Cytotoxic metabolites from the sponge Ianthella basta collected in Papua New Guinea

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CYTOTOXIC METABOLITES FROM THE SPONGE *IANTHELLA BASTA* COLLECTED IN PAPUA NEW GUINEA

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ABSTRACT.—Two new cytotoxic metabolites, bastadin 8 [1] and bastadin 9 [2], have been isolated from the marine sponge *Ianthella basta* collected in Papua New Guinea.

Marine invertebrates are currently the focus of an intense worldwide search for new cytotoxic and antineoplastic agents (1,2). In our laboratories (3), we have screened extracts of marine invertebrates collected in Papua New Guinea for in vitro cytotoxicity against the L-1210 leukemia cell line and in vivo antineoplastic activity against the P-388 leukemia cell line (4). Our bioassay program revealed that MeOH extracts of the sponge *Ianthella basta* (Pallas) (Order Verongidae) exhibit significant in vitro cytotoxicity but no in vivo antineoplastic activity. We now report the isolation of two new cytotoxic metabolites, bastadin 8 [1] and bastadin 9 [2], from *I. basta*.

Samples of *I. basta* were collected by hand using scuba on reefs near Madang, Papua New Guinea. The sponge was identified by Dr. R.M. van Soest, Institute Voor Taxonomische Zoologie, University of Amsterdam. Voucher specimens are incorporated in the Porifera collection of the Zoological Museum of Amsterdam. Freshly collected sponge was immediately frozen on site using dry ice and kept frozen until examined. Thawed sponge was homogenized in a Waring blender with MeOH and extracted at room temperature for 2 days. The MeOH extract was concentrated in vacuo, and the resulting residue was suspended in H_2O and sequentially extracted with hexanes, CH_2Cl_2, and EtOAc. Both the CH_2Cl_2- and EtOAc-soluble fractions contained mixtures of bastadins. Separation of the mixtures was accomplished by sequential application of Sephadex LH 20 [MeOH-CH_2Cl_2 (1:1)], Si gel centrifugal [CH_2Cl_2-MeOH (40:1)], and normal phase high performance liquid [hexane-EtOAc (1:1)]

1 R=H, X=H, Y=Br
2 R=H, X=Br, Y=H
5 R=Me, X=H, Y=Br
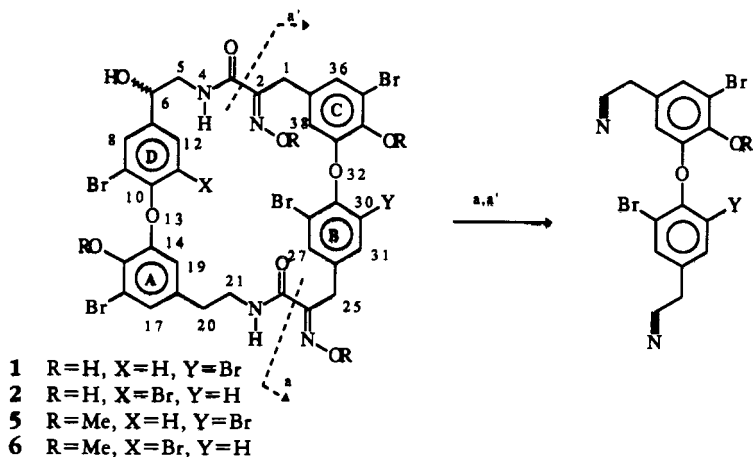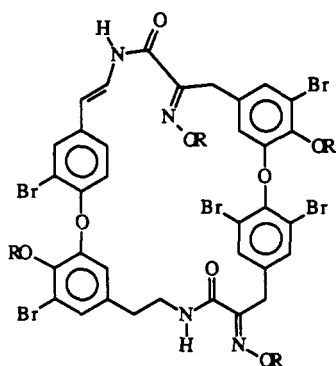6 R=Me, X=Br, Y=H

FIGURE 1. Major mass spectral fragmentation pathway for the bastadins.

3   R=H
4   R=Me

chromatographies to give pure samples of the known bastadins 4 [3], 5, 6, and 7 (4) and the new bastadins 8 [1] and 9 [2]. The previously described bastadins 4 [3], 5, 6, and 7 were identified by comparing their spectral data to the literature values (4).

