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A short practical approach to 24*R*,25-dihydroxyvitamin D_3 ☆

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ABSTRACT

A synthesis of the vitamin D_3 metabolite 24*R*,25-dihydroxyvitamin D_3 (**1**) by Lythgoe's Wittig–Horner approach is described. The key step of the synthesis is the stereocontrolled introduction of the 24-hydroxyl group by a palladium(0)-induced [3,3]-sigmatropic rearrangement on a 22*R*-allylic acetate (**7**).

1. Introduction

The principal pathway of vitamin D_3 metabolism involves hydroxylation of vitamin D_3 (Fig. 1) in the liver and a second hydroxylation of the resulting 25-hydroxyvitamin D_3 [25(OH)D_3] either at C1 or C24 in the kidney to produce 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$] or 24*R*,25-dihydroxyvitamin D_3 [**1**, 24*R*,25(OH)$_2D_3$] [1–5]. While 1α,25(OH)$_2D_3$ is considered the hormonally active form of vitamin D_3 responsible for the regulation of gene transcription in over 30 target organs, only a few biological actions have also been attributed to 24*R*,25(OH)$_2D_3$ [6–9], namely activation of extracellular signal-related kinase phosphorylation [10], regulation of bone fracture healing [6,10], inhibition of rapid actions of 1α,25(OH)$_2D_3$ on stimulation of calcium transport in perfused duodena, as well as activation of protein kinases C and A [11], mediation of rapid non-genomic responses in intestinal cells [12,13], regulation of cartilage and bone via autocrine mechanisms [14], and inhibition of colon carcinogenesis [15]. 24*R*,25-Dihydroxyvitamin D_3 (**1**) are also intermediates on a catabolic pathway finally leading to excretion of calcitroic acid [4].

A few syntheses of 24*R*,25-dihydroxyvitamin D_3 have been carried out in the past by the biomimetic classical route [16–26]. In 1998 Stepanenko and Wicha [27] described the synthesis of this metabolite using an optically active hydroxylactone to build the side chain. Shortly afterwards, an alternative route to 24*R*,25(OH)$_2D_3$ was completed by Kütner and co-workers [28] using a diastereoselective α-hydroxylation of a side chain ester as the key step. In 2002 Sarandeses described a synthesis of 24*R*,25-dihydroxyvitamin D_3 using an ultrasonically induced aqueous conjugate addition of an iodide to a dioxolanone or oxazolidinone to generate the stereocenter at C24 [29a,30] and Fernández et al. developed a method to construct the side chain using aminoacids [31].

We disclose here a short and practical synthesis of 24*R*,25-dihydroxyvitamin D_3 (**1**) that involves the construction of the vitamin D triene system employing Lythgoe's Wittig–Horner approach [32] (coupling between ketone **2** and the phosphine oxide anion **3**) (Fig. 2).

2. Results and discussion

Our synthesis of 24*R*,25-dihydroxyvitamin D_3 (**1**) uses aldehyde **4**, which can be prepared in 50% yield from commercially available vitamin D_2 [33]. The addition of alkenyl lithium reagent **5** [34] to aldehyde **4** proceeded stereoselectively to afford, after medium pressure liquid chromatography separation from the minor isomer, the known alcohol **6a** in 75% yield (Fig. 3) [34]. Alcohol **6a** was then acetylated in the usual way to the corresponding acetate **7** in 93% yield. Treatment of **7** with a catalytic amount

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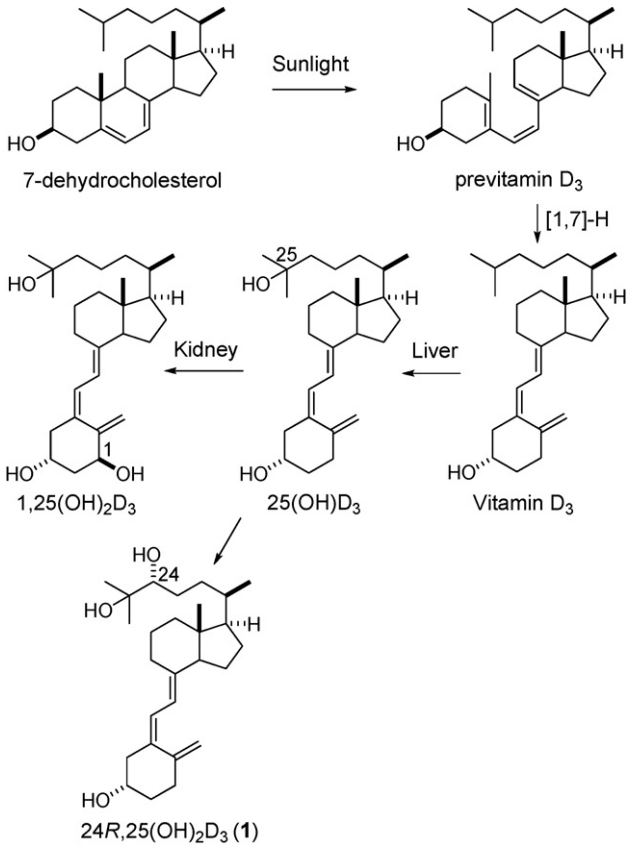

