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Chemoenzymatic synthesis and biological evaluation of C-3 carbamate analogues of 1α,25-dihydroxyvitamin D_3

Vicente Gotor-Fernández,[a] Susana Fernández,[a] Miguel Ferrero,[a] Vicente Gotor,[a,*] Roger Bouillon[b] and Annemieke Verstuyf[b]

[a]*Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, 33071 Oviedo, Spain*
[b]*Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Gasthuisberg, B-3000 Leuven, Belgium*

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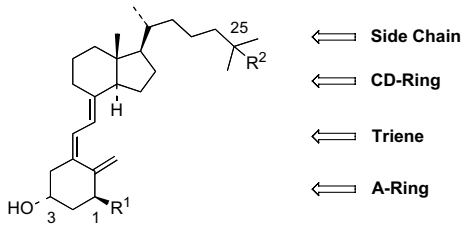
Abstract—The synthesis of new analogues of 1α,25-dihydroxyvitamin D_3 containing a carbamate function at the A-ring fragment has been described using the cross-coupling approach. The carbamate group was selectively introduced at the C-3 position by regioselective enzymatic alkoxycarbonylation of A-ring enyne **3** and subsequent treatment with ammonia, amines, amino alcohols, and amino acids. Biological studies to evaluate the potency of all five of these carbamate analogues were performed and demonstrated very low binding affinity for the vitamin D receptor compared with 1α,25-dihydroxyvitamin D_3. Moreover, all the carbamate analogues were less active than 1α,25-dihydroxyvitamin D_3 in inhibiting cell proliferation or stimulating cell differentiation. Of all the five analogues, the 3-*O*-carbamoyl-1α,25-$(OH)_2$-D_3 analogue **10a** was the most potent one in vitro. However, all investigated carbamate analogues demonstrated lower calcemic effects in vivo than the parent compound.

1. Introduction

The seco-steroid 1α,25-dihydroxyvitamin D_3 [calcitriol, 1α,25-$(OH)_2$-D_3 (**1**), Fig. 1], the biologically active form of vitamin D_3 (**2**), functions as a regulator of calcium and phosphorous homeostasis, and also shows a broad spectrum of biological activities such as modulation of cell proliferation and differentiation.[1] Unfortunately, the therapeutic value of 1α,25-$(OH)_2$-D_3 has been limited by inherent toxicity due to adverse calcium absorption and mobilization effects.[1c,d] For this reason, there is a continued effort stimulated by medical needs to design analogues of the natural hormone with dissociation of effects on cell differentiation compared with their calcemic effects.[2]



1, $R^1 = R^2 =$ OH; 1α,25-$(OH)_2$-D_3
2, $R^1 = R^2 =$ H; Vitamin D_3

Figure 1.

Carbamate derivatives are compounds of considerable interest in some areas of medicinal chemistry.[3] This functional group is present in antineoplastic agents such as mitomycins[4] and bleomycins.[5] The alkaloid physostigmine, which has a carbamate moiety in its structure, is under investigation as potential drug in the therapy of Alzheimer's disease.[6] Also, potent inhibitors of HIV-1 protease posses this group in their structures.[7] An important application of the carbamate group is to increase the permeability across the cellular membranes. Thus, certain β-adrenergic blockers are introduced into cells as carbamate derivatives.[8] Recently, Liu and co-workers have described the synthesis of amino and polyamino carbamate analogues of vitamin D_2 and vitamin D_3 as useful cationic lipids for gene delivery in vitro.[9]

Most analogues of 1α,25-$(OH)_2$-D_3 involved modifications of the side chain, while modifications of the A-ring

* Corresponding author. Tel./fax: +34-98-510-3448; e-mail: vgs@sauron.quimica.uniovi.es

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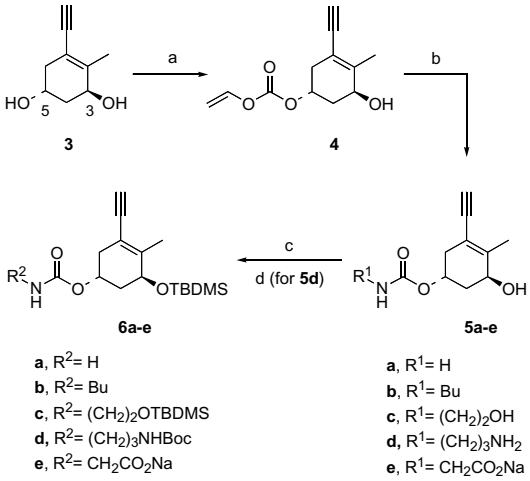
are less extensive due to its more complicated synthetic routes required to obtain the adequate precursors. The A-ring fragment possesses two hydroxyl groups of similar reactivity, and as a result it is very difficult to discern between these two groups from a chemical point of view. However, we have applied the synthetic potential of enzymes in this field to selectively modify polyfunctionalized compounds.[10]

Taking into account the interesting features showed by the carbamate moiety, here we report the chemoenzymatic synthesis of C-3 carbamates analogues of the steroid hormone 1α,25-$(OH)_2$-D_3. Furthermore, different hydrophilic groups such as alcohol, amino, and acid (from an amino acid) were introduced at the end of the alkyl chain of the carbamate function. These interesting products can provide information about the mode of action of 1α,25-$(OH)_2$-D_3 and the influence of the several functional groups of the molecule in the biological responses associated to the hormone. Amino acids linked to vitamin D_3 would be promising products.



Scheme 1. Reagents and conditions: (a) **CAL-B**, acetone *O*-[(vinyloxy)carbonyl]oxime, toluene, 30 °C, 4 h (quantitative); (b) R^1NH_2, THF (DMF for **5e**), 60 °C [18 h (quantitative) for **5a**, 22 h (91%) for **5b**, 48 h (80%) for **5c**, 48 h (76%) for **5d**, 48 h (87%) for **5e**]; (c) TBDMSCl, imidazol, DMAP, CH_2Cl_2, rt, 12 h (91% for **6a**, 97% for **6b**, 86% for **6c**, 88% for **6e**); (d) Boc_2O, $CHCl_3$, $NaHCO_3$ (aq), rt, 3 h (67% for **6d**, steps c and d).

2. Results and discussion

To synthesize the C-3 carbamate derivatives we used a convergent route[11] based on the palladium-catalyzed coupling of an A-ring enyne with the CD-ring vinyl triflate fragment, which are separately synthesized. In this approach, a dienyne is semihydrogenated to a previtamin structure that undergoes rearrangement to the corresponding vitamin D analogue.

Previously, we have reported[12] the synthesis of several carbamate derivatives of the 1α,25-$(OH)_2$-D_3 A-ring precursor through a two-step chemoenzymatic process (Scheme 1). First, enzymatic alkoxycarbonylation reaction of synthon **3**[13] with acetone *O*-[(vinyloxy)carbonyl] oxime catalyzed by *Candida antarctica* lipase B (CAL-B) gave rise selectively to carbonate **4** in quantitative yield. This versatile vinylcarbonate reacts with ammonia, amines, amino alcohols, diamines, and amino acids to obtain the corresponding 1α,25-$(OH)_2$-D_3 A-ring C-5[14] carbamate synthons **5a**–**e**. In order to improve the yield of the coupling reaction, enynes **5** were conveniently protected as silyl ethers with *tert*-butyldimethylsilyl chloride (TBDMSCl) given place to derivatives **6**.