Bastadin 8 [1] was isolated as a white amorphous solid. A preliminary examination of the ^{1}H-nmr data obtained for 1 (Table 1) indicated that the new metabolite was closely related to the previously described bastadin 4 [3] (5). Because 3 had been most fully characterized as its tetramethyl derivative 4 (5), bastadin 8 was converted to its tetramethyl derivative 5 by reaction with MeI and K_2CO_3 in DMF (5). The molecular

TABLE 1. ^{1}H-nmr Data (400 MHz) for Bastadin 8 [1] and Bastadin 9 [2].[a]

Proton	Compound	
	1	2
	δ	δ
H-1	3.80 (d, 12.9), 3.88 (d, 12.9)	3.72 (d, 12.9), 3.78 (d, 12.9)
H-4	7.04 (t, 6.1)[b]	7.08 (t, 6.1)[b]
H-5	3.55 (m): 3.46 (m)	3.41 (m): 3.63 (m)
H-6	4.82 (t, 6.1)	4.80 (t, 6.1)
H-8	7.70 (d, 2.0)	7.58 (br s)
H-11	6.97 (d, 8.3)	—
H-12	7.21 (dd, 2.0, 8.3)	7.58 (br s)
H-17	6.33 (d, 1.8)[c]	6.13 (d, 1.8)[c]
H-19	7.08 (d, 1.8)[c]	7.03 (d, 1.8)[c]
H-20	2.70 (m)	2.66 (m)
H-21	3.40 (m)	3.36 (m)
H-22	7.28 (t, 6.1)[b]	7.14 (t, 6.1)[b]
H-25	3.69 (d, 12.6), 3.60 (d, 12.6)	3.89 (d, 13.0), 3.81 (d, 13.0)
H-27	7.47 (s)	7.52 (d, 1.8)
H-30	—	6.78 (d, 8.4)
H-31	7.47 (s)	7.14 (dd, 2.0, 8.4)
H-36	7.16 (d, 1.8)[d]	7.26 (d, 1.8)[d]
H-38	6.50 (d, 1.8)[d]	6.64 (d, 1.8)[d]
Ar-OH's	10.50 (br s)	10.73 (br s)
C=N-OH's	11.65 (br s)	11.27 (br s)

[a]Recorded in CDCl$_3$ with one drop DMSO-d_6. Chemical shifts are in ppm from internal TMS.
[b-d]These assignments may be interchanged.

formula of tetramethylbastadin 8 [5], an optically active white amorphous solid, was determined from its hreims which showed a parent ion cluster at m/z 1086, 1088, 1090, 1092, 1094, and 1096 Da (1089.8282, $C_{38}H_{35}N_4O_9{}^{79}Br_3{}^{81}Br_2$; $\Delta m - 0.2$ mmu). The ^{1}H- and ^{13}C-nmr spectra of the tetramethyl derivative 5 (Table 2 and Experimental) revealed that bastadin 8 did not contain the $\Delta^{5,6}$ olefin functionality found in bastadin 4. This observation, coupled with fact that the molecular formula of tetramethylbastadin 8 [5] differed from the molecular formula of tetramethylbastadin 4 [4] by the extra elements of H_2O, suggested that bastadin 4 [3] was simply the dehydration product of bastadin 8 [1]. In concert with this proposal, ^{1}H double resonance and difference nOe experiments conducted on bastadin 8 identified four aromatic ring systems and their attached side chains as shown. The chemical shifts of the resonances assigned to these fragments in tetramethylbastadin 8 were in close agreement with those assigned to tetramethylbastadin 4 (Table 2) except for the resonances assigned to the protons on C-5 and C-6, which had chemical shifts appropriate for a saturated two-carbon fragment containing a benzylic alcohol at C-6 (^{13}C nmr δ 72.4, d).

Figure 1 shows the major mass spectral fragmentation pathway for the bastadins. The cleavage labelled aa', which gives information on the total number of bromine atoms in rings B and C, usually leads to one of the most intense fragment ions in the ei mass spectra of this family of compounds (5). Prominent fragment ion clusters at m/z 1068, 1070, 1072, 1074, 1076, 1078 $[M - H_2O]^+$, and 512/514/516/518 (aa': hreims 513.8370, $C_{17}H_{11}N_2O_2{}^{79}Br_2{}^{81}Br$, $\Delta M -0.9$ mmu) in the ei mass spectrum of tetramethylbastadin 8 [5] were consistent with the assigned structure. We have assumed that the oxime functionalities in 5 have the same configuration as that determined by X-ray diffraction analysis for bastadin 4 [3] (5).

