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Dolastatin 11 conformations, analogues and pharmacophore

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Abstract—Twenty analogues of the natural antitumor agent dolastatin 11, including majusculamide C, were synthesized and tested for cytotoxicity against human cancer cells and stimulation of actin polymerization. Only analogues containing the 30-membered ring were active. Molecular modeling and NMR evidence showed the low-energy conformations. The amide bonds are all trans except for the one between the Tyr and Val units, which is cis. Since an analogue restricted to negative 2-3-4-5 angles stimulated actin polymerization but was inactive in cells, the binding conformation (most likely the lowest-energy conformation in water) has a negative 2–3–4–5 angle, whereas a conformation with a positive 2–3–4–5 angle (most likely the lowest energy conformation in chloroform) goes through cell walls. The highly active *R* alcohol from borohydride reduction of dolastatin 11 is a candidate for conversion to prodrugs.
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1. Introduction

The isolation from the sea hare *Dolabella auricularia*, structure determination, and strong antitumor activity of dolastatin 11 (**1**) were reported in 1989.[1] Its synthesis was reported in 1997[2] and its cytotoxicity was reported to involve disruption of microfilaments by stimulation of the polymerization of actin in 2001.[3] An X-ray fiber diffraction study of the F-actin/dolastatin 11 complex showed dolastatin 11 (**1**) to stabilize microfilaments by binding to two strands of F-actin.[4] We now report the synthesis of 20 analogues (**2–21**) of dolastatin 11 (**1**) and the results of testing them for cytotoxicity against human tumors and for stimulation of actin polymerization. These results are interpreted in terms of the main conformations which appear to be present from NMR studies and molecular modeling.

Keywords: Dolastatin 11; Pharmacophore; Depsipeptide; Anticancer.
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2. Conformations of dolastatin 11 (1)

NMR evidence from NOEs (Table 1) indicated that in the predominant form of dolastatin 11 (**1**) in chloroform solution, the ester bond and all of the amide bonds are trans except for the amide bond between the Tyr and Val units, which is cis from the very strong NOE observed between the Tyr and Val α-H's. The NOE peaks showing these configurations are in italics in Table 1.

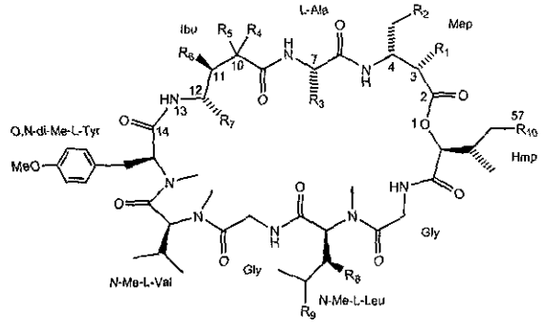
Molecular modeling calculations on dolastatin 11 (**1**) with the configurations of the amide and ester bonds restricted to those determined by NMR were performed for chloroform and water solutions: chloroform to understand the conformations in the NMR solvent (deuterochloroform; not soluble enough in D_2O for NMR) which may also be favored for dolastatin 11 and analogues in the cell wall, and water because the calculations in water should apply well to the conformations favored in blood and intracellular fluid. Fifteen conformations (C1–C15) were calculated to be within 10 kJ/mol of the lowest-energy form in chloroform

Structures of dolastatin 11 (**1**) and derivatives **2**–**21**[a]

Compound	Map		Ala	Ibu				Leu		Hmp
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Dolastatin 11 (**1**)	Me	Et	Me	Me	Me	=O	Me	H	Me	Me
Map analogues										
3-Nor (**2**)	H									
3,4-Bis-nor (**3**)	H	H								
R,R-Acc (3-epimer) (**4**)	[b]$CH_2CH_2CH_2$									
S,S-Acc (4-epimer) (**5**)	$CH_2CH_2CH_2$[b]									
Ala analogues										
7-Epi (**6**)			[b]							
7-Nor (**7**)			H							
Ibu analogues										
10-Nor (**8**)				H						
10,10-Bis-nor (**9**)				H	H					
11R-OH (**10**)						OH				
11R-OAc (**11**)						OAc				
12-Epi (**12**)							[b]			
Leu analogue										
majusculamide C (**13**)								Me	H	
Hmp analogue										
57-Nor (**14**)										H
Map, Ala analogue										
3,4,7-Tris-nor (**15**)	H	H	H							
Map, Ibu analogue										
3,4-Bis-nor-11-OH (**16**)	H	H				OH				
Ala, Ibu analogue										
7-Nor-11-OH (**17**)			H			OH				
Map, Ala, Ibu analogue										
3,4,7-Tris-nor-11-OH (**18**)	H	H	H			OH				
Open analogues										
1,2-Seco (**19**)										
13,14-Seco (**20**)										
Unit-deletion analogue										
des-Hmp (**21**)										

Only the groups which differ from those in dolastatin 11 (**1**) are shown for the derivatives.
[a] Acc = 2-aminocyclopentanecarboxylic acid; Hmp = (2*S*,3*S*)-2-hydroxy-3-methylpentanoic acid; Ibu = (*S*)-4-amino-2,2-dimethyl-3-oxopentanoic acid; Map = (2*S*,3*R*)-2-methyl-3-aminopentanoic acid.
[b] Epimer at this center.

(C1), and eighteen (W1–W18) to the lowest conformation in water (W1); eight were common to both media.



These 25 conformations included eight different conformations of the 30-membered ring, listed in Table 2, with the others involving different side-chain rotations, as shown in Tables 3 and 4. The two lowest-energy conformations calculated in chloroform (C1and C2) and the lowest (W1) in water are depicted in Figure 1. From Figure 1 and Table 2, it can be seen that the 15–16–17–18 cis-amide bond between Tyr and Val and the 28–29–30–1 bond in the Hmp unit have angles close to zero in all of these conformations, and that there is not much variation in the conformation of the shorter (13-atom) Val-Gly1-Leu-Gly2 connecting chain; the largest change in this shorter chain comes in C2, where the 25–26–27–28 and 26–27–28–29 angles in Gly2 are 109° and −110°, respectively. This shorter chain of ring atoms is nearly

Table 1. NOE peaks of dolastatin 11 (**1**) and ROESY peaks of 7-epidolastatin 11 (**6**)

Proton[a]	Proton #	NOE peaks in **1**	ROESY peaks in **6**
Map-α-CH	3	3a, 4, 4a′, 4b	3a, 4
Map-β-CH_3	3a	3, 4, 5	3, 4, 4a′, 5, 28
Map-β-CH	4	3, 3a, 4a, 4a′, 4b	3, 3a, 4a, 4a′, 4b, 8
Map-γ-CH_2	4a	4, 4b, 5	4, 4a′, 4b, 5
Map-γ-CH_2	4a′	3, 4, 4b	3a, 4, 4a, 4b
Map-δ-CH_3	4b	3, 4, 4a, 4a′, *5*	4, 4a, 4a′, 5
Map-NH	5	3a, 4a, 4b, *7*	3a, 4a, 4b, *7*, 28
Ala-α-CH	*7*	*5*,[b] 7a, 8	*5*, 7a
Ala-β-CH_3	7a	7, 8	7, 8
Ala-NH	8	7, 7a, *10b*	4, 7a, *10a*, *10b*
Ibu-β-CH_3	10a	12	*8*, 10b
Ibu-β-CH_3	10b	*8*	*8*, 10a, 12
Ibu-γ-CH	12	10a, 12a, 13	10b, 12a, 13
Ibu-δ-CH_3	12a	12, 13	12
Ibu-NH	13	12, 12a, *15*	12, *15*, 19a
Tyr-α-CH	15	*13*, 15a, 15a′, 15c, *18*	*13*, 15a 15a′, 15c, *18*
Tyr-β-CH_2	15a	15, 15a′, 15c	15, 15a′, 15c, 18
Tyr-β-CH_2	15a′	15, 15a, 15c	15, 15a, 15c
Tyr-δ-CH	15c	15, 15a, 15a′, 15d, 16a, 18, 18c	15, 15a, 15a′, 15d, 16a, 18, 18b
Tyr-ε-CH	15d	15c, 15f, 18c	15c, 15f, 18b
OCH_3	15f	15d	15d
Tyr-NCH_3	16a	15c	15c
Val-α-CH	18	*15*, 15c, 18a, 18b, 18c	*15*, 15a, 15c, 18b, 18c
Val-β-CH	18a	18, 18b, 18c, 19a	18b, 18c, 19a
Val-γ-CH_3	18b	18, 18a, 19a	15c, 15d, 18, 18a, 18c, 19a
Val-γ-CH_3	18c	15c, 15d, 18, 18a	18, 18a, 18b, 19a
Val-NCH_3	19a	18a, 18b, *21a*, *21b*	13, 18a, 18b, 18c, *21a*, *21b*
Gly^1-α-CH_2	21a	*19a*, 21b, 22	*19a*, 21b, 22
Gly^1-α-CH_2	21b	*19a*, 21a, 22	*19a*, 21a, 22
Gly^1-NH	22	21a, 21b, *24*	21a, 21b, *24*
Leu-α-CH	24	*22*, 24a, 24a′, 24b, 24c	*22*, 24a, 24a′, 24c
Leu-β-CH_2	24a	24, 24a′, 24b, 24c, 24d, 25a	24, 24a′, 24c
Leu-β-CH_2	24a′	24, 24a, 24b, 24c, 24d, 25a	24, 24a, 24b, 24d, 25a
Leu-γ-CH	24b	24, 24a, 24a′	24a′, 24c, 24d, 25a
Leu-δ-CH_3	24c	24, 24a, 24a′	24, 24a, 24b
Leu-δ-CH_3	24d	24a, 24a′	24a′, 24b
Leu-NCH_3	25a	24a, 24a′, *27a*	24a′, 24b, *27a*, *27b*
Gly^2-α-CH_2	27a	*25a*, 27b, 28	*25a*, 27b, 28
Gly^2-α-CH_2	27b	27a, 28	*25a*, 27a, 28
Gly^2-NH	28	27a, 27b, *30*	3a, 5, 27a, 27b, *30*
Hmp-α-CH	30	*28*, 30a, 30b, 30b′, 30c, 30d	*28*, 30a, 30b, 30c, 30d
Hmp-β-CH	30a	30, 30b, 30b′, 30c, 30d	30, 30b, 30b′, 30c, 30d
Hmp-γ-CH_2	30b	30, 30a, 30b′, 30c, 30d	30, 30a, 30b′, 30c, 30d
Hmp-γ-CH_2	30b′	30, 30a, 30b, 30c, 30d	30a, 30b, 30c, 30d
Hmp-δ-CH_3	30c	30, 30a, 30b, 30b′	30, 30a, 30b, 30b′
Hmp-γ-CH_3	30d	30, 30a, 30b, 30b′	30, 30a, 30b, 30b′

[a] Within CH_2s and $C(CH_3)_2$s, upfield absorbing group is listed first.
[b] *Italicized* numbers in the table indicate the main peaks which show amide configurations.

extended to maximum length, with most angles near 180°. In contrast, the longer (17-atom) Hmp-Map-Ala-Ibu-Tyr chain shows much variation among these eight ring conformations. The conformations divide into two groups (+ and −) based on the sign of the 2–3–4–5 angle (around the only sp^3-sp^3 bond in the large ring), which is either about +60° or −60°; the significance of this is discussed below in the section on analogues.

