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The Cephalostatins. 23. Conversion of Hecogenin to a Steroidal 1,6-Dioxaspiro[5.5]nonane Analogue for Cephalostatin 1[1]

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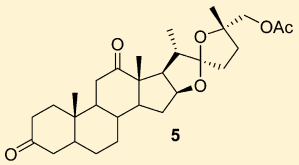
ABSTRACT: Cephalostatin 1 (**1**) has proved to be a remarkably potent cancer cell growth inhibitor. Since this steroidal alkaloid constituent of the marine worm *Cephalodiscus gilchristi* possesses a complex structure, providing preclinical supplies by total synthesis continues to be challenging. Therefore, syntheses of less complex structural modifications of this important pyrazine have also received substantial attention. Herein are summarized the synthesis of [5.5]spiroketal **5**, a simplified right-side steroidal unit of **1**, in seven steps from hecogenin acetate (**11**) with an overall yield of 4.6%. Consistent with other SAR studies, such reduction in structural complexity compared to **1** led to loss of cancer cell growth inhibitory activity against the P388 lymphocytic leukemia cell line.



After 15 years of challenging research directed at isolation and structural elucidation of the anticancer-active constituents of the marine worm *Cephalodiscus gilchristi*,[1] we reported the discovery of cephalostatin 1 (**1**) in 1988 in 8.4×10^{-4}% yield.[2] Cephalostatin 1 consists of two highly oxygenated hexacyclic steroid monomers linked by a pyrazine core.[3] The complete structural assignment was accomplished by X-ray crystallographic analysis. An additional 18 constituents, designated cephalostatins 2−19, were later discovered in *C. gilchristi* by our research group.[4−11] Subsequently, another 27 related members were identified from *Ritterella tokioka* by Fusetani and colleagues and designated the ritterazines.[12−15] The novel trisdecacyclic alkaloid **1** proved to be one of the most powerful cancer cell growth inhibitors ever evaluated by the U.S. National Cancer Institute (NCI), with a P388 lymphocytic leukemia cell value of $ED_{50} < 10^{-7}$ μg/mL and an average GI_{50} against the NCI 60 human cancer cell lines of 1.8 nM.[2]

Elucidation of the novel biological mechanisms of action of **1** has also been challenging. Cephalostatin 1 (**1**) was found to promote apoptosis in cancer cells by stimulating the release of the mitochondrial protein Smac (second mitochondria-derived activator of caspase) into the cellular cytosol by dissipating the mitochondrial membrane potential.[16] Those pioneering investigations by Vollmar and colleagues have continued to be very successful and are providing great insight into the complexity leading to apoptosis of cancer cells.[17−20] Other surprising advances were made by Shair and co-workers revealing that cancer cells depend on oxysterol-binding proteins OSBP and OSBP-related protein 4L (ORP4L), which are targeted by **1**.[21]

Impressively, **1** yielded to total synthesis in 1998 by Fuchs and co-workers using a 65-step convergent strategy.[22] More recently, an enantioselective route was reported by Shair and colleagues.[23] A number of research groups have undertaken the synthesis of cephalostatin analogues in an effort to provide synthetically accessible biosteroidal derivatives with promising cancer cell growth inhibition.[24−32] One of these modifications, hybrid 25-*epi*-ritterostatin G_N1_N (**2**), has proved to be the most promising owing to its subnanomolar activity (GI_{50} 0.48 nM) against the NCI-60 cell line.[24] Not only was **2** more cytotoxic than the natural isomer (ritterostatin G_N1_N; GI_{50} 14 nM), but its convergent synthesis yielded a more accessible structure. As part of prior research directed at less complex modifications of **1**, we prepared bis-steroidal pyrazines such as **3**[25] and **4**.[27] However, **3** yielded unremarkable cancer cell growth inhibition, but more significant (ED_{50} 1.8 μg/mL) activity for **4** against the P388 lymphocytic leukemia cell line was observed.[25,27]

Recent SAR studies[24−32] and observation of the cephalostatins and their analogues revealed that the right (otherwise known as the north or eastern) half of **1** not only is the most common unit in the cephalostatin family but is also associated with the most potent antitumor activity. Therefore, we undertook the synthesis of a right-side monomer with a 1,6-dioxaspiro[5.5]nonane side chain (**5**) in the present study that was structurally close to the right unit of **1** without producing another already reported analogue. The monomeric unit (cf., **5**) closely resembles the right half of a number of ritterazines discovered by Fusetani and colleagues in the marine tunicate *R. tokioka*.[12−15] These include F (**6**), I (**7**), Q (**8**), S (**9**), and Y (**10**), with the notable exception that these constituents possess a C/D *cis* configuration. By employing a more **1**-like C/D *trans* orientation, an increase in biological activity might be anticipated. In this regard, **9** is a symmetrical dimer possessing C/D *cis* geometry. An additional difference is the presence of a 26-hydroxy group moiety in **5** indicative of **1**, but not found in the ritterazines. The present study is the first segment of a larger initiative to synthesize **5** and then symmetrical and asymmetrical pyrazine variations. We therefore chose **5** as our target because of its similarity to the right unit of **1** as well as its more readily accessible structure owing to its less oxygenated nature.

