Steroids 77 (2012) 276–281

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Transetherification-mediated E-ring opening and stereoselective "Red-Ox" modification of furostan

Young Cheun, Myong Chul Koag, Yi Kou, Zachary Warnken, Seongmin Lee *

The Division of Medicinal Chemistry, College of Pharmacy, University of Texas at Austin, Austin, TX 78712, United States

ARTICLE INFO

Article history:
Received 7 October 2011
Received in revised form 5 December 2011
Accepted 6 December 2011
Available online 17 December 2011

Keywords:
E-ring opening
Cephalostatin
Transetherification

ABSTRACT

We have developed a novel E-ring opening method for furostan, and applied it to prepare D-ring modified steroids, which can be used to synthesize cephalostatin analogs.

1. Introduction

Cephalostatins and ritterazines are bis-steroidal pyrazine marine natural products isolated from different *phyla*; 19 cephalostatins from *Cephalodiscus gilchristi* and 27 ritterazines from *Ritterela tokioka* [1–3]. The 45 members of cephalostatin family display extreme antiproliferative activities against various human tumors, for example cephalostatin 1 **1** showing avg. 1.8 nM of GI_{50} values in the NCI-60 cancer cell lines. Currently, the target biomolecule and the mechanism of action of these anticancer steroids are still poorly understood. The fingerprint of cephalostatin bioactivities in the 60-tumor panel is quite different from those of known antitumor agents, potentially indicating a new mode of action [4–8]. Semiempirical calculations for rationalizing the SAR of natural cephalostatins/ritterazines and their analogues show a strong correlation between bioactivity and enthalpy of oxacarbenium ion formation [3], implicating a potential role for the cephalostatins' spiroketal moiety in the E/F rings as a latent precursor of oxacarbenium ion (e.g., E-ring oxacarbenium ion **2**), which can react with bionucleophiles (e.g., DNA) to exert their bioactivities (Fig. 1) [9].

Majority of cephalostatins possess molecular architectures highly functionalized at D, E, and F-rings. For example, D–F ring of north unit of cephalostatin 1 **1** contain a 5/5 spiroketal moiety, primary, secondary, and tertiary alcohols, and six contiguous stereocenters (Fig. 1). Due largely to these molecular complexities in D–F ring, synthesis of cephalostatins has been very challenging, as is evidenced in that earlier syntheses of north unit of cephalostatin 1 **1** required over 30 synthetic steps from starting materials bearing A–C ring of the unit [10–12]. These syntheses involved deletion of 6 carbon atoms from hecogenin acetate via Marker degradation or addition of 6 carbon atoms to *trans*-androsterone via Sonogashira coupling. In conjunction our efforts to develop efficient synthetic routes for cephalostatins, we have embarked "Red-Ox" strategy where we seek to prepare cephalostatins by using multiple reductions and oxidations from a commercially available hecogenin acetate **3** with retention of all the 27 carbon atoms in the starting material (Fig. 2).

For cephalostatin synthesis studies, methods that enable rapid elaboration of D–F ring with an intact steroid skeleton would be of great use. One such method would be ring opening of steroidal spiroketals (spirostans) and cyclic ethers (furostans). Currently, various ring-opening protocols have been developed to modify spirostans and furostans. Among them are Zn/HCl [13], $K_2Cr_2O_7$/AcOH [14], DMDO/Ac_2O or TMSI [15,16], PPh_3/I_2/Lewis acid [17], and trifluoroacetyltrifluoro methanesulfonate [18]. We also reported CrO_3/Bu_4NIO_4-mediated opening of E-ring leading to the formation of a diketone [19], which was further "Red-Ox" functionalized to provide an analog **4** of north unit of cephalostatin 1 **1** (Fig. 3) [20]. Herein, we describe another novel E-ring cleavage method, and its application to the synthesis of D-ring modified steroids.

2. Experimental

2.1. General methods

Reagents, such as triethylsilane, borontrifluoride etherate, iodine, purchased from Aldrich Chemical Company Inc., were used

* Corresponding author. Tel.: +1 512 471 1785; fax: +1 512 471 4726.
E-mail address: SeongminLee@mail.utexas.edu (S. Lee).

0039-128X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.12.015

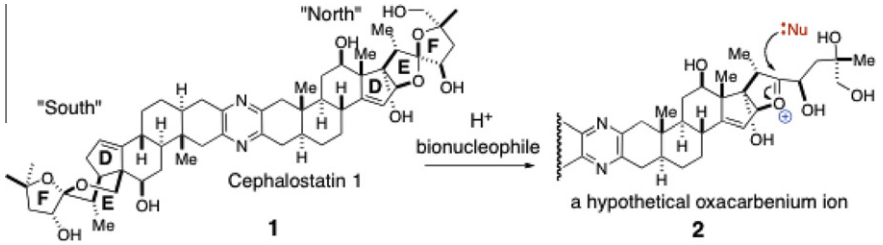


Fig. 1. Anticancer bissteroidal pyrazine cephalostatin 1 **1** and its potential chemical mode of action.

