JOURNAL OF NATURAL PRODUCTS

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The Cephalostatins. 21. Synthesis of Bis-steroidal Pyrazine Rhamnosides[1]

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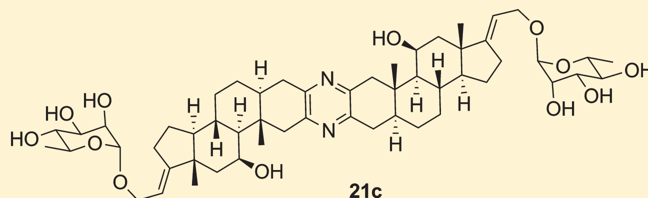
Supporting Information

ABSTRACT: The synthesis of bis-steroidal pyrazines derived from 3-oxo-11,21-dihydroxypregna-4,17(20)-diene (**4**) and glycosylation of a D-ring side chain with α-L-rhamnose have been summarized. Rearrangement of steroidal pyrazine **10** to **14** was found to occur with boron triflouride etherate. Glycosylation of pyrazine **10** using 2,3,4-tri-*O*-acetyl-α-L-rhamnose iodide led to 1,2-orthoester-α-L-rhamnose pyrazine **17b**. By use of a persilylated α-L-rhamnose iodide as donor, formation of the orthoester was avoided. Bis-steroidal pyrazine **10** and rhamnosides **17b** and **21c** were found to significantly inhibit cancer cell growth in a murine and human cancer cell line panel. Pyrazine **9** inhibited growth of the nosocomial pathogen *Enterococcus faecalis*.



In 1988, we reported the isolation and X-ray crystal structure determination of cephalostatin 1 (**1**), a complex tridecacyclic pyrazine from the South African marine tube worm *Cephalodiscus gilchristi*.[2a] Cephalostatin 1 was found to exhibit an extraordinarily powerful cytostatic activity (GI_{50} 1.2−4.2 nM) against the U. S. National Cancer Institute (NCI) human cancer cell line panel.[3,4] To date, we have isolated and determined the structures of 19 cephalostatins,[2b] which were later augmented by 25 structurally related ritterazines from the Japanese tunicate *Ritterella tokioka* that were isolated and characterized by the Fusetani group.[5] A new total synthesis of cephalostatin 1,[4a] the 2009 review by Fuchs,[4b] and important summaries by Vollmar,[4c] Moser,[4d] Winterfeldt,[4e] and Morzycki[4f] provide a very comprehensive view of the chemical and biological advances inspired by these remarkably powerful anticancer marine natural products.

The low bioavailability and biological importance (including a unique apoptotic/mitochondria mechanism) of the cephalostatins and ritterazines, in combination with the challenging molecular architecture, has stimulated a broad spectrum of organic synthesis efforts in a number of laboratories. These include the classic (65 steps) first total synthesis of cephalostatin 1 by Fuchs,[6] a second enantioselective synthesis by Shair,[4a] and a variety of simpler structural modifications.[4,7] Many of the analogues synthesized have been shown to be only weakly cytotoxic, even the ones that closely resemble the cephalostatins by incorporating a spiroketal unit. Important insights into the mechanism of action of cephalostatin 1 and other members of the series were not revealed until Vollmar[1b,4c] and colleagues discovered a new mode of apoptosis events. Cephalostatin 1 was found to induce a novel pathway of mitochondrial activation that selectively releases Smac/DIABLO (second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with a low isoelectric point), leading to shrunken mitochondria and apoptosis. In contrast, certain other anticancer drugs, such as staurosporine, that affect the mitochondria cause swelling and apoptosis.[1b]

The cephalostatins, ritterazine B,[5b] and schweinfurthin A[8a] share some structural features and cancer cell line inhibitory activity with certain polyhydroxylated steroids found in nature, particularly the steroidal glycoside OSW-1 (**2**),[8b−e] a saponin that belongs to a large family of glycoconjugates with a wide spectrum of biological and pharmacological activities.[9a−c] OSW-1 (**2**) has been considered structurally analogous to a masked spiroketal, and it displays a profile and potency similar to cephalostatin 1 (**1**): an NCI COMPARE analysis of **2** showed a reasonable correlation with **1** of 0.6−0.83.[8d,10a] The structure of OSW-1 (**2**) is related to that of cephalostatin 1 (**1**) through an intermediate formed by loss of the disaccharide unit and water.[10b] However, SAR studies imply that the sugar is important for cytotoxic activity.[10c] While the OSW-1 disaccharide unit is composed of L-arabinose and D-xylose, other steroid/triterpene-type cancer cell line growth inhibitors such as solamargine (**3**), a steroidal alkaloid glycoside,[11] and the pentacyclic triterpene hederacolchiside A_1[12] are α-L-rhamnopyranosides. A selection of recent examples include α-L-rhamnopyanose glycosides of the steroidal sapogenin diosgenin[13a] and the lupane pentacyclic triterpene betulinic acid.[13b] Because of the increasing evidence that the sugar groups of active steroidal glycosides play a key role[14] in their biology and as part of our investigation into structural modifications of compound **1** with cancer cell growth inhibitory activity, we undertook the syntheses of bis-steroidal pyrazines related to cephalostatin 1 that have a C-17 side chain designed for coupling with an α-L-rhamnose donor.

Received: May 13, 2011
Published: September 07, 2011

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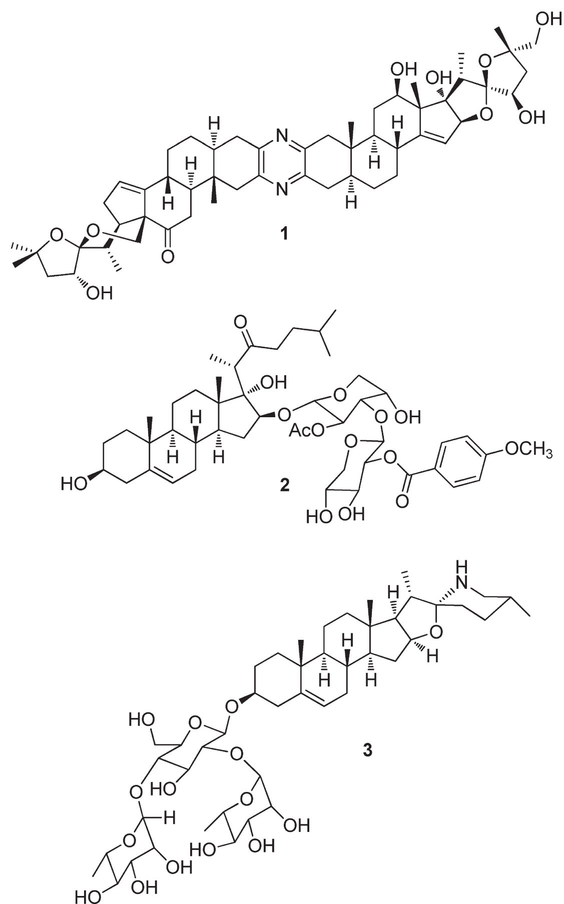
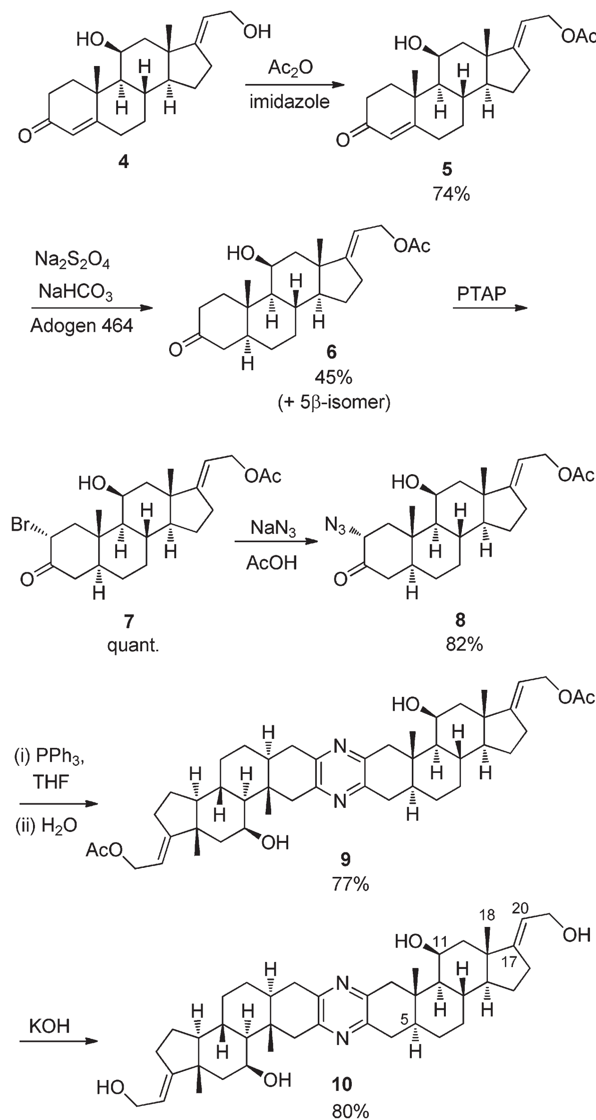


Scheme 1



RESULTS AND DISCUSSION

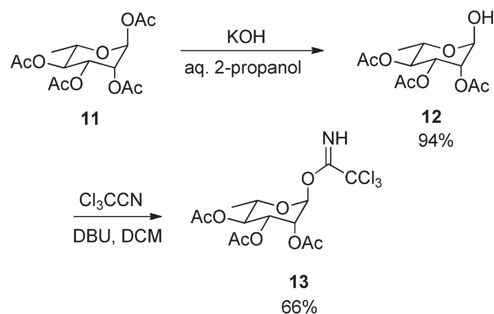
Selective acetylation of the C-21 alcohol group of 3-oxo-11,21-dihydroxypregna-4,17(20)-diene (**4**) was accomplished with acetic anhydride/imidazole to afford acetate **5** (74% yield). Regioselective reduction of the $\Delta^{4,5}$ bond with sodium dithionite using phase transfer catalysis afforded a mixture of 5α/*β*-isomers (75% yield, α-isomer in slight excess). The 5α-isomer (**6**) was isolated (45% yield) by fractional recrystallization from acetone (Scheme 1).

