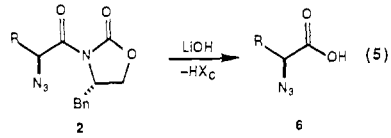
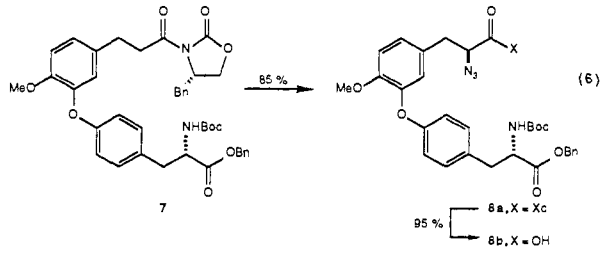
experiments that have not been fully optimized, we have found that the sodium enolate derived from **1** affords good yields of α-diazoimides **3** upon reaction with *p*-nitrobenzenesulfonyl azide, (PNBSA).[18] Thus, the sodium enolate derived from **1** (R = CH_2Ph) and NaHMDS, on treatment with 1.2 equiv of PNBSA (THF, −78 °C, 30 min), afforded an 85% yield of diazocarboximide **3** (R = CH_2Ph) after quenching the reaction with a pH 7 phosphate buffer.

The racemization-free removal of the chiral auxiliary from the α-azido imides **2** has proven to be a facile process (eq 5). Relevant hydrolysis and transesterification studies carried out on the α-azido carboximides diastereomeric to **2** have already been reported.[4] In a similar fashion, the hydrolysis of **2c** (R = CH_2Ph), chosen as a representative unhindered substrate, with 2.0 equiv of LiOH (3:1 THF–H_2O, 0.05 M in **2**, 0 °C, 30 min)[4] afforded the (2*S*) azido acid **6c** (R = CH_2Ph) in quantitative yield with no detectable racemization.[19] Even the highly racemization prone azide **2d** (R = Ph), when hydrolyzed under the above conditions, afforded a quantitative yield of (2*S*)-azidophenylacetic acid (**6d**) (R = Ph) having a minimum enantiomeric purity of 99.5%. In the most sterically demanding case, treatment of **2f** (R = CMe_3) with LiOH as described above (0 °C, 1.5 h) resulted in predominant attack at the oxazolidone carbonyl. However, a dramatic improvement in selectivity was achieved in the hydrogen peroxide-mediated hydrolysis[20] of this substrate. Thus, a 0.05 M solution of **2f** in 3:1 THF–H_2O containing 4 equiv of H_2O_2 was treated with 2.0 equiv of LiOH (0 °C, 30 min) to afford the enantiomerically pure acid **6f**, isolated in 98% yield by a simple extraction procedure, along with a 98% recovery of the chiral auxiliary. This hydrolysis procedure has proven to be the most generally useful protocol yet discovered for regioselective carboximide hydrolysis.[21]



One of the distinct advantages of this azidation reaction is its applicability to the construction of polyfunctional amino acids. The example shown below serves to illustrate this point (eq 6).



Treatment of **7** with 2.2 equiv of KHMDS followed by 1.1 equiv of trisyl azide according to the conditions detailed above afforded an 85% yield of the diastereomerically pure azide **8a** after chromatographic purification. Finally, **8a** may be selectively hydrolyzed to the fully differentiated isodityrosine derivative **8b** without concomitant benzyl ester hydrolysis in 96% yield by using the basic hydrogen peroxide procedure described above.[22]

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Supplementary Material Available: Detailed general procedures for azidation and hydrolysis as well as full spectral data (IR, ^1H NMR, ^{13}C NMR) are provided (4 pages). Ordering information is given on any current masthead page.