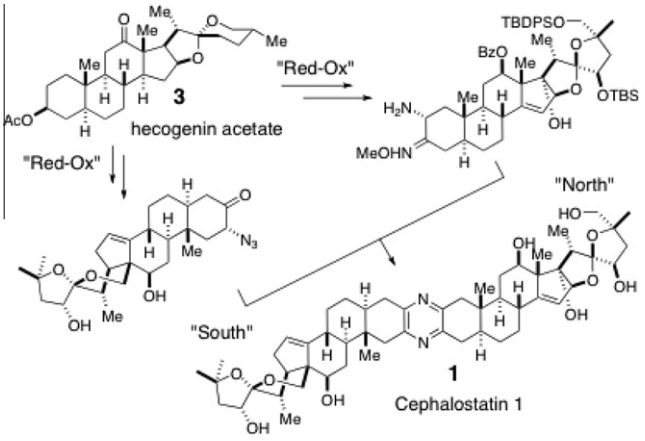


Fig. 2. "Red-Ox" strategy for cephalostatin 1 **1** synthesis.

as received. Acetonitrile, methylene chloride, pyridine, triethylamine, and *N*,*N*-diisopropylamine were distilled from calcium hydride: Methanol was distilled from magnesium turnings: THF was distilled from Na/benzoquinone. *N*-Bromosuccinimide was recrystallized from boiling water. Sodium sulfate (Na_2SO_4) was anhydrous. All chromatographic and workup solvents were distilled.

Unless otherwise indicated, all reactions were carried out under a positive pressure of argon in anhydrous solvents and the reaction flasks were fitted with rubber septa for the introduction of substrates and reagents via syringe. Progress of reactions was monitored by thin layer chromatography (TLC) in comparison with the starting materials. All TLC analyses were carried out on Merck Silica Gel 60 F254 TLC plates, thickness of 0.25 mm. The plates were visualized by ultraviolet illumination at 254 nm and immersion in visualizing solution. The two commonly employed TLC visualizing solutions were: (i) *p*-anisaldehyde solution (1350 mL absolute ethanol, 50 mL concentrated H_2SO_4, 37 mL *p*-anisaldehyde), and (ii) permanganate solution (weight percents of 1% $KMnO_4$ and 2% Na_2CO_3 in water).

Analytical samples were obtained from flash silica gel chromatography, using silica gel of 230–400. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 500 (500 MHz). NMR spectra were determined in chloroform-d_1 ($CDCl_3$) and are reported in parts per million (ppm) from the residual chloroform (7.24 and 77.0 ppm) and benzene (7.16 and 128.39 ppm) standard, respectively. Peak multiplicates in ^{1}H-NMR spectra, when reported, are abbreviated as s (singlet), d (doublet), t (triplet), m (multiplet), and/or ap (apparent) and/or br (broad). Mass spectra were all obtained on either a JEOL AX-505 or a JEOL SX-102.

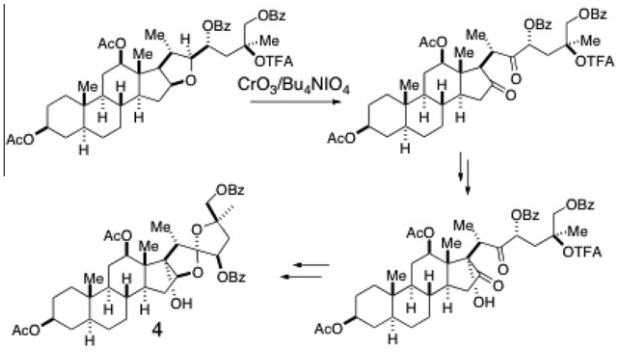


Fig. 3. Synthesis of an analog **4** of north unit of cephalostatin 1 **1** via an oxidative E-ring opening and "Red-Ox" elaboration of a furostan.

2.2. Chemical synthesis

2.2.1. Lumihecogenin acetate (5)

Hecogenin acetate **3** (25.4 g, 53.8 mmol) was dissolved in dichloromethane and was argonated. The mixture was irradiated with a 500 W Hanovia medium-pressure Hg lamp for 2 days at an ambient temperature, concentrated under reduced pressure, and subjected to silica gel chromatography to give an aldehyde **5** (21.3 g, 83%) as white solids.

^{1}H NMR (300 MHz, C_6D_6) δ 9.34 (1H, s, CHO), 4.86 (1H, m, C3-H), 3.68 (2H, s), 2.52–2.88 (2H, m, C26-H), 2.34 (1H, m), 1.86 (3H, s, C3-OAc), 1.66 (3H, s), 1.35 (3H, d), 0.74 (3H, d), 0.56 (3H, s); ^{13}C NMR (75 MHz, C_6D_6) δ 198.8, 168.9, 136.2, 106.0, 78.7, 72.6, 67.2, 48.6, 45.5, 42.5, 39.0, 37.9, 37.2, 36.0, 32.9, 31.1, 30.7, 29.4, 29.0, 28.4, 21.7, 17.8, 13.9, 12.4; LRMS (ESI) 472 (M+); HRMS (ESI) calculated for $C_{29}H_{45}O_5$ (M + H) 473.3267, found 473.3259.