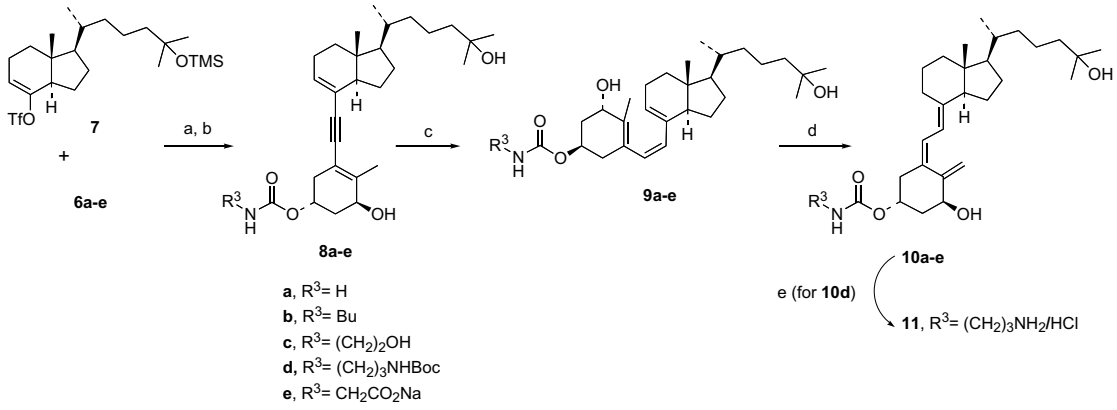
The synthesis of 3-*O*-carbamoyl-1α,25-$(OH)_2$-D_3 (**10a**) (Scheme 2) starts with the coupling of A-ring precursor **6a** and vinyl triflate **7**, prepared according to the published procedure,[15] in the presence of catalytic amount of bis(triphenylphosphine)palladium(II) acetate–copper(I) iodide. The protected dienyne was unstable, and for this reason desilylation with tetrabutylammonium fluoride (TBAF) was performed immediately to afford dienyne **8a** in 90% yield (for coupling and desilylation steps).

Catalytic hydrogenation of diol **8a** in methanol, in the presence of Lindlar catalyst and quinoline poison, generated previtamin **9a**. Reaction was carefully monitored by TLC to avoid over-reduction. A critical factor is the state of the catalyst; so previously it was pre-dried at 60 °C in vacuum. Thermolysis of compound **9a** at 80 °C for 4 h afforded 3-*O*-carbamoyl-1α,25-$(OH)_2$-D_3 (**10a**) in 62% yield from **8a**.

Similarly, 3-*O*-[*N*-(butyl)carbamoyl]-1α,25-$(OH)_2$-D_3 (**10b**) and 3-*O*-[*N*-(2-hydroxyethyl)carbamoyl]-1α,25-$(OH)_2$-D_3 (**10c**) were synthesized from A-ring precursors **6b** and **6c**, respectively.

Firstly, the preparation of *N*-(3-aminopropyl)carbamoyl derivative **11** was carried out with the A-ring synthon in an unprotected state, but only low yields were obtained. The presence of a free amino group led to difficulties in the isolation and purification of the products; for example, it was necessary to use aqueous ammonia for purification by flash chromatography. Accordingly, the terminal amino group in ring A was protected as the *tert*-butyloxycarbonyl (Boc) derivative to afford derivative **6d**. Excellent yields were obtained in the coupling reaction between this A-ring fragment and the enol triflate **7**. Subsequent catalytic hydrogenation, isomerization, and deprotection of the amino group with HCl in ethanol afforded 3-*O*-[*N*-(3-aminopropyl)carbamoyl]-1α,25-$(OH)_2$-D_3 (**11**), which was isolated as its hydrochloride salt.

For the synthesis of 3-*O*-[*N*-(carboxymethyl)carbamoyl]-1α,25-$(OH)_2$-D_3 sodium salt (**10e**) a larger amount of $Pd(PPh_3)_2(OAc)_2$ and CuI was needed to improve the yield of the cross-coupling reaction of **7** and **6e**. Moreover, the thermolysis process was performed at lower temperature (65 °C) and in less reaction time (3 h).



Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)(OAc)_2$, CuI, Et_2NH, DMF, rt, 1 h; (b) Bu_4NF, THF, rt, 12 h (90% for **8a**, 71% for **8b**, 90% for **8c**, 96% for **8d**, 61% for **8e**, two steps); (c) H_2, Lindlar cat., quinoline, MeOH, rt, 1 h; (d) acetone, 80 °C, 4 h (65 °C, 3 h for **9e**) (62% for **10a**, 54% for **10b**, 71% for **10c**, 54% for **10d**, 62% for **10e**, two steps); (e) HCl(g), EtOH, rt, 30 min (69%).

3. Biological evaluation

Analogues **10a**–**c**, **10e**, and **11** were evaluated in vitro in terms of their ability to bind to the calf thymus vitamin D receptor in comparison to the natural hormone and to inhibit the cell proliferation (MCF-7, keratinocytes) or to induce cell differentiation (HL 60) (Table 1). The relative affinity of the analogues was calculated from their concentration required for 50% displacement of [^{3}H]1α,25-$(OH)_2$-D_3 from the receptor protein compared with the activity of 1α,25-$(OH)_2$-D_3 (assigned a value of 100 by definition). In this assay, all the analogues were less effective than 1α,25-$(OH)_2$-D_3 for binding to the VDR. As shown in Table 1, the *N*-(2-hydroxyethyl)carbamoyl derivative **10c** was at least three times more potent than the other analogues.

As a measure of cell proliferation, [^{3}H]-thymidine incorporation of MCF-7 and keratinocytes was determined after a 72 h incubation period with various concentrations of 1α,25-$(OH)_2$-D_3, analogues or vehicle (arachis oil). The most active analogue to inhibit MCF-7 cell proliferation was the carbamate analogue **10a**, however this compound was 10 times less potent than 1α,25-$(OH)_2$-D_3. The analogue **10c** inhibited the MCF-7 cell proliferation only at the highest concentration of 10^{-6} M and was not able to inhibit the cell proliferation for 50% but only for 30% whereas 1α,25-$(OH)_2$-D_3 could inhibit the cell proliferation for 80% at this concentration (Table 1, Fig. 2A). The other analogues **10b**, **10e**, and **11** demonstrated no effects on the MCF-7 cell proliferation. All the evaluated analogues inhibited the proliferation of keratinocytes but all were less potent (1.3–11-fold) than the parent compound (Table 1, Fig. 2B).

Table 1. Biological activity of carbamate analogues of 1α,25-$(OH)_2$-D_3

Compound	VDR (%)	MCF-7 (%)	Keratinocytes (%)	HL 60 (%)
1α,25-$(OH)_2$-D_3	100	100	100	100
10a	<1	10	75	5
10b	<1	0	55	0
10c	3	4	65	0
10e	<1	0	20	2
11	<1	0	9	0

Summary of the in vitro effects of carbamate analogues of 1α,25-$(OH)_2$-D_3 on receptor binding (VDR), HL 60 differentiation, MCF-7, and keratinocyte proliferation. The in vitro effect is expressed as percentage activity (at EC_{50} or EC_{30} if analogues do not reach EC_{50}) in comparison with 1α,25-$(OH)_2$-D_3 (= 100% activity).

Differentiation of HL 60 cells (ATCC) was measured by the nitro blue tetrazolium reduction assay after a 72 h incubation period in presence of 1α,25-$(OH)_2$-D_3, analogues or vehicle.[16] Again all the analogues were less active than 1α,25-$(OH)_2$-D_3 (Table 1). The analogues **10a** and **10e** induced only differentiation at 10^{-6} M of, respectively, 50% and 30% whereas nearly 100% of the HL 60 cells were differentiated after incubation with 10^{-6} M 1α,25-$(OH)_2$-D_3 (Fig. 2C).