Methods for the synthesis of symmetrical bis-steroidal pyrazines are now well established and generally involve reduction of a 2-azido-3-oxo steroid.[7e,g] Therefore, bromination of 3-ketone **6** with phenyltrimethylammonium perbromide (PTAP) to give the 2-bromo-3-oxo steroid **7** in quantitative yield was first carried out. Conversion of bromide **7** to 2-azido-3-oxo **8** was accomplished by reaction with sodium azide in DMF, acetic acid being added to the reaction medium to inhibit decomposition of azide **8** to a 2-enamino-3-ketone.[7h]

The reduction of 2-azido-3-oxo steroids commonly used in the synthesis of bis-steroidal pyrazines has generally utilized direct hydrogenation or triphenylphosphine (Staudinger reaction) for conversion to a 2-amino-3-oxo intermediate, which in turn undergoes self-condensation to a dihydropyrazine. Subsequent oxidation of the dihydropyrazine affords the desired pyrazine. The Staudinger reaction was chosen for the reduction of azide **8** in order to avoid hydrogenation of the exocyclic double bond at C-17, which had been found to be readily reduced during a small-scale hydrogenation of azide **8**. Thus, treatment (20 h) of azide **8** (in THF) with triphenylphosphine in THF, followed by addition of water and exposure to air, gave the C-2-symmetrical pyrazine **9** in 77% yield. In previous reports of triphenylphosphine-induced dimerization of 3-azido-2-oxo steroids,[7f−h] the azide and triphenylphosphine were first mixed, and several minutes later tetrahydrofuran was added. When we repeated those conditions, the yields of the pyrazine were consistently lower (23−40%) than when we used the THF solutions of the reactants.

Attempts to crystallize pyrazine **9** were unsuccessful and instead gave precipitates or moist glassy foams. However, the pyrazine was easily identified by NMR spectroscopy, which shows the disappearance of the H-3 signals and two new signals at δ 148 in the carbon spectrum, typical of pyrazine carbon atoms. Deacetylation of pyrazine **9** with potassium carbonate in aqueous methanol gave diol **10** in low yield (10−32%). However, use of KOH in methanol considerably increased the yield (80%). Because of its low solubility in some organic solvents, the extraction of **10** from the aqueous layer was monitored by TLC. Pyrazine **10** was considered ideally suited for glycosylation reactions, with four available OH groups. Since the 11*β*-alcohol groups were expected to be considerably hindered by the C-18

Scheme 2



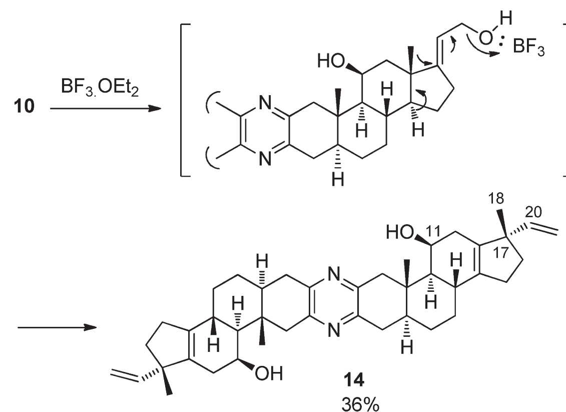
angular methyl groups, it was assumed that they would provide regioselectivity in a glycosylation step.

Next, we converted α-L-rhamnose to a trichloroacetimidate derivative. This type of reagent is widely used in carbohydrate syntheses and in glycosylation of steroids such as OSW-1.[14−16] Peracetylated α-L-rhamnose (**11**)[17] was obtained in 95% yield from α-L-rhamnose, and the anomeric acetate was selectively hydrolyzed with KOH[18] to give hemiacetal **12**.[19] Reaction of **12** with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene afforded 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**13**)[19] in 59% overall yield (Scheme 2).

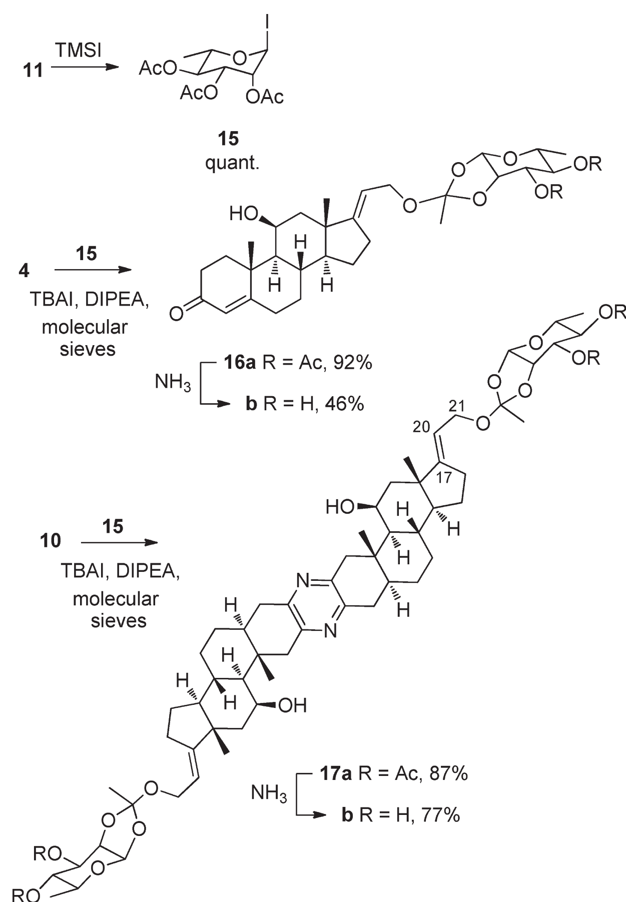
Boron trifluoride-catalyzed coupling of trichloroacetimidate **13** with pyrazine **10** gave a very complex mixture. Initially, this was thought to arise from some lack of selectivity between the allylic and 11*β* alcohols. The reaction mixture NMR spectrum showed the disappearance of the signal for the allylic proton (H-20) and a new set of signals at low field (δ ~5.8). That analysis suggested possible rearrangement of the steroid skeleton. Steroids with a (17)20 exocyclic double bond or possessing a good leaving group at C-21 are prone to Wagner−Meerwein rearrangement under acidic conditions with concomitant loss of water[20,21] and migration of the C-18 angular methyl group to C-17.[21] The main component isolated from the glycosylation reaction mixture was analyzed. The ^{13}C NMR spectrum showed two extra sp^2-hybridized carbons at δ 135.8 and 138.3, and a low-field signal appeared at δ 5.79 in the ^{1}H NMR spectrum. The NMR analyses augmented by HRMS data allowed assignment of structure **14**. Presumably, the rearrangement proceeds through a BF_3-mediated loss of the C-21 OH followed by migration of the 18-methyl group (Scheme 3). The nature of this concerted rearrangement indicates that the migrating methyl group should reside on the *β*-face of the steroid skeleton. The relative configuration of olefin **14** was established by comparison with the product from an analogous $BF_3 \cdot Et_2O$-promoted rearrangement of dienediol **4**. Glycosylation of pyrazine **10** with trichloroacetimidate **13** was repeated using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the activator, but the only product isolated was again the product of rearrangement (**14**). That result clearly pointed to the need for a glycosylation method employing neutral or basic conditions.

Carbohydrate iodides have been used in glycosylations and found to be efficient glycosyl donors under basic catalysis in the presence of diisopropylethylamine (DIPEA) and tetrabutylammonium iodide (TBAI).[22] Therefore, we prepared 2,3,4-*O*-acetyl-α-L-rhamnopyranosyl iodide **15**[23] from α-L-rhamnose peracetate (**11**) (Scheme 4). That was easily accomplished by selective substitution of the anomeric acetate by iodide using iodotrimethylsilylane

Scheme 3



Scheme 4



(TMSI).[22] Next, dienediol **4** was used as a model compound for coupling rhamnose iodide **15** with pyrazine **10**. Condensation of **15** with alcohol **4** in the presence of DIPEA and TBAI gave rhamnoside **16a** in 92% yield. The appearance of clear ^{1}H NMR signals from the H-20 and H-21 protons confirmed that rearrangement of the steroid nucleus had not occurred. Another important feature of the ^{1}H NMR spectrum was a shift to higher field of one of the rhamnoside acetate signals, from their normal values of δ 2.0−2.5 to δ 1.74. Such a chemical shift is characteristic of methyl

orthoesters, which are sometimes formed between acceptors with low reactivity and carbohydrate donors with ester-protecting groups at C-2. Identification of the site of glycosylation was inferred from a shift of the H-21 protons to higher field, while the signal for H-11α remained similar to that in alcohol **4**. Orthoester **16a** slowly decomposed to dienediol **4** and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside even when kept at 0 °C, possibly through the presence of small traces of acid or moisture that catalyze the reverse reaction.

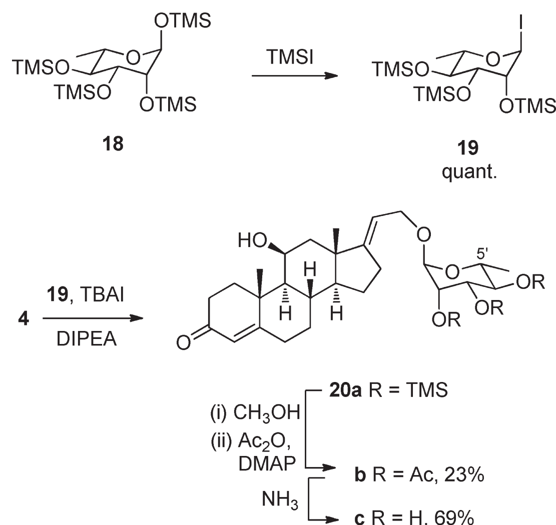
Treatment of 1,2-orthoesters of type **16a** with a catalytic amount of TMSOTf is known to promote rearrangement of the orthoester to a 2,3,4-glycoside.[24] The major drawback in cleavage of **16a** was clearly the potential sensitivity of this steroid skeleton to acidic conditions. As predicted, when orthoester **16a** was treated with a catalytic amount of TMSOTf only, a complex mixture was obtained, and the ^{1}H NMR spectrum showed signals resulting from the C-18 rearrangement. Other Lewis acids such as $Cu(OTf)_2$[25] gave results similar to TMSOTf, and by contrast $Yb(OTf)_3$ promoted no reaction even at 0.5 equivalents. When the orthoester (**16a**) was deacetylated with NaOMe in MeOH, triol **16b** was obtained in only 12% recovery. However, with use of ethanolic ammonia, triol **16b** was realized in 46% yield. Interestingly, **16b** proved to be more stable than its parent (**16a**), as noted from a ^{1}H NMR spectrum recorded following several months of storage at ambient temperatures.

