Synthesis of 25-Hydroxy- and 1α,25-Dihydroxyvitamin D_3 from Vitamin D_2 (Calciferol)

David R. Andrews, Derek H. R. Barton, Robert H. Hesse,* and Maurice M. Pechet

Research Institute for Medicine and Chemistry, Cambridge, Massachusetts 02142

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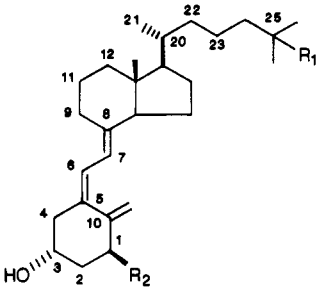
Efficient procedures for the protection and deprotection of the triene system of vitamin D_2 (calciferol) have been developed and applied to the synthesis of the important metabolites 25-hydroxy- and 1α,25-dihydroxyvitamin D_3.

25-Hydroxyvitamin D_3 (**1**) is the major circulating metabolite of vitamin D_3 (**2**).[1] The 25-hydroxy group is

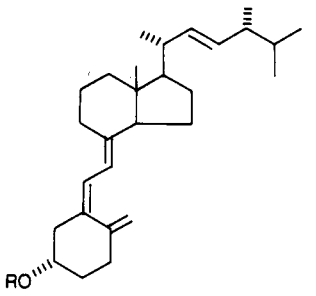


1: R^1=OH, R^2=H
2: R^1= R^2= H
3: R^1= R^2= OH
4: R^1 = H, R^2=OH

essential for enzymatic hydroxylation to 1α,25-dihydroxyvitamin D_3 (**3**), which is thought to be the hormonal form of the vitamin. The introduction of the 1α-hydroxy substituent is an essential metabolic event for the action of vitamin D on bone.[1] 1α,25-Dihydroxyvitamin D_3 (**3**), together with the synthetic analogue 1α-hydroxyvitamin D_3 (**4**), has been studied clinically in several disease states such as chronic renal failure, hypoparathyroidism, osteoporosis, and neonatal hypocalcemia.[2]

The obvious medicinal importance of these and other analogues and metabolites of vitamin D_3 has stimulated a great deal of interest in the problems involved in their partial[3] and total[4] synthesis, and several elegant solutions have materialized.

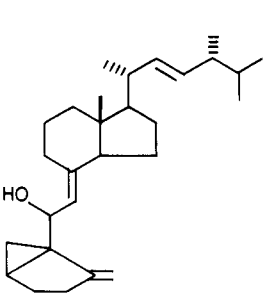
In principle, an economic synthesis starting from the readily available calciferol (vitamin D_2; **5**), via a sequence



5: R=H
12: R=CH_3CO

involving protection of the triene system, selective cleavage of the isolated side-chain double bond, and subsequent introduction of the required 25-hydroxy side chain, would have merit. We describe herein the application of this approach to the partial syntheses of 25-hydroxy- and 1α,25-dihydroxyvitamin D_3.

The concept of triene protection to allow chemical modification of the vitamin D system has received relatively little attention. Mazur has prepared the 6(*R*)-hydroxy-3,5-cyclovitamin D (**6**) by solvolysis of vitamin D



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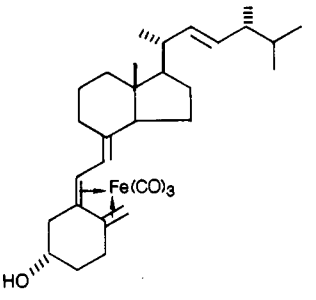


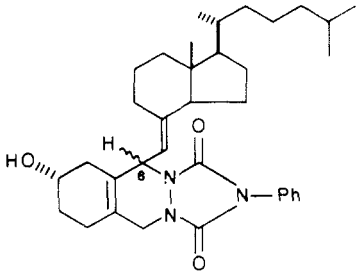
7: α-$Fe(CO)_3$
8: β-$Fe(CO)_3$

tosylate in aqueous acetone.[5] Deprotection under acidic conditions regenerated the triene system in modest yield as a mixture of the 5,6-cis and -trans isomers. We have shown previously that treatment of vitamin D_2 with nonacarbonyldiiron leads to the formation of the tricarbonyl complexes **7** and **8** (2:1), which could be efficiently deprotected under very mild conditions using ferric chloride.[6] Clearly, neither of these systems would be compatible with the proposed oxidative cleavage of the side-chain double bond of vitamin D_2.

(1) Deluca, H. F.; Paaren, H. F.; Schnoes, H. K. *Top. Curr. Chem.* **1979**, *83*, and references there cited.
(2) Jones, H.; Rasmusson, G. H. *Fortschr. Chem. Org. Naturst.* **1980**, *39*, 63 and references there cited.
(3) For example, see: Barton, D. H. R.; Hesse, R. H.; Pechet, M. M.; Rizzardo, E. *J. Am. Chem. Soc.* **1973**, *95*, 2748; *J. Chem. Soc., Chem. Commun.* **1974**, 203. Takayama, H.; Ohmori, N.; Yamada, S. *Tetrahydron Lett.* **1980**, *21*, 5027. Partridge, J. J.; Shivey, S.-J.; Chadha, N. K.; Baggiolini, E. G.; Blount, J. F.; Uskokovic, M. R. *J. Am. Chem. Soc.* **1981**, *103*, 1253. Ishiguro, M.; Koizumi, N.; Yasuda, N.; Ikekawa, N. *J. Chem. Soc., Chem. Commun.* **1981**, 115. Meadows, J. O.; Williams, D. H. *Ibid.* **1980**, *21*, 4377. Ikekawa, N.; Hirano, Y.; Ishiguro, M.; Oshida, J.-I.; Eguchi, T.; Miyasaki, S. *Chem. Pharm. Bull.* **1980**, *28*, 2852 and references there cited.
(4) Lythgoe, B. *Chem. Soc. Rev.* **1980**, *9*, 449. Kocienski, P. J.; Lythgoe, B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1400. Lythgoe, B.; Waterhouse I. *Ibid.* **1980**, 1405. Lythgoe, B.; Roberts D. A. *Ibid.* **1980**, 892. Kocienski, P. J.; Lythgoe, B.; Roberts, D. A. *Ibid.* **1980**, 897. Trost, B. M.; Bernstein, P. R.; Funfshilling, P. C. *J. Am. Chem. Soc.* **1979**, *101*, 4378. Grieco, P. A.; Takigawa, T.; Moore, D. R. *Ibid.* **1979**, *101*, 4380. Hammond, M. L.; Mourino, A.; Okamura, W. H. *Ibid.* **1978**, *100*, 4907. Condran, P., Jr.; Okamura, W. H. *J. Org. Chem.* **1980**, *45*, 4011. Cordran, P., Jr.; Hammond, M. L.; Mourino, A.; Okamura, W. H. *J. Am. Chem. Soc.* **1980**, *102*, 6259. Mourino, A.; Lewicka-Piekut, S.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1980**, *45*, 4015. Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Uskoković, M. R. *J. Am. Chem. Soc.* **1982**, *104*, 2945. Johnson, W. S.; Elliott, J. D.; Hanson, G. J. *Ibid.* **1984**, *106*, 1138 and references there cited.
(5) Sheves, M.; Mazur, Y. *J. Am. Chem. Soc.* **1975**, *97*, 6249; *Tetrahedron Lett.* **1975**, 1913; *Ibid.* **1976**, 2987. Sheves, M.; Sialom, B.; Mazur, Y. *J. Chem. Soc., Chem. Commun.* **1978**, 554.
(6) Barton, D. H. R.; Patin, H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 829.

0022-3263/86/1951-4819$01.50/0

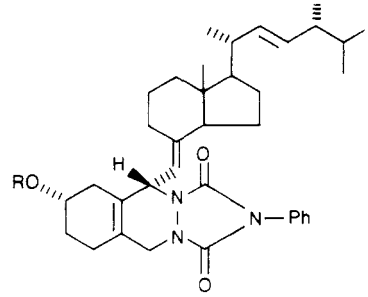
Aberhart, however, has shown that the Diels–Alder reaction of vitamin D_3 (**2**) with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) at 0 °C in ethyl acetate gives rise to a mixture of adducts **9** and **10** in the ratio 95:5.[7] In an



9: 6α-H
10: 6β-H

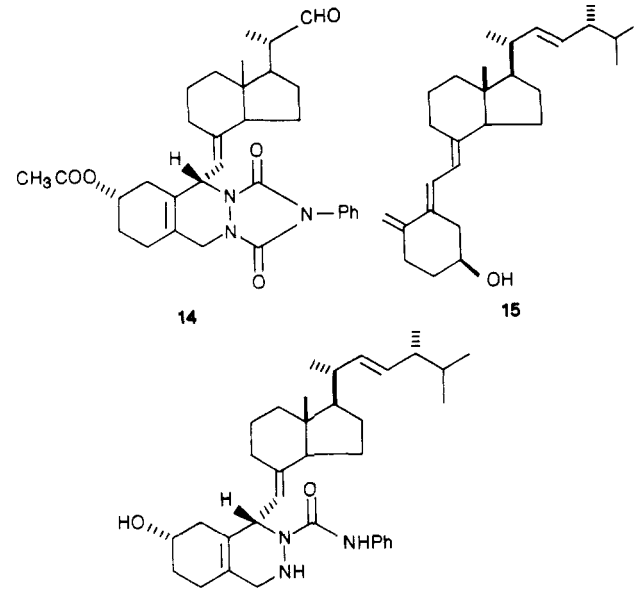
independent study, Zbiral[8] found that addition of PTAD to vitamin D_3 (**2**) in dichloromethane at 0 °C gave a 3:1 mixture of adducts **9** and **10**. We initially undertook to examine the previously unreported PTAD adducts of vitamin D_2 (**5**).

Addition of PTAD to vitamin D_2 (**5**), according to the procedure of Aberhart,[7] afforded a major product in 86% yield after crystallization. This material was strongly dextrorotary [$[\alpha]_D$ +208°] and was therefore assigned as the C-6 (*S*)-isomer **11** resulting from addition of the dienophile to the β face, in accordance with the revised assignment of Zbiral.[8b] The mother liquors were not examined further for the presence of the minor isomer.



