PREPARATION OF (22*S*)- AND (22*R*)-24-HOMO-26,26,26,27,27,27-HEXAFLUORO-1,22,25-TRIHYDROXY-24-YNE-VITAMIN D_3

Yutaka OHIRA,[a] Takeo TAGUCHI,*[,a] Katsuhiko ISEKI,[b] and Yoshiro KOBAYASHI[b]

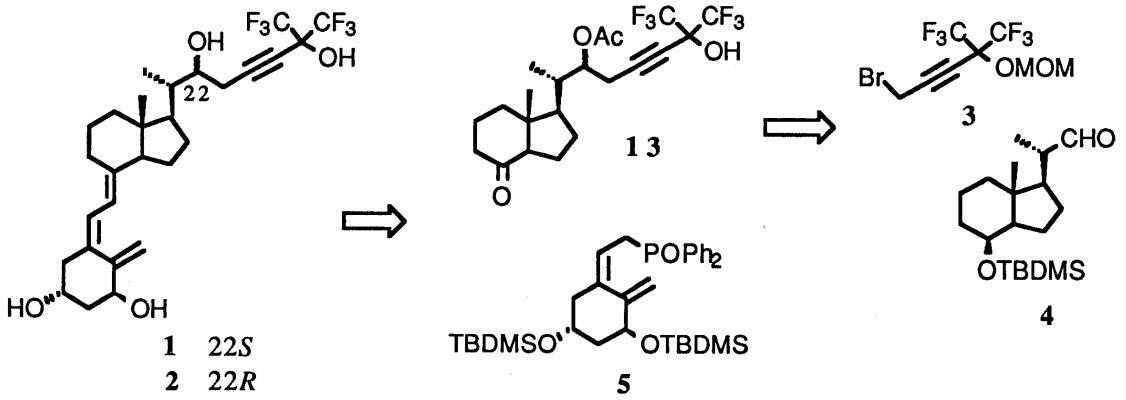
Tokyo College of Pharmacy,[a] 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan and MEC Laboratory, Daikin Industries, Ltd.,[b] 3 Miyukigaoka, Tsukuba, Ibaraki 305, Japan

A convergent synthesis of the titled fluorinated analogs of the 1,25-dihydroxyvitamin D_3, which modulates butyrate-induced differentiation of HT-29 human colonic carcinoma cells with very little effect on bone calcium mobilization, is reported.

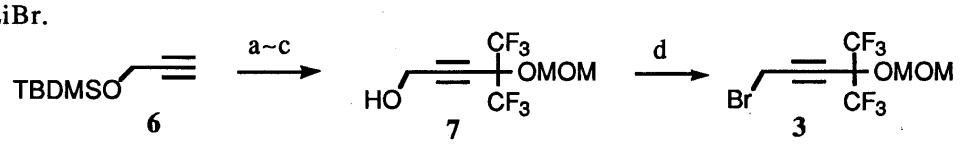
KEYWORDS vitamin D_3; vitamin D_3 fluoro analog; calcium-regulating activity; Wittig reaction; cell differentiation

It is well known that 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] is a hormonal metabolite of vitamin D_3, which regulates calcium and phosphorous metabolism.[1] Since its discovery, extensive efforts have been made to synthesize a number of analogs of vitamin D_3 with the aim of increasing and/or separating the biological activities.[2] It was demonstrated that 24,24-difluoro-[3] and 26,26,26,27,27,27-hexafluoro analogs[4] of 1,25(OH)$_2$D$_3$ are 5~10 times more active than 1,25(OH)$_2$D$_3$ in various vitamin D bioassays. It was also reported that the active forms of vitamin D_3, 1,25(OH)$_2$D$_3$[5] as well as the fluoro analogs[6], show the ability to induce differentiation of malignant cells. Although there is an important possibility that those active forms of vitamin D_3 might be effective in modulating malignant cell growth and differentiation *in vivo*, problems would be encountered by inducing severe hypercalcemia at the concentrations required for cell differentiation. Therefore, analogs having a strong effect on cell differentiation without showing calcium-regulating activity would be desirable for possible therapeutic use.[7-9] Among the reported analogs with structural alterations in 1,25(OH)$_2$D$_3$ in this line, 24- and 26-homo analogs[8] and 23-yne analogs[9] are classes of compounds which show effects on cell differentiation with decreased calcium-regulating activity. It was also reported that 22-hydroxylated vitamin D_3 exerts no calcium-regulating activity.[10]