Fig. 1. Biosynthesis and metabolism of vitamin D_3.

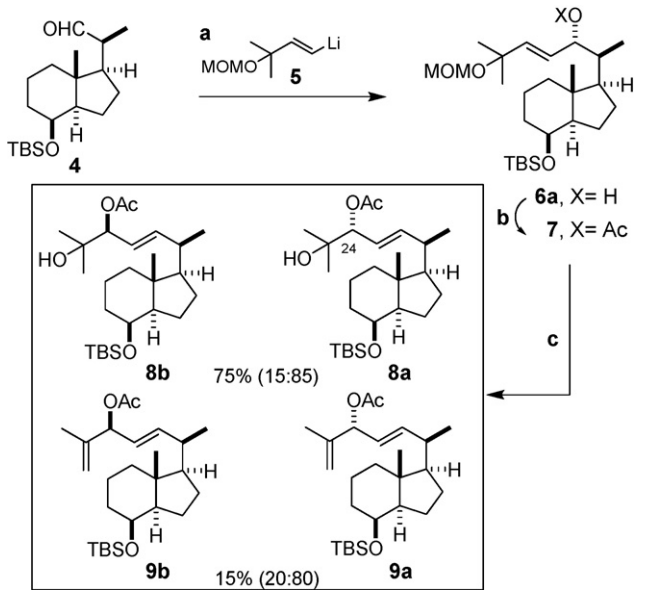

Fig. 3. (a) THF, −78 °C (75%); (b) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C, 30 min (93%); (c) $(CH_3CN)_2PdCl_2$ (8 mol%), THF, rt, 48 h.

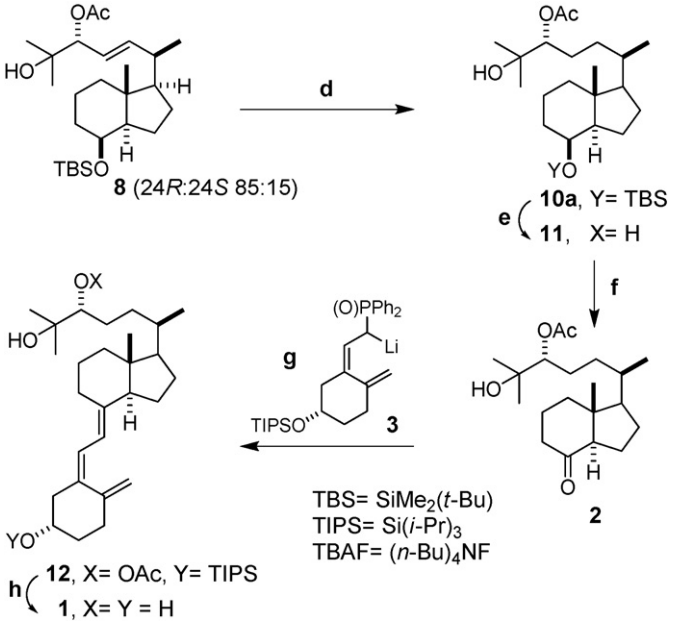

Fig. 4. Synthesis of 24*R*,25(OH)$_2$D$_3$. (d) H_2, PtO_2, EtOAc (85%); (e) HF (48%), CH_3CN–CH_2Cl_2, 72 h (93%); (f) PDC, CH_2Cl_2 (92%); (g) THF, −78 °C (96%); (h) TBAF, THF; NaOMe, THF (88%).

of bis(acetonitrile)palladium(II) chloride in THF [35] afforded an inseparable mixture of acetates **8a** and **8b** in 75% yield (ratio 85:15) together with the elimination products **9a** and **9b** in 15% yield (ratio 80:20) (Fig. 3). A formal [3,3]-sigmatropic-type rearrangement has been previously proposed for the palladium(0)-assisted rearrangement of allylic acetates [36].