11: R=H
13: R=CH_3CO

A similar reaction was observed for the derived acetate **12** of vitamin D_2, which gave the corresponding PTAD adduct **13** in 85% yield. On treatment with ozone (1.5 equiv) in acetone at −78 °C in the presence of tetracyanoethylene, acetate **13** afforded the corresponding aldehyde **14** (42%), together with unreacted starting material (50%). However, deprotection of adduct **11** under the conditions reported for the analogous adducts **9** and **10**, namely (a) potassium hydroxide in refluxing aqueous ethylene glycol[7] or (b) potassium hydroxide in refluxing butanol,[8] gave 5,6-*trans*-vitamin D_2 (**15**) in very poor yield (15%), together with substantial quantities of the semihydrolyzed product **16**. Similar results were obtained with potassium hydroxide in refluxing toluene in the presence of dibenzo-18-crown-6 or sodium hydroxide in refluxing methanol. Reaction of adduct **11** with potassium *tert*-butoxide (6 equiv) and water (2 equiv) in ether at room temperature[9] led to the formation of semihydrolyzed product **16** in greater than 80% yield. Under these conditions, the PTAD adducts of vitamin D_3 behaved simi-

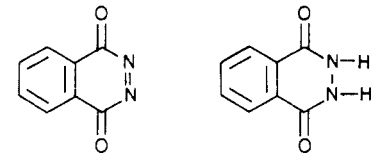


14 **15**

16

larly. Clearly, under basic reaction conditions, deprotonation of the product **16** can lead to the formation of a charged intermediate that would be resistant to further nucleophilic attack. Interestingly, subsequent reports[10,11] in the literature have described the successful regeneration of dienes from various PTAD adducts by basic hydrolysis followed by an oxidation step. In one case, the oxidation of a semihydrolyzed adduct has led to diene formation.[12]

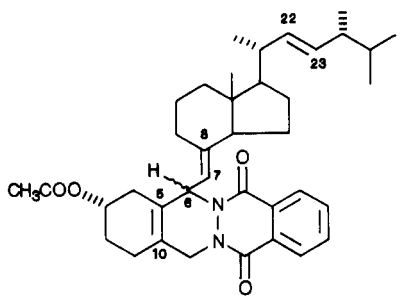
Phthalazine-1,4-dione (**17**), which is readily prepared by oxidation of the commercially available phthalhydrazide (**18**), is an extremely reactive dienophile[13–15] that has been



17 **18**

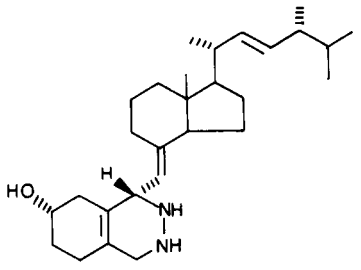
used to protect the diene system of ergosterol.[16,17] We have found that the addition of a solution of lead tetraacetate in dichloromethane to a well-stirred mixture of vitamin D_2 acetate (**12**) and phthalhydrazide (**18**) in dichloromethane at 0 °C gives rise to the adducts **19** and **20** (2:1) in 82% yield. The strongly dextrorotary major product [$[\alpha]_D$ + 343°], isolable by careful crystallization from ethyl acetate, was assigned as the 6(*S*) isomer **19** by analogy with the stereochemical assignments of the previously described PTAD adducts. Subsequent chromatography of the mother liquors afforded a strongly levorotory product [$[\alpha]_D$ −306°], which was similarly assigned as the 6(*R*) isomer **20**. Attempted deprotection of the adduct **19** by basic hydrolysis predictably gave rise to complex, polar product mixtures. However, treatment with anhydrous hydrazine in refluxing ethanol gave in quantitative yield a single, more polar product, most probably

(7) Aberhart, D. J.; Hsu, A. C.-T. *J. Org. Chem.* **1976**, *41*, 2098.
(8) (a) Reischl, W.; Zbiral, E. *Justus Liebigs Ann. Chem.* **1978**, 745. (b) Kraty, C.; Reischl, W.; Altmann, E.; Zbiral, E. *Monatsh. Chem.* **1982**, *113*, 439.
(9) Gassman, P. G.; Hodgson, P. K. G.; Baichuris, R. J. *J. Am. Chem. Soc.* **1976**, *98*, 1275.
(10) Kaufmann, D.; de Meijere, A. *Tetrahedron Lett.* **1979**, 779.
(11) Sato, T.; Ito, S. *Tetrahedron Lett.* **1979**, 1051.
(12) Askani, R.; Wieduwitt, M. *Tetrahedron Lett.* **1979**, 925.
(13) Clement, R. A. *J. Org. Chem.* **1960**, *25*, 1724.
(14) Clement, R. A. *J. Org. Chem.* **1962**, *27*, 1115.
(15) Kealy, T. L. *J. Am. Chem. Soc.* **1962**, *84*, 966.
(16) Bogoslovskii, N. A.; Litwinova, G. E.; Samokhvalov, G. I. *J. Gen. Chem. USSR* **1978**, *48*, 828.
(17) Bogoslovskii, N. A.; Litvinova, G. E.; Bekker, A. R.; Filippova, T. M.; Samokhvalov, G. I. *J. Gen. Chem. USSR* **1978**, *48*, 818.
(18) Barton, D. H. R.; Ley, S. V.; Meerholz, C. A. *J. Chem. Soc., Chem. Commun.* **1979**, 755; *Tetrahedron* **1981**, *37*, 213.



19: 6β-H
20: 6α-H

the hydrazine **21**. Subsequent oxidation of the crude product with aqueous sodium periodate led, via the intermediacy of the azo compound **22**, to the formation of 5,6-*trans*-vitamin D_2 (**15**) in 77% yield. Unfortunately, this reaction required a large excess of oxidant (30 equiv), presumably due to the presence of excess hydrazine. Since all attempts to remove this contaminant were unsuccessful, an alternative oxidant was sought.



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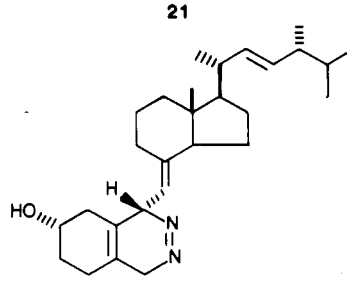

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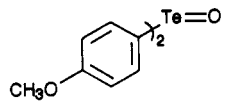
The recently described mild oxidizing agent dianisyl telluroxide (**23**)[18] was found to oxidize cleanly the hydra-

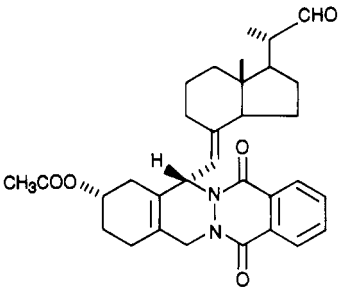


23

zine derivative **21** in dichloromethane at room temperature, to give 5,6-*trans*-vitamin D_2 (**15**) in greater than 90% yield. Moreover, by performing the oxidation of compound **21** with only 1 equiv of dianisyl telluroxide and 30 equivalents of 1,2-dibromotetrachloroethane in a two-phase system of dichloromethane and aqueous potassium carbonate, the reaction was complete after 5 h at room temperature. The isolated yields of pure 5,6-*trans*-vitamin D_2 (**15**) obtained in this way were of the order of 90%.

The 6(*R*)-phthalazine adduct **20**, when subjected to these deprotection conditions, behaved in the anticipated manner, giving rise to 5,6-*trans*-vitamin D_2 (**15**) in excellent yield. However, the PTAD adduct **11**, when treated with anhydrous hydrazine in refluxing ethanol, failed to give any of the steroidal hydrazine **21**.

With the protection and deprotection sequences for the triene system secured, the oxidative cleavage of the side chain was examined. Treatment of the phthalazine adduct **19** in dichloromethane with ozone at −78 °C, followed by addition of triphenylphosphine, afforded the required aldehyde **24** in greater than 80% yield after crystallization.

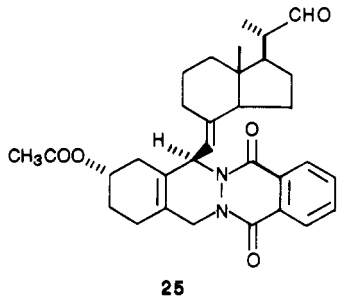


24

However, this yield was only attainable on a small scale (200 mg); when the reaction was performed on a larger scale (4–5 g), the aldehyde **24** could only be isolated in 55% yield. Clearly, the longer reaction times required for the large-scale reactions were resulting in the decomposition of intermediate ozonides with the formation of polar uncharacterized products. Attempts to introduce ozone at a faster rate led only to nonselective and/or over-oxidation. However, use of a Vibromixer permitted the introduction of ozone at a rate of 1 mmol/min without concomitant over-oxidation, and routine yields of aldehyde **24** of about 65% could be obtained after chromatography and crystallization. Alternative reductants were also investigated in an attempt to avoid chromatography. Use of dimethyl sulfide resulted in a decrease in the yield of aldehyde (55%).

When the ozonolysis was carried out in a mixture of dichloromethane and methanol (3:1), improved yields of between 75 and 85% were obtained after crystallization. This result perhaps reflects the greater stability of methoxy hydroperoxide intermediates.[19] Comparison of the ^1H NMR spectrum of the aldehyde **24** with that of a sample deliberately epimerized at C-20 by treatment with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) in benzene–methanol at room temperature clearly and unambiguously demonstrated that epimerization at C-20 had not taken place during its preparation. Aldehyde **24** was therefore assigned the "natural" 20(*S*) configuration required for subsequent transformations.

Finally, in a separate experiment, it was found that the 6(*R*)-phthalazine adduct **20** behaved in an analogous manner. Thus, ozonolysis furnished cleanly and in high yield the corresponding 20(*S*) aldehyde **25**.



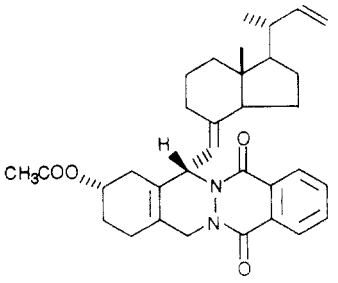
25

With the aldehyde **24** in hand, our next objective was to construct the 25-hydroxycholestane side chain. Several efficient procedures for the conversion of a C-22 aldehyde to the required side-chain product exist in the literature,[20-23] all of which introduce side-chain unsaturation at

(19) Knowles, W. S.; Thompsn, Q. E. *J. Org. Chem.* **1960**, *25*, 1031.
(20) Partridge, J. J.; Faber, S.; Uskokovic, M. R. *Helv. Chim. Acta* **1974**, *57*, 764.

an intermediate stage, via, for example, a Wittig reaction. Clearly, the success of such an approach in our case was dependant on two factors: first, the stability of the phthalazine protecting group under the conditions of the Wittig reaction; second, the selective saturation of the thus formed side-chain double bond in the presence of the 5(10) and 7(8) double bonds.

Although the phthalazine-1,4-dione adduct of ergosterol had previously been shown to be compatible with a nonstabilized Wittig reagent,[17] it was considered worthwhile to confirm this result on the aldehyde **24**. Indeed, treatment with methylenetriphenylphosphorane in diethyl ether at room temperature led to the formation of the expected methylene side-chain product **26** in 75% yield.



