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Synthesis of 14′,15′-dehydro-ritterazine Y via reductive and oxidative functionalizations of hecogenin acetate

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ABSTRACT

An analog of ritterazine Y was synthesized from hecogenin acetate in 23 steps via functional group manipulations of hecogenin acetate. Preparation of the north G and south Y units and the late stage Guo–Fuchs asymmetric coupling of the both units afforded the ritterazine Y analog.

1. Introduction

The cephalosatin/ritterazine family comprises of 45 members of bissteroidal pyrazine marine natural products; 19 cephalostains (1–19) from the marine tube worm *Cephalodiscus gilchristi* [1–4] and 26 ritteazines (A–Z) from the tunicate *Ritterella tokioka* [5,6]. They are structurally related, featuring two C_{27} sterodial units (referred to as north and south, Fig. 1) and a central pyrazine ring. Cephalostatin 1 **1** and ritterazine B **2**, the two most potent members of the family, are among the most powerful anticancer agents ever tested by the NCI with avg. GI_{50} 1.8 and 3.2 nM, respectively, in the NCI 60 cancer cell lines. The cytotoxicity profiles of these anticancer agents had no significant correlation to any molecule of known mechanism of action. Cephalostatin 1 **1** has shown to induce apoptosis in a novel mitochondrial pathway [7–11]. Interestingly, cephalostatin 1 **1** and ritterazine B **2** are about ~50- to 250-fold more cytotoxic to cells with mutations in p21 tumor suppressor gene [12]. A cephalostatin analog has also shown selective cytotoxicity to cells with mutations in ras tumor suppressor genes [13], which is important in the etiology of many cancers, implicating cephalostatin/ritterazine as potential "synthetic lethal" anticancer agents [12]. Another class of anticancer steroids exhibiting cephalostatin-like activity is OSW-1 **3**, a mono-steroidal glycoside from the bulb of *Ornithogalum saundersiae* [14–17]. Surprisingly, the cytotoxicity profile of OSW-1 **3** was very similar to that of cephalostatins with correlation coefficient of 0.60–0.83, suggesting that they share the same mechanism of action. Indeed, very recently, it has shown that both cephalostatin 1 and OSW-1 inhibit novel anticancer targets called oxysterol-binding protein (OSBP) and OSBP-related proteins (ORP) [12,18,19]. These highly oxygenated steroidal anticancer agents tightly bind OSBP and reduce OSBP level via proteasome-dependent degradation [12].

Because of medical significance of cephalostatins and ritterazines, many research groups including us reported the synthesis of the natural bissteroidal pyrazines (e.g., cephalostatin 1 and 7, ritterazine K and M) and their analogs [20–28]. Ritterazine Y **4** is an interesting molecule, because, although it lacks 7′-OH and 17′-OH functionalities of ritteazine B **2** (Fig. 2), it has been shown to exhibit considerable cytotoxicity against P388 murine cancer cell line [5]. In conjunction with efforts to develop efficient synthetic routes for cephalostatins and ritterazines, the Fuchs laboratory has embarked on a "reduction/oxidation" strategy [29,30] where they seek to prepare target compounds using multiple reductive/oxidative functionalizations of commercially available hecogenin acetate **6** with retention of all the 27 carbon atoms in the starting material (Fig. 3). Herein, we report the synthesis of 14′,15′-dehydro-ritterazine Y **5** via reductive/oxidative functionalizations of hecogenin acetate, which involves the synthesis of the north G and the south Y hemispheres and an asymmetric coupling of the both hemispheres.

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

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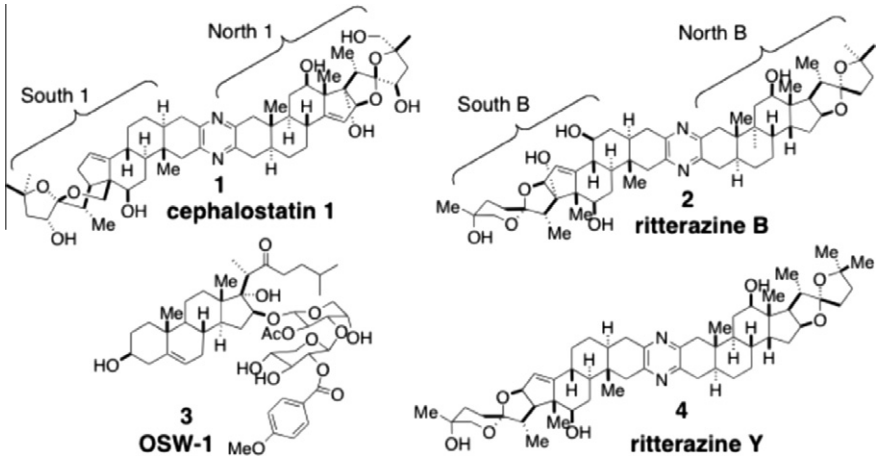

Fig. 1. Anticancer steroids. Cephalostatin 1, ritterazines B and Y, and OSW-1.

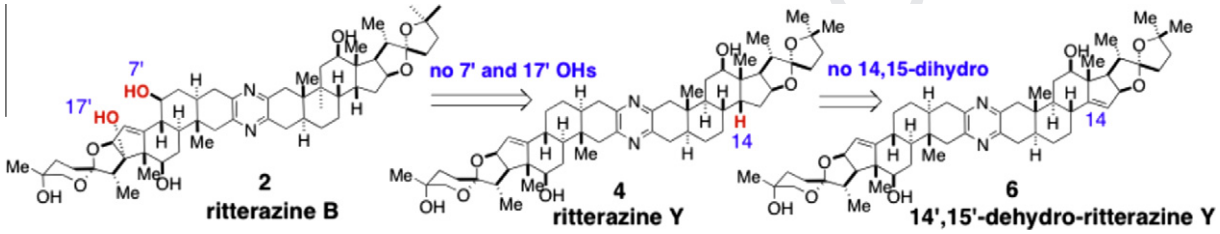

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Fig. 2. Anticancer steroids. Cephalostatin 1, ritterazines B and Y, and OSW-1.

