Pergamon

Tetrahedron Vol. 50, No. 42, pp. 12097-12108, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00765-9

The Dolastatins 20. A Convenient Synthetic Route to Dolastatin 15[1a]

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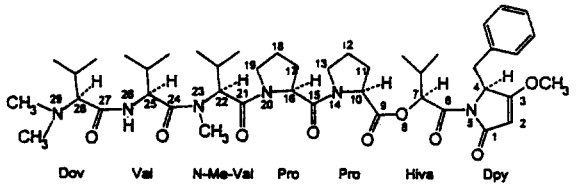
ABSTRACT. A segment synthetic strategy was utilized for obtaining the *Dolabella auricularia* (Indian Ocean sea hare) depsipeptide dolastatin 15. Reaction of protected (S)-Hiva-(S)-Phe 2c with isopropenyl chloroformate followed by Meldrum's ester, cyclization (2c → 3a) of the product in toluene and finally methylation afforded the key (S)-dolapyrrolidine (Dpy) derivative 3b. Condensation of tripeptide 8 with the three unit Dpy segment 5b followed by deprotection and coupling (diethyl phosphorocyanidate) led to dolastatin 15 in 11% overall yield. The powerful and selective activity of dolastatin 15 against the U.S. National Cancer Institute's panel of human cell lines has been summarized.

The rapidly intensifying search for biologically and medicinally important marine organism constituents has attracted a great deal of interest world-wide.[2] Not unexpectedly[3] a reassuring number of marine animals, plants and microorganisms are being found to produce promising anticancer substances of unprecedented structural types.[2,4] Illustrative of this observation has been the Indian Ocean sea hare *Dolabella auricularia*. Very importantly this marine mollusc has been found to be a remarkable reservoir of antineoplastic and/or cytostatic substances which we have designated the dolastatins.[1a] The dolastatins were found to be peptides possessing a number of hitherto unknown amino acids. For example dolastatin 15 (1) was found to contain a new pyrrolidone amino acid designated dolapyrrolidone.[5]



1, Dolastatin 15

Dolastatin 15 (1) has been found to strongly and selectively inhibit (TGI < 10^{-9} μg/mL) the growth of thirteen human cancer cell lines included in the U. S. National Cancer Institute human cell line panel.[6] Originally we isolated 6.2 mg (4 x 10^{-7}% yield) of dolastatin 15 from 1,600 kg (wet wt.) of the sea hare.[5] Since dolastatin 15 has been selected for eventual clinical trial a practical total synthesis was urgently required. In 1989 we completed the first total synthesis[7] of this promising new depsipeptide and now report modifications in

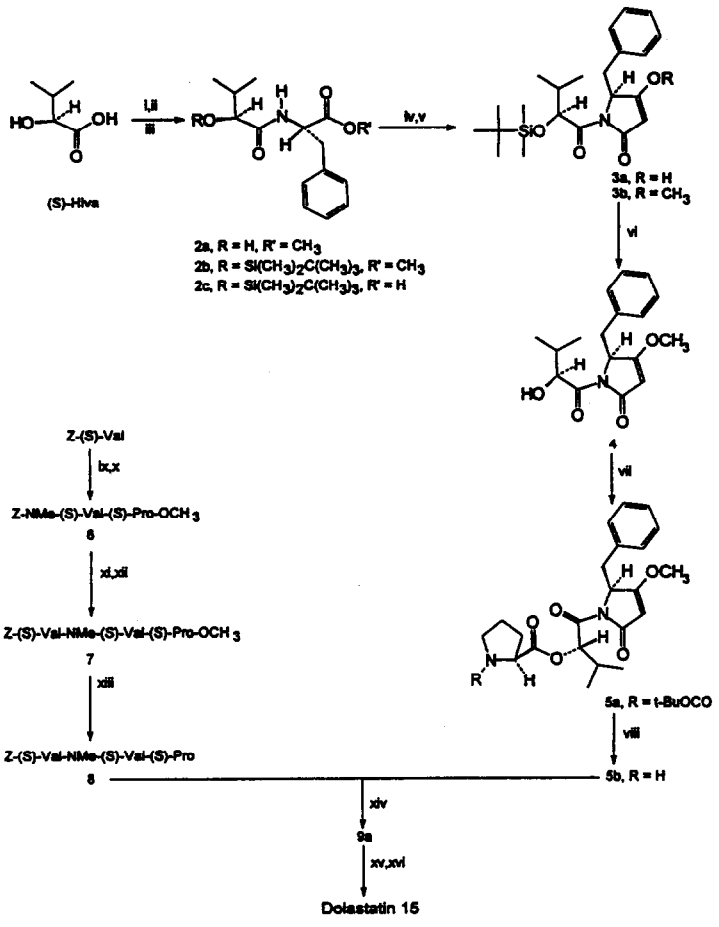
this synthetic route which have resulted in a very practical method for preparing natural (-)-dolastatin 15 in quantity.

Structurally, dolastatin 15 is derived from (S)-dolapyrrolidone (Dpy),[7] (S)-2-hydroxy-isovaleric acid (Hiva), dolavaline (Dov),[8] two units of proline, valine, and N-methyl-valine. Dolapyrrolidone falls in a class of modified amino acids presumably derived biosynthetically from phenylalanine through a two carbon condensation. Natural products containing a glycine derived pyrrolidone C-terminus have previously been found in *Streptomyces* (e.g. the antibiotic althiomycin[9]), the blue green algae components malyngamide,[10] and pukeleimide.[11] Dysidin,[12] a constituent of both sponges and blue green algae, contains a valine derived pyrrolidone C-terminus. Very recently a hexachloro metabolite, dysidamide[13] was isolated from a *Dysidea* species of sponge. Hiva is found to be incorporated in the Hip unit of the potent tunicate didemnins.[14]

In order to start the original synthesis in an orderly fashion, it was assumed that all of these amino acids have the L (S)-stereochemistry as found in dolastatin 10.[8] The challenge of realizing racemizaton-free coupling of Dpy with Hiva was apparent due to the non-nucleophilic nature of the Dpy nitrogen. In order to avoid total racemization encountered in preliminary experiments[15a] involving generation of the nitrogen anion with sodium hydride in tetrahydrofuran,[15] it was decided to synthesize (*S*)-Hiva-(*S*)-Phe-OMe (2a) (see Scheme 1) and conduct a ring closure of the protected derivative (2c) to afford a blocked Hiva-Dpy (3a). In turn (*S*)-Hiva was prepared from (*S*)-valine through a well known[15b,c] diazotation procedure with retention of configuration. The (*S*)-Hiva was coupled with Phe-OMe hydrochloride using diethyl phosphorocyanidate (DEPC) in the presence of N-methylmorpholine to give (*S*)-Hiva-(*S*)-Phe-OMe (2a). The Hiva-Phe-OMe was further protected (in excellent yield) using t-butyldimethylsilyl choride in the presence of imidazole.[16-18] Cleavage (2b → 2c) of the methyl ester was performed using mild alkaline conditions.

