Dolastatins. 26. Synthesis and Stereochemistry of Dolastatin 11[1a]

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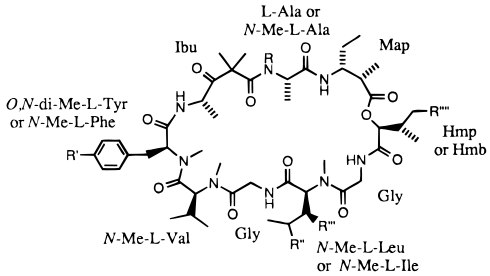
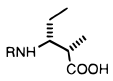
Abstract: The first synthesis of dolastatin 11, a potent antineoplastic agent from the sea hare *Dolabella auricularia*, confirmed the proposed structure and established the last configuration in this natural product and in dolastatin 12, majusculamide C, and 57-normajusculamide C.

Dolastatins 11 (**1**) and 12 (**2**), isolated from the sea hare *Dolabella auricularia*, inhibit growth of the murine P388 lymphocytic leukemia with ED_{50} 2.7×10^{-3} and 7.5×10^{-2} μg/mL, respectively.[1b] They were characterized largely by NMR and MS, with the configurations except in the Ibu and Map units based on the striking similarities of their NMR parameters to those of majusculamide C (**3**)[2] and 57-normajusculamide C (**4**),[3] closely-related antifungal agents from the blue-green alga *Lyngbya majuscula*. The Map configurations were determined to be 2*S*,3*R* in **1**−**4** by NMR and optical rotatory dispersion (ORD) comparison of synthetic (2*R*,3*S*)-Map with (2*S*,3*R*)-Map (**5**) from the degradation of majusculamide C (**3**).[4] Until the present work, the stereochemistry was unknown at the stereogenic center in Ibu, since the aminoketone obtained from this segment of majusculamide C (**3**) was racemic after hydrolysis with HCl.[2] We herein report the first dolastatin 11 (**1**) synthesis. The strategic approach was similar to Shioiri's convergent syntheses of didemnins.[5]

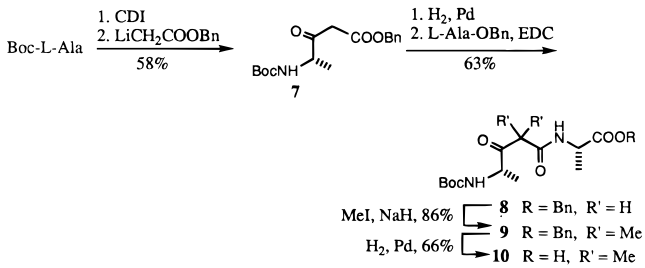


	R	R'	R''	R'''	R''''
1 dolastatin 11	H	OMe	Me	H	Me
2 dolastatin 12	Me	H	Me	H	Me
3 majusculamide C	H	OMe	H	Me	Me
4 57-normajusculamide C	H	OMe	H	Me	H



5 R = H
6 R = Boc

Scheme 1



The synthesis of the natural (2*S*,3*R*)-Map stereoisomer (**5**) has been reported by two routes.[6,7] We first made it using the five-step method described for its enantiomer[4] with minor changes. Later, we used the eight-step procedure of Jefford and McNulty,[7] because their synthesis, though longer, has nonvolatile, nonazidic intermediates. Map (**5**) was readily converted to Boc-Map (**6**) for incorporation of this valuable material late in the synthesis.

The other new amino acid, Ibu (see structure **1**), was prepared in dipeptide **9** as shown in Scheme 1. Attempts to obtain Boc-L-Ibu by having the acylimidazole prepared from Boc-L-Ala react with the dianion from isobutyric acid[8] failed, presumably for steric reasons. In the successful route, the acylimidazole derivative of Boc-L-Ala was allowed to react with benzyl lithioacetate[9] to give β-ketoester **7**, which was hydrogenolyzed at 0 °C to the β-ketoacid. This carboxylic acid was coupled with L-Ala-*O*-Bn without isolation to give dipeptide **8**, which was dimethylated to complete the Ibu unit in dipeptide **9**. The dimethylation yield was much better (86 *vs* 35%) when the two equivalents of sodium hydride were added one at a time with a 45 min interval. Benzyl group removal gave highly crystalline dipeptide **10** for incorporation late in the synthesis.

