Chemistry of Renieramycins. Part 14: Total Synthesis of Renieramycin I and Practical Synthesis of Cribrostatin 4 (Renieramycin H)

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Abstract: The first total synthesis of (±)-renieramycin I, which was isolated from the Indian bright blue sponge Haliclona cribricuts, is described. The key step is the selenium oxide oxidation of pentacyclic bis-p-quinone derivative (3) stereo- and regioselectively. We also report a large-scale synthesis of cribrostatin 4 (renieramycin H) via the C3-C4 double bond formation in an early stage based on the Avendaño’s protocol, from readily available 1-acetyl-3-(3-methyl-2,4,5-trimethylphenyl)methyl-piperazine-2,5-dione (8) in 18 steps (8.3% overall yield). The synthesis provides unambiguous evidence supporting the original structure of renieramycin I.

Keywords: cribrostatin 4 (renieramycin H); renieramycin I; total synthesis; structural determination; selenium oxide oxidation; marine natural product

1. Introduction

Many tetrahydroisoquinoline antitumor natural products, such as renieramycins, saframycins, and euteinascidins, have attracted considerable interest due to their extraordinary structures and meager availability in nature, as well as their potent antitumor activity [1,2]. Among them, renieramycins H (1h) and I (1i) were isolated from the methanol extract of the Indian bright blue sponge Haliclona...
cribricutis collected from the intertidal region of Okha, Gujarat, in 1988 [3]. Original structures 1h and 1i were given the names renieramycins H and I, respectively. Thereafter, we revised the structure of renieramycin H to that of cribrostatin 4 (2) [4–6], which was independently isolated from the blue sponge Cribrochalina sp. collected from reef passages in the Republic of Maldives, based on 13C NMR studies of several semi-synthetic models (Figure 1) [7,8]. Cribrostatin 4 (2) has attracted the interest of several medicinal chemistry experts because of its unique structure and cytotoxicity despite the lack of the hemiaminal or aminonitrile function at C-21. Three total syntheses of 2 have been reported [9–11]. Recently, we completed a 21-step stereocontrolled total synthesis of (+)-2 from 1-acetyl-3-(3-methyl-2,4,5-trimethylphenyl)methyl-piperazine-2,5-dione (8) in 3.4% overall yield [12,13]. Furthermore, we have accomplished the total synthesis of renieramycin G (1g) [14,15]. The availability of 1g and 2 has enabled us to prepare several renieramycin derivatives having a lactam carbonyl to understand the molecular basis of their impressive cytotoxicity profiles. We present herein an alternative large-scale approach for the total synthesis of 2. This approach might yield a variety of novel analogs of cribrostatin 4 (2), as well as C3-C4 unsaturated bis-p-quinone derivatives, such as renieramycin I (1i), for detailed studies of structure activity relationships (SARs) of these classes of antitumor marine natural products.

![Figure 1](image-url) Structures of bis-1,2,3,4-tetrahydroisoquinoline marine natural products.

2. Results

The most serious problem in our previous cribrostatin 4 (2) synthesis was that 1-epi-pentacyclic alcohol (4) (Chart 1) might be formed, and the undesired stereochemistry had to be converted into the natural one at C-1 position via enolate formation through several cycles. Avendaño et al. reported that the stereocenter at C-3 of 1,3-trans-compound 5 [16] could be transformed into corresponding 1,3-cis-compound 7 via unsaturated compound 6 through regioselective radical bromination, followed by hydrogenation from the less hindered α-face in good yield [17]. They applied this protocol to the preparation of pentacyclic phthalascidin analogs [18]. We were very interested in this procedure for constructing the 1,3-cis relationships of renieramycins (Scheme 1) [19].
Scheme 1. Epimerization at C-3 through regioselective bromination at C-3 position and reduction sequences by Avendaño and co-workers.

Based on Avendaño’s protocol, we designed an alternative synthetic plan that involves the key transformations outlined in Scheme 2: (1) construction of tricyclic compound 9 having an $\alpha,\beta$-unsaturated amide carbonyl from readily available compound 8 [20]; (2) condensation of 9 with benzaldehyde derivative and subsequent regio- and stereospecific hydrogenation leading to compound 11; (3) construction of pentacyclic framework and conversion of ester into our intermediate 12, which can be transformed into cribrostatin 4 intermediate 3 [13] (Scheme 2).

Scheme 2. Strategy for practical synthesis of compound 3, which will be converted into 1i and 2.

According to the results of our previous studies [21,22], treatment of 8 with trimethylsilyl chloride (TMSCl) in the presence of triethylamine (TEA) in CH$_2$C1$_2$ gave O-trimethylsilyl lactim intermediate 14, which was treated with 2,2-diethoxyethyl benzoate in the presence of trimethylsilyl
triflate (TMSOTf) and acetic anhydride to give **15** as an inseparable mixture of diastereomers (**15a**: **15b** = 10:3) in 92% yield. After exerting a great deal of effort to separate this mixture by column chromatography several times, we obtained both isomers in their pure forms, and detailed 2D NMR studies confirmed the structures of **15a** (minor) and **15b** (major). The NMR spectrum of **15a** displayed H-1 and H-3 proton signals at δ 6.20 and δ 4.70, respectively, whereas the NMR spectrum of **15b** showed H-1 and H-3 proton signals appearing at δ 6.15 and δ 4.07, respectively. An observable nuclear Overhauser enhancement (NOE) between H-3 and H-22 revealed that compound **15a** has the trans form (Scheme 3).

We then studied the conversion of **15** into unsaturated compound **9**, which is the first key step of our synthesis. A preliminary experiment was carried out using major isomer **15a**. According to the typical conditions of Avendaño et al. [17], **15a** was treated with N-bromosuccinimide (NBS: 1.0 equiv.) and 2,2′-azobisisobutyronitrile (AIBN: 0.1 equiv.) in CCl₄ at 80 °C for 6 h to generate **9** (55%) and **16** (4%) plus unreacted **15a** (33%). The ¹H NMR spectrum of **9** showed an H-4 olefinic proton signal that appeared as a singlet at δ 7.47. The ¹H NMR spectrum of **16** showed characteristic AB type doublet proton signals at δ 4.60 and δ 4.57 along with the H-4 olefinic singlet proton signal at δ 7.41. Accordingly, **16** might be a product of over-reaction product at C-6 aromatic methyl group. After extensive investigation of the reaction conditions, we found that the yield of our target **9** could be improved by slightly lowering the reaction temperature (60 °C) and excluding AIBN. Thus, the reaction of **15a** with NBS (2 equiv.) in CCl₄ at 60 °C for 6 h gave **9** (69%) and **16** (13%). It was extremely difficult to separate **9** and **16** in a large scale using silica gel column chromatography. However, catalytic reduction of the above mixture using 10% Pd/C in 2-propanol and DMF at 25 °C for 11 h gave **9** as the sole product in 82% overall yield. Accordingly, the transformation of **15a** into **9** without any purification of the intermediates was found to be the best choice in terms of overall yield (**9** in 71% yield in four steps).

With key intermediate **9** in hand, we next looked into ways to design a practical transformation of **9** into **12**, which was the key intermediate in our previous total synthesis of cribrostatin 4 (2) (Schemes 4 and 5). Condensation of **9** with benzaldehyde derivative **17** [20] in the presence of potassium tert-butoxide gave (Z)-arylidene-piperazinedione **10** in 70% yield. Catalytic hydrogenation of the
trisubstituted double bond of 10 over 10% Pd on carbon in MeOH at 25 °C proceeded chemoselectively to give desired 11a (72%) along with 11b (21%). Detailed 2D NMR studies were performed to confirm the structures of 11a and 11b. The NMR spectrum of 11a displayed H-1 and H-13 proton signals at δ 6.53 and δ 4.34, respectively, whereas the NMR spectrum of 11b had H-1 and H-13 proton signals appearing δ 6.43 and δ 4.45, respectively. An NOE between H-1 and H-13 proton signals was observed in 11a but not 11b. Thus, the hydrogenation of 10 obviously occurred stereoselectively from the α-face to generate H-1 and H-13 cis isomer 11a. It is proposed that the steric hindrance due to the C-8 methoxy group and the C-21 carbonyl group was responsible for the β-axial orientation of C-1 substituent as shown in conformer X of 10.

Scheme 4. Preparation of key intermediate 11a.

The piperazinedione ring of 11a was activated by introducing a 2-propyloxycarbonyl group to give imide 18 in 96% yield. Chemoselective reduction of 18 in the conventional manner afforded a hemiaminal, which was treated with formic acid at 25 °C for 0.5 h to afford 19 [23] in 82% yield. Deprotection of 19 with TFA and H2SO4 gave secondary amine 20, which was transformed into 21 by reductive methylation in high yield. Hydrolysis of 21 with 10 N aqueous LiOH in THF/MeOH at 25 °C for 8 h gave primary alcohol 12 in 97% yield, which is identical to the intermediate in our previous total synthesis [13].

