Brasilicardin A. A Novel Tricyclic Metabolite with Potent Immunosuppressive Activity from Actinomycete *Nocardia brasiliensis*

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A novel tricyclic metabolite, brasilicardin A (**1**), with potent immunosuppressive activity has been isolated from the cultured broth of the actinomycete *Nocardia brasiliensis* IFM 0406, and the structure including absolute stereochemistry was established on the basis of spectroscopic data, chemical means, and X-ray analysis. Brasilicardin A (**1**) is the first tricyclic compound consisting of an anti/syn/anti-perhydrophenanthrene skeleton with a rhamnose, an *N*-acetylglucosamine, and an amino acid moiety.

In our investigations on new bioactive metabolites from pathogenic actinomycete *Nocardia* strains, we have reported an anthracycline,[2] benz[*a*]anthraquinones,[3] and an indole alkaloid[4] with cytotoxic and antimicrobial activities isolated from *N. brasiliensis* IFM 0075 and IFM 0089. We recently isolated a new 32-membered macrolide, brasilinolide A, possessing immunosuppressive activity from *N. brasiliensis* IFM 0406.[5] Further search for unique bioactive metabolites from the same strain resulted in the isolation of brasilicardin A (**1**), a novel tricyclic metabolite with potent immunosuppressive activity, consisting of an anti/syn/anti-perhydrophenanthrene skeleton with a rhamnose, an *N*-acetylglucosamine, and an amino acid moiety. The structure containing the relative stereochemistry of **1** was elucidated on the basis of 2D NMR data and chemical means. The presence of L-rhamnose and D-glucosamine was identified by GC and amino acid analyses and optical rotations of hydrolysis products of **1**. The absolute stereochemistry of **1** was established by modified Mosher's methods and CD exciton chirality applied for derivatives of the hydrolysis product (**2**) of **1**, as well as X-ray analysis for the 4-bromobenzoyl derivative of **2**. In this paper we describe the isolation, structure elucidation, and biological activities of **1**.

Results and Discussion

The actinomycete *N. brasiliensis* IFM 0406 was grown in the broth [glycerol (2.0%), polypeptone (1.0%), and meat extract (0.5%) in H_2O, pH 7.0]. Cultures were incubated at 32 °C for 4 days with stirring at 250 rpm. The supernatant of the fermentation broth was passed through a Diaion HP-20 column, which was subsequently eluted with MeOH. The MeOH-soluble portion was suspended in H_2O and then partitioned with $CHCl_3$. The aqueous layer was chromatographed on a DEAE-Toyopearl 650M column and then subjected to a CM Toyopearl 650M column. The active fraction was further purified by reversed-phase HPLC to give brasilicardin A (**1**, 3.6 mg).

HRFABMS analysis of brasilicardin A (**1**), a colorless amorphous solid ($[\alpha]^{30}_D$ +15.0°), revealed the molecular formula to be $C_{45}H_{68}N_2O_{16}$ [*m*/*z* 893.4646 $(M + H)^+$, Δ −0.1 mmu]. Interpretation of ^1H and ^{13}C NMR data (Table 1) of **1** in CD_3OD indicated the presence of one carboxyl, one ester, and one amide carbonyl, one trisubstituted olefin, two hemiacetal carbons, 10 oxymethines, three sp^3 quaternary carbons, two aromatic quaternary carbons, five sp^3 methines, four sp^2 methines, one oxymethylene, five sp^3 methylenes, and one methoxy, one acetyl, and six methyls. Two anomeric protons (δ_H 5.06 and 4.58) and carbons (δ_C 103.62 and 104.54) indicated that **1** possessed two sugar moieties. The presence of 3-hydroxybenzoate was revealed by the proton signals at δ_H 7.51 (t, J = 1.4 Hz, H-9′), 7.10 (dd, J = 7.6 and 1.4 Hz, H-11′), 7.38 (t, J = 7.6 Hz, H-12′), and 7.59 (dd, J = 7.6 and 1.4 Hz, H-13′), and a fragment ion peak at *m*/*z* 121 [(HO)PhCO] in the FABMS spectrum. UV absorptions at 212 (ε 15000) and 239 (5200) nm also supported the presence of the benzoate group. Detailed analysis of the $^1H-^1H$ COSY spectrum of **1** implied connectivities of C-1−C-3, C-5−C-7, C-9−C-12, C-14−C-17, C-1′−C-6′, and C-1″−C-6″. HMBC correlations of H_3-19 and H_3-20 to C-3, C-4, and C-5 revealed that both methyl groups (Me-19 and Me-20) were attached to C-4. The presence of Me-21 at C-10 and Me-22 at C-8 was indicated by HMBC correlations of H_3-21 to C-1, C-5, C-9, and C-10 and H_3-22 to C-7, C-8, C-9, and C-14. The $^1H-^{13}C$ long-range correlations of H_3-23 to C-12, C-13, and C-14

