Organic Process Research & Development **2007**, *11*, 200–205

Practical Synthesis of a Heterocyclic Immunosuppressive Vitamin D Analogue

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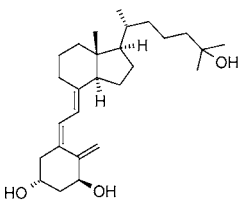
Abstract:

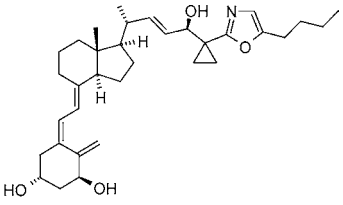
1α,25-Dihydroxyvitamin D_3 (calcitriol) 1 and synthetic analogues thereof are highly potent compounds with a wide range of pharmacological activity making them of great interest for the pharmaceutical industry. Herein we report an improved synthesis of the calcitriol analogue 2, which features a novel oxazole-containing side chain. The crucial part of the synthesis was the development of a practical route to the β-keto phosphonate 28, allowing an easy introduction of the unnatural side chain by a Wittig Horner reaction.

Introduction

The hormonally active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (**1**, calcitriol), has a remarkably wide range of pharmacological activities. The discovery that calcitriol (**1**) possesses next to its well-known calciotropic activity,[1] additional immunosuppressive,[2] cellular proliferation inhibiting, and cellular differentiation inducing[3] activities has resulted in a very active search for synthetic analogues featuring dissociation of the calcemic from the other pharmacological effects.[4] Many of the analogues found represent modifications in the side chain. Among them, the calcitriol analogue ZK 191784 (**2**), which bears an oxazole unit in the side chain, has been shown to be a very promising compound for the treatment of psoriasis.[5] This compound has a therapeutic advantage over 1α,25-dihydroxyvitamin D_3 (**1**) by inducing immunosuppressive effects without causing hypercalcemia.



1 (calcitriol)

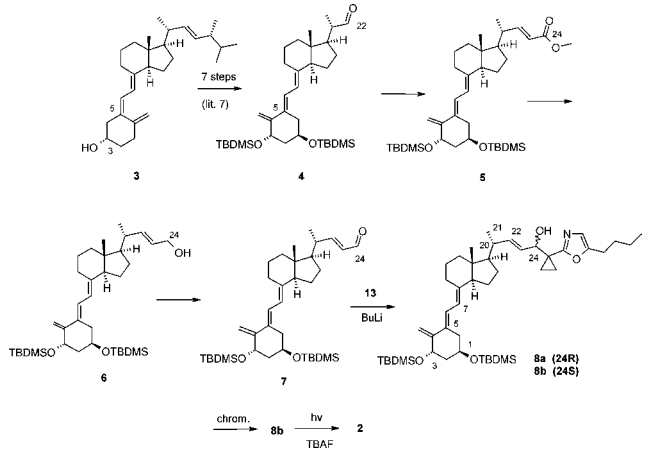


2 (ZK 191784)

In general, the assembly of vitamin D analogues can be envisioned either by starting from steroid precursors or by total synthesis.[6] Several years ago we assessed both options and decided to build up our calcitriol technology platform based on the aldehyde **4** which we manufacture on a multikilogram scale (30% yield from **3**; see Scheme 1) starting from vitamin D_2 (**3**) in analogy to a known literature protocol.[7]

Aldehyde **4** allows synthesis of a broad variety of calcitriol derivatives with differently substituted side chains by using Wittig-type reactions. In particular, the medicinal chemistry route for the preparation of **2** utilizes a Wittig–Horner reaction and subsequent cyclopropyl lithium addition as key steps towards introduction of the desired side chain. In particular, reaction of **4** with methyl (dimethyl)phosphinylacetate yielded the 24-ester **5**, which was converted to the corresponding 24-alcohol **6** by DIBAH reduction. Subsequent Swern oxidation[8] of the allylic alcohol **6** led to the 24-enal **7**. The generation of the side chain was performed by addition of the lithiated cyclopropane derivative obtained by metal–halogen exchange between BuLi and oxazolyl bromide **13**[9] (Scheme 1). Unfortunately, the addition is nonstereoselective, and the 1:1 mixture of the 24-alcohols **8a**/**8b** obtained demands for separation of the diastereomers by chromatography.

Scheme 1. **Research synthesis of ZK 191784 (2)**



A key intermediate in the preparation of oxazole **13** was 1-aminohexan-2-one **10** which synthesis comprises the α-nitrosation of ketoester **9** followed by reduction of the