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(1) (a) For the previous contribution in this series refer to Antineoplastic Agents. 383: G. P. Kalemkerian, X. Ou, S. K. Madan, and G. R. Pettit, manuscript in preparation. (b) Pettit, G. R.; Kamano, Y.; Kizu, H.; Dufresne, C.; Herald, C. L.; Bontems, R.; Schmidt, J. M.; Boettner, F. E.; Nieman, R. A. *Heterocycles* **1989**, *28*, 553.
(2) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236.
(3) Mynderse, J. S.; Hunt, A. H.; Moore, R. E. *J. Nat. Prod.* **1988**, *51*, 1299.
(4) Bates, R. B.; Gangwar, S. *Tetrahedron: Asymmetry* **1993**, *4*, 69.
(5) Hamada, Y.; Kondo, Y.; Shibata, M.; Shioiri, T. *J. Am. Chem. Soc.* **1989**, *111*, 669.
(6) Davies, S. G.; Ichihara, O.; Walters, I. A. S. *Synlett* **1994**, 117.
(7) Jefford, C. W.; McNulty, J. *Helv. Chim. Acta* **1994**, *77*, 2142.
(8) Pfeffer, P. E.; Silbert, L. S. *Liebig's Ann. Chem.* **1970**, *35*, 262.
(9) Benzyl lithioacetate was prepared by the procedure used by Shioiri[5] to make ethyl lithioacetate.

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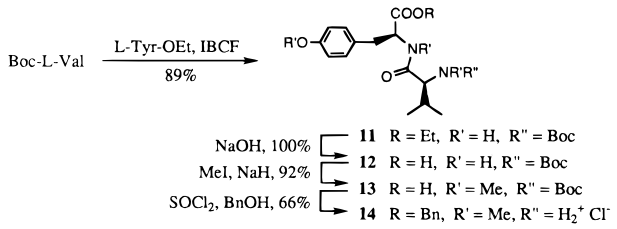
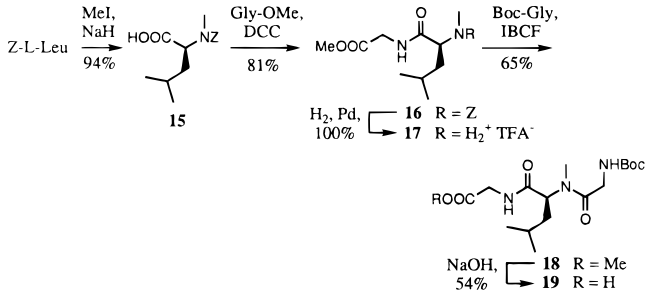
Scheme 2



Scheme 3



Because its three methyl groups attached to heteroatoms could be added simultaneously, dipeptide **14** was synthesized as a unit as shown in Scheme 2. To provide convergence in the synthesis, the Gly-*N*-Me-Leu-Gly tripeptide **19** was synthesized as shown in Scheme 3. Scheme 4 shows the assembly of the units used to synthesize dolastatin 11 (**1**). The hydroxyacid Hmp (**22**) was prepared from L-Ile by treatment with nitrous acid under conditions used on L-Leu[10] followed by low-temperature recrystallization (a previous preparation[2] gave a mixture of diastereomers which was separated by HPLC).

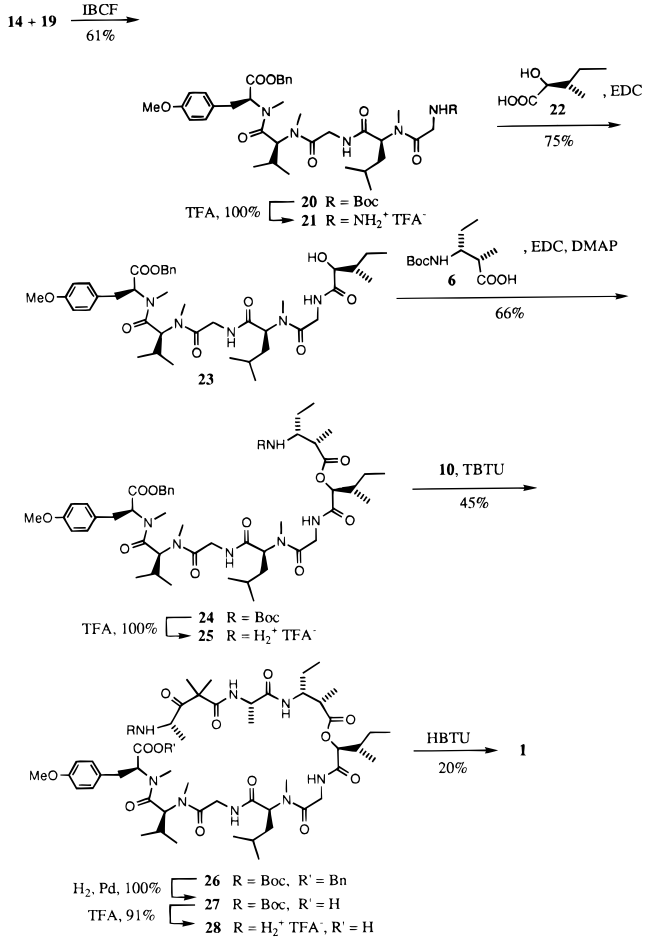
The linear nonadepsipeptide **26** was first prepared from hexadepsipeptide **23** and a tripeptide acid derived from Map (**5**) and dipeptide **10**. Since this first approach gave the ester bond in only a 10% yield, it was replaced by the route in Scheme 4, which gave a 66% yield of ester **24** from acid **6** and alcohol **23** and was not so wasteful of the valuable Map and Ibu units.