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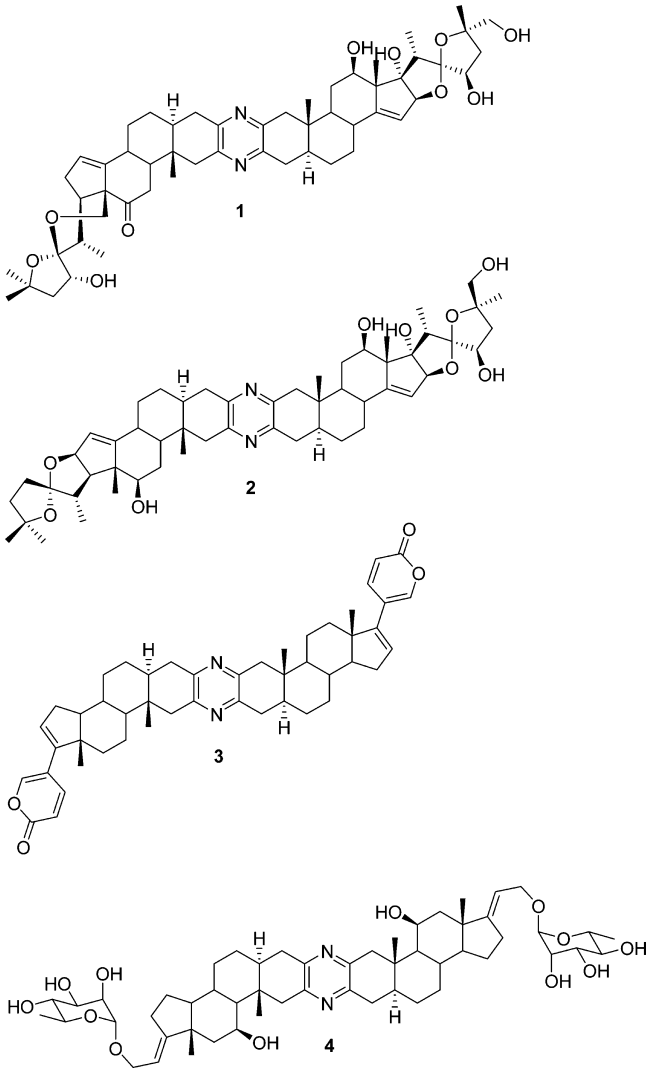
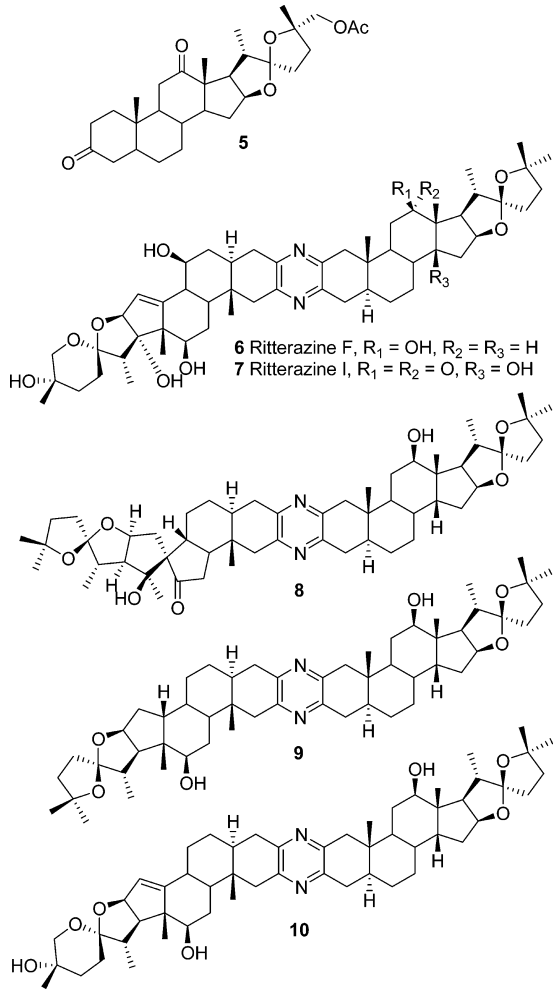
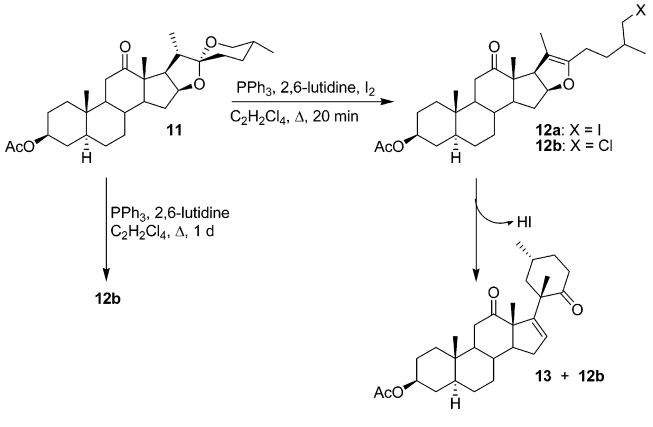