Table 5 shows nitrogen to carbonyl oxygen distances for likely intramolecular hydrogen bonds in the eight 30-membered ring conformations; not shown in Table 5 are the 2.24–2.36Å distances between Gly-2-N and O1 (the non-carbonyl ester oxygen) which probably provide similar five-membered ring hydrogen bonds in all eight conformations. C2, with its four 10- to 15-membered cross-ring hydrogen bonds of the β-sheet type, is very different from all of the others. C7 differs from C4 only in the Ibu-NH to Ibu-1-CO bond, C15 from C1 only in the Gly-1-NH to Ibu-1-CO bond, W1 from C3 only in the Map-NH to Gly-2-CO bond, and W5 from W1 largely in the Gly-1-NH to Ibu-1-CO bond.

All of the NMR evidence, including the NOE's shown in Table 1 for dolastatin 11 (**1**) and strong additional evidence from the spectra of restricted rotation derivatives given below, is consistent with the view that its predom-

Table 2. Thirty-membered ring torsion angles for dolastatin 11 (1) conformations C1–C4, C7, C15, W1, and W5

Unit	Angle	C1	C2	C3	C4	C7	C15	W1	W5
Map	1–2–3–4	−122	−42	−30	−23	−21	−115	14	21
	2–3–4–5	76	−86	−81	−64	−65	69	−79	−85
	3–4–5–6	82	88	99	147	149	76	125	120
	4–5–6–7	173	174	171	168	170	171	173	180
Ala	5–6–7–8	95	99	168	127	124	106	127	133
	6–7–8–9	−157	−155	−167	−167	−168	−164	−164	−158
	7–8–9–10	172	173	−166	180	178	171	−177	−174
	8–9–10–11	−159	72	141	112	165	154	126	129
Ibu	9–10–11–12	−93	39	−50	−47	−77	−68	−46	−47
	10–11–12–13	56	131	109	96	64	71	109	108
	11–12–13–14	−146	−158	−153	−163	−153	−158	−158	−157
	12–13–14–15	178	171	175	−179	−175	−178	180	180
Tyr	13–14–15–16	126	110	76	62	107	100	73	89
	14–15–16–17	−126	−121	−125	−139	−128	−135	−130	−136
	15–16–17–18	−2	0	−11	−5	−7	0	−6	−8
Val	16–17–18–19	80	103	85	94	84	81	86	78
	17–18–19–20	−129	−106	−133	−127	−137	−140	−131	−137
	18–19–20–21	178	−175	−179	−176	−175	−176	−179	173
Gly-1	19–20–21–22	−163	−158	−150	−167	−176	−176	−151	−150
	20–21–22–23	−170	−175	−148	−178	−176	−176	−147	177
	21–22–23–24	172	−177	175	176	180	−179	177	−179
Leu	22–23–24–25	117	109	120	113	109	120	120	158
	23–24–25–26	−150	−126	−147	−137	−133	−121	−143	−126
	24–25–26–27	−177	178	−178	177	180	−178	176	−179
Gly-2	25–26–27–28	−174	109	−175	−170	−170	−172	−160	−166
	26–27–28–29	−177	−110	−164	−170	−171	−175	179	179
	27–28–29–30	−179	177	171	172	171	178	179	179
Hmp	28–29–30–1	−1	−10	−7	−7	−8	−1	−32	−35
	29–30–1–2	−82	−69	−68	−71	−71	−81	−72	−73
	30–1–2–3	−173	−169	−176	176	176	−172	171	173

Table 3. Energies (kJ/mol > minimum in that solvent), sign of angle 2–3–4–5, 30-membered ring type, and side chain conformations of the 25 conformations calculated to be within 10 kJ/mol of the minima for dolastatin 11 (1) in chloroform (C) and water (W); see Table 4 for key to side chain rotation numbers

Conformation	2–3–4–5 Sign	Ring type	Side chain rotations						
			Map	Tyr	Val	Leu		Hmp	
			χ^1	χ^1	χ^1	χ^1	χ^2	χ^1	χ^2
C1 (0) = W2 (1.6)	+	C1	1	1	1	1	1	1	1
C2 (2.2) = W18 (9.9)	−	C2	1	2	1	1	1	1	1
C3 (5.4) = W3 (2.7)	−	C3	1	2	1	1	1	1	2
C4 (7.7) = W4 (5.1)	−	C4	2	2	1	2	2	1	1
C5 (7.9) = W11 (8.3)	+	C1	1	1	1	1	1	1	3
C6 (8.0)	+	C1	1	1	1	1	3	1	1
C7 (8.3)	−	C7	2	1	1	2	2	1	1
C8 (8.3)	−	C7	1	1	1	1	2	1	1
C9 (8.4) = W8 (7.1)	−	C7	1	1	1	1	1	2	1
C10 (8.5) = W14 (9.1)	−	C7	1	1	2	1	1	1	1
C11 (9.1)	−	C7	1	2	1	2	2	1	1
C12 (9.3)	−	C3	1	1	1	1	1	3	1
C13 (9.4)	+	C1	1	1	1	1	1	3	1
C14 (9.7)	−	C7	2	2	1	1	1	1	1
C15 (9.8) = W7 (6.8)	+	C15	1	1	2	2	2	1	1
W1 (0)	−	W1	1	2	1	1	1	2	1
W5 (5.2)	−	W5	1	2	2	2	2	2	1
W6 (6.0)	−	W1	1	2	1	1	1	1	3
W9 (7.1)	+	C15	1	1	1	2	2	2	1
W10 (7.9)	−	W5	3	2	1	2	2	1	1
W12 (8.9)	−	C7	1	1	1	1	3	1	1
W13 (9.1)	−	W1	2	2	1	1	1	2	1
W15 (9.6)	−	W1	2	1	1	2	2	2	3
W16 (9.6)	−	W1	2	2	1	1	1	2	3
W17 (9.9)	+	C15	1	2	2	2	2	2	3

Table 4. Key to side chain rotation numbers in Table 3

Unit	1	2	3
Map	Far Me anti to CH	Far Me anti to N	Far Me anti to H
Tyr χ^1	Ar anti to CO	Ar anti to N	—
Val χ^1	H's anti	H anti to N	—
Leu χ^1	*i*-Pr anti to N	*i*-Pr anti to CO	—
χ^2	Me anti to CH	Me′ anti to CH	H anti to CH
Hmp χ^1	Et anti to H	Et anti to CO	Et anti to OCO
χ^2	Far Me anti to CH	Far Me anti to Me	Far Me anti to H

inant conformation in chloroform solution is C1. It was not possible to determine its preferred conformation(s) in water by NMR due to its low solubility.

3. Synthesis of dolastatin 11 (1) analogues 2–21

Analogues **2–21** were made by syntheses following that of dolastatin 11 (**1**)[2] with three improvements: (1) In the last step in the preparation of Map, the *p*-toluenesulfonyl group was removed not with HBr but with sodium in liquid ammonia. (2) In the workup of the preparation of N-Me-L-Val-O,N-diMe-L-Tyr-OBn·HCl, evaporation of most of the benzyl alcohol and addition of ether caused the product to crystallize and avoided multiple extractions and lyophilization. (3) Some of the final cyclizations were carried out at higher dilution (Procedure B, Experimental Section), doubling the yield from the earlier procedure (Procedure A).[2]

The NMR data on these analogues are summarized in Table 6. Analogue **20**, the last intermediate in the dolastatin 11 (**1**) synthesis, is not included; its NMR parameters were reported earlier.[2] The spectra of analogues **19** and **20**, which lack the 30-membered ring but have three *N*-methyl amide groupings, are complicated by the occurrence of significant amounts of up to eight rotamers; when the 30-membered ring is closed, as noted earlier, the 16,17-cis, 19,20-trans, 25,26-trans rotamer predominates greatly over the others, making the spectra much easier to interpret. 2D NMR studies were carried out on analogues **6** and **7**, and the assignments for the other analogues were made where possible by comparisons with these and the spectra of dolastatin 11 (**1**). 'Obsc' means the absorption was obscured and asterisks mean the assignments may be reversed with

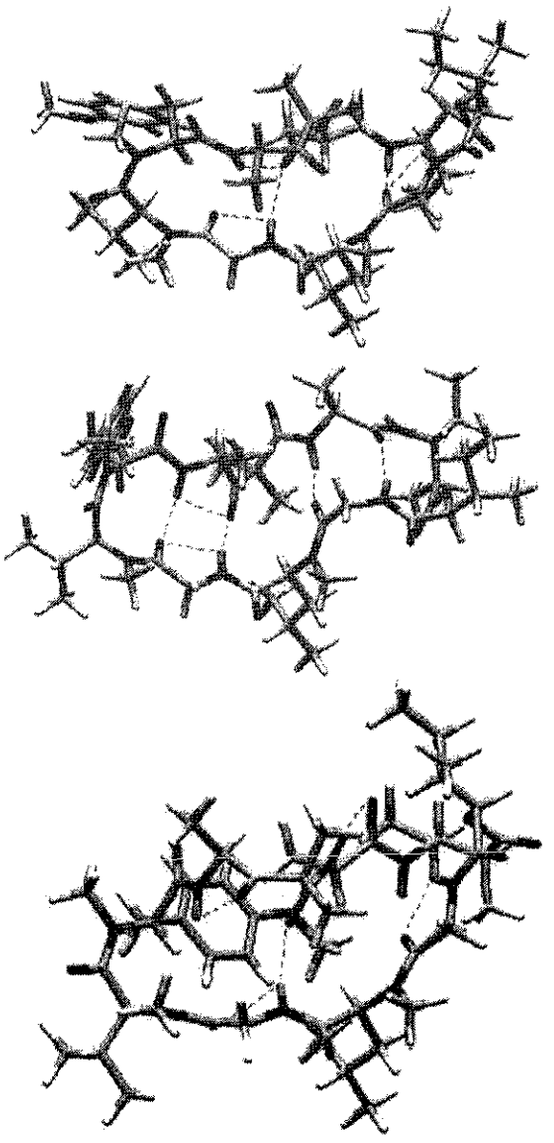


Figure 1. The two lowest-energy conformations calculated for dolastatin 11 (**1**) in chloroform (C1, top; C2, middle) and the lowest in water (W1, bottom). Dashed lines represent intramolecular hydrogen bonds.

