stereochemistry for **10a,b** was assigned by the diaxial coupling observed between the bridgehead and the neighboring methine proton (**10a**, J = 10.7 Hz; **10b**, J = 10.8 Hz).

Notably, our methodology provides a new a regiocontrolled route into nine- and ten-membered-ring unsaturated lactams via a charge accelerated variant of the aza Claisen rearrangement starting from simple monocyclic precursors. Furthermore, the regio- and stereocontrolled introduction of new heteroatom functionality into bicyclic compounds from macrocyclic precursors provides a variety of opportunities for the elaboration of additional functionality.[18] Application of this methodology to the synthesis of bioactive compounds is currently being pursued.

Acknowledgment. This work was supported by funds from Utah State University and the Donors of the Petroleum Research Fund, administered by the American Chemical Society. The X-ray structural information was obtained by Dr. Atta Ariff, Department of Chemistry, University of Utah.

Supplementary Material Available: Synthetic procedures and analytical data for **6a**, **6b**, **8a**, **8b**, **9b**, and **10a-c** and listings of crystallographic details, ORTEP, bond distances, bond angles, torsion angles, and positional and displacement parameters for **8b** and **9a** (25 pages); listing of observed and calculated structure factors for **8b** and **9a** (11 pages). Ordering information is given on any current masthead page.

(18) For purposes of future synthetic applications, both chlorine atoms can be readily cleaved from the macrocyclic lactams **6a** and **8a** by using Zn/Ag: Heathcock, C. H.; Clark, R. D. *J. Org. Chem.* **1972**, *38*, 3658.

Antineoplastic Agents. 220. Synthesis of Natural (-)-Dolastatin 15[1]

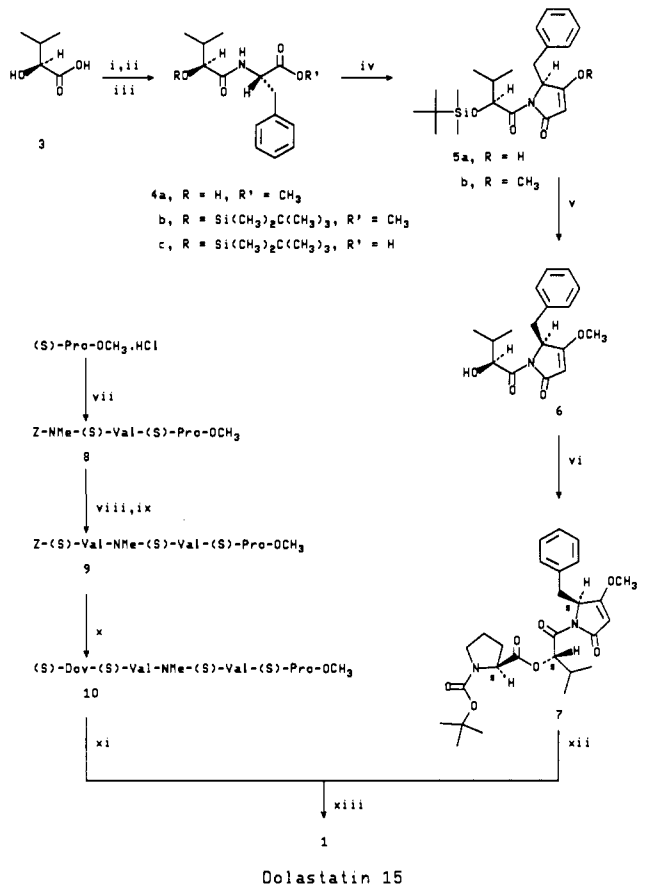
George R. Pettit,* Delbert L. Herald, Sheo Bux Singh, Timothy J. Thornton, and Jeffrey T. Mullaney

Cancer Research Institute and Department of Chemistry Arizona State University, Tempe, Arizona 85287-1604

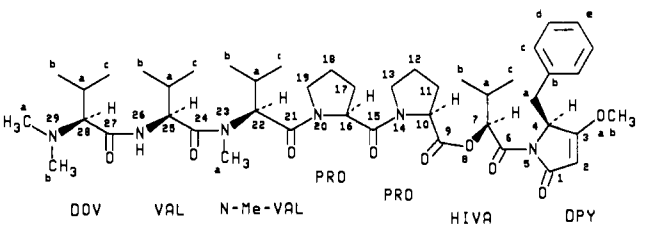
Received December 19, 1990

The discovery and synthesis[2] of potentially useful antineoplastic peptides comprise one of the most essential and promising approaches to new types of anticancer drugs. Of special interest here are the dolastatins,[2d,g,3] an unprecedented series of linear and cyclic antineoplastic and/or cytostatic peptides isolated from the Indian Ocean sea hare *Dolabella auricularia*.[3] Presently dolastatins 10[2d,3e] and 15 (**1**)[3a] represent the two most important members. While dolastatin 10 has recently yielded to total synthesis,[2d] the corollary problem of deducing the absolute configuration (seven chiral centers) of dolastatin 15 (**1**) and devising a total synthesis has remained urgent. We now report the absolute configuration of dolastatin 15 (**1**) and total synthesis of the correct natural (-)-isomer from among 128 possibilities.