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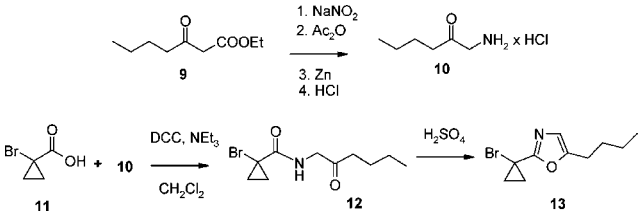
(1) Christakos, S.; Raval-Pandya, M.; Wernyj, R. P.; Yang, W. *Biochem. J.* **1996**, *316*, 361–371.
(2) Casteels, K.; Bouillon, R.; Waer, M.; Mathieu, C. *Curr. Opin. Nephrol. Hypertens.* **1995**, *4*, 313–318.
(3) Van Leewen, J. P. T. M.; Pols, H. A. P. In *Vitamin D: Anticancer and Differentiation in Vitamin D*; Feldman, D., Glorieux, F. H., Pike, J. W., Eds.; Academic Press: San Diego, CA, 1997; pp 1089–1105.
(4) Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocrinol. Rev.* **1995**, *16*, 200–257.
(5) Zuegel, U.; Steinmeyer, A.; Giesen, C.; Asadullah, K. *J. Invest. Dermatol.* **2002**, *119* (6), 1434–1442.
(6) Zhu, G. D.; Okamura, W. H. *Chem. Rev.* **1995**, *95*, 1877–1952.
(7) (a) Andrews, D. R.; Barton, D. H. R.; Hesse, R. H.; Pechet, M. M. *J. Org. Chem.* **1986**, *51*, 4819–4828. (b) Calverly, M. R. *Tetrahedron* **1987**, *43*, 4609–4619.
(8) Steinmeyer, A.; Schwarz, K.; Haberey, M.; Langer, G.; Wiesinger, H. *Steroids* **2001**, *66*, 257–266.
(9) Giesen, C.; Haberey, M.; Fähnrich, M.; Schwarz, K.; Steinmeyer, A. WO 97/41096, 1997.

Published on Web 01/27/2007

10.1021/op060130d CCC: $37.00

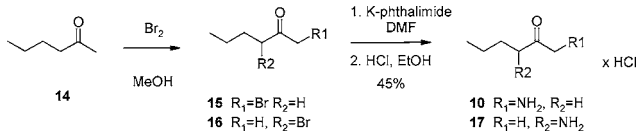
nitroso intermediate using Zn or $SnCl_2$.[10] The condensation of α-bromo acid **11**[11] with 1-aminohexanone **10** (as hydrochloride) in the presence of dicyclohexyl carbodiimide (DCC) produced keto amide **12** which was finally cyclized to oxazole **13** (Scheme 2).

Scheme 2



Attempted preparation of aminohexanone **10** by Gabriel synthesis failed because of the nonregioselective bromination of hexan-2-one **14** leading to nonseparable mixture of the bromo isomers (**15**/**16**) and amino isomers (**10**/**17**)[12] (Scheme 3).

Scheme 3



Results and Discussion

Due to the safety issues related to the nitroso intermediate and the low efficiency of the long linear medicinal chemistry route, we looked for a safe and more convergent synthesis alternative. Consequently, we focused on a Horner−Wittig olefination approach based on the reaction of the 22-aldehyde **4** with the oxazole-phosphonate **28**. This strategy would allow for a direct introduction of the side chain thereby circumventing the handling of the unstable intermediates **6** and **7**. The required oxazoles **27** and **28** should be easily accessible from common precursors 1,2-hexene oxide **19** and methyl-1,1-cyclopropane dicarboxylate **22** (Scheme 4).

The synthesis of the side chain building block **28** started with the reaction of 1,2-hexene oxide **19** and dibenzylamine[13] in methanol. For the initial route finding and upscaling of the single steps of the phosphonate **28** we were surprised by some unexpected results.

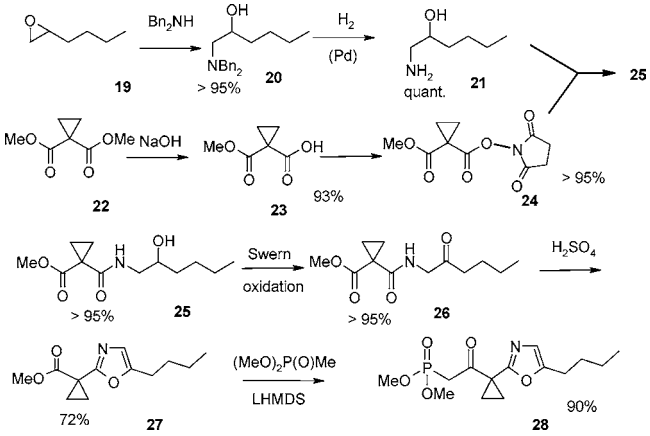
In first experiments, aminolysis of the oxirane **19** with a large excess of ammonia afforded mainly peralkylated products. Lithium trifluoromethanesulfonate[14] as an additive gave slightly more of the monoalkylated product, but the reaction showed insufficient selectivity. Even when benzylamine was applied, the dominant product was the bisalkylated amine **20b**. Finally, we used dibenzylamine to generate the monoalkylated product **20a**. Table 1 shows the results of the 1,2-epoxy-hexane (**19**) reaction with different amines (Scheme 5).