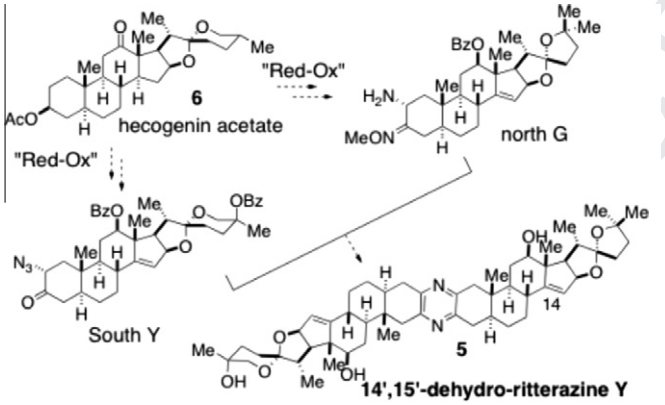

Fig. 3. Strategy for synthesis of 14′,15′-dehydro-ritterazine Y form hecogenin acetate.

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-5α-spirostan-14-ene (**10**)

To a solution of ketone **9** (12.7 g, 27.0 mmol) in THF/MeOH (1:1, 135 mL/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 four hours. 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 partitioned between ethylacetate (200 mL) and water. The organic layer was washed with brine, dried over Na_2SO_4, concentrated, and subjected to sgc to give C12-β alcohol (11.0 g, 87%) 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 **10** (12.6 g, 94%) as white solids (mp, 171–173 °C).

^1H 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,

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$CDCl_3$) 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 [25].

2.2.2. 3β-Acetoxy-12β-benzyloxy-5α-furostan-26-hydroxy-14-ene (**11**)

To a CH_2Cl_2 solution of benzoate **10** (5.76 g, 10 mmol) and triethylsilane (3.19 mL, 20 mmol) was added dropwise CH_2Cl_2 (100 mL) solution of boron trifluroide 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 **11** (5.40 g, 94%) as a clear oil.

^{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 [25].

2.2.3. 3β-Acetoxy-12β-benzyloxy-5α-furostan-14,26-diene (**12**)

A primary alcohol **11** (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 **12** (3.96 g, 78%) as white solids (mp, 108–110 °C).

^{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 [25].

2.2.4. 3β-Acetoxy-12β-benzyloxy-5α-furostan-25-hydroxy-14-ene (**13**)

To terminal olefin **12** (560 mg, 1 mmol) in 6 ml of THF wrapped with aluminum foil, was added $PhI(OAc)_2$ (350.5 mg, 1.1 mmol) in 4 ml of H_2O and 6 ml of THF. The reaction proceeded at room temperature overnight in dark, and was added $NaBH_4$ (370 mg, 10 mmol) afterwards. After 2 more hours, the reaction was quenched with 5 N ammonium chloride solution. Then mixture was extracted with 30 ml ethyl acetate for 3 times and organic layer was washed with brine. After drying over sodium sulfate, the organic layer was concentrated and taken to silica gel column chromatography using a gradient of 4:1 to 1:1 of ethyl acetate to hexane to elute. The final product was collected, concentrated and dried by vacuum to give tertiary alcohol **13** (381 mg, 66%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00–7.97 (2H, m), 7.52–7.48 (1H, m), 7.41–7.28 (1H, m), 5.40 (1H, s), 4.78 (1H, d, J = 5.1 Hz), 4.19 (1H, d, J = 7.8 Hz), 4.64 (1H, m), 4.55 (1H, m), 2.20–2.11 (1H, m), 2.09–1.97 (1H, m), 1.94 (3H, s), 1.18 (3H, s), 1.13 (6H, s), 0.83 (3H, s), 0.80 (3H, s); ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.4, 170.3, 165. 7, 165.6, 157.0, 153.9, 132.9, 132.8, 130.3, 130.1, 129.3, 128.4, 128.3, 120.5, 119.9, 87.4, 86.8, 86.2, 85.9, 81.5, 78.5, 73.1, 69.9, 69.9, 59.3, 55.8, 51.8, 51.6, 50.4, 50.3, 44.2, 44.0, 41.1, 40.8, 40.5, 36.4, 36.3, 35.7, 35.5, 34.5, 34.3, 34.0, 33.6, 31.4, 29.3, 29.2, 29.2, 27.9, 27.4, 27.2; HRMS for $C_{30}H_{50}O_6$ (M+H) calcd: 579.3680, found: 579.3683.

2.2.5. 3β-Acetoxy-12β-benzyloxy-5α-spirostan-14-ene (**14**)

To a CH_2Cl_2 solution of $PhI(OAc)_2$ (423.5 mg, 1.3 mmol) and iodine (333.5 mg, 1.3 mmol) was added tertiary alcohol **13** (380.5 mg, 0.66 mmol) in CH_2Cl_2 dropwise at 0 °C, and then the resulting mixture was stirred for 3 h. The reaction mixture was quenched by aqueous saturated $Na_2S_2O_3$, extracted with dichloromethane (3×30 ml), washed by the brine, dried over sodium sulfate, and subjected to silica gel column chromatography to give 5/5 spiroketal **14** (262 mg, 69%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03–8.00 (2H, m), 7.56–7.51 (1H, m), 7.44–7.39 (2H, m), 5.43 (1H, s), 4.93 (1H, d, J = 6.6 Hz), 4.66–4.64 (1H, m), 4.62–4.57 (1H, m), 2.46–2.41 (1H, m), 1.99 (3H, s), 1.32 (3H, s), 1.22 (6H, s), 1.09 (3H, s), 0.87 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 165.7, 156.0, 132.7, 130.5, 129.3, 128.2, 120.5, 117.2, 84.1, 81.8, 81.4, 73.2, 55.9, 52.1, 51.4, 44.1, 41.1, 37.1, 36.4, 35.9, 33.9, 33.7, 33.1, 29.9, 29.4, 28.3, 28.0, 27.1, 26.4, 21.3, 15.0, 14.0, 11.9. HRMS for $C_{30}H_{48}O_6$ (M+H) calcd: 577.3524, found: 577.3522.

