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ANTINEOPLASTIC AGENTS, 326. THE STEREOCHEMISTRY OF BASTADINS 8, 10, AND 12 FROM THE BISMARCK ARCHIPELAGO MARINE SPONGE *IANTHELLA BASTA*[1]

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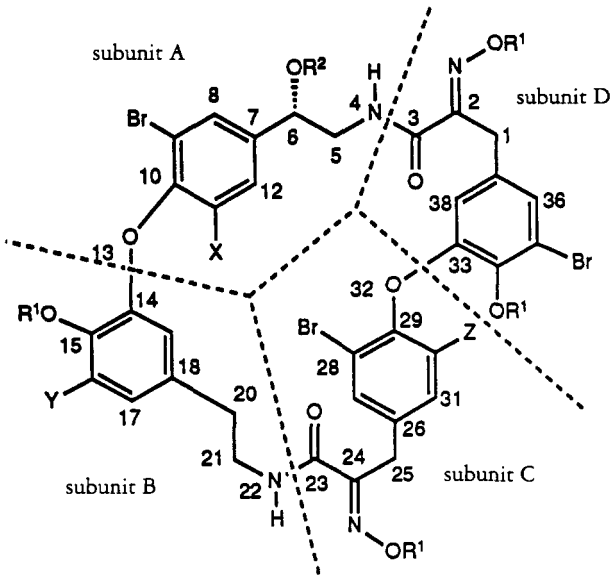
ABSTRACT.—An investigation of cancer cell-growth inhibitory constituents of the Papua New Guinea marine sponge *Ianthella basta* led to isolation of the C-6 hydroxybastadins 8 [**1**], 10 [**2**], and 12 [**3**]. The absolute stereochemistry (6*S*) of each bastadin (or its tetramethyl ester derivative) was determined by means of the Mosher-Trost method. Bastadins 10 [**2**] and 12 [**3**] were found to significantly inhibit the growth of a selection of human cancer cell lines. Bastadins 8, 10, and 12 inhibited growth of the Gram-positive opportunists *Staphylococcus aureus* and *Enterococcus faecalis*.

Interest in the marine Porifera genus *Ianthella* (class Demospongiae, order Verongida, family Ianthellidae) gained momentum in 1970 with the isolation of an imidazole-type hypotensive agent (2), and the antibacterial aeroplysinin (3) from two Caribbean Verongid (4) sponges. Except for several studies of the carotenoid constituents (5–7) of *Ianthella basta* (Pallas, 1776) all subsequent investigations have been focused on its tyrosine-derived biosynthetic products. The pioneering studies of Wells and colleagues (8,9) of the Australian *I. basta* conducted in the late 1970s led to isolation and structure determination of bastadins 1–7. These bastadins were found to show in vitro activity against Gram-positive bacteria (9). Soon thereafter bastadin 6 was synthesized (10). In 1990, *I. basta* from Guam was shown by Schmitz (11) to contain bastadins 8–11 (as well as bastadins 2–6), and Andersen reported (12) the isolation of bastadins 8 and 12 (13) from a Papua New Guinea collection. Bastadin 13, which contained a novel cyclization pattern, was reported from an Australian *I. basta* by Capon and coworkers (14) in 1991. The preceding number assignments for bastadins 12 and 13 follow those recommended by Scheuer (13) who found bastadin 14 in a sample of the Verongid sponge *Psammaplysilla purpurea* (Carter) collected in the Federated States of Micronesia (Pohnpei). The next addition, bastadin 15, was discovered (15) in an Australian *Ianthella* sp., while bastadins 16 and 17 were isolated from an Indonesian *I. basta* (16). The 34-sulfated derivative of bastadin 13 has also been reported (17).

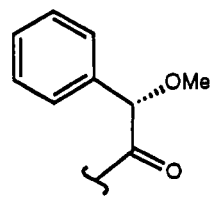
RESULTS AND DISCUSSION

As part of our 1981 Papua New Guinea expedition southeast of Port Moresby, we collected *I. basta*. An *i*-PrOH extract was found to be active against the U.S. National Cancer Institute's P-388 murine lymphocytic leukemia (PS system). In another Papua New Guinea expedition to New Ireland (Bismarck Archipelago) in 1983, *I. basta* was located in quantity and recollected (100 kg wet wt). While the initial EtOH extract of this collection proved to be less active (PS 20% life extension at 200 mg/kg) than its 1981 counterpart, it did show useful P-388 cell line activity (ED_{50} 0.23 μg/ml) and separation was guided using this bioassay. Employing a series of solvent partition (18), gel permeation, and partition chromatographic techniques (19), a P-388-active CH_2Cl_2-soluble fraction led to bastadins 8 [**1**], 10 [**2**], and 12 [**3**] in 3.0×10^{-4}, 3.8×10^{-4}, and 2.0×10^{-4}% yields, respectively. Identification of these compounds was performed by

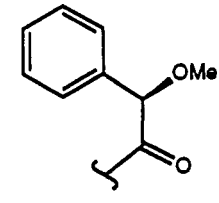
[1]For contribution 325, see Pettit *et al.* (1).



	X	Y	Z	R^1	R^2
1	H	Br	Br	H	H
2	H	Br	H	H	H
3	Br	Br	H	H	H
4	H	Br	H	Me	H
5	H	Br	H	Me	(*S*)-MPA
6	H	Br	H	Me	(*R*)-MPA
7	H	Br	Br	Me	H
8	Br	Br	H	Me	H
9	H	Br	Br	Me	(*S*)-MPA
10	H	Br	Br	Me	(*R*)-MPA
11	Br	Br	H	Me	(*S*)-MPA
12	Br	Br	H	Me	(*R*)-MPA



(*S*)-MPA



(*R*)-MPA

spectroscopic methods and by comparison with authentic samples. Overall, there was good agreement between the ^{1}H- and ^{13}C-nmr data recorded here and those previously reported for bastadin 10 (11). Our 2D nmr analyses employing a larger (only 2.7 mg of an oil was originally available, 11) specimen of bastadin 10 revealed that some of the ^{13}C-nmr resonances originally assigned (11) by comparison with bastadins 4 and 8 required reassignment.

