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Synthesis of CD-ring modified 1α,25-dihydroxy Vitamin D Analogues : C-ring Analogues

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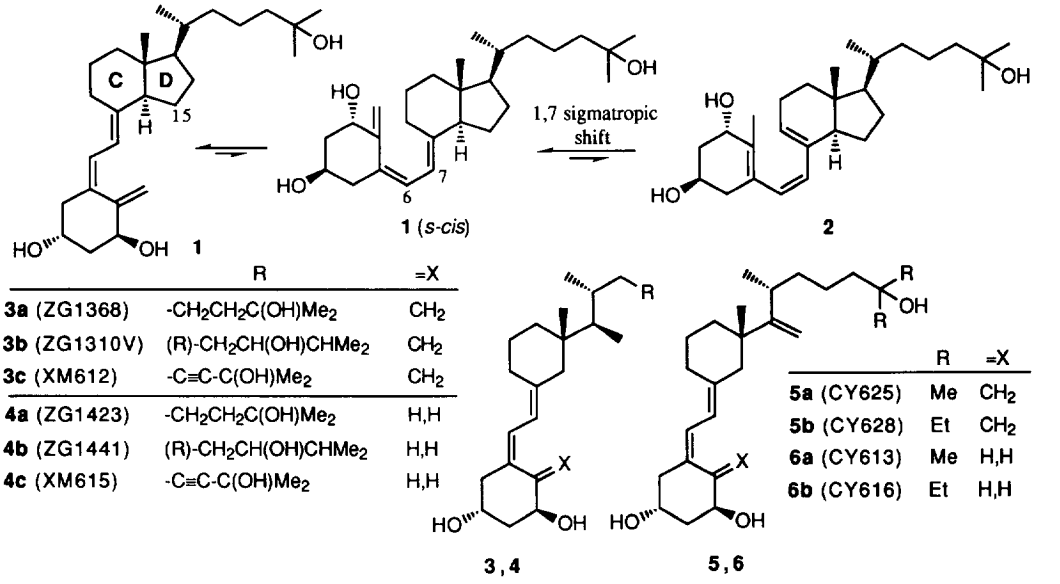
***Abstract** : Vitamin D analogues, characterized by the absence of a D-ring (C-ring analogues) are described.* Copyright © 1996 Elsevier Science Ltd

The finding that 1α,25-dihydroxy vitamin D_3 (**1** ; 1α,25$(OH)_2D_3$) is active in the regulation of cell proliferation and differentiation, next to its classical role in calcium-bone homeostasis, has led in recent years to the development of analogues which can dissociate the cell differentiating effects from the calcemic effects.[1,2] Among the different portions of the vitamin D skeleton especially structural modifications of the side-chain and of the A-ring have been studied in the past.[3]



