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Synthesis of bis-diosgenin pyrazine dimers: New cephalostatin analogs

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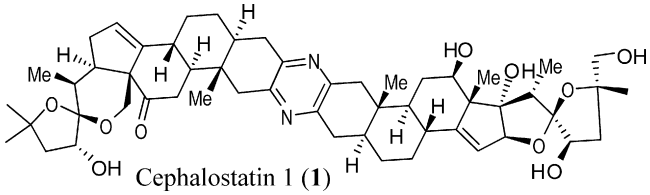
ABSTRACT

A convenient synthesis for bis-diosgenin pyrazine dimers, cephalostatin analogues is reported. These symmetrical dimeric steroid-pyrazines were obtained by the classical condensation of α-amino ketones, the most efficient method for pyrazine ring construction.

1. Introduction

Many classes of steroids exhibit antitumor properties with receptor binding and DNA binding that are important steps for many antitumor steroids [1]. Hormonal steroids have been shown to be active against neoplasms of the colon, breast and other cell lines [2]. Steroids have been used as delivery agents for DNA-active cytotoxic units such as alkylating agents [3]. On the other hand, dimeric steroids were considered more of a novelty than anything else, until such dimeric steroid systems were found in nature [4]. Evidence that the dimerization of the steroid skeleton leads to unique characteristics and applications has gradually began to emerge in different areas. Many dimeric and oligomeric steroids exhibit micellular, detergent and liquid crystal behavior [5,6].

Steroidal dimers have been used as catalysts for many types of reactions [7] and many lead to new phamacologically active steroids [8]. For example, the cephalostatin (**1**) is one of dimeric steroids that are among the most powerful anticancer agent ever tested by the National Cancer Institute [9,10]. This exceptional activity of cephalostatins has led to interest in the synthesis of the compounds and their analogs as potential antitumor agents. The synthesis of dimeric steroid-pyrazine marine alkaloids and the isolation of these steroid derivatives from natural products have been reviewed [11].



Cephalostatin 1 (**1**)

Cephalostatin (**1**) is extremely potent against the NCI primary assay, the marine lymphocyctic leukemia P388, with an ED_{50} value of 10^{-7} to 10^{-9} μg mL^{-1}. Pettit's group has characterized 16 other cephalostatins [9], all containing the novel structure framework of two steroid units linked at the A ring by

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a pyrazine [12]. A steroid pyrazine dimer from an unexpected source was also reported in literature [13].

Many different routes were reported for the construction of the pyrazine-based dimeric steroids. Some of them allow the synthesis of unsymmetrical dimers [14,15]. The symmetrical dimeric steroid-pyrazines can be obtained by the classical condensation of α-amino ketones that is, actually, the most efficient method of pyrazine rings construction. The intermediate steroidal 2α-amino-3-ketones undergo spontaneous dimerization to a mixture of dihydropyrazines, which are then oxidized by air [16] to give the pyrazine dimer.

In this study we start from diosgenin (**2**), a readily available and cheap natural steroid to synthesize four new symmetrical bis-steroidal pyrazine dimers that are analogs of cephalostatins.

2. Experimental

2.1. General remarks

Melting points (mp) were determined on electrothermal digital melting point apparatus and are uncorrected. All the starting materials and reagents were obtained from commercial source and were used without further purification. FT-IR spectra were recorded on a Nicolet-Impact 410 spectrophotometer. Both ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 instruments. The chemical shifts (δ) are reported in ppm relative to TMS used as an internal standard.

2.2. Chemical synthesis

2.2.1. *(25R)-5-en-spirosta-3-one* (3)

Pyridinum chloro chromate (1.7 g, 4.0 mmol) was added to a mixture of powdered $CaCO_3$ (4.0 g, 4.0 mmol) and **2** (2.0 g, 4.8 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was stirred for 30 min. The reaction mixture was diluted with diethyl ether (50 mL) and filtered through a short column of florisil. The solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (10% ethyl acetate in hexane) to give a ketone **3** as a white solid. Yield: 1.6 g (80%), mp 193–194 °C, lit. mp 194–196 °C [17]. IR (KBr): 2961, 1726 and 1681 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.3 (s, 1H, C6), 4.4 (dt, 1H, C16-α), 3.48 (m, 2H, C26), 3.38 (t, 2H, C26). ^{13}C NMR ($CDCl_3$): δ 211 (C3), 140.8 (C5), 121 (C6), 80.7 (C17), 66.7 (C26), 109 (C22), 62.7 (C17).

