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The Cephalostatins. 22. Synthesis of Bis-steroidal Pyrazine Pyrones[1]

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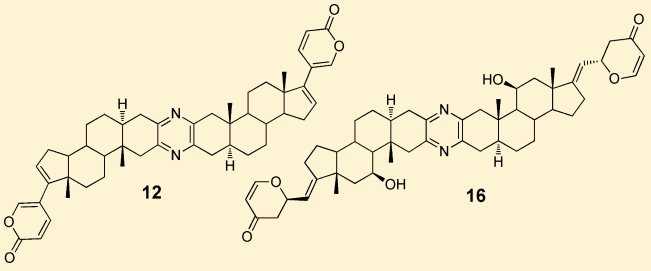
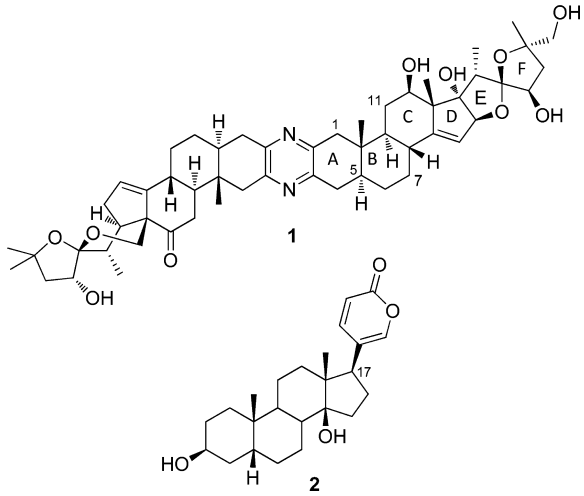
Supporting Information

ABSTRACT: Cephalostatin 1 (**1**), a remarkably strong cancer cell growth inhibitory trisdecacyclic, bis-steroidal pyrazine isolated from the marine tube worm *Cephalodiscus gilchristi*, continues to be an important target for practical total syntheses and a model for the discovery of less complex structural modifications with promising antineoplastic activity. In the present study, the cephalostatin E and F rings were greatly simplified by replacement at C-17 with an α-pyrone (in **12**), typical of the steroidal bufodienolides, and by a dihydro-γ-pyrone (in **16**). The synthesis of pyrazine **12** from 5α-dihydrotestosterone (nine steps, 8% overall yield) provided the first route to a bis-bufadienolide pyrazine. Dihydro-γ-pyrone **16** was synthesized in eight steps from ketone **13**. While only insignificant cancer cell growth inhibitory activity was found for pyrones **12** and **16**, the results provided further support for the necessity of more closely approximating the natural D–F ring system of cephalostatin 1 in order to obtain potent antineoplastic activity.



The isolation and structure of cephalostatin 1 (**1**), a powerful anticancer constituent of the Indian Ocean colonial marine tube worm *Cephalodiscus gilchristi* Ridewood (Cephalodiscidae), was summarized by us[2a] 24 years ago. Subsequently, **1** became the prototype of the cephalostatins[2] and ritterazines, which together make up a unique family of 45 highly oxygenated bis-steroidal pyrazines.[3] These marine invertebrate constituents exhibit powerful cancer cell growth inhibitory behavior (e.g., murine P388 lymphocytic leukemia cell line, ED_{50} 10^{-7} μg/mL; NCI 60 human cancer cell lines, GI_{50} 1.8 nM).[2] The availability of the cephalostatins and ritterazines from their only known natural sources, *C. gilchristi* and the marine tunicate *Ritterella tokioka*, is still extremely limited. As a result, in vivo anticancer evaluation of these very promising natural products and subsequent preclinical development have been greatly restricted.

The outstanding antineoplastic potency together with the new and challenging molecular architecture of **1** and poor availability from natural sources (~10^{-6}% yields from *C. gilchristi*) soon led to synthetic approaches by a number of research groups. By 1998, Fuchs and colleagues had completed the first total synthesis of **1** (in 65 synthetic steps)[4a] and of two additional members of the cephalostatin family (7 and 12) and ritterazine K.[4b] However, owing to the complexity of the targets, only very small amounts of these substances were produced and in very low overall yields (2 mg of **1**, 10^{-5}%, for instance),[4a] not suitable to supply sufficient samples at reasonable cost for extended biological evaluation. However, the recent enantioselective total synthesis of cephalostatin 1 by Shair[3c] does offer a potentially useful approach to scale-up and represents a splendid contribution. Meanwhile, a number of SAR studies concerned with the cephalostatins were initiated by various research groups in an effort to discover the minimum pharmacophore required to maintain potent cancer cell growth inhibitory behavior.[5,6]



The urgency of the practical syntheses and structural simplification endeavor was elevated when Vollmar and colleagues[3e,7] began to elucidate the unique mechanism of cephalostatin 1 (**1**). Because of their detailed research results, it is clear that **1** evokes a new cytochrome *c*-independent apoptosis signaling pathway. This is in contrast to most of the well-known anticancer drugs, which act in a cytochrome *c*-dependent route.[3e,7b–d] The lack of cytochrome *c* release by **1**

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indicates that it induces aopotosis in cancer cells via caspase-9 activation without formation of an apoptosome (complex of cytochrome *c*, procaspase-9, and the cytosolic factor Apaf-1). As part of this unique apoptosis mechanism,[7] **1** has been found to selectively release mitochondrial Smac (second mitochondria-derived activator of caspases), necessary for caspase-9 and caspase-2 activations.[7a] Furthermore, **1** produces an endoplasmic reticulum stress response, inducing caspase-4, which activates the caspase-9 route to apoptosis. The new pathway is marked by structural changes in the mitochondria.[7b] Current research results[7a] strongly indicate that the action of **1** on cancer cells is very complex and that further mechanistic investigation will provide many new insights of importance to cancer biology and further preclinical development of the cephalostatins. Shair and colleagues[3a] have recently reported that cephalostatin 1 (**1**) is one of a group of molecules that target oxysterol binding protein and its closest paralog and are useful as probes to reveal the functions of these proteins.