Scheme 5. Construction of pentacyclic primary alcohol 12.
The conversion of 12 into 22 was accomplished by partial demethylation with boron tribromide (BBr₃), followed by oxidative demethylation to give bis-p-quinone 22 in 67% yield (Scheme 6). Acylation of 22 with in situ prepared angeloyl chloride in dichloromethane gave common intermediate 23 in 84% yield. Encouraged by the results of our extensive model studies, including the transformation of several natural products [24–26], the introduction of a methoxy group to the C-14 position of 23 was achieved using 10 equiv. of SeO₂ in a mixture of methanol and dioxane at 100 °C for six days to give 1i in 43% yield along with secondary alcohol 24 in 29% yield. The orientation of the methoxy group of 1i was assigned on the basis of the signal of 14-H (δ 4.34, d, J = 1.4 Hz). The spectroscopic properties of synthetic 1i were in complete accord with those of natural renieramycin I (1i) [27].

Scheme 6. Transformation of compound 12 into renieramycin I (1i) and cribrostatin 4 (2)
through compound 23.

3. Experimental Section

IR spectra were obtained with a Shimadzu Prestige 21/IRAffinity-1 FT-IR spectrometer. ¹H- and ¹³C-NMR spectra were recorded on a JEOL JNM-ECA 500 FT NMR spectrometer at 500 MHz for ¹H and 125 MHz for ¹³C; a JEOL JNM-AL 400 NMR spectrometer at 400 MHz for ¹H and 100 MHz for ¹³C; and a JEOL JNM-AL 300 NMR spectrometer at 300 MHz for ¹H and 75 MHz for ¹³C (ppm, J in Hz with TMS as internal standard). All proton and carbon signals were assigned by extensive NMR measurements using COSY, HMBC, and HMQC techniques. Mass spectra were recorded on a JEOL JMS 700 instrument with a direct inlet system operating at 70 eV. Elemental analyses were conducted on a YANACO MT-6 CHN CORDER elemental analyzer.
3.1. ((6R*,11aR*)-2-Acetyl-7,8,10-trimethoxy-9-methyl-1,4-dioxo-1,3,4,6,11,11a-hexahydro-2H-pyrazino-(1,2-b)isoquinolin-6-yl)methyl Benzoate (15a) and ((6R*,11aS*)-2-acetyl-7,8,10-trimethoxy-9-methyl-1,4-dioxo-1,3,4,6,11,11a-hexahydro-2H-pyrazino(1,2-b)isoquinolin-6-yl)methyl Benzoate (15b)

TMSCl (498 μL, 3.9 mmol) was added to a stirred solution of 8 (1.05 g, 3.0 mmol) in dichloromethane (18 mL) and TEA (544 μL, 3.9 mmol), and stirring was continued at 25 °C for 2 h. A solution of 2,2-diethoxyethyl benzoate [28] (785.8 mg, 3.3 mmol) in dichloromethane (12 mL) followed by TMSOTf (2.71 mL, 15 mmol) was added dropwise for 5 min each, and then Ac₂O (283.6 μL, 3.0 mmol) was added in one portion at 25 °C and the reaction mixture was stirred for 4 h. The reaction mixture was diluted with saturated NaHCO₃ solution (100 mL) and extracted with CHCl₃ (100 mL × 3). The combined extracts were washed with brine (100 mL), dried, and concentrated in vacuo. The residue was subjected to column chromatography with ethyl acetate–hexane (1:2) to give 15 (1.37 g, 92%, 15a:15b = 10:3) as a colorless amorphous powder, which was an inseparable mixture of diastereomers. Each authentic sample was obtained by chromatography on preparative layer silica gel plates (Merck 5715).

Compound 15a: 1H-NMR (400 MHz, CDCl₃) δ: 7.99 (2H, m, Ar-H), 7.57 (1H, m, Ar-H), 7.44 (2H, m, Ar-H), 6.20 (1H, dd, J = 9.5, 3.9 Hz, C6-H), 4.75 (1H, dd, J = 11.7, 9.5 Hz, C12-H), 4.70 (1H, dd, J = 9.8, 5.4 Hz, C11a-H), 4.52 (1H, dd, J = 11.7, 9.3 Hz, C12-H), 4.36 (1H, d, J = 18.0 Hz, C3-H), 4.31 (1H, d, J = 18.0, C3-H), 3.95 (3H, s, C7-OMe), 3.80 (3H, s, C8-OMe), 3.72 (3H, s, C10-OMe), 3.40 (1H, dd, J = 16.6, 5.4 Hz, C11-Hα), 3.14 (1H, dd, J = 16.6, 9.8 Hz, C11-Hβ), 2.59 (3H, s, COCH₃), 2.21 (3H, s, C9-Me). 13C-NMR (100 MHz, CDCl₃) δ: 171.7 (s, COCH₃), 167.9 (s, C1), 166.6 (s, OCOPh), 163.2 (s, C4), 152.4 (s, C10), 150.6 (s, C8), 146.2 (s, C7), 133.3 (d, Ph), 129.7 (d, Ph × 2), 129.6 (s, Ph), 128.5 (d, Ph × 2), 125.9 (s, C9), 122.6 (s, C6a), 121.4 (s, C10a), 63.6 (t, C12), 60.6 (q, C7-OCH₃), 60.3 (q, C10-OCH₃), 60.0 (q, C8-OCH₃), 54.0 (t, C11a), 48.3 (d, C6), 45.7 (t, C3), 27.2 (q, COCH₃), 60.0 (q, C9-CH₃). FT-IR (KBr) cm⁻¹: 1717, 1686, 1672, 1275, 714. EI-MS m/z (%): 496 (M⁺, 11), 361 (100), 319 (13), 234 (15), 204 (7), 105 (7). HR-EI-MS: calcd for C₂₆H₂₈N₂O₈, 496.1846, found: 496.1841.

Compound 15b: 1H-NMR (400 MHz, CDCl₃) δ: 7.93 (2H, m, Ar-H), 7.54 (1H, m, Ar-H), 7.42 (2H, m, Ar-H), 6.15 (1H, dd, J = 7.0, 4.6 Hz, C6-H), 5.10 (1H, d, J = 16.0 Hz, C3-H), 4.55 (1H, dd, J = 11.5, 7.0 Hz, C12-H), 4.37 (1H, dd, J = 11.5, 4.6 Hz, C12-H), 4.07 (1H, dd, J = 12.2, 4.9 Hz, C11a-H), 3.94 (3H, s, C7-OMe), 3.79 (3H, s, C8-OMe), 3.78 (1H, d, J = 16.0 Hz, C3-H), 3.68 (1H, dd, J = 15.9, 4.9 Hz, C11-Hα), 3.64 (3H, s, C10-OMe), 3.05 (1H, dd, J = 15.9, 12.2 Hz, C11-Hβ), 2.59 (3H, s, COMe), 2.22 (3H, s, C9-Me). 13C-NMR (100 MHz, CDCl₃) δ: 170.9 (s, COCH₃), 168.7 (s, C1), 166.4 (s, OCOPh), 166.1 (s, C4), 151.7 (s, C10), 150.6 (s, C8), 146.2 (s, C7), 133.2 (d, Ph × 2), 129.7 (s, Ph), 129.6 (d, Ph), 128.4 (d, Ph × 2), 126.1 (s, C9), 123.6 (s, C6a), 121.8 (s, C10a), 66.1 (t, C12), 61.0 (q, C10-OCH₃), 60.7 (q, C7-OCH₃), 60.0 (q, C8-OCH₃), 56.3 (d, C11a), 48.5 (d, C6), 45.6 (t, C3), 26.9 (q, COCH₃), 22.8 (t, C11), 9.4 (q, C9-CH₃). FT-IR (KBr) cm⁻¹: 1721, 1707, 1692, 1412, 1368, 1273. EI-MS m/z (%): 496 (M⁺, 10), 361 (100), 319 (11), 234 (13), 204 (7), 105 (7). HR-EI-MS: calcd for C₂₆H₂₈N₂O₈, 496.1846, found: 496.1841.
NBS (106.8 mg, 0.6 mmol) was added to a stirred solution of 15a (150.0 mg, 0.3 mmol) in dry CCl₄, and the reaction mixture was heated at 60 °C for 6 h. The reaction mixture was filtered through a short pad of Celite, and the filtrate was washed with CCl₄. The combined filtrates were concentrated in vacuo, and the residue was purified by flash chromatography on silica gel with ethyl acetate–hexane (1:3) as solvent to give 9 (102.0 mg, 69%) and 16 (17.7 mg, 13%).