RESULTS AND DISCUSSION

Fuchs in 1999 reported the concurrent degradation and halogenation of hecogenin acetate (**11**) to afford 26-iodide **12a** employing triphenylphosphine, iodine, and 2,6-lutidine in tetrachloroethane (Scheme 1).[33] Also reported by Fuchs was the formation of chloride **12b** (25:1 **12a**:**12b**) and rearrangement byproduct 12,22-dione **13** (5%). When we used the Fuchs degradation protocol with **11**, it afforded exclusively **12b** and **13**. Presumably, **12b** arises by base-promoted elimination of HCl from the solvent (1,1,2,2-tetrachloroethane) at elevated temperature and subsequent nucleophilic substitution by chloride. Competitive halogenation by iodine provides **12a**, thus giving a mixture of 26-halides **12a** and **12b**. Owing to the highly labile nature of primary iodides, **12a** presumably undergoes intramolecular nucleophilic substitution to yield, following elimination of the 17α-H, dione **13**. In an effort to avoid a mixture of two compounds, reaction conditions were modified to provide exclusively **12b** by removing iodine from the procedure, which, as anticipated, eliminated the formation of **13** and provided **12b** in 46% yield from **11**.

Scheme 1. Degradation of Hecogenin Acetate (11) with Iodine to Yield a Mixture of 12b and 13 and without Iodine to Provide Exclusively 12b



Saponification of the 3β-acetate moiety of **11** with KOH in methanol provided hecogenin (**14**, Scheme 2) in quantitative yield (99%). Subsequent oxidation to afford 3-oxo-hecogenin (**15**) employing the Jones reagent also proceeded in quantitative yield (99%). Degradation and concurrent halogenation of the spiroketal unit of **15** to give 3-oxo-26-chloride **16** by use of the previously mentioned (Scheme 1) modified Fuchs procedure was accomplished in moderate yield (54%). Base-promoted dehydrohalogenation of **16** using potassium *tert*-butoxide yielded 3,12-dioxo-20(22),25-diene **17** in good yield (72%). Sharpless[34] dihydroxylation of **17** afforded $\Delta^{20(22)}$-25(*R*,*S*),26-diols **18*R*** and **18*S*** in a combined yield of 49%. Employment of the AD mix α reagent was necessary to obtain the desired C-25*S* isomer (**18*S***), corresponding to the

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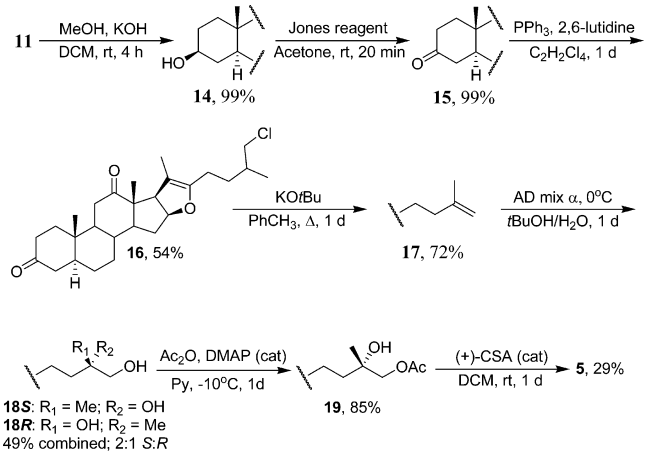
Scheme 2. Route to [5.5]Spiroketal 5



stereochemistry (confirmed by 2D ROESY NMR) of **1** at carbon 25.[2] Separation of the mixture of *R* and *S* isomers using silica gel chromatography (*i*-PrOH−CH_2Cl_2 as eluent) provided an enantiomeric ratio of 2:1 (**18*S***:**18*R***). Selective acetylation of the primary alcohol at C-26 of **18*S*** with acetic anhydride, 4-dimethylaminopyridine (DMAP), and pyridine provided 26-acetoxy-25*S*-alcohol **19** in good yield (85%).

The acetate-protecting group was of particular utility owing to the ease of removal at a later stage, a generally favorable effect on achieving crystalline solids, and its relatively small size. Bulky protecting groups (TBDMS, TES, TIPS, TBDPS, trityl, etc.) were a concern owing to unwanted steric hindrance in subsequent reactions. Acid-catalyzed spirocyclization[35] of **19** with (+)-(1*S*)-camphor-10-sulfonic acid [(+)-CSA] in CH_2Cl_2 provided 20*S*,22*S*,25*S*-[5.5]spiroketal **5** in 29% yield along with 7% of the 20*R*,22*R*,25*S* isomer. The overall yield to **5** from **11** in seven steps was 4.6%. Employment of the chiral acid (+)-CSA was necessary to obtain the desired 20*S*,22*S* isomer, which was obtained in a favorable ratio of 4:1 (20*S*,22*S*:20*R*,22*R*; separated by HPLC) and corresponded to the stereochemistry (confirmed by 2D ROESY NMR) of **1** at carbons 20 and 22.[2]

High-resolution (atmospheric pressure chemical ionization, $APCI^+$) mass spectrometric characterization revealed identical molecular ions for both **18** and **19** corresponding to the following fragments: *m*/*z* 409.27444 [M + H − $2H_2O$] for diol **18** and *m*/*z* 409.27428 [M + H − H_2O − OAc] for **19**. Spiroketal **5**, by contrast, gave an intact molecular ion corresponding to *m*/*z* 487.30608 [M + H] and a fragment at *m*/*z* 427.26542 [M + H − OAc], which was expected based on observation of previous spectra (cf., **19**).

