Pergamon

Tetrahedron 58 (2002) 10293–10299

TETRAHEDRON

A stereoselective synthesis of squalamine

Xiang-Dong Zhou,† Feng Cai and Wei-Shan Zhou*

Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Lu, Shanghai 200032, People's Republic of China

Received 30 August 2002; revised 7 October 2002; accepted 31 October 2002

Abstract—Squalamine (**1**) was synthesized stereoselectively in 14 steps and 19% overall yield from 3-keto-5α-chenodeoxycholanate (**2**) by using a modified Sharpless asymmetric dihydroxylation as the key step. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Squalamine, 3β-*N*-1-{*N*-[3-(4-aminobutyl)]-1,3-diaminopropane}-7α, 24*R*-dihydroxy-5α-cholestane-24-sulfate (**1**) (Fig. 1), the first example of an aminosterol, was isolated by Zasloff[1] in minute quantities from the stomach of the dogfish shark *Squalus acanthias* in 1993.[2a] Squalamine, whose structure was determined by ^{1}H NMR and ^{13}C NMR spectroscopy and FAB mass spectrometry,[2,3a] possesses both amphipathic and zwitterionic properties, due to the lipophilic steroidal skeleton, the hydophilic 3β-spermine and 24*R*-sulfate, and the cationic spermidine and anionic 24-sulfate moieties.

Squalamine displays potent bactericidal activities against both Gram-negative and Gram-positive bacteria.[2] However, the most significant property is its anti-angiogenic activity,[4a] which first led to the development of squalamine for cancer chemotherapy.[4b] In particular, the combination of squalamine and cisplatin was found to be highly active against human cancer cells.[4d] Squalamine also has activity against age-related macular degeneration, malaria, obesity and asthma.[4c] Coupled with this promising biological activity, the recent discovery of several analogues of squalamine from the stomach of the dogfish shark *S. acanthias*,[5] has ushered in a new era of research on squalamine.

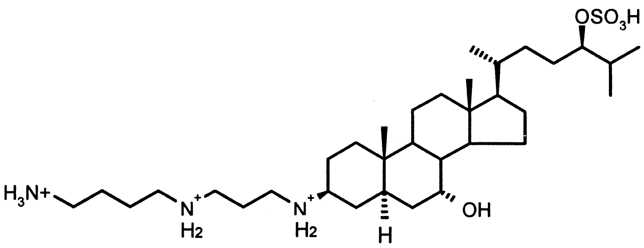


Figure 1. Squalamine (**1**).

This compound cannot be obtained in a large amount from natural sources. Therefore, much effort has been expended in synthesizing it and its analogues. Moriarty's[3b] and Pechulis'[6] groups have synthesized squalamine in racemic form, and the former group also synthesized the 24-*R*-hydroxy side chain.[3a] Kinney[7] and his co-workers also have obtained good results. Although, this group obtained the 24-*R*-hydroxy compounds in 91% d.e., the undesired 24-*S*-isomer had to be removed in the last step by HPLC. Herein, we report a highly stereoselective new synthetic route to squalamine by using an improved Sharpless catalytic asymmetric dihydroxylation[8]as a key step. Thus, the 24-*R*-hydroxy group was introduced[9] in 100% d.e. and the pure squalamine was obtained in an overall yield of 19%.

2. Results and discussion

The starting material, methyl 3-keto-5α-chenodeoxycholanate **2**, was prepared from methyl chenodeoxycholanate according to the literature.[10] In addition, our group also developed a method[11] to prepare it from methyl hyodeoxycholanate (Me-HDCA), which is readily available in China. Our synthesis of squalamine was divided into three stages. First, compound **7** with the 24-*R*-hydroxyl group was prepared. Then, the side chain on ring D was constructed through the synthesis of compound **11**. Finally, the spermidine side chain was connected to ring A, providing the target compound.

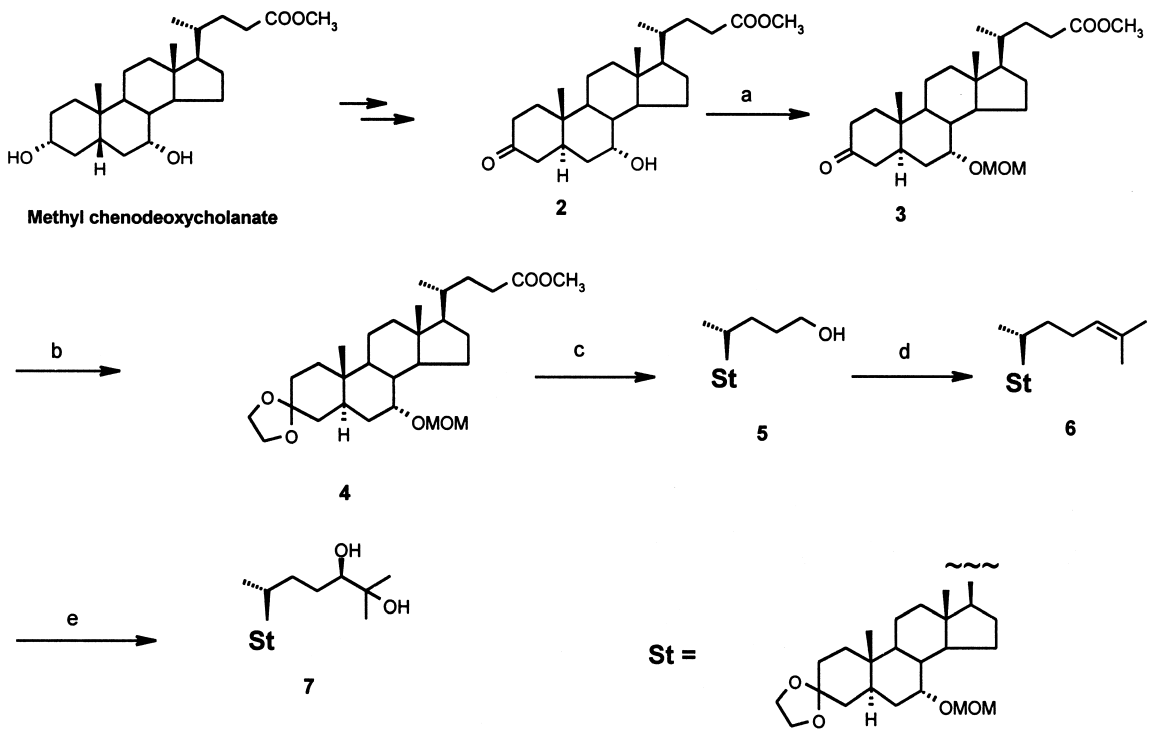
As depicted in Scheme 1, the 7α-hydroxyl group of **2** was converted by treatment with dimethoxymethane in the presence of P_2O_5 in chloroform into the 7α-methoxymethyl ether **3** (94%). Then the 3-keto group was protected with

Keywords: squalamine; improved Sharpless AD; aminosterol; stereoselective.