The Isolation and Structure of a Remarkable Marine Animal Antineoplastic Constituent: Dolastatin 10[1a]

George R. Pettit,* Yoshiaki Kamano, Cherry L. Herald, Albert A. Tuinman, Fred E. Boettner,[1b] Haruhisa Kizu, Jean M. Schmidt, Lubomir Baczynskyj,[1c] Kenneth B. Tomer,[1d] and Roger J. Bontems

Cancer Research Institute and Department of Chemistry, Arizona State University Tempe, Arizona 85287

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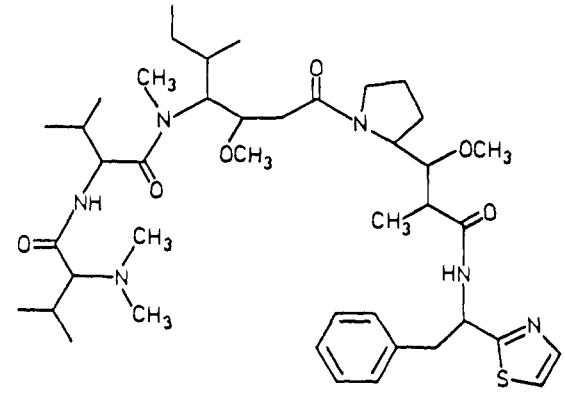
Exceptionally potent biological properties exhibited by the sea hare *Dolabella auricularia* have been recorded for nearly two millennia.[2] In 1972 we found Indian Ocean specimens of this captivating sea hare to yield extracts that proved very effective (over 100% increase in life span) against the U.S. National Cancer Institute's (NCI) murine P388 lymphocytic leukemia (PS system).[3] Subsequently, we isolated nine new (and powerful) cell growth inhibitory and/or antineoplastic peptides designated dolastatins 1–9[2a,b] and two cytotoxic terpenes.[2c] Due to the dolastatins potency, the sea hare seems to require only vanishingly small quantities (ca. ~1 mg each from 100 kg),[2b] making isolation and structural elucidation[2a] of these peptides exceptionally challenging. Now we are pleased to report that our 15-year effort directed at discovering the most important *Dolabella auricularia* antineoplastic constituents has resulted in isolation and structural determination of a unique linear pentapeptide herein named dolastatin 10 (**1**). To our knowledge, dolastatin 10 is the most active



DOLASTATIN 10

1

(17) Previous attempts to effect direct diazo transfer to related imide enolates were unsuccessful: Doyle, M. P.; Dorow, R. L.; Terpstra, J. W.; Rodenhouse, R. A. *J. Org. Chem.* **1985**, *50*, 1663–1666.

(18) The scope of this reaction with other enolates is currently under active investigation.

(19) The optical purity of the azido acids was determined by capillary gas chromatographic analysis of their derived (+)-MTPA-amide methyl esters as previously described in ref 2 and 4.

(20) The enhanced reactivity of hydroperoxide over hydroxide in active ester hydrolysis is well-documented: Jencks, W. P.; Gilcrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622–2637.

(21) For additional examples documenting the scope of this method, see: Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.*, submitted for publication.

(22) This experiment was carried out by J. A. Ellman in this laboratory.

(1) (a) Contribution 136 of the series "Antineoplastic Agents". Consult the following for part 135: Dell'Aquilla, M. L.; Nguyen, H. T.; Herald, C. L.; Pettit, G. R.; Blumberg, P. M. *J. Biol. Chem.*, in press. (b) Polysciences, Inc., Paul Valley Industrial Park, Warrington, PA 18976. (c) Physical and Analytical Chemistry, The Upjohn Co., Kalamazoo, MI 49001. (d) Midwest Center for Mass Spectrometry, The University of Nebraska–Lincoln, Lincoln, NB 68588.

(2) For leading references refer to the following: (a) Pettit, G. R.; Kamano, Y.; Brown, P.; Gust, D.; Inoue, M.; Herald, C. L. *J. Am. Chem. Soc.* **1982**, *104*, 905. (b) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L.; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. J. *J. Nat. Prod.* **1981**, *44*, 482. (c) Pettit, G. R.; Herald, C. L.; Ode, R. H.; Brown, P.; Gust, D. J.; Michel, C. *J. Nat. Prod.* **1980**, *43*, 752.

