Tetrahedron 71 (2015) 4296–4303

Contents lists available at ScienceDirect

Tetrahedron

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

Asymmetric total synthesis of three stereoisomers of (−)-renieramycin G and their cytotoxic activities

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ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 17 April 2015
Accepted 20 April 2015
Available online 6 May 2015

Keywords:
(−)-Renieramycin G
Stereoisomers
Cytotoxicity
Topological configuration

ABSTRACT

(−)-Renieramycin G, a marine antitumor natural product, is a typical member of the bis-tetrahydroisoquinoline alkaloid family. In this paper, an efficient protocol of asymmetric total synthesis of its three stereoisomers, (+)-renieramycin G, 11,13-*epi*-(+)-renieramycin G and 11,13-*epi*-(−)-renieramycin G was established by the use of a combination of L- and/or D-tyrosine as the starting materials. The preliminary cytotoxicity assays tested on human cancer cell lines revealed that the L-shaped topological configuration of (−)-renieramycin G played a critical role in its cytotoxic activity.

1. Introduction

The pharmacological activities of chiral drugs depend mainly on their interaction with drug targets, most of which are also chiral such as antibodies, enzymes, DNA. The stereoisomers of chiral drugs, possessing complex three-dimensional structures, may display different biological and pharmacological activities in chiral living organisms.[1–3] Possessing several chiral centers, bis-tetrahydroisoquinoline alkaloids such as ecteinascidin 743, saframycin A, renieramycin G, and jorumycin, have attracted considerable interest due to their unique structures and potent biological activities.[4–6] Generally, hemiaminal or aminonitrile at C-21 position are believed to be the essential functional groups for their potent biological activities through the formation of covalent bonds to DNA and possibly other biomacromolecules (Fig. 1).[4]

However, (−)-renieramycin G, a typical member of bis-tetrahydroisoquinoline alkaloids, still retained moderate cytotoxicity (MIC: 0.5 and 1.0 μg/mL against KB and LoVo cell lines, respectively) despite lacking the essential functional groups at C-21 position.[7] Interestingly, Williams and co-workers reported that 3-*epi*-(−)-jorumycin exhibited an approximate 2–3 orders of magnitude decrease in the cytotoxicity compared with natural product (−)-jorumycin, while 3-*epi*-(−)-renieramycin G and (−)-renieramycin G displayed equipotent cytotoxicity activities against the A549 and HCT-116 cell lines.[8a] This finding attracted us to investigate the stereochemical effect on the antitumor activity of (−)-renieramycin G, which would be helpful to further understand the mechanisms of its cytotoxic effect. To date, six total synthesis of (−)-renieramycin G have been reported,[8–13] but less attention has

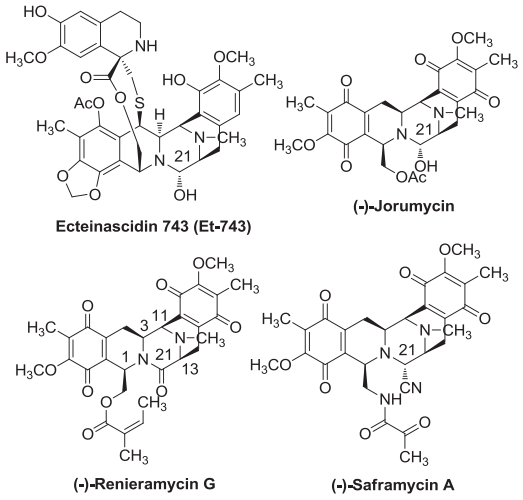

Fig. 1. Structures of the bis-tetrahydroisoquinoline alkaloids.

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http://dx.doi.org/10.1016/j.tet.2015.04.064
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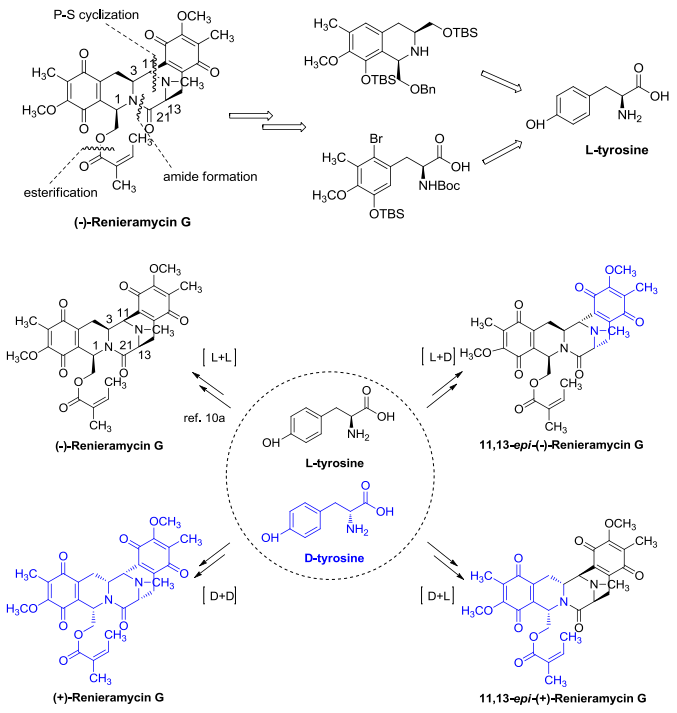
been paid to the synthesis of its stereoisomers. Williams et al. have firstly developed a convergent asymmetric synthesis of 3-*epi*-(−)-jorumycin and 3-*epi*-(−)-renieramycin G.[8b] Lemaire has reported the synthesis of 3-*epi*-pentacyclic framework using bis-tetrahydroisoquinoline as a key intermediate.[14] In addition, Saito[12] and Fukuyama[15] have described the synthesis of 1-*epi*-pentacyclic framework.

Previously, we reported an efficient approach to asymmetrically synthesize the bis-tetrahydroisoquinoline alkaloids including (−)-renieramycin G, (−)-saframycin A, and (−)-jorumycin and their analogs using L-tyrosine as the chiral starting material.[10] Apparently, this methodology can be extended to the synthesis of the stereoisomers of the bis-tetrahydroisoquinoline alkaloids simply by the combinatorial use of L- and/or D-tyrosine as the chiral starting materials (Scheme 1). It should be noted that although there would theoretically exist eight pairs of stereoisomers of (−)-renieramycin G, which bears four chiral centers, four pairs could actually be achieved since the intramolecular Pictet–Spengler reaction afforded solely the 11,13-cis-diastereomer as is required by the rigidity of the pentacyclic skeleton. Herein we would report the synthesis and cytotoxicity of three stereoisomers of (−)-renieramycin G, namely (+)-renieramycin G, 11,13-*epi*-(+)-renieramycin G, and 11,13-*epi*- (−)-renieramycin G. Furthermore, the preliminary stereochemical effect on the antitumor activity of the renieramycin G isomers was disclosed.



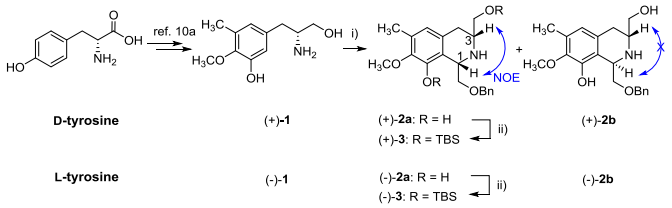
Scheme 1. Retro-synthesis of (−)-renieramycin G[10a] and the synthesis of its three stereoisomers by the combinatorial use of L- and/or D-tyrosine as the chiral starting materials.

