JOC The Journal of Organic Chemistry

Article

Stereoselective Total Synthesis of (-)-Renieramycin T

Masashi Yokoya, Ryoko Toyoshima, Toshihiro Suzuki, Vy H. Le, Robert M. Williams, and Naoki Saito

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.6b00327 • Publication Date (Web): 28 Mar 2016

ACS Publications

The Journal of Organic Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Stereoselective Total Synthesis of (−)-Renieramycin T

Masashi Yokoya,*† Ryoko Toyoshima,† Toshihiro Suzuki,† Vy H. Le,‡ Robert M. Williams,*‡ and Naoki Saito†

†Graduate School of Pharmaceutical Sciences, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

‡Department of Chemistry Colorado State University Fort Collins, Colorado 80523 and The University of Colorado Cancer Center, Aurora, Colorado 80045

*yokoya@my-pharm.ac.jp

*robert.williams@colostate.edu

Abstract

A stereoselective total synthesis of (−)-Renieramycin T (**1t**) from a key tetrahydroisoquinoline intermediate previously utilized in our formal total synthesis of Ecteinascidin 743 is described. The synthesis features a concise approach for construction of the pentacyclic framework using a Pictet-Spengler cyclization of bromo-substituted carbinolamine **17**, which obviates the regioselectivity problem of the Pictet-Spengler cyclization. The results of cytotoxicity studies are also presented.

Introduction

The Renieramycins and Ecteinascidins are 1,2,3,4-tetrahydroisoquinoline (THIQ) marine natural products that are structurally and biologically related to other tetrahydroisoquinoline-based natural products, including the Saframycins, Bioxalomycins, Jorumycin, Tetrazomine and Quinocarcin, among others.[1] Ecteinascidin 743 (**2a**:

Yondelis®) has been demonstrated to possess the most potent cytotoxic activity in this family and it has been approved and marketed in eighty countries worldwide for the treatment of human soft tissue sarcoma and is undergoing additional clinical trials in other countries.[2] In our continuing chemical studies on THIQ marine natural products, we succeeded in identifying the trace metabolites Renieramycin T (**1t**) along with Renieramycin U (**1u**) from the Thai blue sponge *Xestospongia* sp. that was pretreated with KCN (Figure 1).[3] These natural substances were also discovered from the Philippine blue sponge *Xestospongia* sp., growing in the vicinity of Puerto Galera, Oriental Mindoro, Mindoro Island along with three new Renieramycin type compounds Renieramycins W−Y (**1w**−**y**).[4] Compounds **1t**, **1u** and **1x** possess a highly functionalized aromatic A ring, which bear the same substitution pattern as that of the Ecteinascidins, and are the first examples of Ecteinascidin–Renieramycin hybrids from natural sources. The structural similarity of **1t** and **2a** suggested that a comparison of the anti-cancer activities of these natural products might be informative. However, the severely limited supply of these new Renieramycins from marine organisms has thus far precluded detailed biological evaluation. To date, several natural Renieramycins have been successfully synthesized by several laboratories,[5] and we also reported the enantioselective total synthesis of Renieramycin G, Cribrostatin 4 (Renieramycin H) and Renieramycin I.[6]

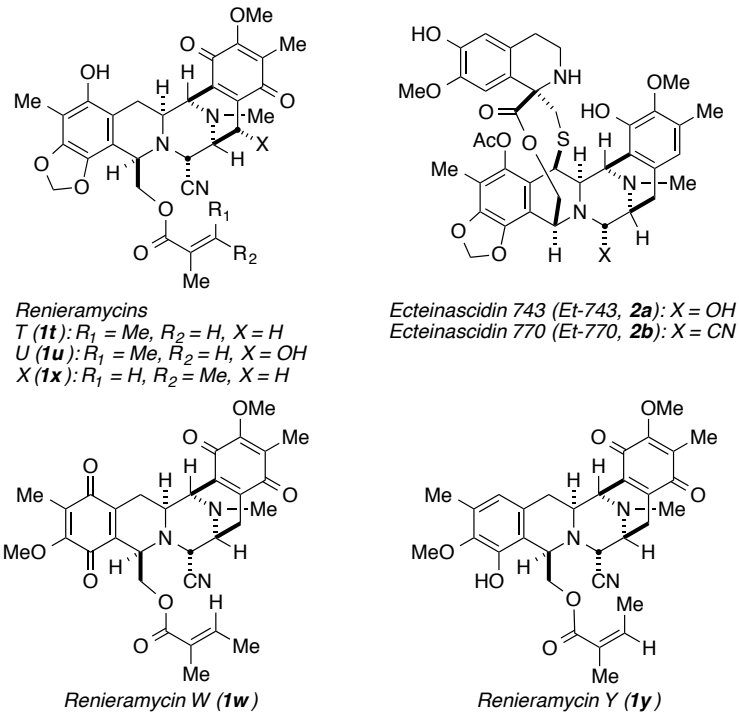
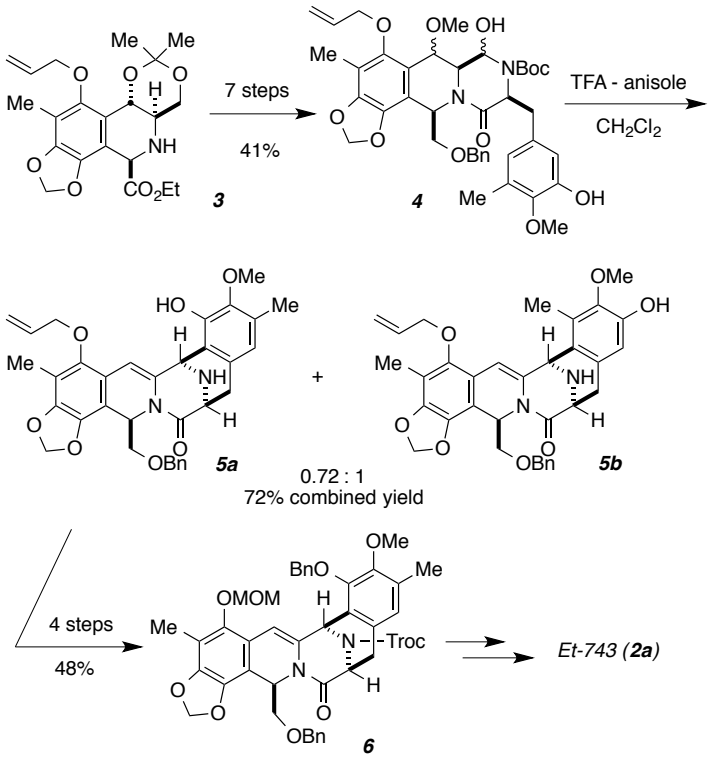


Figure 1. Antitumor THIQ marine natural products.

We previously reported a formal synthesis of **2a** (Scheme 1)[7] that commences with 1,3-*cis* tetrahydroisoquinoline **3**, which was obtained via an intramolecular 6-*endo* radical closure on a glyoxalimine, and was then coupled with a tyrosine derivative and further manipulated to provide **4**. Pentacyclic framework formation of **4** provided an unfavorable 0.72 : 1 ratio of regioisomers **5a** : **5b**. Following chromatographic separation, desired species **5a** was converted into compound **6** which intersects intermediates reported in the formal total synthesis of Et-743 by the Danishefsky laboratory,[8] which further relayed into the total synthesis of Et-743 reported by Fukuyama and co-workers.[9] We envisioned that this general strategy could be improved upon, particularly the non-regioselective Pictet-Spengler cyclization, to enable a more practical and efficient total synthesis of

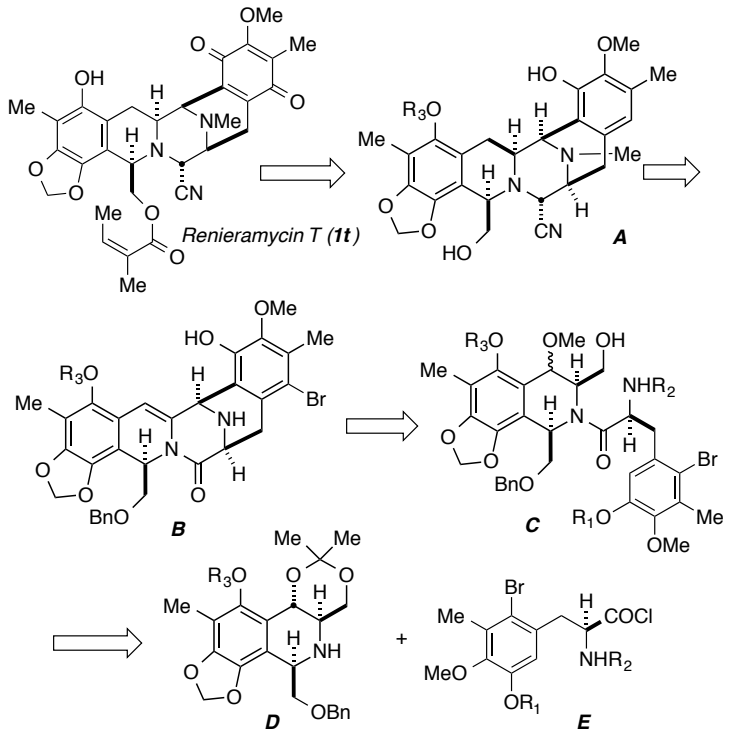
Et-743 and congeners. Herein, we report the successful realization of this goal to an enantioselective total synthesis of Renieramycin T (**1t**).



Scheme 1. Formal synthesis of Et-743.

As shown in Scheme 2, we envisioned that the final steps in the synthesis of Renieramycin T, would involve an esterification of the primary alcohol to install the angelate and late-stage oxidation to the quinone. An additional challenge, is the planned saturation of the alkene in dihydroisoquinoline **B** to the tetrahydroisoquinoline (**A**) that has proven hard and in some instances, impossible on structurally-related substrates under hydrogenation conditions. To overcome the regioselectivity problem encountered in our

first-generation synthesis, we designed substrate **C**, which has a bromine atom *para*- to the phenolic residue that would obviate the regioselectivity problem (*vide supra*). The construction of **C** was planned to proceed through the coupling of tetrahydroisoquinoline (**D**) and the bromo-substituted tyrosine derivative (**E**).

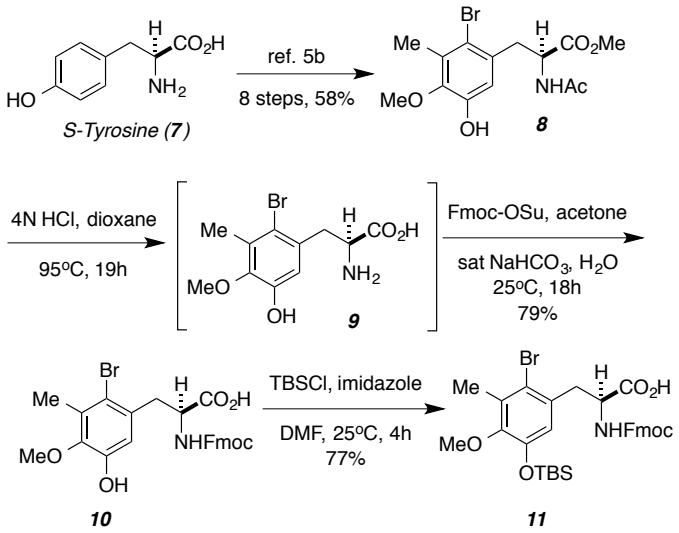


Scheme 2. Retrosynthetic analysis of Renieramycin T.