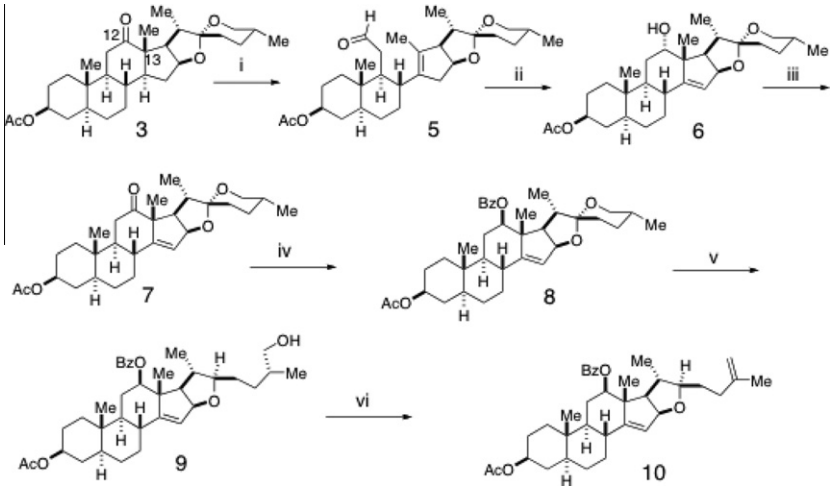
2.2.2. 3β-Acetoxy-12a-hydroxy-5α-spirostan-14-ene (6)

To a CH_2Cl_2 solution of lumihecogenin acetate **5** (12.5 g, 26.4 mmol) was added zinc chloride (7.18 g, 52.8 mmol, 2 equiv) in one portion, and the resulting mixture was stirred at an ambient temperature. After 2 h, the reaction mixture was quenched by adding saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and subjected to silica gel chromatography to give a homoallylic alcohol **6** (9.02 g, 72%). ^{1}H NMR and mass spectral data are consistent with known values [21].

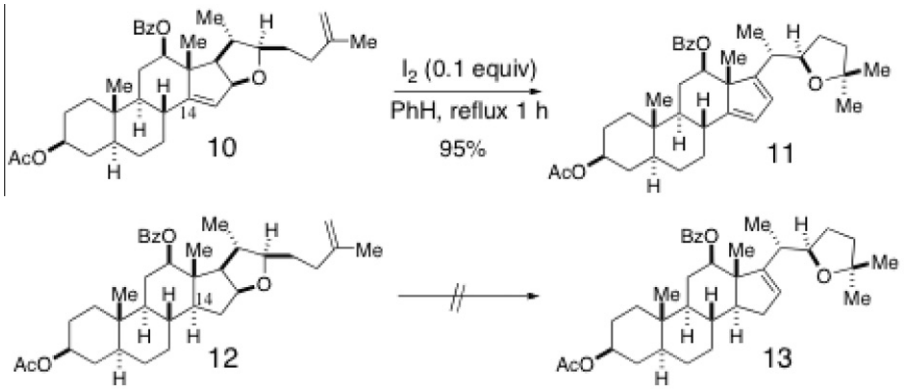
2.2.3. 3β-Acetoxy-12-keto-5α-spirostan-14-ene (7)

To an acetone (125 mL) solution of a homoallylic alcohol **6** (5.94 g, 12.5 mmol) was added dropwise Jones' reagent at 0 °C, and the resulting mixture was stirred for 10 min at the same temperature. The reaction mixture was quenched by adding saturated sodium thiosulfate, was concentrated *in vacuo,* and extracted with ethyl acetate. The organic layer was collected, dried over anhydrous Na_2SO_4, concentrated by using rotary evaporator, and subjected to silica gel chromatography to give ketone **7** (5.27 g, 89%).

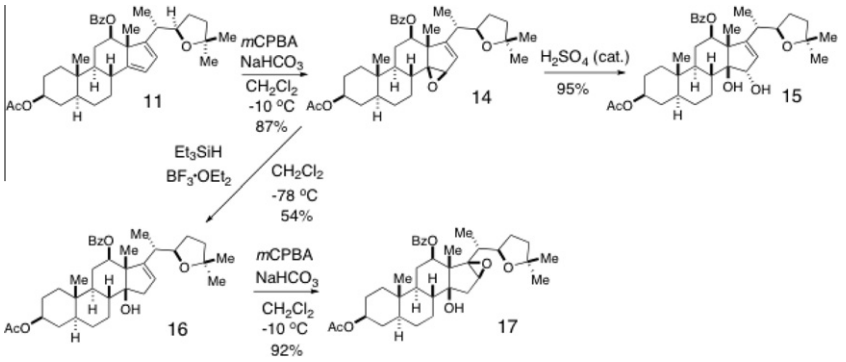
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.45 (1H, br), 4.77 (1H, dd, J = 8 and 2 Hz), 4.68 (1H, m), 3.49 (1H, dd, J = 10.9 and 3.0 Hz), 3.41 (1H, d,



Scheme 1. Preparation of a steroidal cyclic ether bearing Δ^{14} olefin moiety: (i) hv, CH_2Cl_2, 2 days, (ii) $ZnCl_2$, CH_2Cl_2, 25 °C, 60% two steps, (iii) Johns reagent, acetone, 0 °C 10 min, 89% (iv) $CeCl_3$, $NaBH_4$, THF/MeOH, 0 °C 5 h; BzCl, pyridine, 25 °C 6 h, 82% (v) Et_3SiH, BF_3●OEt_2, CH_2Cl_2, 18 h, 94%, (vi) PPh_3, I_2, imidazole, THF; DBU, DMF, 25 °C 12 h, 78%.