Table 5. Intramolecular hydrogen bonding (with N to carbonyl O distances in Angstroms) in the eight lowest-energy 30-membered ring conformations of dolastatin 11 (**1**); weak bonds (2.7–3.1 Å) are in parentheses

Conformation	Map-N to	Ala-N to		Ibu-N to			Gly-1-N to			Gly-2-N to	
	Gly-2	Ala	Gly-2	Ibu-1	Ibu-3	Gly-1	Gly-1	Ibu-1	Ibu-3	Gly-2	Ala
H-bond ring size →	12	5	15	7	5	10	5	16	14	5	11
C1 = W2	1.81	(2.84)	—	1.97	—	(3.03)	2.16	2.08	—	2.08	—
C2 = W18	—	(2.83)	1.86	—	2.29	1.89	2.30	—	1.83	(2.90)	1.76
C3 = W3	—	2.12	—	(2.90)	2.63	—	2.43	1.99	—	2.21	2.10
C4 = W4	1.98	2.41	—	(2.88)	(2.89)	—	2.17	(2.79)	—	2.12	—
C7	1.95	2.44	—	2.02	—	—	2.10	(2.79)	—	2.11	—
C15 = W7	1.81	(2.84)	—	2.03	—	—	2.14	(2.90)	—	2.12	—
W1	2.69	2.43	—	(3.08)	2.63	—	2.50	1.95	—	2.10	—
W5	—	2.37	—	(3.01)	2.66	—	2.15	—	—	2.10	—

Table 6. ^{1}H NMR shifts (δ, $CDCl_3$), multiplicities, and coupling constants (J, in parentheses) for compounds **1–19** and **21**

	Dolastatin 11 (**1**)	3-Nor (**2**)	3,4-Bis-nor (**3**)	*R,R*-Acc (**4**)	*S,S*-Acc (**5**)	7-Epi (**6**)	7-Nor (**7**)
Map							
α-CH	2.79qd(7,2.5)	CH_2 2.33dd(14.5,11) 2.73dd(14.5,2.5)	CH_2 2.62ddd(16,10,2) 2.74ddd(16,8,3)	3.33q(8)	2.49q(8)	3.03 m	2.83 m
β-CH_3	1.10d(7)	—	—	CH_2 1.77,2.17m	CH_2 1.80,2.10m	1.23d(7)	1.15d(7)
β-CH	4.47m	4.91m	CH_2 3.60,3.85m	4.90m	4.82m	3.77m	4.24m
γ^1-CH_2	1.46m	1.46m	—	2.18m	2.20m	1.25m	1.38m
γ^2-CH_2	1.56m	1.56m	—	2.18m	2.20m	1.76dqd(14,7,3)	1.63m
δ-CH_3	0.93t(7.5)	0.95t(7)	—	CH_2 1.50m	CH_2 1.50m	0.98t(7)	0.96t(7.5)
NH	7.09d(10.5)	7.09d(9)	7.47br t(7)	7.31m	6.75d(9)	8.17d(7)	7.42d(9)
Ala							
α-CH	4.44p(7)	4.44p(6.5)	4.65p(7)	4.63p(7)	4.45p(6.5)	4.56p(7)	CH_2 3.59dd(16,5) 4.05dd(16,6)
β-CH_3	1.07d(7)	1.07d(7)	1.25d(7)	1.22d(7)	1.07d(6.5)	1.25d(7)	—
NH	7.78d(8.5)	7.79d(8.5)	7.18d(7)	6.99d(7)	7.83d(9)	6.78d(8)	7.53t(5.5)
Ibu							
β^1-CH_3	1.44s	1.45s	1.38s	1.30s	1.43s	1.39s	1.45s
β^2-CH_3	1.49s	1.48s	1.57s	1.49s	1.43s	1.57s	1.53s
γ-CH	4.91p(7)	4.93p(7)	4.98p(7)	4.88p(7)	4.93p(7)	4.88p(6.5)	4.89p(7)
δ-CH_3	1.13d(7)	1.13d(7)	1.30d(7)	1.41d(7)	1.09d(7)	1.29d(6.5)	1.20d(7)
NH	7.15d(9)	7.13d(9)	6.49d(7.5)	6.45d(8)	7.30d(7)	6.50d(7)	6.72d(7)
Tyr							
α-CH	5.11dd(8.5,6.5)	5.12dd(8,6.5)	5.13dd(10.5,5)	5.05d(9,4)	5.08t(7.5)	5.07dd(11,4)	5.08dd(9.5,5)
β^1-CH_2	2.82dd(14,8.5)	2.84dd(14.5,8)	2.79dd(15,10.5)	2.75dd(14,9)	2.84dd(15,8)	2.76dd(14.5,11)	2.81dd(14.5,9.5)
β^2-CH_2	3.25dd(14,6.5)	3.25dd(14.5,6.5)	3.37dd(15,5)	3.38dd(14,4)	3.24dd(15,7)	3.43dd(14.5,4)	3.34dd(14.5,5)
δ-Ar	7.14d(8.5)	7.15d(8.5)	7.14br d(8.5)	7.14d(9)	7.14d(8.5)	7.16d(8.5)	7.15d(8.5)
ε-Ar	6.81d(8.5)	6.82d(8.5)	6.82br d(8.5)	6.82d(9)	6.82d(8.5)	6.83d(8.5)	6.83d(8.5)
OCH_3	3.75s	3.76s	3.75s	3.75s	3.76s	3.76s	3.77s
NCH_3	2.96s	2.96s	2.95s*	2.92s*	3.00s*	2.89s	2.93s*
Val							
α-CH	4.78d(10.5)	4.79d(10.5)	4.59d(10.5)	4.60d(11)	4.88d(10.5)	4.53d(10.5)	4.64d(10.5)
β-CH	2.23m	2.24m	2.16dh(10.5,6.5)	2.16d(11)	2.23m	2.15dh(10.5,6.5)	2.20dh(10.5,6.5)
γ^1-CH_3	0.37d(6.5)	0.38d(6.5)	0.17d(6.5)	0.22d(7)	0.45d(6.5)	0.17d(6.5)	0.28d(6.5)
γ^2-CH_3	0.74d(6.5)	0.75d(6.5)	0.65d(6.5)	0.66d(6.5)	0.78d(6.5)	0.63d(6.5)	0.68d(7)
NCH_3	2.95s	2.94s	2.89s*	2.90s*	2.98s*	2.87s	2.91s*
Gly-1							
α^1-CH_2	3.60dd(18,2)	3.60dd(17.5,1.5)	3.75dd(17,2.5)	3.96dd(17.5,3)	3.65dd(16,2)	3.86dd(17.5,3.5)	3.79dd(18,3)
α^2-CH_2	4.42dd(18,7.5)	4.42dd(17.5,7.5)	4.48dd(17,7)	4.24dd(17.5,4)	4.40dd(16,8)	4.12dd(17.5,7)	4.42dd(18,6.5)
NH	7.41dd(7.5, 2)	7.42dd(7.5,1.5)	6.84dd(7,2.5)	7.12m*	7.59dd(8,2)	6.94dd(7,3.5)	7.09dd(6.5,3)
Leu							
α-CH	5.37dd(11,5)	5.37dd(11,5)	5.20dd(11.5,5)	5.26dd(9.5,6.5)	5.42dd(11,5)	5.35dd(11.5,4.5)	5.32dd(11.5,5)
β^1-CH_2	1.62m	1.62m	1.80ddd(13.5,8,5)	1.78m	1.62m	1.73ddd(14.5,10,4.5)	1.65ddd(15,10,5)
β^2-CH_2	1.87ddd(13.5,11,4)	1.89ddd(13.5,11,4)	1.92ddd(13.5,11.5,4)	1.96m	1.87m	1.96ddd(14.5,11.5,4)	1.93ddd(15,11.5,4)
γ-CH	1.56m	1.56m	Obsc	1.55m	1.55m	1.54m	1.56m
δ^1-CH_3	0.92d(6.5)	0.94d(6)	0.97d(6.5)	0.96d(6.5)	0.91d(6.5)	0.97d(6.5)	0.96d(6)

(*continued on next page*)

Table 6 (*continued*)