**Compound 9**: 1H-NMR (400 MHz, CDCl₃) δ: 7.91 (2H, m, Ar-H), 7.55 (1 H, m, Ar-H), 7.47 (1H, s, C11-H), 7.42 (2H, m, Ar-H), 6.47 (1H, dd, J = 7.7, 3.8 Hz, C6-H), 4.93 (1H, d, J = 17.6 Hz, C3-H), 4.52 (1H, dd, J = 11.7, 7.7 Hz, C12-H), 4.27 (1H, dd, J = 11.7, 3.8 Hz, C12-H), 3.96 (3H, s, C7-OMe), 3.92 (1H, d, J=17.6 Hz, C3-H), 3.85 (3H, s, C8-OMe), 3.71 (3H, s, C10-OMe), 2.61 (3H, s, COMe), 2.21 (3H, s, C9-Me). 13C-NMR (100 MHz, CDCl₃ δ: 171.8 (s, COCH₃), 166.3 (s, OCOPh), 162.7 (s, C4), 160.7 (s, C1), 154.1 (s, C8), 152.4 (s, C10), 146.0 (s, C7), 133.3 (d, Ph), 129.6 (d, Ph × 2), 129.4 (s, Ph), 128.5 (d, Ph × 2), 126.7 (s, C9), 125.8 (s, C11a or C10a), 121.5 (s, C6a), 118.9 (s, C11a or C10a), 114.7 (d, C11), 64.3 (t, C12), 62.1 (q, C10-OC₆H₅), 60.8 (q, C7-OC₆H₅), 60.2 (q, C8-OC₆H₅), 47.9 (d, C6), 45.2 (t, C3), 26.7 (q, COCH₃), 9.4 (q, C9-CH₂Br). FT-IR (KBr) cm⁻¹: 1701, 1416, 1393, 1368, 1269, 1215. EI-MS m/z (%): 494 (M⁺, 14), 359 (100), 260 (84), 232 (38), 217 (6), 202 (5), 105 (6). HR-EI-MS: calcd for C₂₆H₂₆N₂O₈, 494.1689, found: 494.1686.

**Compound 16**: 1H-NMR (400 MHz, CDCl₃) δ: 7.90 (2H, m, Ar-H), 7.55 (1H, m, Ar-H), 7.42 (2H, m, Ar-H), 7.41 (1H, s, C11-H), 6.49 (1H, dd, J = 7.7 Hz, 3.8 Hz, C6-H), 4.94 (1H, d, J = 17.6 Hz, C3-H), 4.60 (1H, d, J = 9.1 Hz, C9-CH₂Br), 4.57 (1H, d, J = 9.1 Hz, C9-CH₂Br), 4.55 (1H, dd, J = 11.6 Hz, 7.7 Hz, C12-H), 4.30 (1H, dd, J = 11.6 Hz, 3.8 Hz, C12-H), 4.03 (3H, s, C8-OMe), 3.97 (3H, s, C7-OMe), 3.90–3.95 (1H, overlapped, C3-H), 3.90 (3H, s, C10-OMe), 2.62 (3H, s, COCH₃). 13C-NMR (100 MHz, CDCl₃) δ: 171.8 (s, COCH₃), 166.3 (s, OCOPh), 162.7 (s, C4), 160.4 (s, C1), 153.9 (s, C8), 152.1 (s, C10), 146.0 (s, C7), 133.4 (d, Ph), 129.6 (d, Ph × 2), 129.3 (s, Ph), 128.5 (d, Ph × 2), 127.3 (s, C9), 126.3 (s, C10a or C11a), 125.1 (s, C6a), 119.4 (s, C10a or C11a), 113.6 (d, C11), 64.2 (t, C12), 63.4 (q, C10-OC₆H₅), 60.7 (q, C7-OC₆H₅ and C8-OC₆H₅), 47.9 (d, C6), 45.2 (t, C3), 26.7 (q, COCH₃), 21.5 (t, C9-CH₂Br). FT-IR (KBr) cm⁻¹: 1701, 1368, 1292, 1269, 1211, 1088, 1072, 1043, 1028, 1007, 964, 459. EI-MS m/z (%): 494 (M⁺, 14), 359 (100), 260 (84), 232 (38), 217 (6), 202 (5), 105 (6). HR-EI-MS: calcd for C₂₆H₂₅BrN₂O₈, 572.0794, found: 572.0796.

3.3. (2-Acetyl-7,8-10-trimethyl-1,4-dioxo-1,3,4,6-tetrahydro-2H-pyrazino(1,2-b)isoquinoline-6-yl)methyl Benzoate (9) from 8 in Four Steps

TMSCl (498 μL, 3.9 mmol) was added to a stirred solution of 8 (1.05 g, 3.0 mmol) in dichloromethane (18 mL) and TEA (544 μL, 3.9 mmol), and stirring was continued at 25 °C for 2 h. A solution of 2,2-diethoxyethyl benzoate (785.8 mg, 3.3 mmol) in dichloromethane (12 mL) followed by TMSOTf (2.71 mL, 15 mmol) was added dropwise respectively over 5 min. Then, Ac₂O (283.6 μL, ...
3.0 mmol) was added at 25 °C and the reaction mixture was stirred for 4 h. The reaction mixture was diluted with saturated NaHCO₃ solution (100 mL) and extracted with CHCl₃ (100 mL × 3). The combined extracts were washed with brine (100 mL), dried, and concentrated in vacuo. The residue was subjected to column chromatography with ethyl acetate–hexane (1:2) to give 15 (1.37 g, 92%, 15a:15b = 10:3) as a colorless amorphous powder. Diastereomeric mixture 15 was dissolved in CCl₄ (90 mL) and NBS (106.8 mg, 5.46 nmol) was added at 25 °C, and the reaction mixture was heated at 60 °C for 3 h. The reaction mixture was filtered through a short pad of Celite, and the filtrate was washed with CCl₄. The combined filtrates were concentrated in vacuo, and the residue was used in the next step without further purification. The above residue was dissolved in 2-propanol/DMF (1:0.2) (72 mL) and was hydrogenated over 10% Pd/C (980 mg) at 25 °C for 11 h. The catalyst was removed by filtration and washed with CHCl₃ and MeOH. The combined filtrates were diluted with H₂O (100 mL) and extracted with CHCl₃ (100 mL × 3). The combined extracts were washed with brine (100 mL), dried, and concentrated in vacuo. The residue was subjected to column chromatography with ethyl acetate–hexane (1:4) to give 9 (1.05 g, 71% overall yield, 4 steps) as a colorless amorphous powder.

3.4. (Z)-(7,8,10-Trimethoxy-9-methyl-1,4-dioxo-3-(2,4,5-trimethoxy-3-methylbenzylidene)-1,3,4,6-tetra-hydro-2H-pyrazino(1,2-b)isoquinolin-6-yl)methyl Benzoate (10)

A solution of t-BuOK in t-BuOH (1 M, 2.4 mL, 2.4 mmol) was added to a solution of 9 (988 mg, 2.0 mmol) and 2,4,5-trimethoxybenzaldehyde (17) (420 mg, 2.0 mmol) in CH₂Cl₂ (20 mL) over 1 h at 0 °C, and the reaction mixture was stirred at 25 °C for 2 h. The reaction mixture was diluted with saturated NH₄Cl (100 mL) and extracted with CH₂Cl₂ (100 mL × 3). The combined extracts were washed with brine (100 mL), dried, and concentrated in vacuo. The residue was subjected to column chromatography with ethyl acetate–hexane (1:2) to give 10 (901 mg, 70%) as a pale yellow amorphous powder.