Dolapyrrolidone derivative 3a was synthesized <u>via</u> acylation[19] of Meldrum's ester as follows. Isopropenyl chloroformate was found to give the best results of several mixed carbonic anhydrides derived from carboxylic acid 2c, as reported,[20] when used in the presence of five molar equivalents of 4-dimethylaminopyridine. After removal of base using 10% aqueous $KHSO_4$ the Meldrum's ester adduct was heated in refluxing toluene to afford pyrrolidone 3a in 68% yield. Methylation of the tautomeric mixture (3a) using dimethylsulfate and potassium carbonate in tetrahydrofuran gave methyl vinyl ether 3b, without any detectable C-alkylated product. Interestingly, some racemization was detected at the Phe center to give an approximate product ratio of 7.4:1 S,S: *S,R* TBDMS-Hiva-Dpy (3b). The *S,R* isomer side-product was easily removed using a column chromatographic separation. The yield of pyrrolidone 3a fell dramatically to 21% and lower when the Meldrum's ester adduct was cyclized in methanol,[20] and this may have been due to simple β-keto ester formation.

Currently a variety of reagents are available for removal of silyl groups[16-18] and we earlier reported[7] successful (90% yield) cleavage of the silyl ether 3b using pyridinium



Scheme 1

(i) Phe-OMe·HCl, diethyl phosphorocyanidate (DEPC), NMM, CH_2Cl_2; (ii) TBDMS chloride, imidazole, DMF; (iii) 1 N NaOH, CH_3CH_2OH-H_2O; (iv) Meldrum's ester, 4-DMAP, $ClCO_2C(CH_3)=CH_2$, CH_2Cl_2; toluene, Δ; (v) K_2CO_3, $(CH_3O)_2SO_2$, THF; (vi) TFA; (vii) Boc-(S)-Pro, DCCI, 4-pyrrolidinopyridine, CH_2Cl_2; (viii) TFA, CH_2Cl_2; (ix) NaH, CH_3I, THF; (x) (S)-Pro-OCH_3·HCl, DEPC, TEA, DME; (xi) H_2, 10% Pd/C, EtOAc-CH_3OH; (xii) Z-(S)-Val, $(CH_3)_3CCOCl$, NMM, CH_2Cl_2; (xiii) 1 N NaOH, CH_3OH-H_2O; (xiv) DEPC, TEA, CH_2Cl_2, 0°C; (xv) H_2, 10% Pd/C, ETOAc-CH_3OH; (xvi) (S)-Dov, DEPC, TEA, CH_2Cl_2, 0°C.

polyhydrogen fluoride. Later we discovered that the use of trifluroacetic acid led to quantitative yields. In contrast, tetrabutylammonium fluoride in tetrahydrofuran solution gave only poor results. Esterification of alcohol 4 with Boc-(S)-Pro using dicyclohyxylcarbodiimide (DCCI) with 4-pyrrolidinopyridine gave the desired depsipeptide (5a) in excellent yield (92%). Depsipeptide 5a was shown by X-ray crystal structure determinaton to have the chirality required for conversion to dolastatin 15.[7] Removal of the Boc-protecting group was accomplished in quantitative yield using trifluoroacetic acid to provide amine 5b.

Since proline coupling is usually racemization free, a segment condensation approach based on coupling at Pro was adopted for completing the synthesis of dolastatin 15. In addition, it was found that consistently higher yields were obtained by using the overall strategy of coupling the two tripeptide units 8 and 5b followed by final condensation with Dov, rather than coupling the tetrapeptide (Dov-(S)-Val-Nme-(S)-Val-(S)-Pro) with the depsipeptide 5b at the Pro-Pro linkage, as previously reported.[7] Condensation of N-Z,N-Me-(S)-Val[21] with (S)-Pro-OMe was realized employing the DEPC coupling procedure to give dipeptide (6) in 77% yield. Cleavage of the carbobenzoxy protecting group by hydrogenolysis was followed by coupling with the mixed anhydride prepared from pivaloyl chloride and Z-(S)-Val to afford (83%) tripeptide 7. The pivaloyl anhydride procedure has previously been described in our dolastatin 10 synthesis[8] at an analogous location involving coupling of an N-Me-amino acid. A similar step appears in synthesis of the immunosuppressive peptide cyclosporine.[22]

The methyl ester group of tripeptide 7 was removed using dilute base and the resulting carboxylic acid 8 was coupled with depsipeptide 5b using DEPC to give Z-protected depsipeptide 9a. Deprotection by hydrogenolysis yielded the corresponding amine (9b) which was then coupled (DEPC) with dimethyl valine (Dov). Dolastatin 15 (1) was obtained in excellent yield (97%) by silica gel column chromatographic purification. Final purification by recrystallization gave finely divided colorless crystals identical with natural (-)-dolastatin 15 (1). Identity was confirmed by results of high field (400 MHz) ^{1}H-NMR, ^{13}C-NMR, and mass spectral comparison combined with biological, detailed high performance liquid (and thin layer) chromatographic, and optical rotation results.

Dolastatin 15 (1) was found to strongly inhibit progression of an important series of human cancer cell lines among the U.S. National Cancer Institute's disease oriented panel.[23-25] Remarkable potency (TGI $\log_{10}$ -7 to -9) and selectivity was exhibited against non-small cell lung (NCI-H23), NCI-H552), small cell lung (DMS-114, DMS-273), colon (COLO-205, HCC2998, HT29, KM-20L2), brain (SF-295, SF-539), melanoma (SK-MEL-2, SK-MEL-5), ovary (OVCAR-3), renal (SN12K1) cancers and a leukemia (HL-60TB). A number of human cancer xenograft studies and other preclinical research objectives are in progress and results will be summarized in a future report.