* Corresponding author. Tel.: +86-21-6416-3300; fax: +86-21-6416-6128; e-mail: zhws@pub.sioc.ac.cn

† Present address: Department of Chemistry, The Third Millitary Medical University, Chongqing 400038, People's Republic of China.

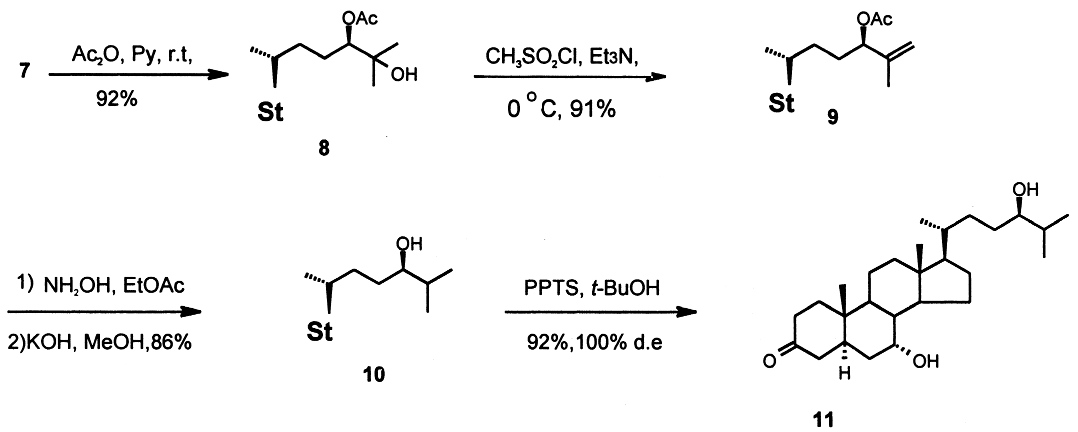
PII: S0040-4020(02)01413-8



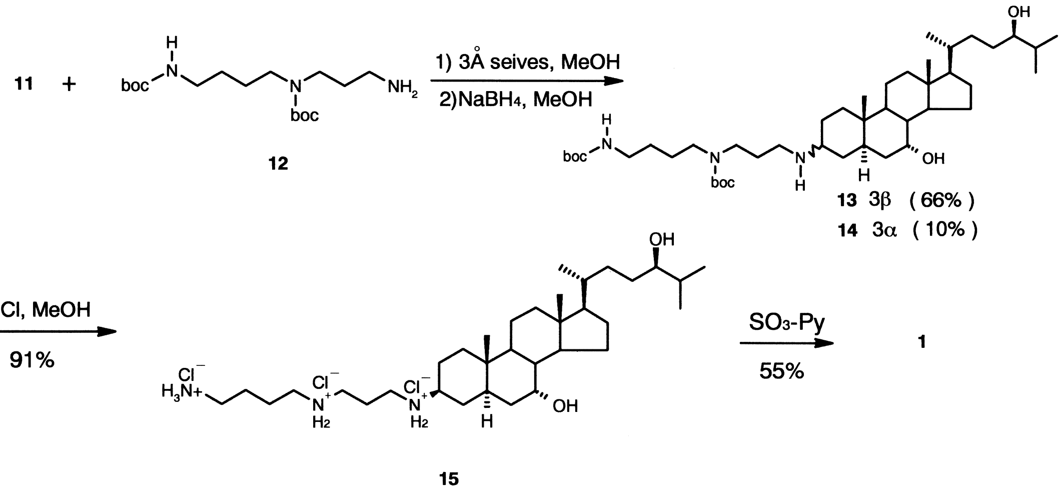
Scheme 1. (a) $CH_3OCH_2OCH_3$, P_2O_5, $CHCl_3$, room temperature, 94%; (b) ethylene glycol, PTSA, benzene, △ 96%; (c) $LiAlH_4$, THF, room temperature 94%; (d) (1), $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C, (2) BuLi, $Ph_3P^+CH(CH_3)_2I^-$, THF, room temperature, 91% for two steps; (e) $(DHQD)_2PHAL$, $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, *t*-butanol–methyl *t*-butyl ether–H_2O (2.5:3:2.5), room temperature, 97%, 100% d.e.

ethylene glycol to give the ethylene acetal **4** (96%). The side chain ester in **4** was reduced with $LiAlH_4$ to the 24-alcohol **5** (94%), Swern oxidation of which followed by Wittig olefination with isopropyltriphenylphosphonium iodide and BuLi in THF at room temperature gave the desmosteroid derivative **6** in 91% overall yield. Compound **6** could be used as a key intermediate for transformation into squalamine and its cognates,[5] for example, 24-one-26-cysteine squalamine, Δ^{25}-24-one squalamine, etc. In the course of our synthesis of squalamine, the key step is the stereoselective introduction of the 24*R*, 25-dihydroxy group into the side chain of **6** via an improved Sharpless catalytic asymmetric dihydroxylation.[8] Dihydroxylation of **6** with $(DHQD)_2PHAL$ and $K_2OsO_2(OH)_4$ in *tert*-BuOH-methyl *tert*-butyl ether–H_2O (2.5:3:2.5) solvent system gave 24*R*,25-dihydroxy compound **7** in 6 h in 97% yield and with 100% d.e. This solvent system has the advantages over the usual solvent system (1:1 *t*-BuOH–H_2O[12] or 1.5:1 *t*-BuOH–H_2O[13]) in the AD reaction in that it can greatly shorten the reaction time and increase the diastereoselectivity due to the larger solubility of steroids therein. Thereby 24-*R*-hydroxyl group was successfully introduced with complete stereoselectivity.