**Compound 10:** ¹H-NMR (400 MHz, CDCl₃) δ: 9.40 (1H, s, N-H), 7.93 (2H, m, Ar-H), 7.49 (1H, m, Ar-H), 7.36 (2H, m, Ar-H), 7.31 (1H, s, C11-H), 6.94 (1H, s, C3a-H), 6.68 (1H, dd, J = 6.8, 3.9 Hz, C6-H), 6.62 (1H, s, C6'-H), 4.52 (1H, dd, J = 11.7, 6.8 Hz, C12-H), 4.45 (1H, dd, J = 11.7, 3.9 Hz, C12-H), 3.98 (3H, s, C7-OMe), 3.84 (3H, s, C8-Ome), 3.83 (3H, s, C4'-Ome), 3.83 (3H, s, C5'-Ome), 3.69 (3H, s, C10-Ome), 3.58 (3H, s, C2'-Ome), 2.24 (3H, s, C3'-Me), 2.19 (3H, s, C9-Me). ¹³C-NMR (100 MHz, CDCl₃) δ: 166.1 (s, OOPh), 157.4 (s, C4), 156.5 (s, C1), 153.3 (s, C8), 151.8 (s, C10), 149.6 (s, C5'), 149.2 (s, C2'), 149.0 (s, C4'), 146.0 (s, C7), 132.9 (d, Ph), 129.7 (s, Ph), 129.7 (d, Ph × 2), 128.2 (d, Ph × 2), 126.6 (s, C3'), 126.4 (s, C9), 126.2 (s, C11a), 125.4 (s, C3), 121.6 (s, C1'), 120.6 (s, C6a), 119.1 (s, C10a), 114.0 (d, C3a), 111.9 (d, C6'), 110.8 (d, C11), 65.0 (t, C12), 62.0 (q, C10-OCH₃), 60.9 (q, C2'-OCH₃), 60.7 (q, C7-OCH₃), 60.4 (q, C4'-OCH₃), 60.1 (q, C8-OCH₃), 55.9 (q, C5'-OCH₃), 48.7 (d, C6), 9.5 (q, C3'-CH₃), 9.3 (q, C9'-CH₃). FT-IR (KBr) cm⁻¹: 3246, 1724, 1688, 1628, 1489, 1468, 1452, 1414, 1387, 1369, 1323, 1269, 1248, 1090, 1067, 1003, 712. EI-MS m/z (%): 644 (M⁺, 7), 509 (100), 481 (5), 232 (10). HR-EI-MS: calcd for C₃₅H₃₆N₂O₁₀, 644.2370, found: 644.2369.
A solution of 10 (644.0 mg, 1.0 mmol) in MeOH (50 mL) was hydrogenated over 10% Pd/C (213.0 mg) at 25 °C for 22 h. The catalyst was removed by filtration and washed with CHCl3 and MeOH. The combined filtrate was concentrated in vacuo. The residue was subjected to column chromatography with ethyl acetate–hexane (1:2) to give 11b (137.0 mg, 21%) as a pale yellow amorphous powder, and with ethyl acetate–hexane (2:1) to give 11a (463.0 mg, 72%) as a pale yellow amorphous powder.

Compound 11a: 1H-NMR (400 MHz, CDCl3) δ: 8.00 (2H, m, Ar-H), 7.53 (1H, m, Ar-H), 7.42 (2H, m, Ar-H), 7.25 (1H, s, C11-H), 6.53 (1H, dd, J = 7.5, 4.2 Hz, C6-H), 6.51 (1H, s, C6'-H), 6.22 (1H, s, N-H), 4.45 (1H, dd, J = 11.4, 7.5 Hz, C12-H), 4.34 (1H, dd, J = 10.3, 3.3 Hz, C3-H), 4.31 (1H, dd, J = 11.4, 4.2 Hz, C12-H), 3.97 (3H, s, C7-OMe), 3.82 (3H, s, C8-OMe), 3.80 (3H, s, C5'-OMe), 3.78 (3H, s, C4'-OMe), 3.67 (3H, s, C10-OMe), 3.64 (3H, s, C2'-OMe), 3.52 (1H, dd, J = 13.8, 3.3 Hz, C3a-H), 2.73 (1H, dd, J = 13.8, 10.3 Hz, C3a-H), 2.20 (3H, s, C3'-Me), 2.19 (3H, s, C9-Me). 13C-NMR (100 MHz, CDCl3) δ: 166.1 (s, OCOPh), 165.4 (s, C4), 149.7 (s, C5'), 147.6 (s, C4'), 146.0 (s, C7), 133.1 (d, Ph), 129.9 (s, Ph), 128.3 (d, Ph × 2), 126.4 (s, C9), 126.3 (s, C3'), 123.7 (s, C1'), 121.2 (s, C6a), 119.1 (s, C10a), 111.5 (d, C6a), 110.7 (d, C11), 64.6 (t, C12), 62.0 (q, C10-OCH3), 60.8 (q, C7-OCH3), 60.6 (q, C2'-OCH3), 60.2 (q, C4'-OCH3), 60.1 (q, C8-OCH3), 56.0 (q, C5'-OCH3) 55.3 (d, C3), 47.8 (d, C6), 33.5 (t, C3a), 9.7 (q, C3'-CH3), 9.4 (q, C9-CH3).

FT-IR (KBr) cm⁻¹: 3391, 1724, 1690, 1628, 1489, 1468, 1414, 1379, 1321, 1271, 1121, 1088. EI-MS m/z (%): 646 (M⁺, 14), 511 (100), 509 (13), 483 (36), 260 (8), 232 (17), 195 (10). HR-El-MS: calced for C35H38N2O10, 646.2527, found: 646.2523.

Compound 11b: 1H-NMR (400 MHz, CDCl3) δ: 7.93 (2H, m, Ar-H), 7.52 (1H, m, Ar-H), 7.45 (2H, m, Ar-H), 7.25 (1H, s, C11-H), 6.53 (1H, dd, J = 7.5, 4.2 Hz, C6-H), 6.51 (1H, s, C6'-H), 6.22 (1H, s, N-H), 4.45 (1H, dd, J = 11.4, 7.5 Hz, C12-H), 4.34 (1H, dd, J = 10.3, 3.3 Hz, C3-H), 4.31 (1H, dd, J = 11.4, 4.2 Hz, C12-H), 3.98 (3H, s, C7-OMe), 3.82 (3H, s, C8-OMe), 3.80 (3H, s, C5'-OMe), 3.78 (3H, s, C4'-OMe), 3.67 (3H, s, C10-OMe), 3.64 (3H, s, C2'-OMe), 3.52 (1H, dd, J = 13.8, 3.3 Hz, C3a-H), 2.73 (1H, dd, J = 13.8, 10.3 Hz, C3a-H), 2.20 (3H, s, C3'-Me), 2.19 (3H, s, C9-Me). 13C-NMR (100 MHz, CDCl3) δ: 166.2 (s, OCOPh), 165.0 (s, C4), 160.7 (s, C7), 153.2 (s, C8), 151.8 (s, C10), 150.9 (s, C2'), 149.7 (s, C5'), 147.6 (s, C4'), 146.0 (s, C7), 133.1 (d, Ph), 129.9 (d, Ph × 2), 129.8 (s, Ph), 128.3 (d, Ph × 2), 126.4 (s, C9), 126.3 (s, C3'), 123.7 (s, C1'), 121.2 (s, C6a), 119.1 (s, C10a), 111.5 (d, C6a), 110.7 (d, C11), 64.6 (t, C12), 62.0 (q, C10-OCH3), 60.8 (q, C7-OCH3), 60.6 (q, C2'-OCH3), 60.2 (q, C4'-OCH3), 60.1 (q, C8-OCH3), 56.0 (q, C5'-OCH3) 55.3 (d, C3), 47.8 (d, C6), 33.5 (t, C3a), 9.7 (q, C3'-CH3), 9.4 (q, C9-CH3).

FT-IR (KBr) cm⁻¹: 3300, 1724, 1692, 1628, 1487, 1468, 1414, 1379, 1321, 1271, 1119, 1088. El-MS m/z (%): 646 (M⁺, 11), 511 (100), 483 (39), 232 (16), 195 (9). HR-El-MS: calced for C35H38N2O10, 646.2527, found: 646.2521.
3.6. Isopropyl ((3S*,6R*)-7,8,10-Trimethoxy-9-methyl-1,4-dioxo-3-(2,4,5-trimethoxy-3-methylbenzyl)-1,3,4,6-tetrahydro-2H-pyrazino(1,2-b)isoquinolin-6-yl)methyl Benzoate (18)

A solution of 11a (16.47 g, 25 mmol), TEA (28.10 mL, 200 mmol), and DMAP (12.21 g, 100 mmol) in dichloromethane (400 mL) was cooled with ice water, and isopropyl chloroformate (40.08 mL, 350 mmol) was added dropwise over 30 min. The reaction mixture was stirred at 25 °C for 4 h. The organic layer was diluted with dichloromethane (500 mL), washed with 1 M aqueous HCl (500 mL × 2) and then water (500 mL), dried, and concentrated in vacuo to give a residue. The residue was subjected to column chromatography with ethyl acetate–hexane (2:5) to give 18 (17.5 g, 96%) as a yellow amorphous powder.

