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Antineoplastic Agents. 590. X-ray Crystal Structure of Dolastatin 16 and Syntheses of the Dolamethylleuine and Dolaphenvaline Units†

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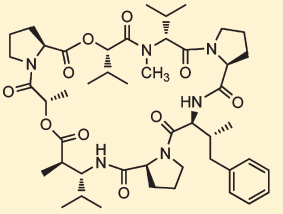
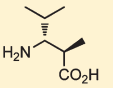
Supporting Information

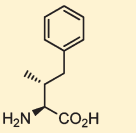
ABSTRACT: Three advances necessary to bring dolastatin 16 (**1**) into full-scale preclinical development as an anticancer drug have been accomplished. The X-ray crystal structure of dolastatin 16 has been solved, which allowed stereoselective syntheses of its two new amino acid units, dolamethylleuine (Dml) and dolaphenvaline (Dpv), to be completed. The X-ray crystal structures of synthetic *Z*-Dml and TFA-Dpv have also been completed.



1, dolastatin 16



2, dolamethylleuine

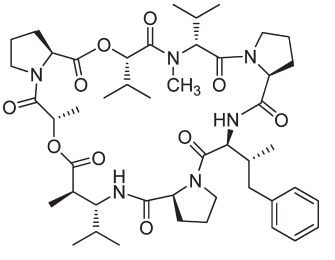


3, dolaphenvaline

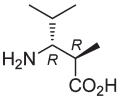
Very early in the discovery of the biologically remarkable and structurally unique peptides from the sea hare *Dolabella auricularia*, which we designated dolastatins, it became clear that certain members (e.g., 10−15) exhibited a variety of important properties that include anticancer[2] and antifungal activities.[3] Indeed, dolastatin 10 and three structural modifications are currently in human cancer phase II and phase III clinical trials.[2a] Two derivatives of dolastatin 15 are also in cancer clinical trials (phase I−II).[2a]

When we extended our field collections of *D. auricularia* from the Indian Ocean to the Western Pacific (Papua New Guinea and the Philippines), we were able to expand the dolastatin series to 16−19.[4] Dolastatin 16 (**1**)[4a] especially proved to be an exceptionally potent inhibitor of cancer cell growth and a candidate for further development. However, the latter important initiative has been delayed by the need for unequivocal configurational assignments and a practical total synthesis of dolastatin 16. We are pleased to report herein the X-ray crystal structure of dolastatin 16 (**1**) and syntheses of the new amino acid units dolamethylleuine (**2**) and dolaphenvaline (**3**).

Other options for obtaining certain dolastatin members appeared likely some 35 years ago when we considered[2d] that *Dolabella* species derived nutrition by consuming marine microalgae and that such exogenous sources might be providing the dolastatins or intermediates. This expectation has been amply realized over the past decade by the isolation of dolastatins 10−16[4a,5] or close analogues from the cyanobacterium *Lyngbya majuscula* and other such microalgae. Thus, fermentation methods using marine cyanobacteria may eventually be competitive with total syntheses for scale-up production of new anticancer drugs in the family. At present, the yields from these initial experiments remain very low, and for the foreseeable future the provision of dolastatin 16 for cancer clinical trial development



1



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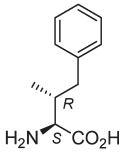

3

will require a practical total synthesis for scale-up production. However, the microalgae investigations continue to be very productive and promising for the future.

