 382.7 $\mu$mol) in THF (1.6 mL) was added via cannula, and stirring was continued at room temperature for 1 h. Water was added, and the mixture was extracted with ethyl acetate, dried ($MgSO_4$), and concentrated. The residue was applied on a silica Sep-Pak cartridge (5 g) and eluted with hexane/ethyl acetate (95:5) to afford compound **33** (91 mg, 73%).

**(3*R*,5*R*)-5-[(*tert*-Butyldimethylsilyl)oxy]-1,3-dihydroxy-4-methylenecyclohexanecarboxylic Acid Methyl Ester (34).** A solution of lactone **33** (330 mg, 1.01 mmol) was vigorously stirred in methanolic sodium methoxide solution (0.04 M; 10 mL, 0.4 mmol) at room temperature for 17 h under argon. Water was added, and the mixture was extracted with ethyl acetate, dried ($Na_2SO_4$), and concentrated. The residue was applied on a silica Sep-Pak cartridge (5 g) and eluted with hexane/ethyl acetate (7:3) to give the diol **34** (253 mg, 79%) as a colorless oil.

**(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-4-methylenecyclohexanecarboxylic Acid Methyl Ester (35).** 2,6-Lutidine (191 $\mu$L, 1.65 mmol) was dropwise added to a stirred solution of the diol **34** (274 mg, 865.8 $\mu$mol) in anhydrous methylene chloride (4.5 mL) at −40 °C followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (300 $\mu$L, 1.3 mmol). The stirring was continued at −40 °C for 1 h, and saturated $NaHCO_3$ was added. Cooling bath was removed, and the reaction mixture was allowed to warm up slowly to room temperature. The mixture was extracted with methylene chloride, and combined organic layers were washed with 5% HCl and water, dried ($Na_2SO_4$), and concentrated. The residue was applied on a silica Sep-Pak cartridge (10 g) and eluted with hexane/ethyl acetate (93:7) to give compound **35** (353.5 mg, 95%) as a colorless oil.

**(3*R*,5*R*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methylene-cyclohex-1-enecarboxylic Acid Methyl Ester (36).** To a stirred solution of alcohol **35** (326 mg, 756.8 $\mu$mol) in anhydrous carbon tetrachloride (8.2 mL) was added a solution of bis[$\alpha$,$\alpha$-bis-(trifluoromethyl)benzyloxy]diphenylsulfur (752 mg, 1.12 mmol) in anhydrous carbon tetrachloride (6 mL) at room temperature under argon. Reaction was stirred for 30 min, water was added, and the mixture was extracted with methylene chloride. The organic phase was


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dried ($Na_2SO_4$) and concentrated. The resulting residue was applied on a silica Sep-Pak cartridge (5 g) and eluted with hexane/diethyl ether (98:2) to give the desired product contaminated by dehydrating reagent. Further purification on preparative TLC plates (silica gel $60F_{254}$, 20 cm × 20 cm, layer thickness 250 nm) using hexane/diethyl ether (92:8) afforded unsaturated ester **36** (276 mg, 90%) as a colorless oil.

**(3a*R*,4*R*,6*R*,7a*R*)-4,6-Bis[(*tert*-butyldimethylsilyl)oxy]-5-methylene-3,3a,4,5,6,7-hexahydroindazole-7a-carboxylic Acid Methyl Ester (37).** Solution of diazomethane in diethyl ether [2.7 mL (prepared according to the procedure of Arndt)][24] was added to a solution of the ester **36** (264 mg, 639.7 μmol) in anhydrous ethyl ether (1 mL) at room temperature. Reaction mixture was protected from light and stirred for 2 h. Solvent was evaporated, a residue dissolved in hexane, applied on a silica Sep-Pak cartridge (5 g), and eluted with hexane/ethyl acetate (97:3) to give bicyclic adduct **37** (288 mg, 99%) as colorless oil.

