Facile Orthoester Formation in a Model Compound of the Taxol Oxetane: Are Biologically Active Epoxy Esters, Orthoesters, and Oxetanyl Esters Latent Electrophiles?

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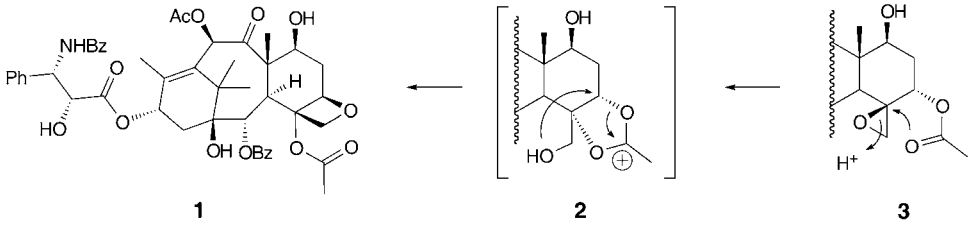
Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday.

A steroidal oxetanyl ester was synthesized in eight steps as a biomimetic model of taxol oxetane. The model compound was surprisingly reactive under acidic conditions, rearranging in the absence of H_2O to a [2.2.1]-bicyclic orthoester. Both the oxetanyl ester and the orthoester readily hydrolyze to produce the same triol monoacetate. On the basis of the oxetanyl ester/orthoester rearrangement, a novel biochemical function is suggested for the epoxy esters and oxetanyl esters found in taxoids whereby dioxonium ions, generated from these functional groups, react with cellular proteins to form mixed orthoesters or ethers. A similar process could be involved in the mechanism of action of natural orthoesters such as resiniferatoxin.

Introduction. – The oxetane ring and its associated β-AcO group are considered important molecular features for the anticancer activity of taxol[1]) (**1**; for a review, see [1]), a valuable medicinal natural product found in the yew tree *Taxus brevifolia* [2]. Co-occurring epoxy alcohols and esters (*e.g.*, **3**, *Scheme 1*) are considered to represent the biosynthetic precursors of the oxetane system, and a variety of possible mechanisms have been proposed for this interconversion [3].

Scheme 1. *Proposed Biosynthesis of Taxol* (**1**) *Oxetane by Epoxy-Ester/Cyclic-Ether Rearrangement*



1 **2** **3**

We assume that the biosynthetic reaction proceeds *via* an acid-catalyzed epoxy-ester rearrangement of the type we have recently investigated in the formation of [3.2.1]-bicyclic [4] and [2.2.1]-bicyclic [5] orthoesters. By our proposed mechanism (*Scheme 1*), intramolecular nucleophilic displacement with inversion at the spiro-epoxide center gives rise to a dioxonium-ion intermediate (**2**). Subsequent intramolecular displacement of the acetoxonium ion by the newly generated OH group

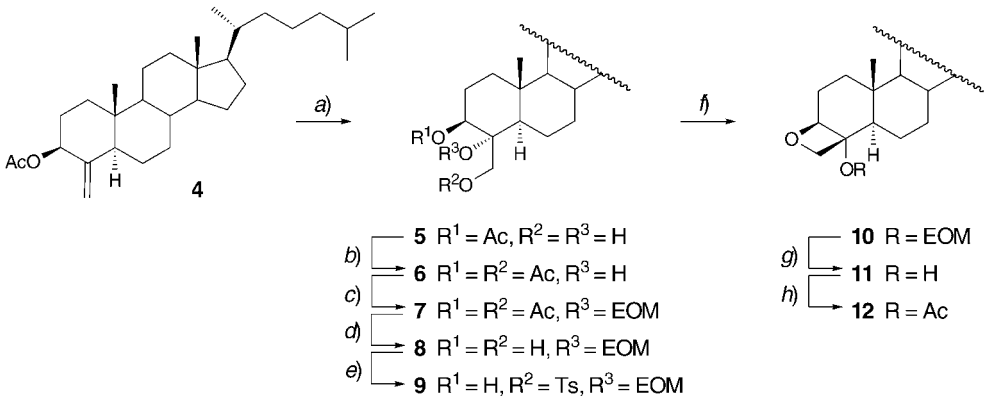
[1]) *Taxol* is a registered trademark of *Bristol-Myers Squibb Co.*, Princeton, NJ.

gives rise to the oxetane ring, again with inversion of configuration. The second step of this rearrangement is analogous to the mechanism of cyclic-ether formation from bicyclic orthoesters, a reaction that typically produces tetrahydrofurans [4c][6], but which may also extend to oxetanes [7].

As part of a biomimetic study to explore this rearrangement, a steroidal model of the taxol oxetane was required as a reference compound. This compound proved to be remarkably reactive, undergoing facile rearrangement in the presence of acid to a [2.2.1]-bicyclic orthoester.

Results. – The model oxetane **12** was obtained from the known steroid **4** [8] in eight steps (56% overall yield, *Scheme 2*) by means of a modification of a strategy developed for the synthesis of taxol [9].

