A Short Route to Cephalostatin Analogues

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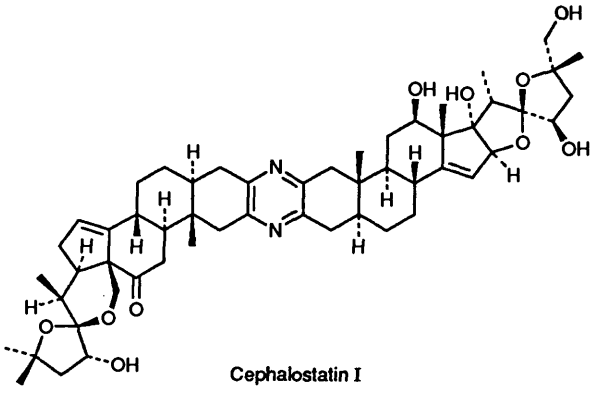
Starting from hecogenin a short route to non-symmetric pyrazino-bis-steroids is described.

The limited availability of the powerful cytotoxins cephalostatins[1,2] persuaded us to attempt the synthesis of the corresponding pyrazino-bis-steroids. The steroid part of cephalostatin I is quite similar to that of hecogenin **2a** although, remarkably, it is a dissymmetric molecule and has a $\Delta^{14,15}$ double bond.

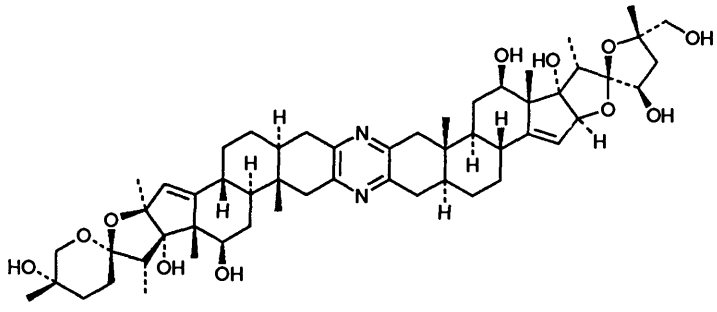
In synthesizing the title compounds a decision was necessary as to whether a direct approach to non-symmetric pyrazine synthesis[3,4] should be attempted or whether symmetric compounds should be prepared and dissymmetry introduced at a later stage. We decided on the latter course. Although Heathcock[5] and Fuchs[6] recently reported their success in preparing both symmetric and non-symmetric pyrazino-bis-steroids the results disclosed here lead not only to non-symmetric compounds but also ones which for the first time contain a $\Delta^{14,15}$-double bond.

Our starting material was the diketone **3a**, a $\Delta^{14,15}$-hecogenin derivative. This compound, readily available from hecogenin by a photoprocess[7,8] followed by an oxidation, is, of course, well suited to selective transformations at the two carbonyl groups. Standard bromination techniques gave **3b** which, on subsequent nucleophilic substitution, was easily converted into the azide **3c**. Although azides have generally been used to form amines, α-keto azides are known to decompose easily to form imino ketones or enamino ketones on treatment with base.[6,9] In our work this led to the enamino ketone **4** (90%), which, as expected, showed no tendency to pyrazine formation. Hydrogenation, however, gave the cephalostatin analogue **5a** spontaneously in good yield and provided crystalline material (64%) after purification. Standard reduction conditions (sodium borohydride–MeOH, 70%) followed by acetylation then gave the corresponding 12,12′β-diacetate **5b** (60%).

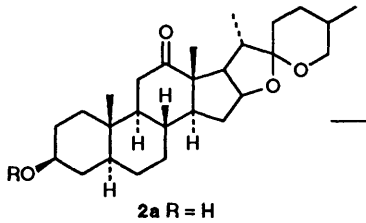
Since we now had two compounds available for selective transformations at the 12,12′-positions we generated from the diketone **5a** the non-symmetric monoenolates. Treatment of these with pivaloyl chloride in the presence of potassium hexamethyldisilazanide (3.2 equiv.) as the proton-accepting species in dry THF gave a 1:2 mixture of the bis-enol pivalate



