Bioorganic & Medicinal Chemistry 17 (2009) 4548–4558

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Chemistry of renieramycins. Part 8: Synthesis and cytotoxicity evaluation of renieramycin M–jorunnamycin A analogues ☆

Kornvika Charupant [a], Naomi Daikuhara [b], Emi Saito [b], Surattana Amnuoypol [a], Khanit Suwanborirux [a,*], Takashi Owa [c], Naoki Saito [b,*]

[a] Department of Pharmacognosy and Pharmaceutical Botany, Center for Bioactive Natural Products from Marine Organisms and Endophytic Fungi (BNPME), Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand
[b] Graduate School of Pharmaceutical Sciences, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan
[c] Discovery Research Laboratories II, Integrative Oncology Area, Eisai Co., Ltd, 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan

ARTICLE INFO

Article history:
Received 4 April 2009
Revised 1 May 2009
Accepted 2 May 2009
Available online 8 May 2009

Keywords:
Renieramycin
Marine natural product
Cytotoxicity
Structure–activity relationship study

ABSTRACT

Twenty-four ester analogues of renieramycin M (**1m**) were prepared from jorunnamycin A (**3a**), which was easily transformed from marine natural **1m** in three steps. These analogues, along with **1m** itself, cyanojorumycin (**2b**), and jorunnamycins A (**3a**) and C (**3b**), were evaluated in vitro for cytotoxicity by measuring IC_{50} values through the 3-(4,5-dimethyltriazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay using human HCT116 colon carcinoma and MDA-MB-435 breast carcinoma cell lines. Nitrogen-containing heterocyclic ester derivatives **9a–f** showed similar in vitro cytotoxicity to **1m**, whereas the other derivatives were slightly less cytotoxic than **1m**. 2′-Pyridinecarboxylic acid ester derivative (**9c**) exhibited a threefold increase in cytotoxicity relative to **1m**.

1. Introduction

Bisisoquinolinequinone natural products and their reduced forms have attracted considerable interest over the past 30 years due to their potent biological properties (Fig. 1).[2] The most bioactive member of this family, ecteinascidin 743 (Yondelis™, trabectedin), has a unique mechanism of action that is based on its binding to the minor groove of DNA to interfere with cell division, activated transcription, and DNA repair.[3–6] The remarkable preclinical and clinical results of ecteinascidin 743 have stimulated further research of other antitumor agents, such as phthalascidin (Pt 650)[7,8] and QAD[9] (Fig. 2).

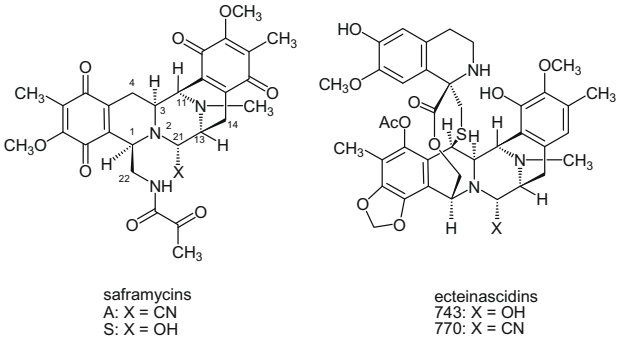


Figure 1. Structures of representative isoquinoline natural products.

Following the discovery of renieramycins A–D (**1a–d**) from the Mexican sponge *Reniera* sp. by Frincke and Faulkner in 1982,[10] ten marine natural renieramycins were isolated from marine sponges belonging to genera *Reniera*,[11] *Xestospongia*,[12] *Haliclona*,[13,14] *Cribrochalina*,[15,16] and *Neopetrosia*.[17] An additional related compound, jorumycin (**2a**), which contains an acetyl ester instead of an angelate ester, was isolated from the skin and the mucus of the

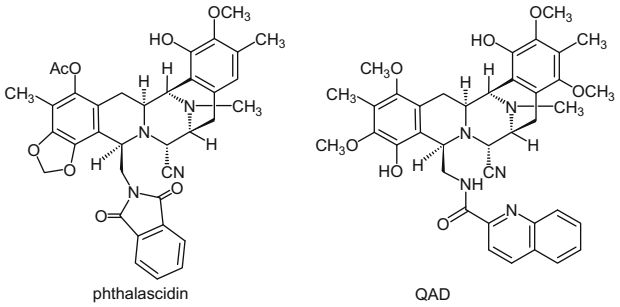


Figure 2. Structures of synthetic analogues possessing high cytotoxicity.

☆ See Ref. 1.

* Corresponding authors. Tel./fax: +66 2 218 8357 (K.S.), tel./fax: +81 42 495 8794 (N.S.).
E-mail addresses: skhanit@chula.ac.th (K. Suwanborirux), naoki@my-pharm.ac.jp (N. Saito).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.05.009

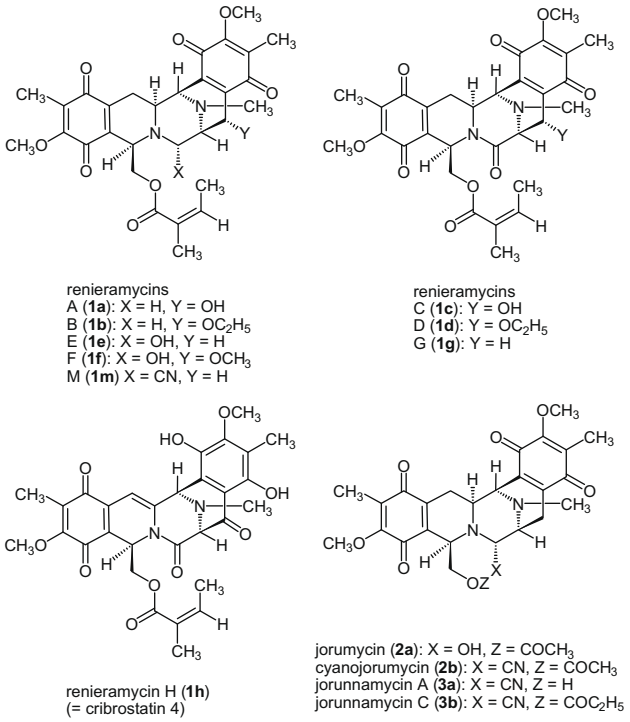


Figure 3. Structures of marine natural renieramycins.

nudibranch *Jorunna funebris* (Fig. 3).[18] The novel structures of renieramycins, together with their remarkable biological activities and lack of availability from natural sources, have made these compounds extremely attractive and important synthetic targets. The first total synthesis of renieramycin A (**1a**) was accomplished by Fukuyama in 1990.[19] To date, two total syntheses of renieramycin G (**1g**),[20,21] three total syntheses of renieramycin H (**1h**: cribrostatin 4),[22–24] and one total synthesis of **2a** have been published.[21] However, the structure–activity relationships of renieramycins are relatively unexplored because most synthetic approaches have focused on their total synthesis.

As part of our search for new metabolites via the isolation and characterization of biologically active compounds from Thai marine animals, we have reported the isolation and structure elucidation of renieramycin M (**1m**) in gram scale from the Thai sponge *Xestospongia* sp. by pretreatment with potassium cyanide.[25,26] The availability of **1m** has enabled us to prepare and evaluate new members of this class of compounds. We succeeded in semisynthetically preparing **2a** from **1m** via deangeloylrenieramycin M (**3a**: named jorunnamycin A),[27] which was isolated from the mantles and egg ribbons of the KCN-pretreated Thai nudibranch *J. funebris*.[28] Preliminary biological evaluation of **2a** and jorunnamycins A (**3a**) and C (**3b**) revealed growth inhibition at micromolar concentrations when tested with two human carcinoma cell lines (HCT-116 and QG 56). Therefore, this preliminary biological evaluation established the basis for conducting new investigations of this class of antitumor marine alkaloids with focus on the effect of the variation of the ester side chain at C-22.

In this paper, we report significant results gained from the extension of our initial investigation and the results of cytotoxicity evaluation of the ester analogues at C-22.

2. Chemistry

Synthesis of the ester analogues at C-22 of **1m** was accomplished by acylation of readily available compound **3a**, which was prepared in 45–54% yield through our previously reported three-step procedure for **1m**, namely, hydrogenation, hydride reduction, and air oxidation (Scheme 1).[27]

We first prepared the isomers of the ester side chain at C-22 of **1m**, such as **4a** and **4b**. Acylation of **3a** with DMAP and mixed anhydride **5a**[29] at 45 °C for 7 h gave **4a** in 38% yield. Treatment of **3a** with carbonate **5b**[30] in pyridine at −17 °C for 1 h afforded **4b** in 81% yield. Next, six aliphatic esters **6a–f** were prepared from **3a** with the anhydride[31] or the acid chloride in pyridine at -17 °C in 30–65% yields. Because commercially available racemic acid chloride was used to prepare ester **6d**, we obtained **6d** as an inseparable 1:1 mixture of epimers. Furthermore, five alicyclic compounds **7a–e** were prepared by esterifying **3a** with acid chloride or carbonate[30] following the same procedure as above in 58–80% yields. Ester **7e** was also obtained as an inseparable 1:1 mixture of diastereomers. Finally, we synthesized aromatic ester derivatives **8a–e** (39–83% yields) and nitrogen-containing heterocycles **9a–f** (34–67% yields).

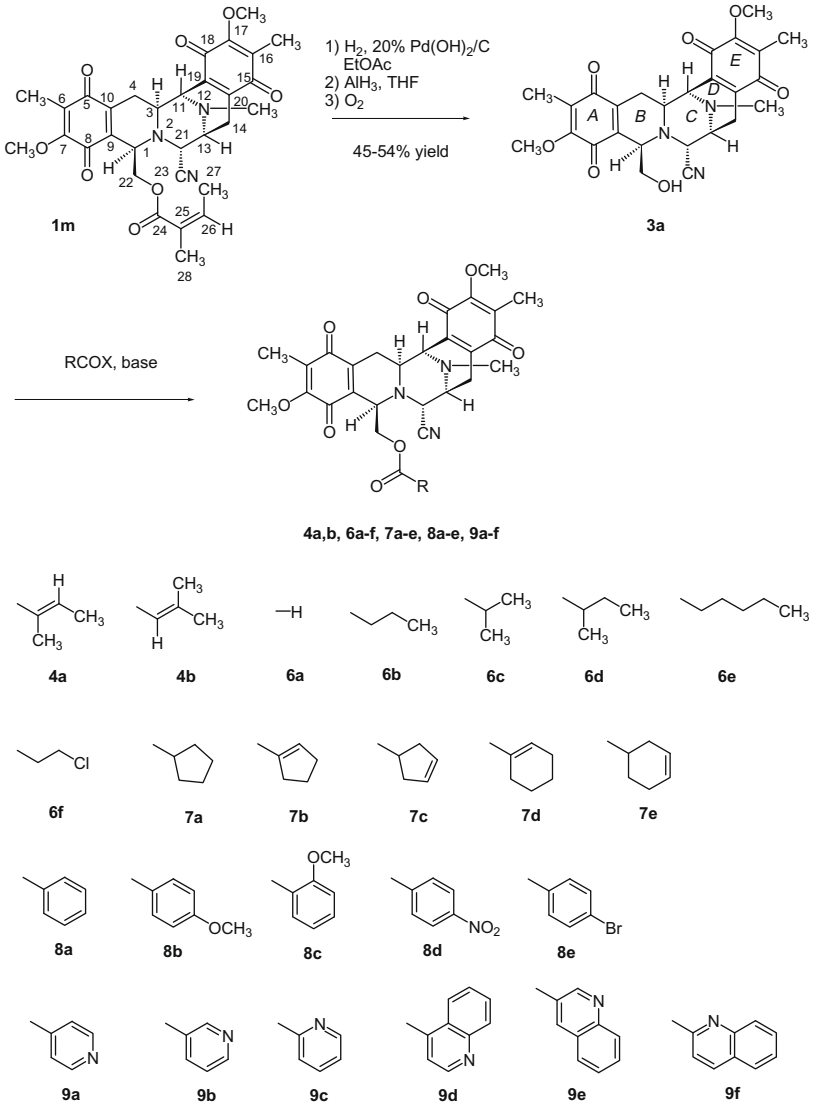
All the compounds were characterized by IR, mass, and ^{1}H and ^{13}C NMR measurements. All proton and carbon signals were assigned by ^{1}H–^{1}H, ^{1}H–^{13}C COSY, and a series of ^{1}H detected two-dimensional heteronuclear multiple-bond correlation (HMBC) experiments. Signals of 16-CH_3 and 17-OCH_3 protons of aromatic ester derivatives **8a–e** and **9a–f** were shifted upfield compared to those of aliphatic, acyclic and cyclic derivatives, **4a,b**, **6a–f**, and **7a–f** (Table 1). It was confirmed that both protons of quinone ring E might be kept in the aromatic ring of the side chain.