	Dolastatin 11 (**1**)	3-Nor (**2**)	3,4-Bis-nor (**3**)	*R,R*-Acc (**4**)	*S,S*-Acc (**5**)	7-Epi (**6**)	7-Nor (**7**)
δ^2-CH_3	0.98d(6.5)	1.00d(6)	1.03d(6.5)	1.01d(6.5)	0.99d(6.5)	1.02d(6.5)	1.00d(6.5)
NCH_3	3.14s	3.16s	3.09s	3.00s	3.22s	3.05s	3.08s
Gly-2							
α^1-CH_2	3.58dd(16,4.5)	3.58dd(16,5)	3.62dd(17.5,4)	3.78dd(16.5,4)	3.50dd(17,4.5)	4.01dd(18,3.5)	3.76dd(17,5.5)
α^2-CH_2	4.46dd(16,7)	4.46dd(16,7)	4.22dd(17.5,7.5)	4.26dd(16.5,4)	4.59dd(17,7)	4.24dd(18,5)	4.38dd(17,6)
NH	7.35dd(7,4.5)	Obsc	7.33dd(7.5,4)	7.23m*	7.19m	7.14m	7.15t(6)
Hmp							
α-CH	5.19d(3.5)	5.24d(3)	5.18 d(4)	5.30d(3.5)	5.15d(3)	5.36d(3)	5.20d(3)
β-CH	2.07m	2.07m	2.07m	2.08m	2.06m	2.08m	2.07m
γ^1-CH_2	1.23m	1.22m	1.25m	1.17m	1.27m	1.19m	1.27m
γ^2-CH_2	1.47m	1.47m	1.48m	1.48m	1.47m	1.42m	1.47m
γ-CH_3	0.89d(7)	0.89d(8)	0.95d(7)	0.91d(7)	0.90d(7)	0.89d(6.5)	0.89d(6.5)
δ-CH_3	0.88t(7.5)	0.88t(7)	0.90t(7.5)	0.88t(7.5)	0.90t(7.5)	0.87t(7.5)	0.89t(7.5)
	12-Epi-7-nor (**7a**)	10-Nor (**8**)	10,10-Bis-nor (**9**)	11*R*-OH (**10**)	11*R*-OAc (**11**)	12-Epi (**12**)	Majusculamide C (**13**)
Map							
α-CH	2.80m	Obsc	2.88qd(7,2.5)	2.73qd(7,2.5)	3.12qd(7.5,2.5)	2.75m	2.80qd(8,2.5)
β-CH_3	1.15d(7)	1.16d(7)	1.16d(7)	1.14d(7.5)*	1.26d(7)	1.12d(7)	1.11d(7)
β-CH	4.37m	4.53m	4.22m	4.66m	3.71m	Obsc	4.52m
γ^1-CH_2	Obsc	Obsc	Obsc	Obsc	Obsc	Obsc	1.46m
γ^2-CH_2	Obsc	Obsc	Obsc	Obsc	1.78dqd(14,7.5,3)	Obsc	1.56m
δ-CH_3	0.94t(7)	0.88-0.95	0.96t(7)	0.94t(7.5)	0.94t(7.5)	0.95t(7)	0.94t(7.5)
NH	6.95d(10)	7.04d(8)*	6.98br d(4)*	6.72d(9)	7.72d(6.5)	6.91d(10)	7.08d(9.5)
Ala							
α-CH	CH_2 3.56dd(16,6) 4.12dd(16,6)	4.50p(7)*	4.54m	4.45p(7)	4.73p(6.5)	4.61p(7)	4.44p(6.5)
β-CH_3	—	1.20d(7.5)*	1.27d(7)*	1.08d(7)*	1.01d(6.5)	1.08d(7)	1.06d(6)
NH	7.53t(6)	7.62d(8)	Obsc	7.48d(7.5)	7.00d(6.5)	7.52d(8)	7.80d(8.5)
Ibu							
β-CH	—	—	—	3.54br s	4.84d(2.5)	—	—
β^1-CH_3	1.46s	1.19d(7)*	α-CH_2 3.29d(15)	1.17s	1.11s	1.52s	1.49s
β^2-CH_3	1.55s	α-CH obsc	α-CH_2 3.32d(15)	1.38s	1.29s	1.52s	1.51s
γ-CH	4.90p(7)	4.50p(7)*	4.54m	4.18br p(6.5)	4.01br p(6.5)	4.98p(6.5)	4.93p(7)
δ-CH_3	1.25d(6.5)	1.33d(7)*	1.34d(7.5)*	0.81d(6.5)	1.50d(6.5)	1.22d(6.5)	1.14d(7)
NH	7.28d(8)	Obsc	6.91d(7)*	7.32d(10.5)	6.66d(8.5)	7.31d(8)	7.34d(5.5)
Ac	—	—	—	—	2.14s	—	—
Tyr							
α-CH	4.98m	5.08t(7.5)	5.20t(7.5)	4.92t(7)	4.95dd(11,3)	5.04t(8)	5.16t(7.5)
β^1-CH_2	2.80m	2.71dd(14,7)	2.75dd(14,8)	2.89dd(14,8)	2.71dd(14.5,11)	2.76-2.79m	2.82dd(14,7.5)
β^2-CH_2	3.24dd(14,8)	3.50dd(14,8)	3.48dd(14,7)	3.20dd(14,6.5)	3.44dd(14.5,3)	3.27dd(13.5,8)	3.26dd(14,7)
δ-Ar	7.15d(8.5)	7.21d(8)	7.19d(8.5)	7.15d(8.5)	7.15d(8.5)	7.14d(9)	7.15d(8.5)
ε-Ar	6.83d(8.5)	6.92d(8)	6.87d(8.5)	6.83d(8.5)	6.81d(8.5)	6.83d(9)	6.82d(8.5)
OCH_3	3.77s	3.86s	3.80s	3.77s	3.74s	3.77s	3.76s
NCH_3	3.00s*	2.88s*	2.99s*	3.02s*	2.81s*	2.99s	2.97s

Val							
α-CH	4.95d(10.5)	4.93d(10.5)	4.83d(11)	4.83d(10.5)	4.48d(10.5)	4.89d(10.5)	4.81d(11.5)
β-CH	2.28dh(10.5,6.5)	2.31m	2.27m	2.23m	2.14m	2.28m	2.24m
γ^1-CH_3	0.50d(6.5)	0.65d(6.5)	0.47d(6.5)	0.40,0.29d(6.5)	0.14d(6.5)	0.57d(6.5)	0.42d(6.5)
γ^2-CH_3	0.79d(6.5)	0.81d(6.5)	0.76d(6.5)	0.74,0.62d(6.5)	0.62(6.5)	0.81d(6.5)	0.76d(7)
NCH_3	2.98s*	2.95s*	2.90s*	2.98s*	2.85s*	2.93s	2.96s
Gly^1							
α^1-CH_2	3.65dd(17,2)	3.67dd(18,2)*	3.76-3.82m	3.48dd(17.5,2)	3.91dd(18,2)	3.65dd(18,2)	3.93dd(17,2)
α^2 -CH_2	4.42dd(17,7)*	4.43dd(18,7)	4.28dd(17.5,6.5)*	4.52dd(17.5,8)	4.29dd(18,6)	4.44dd(18,7.5)	4.63dd(17,8)
NH	7.12m	Obsc	7.15br t(4)*	7.01br d(8)	7.00br d(6)	Obsc	7.60br d(8)
Leu							
α-CH	5.20dd(10,5)	5.36dd(9,6.5)	5.13dd(11,5)	5.08dd(10,5)	5.39dd(11,5)	5.20dd(10.5,5.5)	4.91d(10.5)
β^1-CH_2	1.62m	Obsc	Obsc	Obsc	1.70ddd(14.5,10,4)	Obsc	CH 2.08m
β^2-CH_2	1.82ddd(13,10,5)	1.72m	1.80ddd(14,11,4)	1.81ddd(14.5,10,4)	1.91ddd(14.5,11,3.5)	1.81ddd(13,10.5,4)	–
γ-CH	Obsc	Obsc	Obsc	Obsc	1.78m	Obsc	CH_2 1.08,1.43m
δ^1-CH_3	0.87–0.90	0.88–0.95	Obsc	0.90d(6.5)	0.94d(6.5)	0.89–0.94	0.92d(6.5)
δ^2-CH_3	0.95d(6.5)	0.88–0.95	Obsc	0.98d(6)	0.98d(6.5)	0.89–0.94	γ-CH_3 1.05d(6.5)
NCH_3	3.06s	3.04s	3.04s	3.07s	2.94s	3.10s	3.23s
Gly^2							
α^1-CH_2	3.80dd(17,2)	3.69dd(17.5,2)*	3.76-3.82m	3.90dd(16,5.5)	4.12dd(17,3.5)	3.71dd(16.5,4.5)	3.56dd(16,7)
α^2-CH_2	4.34dd(17,7)*	4.50–4.54m	4.38dd(17,6.5)*	4.37dd(16,6)	4.34dd(17,7.5)	4.48dd(16.5,7)	4.45dd(16,6.5)
NH	7.22m	Obsc	7.11br t(6)*	7.39t(6)	7.15dd(7.5,3.5)	7.34dd(7,4.5)	7.38t(6.5)
Hmp							
α-CH	5.28d(3)	5.22d(4)	5.22d(3)	5.35d(3)	5.32d(3.5)	5.27d(3)	5.21d(3.5)
β-CH	2.08m	1.99m	2.08m	2.09m	2.04m	2.07m	2.09m
γ^1-CH_2	Obsc	Obsc	Obsc	1.22m	1.22m	Obsc	1.25m
γ^2-CH_2	1.49m	Obsc	Obsc	Obsc	1.44m	Obsc	1.44m
γ-CH_3	0.87-0.90	0.88-0.95	0.90d(7.5)	0.88d(7)	0.90d(7)	0.89d(7.5)	0.90d(6.5)
δ-CH_3	0.87-0.90	0.88-0.95	0.90t(7.5)	0.88t(7.5)	0.88t(7.5)	0.88t(7.5)	0.89t(7)
	57-Nor (**14**)	3,4,7-Tris-nor (**15**)	3,4-Bis-nor-11-OH (**16**)	7-Nor-11-OH (**17**)	3,4,7-Tris-nor-11-OH (**18**)	1,2-Seco (**19**)	des-Hmp (**21**)
Map							
α-CH	2.81m	CH_2 2.62ddd(16.5,8,2.5) 2.70ddd(16.5,9,3)	CH_2 2.65,2.66m	Obsc	CH_2 2.66m	Obsc	2.72qd(7,4)
β-CH_3	1.11d(7)	—	—	1.17d(7)	—	Obsc	1.41d(7)*
β-CH	4.50m	CH_2 3.52,3.95m	CH_2 3.65,3.74m	4.30m	CH_2 Obsc	Obsc	Obsc
γ^1-CH_2	Obsc	—	—	Obsc	—	Obsc	Obsc
γ^2-CH_2	Obsc	—	—	Obsc	—	Obsc	Obsc
δ-CH_3	0.95t(7.5)	—	—	0.89–0.93	—	0.85–1.05	0.88t(7.5)
NH	7.08d(10)	7.38d(9)	7.56t(6)	7.40d(9)	Obsc	6.28d(8)*	6.72d(7)*
Ala							
α-CH	4.45p(7)	CH_2 3.84dd(17,4) 4.18dd(17,4)	4.70p(7)	CH_2 3.64dd(15.5,5.5) 4.03dd(15.5,5.5)	CH_2 3.61dd(16.5,3) 4.20dd(16.5,6.5)	Obsc	4.41p(7)
β-CH_3	1.08d(7)	—	1.21d(6.5)	—	—	Obsc	1.19d(7)*
NH	7.74d(8.5)	6.99br t(4)	6.78d(8)	7.41br s	Obsc	7.32,7.40m*	7.50d(8.5)