EXPERIMENTAL

The amino acids and derivatives S-phenylalanine methyl ester hydrochloride, S-proline methyl ester hydrochloride, N-Boc-(S)-proline, S-valine, and Z-S-valine, were employed as

obtained from Sigma-Aldrich Co. Other reagents were also obtained from this company or Lancaster Synthesis. Solvents were redistilled and solvent extracts of aqueous solutions, unless otherwise noted, were dried, unless otherwise noted, over anhydrous magnesium sulfate. Evaporation of solvents was performed under reduced pressure on a Buchi rotary evaporator. Ether refers to diethyl ether, THF to tetrahydrofuran, DMF to dimethylformamide, DME to ethylene glycol dimethyl ether and EtOAc to ethyl acetate. The THF was distilled from lithium aluminum hydride prior to use.

Analtech silica gel GF (0.25 mm) plates were used for thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) and developed with either 3% ceric sulfate in 3N sulfuric acid spray and/or iodine vapor. Stationary phases used for gravity or flash column chromatography were E. Merck (Darmstadt) silica gel (70-230 mesh, for gravity column and 40-63 for flash column). The HPLC analyses were performed using a reverse phase Phenomenex Ultremex 5 C_8 column (100 x 4.6 mm) and an analytical Gilson HPLC (306B, 811, 7 x 302), equipped with a Rheodyne injection valve (7125 with a 20 μl loop), working pressure ~94-101 bar.

Melting points were observed with an Electrothermal digital-melting-point apparatus, model IA9200. Optical rotation measurements were recorded using a Perkin-Elmer 241 polarimeter. The ultraviolet spectra were obtained in methanol solution with a Hewlett-Packard 8450A UV/vis spectrophotometer. A Mattson 2020 Galaxy FT spectrophotometer was employed for infrared measurements. Tetramethylsilane, residual chloroform (7.256 ppm) or dichloromethane (5.32 ppm) was used as an internal reference in all nuclear magnetic resonance measurements determined with Bruker AM 400 (^{1}H, ^{13}C) or Varian AM 300 Gemini instruments. Chemical shifts were recorded in ppm and peak multiplicities not designated in full are thus: s, singlet; d, doublet; t, triplet; dd, double-doublet, dt, double-triplet, bd, broad-doublet; m, multiplet. Deuteriochloroform was used as the NMR solvent unless otherwise mentioned. The HREI and SP-SIMS (FAB) mass spectra were recorded with a Kratos MS 50 instrument in the NSF regional mass spectrometry facility at the University of Nebraska. Elemental analyses were determined by Dr. A. W. Spang (Spang Microanalytical Laboratory, Eagle Harbor, MI).

(S)-Hiva-(S)-Phe-OMe (2a). To a stirred and cooled (0°C) solution composed of (S-2-hydroxy-isovaleric acid[25] (5 g, 42.3 mmol), (*S*)-phenylalanine methyl ester hydrochloride (9.12 g, 42.3 mmol), and 4-methylmorpholine (9.3 mL, 84.6 mmol) in dry CH_2Cl_2 (100 mL) was added diethyl phosphorocyanidate (6.4 mL, 42.3 mmol). After 2 h, the solution was washed with water (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The oily residue was dissolved in CH_2Cl_2 (5 mL) and placed on a column of silica gel (4x37 cm) and eluted with 1:4 EtOAc-CH_2Cl_2. The appropriate fractions were combined and concentrated under reduced pressure to give an oil 7.17 g (61%). An aliquot was recrystallized (3X) from toluene-hexane to give colorless needles: mp 73°C; $[\alpha]^{23}D$ = -24° (c=0.2, $CHCl_3$); EIMS (m/z): 279 (3.4, M^+); 178 (6.8, M^+-Hiva); 120 (79); 88 (100%); NMR (300 MHz) δ 0.74 (d, J=7.0 Hz, 3H, Val CH_3), 0.97 (d, J=7.0 Hz, 3H, Val CH_3), 2.08 (m, 1H, Val CH β), 2.66 (bd, J=5.1, 1 Hz, 1H, OH), 3.12 (m, 2H, phenyl CH_2), 3.73 (s, 3H, OCH_3), 3.95 (m, 1H, Val CH α), 4.92 (m, 1H, Phe CH α), 6.85 (bd, J=7.9 Hz,

1H, amide NH), 7.15 (m, 2H, 2 x phenyl H), 7.29 (m, 3H, 3 x phenyl H).

Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.49; H, 7.58; N, 5.01. Found: C, 64,62; H, 7.71; N, 5.14.

O-t-Butyldimethylsilyl-(S)-Hiva-(S)-Phe-OMe (2b). To a stirred solution of (S)-Hiva-(S)-Phe-OMe (2a), 2.79 g, 10 mmol) and imidazole (2.03 g, 30 mmol in dry DMF (30 mL) was added t-butyldimethylsilyl chloride (2.26 g, 15 mmol). After 18 h in the absence of moisture at 40°C, ether (200 mL) was added. The solution was washed with water (2 x 100 mL), dried and concentrated to an oil. The residual oil was dissolved in CH_2Cl_2 (5 mL) and applied to a column of silica and fractions eluted with CH_2Cl_2. The appropriate fractions were combined and concentrated to give a clear oil (3.42 g, 87%): $[\alpha]^{24}_D$ -40° (c=0.2, $CHCl_3$); NMR (300 MHz) δ 0.02 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.74 (d, J=6.9 Hz, 1H, Val CH_3), 0.87 (s, 9H, t-Bu), 0.89 (d, J=6.9 Hz, 3H, Val CH_3), 2.05 (m, 1H, Val CHβ), 3.10 (m, 2H, CH_2), 3.70 (s, 3H, OCH_3),3.93 (d, J=3.3 Hz, 1H, Val CHα), 4.96 (m, 1H, Phe CHα), 6.92 (bd, J=8.5 Hz, 1H, amide NH), 7.14 (m, 2H, 2 x phenyl H), 7.27 (m, 3H, 3 x phenyl H); MS m/z 393 (0.7, M^+), 378 (1.4, M^+-CH_3), 362 (0.5, M^+-CH_3)2), 336 (100, M^+-t-Bu)%. Anal. Calcd for $C_{21}H_{35}NO_4Si$: C, 64.08; H, 8.96; N, 3.56. Found: C, 63.82; H, 8.77; N, 3.75.

