Biosynthetic Studies of Marine Lipids. 39.[1] 19-Norsterols: The Course of C-19 Methyl Elimination

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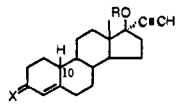
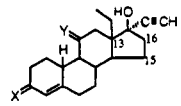
Abstract: The biosynthesis of 19-norstanols in the Mediterranean sponge, *Axinella polypoides*, was investigated through the use of radiotracer experiments. It was found that the conversion of cholesterol (**7**) to 19-nor-5α-cholestan-3β-ol (**8**) involved oxidation at C-3 with the distribution of the abstracted hydride from the 3α-position of dietary cholesterol into all of the 19-norstanols of the native mixture. Furthermore, while the efficiency of conversion of Δ^5-19-oxygenated sterol precursors 19-hydroxycholesterol (**9**) and 3β-hydroxycholest-5-en-19-oic acid (**10**) to **8** was low, the efficiency of the conversion of 19-hydroxycholest-4-en-3-one (**23**) to **8** was high, suggesting that the principal pathway for 19-norstanol biosynthesis involves oxidative isomerization of a dietary Δ^5-3β-hydroxy sterol to the Δ^4-3-ketone before oxidation at C-19. It was also shown that the conversion of cholesterol to 19-nor-5α-cholestan-3β-ol involves the stereospecific loss of the 4β-hydrogen atom. It was further determined that the biological demethylation pathway is suppressed for dietary sterols bearing an unconventional configuration at C-20 in the hydrocarbon side chain.

All steroid oral contraceptives, currently used by at least 50 million women worldwide, are based on two progestins—norethindrone (**1**)[3] and norgestrel (**2**)[4]—or slight structural variants thereof (e.g., 3-deoxo (**3**),[5] 17-acetate (**4**),[6] Δ^{15}-dehydro (**5**),[7] 11-methylene (**6**)[8]). Their common and most distinctive structural feature is the absence of the 19-methyl group attached to C-10 in conventional steroids.



1 X=O; R=H
3 X=H_2; R=H
4 X=O; R=OAc



2 X=O; Y=H_2
5 X=O; Y=H_2; Δ^{15}
6 X=H_2; Y=CH_2

Initially such 19-norsteroids were produced[3] by Birch reduction of the ring A aromatic estrogenic steroids. A second partial synthesis[9] was based on functionalization of the 19-methyl group via a suitable 6β-hydroxy intermediate, followed by elimination of the oxygenated C-19 substituents via vinylogous activation of the Δ^4-3-keto intermediate. These two partial syntheses[3,9] were the basis of the initial industrial processes whereby these 19-norsteroids were introduced into medical practice.

Partial synthesis meant that the ultimate starting material was either the sapogenin diosgenin or a plant sterol (e.g., sitosterol). For reasons described elsewhere,[10] sudden price escalation of diosgenin, based partly on political factors operating in Mexico (a type of steroid-OPEC strategy that failed), made total synthesis an attractive alternative; at present, the bulk of norethindrone (**1**) and its congeners is produced commercially by total synthesis.[10a] Norgestrel (**2**) and its relatives were always prepared by total synthesis[4,10] because there are no naturally occurring steroid precursors possessing an angular C-13 ethyl substituent—the distinctive feature of norgestrel (**2**).

At the time that 19-norsteroids were first synthesized,[3,11] no naturally occurring, nonaromatic 19-norsteroids were known. Since then, 19-norandrostenes have been shown[12] to be converted in vivo to the natural estrogens, and other 19-norsteroids, such as 19-nordeoxycorticosterone[13] (first synthesized[14] in 1953), have also been isolated. However, they are present only in trace quantities. Naturally occurring 19-norsteroidal precursors, therefore, were never potential starting materials candidates for alternate partial syntheses of oral contraceptives.

In 1974 the theoretical picture for partial syntheses of 19-norsteroids changed dramatically. A natural source—in this instance a marine sponge (*Axinella polypoides*)—turned out[15] to be a rich source of 19-norstanols, notably 19-nor-5α-cholestanol. A subsequent, more detailed examination[16] of this Mediterranean sponge showed that a wide mixture of 19-norstanols with different hydrocarbon side chains occurred in this sponge. In fact, other marine organisms have been shown[17] to contain the even more attractive Δ^5-3β-hydroxy or Δ^4-3-keto 19-norsterols, albeit in trace quantities. It is conceivable that the search for such Δ^5- or Δ^4-3-oxygenated 19-norsterols may uncover richer sources but, at present, we felt that the most appropriate focus was on the sponge, *A. polypoides*. Our detailed analysis[16] showed that 42% of the 19-norstanols were Δ^{22} unsaturated, which represent the ideal intermediates for partial synthesis of 19-norsteroid hormones. As pointed out elsewhere,[18] in principle it would only be necessary to ozonize the total sterol mixture of *A. polypoides* and separate the resulting 19-nor-22-aldehyde from unreacted 19-norstanols. Standard degradation of the 22-aldehyde would then lead to

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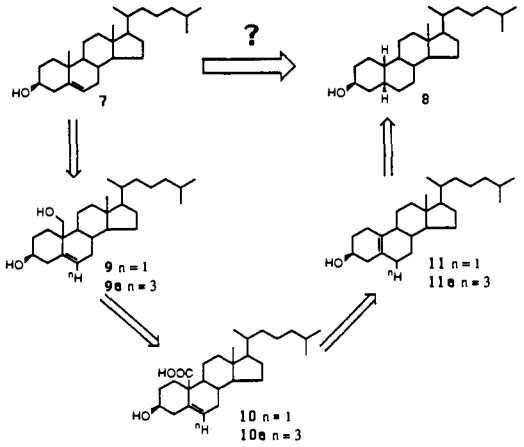
Scheme I. Possible Route for the Biosynthesis of 19-Nor-5α-cholestan-3β-ol (**8**) Not Involving Oxidation at C-3



19-norpregnanes or -androstanes of biological and economic significance.

It should immediately be recognized that these are only hypothetical routes to oral contraceptives and that major hurdles would have to be overcome to convert them into reality. First and foremost, even if a sponge, or other marine invertebrate, could be found that is rich in 19-norsterols with unsaturated side chains, it would be ecologically unacceptable to harvest them, considering their slow growth and capacity for regeneration. What would be needed is to establish the biosynthetic mechanism and to isolate the enzyme system(s) responsible for the sponge's efficient conversion of Δ^5-3β-hydroxy sterols into their 19-nor equivalents. If this can be done and if an immobilized enzyme system could be constructed that works efficiently on a substrate such as stigmasterol, then a potentially economical route to oral contraceptives of the norethindrone (**1**) class could be developed.

The Italian group[15] not only was the first to have observed the natural occurrence of 19-norstanols in sponges but they also carried out the first biosynthetic experiments[19] by feeding radiolabeled precursors. Their results can be summarized as follows: (1) ^{14}C-labeled acetate was not found to be converted to 19-norstanols;[19a] (2) radiolabeled cholesterol (**7**) was efficiently converted to 19-norstanols;[19a] (3) partial loss of the C-3 hydrogen atom was observed in the conversion of cholesterol (**7**) to 19-nor-5α-cholestan-3β-ol (**8**);[19b] (4) the hydrogen atoms in the 4β-position as well as those at C-7 were found to be retained during this conversion;[19b] and (5) a competitive uptake experiment showed that the nuclear unsaturation of Δ^5-3β-hydroxy sterols was necessary for C-19 demethylation to occur.[19b]

These results seemed to suggest the nonintermediacy of 3-keto steroids in the biosynthesis of 19-norstanols. While the lack of incorporation of dietary acetate into sterol would suggest the sponge's inability to produce sterols de novo, subsequent work[20] with labeled squalene has demonstrated that this is not the case and that *A. polypoides* is capable of both de novo sterol biosynthesis as well as the utilization of dietary sterols.

Nuclear Transformations Involved in the Conversion of Cholesterol (7) to 19-Nor-5α-cholestan-3β-ol (8): First-Generation Radiotracer Studies. Because the original studies[20] on the biosynthesis of 19-norstanols by *A. polypoides* seemed to contraindicate oxidation at C-3 during the conversion of **7** to **8**, we assumed the operation of a "Δ^5-pathway" for this conversion (Scheme I), drawing upon analogies with the well-studied steroid demethylations in terrestrial sterol and steroid hormone biosynthesis.[21]

Knowing that these demethylations always involve loss of carbon as either formic acid or CO_2, we anticipated that consecutive hydroxylation of C-19 by a cytochrome P-450 dependent mixed-function oxidase would result in 19-hydroxy and 19-oic sterols **9** and **10**, respectively, through the intermediacy of a 19-oxo species.[22] Loss of C-19 as CO_2 would be facilitated by the nuclear double bond which would assist in C-10–C-19 bond cleavage by migration to the $\Delta^{5(10)}$-position in what can be considered to be formally a [1,5] hydrogen shift. The expectation that C-19 is lost as CO_2 rather than formic acid comes from analogy to terrestrial sterol biosynthesis in which loss of carbon as formic acid (i.e., C-32 from lanosterol and C-19 from testosterone) results in the introduction of an additional nuclear double bond into the substrate,[23] whereas this additional degree of oxidation manifests itself in the released CO_2 during loss of the *gem*-dimethyls C-30 and C-31 in the biosynthesis of cholesterol from lanosterol.[24] Reduction of the $\Delta^{5(10)}$ double bond of the resulting 19-norcholest-5(10)-en-3β-ol (**11**) would then produce the 19-norstanol **8**. Typically, reduction of such a nuclear double bond would entail oxidation at C-3[25] followed by isomerization to the Δ^4-3-ketone[26] and conjugate reduction by a steroid 5α-reductase.[27] The requirement for no oxidation to occur at C-3 during this conversion,[19b] however, would necessitate the existence of an as yet unknown mechanism for Δ^5 ($\Delta^{5(10)}$) reduction.

[6-^{3}H]Cholest-5-ene-3β,19-diol (**9a**), [6-^{3}H]-3β-hydroxycholest-5-en-19-oic acid (**10a**), and [6-^{3}H]-19-norcholest-5-(10)-en-3β-ol (**11a**) were synthesized using the procedures of Tanabe et al.[28] The three precursors (20 μCi each) were incorporated with freshly harvested specimens of *A. polypoides* as previously described.[19a] The results of these incorporation experiments are shown in Table I.

Table I. Results of First-Generation Precursor Feeding Experiments

precursor	administered	recovered[a]	recovered precursor[b]	8[b]	specific activity[c]
9a	20 μCi	4.2×10^6 (9.5%)	5.1×10^5 (12%)	1.9×10^6 (45%)	1.5×10^7
10a	20 μCi	1.3×10^6 (3%)	8.8×10^4 (7%)	2.7×10^5 (21%)	1.9×10^6
11a	20 μCi	8.9×10^6 (20%)	2.3×10^6 (26%)	2.2×10^6 (25%)	6.0×10^7

[a] In dpm; as a percentage of administered activity. [b] In dpm; as a percentage of recovered activity. [c] In dpm/mmol.

In all three experiments, the total recovered radioactivity was in the range of 3–20% of the administered activity. In addition, all three experiments reveal that the respective precursors were converted to 19-nor-5α-cholestan-3β-ol (**8**) in radiochemical yields of 45%, 21%, and 25% for precursors **9a**, **10a**, and **11a**, respectively. Untransformed precursor was also recovered in each case. The specific activities of the biosynthesized 19-nor-5α-cholestan-3β-ol (**8**) in each case were found to be 1 order of magnitude lower (and in the case of **10a**, 2 orders of magnitude lower) than those that have been observed for the conversion of cholesterol[19a] as well as 3-keto steroid precursors (vide infra). In addition, in the case of precursor **11a** (Table I), spurious radioactivity was found in many of the chromatographic fractions during purification

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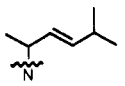
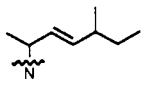
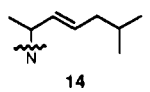
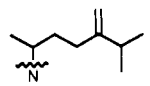
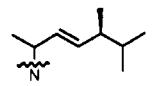
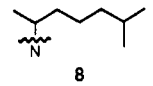
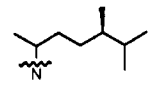
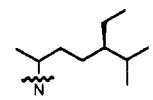
Table II. Results of Incorporation of [3α-^{3}H,4-^{14}C]Cholesterol

sterol (N = 19-nor-5α-stan-3β-ol)	total activity[a] ^{3}H	total activity[a] ^{14}C	specific activity[b] alcohol[c]	specific activity[b] ketone[d]	^{3}H:^{14}C ratio alcohol	^{3}H:^{14}C ratio ketone[d]
 12	0.62	0	6.0	background		
 13	1.1	0	12	background		
 14	1.9	0	7.2	background		
 15	0.92	0	13	background		
 16	3.3	0	7.9	background		
 8	3.0	89	1.7	53[e]	0.02	<0.01
 17	9.6	1.1	7.5	0.17[e]	17	<0.01
 17	4.3	0	8.4	background		

[a] In dpm × 10^5. [b] In dpm/mmol × 10^6 (as the acetate). [c] ^{3}H activity. [d] Resulting from PCC oxidation of the alcohol. [e] ^{14}C activity.

of the native 19-norstanols, leading to the suspicion that other metabolic processes were operating on the precursor ([6-^{3}H]-19-norcholest-5(10)-en-3β-ol (**11a**)), which appeared to be distinct from those of 19-demethylation.

Reinvestigation of the Lability of the Hydrogen Atom at C-3 in the Conversion of Cholesterol (7) to 19-Nor-5α-cholestan-3β-ol (8). Preliminary studies[19b] on the biosynthesis of 19-norstanols in *A. polypoides* showed the partial retention of tritium when doubly labeled [3α-^{3}H,4-^{14}C]cholesterol was fed to the sponge. The Italian investigators concluded that either a "compartmentalized pool" of NADPH was responsible for this partial retention or that there might be two distinct metabolic pathways leading to C-19 demethylation: one involving oxidation at C-3, the other proceeding without oxidation at C-3. Because of the distinctly low specific activities reported for our first-generation precursors (Table I), we concluded that the transformation of **7** to **8** must involve a pathway which is more efficient than that postulated in Scheme I.

Doubly labeled [3α-^{3}H,4-^{14}C]cholesterol (^{3}H:^{14}C activity ratio = 0.46) was fed to *A. polypoides* in a repetition of the original experiment.[19b] The dried sponge (23 g) was extracted in the usual way and purified by chromatography to provide 235 mg of native 19-norstanols displaying a ^{3}H:^{14}C activity ratio of 0.44—nearly identical to that of the administered precursor. This result would appear to indicate that there was complete retention of the tritium label during C-19 demethylation. Unlike the investigators who originally performed this experiment, we had at our disposal the aid of reverse-phase HPLC for a more detailed analysis of the native 19-norstanol mixture. The results of this analysis are shown in Table II.

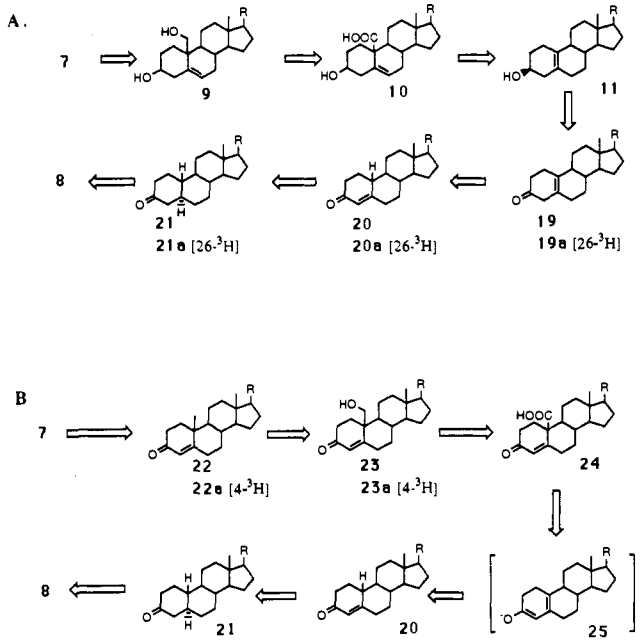
Recovered 19-nor-5α-cholestan-3β-ol (**8**) possessed a ^{3}H:^{14}C activity ratio of 0.02, representing a 96% loss of tritium in the conversion of [3-^{3}H,4-^{14}C]cholesterol to **8**. Interestingly, however, we also observed that rather than being completely expelled from the organism, this "lost" tritium was found to have been incorporated into each of the native 19-norstanols of *A. polypoides* (Table II). Oxidation of each of the free 19-norstanols with PCC resulted in the complete loss of radioactivity and demonstrated that the incorporated tritium was located at C-3 in the native 19-norstanols. The specific activities of all of the tritiated 19-norstanols were found to be nearly identical, suggesting that they all were tritiated by a common mechanism.

Clearly the native 19-norstanols bearing side chain alkylation at C-24 are not biosynthetically derived from the precursor (i.e., cholesterol). The explanation for the distribution of the C-3 hydrogen (tritium) atom to all of the native 19-norstanols most likely comes from the first of the hypotheses put forth by Minale et al.:[19b] a "compartmentalized pool" of pyridine dinucleotide, as the cofactor of a 3-keto steroid oxido-reductase, is responsible for accepting the C-3 hydrogen of **7** in the form of hydride and reintroducing it at C-3 during reduction of a 3-keto 19-norsteroid species. This theory, of course, implies that a single oxido-reductase operates on both dietary as well as 19-norsteroids.