Scheme 2. *Synthesis of Model Oxetane* **12**

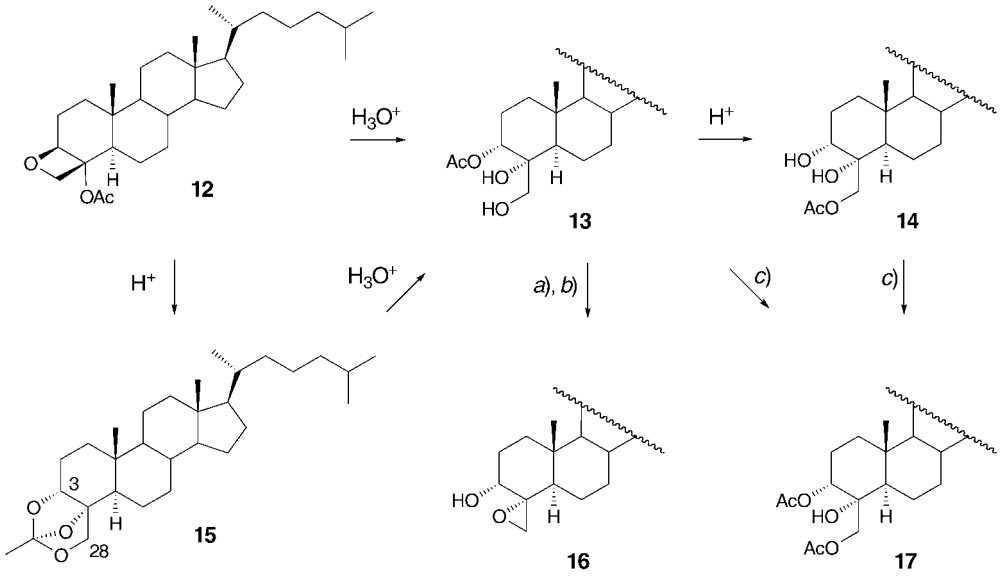


a) OsO_4, Py, Et_2O; 84%. *b*) Ac_2O, Py, DMAP, CH_2Cl_2; 99%. *c*) i-Pr_2NEt, $EtOCH_2Cl$, reflux; 95%. *d*) $LiAlH_4$, Et_2O; 98%. *e*) TsCl, Py, DMAP; 89%. *f*) NaH, THF, reflux. *g*) cat. H_2SO_4, MeOH/H_2O 4:1, reflux; 83% (2 steps). *h*) Ac_2O, Py, DMAP, CH_2Cl_2; 98%. Abbrev.: DMAP = 4-(dimethylamino)pyridine, EOM = ethoxymethyl, Py = pyridine, Ts = tosyl.

Oxetane **12** partially decomposed during an NMR measurement in $CDCl_3$, hydrolyzing to the secondary acetate **13** (*Scheme 3*). Upon standing in 6.6 mM trifluoroacetic acid (TFA) in $CDCl_3$, the initial hydrolysis product (**13**) equilibrated to a 1:4 mixture of acetates **13** and **14**, favoring the primary acetate **14**. Acetylation of either **13** or **14** yielded the diacetate **17**. The inversion of configuration at C(3) of the hydrolysis products was established by the conversion of **13** to the known epoxy alcohol **16** [10].

Under anhydrous conditions, acidic treatment (7.9 mM TFA in anh. $CDCl_3$) resulted in the rearrangement of oxetane **12** to the [2.2.1]-bicyclic orthoester **15** (*Scheme 3*). This process was followed by ^{1}H-NMR *via* the gradual replacement ($t_{1/2}$ = 70 min, 25°) of the AcO Me signal at 2.05 ppm with that of an orthoacetate Me signal at 1.71 ppm. Orthoester **15** proved very susceptible to hydrolysis, typically decomposing to mixtures of **13** and **14**. However, only the secondary acetate **13** was observed in a sample of **15**

Scheme 3. *Reactions of Model Oxetane* **12**



a) MsCl, Py; 97%. *b*) K_2CO_3, MeOH; quant. *c*) Ac_2O, Py, quant. Abbrev.: Ms = mesyl (methanesulfonyl chloride), Py = pyridine.

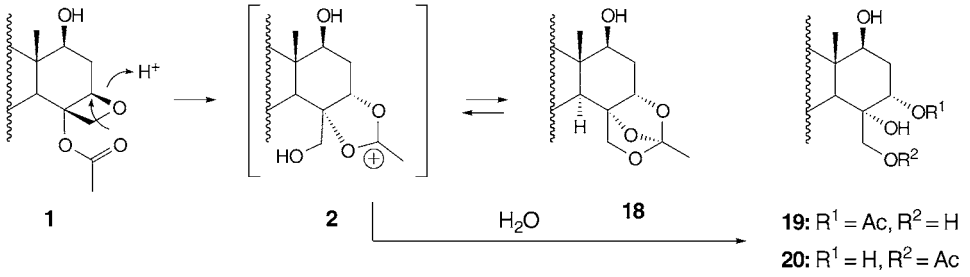
that had been frozen in C_6D_6 for four weeks. Despite the reactivity of the orthoester, it was possible to isolate pure **15** in 70% yield by means of silica-gel chromatography, scrupulously avoiding acidic conditions by addition of Et_3N to the solvent. The presence of an orthoester was confirmed by an HMBC-NMR experiment, which showed correlations of H–C(3) (3.97 ppm), H–C(28) (3.70 ppm), and the orthoacetate Me (1.71 ppm) with a ^{13}C-NMR signal (diagnostic for an orthoester) at 118.1 ppm. In addition, HR-EI-MS showed a molecular ion at *m/z* 458.3752 (M^+, $C_{30}H_{50}O_3^+$; calc. 458.3762).

In contrast to the steroidal model **15**, the oxetane ring of taxol (**1**) proved to be quite resistant. When treated with 1M TFA in $CDCl_3$, **1** remained largely unchanged after 3 h at 25°.

Discussion. – The intermediacy of [2.2.1]-bicyclic orthoesters of type **18** has long been proposed in *Lewis* acid catalyzed hydrolysis of taxoid oxetanes such as **1** [11]. However, this is unnecessary, since direct hydrolysis of the intermediate dioxonium ion **2** will lead to the observed hydrolysis products **19** and **20** (*Scheme 4*). To date, no taxoid [2.2.1]-bicyclic orthoesters **18** have been reported. However, we believe that, by applying the precautions used to prepare the model orthoester **15** (strictly anhydrous reaction conditions and purification under basic conditions), it should also be possible to prepare such compounds.