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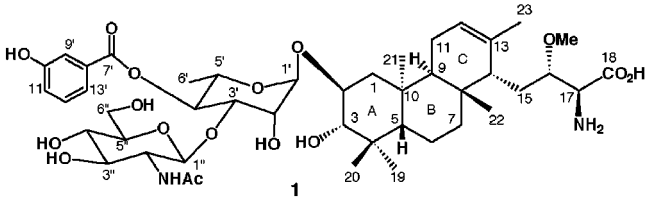
Table 1. ^{1}H and ^{13}C NMR Data of Brasilicardin A (1) in CD_3OD

position	^{1}H[a]		J (Hz)	^{13}C[a]		HMBC[b] (^{1}H)
1(a)	1.49	m		44.53	t	21
1(b)	1.82	m				
2	3.73	t	9.0	80.31	d	3
3	3.06	d	9.7	83.83	d	2, 19, 20
4				41.70	s	3, 19, 20
5	1.69	m		19.22	d	6b, 9, 19, 20, 21
6(a)	1.69	m		46.75	t	
6(b)	1.78	m				
7	1.41	m		31.78	t	6b, 22
8				39.05	s	9, 14, 15, 22
9	1.32	m		47.87	d	11b, 12, 14, 21, 22
10				38.09	s	1a, 9, 21
11	1.93	brs		27.57	t	9
12	5.39	brs		124.08	d	11b, 14, 23
13				139.04	s	11b, 14, 23
14	1.61	m		52.81	d	12, 22, 23
15(a)	1.41	m		32.46	t	14, 16
15(b)	1.49	m				
16	3.81	dd	11.4, 3.3	81.07	d	14, 17
OMe	3.53	s		58.90	q	16
17	4.46	d	3.5	55.36	d	
18				170.62	s	17
19	0.97	s		17.82	q	18
20	1.04	s		29.74	q	18
21	1.14	s		29.42	q	9
22	1.09	s		23.06	q	14
23	1.69	s		23.13	q	12
1′	5.06	d	1.1	103.62	d	2
2′	4.46	dd	3.1, 1.1	72.60	d	1′
3′	4.12	dd	9.8, 3.1	80.35	d	1′, 2′
4′	5.31	t	9.8	74.64	d	2′, 3′, 6′
5′	4.05	dq	9.8, 6.2	68.58	d	1′, 4′, 6′
6′	1.16	d	6.2	18.30	q	4′
7′				167.50	s	4′
8′				132.96	s	9′, 12′
9′	7.51	t	1.4	117.94	d	10′, 13′
10′				159.50	s	9′, 12′
11′	7.10	dd	7.6, 1.4	122.04	d	13′
12′	7.38	t	7.6	131.38	d	
13′	7.59	dd	7.6, 1.4	122.50	d	9′, 11′
1″	4.58	d	8.5	104.54	d	3′
2″	3.58	dd	10.0, 8.5	58.02	d	
NHAc	1.54	s		23.42	q	
				174.55	s	2″
3″	3.41	dd	10.0, 8.1	75.80	d	2″
4″	3.35	m		72.33	d	3″, 6″b
5″	3.33	m		78.30	d	6″a
6″(a)	3.73	d	11.6	63.07	t	
6″(b)	3.93	dd	11.6, 1.6			

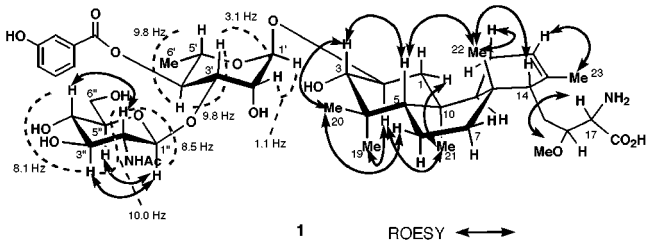
[a] δ in ppm. [b] Delay time (Δ) for C−H long-range coupling was set to 50 ms.

Chart 1



1

revealed that a vinyl methy group (Me-23) was attached to C-13. These spectral data suggested that **1** possessed a perhydrophenanthrene skeleton (Chart 1).[6] A methoxy group (δ_H 3.53) was attached at C-16 judging from an HMBC correlation between H-16 (δ_H 3.81) and the methoxy carbon (δ_C 58.90). The presence of a carboxyl group at C-17 was indicated by an HMBC correlation of



1 ROESY ⟷

Figure 1. Relative stereochemistry of brasilicardin A (**1**).