In the case of the original experiments,[19b] the crude native 19-norstanol mixture showed a ^{3}H:^{14}C activity ratio which was 40% that of the precursor. In our case, these two ratios were nearly identical. This discrepancy is readily explained by assuming a sterol turnover rate on the order of 0.5 month.[29] If all of the administered ^{14}C is retained by biosynthesized **8** but the administered ^{3}H is distributed evenly among all of the approximately 15 19-norstanols of the native mixture,[16] then we would expect to observe a steady decrease in the total ^{3}H:^{14}C activity ratio as

(29) For a discussion of turnover rates and half-lives of sterol in humans, see: Kritchevsky, D. *Cholesterol*; John Wiley and Sons: New York, 1958; pp 88–92.

Scheme II. Pathways for C-19 Demethylation That Involve Oxidation at C-3 (R = C_8H_{17})



a function of incubation time as well as the seasonal metabolism of the individual animal due to a constant turnover rate for all of the native 19-norstanols.

Table II also indicates the presence of ^{14}C in the HPLC fraction corresponding to 24(*R*)-methyl-19-nor-5α-cholestan-3β-ol (**17**). This sterol is not biosynthetically derived from the precursor, and the true source of this activity was demonstrated (vide infra) to be due to contamination by 5α-cholestan-3β-ol (dihydrocholesterol, DHC), the product of simple biological reduction of the nuclear double bond of the precursor Δ^5-3β-hydroxy sterol without further loss of the angular methyl group.

Second-Generation Radiotracer Studies: 3-Keto Steroids. With the knowledge that removal of C-19 from dietary cholesterol involves an oxidative step at C-3, two possible biosynthetic routes become evident based on analogy to similar transformations in terrestrial organisms.

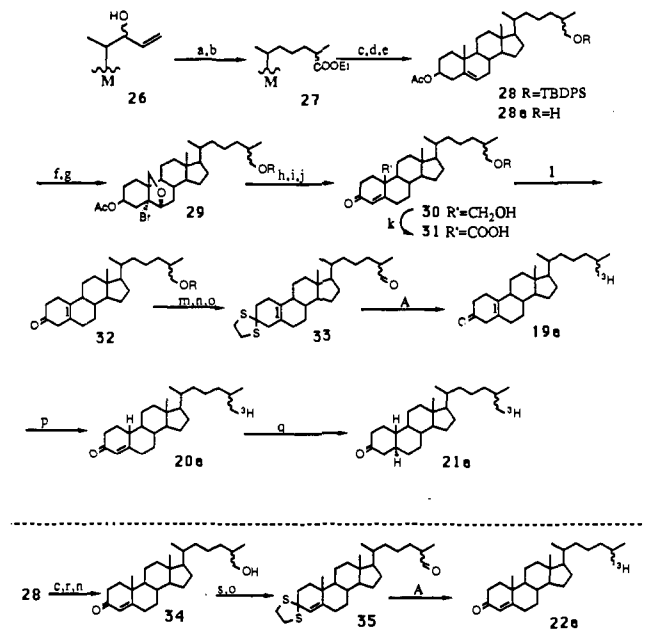
The first route (Scheme IIA) makes use of an oxidative step at C-3 to assist in the reduction of the nuclear double bond. It is known that reduction of the Δ^5 double bond of sterols always involves oxidation at C-3 followed by isomerization of the double bond to the Δ^4-position and conjugate reduction by a 5α-reductase.[25,27] The same may be true for reduction of the homoallylic double bond of 19-norcholest-5(10)-en-3β-ol (**11**, Scheme IIA). Oxidation to the corresponding 3-keto steroid (**19**) followed by isomerization[26] to 19-norcholest-4-en-3-one (**20**) and conjugate reduction would provide saturated 3-keto 19-norsteroid **21**, which may be readily reduced by the 3-keto steroid oxido-reductase to yield 19-norstanol **8**.

The second hypothetical pathway (Scheme IIB) makes use of oxidation at C-3 not only to aid in the reduction of the nuclear double bond but also to assist in decarboxylation as part of a steroidal vinylogous β-keto acid. Oxidation at C-3 followed by double bond isomerization results in cholest-4-en-3-one (**22**), a known intermediate in the biological conversion of cholesterol to 5α-cholestan-3β-ol.[30] Hydroxylation at C-19 initially results in 19-hydroxycholest-4-en-3-one (**23**) and eventually the keto acid **24**. Enzymatic or nonenzymatic decarboxylation of **24** may result in a dienolate species (**25**), which can collapse to 19-nor enone **20**. Conjugate reduction followed by reduction at C-3 as described above provides the native 19-norstanol **8**.

The putative intermediate **19** is unique to pathway A, while **22**, **23**, and **24** are unique to pathway B. Radiolabeled analogues

(30) Smith, A. G.; Brooks, C. J. W. *Biochem. J.* **1977**, *167*, 121–129. (b) Rosenfeld, R. S.; Zumoff, B.; Hellman, L. *J. Lipid Res.* **1967**, *8*, 16–23.

Scheme III. Synthesis of 3-Keto Steroid Precursors **19a**, **20a**, **21a**, and **22a**[a,b] (M = 3α,5-cyclo-6β-methoxy-5α-stanane, R = *tert*-Butyldiphenylsilyl (TBDPS))



[a] (a) $(EtO)_3CCH_2CH_3$. (b) H_2/Pd. (c) LAH. (d) TBDPSCl/imidazole. (e) AcOH/reflux. (f) NBS/$HClO_4$. (g) $Pb(OAc)_4/I_2/h\nu$. (h) KOH. (i) Jones' Ox., then NaOAc. (j) Zn. (k) Jones' Ox. (l) TEA/reflux. (m) $(TMSSCH_2)_2$/TMSOTf. (n) TBAF. (o) PCC. (p) TsOH. (q) Li/NH_3. (r) PCC, then HCl. (s) $(TMSSCH_2)_2/ZnI_2$. [b] A = (i) NaB^3H_4. (ii) TsCl/pyr. (iii) $NaBH_4$/DMSO. (iv) PhI-$(OOCCF_3)$.

19, **22**, and **23** were thus the targets for our next set of radiotracer studies. In addition, we prepared radiolabeled analogues of **20** and **21** for administration to *A. polypoides*. Due to facile decarboxylation, the vinylogous β-keto acid **24** was deemed to be an unacceptable substrate for an incorporation experiment.

Synthesis of Radiolabeled 3-Keto Steroid Precursors. Because the biosynthesis of 19-norstanols from dietary sterols by the sponge involves nuclear transformations, we decided to incorporate the radiolabel into the hydrocarbon side chain. The synthesis of 26-tritio-**19**, -**20**, -**21**, and -**22** is shown in Scheme III. The scheme is designed around the use of NaB^3H_4 for the introduction of tritium at a point as close to the end of the synthesis as possible.

Treatment of epimeric allylic alcohols **26**[31] with triethyl orthopropionate in refluxing xylenes led to a Claisen product, which was readily reduced by catalytic hydrogenation to afford C-25 epimeric ethyl esters **27**. Reduction to the corresponding alcohol with LAH and protection with *tert*-butylchlorodiphenylsilane (TBDMSCl) resulted in a silyl ether which underwent retro i-steroid rearrangement in refluxing glacial acetic acid to provide Δ^5-3β-acetoxy steroid **28**.

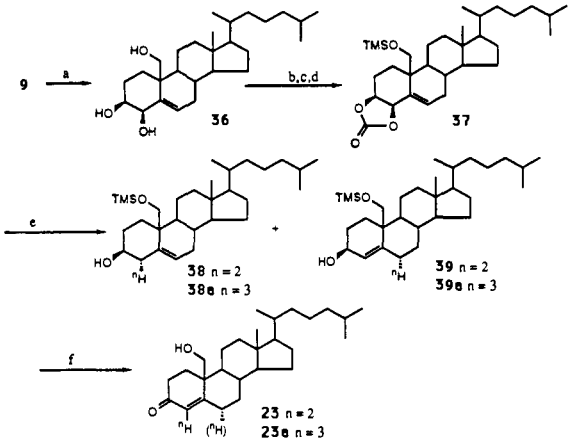
Functionalization of C-19 was accomplished in the standard way.[9,32] Thus, treatment of olefin **28** with *N*-bromosuccinimide (NBS) and perchloric acid resulted in a bromohydrin that, after careful chromatographic purification, was treated with $Pb(OAc)_4$ and I_2 and irradiated with visible light to provide 6β,19-epoxy steroid **29**. Conversion to the 19-hydroxy-Δ^4-3-ketone by saponification, oxidation, and dehydrobromination followed by reduction with zinc powder provided hydroxy enone **30**. Oxidation to keto acid **31** was followed by decarboxylation in refluxing triethylamine (TEA) to provide 3-keto-$\Delta^{5(10)}$ 19-norsteroid **32**.

Protection as the ethylene thioketal was accomplished with 1,2-bis[(trimethylsilyl)thio]ethane[33] and catalyzed by trimethylsilyl

(31) Walkup, R. D.; Anderson, G. D.; Djerassi, C. *Tetrahedron Lett.* **1979**, *9*, 767–770.

(32) For general reviews, see: Heusler, H.; Kalvoda, J. In *Organic Reactions in Steroid Chemistry*; Fried, J., Edwards, J. A., Eds.; Van Nostrand Reinhold: New York, 1972; Vol. 2, Chapter 12.

Scheme IV. Synthesis of [4-^{3}H]-19-Hydroxycholest-4-en-3-one[a]



[a](a) SeO_2. (b) $CO(Im)_2$. (c) KOH. (d) BSA. (e) NaB^3H_4/$(Ph_3P)_4Pd$/dppe. (f) PCC, then HCl.

trifluoromethanesulfonate (TMSOTf) as described by Noyori[34] without isomerization of the $\Delta^{5(10)}$ double bond. Silyl ether cleavage with tetra-*n*-butylammonium fluoride (TBAF) followed by oxidation with PCC resulted in 26-oxo thioketal **33**. Labeling was accomplished using a protocol ("A", Scheme III) consisting of reduction at C-26 with NaB^3H_4 followed by hydrogenolysis of the tosylate of the resulting primary alcohol with $NaBH_4$ in DMSO.[35] These steps were necessary to optimize the incorporation of ^{3}H at C-26. Simple reduction of the tosylate with NaB^3H_4 in DMSO resulted in >99% recovery of starting material, even after 36 h of reaction time. This was most likely due to the low purity of the reagent as well the impracticality of using it in excess. Oxidative deprotection of the ethylene thioketal using the method of Stork[36] resulted in [26-^{3}H]-19-norcholest-5(10)-en-3-one (**19a**) without any trace of double bond migration, as determined by 400-MHz ^{1}H NMR spectral examinations of the unlabeled analogue.

The $\Delta^{5(10)}$-ketone **19a** was readily transformed into its Δ^4 isomer (**20a**) by treatment with toluenesulfonic acid (TsOH) in hot methanol.[28,37] Reduction with lithium in ammonia provided the saturated 3-keto 19-norsteroid **21a**.

[26-^{3}H]-Cholest-4-en-3-one was prepared by subjecting 26-oxo thioketal **35** to labeling protocol A (Scheme III). The synthesis of **35** was readily accomplished from the Δ^5-3β-acetoxy steroid **28** by saponification, PCC oxidation, and acid-catalyzed double bond isomerization to give 26-[(*tert*-butyldimethylsilyl)oxy]-cholest-4-en-3-one, which was desilylated with TBAF to give the known hydroxy enone **34**.[38] Thioketal formation catalyzed by ZnI_2[33] and PCC oxidation provided **35**.

Upon prolonged exposure to air, 19-nor-$\Delta^{5(10)}$-3-ketone **19** was seen to undergo autoxidation to the 3-keto-Δ^4-nor-10-hydroperoxide.[39] Nevertheless, **19a** was placed under argon in a sealed ampoule and sent to Italy for incorporation. It was hoped that once taken up by the sponge, autoxidation would be negligible.

The synthesis of radiolabeled 19-hydroxycholest-4-en-3-one was different from those described above and involved labeling at C-4 using a procedure for the stereospecific introduction of tritide into the 4α-position of Δ^5-3β-hydroxy steroids.[40] The synthesis is shown in Scheme IV.

Table III. Results of Incorporation Experiments Involving 3-Keto Steroid Precursors **19a**, **20a**, **21a**, **22a**, and **23a**

	precursor				
radioactivity	**22a**	**20a**	**19a**	**21a**	**23a**
administered	30 μCi	32 μCi	42 μCi	18 μCi	37 μCi
recovered (%)[a]	2.9×10^7 (44%)	2.4×10^7 (34%)	3.7×10^6 (4%)	1.5×10^7 (38%)	4.2×10^7 (51%)
precursor (%)[b]	2.7×10^6 (9.3%)	4.6×10^5 (2%)	0	6.6×10^5 (5%)	1.9×10^6 (5%)
8[b]	1.9×10^7 (65%)	1.2×10^7 (50%)	4.6×10^5 (12%)	1.1×10^8 (73%)	2.2×10^7 (53%)
5α-cholestan-3β-ol[b]	5.7×10^5 (2%)				
11			3.6×10^4 (1%)		
21		8.5×10^4 (0.3%)	0		
specific activity of **8**[c]	5.9×10^8	3.2×10^8	1.2×10^7	6.0×10^8	8.6×10^8

[a] Total recovered activity in dpm (as a percent of administered activity). [b] Activity in dpm (as a percent of recovered activity). [c] In dpm/mmol.

Selenium dioxide oxidation[41] of cholest-5-ene-3β,19-diol (**9**) resulted in triol **36**. Derivatization with carbonyldiimidazole[42] followed by mild hydrolysis of the resulting 19-imidazole carbamate 3,4-cyclic carbonate provided the 3,4-cyclic carbonate derivative of triol **36**, which could be silylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in refluxing $CHCl_3$ to give the TMS derivative **37**. Reduction of this carbonate as described[40] with NaB^3H_4 and catalytic Pd(0) resulted in a 5:1 mixture of Δ^5 and Δ^4 olefins **38** and **39**, tritiated in the 4α- and 6α-positions, respectively. While these two compounds could be separated by careful preparative TLC purification, it was more convenient to use the mixture for the subsequent transformations. Oxidation with PCC followed by treatment with cold, dilute ethereal HCl resulted in desilylated hydroxy enone **23a**, tritiated at C-4 and nominally at C-6. The acid-catalyzed double bond isomerization has been shown to occur with the stereospecific loss of the 4β-hydrogen atom.[43] Measurement of the specific activity before and after isomerization showed that this was indeed the case.

Results of Feeding Experiments Involving Precursors 19a, 20a, 21a, 22a, and 23a. The five tritiated precursors were fed to *A. polypoides* and the results are presented in Table III. The results of the feeding experiment involving the unstable $\Delta^{5(10)}$-3-ketone **19a** show that this precursor did not survive the conditions of the incorporation procedure. Of the administered activity (42 μCi), only 4% was recovered in the extracted total lipids. Purification of the native sterols showed that no **19a** was present. Radioactivity was found, however, in chromatographic fractions that had the same R_f as the autoxidation product of **19a**. While some conversion to the 19-norstanol **8** was observed (12% of the recovered activity), the specific activity of this compound was found to be unusually low, suggesting inefficient conversion to **8**. While the decomposition of precursor **19a** has precluded any accurate determination of the role of **19** in the conversion of **7** to **8**, careful analysis of the native 19-norstanol mixture did reveal the presence of radiolabeled 19-norcholest-5(10)-en-3β-ol (**11**), which could only arise from the biological reduction of the precursor. This fact supports our finding that the C-3 oxido-reductase system in C-19 demethylation has a low substrate specificity.

The remaining four feeding experiments all show not only high levels of recovered radioactivity but also high levels of conversion to **8** (50–73% of the recovered activity). More importantly, however, these high levels of conversion are accompanied by high specific activities—on the order of 10^8 dpm/mmol. This last fact is suggestive of efficient conversion of the respective precursors to **8** and implies that they are obligatory intermediates in the biosynthesis of **8**.

Trace levels of 19-nor-5α-cholestan-3-one (**21**) were found in the extract of the feeding experiment involving labeled 19-nor-

(33) Evans, D. A.; Truesdale, L. K.; Grimm, K. G.; Nesbitt, S. L. *J. Am. Chem. Soc.* **1977**, *99*, 5009–5017.
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(35) Hutchins, R. O.; Hoke, D.; Keogh, J.; Koharski, D. *Tetrahedron Lett.* **1969**, *40*, 3495–3498.
(36) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 287–290.
(37) Hagiwara, H.; Noguchi, S.; Nishikawa, M. *Chem. Pharm. Bull.* **1960**, *8*, 84–85.
(38) Tschesche, R.; Brennecke, H. R. *Chem. Ber.* **1979**, *112*, 2680–2691.
(39) This product has been noted previously as the product of autoxidation: DiNunno, C. M.; Burdett, J. E.; Rao, P. N.; Kim, H. K.; Blye, R. P. *Steroids* **1983**, *42*, 401–408.
(40) Rabinowitz, M. H. *Tetrahedron Lett.*, in press.
(41) Rosenheim, O.; Starling, W. W. *J. Chem. Soc.* **1937**, 377–384.
(42) Kutney, J. P.; Ratcliffe, A. H. *Synth. Commun.* **1975**, *5*, 47–52.
(43) de la Mare, P. B. D.; Wilson, R. D. *J. Chem. Soc., Perkin Trans. 2* **1977**, 157–162.

