JOC Article

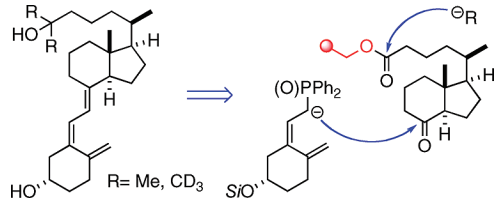
Synthesis of 25-Hydroxyvitamin D_3 and 26,26,26,27,27,27-Hexadeutero-25-hydroxyvitamin D_3 on Solid Support†

Daniel Nicoletti, Antonio Mouriño, and Mercedes Torneiro*

Departamento de Química Orgánica y Unidade Asociada al CSIC, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

mercedes.torneiro@usc.es

Received March 11, 2009



A convenient five-step route to 25-hydroxylated vitamin D_3 compounds on (hydroxymethyl)polystyrene support is reported. A CD-side chain fragment was anchored to the solid phase through an ester group at C25 and coupled to an A ring building block to assemble the vitamin D triene system by the Wittig−Horner approach. Deprotection of the hydroxy group was carried out on the support, prior to functionalization at C25. The title compounds were released from the resin in excellent global yield by nucleophilic attack on the ester carbonyl group using commercially available organometallic reagents. This key last step offers an opportunity for the efficient generation of 26,27-labeled compounds and also for diversification at the side chain without need for a pool of side chain fragments.

Introduction

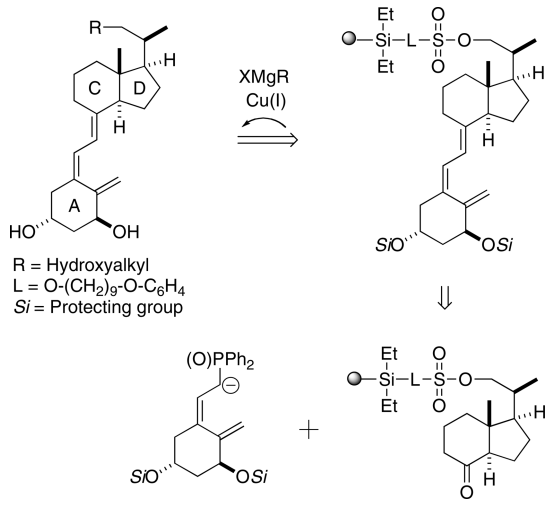
Vitamin D_3 undergoes two successive hydroxylations, first in the liver at C25 to produce 25-hydroxyvitamin D_3 (**1a**) and then in the kidney at C1 to generate 1,25-dihydroxyvitamin D_3 (**1b**), the active form of vitamin D_3, which regulates a plethora of biological functions including calcium homeostasis, bone mineralization, proliferation and differentiation of various types of cells, and immune modulation.[1−3] The antiproliferative-prodifferentiating activities of the secosteroid hormone **1b** have suggested its potential use in the treatment of various cancers (breast, colon) and skin diseases.[4,5] However, its therapeutic application has been limited by the parallel induction of hypercalcemic effects.[2,6,7] Efforts aimed at developing vitamin D analogues with strong cell-differentiating ability and low calcemic action have led to the synthesis of more than 3000 vitamin D analogues.[7,8] However, only a few of these have successfully made it to the market,[9] for example, calcipotriol (MC903, Daivonex), which is currently used for the treatment of psoriasis.[10,11] Successful convergent synthetic strategies to access these compounds have been reported.[12−14] However, aside from the pioneering work of Takahashi and co-workers,[15,16] studies on the syntheses of new vitamin D analogues involving

† Dedicated to Prof. Josep Font on the occasion of his retirement.

(1) Bouillon, R.; Norman, A. W.; Pasqualini, J. R. Proceedings of the 13th and 12th Workshops on Vitamin D. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 201–822; **2004**, *89−90*, 1−633 and the previous 11 volumes in this series.
(2) *Vitamin D*, 2nd ed.; Feldman D., Pike, J. W., Glorieux, F. H., Eds.; Academic Press: New York, 2005.
(3) Jones, G.; Strugnell, S. A.; DeLuca, H. F. *Physiol. Rev.* **1998**, *78*, 1193–1231.
(4) Abe, E.; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4990–4994.
(5) Colston, K.; Colston, M. J.; Feldman, D. *Endocrinology* **1981**, *108*, 1083–1086.
(6) Ettinger, R. A.; DeLuca, H. F. *Adv. Drug Res.* **1996**, *28*, 269–312.
(7) Carlberg, C.; Mouriño, A. *Expert Opin. Ther. Pat.* **2003**, *13*, 761–772.
(8) Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200–256.
(9) Kabat, M. M.; Radinov, R. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 808–833.
(10) Jain, S.; Sehgal, V. N. *Int. J. Dermatol.* **1997**, *36*, 255–258.
(11) Calverley, M. J. *Tetrahedron* **1987**, *43*, 4609–4619.
(12) Dai, H.; Posner, G. H. *Synthesis* **1994**, 1383–1398. Zhu, G.-D.; Okamura, W. H. *Chem. Rev.* **1995**, *95*, 1877–1952.
(13) Posner, G. H.; Kahraman, M. *Eur. J. Org. Chem.* **2003**, 3889–3895.
(14) Krause, S. Schmalz H.-G. In *Organic Synthesis Highlights IV*; Schmalz, H.-G., Ed.; Wiley-VCH: Weinheim, 2000; pp 212−217.
(15) Doi, T.; Hijikuro, I.; Takahashi, T. *J. Am. Chem. Soc.* **1999**, *121*, 6749–6750.
(16) Hijikuro, I.; Doi, T.; Takahashi, T. *J. Am. Chem. Soc.* **2001**, *123*, 3716–3722.