Compound 18: 1H-NMR (400 MHz, CDCl3) \( \delta \): 8.00 (2H, m, Ar-H), 7.48 (1H, m, Ar-H), 7.34 (2H, m, Ar-H), 7.19 (1H, s, C11-H), 6.53 (1H, t, \( J = 3.4 \) Hz, C6-H), 5.24 (1H, t, \( J = 6.1 \) Hz, C3-H), 4.96 (1H, sept, \( J = 6.3 \) Hz, CO2CH(CH3)2), 4.65 (1H, dd, \( J = 13.7 \) Hz, C3a-H), 3.80 (3H, s, C7-OMe), 3.80 (3H, s, C4′-OMe or C5′-OMe), 3.78 (3H, s, C8-OMe), 3.63 (3H, s, C4′-OMe or C5′-OMe), 3.59 (3H, s, C2′-OMe), 3.44 (3H, s, C10-OMe), 3.34 (1H, dd, \( J = 13.7 \) Hz, C3a-H), 2.10 (3H, s, C9-Me), 2.09 (3H, s, C3′-Me), 1.28 (3H, d, \( J = 6.3 \) Hz, CO2CH(CH3)2), 1.20 (3H, d, \( J = 6.3 \) Hz, CO2CH(CH3)2). 13C-NMR (100 MHz, CDCl3) \( \delta \): 165.7 (s, OCOPh), 163.4 (s, C4), 157.6 (s, C1), 153.4 (s, C8), 151.7 (s, CO2CH(CH3)2), 151.6 (s, C10), 151.4 (s, C2′), 149.0 (s, C4′ or C5′), 147.4 (s, C4′ or C5′), 145.8 (s, C7), 133.0 (d, Ph), 129.7 (d, Ph × 2), 129.6 (s, Ph), 128.3 (d, Ph × 2), 126.8 (s, C11a or C10a), 126.2 (s, C9), 125.6 (s, C3′), 122.5 (s, C1′), 120.0 (s, C6a), 119.3 (s, C11a or C12), 124.7 (d, C6′), 111.6 (d, C11), 71.8 (d, CO2CH(CH3)2), 66.3 (t, C12), 61.8 (q, C10-OC3H), 60.6 (q, C2′-OC3H), 60.5 (q, C7-OC3H), 60.0 (q, C4′-OC3H or C5′-OC3H), 59.4 (d, C3), 55.9 (q, C4′-OC3H or C5′-OC3H), 49.9 (d, C6), 35.8 (t, C3a), 21.6 (q, CO2CH(CH3)2), 21.5 (q, CO2CH(CH3)2), 9.7 (q, C3′-CH3), 9.2 (q, C9-CH3). FT-IR (KBr) cm\(^{-1}\): 2938, 1722, 1684, 1468, 1418, 1391, 1375, 1344, 1271, 1252, 1107, 1092, 1072, 712. EI-MS m/z (%): 732 (M+, 12), 597 (100), 569 (19), 555 (6), 511 (8), 483 (21), 415 (9), 260 (13), 232 (19), 195 (13). HR-EI-MS: calcd for C39H44N2O12, 732.2894, found: 732.2889.

3.7. Isopropyl (6S*,9R*,15R*)-9-((benzoyloxy)methyl)-1,2,4,10,11,13-hexamethoxy-3,12-dimethyl-7-oxo-6,7,9,15-tetrahydro-5H-6,15-epiminobenzo(4,5)azocino(1,2-b)isoquinoline-16-carboxylate (19)

A stirred solution of 18 (402.3 mg, 0.55 mmol) in THF (36 mL) was cooled with ice water and lithium tri-tert-butoxaluminohydride (1.12 g, 4.4 mmol) was added over 10 min. After continued stirring at 25 °C for 30 min, anhydrous Na2SO4 (2 g) was added and the reaction mixture was quenched with water. The reaction mixture was filtered through Celite pad and then, the filtrate was diluted with brine (200 mL) and extracted with CHCl3 (3 × 200 mL). The combined extracts were washed with brine (200 mL), dried, and concentrated in vacuo to give a residue, which was used in the next step without further purification. A solution of the residue as above in formic acid (36 mL) was stirred at 25 °C for 30 min. The reaction mixture was filtered through Celite pad and then, the filtrate was diluted with saturated aqueous NaHCO3 solution (80 mL) and extracted with CHCl3 (3 × 80 mL). The combined extracts were washed with brine (80 mL), dried, and concentrated in vacuo to give a residue.
The residue was subjected to column chromatography with ethyl acetate–hexane (1:3) to give 19 (322.7 mg, 82%) as a pale yellow amorphous powder.

Compound 19: $^1$H-NMR (400 MHz, DMSO, 140 °C) δ: 7.58 (2H, m, Ar-H), 7.54 (1H, m, Ar-H), 7.41 (2H, m, Ar-H), 6.26 (1H, dd, $J = 8.1, 4.2$ Hz, C9-H), 5.93 (1H, d, $J = 1.5$, C15-H), 4.98 (1H, m, 1.5 Hz, C6-H), 4.86 (1H, sept, $J = 6.2$ Hz, CO$_2$CH(CH$_3$)$_3$), 3.92 (1H, dd, $J = 11.5$, 4.2 Hz, C16-H), 3.87 (3H, s, C2-OMe or C11-OMe), 3.85 (1H, dd, $J = 11.5$, 8.1 Hz, C16-H), 3.75 (3H, s, C4-OMe or C13-OMe), 3.73 (3H, s, C2-OMe or C11-OMe), 3.67 (3H, s, C4-OMe or C13-OMe), 3.51 (3H, s, C1-OMe), 3.09 (1H, br d, $J = 17.6$, C5-H), 3.04 (1H, dd, $J = 17.6$, 3.9 Hz, C5-H), 2.15 (3H, s, C3-Me or C12-Me), 1.98 (3H, s, C3-Me or C12-Me), 1.23 (3H, d, $J = 6.2$ Hz, CO$_2$CH(CH$_3$)$_3$), 1.21 (3H, d, $J = 6.2$ Hz, CO$_2$CH(CH$_3$)$_3$). $^{13}$C-NMR (100 MHz, DMSO, 140 °C) δ: 165.4 (s, C7), 164.4 (s, OCOPh), 152.1 (s, CO$_2$CH(CH$_3$)$_3$), 151.7 (s, C1), 150.2 (s, C2 or C11), 149.2 (s, C4 and C13), 145.0 (s, C2 or C11), 144.7 (s, C10), 132.3 (s, C14a), 131.9 (d, Ph), 128.7 (s, Ph), 128.3 (d, Ph × 2), 127.5 (d, Ph × 2), 124.5 (s, C3 or C12), 124.0 (s, C3 or C12), 123.7 (s, C4a), 119.9 (s, C15a), 118.8 (s, C13a), 118.2 (s, C9a), 100.0 (d, C14), 68.8 (d, CO$_2$CH(CH$_3$)$_3$), 62.6 (t, C16), 60.3 (q, C4-CH$_3$ or C13-CH$_3$), 59.8 (q, C2-CH$_3$ or C10-CH$_3$ or C11-CH$_3$), 59.2 (q, C2-CH$_3$ or C10-CH$_3$ or C11-CH$_3$), 59.1 (q, C2-CH$_3$ or C10-CH$_3$ or C11-CH$_3$), 58.9 (q, C4-CH$_3$ or C13-CH$_3$), 58.6 (q, C1-CH$_3$), 52.4 (q, CO$_2$CH(CH$_3$)$_3$), 8.24 (q, C3-CH$_3$ or C12-CH$_3$), 8.18 (q, C3-CH$_3$ or C12-CH$_3$). FT-IR (KBr) cm$^{-1}$: 1717, 1707, 1686, 1647, 1466, 1414, 1362, 1344, 1298, 1269, 1109, 1070, 1007, 964, 712. EI-MS m/z (%): 716 (M$^+$, 22), 581 (100), 553 (67), 234 (21). HR-EI-MS: calcd for C$_{39}$H$_{44}$N$_2$O$_{11}$, 716.2945, found: 716.2942.

3.8. ((6S*,9R*,15R*)-1,2,4,10,11,13-hexamethoxy-3,12-dimethyl-7-oxo-6,7,15-tetrahydro-5H-6,15-epiminobenzo(4,5)azocino(1,2-b)isoquinolin-9-yl)methyl Benzoate (20)

Concentrated H$_2$SO$_4$ (1.7 mL) was added to a stirred solution of 19 (322.7 mg, 0.45 mmol) in TFA (34 mL) at 0 °C over 5 min, and the reaction mixture was stirred at 25 °C for 4 h. The reaction mixture was poured into water (40 mL) at 0 °C, basified with concentrated NH$_4$OH, and then extracted with CHCl$_3$ (3 × 100 mL). The combined extracts were washed brine (100 mL), dried, and concentrated in vacuo to give a residue. The residue was subjected to column chromatography with ethyl acetate–hexane (1:3) to give 20 (267.6 mg, 94%) as a pale yellow amorphous powder.