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0002-7863/87/1509-6883$01.50/0

(lowest dose) antineoplastic substance presently known and has shown, e.g., a 17–67% curative response at 3.25–26 μg/kg against the NCI human melanoma xenograph (nude mouse), 42–138% life extension at 1.44–11.1 μg/kg using the B16 melanoma, and 69–102% life extension at 1–4 μg/kg against the PS leukemia (ED_{50} 4.6 × 10^{-5} μg/mL)!

A combined ethanol–2-propanol extract of *D. auricularia* (~ 1000 kg wet, collected in 1982) was concentrated to an active methylene chloride fraction by a series of solvent partition steps.[4] Extensive column chromatographic separation (steric exclusion and partition on Sephadex, partition and adsorption on silica gel and HPLC) using gradient elution techniques guided by PS bioassay led to 28.7 mg of pure dolastatin 10 (**1**) as a colorless amorphous powder (from methylene chloride–methanol): $C_{42}H_{68}N_6O_6S$ by HREIMS (M^+ obsd. av. 784.4899, calcd for 784.4921); mp 107–112 °C; $[\alpha]^{29}_D$ −68° (*c* 0.01, methanol); R_f 0.43 in 90:10:0.8:0.2 CH_2Cl_2–CH_3OH–H_2O–NH_4OH; UV λ_{max} (CH_3OH) 216 (ε 20 180) and 242 (ε 3609) nm.

Vigorous hydrolysis of dolastatin 10 under acidic (6 N HCl; 110 °C; 24, 48, and 70 h) or basic (aqueous $Ba(OH)_2$, 120 °C, 20 h) conditions followed by amino acid analyses of the products consistently gave evidence of valine and phenylalanine. The latter observation was not in complete accord with initial NMR spectral data and was a source of concern until a structure for the new masked Phe named dolaphenine (cf., Figure 1) was deduced. Meanwhile, dolastatin 10 was found refractory to simple acetylation and saponification reactions suggesting a cyclic peptide[5] but eliminating a depsipeptide structure. Compelling evidence for a linear pentapeptide structure was obtained by partial hydrolysis (in addition to above acidic and basic conditions, hydrolysis with 1:1 concentrated HCl and propionic acid for 15 min at 160 °C was performed) followed by conversion of the products to *N*-trifluoroacetyl butyl esters corresponding to units Dov-Val, Dil, Dap, and Doe (see Figure 1) minus loss of the two methoxy groups (from Dil and Dap) and addition of water (to Dap). By means of detailed gas chromatographic separation followed by HREI mass spectrometry (Kratos MS-80, HREI- and CI-modes) the C-terminal unit Doe was found to lack a butyl ester and the N-terminal segment (Dov-Val) a trifluoroacetyl group. At this point extensive high resolution mass and ^1H and ^{13}C NMR (400 MHz using ^1H-^1H-COSY,[6,7] 2D-J resolved,[8] and ^1H-^{13}C-2D shift correlated methods[9,10]) spectral studies (Table I) had already been analyzed and structures ascertained for the four hitherto unknown (in nature) amino acid constituents leaving only the correct sequence in question.

The dolastatin 10 sequence was unequivocally assigned on the basis of SP-SIMS[11] measurements in conjunction with the collision activated (induced) decomposition (CAD or MS/MS or tandem mass spectrometry with a Kratos MS-50-3F)[12] of the SP-SIMS ions (and fragments resulting from decarbonylation, see Figure 1[12] and Table II). The SP-SIMS ion at *m/z* 227 yields *m/z* 100 as nearly the only CAD fragment ion indicating good stability for the immonium ion $(CH_3)_2N^+$ =$CHCH(CH_3)_2$. Of the two final sequences namely Dov-Val-Dap-Dil-Doe or Dov-Val-Dil-