10.1021/jo900524u CCC: $40.75
Published on Web 06/03/2009

SCHEME 1. Takahashi's Strategy to Vitamin D_3 Analogues



the construction of the labile vitamin D triene system on solid support are unknown.[17] Takahashi's approach uses the Lythgoe's powerful Wittig−Horner strategy[18−20] to build the vitamin D triene system by coupling a phosphine oxide anion (A-ring fragment) with a solid supported ketone (CD-fragment), anchored to PS-DES resin via a sulfonated linker. Subsequently a Cu(I)-catalyzed Grignard displacement of the resin can be used to introduce the side chain fragment (Scheme 1). Final deprotection of analogues is accomplished in solution.[16]

As part of a broader research program to develop effective synthetic pathways to bioactive vitamin D analogues, we disclose here a convenient five-step method that involves the construction of the vitamin D triene system employing Lythgoe's Wittig−Horner approach on solid phase to generate 25-hydroxyvitamin D_3 (**1a**) and their analogues functionalized at C25[21] (Scheme 2). It was assumed that the target compounds could be released from the deprotected polymer-bound vitamin D_3 intermediate **2** by nucleophilic attack on the ester carbonyl group using a variety of commercially available organometallic reagents. Moreover, this key step offers an opportunity for the generation of 26,27-labeled compounds (^{11}C, ^{14}C, ^{3}H) for biomedical studies or standards (^{13}C, ^{2}H) for quantification of vitamin D_3 metabolites by mass spectrometry.

We hoped that the desilylation step could be performed after formation of the triene system without problem. This operation, which is of particular importance in the pathway to labeled materials, would be intriguing employing silylated linkers. Another key step includes the formation of the vitamin D triene system by Wittig−Horner coupling between phosphine anion **4** and polymer-linked ketone **3** because the resulting solid-supported intermediate could serve as a handle for diversity-oriented synthesis. At this point we were optimist that possible basic species generated upon workup would not hydrolyze the ester group. Access to ketone **3** would be carried out by selective esterification of carboxylic acid **5** with simple commercial resins containing a primary alcoholic hydroxy group. This approach would also serve to access other known bioactive or antagonist vitamin D_3 analogues by simple adaptation of the corresponding carboxylated side chain.

Results and Discussion

As representative targets we chose 25-hydroxyvitamin D_3 (25-OH-D_3, **1a**) and 26,26,26,27,27,27-hexadeutero-25-hydroxyvitamin D_3 (**1c**). 25-OH-D_3 is the most abundant vitamin D metabolite in circulation and serves as an indicator of nutritional vitamin D status[22] and in the treatment of several deseases.[1] The hexadeuterated analogue **1c** is used as an internal standard in the quantification of circulating 25-OH-D_3.[23,24] Our preliminary studies on the formation of the ester linkage began with hydroxyester **7** (Scheme 3), which was easily prepared from commercial vitamin D_2 in three steps.[25,26] Silylation of the secondary hydroxy group followed by hydrolysis of the ester provided the desired carboxylic acid **9**, which was anchored directly to (hydroxymethyl)polystyrene resin (**6**) employing 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) and *N*-methylimidazole (MeIm) to activate the carboxylic group.[27]

The loading yield of the anchored CD-side chain fragment **10** was 100%,[28] and the excess unreacted acid **9** (1 equiv) was recovered by filtration. IR and ^{13}C NMR spectra of resin **10** confirmed the presence of the ester linkage and the disappearance of the free hydroxy groups by comparison with spectra of unloaded resin **6** and partially loaded resin **10b** obtained by reaction of **6** with 0.6 equiv of **9**. At this point we decided to test the feasibility of the final releasing step of the synthesis before building the vitamin D triene system. Addition of methyllithium provided **11a** in 93% yield, while addition of trideutero-methylmagnesium iodide produced the tertiary alcohol **11b** in 96% yield. Both alcohols were analytically pure by ^{1}H and ^{13}C NMR spectral analysis. Desilylation of **10** with aqueous hydrogen fluoride provided the polymer-bound alcohol **12** in 97% yield.[29]

At this stage and as discussed on the retrosynthetic plan, we considered the possibility of preparing hydroxyester **12** directly from acid **5**, thus avoiding the protection−deprotection steps.