Table 1. **Aminolysis of 1,2-epoxy-hexane (19) with various amines**[a]

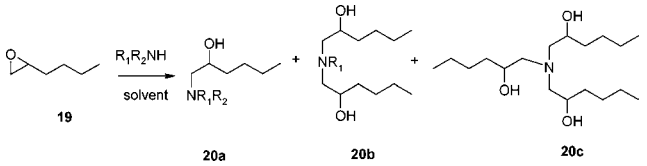
expt	R_1R_2NH R_1/R_2 =	solvent	conditions	**20a** (%)	**20b** (%)	**20c** (%)
1	H/H	MeOH	24 h/rt	5	9	25
2	Bn/H	MeOH	24 h/rt	23	39	
3	Bn/Bn	MeOH	24 h/rt	>95		
4	Bn/Bn	MeOH	6 h/40 °C	>95		
5	Bn/Bn	THF	48 h/rt	80		
6	Bn/Bn	EtOH	24 h/rt	95		

[a] Conditions: 1 equiv of 1,2-epoxy-hexane, 1.1 equiv of amine, 10 mmol scale, c = 4.0 mol/L, in MeOH, rt.

Scheme 4. **Synthesis scheme for β-keto phoshonate 28**

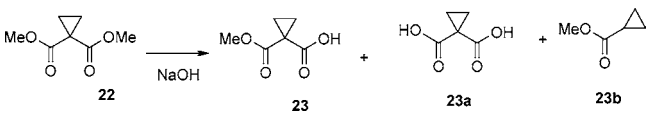


Scheme 5



Methanolic solution of the dibenzylated amino-alcohol **20** was then hydrogenolysed using 10% Pd on charcoal as catalyst to generate the deprotected amine **21**. Separately, the malonyl unit was built up starting from commercially available dimethyl cyclopropylmalonate **22**. Selective saponification to the mono ester **23** (Scheme 6) required optimization of the reaction parameters. Not surprisingly, an excess of NaOH gave more of dicarboxylic acid **23a**, and higher temperatures gave more of decarboxylated product **23b**. The results are shown in Table 2.

Scheme 6



For workup, the aqueous solution was stirred with hexanes at pH > 7 for the extraction of the starting material **22**. Then,

(10) Pascual, V.; Pulido, A.; An. R. *Soc. Esp. Fis. Quim.* **1947**, *43*, 391; *Chem. Abstr.* **1948**, 129.

(11) Barnier, J. P.; Rousseau, G.; Conia, J.-M. *Synthesis* **1983**, *11*, 915−916.

(12) (a) The bromination of 2-hexanone gives besides 1-bromohexan-2-one also the 3-bromo regioisomer: Thomas, A. P.; Allott, C. P.; Gibson, K. H.; Major, J. S.; Masek, B. B.; Oldham, A. A.; Ratcliffe, A. H.; Roberts, D. A.; Russell, S. T.; Thomason, D. A. *J. Med. Chem.* **1992**, *35*, 877−885. (b) For the synthesis of 1-amino-2-hexan-2-one by Gabriel synthesis, see: Brown, P.; Davies, D. T.; O'Hanlon, P. J.; Wilson, J. M. *J. Med. Chem.* **1996**, *39*, 446−457.

(13) The reaction of epoxide **19** with ammonia resulted in a mixture of mono-, bis-, and tris(2-hydroxyhex-1-yl)amines; benzylamine and **19** led to a 1:1 mixture of the mono- and bis(2-hydroxyhex-1-yl)benzylamines.

(14) Auge, J.; Leroy, F. *Tetrahedron Lett.* **1996**, *37*, 7715−7716.

Table 2. **Results for saponification of diester 22**[a]

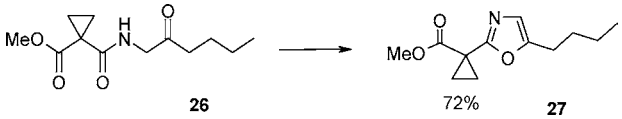
expt	equiv of NaOH	conditions	**23** (%)	**23a** (%)	**23b** (%)	**22** (%)
1	1	2 h/40 °C	90.0	0.2	0.1	9.7
2	1.05	2 h/40 °C	93.8	0.3	0.1	5.2
3	1.1	2 h/40 °C	97.4 (96)[b]	1.3	0.1	1.2
4	1.1	2 h/60 °C	91.7	0.3	7	1
5	1.2	2 h/40 °C	87.0	11.6	1.3	0.1

[a] Experiments were performed on a 100 mmol scale; 15.8 g of educt **22** in 84 mL of water; NaOH solution was freshly prepared from pellets; GC values. [b] Isolated yield.

the aqueous phase was acidified to pH 3.5, and the product **23** was extracted with ethyl acetate. The product **23a** is thermally labile; it partially decomposes at 60 °C to cyclopropylester **23b** (entry 4, Table 2). The saponification of diester **22** by 1.1 equiv NaOH produces the mono ester **23** in 96% isolated yield.