Methylation of bastadin 10 [**2**] using CH_3I/K_2CO_3 in DMF yielded an optically active tetramethyl ether [**4**]. Examination of the ^{1}H-nmr spectrum recorded in CD_2Cl_2[2] indicated that the benzylic methylene resonances H_2-1 (δ 3.74, d, J=13 Hz; 3.76, d, J=13.0 Hz) and H_2-25 (δ 3.80, br s) were sufficiently separated to unambiguously confirm the structure (Table 1). HMBC correlations (Table 1) from NH-4 (δ 6.90, t, J=6.0 Hz) and H_2-1 to C-3 (163.5 ppm) allowed subunits A and D to be linked, while correlations from NH-22 (δ 6.77, t, J=6.0 Hz) and H_2-25 to C-23 (162.5 ppm) allowed subunits B and C to be linked. Thus, the structure for bastadin 10 [**2**] was proven to be identical to that proposed by Schmitz (11, 20).

With the overall structure of bastadin 10 [**2**] firmly secured, the absolute stereochemistry of the C-6 hydroxy group and absolute configuration were investigated using the improved Mosher-Trost method (21), which allowed the absolute stereochemistry of a secondary alcohol to be determined by comparing the ^{1}H-nmr spectra of (*R*)- and (*S*)-α-methoxyphenylacetic acid (MPA) derivatives (21–25). By examining models (Figure 1) of the (*R*)- and (*S*)-MPA diastereomers of both (6*R*)- and (6*S*)-bastadin 10

[2]The ^{1}H-nmr resonances for H_2-1 and H_2-25 (δ 3.78, br s) were coincident in $CDCl_3$.

TABLE 1. Nmr Spectral Assignments for **4** in CD_2Cl_2.

Position	^1H δ (m, *J*) (500 MHz)	^{13}C (ppm) (100 MHz)	COSY[a] (500 MHz)	HMBC (500 MHz)
1	3.74 (d, 13.0) 3.76 (d, 13.0)	29.0	(H-36, H-38)	C-2,-3,-36,-37,-38
2	—	151.2		
3	—	163.5		
N-4	6.90 (t, 6.0)	—	H-5a,b	C-3,-5
5	3.42 (m) 3.67 (ddd, 4.5, 7.0, 14.0)	47.3	NH-4, H-5b, H-6 NH-4, H-5a, H-6	C-4
6	4.82 (ddd, 4.5, 6.5)	72.6	H-5a,b (H-8)	C-8,-12
OH-6	2.92 (br s)	—		
7	—	140.0		
8	7.67 (d, 2.0)	131.6	H-12 (H-6)	C-6,-9,-10,-12
9	—	115.0		
10	—	152.6		
11	6.96 (d, 8.5)	120.8	H-12	C-7,-9,-10
12	7.27 (dd, 2.0, 8.5)	127.1	H-8, H-11	C-6,-8,-9,-10
14	—	150.8		
15	—	146.1		
16	—	118.3		
17	7.15 (d, 2.0)	128.1	H-19 (H_2-20)	C-15,-16,-19,-20
18	—	136.6		
19	6.54 (d, 2.0)	118.1	H-17 (H_2-20)	C-14,-15,-16,-17,-20
20	2.68 (t, 6.0)	35.3	H_2-21 (H-17, H-19)	C-17,-18,-19,-21
21	3.42 (m)	40.4	H_2-20, NH-22	C-20
N-22	6.77 (t, 6.0)	—	H_2-21	C-21,-23
23	—	162.5		
24	—	151.4		
25	3.80 (br s)	29.2	(H-27, H-31)	C-23,-24,-26,-27,-31
26	—	133.9		
27	7.55 (d, 2.0)	134.7	H-31 (H_2-25)	C-25,-28,-29,-31
28	—	114.1		
29	—	151.8		
30	6.71 (d, 8.5)	119.7	H-31	C-26,-28,-29
31	7.16 (dd, 2.0, 8.5)	130.1	H-27, H-30 (H_2-25)	C-25,-27,-28
33	—	150.2		
34	—	146.6		
35	—	118.0		
36	7.26 (d, 2.0)	129.0	H-38 (H_2-1)	C-1,-34,-35,-38
37	—	133.9		
38	6.67 (d, 2.0)	119.4	H-36 (H_2-1)	C-1,-33,-34,-35,-36
OCH_3-15	3.93 (s)	61.42		C-15
OCH_3-34	3.88 (s)	61.37		C-34
$NOCH_3$-2	4.00 (s)[b]	63.4		
$NOCH_3$-24	3.76 (s)[b]	63.3		

[a]Long-range COSY correlations inside brackets.
[b]Assignments may be interchanged.

tetramethyl ether, the observation of a positive $\Delta\delta$ ($=\delta_S-\delta_R$) value for the aromatic region and negative $\Delta\delta$ value for the amide region would be consistent with (6*S*) stereochemistry. Conversely, a negative $\Delta\delta$ value for the aromatic region and positive $\Delta\delta$ value for the amide region would have been consistent with (6*R*) stereochemistry. The (*R*)- and (*S*)-MPA esters **5** and **6** of bastadin 10 tetramethyl ether were prepared using MPA, DCCI, and DMAP in dry CH_2Cl_2. The ^1H-nmr spectra of esters **5** and **6** were recorded

