J. Nat. Prod. **1996,** *59,* 1121−1127

Bastadin 20 and Bastadin *O*-Sulfate Esters from *Ianthella basta*: Novel Modulators of the Ry_1R FKBP12 Receptor Complex

Melanie A. Franklin,† Sharron G. Penn,† Carlito B. Lebrilla,† Tien H. Lam,‡ Isaac N. Pessah,‡ and Tadeusz F. Molinski*,†

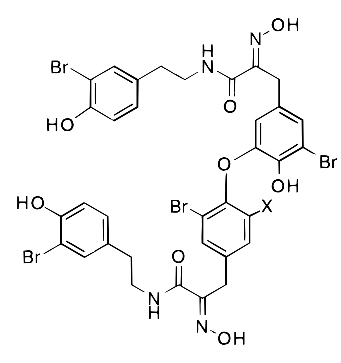
Departments of Chemistry and Molecular Biosciences, University of California, Davis, California 95616

Received June 7, 1996⊗

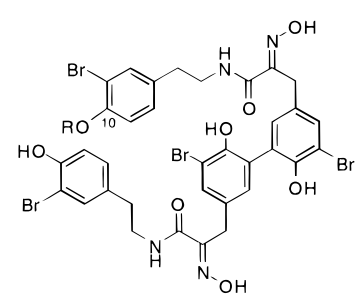
New compounds bastadin 20 (**9**), 15,34-*O*-disulfatobastadin 7 (**10**), and 10-*O*-sulfatobastadin 3 (**11**) were isolated from *Ianthella basta* collected in Exmouth Gulf, Western Australia. Compounds **10** and **11** exhibited moderate differential activity as SR Ca^{2+} channel agonists (EC_{50} 13.6 and 100 μM, respectively) of the Ry_1R FKBP12 complex, while the potency of **9** was almost half that of **10** (EC_{50} 20.6 μM). The problem of dereplication of bastadins was addressed using ^{1}H-NMR "fingerprinting" of MeO signals in the corresponding permethyl bastadin derivatives.

The bastadins are a family of highly modified tetrapeptides occurring in the marine sponges *Ianthella* spp.[1] and *Psammaplysilla purpurea*.[2] The first seven members of the series were described by Kazlauskus *et al*. in 1981;[1] however, since then more than a dozen analogues have been reported.[2−8] Most bastadins are macrocyclic. The structure of the bastadin macrocycle can be dissected into a heterodimer of two "hemibastadin" units. Hemibastadins, in turn, are composed of a brominated tyramine linked to a brominated dehydrotyrosine (α-oximino group) by an amide bond. Two units of hemibastadins constitute the "northern" and "southern" hemispheres of the macrocycle and are bonded through catechol ether linkages, formed by phenolic coupling of the *p*-hydroxyl groups. The most common macrocyclic bastadin carbon skeleton is 13,32-dioxa-4,-22-diazabastarane[1] (abbreviated in this paper as "bastarane").

Bastadins 1−7 (**1**−**7**) exhibited moderate antibacterial activity, while other bastadins have been shown to have in vitro cytotoxic activity against human tumor cell lines[2,3] or antiinflammatory activity (Chart 1).[3] We, have recently shown[8] that bastadin 5 (**5**) induces a large release of Ca^{2+} ions (EC_{50} 2 μM) from Ca^{2+} stores within the sarcoplasmic reticulum (SR) of fast-twitch skeletal muscle through the SR Ca^{2+} channel, a >2000 kDa tetrameric transmembrane protein that gates Ca^{2+} in response to excitation. The conformational changes induced in the Ca^{2+} channel by binding of bastadin 5 and release of Ca^{2+} can be monitored by concomitant binding of the plant alkaloid ryanodine to the Ca^{2+} channel-receptor complex (Ry_1R). Competition experiments have shown that **5** does not bind to the known effector sites on Ry_1R (caffeine, ATP, Ca^{2+}, Mg^{2+}).[8] Instead, **5** appears to be involved with the site that binds another component, FKBP12, a small polypeptide (12 kDa) that is better known as an important link in the intracellular signal transduction cascade leading to interleukin-2-induced T-lymphocyte recruitment.[9−11] Binding of the immunosuppressant drug FK506 to FKBP12 blocks calcineurin-dependent induced T-cell activation. Collins[12] and others[13,14] have shown that FKBP12 is also a constitutive, functional component of the Ry_1R complex and, more recently, the IP_3 dependent Ca^{2+} channel located in the endoplasmic reticulum (ER) within the cytosol.[15,16] The detailed relationship of the FKBP12 binding site to the bastadin 5 binding site in Ry_1R is not yet known. The importance of FKBP12 association with the Ry_1R Ca^{2+} channel complex and the remarkable activity of bastadin 5 prompted us to investigate the Ca^{2+} channel modulatory activity of other constituents of *I. basta*. In this paper we report the isolation of two new analogues from *I. basta*—bastadin 19 (9-debromobastadin 13, **8**),[8] bastadin 20 (**9**), and the novel sulfate half-esters 15,34-*O*-bis-sulfatobastadin 7 (**10**) and 10-*O*-sulfatobastadin 3 (**11**). The structures were determined by analysis of ^{1}H- and ^{13}C-NMR spectra, 2D-NMR spectra, matrix assisted laser desorption ionization Fourier transform mass spectrometry (MALDI FTMS) and chemical correlation. Compounds **8**−**10** show varying activity as agonists of the SR Ca^{2+} channel but are less active than **5**.