Compound 20: $^1$H-NMR (400 MHz, CDCl$_3$) δ: 7.70 (2H, m, Ar-H), 7.49 (1H, m, Ar-H), 7.38 (2H, m, Ar-H), 6.41 (1H, dd, $J = 8.1, 5.2$ Hz, C9-H), 6.26 (1H, s, C14-H), 4.99 (1H, s, C15-H), 4.12 (1H, br d, $J = 6.3$ Hz, C6-H), 3.95–3.89 (2H, overlapped, C16-H), 3.90 (3H, s, C10-OMe), 3.87 (3H, s, C1-OMe), 3.76 (3H, s, C13-OMe), 3.76 (3H, s, C11-OMe), 3.67 (3H, s, C2-OMe), 3.44 (3H, s, C4-OMe), 3.19 (1H, dd, $J = 17.0$, 1.3 Hz, C5-H), 3.07 (1H, dd, $J = 17.0$, 6.3 Hz, C5-H), 2.19 (3H, s, C12-Me), 1.88 (3H, s, C3-Me). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ: 168.5 (s, C7), 166.0 (s, OCOPh), 152.5 (s, C4), 150.8 (s, C11), 149.9 (s, C2), 149.8 (s, C13), 146.3 (s, C1), 146.0 (s, C10), 136.1 (s, C13a or C14a), 132.5 (d, Ph), 129.6 (d, Ph), 129.4 (s, Ph), 128.3 (d, Ph), 125.7 (s, C12), 125.3 (s, C15a), 125.0 (s, C3), 121.5 (s, C4a), 120.4 (s, C13a or C14a), 119.5 (s, C9a), 99.8 (d, C14), 63.4 (t, C16), 61.4 (q, C13-CH$_3$), 60.8 (q, C10-CH$_3$), 60.2 (q, C11-CH$_3$), 59.9
(q, C2-OCH₃), 59.3 (q, C4-OCH₃), 53.9 (d, C6), 50.1 (d, C15), 45.7 (d, C9), 29.0 (t, C5), 9.3 (q, C12-CH₃), 9.1 (q, C3-CH₃). FT-IR (KBr) cm⁻¹: 2938, 1722, 1680, 1638, 1466, 1412, 1362, 1271, 1248, 1115, 1070, 1099, 964, 712. EI-MS m/z (%): 630 (M⁺, 22), 495 (48), 467 (100), 234 (39), 204 (18). HR-EI-MS: calcd for C₃₅H₃₈N₂O₉, 630.2577, found: 630.2581.

3.9. ((6S*,9R*,15R*)-1,2,4,10,11,13-hexamethoxy-3,12,16-trimethyl-7-oxo-6,7,9,15-tetrahydro-5H-6,15-epiminobenzo(4,5)azocino(1,2-b)isoquinolin-9-yl)methyl Benzoate (21)

A 37% aqueous solution of formaldehyde (6 mL) was added to a stirred solution of 20 (251.2 mg, 0.4 mmol) in formic acid (7.0 mL) at 60 °C, and the reaction mixture was heated at 70 °C for 1 h. The reaction mixture was diluted with 5% aqueous NaHCO₃ solution (80 mL) and extracted with CHCl₃ (3 × 80 mL). The combined extracts were washed with brine (80 mL), dried, and concentrated in vacuo to give a residue. The residue was subjected to column chromatography with ethyl acetate–hexane (1:1) to give 21 (247.3 mg, 96%) as a colorless amorphous powder.

Compound 21: ¹H-NMR (400 MHz, CDCl₃) δ: 7.71 (2H, m, Ar-H), 7.49 (1H, m, Ar-H), 7.38 (2H, m, Ar-H), 6.43 (1H, t, J = 6.4 Hz, C9-H), 6.30 (1H, s, C14-H), 5.63 (1H, s, C15-H), 3.92 (3H, s, C11-OCH₃), 3.91 (2H, d, J = 6.4 Hz, C17-H), 3.87 (3H, s, C10-OCH₃), 3.76 (6H, s, C11-OMe and C13-OMe), 3.68 (1H, overapped, C6-H), 3.67 (3H, s, C2-OMe), 3.32 (1H, dd, J = 17.6 Hz, C5-H), 3.11 (1H, br d, J = 17.6 Hz, C5-H), 2.55 (3H, s, N-Me), 2.20 (3H, s, C12-Me), 1.89 (3H, s, C3-Me). ¹³C-NMR (100 MHz, CDCl₃) δ: 168.1 (s, C7), 166.0 (s, OCOPh), 152.4 (s, C4), 150.7 (s, C11), 149.8 (s, C2), 149.5 (s, C13), 146.1 (s, C1), 145.9 (s, C10), 132.7 (s, C2), 132.5 (d, Ph), 129.6 (d, Ph × 2), 129.3 (s, Ph), 128.3 (d, Ph × 2), 126.0 (s, C15a), 125.7 (s, C12), 124.7 (s, C5), 121.1 (s, C4a), 119.9 (s, C13a), 119.6 (s, C9a), 102.7 (d, C14), 63.6 (t, C17), 61.3 (q, C11-OCH₃ or C13-OCH₃), 60.7 (q, C10-OCH₃), 60.5 (d, C6), 60.1 (q, C1-OCH₃), 60.0 (q, C11-OCH₃ or C13-OCH₃), 59.8 (q, C2-OCH₃), 59.2 (q, C4-OCH₃), 56.8 (d, C15), 45.5 (d, C9), 41.6 (q, N-CH₃), 29.2 (t, C5), 9.2 (q, C12-CH₃), 9.0 (q, C3-CH₃). FT-IR (KBr) cm⁻¹: 1722, 1678, 1638, 1464, 1412, 1358, 1341, 1271, 1126, 1113, 1099, 1067, 1007, 712. EI-MS m/z (%): 644 (M⁺, 17), 509 (20), 481 (100), 248 (37), 218 (15). HR-EI-MS: calcd for C₃₆H₄₀N₂O₉, 644.2734, found: 644.2733.

3.10. (6S*,9R*,15R*)-9-(hydroxymethyl)-1,2,4,10,11,13-hexamethoxy-3,12,16-trimethyl-5,6,9,15-tetrahydro-7H-6,15-epiminobenzo(4,5)azocino(1,2-b)isoquinolin-7-one (12)

A 10 M aqueous solution of lithium hydroxide monohydrate (77 μL, 0.77 mmol) was added to a stirred solution of 21 (226.3 mg, 0.35 mmol) in THF (2.0 mL) and MeOH (0.7 mL), and stirring was continued at 25 °C for 8 h. The reaction mixture was diluted with water (80 mL), and the mixture was extracted with CHCl₃ (3 × 80 mL). The combined extracts were washed with brine (80 mL), dried, and concentrated in vacuo to give a residue. The residue was subjected to column chromatography with ethyl acetate–hexane (2:1) to give 12 (184.1 mg, 97%) as a colorless amorphous powder.

Compound 12: ¹H-NMR (400 MHz, CDCl₃) δ: 6.27 (1H, s, C14-H), 6.08 (1H, dd, J = 7.9, 5.1 Hz, C9-H), 4.70 (1H, brs, C15-H), 3.88 (3H, s, OMe), 3.86 (3H, s, OMe), 3.78 (3H, s, OMe), 3.77 (3H, s, OMe), 3.75 (3H, s, OMe) 3.72 (1H, br t, C6-H), 3.66 (3H, s, OMe), 3.32 (1H, dt, J = 11.5, 5.1 Hz, C12-Me), 1.85 (3H, s, C3-Me). ¹³C-NMR (100 MHz, CDCl₃) δ: 168.1 (s, C7), 166.0 (s, OCOPh), 152.4 (s, C4), 150.7 (s, C11), 149.8 (s, C2), 149.5 (s, C13), 146.1 (s, C1), 145.9 (s, C10), 132.7 (s, C2), 132.5 (d, Ph), 129.6 (d, Ph × 2), 129.3 (s, Ph), 128.3 (d, Ph × 2), 126.0 (s, C15a), 125.7 (s, C12), 124.7 (s, C5), 121.1 (s, C4a), 119.9 (s, C13a), 119.6 (s, C9a), 102.7 (d, C14), 63.6 (t, C17), 61.3 (q, C11-OCH₃ or C13-OCH₃), 60.7 (q, C10-OCH₃), 60.5 (d, C6), 60.1 (q, C1-OCH₃), 60.0 (q, C11-OCH₃ or C13-OCH₃), 59.8 (q, C2-OCH₃), 59.2 (q, C4-OCH₃), 56.8 (d, C15), 45.5 (d, C9), 41.6 (q, N-CH₃), 29.2 (t, C5), 9.2 (q, C12-CH₃), 9.0 (q, C3-CH₃). FT-IR (KBr) cm⁻¹: 1722, 1678, 1638, 1464, 1412, 1358, 1341, 1271, 1126, 1113, 1099, 1067, 1007, 712. EI-MS m/z (%): 644 (M⁺, 17), 509 (20), 481 (100), 248 (37), 218 (15). HR-EI-MS: calcd for C₃₆H₄₀N₂O₉, 644.2734, found: 644.2733.
C17-H), 3.22 (1H, dt, $J = 11.5, 7.9$ Hz, C17-H), 3.21 (2H, d, $J = 4.4$ Hz, C5-H), 2.57 (3H, s, N-Me), 2.19 (3H, s, C12-Me), 2.18 (3H, s, C3-Me), 1.37 (1H, t, $J = 6.1$ Hz, -OH). 13C-NMR (100 MHz, CDCl3) $\delta$: 169.1 (C7), 152.6 (C4), 150.7 (C11), 150.1 (C2), 149.7 (C13), 146.3 (C1), 145.8 (C10), 132.8 (C14a), 126.0 (C15a), 125.4 (C12), 125.1 (C3), 121.1 (C4a), 120.6 (C9a), 119.5 (C13a), 102.9 (14), 64.6 (C17), 61.3 (-OCH3), 60.1 (-OCH3), 60.0 (-OCH3), 59.8 (-OCH3), 56.5 (C15), 49.1 (C9), 41.6 (N-CH3), 34.2 (C5), 9.4 (-CH3), 9.2 (-CH3). FT-IR (KBr) cm$^{-1}$: 3468, 2940, 1672, 1636, 1466, 1412, 1341, 1248, 1113, 1065, 1007, 964. EI-MS m/z (%): 540 (M+, 9), 509 (27), 481 (100), 248 (51). HR-EI-MS: calcd for C$_{29}$H$_{36}$N$_2$O$_8$, 540.2472, found: 540.2473.