The three most obvious challenges to finding a useful synthesis of dolastatin 16, namely, an X-ray crystal structure to confirm the configuration and convenient stereoselective syntheses of the new amino acid units **2** and **3**, have been met as follows. Dolastatin 16 was originally isolated (in 3.1×10^{-7}% yield) as an amorphous powder, and a long period of attempts at crystallization were unsuccessful. Eventually, we found that very slow (over three years) crystal formation from acetonitrile and water provided X-ray quality crystals. Structurally, dolastatin 16 is a

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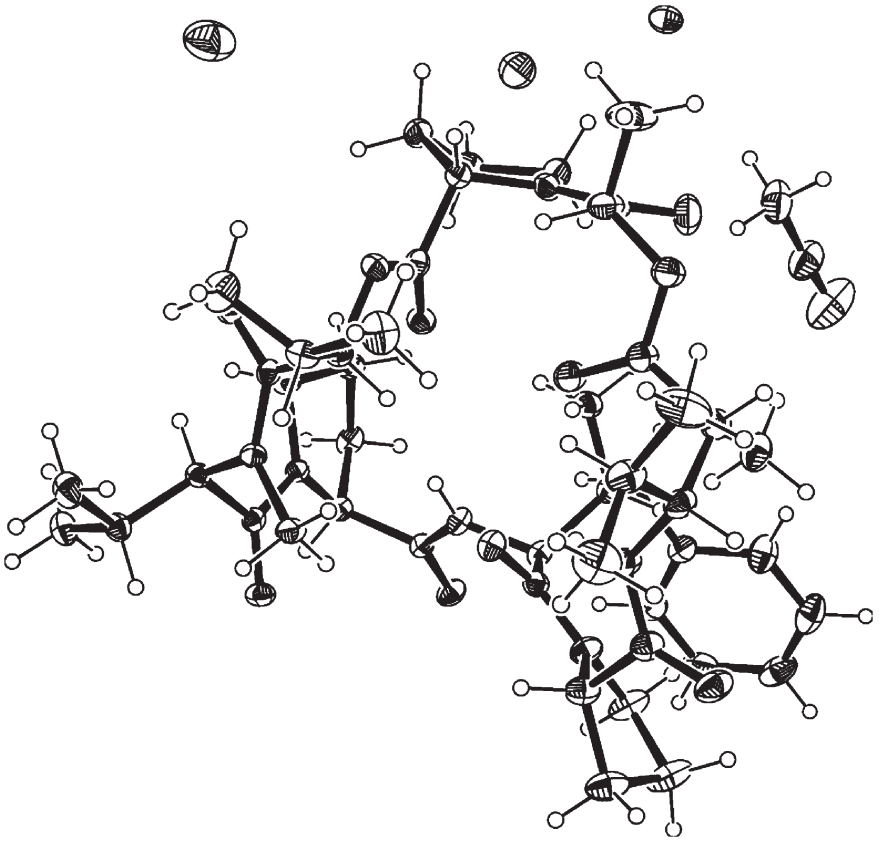
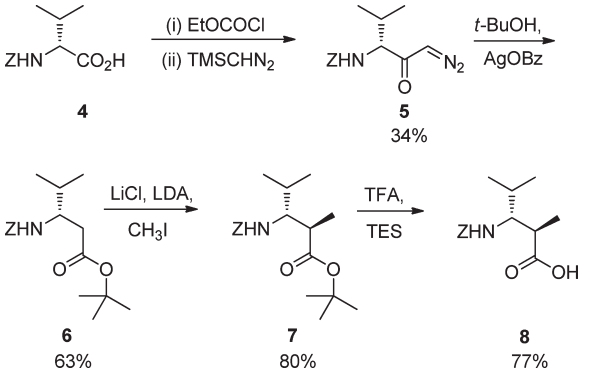


Figure 1. X-ray structure of dolastatin 16 (**1**). The atoms of this cyclic depsipeptide and solvent (one acetonitrile and three water) molecules are displayed as 30% probability thermal ellipsoids.