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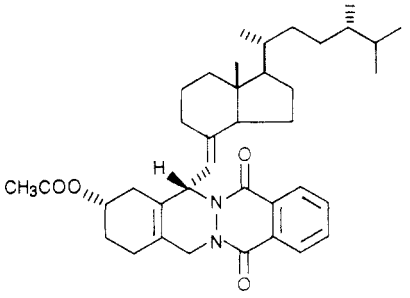
The adduct **19** was then chosen as a suitable model substrate to examine the feasibility of selective saturation of the side-chain double bond. Hydrogenation at room temperature and atmospheric pressure over 10% Pd on charcoal or in the presence of tris(triphenylphosphine)-rhodium(I) chloride proved to be ineffective. However, the use of a 5% Pt on charcoal catalyst afforded, in quantitative yield, the 22,23-dihydro product **27**. Remarkably, the 5(10) and 7(8) double bonds were completely unreactive under these conditions even over prolonged reaction times. The isomeric 6(*R*) adduct **20** behaved similarly.



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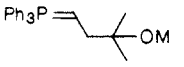
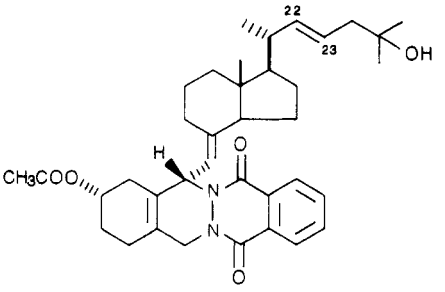
The route adopted for the construction of the required functionalized 25-carbon framework was originally described by Salmond[23] who prepared the ylide **28** by sequential treatment of methylenetriphenylphosphorane with isobutylene epoxide in tetrahydrofuran, followed by 1 equiv of *n*-butyllithium. However, in our hands, addition of the aldehyde **24** in benzene to a solution of the ylide **28** (1.5 equiv) in tetrahydrofuran, prepared in situ according to Salmond's procedure,[23] gave the desired product **30** in only 30–35% yield. Initially, it was thought that the presence of the alkoxide function of the ylide **28** was responsible for the poor yields. Use of the trimethylsilyl derivative **29**,[23] however, failed to bring about any improvement. Clearly these results reflected the low purity of the ylides used. Indeed, acidification of the ylide with dry hydrogen bromide afforded a mixture of the required hydroxyphosphonium salt **31** and methyltriphenylphosphonium bromide (**32**) (4:1).



28: M = Li
29: M = trimethylsilyl (Me_3Si)



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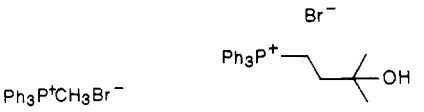
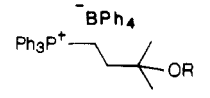


32 **31**

A simple modification of the method of Salmond was therefore developed as follows. Formation of the ylide mixture containing ylide **28** in tetrahydrofuran, using phenyllithium as base, followed by addition of 0.3 equiv of benzophenone, and subsequent acidification with 1.1 equiv of hydrobromic acid gave the crude phosphonium salt **31** (58%). This material resisted all attempts at crystallization. However, treatment of an alcoholic solution with a concentrated aqueous solution of sodium tetraphenylborate (1 equiv) resulted in the quantitative precipitation of the pure but amorphous salt **33**. Phosphonium tetraphenylborates have previously been shown to be compatible with Wittig chemistry.[24]

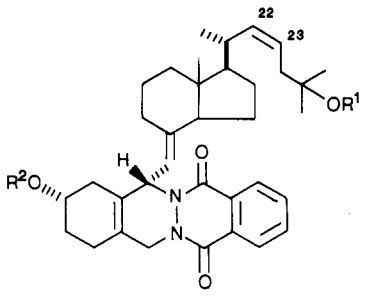


33: R = H
34: R = triethylsilyl (TES)

Addition of phenyllithium (2 equiv) to the phosphonium salt **33** in tetrahydrofuran at room temperature generated the corresponding ylide **28**, which on treatment with the aldehyde **24** afforded the olefin **30** in 47% yield. In view of the high yield (75%) obtained in the reaction of aldehyde **24** with methylenetriphenylphosphorane, it was concluded that the poorer yield obtained in this case was due to cleavage of the phthalazine group caused by alkoxide ion. Accordingly, the phosphonium salt **33** was treated in dichloromethane with triethylsilyl chloride in the presence of imidazole to give the corresponding silyl ether in 77% yield. Subsequent anion exchange with sodium tetraphenylborate furnished the crystalline phosphonium tetraphenylborate (**34**) (92%).

As predicted, generation of the corresponding ylide in tetrahydrofuran with phenyllithium followed by addition of the aldehyde **24** as before resulted in the formation of the Wittig product **35** in good yield (75%). The silyl protecting group was removed in 85% yield by treatment with acetic acid–water–tetrahydrofuran (8:1:1), to give the

(21) Salmond, W. G.; Sobala, M. C.; Maisto, K. D. *Tetrahedron Lett.* **1977**, 1237.
(22) Salmond, W. G.; Sabala, M. C. *Tetrahedron Lett.* **1977**, 1695.
(23) Salmond, W. G.; Barta, M. A.; Havens, J. L. *J. Org. Chem.* **1978**, *43*, 790.
(24) Bissing, D. E. *J. Org. Chem.* **1965**, *30*, 1296.

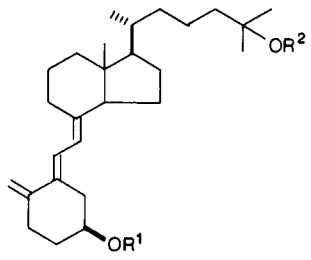


35: R^1 = TES, R^2 = CH_3CO
36: R^1 = H, R^2 = CH_3CO
37: R^1 = H, R^2 = H, 22,23-dihydro

corresponding alcohol **36** (75%). Alcohols **30** (mp 175–177 °C) and **36** (mp 182–184 °C) were tentatively assigned as the trans and cis geometrical isomers, respectively, by analogy with the assignments of Salmond.[23] However, since hydrogenation of the side-chain double bond was the next step, a definite stereochemical assignment was not considered to be critical.

Hydrogenation of the alcohol **36** over a 5% Pt on carbon catalyst (substrate:catalyst = 3:1) followed by saponification of the C-3 acetate afforded the saturated diol **37** in 90% yield after crystallization.

Deprotection of the adduct **37** under the conditions developed before gave after column chromatography and further purification by preparative layer chromatography the expected 25-hydroxy-5,6-*trans*-vitamin D_3 (**38**) in 90%

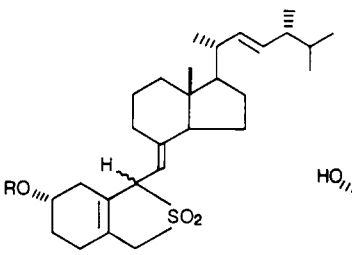


38: R^1 = R^2 = H
39: R^1 = 3,5-dinitrobenzoyl, R^2 = H

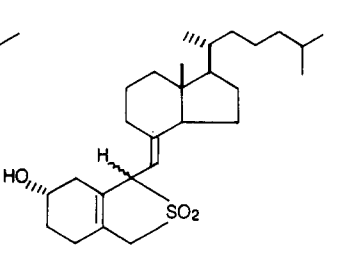
yield: UV λ_{max} 273 nm (21 500); $[\alpha]_D$ +160°; ^1H NMR δ 6.58 and 5.83 (AB q, J = 11 Hz, C-6, -7H), 4.97 (s, C-19H), 4.67 (s, C-19H), 3.85 (m, W = 14 Hz, C-3H), 1.22 (s, C-$26H_3$, -$27H_3$), 0.95 (br s, C-$21H_3$), 0.55 (s, C-$18H_3$). Further characterization was obtained by treatment with 3,5-dinitrobenzoyl chloride (1 equiv), which afforded the corresponding crystalline 3-(3′,5′-dinitrobenzoate) derivative **39**.

Subsequent photoisomerization[25] of the trans isomer **38** in the presence of anthracene[26] as triplet sensitizer gave rise to the known human metabolite 25-hydroxyvitamin D_3 (**1**) (74%), isolated as a monohydrate[27] from aqueous acetone: UV λ_{max} 262 nm (19 060); $[\alpha]_D$ +77°; ^1H NMR δ 6.25 and 6.1 (AB q, J = 11 Hz, C-6H, -7H), 5.05 (s, C-19H), 4.83 (s, C-19H), 3.9 (m, W = 18 Hz, C-3H), 1.27 (s, C-$26H_3$, -$27H_3$), 0.95 (br s, C-$21H_3$), 0.55 (s, C-$18H_3$). The melting point of this product was undepressed on admixture with an authentic sample supplied by Roussel-Uclaf.

During the course of our investigations, several reports appeared concerning the preparation and subsequent thermolysis of the sulfur dioxide adducts of vitamins D_2 and D_3.[28-30] These adducts, **40** and **41**, are readily pre-



40: R = H
44: R = TES



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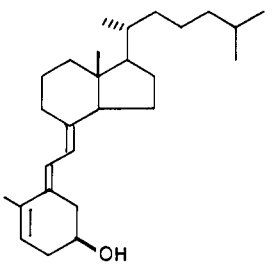

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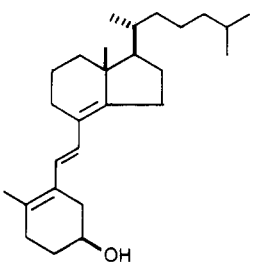

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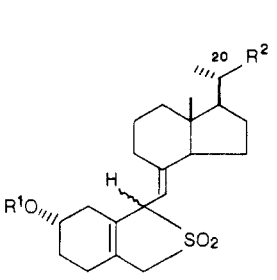
pared in quantitative yield as equimolar mixtures of the 6(R) and 6(S) epimers. Yamada and Takayma[28] have reported that thermolysis of the adducts **40** derived from vitamin D_2 (**5**) in refluxing ethanol gave rise to 5,6-*trans*-vitamin D_2 (**15**) together with traces of the 5,6-cis isomer. Under the same conditions, however, the vitamin D_3 adducts **41** apparently afforded a 1:1 mixture of vitamin D_3 and its 5,6-trans isomer. In contrast, Reischl and Zbiral[29] thermolyzed the same vitamin D_3 adducts in refluxing benzene and isolated the isovitamin D_3 (**42**) and the isotachysterol$_3$ (**43**). The formation of these products was rationalized in terms of nonconcerted mechanisms.[29] When the reaction was carried out in hot methanolic potassium hydroxide, however, a good yield of 5,6-*trans*-vitamin D was obtained. We favor an alternative mechanism involving initially a concerted retro Diels–Alder reaction to give the 5,6-trans vitamin, which in a nonpolar solvent can then undergo the known acid-catalyzed rearrangement to the observed products **42** and **43**.[31] Clearly, under basic conditions this rearrangement cannot take place. Consistent with this theory, we have found that in all the cases we have examined, the thermolysis of sulfur dioxide adducts of calciferol in reluxing alcohol containing sodium hydrogen carbonate gave the 5,6-*trans*-vitamin D in yields of 80–90%. Since this method of triene protection seemed to possess certain advantages over the phthalazine protecting group, we have applied it to the synthesis of 25-hydroxy- and 1α,25-dihydroxyvitamin D_3. As an alternative to the Wittig methodology described above we have also examined the direct displacement of C-20 primary tosylates by Grignard reagents in the presence of copper(I) salts,[32,33] as a means of constructing the required hydroxylated side chain.