Table I. Dolastatin 10 Correlated ^{13}C and ^1H NMR Assignments in Dichloromethane-d_2 Solution[a]

structure assgnmnt	13C (mult)	chemical shift, ppm ^1H (mult, *J* (Hz), intgrtn)	NOE
2	170.51 (s)		
4	142.77 (d)	7.717 (d, 3.3, 1 H)	
5	118.76 (d)	7.254 (d, 3.3, 1 H)	
6	53.02 (d)	5.516 (ddd, 5.7, 7.2, 9.3, 1 H)	
6a	41.48 (t)	3.399 (dd, 5.7, 14, 1 H) 3.256 (dd, 5.7, 14, 1 H)	
6b1	137.74 (s)		
6b2, 6b6	128.74 (d) × 2	7.243 (d, 7.9, 2 H)	
6b3, 6b5	129.80 (d) × 2	7.214 (dd, 7.9, 9.2, 2 H)	
6b4	127.02 (d)	7.194 (t, 9.2, 1 H)	
7		7.256 (t, 7.6, 1 H)	9, 9a, 10, 10ab, 11
8	175.67 (s)		
9	44.79 (d)	2.282 (quintet, 7.2, 1 H)	7
9a	14.49 (q)	1.085 (d, 7.1, 3 H)	
10	82.05 (d)	3.845 (dd, 2.0, 8.2, 1 H)	7
10ab	60.89 (q)	3.309 (s, 3 H)	6, 7
11	59.86 (d)	3.985 (m, 1 H)	
12	25.00 (t)	1.804 (ddd, 5.5, 7.0, 19, 1 H) 1.600 (ddd, 7, 9.2, 19, 1 H)	
13	25.45 (t)	1.446 (ddd, 4.7, 7, 19, 1 H) 1.715 (ddd, 4.7, 7.8, 12.7, 1 H)	
14	48.03 (t)	3.401 (dd, 7.8, 10, 1 H) 3.390 (m, 1 H)[b]	17
16	174.01 (s)		
17	38.11 (t)	2.394 (ABq, 9.0, 2 H)	14
18	78.86 (d)	4.122 (br t, 8.7, 1 H)	
18ab	58.16 (q)	3.313 (s, 3 H)	22
19	54.11 (d)	3.26–3.39 (1 H)[c]	
19a	33.62 (d)	1.680 (1 H)[b]	
19b	26.25 (t)	1.370 (br m, 1 H) 1.000 (br m, 1 H)[b]	
19c	10.92 (q)	0.823 (t, 7.4, 3 H)	
19d	19.82 (q)	1.003 (d, 6.8, 3 H)	
20a	30.09 (q)	3.012 (s, 3 H)	22
21	171.39 (s)		
22	54.20 (d)	4.761 (dd, 6.5, 8.8, 1 H)	18, 18ab, 20, 19
22a	31.42 (d)	1.983 (sextet, 6.7, 1 H)	
22b	18.18 (q)	0.941 (d, 6.8, 3 H)	
22c	16.09 (q)	0.977 (d, 6.8, 3 H)	
23		6.861 (d, 8.9, 1 H)	25, 25bc
24	172.44 (s)		
25	76.77 (d)	2.454 (d, 6.9, 1 H)	23
25bc	49.92 (q) × 2	2.262 (s, 6 H)	23
26	28.08 (d)	2.073 (sextet, 6.7, 1 H)	
27	20.24 (q)	0.964 (d, 6.8, 3 H)	
28	18.18 (q)	0.902 (d, 6.8, 3 H)	

[a] Residual $CHDCl_2$ as internal reference (5.32 ppm). [b] Overlapping signal. [c] Signal assigned from NOE data.

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(5) Another guide pointing to a cyclic peptide appeared from pronounced changes in the ^1H NMR spectra (at 400 MHz Bruker AM-400) with changing concentration and solvent presumably due to conformational changes (from 3:1 in CD_2Cl_2 → 2:3 in CD_3OD) in the ring system. Later these interesting chemical shifts in the dolaphenine 4-, 5-, and 6-H and the Val 22-H signals were interpreted as possibly reflecting quasi-cyclic conformers due to hydrogen bonding involving N-7, N-23, and/or N-25a. At present these observations could also be interpreted on the basis of chemical shifts emanating from restricted rotations from N and O methyl groups.