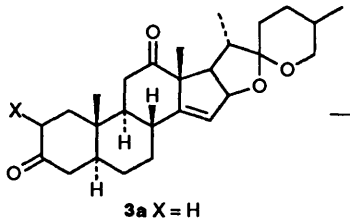
Cephalostatin I



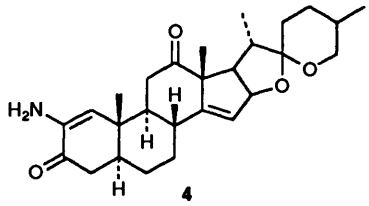
Cephalostatin VII



2a R = H
b R = Ac



3a X = H
b X = Br
c X = N_3



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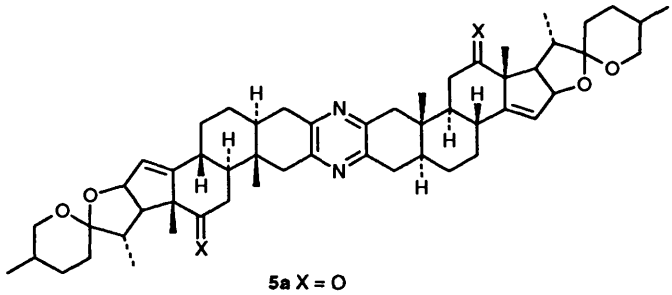
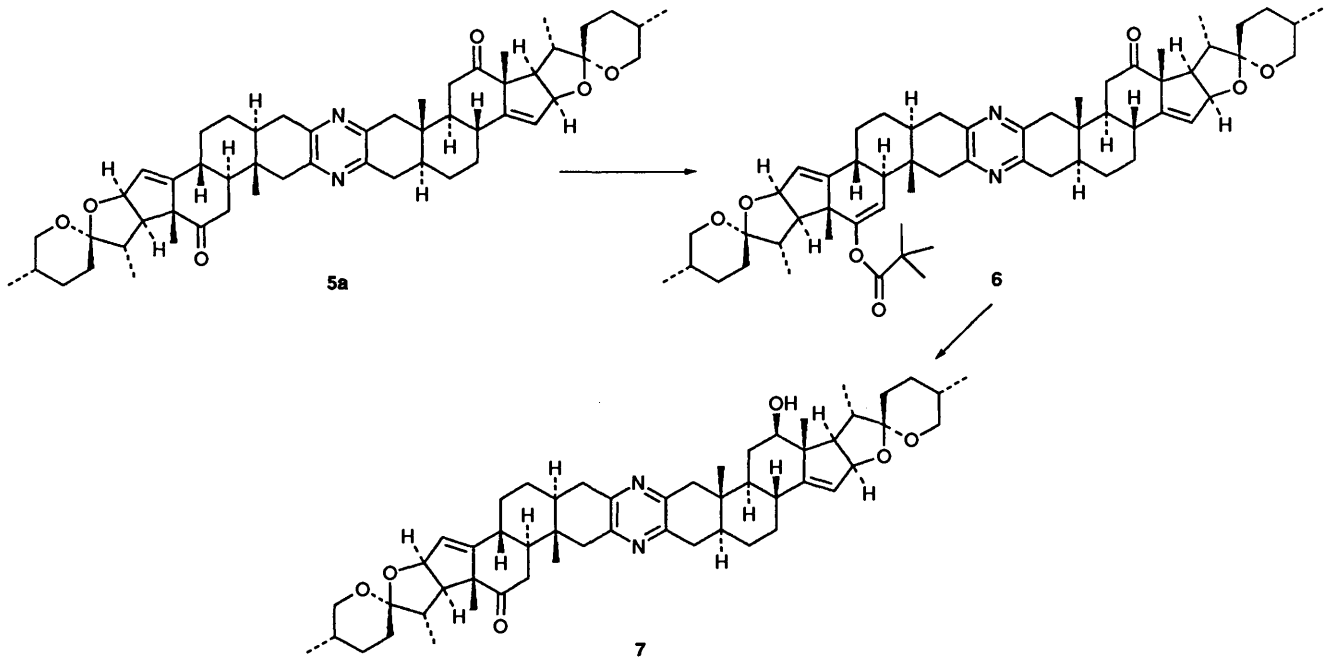


5a X = O
b X = αH, βOAc



and the desired monopivalate **6**. The two compounds were easily separated by flash chromatography, which provided **6** (40%).

Borohydride reduction of **6** followed by hydrolysis of the enol pivalate yielded the hydroxy ketone **7** (80%), steroid rings of which were substituted in a similar way to those in cephalostatin I. Data on the biological activity of this material and related compounds will be published elsewhere.