Results and Discussion

Our synthesis commences with methyl ester **8** which can be prepared from commercially available L-tyrosine according to a published procedure (Scheme 3).[5b] The brominated phenol **8** was submitted to hydrolysis under acidic conditions to provide amino acid **9**, that was subsequently Fmoc-protection to afford carboxylic acid **10** in 79% yield

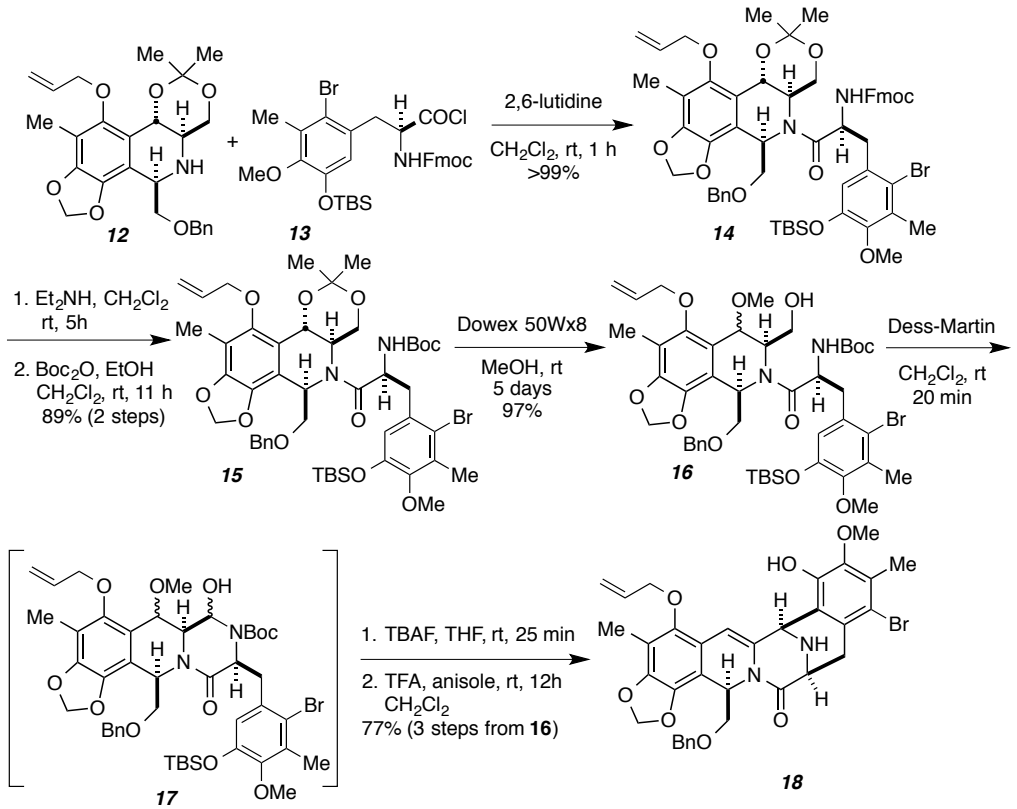
over the two steps. Finally, TBS protection of the phenolic group afforded carboxylic acid **11** which could used as a right hand segment of **1t**.



Scheme 3. Preparation of Tyrosine derivative **11**.

With the bromo-protected acid **11** in hand, our efforts were directed toward the preparation of a pentacyclic compound according to our previously reported procedure (Scheme 4).[7] Acylation of tetrahydroisoquinoline **12** was achieved via the *N*-Fmoc-protected amino acid chloride **13** to give amide **14** without epimerization. Treatment of **14** with diethylamine provided the corresponding primary amine, which was subjected to Boc-protection providing amide **15** in 89% yield. Deprotection of the acetonide group from **15** was accomplished with Dowex 50W-X8 cationic resin in methanol. Instead of providing the expected 1,3-diol product, this product incorporated methanol at the benzylic position via the incipient *ortho*-quinonemethide species. The

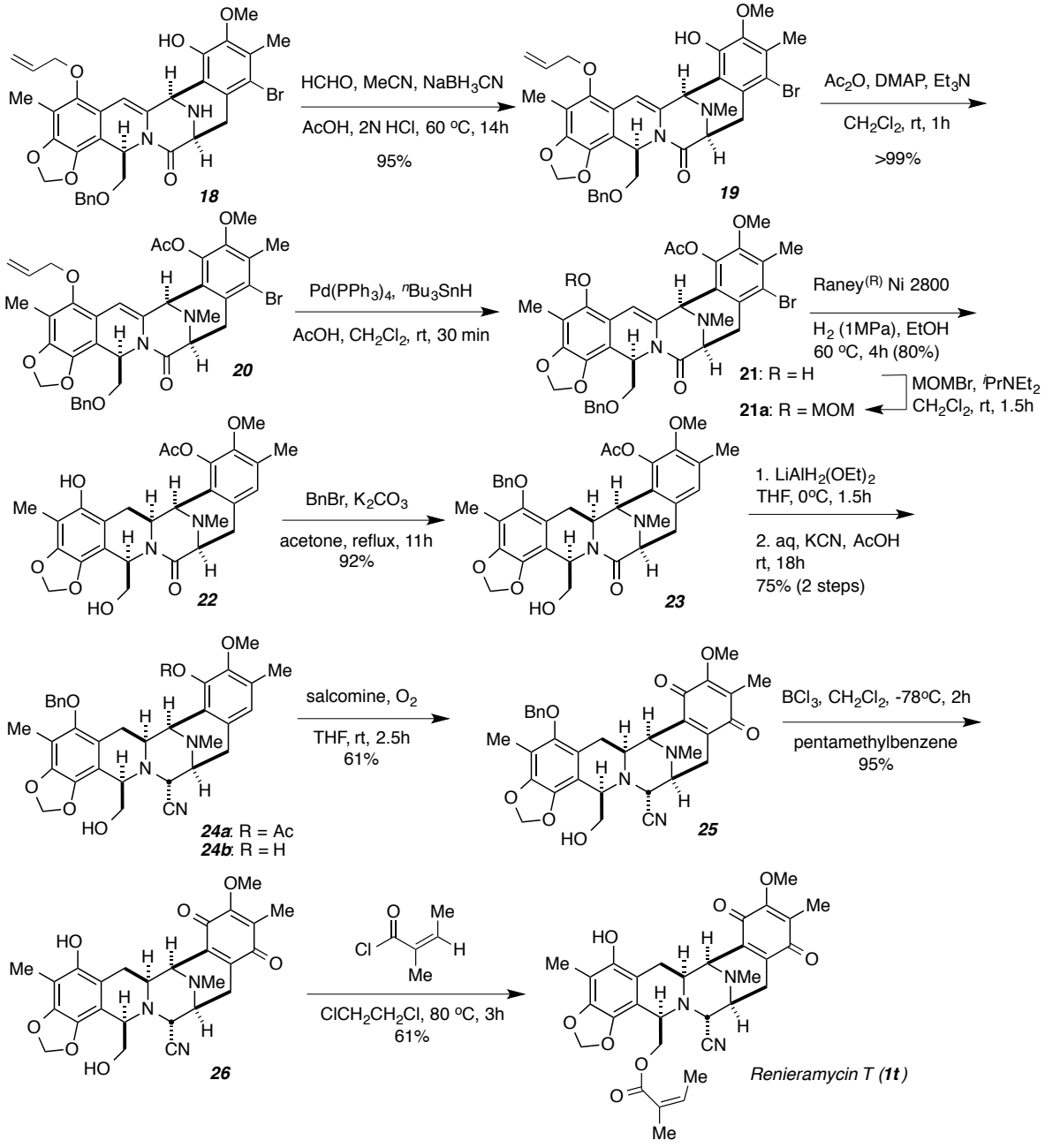
^{1}H-NMR spectra of methyl ether **16** was extremely complex due to amide and carbamate rotamers, which was more clearly resolved with increased temperature (140 °C), and was identified a single diastereomer. The relative stereochemistry of the benzylic methoxy group, being ultimately inconsequential, was not definitively assigned. With the alcohol **16** in hand, oxidation with Dess-Martin periodinane afforded the hemi-aminal compound **17**, whose ^{1}H-NMR spectra was also complicated by amide and carbamate rotamers. Hemi-aminal **17** was treated with TBAF to remove the TBS ether and the obtained crude material was directly treated with TFA in CH_2Cl_2 in the presence of anisole to afford the desired pentacyclic compound **18** (77% yield, three steps).



Scheme 4. Construction of pentacyclic core.

The final stages of the synthesis of Renieramycin T are illustrated in Scheme 5. Reductive amination of **18** resulted in *N*-methylation providing **19** in 95% yield. After introduction of an acetyl residue at the phenolic hydroxyl of **19**, removing the allyl group of **20** with tributyltin hydride, $(Ph_3P)PdCl_2$, and AcOH in THF afforded phenol **21**. Although compound **21** was homogeneous from TLC analysis, its ^{1}H-NMR spectrum is extremely complex and the structure was confirmed after conversion to the corresponding MOM ether **21a**. Reduction of the double bond of **21** under the action of hydrogen (1 MPa) on Raney® nickel,[10] proceeded stereoselectively delivering the desired relative stereochemistry which concomitantly resulted in cleavage of benzyl group and removal of the bromine atom to furnish **22** in 80% yield. After benzylation of the phenolic hydroxyl group of **22** gave **23**, we next investigated the conversion of lactam **23** into amino nitrile **24b** by following the procedure previously published in our model study.[11] The partial reduction of the lactam carbonyl of **23** with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al®) in THF, followed by the addition of aqueous KCN and AcOH unfortunately led to the recovery of unreacted **23**. After the extensive investigation, we found that the partial reduction of **23** with 5 equiv of $LiAlH_2(OEt)_2$,[12] followed by treatment with KCN and AcOH gave **24a** in 39% yield along with recovered **23** (38%). Increasing the amount of $LiAlH_2(OEt)_2$ (15 equiv) afforded desired **24b** in 75% yield. Oxidation of **24b** with O_2 in the presence of salcomine gave quinone **25** in 61% yield. The *O*-debenzylation of **25** under hydrogenolysis conditions (10% Pd/C, H_2) resulted in the reduction of the quinone to the corresponding hydroquinone, which was easily oxidized and restored to starting material **25** during the work up. On the other hand, the debenzylation was achieved smoothly with BCl_3 in the

presence of pentamethylbenzene at −78 °C to furnish the desired phenol **26** in 95% yield.[13] Finally, esterification of **26** with angeloyl chloride in 1,2-dichloroethane at 80 °C afforded (−)-Renieramycin T (**1t**). The spectroscopic data of synthetic **1t** are consistent with those for the natural product.



Scheme 5. Construction of Renieramycin T.

We reported that natural Renieramycin T (**1t**) showed moderate cytotoxicity to four human cancer cell lines, human colon carcinoma (HCT116), human lung carcinoma (QG56), human pancreatic adenocarcinoma (AsPC1) and human ductal breast epithelial tumor (T74D) with IC_{50} values of 0.039, 0.077, 0.098 and 0.0047 μM, respectively.[3] The compounds synthesized here, including natural Ecteinascidin 770 (**2b**),[14] were evaluated in terms of their inhibitory activity against two human cancer cell lines (Table 1).[15] The data revealed that the introduction of the cyano group at C-21 significantly enhances the *in vitro* cytotoxic activity within this series of compounds as expected. Moreover, we found that the introduction of a benzyl ether in the A-ring and angelate ester at B-ring side chain slightly increase the cytotoxicity to the both cell lines.