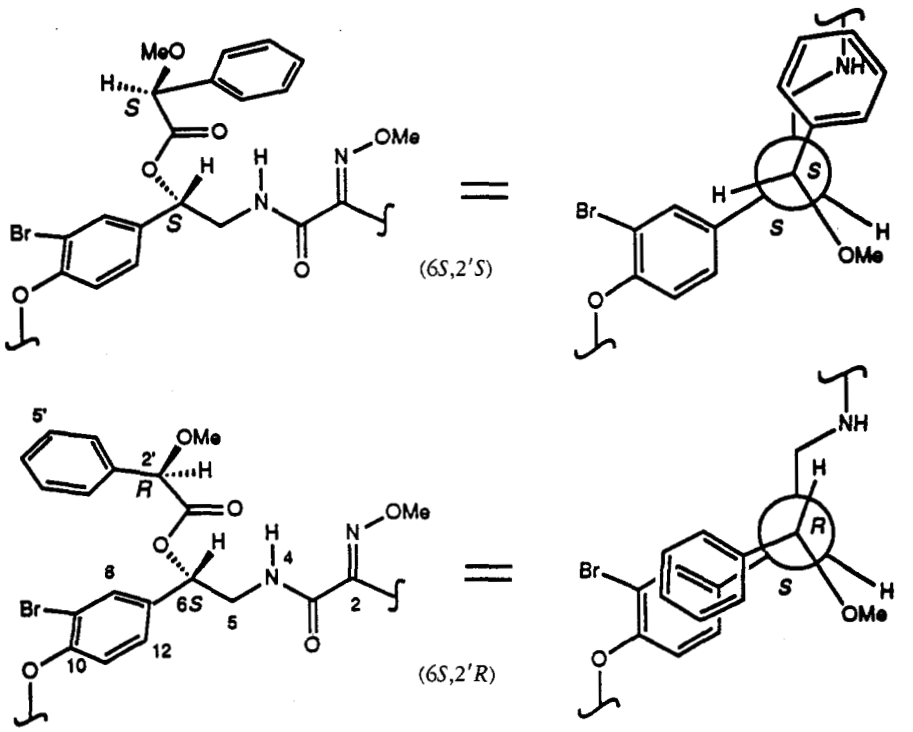


FIGURE 1. Assumed conformational preference of the (6*S*,2'*S*)- and (6*S*,2'*R*)-MPA derivatives [**5** and **6**] of bastadin 10 tetramethyl ether.

in $CDCl_3$ and fully assigned by examination of their COSY and TOCSY spectra. The ^{1}H-nmr spectra of esters **5** and **6** contained one set of distinct signals that indicated bastadin 10 [**2**] was a single enantiomer. Application of the Mosher-Trost method (23) to esters **5** and **6** indicated that bastadin 10 [**2**] contained a (6*S*)-hydroxy group and the corresponding absolute stereochemistry (Table 2, positive $\Delta\delta$ values for H-8, H-11, and H-12 and negative $\Delta\delta$ values for H-5_a, H-5_b, and NH-4).

TABLE 2. Comparison of the ^{1}H-Nmr Chemical Shifts ($\Delta\delta=\delta_S-\delta_R$) of the (*S*) and (*R*) MPA Esters, Compounds **5** and **6**, **9** and **10**, and **11** and **12**.

MPA esters	H-8			H-11			H-12		
	δ_S	δ_R	$\Delta\delta$	δ_S	δ_R	$\Delta\delta$	δ_S	δ_R	$\Delta\delta$
5 and **6**	7.50	7.16	+0.34	6.88	6.78	+0.10	7.19	6.94	+0.25
9 and **10**	7.49	7.12	+0.37	6.89	6.79	+0.10	7.20	6.94	+0.26
11 and **12**	n/o[a]	n/o[a]	—	—	—	—	n/o[a]	n/o[a]	—
MPA esters	H-5a			H-5b			NH-4		
	δ_S	δ_R	$\Delta\delta$	δ_S	δ_R	$\Delta\delta$	δ_S	δ_R	$\Delta\delta$
5 and **6**	3.44	3.51	−0.07	3.60	3.72	−0.12	6.55	6.69	−0.14
9 and **10**	3.50	3.52	−0.02	3.50	3.66	−0.16	6.47	6.59	−0.12
11 and **12**	3.48	3.50	−0.02	3.68	3.78	−0.10	6.66	6.80	−0.14

[a]n/o=not observed.

The tetramethyl ethers of bastadin 8 [**7**] and bastadin 12 [**8**] were also prepared using CH_3I/K_2CO_3 in DMF. Application of the Mosher-Trost (23) method to the (*S*)- and (*R*)-MPA derivatives [**9** and **10**] of bastadin 8 tetramethyl ether indicated that bastadin 8 [**1**] was the (6*S*) enantiomer (Table 2). Esters **9** and **10** exhibited positive $\Delta\delta$ values for H-8, H-11, and H-12 and negative $\Delta\delta$ values for H-5_a, H-5_b, and NH-4.

Extension of the Mosher-Trost technique (23) to the (*S*)- and (*R*)-MPA derivatives [**11** and **12**] of bastadin 12 tetramethyl ether was not as direct. Negative $\Delta\delta$ values for H-5a, H-5b, and NH-4 (Table 2) were observed, which were consistent with (6*S*) absolute stereochemistry. However, resonances associated with the aromatic region (H-8 and H-11) were not observed at room temperature. In bastadin 12 [**3**] and its methyl ether derivative **8**, the aromatic methines H-8 and H-11 were observed as broad resonances (11). Interestingly, the ^{1}H-nmr chemical shifts associated with the MPA phenyl group were observed as a broad five-proton singlet in all the (6*S*,2′*R*) diastereomers [**6**, **10**, and **12**], while those for all the (6*S*,2′*S*) diastereomers [**5**, **9**, and **11**] were observed as separate two-proton and three-proton multiplets.