Bastadin 9 [2], isolated as a white amorphous solid, was likewise converted to its tetramethyl ether 6 for characterization. Tetramethyl bastadin 9 [6], obtained as an optically active white amorphous solid, showed a parent ion cluster at m/z 1086, 1088, 1090, 1092, 1094, and 1096 in the hreims appropriate for a molecular formula (1091.8294, $C_{38}H_{35}N_4O_9{}^{79}Br_2{}^{81}Br_3$, ΔM 3.0 mmu) isomeric with tetramethylbastadin 8 [5]. The similarity of the aliphatic resonances in the ^{1}H-nmr spectra of bastadin 8 [1] and bastadin 9 [2] (Table 1) and in the ^{1}H- and ^{13}C-nmr spectra of tetramethylbastadin 8 [5] and tetramethylbastadin 9 [6] (Table 2 and Experimental) indicated that compounds 1 and 2 had the same 6-hydroxy-13,32-dioxa-4,22-diazabastarane macrocyclic skeleton. A very broad two-proton resonance appeared in the aromatic region (δ 7.65) of the ^{1}H-nmr spectrum of tetramethylbastadin 9 recorded in $CDCl_3$ at room temperature. Recording the ^{1}H-nmr spectrum at $-30°$ converted this broad signal into a pair of sharp one-proton resonances at δ 7.43 (d, $J = 1.8$ Hz) and 7.81 (d, $J = 1.8$ Hz). Irradiation of the carbinol methine proton in 6 (H-6, δ 4.86 at $-30°$) induced nOe's in these two aromatic resonances, demonstrating that the two-carbon fragment containing the benzylic hydroxyl was adjacent to this ring. Only a 9,11-dibromo ring D would have the symmetry and ethylamine side chain attachment required to explain both the coalescence and nOe results described above. NOe and double resonance experiments revealed that ring B in 6 (Table 2) was only trisubstituted and therefore lacked the C-30 bromine substituent found in 5. A prominent fragment ion cluster at m/z 434/436/438 (1:2:1) in the eims of 6, resulting from the general aa' cleavage shown in Figure 1, was consistent with the bromination pattern indicated by the ^{1}H-nmr data. The chemical ionization mass spectra of both tetramethyl bastadins 8 [5] and 9 [6] showed intense $[M + Br]^+$ ion clusters, $[M]^+$ ion clusters, base peaks at m/z 79/81, and very few other fragment ions. The same interesting addition of Br was exhibited in the cims by the known compound tetramethyl bastadin 4 [4].

Bastadins 8 [1] and 9 [2] are the first bastadins to have the 6-hydroxy substituent

TABLE 2. ¹H-nmr Data (400 MHz) for Tetramethylbastadin 4 [4], Tetramethylbastadin 8 [5], and Tetramethylbastadin 9 [6]. Chemical shifts are in ppm from internal TMS.