In the second stage (Scheme 2), compound **7** was acetylated by using acetic anhydride and pyridine to give the acetate **8** (92%). Dehydration of the 25-*tert*-hydroxy group in **8** with methanesulfonyl chloride and triethylamine gave the Δ^{25}-24*R*-acetoxyl-compound **9** (91%). This result is better than that obtained from the reaction in which DMAP is used as a catalyst, as described in our preliminary communication.[9] Diimide reduction[14] of **9** followed by hydrolysis with potassium hydroxide in methanol afforded compound **10** (86%). Removal of the 7α-MOM and 3-acetal



Scheme 2.



Scheme 3.

protecting groups of **10** with PPTS in *tert*-BuOH gave **11** in 92% yield.

With the steroidal skeleton in hand, our attention was turned to the introduction of the spermidine side chain into ring A (Scheme 3). This was achieved via a reductive amination of compound **11** with the protected spermidine **12**[15] utilizing sodium borohydride as the reducing agent, and gave a mixture of the aminosterols **13** and **14**. These were separated with flash chromatography with silica gel to give the 3β-aminosterol **13** (66%) and the 3α-aminosterol **14** (10%). Removal of the Boc protecting groups of **13** by treatment with a solution of HCl in MeOH gave **15** as the hydrochloric acid salt (91%), which without further purification was treated with sulfur trioxide–pyridine complex in pyridine to give squalamine in 55% yield. This synthetic squalamine obtained from 3β-aminosterol **13** was identical to natural squalamine[5] according to ^{1}H NMR and ^{13}C NMR spectroscopy, and HRMS (ESI).

3. Conclusion

There are two key elements in our synthesis of squalamine. The first one is the highly stereoselective introduction of the 24*R*-hydroxy group into the key intermediate, desmosteroid derivative **6**, via an improved Sharpless asymmetric dihydroxylation in 100% d.e. The second is the reductive replacement of the 3-keto group in ring A by the spermidine side chain to give a mixture of 3β-aminosterol **13** and 3α-aminosterol **14** in a ratio of 6:1.

Another novel synthetic route to squalamine and its analogues is in progress.

4. Experimental

4.1. General

All melting points are uncorrected. IR spectra were recorded with FT-IR apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz in $CDCl_3$ except those noted. Chemical shifts are reported in ppm relative to TMS as internal standard. Mass spectra were recorded by EI or ESI methods. Flash column chromatography was carried out with silica gel (300–400 mesh). THF was distilled over sodium and dichloromethane was distilled over CaH_2. The d.e. value was determined by HPLC analysis on Inersil ODS-3 column with CH_3CN-H_2O as an eluent.

4.1.1. Methyl 7α-methoxymethyl-5α-cholanate-3-one (3). To a solution of 3-keto-5α-chenodeoxycholanate **2** (50 mg, 0.12 mmol) in dry chloroform (1 mL) and dimethoxymethane (0.23 mL, 2.6 mmol) was added phosphorus pentoxide (100 mg, 0.7 mmol) with stirring at room temperature. After being stirred for 1 h, the mixture was filtered through a pad of silica gel and washed with chloroform (3×30 mL). Removal of the solvent in vacuo afforded a light yellow oil (79 mg), which was purified by flash chromatography (pet. ether/ethyl acetate 8:l) to afford pure **3** (52 mg, 94%). Recrystallization from methanol gave colorless needles; mp 147–148°C; $[\alpha]_D^{20}=-3$ (*c* 0.15, $CHCl_3$); MS-EI (*m/z*): 416 (M^+-CH_3OH), 386 ($M^+-HOCH_2OCH_3$); IR (cm^{-1}): 1741 ($COOCH_3$); ^{1}H NMR δ 0.67 (3H, s, 18-CH_3), 0.92 (3H, d, J=6.3 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.10–1.20 (3H, m), 1.22–1.45 (11H, m), 1.52–1.68 (5H, m) 1.72–2.20 (5H, m), 2.30–2.50 (3H, m), 3.35 (3H, s, OCH_3), 3.69 (3H, s, $COOCH_3$), 4.60 (1H, d, A of AB, J=6.9 Hz, $-OCH_2O-$), 4.67 (1H, d, B of AB, J=6.9 Hz, $-OCH_2O-$). Anal. calcd for $C_{27}H_{44}O_5$: C, 72.28%; H, 9.89%. Found C, 72.22%; H, 9.81%.

4.1.2. Methyl 7α-methoxymethyl-3-dioxolane-5α-cholanate (4). To a solution of **3** (183 mg, 0.41 mmol) in 20 mL benzene was added ethylene glycol (25 mL) and *p*-toluenesulfonic acid (500 mg). The resulting solution was refluxed for 3 h with use of a Dean–Stark trap to remove water, and then cooled to room temperature. The solvent was removed in vacuo then extracted with ethyl acetate (3×30 mL). The organic layer was washed with sodium bicarbonate solution (2×10 mL), distilled water (10 mL) and brine (10 mL), respectively. The organic layer was dried over Na_2SO_4, and the solvent was removed in vacuo, and the residue was purified by flash chromatography (pet. ether/ethyl acetate 10:1) to afford pure compound **4** (193 mg, 96%, white solid); mp 100–102°C; $[\alpha]_D^{20}=-9.6$ (*c* 0.65, $CHCl_3$); MS-EI (*m/z*): 492 (M^+), 460 (M^+-CH_3OH), 432 ($M^+-HOCH_2OCH_3$); IR (cm^{-1}): 1734 ($COOCH_3$); ^1H NMR δ 0.64 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 0.92 (3H, d, J=6.3 Hz, 21-CH_3), 1.02–1.70 (11H, m), 1.80–2.10 (3H, m), 2.20–2.40 (2H, m), 3.36 (3H, s, OCH_3), 3.64 (1H, m, 7β-H), 3.66 (3H, s, $COOCH_3$), 3.92 (4H, s, OCH_2CH_2O), 4.60 (1H, d, A of AB, J=6.9 Hz, $-OCH_2O-$), 4.69 (1H, d, B of AB, J=6.9 Hz, $-OCH_2O-$); HRMS calcd for $C_{29}H_{48}O_6$: 492.3451. Found 492.3454.