Previously, bastadins 4 (11), 8 (11,12), 9 (11,12), and 12 (12) were found to inhibit (ED_{50} 2 to 5 μg/ml) growth of the P-388 and L-1210 murine leukemia cell lines, and bastadins 4, 8, and 9 also displayed anti-inflammatory activity (mouse ear assay, 11). Bastadin 14 (13) showed comparable cytotoxicity and inhibited dihydrofolate reductase and topoisomerase II enzyme systems. Comparison of bastadins 8, 10, and 12 against a minipanel of human cancer cell lines (Table 3) showed the murine P-388 leukemia line to be the most resistant to each and bastadin 12 the most effective overall. The tetramethyl ether derivatives of bastadins 8, 10, and 12 resulted in a dramatic reduction in cell-growth inhibitory activity.

TABLE 3. Cell-Growth Inhibitory Activity of **1–3** Against the P-388 Cell Line and a Selection of Human Cancer Cell Lines.

Cancer cell line	**1**	**2**	**3**
	ED_{50}/IC_{50} (μg/ml)	ED_{50}/IC_{50} (μg/ml)	ED_{50}/IC_{50} (μg/ml)
Murine leukemia (P-388)	22.6	>10	9.1
Ovarian (OVCAR-3)	>10	2.9	2.0
CNS (SF-295)	>10	3.2	2.4
Renal (A498)	7.0	4.5	2.1
Lung (NCI-H460)	>10	3.4	2.3
Colon (KM20L2)	>10	2.0	2.1
Melanoma (SKMEL-5)	9.9	3.7	2.2

Several bastadins are reportedly antimicrobial. Bastadin 13 (Australian *I. basta*), for example, has a mic of 6 μg/ml for the Gram-positive bacterium *Bacillus subtilis* (16). Although no data were presented, Wells and colleagues (9) noted the antimicrobial activity of bastadins 1–7 (Australian *I. basta*) for Gram-positive bacteria. We investigated the potential antibacterial and antifungal activities of bastadins 8, 10 and 12 from the Bismarck Archipelago *I. basta*. All three compounds inhibited growth of the Gram-positive opportunists *Staphylococcus aureau* and *Enterococcus faecalis* (Table 4). At up to 100 μg/disk, these compounds exhibited no antimicrobial activity against the Gram-negative bacteria *Escherichia coli* and *Neisseria gonorrhoeae*, or the fungi *Candida albicans* and *Cryptococcus neoformans*.

TABLE 4. Minimum Inhibitory Concentrations (μg/disk) of Bastadins 8, 10 and 12 for Bactria and Fungi.

Compound(s)	*E. coli*	*N. gonorrhoeae*	*E. faecalis*	*S. aureua*	*C. albicans*	*C. neoformans*
Bastadin 8	[a]	[a]	<50	0.195–0.39	[a]	[a]
Bastadin 10	[a]	[a]	6.25–12.5	1.56–3.12	[a]	[a]
Bastadin 12	[a]	[a]	50–100	6.25–12.5	[a]	[a]

[a]No inhibition at 100 mg/disk.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents were distilled prior to use. Sephadex LH-20 (particle size 25–100 μm) was obtained from Pharmacia Fine Chemical AB, Uppsala, Sweden. Si gel (Kieselgel 60, mesh 230–400), was obtained from E. Merck. Tlc plates (GF Uniplate, Analtech, Inc., Newark, DE) were viewed under shortwave uv light (254 nm) and developed with I_2 vapor. For hplc separations, a Phenomenex Prepex (25 cm×10.0 mm, 5–20 μm) normal-phase column was used with an Altex solvent metering pump (Model 110A) and a Gilson Holochrome uv detector set at 276 nm.

Optical rotations were measured using a Perkin-Elmer model 241 polarimeter. Uv spectra were recorded with a Hewlett-Packard 8450 uv-vis spectrometer, while ir spectra were recorded employing a Mattson Instruments 2020 Galaxy Series Ft-ir. Nmr spectra were determined with either a Varian Unity-500, Bruker AM-400, Varian Unity Plus-400, or a Varian Gemini 300 and were referenced to the residual proton solvent resonance (CD_2Cl_2: δ 5.32 and 53.8 ppm; $CDCl_3$: δ 7.26 and 77.0 ppm; DMSO-d_6: δ 2.49 and 39.5 ppm). Mass spectra were recorded at the Midwest Center for Mass spectrometry at the University of Nebraska-Lincoln.

ANIMAL MATERIAL.—During a June 1981 collection expedition to Papua New Guinea, a bluish purple sponge (ca. 20 cm in height) was collected by scuba at depths of 2 to 10 m at Idler Bay Point, 5 km west along the coast from Moresby Bay. The sponge was identified as *Ianthella basta* (Pallas, 1776) (Porifera; Demospongiae; Verongida; Ianthellidae) and a voucher specimen is maintained at the Arizona State University Cancer Research Institute.

The large-scale recollection (100 kg) of *Ianthella basta* was made in the Ysabel Channel on the East Coast of New Hanover, Papua New Guinea in 1983.

EXTRACTION AND ISOLATION.—The shipping *i*-PrOH-H_2O solution was drained from the containers, concentrated to H_2O by vacuum distillation, and partitioned with CH_2Cl_2. Evaporation of the CH_2Cl_2-soluble material gave a 209-g fraction that showed an ED_{50} value of 42 μg/ml (P-388 cell line). The sponges were re-extracted twice with CH_3OH-CH_2Cl_2 (1:1). The CH_3OH-CH_2Cl_2 extract was collected and H_2O added until two phases were observed. The CH_2Cl_2 layer was concentrated to yield a 1.037 kg residue (P-388, ED_{50} 13 μg/ml). The CH_2Cl_2-soluble fraction from the shipping solution and the CH_3OH/CH_2Cl_2 extractions were combined and partitioned between hexane and CH_3OH-H_2O (9:1). The P-388-inactive hexane fraction was removed. The MeOH solution was adjusted to CH_3OH-H_2O (3:2), extracted with CH_2Cl_2, and the chlorocarbon fraction was concentrated to yield 314 g.