Scheme 2. E-ring opening of furostan via transetherification.



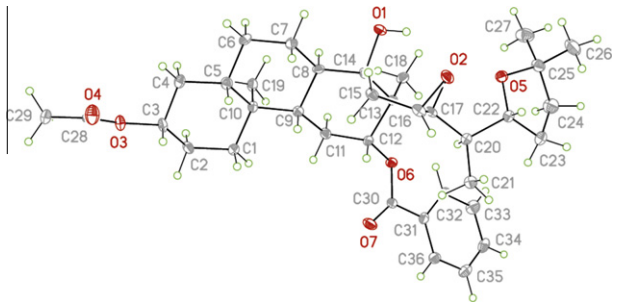
Scheme 3. "Red-Ox" modification of a steroidal D-ring diene **11.**

J = 11.1 Hz), 3.33 (1H, d, *J* = 8.7 Hz), 2.56 (1H, d, *J* = 14.3 Hz), 2.46 (1H, d, *J* = 11 Hz), 2.35 (1H, dd, *J* = 14.6, 4.6 Hz), 2.03 (3H, s), 1.99 (3H, d, *J* = 7 Hz), 1.26 (3H, s), 1.04 (d, *J* = 6.9 Hz), 0.95 (3H, s), 0.81 (3H, d, *J* = 6.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 213.4, 170.5, 154.6, 119.8, 106.9, 83.8, 72.9, 66.9, 62.1, 53.4, 49.6, 44.0, 43.9, 37.2, 36.1, 36.1, 33.6, 34.0, 31.1, 30.2, 29.3, 27.8, 28.7, 27.1, 21.3, 19.8, 17.0, 13.7, 11.6.

^{1}H and ^{13}C NMR spectral data are consistent with known values [22].

2.2.4. 3β-Acetoxy-12β-benzyloxy-5α-spirostan-14-ene ***(8)***

To a solution of ketone **7** (12.7 g, 27.0 mmol) in THF/MeOH (1:1, 135/135 mL) was added cerium chloride heptahydrate (7.03 g, 18.9 mmol) at 0 °C and the mixture was stirred for 30 min at the same temperature. Sodium borohydride (1.53 g, 40.5 mmol) was added portionwise over 1 h and the resulting mixture was stirred for additional 4 h. The reaction was quenched by adding water (250 mL) to give precipitates, which were removed by filtration. The filtrate was concentrated under reduced pressure, and



Scheme 4. ORTEP view of the X-ray structure of an epoxyalcohol **17.**

partitioned between ethylacetate (200 mL) and water. The organic layer was washed with brine, dried over Na_2SO_4, concentrated, and subjected to silica gel chromatography to give C12-β alcohol (11.0 g, 87%) **8** and C12-α alcohol (1.04 g, 8%). To a pyridine (120 mL) solution of the C12-β alcohol (11.0 g, 23.3 mmol) was added benzoyl chloride (4.89 g, 35.0 mmol) and the resulting mixture was stirred for 6 h. The reaction was quenched by adding saturated aqueous sodium bicarbonate. The resulting mixture was concentrated *in vacuo*, and subjected to silica gel column chromatography to provide benzoate **8** (12.6 g, 94%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–8.05 (5H, m), 5.46 (1H, s, C14–H), 4.86 (1H, d, C12–H), 4.59–4.74 (2H, m, C3–H, C16–H), 3.32–3.50 (2H, m, C26–H), 2.43 (1H, t), 2.12 (1H,), 1.99 (3H, s, C3-OAc), 1.22 (3H, s), 0.86 (3H, d), 0.84 (3H, s), 0.74 (3H, d); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 165.7, 156.5, 132.4, 130.4, 129.4, 128.1, 120.2, 106.6, 84.3, 81.6, 73.0, 66.2, 55.7, 52.0, 51.2, 44.2, 36.6, 36.0, 31.0, 30.0, 29.2, 28.7, 27.2, 26.5, 21.0, 17.0, 14.8, 13.7, 12.0.

^{1}H and ^{13}C NMR spectral data are consistent with known values [23].