**[(3′*R*,5′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-2′-methyl-4′-methylenecyclohex-1′-enyl]methanol (30) and [(1′*S*,3′*R*,4′*S*,′5*R*,6′*R*)-3′,5′-Bis[(*tert*-butyldimethylsilyl)oxy]-4′-[(trimethylsilyl)oxy]bicyclo[4.1.0]hept-1-yl]methanol (38).** A solution of compound **37** (39 mg, 162.8 μmol) in freshly distilled anhydrous DMF (1.7 mL) was stirred at 125 °C for 6 h under argon. Heating bath was removed, water was added, and the mixture was extracted with hexane, dried ($Na_2SO_4$), and concentrated. The crude product was applied on a silica Sep-Pak cartridge (2 g) and eluted with hexane/diethyl ether (97:3). Removal of the solvents gave an oily residue (25 mg) that was dissolved in toluene/methylene chloride (2:1, 3 mL). To this solution diisobutylaluminum hydride (1.0 M in toluene; 260 μL, 260 μmmol) was slowly added at −78 °C under argon and stirred for 2 h. The mixture was quenched by a slow addition of potassium−sodium tartrate (2 N, 4 mL), aqueous HCl (2 N, 4 mL), and $H_2O$ (16 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated. The residue was applied on a silica Sep-Pak cartridge (2 g) and eluted with hexane/ethyl acetate (98:2) to give the allylic alcohol **30** (14 mg, 60%) and bicyclic product **38** (8 mg, 34%).

**1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methylene-25-[(triethylsilyl)oxy]-9,10-secocholesta-5(10),8-dien-6-yne (39).** To a solution of dienyne **14** (8 mg, 20.4 μmol) and triflate **15** (8.4 mg, 15.9 μmol) in anhydrous DMF (200 μL) were added CuI (0.45 mg, 2.37 μmol), $(PPh_3)_2Pd(OAc)_2$ (0.34 mg, 0.45 μmol), and $Et_2NH$ (159 μL) at room temperature under argon. After 45 min the mixture turned deep reddish-brown. Water was added, and the mixture was extracted with hexane, dried ($MgSO_4$), and concentrated. The residue was applied on a silica Sep-Pak cartridge (2 g) and eluted with hexane to afford trienyne **39** (8.3 mg, 92%) and recovered dienyne **14** (2.2 mg).

**(20*S*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methylene-25-[(triethylsilyl)oxy]-9,10-secocholesta-5(10),8-dien-6-yne (40).** Sonogashira reaction of dienyne **14** and triflate **16**, performed according to the procedure described above for the coupling of **14** and **15**, gave the trienyne **40** (54%).

**1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methylene-9,10-secocholesta-5(10),8-dien-6-yne (41).** Sonogashira reaction of triflate **17** and the dienyne **14**, performed analogously as described above for the coupling of **14** and **15**, gave trienyne **41** (84%).

**(20*S*)-1α,3β-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methylene-9,10-secocholesta-5(10),8-dien-6-yne (42).** Sonogashira reaction of the triflate **18** and the dienyne **14** was performed analogously as described above for the coupling of **14** and **15** to afford trienyne **42** (98%).

**1α-[(*tert*-Butyldimethylsilyl)oxy]-2-methylene-25-[(triethylsilyl)oxy]vitamin $D_3$ *tert*-Butyldimethylsilyl Ether (47).** To a solution of the trienyne **39** (8.3 mg, 10.8 μmol) in hexane (3 mL) and quinoline (2 μL) was added Lindlar catalyst (25 mg), and the mixture was stirred at room temperature under a positive pressure of hydrogen. Lindlar catalyst was added twice during 2.5 h (in 20 mg portions) and then the mixture was applied on a silica Sep-Pak cartridge (2 g) and eluted with hexane/ether (98:2) to give the silylated previtamin **43** (5.8 mg, 70%). The previtamin was then dissolved in anhydrous hexane (3 mL) and stirred at 60 °C for 14 h under argon. Solvent was evaporated and residue was applied on a silica Sep-Pak cartridge (2 g) and eluted with hexane/diethyl ether (99.6:0.4) to give protected vitamin D compound **47** (5.8 mg, 100%).

**(20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-methylene-25-[(triethylsilyl)oxy]vitamin $D_3$ *tert*-Butyldimethylsilyl Ether (48).** Hydrogenation of trienyne **40**, performed according to the procedure described above for **39**, gave silylated previtamin **44** (84%). Compound **44** was then subjected to the analogously performed thermal isomerization to give protected vitamin **48** (70%).

**1α-[(*tert*-Butyldimethylsilyl)oxy]-2-methylenevitamin $D_3$ *tert*-Butyldimethylsilyl Ether (49).** Hydrogenation of trienyne **41** was performed analogously as described above for **39**. The obtained silylated previtamin **45** (83%) was then subjected to the thermal isomerization to give protected vitamin **49** (70%).