The sulfur dioxide adducts **40** of vitamin D_2 were readily prepared by passing sulfur dioxide through a well-stirred mixture of vitamin D_2 (**5**) in benzene and water, according to the procedure of Zbiral.[29] Subsequent treatment with triethylsilyl chloride and imidazole in dichloromethane furnished the corresponding 3-triethylsilyl ethers **44** in 77% overall yield from vitamin D_2. As anticipated in view

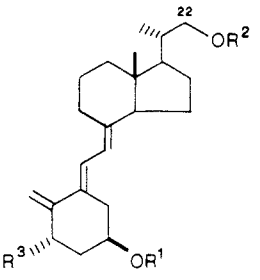
(25) Gielen, J. W. J.; Koolstra, R. N.; Jacobs, H. J. C.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1980**, *99*, 306.
(26) Downs, C. R. U.S. Patent 1 303 639; *Chem. Abstr.* **1919**, *13*, 1862.
(27) Toan, T.; Ryan, R. C.; Simon, G. L.; Calabrese, J. L.; Dahl, L. F.; Deluca, H. F. *J. Chem. Soc., Perkin Trans. 2* **1977**, 393.
(28) Yamada, S.; Takayama, H. *Chem. Lett.* **1979**, 583.
(29) Reischl, W.; Zbiral, E. *Helv. Chim. Acta* **1979**, *62*, 1763.
(30) Reischl, W.; Zbiral, E. *Monatsh.* **1979**, *110*, 1463.
(31) Inhoffen, H. H.; Quinkert, G.; Hess, J. J.; Erdmann, H. M. *Chem. Ber.* **1956**, *89*, 2273.
(32) Fouquet, G.; Schlosser, M. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 82.
(33) Posner, G. *Org. React.* **1975**, *22*, 287.

of the strongly electron-withdrawing nature of the SO_2 moiety, the 7(8) double bond was found to be inert to ozonolysis. Thus, the adduct mixture **44**, on treatment with ozone at −78 °C in dichloromethane–methanol, followed by addition of triphenylphosphine and subsequent basic workup, gave the C-22 aldehyde **45**, epimeric at C-6, in about 80% yield. The stereochemical integrity at C-20 was confirmed by subsequent transformations (see below).

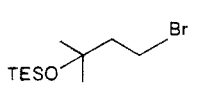
Reduction of the aldehyde **45** with sodium borohydride in ethanolic benzene afforded a mixture of the corresponding C-22 alcohols **46** in essentially quantitative yield. Thermolysis of this material in refluxing ethanol containing sodium hydrogen carbonate gave the required product **47** (47%) and the 3,22-bis(triethylsilyl ether) **48** (20%). The presence of a substantial amount of the 3,22-diol derivative **49** was also indicated by TLC analysis, but this compound was not isolated. These alternative products evidently resulted from the occurrence of trans-silylation reactions under the reaction conditions.



45: R^1 = TES, R^2 = CHO
46: R^1 = TES, R^2 = CH_2OH
51: R^1 = TES, R^2 = CH_2OTs



47: R^1 = TES, R^2 = H, R^3 = H
48: R^1 = TES, R^2 = TES, R^3 = H
49: R^1 = H, R^2 = H, R^3 = H
50: R^1 = TES, R^2 = Ts, R^3 = H
55: R^1 = TES, R^2 = Ts, R^3 = OH



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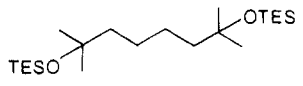

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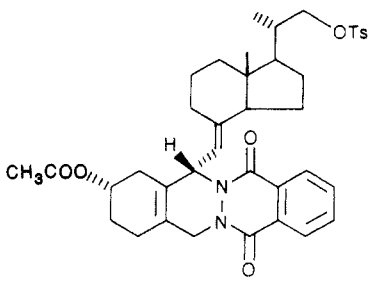

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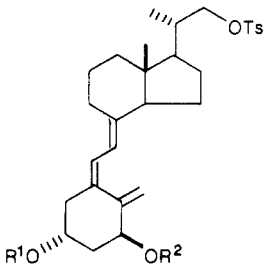
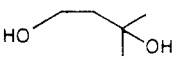
The reaction of alcohol **47** with *p*-toluenesulfonyl chloride in pyridine at room temperature for 16 h resulted in a rather complex mixture of products, from which the required *p*-toluenesulfonate **50** was isolated by chromatography in only 58% yield.

The calciferol derivative **50** could however be prepared more efficiently by an alternative procedure. Thus, the mixture of epimeric sulfur dioxide adducts derived from the C-21 alcohol **46**, on standing in pyridine containing *p*-toluenesulfonyl chloride at 5 °C, was cleanly converted to a less polar mixture of the corresponding *p*-toluenesulfonates **51**. Under these milder conditions, pyridinium hydrochloride crystallized from the reaction mixture, thereby suppressing chloride ion induced desilylation. Subsequent thermolysis in refluxing ethanol containing sodium hydrogen carbonate in the usual way afforded the desired product **50** in 70% overall yield from alcohol **46**. Sequential treatment of the Grignard reagent derived from bromide **52** in tetrahydrofuran, with copper(I) iodide (1.5 equiv, 0 °C, 30 min) followed by the *p*-toluenesulfonate **50** (room temperature, 40 min), gave after chromatography a nonpolar steroidal product contaminated with traces of a triethylsilyl ether, most probably the coupled product **53**. The crude product mixture was desilylated on treatment with tetra-*n*-butylammonium fluoride in refluxing tetrahydrofuran to give the previously described diol **38** in 85% overall yield. Interestingly, under the same conditions, *p*-toluenesulfonate **54** gave a complex mixture of polar products, most plausibly due to degradation of the phthalazine protecting group. Subsequent photoisomerization of diol **38** in the presence of anthracene as before afforded 25-hydroxyvitamin D_3 (**1**). This alternative route to 25-hydroxyvitamin D_3 (**1**) from vitamin D_2 (**5**) proceeded with an overall yield of 25%.

We have recently developed an efficient procedure for the direct introduction of the physiologically important 1α-hydroxy substituent into the vitamin D molecule, which proceeds with high regio- and stereoselectivity.[34] By this method allylic hydroxylation of *p*-toluenesulfonate **50** (*N*-methylmorpholine *N*-oxide, selenium dioxide, dichloromethane–methanol, reflux) gave the 1α-hydroxy product **55** (57%), which was photoisomerized to the corresponding 5,6-cis isomer **56** in 88% yield. Subsequent protection of



56: R^1 = TES, R^2 = H
57: R^1 = R^2 = TES



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the 1α-hydroxy substituent (triethylsilyl chloride, imidazole, dichloromethane) to give **57** followed by treatment with the cuprate reagent as before gave rise to a less polar steroidal product, again contaminated with the silyl ether **53**. Desilylation was effected by treatment with excess tetra-*n*-butylammonium fluoride in tetrahydrofuran to afford a mixture of 1α,25-dihydroxyvitamin D_3 (**3**) (63%) and what appeared to be isopentanediol (**58**). Crystallization from acid-free chloroform gave a pure sample of the known crystalline chloroform solvate of 1α,25-dihydroxyvitamin D_3 (**3**).

Experimental Section

Melting points were determined on a Kofler block (Mel-Temp or Fisher-Johns apparatus) and are uncorrected. Optical rotations were recorded at room temperature on a Rudolph photoelectric polarimeter and refer to chloroform solutions (unless otherwise stated). IR spectra were recorded on a Perkin-Elmer 137 infracord spectrophotometer and are reported for KBr disks (unless otherwise stated). UV spectra were recorded with a Cary 11 spectrophotometer and are reported for ethanol solutions. NMR spectra were recorded with a Varian T60 spectrometer for solutions in $CDCl_3$ (unless otherwise stated); chemical shifts are reported downfield from internal Me_4Si (δ). Medium-pressure column chromatography was carried out with Merck silica gel 60H. Thin-layer chromography (TLC) was carried out on 250 silica gel GHLF Uniplates, and preparative layer chromatography (PLC) on 1-mm silica gel GF-254 Analtech Uniplates. Reactions on calciferol substrates were routinely performed under an argon atmosphere.

6(*S*),19-(4-Phenyl-3,5-dioxo-1,2,4-triazolidine-1,2-diyl)-3β-hydroxy-9,10-secoergosta-5(10),7(*E*),22(*E*)-triene (11). To ergocalciferol (5 g) in ethyl acetate (150 mL) at 0 °C under an argon atmosphere was added 4-phenyl-1,2,4-triazoline-3,5-dione

(2.4 g, 1.1 equiv) in ethyl acetate (150 mL) over 45 min. After a further 1 h, some of adduct **11** had precipitated. The mixture was filtered and the filtrate passed down a neutral alumina column. Elution with hexane–ethyl acetate gave the remainder of the product.

Crystallization from alcohol gave the *title compound*: 6.2 g (86%); mp 99 °C; $[\alpha]_D$ +208° (*c* 0.76); ^{1}H NMR δ 7.48 (s, 5 H, aryl), 5.22 (m, *W* = 10 Hz, C-22H, -23H), 4.98 and 4.73 (AB q, *J* = 10 Hz, C-H, -7H), 4.2 and 3.85 (AB q, *J* = 15 Hz, C-19H_2), 4.1 (m, C-3H), 0.53 (s, C-19H_3); IR ν_{max} ($CHCl_3$) 3700 (br), 2950 (s), 1775 (m), 1710 (s), 1425 (s) cm^{-1}; MS *m/e* 571. Anal. Calcd for $C_{36}H_{49}O_3N_3$: C, 75.62; H, 8.64; N, 7.35. Found: C, 75.63; H, 8.62; N, 7.36.

Similarly prepared from ergocalciferol acetate (**12**) in 85% yield was the corresponding acetate **6(*S*),19-(4-phenyl-1,2,4-triazolidine-1,2-diyl)-3β-acetoxy-9,10-secoergosta-5(10),7(*E*),22(*E*)-triene (13)**: crystallized from ethanol; mp 85°C; $[\alpha]_D$ +183° (*c* 0.82); δ 7.48 (s, 5 H, aryl), 5.22 (m, *W* = 12 Hz, C-3H, C-3H, -22H, -23H), 4.98 and 4.73 (AB q, *J* = 10 Hz, C-6H, -7H), 4.2 and 3.85 (AB q, *J* = 16 Hz, C-19H_2), 2.0 (s, OAc), 0.53 (s, C-18H_3); IR ν_{max} ($CHCl_3$) 2950 (s), 2900 (sh), 1725 (s), 1420 (m) cm^{-1}; MS *m/e* 613. Anal. Calcd for $C_{38}H_{51}O_4N_3$: C, 74.35; H, 8.83; N, 6.85. Found: C, 74.18; H, 8.11; N, 6.65.