2.2.2. *(25R)-5-en-2α-bromo-spirosta-3-one* (4)

To a solution of ketone **3** (0.2 g, 0.48 mmol) in THF (20 mL) at room temperature, phenyl trimethylammonium perbromide (PTAB, 0.23 g, 0.63 mmol, 1.3 equiv.) in THF (7 mL) was added rapidly. The resulting orange solution deposited copious precipitate and faded to a beige color within 6 min. The solution quenched with brine solution (10 mL), extract with $CHCl_3$ (2 mL × 20 mL), dried over Na_2SO_4, concentrated and purified by column chromatography (15% ethyl acetate/hexane) to give a white solid of **4**. Yield: 0.26 g (72%), mp 160–163 °C. IR (KBr, cm^{-1}): 2946, 1681, 1619, 797. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.5 (1H, dd, J = 7.1, 6.2 Hz, H2β), 4.36 (1H, dt, J = 7.6, 7.2, H16α), 3.46 (1H, m, H26β), 3.3 (1H, t, J = 10.8 Hz, H26α). ^{13}C NMR (400 MHz, $CDCl_3$): δ 204 (C3), 121 (C6), 109 (C22), 80.6 (C16), 66.7 (C26), 62.7 (C17), 54 (C2). Anal. Calcd. for $C_{27}H_{39}BrO_3$: C, 65.98; H, 8.00. Found: C, 65.56; H, 7.86.

2.2.3. *Di(25R-5-en -spirostano[2,3-b:2′,3′-e]) pyrazine* (5)

The bromo ketone **3** (0.4 g, 0.81 mmol) was dissolved in DMF (50 mL) and few mg of KI was added followed by addition of NaN_3 (0.52 g, 8.1 mmol, 10 equiv.). The mixture was stirred for 3 h at 50 °C. The solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL). The resulting solution was washed with brine solution (2 mL × 20 mL), dried over Na_2SO_4 and evaporated to give azido ketone intermediate. To the intimate mixture of azido ketone in THF (10 mL) was added triphenylphosphine (0.7 g, 2.64 mmol, 3 equiv.) under N_2. The solution was stirred for 5 h under N_2 until evolving gas from the solution was ceased. Then THF (5 mL) and water (0.2 mL, 11 mmol) were added and the reaction mixture was stirred overnight. After concentration, the yellow residue was azeotrope with toluene and absolute ethanol (10 mL) and TsOH (catalytic amount) were added. The orange mixture was stirred vigorously at room temperature under atmospheric pressure for further 2 days. The fine solid was filtered over a bed of celite and washed with $CHCl_3$ followed by evaporation to give pure dimeric pyrazine **5**. Yield: 0.12 g (70%), mp 290 °C (d). IR (KBr, cm^{-1}): 2970, 1660 and 1412. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.4 (1H, t, H6), 4.4 (1H, dt, 16α), 3.4 (2H, m, H-26). ^{13}C NMR (400 MHz, $CDCl_3$): δ 144, 143 (pyrazine carbons), 140 (C6), 109 (C22), 81 (C16), 67 (C26), 63 (C17). Anal. Calcd. for $C_{54}H_{76}N_2O_4$: C, 79.37; H, 9.37; N, 3.43. Found: C, 79.06; H, 9.14; N, 3.09.

2.2.4. *(25R)-5α-spirosta-3-one* (6)

A solution of **3** (1.0 g, 2.4 mmol) in ethyl acetate (30 mL) was stirred with 10% palladium on carbon (10 mg) under hydrogen at 40 °C for 24 h. The reaction mixture was filtered over a bed of celite and the filtrate was evaporated under reduced pressure. The residue was crystallized from methanol to give **6**. Yield: 0.90 g (90%), mp: 155–158 °C. IR (KBr): 2945, 1718 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.37 (dt, 1H, C16-αH, J = 7.6, 7.2 Hz), 3.46 (m, 2H, C26), 3.32 (t, 2H, J = 10.8 Hz, C26,). ^{13}C NMR (200 MHz, $CDCl_3$: δ 210 (C3), 109 (C22), 80.7 (C16), 66.7 (C26), 62.7 (C17).