Scheme 1



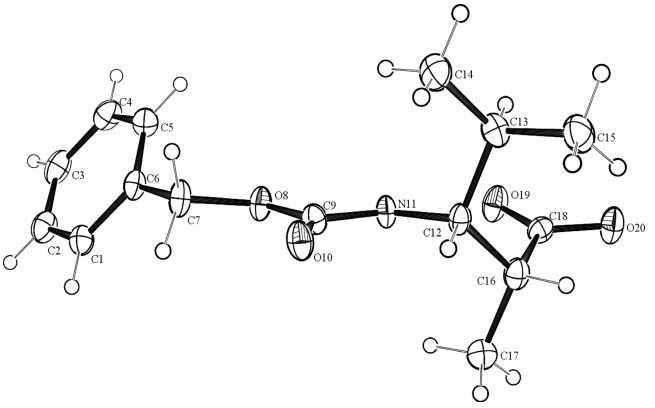


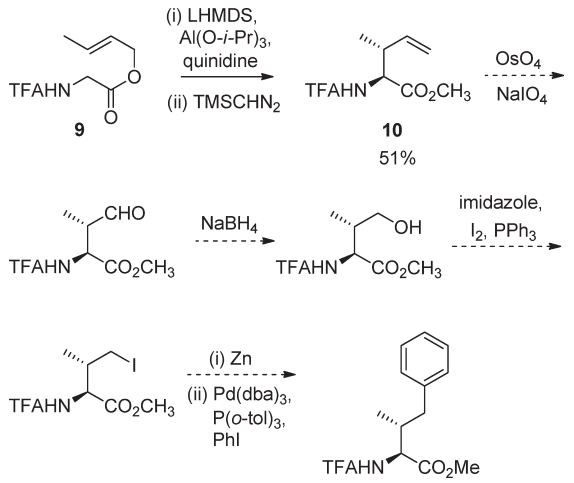
Figure 2. X-ray structure of *N*-*Z*-dolamethylleuine (**8**). Atoms are displayed as 30% probability thermal ellipsoids.

cyclodepsipeptide containing two new amino acids, dolamethylleuine (Dml, **2**), a β-amino acid, and dolaphenvaline (Dpv, **3**). As reported previously,[4a] the structure of **1** without assignment of the configuration of the novel amino acids was achieved by high-field NMR and tandem MS/MS mass spectroscopic interpretations. X-ray crystallographic analysis of **1** has now confirmed its cyclodepsipeptide structure and permitted the configurational assignments of the novel amino acids as 2*R*,3*R* for **2** and 2*S*,3*R* for **3** (Figure 1).

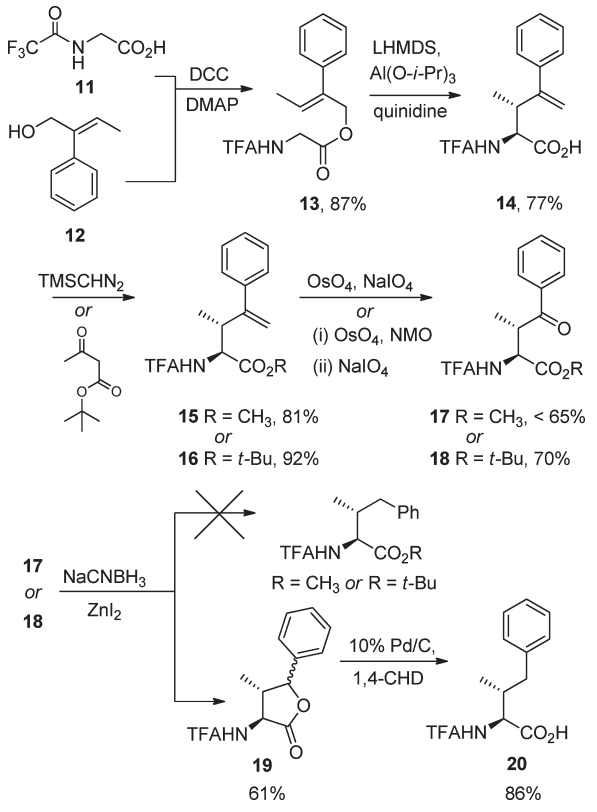
Synthesis of the β-amino acid dolamethylleuine **2** as its *Z*-protected synthon was carried out in four steps as outlined in Scheme 1 (13% overall yield). With *Z*-*R*-valine (**4**) as substrate, the Arndt−Eistert reaction[6] followed by a Wolff rearrangement[7] of the resulting diazoketone **5** afforded the protected β-amino acid **6**. Methylation at the α-position was accomplished stereoselectively with LDA and iodomethane to afford **7**.[6,8] Deprotection of the *tert*-butyl ester by use of trifluoroacetic acid (TFA) and triethylsilane (TES)[9] in DCM provided *Z*-Dml (**8**). This crystalline acid was subjected to X-ray crystallography, which confirmed the desired configuration (Figure 2).