(17) For the synthesis of vitamin D-related compounds on solid support not involving the construction of the vitamin D triene system, see: Hanazawa, T.; Wada, T.; Masuda, T.; Okamoto, S.; Sato, F. *Org. Lett.* **2001**, *3*, 3975–3977. D'herde, J. N.; De Clercq, P. J. *Molecules* **2006**, *11*, 655–660.

(18) Lythgoe, B.; Roberts, D. A.; Waterhouse, I. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2608–2612.

(19) Lythgoe, B. *Chem. Soc. Rev.* **1980**, *9*, 449–475.

(20) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098–3108.

(21) Elongation of the side chain at positions C26 and C27 increases the biological activity of the vitamin D hormone: Honda, A.; Mori, Y.; Otomo, S.; Ishizuka, S.; Ikekawa, N. *Steroids* **1991**, *56*, 142–147. Miyahara, T.; Harada, M.; Miyata, M.; Sugure, A.; Ikemoto, Y.; Takamura, T.; Higuchi, S.; Otomo, S.; Kozuka, H.; Ikekawa, N. *Calcif. Tissue Int.* **1992**, *51*, 218–222. For recent examples of active analogues modified at C25, see: Shimizu, M.; Miyamoto, Y.; Takaku, H.; Matsuo, M.; Nakabayashi, M.; Masuno, H.; Udagawa, N.; DeLuca, H. F.; Ikura, T.; Ito, N. *Bioorg. Med. Chem.* **2008**, *16*, 6949–6964, and references therein. Yoon, J. S.; Kim, J. Y.; Park, H. K.; Kim, E. S.; Ahn, K. S.; Yoon, S. S.; Cho, C. G.; Kim, B. K.; Lee, Y. Y. *Int. J. Oncol.* **2008**, *32*, 387–396. Brown, A. J.; Slatopolsky, E. *Mol. Aspects Med.* **2008**, *29*, 433–452. Sundaram, S.; Beckman, M. J.; Bajwa, A.; Wei, J.; Smith, K. M.; Posner, G. H.; Gewirtz, D. A. *Mol. Cancer Ther.* **2006**, *5*, 2806–2814.

(22) Hollis, B. W.; Wagner, C. L.; Drezner, M. K.; Binkley, N. C. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 631–634.

(23) Maunsell, Z.; Wright, D. J.; Rainbow, S. J. *Clin. Chem.* **2005**, *51*, 1683–1690.

(24) Tsugawa, N.; Suhara, Y.; Kamao, M.; Okano, T. *Anal. Chem.* **2005**, *77*, 3001–3007.

(25) Mascareñas, J. L.; Pérez-Sestelo, J.; Mouriño, A. *Tetrahedron Lett.* **1991**, *32*, 2813–2816.

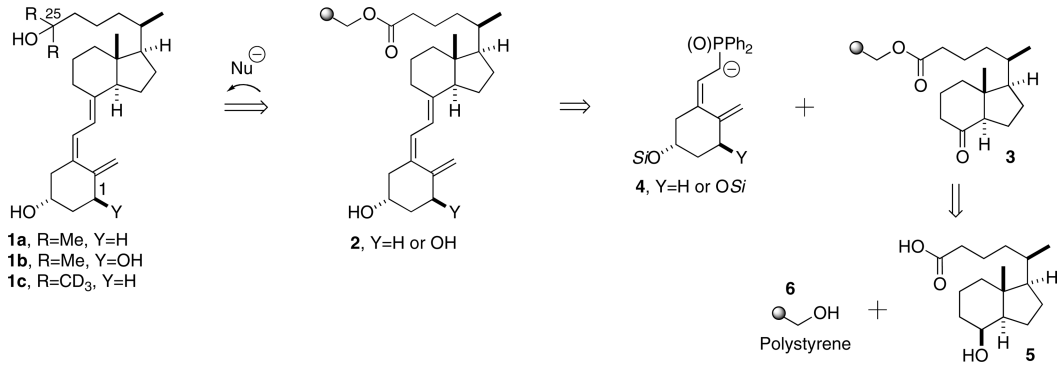
(26) Gregorio, C.; Eduardo, S.; Rodrigues, L. C.; Fraga, R.; Riveiros, R.; Maestro, M.; Mouriño, A. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 227–230.

(27) Blankemeyer-Menge, B.; Nimtz, M.; Frank, R. *Tetrahedron Lett.* **1990**, *31*, 1701–1704.