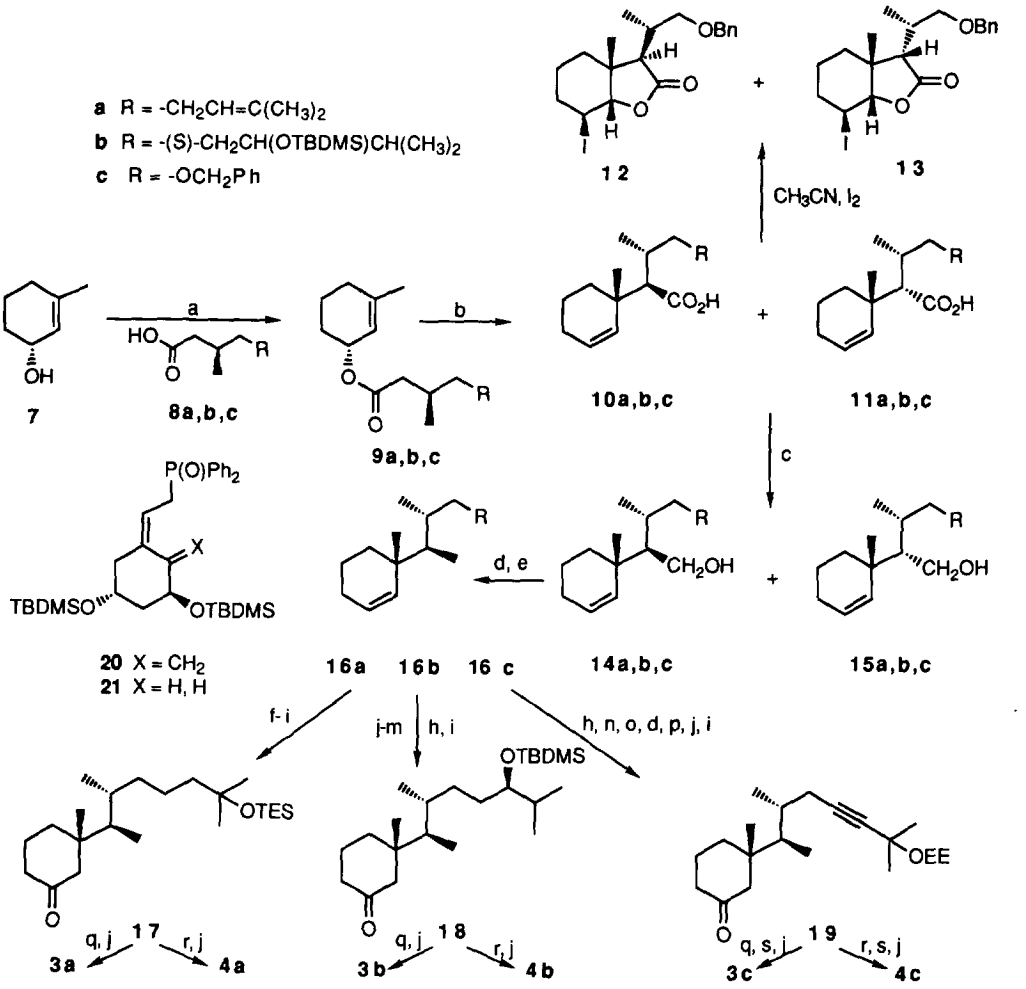
	R	=X
3a (ZG1368)	$-CH_2CH_2C(OH)Me_2$	CH_2
3b (ZG1310V)	(R)-$-CH_2CH(OH)CHMe_2$	CH_2
3c (XM612)	$-C{\equiv}C-C(OH)Me_2$	CH_2
4a (ZG1423)	$-CH_2CH_2C(OH)Me_2$	H,H
4b (ZG1441)	(R)-$-CH_2CH(OH)CHMe_2$	H,H
4c (XM615)	$-C{\equiv}C-C(OH)Me_2$	H,H

	R	=X
5a (CY625)	Me	CH_2
5b (CY628)	Et	CH_2
6a (CY613)	Me	H,H
6b (CY616)	Et	H,H

SCHEME 1

Some years ago, we have embarked on an extensive study of the structure-function relationship with the focus on the least studied part of the molecule, i.e. the central CD-ring regio.[4] In the present paper we wish to

describe the synthesis of analogues that lack the ring closed five-membered ring of the CD-ring skeleton, i.e., the "C-ring" analogues **3**, **4**, **5** and **6**.[5]



(a) DCC, DMAP, CH_2Cl_2 (for **8a** : 91 %; for **8b** : 96 %; for **8c** : 96 %); (b) for **9a** : LDA, THF-HMPA; TBDMSCl; reflux 16 h (58 %); for **9b** : LICA, THF-HMPA (8/3); TBDMSCl, reflux 18 h (67 %); for **9c** : LDA, THF-HMPA; TBDMSCl, reflux 16 h (78 %); (c) LAH, THF (for **a** : 86 %; for **b** : 80 %(after prior conversion to the corresponding methyl ester); for **c** : 90 %); (d) TsCl, pyridine (>95 %); (e) LAH, THF (>95 %); (f) $Hg(OAc)_2$, NaOH; $NaBH_4$ (67 %); (g) TESCl, DMAP, imidazole, DMF (92 %); (h) 9-BBN, H_2O_2 (for **a** : 95 %; for **b** : 92 %; for **c** : 95 %); (i) PDC (for **a** : 80 %; for **b** : 92 %; for **c** : 85 %); (j) TBAF, THF, 30°C (~90 %); (k) PPh_3, DEAD, p-NO_2PhCOOH (68 %); (l) K_2CO_3, KOH; (m) TBDMSCl, imidazole, DMAP, DMF (97 %); (n) TBDPSCl, imidazole, DMAP, DMF (95 %); (o) $Pd(OH)_2$/C, EtOH, cyclohexene, reflux (95 %); (p) NaH, DMSO, HC≡C-C(OEE)Me_2 (68 %); (q) **20**, n-BuLi; THF, -78°C (for **3a** : 88 %; for **3b** : 93 %; for **3c** : 86 %); (r) **21**, n-BuLi; THF, -78°C (for **4a** : 85 %; for **4b** : 88 %; for **4c** : 89 %); (s) PPTS, CH_2Cl_2, r.t. (95 %).