3. Biology

Twenty-four ester analogues of **1m**, along with **1m** itself, cyanojorumycin (**2b**), **3a**, and **3b**, were tested for cytotoxicity to representative human tumor cell lines, HCT116 colon carcinoma and MDA-MB-453 breast carcinoma cell lines, at doses ranging from 100 nM to 1.4 nM. The results are shown in Table 1. **4a** and **4b**, which possessed isomeric groups at the side chain, showed threefold lower cytotoxicity than **1m**. We are very interested in **1m** analogues that possessed elongated aliphatic ester groups in the side chain, because both **2b** and **3b** exhibited high cytotoxicity similar to **1m**. However, **6b–e** possessing long aliphatic alkyl groups at the side chain and **6a** with the formyl ester side chain showed significantly decreased cytotoxicity. Furthermore, **7a–e** and **8a–e**, which possessed, respectively, a variety of aliphatic rings and a variety of monosubstituted phenyl groups, also showed decreased cytotoxicity. In contrast, nitrogen-containing heterocyclic ester derivatives (**9a–f**) had similar activity to **1m**. It is noteworthy that 2′-pyridinecarboxylic acid ester derivative (**9c**) was approximately threefold more cytotoxic than **1m**. Although a general structure–activity relationship of the ester analogues of **1m** with the anticancer effect could not be summarized from these data, these data show that the side chain of the ester group appears to be somewhat relevant for the biological activity.

4. Conclusion

Twenty-four analogues of **1m** were prepared from readily available **3a** and were evaluated in vitro for cytotoxic activities against both human HCT116 colon carcinoma and MDA-MB-435 breast carcinoma cell lines. For good activity, the hydroxyl group at C-22 has to be acylated by a small group, such as an acetyl or a propionyl group, whereas a formyl group dramatically decreased the cytotoxicity. Ester analogues having an aliphatic ring or a substituted phenyl group were also slightly less cytotoxic than **1m**. In contrast, it is advantageous to introduce a nitrogen-containing heterocyclic ring, such as pyridine or quinoline. Interestingly, **9c** and **2b** displayed a low nanomolar cytotoxicity profile. This is a somewhat unexpected result and is currently under investigation.



Scheme 1. Preparation of renieramycin M analogues.

We have witnessed in recent years a number of dramatic developments in the preparation of synthetic analogues and their antiproliferative activities.[32–37] Second-generation synthetic analogues **10a** and zalypsis (**10b**) were evaluated by a Harvard University group[38] and Pharma Mar,[39] respectively (Fig. 4). Our findings show that the potent antitumor activity of our analogue **9c** is promising and further effort to explore the therapeutic potential of other renieramycin analogues is under way.

5. Experimental

Optical rotations were measured with a Horiba—SEPA polarimeter. IR spectra were obtained with a Shimadzu Prestige 21/IRA Affinity-1 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on a JEOL–JNM–LA 500 FT NMR spectrometer (ppm, *J* in hertz with TMS as internal standard). Mass spectra were recorded on a JEOL JMS 700 instrument with a direct inlet system operating at 70 eV.

5.1. Tiglic acid ester 4a

A solution of mixed anhydride **5a**[29] was prepared by dissolving tiglic acid (40 mg, 0.4 mmol) in CH_2Cl_2 (0.6 mL) with DBU (5.98 μL, 0.04 mmol) and 2,4,6-trichlorobenzoyl chloride (62.5 μL, 0.4 mmol) at 0 °C for 1 h. A solution of **3a** (18.0 mg, 0.0365 mmol) with DMAP (0.89 mg, 7.3 mmol) in CH_2Cl_2 (1.4 mL) was added to the above mixed anhydride solution **5a** at 0 °C over 5 min. The reaction mixture was stirred at 45 °C for 7 h and then poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (105.7 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **4a** (8.0 mg, 38.1%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 61.7 (*c* 0.17, $CHCl_3$); IR (KBr) 2928, 2360, 1706, 1655, 1618, 1448, 1375, 1236, 1149, 1080, 957, 883, 731 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.48 (1H, qq, *J* = 7.2, 1.5 Hz, 3′-H), 4.58 (1H, dd, *J* = 12.3, 3.5 Hz, 22-Ha), 4.04–3.96 (4H, overlapped, 21-H, 1-H, 11-H, 22-Hb), 4.02 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 3.39 (1H, br d, *J* = 7.7 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.0, 2.8 Hz, 3-H), 2.91 (1H, dd, *J* = 16.9, 2.8 Hz, 4-Hα), 2.74 (1H, dd, *J* = 21.1, 7.7 Hz, 14-Hα), 2.31 (1H, d, *J* = 21.1 Hz, 14-Hβ), 2.27 (3H, s, NCH_3), 1.96 (3H, s, 16-CH_3), 1.93 (3H, s, 6-CH_3), 1.66 (3H, dq, *J* = 7.2, 1,2 Hz, 3′-CH_3), 1.58 (3H, dq, J = 1.5, 1.2 Hz, 2′-CH_3), 1.32 (1H, ddd, *J* = 16.9, 11.6, 2.4 Hz, 4-Hβ); FABMS *m/z* 576 (MH^+); HRFABMS *m/z* 576.2348 (MH^+, calcd for $C_{31}H_{34}N_3O_8$, 576.2346).

Table 1
Cytotoxicity to two carcinoma cell lines along with selected ^{1}H NMR spectral data of renieramycins and related analogues

Entry	Compound	R	Cytotoxicity IC_{50} ± SD nM		^{1}H NMR ($CDCl_3$) δ			
					CH_3		OCH_3	
			HCT116[a]	MDA-MB-435[a]	6	16	7	17
1	**1m**	$COC(CH_3)$=$CHCH_3$ (Renieramycin M: *Z*-form	9.4 ± 1.6	3.8 ± 0.54	1.90	1.94	3.99	4.02
2	**3a**	H (Jorunnamycin A)	1.3×10^2 ± 8.9	2.9×10^2 ± 4.8	1.93	1.93	3.98	4.03
3	**2b**	$COCH_3$ Cyanojorumycin	3.2 ± 0.47	1.4 ± 0.071	1.94	1.96	3.99	4.01
4	**3b**	$COCH_2CH_3$ Jorunnamycin C	11 ± 0.37	4.2 ± 0.087	1.95	1.95	4.01	4.01
5	**4a**	$COC(CH_3)$=$CHCH_3$ (*E*-form)	34 ± 0.33	11 ± 0.33	1.93	1.96	3.99	4.02
6	**4b**	$COCH$=$C(CH_3)_2$	27 ± 0.31	11 ± 0.31	1.92	1.95	4.00	4.01
7	**6a**	CHO	38 ± 1.9	38 ± 1.6	1.95	1.96	4.01	4.02
8	**6b**	$COCH_2CH_2CH_3$	34 ± 1.4	12 ± 0.41	1.95	1.95	4.01	4.02
9	**6c**	$COCH(CH_3)_2$	22 ± 1.5	10 ± 0.12	1.94	1.94	4.00	4.02
10	**6d**[b]	$COCH(CH_3)CH_2CH_3$	33 ± 0.89	12 ± 0.31	1.94	1.94	4.01	4.01
11	**6e**	$COCH_2CH_2CH_2CH_2CH_3$	1.0×10^2 ± 2.4	40 ± 0.63	1.95	1.95	4.01	4.02
12	**6f**	$COCH_2CH_2Cl$	16 ± 0.70	12 ± 0.47	1.95	1.96	4.01	4.03
13	**7a**	CO-Cyclopentyl	93 ± 5.2	30 ± 0.51	1.93	1.93	4.02	4.02
14	**7b**	CO-1-Cyclopentenyl	33 ± 1.1	13 ± 0.72	1.94	1.95	4.00	4.02
15	**7c**	CO-3-Cyclopentenyl	33 ± 2.2	13 ± 0.70	1.94	1.95	4.00	4.02
16	**7d**	CO-1-Cyclohexenyl	49 ± 2.0	29 ± 0.26	1.94	1.95	4.01	4.02
17	**7e**[b]	CO-3-Cyclohexenyl	43 ± 0.74	27 ± 3.1	1.93	1.95	4.00	4.02
18	**8a**	COC_6H_5	25 ± 1.3	8.6 ± 0.42	1.97	1.69	4.03	3.67
19	**8b**	$COC_6H_4OCH_3$-4	26 ± 1.7	8.9 ± 0.76	1.97	1.72	4.03	3.71
20	**8c**	$COC_6H_4OCH_3$-2	34 ± 0.89	11 ± 0.37	1.95	1.48	4.00	3.62
21	**8d**	$COC_6H_4NO_2$-4	26 ± 1.5	9.4 ± 0.81	1.97	1.69	4.02	3.77
22	**8e**	COC_6H_4Br-4	38 ± 1.2	13 ± 0.50	2.00	1.76	4.05	3.78
23	**9a**	CO-4-Pyridyl	10 ± 0.78	4.4 ± 0.12	2.00	1.76	4.04	3.76
24	**9b**	CO-3-Pyridyl	7.9 ± 1.1	3.5 ± 0.11	1.99	1.74	4.04	3.77
25	**9c**	CO-2-Pyridyl	3.3 ± 0.15	1.4 ± 0.031	1.96	1.69	4.01	3.85
26	**9d**	CO-4-Quinolynyl	10 ± 0.33	4.1 ± 0.095	2.02	1.16	4.05	3.65
27	**9e**	CO-3-Quinolynyl	10 ± 0.35	3.8 ± 0.052	2.03	1.31	4.06	3.44
28	**9f**	CO-2-Qunolynyl	11 ± 0.14	4.2 ± 0.12	2.02	1.42	4.03	3.28

[a] HCT116: human colon carcinoma; MDA-MB-435: human breast carcinoma.
[b] An inseparable 1:1 mixture of diastereomers.

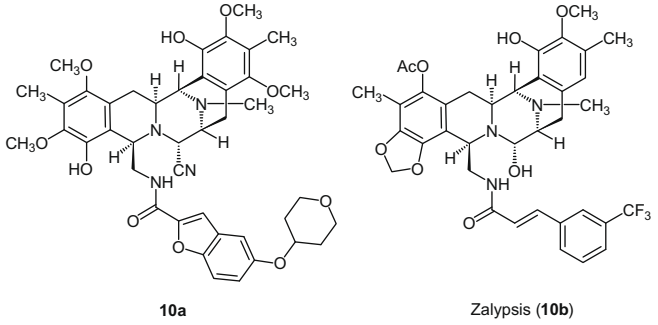


Figure 4. Structures of second-generation synthetic analogues.

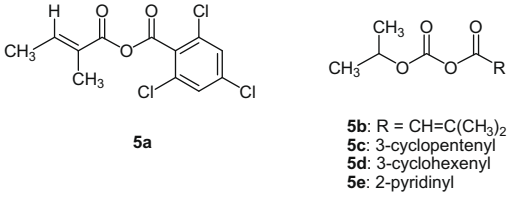


Figure 5. Structures of mixed anhydrides.

5.2. 3,3-Dimethylacryloyl ester 4b

Carbonate **5b**[30] (27.3 mg, 0.147 mmol) was added to a stirred solution of **3a** (5.0 mg, 0.01 mmol) in pyridine (0.2 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (24.4 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **4b** (4.7 mg, 80.6%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 61.6 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 2928, 2854, 2228, 1715, 1652, 1615, 1456, 1417, 1374, 1261, 801 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.30 (1H, br s, 2′-H), 4.53 (1H, dd, *J* = 11.4, 3.1 Hz, 22-Ha), 4.06 (1H, d, *J* = 2.3 Hz, 21-H), 4.01 (3H, s, OCH_3), 4.01 (1H, overlapped, 11-H), 4.00 (3H, s, OCH_3), 4.00 (1H, overlapped, 1-H), 3.90 (1H, dd, *J* = 11.4, 3.4 Hz, 22-Hb), 3.37 (1H, ddd, *J* = 7.5, 2.3, 0.5 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.1, 2.5 Hz, 3-H), 2.91 (1H, dd, *J* = 17.1, 2.5 Hz, 4-Hα), 2.73 (1H, dd, *J* = 21.0, 7.5 Hz, 14-Hα), 2.30 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.27 (3H, s, *N*CH_3), 2.01 (3H, s, 4′-H_3), 1.95 (3H, s, 16-CH_3), 1.92 (3H, s, 6-CH_3), 1.81 (3H, s, 3′-CH_3), 1.34 (1H, ddd, *J* = 17.1, 11.6, 2.4 Hz, 4-Hβ), 0.82 (3H, t, *J* = 7.3 Hz, 4′-H_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.9 (C-15), 185.5 (C-5), 182.5 (C-18), 181.0 (C-8), 165.4 (OCOR), 158.7 (C-3′), 155.6 (C-7), 155.2 (C-17), 142.1 (C-20), 141.8 (C-10), 135.7 (C-9), 134.9 (C-19), 128.5 (C-6), 128.4 (C-16), 117.0 (CN), 114.8 (C-2′), 62.1 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.8 (C-21), 56.1 (C-1), 54.6 (C-13), 54.4 (C-3), 54.4 (C-11), 41.5 (*N*CH_3), 27.4 (3′-CH_3), 25.4 (C-4), 21.1 (C-14), 20.2 (C-4′), 8.7 (16-CH_3), 8.7 (6-CH_3); EIMS m/*z* (% intensity) 575 (M^+, 8), 464 (5), 260 (9), 220 (100), 218 (24), 204 (10), 176 (6); HREIMS *m/z* 575.2268 (M^+, calcd for $C_{31}H_{33}N_3O_8$, 575.2268).