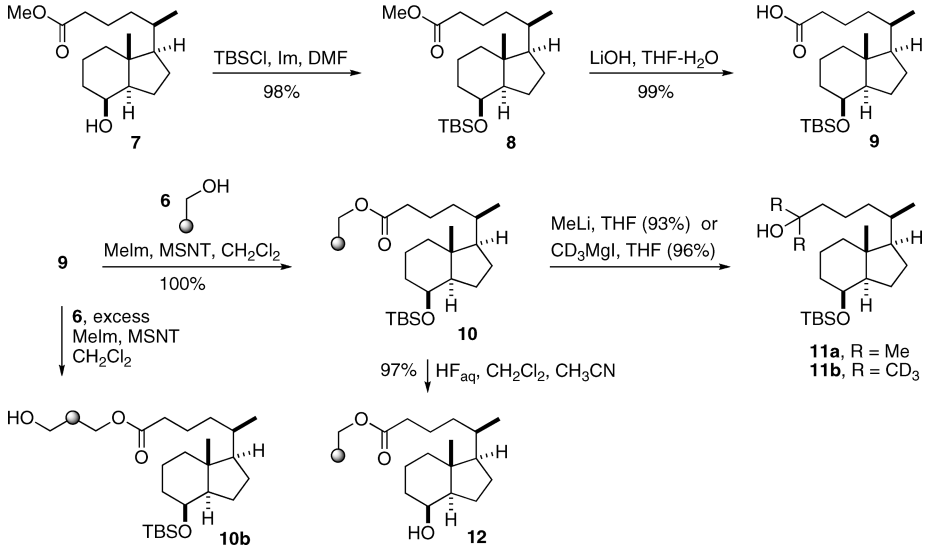
(28) The solid-phase reactions could be conveniently monitored by ^{13}C NMR spectrometry of suspended resins ($CDCl_3$). See Supporting Information. The yields of solid-phase reactions were determined by gravimetric analysis of the supported product.

(29) We attribute the 3% wastage of this step to manipulative loss of resin.

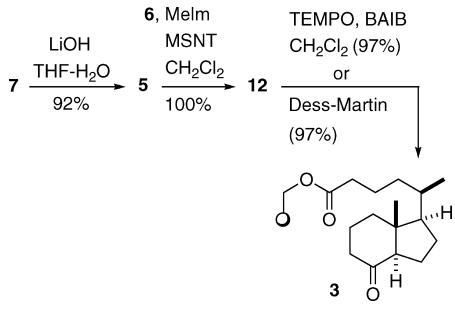
SCHEME 2. Retrosynthetic Analysis of Vitamin D_3 Metabolites and Analogues on Solid Support



SCHEME 3. Synthesis of the Solid-Supported CD-Side Chain Upper Fragment 12



SCHEME 4. Shorter Route to 12 and Oxidation



The hydroxyacid **5** was prepared by saponification of ester **7** as previously described (Scheme 4)[26] and then was treated with resin **6** under the above reaction conditions to afford polymer-supported **12** (92% yield for the two steps). Importantly, the ^1H and ^{13}C NMR spectra of the product were identical to those of the same compound obtained by the longer synthetic route. The excess of hydroxyacid **5** (2 equiv) was recovered by filtration. Attempts to prepare the solid-supported ketone **3**, required for the Wittig−Horner coupling, by oxidation of **12** with pyridinium dichromate resulted in a dark-colored material. The IR spectrum shows the presence of carbonyl groups; however, no ^{13}C NMR spectrum could be obtained, probably because of contamination with chromium species. Fortunately, oxidation of **12** with a catalytic amount of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical) in the presence of DAIB (diacetoxyiodobenzene)[30] or with Dess−Martin periodinane[31] delivered the desired polymer-supported ketone **3** in high yield (89% overall yield from **7**, three steps).[29]

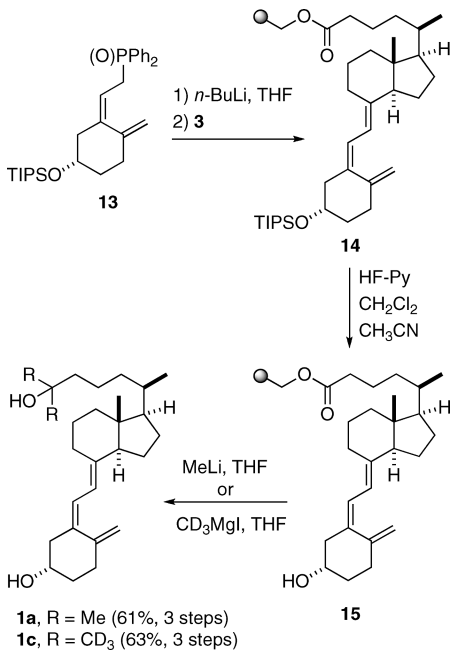
With the upper-keto fragment **3** in hand, we were ready to install the vitamin D triene system. Toward this end, the Wittig−Horner reaction proceeded stereoselectively to join ketone **3** (Scheme 5) to the anion of phosphine oxide **13**[26] (5 equiv) to produce the desired immobilized vitamin **14**. The excess of phosphine oxide was recovered by filtration. Desilylation of **14** with hydrogen fluoride pyridine complex in CH_2Cl_2/CH_3CN gave the target alcohol **15**. The ester linkage allows for an early deprotection in the solid phase that avoids the practical difficulties associated with a solution-phase deprotection after cleavage from the resin in the case of diversity-oriented synthesis[16] and prevents the contamination of the final compounds with silyl species, which is of primary importance in the case of labeled analogues.