	Compound					
Proton	4 (CDCl$_3$) δ	5 (CDCl$_3$) δ	5 nOe[a]	6 (CDCl$_3$) δ	6 (1:2 CDCl$_3$/C$_6$D$_6$) δ	6 (1:2 CDCl$_3$/C$_6$D$_6$) nOe[a]
H-1	3.68 (s, 2H)	3.69 (s, 2H)	H-36, H-38	3.80 (s, 2H)	3.73 (s, 2H)	H-36, H-38
H-4	8.32 (d, J=11.2)	6.86 (t, J=6.1)		6.73 (t, J=6.1)^b	6.26 (t, J=6.1)^b	
H-5	7.38 (dd, J=11.2, 14.7)	3.38 (m), 3.70 (m)		3.38 (m, 2H)	2.94 (m), 3.32 (m)	
H-6	6.18 (d, J=14.7)	4.86 (t, J=6.0)	H-4, H-5, OH-6, H-8, H-12	4.86 (dd, J=3.2, 7.5)	4.37 (br d, J=7.3)	H-5, OH-6, H-8, H-12
H-8	7.60 (d, J=2.0)	7.70 (d, J=2.0)		7.65 (br s)	7.30–7.60 (br s)	
H-11	6.99 (d, J=8.4)	6.97 (d, J=8.4)				
H-12	7.39 (dd, J=2.0, 8.4)	7.27 (dd, J=2.0, 8.4)		7.65 (br s)	7.30–7.60 (br s)	
H-17	6.65 (d, J=1.9)^b	6.65 (d, J=1.9)^b		7.08 (d, J=1.9)^c	6.86 (d, J=1.9)^c	
H-19	7.13 (d, J=1.9)^b	7.14 (d, J=1.9)^b		6.15 (d, J=1.9)^c	6.30 (d, J=1.9)^c	
H-20	2.81 (t, J=5.8)	2.74 (m, 2H)	H-17, H-19, H-21, H-21'	2.69 (m, 2H)	2.19 (m, 2H)	H-17, H-19, H-21, H-21'
H-21	3.57 (q, J=6.0)	3.55 (m), 3.46 (m)		3.38 (m, 2H)	3.10 (m), 2.94 (m)	
H-22	6.59 (t, J=6.2)	6.75 (t, J=6.1)		7.07 (t, J=6.1)^b	6.70 (t, J=6.0)^b	
H-25	3.73 (s, 2H)	3.77 (d, J=13.2), 3.84 (d, J=13.2)	H-27, H-31	3.84 (d, J=13.0); 3.78 (d, J=13.0)	3.69 (s, 2H)	H-27, H-31
H-27	7.50 (s)	7.53 (s)		7.52 (d, J=2.0)	7.60 (d, J=2.0)	
H-30				6.65 (d, J=8.4)	6.64 (d, J=8.4)	
H-31	7.50 (s)	7.53 (s)		7.14 (dd, J=2.0, 8.4)	7.13 (dd, J=2.0, 8.4)	
H-36	7.11 (d, J=1.9)^c	7.16 (d, J=1.9)^c		7.29 (d, J=2.0)^d	7.28 (d, J=2.0)^d	
H-38	6.25 (d, J=1.9)^c	6.28 (d, J=1.9)^c		6.70 (d, J=2.0)^d	6.78 (d, J=2.0)^d	
6-OH		2.90 (br s)		3.21 (br s)	2.70 (br s)	
OMe	4.01 (s, 12H)	4.02 (s, 3H), 4.01 (s, 3H), 3.98 (s, 3H), 3.70 (s, 3H)		4.05 (s, 3H), 4.01 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H)	4.00 (s, 3H), 3.80 (s, 3H), 3.65 (s, 3H), 3.58 (s, 3H)	

[a] Resonance in proton column irradiated.
[b-d] May be interchanged within a column.

on the 13,32-dioxa-4,22-diazabastarane macrocycle. The tetramethyl derivatives of both **1** and **2** (i.e., **5** and **6**) are optically active (Experimental), ruling out any possibility that the C-6 benzylic hydroxylation resulted from air oxidation during workup. The bastadins all show in vitro L-1210 cytotoxicity with ED_{50} of ≈ 5 µg/ml. This is the first report of cytoxicity by the bastadins.

EXPERIMENTAL

COLLECTION AND ISOLATION.—The fan-shaped sponge was collected by hand using scuba on reefs at a depth of 10 to 15 m off Madang, Papua New Guinea, in April of 1986 and November of 1987. The samples were frozen with dry ice immediately after collection and preserved at $-20°$ until workup. Thawed sponge (275 g) was homogenized in a Waring blender with MeOH (1 liter) and extracted for 2 days. Filtration followed by solvent removal under vacuum at room temperature afforded a red aqueous suspension which was diluted with H_2O to 200 ml and sequentially extracted with hexanes (300 ml × 3), CH_2Cl_2 (300 ml × 3), and EtOAc (300 ml × 3). The three organic-soluble fractions were separately dried over anhydrous Na_2SO_4, and the solvents were removed under vacuum. Tlc and ^1H nmr indicated that both the CH_2Cl_2-soluble fraction (750 mg) and the EtOAc-soluble fraction (400 mg) contained bastadins.

The CH_2Cl_2-soluble fraction (750 mg) was chromatographed on Sephadex LH 20, using MeOH-CH_2Cl_2 (1:1) as an eluent, to yield four fractions pooled according to tlc [silica, CH_2Cl_2-MeOH (10:1)]. The first fraction contained mainly fat, carotenoids, and steroids, whereas the following ones were found to contain bastadins. The second fraction was chromatographed on preparative radial Si gel tlc CH_2Cl_2-MeOH (40:1) and normal phase hplc hexane-EtOAc (1:1) to give pure bastadins 5 (19 mg) and 6 (30 mg). The third fraction was purified by the same procedure to give bastadin 8 [**1**] (10 mg) and bastadin 9 [**2**] (11 mg). The fourth fraction was methylated with MeI and anhydrous K_2CO_3 in DMF and fractionated on preparative radial Si gel tlc hexane-CH_2Cl_2 (1:9) to give tetramethylbastadin 4 [**4**] (20 mg) and tetramethylbastadin 7 (12 mg).