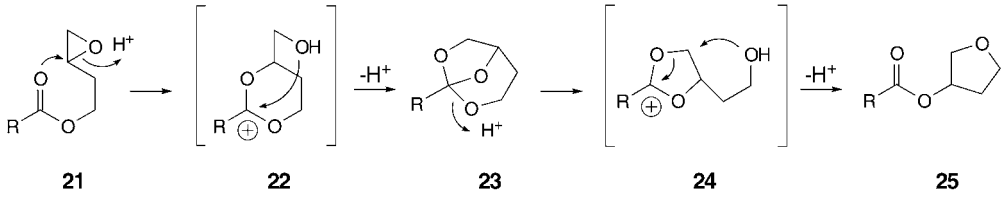
The isomerization of the steroidal oxetane **12** to the orthoester **15** has implications for the proposed biosynthesis of taxol (**1**). We have recently shown that the formation

Scheme 4. *Reactions of Taxol* (**1**) *Oxetane*



of tetrahydrofurans of type **25** by the rearrangement of the corresponding epoxy esters **21** proceeds *via* the [3.2.1]-bicyclic orthoesters **23** and the two different dioxonium-ion intermediates **22** and **24** (*Scheme 5*) [4c] (see also [6b]). If the taxoid orthoesters **18**, like the model orthoester, prove to be thermodynamically more stable than the corresponding oxetanes (*e.g.*, **1**), then the proposed biosynthetic epoxy ester/cyclic ether rearrangement of taxoids (*Scheme 1*) will, in contrast to the formation of tetrahydrofurans, not involve the intermediacy of orthoesters, but should proceed directly *via* the dioxonium ion **2**.

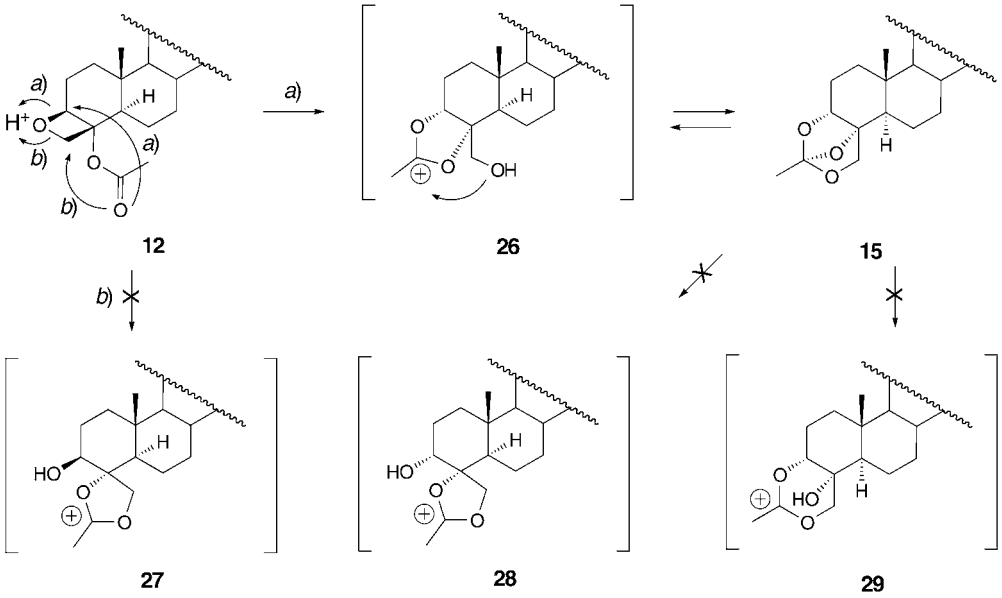
Scheme 5. *Epoxy Ester/Orthoester/Cyclic-Ether Rearrangement*



The disparity in the reactivity of taxol (**1**) compared to the model oxetane **12** is an interesting result. Perhaps the enhanced reactivity of **12** relative to **1** is due to increased ring strain imparted by the more-rigid steroidal system. Alternatively, **1** may be stabilized relative to **12** by the influence of a nearby functional group in the taxoid system that somehow stabilizes the oxetanyl ester towards acid-catalyzed rearrangement. However, while the reactivities of **1** and **12** are very different, both yield the same types of hydrolysis products (**13**/**14** and **19**/**20**, resp.). These products are best rationalized by the intermediacy of dioxonium ions **2** and **26**, which are generated *via* 5-*exo* ring closure with inversion of configuration at the secondary center of the oxetane. In both cases, despite steric hindrance, oxetane-ring opening takes place at the secondary (*Scheme 6*, Path *a*) instead of the primary center (Path *b*). No evidence for the formation of an alternative dioxonium ion **27**, with retention of configuration, was detected. This is a similar pattern to that seen in epoxides, where enhanced reactivity at the more-substituted site is believed to be due to the ability of electron-donating alkyl groups to stabilize a partial positive charge in a 'borderline S_N2 mechanism' [12].

Dioxonium ion **26** is also the most likely intermediate in the hydrolysis of orthoester **15**, based on the initial formation of secondary acetate **13**. The two alternative dioxonium ions (**28** and **29**) are less likely intermediates, since they would be expected to hydrolyze to the more stable primary acetate **14** (*Scheme 6*).