2.2.6. 3β-Hydroxy-12β-benzyloxy-5α-spirostan-14-ene (**15**)

To acetate **14** (250 mg, 0.43 mmol) in 10 ml MeOH was added K_2CO_3 (120 mg, 0.87 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was quenched by adding saturated aqueous ammonium chloride, extracted with ethyl acetate (3×30 ml), and washed with brine. The combined organic layer was dried over sodium sulfate, concentrated under reduced pressure, and subjected to silica gel column chromatography to give alcohol **15** (185 mg, 80%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–7.99 (2H, m), 7.58–7.50 (1H, m), 7.43–7.38 (2H, m), 5.42 (1H, s), 4.94–4.91 (1H, m), 4.60–4.55 (1H, m), 3.61–3.50 (1H, m), 2.42 (1H, t, J = 9.0 Hz), 2.19–1.97 (2H, m), 1.95 (1H, s), 1.31 (3H, s), 1.22 (6H, s), 1.08 (3H, s), 0.84 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9 156.2, 132.8, 130.6, 129.4, 128,3, 120.4, 117.3, 84.2, 81.9, 81.6, 70.9, 55.9, 52.3, 51.5, 44.4, 41.2, 37.8, 37.1, 36.7, 36.0, 34.6, 34.1, 33.1, 31.5, 31.2, 29.9, 28.3, 29.6, 28.2, 29.5, 26.5, 25.2, 22.6, 20.6, 15.0, 14.1, 14.0, 12.0; HRMS for $C_{34}H_{46}O_5$ (M+Na) calcd: 557.3238, found: 557.3237.

2.2.7. 12β-Benzyloxy-3-keto-5α-spirostan-14-ene (**16**)

To the secondary alcohol **15** (180 mg, 0.34 mmol) in acetone was added Jones reagent (0.23 ml, 0.31 mmol) dropwise at 0 °C. The reaction was quenched after 10 min with saturated $Na_2S_2O_3$ solution, and then extracted with ethyl acetate (3×30 ml). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and purified by silica gel column chromatography to give ketone **16** (157 mg, 87%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–7.99 (2H, m), 7.56–7.52 (1H, m), 7.44–7.39 (2H, m), 5.44 (1H, s), 4.95–4.91 (1H, m), 4.62–4.57 (1H, m), 2.47–2.41 (1H, m), 2.33–2.19 (2H, m), 2.16–1.95 (3H, m), 1.30 (3H, s), 1.23 (6H, d, J = 6.9 Hz), 1.08 (3H, s), 1.03 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.1, 165.8, 155.5, 132.9, 130.5, 129.4, 128.3, 121.0, 117.3, 84.1, 82.0, 81.3, 55.9, 51.7, 51.5, 46.0,

44.3, 41.2, 38.0, 37.8, 37.1, 36.0, 34.6, 33.9, 33.1, 31.5, 29.9, 29.6, 29.2, 28.4 26.64 25.2, 22.6, 15.0, 14.0, 11.2; HRMS for $C_{34}H_{44}O_5$ (M+Na) calcd: 555.3081, found: 555.3081.

*2.2.8. 2α-Bromo-3-keto-12β-benzyloxy-5α-spirostan-14-ene (**17**)*

To the ketone **16** (142 mg, 0.27 mmol) in 5 ml of THF was added PTAB (91.0 mg, 0.24 mmol) at 0 °C. After 30 min, the reaction was quenched with saturated $Na_2S_2O_3$ solution and extracted with ethyl acetate (3×30 ml). The combined organic layer was washed with brine, dried over sodium sulfate, concentrated under reduced pressure, and subjected to silica gel column chromatography to give bromoketone **17** (125 mg, 77%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–7.99 (2H, m), 7.56–7.51 (1H, m), 7.44–7.39 (2H, m), 5.45 (1H, s), 4.94–4.91 (1H, m) 4.70–4.63 (1H, m), 4.62–4.56 (1H, m), 2.60–2.50 (1H, m), 2.61–2.38 (5H, m), 2.16–2.05 (1H, m), 1.30 (3H, s), 1.23 (6H, d, *J* = 6 .0 Hz), 1.11 (3H, s), 1.08 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.4, 165.8, 154.8, 133.0, 130.3, 129.34, 128.3, 121.9, 117.3, 84.0, 82.0, 80.9, 55.9, 53.5, 51.7, 51.5, 51.3, 50.9, 46.8, 43.5, 41.1, 39.1, 38.0, 37.1, 36.0, 34.6, 34.4, 33.4, 33.1, 31.5, 29.9, 29.6, 28.9, 28.3, 27.8, 26.7, 25.2, 22.6, 20.6, 15.0, 14.1, 14.0, 11.8, 11.4; HRMS for $C_{34}H_{43}BrO_5$ (M+Na) calcd: 633.2186, found: 633.2184.

