Studies on the A-Ring Diastereomers of 1α,25-Dihydroxyvitamin D_3[1a]

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The three A-ring diastereomers **3b** (compound HL), **4a** (compound HJ), and **4b** (compound HH), of the steroid hormone 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2$-D_3, **3a**, compound C) have been synthesized and biologically evaluated. (*R*)-Carvone was converted in seven steps to the enantiomerically pure A-ring enyne **7a**. Palladium-catalyzed cross-coupling of the latter with the CD-ring triflate **8** resulted in silyloxy dienyne **10**, which was converted in three steps to 1β,25-$(OH)_2$-3-epi-D_3 (**4b**). Oxidation of the latter with Dess–Martin reagent afforded trienone **6c**, which upon reduction with sodium borohydride followed by thermolysis generated the 1α-epimer **4a**. An identical sequence converted 1α,25-$(OH)_2$-D_3 to its 1β-epimer **3b** via trienone **5c**. Reduction of the latter with sodium triacetoxyborohydride followed by thermal isomerization regenerated the hormone **3a**. Relative competitive indices (RCIs) of these analogues, which reflect their ability to bind to the chick intestinal nuclear receptor under in vitro conditions, were determined. Analogues **3b**, **4a**, and **4b** had RCI values of 0.8 ± 0.1%, 24.0 ± 4.5%, and 0.22 ± 0.01%, respectively, in comparison to 1α,25-$(OH)_2$-D_3 whose value is 100% by definition. In addition, in vivo biological evaluation of these analogues was performed to determine their ability to induce intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) in vitamin D deficient chicks. Analogue **4a** was effective in stimulating ICA and BCM whereas analogues **3b** and **4b** exhibited little potency in eliciting these biological effects.

Introduction

Metabolic activation of vitamin D_3 (**1**) involves hydroxylation in the liver to form 25-hydroxyvitamin D_3 (25-OH-D_3, **2**) followed by hydroxylation in the kidney to produce 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2$-D_3,**3a**), the hormonally active form of vitamin D (Scheme I).[2] The latter metabolite functions as a classical steroid hormone to induce its classical physiological effects, intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) via genomic mechanisms.[2] In addition to its role as a regulator of calcium homeostasis, 1α,25-$(OH)_2$-D_3 has recently been shown to promote normal cell differentiation and proliferation[3] as well as to generate a variety of biological responses through nongenomic mechanisms.[4] This has led to a renewed interest in vitamin D_3 research due to its potential use as a therapeutic agent in cancer treatment[3] and in the treatment of psoriasis.[5] However, the use of 1α,25-$(OH)_2$-D_3 itself for the treatment of malignancy resulted in toxic hypercalcemia.[3c,d] Accordingly, one of the goals of our current vitamin D research is to find analogues with high potency in inducing cell differentiation with low calcitropic effects.[6]

The analogues **3b**, **4a**, and **4b** described in this study are the three A-ring alcohol diastereomers of the natural metabolite **3a**. These analogues should be useful in probing the A-ring stereochemical requirements for binding between the ligand and the hormone receptor. Furthermore, these analogues might also be used to elucidate the stereochemical requirements of 1α,25-$(OH)_2$-D_3 in its other biochemical effects such as cell differentiation and other noncalcitropic functions. It is the purpose of this article to describe methods for preparing the diastereomers of 1α,25-$(OH)_2$-D_3 and to report on their biological profile.

Results and Discussion

We envisaged that the synthesis of **4b** could be achieved by thermolysis of the previtamin **6b** obtained by a convergent route[7] from the A-ring synthon **7a**[8] and the known CD-triflate **8**.[9] A highly efficient synthesis of the enantiomer of the A-ring synthon **7a** has been previously reported from this laboratory.[8] This procedure employed (*S*)-carvone as the starting material, and we now report the synthesis of the enantiomer of this previously reported A-ring fragment from (*R*)-carvone. The latter was stereoselectively epoxidized with H_2O_2 and NaOH to afford

(1) (a) This is paper 45 in the series Studies of Vitamin D (Calciferol) and its Analogues. For part 44, see: Okamura, W. H.; Elnagar, H. Y.; Ruther, M.; Dobreff, S. *J. Org. Chem.* **1993**, *58*, 600. (b) Department of Chemistry. (c) Department of Biochemistry and the Division of Biomedical Sciences.

(2) For leading references see: (a) Norman, A. W., Bouillon, R., Thomasset, M., Eds. *Vitamin D: Gene Regulation, Structure–Function Analysis and Clinical Application*; Walter de Gruyter and Co.: Berlin, 1991. (b) Norman, A. W. *Vitamin D: The Calcium Homeostatic Steroid Hormone*; Academic Press: New York, 1979.

(3) Differentiation of leukemia cells: (a) Honma, Y.; Hozumi, M.; Abe, E.; Konno, K.; Fukushima, M.; Hata, S.; Nishii, Y.; DeLuca, H. F.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 201. (b) Koeffler, H. P.; Amatruda, T.; Ikekawa, N.; Kobayashi, Y.; DeLuca, H. F. *Cancer Res.* **1984**, *44*, 5624. (c) Munker, R.; Norman, A. W.; Koeffler, H. P. *J. Clin. Invest.* **1986**, *78*, 424. (d) Koeffler, H. P.; Hirji, K.; Itri, L.; Southern California Leukemia Group. *Cancer Treat. Rep.* **1985**, *69*, 1399.

(4) (a) Farach-Carson, M. C.; Sergeev, I.; Norman, A. W. *Endocrinology* **1991**, *129*, 1876. (b) Zhou, L.-X.; Nemere, I.; Norman, A. W. *J. Bone Min. Res.* **1992**, *7*, 457. (c) Nemere, I.; Yoshimoto, Y.; Norman, A. W. *Endocrinology* **1984**, *115*, 1476.