Scheme 6. *Alternative Dioxycarbenium Ions in the Reaction Mechanisms of* **12** *and* **15**

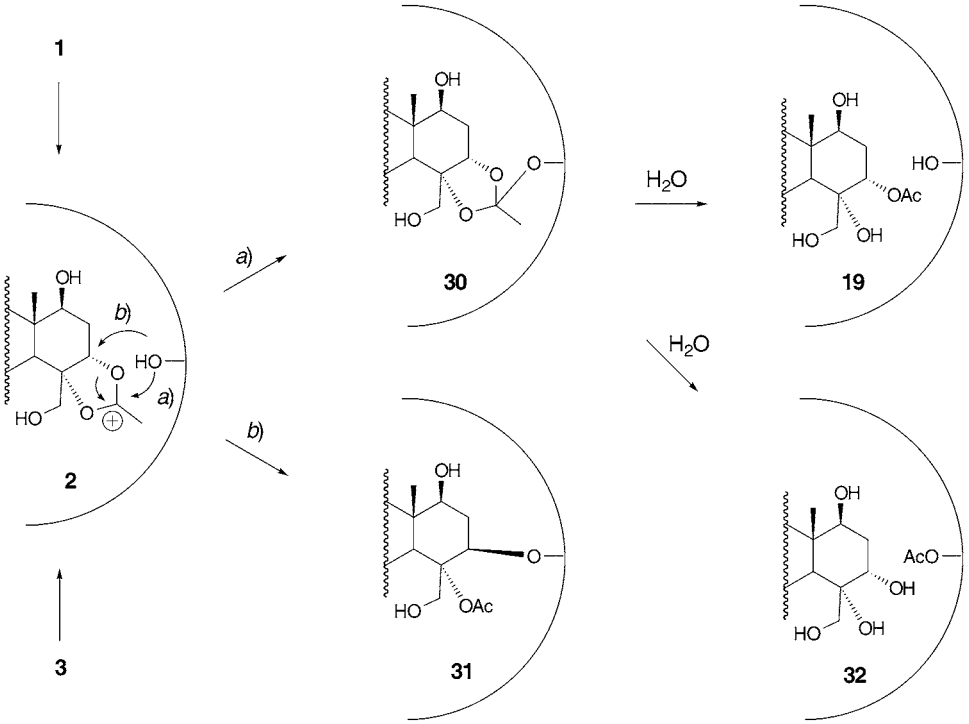


It is interesting to speculate on the biological implications of the rearrangement of the model oxetane **12** to the orthoester **15**. Although the oxetanyl ester of taxol (**1**) is relatively stable, it could be activated in a protein-bound form by conformational distortion or changes in H-bonding to more closely approximate the high reactivity seen with the model compound **12**. The generated dioxonium ion **2** is a reactive species that might react with a protein OH group to form a mixed taxoid–protein orthoester (**30**, *Scheme 7*). This type of covalent linkage would have limited stability, being susceptible to hydrolysis, leading to the generation of ring-opened products, either with retention of the Ac group by the taxoid (*e.g.*, **19**) or with transfer to the protein (**32**). Alternatively, the protein nucleophile could displace the acetoxonium group leading to the formation of a more-stable ether linkage (**31**) (S- and N-nucleophiles are also possible). The same scenario also applies to taxoids having the epoxy ester substructure **3**.

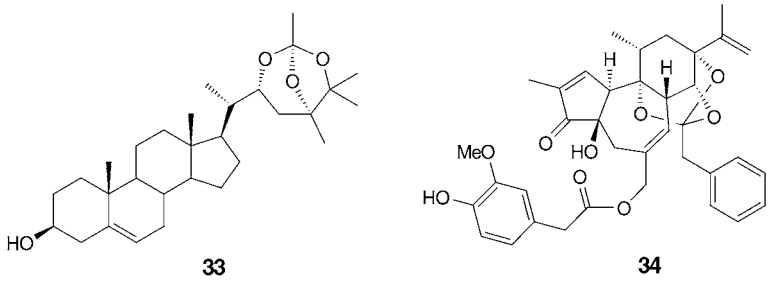
It is unlikely that the latent electrophilicity of the oxetane ester is of relevance to the anticancer mechanism of taxol in tubulin binding, which has been shown to be noncovalent based on the ability of taxol to displace [^{3}H]-labeled taxol from microtubules [13]. However, the biological action remains unknown for the majority of taxoids that do not bind to tubulin and are generated by the yew tree as chemical defenses against insects [14]. Little is also known about the binding mechanism of taxol (**1**) itself in its interactions with nontubulin proteins [15]. In these cases, it is possible

that the oxetanyl esters and epoxy esters of taxoids function as latent electrophiles capable of reacting with cellular proteins.

Scheme 7. *Possible Reactions of Taxoid Oxetanyl Esters* (**1**) *and Epoxy Esters* (**3**) *with Proteins*



The speculation that taxoids containing epoxy esters and oxetanyl esters can act as latent electrophiles raises the possibility that other natural products with similar functionalities might act in the same way. The orthoester moieties of both the GABAergic antagonist petuniasterone D (**33**) [16] and the potent vanilloid-receptor ligand resiniferatoxin (**34**) [17] have been shown to be essential for their biological activities. These biologically active orthoesters can also be regarded as latent dioxonium ions, potentially capable of undergoing covalent reactions with proteins.



We thank Dr. *Jeffrey M. Evans* (*Bristol-Myers Squibb*) for providing a sample of taxol.

Experimental Part

General. TLC was performed on aluminum-backed plates coated with a 0.25-mm layer of silica gel *60 F_{254}*. ^{1}H- and ^{13}C-NMR spectra were acquired at 300 and 75 MHz, resp., using $CDCl_3$ as the solvent, unless specified otherwise; chemical shifts δ in ppm rel. to Me_4Si, coupling constants J in Hz. Only selected ^{1}H-NMR signals relevant to the structural alterations of the steroidal skeleton are given.