4.1.3. 7α-Methoxymethyl-3-dioxolane-5α-cholane-24-ol (5). A solution of **4** (1.2 g, 2.44 mmol) in dry tetrahydrofuran (20 mL) was added to a suspension of lithium aluminum hydride (114 mg, 3.0 mmol) in dry tetrahydrofuran (30 mL) under argon over 3 h. The reaction mixture was stirred for an additional hour before being quenched with $Na_2SO_4\cdot 10H_2O$, filtered through a pad of celite, and washed with ethyl acetate. Removal of the solvent in vacuo and purification by flash chromatography (pet. ether/ethyl acetate 6:1) afforded pure compound **5** (1.09 g, 94%, white solid); mp 133–135°C; $[\alpha]_D^{20}=-11.7$ (*c* 0.4, $CHCl_3$); MS-EI (*m/z*): 464 (M^+), 420 (M^+-CO_2), 403 ($M^++1-HOCH_2OCH_3$); IR (cm^{-1}): 3317 (OH); ^1H NMR δ 0.65 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3) 0.91 (3H, d, J=6.4 Hz, 21-CH_3), 1.02–1.20 (4H, m), 1.22–1.60 (10H, m), 1.62–1.80 (10H, m), 1.80–2.10 (3,H, m), 3.37 (3H, s, OCH_3), 3.60 (3H, m, 7β-H and 24-H), 3.92 (4H, s, OCH_2CH_2O), 4.60 (1H, d, A of AB, J=6.9 Hz, $-OCH_2O-$), 4.69 (1H, d, B of AB, J=6.9 Hz, $-OCH_2O-$). Anal. calcd for $C_{28}H_{48}O_5$: C, 72.37%; H, 10.41%. Found C, 72.55%; H, 10.52%.

4.1.4. Δ^{24},7α-Methoxymethyl-3-dioxolane-5α-cholestene (6). Dimethyl sulfoxide (0.42 mL, 5 mmol) was added dropwise to a solution of oxalyl chloride (0.23 mL, 2.5 mmol) in dry dichloromethane (2 mL) at −78°C. The solution was stirred in a dry ice–acetone bath under argon over 35 min. A solution of **5** (525 mg, 1.13 mmol) in dichloromethane (3 mL) was added dropwise over 2 min. The stirring was continued for an additional 1 h. When triethylamine (1.6 mL) was added dropwise to the reaction mixture, a large amount of white solid appeared. After being stirred for 10 min, the reaction mixture was allowed to warm to room temperature and washed in turn with sat. NH_4Cl solution (2×5 mL), sat. $NaHCO_3$ solution (5 mL), brine (10 mL) and dried over sodium sulfate. Removal of the solvent in vacuo afforded yellow solid (570 mg). Without chromatography, it was directly used for the next step. Thus, *n*-butyl lithium (1.6 M in hexane, 2.2 mL) was added to a suspension of isopropyltriphenylphosphonium iodide (1.25 g, 2.9 mmol) in dry tetrahydrofuran (6 mL) at room temperature, giving a deep red solution, to which the compound (570 mg) from last step in dry tetrahydrofuran (6 mL) was added to it. After being stirred for 2 h, the reaction was quenched with ethyl acetate (20 mL), and a large amount of white solid appeared. The mixture was filtered through a pad of celite and washed with ethyl acetate (20 mL). The filtrate was then washed with sat. NH_4Cl (2×5 mL) and brine (10 mL), respectively, and dried over sodium sulfate. Removal of the solvent in vacuo and purification by flash chromatography (pet. ether/ethyl acetate 10:1) afforded **6** (504 mg, 91.3%, white solid); mp 111–113°C; $[\alpha]_D^{20}=-19$ (*c* 0.12, $CHCl_3$); MS-EI (*m/z*): 456 ($M^+-HOCH_2OCH_3$), 426 ($M^+-HOCH_2OCH_3$); ^1H NMR δ 0.64 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 0.93 (3H, d, J=6.5 Hz, 21-CH_3), 1.02–1.40 (11H, m), 1.52–1.70 (18H, m), 1.82–2.10 (4H, m), 3.38 (3H, s, OCH_3), 3.61 (1H, m, 7β-H), 3.92 (4H, s, $-OCH_2CH_2O-$), 4.61 (1H, d, A of AB, J=6.9 Hz, $-OCH_2O-$), 4.69 (1H, d, B of AB, J=6.9 Hz, $-OCH_2O-$), 5.07 (1H, t, 24-H); ^{13}C NMR δ 130.96, 125.35, 109.41, 96.18, 75.36, 64.18, 64.15, 56.11, 55.69, 50.11, 45.94, 42.64, 39.90, 39.51, 37.68, 36.50, 36.20, 35.88, 35.75, 35.59, 33.57, 31.29, 28.26, 25.78, 24.86, 23.84, 21.11, 18.72, 17.71, 11.90, 10.60. Anal. calcd for $C_{31}H_{52}O_4$: C, 76.18%; H, 10.72%. Found C, 76.30%; H, 11.01%.