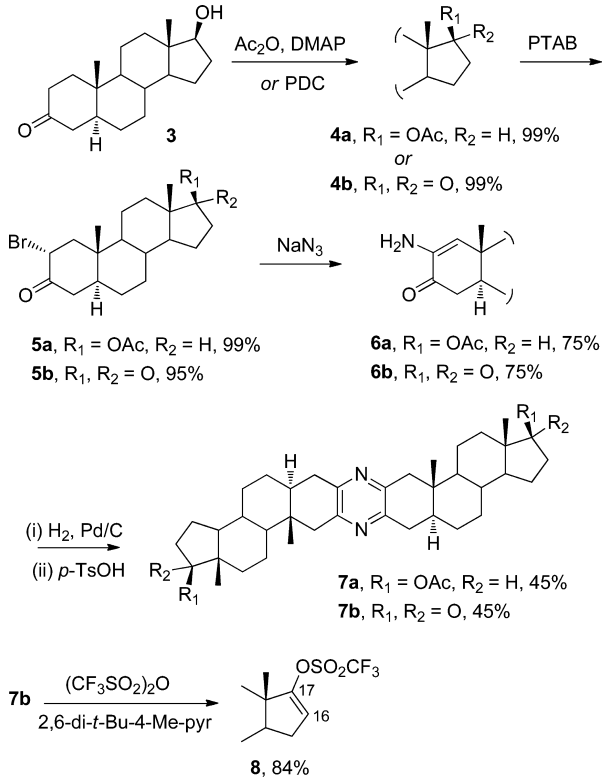
In order to extend our SAR studies[1] of **1**, we next focused on examining an extension of the C-17 side chain of a bis-steroidal pyrazine with a 5-substituted α-pyrone system characteristic of the bufadienolides, a large family of broadly bioactive animal and plant steroids originally isolated from toad venom. Certain members of the bufadienolides possess significant antineoplastic behavior,[8] typified by bufalin (**2**),[8b−d] and a broad spectrum of other biological activities, including regulation of hypertension.[8e,f] Also, in an effort to keep synthetic complexity to a minimum, a symmetrical C-17-pyrone bis-steroidal pyrazine was targeted. Cephalostatin 12[2f] is the only natural C_2-symmetric cephalostatin.

RESULTS AND DISCUSSION

Initial efforts (Scheme 1)[9] were directed at the formation of a simple symmetric pyrazine derived from 3-oxo-17β-hydroxy-5α-androstane (**3**, 5α-dihydrotestosterone) that would be suitable for condensation with the required pyran-2-one synthon, according to the method of Liu and Meinwald.[10] Acylation of **3** afforded **4a**, and subsequent conversion of **4a** to **5a** employing phenyltrimethylammonium tribromide (PTAB)[9e] was readily accomplished (98% yield from **3**). Transformation of **5a** to **6a** with NaN_3[9c,d] and catalytic NaI[9f] in dimethylformamide (DMF) proceeded well (75% yield). Direct dimerization of **6a** to give symmetric pyrazine **7a** was attempted with *p*-toluenesulfonic acid (*p*-TsOH)[9b,c] as catalyst, but a dehydropyrazine was formed instead. Subsequent attempts at conversion to **7a** with O_2 and again with 10% Pd/C proved ineffective. Hence, an alternate route was chosen in which **6a** was first reduced catalytically with H_2 and 10% Pd/C in toluene at 40 psi for 2 h.[10] Subsequent acid-catalyzed dimerization with *p*-TsOH in EtOH provided **7a** in moderate yield (45%), with an overall yield of 32% from **3**.

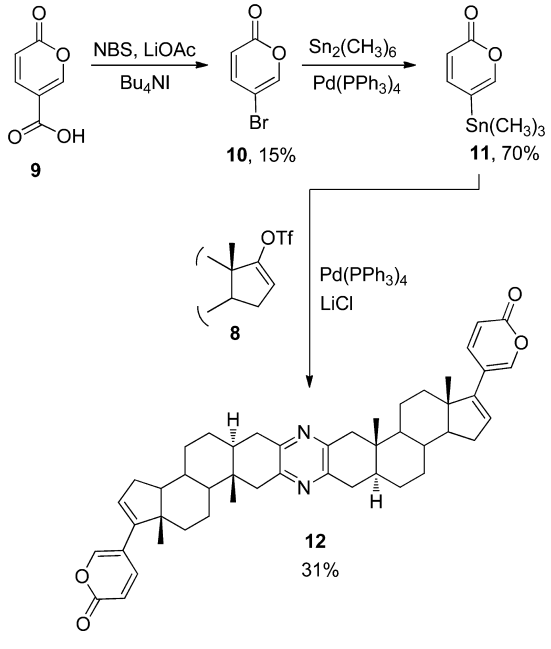
Rather than use **7a** as a substrate to obtain the required 17-oxo derivative, it was more practical to repeat the sequence depicted in Scheme 1 with 3,17-dioxo-5α-androstane (**4b**) as starting material, which was prepared by oxidation of **3** using pyridinium dichromate. Subsequent bromination to afford **5b**, followed by amination (**6b**) and dimerization, yielded 17,17′-dioxopyrazine **7b** in five steps from **3** in an overall yield of 28%. Efforts at transformation of ketone **7b** to 16,16′-dienyl-17,17′-ditriflate **8** using lithium diisopropylamide (LDA)[10] as base resulted in decomposition, but the hindered base 2,6-di-*tert*-butyl-4-methylpyridine proved very effective as a substitute for LDA in promoting the enolization of **7b**, which provided **8** in good yield (84%) as a substrate for subsequent condensation with a trimethylstannyl derivative.