2. Results and discussion

2.1. Synthesis of 1,3-*cis*-tetrahydroisoquinoline (+)/(−)-2a

The synthetic routes basically followed our previously reported procedures.[10] In this work, 1,3-*cis*-tetrahydroisoquinoline (+)/(−)-**2a**, prepared from L or D-tyrosine was used to synthesize the three stereoisomers of (−)-renieramycin G. The highly diastereoselective Pictet–Spengler cyclization reaction afforded a pair of diastereomers (**2a**/**2b**=7:1). Tetrahydroisoquinolines (+)/(−)-**2a** were then transformed into the corresponding TBS ethers (+)/(−)-**3** with TBSCl and imidazole in DMF. The absolute stereochemistry of the C-1 carbon of (+)/(−)-**2a**/**2b** was determined by their NOESY spectra.

A strong correlation was observed between C-1 H and C-3 H in NOESY thus indicating a cis relationship of the C-1 H and C-3 H in compounds (+)/(−)-**2a**. In contrast, the NOE effect between C-1 H and C-3 H in the trans isomer (+)/(−)-**2b** was absent (Scheme 2). The absolute configurations of compounds (+)/(−)-**2a**/**2b** were further confirmed through their circular dichroism (CD) spectra, which showed perfect mirror images, respectively (Fig. 2).



Scheme 2. Synthesis of compounds (+)/(−)-**3**: (i) $BnOCH_2CHO$, HOAc, 4 Å M.S., CH_2Cl_2/TFE (7:1), 0 °C, 7 h; (ii) TBSCl, imidazole, DMF, rt, 12 h.

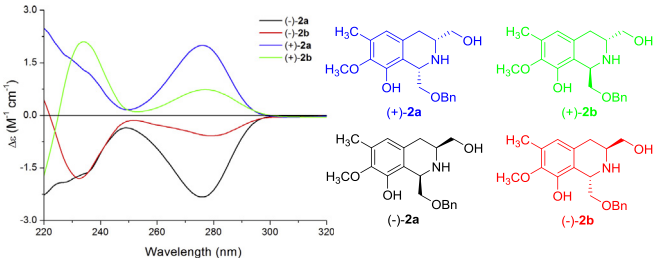


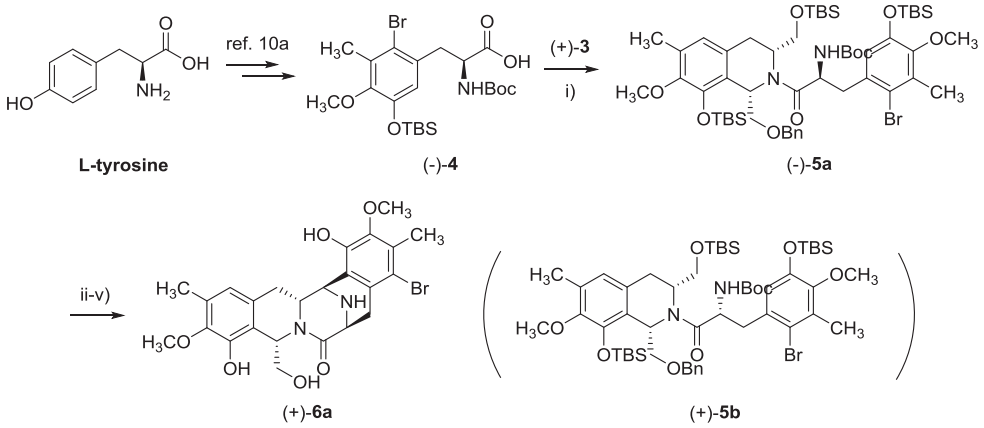
Fig. 2. CD spectra of compounds (+)/(−)-**2a**/**2b**.

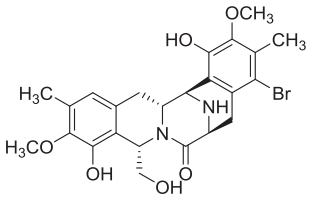
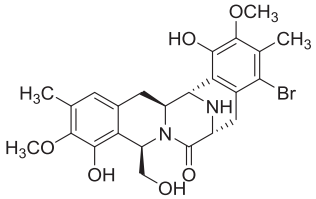
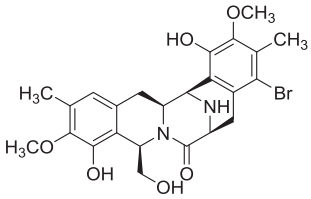
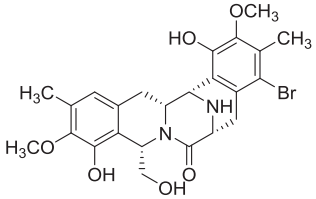
2.2. Synthesis of the pentacyclic skeletons (+)/(−)-6a/6b

Tyrosine derivatives (+)/(−)-**4** were obtained as illustrated in our previous synthesis of (−)-renieramycin G.[10a] Condensation of tetrahydroisoquinoline precursors (+)/(−)-**3** with tyrosine derivatives (+)/(−)-**4** in the presence of BOPCl and Et_3N gave four chiral amides (+)/(−)-**5a**/**5b**. Subsequent deprotection of the TBS ethers followed by oxidation with Dess–Martin periodinane and then removal of the aryl TBS group with TBAF gave the cyclization precursors. Transformation of the cyclization precursors into pentacyclic frameworks was achieved by the intramolecular Pictet–Spengler condensation reaction. Treatment of the cyclization precursors with trifluoromethanesulfonic acid (TFSA) afforded the corresponding pentacyclic frameworks (+)/(−)-**6a**/**6b** as single stereoisomers (Scheme 3).[10]

2.3. Synthesis of the stereoisomers of (−)-renieramycin G

With the pentacyclic frameworks (+)/(−)-**6a**/**6b** in hand, efforts were then directed toward the synthesis of the stereoisomers of (−)-renieramycin G. Reductive methylation of compounds (+)/(−)-**6a** and (+)-**6b** with 37% aqueous formaldehyde solution and sodium cyanoborohydride in the presence of acetic acid gave compounds (+)/(−)-**7a** and (+)-**7b**, respectively, which were subsequently subjected to catalytic hydrogenation via H_2 (50 psi) and $Pd(OH)_2$/C to afford alcohols (+)/(−)-**8a** and (+)-**8b**. Salcomine oxidation of (−)-**8a** gave quinone **9** in 71% yield. Acylation of the primary alcohol group with angelic acid was achieved under mild Yamaguchi conditions to afford the stereoisomer of 11,13-*epi*-(−)-renieramycin G in 59% yield. In order to improve the synthetic efficiency of the last two steps, we adopted a different sequence



Coupling partners	product	Coupling partners	product
(+)-**3** (−)-**4**	 (+)-**6a**	(−)-**3** (+)-**4**	 (-)-**6a**
(−)-**3** (−)-**4**	 (-)-**6b**[10d]	(+)-**3** (+)-**4**	 (+)-**6b**

Scheme 3. Synthesis of pentacyclic frameworks (+)/(−)-**6a**/**6b**: (i) BOPCl, Et_3N, CH_2Cl_2, rt, 72 h, 86%; (ii) HCOOH, H_2O, THF, rt, 10 h, 90%; (iii) Dess–Martin periodinane, CH_2Cl_2, rt, 2 h, 91%; (iv) TBAF, THF, 0 °C, 1 h, 90%; (v) TFSA, rt, 2 h, 80%.