Table 1. Cytotoxicities of Renieramycin T and related compounds to human cancer cell lines

	IC_{50} ± SD (μM)	
Compound	HCT116[a]	DU145[a]
18	> 3	> 3
19	> 3	> 3
20	> 3	> 3
21	> 3	> 3
22	> 3	> 3
23	> 3	> 3
24b	1.3 ± 0.1	1.6 ± 0.1
25	0.8 ± 0.08	0.6 ± 0.03
26	2.6 ± 0.06	2.1 ± 0.09
Renieramycin T (**1t**)	$(9.6 \pm 0.4) \times 10^{-2}$	$(8.2 \pm 0.4) \times 10^{-2}$
Ecteinascidin 770 (**2b**)	$(3.2 \pm 0.3) \times 10^{-3}$	$(4.8 \pm 0.7) \times 10^{-3}$

[a] HCT116 = human colon carcinoma; DU145 = human prostate carcinoma

Conclusions

An enantioselective total synthesis of Renieramycin T has been accomplished in seventeen steps from readily available tetrahydroisoquinoline (**12**) and L-tyrosine derivative (**11**). Our synthesis features a concise approach for construction of the pentacyclic framework using the intramolecular Pictet-Spengler cyclization of compound **17**, which has the bromine atom *para*- to the phenolic hydroxyl group obviating the previous regioselectivity problem. We confirm that the introduction of a cyano group at the C-21 position is necessary for cytotoxicity and is consistent with the accepted modes of DNA-alkylation by this family of tetrahydroisoquinoline alkaloids.[1] Moreover, the protection of phenol hydroxy group at A-ring and acylation of alcohol at C-1 side chain enhanced the cytotoxicity of these compounds. We are currently exploring a more practical synthetic route that could be applied on larger scale to supply Renieramycin T for SAR studies.

Experimental Section

General procedures. ^{1}H- and ^{13}C-NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C; at 400 MHz for ^{1}H and 100 MHz for ^{13}C; at 300 MHz for ^{1}H and 75 MHz for ^{13}C (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 with a direct inlet system operating at 70 eV. High-resolution mass spectra were obtained on a double-focusing high-resolution magnetic-sector mass analyzer operating in a fast atom bombardment (FAB) mode or an electron impact (EI)

mode.

(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-bromo-5-hydroxy-4-methoxy-3-methylphenyl)propanoic acid (10). To a solution of compound **8** (15.65 g, 43.5 mmol) in dioxane (250 mL) was added 4N HCl (250 mL) and then heated at 95 °C for 19 h. The solvent was removed and the residue was suspended in saturated aqueous $NaHCO_3$ (900 mL) and acetone (450 mL), and the solution was added Fmoc-OSu (16.12 g, 47.8 mmol). Then the mixture was stirred at 25 °C for 18 h. The solvent was removed and the residue was acidified with 1N HCl aq. and extracted with $CHCl_3$ (3 × 1 L). The combined extracts were washed with water, dried, and concentrated in vacuo, and the residue was subjected to SiO_2 flash column chromatography with MeOH-$CHCl_3$ = 1:19 to afford **10** (17.95 g, 79%) as a colorless amorphous powder.

$[\alpha]_D^{25}$ +0.9 (c 1.0, CH_2Cl_2); IR (KBr) 3393, 3337, 2945, 1701, 1578, 1522, 1474, 1449, 1412, 1339, 1250, 1055, 1005, 760, 741 cm^{-1}; ^1H and ^{13}C NMR spectra are extremely complex due to carbamate rotamers. ^1H NMR (DMSO-d_6, 400MHz, 413 K) δ: 7.82 (2H, d, *J* = 7.8 Hz), 7.77 (2H, d, *J* = 7.8 Hz), 7.39 (2H, t, *J* = 7.8 Hz), 7.32 (2H, t, *J* = 7.8 Hz), 6.91 (1H, s), 6.18 (2H, s), 3.73 (3H, s), 3.72 (1H, overlapped), 3.60 (1H, dd, *J* = 8.3, 5.4 Hz), 3.25 (1H, dd, *J* = 14.6, 5.4 Hz), 2.81 (1H, dd, *J* = 14.6, 8.3 Hz), 2.30 (3H, s); EIMS *m/z* (%): 525 (1), 349 (7), 347 (7), 331 (7), 329 (7), 231 (65), 229 (66), 196 (17), 179 (37), 178 (100), 166 (39), 165 (62), 151 (10); HREIMS *m/z* 525.0784 (M^+, calcd for $C_{26}H_{24}NO_6Br$ 525.0787).

(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-bromo-5-((*tert*-butyldimethylsilyl)oxy)-4-methoxy-3-methylphenyl)propanoic acid (11). To a solution of compound

10 (526 mg, 1 mmol) in DMF (5 mL) was added TBSCl (452 mg, 3 mmol) and imidazole (408 mg, 6 mmol). The solution was stirred at 25 °C for 4 h. After dilution with H_2O (13 mL), the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined extracts were washed with water (2 × 10 mL), brine (10 mL), dried, and concentrated in vacuo, and the residue was subjected to SiO_2 flash column chromatography with MeOH-$CHCl_3$ = 1:49 to afford **11** (578.9 g, 77%) as a colorless amorphous powder.

$[\alpha]_D^{25}$ −2.92 (c 1.0, CH_2Cl_2); IR (KBr) 3325, 2955, 2930, 2859, 1717, 1522, 1472, 1450, 1417, 1341, 1325, 1254, 1231, 1080, 1011, 839 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.73 (2H, d, *J* = 7.1 Hz), 7.53 (2H, d, *J* = 7.1 Hz), 7.37 (2H, t, *J* = 7.1 Hz), 7.27 (2H, t, *J* = 7.1 Hz), 6.68 (1H, s), 5.37 (1H, br d, *J* = 7.6 Hz), 4.73 (1H, m), 4.30 (2H, d, *J* = 7.0 Hz), 4.16 (1H, t, *J* = 7.0 Hz), 3.68 (3H, s), 3.41 (1H, dd, *J* = 14.0, 4.8 Hz), 3.11 (1H, dd, *J* = 14.0, 9.4 Hz), 2.35 (3H, s), 0.97 (9H, s), 0.14 (6H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.0 (s), 156.0 (s), 149.3 (s), 147.9 (s), 143.7 (s), 141.2 (s), 133.3 (s), 131.3 (s), 127.7 (d), 127.1 (d), 125.2 (d), 120.9 (d), 119.9 (s), 119.4 (d), 67.3 (t), 60.1 (q), 54.1 (d), 47.0 (d), 38.2 (t), 25.6 (q), 18.2 (s), 17.1 (q), −4.6 (q); FABMS *m/z* 640 ($[M+H]^+$; HRFABMS *m/z* 640.1738 ($[M+H]^+$, calcd for $C_{32}H_{38}NO_6BrSi$ 640.1730).

(9*H*-Fluoren-9-yl)methyl (*S*)-1-((4*R*,5a*R*,9a*S*)-10-(allyloxy)-4-((benzyloxy)methyl)-8,8,11-trimethyl-4,5a,6,9a-tetrahydro-5H-[1,3]dioxino[5,4-*c*][1,3]dioxolo[4,5-*h*]isoquinolin-5-yl)-3-(2-bromo-5-((*tert*-butyldimethylsilyl)oxy)-4-methoxy-3-methylphenyl)-1-oxopropan-2-yl)carbamate (14). Fmoc amino acid **11** (868 mg, 1.15 mmol) was dissolved in CH_2Cl_2 (8.5 mL) and oxalyl chloride (2.1 mL, 24.6mmol) at room temperature under Ar, to which was added dry DMF (10 μL) drop-wise. After stirring for 1

h, the solution was concentrated and dried under high vacuum. The acid chloride was dissolved in CH_2Cl_2 (6 mL) and cooled to 0 °C. To this was added a solution of THIQ **12** (372 mg, 0.821 mmol) and 2,6-lutidine (133 μL, 1.15mmol) in CH_2Cl_2 (11 mL) drop-wise. The reaction was stirred 3 h, and then quenched with aq. saturated $NaHCO_3$ solution (20 mL) and extracted to $CHCl_3$ (20 mL x 3). The combined extracts were dried over $MgSO_4$, filtered and concentrated (1.23 g, pale brown amorphous), and the residue was subjected to SiO_2 flash column chromatography (*n*-hexane−EtOAc = 3:1) to provide peptide **14** (883 mg, 100%) as a colorless amorphous powder.

Rf = 0.30 (hexanes−EtOAc = 3:1); $[\alpha]_D^{24}$ −30.5 (c 1.0, $CHCl_3$); IR (KBr) 3296, 2930, 2859, 1721, 1647, 1420, 1400, 1252, 1225, 1103, 1011, 839 cm^{-1}; ^1H and ^{13}C NMR spectra are extremely complex due to carbamate rotamers. ^1H-NMR (DMSO-d_6, 400MHz, 413 K) δ: 7.83 (2H, d *J* = 7.6 Hz), 7.61 (2H, t, *J* = 7.6 Hz), 7.38 (2H, td, *J* = 7.6, 4.4 Hz), 7.28-7.22 (7H, m), 6.82 (1H, s, Ar-H), 6.02 (1H, ddt, *J* = 17.4, 10.7, 5.4 Hz, -OAllyl), 5.86 (1H, s, -OCH_2O-), 5.72 (1H, s, -OCH_2O-), 5.60 (1H, t, *J* = 5.9 Hz), 5.33 (1H, d, *J* = 9.8 Hz), 5.30 (1H, dq, *J* = 17.4, 1.7 Hz, -OAllyl), 5.16 (1H, dq, *J* = 10.7, 1.7 Hz, -OAllyl), 4.96 (1H, dd, *J* = 15.6, 7.3 Hz), 4.42 (2H, s), 4.29-4.19 (5H, m), 4.27-4.15 (2H, m, -OAllyl), 4.13 (1H, t, *J* = 7.1 Hz), 3.64 (3H, s, $ArOCH_3$), 3.62-3.51 (2H, m), 3.11 (1H, dd, *J* = 14.0, 7.1 Hz), 3.05 (1H, dd, *J* = 14.0, 7.1 Hz), 2.25 (3H, s, Ar-CH_3), 2.04 (3H, s, Ar-CH_3), 1.40 (3H, s, -$OC(CH_3)_2O$-), 1.33 (3H, s, -$OC(CH_3)_2O$-), 0.99 (9H, s, -OTBS), 0.19 (6H, s, -OTBS); FABMS *m/z* 1075 ($[M+H]^+$; HRFABMS *m/z* 1075.3781 ($[M+H]^+$, calcd for $C_{58}H_{68}N_2O_{11}BrSi$ 1075.3770).

***Tert*-butyl ((*S*)-1-((4*R*,5a*R*,9a*S*)-10-(allyloxy)-4-((benzyloxy)methyl)-8,8,11-**

trimethyl-4,5a,6,9a-tetrahydro-5*H*-[1,3]dioxino[5,4-*c*][1,3]dioxolo[4,5-*h*]isoquinolin-5-yl)-3-(2-bromo-5-((tert-butyldimethylsilyl)oxy)-4-methoxy-3-methylphenyl)-1-oxopropan-2-yl)carbamate (15). Fmoc-Peptide **14** (1.04 g, 966 μmol) was dissolved in CH_2Cl_2 (10 mL) and diethylamine (3.5 mL) was added and stirred for 5 h. TLC (hexanes−EtOAc = 3:1) shows complete consumption of starting material and a clean new spot positive by ninhydrin test. The solution was concentrated and dried under high vacuum. The crude amine was dissolved in EtOH : CH_2Cl_2 (10 : 3.5 mL). Boc_2O (1.1 mL, 4.83 mmol) was added in one portion and the mixture was stirred for 11 h, then concentrated and purified by SiO_2 flash chromatography (hexanes−EtOAc = 6:1). The Boc protected peptide **15** was obtained as a colorless amorphous powder (823 mg, 89%).