Table IV. Results of Feeding Experiments Involving 4α- and 4β-Tritio Precursors[a]

precursor (ratio)	recovered	recovered precursor	DHC	8	8 (PCC)	8 (PCC/KOH)
A: [4α-^{3}H,4-^{14}C]cholesterol (1.0)	0.94	0.98	0.93	0.96	0.90	<0.05
B: [4β-^{3}H,4-^{14}C]cholesterol (0.51)	0.33	0.52	0.28	0.28	0.28	<0.06

[a] The data represent the cpm ratios of ^{3}H:^{14}C.

cholest-4-en-3-one (**20a**), demonstrating the presence of a steroid 5α-reductase in *A. polypoides*. Labeled cholest-4-en-3-one (**22a**) was shown to be converted to a small extent (Table III) to 5α-cholestan-3β-ol (DHC) by *A. polypoides*. 5α-Cholestan-3β-ol has identical chromatographic characteristics as its isomer, 24-(*R*)-methyl-19-nor-5α-cholestan-3β-ol (**17**, the major sterol of the native mixture[16]). Determination of the presence of DHC was accomplished using the original procedure for the isolation of 19-norstanols in *A. polypoides*.[15] Thus, unlabeled DHC was added to the radioactive reverse-phase HPLC fraction corresponding to the mixture of DHC and 24(*R*)-methyl-19-nor-5α-cholestan-3β-ol. Oxidation with PCC followed by bromination, dehydrobromination, and acetylation resulted in a mixture of dienones derived from DHC and phenol acetates derived from 24(*R*)-methyl-19-nor-5α-cholestan-3β-ol. The tritium activity was found to be located exclusively in the dienone fractions. This finding explains the presence of ^{14}C in the 24(*R*)-methyl-19-nor-5α-cholestan-3β-ol (**17**) fraction in the [3α-^{3}H,4-^{14}C]cholesterol feeding experiment (Table II).

The highly efficient conversions of precursors **22** and **23** to **8** in addition to the demonstrated conversion of **20** and **21** to **8** strongly suggests that C-19 demethylation occurs in *A. polypoides* by the "vinylogous β-keto acid route" depicted in Scheme IIB. The fact that Δ^5 precursors **9a** and **10a** were converted to **8** with low efficiency suggests that pathway A, Scheme II, is unlikely to operate in *A. polypoides* and that **9a** and **10a**, while not obligatory intermediates in the conversion of **7** to **8**, are transformed into compounds which are true intermediates of the biosynthetic pathway.

Investigation of the Lability of the 4α- and 4β-Hydrogen Atoms. Once it was established that the Δ^4-3-keto steroids are intermediates in the biosynthesis of 19-norstanols, it followed that their formation involved loss of either the 4α- or 4β-hydrogen atom from dietary Δ^5 sterols. In such isomerizations, the migration of the Δ^5 double bond to the Δ^4-position involves the stereospecific loss of the 4β-hydrogen atom.[25,30a,44] The fact that this hydrogen atom has been shown to be retained during the conversion of cholesterol (**7**) to 19-nor-5α-cholestan-3β-ol (**8**)[19b] presented an inconsistency that we felt merited further investigation.

[4α-^{3}H]Cholesterol[40] and [4β-^{3}H]cholesterol[40] were mixed with appropriate amounts of [4-^{14}C]cholesterol and fed to *A. polypoides*. The results of the feeding experiments involving these doubly labeled precursors are shown in Table IV.

For entry A ([4α-^{3}H,4-^{14}C]cholesterol), 19-nor-5α-cholestan-3β-ol (**8**) was found to have a ^{3}H:^{14}C activity ratio of 0.96—nearly identical to that of the administered precursor (1.0). Oxidation of **8** resulted in little change in this ratio. Equilibration of the resulting ketone with KOH in refluxing methanol resulted in a complete loss of ^{3}H, proving that the tritium label did not migrate from C-4 during metabolism. Additionally, recovered DHC produced from the precursor also showed a ^{3}H:^{14}C activity ratio that was substantially identical to that of the precursor.

The results for the feeding experiment involving [4β-^{3}H,4-^{14}C]cholesterol (entry B, Table IV), on the other hand, indicated that the biosynthesis of **8** involves loss of the 4β-hydrogen atom of cholesterol. The recovered 19-norstanol **8** showed a ^{3}H:^{14}C activity ratio of 0.28, representing a 45% loss of ^{3}H. PCC oxidation of **8** showed no change in the ^{3}H:^{14}C activity ratio, but KOH equilibration of the PCC product resulted in a complete loss of ^{3}H. SeO_2 oxidation of the recovered precursor, which is known to occur with stereospecific loss of the 4β-hydrogen atom,[45] resulted in the complete loss of tritium, proving the high degree of stereospecificity of the tritium label in the precursor.

The DHC recovered in this experiment was found to have lost ^{3}H to the same extent as **8** (^{3}H:^{14}C = 0.28). This result strongly suggests that the oxido-reductase/isomerase system used for C-19 demethylation is the same as that used in the formation of 5α-stanols from dietary Δ^5 sterols.

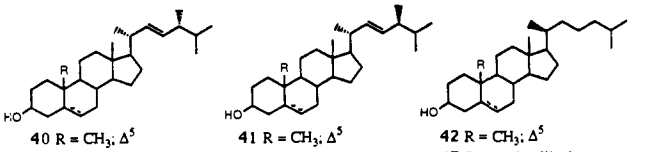
It has been suggested[46] that the 3-oxosteroid-Δ^5-isomerase promotes the intramolecular shift of the 4β-hydrogen atom to the 6β-position. This is apparently not the case for the isomerase of *A. polypoides*. The partial retention of tritium, however, is harder to understand. One possibility is that scrambling of the tritium label is occurring in the intermediate [4β-^{3}H]-Δ^5-3-keto steroid.

Assuming a relatively small energy difference between the chair and boat conformers of ring A[47] of the C-3 oxidation product (i.e., [4β-^{3}H,4-^{14}C]cholest-5-en-3-one), nonenzymatic loss of the axial substituent at C-4 from the chair conformer results in loss of tritium to give a Δ^4-3-ketone, while axial loss at C-4 from the boat conformer results in loss of hydrogen, leading to a 4-tritio-Δ^4-3-ketone. Furthermore, regardless of the position of the chair/boat equilibrium, the assumption that hydrogen abstraction will be faster than tritium abstraction implies a greater ratio of tritiated to untritiated Δ^4-3-ketone than would otherwise be reflected by the equilibrium ratio of the chair and boat conformers. This rationale is supported by the demonstration of a small but measurable loss of ^{3}H from [4α-^{3}H]cholesterol in the conversion to 19-norstanol **8** (entry A, Table IV).

Further support for this comes from the work of de la Mare and Wilson,[43] who showed that in the isomerization of [4β-^{2}H]cholest-5-en-3-one to the Δ^4-3-ketone, after more than 1 half-life, recovered Δ^5-3-ketone showed significant migration of the deuterium label to the 4α-position.

Hydrocarbon Side Chain Requirements for Nuclear Demethylation of Dietary Sterols. All studies of the biosynthesis of 19-norstanols by *A. polypoides* thus far have dealt exclusively with cholesterol and its analogues. Utility of the demethylation pathway for the production of clinically important 19-norsteroids would, of course, require either short side chain Δ^5-3β-hydroxy precursors or dietary sterols with suitably functionalized side chains that would allow for subsequent chemical transformation.

Synthesis and Incorporation of Radiolabeled Crinosterol, Brassicasterol, and 20-Isocholesterol. Both 19-nor-5α-dihydrobrassicasterol (**43**) and 19-nor-5α-dihydrocrinosterol (**44**) are found in the native 19-norstanol mixture of *A. polypoides*.[16] Because they both contain Δ^{22} double bonds and are also present in the sponge in substantial quantities, their biosyntheses from brassicasterol (**40**) and crinosterol (**41**) were examined. In addition, the tolerance for unconventional side chain structure was examined through a feeding experiment involving [6-^{3}H]-20-isocholesterol (**42b**).



40 R = CH_3; Δ^5
43 R = H; 5α-dihydro

41 R = CH_3; Δ^5
44 R = H; 5α-dihydro

42 R = CH_3; Δ^5
45 R = H; 5α-dihydro

a: [26-^{3}H]
b: [6-^{3}H]

(44) (a) Murota, S.; Fereslav, C. C.; Talalay, P. *Steroids* **1971**, *17*, 25–37. (b) Werbin, H.; Chaikoff, L. L. *Biochim. Biophys. Acta* **1964**, *82*, 581–594.

(45) Lockley, W. J. S.; Rees, H. H.; Goodwin, T. W. *J. Labelled Compd. Radiopharm.* **1978**, *15*, 413–423.

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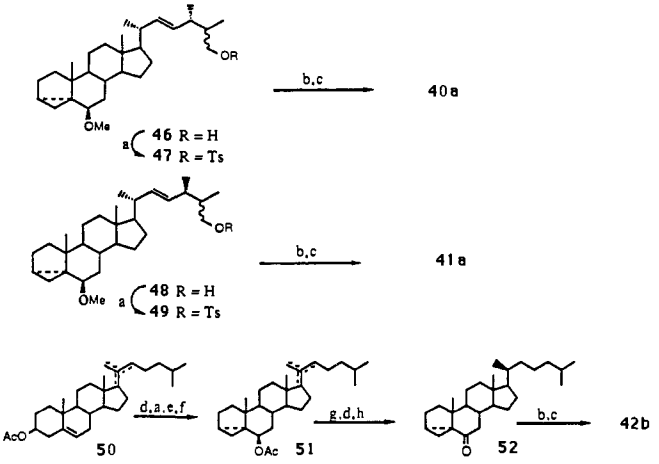
Scheme V. Synthesis of precursors **40a**, **41a**, and **42b**[a]



[a] (a) TsCl/pyr. (b) NaB^3H_4. (c) $HClO_4$. (d) LAH. (e) $NaHCO_3/H_2O$. (f) Ac_2O/pyr. (g) H_2/Pd, then HPLC. (h) Jones' Ox.

Table V. Results of Feeding Experiments Involving Long Side Chain Precursors **40a**, **41a**, and **42b**

precursor (activity)	recovered activity[a]	recovered precursor[b]	19-Nor-5α-stanol[b]	specific activity[c]
40a	4.2×10^6	9.1×10^5	**43**: 2.0×10^6	4.6×10^8
(18 μCi)	(10%)	(22%)	(48%)	
41a	8.4×10^6	2.0×10^6	**44**: 4.0×10^6	2.8×10^8
(24 μCi)	(16%)	(24%)	(47%)	
42b	1.2×10^7	5.8×10^6	**45**: 0	
(27 μCi)	(20%)	(50%)		

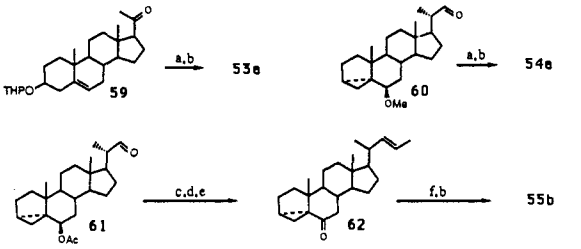
[a] In dpm (as a percent of administered activity). [b] In dpm (as a percent of recovered activity). [c] In dpm/mmol.

The syntheses of **40a**, **41a**, and **42b** are shown in Scheme V. Reduction of the tosylate of (25ξ)-26-hydroxy i-steroid **46**[48] with NaB^3H_4 in hot DMSO resulted in less than 1% conversion to the tritiated product of tosylate cleavage. Addition of $NaBH_4$ and continued heating resulted in the complete reduction of tosylate **47**. Nevertheless, tritiation did occur to a useful extent. Hydrolysis with aqueous mineral acid resulted in **40a**. The same sequence was applied to alcohol **48**[48] to provide **41a**.

The synthesis of tritiated 20-isocholesterol was a variation of the procedure described by Nes.[49a] Thus a mixture of $\Delta^{17(20)}$, Δ^{20}, and $\Delta^{20(22)}$ olefins[49b] was converted to the i-acetates (**51**) by standard procedures[50] and then hydrogenated over palladium on carbon. Separation by reverse-phase HPLC provided pure (20*S*)-20-iso-3α,5-cyclo-5α-cholestan-6β-yl acetate. Tritiation of the corresponding i-ketone **52** followed by acidic hydrolysis provided **42b**.

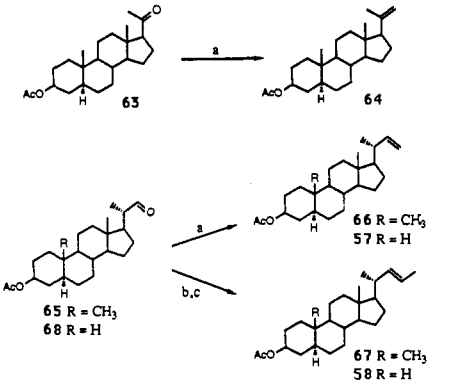
Incorporation of precursors **40a**, **41a**, and **42b** into *A. polypoides* provided some unexpected results (Table V). While both **40a** and **41b** were transformed to their 19-nor-5α-dihydro analogues **43** and **44** in high yields (48% and 47%, respectively) displaying high specific activities, 20-isocholesterol (**42b**) was not transformed to any extent to its 19-nor-5α-dihydro analogue **45**. Instead, in addition to recovered precursor, 16% of the recovered radioactivity was found in tritiated (20*S*)-20-iso-5α-cholestan-3β-ol,[49a,51] the product of simple reduction of the nuclear double bond of the precursor.

Scheme VI. Synthesis of Short Side Chain Precursors **53a**, **54a**, and **55b**[a]



[a] (a) $^{14}CH_2PPh_3$. (b) H_3O^+. (c) CH_3CHPPh_3, then HPLC. (d) LAH. (e) Jones' Ox. (f) NaB^3H_4.

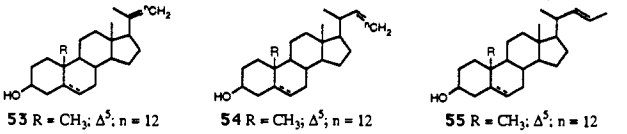
Scheme VII. Synthesis of Cold-Carriers for Short Side Chain Feeding Experiments[a]



[a] (a) CH_2PPh_3. (b) CH_3CHPPh_3. (c) AIBN/PhSH, then HPLC.

This unexpected result suggests that the stereochemistry at C-20 is crucial to overall C-19 demethylation by *A. polypoides* and that this pathway tolerates only conventional stereochemistry at this center. It appears, however, that nuclear saturation of Δ^5-3β-hydroxy sterols (i.e., conversion to the Δ^4-3-ketone followed by reductions at C-5 and C-3) is less substrate-specific and more tolerant of unusual side chain modifications. While this effect may also be attributed to transport phenomena, this hypothesis would require the existence of a 3-keto steroid oxido-reductase/5α-reductase system in this primitive organism that is separate and distinct from those of the demethylation pathway.

Studies of the Conversion of C_{22}, C_{23}, and C_{24} Short Side Chain Sterols. A second set of side chain studies was devised to examine the tolerance of the demethylation apparatus of *A. polypoides* for short side chain sterols. Radiolabeled [21-^{14}C]-20-methylpregna-5,20-dien-3β-ol (**53a**), [23-^{14}C]-24-norchola-5,22-dien-3β-ol (**54a**), and [6-^{3}H]-(22*E*)-chola-5,22-dien-3β-ol (**55b**) were synthesized by the route shown in Scheme VI.



Treatment of the dihydropyran adduct of pregnenolone[52a] (**59**) with $^{14}CH_2PPh_3$[53] followed by acid hydrolysis provided **53a**. Similarly, treatment of aldehyde **60**[54] with the same labeled ylide followed by hydrolysis resulted in **54a**. Treatment of aldehyde **61**[55] with ethylidenetriphenylphosphorane[56] resulted in a mixture

(48) (a) Fujimoto, Y.; Kimura, M.; Khalifa, F. A. M.; Ikekawa, N. *Chem. Pharm. Bull.* **1984**, *32*, 4372–4381. (b) Lang, R. W.; Djerassi, C. *Helv. Chim. Acta* **1982**, *65*, 407–418.

(49) (a) Nes, W. R.; Varkey, T. E.; Krevitz, K. E. *J. Am. Chem. Soc.* **1977**, *99*, 260–262. (b) Nes, W. R.; Varkey, T. E.; Crump, D. R.; Gut, M. *J. Org. Chem.* **1976**, *41*, 3429–3433.

(50) i-Alcohol formation (Steele, J. A.; Mossettig, E. *J. Org. Chem.* **1963**, *28*, 571–572) followed by acetylation with Ac_2O in dry pyridine (see ref 55b).

(51) (a) Chadhuri, N. K.; Nickelson, R.; Williams, J. G.; Gut, M. *J. Org. Chem.* **1969**, *34*, 3767–3774. (b) Nair, G. V.; Mosettig, E. *J. Org. Chem.* **1962**, *27*, 4659–4660. (c) Ibaka, T.; Taga, T.; Shingu, T.; Saito, M.; Nishi, S.; Yamamoto, Y. *J. Org. Chem.* **1988**, *53*, 3947–3952.

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Table VI. Results of Feeding Experiments Involving Short Side Chain Precursors **53a**, **54a**, and **55b**. 19-Norstanols Analyzed as Their Acetates

precursor (activity)	recoverd activity[a]	recovered precursor[b]	19-nor-5α-stanol[b]	5α-stanol[b]
53a (23 μCi)	7.6×10^5 (5%)	4.0×10^4 (5.2%)	**56**: 0	**64**: 2.8×10^5 (37%)
54a (23 μCi)	1.1×10^6 (22%)	1.3×10^5 (12%)	**57**: 1.1×10^5 (10%)	**66**: 6.3×10^4 (5.7%)
55b (40 μCi)	1.3×10^6 (15%)	2.6×10^5 (20%)	**58**: 6.7×10^4 (5%)	**67**: 1.0×10^4 (0.8%)

[a] In dpm (as a percent of administered activity). [b] In dpm (as a percent of recovered activity).

of cis and trans olefins, which could be purified by reverse-phase HPLC. Conversion of the trans isomer to ketone **62** was followed by tritiation at C-6 with NaB^3H_4 and acid hydrolysis to provide **55b**.