2.2.5. *(25R)-2α-bromo-5α-spirosta-3-one* (7)

Procedure as described for **4** gave a white solid of **7**. Yield: 0.18 g (75%), mp 256 °C (decomp), lit. mp 254 °C (decomp) [17]. IR (KBr, cm^{-1}): 2950, 1697, 798. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73 (dd, 1H, C2-βH, J = 6, 7.2, 6.4), 4.37 (dt, 1H, C16-αH, J = 7.6, 7.2), 3.46 (m, 2H, C26), 3.32 (t, 2H, C26, J = 10.8). ^{13}C NMR (400 MHz, $CDCl_3$): δ 201(C3), 109(C22), 80.7 (C16), 67(C26), 62.7(C17), 54 (C2).

2.2.6. *Di(25R-5α-spirostano [2,3-b:2′,3′-e]) pyrazine* (8)

Procedure as described for **5** gave a white solid of pure **8**. Yield: 0.28 g (82%), mp 250 °C (d). IR (KBr, cm^{-1}): 2949, 1412, 1186, 695. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.4 (1H, dt, 16α), 3.5 (2H, m, H-26). ^{13}C NMR (400 MHz, $CDCl_3$: δ 147 (pyrazine carbons), 109 (C22), 80 (C16), 67 (C26),), 66 (C17). Anal. Calcd. for $C_{54}H_{80}N_2O_4$: C, 78.98; H, 9.82; N, 3.41. Found: C, 78.59; H, 9.78; N, 3.15.

2.2.7. *(25R)-4-en-spirosta-3,6-dione (9)*
Freshly prepared Jones reagent was added dropwise to a solution of **2** (2.0 g, 4.82 mmol) in acetone (150 mL) at 10 °C. The reaction mixture was stirred below 20 °C for 30 min. The reaction mixture was extracted with brine solution (2 mL × 20 mL), dried over $NaSO_4$, concentrated. The residue was purified by column chromatography 20% (ethyl acetate/hexane) to give a white solid of pure **9**. Yield: 1.95 g (95%), lit. mp 190–192 °C [21]. IR (KBr, cm^{-1}): 2955, 1687, 1610 and 1456. ^1H NMR (400 MHz, $CDCl_3$): δ 6.3 (s, 1H, C4), 4.36 (dt, 1H, C16-αH), 3.45 (m, 2H, C26), 3.37 (t, 2H, C26). ^{13}C NMR (400 MHz, $CDCl_3$): δ 202 (C3), 199 (C6), 163 (C5), 127 (C4), 109 (C22), 80.6 (C16), 67 (C26), 62.7 (C17), 56 (C14).

2.2.8. *(25R)-4-en-2α-bromo-spirosta-3,6-dione (10)*
Procedure as described for **4** afforded a white solid of **10**. Yield: 0.16 g (67%), mp 187–190 °C. IR (KBr, cm^{-1}): 2946, 1680, 1611, 983. ^1H NMR (400 MHz, $CDCl_3$): δ 6.3 (s, 1H, C4), 4.47 (dt, 1H, C16-αH, J = 6.7, 6.3), 4.44 (d, 1H, C2-βH, J = 3.6), 3.46 (m, 2H, C26), 3.37 (t, 2H, C26, J = 10.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ 198 (C3), 195 (C6), 159 (C5), 129 (C4), 109.5 (C22), 80.6 (C16), 67 (C26), 62.7 (C17), 57 (C14). Anal. Calcd. for $C_{27}H_{37}BrO_4$: C, 64.15; H, 7.38. Found: C, 63.79; H, 7.10.

2.2.9. *Di(25R-6 oxo-4-en-spirostano[2,3-b:2′,3′-e]) pyrazine (11)*
Procedure as described for **8** afforded pure dimeric pyrazine **11**. Yield: 0.11 g (68%), mp 300 °C (d). IR (KBr, cm^{-1}): 2922, 1684, 1607 and 1416. ^1H NMR (400 MHz, $CDCl_3$): δ 6.0 (1H, s, H4), 4.4 (1H, dt, 16α), 3.4 (2H, m, H-26). ^{13}C NMR (400 MHz, $CDCl_3$: δ 146, 145 (pyrazine carbons), 202 (C6), 161 (C5), 129 (C4), 109 (C22), 81 (C16), 67 (C26), 62 (C17). Anal. Calcd. for $C_{55}H_{76}N_2O_6$: C, 76.71; H, 8.89; N, 3.25. Found: C, 76.25; H, 8.77; N, 3.00.