**(20*S*)-1α-[(*tert*-Butyldimethylsilyl)oxy]-2-methylenevitamin $D_3$ *tert*-Butyldimethylsilyl Ether (50).** Hydrogenation of trienyne **42** was performed analogously as described above for **39**. The obtained silylated previtamin **46** (85%) was then subjected to the thermal isomerization to afford protected vitamin **50** (91%).

**1α,25-Dihydroxy-2-methylenevitamin $D_3$ (8).** To a solution of protected vitamin **47** (5.8 mg, 7.5 μmol) in THF (1 mL) was added tetrabutylammonium fluoride (1.0 M in THF; 450 μL, 450 μmol) at room temperature under argon. The stirring was continued for 20 h, brine was added, and the mixture was extracted with ethyl acetate. The organic extracts were dried ($MgSO_4$) and evaporated. The residue was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system; compound **8** (1.28 mg, 40%) was collected at $R_V$ = 36 mL. Analytical sample of the vitamin was obtained after reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (88:12) solvent system ($R_V$ = 33 mL).

**(20*S*)-1α,25-Dihydroxy-2-methylenevitamin $D_3$ (9).** Treatment of protected vitamin **48** with TBAF, performed according to the procedure described above for **47**, gave a product that was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (92:8) solvent system; vitamin **9** (16%) was collected at $R_V$ = 36 mL. Analytical sample of the vitamin was obtained after reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (88:12) solvent system ($R_V$ = 30 mL).

**1α-Hydroxy-2-methylenevitamin $D_3$ (10).** Hydroxyl deprotection of silylated vitamin **49** was performed analogously as described above for **47**. The obtained product was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (95:5) solvent system; vitamin **10** (1.8 mg, 40%) was collected at $R_V$ = 34 mL. Analytical sample of the vitamin was obtained after reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (97:3) solvent system ($R_V$ = 40 mL).

**(20*S*)-1α-Hydroxy-2-methylenevitamin $D_3$ (11).** Hydroxyl deprotection of protected vitamin **50** (11 mg, 17.2 μmol) was performed analogously as described above for **47**. The product was purified by HPLC (9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (95:5) solvent system; vitamin **11** (3.3 mg, 46%) was collected at $R_V$ = 34 mL. Analytical sample of the vitamin was obtained after reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (97:3) solvent system ($R_V$ = 38 mL).

**(5*E*)-1α,25-Dihydroxy-2-methylenevitamin $D_3$ (12).** Treatment of compound **8** in ether with a catalytic amount of iodine (2% of the amount of **8**), while keeping the solution under diffuse daylight for 1 h, resulted in its partial isomerization. A mixture of (5*Z*)- and (5*E*)-isomers **8** and **12** was formed in a ratio of 3:7, respectively. Compounds were separated by reversed-phase HPLC (9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (84:16) solvent system, and analytically pure (5*E*)-isomer **12** was eluted at $R_V$ = 57 mL.

**(5*E*,20*S*)-1α,25-Dihydroxy-2-methylenevitamin $D_3$ (13).** Analogously, as in the case of **8**, iodine-catalyzed isomerization of (5*Z*)-vitamin **9** to the respective (5*E*)-isomer **13** was performed. Analytically


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pure sample of the vitamin **13** was obtained after reversed-phase HPLC (9.4 mm × 25 mm Zorbax Eclipse XDB-C18 column, 4 mL/min) using methanol/water (84:16) solvent system ($R_V$ = 55 mL).

**Biological Studies.** *1. In Vitro Studies.* VDR binding, HL-60 differentiation and 24-hydroxylase transcription assays were performed as previously described.[18,25]

*2. In Vivo Studies. 2.1. Bone Calcium Mobilization and Intestinal Calcium Transport.* Male, weanling Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). The animals were group housed and placed on diet 11 (0.47% Ca) + AEK oil for 1 week followed by diet 11 (0.02% Ca) + AEK oil for 3 weeks. The rats were then switched to a diet containing 0.47% Ca [26] for 1 week followed by 2 weeks on a diet containing 0.02% Ca. Dose administration began during the last week on 0.02% Ca diet. Four consecutive intraperitoneal doses were given approximately 24 h apart. Twenty-four hours after the last dose, blood was collected from the severed neck and the concentration of serum calcium determined as a measure of bone calcium mobilization. The first 10 cm of the intestine was also collected for the intestinal calcium transport analysis using the everted gut sac method.[25]

All animals were managed in accordance with University of Wisconsin standards and protocols for animal care and use. Our experiments were approved by the College of Agricultural and Life Sciences Institutional Animal Care and Use Committee.