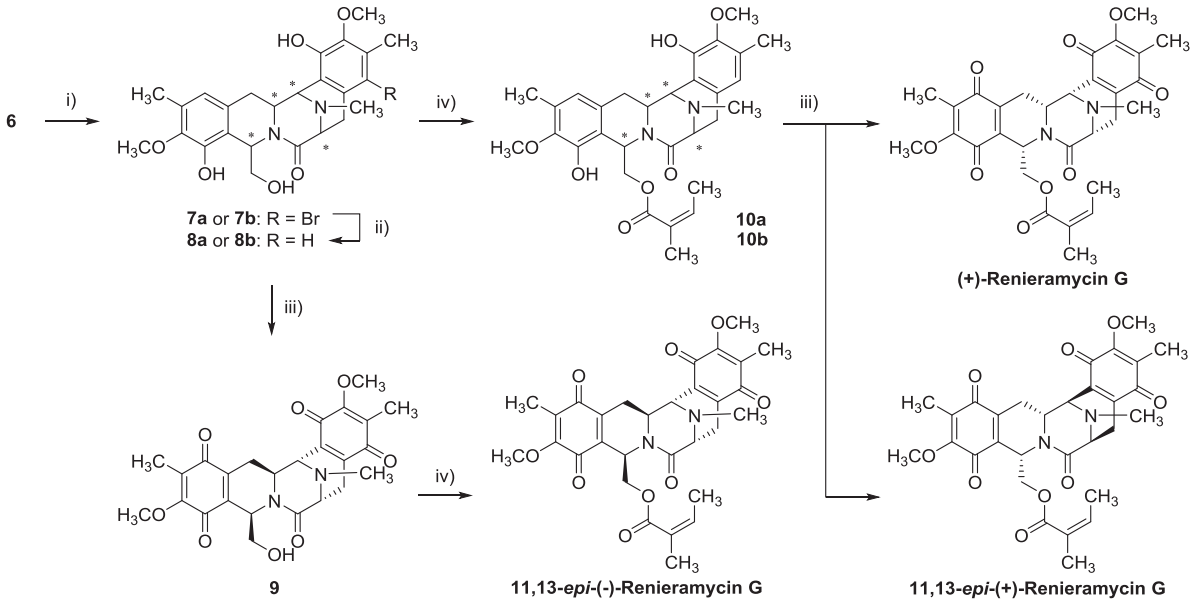
for the synthesis of the other two stereoisomers, namely converting alcohols **8** into the angelica esters before oxidation of the phenol units. Thus, selective acylation of alcohols (+)-**8a**/**8b** with angelic acid, followed by oxidation, gave the stereoisomers 11,13-*epi*-(+)-renieramycin G and (+)-renieramycin G, respectively (Scheme 4). CD spectra were used to determine the absolute configurations of (−)-renieramycin G and its three stereoisomers. As expected for the two pairs of enantiomers, the CD traces exhibited excellent mirror-image relationships, respectively (Fig. 3). The measured optical rotations for the two pairs of (+)/(−)-renieramycin G and 11,13-*epi*-(+)/(−)-renieramycin G were found to be close to each other in the absolute value but with opposite signs.

2.4. Biological evaluations

The cytotoxicity of (−)-renieramycin G and its three stereoisomers against the HCT-8, HELA, A549, KB, BGC-803 cell lines were tested by the standard MTT method (Table 1). Preliminary results indicated that (+)-renieramycin G possessed similar cytotoxicity to that of the natural product (−)-renieramycin G. The pair of 11,13-*epi*-isomers were substantially less cytotoxic and, particularly, 11,13-*epi*-(+)-renieramycin G showed nearly no cytotoxic effects, which implied the importance of the stereochemistry factor for the antitumor activity of these renieramycin G stereoisomers. The stable configuration of the natural product (−)-renieramycin G and the synthetic isomer 11,13-*epi*-(+)-renieramycin G was optimized by DFT calculations. It showed that the topological configuration of the pentacyclic skeleton of (+)/(−)-renieramycin G was L-shaped while that of 11,13-*epi*-isomers are S-shaped (Fig. 4). It is thus reasonable to believe that the decrease of 11,13-*epi*-isomers in their cytotoxic activities might be attributed to the changes of topological configuration of the pentacyclic skeleton from the L-shaped to the S-shaped. Interestingly, a similar study of Williams' group found that the antiproliferative activities of (−)-renieramycin G and 3-*epi*-(−)-renieramycin G against HCT-116 and A549 cancer cell lines were nearly the same. It is in full agreement with our conclusion in that the L-shaped topological configuration of 3-*epi*-(−)-renieramycin G remained almost unchanged despite the C-3 epimerization, which was proved by the X-ray analysis.[8]

3. Conclusions

In summary, a systematic and efficient protocol was established for the synthesis of the stereoisomers of (−)-renieramycin G through the combinatorial use of L- and/or D-tyrosine as the chiral starting materials. The utility was illustrated by the total asymmetric synthesis of three stereoisomers of (−)-renieramycin G, namely (+)-renieramycin G, 11,13-*epi*-(−)-renieramycin G, and 11,13-*epi*-(+)-renieramycin G. This methodology could also be



Scheme 4. Reagents and conditions: (i) HCHO, $NaBH_3CN$, HOAc, CH_3OH, rt, 2 h; (ii) H_2 (50 psi), $Pd(OH)_2/C$, CH_3OH, 12 h; (iii) salcomine, CH_3CN, rt, 2 h; (iv) angelic acid, 2,4,6-trichlorobenzoyl chloride, Et_3N, toluene, 90 °C, 12 h.

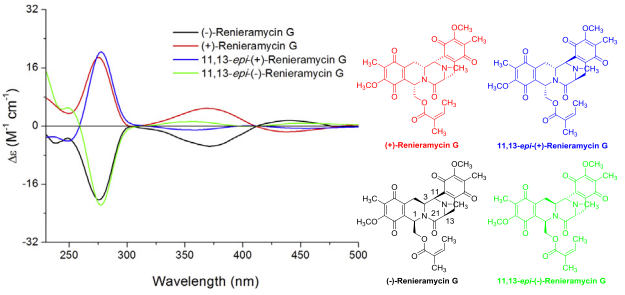


Fig. 3. CD traces of (+)/(−)-renieramycin G and 11,13-*epi*-(+)/(−)-renieramycin G.

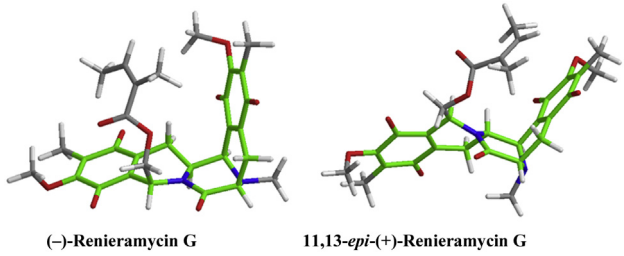


Fig. 4. The stable conformers for (−)-renieramycin G and 11,13-*epi*-(+)-renieramycin G were optimized by DFT calculations.

extended to the asymmetric synthesis of other stereoisomers and analogs of bis-tetrahydroisoquinoline alkaloids. Besides, it was revealed for the first time that the L-shaped topological configuration of the pentacyclic skeleton was of critical importance for the cytotoxicity of renieramycin G stereoisomers.

4. Experimental section

4.1. General

Unless otherwise stated, solvents and reagents were obtained from commercial suppliers and used without further purification. Molecular sieves were activated at 450 °C for 10 h and stored under nitrogen. Anhydrous CH_2Cl_2, THF, Et_3N, DMF, and toluene were distilled and dried over 4 Å molecular sieves prior to use. Optical rotations were measured with a PerkinElmer Polarimeter 341LC at 589 nm and 20 °C. High-resolution mass spectra (HRMS) were obtained on an Agilent LC/MSD TOF or Thermo Exactive Plus spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on Varian Mercury 300, Varian Mercury 400, Bruker AV 500 or Varian INOVA 600 MHz spectrometers as indicated.