Scheme 1. Synthesis of 17,17′-Diacetate 7a and 17,17′-Diketone 7b from 3-Oxo-17β-hydroxy-5α-androstane (3)



Adaptation of the sequence detailed by Cho[11] for decarboxylation/bromination of coumalic acid (**9**, Scheme 2) with *N*-bromosuccinimide (NBS) and lithium acetate led to 5-bromo-2*H*-pyran-2-one (**10**) in low yield (15%), accompanied by a dibrominated byproduct that was obtained in similar yield. By employment of methods analogous to those used to give

Scheme 2. Synthesis of Bis-bufadienolidepyrazine 12



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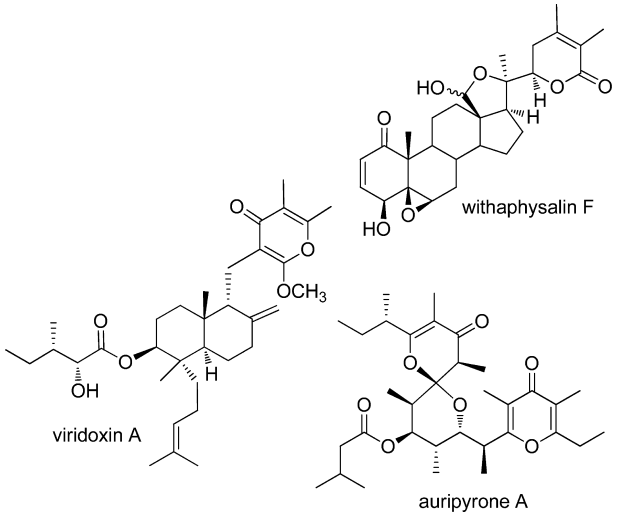
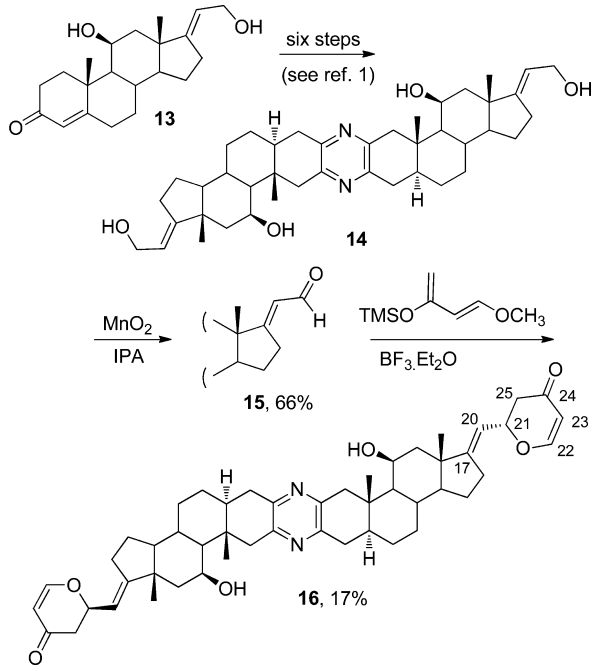
phenylstannates, as described by Liu and Meinwald,[10] treatment of **10** with hexamethylditin and the catalyst $Pd(PPh_3)_4$ yielded 5-(trimethylstannyl)-2*H*-pyran-2-one (**11**) in good yield (70%). Stille coupling[12] of enol triflate **8** and stannyl pyrone **11** employing $Pd(PPh_3)_4$ as catalyst resulted in the first synthesis (31% yield) of a bis-bufadienolide pyrazine (**12**), which was obtained from **3** in 8% overall yield.

In order to extend the bis-steroidal pyrazine 17-pyrone SAR probes, the general approach was extended as follows to a dihydro-γ-pyrone. The rationale for a dihydro-α- or dihydro-γ-pyrone unit at or near the steroid C-17 position receives some support from nature. A cancer cell growth inhibitory series of terrestrial plant constituents that contain a dihydro-α-pyrone ring incorporated at the steroid C-22 position, known as the withaphysalins, of which withaphysalin F[13a] (from *Physalis angulata*) is illustrative, are found in the Solanaceae family. Biologically active γ-pyrone-based natural products have been isolated from plant, fungal, and animal sources (e.g., viridoxin A[13b] from the fungus *Metarhizium flavoviride* and auripyrone A[13c] from the mollusk *Dolabella auricularia*), and interest in the properties of the widespread γ-pyrones has increased recently.[13d,e]



In the next approach to formation of a cephalostatin analogue with a structurally very simple replacement for the E and F rings of **1**, that is, a dihydro-γ-pyrone unit, we utilized pyrazine **14** (prepared in six synthetic steps from keto-alcohol **13** as described in our previous report,[1] which was focused on replacement of the E and F rings with a rhamnoside). Selective oxidation (Scheme 3) of **14** with manganese dioxide afforded dialdehyde **15**. Condensation of **15** with Danishefsky's diene[14] employing a Diels–Alder-type cycloaddition provided, albeit in low yield (17%), the 2,3-dihydro-4-pyrone **16**. The configuration at C-21 on the pyrone rings was deduced from analysis of the ^1H and ^1H–^1H COSY NMR spectra. A pair of doublets at δ 7.38 (J = 6.0 Hz) and 7.35 (J = 6.0 Hz), which were assigned to the C-22 protons of the rings, were coupled with the signal at δ 5.42 (J = 6.0 Hz, H-23). The multiplet at δ 5.20 (H-20) shows a vicinal coupling of 8.5 Hz and an allylic coupling of 2.5 Hz, corresponding to a strong correlation with the multiplet at δ 4.95–5.03 (H-21) and a minor correlation with the signal at δ 2.53–2.68 (H-25). These data are consistent with a structure that includes both (*R*)- and (*S*)-pyrone substituents, resulting from both *exo* and *endo* hetero-Diels–Alder cycloaddition, which gives rise to complex

Scheme 3. Synthesis of Dihydro-4-pyrone 16 from 13



multiplets for each of the C-20 and C-21 protons and a pair of doublets for the two C-22 protons. The signals from the C-23 protons are coincident, and those from H-25 are buried in the steroid skeleton signals.