The lability of orthoester **16a** suggested the possibility that a similar treatment of pyrazine **10** could provide a useful orthoester prodrug modification. Coupling of **10** with rhamnose iodide **15** under the same conditions used for dienediol **4** gave, following a three-day reaction period, orthoester pyrazine **17a** in 87% yield (Scheme 4). TLC showed the formation of an initial intermediate (the monorhamnosylated pyrazine) that slowly disappeared to give rise to the new, less polar **17a**. Deacetylation of orthoester **17a** was realized in ethanolic ammonia solution by simple evaporation of the solvent to yield deprotected pyrazine **17b** in 77% yield. The pyrazine orthoesters **17a** and **17b** were found to be quite stable, in contrast to their monosteroidal counterpart **16**. The increased stability is probably due to the moderate pyrazine basic properties, which inhibit acid-catalyzed orthoester decomposition.

A useful approach to avoid orthoester formation in glycosylations is to use an ether protecting group at C-2. Benzyl[22,26] and trimethylsilyl ether[27] hexose iodides have been reported to participate successfully in glycosylation reactions. The *O*-trimethylsilyl-protected carbohydrate iodides are the simplest and more advantageous to employ, as the TMS group can be easily cleaved by heating at reflux in methanol. Thus, persilylation of anhydrous α-L-rhamnose with chlorotrimethylsilane in the presence of triethylamine in DMF gave persilyl α-L-rhamnose **18**[28] as a clear oil in 87% yield. Reaction with TMSI afforded 2,3,4-*O*-trimethylsilyl-α-L-rhamnopyranosyl iodide (**19**), which was used immediately (Scheme 5).

When dienediol **4** was allowed to react with iodide **19**, followed by cleavage of TMS from the product (**20a**) and acetylation, glycoside **20b** was formed in 23% overall yield. Because of the axially configured 2-position, assignment of the glycoside as α or β using $^{3}J_{1,2}$ coupling constants (0 to ≤3 Hz in ^{1}H NMR) proved difficult. Although the anomeric configuration can be determined by use of ^{1}H-coupled ^{13}C NMR, with the $^{1}J_{CH}$ constants ranging from 170 (for α) to 160 Hz (β),[29] inspection of the coupled spectrum of rhamnoside **20b** did not provide unequivocal evidence. However, as described below, a comparison of the spectroscopic data with those from known substances

Scheme 5



of established anomeric configuration allowed a good correspondence of the ^{1}H and ^{13}C NMR signals of **20b** to an α-configuration, and the assignment was confirmed by synthesis of the α- and β-rhamnosylated pyrazines.

The configuration of the substituent at C-1 has been reported to have a characteristic effect upon the chemical shift of C-5 in rhamnose derivatives.[30] With an α-OAc at C-1, the C-5 signal appears in the range δ 68.5 to 71.0. When C-1 bears a free α-OH, α-OME, or α-OBn, the C-5 signal appears below δ 69.0. If C-1 bears a β-OH or β-OAc, the resonance line of C-5 is found to appear above δ 71.4. These relationships were further supported by spectroscopic data from a pair of rhamnose-containing α- and β- disaccharides, of which the configuration at C-1 was confirmed by their anomeric $^{1}J_{CH}$ coupling constants (172 Hz for the α- and 156 Hz for the β-rhamnoside); the resonance line for C-5 in the α-rhamnoside appears at δ 66.5 and that of the β-rhamnoside at δ 71.0.[31] Rhamnoside **20b** displays a signal for C-5′ at δ 66.5 (H-5′ appears at δ 3.89−3.93 as a multiplet), confirming the presence of the α-rhamnoside in the assigned structure. Compound **20b** was deacetylated with ethanolic ammonia to produce diene **20c**. The ^{13}C NMR signal of C-5′ in rhamnoside **20c** appears at δ 69.8, also consistent with the α-rhamnoside assignment.

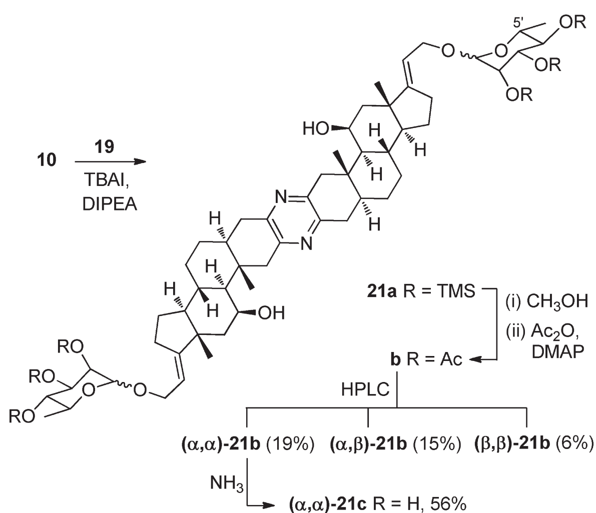
Once acceptable reaction conditions for glycosylation of the alcohol **4** side chain were in hand, pyrazine **10** was coupled with persilylated rhamnose iodide **19** (Scheme 6). The glycosylation of pyrazine **10** was slow at room temperature, probably owing to the low solubility of the pyrazine in CH_2Cl_2, and consumption of all of the starting material required heating at reflux for four days. After the crude product was heated in refluxing MeOH in order to cleave the TMS groups, attempts were made to isolate the deprotected pyrazine rhamnoside directly, but separation of the mixture was not fully achieved using a variety of chromatographic techniques. Analysis of the mixture by HPLC showed the presence of two major components, but their retention times were almost identical. Acetylation of the mixture was performed with acetic anhydride/pyridine to simplify purification. While the acetylated mixture was barely resolved by silica gel TLC, on neutral alumina two spots were clearly distinguished although still with little separation. Following partial separation of the products by column chromatography, complete resolution by

HPLC yielded three pyrazine rhamnosides. The major component isolated in 19% yield (from **10**) was easily identified as the α,α′-dirhamnoside acetate by comparison with **20b** and assigned structure (α,α)-**21b**. The ^{1}H and ^{13}C NMR spectra of this product were almost superimposable on those of **20b**, the resonance line of C-5′ appearing at δ 66.5 and that of H-5′ as a multiplet at δ 3.90−3.95.

A second component obtained in 15% yield was considered to be an unsymmetrical pyrazine because of the appearance in the ^{1}H NMR spectrum of two sets of doublets for the H-1β protons. Also, the rhamnose and steroidal H-20 and H-21 protons appeared duplicated. On the symmetrical pyrazine these H-1β protons resonate at the same frequency. After careful spectroscopic analyses this product was identified as the α,β-dirhamnose (α,β)-**21b**. Again, the resonance signals of H-5′ and C-5′ were diagnostic. Two multiplets for two different H-5′ signals corresponding to different rhamnose residues, one at δ 3.90−3.95 and another at δ 3.46−3.52, were correlated to two signals in the carbon spectrum at δ 66.4 and δ 70.4, respectively. As noted above, these chemical shifts agree with those for an α-rhamnoside and for a β-rhamnoside, respectively.[31] The H-1′ signals for the α-rhamnoside appear at δ 4.79 and for the β-rhamnoside at δ 4.67.

The third reaction product obtained in low yield (6%) was identified as β,β′-dirhamnoside (β,β)-**21b**. Analysis of the NMR spectra of this isomer provided confirmation of the structure and proton assignment for the unsymmetrical (α,β)-**21b**. In order to complete this phase of our cephalostatin 1 structure−activity (SAR) research, the hexaacetate (α,α)-**21b** was deacetylated with ethanolic ammonia to give, following recrystallization from MeOH, deprotected (α,α)-**21c** in 56% yield. Research continues in our laboratory to further study the potential use of tetraol **10** as an intermediate for obtaining more potent structural modifications of cephalostatin 1.

Scheme 6



Biological Activity. The cancer cell growth inhibitory properties were examined using the murine P388 lymphocytic leukemia cell line (Table 1), and selected compounds were tested against a range of human cancer cell lines (Table 2). With the P388 assay, pyrazine **10** displayed the most significant cancer cell growth inhibitory activity, followed by orthoester **16b** and 2-bromosteroid 7. The biological activity of orthoester **16b** is consistent with expectations based on such functional groups in naturally occurring substances, e.g., the marine steroidal orthoester designated orthoesterol B (active against feline leukemia virus and other viruses).[32] Interestingly, bis-steroidal pyrazine orthoester **17b** was less active than the related monosteroid orthoester **16b**. Three of the SAR targets (**10**, **17b**, and α,α-**21c**) were selected for more cancer cell line evaluations employing a representative human cancer cell line minipanel. All three bis-steroidal pyrazines showed significant inhibition of cancer cell growth.

Pyrazines **9**, **10**, and **17b** were evaluated against a panel of 10 bacteria and fungi. Pyrazine **9** selectively inhibited the growth of *Enterococcus faecalis* (minimum inhibitory concentration = 32 μg/mL), an important nosocomial pathogen that frequently causes urinary tract infections, wound infections, endocarditis, and bacteremia. At 64 μg/mL, pyrazines **10** and **17b** were not active in broth microdilution assays.