3.11. ((6S*,9R*,15R*)-1,2,4,10,11,13-hexamethoxy-3,12,16-trimethyl-7-oxo-6,7,9,15-tetrahydro-5H-6,15-epiminobenzo(4,5)azocino(1,2-b)isoquinolin-9-yl)methyl Benzoate (22)

To a stirred solution of 12 (216.0 mg, 0.4 mmol) in CH$_2$Cl$_2$ (24 mL) at −78 °C was added a CH$_2$Cl$_2$ solution of BBr$_3$ (1.0 M, 2.40 mL, 2.4 mmol) over 5 min. Stirring was continued at the same temperature for 1 h, and then at −20 °C for 14.5 h. The reaction mixture was diluted with water (200 mL) and extracted with 5% MeOH in CHCl$_3$ (4 × 200 mL). The combined extracts were washed with 5% NaHCO$_3$ solution (200 mL), dried, and concentrated in vacuo to give a residue. A solution of the above residue in 10 N HNO$_3$ (5.0 mL) was stirred at 25 °C for 30 min. The reaction mixture was diluted with water (150 mL) and extracted with ethyl acetate (3 × 200 mL). The combined extracts were washed with brine (200 mL), dried, and concentrated in vacuo. The residue was subjected to purification by silica gel chromatography with ethyl acetate to give 22 (130.0 mg, 67%) as a dark purple amorphous powder.

Compound 22: 1H-NMR (500 MHz, CDCl$_3$) $\delta$: 6.26 (1H, s, 14-H), 5.96 (1H, dd, $J = 7.1, 4.5$ Hz, 9-H), 4.55 (1H, s, 15-H), 4.02 (3H, s, OCH$_3$), 3.74 (1H, dt, $J = 6.5, 1.5$ Hz, 6-H), 3.50 (1H, dd, $J = 11.4, 4.5$ Hz, 17-H), 3.36 (1H, dd, $J = 11.4, 7.1$ Hz, 17-H), 2.96 (1H, dd, $J = 19.8, 6.5$ Hz, 5-Ha), 2.89 (1H, dd, $J = 19.8, 1.5$ Hz, C5-H$\beta$), 2.51 (3H, s, N-CH$_3$), 1.96 (3H, s, 3-CH$_3$ or 12-CH$_3$), 1.94 (3H, s, 3-CH$_3$ or 12-CH$_3$), 1.62 (1H, br s, OH). 13C-NMR (125 MHz, CDCl$_3$) $\delta$: 186.6 (s, C-4), 185.0 (s, C-13), 180.5 (s, C-10 and C-1), 168.0 (s, C-7), 156.0 (s, C-11), 155.4 (s, C-2), 140.7 (s, C-14a), 140.0 (s, C-4a), 136.5 (s, C-15a), 134.5 (s, C-13a), 129.1 (s, C-3), 127.6 (s, C-12), 125.0 (s, C-9a), 101.8 (d, C-14), 62.9 (t, C-17), 61.1 (q, OCH$_3$), 61.0 (q, OCH$_3$), 59.6 (d, C-6), 54.4 (d, C-15), 48.4 (d, C-9), 41.2 (q, N-CH$_3$), 28.7 (t, C-5), 8.8 (q, Ar-CH$_3$), 8.7 (q, Ar-CH$_3$). FT-IR (KBr) cm$^{-1}$: 3347, 2951, 2855, 1654, 1616, 1568, 1450, 1373, 1310. LR-MS (FAB$^+$): 481 [M + H]$^+$. HR-MS (FAB$^+$): calcd for C$_{25}$H$_{25}$N$_2$O$_8$, 481.1611, found: 481.1623.

3.12. ((6S*,9R*,15R*)-2,11-dimethoxy-3,12,16-trimethyl-4,7,10,13-penta-oxo-1,5,6,7,9,10,13,15-octa-hydro-4H-6,15-epiminobenzo(4,5)azocino(1,2-b)isoquinolin-9-yl)methyl (Z)-2-methylbut-2-enoate (23)

A solution of angelic acid (601.0 mg, 6.0 mmol) in ether (30 mL) was cooled with ice water, and a solution of oxalyl chloride (0.5 mL, 5.9 mmol) in DMF (46.0 μL, 592 mmol) was added dropwise over 5 min. The resulting solution was stirred at 25 °C for 2 h. Then, a solution of 22 (142.0 mg, 0.3 mmol) in...
in CH2Cl2 (15 mL) was added over 5 min. The reaction mixture was concentrated to approximately 3.0 mL with a stream of argon, and CH2Cl2 (8.0 mL) was added. The resulting mixture was stirred at 25 °C for 21 h. The reaction mixture was directly purified by silica gel chromatography with ethyl acetate–hexane (2:1) to afford 23 (139.0 mg, 84%) as a dark purple film.

Compound 23: ¹H-NMR (500 MHz, CDCl3) δ: 6.24 (1H, s, 14-H), 6.12 (1H, dd, J = 5.7, 2.9 Hz, 9-H), 5.92 (1H, qq, J = 7.4, 1.4 Hz, 21-H), 4.50 (1H, br s, 15-H), 4.21 (1H, dd, J = 11.9, 2.9 Hz, 17-H), 3.72 (1H, dt, J = 6.8, 1.4 Hz, 6-H), 2.95 (1H, dd, J = 19.8, 6.8 Hz, 5-Hβ), 2.84 (1H, dd, J = 19.8, 1.4 Hz, 5-H), 2.47 (3H, s, N-CH3), 1.96 (3H, s, 12-CH3), 1.92 (3H, s, 3-CH3), 1.75 (3H, dq, J = 7.4, 1.4 Hz, 21-CH3), 1.57 (1H, quint, J = 1.4 Hz, 20-CH3). ¹³C-NMR (125 MHz, CDCl3) δ: 186.5 (s, C-4), 184.9 (s, C-13), 180.5 (s, C-1), 180.1 (s, C-10), 167.1 (s, C-7 and C-19), 156.2 (s, C-11), 155.2 (s, C-2), 140.6 (s, C-14a), 139.8 (s, C-4a), 139.3 (d, C-21), 136.2 (s, C-15a), 134.6 (s, C-13a), 128.5 (s, C-3), 127.3 (s, C-12), 126.8 (s, C-20), 124.2 (s, C-9a), 101.3 (d, C-14), 62.4 (t, C-17), 61.1 (q, 11-OCH3), 61.0 (q, 2-OCH3), 59.5 (d, C-6), 54.3 (d, C-15), 47.1 (d, C-9), 41.1 (q, N-CH3), 28.3 (t, C-5), 20.2 (q, C-23), 15.5 (q, C-22), 8.7 (q, 3-CH3), 8.6 (q, 12-CH3). FT-IR (KBr) cm⁻¹: 2949, 1653, 1616, 1570, 1458, 1309, 1228, 1153. EI-MS m/z (%): 562 (M⁺, 5), 421 (100), 218 (40). HR-EI-MS: calcd for C₃₀H₃₀N₂O₉, 562.1951, found: 562.1952.