*2.2.9. 2α-Azido-3-keto-12β-benzyloxy-5α-spirostan-14-ene (**18**)*

To bromoketone **17** (120 mg, 0.20 mmol) in 20 ml CH_3NO_2 was added $TMGN_3$ (128 mg, 0.82 mmol) at 0 °C. The mixture was stirred overnight, quenched by addition of water, and extracted with ethyl acetate (3×20 ml). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, concentrated, and subjected to silica gel column chromatography using a gradient of 4:1 to 1:1 of hexane to ethyl acetate to give azido ketone **18** (105 mg, 93%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–7.99 (2H, m), 7.56–7.51 (1H, m), 7.44–7.39 (2H, m), 5.45 (1H, s), 4.92 (1H, d, *J* = 8.2 Hz) 4.68 (1H, dd, *J* = 6.9 and 13.9 Hz), 3.91 (1H, dd, *J* = 6.1 and 13.0 Hz), 2.60–2.50 (1H, m), 2.98 (1H, s), 2.61–2.38 (5H, m), 1.95 (3H, s), 1.30 (3H, s), 1.24 (3H, s), 1.22 (3H, s), 1.08 (3H, s), 0.89 (3H, d, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.5, 165.8, 154.8, 132.9, 130.3, 129.38, 128.3, 121.3, 117.3, 84.0, 82.0, 80.9, 63.5, 55.9, 51.5, 51.4, 46.9, 44.9, 43.4, 41.1, 39.1, 37.2, 37.0, 33.3, 33.0, 31.5, 29.9, 29.6, 28.9, 28.3, 27.8, 26.7, 22.6, 15.0, 14.1, 14.0, 13.9, 12.2.

*2.2.10. 2α-Azido-3-(methoxyimino)-12β-benzyloxy-5α-spirostan-14-ene (**19**)*

To azidoketone **18** (95 mg, 0.17 mmol) in dichloromethane (24 ml) and pyridine (1.6 ml) was added methoxy amine (67 mg, 0.8 mmol), and the mixture was stirred for 4.5 h at room temperature. The mixture was quenched by adding water, extracted with ethyl acetate (3×20 ml), and the combined organic layer was washed with brine. After drying over sodium sulfate and concentration, the residue was subjected to silica gel column chromatography (hexane/EtOAc = 4:1) to give azido methoxime **19** (44 mg, 44%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07–8.04 (2H, m), 7.58 (1H, t, *J* = 7.2 Hz), 7.49–7.44 (2H, m), 5.48 (1H, s), 4.979–4.96 (1H, m), 4.68–4.62 (1H, m), 3.93 (3H, s), 3.91 (1H, s), 3.10–3.04 (1H, m), 2.51–2.45 (1H, t, *J* = 8.7 Hz), 2.18 (1H, s), 2.16–2.10 (1H, m), 2.01–1.98 (2H, m), 1.35 (3H, s), 1.27 (6H, s), 1.13 (3H, s), 0.98 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 155.3, 154.9, 132.9, 130.5, 129.4, 128.4, 121.1, 117.3, 84.05, 82.00, 81.1, 62.0, 57.6, 55.9, 51.7, 51.5, 44.5, 44.2, 41.2, 37.1, 36.9, 34.6, 33.5, 33.12 30.9, 30.0, 29.7, 29.0, 28.4, 27.6, 27.3, 26.5, 25.2, 15.0, 14.0, 12.2; HRMS for $C_{35}H_{46}N_4O_5$ (M+H) calcd: 603.3541, found: 603.3539.

*2.2.11. 2α-Amino-3-(methoxyimino)-12β-benzyloxy-5α-spirostan-14-ene (**20**)*

To azido methoxime **19** (40 mg, 0.07 mmol) in 1.5 ml THF and 75 μl of water was added PPh_3 (52 mg, 0.2 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with water and extracted with ethyl acetate (3×20 ml). The combined organic layer was washed with brine, dried over sodium sulfate, concentrated in vacuo, and the subjected to silica gel column chromatography using to give aminomethoxime **20** (38 mg, 99%) as a white foam.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–8.00 (2H, m), 7.57–7.52 (1H, t, *J* = 7.5 Hz), 7.45–7.40 (2H, m), 4.94 (1H, d, *J* = 7.2 Hz), 4.62–4.57 (1H, dd, *J* = 4.5 and 11.1 Hz), 3.95–3.88 (1H, dd, *J* = 6.3 Hz), 2.98 (1H, s), 2.46–2.40 (1H, m), 2.35–2.24 (1H, m), 1.95 (2H, s), 1.31 (3H, s), 1.23 (6H, d, *J* = 6.3 Hz), 1.08 (3H, s), 0.96 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.5, 165.8, 154.9, 132.9, 130.3, 129.4, 128.3, 121.3, 117.3, 83.96, 82.0, 80.9, 63.5, 55.9, 51.5, 51.4, 46.9, 44.9, 43.4, 41.1, 37.2, 37.1, 33.3, 33.1, 31.5, 29.9, 29.6, 29.0, 28.3, 27.8, 26.7, 22.6, 15.0, 14.1, 14.1, 12.2; HRMS for $C_{35}H_{49}N_2O_5$ (M+H) calcd: 577.3636, found: 577.3635.

