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Synthesis of 23-deoxy-25-*epi* north unit of cephalostatin 1 via reductive and oxidative modifications of hecogenin acetate

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ABSTRACT

An efficient synthesis of the 23-deoxy-25-*epi* north unit of cephalostatin 1 has been achieved in 17 steps via reductive and oxidative functionalizations of hecogenin acetate with an overall yield of 3.8%. This synthesis features transetherification-mediated E-ring opening, D-ring oxidation, hemiketalization-mediated E-ring closure, and stereoselective 5/5-spiroketalization.

1. Introduction

Marine natural products cephalostatins and ritterazines are composed of growing number of bis-steroidal pyrazines that contain two C_{27} steroidal units and a central pyrazine. The cephalostatin/ritterazine family is composed of twenty cephalostatins (1–20) from the marine tube worm *Cephalodiscus gilchristi* and twenty-six ritterazines (A–Z) from the tunicate *Ritterella tokioka* [1–6]. These bissteroidal pyrazine natural products display potent antiproliferative activities against various cancer cell lines. In particular, cephalostatin 1 is among the most powerful anticancer agents ever tested by the National Cancer Institute (NCI), displaying average GI_{50} value of 1.8 nM in the NCI 60-cell line panel [3]. The cytotoxicity profiles of these anticancer agents, which induce apoptosis via mitochondrial-dependent pathway, did not correlate with any molecule of known mechanism of action [7–11]. The bissteroidal pyrazine anticancer agents and structurally unrelated monosteroidal glycoside OSW-1 have been suggested to kill cancer cells by targeting oxysterol-binding proteins (OSBP) and OSBP-related protein 4L and to reduce the cellular level of these proteins in proteasome-dependent manner [12–14].

Due to the medical significance of the bisteroidal pyrazine antineoplastics, several research groups have reported the synthesis of natural cephalostatins and ritterazines [15–23]. Development of a cephalostatin-based anticancer drug, however, has been significantly hampered due to lengthy (>60 steps) synthetic steps that were required to construct the north and south units of the natural cephalostatins and ritterazines [18,19,23]. In order to develop structurally less complex yet extremely potent cephalostatin/ritterazine analogs, a number of research groups have synthesized cephalostatin/ritterazine analogs including hybrid of cephalostatins and ritterazines (hereafter called ritterostatins) [17,23]. In particular, 25-*epi* ritterostatin G_N1_N, which contains the north unit of ritterazine G and the 25-*epi* north unit of cephalostatin 1, was ~30- and 2-fold more cytotoxic than ritterostatin G_N1_N and cephalostatin 1, respectively. The results revealed that structurally complex south 1 unit can be replaced by the readily accessible north G unit without significantly compromising the potency and the stereochemistry at C25 of the north 1 unit is important for bioactivity (Fig. 1) [23].

In conjunction with our on-going efforts to develop cephalostatin analogs that are potent yet structurally less complex than cephalostatin 1 [15,20], herein we report the synthesis of 23-deoxy-25-*epi* north 1 unit **4** via multiple reductions and oxidations of key intermediate **5**, which is readily accessible from commercially available hecogenin acetate **6** (Scheme 1). The synthesis of the 23-deoxy-25-*epi* north 1 unit was accomplished in 17 synthetic steps starting from hecogenin acetate with an overall yield of 3.8%. This synthesis features transetherification-mediated E-ring opening, D-ring oxidation, hemiketalization-induced E-ring closure, and stereoselective 5/5 spiroketalization.