(5) Such analogues are also potentially useful in the treatment of skin disorders such as psoriasis: (a) MacLaughlin, J. A.; Gange, W.; Taylor, D.; Smith, E.; Holick, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5409. (b) Morimoto, S.; Onishi, T.; Imanaka, S.; Yukawa, H.; Kozuka, T.; Kitano, Y.; Yoshikawa, Y.; Kumahara, Y. *Calcif. Tissue Int.* **1986**, *38*, 119.

(6) For example, see: (a) Okamura, W. H.; Palenzuela, J. A.; Plumet, J.; Midland, M. M. *J. Cellular Biochem.* **1992**, *49*, 10. (b) Figadere, B.; Norman, A. W.; Henry, H. L.; Koeffler, H. P.; Zhou, J.-Y.; Okamura, W. H. *J. Med. Chem.* **1991**, *34*, 2452.

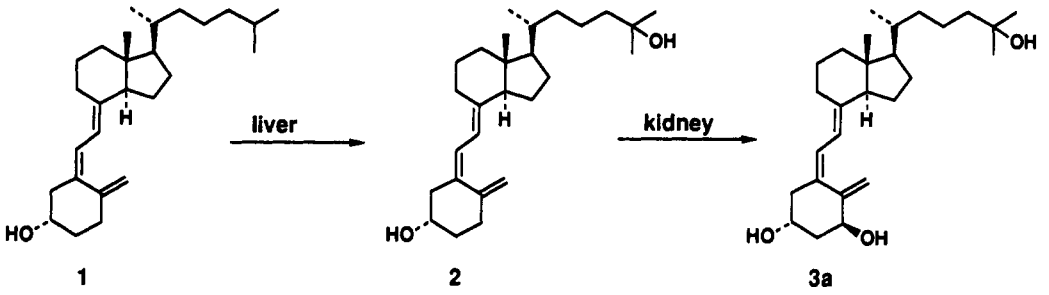
(7) (a) Castedo, L.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1986**, *27*, 1523. (b) Castedo, L.; Mascareñas, J. L.; Mouriño, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1988**, *29*, 1203. (c) Barrack, S. A.; Gibbs, R. A.; Okamura, W. H. *J. Org. Chem.* **1988**, *53*, 1790.

(8) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072.

(9) Curtin, M. L.; Okamura, W. H. *J. Am. Chem. Soc.* **1991**, *113*, 6958.

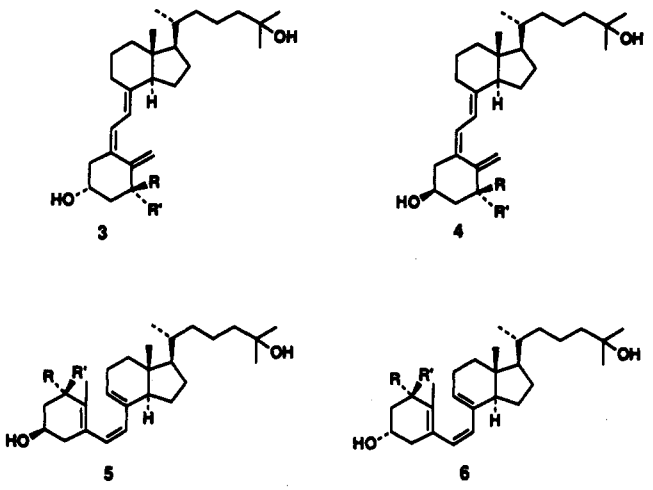
0022-3263/93/1958-1895$04.00/0

Scheme I



1 2 3a

Chart I[a]

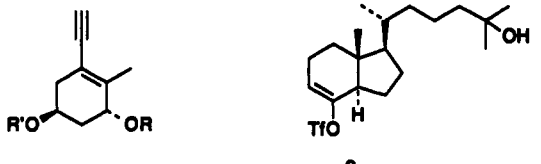


3 4 5 6

a, R = OH; R' = H
b, R = H; R' = OH
c, R,R' = O

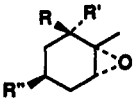
[a] Compound code names (see ref 18 of text): **3a** (compound C); **3b** (compound HL); **4a** (compound HJ); **4b** (compound HH).

Chart II



7a, R = R' = $SiMe_2t$-Bu
b, R = H; R' = Ac
c, R = R' = H

8



9a, R,R' = O; R'' = $C(CH_3)CH_2$
b, R = OH; R' = C_2H; R'' = $C(CH_3)CH_2$
c, R = OAc; R' = C_2H; R'' = $C(CH_3)CH_2$
d, R = R'' = OAc; R' = C_2H

the epoxy ketone **9a**. Treatment of **9a** with lithium acetylide afforded the epoxypropargyl alcohol **9b** with high stereoselectivity, and the esterification of this compound with acetic anhydride generated the epoxypropargyl acetate **9c**. Selective ozonolysis of **9c** followed by acylation and an in situ Creigee rearrangement afforded the epoxypropargyl diacetate **9d**.[10] The latter was converted to enynol **7c** via palladium-catalyzed samarium iodide mediated acetate displacement–epoxide ring opening followed by direct saponification of the intermediate monoacetate **7b**. Protection of the resulting diol afforded the desired A-ring fragment **7a** in good overall yield.

The A- and CD-ring fragments were coupled using bis[triphenylphosphine]palladium(II) acetate–copper(I) iodide catalyst and diethylamine in *N,N*-dimethylformamide.[11] The resulting silyloxy dienyne **10** was deprotected with tetrabutylammonium fluoride to afford the trihydroxydienyne **11**. Catalytic hydrogenation of the triol **11** in methanol, in presence of Lindlar catalyst and quinoline poison, generated the previtamin **6b**. Thermolysis of the latter at 80 °C for 4 h followed by HPLC purification afforded the vitamin **4b**.