SCHEME 2

The deletion of the five-membered ring in the *trans*-fused perhydrindane system should have important consequences as to the composition of the well known 1,7-sigmatropic shift induced equilibrium between

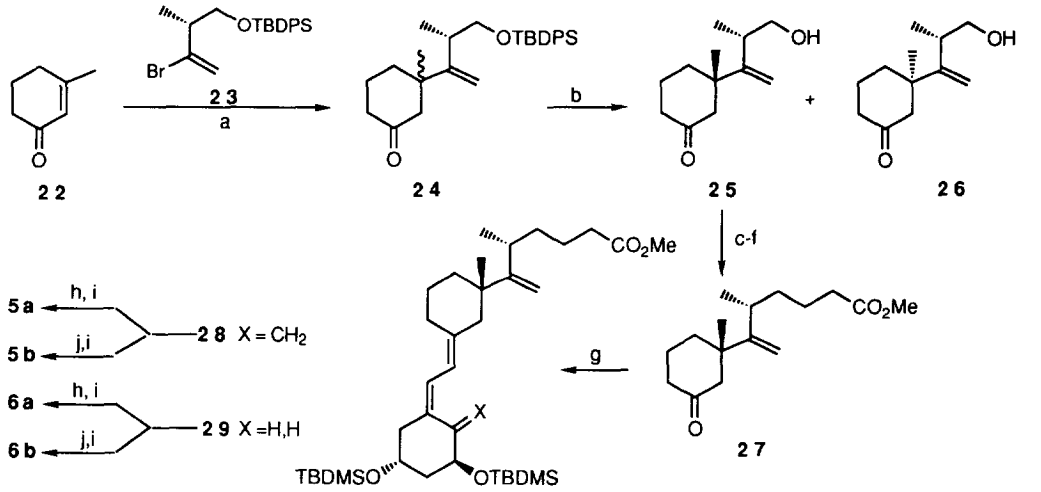
vitamin and previtamin forms.[6] It is well established that the reason why the vitamin form predominates in the natural equilibrium (>90 % at room temperature) is due to the constraint imposed by the *trans*-fusion on the 6-membered C-ring.[6] Hence in the monocyclic case (**3** and **5**) predominance of the previtamin form is expected. Therefore, next to analogues **3** and **5** which possess the natural 19-methylene group, the corresponding 19-nor derivatives (**4** and **6**, respectively) have also been considered. As for the side-chain structure, next to the natural 25-OH chain (**3a**, **4a**, **5a**, **6a**), derivatives characterized by a 24R-OH group (**3b**, **4b**), a 23-yne function (**3c**, **4c**), a formal 16,17-ene bond (**5**, **6**) and a bis-homologation (**5b**, **6b**) are considered.[3]

The synthesis of analogues **3** and **4** rests on the Ireland-Claisen rearrangement of the homochiral **9**. Within the three series **a**, **b** and **c** **9** resulted from the esterification of (R)-3-methyl-2-cyclohexen-1-ol **7** (90 % ee) with the corresponding acid **8**. Whereas **8a** is (R)-(+)-citronellic acid, **8b** (98 % ee) is obtained through a 4-step sequence involving the Bayer-Villiger oxidation of (-)-menthone as first crucial step.[7] (S)-4-Benzyloxy-3-methylbutanoic acid (**8c**) (94 % ee) is obtained from (S)-3-methyl-γ-butyrolactone.[8] The Ireland-Claisen rearrangement is performed on the (Z)-silyl ketone acetal (HMPA is used as cosolvent upon enolate formation) and leads through a boat-like transition state to a diastereomeric mixture strongly in favor of the (R)-17-substituted acid (ratio **10/11** better than 10:1).[9] In the three series the undesired diastereomer is separated after reduction of the acid mixture to the corresponding alcohols **14** and **15**. In the case of the **c**-series the stereochemical assignment was readily established on the stage of the γ-lactones **12** and **13** (obtained from the mixture of **10c** and **11c** with iodine in acetonitrile) : in the minor isomer **13** a strong NOE is observed between the angular methyl group and H-17 (δ = 2.85 ppm, ^3J = 2.9 Hz), while none is observed in the case of isomer **12** (δ = 2.55 ppm, ^3J = 6.8 Hz).