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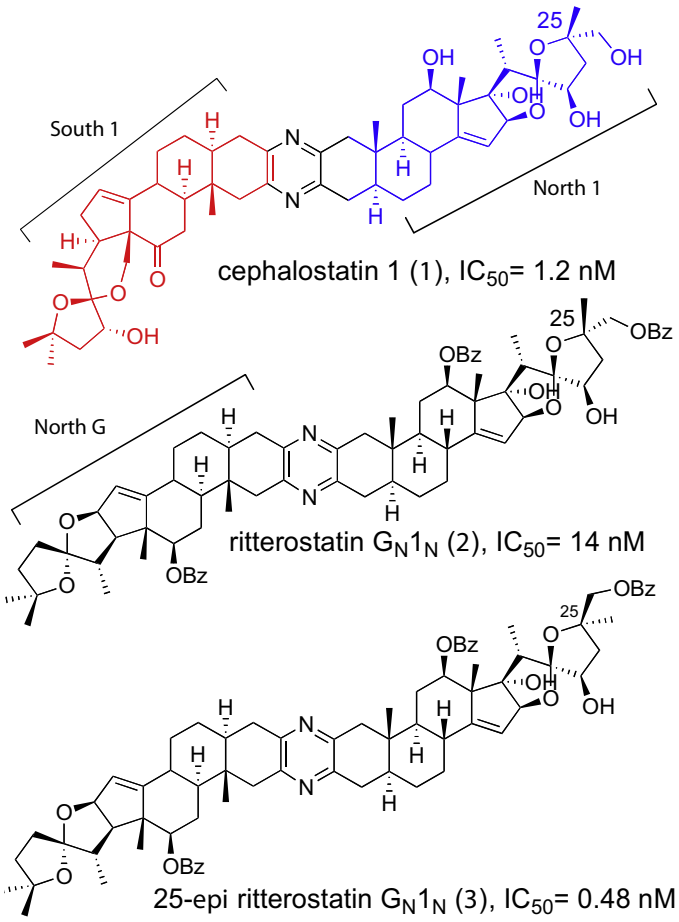


Fig. 1. Structures of cephalostatin 1 **1**, ritterostatin G_N1_N **2** and 25-*epi* ritterostatin G_N1_N **3** and their IC_{50} values in the NCI-60 cancer cell lines.

2. Experimental

2.1. General methods

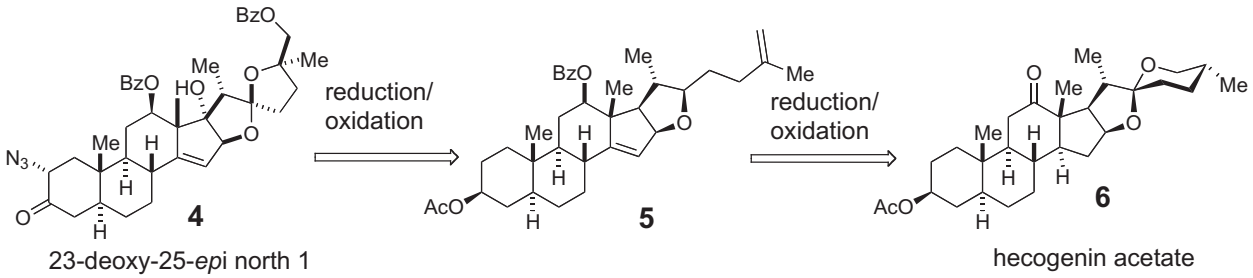
Boron trifluoride etherate (BF_3OEt_2), triethylsilane (Et_3SiH), imidazole, iodine, iodobenzene diacetate ($PhI(OAc)_2$), potassium carbonate, and *N*,*N*-dimethyl formamide (DMF) were purchased from Acros Organics (Geel, Belgium). Methylene chloride (dichloromethane or DCM), tetrahydrofuran (THF), and methanol were purchased from Fisher Chemical (Farlawn, NJ). Triphenylphosphine (PPh_3) was purchased from Alfa Aesar (Ward Hill, MA). Sodium azide was purchase from MP Biomedicals, LLC (Solon, OH). All reactions were performed under positive pressure of argon in anhydrous solvents. Each reaction progress was monitored by thin layer chromatography (TLC). TLC Silica gel 60 F_{254} glass plates from EMD Chemicals Inc. (Darmstadt, Germany) and appropriate solvent systems were used for TLC development. TLC plates were visualized by ultraviolet illumination (254 nm) and *p*-Anisaldehyde solution (4 mL of concentrated sulfuric acid, 800 mL of ethanol, 1.2 mL of acetic acid, and 1.6 mL of *p*-anisaldehyde). Analytical samples were prepared via flash silica gel chromatography. 60 Å silica from Bonna-Agela technologies (Wilmington, DE) was used to purify the products. ^1H and ^{13}C NMR spectra were generated by Varian MERCURY 400 (400 MHz). $CDCl_3$ was used as the NMR standard. Peak multiplicates in ^1H NMR spectra, when reported, were abbreviated as s (singlet), d (doublet), t (triplet), m (multiplet), ap (apparent), and br (broad). High-resolution mass spectrometry data were generated by Agilent 6530 Accurate-Mass Q-TOF LC/MS.