1. *Synthesis of Steroidal Oxetane* **12**. – *3β-Acetoxy-4β-(hydroxymethyl)-5α-cholestan-4α-ol* (**5**). A soln. of **4** [8] (376.6 mg, 0.85 mmol) in Et_2O (13 ml) and pyridine (0.5 ml) was treated at r.t. with a soln. of OsO_4 (250 mg, 0.98 mmol) in benzene (3 ml). After 6 h, the solvents were evaporated under reduced pressure, and the resulting osmate was immediately hydrolyzed by adding pyridine (20 ml), H_2O (15 ml), and Na_2SO_5 (2.5 g, 13.17 mmol). After 12 h at 20°, the soln. was extracted with AcOEt. The org. layer was washed with 10% aq. HCl and brine, dried (Na_2SO_4), and evaporated under reduced pressure. Column chromatography (CC) (SiO_2; AcOEt/hexanes 1:4 and 3:2 afforded 338.7 mg (84%) of **5**. R_f 0.43 (hexanes/AcOEt 2:1). ^{1}H-NMR: 4.74 (*dd*, *J* = 12.3, 4.2, 1 H); 4.03 (*d*, *J* = 11.7, 1 H); 3.47 (*d*, *J* = 11.7, 1 H); 2.08 (*s*, 3 H); 0.89 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.85 (*d*, *J* = 6.6, 3 H); 0.76 (*s*, 3 H); 0.62 (*s*, 3 H). ^{13}C-NMR: 170.5; 83.6; 73.7; 61.8; 56.3; 56.1; 55.8; 54.4; 42.4; 39.8; 39.5; 36.8; 36.5; 36.1; 35.8; 35.2; 32.3; 28.2; 28.0; 25.1; 24.2; 23.8; 22.8; 22.6; 21.4; 21.0; 20.4; 18.6; 14.4; 12.0.

3-β-Acetoxy-4β-(acetoxymethyl)-5α-cholestan-4α-ol (**6**). A soln. of DMAP (4-(dimethylamino)pyridine; 30 mg, 0.25 mmol) in Ac_2O/pyridine 1:2 (1.5 ml) was added to a stirred soln. of **5** (216.7 mg, 0.46 mmol) in anh. CH_2Cl_2 (5 ml) at 20°. After 45 min, the solvents were evaporated under reduced pressure, the residue was taken up in Et_2O and filtered through SiO_2 to afford, after evaporation, 234.1 mg (99%) of **6**. R_f 0.51 (hexanes/AcOEt 2:1). ^{1}H-NMR: 4.69 (*dd*, *J* = 12.1, 4.8, 1 H); 4.52 (*d*, *J* = 12, 1 H); 4.10 (*d*, *J* = 12, 1 H); 2.08 (*s*, 3 H); 2.06 (*s*, 3 H); 0.89 (*d*, *J* = 6.3, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.85 (*d*, *J* = 6.6, 3 H); 0.84 (*s*, 3 H); 0.63 (*s*, 3 H). ^{13}C-NMR: 171.4; 170.6; 81.7; 74.0; 63.7; 56.2; 56.1; 55.9; 55.3; 42.4; 39.7; 39.5; 36.7; 36.6; 36.1; 35.7; 35.1; 32.5; 28.2; 28.0; 25.1; 24.2; 23.8; 22.8; 22.5; 21.2; 21.0; 20.9; 20.8; 18.6; 14.2; 12.0. Anal. calc. for $C_{32}H_{54}O_5$: C 74.08, H 10.49; found: C 73.83, H 10.41.

3β-Acetoxy-4β-(acetoxymethyl)-4α-(ethoxymethoxy)-5α-cholestane (**7**). To a soln. of **6** (234.1 mg, 0.45 mmol) in 10 ml of i-Pr_2NEt was added, dropwise, $EtOCH_2Cl$ (1 ml, 1.02 g, 10.8 mmol) at 20° under N_2. The mixture was stirred for 0.5 h and then refluxed for 1.5 h. It was quenched at 20° with 10% aq. HCl. (100 ml) and extracted with Et_2O. The org. phase was washed with brine and dried (Na_2SO_4). After evaporation of the solvents, the residue was purified by flash chromatography (FC; hexanes/AcOEt 9:1) to afford 240.3 mg (95%) of **7**. R_f 0.41 (hexanes/AcOEt 4:1). ^{1}H-NMR: 4.92 (*dd*, *J* = 12.0, 4.8, 1 H); 4.87 (*d*, *J* = 7.8, 1 H); 4.72 (*d*, *J* = 7.8, 1 H); 4.66 (*d*, *J* = 12.9, 1 H); 4.33 (*d*, *J* = 12.9, 1 H); 3.63 (*m*, 1 H); 3.51 (*m*, 1 H); 2.09 (*s*, 3 H); 2.03 (*s*, 3 H); 1.15 (*t*, *J* = 6.9, 2 H); 0.90 (*s*, 3 H); 0.89 (*d*, *J* = 6.7, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.85 (*d*, *J* = 6.6, 3 H); 0.64 (*s*, 3 H). ^{13}C-NMR: 170.8; 170.1; 89.2; 79.1; 76.9; 64.2; 63.8; 56.6; 56.2; 56.1; 53.2; 42.5; 39.8; 39.5; 37.4; 36.7; 36.6; 36.1; 35.7; 35.4; 32.9; 28.2; 28.0; 25.4; 24.2; 23.8; 22.8; 22.5; 22.0; 21.3; 21.2; 21.0; 18.6; 14.9; 14.2; 12.0.