*2.2.12. 3β-Acetoxy-12β,25-dibenzyloxy-5α-26-OTBS-furostan-14-ene (**22**)*

To a co-solvent of *t*-BuOH (5 mL) and H_2O (5 mL), were added $K_3Fe(CN)_6$ (987 mg, 3 mmol), $(DHQ)_2PHAL$ (78 mg, 10 mol%), K_2OsO_4 (7 mg, 2 mol%), and K_2CO_3 (414 mg, 3 mmol), and the resulting mixture was vigorously stirred for 10 min at 0 °C. Terminal olefin **12** was added to the mixture and then the mixture was stirred at the same temperature. After 18 h, the reaction mixture was quenched by adding saturated aqueous sodium thiosulfate (10 mL). The aqueous layer was extracted with EtOAc (20 mL×3), washed with brine (10 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give diol **21**, which was subjected to the next reaction without further purification. To the diol **21** in dichloromethane (10 mL) were added TBSCl (225 mg), imidazole (391 mg), and DMAP (14 mg), and the resulting mixture was stirred at 25 °C for an hour. The reaction mixture was partitioned between CH_2Cl_2 (20 mL) and water, and the organic layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic solution was dried over anhydrous sodium sulfate, and concentrated to give the monosilylated compound, which was subjected to the next reaction without further purification. To CH_2Cl_2 (10 mL) solution of the mono-protected compound, were added triethylamine (340 μL), $MgBr_2{\cdot}2Et_2O$ (423 mg), and benzoic anhydride (555 mg), and the resulting mixture was stirred at 25 °C for 2 h. The reaction was quenched by adding saturated aqueous sodium bicarbonate, extracted with CH_2Cl_2, dried over anhydrous sodium sulfate, concentrated in vacuo, and subjected to silica gel chromatography to give tertiary benzoate **22** as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10–7.92 (4H, m), 7.59–7.32 (6H, m), 5.47 (1H, s), 4.77 (2H, d, *J* = 8.1 Hz), 4.71–4.63 (2H, m), 3.92–3.85 (1H, m), 3.30–3.28 (1H, m), 2.25–2.20 (1H, m), 2.09 (1H, s), 1.98 (3H, s), 1.51 (3H, s), 1.21 (3H, s), 0.86 (3H, s), 0.81 (9H, s), 0.0 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 170.6, 165.9, 165.5, 157.1, 133.5, 132.9, 132.3, 131.6, 130.4, 130.0, 129.4, 129.3, 128.3, 128.0, 120.1, 87.4, 86.0, 84.7, 81.7, 73.2, 66.1, 59.9, 51.9, 51.7, 44.1, 40.9, 36.4, 35.8, 34.1, 33.7, 32.9, 31.5, 29.4, 28.0, 27.2, 27.1, 26.6, 25.7, 21.3, 21.3, 18.0, 17.1, 16.0, 14.0, 11.9.

*2.2.13. 3β-Acetoxy-12β,26-dibenzyloxy-5α-furostan-26-hydroxy-14-ene (**23**)*

To **22** (388 mg, 0.48 mmol) in CH_2Cl_2 (10 mL) was added dropwise boron trifluoride diethyletherate and the mixture was stirred at °C for an hour. The reaction was quenched by adding aqueous saturated sodium bicarbonate, extracted with CH_2Cl_2 (3×20 mL), concentrated under reduced pressure, and subjected to silica gel

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chromatography to give primary alcohol **23** (238 mg, 71%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03–7.94 (4H, m), 7.58–7.36 (6H, m), 5.45 (1H, s), 4.75–4.63 (4H, m), 3.94 (1H, s), 3.84–3.79(1H, m) 2.22 (1H, t, J = 7.8 Hz), 2.20–2.09 (1H, m), 1.99 (4H, s), 1.48 (3H, s), 1.21 (3H, s), 0.89 (3H, s), 0.83 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 166.9, 165.9, 157.2, 133.0, 130.8, 130.4, 129.6, 129.4, 128.4, 128.3, 120.0, 87.2, 87.0, 86.1, 81.7, 73.2, 68.2, 59.6, 51.9, 51.8, 44.2, 40.8, 36.5, 35.9, 34.1, 33.7, 33.6, 31.5, 29.5, 28.0, 27.2, 26.7, 26.6, 25.2, 22.6, 21.4, 20.7, 16.8, 16.0, 14.1, 11.9; HRMS for $C_{43}H_{55}O_8$(M+H) calcd: 699.3892, found: 699.3883.

2.2.14. 3β-Acetoxy-12β,26-dibenzyloxy-5α-furostan-14-ene ***(24)***

To a dichloromethane/cyclohexane (1 mL/3 mL) solution of iodobenzene diacetate (128 mg, 0.40 mmol) and iodine (101 mg, 0.40 mmol), was added the primary alcohol **23** (140 mg, 0.20 mmol) in dichloromethane (3 mL), and the resulting mixture was stirred at 0 °C for 6 h. The reaction was quenched by adding saturated sodium thiosulfate, extracted with dichloromethane (3×20 mL), concentrated in vacuo, and subjected to silica gel chromatography (hexane/EtOAc = 4:1) to give 5/6 spiroketal **24** (89 mg, 64%) as a white foam.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01–7.95 (4H, m), 7.55–7.24 (6H, m), 5.46 (1H, s), 4.91 (1H, d, J = 8.1 Hz), 4.63 (1H, dd, J = 5.2 and 11.4 Hz), 4.07–4.02 (1H, m), 3.69 (1H, d, J = 12.3 Hz), 2.48 (1H, s), 2.44 (1H, d, J = 8.4 Hz), 2.15–2.08 (1H, m), 1.99 (3H, s), 1.49 (3H, s), 1.20 (6H, s), 0.87 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 165.6, 156.7, 132.9, 132.5, 131.6, 129.4, 129.4, 128.4, 128, 2, 120.1, 107.7, 106.3, 84.9, 81.4, 78.1, 73.2, 66.4, 55.9, 52.2, 51.5, 44.2, 36.5, 36.0, 34.1, 33.8, 30.3, 29.4, 28.1, 27.2, 26.6, 21.6, 21.4, 15.0, 13.6, 12.0; HRMS for $C_{43}H_{55}O_8$(M+H) calcd: 697.3735, found: 697.3728.