Biological evaluation of the synthetic products **19** → **5**, excluding **14** and **15** owing to their similarity to **11**, did not meet the usual criteria for activity against the murine P388 lymphocytic leukemia cell line (summarized in Table 1). Typically, compounds with ED_{50} < 10 μg/mL are considered to be promising leads for further study. However, the P388 cell line results proved useful for eliminating such small structural fragments (cf., **5**) of **1** as high priority targets.

In conclusion, we have completed a facile synthetic route to a key intermediate (**5**) necessary for future preparation of symmetrical and asymmetrical trisdecacyclic bis-steroidal pyrazine modifications of the remarkably promising marine natural product anticancer drug cephalostatin 1 (**1**).

Table 1. Murine P388 Lymphocytic Leukemia Cell Line Results (ED_{50} Values)

compound	μg/mL
1	$<10^{-7}$
5	>10
12b	>100
16	>100
17	14.5
18*S*	23.4
19	19.3

EXPERIMENTAL SECTION

General Experimental Procedures. Ar refers to argon gas, rt to room temperature, and sgc to silica gel chromatography. All solvents were redistilled. Hecogenin acetate (**11**) was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All other chemicals were purchased from either Sigma-Aldrich Corp. or Acros Organics (Thermo Fisher Scientific, Pittsburgh, PA, USA). All chemicals and reagents were used as received.

Reactions were monitored by thin-layer chromatography (TLC) using Analtech silica gel GHLF Uniplates and were developed with either phosphomolybdic acid (10 wt %/wt solution in ethanol) or iodine. Solvent extracts of aqueous solutions were dried over anhydrous magnesium or sodium sulfate. Where appropriate, the crude products were separated by column chromatography using 70−230 mesh ASTM silica gel from E. Merck (Darnstadt, Germany). High-performance liquid chromatography was performed using a Waters 600E HPLC equipped with a Waters 2487λ absorbance detector and a Waters 2410 differential refractometer. The HPLC unit was operated in both analytical and semipreparative modes using typical C_8 and C_{18} columns.

Melting points are uncorrected and were determined with an Electrothermal 9100 apparatus. The IR spectra were obtained using a Thermo-Nicolet (Thermo Fisher Scientific) Avatar 360 Series FT-IR. The ^1H NMR and ^{13}C NMR spectra were recorded employing Varian Gemini 300 and Varian Unity 500 instruments using $CDCl_3$ (TMS internal reference) as solvent unless otherwise noted. Bs refers to broad singlet. High-resolution $APCI^+$ mass spectra were obtained with a JEOL JMS-LCmate mass spectrometer. Elemental analyses were determined by Galbraith Laboratories, Inc. (Knoxville, TN, USA).

*3β-Acetoxy-12-oxo-26-chloro-5α-furostan-20(21)-ene (**12b**).* To a stirred solution of **11** (10.0 g, 21.2 mmol) in 1,1,2,2-tetrachloroethane (100 mL) under Ar at rt were added triphenylphosphine (11.0 g, 41.9 mmol) and 2,6-lutidine (15.0 mL, 129 mmol), and the solution was heated to reflux for 1 d. After cooling to rt, the mixture was concentrated in vacuo and dissolved in CH_2Cl_2−H_2O (1:1; 200 mL). After separation of the phases the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic phase concentrated in vacuo, adsorbed onto silica gel (70−230 mesh), and subjected to sgc (70−230 mesh; 1:48 *i*-PrOH−CH_2Cl_2) to afford an amorphous, light tan solid. Crystallization from acetone−cyclohexane gave 26-chloride **12b** as colorless crystals (4.6 g, 46%): mp 151.1−152.2 °C; IR (neat) ν_{max} 2963, 2918, 2853, 1741, 1728, 1707, 1445, 1366, 1312, 1260, 1196, 1071, 1030, 931, 900, 723, 541 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.67 (2H, m, H-3$_\beta$, H-17$_\alpha$), 3.43 (2H, m, H-26$_{\alpha,\beta}$), 3.25 (1H, d, *J* = 10.2 Hz, H-17$_\alpha$), 2.42 (1H, t, *J* = 13.2 Hz, H-11$_\alpha$), 2.26 (2H, m, H-15$_\alpha$, H-11$_\beta$), 2.10 (2H, m, H-23$_{\alpha,\beta}$), 2.01 (3H, s, −OCOC<u>H</u>$_3$), 1.81 (4H, m), 1.62 (5H, m), 1.57 (3H, s), 1.49 (1H, m), 1.34 (4H, m), 1.29 (2H, m), 1.02 (3H, s), 0.93 (3H, s), 0.91 (3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.6 (C-12), 170.6 (−O<u>C</u>OCH$_3$), 152.0 (C-22), 103.6 (C-20), 82.7 (C-16), 73.1 (C-3), 57.3, 55.7, 55.5, 54.2, 50.9, 44.5, 38.0, 36.3, 36.1, 34.9, 34.2, 33.8, 33.5, 31.3, 28.2, 27.2, 23.2, 21.4 (−OCO<u>C</u>H$_3$), 17.6, 14.0, 11.8, 11.2; HRMS ($APCI^+$) *m*/*z* calcd for $C_{29}H_{43}Cl^{35}O_4$ 490.28499, found 491.29519 [M(Cl^{35}) + H]. Anal. Calcd for $C_{29}H_{43}Cl^{35}O_4$: C 70.92; H 8.83. Found: C 70.78; H 9.18.