4α-(Ethoxymethoxy)-4β-(hydroxymethyl)-5α-cholestan-3β-ol (**8**). $LiAlH_4$ (200 mg, 5.3 mmol) was added to a stirred soln. of **7** (230.5 mg, 0.41 mmol) in 10 ml of anh. Et_2O at 20°. After 15 min, the reaction was quenched by careful addition of 10% aq. HCl acid (20 ml), and the mixture was extracted with Et_2O. The org. layer was washed with brine, dried (Na_2SO_4), and evaporated to afford 207.0 mg (98%) of **8**. R_f 0.43 (hexanes/AcOEt 1:1). ^{1}H-NMR: 5.03 (*d*, *J* = 10.8, 1 H); 5.00 (*d*, *J* = 10.8, 1 H); 4.35 (*d*, *J* = 5.1, OH); 4.22 (*dd*, *J* = 12.3, 5.1, 1 H); 3.82–3.64 (*m*, 4 H); 3.52 (*t*, *J* = 6, OH); 1.24 (*t*, *J* = 7.2, 2 H); 0.89 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.85 (*d*, *J* = 6.6, 3 H); 0.84 (*s*, 3 H); 0.62 (*s*, 3 H). ^{13}C-NMR: 89.6; 82.0; 76.8; 64.1; 63.6; 56.5; 56.4; 56.1; 52.5; 42.4; 39.7; 39.5; 37.4; 36.9; 36.1; 35.7; 35.1; 32.6; 28.2; 28.2; 28.0; 24.2; 23.8; 22.8; 22.5; 20.9; 20.8; 18.6; 15.2; 14.8; 12.0.

4α-(Ethoxymethoxy)-4β-([[(4-methylphenyl)sulfonyl]oxy}methyl)-5α-cholestan-3β-ol (**9**). To a stirred soln. of **8** (200.1 mg, 0.41 mmol) and DMAP (15 mg, 0.12 mmol) in anh. pyridine (15 ml) was added 4-methylbenzenesulfonyl chloride (TsCl; 1.5 g, 7.87 mmol). After 17 h at 20° under N_2, the reaction was quenched by adding H_2O, and the mixture was extracted with hexanes/AcOEt 2:1. Drying (Na_2SO_4), removal of solvents under reduced pressure, and CC (hexanes/AcOEt 19:1, 9:1, and 4:1) afforded 222.7 mg (89%) of **9**. R_f 0.58 (hexanes/AcOEt 2:1). ^{1}H-NMR: 7.80 (*d*, *J* = 8.4, 2 H); 7.34 (*d*, *J* = 8.1, 2 H); 4.68 (*d*, *J* = 10.2, 1 H); 4.66 (*d*, *J* = 10.2, 1 H); 4.53 (*d*, *J* = 12.0, 1 H); 4.29 (*s*, OH); 4.27 (*d*, *J* = 12.0, 1 H); 3.78–3.64 (*m*, 1 H); 3.57–3.47 (*m*, 1 H); 1.18 (*t*, *J* = 6.9, 3 H); 0.88 (*d*, *J* = 6.9, 3 H); 0.86 (*d*, *J* = 6.9, 3 H); 0.85 (*d*, *J* = 6.9, 3 H); 0.76 (*s*, 3 H); 0.63 (*s*, 3 H).

^{13}C-NMR: 144.8; 132.6; 129.7 (2 C); 128.1 (2 C); 88.6; 81.3; 75.8; 68.1; 63.9; 56.7; 56.4; 56.2; 53.7; 42.3; 39.8; 39.5; 37.3; 36.9; 36.1; 35.7; 35.2; 33.1; 28.2; 28.0; 27.0; 24.2; 23.8; 22.8; 22.5; 22.0; 21.6; 20.8; 18.6; 15.0; 14.0; 11.9.

3β,4β-(Epoxymethano)-4α-(ethoxymethoxy)-5α-cholestane (= *(2a*S*,4a*R*,4b*R*,6a*R*,9a*S*,9b*S*,11a*R*,11b*R*)-7-[(1*R*)-1,5-dimethylhexyl]-11b-(ethoxymethoxy)octadecahydro-4a,6a-dimethylcyclopenta[7,8]phenanthro[2,1-*b*]oxete*; **10**). A soln. of **9** (222.7 mg, 0.36 mmol) in anh. THF (10 ml) was added under N_2 to a suspension of NaH (96 mg, 4 mmol) in THF (10 ml) at 20°. After 30 min at reflux, H_2O was added dropwise, and the mixture was extracted with Et_2O. The org. layer was dried (Na_2SO_4) and evaporated under reduced pressure to afford crude **10**. R_f 0.76 (hexanes/AcOEt 2:1). ^{1}H-NMR: 4.94 (*d*, *J* = 8.1, 1 H); 4.91 (*d*, *J* = 7.5, 1 H); 4.78 (*d*, *J* = 7.5, 1 H); 4.45 (*d*, *J* = 14.1, 1 H); 4.43 (*d*, *J* = 14.1, 1 H); 3.65 (*q*, *J* = 6.9, 2 H); 1.21 (*t*, *J* = 6.9, 3 H); 1.18 (*s*, 3 H); 0.90 (*d*, *J* = 6.6, 3 H); 0.87 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.66 (*s*, 3 H). ^{13}C-NMR: 90.2; 83.8; 80.4; 75.4; 63.5; 56.6; 56.2; 55.0; 48.9; 42.4; 39.9; 39.5; 36.1; 36.0; 35.8; 35.4; 33.4; 31.6; 28.2; 28.0; 27.4; 24.2; 23.8; 22.8; 22.6; 20.9; 20.2; 18.6; 15.2; 12.1; 12.0.