5.3. Formic acid ester 6a

A CH_2Cl_2 solution of acetic formic anhydride[31] (1.8 M, 66.7 μL, 0.12 mmol) was added to a stirred solution of **3a** (24.7 mg, 0.05 mmol) in pyridine (1.0 mL) at −17 °C, and the resulting solution was stirred at 0 °C for 24 h. The reaction mixture was diluted with water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with aqueous saturated NaH-

CO_3 solution, dried, and concentrated in vacuo to give a residue (27.5 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **6a** (11.8 mg, 45.2%) as a pale yellow solid. $[\alpha]_D^{22}$ − 92.2 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 2944, 2852, 2229, 1724, 1654, 1619, 1451, 1374, 1310, 1234, 1079, 1021, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.80 (1H, s, HCO), 4.47 (1H, dd, *J* = 11.3, 2.9 Hz, 22-Ha), 4.05 (1H, d, *J* = 2.3 Hz, 21-H), 4.02 (3H, s, OCH_3), 4.02 (1H, overlapped, 1-H), 4.01 (1H, overlapped, 11-H), 4.01 (3H, s, OCH_3), 3.96 (1H, dd, *J* = 11.3, 4.3 Hz, 22-Hb), 3.38 (1H, ddd, *J* = 7.6, 2.3, 1.7 Hz, 13-H), 3.12 (1H, ddd, *J* = 11.6, 3.1, 2.6 Hz, 3-H), 2.94 (1H, dd, *J* = 17.2, 2.6 Hz, 4-Hα), 2.77 (1H, dd, *J* = 21.1, 7.6 Hz, 14-Hα), 2.31 (3H, s, *N*CH_3), 2.25 (1H, d, *J* = 21.1 Hz, 14-Hβ), 1.96 (3H, s, 16-CH_3), 1.95 (3H, s, 6-CH_3), 1.36 (1H, ddd, *J* = 17.2, 11.6, 2.4 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.2 (C-15), 185.3 (C-5), 182.5 (C-18), 181.0 (C-8), 159.8 (HCO), 155.5 (C-7), 155.2 (C-17), 142.2 (C-20), 142.1 (C-10), 135.0 (C-9), 134.9 (C-19), 128.8 (C-6), 128.7 (C-16), 116.8 (CN), 63.5 (C-22), 61.1 (OCH_3), 61.1 (OCH_3), 59.2 (C-21), 55.6 (C-1), 54.6 (C-13), 54.6 (C-3), 54.3 (C-11), 41.5 (*N*CH_3), 25.2 (C-4), 21.3 (C-14), 8.8 (6-CH_3), 8.7 (16-CH_3); EIMS *m/z* (% intensity) 521 (M^+, 10), 495 (0.5), 462 (2), 435 (3), 260 (9), 243 (7), 220 (100), 218 (25), 204 (14), 176 (10); HREIMS *m/z* 521.1792 (M^+, calcd for $C_{27}H_{27}N_3O_8$, 521.1798).

5.4. Butyric acid ester 6b

Butyric anhydride (24.0 μL, 0.147 mmol) was added to a stirred solution of **3a** (5.0 mg, 0.01 mmol) in pyridine (0.2 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 5 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (12.2 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **6b** (3.7 mg, 64.8%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 79.9 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 2935, 2854, 2221, 1738, 1654, 1615, 1456, 1412, 1373, 1235, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.39 (1H, dd, *J* = 11.5, 3.1 Hz, 22-Ha), 4.06 (1H, d, *J* = 2.4 Hz, 21-H), 4.02 (3H, s, OCH_3), 4.01 (1H, overlapped, 11-H), 4.01 (3H, s, OCH_3), 3.99 (1H, br s, 1-H), 3.93 (1H, dd, *J* = 11.5, 3.6 Hz, 22-Hb), 3.38 (1H, br d, *J* = 7.3 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.93 (1H, dd, *J* = 17.4, 2.7 Hz, 4-Hα), 2.76 (1H, dd, *J* = 21.0, 7.3 Hz, 14-Hα), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.29 (3H, s, *N*CH_3), 2.01 (2H, t, *J* = 7.3 Hz, 2′-H_2), 1.95 (3H, s, 16-CH_3), 1.95 (3H, s, 6-CH_3), 1.42 (2H, sept, *J* = 7.3 Hz, 3′-H_2), 1.32 (1H, ddd, *J* = 17.4, 11.6, 2.4 Hz, 4-Hβ), 0.82 (3H, t, *J* = 7.3 Hz, 4′-H_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.1 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 172.6 (OCOR), 155.6 (C-7), 155.2 (C-17), 142.1 (C-20), 141.7 (C-10), 135.5 (C-9), 135.0 (C-19), 128.6 (C-6), 128.5 (C-16), 116.9 (CN), 63.3 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.9 (C-21), 56.0 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (*N*CH_3), 36.0 (C-2′), 25.4 (C-4), 21.2 (C-14), 18.3 (C-3′), 13.6 (C-4′), 8.8 (16-CH_3), 8.6 (6-CH_3); EIMS *m/z* (% intensity) 563 (M^+, 7), 462 (3), 435 (4), 243 (13), 220 (100), 218 (23), 204 (11), 176 (6); HREIMS *m/z* 563.2271 (M^+, calcd for $C_{30}H_{33}N_3O_8$, 563.2268).

5.5. 2′-Methylpropanoic acid ester 6c

Isobutyric anhydride (21.9 μL, 0.735 mmol) was added to a stirred solution of **3a** (24.7 mg, 0.05 mmol) in pyridine (0.9 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 5 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (199.5 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **6c** (12.7 mg, 44.1%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 67.0 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 2963, 2854, 2228, 1732, 1653, 1455, 1411, 1374, 1261, 1190, 1081, 956, 769 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.30 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.07 (1H, d, *J* = 2.1 Hz, 21-H), 4.06 (1H, dd, *J* = 11.6, 3.6 Hz, 22-Hb), 4.04 (1H, overlapped, 11-H), 4.02 (3H, s, OCH_3), 4.01 (1H, br s, 1-H), 4.00 (3H, s, OCH_3), 3.40 (1H, ddd, *J* = 7.6, 2.1, 0.5 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.5, 3.1, 2.8 Hz, 3-H), 2.91 (1H, dd, *J* = 17.4, 2.8 Hz, 4-Hα), 2.78 (1H, dd, *J* = 20.8, 7.6 Hz, 14-Hα), 2.31 (1H, d, *J* = 20.8 Hz, 14-Hβ), 2.30 (1H, overlapped, 2′-H), 2.29 (3H, s, *N*CH_3), 1.94 (3H, s, 16-CH_3), 1.94 (3H, s, 6-CH_3), 1.35 (1H, ddd, *J* = 17.4, 11.5, 2.4 Hz, 4-Hβ), 0.95 (3H, d, *J* = 7.0 Hz, 3′-H_3), 0.93 (3H, d, *J* = 7.0 Hz, 2′-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.3 (C-15), 185.4 (C-5), 182.5 (C-18), 181.0 (C-8), 176.0 (OCOR), 155.7 (C-7), 155.3 (C-17), 142.0 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.7 (C-6), 128.4 (C-16), 116.8 (CN), 63.3 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.8 (C-21), 56.2 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 41.5 (*N*CH_3), 33.8 (C-2′), 25.4 (C-4), 21.2 (C-14), 19.0 (C-3′), 18.5 (2′-CH_3), 8.7 (16-CH_3), 8.6 (6-CH_3); EIMS *m/z* (% intensity) 563 (M^+, 7), 464 (4), 435 (3), 260 (15), 243 (5), 220 (100), 218 (20), 204 (9), 176 (5); HREIMS *m/z* 563.2273 (M^+, calcd for $C_{30}H_{33}N_3O_8$, 563.2268).

5.6. 2′-(*R*/*S*)-Methylbutanoic Acid Ester 6d

(±)-2-Methylbutyryl chloride (18.4 μL, 0.15 mmol) was added to a stirred solution of **3a** (14.8 mg, 0.03 mmol) and DMAP (0.73 mg, 0.006 mmol) in dry CH_2Cl_2 (3 mL) at 0 °C, and the resulting solution was stirred at 0 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (19.6 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave an inseparable mixture of **6d** (7.8 mg, 40.1%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 79.1 (*c* 0.7, $CHCl_3$); IR ($CHCl_3$) 3445, 3281, 2964, 2855, 2229, 1732, 1652, 1615, 1463, 1410, 1373, 1235, 1802 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) *one isomer* δ 4.24 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.12 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Hb), 4.08 (1H, d, *J* = 2.7 Hz, 21-H), 4.03 (1H, br d, *J* = 2.7 Hz, 11-H), 4.01 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.00 (1H, overlapped, 1-H), 3.41 (1H, dd, *J* = 7.3, 1.2 Hz, 13-H), 3.11 (1H, ddd, *J* = 11.4, 2.7, 2.5 Hz, 3-H), 2.90 (1H, dd, *J* = 17.1, 2.5 Hz, 4-Hα), 2.78 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.30 (1H, dd, *J* = 21.0, 1.2 Hz, 14-Hβ), 2.29 (3H, s, *N*CH_3), 2.09 (1H, m, 2′-H), 1.94 (3H, s, 16-CH_3), 1.94 (3H, s, 6-CH_3), 1.35 (1H, ddd, *J* = 17.1, 11.4, 2.7 Hz, 4-Hβ), 1.30 (2H, overlapped, 3′-H_2), 0.89 (3H, d, *J* = 7.0 Hz, 2′-CH_3), 0.88 (3H, t, *J* = 7.2 Hz, 4′-H_3); *other isomer* δ 4.27 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.12 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Hb), 4.08 (1H, d, *J* = 2.7 Hz, 21-H), 4.03 (1H, br d, *J* = 2.4 Hz, 11-H), 4.01 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.00 (1H, overlapped, 1-H), 3.41 (1H, dd, *J* = 7.3, 1.2 Hz, 13-H), 3.11 (1H, ddd, *J* = 11.5, 3.1, 2.7 Hz, 3-H), 2.90 (1H, dd, *J* = 17.1, 2.4 Hz, 4-Hα), 2.78 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.30 (1H, dd, *J* = 21.0, 1.2 Hz, 14-Hβ), 2.29 (3H, s, *N*CH_3), 2.09 (1H, m, 2′-H), 1.94 (3H, s, 16-CH_3), 1.94 (3H, s, 6-CH_3), 1.35 (1H, ddd, *J* = 17.1, 11.3, 2.7 Hz, 4-Hβ), 1.27 (2H, m, 3′-H_2), 0.91 (3H, d, *J* = 7.0 Hz, 2′-CH_3), 0.74 (3H, t, *J* = 7.2 Hz, 4′-H_3); ^{13}C NMR ($CDCl_3$, 125 MHz) *one isomer* δ 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.4 (OCOR), 155.8 (C-7), 155.3 (C-17), 142.0 (C-20), 141.3 (C-10), 135.5 (C-9), 135.2 (C-19), 128.6 (C-6), 128.3 (C-16), 116.8 (CN), 63.4 (C-22), 61.0 (OCH_3), 61.0 (OCH_3), 58.8 (C-21), 56.3 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (*N*CH_3), 40.8 (C-2′), 26.6 (C-3′), 25.4 (C-4), 21.2 (C-14), 16.5 (2′-CH_3), 14.1 (C-4′), 8.7 (16-CH_3), 8.6 (6-CH_3); *other isomer* δ 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.7 (OCOR), 155.8 (C-7), 155.3 (C-17), 142.0 (C-20), 141.3 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.3 (C-16), 116.8 (CN), 63.2 (C-22), 61.0 (OCH_3), 61.0 (OCH_3), 58.8 (C-21), 56.2 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (*N*CH_3), 40.8 (C-2′), 26.2 (C-3′), 25.4

(C-4), 21.2 (C-14), 16.5 (2′-CH_3), 14.1 (C-4′), 8.7 (16-CH_3), 8.6 (6-CH_3); EIMS *m/z* (% intensity) 577 (M^+, 10), 4642 (3), 435 (2), 260 (16), 243 (6), 220 (100), 218 (24), 204 (10), 176 (6); HREIMS *m/z* 577.2424 (M^+, calcd for $C_{31}H_{35}N_3O_8$, 577.2424).