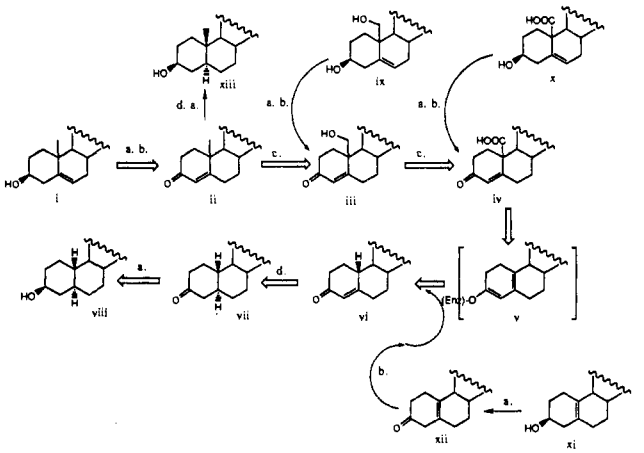
Identification of biological transformation products for feeding experiments involving these three precursors required the synthesis of unlabeled analogues of the expected biosynthetic products (i.e., 19-nor-5α-dihydro and 5α-dihydro analogues of **53**, **54**, and **55**). The syntheses of these compounds followed routes similar to those described in Scheme VI. Thus, methylenation of ketone **63**[57] provided **64** (Scheme VII). Similar treatment of aldehydes **65**[58] and **68** (prepared from acetylation and ozonolysis of the mixture of native 19-norstanols of *A. polypoides*) resulted in methylene olefins **66** and **57**, respectively. Treatment of the same aldehydes with ethylidenetriphenylphosphorane followed by equilibration of the resulting olefins[59] and purification by reverse-phase HPLC resulted in trans olefins **67** and **58**.

The results of the feeding experiments involving precursors **53a**, **54a**, and **55b** are shown in Table VI. The recovered native sterols were analyzed as their acetates. The results clearly show a relationship between side chain length and nuclear demethylation. The C_{22} precursor (**53a**), which has an sp^2 center at C-20, was shown not to be demethylated by the sponge. However, this precursor was transformed to its 5α-dihydro analogue (**64**) in high yield (37% of the recovered activity). Its C_{23} homologue (**54a**) was found to be converted by the sponge to both the 19-nor-5α analogue (**57**) as well as the 5α-dihydro analogue **66**. Each compound was formed in similar amounts (10% and 5.7%, respectively). The final precursor, **55b**, was found to be converted to its 19-nor-5α analogue, **58**, in an amount approximately 1 order of magnitude higher than that of the nuclear reduction product **67**, lending credence to the idea that as the sterol side chain length increases and approaches that of conventional dietary sterols (i.e., C_8 and higher), the ratio of C-19 demethylation to nuclear saturation increases, from a low of 0:1 for C_{22} precursor **53b**, to approximately 100:1 for cholesterol (Table II).

Conclusion

A summary of our findings regarding nuclear transformations involved in the biosynthesis of 19-norstanols from Δ^5-3β-hydroxy sterols is shown in Scheme VIII. The bold arrows represent the obligatory demethylation pathway while the thin arrows represent secondary pathways. Dietary Δ^5-3β-hydroxy sterol i is transformed to Δ^4-3-ketone ii by the actions of a 3β-hydroxy steroid oxido-reductase and 3-oxosteroid-Δ^5-isomerase. Repeated hydroxylation at C-19 by a mixed-function oxidase[60] produces first 19-hydroxy enone iii and then vinylogous β-keto acid iv. Enzymatic or nonenzymatic[61] decarboxylation will produce either an enzyme-

Scheme VIII. Most Likely Pathway for Biosynthesis of 19-Norstanols by *Axinella polypoides*[a]



[a] (a) 3β-Hydroxysteroid oxido-reductase. (b) 3-Oxosteroid-Δ^5-isomerase. (c) 19-Hydroxylase. (d) 4-Ene-5α-reductase.

bound (v) or free dienolate, which may collapse to 19-nor-Δ^4-3-ketone vi. Conjugate reduction by a steroid 5α-reductase followed by oxido-reductase reduction of C-3 provides 19-norstanol viii. This scheme also shows the formation of 5α-stanol xiii by conjugate reduction and C-3 oxido-reductase reduction of Δ^4-3-ketone ii.

The low but nevertheless detectable conversions of cholest-5-en-3β,19-diol (**9**), 3β-hydroxycholest-5-en-19-oic acid (**10**), 19-norcholest-5(10)-en-3β-ol (**11**), and 19-norcholest-5(10)-en-3-one (**19**) to 19-nor-5α-cholestan-3β-ol (**8**) is shown in Scheme VIII (structures ix, x, xi, and xii, respectively) as arising from the actions of a fairly nonspecific 3-keto steroid oxido-reductase and 3-oxosteroid-Δ^5-isomerase.

Evidence for the nonspecificity of the oxido-reductase/isomerase system with respect to the 19-hydroxylase was demonstrated by the complete suppression of nuclear demethylation for sterols with unconventional hydrocarbon side chains (i.e., feeding experiments with precursors **42a** and **53a**) while retaining 5α-stanol biosynthesis activity. This nonspecificity was also demonstrated by the distribution of the ^3H label of [3α-^3H,4-^{14}C]cholesterol into all of the 19-norstanols of the native mixture.

Practical utilization of the results would require the isolation of the 19-hydroxylase enzyme, preferably its attachment to a solid support, and the passage of a suitable Δ^4-3-keto steroid (e.g., derived from stigmasterol) through such a column to yield the corresponding 19-nor-Δ^4-3-ketone. Its subsequent transformation to oral contraceptives of the norethindrone type (**1**) would then be straightforward.

Experimental Section

All reagents and solvents were used as supplied unless otherwise noted. Hexanes, diethyl ether, methanol, and ethyl acetate for chromatography and chloroform used for extraction were distilled through a 30 × 3 cm Vigreaux column before use. Tetrahydrofuran was distilled from benzophenone ketyl. Anhydrous ether was distilled from CaH_2, and anhydrous CH_2Cl_2 was distilled from P_2O_5 prior to use. Pyridine was distilled from KOH under nitrogen and stored in a Schlenk buret over activated 4-Å molecular sieves (4ÅMS). DMSO was distilled under reduced pressure from KOH and stored over 4ÅMS. "Hexanes" refers to a commercially available bulk mixture of C_6H_{14} hydrocarbons. "The usual workup" refers to repeated washing of the reaction mixture with saturated $NaHCO_3$ followed by deionized water and brine with drying over $MgSO_4$.

Sodium borohydride-*t* (sodium borotritide) was purchased from either New England Nuclear or ICN Radiochemicals and used as supplied. [4-^{14}C]Cholesterol (60 mCi/mmol) was purchased from NEN and analyzed by reverse-phase HPLC before use. [^{14}C]Methyliodide was purchased from ICN. All syntheses involving radioactive isotopes were first performed using stable isotopes to obtain satisfactory physical and spectral data.

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR data was recorded on

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(59) Schwartz, M.; Graminski, G. F.; Waters, R. M. *J. Org. Chem.* **1986**, *51*, 260–263.
(60) See: Gower, D. B. in ref 22, Chapter 7, pp 230–292.
(61) The possibility for a nonenzymatic decarboxylation step parallels the reported final step in C_{18} steroid biosynthesis: Goto, J.; Fishman, J. *Science* **1977**, *195*, 80–81.

an IBM FTIR using a 1-mm NaCl cell in CCl_4. Low-resolution mass spectra were recorded with either a Hewlett-Packard 5970 Series quadrupole mass detector connected to a Hewlett-Packard 5890 gas chromatograph fitted with a Hewlett-Packard HP-5 methyl silicone capillary column with a temperature program of 180 to 290 °C at a rate of 10 °C/min or a Hewlett-Packard Model HP 5995 direct inlet mass spectrometer.

The ^1H and ^{13}C NMR spectra were recorded with either a Varian XL400 spectrometer, a Varian Gemini 300, or a Varian Gemini 200 instrument. Spectra were recorded in $CDCl_3$ with chemical shift values in ppm relative to the solvent peak (7.26 ppm for ^1H and 77.0 ppm for ^{13}C). Coupling constants are in units of hertz. UV–vis spectra were recorded with a Hewlett-Packard HP 8450 A spectrophotometer equipped with glass cuvettes (path length = 1 cm).

HPLC analysis was performed with a Waters Associates HPLC unit (M6000 pump, UK 6 injector, R403 differential refractometer detector). For normal-phase HPLC, a single Whatman Partisil M9 10/50 column was employed at a flow rate of 3.0 mL/min with hexanes/EtOAc 96:4 as the mobile phase. Reverse-phase HPLC was performed using two in-line Altex Ultrasphere ODS 5-μm columns at a flow rate of 3.0 mL/min using either purified methanol or an acetonitrile solvent system (CH_3CN/EtOAc/MeOH, 11:4:4) as the mobile phase.

Radioactivity measurements were performed with a Beckman LS 7500 dual-channel liquid scintillation counter with Beckman Ready Organic scintillation cocktail. Conversion of ^3H and ^{14}C cpm data to dpm was performed using the H-number method. ^3H:^{14}C ratios in double labeling experiments were determined by the channel ratios method.

Sponges were collected in the Bay of Naples at a depth of approximately 30 m. Feeding experiments were performed as previously described.[19a] Extraction of the dried animals with chloroform provided the total lipids which were purified by chromatography on Florisil and silica gel until free of pigment. Purification and analysis of the individual 19-norstanols was accomplished by reverse-phase HPLC.

[6-^{3}H]-3β-Hydroxycholest-5-en-19-oic Acid (10a) and [6-^{3}H]Cholest-5-ene-3β,19-diol (9a). 6-Oxo-3α,5-cyclo-5α-cholestan-19-oic acid[28b] (75 mg, 0.18 mmol, 1 equiv) was treated with an ethereal solution of CH_2N_2. The usual workup provided methyl 6-oxo-3α,5-cyclo-5α-cholestan-19-oate as a clear oil (80 mg, quantitative): ^1H NMR (200 MHz) δ 0.650 (s, 3 H, 18-Me), 0.854 (d, 6 H, J = 6.6, 26-, 27-Me), 0.889 (d, 3 H, J = 7.2, 21-Me), 1.512 (dd, 1 H, J = 16, 4.2), 3.686 (s, 3 H, MeO); ^{13}C NMR (50 MHz) δ 11.8, 12.0, 18.4, 22.4, 22.6, 23.6, 24.0, 24.1, 27.8, 28.0, 29.0, 34.1, 35.4, 35.5, 35.9, 39.3, 39.4, 41.6, 42.7, 44.0, 45.9, 51.7, 56.0, 56.7, 59.9, 175.5, 207.9 (one carbon unresolved); MS 428 (M^+, 14), 369 (100), 273 (5), 255 (5), 229 (11), 215 (23); HRMS calcd for $C_{28}H_{44}O_3$ 428.3290, found 428.3284.

Using the procedure of Tanabe et al.,[28b] methyl 6-oxo-3α,5-cyclo-5α-cholestan-19-oate was reduced with 25 mCi of NaB^3H_4 (ICN, 1.6 Ci/mmol, 0.6 mg) to give a mixture of 6α- and 6β-alcohols, 15% of which was hydrolyzed with 10% aqueous dioxane and 50 μL of 72% $HClO_4$ to provide, after purification on a pipet of silica gel (hexanes/ether, 1:1), [6-^{3}H]-3β-hydroxycholest-5-en-19-oic acid (**10a**, 700 μCi, 19% for the two steps).

The remaining 85% of the mixture of 6α- and 6β-alcohols was reduced with excess LAH and hydrolyzed as described above to provide, after purification on a pipet of silica gel (hexanes/ether, 3:1), [6-^{3}H]cholest-5-ene-3β,19-diol (**9a**, 4.2 mCi, 20% for the three steps). The unlabeled analogues **9** and **10** produced by this procedure gave satisfactory ^1H NMR and mass spectra which were the same as those of the authentic materials.[28,62]

[6-^{3}H]-19-Norcholest-5(10)-en-3β-ol (11a). Acid **10a** (260 μCi) was dissolved in 1 mL of a 1:1 mixture of acetic anhydride and pyridine along with a small crystal of DMAP. After 1 h the reaction mixture was pipeted into cold 1 M HCl and extracted with ether. The extract was washed with 5% $NaHCO_3$ and dried over $MgSO_4$ to give an acetate which was purified by chromatography on a pipet of silica gel (hexanes/ether, 15:1). The purified acetate was placed in a 5-mL round-bottom Pyrex flask, placed under water-pump vacuum, and pyrolyzed at 250 °C as described[28b] to give [6-^{3}H]-19-norcholest-5(10)-en-3β-yl acetate (89 μCi, 34% from **10a**), which was converted quantitatively to **11a** by reduction with 2 equiv of LAH in THF.

The identical reaction sequence using unlabeled acid (**10**) resulted in 19-norcholest-5(10)-en-3β-ol (**11**) in 30% overall yield: ^1H NMR (400 MHz) δ 0.673 (s, 3 H, 18-Me), 0.859 (d, 3 H, J = 6.4, 26-Me), 0.863 (d, 3 H, J = 6.4, 27-Me), 4.04 (m, 1 H, 3-H); MS (acetate, DCI-NH_4^+) 432 ($M^+ + NH_4^+$, 18), 354 ($M^+ - CH_3COOH$, 100); MS (EI) 354 ($M^+ - CH_3COOH$, 30), 339 (4), 283 (4), 252 (13), 421 (13), 207 (100).

Ethyl (25ξ)-6β-Methoxy-3α,5-cyclo-5α-cholestan-26-oate (27). C-22 epimeric alcohols **26**[31] (9.4 g, 0.025 mol, 1 equiv) were dissolved in 200 mL of xylenes. Triethyl orthopropionate (40 mL, 0.2 mol, 8 equiv) was added followed by 30 μL of propionic acid, and the solution was refluxed under moisture-free conditions for 3 h. The cooled reaction mixture was washed with 5% $NaHCO_3$ and dried over $MgSO_4$ to give 12 g of a crude product mixture which was chromatographed on silica gel to provide ethyl (25ξ,22*E*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-26-oate (8.7 g, 76%): ^1H NMR (400 MHz) δ 0.712 (s, 3 H, 18-Me), 0.980 and 0.985 (d, 3 H, J = 6.4 and 6.8, diastereomeric 21-Me), 1.015 (s, 3 H, 19-Me), 1.115 (d, 3 H, J = 7.2, 27-Me), 1.246 (t, 3 H, J = 7.2, CH_3CH_2O), 2.29 (m, 1 H, 20-H), 2.43 (m, 1 H, 25-H), 2.764 (m, 1 H, 6-H), 3.318 (s, 3 H, MeO), 4.107 (q, 2 H, J = 7.2, CH_3CH_2O), 5.26 (m, 2 H, 22-, 23-H); ^{13}C NMR (100 MHz) δ 12.4, 13.0, 14.2, 16.48, 16.52, 19.3, 20.61, 20.64, 21.5, 22.7, 24.1, 24.9, 28.58, 28.64, 30.4, 33.3, 35.0, 36.6, 36.7, 39.78, 39.83, 40.09, 40.11, 40.13, 42.7, 43.3, 48.0, 55.8, 56.5, 60.10, 60.13, 82.4, 123.8, 124.0, 139.6 (C=O not observed); MS 456 (M^+, 1), 441 (3), 424 (9), 401 (8), 282 (3), 281 (2), 267 (2), 255 (20), 227 (10), 196 (10), 95 (100).

The olefin (6.7 g, 0.016 mol) was dissolved in 150 mL of hexanes and hydrogenated over PtO_2 at atmospheric pressure for 30 h to give **27** (6.7 g, quantitative): ^1H NMR (400 MHz) δ 0.699 (s, 3 H, 18-Me), 0.886 (d, 3 H, J = 6.4, 21-Me), 1.011 (s, 3 H, 19-Me), 1.129 (d, 3 H, J = 7.2, 27-Me), 1.232 (t, 3 H, J = 7.2, CH_3CH_2O), 2.40 (m, 1 H, 25-H), 2.763 (s, 1 H, 6-H), 3.316 (s, 3 H, MeO), 4.122 (d, 2 H, J = 7.2, CH_3CH_2O); ^{13}C NMR (100 MHz) δ 12.19, 12.27, 13.00, 13.05, 13.09, 18.6, 19.2, 19.3, 21.4, 21.5, 22.7, 23.66, 23.68, 24.13, 24.18, 24.9, 28.27, 28.29, 30.4, 33.31, 33.38, 34.26, 34.9, 35.0, 35.2, 35.6, 35.7, 39.6, 40.21, 40.25, 42.7, 43.3, 47.9, 48.0, 56.13, 56.18, 56.21, 56.4, 56.50, 56.58, 82.3, 82.5, 151.0; MS 458 (M^+, 8), 443 (11), 426 (21), 403 (23), 305 (15), 255 (28), 213 (22), 207 (13), 201 (12), 175 (12), 55 (100); HRMS calcd for $C_{30}H_{50}O_3$ 458.3760, found 458.3756.

(25ξ)-26-[(*tert*-Butyldiphenylsilyl)oxy]cholest-5en-3β-yl Acetate (28). To ester **27** (6.7 g, 0.015 mmol, 1 equiv) in 100 mL of anhydrous ether was added LAH (425 mg, 11 mmol, 0.75 equiv) with stirring. After 30 min at room temperature, the excess reducing agent was destroyed with EtOAc, and the reaction mixture was acidified with 10% H_2SO_4 washed with 5% $NaHCO_3$, and dried over $MgSO_4$ to give 6.7 g of diastereomeric alcohols: ^1H NMR (400 MHz) δ 0.680 (s, 3 H, 18-Me), 0.877 (d, 3 H, J = 6.8, 21-Me), 0.884 (d, 3 H, J = 6.4, 27-Me), 0.986 (s, 3 H, 19-Me), 2.741 (m, 1 H, 6-H), 3.290 (s, 3 H, MeO), 3.36 (m, 1 H, 26-H), 3.45 (m, 1 H, 26-H′); MS 416 (M^+, 7), 401 (8), 384 (14), 361 (15), 255 (8), 213 (10), 55 (100).