4.2. (1*S*,3*R*)-(+)-2a and (1*R*,3*R*)-(+)-2b

To a solution of (+)-**1** (630 mg, 3.0 mmol), HOAc (0.44 mL, 7.5 mmol), and 4 Å molecular sieves (0.60 g) in CH_2Cl_2/TFE (7:1, 12 mL) at 0 °C was added benzyloxyacetaldehyde (1.0 M in CH_2Cl_2, 3.3 mL) dropwise over 60 min under argon. The reaction mixture was then stirred for 6 h at 0 °C and subsequently diluted with

Table 1
Cytotoxic activity of (−)-renieramycin G and its three stereoisomers

Compounds	IC_{50} (μM) values in indicated cell lines[a]				
	HCT-8	HELA	A549	KB	BGC-803
(−)-Renieramycin G	8.91±0.43	3.88±0.32	5.70±0.27	3.50±0.15	5.37±0.36
(+)-Renieramycin G	10.92±0.57	6.92±0.34	9.46±0.43	5.23±0.23	21.73±1.51
11,13-*epi*-(−)-Renieramycin G	84.87±2.76	49.73±2.32	80.96±3.38	35.54±2.75	71.90±3.55
11,13-*epi*-(+)-Renieramycin G	>100	>100	>100	>100	>100
Cisplatin	2.92±0.33	1.43±0.15	1.42±0.03	3.23±0.30	0.83±0.05

[a] Mean value±SD (standard deviation from three experiments).

CH_2Cl_2. The mixture was filtered and the organic phase was washed with saturated aq $NaHCO_3$, dried, and concentrated in vacuo to give a residue that was subjected to chromatography on silica gel (CH_2Cl_2/MeOH=100:2) to give (1*S*,3*R*)-(+)-**2a** as a white solid (750 mg, 73.2%) and (1*R*,3*R*)-(+)-**2b** (100 mg, 9.7%) as a white solid.

*(1S,3R)-(+)-**2a***: mp 132–134 °C; $[\alpha]_D^{20}$ +125 (*c* 2.0, CH_3OH); HRMS calcd for $C_{20}H_{26}NO_4$ $[M+H]^+$ 344.1856, found 344.1846; ^1H NMR (400 MHz, DMSO-d_6): δ 8.65 (s, 1H), 7.42–7.22 (m, 5H), 6.37 (s, 1H), 4.70 (t, *J*=4.8 Hz, 1H), 4.52 (d, *J*=12.4 Hz, 1H), 4.47 (d, *J*=12.4 Hz, 1H), 4.31 (d, *J*=6.0 Hz, 1H), 4.13 (dd, *J*=8.4, 2.4 Hz, 1H), 3.59 (s, 3H), 3.51–3.38 (m, 2H), 3.38–3.30 (m, 1H), 2.69 (m, 1H), 2.46–2.38 (m, 1H), 2.34–2.24 (m, 1H), 2.14 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 146.7, 143.8, 138.7, 132.6, 128.1 (2C), 128.0, 127.3 (2C), 127.2, 121.1, 121.0, 73.9, 72.1, 65.2, 59.9, 53.9, 53.0, 33.0, 15.4.

*(1R,3S)-(−)-**2a***: $[\alpha]_D^{20}$ −102 (*c* 3.0, CH_3OH).

*(1R,3R)-(+)-**2b***: mp 179–181 °C; $[\alpha]_D^{20}$ +0.83 (*c* 1.2, CH_3OH); HRMS calcd for $C_{20}H_{26}NO_4$ $[M+H]^+$ 344.1856, found 344.1847; ^1H NMR (400 MHz, CD_3OD): δ 7.45–7.25 (m, 5H), 6.50 (s, 1H), 4.63 (s, 2H), 4.26 (dd, *J*=10.0, 3.2 Hz, 1H), 3.71 (m, 1H), 3.70 (s, 3H), 3.61 (t, *J*=10.4 Hz, 1H), 3.50 (dd, *J*=10.4, 3.2 Hz, 1H), 3.42 (dd, *J*=10.8, 8.4 Hz, 1H), 3.27 (m, 1H), 2.61 (dd, *J*=16.4, 4.4 Hz, 1H), 2.40 (dd, *J*=16.4, 11.6 Hz, 1H), 2.13 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD): δ 150.0, 146.0, 139.7, 131.4, 129.6, 129.5 (2C), 129.0 (2C), 128.8, 124.9, 115.4, 74.1, 69.6, 66.9, 60.4, 54.7, 48.9, 31.9, 11.6.

*(1S,3S)-(−)-**2b***: $[\alpha]_D^{20}$ −0.67 (*c* 1.5, CH_3OH).

4.3. (1*S*,3*R*)-(+)-3

To a solution of (1*S*,3*R*)-(+)-**2a** (5.0 g, 15.58 mmol) in anhydrous DMF (15 mL) were added imidazole (7.93 g, 116.62 mmol) and TBSCl (8.74 g, 58.27 mmol). The reaction mixture was stirred at room temperature for 24 h. It was then quenched with water and extracted with EtOAc (40 mL×3). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product. Purification by chromatography on silica gel (PET/EtOAc=10:1) give (1*S*,3*R*)-(+)-**3** (7.25 g, 87.1%) as a colorless oil. $[\alpha]_D^{20}$ +58.6 (*c* 3.8, $CHCl_3$); HRMS calcd for $C_{32}H_{54}NO_4Si_2$ $[M+H]^+$ 572.3586, found 572.3581; ^1H NMR (300 MHz, $CDCl_3$): δ 7.28–7.26 (m, 5H), 6.57 (s, 1H), 4.51 (d, *J*=11.7 Hz, 1H), 4.45 (d, *J*=11.7 Hz, 1H), 4.49–4.47 (m, 1H), 4.16 (d, *J*=8.7 Hz, 1H), 3.75–3.68 (m, 2H), 3.62 (s, 3H), 3.62–3.58 (m, 1H), 2.84 (s, 1H), 2.62–2.50 (m, 2H), 2.22 (s, 3H), 0.95 (s, 9H), 0.90 (s, 9H), 0.24 (s, 3H), 0.08 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 147.7, 145.9, 138.7, 133.0, 129.5, 128.2 (2C), 127.6 (2C), 127.3, 125.6, 124.1, 73.4, 73.2, 67.0, 59.9, 54.2, 53.9, 33.3, 26.2 (2C), 25.9 (2C), 25.6, 18.6, 18.3, 15.7, −3.6, −3.7, −4.4, −5.3, −5.3.

*(1R,3S)-(−)-**3***: $[\alpha]_D^{20}$ −47.8 (*c* 7.4, $CHCl_3$).

4.4. (*R*)-(+)-4

(*R*)-(+)-**4** was prepared as previously described.[10a] $[\alpha]_D^{20}$ +12.9 (*c* 10.0, $CHCl_3$); HRMS calcd for $C_{44}H_{72}N_2O_{12}Si_2Br_2Na$ $[2M+Na]^+$ 1057.2883, found 1057.2881; ^1H NMR (500 MHz, $CDCl_3$): δ 6.64 (s, 1H), 4.74 (m, 1H), 3.73 (s, 3H), 3.46 (m, 1H), 2.76 (m, 1H), 2.36 (s, 3H), 1.39 (s, 3H), 1.14 (s, 3H), 1.00 (s, 6H), 0.91 (s, 6H), 0.18 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.6, 131.4, 121.9, 121.0, 119.3, 81.0, 60.1, 53.5, 41.4, 38.4, 28.2, 27.9, 18.2, 17.9, 16.8, −3.6, −4.6.