4.1.5. 7α-Methoxymethyl-3-dioxolane-5α-24*R*,25-dihydroxy-cholestane (7). A solution of $K_3Fe(CN)_6$ (820 mg, 2.48 mmol), K_2CO_3 (350 mg, 2.54 mmol), $CH_3SO_2NH_2$ (80 mg, 0.84 mmol), $(DHQD)_2PHAL$ (40 mg, 9 mol%), and $K_2OsO_2(OH)_4$ (10 mg, 5 mol%) in *t*-butanol–water (1:1, 40 mL) was cooled to 0°C. A solution of **6** (268 mg, 0.55 mmol) in methyl *t*-butyl ether (30 mL) was added dropwise. The resulting mixture was stirred vigorously for 6 h. Then the reaction was quenched at 0°C with sodium sulfite (1.0 g). Stirring was continued for an additional hour. The aqueous layer was extracted with ethyl acetate (3×3 mL). The combined organic layer was washed with 2 M KOH solution (25 mL), 10% HCl solution (10 mL), sat. $NaHCO_3$ solution (10 mL), brine (10 mL), dried over sodium sulfate and evaporated to give the crude product, which was purified by flash chromatography (pet. ether/acetone 2:1) to afford pure **9** (279 mg, 100% d.e., 97%, white solid); mp 167∼169°C, $[\alpha]_D^{23}=+0.6°$ (*c* 4.1, $CHCl_3$); MS-EI (*m/z*): 522 (M^+), 443 ($M^++1-HOCH_2OCH_3-H_2O$); IR (cm^{-1}): 3443 (OH); ^1H NMR δ 0.65 (3H, s, 18-CH_3), 0.88 (3H, s, 19-CH_3), 0.92 (3H, d, J=6.2 Hz, 21-CH_3), 0.90–1.05 (8H, m), 1.07 (3H, s, 26-CH_3), 1.13 (3H, s, 27-CH_3), 1.30–1.7 (16H, m), 1.80–2.10 (2H, m) 3.25 (1H, m, 24-H), 3.39 (3H, s, CH_3O), 3.59 (H, m, 3-H), 3.92 (4H, s, $-OCH_2CH_2O-$), 4.61 (1H, d, A of AB, J=6.9 Hz, $-OCH_2O-$), 4.69 (1H, d, B of AB, J=6.9 Hz, $-OCH_2O-$); ^{13}C NMR δ 109.31, 96.08, 78.78, 75.26, 64.08, 64.05, 55.98, 55.62, 50.00, 45.81, 39.77, 37.55, 36.40, 35.76, 35.64, 34.47, 33.45, 32.86, 31.17, 30.32, 29.69, 28.26, 28.13, 26.55, 23.72, 23.27, 21.00, 18.55, 11.83, 10.51. Anal. calcd for $C_{31}H_{54}O_6$: C, 71.23%; H, 10.41%. Found C, 71.33%; H, 10.21%.

4.1.6. 7α-Methoxymethyl-3-dioxolane-5α-24*R*,25-dihydroxy-cholestan-24-acetate (8). A solution of **7** (118 mg, 0.226 mmol) in acetic anhydride (15 mL) and pyridine (1.5 mL) was stirred at room temperature for 9 h. The reaction mixture was partitioned between ethyl acetate (20 mL) and water. The organic layer was washed with 5% HCl (5 mL), sat. $NaHCO_3$ (5 mL), and brine (2×5 mL), respectively, and dried over sodium sulfate, evaporated in vacuo and purified by flash chromatography (pet. ether/acetone 4:1) to afford pure compound **8** (117 mg, 92%, white solid); mp 76–78°C; $[\alpha]_D^{22}=-5.6$ (*c* 1.6, $CHCl_3$); MS-EI (*m/z*): 564 (M^+), 519 ($M^+-CH_3OCH_2$), 502 ($M^+-CH_3OCH_2OH$); IR (cm^{-1}): 3450 (OH), 1736 (CH_3CO_2); ^1H NMR δ 0.64 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 0.91 (3H, d, J=6.4 Hz, 21-CH_3), 0.90–1.05 (3H, m), 1.20 (3H, s, 26-CH_3), 1.20 (3H, s, 27-CH_3), 1.25–1.45 (12H, m), 1.50–1.85 (9H, m), 1.90–2.00 (3H, m), 2.11 (3H, s,

CH_3CO_2–), 3.38 (3H, s, –OCH_3), 3.59 (1H, s, 7β-H), 3.92 (4H, s, –OCH_2CH_2O–), 4.72 (1H, d, A of AB, *J*=6.9 Hz, –OCH_2O–), 4.76 (1H, d, B of AB, *J*=6.9 Hz, –OCH_2O–), 4.78 (1H, dd, *J*=10.2, 2.4 Hz, 24-H); ^{13}C NMR δ 171.26, 109.27, 96.01, 80.03, 75.17, 72.49, 64.06, 64.02, 55.70, 55.61, 49.96, 45.77, 42.47, 39.70, 39.35, 37.51, 36.35, 35.71, 35.44, 35.32, 33.40, 32.04, 31.13, 28.03, 26.82, 25.68, 24.85, 23.64, 21.06, 20.95, 18.42, 11.76, 10.49; HRMS calcd for $C_{33}H_{56}O_7$: 564.4026. Found 564.4004.

4.1.7. Δ^{25},7α-Methoxymethyl-3-dioxolane-5α-24*R*-hydroxy-cholesten-24-acetate (9). To a solution of **8** (908 mg, 1.61 mmol) and triethylamine (1.92 mL, 138.7 mmol) in dichloromethane (70 mL) stirring at 0°C under argon was added dropwise methanesulfonyl chloride (5.6 mL, 72.2 mmol). After 20 h, water (30 mL) was dropped in. The reaction mixture was extracted with dichloromethane (30×3 mL). The organic lay was washed with brine (20 mL), dried over sodium sulfate and concentrated in vacuo. The residue was purified by flash chromatography (pet. ether/acetone 4:1) to afford pure **9** (658 mg, 75%, white solid, 162 mg **8** was recovered); mp 104–108°C; $[\alpha]_D^{20}=-13$ (*c* 0.23, $CHCl_3$); MS-EI (*m/z*): 546 (M^+), 484 ($M^+-HOCH_2OCH_3$); IR (cm^{-1}): 1737 (CH_3CO_2), 1655 ($C{=}CH_2$), 902 ($C{=}CH_2$); ^1H NMR δ 0.63 (3H, s, 18-CH_3), 0.91 (3H, d, *J*=6.5 Hz, 21-CH_3), 0.81 (3H, s, 19-CH_3), 1.02–1.10 (4H, m), 1.20–1.60 (12H, m) 1.75–1.90 (8H, m), 1.92–2.00 (2H, m), 1.71 (3H, s, 27-CH_3), 2.06 (3H, s, 24-CH_3CO_2), 3.38 (3H, s, –OCH_3), 3.58 (1H, s, 7β-H), 3.92 (4H, s, –OCH_2CH_2O–), 4.64 (1H, d, A of AB, *J*=7.0 Hz, –OCH_2O–), 4.64 (1H, d, B of AB, *J*=7.0 Hz, –OCH_2O–), 4.90 (2H, d, *J*=7.0 Hz, 26-H), 5.11 (1H, t, *J*=6.5 Hz, 24-H); ^{13}C NMR δ 170.48, 143.51, 112.56, 109.34, 96.15, 77.71, 75.31, 64.18, 64.14, 55.78, 55.72, 50.09, 45.90, 42.60, 39.85, 39.45, 37.64, 36.48, 35.85, 35.56, 35.53, 33.53, 31.40, 31.26, 29.19, 28.19, 23.78, 21.31, 21.08, 18.71, 18.26, 11.87, 10.60; HRMS (ESI) calcd for $C_{33}H_{54}O_6$: 546.3920. Found 546.3955.