Besides the in vitro screening, the in vivo calcemic effects of the carbamate analogues were evaluated in mice. All the analogues were much less calcemic than 1α,25-$(OH)_2$-D_3 even at 10–100-fold higher doses (Fig. 3A and B) than 1α,25-$(OH)_2$-D_3 (0.1 μg/kg/d).

4. Conclusions

In summary, we synthesized several carbamate derivatives of 1α,25-$(OH)_2$-D_3 (A-ring modified analogues) through a chemoenzymatic process based in the selective functionalization catalyzed by CAL-B of the C-5 hydroxyl group in A-ring enyne **3**. The versatility of the route allows the introduction of several functional groups at the end of the alkyl chain of the carbamate moiety such as alcohol, amino, or acid. The results of VDR binding affinity indicated that these analogues exhibited less potency than 1α,25-$(OH)_2$-D_3. The most potent analogue to inhibit the cell proliferation of MCF-7 cells or keratinocytes or to stimulate the HL 60 cell differentiation is the analogue 3-*O*-carbamoyl-1α,25-$(OH)_2$-D_3 (**10a**) however this compound is still less potent than the parent compound 1α,25-$(OH)_2$-D_3 but has lower calcemic effects in vivo.

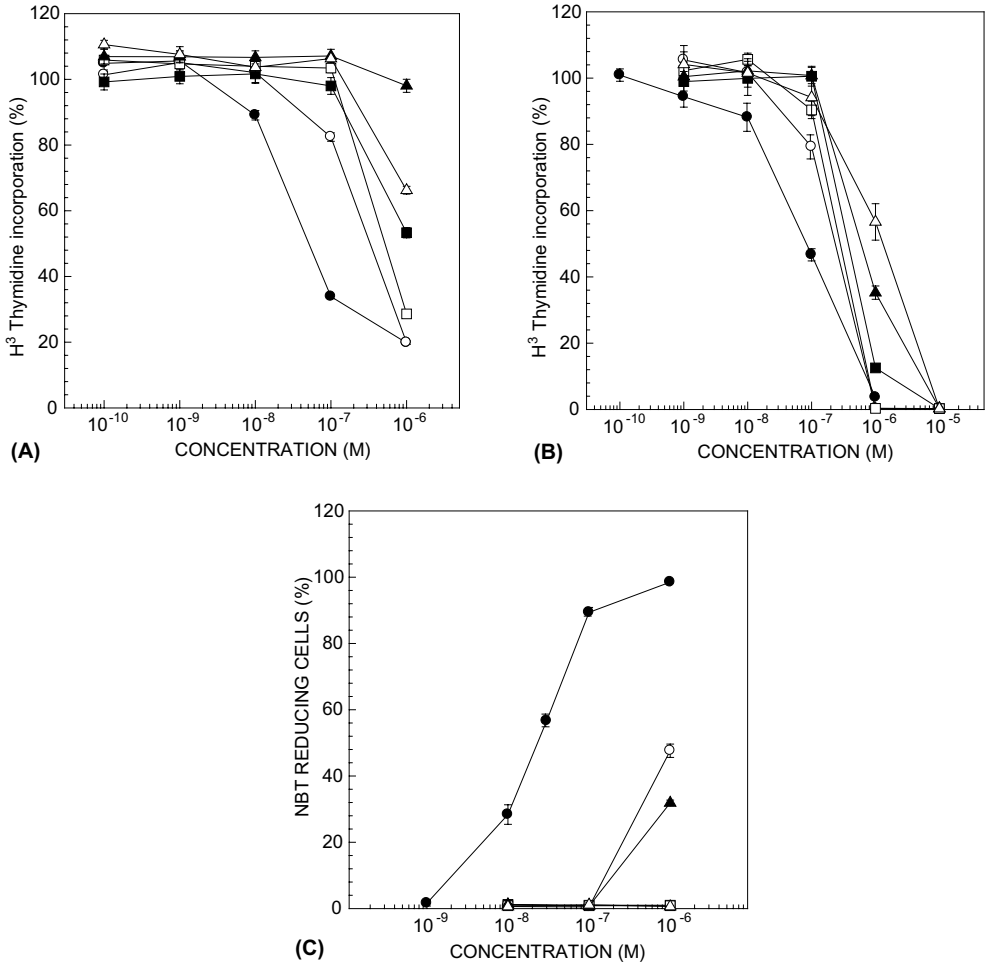


Figure 2. Prodifferentiating and antiproliferating effects of carbamate analogues on: (A) MCF-7; (B) keratinocytes; and (C) HL 60 cells. 1α,25-$(OH)_2$-D_3 (●); **10a** (○); **10b** (■); **10c** (□); **10e** (▲); **11** (△).

5. Experimental section

5.1. General spectroscopic and experimental data

Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on a Infrared Fourier Transform spectrophotometer using NaCl plates or KBr pellets. Flash chromatography was performed using silica gel 60 (230–400 mesh). ^1H, ^{13}C NMR, and DEPT were obtained using AC-200 (^1H, 200.13 MHz and ^{13}C, 50.3 MHz), AC-300 (^1H, 300.13 MHz, and ^{13}C, 75.5 MHz) or DPX 300 (^1H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometers for routine experiments. AMX-400 spectrometer operating at 400.13 and 100.61 MHz for ^1H and ^{13}C, respectively, was used for the acquisition of ^1H–^{13}C heteronuclear correlation experiments. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). EI (70 eV), FAB^+ (nitrobenzyl alcohol as matrix), and ES^+ were used to record mass spectra (MS). EI was used to record HRMS Microanalyses were performed on a Perkin–Elmer model 2400 instrument. HPLC was performed using UV detector and a Spherisorb W, 5 lm silica gel column, 250 × 10 mm.

5.2. Synthesis of carbamates 6a–e

Imidazole (54 mg, 0.793 mmol), DMAP (3.9 mg, 0.032 mmol), and *tert*-butyldimethylsilyl chloride (96 mg, 0.634 mmol) were added to a stirred solution of carbamates **5**[12] (0.317 mmol) in CH_2Cl_2 (2.5 mL) under nitrogen atmosphere. In case of **5c**, which possesses an extra hydroxyl group at the end of the alkyl chain, double amount of reagents were used. The reaction mixture was stirred at room temperature for 12 h. Then, a saturated solution of NH_4Cl was added, and the aqueous phase was extracted three times with CH_2Cl_2. Solvents were evaporated under reduce pressure, and the residue was purified by flash chromatographic column (20% EtOAc/hexane for **6a**, 5% EtOAc/hexane for **6b** and **6c**, 1% NH_3(aq)/MeOH for silyl ether precursor of **6d**, 60% EtOAc/MeOH for **6e**). To a solution of silyl ether precursor of **6d** (39 mg, 0.106 mmol) in $CHCl_3$ (2 mL), were added Boc_2O (26 mg, 0.117 mmol) and 0.1 mL of an aqueous saturated solution of $NaHCO_3$. The reaction mixture was stirred for 3 h at room temperature. After this time, H_2O was added and the aqueous layer was extracted three times with $CHCl_3$. Solvents were evaporated, and the residue was purified by flash