*2.2. Statistical Analysis.* All statistical analyses were done using the SAS mixed model procedures and Tukey's pairwise comparisons (SAS Institute, Inc. Cary, N.C., USA).

**Molecular Modeling.** The molecular mechanics studies were used to establish the energy-minimized structures of the synthesized compounds shown in Figure 2. The calculation of optimized geometries and steric energies was initially carried out using the algorithm from the MM+ HyperChem (release 8.0) software package (Autodesk, Inc.). MM+ is an all-atom force field based on the MM2 functional form. The procedure used for finding the global minimum structures involved the Conformational Search module; these structures were further energy-minimized using the semiempirical AM1 method. Finally, the respective vicinal proton−proton coupling constants in the preferred, energy-minimized conformations were calculated using PCMODEL (release 9.0) software package (Serena Software).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01295.

Purity criteria of the vitamin D analogues **8**−**13**, their $^1$H and $^{13}$C NMR spectra, figures with either competitive binding curves or dose−response curves derived from cellular differentiation and transcriptional assays, and spectral data of the all synthesized compounds (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

1$\alpha$,25-$(OH)_2D_3$, 1$\alpha$,25-dihydroxyvitamin $D_3$; 2MD, (20*S*)-1$\alpha$,25-dihydroxy-2-methylene-19-norvitamin $D_3$; VDR, vitamin D receptor

## ■ REFERENCES


(1) (a) Norman, A. W. *Vitamin D, the Calcium Homeostatic Steroid Hormone*; Academic Press: New York, 1979. (b) Feldman, D., Pike, J. W., Adams, J. S., Eds. *Vitamin D*, 3rd ed.; Elsevier Academic Press: San Diego, CA, 2011.

(2) (a) Jones, G.; Strugnell, S. A.; DeLuca, H. F. Current understanding of the molecular actions of vitamin D. *Physiol. Rev.* **1998**, *78*, 1193−1231. (b) Haussler, M. R.; Whitfield, G. K.; Kaneko, I.; Haussler, C. A.; Hsieh, D.; Hsieh, J. C.; Jurutka, P. W. Molecular mechanisms of vitamin D action. *Calcif. Tissue Int.* **2013**, *92*, 77−98.

(3) (a) Bouillon, R.; Okamura, W. H.; Norman, A. W. Structure−function relationships in the vitamin D endocrine system. *Endocr. Rev.* **1995**, *16*, 200−257. (b) Okamura, W. H.; Zhu, G. D. Chemistry and design: structural biology of vitamin D action. In *Vitamin D*; Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic Press: New York, 1997; pp 937−971. (c) Binderup, L.; Binderup. E.; Godtfredsen, W. O. Development of new vitamin D analogs. In *Vitamin D*; Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic Press: New York, 1997; pp 1027−1043.

(4) Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. New 1$\alpha$,25-dihydroxy-19-norvitamin $D_3$ compounds of high biological activity: Synthesis and biological evaluation of 2-hydroxymethyl, 2-methyl and 2-methylene analogues. *J. Med. Chem.* **1998**, *41*, 4662−4674.

(5) (a) Shevde, N. K.; Plum, L. A.; Clagett-Dame, M.; Yamamoto, H.; Pike, J. W.; DeLuca, H. F. A potent analog of 1$\alpha$,25-dihydroxyvitamin $D_3$ selectively induces bone formation. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 13487−13491. (b) Yamamoto, H.; Shevde, N. K.; Warrier, A.; Plum, L. A.; DeLuca, H. F.; Pike, J. W. 2-Methylene-19-nor-(20*S*)-1,25-dihydroxyvitamin $D_3$ potently stimulates gene-specific DNA binding of the vitamin D receptor in osteoblasts. *J. Biol. Chem.* **2003**, *278*, 31756−31765.