Mazur et al.[12] and others[13] have shown that the oxidation of 1α,25-$(OH)_2$-D_3 with activated MnO_2 affords 1-oxo-25-hydroxyprevitamin D_3 (**5c**), but the yields were low. In this reaction, the C-1 allylic alcohol is oxidized preferentially over C-3 alcohol to produce 1-oxo-25-hydroxyvitamin D_3, which undergoes spontaneous [1,7]-hydrogen shift to afford **5c**. We now report the more efficient conversion of 1α,25-$(OH)_2$-D_3, or its A-ring analogue **4b**, to the corresponding trienones **5c** and **6c**, respectively, using the Dess–Martin reagent.[14] These trienones can in turn serve as synthetic precursors for the other two diasteromers of 1α,25-$(OH)_2$-D_3, namely **3b** and **4a**,[15] respectively. Thus, oxidation of **3a** with the Dess–Martin reagent afforded the corresponding trienone **5c** in 90% yield. Reduction of **5c** in a manner similar to that described earlier[13] with $NaBH_4$ in MeOH resulted in the stereoselective formation of the cis-1,3-diol **5b** in 69% yield. Thermal isomerization of the latter at 80 °C in acetone followed by HPLC separation yielded 1β,25-$(OH)_2$-D_3, **3b** (90%). Similarly, reduction of the ketovitamin **6c** with $NaBH_4$ and subsequent thermolysis of the cis-1,3-diol **6a** generated the required analogue, 1α,25-$(OH)_2$-3-epi-D_3 (**4a**).[15] Alternatively, reduction of **5c** with sodium triacetoxyborohydride afforded the 1,3-trans diol **5a** in a highly stereoselective fashion.[16] The latter procedure provides

(10) Schreiber, S. L.; Liew, W.-F. *Tetrahedron Lett.* **1983**, *24*, 2363.

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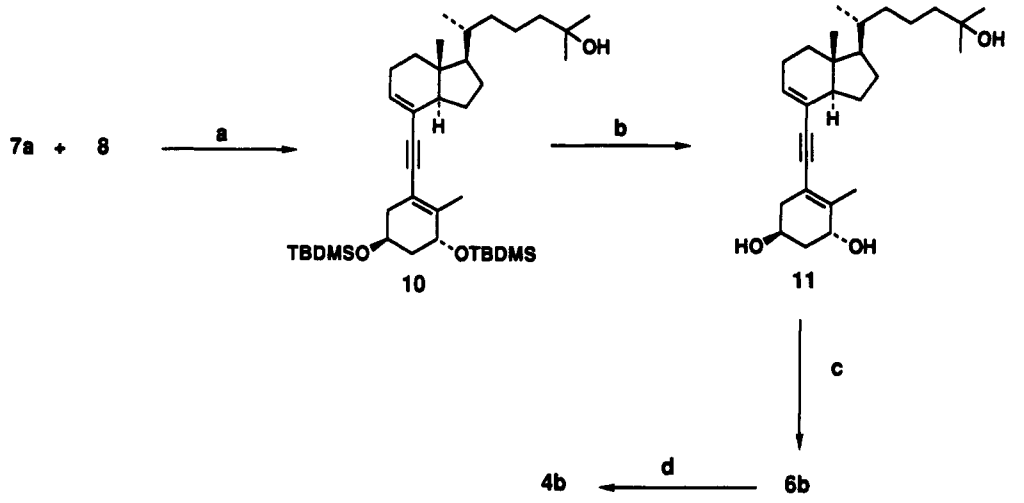
(15) (a) This substance has been reported to have been biosynthetically produced: Holick, S. A.; Holick, M. F.; Frommer, J. E.; Henley, J. W.; Lenz, J. A. *Biochemistry* **1980**, *19*, 3933. See also: (b) Semmler, E. J.; Holick, M. F.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1972**, 4147. The chemical synthesis of the 25-deoxy counterpart has also been reported: (c) Okamura, W. H.; Mitra, M. N.; Pirio, M. R.; Mouriño, A.; Carey, S. C.; Norman, A. W. *J. Org. Chem.* **1978**, *43*, 574. (d) Okamura, W. H.; Pirio, M. R. *Tetrahedron Lett.* **1975**, 4317. (e) Sheves, M.; Berman, E.; Freeman, D.; Mazur, Y. *J. Chem. Soc., Chem. Commun.* **1975**, 643.

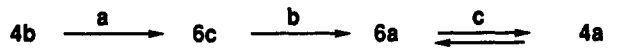
(16) (a) Evans, D. A.; DiMare, M. *J. Am. Chem. Soc.* **1986**, *108*, 2476. (b) Turnbull, M. D.; Hatter, G.; Ledgerwood, D. E. *Tetrahedron Lett.* **1984**, *25*, 5449. (c) Saksena, A. K.; Mangiaracina, P. *Tetrahedron Lett.* **1983**, *24*, 273.

Scheme II[a]



[a] Key: (a) $Pd(PPh_3)_2(OAc)_2$, CuI, DMF (79%); (b) TBAF (76%); (c) H_2, Lindlar, quinoline (95%); (d) acetone, Δ (90%).

Scheme III[a]



[a] Key: (a) Dess–Martin reagent (90%); (b) $NaBH_4$, MeOH (67%); (c) acetone, Δ (56%).

a convenient means of introducing a deuterium or tritium label at the 1β-position of the natural hormone using deuteriated or tritiated sodium triacetoxyborohydride, respectively.[17] Thus, judicious choice of the reducing agent allows for producing either C-1 epimer of 1α,25-$(OH)_2$-D_3 or its other A-ring diastereomers.

Biological Evaluation. With analogues **3b** (compound HL), **4a** (compound HJ), and **4b** (compound HH) in hand, they were evaluated in vitro in terms of their ability to bind to the chick intestinal receptor in comparison to the natural hormone 1α,25-$(OH)_2$-D_3 (**3a**, compound C).[18] In this assay,[19] the analogues are scored in terms of relative competitive indices (RCIs) wherein the value for 1α,25-$(OH)_2$-D_3 is 100% by definition. The RCI values for the four A-ring diastereomers of 1,25-$(OH)_2$-D_3, namely C (**3a**), HL (**3b**), HJ (**4a**), and HH (**4b**), are compared collectively together with selected NMR data in Table I. In yet another comparison, the dienyne 11 (compound HK) and its 1α, 3β-epimer (compound HI, structure not shown),[18] both of which resemble topologically compound HL (**4b**) and C (**3a**), respectively, exhibited RCI values of 0.05 ± 0.01% and 3.1 ± 0.8%, respectively.