After reduction of the diastereomeric mixture of acids **10** and **11**, the obtained alcohols **14** and **15** are separated within each series. The reductive removal of the hydroxy group is further accomplished via tosylation followed by LAH reduction. In each series the necessary 8-ketone function (cf. **17**, **18** and **19**) is obtained *via* oxidative 9-BBN treatment followed by PDC oxidation of the resulting 8-hydroxyl group. The construction of the desired side-chains varies depending on the series; for the **a**-series : conversion of the trisubstituted double bond to the tertiary alcohol using mercuric acetate, NaOH and sodium borohydride; in the **b**-series : an uneventful sequence including a Mitsunobu inversion; in the **c**-series : the introduction of the 3,3-dimethyl-3-ethoxyethyloxy alkyne fragment *via* tosylate substitution. The eventually obtained cyclohexanone derivatives **17**, **18** and **19** are each subjected to the usual Horner-Wittig conditions[10] with phosphine oxides **20**[11] and **21**[12], and lead after removal of the different protective groups to the desired analogues **3a**, **3b**, **3c** and **4a**, **4b**, **4c**.

The synthesis of analogues **5** and **6** with a 17-methylene substituent is based on the conjugate addition of the cuprate derived from enantiopure **23** with 3-methyl-2-cyclohexenone (**22**). The chiral bromide **23** (99 % ee) was obtained from methyl (R)-3-hydroxy-2-methyl propionate[13] in a 4 step sequence involving after hydroxy group protection and reduction to the aldehyde, Gilbert's alkynynation[14] followed by bromination. With **23** in hand, we turned our attention to the conjugate addition. We first studied higher order cuprates ($R^*_2Cu(CN)_2Li_2$) which have been successfully applied in the conjugate addition on 3-methyl-2-cyclohexenone in the presence of BF_3-OEt_2 or Me_3SiCl.[15] This method failed, only formation of dimers derived from **23** were found. Also the Noyori-Hooz method, where a CuI/n-Bu_3P complex is formed from the vinyllithium was unsuccessful.[16] After considerable experimentation we found that only the procedure described by Magnus et

al. led to a viable route (62 % of **24**).[17] The experimental conditions are critical. Reaction of the vinylbromide **23** with 2 eq. t-BuLi in ether/THF at -130°C gave the vinyllithium which was treated immediately with preformed CuI/HMPT complex.[18] The reaction mixture was treated with $BF_3.OEt_2$, followed by the addition of the enone at -78°C, and gradually warmed to -20°C over 18 hours. After deprotection of the alcohol, careful HPLC (Bio-Sil 90-10 column) separation, the two diastereoisomers **25** and **26** (in a ratio of 1/1) were obtained in >95 % de.