2.2.15. 3β,12β,26-Trihydroxy-5α-furostan-14-ene ***(25)***

^{1}H NMR (300 MHz, Pyr-d_5) δ 5.59 (1H, s), 5.21 (1H, d), 4.05 (1H, d), 3.72 (1H, d), 3.47 (1H, m), 3.13 (1H, t), 2.52 (1H, dt),1.32 (3H, d), 1.28 (3H, s), 1.24 (3H, s), 0.82 (3H, s); ^{13}C NMR (75 MHz, Pyr-d_5) δ 158.1 (14′), 119.6 (15′), 107.1 (22′), 85.5 (16′), 79.0 (12′), 70.5 (26′), 70.1 (26′), 66.0 (25′), 56.6 (17′), 53.5 (9′), 53.4 (13′), 45.2 (20′), 44.9, 39.0, 37.3, 36.2 (10′), 34.3, 33.8 (24′), 32.4 (8′), 30.9 (11′), 30.1, 28.8 (7′), 27.7 (6′), 27.0 (23′), 14.4 (21′), 14.1 (18′), 12.3 (19′).

NMR spectra of the south unit of ritterazine Y: ^{1}H NMR (Pyr-d_5) δ 5.23 (1H, s), 4.24 (1H, d), 3.72 (1H, d), 3.50 (1H, m), 3.12 (1H, t), 2.56 (1H, dt), 1.35 (3H, d), 1.25 (3H, s), 1.18 (3H, s), 1.78 (3H, s); ^{13}C NMR (75 MHz, Pyr-d_5) δ 157.7 (14′). 120.5 (15′), 107.1 (22′), 85.4 (16′), 78.7 (12′), 70.2 (26′), 66.0 (25′), 56.4 (17′), 52.7 (9′), 52.6 (13′), 46.2 (1′), 45.2 (20′), 33.8 (24′), 33.1 (8′), 30.9 (11′), 29.7 (7′), 27.0 (27′), 14.5 (21′), 14.0 (18′), 11.8 (19′).

2.2.16. 3-Keto-12β,26-dibenzyloxy-5α-furostan-14-ene ***(26)***

To a well stirred solution of steroidal acetate **24** (88 mg, 0.127 mmol) in 5:1 MeOH/H_2O at 25 °C was added potassium carbonate (17 mg, 0.127 mmol). After stirring for 12 h, the reaction mixture was quenched with saturated NH_4Cl and the solvent was evaporated under reduced pressure to afford crude product. Extraction with EtOAc, washing with brine, drying over anhydrous sodium sulfate, and evaporation under reduced pressure afforded the crude product mixture, which was subjected to silica gel chromatography to give the corresponding alcohol. To acetone solution of the alcohol was added dropwise Jones reagent, and the mixture was stirred for 20 min at 0 °C. The reaction mixture was quenched with saturated aqueous sodium thiosulfate, extracted with ethyl acetate, concentrated *in* vacuo, and subjected to silica gel chromatography (hexane/EtOAc = 2:1) to give ketone **26** (45 mg, 53% over two steps) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01–7.95 (4H, m), 7.56–7.33 (6H, m), 5.48 (1H, s), 4.92 (1H, d, J = 7.8 Hz), 4.65 (1H, dd, J = 5.2 and 10.8 Hz), 4.05 (1H, d, J = 9.9 Hz), 3.64 (1H, d, J = 12.0 Hz), 2.49–2.44 (3H, m), 2.38–2.11 (5H, m), 2.02–2.00 (2H, m), 1.49 (3H, s), 1.24 (3H, s), 0.87 (3H, s), 0.85 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.1, 165.9, 165.6, 156.1, 133.0, 132.5, 131.6, 130.4, 129.4, 129.3, 128.38, 128.2, 120.5, 106.4, 84.8, 81.1, 78.1, 66.4, 55.9, 51.8, 51.5, 46.0, 44.3, 44.2, 38.1, 37.8, 36.1, 34.0, 30.3, 29.2, 28.4, 26.7, 21.6, 15.0, 13.6, 11.2.

2.2.17. 2α-Azido-3-keto-12β,26-dibenzyloxy-5α-furostan-14-ene ***(28)***

To a solution of ketone **26** (45 mg, 0.067 mmol) in THF (1 mL) was added phenyltrimethylammonium tribromide (28 mg, 0.078 mmol) at 0 °C. After stirring for 20 min at the same temperature, the reaction mixture was quenched with saturated aqueous sodium thiosulfate and extracted with ethyl acetate. The combined extract was washed with brine, dried over anhydrous sodium sulfate, concentrated, and subjected to silica gel chromatography to give the corresponding bromoketone **27**. A solution of bromoketone **27** in freshly dried CH_3NO_2 was cooled to 0 °C and $TMGN_3$ was added portionwise for 2 min. The reaction was allowed to warm slowly to 25 °C during 6 h of stirring and partitioned between EtOAc and brine, and organic layer was dried over anhydrous Na_2SO_4 and concentrated to give crude mixture, which was subjected to silica gel chromatography (Hexane/EtOAc = 4:1) to give azidoketone **28** (14 mg, 30% over two steps) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01–7.95 (4H, m), 7.68–7.62 (2H, m), 7.56–7.34 (4H, m), 5.50 (1H, s), 4.06–4.02 (3H, m), 3.68 (1H, d, J = 12.0 Hz) 2.47 (1H, t, J = 8.7 Hz), 2.37 (1H, s), 2.32–2.30 (1H, m), 2.02 (3H, s), 1.49 (3H, s) 1.23 (3H, s), 1.13(3H, s), 0.87 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.1, 165.9, 165.6, 156.1, 133.0, 132.5, 131.6, 130.4, 129. 4, 129.3, 128.38, 128.2, 120.5, 106.4, 84.8, 81.1, 78.2, 66.4, 55.9, 51.8, 51.5, 46.0, 44.3, 44.2, 38.1, 37.8, 36.1, 34.0, 30.3, 29.2, 28.4, 26.7, 21.6, 15.0, 13.6, 11.2; HRMS for $C_{41}H_{47}N_3O_7$(M+Na) calcd: 716.3306, found: 716.3301.