H-17 to C-18, while an amino group was also attached at C-17 (δ_C 55.36) from comparison with α-carbon chemical shifts (δ_C 50−60) of amino acids. ^{1}H−^{1}H coupling constants ($J_{1',2'}$ = 1.1 Hz, $J_{2',3'}$ = 3.1 Hz, $J_{3',4'}$ = 9.8 Hz, and $J_{4',5'}$ = 9.8 Hz) indicated that a sugar moiety (C-1′−C-6′) was rhamnose, while another sugar moiety was assigned as glucosamine on the basis of ^{1}H−^{1}H coupling constants ($J_{1'',2''}$ = 8.5 Hz, $J_{2'',3''}$ = 10.0 Hz, and $J_{3'',4''}$ = 8.1 Hz) and ROESY correlations of 1″/3″, 1″/5″, 2″/4″, 4″/6″a, and 4″/6″b (Figure 1). The presence of the rhamnose was firmly identified on the basis of GC analysis of the trimethylsilyl derivatives of the methanolysis product of **1**, while the presence of a glucosamine was identified by amino acid analysis of the acid hydrolysate of **1**. An HMBC correlation of H-4′ to C-7′ revealed that 3-hydroxybenzoate was attached at C-4′, while an acetyl group (δ_H 1.54) was attached at the amino group of glucosamine by an HMBC correlation of H-2″ (δ_H 3.58) to the acetyl carbonyl (δ_C 174.55). The *N*-acetylglucosamine was connected at C-3′ of the rhamnose from ^{1}H−^{13}C long-range correlation between H-3′ and C-1″. An HMBC correlation between H-2 and C-1′ indicated that the rhamnose was attached at C-2. Stereochemistry at each anomeric position of the sugar moieties was assigned as α for rhamnose and β for glucosamine, respectively, on the basis of the one-bond ^{1}H−^{13}C coupling constants[7] obtained by an INEPT experiment[8] of **1** in CD_3OD (C-1′, $^1J_{C,H}$ = 171.0 Hz; C-1″, $^1J_{C,H}$ = 165.4 Hz)[9] and ^{1}H−^{1}H coupling constants ($J_{1',2'}$ = 1.1 Hz; $J_{1'',2''}$ = 8.5 Hz). The existence of the β-glycoside bond at C-1″ was consistent with ROESY correlation observed between H-1″ and H-5″. Relative stereochemistry of the anti/syn/anti-perhydrophenanthrene skeleton (Figure 1) was elucidated by ROESY data. ROESY correlations of H-2/H_3-19, H-3/H-5, H-3/H_3-20, and H-1b/H_3-21 indicated that ring A had a chair conformation, while a boat conformation of ring B was assigned from ROESY correlation of H-5/H_3-22. Relative stereochemistry of the amino acid moiety (C-16−C-18) was elucidated to be *erythro* between C-16 and C-17 by comparison of ^{1}H−^{1}H coupling constant ($J_{16,17}$ = 2.6 Hz) with those of *O*-methylthreonine ($J_{\alpha,\beta}$ <1 Hz) and *allo*-*O*-methythreonine ($J_{\alpha,\beta}$ = 3.6 Hz). Thus the relative stereostructure of brasilicardin A was assigned to be **1**.

Methanolysis of brasilicardin A (**1**) yielded the aglycone **2**, methyl α-glucosamine (**3**), methyl α-rhamnopyranoside (**4**), and methyl 3-hydroxybenzoate (**5**) (Scheme 1). The

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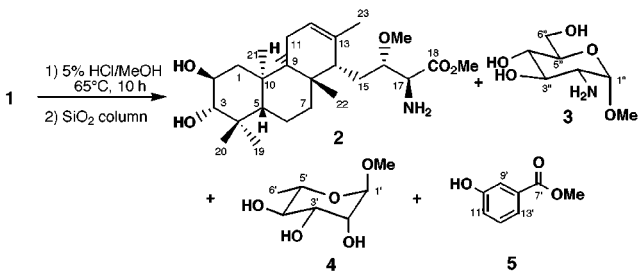
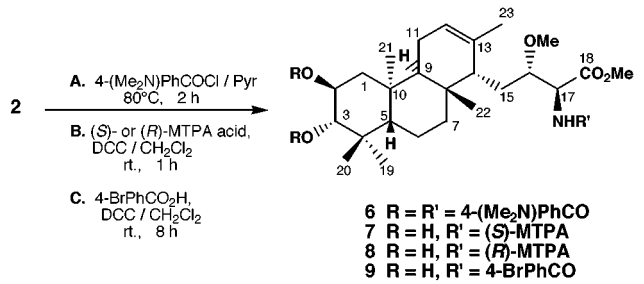
Scheme 1