Coupling of the mono ester **23** with the amino alcohol **21** was performed using an activated ester method. Condensation of **23** with *N*-hydroxysuccinimde in the presence of diisopropyl carbodiimide was directed to activated ester **24**[15] which was then subsequently converted with 1-amino-2-hydroxyhexane **21** to amide **25** in a very good yield.[16] Swern oxidation of alcohol function yielded ketoamide **26** (Scheme 4). A bottleneck in the synthesis was the condensation of amide **26** to oxazole **27**. Stirring of the ketone **26** in sulfuric acid at 40 °C followed by dilution with water gave the product **27** in 40% yield. During optimization it was found that the oxazole is not stable in an acidic aqueous medium when the stirring time for workup was extended. A stability test suggested the product to be more stable in a two-phase system. The best results were found when the reaction and workup are carried out in the two-phase system sulfuric acid−dichloromethane (DCM; see Table 3). Carrying out

Table 3. **Results for the conversion of 26 to oxazole 27**[a]



26 72% **27**

expt	concn of **26** in g/mL H_2SO_4	*T* (°C)	time (h)	cosolvent	yield[b] **27**
1	0.2	45	15		40
2	0.2	Rt	24		17
3	0.33	45	18		30
4	0.2 (Eatons reagent[c])	45	15		20
5	0.33	45	16		72
6	0.2	80	4		
7	0.2	45	16	DCM	71
8	0.33	45	16	DCM	86

[a] 30 mmol scale; for workup water/DCM is added. [b] Isolated yield after chromatographic purification. [c] 10% P_2O_5 in methansulfonic acid.

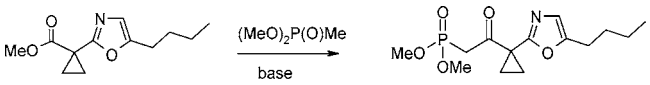
the reaction under phase transfer conditions prevents decomposition of the product and improves the yield of the cyclisation step to 86%.

(15) The condensation of **21** and **23** in the presence of diisopropyl carbodimide instead of DCC gave a lower yield of the amide **25**.

(16) The direct condensation between the ester **22** and alcohol **21** gave a moderate yield (51%) of **25**. 2-Hydroxypyridine catalyses this reaction; in a side reaction also 5% of the 1-amino-hex-2-enyl-ester was formed.

The oxazole ester **27** was purified by short path distillation to a GC purity of 98%. For the synthesis of the phosphonate **28**, we optimized the reaction parameters. In the first step, dimethyl methylphosphonate is deprotonated in THF. In the case of amide bases at −70 °C, the metalated dimethyl methylphosphonate reacts with the ester **27** in a Claisen type reaction to phosphonate **28**. We assert that 2 equiv of the base are optimal. Lithium hexamethyldisilylamide (LiHMDS) as base gave a better yield than lithium diisopropylamide (LDA). The results are shown in Table 4.

Table 4. **Synthesis of phosphonate 28**



27 **28**

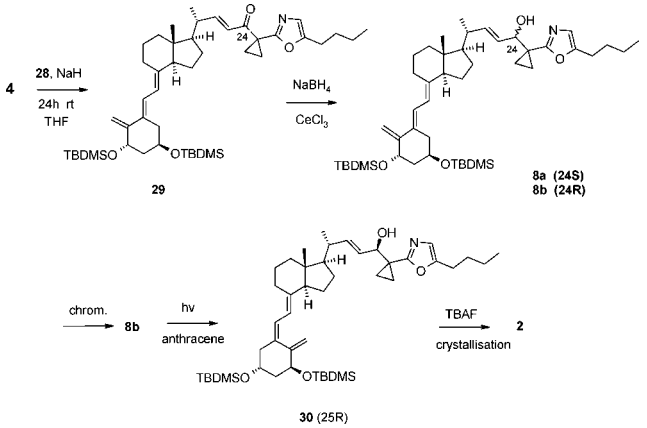
expt	mmol base	base	conditions[a]	yield[b]
1	1	LDA	2 h/−70 °C	21
2	1.5	LDA	2 h/−70 °C	59
3	2	LDA	2 h/−70 °C	81
4	1	LiHMDS	2 h/−70 °C	25
5	1.5	LiHMDS	2 h/−70 °C	63
6	2	LiHMDS	2 h/−70 °C	94
7	2	NaHMDS	2 h/−70 °C	76
8	2	NaH	8 h/−50 °C	65
9	2	KOtBu	8 h/−50 °C	58

[a] 50 mmol scale, $c = 1$ mol/L. [b] Isolated yields after chromatographic purification.

For workup, a stoichiometric amount of acetic acid is added followed by water (pH 6−7). The β-ketophosphonate **28** was purified by chromatography. The overall yield of the synthesis given in Scheme 4 was 52% on a lab scale which is described in the experimental part and even higher (64%) on a 15 kg pilot plant scale.

For the incorporation of the heterocyclic side chain, the β-keto phosphonate **28** was converted into the corresponding Na-salt in THF using sodium hydride (Scheme 7). The reaction with the aldehyde **4** proceeds very smoothly giving after 24 h at rt the 24-ketone **29** in 90% yield We found that the olefination is much faster (1 h, rt) when powdered NaOH is used as base, but unfortunately an epimerisation at C-20 of about 3% was also observed.