Summary. Overall, the synthesis results provide a convenient means of manipulating the stereochemistry at C-1 and C-3 of the hormone 1α,25-dihydroxyvitamin D_3 (compound C). It seems likely that some of the intermediates could allow modification of the carbinol functionality as well as introduction of label.[17] Some of these studies remain for future investigations. This study provides for the first time a complete side by side comparison of the four A-ring carbinol diastereomers of

Table I. Comparison of Physical and Biological Properties

compd	code[a]	relevant ^{1}H-NMR signals	orientation of 1- and 3-hydroxyl groups	RCI values (%)
3a	C	δ 4.25, 4.45, 5.0, 5.30, 6.01, 6.35	1α,3β	100[b]
3b	HL	δ 4.11, 4.36, 5.01, 5.29, 6.05, 6.45	1β,3β	0.8 ± 0.1
4a	HJ	δ 4.05, 4.30, 5.0, 5.29, 6.02, 6.43	1α,3α	24 ● 4.5
4b	HH	δ 4.23, 4.45, 5.0, 5.32, 6.01, 6.39	1β,3α	0.22 ● 0.01

[a] See footnote 18. [b] By definition.

1α,25-dihydroxyvitamin D_3. The relative ability of the diastereomers to compete for the hormonal, chick intestinal receptor further emphasizes the critical importance of the 1α-hydroxyl group to effective binding. This compliments the earlier finding that 3-deoxy-1α,25-dihydroxyvitamin D_3 is the most effective ligand for binding to this same receptor among the three possible dihydroxylated analogues formally derived by removing a single hydroxyl group from the natural hormone, 1α,25-dihydroxyvitamin D_3.[20] A more complete description of the biological evaluation of these diastereomeric substances with respect to genomic and nongenomic actions has in part been described elsewhere.[21]

Experimental Section

1β,25-Dihydroxyvitamin D_3 (3b). Sodium borohydride (38 mg, 1.0 mmol) was reacted with 1-oxo-25-hydroxyprevitamin D_3 (**5c**) (25 mg, 0.06 mmol) in MeOH (2 mL) and then worked up as described below for the preparation of the 1α,3α-diastereomer **4a**. The product was purified by HPLC (10% iPrOH/hexane) to yield after vacuum drying 17 mg (69%) of the previtamin. The latter was dissolved in acetone (1 mL) and placed in a screw-capped vial and heated in a constant temperature bath set at 80 °C for 4 h. It was concentrated in vacuo and purified by HPLC (80% EtOAc/hexane) to afford after vacuum drying 12 mg (70%) of the vitamin as a colorless oil. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.55 (3 H, C_{18}-CH_3, s), 0.94 (3 H, C_{21}-CH_3, d, $J \approx 5.7$ Hz), 1.22 (6 H, $C_{26,27}$-CH_3, s), 2.50 (2 H, m), 2.83 (1 H, m), 4.11 (1 H, m),

(17) Dr. Moriarity has applied our sequence to synthesize C-1 labeled compounds.

(18) The compound code letters (e.g., HL, HJ, HH, and C) are used in the Riverside laboratories for a comprehensive data base of analogues and their biological activities currently under development.

(19) (a) Procsal, D. A.; Okamura, W. H.; Norman, A. W. *Am. J. Clin. Nutrition* **1976**, 1271. (b) Wecksler, W. R.; Norman, A. W. *Methods Enzym.: Vitamins Co-Enzymes* **1980**, *67*, 488. (c) Procsal, D. A.; Okamura, W. H., Norman, A. W. *J. Biol. Chem.* **1975**, *250*, 8382.

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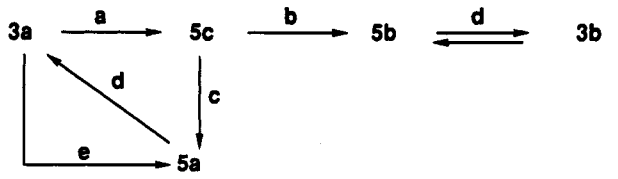
(21) Norman, A. W.; Nemere, I.; Muralidharan, K. R.; Okamura, W. H. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 1450.

Scheme IV[a]



[a] Key: (a) Dess–Martin reagent (90%); (b) $NaBH_4$, MeOH (69%); (c) $NaBH(OAc)_3$ (69%); (d) acetone, Δ (90%); (e) isooctane, reflux, rapid cooling, HPLC separation of the minor isomer.

4.36 (1 H, m), 5.01 (1 H, H_{19}, d, $J \approx 1.5$ Hz), 5.29 (1 H, H_{19}, d, $J \approx 1.2$ Hz), 6.05 and 6.45 (2 H, $H_{6,7}$, AB pattern, $J \approx 11.4$ Hz). UV: (100% EtOH) λ_{max} 264 nm (ε 17 100). This compound has been previously reported.[13]