Table 1. Murine P388 Lymphocytic Leukemia Cell Line Incubation Results

compound	ED_{50} (μg/mL)	compound	ED_{50} (μg/mL)
5	>100	**17a**	>10
6	16.6	**17b**	>10
7	6.6	**20b**	>10
8	12.0	**20c**	>100
9	77.2	(α,α)-**21b**	>10
10	1.5	(α,β)-**21b**	>10
14	>10	(β,β)-**21b**	>10
16a	9.5	(α,α)-**21c**	>10
16b	5.2		

EXPERIMENTAL SECTION

General Experimental Procedures. Unless otherwise noted, all reagents were obtained from commercial suppliers. Except for THF, which was distilled under argon from sodium/benzophenone immediately prior to use, other anhydrous solvents were obtained from commercial suppliers. Triethylamine was distilled under argon from CaH_2. All reactions involving air- or moisture-sensitive reagents were conducted under an atmosphere of argon in septum-sealed flasks. Transfer of reagents was accomplished by standard syringe techniques. Organic extracts of aqueous solutions were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure using a rotary evaporator. Column chromatography (CC) was carried out using Merck 60 silica gel. Reaction mixtures and chromatography fractions were analyzed employing Analtech 250 μm silica gel GHLF plates and developed with phosphomolybdic acid (10% in EtOH). Semipreparative HPLC was performed with a Gibson model 805 HPLC coupled with a

Table 2. Human Cancer Cell Line Evaluation Results (GI_{50}, μg/mL)

compound	BXPC-3 (pancreas)	MCF-7 (breast)	SF-268 (CNS)	NCI-H460 (lung)	KM20L2 (colon)	DU-145 (prostate)
10	1.3	1.3	1.7	2.2	1.7	1.8
17b	2.1	2.8	3.0	3.8	3.3	3.0
(α,α)-**21c**	1.8	1.8	1.8	1.8	1.8	2.0

Gibson model 117 UV detector. Analytical HPLC was conducted with a Hewlett-Packard model 1050 HPLC coupled with a H−P diode-array detector.

Melting points were determined using an Olympus electrothermal melting point unit and are uncorrected. The optical rotation data were recorded with a Perkin-Elmer 241 polarimeter. IR spectra were recorded using a Thermo Nicolet Avatar 360 infrared spectrometer as thin films or solid samples. NMR spectra were measured at 300 (Varian XL-300), 400, or 500 MHz (Varian UNITY INOVA) for ^{1}H and at 100 or 125 MHz for ^{13}C using TMS as an internal reference (*J* values are given in Hz). NMR assignments are based on 2D spectra (COSY and HMQC) obtained using standard software.

Elemental analyses were performed by Galbraith Laboratories. High-resolution mass spectra were obtained with a JEOL LCMate magnetic sector instrument either in FAB mode with a glycerol matrix or by APCI with a polyethylene glycol reference. Fast-atom bombardment mass spectrometry was provided by the Washington University Mass Spectrometry Resource with support from the NIH National Center for Research Resources (Grant No. P41RR0954).

*3-Oxo-11β-hydroxy-21-acetoxypregna-4,17(20)-diene (**5**).* A mixture of dienediol **4** (Upjohn Co., 8.0 g, 24.20 mmol), acetic anhydride (115 mL), and imidazole (3.3 g, 48.4 mmol) in $CHCl_3$ (120 mL) was heated at reflux for 3 h. After cooling, the reaction mixture was diluted with $CHCl_3$ (100 mL), washed with HCl (10%), saturated aqueous $NaHCO_3$, and brine, and dried. The solvent was removed to yield a yellow solid that was crystallized from EtOAc to afford acetate **5** as pale yellow crystals (6.65 g, 74%): mp 190 °C (EtOAc); $[\alpha]^{24}_D$ +148.2 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3409, 1727, 1652, 1245, and 1231 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.17 (3H, s, H-18), 1.45 (3H, s, H-19), 2.06 (3H, s, Ac), 0.98−2.55 (17H, m), 4.41 (1H, br d, *J* 3.3 Hz, H-11α), 4.59−4.72 (2H, m, H-21), 5.23 (1H, tt, *J* 1.8, 7.3 Hz, H20), 5.68 (1H, d, *J* 1.8 Hz, H4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.9, 21.0, 21.1, 23.9, 30.9, 31.0, 32.0, 32.4, 33.8, 34.0, 39.2, 43.9, 46.5, 56.3, 56.7, 60.8, 68.3, 113.3, 122.3, 156.0, 171.1, 172.0, 199.5; MS (APCI) *m/z* 373 [M + H]$^+$; *anal.* C 73.89, H 8.76%, calcd for $C_{23}H_{32}O_4$, C 74.19, H 8.60%.

*3-Oxo-11β-hydroxy-21-acetoxy-5α-pregna-47(20)-ene (**6**).* To a solution of enone **5** (6.65 g, 17.85 mmol) in toluene (120 mL) was added a solution of $Na_2S_2O_4$ (18.65 g, 107.1 mmol)[33] and $NaHCO_3$ (9.00 g, 107.1 mmol) in H_2O (12 mL), followed by Adogen 464 (2.2 mL), and the mixture was vigorously stirred under reflux until most of the starting material was consumed (1−2 h). Longer reaction times at full consumption of the starting material caused partial reduction of the C-3 ketone. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The organic phase was washed with brine and dried, and the solvent was removed. Fractional crystallization from acetone gave the 5α-isomer **6** as colorless needles (3.03 g, 45%): mp 218−219 °C (acetone); R_f 0.31 (17:3 DCM−EtOAc); $[\alpha]^{24}_D$ +56.2 (*c* 1.27, $CHCl_3$); IR (film) ν_{max} 3449, 2912, 1721, 1697, 1656, 1233 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.78−2.53 (19H, m), 1.14 (3H, s), 1.27 (3H, s), 2.06 (3H, s), 4.37 (1H, br t, *J* 3.3), 4.59−4.72 (2H, m), 5.22 (1H, tt, *J* 1.9, 7.5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2, 20.0, 21.1, 24.0, 28.3, 30.7, 31.1, 31.9, 35.8, 38.0, 38.2, 44.10, 44.14, 46.4, 47.7, 57.0, 57.5, 60.8, 68.5, 113.1, 156.4, 171.1, 212.0; MS (FAB) *m/z* 381 [M + Li]$^+$, 375 [M + H]$^+$; *anal.* C 73.65, H 9.47%, calcd for $C_{23}H_{34}O_4$ C 73.76, H 9.15%.

The 5β-isomer (2.69 g, 40%) was obtained as a colorless crystalline powder and identified by its proton spectrum: R_f 0.4 (17:3 DCM−EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03−2.56 (19H, m), 1.15 (3H, s), 1.27 (3H, s), 2.06 (3H, s), 2.64 (1H, t, *J* 14.5, H-5β), 4.34 (1H, br t, *J* 3.3), 4.59−4.73 (2H, m), 5.23 (1H, tt, *J* 1.9, 7.5).

*2α-Bromo-3-oxo-11β-hydroxy-21-acetoxy-5α-pregna-17(20)-ene (**7**).* To a solution of ketone **6** (2.57 g, 6.86 mmol) in dry THF (50 mL) under argon that was cooled to 0 °C was added rapidly phenyltrimethylammonium perbromide via cannula. The resulting orange solution faded rapidly to a pale yellow color, which gradually disappeared with stirring at 0 °C for 20 min. The reaction was terminated with brine (20 mL) and extracted with EtOAc. The organic layer was washed with brine (50 mL), dried, concentrated, and filtered through a pad of silica gel (2:1 hexane−EtOAc as eluent) to provide bromoketone **7** (3.08 g, 99%) as a colorless powder. The analytical sample was obtained by crystallization from hexane−ether (87% yield): mp 155−156 °C; $[\alpha]^{24}_D$ +53.3 (*c* 0.12, $CHCl_3$); IR (film) ν_{max} 3531, 2924, 1725, 1236, 1022, 734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.78−2.49 (17H, m), 1.14 (3H, s), 1.34 (3H, s), 2.06 (3H, s), 2.82 (1H, dd, *J* 6.0, 12.4), 4.35 (1H, br d, *J* 3.2), 4.58−4.71 (2H, m), 4.83 (1H, dd, *J* 6.0, 13.6), 5.23 (1H, tt, *J* 2.0, 7.6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.7, 19.9, 21.1, 24.0, 27.8, 30.3, 31.1, 31.7, 39.1, 43.4, 44.0, 46.4, 48.5, 51.2, 54.4, 56.8, 57.2, 60.8, 68.3, 113.3, 156.2, 171.1, 201.2; MS (APCI) *m/z* 453/455 [M + H]$^+$; *anal.* C 60.71, H 7.70%, calcd for $C_{23}H_{33}O_4Br$, C 60.93, H 7.34%.

*2α-Azido-3-oxo-11β-hydroxy-21-acetoxy-5α-pregna-17(20)-ene (**8**).* To a solution of bromoketone **7** (0.99 g, 2.19 mmol) in DMF (20 mL) were added acetic acid (0.13 mL) and sodium azide (0.543 g, 8.36 mmol). The mixture was stirred at room temperature (rt) for 2.5 h and then poured onto ice−water. The precipitate was collected by filtration, washed with H_2O, and dried in a desiccator over P_2O_5. The crude solid was purified further by dry-column chromatography (eluent: 5−15% EtOAc−toluene) to give pure azidoketone **8** as a colorless foam (0.75 g, 82%): R_f 0.36 (9:1 toluene−EtOAc); mp 58−60 °C; $[\alpha]^{24}_D$ +201.6 (*c* 0.06, $CHCl_3$); IR (film) ν_{max} 3504, 2924, 3103, 1725, 1236, 1022, 734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82−2.52 (17H, m), 1.11 (3H, s), 1.30 (3H, s), 2.03 (3H, s), 4.04 (1H, dd, *J* 5.8, 13.4), 4.33 (1H, dd, *J* 3.3, 6.0), 4.58−4.68 (2H, m), 5.21 (1H, tt, *J* 2.4, 7.6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.1, 19.9, 21.1, 24.0, 27.8, 30.2, 31.1, 31.7, 37.1, 43.2, 44.1, 44.9, 46.4, 48.5, 56.8, 57.3, 60.8, 63.8, 68.4, 113.3, 156.2, 171.1, 205.2. Azide **8** was immediately used in the next reaction.