In this paper, we report the preparation of the titled new fluoro analogs (**1**, **2**), which are highly potent in cell differentiation of HT-29 human colonic carcinoma cells with very little effect on bone calcium mobilization. The structural features of the present fluoro analogs as compared with those of hormonal metabolite are an additional hydroxyl at C-22, one carbon longer in side chain and 24-yne structure. For the preparation of the fluoro analogs (**1**, **2**), we employed a convergent methodology using the three fragments **3**, **4** and **5**. This methodology would provide a general route for a variety of active form of vitamin D_3 and its analogs.



The hydroxyl protected propargyl bromide **3**, the side chain fragment, was readily prepared through the reaction of acetylide with hexafluoroacetone. Conversion of the alcohol **7** to the bromide **3** was achieved by treating **7** with butyllithium followed by addition of MsCl in the presence of LiBr.

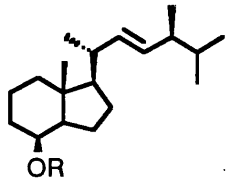


6 **7** **3**

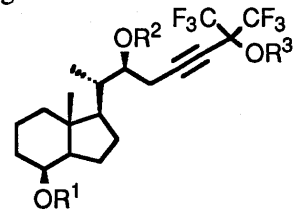
a) BuLi/THF then CF_3COCF_3 (77%) b) NaH, MOMCl/THF (89%)
c) TBAF/THF (79%) d) BuLi, MsCl, LiBr/THF (80%)

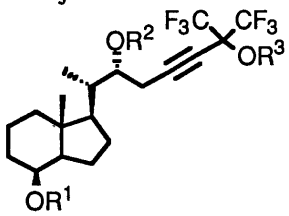
The aldehyde **4**, C-D ring fragment was prepared from vitamin D_2 according to the reported procedures with some modifications.[11,12] Thus, ozonolysis of the silyl ether **9** obtained by treating **8**[12] with TBDMSOTf and 2,6-lutidine (98% yield) was carried out in pyridine and CH_2Cl_2 (1:10 v/v) at -78°C, and subsequent treatment of the reaction mixture with Zn-AcOH provided the aldehyde **4** in 91% yield.[13]

Zinc-mediated coupling reaction of the bromide **3** with **4** was carried out in DMF at room temperature for 30 min to give a diastereomeric mixture of homopropargyl alcohols **10a** (67%) and **10b** (27%), which were readily separated by column chromatography. In this case, reaction proceeded regioselectively, and no allenic product was detected.[14] The *R*-configration at the newly formed chiral center of the minor adduct **10b** was confirmed on the basis of X-ray crystallographic analysis of the acetate **12b** (mp 156~157.5°C) derived from **10b**. Thus, the coupling reaction proceeded through Cram rule to give *S*-isomer **10a** as a major adduct.

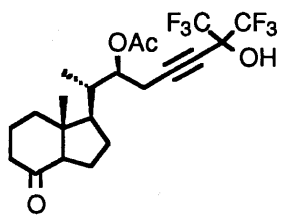


8 R=H
9 R=TBDMS

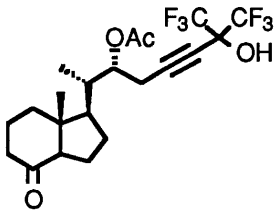




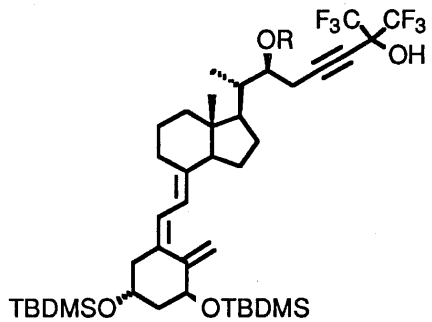
R^1=TBDMS	R^2=H	R^3=MOM	**10a**		**10b**
R^1=TBDMS	R^2=Ac	R^3=MOM	**11a**		**11b**
R^1=H	R^2=Ac	R^3=H	**12a**		**12b**