3.13. Renieramycin I (1i)

A suspension of 23 (15.0 mg, 0.027 mmol) and SeO₂ (29.6 mg, 0.27 mmol) in dioxane (3.0 mL) and MeOH (1.0 mL) was heated at 100 °C for 6 days. The reaction mixture was filtered and the filter cake was washed with ethyl acetate. The combined filtrates were concentrated in vacuo to give a residue. Flash column chromatography on silica gel with ethyl acetate–hexane (2:3) afforded 1i (6.8 mg, 43%) as a dark red film and 24 (4.4 mg, 29%).

Renieramycin I (1i): ¹H-NMR (400 MHz, CDCl3) δ: 6.26 (1H, s, 4-H), 6.07 (1H, dd, J = 5.5, 2.7 Hz, 1-H), 5.92 (1H, qq, J = 7.3, 1.4 Hz, 26-H), 4.54 (1H, d, J = 0.9 Hz, 11-H), 4.34 (1H, d, J = 1.4 Hz, 14-H), 4.16 (1H, dd, J = 12.1, 5.5 Hz, 22-H), 4.06 (3H, s, 7-OCH3), 4.02 (1H, dd, J = 12.1, 2.7 Hz, 22-H), 3.98 (3H, s, 17-OCH3), 3.74 (1H, br t, J = 1.4 Hz, 13-H), 3.62 (3H, s, 14-OCH3), 2.55 (3H, s, N-CH3), 1.96 (3H, s, 6-CH3), 1.94 (3H, s, 16-CH3), 1.73 (3H, dq, J = 7.3, 1.4 Hz, 26-CH3), 1.55 (3H, quint, J = 1.4 Hz, 25-CH3). ¹³C-NMR (100 MHz, CDCl3) δ: 185.8 (s, C-15), 184.7 (s, C-5), 180.9 (s, C-18), 180.1 (s, C-8), 167.1 (s, C-24), 164.1 (s, C-21), 156.1 (s, C-7), 155.2 (s, C-17), 139.2 (d, C-26), 138.6 (s, C-3), 137.4 (s, C-19), 137.0 (s, C-20), 134.2 (s, C-10), 129.3 (s, C-16), 127.4 (s, C-6), 126.7 (s, C-25), 124.9 (s, C-9), 102.2 (d, C-4), 73.4 (d, C-14), 64.7 (d, C-13), 62.6 (t, C-22), 61.2 (q, 7-OCH3), 60.9 (q, 17-OCH3), 59.4 (q, 14-OCH3), 54.5 (d, C-11), 47.3 (d, C-1), 41.7 (q, N-CH3), 20.2 (q, 25-CH3), 15.5 (q, 26-CH3), 8.9 (q, Ar-CH3), 8.7 (q, Ar-CH3). FT-IR (KBr) cm⁻¹: 3429, 2949, 1717, 1684, 1655, 1614, 1570, 1454, 1342, 1306, 1233, 1153, 1096. EI-MS m/z (%): 594 ([M + 2H⁺], 0.7), 593 ([M + H⁺], 2), 592 (M⁺, 6), 479 (19), 452 (26), 451 (100), 421 (20), 248 (25), 218 (11). HR-EI-MS: calcd for C₃₀H₃₀N₂O₁₀, 592.2057, found: 592.2056.
3.14. ((6S*,9R*,15R*)-5-hydroxy-2,11-dimethoxy-3,12,16-trimethyl-1,4,7,10,13-pentaoxo-1,5,6,7,9,10,13,15-octahydro-4H-6,15-epiminobenzo(4,5)azocino(1,2-b)isoquinolin-9-yl) methyl (Z)-2-methylbut-2-enoate (24)

\[
\begin{align*}
\text{1H-NMR (500 MHz, CDCl3)} & \; \delta: 6.28 (1H, s, 4-H), \\
\text{6.09 (1H, dd, J = 7.0 Hz, 14-H), } & \text{4.52 (1H, d, J = 1.1 Hz, 11-H), } \\
\text{4.19 (1H, dd, J = 12.0, 6.0 Hz, 22-H), } & \text{3.75 (1H, dd, J = 1.7, 1.1 Hz, 13-H), } \\
\text{2.88 (1H, br d, J = 7.0 Hz, OH), } & \text{2.55 (3H, s, N-CH3), } \\
\text{2.55 (3H, s, 6-CH3), } & \text{1.96 (3H, s, 6-CH3), } \\
\text{1.75 (3H, dq, J = 7.3, 1.5 Hz, 26-CH3), } & \text{1.56 (3H, quint, J = 1.5 Hz, 25-CH3).}
\end{align*}
\]

\[
\begin{align*}
\text{13C-NMR (125 MHz, CDCl3)} & \; \delta: 186.7 (s, C-15), \\
\text{184.7 (s, C-5), } & \text{181.0 (s, C-18), } \\
\text{180.0 (s, C-8), } & \text{167.1 (s, C-24), } \\
\text{163.9 (s, C-21), } & \text{156.1 (s, C-7), } \\
\text{155.4 (s, C-17), } & \text{139.3 (d, C-26), } \\
\text{138.4 (s, C-10 or C-20), } & \text{138.3 (s, C-10 or C-20), } \\
\text{136.9 (s, C-19), } & \text{134.3 (s, C-3), } \\
\text{128.8 (s, C-16), } & \text{127.4 (s, C-6), } \\
\text{126.7 (s, C-25), } & \text{124.8 (s, C-9), } \\
\text{102.2 (d, C-4), } & \text{67.1 (d, C-13), } \\
\text{64.8 (d, C-14), } & \text{62.3 (t, C-22), } \\
\text{61.1 (q, OCH3), } & \text{61.0 (q, OCH3), } \\
\text{54.7 (d, C-11), } & \text{47.2 (d, C-1), } \\
\text{41.5 (q, N-CH3), } & \text{20.2 (q, 25-CH3), } \\
\text{15.5 (q, 26-CH3), } & \text{8.7 (q, Ar-CH3), } \\
\text{8.7 (q, Ar-CH3).}
\end{align*}
\]

3.15. Cribrostatin 4 (2) via 24

A suspension of 23 (112.4 mg, 0.2 mmol) and SeO₂ (110.9 mg, 1.0 mmol) in dioxane (30 mL) and water (3.0 mL) was heated at 80 °C for 6 h. The reaction mixture was filtered and the filter cake was washed with ethyl acetate (200 mL). The combined filtrates were concentrated \textit{in vacuo} to give a residue (248.3 mg). Flash column chromatography on silica gel (70 g) with hexane–ethyl acetate (1:1) afforded 24 (72.5 mg, 63%) along with recovered 23 (16.2 mg, 14%). Dess–Martin periodinane (DMP, 445.3 mg, 1.05 mmol) was added to a stirred solution of 24 (57.8 mg, 0.1 mmol) in dichloromethane (15 mL), and the mixture was stirred at 25 °C for 3 h. The reaction mixture was diluted with THF (50 mL), saturated aqueous Na₂S₂O₃ solution (50 mL) was added, and the mixture was stirred at 25 °C for 2 h. The reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate (100 mL × 3). The combined extracts were washed with brine (100 mL), dried, and concentrated \textit{in vacuo}. The residue (230 mg) was purified by silica gel (9 g) flash column chromatography with hexane–ethyl acetate (1:2) to give cribrostatin 4 (2: 81.0 mg, 70.0% from 23) as a dark red film. 1H-, 13C NMR, and also IR spectral charts of synthetic renieramycin I and cribrostatin 4 are available in the supplementary information.

4. Conclusions

In summary, we have succeeded in reducing the number of steps in our first version of the total synthesis of cribrostatin 4 through key intermediate 12, from 21 steps in 3.4% overall yield to 18 steps in 8.3% overall yield. The main point of this alternative total synthesis is based on the Avendano’s protocol introducing C3-C4 double bond in the early stage. We have completed the first total synthesis of renieramycin I (1i), and the spectroscopic data provide unambiguous evidence that supports the original structure of the natural product. The development of ways to utilize this approach for the
synthesis of other members of the C3-C4 unsaturated renieramycin family, and the examination of their biological activities to evaluate the mechanism of action, are undergoing in our laboratory.

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Author Contributions

Masashi Yokoya Keiichiro Kobayashi, and Mitsuhiro Sato: conducted the experimental. Naoki Saito and Masashi Yokoya: wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes


16. For simplicity, natural product numbering was used in the manuscript, but IUPAC names and numbers were used in the Experimental.


23. The signal in the NMR spectrum (CDCl₃, at 20 °C) of 19 was not split, which was a mixture of two rotational isomers.


27. We cannot obtain any $^{13}$C NMR spectral data of natural renieramycin I (1i); see reference [3].


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