Compounds **7a,b**, **8**, **12**, and **16** were screened for cancer cell growth inhibitory activity in the murine P388 lymphocytic leukemia cancer cell line (Table 1). The steroidal pyrazine side-chain pyrones **12** (ED_{50} 41 μg/mL, 57 μM) and **16** (ED_{50} 20 μg/mL, 25 μM) did not exhibit significant ($ED_{50} \leq 10$ μg/mL) activity. However, these SAR results added further confirmation of Winterfeldt's[6c–f] early analysis of cephalostatin 1 structural requirements for strong cancer cell growth inhibitory activity, particularly with respect to the need for a C-14 double bond, C-12 oxygenation (alcohol or ketone), and a 17α-hydroxy group for optimum activity. Presumably, there are a number of other less obvious molecular features of the cephalostatin-type steroids that are critical to their quite unique potency against cancer cell growth and mechanisms of biological activity. The uncovering of such structural features is a goal of future SAR investigations of the cephalostatins. Meanwhile, we are further evaluating the bis-steroidal pyrazine pyrones described herein for other biological activities.

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

compound no.	μg/mL	μM
1[2a]	10^{-7}–10^{-9}	10^{-7}–10^{-9}
cephalostatin 12[2f]	0.072	0.076
7a	60	91
7b	68	120
8	35	42
12	41	57
16	20	25

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EXPERIMENTAL SECTION

General Experimental Procedures. 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 spectrometer. The ^{1}H 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$^+$ (atmospheric pressure chemical ionization) mass spectra were obtained with a Jeol JMS-LCmate mass spectrometer. Elemental analyses were determined by Galbraith Laboratories, Inc.

Ether refers to diethyl ether, TEA to triethylamine, DCM to dichloromethane, DMAP to 4-dimethylaminopyridine, Ar to argon gas, and rt to room temperature. All solvents were redistilled. All chemicals were purchased from either Sigma-Aldrich Corp. or Acros Organics (Thermo Fisher Scientific). Reactions were monitored by thin-layer chromatography (TLC) using Analtech silica gel GHLF Uniplates and were visualized with either phosphomolybdic acid (10 wt %/wt solution in EtOH) or iodine. Solvent extracts of aqueous solutions were dried over anhydrous magnesium or sodium sulfate. Where appropriate, the crude products were purified by column chromatography (CC) on silica gel (70−230 mesh ASTM) from E. Merck.

*3-Oxo-17β-acetoxy-5α-androstane (**4a**).* To a stirred solution of dihydrotestosterone (**3**, 1.0 g, 3.45 mmol) and DMAP (cat) in pyridine (10 mL) at rt under Ar was added acetic anhydride (10 mL, 106 mmol) dropwise, and stirring continued for 6 h. The mixture was cooled to 0 °C, H_2O (15 mL) and ether (15 mL) were added, and the phases were separated. After extraction of the aqueous phase with ether (2 × 15 mL), the combined organic phase was washed with HCl (1 M, 2 × 15 mL), $NaHCO_3$ (saturated aqueous, 2 × 15 mL), and H_2O (15 mL). After drying, concentration of the organic phase afforded a colorless, amorphous solid. Crystallization from cyclohexane−acetone provided **4a** as colorless crystals (1.14 g, 99%): mp 155−156 °C [lit.[15] mp 157−158.5 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60 (1H, t, J = 8.8 Hz, H-17α), 2.42 (1H, m, H-2α), 2.32 (1H, t, J = 9.0 Hz, H-2β), 2.21 (1H, t, J = 8.8 Hz, H-4α), 2.10 (1H, m, H-16β), 2.04 (1H, m, H-4β), 2.03 (3H, s, −OCOCH$_3$), 1.75−1.05 (14H), 1.02 (3H, s, CH$_3$), 0.94 (1H, m), 0.81 (3H, s, CH$_3$), 0.76 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$) δ 211.8, 171.2, 82.7, 53.7, 50.6, 49.6, 44.6, 42.6, 38.5, 38.1, 36.8, 35.7, 35.2, 31.2, 28.8, 27.5, 23.5, 21.1, 20.9, 12.1, 11.4; HRMS (APCI$^+$) m/z 333.24144 [M + H]$^+$ (calcd for $C_{21}H_{33}O_3$, 333.24297); anal. C 75.76, H 9.89%, calcd for $C_{21}H_{32}O_3$, C 75.86, H 9.70%.