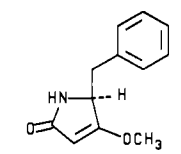
Scheme I[a]



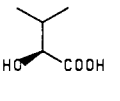
[a] (i) Phe-OMe, diethyl phosphorocyanidate (DEPC), NMM, CH_2Cl_2; (ii) TBDMS chloride, imidazole, DMF; (iii) 1 N NaOH, CH_3OH-H_2O-H_2O; (iv) Meldrum's ester, 4-DMAP, $C_6F_5O_2CCF_3$, CH_2Cl_2; CH_3OH, ΔH; K_2CO_3, $(CH_3O)_2SO_2$, THF; (v) HF-pyridine; (vi) Boc-(*S*)-Pro, DCCI, 4-pyrrolidinopyridine, CH_2Cl_2; (vii) Z-NMe-(*S*)-Val, DEPC, TEA, DME; (viii) H_2, 10% Pd/C, EtOAc-CH_3OH, HCl-ether; (ix) Z-(*S*)-Val, $(CH_3)_3CCOCl$, NMM, $CHCl_3$; (x) (*S*)-Dov-OPfp, H_2, 10% Pd/C, dioxane; (xi) NaOH, dioxane, H_2O, HCl; (xii) TFA, CH_2Cl_2; (xiii) DEPC, TEA, DME, 0 °C → room temperature. Satisfactory elemental and spectral analyses were obtained for each new substance.



1, Dolastatin 15



2, Dolapyrrolidone (Dpy)



3, Hiva

The structure of dolastatin 15,[3a] elucidated via extensive 2D NMR and high resolution mass spectral techniques, was found

* To whom correspondence should be addressed.

(1) Part 219: Pettit, G. R.; Herald, C. L.; Leet, J. E.; Dufresne, C.; Doubek, D. L.; Schmidt, M. H.; Cerny, R. L.; Rutzler, K. *J. Org. Chem.*, submitted.

(2) Cf.: (a) Li, W. R.; Ewing, W. R.; Harris, B. D.; Joullié, M. M. *J. Am. Chem. Soc.* **1990**, *112*, 7659-7672. (b) Ewing, W. R.; Harris, B. D.; Li, W. R.; Joullié, M. M. *Tetrahedron Lett.* **1989**, *30*, 3757-3760. (c) Holzapfel, C. W.; van Zyl, W. J. *Tetrahedron* **1990**, *46*, 649-660. (d) Pettit, G. R.; Singh, S. B.; Hogan, F. M.; Lloyd-Williams, P.; Herald, D. L.; Burkett, D. D.; Clewlow, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 5463. (e) Schmidt, U.; Kroner, M.; Griesser, H. *Tetrahedron Lett.* **1988**, *29*, 4407-4408. (f) Schmidt, U.; Kroner, M.; Griesser, H. *Tetrahedron Lett.* **1988**, *29*, 3057-3060. (g) Pettit, G. R.; Kamano, Y.; Holzapfel, C. W.; van Zyl, W. J.; Tuinman, A. A.; Herald, C. L.; Baczynskyj, L.; Schmidt, J. M. *J. Am. Chem. Soc.* **1987**, *109*, 7581-7582.

(3) (a) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herald, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005. (b) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Bates, R. G.; Schmidt, J. M.; Cerny, R. L.; Kizu, H. *J. Org. Chem.* **1990**, *55*, 2989-2990. (c) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Cerny, R. L.; Herald, D. L.; Schmidt, J. M.; Kizu, H. *J. Am. Chem. Soc.* **1989**, *111*, 5015-5017. (d) 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*, 553-558. (e) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883-6885.

0002-7863/91/1513-6692$02.50/0

to contain the unusual dolapyrrolidone (Dpy, **2**), 2-hydroxyisovaleric acid (Hiva, **3**), dolavaline (Dov[3e]), proline, valine, and *N*-methylvaline units. The paucity of natural product prevented us from determining the absolute configuration of dolastatin 15. On the assumption that the dolastatin 15 amino acids most probably possessed the common L configuration (*S* used in the sequel) found in dolastatin 10,[2d] the total synthesis was initiated on that basis.