Scheme 2



absolute configurations of compounds **3** and **4** were assigned as D and L, respectively, by comparison of optical rotations for **3** ($[\alpha]_D$ +49°) and for **4** ($[\alpha]_D$ −39°) with those of synthetic **3** ($[\alpha]_D$ +49°) and **4** ($[\alpha]_D$ −46°). Since brasilicardin A (**1**) was elucidated to possess a 1,2-diol functionality at C-2 and C-3, the CD exciton chirality method[10] was applied to determine absolute stereochemistry of the perhydrophenanthrene skeleton. The ^{1}H−^{1}H coupling constant ($J_{2,3}$ = 10.3 Hz) between H-2 (δ_H 5.38) and H-3 (δ_H 5.10) in compound **6**, which was derived from compound **2** by 4-dimethylaminobenzoylation (Scheme 2, **A**), indicated that the two 4-dimethylaminobenzoate groups at C-2 and C-3 were trans diequatrially disposed. The CD spectrum of **6** showed well-split intense Cotton effects, $\Delta\epsilon$ +43.8 at 322 nm and $\Delta\epsilon$ −24.6 at 297 nm, indicating that the projection of the two 4-dimethylaminobenzoate groups at C-2 and C-3 should be clockwise. These results allowed assignment of the *S* configurations for C-2 and C-3. To determine the absolute configuration at C-17 of brasilicardin A (**1**), compound **2** was converted into the (*S*)- and (*R*)-2-methoxy-2-trifluoromethylphenylacetamides (MTPA amides) (**7** and **8**, respectively) (Scheme 2, **B**).[11] The values of $\Delta\delta$ [δ(*S*-MTPA amide) − δ(*R*-MTPA amide)] (Figure 2) indicated that the absolute configuration at C-17 of **2** was *S*. Furthermore, compound **2** was converted into the *p*-bromobenzamide (**9**) (Scheme 2, **C**), which was crystallized from EtOH to give prisms of space group $P2_12_12_1$. The crystal structure was solved by the direct method and refined by a full-matrix least-squares method to R = 0.053 and R_w = 0.156 using 1845 ($I > 2\sigma(I)$) observed reflections. The absolute stereostructure of **9** was established by the X-ray analysis as shown in Figure 3, which corresponded to that of **1** elucidated by NMR and CD data. Thus, the absolute stereochemistry of **1** was determined as shown in Chart 1.

Brasilicardin A (**1**) is a novel tricyclic metabolite containing a rhamnose, an *N*-acetylglucosamine, and an amino acid moiety from the broth of *N. brasiliensis* IFM 0406. This is the first isolation of perhydrophenanthrene derivative with two sugar units from natural sources. The anti/syn/anti-perhydrophenanthrene skeleton such as **1** is very rare, although only one example, isoaplysin-20 from a sea hare,[12,13] has been reported. On the other hand, tricyclic diterpenoids and sesterterpenoids possessing an anti/anti/anti-perhydrophenanthrene skeleton have been isolated from a plant[14] and a marine nudibranch.[15] Biosynthetically the anti/syn/anti-perhydrophenanthrene skeleton with a boat form for ring B may be derived from cyclization of (*E*,*E*,*E*)-geranylgeranyl acetate.[6] Brasilicardin A (**1**) exhibited a potent immunosuppressive activity in mouse mixed lymphocyte assay (IC_{50}, 0.07 μg/mL), while the IC_{50} values of cyclosporin A and ascomycin, known immunosuppresive agents, in the assay were 0.016 and 0.04 μg/mL, respectively. Preliminary study suggests that the mode of action of **1** might be different from those of cyclosporin A or FK506. Compound **1** showed cytotoxicity against murine leukemia L1210 cells (IC_{50}, 1.2 μg/mL), human epidermoid carcinoma KB cells (IC_{50}, 1.3 μg/mL), and adriamycin-resistant murine leukemia P388/ADM cells (IC_{50}, 0.22 μg/mL), while **1** exhibited antifungal activity against *Paecilomyces variotti* (MIC, 25 μg/mL). Testing of **1** in the human tumor screen using 38 types of cell lines[16,17] gave a striking pattern of differential cytotoxicity. COMPARE

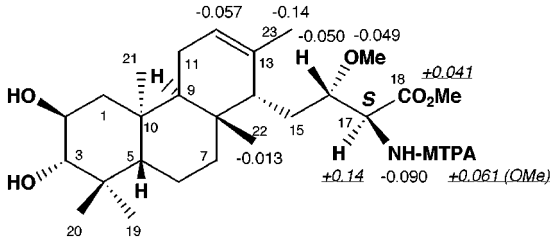


Figure 2. ^{1}H NMR chemical shift differences ($\Delta\delta$) for MTPA amides of compound **2.** $\Delta\delta$ (ppm) = δ[(*S*)-MTPA amide (**7**) − (*R*)-MTPA amide (**8**)].