2.2.5. 3β-Acetoxy-12β-benzyloxy-5α-furostan-26-hydroxy-14-ene ***(9)***

To a CH_2Cl_2 solution of benzoate **8** (5.76 g, 10 mmol) and triethylsialne (3.19 mL, 20 mmol) was added dropwise CH_2Cl_2 (100 mL) solution of borontrifluroide diethyletherate (2.13 g, 15 mmol) over a period of 1 h at 0 °C, and the resulting mixture was stirred for 18 h at 25 °C. The reaction mixture was quenched by slowly adding saturated aqueous sodium bicarbonate, extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and subjected to silica gel chromatography to yield a primary alcohol **9** (5.40 g, 94%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–8.05 (5H, m), 5.44 (1H, s, C14–H), 4.74 (1H, d, C12–H), 4.61–4.72 (2H, m, C3–H, C16–H), 3.42 (2H, m, C26–H), 3.21 (1H, m, C22–H), 2.21 (1H, t), 2.09 (1H, m), 1.99 (3H, s, C3-OAc), 1.24 (3H, s), 0.86 (3H, d), 0.84 (3H, s), 0.79 (3H, d); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 165.7, 157.0, 132.8, 130.2, 129.0, 128.2, 119.9, 87.1, 85.7, 81.6, 73.0, 67.7, 59.2, 51.6, 44.1, 40.8, 36.2, 35.6, 33.9, 33.6, 30.0, 29.8, 29.2, 28.7, 27.9, 27.0, 26.5, 20.8, 16.2, 15.8, 11.8. ^{1}H and ^{13}C NMR spectral data are consistent with known values [23].

*2.2.6. 3β-Acetoxy-12β-benzyloxy-5α-furostan-14, 26-diene (**10**)*

A primary alcohol **9** (5.28 g, 9.13 mmol), triphenyl phosphine (4.78 g, 18.3 mmol), and imidazole (3.13 g, 45.7 mmol) were dissolved in THF (90 mL), iodine (4.60 g, 18.3 mmol) was added over a period of 30 min, and the resulting mixture was stirred for 2 h at an ambient temperature. The reaction mixture was quenched by adding saturated sodium thiosulfate solution, extracted with ethyl acetate, washed with brine, dried over anhydrous sodium thiosulfate, concentrated under reduced pressure to give a crude mixture of the corresponding primary iodide. To a DMF (45 mL) solution of the iodide was added DBU (2.78 mL, 18.3 mmol), and the resulting mixture was stirred at 25 °C. After 12 h, the reaction mixture was partitioned between ethylacetate (450 mL) and water (450 mL). The organic layer was washed with brine and saturated lithium chloride solution, dried over anhydrous sodium sulfate, and concentrated in vacuo, and subjected to a silica gel chromatography to give diene **10** (3.96 g, 78%) as white solids.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–8.02 (5H, m), 5.52 (1H, s), 4.65–4.82 (4H, m), 3.27 (1H, m), 1.99 (3H, s), 1.70 (3H,s), 1.24 (3H, s), 0.95 (3H, s), 0.80 (3H, d, J = 7.1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 165.7, 163.5, 145.7, 133.0, 130.4, 129.4, 128.5, 120.2, 86.3, 85.6, 81.7, 73.3, 59.2, 51.7, 44.2, 41.0, 36.6, 36.4, 35.7, 33.7, 33.1, 31.2, 29.2, 28.7, 27.2, 26.3, 22.4, 21.0, 16.9, 15.8, 12.0. ^{1}H and ^{13}C NMR spectral data are consistent with known values [23].

*2.2.7. 3β-Acetoxy-12β-benzyloxy-5α-furostan-14, 16-diene (**11**)*

To a benzene (10 mL) solution of a terminal olefin **10** (584 mg, 1 mmol) was added iodine (25 mg, 0.1 mmol) at 25 °C, and the resulting mixture was heated under reflux with stirring for 1 h. The reaction was quenched with saturated aqueous sodium thiosulfate, extracted with EtOAc (3 × 30 mL), dried with anhydrous sodium sulfate, concentrated under reduced pressure, and subjected to silica gel column chromatography to give a cyclopentadiene **11** (552 mg, 95%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–8.07 (5H, m), 6.13 (1H, s), 5.92 (1H, s), 4.67 (1H, sep), 4.40 (1H, dd, J = 11.3 Hz, 4.3 Hz), 3.92 (1H, q), 2.58 (1H, m), 2.0 (3H, s), 1.25 (3H, s), 1.17 (3H, s), 1.15 (3H, s), 0.90 (3H, s), 0.80 (3H, d, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 165.9, 160.8, 154.7, 133.2, 131.0, 129.7, 128.7, 124.9, 121.5, 83.3, 80.4, 79.9, 73.6, 57.2, 53.6, 44.5, 39.0, 37.9, 37.2, 36.0, 34.9, 34.1, 29.7, 29.4, 29.0, 28.4, 28.3, 27.8, 27.5, 21.7, 18.8, 13.9, 12.4; LRMS (ESI) 584 (M + Na); HRMS (ESI) calculated for $C_{36}H_{48}O_5$ (M + Na) 584.3472, found 584.3395.

*2.2.8. 3β-Acetoxy-12β-benzyloxy-5α-furostan-14-epoxy-17-ene (**14**)*

To a solution of a diene **11** (238 mg, 0.41 mmol) in dry CH_2Cl_2 (4 mL) were added $NaHCO_3$ (103 mg, 3 equiv.) and *m*CPBA (100 mg, 1.1 equiv.) at −10 °C, and the resulting mixture was stirred for 1 h. The reaction was quenched by adding saturated aqueous $Na_2S_2O_3$, extracted with CH_2Cl_2 (3 × 20 mL), concentrated under reduced pressure, and subjected to silica gel chromatography to provide an allylic epoxide **14** (213 mg, 87%).