2.2.10. *(25R)-5α-spirosta-3,6-dione (12)*
A solution of **9** (1.0 g, 2.35 mmol) in ethyl acetate (30 mL) was stirred with 10 wt% palladium on carbon (10 mg) under hydrogen at 40 °C for 2 days. The reaction mixture was filtered over a bed of celite, the solvent was evaporated in vacuum. Recrystallization in methanol afforded **12**. Yield: 0.8 g (80%), mp 230–231 °C, lit. mp 230–232 °C [21]. IR (KBr, cm^{-1}): 2960, 1708, and 1706. ^1H NMR (400 MHz, $CDCl_3$): δ 4.67 (1H, dd, H2β), 4.36 (1H, dt, 16α), 3.45 (1H, m, H-26β), 3.37 (1H, t, H26α). ^{13}C NMR (400 MHz, $CDCl_3$): δ 204 (C3), 200 (C6), 109 (C22), 81 (C16), 67 (C26), 62 (C17).

2.2.11. *(25R)-2α-bromo-5α-spirosta-3,6-dione (13)*
Procedure as described for **4** gave a white solid of bromodione **13**. Yield: 0.35g (74%), mp 184–187 °C. IR (KBr, cm^{-1}): 2965, 1686 and 1705. ^1H NMR (400 MHz, $CDCl_3$): δ 4.72 (1H, dd, H2β), 4.45 (1H, dt, 16α), 3.46 (1H, m, H-26β), 3.37 (1H, t, H26α). ^{13}C NMR (400 MHz, $CDCl_3$: δ 205 (C3), 201 (C6), 109 (C22), 81 (C16), 67 (C26), 63 (C17). Anal. Calcd. for $C_{28}H_{43}BrO_4$: C, 64.24; H, 8.28. Found C, 63.88; H, 8.15.

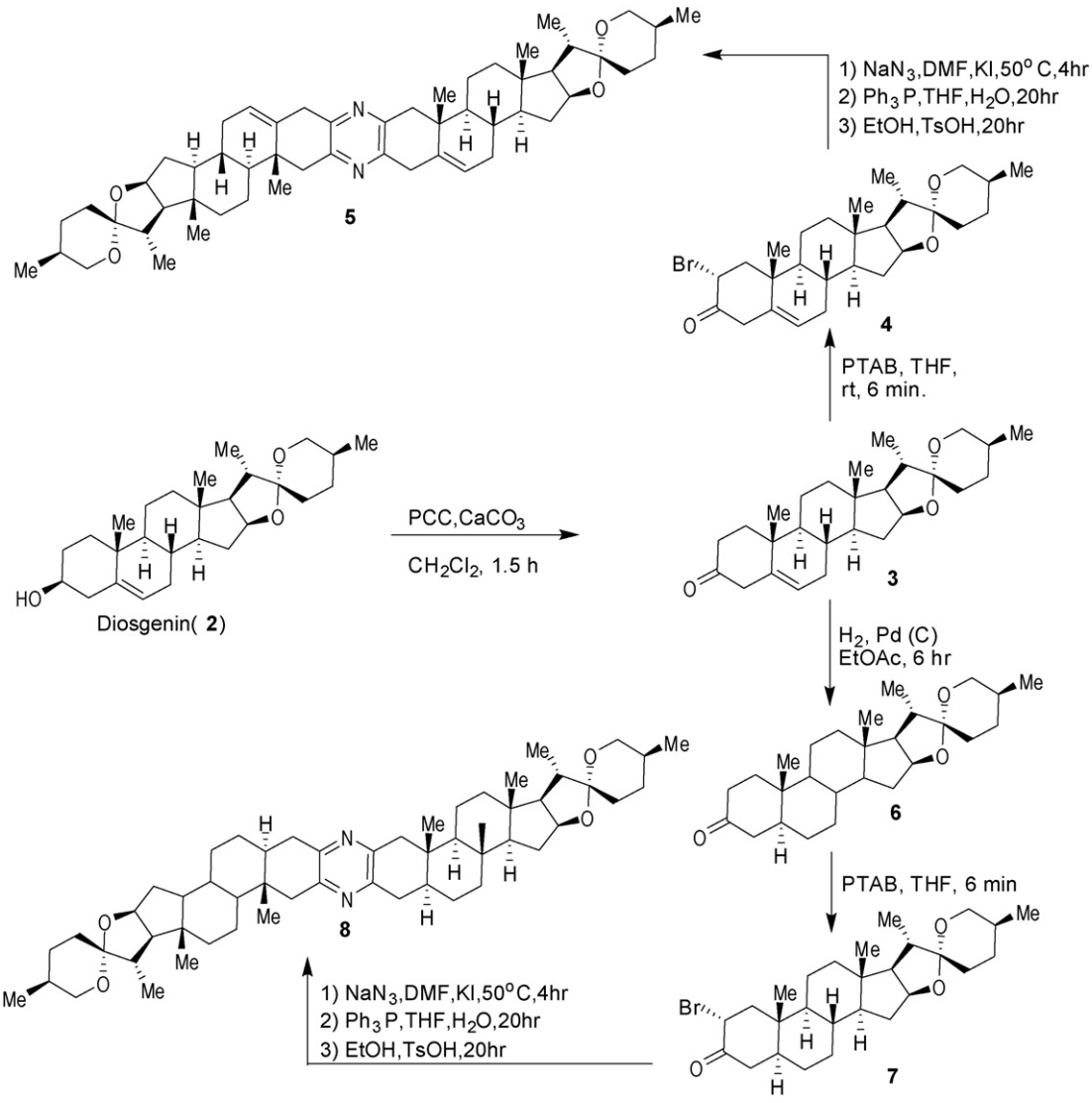
2.2.12. *Di(25R-6 oxo-5α-spirostano[2,3-b:2′,3′-e]) pyrazine (14)*
Procedure as described for **5** afforde pure dimeric pyrazine **14**. Yield: 0.2 g (78%), 280 °C(d). IR (KBr, cm^{-1}): 2950, 1705 and 1407. ^1H NMR (400 MHz, $CDCl_3$): δ 4.4 (1H, dt, 16α), 3.4 (2H, m, H-26). ^{13}C NMR (400 MHz, $CDCl_3$: δ 142, 141 (pyrazine carbons), 203 (C6), 109 (C22), 81 (C16), 67 (C26), 63 (C17). Anal. Calcd. for $C_{55}H_{80}N_2O_6$: C, 76.35; H, 9.32; N, 3.24. Found: C, 75.68; H, 8.97; N, 3.01.