The C-25 epimeric alcohols (2.14 g, 0.00514 mol, 1 equiv) and imidazole (770 mg, 2.2 equiv) were dissolved in 10 mL of DMF. *tert*-Butyldiphenylsilyl chloride (1.5 mL, 1.1 equiv) was added via syringe to the stirred solution under argon. After 1 h, workup[63] provided an oily product which was dried under high vacuum at 50 °C for 24 h to provide a mixture of diastereomeric silyl ethers (3.3 g, 98%). The epimeric silyl ethers (3.03 g, 0.0047 mol) were immediately dissolved in 15 mL of glacial acetic acid and heated at reflux for 40 min. The cooled reaction was pipeted into iced H_2O, and 5 N KOH was added dropwise until the solution was neutral to pH paper. Extracting with ether and drying over $MgSO_4$ provided **28** as a single product which was dried at 45 °C under vacuum for 24 h (2.8 g, 89%): ^1H NMR (400 MHz) δ 0.669 (s, 3 H, 18-Me), 0.892 (d, 3 H, J = 7.2, 21-Me), 0.917 (d, 3 H, J = 6.8, 27-Me), 1.018 (s, 3 H, 19-Me), 1.049 (s, 9 H, Me_3CSi), 2.035 (s, 3 H, CH_3COO), 2.31 (m, 2 H, 4-H_2), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 4.61 (m, 1 H, 3-H), 5.37 (m, 1 H, 6-H), 7.4 (m, 6 H, H_{arom}), 7.67 (m, 3 H, H_{arom}), 7.72 (m, 1 H, H_{arom}).

Silyl ether **28** could be converted to known alcohol **28a** by treatment with 2 equiv of TBAF in THF for a period of 24 h. The free alcohol (**28a**) provided ^1H NMR, ^{13}C NMR, and mass spectra which were in agreement with those published:[64] ^1H NMR (400 MHz) δ 0.658 (s, 3 H, 18-Me), 0.904 (d, 6 H, J = 6.8, 21-, 27-Me), 1.001 (s, 3 H, 19-Me), 2.020 (s, 3 H, CH_3COO), 2.32 (m, 2 H, 4-H_2), 3.40 (m, 1 H, 26-H), 3.50 (m, 1 H, 26-H′), 4.60 (m, 1 H, 3-H), 5.35 (m, 1 H, 6-H); ^{13}C NMR (100 MHz, selected peaks) δ 68.5 (C26), 74.0 (C3), 122.6 (C6), 139.6 (C5), 170.5 (CH_3CO); MS 426 ($M^+ - H_2O$, 2), 384 ($M^+ - CH_3COOH$, 100), 369 (10), 276 (5), 263 (13), 255 (14).

(25ξ)-5α-Bromo-26-[(*tert*-butyldiphenylsilyl)oxy]-6β,19-epoxy-5α-cholestan-3β-yl Acetate (29). Olefin **28** (2.82 g, 0.00413 mol, 1 equiv) was dissolved in 170 mL of freshly distilled ether and stirred at 0 °C. Water (10 mL) was added followed by 8.5 mL of 8% $HClO_4$. *N*-

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Bromosuccinimide (1.03 g, 1.4 equiv, recrystallized from water) was added slowly to the stirred reaction mixture over 30 min. Stirring was continued at 0 °C for another 30 min then at room temperature for an additional hour. The reaction was quenched by the addition of cold 10% sodium thiosulfate. The organic layer was washed with 5% $NaHCO_3$ and brine and dried over $MgSO_4$. The crude product (3.2 g) was purified by silica gel chromatography (hexanes/ether, 10:1) to provide (25ξ)-5α-bromo-26-[(*tert*-butyldiphenylsilyl)oxy]-6β-hydroxy-5α-cholestan-3β-yl acetate (1.6 g, 50%): ^{1}H NMR (400 MHz) δ 0.668 (s, 3 H, 18-Me), 0.881 and 0.887 (d, 3 H, J = 6.8, 21-Me), 0.907 and 0.915 (d, 3 H, J = 6.8, diastereomeric 27-Me), 1.048 (s, 9 H, Me_3CSi), 1.312 (s, 3 H, 19-Me), 2.506 (dd, 1 H, J = 13.6, 10.4, 4α-H), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 4.180 (bs, 1 H, 6-H), 5.47 (m, 1 H, 3-H), 7.4 (m, 6 H, H_{arom}), 7.6 (m, 4 H, H_{arom}); ^{13}C NMR (100 MHz) δ 12.2, 16.8, 18.0, 18.7, 19.3, 21.3, 21.4, 24.0, 26.3, 26.8, 28.2, 30.5, 34.5, 35.1, 35.6, 35.7, 35.8, 38.4, 39.6, 40.3, 42.6, 47.4, 55.7, 56.0, 68.8, 72.1, 75.7, 86.7, 127.5, 129.4, 134.1, 135.6, 170.5.

The unstable bromohydrin (1.4 g, 0.0018 mol, 1 equiv) was added to a suspension of $Pb(OAc)_4$ (4.0 g, 0.009 mol, 5 equiv) and $CaCO_3$ (500 mg) in 250 mL of cyclohexane. To the stirred mixture was added 0.91 g of I_2 (0.0036 mol, 2 equiv), and the reaction flask was fitted with a $CaCl_2$ drying tube and a reflux condenser. The purple reaction mixture was irradiated by a 600-W tungsten–halogen lamp which maintained gentle reflux. After 3 h, the cooled reaction mixture was filtered and washed with 10% sodium thiosulfate and brine and dried over $MgSO_4$ to give 2.53 g of a crude oil, which was chromatographed on silica gel (hexanes/ether, gradient 10:1 to 8:1) to give the 6β,19-epoxy **29** (1.04 g, 75%): ^{1}H NMR (400 MHz) δ 0.687 (s, 3 H, 18-Me), 0.873 and 0.878 (d, 3 H, J = 6.8, diastereomeric 21-Me), 0.909 and 0.917 (d, 3 H, J = 6.8, diastereomeric 27-Me), 1.050 (s, 9 H, Me_3CSi), 2.034 (s, 3 H, CH_3COO), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 3.747 (d, 1 H, J = 8.4, 19-H), 3.923 (d, 1 H, J = 8.4, 19-H′), 4.063 (d, 1 H, J = 4.8, 6-H), 5.20 (m, 1 H, 3-H), 7.4 (m, 6 H, H_{arom}), 7.6 (m, 4 H, H_{arom}); ^{13}C NMR (100 MHz) δ 12.4, 16.8, 17.0, 18.5, 18.6, 19.3, 21.3, 22.6, 23.22, 23.24, 23.27, 23.43, 26.8, 28.2, 32.8, 33.2, 33.6, 35.65, 35.66, 35.7, 36.1, 39.7, 41.3, 45.78, 45.80, 48.61, 48.63, 54.3, 55.9, 67.4, 68.7, 70.0, 74.5, 82.3, 127.5, 129.4, 134.1, 135.6, 170.3; HMRS calcd for $C_{41}H_{56}O_4{}^{81}BrSi$ ($M^+ - t$-C_4H_9) 721.3111, found 721.3101, calcd for $C_{41}H_{56}O_4{}^{79}BrSi$ ($M^+ - t$-C_4H_9) 719.3131, found 719.3094.

(25ξ)-26-[(*tert*-Butyldiphenylsilyl)oxy]-19-hydroxycholest-4-en-3-one (30). Bromo acetate **29** (1.11 g) was dissolved in 40 mL of 1% methanolic KOH and heated at reflux under moisture-free conditions for 30 min. The cooled reaction mixture was poured into 150 mL of iced 0.75 N HCl, extracted with ether, washed with 5% $NaHCO_3$ and brine, and dried over $MgSO_4$ to give 1.10 g of the corresponding 3β-alcohol: ^{1}H NMR (400 MHz) δ 0.684 (s, 3 H, 18-Me), 0.869 and 0.873 (d, 3 H, J = 6.4, diastereomeric 21-Me), 0.905 and 0.912 (d, 3 H, J = 6.4, diastereomeric 27-Me), 1.046 (s, 9 H, Me_3CSi), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 3.727 (d, 1 H, J = 8.4, 19-H), 3.911 (d, 1 H, J = 8.4, 19-H′), 4.067 (d, 1 H, J = 4.4, 6-H), 4.15 (m, 1 H, 3-H), 7.4 (m, 6 H, H_{arom}), 7.6 (m, 4 H, H_{arom}).

The alcohol (1.1 g) was immediately dissolved in 50 mL of acetone and cooled to 0 °C. Jones' reagent (8 N) was added dropwise to the stirred solution until an orange color persisted (ca. 8 mL). Stirring was continued for 1 h at 0 °C, after which time the reaction mixture was poured into cold water and extracted with ether. The ether was removed in vacuo, and the residue was dissolved in 50 mL of methanol and refluxed for 2 h with 500 mg of NaOAc. The reaction mixture was poured into ice water, extracted with ether, and dried over $MgSO_4$ to provide (25ξ)-26-[(*tert*-butyldiphenylsilyl)oxy]-6β,19-epoxycholest-4-en-3-one (910 mg, 96% from **22**): ^{1}H NMR (400 MHz) δ 0.739 (s, 3 H, 19-Me), 0.883 (d, 3 H, J = 6.4, 21-Me), 0.905 and 0.913 (d, 3 H, J = 6.8, 27-Me), 1.046 (s, 9 H, Me_3CSi), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 3.496 (d, 1 H, J = 8.0, 19-H), 4.218 (d, 1 H, J = 8.0, 19-H′), 4.687 (d, 1 H, J = 5.2, 6-H), 5.809 (s, 1 H, 4-H), 7.4 (m, 6 H, H_{arom}), 7.6 (m, 4 H, H_{arom}); ^{13}C NMR (100 MHz) δ 12.3, 17.1, 18.6, 19.3, 23.7, 24.1, 26.5, 26.8, 28.2, 33.3, 33.5, 33.6, 33.7, 35.6, 35.7, 36.1, 39.4, 41.3, 43.4, 46.0, 50.3, 54.7, 55.9, 68.8, 69.0, 75.6, 114.7, 127.5, 129.4, 134.1, 135.6, 172.3, 199.1; UV λ_{max} = 238 nm (log ε = 3.81); HRMS calcd for $C_{39}H_{51}O_3Si$ ($M^+ - t$-C_4H_9) 595.3607, found 595.3612.

(25ξ)-26-[(*tert*-Butyldiphenylsilyl)oxy]-6β,19-epoxycholest-4-en-3-one (850 mg, 1.34 mmol, 1 equiv) was dissolved in 50 mL of DMF/2-propanol (1:1) and stirred with 16 g of activated zinc powder at reflux for 8 h. The cooled reaction mixture was filtered through Celite. The solvents were stripped in vacuo to give 1 g of crude product, which was purified by silica gel chromatography (hexanes/ether, gradient 6:1 to 2:1) to provide the pure 19-alcohol **23** (523 mg, 62%): ^{1}H NMR (400 MHz) δ 0.691 (s, 3 H, 18-Me), 0.879 and 0.885 (d, 3 H, J = 6.4, diastereomeric 21-Me), 0.911 and 0.919 (d, 3 H, J = 6.8, diastereomeric 27-Me), 1.051 (s, 9 H, Me_3CSi), 2.75 (m, 1 H, 4-H), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 3.887 (d, 1 H, J = 10.8, 19-H), 4.073 (d, 1 H, J = 10.8, 19-H′), 5.939 (s, 1 H, 4-H), 7.4 (m, 6 H, H_{arom}), 7.6 (m, 4 H, H_{arom}); ^{13}C NMR (100 MHz) δ 12.0, 16.8, 17.0, 18.6, 19.3, 21.5, 23.3, 24.0, 26.8, 28.1, 32.2, 33.5, 33.6, 35.1, 35.57, 35.61, 35.71, 36.1, 36.2, 39.9, 42.2, 43.9, 53.9, 55.9, 56.1, 65.9, 68.7, 68.9, 126.7, 127.5, 129.4, 134.0, 135.5, 167.5, 200.5; UV λ_{max} = 240 nm (log ε = 3.84); HRMS calcd for $C_{39}H_{53}O_3Si$ ($M^+ - t$-C_4H_9) 597.3764, found 597.3735.

(25ξ)-26-[(*tert*-Butyldiphenylsilyl)oxy]-19-norcholest-5(10)-en-3-one (32). Alcohol **30** (213 mg, 0.325 mmol) was dissolved in 20 mL of acetone, cooled to 0 °C, and titrated with 8 N Jones' reagent. After 2 h of vigorous stirring at 0 °C, the reaction mixture was poured into a cold half-saturated NaCl solution and extracted with ether. The ether layer was repeatedly washed with 5% $NaHCO_3$ and dried over $MgSO_4$ to provide acid **31** (194 mg, 89%): ^{1}H NMR (400 MHz) δ 0.692 (s, 3 H, 18-Me), 0.877 and 0.882 (d, 3 H, J = 6.4, diastereomeric 21-Me), 0.907 and 0.915 (d, 3 H, J = 6.8, diastereomeric 27-Me), 1.048 (s, 9 H, Me_3CSi), 2.63 (bt, 1 H, J = 17), 2.79 (bd, 1 H, J = 14), 2.88 (bt, 1 H, J = 13), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 5.924 (s, 1 H, 4-H), 7.4 (m, 6 H, H_{arom}), 7.6 (m, 4 H, H_{arom}); ^{13}C NMR (100 MHz) δ 12.0, 16.8, 17.0, 18.58, 18.63, 19.3, 22.8, 23.3, 24.0, 26.8, 28.2, 31.1, 32.6, 33.5, 33.6, 34.0, 34.9, 35.66, 35.72, 35.9, 36.1, 39.6, 42.4, 50.6, 53.7, 55.9, 68.8, 68.9, 126.8, 127.5, 129.4, 134.1, 135.6, 163.0, 176.4, 199.4. This rather unstable acid was used immediately for subsequent transformations.

Vinylogous β-keto acid **31** (19 mg, 0.028 mmol) was dissolved in 1 mL of TEA and heated at reflux for 30 min. The solvent was stirred in vacuo and the residue purified by silica gel chromatography (hexanes/ether, 10:1) to provide β,γ-unsaturated ketone **32** (15 mg, 86%): ^{1}H NMR (400 MHz) δ 0.678 (s, 3 H, 18-Me), 0.89 (d, 3 H, J = 6.8, 21-Me), 0.92 (d, 3 H, J = 6.8, 27-Me), 1.050 (s, 9 H, Me_3CSi), 2.68 (AB, 2 H, J = 18, 4-H), 2.77 (AB, 2 H, J = 18, 4-H′), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 7.4 (m, 6 H, H_{arom}), 7.6 (m, 4 H, H_{arom}); ^{13}C NMR (100 MHz) δ 12.2, 17.1, 18.6, 19.3, 23.3, 23.7, 25.5, 26.8, 27.4, 28.3, 30.8, 33.5, 33.6, 35.66, 35.69, 35.7, 36.2, 39.0, 39.1, 40.2, 43.0, 44.6, 45.9, 53.2, 55.0, 55.9, 56.3, 68.8, 69.0, 126.1, 127.5, 129.4, 131.3, 134.1, 135.6, 211.7; HRMS calcd for $C_{42}H_{60}O_2Si$ 624.4363, found 624.4322.

(25ξ)-19-Norcholest-5(10)-ene-3,26-dione 3-(Ethylene thioketal) (33). A solution of ketone **32** (37 mg, 0.060 mmol, 1 equiv) in 2.5 mL of dry CH_2Cl_2 in a 5-mL test tube fitted with a rubber septum was cooled to −78 °C under argon. To the stirred solution was added 2 μL of TMSOTf followed by 1,2-bis[(trimethylsilyl)thio]ethane (17 μL, 1.1 equiv) via syringe. After 4.5 h the reaction mixture was warmed to −23 °C and stirred to an additional 30 min. Quenching with dry pyridine followed by workup[34] provided the ethylene thioketal (35 mg, 83%, pure by analytical TLC): ^{1}H NMR (300 MHz) δ 0.673 (s, 3 H, 18-Me), 0.89 (m, 6 H, 21-, 27-Me), 1.044 (s, 9 H, Me_3CSi), 2.435 and 2.559 (AB, 2 H, J = 17.8, 4-H_2), 3.30 (m, 4 H, $H_{dithiolane}$), 3.46 (m, 2 H, 26-H_2), 7.40 (m, 6 H, H_{arom}), 7.66 (m, 4 H, H_{arom}); ^{13}C NMR (75 MHz) δ 12.0, 16.9, 18.4, 18.5, 19.1, 23.1, 23.1, 23.5, 25.4, 26.7, 26.9, 27.0, 28.2, 30.7, 33.4, 33.5, 35.6, 36.0, 36.1, 38.3, 38.6, 38.7, 38.9, 40.2, 42.9, 45.9, 47.1, 55.2, 56.3, 65.7, 68.8, 69.0, 127.4, 127.7, 129.6, 130.1, 134.4, 135.8; HRMS calcd for $C_{44}H_{64}OSiS_2$ 700.4168, found 700.4152.