t-Butyldimethylsilyl-(S)-Hiva-(S)-Phe (2c). To a vigorously stirred solution of t-butyldimethylsilyl-(S)-Hiva-(S)-Phe-OMe (2b, 3.58 g, 9.1 mmol) in EtOH (20 mL) and water (40 mL) was added sodium hydroxide solution (1.0 N, 18.4 mL, 18.4 mmol). Saponification was conducted at room temperature for 30 min and the clear solution was acidified to pH 3.0 using saturated citric acid solution. The product was extracted with ethyl ether (2 x 50 mL) and the fractions combined, dried, and concentrated to give a solid (3.1 g, 90%). Recrystallization from hexane led to pure colorless crystals: mp 87-88°C; $[\alpha]^{24}_D$ -18° (c=0.2, $CHCl_3$); MS m/z 379 (0.7, M^+), 364 (2, M^+-CH_3), 335 (M^+-CO_2), 322 (100, M^+-t-Bu)%. ^{1}H-NMR (300 MHz) δ 0.04 (s, 6H, 2 x $SiCH_3$), 0.70 (d, J=6.8 Hz,3H, Val CH_3), 0.85 (s, 9H, t-Bu), 0.86 (d, J=6.8 Hz, 3H, Val CH_3), 2.00 (m, 1H, Val CHβ), 3.16 (m, 2H, CH_2), 3.97 (d, J=3.2 Hz, 1H, Val CHα), 4.94 (m, 1H, Phe CHα), 6.94 (bd, J=8.6 Hz, 1H, amide NH), 7.18-7.33 (m, 5H,phenyl), carboxylic acid not seen.

Anal. Calcd for $C_{20}H_{33}NO_4Si$: C, 63.29; H, 8.76; N, 3.69. Found: C, 63.54; H, 9.28; N, 3.89.

O-t-Butyldimethylsilyl-(S)-Hiva-(S)-Dpy (3b). To a stirred and cooled (-10°C) solution of t-butyldimethylsilyl-(S)-Hiva-(S)-Phe (2c, 7.6 g, 20 mmol) in dry CH_2Cl_2 (10 mL) was added 4-dimethylaminopyridine (12.2 g, 0.1 mmol) and Meldrum's ester (3.18 g, 22 mmol). The solution was stirred in the absence of moisture for 2 h at -10°C, and then allowed to rise to 0°C and stirred for an additional 2 h. The reaction mixture was then washed with 10% aqueous $NaHSO_4$ solution (2 x 200 mL), followed by water (100 mL), dried and concentrated to an oil. The oil was dissolved in toluene (150 mL) and the solution heated at reflux for 1 h. Concentration under reduced pressure gave a pale reddish oil which was dissolved in dry THF (150 mL). To this solution was added potassium carbonate (8 g, 58 mmol) and dimethyl sulfate (5 mL, 50 mmol). The mixture was stirred for 18 h and the solution filtered through a bed of celite. Concentration (*in vacuo*) gave an oil which was dissolved in CH_2Cl_2 (10 mL) and placed on a column of silica gel. The chromatography column was eluted with CH_2Cl_2 to

give firstly t-butyldimethylsilyl-(S)-Hiva-(R)-Dpy, a clear oil which later crystallized (0.74 g, 9.2%) followed by the optically pure S,S-product (2c), a clear oil (5.45 g, 68% which soon crystallized: mp 110-111°C; $[\alpha]^{24}_D$ + 165° (c=0.2, $CHCl_3$); MS m/z 417 (2,M^+), 402 (3.6, M^+-CH_3), 360 (100, M^+-t-butyl)%; ^{1}H-NMR (300 MHz) δ 0.10 (s, 3H, $SiCH_3$), 0.84 (d, J=6.9 Hz, 3H, Val CH_3), 0.98 (s, 9H, t-Bu), 1.00 (d, J=6.9 Hz, 3H, Val CH_3), 1.97 (m, 1H, Val CH β), 3.19 (dd, J=3.0, 13.8 Hz, 0.5 Phe CH_2), 3,62 (dd, J=5.3, 13.8 Hz, 0.5 CH_2), 3.80 (s, 3H, OCH_3), 4.76 (dd, J=3.0, 5.3 Hz,H-5), 4.81 (s, 1H,H-3), 5.28 d, J=2.8 Hz, 1H, Val CH), 7.04 (m, 2H, 2 x phenyl H), 7.21 (m, 3H, 3 x phenyl H).

Anal. Calcd for $C_{23}H_{35}NO_4Si$: C, 66.15; H, 8.45; N, 3.35. Found: C, 66.61; H, 8.85; N, 3.42.

(S)-Hiva-(S)-Dpy (4). Trifluoroacetic acid (15 mL) was added to a stirred solution of t-butyldimethylsilyl-(S)-Hiva-(S)-Dpy (3b), 2.59 g, 6.2 mmol) in CH_2Cl_2 (200 mL). After 2 h the solvent was removed under reduced pressure. The oily residue was dissolved in CH_2Cl_2 (10 mL) and chromatographed on a column of silica gel. Elution with CH_2Cl_2-EtOAc (10:1) led to the alcohol as a clear oil (1.88 g, 100%): $[\alpha]^{23}_D$ +285° (c=0.2, $CHCl_3$); MS m/z 303 (0.7%, M^+); ^{1}H-NMR (300 MHz) δ 0.87 (d, J=6.9 Hz, 3H, Val CH_3), 1.08 (d, J=6.9 Hz, 3H, Val CH_3), 2.13 (m, 1H, Val CH β), 3.13 (dd, J=2.8, 13.9 Hz, 0.5 Phe CH_2), 3.65 (dd, J=5.0, 13.9 Hz, 0.5 Phe CH_2), 3.66 (br, 1H, OH), 4.79 (m, 1H, H-5), 4.83 (s, 1H, H-3), 4.85 (d, J=2.9 Hz, 1H, Val CHα), 6.97 (m, 2H, 2 x phenyl), 7.24 (m, 3H, 3 x phenyl).

Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.97; N, 4.61. Found: C, 67.39; H, 7.06; N, 4.65.

Boc-(S)-Pro-(S)-Hiva-(S)-Dpy (5a). A solution of Boc-(S)-proline (2.13 g, 9.89 mmol), (S)-Hiva-(S)-Dpy (4), 2.52 g, 8.29 mmol), DCCI (2.04 g, 9.89 mmol), and 4-pyrrolidinopyridine (1.47 g, 8.29 mmol) in CH_2Cl_2 (25 mL) was stirred at room temperature under an argon atmosphere, overnight. The precipitated dicyclohexylurea was removed by filtration and the filtrate concentrated under reduced pressure to give a yellow oil. A solution of the oil in CH_2Cl_2 (10 mL) was added to a silica gel column and elution performed with CH_2Cl_2-EtOAc (10:1). The appropriate fractions were concentrated to give a crystalline solid (3.83 g, 92%). Recrystallization from toluene-hexane afforded analytically pure crystals: mp 157-158°C; $[\alpha]^{30}_D$ +96° (c=0.19, $CHCl_3$); EIMS (m/z: 500 (5, M^+), 444 (7), 399 (12), 286 (10), 240 (8), 204 (15), 170 (24), 114 (100)%; IR (NaCl) ν_{max} 1749, 1729, 1699, 1629, 1394, 1380, 1367, 1308, 1196, 1168 cm^{-1}, ^{1}H-NMR (300 MHz) two conformers in the ratio of 3:1 δ 0.90 (d, J=6.9 Hz, 3H, Val CH_3), 1.04 (d, J=6.9 Hz, 3H, Val CH_3), 1.42, 1.44 (s, 9H, t-Bu), 1.91 (m, 2H, Pro CH_2), 2.25 (m, 1H, CHβ), 2.25 (m, 2H, Pro CH_2), 3.06 (dd, J=3.3, 13.9 Hz, 1H, 0.5 Phe CH_2), 3.55 (dd, J=5.0, 13.9 Hz, 1H, 0.5 Phe CH_2), 3.59 (m, 1H, 0.5 Pro CH_2), 3.71, 3.74 (s, 1H, OCH_3), 4.36, 4.49 (t, J=6.1 Hz, 1H, CHα), 4.69, 4.71 (s, 1H, H-3), 4.76 dd, J=3.0, 5.0 Hz, 1H, H-5), 5.83, 5.89 (d, J=2.5 Hz, Hiva CHα), 7.08 (m, 2H, phenyl H), 7.18 (m, 3H, phenyl H); ^{13}C-NMR (400 MHz) 15.83 (Hiva-CH_3), 23.46 (Pro-CH_2), 28.31 (t-Bu CH_3), 28.52 (Pro-CH_2), 30.75 (Hiva-CH), 34.94 (Dpy-CH), 46.34 (Pro-CH_2), 58.36 (Dpy-CH), 58.72 (Pro-CH), 60.02 (Dpy-OCH_3), 77.68 (Hiva-CH), 79.93 (Pro-CO), 94.72 (Dpy-CH), 127.02 (Dpy-CH), 128.24 (Dpy-2 x CH), 129.92 (Dpy-2 x CH), 133.98 (Dpy-C-1), 154.02 (Pro-CO) 169.14 (Pro-CO), 169.57 (Hiva-CO), 172.64 (Dpy-CO), 178.42 (Dpy-CO).

Anal. Calcd for $C_{27}H_{36}N_2O_7$: C, 64.78; H, 7.25; N, 5.60. Found: C, 64.90; H, 7.34; N, 5.63.

(S)-Pro-(S)-Hiva-(S)-Dpy (5b). To a cool (ice-bath) and stirred solution of the depsipeptide (5b, 1.48 g, 2.96 mmol) in CH_2Cl_2 (100 mL) was added trifluroacetic acid. One hour later the solvent was removed under reduced pressure; toluene (25 mL) was added and the solution was reconcentrated, under reduced pressure, to give a clear oil. The oil was dissolved in CH_2Cl_2 (25 mL), the solution cooled to 0°C, triethylamine (5 mL) added, and the mixture stirred for five minutes. The solution was then concentrated and the resulting oil dissolved in CH_2Cl_2 and added to a column of silica gel. The product was eluted with 5% EtOH in CH_2Cl_2. The analogous fractions were combined and concentrated to give a clear gum/glass (1.19 g, 100%): $[\alpha]^{23}_D$ + 136° (c = 0.28, $CHCl_3$); EIMS (m/z) 400 (2.8, M^+), 356 (12), 302 (3.2, M^+-Pro), 287 (302-CH_3)%; ^{1}H-NMR (300 MHz) δ 0.94 (d, J=7.0 Hz, 3H, Val CH_3), 1,09 (d, J=7.0 Hz, 3H, Val CH_3), 1.94 (m, 1H, Pro CH_2), 2.27 (m, 1H, Val CHβ), 2.37 (m, 2H, Pro CH_2), 3.07 (dd, J=14.0, 3.5 Hz, 1H, 0.5 Phe CH_2), 3.24 (m, 2H, Pro CH_2), 3.57 (dd, J = 14.0, 4.4 Hz, 1H, 0.5 Phe CH_2), 3.79 (s, 3H, OCH_3), 4.33 (t, J=6.9 Hz, 1H, Pro CHα), 4.76 (s, 1H, H-3), 4.79 (m, 1H, Phe H-5), 5.93 (d, J=2.6 Hz, 1H, Val CHα), 7.10 (m, 2H, 2 x phenyl), 7.24 (m, 3H, 3 x phenyl); ^{13}C-NMR (400 MHz): 15.77, 19.74, 25.16, 28.83, 30.08, 34.85, 46.886, 58.31, 59.43, 59.92, 77.78, 94.69, 126.97, 128.15, 129.84, 133.96, 169.13, 169.43, 174.90, 178.32.

Anal. Calcd for $C_{22}H_{28}N_2O_5$: C, 65.98; H, 7.05; N, 7.00. Found: C, 64.45; H, 7.17; N, 7.57.