Experimental

M.p.s were recorded on a Büchi melting point microscope. UV spectra were measured in methanol on a Beckman 3600 instrument and IR spectra on a Perkin–Elmer 581 spectrometer. ^1H and ^{13}C NMR spectra were recorded on the Bruker AM 200 and δ values are given relative to tetramethylsilane; J values in Hz. Mass spectra were determined with a Finnigan MAT 312 instrument at 70 eV. Elemental analysis were obtained using a Heraeus CHN rapid analyser. For flash chromatography, silica gel (300–600 mesh, Baker) was used at 0.3 bar and all solvents were dried by the usual methods.

(25R)-2-*Amino*-5α-*spirosta*-1,14-*diene*-3,12-*dione* **4**.—The bromo ketone **3b** (500 mg, 0.99 mmol) was dissolved in dimethylformamide (50 cm^3) and sodium azide (700 mg) and sodium iodide (a few mg) were added to the solution. After being stirred for 2 h at 50 °C, the reaction mixture was brought to room temperature and poured into water (20 cm^3) and extracted with Et_2O–Bu^{t}OMe. This extract was washed with brine, dried ($MgSO_4$) and evaporated to yield, after crystallisation, the title compound **4** (407 mg, 80%); λ_{max}(MeOH)/nm 214 and 290; ν_{max}(KBr)/cm^{-1} 3452, 3368, 3060, 1708, 1676 and 1628; δ_H(200 MHz; CD_2Cl_2) 0.79 (3 H, d, J 6), 1.01 (3 H, d, J 7), 1.10 (3 H, s), 1.30 (3 H, s), 2.50 (5 H m), 3.34 (4 H, m), 4.71 (1 H, dd, J 8/2), 5.41 (1 H, tr, J 2) and 5.86 (1 H, s); m/z 439 (M^+, 25%), 325 (35), 310 (19), 136 (25) and 126 (19) (Found: M^+, 439.2722. $C_{27}H_{37}NO_4$ requires M, 439.2708.

Pyrazino[2,3-b;5,6-b′]*bis*[(25R)-5α-*spirost*-14-*ene*]-12,12′-*dione* **5a**.—The enamino ketone **4** (897 mg, 2.04 mmol) was dissolved in ethyl acetate (50 cm^3) and methanol (3 cm^3). Acetic acid and palladium-on-charcoal (10%; 270 mg) were added to the solution which was then hydrogenated at room temperature (TLC-control). After completion of the reaction the solution was filtered and evaporated to give a residue which was purified by chromatography to the pyrazine **5a** (64%); λ_{max}(MeOH)/ nm 211, 288 and 305sh; ν_{max}(KBr)/cm^{-1} 3060, 1712, 1644, 1396, 1376, 1064 and 980; δ_H(200 MHz; $CDCl_3$) 0.80 (3 H, d, J 6), 0.92 (6 H, s), 1.04 (6 H, d, J 7), 1.33 (6 H, s), 3.44 (4 H, m), 4.78 (2 H, dd, 8/2) and 5.49 (2 H, s br); m/z (FAB) 845.5 (M^+, 100%) [Found: C, 76.65; H, 8.6; N, 4.0. $C_{54}H_{72}N_2O_6$ (845.18) requires C, 76.74; H, 8.60; N, 3.31%].

Pyrazino[2,3-b;5,6-b′]*bis*[(25R)-5α-*spirost*-14-*ene*]-12,12′-*diyl Diacetate* **5b**.—The pyrazine dione **5a** (46 mg, 0.0544 mmol) was dissolved in dichloromethane–methanol (1:1; 5 cm^3) and the solution cooled to −78 °C; sodium borohydride (4 mg) was then added to it. After 4 h at −78 °C the excess of borohydride was destroyed with acetone (1 cm^3) and the mixture allowed to reach room temperature. It was then diluted with dichloromethane, washed with aqueous NaOH (1 mol dm^{-3}) and brine, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography. The resulting product was dissolved in dry pyridine (2 cm^3) and after addition of dimethylaminopyridine (a few mg) and acetic anhydride (0.022 cm^3), the solution was refluxed for 3 h. It was then brought to room temperature and poured into ice–water and extracted with Et_2O–Bu^{t}OMe. The extract was washed with HCl (1 mol dm^{-3}) and brine, dried ($MgSO_4$) and evaporated. The residue was separated by flash chromatography to yield the diacetate **5b** (31 mg; 60%); λ_{max}(MeOH)/nm 210, 287 and 305sh; ν_{max}(KBr)/cm^{-1} 3060, 1751, 1741, 1400, 1375, 1065 and 982; δ_H(200 MHz; $CDCl_3$) 0.81 (6 H, d, J 6), 0.85 (6 H, s), 1.00 (6 H, d, J 7), 1.10 (6 H, s), 2.06 (6 H, s), 3.43 (4 H, m), 4.43 (2 H, dd, J 11/5), 4.87 (2 H, dd, J 8/2) and 5.48 (2 H, s br); m/z (FAB) 933 (M^+, 100%) [Found: C, 74.1; H, 8.35; N, 3.6. $C_{58}H_{80}N_2O_8$ (933.279) requires C, 74.64; H, 8.64; N, 3.00%].