Dolaphenvaline (**3**) was later reported by Scheuer[10] as a constituent of kulokekahilide-1, a cyclodepsipeptide from the cephalaspidean mollusk *Philinopsis speciosa*. As part of the

Scheme 2



Scheme 3



structure elucidation of kulokekahilide-1, all four diastereoisomers of dolaphenvaline were prepared via a non-stereospecific approach. Since we required a stereocontrolled synthesis, an attractive approach to inducing the required chirality appeared to be the Claisen rearrangement of allylic esters of protected amino acids in the presence of chiral ligands.[11,12] The reported rearrangement of allyl ester **9** followed by methylation led to the γ,δ-unsaturated amino acid methyl ester **10** in high yield, with excellent stereoselectivity and reproducibility.[12] We repeated

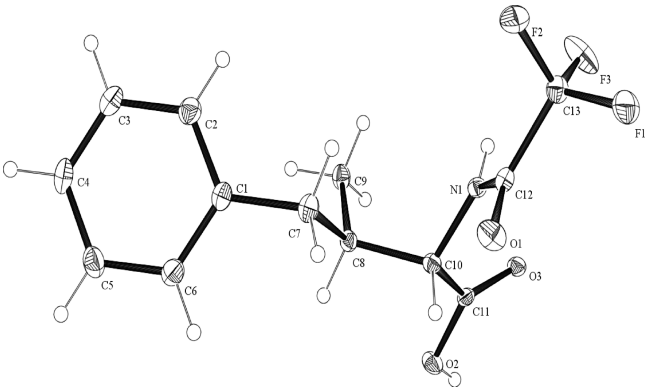


Figure 3. X-ray structure of *N*-trifluoroacetyldolaphenvaline (**20**). Atoms are displayed as 30% probability thermal ellipsoids.

that sequence, and **10** seemed to be a viable starting point for the synthesis of *N*-trifluoroacetyldolaphenvaline, as outlined in Scheme 2.

However, while this approach appeared feasible on a pilot scale, it had deficiencies in terms of lack of convergence and potential scale-up problems. Therefore, we explored the more convergent approach outlined in Scheme 3, beginning with the DCC/DMAP-mediated condensation of *N*-trifluoroacetylglycine (**11**) with **12**[13] to provide allylic ester **13** in 87% yield. Claisen rearrangement of **13** with LHMDS in the presence of $Al(O\text{-}i\text{-}Pr)_3$ and quinidine afforded the γ,δ-unsaturated amino acid **14**. After methylation with $TMSCHN_2$, methyl ester **15** was subjected to oxidative cleavage of the double bond by reaction with OsO_4 followed by $NaIO_4$ to afford ketone **17** in reasonable yield. However, this material was contaminated by a difficultly separable byproduct tentatively identified as the intermediate diol on the basis of its NMR spectrum. Despite extensive experimentation, it was not feasible either to drive this reaction to completion or to obtain a pure sample of **17**. Attempts to selectively remove the ketone group of **17** via the $NaCNBH_3/ZnI_2$[14] procedure did not lead to the desired reduced product but rather a mixture of lactones (**19**) that presumably arise from cyclization of an intermediate alcohol.