The acid/base-sensitive ketone-containing Ibu unit was incorporated late in the synthesis to avoid epimerization. An indication that its chiral center would survive the removal of the Boc group in the penultimate reaction was that majusculamide C (**3**) was recovered unchanged upon treatment with CF_3-COOD under the conditions of Boc deprotection.

The cyclization of depsipeptide **28** to dolastatin 11 (**1**) went best (20−24%) employing HBTU as the peptide bond-forming reagent;[11] with BOP-Cl[12] at high dilution, the yield was only 13%.

The synthetic procedure developed to obtain dolastatin 11 (**1**) offers the first practical route to this important anticancer drug prospect;[13] the yield from sea hares was only (3×10^{-6})%,[1b] compared to 0.7−1.7% from the starting materials in Schemes 1−3. The synthesis required no chromatography in the early stages; only dolastatin 11 (**1**) and late intermediates **24** and **26** were purified by chromatography (HPLC). Key intermediates **6**, **10**, **14**, **19**, and **22** were readily purified by crystallization. The synthesis confirmed the proposed structure of dolastatin 11 (**1**)[1b] and established for the first time the *S* configuration for the Ibu unit in this natural product as well as

Scheme 4



in the closely related depsipeptides dolastatin 12 (**2**),[1b] majusculamide C (**3**),[2] and 57-normajusculamide C (**4**).[3]

Experimental Section

Solvent and reagent abbreviations used are Bn = benzyl, Boc = *tert*-butyloxycarbonyl, BOP-Cl = bis(2-oxo-3-oxazoladinyl)phosphinic chloride,[12] CDI = carbonyldiimidazole, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DCU = *N*,*N*′-dicyclohexylurea, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate,[11] Hmb = (*S*)-2-hydroxy-3-methylbutanoic acid, Hmp = (2*S*,3*S*)-2-hydroxy-3-methylpentanoic acid, HOBT = 1-hydroxybenzotrizole, IBCF = isobutyl chloroformate, Ibu = (*S*)-4-amino-2,2-dimethyl-3-oxopentanoic acid, LDA = lithium diisopropylamide, Map = (2*S*,3*R*)-3-amino-2-methylpentanoic acid, NMM = *N*-methylmorpholine, TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate,[14] TFA = trifluoroacetic acid, and Z = benzyloxycarbonyl.

Reagents were purchased from Sigma-Aldrich Co. except for BOP-Cl, which was from TCI America, Portland, OR. Most reactions were carried out under argon, and solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Column chromatography employed silica gel (70−230 mesh) from E. Merck (Darmstadt). HPLC purifications used a 21.4 mm i.d. × 25 cm reversed-phase C-18 column and elution with water−acetonitrile mixtures of from 0 to 100% acetonitrile over 30 min, except for the purification of dolastatin 11 (**1**), for which a 50 min protocol was used. Melting points were uncorrected. NMR spectra were obtained at 250−500 MHz in $CDCl_3$ unless otherwise noted, with TMS as an internal standard.

(2*S*,3*R*)-Map (5). Method A. (3*S*,4*R*)-4-Methyl-5-hexen-3-ol[4] was converted to (2*S*,3*R*)-Map (**5**) by the method used to make its enantiomer[4] in comparable yields with the following changes. In the

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(11) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, 572.

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(13) Dolastatin 11 (**1**) was recently placed in preclinical development by the National Cancer Institute (Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812).