*2α-Bromo-3-oxo-17β-acetoxy-5α-androstane (**5a**).* To a stirred solution of **4a** (0.50 g, 1.50 mmol) in THF (5 mL) at 0 °C under Ar was added phenyltrimethylammonium tribromide (0.59 g, 1.56 mmol), and the solution immediately turned orange. After 20 min stirring, a tan precipitate formed, which dissolved upon addition of H_2O (5 mL). After extraction with DCM (2 × 5 mL) the combined organic phase was concentrated in vacuo, dissolved in minimal DCM, and subjected to CC (3:7 EtOAc−*n*-hexane) to yield a light tan, amorphous solid. Crystallization from cyclohexane−acetone gave **5a** as colorless crystals (0.61 g, 99%): mp 171.8−172.4 °C; IR (neat) ν_{max} 1733 (C═O, ketone and ester) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.73 (1H, q, J = 8.8 Hz, H-2β), 4.58 (1H, t, J = 8.8 Hz, H-17α), 2.64 (1H, q, J = 8.8 Hz, H-1α), 2.42 (2H, m, H-4α,β), 2.17 (1H, m, H-16β), 2.03 (3H, s, OCOCH$_3$), 1.80 (1H), 1.78−1.20 (14H), 1.08 (3H, s, CH$_3$), 0.98 (1H, m), 0.85 (1H, m), 0.79 (3H, s, CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.9, 171.1, 82.5, 54.3, 53.5, 51.6, 50.4, 47.4, 43.8, 42.6, 39.0, 36.6, 34.7, 31.0, 28.2, 27.5, 23.5, 21.1, 21.0, 12.1; HRMS (APCI$^+$) m/z 411.15272 [M + H]$^+$ (calcd for $C_{21}H_{32}{}^{79}BrO_3$, 411.15348); anal. C 61.42, H 7.56%, calcd for $C_{21}H_{31}BrO_3$, C 61.37, H 7.60%.

*2-Amino-3-oxo-17β-acetoxy-5α-androstan-1-ene (**6a**).* To a stirred solution of **5a** (0.50 g, 1.22 mmol) in DMF (10 mL) at rt under Ar were added NaN_3 (1.00 g, 15.4 mmol) and NaI (cat). The suspension was heated to 60 °C for 2 h and then cooled to rt, H_2O (10 mL) was added, and the solution was extracted with ether (5 × 10 mL). The combined organic phase was concentrated in vacuo, and separation (CC, 1% TEA in 7:3 *n*-hexane−EtOAc) yielded a yellow, amorphous solid. Crystallization from cyclohexane−acetone gave **6a** as light yellow needles (0.46 g, 75%): mp 149.8−150.5 °C; IR (neat) ν_{max} 1739, 1715 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09 (1H, s, H-1), 4.61 (1H, t, J = 8.8 Hz, H-17α), 3.43 (2H, bs, NH$_2$), 2.41 (1H, t, J = 8.8 Hz, H-4α), 2.26 (1H, dd, J = 8.8 Hz, H-4β), 2.16 (1H, m, H-16β), 2.04 (3H, s, OCOCH$_3$), 1.91 (1H, m, H-5α), 1.80−1.20 (14H), 1.08 (3H, s, CH$_3$), 0.98 (1H, m), 0.85 (1H, m), 0.79 (3H, s, CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.7, 171.7, 137.7, 126.5, 82.6, 51.2, 50.7, 44.8, 42.8, 40.2 (C-4), 38.1, 36.8, 35.3, 30.9, 27.5, 27.2, 27.1, 23.4, 21.1, 20.9, 13.9, 12.2; HRMS (APCI$^+$) m/z 346.23829 [M + H]$^+$ (calcd for $C_{21}H_{32}NO_3$, 346.23822); anal. C 73.32, H 9.11%, calcd for $C_{21}H_{31}NO_3$, C 73.01, H 9.04%.

*Bis(17β-acetoxy-5α-androstan[3,2-e,3′,2′])pyrazine (**7a**).* To a stirred solution of amine **6a** (0.50 g, 1.66 mmol) in toluene (10 mL) was added 10% Pd/C (0.28 g, 15 mol % Pd) in a heavy-walled hydrogenation flask. The flask was purged with H_2 (4×) before hydrogenation at 40 psi for 2 h. The black suspension was collected by filtration and washed with DCM (10 mL), and concentration of the organic phase in vacuo provided the 2α-amino ketone[3b,9a,b] intermediate as a brown solid, which was immediately used for dimerization without further purification. The 2α-amino ketone was dissolved in EtOH (5 mL), and *p*TsOH (cat) was added. After stirring for 48 h, the reaction solution was filtered through a pad of silica gel and washed with EtOAc (100 mL). The organic phase was concentrated in vacuo, and fractionation (CC, 1% TEA in 7:3 *n*-hexane−EtOAc) yielded an amorphous solid. Cystallization from cyclohexane−acetone provided **7a** as colorless needles (0.24 g, 45%): mp 240 °C (dec); IR (neat) ν_{max} 1738, 1717 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.61 (1H, t, J = 8.8 Hz, H-17α), 2.91 (1H, d, J = 8.8 Hz, H-1α), 2.71 (1H, dd, H-4α), 2.55 (2H, m, H-1β, H-4β), 2.16 (1H, m, H-16β), 2.04 (3H, s, OCOCH$_3$), 1.81−1.20 (14H), 1.08 (3H, s, CH$_3$), 0.98 (1H, m), 0.85 (1H, m), 0.79 (3H, s, CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2, 133.6, 127.1, 82.7, 52.0, 50.8, 50.6, 43.5, 42.8, 42.5, 41.7, 39.9, 38.3, 36.9, 35.6, 35.4, 30.9, 27.5, 27.0, 23.5, 21.2, 21.1; HRMS (APCI$^+$) m/z 657.46041 [M + H]$^+$ (calcd for $C_{42}H_{61}N_2O_4$, 657.46313); anal. C 76.94, H 9.11, N 4.31%, calcd for $C_{42}H_{60}N_2O_4$, C 76.79, H 9.21, N 4.26%.