Scheme 7



The next step, the reduction of ketone **29**, appears to be more challenging. The results are summarized in Table 5. Luche reduction[17] of **29** using $NaBH_4/CeCl_3 \cdot 7H_2O$ led to a mixture of the 24R/24S diastereoisomers **8a/8b** in a 1:1 ratio. Despite the excellent regioselectivity, the Luche reduction is a method which still suffers from a lack of stereoselectivity. In an attempt to circumvent this problem, different reaction methods and conditions were tested (Table 5). Unfortunately, some increase in stereoselectivity achieved was overweigthed by the loss in regioselectivity, resulting in a lack of any yield improvement. In particular, asymmetric hydrogenation with the S-BINAP-DAIPEN-Ru system[18] resulted in the reduction of the 22,23-double bond rather than the sterically hindered 24-carbonyl group. The diastereoisomers **8a/8b** were separated by chromatography on silica gel, whereas the desired 24S isomer **8b** is the more polar component (SiO_2, eluent MTBE-hexane). Recycling of the 24R isomer **8a** was achieved by oxidation with manganese dioxide (10−15 equiv of MnO_2 in THF at 22 °C) back to the ketone **29**.

Table 5. **Results of Reduction Experiments for Ketone 29**

expt	reagent	solvent	ratio **8a/8b** (24S/24R)	yield **8a/8b**
1	$NaBH4/CeCl_3 \cdot 7H_2O$	EtOH	49:51	92
2	L-selectride	THF	78:22	79
3	$Na(OMe)_3H$	THF	25:75	63
4	Bakers yeast	H_2O		
	S-BINAP-DAIPEN-Ru	EtOH		

The final transformation of **2** was accomplished by UV-irradiation of **8b** in dichloromethane in the presence of 0.5 equiv of anthracene with a Heraeus TQ 150 Hg lamp which gives the desired 5Z isomer **30**. Finally, deprotection of **30** with tetrabutyl-ammonium fluoride (TBAF) in tetrahydrofuran gave calcitriol **2**, which was obtained after chromatography on silica gel and crystallization from ethyl acetate in highly pure form as white crystals (mp 98 °C).

Conclusion

For the calcitriol analogue ZK 191784 the building block **28** was prepared in a robust synthesis. This fragment was used for the side chain construction of secosteroid **29** in a more convergent synthesis. Also the reaction conditions were optimized by empirical methods. In addition, a practical and scaleable synthesis for the preparation of the calcitriol analogue ZK 191784 has been developed.

Experimental Section

1-(*N*,*N*-Dibenzylamino)-2-hydroxyhexane (20): 100 g (1 mol) of 1,2-hexene oxide **19** was dissolved in 0.5 L of methanol at 0 °C. 197.3 g (1 mol) of *N*,*N*-dibenzylamine was added over 10 min. The solution was stirred at 22 °C for 72 h. Thin layer chromatography (TLC) monitoring showed complete conversion. The solution was used directly in the following step without isolation of the product **20**.

(17) Gemal, A. L.; Luche, J.-L. *J. Am. Chem. Soc*. **1981**, *103*, 5454−5459.
(18) Okhuma, N.; Ishii, D.; Takeno, H.; Noyori, R. *J. Am. Chem. Soc.* **2000**, *122*, 6510−6511.

20: $C_{20}H_{27}NO$, mw 297.44; MS (CI−NH_3, 70 eV): $m/z = 298$ $[M + H]^+$.

1-Amino-2-hydroxy-hexane (21): The previous solution of compound **20** (1 mol, diluted with additional 0.5 L of methanol) was hydrogenolysed at 60 °C and 20 bar in the presence of 50 g of Pd (10% on charcoal) until 45 L of H_2 were absorbed. After the solution cooled at 20 °C the catalyst was filtered off and washed with 500 mL of methanol. Evaporation of solvent yielded 1-amino-2-hydroxy-hexane (**21**) (109.8 g, 93% yield). The GC purity of the product was 99%.

21: $C_6H_{15}NO$, mw 117.19, MS (CI−NH_3, 70 eV): $m/z = 118$ $[M + H]^+$.

Cyclopropane-1,1-dicarboxylic Acid Methylester (23): 150 g (0.95 mol) of cyclopropane-1,1-dicarboxylic acid dimethylester **22** was dissolved in 1050 mL of methanol. Sodium hydroxide (40 g, 1 mol) in 115 mL of water was added to the methanol solution and stirred at 22 °C for 24 h. 70 mL of methanol were distilled off, and 160 mL of water were added. The solution was then stirred with 50 mL of hexanes (for extraction of the diester **22**). The water solution was acidified with HCl (37%) and extracted with ethyl acetate. After drying and evaporation of the organic phase, the yield of the monoester **23** was 127 g (93% of theory). The GC purity is 99%.

23: $C_6H_8O_4$, mw 144.13, MS (CI−NH_3, 70 eV): $m/z = 145$ $[M + H]^+$; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 1.75-1.88$ (m, 4 H), 3,82 (s, 3H), 4.1.