5.7. Hexanoic acid ester 6e

Hexanoic anhydride (69.0 μL, 0.298 mmol) was added to a stirred solution of **3a** (9.9 mg, 0.02 mmol) and DMAP (0.24 mg, 0.002 mmol) in pyridine (0.4 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1.5 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (68.8 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **6e** (7.0 mg, 58.4%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 84.7 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 2932, 2857, 2229, 1738, 1652, 1615, 1456, 1417, 1373, 1235, 803 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.38 (1H, dd, *J* = 11.6, 3.1 Hz, 22-Ha), 4.06 (1H, d, *J* = 2.0 Hz, 21-H), 4.02 (1H, overlapped, 11-H), 4.02 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 3.99 (1H, br s, 1-H), 3.91 (1H, dd, *J* = 11.6, 4.0 Hz, 22-Hb), 3.38 (1H, ddd, *J* = 7.3, 2.0, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.93 (1H, dd, *J* = 17.1, 2.7 Hz, 4-Hα), 2.76 (1H, dd, *J* = 20.7, 7.3 Hz, 14-Hα), 2.31 (1H, d, *J* = 20.7 Hz, 14-Hβ), 2.29 (3H, s, *N*CH_3), 2.02 (2H, t, *J* = 8.3 Hz, 2′-H_2), 1.95 (3H, s, 16-CH_3), 1.95 (3H, s, 6-CH_3), 1.40 (2H, sextet, *J* = 8.3 Hz, 3′-H_2), 1.31 (H, ddd, *J* = 17.1, 11.6, 2.4 Hz, 4-Hβ), 1.23 (2H, m, 5′-H_2), 1.16 (2H, m, 4′-H), 0.85 (3H, t, *J* = 7.3 Hz, 6′-H_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.1 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 172.8 (OCOR), 155.6 (C-7), 155.2 (C-17), 142.2 (C-20), 141.7 (C-10), 135.5 (C-9), 135.0 (C-19), 128.6 (C-6), 128.5 (C-16), 116.9 (CN), 63.5 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 59.0 (C-21), 55.9 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (*N*CH_3), 34.0 (C-2′), 31.2 (C-4′), 25.3 (C-4), 24.4 (C-3′), 22.5 (C-5′), 21.2 (C-14), 13.8 (C-6′), 8.8 (16-CH_3), 8.6 (6-CH_3); EIMS *m/z* (% intensity) 591 (M^+, 5), 464 (3), 435 (3), 260 (10), 243 (100), 220 (69), 204 (10); HREIMS m/*z* 591.2578 (M^+, calcd for $C_{32}H_{37}N_3O_8$, 591.2581).

5.8. 3′-Chloropropanoic acid ester 6f

3-Chloropropanoyl chloride (105.2 μL, 1.10 mmol) was added to a stirred solution of **3a** (37.0 mg, 0.075 mmol) and DMAP (0.92 mg, 0.0075 mmol) in pyridine (1.5 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (170.7 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **6f** (13.4 mg, 29.8%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 79.6 (*c* 0.7, $CHCl_3$); IR ($CHCl_3$) 3446, 2944, 2853, 2229, 1737, 1654, 1449, 1417, 1374, 1311, 1235, 1150, 1080, 966, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.39 (1H, dd, *J* = 11.6, 3.1 Hz, 22-Ha), 4.10 (1H, dd, *J* = 11.6, 3.7 Hz, 22-Hb), 4.07 (1H, d, *J* = 3.1 Hz, 21-H), 4.03 (1H, overlapped, 11-H), 4.03 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.01 (1H, br s, 1-H), 3.53 (2H, m, 3′-H_2), 3.40 (1H, ddd, *J* = 7.4, 1.8, 0.5 Hz, 13-H), 3.11 (1H, ddd, *J* = 11.6, 3.1, 2.5 Hz, 3-H), 2.92 (1H, dd, *J* = 17.4, 2.5 Hz, 4-Hα), 2.76 (1H, dd, *J* = 21.0, 7.4 Hz, 14-Hα), 2.53 (2H, m, 2′-H_2), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.30 (3H, s, *N*CH_3), 1.96 (3H, s, 16-CH_3), 1.95 (3H, s, 6-CH_3), 1.37 (H, ddd, *J* = 17.4, 11.6, 2.6 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.3 (C-15), 185.3 (C-5), 182.4 (C-18), 181.0 (C-8), 169.5 (OCOR), 155.6 (C-7), 155.4 (C-17), 142.0 (C-20), 141.8 (C-10), 135.1 (C-9), 135.1 (C-19), 128.7 (C-6), 128.5 (C-16), 116.8 (CN), 63.8 (C-22), 61.1 (OCH_3), 61.1 (OCH_3), 58.8 (C-21), 55.9 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 41.5 (*N*CH_3), 38.5 (C-3′), 37.4 (C-2′), 25.4 (C-4), 21.2 (C-14), 8.8 (16-CH_3), 8.6 (6-CH_3); EIMS *m/z* (% intensity) 583 (M^+, 3), 547 (5), 462 (3), 435 (1), 260 (7), 220 (100), 218 (24), 204 (10), 176 (6); HREIMS *m/z* 583.1728 (M^+, calcd for $C_{29}H_{30}ClN_3O_8$, 583.1722).

5.9. Cyclopentanecarboxylic acid ester 7a

Cyclopentanecarbonyl chloride (35.7 μL, 0.294 mmol) was added to a stirred solution of **3a** (9.9 mg, 0.02 mmol) and DMAP (0.24 mg, 0.002 mmol) in pyridine (0.4 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (41.5 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **7a** (9.8 mg, 78.8%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 66.6 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 3444, 3277, 2960, 2855, 2228, 1732, 1652, 1615, 1455, 1411, 1373, 1261, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.30 (1H, dd, *J* = 11.3, 2.7 Hz, 22-Ha), 4.08 (1H, d, *J* = 2.1 Hz, 21-H), 4.07 (1H, overlapped, 11-H), 4.02 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.02 (1H, overlapped, 22-Hb), 3.99 (1H, br s, 1-H), 3.40 (1H, ddd, *J* = 7.3, 2.1, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.1, 2.5 Hz, 3-H), 2.91 (1H, dd, *J* = 17.1, 2.5 Hz, 4-Hα), 2.78 (1H, dd, *J* = 20.8, 7.5 Hz, 14-Hα), 2.43 (1H, quintet, *J* = 7.5 Hz, 1′-H), 2.30 (1H, d, *J* = 20.8 Hz, 14-Hβ), 2.29 (3H, s, *N*CH_3), 1.93 (3H, s, 16-CH_3), 1.93 (3H, s, 6-CH_3), 1.66 (2H, m), 1.65 (2H, m), 1.52 (1H, m), 1.50 (2H, m), 1.46 (1H, m), 1.35 (H, ddd, *J* = 17.1, 11.6, 2.6 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.7 (OCOR), 155.7 (C-7), 155.3 (C-17), 142.1 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.3 (C-16), 116.8 (CN), 63.3 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.8 (C-21), 56.3 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 43.8 (C-1′), 41.5 (*N*CH_3), 30.1 (C-2′), 29.7 (C-5′), 25.6 (C-3′), 25.5 (C-4′), 25.4 (C-4), 21.2 (C-14), 8.7 (16-CH_3), 8.6 (6-CH_3); EIMS *m/z* (% intensity) 589 (M^+, 9), 462 (4), 260 (11), 243 (47), 220 (100), 218 (24), 204 (12); HREIMS *m/z* 589.2427 (M^+, calcd for $C_{32}H_{35}N_3O_8$, 589.2424).

5.10. 1′-Cyclopentenecarboxylic acid ester 7b

1-Cyclopentenecarbonyl chloride (57.6 mg, 0.440 mmol) was added to a stirred solution of **3a** (14.8 mg, 0.03 mmol) and DMAP (0.37 mg, 0.003 mmol) in pyridine (0.6 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 2.5 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (66.4 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **7b** (11.9 mg, 60.5%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 75.5 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 3443, 3279, 2961, 2854, 2228, 1714, 1652, 1447, 1373, 1235, 801 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.75 (1H, dt, *J* = 6.4, 2.1 Hz, 2′-H), 4.55 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.05 (1H, d, *J* = 2.0 Hz, 21-H), 4.02 (1H, overlapped, 1-H), 4.02 (3H, s, OCH_3), 4.01 (1H, overlapped, 22-Hb), 4.00 (3H, s, OCH_3), 4.00 (1H, overlapped, 11-H), 3.38 (1H, ddd, *J* = 7.6, 2.0, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.3, 3.1, 2.7 Hz, 3-H), 2.91 (1H, dd, *J* = 17.4, 2.7 Hz, 4-Hα), 2.74 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.38 (2H, m, 3′-H_2), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.27 (3H, s, *N*CH_3), 2.27 (2H, overlapped, 5′-H_2), 1.95 (3H, s, 16-CH_3), 1.94 (3H, s, 6-CH_3), 1.84 (2H, quintet, *J* = 7.6 Hz, 4′-H_2), 1.35 (H, ddd, *J* = 17.4, 11.3, 2.1 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.0 (C-15), 185.5 (C-5), 182.5 (C-18), 181.0 (C-8), 164.1 (OCOR), 155.7 (C-7), 155.1 (C-17), 144.7 (C-2′), 142.2 (C-20), 141.8 (C-10), 135.8 (C-9), 135.5 (C-19), 134.8 (C-1′), 128.5 (C-6), 128.4 (C-16), 117.0 (CN), 62.3 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.7 (C-21), 56.4 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 43.0 (C-1′), 41.5 (*N*CH_3), 33.3 (C-3′), 31.2 (C-5′), 25.4 (C-4),

22.9 (C-4′), 21.1 (C-14), 8.8 (16-CH_3), 8.7 (6-CH_3); EIMS *m/z* (% intensity) 587 (M^+, 10), 462 (4), 260 (10), 220 (100), 218 (24), 204 (10), 176 (6); HREIMS *m/z* 587.2267 (M^+, calcd for $C_{32}H_{33}N_3O_8$, 587.2268).

5.11. 3′-Cyclopentenecarboxylic acid ester 7c

Carbonate **5c**[30] (87.4 mg, 0.440 mmol) was added to a stirred solution of **3a** (14.8 mg, 0.03 mmol) and DMAP (0.37 mg, 0.003 mmol) in pyridine (0.6 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (84.4 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **7c** (12.5 mg, 64.4%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 82.5 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 2942, 2855, 2228, 1732, 1652, 1615, 1456, 1410, 1374, 1261, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) *δ* 5.53 (2H, m, 3′-H and 4′-H), 4.36 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.08 (1H, d, *J* = 2.1 Hz, 21-H), 4.05 (1H, dd, *J* = 11.6, 3.7 Hz, 22-Hb), 4.02 (1H, overlapped, 11-H), 4.02 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 4.00 (1H, overlapped, 1-H), 3.40 (1H, ddd, *J* = 7.4, 2.1, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.3, 3.1, 2.5 Hz, 3-H), 2.91 (1H, dd, *J* = 17.3, 2.5 Hz, 4-Hα), 2.84 (1H, quintet, *J* = 7.0 Hz, 1′-H), 2.81 (2H, t, *J* = 7.0 Hz, 2.74 (1H, dd, *J* = 21.0, 7.4 Hz, 14-Hα), 2.41 (2H, m, 3′-H_2), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.28 (3H, s, *N*CH_3), 1.95 (3H, s, 16-CH_3), 1.94 (3H, s, 6-CH_3), 1.35 (H, ddd, *J* = 17.3, 11.3, 2.7 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.0 (OCOR), 155.7 (C-7), 155.2 (C-17), 142.1 (C-20), 141.6 (C-10), 135.4 (C-9), 135.0 (C-19), 129.1 (C-3′), 128.6 (C-4′), 128.4 (C-6), 128.4 (C-16), 116.8 (CN), 63.5 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.9 (C-21), 56.3 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 41.6 (C-1′), 41.5 (*N*CH_3), 36.3 (C-2′), 35.7 (C-5′), 25.4 (C-4), 21.2 (C-14), 8.7 (16-CH_3), 8.7 (6-CH_3); EIMS *m/z* (% intensity) 587 (M^+, 10), 462 (3), 260 (9), 220 (100), 218 (26), 204 (12), 176 (8); HREIMS *m/z* 587.2267 (M^+, calcd for $C_{32}H_{33}N_3O_8$, 587.2268).