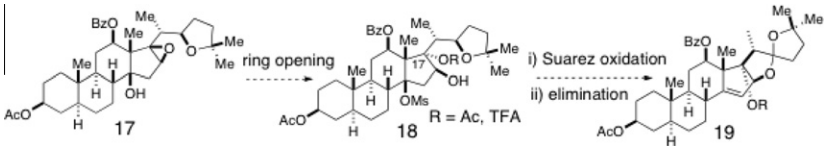
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–8.02 (5H, m), 5.99 (1H, s), 4.76 (1H, dd, J = 11.6 Hz, 4.0 Hz), 4.67 (1H, m), 3.75 (1H, m), 3.71 (1H, s), 2.34 (1H, m), 1.99 (3H, s), 1.35 (3H, s), 1.14 (3H, s), 1.14 (3H, s), 0.80 (3H, d, J = 7.1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 165.7, 163.5, 133.0, 130.4, 129.4, 128.5, 124.8, 82.3, 80.6, 80.3, 73.3, 70.5, 60.2, 52.7, 47.6, 44.2, 38.6, 36.6, 36.4, 35.7, 33.7, 33.1, 28.7, 28.7, 28.1, 27.7, 27.2, 27.1, 26.3, 21.4, 17.4, 12.1, 12.0; MS (ESI) 599 (M + Na); HRMS (ESI) calculated for $C_{36}H_{48}O_6$ (M + Na) 599.3343, found 599.3346.

*2.2.9. 3β-Acetoxy-12β-benzyloxy-5α-furostan-14,15-dihydroxy-16-ene (**15**)*

Allylic epoxide **14** (40 mg, 0.069 mmol) was dissolved in 5:1 mixture of acetone and H_2O, and the catalytic amount of H_2SO_4 was added to the solution. After stirring for 1 min at 25 °C, the reaction was quenched with saturated $NaHCO_3$, extracted with EtOAc (3 × 30 mL), and dried over Na_2SO_4. Concentration and flash chromatography on silica gel provided a diol **15** (39 mg, 95%).

*2.2.10. 3β-Acetoxy-12β-benzyloxy-5α-furostan-14-hydroxy-17-ene (**16**)*

To a CH_2Cl_2 solution of an epoxide **14** (57 mg, 0.10 mmol) and triethylsilane (80 μL, 5 equiv.) was added dropwise borontrifluoride diethyletherate (42 μL, 3 equiv.) at −78 °C, and the mixture



Scheme 5. Blueprint for synthesis of north unit **19** of a cephalostatin analog starting from the epoxy alcohol **17.**

was stirred for additional 3 h at the same temperature. The reaction mixture was then poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 20 mL), washed with brine, and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residue was subjected to silica gel chromatography to give a tertiary alcohol **16** (31 mg, 54%) and unreacted starting material (22 mg, 39%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–8.03 (5H, m), 5.53 (1H, s), 4.68–4.73 (2H, m), 3.66–3.71 (1H, m), 2.64 (1H, d, *J* = 16.4 Hz), 2.01 (3H, s), 1.31 (3H, s), 1.21 (3H, s), 1.13 (3H, s), 0.84 (3H, s), 0.72 (3H, d, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 166.1, 157.1, 133.1, 130.8, 129.6, 128.6, 121.1, 87.2, 87.1, 82.0, 80.6, 73.7, 56.6, 46.8, 44.5, 39.9, 38.8, 38.8, 38.2, 37.1, 35.9, 34.0, 31.2, 29.1, 28.4, 28.2, 27.5, 26.1, 21.7, 18.0, 12.4, 9.4; MS (ESI) 602 (M + Na); HRMS (ESI) calculated for $C_{36}H_{50}O_6$ (M + Na) 601.3500, found 601.3500.

*2.2.11. 3β-Acetoxy-12β-benzyloxy-5α-furostan-14-hydroxy-16-epoxide (**17**)*

To a CH_2Cl_2 solution of an olefin **14** (11 mg, 0.018 mmol) were added $NaHCO_3$ (3 equiv.) and *m*CPBA (14 mg, 3 equiv.) at −10 °C, and the resulting mixture was stirred for 5 h. The reaction was quenched by adding saturated aqueous $Na_2S_2O_3$, extracted with CH_2Cl_2 (3 × 20 mL), concentrated under reduced pressure, and subjected to silica gel chromatography to provide a trisubstituted epoxide **17** (10 mg, 92%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–8.00 (5H, m), 4.67 (1H, dd, *J* = 11.6 Hz, 3.8 Hz), 4.61 (1H, m), 3.99 (1H, q, *J* = 6.8 Hz), 3.66 (1H, s), 3.43 (1H, s), 2.36 (1H, m), 1.92 (3H, s), 1.87 (3H, s), 1.33 (3H, s), 1.11 (3H, s), 1.09 (3H, s), 0.71 (3H, s), 0.56 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173656 33329428285795 78.6, 76.2, 74.9, 73.1, 58.6, 51.3, 45.0, 43.9, 39.7, 38.6, 36.6, 35.7, 35.3, 34.5, 33.5, 28.5, 28.2, 28.0, 27.1, 27.0, 26.4, 21.2, 12.1, 11.8, 9.4; MS (ESI) 618 (M + Na); HRMS (ESI) calculated for $C_{36}H_{50}O_7$ (M + Na) 617.3450, found 617.3455.