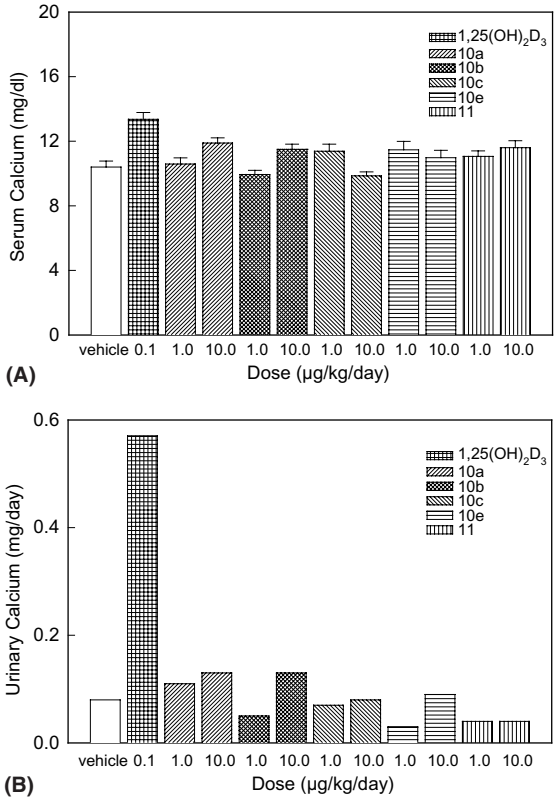


Figure 3. In vivo biological activity of carbamate analogues determined by measuring serum and urine calcium levels in mice after intraperitoneally injections after 7 consecutive days. Mice were injected with vehicle (arachis oil), 1α,25-$(OH)_2$-D_3 (0.1 μg/kg/d) or analogues (1 or 10 μg/kg/d).

chromatography column (20% EtOAc/hexane) to afford **6d**. Yields are given in Scheme 1.

5.2.1. (3*S*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-5-(carbamoyloxy)-1-ethynyl-2-methylcyclohex-1-ene (6a). R_f (20% EtOAc/hex): 0.3; Mp: 114–116 °C; IR (KBr): *v* 3456, 3282, 2955, 2931, 2859, 2095, 1696, 1607, 1407, 1363, 1075, and 837 cm^{-1}; ^1H NMR (MeOH-d_4, 300 MHz): δ 0.32 (s, 6H, 2*Me*Si), 1.11 (s, 9H, Me_3CSi), 2.07 (m, 1H, H_4), 2.10 (s, 3H, H_9), 2.17 (m, 1H, H_4), 2.33 (d, 1H, H_6, $^2J_{HH}$ 17.3 Hz), 2.72 (d, 1H, H_6, $^2J_{HH}$ 17.2 Hz), 3.63 (s, 1H, H_8), 4.51 (t, 1H, H_3, $^3J_{HH}$ 4.9 Hz), and 5.15 (m, 1H, H_5); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ −4.1 (*Me*Si), −3.7 (*Me*Si), 19.4 (C_9), 19.6 (Me_3*C*Si), 26.8 (Me_3CSi), 37.0 (C_6), 38.6 (C_4), 68.8 (C_5), 70.6 (C_3), 82.3 (C_8), 84.4 (C_7), 115.1 (C_1), 144.9 (C_2), and 159.8 (C_{10}); MS (EI, *m*/*z*): 252 [$(M - {}^tBu)^+$, 100%], 249 (21), 248 (62), 234 (16), and 233 (56); HRMS (*m*/*z*) Calcd for $C_{16}H_{27}NO_3Si$: 309.1760. Found: 309.1753; Anal Calcd (%) for $C_{16}H_{27}NO_3Si$: C, 61.75; H, 8.52; N, 4.53. Found: C, 62.1; H, 8.8; N, 4.5.

5.2.2. (3*S*,5*R*)-5-[*N*-(Butylcarbamoyl)oxy]-3-[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-2-methylcyclohex-1-ene (6b). R_f (5% EtOAc/hex): 0.2; Mp: 43–45 °C; IR (NaCl): *v* 3314, 2952, 2932, 2859, 2095, 1696, 1530, 1464, 1363, 1071, and 836 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz): δ 0.10 (s, 6H, 2*Me*Si), 0.90 (s, 9H, Me_3CSi), 0.92 (t, 3H, H_{14}, $^3J_{HH}$ 7.4 Hz), 1.34 (m, 2H, H_{13}), 1.46 (m, 2H, H_{12}), 1.87 (m, 1H, H_4), 1.94 (s, 3H, H_9), 1.96 (m, 1H, H_4), 2.19 (d, 1H, H_6, $^2J_{HH}$ 17.6 Hz), 2.59 (d, 1H, H_6, $^2J_{HH}$ 17.6 Hz), 3.05 (s, 1H, H_8), 3.15 (apparent q, 2H, H_{11}, $^3J_{HH}$ 6.5 Hz), 4.24 (m, 1H, H_3), and 5.06 (m, 1H, H_5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ −4.9 (*Me*Si), −4.4 (*Me*Si), 13.7 (C_{14}), 18.0 (Me_3*C*Si), 18.5 (C_9), 19.8 (C_{13}), 25.7 (Me_3CSi), 32.0 (C_{12}), 35.2 (C_6), 36.8 (C_4), 40.6 (C_{11}), 67.3 (C_5), 68.4 (C_3), 79.8 (C_8), 83.3 (C_7), 112.9 (C_1), 144.2 (C_2), and 155.8 (C_{10}); MS (FAB^+, *m*/*z*): 388 [$(M+Na)^+$, 30%], 364 [$(M - H)^+$, 3], 308 (12), 234 (38), and 73 (100); Anal Calcd (%) for $C_{20}H_{35}NO_3Si$: C, 65.71; H, 9.66; N, 3.83. Found: C, 65.9; H, 9.7; N, 3.8.

5.2.3. (3*S*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-5-[*N*-[[2-(*tert*-butyldimethyl-silyl)oxyethyl]-carbamoyl]oxy]-1-ethynyl-2-methylcyclohex-1-ene (6c). R_f (5% EtOAc/hex): 0.2; Colorless oil; IR (NaCl): *v* 3361, 3314, 2953, 2930, 2857, 2094, 1722, 1512, 1471, 1360, 1072, and 835 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.05 (s, 6H, 2*Me*Si), 0.09 (s, 6H, 2*Me*Si), 0.88 (s, 9H, Me_3CSi), 0.89 (s, 9H, Me_3CSi), 1.87 (m, 2H, H_4), 1.93 (s, 3H, H_9), 2.17 (d, 1H, H_6, $^2J_{HH}$ 16.0 Hz), 2.59 (d, 1H, H_6, $^3J_{HH}$ 17.2 Hz), 3.04 (s, 1H, H_8), 3.27 (q, 2H, H_{11}, $^3J_{HH}$ 10.4 Hz), 3.66 (t, 2H, H_{12}, $^3J_{HH}$ 5.1 Hz), 4.23 (t, 1H, H_3, $^3J_{HH}$ 5.3 Hz), and 5.00 (m, 1H, H_5); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ −5.4 (Me_2Si), −4.9 (*Me*Si), −4.4 (*Me*Si), 17.9 (Me_3*C*Si), 18.2 (Me_3*C*Si), 18.6 (C_9), 25.7 (Me_3CSi), 25.8 (Me_3CSi), 35.2 (C_6), 36.8 (C_4), 43.1 (C_{11}), 62.0 (C_{12}), 67.3 (C_5), 68.5 (C_3), 79.8 (C_8), 83.2 (C_7), 112.9 (C_1), 144.0 (C_2), and 155.8 (C_{10}); MS (EI, *m*/*z*): 410 [$(M - {}^tBu)^+$, 4%], 249 (12), 248 (13), 162 (40), 117 (20), 115 (24), 100 (17), and 75 (100); HRMS (*m*/*z*) Calcd for $C_{20}H_{36}NO_4Si_2$ ($M - {}^tBu$): 410.2183. Found: 410.2176; Anal Calcd (%) for $C_{24}H_{45}NO_4Si_2$: C, 61.62; H, 9.47; N, 2.99. Found: C, 61.8; H, 9.4; N, 3.1.