5.12. 1′-Cyclohexenecarboxylic acid ester 7d

1-Cyclopentenecarbonyl chloride (63.7 mg, 0.440 mmol) was added to a stirred solution of **3a** (14.8 mg, 0.03 mmol) and DMAP (0.37 mg, 0.003 mmol) in pyridine (0.6 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 2 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (31.3 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **7d** (11.9 mg, 58.4%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 60.9 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 2935, 2856, 2228, 1714, 1652, 1615, 1456, 1261, 801 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) *δ* 6.62 (1H, dt, *J* = 6.4, 2.1 Hz, 2′-H), 4.50 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.04 (1H, br s, 21-H), 4.02 (1H, overlapped, 1-H), 4.02 (3H, s, OCH_3), 4.01 (1H, overlapped, 22-Hb), 4.01 (3H, s, OCH_3), 4.00 (1H, overlapped, 11-H), 3.38 (1H, ddd, *J* = 7.6, 1.8, 0.6 Hz, 13-H), 3.09 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.92 (1H, dd, *J* = 17.1, 2.7 Hz, 4-Hα), 2.75 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.28 (3H, s, *N*CH_3), 2.06 (2H, m, 3′-H_2), 1.95 (2H, overlapped, 6′-H_2), 1.95 (3H, s, 16-CH_3), 1.94 (3H, s, 6-CH_3), 1.94 (2H, overlapped, 5′-H_2), 1.51 (2H, m, 4′-H_2), 1.32 (H, ddd, *J* = 17.1, 11.6, 2.7 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* 186.1 (C-15), 185.5 (C-5), 182.5 (C-18), 181.0 (C-8), 166.5 (OCOR), 155.7 (C-7), 155.1 (C-17), 142.2 (C-20), 141.7 (C-10), 140.6 (C-2′), 135.6 (C-9), 134.8 (C-19), 129.6 (C-1′), 128.4 (C-6), 128.3 (C-16), 116.9 (CN), 62.6 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.8 (C-21), 56.4 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (*N*CH_3), 25.7 (C-3′), 25.4 (C-4), 24.0 (C-6′), 21.2 (C-5′), 21.1 (C-14), 8.8 (16-CH_3), 8.8 (6-CH_3); EIMS *m/z* (% intensity) 601 (M^+, 10), 464 (6), 260 (12), 220 (100), 218 (27), 204 (12), 176 (8); HREIMS *m/z* 601.2427 (M^+, calcd for $C_{33}H_{35}N_3O_8$, 601.2424).

5.13. (*R*/*S*)-3′-Cyclohexenecarboxylic acid ester 7e

Carbonate **5d**[30] (31.2 mg, 0.147 mmol) was added to a stirred solution of **3a** (5.0 mg, 0.01 mmol) and DMAP (0.12 mg, 0.001 mmol) in pyridine (0.2 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (53.4 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave an inseparable mixture of **7e** (5.0 mg, 80.4%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 68.8 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) 2929, 2841, 2229, 1731, 1646, 1613, 1451, 1374, 1233, 798 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) *one isomer* *δ* 5.78 (2H, br s, 3′-H, 4′-H), 4.42 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.08 (1H, br s, 21-H), 4.07 (1H, dd, *J* = 11.6, 3.1 Hz, 22-Hb), 4.02 (3H, s, OCH_3), 4.01 (1H, overlapped, 11-H), 4.01 (3H, s, OCH_3), 4.00 (1H, overlapped, 1-H), 3.40 (1H, ddd, *J* = 7.6, 1.8, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.90 (1H, dd, *J* = 17.1, 2.7 Hz, 4-Hα), 2.77 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.28 (3H, s, *N*CH_3), 2.26 (1H, m, 1′-H), 2.04 (1H, m, 2′-H), 2.01 (1H, m, 6′-H), 1.95 (3H, s, 16-CH_3), 1.94 (1H, m, 2′-H), 1.93 (3H, s, 6-CH_3), 1.87 (1H, m, 6′-H), 1.35 (2H, m, 5′-H_2), 1.34 (H, ddd, *J* = 17.1, 11.6, 2.7 Hz, 4-Hβ); *other isomer* *δ* 5.60 (1H, m, 3′-H), 5.51 (1H, m, 4′-H), 4.35 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.13 (1H, dd, *J* = 11.6, 3.1 Hz, 22-Hb), 4.08 (1H, d, *J* = 2.2 Hz, 21-H), 4.02 (3H, s, OCH_3), 4.01 (1H, overlapped, 11-H), 4.00 (3H, s, OCH_3), 4.00 (1H, overlapped, 1-H), 3.40 (1H, ddd, *J* = 7.6, 2.2, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.90 (1H, dd, *J* = 17.1, 2.7 Hz, 4-Hα), 2.77 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.28 (3H, s, *N*CH_3), 2.26 (1H, m, 1′-H), 2.04 (1H, m, 2′-H), 2.01 (1H, m, 6′-H), 1.95 (3H, s, 6-CH_3), 1.94 (1H, m, 2′-H), 1.93 (3H, s, 16-CH_3), 1.87 (1H, m, 6′-H), 1.35 (2H, m, 5′-H_2), 1.34 (H, ddd, *J* = 17.1, 11.6, 2.7 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) *one isomer* *δ* 186.2 (C-15), 185.5 (C-5), 182.4 (C-18), 180.9 (C-8), 174.8 (OCOR), 155.8 (C-7), 155.3 (C-17), 142.1 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.3 (C-16), 126.9 (C-3′), 124.8 (C-4′), 116.8 (CN), 62.7 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.6 (C-21), 56.4 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (*N*CH_3), 39.2 (C-1′), 27.7 (C-6′), 25.5 (C-4), 25.2 (C-5′), 24.3 (C-2′), 21.1 (C-14), 8.7 (16-CH_3), 8.7 (6-CH_3); *other isomer* *δ* 186.2 (C-15), 185.5 (C-5), 182.4 (C-18), 181.0 (C-8), 174.8 (OCOR), 155.8 (C-7), 155.3 (C-17), 142.1 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.4 (C-16), 126.6 (C-3′), 124.6 (C-4′), 116.8 (CN), 62.8 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.6 (C-21), 56.4 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (*N*CH_3), 39.1 (C-1′), 27.7 (C-6′), 25.5 (C-4), 25.2 (C-5′), 24.3 (C-2′), 21.2 (C-14), 8.7 (16-CH_3), 8.6 (6-CH_3); EIMS *m/z* (% intensity) 601 (M^+, 9), 462 (3), 260 (11), 220 (100), 218 (22), 204 (10), 176 (5); HREIMS *m/z* 601.2420 (M^+, calcd for $C_{33}H_{35}N_3O_8$, 601.2424).

5.14. Benzenecarboxylic acid ester 8a

Benzoyl chloride (72.8 μL, 0.63 mmol) was added to a stirred solution of **3a** (21.4 mg, 0.043 mmol) and DMAP (0.49 mg, 0.0043 mmol) in pyridine (0.8 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1.5 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (103.7 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1)

as eluent gave **8a** (10.2 mg, 39.4%) as a pale yellow amorphous powder. $[\alpha]_D^{22}$ − 66.2 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) 3445, 2926, 2853, 2229, 1721, 1652, 1616, 1452, 1373, 1272, 1117, 956, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.60 (2H, dd, *J* = 7.7, 1.2 Hz, 2′-H, 6′-H), 7.50 (1H, dt, *J* = 7.7, 1.2 Hz, 4′-H), 7.31 (2H, t, *J* = 7.7 Hz, 3′-H, 5′-H), 5.00 (1H, dd, *J* = 12.0, 3.1 Hz, 22-Ha), 4.12 (1H, br s, 21-H), 4.06 (1H, d, *J* = 12.0 Hz, 22-Hb), 4.06 (1H, overlapped, 1-H), 4.03 (3H, s, 7-OCH_3), 3.97 (1H, br d, *J* = 2.3 Hz, 11-H), 3.67 (3H, s, 17-OCH_3), 3.44 (1H, ddd, *J* = 7.2, 1.8, 0.6 Hz, 13-H), 3.12 (1H, ddd, *J* = 11.4, 2.4, 2.3 Hz, 3-H), 2.88 (1H, dd, *J* = 17.4, 2.4 Hz, 4-Hα), 2.74 (1H, dd, *J* = 21.1, 7.2 Hz, 14-Hα), 2.40 (1H, d, *J* = 21.1 Hz, 14-Hβ), 2.25 (3H, s, *N*CH_3), 1.97 (3H, s, 6-CH_3), 1.69 (3H, s, 16-CH_3), 1.27 (H, ddd, *J* = 17.4, 11.4, 2.4 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.4 (C-15), 185.1 (C-5), 181.6 (C-18), 180.8 (C-8), 165.4 (OCOR), 155.7 (C-7), 154.7 (C-17), 142.0 (C-20), 141.7 (C-10), 140.6 (C-2′), 135.3 (C-9), 135.3 (C-19), 133.1 (C-4′), 130.0 (C-1′), 129.2 (C-2′, C-6′), 129.0 (C-6), 128.6 (C-16), 128.4 (C-3′, C-5′), 116.6 (CN), 61.9 (C-22), 61.2 (OCH_3), 60.9 (OCH_3), 58.0 (C-21), 56.8 (C-1), 54.7 (C-13), 54.3 (C-3), 53.9 (C-11), 41.4 (*N*CH_3), 25.5 (C-4), 21.8 (C-14), 9.0 (6-CH_3), 9.0 (16-CH_3); EIMS *m/z* (% intensity) 597 (M^+, 10), 464 (3), 435 (3), 368 (5), 260 (13), 243 (19), 220 (100), 218 (25), 204 (11), 105 (24); HREIMS *m/z* 597.2115 (M^+, calcd for $C_{33}H_{31}N_3O_8$, 597.2111).

5.15. 4′-Methoxybenzenecarboxylic acid ester 8b

4-Methoxybenzoyl chloride (20.2 μL, 0.15 mmol) was added to a stirred solution of **3a** (5.0 mg, 0.01 mmol) and DMAP (0.12 mg, 0.001 mmol) in pyridine (0.2 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 2.5 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (158.9 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **8b** (2.8 mg, 44.0%) as a pale yellow amorphous powder. $[\alpha]_D^{27}$ − 51.3 (*c* 0.11, $CHCl_3$); IR ($CHCl_3$) 2927, 2854, 2229, 1715, 1652, 1512, 1455, 1372, 1258, 1167, 1027, 802, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.55 (2H, d, *J* = 8.8 Hz, 2′-H, 6′-H), 6.77 (2H, d, *J* = 8.8 Hz, 3′-H, 5′-H), 4.97 (1H, dd, *J* = 12.1, 3.1 Hz, 22-Ha), 4.07 (1H, d, *J* = 2.0 Hz, 21-H), 4.03 (2H, d, overlapped, 22-Hb, 1-H), 4.03 (3H, s, 7-OCH_3), 3.97 (1H, br s, 11-H), 3.81 (3H, s, OCH_3), 3.71 (3H, s, 17-OCH_3), 3.36 (1H, ddd, *J* = 7.2, 2.0, 0.6 Hz, 13-H), 3.06 (1H, ddd, *J* = 11.4, 2.7, 2.2 Hz, 3-H), 2.86 (1H, dd, *J* = 17.2, 2.2 Hz, 4-Hα), 2.69 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.34 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.19 (3H, s, *N*CH_3), 1.97 (3H, s, 6-CH_3), 1.72 (3H, s, 16-CH_3), 1.30 (H, ddd, *J* = 17.2, 11.7, 2.2 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.6 (C-15), 185.3 (C-5), 182.0 (C-18), 180.9 (C-8), 165.9 (OCOR), 163.2 (C-4′), 155.7 (C-7), 154.7 (C-17), 142.1 (C-20), 141.9 (C-10), 135.5 (C-9), 135.3 (C-19), 131.0 (C-2′, C-6′), 128.5 (C-6), 128.2 (C-16), 121.4 (C-1′), 116.9 (CN), 113.7 (C-3′, C-5′), 61.6 (C-22), 61.2 (7-OCH_3), 60.7 (17-OCH_3), 58.4 (C-21), 56.9 (C-1), 55.4 (4′-OCH_3), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (*N*CH_3), 25.7 (C-4), 21.2 (C-14), 9.0 (6-CH_3), 9.0 (16-CH_3); EIMS *m/z* (% intensity) 627 (M^+, 12), 464 (7), 435 (2), 368 (10), 260 (17), 243 (20), 220 (100), 218 (25), 204 (11), 152 (27), 135 (56); HREIMS *m/z* 627.2220 (M^+, calcd for $C_{34}H_{33}N_3O_9$, 627.2217).