1α,25-Dihydroxy-3-epivitamin D_3 (4a). Sodium borohydride (38 mg, 1.0 mmol) was added to an ice-cold solution of 1-oxo-25-hydroxy-3-epiprevitamin D_3 (**6c**) (25 mg, 0.06 mmol) in MeOH (2 mL). After the reaction mixture was stirred for 1 h, TLC (75% ethyl acetate/hexane) showed complete disappearance of starting material. The mixture was extracted three times with ether, and the ether extract was dried ($MgSO_4$) and then concentrated in vacuo. The crude product was purified by HPLC (10% iPrOH/hexane) to yield 17 mg (69%) of the previtamin. The latter dissolved in acetone (1 mL) was placed in a screw-capped vial and heated for 4 h in a constant temperature bath set at 80 °C. The reaction solution was concentrated in vacuo, and then the residue was purified by HPLC (10% iPrOH/hexane) to afford after vacuum drying 15 mg (90%) of the vitamin as a colorless oil. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.54 (3 H, C_{18}-CH_3, s), 0.93 (3 H, C_{21}-CH_3, d, $J \approx 6.2$ Hz), 1.21 (6 H, $C_{26,27}$-CH_3, s), 2.43 (1 H, $H_{4\beta}$, dd, $J \approx 13.5$, 5.5 Hz), 2.56 (1 H, $H_{4\alpha}$, dd, $J \approx 13.5$, 2.9 Hz), 2.83 (1 H, $H_{9\beta}$, dd, $J \approx 11.8$, 3.0 Hz), 4.0–4.1 (1 H, H_3, m), 4.25–4.35 (1 H, H_1, m), 5.0 (1 H, H_{19}, narrow m), 5.29 (1 H, H_{19}, narrow m), 6.02 and 6.43 (2 H, $H_{6,7}$, AB pattern, $J \approx 11.3$ Hz). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ 12.0, 18.8, 20.8, 22.2, 23.5, 27.7, 29.1, 29.2, 29.4, 36.1, 36.4, 40.5, 40.7, 44.4, 45.5, 45.9, 56.3, 56.5, 68.2, 71.1, 73.2, 112.9, 117.0, 125.6, 131.6, 143.2, 147.2. IR: (CCl_4) δ 3018 (OH, br, s), 2965 (sp^3 CH, br, s), 1377 (s), 1215 (s), 668 (m) cm^{-1}. UV: (95% EtOH) λ_{max} 264 nm (ε 16 900). HRMS: m/z 416.3279 (calcd. for $C_{27}H_{44}O_3$, 416.3292). MS: m/z 416 (19, M), 398 (28, M–H_2O), 380 (10, M–2H_2O), 330 (3), 285 (12), 251 (7), 227 (6), 152 (base, A-ring portion due to $C_{7,8}$-cleavage), 134 (73, m/z 152-H_2O), 107 (26), 95 (26), 81 (27), 55 (30).

1β,25-Dihydroxy-3-epivitamin D_3 (4b). A stirred mixture of dienyne **11** (27 mg, 0.065 mmol), Lindlar catalyst (27 mg), and quinoline (308 μl, 0.17 M in hexanes) in methanol (2.5 mL) was exposed to a positive pressure of hydrogen gas for 22 min. The mixture was filtered and concentrated to afford a residual oil which was purified by flash chromatography (elution with 50% ethyl acetate–hexane followed by 90% ethyl acetate–hexane) to afford 27 mg of the crude previtamin. ^{1}H-NMR analysis of the latter material showed the complete absence of starting material. A solution of the crude previtamin (27 mg, 0.065 mmol) in acetone (1 mL) was placed in a screw-capped vial and heated for 4 h in a constant temperature bath set at 80 °C. The residue was concentrated under vacuum and purified by HPLC (85% ethyl acetate–hexane, 4 mL/min, Rainin Dynamax 60A column) to afford after vacuum drying 15 mg (56%) of the vitamin as a colorless oil. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.54 (3 H, C_{18}-CH_3, s), 0.93 (3 H, C_{21}-CH_3, d, $J \approx 6.0$ Hz), 1.21 (6 H, $C_{26,27}$-CH_3, s), 2.30 (1 H, $H_{4\beta}$, dd, $J \approx 13.0$, 7.5 Hz), 2.62 (1 H, $H_{4\alpha}$, dd, $J \approx 13.0$ Hz, 3.7 Hz), 2.82 (1 H, $H_{9\beta}$, dd, $J \approx 11.8$, 3.0 Hz), 4.15–4.30 (1 H, H_3, m), 4.40–4.50 (1 H, H_1, m), 5.00 (1 H, H_{19}, narrow m), 5.32 (1 H, H_{19}, narrow m), 6.01 and 6.39 (2 H, $H_{6,7}$, AB pattern, $J \approx 11.4$ Hz). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ 12.0, 18.8, 20.8, 22.3, 23.6, 27.6, 29.1, 29.2, 29.4, 29.7, 36.1, 36.4, 40.5, 42.8, 44.4, 45.5, 45.9, 56.3, 56.5, 66.8, 71.4, 112.6, 117.0, 125.0, 132.7, 143.3, 147.3. IR: (CCl_4) ν 3357 (OH, br s), 2944 (sp^3CH, br s), 1377 (s), 1216 (s), 1053 (s), 667 (s) cm^{-1}. UV: (95% EtOH) λ_{max} 264 nm (ε 17 000). HRMS: m/z 416.3288 (calcd for $C_{27}H_{44}O_3$, 416.3292). MS: m/z 416 (21, M), 398 (72, M–H_2O), 380 (36, M–2H_2O), 362 (3), 329 (3), 285 (11), 251 (10), 227 (9), 197 (8), 152 (29, A-ring portion after $C_{7,8}$-cleavage), 134 (base, m/z 152–H_2O).