Finally, exposure of **15** to methyllithium in THF delivered, after simple purification, the desired 25-hydroxyvitamin D_3 metabolite (**1a**) in 61% yield from the solid-supported CD-moiety **3**. Alternatively, treatment of **15** with CD_3MgI in THF produced the hexadeuterated analogue **1c** in 63% yield (3 steps).

In summary, a practical five-step solid-phase synthetic method for the preparation of 25-hydroxyvitamin D_3 metabolite and

(30) De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. *J. Org. Chem.* **1997**, *62*, 6974–6977.
(31) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.

SCHEME 5. Synthesis of 25-Hydroxyvitamins 1a and 1c



26,26,26,27,27,27-d_6-25-hydroxyvitamin D_3 has been developed. Key steps of the synthesis include (1) the use of inexpensive (hydroxymethyl)polystyrene resin; (2) the immobilization of the CD-side chain fragment through an ester function at C25; (3) the construction of the vitamin D triene system on solid support; and (4) the possibility of diversification of the side chain at C25, including the preparation of labeled material, in the last step of the synthesis, with concomitant release from the resin. The strategy is amenable to diversity-oriented synthesis by combining different CD-system and A-ring building blocks. Diversification at the side chain can be carried out using commercial organometallic reagents during the release step, avoiding the preparation of a pool of different side chain fragments. This report expands the generality of Lythgoe's Wittig−Horner approach to prepare vitamin D metabolites and analogues on solid support.

Experimental Section

Methyl 8β-[(*tert*-Butyldimethylsilyl)oxy]-de-A,B-cholane-24-carboxylate (8). Imidazole (1.24 g, 18.2 mmol) and TBSCl (2.20 g, 14.6 mmol) were consecutively added to a solution of ester **7**[25,26] (1.03 g, 3.65 mmol) in dry DMF (12 mL). The reaction mixture was stirred at 60 °C for 48 h. The reaction was quenched with brine (70 mL), and the aqueous phase was extracted successively with hexanes (3 × 60 mL) and Et_2O (1 × 80 mL). The combined organic phase was washed with H_2O (3 × 30 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (12 × 3.5 cm, 0−5% EtOAc/hexanes) to give ester **8**[32] (1.42 g, 98%) as a white solid. R_f = 0.5 (5% EtOAc/hexanes).

8β-[(*tert*-Butyldimethylsilyl)oxy]-de-A,B-cholane-24-carboxylic Acid (9). An aqueous solution of LiOH (5 mL, 2 M, 10 mmol) was added to a solution of ester **8** (0.500 g, 1.26 mmol) in THF (30 mL), and the reaction mixture was stirred at reflux for 5 h. To the mixture at room temperature was added aqueous HCl (10%) until pH = 2. After stirring for 5 min, the mixture was extracted with Et_2O (3 × 50 mL), and the combined organic phase was washed with brine (1 × 50 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (12 × 2 cm, 20% EtOAc/hexanes) to give acid **9**[32] as a white powder (0.477 g, 99%). R_f = 0.2 (20% EtOAc/hexanes).

Polymer-Bound (*tert*-Buthyldimethylsilyl) Ether 10. A mixture of acid **9** (0.343 g, 0.896 mmol), MeIm (0.17 mL, 1.80 mmol), and MSNT (0.267 g, 0.901 mmol) in dry CH_2Cl_2 (3 mL) was stirred until a homogeneous solution was obtained, and then dry THF (3 mL) was added. The resulting solution was added in two portions via syringe over hydroxymethylpolystyrene resin (**6**, 0.450 g, 1.0 mmol/g loading, 0.450 mmol, previously dried for 12 h under high vacuum) in a reaction vessel, and the mixture was shaken for 2 h under argon atmosphere. The resin was filtered and washed with CH_2Cl_2 (20 mL). The filtrate was concentrated, and the residue was purified by flash chromatography (12 × 2 cm, 20% EtOAc/hexanes) to recover unreacted excess **9** (161 mg, 94%). The resin was successively washed with CH_2Cl_2 (6 × 2 mL), DMF (2 mL), MeOH (4 × 2 mL), dioxane (3 × 2 mL), DMF (2 mL), MeOH (3 × 2 mL), and CH_2Cl_2 (6 × 2 mL). The loaded resin was air-dried and high-vacuum-dried for 12 h to give **10** (0.613 g, 0.733 mmol/g, 0.450 mmol, 100%). ^{13}C NMR (63 MHz, $CDCl_3$) δ 173.6, 144.9, 138.7, 127.7, 125.7, 69.4, 66.0, 56.4, 53.0, 45.8, 44.0, 42.1, 40.6, 35.0, 34.5, 27.3, 25.8, 23.5, 21.5, 18.6, 17.7, 13.8, −4.7, −5.1. IR (KBr) 3026, 2927, 2853, 1737 (CO), 1451, 1251, 1163, 1025, 835, 758, 698, 539 cm^{-1}.