With the intent of avoiding lactone formation, the use of the *tert*-butyl ester for carboxyl protection was explored. Reaction of **14** with *tert*-butyl acetoacetate and a catalytic amount of H_2SO_4 in a sealed vessel[15] gave *tert*-butyl ester **16**. An attempt to carry out the oxidative cleavage reaction via the procedure used for **15** failed with **16** as the substrate. However, a two-step procedure using *N*-methylmorpholine *N*-oxide (NMO) as co-oxidant[16] was successful and yielded **18** cleanly in 70% yield. An unexpected bonus of the *tert*-butyl ester approach is that both **16** and **18** proved to be nicely crystalline solids, whereas methyl esters **15** and **17** were obtained as oils. Interestingly, the NMO-mediated oxidative cleavage approach successful with **16** failed with the methyl ester analogue **15**. With the *tert*-butyl ester ketone **18** in hand, the $NaCNBH_3/ZnI_2$[14] deoxygenation was attempted. Again, as with methyl ester **17**, the lactone mixture **19** was the main product. However, since the epimeric lactones (**19**) were an intermediate reduction product, the reductive process was completed via transfer hydrogenolysis of **19** with 1,4-cyclohexadiene and Pd/C to afford protected dolaphenvaline **20** (22.6% overall yield via **16**). This crystalline acid was subjected to X-ray

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crystallography, which confirmed the desired configuration (Figure 3).

The unequivocally established configuration as well as the preceding stereoselective syntheses of protected Dml and Dpv have allowed our total synthetic approaches to scale-up preparation of dolastatin 16 (**1**) to proceed nicely, and this will be reported when complete.

EXPERIMENTAL SECTION

General Experimental Procedures. All starting reagents were used as purchased unless otherwise stated. Reactions were monitored by TLC on Analtech silica gel GHLF uniplates visualized under long- and short-wave UV irradiation and stained with H_2SO_4/heat, phosphomolybdic acid/heat, or $KMnO_4$/heat. Solvent extracts were dried over anhydrous sodium sulfate. Where appropriate, the crude products were separated by flash chromatography on silica gel (230−400 mesh ASTM) from E. Merck.

Melting points are uncorrected and were determined employing an Electrothermal Mel-Temp apparatus. The ^{1}H and ^{13}C NMR spectra were recorded employing Varian Gemini 300, Varian Unity 400, or Varian Unity 500 instruments in $CDCl_3$ unless otherwise indicated. HRMS data were recorded with a JEOL LCmate or JEOL GCmate mass spectrometer. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN. X-ray structure analyses were performed on a Bruker AXS Smart 600 diffractometer. The X-ray data have been submitted as Supporting Information.[17] Descriptions of the X-ray techniques utilized in our laboratory have been previously described.[18]

1-Diazo-2-oxo-(3R)-3-benzyloxycarbonylamino-4-methylpentane (***5***). A solution of *Z*-*R*-valine (**4**, 1.01 g, 3.98 mmol) and TEA (0.57 mL, 415 mg, 4.11 mmol) in THF (20 mL) under N_2 was cooled to −15 °C. Ethyl chloroformate (0.39 mL, 446 mg, 4.11 mmol) in THF (4 mL) was added, and the solution stirred at −15 °C for 30 min. The solution was filtered and the precipitate washed with THF (10 mL). The combined filtrate and washings were diluted with acetonitrile (20 mL) and cooled to 0 °C under N_2. Trimethylsilyldiazomethane (4 mL of a 2 M solution in hexane, 8 mmol) was added, and the solution stirred at ambient temperature for 18 h. The reaction mixture was diluted with ether (80 mL), washed successively with 10% citric acid (50 mL), saturated $NaHCO_3$ (50 mL), and 5 M NaCl (20 mL), dried, evaporated, and co-evaporated with toluene (15 mL). The residue was separated by chromatography on silica gel (30 g, 7:3 hexane−EtOAc) to afford 0.37 g (34%) of **5** as a pale yellow solid: mp 68−69 °C; R_f 0.21 (4:1 hexane−EtOAc); $[\alpha]^{23}_D$ +25 (*c* 1.10, $CHCl_3$); ^{1}H NMR δ 7.33 (5H, m), 5.39 (2H, br s), 5.11 (2H, s), 4.13 (1H, m), 2.09 (1H, heptet), 0.99 (3H, d), 0.89 (3H, d); *anal.* C 61.31, H 6.56, N 14.93%, calcd for $C_{14}H_{17}N_3O_3$, C 61.08, H 6.22, N 15.26%.