5.16. 2′-Methoxybenzenecarboxylic acid ester 8c

2-Methoxybenzoyl chloride (91.9 μL, 0.62 mmol) was added to a stirred solution of **3a** (20.6 mg, 0.042 mmol) and DMAP (0.49 mg, 0.004 mmol) in pyridine (0.8 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (122.4 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **8c** (14.3 mg, 54.9%) as a pale yellow amorphous powder. $[\alpha]_D^{27}$ − 45.6 (*c* 0.11, $CHCl_3$); IR ($CHCl_3$) 3467, 2944, 2850, 2229, 1704, 1654, 1618, 1491, 1461, 1373, 1267, 1236, 1148, 759 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.50 (1H, dd, *J* = 7.7, 1.6 Hz, 6′-H), 7.41 (1H, dt, *J* = 7.7, 1.6 Hz, 4′-H), 6.88 (1H, t, *J* = 7.7 Hz, 3′-H), 6.85 (1H, d, *J* = 7.7 Hz, 3′-H), 5.00 (1H, dd, *J* = 11.4, 2.1 Hz, 22-Ha), 4.26 (1H, br s, 21-H), 4.06 (2H, br s, 1-H, 11-H), 4.00 (3H, s, 7-OCH_3), 3.98 (1H, d, *J* = 11.4 Hz, 22-Hb), 3.80 (3H, s, OCH_3), 3.62 (3H, s, 17-OCH_3), 3.36 (1H, br, 13-H), 3.22 (1H, ddd, *J* = 11.5, 2.7, 2.4 Hz, 3-H), 2.90 (1H, dd, *J* = 17.1, 2.4 Hz, 4-Hα), 2.76 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.50 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.35 (3H, s, *N*CH_3), 1.95 (3H, s, 6-CH_3), 1.59 (H, ddd, *J* = 17.1, 11.5, 3.6 Hz, 4-Hβ), 1.48 (3H, s, 16-CH_3),; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.5 (C-15), 185.2 (C-5), 182.0 (C-18), 180.9 (C-8), 165.7 (OCOR), 158.2 (C-2′), 155.7 (C-7), 154.4 (C-17), 142.1 (C-20), 141.8 (C-10), 135.4 (C-9), 135.4 (C-19), 133.9 (C-4′), 132.6 (C-6′), 128.6 (C-6), 128.4 (C-16), 120.2 (C-5′), 118.5 (C-1′), 116.8 (CN), 112.0 (C-3′), 62.4 (C-22), 61.2 (7-OCH_3), 60.9 (17-OCH_3), 58.0 (C-21), 56.4 (C-1), 55.6 (4′-OCH_3), 54.7 (C-13), 54.5 (C-3), 54.0 (C-11), 41.5 (*N*CH_3), 25.3 (C-4), 21.2 (C-14), 9.0 (6-CH_3), 8.6 (16-CH_3); FABMS *m/z* 628 (MH^+); HRFABMS *m/z* 628.2298 (MH^+, calcd for $C_{34}H_{34}N_3O_9$, 628.2295).

5.17. 4′-Nitrobenzenecarboxylic acid ester 8d

4-Nitrobenzoyl chloride (109.5 μL, 0.59 mmol) was added to a stirred solution of **3a** (20.4 mg, 0.04 mmol) and DMAP (0.49 mg, 0.004 mmol) in pyridine (0.8 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (104.1 mg). Chromatography on a silica gel column with hexane–ethyl acetate (5:1) as eluent gave **8d** (13.0 mg, 48.6%) as a pale yellow amorphous powder. $[\alpha]_D^{30}$ − 316 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) 2929, 2853, 2229, 1727, 1654, 1613, 1528, 1449, 1374, 1348, 1273, 1149, 1016, 956 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.17 (2H, d, *J* = 8.5 Hz, 3′-H, 5′-H), 7.80 (2H, d, *J* = 8.5 Hz, 2′-H, 6′-H), 4.94 (1H, dd, *J* = 11.7, 2.4 Hz, 22-Ha), 4.19 (1H, d, *J* = 11.7 Hz, 22-Hb), 4.11 (2H, overlapped, 21-H, 1-H), 4.02 (3H, s, 7-OCH_3), 3.96 (1H, br s, 11-H), 3.77 (3H, s, 17-OCH_3), 3.47 (1H, ddd, *J* = 7.3, 1.8, 0.6 Hz, 13-H), 3.14 (1H, ddd, *J* = 11.1, 2.7, 2.0 Hz, 3-H), 2.91 (1H, dd, *J* = 17.3, 2.0 Hz, 4-Hα), 2.73 (1H, dd, *J* = 21.2, 7.3 Hz, 14-Hα), 2.33 (1H, d, *J* = 21.2 Hz, 14-Hβ), 2.26 (3H, s, *N*CH_3), 1.97 (3H, s, 6-CH_3), 1.69 (3H, s, 16-CH_3), 1.27 (H, ddd, *J* = 17.3, 11.1, 2.0 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.3 (C-15), 185.0 (C-5), 181.5 (C-18), 180.7 (C-8), 163.6 (OCOR), 155.5 (C-7), 154.6 (C-17), 150.4 (C-4′), 142.0 (C-20), 141.5 (C-10), 135.0 (C-9), 134.2 (C-19), 134.2 (C-1′), 130.4 (C-2′, C-6′), 128.8 (C-6), 127.9 (C-16), 123.6 (C-3′, C-5′), 116.4 (CN), 63.3 (C-22), 61.3 (7-OCH_3), 61.0 (17-OCH_3), 58.0 (C-21), 56.5 (C-1), 54.6 (C-13), 54.4 (C-3), 54.0 (C-11), 41.4 (*N*CH_3), 25.5 (C-4), 21.9 (C-14), 9.1 (6-CH_3), 8.9 (16-CH_3); FABMS *m/z* 643 (MH^+); HRFABMS *m/z* 643.2037 (MH^+, calcd for $C_{33}H_{31}N_4O_{10}$, 643.2030).

5.18. 4′-Bromobenzenecarboxylic Acid Ester 8e

4-Bromobenzoyl chloride (64.5 mg, 0.44 mmol) was added to a stirred solution of **3a** (9.9 mg, 0.02 mmol) and DMAP (0.24 mg, 0.002 mmol) in pyridine (0.4 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 1 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (56.4 mg). Chromatography on a

silica gel column with hexane–ethyl acetate (5:1) as eluent gave **8e** (11.2 mg, 83.0%) as a pale yellow amorphous powder. $[\alpha]_D^{30}$ − 85.9 (*c* 0.19, $CHCl_3$); IR (KBr) 2924, 2361, 2341, 1717, 1653, 1616, 1456, 1373, 1271, 1234, 1070, 1012, 957, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.49 (2H, d, *J* = 9.5 Hz, 2′-H, 6′-H), 7.47 (2H, d, *J* = 9.5 Hz, 3′-H, 5′-H), 5.02 (1H, dd, *J* = 11.3, 2.5 Hz, 22-Ha), 4.09 (1H, dd, *J* = 6.7, 2.5 Hz, 1-H), 4.07 (1H, br s, 21-H), 4.05 (1H, overlapped, 22-Hb), 4.05 (3H, s, 7-OCH_3), 3.94 (1H, br d, *J* = 2.8 Hz, 11-H), 3.78 (3H, s, 17-OCH_3), 3.38 (1H, ddd, *J* = 7.4, 1.8, 0.6 Hz, 13-H), 3.08 (1H, ddd, *J* = 11.3, 2.9, 2.6 Hz, 3-H), 2.89 (1H, dd, *J* = 17.4, 2.6 Hz, 4-Hα), 2.70 (1H, dd, *J* = 21.1, 7.4 Hz, 14-Hα), 2.34 (1H, d, *J* = 21.1 Hz, 14-Hβ), 2.21 (3H, s, *N*CH_3), 2.00 (3H, s, 6-CH_3), 1.76 (3H, s, 16-CH_3), 1.26 (H, ddd, *J* = 17.4, 11.3, 2.0 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.8 (C-15), 185.5 (C-5), 182.2 (C-18), 181.1 (C-8), 164.8 (OCOR), 155.7 (C-7), 155.0 (C-17), 142.1 (C-20), 142.0 (C-10), 135.4 (C-9), 134.5 (C-19), 131.9 (C-2′, C-6′), 130.9 (C-3′, C-5′), 128.7 (C-1′), 128.5 (C-4′), 128.1 (C-6), 128.0 (C-16), 116.9 (CN), 62.0 (C-22), 61.2 (7-OCH_3), 60.8 (17-OCH_3), 58.3 (C-21), 56.8 (C-1), 54.6 (C-13), 54.3 (C-3), 54.1 (C-11), 41.4 (*N*CH_3), 25.6 (C-4), 20.9 (C-14), 8.8 (6-CH_3), 8.7 (16-CH_3); FABMS *m/z* 676 (MH^+); HRFABMS *m/z* 676.1300 (MH^+, calcd for $C_{33}H_{31}BrN_3O_{10}$, 676.1294).

5.19. 4′-Pyridinecarboxylic acid ester 9a

4-Pyridinecarbonyl chloride-hydrochloride (52.3 mg, 0.29 mmol) was added to a stirred solution of **3a** (9.9 mg, 0.10 mmol) and triethylamine (0.28 μL, 0.002 mmol) in pyridine (1.0 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (79.2 mg). Chromatography on a silica gel column with hexane–ethyl acetate (3:1) as eluent gave **9a** (6.8 mg, 56.8%) as a pale yellow amorphous powder. $[\alpha]_D^{16}$ − 66.1 (*c* 0.5, $CHCl_3$); IR (KBr) 2934, 2854, 2337, 2330, 1728, 1655, 1616, 1591, 1456, 1420, 1373, 1281, 1236, 1192, 1026, 957 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.62 (2H, d, *J* = 8.3 Hz, 2′-H, 6′-H), 7.44 (2H, d, *J* = 8.3 Hz, 3′-H, 5′-H), 4.99 (1H, dd, *J* = 11.7, 2.9 Hz, 22-Ha), 4.17 (1H, dd, *J* = 11.7, 2.1 Hz, 22-Hb), 4.10 (2H, m, 1-H, 21-H), 4.04 (3H, s, 7-OCH_3), 3.95 (1H, br d, *J* = 2.2 Hz, 11-H), 3.76 (3H, s, 17-OCH_3), 3.40 (1H, ddd, *J* = 7.6, 1.8, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.6, 3.1, 2.4 Hz, 3-H), 2.92 (1H, dd, *J* = 17.4, 2.4 Hz, 4-Hα), 2.71 (1H, dd, *J* = 20.9, 7.6 Hz, 14-Hα), 2.32 (1H, d, *J* = 20.9 Hz, 14-Hβ), 2.23 (3H, s, *N*CH_3), 2.00 (3H, s, 6-CH_3), 1.74 (3H, s, 16-CH_3), 1.25 (H, ddd, *J* = 17.4, 11.6, 2.4 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.9 (C-15), 185.4 (C-5), 182.1 (C-18), 181.1 (C-8), 164.1 (OCOR), 155.6 (C-7), 154.9 (C-17), 150.7 (C-2′, C-6′), 142.1 (C-20), 142.1 (C-10), 136.2 (C-4′), 135.2 (C-9), 134.6 (C-19), 128.8 (C-6), 128.2 (C-16), 122.5 (C-3′, C-5′), 116.8 (CN), 62.8 (C-22), 61.2 (7-OCH_3), 60.9 (17-OCH_3), 58.4 (C-21), 56.6 (C-1), 54.5 (C-13), 54.3 (C-3), 54.1 (C-11), 41.4 (*N*CH_3), 25.5 (C-4), 21.0 (C-14), 8.9 (6-CH_3), 8.7 (16-CH_3); FABMS *m/z* 599 (MH^+); HRFABMS *m/z* 599.2133 (MH^+, calcd for $C_{32}H_{31}N_4O_8$, 599.2142).