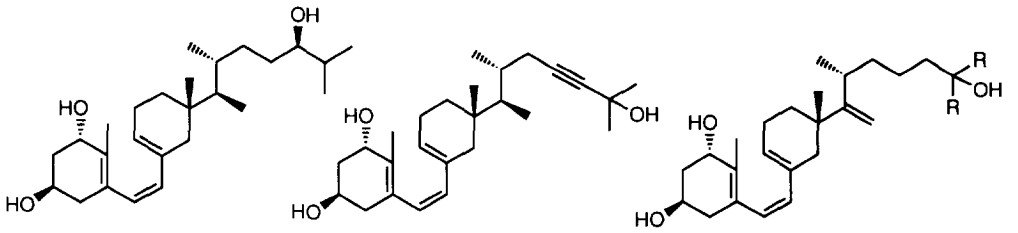


(a) (*i*) 2 eq t-BuLi, THF, -130°C; (*ii*) CuI, HMPT, -78°C; (*iii*) $BF_3.OEt_2$, -78°C; (*iv*) 3-methyl-2-cyclohexenone, -78°C → -18°C, 16 h; (b) TBAF, THF, r.t., 2 h; (c) Ph_3P, imidazole, I_2, THF, 0°C → r.t., 6 h; (d) $NaBH_4$, CH_2Cl_2/MeOH (1:1), -78°C → -30°C; 5 h; (e) CuI, Zn dust, CH_2=CH-$COOCH_3$, EtOH/H_2O (7:3),))), -10°C; (f) PDC, CH_2Cl_2, 0°C → r.t., 24 h; (g) for **28** : n-BuLi, **20**, THF, -78°C → -20°C, 4 h (85 %); for **29** : same procedure with **21** (65 %); (h) MeMgBr, THF, r.t., 2 d (98 %); (i) TBAF, THF, 0°C → r.t., 2 d (90 %); (j) EtMgBr, THF, r.t., 2 d (89 %).

SCHEME 3

For each, only one C-18 methyl signal was observed in the 500 MHz ^1H NMR in the presence of Eu(hfc)$_3$ (with control on the racemate). The stereochemistry of the two C-13 epimers was determined by circular dichroism measurement for which the opposite Cotton effects were observed. The desired (S)-epimer **25** shows a negative Cotton effect ($\Delta\varepsilon$ -0.02 at 289 nm, CH_3CN). The further elaboration of the side-chain involves conversion of the primary alcohol **25** into the iodide followed by the ultrasonically induced addition with methyl acrylate.[19] This step was performed on the alcohol (obtained after reduction of the cyclohexanone) which was then oxidized back to ketone **27**. Finally, the cyclohexanone **27** was coupled with the two A-ring phosphine oxides **20** and **21**. The 25-OH substituted analogues were eventually obtained *via* reaction with MeMgBr (**a**-series) and EtMgBr (**b**-series) in THF, followed by deprotection.

As expected, upon standing in acetone the "natural" methylene substituted derivatives **3b**, **3c** and **5a**, **5b** all lead to equilibria in which the corresponding previtamin forms **30**, **31**, **32** and **33**, respectively, predominates; e.g., starting from **3c** at 24°C the equilibrium **3c/31** (77:23) is reached after ca 6 days.



30 (ZG 1310 B) **31** (XM 617) **32** (CY 626) R = Me
33 (CY 629) R = Et

SCHEME 4

The affinity of the C-ring analogues of 1α,25-$(OH)_2D_3$ to the pig intestinal mucosa vitamin D receptor was evaluated as described previously.[20] The relative affinity of the analogues was calculated from their concentration needed to displace 50 % of [^{3}H]1α,25-$(OH)_2D_3$ from its receptor compared with the activity of 1α,25-$(OH)_2D_3$ (assigned a value of 100 %).

The biological evaluation was determined *in vitro* on different cell lines (HL60, MCF-7, MG-63, keratinocytes).[3] The *in vivo* calcemic effect of the C-ring analogs was tested in vitamin D-replete normal NMRI Mice. The C-ring analogue with the natural side-chain as in 1α,25-$(OH)_2D_3$ (ZG1368) displayed about 60 % of the VDR affinity and this compound showed an antiproliferative and prodifferentiating activity 10 (HL60) to 60 (MCF-7, keratinocytes) times that of 1α,25-$(OH)_2D_3$ and was 2 times less calcemic than 1α,25-$(OH)_2D_3$. Other modifications of the side chain (ZG1310V, XM612) also improved the antiproliferative activity of 1α,25-$(OH)_2D_3$ and decreased the calcemic activity. The introduction of a double bond between carbon 16 and carbon 17 (CY625) increased the antiproliferative activity of 1α,25-$(OH)_2D_3$ (2 times) and showed poor calcemic effects (1 % compared to 1α,25-$(OH)_2D_3$). The C-ring analogue with the natural side-chain as in 1α,25-$(OH)_2D_3$ but lacking carbon 19 (ZG1423) was less potent than the compound with a normal A-ring but other 19-nor C-ring analogues were equipotent or even superagonist compared to 1α,25-$(OH)_2D_3$ with a poor calcemic activity (≤20 % compared to 1α,25-$(OH)_2D_3$). Further details of the biological activity of the C-ring analogues will be published elsewhere.