This compound was dissolved in 2 mL of dry THF and stirred with 150 μL of a 1 M solution of TBAF. After 18 h the usual workup provided (25ξ)-26-hydroxy-19-norcholest-5(10)-en-3-one 3-(ethylene thioketal) (21 mg, 76%): ^{1}H NMR (300 MHz) δ 0.680 (s, 3 H, 18-Me), 0.911 (d, 3 H, J = 6.8, 21-Me), 0.919 (d, 3 H, J = 6.8, 27-Me), 2.373 and 2.621 (AB, 1 H, J = 17.8, 4-H_2), 3.32 (m, 4 H, $H_{dithiolane}$), 3.41 (m, 1 H, 26-H), 3.50 (m, 1 H, 26-H′); ^{13}C NMR (75 MHz) δ 12.0, 16.5, 18.4, 18.5, 23.19, 23.23, 23.5, 25.4, 26.8, 26.9, 28.2, 30.7, 33.3, 33.5, 35.5, 35.6, 36.0, 36.1, 38.2, 38.5, 38.7, 38.9, 40.2, 42.9, 45.9, 47.1, 55.2, 56.2, 65.7, 68.3, 69.5, 127.4, 130.1; MS 462 (M^+, 4), 197 (3), 145 (9), 131 (42), 118 (100).

The 3-(ethylene thioketal) (5.8 mg, 0.012 mmol, 1 equiv) was dissolved in 1 mL of dry CH_2Cl_2 and stirred at room temperature with pyridinium chlorochromate (PCC, 6 mg, 2.3 equiv). After 1 h, dry ether was added and the solids were filtered through a pipet of Florisil to provide, after preparative TLC purification, **33** (3.8 mg, 70%), which gave a positive DNP test: ^{1}H NMR (400 MHz) δ 0.678 (s, 3 H, 18-Me), 0.786 (d, 3 H, J = 6.8, 21-Me), 1.087 (d, 3 H, J = 6.8, 27-Me), 2.434 and 2.560 (AB, 2 H, J = 16.8, 4-H_2), 3.30 (m, 4 H, $H_{dithiolane}$), 9.609 (d, 1 H, J = 1.6, CHO); MS (dec) 279 (12), 167 (53), 149 (100), 132 (3), 113 (11), 55 (10); HRMS calcd for $C_{28}H_{44}OS_2$ 460.2834, found 460.2819.

(25ξ)-26-Hydroxycholest-4-en-3-one (34). To acetate **28** (620 mg, 0.91 mmol, 1 equiv) in 30 mL of dry ether was added LAH (20 mg, 0.65 equiv) with stirring. After 1 h the excess LAH was destroyed with EtOAc, and the mixture was acidified with 1 N HCl. The ether layer was worked up as usual to give an alcohol (^{1}H NMR (400 MHz) δ 0.670 (s, 3 H, 18-Me), 1.047 (s, 3 H, 19-Me), 2.27 (m, 2 H, 4-H_2), 3.43 (m, 1 H, 26-H), 3.49 (m, 1 H, 26-H′), 3.51 (m, 1 H, 3-H), 5.35 (m, 1 H,

6-H)), which was dissolved in 20 mL of CH_2Cl_2 and stirred with 290 mg of PCC (1.5 equiv) for 6 h. Addition of ether and filtration of the solids through Florisil provided a crude Δ^5-3-ketone (410 mg), which was immediately dissolved in 20 mL of dry ether and cooled to 0 °C. Anhydrous ethereal HCl (0.5 mL, 0.8 N) was added dropwise and the reaction was stirred at 0 °C for 45 min. Quenching with 5% $NaHCO_3$ and drying of the ether layer over $MgSO_4$ provided 400 mg of the Δ^4-3-ketone as an oil: ^{1}H NMR (200 MHz) δ 0.697 (s, 3 H, 18-Me), 0.90 (m, 6 H, 21-, 27-Me), 1.046 (s, 9 H, Me_3CSi), 1.176 (s, 3 H, 19-Me), 3.46 (m, 2 H, 26-H_2), 5.722 (s, 1 H, 4-H), 7.38 (m, 6 H, H_{arom}), 7.65 (m, 4 H, H_{arom}).

The enone (400 mg, 0.63) was dissolved in 10 mL of THF and stirred at room temperature with 1.22 mL of a 1 M solution of TBAF for 22 h. Acidic workup provided 420 mg of a yellow oil, which was chromatographed on silica gel (hexanes/ether, 2:1) to give (25ξ)-26-hydroxycholest-4-en-3-one (**34**, 250 mg, 70% from **28**) whose NMR and mass spectra matched those of the known 25(*R*) epimer:[38] ^{1}H NMR (400 MHz) δ 0.691 (s, 3 H, 18-Me), 0.897 and 0.899 (d, 3 H, J = 6.8, diastereomeric 21-Me), 0.903 and 0.907 (d, 3 H, J = 6.8, diastereomeric 27-Me), 1.165 (s, 3 H, 19-Me), 3.40 (m, 1 H, 26-H), 3.48 (m, 1 H, 26-H′), 5.709 (s, 1 H, 4-H); MS 400 (M^+, 29), 385 (3), 382 (2), 358 (6), 277 (12), 271 (5), 229 (18), 211 (7), 124 (100).

(25ξ)-Cholest-4-ene-3,26-dione 3-(Ethylene thioketal) (35). To enone **34** (13 mg, 0.325 mmol, 1 equiv) in 2 mL of dry $CHCl_3$ was added 1 mg of anhydrous ZnI_2 under argon. 1,2-Bis[(trimethylsilyl)thio]ethane (100 μL, 95 mmol, 285 equiv) was added via syringe, and the reaction was stirred under argon at room temperature for 38 h. An additional 1 mg of ZnI_2 was added and stirring continued for a total of 72 h. The reaction mixture was diluted with CH_2Cl_2 and worked up[33] to provide (25ξ)-26-hydroxycholest-4-en-3-one (3-(ethylene thioketal), which was purified by preparative TLC to give the pure thioketal (12.3 mg, 80%): ^{1}H NMR (400 MHz) δ 0.662 (s, 3 H, 18-Me), 0.89 (d, 3 H, J = 7.0), 0.91 (d, 3 H, J = 6.4), 1.006 (s, 3 H, 19-Me), 3.22 (m, 1 H, $H_{dithiolane}$), 3.36 (m, 3 H, $H_{dithiolane}$), 3.41 (m, 1 H, 26-H), 3.50 (m, 1 H, 26-H′), 5.469 (s, 1 H, 4-H); ^{13}C NMR (100 MHz) δ 11.9, 16.5, 16.7, 18.5, 18.6, 21.2, 23.38, 23.44, 24.2, 28.2, 32.1, 32.7, 33.5, 33.6, 35.7, 35.8, 36.1, 36.2, 36.6, 37.3, 38.1, 39.5, 39.8, 40.0, 42.4, 54.1, 55.9, 56.1, 65.9, 68.3, 68.5, 123.8, 146.8; MS 384 ($M^+ - C_2H_4S_2$, 35), 369 (5), 351 (6), 300 (34), 271 (20), 255 (28), 55 (100); HRMS calcd for $C_{29}H_{48}OS_2$ 476.3147, found 476.3139.

(25ξ)-26-Hydroxycholest-4-en-3-one 3-(ethylene thioketal) (11.3 mg, 0.0237 mmol, 1 equiv) was dissolved in 0.5 mL of dry CH_2Cl_2 and stirred with PCC (7.6 mg, 0.0356 mmol, 1.5 equiv) at room temperature for 80 min. Workup as described above provided 9.0 mg of aldehyde **35** (80%): ^{1}H NMR (400 MHz) δ 0.661 (s, 3 H, 18-Me), 0.891 (d, 3 H, J = 6.8, 21-Me), 1.005 (s, 3 H, 19-Me), 1.085 (d, 3 H, J = 6.8, 27-Me), 3.22 (m, 1 H, $H_{dithiolane}$), 3.36 (m, 3 H, $H_{dithiolane}$), 5.470 (s, 1 H, 4-H), 9.607 (d, 1 H, J = 2.0, CHO); ^{13}C NMR (75 MHz) δ 11.7, 18.3, 18.4, 21.1, 23.3, 24.0, 28.0, 30.9, 32.0, 32.5, 35.47, 35.50, 35.64, 35.69, 35.8, 36.5, 37.1, 37.9, 39.4, 39.7, 39.8, 42.4, 46.3, 54.0, 55.91, 55.97, 65.8, 124.1, 147.0, 205.9; MS 474 (M^+, 2), 446 (2), 414 (2), 319 (2), 287 (4), 211 (11), 207 (20), 105 (100); HRMS calcd for $C_{29}H_{46}OS_2$ 474.2990, found 474.2980.

Labeling Protocol A (Scheme III). To a solution of the aldehyde (**33**, or **35**, 2 mg, 0.004 mmol) in 0.5 mL of ethanol was added 10 mCi of NaB^3H_4 (ICN, 1.4 Ci/mmol). The reaction was magnetically stirred for 13 h, after which time wet ether was added and the solvents were stripped with a stream of N_2. The residue was taken up in ether and filtered through a plug of silica. After removal of the ether (N_2), the [26-^{3}H]-26-hydroxy thioketal was taken up in 0.5 mL of dry pyridine and stirred with 5 mg (6 equiv) of toluenesulfonyl chloride and a small crystal of DMAP. After 22 h, the solvent was blown down (N_2) and the residue chromatographed on a pipet of silica gel (hexanes/ether, 3:1) to give the tosylate (analytical TLC R_f 0.58), which was dried overnight in a vacuum dessicator.

To the dried tosylate in a 1-mL Reacti-vial was added 0.4 mL of DMSO followed by 5 mg of $NaBH_4$. The sealed vials were heated at 85 °C for 3 h, after which time a single product was observed by analytical TLC. Cold water was added to the cooled reaction mixtures, and the 26-^{3}H-labeled thioketal was extracted repeatedly with hexane. The combined organic layers were washed with brine and dried over $MgSO_4$.

Addition of [bis(trifluoroacetoxy)iodo]benzene[36] (5 mg, 3 equiv) to the thioketal in 0.7 mL of 10% aqueous ethanol was followed by stirring at room temperature for 10 min, after which time the reaction mixture was pipeted into a saturated $NaHCO_3$ solution and extracted with ether to afford the desired [26-^{3}H]-3-keto steroid (**19a** or **22a**), which was purified by chromatography on a pipet of silica gel (hexanes/ether, 5:1 to **22a** and 6:1 for **19a**).

[26-^{3}H]Cholest-4-en-3-one (22a). Aldehyde **35** was subjected to labeling protocol A to give 300 μCi of crystalline **22a** (3% radiochemical yield), which tested positive on treatment with DNP. The ^{1}H NMR and mass spectra of the unlabeled analogue were identical to those of authentic cholest-4-en-3-one.

[26-^{3}H]-19-Norcholest-5(10)-en-3-one (19a). Aldehyde **33** was subjected to labeling protocol A to provide β,γ-unsaturated ketone **19a** (1045 μCi, 10% radiochemical yield), which tested positive upon treatment with DNP. The ^{1}H NMR and mass spectra of the unlabeled analogue were identical to those of the authentic material:[65] ^{1}H NMR (400 MHz) δ 0.680 (s, 3 H, 18-Me), 0.859 (d, 3 H, J = 6.4, 26-Me), 0.863 (d, 3 H, J = 6.8, 27-Me), 0.908 (d, 3 H, J = 6.4, 21-Me), 2.69 and 2.76 (AB, 2 H, J = 18, 4-H_2); MS 370 (M^+, 47), 355 (15), 257 (34), 247 (13), 215 (58), 213 (20), 55 (100).

[26-^{3}H]-19-Norcholest-4-en-3-one (20a). β,γ-Unsaturated ketone **19a** (59 μCi) was dissolved in 0.5 mL of 0.005 M methanolic toluenesulfonic acid and heated at reflux for 15 min.[28b] The cooled reaction mixture was washed with saturated $NaHCO_3$ and dried over $MgSO_4$ to provide, after chromatography on a pipet of silica gel (hexanes/ether 3:1), α,β-unsaturated ketone **10a** (54 μCi, 92%). The ^{1}H NMR and mass spectra of the unlabeled compound were identical to those of the authentic material:[28b,65,66] ^{1}H NMR (400 MHz) δ 0.716 (s, 3 H, 18-Me), 0.858 (d, 3 H, J = 6.6, 26-Me), 0.863 (d, 3 H, J = 6.6, 27-Me), 0.909 (d, 3 H, J = 6.5, 21-Me), 5.818 (s, 1 H, 4-H); MS 370 (M^+, 54), 231 (19), 215 (40), 197 (11), 187 (13), 173 (19), 163 (11), 55 (100).

[26-^{3}H]-19-Nor-5α-cholestan-3-one (21a). Enone **20a** (130 μCi) was mixed with 0.6 mg of unlabeled 19-norcholest-4-en-3-one,[65] dissolved in 0.5 mL of anhydrous THF, and added dropwise to a stirred solution of Li wire (5 mg) in 1 mL of redistilled liquid ammonia at −78 °C under argon. After 20 min, the reaction mixture was quenched with a saturated solution of NH_4Cl, and the ammonia was allowed to evaporate at room temperature. The product was extracted with ether and dried over $MgSO_4$. The crude product was taken up in 1.0 mL of freshly distilled CH_2Cl_2 and stirred with 10 mg of PCC for 2 h. Addition of ether and filtration of solids provided a crude oil, which was chromatographed on a pipet of silica gel (hexanes/ether, 5:1) to provide pure ketone **21a** (R_f = 0.52, 71 μCi, 55% yield), which gave a positive DNP test. The ^{1}H NMR and mass spectra of the unlabeled analogue (**21**) were identical to those of the ketone resulting from PCC oxidation of 19-nor-5α-cholestan-3β-ol isolated from *Axinella polypoides*: ^{1}H NMR (400 MHz) δ 0.686 (s, 3 H, 18-Me), 0.859 (d, 3 H, J = 6.6, 26-Me), 0.863 (d, 3 H, 27-Me), 0.904 (d, 3 H, J = 6.6, 21-Me), 2.083 (t, 1 H, J = 13.5); MS 372 (M^+, 19), 232 (6), 217 (100), 203 (11), 199 (8), 162 (31); HRMS calcd for $C_{26}H_{44}O$ 372.3392, found 372.3395.

[4-^{3}H]-19-Hydroxycholest-4-en-3-one (23a). 19-Hydroxycholesterol[62] (**9**, 173 mg, 0.43 mmol) was dissolved in 3 mL of toluene and added to an 80 °C solution of SeO_2 (81 mg) in 3 mL of glacial acetic acid containing 0.5 mL of H_2O.[41] The reaction mixture was stirred at reflux for 1.5 h. NaOAc (350 mg) was added and the reaction mixture was warmed at 80 °C for 5 min. Filtration of the cooled solution through Celite and dilution with ether was followed by washing with a saturated $NaHCO_3$ solution until evolution of CO_2 ceased. Silica gel chromatography of the resulting crude oil (hexanes/ethyl acetate, 1:1) resulted in cholest-5-ene-3β,4β,19-triol (**36**) as a white solid (76 mg, 42%): mp 182–183 °C; ^{1}H NMR (400 MHz) δ 0.694 (s, 3 H, 18-Me), 0.853 (d, 3 H, J = 6.6, 26-Me), 0.858 (d, 3 H, J = 6.6, 27-Me), 0.899 (d, 3 H, J = 6.5, 21-Me), 3.61 (m, 1 H, 3-H), 3.695 and 3.815 (AB, 2 H, J = 10.8, 19-H_2), 4.122 (dd, 1 H, J = 3.4, 1.1, 4-H), 5.952 (dd, 1 H, J = 4.7, 2.6, 6-H); ^{13}C NMR (100 MHz) δ 12.0, 18.7, 21.1, 22.5, 22.8, 23.8, 24.1, 26.2, 28.0, 28.2, 31.9, 32.5, 33.9, 35.7, 36.1, 39.5, 39.8, 40.7, 42.3, 50.4, 56.0, 57.6, 66.1, 71.9, 76.5, 133.1, 137.9; HRMS calcd for $C_{27}H_{44}O_2$ ($M^+ - H_2O$) 400.3341, found 400.3349.

Triol **36** (70 mg, 0.17 mmol, 1 equiv) was dissolved in 1 mL of toluene and refluxed with carbonyldiimidazole[42] (135 mg, 0.83 mmol, 5 equiv) for 12 h. Washing with 0.5 N HCl, 5% $NaHCO_3$, and brine followed by drying over $MgSO_4$ provided cholest-5-ene-3β,4β,19-triol 3,4-cyclic carbonate 19-imidazole carbamate (72 mg, 83%): ^{1}H NMR (400 MHz) δ 0.629 (s, 3 H, 18-Me), 0.849 (d, 3 H, J = 6.8, 26-Me), 0.854 (d, 3 H, J = 6.4, 27-Me), 0.884 (d, 3 H, J = 6.4, 21-Me), 4.487 and 4.563 (AB, 2 H, J = 11.6, 19-H_2), 4.83 (m, 1 H, 3-H), 5.061 (d, 1 H, J = 7.8, 4-H), 6.18 (m, 1 H, 6-H), 7.08 (s, 1 H, $H_{imidazole}$), 7.39 (s, 1 H, $H_{imidazole}$), 8.08 (s, 1 H, $H_{imidazole}$); ^{13}C NMR (100 MHz) δ 12.0, 18.6, 21.9, 22.5, 22.8, 23.8, 23.9, 24.2, 24.3, 28.0, 28.1, 31.1, 32.1, 35.6, 36.1, 39.4, 39.6, 39.7, 42.5, 46.6, 56.0, 57.3, 69.6, 76.0, 81.8, 97.7, 117.1, 129.6, 130.8, 136.9, 138.3, 148.1.

The imidazole carbamate (60 mg, 0.12 mmol) was dissolved in 10 mL of 1% aqueous acetone and stirred at room temperature with a small crystal of KOH. After 2.5 h at room temperature, the reaction mixture was pipeted into cold H_2O and acidified with 1 N HCl. Extraction with

(65) Dannenberg, H.; Neumann, H.-G.; Dannenberg-von Dresler, D. *Liebigs Ann. Chem.* **1964**, *674*, 152–167. The 19-norcholest-4-en-3-one produced by this procedure has the same mass spectrum as that of the material reported by Popov et al., ref 66.