Partially loaded resin **10b** was prepared to corroborate the absence of free hydroxyl groups by the disappearance of the signals of CH_2OH moieties in the ^{13}C NMR spectrum of the above loaded resin **10**.

Partially Loaded Resin 10b. A mixture of acid **9** (25 mg, 0.065 mmol, 0.64 equiv), MeIm (10 μL, 0.12 mmol, 1,2 equiv), and MSNT (19 mg, 0.065 mmol, 0.64 equiv) in dry CH_2Cl_2 (3 mL) was stirred until a homogeneous solution was obtained. Dry THF (3 mL) was added. The resulting solution was added in two portions via syringe over hydroxymethylpolystyrene resin **6** (102 mg, 1.0 mmol/g loading, 0.102 mmol, previously dried for 12 h under high vacuum) in a reaction vessel. The mixture was shaken for 2 h under argon atmosphere. The resin was filtered and washed successively with CH_2Cl_2 (6 × 2 mL), DMF (2 mL), MeOH (4 × 2 mL), dioxane (3 × 2 mL), DMF (1 × 2 mL), MeOH (3 × 2 mL), and CH_2Cl_2 (6 × 2 mL). The washed resin was air-dried and high-vacuum-dried for 12 h to give partially loaded resin **10b** (∼50%). ^{13}C NMR (63 MHz, $CDCl_3$) δ 173.6, 144.9, 138.7, 127.7, 125.7, 69.4, 66.0 ($RCOOCH_2$-resin-loaded), 65.1 ($HOCH_2$-resin-unloaded), 56.4, 53.0, 45.8, 44.0, 42.1, 40.6, 35.0, 34.5, 27.3, 25.8, 23.5, 21.5, 18.6, 17.7, 13.8, −4.7, −5.1.

8β-[(*tert*-Butyldimethylsilyl)oxy]-de-A,B-cholestan-25-ol (11a). A solution of MeLi in Et_2O (0.40 mL, 1.6 M, 0.64 mmol) was added to a suspension of resin **10** (100 mg, 0.733 mmol/g, 0.0733 mmol) in dry THF (3 mL) at −78 °C in a cylindrical flask. The mixture was shaken for 12 h with slow warming to −40 °C. The reaction was quenched at −78 °C by the addition of a few drops of H_2O. The mixture was filtered, and the solids were washed with CH_2Cl_2 (5 × 2 mL) and Et_2O (5 × 2 mL). The filtrate was washed with brine (10 mL), and the organic layer was dried, filtered and concentrated in vacuum to give alcohol **11a**[33] (27 mg, 93%) as a colorless oil.

Polymer-Bound Alcohol 12 from Resin 10. Resin **10** (0.600 g, 0.733 mmol/g, 0.440 mmol) was placed into a HF-resistant vial and suspended in dry CH_2Cl_2 (3 mL) and dry CH_3CN (3 mL). After 30 min, aqueous HF (0.30 mL, 48−50%) was added, and the mixture was shaken for 72 h. Unreacted HF was quenched with DIPEA (0.5 mL). After shaking for 5 min the resin was filtered and washed successively with CH_2Cl_2/DIPEA (1:1, 4 mL), CH_2Cl_2 (14 mL), CH_2Cl_2/DIPEA (1:1, 4 mL), CH_2Cl_2 (3 × 4 mL), DMF (2 mL), dioxane (6 × 2 mL), DMF (2 mL), and CH_2Cl_2 (6 × 2 mL). The resin was successively air-dried and high-vacuum-dried

(32) Nemoto, H.; Ando, M.; Fukumoto, K. *Tetrahedron Lett.* **1990**, *31*, 6205–6208.

(33) Fall, Y.; Vitale, C.; Mouriño, A. *Tetrahedron Lett.* **2000**, *41*, 7337–7340.

for 12 h to give resin loaded with alcohol **12** (0.533 g, 0.800 mmol/g, 97%). ^{13}C NMR (63 MHz, $CDCl_3$) δ 173.6, 145.3, 127.6, 125.6, 69.2, 66.0, 56.2, 52.2, 45.8, 44.0, 41.8, 40.3, 35.0, 33.5, 27.1, 22.5, 21.4, 18.5, 17.4, 13.5. IR (KBr) 3440 (OH), 2923, 2873, 2855, 1732 (CO), 1491, 1159, 756, 698, 540 cm^{-1}.