1α,25-Dihydroxyprevitamin D_3 (5a). A solution of 20 mg (0.048 mmol) of 1-oxo-25-hydroxyprevitamin D_3 (**5c**) in 0.5 mL of anhydrous THF (containing 100 μL of glacial acetic acid, 0.6 mmol) was added via cannula to a stirred suspension of sodium triacetoxyborohydride (85 mg, 0.4 mmol, 8 equiv) in THF (0.5 mL) containing 100 μL of AcOH (0.6 mmol) under N_2 at room temperature. Two additional portions of $Na(OAc)_3BH$ (42 mg each) were added after 60 and 90 min, respectively. After 120 min (total reaction time), TLC indicated complete disappearance of starting material. The mixture was poured onto ice-cooled brine and extracted with EtOAc (3 × 20 mL). The organic extracts were dried ($MgSO_4$) and evaporated to dryness. The resulting oil was purified by HPLC (Rainin Macro Column, 3 mL/min) using ethyl acetate/hexane (9:1) to afford, in order of elution, 1α,25-dihydroxyvitamin D_3 (2.4 mg, retention time ~18 min) and 1α,25-dihydroxyprevitamin D_3 (11.4 mg, retention time ~25 min). The combined yield was 69%. The material was identical to that reported elsewhere.[9] ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.71 (3 H, C_{18}-CH_3, s), 0.96 (3 H, C_{21}-CH_3, d, $J \approx 6.6$ Hz), 1.23 (6 H, $C_{26,27}$-2CH_3, s), 1.78 (3 H, C_{19}-CH_3, br s), 2.51 (1 H, apparent dd, $J \approx 14$, 4.2 Hz), 4.06 (1 H, $H_{3\alpha}$, m), 4.20 (1 H, $H_{1\beta}$, m), 5.50 (1 H, H_9, m), 5.78 and 5.92 (2 H, $H_{6,7}$, AB pattern, $J \approx 12.3$ Hz). UV: (95% EtOH) λ_{max} 260 nm (ε 8700).

1-Oxo-25-hydroxyprevitamin D_3 (5c). A solution (obtained by gently warming at 35 °C the originally obtained suspension) of 20 mg (0.05 mmol) of 1α,25-dihydroxyvitamin D_3 in 4 mL of anhydrous CH_3CN was added dropwise to a well-stirred suspension of Dess–Martin reagent (26 mg, 0.065 mmol) in CH_3CN (4 mL) under argon at room temperature. After 60 min of stirring at room temperature, an additional 6 mg (0.3 molar equiv) of oxidant was added in one portion and stirring was maintained for another 60 min. Ether (10 mL) was added, and the resulting mixture was washed with a 1:1 mixture of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution (20 mL). The organic layer was then dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using hexane/ethyl acetate (1:3) to afford 17.5 mg (88% yield) of 1-oxo-25-hydroxyprevitamin D_3 (**5c**). This substance was prepared in lower yield (<40%) using MnO_2 by previously described procedures.[12,13a] ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.72 (3 H, C_{18}-CH_3, s), 0.97 (3 H, C_{21}-CH_3, d, $J \approx 6.6$ Hz), 1.23 (6 H, $C_{26,27}$-2CH_3, s), 1.80 (3 H, C_{19}-CH_3, s), 4.17 (1 H, H_3, m), 5.50 (1 H, H_9, m), 6.04 and 6.14 (2 H, $H_{6,7}$, AB pattern, $J \approx 11.7$ Hz). ^{13}C-NMR (75 MHz): ($CDCl_3$) δ 11.2, 11.7, 18.7, 20.8, 23.3, 25.1, 28.4, 29.2, 29.3, 35.9, 36.1, 36.4, 38.8, 42.1, 44.4, 47.0, 50.6, 54.3, 67.0, 71.1, 71.2, 127.3, 132.5, 134.1, 136.4, 151.2, 197.7. UV: (95% EtOH) λ_{max} 240 nm (ε 15 000), 300 nm (ε 11 800); (ether) λ_{max} 234 nm (ε 15 100), 288 nm (ε 11 200).

1-Oxo-25-hydroxy-3-epiprevitamin D_3 (6c). 1β,25-Dihydroxy-3-epivitamin D_3 (**4b**, 28.0 mg, 0.067 mmol) was added to the Dess–Martin periodinane reagent (40 mg, 0.10 mmol) in dry CH_3CN (12 mL). The reaction mixture was stirred at room temperature for 60 min under argon. The resulting bright yellow solution was diluted with ether and washed with a 1:1 mixture (v/v) of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution (20 mL). The organic layer was then dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using 1:3 hexane/ethyl acetate to afford after vacuum drying 25 mg (90%) of 1-oxo-25-hydroxy-3-epiprevitamin D_3 as a pale yellow oil, which was sufficiently pure for spectral characterization and further reaction. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.71 (3 H, C_{18}-CH_3), 0.96 (3 H, C_{21}-CH_3, d, $J \approx 6.6$ Hz), 1.21 (6 H, $C_{26,27}$-2CH_3, s), 1.78 (3 H, C_{19}-CH_3, s), 2.4–2.6 (1 H, m), 2.70–2.85 (1 H, m), 4.16 (1 H, H_3, m), 5.47 (1 H, H_9, m), 6.05 and 6.11 (2 H, $H_{6,7}$, AB pattern, $J \approx 11.7$ Hz). UV: (95% EtOH) λ_{max} 242 nm (ε 10 000), 298 nm (ε 11 200). HRMS: (CI, NH_3) m/z 414.3145 (calcd for $C_{27}H_{42}O_3$, 414.3136). MS: (CI, NH_3) m/z 415 (15, MH), 414 (7, M), 396 (86, M – H_2O), 379 (base, MH–2H_2O), 363 (4), 338 (2), 323 (3), 295 (2), 267 (10), 253 (4), 239 (3), 213 (6), 199 (4), 171 (9), 157 (6), 135 (3), 121 (4), 107 (3), 95 (6), 81 (4), 69 (2).

(3*R*,5*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-ethynyl-2-methylcyclohex-1-ene (7a). The enantiomer of this compound was synthesized without isolation of intermediates as previously reported.[8] Epoxy diacetate **9d** (900 mg, 3.63 mmol) was treated with SmI_2 (8.5 mmol) and $Pd(PPh_3)_4$ (98 mg, 0.084