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References and notes

1. Bouillon, R.; Van Baelen, H. *Saudi Med. J.* **1989**, *10*, 260.
2. DeLuca, H.F.; Burmester, J.; Darwish, H.; Krisinger, J. *Comprehensive Medicinal Chemistry*, Pergamon Press, New York **1990**, *vol. 3*, 1129.
3. Bouillon, R.; Okamura, W.H.; Norman, A.W. *Endocrine Reviews* **1995**, *16*, 200.
4. (a) Bouillon, R.; De Clercq, P.; Pirson, P.; Vandewalle, M. *Novel structural analogues of vitamin D;* patent PCT/EP 93.202037.3; priority date 09-07-1993; (b) A similar concept was reported by A. Kutner at

the 9th Vitamin D Workshop, Orlando, USA (28/04 - 02/05 1994). See Kutner, A.; Wilson, S.R.; Zhao, H.; Fitak, H. Chodymski, H.; Halkes, S.J. Polish Patent Appl. No P302010, 1994.

5. For the synthesis of "E-ring" analogues, see : Sabbe, K.; D'Hallewyn, C.; De Clercq, P.; Vandewalle, M.; Bouillon, R.; Verstuyf, A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1697.
6. For a review, see : Norman, A. W. *Vitamin D, the Calcium Homeostatic Steroid Hormone*, Academic Press: New York, **1979**; pp. 37. Havinga, E. *Experientia* **1973**, *29*, 1181.
7. Grieco, P.A.; Yokoyama, Y.; Gilman, S.; Ohfune, Y. *J. Chem. Soc.* **1977**, 870.
8. Chapleo, C. B.; Hallett, P.; Lythgoe, B.; Waterhouse, I.; Wright, P. W. *J. Chem. Soc. Perkin I*, **1977**, 1211.
9. Ireland, R. E.; Wipf, P.; Jia-Ning Xiang *J. Org. Chem.* **1991**, *56*, 3572.
10. Lythgoe, B.; Moran, T.A.; Nambudiry, M.E.N.; Tideswell, J.; Wright, P.W. *J. Chem. Soc., Perkin Trans I* **1978**, 590; Kocienski, P.J.; Lythgoe, V. *J. Chem. Soc., Perkin Trans. I* **1978**, 1290.
11. Baggiolini, E.G.; Iacobelli, J.A.; Hennessy, B.M.; Batcho, A.D.; Sereno, J.F.; Uskokovic, M.R. *J. Org. Chem.* **1986**, *51*, 3098.
12. (a) Perlman, K.L.; Sicinski, R.R.; Schnoes, H.K.; DeLuca, H.F. *Tetrahedron Lett.* **1990**, *31*, 1823; (b) Perlman, K.L.; Swenson, R.E.; Paaren, H.E.; Schnoes, H.K.; DeLuca, H.F. *Tetrahedron Lett.* **1991**, *32*, 7663.
13. Corey, E. J. *J. Am. Chem. Soc.* **1972**, *94*, 6190; also see : Roush, W. R.; Palkowitz, A. D.; Palmer, M. A. *J. Org. Chem.* **1987**, *52*, 316.
14. Gilbert, J. C.; Weerasooriya, U. *J. Org. Chem.*, **1979**, *44*, 4997.
15. Lipshutz, B. H.; Wilhelm, R. S.; Kozlowki, J. *Tetrahedron Lett.* **1982**, *23*, 3755; Lipshutz, B. H.; Wilhelm, R. S.; Kozlowski, J. *J. Org. Chem.* **1984**, *48*, 3938.
16. Noyori, R.; Suzuki, M.; Kawagishi, T. *Tetrahedron Lett.* **1980**, *21*, 1249; Hooz, J.; Layton, R. B. *Can. J. Chem.* **1970**, *48*, 1626.
17. Magnus, P.; Quaglioto, D. *J. Org. Chem.* **1985**, *50*, 1621; Magnus, P. private communication.
18. Semmelhack, M. F.; Yamashita, A.; Tomesch, J. C.; Hirotsu, K. *J. Am. Chem. Soc.* **1978**, *100*, 5565.
19. Pérez-Sestelo, J.; Mascareñas, J.L.; Mouriño, A. *Tetrahedron Lett.* **1994**, 275.
20. Bouillon, R.; Allewaert, K.; Van Leeuwen, J.P.T.M.; Tan, B.K.; Xiang D.Z.; De Clercq, P.; Vandewalle, M.; Pols, H.A.P.; Bos, M.P.; Van Baelen, H.; Birkenhäger, J.C. *J. Biol. Chem.* **1992**, *267*, 3044.

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