*2.2.18. 14′,15′-dehydro-ritterazine Y (****5****)*

To a solution of azidoketone **28** (14 mg, 0.020 mmol) and alpha-aminomethoxime **20** (15 mg) in 10 mL benzene was added dichlorodibutylstannane (cat.) and polyvinyl pyridine (20 mg). The reaction flask was equipped with a Dean–Stark trap, and the mixture was heated at reflux for 3 h (2 mL of fresh benzene was added twice to maintain the solvent level in the reaction vessel), at which time TLC indicated no remaining azidoketone **28**. The reaction mixture was cooled and filtered, and the solids were washed with CH_2Cl_2. Evaporation of the filtrate and silica gel chromatography of the residue gave the protected ritterazine Y analog, which was subjected to a global deprotection with KOH to give the desired Δ^{14}-ritterazine Y **5** (4.2 mg, 24%) as a white foam after chromatography (EtOAc/MeOH = 10:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (1H, s), 5.39 (1H, s), 5.14 (1H, br), 5.09 (1H, br), 4.93 (1H, d, J = 8.5 Hz), 4.85 (1H, d, J = 8.5 Hz), 3.79 (1H, s), 3.31 (2H, m), 2.90–2.76 (4H, m), 2.62–2.49 (3H, m), 2.46 (1H, s), 2.38 (1H, s), 1.25 (3H, s), 1.22 (3H, s), 1.18 (3H, s), 1.11 (3H, s), 1.09 (3H, d, J = 6.8 Hz), 1.02 (6H, s), 0.86 (3H, d, J = 5.5 Hz), 0.84 (3H, s); HRMS for (M+H) calcd: 863.5574, found: 863.5575.

3. Results and discussion

To prepare 14′,15′-dehydro-ritterazine Y, we first synthesized terminal olefin **12** from hecogenin acetate **6** by using synthetic procedures similar to those for ritterazine M (Scheme 1) [30]. Conversion of hecogenin acetate **6** into lumihecogenin acetate **7** via the Welzel photolysis [23,30,31] followed by functional group

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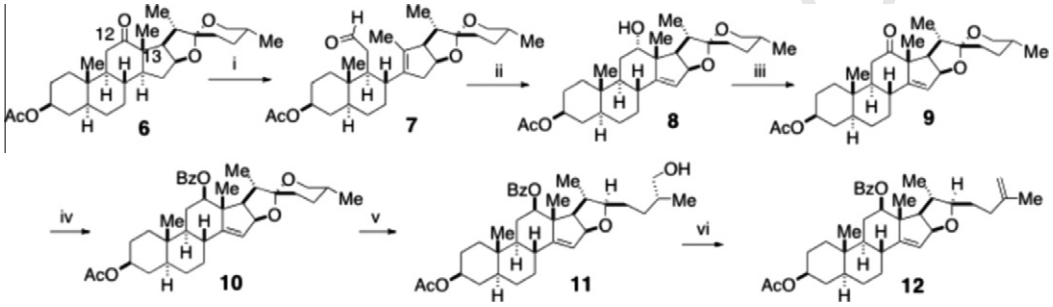
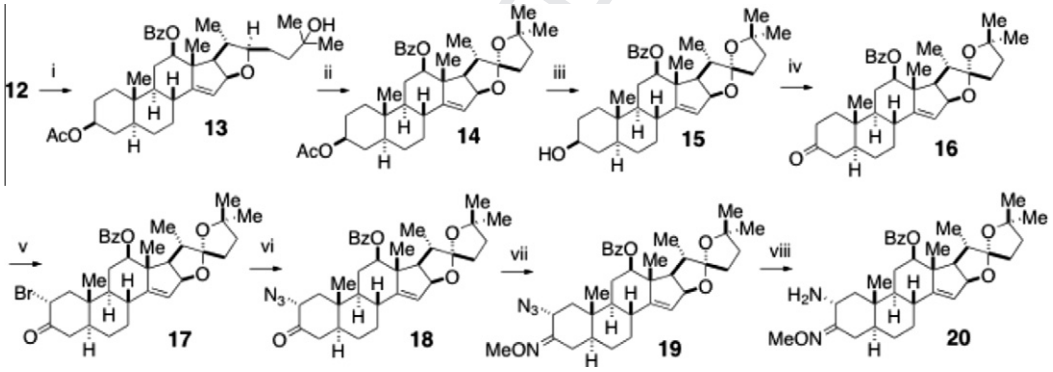
manipulations of **7** afforded diene **12** [30], which was then transformed into 14′,15′-dehydro-ritterazine Y north hemisphere **20** (Scheme 2) by adopting procedures developed by the Fuchs and Shair groups [25,26,33].

The synthesis of the 14′,15′-dehydro-ritterazine Y south hemisphere **28** began with oxidative modification of the terminal olefin **12** that was used in the synthesis of 14′,15′-dehydro-ritterazine Y north hemisphere **20** (Scheme 3). Subjection of the terminal olefin **12** into sharpless asymmetric dihydroxylation produced C25,26-diol **21** without affecting Δ^{14} olefin moiety. For the reestablishment of the 5/6 spiroketal (1,6-dioxaspiro[4,5]decane), it was necessary to protect the tertiary alcohol in **21** to prevent oxidative cleavage of the 1,2-diol. Sequential protection of the primary alcohol with TBDMS group and the tertiary alcohol with benzoyl group and removal of TBDMS group with boron trifluoride diethyletherate provided 26-alcohol **23**. Hypoiodite-mediated alkoxy radical cyclization of the primary alcohol **23** stereoselectively provided **24** with establishment of 5/6 spiroketal. The stereochemistry of 5/6 spiroketal **24** was assessed by comparison of ^{1}H and ^{13}C NMR data of triol **25** and the south unit of ritterazine Y (see Fig. 4 and Section 2.2.15). The carbon NMR chemical shifts of C16, 22, and 25 of triol **25** matched well with C16′, 22′, and 25′ of the south unit of ritterazine Y, suggesting triol **25** has the same spiroketal stereochemistry as that of the ritterazine Y south hemisphere. Having established the desired spiroketal stereochemistry, we then converted 3-acetate **24** into azidoketone **28**, the south Y unit, employing the similar procedures that were used for the preparation of the north G unit (Scheme 2).