Crystals of **17** grew as colorless needles by slow evaporation from diethyl ether. The crystal had approximate dimensions of 0.30 × 0.05 × 0.05 mm. The data were collected on a Rigaku AFC12 diffractometer with a Saturn 724 + CCD using a graphite monochromator with MoKa radiation (*l* = 0.71075 Å). A total of 1688 frames of data were collected using w-scans with a scan range of 0.5° and a counting time of 30 s per frame. The data were collected at 100 K using a Rigaku XStream low temperature device.

Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	*a* = 10.3691(5) Å	*a* = 90°
	b = 10.9021(6) Å	*b* = 90°
	c = 28.0269(15) Å	*g* = 90°

3. Results and discussion

Synthesis of a substrate for the E-ring opening study started with photolysis of hecogenin acetate **3** (Scheme 1). Irradiation of hecogenin acetate with 1000 W mercury vapor lamp cleaved C12-C13 regioselectively to give lumihecogenin acetate **5** [24], which was subjected to zinc bromide-catalyzed ene reaction to provide a 12-α homoallylic alcohol **6** stereoselectively. Stereochemistry conversion at C12 was achieved by Johns oxidation of the alcohol **6** followed by Luche reduction of the corresponding ketone **7**. The 12-β alcohol (not shown) was protected with benzoyl group, and then the benzoate **8** was treated with triethylsilane and borontrifluoride-etherate to furnish a F-ring-opened product **9** stereoselectively. The conversion of the primary alcohol **9** into iodide followed by DBU-assisted elimination of the iodide afforded a terminal olefin **10** in 78% yield.

With a 14,26-diene **10** in hand, we surveyed various reaction conditions to effect E-ring opening, and finally found that E-ring tetrahdyrofuran underwent smooth opening under the influence of catalytic amount of iodine to provide a cyclopentadiene **11** with concomitant formation of F-ring tetrahydrofuran [25] (Scheme 2). This transetherification appears to be driven by cyclopentadiene formation because a cyclic ether **12** lacking Δ14 olefin moiety did not undergo E-ring opening under the same reaction conditions and other ring opening conditions.

Having developed a novel E-ring opening method, we next explored "Red-OX" modifications of steroidal D-ring diene **11** (Scheme 3). Treatment of the diene **11** with *m*CPBA affected Δ14-olefin moiety in a regio- and stereoselective manner to give a β-epoxide **14**. The selective formation of β-epoxide is attributed to preferential approach of the oxidant towards the convex face of C–D ring. When the allylic epoxide **14** was treated with sulfuric acid, oxirane ring was readily cleaved to furnish a *trans*-1,2-diol **15**, of which stereochemistry was determined by a single crystal X-ray crystallography. When the allylic epoxide **14** was subjected to a triethylsilane-mediated reduction, regioselective cleavage of oxirane ring took place smoothly at −78 °C to afford a tertiary alcohol **16**. Oxidation of a trisubstituted olefin moiety in **16** with *m*CPBA in a $NaHCO_3$-buffered medium furnished β-epoxide **17** exclusively. A single crystal X-ray structure of epoxyalcohol **17** led to unambiguous determination of stereochemistries at C14, 16, and 17 (Scheme 4).

We expect that the epoxy alcohol **17** may be used as an important intermediate in the synthesis of cephalostatin analogs. For example, mesylation of the epoxy alcohol **17** followed by S_N1 nucleophilic opening of the oxirane ring would give a C17 substituted alcohol **18**, which can be subjected to an alkoxy radical cyclization (e.g. Suarez oxidation) followed by E2 elimination to provide a north unit **19** of cephalostatin analogs (Scheme 5).

In summary, we have developed transetherification-mediated E-ring opening method, and elaborated a D-ring diene via "Red-Ox" modifications. Further investigations for applying these methods to cephalostatin analogs synthesis are underway, and the results will be reported in due course.

Acknowledgements

This research was supported by Robert A. Welch Foundation (Grant No. F-1741).

References

[1] Pettit GR, Inoue M, Herald DL, Krupa TS. Antineoplastic agents 147. Isolation and structure of the powerful cell growth inhibitor cephalostatin 1. J Am Chem Soc 1988;110:2006.