(6) (a) Ke, H. Z.; Qi, H.; Crawford, D. T.; Simmons, H. A.; Xu, G.; Li, M.; Plum, L.; Clagett-Dame, M.; DeLuca, H. F.; Thompson, D. D.; Brown, T. A. A new vitamin D analog, 2MD, restores trabecular and cortical bone mass and strength in ovariectomized rats with established osteopenia. *J. Bone Miner. Res.* **2005**, *20*, 1742−1755. (b) Plum, L. A.; Fitzpatrick, L. A.; Ma, X.; Binkley, N.; Zella, J. B.; Clagett-Dame, M.; DeLuca, H. F. 2MD, a new anabolic agent for osteoporosis treatment. *Osteoporosis Int.* **2006**, *17*, 704−715. (c) DeLuca, H. F. The development of a bone- and parathyroid-specific analog of vitamin D: 2-methylene-19-nor-(20*S*)-1$\alpha$,25-dihydroxyvitamin $D_3$. *BoneKEy Rep.* **2014**, 514.

(7) DeLuca, H. F.; Bedale, W.; Binkley, N.; Gallagher, J. C.; Bolognese, M.; Peacock, M.; Aloia, J.; Clagett-Dame, M.; Plum, L. The vitamin D analogue 2MD increases bone turnover but not BMD in postmenopausal women with osteopenia: Results of a 1-year phase 2 double-blind, placebo-controlled, randomized clinical trial. *J. Bone Miner. Res.* **2011**, *26*, 538−545.

(8) (a) Sicinski, R. R. 2-Alkylidene analogs of 19-nor-1$\alpha$,25-$(OH)_2D_3$: Synthesis and biological activity. *Polish J. Chem.* **2006**, *80*, 573−585. (b) Glebocka, A.; Chiellini, G. A-Ring analogs of 1,25-dihydroxyvitamin $D_3$. *Arch. Biochem. Biophys.* **2012**, *523*, 48−57.

(9) Sibilska, I. K.; Barycka, K. M.; Sicinski, R. R.; Plum, L. A.; DeLuca, H. F. 1-Desoxy analog of 2MD: synthesis and biological activity of (20*S*)-25-hydroxy-2-methylene-19-norvitamin $D_3$. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 51−55.

(10) DeLuca, H. F.; Sibilska, I. K.; Plum, L. A.; Clagett-Dame, M.; Sicinski, R. R. 3-Desoxy-2-methylene-19-nor-vitamin D analogs and their uses. U.S. Patent Application 2012/0322775 A1, Dec 20, 2012.

(11) Sibilska, I. K.; Sicinski, R. R.; Plum, L. A.; DeLuca, H. F. Synthesis and biological activity of 25-hydroxy-2-methylene-vitamin $D_3$ compounds. *J. Steroid Biochem. Mol. Biol.* **2013**, *136*, 17−22.