*Bis-steroidal Pyrazine **9**.* To a solution of azidoketone **8** (1.45 g, 3.49 mmol) in dry THF (40 mL) was added (via cannula) triphenylphosphine (2.75 g, 10.5 mmol) in dry THF (15 mL) under argon, and the mixture was stirred at rt for 20 h. Water was added (1.4 mL) and stirring was continued for another 24 h at rt, with exposure to air. The reaction mixture was then concentrated and co-evaporated twice with toluene to remove H_2O. The crude residue was separated by flash chromatography on silica gel [eluent: 3:2 toluene−EtOAc (R_f 0.35)] to give pyrazine **9** (0.99 g, 77%) as a colorless glassy foam: mp 185−190 °C (EtOAc); $[\alpha]^{24}_D$ +75.6 (*c* 0.80, $CHCl_3$); IR (film) ν_{max} 3316, 2925, 1737, 1443, 1401, 1240, 1023, 733 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85−2.52 (28H, m), 1.10 (6H, s, H-18), 1.16 (6H, s, H-19), 2.06 (6H, s, Ac), 2.58−2.66 (4H, m, H-4β, H-1α), 2.81 (2H, dd, *J* 5.4, 17.8, H-4α), 3.11 (2H, d, *J* 16.0, H-1β), 4.50 (2H, br s, H-11), 4.63−4.72 (4H, m, H-21), 5.22 (2H, t, *J* 7.2, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.4, 19.9, 21.1, 24.0, 27.9, 30.7, 31.2, 32.0 35.1, 35.9, 42.5, 44.0, 45.2, 46.5, 57.2, 57.4, 60.9, 68.4, 113.1, 148.4, 148.7, 156.6, 171.1; HRMS (APCI$^+$) *m/z* 741.4880 [M + H]$^+$ (calcd for $C_{46}H_{65}N_2O_6$, 741.4843); *anal.* C 72.51, H 8.76, N 3.74%, calcd for $C_{46}H_{64}N_2O_6 \cdot H_2O$, C 72.79, H 8.76, N 3.69%.

*Bis-steroidal Pyrazine **10**.* To a solution of pyrazine **9** (165 mg, 0.222 mmol) in CH_3OH−DCM (15 mL, 1:1) was added powdered KOH (0.040 g, 0.713 mmol), and the mixture was stirred at reflux for 2 h (monitored by TLC). The reaction mixture was concentrated, and the residue was partitioned between brine (20 mL) and EtOAc (20 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc until TLC showed complete extraction. The combined organic phase was successively washed with 1% HCl, saturated $NaHCO_3$, and brine and dried. Removal of solvent gave pyrazine **10** (0.116 g, 80%) as a colorless solid. The analytical sample was obtained by crystallization from MeOH: R_f 0.30 (2:3 toluene−acetone); mp 350 °C (dec); $[\alpha]^{24}_D$ +71.0 (*c* 0.10, CH_3OH); IR (solid) ν_{max} 3383, 2917, 1401 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 0.94−2.01 (22H, m),

1.36 (6H, s), 2.32 (2H, dd, *J* 9.0, 16.6), 2.55 (2H, dd, *J* 9.0, 16.6), 2.74 (2H, d, *J* 13.6), 2.82 (2H, dd, *J* 4.8, 12.4, H-4β), 2.89 (2H, d, *J* 16.4, H-1α), 3.03 (2H, dd, *J* 5.6, 18.0, H-4α), 3.62 (2H, d, *J* 16.0, H-1β), 4.60−4.70 (6H, m, H-11, H-21), 5.64 (1H, s, OH, exch. with D_2O), 5.65 (1H, s, OH, exch. with D_2O), 5.67 (2H, t, *J* 6.8, H-20), 6.01 (2H, br s, OH, exch. with D_2O); ^{13}C NMR (C_5D_5N, 100 MHz) δ 14.5, 20.1, 24.5, 28.5, 31.3, 31.5, 32.5, 35.9, 36.3, 42.8, 44.4, 45.6, 57.8 (2C), 58.4, 67.7, 121.4, 148.7, 149.3, 151.4; HRMS (APCI$^+$) *m/z* 657.46035 [M + H]$^+$ (calcd for $C_{42}H_{61}N_2O_6$, 657.46314); *anal.* C 73.06, H 9.23, N 4.00%, calcd for $C_{42}H_{61}N_2O_6 \cdot 2CH_3OH$, C 73.29, H 9.51, N 3.89%.

Bis-steroidal Pyrazine ***14***. A mixture of pyrazine **10** (98 mg, 0.149 mmol), trichloroacetimidate **13**[14,19] (143 mg, 0.328 mmol), and freshly activated molecular sieves (4 Å, powder, 0.4 g) in anhydrous CH_2Cl_2 (20 mL) was stirred under argon for 15 min. Next, $BF_3 \cdot OEt_2$ (8.0 μL, 0.05 mmol) was added, and the mixture stirred at 0 °C for 2 h. After 24 h at rt, the starting material was still the main component (by TLC) of the mixture, and more $BF_3 \cdot OEt_2$ (8.0 μL, 0.05 mmol) was added. Further addition of $BF_3 \cdot OEt_2$ (33 μL, 0.26 mmol), totaling 1.1 equiv in relation to imidate and 2.4 equiv to pyrazine, was required for the starting material to be completely consumed. The mixture was separated from the molecular sieves, diluted with $CHCl_3$, and washed with aqueous $NaHCO_3$ to pH 8 and then with brine to neutrality. The organic phase was dried, filtered, and evaporated. The residue was separated by CC on silica gel using 2:3 toluene−EtOAc as eluent to give pyrazine **14** (36%) as a film: IR (film) ν_{max} 3377, 2923, 1446, 1399, 1370, 1244, 1078, 1054 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.86−2.41 (22H, m), 1.05 (6H, s), 1.09 (6H, s), 2.57 (2H, d, *J* 16.0, H-1α), 2.60−2.66 (2H, m), 2.77 (2H, dd, *J* 5.5, 17.5, H-4α), 3.12 (2H, d, *J* 17.0, H-1β), 4.45 (2H, s), 4.89−4.93 (4H, m), 5.79 (2H, dd, *J* 10.0, 17.5); ^{13}C NMR (C_5D_5N, 100 MHz) δ 13.4, 22.9, 28.7, 29.5, 30.3, 31.1, 31.3, 33.8, 35.2, 35.9, 38.6, 42.3, 46.1, 51.7, 55.0, 65.3, 110.7, 135.8, 138.3, 145.7, 148.7, 148.8; HRMS (APCI$^+$) *m/z* 621.44219 [M + H]$^+$ (calcd for $C_{42}H_{57}N_2O_2$, 621.44201).

*1,2-Orthoester-3,4-di-O-acetyl-α-L-rhamnoside (**16a**)*. To a solution of tetra-*O*-acetyl-α-L-rhamnose (**11**, 0.84 g, 2.53 mmol) in DCM (20 mL) cooled to 0 °C under a flow of argon was added TMSI (0.4 mL, 2.81 mmol). The reaction was allowed to proceed for 2 h at 0 °C. The solvent was removed and the residue azeotroped with anhydrous toluene until a colorless distillate persisted, in order to remove the trimethylsilyl acetate product. The resulting pale yellow oil identified by ^{1}H NMR as iodide **15**[23] (0.99 g, 98%) was redissolved in anhydrous DCM (10 mL) and added via cannula to a stirred solution of steroid **4** (0.41 g, 1.25 mmol), DIPEA (0.43 mL, 2.50 mmol), TBAI (0.92 g, 2.50 mmol), and freshly activated molecular sieves (4 Å, 0.2 g) in anhydrous DCM (50 mL). The solution was stirred for 21 h at reflux, and the solvent was evaporated. The resulting oil was subjected to flash chromatography (eluent: 1:1 toluene−EtOAc, R_f 0.39) to afford orthoester **16a** (0.693 g, 92%): IR (film) ν_{max} 3507, 2941, 1751, 1665, 1230, 1055 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96−2.54 (14H, m), 1.15 (3H, s, H-18), 1.23 (3H, d, *J* 6.0, H-6′), 1.46 (3H, s, H-19), 1.74 (3H, s, CH_3CO_3), 2.08 (3H, s, Ac), 2.13 (3H, s, Ac), 3.45−3.54 (1H, m, H-5′), 4.08 (2H, dd, *J* 3.0, 7.5, H-21), 4.36 (1H, br t, *J* 3.0, H-11), 4.71 (1H, dd, *J* 2.5, 4.0, H-2′), 4.95−5.10 (2H, m, H-3′,4′), 5.20 (1H, t, *J* 7.5, H-20), 5.41 (1H, d, *J* 2.1, H-1′), 5.68 (1H, s, H-4); ^{13}C NMR (CD_2Cl_2, 125 MHz) 17.6, 20.3, 21.0, 21.2, 21.5, 24.4, 25.4, 31.3, 31.5, 32.4, 32.9, 34.2, 35.3, 39.6, 44.2, 46.6, 56.7, 57.2, 58.8, 68.7, 69.2, 70.5, 72.0, 77.0, 97.7, 115.2, 122.4, 138.3, 155.1, 170.1, 172.5, 199.3; HRMS (APCI$^+$) *m/z* 603.31506 [M + H]$^+$ (calcd for $C_{33}H_{47}O_{10}$, 603.31688).