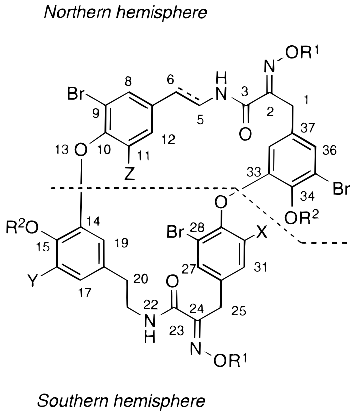
1 Bastadin 1 X = H,
2 Bastadin 2 X = Br

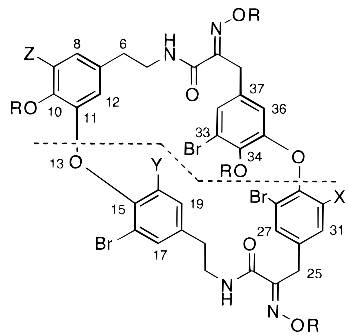


3 Bastadin-3 R = H,
11 10-sulfatobastadin-3 R = SO_3Na

* To whom correspondence should be addressed. Phone: (916) 752-6358. FAX: (916) 752-8995. E-mail: tfmolinski@ucdavis.edu.
† Department of Chemistry.
‡ Department of Molecular Biosciences.
⊗ Abstract published in *Advance ACS Abstracts*, November 15, 1996.

Chart 1





		R^1	R^2	X	Y	Z	C5,6
4	Bastadin 4	H	H	Br	Br	H	Δ
5	Bastadin 5	H	H	Br	Br	H	-
17	Bastadin 4 tetra-*O*-Me	Me	Me	Br	Br	H	Δ
15	Bastadin 5 tetra-*O*-Me	Me	Me	Br	Br	H	-
6	Bastadin 6	H	H	Br	Br	Br	-
18	Bastadin 6 tetra-*O*-Me	Me	Me	Br	Br	Br	-
7	Bastadin 7	H	H	H	Br	H	Δ
10	15,34-*O*-disulfatobastadin 7	H	SO_3Na	H	Br	H	Δ
16	Bastadin 15 tetra-*O*-Me	Me	Me	H	Br	Br	-
12	Bastadin 18	H	H	H	Br	Br	-
21	Bastadin 14	H	H	H	Br	Br	Δ
22	Bastadin 14 tetra-*O*-Me	Me	Me	H	Br	Br	Δ

		R	X	Y	Z
8	Bastadin 19	H	Br	H	Br
13	Bastadin 19 tetra-*O*-Me	Me	Br	H	Br
9	Bastadin 20	H	H	H	Br
14	Bastadin 20 tetra-*O*-Me	Me	H	H	Br
19	'*iso*-Bastadin 6'	H	Br	Br	Br
20	'*iso*-Bastadin 6' tetra-*O*-Me	Me	Br	Br	Br

Results and Discussion

A sample of *Ianthella basta* Pallas 1766 (Ianthellidae), collected in Exmouth Gulf, Western Australia, was lyophilized and exhaustively extracted with MeOH. The solvent extract was separated with monitoring of Ry_1R binding activity.[8] Partitioning of the MeOH extract against solvents of increasing polarity concentrated most of the activity in the *n*-BuOH fraction. The active fraction was separated by sequential chromatography using gel-permeation (Sephadex LH20, MeOH elution), Si gel flash chromatography (20−100% MeOH/$CHCl_3$) and finally reversed-phase HPLC (60−80% MeOH/H_2O) to give new compounds bastadin 20 (**9**), 15,34-*O*-bis-sulfatobastadin 7 (**10**), and 10-*O*-sulfatobastadin 3 (**11**) in addition to known analogues bastadins 3−7 (**3**−**7**),[1] 10,[3] 18 (**12**),[7] and 19 (**8**). The known compounds were identified by comparison of ^{1}H-NMR, ^{13}C-NMR, and MS data with literature values, while the assignment of structure **8** appears in our preceding paper.[8]

Bastadin 20 (**9**, 28-debromobastadin 19) gave a FABMS with extensive fragmentation and low intensity parent ions peaks. Accurate mass measurement of **9** was further complicated by multiple isotope peaks of the parent ion due to the presence of four Br atoms. We had previously employed MALDI FTMS to mitigate these problems and provide parent ion mass spectra with little fragmentation, good signal-to-noise, and reliable accurate mass determination of the lowest mass parent isotopomers peak.[17] MALDI FTMS spectrum of **9** (Figure 1) gave a strong sodiated parent ion (M + Na$^+$, *m/z* 958.8588, Δmmu 5) leading to a formula $C_{34}H_{28}Br_4N_4O_8$, which is isomeric with bastadins 9, 13, and 18.

The ^{1}H-NMR data for **9** did not match that of bastadin 9 and 13, but were similar to those of bastadin 18 (**12**). The HPLC retention times of **9** and **12**,[18] however, were very different and suggested a novel structure for the latter. Analysis of the ^{1}H-NMR, COSY, and HMQC

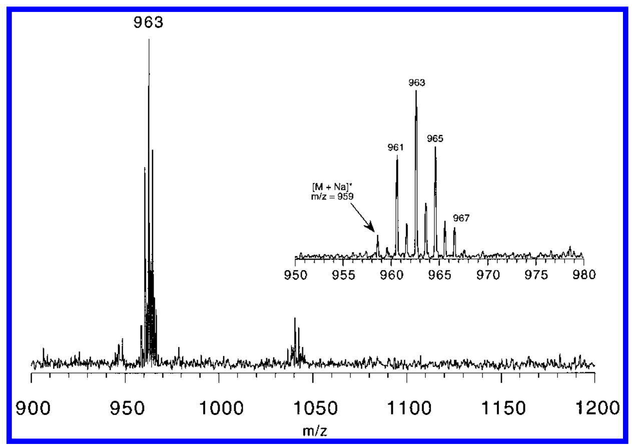


Figure 1. MALDI FTMS of bastadin 20 (**9**) with a matrix of 2,5-dihydroxybenzoic acid (0.3 M in EtOH). Insert shows M + Na$^+$ parent ion. Other conditions are reported elsewhere.[17] Accurate mass determination (*m/z* 958.8588, Δmmu 5) was made from another measurement (not shown) with gramicidin S (MH$^+$ *m/z* 1141.7138) as internal calibrant.