Cyclopropane-1,1-dicarboxylic Acid Methyl *N*-Succinimid-1-yl Ester (24): 105.5 g (0.68 mol) of methyl-1,1-cyclopropanedicarboxylate **23** and 78.26 g (0.68 mol) of *N*-hydroxysuccinimide were dissolved in 400 mL of toluene and then evaporated to dryness (water content < 0.5%). The residue was dissolved in 500 mL of dichloromethane, and 94.24 g (0.748 mol) of diisopropylcarbodiimide in 100 mL of dichloromethane were added at −10 °C to the solution. The reaction was completed after 4 h at 20 °C, then cooled at 0 °C (for a better precipitation of the diisopropyl urea), and stirred for an additional 20 h. The precipitate was filtered off and washed with 50 mL of cold dichloromethane. Evaporation of the filtrate yielded 184 g of crude cyclopropane-1,1-dicarboxylic acid methyl *N*-succinimid-1-yl ester (**24**).

24: $C_{10}H_{11}NO_6$, mw 241.2, MS (CI−NH_3, 70 eV): $m/z = 242$ $[M + H]^+$; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 1.75-1.85$ (m, 4 H), 2.83 (m, 4 H), 3,83 (s, 3H).

1-(2-Hydroxy-hexylcarbamoyl)cyclopropanecarboxylic Acid Methyl Ester (25): 79.8 g (0.68 mol) of 1-amino-2-hydroxy-hexane **21** (crude) were dissolved in 200 mL of tetrahydrofuran (THF) at 15 °C. 184 g of crude cyclopropane-1,1-dicarboxylic acid methyl *N*-succinimid-1-yl ester (**24**) (0.68 mol) dissolved in 200 mL of tetrahydrofuran were added, and the reaction stirred 24 h at 22 °C. The THF was distilled off, and the product was extracted with methyl *tert*-butyl ether. The yield was 195 g of **25** as crude product (100% yield).

25: $C_{12}H_{21}NO_4$, mw 243.31, MS (CI−NH_3, 70 eV): $m/z = 244$ $[M - H^+]$; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.9$ (t,

3H), 1.25−1.5 (m, 6H), 1.55−1.75 (m, 4H), 3.02 (s, 1H), 3.15−3.55 (m, 3H), 3.7 (s, 3H) 4.5 (m, 1H).

1-(2-Oxo-hexylcarbamoyl)cyclopropanecarboxylic Acid Methyl Ester (26): A solution of 35.8 mL (0.5 mol) of DMSO in 100 mL of dichloromethane was cooled to −40 °C, and 19.8 mL (0.23 mol) of oxalyl chloride in 198 mL of dichloromethane (precooled to −40 °C) were slowly added. After 10 min of stirring, a solution of 51.6 g (0.18 mol) 1-(2-hydroxy-hexylcarbamoyl)cyclopropanecarboxylic acid methyl ester **25** (crude, content 83%, dissolved in 198 mL of dichloromethane) was slowly added over a period of 40 min. The reaction was stirred for 30 min at −40 °C. Then 69.8 mL (0.5 mol) of triethylamine were added followed by 300 mL of water. Extraction with dichloromethane and evaporation of the solvent yielded 44.72 g (100% yield) of crude **26** as an oil.

26: $C_{12}H_{19}NO_4$, mw 241.29 MS (CI−NH_3, 70 eV): m/z = 242 [M − H^+]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.9 (t, 3H), 1.25−1.43 (m, 2H), 1.55−1.74 (m, 6H), 2.45 (m, 2H), 3.7 (s, 3H), 4.15−4.2 (m, 2H), 9,3 (s, 1H).

1-(5-Butyl-oxazol-2-yl)cyclopropylcarboxylic Acid Methyl Ester (27): 44 g (0.18 mol) of compound **26** in 100 mL of dichloromethane were slowly added to 132 mL of concd sulfuric acid at 20−30 °C. The reaction was stirred for 14 h at 40 °C. For workup the solution was poured onto 200 mL of ice−water and 200 mL of dichloromethane. Phase separation and evaporation of the solvent yielded 45 g of crude product, which gave after distillation at 78 °C/1 mbar 28.77 g of compound **27** (72% yield) in a GC purity of 98%.

27: $C_{12}H_{17}NO_3$, mw 223.17, MS (CI−NH_3, 70 eV): m/z = 224 [M−H^+]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.95 (t, 3H), 1.3−1.7 (m, 8H), 2.58−2.67 (m, 2H), 3.7 (s, 3H), 6.64 (s, 1H)

{**2-[1-(5-Butyl-oxazol-2-yl)cyclopropyl]-2-oxoethyl**}-**phosphonic Acid Dimethylester (28):** Under an inert atmosphere of nitrogen, to 41.9 mL (198 mmol) of dry hexamethyldisilazane (HMDS) dissolved in 155 mL of tetrahydrofuran was added 124 mL (0.198 mol) of butyllithium (1.6 M in hexanes) at −50 °C and stirred for 15 min. Then 12.33 g (99 mmol) of dimethyl methylphosphonate in 4 mL of tetrahydrofuran was added at −50 °C to the solution. After 30 min 22.2 g of 1-(5-butyl-oxazol-2-yl)-cyclopropylcarboxylic acid methyl ester **27** (99 mmol) dissolved in 30 mL of THF were added, and the reaction was stirred for over 2 h. The temperature came up to 0 °C. Then 20 mL of acetic acid were added followed by 80 mL of water. The product **28** was extracted with ethyl acetate and purified by chromatography on silica gel with ethyl acetate−hexanes as eluent. Evaporation of the product fractions gave 28.05 g (90% yield) of phosphonate **28** as a colourless oil.