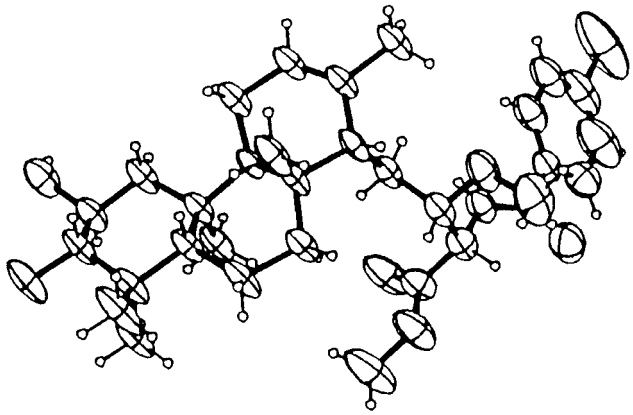


Figure 3. Perspective ORTEP drawing of the X-ray structure of **9**.

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pattern-recognition analyses of the mean-graph profiles of **1** did not reveal any significant correlations to the profiles of known antitumor compounds contained in the standard agent database.[16,17] The mean panel GI_{50} concentration of **1** was approximately 0.43 μM. Detailed bioactivities of **1** will be reported elsewhere.

Experimental Section

Cultivation. The voucher specimen (*Nocardia brasiliensis* IFM 0406, deposit No. FERM BP-5498)[18] was deposited at the Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University. The actinomycete *N. brasiliensis* IFM 0406 was grown in the broth [glycerol (2.0%), polypeptone (1.0%), and meat extract (0.5%) in H_2O, pH 7.0]. Cultures were incubated in a 150 L jar fermentor at 32 °C for 4 days with stirring at 250 rpm and 150 L/min aeration rate and were centrifuged.

Extraction and Separation. The supernatant of the fermentation broth (15 L) was passed through a Diaion HP-20 column (5 × 30 cm) and washed with 2 M NaCl(aq) (4 L) and H_2O (4 L) and then eluted batchwise with MeOH/H_2O (1:1, 4 L) and MeOH (2 L). The eluant with MeOH was suspended in H_2O and partitioned with $CHCl_3$. The aqueous layer was chromatographed on a DEAE-Toyopearl 650M column (2.5 × 10 cm) eluted with 20 mM Tris-HCl (pH 8). The active fraction (32 mg) was subjected to a CM Toyopearl 650M column (2.5 × 10 cm) eluted with 20 mM NaOAc−HCl (pH 4). The fraction (17 mg) was further purified by reversed-phase HPLC (Capcell pak C_{18} SG120, Shiseido Co. Ltd., 3 × 25 cm, flow rate 3.0 mL/min, 18%→42% MeCN/H_2O containing 0.15% TFA) to give brasilicardin A (**1**, 3.6 mg).

Brasilicardin A (1): A colorless amorphous solid: mp 270−273 °C; $[\alpha]^{30}_D$ +15.0° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3432, 2934, 1676, 1454, 1378, 1291, 1203, 1075, 893, 839, 801, 755, 722, and 570 cm^{-1}; UV (MeOH) λ_{max} 212 (ϵ 15000), 239 (5200), and 300 (1900) nm; ^1H and ^{13}C NMR (see Table 1); FABMS (positive, glycerol matrix) *m/z* 893 $(M + H)^+$ and 121; HR-FABMS *m/z* 893.4646 $(M + H)^+$, calcd for $C_{45}H_{69}N_2O_{16}$, 893.4647; HMBC correlations (see Table 1); ROESY correlations (CD_3OD, H/H) 1b/21, 2/19, 2/21, 2/1′, 3/5, 3/20, 5/22, 6b/20, 11/22, 12/23, 14/17, 14/22, 16/16-OMe, 17/16-OMe, 4′/6′, 1″/3″, 1″/5″, 2″/4″, 4″/6″a, and 4″/6″b.

Sugar Analysis of 1 by GC. Compound **1** (0.5 mg) was dissolved in 5% HCl/MeOH (0.5 mL) and heated at 65 °C for 15 h in a sealed tube. After evaporation of the solvent by a stream of nitrogen, the residue was dissolved in pyridine (50 μL) and treated with hexamethyldisilazane (10 μL) and trimethylsilyl chloride (5 μL) at room temperature for 30 min. Solvent was removed by a nitrogen stream, and the residue dissolved in hexane was used for GC analysis [1.5% OV-17 glass column (3 mm × 2 m); N_2 as a carrier gas; the program rate 120−200 °C at 0.5 °C/min], showing a peak at t_R 4.4 min, which corresponded to that of rhamnose (4.4 min) but not fucose (5.3 min). The TMS/Me derivatives of 6-deoxyhexoses, rhamnose and fucose, were prepared as authentic specimens.