spectra of **9** (Table 1) allowed the identification of four ^{1}H spin networks belonging to two pairs of similar 2,4-disubstituted phenolic ether and 2,4-disubstituted-catechol ether groups by analysis of *ortho* ($\sim J$ = 8.5 Hz) and *meta* ($\sim J$ = 2 Hz) ^{1}H spin couplings. In addition, **9** contained two ethylamido side chains and two α-hydroximino carboxamides (CH_2 groups, C1 δ 3.63, s, 2H, 28.1, t; 3.88, s, 2H, 28.3, t). Upfield aryl protons (δ ~ 6.5−6.8 ppm) were assigned to positions *ortho* or *para* to phenoxyl oxygens, and the four bromines were placed at remaining substitution sites. This immediately eliminated isomers with the 2,6-dibromophenoxy group found in bastadins 9 and 13; however, the identity of the carbon side chains on the benzene rings were not yet apparent. An HMBC spectrum of **9** (optimized for J_{CH} = 8 Hz) showed self-consistent three-bond correlations ($^3J_{CH}$) from benzylic ^{13}C signals (C1, C6, C20, C25) to the respective *ortho* aryl proton signals (see Table

Table 1. NMR Data (d_6-DMSO) for Bastadin 20 (**9**), 15,34-*O*-Disulfatobastadin 7 (**10**), and Bastadin 7 (**7**)[a]

atom	^{13}C NMR (δ(**9**)[b]	^{1}H NMR (**9**) δ (mult., *J* Hz, Int.)[b]	COSY (**9**)[b]	HMBC (**9**)[b]	^{13}C NMR δ (**10**)	^{1}H NMR (**10**) δ (mult., *J* Hz, int.)[g]	^{13}C NMR δ (**7**)	^{1}H NMR (**7**) δ (mult., *J* Hz, int)
1	28.1	3.63 (s, 2H)	H36	H36, H38	27.8	3.74 (s, 2H)	27.5	3.71 (s, 2H)
2	151.3			H1, N^{2}OH	151.2[e]		150.5	
3	162.9			H1, H5	161.2		161.3	
4		6.75 (t, $J = 6$, 1H)	H5			10.26 (d, $J = 10$, 1H)		10.29 (d, $J = 10$, 1H)
5	40.4	3.39 (m, 2H)	H4, H6	H6	124.1	7.32 (dd, $J = 10, 14$, 1H)	124.3	7.34 (dd, $J = 10, 15$, 1H)
6	34.4	2.62 (t, $J = 6.5$, 1H)	H5	H5, H8, H12	111.4	6.40 (d, $J = 14$, 1H)	111.1	6.42 (d, $J = 15$, 1H)
7	131.6			H5, H6	134.6		135.0	
8	127.5	7.06 (d, $J = 2$, 1H)	H12	H6, H12	129.6	7.62 (d, $J = 2$, 1H)	129.9	7.67 (d, $J = 1.9$, 1H)
9	110.3			H8	114.9		114.7	
10	144.7			H8, H12	151.3[e]		150.8	
11	143.2			H12	122.7	7.01 (d, $J = 8.5$, 1H)	121.9	6.99 (d, $J = 8.8$, 1H)
12	117.2	6.44 (d, $J = 2$, 1H)	H8	H6, H8	125.6	7.42 (dd, $J = 8.5, 2$ 1H)	125.4	7.45 (dd, $J = 8.8, 1.9$, 1H)
14	151.4			H16, H18, H19	151.4		145.7	
15	114.1			H16, H19	139.6		142.9	
16	133.7	7.41 (d, $J = 2$, 1H)	H18	H18, H20	118.8		110.4	
17	136.8			H19, H20	127.2	7.12 (d, $J = 2$, 1H)	127.3	7.20 (d, $J = 2$, 1H)[f]
18	129.3[c]	7.06 (dd, $J = 2, 8$, 1H)	H16, H19	H16, H20	137.1		131.5	
19	120.4	6.84 (d, $J = 8$, 1H)	H18		116.6	6.39 (d, $J = 2$, 1H)	116.0	6.43 (d, $J = 2$, 1H)[f]
20	34.6	2.77 (t, $J = 6.5$, 2H)	H21	H16, H21	33.0	2.71 (t, $J = 6$, 2H)	33.1	2.64 (t, $J = 6$, 2H)
21	40.2	3.54 (m, 2H)	H20, H22	H20	38.7	3.27 (m, 2H)	38.7	3.24 (m, 2H)
22		6.97 (t, $J = 6$, 1H)	H21			7.85 (t, $J = 6.0$, 1H)		
23	163.4			H21, H25	163.2		163.2	
24	151.9			H25, N^{24}OH	151.0		150.9	
25	28.3	3.88 (s, 2H)	H27	H27	29.0	3.43 (s, 2H)	28.8	3.50 (s, 2H)
26	134.5			H30	133.9		134.4	
27	134.2	7.54 (d, $J = 2$, 1H)	H25, H31	H31, H25	132.6	7.38 (d, $J = 2$, 1H)	133.4	7.44 (d, $J = 1.9$, 1H)
28	114.1			H27, H30	112.8		112.9	
29	151.1			H27, H30, H31	151.5		151.5	
30	120.2	6.83 (d, $J = 8.5$, 1H)	H31		120.2	6.70 (d, $J = 8.5$, 1H)	119.2	6.66 (d, $J = 8.3$, 1H)
31	129.3[c]	7.13 (dd, $J = 2, 8.5$, 1H)	H27, H30	H27, H25	129.8	7.02 (dd, $J = 2, 8.5$, 1H)	130.2	7.10 (dd, $J = 8.3, 1.9$, 1H)
33	142.8			H38	151.4		145.0	
34	144.6			H36, H38	140.5		143.6	
35	109.8			H36	119.0		110.8	
36	128.1	7.20 (d, $J = 2$, 1H)	H1, H38	H1, H38	128.1	7.23 (d, $J = 2$, 1H)	128.2	7.09 (d, $J = 1.5$, 1H)
37	129.3[c]			H1	133.6		128.5	
38	117.8	6.53 (d, $J = 2$, 1H)	H36	H1, H36	117.9	6.40 (d, $J = 2$, 1H)	117.3	6.40 (d, $J = 1.5$, 1H)
N^{2}OH		10.89 (s, 1H)				12.13 (s, 1H)		11.99 (s, 1H)
N^{24}OH		11.24 (s, 1H)				11.94 (s, 1H)		12.09 (s, 1H)
10-OH[d]	6.70	(br s)						9.89 (s, 2H)
34-OH[d]	7.65	(br s)						9.89 (s, 2H)

[a] ^{13}C multiplicities confirmed by DEPT and HMQC. [b] $CDCl_3$ with drop of d_6-DMSO, COSY (300 MHz), ^{13}C NMR (100 MHz); ^{1}H NMR, HMQC, and HMBC (500 MHz). [c] Overlap. [d,e] Interchangeable. [f] Assignments revised from Kazlauskus et al. (1) based on HMQC, HMBC, and COSY experiments.