13a



13b



14a R=Ac
15a R=H

Each isomer (**10a** or **10b**) was converted to the ketone (**13a** or **13b**) to react with the phosphine oxide **5**. Thus, acetylation of **10a** (Ac_2O, Py, cat. DMAP, 88% yield) followed by deprotection of both TBDMS and MOM groups of **11a** [5% HCl-AcOH-CH_2Cl_2 (1:6:6 v/v), reflux, 5h] gave the diol **12a** (53% yield), which in turn was treated with PCC (CH_2Cl_2, rt, 2.5h) to afford the ketone **13a** in quantitative yield. Lithium salt of the phosphine oxide formed by deprotonation of **5**[15] (10 eq) with butyllithium (10 eq) in THF at -78°C was reacted with **13a** (1 eq) in the same solvent at -78°C for 1 min, then at room temperature for 1h to afford **14a**, which was further saponified with 5% LiOH to give the bissilyl ether **15a** in 75% yield based on **13a**. Desilylation of **15a** was effected by Dowex 50W-X4 in CH_3OH to give the final vitamin D_3 form of (22*S*)-isomer **1**[16] in 93% yield. According to procedures similar to those described above, (22*R*)-isomer **2**[17] was also synthesized from **10b**.

As preliminary results of biological assays, both **1** and **2** showed over 10 times more activity than $1,25(OH)_2D_3$ in butyrate-induced differentiation of HT-29 human colonic carcinoma cells, whereas these fluoro analogs did not show any activity in bone calcium mobilization in rats. Details will be reported elsewhere soon.

ACKNOWLEDGEMENT The authors are grateful to Prof. N. Ikekawa of Iwaki-Meisei University for helpful discussion, to Dr. Y. Tanaka of Albany Medical College, Albany, NY, to Dr. M. Kawai and Dr. S. Unten of Aichi Medical University for biological assays, and to Dr. M. Shiro of Rigaku Corporation for X-ray crystallographic analysis.