Methanolysis of 1. Compound **1** (2.0 mg) was dissolved in 5% HCl/MeOH (1.0 mL) and heated at 65 °C for 10 h in a sealed tube. After evaporation of the solvent by a stream of nitrogen, the residue was separated by a silica gel column ($CHCl_3$/MeOH, 14:1 → BuOH/AcOH/H_2O, 8:1:1) to afford compounds **2** (0.6 mg, yield 61%), **3** (0.3 mg), **4** (0.2 mg), and **5** (0.2 mg).

Compound 2: A colorless amorphous solid; $[\alpha]^{26}_D$ +58° (*c* 0.1 MeOH); IR (film) ν_{max} 3369, 2929, 1735, 1560, 1438, 1377, 1261, 1101, 802, and 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.30 (1H, s, H-12), 3.89 (1H, d, J = 2.6 Hz, H-17), 3.75 (3H, s, MeO), 3.72 (1H, td, J = 9.5 and 5.2 Hz, H-2), 3.42 (1H, m, H-16), 3.41 (3H, s, MeO), 2.95 (1H, d, J = 9.5 Hz, H-3), 1.89 (1H, m, H-11a), 1.86 (3H, s, H-23), 1.80 (1H, m, H-11b), 1.72 (1H, m, H-6a), 1.71 (1H, m, H-14), 1.70 (1H, m, H-1a), 1.60 (1H, m, H-6b), 1.55 (1H, m, H-7a), 1.54 (1H, m, H-5), 1.52 (1H, m, H-14), 1.42 (1H, m, H-15a), 1.35 (1H, m, H-15b), 1.34 (1H, m, H-1b), 1.28 (1H, m, H-7b), 1.24 (1H, m, H-9), 1.05 (3H, s, H-21), 0.99 (6H, s, H-20 and H-22), and 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 173.91 (s, C-18), 137.79 (s, C-13), 121.97 (d, C-12), 83.88 (d, C-3), 82.97 (d, C-16), 69.47 (d, C-2), 57.88 (q, MeO), 55.35 (d, C-17), 51.98 (q, MeO), 50.98 (d, C-14), 45.95 (d, C-9), 43.23 (t, C-1), 43.08 (t, C-6), 39.56 (s, C-4), 37.26 (s, C-8), 36.54 (s, C-10), 30.79 (t, C-15), 29.93 (t, C-7), 28.61 (q, C-20), 28.26 (q, C-21), 26.02 (t, C-11), 22.59 (q, C-22), 22.26 (q, C-23), 17.65 (d, C-5), and 16.64 (q, C-19); EIMS *m/z* 437 (M^+), 378, 349, 331, 317, 299, 281, 207, and 89; HREIMS *m/z* 378.2972 $(M - CO_2Me)^+$, calcd for $C_{23}H_{40}NO_3$, 378.3008.

Tris-4-dimethylaminobenzoyl Derivative (6) from 2. The mixture of compound **2** (0.2 mg) and excess 4-dimethylaminobenzoyl chloride in dry pyridine (0.2 mL) was heated at 80 °C for 2 h. After complete removal of pyridine in vacuo, the residue was purified by a silica gel column (hexane/acetone, 5:2) to give compound **6** (0.2 mg, yield 50%), a colorless amorphous solid: $[\alpha]^{27}_D$ +94.0° (*c* 0.085, MeOH); IR (KBr) ν_{max} 1739, 1702, 1608, 1530, and 1447 cm^{-1}; UV (hexane) λ_{max} 310 (ϵ 39400) and 224 (14100) nm; CD (hexane) 322 ($\Delta\epsilon$ +43.8) 309 (0), and 297 (−24.6) nm; ^1H NMR ($CDCl_3$) δ 7.85 (2H, d, J = 10.0 Hz, 4-(Me_2N)Bz), 7.78 (2H, d, J = 8.9 Hz, 4-(Me_2N)-Bz), 7.70 (2H, d, J = 9.0 Hz, 4-(Me_2N)Bz), 6.65 (2H, d, J = 9.0 Hz, 4-(Me_2N)Bz), 6.57 (2H, d, J = 8.9 Hz, 4-(Me_2N)Bz), 6.55 (2H, d, J = 10.0 Hz, 4-(Me_2N)Bz), 5.38 (1H, td, J = 10.3 and 5.3 Hz, H-2), 5.35 (1H, m, H-17), 5.30 (1H, brs, H-12), 5.15 (1H, m, H-16), 5.10 (1H, d, J = 10.3 Hz, H-3), 3.79 (3H, s, MeO), 3.50 (3H, s, MeO), 3.09 (3H, s, NMe_2), 3.08 (3H, s, MeN), 3.07 (3H, s, MeN), 3.06 (3H, s, MeN), 3.05 (3H, s, MeN), 3.04 (3H, s, MeN), 2.00 (1H, m, H-11a), 1.85 (1H, m, H-11b), 1.82 (1H, m, H-1a), 1.81 (1H, m, H-14), 1.75 (1H, m, H-6a), 1.63 (3H, s, H-23), 1.58 (1H, m, H-5), 1.52 (1H, m, H-7a), 1.42 (1H, m, H-15a), 1.35 (1H, m, H-15b), 1.34 (1H, m, H-1b), 1.32 (1H, m, H-6b), 1.28 (1H, m, H-7b), 1.24 (1H, m, H-9), 1.20 (3H, s, H-21), 1.15 (3H, s, H-19), 1.05 (3H, s, H-22), and 0.95 (3H, s, H-20); FDMS *m/z* 878 (M^+); HRFDMS *m/z* 878.5161 (M^+), calcd for $C_{52}H_{70}N_4O_8$, 878.5194.