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preparation of (2*S*,3*R*)-3-azido-2-methylpentanoic acid, the aqueous layer was saturated with NaCl before extraction with ether. The hydrogenation which gave Map (**5**) was conducted in absolute ethanol rather than ethyl acetate. After the reaction, the solution was filtered and the filter paper was washed with ethanol (5 mL) and water (50 mL). Concentration to 0.5 mL by rotary evaporation led (3 d) to crystals. Washing (2 × 3 mL) with hot absolute ethanol gave colorless crystals, mp 206−209 °C dec. Anal. Calcd for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68. Found: C, 55.12; H, 10.20; N, 10.71. The hydrochloride had $[\alpha]^{25}_D$ +14.1 (c 0.064, H_2O).

Method B. The procedure of Jefford and McNulty[7] was used but on a 10−20 times larger scale with the following changes. The (2*S*,3*S*)-2-methyl-3-(tosylamino)butano-4-lactone was purified by recrystallization from methanol rather than by chromatography. Ethyl (2*S*,3*S*)-4-iodo-2-methyl-3-(tosylamino)butanoate was purified by recrystallization from ethyl acetate−hexanes rather than by chromatography, but the overall yield was better if this material was used without either purification. The tosyl group was removed from (2*S*,3*R*)-2-methyl-3-(tosylamino)pentanoic acid in 90% yield by heating in a pressure bottle at 65 °C with 33% HBr in acetic acid for 3 d rather than by refluxing with 48% aqueous HBr at atmospheric pressure for 90 min. (2*S*,3*R*)-Map (**5**) was obtained from its hydrobromide not with propylene oxide, but by adjusting the pH to 6.6 with 4 N NaOH, evaporating the water, triturating the residue with ethanol, and evaporating.

Benzyl (4*S*)-*N*-Boc-4-amino-3-oxopentanoate (7). To a cooled (0 °C), stirred solution of Boc-L-Ala (2.0 g, 11 mmol) in THF (12 mL) was added CDI (1.95 g, 12 mmol), and the solution was stirred at 0 °C for 30 min and at 25 °C for 2 h. An LDA solution was prepared by slow addition at −78 °C of 1.6 M *n*-BuLi (20.7 mL, 33 mmol) to dry THF (30 mL) and diisopropylamine (4.62 mL, 33 mmol, dried over molecular sieves). The mixture was warmed to 0 °C for 15 min and recooled to −78 °C. Benzyl acetate (4.77 mL, 33 mmol, dried over molecular sieves) was added, the solution was stirred for 1.25 h at −78 °C, and the acylimidazole solution prepared above was cannulated dropwise into this solution. After stirring at −78 °C for an additional 15 min, 1 N hydrochloric acid (33 mL) was added and the mixture was warmed to 0 °C, acidified to pH 3 with citric acid, and extracted with ethyl acetate (3 × 100 mL). The combined solvent extract was washed successively with 100 mL portions of 5% $NaHCO_3$ and brine. Evaporation of solvent and recrystallization in a freezer from ethyl acetate−hexanes (2.5:100 mL) gave ketoester **7** (2.08 g, 58%): mp 51−53 °C: $[\alpha]^{20}_D$ −9.3° (*c* 0.4, $CHCl_3$); ^{1}H NMR δ 1.33 (3H, d, J = 7.2 Hz), 1.44 (9H, s), 3.60 and 3.62 (2H, d, J = 16.0 Hz), 4.35 (1H, p, J = 7.2 Hz), 5.1 (1H, m), 5.18 (2H, br s), 7.36 (5H, m). Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.60; H, 7.28; N, 4.33.