1). COSY analysis provided sequential homonuclear vicinal coupling assignments along the phenylethylamido chains (NH22 to H20 and NH4 to H6) and weak benzylic couplings ($J < 1$ Hz) from CH_2 singlets at C1 and C25 to the *ortho* aryl ring protons H36,38 and H27, 31, respectively. The foregoing data unambiguously secured the complete substitution patterns around the four phenyl rings. Finally, the presence of four broad exchangeable singlets in the ^{1}H-NMR spectrum ($CDCl_3$; d_6-DMSO) and conversion of **9** to tetra-*O*-methyl ether **13** (CH_3I, K_2CO_3, DMF, silica HPLC purification, 1:1 EtOAc/*n*-hexane, MALDI FTMS *m/z* 1014.9143, Δmmu 2.1; δ 3.61, s; 3.91, s, 4.01, s; 4.04, s, 4 × OMe) provided evidence for two phenolic and two oxime OH groups.

Bastadin 20 (**9**) appeared to have aryl ring substitution patterns identical to **12**, so it only remained for us to establish the positions of the C−O phenolic ether couplings. This we did by linking the substituted aryl rings on each side of side-chains within each of the northern and southern hemispheres by heteronuclear correlation of the ethylamido groups through the respective amide carbonyls (δ 162.9, s, C3; δ 163.4, C23). HMBC correlations were observed in the northern hemisphere between the ethylamido group proton signal H5 (δ 3.39, m, 2H) and the carboxyl group identified as C3 (δ 162.9, s, C═O), which was further correlated to the singlet at H1 (δ 3.63, s, 2H). Similarly, the southern hemisphere components were linked by correlations from H21 (δ 3.54, m, 2H) and H25 (δ 3.63, s, 2H) to the carboxyl group C23 (δ 163.4, s). Because the foregoing analysis of **9** gave a structure with two catechol units in the same hemisphere as opposed to the more common catechol−phenol hemisphere, bastadin 20 (**9**) has the isomeric isobastarane skeleton. The only other bastadins with this isomeric ring closure are bastadin 19,[8] bastadin 13 (originally named bastadin 12[2,4]), 32-*O*-sulfatobastadin 13,[6] and a synthetic hexabromo "isobastadin 6".[19,20]

15,34-*O*-Disulfatobastadin 7 (**10**) and 10-*O*-sulfatobastadin 3 (**11**) were each isolated from late-eluting Sephadex LH20 fractions and obtained pure by elution through a reversed-phase cartridge with 70% MeOH/H_2O.[21] ^{1}H-NMR data of **10** were almost identical to those of bastadin 7 (**7**). The presence of mutually coupled signals due to a disubstituted double bond (δ 6.40, d, $J = 14$ Hz, H6; 7.32, dd, 1H, $J = 14, 10$ Hz, H5) suggested that **10** contained the *E* Δ^5 enamide also present in bastadins 4, 7, 11, and 14. MALDI of **10** gave a parent ion corresponding to $M + K_3^+$ (*m/z* 1208.6481, Δmmu 10.6) implying a formula of $C_{34}H_{24}Br_4N_4O_{14}S_2Na_2$ for native **10**,[21] and sulfation of two phenolic or oxime OH's [Ar − $(OSO_3Na)_2$]. A sample of compound **10** was hydrolyzed (2 M HCl aqueous MeOH, 50 °C, 30 min) and the product partitioned into H_2O-soluble and

EtOAc-soluble material. The EtOAc-soluble material gave a single peak on HPLC (C18 reversed-phase, 65:35 MeOH/H_2O) with a retention time and MALDI spectrum (M + Na^+ *m/z* 956.8382, $C_{34}H_{26}Br_4N_4O_8$, Δmmu 0) identical to that of authentic **7**. Upon addition of a drop of $BaCl_2$ (1 M aqueous) to the H_2O-soluble hydrolysate a fine white precipitate of $BaSO_4$ formed, confirming the presence of sulfate.

The OSO_3Na groups were located at C15 and C34 phenolic oxygens using the following arguments. Bastadin 7 has four exchangeable OH groups, but only two OH groups were observed in the ^{1}H-NMR spectrum of **10** (d_6-DMSO, δ 12.13, s; 11.94, s). These lowfield exchangeable signals are assigned to oxime OH's in the bastadin series[1,4] and are always seen downfield of OH's of bastadin phenolic OH's (δ ~ 9.5−10 ppm), suggesting that C15 and C34 OH's were sulfated. Direct comparison of the ^{13}C-NMR spectra of **10** and **7** (Table 1) showed upfield ^{13}C shifts for carbons *ipso* to the OSO_3Na group (C15, δ 139.6, Δδ −3.3 ppm; C34, 140.5, Δδ −3.1 ppm) and downfield shifts for carbons *ortho* and *para* to the substituent (C16, 118.8, Δδ +8.4; C14, 151.4, Δδ +5.7; C35, 119.0, Δδ +8.2; C33, 151.4, Δδ +6.4). These chemical shift changes are characteristic upon sulfation of phenolic OH as also seen in 32-*O*-sulfatobastadin 13.[6]