Ozonolysis of Adduct 13. The adduct **13** (250 mg) in acetone (10 mL) containing tetracyanoethylene (55 mg, 1 equiv) at −78 °C was treated with ozone for 3 min (approximately 1.5 equiv). The system was purged with argon while warming to room temperature. The product mixture was separated by PLC to give 130 mg of starting material (NMR) and the aldehyde **14**: 90 mg (84%); white foam; ^{1}H NMR δ 9.55 (d, *J* = 3.75 Hz, C-22H), 7.45 (s, 5 H, aryl), 5.15 (m, *W* = 12 Hz, C-3H), 4.92 and 4.82 (AB q, *J* = 10 Hz, C-6H, -7H), 4.18 and 4.70 (AB q, *J* = 16 Hz, C-19H_2), 2.0 (s, OAc), 1.12 (d, *J* = 7 Hz, C-21H_3), 0.57 (s, C-18H_3).

Semihydrolyzed Adduct 16. To the adduct **11** (200 mg) in ether (10 mL) containing water (6 μL, 2 equiv) was added potassium *tert*-butoxide (270 mg, 6.5 equiv). After stirring at room temperature for 2 h, the mixture was added to ice water and diluted with ether. The ethereal solution was washed with water–brine and dried. Filtration and evaporation gave the *semihydrolyzed adduct 16*: 163 mg (85%); crystallized from CH_2Cl_2–hexane, mp 164 °C; $[\alpha]_D$ +297° (*c* 1.1); UV λ_{max} 243 nm (24 800), 275 (1240); ^{1}H NMR δ 8.45 (s, exchanges with D_2O, NH), 7.7–6.85 (m, 5 H, aryl), 5.15 and 4.85 (AB q, *J* = 10 Hz, C-6H, -7H), 5.18 (m, *W* = 10 Hz, C-22H, -23H), 4.0 (m, *W* = 22 Hz, C-3H, -19H_2), 0.52 (s, C-18H_3); IR ν_{max} ($CHCl_3$) 3500 (br), 2950 (s), 2900 (sh), 1660 (s), 1590 (m), 1525 (s), 1440 (s) cm^{-1}; MS *m/e* 545. Anal. Calcd for $C_{35}H_{51}N_3O_2$: C, 77.02; H, 9.42; N, 7.70; O, 5.86. Found: C, 76.81; H, 9.42; N, 7.50; O, 6.04.

Reaction of Calciferol Acetate (12) with Phthalazine-1,4-dione (17). Phthalhydrazide (**18**; 10 g, 2.5 equiv) was suspended in a solution of calciferol acetate (**12**; 10 g) in dry CH_2Cl_2 (200 mL). The efficiently mixed mixture was cooled to 0 °C, and a solution of lead tetraacetate (20 g) in dry CH_2Cl_2 (100 mL) and acetic acid (1 mL) was added dropwise. The reaction was monitored by TLC. Upon completion, the residual phthalhydrazide was filtered off. Aqueous workup followed by careful crystallization from ethyl acetate gave **6(*S*),19-(*N*,*N*′-phthalhydrazido)-3β-acetoxy-9,10-secoergosta-5(10),7(*E*),22(*E*)-triene (19)**: 7.4 g (54%); mp 202–203 °C; $[\alpha]_D$ +343° (*c* 1.02); UV λ_{max} 238 nm (38 250), 312 (11 300); ^{1}H NMR δ 8.3 (m, *W* = 12 Hz, 2 H, aryl), 7.8 (m, *W* = 10 Hz, 2 H, aryl), 5.9 (d, *J* = 10 Hz, C-7H), 5.08 (m, *W* = 10 Hz, C-3H, -22H, -23H), 4.78 and 4.22 (AB q, *J* = 18 Hz, C-19H_2), 4.75 (d, *J* = 10 Hz, C-6H), 2.0 (s, OAc), 0.13 (s, C-18H_3); IR ν_{max} 2950 (s), 2900 (sh), 1750 (s), 1660 (s), 1610 (m), 1380 (m), 1355 (m), 1250 (s) cm^{-1}; MS *m/e* 598. Anal. Calcd for $C_{38}H_{50}O_4N_2$: C, 76.22; H, 8.42; N, 4.68. Found: C, 75.92; H, 8.30; N, 4.61. The mother liquors were chromatographed on silica gel to give 3.6 g (26%) of essentially pure **6(*S*),19-(*N*,*N*′-phthalhydrazido)-3β-acetoxy-9,10-secoergosta-5(10),7-(*E*),22(*E*)-triene (20)**: mp 114–116 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ 306° (*c* 0.64); ^{1}H NMR δ 8.3 (m, *W* = 12 Hz, 2 H, aryl), 7.8 (m, *W* = 10 Hz, 2 H, aryl), 6.0 (d, *J* = 10 Hz, C-7H), 5.2 (m, *W* = 10 Hz, C-3H, -22H, -23H), 4.83 (d, *J* = 10 Hz, C-6H), 4.78 and 4.23 (AB q, *J* = 18 Hz, C-19H_2), 2.17 (s, OAc), 0.65 (s, C-18H_3); IR ν_{max} 2950 (s), 2900 (sh), 1660 (s), 1610 (m), 1380 (m), 1355 (m), 1250 (s) cm^{-1}; MS *m/e* 598.

General Procedure for the Ozonolysis of the Ergosterol Side Chain. The adduct (4–5 g) in CH_2Cl_2 (180 mL) and methanol (60 mL) was cooled to −78 °C. The efficiently mixed solution was treated with an ozone–oxygen mixture (approximately 1 mmol of O_3/min) for 8–12 min (TLC control) and then thoroughly purged with dry argon for approximately 5 min. Triphenylphosphine (2.5–3 g) was added, and the mixture, after approximately 30 min at −78 °C (TLC monitoring of the breakdown of the methoxy hydroperoxide intermediates) was shaken with 5% aqueous $NaHCO_3$ (to prevent dimethyl acetal formation) and allowed to warm to room temperature. The layers were separated and the organic solution dried. Chromatography through silica gel (40–50 g) gave the aldehyde (75–86%) free from any of the C-20 (*R*) epimer (NMR).

6(*S*),19-(*N*,*N*′-Phthalhydrazido)-3β-acetoxy-20(*S*)-formyl-9,10-secopregna-5(10),7(*E*)-diene (24): prepared according to the general procedure; mp 192–193 °C (CH_2Cl_2–ether); $[\alpha]_D$ 382° (*c* 1.235); ^{1}H NMR δ 9.55 (d, *J* = 3 Hz, C-22H), 8.3 (m, *W* = 12 Hz, 2 H, aryl), 7.8 (m, *W* = 10 Hz, 2 H, aryl), 5.9 (d, *J* = 10 Hz, C-7H), 5.17 (m, C-3H), 4.78 and 4.22 (AB q, *J* = 18 Hz, C-19H_2), 4.75 (d, *J* = 10 Hz, C-6H), 2.07 (s, OAc), 1.07 (d, *J* = 7 Hz, C-21H_3), 0.22 (s, C-18H_3); IR ν_{max} ($CHCl_3$) 2950 (m), 2900 (sh), 1740 (s), 1645 (s), 1610 (m), 1370 (m), 1350 (m) cm^{-1}; MS *m/e* 530. Anal. Calcd for $C_{32}H_{38}O_5N_2$: C, 72.43; H, 7.22; N, 5.28. Found: C, 72.13; H, 7.12; N, 5.20.

6(*S*),19-(*N*,*N*′-Phthalhydrazido)-3β-acetoxy-20(*S*)-ethenyl-9,10-secopregna-5(10),7(*E*)-diene (26). Methyltriphenylphosphonium bromide (600 mg, 1.2 equiv) was suspended in THF (6 mL). *n*-Butyllithium (1.5 M solution, 0.15 mL) was added. To the resulting orange solution was added the aldehyde **24** (100 mg) in benzene (6 mL) quickly. After a further 10 min, water was added and the mixture extracted with CH_2Cl_2. Acid workup followed by purification by PLC gave 75 mg (75%) of the *title compound*: mp 173–175 °C (CH_2Cl_2–ether); $[\alpha]_D$ +386° (*c* 0.86); ^{1}H NMR δ 8.3 (m, *W* = 12 Hz, 2 H, aryl), 7.8 (m, *W* = 10 Hz, 2 H, aryl), 5.9 (d, *J* = 10 Hz, C-7H), 5.6–4.8 (m, C-3H, -22H, -23H_2), 4.78 and 4.21 (AB q, *J* = 7 Hz, C-21H_3), 0.17 (s, C-18H_3); IR ν_{max} 2950 (m), 1740 (s), 1650 (s), 1610 (m), 1370 (s), 1350 (s), 1260 (s), 1230 (s) cm^{-1}; MS *m/e* 528. Anal. Calcd for $C_{33}H_{40}O_4N_2$: C, 74.97; H, 7.63; N, 5.30. Found: C, 75.03; H, 7.72; N, 5.21.

6(*S*),19-(*N*,*N*′-Phthalhydrazido)-3β-acetoxy-9,10-secoergosta-5(10),7(*E*)-diene (27). Compound **19** (100 mg), 5% Pt/C (100 mg), and $NaHCO_3$ (50 mg) were stirred together under a hydrogen atmosphere for 16 h, in a mixture of benzene (5 mL) and alcohol (5 mL). Filtration through Celite gave the saturated *side-chain compound* **27** essentially quantitatively: mp 175–177 °C (ethyl acetate); $[\alpha]_D$ +347° (*c* 0.83); ^{1}H NMR δ 8.3 (m, *W* = 12 Hz, 2 H, aryl), 7.8 (m, *W* = 10 Hz, 2 H, aryl), 5.9 (d, *J* = 10 Hz, C-7H), 5.08 (m, C-3H), 4.78 and 4.22 (AB q, *J* = 18 Hz, C-19H_2), 4.75 (d, *J* = 10 Hz, C-6H), 2.03 (s, OAc), 0.14 (s, C-18H_3); IR ν_{max} 2950 (s), 2900 (sh), 1750 (s), 1650 (s), 1610 (m), 1370 (s), 1350 (s), 1250 (s), 1030 (s) cm^{-1}; MS *m/e* 600. Anal. Calcd for $C_{38}H_{52}O_4N_2$: C, 75.96; H, 8.72; N, 4.66. Found: C, 75.68; H, 8.73; N, 4.73.