3β,4β-(Epoxymethano)-5α-cholestan-4α-ol (= *(2a*S*,4a*R*,4b*R*,6a*R*,9a*S*,9b*S*,11a*R*,11b*R*)-7-[(1*R*-1,5-dimethylhexyl)-11b-hydroxyoctadecahydro-4a,6a-dimethylcyclopenta[7,8]phenanthro[2,1-*b*]oxete*; **11**). Removal of the ethoxymethyl group was accomplished by dissolving **10** in MeOH/H_2O 4:1 (15 ml) and adding a 1% soln. of H_2SO_4 in THF (2 ml). The resulting mixture was stirred at reflux for 2.5 h and then quenched with pyridine (0.5 ml). Partial evaporation under reduced pressure gave an aq. soln., which was extracted with Et_2O. The org. layer was dried (Na_2SO_4), and the solvent was removed under reduced pressure. FC (SiO_2; AcOEt/hexanes 1:9 and 1:4) afforded 123.7 mg (83% from **9**) of **11**. R_f 0.41 (hexanes/AcOEt 2:1). ^{1}H-NMR: 4.75 (*dd*, *J* = 9.0, 2.7, 1 H); 4.54 (*d*, *J* = 7.5, 1 H); 4.30 (*d*, *J* = 7.5, 1 H); 1.15 (*s*, 3 H); 0.90 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.66 (*s*, 3 H). ^{13}C-NMR: 87.8; 78.1; 75.4; 56.6; 56.2; 54.8; 52.3; 42.4; 39.9; 39.5; 36.1; 35.9; 35.8; 35.3; 33.3; 31.6; 28.2; 28.0; 27.2; 24.1; 23.8; 22.8; 22.5; 20.8; 20.4; 18.6; 12.0; 11.7. Anal. calc. for $C_{28}H_{48}O_2$: C 80.71, H 11.61; found: C 80.56, H 11.70.

4α-Acetoy-3β,4β-(epoxymethano)-5α-cholestane (= *(2a*S*,4a*R*,4b*R*,6a*R*,9a*S*,9b*S*,11a*R*,11bR)-11b-acetoxy-7-(1*R*-1,5-dimethylhexyl)octadecahydro-4a,6a-dimethylcyclopenta[7,8]phenanthro[2,1-*b*]oxete*; **12**). A soln. of **11** (113.1 mg, 0.27 mmol) in CH_2Cl_2 (7 ml) and pyridine (2 ml) was treated with Ac_2O (0.9 ml) and DMAP (15 mg, 0.12 mmol). After 16 h at 20°, the mixture was partitioned between Et_2O and H_2O. The org. layer was filtered through SiO_2 and dried (Na_2SO_4). Solvents were removed under reduced pressure to afford 121.8 mg (98%) of **12**. R_f 0.43 (hexanes/AcOEt 9:1). ^{1}H-NMR (600 MHz): 5.03 (*d*, *J* = 8.5, 1 H); 4.42 (br. *s*, 2 H); 2.25 (*m*, 1 H); 2.05 (*s*, 3 H); 1.98 (*dt*, *J* = 12.6, 3.4, 1 H); 1.74 (*dd*, *J* = 13.2, 3.4, 1 H); 1.20 (*s*, 3 H); 0.91 (*d*, *J* = 6.5, 3 H); 0.87 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.67 (*s*, 3 H). ^{13}C-NMR: 169.7; 84.0; 81.8; 73.9; 56.5; 56.2; 54.4; 47.4; 42.4; 39.9; 39.5; 36.3; 36.2; 35.8; 35.3; 32.9; 31.5; 28.2; 28.0; 27.4; 23.8; 22.8; 22.6; 21.8; 20.8; 20.2; 18.6; 12.1; 11.7. Anal. calc. for $C_{30}H_{50}O_3$: C 78.55, H 10.99; found: C 78.44, H 11.23.

2. *Reactions of Model Steroidal Oxetane* **12**. – 2.1. *Hydrolysis of* (**12**). *3α-Acetoxy-4β-(hydroxymethyl)-5α-cholestan-4α-ol* (**13**). According to NMR measurement of **12** (5.6 mg, 0.01 mmol) in alumina-filtered $CDCl_3$ (0.75 ml), 20% of the starting oxetane was converted to a new compound after 20 min (^{1}H-NMR). Evaporation of the solvent with a stream of N_2 followed by prep. TLC (SiO_2; hexanes/AcOEt 9:1) afforded starting material (4.4 mg, 79%) and **13** (1.1 mg, 20%). ^{1}H-NMR: 5.21 (*t*, *J* = 2.4, 1 H); 3.63 (br. *s*, 2 H); 2.32 (*s*, OH); 2.14 (*s*, 3 H); 0.90 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.80 (*s*, 3 H); 0.64 (*s*, 3 H). ^{13}C-NMR: 170.9; 74.4; 72.2; 62.8; 56.5; 56.2; 55.9; 50.7; 42.4; 39.8; 36.8; 36.2; 35.8; 35.2; 32.5; 31.8; 28.2; 28.0; 24.2; 23.8; 23.6; 22.8; 22.6; 21.4; 20.9; 20.2; 18.7; 14.1; 12.0.

2.1.1. *Stereochemical Assignment of* **13**. *3α-Acetoxy-4β-[[(methylsulfonyl)oxy]methyl]-5α-cholestan-4α-ol*. A soln. of **13** (1.5 mg, 0.003 mmol) in anh. pyridine (0.5 ml) was treated with methanesulfonyl chloride (MsCl; 10 µl, 0.13 mmol) at 20° under N_2. After 0.5 h, the reaction was quenched by adding 10% aq. HCl (1 ml), and the mixture was extracted with Et_2O. The org. layer was filtered through a pad of SiO_2, evaporated with a stream of N_2, and purified by prep. TLC (SiO_2; hexanes/AcOEt 2:1) to afford 1.7 mg (97%) of the mesylate (precursor of **16**). ^{1}H-NMR: 5.17 (*t*, *J* = 2.4, 1 H); 4.43 (*d*, *J* = 10.8, 1 H); 4.21 (*d*, *J* = 10.8, 1 H); 3.10 (*s*, 3 H); 2.34 (*s*, OH); 2.14 (*s*, 3 H); 0.90 (*d*, *J* = 6.6, 3 H); 0.87 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.84 (*s*, 3 H); 0.64 (*s*, 3 H).