*(S)-(−)-**4***: $[\alpha]_D^{20}$ −9.4 (*c* 1.2, CH_3OH).[10a]

4.5. Amide (−)-5a

To a solution of (1*S*,3*R*)-(+)-**3** (8.30 g, 14.50 mmol) in anhydrous CH_2Cl_2 (200 mL) were added Et_3N (5.20 mL, 37.70 mmol), and (*S*)-(−)-**4** (9.74 g, 18.85 mmol), followed by BOPCl (4.80 g, 18.85 mmol) in two batches. The resulting mixture was then warmed to ambient temperature and stirred for 72 h. The reaction mixture was quenched with 1 N aq HCl (20 mL) and diluted with CH_2Cl_2 (20 mL). The organic phase was separated, washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (PET/EtOAc=20:1) to afford amide (−)-**5a** (13.37 g, 86.0%) as a colorless oil. $[\alpha]_D^{20}$ −2.0 (*c* 0.1, CH_3OH); HRMS calcd for $C_{54}H_{88}BrN_2O_9Si_3$ $[M+H]^+$ 1071.4976, found 1071.4988; ^1H NMR (500 MHz, $CDCl_3$): δ 7.30–7.16 (m, 5H), 6.63 (s, 1H), 6.57 (s, 1H), 6.23 (m, 1H), 5.57 (s, 1H), 5.25–5.17 (m, 2H), 4.57 (m, 1H), 4.52–4.45 (m, 2H), 4.32 (m, 1H), 3.88 (m, 1H), 3.65 (s, 3H), 3.62 (s, 3H), 3.22 (m, 1H), 3.10 (m, 1H), 2.92 (m, 2H), 2.73 (m, 1H), 2.35 (s, 1H), 2.25 (s, 3H), 2.23 (s, 3H), 1.44 (s, 9H), 1.26 (s, 9H), 1.02 (s, 18H), 0.32 (s, 3H), 0.19 (s, 3H), 0.18 (s, 3H), 0.13 (s, 3H), 0.07 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 171.2, 154.8, 154.5, 148.8, 148.7, 147.8, 145.3, 138.6, 132.5, 131.6, 131.3, 128.4, 128.2, 127.4, 127.3, 127.2 124.1, 123.3, 121.9, 119.5, 79.0, 72.9, 70.8, 64.9, 64.7, 60.2, 59.9, 52.6, 51.9, 49.6, 41.4, 37.4, 29.6, 29.4, 28.0, 26.5, 26.2, 25.9 (3C), 25.6 (3C), 18.7, 18.2, 18.1, 16.9, 15.9, −3.7, −4.2, −4.5, −5.3, −5.5.

4.6. Amide (−)-5b

Following the general reaction protocol, (1*S*,3*R*)-(+)-**3** (7.50 g, 13.13 mmol) was reacted with BOPCl (4.34 g, 17.08 mmol), Et_3N (4.70 mL, 34.06 mmol), and (*R*)-(+)-**4** (8.80 g, 17.03 mmol) in CH_2Cl_2 (180 mL), which provided amide (−)-**5b** (11.31 g, 80.5%) as a colorless oil. $[\alpha]_D^{20}$ −10.2 (*c* 1.5, $CHCl_3$); HRMS calcd for $C_{54}H_{88}BrN_2O_9Si_3$ $[M+H]^+$ 1071.4976, found 1071.4978; ^1H NMR (500 MHz, $CDCl_3$): δ 7.32–7.16 (m, 5H), 6.63 (s, 1H), 6.59 (s, 1H), 6.09 (dd, *J*=6.5, 4.0 Hz, 1H), 5.53 (d, *J*=8.5 Hz, 1H), 5.08 (dd, *J*=14.0, 9.0 Hz, 1H), 4.55 (d, *J*=12.0 Hz, 1H), 4.37 (d, *J*=12.0 Hz, 1H), 3.73–3.67 (m, 2H), 3.64 (s, 3H), 3.62 (s, 3H), 2.99 (dd, *J*=13.5, 5.0 Hz, 1H), 2.92–2.85 (m, 1H), 2.76 (dd, *J*=13.0, 9.0 Hz, 1H), 2.65 (dd, *J*=16.0, 7.0 Hz, 1H), 2.36–2.32 (m, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 1.32 (s, 6H), 1.22 (s, 3H), 1.02 (s, 9H), 0.98 (s, 9H), 0.92 (s, 3H), 0.85 (s, 6H), 0.27 (s, 3H), 0.18 (s, 3H), 0.14 (s, 3H), 0.10 (s, 3H), 0.003 (s, 3H), −0.043 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.5, 154.5, 149.0, 147.8, 147.4, 145.2, 138.7, 132.6, 132.0, 130.4, 128.7, 128.3, 127.5, 127.2, 127.1, 124.4, 123.5, 123.3, 121.9, 119.4, 78.7, 72.8, 71.9, 65.6, 60.3, 60.0, 59.8, 52.6, 50.6, 49.1, 41.3, 29.6, 28.2, 26.2 (3C), 25.9 (3C), 25.6 (3C), 18.7, 18.2, 16.9, 15.8, 14.2, −0.02, −3.6, −3.7, −4.2, −4.5, −4.6, −5.3.

4.7. General protocol for the preparation of pentacyclic framework (1*S*,3*R*,11*R*,13*S*)-(+)-6a

(1) The starting amide (−)-**5a** (8.60 g, 8.00 mmol) was dissolved in THF/HCOOH/H_2O (6:3:1, 130 mL) and stirred at room temperature for 10 h. The mixture was then concentrated in vacuo, dissolved in EtOAc, washed with saturated aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and re-concentrated in vacuo. Flash chromatography on silica gel (PET/EtOAc=8:1) afforded primary alcohol (6.90 g, 89.8%) as a white solid.

(2) To a solution of the primary alcohol (4.10 g, 4.28 mmol) from the previous step dissolved in CH_2Cl_2 (100 mL) was added Dess–Martin periodinane (3.20 g, 7.52 mmol). The reaction mixture was stirred at room temperature for 2 h under argon and then washed with saturated aq $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel (PET/EtOAc=10:1) afforded the hemiaminal product (3.70 g, 90.5%) as a white solid.

(3) To a solution of the hemiaminal product (1.83 g, 1.91 mmol) in THF (40 mL) at 0 °C was added TBAF (1.0 M in THF, 5.73 mL). The reaction mixture was stirred at 0 °C for 1 h and then quenched with saturated aq NH_4Cl and extracted with EtOAc (40 mL×3). The combined organic phase was washed with brine, dried

over anhydrous Na_2SO_4, and concentrated in vacuo to provide the crude product. Flash chromatography on silica gel (PET/EtOAc=1:1) afforded the cyclization precursor (1.25 g, 89.9%) as a white solid.

(4) To a solution of the cyclization precursor (970 mg, 1.33 mmol), under argon, was added trifluoromethanesulfonic acid (8 mL). The reaction mixture was stirred for 2 h at room temperature and quenched with saturated aq $NaHCO_3$ and extracted with EtOAc (20 mL×3). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH=100:2) to afford (1*S*,3*R*,11*R*,13*S*)-(+)-**6a** (550 mg, 79.6%) as a white solid. Mp 183–185 °C; $[\alpha]_D^{20}$ +198 (*c* 0.5, CH_3OH); HRMS calcd for $C_{24}H_{28}BrN_2O_6$ $[M+H]^+$ 519.1125, found 519.1125; ^1H NMR (500 MHz, DMSO-d_6): δ 9.20 (s, 1H), 8.79 (s, 1H), 6.47 (s, 1H), 5.00 (s, 1H), 4.41 (dd, *J*=8.5, 5.0 Hz, 1H), 4.32 (s, 1H), 4.20 (ddd, *J*=11.0, 4.5, 2.5 Hz, 1H), 3.78 (s, 1H), 3.58 (s, 3H), 3.55 (s, 3H), 3.32–3.29 (m, 1H), 3.21 (t, *J*=13.0 Hz, 1H), 2.94 (d, *J*=11.5 Hz, 1H), 2.77–2.73 (m, 2H), 2.60 (d, *J*=13.0 Hz, 1H), 2.22 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.8, 145.9, 145.1, 144.7, 144.2, 134.9, 129.3, 128.1, 127.0, 120.4, 119.9, 115.7, 61.9, 61.0, 60.5, 59.9, 54.8, 54.6, 53.6, 49.5, 36.3, 32.2, 16.2, 15.3.