tert-Butyl (3S)-3-Z-amino-4-methylpentanoate (***6***). Diazo derivative **5** (0.602 g, 2.19 mmol) was dissolved in *t*-BuOH (9 mL) under N_2 at 70 °C. Silver benzoate (80.2 mg, 0.35 mmol) in TEA (0.94 mL, 685 mg, 6.70 mmol) was added dropwise, and the mixture stirred at 70 °C in the dark for 4 h. The mixture was allowed to cool and was filtered through Celite, and the solvent was evaporated. The residue was partitioned between EtOAc (100 mL) and saturated $NaHCO_3$ (20 mL). The organic phase was separated, washed with saturated $NaHCO_3$ (20 mL), H_2O (20 mL), and 5 M NaCl (20 mL), and dried, and the solvent was evaporated. The residue was chromatographed (silica gel, 23 g; 9:1 hexane−acetone) to provide 0.443 g (63%) of **6** as a colorless oil: R_f 0.46 (5:1 hexane−acetone); $[\alpha]^{23}_D$ +22 (*c* 1.20, $CHCl_3$); ^{1}H NMR δ 7.34 (5H, m), 5.12 (1H, d), 5.09 (2H, s), 3.81 (1H, qt), 2.45 (1H, dd, *J* = 5, 15 Hz), 2.37 (1H, dd, *J* = 7, 15 Hz), 1.81 (1H, m), 1.42 (9H, s), 0.93 (3H, d, *J* = 3 Hz), 0.91 (3H, d, *J* = 3 Hz); ^{13}C NMR δ 170.5, 155.5, 136.2, 127.9, 127.5, 80.4, 66.0, 53.3, 37.9, 31.5, 27.5, 18.7, 18.0; HRMS *m*/*z* 322.2041 $[M + H]^+$ (calcd for $C_{18}H_{28}NO_4$, 322.2018); *anal.* C 67.05, H 8.44, N 4.40%, calcd for $C_{18}H_{27}NO_4$, C 67.26, H 8.47, N 4.36%.

tert-Butyl (2R,3R)-3-N-Z-amino-2,4-dimethylpentanoate (***7***). To a stirred mixture of dipyridyl indicator, LiCl (0.77 g, 18 mmol), and diisopropylamine (2.0 mL, 14 mmol) in THF (30 mL) at −78 °C under N_2 was added BuLi (1.6 M solution in hexane, 8.75 mL, 14 mmol) dropwise until the mixture turned a wine-red color. The mixture was stirred at −78 °C for 15 min, and **6** (1.90 g, 5.9 mmol) in THF (15 mL) was added. The reaction mixture was stirred for 1 h, followed by the addition of iodomethane (1.9 mL, 31 mmol). Stirring was continued for 21 h at ambient temperature. The reaction was terminated by the addition of saturated NH_4Cl (30 mL), and the mixture was extracted with EtOAc (150 mL). The extract was washed with 10% $Na_2S_2O_3$ (30 mL), and the washing was back-extracted with EtOAc (100 mL). The organic solutions were combined, dried, and evaporated. The residue was further separated by chromatography on silica gel (60 g, 9:1 hexane−acetone) to yield 1.60 g (80%) of **7** as a colorless oil: R_f 0.44 (9:1 hexane−acetone); $[\alpha]^{23}_D$ +22 (*c* 0.70, $CHCl_3$); ^{1}H NMR δ 7.33 (5H, m), 5.62 (1H, d, *J* = 7.2 Hz), 5.10 (2H, s), 3.44 (1H, m), 2.66 (1H, m), 1.71 (1H, m), 1.42 (9H, s), 1.18 (3H, d, *J* = 7.2 Hz), 0.96 (3H, d, *J* = 6.6 Hz), 0.92 (3H, d, *J* = 6.6 Hz); ^{13}C NMR δ 174.6, 156.4, 136.4, 127.9, 80.3, 65.9, 59.0, 40.7, 31.4, 27.5, 19.3, 18.7, 15.3; HRMS *m*/*z* 336.2155 $[M + H]^+$ (calcd for $C_{19}H_{30}NO_4$, 336.2175); *anal.* C 68.16, H 8.91, N 4.47%, calcd for $C_{19}H_{29}NO_4$, C 68.03, H 8.71, N 4.18%.