2.2. Chemical synthesis

2.2.1. 3β-Acetoxy-12β-benzyloxy-22-hydroxy-5α-furostan-14,16-diene (**8**)

To a solution of the iodide **7a** (6 g, 8.75 mmol) in 100 mL of ethanol was added zinc powder (5.6 g, 87.5 mmol) followed by AcOH (5.6 mL, 94 mmol). The reaction mixture was heated to 95 °C and stirred for 3 h at this temperature. Check TLC for the completion of reaction, then the reaction mixture was diluted with 50 mL of EtOAc and filtered. The filtrate was concentrated to remove excess acetic acid, then the residue was diluted with EtOAc (100 mL). It was washed with 50 mL of saturated aqueous $NaHCO_3$, brine (50 mL), dried ($MgSO_4$), and concentrated in vacuum. The resulting product was purification by flash chromatography (petroleum ether–EtOAc, 7:3) afforded **8** as white solid. (4.4 g, 89%; m.p. 171–172 °C).

R_f = 0.5 (petroleum ether–EtOAc, 4:1); ^1H NMR (400 MHz, $CDCl_3$): 8.08 (dd, *J* = 8.6, 1.6, 2H), 7.59 (td, *J* = 7.3, 1.7 Hz, 1H), 7.47 (td, *J* = 7.3, 1.7 Hz, 2H), 6.20 (d, *J* = 2.0 Hz, 1H), 5.97 (t, *J* = 2.0 Hz, 1H), 4.75 (m, 1H), 4.68 (m, 1H), 4.64 (m, 9H), 4.46 (dd, *J* = 11.3, 4.3, 1H), 3.56 (tt, *J* = 8.6, 2.0, 1H), 2.58 (dq, *J* = 8.6, 7.0, 1H), 2.27–2.18 (m, 1H), 2.18–2.11 (m, 1H), 2.11–2.05 (m, 1H), 2.05–1.97 (m, 1H), 2.02 (s, 3H), 1.85–1.76 (m, 2H), 1.76–1.65 (m, 2H), 1.57–1.50 (m, 1H), 1.52–1.45 (m, 2H), 1.45–1.40 (m, 2H), 1.42–1.32 (m, 2H), 1.28 (m, 3H), 1.28–1.19 (m, 2H), 1.10 (td, *J* = 13.7, 3.5, 1H), 0.93 (s, 3H), 0.88–0.82 (m, 1H), 0.80 (d, *J* = 6.7 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 170.6 (—C=O—, OAc), 165.6 (—C=O, Bz), 160.1 (—C14—), 156.1 (—C17—), 146.0 (—C25—), 133.1 (—CH—, Bz), 130.5 (—CH—, Bz), 129.4 (—C15H—), 128.5 (—CH—, Bz), 124.9 (—C16H—), 120.8 (—CH—, Bz), 109.8 (—C26H—), 79.2 (—C12H—), 75.4 (—C22H—), 73.3 (—C3H—), 57.0 (—C13—), 53.1 (—C10—), 44.3 (—C20H—), 39.2 (—C5H—), 37.0 (—C8H—), 35.8 (—C9H—), 34.8 ($—C4H_2—$), 33.8 ($—C23H_2—$), 33.5 ($—C1H_2—$), 31.9 ($—C24H_2—$), 29.2 ($—C2H_2—$), 28.1 ($—C6H_2—$), 27.4 ($—C18H_3—$), 27.2 ($—C11H_2—$), 22.5 ($—C7H_2$), 21.4 ($—C27H_3—$), 19.7 ($—CH_3—$, OAc), 14.3 ($—C21H_3—$), 12.2 ($—C19H_3—$); MS (ESI): *m*/*z* = 583 [M+Na]$^+$. HRMS: calcd. for $C_{36}H_{48}O_5Na$ [M+Na]$^+$: 583.3399: found: 583.3401.8.



Scheme 1. Retrosynthetic analysis of the 23-deoxy-25-*epi* north 1 unit.