28: $C_{14}H_{22}NO_5P$, mw 315.3, MS (CI−NH_3, 70 eV): m/z = 316 [M − H^+]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.93 (t, 3H), 1.33−1.75 (m, 8 H), 2.65 (m, 2H), 3.54 (d, 2H, J = 25 Hz), 3.75 (d, 6H, J = 15 Hz), 6.69 (s, 1H).

(5*E*,7*E*,22*E*)-(1*S*,3*R*)-1,3-Bis(*tert*-butyldimethylsilyloxy)-24-oxo-25-(5-butyloxazol-2-yl)-26,27-cyclo-9,10-secocholesta-5,7,10(19),22-tetraen (29). To 4.84 g of sodium hydride (121 mmol, 60% of a dispersion in paraffine) in 100 mL of THF at 0 °C were added slowly 42 g (133 mmol) of **28** dissolved in 250 mL of THF. The solution was stirred for 1 h at rt, and then 65.9 g (115 mmol) of (5*E*,7*E*)-(1*S*,3*R*)-1,3-bis(*tert*-butyldimethylsilyloxy)-9,10-secopregna-5,7,10(19)-triene-20-carbaldehyde (**4**) dissolved in 400 mL of THF were added. The solution was stirred 1 h at 20 °C and 19 h at 50 °C. The reaction was monitored by thin layer chromatography (TLC) on SiO_2 plates (eluent ethyl acetate/hexane 1:9 v/v), R_f = 0.15 (product **29**), R_f = 0.53 (educt **4**). 6 g (100 mmol) of acetic acid in 50 mL of THF were added for workup followed by 1 L of water, with citric acid used to adjust pH to 7. The product was extracted twice with 500 mL of methyl *tert*-butylether and evaporated to dryness. The crude material was purified by chromatography on silica gel by elution with hexanes and an increasing gradient of ethyl acetate (0−15%). The evaporation of the fractions gave 79.5 g of **29** in 90% yield.

29: $C_{46}H_{75}NO_4Si_2$, mw 762,29; MS (CI−NH_3, 70 eV): m/z = 762 [M − H^+], 730 [(M−TBDMSOH)−H^+]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.04 (s, 6H), 0.05 (s, 6H), 0.5 (s, 3H, H-19), 0.9 (s, 9H), 0.95 (s, 9H), 1.05 (d, 3H), 1.1−2.1 (m), 2.55 (m, 1H), 2.65 (m, 2H), 2.85 (m, 1H), 4.2 (m, 1H, H-3), 4.5 (m, 1H, H-1), 4.95 (d, 2H, H-19), 5.8 (d, 1H, H-7), 6.1 (d, 1H, H22), 6.47 (d, 1H, H-6), 6.65 (s, 1H, H-4′), 6.75 (m, 1 H, H-23).

(5*Z*,7*E*,22*E*)-(1*S*,3*R*,24*R*)-1,3-Bis(*tert*-butyl-dimethyl-silyloxy)-24-hydroxy-25-(5-butyloxazol-2-yl)-26,27-cyclo-9,10-secocholesta-5,7,10(19),22-tetraene (8b). 48.4 g (63.5 mmol) of compound **29** were dissolved in 240 mL of THF and 200 mL of methanol. 3.29 g (8.8 mmol) of $CeCl_3$-heptahydrate were added and stirred for 15 min. 3.68 g (97 mmol) of $NaBH_4$ were dissolved in 22 mL of water and dropped at 5 °C to the solution of the enone. The reaction was monitored by TLC (SiO_2 plates, hexane/ethyl acetate 3:1 v/v, R_f = 0.55 (for educt **29**, 0.35 for **8a** (24R) and 0.31 for **8b** (24S))). For quenching and workup 4 mL of acetone were added followed by 145 mL of water. After adjusting pH to 7 using acetic acid, the product was extracted with methyl *tert*-butylether. After phase separation and evaporation to dryness the diastereomeric alcohols were separated by chromatography on Merck silicagel with hexanes−ethyl acetate as eluent, starting with 10% ethyl acetate/90% hexane as a gradient up to 20% ethyl acetate. The first fraction was the 24S-alcohol **8a**, the second fraction was the desired 25R-alcohol **8b**. The evaporation of the fractions gave 26 g of **8b** as a white solid. The 24S isomer **8a** (22 g) was oxidized by MnO_2 (10 equiv) in DCM to ketone **29** which was reduced by the previously described method giving after purification by chromatography an additional 8.5 g of compound **8b** in 71% yield.