(66) Popov, S.; Carlson, R. M. K.; Djerassi, C. *Steroids* **1983**, *41*, 537–548.

ether followed by washing with 5% $NaHCO_3$ and drying over $MgSO_4$ gave a crude product that was purified by chromatography on silica gel (hexanes/ether, gradient 5:1 to 2:1) to afford cholest-5-ene-3β,4β,19-triol 3,4-cyclic carbonate (47 mg, 88%): ^{1}H NMR (300 MHz) δ 0.738 (s, 3 H, 18-Me), 0.853 (d, 3 H, J = 6.6, 26-, 27-Me), 3.685 and 3.723 (AB, 2 H, J = 11.9, 19-H_2), 4.79 (m, 1 H, 3-H), 5.023 (d, 1 H, J = 7.7, 4-H), 6.19 (m, 1 H, 6-H); HRMS calcd for $C_{28}H_{44}O_4$ 444.3240, found 444.3255.

The 3,4-cyclic carbonate (40 mg, 0.09 mmol) was dissolved in 2 mL of anhydrous $CHCl_3$ and refluxed for 10 h with 100 μL of *N,O*-bis(trimethylsilyl)acetamide. The reaction mixture was washed with a 5% solution of $NaHCO_3$ and then brine and dried over $MgSO_4$. Purification on two columns of silica gel (hexanes/ether, 5:1), which was necessary to remove all traces of acetamide, provided 19-[(trimethylsilyl)oxy]-cholest-5-ene-3β,4β-diol cyclic carbonate (**37**) as a white semicrystalline solid (25 mg, 55%): mp 94–95 °C; ^{1}H NMR (200 MHz) δ 0.072 (s, 9 H, Me_3Si), 0.719 (s, 3 H, 18-Me), 0.855 (d, 6 H, 26-, 27-Me), 0.907 (d, 3 H, J = 6.4, 21-Me), 3.461 and 3.513 (AB, 2 H, J = 10.7, 19-H_2), 4.75 (m, 1 H, 3-H), 5.485 (d, 1 H, J = 7.6, 4-H), 6.06 (m, 1 H, 6-H); ^{13}C NMR (50 MHz) δ −0.9, 11.8, 18.5, 21.3, 22.4, 22.6, 23.6, 23.9, 24.3, 24.7, 27.8, 28.1, 31.3, 31.8, 35.6, 36.0, 39.4, 39.9, 40.8, 42.5, 47.5, 55.9, 57.4, 64.9, 76.5, 82.7, 131.3, 137.7 (C=O not observed).

19-[(Trimethylsilyl)oxy]cholest-5-ene-3β,4β-diol 3,4-cyclic carbonate (**37**, 5 mg, 0.01 mmol) was reduced with NaB^3H_4 (NEN, 0.6 Ci/mmol, 0.2 mg), triphenylphosphine, and $(Ph_3P)_4Pd$ at 80 °C as described[40] to give, after purification on a pipet of silica gel (hexanes/ether, 5:1), a mixture of [4α-^{3}H]-19-[(trimethylsilyl)oxy]cholest-5-en-3β-ol (**38a**) and [6α-^{3}H]-19-[(trimethylsilyl)oxy]cholest-4-en-3β-ol (**39a**), which were dried overnight in a vacuum dessicator. Approximately 80% of this mixture was stirred with 5 mg of PCC and 10 mg of NaOAc in 1 mL of CH_2Cl_2 for 2 h. Ether was added and the solids filtered through Florisil. The resulting Δ^5-3-ketone (dried for 2 h in a vacuum dessicator) was dissolved in anhydrous ether containing 100 μL of 0.8 N ethereal HCl. The usual workup resulted in a crude product, which was purified on a pipet of silica gel (hexanes/ether, 3:1) to give **23a**, tritiated at C-4 and nominally at C-6 (370 μCi, 15% radiochemical yield). The [4α-^{2}H] analogue, **38**, was produced by the same route using NaB^2H_4 and purified by preparative TLC: ^{1}H NMR (400 MHz) δ 0.072 (s, 9 H, Me_3Si), 0.699 (s, 3 H, 18-Me), 0.857 (d, 3 H, J = 6.6, 26-Me), 0.861 (d, 3 H, J = 6.6, 27-Me), 0.909 (d, 3 H, J = 6.5, 21-Me), 2.19 (m, 1.10 H, 4-H_2), 3.521 (d, 1 H, J = 10.6, 19-H), 3.55 (m, 1 H, 3-H), 3.761 (d, 1 H, J = 10.6, 19-H′), 5.58 (m, 1 H, 6-H); HRMS calcd for $C_{30}H_{51}{}^2HOSi$ (M^+ − H_2O) 457.3850, found 457.3849.

The remaining 20% of the mixture of **38a** and **39a** (170 μCi) was dissolved in 2.0 mL of ether, and a 10-μL aliquot was removed and mixed with 3 mg of an unlabeled mixture of **38** and **39**. This mixture (specific activity = 277 μCi/mmol) was oxidized with PCC and epimerized with ethereal HCl as described in the synthesis of **34** to give, after silica gel chromatography, [4-^{3}H]-19-hydroxycholest-4-en-3-one (**23a**, specific activity = 263 μCi/mmol). The [4-^{2}H] analogue, **23**, was synthesized in the same way starting from **38**. Compound **23**: mp 140–141 °C; HRMS calcd for $C_{27}H_{43}{}^2HO_2$ 401.3404, found 401.3385. The 400 MHz ^{1}H NMR spectrum of **40a** was the same as that of 19-hydroxycholest-4-en-3-one,[65] except δ 5.953 (s, 0.28 H, 4-H).

[26-^{3}H]Brassicasterol (40a). C-25 epimeric alcohols[48a] **46** (106 mg, 0.248 mmol) were dissolved in 2 mL of dry pyridine and allowed to stand in the dark overnight with 140 mg (3 equiv) of tosyl chloride. After acidification and the usual workup, the crude diastereomeric tosylates were purified by column chromatography on silica gel (hexanes/ether, 9:1) to afford the C-25 epimeric tosylates **47** as a clear oil, which solidified upon standing (110 mg, 76%): ^{1}H NMR (400 MHz) δ 0.695 (t, 3 H, 18-Me), 0.779 (d, 3 H, J = 7.0, 26-Me), 0.899 (d, 3 H, J = 4.8), 0.916 (d, 3 H, J = 4.3), 1.017 (s, 3 H, 19-Me), 2.15 (m, 1 H, 24-H), 2.447 (s, 3 H, MeAr), 2.768 (m, 1 H, 6-H), 3.322 (s, 3 H, MeO), 3.78 (m, 1 H, 26-H), 3.868 (dd, 0.75 H, J = 9.2, 6.6, 26-H), 3.96 (dd, 0.25 H, J = 9.2, 6.6, 27-H), 5.06 (m, 2 H, 22-, 23-H); ^{13}C NMR (100 MHz) δ 12.3, 12.4, 13.1, 18.2, 19.3, 20.8, 21.4, 21.7, 22.7, 24.1, 24.9, 28.6, 30.5, 33.3, 35.1, 35.2, 37.2, 37.7, 40.1, 42.7, 43.4, 48.0, 55.8, 56.6, 73.8, 82.3, 127.9, 128.4, 129.8, 130.4, 133.1, 137.1; HRMS calcd for $C_{36}H_{54}O_4S$ 582.3743, found 582.3722.

Epimeric tosylates **47** (0.7 mg, 1.2 μmol) were placed in a 2.0-mL Reactivial and dissolved in 1.5 mL of dry DMSO. A small crystal of NaB^3H_4 (0.2 mg, 1CN, 800 μCi, 1.6 Ci/mmol) was added with a spatula. The reaction vial was flushed with argon, tightly sealed, and heated at 80 °C for 5 h. Partial reduction was observed by analytical TLC. Sodium borohydride (1 mg, excess) was added to complete the reduction, and the reaction was continued for an additional hour at which time all starting material appeared to be consumed by TLC. The solvent was stripped with a stream of N_2 at 80 °C, and the residue was taken up in 1 mL of 10% aqueous dioxane and 25 μL of 72% $HClO_4$, sealed, and heated at 80 °C for 1 h. The acid was neutralized with a few drops of saturated $NaHCO_3$ and the solvent blown down with N_2. The residue, which appeared pure by TLC, was purified on a pipet column of silica gel (hexanes/ether, 9:1) to provide 36 μCi of crystalline **40a** (5% radiochemical yield), mp 147–148 °C (lit[48a] mp 145–147 °C); the 400-MHz ^{1}H NMR values were identical to those reported.[48] The spectrum itself was superimposable upon that of the authentic natural product (laboratory files).

[26-^{3}H]Crinosterol (41a). C-25 epimeric alcohols **48**[48a] (73 mg) were treated with 3 equiv of tosyl chloride as described above to give the epimeric tosylates **49** (89 mg, 90%): ^{1}H NMR (400 MHz) δ 0.691 (s, 3 H, 18-Me), 0.774 (d, 3 H, J = 7.2, 28-Me), 0.907 (d, 3 H, J = 6.8, 27-Me), 0.931 (d, 3 H, J = 6.4, 21-Me), 1.022 (s, 3 H, 19-Me), 2.458 (s, 3 H, MeAr), 2.76 (m, 1 H, 6-H), 3.332 (s, 3 H, MeO), 3.81 (m, 2 H, 26-H_2), 5.00 (m, 2 H, 22-, 23-H); ^{13}C NMR (100 MHz) δ 12.1, 12.4, 13.1, 18.6, 19.3, 20.7, 21.4, 21.7, 22.7, 24.2, 24.9, 29.0, 30.5, 33.3, 35.1, 37.3, 37.8, 40.2, 40.4, 42.6, 43.4, 48.0, 55.7, 56.6, 74.0, 82.3, 127.9, 128.4, 129.4, 129.8, 137.3, 138.2, 144.5; HRMS calcd for $C_{36}H_{54}O_4S$ 582.3743, found 582.3711.

Epimeric tosylates **49** (0.2 mg) were tritiated with 0.2 mg of NaB^3H_4 and converted to [26-^{3}H]crinosterol (**41a**) as described above to give 73 μCi of **41a** (10% radiochemical yield after chromatography), mp 153–154 °C (lit[48a] mp 155–157 °C). The ^{1}H NMR (400 MHz) values were identical to the reported values.[48] The spectrum itself was identical to that of the natural product (laboratory files).

[6-^{3}H]-20-Isocholesterol (42b). The mixture of olefins **50**[49b] (390 mg, 0.91 mmol) was taken up in 7 mL of 1% methanolic KOH and refluxed for 1 h. Acidification of the reaction mixture followed by extraction with ether and the usual workup afforded 353 mg of free alcohols (91%), which were converted to their i-acetates (**51**) according to literature procedure.[50,55] The resulting olefins **51** (330 mg, 85%, MS 366 (M^+ − CH_3COOH, 36), 351 (22), 253 (44), 213 (48), 199 (39), 159 (57), 145 (97), 91 (100)) were immediately dissolved in 10 mL of hexane and hydrogenated at atmospheric pressure over 5% palladium on carbon for 48 h. Filtration of the catalyst through Celite and concentration in vacuo afforded 210 mg of a 1:1 mixture of 20-isocholesterol i-acetate and cholesterol i-acetate. Reverse-phase HPLC purification (methanol) of 30 mg of this mixture resulted in 3α,5-cyclo-5α-cholestan-6β-yl acetate[67] (11.9 mg, 0.027 mmol, 22% from **51**) and (20*S*)-20-iso-3α,5-cyclo-5α-cholestan-6β-yl acetate (12.1 mg, 0.028 mmol, 22% from **51**) as a clear oil: ^{1}H NMR (400 MHz) δ 0.719 (s, 3 H, 18-Me), 0.814 (d, 3 H, J = 6.4, 21-Me), 0.866 (d, 6 H, J = 6.4, 26-, 27-Me), 1.003 (s, 3 H, 19-Me), 2.048 (s, 3 H, CH_3COO), 4.50 (m, 1 H, 6-H); ^{13}C NMR (100 MHz) δ 12.1, 12.4, 18.7, 19.3, 21.6, 22.6, 22.7, 23.9, 24.12, 24.16, 25.0, 28.1, 30.6, 33.1, 35.2, 35.3, 35.6, 36.3, 39.4, 40.0, 42.7, 42.9, 47.5, 55.8, 56.3, 76.2, 170.9 (2 carbons unresolved); MS 428 (M^+, 0.2), 371 (4), 368 (46), 353 (9), 331 (1), 260 (17), 255 (21), 147 (100).

(20*S*)-20-Iso-3α,5-cyclo-5α-cholestan-6β-yl acetate (6 mg, 0.014 mmol) was dissolved in 0.5 mL of THF and stirred at room temperature with LAH (1 mg, 2 equiv) to give, after the usual workup, 5 mg of an alcohol (HRMS calcd for $C_{27}H_{46}O$ 386.3549, found 386.3535), which was dissolved in 5 mL of acetone, titrated at 0 °C with 8 N Jones' reagent, and allowed to stir for 1 h. The reaction was pipeted into a cold, saturated $NaHCO_3$ solution and extracted with ether to give, after drying over $MgSO_4$ and purification by preparative thin-layer chromatography, (20*S*)-20-iso-3α,5-cyclo-5α-cholestan-6-one (**52**, 5 mg, 93% from the acetate): ^{1}H NMR (300 MHz) δ 0.708 (s, 3 H, 18-Me), 0.825 (d, 3 H, J = 6.4, 21-Me), 0.871 (d, 6 H, J = 6.6, 26-, 27-Me), 1.002 (s, 3 H, 19-Me), 2.43 (m, 1 H, CHC=O); MS 384 (M^+, 66), 369 (27), 366 (23), 356 (17), 355 (17), 243 (14), 229 (20), 207 (28), 161 (36), 136 (100); ^{13}C NMR (75 MHz) δ 11.4, 12.0, 18.4, 19.4, 22.4, 22.5, 22.70, 22.71, 23.8, 25.7, 27.8, 27.9, 33.3, 34.6, 35.0, 35.1, 35.5, 39.3, 39.5, 42.6, 44.7, 46.0, 46.2, 46.7, 55.6, 57.0, 210.2; HRMS calcd for $C_{27}H_{44}O$ 384.3392, found 384.3382.

(20*S*)-20-Iso-3α,5-cyclo-5α-cholestan-6-one (**52**, 1.7 mg, 0.0044 mmol) was dissolved in 1 mL of 2-propanol and stirred at room temperature with NaB^3H_4 (0.2 mg, ICN, 700 μCi, 1.4 Ci/mmol). After 16 h analytical TLC showed no starting material. The solvent was stripped with a stream of nitrogen, and the residue was taken up in wet ether and filtered through a plug of silica. The ether was stripped, and the residue was taken up in 1 mL of 10% aqueous dioxane and heated for 1.5 h at 80 °C with 50 μL of 72% $HClO_4$ in a sealed high-pressure vial. Solid $NaHCO_3$ (1 mg) was added, and the solvents were stripped. The crude sterol was taken up in ether, washed with 5% $NaHCO_3$, and then dried over $MgSO_4$. Chromatography on a pipet of silica gel gave [6-^{3}H]-(20*S*)-20-isocholesterol (**42b**) (909 μCi, 12% radiochemical yield). Unlabeled (20*S*)-20-isocholesterol was made by the identical procedure

(67) Wallis, E. S.; Fernholz, E.; Gephart, F. T. *J. Am. Chem. Soc.* **1937**, *59*, 137–140.

using NaB^1H_4, mp 153–154 °C (lit[49a,68] mp 153–154 °C); ^1H NMR and mass spectral data were identical to those reported.[49a,68]

(20*S*)-20-Iso-5α-cholestan-3β-ol. The mixture of olefins **50**[49b] (95 mg, 0.22 mmol) was dissolved in 8 mL of hexanes and hydrogenated at atmospheric pressure over 5% palladium on carbon for 30 h to give 75 mg of an amorphous white solid consisting of a 1:1 mixture of 5α-cholestan-3β-yl acetate and (20*S*)-20-iso-5α-cholestan-3β-yl acetate. From this mixture 50 mg (0.12 mmol) was immediately dissolved in dry ether and reduced with 10 mg of LAH (2 equiv) to provide, after acidification and the usual workup, 45 mg of the free 3β-alcohols. A 20-mg sample of this mixture was purified by reverse-phase HPLC (methanol) to give 9 mg of 5α-cholestan-3β-ol (0.023 mmol, 35% from **50**) and (20*S*)-20-iso-5α-cholestan-3β-ol (9.1 mg, 0.023 mmol, 35% from **50**): mp 160 °C (lit mp 160–162 °C,[51a] 160–161 °C[51b]); ^1H NMR (400 MHz) δ 0.640 (s, 3 H, 18-Me), 0.798 (s, 3 H, 19-Me), 0.804 (d, 3 H, J = 5.0, 21-Me), 0.860 (d, 6 H, J = 6.7, 26-, 27-Me), 1.930 (dt, 1 H, J = 12.3, 3.3), 3.58 (m, 1 H, 3-H); MS 388 (M^+, 15), 373 (5), 262 (7), 248 (6), 233 (72), 219 (11), 215 (100).