10-*O*-Sulfatobastadin 3 (**11**) was isolated as a colorless powder and gave a formula of $C_{34}H_{29}Br_4N_4O_{11}SNa$ corresponding to the sodium salt of a monosulfate half-ester (MALDI, M + Na_2^+, *m/z* 1062.8130, Δmmu 4.8). The conjugate acid of **11** has a formula that corresponds to the addition of the elements of SO_3 to bastadin 1 or the isomeric symmetrical C−C-coupled dimer bastadin 3 (**3**) ($C_{34}H_{30}Br_4N_4O_8$).[1] The ^{1}H-NMR spectrum of **11** (d_6-DMSO) suggested pseudosymmetry because two discrete, almost overlapping families of spins were evident. Upon standing at 30 °C for 24 h in d_6-DMSO $CDCl_3$ containing trace moisture, **11** spontaneously hydrolyzed, giving a compound with half the number of ^{1}H- and ^{13}C-NMR signals whose chemical shifts were identical to those of authentic **3**. The H_2O-soluble fraction of the hydrolysate from **11** contained SO_4^{2-} as shown by formation of a $BaSO_4$ precipitate upon addition of aqueous $BaCl_2$. Interpretation of HMQC, HMBC, and COSY NMR data of **11** corroborated the C−H connectivities and allowed assignment of most of the signals (see Table 2). As with disulfate **10**, compound **11** showed characteristic changes in ^{13}C-NMR chemical shifts that allowed placement of the sulfate group, this time at C10 (Table 2). Thus, **11** is the Na salt of 10-*O*-sulfatobastadin 3.

For purposes of characterization, bastadins 19 (**8**) and 20 (**9**) were exhaustively methylated (MeI, K_2CO_3, DMF) to afford the corresponding *O*-tetramethyl ethers **13** and **14**, respectively, which were readily distinguished from reported bastadin tetramethyl ethers (e.g., bastadin 5 *O*-tetramethyl ether, **15**[1] and bastadin 15 tetra-*O*-methyl ether, **16**[5]) by ^{1}H NMR (Table 3).

Table 2. NMR Data (d_6-DMSO) for 13-*O*-Sulfatobastadin 3 (**11**)[a]

position	^{13}C NMR δ	^{1}H NMR δ (mult., *J* Hz, int)	COSY	HMBC
1	27.8	3.69 (s, 2H)		H36, H38
2	152.0			H1, N2-OH
3[b]	163.1			H1, H4
4		7.89 (t, *J* = 5.5, 1H)	H5	
5[c]	39.9	3.30 (m, 2H)	H4, H6	H6
6	33.7	2.68 (t, *J* = 7, 1H)	H5	H12
7	135.4			H6, H11
8	132.3	7.36 (d, *J* = 2, 1H)	H12	H6, H12
9	113.8			H8, H11
10	148.9			H8, H11, H12
11	121.2	7.47 (d, *J* = 8.5, 1H)	H12	
12	128.6	7.09 (dd, *J* = 2, 8.5, 1H)	H8, H11	H6, H8
13				
14	109.0			H16, H19
15[d]	152.1			H19, H17
16	116.2	6.76 (d, *J* = 8, 1H)	H17	
17	128.7	6.88 (dd, *J* = 2, 8, 1H)	H16, H19	H19, H20
18	131.4			H16, H20
19	132.5	7.25 (d, *J* = 2, 1H)	H17	H20
20	33.5	2.62 (t, *J* = 7, 2H)	H21	
21[c]	39.8	3.30 (m, 2H)	H20, H22	H20
22		7.87 (t, *J* = 5.5, 2H)	H21	
23[b]	163.0			H22, H25
24	152.0			H25, N^{24}OH
25	27.8	3.69 (s, 2H)		H27, H31
26	131.4			H25, H31
27	132.3	7.22 (br s, 2H)	H31	H25
28[e]	112.4			H27
29[d]	152.2			H27, H31
30	128.2			H31
31	130.7	6.96 (d, *J* = 2, 1H)	H27	H25
32				
33	128.2			H38
34[d]	152.2			H36, H38
35[e]	112.3			H36
36	132.3	7.22 (br s, 2H)	H38	H1
37	131.4			H1, H38
38	130.7	6.97 (d, *J* = 2, 1H)	H36	H1
2-NOH[f]		11.75 (s, 1H)		
24-NOH[f]		11.69 (s, 1H)		
15-OH		10.00 (s, 1H)		
29-OH		8.90 (br s)		
34-OH		8.90 (br s)		

[a] COSY (300 MHz), ^{13}C (75 MHz), HMQC and HMBC (500 MHz); ^{13}C multiplicities were assigned from DEPT or HMQC spectra. [b,c,d,e,f] Assignments with same superscript are interchangeable.

Table 3. Selected ^{1}H-NMR Data of Bastadin Tetra-*O*-methyl Ethers[a]

number	parent bastadin	δ of MeO groups ($CDCl_3$)				reference
17	4	4.01	4.01	4.01	4.01	1, 4
15	5	4.02	3.98	3.94	3.52	1
18	6	4.06	4.03	4.02	3.61	1, 20
20	"isobastadin 6"	4.08	4.04	4.02	3.92	20
	8	4.02	4.01	3.97	3.70	3
	9	4.05	4.00	3.90	3.49	3
	11	4.01	4.01	3.93	3.92	3
	12	4.05	4.01	3.89	3.87	4
	13	4.04	4.01	3.81	3.48	23
22	14	4.10	4.05	4.03	3.83	2
16	15	4.03	4.01	3.89	3.73	5
13	19	4.04	4.01	3.91	3.61	8, this work
14	20	4.00	3.92	3.91	3.72	this work

[a] Bastadins 9, 12, and 13 have been renumbered from the original references. See comments by Scheuer *et al.*[2]