1,2-Orthoester α-L-Rhamnoside ***16b***. A solution of acetate **16a** (100 mg, 0.166 mmol) in ethanolic ammonia (20 mL, saturated at −10 °C) was left standing overnight at 0 °C and then evaporated to dryness. The crude residue was separated by CC on silica gel (eluent: EtOAc, R_f 0.38) to yield alcohol **16b** (40 mg, 46%) as a colorless powder: mp 124−126 °C ($CHCl_3$); $[\alpha]^{24}_D$ +64.3 (*c* 0.60, $CHCl_3$); IR (film) ν_{max} 3444, 2930, 1656, 1104, 1071, 1050, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88−2.51 (17H, m), 1.18 (3H, s, H-18), 1.34 (3H, d, *J* 6.0, H-6′), 1.46 (3H, s, H-19), 1.72 (3H, s, CH_3CO_3), 3.15 (1H, br, exch. D_2O), 3.28−3.37 (1H, m, H-5′), 3.50 (1H, pseudo-t, *J* 9.3, H-4′), 3.69 (1H, br, exch. D_2O), 3.75 (1H, dd, *J* 3.9, 9.3, H-3′), 4.02−4.23 (2H, m, H-21), 4.40 (1H, br d, *J* 2.7, H-11), 4.56 (1H, dd, *J* 2.4, 4.2, H-2′), 5.27 (1H, t, *J* 7.8, H-20), 5.41 (1H, d, *J* 2.4, H-1′), 5.69 (1H, s, H-4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.6, 20.2, 21.1, 23.9, 30.8, 31.2, 32.0, 32.4, 33.8, 35.0, 39.2, 40.9, 44.3, 46.5, 56.3, 56.6, 68.0, 68.5, 69.9, 73.5, 92.3, 115.6, 122.4, 138.2, 156.6, 172.0, 199.5; HRMS (APCI$^+$) *m/z* 331.22453 [M − Rha]$^+$ (calcd for $C_{21}H_{31}O_3$, 331.22733).

Bis-steroidal Pyrazine α-L-Rhamnoside ***17a***. Pyrazine **17a** was prepared from iodide **14** (0.363 g, 0.91 mmol) and pyrazine **10** (44 mg, 0.067 mmol) by following the procedure for synthesis of 1,2-orthoester **16a**. Isolation by CC on silica gel (eluent: 2:3 toluene−EtOAc, R_f 0.22) gave orthoester **17a** (70 mg, 87%) as a film: IR (film) ν_{max} 3404, 2920, 1750, 1455, 1375, 1228, 1055 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87−2.29 (30H, m), 1.06 (6H, s, H-18), 1.11 (6H, s, H-19), 1.23 (6H, d, *J* 6.6 Hz, H-6′), 1.75 (6H, s, CH_3CO_3), 2.08 (6H, s, Ac), 2.13 (6H, s, Ac), 2.46 (2H, dd, *J* 8.7, 17.7, H-4β), 2.58 (2H, d, *J* 16.0, H-1α), 2.76 (2H, dd, *J* 5.3, 17.7, H-4α), 3.09 (2H, d, *J* 16.0, H-1β), 3.45−3.54 (2H, m, H-5′), 4.06−4.16 (4H, m, H-21), 4.42 (2H, br s, H-11), 4.69 (2H, dd, *J* 2.7, 3.9, H-2′), 4.97−5.12 (4H, m, H-3′, H-4′), 5.21 (2H, t, *J* 7.2, H-20), 5.38 (2H, d, *J* 2.7, H-1′); ^{13}C NMR ($CDCl_3$, 100 MHz) 14.5, 17.6, 20.2, 20.9, 21.0, 24.5, 25.3, 28.4, 31.2, 31.6, 32.5, 35.3, 36.2, 42.8, 44.2, 45.2, 46.8, 57.7, 57.8, 59.0, 68.8, 69.2, 70.6, 71.8, 76.9, 97.6, 115.1, 124.3, 148.7, 149.1, 155.4, 170.2, 171.2; HRMS (APCI$^+$) *m/z* 1201.6468 [M + H]$^+$ (calcd for $C_{66}H_{93}N_2O_{18}$, 1201.6424).

Bis-steroidal Pyrazine 1,2-Orthoester α-L-Rhamnoside ***17b***. Deacetylation of **17a** (70 mg, 0.058 mmol) in ethanolic ammonia, according to the procedure for alcohol **16b**, gave pyrazine **17b** (46 mg, 77%) as a colorless solid following evaporation of the solvent: mp 205−208 °C (dec); $[\alpha]^{24}_D$ +3.0 (*c* 0.1, pyridine); IR (film) ν_{max} 3354, 2909, 1446, 1399, 1082, 1007 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 0.92−1.98 (22H, m), 1.35 (6H, s, H-18), 1.44 (6H, s, H-19), 1.61 (6H, d, *J* 6.4, H-6′), 1.99 (6H, s, CH_3CO_3), 2.27 (2H, dd, *J* 8.7, 16.7), 2.50 (2H, dd, *J* 8.7, 16.7), 2.75−2.84 (4H, m, includes H-4β), 2.88 (2H, d, *J* 16.4, H-1α), 3.03 (2H, dd, *J* 5.6, 18.0, H-4α), 3.58, 4.05 (2H, pseudo-t, *J* 9.2, H-4′), 4.20 (2H, dd, *J* 4.0, 9.2, H-3′), 4.54−4.61 (4H, m, H-21, 4.66 (2H, br s, H-11), 4.89 (2H, dd, *J* 2.6, 3.8, H-2′), 5.45 (2H, t, *J* 6.8, H-20), 5.59 (2H, br s, OH, exch. D_2O), 5.62 (2H, d, *J* 1.6, H-1′); ^{13}C NMR (C_5D_5N, 100 MHz) δ 14.5, 18.4, 20.1, 24.4, 26.3, 28.4, 31.3, 31.4, 32.5, 35.9, 36.3, 42.8, 44.6, 45.6, 46.5, 57.5, 57.6, 58.8, 67.6, 72.0, 73.1, 73.2, 81.4, 98.2, 115.9, 124.1, 148.7, 149.4, 154.4; HRMS (Q-TOF, ESI) *m/z* 1033.5983 [M + H]$^+$ (calcd for $C_{58}H_{85}N_2O_{14}$, 1033.6001).

*3-Oxo-11β-hydroxy-21-O-(2′,3′4′-tri-O-acetyl-α-L-rhamnosyl)-pregna-4,17(20)-diene (**20b**)*. TMSI (0.5 mL, 3.5 mmol) was added to a 0.1 M solution of 1,2,3,4-tetra-*O*-trimethylsilyl-α-L-rhamnose (**18**, 1.4 g)[30] in anhydrous DCM at 0 °C. The solution was stirred under argon at rt for 10 min and then concentrated in vacuo. The residual water was azeotroped with anhydrous toluene until the distillate color remained pale yellow. The crude product (iodide **19**, 1.51 g) was dissolved in dry DCM (5 mL) and cannulated into a stirred mixture of dienediol **4** (0.51 g, 1.54 mmol), TBAI (1.15 g, 3.11 mmol), DIPEA (0.54 mL, 3.11 mmol), molecular sieves (4 Å, 0.5 g), and anhydrous DCM (30 mL). The mixture was stirred at rt under argon for 24 h, and the solvent was evaporated in vacuo. EtOAc was added to the crude residue, and the mixture was cooled in an ice bath to precipitate TBAI, which was removed by filtration. The filtrate was concentrated, and the residue was dissolved in CH_3OH (50 mL). The solution was heated at reflux for 4 h, cooled, and concentrated. The methanolic mixture was made neutral with triethylamine, the solvent was evaporated in vacuo, and the residue was dissolved in pyridine (20 mL). Acetic anhydride (4 mL) and DMAP (catalytic) were added, and the reaction mixture was stirred for 10 h. Pyridine was removed by azeotroping with

toluene, and the residue was purified by flash chromatography (eluent: 7:3 DCM−EtOAc, R_f 0.30) to give rhamnosylated pregnene **20b** as a foam (0.213 g, 23%): mp 108−110 °C; $[\alpha]^{24}_{D}$ +57.1 (*c* 0.14, $CHCl_3$); IR (film) ν_{max} 3469, 2936, 1750, 1667, 1372, 1225, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.99−2.56 (17H, m), 1.19 (3H, s, H-18), 1.22 (3H, d, *J* 6.4 Hz, H-6′), 1.46 (3H, s, H-19), 1.99 (3H, s, Ac), 2.05 (3H, s, Ac), 2.16 (3H, s, Ac), 3.89−3.93 (1H, m, H-5′), 4.10 (1H, dd, *J* 7.0, 11.6, H-21), 4.18 (1H, dd, *J* 7.0, 11.6, H-21), 4.41 (1H, br s, H-11), 4.77 (1H, d, *J* 0.8, H-1′), 5.08 (1H, t, *J* 10.0 Hz, H-4′), 5.22−5.24 (2H, m, H-2′, H-20 obscured by H-2′), 5.32 (1H, dd, *J* 3.6, 10.0, H-3′), 5.68 (1H, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.01, 24.0, 31.0, 32.0, 32.5, 33.8, 34.9, 39.2, 44.0, 46.0, 56.3, 56.6, 63.8, 66.5, 68.4, 69.1, 70.2, 71.1, 96.7, 114.2, 122.3, 155.9, 170.0, 170.1, 170.5, 172.1, 199.5; HRMS (FAB) *m/z* 609.3236 [M + Li]$^+$ (calcd for $C_{33}H_{46}LiO_{10}$, 609.3251).

*3-Oxo-11β-hydroxy-21-O-(α-L-rhamnosyl)pregna-4,17(20)-diene (**20c**).* Deacetylation of rhamnoside **20b** (100 mg, 0.166 mmol) in ethanolic ammonia followed the procedure for obtaining triol **16b**. Isolation of product by CC on silica gel (eluent: 9:1 DCM−CH_3OH, R_f 0.31) led to tetraol **20c** (55 mg, 69%) as a foam: mp 100−105 °C (CH_3OH); $[\alpha]^{24}_{D}$ +42.0 (*c* 0.35, CH_3OH); IR (film) ν_{max} 3424, 2929, 1664, 1070, 1042; ^{1}H NMR (C_5D_5N, 500 MHz) 0.86−2.69 (17H, m), 1.40 (3H, s, H-18), 1.57 (3H, s, H-19), 1.62 (3H, d, *J* 6.0, H-6′), 4.24−4.29 (3H, m, H-5′, H-4′, H-21), 4.51 (2H, dd, *J* 3.5, 9.0, H-3′, H-11), 4.55 (1H, dd, *J* 1.5, 3.5, H-2′), 4.66 (1H, dd, *J* 7.2, 11.7, H-21), 5.33 (1H, d, *J* 1.0, H-1′), 5.40 (1H, t, *J* 7.0, H-20), 5.68 (1H, br s, OH, exch. D_2O), 5.68 (1H, s, H-4), 6.80 (2H, br, OH, exch. D_2O); ^{13}C NMR (C_5D_5N, 125 MHz) 18.6, 20.2, 21.0, 22.8, 24.3, 31.2, 31.4, 32.1, 32.9, 34.3, 34.9, 39.6, 44.4, 46.8, 56.5, 57.1, 63.4, 67.5, 69.8, 72.4, 72.8, 74.0, 100.7, 116.4, 122.4, 154.1, 172.1, 198.5; HRMS (APCI$^+$) *m/z* 477.2845 [M + H]$^+$ (calcd for $C_{27}H_{41}O_7$ 477.2852).