Rf = 0.56 (Hexanes−EtOAc = 3:1); $[\alpha]_D^{23}$ −37.0 (c 0.9, $CHCl_3$); IR (KBr) 3308, 2932, 2859, 1717, 1653, 1497, 1472, 1366, 1252, 1167, 1117, 839 cm^{-1}; ^1H and ^{13}C NMR spectra are extremely complex due to carbamate rotamers; ^1H-NMR (DMSO-d_6, 400MHz, 413 K) δ: 7.30-7.19 (5H, m, -OBn), 6.79 (1H, s, Ar-H), 6.28 (1H, br s), 6.08 (1H, m, -OAllyl), 5.93 (1H, br s, -OCH_2O-), 5.88 (1H, br s, -OCH_2O-), 5.56 (1H, br t, *J* = 5.7 Hz), 5.36 (1H, br d, *J* = 9.4 Hz), 5.33-5.27 (1H, m, -OAllyl), 5.19-5.15 (1H, m, -OAllyl), 4.88 (1H, br dd, *J* = 15.1, 8.2 Hz), 4.45 (3H, s, Ar-OCH_3), 4.28-4.17 (2H, m, -OAllyl), 3.67 (3H, br s), 3.64 (1H, br s), 3.62-3.58 (1H, m), 3.53 (2H, br d, *J* = 8.2 Hz), 3.05 (1H, dd, *J* = 13.2, 6.4 Hz), 2.95 (1H, dd, *J* = 13.2, 7.8 Hz), 2.27 (3H, s, $ArCH_3$), 2.07 (3H, s, $ArCH_3$), 1.40 (3H, s, -$OC(CH_3)_2O$-), 1.34 (3H, s, -$OC(CH_3)_2O$-), 1.32 (9H, s, -Boc), 1.02 (9H, s, -OTBS), 0.22 (6H, s, -OTBS); FABMS *m/z* 953 $[M+H]^+$; HRFABMS *m/z* 953.3612 ($[M+H]^+$, calcd for $C_{48}H_{66}N_2O_{11}BrSi$ 953.3614).

***Tert*-butyl ((2*S*)-1-((7*R*,9*R*)-5-(allyloxy)-9-((benzyloxy)methyl)-7-(hydroxymethyl)-6-methoxy-4-methyl-6,9-dihydro-[1,3]dioxolo[4,5-*h*]isoquinolin-8(7*H*)-yl)-3-(2-bromo-5-((*tert*-butyldimethylsilyl)oxy)-4-methoxy-3-methylphenyl)-1-oxopropan-2-yl)carbamate (16).** The Boc protected peptide **15** (189 mg, 198 μmol) was dissolved in dry MeOH (3.4 mL) to which was added Dowex 50W-X8 resin (190 mg). The reaction was stirred for 5 days and then passed through a Celite pad (eluting with MeOH and CH_2Cl_2) and the filtrate was concentrated and purified by SiO_2 flash chromatography (*n*-hexane−EtOAc = 3:1) to provide methyl ether **16** (140 mg, 97%) as colorless amorphpus powder. Rf = 0.38 (Hexanes−EtOAc = 2:1); $[\alpha]_D^{23}$ +44.0 (c 1.0, $CHCl_3$); IR (KBr) 3435, 2932, 2889, 1717, 1647, 1472, 1422, 1098, 841 cm^{-1}; ^1H and ^{13}C NMR spectra are extremely complex due to carbamate rotamers; ^1H-NMR (DMSO-d_6, 400MHz, 413 K) δ: 7.30-7.19 (5H, m, -OBn), 6.86 (1H, s, Ar-H), 6.18-6.08 (2H, m), 5.91 (2H, br s, -OCH_2O-), 5.60 (1H, br s,), 5.41 (1H, br d, *J* = 16.9 Hz, -OAllyl), 5.25 (1H, br d, *J* = 10.3 Hz, -OAllyl), 5.08-4.95 (1H, br s), 4.70 (1H, br s), 4.60-4.52 (1H, m), 4.51-4.44 (1H, m), 4.43-4.35 (2H, m), 4.34-4.29 (1H, m), 4.16-4.06 (1H, m), 4.02-3.91 (1H, m), 3.85 (1H, m), 3.69 (3H, s, $ArOCH_3$), 3.61-3.35 (2H, m), 3.25 (3H, s, -OCH_3), 3.08-2.89 (1H, m), 2.31 (3H, s, $ArCH_3$), 2.14 (3H, s, $ArCH_3$), 1.30 (9H, br s, -Boc), 1.01 (9H, s, -OTBS), 0.21 (6H, s, -OTBS); FABMS *m/z* 927 $[M+H]^+$; HRFABMS *m/z* 927.3455 ($[M+H]^+$, calcd for $C_{46}H_{64}N_2O_{11}BrSi$ 927.3457).

(7*R*,13*S*,16*R*)-5-(Allyloxy)-16-((benzyloxy)methyl)-11-bromo-8-hydroxy-9-methoxy-4,10-dimethyl-7,12,13,16-tetrahydro-14*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinolin-14-one (18). The Boc protected peptide **16** (385 mg, 415 μmol) was dissolved in dry CH_2Cl_2 (9 mL) to which was added Dess-Martin (352 mg, 830

μmol). The reaction was stirred for 20 min, and then quenched with aqueous saturated $Na_2S_2O_3$ solution (9 mL) and then diluted with aqueous saturated $NaHCO_3$ solution (9 mL). The mixture was extracted to Et_2O (3 × 50 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated (401 mg, colorless amorphous powder). The obtained material was dissolved in THF (9 mL), to which was added TBAF (1.0 M in THF, 415 μL, 415 μmol) with stirring. The reaction was stirred for 25 min., and then the reaction mixture was filtered through a short pad of SiO_2 and eluted with EtOAc. The filtrate was concentrated (354 mg, pale yellow amorphous). The obtained material was dissolved in CH_2Cl_2 (5 mL), to which was added TFA (5 mL) and anisole (451 μL, 4.15 mmol) with stirring. The reaction was stirred for 12 h, and then the reaction mixture was diluted with CH_2Cl_2 (50 mL) and quenched with saturated $NaHCO_3$ solution (50 mL) and extracted to $CHCl_3$ (3 × 50 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated. The residue was purified by SiO_2 flash chromatography (^{i}PrOH−*n*-Hexane = 1:6) to provide pentacyclic compound **18** (210 mg, 77%) as a yellow amorphous powder.

Rf = 0.39 (20% ^{i}PrOH in Hexanes); $[\alpha]_D^{23}$ +95.02 (c 1.0, $CHCl_3$); IR (KBr) 3306, 2936, 2862, 1672, 1634, 1456, 1408, 1364, 1287, 1236, 1111, 1088, 984 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.26-7.16 (m, 3H, Ph), 6.95-6.93 (m, 2H, Ph), 6.23 (s, 1H, 6-H), 6.14 (ddt, *J* = 16.0, 10.4, 5.6 Hz, 1H, $OCH_2C\underline{H}=CH_2$), 6.03 (dd, *J* = 6.8, 4.9 Hz, 1H, 16-H), 5.87 (d, *J* = 1.2 Hz, 1H, 2-H), 5.84 (d, *J* = 1.2 Hz, 1H, 2-H), 5.47 (dq, *J* = 17.2, 1.6 Hz, 1H, $OCH_2CH=C\underline{H_2}$), 5.31 (dq, *J* = 10.5, 1.5 Hz, 1H, $OCH_2CH=C\underline{H_2}$), 4.96 (s, 1H, 7-H), 4.32 (tt, *J* = 5.3, 1.3 Hz, 1H, $OC\underline{H_2}CH=CH_2$), 4.14 (d, *J* = 6.1 Hz, 1H, 13-H), 4.00 (d, *J* = 12.2 Hz, 1H, OBn), 3.86 (d, *J* = 12.2 Hz, 1H, OBn), 3.63 (s, 3H, 9-OCH_3), 3.21 (dd, *J* = 10.5, 4.9 Hz,

1H, 18-H), 3.20 (dd, *J* = 17.4, 1.5 Hz, 1H, 12-H), 3.13 (dd, *J* = 10.6, 6.8 Hz, 1H, 18-H), 3.13 (dd, *J* = 17.4, 6.7 Hz, 1H, 12-H), 2.27 (s, 3H, 10-CH_3), 2.12 (s, 3H, 4-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.3 (s, C-14), 147.5 (s, C-5), 145.6 (s, C-3), 144.9 (s, C-8), 143.9 (s, C-9), 139.5 (s, C-1), 138.3 (s, Ph), 133.7 (d, $OCH_2\underline{C}H{=}CH_2$), 133.7 (s, C-6a), 130.3 (s, C-11), 129.1 (s, C-11a), 128.1 (s, Ph), 127.0 (s, Ph), 126.8 (s, Ph), 121.2 (s, C-7a), 118.0 (s, C-10), 117.6 (d, $OCH_2CH{=}\underline{C}H_2$), 117.2 (s, C-5a), 112.8 (s, C-4), 108.6 (s, C-16a), 101.3 (t, C-2), 100.5 (d, C-6), 75.1 (t, $O\underline{C}H_2CH{=}CH_2$), 72.7 (d, Bn), 70.0 (t, C-18), 61.1 (q, 9-OCH_3), 54.4 (d, C-13), 49.8 (d, C-7), 46.9 (d, C-16), 35.6 (t, C-12), 16.6 (q, 10-CH_3), 9.3 (q, 4-CH_3); EIMS *m/z* (%): 660 (M^+, 27), 541 (72), 539 (67), 513 (100), 511 (97), 433 (46), 270 (49), 268 (50), 257 (74), 203 (45); HREIMS *m/z* 660.1467 (M^+, calcd for $C_{34}H_{33}N_2O_7Br$: 660.1471).

(7*R*,13*S*,16*R*)-5-(Allyloxy)-16-((benzyloxy)methyl)-11-bromo-8-hydroxy-9-methoxy-4,10,17-trimethyl-7,12,13,16-tetrahydro-14*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinolin-14-one (19). To a stirred solution of amine **18** (119 mg, 180 μmol) in MeCN (6.0 mL) was added 37% aqueous solution of HCHO (265 μL, 3.6 mmol). The reaction mixture was stirred for 15 min, after which $NaCNBH_3$ (113 mg, 1.80 mmol) was added. The reaction mixture was stirred for 15 min, after which AcOH (103 μL, 1.80 mmol) was added dropwise over 3 min. The reaction mixture was stirred for 5 min, after which 2N HCl (10 mL) was added one portion. The reaction was heated to 60 °C and was stirred for 14 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and quenched with saturated $NaHCO_3$ solution (50 mL) and extracted to CH_2Cl_2 (3 × 50 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated (120 mg, pale yellow

amorphous). The residue was purified by SiO_2 flash chromatography (*n*-hexane−EtOAc = 3:1) to provide *N*-methyl compound **19** (114 mg, 95%) as a pale yellow amorphous powder.

Rf = 0.44 (n-hexane−EtOAc = 1:1); $[\alpha]_D^{23}$ +78.4 (c 1.0, $CHCl_3$); IR (KBr) 2940, 2860, 1776, 1676, 1634, 1458, 1408, 1368, 1287, 1236, 1192, 1128, 1088, 997 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.24−7.16 (m, 3H, Bn-H), 6.95 (d, *J* = 6.4 Hz, 2H, Bn-H), 6.28 (s, 1H, 6-H), 6.15 (ddt, *J* = 17.2, 10.5, 5.7 Hz, 1H, $OCH_2CH{=}CH_2$), 6.07 (dd, *J* = 7.1, 4.1 Hz, 1H, 16-H), 5.88 (d, , *J* = 1.4 Hz, 1H, 2-H), 5.85 (d, , *J* = 1.4 Hz, 1H, 2-H), 5.79 (br s, 1H, 8-OH), 5.45 (dq, *J* = 17.1, 1.4 Hz, 1H, $OCH_2CH{=}CH_2$), 5.31 (dq, *J* = 10.4, 1.4 Hz, 1H, $OCH_2CH{=}CH_2$), 4.62 (d, *J* = 1.1 Hz, 1H, 7-H), 4.33 (dt, *J* = 5.7, 1.4 Hz, 2H, $OCH_2CH{=}CH_2$), 4.00 (d, *J* = 12.4 Hz, 1H, Bn), 3.82 (d, *J* = 12.4 Hz, 1H, Bn), 3.73 (td, *J* = 4.8, 1.1 Hz, 1H, 13-H), 3.64 (s, 3H, 9-OMe), 3.20 (dd, *J* = 10.5, 4.1 Hz, 1H, 18-H), 3.17 (d, *J* = 4.8 Hz, 2H, 12-H), 3.11 (dd, *J* = 10.5, 7.1 Hz, 1H, 18-H), 2.55 (s, 3H, N-Me), 2.27 (s, 3H, 10-Me), 2.13 (s, 3H, 4-Me); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.7 (s, C-14), 147.4 (s, C-5), 145.7 (s, C-3), 144.9 (s, C-8), 143.9 (s, C-9), 139.5 (s, C-1), 138.3 (s, Bn), 133.7 (d, $OCH_2CH{=}CH_2$), 130.4 (s, C-6a), 130.2 (s, C-10), 128.9 (s, C-11a), 128.1 (d, Bn), 127.1 (d, Bn), 126.9 (d, Bn), 121.2 (s, C-7a), 117.8 (s, C-11 and t, $OCH_2CH{=}CH_2$, overlapped), 117.0 (s, C-5a), 112.9 (s, C-4), 108.7 (s, C-16a), 103.4 (d, C-6), 101.4 (t, C-2), 75.2 (t, $OCH_2CH{=}CH_2$), 72.7 (t, Bn), 70.2 (t, C-18), 61.2 (q, 9-OMe), 61.0 (d, C-13), 56.4 (d, C-7), 46.8 (d, C-16), 41.3 (q, *N*-Me), 35.3 (t, C-12), 16.7 (q, 10-Me), 9.3 (q, 4-Me); EIMS *m/z* (%): 674 (M^+, 22%), 676 (M^++2, 24), 555 (29), 553 (28), 527 (100), 525 (95), 486 (14), 484 (13), 284 (81), 282 (79), 269 (20), 267 (20); HREIMS *m/z* 674.1624 (M^+, calcd for $C_{35}H_{35}N_2O_7Br$:

674.1628).