11′,12′-*Didehydro*-12′-*pivaloyloxypyrazino*[2,3-b;5,6-b′]*bis*-[(25R)-5α-*spirost*-14-*en*-12-*one*] **6**.—The pyrazinedione **5a** (100 mg) was dissolved in dry tetrahydrofuran (7 cm^3) and, under an argon atmosphere, potassium hexamethyldisilazanide (3.2 equiv.) was added at −78 °C. After 10 min pivaloyl chloride (0.16 cm^3) was added to the reaction mixture which was then

poured into aqueous citric acid (20%; 15 cm^3) and extracted with Et_2O–Bu^tOMe. The extract was washed with aqueous sodium hydrogen carbonate and brine, evaporated and separated by flash chromatography to yield the title compound **6** (44 mg, 40%); ν_{max}(MeOH)/nm 208, 288 and 305sh; ν_{max}(KBr)/cm^{-1} 3060, 1740, 1708, 1668, 1396 and 1132; δ_H(200 MHz, $CDCl_3$) 0.80 (6 H, d, *J* 6), 0.89 (3 H, s), 0.92 (3 H, s), 1.05 (6 H, d/d, each *J* 7), 1.22 (3 H, s), 1.28 (9 H, s), 1.33 (3 H, s), 3.45 (4 H, m), 4.81 (2 H, dd/dd, each *J* 8/2), 5.21 (1 H, s br) and 5.49 (1 H, m); *m/z* (FAB) 929.7 (M^+, 100%) [Found: C, 76.2; H, 8.7; N, 3.45. $C_{59}H_{80}N_2O_7$ (929.298) requires C, 76.25; H, 8.67; N, 3.01%].

12β-*Hydroxypyrazino*[2,3-b;5,6-b′]*bis*[(25R)-5α-*spirost*-14-*en*-12′-*one*] **7**.—The enol pivalate **6** (63 mg, 0.0678 mmol) was dissolved in dichloromethane–methanol (1:1; 8 cm^3) and the solution cooled to −78 °C. Sodium borohydride was then added to it. After 3 h at −78 °C the excess of borohydride was destroyed with acetone (1.5 cm^3) and the mixture allowed to reach room temperature. It was then diluted with dichloromethane, washed with aq. NaOH (1 mol dm^{-3}), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (silica, light petroleum–ethyl acetate, 1:1). The product was then dissolved in dichloromethane–methanol (1:1; 4 cm^3) and, after the addition of KOH (67 mg) in water (0.5 cm^3), was refluxed for 12 h. The reaction mixture was then treated with aqueous citric acid (20%; 5 cm^3) and extracted with dichloromethane. The extract was washed with aqueous sodium hydrogen carbonate and brine, dried ($MgSO_4$) and evaporated. On chromatography (silica, light petroleum–ethyl acetate, 1:1) the residue gave the hydroxy ketone **7** (48 mg, 78%); ν_{max}(MeOH)/nm 208, 288 and 305sh; ν_{max}(KBr)/cm^{-1} 3443, 3060, 1715, 1645 and 1399; δ_H(200 MHz; CD_2Cl_2) 0.8 (6 H, d, *J* 6), 0.85 (3 H, s) 0.90 (3 H, s), 1.01 (9 H, d, *J* 7), 1.31 (3 H, s), 3.36 (6 H, m), 4.73 (1 H, dd, *J* 8/2), 4.83 (1 H, dd, *J* 8/2), 5.37 (1 H, tr, *J* 1) and 5.42 (1 H, tr, *J* 1); *m/z* (FAB) 929.7 (M^+, 100%) [Found: C, 76.7; H, 8.8. $C_{54}H_{74}N_2O_6$ (847.196) requires C, 76.56; H, 8.80%].

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