Z-NMe-(S)-Val-(S)-Pro-OMe (6). A stirred solution of (S)-proline methyl ester hydrochloride (0.94 g, 5.66 mmol) and Z-NMe-(S)-valine[21] (15, 1.5 g, 5.66 mmol) in DME (40 mL was treated with diethyl phosphorocyanidate (0.92 mL, 6 mmol) and triethylamine (1.59 mL, 11.32 mmol). The mixture was stirred under argon at 0°C for 2 h and then 6 h at room temperature. Upon addition of water (100 mL) the product was extracted with EtOAc (3 x 100 mL). The combined extracts were successively washed with 5% hydrochloric acid (2 x 100 mL), water (100 mL), saturated $NaHCO_3$ solution (2 x 100 mL), water (100 mL), and dried. The solvent was removed under reduced pressure and dipeptide 6 crystallized as needles from toluene-hexane (1.65 g, 77%); mp 104-105°C; $[\alpha]^{30}_D$ -144° c=0.2, $CHCl_3$); EIMS (m/z) 376 (M^+, 8%), 220 (30), 176 (53), 128 (10), 91 (100); IR (NaCl) ν_{max} 2961, 1748, 1697, 1649, 1437, 1396, 1304, 1197, 1176, 1164 cm^{-1}H-NMR (300 MHz) δ, two major conformers in the ratio of 1:3: 0.85, 0.87 (d, J=6.6 Hz, 3H, Val CH_3), 0.93, 0.99 (d, J=6.5 Hz, 3H, Val CH_3), 1.80-2.04, 2.17-2.23 (m, 4H, 2 x Pro CH_2), 2.26 (m, 1H, Val CHβ), 2.92, 2.94 (s, 3H, N-CH_3), 3.67-3.73 (m, 1H, 0.5 Pro CH_2), 3.68, 3.72 (s, 3H, OCH_3), 3.89 dt, J=10, 6.8 Hz, 1H, Pro CHα), 4.36, 4.62 (d, J=11 Hz, 1H, Val CHα), 4.43 (dd, J=8.6, 3.8 Hz, Pro CHα). 5.03-5.28 (m, 2H, benzyl CH_2), 7.27-7.36 (m, 5H, phenyl); ^{13}C-NMR (400 MHz): 18.60 (Val CH_3), 18.79 (Val CH_3), 24.84 (Pro CH_2), 27.58 (Val CH), 27.59 (Pro CH_2), 29.12 (N-CH_3), 47.25 (Pro CH_2), 52.02 (Pro CH), 58.85 (Val CH), 61.79 (Pro OCH_3), 67.26 (ArCH_2O), 127.53, 127.89, 128.46 (ArCH), 136.60 (Val CO), 157.11, 169.72 (Val CO), 172.49 (Pro CO).

Anal. Calcd for $C_{20}H_{28}N_2O_5$: C, 63.81; H, 7.50; N, 7.44. Found: C.63.79; H, 7.48; N, 7.47.

Z-(S)-Val-NMe-(S)-Val-(S)-Pro-OMe (7). A mixture of dipeptide 6 (1.15 g, 3.05 mmol) in EtOAc-methanol (3:1) and 10% palladium/carbon (0.20 g) was vigorously stirred in a hydrogen

atmosphere for 4 h. The solution was filtered and the filtrate concentrated to the clear, oily amine. Pivaloyl chloride (0.75 mL, 6.12 mmol) and N-methylmorpholine (1.34 mL, 12.24 mmol) were added to a vigorously stirred and cooled (-23°C) solution of Z-(S)-valine (1.54 g, 6.12 mmol) in CH_2Cl_2 (20 mL). The solution was stirred (under argon) for 3 h at the same temperature and the dipeptide 6 hydrogenation product was added. After stirring at -23°C for 4 h and at room temperature for 24 h, CH_2Cl_2 (100 mL) was added. The solution was washed with saturated citric acid (3 x 40 mL), water (20 mL), saturated $NaHCO_3$ solution (2 x 40 mL), and finally water (40 mL). After drying and concentrating, the clear oil was dissolved in CH_2Cl_2 (10 mL) and applied to a column of silica gel. Upon eluting with CH_2Cl_2-EtOAc (4:1) corresponding fractions were combined and concentrated to yield tripeptide 7 (1.2 g, 83%) as a colorless glass; $[\alpha]^{30}_D$ - 145° (c=0.26, $CHCl_3$); EIMS (m/z): 475 M^+, 5%, 444 (2), 346 (12), 206 (14), 162 (20), 91 (100); IR (NaCl) ν_{max} 3300, 2963, 17.49, 1720, 1637, 1437, 1260, 1234, 1216, 1198, 1176 cm^{-1} NMR (300 MHz) δ 0.75 (d, J=6.6 Hz, 3H, Val CH_3), 0.86 (d, J=6.6 Hz, 3H, NMe-Val CH_3), 0.91 (d, J=6.6 Hz, 3H, Val CH_3), 1.81-2.04 (m, 4H, Pro 2 x CH_2), 2.17 (m, 1H, Val CHβ), 2.28 (m, 1H, NMe-Val CHβ), 3.21 (s,3H,N-CH_3) 3.66 (m, 1H, Pro 0.5 CH_2), 3.70 (s, 3H, Pro OCH_3), 3.91 (m, 1H, Pro 0.5 CH_2), 4.37 (dd, J=8.2, 5.7 Hz, 1H, Pro CHα), 4.49 (dd, J=9.2, 6.4 Hz, 1H, Val CHα), 5.04 (d, J=11.2 Hz, 1H, NMe-Val CHα), 5.07 (s, 2H, benzyl CH_2), 5.46 (d, J=9.2 Hz, 1H, Val NH), 7.31 (m, 5H, phenyl); ^{13}C NMR (400 MHz): 17.31, 19.39, (Val CH_3's), 18.55, 18.80 (NMe-Val CH_3's), 25.01, 29.22, 47.37 (Pro CH_2's), 27.29 (NMe-Val CH), 30.57 (N-CH_3), 31.05 (Val CH), 52.12 (Pro CHα), 56.05 (Val CH), 58.85 (NMe-Val CH), 59.27 (Pro OCH_3), 66.85 ($ArCH_2O$), 127.90, 128.07, 128.48, 136.41 (Val ArC's), 156.39, 173.11 (Val CO's), 169.40 (NMe-Val CO), 172.43 (Pro CO).