(7*R*,13*S*,16*R*)-5-(Allyloxy)-16-((benzyloxy)methyl)-11-bromo-9-methoxy-4,10,17-trimethyl-14-oxo-7,13,14,16-tetrahydro-12*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinolin-8-yl Acetate (20). To a stirred solution of amine **19** (191 mg, 283 μmol) in CH_2Cl_2 (7 mL) was added DMAP (6.9 mg, 56.7 μmol) and Ac_2O (134 μL, 1.42 mmol) at room temperature. The reaction mixture was stirred for 1 h, after which the reaction mixture was diluted with CH_2Cl_2 (50 mL) and quenched with saturated $NaHCO_3$ solution (50 mL) and was extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated (209 mg, pale yellow amorphous). The residue was purified by SiO_2 flash chromatography (MeOH−$CHCl_3$ 1:49) to provide the acetate **20** (203 mg, 100%) as a pale yellow amorphous powder.

Rf = 0.29 (MeOH−$CHCl_3$ 1:49); $[\alpha]_D^{23}$ +91.5 (c 1.0, $CHCl_3$); IR (KBr) 2940, 2860, 1776, 1676, 1458, 1408, 1368, 1287, 1236, 1192, 1128, 1088, 997 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.26−7.14 (m, 3H, Bn-H), 7.02 (d, *J* = 7.8 Hz, 2H, Bn-H), 6.10 (m, 1H, $OCH_2C\underline{H}=CH_2$), 6.09 (dd, *J* = 10.3, 4.8 Hz, 1H, 16-H), 6.05 (s, 1H, 6-H), 5.87 (d, *J* = 1.4 Hz, 1H, 2-H), 5.85 (d, *J* = 1.4 Hz, 1H, 2-H), 5.47 (dt, *J* = 17.2, 1.6 Hz, 1H, $OCH_2CH=C\underline{H_2}$), 5.32 (d, *J* = 10.5 Hz, 1H, $OCH_2CH=C\underline{H_2}$), 4.41 (s, 1H, 7-H), 4.34 (dd, *J* = 12.7, 5.0 Hz, 1H, $OC\underline{H_2}CH=CH_2$), 4.26 (dd, *J* = 12.7, 5.4 Hz, 1H, $OC\underline{H_2}CH=CH_2$), 3.97 (d, *J* = 12.4 Hz, 1H, Bn), 3.74 (t, *J* = 4.2 Hz, 1H, 13-H), 3.72 (d, *J* = 12.4 Hz, 1H, Bn), 3.67 (s, 3H, 9-OMe), 3.19 (d, *J* = 4.2 Hz, 2H, 12-H), 3.18 (dd, *J* = 10.3, 4.8 Hz, 1H, 18-H), 3.08 (t, *J* = 10.3 Hz, 1H, 18-H), 2.53 (s, 3H, *N*-Me), 2.38 (s, 3H, OAc), 2.28 (s, 3H, 10-CH_3), 2.12 (s, 3H, 4-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.8 (s, OAc), 167.6 (s, C-14), 148.9 (s, C-9),

147.4 (s, C-5), 146.0 (s, C-3), 140.7 (s, C-8), 139.6 (s, C-1), 138.4 (s, Bn), 133.6 (d, $OCH_2\underline{C}H{=}CH_2$), 132.4 (s, C-10), 130.3 (s, C-6a), 129.3 (d, C-11a), 128.1 (d, Bn), 127.2 (s, C-7a), 127.0 (d, Bn), 126.9 (d, Bn), 125.4 (s, C-11), 117.3 (t, $OCH_2CH{=}\underline{C}H_2$), 116.3 (s, C-5a), 112.9 (s, C-4), 108.7 (s, C-16a), 103.0 (d, C-6), 101.5 (t, C-2), 74.8 (t, $O\underline{C}H_2CH{=}CH_2$), 72.4 (t, Bn), 69.6 (t, C-18), 61.0 (q, 9-OMe), 60.9 (d, C-13), 57.1 (d, C-7), 46.5 (d, C-16), 41.2 (q, *N*-Me), 35.5 (t, C-12), 20.9 (s, OAc), 16.7 (q, 10-CH_3), 9.2 (q, 4-CH_3); EIMS *m/z* (%): 716 (M^+, 19%), 718 (M^++2, 21), 597 (31), 595 (29), 569 (100), 567 (96), 528 (23), 526 (21), 326 (22), 324 (23), 284 (46), 282 (48).; HREIMS *m/z* 716.1736 (M^+, calcd for $C_{37}H_{37}N_2O_8Br$: 716.1733).

(7*R*,13*S*,16*R*)-16-((Benzyloxy)methyl)-11-bromo-5-hydroxy-9-methoxy-4,10,17-trimethyl-14-oxo-7,13,14,16-tetrahydro-12*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinolin-8-yl Acetate (21). To a mixture of **20** (201 mg, 280 μmol), AcOH (48 μL, 841 μmol) and $Pd(PPh_3)_4$ (16.2 mg, 14 μmol) in CH_2Cl_2 (20 mL) was added Bu_3SnH (151 μL, 561 μmol) and stirred at room temperature for 30 min. The reaction mixture was concentrated and the residue was dissolved in Et_2O. The mixture was filtered through a short pad of celite, and the obtained filtrate was concentrated again. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$ solution (30 mL) and extracted to $CHCl_3$ (3 × 50 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated (430 mg, pale yellow amorphous). The residue was purified by SiO_2 flash chromatography (MeOH−$CHCl_3$ 1:99) to provide phenol **21** (189 mg, 99%) as a pale orange amorphous powder.

Rf = 0.33 (MeOH−$CHCl_3$ = 1:19); $[\alpha]_D^{22}$ −189.5 (c 0.9, $CHCl_3$); IR (KBr) 3391, 2941, 2872,

1776, 1636, 1616, 1460, 1435,1420,1369, 1292, 1234, 1192, 1125, 1094, 758 cm^{-1}; ^1H and ^{13}C NMR spectra are extremely complex due to nebulous reasons. This structure was confirmed after the protection with MOM ether shown below. EIMS *m/z* (%): 676 (M^+, 23%), 678 (M^++2, 24), 557 (16), 555 (16), 529 (100), 527 (97), 484 (39), 482 (40); HREIMS *m/z* 676.1419 (M^+, calcd for $C_{34}H_{33}N_2O_8Br$ 676.1420).

(7*R*,13*S*,16*R*)-16-((Benzyloxy)methyl)-11-bromo-9-methoxy-5-(methoxymethoxy)-4,10,17-trimethyl-14-oxo-7,13,14,16-tetrahydro-12*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinolin-8-yl acetate (21a). To a solution of **21** (74.4 mg, 109.9 μmol) in CH_2Cl_2 (8.5 mL) was added $^iPr_2NEt$ (48 μL, 274.7 μmol, 2.5 eq.) and MOMBr (22.5 μL, 274.7 μmol, 2.5 eq.) and stirred at 25 °C for 1.5 h. The reaction material was diluted in CH_2Cl_2 (20 mL) and washed with saturated $NaHCO_3$ solution (20 mL) and extracted to $CHCl_3$ (3 × 20 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated (72.2 mg, pale yellow amorphous). The residue was purified by SiO_2 flash chromatography (*n*-Hexane−AcOEt = 1:1) to provide compound **21a** (66.8 mg, 84%) as a pale yellow amorphous powder.

$[\alpha]_D^{25}$ +97.4 (c 0.9, $CHCl_3$); IR (KBr) 2940, 2903, 1776, 1674, 1636, 1458, 1435, 1368, 1287, 1238, 1192, 1128, 1053, 968 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.26−7.14 (m, 3H, Bn-H), 7.01 (d, *J* = 7.1 Hz, 2H, Bn-H), 6.11 (s, 1H, 6-H), 6.09 (dd, *J* = 8.9, 4.8 Hz, 1H, 16-H), 5.87 (d, *J* = 1.4 Hz, 1H, 2-H), 5.85 (d, , *J* = 1.4 Hz, 1H, 2-H), 4.96 (d, *J* = 5.7 Hz, 1H, $OC\underline{H_2}OCH_3$), 4.93 (d, *J* = 5.7 Hz, 1H, $OC\underline{H_2}OCH_3$), 4.42 (s, 1H, 7-H), 3.97 (d, *J* = 12.4 Hz, 1H, Bn), 3.73 (d, *J* = 12.4 Hz, 1H, Bn), 3.72 (m, 1H, 13-H), 3.67 (s, 3H, 9-OCH_3), 3.61 (s, 3H, $OCH_2OC\underline{H_3}$), 3.19 (d, *J* = 4.6 Hz, 2H, 12-H), 3.18 (dd, *J* = 10.3, 4.8 Hz, 1H, 18-H),

3.09 (dd, *J* = 10.3, 8.9 Hz, 1H, 18-H), 2.53 (s, 3H, *N*-Me), 2.41 (s, 3H, OAc), 2.28 (s, 3H, 10-CH_3), 2.14 (s, 3H, 4-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.8 (s, OAc), 167.5 (s, C-14), 148.8 (s, C-9), 146.1 (s, C-5), 146.0 (s, C-3), 140.7 (s, C-8), 139.8 (s, C-1), 138.4 (s, Bn), 132.4 (s, C-10), 130.3 (s, C-6a), 129.3 (s, C-11a), 128.1 (d, Bn), 127.2 (s, C-7a), 126.9 (d, Bn x 2, overlapped), 125.4 (s, C-11), 116.6 (s, C-5a), 113.0 (s, C-4), 108.8 (s, C-16a), 103.3 (d, C-6), 101.5 (t, C-2), 100.1 (t, O$\underline{C}H_2OCH_3$), 72.4 (t, Bn), 69.5 (t, C-18), 60.9 (q, 9-OCH_3 and d, C-13, overlapped), 57.9 (q, $OCH_2O\underline{C}H_3$), 57.1 (d, C-7), 46.5 (d, C-16), 41.2 (q, *N*-Me), 35.6 (t, C-12), 20.7 (q, OAc), 16.7 (q, 10-CH_3), 9.6 (q, 4-CH_3); EIMS *m/z* (%): 720 (M^+, 19%), 722 (M^++2, 21), 601 (21), 599 (20), 573 (100), 571 (97), 326 (12), 324 (12), 284 (31), 282 (32); HREIMS *m/z* 720.1678 (M^+, calcd for $C_{36}H_{37}N_2O_9Br$ 720.1682).