*3β-Hydroxy-5α-Hecogenin (**14**).* To a stirred solution of hecogenin acetate (**11**, 5.0 g, 10.6 mmol) in CH_2Cl_2 (25 mL) at rt was added a

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10% (w/w) methanolic KOH (30 mL) solution dropwise. After stirring for 18 h, CH_2Cl_2 (100 mL) was added and the white precipitate dissolved. Water (100 mL) was then added and the phases were separated. The organic phase was washed with HCl (1 M, 100 mL), $NaHCO_3$ (saturated aqueous, 100 mL), and water (100 mL) and dried. Concentration in vacuo and recrystallization from acetone−cyclohexane gave the 3β-alcohol **14** (4.5 g, 99%) as colorless crystals: mp 262.1−262.8 °C; IR (neat) ν_{max} 3521, 3450, 3306, 3240, 2952, 2921, 2856, 1703, 1449, 1374, 1345, 1242, 1176, 1157, 1135, 1097, 1073, 1040, 1036, 1007, 979, 953, 918, 862, 778, 668, 605 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.35 (1H, m, H-16$_\alpha$), 3.59 (1H, m, H-3$_\alpha$), 3.48 (1H, m, H-26$_\beta$), 3.34 (1H, t, J = 10.8 Hz, H-26$_\alpha$), 2.51 (1H, t, J = 6.9 Hz, H-17$_\alpha$), 2.40 (1H, t, J = 14.4 Hz, H-11$_\beta$), 2.22 (1H, m, H-11$_\alpha$), 2.11 (1H, m, H-15$_\alpha$), 1.91 (1H, m), 1.78 (3H, m), 1.59 (7H, m), 1.44 (4H, m), 1.36 (3H, m), 1.28 (1H, m), 1.11 (2H, m), 1.07 (3H), 1.04 (3H), 0.90 (3H), 0.78 (3H, d, J = 6.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.1 (C-12), 108.7 (C-22), 78.7 (C-16), 70.3 (C-3), 66.4 (C-26), 55.3, 55.0, 54.6, 53.0, 44.1, 41.7, 37.3, 37.2, 36.0, 35.6, 33.8, 31.0, 30.9, 30.8, 30.7, 30.6, 29.6, 27.8, 16.6, 15.5, 12.7, 11.4; HRMS (APCI$^+$) m/z calcd for $C_{27}H_{42}O_4$ 430.30831, found 431.31643 [M + H].

*3-Oxo-5α-Hecogenin (**15**).* To a stirred suspension of **14** (1.0 g, 2.32 mmol) in acetone (50 mL) at rt was added Jones reagent (1.94 M, 25 mL). After stirring for 20 min, isopropyl alcohol (2 mL) was added and the suspension turned from green to blue. After stirring an additional 5 min, H_2O (30 mL) and CH_2Cl_2 (50 mL) were added, and, following separation of the phases, the aqueous phase was extracted with CH_2Cl_2 (1 × 30 mL). The combined organic phase was then dried, concentrated in vacuo, and recrystallized from acetone−cyclohexane to provide 3,12-dione **15** (0.98 g, 99%) as colorless crystals: mp 222.5−223.2 °C; IR (neat) ν_{max} 2950, 2927, 2859, 1710, 1703, 1452, 1419, 1377, 1345, 1237, 1176, 1156, 1132, 1097, 1074, 1056, 1008, 980, 941, 920, 863, 778, 668, 609 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.35 (1H, m, H-16$_\alpha$), 3.48 (1H, m, H-26$_\beta$), 3.33 (1H, t, J = 10.8 Hz, H-26$_\alpha$), 2.51 (1H, t, J = 6.9 Hz, H-17$_\alpha$), 2.40 (2H, m, H-11$_\beta$, 2$_\alpha$), 2.31 (3H, m, H-2$_\beta$, H-4$_{\alpha,\beta}$), 2.22 (1H, m, H-11$_\alpha$), 2.11 (1H, m, H-15$_\alpha$), 1.91 (1H, m), 1.78 (2H, m), 1.59 (5H, m), 1.44 (4H, m), 1.36 (3H, m), 1.28 (1H, m), 1.11 (2H, m), 1.07 (3H), 1.04 (3H), 0.90 (3H), 0.78 (3H, d, J = 6.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.1 (C-12), 210.8 (C-3), 108.7 (C-22), 78.6 (C-16), 66.4 (C-26), 54.9, 54.6, 54.4, 53.1, 45.7, 43.9, 41.7, 37.3, 35.7, 33.8, 33.8, 30.9, 30.7, 29.7, 28.3, 28.0, 27.5, 27.1, 16.6, 15.5, 12.7, 10.6; HRMS (APCI$^+$) m/z calcd for $C_{27}H_{40}O_4$ 428.29266, found 429.30102 [M + H].