Catalytic hydrogenation of mixture of acetates **8** followed by purification by flash-chromatography provided alcohol **10a** (85%), which was desilylated with hydrofluoric acid to diol **11** (93%). Pyridinium dichromate oxidation of **11** gave the desired ketone **2** (92%), which was coupled with phosphine oxide anion **3** to afford [32], after desilylation and saponification, the desired metabolite 24*R*,25-dihydroxyvitamin D_3 (**1**) [29b] in 82% yield (Fig. 4).

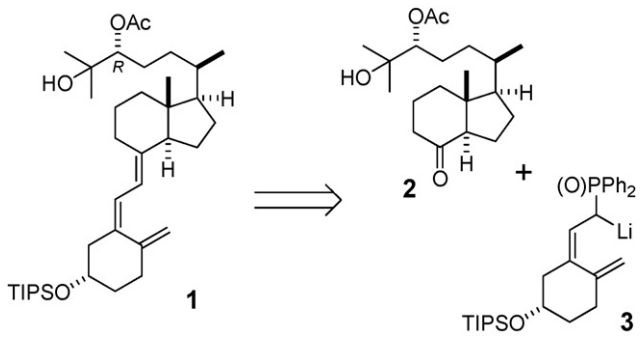

Fig. 2. Synthetic strategy (Wittig–Horner approach).

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References

[1] R. Bouillon, A.W. Norman, J.R. Pasqualini (Eds.), Proceedings of the 12th and 13th Workshop on Vitamin D, J. Steroid Biochem. Mol. Biol. 103 (2007) 201–822, 89–90 (2004) 1–633, and the previous 11 volumes in this series.