8b: $C_{46}H_{77}NO_4Si_2$, mw 7642,289; MS (CI−NH_3, 70 eV): m/z = 764 [M − H^+]; 732 [(M−TBDMSOH)−H^+]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.04 (s, 6H), 0.045 (s, 6H), 0.53 (s, 3H, H-19), 087 (s, 9H), 0.91 (s, 9H), 1.03 (d, 3H), 1.1−1.6 (m), 1.85−2.1 (m), 2.3 (m, 1H), 2.57 (m, 2H), 2.87 (m, 1H), 4.1 (m, 1H, H-24), 4.2 (m, 1H, H-3), 4.5 (m,

1H, H-1), 4.95 (m, 2H, H-19), 5.5 (m, 2h, H22,H-23), 5.9 (m, 1H, H-7), 6.45 (d, 1H, H-6), 6.6 (s, 1H, H-4′).

(5*Z*,7*E*,22*E*)-(1*S*,3*R*,24*R*)-1,3-Bis(*tert*-butyl-dimethyl-silyloxy)-24-hydroxy-25-(5-butyloxazol-2-yl)-26,27-cyclo-9,10-secocholesta-5,7,10(19),22-tetraene (30). 0.7 g of anthracene (3.9 mmol) and 5 g (6.5 mmol) of compound **29** in 250 mL of dichloromethane were stabilized with 0.4 mL of triethylamine. The solution was degassed with nitrogen and irradiated with a Heraeus TQ 150 Hg lamp at 10 °C for 30 min while nitrogen was purged. The 5E−5Z isomerization was monitored by TLC and HPLC until the ratio **30:8b** is 97:3. Irradiation was performed in four portions (each 5 g) of a total 20 g (26 mmol) which gave after irradiation and workup by evaporation of solvent 22.5 g (100% yield) of the 5Z form **30** in a Z/E ratio of 97:3.

30: $C_{46}H_{77}NO_4Si_2$, mw 764,289; MS (CI−NH_3, 70 eV): m/z = 764 [M − H^+]; 732 [(M−TBDMSOH)−H^+]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.04 (s, 6H), 0.044 (s, 6H), 0.54 (s, 3H, H-19), 085 (s, 9H), 0.97 (s, 9H), 1.05 (d, 3H), 1.1−2.1 (m), 1.85−2.1 (m), 2.2 (m, 1H), 2.45 (m,1H), 2.53 (m, 2H), 2.83 (m, 1H), 4.15 (m, 2H, H-24), 4.35 (m, 1H, H-3), 4.85 (m, 1H, H-1), 5.16 (m, 1H), 5.5 (m, 2H), 6.0 (m, 1H, H-7), 6.22 (d, 1H, H-6), 6.55 (s, 1H, H-4′).

(5*Z*,7*E*,22*E*)-(1*S*,3*R*,24*R*)-25-(5-Butyloxazol-2-yl)-26,27-cyclo-9,10-secocholesta-5,7,10(19),22-tetraene-1,3,24-triol (2). 22 g (26 mmol) of crude **30** were dissolved in 200 mL of THF at 22 °C. 37 g (120 mmol) of tetrabutylammonium fluoride trihydrate were added, and the solution was stirred for 7 h at 20 °C. The completion of reaction was monitored by TLC (SiO_2 plates, eluent 100% ethyl acetate). To work up, 500 mL of water were added, and the product was extracted three times with 500 mL of ethyl acetate. The organic layer was washed with 100 mL of water. The crude product was purified by chromatography on silica gel using ethyl acetate/hexane as eluent starting from a ratio of 60/40 by a linear gradient up to 100/00. The first fraction gave 0.5 g of the previtamin form, followed by product fractions. Evaporation of the main fractions gave 11.2 g of the product **2**. For recrystallization the purified product **2** was dissolved in 112 mL of ethanol under nitrogen; to the solution 220 mL of water were slowly added at 22 °C over a period of 3 h. After stirring the solution for 16 h at 3 °C, the precipitate was filtered off and washed twice with 15 mL of cold water. The crystals were dried over 72 h at 1 mbar; 10.45 g of product **2** (75%) of a white solid, mp 108 °C (dec). The product **2** shows an HPLC purity of 99%, column 4.6 mm × 150 mm Waters X-Terra RP C8, 3.5 μ, flow 1 mL/min, det. UV (220 nm), eluent: acetonitrile/water/methanol 36:10:54, t_R = 45 min (**2**).

2: $C_{34}H_{49}NO_4$, mw 535,764; MS (CI−NH_3, 70 eV): m/z = 535 [M − H^+]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.53 (s, 3H, H-19), 0.9 (t, 3H), 1.00 (d, 3H, H-21), 1.1−1.8 (m), 1.85−2.1 (m), 2.3 (m), 2.55 (m), 2.8 (m, 1H), 2.83 (m, 1H), 4.1 (m), 4.2 (m, 1H), 4.4 (m), 5.0 (m, 1H, H-1), 5.3 (s, 1H), 5.4−5.6 (m, 2H), 6.0 (m, 1H, H-7), 6.38 (d, 1H, H-6), 6.57 (s, 1H, H-4′).

Acknowledgment

We thank Ute Imbery, Anh-Thu Nguyen, and Peter Fabian for technical assistance, Joerg Lorenz for spectral analysis, and Helmut Dahl and Andreas Steinmeyer for helpful discussions.

Received for review June 29, 2006.

OP060130D