Discussion

Examination of bastadin structures evokes a common biogenesis beginning with the precursor 3,5-dibromotyrosine. Labeling experiments carried out by Rinehart and Carney[22] confirm incorporation of [^{14}C] 3,5-dibromotyrosine into metabolites from a Verongid sponge that are related to bastadins, while Jaspars and Crews[7] have elaborated a putative biosynthetic tree for the biosynthesis of bastadins from 3,5-dibromotyrosine. Structural modifications that give rise to different bastadins include variable Br content, hydroxylation at C6, introduction of Δ^5 and, less commonly, closure of the northern and southern hemispheres at alternative free phenolic OH groups to generate the isomeric isobastarane skeleton. Sulfation of one or more free phenolic OH groups generates a family of sulfate half-esters, but only three have been reported, including the new compounds **10** and **11** described here. With the prevalence of the 2,6-dibromophenyl ether moiety in several bastadin structures, we were mindful of the possibility of atropoisomerism, but to date this has not been observed in any cyclic bastadins at room temperature, although Miao and Andersen[4] report NMR evidence for restricted rotation in bastadin 12 (originally named bastadin 9) at −30 °C. Permutation of all of structural possibilities within a common tetrameric framework gives rise to a number of possible structures that exceeds the count of known bastadins.

No doubt, additional bastadins will be found that will fill the gaps in the current list; however, these new compounds will become increasingly difficult to identify and dereplicate. Bastadins are often spectroscopically similar to one another, but we have found that MALDI mass spectrometry provides a particular advantage in accurately determining molecular masses for formula determination of these highly brominated compounds.[17] The isomer problem, however, remains. Rapid determination of the presence of the bastarane or isobastarane skeleta has become important because we have found the Ca^{2+} channel activity of the bastadins is highly dependent upon this constitutional isomerism. Unfortunately, *^{1}H and ^{13}C NMR alone are insufficient to discriminate bastarane and isobastarane isomers*. For example, the structure of bastadin 5 (**5**), the most potent Ry_1R Ca^{2+} channel modulator among the natural bastadins tested to date (EC_{50} 2 μM) is almost impossible to distinguish from bastadin 19, the inactive isobastarane isomer of **5** ($EC_{50} > 100$ μM),[8] by ^{1}H or ^{13}C NMR alone without recourse to 2D heteronuclear experiments. A characteristic EIMS double fragmentation in cyclic bastadins that cleaves at the two amide bonds to provide “east−west” hemisphere fragment ions has been used to assign structure,[1,3,4] but this fragment ion is not always apparent. Because members of the isobastarane series may have widely different activities in the Ry_1R binding assay, *it is essential to identify accurately both known and new bastadin analogues* before any conclusions can be drawn from structure−activity relationship studies.

Microscale preparation of permethyl derivatives provides a partial solution to the problem by allowing “fingerprinting” of the MeO signals by ^{1}H NMR (see Table 3). Exhaustive methylation of a small sample of bastadin (1−10 mg), either by addition of excess ethereal CH_2N_2 or treatment with MeI and K_2CO_3 in dry DMF followed by HPLC purification, provides the nonpolar tetra-*O*-methyl ether in good-to-excellent yields. Permethylation is easily verified by ^{1}H NMR (presence of four MeO signals), while the chemical shift pattern of the MeO signals, measured in $CDCl_3$, can be matched reliably against those of previously reported bastadin tetra-*O*-methyl ethers. For example, the ^{1}H-NMR spectra of three isomers—bastadin 5, bastadin 15,[5] and bastadin 19—are very similar in CD_3OD or d_6-DMSO; however, the ^{1}H NMR MeO signals of the corresponding tetramethyl ethers **15**,[1] **16**[5], and **13** are readily distinguishable in $CDCl_3$ (see Table 3).

Specific assignments for MeO signals are difficult and have been made for only two of the compounds in Table 3. Nevertheless, a useful trend is apparent. The ^{1}H signals from the two lowest-field MeO groups always appear within a narrow range (δ 3.99−4.10 ppm), while the highest-field MeO signals (last two columns of δs, Table 3) display hypervariable chemical shifts that lie within the range δ 3.48−4.01 ppm and are most likely assigned to the catechol MeO groups. For example, the two highfield MeO groups have been assigned to methoximine groups (C=NOMe) in bastadin 13[23] and are most likely the same in bastadin 9. The assignment of δ 3.90 (s, 3H) to the C15 OMe in bastadin 9 tetra-*O*-methyl ether (NOE and INAPT data)[3] allows the remaining highfield OMe group (δ 3.49, s, 3H) to be located at C34 of the catechol subunit in the northern hemisphere.

The hypervariable MeO signal improves the reliability of fingerprinting by MeO chemical shifts in bastadin methyl ethers. Although no consistent pattern has emerged in MeO chemical shift fingerprinting that would allow empirical assignment of the bastarane or isobastarane macrocycle to new compounds, the anisotropic environment of the macrocycle and the sensitivity of the MeO chemical shifts to local ring currents makes it highly unlikely that two bastadins will have the same set of MeO ^{1}H-NMR signals in $CDCl_3$. We would like to encourage routine reporting of ^{1}H-NMR data of bastadin tetra-*O*-methyl ethers to assist in systematic dereplication of known bastadins in an expanding inventory.

Biological Activity

The new compounds were all less active than bastadin 5 as SR Ca^{2+} channel agonists in the [^{3}H] ryanodine binding assay (EC_{50} 2 μM).[8] Compounds **10** and **11** exhibited moderate differential activity as SR Ca^{2+} channel agonists (EC_{50} 13.6 and 100 μM, respectively), while the potency of **9** (EC_{50} 20.6 μM) was significantly less than that of **10**. The sulfate esters of bastadins tended to show higher overall efficacy than the parent phenols. A full account of bastadin structure−activity relationships will be reported elsewhere.

In summary, the structure of bastadin **20** was defined as the isobastarane isomer of bastadin 18 (**12**). Compounds **10** and **11**, together with 32-*O*-sulfatobastadin 13,[6] are the only known examples of bastadin sulfate esters. Compound **10** is the first bastadin disulfate ester, and it is likely that additional sulfate esters will be found in the most polar fractions of *Ianthella basta* extracts.