Synthesis of the key Dpy (**2**) intermediate **6** was achieved as summarized in Scheme I. The hydroxyisovaleric acid (Hiva) component was obtained from (*S*)-Val via a diazotization sequence.[4] Condensation of unprotected (*S*)-Hiva with (*S*)-Phe-OMe, employing diethyl phosphorocyanidate, proceeded well to afford (60%) (*S*)-Hiva-(*S*)-Phe-OMe (**4a**) as needles from toluene–hexane, melting at 70 °C: $[\alpha]_D$ −45° (*c*, 0.002). Protection (**4a** → **4b**, oil, 87% yield)[5] employing *tert*-butyldimethylsilyl chloride, saponification (**4b** → **4c**), and reaction of the resulting carboxylic acid (**4c**, mp 87–8 °C from hexane, 90% yield) with pentafluorophenyl trifluoroacetate followed by Meldrum's ester[6] gave pyrrolidone **5a** as a viscous oil. Methylation with dimethyl sulfate provided (21% overall from **4c**) the corresponding methyl ether (**5b**). Cleavage of the silyl group with pyridinium polyhydrogen fluoride gave (90%) (*S*)-Hiva-(*S*)-Dpy (**6**) as an oil: $[\alpha]_D$ +285° (*c*, 0.002). Esterification[7] of alcohol **6** with Boc-(*S*)-Pro gave (74% yield) depsipeptide **7** [(mp 157–158 °C, $[\alpha]_D$ +96.2° (*c*, 1.85, $CHCl_3$)]. In order to unequivocally establish the absolute configuration of depsipeptide **7**, a specimen crystallized from acetone–hexane was subjected to X-ray crystal structure analysis (see the supplementary material).[8-11] Absolute stereochemical assignments (Figure 1, supplementary material) to the three chiral centers were made, based upon the known stereochemical configuration of (*S*)-Pro. The stereochemical designations for the three chiral centers were determined to be C-4(*S*), C-7(*S*), and C-10(*S*).

Tetrapeptide **10** was prepared by starting with dipeptide **8** [(77%, needles from ethyl acetate–hexane, mp 100–2 °C, $[\alpha]_D$ −144.6° (*c*, 2.04, $CHCl_3$)] using the procedure outlined in Scheme I. Hydrogenolysis followed by a pivaloyl mixed anhydride peptide bond forming step gave tripeptide **9** [83%, oil $[\alpha]_D$ −145° (*c*, 2.56, $CHCl_3$)]. Subsequent hydrogenolysis and in situ reaction with (*S*)-Dov-OPfp led to tetrapeptide **10** [(84%, amorphous powder from acetone–hexane, mp 137–40 °C, $[\alpha]_D$ −180° (*c*, 2.08, $CHCl_3$)]. Finally, the carboxylic acid obtained by saponification of methyl ester **10** was condensed with the amine derivative of proline **7** to give, following separation on Sephadex LH-20 (2:2.5:7.5, hexane–methylene chloride–methanol), natural (−)-dolastatin 15 (**1**, 68% yield as an amorphous powder from acetone–hexane), mp 112–4 °C, $[\alpha]_D$ −48.2° (*c*, 0.11, CH_3OH), identical by TLC and ^1H and ^{13}C NMR with an authentic sample. Synthetic dolastatin 15 gave the same (ED_{50} 10^{-3} μg/mL) potent activity against the P388 lymphocytic leukemia routinely obtained with the natural product.

The first total synthesis of natural (−)-dolastatin 15 herein summarized has firmly established the overall absolute configuration (all chiral centers *S*). Synthesis of potentially useful structural and chiral modifications of (−)-dolastatin 15 (**1**) and a broad evaluation of biological properties are in progress. Presently (−)-dolastatin 15 is undergoing preclinical development as a potential anticancer drug.

Acknowledgment. Very helpful financial support was provided by Outstanding Investigator Grant CA44344-01A1 and Grants CA-16049-10-12 awarded by the National Cancer Institute, DHHS; the Fannie E. Rippel Foundation; the Arizona Disease Control Research Commission; Herbert K. and Dianne Cummings (The Nathan Cummings Foundation, Inc.); Eleanor W. Libby; the Waddell Foundation (Donald Ware); Lotte Flugel; and NSF equipment Grants CHE-8211164, CHE-8620177 (to the University of Nebraska, Midwest Center for Mass Spectrometry), and CHE-8409644. We also thank Drs. F. Hogan, Y. Kamano, and T. R. Kasturi for assistance.