The latter CH_2Cl_2 fraction in MeOH was chromatographed on a column of Sephadex LH-20. The P-388-active fractions (41 g) were eluted last. A portion (11.8 g) of this fraction was rechromatographed again on Sephadex LH-20 and eluted with CH_3OH to provide an array of active fractions. Examination of these fractions by ^{1}H-nmr spectroscopy revealed resonances consistent with bastadin-type structures (see below). One of these fractions proved to be a rich source of the C-6 hydroxy-bastadins (3.26 g) and was chromatographed on a Sephadex LH-20 column using CH_2Cl_2-CH_3OH (3:2) as eluent. The resulting 1.55 g P-388-active fraction was subjected to chromatography on Sephadex LH-20 employing hexane-toluene-CH_3OH (2:1:1) to provide 1.2 g of a P-388-active fraction. Si gel cc of a 0.41-g aliquot of this fraction using CH_2Cl_2-*i*-PrOH (97:3) as eluent afforded 0.11 g (3.8×10^{-4}% yield) of bastadin 10 [**2**] and a 0.16-g mixture of bastadins 8 [**1**] and 12 [**3**]. A 15.6-mg sample of the **1** and **3** mixture was separated by normal-phase hplc (45% EtOAc in hexane; 2.0 ml/min) to yield 8.4 mg (3.0×10^{-4}% yield) of pure bastadin 8 [**1**] and 6.0 mg (2.0×10^{-4}% yield) of bastadin 12 [**3**], which were identical with authentic samples (10,11).

Bastadin 10 [**2**].—Colorless solid; ^{13}C nmr (DMSO-d_6, 100 MHz) δ 27.6 (C-1), 28.5 (C-25), 33.5 (C-20), 40.6 (C-21), 47.0 (C-5), 70.2 (C-6), 110.5 (C-35), 110.7 (C-16), 113.3 (C-9), 113.3 (C-28), 117.4 (C-12, C-19, C-38), 119.9 (C-30), 120.0 (C-11), 126.8 (C-12), 127.7 (C-17), 128.0 (C-36), 128.8 (C-36), 129.6 (C-31), 130.6 (C-8), 131.7 (C-18), 133.5 (C-27), 134.5 (C-26), 141.0 (C-7), 143.4 (C-2, C-15, C-34), 145.0 (C-14, C-33), 150.8 (C-24), 151.2 (C-2, C-29), 151.7 (C-10), 162.9 (C-3), 163.1 (C-23); identical by tlc with an authentic sample.

6(S)-2,15,24,34-Tetramethoxy-bastadin 8 [**7**].—A mixture of bastadin 8 [**1**] (11.3 mg), K_2CO_3 (60 mg), and CH_3I (0.75 ml) in dry DMF (0.75 ml) was stirred at room temperature for 2 days. The reaction mixture was poured into H_2O (20 ml) and extracted with Et_2O (2×20 ml). The combined ethereal extract was washed with H_2O (2×20 ml), dried (Na_2SO_4), and the solvent was removed *in vacuo*. Normal-phase hplc (CH_3OH-CH_2Cl_2, 99:1, 1.5 ml/min) provided the tetramethyl ether **7** as a colorless solid (7.2 mg, 60%): $[\alpha]^{23}D$ +10.6° (*c*=1.31, CH_2Cl_2) [lit. (12), $[\alpha]D$ +12° (*c*=0.61, CH_2Cl_2)]; identical in $[\alpha]D$, ^1H-nmr, and tlc R_f data to an authentic sample (11,12).

6(S)-2,15,24,34-Tetramethoxy-bastadin 10 [**4**].—The preceding experiment (cf. 8) was repeated (1-day reaction time) with bastadin 10 [**2**] (30 mg), K_2CO_3 (100 mg), and CH_3I (1 ml) in dry DMF (2 ml). Si gel cc elution with CH_2Cl_2-EtOAc (85:15) led to ether **4** (23 mg, 72%) as a colorless solid: $[\alpha]^{23}D$ +6.7° (*c*=1.69, CH_2Cl_2); uv (CH_2Cl_2) λ max (log ε) 235 (4.54), 276 (3.87) nm; ir ν max (NaCl, film) 3405 (br), 1672, 1523, 1485, 1236, 1045 cm^{-1}; ^1H-nmr data (CD_2Cl_2, 500 MHz), see Table 1; ^{13}C-nmr data (CD_2Cl_2, 100 MHz), see Table 2; fabms (3-NBA matrix) *m/z* 1009/1011/1013/1015/1017/ $[M+H]^+$, 991/993/995/997/999 ($[M+H]^+-H_2O$), 977/979/981/983/985 ($[M+H]^+-CH_3OH$); hrfabms *m/z* 990.9188 ($C_{38}H_{35}N_4O_8{}^{79}Br_4$, calcd 990.9200), 992.9161 ($C_{38}H_{35}N_4O_8{}^{79}Br_3{}^{81}Br$, calcd 992.9180), 994.9158 ($C_{38}H_{35}N_4O_8{}^{79}Br_2$, calcd 994.9160), 996.9155 ($C_{38}H_{35}N_4O_8{}^{79}Br^{81}Br_3$, calcd 996.9140), 998.9135 ($C_{38}H_{35}N_4O_8{}^{81}Br_4$, calcd 998.9120).

6(S)-2,15,24,34-Tetramethoxy-bastadin 12 [**8**].—The tetramethylation of bastadin 12 [**3**] (11.6 mg) with CH_3I (1 ml) and K_2CO_3 (80 mg), in dry DMF (1 ml) was conducted as described above for ether **7**. Normal-phase hplc (CH_2Cl_2-CH_3OH, 99:1, 1.5 ml/min) afforded ether **8** as a colorless solid (6.6 mg, 54%): $[\alpha]^{23}D$ +4.5° (*c*=0.65, CH_2Cl_2) [lit. (12) $[\alpha]D$ +2.7° (*c*=0.77, CH_2Cl_2)]; identical in tlc R_f, ^1H-nmr, and $[\alpha]D$ data to an authentic sample (12).