mmol) and the resulting hydroxy acetate was hydrolyzed with 0.2 M sodium methoxide in methanol at 0 °C. The diol obtained was then treated with *tert*-butyldimethylsilyl chloride (1.45 g, 9.6 mmol) and imidazole (1.27 g, 18.6 mmol) in DMF to afford 634 mg (70%) of the desired product as a colorless oil. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.06 (6 H, $Si(CH_3)_2$, s), 0.10 (6 H, $Si(CH_3)_2$, s), 0.88 (9 H, t-Bu, s), 0.90 (9 H, t-Bu, s), 1.68 (1 H, H_4, ddd, $J \approx 12.9, 9.6, 4.5$ Hz), 1.83 (1 H, H_4, dt, $J \approx 13.0, 3.3$ Hz), 1.92 (3 H, C_2-CH_3, br s), 2.08 (1 H, H_6, m), 2.42 (1 H, H_6, dd, $J \approx 16.8, 4.2$ Hz), 3.05 (1 H, sp-CH, s), 4.10 (1 H, H_5, m), 4.21 (1 H, H_3, apparent t, $J \approx 3.9$ Hz). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ −4.8, −4.7, −4.6, −4.3, 18.0, 18.1, 19.0, 25.8, 25.9, 39.4, 41.1, 64.1, 69.7, 79.6, 83.8, 114.1, 143.4. Specific rotation: $[\alpha]^{25}_D$ +80° (*c* 0.1, $CHCl_3$) [data for the enantiomer: lit.[8] $[\alpha]^{25}_D$ −102.5 °(*c* 0.4, $CHCl_3$), lit.[22] $[\alpha]^{22}_D$ −90 °($CHCl_3$)].

25-Hydroxy-de-*A,B*-cholest-8-en-8-yl Trifluoromethanesulfonate (8). This compound was prepared as previously reported.[9] ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.75 (3 H, C_{18}-CH_3, s), 0.94 (3 H, C_{21}-CH_3, d, $J \approx 6.3$ Hz), 1.21 (6 H, C_{26} and C_{27}, $2CH_3$, s), 1.7–1.8 (1 H, m), 1.9–2.1 (2 H, m), 2.25–2.35 (2 H, m), 2.4–2.5 (1 H, m), 5.56 (1 H, H_9, ddd, $J \approx 3.3, 3.3, 3.3$ Hz). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ 11.3, 18.6, 20.7, 21.4, 23.8, 28.3, 29.2, 29.3, 34.8, 35.9, 36.2, 44.2, 45.2, 50.1, 54.2, 71.0, 116.0, 118.5 (q, $J \approx 320.3$ Hz), 149.8.

(1*R*,4*R*,6*R*)-1-Methyl-4-isopropenyl-7-oxabicyclo[4.1.0]heptan-2-one (9a). Treatment[8] of (*R*)-carvone (60.11 g) with 30% H_2O_2 and 6 M NaOH in methanol afforded 59.52 g (90%) of the desired product as a colorless oil. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 1.37 (3 H, C_1-CH_3, s), 1.68 (3 H, vinyl CH_3, s), 1.86 (1 H, H_5, dd, $J \approx 14.7, 11.1$ Hz), 1.97 (1 H, H_3, dd, $J \approx 17.4, 11.7$ Hz), 2.36 (1 H, H_5, m), 2.55 (1 H, H_3, m), 2.68 (1 H, H_4, m), 3.40 (1 H, H_6, d, $J \approx 2.4$ Hz), 4.68 and 4.75 (2 H, vinylic H's, two br s). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ 15.1, 20.4, 28.6, 34.8, 41.0, 58.6, 61.1, 110.3, 146.1, 205.2.

(1*R*,2*S*,4*R*,6*R*)-2-Ethynyl-2-hydroxy-4-isopropenyl-1-methyl-7-oxabicyclo[4.1.0]heptane (9b). Treatment[8] of **9a** (10.69 g, 64.3 mmol) with lithium acetylide (96.4 mmol) in THF at −78 °C afforded 10.38 g (84%) of the desired product which was purified by crystallization (mp 56–57 °C) from boiling pentane. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 1.5–1.8 (2 H, $2H_3$, m), 1.58 (3 H, C_1-CH_3, s), 1.69 (3 H, vinyl CH_3, s), 2.05 (1 H, dt, $J \approx 13.7, 1.9$ Hz), 2.1–2.3 (2 H, m), 2.58 (1 H, sp-CH, s), 3.00 (1 H, s), 3.34 (1 H, narrow m), 4.69 and 4.75 (2 H, 2 vinyl H, two br s). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ 20.6, 20.8, 30.0, 31.7, 43.8, 61.5, 63.8, 67.8, 73.5, 85.0, 109.9, 147.0.

(1*R*,2*S*,4*R*,6*R*)-2-Acetoxy-2-ethynyl-4-isopropenyl-1-methyl-7-oxabicyclo[4.1.0]heptane (9c). Treatment[8] of epoxypropargyl alcohol **9b** (15.0 g, 78.0 mmol) with acetic anhydride (46 mL, 0.5 mmol), 4-(dimethylamino)pyridine (1.86 g), and triethylamine (212 mL) afforded 16.5 g (90%) of the desired epoxypropargyl acetate **9c** as a colorless oil. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 1.5–1.7 (2 H, m), 1.70 (3 H, vinyl CH_3, br s), 1.72 (3 H, C_1-CH_3, s), 2.08 (3 H, Ac, s), 2.15–2.35 (3 H, m), 2.66 (1 H, sp-CH, s), 3.07 (1 H, H_6, narrow m), 4.70 and 4.74 (2 H, 2 vinyl H, two br s).

(1*R*,2*S*,4*R*,6*R*)-2,4-Diacetoxy-2-ethynyl-1-methyl-7-oxabicyclo[4.1.0]heptane (9d). Ozonolysis of the acetate **9c** (4.39 g, 18.7 mmol) at −78 °C in methylene chloride (320 mL) and methanol (75 mL) followed by workup and then treatment with *p*-nitrobenzoyl chloride (9.6 g, 51.6 mmol) in methylene chloride and pyridine afforded 3.53 g (75%) of the crystalline diacetate (mp 115 °C).[8] ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 1.61 (3 H, C_1-CH_3, s), 1.96 (1 H, dd, $J \approx 13.8, 3.0$ Hz), 2.0–2.1 (1 H, m), 2.01 (3 H, Ac, s), 2.07 (3 H, Ac, s), 2.37 (1 H, dd, $J \approx 16.2, 6.3$ Hz), 2.69 (1 H, sp-CH, s), 2.85 (1 H, dd, $J \approx 11.1, 5.1$ Hz), 3.16 (1 H, H_6, d, $J \approx 3.3$ Hz), 5.00 (1 H, H_4, m). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ 18.3, 21.0, 21.4, 28.8, 35.0, 59.6, 60.3, 65.6, 74.3, 75.8, 81.5, 168.8, 169.8.