Similar treatment of the isomeric C-6 (*S*) compound **20** gave the corresponding saturated side-chain product (NMR).

[3-[(Triethylsilyl)oxy]-3-methylbut-1-yl]triphenylphosphonium Tetraphenylborate (33). To methyltriphenylphosphonium bromide (3 g) suspended in THF (40 mL) was added phenyllithium (1 equiv, 6 mL of a 1.5 M solution). After 15 min isobutylene epoxide (1 mL, 1.25 equiv) was added followed, after a further 5 min, by a second addition of phenyllithium (1 equiv). To this mixture was added benzophenone (1 g, approximately 0.3 equiv). After stirring for 20 min, the reaction was quenched with 48% aqueous HBr until just acidic (litmus paper). The organic solvent was removed on a rotary evaporator, water was added, the aqueous layer was washed with ether, and the layers were separated. The water was removed (rotary evaporator) and the resulting oil taken up in CH_2Cl_2. Aqueous workup gave the phosphonium salt **31**: 3.1 g (58%); oil; ^{1}H NMR 8.17–7.67 (m, 15 H, aryl), 5.37 (br s, OH), 3.8 (m, *W* = 32 Hz, C-1H_2), 1.8 (m, *W* = 22 Hz, C-2H_2), 1.28 [s, $(CH_3)_2$]; IR ν_{max} ($CHCl_3$) 3450 (s), 3000 (s), 1590 (sh), 1440 (s) cm^{-1}.

To the phosphonium salt **31** (3.7 g) in CH_2Cl_2 (70 mL) was added imidazole (3.4 g) followed by triethylsilyl chloride (5 mL). After 40 h of stirring at room temperature, water was added and the mixture diluted with CH_2Cl_2. The CH_2Cl_2 solution after an acid workup was evaporated and the oily residue partitioned between water and hexane–ether. The water was evaporated and the residue taken up in CH_2Cl_2, which was washed with brine and dried to give on evaporation the bromide salt [3.6 g (77%)] as an oil.

To this bromide salt (3.6 g) in 95% ethanol (50 mL) was added dropwise, with stirring, a solution of sodium tetraphenylborate (2.5 g, 1.1 equiv) in water (20 mL). An oily residue was formed that solidified on continued stirring. Filtration gave the *salt* **34** [4.78 g (92%)] as a white, amorphous, nonhygroscopic solid that was recrystallized from acetone–hexane–ethanol: mp 150–151 °C; ^{1}H NMR (acetone-d_6) δ 8.2–6.8 (m, 35 H, aryl), 3.53 (m, W = 34 Hz, C-1H$_2$), 1.8 (m, W = 24 Hz, C-2H$_2$), 1.33 (s, (CH$_3$)$_2$), 1.25–0.5 (m, 15 H, SiEt$_3$); IR ν_{max} 3100 (s), 2950 (s), 1580 (m), 1490 (s), 1440 (s), 1110 (s), 1020 (s) cm^{-1}. Anal. Calcd for $C_{53}H_{60}BOPSi$: C, 81.31; H, 7.73; P, 3.96. Found: C, 81.41; H, 7.73; P, 3.93.

6(*S*),19-(*N*,*N*′-Phthalhydrazido)-3β-acetoxy-25-hydroxy-9,10-secocholesta-5(10),7(*E*),22(*E*)-triene (30). Method A. To methyltriphenylphosphonium bromide (2.898 g) suspended in THF (32 mL) at 0 °C was added butyllithium (2.03 M, 4 Ml). Isobutylene epoxide (720 μL, 1 equiv) was slowly added. After a further 15 min, butyllithium (4 mL) was added. To 3 mL of this solution was added the aldehyde **24** (300 mg) in benzene (10 mL). The red color was quickly discharged. Water was added and the mixture extracted with CH_2Cl_2. After acid workup the major product was isolated by PLC to give **30**, 105 mg (31%).

Method B. The salt **31** (628 mg) was suspended in ether (15 mL) and THF (10 mL). Butyllithium was added dropwise until a stable color was formed, and then 0.75 mL (2 equiv for steroid, 1 equiv for P compound) was added. To this mixture was added the aldehyde **24** (400 mg) in benzene (6 mL) (approximately 5 min). After the addition, water was added and the mixture extracted with CH_2Cl_2. Workup as above gave **30**, 155 mg (34%).

Method C. The phosphonium salt **33** (280 mg, 1.5 equiv) was dissolved in THF (15 mL) at 0 °C. Phenyllithium (3 equiv) was added. The aldehyde **24** (150 mg, 1 equiv) in benzene (6 mL) was added quickly. TLC showed no change during 30 min, and so water was added. Workup as above and isolation by PLC gave *the product* **30**: 80 mg (47%); mp 175–177 °C (CH_2Cl_2–ether); $[\alpha]_D$ +347° (*c* 0.83); ^{1}H NMR δ 8.3 (m, W = 12 Hz, 2 H, aryl), 7.8 (m, W = 10 Hz, 2 H, aryl), 5.9 (d, J = 10 Hz, C-7H), 5.27 (m, W = 10 Hz, C-3H, -22H, -23H), 4.78 and 4.21 (AB q, J = 18 Hz, C-19H$_2$), 4.75 (d, J = 10 Hz, C-6H), 2.03 (s, OAc), 1.15 (s, C-26H$_3$, -27H$_3$), 0.97 (d, J = 7 Hz, C-21H$_3$), 0.17 (s, C-18H$_3$); IR ν_{max} 3800 (m), 2950 (s), 2900 (sh), 1750 (s), 1650 (s), 1610 (m), 1370 (s), 1350 (s), 1240 (s), 965 (m) cm^{-1}; MS m/e 600. Anal. Calcd for $C_{37}H_{48}O_5N_2$: C, 73.97; H, 8.05; N, 4.66. Found: C, 73.94; H, 8.17; N, 4.59.

6(*S*),19-(*N*,*N*′-Phthalhydrazido)-3β-acetoxy-25-hydroxy-9,10-secocholesta-5(10),7(*E*),22(*Z*)-triene (36). To the phosphonium salt **34** (1.9 g) in THF (30 mL) was added phenyllithium (1.5 M solution, 1.7 mL, 1 equiv). After a few minutes, the aldehyde **24** (1 g) in benzene (35 mL) was added dropwise over about 1 min. After a further 3 min, water was added and the mixture diluted with CH_2Cl_2 and given an acid workup. The reaction was repeated as above, and the combined products were chromatographed to yield 2.12 g (78%) of a crude, yellow product.

The above mixture (1.4 g) was treated with AcOH–H_2O–THF (8:1:1) (10 mL) for 1.5 h. Dilution with CH_2Cl_2 followed by aqueous workup, chromatography, and crystallization gave 1 g of product (85%). Further crystallization from CH_2Cl_2–ether afforded the *title compound*: mp 182–184 °C; $[\alpha]_D$ +339° (*c* 0.84); ^{1}H NMR δ 8.3 (m, W = 12 Hz, 2 H, aryl), 7.8 (m, W = 10 Hz, 2 H, aryl), 5.9 (d, J = 10 Hz, C-7H), 5.27 (m, W = 12 Hz, C-3H, -22H, -23H), 4.78 and 4.21 (AB q, J = 18 Hz, C-19H$_2$), 4.75 (d, J = 10 Hz, C-6H), 2.03 (s, OAc), 1.17 (s, C-26H$_3$, -27H$_3$), 0.9 (d, J = 7 Hz, C-21H$_3$), 0.17 (s, C-18H$_3$); IR ν_{max} 3650 (m), 2950 (s), 2900 (sh), 1750 (s), 1650 (s), 1610 (m), 1370 (s), 1350 (s), 1240 (s) cm^{-1}; MS m/e 600. Anal. Calcd for $C_{37}H_{48}O_5N_2$: C, 73.97; H, 8.05; N, 4.66. Found: C, 74.10; H, 8.15; N, 4.47.

6(*S*),19-(*N*,*N*′-Phthalhydrazido)-3,25-dihydroxy-9,10-secocholesta-5(10),7(*E*)-diene (37). The unsaturated side-chain compound **36** (450 mg) in benzene (5 mL) and ethanol (5 mL) containing $NaHCO_3$ (100 mg) and 5% Pt/C (150 mg) was stirred under a hydrogen atmosphere for 24 h. The mixture was filtered through Celite and the solvent removed. To the residue, in benzene (10 mL), was added NaOH in methanol (1.25 M solution, 2 mL) and the mixture stirred for 20 min at room temperature. Acid workup and crystallization from CH_2Cl_2–ether afforded 380 mg (91%) of the *title compound* **37**: mp 174–177 °C; $[\alpha]_D$ +408° (*c* 0.825); ^{1}H NMR δ 8.3 (m, W = 12 Hz, 2 H, aryl), 7.8 (m, W = 10 Hz, 2 H, aryl), 5.9 (d, J = 10 Hz, C-7H), 4.78 and 4.22 (AB q, J = 18 Hz, C-19H$_2$), 4.75 (d, J = 10 Hz, C-6H), 4.11 (m, C-3H), 1.22 (s, C-26H$_3$, -27H$_3$), 0.87 (br s, C-21H$_3$), 0.18 (s, C-18H$_3$); IR ν_{max} 3550 (s), 2900 (sh), 1650 (s), 1610 (m), 1370 (s), 1350 (s) cm^{-1}. Anal. Calcd for $C_{35}H_{48}O_4N_2$: C, 74.96; H, 8.63; N, 5.00. Found: C, 74.65; H, 8.66; N, 5.06.

General Procedure for the Conversion of Phthalazine-1,4-dione Adducts to the Corresponding 5(*E*),7(*E*),10(9)-Triene System. The adduct (200–600 mg) was refluxed overnight, under argon in ethanol (10 mL) and hydrazine (3 mL). After the mixture was cooled to room temperature, the solvents were removed under reduced pressure and the resulting solid taken up in water (30 mL) and CH_2Cl_2 (30 mL). To this two-phase system under argon was added dianisyl telluroxide (150–450 mg), K_2CO_3 (6 g), and 1,2-dibromotetrachloroethane (3 g) and the mixture stirred for approximately 5 h (TLC control). After acid workup the mixture was chromatographed through silica gel and the product removed from traces of tellurium oxidant by PLC to give the desired vitamin D compound in 85–93% yield.

3β,25-Dihydroxy-9,10-secocholesta-5(*E*),7(*E*),10(19)-triene (38). Prepared from the adduct **37** (200 mg) as described in the general procedure, this gave the *title compound* **38**: 131 mg (92%); mp 79–81 °C (ether–hexane); $[\alpha]_D$ +160° (*c* 0.735); UV λ_{max} 273 nm (21 500); ^{1}H NMR δ 6.5 and 5.83 (AB q, J = 11 Hz, C-6H, -7H), 4.97 (s, C-19H), 4.67 (s, C-19H), 3.85 (m, W = 14 Hz, C-3H), 1.22 (s, C-26H$_3$, -27H$_3$), 0.95 (br s, C-21H$_3$), 0.55 (s, C-18H$_3$); IR ν_{max} 3400 (m), 2950 (s), 1620 (w); MS m/e 400. Anal. Calcd for $C_{27}H_{44}O_2\cdot H_2O$: C, 77.46; H, 11.07. Found: C, 77.50; H, 10.99.