3. Results and discussion

The structure of cephalostatin (**1**) is in essence two substituted cholestane skeletons connected at the C-2 and -3 positions by two nitrogen atoms to form a pyrazine ring. They have been synthesized by the dimerization of two α-amino ketosteroids [14,15]. Initially, the synthesis of dimer **5**, which has a double bond at C5-6, was carried out, as shown in Scheme 1. Oxidation of diosgenin (**2**) with PCC to give the known ketone **3** [17]. The next step was to introduce an amino group or its equivalent (N_3 or NO_2) at C2 of this ketone. This aim was achieved by bromination of the carbonyl group followed by substitution of the bromide by an azido group. Therefore, selective bromination of ketone **3** by phenyl trimethylammonium perbromide (PTAB) gave the α-bromo ketone **4** as the thermodynamic product. The ^1H NMR spectrum for product **4** shows doublet of doublet peak at 4.5 ppm for β proton at C2 and doublet of triplet peak at 4.36 ppm for one α proton at C16. The ^{13}C NMR spectrum shows a new peak at 204 ppm for carbonyl carbon, 121 ppm for C6 and a peak at 54 ppm for C2.

The dimerization of the C-5 unsaturated-2α-bromoketone **4** involved two steps to achieve the formation of the pyrazine dimer **5**. The first step is the substitution of bromo atom with an azido group using sodium azide in the presence of catalytic amount of sodium iodide. This keto azide is not stable at room temperature and it easily converts into enamino ketone (hygroscopic) which failed to dimerize under different conditions. Therefore, the azido intermediate was not isolated but it was reduced to an α-amino ketone by triphenylphosphine in dry THF followed by addition of water to hydrolyzed the aza-wittig intermediate. The TLC of the reduction process afforded a mixture of products. Thus, the intermediate α-amino ketone was not isolated. It was subjected to dimerization conditions by stirring in ethanol and toluene containing a catalytic amount of toluenesulfonic acid and open to the atmosphere to facilitate aromatization to yield the C-5 unsaturated pyrazine dimer **5** in good overall yield. The ^1H NMR spectrum shows a triplet peak at 5.4 ppm for C6 proton and a doublet of triplet peak at 4.4 ppm for one α proton at C16. The ^{13}C NMR spectrum shows two peaks at 143 and 144 ppm characteristics for the pyrazine carbons.