Methyl 8β-[(*tert*-Butyldimethylsilyl)oxy]-de-A,B-cholane-24-carboxylate (5). An aqueous solution of LiOH (9 mL, 2 M, 18 mmol) was added to a solution of ester **7** (0.60 g, 2.12 mmol) in dry THF (36 mL). The reaction mixture was stirred at reflux for 5 h under argon and then allowed to reach room temperature. An aqueous solution of HCl (10%) was added until pH = 2. The mixture was stirred for 5 min and then extracted with Et_2O (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (12 × 2 cm, 20% EtOAc /hexanes) to give **5**[26] as a white powder (0.53 g, 92%). R_f = 0.2 (20% EtOAc/hexanes).

Polymer-Bound Alcohol 12 from Acid 5. A mixture of acid **5** (0.088 g, 0.33 mmol), MeIm (65 μL, 0.66 mmol), and MSNT (0.085 g, 0.33 mmol) in dry CH_2Cl_2 (3 mL) was stirred until an homogeneous solution was obtained. Dry THF (3 mL) was added. The resulting solution was added in two portions via syringe to hydroxymethylpolystyrene resin (**6**, 0.105 g, 1.0 mmol/g, 0.105 mmol, previously dried for 12 h under high vacuum). The mixture was shaken for 90 min under argon atmosphere. The resin was filtered and washed with CH_2Cl_2 (20 mL). The filtrate was concentrated, and the residue was purified by flash chromatography (12 × 1 cm, 10−30% EtOAc/hexanes) to recover the unreacted excess of 5 (58 mg, 97%). The resin was washed successively with CH_2Cl_2 (6 × 2 mL), DMF (2 mL), MeOH (2 × 2 mL), dioxane (3 × 2 mL), DMF (2 mL), MeOH (2 × 2 mL), and CH_2Cl_2 (6 × 2 mL). The resin was air-dried and high-vacuum-dried for 12 h to give resin loaded with alcohol **12** (0.131 g, 0.800 mmol/g, 100%). The ^{13}C NMR spectrum was identical to the spectrum of the resin **12** obtained from **10**.

Polymer-Bound Ketone 3. Method A: TEMPO (13.1 mg, 0.084 mmol) and DAIB (0.300 g, 0.93 mmol) were successively added to a suspension of resin loaded with alcohol **12** (0.168 g, 0.800 mmol/g, 0.134 mmol) in dry CH_2Cl_2 (5 mL). After shaking in the darkness for 18 h at room temperature, the resin was filtered and washed successively with CH_2Cl_2 (2 × 4 mL), MeOH (4 mL), aq $KHCO_3$ (3 × 4 mL), H_2O (3 × 4 mL), MeOH (4 × 4 mL), and CH_2Cl_2 (3× 4 mL). The resin was nitrogen-dried and high-vacuum-dried for 12 h to give the solid-supported ketone **3** (0.163 g, 0.801 mmol/g, 97%). ^{13}C NMR (63 MHz, $CDCl_3$) δ 211.8, 145.1, 127.7, 125.6, 66.0, 61.9, 56.3, 49.8, 45.8, 44.0, 40.3, 38.9, 35.2, 34.6, 30.6, 27.5, 24.0, 21.4, 19.0, 18.7, 12.5. IR (KBr) 3025, 2923, 1734 (CO-25), 1714 (CO-8), 1601, 1311, 1450, 1097, 757, 698, 540 cm^{-1}. Method B: Dess−Martin periodinane (0.40 g, 0.94 mmol) was added to a suspension of resin **12** (0.200 g, 0.800 mmol/g, 0.16 mmol) in dry CH_2Cl_2 (6 mL) and dry pyridine (0.2 mL). The reaction mixture was shaken in the dark at rt for 18 h. The resin was filtered and washed successively with THF/($NaHCO_3$−$Na_2S_2O_3$)aq (3:1, 2 × 4 mL), THF/H_2O (3:1, 2 × 4 mL), MeOH (2× 4 mL), THF/H_2O (3:1, 2 × 4 mL), MeOH (2× 4 mL), and Et_2O (3× 4 mL). The resin was nitrogen-dried and high-vacuum-dried for 12 h to give 3 (0.194 g, 0.801 mmol/g, 97%), whose ^{13}C NMR spectrum was identical to the spectrum of the resin prepared by method A.