3β-(3,5-Dinitrobenzoic acid ester) 39. The crude **38** (125 mg) in pyridine (5 mL) was treated with 3,5-dinitrobenzoyl chloride (85 mg, 1.1 equiv). Water was added and the mixture diluted with ether. After acid workup, the ester **39** was isolated by PLC: 129 mg (70%); mp 105–107 °C (ether–hexane); $[\alpha]_D$ +168° (*c* 0.97); ^{1}H NMR δ 9.13 (m, 3 H, aryl), 6.62 and 5.82 (AB q, J = 11 Hz, C-6H, -7H), 5.3 (m, W = 14 Hz, C-3H), 5.07 (s, C-19H), 4.77 (s, C-19H), 1.23 (s, C-26H$_3$, -27H$_3$), 0.93 (br s, C-21H$_3$), 0.43 (s, C-18H$_3$); IR ν_{max} 3550 (m), 2950 (s), 2900 (sh), 1750 (s), 1640 (w), 1550 (s), 1350 (s), 1275 (s) cm^{-1}. Anal. Calcd for $C_{34}H_{46}N_2O_7$: C, 68.66; H, 7.80; N, 4.71. Found: C, 68.62; H, 7.85; N, 4.65.

3β,25-Dihydroxy-9,10-secocholesta-5(*Z*),7(*E*),10(19)-triene (1). A solution of the 5,6-trans compound **38** (126 mg) in benzene (30 mL) containing triethylamine (2 drops) and anthracene (25 mg) was thoroughly degassed. A Hanovia lamp (No. 654A36) was placed such that the outside of the water-cooled jacket was 15 cm from the reaction vessel. The mixture was irradiated for 25 min and the 5,6-cis compound **1** isolated by PLC: 93 mg (74%); mp 98–100 °C (acetone–water); $[\alpha]_D$ +77° (*c* 0.26); UV λ_{max} 262 nm (19 060); ^{1}H NMR δ 6.25 and 6.1 (AB q, J = 11 Hz, C-6H, -7H), 5.05 (s, C-19H), 4.83 (s, C-19H), 3.9 (m, W = 18 Hz, C-3H), 1.27 (s, C-26H$_3$, -27H$_3$), 0.95 (br s, C-21H$_3$), 0.55 (s, C-18H$_3$); IR ν_{max} 3500 (s), 2950 (s), 2900 (sh), 1640 (w), 1480 (m), 1380 (m), 1055 (s), cm^{-1}. Anal. Calcd for $C_{27}H_{44}O_2\cdot H_2O$: C, 77.46; H, 11.08. Found: C, 77.29; H, 11.08. The melting point of an authentic sample supplied by Roussel Uclaf, Inc. (Romainville, France), did not depress on mixing.

3β-(3,5-Dinitrobenzoic acid ester) 39: prepared as previously described (see above); mp 149–150 °C (ether–hexane); $[\alpha]_D$ +90° (*c* 0.6). Anal. Calcd for $C_{34}H_{46}N_2O_7$: C, 68.66; H, 7.80; N, 4.71. Found: C, 68.94; H, 7.80; N, 4.52.

SO_2 Adducts of 3β-Hydroxy-9,10-secoergosta-5(*Z*),7(*E*),10(19),22(*E*)-tetraene (40). Sulfur dioxide was slowly passed through a well-stirred mixture of benzene (100 mL) and water (50 mL) containing calciferol (5 g), for a total of 3.5 h. After this time, air was passed through the mixture for approximately 20 min, ether and brine were added, and the layers were separated. Aqueous workup gave the known sulfur dioxide adducts **40** that

were used without further purification.

3β-Hydroxy-9,10-secoergosta-5(*E*),7(*E*),10(19),22(*E*)-tetraene (15). The SO_2 adducts **40** in methanol (125 mL) were refluxed in the presence of $NaHCO_3$ (5 g) for 2.5 h. The mixture was concentrated and then partitioned between ether and water. The ethereal solution was dried and the solvent removed to give the known 5,6-*trans*-calciferol (**15**): 4.3 g (86%); ^1H NMR and UV spectra, identical with that of an authentic sample.

SO_2 Adducts of 3β-[(Triethylsilyl)oxy]-9,10-secoergosta-5(*E*),7(*E*),10(19),22(*E*)-tetraene (44). The crude mixture of sulfur dioxide adducts of calciferol (prepared from 5 g of calciferol as described previously), in CH_2Cl_2 (40 mL), containing imidazole (4 g) was stirred with triethylsilyl chloride (3.5 mL). After about 30 min, the reaction was worked up as described for **40** to give, after chromatography, 5.3 g (74% from calciferol) of **44** as an oil: ^1H NMR δ 5.22 (m, W = 9 Hz, C-22H, -23H), 4.64 (m, W = 10 Hz, C-6H, -7H), 4.02 (m).

SO_2 Adducts of 3β-[(Triethylsilyl)oxy]-20(*S*)-formyl-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (45). The vitamin D_2 adduct **44** (4.7 g) was treated with ozone as described in the general procedure to give, after chromatography, the title compound **45**: 82%; IR ν_{max} (film) 2950 (s), 2900 (sh), 1735 (s), 1660 (w), 1460 (m), 1380 (m), 1310 (s), 1150 (m) cm^{-1}; ^1H NMR δ 9.57 (m, C-22H), 4.67 (m, W = 12 Hz, C-6H), 3.97 (m, W = 16 Hz, C-3H), 3.65 (br s, C-19H_2), 1.15 (d, J = 6 Hz, C-21H_3).

SO_2 Adducts of 3-[(Triethylsilyl)oxy]-20(*S*)-(hydroxymethyl)-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (46). Aldehyde **45** (2.5 g) in benzene (60 mL) was added dropwise to sodium borohydride (0.8 g) in ethanol (20 mL) over a period of 15–20 min. The excess reducing agent was carefully quenched with dilute hydrochloric acid and the mixture diluted with dichloromethane. Aqueous workup afforded the title compound **46** in 90% yield: IR ν_{max} (thin film) 3550 (br), 2950 (s), 2900 (sh), 1660 (w), 1460 (m), 1380 (m), 1305 (s), 1240 (m), 1155 (m) cm^{-1}; ^1H NMR δ 4.63 (m, W = 12 Hz, C-6H, -7H), 3.93 (m, W = 16 Hz, C-3H), 3.77–3.17 (7, C-19H_2, -22H_2).

3β-[(Triethylsilyl)oxy]-20(*S*)-(hydroxymethyl)-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (47). Adducts **46** (3 g) were stirred in refluxing methanol (50 mL) containing $NaHCO_3$ (3 g) for 2.5 h. Workup gave the derivative **47**: 47%; UV λ_{max} 273 nm; δ 6.43 and 5.7 (AB q, J = 11 Hz, C-6H, -7H), 4.9 (s, C-19H), 4.6 (s, C-19H), 4.03–3.13 (m, C-4H, -22H_2).

3β-[(Triethylsilyl)oxy]-20(*S*)-[[(*p*-tolylsulfonyl)oxy]-methyl]-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (50). Method A. To the hydroxy compound **47** (400 mg) in pyridine (5 mL) was added *p*-toluenesulfonyl chloride (350 mg) and the mixture stirred overnight at room temperature. Water was added and the mixture diluted with ether. Acid workup gave, after purification by PLC, 310 mg (58%) of the required *p*-toluenesulfonate **50**: ^1H NMR δ 7.73 (d, J = 8 Hz, 2 H, aryl), 7.28 (d, J = 8 Hz, 2 H, aryl), 6.43 and 5.81 (AB q, J = 11 Hz, C-6H, -7H), 4.92 (s, C-19H), 4.63 (s, C-19H), 4.2–3.57 (m, C-3H, -22H_2), 2.48 (s, aryl CH_3); IR ν_{max} (film) 2960 (s), 2900 (sh), 1600 (w), 1460 (m), 1360 (s), 1190 (s), 1175 (s), 1090 (s) cm^{-1}.

Method B. The crude SO_2 adducts of 3β-[(triethylsilyl)-oxy]-20(*S*)-hydroxy-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (**46**) (3.2 g) was stirred overnight in pyridine (40 mL) at 5 °C with *p*-toluenesulfonyl chloride (4 g). The reaction was cooled to 0 °C, water was added, and after a few minutes, the mixture was diluted with Et_2O. After an acid workup, the crude oily product **51** was taken up in ethanol (100 mL) and refluxed in the presence of $NaHCO_3$ (4 g) for 1 h. The mixture was concentrated and partitioned between CH_2Cl_2–water–brine. The organic solution was dried and chromatographed to give 2.64 g (70%) of the required derivative **50**; NMR and IR, identical with that of the product obtained by method A.

1α-Hydroxy-3β-[(triethylsilyl)oxy]-20(*S*)-[[(*p*-tolylsulfonyl)oxy]methyl]-9,10-secopregna-5(*E*),7(*E*),10(19)-triene (55). Selenium dioxide (56 mg) was stirred in acetonitrile (3.5 mL) for 45 min. *N*-Methylmorpholine *N*-oxide (NMO) (280 mg) was stirred in CH_2Cl_2 (3.5 mL) in the presence of anhydrous $MgSO_4$ for 30 min. The NMO solution was filtered into a solution of the compound **50** (308 mg) in 1,2-dichloroethane (3.5 mL) and the mixture warmed to reflux. To this was added the SeO_2–C_6H_5CN mixture, and refluxing was continued for a further 5.5 min. The reaction mixture was cooled in an ice bath, diluted with CH_2Cl_2, and worked up to give 180 mg (57%) of the 1α-hydroxy compound **55**: ^1H NMR δ 7.73 (d, J = 8 Hz, 2 H, aryl), 7.28 (d, J = 8 Hz, 2 H, aryl), 6.43 and 5.81 (AB q, J = 11 Hz, C-6H, -7H), 5.03 (s, C-19H), 4.93 (s, C-19H), 4.63–3.6 (m, C-1H, 3 H, 22H_2), 2.48 (s, aryl CH_3).