BASTADINS 8 [**1**] AND 9 [**2**].—Both were obtained as white amorphous solids: ^1H nmr see Table 1.

METHYLATION OF BASTADINS 8 [**1**] AND 9 [**2**].—Bastadin 8 [**1**] (10 mg) was stirred with MeI (0.5 ml, passed through a pre-flamed basic alumina plug) and anhydrous K_2CO_3 (0.3 g) in DMF (5 ml) at room temperature for 18 h. The solvent was removed under vacuum to give a yellow residue which was suspended in CH_2Cl_2 and filtered. Purification of the CH_2Cl_2-soluble material on normal phase hplc using hexane-EtOAc (6:4) as the solvent system gave 10 mg of tetramethylbastadin 8 [**5**]. Treatment of bastadin 9 [**2**] (10 mg) in the same procedure gave 9 mg of tetramethylbastadin 9 [**6**].

TETRAMETHYLBASTADIN 8 [**5**].—White amorphous solid: $[\alpha]D + 12°$ ($c = 0.61$, CH_2Cl_2); ir (film) 3402, 2974, 2932, 2825, 1669, 1487 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr (75 MHz, $CDCl_3$) δ 28.63 (t), 29.06 (t), 35.02 (t), 39.65 (t), 47.17 (t), 60.96 (q), 61.26 (q), 62.82 (q), 63.34 (q), 72.43 (d), 113.92 (d), 114.60 (s), 117.86 (d), 117.99 (s, 2C), 118.04 (s), 120.13 (d), 126.55 (d), 127.47 (d), 127.93 (d), 131.14 (d), 132.72 (s), 133.74 (d, 2C), 135.57 (s), 136.39 (s), 139.20 (s), 144.44 (s), 146.40 (s), 146.71 (s), 149.93 (s), 150.16 (s), 150.28 (s), 150.79 (s), 152.36 (s), 162.13 (s), 163.33 (s) ppm; lreims *m/z* (rel. int.) 1096 (0.4), 1094 (2.7), 1092 (5.8), 1090 (7.1), 1088 (4.7), 1086 (1.7), 1078 (2.6), 1076 (10.6), 1074 (19.3), 1072 (18.1), 1070 (9.4), 1068 (2.0), 1065 (4.8), 1063 (18.5), 1061 (33.1), 1059 (34.0), 1057 (17.5), 1055 (4.5), 1047 (2.5), 1045 (7.1), 1043 (11.2), 1041 (10.5), 1039 (5.1), 1037 (1.6), 1033 (2.3), 1031 (3.7), 1029 (4.2), 1027 (3.6), 1025 (2.5), 1023 (1.3), 1015 (2.8), 1013 (4.7), 1011 (5.5), 1009 (3.5), 1007 (1.6), 682 (0.7), 680 (2.2), 678 (2.3), 676 (1.3), 651 (1.8), 649 (5.5), 647 (5.3), 645 (1.9), 606 (0.5), 604 (0.8), 602 (0.9), 593 (0.9), 591 (2.4), 590 (1.9), 589 (2.3), 574 (10.1), 572 (10.8), 570 (4.6), 518 (19.3), 516 (53.4), 514 (56.8), 512 (23.8), 456 (12.5), 454 (16.6), 452 (15.2), 450 (7.8), 422 (15.1), 420 (26.7), 418 (13.9), 410 (14.9), 408 (11.5), 397 (12.6), 395 (13.1), 393 (7.2), 356 (18.0), 354 (26.9), 240 (5.3), 238 (5.4), 236 (3.0), 212 (2.8), 210 (4.5), 208 (2.8), 169 (18.7), 82 (37.0), 80 (37.6), 44 (39.3), 31 (91.9), 29 (100.0); hreims *m/z* 1089.8282 (calcd 1089.8284 for $C_{38}H_{35}N_4O_9{}^{79}Br_3{}^{81}Br_2$); cims *m/z* $[M + Br]^+$ 1178 (1.3), 1176 (15.2), 1174 (15.8), 1172 (26.0), 1170 (20.3), 1168 (4.4), 1096 (34.8), 1094 (85.4), 1092 (100.0), 1090 (84.8), 1088 (27.9), 1086 (2.5).