*(1R,3S,11S,13R)-(−)-**6a***: $[\alpha]_D^{20}$ −171.2 (*c* 2.6, CH_3OH).

4.8. (1*S*,3*R*,11*S*,13*R*)-(+)-6b

Following general protocol for step 1, the starting amide (−)-**5b** (7.40 g, 6.92 mmol) dissolved in THF–HCOOH–H_2O (6:3:1, 110 mL) and stirred at room temperature for 10 h. Workup and purification provided the pure alcohol (5.63 g, 84.9%) as a white solid.

Following the general protocol for step 2, the primary alcohol (5.20 g, 5.43 mmol) was dissolved in CH_2Cl_2 (130 mL) and reacted with Dess–Martin periodinane (4.03 g, 9.50 mmol) for 2 h. Workup and purification provided the hemiaminal product (4.31 g, 83.0%) as a white solid.

Following the general protocol for step 3, the hemiaminal product (2.10 g, 2.20 mmol) was dissolved in THF (46 mL) and reacted with TBAF (1.0M in THF, 6.58 mL) for 1 h. Workup as in general procedure afforded the cyclization precursor (1.45 g, 90.6%) as a white solid.

Following the general protocol for step 4, the cyclization precursor (1.20 g, 1.65 mmol) was treated with trifluoromethanesulfonic acid (10 mL) under argon and warmed to rt over 2 h. Workup and purification afforded (1*S*,3*R*,11*S*,13*R*)-(+)-**6b** (710 mg, 83.1%) as a white solid. Mp 165–167 °C; $[\alpha]_D^{20}$ +81.1 (*c* 2.6, CH_3OH); HRMS calcd for $C_{24}H_{28}BrN_2O_6$ $[M+H]^+$ 519.1125, found 519.1100; ^1H NMR (500 MHz, DMSO-d_6): δ 9.21 (s, 1H), 8.71 (s, 1H), 6.46 (s, 1H), 5.47 (t, *J*=5.0 Hz, 1H), 4.44 (s, 1H), 4.32 (t, *J*=5.0 Hz, 1H), 3.81–3.76 (m, 2H), 3.62 (s, 3H), 3.61 (s, 3H), 3.19 (m, 1H), 3.05 (m, 1H), 2.95–2.91 (m, 1H), 2.83 (m, 2H), 2.36–2.31 (m, 1H), 2.28 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.2, 146.4, 146.0, 144.5, 144.4, 132.8, 129.8, 129.7, 128.9, 122.0, 119.8, 119.6, 116.0, 63.5, 61.0, 60.3, 59.8, 53.2, 50.5, 48.3, 36.0, 32.1, 16.5, 15.5.

4.9. (1*S*,3*R*,11*R*,13*S*)-(+)-7a

To a stirred solution of (1*S*,3*R*,11*R*,13*S*)-(+)-**6a** (425 mg, 0.82 mmol) in 40 mL CH_3OH were added HCHO (37%, 4.40 mL), $NaBH_3CN$ (520 mg, 8.2 mmol), and HOAc (7.90 mL). The reaction mixture was stirred for 2 h at room temperature and concentrated in vacuo. The residue was dissolved in EtOAc, washed with saturated aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and re-concentrated in vacuo. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH=100:1) to afford (1*S*,3*R*,11*R*,13*S*)-(+)-**7a** (414 mg, 94.8%) as a white solid. Mp 138–140 °C; $[\alpha]_D^{20}$ +153.0 (*c* 0.1, CH_3OH); HRMS calcd for $C_{25}H_{30}BrN_2O_6$ $[M+H]^+$ 533.1282, found 533.1282; ^1H NMR (500 MHz, $CDCl_3$): δ 6.52 (s, 1H), 5.20 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.99 (d, *J*=11.0 Hz, 1H), 3.90 (d, *J*=5.5 Hz, 1H), 3.79 (dd, *J*=11.5, 4.0 Hz, 1H), 3.71 (s, 3H), 3.65 (s, 3H), 3.35 (t, *J*=13.0 Hz, 1H), 3.21 (d, *J*=11.5 Hz, 1H), 2.98–2.88 (m, 2H), 2.80 (d, *J*=14.5 Hz, 1H), 2.45 (s, 3H), 2.32 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.6, 145.7, 145.0, 144.2, 144.1, 133.5, 130.2, 129.0, 128.0, 121.7, 121.3, 119.5, 117.7, 68.1, 62.5, 61.2, 60.7, 60.1, 58.7, 56.1, 39.3, 36.8, 26.2, 16.6, 15.7.

*(1R,3S,11S,13R)-(−)-**7a***: $[\alpha]_D^{20}$ −148.2 (*c* 53.9, CH_2Cl_2).

4.10. (1*S*,3*R*,11*S*,13*R*)-(+)-7b

Following the general reaction protocol (1*S*,3*R*,11*S*,13*R*)-(+)-**6b** (500 mg, 0.97 mmol) was reacted with HCHO (37%, 5.18 mL), $NaBH_3CN$ (612 mg, 9.71 mmol), and HOAc (9.29 mL) in CH_3OH (47 mL). Workup and purification afforded (1*S*,3*R*,11*S*,13*R*)-(+)-**7b** (460 mg, 89.6%) as a white solid. Mp 155–157 °C; $[\alpha]_D^{20}$ +55.7 (*c* 5.3, CH_2Cl_2); HRMS calcd for $C_{25}H_{30}BrN_2O_6$ $[M+H]^+$ 533.1282, found 533.1292; ^1H NMR (400 MHz, $CDCl_3$): δ 6.51 (s, 1H), 6.17 (s, 1H), 6.05 (s, 1H), 5.79–5.73 (m, 1H), 4.31 (s, 1H), 4.04 (d, *J*=12.0 Hz, 1H), 3.81––3.72 (m, 7H), 3.46 (m, 1H), 3.38 (m, 1H), 3.12–2.96 (m, 3H), 2.42 (s, 3H), 2.39 (m, 1H), 2.37 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.7, 145.7, 145.5, 144.2, 143.9, 133.2, 130.8, 129.7, 129.5, 120.9 (2C), 118.2, 118.1, 68.2, 61.3, 60.7, 60.0, 58.9, 55.3, 52.7, 39.9, 31.8, 31.1, 16.8, 15.7.

4.11. (1*S*,3*R*,11*R*,13*S*)-(+)-8a

A solution of (1*S*,3*R*,11*R*,13*S*)-(+)-**7a** (361 mg, 0.68 mmol) in CH_3OH (30 mL), HOAc (2.0 mL), and $Pd(OH)_2$ (moist, Pd content 20%, 340 mg) were placed in a Parr apparatus. The vessel was filled with hydrogen gas at 50 psi and the reaction mixture was vigorously stirred for 10 h at room temperature. The mixture was filtered and concentrated in vacuo. The residue was dissolved in EtOAc, washed with saturated aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and re-concentrated in vacuo. The crude product was purified by column chromatography on silica gel (PET/EtOAc=1:2) to afford (1*S*,3*R*,11*R*,13*S*)-(+)-**8a** (287 mg, 93.2%) as a white solid. Mp 244–246 °C; $[\alpha]_D^{20}$ +115.0 (*c* 0.1, CH_3OH); HRMS calcd for $C_{25}H_{31}N_2O_6$ $[M+H]^+$ 455.2177, found 455.2190; ^1H NMR (500 MHz, DMSO-d_6): δ 8.83 (s, 1H), 8.77 (s, 1H), 6.46 (s, 1H), 6.39 (s, 1H), 5.01 (s, 1H), 4.50 (s, 1H), 4.14 (s, 1H), 4.08 (s, 1H), 3.58 (s, 3H), 3.54 (s, 3H), 3.40 (d, *J*=8.5 Hz, 1H), 3.31–3.26 (m, 2H), 2.98 (dd, *J*=17.5, 6.5 Hz, 1H), 2.89 (d, *J*=7.2 Hz, 1H), 2.66 (d, *J*=14.0 Hz, 1H), 2.56 (d, *J*=17.0 Hz, 1H), 2.42 (s, 3H),2.16 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (125 MHz, DMSO): δ 172.0, 146.2, 146.1, 144.2, 144.0, 135.3, 128.9, 128.2, 127.1, 122.7, 120.5, 119.8, 62.5, 61.0, 59.9, 59.8, 59.1, 55.4, 54.6, 37.1, 24.6, 15.4, 15.3.