5.20. 3′-Pyridinecarboxylic acid ester 9b

3-Pyridinecarbonyl chloride-hydrochloride (67.4 mg, 0.38 mmol) was added to a stirred solution of **3a** (12.7 mg, 0.26 mmol) and triethylamine (0.36 μL, 0.026 mmol) in pyridine (1.0 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (16.1 mg). Chromatography on a silica gel column with hexane–ethyl acetate (3:1) as eluent gave **9b** (5.6 mg, 36.4%) as a pale yellow amorphous powder. $[\alpha]_D^{16}$ − 57.8 (*c* 0.3, $CHCl_3$); IR (KBr) 2933, 2854, 2338, 2320, 1728, 1655, 1616, 1591, 1456, 1420, 1373, 1281, 1236, 1192, 1026, 957 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.83 (1H, m, 2′-H), 8.74 (1H, dd, *J* = 4.9, 1.6 Hz, 6′-H), 7.93 (1H, dd, *J* = 7.9, 2.0 Hz, 4′-H), 7.32 (1H, ddd, *J* = 7.9, 4.9, 0.9 Hz, 5′-H), 5.01 (1H, dd, *J* = 11.8, 2.9 Hz, 22-Ha), 4.15 (1H, dd, *J* = 11.8, 2.3 Hz, 22-Hb), 4.11 (2H, m, 1-H, 21-H), 4.04 (3H, s, 7-OCH_3), 3.96 (1H, br s, 11-H), 3.77 (3H, s, 17-OCH_3), 3.40 (1H, ddd, *J* = 7.6, 1.8, 0.6 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.3, 3.1, 2.4 Hz, 3-H), 2.92 (1H, dd, *J* = 17.2, 2.4 Hz, 4-Hα), 2.71 (1H, dd, *J* = 21.1, 7.6 Hz, 14-Hα), 2.35 (1H, d, *J* = 21.1 Hz, 14-Hβ), 2.23 (3H, s, *N*CH_3), 1.99 (3H, s, 6-CH_3), 1.74 (3H, s, 16-CH_3), 1.24 (H, ddd, *J* = 17.2, 11.3, 2.8 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.9 (C-15), 185.3 (C-5), 182.2 (C-18), 181.1 (C-8), 164.3 (OCOR), 155.6 (C-7), 155.0 (C-17), 153.6 (C-2′), 150.5 (C-6′), 142.1 (C-20), 142.0 (C-10), 136.9 (C-4′), 135.2 (C-9), 134.6 (C-19), 128.9 (C-6), 128.3 (C-16), 125.1 (C-3′), 123.4 (C-5′), 116.8 (CN), 62.4 (C-22), 61.2 (OCH_3), 60.9 (OCH_3), 58.4 (C-21), 56.6 (C-1), 54.5 (C-13), 54.3 (C-3), 54.1 (C-11), 41.4 (*N*CH_3), 25.5 (C-4), 21.0 (C-14), 8.9 (6-CH_3), 8.7 (16-CH_3); FABMS *m/z* 599 (MH^+); HRFABMS *m/z* 599.2148 (MH^+, calcd for $C_{32}H_{31}N_4O_8$, 599.2142).

5.21. 2′-Pyridinecarboxylic acid ester 9c

Carbonate **5e**[30] (60.0 mg, 0.31 mmol) was added to a stirred solution of **3a** (9.8 mg, 0.02 mmol) and triethylamine (0.28 μL, 0.002 mmol) in pyridine (1.0 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (14.9 mg). Chromatography on a silica gel column with hexane–ethyl acetate (3:1) as eluent gave **9c** (4.8 mg, 40.1%) as a pale yellow amorphous powder. $[\alpha]_D^{16}$ − 87.9 (*c* 0.3, $CHCl_3$); IR (KBr) 2941, 2853, 2361, 2343, 1724, 1655, 1616, 1587, 1439, 1412, 1373, 1304, 1236, 1190, 1136, 1080, 1045, 1024, 957 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.52 (1H, ddd, *J* = 4.8, 1.8, 0.9 Hz, 6′-H), 7.84 (1H, dt, *J* = 7.3, 1.2 Hz, 3′-H), 7.77 (1H, ddd, *J* = 8.3, 7.3, 1.8 Hz, 4′-H), 7.43 (1H, ddd, *J* = 8.3, 4.8, 1.2 Hz, 5′-H), 4.92 (1H, dd, *J* = 11.3, 3.1 Hz, 22-Ha), 4.25 (1H, d, *J* = 2.0 Hz, 21-H), 4.15 (1H, ddd, *J* = 3.8, 3.1, 2.2 Hz, 1-H), 4.07 (1H, dd, *J* = 11.3, 3.8 Hz, 22-Hb), 4.01 (3H, s, 7-OCH_3), 3.95 (1H, br s, 11-H), 3.85 (3H, s, 17-OCH_3), 3.38 (1H, ddd, *J* = 7.4, 2.0, 0.6 Hz, 13-H), 3.11 (1H, ddd, *J* = 11.6, 3.1, 2.6 Hz, 3-H), 2.87 (1H, dd, *J* = 17.1, 2.6 Hz, 4-Hα), 2.68 (1H, dd, *J* = 21.0, 7.4 Hz, 14-Hα), 2.41 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.23 (3H, s, *N*CH_3), 1.96 (3H, s, 6-CH_3), 1.69 (3H, s, 16-CH_3), 1.63 (H, ddd, *J* = 17.1, 11.6, 2.5 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.9 (C-15), 185.5 (C-5), 182.2 (C-18), 181.2 (C-8), 164.7 (OCOR), 155.5 (C-7), 154.8 (C-17), 149.8 (C-6′), 147.4 (C-2′), 142.9 (C-10), 142.1 (C-20), 136.9 (C-4′), 135.0 (C-9), 134.6 (C-19), 128.8 (C-6), 127.9 (C-16), 126.9 (C-5′), 125.3 (C-3′), 117.2 (CN), 62.3 (C-22), 61.1 (OCH_3), 60.9 (OCH_3), 58.9 (C-21), 56.1 (C-1), 54.8 (C-3), 54.6 (C-13), 54.4 (C-11), 41.4 (*N*CH_3), 25.5 (C-4), 20.9 (C-14), 8.9 (6-CH_3), 8.7 (16-CH_3); FABMS *m/z* 599 (MH^+); HRFABMS *m/z* 599.2141 (MH^+, calcd for $C_{32}H_{31}N_4O_8$, 599.2142).

5.22. 4′-Quinolinecarboxylic acid ester 9d

4-Quinolinecarbonyl chloride (169.0 mg, 0.88 mmol) was added to a stirred solution of **3a** (30.0 mg, 0.06 mmol) and DMAP (0.73 mg, 0.006 mmol) in pyridine (1.2 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (170.4 mg). Chromatography on a silica gel column with hexane–ethyl acetate (2:1) as eluent gave **9d** (26.1 mg, 67.1%) as a pale yellow amorphous

powder. $[\alpha]_D^{16}$ − 88.1 (*c* 0.6, $CHCl_3$); IR (KBr) 2928, 2850, 2351, 1725, 1655, 1618, 1459, 1374, 1236, 1148, 1019, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.90 (1H, d, *J* = 4.3 Hz, 2′-H), 8.58 (1H, d, *J* = 8.3 Hz, 5′-H), 8.16 (1H, d, *J* = 8.3 Hz, 8′-H), 7.77 (1H, t, *J* = 8.3 Hz, 7′-H), 7.57 (1H, t, *J* = 8.3 Hz, 6′-H), 7.49 (1H, d, *J* = 4.3 Hz, 2′-H), 5.10 (1H, dd, *J* = 11.7, 2.9 Hz, 22-Ha), 4.30 (1H, dd, *J* = 11.7, 2.9 Hz, 22-Hb), 4.17 (2H, m, 1-H, 21-H), 4.05 (3H, s, 7-OCH_3), 3.97 (1H, br s, 11-H), 3.65 (3H, s, 17-OCH_3), 3.40 (1H, ddd, *J* = 7.4, 1.8, 0.6 Hz, 13-H), 3.16 (1H, ddd, *J* = 11.4, 3.1, 2.5 Hz, 3-H), 2.95 (1H, dd, *J* = 17.7, 2.5 Hz, 4-Hα), 2.67 (1H, dd, *J* = 21.0, 7.4 Hz, 14-Hα), 2.38 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.22 (3H, s, *N*CH_3), 2.02 (3H, s, 6-CH_3), 1.41 (H, ddd, *J* = 17.7, 11.4, 2.7 Hz, 4-Hβ), 1.16 (3H, s, 16-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.5 (C-15), 185.4 (C-5), 182.2 (C-18), 181.1 (C-8), 165.1 (OCOR), 155.8 (C-7), 154.7 (C-17), 149.4 (C-2′), 149.4 (C-8′a), 149.1 (C-4′a), 142.0 (C-20), 141.9 (C-10), 135.5 (C-9), 134.5 (C-19), 133.4 (C-4′), 130.2 (C-7′), 130.1 (C-8′), 128.8 (C-6), 128.6 (C-6′), 128.1 (C-16), 127.4 (C-4′a), 125.4 (C-5′), 122.0 (C-3′), 116.8 (CN), 62.9 (C-22), 61.2 (OCH_3), 60.7 (OCH_3), 58.4 (C-21), 56.6 (C-1), 54.6 (C-13), 54.2 (C-3), 54.1 (C-11), 41.4 (*N*CH_3), 25.7 (C-4), 21.0 (C-14), 8.9 (6-CH_3), 7.8 (16-CH_3); FABMS *m/z* 649 (MH^+); HRFABMS *m/z* 649.2296 (MH^+, calcd for $C_{36}H_{33}N_4O_8$, 649.2298).

5.23. 3′-Quinolinecarboxylic acid ester 9e

3-Quinolinecarbonyl chloride (169 mg, 0.88 mmol) was added to a stirred solution of **3a** (10.8 mg, 0.022 mmol) and DMAP (0.27 mg, 0.0022 mmol) in pyridine (1.0 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 6 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (11.4 mg). Chromatography on a silica gel column with hexane–ethyl acetate (2:1) as eluent gave **9e** (4.9 mg, 34.4%) as a pale yellow amorphous powder. $[\alpha]_D^{16}$ − 133.1 (*c* 0.3, $CHCl_3$); IR (KBr) 3447, 2945, 2355, 2343, 2330, 1724, 1653, 1618, 1570, 1497, 1447, 1420, 1375, 1286, 1259, 1234, 1198, 1130, 1110, 1080, 1047, 1022, 966, 876, 841, 793 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.02 (1H, d, *J* = 2.1 Hz, 2′-H), 8.50 (1H, d, *J* = 2.1 Hz, 4′-H), 8.14 (1H, dd, *J* = 8.9, 1.2 Hz, 8′-H), 7.87 (1H, dd, *J* = 5.5, 1.2 Hz, 5′-H), 7.86 (1H, ddd, *J* = 8.9, 7.6, 1.2 Hz, 7′-H), 7.65 (1H, ddd, *J* = 7.9, 7.6, 1.2 Hz, 6′-H), 5.18 (1H, dd, *J* = 11.4, 2.1 Hz, 22-Ha), 4.16 (1H, dd, *J* = 11.4, 2.1 Hz, 22-Hb), 4.13 (2H, m, 1-H, 21-H), 4.06 (3H, s, 7-OCH_3), 3.95 (1H, br s, 11-H), 3.44 (3H, s, 17-OCH_3), 3.40 (1H, ddd, *J* = 7.4, 1.8, 0.6 Hz, 13-H), 3.12 (1H, ddd, *J* = 11.4, 3.1, 2.3 Hz, 3-H), 2.93 (1H, dd, *J* = 17.5, 2.3 Hz, 4-Hα), 2.69 (1H, dd, *J* = 21.1, 7.6 Hz, 14-Hα), 2.38 (1H, d, *J* = 21.1 Hz, 14-Hβ), 2.20 (3H, s, *N*CH_3), 2.03 (3H, s, 6-CH_3), 1.33 (H, ddd, *J* = 17.5, 11.4, 2.6 Hz, 4-Hβ), 1.31 (3H, s, 16-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.7 (C-15), 185.4 (C-5), 182.0 (C-18), 181.2 (C-8), 164.4 (OCOR), 155.6 (C-7), 154.8 (C-17), 149.2 (C-2′), 149.2 (C-4′a), 147.3 (C-8′a), 142.1 (C-10), 141.9 (C-20), 139.1 (C-4′), 135.4 (C-9), 134.5 (C-19), 132.4 (C-7′), 129.4 (C-8′), 129.3 (C-5′), 128.1 (C-6), 127.9 (C-6′), 126.5 (C-16), 121.9 (C-3′), 116.9 (CN), 62.0 (C-22), 61.2 (OCH_3), 60.4 (OCH_3), 58.2 (C-21), 56.7 (C-1), 54.5 (C-13), 54.2 (C-3), 54.0 (C-11), 41.4 (*N*CH_3), 25.7 (C-4), 20.9 (C-14), 8.9 (6-CH_3), 8.0 (16-CH_3); FABMS *m/z* 649 (MH^+); HRFABMS *m/z* 649.2295 (MH^+, calcd for $C_{36}H_{33}N_4O_8$, 649.2298).