4.1.8. 7α-Methoxymethyl-3-dioxolane-5α-24*R*-hydroxy-cholestane (10). To a suspension of $NH_2OH\cdot HCl$ (3.59 g, 51.7 mmol) in DMF (10 mL) with stirring at 0°C was added KOH (85%, 3.41 g, 51.7 mmol). After being stirred for 30 min, the mixture was filtered and the solid was washed with DMF (ca. 2 mL). The combined filtrate was cooled to 0°C, and then ethyl acetate (2.22 mL, 22.6 mmol) was added dropwise. After being stirred for an additional 30 min, the solution was added dropwise to another flask containing **9** (413 mg, 0.756 mmol) with stirring at 90–95°C. After being stirred for 10 h, the mixture was cooled to room temperature, and water (15 mL) was added. The mixture was then extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with water (15 mL), 5% HCl (15 mL), sat. $NaHCO_3$ (20 mL), and brine (20 mL), respectively, and then was dried over sodium sulfate and evaporated to afford the crude product. It was dissolved in methanol (20 mL) containing water (1 mL) and KOH (400 mg) and refluxed for 5 h. After removal of solvents in vacuo, the residue was dissolved in ethyl acetate (50 mL), washed with water (2×10 mL) and brine (2×10 mL), respectively, and dried over sodium sulfate, evaporated and purified by flash chromatography (pet. ether/ethyl acetate 6:1) to afford pure **10** (329 mg, 86%, 100% d.e., white solid.); mp 110–112°C; $[\alpha]_D^{24}=-0.3$ (*c* 0.91, $CHCl_3$); MS-EI (*m/z*): 506 (M^+), 461 ($M^+-CH_2OCH_3$), 444 ($M^+-HOCH_2OCH_3$); IR (cm^{-1}): 3487 (OH); ^1H NMR δ 0.65 (3H, s, 18-CH_3), 0.68 (3H, d, *J*=6.8 Hz, 21-CH_3), 0.81 (3H, s, 19-CH_3), 1.01 (3H, s, 26-CH_3), 1.10–1.80 (22H, m), 1.90–2.10 (6H, m), 2.20–2.40 (3H, m), 3.33 (1H, t, *J*=4.5 Hz, 24-H), 3.35 (3H, s, OCH_3), 3.59 (1H, s, 7β-H), 3.92 (4H, s, –OCH_2CH_2O–), 4.61 (1H, d, A of AB, *J*=6.9 Hz, –OCH_2O–), 4.69 (1H, d, B of AB, *J*=6.9 Hz, –OCH_2O–); ^{13}C NMR δ 109.40, 96.14, 75.32, 64.18, 64.14, 56.01, 55.71, 50.09, 45.90, 42.61, 39.86, 39.48, 37.65, 36.48, 35.85, 35.83, 35.57, 33.63, 32.13, 31.26, 30.69, 29.78, 28.33, 23.80, 21.09, 19.00, 18.73, 17.32, 11.91, 10.60, 1.10; HRMS calcd for $C_{31}H_{54}O_5$: 506.3971. Found 506.3922.

4.1.9. 7α,24*R*-Dihydroxy-5α-cholestane-3-one (11). A mixture of **10** (300 mg, 0.593 mmol) and PPTS (400 mg, 1.6 mmol) in *t*-butanol (30 mL) was refluxed for 10 h. Removal of the solvent in vacuo and purification by flash chromatography (pet. ether/acetone 4:1) afforded pure **11** (white prism crystal, 228 mg, 92%); mp 149–151°C (lit.[7e] 151–153°C), $[\alpha]_D^{20}=+22.6$ (*c* 0.32, $CHCl_3$); IR (cm^{-1}): 3447 (–OH), 1707 (3-one); ^1H NMR δ 0.70 (3H, s, 18-CH_3), 0.91 (9H, m, 19-CH_3), 1.01 (3H, s), 1.10–1.70 (18H, m), 1.90–2.10 (7H, m), 2.20–2.30 (2H, m), 2.30–2.40 (2H, m), 3.32 (1H, m, 24-H) 3.86 (1H, m, 7β-H); MS-EI (*m/z*): 419 (4.8%, [M^++1]), 400 (24.2%, [M^+-H_2O]), 382 (33.4%, [M^+-2H_2O]); ^{13}C NMR δ 211.57, 77.97, 67.55, 56.05, 50.47, 45.24, 44.14, 42.68, 39.51, 39.44, 39.08, 38.16, 38.12, 36.57, 35.69, 33.61, 32.00, 30.92, 30.55, 28.24, 23.67, 21.22, 18.90, 18.60, 17.25, 11.88, 10.46; HRMS calcd for $C_{27}H_{46}O_3$: 418.3447. Found 418.3419.

4.1.10. 3β-[5,10Bis(*tert*-butoxycarbonyl)-1,5,10-triazadecyl]-5α-7α,24*R*-dihydroxy-cholestane (13) and 3α-[5,10-bis(*tert*-butoxycarbonyl)-1,5,10-triazadecyl]-5α-7α,24*R*-dihydroxy-cholestane (14). A mixture of compound **11** (32 mg, 0.076 mmol), amino compound **12** (95 mg, 0.28 mmol) and 3 Å molecular sieves (0.35 g) in absolute methanol (3 mL) was stirred for 18 h at room temperature under argon. $NaBH_4$ (40 mg) was added and the solution stirred for 4 h at −78°C. Then acetic acid (20 mL) was dropped until pH=7.0 to quench the reaction. The mixture was filtered through celite, the cake was washed well with MeOH and CH_2Cl_2. Removal of the solvent in vacuo and purification by flash chromatography (pet. ether/ethyl acetate/triethyl amine 6:4:1) afforded 3β-compound **13** (38 mg, 66%, colorless oil) and 3α-compound **14** (6 mg, 10%, colorless oil).