Anal. Calcd for $C_{25}H_{37}N_3O_6$: C, 63.14; H, 7.86; N, 8.84. Found: C, 63.05; H, 8.04; N, 8.77.

Z-(S)-Val-NMe-(S)-Val-(S)-Pro (8). The Z-tripeptide 7 (0.95 g, 2 mmol) was stirred for 2 h in a solution of 1N sodium hydroxide (3 mL, 3 mmol), water (10 mL), and ethanol (10 mL). The clear solution was concentrated to half its volume and acidified to pH 3.0 using 1N hydrochloric acid. The organic material was extracted using EtOAc (3 x 25 mL) and the combined extracts were washed with water (50 mL), dried and concentrated to a clear glass (0.92 g, 100%): $[\alpha]^{23}_D$ -145° (c=0.26, $CHCl_3$); ^1H NMR (300 MHz) δ 0.83-0.98 (m, 12H, 4 x Val CH_3), 1.87-2.08 (m, 4H, 2 x Pro CH_2), 2.32 (m, 2H, 2 x Val CHβ), 3.15 (s, 3H, N-CH_3), 3.67 (m, 1H, 0.5 Pro CH_2), 3.91 (m, 1H, 0.5 Pro CH_2), 4.52 (m, 1H, Pro CHα), 5.08 (d, J=11.1 Hz, 1H, NMe-Val CHα), 5.09 (s, 2H, Phe CH_2), 5.58 (d, J=9.4 Hz, 1H, NH), 7.35 (s, 5H, phenyl), carboxylic acid not seen: ^{13}C NMR (400 MHz), 17.51, 18.32, 18.67, 19.14, 24.74, 27.16, 28.76, 30.85, 47.37, 56.17, 58.82, 59.32, 66.66, 127.66, 127.74, 127.85, 128.29, 136.36, 156.45, 169.58, 173.75 and 174.78.

Z-(S)-Val-NMe-(S)-Val-(S)-Pro-(S)-Pro-(S-Hiva-(S)-Dpy (9a). To a cooled (0°C) and stirred solution of tripeptide 8 (0.39 g, 0.97 mmol), depsipeptide 5b (0.46 g,1 mmol) and triethylamine (0.27 mL, 2 mmol) in CH_2Cl_2 (25 mL) was slowly added diethyl phosphorocyanidate (0.167 mL, 1.1 mmol). The dry solution was stirred for 18 h, concentrated to an oil, dissolved in CH_2Cl_2 (5 mL) and applied to a column of silica gel. Elution with 5% ethanol

in CH_2Cl_2 solution led to a clear glass (0.73 g, 89%) $[\alpha]^{23}_D$ - 65° (c=0.26, $CHCl_3$); FAB MS m/z): 984 [M+H$^+$]. ^{1}H-NMR (300 MHz) δ 0.82-0.94 (m, 12H, 4 x Val CHβ), 3.05 (dd, J=14.0, 3.5, Hz, 1H, 0.5 Phe CH_2), 3.16 (s, 3H, N-CH_3), 3.54 (dd, J=14.0, 4.3 Hz, 1H, 0.5 Phe CH_2), 3.60 (m, 1H, 0.5 Pro CH_2), 3.76 (s, 3H, OCH_3), 3.77 (m, 2H, Pro CH_2), 3.92 (m, 1H, 0.5 Pro CH_2), 4.53 (m, 1H, CHα), 4.64 (m, 1H, CHα), 4.73 (s, 1H, H-3), 4.78 (m, 1H, H-5), 4.85 (dd, J=8.6, 2.5 Hz, 1H, CHα), 5.08 (m, 1H, NMe-Val CHα), 5.10 (s, 2H, Z-Val CH_2), 5.46 (bd, J=9.5 Hz, 1H, NH), 5.90 (d, J=2.7 Hz, 1H, Hiva CHα), 7.14-7.24 (m, 5H, phenyl), 7.35 (s, 5H, 5 x Z-Val phenyl); ^{13}C NMR (400 MHz): 15.80, 17.30, 18.65, 19.18, 19.47, 19.81, 24.61, 24.69, 27.46, 28.36, 28.53, 28.87, 30.66, 31.17, 34.88, 46.37, 47.77, 56.04, 58.08, 58.30, 59.39, 59.87, 66.85, 77.84, 94.73. 126.98, 127.90, 128.12, 128.49, 129.98, 134.175, 136.45, 156.42, 169.24, 169.24, 169.47, 170.18, 171.44, 172.95, 178.18.

Anal. Calcd for $C_{46}H_{61}N_5O_{10}$: C, 65.46; H, 7.29; N, 8.30. Found: C, 64.80 H, 7.32; N, 8.22.

(S)-Val-NMe-(S)-Val-(S)-Pro-(S)-Pro-(S)-Hiva-(S)-Dpy (9b). A mixture of the Z-peptide (9a, 0.83 g, 0.986 mmol) and 10% Pd/C (0.8 g) was stirred vigorously in EtOAc (20 mL) under a hydrogen atmosphere (balloon pressure) for 18 h. After filtration, the clear solution was concentrated, the residue dissolved in CH_2Cl_2 (5 mL), added to a column of silica gel and the product eluted with 5% ethanol in CH_2Cl_2 to afford a clear glass (0.655 g, 96%); $[\alpha]^{24}_D$ -49.6° (c=0.26, $CHCl_3$); ^{1}H-NMR (300 MHz) δ 0.87-0.98 (m, 12H, 4 x Val CH_3), 1.06-1.09 (m, 6H, 2 x Val CH_3), 1.87-2.44 (m,11H, 4 x Pro CH_2, 3 x Val CHβ), 3.06 (dd, J=14.2, 3.5 Hz, 1H, 0.5 Phe ArCH_2), 3.09 (s, 3H, N-CH_3), 3.53 (dd, J=14.2, 4.4 Hz, 1H, 0.5 Phe CH_2), 3.62 (m, 1H, 0.5 Pro CH_2), 3.76 (s, 3H, OCH_3), 3.77 (m, 2H, Pro CH_2), 3.85 (m, 1H, 0.5 Pro CH_2), 4.64 (m, 1H, CHα), 4.72 (s, 1H, H-3), 4.78 (t, J=3.9 Hz, 1H, H-5), 4.85 (dd, J=8.6, 2.6 Hz, 1H, CHα)m 4,96 (m, 1H, CHα), 5.16 (d, J=11.1 Hz, 1H, NMe-Val CHα), 5.90 (d, J=2.7 Hz, 1H, Hiva CHα), 7.14-7.23 (m, 5H, phenyl); ^{13}C-NMR (400 MHz: 15.80, 16.70, 18.82, 19.26, 19.86, 20.00, 24/63, 24.73, 27.50, 28.39, 28.86, 30.38, 31.72, 34.87, 46.38, 47.80, 56.68, 58.10, 58.27, 58.34, 59.33, 59.87, 77.86, 94.76, 127.01, 128.15, 130.00, 134.16, 169.30, 169.50, 170.26, 171,.46, 178.20.