(*continued on next page*)

Table 6 (*continued*)

	57-Nor (**14**)	3,4,7-Tris-nor (**15**)	3,4-Bis-nor-11-OH (**16**)	7-Nor-11-OH (**17**)	3,4,7-Tris-nor-11-OH (**18**)	1,2-Seco (**19**)	des-Hmp (**21**)
Ibu							
β-CH	—	—	3.53dd(5,2.5)	3.56d(2)	3.55m	—	—
β^1-CH_3	1.45s	1.41s	1.17s	1.20s	1.21,1.16s	Obsc	1.34s
β^2-CH_3	1.51s	1.59s	1.31s	1.34s	1.30,1.21s	Obsc	1.57s
γ-CH	4.92p(7)	4.94p(7)	4.01br p(7)	4.05br p(7)	4.05m	Obsc	4.95p(7)
δ-CH_3	1.14d(7)	1.26d(7)	0.88,1.08d(6.5)	0.89–0.93	0.89–0.98,1.08d(7)	Obsc	1.16d(7)*
NH	7.14m	6.44d(7)	Obsc	6.65d(8.5)	Obsc	6.47,6.54m*	6.83d(6)*
Tyr							
α-CH	5.13t(7.5)	5.10dd(10.5,4)	4.89dd(9,6)	4.86dd(8,6.5)	4.86m	Obsc	5.04dd(9,6)
β^1-CH_2	2.83dd(14,8)	2.79dd(14.5,10.5)	2.87dd(14,9)	Obsc	Obsc	Obsc	2.75dd(14.5,9)
β^2-CH_2	3.26dd(14,7)	3.38dd(14.5,4)	3.25dd(14,6)	3.24dd(14,6)	3.27,3.18dd(14,6)	Obsc	3.44dd(14.5,6)
δ-Ar	7.15d(8.5)	7.13d(8.5)	7.15d(8.5)	7.15d(8.5)	7.15,7.13d(8.5)	7.10-7.13d(8.5)	7.15d(8.5)
ε-Ar	6.82d(8.5)	6.82d(8.5)	6.83d(8.5)	6.83d(8.5)	6.83d(8.5)	6.83d(8.5)	6.84d(8.5)
OCH_3	3.76s	3.74s	3.76s	3.77s	3.76,3.77s	3.74s	3.76s
NCH_3	2.96s*	2.93	2.99,2.93s*	3.00,2.85s*	2.98,2.95s*	2.95,2.98s*	2.95s*
Val							
α-CH	4.80d(10.5)	4.58d(10.5)	4.85d(10)	4.80d(10.5)	4.85,4.72d(10.5)	5.10d(10.5)	4.67d(10.5)
β-CH	2.25dh(10.5,6.5)	2.15dh(10.5,6.5)	2.22dh(10,6.5)	2.20dh(10.5,6.5)	2.22m	Obsc	2.22m
γ^1-CH_3	0.39d(6.5)	0.16d(6.5)	0.39,0.27d(6.5)	0.38,0.16d(6.5)	0.42,0.40d(6.5)	0.27,0.48d(6.5)	0.37d(6.5)
γ^2-CH_3	0.76d(6.5)	0.63d(6.5)	0.73,0.67d(6.5)	0.73,0.60d(6.5)	0.75,0.71d(6.5)	0.68,0.70,0.75d(6.5)	0.70d(6.5)
NCH_3	2.98s*	2.88s	2.94,2.89s*	2.96,2.84s*	2.97,2.91s*	2.88,2.89,2.90s*	2.94s*
Gly^1							
α^1-CH_2	3.61dd(18,2)	3.81dd(17.5,2)	3.75m*	3.69dd(17.5,2)	3.69dd(17.5,2)	Obsc	3.94dd(17.5, 2)
α^2-CH_2	4.42dd(18,7.5)	4.42dd(17.5,6.5)	4.44dd(17.5,6.5)	4.36dd(17.5,6.5)*	4.35,4.45dd(17.5,8)	Obsc	4.37dd(17.5, 7)
NH	7.41br d(7.5)	6.75dd(6.5,2)	6.96m*	7.01m*	Obsc	6.90,6.95d(7)*	7.06br d(7)
Leu							
α-CH	5.38dd(11,5)	5.24dd(11,5)	5.38dd(10.5,6)	5.28dd(10,4.5)	5.39,5.36dd(10,5)	Obsc	5.38dd(10,6)
β^1-CH_2	1.65m	1.81ddd(14.5,9.5,5)	1.69ddd(15,9,6)	1.60ddd(14.5,10.5,5.5)	Obsc	Obsc	Obsc
β^2-CH_2	1.88ddd(14,11,4.5)	1.93ddd(14.5,11,4.5)	1.86ddd(15,10.5,5)	1.85ddd(14.5,10,4)	Obsc	Obsc	Obsc
γ-CH	Obsc	Obsc	Obsc	Obsc	Obsc	Obsc	Obsc
δ^1-CH_3	0.94d(7)*	0.97d(6.5)*	0.93d(6.5)*	0.89–0.93	0.89–0.98	0.85–1.05	0.92d(6.5)
δ^2-CH_3	1.00d(6.5)	1.03d(6.5)	0.99d(6.5)	0.98d(6.5)	0.89–0.98	0.85–1.05	0.98d(6.5)
NCH_3	3.16s	3.06s	3.01,2.97s*	3.02,2.91s*	3.02,2.98s	3.02,3.06s	3.01s
Gly^2							
α^1-CH_2	3.59dd(16,4.5)	3.64dd(17.5,4)	3.87dd(17.5,2)*	3.83dd(17,4)	Obsc	Obsc	3.55dd(17,3)
α^2-CH_2	4.47dd(16,7)	4.19dd(17.5,7)	4.29dd(18,6)	4.33dd(17,6)*	4.28,4.35dd(18,6)	Obsc	4.70dd(17,9)
NH	7.34dd(7,4.5)	7.69dd(7,4)	7.13m*	7.17m*	Obsc	6.69d(7)*	6.59br d(9)
Hmp							
α-CH	5.15d(3.5)	5.11d(4)	5.30d(2.5)	5.26d(4)	5.13d(4.5)	Obsc	—
β-CH	2.34hd(7,3.5)	2.07m	2.08m	2.05m	2.08m	Obsc	—
γ^1-CH_2	—	1.25m	Obsc	Obsc	Obsc	Obsc	—
γ^2-CH_2	CH_3 0.93d(7)	1.49m	Obsc	Obsc	Obsc	Obsc	—
γ-CH_3	0.93d(7)*	0.96d(7)*	0.95d(6.5)*	0.89–0.93	0.89–0.98	0.85–1.05	—
δ-CH_3	—	0.91t(7.5)	0.90t(7.5)	0.89t(7.5)	0.89–0.98	0.85–1.05	—

similar ones in the same column. All analogues were characterized by mass as well as NMR spectra.

About half as much of the 7-epimer **6** as dolastatin 11 (**1**) was formed as a by-product in the dolastatin 11 (**1**) synthesis. It was separated by HPLC; retention times for 7-epidolastatin 11 (**6**) and dolastatin 11 (**1**) were 43.2 and 45.2 min, respectively. To establish its identity, this by-product was also synthesized starting with Boc-D-Ala for the Ala unit.

The alcohols **10** and **16–18** were made by sodium borohydride reduction of the corresponding ketones (**1**, **3**, **7**, and **15**). The method of Schmidt et al.[5] using ether/ethanol at −20 °C was generally used; the method of Winterfeldt et al.[6] gave similar results. In each case, a new multiplet appeared in the NMR at δ3.53–3.56 for the Ibu-β-CH proton, and large changes in chemical shifts occurred for the other Ibu protons. In the reduction of dolastatin 11 (**1**), 3,4-bis-nordolastatin 11 (**3**), and 7-nordolastatin (**7**), one stereoisomer predominated to the extent of at least 80%, presumably the 11*R* stereoisomer since the ketonic carbonyl carbon is much more accessible from the side which gives the 11*R* alcohol in the low-energy conformations. In contrast, 3,4,7-tris-nordolastatin (**15**) gave about equal amounts of both stereoisomers, and the mixture was tested in this case. In Table 6, the NMR parameters of alcohols are given first for the stereoisomer believed to be 11*R*, and then the most characteristic peaks are given for what is presumed to be the 11*S* stereoisomer.

4. Biological testing of dolastastin 11 (1) and analogues 2–21

A summary of the results is given in Table 7. All analogues were tested against six human cancer cell lines; the cell lines used most often are listed. Where it was measured, the activity against P388 murine leukemia is given as well. All analogues were also tested for their ability to stimulate the polymerization of actin.[3] These results will be discussed in the next section.

The results of testing dolastatin 11 (**1**) and analogue **3** against about 60 human cancer cell lines are given in Table 8. Dolastatin 11 (**1**) shows GI_{50}s between 1.3 nM (NCI-H460 lung cancer) and 2.8 mM, whereas 3,4-bis-nordolastatin 11 (**3**) has GI_{50}s between 12 nM (COLO 205 colon cancer) and 15 mM. Surprisingly, the analogue **3** has the best LC_{50}, 0.24 mM, again with COLO 205 colon cancer.

5. Design and SAR of dolastatin 11 (1) analogues

Analogues **19–21**, which lack the 30-membered ring, were inactive in all tests, suggesting that this large ring may be necessary for activity. Compound **19** is the hydroxy acid obtained by hydrolysis of dolastatin 11 (**1**) with mild alkali. Acyclic nonadepsipeptide **20** is the last intermediate in the synthesis of dolastatin 11 (**1**). des-Hmp-dolastatin 11 (**21**), with a 27-membered ring, was prepared by omitting the step in which Hmp is added. All other analogues had the 30-membered ring and showed at least some activity.

The synthesis of majusculamide C (**13**), isolated from the blue-green alga *Lyngbya majuscula*,[7] verified that it differs from dolastatin 11 (**1**) only in the substitution of Ile for Leu. It was as active as dolastatin 11 (**1**) in most tests, but did not bind quite as well to actin.[3]

The Hmp analogue 57-nordolastatin 11 (**14**, analogous to 57-normajusculamide C,[8] which accompanies majusculamide C (**13**) in *L. majuscula*) was almost as active as dolastatin 11 (**1**).