TETRAMETHYLBASTADIN 9 [**6**].—White amorphous solid: $[\alpha]D + 2.7°$ ($c = 0.77$, CH_2Cl_2); ir (film) 3327, 2932, 2857, 1669, 1487 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr (75 MHz, $CDCl_3$) δ 28.58 (t), 28.99 (t), 34.77 (t), 39.59 (t), 47.31 (t), 61.08 (q), 61.16 (q), 63.17 (q) (2C coincidental overlap), 72.26 (d), 112.72 (d), 113.38 (s), 117.87 (s), 118.37 (s, 2C), 118.54 (d), 119.60 (d), 126.57 (d), 129.24 (d), 129.71 (d), 130.63 (d, 2C), 133.07 (s), 133.15 (s), 134.22 (d), 135.35 (s), 142.10 (s), 144.46 (s), 146.83 (s), 147.49 (s), 149.52 (s), 150.17 (s), 150.74 (s), 151.05 (s), 151.81 (s), 162.26 (s), 163.63 (s) ppm; lreims *m/z* (rel. int.) 1096 (0.5), 1094 (2.0), 1092 (4.1), 1090 (4.2), 1088 (2.4), 1086 (0.7), 1078

(1.0), 1076 (3.7), 1074 (7.2), 1072 (6.5), 1070 (3.4), 1068 (0.8), 1065 (1.1), 1063 (2.3), 1061 (5.6), 1059 (5.3), 1057 (3.0), 1055 (0.8), 1047 (1.1), 1045 (3.2), 1043 (5.6), 1041 (5.0), 1039 (2.9), 1037 (0.7), 1015 (1.3), 1013 (2.2), 1011 (2.7), 1009 (2.1), 1007 (0.9), 648 (0.4), 632 (0.8), 630 (0.6), 602 (3.1), 600 (5.7), 598 (2.8), 592 (1.2), 590 (1.6), 571 (8.0), 569 (13.9), 567 (7.6), 551 (2.0), 549 (5.1), 547 (4.9), 545 (2.3), 536 (2.5), 534 (3.6), 532 (4.6), 530 (3.3), 438 (21.1), 436 (40.4), 434 (20.8), 412 (12.9), 411 (14.1), 410 (19.6), 409 (14.0), 408 (10.0), 342 (20.5), 340 (18.3), 318 (5.6), 316 (13.8), 314 (10.4), 276 (22.5), 261 (8.8), 169 (9.2), 115 (9.4), 82 (41.5), 80 (42.1), 31 (73.6), 29 (100.0); hreims m/z 1089.8312 (calcd 1089.8284 for $C_{38}H_{35}N_4O_9{}^{79}Br_3{}^{81}Br_2$);cims (showed one extra Br) $[M + Br]^+$ 1178 (12.7), 1176 (37.3), 1174 (72.2), 1172 (92.9), 1170 (56.4), 1168 (21.8), 1166 (1.2), 1096 (21.0), 1094 (52.0), 1092 (74.2), 1090 (54.0), 1088 (22.6), 1086 (2.4).

ACKNOWLEDGMENTS

LITERATURE CITED

1.   M. Suffness and J. Thompson, in: "Biomedical Importance of Marine Organisms." Ed. by D.G. Fautin, California Academy of Sciences, San Francisco, 1988, pp. 151–157.
2.   M.H.G. Munro, R.T. Luibrand, and J.W. Blunt, in: "Bioorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, Berlin, 1987, pp. 93–176.
3.   E.D. de Silva, R.J. Andersen, and T.M. Allen, *Tetrahedron Lett.*, **31**, 489 (1990).
4.   R. Kumar, L.I. Weibe, T.L. Hall, E.E. Knaus, D.R. Tovell, D.L. Tyrell, T.M. Allen, and R. Fathi-Afshar, *J. Med. Chem.*, **32**, 941–944 (1989).
5.   R. Kazlauskas, R.O. Lidgard, P.T. Murphy, R.J. Wells, and J.F. Blount, *Aust. J. Chem.*, **34**, 765 (1981).

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