Registry No. 1, 123884-00-4; **3**, 17407-55-5; **4a**, 134816-19-6; **4b**, 134816-26-5; **4c**, 134816-27-6; **5b**, 134816-20-9; **6**, 134816-21-0; **7**, 134816-22-1; **8**, 134816-23-2; **9**, 134816-24-3; **10**, 134816-25-4; Val, 72-18-4; Phe-OMe·HCl, 7524-50-7; Boc-Pro, 15761-39-4; Pro-OMe·HCl, 2133-40-6; (Z)-MeVal, 42417-65-2; MeVal-Pro-OMe·HCl, 134816-28-7; (Z)-Val, 1149-26-4; Dov-OC_6F_5, 97800-78-7; Dov-Val-MeVal-Pro-OH, 134816-29-8; Meldrum's acid, 2033-24-1.

Supplementary Material Available: Experimental section for synthesis of dolastatin 15 and crystal structure details, tables of bond distances and angles, and positional parameters for Dpy derivative **7** (22 pages). Ordering information is given on any current masthead page.

(4) (a) Kim, H.-O.; Olsen, R. K.; Choi, O.-S. *J. Org. Chem.* **1987**, *52*, 4532–4536. (b) Cook, A. H.; Cox, S. F.; Farmer, T. H. *J. Chem. Soc.* **1949**, 1022.

(5) (a) Shekhani, M. S.; Khan, K. M.; Mahmood, K.; Shah, P. M.; Malik, S. *Tetrahedron Lett.* **1990**, *31*, 1669–1670. (b) Corey, E. J.; Venkateswarlu, A. J. *Am. Chem. Soc.* **1972**, *94*, 6190.

(6) Jouin, P.; Castro, B.; Nisato, D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1177–1182.

(7) Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, *46*, 4475–4478.

(8) Crystal data: intermediate **7**, $C_{27}H_{36}N_2O_7$, orthorhombic, space group $P2_12_12_1$, with a = 10.9247 (10) Å, b = 12.8687 (24) Å, c = 20.0358 (24) Å, $\alpha = \beta = \gamma = 90°$, V = 2816.77 Å^3, ρ_{obsd} = 1.160 g cm^{-3}, ρ_{calcd} = 1.181 g cm^{-3} for Z = 4.

(9) North, A. C.; Phillips, D. C.; Mathews, F. S. *Acta Crystallogr., Sect. A* **1968**, *24*, 351.

(10) Sheldrick, G. M., Institut für Anorganische Chemie der Universität Göttingen, Tammannstr. 4, D-3400 Göttingen, Federal Republic of Germany.

(11) Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W. *Crystals User Guide*; Chemical Crystallography Laboratory: University of Oxford, Oxford, England, 1985.

Antineoplastic Agents. 224. Isolation and Structure of Neristatin 1[1a]

George R. Pettit,* Feng Gao, Delbert L. Herald, Peter M. Blumberg,[1b] Nancy E. Lewin,[1b] and Ronald A. Nieman

Cancer Research Institute and Department of Chemistry Arizona State University, Tempe, Arizona 85287-1604

Received March 6, 1991

The marine bryozoan *Bugula neritina* has been found to contain a unique series of closely related macrocyclic (22-membered) lactones now known as the bryostatins.[2,3] Because of their very selective antineoplastic and cytostatic activity, potent influence on protein kinase C biochemical pathways, antitumor promoter effects, and stimulation of bone marrow progenitor cells to form colonies (GM-CSF activity), bryostatin 1 (**1**)[4] has been selected for clinical evaluation. Discovery and study of bryostatin biosynthetic precursors or degradation products has been considered necessary to gain further mechanistic and structure/activity insights. We now report the isolation and structural elucidation of the first such example, herein designated neristatin 1 (**2**).

A 1000-kg (approximate damp weight) re-collection (1986, Gulf of Mexico coast of Florida) of *B. neritina* Linnaeus was extracted with 2-propanol. Initial solvent partitioning and steric exclusion chromatographic procedures were conducted as previously described for the closely related bryozoan *Amathia convoluta*.[5] Separation was guided by bioassay employing the P388 lymphocytic leukemia cell line with a combination of gel permeation (and partition on Sephadex LH-20) and partition (silica gel)

(1) (a) Part 223: Kantoci, D.; Pettit, G. R.; Cichacz, Z. *J. Liq. Chromatogr.*, in press. (b) Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD 20892.

(2) Pettit, G. R. The Bryostatins. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Ed.; Springer-Verlag: New York, in press.

(3) Suffness, M.; Newman, D. J.; Snader, K. *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer-Verlag: New York, 1989; Vol. 3, p 132.

(4) Pettit, G. R.; Leet, J. E.; Herald, C. L.; Kamano, Y.; Boettner, F. E.; Baczynsky, L.; Nieman, R. A. *J. Org. Chem.* **1987**, *52*, 2854.

(5) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, *41*, 985.

0002-7863/91/1513-6693$02.50/0