Experimental Section

Extraction and Isolation. *Ianthella basta* was collected by hand using scuba at Stuart's Shoal, Exmouth Gulf, Western Australia (22°, 8′S; 114°, 8′E) at a depth of −5 m and frozen at −20 °C until needed. The sponge was identified by Mary Kay Harper, Scripps Institution of Oceanography, and a voucher specimen is archived in the Department of Chemistry, University of California, Davis. The sponge (133.6 g wet wt) was lyophilized (28.7 g dry wt) and extracted with MeOH (800 mL) overnight. After decanting the MeOH solution, the extraction was repeated (3 days) and the combined MeOH extracts were filtered, diluted with

H_2O (10%), and subjected to sequential solvent partitioning with *n*-hexane (500 mL), $CHCl_3$ (500 mL), and *n*-BuOH (20 mL) as described previously.[24] The solvent fractions were assayed for ryanodine binding activity to Ry_1R^8, and the majority of the activity was located in the *n*-BuOH fraction. Evaporation of the *n*-BuOH fraction gave a dark red solid (1.70 g) that was eluted in two batches (0.85 g × 2) on a Sephadex LH-20 column (2.5 × 120 cm, MeOH) and monitored by UV absorbance at 250 nm. The bands eluting from the Sephadex LH20 column were further purified by chromatography (Si gel flash chromatography, gradient of 0−100% MeOH in $CHCl_3$; Sephadex LH20, elution with MeOH; reversed-phase HPLC, Microsorb C_{18}, 1 × 30 cm, 60−80% MeOH/H_2O) to yield bastadin 3 (**3**, 0.011% dry wt), 4 (**4**, 0.028% dry wt), 5 (**5**, 0.0052% dry wt), 6 (**6**, 0.023% dry wt), 7 (**7**, 0.050% dry wt), 10 (0.013% dry wt), 18 (**12**, 0.0084% dry wt), 19 (**8**, 0.119% dry wt), 20 (**9**, 0.012% dry wt), 15,34-*O*-bis-sulfatobastadin 7 (**10**, 0.037% dry wt) and 10-*O*-sulfatobastadin 3 (**11**, 0.028% dry wt).

Bastadin 20 (9): 6.9 mg, colorless solid; $C_{34}H_{28}Br_4N_4O_8$; UV (MeOH) λ_{max} 209 (ϵ 84 400), 279 (5550); IR (ZnSe, film) ν 3400−3000, 2921, 2852, 1660, 1531, 1486, 1424, 1262, 1234, 1180, 989 cm^{-1}; MALDI FTMS *m/z* (M + Na^+) 958.8588 (calcd for $C_{34}H_{28}Br_4N_4O_8Na$, 958.8538); ^1H and ^{13}C NMR, Table 1.

15,34-*O*-Disulfatobastadin 7 (10): 21.1 mg, yellow solid; $C_{34}H_{25}Br_4N_4O_{14}S_2Na_2$; UV (MeOH) λ_{max} 207 (ϵ 82 300), 321 (12 400); IR (ZnSe, film) ν 3300−2860, 1670, 1653, 1571, 1523, 1482, 1418, 1277, 1240, 1051, 1026, 1005 cm^{-1}; MALDI FTMS *m/z* (M + K_3^+) 1208.6481 (calcd for $C_{34}H_{24}Br_4N_4O_{14}S_2K_3$, 1208.6375); ^1H and ^{13}C NMR, Table 1.

10-*O*-Sulfatobastadin 3 (11): 16.6 mg, white powder; $C_{34}H_{29}Br_4N_4O_{11}SNa$; UV (MeOH) λ_{max} 209 (ϵ 84 400), 279 (5550); IR (ZnSe, film) ν 3400−3000, 2921, 2852, 1660, 1531, 1486, 1424, 1262, 1234, 1180, 989 cm^{-1}; MALDI FTMS *m/z* (M + Na_2^+) 1062.8130 (calcd for $C_{34}H_{29}Br_4N_4O_{11}SNa_2$, 1062.8082); ^1H and ^{13}C NMR, Table 2.

Bastadin 19 Tetra-*O*-methyl Ether (13). A solution of bastadin 19 (**8**) (16.2 mg, 0.016 mmol) in DMF (4 mL), was stirred at room temperature with K_2CO_3 (200 mg, 1.44 mmol) and MeI (200 μL, 3.21 mmol) for 18 h. After evaporation of the volatiles, the residue was triturated with CH_2Cl_2, filtered, and the filtrate concentrated. The crude product (10.0 mg) was purified by silica HPLC (1:1 EtOAc/hexane) to give the pure bastadin 19 tetra-*O*-methyl ether (**13**) as a colorless powder (8.9 mg, 53%); UV (MeOH) λ_{max} 207 (ϵ 97 000), 276 (4500); IR (NaCl film), ν 3400−3200, 2935, 2868, 1663, 1489, 1256, 1046 cm^{-1}; HRFAB *m/z* (MH^+) 1070.8372 (calcd for $C_{38}H_{36}Br_5N_4O_8$, 1070.8449); ^1H NMR ($CDCl_3$, 300 MHz), Table 3.

Bastadin 20 Tetra-*O*-methyl Ether (14). Bastadin 20 (**8**) (3.0 mg, 3 μmol) was methylated in a similar manner to bastadin 19 (DMF, 1 mL; K_2CO_3, 30 mg, 0.217 mmol; MeI, 200 μL, 3.21 mmol, 18 h). Workup gave a product (2.6 mg) that was subjected to purification by silica HPLC (50:50 EtOAc/hexane) and afforded bastadin 20 tetra-*O*-methyl ether (**14**) as a white powder (0.9 mg, 30%); UV (MeOH) λ_{max} 210 (ϵ 112 000), 271 (7400); IR (ZnSe film) ν 3400−3300, 2959, 2930, 2851, 1728, 1486, 1286, 1274, 1123 cm^{-1}; MALDI FTMS *m/z* (M + Na^+) 1014.9143 (calcd for $C_{38}H_{36}Br_4N_4O_8Na$, 1014.9164); ^1H NMR ($CDCl_3$, 300 MHz), Table 3.