*3,17-Dioxo-5α-androstane (**4b**).* To a stirred solution of dihydrotestosterone (**3**, 1.0 g, 3.44 mmol) in DCM (10 mL) at rt under Ar was added pyridinium dichromate (2.10 g, 5.58 mmol). After stirring for 2 h, the black suspension was collected by vacuum filtration through a short pad of silica gel with EtOAc (100 mL) as eluent. The organic extract was concentrated in vacuo, and purification (CC, 3:2 *n*-hexane−EtOAc) led to a colorless, amorphous solid. Crystallization from cyclohexane−acetone provided **4b** as colorless crystals (0.98 g, 99%): mp 134.1−134.5 °C [lit.[16] mp 133.5−134.0 °C]; IR (neat) ν_{max} 1742, 1739 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (1H, m, H-2α), 2.32 (1H, t, J = 9.0 Hz, H-2β), 2.21 (1H, t, J = 8.8 Hz, H-4α), 2.10 (1H, m, H-16β), 2.04 (1H, m, H-4β), 1.82 (1H, m, H-16α), 1.76−1.11 (14H), 1.03 (3H, s, CH$_3$), 0.94 (1H, m), 0.88 (3H, s, CH$_3$), 0.76 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.9 (C-17), 210.9 (C-3), 53.4, 50.7, 47.2, 46.1, 44.0, 37.9, 37.5, 35.3, 34.5, 31.0, 30.0, 28.1, 21.2, 20.2, 13.3, 10.9; HRMS (APCI$^+$) m/z 289.2165 [M + H]$^+$ (calcd for $C_{19}H_{29}O_2$, 289.2168); anal. C 79.28, H 9.95%, calcd for $C_{19}H_{28}O_2$, C 79.12, H 9.78%.

*2α-Bromo-3,17-dioxo-5α-androstane (**5b**).* To a stirred solution of **4b** (1.5 g, 5.20 mmol) in THF (15 mL) at 0 °C under Ar was added PTAB (2.20 g, 5.85 mmol). The solution immediately turned orange and was stirred for 20 min. A tan precipitate formed that dissolved on addition of H_2O (10 mL). After extraction with DCM (2 × 15 mL), the combined organic phase was concentrated in vacuo, and purification (CC, 7:3 *n*-hexane−EtOAc) yielded a light tan, amorphous solid. Crystallization from cyclohexane−acetone gave **5b** as colorless crystals (1.81 g, 95%): mp 136.4−136.7 °C; IR (neat) ν_{max} 1733, 1716 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.73 (1H, q, J = 8.8 Hz, H-2β), 2.65 (1H, q, J = 8.8 Hz, H-1β), 2.45 (3H, m, H-4α, H−16β, H-16α), 2.11 (1H, t, H-1α), 1.96 (1H), 1.89−1.18 (14H), 1.11

(3H, s, CH_3), 0.94 (1H, m), 0.88 (3H, s, CH_3), 0.76 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.9 (C-17), 211.1 (C-3), 54.3 (C-2), 53.5, 53.2, 51.0, 50.5, 47.1, 46.9, 43.3, 35.2, 34.0, 30.1, 29.8, 27.6, 21.2, 20.3, 17.2, 13.3, 11.6; HRMS (APCI$^+$) m/z 367.1321 $[M + H]^+$ (calcd for $C_{19}H_{28}{}^{79}BrO_2$ 367.1273); anal. C 62.21, H 7.39%, calcd for $C_{19}H_{27}BrO_2$, C 62.13, H 7.41%.

*2-Amino-3,17-dioxo-5α-androstan-1-ene (**6b**).* To a stirred solution of **5b** (1.5 g, 4.08 mmol) in DMF (15 mL) at rt under Ar were added NaN_3 (2.70 g, 41.5 mmol) and NaI (cat), and the suspension was heated to 60 °C for 2 h. After cooling to rt, the mixture was diluted with H_2O (15 mL) and extracted with ether (5 × 20 mL). The combined organic phase was concentrated in vacuo, and the residue was purified (CC, 1% TEA in 7:3 *n*-hexane−EtOAc) to yield a yellow, amorphous solid. Crystallization from cyclohexane−acetone gave **6b** as light yellow needles (0.92 g, 75%): mp 135.3−175.9 °C; IR (neat) ν_{max} 1734, 1700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.07 (1H, s, H-1), 3.45 (2H, bs, NH_2), 2.45 (3H, m, H-4α, H-16β, H-16α), 2.11 (1H, H-16β), 1.94−1.16 (14H), 1.00 (3H, s, CH_3), 0.94 (1H, m), 0.88 (3H, s, CH_3), 0.76 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.1 (C-17), 195.0 (C-3), 137.4 (C-1), 125.4 (C-2), 50.9, 50.8, 47.3, 44.3, 39.6, 37.7, 35.3, 34.6, 31.0, 29.7, 26.6, 21.1, 20.2, 13.4, 11.9; HRMS (APCI$^+$) m/z 302.2120 $[M + H]^+$ (calcd for $C_{19}H_{28}NO_2$, 302.2120); anal. C 75.42, H 9.13, N 4.56%, calcd for $C_{19}H_{27}NO_2$, C 75.71, H 9.03, N 4.65%.