[(4*S*)-*N*-Boc-4-amino-3-oxopentanoyl]-L-Ala-*O*-Bn (8). A solution of ketoester **7** (6.0 g, 18.7 mmol) in CH_2Cl_2 (200 mL) was stirred with 5% Pd/C (2 g) under hydrogen (1 atm) at −5 to 0 °C for 3 h (reaction appeared complete by TLC). The cold mixture was quickly (to avoid decarboxylation of the intermediate β-ketoacid) filtered through Celite into a stirred mixture of alanine benzyl ester hydrochloride (4.03 g, 18.7 mmol) and NMM (2.05 mL, 18.7 mmol) in CH_2Cl_2 (200 mL) at 0 °C. To this mixture was added EDC (3.95 g, 20.6 mmol). After stirring at 0 °C for 3 h and 25 °C for 16 h, the solvent was evaporated, the residue was dissolved in ethyl acetate (300 mL), and the solution was washed successively with 100 mL portions of 10% citric acid, water, saturated $NaHCO_3$, and brine. Filtration, evaporation, and one recrystallization from ethyl acetate−hexanes (25:500 mL) gave dipeptide **8** (4.65 g, 63%): mp 82−85 °C; $[\alpha]^{20}_D$ −4.6° (*c* 2.0 $CHCl_3$); ^{1}H NMR δ 1.31 (3H, d, J = 7.2 Hz), 1.44 (9H, s), 1.44 (3H, d, J = 7.2 Hz, Ala-Me), 3.51 and 3.54 (2H, d, J = 16.0 Hz), 4.30 (1H, p, J = 7.2 Hz), 4.61 (1H, p, J = 7.2 Hz, Ala-CH), 5.17 (1H, m), 5.17 and 5.18 (2H, d, J = 12.5 Hz), 7.35 (5H, m), 7.49 (1H, m). Anal. Calcd for $C_{20}H_{28}N_2O_6$: C, 61.21; H, 7.19; N, 7.14. Found: C, 61.44; H, 7.28; N, 7.32.

Boc-Ibu-L-Ala-*O*-Bn (9). To a stirred solution of dipeptide **8** (5.5 g, 14 mmol), iodomethane (60 mL, 420 mmol), and THF (500 mL) at 0 °C was added with gas evolution 60% sodium hydride in mineral oil (562 mg, 14 mmol). After 45 min at 0 °C, an additional 562 mg portion of NaH was added. After stirring at 0 °C for 6 h and 25 °C for 16 h, the solvent was evaporated and the residue dissolved in ethyl acetate (300 mL). Washing with brine (2 × 100 mL) and evaporating gave *gem*-dimethyl derivative **9** (5.05 g, 86%) contaminated with 0.45 g of mineral oil which was not removed until the next step: ^{1}H NMR δ 1.24 (3H, d, J = 7.1 Hz), 1.40 (3H, d, J = 7.2 Hz, Ala-Me), 1.42 (9H, s), 1.45 (6H, s), 4.60 (1H, p, J = 7.1 Hz), 4.71 (1H, p, J = 7.2 Hz, Ala-CH), 5.10 (1H, m), 5.14 and 5.20 (2H, d, J = 12.1 Hz), 6.78 (1H, m), 7.35 (5H, m). MS(EI), *m*/*z* 347 (M − *t*-BuO).

Boc-Ibu-L-Ala-Map-Hmp-Gly-*N*-Me-L-Leu-Gly-*N*-Me-L-Val-*O*,*N*-diMe-L-Tyr-*O*-Bn (26). A solution of TFA salt **25** (70 mg, 0.0796 mmol), dipeptide **10** (26 mg, 0.0796 mmol), TBTU (127 mg, 0.398 mmol), and *N*,*N*-diisopropylethylamine (69 μL, 0.40 mmol) in DMF (2 mL) was stirred for 20 h. Product separation by HPLC gave depsipeptide **26** (43 mg, 45%): ^{1}H NMR (16 rotamers) δ 0.21, 0.55, 0.71 (d, J = 6.0−6.3 Hz, Val Me's), 1.42 (9H, s), 2.37−3.05 (9H, s), 3.75 (3H, s), 5.13 and 5.21 (2H, d, J = 12.5 Hz), 6.73, 6.84, 7.07, and 7.12 (4H, m), 7.34 (5H, br s), FABMS *m*/*z* 1193 $[M + 1]^+$.

Dolastatin 11 (1). A mixture of the salt **28** (141 mg, 0.125 mmol), DMF (60 mL), HBTU (213 mg, 0.623 mmol), and triethylamine (34 μL, 0.249 mmol) was stirred for 7 h at 25 °C under argon. Evaporation of the solvent and separation of the residue by HPLC gave dolastatin 11 (**1**; 25 mg, 20% yield, retention time 45 min): $[\alpha]^{25}_D$ −143 (*c* 0.33, CH_2Cl_2); FABMS *m*/*z* 985 $[M + 1]^+$. The synthetic dolastatin 11 (**1**) was identical with the natural product by comparison of ^{1}H and ^{13}C NMR spectra, HPLC, tlc, and cancer cell line activity.

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Supporting Information Available: Preparations of compounds **6**, **10**−**25**, **27**, and **28**, proton NMR spectra of natural and synthetic dolastatin 11 (**1**) and intermediates **5**−**28**, and carbon NMR shifts of natural and synthetic **1** (39 pages). See any current masthead page for ordering and Internet access instructions.

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