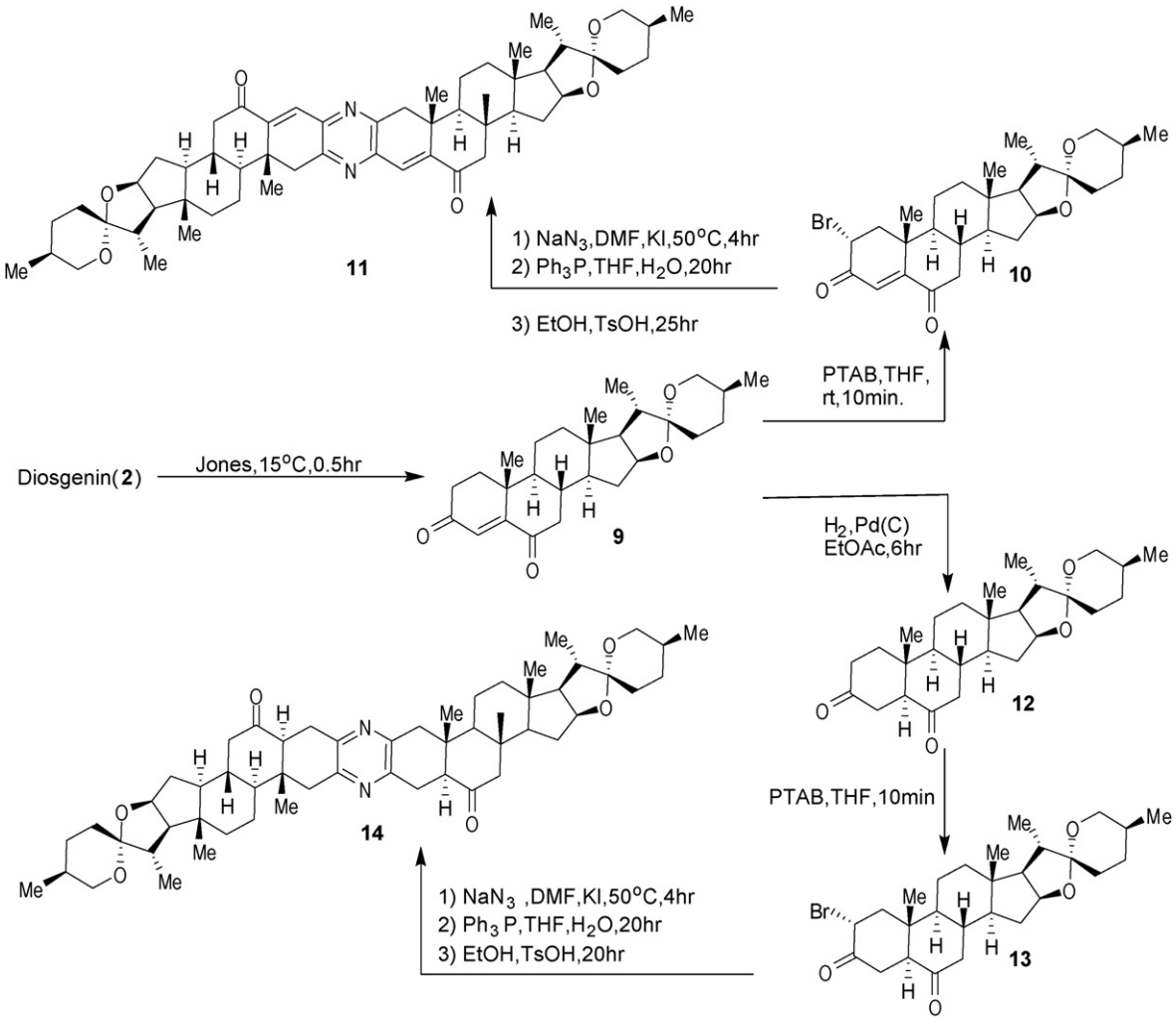
Since cephalostatin **1** has a saturated ring attached on either side of the pyrazine ring, the next target structure was the saturated symmetrical bis-steroidal pyrazine dimer **8**. This dimer lacks the double bond at C5. Therefore, hydrogenation of ketone **3** using palladium on carbon afforded product **6** in excellent yield. In the ^1H NMR spectrum the peak at 5.3 ppm which was characteristics for the C6 hydrogen disappeared which was in good agreement with literature [18]. Selective bromination of ketone **6** by phenyl trimethylammonium perbromide (PTAB) furnished α-bromo ketone **7** as the thermodynamic product. The ^1H NMR spectrum of **7** shows a doublet of doublet peaks at 4.73 ppm (J = 6, 7.2 Hz) for the β proton at C2, and the ^{13}C NMR spectrum shows the car-