6(S)-[α-(S)-Methoxyphenylacetyl]-2,15,24,34-tetramethoxy-bastadin 8 [**9**].—A mixture of bastadin 8 tetramethyl ether [**7**] (5.4 mg), 4-dimethylaminopyridine (DMAP) (9 mg), 1,3-dicyclohexylcarbodiimide (DCCI) (10 mg) and α-(*S*)-methoxyphenylacetic acid (MPA) (6 mg) in dry CH_2Cl_2 (0.5 ml) was stirred under Ar for 4 h. The solution was filtered, the filtrate concentrated to dryness (rotary evaporation), and the residue redissolved in CH_2Cl_2. The CH_2Cl_2 solution was chromatographed on Si gel (Pasteur pipette column) and eluted with hexane-EtOAc (4:1→1:1) (5 ml each) and finally EtOAc (5 ml). The bastadin 8α-methoxyphenylacetate [**9**], contaminated by 1,3-dicyclohexylurea, eluted with the 1:1 fractions. Normal-phase hplc (45% EtOAc in hexane; 1.5 ml/min) gave pure **9** as a colorless solid (5.7 mg, 93%): $[\alpha]^{26}D$ +19.0° (*c*=0.57, CH_2Cl_2); uv (CH_2Cl_2) λ max (log ε) 233 (4.50), 275 sh (3.78) nm; ir ν max (NaCl, film) 3408, 2936, 1751, 1674, 1522, 1487, 1454, 1418, 1246, 1045, 999 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 2.73 (2H, m, H_2-20), 3.40 (3H, s, OCH_3-2')[3], 3.50 (4H, m, H_2-5, H_2-21), 3.61 (2H, br s, H_2-1), 3.70 (3H, s, OCH_3-34), 3.75 (1H, d, *J*=13.2 Hz, H-25a), 3.80 (1H, d, *J*=13.2 Hz, H-25b), 3.94 (3H, s, OCH_3-15)[3], 4.00 (3H, s, $NOCH_3$-24)[3], 4.01 (3H, s, $NOCH_3$-2)[3], 4.81 (1H, s, H-2'), 5.79 (1H, dd, *J*=6.1 and 6.1 Hz, H-6), 6.30 (1H, d, *J*=1.9 Hz, H-38), 6.47 (1H, t, *J*=6.4 Hz, NH-4), 6.71 (1H, d, *J*=2.0 Hz, H-19), 6.77 (1H, t, *J*=6.4 Hz, NH-22), 6.89 (1H, d, *J*=8.4 Hz, H-11), 7.12 (1H, d, *J*=1.9 Hz, H-36), 7.17 (1H, d, *J*=2.0 Hz, H-17), 7.20 (1H, dd, *J*=2.0 and 8.4 Hz, H-12), 7.35 (3H, m, H-5', H-6', H-7'), 7.42 (2H, m, H-4', H-8'), 7.49 (1H, d, *J*=2.0 Hz, H-8), 7.52 (2H, s, H-27, H-31); fabms (3-NBA/NaI) *m/z* $[M+Na]^+$ 1257/1259/1261/1263/1265/1267; hrfabms *m/z* 1256.8768 ($C_{47}H_{43}N_4O_{11}{}^{79}Br_5$ Na, calcd 1256.8742).

6(S)-[α-(R)-Methoxyphenylacetyl]-2,15,24,34-tetramethoxy-bastadin 8 [**10**].—The preceding acetylation (cf. **9**) was repeated with bastadin 8 tetramethyl ether [**7**], (12.4 mg), DMAP (10 mg), DCCI (10 mg), and (*R*)-MPA (13 mg) in dry CH_2Cl_2 (0.5 ml). Isolation (chromatography) of the product gave acetate **10** as a colorless solid (11.7 mg, 83%): $[\alpha]^{27}D$ +3.3° (*c*=1.10, CH_2Cl_2); uv (CH_2Cl_2) λ max (log ε) 232 (4.51), 275 sh (3.64) nm; ir ν max (NaCl, film) 3408, 2938, 1755, 1674, 1522, 1487, 1454, 1418, 1246, 1045, 999 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 2.71 (2H, m, H_2-20), 3.41 (3H, s, OCH_3-2'), 3.48 (2H, m, H_2-21), 3.52 (1H, m, 5-Ha/H-5b), 3.65 (3H, s, OCH_3-34), 3.66 (1H, m, 5-Hb), 3.67 (2H, br s, H_2-1), 3.77 (1H, d, *J*=12.6 Hz, H-25a), 3.82 (1H, d, *J*=12.6 Hz, H-25b), 3.93 (3H, s, OCH_3-15)[3], 4.00 (3H, s, $NOCH_3$-24)[3], 4.02 (3H, s, $NOCH_3$-2)[3], 4.82 (1H, s, H-2'), 5.78 (1H, dd, *J*=5.6 and 5.6 Hz, H-6), 6.30 (1H, d, *J*=1.9 Hz, H-38), 6.59 (1H, t, *J*=6.4 Hz, NH-4), 6.64 (1H, d, *J*=2.0 Hz, H-19), 6.77 (1H, t, *J*=6.4 Hz, NH-22), 6.79 (1H, d, *J*=8.4 Hz, H-11), 6.94 (1H, dd, *J*=2.0 and 8.4 Hz, H-12), 7.12 (1H, d, *J*=2.0 Hz, H-8), 7.15 (1H, d, *J*=2.0 Hz, H-17), 7.17 (1H, d, *J*=2.0 Hz, H-36), 7.35 (5H, br s, H-4' to H-8'), 7.52 (2H, s, H-27, H-31); fabms (3-NBA/NaI) *m/z* $[M+Na]^+$ 1257/1259/1261/1263/1265/1267; hrfabms *m/z* 1256.8757 ($C_{47}H_{43}N_4O_{11}{}^{79}Br_5$ Na, calcd 1256.874).