[2] Fukuzawa S, Matsunaga S, Fusetani N, Ritterazine A. A highly cytotoxic dimeric steroidal alkaloid, fromthe tunicate ritterealla tokioka. J Org Chem 1994;59:6164–6.
[3] Lee S, LaCour TG, Fuchs PL. Chemistry of trisdecacyclic pyrazine antineoplastics: the cephalostatins and ritterazines. Chem Rev 2009;109:2275–314.
[4] Burgett AW, Poulsen TB, Wangkanont K, Anderson DR, Kikuchi C, Shimada K, Okubo S, Fortner KC, Mimaki Y, Kuroda M, Murphy JP, Schwalb DJ, Petrella EC, Cornella-Taracido I, Schirle M, Tallarico JA, Shair MD. Natural products reveal cancer cell dependence on oxysterol-binding proteins. Nat Chem Biol 2011;7:639–47.
[5] Kumar KA, La Clair JJ, Fuchs PL. Synthesis and evaluation of a fluorescent ritterazine-cephalostatin hybrid. Org Lett 2011;13:5334–7.
[6] Rudy A, López-Antón N, Barth N, Pettit GR, Dirsch VM, Schulze-Osthoff K, Rehm M, Prehn JH, Vogler M, Fulda S, Vollmar AM. Role of Smac in cephalostatin-induced cell death. Cell Death Differ 2008;15:1930–40.
[7] Rudy A, López-Antón N, Dirsch VM, Vollmar AM. The cephalostatin way of apoptosis. J Nat Prod 2008;71:482–6.
[8] Müller IM, Dirsch VM, Rudy A, López-Antón N, Pettit GR, Vollmar AM. Cephalostatin 1 inactivates Bcl-2 by hyperphosphorylation independent of M-phase arrest and DNA damage. Mol Pharmacol 2005;67:1684–9.
[9] Kim YC, Che QM, Gunatilaka AA, Kingston DG. Bioactive steroidal alkaloids from Solanum umbelliferum. J Nat Prod 1996;59:283–5.
[10] Fortner KC, Kato D, Tanaka Y, Shair MD. Enantioselective synthesis of (+)-cephalostatin 1. J Am Chem Soc 2010;132:275–80.
[11] Kim S, Sutton SC, Guo C, LaCour TG, Fuchs PL. Synthesis of the North 1 Unit of the Cephalostatin Family from Hecogenin Acetate. J Am Chem Soc 1999;121:2056–70.
[12] LaCour TG, Guo C, Bhandaru S, Boyd MR, Fuchs PL. Interphylal product splicing: the first total syntheses of cephalostatin 1, the north hemisphere of Ritterazine G, and the highly active hybrid analogue, Ritterostatin G_N1_N. J Am Chem Soc 1998;120:692–707.
[13] Zachis M, Rabi JA. The Clemensen reaction of tigogenin A reinvestigation. Tetrahedron Lett 1980;21:3735–8.
[14] Basler S, Brunck A, Jautelat A, Winterfeldt E. Synthesis of Cytostatic Tetradecacyclic Pyrazines and a Novel Reduction-Oxidation Sequence for Spiroketal Opening in Sapogenins. Helv Chim Acta 2000; 83; 1854.
[15] Bovicelli P, Lupattelli P, Fracassi D, Mincione E. Sapogenins and Dimethyldioxirane: a New Entry to Cholestanes Functionalized at the Side Chain. Tetrahedron Lett 1994;35:935–8.
[16] Li M, Yu B. Facile conversion of spirostan saponin into furostan saponin: synthesis of methyl protodioscin and its 26-Thio-analogue. Organic Lett 2006;8:2679–82.
[17] LaCour TG, Tong Z, Fuchs PL. Consequences of acid catalysis in concurrent ring opening and halogenation of spiroketals. Org Lett 1999;1:1815–8.
[18] Lee JS, Fuchs PL. Efficient protocol for ring opening of spiroketals using trifluoroacetyl trifluoromethanesulfonate (TFAT). Org Lett 2003;5:3619–22.
[19] Lee S, Fuchs PL. An efficient C–H oxidation protocol for α-hydroxylation of cyclic steroidal ethers. Org Lett 2004;6:1437–40.
[20] Lee S, Jamieson D, Fuchs PL. Synthesis of C14, 15-dihydro-C22, 25-epi north unit of Cephalostatin 1 via "Red-Ox" modifications of hecogenin acetate. Org Lett 2009;11:5–8.
[21] Jauetlat R, Muller-Fahrnow A, Winterfeldt E. A novel oxidative cleavage of the steroidal skeleton. Chem Eur J 1999;5:1226–33.
[22] Welzel P, Janssen B, Duddeck H. B-Hydroxysteroids, II. The Prins reaction of lumihecogenin acetate. Liebigs Annalen der Chemie 1981;3:546–64.
[23] M. Lee S, LaCour TG, Lantrip D, Fuchs PL. Redox refunctionalization of steroid spiroketals. Structure correction of ritterazin. Org Lett 2002; 4: 313–316.
[24] Bladon P, McMeekin W, Williams IA. Steroids derived from hecogenin. Part III. The photochemistry of hecogenin acetate. J Chem Soc 1963:5727–37.
[25] Lee JS, Fuchs PL. A Biomimetically inspired, efficient synthesis of the south 7 Hemisphere of Cephalostatin 7. J Am Chem Soc 2005;127:13122–3.