Scheme 1 – Synthesis of dimers 5 and 8.

bonyl carbon peak at 201 ppm and a new peak at 54 ppm for C2. The dimerization of α-bromo ketone **7** employing the same methodology as that for dimer **5** furnished C-5 saturated pyrazine dimer **8**. The ^{1}H NMR spectrum shows a doublet of triplet at 4.4 for 16α-H and a multiplet at 3.5 for H-26. The ^{13}C NMR spectrum shows a peak at 147 ppm for pyrazine carbons.

Oxysterols possess powerful biological activities. Some of their effects on the regulation of key enzymes are similar to those of cholesterol, but are much more potent. One of the critical properties of oxysterols is their ability to pass lipophilic membranes at a high rate. Dramatic example of an oxysterol's hydroxyl group were observed on both the rate of transport of oxysterols between lipophilic compartments and the in vivo kinetics of deuterium labeled oxysterols [19]. Therefore, in order to increase the polarity of the next dimer, we decided to increase the oxygen content by further oxidation of the diosgenin (**2**) using Jones reagent. The 4-ene-3,6-dione **9** was prepared in excellent yield. The ^{1}H NMR spectrum for compound **9** shows a new singlet peak at 6.3 ppm for the C4 proton, and the ^{13}C NMR spectrum shows two new peaks at 202 and 199 ppm for carbonyl groups at C3 and C6, respectively. It also shows new peaks at 163 and 127 ppm for C5 and C4, respectively, that is in good agreement with literature values [20,21]. Selective bromination at C2 with PTAB give α-bromo dione due to the steric factor between the bulk group of reagent and the β methyl group at C10 and afforde the bromodione **10** in good yield. The ^{1}H NMR shows singlet peak at 6.3 ppm for the proton on C-4 and doublet at 4.44 ppm (J = 3.6 Hz) for C2-βH. The ^{13}C NMR shows peaks at 198, 195,159 and 129 ppm for C3, C6, C5 and C4, respectively. The dimerization of compound **10** using the same methodology as that for dimer **5** furnished the 4-ene-6-one pyrazine dimer **11** (Scheme 2). The ^{1}H NMR spectrum shows singlet peak at 6.0 ppm for C4 proton and doublet of triplet peak at 4.4 ppm for one α proton at C16. The ^{13}C NMR spectrum shows two peaks at 145 and 146 ppm characteristics for the pyrazine carbons and three peaks at 202, 161 and 129 ppm characteristics for C6, C5 and C4, respectively.



Scheme 2 – Synthesis of dimers 11 and 14.

The last dimer, dimer **14** was synthesized in order to more closely duplicate the structure of cephalostatin (**1**). The endione **9** was reduced to the dione **12** by hydrogenation using palladium on carbon. The dione **12** was characterized by ^{1}H NMR. The doublet of doublets at 6.3 ppm for H4 was disappeared and the ^{13}C NMR spectrum shows peaks at 200, 204 ppm characteristics for C3, C6, respectively [21]. Selective bromination at C2 gives the bromodione **13** in good yield. The ^{1}H NMR spectrum shows a doublet of doublets at 4.72 ppm characteristics for H2β. The ^{13}C NMR spectrum shows peaks at 205, 201 ppm characteristics for C3, C6, respectively Finally, the dimerization of compound **13** using same methodology as that for dimer **5** afforded dimer **14**. The ^{1}H NMR spectrum shows a doublet of triplet peak at 4.4 ppm for one α proton at C16 and a multiplet at 3.4 ppm for H-26. The ^{13}C NMR spectrum shows two peaks at 142 and 141 ppm characteristics for the pyrazine carbons and three peaks at 203, 109 and 67 ppm characteristics for C6, C22 and C26, respectively. Samples of the prepared dimers (**5**, **8**, **11**, **14**) will be submitted for biological testing.