1α-Hydroxy-3β-[(triethylsilyl)oxy]-20(*S*)-[[(*p*-tolylsulfonyl)oxy]methyl]-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (56). The corresponding 5(*E*) compound **55** (225 mg) in benzene (35 mL) containing triethylamine (3 drops) was irradiated as described for compound **38** with anthracene (45 mg) as triplet sensitizer for 30 min to give, after PLC, 185 mg (82%) of the *title compound* **56**: UV λ_{max} 263 nm, 216; ^1H NMR δ 7.73 (d, J = 8 Hz, 2 H, aryl), 7.3 (d, J = 8 Hz, 2 H, aryl), 6.28 and 5.98 (AB q, J = 11 Hz, C-6H, -7H), 5.28 (s, C-19H), 4.92 (s, C-19H), 4.55–3.58 (m, C-1H, -3H, -22H_2), 2.45 (s, aryl CH_3).

1α,3β-Dihydroxy-20(*S*)-[[(*p*-tolylsulfonyl)oxy]methyl]-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene. The silyl ether **56** (185 mg) in THF (5 mL) containing n-Bu_4NF (1 M solution in THF, 0.32 mL) was stirred for 15 min at room temperature. Dilution with CH_2Cl_2, aqueous workup, and purification by PLC gave the diol: 110 mg (73%); UV λ_{max} 263 nm (17 427), 216 (18 672); ^1H NMR δ 7.68 (d, J = 8 Hz, 2 H, aryl), 7.23 (d, J = 8 Hz, 2 H, aryl), 6.28 and 5.97 (AB q, J = 11 Hz, C-6H, -7H), 5.27 (s, C-19H), 4.93 (s, C-19H), 4.57–3.6 (m, C-1H, -3H, -22H_2), 2.45 (s, aryl CH_3), 1.05 (d, J = 6 Hz, C-21H_3), 0.52 (s, C-18H_3).

3β,25-Dihydroxy-9,10-secocholesta-5(*E*),7(*E*),10(19)-triene (38). Magnesium turnings were washed with diluted HCl–water–acetone–ether and dried in vacuo for 24 h. The bromide **52** (1 g) in freshly distilled (from $LiAlH_4$) THF (10 mL) containing magnesium metal (82 mg) was refluxed for 2 h.

Cuprous iodide (100 mg) was placed in a flask and purged with argon, while cooling to 0 °C. To this was added the above Grignard solution (5 mL), and the purple mixture stirred for an additional 30 min at 0 °C. A solution of the *p*-toluenesulfonate **50** (200 mg) in ether (2 mL) was added and the mixture stirred for 40 min at room temperature. Water was added and the reaction mixture extracted with ether. After an acid workup, the nonpolar product was isolated by PLC contaminated with large quantities of low molecular weight alkyl residues. This mixture was stirred with n-Bu_4NF (1 M solution in THF, 2 mL) in refluxing THF (5 mL) for 2 h. Dilution with CH_2Cl_2 followed by aqueous workup and purification by PLC gave 110 mg (82% from the *p*-toluenesulfonate **50**) of this previously described diol **38**.

3β,25-Dihydroxy-9,10-secocholesta-5(*Z*),7(*E*),10(19)-triene (1). The above product **38** (100 mg) in benzene (30 mL) and triethylamine (3 drops) containing anthracene (25 mg) was thoroughly degassed and irradiated for 1 h as described above to give, after purification by PLC, the 5(*Z*) compound **1**, 90 mg (82%). The physical and spectral properties of this material were in all respects identical with that of the product obtained via the phthalazine adduct **19**. A mixed melting point determination of this material and an authentic sample, suppled by Roussel Uclaf, Inc. (Romainville, France), was undepressed.

1α,3β-Bis[(triethylsilyl)oxy]-20(*S*)-[[(*p*-tolylsulfonyl)-oxy]methyl]-9,10-secopregna-5(*Z*),7(*E*),10(19)-triene (57). *p*-Toluenesulfonate **56** (105 mg) in CH_2Cl_2 (5 mL) containing imidazole (75 mg) and triethylsilyl chloride (45 μL) was stirred at room temperature for about 15 min. Water was added and the mixture diluted with CH_2Cl_2. Acid workup gave the nonpolar bis(silyl ether) **57**, which was used without further purification.

1α,3β,25-Trihydroxy-9,10-secocholesta-5(*Z*),7(*E*),10(19)-triene (3). To the alkylcopper reagent at 0 °C prepared exactly as described above was added a solution of the above *p*-toluenesulfonate **57** in THF (3 mL) and the mixture stirred at room temperature for 25 min. Workup and purification as above for **38** gave the tris(triethylsilyl) derivative contaminated with large quantities of low molecular weight alkyl residues. This mixture was treated with n-Bu_4NF (1 M solution in THF, 4 mL) in THF (5 mL) for 20 min at room temperature followed by 1.5 h at reflux to give, after the usual workup and purification by PLC, a mixture of the steroidal triol **3** [38 mg (63%) from **56**] contaminated with isopentanediol (**58**), 10 mg. Dissolution of this mixture in $CHCl_3$ gave the required product **1** as its crystalline $CHCl_3$ solvate: 25 mg; mp 99–105 °C; $[\alpha]_D$ (Et_2O) +35° (c 0.86); UV λ_{max} 264 nm (16 820); ^1H NMR δ (acetone-d_6) 8.07 (s, $CHCl_3$), 6.35 and 6.18 (AB q, J = 12 Hz, C-6H, -7H), 5.38 (s, C-19H), 4.93

(s, C-19H), 4.7-4.07 (m, C-1H, -3H), 1.2 (s, C-26H_3, -27H_3), 1.0 (br s, C-21H_3), 0.6 (s, C-18H_3); IR ν_{max} 3500 (s), 2950 (s), 2900 (sh), 1640 (w), 1480 (m), 1440 (m), 1380 (m), 1360 (m), 1140 (m), 1050 (s) cm^{-1}.

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A Short, Stereoselective Synthesis of the Lactone Precursor to 2*R*,4*S*,5*S* Hydroxyethylene Dipeptide Isosteres†

Andrew H. Fray, Robert L. Kaye, and Edward F. Kleinman*

Department of Medicinal Chemistry, Central Research, Pfizer Inc., Groton, Connecticut 06340

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Lactone **1**, a precursor to the 2*R*,4*S*,5*S* hydroxyethylene dipeptide isostere unit, was synthesized steroselectively in four steps from *N*-Boc-L-leucinal in 13% overall yield. Peptides containing hydroxyethylene dipeptide isosteres with this chirality are potent inhibitors of aspartyl proteases. Addition of the lithium salt of ethyl propiolate to *N*-Boc-L-leucinal (**3**) afforded hydroxy acetylenic esters **4** as a mixture of diastereomers. Reduction of the acetylenic function of **4** and subsequent lactonization gave a readily separable 4.5:1 mixture of the desired 4*S* lactone **2** and the 4*R* lactone **5**. Direct alkylation of **2** with methallyl bromide and lithium hexamethyldisilazide as base yielded the trans lactone **10**, which was catalytically reduced to **1**. The structure of lactone **10** was confirmed by X-ray analysis.

During recent years, there has been a growing interest in the use of enzyme inhibitors as therapeutic agents.[1] One class of proteolytic enzymes that has received particular attention in this regard is the aspartyl proteases (or acid proteases),[2] which includes pepsin and the blood pressure regulating enzyme renin.[3] These enzymes cleave between two internal hydrophobic amino acid residues and have, as the catalytic apparatus, two aspartyl groups in the active site. Recently, a potent series of synthetic peptide inhibitors of aspartyl proteases has been reported that contain a dipeptide mimic known as the "hydroxyethylene dipeptide isostere".[4,5] We describe here an improved, stereoselective synthesis of this isostere unit in its lactone form.

As shown in Figure 1, the hydroxyethylene dipeptide isostere is a 5-aminopentanoic acid derivative which is derived by replacing the amide linkage of the dipeptide residue surrounding the cleavage site of the substrate by the hydroxyethylene moiety of statine, the unusual amino acid found in the naturally occurring pentapeptide inhibitor pepstatin[6] (Iva-Val-Val-Sta-Ala-Sta). The configurations of the three chiral centers of the isostere correspond with the L-amino acids of the peptide substrate and the 3*S* configuration of statine and are thus assigned 2*R*,4*S*,5*S*. The 4*S* configuration is believed to be especially crucial to inhibition since pepstatin analogues with (3*R*)-statine are over 100-fold less potent inhibitors than those with (3*S*)-statine.[7] The possibility that pepstatin may be a transition-state analogue,[8] which is based on the close resemblance between the hydroxyethylene group and the putative tetrahedral intermediate of proteolytic hydrolysis, is discussed by Rich in an excellent review of pepstatin binding.[9]

Syntheses of the hydroxyethylene dipeptide isostere unit were first reported by Szelke[4] and Rich[5] in 1983 and, more recently, by Evans.[10] Of the three routes, which are summarized in Scheme I, the Evans synthesis requires the fewest number of steps, but in terms of stereocontrol, only Rich's synthesis is stereoselective at C-2 and C-4. In Rich's synthesis, the C-2 chiral center is introduced as part of an optically active C-1–C-3 fragment which is added as a Grignard reagent to *N*-Boc-L-leucinal to provide a 4:1 epimeric mixture of the C-4 alcohols enriched in the desired 4*S* diastereomer. Separation of the two diastereomers is postponed until the isostere is incorporated into the peptide. A drawback of this synthesis, however, is the use of excess (2.5 equiv) optically active Grignard reagent, which is prepared in four steps from the chiaral oxazolidinone derived from (1*S*,2*R*)-norephedrine.[11]

† This manuscript is dedicated to Professor Clayton H. Heathcock on the occasion of his 50th birthday.

(1) *Enzyme Inhibitors as Drugs*; Sandler, M., Ed.; University Park: Baltimore, 1980.
(2) *Acid Proteases: Structure, Function, and Biology*; Tang, J., Ed.; Plenum: New York, 1977.
(3) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313.
(4) (a) Szelke, M.; Jones, D. M.; Hallett, A. European Patent Application EP 45 665, 1982; *Chem. Abstr.* **1982**, *97*, 39405p. (b) Szelke, M.; Jones, D. M.; Atrash, B.; Hallet, A.; Leckie, B. J. *Proc. Am. Pept. Symp. 8th* **1983**, 579. (c) Leckie, B. J.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Szelke, M.; Tree, M. *Scott. Med. J.* **1984**, *29*, 125.
(5) (a) Rich, D. H.; Salituro, F. G.; Holliday, M. W. *Proc. Am. Pept. Symp., 8th* **1983**, 511. (b) Holliday, M. W.; Rich, D. H. *Tetrahedron Lett.* **1983**, *24*, 4401.
(6) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsuzaki, M.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1970**, *23*, 259.
(7) Rich, D. H.; Sun, E. T. O.; Ulm, E. *J. Med. Chem.* **1980**, *23*, 27.
(8) Wolfenden, R. *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 271.
(9) Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263.
(10) Evans, B. E.; Rittle, K. E.; Homnick, C. F.; Springer, J. P.; Hirshfield, J.; Veber, D. F. *J. Org. Chem.* **1985**, *50*, 4615.

0022-3263/86/1951-4828$01.50/0