The other analogues involved changes in the Map, Ala, and Ibu units. Dolastatin 11 (**1**) stereoisomers 7-epidolastatin 11 (**6**), and 12-epidolastatin 11 (**12**) were prepared in an effort to learn the identity of the stereoisomer formed in about a 1:2 ratio with dolastatin 11 (**1**) in the synthesis. It proved to be 7-epidolastatin 11 (**6**), indicating that considerable epimerization occurs when the Ala-Map amide bond is made. We have not been able to reduce the amount of epimerization in this step of the synthesis of dolastatin 11 (**1**) itself by changing the coupling agent, but have avoided this type of by-product in some derivatives by removing the Ala chiral center as described below. It is not known why this epimerization occurs; it may be related to Map being a β-amino acid and/or Hmp being a hydroxy acid. The formation of 7-epidolastatin 11 (**6**) reduces the yield of dolastatin 11 (**1**) and necessitates their separation by HPLC (as noted earlier, 7-epidolastatin 11 (**6**) comes off 2 min before dolastatin 11 (**1**) under our best conditions). Dolastatin 11 (**1**) epimers **6** and **12** unexpectedly had greatly reduced activity compared to dolastatin 11 (**1**); possible reasons will be discussed in the next section.

3,4-Bis-nordolastatin 11 (**3**), in which β-alanine replaces Map, was prepared with a view to shortening the synthesis by the eight steps used to prepare Map. Because it would be much more economical to synthesize than dolastatin 11 (**1**), analogue **3** was chosen for further testing. It binds strongly to actin, and on the average has about 20% of the activity against 60 human tumors as dolastatin 11 (**1**; Table 8). It was not accompanied by an appreciable amount of a 7-epi compound, possibly because the Ala-Map amide bond is formed much faster without steric hindrance from a 4-ethyl group.

3-Nordolastatin 11 (**2**) was prepared by leaving out a methylation step in the preparation of Map. It is intermediate between dolastatin 11 (**1**) and 3,4-bis-nordolastatin 11 (**3**) in activity showing that both the 4-ethyl group and the 3-methyl group contribute to the activity.

7-Nordolastatin 11 (**7**) was synthesized to avoid the problem of getting a 7-epimer. It had strong cytotoxic activity, but was unfortunately accompanied by about 30% of 12-epi-7-nordolastatin (**7a**), whose identity was established by an independent synthesis starting from Boc-D-Ala for the Ibu unit. The 12-epimer appears in this synthesis and not in the synthesis of dolastatin 11

Table 7. Biological testing results on **1–21**: actin binding, murine leukemia, and six human tumors

Compound	Activity summaries		EC_{50} μM bind actin	ED_{50} (μg/mL)	GI_{50}, human cancer cell lines (μg/mL)					
	Actin	Cells		P388 leukemia	NCI-H460 lung-nsc	OVCAR-3 ovarian	BXPC-3 pancreas-a	SF-295 CNS	KM20L2 colon	DU-145 prostate
Dolastatin 11 (**1**)	V strong	V strong	9.5	0.0027	<0.001	0.0075		0.034	0.0047	
					0.0013[a]	0.023[a]		0.13[a]		0.22[a]
Map analogues										
3-Nor (**2**)	Strong	Strong	22		0.014		0.092		0.063	0.34
3,4-Bis-nor (**3**)	Strong	Medium	32	0.017	0.097		0.66			>1
					0.076[a]	0.62[a]		0.31[a]		2.8[a]
R,R-Acc (3-epimer) (**4**)	Strong	Weak	23		8.7		26.0		8.7	12.5
S,S-Acc (4-epimer) (**5**)	None	Weak	>50		7.0		>10		7.4	3.8
Ala analogues										
7-Epi (**6**)	None	Weak	>50	0.24	0.43	3.4	2.9	3.5	1.3	3.4
7-Nor (**7**)	Strong	Strong	21	0.018	<0.001	0.70	0.36	0.38	0.063	0.64
Ibu analogues										
10-Nor (**8**)	None	Medium	>50	0.039	0.058	0.90	0.46	0.51	0.38	0.63
10,10-Bis-nor (**9**)	Strong	Medium	23	0.042	0.021	0.59	0.37	0.42	0.23	0.48
11*R*-OH (**10**)	Medium	Strong	42	0.011	0.0003	0.18	0.075	0.16	0.039	0.25
11*R*-OAc (**11**)	Medium	Medium	41	0.050	0.032	0.63	0.41	0.45	0.27	0.37
12-Epi (**12**)	None	Weak	>50	0.28	0.44	4.1	3.4	3.6	2.9	2.4
Leu analogue										
Majusculamide C (**13**)	Strong	V strong	19	0.0033	<0.001	<0.001		0.033	<0.001	
Hmp analogue										
57-Nor (**14**)	Strong	Strong	22		0.0048		0.030		0.014	0.19
Map, Ala analogue										
3,4,7-Tris-nor (**15**)	Strong	Weak	20		3.5		24.9		10.4	>10
Map, Ibu analogue										
3,4-Bis-nor-11-OH (**16**)	Strong	Weak	21		3.8		9.2		5.7	>10
Ala, Ibu analogue										
7-Nor-11-OH (**17**)	Strong	Medium	20		0.16		0.59		0.43	2.9
Map, Ala, Ibu analogue										
3,4,7-Tris-nor-11-OH (**18**)	Strong	Weak	20		27.8		>10		4.7	>10
Open analogues										
1,2-Seco (**19**)	None	None	>50	>10	>1	>1	>1	>1	>1	>1
13,14-Seco (**20**)	None	None	>50		>10		>10		>10	>10
Unit-deletion analogue										
des-Hmp (**21**)	None	None	>50	>10	>10	>10	>10	>10	>10	>10

[a] Results from the National Cancer Institute.

(**1**) and most of its analogues because most of the intermediates for the Ibu-Ala unit in the latter syntheses are crystalline, whereas those for the Ibu-Gly unit in the former are not crystalline; thus, products of partial epimerization α to the ketone carbonyl group are removed by recrystallization in the latter cases but not in the former case.

3,4,7-Tris-nordolastatin 11 (**15**) combines the above simplifications in the Map and Ala units. While it bound well to actin, it had only weak activity against cancer cells, either because it does not get through the cell wall efficiently or because it is metabolized much more readily than dolastatin 11 (**1**). Three other analogues (**4**, **16**, and **18**) have strong actin-binding activity but only weak activity in cells. **15**, **16**, and **18** lack two or three methyl groups, and might well be metabolized much more rapidly than dolastatin 11 (**1**; hydrolysis of an amide or ester bond would presumably cause loss of activity as noted above). Analogue **4** still has all the carbon atoms of dolastatin 11 (**1**) and should not be much more readily metabolized than dolastatin 11 (**1**), so we postulate that it is inactive in cells because it does not get through cell walls efficiently, as discussed further below.

10-Nordolastatin 11 (**8**) and 10,10-bis-nordolastatin 11 (**9**) were made by omitting one or two methylation steps, respectively. They had only medium activity, suggesting that it is worthwhile to put in both methyl groups.

Derivatives **4** and **5**, in which (*R,R*)- and (*S,S*)-2-aminocyclopentanecarboxylic acids (Acc), respectively, replace Map, were synthesized as analogues with restricted

Table 8. In vitro testing results for dolastatin 11 (1) and 3,4-bis-nordolastatin 11 (3), molar units