*3,12-Dioxo-26-chloro-5α-furostan-20(22)-ene (**16**).* To a stirred solution of **15** (0.80 g, 1.87 mmol) and triphenylphosphine (1.30 g, 4.96 mmol) in 1,1,2,2-tetrachloroethane (20 mL) at rt under Ar was added 2,6-lutidine (3.4 mL, 29.2 mmol), and the solution heated to reflux for 5 d. After cooling to rt, the reaction was concentrated in vacuo and dissolved in CH_2Cl_2−H_2O (1:1; 40 mL). After separation of the phases the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic phase concentrated in vacuo, adsorbed onto silica gel (gravity), and subjected to sgc (gravity, 1:48 *i*-PrOH−CH_2Cl_2) to obtain an amorphous, light tan solid. Recrystallization from acetone−cyclohexane yielded 26-chloride **16** as colorless needles (0.45 g, 54%): mp 162.6−163.1 °C; IR (neat) ν_{max} 2952, 2923, 2854, 1711, 1704, 1448, 1419, 1378, 1313, 1273, 1227, 1197, 1172, 1127, 1069, 1045, 985, 930, 857, 729, 613 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.66 (1H, m, H-16$_\alpha$), 3.40 (2H, m, H-26$_{\alpha,\beta}$), 3.22 (1H, d, J = 10.2 Hz, H-17$_\alpha$), 2.48 (1H, t, J = 6.9 Hz, H-11$_\alpha$), 2.25 (4H, m, H-11$_\beta$, H-2$_\alpha$, H-15$_\alpha$, H-4$_\alpha$), 2.11 (2H, m, H-23$_{\alpha,\beta}$), 1.82 (5H, m), 1.58 (6H, m), 1.35 (2H, m), 1.18 (1H, m), 1.07 (3H, s), 0.98 (3H, s), 0.96 (3H, s), 0.92 (3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.4 (C-12), 210.6 (C-3), 152.2 (C-22), 103.7 (C-20), 82.6 (C-16), 73.3 (C-3), 57.4, 55.6, 55.4, 54.3, 50.9, 44.5, 38.0, 37.3, 37.1, 34.9, 34.2, 33.8, 33.5, 31.3, 28.2, 27.2, 23.2, 17.6, 14.0, 11.8, 11.2; HRMS (APCI$^+$) m/z calcd for $C_{27}H_{39}Cl^{35}O_3$ 446.25877, found 447.26564 [M(Cl^{35}) + H]. Anal. Calcd for $C_{27}H_{39}Cl_{35}O_4$: C 72.54; H 8.79. Found: C 72.58; H 9.14.

*3,12-Dioxo-5α-furostan-20(22),25-diene (**17**).* To a stirred solution of **16** (11.5 g, 23.4 mmol) in toluene (600 mL) at rt under Ar was added potassium *tert*-butoxide (28.5 g, 254 mmol). After heating at reflux for 2 d, the reaction mixture was cooled to rt and HCl (1M, 100 mL) added dropwise. After stirring for another 10 min the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phase was then concentrated in vacuo, and the residue dissolved in minimal CH_2Cl_2 and subjected to sgc (gravity, 3:97 acetone−CH_2Cl_2) to deliver an amorphous, colorless solid. Recrystallization from acetone−*n*-hexane afforded diene **17** as colorless needles (6.96 g, 72%): mp 132.3−132.9 °C; IR (neat) ν_{max} 2931, 2920, 2852, 1710, 1704, 1447, 1416, 1379, 1315, 1273, 1214, 1172, 1128, 1096, 1069, 1033, 884, 860, 741, 730, 613, 612 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.69 (2H, m, H-26$_{\alpha,\beta}$), 3.26 (1H, d, J = 10.2 Hz, H-17$_\alpha$), 2.52 (1H, t, J = 13.2 Hz, H-11$_\alpha$), 2.30 (2H, m, H-2$_\alpha$, H-4$_\alpha$), 2.24 (2H, m, H-15$_\alpha$, H-11$_\beta$), 2.20 (2H, m, H-2$_\beta$, H-4$_\beta$), 2.17 (2H, m, H-23$_{\alpha,\beta}$), 1.87 (3H, m), 1.61 (4H, m), 1.72 (3H, s), 1.59 (3H, s), 1.42 (4H, m), 1.16 (2H, m), 1.10 (3H, s), 0.96 (3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.1 (C-12), 210.8 (C-3), 152.0 (C-22), 145.2 (C-25), 110.2 (C-26), 103.5 (C-20), 82.6 (C-16), 57.3, 55.5, 55.4, 54.4, 44.7, 38.2, 37.8, 37.5, 37.1, 35.1, 34.2, 33.9, 31.8, 31.2, 28.2, 24.4, 22.2, 14.0, 11.9, 11.2; HRMS (APCI$^+$) m/z calcd for $C_{27}H_{38}O_3$ 410.28210, found 411.29094 [M + H]. Anal. Calcd for $C_{27}H_{38}O_3$: C 78.98; H 9.33. Found: C 78.76; H 9.20.