(4R)-Spiro[5α-cholestan-4,2′-oxiran]-3α-ol (**16**). Treatment of the above mesylate (1.7 mg, 0.003 mmol) with K_2CO_3 in MeOH at 20° overnight gave, after evaporation of the solvent with a stream of N_2, extraction of the residue with Et_2O, and filtration through SiO_2, **16** [10] in quant. yield: ^{1}H-NMR: 3.36 (br. *s*, 1 H); 2.87 (*d*, *J* = 4.5, 1 H); 2.61 (*d*, *J* = 4.5, 1 H); 0.90 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.83 (*s*, 3 H); 0.65 (*s*, 3 H).

2.1.2. *Isomerization of* **13** *to* **14**. *4β-(Acetoxymethyl)-5α-cholestane-3α,4α-diol* (**14**). A soln. of **13** (3.6 mg, 0.008 mmol) in $CDCl_3$ (0.75 ml) was treated with 10 µl of a 0.5M soln. of trifluoroacetic acid (TFA) in $CDCl_3$ (final conc.: 6.6 mM TFA). After 3.75 h at 20°, the solvents were evaporated with a stream of N_2 to afford a 1:4

mixture of **13** and **14** in quant. yield. Prep. TLC (SiO_2; hexanes/AcOEt 9:1) gave pure **14**. ^{1}H-NMR: 4.23 (*d*, *J* = 12, 1 H); 3.98 (*d*, *J* = 12, 1 H); 3.69 (*t*, *J* = 2.4, 1 H); 2.45 (*s*, OH); 2.38 (*s*, OH); 2.01 (*s*, 3 H); 0.79 (*d*, *J* = 6.6, 3 H); 0.76 (*d*, *J* = 6.6, 3 H); 0.76 (*d*, *J* = 6.6, 3 H); 0.74 (*s*, 3 H); 0.54 (*s*, 3 H). ^{13}C-NMR: 171.1; 74.3; 68.4; 65.4; 56.4; 56.1; 55.9; 48.6; 42.4; 39.8; 39.5; 36.7; 36.2; 35.8; 35.1; 32.5; 30.7; 28.2; 28.0; 25.0; 24.2; 23.8; 22.8; 22.6; 20.9; 20.8; 20.4; 18.7; 14.1; 12.0.

3α-Acetoxy-4β-(acetoxymethyl)-5α-cholestan-4α-ol (**17**). Acetylation of either **13** or **14** with Ac_2O/pyridine gave **17** in quant. yield. ^{1}H-NMR: 5.09 (*t*, *J* = 2.8, 1 H); 4.38 (*d*, *J* = 11.7, 1 H); 4.01 (*d*, *J* = 11.7, 1 H); 2.30 (*s*, OH); 2.13 (*s*, 3 H); 2.13 (*s*, 3 H); 0.90 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.86 (*d*, *J* = 6.6, 3 H); 0.86 (*s*, 3 H); 0.64 (*s*, 3 H). ^{13}C-NMR: 171.5; 171.0; 73.5; 71.4; 65.1; 56.4; 56.2; 55.9; 50.1; 42.4; 39.7; 39.5; 36.7; 36.1; 35.8; 35.1; 32.4; 31.6; 28.2; 28.0; 24.1; 23.8; 23.7; 22.8; 22.6; 21.4; 20.9; 20.8; 20.2; 18.7; 14.2; 12.0. Anal. calc. for $C_{32}H_{54}O_5$: C 74.08, H 10.49; found: C 73.95, H 10.55.

2.2. *Rearrangement of* **12**. *Steroidal Orthoester* **15**. A soln. of **12** (12.1 mg, 0.03 mmol) in anh. $CDCl_3$ (0.75 ml) was treated with 10 µl of a 0.5M soln. of TFA in $CDCl_3$ (final conc.: 6.6 mM TFA) at 25° under N_2. After 150 min, the reaction was quenched with 10 µl of Et_3N, and the mixture was directly applied to a prep. TLC plate of SiO_2 (SiO_2, pretreated with 5% Et_3N in Et_2O). Development with 2% Et_3N in hexanes and elution with 2% Et_3N in Et_2O gave a 1:2 mixture of **13** and **14** (1.5 mg, 12%), and orthoester **15** (8.4 mg, 70%). ^{1}H-NMR (600 MHz, C_6D_6): 3.89 (*dd*, *J* = 6.1, 11.4, 1 H); 3.69 (*d*, *J* = 6.2, 1 H); 3.57 (*d*, *J* = 6.2, 1 H); 1.86 (*s*, 3 H); 1.01 (*d*, *J* = 6.5, 3 H); 0.93 (*d*, *J* = 6.6, 3 H); 0.93 (*d*, *J* = 6.6, 3 H); 0.63 (*s*, 3 H); 0.48 (*s*, 3 H). EI-MS: 458 (10, M^+), 443 (9), 416 (18), 399 (31), 398 (100), 370 (48), 243 (62), 95 (37). HR-EI-MS: 458.3752 (M^+, $C_{30}H_{50}O_3^+$; calc. 458.3762).

Hydrolysis of Orthoester **15**. A soln. of **15** (3.5 mg, 0.008 mmol) in $CDCl_3$ (0.75 ml) was treated with 10 µl of a 10% soln. of TFA in H_2O. After 10 min at 25°, ^{1}H-NMR (600 MHz) showed a *ca.* 1:1 mixture of **13** and **14**. Spontaneous hydrolysis was observed in a sample of **15** that had been frozen in C_6D_6 for four weeks. In this case, only the secondary acetate **13** was observed.

3. *Acid Treatment of Taxol* (**1**). Compound **1** (10.2 mg, 0.012 mmol) was treated in 0.8 ml of a 1M soln. of TFA in $CDCl_3$ at 25° and under N_2. After 3 h, the reaction was quenched with Et_3N, and the mixture was evaporated with a stream of N_2 to recover mainly starting material.

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