*Bis(17-oxo-5α-androstan[2,3-b:2′,3′-e])pyrazine (**7b**).* To a stirred solution of **6b** (0.50 g, 1.66 mmol) in toluene (10 mL) was added 10% Pd/C (0.28 g, 15 mol % Pd) in a heavy-walled hydrogenation flask. The flask was purged with H_2 (4×) before hydrogenation at 40 psi for 2 h. The black suspension was then removed by filtration and washed with DCM (10 mL). The combined organic extract was concentrated in vacuo to yield the 2α-amino ketone intermediate as a brown solid, which was immediately used without further purification for dimerization. The amine (1.66 mmol) was dissolved in EtOH (5 mL), and *p*TsOH (cat) added. After stirring for 48 h, the reaction solution was filtered (vacuum) through a short pad of silica gel and washed with EtOAc (100 mL). The solvent extract was concentrated in vacuo, adsorbed onto silica gel, and subjected to CC (7:3 *n*-hexane−EtOAc, 1% TEA) to give an amorphous solid. Crystallization from cyclohexane−acetone provided **7b** as colorless needles (0.19 g, 45%): mp 235 °C (dec); IR (neat) ν_{max} 1737 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.93 (1H, d, J = 8.8 Hz, H-1α), 2.68 (1H, dd, H-4α), 2.50 (3H, m, H-1β, H-4β, H-16β), 2.19 (1H, m, H-16α), 1.88−0.96 (16H), 0.88 (3H, s, CH_3), 0.85 (1H, m), 0.82 (3H, s, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.4 (C-17), 133.6 (C-2), 127.1 (C-3), 53.3, 50.8, 47.1, 45.4, 41.9, 41.3, 35.3, 34.9, 34.4, 33.3, 32.6, 31.1, 29.9, 27.6, 24.2, 21.3, 20.0, 13.2, 11.5; HRMS (APCI$^+$) m/z 569.4158 $[M + H]^+$ (calcd for $C_{38}H_{53}N_2O_2$, 569.4107); anal. C 80.31, H 9.11, N 4.86%, calcd for $C_{38}H_{52}N_2O_2$, C 80.24, H 9.21, N 4.92%.

*Bis(17-triflyloxy-Δ^{16}-5α-androstan[2,3-b:2′,3′-e])pyrazine (**8**).* To a stirred solution of **7b** (0.40 g, 0.70 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (0.44 g, 2.14 mmol) in DCM (4 mL) at −10 °C under Ar was added (dropwise) triflic anhydride (0.36 mL, 2.14 mmol). After 18 h, H_2O (8 mL) and DCM (4 mL) were added, and the aqueous phase was extracted with DCM (2 × 8 mL). The combined organic phase was concentrated in vacuo, and silica gel separation (CC, 1:1 *n*-hexane−EtOAc) yielded **8** as an amorphous, light yellow solid (0.49 g, 84%): mp 190 °C (dec); ^1H NMR (300 MHz, $CDCl_3$) δ 5.77 (1H, m, H-16), 2.91 (1H, d, J = 8.8 Hz, H-1α), 2.71 (1H, dd, H-4α), 2.50 (2H, m, H-1β, H-4β), 1.81−1.11 (16H), 1.00 (3H, s, CH_3), 0.94 (1H, m), 0.84 (3H, s, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.7 (C-17), 152.0 (OSO_2CF_3), 135.6 (C-2), 129.1 (C-3), 113.9 (C-16), 53.6, 53.5, 45.1, 44.3, 41.4, 35.3, 34.9, 32.8, 32.2, 29.9, 29.6, 28.0, 27.5, 19.9, 14.7, 11.4; HRMS (APCI$^+$) m/z 833.3094 $[M + H]^+$ (calcd for $C_{40}H_{51}F_6N_2O_6S_2$, 833.3093).

*5-Bromo-2H-pyran-2-one (**10**).* To a stirred solution of coumalic acid (**9**, 10.0 g, 71.4 mmol) in CH_3CN−H_2O (9:1; 400 mL) at rt were added LiOAc (5.60 g, 84.9 mmol), NBS (19.0 g, 107 mmol), and Bu_4NI (1.0 g, 4 mol %). After the mixture was stirred for 10 days, H_2O (250 mL) and DCM (250 mL) were added, and the aqueous phase was extracted with DCM (2 × 100 mL). The combined solvent extract was concentrated in vacuo, and separation of the residue (CC, 9:1 *n*-hexane−EtOAc) led to **10** as a light tan, crystalline solid (1.9 g, 15%); mp 39.4−39.9 °C; IR (neat) ν_{max} 1738 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.63 (1H, dd, J = 2.7, 1.2 Hz, H-6), 7.32 (1H, dd, J = 9.9, 2.7 Hz, H-4), 6.28 (1H, dd, J = 1.2, 9.9 Hz, H-3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 171.0 (C-2), 146.7 (C-4), 133.8 (C-6), 120.9 (C-3), 100.6 (C-5).

*5-(Trimethylstannyl)-2H-pyran-2-one (**11**) (ref 10).* To a stirred solution of pyrone **10** (0.70 g, 4.00 mmol) in THF (7 mL) at rt under Ar were added $Pd(PPh_3)_4$ (0.20 g, 0.173 mmol) and hexamethylditin (5.0 g, 15.3 mmol), and the solution was heated to reflux for three days. The black mixture was cooled to rt and concentrated in vacuo. Separation of the residue (CC, 3:17 EtOAc−*n*-hexane) yielded **11** as a colorless oil (0.73 g, 70%): IR (neat) ν_{max} 1736 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.23 (1H, dd, J = 2.4, 9.3 Hz, H-4), 7.17 (1H, dd, J = 1.8, 2.4 Hz, H-6), 6.26 (1H, dd, J = 1.8, 9.3 Hz, H-3), 0.25 (9H, s, $(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0 (C-2), 146.7 (C-4), 134.6 (C-6), 120.9 (C-3), 111.8 (C-5), −2.1 (3 × $-CH_3$).