(6a*S*,7*R*,13*S*,16*R*)-5-Hydroxy-16-(hydroxymethyl)-9-methoxy-4,10,17-trimethyl-14-oxo-6,6a,7,13,14,16-hexahydro-12*H*-7,13-epiminobenzo[4,5]azocino[1,2-b][1,3]dioxolo[4,5-*h*]isoquinolin-8-yl Acetate (22). To a solution of **21** (62.8 mg, 92.7 μmol) in EtOH (4 mL) was added a slurry of Raney Ni 2800 (530 mg of commercially available water slurry, washed with absolute EtOH 3 x 1 mL) and suspended with EtOH (7 mL). The reaction mixture was stirred under H_2 (1 MPa) at 60 °C for 4 h. The reaction mixture was diluted with EtOAc (25 mL) and 1.2 M Rochell's salt aq. (25 mL), and the mixture was stirred for 2 h. The reaction mixture was filtered through a short pad of Celite, rinsed with $CHCl_3$. The filtrate was extracted with $CHCl_3$ (3 × 25 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated. By checking the TLC analysis, a starting material **21** was still remained in the residue, the obtained residue was dissolved in EtOH (4 mL) and was added a slurry of Raney Ni 2800 (530 mg of commercially available water

slurry, washed with absolute EtOH (3 x 1 mL) and suspended with EtOH (7 mL). The reaction mixture was stirred under H_2 (1 MPa) at 60 °C for 4 h. The reaction mixture was diluted with EtOAc (25 mL) and 1.2 M Rochell's salt aq. (25 mL), and the mixture was stirred for 2 h. The reaction mixture was filtered through a short pad of Celite, washed with $CHCl_3$. The combined filtrates were extracted with $CHCl_3$ (3 × 25 mL). The combined extarcts were dried over Na_2SO_4, filtered and concentrated. The residue was purified by SiO_2 flash chromatography (*i*-PrOH−*n*-Hexane = 1:2) to provide compound **22** (37.8 mg, 80%) as a pale yellow gummy solid.

$[\alpha]_D^{23}$ −122.2 (c 0.7, $CHCl_3$); IR (KBr) 3374, 2934, 1773, 1636, 1437, 1238, 1196, 1103, 1061, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.90 (s, 1H, 11-H), 5.93 (s, 1H, 2-H), 5.88 (s, 1H, 2-H), 5.46 (dd, *J* = 6.8, 3.9 Hz, 1H, 16-H), 3.86 (br d, *J* = 10.8 Hz, 1H, 6a-H), 3.85 (br s, 1H, 7-H), 3.75 (d, *J* = 6.8 Hz, 1H, 13-H), 3.73 (s, 3H, 9-OMe), 3.47 (dd, *J* = 11.2, 3.9 Hz, 1H, 18-H), 3.31 (dd, *J* = 11.2, 6.8 Hz, 1H, 18-H), 3.27 (br d, *J* = 16.6 Hz, 1H, 6-H), 3.24 (dd, *J* = 18.0, 6.8 Hz, 1H, 12-H), 2.89 (d, *J* = 18.0 Hz, 1H, 12-H), 2.44 (s, 3H, OAc), 2.39 (s, 3H, *N*-Me), 2.28 (s, 3H, 10-CH_3), 2.12 (s, 3H, 4-CH_3), 2.10 (m, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 172.7 (s, C-14), 168.6 (s, OAc), 148.5 (s, C-9), 144.9 (s, C-3), 144.6 (s, C-5), 142.5 (s, C-8), 137.6 (s, C-1), 132.1 (s, C-10), 129.1 (s, C-11), 128.9 (d, C-11a), 121.6 (s, C-7a), 114.7 (s, C-5a), 112.7 (s, C-16a), 106.7 (s, C-4), 101.2 (t, C-2), 68.2 (t, C-18), 60.6 (q, 9-OMe), 59.7 (d, C-6a), 59.6 (d, C-13), 56.4 (d, C-7), 52.8 (d, C-16), 39.5 (q, *N*-Me), 27.3 (t, C-12), 25.8 (t, C-6), 20.9 (s, OAc), 16.0 (q, 10-CH_3), 8.9 (q, 4-CH_3); EIMS *m/z* (%): 510 (M^+, 20%), 479 (26), 247 (21), 246 (100), 204 (54), 189 (16); HREIMS *m/z* 510.1999 (M^+, calcd for $C_{27}H_{30}N_2O_8$ 510.2002).

(6a*S*,7*R*,13*S*,16*R*)-5-(Benzyloxy)-16-(hydroxymethyl)-9-methoxy-4,10,17-trimethyl-14-oxo-6,6a,7,13,14,16-hexahydro-12*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinolin-8-yl Acetate (23). To a solution of **22** (51.3 mg, 101 μmol) in acetone (15 mL) was added BnBr (17.9 μL, 151 μmol) and K_2CO_3 (41.7 mg, 301 μmol), and the reaction mixture was stirred for 12 h at 60 °C. The reaction mixture was filtered to remove inorganic materials and the filtrate was concentrated in vacuo. The residue was purified by SiO_2 flash chromatography (MeOH−$CHCl_3$ = 1:49) to provide benzyl ether **23** (55.5 mg, 92%) as a pale yellow gummy solid.

$[\alpha]_D^{25}$ −89.9 (c 0.3, $CHCl_3$); IR (KBr) 3011, 2940, 1771, 1636, 1439, 1369, 1236, 1196, 1105, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.48−7.34 (m, 5H, Bn-H), 6.89 (s, 1H, 11-H), 5.99 (d, *J* = 1.2 Hz, 1H, 2-H), 5.93 (d, *J* = 1.2 Hz, 1H, 2-H), 5.56 (dd, *J* = 6.4, 4.0 Hz, 1H, 16-H), 4.78 (d, *J* = 1.6 Hz, 1H, Bn), 4.75 (d, *J* = 1.6 Hz, 1H, Bn), 3.86 (dt, *J* = 12.5, 2.9 Hz, 1H, 6a-H), 3.73 (d, *J* = 2.9 Hz, 1H, 7-H), 3.72 (d, *J* = 6.7 Hz, 1H, 13-H), 3.71 (s, 3H, 9-OCH_3), 3.51 (ddd, *J* = 11.0, 5.5, 4.0 Hz, 1H, 18-H), 3.34 (ddd, *J* = 11.0, 6.4, 5.5 Hz, 1H, 18-H), 3.26 (dd, *J* = 15.0, 2.9 Hz, 1H, 6-H), 3.22 (dd, *J* = 17.7, 6.7 Hz, 1H, 12-H), 3.14 (t, *J* = 5.5 Hz, 1H, 18-OH), 2.87 (d, *J* = 17.7 Hz, 1H, 12-H), 2.34 (s, 3H, *N*-Me), 2.28 (s, 3H, 10-CH_3), 2.18 (s, 3H, 4-CH_3), 2.11 (dd, *J* = 15.0, 12.5 Hz, 1H, 6-H) , 2.00 (s, 3H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 172.9 (s, C-14), 168.6 (s, OAc), 148.5 (s, C-9), 148.1 (s, C-5), 145.3 (s, C-3), 142.4 (s, C-8), 139.9 (s, C-1), 137.2 (s, Bn), 131.9 (s, C-10), 129.0 (s, C-11a), 129.0 (d, C-11), 128.6 (d, Bn), 128.2 (d, Bn), 127.4 (d, Bn), 122.0 (s, C-7a), 121.1 (s, C-5a), 112.9 (s, C-16a), 112.6 (s, C-4), 101.2 (t, C-2), 75.4 (t, Bn), 68.3 (t, C-18), 60.5 (q, 9-OMe), 59.7 (d, C-13), 59.6 (d, C-6a), 56.3 (d, C-7), 52.8 (d, C-16), 39.6 (q, *N*-Me),

27.4 (t, C-12), 26.2 (t, C-6), 20.4 (q, Ac), 15.9 (q, 10-CH_3), 9.4 (q, 4-CH_3); EIMS *m/z* (%): 600 (M^+, 15%), 569 (41), 509 (21), 247 (38), 246 (100), 204 (60), 189 (16); HREIMS *m/z* 600.2471 (M^+, calcd for $C_{34}H_{36}N_2O_8$ 600.2472).

(6a*S*,7*R*,13*S*,14*R*,16*R*)-5-(Benzyloxy)-14-cyano-16-(hydroxymethyl)-9-methoxy-4,10,17-trimethyl-6,6a,7,13,14,16-hexahydro-12*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinolin-8-yl Acetate (24a). To a solution of **23** (5.5 mg, 9.2 μmol) in THF (0.4 mL) at 0 °C was slowly added $LiAlH_2(OEt)_2$ (0.2 mol/L in Et_2O, 230 μL, 45.8 μmol) over 10 min. The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched with AcOH (12.0 μL, 200 μmol), followed by the addition of KCN (4.8 mol/L in H_2O, 11.5 μL, 55 μmol), Na_2SO_4 (67 mg) and celite, and stirring was continued for 4.5 h at 25 °C. The reaction mixture was filtered through a Celite pad, and concentrated in vacuo to give a residue (7.2 mg), which was purified by SiO_2 flash chromatography (*n*-hexane−EtOAc = 1:1) to give acetate **24a** (2.2 mg, 39%) as a pale yellow gummy solid, and with *n*-hexane−EtOAc = 1:5 to provide **23** (2.1 mg, 38% recovery) as a pale yellow gummy solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.50-7.39 (5H, m, Bn-H), 6.86 (1H, s, 4-H), 5.96 (1H, d, *J* = 1.4 Hz, 2-H), 5.91 (1H, d, *J* = 1.4 Hz, 2-H), 4.75 (1H, d, *J* = 11.3 Hz, -OC$\underline{H_2}$Ph), 4.65 (1H, d, *J* = 11.3 Hz, -OC$\underline{H_2}$Ph), 4.07 (1H, d, *J* = 1.9 Hz, 14-H), 4.01 (1H, t, *J* = 3.1 Hz, 16-H), 3.70-3.64 (2H, m, 11-H, 17-H), 3.68 (3H, s, -OCH_3), 3.46 (1H, td, *J* = 10.1, 3.1 Hz, 17-H), 3.39 (1H, dt, *J* = 7.7, 1.9 Hz, 13-H), 3.29 (1H, dt, *J* = 11.9, 2.6 Hz, 6a-H), 3.13 (1H, dd, *J* = 18.1, 7.7 Hz, 12-H), 3.09 (1H, dd, *J* = 14.4, 2.6 Hz, 6-H), 2.55 (1H, d, *J* = 18.1 Hz, 12-H), 2.28 (3H, s, N-Me), 2.26 (3H, s, 10-CH_3), 2.16 (3H, s, 4-CH_3), 2.02 (3H, s, OAc),

1.94 (1H, dd, *J* = 14.4, 11.9 Hz, 6-H), 1.77 (1H, dd, *J* = 10.1, 3.1 Hz, 17-OH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 168.8 (-O<u>C</u>OCH_3), 148.1 (C-5), 148.1 (C-9), 144.7 (C-3), 142.5 (C-8), 139.2 (C-1), 137.4 (C-2'), 131.4 (C-10), 129.8 (C-11a), 128.6 (C-4'), 128.0 (C-5'), 127.6 (C-11), 127.3 (C-3'), 123.4 (C-7a), 120.5 (C-5a or C-16a), 117.5 (CN), 113.2 (C-5a or C-16a), 112.4 (C-4), 101.3 (C-2), 74.8 (C-1'), 63.7 (C-17), 60.5 (-OCH_3), 60.1 (C-14), 58.2 (C-16), 57.7 (C-7), 56.5 (C-6a), 55.2 (C-13), 41.6 (N-Me), 26.2 (C-6), 25.6 (C-12), 20.3 (-OCO<u>C</u>H_3), 15.9 (10-CH_3), 9.3 (4-CH_3). FABMS *m/z* 612 $[M+H]^+$; HRFABMS *m/z* 612.2716 ($[M+H]^+$, calcd for $C_{35}H_{38}N_3O_7$ 612.2711).