*3,12-Dioxo-(25R,25S)-dihydroxy-5α-furostan-20(22)-ene (**18S, 18R**).* To a stirred suspension of AD mix α (137 mg, 1.41 g AD mix α/mmol olefin) at 0 °C in *t*-BuOH−H_2O (1:1; 3 mL) was added **17** (40 mg, 0.097 mmol). After vigorous stirring in the dark for 8 h at 0 °C, the suspension was allowed to equilibrate to rt. After stirring for an additional 1 d, the suspension was cooled to 0 °C and Na_2SO_3 (40 mg) added. After stirring for an additional 2 h, CH_2Cl_2−H_2O (1:1; 6 mL) was added and the phases were separated. After separation of the phases the aqueous phase was extracted with CH_2Cl_2 (2 × 3 mL) and the combined organic phase concentrated in vacuo, dissolved in minimal CH_2Cl_2, and subjected to sgc (gravity, 1:39 *i*-PrOH−CH_2Cl_2) to afford a mixture of **18*R*** and **18*S*** diols (21 mg, 49% combined) as an amorphous, colorless solid. Separation of the diol mixture, dissolved in minimal CH_2Cl_2, utilizing sgc (gravity, 1:39 *i*-PrOH−CH_2Cl_2, performed twice) afforded 14 mg (33% overall) of **18*S*** and 7 mg (17% overall) of **18*R*** as amorphous, colorless solids in a ratio of 2:1 (*S*:*R*). Recrystallization from acetone−cyclohexane afforded colorless, twinned crystals: mp 152.4−152.9 °C; IR (neat) ν_{max} 3342, 3274, 3243, 2931, 2920, 2852, 1710, 1704, 1447, 1416, 1379, 1315, 1273, 1214, 1172, 1128, 1096, 1069, 1033, 884, 860, 741, 730, 613, 612 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.26 (1H, d, J = 10.2 Hz, H-17$_\alpha$), 2.52 (1H, t, J = 13.2 Hz, H-11$_\alpha$), 2.30 (2H, m, H-2$_\alpha$, H-4$_\alpha$), 2.24 (2H, m, H-15$_\alpha$, H-11$_\beta$), 2.20 (2H, m, H-2$_\beta$, H-4$_\beta$), 2.17 (2H, m, H-23$_{\alpha,\beta}$), 1.87 (3H, m), 1.61 (4H, m), 1.72 (3H, s), 1.59 (3H, s), 1.42 (4H, m), 1.16 (2H, m), 1.10 (3H, s), 0.96 (3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.1 (C-12), 210.8 (C-3), 152.0 (C-22), 103.5 (C-20), 82.6 (C-16), 76.5 (C-26), 72.0 (C-25), 57.9, 55.9, 55.6, 54.7, 44.7, 38.2, 37.8, 37.5, 37.1, 35.1, 34.2, 33.9, 31.8, 31.2, 28.2, 23.9, 22.2, 14.0, 11.9, 11.2; HRMS (APCI$^+$) m/z calcd for $C_{27}H_{40}O_5$ 444.28758, found 409.27444 [M + H − $2H_2O$].

*3,12-Dioxo-25-hydroxy-26-acetoxy-5α-furostan-20(22)-ene (**19**).* To a stirred solution of **18*S*** (25 mg, 0.051 mmol) and DMAP (cat.) at −10 °C under Ar in CH_2Cl_2−pyridine (3 mL; 2:1) was added dropwise acetic anhydride (0.50 mL, 5.32 mmol). The solution, after gradually equilibrating to rt for 22 h, was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2 (3 mL) and extracted with HCl (1 M, 3 × 3 mL) and H_2O (1 × 3 mL). The organic phase was dried, concentrated in vacuo, dissolved in minimal CH_2Cl_2, and subjected to sgc (gravity, 1:39 *i*-PrOH−CH_2Cl_2) to give **19** as an amorphous, colorless solid (23 mg, 85%): mp 166.7−167.9 °C; IR (neat) ν_{max} 3279, 3243, 2935, 2930, 2859, 1710, 1702, 1447, 1430, 1416, 1379, 1350, 1315, 1273, 1240, 1214, 1172, 1128, 1096, 1069, 1033, 884, 860, 741, 730, 613, 612 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.26 (1H, d, J = 10.2 Hz, H-17$_\alpha$), 2.52 (1H, t, J = 13.2 Hz, H-11$_\alpha$), 2.30 (2H, m, H-2$_\alpha$, H-4$_\alpha$), 2.24 (2H, m, H-15$_\alpha$, H-11$_\beta$), 2.20 (2H, m, H-2$_\beta$, H-4$_\beta$) 2.17 (2H, m, H-23$_{\alpha,\beta}$), 2.03 (3H, s, −OCOC$\underline{H}_3$), 1.87 (3H, m), 1.61 (4H, m), 1.72 (3H, s) 1.59 (3H, s), 1.42 (4H, m), 1.16 (2H, m), 1.10 (3H, s), 0.96 (3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.1 (C-12), 210.8 (C-3), 171.0 (-O$\underline{C}$OCH$_3$), 152.0 (C-22), 103.5 (C-20), 82.6 (C-16),