REFERENCES AND NOTES

1) H. F. Deluca and H. K. Schnoes, *Ann. Rev. Biochem.*, **45**, 631 (1976); *idem, ibid.*, **52**, 411 (1983).
2) N. Ikekawa, *Medicinal Res. Rev.*, **7**, 333 (1987).
3) a)Y. Tanaka, H. F. DeLuca, H. K. Schnoes, N. Ikekawa, and Y. Kobayashi, *Arch. Biochem. Biophys.*, **199**, 473 (1980); b) B. D. Kabakoff, N. C. Kendrick, D. Faber, H. F. DeLuca, S. Yamada, and H. Takayama, *ibid.*, **251**, 582 (1982); c) S. Okamoto, Y. Tanaka, H. F. DeLuca, Y. Kobayashi, and N. Ikekawa, *Am. J. Physiol.*, **244**, E159 (1983).
4) P. H. Stern, T. Marveas, Y. Tanaka, H. F. DeLuca, N. Ikekawa, and Y. Kobayashi, *Arch. Biochem. Biophys.*, **229**, 348 (1984).
5) a) C. Miura, E. Abe, T. Kuribayashi, H. Tanaka, K. Konno, Y. Nishi, and T. Suda, *Biochem. Biophys. Res. Commun.*, **102**, 937 (1981); b) H. Tanaka, E. Abe, C. Miura, T. Kuribayashi, K. Konno, Y. Nishi and T. Suda, *Biochem. J.*, **204**, 713 (1982).
6) a) H. P. Koeffler, T. Amatruda, N. Ikekawa, Y. Kobayashi, and H. F. DeLuca, *Cancer Res.*,**44**, 5624 (1985); b) M. Inaba, S. Okuno, Y. Nishizawa, K. Yukioka, S. Otani, I. Matsui-Yusa, S. Morisawa, H. F. DeLuca, and H. Morii, *Arch. Biochem. Biophys.*, **258**, 421 (1987); c) Y. Tanaka, K. K. Bush, T. Eguchi, N. Ikekawa, T. Taguchi, Y. Kobayashi, and P. J. Higgins, *ibid.*, **276**, 415 (1990).
7) Some analogs having an effect on cell differentiation with little or no calcium-regulating activity have been reported. a) L. Bindrerup, E. Bramm, *Biochem. Pharmacol.*, **37**, 889 (1988); b) J. Abe, M. Morikawa, K. Miyamoto, S. Kaiho, M. Fukushima, C. Miyaura, A. Abe, T. Suda, and Y. Nishii, *FEBS. Lett.*, **226**, 58 (1987).
8) a) V. K. Ostrem, Y. Tanaka, J. Prahl, H. F. DeLuca, and N. Ikekawa, *Proc. Natl. Acad. Sci. USA*, **84**, 2610 (1987); b) V. K. Ostrem, W. F. Lau, S. H. Lee, K. Perlman, J. Prahl, H. K. Schnoes, H. F. DeLuca, and N. Ikekawa, *J. Biol. Chem.*, **262**, 14164 (1987); c) T. Koizumi, Y. Nakao, S. Ishizuka, J. Oshida, N. Hara, N. Ikekawa, and T. Fujita, *Arch. Biochem. Biophys.*, **276**, 310 (1990).
9) J. -Y. Zhou, A. W. Norman, M. Akashi, D. -L. Chen, M. R. Uskokovic, J. M. Aurrecoechea, W. G. Dauben, W. H. Okamura, and H. P. Koeffler, *Blood*, **78**, 75 (1991) and its references.
10) H. F. DeLuca, *Nutr. Rev.*, **37**, 161 (1979).
11) Y. Wang, H. -S. Ting, J. J. Huang, Y. -C. Chow, and Y. -T. Huang, *Acta. Chim. Sinica*, **24**, 126 (1958).
12) H. T. Toh and W. H. Okamura, *J. Org. Chem.*, **48**, 1414 (1983).
13) Ozonolysis of **9** without pyridine proceeded sluggishly to give a mixture. D. A. Shepherd, R. A. Donia, J. A. Campbell, B. A. Johnson, R. P. Holysz, G. Slomp, J. E. Stafford, R. L. Pederson, and A. C. Ott, *J. Am. Chem. Soc.*, **77**, 1212 (1955).
14) J. -L. Moreau, *Bull. Soc. Chim. Fr.*, **1975**, 1248.
15) S. Hatakeyama, H. Numata, K. Osanai, and S. Takano, *J. Org. Chem.*, **54**, 3515 (1989).
16) **1**: $[\alpha]_D^{20}$=+16.7° (c 0.54, CH_3OH); λ_{max}^{EtOH}264nm (ε 15272). ^{1}H-NMR(CD_3OD) δ; 0.58 (s, 3H), 0.91 (d, J=5.6Hz, 3H), 3,90 (m, 1H), 4.12 (m, 1H), 4.36 (m, 1H), 4.91 (brs, 1H), 5.30 (brs, 1H), 6.10 (d, J=11Hz, 1H), 6.34 (d, J=11Hz, 1H); ^{19}F-NMR(CD_3OD, relative to $CFCl_3$) δ; -77.58 (s), -77.60 (s).
17) **2**: ^{1}H-NMR(CD_3OD) δ; 0.56 (s, 3H), 0.95 (d, J=6Hz, 3H), 2.59 (dd, J=11Hz and 3Hz, 1H), 2.85 (dd, J=11Hz and 3Hz, 1H), 3.94 (m, 1H), 4.23 (m, 1H), 4.43 (m, 1H), 5.00 (brs, 1H), 5.33 (brs, 1H), 6.02 (d, J=11Hz, 1H), 6.37 (d, J=11Hz, 1H).

(Received March 11, 1992)