6-(S)-[α-(S)-Methoxyphenylacetyl]-2,15,24,34-tetramethoxy-bastadin 10 [**5**].—The general esterification (see **9**) reaction was repeated with a mixture of bastadin 10 tetramethyl ether [**4**] (4.0 mg), DMAP (5

[3]Assignments with identical superscripts may be interchanged.

mg), DCCI (8 mg), and (*S*)-MPA (4 mg) in dry CH_2Cl_2 (0.5 ml). Chromatographic separation of the products led to acetate **5** as a colorless solid (4.0 mg, 97%): $[\alpha]^{26}D$ +9.0° (*c*=0.40, CH_2Cl_2); uv (CH_2Cl_2) λ max (log ε) 233 (4.55), 275 sh (3.82) nm; ir ν max (NaCl, film) 3408, 2936, 1751, 1674, 1522, 1487, 1454, 1417, 1285, 1236, 1045 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 2.67 (2H, m, H_2-20), 3.41 (3H, s, OCH_3-2′), 3.44 (1H, m, H-5a), 3.49 (2H, m, H_2-21), 3.60 (1H, m, H-5b), 3.68 (1H, d, *J*=13.0 Hz, H-1a), 3.73 (1H, d, *J*=13.0 Hz, H-1b), 3.74 (1H, d, *J*=13.5 Hz, H-25a), 3.82 (1H, d, *J*=13.5 Hz, H-25b), 3.83 (3H, s, OCH_3-34)[3], 3.87 (3H, s, $NOCH_3$-24)[3], 3.94 (3H, s, OCH_3-15)[3], 4.00 (3H, s, $NOCH_3$-2)[3], 4.82 (1H, s, H-2′), 5.79 (1H, dd, *J*=4.6 and 7.6 Hz, H-6), 6.50 (1H, d, *J*=2.0 Hz, H-19), 6.55 (1H, t, *J*=6.6 Hz, NH-4), 6.60 (1H, d, *J*=8.4 Hz, H-30), 6.75 (1H, d, *J*=1.9 Hz, H-38), 6.76 (1H, t, *J*=6.6 Hz, NH-22), 6.88 (1H, d, *J*=8.4 Hz, H-11), 7.10 (1H, dd, *J*=2.0 and 8.4 Hz, H-31), 7.12 (1H, d, *J*=2.0 Hz, H-17), 7.19 (1H, dd, *J*=2.0 and 8.4 Hz, H-12), 7.24 (1H, d, *J*=1.9 Hz, H-36), 7.35 (3H, m, H-5′, H-6′, H-7′), 7.44 (2H, m, H-4′, H-8′), 7.50 (1H, d, *J*=2.0 Hz, H-27), 7.50 (1H, d, *J*=1.9 Hz, H-8); fabms (3-NBA/NaI) *m/z* $[M+Na]^-$ 1179/1181/1183/1185/1187; hrfabms *m/z* 1178.9650 ($C_{47}H_{44}N_4O_{11}{}^{79}Br_5$ Na, calcd 1178.9647).

6(S)-[α-(R)-*Methoxyphenylacetyl*]-*2,15,24,34-tetramethoxy-bastadin 10* [**6**].—The preceding esterification was repeated with bastadin 10 tetramethyl ether [**4**] (5.1 mg), DMAP (8 mg), DCCI (10 mg), and (*R*)-MPA (5 mg) in dry CH_2Cl_2 (0.5 ml). Chromatographic separation afforded ester **6** as a colorless solid (4.2 mg, 72%): $[\alpha]^{26}D$ −6.9° (*c*=0.42, CH_2Cl_2); uv (CH_2Cl_2) λ max (log ε) 230 (4.52), 275 sh (3.68) nm; ir ν max (NaCl, film) 3406, 2935, 1755, 1674, 1522, 1485, 1418, 1236, 1045 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 2.65 (2H, m, H_2-20), 3.42 (3H, s, OCH_3-2′), 3.48 (2H, m, H_2-21), 3.51 (1H, m, H-5a), 3.72 (1H, m, H-5b), 3.75 (2H, m, H_2-1), 3.78 (2H, m, H_2-25), 3.79 (3H, s, OCH_3-34),[3] 3.88 (3H, s, $NOCH_3$-24)[3], 3.93 (3H, s, OCH_3-15)[3], 4.01 (3H, s, $NOCH_3$-2)[3], 4.83 (1H, s, H-2′), 5.77 (1H, dd, *J*=4.6 and 6.7 Hz, H-6), 6.46 (1H, d, *J*=2.0 Hz, H-19), 6.63 (1H, d, *J*=8.4 Hz, H-30), 6.69 (1H, t, *J*=6.6 Hz, NH-4), 6.73 (1H, d, *J*=1.9 Hz, H-38), 6.77 (1H, t, *J*=6.6 Hz, NH-22), 6.78 (1H, d, *J*=8.4 Hz, H-11), 6.94 (1H, dd, *J*=2.0 and 8.4 Hz, H-12), 7.11 (1H, d, *J*=2.0 Hz, H-17), 7.12 (1H, dd, *J*=2.0 and 8.4 Hz, H-31), 7.16 (1H, d, *J*=1.9 Hz, H-8), 7.27 (1H, d, *J*=1.9 Hz, H-36), 7.35 (5H, br s, H-4′ to H-8′), 7.50 (1H, d, *J*=2.0 Hz, H-27); fabms (3-NBA/NaI) *m/z* $[M+Na]^-$ 1179/1181/1183/1185/1187; hrfabms *m/z* 1178.9596 ($C_{47}H_{44}N_4O_{11}{}^{79}Br_4$ Na, calcd 1178.9637).