Panel	Cell line	Dolastatin 11 (1)			3,4-Bis-nordolastatin 11 (3)		
		GI50	TGI	LC50	GI50	TGI	LC50
Leukemia	CCRF-CEM	4.0E−08	>1.0E−05	>1.0E−05	3.2E−08	1.2E−05	>5.0E−05
	HL-60(TB)	1.4E−08	>1.0E−05	>1.0E−05			
	K-562	8.9E−07	>1.0E−05	>1.0E−05	4.7E−08	>5.0E−05	>5.0E−05
	MOLT-4	1.5E−06	>1.0E−05	>1.0E−05	1.5E−05	>5.0E−05	>5.0E−05
	RPMI-8226	2.7E−07	>1.0E−05	>1.0E−05	4.3E−08	7.2E−06	>5.0E−05
	SR		>1.0E−05	>1.0E−05	4.6E−07	1.3E−05	>5.0E−05
Lung	A549/ATCC	1.9E−08	6.3E−06	>1.0E−05	6.4E−07	>5.0E−05	>5.0E−05
	EKVX	2.1E−07	>1.0E−05	>1.0E−05	3.4E−06	3.2E−05	>5.0E−05
	HOP-62				1.3E−06	5.4E−06	2.9E−05
	HOP-92	2.1E−07	8.2E−07	>1.0E−05	5.1E−07	3.0E−06	>5.0E−05
	NCI-H226	3.1E−07	7.1E−06	>1.0E−05	5.1E−06	2.9E−05	>5.0E−05
	NIC-H23	3.7E−07	>1.0E−05	>1.0E−05	2.8E−07	>5.0E−05	>5.0E−05
	NCI-H322M	4.1E−07	4.7E−06	>1.0E−05	7.3E−06	>5.0E−05	>5.0E−05
	NCI-H460	1.3E−09	>1.0E−05	>1.0E−05	3.1E−08	>5.0E−05	>5.0E−05
	NCI-H522				2.6E−08		>5.0E−05
Colon	COLO 205	4.9E−09		>1.0E−05	1.2E−08	5.5E−08	2.4E−07
	HCC-2998	2.3E−08	>1.0E−05	>1.0E−05	8.2E−08	>5.0E−05	>5.0E−05
	HCT-116	1.7E−07	>1.0E−05	>1.0E−05	2.9E−08	>5.0E−05	>5.0E−05
	HCT-15	6.7E−07	>1.0E−05	>1.0E−05	1.8E−06	>5.0E−05	>5.0E−05
	HT29	2.2E−08	>1.0E−05	>1.0E−05	1.9E−06	1.4E−05	>5.0E−05
	KM12	3.9E−07	>1.0E−05	>1.0E−05		>5.0E−05	>5.0E−05
	SW-620	2.7E−06	>1.0E−05	>1.0E−05	4.9E−08	>5.0E−05	>5.0E−05
CNS	SF-268	1.0E−07	>1.0E−05	>1.0E−05	2.1E−07	5.9E−06	3.8E−05
	SF-295	1.3E−07	1.5E−06	>1.0E−05	2.7E−07	2.8E−05	>5.0E−05
	SF-539	1.3E−08	4.1E−08	6.7E−07	2.3E−07	>5.0E−05	>5.0E−05
	SNB-19	2.8E−07	7.7E−07	>1.0E−05	2.8E−07	1.4E−05	>5.0E−05
	SNB-75	1.8E−07	2.1E−06	>1.0E−05	3.6E−07	3.1E−06	4.6E−05
	U251	2.7E−08	>1.0E−05	>1.0E−05	4.8E−08	9.7E−07	8.0E−06
Melanoma	LOX IMVI				2.0E−07	1.3E−05	>5.0E−05
	MALME-3M	1.2E−07	>1.0E−05	>1.0E−05	3.5E−07	1.8E−05	>5.0E−05
	M14				1.2E−06	2.8E−05	>5.0E−05
	SK-MEL-2	8.3E−08	5.6E−07	>1.0E−05	1.8E−07	3.1E−06	>5.0E−05
	SK-MEL-28	7.3E−08	>1.0E−05	>1.0E−05	7.0E−08	2.6E−05	>5.0E−05
	SK-MEL-5	2.9E−08	2.5E−07	>1.0E−05	6.9E−08	2.3E−07	7.3E−06
	UACC-257	7.9E−08	6.9E−06	>1.0E−05	1.6E−07	1.4E−05	>5.0E−05
	UACC-62	1.4E−07	>1.0E−05	>1.0E−05	2.9E−07	>5.0E−05	>5.0E−05
Ovarian	IGROVI	4.3E−08	9.7E−07	>1.0E−05	5.4E−07	3.4E−06	>5.0E−05
	OVCAR-3	2.3E−08	1.1E−07	3.4E−06	6.4E−07	1.6E−05	>5.0E−05
	OVCAR-4	8.7E−08	8.1E−07	>1.0E−05	8.1E−08	7.9E−07	>5.0E−05
	OVCAR-5	1.2E−06	>1.0E−05	>1.0E−05	2.6E−06	>5.0E−05	>5.0E−05
	OVCAR-8	2.2E−07	>1.0E−05	>1.0E−05	3.8E−06	1.9E−05	>5.0E−05
	SK-OV-3	2.5E−07	3.0E−06	>1.0E−05			
Renal	786-0				1.3E−06	9.4E−06	>5.0E−05
	A498	4.4E−07	>1.0E−05	>1.0E−05	2.2E−06	8.0E−06	2.6E−05
	ACHN	3.5E−08	3.5E−07	>1.0E−05	2.6E−06	2.9E−05	>5.0E−05
	CAKI-1	4.3E−08	1.7E−07	6.1E−07	1.6E−06	6.6E−06	2.0E−05
	RXF 393	2.5E−08	6.1E−08	7.8E−07	6.7E−07	1.9E−06	5.8E−06
	SN12C	3.0E−07	>1.0E−05	>1.0E−05	2.4E−07	>5.0E−05	>5.0E−05
	TK-10	1.1E−07	3.9E−07	2.6E−06	1.1E−06	5.3E−06	3.5E−05
	UO-31	1.7E−07	3.8E−07	8.7E−07	7.8E−06	1.7E−05	3.5E−05
Prostate	PC-3	1.4E−07	4.7E−06	>1.0E−05	2.0E−07	2.0E−05	>5.0E−05
	DU-145	2.2E−07	>1.0E−05	>1.0E−05	2.1E−06	3.1E−05	>5.0E−05
Breast	MCF7	8.8E−08	6.8E−07	>1.0E−05	2.5E−07	>5.0E−05	>5.0E−05
	NCI/ADR-RES	2.8E−06	>1.0E−05	>1.0E−05	2.1E−05	>5.0E−05	>5.0E−05
	MDA-MB-231/ATCC	2.9E−08	1.1E−06	>1.0E−05	1.7E−07	7.4E−06	>5.0E−05
	HS 578T	4.0E−07	>1.0E−05	>1.0E−05	6.0E−07	1.6E−05	>5.0E−05
	MDA-MB-435	9.1E−08	>1.0E−05	>1.0E−05	5.0E−07	3.6E−05	>5.0E−05
	MDA-N	1.1E−07	>1.0E−05	>1.0E−05	3.7E−07	>5.0E−05	>5.0E−05
	BT-549	2.1E−07	4.5E−07	1.0E−06	2.6E−06	1.1E−05	4.6E−05
	T-47D	4.6E−07	>1.0E−05	>1.0E−05			

rotation about the 2–3–4–5 bond in the Map unit. *R,R*-Acc is restricted to values of −60° to −180° for this bond, whereas *S,S*-Acc has values of +60° to +180°. The *S,S*-Acc derivative **5** should be a good model for

the C1 and C15 conformations (with values of +76° and +69°, respectively), especially the former, which is calculated to be the lowest energy conformation of **5** as well as **1**. The *R,R*-Acc derivative **4** should serve as a model for the other low-energy conformations: C2–C4, C7, W1, and W5, which have values from −64° to −86°. When all efforts to resolve a mixture of *R,R*- and *S,S*-Acc[9] failed, the racemic mixture was carried through the synthesis with the hope that the final cyclic depsipeptides **4** and **5** could be separated and identified by NMR; fortunately, this was the case. Surprisingly, neither **4** nor **5** was very active in cells. However, the *R,R* derivative **4** bound very strongly to actin, whereas the *S,S* stereoisomer **5** was inactive. The most likely interpretation of these results is that dolastatin 11 (**1**) goes into cells in an *S,S*-type conformation but binds in an *R,R*-type conformation. If this is correct, an analogue restricted to one of these conformations will not be a suitable drug, and only analogues flexible enough to take both conformations have drug potential.

In which conformation does dolastatin 11 (**1**) go through the cell membrane? Probably C1 = W2, which is by far the lowest energy conformation with a positive value for angle 2–3–4–5 and was calculated to be the lowest-energy conformation for most close derivatives as well as for dolastatin 11 (**1**) in chloroform.

Which is the binding conformation? There are several strong candidates with negative values for angle 2–3–4–5: W1 (with the lowest calculated energy in water), C2 = W18, C3 = W3, and C4 = W4. This will be discussed further in the next section.

Alcohols **10** and **16–18** were prepared to learn their activities and to provide a handle for the preparation of prodrugs. All four bind strongly to actin. The three lacking alkyl groups have greatly reduced activity in cells, but **10**, from reduction of dolastatin 11 (**1**), retains strong activity in cells (it is better than dolastatin 11 (**1**) against NCI-H460 lung cancer, with GI_{50} 0.3 nM). The acetate **11** made from alcohol **10** is a possible prodrug for alcohol **10**.

6. Conformations of the analogues

The NMR data in Table 6 and molecular mechanics calculations on some of the analogues provide information about their preferred conformations. The most revealing analogues are **4** and **5**, which as noted above are limited to − and + values of the 2–3–4–5 angle, respectively. The *only* low-energy conformation calculated for **5** is like C1 (Fig. 2 shows a superposition of the low-energy conformations of **1** and **5**), and since the NMR shifts for dolastatin 11 (**1**) are similar to those of **5** (Table 6), it is likely that they share this as their predominant conformation. Many of the shifts for dolastatin 11 (**1**) are displaced slightly toward those of **4**, consistent with the presence of small amounts of conformations which, like **4**, have negative values for the 2–3–4–5 angle.

Most of the derivatives (**2**, **5**, **8–10**, **12–14**, and **16–18**) had shifts, which suggest that they are similar in conformation to dolastatin 11 (**1**). Molecular mechanics calculations on many of these derivatives supported this view. In contrast, two derivatives which clearly appear to be like **4**, with predominantly negative angle 2–3–4–5 values, are **6** and **11**, in which the two main strands of the depsipeptide are pushed apart by a methyl group and an acetate group, respectively, destabilizing conformation C1. 3,4-Bis-nordolastatin 11 (**3**) and 3,4,7-tris-nordolastatin 11 (**15**) also favor forms with negative 2–3–4–5 angles from their Val and Ibu shifts, but some of the shifts in their other amino acids suggest that **3** and **15** do not share the same make-up of such forms as do **4**, **6**, and **11**. A ROESY spectrum of 7-epidolastatin 11 (**6**, Table 1) showed that it has the same amide configurations as dolastatin 11 (**1**).

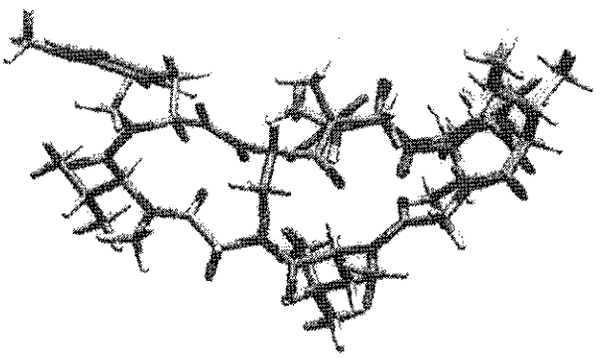


Figure 2. Superposition of the low-energy conformations calculated for **1** and **5** in chloroform.

The predominant conformation of **4**, **6**, and **11** in chloroform is very likely C2 (the lowest-energy calculated conformation for dolastatin 11 (**1**) having a negative 2–3–4–5 angle) for several reasons. First, of the low-energy conformations, only this one has significant changes from C1 in the angles of the 30-membered ring in the Map, Ibu, Val, and Gly2 units (Table 2) and in the Tyr side chain (Table 3) to account for the large differences between the NMR spectra of **1** and the **4**, **6**, **11** group. Second, the NH proton shift changes observed in going from dolastatin 11 (**1**) to 7-epidolastatin 11 (**6**) favor a C2-like conformation for **6**: the Map-NH absorption moves downfield by 1.1 ppm (from strong intramolecular H-bonding in **1** to none in **6**), whereas the other four NH absorptions move upfield due to stronger intramolecular H-bonds in **6** (Table 5). Third, conformation C2 was the lowest-energy conformation calculated for **4**, **6**, and **11** in chloroform (Table 9).

Most of the chemical shifts of 7-nordolastatin (**7**) are about halfway between those of dolastatin 11 (**1**) and 7-epidolastatin 11 (**6**).