(1*R*, 3*S*, 11*S*, 13*R*)-(−)-**8a**: $[\alpha]_D^{20}$ −92.8 (*c* 44.6, CH_2Cl_2).

4.12. (1*S*,3*R*,11*S*,13*R*)-(+)-8b

Following the general reaction protocol, (1*S*,3*R*,11*S*,13*R*)-(+)-**7b** (300 mg, 0.56 mmol) was reacted with $Pd(OH)_2$ (moist, Pd content 20%, 283 mg), HOAc (1.75 mL) and hydrogen gas at 50 psi in CH_3OH (25 mL), which provided (1*S*,3*R*,11*S*,13*R*)-(+)-**8b** (230 mg, 89.8%) as a white solid. Mp 147–149 °C; $[\alpha]_D^{20}$ +75.5 (*c* 2.0, CH_2Cl_2); HRMS calcd for $C_{25}H_{31}N_2O_6$ $[M+H]^+$ 455.2177, found 455.2173; ^1H NMR (400 MHz, $CDCl_3$): δ 6.52 (s, 1H), 6.50 (s, 1H), 6.23 (s, 1H), 6.04 (s, 1H), 5.81–5.75 (m, 1H), 4.30 (s, 1H), 4.07 (d, *J*=11.2 Hz, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 3.74–3.70 (m, 1H), 3.54–3.43 (m, 1H), 3.39–3.31 (m, 1H), 3.22 (dd, *J*=17.2, 6.8 Hz, 1H), 3.01 (dd, *J*=15.2, 2.0 Hz, 1H), 2.92 (d, *J*=17.6 Hz, 1H), 2.47 (s, 3H), 2.38 (m, 1H), 2.26 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 146.5, 145.5, 144.2, 143.5, 132.2,

129.5, 122.3 (2C), 120.9 (2C), 118.2, 68.0, 60.8, 60.7, 59.7, 58.7, 55.3, 52.7, 39.9, 31.7, 30.9, 15.8, 15.7.

4.13. (1*R*,3*S*,11*S*,13*R*)-(−)-9

To a solution of (1*R*,3*S*,11*S*,13*R*)-(−)-**8a** (14.6 mg, 0.032 mmol) in CH_3CN (4.0 mL) was added salcomine (19.5 mg, 0.06 mmol) at room temperature and the reaction mixture was stirred in air for 2 h. The mixture was filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (PET/EtOAc=1:1) to afford (1*R*,3*S*,11*S*,13*R*)-(−)-**9** (11.0 mg, 71.0%) as a yellow solid. $[\alpha]_D^{20}$ −256.2 (*c* 9.7, CH_2Cl_2); HRMS calcd for $C_{25}H_{27}N_2O_8$ $[M+H]^+$ 483.1762, found 483.1745; ^1H NMR (400 MHz, $CDCl_3$): δ 4.87 (s, 1H), 4.24–4.13 (m, 1H), 4.00 (s, 3H), 3.99 (s, 3H), 3.91 (s, 1H), 383–3.78 (m, 1H), 375–3.67 (m, 2H), 3.04 (d, *J*=16.4 Hz, 1H), 2.96 (d, *J*=11.6 Hz, 1H), 2.93–2.83 (m, 1H), 2.77–2.70 (m, 2H), 2.41 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 186.4, 185.8, 182.5, 181.4, 172.5, 155.8, 155.4, 143.2, 139.4, 137.8, 136.9, 129.5, 128.4, 63.7, 61.0, 59.2, 58.9, 58.2, 54.0, 39.2, 30.9, 29.6, 20.1, 8.8 (2C).

4.14. 11,13-*epi*-(−)-Renieramycin G

To a solution of angelica acid (6.6 mg, 0.066 mmol) in anhydrous toluene (1 mL) at 0 °C were added 2,4,6-trichlorobenzoyl chloride (11 μL, 0.07 mmol) and Et_3N (10 μL, 0.07 mmol) under argon. After stirring for 2 h at room temperature, the solution of (1*R*,3*S*,11*S*,13*R*)-(−)-**9** (15 mg, 0.031 mmol) in anhydrous toluene (4 mL) was added to the reaction mixture and then the reaction mixture was stirred for 48 h at 90 °C. After cooled to room temperature, the reaction mixture was quenched with saturated aq NH_4Cl, extracted with EtOAc, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel to afford *11,13-epi-(−)-renieramycin G* (10.4 mg, 59.3%) as a yellow solid. $[\alpha]_D^{20}$ −123.3 (*c* 0.6, CH_2Cl_2); HRMS calcd for $C_{30}H_{33}N_2O_9$ $[M+H]^+$ 565.2181, found 565.2185; ^1H NMR (400 MHz, $CDCl_3$): δ 6.03 (dq, *J*=7.2, 1.2 Hz, 1H), 5.22 (s, 1H), 5.13 (dd, *J*=12.0, 2.4 Hz, 1H), 4.61 (dd, *J*=12.0, 2.4 Hz, 1H), 4.06 (s, 3H), 4.00 (m, 1H), 3.98 (s, 3H), 3.84 (s, 1H), 3.13 (d, *J*=16.8 Hz, 1H), 2.95 (d, *J*=10.4 Hz, 1H), 2.85 (s, 2H), 2.71 (m, 1H), 2.56 (s, 3H), 1.94 (s, 6H), 1.84 (dq, *J*=7.2, 1.6 Hz, 3H), 1.74 (t, *J*=1.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 186.3, 185.4, 182.2, 180.6, 167.2, 156.4, 155.3, 142.9, 139.8, 139.2, 137.6, 137.0, 129.5, 127.9, 126.8, 61.0, 60.7, 58.8, 58.4, 53.9, 53.2, 39.8, 29.4, 21.6, 20.5, 15.5, 8.7 (2C).

4.15. (1*S*,3*R*,11*R*,13*S*)-(+)-10a

To a solution of angelica acid (16.5 mg, 0.165 mmol) in anhydrous toluene (1 mL) at 0 °C were added 2,4,6-trichlorobenzoyl chloride (25.8 μL, 0.165 mmol) and Et_3N (22.9 μL, 0.165 mmol) under argon. After stirring for 2 h at room temperature, the solution of (1*S*,3*R*,11*R*,13*S*)-(+)-**8a** (30 mg, 0.066 mmol) in anhydrous toluene (4 mL) was added to the reaction mixture and then the reaction mixture was stirred for 48 h at 90 °C. After cooled to room temperature, the reaction mixture was quenched with saturated aq NH_4Cl, extracted with EtOAc, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel to afford (1*S*,3*R*,11*R*,13*S*)-(+)-**10a** (26 mg, 73.4%) as a white solid. $[\alpha]_D^{20}$ +118.5 (*c* 2.0, CH_2Cl_2); HRMS calcd for $C_{30}H_{37}N_2O_7$ $[M+H]^+$ 537.2595, found 537.2609; ^1H NMR (300 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6.46 (s, 1H), 5.94–5.89 (m, 1H), 5.89 (s, 1H), 5.71 (s, 1H), 5.51 (s, 1H), 5.04 (d, *J*=11.7 Hz, 1H), 4.57 (d, *J*=10.8 Hz, 1H), 4.17 (s, 1H), 3.76–3.73 (m, 1H), 3.73 (s, 3H), 3.71 (s, 3H), 3.32 (t, *J*=12.9 Hz, 1H), 3.20 (d, *J*=12.0 Hz, 1H), 3.10 (dd, *J*=17.1, 6.6 Hz, 1H), 2.88–2.79 (m, 2H), 2.55 (s, 3H), 2.25 (s, 3H), 2.21 (s, 3H), 1.79 (d, *J*=7.5 Hz, 3H), 1.72 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.4, 145.5, 145.3, 143.7, 143.3, 137.4, 135.2, 128.6, 127.9, 122.0, 121.0, 118.2, 62.8, 62.3, 60.7 (2C), 59.9, 55.9, 52.2, 40.2, 37.3, 30.9, 29.7, 25.1, 20.4, 15.7, 15.4.