Microhydrolysis of 15,34-*O*-Disulfatobastadin 7 (10). A sample of **10** (3 mg, 2.6 μmol) was hydrolyzed with 2 M HCl in MeOH at 50 °C for 30 min. The solution was evaporated and redissolved in 10% MeOH/H_2O, centrifuged, and the supernatant (~50 μL) added to one drop of $BaCl_2$ (aqueous 1.0 M) giving a white precipitate of $BaSO_4$. Control experiments with standard sulfate solutions established the level of detection as ~0.05 μmol SO_4^{2-}. The residue from centrifugation was partitioned between H_2O (1 mL) and EtOAc (1 mL), and the EtOAc layer was concentrated to dryness. The residue was analyzed by reversed-phase HPLC (C_{18} Microsorb 3 μm, 4.7 mm × 30 cm, 65:35 MeOH/H_2O containing 0.1% TFA, 1.0 mL/min) and gave a single peak with a retention time (20 min) identical with that of authentic **7**. MALDI FTMS *m/z* (M + Na^+) 956.8382 (calcd for $C_{34}H_{26}Br_4N_4O_8Na$, 956.8382).

Microhydrolysis of 10-*O*-Sulfatobastadin 3 (11). 10-*O*-Sulfatobastadin 3 (**11**, 6.0 mg, 6 μmol) hydrolyzed spontaneously on standing in 1:3 d_6-DMSO/$CDCl_3$ (0.5 mL, trace moisture, 30 °C, 24 h) giving a compound with ^1H- and ^{13}C-NMR spectra identical with those of **3**. ^1H NMR (d_6-DMSO), see Table 2. The solution was evaporated, resuspended in H_2O, and centrifuged. Addition of one drop of $BaCl_2$ (1M aqueous) to the supernatant produced a white precipitate of $BaSO_4$.

Acknowledgments. We are grateful to Mary Kay Harper, Scripps Institution of Oceanography, for identification of the sponge and to Drs. Jeff de Ropp and Philip Searle for their kind assistance with HMQC and HMBC experiments. T. F. M. gratefully acknowledges an American Cyanamid Faculty Award. This work was partially funded by the National Institutes of Health (AI 31660) and Office of Research, University of California, Davis. The 500 MHz NMR spectrometer was partially funded through NIH ISIO-RR04795 and NSF BBS88-04739. This paper is funded in part by a grant from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under grant number NOAA NA36RG0537, project number R/MP-57A through the California Sea Grant College Program, and in part by the California State Resources Agency. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. The U.S. government is authorized to reproduce and distribute for governmental purposes.

References and Notes

(1) Kazlauskus, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1981**, *34*, 765−786.
(2) Carney, J. R.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod.* **1993**, *56*, 153−157.
(3) Pordesimo, E. O.; Schmitz, F. J. *J. Org. Chem.* **1990**, *55*, 4704−4709.
(4) Miao, S.; Andersen, R. J. *J. Nat. Prod.* **1990**, *53*, 1441−1446.
(5) Dexter, A. F.; Garson, M. J.; Hemling, M. E. *J. Nat. Prod.* **1993**, *56*, 782−786.
(6) Gulavita, N. K.; Wright, A. E.; McCarthy, P. J.; Pomponi, S. A.; Kelly-Borges, M.; Chin, M.; Sills, M. A. *J. Nat. Prod.* **1993**, *56*, 1613−1617.

(7) Jaspars, M.; Rali, T.; Laney, M.; Schatzman, R. C.; Diaz, M. C.; Schmitz, F. J.; Pordesimo, E. O.; Crews, P. *Tetrahedron* **1994**, *50*, 7367−7374.
(8) Mack, M.; Molinski, T. F.; Buck, E. D.; Pessah, I. N. *J. Biol. Chem*. **1994**, *269*, 23 236−23 349.
(9) Schreiber, S. L. *Science* **1991**, *251*, 283−287.
(10) Schreiber, S. L.; Crabtree, G. R. *Immunol. Today* **1992**, *13*, 136−142.
(11) Clardy, J. *Proc. Nat. Acad. Sci. U.S.A.* **1995**, *92*, 56−61.
(12) Collins, J. H. *Biochem. Biophys. Res. Commun*. **1991**, *178*, 1288−1290.
(13) Jayaraman, T.; Brillantes, A. M.; Timerman, A. P.; Fleischer, S.; Erdjument-Bromage, H.; Tempst, P.; Marks, A. R. *J. Biol. Chem*. **1992**, *267*, 9474−9477.
(14) Ma, J.; Bhat, M. B.; Zhao, J. *Biophysics J*. **1995**, *69*, 2398−2404.
(15) Chen, S. R.; Zhang, L.; MacLennan, D. H. *Proc. Nat. Acad. Sci. U.S.A.* **1994**, *91*, 11 953−11 957.
(16) Cameron, A. M.; Steiner, J. P.; Roskams, A. J.; Ali, S. M.; Ronnett, G. V.; Snyder, S. H. *Cell* **1995**, *83*, 463−472.
(17) Wu, J. Y.; Fannin, S. T.; Franklin, M. A.; Molinski, T. F.; Lebrilla, C. A. *Anal. Chem*. **1995**, *67*, 3788−3792.
(18) We are grateful to Drs. Phil Crews and Marcel Jaspars, University of California, Santa Cruz, for an authentic sample of bastadin 18.
(19) Nishiyama, S.; Suzuki, T.; Yamamura, S. *Chem. Lett*. **1982**, 1851−1852.
(20) Nishiyama, S.; Suzuki, T.; Yamamura, S. *Tetrahedron Lett*. **1982**, *23*, 3699−3702.
(21) The sulfate half-esters are formulated as Na^+ salts on the expectation of the most common counterion obtained by extraction in contact with seawater.
(22) Carney, J. R.; Rinehart, K. L. *J. Nat. Prod*. **1995**, *58*, 971−985.
(23) Butler, M. S.; Lim, T. K.; Capon, R. J. *Aust. J. Chem*. **1991**, *44*, 287−296.
(24) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126−8131.

NP960507G