What is the most likely binding conformation of dolastatin to F-actin? Since *R,R*-Acc derivative **4** bound strongly to actin, the binding conformation must have a negative 2-3-4-5 angle, which rules out C1. The low-energy candidates with angle 2–3–4–5 negative are C2–C4 and W1. Though C2 is apparently the conformation that is seen in *chloroform* solution by NMR for

Table 9. Energies (kJ/mol) calculated for conformations of dolastatin 11 (**1**) and three derivatives which favor a conformation with a negative 2–3–4–5 angle in *chloroform* solution

Conformation→	C1 = W2	C2 = W18	C3 = W3	C4 = W4	W1
2–3–4–5 Sign→	+	–	–	–	–
D11 (**1**)	0	2.2	5.4	7.7	25.9
R,R-Acc (**4**)	Impossible	0	8.8	2.7	8.8
7-Epi (**6**)	7.2	0	1.1	14.6	12.0
11R-OAc (**11**)	27.3	0	4.5	34.4	4.6

Table 10. Energies (kJ/mol) calculated for conformations of dolastatin 11 (**1**) and selected derivatives in *water*

Conformation→	C1 = W2	C2 = W18	C3 = W3	C4 = W4	W1
2–3–4–5 Sign→	+	–	–	–	–
D11 (**1**)	1.6	9.9	2.7	5.1	0
3-Nor (**2**)	0	29.5	4.8	23.0	1.1
3,4-Bis-nor (**3**)	0	20.7	11.9	6.7	8.9
R,R-Acc (**4**)	Impossible	16.2	4.9	0	3.6
7-Epi (**6**)	2.5	8.3	0	5.2	1.2
11R-OH (**8**)	0	38.5	26.9	6.2	16.9
11R-OAc (**11**)	14.0	12.3	0	19.1	19.0
12-Epi (**12**)	19.4	0	14.6	9.6	10.3

derivatives like **4**, **6**, and **11**, it is calculated to be much higher in energy than the others in the intracellular fluid, *water* (Table 10), and thus is unlikely to be the binding conformation. We favor W1 since it is the lowest energy conformation in water for the most active compound (**1**) and is of low energy in the other highly active compounds, but C3 and C4 are also of fairly low energies in most strongly active derivatives and are reasonable possibilities as well.

From most evidence, the binding side of the molecule appears to be approximately the side from which the molecule is usually viewed (**1** and Fig. 1), since omitting the 'backside' 3- and 7-methyl and 4-ethyl groups and making a five-membered ring in the Map unit (**4**) do not decrease the binding much (Table 7). However, 10,10-bis-nordolastatin 11 (**9**), which lacks two methyl groups on the front side in W1, also binds strongly. One other piece of evidence: 7-epidolastatin 11 (**6**) has low-energy C1 and W1 conformations, yet is not very active; this may indicate that the 7-methyl group of dolastatin 11 (**1**) is involved with the binding site.

7. Summary

Twenty analogues of the natural antitumor agent dolastatin 11 (**1**), including the natural product majusculamide C (**13**), were synthesized and tested for cytotoxicity against human cancer cells and stimulation of actin polymerization. Analogues lacking the 30-membered ring (**19–21**) were inactive. The simplified analogue **3** in which β-alanine replaces Map retained good activity, as did the 11*R* alcohol **10** from borohydride reduction of dolastatin 11 (**1**). The epimers of dolastatin 11 in the Ala and Ibu units (**6** and **12**) had greatly reduced activity. Molecular modeling and NMR evidence were used to study the low-energy conformations. The amide bonds are all trans except for the one between the Tyr and Val units, which is cis. Cyclic 3*S*,4*S* analogue **5** with restricted rotation about the 3,4 bond, which modeled the lowest-energy conformation of dolastatin 11 in chloroform (C1), was inactive in cells and with actin. Its 3*R*,4*R* stereoisomer **4**, which modeled most of the other low-energy conformations, strongly stimulated actin polymerization but was inactive in cells. Thus, the lowest-energy conformation in chloroform (C1) is not the binding conformation, but may well be the conformation in which these molecules go through cancer cell walls. The binding conformation is most likely W1. The binding side of the molecule is probably the side from which the molecule is usually viewed. Thus, an active derivative should have a low-energy C1 conformation to go through the cell wall, a low-energy W1 conformation to bind, and the right shape to bind strongly.

8. Experimental section

NMR spectra were obtained on Bruker spectrometers at 250, 500, or 600 MHz in $CDCl_3$ unless otherwise stated. Mass spectra were obtained on JOEL HX100A (FAB) and Finnigan LCQ spectrometers (ESI). HPLC was performed on a Rainin instrument eluting with water–acetonitile with UV detection. Unless otherwise stated, all reagents and solvents were from Sigma–Aldrich except 1-cyclopentenecarboxylic acid, which was from Alfa–Aesar Co. Most reactions were carried out under argon. Solvent extracts were dried over anhydrous magnesium sulfate before solvent evaporation. HPLC purifications were done on a 21.4 mm ID × 25 cm reverse-phase C-18 column with elution with water-acetonitrile mixtures from 0% to 100% acetonitrile over 40 min at 8 mL/min, except that a 30 min protocol at 3 mL/min was used for difficult separations; the UV detector was set at 280 nm unless otherwise specified.

8.1. Analogue synthesis

Analogues **2–22** were synthesized using the synthetic route developed for dolastatin 11 (**1**),[2] except for the three significant improvements in that synthesis which are included below. The other new reactions are included in the supplementary material.

8.1.1. (2*S*,3*R*)-2-Methyl-3-aminopentanoic acid (Map). Ammonia (850 mL) was distilled into a solution of *N*-tosyl-Map (17 g, 60 mmol) in THF (50 mL) at −70 °C. Sodium (8.2 g, 356 mmol) was added in two portions over 30 min. After 30 min more, the blue soln was warmed to −30 °C for 30 min. The blue color was eliminated by adding solid NH_4Cl, sodium was destroyed by adding water (cautiously!), and the ammonia was allowed to evaporate. The residue was rinsed into a crystallizing dish with NaOH soln (3 M, 25 mL) and water (100 mL), and water and ammonia were evaporated in a hood. The pH was adjusted to 1 with concd HCl and the solution was washed with CH_2Cl_2 (4 × 50 mL). The pH was adjusted to 6.5 with NaOH pellets and the solution was evaporated in a hood. The residual crystals were triturated with EtOH (5 × 60 mL). The soln was filtered, the EtOH was evaporated in a hood, and the residue was dried in a vacuum dessicator to give Map (7.8 g, 100%). As Map is not easily purified by recrystallization, it was converted to Boc-Map, which was easily recrystallized.[2]

8.1.2. N-Me-L-Val-O,N-diMe-L-Tyr-OBn·HCl. Thionyl chloride (50 mL) was added dropwise to a solution of Boc-N-Me-L-Val-O,N-diMe-L-Tyr-OH (10 g, 23.7 mmol) in benzyl alcohol (200 mL) at 0 °C. After heating at 55 °C for 1 h and evaporation in a hood to 1/4 of the volume, ether (150–200 mL) was added until cloudiness occurred. After 16 h at −20 °C, the crystals were filtered and dried in a vacuum dessicator over P_2O_5, giving N-Me-L-Val-O,N-diMe-L-Tyr-OBn·HCl (8.3 g, 78%).

8.1.3. Dolastatin 11 (1), procedure B. To Ibu-L-Ala-Map-Hmp-Gly-NMe-L-Leu-Gly-NMe-L-Val-O,N-di-Me-L-Tyr-OH·TFA (16.7 mg, 0.015 mmol) in DMF (1.5 mL) was added a solution of HBTU (34 mg, 0.090 mmol) and Et_3N (17 μL, 0.120 mmol) in DMF (1.5 mL) dropwise with stirring over 30 min. After 6 h, the solvent was evaporated in an open dish and the residue was triturated with CH_3CN for HPLC, which gave dolastatin 11 (**1**, 6.5 mg, 44%), after separation from 7-epi-dolastatin (**6**, 4.0 mg, 27%).

8.2. Molecular modeling

Molecular modeling studies involving Monte Carlo calculations and energy minimizations were performed using MacroModel[10] (v. 6.0 and 7.0) on an IBM RS/6000 590 with 66 MHz POWER2 architecture and 256MB RAM. Data workup used SGI Indigo2 GUI-Extreme Graphics, 150 MHz MIPS R4400/R4010 FPU and 64MB RAM. Graphics used 50 MHz MIPS R400/R4010 FPU 48MB RAM. An average of 100 structures/day were generated and minimized. Amide bonds were locked in the trans configuration except for the Tyr-Val bond, which was locked cis.

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Supplementary data

Yield, NMR, and MS data for the intermediates and analogues. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.04.040.

References and notes

1. Pettit, G. R.; Kamano, Y.; Kizu, H.; Dufresne, C.; Herald, C. L.; Bontems, R. J.; Schmidt, J. M.; Boettner, F. E.; Nieman, R. A. *Heterocycles* **1989**, *28*, 553.
2. Bates, R. B.; Brusoe, K. G.; Burns, J. J.; Caldera, S.; Cui, W.; Gangwar, S.; Gramme, M. R.; McClure, K. J.; Rouen, G. P.; Schadow, H.; Stessman, C. C.; Taylor, S. R.; Vu, V. H.; Yarick, G. V.; Zhang, J.; Pettit, G. R.; Bontems, R. *J. Am. Chem. Soc.* **1997**, *119*, 2111.
3. Bai, R.; Verdier-Pinard, P.; Gangwar, S.; Stessman, C. C.; McClure, K. J.; Sausville, E. A.; Pettit, G. R.; Bates, R. B.; Hamel, E. *Mol. Pharmacol.* **2001**, *59*, 462.
4. Oda, T.; Crane, Z. D.; Dicus, C. W.; Sufi, B. A.; Bates, R. B. *J. Mol. Biol.* **2003**, *328*, 319.
5. Schmidt, U.; Griesser, H.; Haas, G.; Kroner, M.; Riedl, B.; Schumacher, A.; Sutoris, F.; Haupt, A.; Emling, F. *J. Pept. Res.* **1999**, *54*, 146.
6. Winterfeldt, E.; Nelke, J. M.; Korth, T. *Chem. Ber.* **1971**, *104*, 802.
7. Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236.
8. Mynderse, J. S.; Hunt, A. H.; Moore, R. E. *J. Nat. Prod.* **1988**, *51*, 1299.
9. Yamazaki, T.; Zhu, Y.-F.; Probstl, A.; Chadha, R. K.; Goodman, M. *J. Org. Chem.* **1991**, *56*, 6644.
10. MacroModel, Interactive Molecular Modeling System. Version 6.0, Department of Chemistry, Columbia University, New York, NY, 1977.