5.2.4. (3*S*,5*R*)-5-[N^1-[3-(N^2-Boc-aminopropyl)carbamoyl]oxy]-3-[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-2-methylcyclohex-1-ene (6d). R_f (25% EtOAc/hex): 0.2; Colorless oil; IR (NaCl): *v* 3347, 3311, 2953, 2931, 2858, 2094, 1696, and 1516 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.09 (s, 6H, 2*Me*Si), 0.88 (s, 9H, Me_3CSi), 1.42 (s, 9H, Me_3C), 1.90 (m, 2H, H_4), 1.93 (s, 3H, H_9), 2.16 (d, 1H, H_6, $^3J_{HH}$ 5.9 Hz), 2.58 (dd, 1H, H_6, $^2J_{HH}$ 16.7, $^3J_{HH}$ 2.8 Hz), 3.04 (s, 1H, H_8), 3.18 (m, 4H, $2H_{11}$+$2H_{13}$), 4.22 (m, 1H, H_3), 4.88 (s, 2H, 2NH), and 5.05 (m, 1H, H_5); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ −4.9 (*Me*Si), −4.5 (*Me*Si), 17.9 (Me_3*C*Si), 18.5 (C_9), 25.7 (Me_3CSi), 28.3 (Me_3C), 30.6 (C_{12}), 35.2 (C_6), 36.8 (C_4), 37.1 (C_{13}), 37.50 (C_{11}), 67.4 (C_5), 68.5 (C_3), 79.2 (Me_3*C*), 79.8 (C_8), 83.2 (C_7), 112.9 (C_1), 144.0 (C_2), 156.2 (C_{10}), and 156.3 (C_{14}); MS (EI, *m*/*z*): 409 [$(M - {}^tBu)^+$, 20%], 335 (2), 309 (5), 249 (16), 248 (62), 115 (35), and 75 (100); HRMS (*m*/*z*) Calcd for $C_{20}H_{33}N_2O_5Si$ ($M - {}^tBu$): 409.2159. Found: 409.2163; Anal Calcd (%) for $C_{24}H_{42}N_2O_5Si$: C, 61.76; H, 9.08; N, 6.01. Found: C, 61.8; H, 9.2; N, 6.1.

5.2.5. (3*S*,5*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-5-[*N*-[(carboxymethyl)carbamoyl]oxy]-1-ethynyl-2-methylcyclohex-1-ene sodium salt (6e). R_f (70% EtOAc/MeOH): 0.2;

Mp: 148–150 °C; IR (KBr): ν 3420, 3313, 2957, 2931, 2858, 2096, 1701, 1595, 1406, 1362, 1257, 1073, and 837 cm^{-1}; ^1H NMR (MeOH-d_4, 300 MHz): δ 0.32 (s, 6H, 2*Me*Si), 1.11 (s, 9H, Me_3CSi), 2.10 (m, 2H, H_4), 2.11 (s, 3H, H_9), 2.36 (dd, 1H, H_6, $^2J_{HH}$ 16.4, $^3J_{HH}$ 6.1 Hz), 2.74 (dd, 1H, H_6, $^2J_{HH}$ 17.1, $^3J_{HH}$ 6.0 Hz), 3.64 (s, 1H, H_8), 3.88 (s, 2H, H_{11}), 4.51 (m, 1H, H_3), and 5.17 (m, 1H, H_5); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ −4.2 (*Me*Si), −3.7 (*Me*Si), 19.4 (Me_3*C*Si), 19.5 (C_9), 26.8 (Me_3CSi), 37.1 (C_6), 38.7 (C_4), 45.8 (C_{11}), 69.1 (C_5), 70.6 (C_3), 82.3 (C_8), 84.5 (C_7), 115.1 (C_1), 144.8 (C_2), 158.8 (C_{10}), and 178.0 (C_{12}); MS (ES^+, *m/z*): 413 [$(MH+Na)^+$, 100%] and 390 [$(MH)^+$, 12]; Anal Calcd (%) for $C_{18}H_{28}NNaO_5Si$: C, 55.50; H, 7.25; N, 3.6. Found: C, 55.8; H, 7.1; N, 3.3.

5.3. Synthesis of dienynes 8a–e

CuI (1.7 mg, 0.009 mmol), $Pd(PPh_3)_2(OAc)_2$ (2.0 mg, 0.003 mmol), and Et_2NH (0.55 mL) were added to a stirred solution of **6** (0.097 mmol) and **7** (43 mg, 0.088 mmol) in DMF (0.55 mL) under nitrogen atmosphere. In order to improve the yield in the coupling reaction of **8e**, 0.2 equiv of CuI and 0.15 equiv of $Pd(PPh_3)_2(OAc)_2$ were used. After 1 h at room temperature, the resulting mixture was diluted with H_2O, and the aqueous phase was extracted three times with Et_2O. The organic layer was dried (Na_2SO_4) and evaporated to dryness to give a crude, which was sufficiently pure for the next step. TBAF (0.44 mL, 0.441 mmol, 1.0 M in THF) was added dropwise to a solution of this crude in THF (3 mL) at 0 °C and the reaction was stirred for 12 h at room temperature. THF was evaporated and the residue was poured into water/EtOAc. The aqueous layer was extracted with EtOAc, solvents were evaporated, and the residue was purified by flash chromatography column (20% ^{i}PrOH/hexane for **8a**, 40% EtOAc/hexane for **8b**, 20% ^{i}PrOH/hexane for **8c**, 65% EtOAc/hexane for **8d**, 50% EtOAc/MeOH for **8e**). Yields are given in Scheme 2.

5.3.1. 3-*O*-Carbamoyl-6,7-dehydro-1α,25-dihydroxyvitamin D_3 (8a). R_f (20% ^{i}PrOH/hex): 0.3; Mp: 138–140 °C; IR (KBr): ν 3387, 2951, 2872, 2187, 1700, 1541, 1458, 1377, and 1269 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H, H_{18}), 0.94 (d, 3H, H_{21}, $^3J_{HH}$ 6.4 Hz), 1.20 (s, 6H, $3H_{26}$+$3H_{27}$), 1.00–2.65 (m, 21H, $2H_2$+$2H_4$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$), 1.99 (s, 3H, H_{19}), 4.21 (t, 1H, H_1, $^3J_{HH}$ 4.6 Hz), 4.79 (s, 4H, 2NH+2OH), 5.00 (m, 1H, H_3), and 5.96 (m, 1H, H_9); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 11.0, 18.5, 18.6, 20.7, 24.1, 25.1, 27.9, 29.1, 29.3, 35.6, 25.8, 36.0, 36.2, 36.5, 41.7, 44.2, 49.9, 54.6, 67.5, 68.5, 71.1, 87.1, 93.4, 115.2, 122.2, 133.8, 139.8, and 156.4; ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 11.0, 18.5, 18.6, 20.7, 24.1, 25.1, 27.9, 29.1, 29.3, 35.6, 25.8, 36.0, 36.2, 36.5, 41.7, 44.2, 49.9, 54.6, 67.5, 68.5, 71.1, 87.1, 93.4, 115.2, 122.2, 133.8, 139.8, and 156.4; MS (ES^+, *m/z*): 480 [$(M+Na)^+$, 100%] and 413 (40); Anal Calcd (%) for $C_{28}H_{43}NO_4$: 73.47; H, 9.48; N, 3.06. Found: C, 73.3; H, 9.5; N, 3.1.