*Bis-steroidal Pyrazine α-L-Rhamnoside **21b**.* A solution of 2,3,4-tri-*O*-trimethylsilyl-α-L-rhamnopyranoside iodide (**19**, 0.325 g, 0.66 mmol) in anhydrous DCM (10 mL) was added via cannula to a stirred mixture of pyrazine **10** (98 mg, 0.15 mmol), TBAI (0.244 g, 0.66 mmol), DIPEA (115 μL, 0.66 mmol), and molecular sieves (4 Å, 0.2 g) in anhydrous DCM (60 mL). The mixture was stirred under argon for 24 h at rt and at reflux for 4 days. Desilylation of the crude residue was followed by an unsuccessful attempt to isolate the deprotected product (**21c**). Therefore, the octaol-containing residue was acetylated as described for preparation of triacetate **20b**. The crude product was separated on a silica gel plug by elution with 7:3 DCM−EtOAc to give a mixture of acetylated pyrazines (85 mg, 47%). Further separation was performed by reversed-phase HPLC (C18; eluent: 93:7 CH_3OH-H_2O) to give three bis-steroidal pyrazines.

The first compound to elute was the β,β′-dirhamnoside (β,β)-**21b** (11 mg, 6%): mp 167−169 °C; $[\alpha]^{24}_{D}$ +60.0 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.92−2.51 (28H, m), 1.09 (6H, s, H-19), 1.11 (6H, s, H-18), 1.29 (6H, d, *J* 6.5, H-6′), 1.99 (6H, s, Ac), 2.06 (6H, s, Ac), 2.18 (6H, s, Ac), 2.63 (2H, dd, *J* 12.5, 17.5, H-4β), 2.65 (2H, d, *J* 17.5, H-1α), 2.80 (2H, dd, *J* 5.5, 18.0, H-4α), 3.12 (2H, d, *J* 16.5, H-1β), 3.46−3.52 (2H, m, H-5′), 4.27 (2H, dd, *J* 6.0, 12.0, H-21), 4.39 (2H, dd, *J* 8.0, 12.0, H-21), 4.49 (2H, br s, H-11), 4.67 (2H, d, *J* 1.0, H-1′), 5.01 (2H, dd, *J* 3.5, 10.0, H-3′), 5.07 (2H, pseudo-t, *J* 9.5, 10.0, H-4′, 5.15 (2H, t, *J* 7.0, H-20); 5.43 (2H, dd, *J* 1.0, 3.5, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.5, 17.6, 20.2, 20.7, 20.8, 21.0, 24.0, 28.0, 30.9, 31.3, 32.1, 35.2, 35.9, 42.6, 43.9, 45.3, 45.8, 57.3, 57.6, 64.5 (C-21), 68.7 (C-11), 69.4 (C-2′), 70.5 (C-5), 70.8 (C-4′), 71.3 (C-3′), 96.1 (C-1′), 114.5, 148.4, 148.7, 156.1, 169.8, 170.2, 170.6.

The second product to elute was the unsymmetrical isomer (α,β)-**21b** (27 mg, 15%): mp 175−178 °C; $[\alpha]^{24}_{D}$ +26.2 (*c* 0.08, $CHCl_3$); IR (film) ν_{max} 3356, 2925, 1748, 1219, 1077, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.92−2.54 (28H, m), 1.09 (3H, s, H-19″), 1.10 (3H, H-19′), 1.11 (3H, s, H-18″), 1.16 (3H, s, H-18′), 1.23 (3H, d, *J* 6.5, H-6′), 1.29 (3H, d, *J* 6.5, H-6″), 1.99 (6H, s, Ac), 2.05 (3H, s, Ac), 2.06 (3H, s, Ac), 2.16 (3H, s, Ac), 2.18 (3H, s, Ac), 2.59−2.67 (4H, m, H-1α, H-4β), 2.80 (2H, dd, *J* 4.5, 17.5, H-4α), 3.12 (1H, d, *J* 16.5, H-1β′), 3.14 (1H, d, *J* 16.5, H-1β″), 3.46−3.52 (1H, m, H-5″), 3.90−3.95 (2H, m, H-5′), 4.08 (1H, dd, *J* 7.5, 11.0, H-21′), 4.25−4.29 (2H, m, H-21′, 21″), 4.39 (1H, d, *J* 8.0, 12.0, H-21″), 4.49 (2H, br s, H-11), 4.67 (1H, s, H-1″), 4.79 (1H, d, *J* 1.5, H-1′), 5.01 (1H, dd, *J* 3.0, 10.5, H-3″), 5.01−5.10 (2H, m, H-4′,4″), 5.15 (1H, t, *J* 7.0, H-20″), 5.22 (1H, t, *J* 7.0, H-20′), 5.24 (1H, dd, *J* 1.5, 3.5, H-2′), 5.33 (1H, dd, *J* 3.5, 10.0, H-3′), 5.43 (1H, d, *J* 3.0, H-2″); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.42, 14.49, 17.4, 17.6, 20.0, 20.2, 20.6, 20.76, 20.82, 20.85, 21.0, 24.0, 28.0, 30.9, 31.2, 32.1, 35.2, 35.9, 42.6, 43.9, 44.1, 45.3, 45.8, 46.1, 57.2, 57.3, 57.4, 57.6, 64.0, 64.6, 66.4, 68.6, 68.7, 69.1, 69.4, 70.3, 70.5, 70.8, 71.2, 71.3, 96.1, 96.8, 114.1, 114.5, 148.4, 148.9, 156.1, 156.2, 169.8, 170.0, 170.2, 170.4, 170.6.

The last substance to elute was the α,α′-dirhamnoside (α,α)-**21b** (35 mg, 19%): mp 188−190 °C; $[\alpha]^{24}_{D}$ +15.7 (*c* 0.70, $CHCl_3$); IR (film) ν_{max} 3360, 2912, 1751, 1223, 1051 cm^{-1}; ^{1}H NMR (500 MHz) δ 0.93−2.54 (28H, m), 1.10 (6H, s, H-18), 1.16 (3H, s, H-19), 1.23 (6H, d, *J* 6.5, H-5′), 1.99 (6H, s, Ac), 2.05 (6H, s, Ac), 2.16 (6H, s, Ac), 2.62 (2H, d, *J* 12.5 Hz, 18.0, H-4β), 2.65 (2-H, d, *J* 16.5, H-1α), 2.80 (2H, dd, *J* 5.5, 18.0, H-4α), 3.14 (2H, d, *J* 16.5, H-1β), 3.90−3.95 (2H, m, H-5′), 4.08 (2H, dd, *J* 7.5, 11.0, H-21), 4.27 (2H, dd, *J* 7.5, 11.0, H-21), 4.49 (2H, br s, H-11), 4.78 (1H, d, *J* 1.5, H-1′), 5.08 (1H, pseudo-t, *J* 9.5, 10.5, H-4′), 5.22 (2H, t, *J* 7.5, H-20), 5.24 (2H, dd, *J* 1.5, 3.5, H-2′), 5.33 (2H, dd, *J* 3.5, 10.0, H-3′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.5, 17.4, 20.0, 20.7, 20.8, 21.0, 24.0, 28.0, 29.7, 30.9, 31.2, 32.1, 35.2, 35.9, 42.6, 44.1, 45.2, 46.1, 57.2, 57.4, 64.0 (C-21), 66.5 (C-5′), 68.6 (C-11), 69.1 (C-3′), 70.3 (C-4′), 71.2 (C-2′), 96.8 (C-1′), 114.1, 148.4, 148.8, 156.2, 170.0 (2C), 170.4; HRMS (APCI$^+$) *m/z* 1201.638 [M + H]$^+$ (calcd for $C_{66}H_{93}N_2O_{18}$, 1201.642); *anal.* C 62.59, H 7.52, N 2.20%, calcd for $C_{66}H_{92}N_2O_{18}.4H_2O$, C 62.25, H 7.91, N 2.20%.

*Bis-steroidal Pyrazine α-L-Rhamnoside Dimer (α,α)-**21c**.* Deacetylation of α,α-**21b** (22 mg, 0.018 mmol) in ethanolic ammonia by the procedure for obtaining triol **16b** and isolation of product by crystallization from MeOH led to octaol **21c** (10 mg, 56%) as colorless needles: mp 280 °C (dec) (CH_3OH); $[\alpha]^{24}_{D}$ −22.0 (*c* 0.05, pyridine); IR (film) ν_{max} 3406, 2927, 1645, 1404, 1036 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 0.91−2.50 (28H, m), 1.34 (6H, s, H-18), 1.41 (6H, s, H-19), 1.63 (6H, d, *J* 5.6, H-6′), 2.75 (2H, dd, *J* 5.6, 18.0, H-1α), 2.87 (2H, d, *J* 16.4, H-4β), 3.01 (2H, dd, *J* 5.6, 18.0, H-1β), 3.59 (2H, d, *J* 16.4, H-4α), 4.27−4.31 (6H, m, H-4′, H-5′, H-21), 4.25−4.54 (2H, d, *J* 3.2, 8.4, H-3′), 4.56 (2H, s, H-2), 4.68−4.71 (4H, m, H-11, H-21), 5.35 (2H, s, H-1′), 5.42 (2H, t, *J* 6.8, H-20), 5.71 (2H, d, *J* 2.8, OH, exch. D_2O), 6.63 (3H, br, OH, exch. D_2O), 6.81 (2H, br, OH, exch. D_2O); ^{13}C NMR (C_5D_5N, 100 MHz) δ 14.6, 18.6, 20.2, 24.5, 28.5, 31.3, 31.6, 32.5, 35.9, 36.4, 42.8, 44.5, 45.6, 47.0, 57.6 (2), 63.5, 67.7, 69.8, 72.5, 72.9, 74.1, 100.8, 116.3, 148.8, 149.4, 154.5; HRMS (APCI$^+$) *m/z* 949.5773 [M + H]$^+$ (calcd for $C_{54}H_{81}N_2O_{12}$, 949.5789).