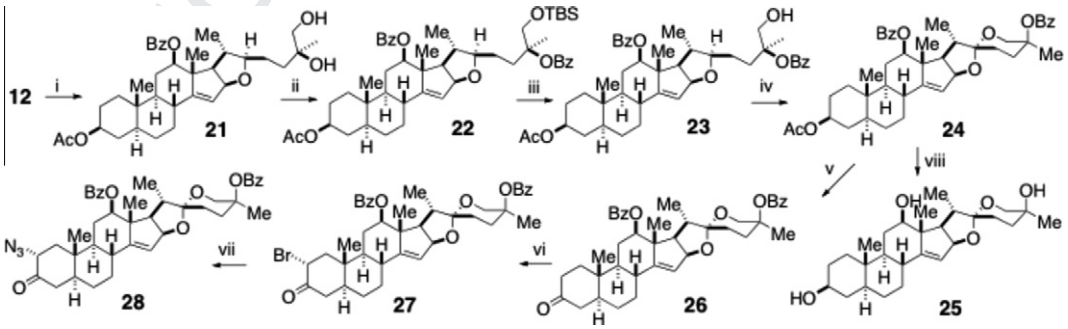
The final stages of 14′,15′-dehydro-ritterazine Y **5** involve the Guo–Fuchs asymmetric pyrazine coupling [34] of the north G and south Y units and the global removal of the protecting groups,



Scheme 1. Preparation of terminal olefin **12**: (i) hv (450 W), CH_2Cl_2, (ii) $ZnCl_2$, CH_2Cl_2, 25 °C, 60% two steps, (iii) Jones 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. Synthesis of the 14′,15′-dehydro-ritterazine Y north hemisphere **20**. (i) $Hg(OAc)_2$, THF/H_2O; $NaBH_4$, 63% (ii) $PhI(OAc)_2$, I_2, CH_2Cl_2, 69% (iv) K_2CO_3, MeOH, 80% (v) CrO_3, H_2SO_4, acetone, 87% (v) phenyltrimethylammonium tribromide, THF, 77% (vi) tetramethylguanidinium azide, nitromethane, 93% (vii) $MeONH_2$, 44% (viii) triphenylphosphine, THF, H_2O, 99%.



Scheme 3. Synthesis of the 14′,15′-dehydro-ritterazine Y south hemisphere **28**. (i) AD-mix α (ii) TBSCl, imidazole, CH_2Cl_2; $MgBr_2$ Et_2O, TEA, CH_2Cl_2 (iii) BF_3 OEt_2, CH_2Cl_2, 71% (iv) $PhI(OAc)_2$, I_2, CH_2Cl_2, 64% (v) K_2CO_3, MeOH; CrO_3, H_2SO_4, acetone, 53% (vi) phenyltrimethylammonium tribromide, THF (vii) tetramethylguanidinium azide, nitromethane, 30% over two steps.

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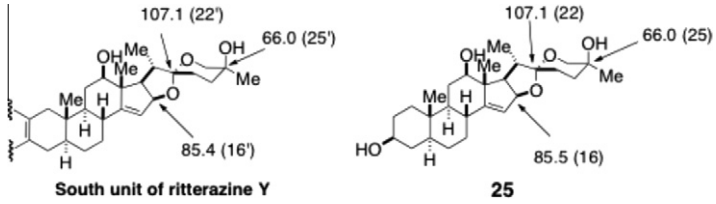

Fig. 4. Comparison of carbon NMR chemical shifts of the south unit of ritterazine Y and triol **25**. Chemical shifts for C16, 22, and 25 of the ritterazine Y south unit and C16′, 22′, and 25′ of the triol **25** are shown in ppm.

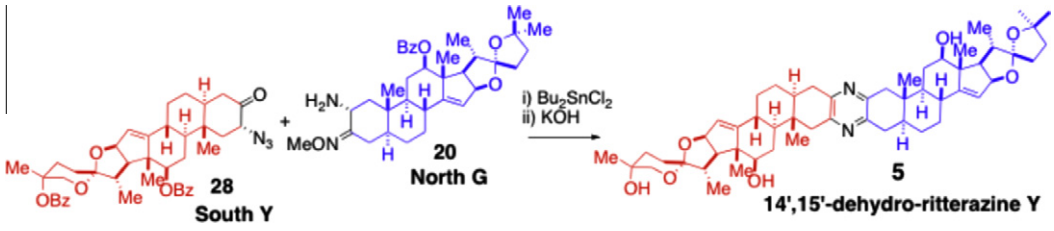

Fig. 5. Synthesis of 14′,15′-dehydro-ritterazine Y **5** via Guo–Fuchs asymmetric pyrazine coupling.

which provided the desired ritterazine Y analog **5** (Fig. 5). In summary, we have prepared 14′,15′-dehydro-ritterazine Y **5** in 23 steps via reductive/oxidative modifications of commercially available hecogenin acetate. The synthesis of the north G and south Y units involve the use of a common intermediate **12**, which enabled the facile construction of the both units. The bioactivity test of this novel bissteroidal pyrazine **5** and synthesis of derivatives of 14′,15′-dehydro-ritterazine Y are in progress and their results will be reported elsewhere in due course.

4. Uncited reference

Q3 [32].

Acknowledgment

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

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