[2] D. Feldman, J.W. Pike, F.H. Glorieux (Eds.), Vitamin D, 2nd ed., Academic Press, New York, 2005.
[3] C. Carlberg, A. Mouriño, New vitamin D receptor ligands, Expert Opin. Ther. Patents 13 (2003) 761–772.
[4] G. Jones, S.A. Strugnell, H.F. DeLuca, Current understanding of the molecular actions of vitamin D, Physiol. Rev. 78 (1998) 1193–1231.
[5] R. Bouillon, W.H. Okamura, A.W. Norman, Structure–function relationships in the vitamin D endocrine system, Endocr. Rev. 16 (1995) 200–257.
[6] For a review on actions and biological activity of 24*R*,25-dihydroxyvitamin D3, see: H.L. Henry, Vitamin D, in: H.M. Goodman (Ed.), Handbook of Physiology, Section 7: The Endocrine System, Oxford University Press, New Work, NY, 2000, pp. 699–718.
[7] A.W. Norman, W.H. Okamura, J.E. Bishop, H.L. Henry, Update on biological actions of 1α, 25(OH)$_2$-vitamin D$_3$ (rapid effects) and 24*R*,25(OH)$_2$-vitamin D$_3$, Mol. Cell. Endocrinol. 197 (2002) 1–13.
[8] B.D. Boyan, D.D. Dean, V.L. Sylvia, Z. Schwartz, Steroid hormone action in musculoskeletal cells involves membrane receptor and nuclear receptor mechanisms, Connect. Tissue Res. 44 (2003) 130–135.
[9] Z. Schwartz, V.L. Sylvia, D.D. Dean, B.D. Barbara, Cell maturation specific regulation of the PKC signaling pathway by 1α,25-(OH)$_2$D$_3$ and 24*R*,25-(OH)$_2$D$_3$ in growth plate chondrocytes, Biomed. Health Res. 54 (2002) 25–35.
[10] E. Inoue, Y. Ishimi, J. Yamauchi, Differential regulation of extracellular signal-related kinase phosphorylation by vitamin D$_3$ analogs, Biosci. Biotechnol. Biochem. 72 (2008) 246–249.
[11] E.-G. Seo, T.A. Einhorn, A.W. Norman, 24*R*,25-dihydroxyvitamin D$_3$: an essential vitamin D$_3$ metabolite for both normal bone integrity and healing of tibial fracture in chicks, Endocrinology 138 (1997) 3864–3872.
[12] I. Nemere, D. Yazzie-Atkinson, D.O. Johns, D. Larsson, Biochemical characterization and purification of a binding protein for 24,25-dihydroxyvitamin D$_3$ from chick intestine, J. Endocrinol. 172 (2002) 211–219.
[13] D. Larsson, I. Nemere, K. Sundell, Putative basal lateral membrane receptors for 24,25-dihydroxyvitamin D$_2$ in carp and Atlantic cod enterocytes: characterization of binding and effects on intracellular calcium regulation, J. Cell. Biochem. 83 (2001) 171–186.
[14] B.D. Boyan, V.L. Sylvia, D.D. Dean, Z. Schwartz, 24,25-(OH)$_2$D$_3$ regulates cartilage and bone via autocrine and endocrine mechanisms, Steroids 66 (2001) 363–374.
[15] T. Taniyama, H. Wanibuchi, E.I. Salim, Y. Yano, S. Otani, Y. Nishizawa, H. Morii, S. Fukushima, Chemopreventive effect of 24*R*,25-Dihydroxyvitamin D$_3$ in N,N'-dimethylhydrazine-induced rat colon carcinogenesis, Carcinogenesis 21 (2000) 173–178.
[16] H.Y. Lam, H.K. Schnoes, H.F. DeLuca, T.C. Chen, 24,25-Dihydroxyvitamin D$_3$. Synthesis and biological activity, Biochemistry 12 (1973) 4851–4855.
[17] J. Redel, P. Bell, F. Delbarre, E. Kodicek, Synthesis of dihydroxy-24,25-cholecalciferol, a polar metabolite of vitamin D$_3$, C. R. Seances Acad. Sci. D 279 (1974) 529–531.
[18] M. Seki, N. Koizumi, M. Morisaki, N. Ikekawa, Synthesis of active forms of vitamin D. VI. Synthesis of (24*R*)- and (24*S*)-24,25-dihydroxyvitamin D$_3$, Tetrahedron Lett. 16 (1975) 15–18.
[19] S.C. Eyley, D.H. Williams, Synthesis of 25-hydroxyvitamin D$_3$ and 25,26-dihydroxyprovitamin D$_3$, J. Chem. Soc., Perkin Trans. I (1976) 731–735.
[20] J. Redel, N. Bazely, F. Delbarre, Y. Calando, Synthesis of the 24*R* and 24*S* diastereosiomers of 24,25-dihydroxycholecalciferol [24,25(OH)$_2$D$_3$], C. R. Seances Acad. Sci. D 283 (1976) 857–860.
[21] G.M. Segal, I.V. Torgov, Preparation of 25-hydroxycholesterol and 24,25-dihydroxycholesterol, Bioorganicheskaya Khimiya 5 (1979) 1668–1676.
[22] H. Takayama, M. Ohmori, S. Yamada, Facile, stereoselective synthesis of (24*R*)-24,25-dihydroxyvitamin D$_3$ usind D-glyceric acid as a chiral synthon, Tetrahedron Lett. 21 (1980) 5027–5028.
[23] M Ishiguro, N. Koizumi, M. Yasuda, N. Ikekawa, Stereoselective introduction of hydroxy-groups into the 24,25-, and 26-positions of the cholesterol side chain, J. Chem. Soc., Chem. Commun. (1981) 115–117.