(6a*S*,7*R*,13*S*,14*R*,16*R*)-5-(Benzyloxy)-8-hydroxy-16-(hydroxymethyl)-9-methoxy-4,10,17-trimethyl-6,6a,7,13,14,16-hexahydro-12*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinoline-14-carbonitrile (24b). To a solution of **23** (11 mg, 18.3 μmol) in THF (0.6 mL) at 0 °C was slowly added $LiAlH_2(OEt)_2$ (0.2 mol/L in Et_2O, 1.4 mL, 274.9 μmol) over 10 min. The reaction mixture was stirred for 1.5 h at 0 °C. The reaction mixture was quenched with AcOH (22.0 μL, 385μmol), followed by the addition of KCN (4.8 mol/L in H_2O, 23 μL, 110 μmol), Na_2SO_4 (135 mg) and Celite, and stirring was continued for 18 h at 25 °C. The reaction mixture was diluted with saturated $NaHCO_3$ solution (20 mL) and extracted to $CHCl_3$ (3 × 20 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated (11.4 mg). The residue was purified by SiO_2 flash chromatography (*n*-hexane−EtOAc = 1:1) to provide alcohol **24b** (7.8 mg, 75%) as a pale yellow gummy solid.

$[\alpha]_D^{24}$ +37.0 (c 0.8, $CHCl_3$); IR (KBr) 3510, 2928, 2872, 1456, 1433, 1233, 1105, 1065, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.49-7.39 (m, 5H, Bn-H), 6.47 (s, 1H, 11-H), 5.94 (d,

J = 1.5 Hz, 1H, 2-H), 5.89 (d, *J* = 1.5 Hz, 1H, 2-H), 5.50 (s, 1H, 8-OH), 4.71 (d, *J* = 11.2 Hz, 1H, Bn), 4.67 (d, *J* = 11.2 Hz, 1H, Bn), 4.13 (dd, *J* = 2.6, 1.0 Hz, 1H, 7-H), 4.04 (d, *J* = 2.5 Hz, 1H, 14-H), 3.99 (t, *J* = 3.2 Hz, 1H, 16-H), 3.69 (s, 3H, 9-OCH_3), 3.66 (dt, *J* = 9.0, 3.2 Hz, 1H, 18-H), 3.44 (ddd, *J* = 10.2, 9.0, 3.2 Hz, 1H, 18-H), 3.36 (ddd, *J* = 7.6, 2.5, 1.0 Hz, 1H, 13-H), 3.30 (dt, *J* = 10.5, 2.6 Hz, 1H, 6a-H), 3.24 (dd, *J* = 15.6, 2.6 Hz, 1H, 6-H), 3.10 (dd, *J* = 18.1, 7.6 Hz, 1H, 12-H), 2.49 (d, *J* = 18.1 Hz, 1H, 12-H), 2.34 (s, 3H, *N*-Me), 2.24 (s, 3H, 10-CH_3), 2.14 (s, 3H, 4-CH_3), 1.87 (dd, *J* = 15.6, 10.5 Hz, 1H, 6-H), 1.84 (dd, *J* = 10.2, 3.2 Hz, 1H, 18-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 148.4 (s, C-5), 146.7 (s, C-8), 144.5 (s, C-3), 143.0 (s, C-9), 139.0 (s, C-1), 137.5 (s, Bn), 130.1 (s, C-11a), 129.1 (s, C-10), 128.5 (d, Bn), 128.0 (d, Bn), 127.9 (d, Bn), 121.2 (s, C-5a or C-16a), 120.9 (d, C-11), 117.7 (s, CN), 116.7 (s, C-7a), 113.4 (s, C-5a or C-16a), 112.4 (s, C-4), 101.2 (t, C-2), 75.2 (t, Bn), 63.5 (d, C-18), 60.6 (q, 9-OCH_3), 60.0 (d, C-14), 58.1 (t, C-16), 56.8 (d, C-6a), 56.6 (d, C-7), 55.3 (d, C-13), 41.7 (q, *N*-Me), 26.2 (t, C-6), 25.7 (t, C-12), 15.7 (q, 10-CH_3), 9.4 (q, 4-CH_3); FABMS *m/z* 570 $[M+H]^+$; HRFABMS *m/z* 570.2604 ($[M+H]^+$, calcd for $C_{33}H_{36}N_3O_6$ 570.2604).

(6a*S*,7*R*,13*S*,14*R*,16*R*)-5-(Henzyloxy)-16-(hydroxymethyl)-9-methoxy-4,10,17-trimethyl-8,11-dioxo-6,6a,7,8,12,13,14,16-octahydro-11*H*-7,13-epiminobenzo[4,5] azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinoline-14-carbonitrile (25). To a solution of **24b** (19.5 mg, 34.3 μmol) in THF (2.0 mL) was added salcomine (11.0 mg, 34.3 μmol) at 25 °C, and the mixture was stirred for 2.5 h under O_2 atmosphere. The reaction mixture was filtered through a cellulose pad and washed with EtOAc. The filtrate was concentrated in vacuo, and the residue (20.5 mg) was purified by SiO_2 flash chromatography

(AcOEt−Benzene 1:5) to provide quinone **25** (12.5 mg, 61%) as a yellow gummy solid. $[\alpha]_D^{23}$ +49.6 (c 0.8, $CHCl_3$); IR (KBr) 3021, 2930, 2879, 1653, 1612, 1456, 1431, 1305, 1107, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.50-7.36 (m, 5H, Bn-H), 5.98 (d, *J* = 1.1 Hz, 1H, 2-H), 5.90 (d, *J* = 1.1 Hz, 1H, 2-H), 4.66 (d, *J* = 10.8 Hz, 1H, Bn), 4.60 (d, *J* = 10.8 Hz, 1H, Bn), 4.15 (d, *J* = 2.5 Hz, 1H, 14-H), 4.04 (t, *J* = 4.2 Hz, 1H, 16-H), 4.01 (br d, *J* = 2.3 Hz, 1H, 7-H), 3.94 (s, 3H, 9-OCH_3), 3.71 (br d, *J* = 10.9 Hz, 1H, 18-H), 3.54-3.48 (m, 1H, 18-H), 3.39 (dd, *J* = 7.5, 2.5 Hz, 1H, 13-H), 3.18 (dt, *J* = 12.0, 2.3 Hz, 1H, 6a-H), 3.04 (dd, *J* = 15.1, 2.3 Hz, 1H, 6-H), 2.82 (dd, *J* = 21.0, 7.5 Hz, 1H, 12-H), 2.30 (s, 3H, *N*-Me), 2.29 (d, *J* = 21.0 Hz, 1H, 12-H), 2.16 (s, 3H, 14-CH_3), 1.95 (s, 3H, 10-H), 1.66 (dd, *J* = 15.1, 12.0 Hz, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 186.5 (s, C-11), 182.5 (s, C-8), 155.3 (s, C-9), 148.1 (s, C-5), 144.9 (s, C-3), 141.3 (s, C-11a), 139.2 (s, C-1), 136.7 (s, Bn), 136.2 (s, C-7a), 128.6 (d, Bn), 128.6 (s, C-10), 128.5 (d, Bn), 128.3 (d, Bn), 120.6 (s, C-5a), 117.4 (s, CN), 112.6 (s, C-4 and C-16a overlapped), 101.3 (t, 2-C), 75.4 (t, Bn), 65.2 (t, C-18), 60.9 (q, 9-OCH_3), 59.8 (d, C-14), 58.5 (d, C-16), 55.9 (d, C-6a), 54.8 (d, C-7 or C-13), 54.7 (d, C-7 or C-13), 41.5 (q, *N*-Me), 27.7 (t, C-6), 21.5 (t, C-12), 9.4 (q, 4-CH_3), 8.7 (q, 10-CH_3); FABMS *m/z* 584 $[M+H]^+$; HRFABMS *m/z* 584.2399 ($[M+H]^+$, calcd for $C_{33}H_{34}N_3O_7$ 584.2397).

(6a*S*,7*R*,13*S*,14*R*,16*R*)-5-Hydroxy-16-(hydroxymethyl)-9-methoxy-4,10,17-trimethyl-8,11-dioxo-6,6a,7,8,12,13,14,16-octahydro-11*H*-7,13-epiminobenzo[4,5]azocino[1,2-*b*][1,3]dioxolo[4,5-*h*]isoquinoline-14-carbonitrile (26). To a solution of **25** (4.4 mg, 7.54 μmol) and pemtamethyl benzene (11.2 mg, 75.4 μmol) in CH_2Cl_2 (1 mL) was added BCl_3 (1.0 mol/L in CH_2Cl_2, 38 μL, 37.7 μmol) at −78 °C and the mixture was stirred for 2 h. The

mixture was diluted with CH_2Cl_2 (1 mL) and quenched with saturated $NaHCO_3$ solution (1 mL) and was extracted with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated to give a residue (8.4 mg), which was purified by SiO_2 flash chromatography (MeOH−$CHCl_3$ 1:49) to provide phenol **26** (3.5 mg, 95%) as a pale yellow amorphous powder.

$[\alpha]_D^{26}$ +59.1 (c 0.14, CH_3OH); IR (KBr) 3431, 3368, 3277, 2928, 2886, 2851, 1653, 1616, 1462, 1435, 1308, 1233, 1146, 1103 cm^{-1}; ^1H NMR (pyridine-d5, 500 MHz) δ: 5.80 (d, *J* = 1.4 Hz, 1H, 2-H), 5.72 (d, *J* = 1.4 Hz, 1H, 2-H), 5.10 (d, *J* = 2.2 Hz, 1H, 14-H), 4.57 (dd, *J* = 8.4, 3.0 Hz, 1H, 16-H), 4.25 (br s, 1H, 7-H), 4.19 (dd, *J* = 10.2, 3.0 Hz, 1H, 18-H), 3.83 (s, 3H, 9-OCH_3), 3.72-3.69 (m, 2H, 6-H and 18-H, overlapped), 3.66 (dt, *J* = 10.7, 2.9 Hz, 1H, 6a-H), 3.49 (dt, *J* = 7.1, 2.2 Hz, 1H, 13-H), 2.96 (dd, *J* = 20.7, 7.1 Hz, 1H, 12-H), 2.85 (d, *J* = 20.7 Hz, 1H, 12-H), 2.35 (s, 3H, 4-CH_3), 2.26 (dd, *J* = 15.0, 10.7 Hz, 1H, 6-H), 2.21 (s, 3H, *N*-Me), 1.86 (s, 3H, 10-CH_3); ^{13}C NMR (pyridine-d_5, 125 MHz) δ: 186.8 (s, C-11), 183.1 (s, C-8), 155.7 (s, C-9), 147.4 (s, C-5), 145.0 (s, C-3), 142.8 (s, C-11a), 137.1 (s, C-1), 136.6 (s, C-7a), 128.7 (s, C-10), 119.2 (d, CN), 116.4 (s, C-5a or C-16a), 113.7 (s, C-5a or C-16a), 108.3 (s, C-4), 101.0 (t, C-2), 68.5 (t, C-18), 61.9 (d, C-14), 60.7 (q, 9-OCH_3), 59.9 (d, C-16), 57.7 (d, C-6a) 55.9 (d, C-7), 55.4 (d, C-13), 41.3 (q, *N*-Me), 28.1(t, C-6), 22.0 (t, C-12), 10.1 (q, 4-CH_3), 8.7 (q, 10-CH_3); FABMS *m/z* 494 $[M+H]^+$; HRFABMS *m/z* 494.1937 ($[M+H]^+$, calcd for $C_{26}H_{28}N_3O_7$ 494.1927).