4.16. (1*S*,3*R*,11*S*,13*R*)-(+)-10b

Following the general reaction protocol, angelica acid (8.3 mg, 0.083 mmol) was activated by 2,4,6-trichloro-benzoyl chloride (13.0 μL, 0.083 mmol) and Et_3N (12.0 μL, 0.087 mmol) in anhydrous toluene (4 mL) and then reacted with (1*S*, 3*R*, 11*S*, 13*R*)-(+)-**8b** (15 mg, 0.033 mmol), which provided (1*S*,3*R*,11*S*,13*R*)-(+)-**10b** (10.2 mg, 57.6%) as a white solid. $[\alpha]_D^{20}$ +100.5 (*c* 4.0, CH_2Cl_2); HRMS calcd for $C_{30}H_{37}N_2O_7$ $[M+H]^+$ 537.2595, found 537.2593; ^1H NMR (300 MHz, $CDCl_3$): δ 6.56–6.44 (m, 2H), 5.94–5.70 (m, 4H), 4.33–4.21 (m, 3H), 4.00 (d, *J*=12.3 Hz, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 3.66 (d, *J*=6.9 Hz, 1H), 3.17 (dd, *J*=17.4, 7.2 Hz, 1H), 2.98 (m, 1H), 2.85 (m, 1H), 2.51 (m, 1H), 2.44 (s, 3H), 2.24 (s, 3H), 2.23 (s, 3H), 1.64 (d, *J*=7.2 Hz, 3H), 1.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.1, 146.3, 145.6, 143.8, 143.1, 133.4, 129.1, 127.5, 122.2, 122.1, 120.7 (2C), 117.5, 64.1, 60.6 (2C), 59.9, 58.2, 55.2, 49.1, 40.2, 32.0, 28.4, 20.0, 15.7 (2C), 15.3.

4.17. 11,13-*epi*-(+)-Renieramycin G

To a solution of (1*S*,3*R*,11*R*,13*S*)-(+)-**10a** (6.5 mg, 0.012 mmol) in CH_3CN (1.5 mL) was added salcomine (3.9 mg, 0.012 mmol) at room temperature and the reaction mixture was stirred in air for 2 h. The mixture was filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (PET/EtOAc=1:1) to afford 11,13-*epi-(+)-renieramycin G* (4.0 mg, 58.5%) as a yellow solid. $[\alpha]_D^{20}$ +122.0 (*c* 0.2, CH_2Cl_2); HRMS calcd for $C_{30}H_{33}N_2O_9$ $[M+H]^+$ 565.2181, found 565.2160; ^1H NMR (500 MHz, $CDCl_3$): δ 6.05–6.00 (m, 1H), 5.19 (s, 1H), 5.17 (s, 1H), 4.58 (dd, *J*=13.0, 3.0 Hz, 1H), 4.05 (s, 3H), 3.97 (s, 3H), 3.94 (s, 1H), 3.76–3.74 (m, 1H), 3.08 (d, *J*=17.0 Hz, 1H), 2.90 (dd, *J*=11.0, 2.0 Hz, 1H), 2.76–2.74 (m, 2H), 2.67 (ddd, *J*=17.0, 11.0, 2.0 Hz, 1H), 2.46 (s, 3H), 1.94 (s, 6H), 1.84 (dd, *J*=7.5, 1.5 Hz, 3H), 1.74 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 186.5, 185.6, 182.5, 180.7, 172.7, 167.2, 156.4, 155.4, 143.2, 139.6, 139.5, 138.0, 137.1, 129.4, 127.8, 126.9, 61.0 (2C), 60.7, 58.9, 58.6, 54.1, 53.2, 39.7, 29.5, 21.1, 20.5, 15.5, 8.7, 8.6.

4.18. (+)-Renieramycin G

Following the general reaction protocol, (1*S*,3*R*,11*S*,13*R*)-(+)-**10b** (6.0 mg, 0.011 mmol) was reacted with salcomine (8.0 mg, 0.025 mmol) in CH_3CN (4.0 mL). Workup and purification afforded (+)-renieramycin G (4.1 mg, 65.0%) as a yellow solid. $[\alpha]_D^{20}$ +141.7 (*c* 0.6, CH_2Cl_2); HRMS calcd for $C_{30}H_{33}N_2O_9$ $[M+H]^+$ 565.2181, found 565.2167; ^1H NMR (400 MHz, $CDCl_3$): δ 5.89 (dq, *J*=7.2, 1.2 Hz, 1H), 5.46 (d, *J*=1.6 Hz, 1H), 4.69 (dd, *J*=11.6, 2.4 Hz, 1H), 4.40 (dd, *J*=11.6, 2.4 Hz, 1H), 4.16 (br s, 1H), 4.05 (s, 3H), 4.00 (s, 3H), 3.90 (br s, 1H), 3.74 (d, *J*=5.2 Hz, 1H), 3.07 (dd, *J*=16.8, 2.4 Hz, 1H), 2.90 (dd, *J*=20.8, 6.4 Hz, 1H), 2.76 (d, *J*=20.4 Hz, 1H), 2.40 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.69 (dq, *J*=7.2, 1.6 Hz, 3H), 1.52 (t, *J*=1.6 Hz, 3H), 1.47 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 186.0, 185.1, 182.1, 180.1, 170.0, 166.8, 156.1, 155.4, 143.2, 141.9, 141.2, 139.7 (2C), 136.0, 129.0, 128.0, 126.5, 62.8 (2C), 61.0, 58.9, 55.8, 53.1, 50.3, 39.8, 25.4, 23.7, 20.3, 15.4, 8.7 (2C).

(−)-Renieramycin G: $[\alpha]_D^{20}$ −148.8 (*c* 0.2, CH_2Cl_2).[10a]

4.19. Cytotoxicity assay

Each sample was tested in vitro against five different cell lines, including HCT-8 (human colon cancer cell line), HELA (Hela human cervical cancer cell line), A549 (human lung adenocarcinoma

epithelial cell line), KB (human oral epidermoid carcinoma cell line), and BGC-803 (human gastric adenocarcinoma cell line). Exponentially growing cells were, respectively, seeded at 1500 cells per well and 3000 cells per well, into 96-well plates and precultured for one day. All tested samples were dissolved in DMSO to make a 20 mM stock solution and further diluted with the culture medium. The cell lines were treated with each sample at concentrations of tenfold dilution ranging from 100 to 0.1 μM and incubated for an additional 3 days. MTT assay was performed to measure the concentration required to inhibit cell growth by 50% (IC_{50} value) in triplicate. The results were measured with a TECAN absorbance microplate reader at 540 nm.

Acknowledgements

This research was financially supported by Beijing Key Laboratory of Active Substance Discovery and Druggability Evaluation.

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.04.064.

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