[21-^{14}C]-20-Methylpregna-5,20-dien-3β-ol (53a). To 5 mg of (0.012 mmol) $^{14}CH_3PPh_3{}^+I^-$ (700 μCi, 58 mCi/mmol prepared[53] from $^{14}CH_3I$ (ICN)) in 1 mL of anhydrous THF in a 2.0-mL Reacti-vial fitted with a Teflon screw-cap and a resealable inlet for a syringe needle was added 8.5 μL of a 1.6 M solution of *n*-butyllithium (1.1 equiv) via syringe. The yellow solution was stirred for 15 min under argon. Pregnenolone 2′-tetrahydropyranyl ether[52a] (14 mg, 0.036 mmol, 3 equiv) in 0.5 mL of THF was added via syringe to the stirred ylide solution. After 16 h under argon, the reaction was quenched by the cautious addition of H_2O. The THF was evaporated with a stream of N_2, and the residue was taken up in 2 mL of 10% aqueous dioxane and heated at 90 °C with 1 mg of toluenesulfonic acid for 1 h. Saturated $NaHCO_3$ was added to the cooled reaction vial, and the crude sterol was extracted with hexanes and dried over $MgSO_4$. Chromatography on a pipet of silica gel (hexanes/ether, 4:1) gave sterol **53a**[52] (252 μCi, 36% radiochemical yield): ^1H NMR (400 MHz) δ 0.586 (s, 3 H, 18-Me), 1.012 (s, 3 H, 19-Me), 1.761 (s, 3 H, 21-Me), 3.53 (m, 1 H, 3-H), 4.710 (bs, 1 H, C=CH_2), 4.850 (bs, 1 H, C=CH_2), 5.35 (m, 1 H, 6-H).

[23-^{14}C]-24-Norchola-5,22-dien-3β-ol (54a). Aldehyde **60**[54] (8 mg, 0.018 mmol, 2 equiv) was treated with $^{14}CH_2PPh_3$ (0.014 mmol) as described for the synthesis of **53a** to provide, after acid hydrolysis and silica gel chromatography, compound **54a**[69] (233 μCi, 27% radiochemical yield): ^1H NMR (400 MHz) δ 0.705 (s, 3 H, 18-Me), 1.010 (s, 3 H, 19-Me), 1.035 (d, 3 H, J = 6.8, 21-Me), 2.28 (m, 1 H, 20-H), 3.50 (n, 1 H, 3-H), 4.813 (dd, 1 H, J = 10, 2.2, *cis*-C=CH_2), 4.900 (dd, 1 H, J = 16, 2.0, *trans*-C=CH_2), 5.35 (m, 1 H, 6-H), 5.664 (ddd, 1 H, J = 17, 10, 8.4, 22-H_{vinyl}); MS 310 ($M^+ - CH_3COOH$, 100), 255 (17), 202 (8), 189 (16), 159 (16), 147 (17), 105 (28), 91 (29), 81 (37).

[6-^{3}H]-(22*E*)-Chola-5,22-dien-3β-ol (55b). Following published procedures,[56] aldehyde **61**[55] (200 mg, 0.55 mmol, 1 equiv) was converted with ethylidenetriphenylphosphorane to a mixture of olefins. The crude product mixture was chromatographed on silica gel (hexanes/ether, 15:1), providing 150 mg of a mixture of cis and trans alkenes in a ratio of 10:1 as determined by HPLC analysis. Reverse-phase HPLC purification (methanol) of 120 mg of this mixture resulted in the recovery of 10 mg of the trans olefin: ^1H NMR (400 MHz) δ 0.735 (s, 3 H, 19-Me), 0.997 (d, 3 H, 21-Me), 1.008 (s, 3 H, 19-Me), 1.611 (d, 1 H, J = 5.1, 24-Me), 2.046 (s, 3 H, CH_3COO), 4.50 (m, 1 H, 6-H), 5.29 (m, 2 H, 22-, 23-H); HRMS calcd for $C_{26}H_{40}O_2$ 384.3028, found 384.3013.

The trans olefin (9.2 mg, 0.024 mmol, 1 equiv) was reduced with LAH (2 mg, 0.048 mmol, 2 equiv) in 3 mL of dry ether. The usual workup provided an alcohol that was immediately dissolved in 5-mL of acetone and titrated with 8 N Jones' reagent at 0 °C. Destruction of excess oxidant with methanol followed by the usual aqueous workup and chromatography on a pipet of silica gel (hexanes/ether, 10:1) resulted in 7.4 mg of (22*E*)-3α,5-cyclo-5α-chola-5,22-dien-6-one (**62**) as a colorless oil (91% from the i-acetate): ^1H NMR (200 MHz) δ 0.762 (s, 3 H, 18-Me), 0.954 (d, 3 H, J = 6.6, 21-Me), 1.608 (d, 3 H, J = 5.2, 24-Me), 5.25 (m, 2 H, 22-, 23-H); MS 340 (M^+, 100), 298 (44), 271 (24), 269 (16), 207 (10), 161 (26), 138 (42), 135 (40), 123 (50), 121 (64).

Reduction of this ketone (3.7 mg, 4 equiv) with NaB^3H_4 (ICN, 0.1 mg, 1.6 Ci/mmol) as described for the synthesis of [6-^{3}H]-20-isocholesterol was followed by the addition of NaB^1H_4 to complete the reduction and then hydrolysis. Purification by column chromatography (hexanes/ether, 6:1) resulted in **55b**[56] (284 μCi, 71% radiochemical yield): ^1H NMR (400 MHz) δ 0.684 (s, 3 H, 18-Me), 0.995 (d, 3 H, J = 7.5, 21-Me), 1.005 (s, 3 H, 19-Me), 1.609 (d, 3 H, J = 5.2, 24-Me), 3.53 (m, 1 H, 3-H), 5.28 (m, 2 H, 22-, 23-H), 5.35 (m, 1 H, 6-H); MS 342 (M^+, 27), 327 (5), 324 (6), 309 (5), 300 (17), 273 (7), 255 (19), 69 (100).

20-Methyl-5α-pregn-20-en-3β-yl Acetate (64). Compound **64** was prepared by treating ketone **63**[57] (21 mg, 0.058 mmol) with methylenetriphenylphosphorane using the procedure of Dusza and Bergmann.[52a] Purification by silica gel chromatography (hexanes/ether, 10:1) afforded **64** (15 mg, 71%): ^1H NMR (400 MHz) δ 0.552 (s, 3 H, 18-Me), 0.820 (s, 3 H, 19-Me), 1.746 (s, 3 H, 21-Me), 2.019 (s, 3 H, CH_3COO), 4.68 (m, 1 H, 3-H), 4.696 and 4.839 (bs, 1 H, C=CH_2); ^{13}C NMR (100 MHz) δ 12.2, 12.9, 21.1, 21.2, 21.5, 24.7, 25.4, 27.5, 28.5, 31.9, 34.0, 35.5, 35.8, 36.7, 38.8, 43.3, 44.7, 54.3, 56.2, 57.3, 73.7, 110.6, 145.7, 170.7; MS 358 (M^+, 49), 283 (12), 275 (10), 229 (35), 215 (100), 201 (51); HRMS calcd for $C_{24}H_{38}O_2$ 358.2871, found 358.2867.

24-Nor-5α-chol-22-en-3β-yl Acetate (66). Aldehyde **65**[58] (10.3 mg, 0.027 mmol) was treated with 1.2 equiv of methylenetriphenylphosphorane using the procedure of Bergmann and Dusza.[52a] Preparative TLC purification afforded alkene **66**[70] (8 mg, 79%): ^1H NMR (400 MHz) δ 0.670 (s, 3 H, 18-Me), 0.816 (s, 3 H, 19-Me), 1.017 (d, 3 H, J = 6.6, 21-Me), 2.018 (s, 3 H, CH_3COO), 4.68 (m, 1 H, 3-H), 4.804 (dd, 1 H, J = 10, 1.8, *cis*-C=CH_2), 4.888 (dd, 1 H, J = 16.6, 1.2, *trans*-C=CH_2), 5.655 (ddd, 1 H, J = 18, 9.4, 7.7, 22-H_{vinyl}); ^{13}C NMR (100 MHz) δ 12.2, 20.1, 21.2, 21.5, 24.2, 27.5, 28.4, 28.6, 31.9, 34.0, 35.5, 36.7, 39.8, 41.2, 42.6, 44.6, 54.2, 55.5, 56.4, 73.7, 111.5, 145.3, 170.9 (2 carbons unresolved); MS 372 (M^+, 2), 357 (3), 344 (8), 315 (26), 312 (23), 257 (71), 255 (13), 215 (34), 201 (17), 107 (100); HRMS calcd for $C_{25}H_{40}O_2$ 372.3028, found 372.3035.

(22*E*)-5α-Chol-22-en-3β-yl Acetate (67). Aldehyde **65** (10.2 mg, 0.027 mmol) was treated with ethylidenetriphenylphosphorane (1.5 equiv) as described in the synthesis of **55b**[56] to give 4.0 mg of a mixture of olefins, which were immediately dissolved in 1 mL of dry benzene and refluxed under argon with 10 μL of thiophenol and 1 mg of AIBN[59] to give 4.0 mg of a mixture of cis/trans olefins in a ratio of 1:3.2 as determined by HPLC analysis. Purification by reverse-phase HPLC (methanol) afforded the pure trans olefin **67** (3 mg, 29% from the aldehyde): ^1H NMR (400 MHz) δ 0.650 (s, 3 H, 18-Me), 0.812 (s, 3 H, 19-Me), 0.977 (d, 3 H, J = 6.4, 21-Me), 1.604 (d, 3 H, J = 5.3, 24-Me), 2.019 (s, 3 H, CH_3COO), 4.68 (m, 1 H, 3-H), 5.27 (m, 2 H, 22-, 23-H); ^{13}C NMR (100 MHz) δ 12.2, 17.9, 20.6, 21.1, 21.5, 24.1, 27.4, 28.5, 28.6, 31.9, 34.0, 35.4, 36.7, 39.8, 40.0, 44.6, 54.2, 55.6, 56.0, 56.4, 73.7, 121.7, 138.1, 170.7 (2 carbons unresolved); MS 386 (M^+, 6), 344 (62), 329 (14), 315 (37), 257 (64), 255 (16), 215 (20), 107 (100); HRMS calcd for $C_{26}H_{42}O_2$ 386.3185, found 386.3174.

20(*S*)-Formyl-19-nor-5α-pregnan-3β-yl Acetate (68). The purified native 19-norstanol mixture of *A. polypoides* (210 mg) was acetylated (pyridine/acetic anhydride, 1:1, 10 mL), poured into ice water, and worked up as usual to give 240 mg of acetates, which were dissolved in 25 mL of CH_2Cl_2 (1% pyridine) and ozonized at −78 °C for 5 min. Decomposition of the ozonide and excess ozone at −78 °C with dimethyl sulfide (3 mL) was followed by silica gel chromatography (hexanes/ether, gradient 95:5 to 90:10) to provide 45 mg of aldehyde **68** (positive DNP test, 0.13 mmol, 48% assuming 50% Δ^{22} stanols in the native mixture[16]): ^1H NMR (400 MHz) δ 0.708 (s, 3 H, 18-Me), 1.114 (d, 3 H, J = 6.8, 21-Me), 2.020 (s, 3 H, CH_3COO), 2.35 (m, 1 H, 20-H), 4.34 (m, 1 H, 3-H), 9.561 (d, 1 H, J = 3.4, CHO); ^{13}C NMR (100 MHz) δ 12.4, 13.4, 21.5, 24.4, 25.9, 27.0, 28.2, 30.8, 31.8, 33.4, 39.3, 39.6, 40.9, 41.2, 43.3, 46.2, 47.8, 49.5, 51.2, 54.8, 73.1, 170.7, 205.2 (1 carbon unresolved); MS 360 (M^+, 14), 300 (100), 272 (13), 216 (41), 201 (61), 199 (18), 187 (10); HRMS calcd for $C_{23}H_{36}O_3$ 360.2664, found 360.2664.

19,24-Bisnor-5α-chol-22-en-3β-yl Acetate (57). Aldehyde **68** (11 mg, 0.031 mmol, 1 equiv) was treated with methylenetriphenylphosphorane (3 equiv) using the procedure of Bergmann and Dusza[52a] to afford, after reverse-phase HPLC purification, alkene **57** (10, mg, 90%): ^1H NMR (400 MHz) δ 0.684 (s, 1 H, 18-Me), 1.021 (d, 3 H, J = 6.4, 21-Me), 2.019 (s, 3 H, CH_3COO), 4.68 (m, 1 H, 3-H), 4.807 (dd, 1 H, J = 10, 2.0, *cis*-C=CH_2), 4.892 (dd, 1 H, J = 17, 2.0, *trans*-C=CH_2), 5.661 (ddd, 1 H, J = 19, 9.0, 7.7, 22-H_{vinyl}); MS 358 (M^+, 3), 343 (5), 301 (36), 298 (4), 243 (100), 241 (15), 201 (21), 147 (31); HRMS calcd for $C_{24}H_{38}O_2$ 358.2872, found 358.2886.

(22*E*)-19-Nor-5α-chol-22-en-3β-yl Acetate (58). Aldehyde **68** (18 mg, 0.050 mmol, 1 equiv) was treated with ethylidenetriphenylphosphorane (2.5 equiv) as described[56] above to give 20 mg of a crude mixture of olefins. A portion of this mixture (8 mg) was equilibrated with thiophenol (10 μL) and AIBN (1 mg) in refluxing benzene (1 mL)[59] to give 7.0 mg of a 4:1 mixture of trans and cis olefins, respectively, which were purified by reverse-phase HPLC (methanol) to give the pure trans isomer **58** (5.1 mg, 68% from **64**): ^1H NMR (400 MHz) δ 0.664 (s, 3 H, 18-Me), 0.981 (s, 3 H, J = 6.8, 21-Me), 1.605 (d, 3 H, J = 5.2,

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24-Me), 2.018 (s, 3 H, CH_3COO), 4.68 (m, 1 H, 3-H), 5.27 (m, 2 H, 22-, 24-H); MS 372 (M^+, 5), 357 (3), 330 (43), 315 (24), 301 (35), 270 (32), 243 (100), 201 (19); IR (cm^{-1}) 1733 (C=O), 972.7 (C=C trans);[56b] HRMS calcd for $C_{25}H_{40}O_2$ 372.3028, found 372.3013.

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Nonenzymatic Template-Directed Synthesis on Oligodeoxycytidylate Sequences in Hairpin Oligonucleotides

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Abstract: We have developed a novel method for studying template-directed synthesis in hairpin oligonucleotides. An unpaired segment at the 5′-terminus of the hairpin acts as an intramolecular template for the extension of the paired 3′-terminus. Products are analyzed by denaturing gel electrophoresis of [^{32}P]-labeled hairpins. Using this system, we have studied the synthesis of oligoguanylates on an oligodeoxycytidylate template. We find that guanosine 5′-phosphoro(2-methyl)imidazolide adds efficiently to a terminal riboguanylate residue at temperatures in the range 0–37 °C but not at 50 °C. At 0 °C, the half-time for addition of the first G residue is about 3 h, and the reaction rate is independent of pH in the range 6.5–8.0. The first addition reaction results in the formation of a predominantly 3′–5′-internucleotide bond. When the 3′-terminal riboguanylate residue is placed by a deoxyguanylate residue, the half-time for the first addition increases from about 3 to about 30 h.

Introduction

The replication of nucleic acids and the transcription of DNA are two of the fundamental processes that allow living systems to transfer and utilize genetic information. Both involve the syntheses of 3′–5′-linked polynucleotides on complementary templates, using nucleoside 5′-triphosphates as substrates. In contemporary living systems these syntheses are catalyzed by DNA and RNA polymerases. The base sequence of the template dictates the sequence of the polynucleotide product through Watson–Crick base pairing.

These biochemical processes can be modeled in much simpler chemical systems. Polyribo- or polydeoxyribonucleotides can act as templates and catalyze the nonenzymatic synthesis of complementary oligonucleotides from activated nucleoside 5′-phosphates. Poly(C) or poly(dC), for example, catalyzes the polymerization of guanosine 5′-phosphoro(2-methyl)imidazolide (2-MeImpG), producing a mixture of 3′–5′-linked guanylic acid oligomers (oligo(G)s) up to at least 30 nucleotides in length.[1] When the template reaction on a poly(C) template is carried out in the presence of the four ribonucleoside 5′-phosphoroimidazolides, only the complementary base, the guanosine nucleotide, is incorporated in the polymer in significant amounts.[2] Templates containing both cytidine and guanosine nucleotides direct the synthesis of the 3′–5′-linked complementary oligomers; r(CCGCC), for example, directs the synthesis of r(GGCGG)[3] and similarly d(CCCGCCCGCCCGCC) directs the synthesis of r-(GGCGGGCGGGCGGG).[4]

While the oligomerization of 2-MeImpG on poly(C) or an oligocytidylate template is a very efficient reaction, most other template reactions are less efficient. Polyuridylic acid directs the synthesis of oligo(A)s from adenosine 5′-phosphoroimidazolide, but the reaction is not very efficient and leads to products that are mainly 2′–5′-linked.[5] Poly(A) does not form a stable double helix with uridine mononucleotides, and it does not catalyze the polymerization of activated uridine 5′-phosphate derivatives, while oligo(G)s or poly(G) form very stable quadruplexes through Hoogsteen pairing and, therefore, do not influence the chemistry of monomeric activated cytidine 5′-phosphate derivatives. The template-directed reactions of nucleotides and oligonucleotides have been reviewed.[6]

Template-directed reactions have usually been carried out by incubating an aqueous solution of an activated ribonucleotide, often the nucleoside 5′-phosphoro(2-methyl)imidazolide (2-MeImpN), at a concentration of about 0.1 M with a roughly stoichiometric amount of template oligomer.[6] The high concentrations of activated mononucleotides that are required in these reactions lead to the synthesis of significant amounts of products that are formed independently of the template, mainly short oligomers containing phosphodiester and pyrophosphate bonds. In analyzing the products by HPLC, these side products tend to mask the low molecular weight products formed in the template-directed reaction. This masking effect becomes more serious as the concentration of the template is reduced. Another difficulty is that nonenzymatic template-directed reactions, unlike most enzyme-catalyzed processes, do not always initiate at a unique site on the template. Initiation can occur at internal positions on the template,

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