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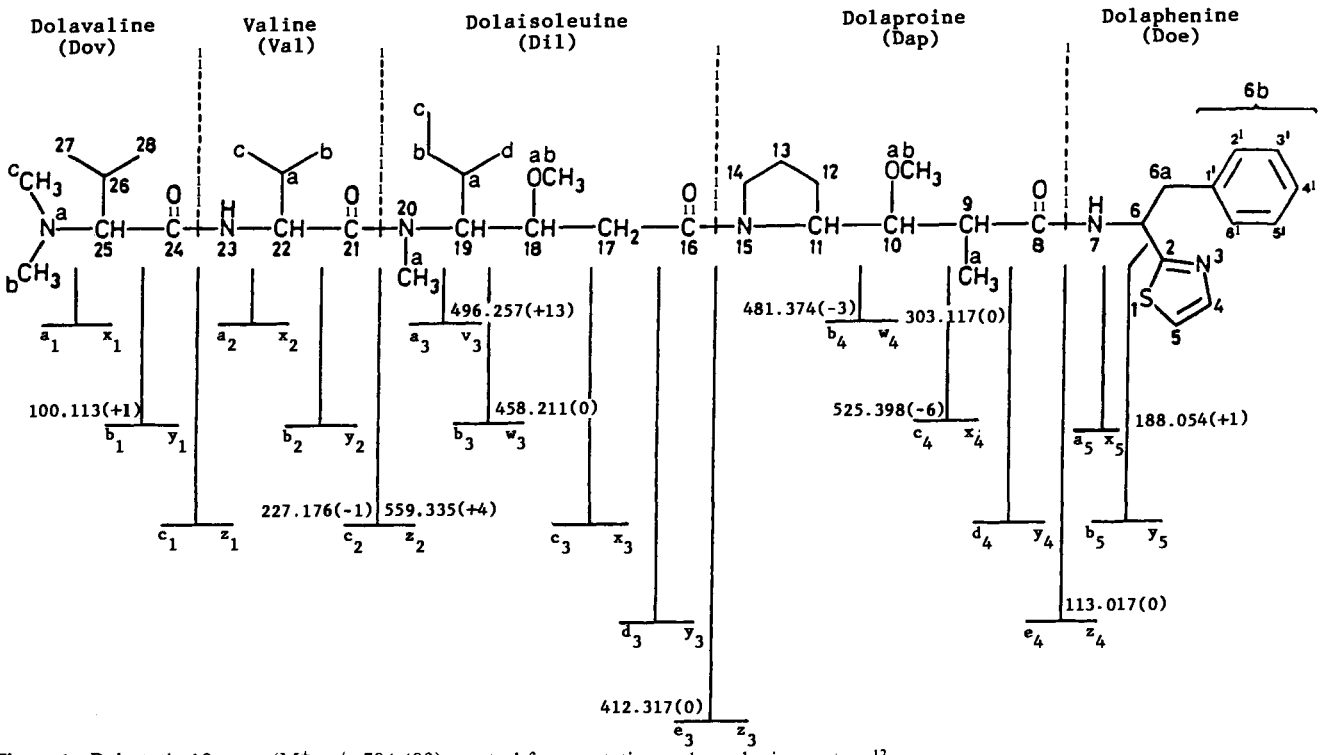

Figure 1. Dolastatin 10 mass (M^+ *m/e* 784.489) spectral fragmentation and numbering system.[12]