[24] N. Koizumi, M. Ishiguro, M. Yasuda, N. Ikekawa, Stereoselective introduction of hydroxy groups into the cholesterol side chain. Preparation of (24*R*)- and (24*S*)-24,25-dihydroxy- and (25*R*)- and (25*S*)-25,26-dihydroxyvitamin D$_3$ by asymmetric synthesis, J. Chem. Soc., Perkin Trans. I (1983) 1401–1410.
[25] M. Seki, J. Rubio-Lightbourn, M. Morisaki, N. Ikekawa, Synthesis of active forms of vitamin D. IV. Synthesis of 24,25- and 25,26-dihydroxycholesterols, Chem. Pharm. Bull. 21 (1973) 2783–2785.
[26] A. Fürst, L. Labler, W. Meier, Synthesis of 24*R*,25-dihydroxycholecalciferol. A stereoselective synthesis of 24*R*,25-dihydroxycholesterol, in: A.W. Norman, K. Schaefer, H.-G. Grigoleit, D.V. Herrath (Eds.), Vitamin D, Chemical, Biochemical and Clinical Update, Walter de Gruyter, Berlin/New York, 1985, pp. 733–738.
[27] W. Stepanenko, J. Wicha, Enantioselective synthesis of 24,25-dihydroxy vitamin D$_3$ northern portion from (*S*)-3- hydroxy-2,2-dimethylcyclohexane-1-one. Remote asymmetric induction in an acid-catalysed conjugate addition, Tetrahedron Lett. 39 (1998) 885–888.
[28] M. Odrzywolska, M. Chodynski, J. Zorgdrager, J.-P. Van de Velde, A. Kütner, Diastereoselective synthesis, binding affinity for vitamin D receptor, and chiral stationary phase chromatography of hydroxy analogs of 1,25-dihydroxycholecalciferol and 25-dihydroxycholecalciferol, Chirality 11 (1999) 701–706.
[29] (a) J. Pérez-Sestelo, I. Cornella, O. de Uña, A. Mouriño, L.A. Sarandeses, Stereoselective convergent synthesis of 24,25-dihydroxyvitamin D$_3$ metabolites: a practical approach, Chem. Eur. J. 8 (2002) 2747–2752;
(b) The identity of the synthetized 24*R*,25-dihydroxyvitamin D$_3$ (**1**) was established by comparison of its R_f, ^{1}H and ^{13}C-NMR data with a reference sample from our laboratory.
[30] I. Cornella, R.M. Suárez, A. Mouriño, J. Pérez-Sestelo, L.A. Sarandeses, Stereoselective convergent synthesis of 24-substituted metabolites and analogues of vitamin D, J. Steroid Biochem. Mol. Biol. 89–90 (2004) 19–23.
[31] C. Fernández, Z. Gándara, G. Gómez, B. Covelo, Y. Fall, D- and L-serine, useful synthons for the synthesis of 24-hydroxyvitamin D$_3$ metabolites. A formal synthesis of 1α,24*R*,25-(OH)$_3$-D$_3$, 24*R*,25-(OH)$_2$-D$_3$ and 24*S*,25-(OH)$_2$-D$_3$, Tetrahedron Lett. 48 (2007) 2939–2942.
[32] (a) E.G. Baggiolini, J.A. Iacobelli, B.M. Hennessy, M.R. Uskokovic, Stereoselective total synthesis of 1α, 25-dihydroxycholecalciferol, J. Am. Chem. Soc. 104 (1982) 2945–2948;
(b) B. Lythgoe, T.A. Moran, M.E.N. Nambudiry, J. Tideswell, P.W. Wrigth, Calciferol and its relatives. Part 22. A direct total synthesis of vitamin D$_2$ and vitamin D$_3$, J. Chem. Soc., Perkin Trans. 1 (1978) 590–595.
[33] B. Fernández, J.A. Martínez, J.R. Granja, L. Castedo, A. Mouriño, Synthesis of hydrindan derivatives related to vitamin D, J. Org. Chem. 57 (1992) 3173–3178.
[34] M. Torneiro, Y. Fall, L. Castedo, A. Mouriño, A. Short, Efficient copper-mediated synthesis of 1α,25-dihydroxyvitamin D$_2$ (1α,25-dihydroxyergocalciferol) and C-24 analogs, J. Org. Chem. 62 (1997) 6344–6352.
[35] P. Grieco, T. Takigawa, S.L. Bongers, H. Tanaka, Complete transfer of chirality in the [3,3]-sigmatropic rearrangement of allylic acetates catalyzed by palladium(II). Application to stereocontrolled syntheses of prostaglandins possessing either the C-15(*S*) or C-15(*R*) configuration, J. Am. Chem. Soc. 102 (1980) 7587–7588.
[36] (a) S. Takatsuto, M. Ishiguro, N. Ikekawa, Chirality transfer in the cholesterol side chain; synthesis of (24*R*)- and (24*S*)-24 cholesterol, J. Chem. Soc., Chem. Commun. (1982) 258–260;
(b) M. Kawatsura, Y. Uozumi, M. Ogasawara, T. Hayashi, Palladium-catalyzed asymetric reduction of racemic allylic esters with formic acid: effects of phosphine ligands on isomerization of π-allylpalladium intermediates and enantioselectivity, Tetrahedron 56 (2000) 2247–2257;
(c) B.M. Trost, D.L. Van Vranken, Asymetric transition metal-catalyzed allylic alkylations, Chem. Rev. 96 (1996) 395–422.