Preparation of (*S*)- or (*R*)-MTPA Amide (7 or 8) of Compound 2. A solution of compound **2** (0.16 mg) in CH_2Cl_2 (30 μL) was treated with DCC (0.15 mg) and (*S*)-MTPA acid (0.17 mg), and then the mixture was stirred at room temperature for 1 h. The reaction mixture containing the precipitates of urea was concentrated, and the residue was separated by preparative SiO_2 TLC (hexane/EtOAc, 1:1) to afford the (*S*)-MTPA amide (**7**, 0.16 mg) of **2**. The (*R*)-MTPA amide (**8**) of **2** was also prepared from **2** and (*R*)-MTPA acid by the same procedure as **7**.

Compound 7: A colorless oil; $[\alpha]^{27}_D$ +104° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3432, 1742, 1707, 1631, 1561, 1516, and 1450 cm^{-1}; UV (MeOH) λ_{max} 207 (ϵ 7500) nm; ^1H NMR ($CDCl_3$) δ 7.592 (2H, m, Ph), 7.363 (3H, m, Ph), 7.205 (1H, brs, NH-17), 5.265 (1H, s, H-12), 5.207 (1H, d, J = 2.6 Hz, H-17), 3.779 (3H, s, CO_2Me-18), 3.697 (1H, td, J = 9.5 and 5.2 Hz, H-2), 3.544 (1H, m, H-16), 3.494 (3H, s, MeO−MTPA), 3.394 (3H, s, MeO-16), 2.957 (1H, d, J = 9.5 Hz, H-3), 1.89 (1H, m, H-11a), 1.80 (1H, m, H-11b), 1.72 (1H, m, H-6a), 1.71 (1H, m, H-14), 1.70 (1H, m, H-1a), 1.60 (1H, m, H-6b), 1.55 (1H, m, H-7a), 1.54 (1H, m, H-5), 1.479 (3H, s, H-23), 1.42 (1H, m, H-15a), 1.35 (1H, m, H-15b), 1.34 (1H, m, H-1b), 1.28 (1H, m, H-7b), 1.24 (1H, m, H-9), 1.05 (3H, s, H-21), 0.99 (3H, s, H-20), 0.974 (3H, s, H-22), and 0.92 (3H, s, H-19); EIMS *m/z* 653 (M^+) and 621 $(M - MeOH)^+$; HREIMS *m/z* 653.3544 (M^+), calcd for $C_{35}H_{50}NO_7F_3$, 653.3539.

Compound 8: A colorless oil; $[\alpha]^{30}_D$ +76° (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3431, 1742, 1705, 1627, 1577, 1516, and 1450 cm^{-1}; UV (MeOH) λ_{max} 207 (ϵ 6400) nm; ^1H NMR ($CDCl_3$) δ 7.549 (2H, m, Ph), 7.405 (3H, m, Ph), 7.295 (1H, brs, NH-17), 5.322 (1H, s, H-12), 5.065 (1H, d, J = 2.6 Hz, H-17), 3.739 (3H, s, CO_2Me-18), 3.699 (1H, td, J = 9.5 and 5.2 Hz, H-2), 3.593 (1H, m, H-16), 3.433 (3H, s, MeO−MTPA), 3.443 (3H, s, MeO-16), 2.961 (1H, d, J = 9.5 Hz, H-3), 1.89 (1H, m, H-11a), 1.80 (1H, m, H-11b), 1.72 (1H, m, H-6a), 1.71 (1H, m, H-14), 1.70 (1H, m, H-1a), 1.624 (3H, s, H-23), 1.60 (1H, m, H-6b),