*Bis(5α-bufa-16,20(21),22-trienolide-[2,3-b:2′,3′-e])pyrazine (**12**).* To a stirred solution of triflate **8** (0.90 g, 1.08 mmol) and pyrone **11** (0.64 g, 2.45 mmol) in THF (10 mL at rt under Ar) were added LiCl (0.68 g, 16.0 mmol) and $Pd(PPh_3)_4$ (0.220 g, 0.190 mmol), and the mixture was heated under reflux for three days. After the solution had cooled to rt, brine (10 mL) and DCM (10 mL) were added successively, and the aqueous phase was extracted with DCM (3 × 10 mL). The combined organic phase was concentrated in vacuo, and the residue was separated (CC, 1:20 acetone−*n*-hexane) to afford **12** as an amorphous solid. Crystallization from cyclohexane−acetone provided bis-bufadienolide **12** as colorless needles (0.24 g, 31%): mp 242 °C (dec); IR (neat) ν_{max} 1722 cm^{-1}; ^1H NMR (126 MHz, $CDCl_3$) δ 7.25 (1H, dd, J = 2.4, 9.3 Hz, H-22), 7.19 (1H, dd, J = 1.8, 2.4 Hz, H-21), 6.46 (1H, dd, J = 1.8, 9.3 Hz, H-23), 5.87 (1H, m, H-16), 2.92 (1H, d, J = 8.8 Hz, H-1α), 2.70 (1H, dd, H-4α), 2.49 (2H, m, H-1β, H-4β), 1.84−1.11 (16H), 0.95 (3H, s, CH_3), 0.91 (1H, m), 0.85 (3H, s, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 146.9, 145.3, 135.1, 129.7, 127.7, 123.8, 120.9, 113.1, 53.9, 53.1, 45.5, 44.1, 41.9, 35.7, 34.4, 32.5, 32.1, 29.6, 29.1, 28.6, 27.1, 19.1, 14.2, 11.5; HRMS (APCI$^+$) m/z 725.4317 $[M + H]^+$ (calcd for $C_{48}H_{57}N_2O_4$, 725.4318); anal. C 79.36, H 7.92, N 3.79%, calcd for $C_{48}H_{56}N_2O_4$, C 79.52, H 7.79, N 3.86%.

*Aldehyde **15**.* To a solution of alcohol **14**[1] (120 mg, 0.183 mmol) in 2-propanol (10 mL) was added MnO_2 (0.4 g, 4.58 mmol), and the mixture was stirred at rt. After 24 h the mixture was filtered through Celite, which was washed with DCM. Removal of solvent from the combined filtrate yielded the crude product as a solid. Purification (CC, 30% DCM−MeOH) and crystallization from DCM−EtOAc yielded **15** (80 mg, 0.122 mmol, 66%): mp 250 °C (dec); $[\alpha]^{25}{}_D$ +29.8 (*c* 0.48, DCM); IR (film) ν_{max} 3368, 3275, 2915, 1666, 1400, 1153 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.11 (2H, d, J = 8.7 Hz, H-21), 5.80 (2H, d, J = 8.7 Hz, H-20), 4.57 (2H, s, H-11), 3.13 (2H, d, J = 16.2 Hz, H-1β), 2.86 (2H, dd, J = 5.6, 18.2 Hz, H-4α), 2.74−2.58 (12H, m), 1.35 (6H, s), 1.13 (6H, s), 2.05−0.94 (22H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.7 (C-21), 179.1 (C-17), 148.6 (C-2/3), 148.5 (C-2/3), 123.4 (C-20), 67.8 (C-11), 56.94, 56.87, 48.1, 46.6, 45.0, 42.2, 35.9, 34.8, 33.1, 32.0, 30.7, 27.8, 23.8, 21.5, 14.4; HRMS (APCI$^+$) m/z 653.4236 $[M + H]^+$ (calcd for $C_{42}H_{57}N_2O_4$, 653.4318); anal. C 70.99, H 9.06, N 3.89%, calcd for $C_{42}H_{56}N_2O_4{\cdot}4CH_3OH$, C 70.74, H 9.29, N 3.59%.

*Bis-dihydro-4-pyrone **16**.* To a solution of aldehyde **15** (15 mg, 0.023 mmol) and Danishefsky's diene (10 μL, 0.051 mmol)[14] at −78 °C in DCM (10 mL) under Ar was added $BF_3{\cdot}Et_2O$ (12 μL, 0.012 mmol). The reaction mixture was stirred for 2 h at −78 °C and then allowed to equilibrate to rt. After 48 h, no change was detectable by TLC, and an additional 12 μL of $BF_3{\cdot}Et_2O$ was added. The reaction mixture was stirred at rt for 14 days and then quenched with $NaHCO_3$ (saturated aqueous) and extracted with EtOAc. Purification (CC, 7:3 acetone−DCM) led to pyrazine **16** (3 mg, 17%): $[\alpha]^{25}{}_D$ +106.2 (*c* 0.10, DCM); IR (neat) ν_{max} 3363 (OH), 1672, 1401 (pyrazine ring) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.38 (1H, d, J = 6.0 Hz, H-22), 7.35 (1H, d, J = 6.0 Hz, H-22), 5.42 (2H, dd, J = 1.0, 6.5 Hz, H-23), 5.20 (2H, m, J = 2.5, 8.5 Hz, H-20), 5.03−4.95 (2H, m, H-21), 4.55

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(2H, s, H-11), 3.13 (2H, d, J = 16 Hz, H-1β), 2.82 (2H, bd, J = 17 Hz), 2.68−2.53 (4H, m, H-25), 2.44−2.36 (2H, m), 2.31−2.21 (2H, m), 2.06−1.83 (6H, m), 1.68−1.42 (8H, m), 1.14−1.05 (10H, m), 0.98−0.83 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.3 (C-24), 163.4 (C-22), 148.7 (C-2/3 *or* C-17), 148.4 (C-2/3 *or* C-17), 119.2 (C-20), 106.9 (C-23), 68.3, 67.9, 57.6, 54.6, 45.6, 45.1, 44.2, 42.5, 36.0, 34.9, 32.1, 31.0, 29.7, 27.9, 27.4, 24.2, 20.4, 14.4; HRMS (APCI$^+$) m/z 789.4840 [M + H]$^+$ (calcd for $C_{50}H_{65}N_2O_6$, 789.4843).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra and a COSY spectrum of compound **16**.[17] This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(17) The spectra of the other compounds characterized in this report are no longer available.