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REFERENCES

[1] Dubowchik GM, Firesone RA. The synthesis of branched steroidal prodrugs of nitrogen mustard for antitumor targeting via reconstituted LDL. Tetrahedron Lett 1994;35:4523–6.
[2] Delbarre A, Oberlin R, Roques B. Ellipticine derivatives with an affinity to the estrogen receptor. An approach to develop intercalating drugs with a specific effect on the hormone-dependent breast cancer. J Med Chem 1985;28:752–61.
[3] Thmas GL, Guo C, Boyd MR, Fuchs PL. Outer-ring stereochemical modulation of cytotoxicity in cephalostatin. Org Lett 2000;2(1):33–6.

[4] Gan Y, Wientjes MG, Au JL-S. Expression of basic fibroblast growth factor correlates with resistance to paclitaxel in human patient tumors. Pharm Res 2006;23(6):1324–31.
[5] Mukhopadhyay S, Maitra U. Chemistry and biology of bile acids. Curr Sci 2004;87(12):1666–83.
[6] Matile J, Beroua N, Nakanishi K, Wood R. Structural studies by exciton coupled circular dichroism over a large distance: porphyrin derivatives of steroids, dimeric steroids, and brevetoxin B. J Am Chem Soc 1996;118:5198–206.
[7] Guthre JP, Cossa J, Darson BA. A water soluble dimeric steroid with catalytic properties. Rate enhancements from hydrophobic binding. Can J Chem 1986;64:2456–69.
[8] Schmidt A, Beckert A, Weiss RD. Simple procedure for reductive coupling of steroids with a cross-conjugated dienone system. Tetrahedron Lett 1992;33:4299–300.
[9] Pettit JM, Xu JP, Schmidt JM. Isolation and structure of the exceptional Pterobranchia human cancer inhibitors cephalostatins 16 and 1. Bioorg Med Chem Lett 1995;5:2027–30.
[10] Camen B, Raimunda F, Perez-Martin I, Tierry P, Ernesto S. A convenient synthesis of C-22 and sterioisomers of cephalostatin North 1 side chain from spirostan sapogenin. Org Lett 2002;4(8):1295–8.
[11] Yuexian L, Dias JR. Dimeric and oligomeric steroids. Chem Rev 1997;97:283–304.
[12] Ganesan A. The dimeric steroid-pyrazine marine alkaloids: challenges for isolation, synthesis, and biological studies. Angew Chem Int Ed Engl 1996;35:611–4.
[13] Fukuzawa S, Matsunaga S, Fusetani N, Ritterazine A. A highly cytotoxic dimeric steroidal alkaloid, from the tunicate *Ritterella tokioka*. J Org Chem 1994;59: 6164–6.
[14] Ganesan A. When two steroids are better than one: the dimeric steroid-pyrazine marine alkaloids. Stud Nat Prod Chem 1996;18:875–906.
[15] Drogemuller M, Flessner T, Jautelat R, Scholzf U, Winterfeldt E. Synthesis of cephalostatin analogues by symmetrical and non-symmetrical routes. Eur J Org Chem 1998:2811–31.
[16] LotowSki Z, Gryszkiewicz A, Borowiecka B, Nikitiuk A, Morzycki JW. A facile synthesis of symmetrical dimeric steroid-pyrazines. Chem Res (S) 1999:662–3.
[17] Rüchardt C, Gerst M, Ebenhoch J. Nichtkatalysierte transferhydrierung und transferhydrogenolyse: neue Wege der wasserstoffübertragung. Angew Chem 1997;109:1474–98.
[18] Zawistoski, Michael P, Kiplinger, Jeffrey P, McCarthy PA. Synthesis of [2,2,3α,4,4-D5]CP-88,818 (tiqueside), an internal standard for a quantitative HPLC/MS assay system. Tetrahedron 1993;49(22):4799–808.
[19] Meaney S, Bodin K, Diczfalusy U, Björkhem I. On the rate of translocation in vitro and kinetics in vivo of the major oxysterols in human circulation: critical importance of the position of the oxygen function. J Lipid Res 2002;44:2130–5.
[20] Bogdan AS, Dragana RM, Dosen-Micovic L. Oxidation of steroidal 5-en-3β-ols with Jones reagent in ether. Steroids 1994;59(5):330–4.
[21] Romo J, Rosenkranz G, Djerassi C, Sondheimer F, Syntex SA, Steroids LV. Steroidal sapogenins. Part XXXV. Chemical introduction of the 6β-hydroxy group into steroidal Δ4-3-ketones by a two step sequence. J Org Chem 1954;19:1509–15.