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1.55 (1H, m, H-7a), 1.54 (1H, m, H-5), 1.42 (1H, m, H-15a), 1.35 (1H, m, H-15b), 1.34 (1H, m, H-1b), 1.28 (1H, m, H-7b), 1.24 (1H, m, H-9), 1.05 (3H, s, H-21), 0.99 (3H, s, H-20), 0.987 (3H, s, H-22), and 0.92 (3H, s, H-19); EIMS *m/z* 653 (M^+) and 621 ($M - MeOH)^+$; HREIMS *m/z* 621.3248 $(M\text{-}MeOH)^+$, calcd for $C_{34}H_{46}NO_7F_3$, 621.3277.

4-Bromobenzoyl Derivative (9) from 2. A solution of compound **2** (1.6 mg) in CH_2Cl_2 (100 μL) was treated with DCC (1.9 mg) and 4-bromobenzoic acid (1.9 mg), and the mixture was stirred at room temperature for 8 h. The reaction mixture containing the precipitates of urea was concentrated, and the residue was separated by preparative SiO_2 TLC ($CHCl_3$/MeOH, 12:1) to afford compound **9** (1.6 mg, yield 70%), a colorless plate (from EtOH): mp 286−287 °C; $[\alpha]^{27}_D$ +118° (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3435, 1741, 1648, 1592, 1540, 1482, and 1439 cm^{-1}; UV (MeOH) λ_{max} 241 (ϵ 16900) and 210 (15500) nm; ^1H NMR ($CDCl_3$) δ 7.68 (2H, d, J = 8.5 Hz, 4-BrBz), 7.59 (2H, d, J = 8.5 Hz, 4-BrBz), 6.63 (1H, d, J = 8.0 Hz, NH-17), 5.33 (1H, brs, H-12), 5.17 (1H, dd, J = 8.1 and 3.5 Hz, H-16), 3.80 (3H, s, CO_2Me-18), 3.71 (1H, td, J = 9.5 and 4.3 Hz, H-2), 3.62 (1H, dd, J = 8.0 and 3.5 Hz, H-17), 3.48 (3H, s, MeO-16), 2.97 (1H, d, J = 9.5 Hz, H-3), 1.91 (1H, brd, J = 17.0 Hz, H-11a), 1.83 (1H, brdd, J = 17.0 and 13.5 Hz, H-11b), 1.74 (1H, m, H-6a), 1.72 (1H, m, H-14), 1.71 (1H, m, H-1a), 1.60 (1H, m, H-6b), 1.55 (3H, s, H-23), 1.55 (1H, m, H-7a), 1.54 (1H, m, H-5), 1.42 (1H, m, H-15a), 1.35 (1H, m, H-15b), 1.34 (1H, m, H-1b), 1.28 (1H, m, H-7b), 1.25 (1H, m, H-9), 1.05 (3H, s, H-21), 1.00 (3H, s, H-20), 0.99 (3H, s, H-22), and 0.90 (3H, s, H-19); EIMS *m/z* 621 and 619 (M^+, 1:1); HREIMS *m/z* 619.2512 (M^+), calcd for $C_{32}H_{46}NO_6{}^{79}Br$, 619.2509.

X-ray Crystallography of 9. Compound **9** was obtained as a colorless prism from EtOH. Compound **9** was crystallized as an orthorhombic system, space group $P2_12_12_1$ with one molecule per asymmetric unit. Cell constants were a = 12.431(1) Å, b = 21.490(3) Å, c = 12.094(3) Å, β = 90.00°, and V = 3230.9(7) Å^3. All unique reflections with $3° < 2\theta < 126°$ were collected on a Rigaku AFC-5 diffractometer using graphite-monochromated Cu Kα radiation and employing $\omega-2\theta$ scan mode. In 2941 collected reflections 1845 reflections ($I > 2\sigma(I)$) were judged as observed and used for the structure determination and refinement. The structure was solved by direct methods and refined by a full-matrix least-squares method with anisotropic thermal parameters [SHELXL93 program].[19] The positions of H atoms were obtained from a difference Fourier map and were included in the final refinement. The residual factors were R = 0.053 and R_w = 0.156 for 1845 observed reflections.[20]

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Supporting Information Available: All spectra of **1**, NMR spectra of compounds **2**, **6**, **7**, **8**, and **9**, and X-ray crystallographic data of **9** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(20) Lists of structure factors, anisotropic distancement parameters, H atom coordinates, and complete geometry have been deposited with the IUCr (Reference: AS1088).