6(S)-[α-(S)-*Methoxyphenylacetyl*]-*2,15,24,34-tetramethoxy-bastadin 12* [**11**].—A mixture of bastadin 12 tetramethyl ether [**8**] (1.8 mg), DMAP (5 mg), DCCI (8 mg), and (*S*)-MPA (3 mg) in dry CH_2Cl_2 (0.5 ml) was allowed to react as summarized above (refer to **6**). Separation by normal-phase hplc (hexane-EtOAc, 3:2, 1.5 ml/min) gave ester **11** as a colorless solid (1.0 mg, 48%): $[\alpha]^{26}D$ +16° (*c*=0.10, CH_2Cl_2); uv (CH_2Cl_2) λ max (log ε) 233 (4.55), 275 (sh) (3.76) nm; ir ν max (NaCl, film) 3400, 2932, 1746, 1672, 1524, 1487, 1454, 1418, 1273, 1236, 1045 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 2.73 (2H, m, H_2-20), 3.35 (2H, m, H_2-21), 3.42 (3H, s, OCH_3-2′), 3.48 (1H, m, H-5a), 3.68 (1H, m, H-5b), 3.75 (4H, m, H_2-1, H_2-25), 3.86 (3H, s, OCH_3-15), 3.89 (3H, s, OCH_3-34)[3], 4.01 (3H, s, $NOCH_3$-24)[3], 4.02 (3H, s, $NOCH_3$-2)[3], 4.84 (1H, s, H-2′), 5.78 (1H, dd, *J*=3.9 and 8.6 Hz, H-6), 6.07 (1H, d, *J*=1.9 Hz, H-19), 6.53 (1H, d, *J*=8.4 Hz, H-30), 6.66 (1H, t, *J*=6.0 Hz, NH-4), 6.73 (1H, t, *J*=6.0 Hz, NH-22), 6.76 (1H, d, *J*=2.0 Hz, H-38), 7.06 (1H, d, *J*=1.9 Hz, H-17), 7.09 (1H, dd, *J*=2.0 and 8.4 Hz, H-31), 7.27 (1H, d, *J*=2.0 Hz, H-36), 7.29–7.73 (5H, m, H-4′ to H-8′), 7.48 (1H, d, *J*=2.0 Hz, H-27); fabms (3-NBA/NaI) *m/z* $[M+Na]^+$ 1257/1259/1261/1263/1265/1267; hrfabms *m/z* 1256.8729 ($C_{47}H_{43}N_4O_{11}{}^{79}Br_5$, calcd 1256.8742).

6(S)-[α-(R)-*Methoxyphenylacetyl*]-*2,15,24,34-tetramethoxy-bastadin 12* [**12**].—The acetate **11** sequence was repeated with bastadin 12 tetramethyl ether [**8**] (4.8 mg), DMAP (5 mg), DCCI (8 mg), and (*R*)-MPA (5 mg) in dry CH_2Cl_2 (0.5 ml). Chromatographic separation (cf. **9**) gave ester **12** as a colorless solid (4.7 mg, 86%): $[\alpha]^{27}D$ −4.3° (*c*=0.47, CH_2Cl_2); uv (CH_2Cl_2) λ max (log ε) 233 (4.53), 275 (sh) (3.73) nm; ir ν max (NaCl, film) 3400, 2936, 1757, 1674, 1524, 1487, 1454, 1418, 1271, 1236, 1045 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 2.73 (2H, m, H_2-20), 3.35 (2H, m, H_2-21), 3.40 (3H, s, OCH_3-2′), 3.50 (1H, m, H-5a), 3.70 (3H, s, OCH_3-15), 3.78 (1H, m, H-5b), 3.80 (4H, m, H_2-1, H_2-25), 3.94 (3H, s, OCH_3-34)[3], 4.00 (3H, s, $NOCH_3$-24)[3], 4.01 (3H, s, $NOCH_3$-2)[3], 4.81 (1H, s, H-2′), 5.77 (1H, dd, *J*=3.9 and 7.6 Hz, H-6), 6.03 (1H, d, *J*=1.9 Hz, H-19), 6.57 (1H, d, *J*=8.4 Hz, H-30), 6.74 (1H, d, *J*=2.0 Hz, H-38), 6.76 (1H, t, *J*=6.0 Hz, NH-22), 6.80 (1H, t, *J*=6.0 Hz, NH-4), 7.05 (1H, d, *J*=1.9 Hz, H-17), 7.10 (1H, dd, *J*=2.0 and 8.4 Hz, H-31), 7.30 (1H, d, *J*=2.0 Hz, H-36), 7.37 (5H, br s, H-4′ to H-8′), 7.48 (1H, d, *J*=2.0 Hz, H-27); fabms (3-NBA/NaI) *m/z* $[M+Na]^+$ 1257/1259/1261/1263/1265/1267; hrfabms *m/z* 1256.8745 ($C_{47}H_{43}N_4O_{11}{}^{79}Br_5$ Na, calcd 1256.8742).

ANTIMICROBIAL SUSCEPTIBILITY TESTING.—Antimicrobial disk susceptibility tests were performed according to the method established by the National Committee for Clinical Laboratory Standards (26). Mueller-Hinton agar was used for susceptibility testing of *Staphylococcus aureus* (ATCC #29213), *Enterococcus faecalis* (ATCC #29212), and *Escherichia coli* (ATCC #25922); Gonococcal Typing agar for *Neisseria gonorrhoeae* (ATCC #49226); and YM agar for *Candida albicans* (ATCC #90028) and *Cryptococcus neoformans* (ATCC #90112). Bastadins 8, 10 and 12 were reconstituted in sterile DMSO, and twofold dilutions applied

to sterile 6-mm disks. Zones of inhibition were recorded after 16 h for bacterial cultures and 42 h for fungal cultures. Results are the average of two experiments. Due to a paucity of bastadin 8, the absolute mic could not be determined.

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