Table II. Collision-Induced Dissociations[12] of Selected Higher Masses from the SP-SIMS[11] Fragmentation of Dolastatin 10

parent ions			
785 M + H	559 z_2 + 2 H	458 w_3	412 e_3
Daughter Ions[a,b]			
753 M + H − CH_3OH	188[c] x_5	426 w_3 − CH_3OH	100 b_1
100 b_1 and/or (z_2/b_3) + H	205 z_4	86 (w_3/e_3)	214 (y_2/e_3) + H
188[c] x_5	374 z_3 + 2 H	188 x_5	380 e_3 − CH_3OH
559 z_2 + 2 H	100 (z_2/b_3) + H	356	352 d_3 − CH_3OH
154 (z_2/e_3) + H − CH_3OH	154 (z_2/e_3) + H − CH_3OH	205 z_4 + 2 H	311 (y_1/e_3) − H
205 z_4 + 2 H	170 (z_3/e_4) + H	170 (z_3/e_4) + H	253
458 w_3	138 (z_3/e_4) + H − CH_3OH	138 (z_3/e_4) + H − CH_3OH	227 c_2
170 (z_3/e_4) + H	527 z_2 + 2H − CH_3OH	303 w_4	368 (x_1/e_3)

[a] See Figure 1 for the letter designations. [b] The eight strongest derivative ions are listed in order of decreasing abundances. [c] The very abundant *m/z* 188 ion seems to mask a *m/z* 186 ((z_2/e_3) + H), but its derived ion appears at *m/z* 154.

Dap-Doe (**1**) the latter was found correct by CAD in conjunction with HREIMS (Figure 1 and Table II) experiments by using the intact peptide. The final confirmation of structure **1** for dolastatin 10 was obtained by H-[^{1}H]-NOE difference[13] NMR experiments as summarized in Table I. The amino acid sequence of dolastatin 10 was thereby established. The NOE experiments in CD_2Cl_2 combined with 2D-NMR COSY techniques.[6,7] in three solvents (C_6D_6, CD_2Cl_2, and CD_3OD) allowed recognition of Dil as an Ile rather than a Leu derivative. In addition, L-Dov-OMe[14] and racemic Doe·HBr were synthesized, and the respective ^{1}H and ^{13}C NMR data were found to nicely approximate chemical shifts assigned to their counterparts in dolastatin 10.

Interestingly, the Dil unit is an *O*-methyl Ile-class relative of the Leu-type amino acid statine (3-hydroxy-4-amino-6-methylheptanoic acid) found in the marine tunicate antineoplastic components didemnins A–C (3*R*,4*S*)[15] and the lower plant acid protease inhibitor (e.g., of renin and pepsin) pepstatin (3*S*,4*S*).[16] A 2-methyl Ala-analogue appears in the anticancer antibiotic drug bleomycin (3*S*,4*R*).[17] The significance of such amino acid derived aldol condensation products in anticancer drug design has been greatly increased by discovery of the extraordinary dolastatin 10. Presently we are vigorously pursuing experiments focused upon determining the relative and absolute configuration of dolastatin 10 in conjunction with total synthesis and structural modifications.

Acknowledgment. For providing the financial resources necessary to complete this investigation, we thank with pleasure the Fannie E. Rippel Foundation, Eleanor W. Libby, the Waddell Foundation (Donald Ware), the Robert B. Dalton Endowment Fund, the Arizona Disease Control Research Commission, Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), the Upjohn Co., Herbert K. and Dianne Cummings (The Nathan Cummings Foundation, Inc.), Lotte Flugel, Polly J. Trautman, the ARCS Foundation, and PHS Grant CA-16049-01-11 awarded by the National Cancer Institute, DHHS. Other very helpful assistance was provided by Drs. B. J. Abbott, D. L. Doubek, J. L. Hartwell, R. C. Kelly, L. W. Knapp, P. Lohavanijaya, R. A. Nieman, S. B. Singh, M. Suffness, W. Wierenga, H. B. Wood, Messrs. C. Michel, P. Jauffret, and P. L. Daschner, the Smithsonian Institution, NSF Grant CHE-8211164 to the University of Nebraska, Midwest Center for Mass Spectrometry, and NSF Grant CHE-8211164–8620177.

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