Antimicrobial Susceptibility Testing. Pyrazines **9**, **10**, and **17b** were evaluated against the bacteria *Stenotrophomonas maltophilia* ATCC 13637, *Micrococcus luteus* Presque Isle 456, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 6303, and *Neisseria gonorrheae* ATCC 49226 and the fungi *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 90112, according to established broth microdilution susceptibility assays.[34,35] Each substance was reconstituted in a small volume of sterile DMSO and diluted in the appropriate medium immediately prior to susceptibility experiments. The minimum inhibitory concentration was defined as the lowest concentration of compound that inhibited all visible growth of the test organism (optically clear). Assays were repeated on separate days.

■ ASSOCIATED CONTENT

S Supporting Information. ^{1}H NMR spectra of compounds **10**, **17b**, and **21c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We appreciate the very necessary financial support provided by grants RO1CA90441-02-05 and 5R01CA90441-07 from the Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute, DHHS; the Arizona Biomedical Research Commission; the Robert B. Dalton Endowment Fund; Dr. Alec D. Keith; the J. W. Kieckhefer Foundation; the Margaret T. Morris Foundation; and the Fannie E. Rippel Foundation. For other assistance, we are pleased to thank Drs. J.-C. Chapuis and F. Hogan, as well as M. Dodson, C. Weber, and L. Williams.

■ DEDICATION

This paper is dedicated to the memory of Professor Georgy B. Elyakov (1929−2005), a pioneering explorer of the oceans and of the organic chemistry of bioactive marine organism constituents.

■ REFERENCES

(1) (a) Antineoplastic Agents series part 562. For the preceding contribution refer to Pettit, G. R.; Smith, T. H.; Feng, S.; Knight, J. C.; Tan, R.; Pettit, R. K.; Hinrichs, P. A. *J. Nat. Prod.* **2007**, *70*, 1073–1083. (b) For "The Cephalostatins 20" see: Dirsch, V. M.; Muller, I. M.; Eichhorst, S. T.; Pettit, G. R.; Kamano; Inoue, M.; Xu, J. P.; Ichihara, Y.; Wanner, G.; Vollmar, A. M. *Cancer Res.* **2003**, *63*, 8869–8876.

(2) (a) Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006–2007. (b) Pettit, G. R.; Tan, R.; Xu, J. P.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 955–958.

(3) Chang, L.-C.; Tsai, T. R.; Wang, J.-J.; Lin, C.-N.; Kuo, K.-W. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 21–25.

(4) (a) Fortner, K. C.; Kato, D.; Tanaka, Y.; Shair, M. D. *J. Am. Chem. Soc.* **2010**, *132*, 275–280. (b) Lee, S.; LaCour, T. G.; Fuchs, P. L. *Chem. Rev.* **2009**, *109*, 2275–2314. (c) Rudy, A.; López-Antón, N.; Dirsch, V. M.; Vollmar, A. M. *J. Nat. Prod.* **2008**, *71*, 482–486. (d) Moser, B. R. *J. Nat. Prod.* **2008**, *71*, 487–491.(e) Flessner, T.; Jautelat, R.; Scholz, U.; Winterfeldt, E. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Falk, H., Kirby, G. W., Eds.; Springer: Vienna, 2004; Vol. 87, pp 1−80. (f) Gryszkiewicz-Wojtkielewicz, A.; Jastrzebska, I.; Morzycki, J. W. *Curr. Org. Chem.* **2003**, *7*, 1257–1277.

(5) (a) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1994**, *59*, 6164–6166. (b) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, *60*, 608–614. (c) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 4484–4491.

(6) LaCour, T. G.; Guo, C.; Bhandaru, S.; Boyd, M. R.; Fuchs, P. L. *J. Am. Chem. Soc.* **1998**, *120*, 692–707.

(7) (a) Poza, J. J.; Rodríguez, J.; Jiménez, C. *Bioorg. Med. Chem.* **2010**, *18*, 58–63. (b) Lee, S.; Jamieson, D.; Fuchs, P. L. *Org. Lett.* **2009**, *11*, 5–8. (c) Banerjee, A.; Sergienko, E.; Vasile, S.; Gupta, V.; Vuori, K.; Wipf, P. *Org. Lett.* **2009**, *11*, 65–68. (d) Nawasreh, M. *Bioorg. Med. Chem.* **2008**, *16*, 255–265. (e) Shawakfeh, K. Q.; Al-Said, N. H.; Al-Zoubi, R. M. *Steroids* **2008**, *73*, 579–584. (f) Kramer, A.; Ullmann, U.; Winterfeldt, E. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2865–2867. (g) Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828–6839. (h) Černý, I.; Pouzar, V.; Buděšnský, M.; Drašar, P. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1597–1608.

(8) (a) Beutler, J. A.; Shoemaker, R. H.; Johnson, T.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 1509–1512. (b) Tsubuki, M.; Matsuo, S.; Honda, T. *Tetrahedron Lett.* **2008**, *49*, 229–232. (c) Shi, B.; Wu, H.; Yu, B.; Wu, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 4324–4327. (d) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2002**, *124*, 6576–6583. (e) Kubo, A.; Mimaki, Y.; Terao, M.; Sashida, Y.; Hirano, T.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1992**, *31*, 3969.

(9) (a) Deng, L.; Wu, H.; Yu, B.; Jiang, M.; Wu, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2781–2785. (b) Morzycki, J. W.; Wojtkielewicz, A.; Wołczyński, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3323–3326. (c) Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: Cambridge, UK, 1995. (d) Waller, G. R. In *Advances in Experimental Medicine and Biology*; Waller, G. R., Yamaskai, K., Eds.; Plenum Press: New York, 1996; Vol. 404.

(10) (a) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633–636. (b) Guo, C.; LaCour, T. G.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 419–424. (c) Wojtkielewicz, A.; Długosz, M.; Maj, J.; Morzycki, J. W.; Nowakowski, M.; Renkiewicz, J.; Strnad, M.; Swaczynová, J.; Wilczewska, A.; Wójcik, J. *J. Med. Chem.* **2007**, *50*, 3667–3673.

(11) Puri, R.; Wong, T. C.; Puri, R. K. *J. Nat. Prod.* **1994**, *57*, 587–596.

(12) Yan, M.-C.; Liu, Y.; Lu, W.-X.; Wang, H.; Sha, Y.; Cheng, M.-S. *Carbohydr. Res.* **2008**, *343*, 780–784.

(13) (a) Yokosuka, A.; Mimaki, Y. *Phytochemistry* **2008**, *69*, 2724–2730. (b) Gauthier, C.; Legault, J.; Lavoie, S.; Rondeau, S.; Tremblay, S.; Pichette, A. *Tetrahedron* **2008**, *64*, 7386–7399.

(14) Pellissier, H. *Tetrahedron* **2004**, *60*, 5123–5162.

(15) van Steijn, A. M. P.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1991**, *211*, 261–277.

(16) (a) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2001**, *123*, 3369–3370. (b) Deng, S.; Yu, B.; Lou, Y.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 202–208.

(17) Bebault, G. M.; Dutton, G. G. S.; Warfield, C. K. *Carbohydr. Res.* **1974**, *34*, 174–179.

(18) Watanabe, K.; Itoh, K.; Araki, Y.; Ishido, Y. *Carbohydr. Res.* **1986**, *154*, 165–176.

(19) Larson, D. P.; Heathcock, C. H. *J. Org. Chem.* **1997**, *62*, 8406–8418.

(20) (a) Kohen, F.; Mallory, R. A.; Scheer, I. *J. Org. Chem.* **1971**, *36*, 716–718. (b) Ouannes, C.; Dvolaitzky, M.; Jacques, J. *Bull. Soc. Chim. Fr.* **1964**, 776.

(21) Loughhead, D. G. *J. Org. Chem.* **1985**, *50*, 3931–3934.

(22) Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61–69.

(23) Mukhopadhyay, B.; Kartha, K. P. R.; Russel, D. A.; Field, R. A. *J. Org. Chem.* **2004**, *22*, 7758–7760.

(24) Wang, W.; Kong, F. *J. Org. Chem.* **1999**, *64*, 5091–5095.

(25) Pleuss, N.; Kunz, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 3174–3176.

(26) Lam, S. N.; Gervay-Hague, J. *Carbohydr. Res.* **2002**, *337*, 1953–1965.

(27) (a) Ferrières, V.; Roussel, M.; Gelin, M.; Plusquellec, D. *J. Carbohydr. Chem.* **2001**, *20*, 855–865. (b) Bhat, A. S.; Gervay-Hague, J. *Org. Lett.* **2001**, *3*, 2081–2084.

(28) Streefkerk, D. G.; de Bie, M. J. A.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1974**, *38*, 47–59.

(29) (a) Perlin, A. S.; Casu, B. *Tetrahedron Lett.* **1969**, *34*, 2921–2924. (b) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297. (c) Pozsgay, V.; Jennings, H. J. *J. Org. Chem.* **1988**, *53*, 4042–4052.

(30) Pozsgay, V.; Neszmélyi, A. *Carbohydr. Res.* **1980**, *80*, 196–202.

(31) Chauvin, A. L.; Nepogodiev, S. A.; Field, R. A. *Carbohydr. Res.* **2004**, *339*, 21–27.

(32) Giner, J.-L.; Faraldos, J. A. *J. Org. Chem.* **2002**, *67*, 2717–2720.

(33) Akamanchi, K. G.; Patel, H. C.; Meenakshi, R. *Synth. Commun.* **1992**, *22*, 1655–1660.

(34) NCCLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard*, 5th ed.; NCCLS document M7-A5; NCCLS: Wayne, PA, 2000.

(35) NCCLS. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard*, 2nd ed.; NCCLS document M27-A2; NCCLS: Wayne, PA, 2002.