5.24. 2′-Quinolinecarboxylic acid ester 9f

2-Quinolinecarbonyl chloride (149 mg, 0.78 mmol) was added to a stirred solution of **3a** (9.8 mg, 0.02 mmol) and DMAP (0.24 mg, 0.0022 mmol) in pyridine (1.0 mL) at −17 °C, and the resulting solution was stirred at −17 °C for 3 h. The reaction mixture was poured into water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with brine, dried, and concentrated in vacuo to give a residue (30.8 mg). Chromatography on a silica gel column with hexane–ethyl acetate (3:1) as eluent gave **9f** (9.0 mg, 69.4%) as a pale yellow amorphous powder. $[\alpha]_D^{16}$ − 133.7 (*c* 0.3, $CHCl_3$); IR (KBr) 3649, 2943, 2847, 2359, 2343, 1724, 1654, 1616, 1560, 1506, 1458, 1412, 1373, 1312, 1294, 1281, 1236, 1211, 1190, 1138, 1109, 1080, 1047, 964, 842, 796 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.23 (1H, d, *J* = 8.4 Hz, 4′-H), 7.95 (1H, dd, *J* = 8.3, 1.2 Hz, 8′-H), 7.88 (1H, d, *J* = 8.4 Hz, 3′-H), 7.87 (1H, dd, *J* = 8.3, 1.4 Hz, 5′-H), 7.77 (1H, ddd, *J* = 8.3, 7.0, 1.4 Hz, 7′-H), 7.67 (1H, ddd, *J* = 8.3, 7.0, 1.2 Hz, 6′-H), 5.19 (1H, dd, *J* = 11.3, 2.8 Hz, 22-Ha), 4.24 (1H, d, *J* = 2.1 Hz, 21-H), 4.17 (1H, dt, *J* = 2.8, 2.5 Hz, 1-H), 4.04 (1H, dd, *J* = 11.3, 2.5 Hz, 22-Hb), 4.03 (3H, s, 7-OCH_3), 3.91 (1H, br d, *J* = 2.0 Hz, 11-H), 3.38 (1H, ddd, *J* = 7.4, 2.1, 0.6 Hz, 13-H), 3.28 (3H, s, 17-OCH_3), 3.13 (1H, dt, *J* = 11.4, 2.0 Hz, 3-H), 2.95 (1H, dd, *J* = 17.4, 2.0 Hz, 4-Hα), 2.65 (1H, dd, *J* = 21.0, 7.4 Hz, 14-Hα), 2.44 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.18 (3H, s, *N*CH_3), 2.08 (H, ddd, *J* = 17.4, 11.4, 2.4 Hz, 4-Hβ), 2.02 (3H, s, 6-CH_3), 1.42 (3H, s, 16-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.9 (C-15), 185.6 (C-5), 181.9 (C-18), 181.4 (C-8), 164.9 (OCOR), 155.5 (C-7), 154.1 (C-17), 147.3 (C-4′a), 147.3 (C-8′a), 143.7 (C-10), 142.0 (C-20), 137.2 (C-4′), 134.9 (C-9), 134.4 (C-19), 130.6 (C-7′), 130.6 (C-8′), 129.1 (C-2′), 128.9 (C-6′), 128.9 (C-6), 127.5 (C-5′), 126.6 (C-16), 120.9 (C-3′), 117.2 (CN), 63.6 (C-22), 61.1 (OCH_3), 60.1 (OCH_3), 58.7 (C-21), 56.5 (C-1), 54.7 (C-3), 54.6 (C-13), 54.2 (C-11), 41.4 (*N*CH_3), 25.6 (C-4), 20.9 (C-14), 8.9 (6-CH_3), 8.4 (16-CH_3); FABMS *m/z* 649 (MH^+); HRFABMS *m/z* 649.2293 (MH^+, calcd for $C_{36}H_{33}N_4O_8$, 649.2298).

6. Preparation of sample solutions and cell cultures

Each sample was prepared as a 20 mM stock solution that was dissolved in DMSO and added to the cells with less than 1% DMSO in the final drug dilution with culture medium. HCT116 colon carcinoma and MDA-MB-453 breast carcinoma cell lines were cultured in PMP11640 (Sigma) containing supplements: 10% penicillin (100 U/mL) and streptomycin (100 U/mL). Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

7. Cell growth inhibition assay (IC_{50})

HCT116 colon carcinoma and MDA-MB-453 breast carcinoma cell lines were, respectively seeded at 1500 cells per well and 3000 cells per well, in 96-well plates 24 h before the experiments. The cell lines were treated with each sample at concentrations of threefold dilution ranging from 100 nM to 0.41 nM and incubated for 3 days in a cell culture incubator. MTT colorimetric assay was performed to measure 50% cell growth inhibition (IC_{50}) relative to untreated control cells. MTT assay results were read in a TECAN absorbance microplate reader by measuring absorbance at 540 nm. All determinations were carried out in triplicate.

Acknowledgments

K.C. was sponsored by the Thailand Research Fund (TRF) for the 2003 Royal Golden Jubilee (RGJ) Ph. D. Program Scholarship (PHD/0157/2546). BNPME was supported by the Grant for Center of Excellence from the Commission of Higher Education, Thailand. This research was partially supported by the Japan Society for the Promotion of Science (JSPS) AA Scientific Platform Program and a grant from the High-Tech Research Center Project, the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (S0801043). We are grateful to JSPS and the National Re-

search Cooperation of Thailand (NRCT) for supporting the collaboration between Thai and Japanese researchers in this work.

References and notes

1. Part 7: Daikuhara, N.; Tada, Y.; Yamaki, S.; Charupant, K.; Amnuoypol, S.; Suwanborirux, K.; Saito, N. *Tetrahedron Lett*, in press.
2. Scott, J. D.; Williams, R. M. *Chem. Rev.* **2002**, *102*, 1669.
3. Rinehart, K. L. *Med. Drug. Rev.* **2000**, *20*, 1.
4. Friedman, D.; Hu, Z.; Kolb, E. A.; Gorfain, B.; Scotto, K. W. *Cancer Res.* **2002**, *62*, 3377.
5. Takebayashi, Y.; Pourquier, P.; Zimonic, D. B.; Nakayama, K.; Emmert, S.; Ueda, T.; Urasaki, Y.; Kanzaki, A.; Akiyama, S.; Popescu, N.; Kraemer, K. H.; Pommier, Y. *Nat. Med.* **2001**, *7*, 961.
6. Aune, G. J.; Furuta, T.; Pommier, Y. *Anticancer Drug* **2002**, *13*, 545.
7. Martinez, E. J.; Owa, T.; Schreiber, S. L.; Corey, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3496.
8. Martinez, E. J.; Corey, E. J.; Owa, T. *Chem. Biol.* **2001**, *8*, 1151.
9. Plowright, A. T.; Schaus, S. E.; Myers, A. G. *Chem. Biol.* **2002**, *9*, 607.
10. Frincke, J. M.; Faulkner, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 265; see: Additions and corrections: Frincke, J. M.; Faulkner, D. J. *J. Am. Chem. Soc.* **1983**, *105*, 5004.
11. He, H.; Faulkner, D. J. *J. Org. Chem.* **1989**, *54*, 5822.
12. Davidson, B. S. *Tetrahedron Lett.* **1992**, *33*, 3721.
13. Parameswarn, P. S.; Naik, C. G.; Kamat, S. Y.; Pramanik, B. N. *Indian J. Chem.* **1998**, *37B*, 1258.
14. We reported the reassignment of the previously reported structure of renieramycin H (**1h**) and showed that it corresponded to cribrostatin VI: Saito, N.; Sakai, H.; Suwanborirux, K.; Pummangura, S.; Kubo, A. *Heterocycles* **2001**, *53*, 21.
15. Pettit, G. R.; Collins, J. C.; Herald, D. L.; Doubek, D. L.; Boyd, M. R.; Schmidt, J. M.; Hooper, J. N. A.; Trackett, L. P. *Can. J. Chem.* **1992**, *70*, 1170.
16. Pettit, G. R.; Knight, J. C.; Collins, J. C.; Herald, D. L.; Pettit, R. K.; Boyd, M. R.; Young, V. G. *J. Nat. Prod.* **2000**, *63*, 793.
17. Oku, N.; Matsunaga, S.; Van Soest, R. W. M.; Fusetani, N. J. *J. Nat. Prod.* **2003**, *66*, 1136.
18. Fontana, A.; Cavaliere, P.; Wahidulla, S.; Naik, C. G.; Cimino, G. *Tetrahedron* **2000**, *56*, 7305.
19. Fukuyama, T.; Linton, S. D.; Tun, M. M. *Tetrahedron Lett.* **1990**, *31*, 5989.
20. Magnus, P.; Matthews, K. S. *J. Am. Chem. Soc.* **2005**, *127*, 12476.
21. Lane, J. W.; Chen, Y.; Williams, R. M. *J. Am. Chem. Soc.* **2005**, *127*, 12684.
22. Chan, C.; Heid, R.; Zheng, S.; Guo, J.; Zhou, B.; Furuuchi, T.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 4596.
23. Vincent, G.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1517.
24. Chen, X.; Zhu, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 3962.
25. Suwanborirux, K.; Amnuoypol, S.; Plubrukarn, A.; Pummangura, S.; Kubo, A.; Tanaka, C.; Saito, N. *J. Nat. Prod.* **2003**, *66*, 1441.
26. Amnuoypol, S.; Suwanborirux, K.; Pummangura, S.; Kubo, A.; Tanaka, C.; Saito, N. *J. Nat. Prod.* **2004**, *67*, 1023.
27. Saito, N.; Tanaka, C.; Koizumi, Y.; Suwanborirux, K.; Amnuoypol, S.; Pummangura, S.; Kubo, A. *Tetrahedron* **2004**, *60*, 3873.
28. Charupant, K.; Suwanborirux, K.; Amnuoypol, S.; Saito, E.; Kubo, A.; Saito, N. *Chem. Pharm. Bull.* **2007**, *55*, 81.
29. Veitch, G. E.; Beckmann, E.; Burke, B. J.; Boyer, A.; Ayats, C.; Ley, S. V. *Angew. Chem. Int. Ed.* **2007**, *46*, 7633–7635. Compound **5a**: ^{1}H NMR ($CDCl_3$) δ 7.41 (2H, s, ArH), 6.31 (1H, qq, J = 7.3, 1.5 Hz, =*C*HCH$_3$), 2.07 (3H, dq, J = 1.5, 1.2 Hz, COC(CH$_3$)=C), 1.95 (3H, dq, J = 7.3, 1.2 Hz, =CHC*H*$_3$).
30. *Carbonate **5b** was prepared as follows*: A suspension of 3-methylcrotonic acid (201.3 mg, 2.0 mmol) and K_2CO_3 (276.4 mg, 2.0 mmol) in CH_2Cl_2 was stirred and isopropyl chloroformate (227.8 μL, 2.0 mmol) was added dropwise at 0 °C for 5 min. The mixture was stirred at 25 °C for 17 h and filtered thereafter. The filter cake was carefully washed with CH_2Cl_2 (50 mL) and the combined filtrates were concentrated in vacuo to give **5b** as a pale yellow oil. Carbonates **5c**, **5d**, and **5e** were also prepared with the same procedure but using 3-cyclopentenoic acid, 3-cyclohexenoic acid, and 2-pyridinecarboxylic acid, respectively (Fig. 5).
31. Muramatsu, I.; Murakami, M.; Yoneda, T.; Hagitani, A. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 244–246. also see: *Org. Synth. Coll.* Vol. VI, pp 8–10.
32. Lane, J. W.; Estevez, A.; Mortara, K.; Callan, O.; Spencer, J. R.; Williams, R. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3180.
33. Ceballos, P. A.; Perez, M.; Cuevas, C.; Francesch, A.; Manzanares, I.; Echavarren, A. M. *Eur. J. Org. Chem.* **2006**, 1926.
34. Aubry, S.; Pellet-Rostaing, S.; dit Chabert, J. F.; Ducki, S.; Lemaire, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2598.
35. Gonzălez, J. F.; de la Cuesta, E.; Avendaño, C. *Bioorg. Med. Chem.* **2007**, *15*, 112.
36. Ortn, I.; Gonzălez, J. F.; de la Cuesta, E.; Manguan-Garćia, C.; Perona, R.; Avendaño, C. *Bioorg. Med. Chem.* **2008**, *16*, 9065.
37. Wright, B. J.; Chan, C.; Danishefsky, S. L. *J. Nat. Prod.* **2008**, *71*, 409.
38. Spencer, J. R.; Sendzik, M.; Oeh, J.; Sabbatini, P.; Dalrymple, S. A.; Magill, C.; Kim, H. M.; Zhang, P.; Squires, N.; Moss, K. G.; Sukbuntherng, J.; Graupe, D.; Eksterowicz, J.; Young, P. R.; Myers, A. G.; Green, M. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4884.
39. http://www.pharmamar.com/en/pipeline/zalypsis.cfm.