Compound **13**. ^1H NMR δ 0.66 (3H, s, 18-CH_3), 0.79 (3H, s, 19-CH_3), 0.91 (9H, m, 21-, 26-, 27-CH_3), 1.02–1.80 (50H, m, including 18H of BOC), 1.90–2.00 (3H, m), 3.1–3.4 (7H, m, NCH and 3α-H), 3.48 (1H, t, *J*=6.0 Hz), 3.54 (1H, t, *J*=4.5 Hz), 3.73 (1H, t, *J*=4.4 Hz), 3.82 (1H, br); ^1H NMR (CD_3OD) δ 0.74 (3H, s, 18-CH_3), 0.87 (3H, s, 19-CH_3), 0.93 (3H, s), 0.95 (3H, s), 0.98 (3H, d, *J*=6 Hz), 1.12–1.60 (44H, m, including 18H of BOC), 1.72–1.90 (7H, m), 1.92–2.10 (2H, m), 3.06–3.26 (9H, m, NCH), 3.82 (1H, br, 7β-H); ^{13}C NMR δ 156.00, 79.34, 76.82, 76.68, 71.83, 71.03, 67.70, 61.59, 57.23, 56.03, 50.55, 46.55, 45.84, 42.60, 40.15, 39.54, 39.48, 37.45, 37.06, 36.55, 36.00, 35.69, 34.36, 33.56, 32.04, 30.50, 29.61, 28.38, 28.20, 27.36, 23.57, 20.86, 19.24, 18.88, 18.57, 17.29, 11.82, 11.20. MS (ESI): 770.8 [M+Na^+], 748.8 [M+H^+], 747.8 [M^+]; HRMS (ESI) calcd for $C_{44}H_{82}N_3O_6$: 748.6203. Found 748.6187.

Compound **14**. ^{1}H NMR δ 0.66 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 0.92 (9H, m, 21-, 26-, 27-CH_3), 1.12–1.80 (44H, m, including 18H of BOC), 1.92–2.10 (3H, m), 2.62–2.70 (3H, m), 3.05–3.35 (9H, m, NCH and 3β-H), 3.49 (1H, t, *J*=6.2 Hz), 3.54 (1H, t, *J*=4.5 Hz), 3.75 (1H, t, *J*=4.6 Hz), 3.81 (1H, br); ^{13}C NMR δ 125.51, 67.79, 61.89, 56.07, 50.63, 46.60, 42.57, 39.65, 39.50, 35.76, 33.57, 32.05, 31.93, 30.61, 30.33, 29.70, 29.66, 28.42, 28.21, 27.38, 23.61, 18.90, 18.62, 17.26, 14.86, 11.89, 10.51; MS (ESI): 770.8 [M+Na^+], 748.8 [M+H^+], 747.8 [M^+]; HRMS (ESI) calcd for $C_{44}H_{82}N_3O_6$: 748.6203. Found 748.6172.

4.1.11. 3β-(1,5,10-Triazadecyl)-5α-7α,24*R*-dihydroxy-cholestane trihydrochloride (15). Compound **13** (29 mg, 0.04 mmol) was dissolved in a solution of HCl in MeOH (dry HCl gas was dissolved in 6 mL MeOH until pH <1). The solution was stirred about 29 h at room temperature. The solvent was removed in vacuo. The desired product **15** as the salt of HCl (white solid, 23 mg, 90.7%) was obtained, it was pure from TLC detection and could be used without further purification. ^{1}H NMR (CD_3OD) δ 0. 74 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 0.92 (3H, s), 0.95 (3H, s), 0.98 (3H, d, *J*=6 Hz), 0.99–1.11 (3H, m), 1.15–1.20 (3H, m), 1.20–1.50 (13H, m), 1.60–1.80 (8H, m), 1.80–1.90 (3H, m), 2.02–2.10 (12H, m), 3.03–3.25 (9H, m, NCH), 3.83 (1H, br, 7β-H); ^{13}C NMR (CD_3OD) δ 77.79, 68.34, 58.94, 57.64, 51.70, 46.75, 46.09, 43.67, 42.96, 40.92, 40.12, 38.52, 37.62, 37.08, 36.78, 34.91, 33.38, 32.02, 31.49, 30.78, 29.32, 25.94, 25.64, 24.43, 24.47, 24.32, 22.05, 19.48, 19.21, 18.03, 12.31, 11.54; HRMS (ESI) calcd for $C_{34}H_{66}N_3O_2$: 548.5155. Found 548.5150.

4.1.12. Squalamine (1). Compound **15** (19 mg, 0.035) and SO_3–pyridine complex (11 mg, 0.07 mmol, 2 equiv.) was added to a flask, flushed with argon. Dry pyridine (1 mL) was added, and the solution was warmed to 40°C in an oil bath and stirred 2 h. MeOH (1 mL) was added to quench the reaction. The flask was removed from the oil bath, and the mixture was stirred for 15 min. The solution was concentrated in vacuo, and the residue was resuspended in MeOH and filtered through a pad of celite. Flash chromatography (CH_2Cl_2/MeOH/NH_4OH 14:4:1) gave the desired product **1** (10.0 mg, 55%, white solid). MS/ESI: 628.6 $[M+1]^+$; ^{1}H NMR (CD_3OD) δ 0.75 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 0.96 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.10–1.3 (8H, m), 1.40–1.70 (10H, m), 1.80–2.00 (8H, m), 2.10–2.30 (5H, m), 3.04 (2H, t, *J*=7.4 Hz, NCH), 3.11–3.25 (7H, m, NCH), 3.84 (1H, s, 7β-H), 4.13 (1H, dd, *J*=10.0 Hz, 24H); ^{13}C NMR (CD_3OD) δ 86.60, 68.52, 59.20, 57.57, 51.93, 48.50, 46.86, 46.13, 43.88, 43.11, 41.00, 40.24, 38.70, 37.92, 37.59, 37.38, 36.99, 32.70, 32.17, 32.09, 29.28, 28.02, 26.01, 25.74, 24.61, 24.50, 24.39, 22.16, 19.56, 18.58, 18.44, 12.58, 11.79; HRMS (ESI) calcd for $C_{34}H_{66}N_3O_5S$: 628.4723. Found 628.4718.