5.3.2. 3-*O*-(*N*-Butylcarbamoyl)-6,7-dehydro-1α,25-dihydroxyvitamin D_3 (8b). R_f (40% EtOAc/hex): 0.1; Mp: 74–76 °C; IR (KBr): ν 3348, 2964, 2872, 2187, 1703, 1535, 1466, 1379, and 1253 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.70 (s, 3H, H_{18}), 0.92 (m, 6H, $3H_{21}$+$3H_{32}$), 1.21 (s, 6H, $3H_{26}$+$3H_{27}$), 1.11–2.70 (m, 25H, $2H_2$+$2H_4$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$+$2H_{30}$+$2H_{31}$), 1.99 (s, 3H, H_{19}), 3.18 (m, 2H, H_{29}), 4.24 (m, 1H, H_1), 4.63 (m, 1H, NH), 5.03 (m, 1H, H_3), and 5.98 (m, 1H, H_9); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 10.9, 13.6, 18.4, 18.6, 19.8, 20.6, 20.7, 24.0, 25.1, 27.9, 29.0, 29.2, 31.9, 35.8, 36.0, 36.2, 36.7, 40.5, 41.7, 44.2, 49.9, 54.6, 67.0, 68.4, 71.0, 87.3, 93.1, 115.0, 122.3, 133.5, 140.1, and 155.9; MS (FAB^+, *m/z*): 536 [$(M+Na)^+$, 97%], 496 [$(M-OH)^+$, 10], 413 (12), 396 (90), 379 (30), and 361 (42); Anal Calcd (%) for $C_{32}H_{51}NO_4$: C, 74.80; H, 10.01; N, 2.73. Found: C, 74.6; H, 10.1; N, 2.9.

5.3.3. 6,7-Dehydro-3-*O*-[*N*-(2-hydroxyethyl)carbamoyl]-1α,25-dihydroxyvitamin D_3 (8c). R_f (20% ^{i}PrOH/hex): 0.1; Mp: 129–131 °C; IR (KBr): ν 3435, 3202, 2957, 2872, 2186, 1702, 1677, and 1355 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H, H_{18}), 0.93 (d, 3H, H_{21}, $^3J_{HH}$ 6.2 Hz), 1.24 (s, 6H, $3H_{26}$+$3H_{27}$), 1.00–2.30 (m, 21H, $2H_2$+$2H_4$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$), 1.96 (s, 3H, H_{19}), 2.57 (d, 1H, H_4, $^2J_{HH}$ 13.6 Hz), 3.28 (m, 2H, H_{29}), 3.65 (m, 2H, H_{30}), 4.19 (m, 1H, H_1), 4.99 (m, 1H, H_3), 5.46 (m, 1H, NH), and 5.94 (d, 1H, H_9, $^3J_{HH}$ 2.6 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 11.0, 18.5, 18.6, 20.8, 24.1, 25.1, 25.2, 27.9, 29.0, 29.2, 35.8, 36.0, 36.3, 36.7, 41.7, 43.2, 44.2, 49.9, 54.6, 61.7, 67.4, 68.4, 71.1, 87.2, 96.3, 115.1, 122.3, 133.7, 140.0, and 156.7; MS (ES^+, *m/z*): 524 [$(M+Na)^+$, 100%] and 540 [$(M+K)^+$, 43]; Anal Calcd (%) for $C_{30}H_{47}NO_5$: C, 71.82; H, 9.44; N, 2.79. Found: 71.6; H, 9.5; N, 2.8.

5.3.4. 3-*O*-[N^1-(3-N^2-Boc-aminopropyl)carbamoyl]-6,7-dehydro-1α,25-dihydroxyvitamin D_3 (8d). R_f (65% EtOAc/hex): 0.3; Mp: 68–70 °C; IR (KBr): ν 3405, 2964, 2880, 2191, 1690, 1530, 1451, and 1366 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H, H_{18}), 0.93 (d, 3H, H_{21}, $^3J_{HH}$ 6.4 Hz), 1.20 (s, 6H, $3H_{26}$+$3H_{27}$), 1.42 (s, 9H, Me_3C), 1.98 (s, 3H, H_{19}), 2.58 (dd, 1H, H_4, $^2J_{HH}$ 16.7, $^3J_{HH}$ 3.3 Hz), 1.00–2.20 (m, 22H, $2H_2$+H_4+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$+$2H_{30}$), 3.16 (m, 4H, H_{29}+$2H_{31}$), 4.23 (m, 1H, H_1), 4.94 (m, 2H, 2NH), 5.22 (m, 1H, H_3), and 5.95 (d, 1H, H_9, $^3J_{HH}$ 3.1 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 11.0, 18.4, 18.6, 20.8, 24.1, 25.1, 27.9, 28.3 (3C), 29.1, 29.3, 30.5, 35.8, 36.1, 36.3, 36.7, 37.1, 37.5, 41.8, 44.3, 49.9, 54.6, 67.1, 68.5, 71.0, 79.2, 87.2, 93.3, 115.3, 122.3, 133.7, 139.9, and 156.3 (2C); MS (ES^+, *m/z*): 637 [$(M+Na)^+$, 100%] and 638 [$(MH+Na)^+$, 47]; Anal Calcd (%) for $C_{36}H_{58}N_2O_6$: C, 70.31; H, 9.51; N, 4.56. Found: C, 70.3; H, 9.5; N, 4.6.

5.3.5. 3-*O*-[*N*-(Carboxymethyl)carbamoyl]-6,7-dehydro-1α,25-dihydroxyvitamin D_3 sodium salt (8e). R_f (60% EtOAc/MeOH): 0.3; Mp: 130–132 °C; IR (KBr): ν 3420, 2963, 2940, 2878, 2185, 1701, 1686, 1596, 1406, and 1279 cm^{-1}; ^1H NMR (MeOH-d_4, 300.13 MHz): δ 0.92

(s, 3H, H_{18}), 1.18 (d, 3H, H_{21}, $^3J_{HH}$ 6.6 Hz), 1.37 (s, 6H, $3H_{26}$+$3H_{27}$), 1.00–2.60 (m, 20H, $2H_2$+H_4+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$), 2.14 (s, 3H, H_{19}), 2.74 (d, 1H, H_4, $^2J_{HH}$ 13.6 Hz), 3.86 (s, 2H, H_{29}), 4.41 (m, 1H, H_1), 5.20 (m, 1H, H_3), and 6.10 (d, 1H, H_9, $^3J_{HH}$ 3.1 Hz); ^{13}C NMR (MeOH-d_4, 100.6 MHz): δ 11.9, 19.3, 19.7, 22.4, 25.9, 26.6, 29.6 (2C), 29.8, 37.5, 37.7, 38.0, 38.2, 38.4, 43.5, 45.8, 46.1, 51.9, 56.6, 69.1, 69.5, 72.0, 89.0, 94.5, 116.7, 124.4, 134.6, 141.8, 158.8, and 177.7; MS (ES^+, *m/z*): 560 [$(M+Na)^+$, 6%], 538 [$(MH)^+$, 5], 413 (16), 242 (100); Anal Calcd (%) for $C_{30}H_{44}NNaO_6$: C, 67.00; H, 8.25; N, 2.61. Found: C, 66.8; H, 8.4; N, 2.7.