Renieramycin T (1t). To a solution of angelic acid (31.3 mg, 313 μmol) in Et_2O (1.6 mL) at 0 °C was added oxalyl chloride (26.4 μL, 308 μmol), and DMF (1.2 μL, 15.4 μmol). The resulting solution was stirred at 25 °C for 2 h and then a solution of compound **26** (3.8 mg,

7.70 μmol) in CH_2Cl_2 (400 μL) was added. The mixture was concentrated with a stream of air and $ClCH_2CH_2Cl$ (800 μL) was then added. The reaction was stirred at 80 °C for 3 h. The mixture was quenched with saturated $NaHCO_3$ solution (20 mL) and was extracted with 5% MeOH in $CHCl_3$ (3 × 20 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated in vacuo to give a residue (8.4 mg), which was purified by SiO_2 flash chromatography (EtOAc−n-Hexane 1:2) to provide Renieramycin T (2.7 mg, 61%) as a pale yellow gummy solid.

$[\alpha]_D^{23}$ −16.5 (c 0.23, $CHCl_3$); IR (KBr) 3429, 3292, 2926, 2853, 1713, 1653, 1616, 1460, 1435, 1375, 1308, 1233, 1152, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ: 6.00 (qq, *J* = 7, 2 Hz, 1H, 3'-H), 5.92 (d, *J* = 2 Hz, 1H, -OCH_2O-), 5.85 (d, *J* = 2 Hz, 1H, -OCH_2O-), 4.55 (br s, 1H, 5-OH), 4.41 (dd, *J* = 11, 4 Hz, 1H, 22-H), 4.16 (dd, *J* = 5, 4 Hz, 1H, 1-H), 4.11 (d, *J* = 2 Hz, 1H, 21-H), 4.00 (dd, *J* = 3, 1 Hz, 1H, 11-H), 3.99 (dd, *J* = 11, 5 Hz, 1H, 22-H), 3.98 (s, 3H, 17-OCH_3), 3.37 (ddd, *J* = 7, 2, 1 Hz, 1H, 13-H), 3.24 (ddd, *J* = 12, 3, 2 Hz, 1H, 3-H), 2.87 (dd, *J* = 15, 2 Hz, 1H, 4-H), 2.75 (dd, *J* = 21, 7 Hz, 1H, 14-H), 2.30 (d, *J* = 21 Hz, 1H, 14-H), 2.29 (s, 3H, *N*-Me), 2.11 (s, 3H, 6-CH_3), 1.94 (s, 3H, 16-CH_3), 1.85 (dq, *J* = 7, 2 Hz, 1H, 4-H), 1.69 (dq, *J* = 2, 2 Hz, 3H, 2'-CH_3), 1.67 (dq, *J* = 15, 12 Hz, 3H, 4'-H) ; ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 186.1 (s, C-15), 182.8 (s, C-18), 167.1 (s, C-1'), 155.4 (s, C-17), 144.9 (s, C-7), 144.7 (s, C-5), 141.8 (s, C-20), 139.7 (d, C-3'), 136.8 (s, C-8), 135.7 (s, C-19), 129.0 (s, C-16), 126.8 (s, C-2'), 117.4 (s, CN), 113.1 (s, C-10), 112.1 (s, C-9), 106.2 (s, C-6), 101.7 (t, OCH_2O), 64.6 (t, C-22), 60.9 (q, 17-OCH_3), 59.8 (d, C-21), 56.4 (d, C-1), 56.2 (d, C-3), 54.9 (d, C-11), 54.8 (d, C-13), 41.4 (q, *N*-Me), 26.8 (t, C-4), 21.2 (t, C-14), 20.5 (q, 2'-CH_3), 15.7 (q, C-4'), 8.8 (q, 6-CH_3), 8.7 (q, 16-CH_3); EIMS *m/z* (%):575 (M^+,

32), 462 (18), 260 (24), 243 (13), 221 (43), 220 (100), 219 (16), 218 (21); HREIMS *m/z* 575.2265 (M^+, calcd for $C_{31}H_{33}N_3O_8$, 575.2268).

Acknowledgments

The Authors wish to dedicate this paper to the 60th birthday of Professor Stuart L. Schreiber of the Board Institute of Harvard and MIT. This work supported by the Japan Society for the Promotion of Science (JSPS) Grant-in Aid for Scientific Research (C) (No. 15K07873 to N.S.) and the National Institutes of Health (grant 1RO1 CA085419 to R.M.W.).

Supporting Information: The Supporting Information is available free of charge on the ACS Publications website at DOI:

NMR spectra for all new compounds including natural and synthetic Renieramycin T (PDF).

References

(1) (a) Scott, J. D.; Williams, R. M. *Chem. Rev.* **2002**, *102*, 1669–1730. (b) Siengalewicz, P.; Rinner, U.; Mulzer, J. *Chem. Soc. Rev.* **2008**, *37*, 2676. (c) Cuevas, C.; Francesch, A. *Nat. Prod. Rep.* **2009**, *26*, 322. (d) Avendaño, C.; de la Cuesta, E. *Chem. - A Eur. J.* **2010**, *16*, 9722–9734. (e) Le, V. H.; Inai, M.; Williams, R. M.; Kan, T. *Nat. Prod. Rep.* **2015**, *32*, 328–347.

(2) (a) Lebedinsky, C.; Gómez, J.; Park, Y. C.; Nieto, A.; Soto-Matos, A.; Parekh, T.; Alfaro, V.; Roy, E.; Lardelli, P.; Kahatt, C. *Cancer Chemother. Pharmacol.* **2011**, *68*,

1223–1231. (b) Baruchel, S.; Pappo, A.; Krailo, M.; Baker, K. S.; Wu, B.; Villaluna, D.; Lee-Scott, M.; Adamson, P. C.; Blaney, S. M. *Eur. J. Cancer* **2012**, *48*, 579–585. (c) Cioffi, A.; Italiano, A. *Expert Opin. Drug Metab. Toxicol.* **2012**, *8*, 113–122. (d) Le Cesne, A.; Yovine, A.; Blay, J.-Y.; Delaloge, S.; Maki, R. G.; Misset, J.-L.; Frontelo, P.; Nieto, A.; Jiao, J. J.; Demetri, G. D. *Invest. New Drugs* **2012**, *30*, 1193–1202.

(3) Daikuhara, N.; Tada, Y.; Yamaki, S.; Charupant, K.; Amnuoypol, S.; Suwanborirux, K.; Saito, N. *Tetrahedron Lett.* **2009**, *50*, 4276–4278.

(4) Tatsukawa, M.; Punzalan, L. L. C.; Magpantay, H. D. S.; Villaseñor, I. M.; Concepcion, G. P.; Suwanborirux, K.; Yokoya, M.; Saito, N. *Tetrahedron* **2012**, *68*, 7422–7428.

(5) synthesis of Renieramycin A: (a) Fukuyama, T.; Linton, S. D.; Tun, M. M. *Tetrahedron Lett.* **1990**, *31*, 5989–5992. synthesis of Renieramycin G: (b) Liao, X. W.; Liu, W.; Dong, W. F.; Guan, B. H.; Chen, S. Z.; Liu, Z. Z. *Tetrahedron* **2009**, *65*, 5709–5715. (c) Wu, Y. C.; Zhu, J. *Org. Lett.* **2009**, *11*, 5558–5561. (d) Magnus, P.; Matthews, K. S. *Tetrahedron* **2012**, *68*, 6343–6360. (e) Du, E.; Dong, W.; Guan, B.; Pan, X.; Yan, Z.; Li, L.; Wang, N.; Liu, Z. *Tetrahedron* **2015**, *71*, 4296–4303. synthesis of Renieramycin H (cribrostatin 4): (f) Chan, C.; Heid, R.; Zheng, S.; Guo, J.; Zhou, B.; Furuuchi, T.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 4596–4598. (g) Chen, X.; Zhu, J. *Angew. Chemie Int. Ed.* **2007**, *46*, 3962–3965.

(6) (a) Lane, J. W.; Chen, Y.; Williams, R. M. *J. Am. Chem. Soc.* **2005**, *127*, 12684–12690. (b) Vincent, G.; Williams, R. M. *Angew. Chemie Int. Ed.* **2007**, *46*, 1517–1520. (c) Yokoya, M.; Shinada-Fujino, K.; Saito, N. *Tetrahedron Lett.* **2011**, *52*, 2446–2449. (d) Yokoya, M.; Ito, H.; Saito, N. *Tetrahedron* **2011**, *67*, 9185–9192. (e) Yokoya, M.;

Shinada-Fujino, K.; Yoshida, S.; Mimura, M.; Takada, H.; Saito, N. *Tetrahedron* **2012**, *68*, 4166–4181. (f) Yokoya, M.; Kobayashi, K.; Sato, M.; Saito, N. *Mar. Drugs* **2015**, *13*, 4915–4933.

(7) (a) Fishlock, D.; Williams, R. M. *Org. Lett.* **2006**, *8*, 3299–3301. (b) Fishlock, D.; Williams, R. M. *J. Org. Chem.* **2008**, *73*, 9594–9600.

(8) Zheng, S.; Chan, C.; Furuuchi, T.; Wright, B. J. D.; Zhou, B.; Guo, J.; Danishefsky, S. J. *Angew. Chemie - Int. Ed.* **2006**, *45*, 1754–1759.

(9) Endo, A.; Yanagisawa, A.; Abe, M.; Tohma, S.; Kan, T.; Fukuyama, T. *J. Am. Chem. Soc.* **2002**, *124*, 6552–6554.

(10) (a) Fukuyama, T.; Yang, L.; Ajeck, K. L.; Sachleben, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 3712–3713. (b) Scott, J. D.; Williams, R. M. *Angew. Chemie Int. Ed.* **2001**, *40*, 1463–1465.

(11) Nakai, K.; Kubo, K.; Yokoya, M.; Saito, N. *Tetrahedron* **2014**, *70*, 6529–6545.

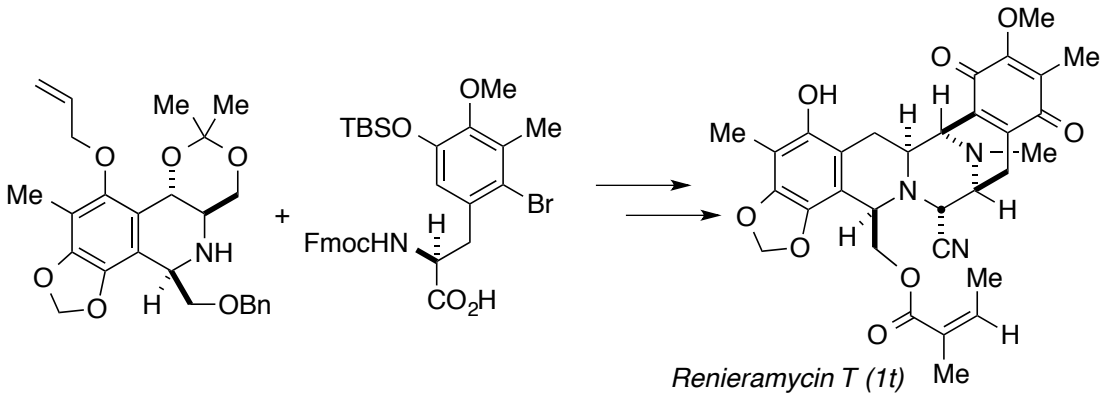
(12) Brown, H. C.; Tsukamoto, A. *J. Am. Chem. Soc.* **1959**, *81*, 502–503.

(13) Okano, K.; Tokuyama, H.; Fukuyama, T. *J. Am. Chem. Soc.* **2006**, *128*, 7136–7137.

(14) Suwanborirux, K.; Charupant, K.; Amnuoypol, S.; Pummangura, S.; Kubo, A.; Saito, N. *J. Nat. Prod.* **2002**, *65*, 935-937.

(15) A single-cell suspension of each cell line (2 × 103 cells/well) was added to the serially diluted test compounds in a microplate. Then, the cells were cultured for 4d. Cell growth was measured with a cell counting kit (DOJINDO, Osaka, Japan). IC_{50} was expressed as the concentration at which cell growth was inhibited by 50% compared with the untreated control.

TOC graphic



Renieramycin T (1t)