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1β-[(*tert*-Butyldimethylsilyl)oxy]-6,7-dehydro-25-hydroxy-3-epiprevitamin D_3 *tert*-Butyldimethylsilyl Ether (10). To a mixture of enol triflate **8** (80 mg, 0.2 mmol) and enyne **7a** (84 mg, 0.22 mmol) in diethylamine (1 mL) and dimethylformamide (1 mL) was added CuI (4.8 mg, 0.003 mmol) and bis[triphenylphosphine]palladium(II) acetate (5.0 mg, 0.007 mmol). The reaction mixture was stirred at room temperature for 1.5 h under argon. Ether was added, and the mixture was washed with H_2O (3 × 5 mL), dried ($MgSO_4$), and evaporated in vacuo. The crude dark brown oil was purified by flash chromatography (10% ethyl acetate–hexane) to afford after vacuum drying 102 mg (79%) of the dienyne as a viscous oil, which was sufficiently pure for the next step. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.06 (6 H, $SiCH_3$, s), 0.09 (6 H, $SiCH_3$, s), 0.70 (3 H, C_{18}-CH_3, s), 0.88 (18 H, Si-t-Bu, two s), 0.95 (3 H, C_{21}-CH_3, d, $J \approx 6.6$ Hz), 1.21 (6 H, $C_{26,27}$-$2CH_3$, s), 1.89 (3 H, C_{19}-CH_3, s), 2.45 (1 H, C_{14}-H, dd, $J \approx 16.5, 4.5$ Hz), 4.0–4.1 (1 H, H_3, br m), 4.18 (1 H, H_1, m), 5.96 (1 H, H_9, d, $J \approx 3.0$ Hz). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ −4.8, −4.7, −4.6, −4.3, 11.1, 18.0, 18.1, 18.7, 19.1, 20.8, 24.2, 25.2, 25.8, 25.9, 28.0, 29.2, 29.4, 35.9, 36.2, 36.4, 39.8, 41.3, 41.9, 44.4, 50.2, 54.7, 64.2, 70.0, 71.1, 88.1, 92.4, 115.5, 122.6, 133.2, 140.3. A satisfactory mass spectrum of this substance could not be obtained. It was best characterized as the corresponding deprotected alcohol.

1β,25-Dihydroxy-6,7-dehydro-3-epiprevitamin D_3 (11). To a solution of dienyne **10** (76 mg, 0.12 mmol) in 5 mL of THF under argon was added tetrabutylammonium fluoride (0.6 mL of 1.0 M solution in THF, 0.6 mmol). The reaction mixture was stirred at room temperature in the dark for 12 h. It was diluted with ethyl acetate and washed with brine (2 × 10 mL). The aqueous layer was extracted with ethyl acetate (2 × 10 mL), and the combined organic layer was dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography of the residual oil (elution with 50% ethyl acetate–hexane followed by 90% ethyl acetate–hexane) afforded after vacuum drying 38 mg (76%) of the triol as a colorless oil, which was sufficiently pure for characterization and further reaction. ^{1}H-NMR (300 MHz): ($CDCl_3$) δ 0.69 (3 H, C_{18}-CH_3, s), 0.95 (3 H, C_{21}-CH_3, d, $J \approx 6.6$ Hz), 1.21 (6 H, $C_{26,27}$-CH_3, s), 1.98 (3 H, C_{19}-CH_3, br s), 2.54 (1 H, H_{14}, dd, $J \approx 16.0, 4.0$ Hz), 4.04–4.12 (1 H, H_3, br m), 4.23–4.28 (1 H, H_1, narrow m), 5.97–5.98 (1 H, H_9, narrow m). ^{13}C-NMR (75.5 MHz): ($CDCl_3$) δ 11.1, 18.7, 18.8, 20.8, 24.2, 25.2, 28.0, 29.2, 29.3, 35.9, 36.2, 36.4, 39.2, 40.0, 41.9, 44.3, 50.1, 54.7, 63.4, 69.3, 71.1, 87.5, 93.4, 116.2, 122.4, 133.8, 139.4. UV: (95% EtOH) λ_{max} 272 nm (ε 14 400), 286 nm (ε 11 000). HRMS: (FAB, NBA matrix) *m/z* 414.3146 (calcd for $C_{27}H_{42}O_3$, 414.3134). MS: (FAB, NBA matrix) *m/z* 414 (15, M), 413 (11), 397 (base, M–OH), 379 (11), 363 (3), 341 (3), 323 (2), 267 (6), 255 (3), 237 (3), 197 (7), 179 (10), 165 (19).

1α,25-$(OH)_2$-D_3 Receptor Steroid Competition Assay. A measure of competitive binding to the chick intestinal 1α,25-$(OH)_2$-D_3 receptor was performed by using the hydroxylapatite batch assay.[19] Increasing amounts of 1α,25-$(OH)_2$-D_3 or analogue were added to a standard amount of [^{3}H]-1α,25-$(OH)_2$-D_3 and incubated with chick intestinal cytosol. The relative competitive index (RCI) for the analogues was determined by plotting the percent maximum 1α,25-$(OH)_2$-D_3 bound × 100 on the ordinate versus [competitor]/[1α,25-$(OH)_2$-[^{3}H]D_3] on the abscissa. The slope of the line obtained for a particular analogue is divided by the slope of the line obtained for 1α,25-$(OH)_2$-D_3; multiplication of this value by 100 gives the RCI value. By definition, the RCI for 1α,25-$(OH)_2$-D_3 is 100.

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Supplementary Material Available: Spectral and analytical data (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.