Acknowledgements

We thank Professor Li-Jun Xia for performing the HPLC analysis. We thank Mr Ji-Ming Sheng and Ms Qun-Yan Pan for preparation of the starting material, and Mr Wei Gong for performing the ^{1}H ^{1}H COSY and ^{13}C NMR spectroscopy.

References

1. Stone, R. *Science* **1993**, *259*, 1125.
2. (a) Moore, K. S.; Wehrli, S.; Roder, H.; Rogers, M.; Forrest, J. N.; McCrimmon, J. D.; Zasloff, M. *Proc. Natl Acad. Sci. USA* **1993**, *90*, 1354–1358. (b) Wehrli, S. L.; Moore, K. S.; Roder, H.; Durell, S.; Zasloff, M. *Steroids* **1993**, *58*, 370–378.
3. (a) Moriarty, R. M.; Enache, L. A.; Kiney, W. A.; Allen, C. S.; Canary, J. W.; Tuladhar, S. M.; Guo, L. *Tetrahedron Lett.* **1995**, *36*, 5139–5142. (b) Moriarty, R. M.; Tuladhar, S. M.; Guo, L.; Wehrli, S. *Tetrahedron Lett.* **1994**, *35*, 8103–8106.
4. (a) Sills, Jr. A. K.; Williams, J. I.; Tyler, B. M.; Epstein, D. S.; Sipos, E. P.; Davis, J. D.; McLane, M. P.; Pitchford, S.; Cheshire, K.; Gannon, F. H.; Kinney, W. A.; Chao, T. L.; Donowitz, M.; Laterra, J.; Zasloff, M.; Brem, H. *Cancer Res.* **1998**, *58*, 2784–2792. (b) Hinde, A.; Ramster, B. *Drug Discovery Today* **2000**, *5*, 489–491. (c) Senior, K. *Drug Discovery Today* **2000**, *5*, 267–268. (d) Williams, J. I.; Weitman, S.; Gonzalez, C. M.; Jundt, C. H.; Marty, J.; Stringer, S. D.; Holroyd, K. J.; Mclane, M. P.; Chen, Q.; Zasloff, M.; Von-Hoff, D. D. *Clin. Cancer Res.* **2001**, *7*, 724–733.
5. Rao, M. N.; Shinnar, A. E.; Noecker, L. A.; Chao, T. L.; Feibush, B.; Snyder, B.; Sharkansky, I.; Sarkahian, A.; Zhang, X. H.; Jones, S. R.; Kinney, W. A.; Zasloff, M. *J. Nat. Prod.* **2000**, *63*, 631–635.
6. Pechulis, A. D.; Bellevuell, C. L. C.; Trapp, S. G.; Fojtik, J. P.; McKitty, A. A.; Frye, L. L. *J. Org. Chem.* **1995**, *60*, 5121–5126.
7. (a) Rao, M. N.; McGuigan, M. A.; Zhang, X. H.; Ze'ev, S.; Kinney, W. A.; Bulliard, M.; Laboue, B.; Lee, N. E. *J. Org. Chem.* **1997**, *62*, 4541–4545. (b) Jones, S. R.; Selinsky, B. S.; Rao, M. N.; Zhang, X. H.; Kinney, W. A.; Tham, F. S. *J. Org. Chem.* **1998**, *63*, 3786–3789. (c) Zhang, X. H.; Rao, M. N.; Jones, S. R.; Shao, B.; Feibush, P.; McGuigan, M.; Tzodikov, N.; Feibush, B.; Sharkansky, I.; Snyder, B.; Mallis, L. M.; Sarkahian, A.; Wilder, S.; Turse, J. E.; Kinney, W. A. *J. Org.Chem.* **1998**, *63*, 8599–8603. (d) Weis, A. L.; Bakos, T.; Alferiev, I.; Zhang, X. H.; Shao, B.; Kinney, W. A.; Williams, A. *Tetrahedron Lett.* **1999**, *40*, 4863–4864. (e) Kinney, W. A.; Zhang, X. H.; Williams, J. I.; Johnston, S.; Michalak, R. S.; DeshPande, M.; Dostal, L.; Rosazza, J. P. N. *Org. Lett.* **2000**, *2*, 2921–2922.
8. (a) Kolb, H. C.; van Nieumenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483–2549. (b) Zhou, X. D.; Zhou, W. S. Chinese Patent ZL99124007.3.
9. Zhou, X. D.; Cai, F.; Zhou, W. S. *Tetrahedron Lett.* **2001**, *42*, 2537–2539.
10. Iida, T.; Nishida, S.; Chang, F. C.; Niwa, T.; Goto, J.; Nambara, T. *Chem. Pharm. Bull.* **1993**, *41*, 763–765.
11. Unpublished work.
12. Sharpless dihydroxylation of (22*E*)-3α,5-cyclo-5α-ergost-22-en-6-one with DHQD-PHN in 1:1 *t*-BuOH–H_2O proceeded in 4–6 days to give a mixture of (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy and (22*S*,23*S*,24*R*)-2α,3α,22, 23-tetrahydroxy

compounds in 78% yield and with 93:7 diastereo-selectivity Huang, L. F.; Zhou, W. S.; Sun, L. Q.; Pan, X. F. *J. Chem. Soc., Perkin Trans. 1* **1993**, *29*, 1683–1686.
13. Sharpless dihydroxylation of desmosterol with $(DHQD)_2$-PYDZ in 1.5:1 *t*-BuOH–H_2O proceeded in 52 h to give 24(*R*), 25-dihydroxycholesterol in 83% yield and with 96:4 diastereoselectivity Corey, E. J.; Grogan, M. J. *Tetrahedron Lett.* **1998**, *39*, 9351–9354.
14. Wade, P. A.; Amin, N. V. *Synth. Commun.* **1982**, *12*, 287.
15. Goodnow, Jr. R.; Konno, K.; Niwa, M.; Niwa, M.; Kallimopoulos, T.; Bukownik, R.; Lenares, D.; Nakanishi, K. *Tetrahedron* **1990**, *46*, 3267–3286.