5.4. Synthesis of C-3 carbamate analogues of 1α,25-dihydroxyvitamin D_3 10a–d

A flask containing Lindlar catalyst (50 mg, 0.024 mmol, pre-dried at 60 °C during 4 h in vacuo) was exposed to a positive pressure of hydrogen gas (balloon). A mixture of dienyne **8** (0.039 mmol) and quinoline (14 μL, 0.024 mmol, 0.17 M in hexane) in deoxygenated MeOH (2.3 mL) was added and the reaction was stirred for 1 h at room temperature. The mixture was filtered on Celite and concentrated to afford crude **9**. ^{1}H NMR analysis of the latter material showed signals of the compound **9**. A solution of the crude previtamin **9** in acetone (3 mL) was placed in a screw-capped vial and heated for 4 h in a constant temperature bath set at 80 °C. For **9e**, thermolysis was performed at 65 °C during 3 h. The residue was concentrated under vacuum and purified by flash chromatography column (15% ^{i}PrOH/hexane for **10a** and **10c**, 40% EtOAc/hexane for **10b**, 60% EtOAc/hexane for **10d**, 50% ^{i}PrOH/hexane for **10e**). Further purification by HPLC (Spherisorb W, 5 μm silica gel column, 250 × 10 mm, 4 mL/min, 12% ^{i}PrOH/hexane for **10a** and **10c**, 30% EtOAc/hexane for **10b** and **10d**, 50% ^{i}PrOH/hexane for **10e**).

5.4.1. 3-*O*-Carbamoyl-1α,25-dihydroxyvitamin D_3 (10a). R_f (15% ^{i}PrOH/hex): 0.2; Mp: 98–100 °C; IR (KBr): *v* 3396, 2947, 2871, 1718, 1604, 1397, 1327, and 1262 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.54 (s, 3H, H_{18}), 0.93 (d, 3H, H_{21}, $^3J_{HH}$ 6.2 Hz), 1.21 (s, 6H, $3H_{26}$+$3H_{27}$), 1.00–2.70 (m, 22H, $2H_2$+H_4+$2H_9$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$), 2.81 (dd, 1H, H_4, $^2J_{HH}$ 11.5, $^3J_{HH}$ 4.0 Hz), 4.39 (c, 1H, H_1, $^3J_{HH}$ 8.8 Hz), 4.63 (s, 4H, 2NH+2OH), 5.01 (s, 1H, H_{19}), 5.10 (m, 1H, H_3), 5.35 (s, 1H, H_{19}), 6.02 (d, 1H, H_7, $^3J_{HH}$ 11.6 Hz), and 6.34 (d, 1H, H_6, $^3J_{HH}$ 11.5 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 11.9, 18.7, 20.7, 22.2, 23.5, 27.6, 29.0, 29.1, 29.3, 36.0, 36.3, 39.9, 40.4, 41.6, 44.3, 45.9, 56.3, 56.4, 70.2, 70.4, 71.1, 111.4, 116.9, 124.5, 132.5, 143.2, 147.4, and 156.2; MS (ES^+, *m/z*): 482 [$(M+Na)^+$, 100%]; Anal Calcd (%) for $C_{28}H_{45}NO_4$: C, 73.15; H, 9.87; N, 3.05. Found: C, 73.4; H, 9.7; N, 3.1.

5.4.2. 3-*O*-(*N*-Butylcarbamoyl)-1α,25-dihydroxyvitamin D_3 (10b). R_f (40% EtOAc/hex): 0.2; Mp: 126–128 °C; IR (KBr): *v* 3360, 2943, 2872, 1695, 1531, 1444, 1377, and 1259 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.54 (s, 3H, H_{18}), 0.92 (d, 3H, H_{21}, $^3J_{HH}$ 6.1 Hz), 1.20 (s, 6H, $3H_{26}$+$3H_{27}$), 1.20–2.90 (m, 29H, $2H_2$+H_4+$2H_9$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$), 3.16 (m, 2H, H_{29}), 4.36 (m, 1H, H_1), 4.65 (s, 1H, NH), 5.00 (s, 1H, H_{19}), 5.10 (m, 1H, H_3), 5.35 (s, 1H, H_{19}), 6.03 (d, 1H, H_7, $^3J_{HH}$ 11.3 Hz), and 6.32 (d, 1H, H_6, $^3J_{HH}$ 11.3 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 11.9, 13.7, 18.8, 19.8, 20.7, 22.2, 23.6, 27.6, 29.1 (2C), 29.3, 31.9, 36.0, 36.3, 40.2, 40.4, 40.6, 41.8, 44.3, 45.9, 56.2, 56.4, 69.8, 70.2, 71.1, 111.0, 117.0, 124.3, 133.0, 143.0, 147.7, and 155.9; MS (ES^+, *m/z*): 538 [$(M+Na)^+$, 100%]; HRMS (*m/z*) Calcd for $C_{32}H_{52}NO_3$ (M − OH): 498.3947. Found: 498.3923; Anal Calcd (%) for $C_{32}H_{53}NO_4$: C, 74.51; H, 10.36; N, 2.72. Found: C, 74.4; H, 10.5; N, 2.6.

5.4.3. 3-*O*-[*N*-(2-Hydroxyethyl)carbamoyl]-1α,25-dihydroxyvitamin D_3 (10c). R_f (20% ^{i}PrOH/hex): 0.2; Mp: 95–97 °C; IR (KBr): *v* 3419, 2946, 2872, 1700, 1538, 1376, and 1262 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.53 (s, 3H, H_{18}), 0.92 (d, 3H, H_{21}, $^3J_{HH}$ 6.3 Hz), 1.20 (s, 6H, $3H_{26}$+$3H_{27}$), 1.00–2.90 (m, 21H, $2H_2$+$2H_4$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$), 3.31 (m, 2H, H_{30}), 3.68 (m, 1H, H_{29}), 4.35 (q, 1H, H_1, $^3J_{HH}$ 8.5 Hz), 5.00 (s, 1H, H_{19}), 5.11 (m, 1H, H_3), 5.24 (m, 1H, NH), 5.34 (s, 1H, H_{19}), 6.02 (d, 1H, H_7, $^3J_{HH}$ 10.9 Hz), and 6.32 (d, 1H, H_6, $^3J_{HH}$ 11.4 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 11.9, 18.7, 20.7, 22.2, 23.5, 27.6, 29.1 (2C), 29.3, 36.0, 36.3, 40.1, 40.4, 41.7, 43.3, 44.3, 45.9, 56.3, 56.5, 62.3, 70.2, 70.4, 71.1, 111.3, 117.0, 124.5, 132.7, 143.1, 147.4, and 156.7; MS (ES^+, *m/z*): 526 [$(M+Na)^+$, 100%], and 242 (42); Anal Calcd (%) for $C_{30}H_{49}NO_5$: C, 71.52; H, 9.81; N, 2.78. Found: C, 71.7; H, 9.9; N, 2.5.