Anal. Calcd for $C_{38}H_{55}N_5O_8$: 1.5 H_2O; C. 63.67, H, 8.16; N, 9.77. Found: C, 63.49; H, 7.94; N. 9.67.

(S)-Dov-(S)-Val-(S)-NMe-Val-(S)-Pro-(S)-Pro-(S)-Hiva-(S)-Dpy (Dolastatin 15, 1). To a cooled (0°) and stirred solution (dry) of 9b (3.85 g, 5.58 mmol), dimethylvaline (Dov,[8] 0.99 g, 6.8 mmol), and triethylamine (0.97 mL, 6.8 mmol) in CH_2Cl_2 (100 mL) was slowly added diethyl phosphorocyanidate (1.02 mL, 6.8 mmol). After 2 h the clear solution was concentrated, dissolved in CH_2Cl_2 (15 mL), applied to a column of silica gel and the dolastatin 15 eluted with 5% ethanol in CH_2Cl_2 to provide a clear glass (4.54 g, 97%) which was identical (see above) to natural (-)-dolastatin 15. In some cases further purification of dolastatin 15 was required and achieved by applying 500 mg aliquots in 5 mL of hexane-acetone (1:1) to a Lobar pre-packed column (Size B, Si 60) and eluting with hexane-acetone (1:1) at 10 psi. The appropriate fractions were collected and concentrated under reduced pressure. With less tenacious impurities rapid gel permeation chromatography in methanol on a column of Sephadex LH-20 proved useful. Recrystallization from toluene-hexane afforded

analytically pure dolastatin 15 (1) as colorless crystals: mp 175-175.5°C; HPTLC-SiO_2 plate, Hexane:acetone-2:3, R_f = 0.31; HPLC-Phenomenex Ultramex 3 C_8 column (100 cm x 4.6 mm), R_t 3.8 min, MeOH:buffer(0.05M KH_2PO_4)-3:1, flow rate 1 ml/min; $[\alpha]^{24}_D$ -77° (c=0.2, CH_3OH); UV (in CH_3OH) (ϵ) λ_{max} 208nm (49,700), 240nm (19,500); FAB MS (m/z): 838 (13.7, M+H$^+$), 498 (100); 340 (93.6)%; IR (KBr) 3587, 3383, 2964, 2876, 1732, 1631, 1446, 1307, 1186, cm^{-1}, ^{1}H-NMR (300 MHz) δ 0.78 (t, J=6.6 Hz, 3H, Val CH_3), 0.92-0.95 (m, 12H, 4 x Val CH_3), 0.99-1.09 (m, 9H, 3 x Val CH_3), 1.82-2.45 (m, 12H, 4 x Pro CH_2, 4 x CHβ), 2.26 (s, 6H, 2 x CH_3, 2.46 (bd, J=6.1 Hz, 1H, Dov CHα), 3.04 (dd, J=14.0, 3.5 Hz, 1H, 0.5 Phe CH_2). 3.18 (s, 3H, N-CH_3), 3.54 (dd, J=14.0, 4.5 Hz, 0.5 Phe CH_2), 3.61 (m, 1H, 0.5 Pro CH_2), 3.76 (s, 3H, OCH_3), 3.77 (m, 2H, Pro CH_2), 3.80 (m, 1H, 0.5 Pro CH_2), 4.65 (m, 1H, CHα), 4.73 (s, 1H, H3) 4.79 (m, 2H, 2 x CHα), 4.84 (dd, J=8.7, 2.7 Hz, 1H, H-5), 1H, H-5), 5.14 (d, J=11.1 Hz, NMe-Val CHα), 5.90 (d, J=2.6 Hz, 1H, Hiva CHα), 6.92 (bd, J=8.9 Hz, 1H, NH), 7.14-7.23 (m, 5H, phenyl); ^{13}C NMR (400 MHz): 15.76, 17.61, 18.08, 18.51, 19.13, 19.55, 19.81, 20.16, 24.66, 27.27, 27.65, 28.35, 28.52, 28.83, 30.67, 31.08, 34.84, 42.94, 46.35, 47.79, 53.61, 58.03, 58.23, 59.16, 59.83, 77.82, 94.71, 125.27, 126.96, 128.11, 128.20, 129.00, 129.96, 134.15, 169.10, 169.25, 169.45, 170.18, 171.43, 171.78, 172.95, 178.16.

Anal. Calcd for $C_{45}H_{68}N_6O_9$: C, 64.57; H, 8.19; N, 10.04. Found: C, 64.16; H, 7.99; N, 9.82.

Another synthesis of dolastatin 15 was reported in 1992 by Poncet,[26] and the results suggest that the [α] and mp values for the depsipeptide are very sensitive to minor impurities.

ACKNOWLEDGMENTS

Very necessary financial assistance was provided by the Outstanding Investigator Grant CA44344-01A1-05 awarded by the NCI, DHHS, the Arizona Disease Control Research Commission, Eleanor W. Libby, the Waddell Foundation (Donald Ware), Herbert K. and Diane Cummings (The Nathan Cummings Foundation, Inc.), the Eagles Art Ehrmann Cancer Fund, Polly Trautman, and Lotte Flugel. We also wish to thank Drs. D. D. Burkett, C. L. Herald, F. Hogan-Pierson and S. B. Singh for other assistance.

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(*Received in USA* 28 *June* 1994; *revised* 29 *August* 1994; *accepted* 30 *August* 1994)