PREPARATION OF (22S)- AND (22R)-24-HOMO-26,26,26,27,27,27-HEXAFLUORO-1,22,25-TRIHYDROXY-24-YNE-VITAMIN D₃

Yutaka OHIRA,¹ Takeo TAGUCHI,ᵃ,ᵃ Katsuhiko ISEKI,ᵇ and Yoshiro KOBAYASHIᵇ
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 D₃, which modulates butyrate-induced differentiation of HT-29 human colonic carcinoma cells with very little effect on bone calcium mobilization, is reported.

**KEYWORDS** vitamin D₃; vitamin D₃ fluoro analog; calcium-regulating activity; Wittig reaction; cell differentiation

It is well known that 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] is a hormonal metabolite of vitamin D₃, which regulates calcium and phosphorous metabolism.¹) Since its discovery, extensive efforts have been made to synthesize a number of analogs of vitamin D₃ 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 analogs⁴) of 1,25(OH)₂D₃ are 5–10 times more active than 1,25(OH)₂D₃ in various vitamin D bioassays. It was also reported that the active forms of vitamin D₃, 1,25(OH)₂D₃ ⁵) as well as the fluoro analogs⁶), show the ability to induce differentiation of malignant cells. Although there is an important possibility that those active forms of vitamin D₃ 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)₂D₃ in this line, 24- and 26-homo analogs⁸) and 23-yno analogs⁹) are classes of compounds which show effects on cell differentiation with decreased calcium-regulating activity. It was also reported that 22-hydroxylated vitamin D₃ 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-yno 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 D₃ and its analogs.
The hydroxyl protected propargyl bromide 3, the side chain fragment, was readily prepared through the reaction of acetyl 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.

\[
\begin{align*}
\text{TBDMSO} & \xrightarrow{\text{a-c}} \text{HO} & \xrightarrow{\text{d}} \text{Br} \\
6 & \xrightarrow{\text{CF}_3\text{COCF}_3 (77\%)} \xrightarrow{\text{NaH, MOMCl/THF (89\%)}} \xrightarrow{\text{BuLi, MsCl, LiBr/THF (80\%)}} \text{7} \\
\end{align*}
\]

a) BuLi/THF then CF\(_3\)COCF\(_3\) (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 TBDMOSOTf and 2,6-lutidine (98\% yield) was carried out in pyridine and CH\(_2\)Cl\(_2\) (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 10\(_a\) (67\%) and 10\(_b\) (27\%), which were readily separated by column chromatography. In this case, reaction proceeded regioselectively, and no allenic product was detected.\(^{14}\) The R-configuration at the newly formed chiral center of the minor adduct 10\(_b\) was confirmed on the basis of X-ray crystallographic analysis of the acetate 12b (mp 156–157.5°C) derived from 10\(_b\). Thus, the coupling reaction proceeded through Cram rule to give S-isomer 10\(_a\) as a major adduct.

\[
\begin{align*}
8 & \quad R=H \\
9 & \quad R=\text{TBDMS} \\
R^1=\text{TBDMS} & \quad R^2=H \quad R^3=\text{MOM} & \quad 10\(_a\) \\
R^1=\text{TBDMS} & \quad R^2=\text{Ac} \quad R^3=\text{MOM} & \quad 10\(_b\) \\
R^1=H & \quad R^2=\text{Ac} \quad R^3=H & \quad 11\(_a\) \\
R^1=H & \quad R^2=\text{Ac} \quad R^3=H & \quad 11\(_b\) \\
12\(_a\) & \quad \quad \quad \quad \quad \quad R^1=\text{Ac} \\
12\(_b\) & \quad \quad \quad \quad \quad \quad R^1=\text{H} \\
\end{align*}
\]

13\(_a\)

13\(_b\)

14\(_a\)

14\(_b\)

15\(_a\)

15\(_b\)

Each isomer (10\(_a\) or 10\(_b\)) was converted to the ketone (13\(_a\) or 13\(_b\)) to react with the phosphine oxide 5. Thus, acetylation of 10\(_a\) (Ac\(_2\)O, Py, cat. DMAP, 88\% yield) followed by deprotection of both TBDMS and MOM groups of 11\(_a\) [5\% HCl-AcOH-CH\(_2\)Cl\(_2\) (1:6:6 v/v), reflux, 5h] gave the diol 12\(_a\) (53\% yield), which in turn was treated with PCC (CH\(_2\)Cl\(_2\), rt, 2.5h) to afford the ketone 13\(_a\) 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 13\(_a\) (1 eq) in the same solvent at -78°C for 1 min, then at room temperature for 1h to afford 14\(_a\), which was further saponified with 5\% LiOH to give the bis-silyl ether 15\(_a\) in 75\% yield based on 13\(_a\). Desilylation of 15\(_a\) was effected by Dowex 50W-X4 in CH\(_3\)OH to give the final vitamin D\(_3\) form of (22S)-isomer 11\(_b\) in 93\% yield. According to procedures similar to those described above, (22R)-isomer 217) was also synthesized from 10\(_b\).
As preliminary results of biological assays, both 1 and 2 showed over 10 times more activity than 1,25(OH)_{2}D_{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

16) 1: [α]D_{20}=+16.7⁰ (c 0.54, CH3OH); λ_{max}^{EiOH}264nm (ε 15277). 1H-NMR(CD2OD) 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); 19F-NMR(CD2OD, relative to CFCl3) δ: -77.58 (s), -77.60 (s).
17) 2: 1H-NMR(CD2OD) δ: 0.56 (s, 3H), 0.95 (d, J=6Hz, 3H), 2.59 (dd, J=J11Hz 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)