5.4.4. 3-*O*-[N^1-(3-N^2-Boc-aminopropyl)carbamoyl]-1α,25-dihydroxyvitamin D_3 (10d). R_f (60% EtOAc/hex): 0.4; Colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.54 (s, 3H, H_{18}), 0.93 (d, 3H, H_{21}, $^3J_{HH}$ 6.3 Hz), 1.21 (s, 6H, $3H_{26}$+$3H_{27}$), 1.42 (s, 9H, Me_3C), 1.00–2.90 (m, 25H, $2H_2$+$2H_4$+$2H_9$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$+$2H_{30}$), 3.17 (m, 4H, $2H_{29}$+$2H_{31}$), 4.38 (m, 1H, H_1), 4.87 (s, 2H, 2NH), 5.01 (s, 1H, H_{19}), 5.07 (m, 1H, H_3), 5.34 (s, 1H, H_{19}), 6.03 (d, 1H, H_7, $^3J_{HH}$ 12.0 Hz), and 6.32 (d, 1H, H_6, $^3J_{HH}$ 11.1 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 11.9, 18.7, 20.7, 22.2, 23.5, 27.6, 28.3 (3C), 29.0, 29.1, 29.3, 30.5, 36.0, 36.3, 37.1, 37.5, 40.1, 40.4, 41.8, 44.3, 45.9, 56.3, 56.4, 70.1, 70.2, 71.0, 79.2, 111.3, 117.0, 124.4, 132.8, 143.0, 147.4, and 156.3 (2C).

5.4.5. 3-*O*-[*N*-(Carboxymethyl)carbamoyl]-1α,25-dihydroxyvitamin D_3 sodium salt (10e). R_f (80% EtOAc/MeOH): 0.1; Mp: 140–142 °C; IR (KBr): *v* 3407, 2945, 2868, 1701, 1597, 1403, 1379, and 1279 cm^{-1}; ^{1}H NMR (MeOH-d_4, 200 MHz): δ 0.77 (s, 3H, H_{18}), 1.16 (d, 3H, H_{21}, $^3J_{HH}$ 6.0 Hz), 1.36 (s, 6H, $3H_{26}$+$3H_{27}$), 1.00–2.80 (m, 22H, $2H_2$+H_4+$2H_9$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$), 3.04 (d, 1H, H_4, $^2J_{HH}$ 11.4 Hz), 3.89 (s, 2H, H_{29}), 4.52 (m, 1H, H_1), 5.12 (s, 1H, H_{19}), 5.26 (m, 1H, H_3), 5.55 (s, 1H, H_{19}), 6.26 (d, 1H, H_7, $^3J_{HH}$ 10.8 Hz), and 6.49 (d, 1H, H_6, $^3J_{HH}$ 11.4 Hz); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 12.9, 19.9, 22.4, 23.9, 25.2, 29.2, 29.6, 29.8, 30.5, 38.0,

41.7, 42.4, 43.3, 45.5, 45.8, 47.5, 52.6, 58.1, 58.5, 71.0, 72.0, 72.2, 111.8, 119.3, 125.4, 135.7, 143.4, 150.3, 158.8, and 177.1; MS (ES^+, *m/z*): 540 [$(MH)^+$, 43%], and 413 (100); Anal Calcd (%) for $C_{30}H_{46}NNaO_6$: C, 66.75; H, 8.60; N, 2.60. Found: C, 66.9; H, 8.8; N, 2.5.

5.5. Synthesis of 3-*O*-[*N*-(aminopropyl)carbamoyl]-1α,25-dihydroxyvitamin D_3 hydrochloride (11)

A solution of **10d** (16.6 mg, 0.027 mmol) in EtOH (1 mL) was added to a solution of HCl/EtOH [12 mL, generated by bubbled HCl(g) in EtOH]. The reaction was stirred for 0.5 h, then the EtOH was evaporated and the residue was washed with Et_2O, affording **11** as a white solid (69%). Mp: 150–152 °C; IR (KBr): *ν* 3422, 2942, 2868, 1701, 1655, 1542, 1377, and 1263 cm^{-1}; ^1H NMR (MeOH-d_4, 200 MHz): *δ* 0.77 (s, 3H, H_{18}), 1.16 (d, 3H, H_{21}, $^3J_{HH}$ 6.0 Hz), 1.36 (s, 6H, $3H_{26}$+$3H_{27}$), 1.00–3.00 (m, 25H, $2H_2$+$2H_4$+$2H_9$+$2H_{11}$+$2H_{12}$+H_{14}+$2H_{15}$+$2H_{16}$+H_{17}+H_{20}+$2H_{22}$+$2H_{23}$+$2H_{24}$+$2H_{30}$), 3.10–3.50 (m, 4H, $2H_{29}$+$2H_{31}$), 4.51 (m, 1H, H_1), 5.12 (s, 1H, H_{19}), 5.26 (m, 1H, H_3), 5.55 (s, 1H, H_{19}), 6.24 (d, 1H, H_7, $^3J_{HH}$ 10.8 Hz), and 6.48 (d, 1H, H_6, $^2J_{HH}$ 11.4 Hz); ^{13}C NMR (MeOH-d_4, 75.5 MHz): *δ* 12.8, 19.9, 22.4, 23.8, 25.2, 27.1, 29.2, 29.6, 29.8, 30.5, 37.9, 38.2, 38.4, 41.6, 42.4, 43.3, 45.8, 47.5, 58.1, 58.5, 71.0, 72.0, 72.4, 112.0, 119.2, 125.4, 131.8, 135.6, 150.2, and 159.8; MS (ES^+, *m/z*): 567 [$(M - Cl)^+$, 100%] and 499 (28); Anal Calcd (%) for $C_{31}H_{53}ClN_2O_4$: C, 67.35; H, 9.67; N, 5.07. Found: C, 67.5; H, 9.8; N, 4.7.

5.6. In vitro and in vivo biological evaluation

5.6.1. VDR binding assays. Vitamin D receptor binding assays were performed with calf thymus cells. These VDRs were saturated with [^3H]-calcitriol. Then solutions of vitamin D analogues were added whereby tritiated calcitriol was removed from the VDR. Affinity toward the VDR was measured by counting the amount of radioactive calcitriol that remains specifically bound to the VDR.

5.6.2. Cell proliferation assays. As a measure of cell proliferation, [^3H]-thymidine incorporation of breast cancer MCF-7 (ATCC, Rockville, MD) and human keratinocytes were determined after a 72 h incubation period with various concentrations of 1α,25-$(OH)_2$-D_3, analogues or vehicle as described previously.[16]

5.6.3. Cell differentiation assays. Differentiation of promyeolocytic HL 60 leukemia cells (ATCC) was measured by the nitro blue tetrazolium reduction assay after a 72 h incubation period in presence of 1,25-$(OH)_2$-D_3, analogues or vehicle.[16]

5.6.4. In vivo calcemic activity. Eight weeks old, male, NMRI mice were obtained from the Proefdierencentrum of Leuven (Belgium) and fed with a vitamin D-replete diet (1% calcium, 1% phosphate, and 2500 units vitamin D/kg; Hope Farms, Woerden, The Netherlands). The calcemic effects of the analogues were tested by daily injections intraperitoneally of 1α,25-$(OH)_2$-D_3 (0.1 μg/kg/d), analogues (1 or 10 μg/kg/d), or vehicle (arachis oil) for 7 consecutive days to groups of six mice. Serum and urinary calcium were measured as calcemic parameters using a commercially available kit (Sigma Diagnostics).

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14. Note that α (down) and β (up) have their usual definitions for the structures of vitamin D_3 and its analogues. However the A-ring synthon **3** and its derivatives has different numbering, compare Figure 1 with Scheme 1. Thus, carbons 1 and 3 in vitamin D structures correspond with carbons 3 and 5 in the A-ring, respectively.
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