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79.9 (C-26), 68.7 (C-25), 57.9, 55.9, 55.6, 54.7, 44.7, 38.2, 37.8, 37.5, 37.1, 35.1, 34.2, 33.9, 31.8, 31.2, 28.2, 23.9, 22.2, 21.4 (−OCO<u>C</u>H$_3$), 14.0, 11.9, 11.2; HRMS (APCI$^+$) m/z calcd for $C_{29}H_{42}O_6$ 486.29814, found 409.27428 [M + H − H_2O − $OCOCH_3$].

(1S)-(+)-Camphor-10-sulfonic acid. A sample of amorphous (+)-CSA, obtained from the Sigma Chemical Company (St. Louis, MO, USA), was recrystallized from hot ethyl acetate to provide (+)-CSA as colorless needles: mp 194.9−195.8 °C (dec) (lit.[36] mp 193−195 °C (dec)); ^{1}H NMR (300 MHz, D_2O) δ 3.13 (1H, d, J = 15.3 Hz, −C<u>H</u>$_2$SO$_3$H), 2.71 (1H, d, J = 15.3 Hz, −C<u>H</u>$_2$SO$_3$H), 2.25 (2H, m, H-3, H-3), 2.00 (1H, t, J = 4.2 Hz, H-6), 1.89 (1H, m, H-4), 1.83 (1H, m, H-6), 1.49 (1H, m, H-5), 1.30 (1H, m, H-5), 0.89 (3H, s, −C<u>H</u>$_3$), 0.68 (3H, s, −C<u>H</u>$_3$).

3,12-Dioxo-23-desmethylene-25-methyl-26-acetoxy-5α-spirostane (5). To a stirred solution of alcohol **19** (90 mg, 0.185 mmol) in CH_2Cl_2 (4 mL) under Ar at rt was added (1S)-(+)-CSA (10 mg, 23 mol %). After stirring for 24 h, the reaction mixture was concentrated in vacuo, dissolved in minimal CH_2Cl_2, and subjected to sgc (gravity, 1:49 *i*-PrOH−DCM) to give spiroketal **5** as an amorphous solid (26 mg, 29%); IR (neat) ν_{max} 2964, 2922, 2865, 1733, 1708, 1497, 1375, 1236, 1171, 1091, 1074, 1046, 901, 888, 842, 646, 628 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.72 (1H, m, H-16$_\alpha$), 4.60 (2H, s, H-26$_{\alpha,\beta}$), 2.53 (1H, t, J = 6.9 Hz, H-17$_\alpha$), 2.43 (2H, m, H-11$_\beta$, H-2$_\alpha$), 2.33 (3H, m, H-2$_\beta$, H-4$_{\alpha,\beta}$), 2.21 (1H, m, H-11$_\alpha$), 2.11 (1H, m, H-15$_\alpha$), 2.08 (3H, s, −OCOC<u>H</u>$_3$), 1.91 (1H, m), 1.78 (2H, m), 1.59 (5H, m), 1.44 (4H, m), 1.36 (3H, m), 1.28 (1H, m), 1.11 (2H, m), 1.07 (3H), 1.04 (3H), 0.90 (3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 213.2 (C-12), 210.5 (C-3), 171.0 (−O<u>C</u>OCH$_3$), 108.6 (C-22), 77.7 (C-26), 69.1 (C-25), 67.6 (C-16), 54.9, 54.6, 54.4, 53.1, 45.7, 43.9, 41.7, 37.3, 35.7, 33.8, 33.8, 30.9, 30.7, 29.7, 28.3, 28.0, 27.5, 27.1, 16.6 (−OCO<u>C</u>H$_3$), 15.5, 12.7, 10.6; HRMS (APCI$^+$) m/z calcd for $C_{29}H_{42}O_6$ 486.29814, found 487.30608 [M + H], 427.26542 [M + H − $OCOCH_3$].

Cancer Cell Line Procedure. Mouse leukemia P388 cells[37] were incubated for 24 h in a 10% horse serum/Fisher medium followed by a 48 h incubation with serial dilutions of the compounds. Cell growth inhibition (ED_{50}) was then calculated using a Z1 Beckman/Coulter particle counter.

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Notes

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