Polymer-Bound Protected Vitamin 14. A solution of phosphine oxide **13**[26] (0.400 g, 0.809 mmol, high-vacuum-dried over P_2O_5 for 72 h) in dry THF (3 mL) at −78 °C was treated under Ar with a solution of *n*-BuLi in hexanes (0.31 mL, 2.5 M, 0.78 mmol). After stirring for 60 min the deep red solution of the anion of **13** was added to a suspension of resin **3** (0.200 g, 0.801 mmol/g, 0.160 mmol, high-vacuum-dried over P_2O_5 for 72 h) in dry THF (3 mL) at −78 °C. The reaction mixture was shaken in the dark for 6 h and then allowed to reach −40 °C. THF (5 mL) was added, and the resin beads were filtered and washed successively with CH_2Cl_2 (3 × 3 mL), Et_2O (3 × 3 mL), MeOH (3 × 3 mL), Et_2O (3 × 3 mL), CH_2Cl_2 (3 × 3 mL), and Et_2O (3 mL). The combined filtrate was concentrated to recover the unreacted excess of phosphine oxide **13** (0.240 g, 75%, after flash chomatography). The remaining resin was nitrogen-dried and then high-vacuum-dried to give protected vitamin-loaded resin **14** (0.243 g). ^{13}C NMR (63 MHz, $CDCl_3$) δ 144.9, 127.7, 125.7, 121.4, 117.9, 111.9, 70.3, 66.0, 56.2, 47.0, 45.7, 40.4, 36.5, 35.9, 35.2, 32.6, 28.9, 27.7, 23.5, 22.3, 21.6, 18.7, 18.1, 12.4.

Polymer-Bound Vitamin 15. Hydrogen fluoride pyridine (HF-Py, 0.35 mL) was added to a suspension of resin **14** (0.238 g, high-vacuum-dried from P_2O_5 for 72 h) in dry CH_2Cl_2 (1.5 mL) and dry CH_3CN (1.5 mL). The resulting mixture was shaken at rt in the dark for 48 h. The reaction was quenched by the adition of diisopropylethylamine (DIPEA, 0.5 mL), and the resin was filtered and washed successively with with CH_2Cl_2/DIPEA (1:1, 4 mL), CH_2Cl_2 (4 mL), CH_2Cl_2/DIPEA (1:1, 4 mL), CH_2Cl_2 (3 × 4 mL), DMF (2 mL), dioxane (6× 2 mL), DMF (2 mL), and CH_2Cl_2 (6× 2 mL). The resin was high-vacuum-dried to afford resin loaded with vitamin D_3 ester **15** (0.195 g), which was directly submitted to next reactions.

25-Hydroxyvitamin D_3 (1a). A solution of MeLi in Et_2O (0.7 mL, 1.4 M, 1 mmol) was added to a suspension of resin **15** (0.101 g, high-vacuum-dried over P_2O_5 for 72 h) in dry THF (3 mL) at −78 °C. The mixture was slowly warmed to −40 °C for 12 h in the dark. The reaction was quenched at −78 °C by the addition of a few drops of MeOH. The resin was filtered and washed with Et_2O (10 mL). The solution was washed with brine (5 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (8 × 1 cm, 40% Et_2O/hexanes) to afford metabolite **1a**. [20 mg, 0,05 mmol, 61% from resin **3**, *Rf* = 0.2 (30% EtOAc/hexanes), 59% from resin **6**]. Spectral data were identical to those of an authentic sample.[25]

26,26,26,27,27,27-Hexadeutero-25-hydroxyvitamin D_3 (1c). A solution of CD_3MgI in Et_2O (0.9 mL, 1.0 M, 0.9 mmol) was added to a suspension of resin **15** (94 mg, high-vacuum-dried over P_2O_5 for 72 h) in dry THF (3 mL) at −78 °C. The mixture was slowly warmed to rt for 12 h in the dark. The reaction was quenched at 0 °C by the addition of a few drops of H_2O. The resin was filtered and washed with Et_2O (10 mL). The solution was washed with saturated NH_4Cl (10 mL) and brine (5 mL), dried, filtered, and concentrated. The residue was purified by flash chromatography (8 × 1 cm, 40% Et_2O/hexanes) to afford labeled metabolite **1c** [19 mg, 0.047 mmol, 63% from resin **3**, 61% from resin **6**]. Spectral data were identical to those of an authentic sample.[25]

Acknowledgment. We thank the Spanish Ministry of Education and Science (projects SAF2001-3187 and SAF2004-1885) and Xunta de Galicia (INCITE08PXIB-209130PR and ACEUIC-2006/XA050) for financial support, Zaida Rodríguez for preliminary experimentation, and Solvay Pharmaceuticals BV (Weesp, The Netherlands) for the gift of vitamin D_2. D. N. thanks the Argentine National Council for Scientific Research (CONICET) for a postdoctoral fellowship.

Supporting Information Available: General experimental procedures. Experimental description for labeled compound **11b**. Copies of ^{13}C NMR spectra for solid-supported compounds **3**, **10**, **10b**, **12**, and **14**. Copies of ^{1}H and ^{13}C NMR spectra for compounds **8**, **9**, **11a**, **11b**, **5**, **1a** and **1c**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO900524U