DOI: 10.1021/acs.jmedchem.5b01295
*J. Med. Chem.* XXXX, XXX, XXX−XXX

(12) Sibilska, I. K.; Sicinski, R. R.; Ochalek, J. T.; Plum, L. A.; DeLuca, H. F. Synthesis and biological activity of 25-hydroxy-2-methylene-vitamin $D_3$ analogues monohydroxylated in the A-ring. *J. Med. Chem.* **2014**, *57*, 8319−8331.
(13) Sibilska, I. K.; Szybinski, M.; Sicinski, R. R.; Plum, L. A.; DeLuca, H. F. Highly potent 2-methylene analogs of 1$\alpha$,25-dihydroxyvitamin $D_3$: Synthesis and biological evaluation. *J. Steroid Biochem. Mol. Biol.* **2013**, *136*, 9−13.
(14) Perlman, K. L.; Swenson, R. E.; Paaren, H. E.; Schnoes, H. K.; DeLuca, H. F. Novel synthesis of 19-nor-vitamin D compounds. *Tetrahedron Lett.* **1991**, *32*, 7663−7666.
(15) (a) Cleophax, J.; Leboul, J.; Mercier, D.; Gaudemer, A.; Dov Gero, S. Voie d'acces facile aux derives de l'acide shikimique et epi-4-shikimique. *Bull. Soc. Chom. Fr.* **1973**, 2992−2995. (b) Ulibarri, G.; Nadler, W.; Skrydstrup, T.; Audrain, H.; Chiaroni, A.; Riche, C.; Grierson, D. S. Construction of the bicyclic core structure of the enediyne antibiotic Esperamicin-$A_1$ in either enantiomeric form from (−)-quinic acid. *J. Org. Chem.* **1995**, *60*, 2753−2761. (c) Barco, A.; Benetti, S.; De Risi, C.; Marchetti, P.; Pollini, G. P.; Zanirato, V. *D*-(−)-Quinic acid: a chiron store for natural product synthesis. *Tetrahedron: Asymmetry* **1997**, *8*, 3515−3545.
(16) Desmaele, D.; Tanier, S. Nouvelle synthese du cycle a du 1*S*-hydroxycholecalciferol a partir de l'acide quinique. *Tetrahedron Lett.* **1985**, *26*, 4941−4944.
(17) (a) Gothelf, K. V.; Jorgensen, K. A. Asymmetric 1,3-dipolar cycloaddition reactions. *Chem. Rev.* **1998**, *98*, 863−910. (b) Padwa, A., Pearson, W. H., Eds. *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry toward Heterocycles and Natural Products*; John Wiley & Sons, Inc.: Hoboken, NJ, 2003; Vol. *59*, p 539.
(18) Glebocka, A.; Sicinski, R. R.; Plum, L. A.; Clagett-Dame, M.; DeLuca, H. F. New 2-alkylidene 1$\alpha$,25-dihydroxy-19-norvitamin $D_3$ analogues of high intestinal activity: synthesis and biological evaluation of 2-(3′-alkoxypropylidene) and 2-(3′-hydroxypropylidene) derivatives. *J. Med. Chem.* **2006**, *49*, 2909−2920.
(19) (a) Sicinski, R. R.; Perlman, K. L.; DeLuca, H. F. Synthesis and biological activity of 2-hydroxy and 2-alkoxy analogues of 1$\alpha$,25-dihydroxy-19-norvitamin $D_3$. *J. Med. Chem.* **1994**, *37*, 3730−3738. (b) Maestro, M. A.; Sardina, F. J.; Castedo, L.; Mourino, A. *J. Org. Chem.* **1991**, *56*, 3582−3587. (c) DeLuca, H. F.; Sicinski, R. R.; Grzywacz, P. K. (20*S*)-1$\alpha$-Hydroxy-2$\alpha$-methyl and 2$\beta$-methyl-19-nor-vitamin $D_3$ and their uses. U.S. Patent 6,846,811 B2, Jan 25, 2005.
(20) Hayashi, R.; Fernández, S.; Okamura, W. H. An 8$\pi$ electron electrocyclization leading to a 9,19-methano-bridged analogue of 1$\alpha$,25-dihydroxyvitamin $D_3$. *Org. Lett.* **2002**, *4*, 851−854.
(21) Mascareñas, J. L.; Sarandeses, L. A.; Castedo, L.; Mouriño, A. Palladium-catalysed coupling of vinyl triflates with enynes and its application to the synthesis of 1$\alpha$,25-dihydroxyvitamin $D_3$. *Tetrahedron* **1991**, *47*, 3485−3498.
(22) Verloop, A.; Koevoet, A. L.; Van Moorselaar, R.; Havinga, E. Studies on vitamin D and related compounds IX: Remarks on the iodine-catalyzed isomerisations of vitamin D and related compounds. *Rec. Trav. Chim.* **1959**, *78*, 1004−1014.
(23) Chen, Y.; Gao, L.-J.; Murad, I.; Verstuyf, A.; Verlinden, L.; Verboven, C.; Bouillon, R.; Viterbo, D.; Milanesio, M.; Van Haver, D.; Vandewalle, M.; De Clercq, P. J. Synthesis, biological activity, and conformational analysis of CD-ring modified trans-decalin 1$\alpha$,25-dihydroxyvitamin D analogs. *Org. Biomol. Chem.* **2003**, *1*, 257−267.
(24) Arndt, F. Nitrosomethylurea. *Org. Synth.* **1935**, *15*, 48−50.
(25) Chiellini, G.; Grzywacz, P.; Plum, L. A.; Barycki, R.; Clagett-Dame, M.; DeLuca, H. F. Synthesis and biological properties of 2-methylene-19-nor-25-dehydro-1$\alpha$-hydroxyvitamin $D_3$-26,23-lactones-weak agonists. *Bioorg. Med. Chem.* **2008**, *16*, 8563−8573.
(26) Suda, T.; DeLuca, H. F.; Tanaka, Y. Biological activity of 25-hydroxyergocalciferol in rats. *J. Nutr.* **1970**, *100*, 1049−1052.


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