(2R,3R)-3-Z-Amino-2,4-dimethylpentanoic Acid (***8***). To a solution of **7** (1.6 g, 4.8 mmol) in DCM (9.9 mL) under N_2 was added a mixture of trifluoroacetic acid (4.6 mL, 62 mmol) and triethylsilane (1.9 mL, 12 mmol). Stirring was continued for 4 h at ambient temperature. Solvents were removed and the residue co-evaporated with toluene (2 × 30 mL). The residue was dissolved in EtOAc (100 mL) and extracted with 6% $NaHCO_3$ (4 × 40 mL). The aqueous extracts were combined, acidified (pH 2) with 6 N HCl, and extracted with EtOAc (3 × 40 mL). The organic extracts were combined, washed with 5 M NaCl (20 mL), dried, and evaporated to provide a colorless solid that crystallized from 2-propanol−water to provide *Z*-Dml (**8**, 1.0 g, 77%) as colorless crystals: mp 135 °C; R_f 0.59 (50:50:1 hexane−acetone−HOAc); $[\alpha]^{25}_D$ +35 (*c* 0.86, $CHCl_3$); ^{1}H NMR δ 7.34 (5H, m), 5.61 (1H, d, *J* = 10.5 Hz), 5.11 (2H, s), 3.46 (1H, m), 2.83 (1H, m), 1.77 (1H, m), 1.25 (3H, d, *J* = 7.2 Hz), 0.96 (3H, d, *J* = 6.6 Hz), 0.93 (3H, d, *J* = 6.6 Hz); ^{13}C NMR δ 179.4, 156.6, 136.2, 127.9, 127.5, 127.4, 66.1, 58.8, 39.8, 19.4, 18.8, 15.3; HRMS *m*/*z* 280.1558 $[M + H]^+$ (calcd for $C_{15}H_{22}NO_4$, 280.1549); *anal.* C 64.69, H 7.73, N 4.96%, calcd for $C_{15}H_{21}NO_4$, C 64.50, H 7.58, N 5.01%.

(E)-2-Phenylbut-2-enyl 2-(2,2,2-Trifluoroacetamido)acetate (***13***). To a suspension of *N*-trifluoroacetylglycine (**11**, 3.68 g, 21.50 mmol) and (*E*)-2-phenyl-2-buten-1-ol (**12**, 2.68 g, 19.32 mmol) in DCM (60 mL) at −40 °C under N_2 was added via cannula a solution of dicyclohexylcarbodiimide (4.43 g, 21.50 mmol) and 4-dimethylaminopyridine (0.269 g, 2.15 mmol) in DCM (60 mL). The solution was stirred at ambient temperature for 18 h and filtered, and the precipitate was washed with DCM (2 × 40 mL). The combined filtrate and washing was washed with 10% citric acid (2 × 25 mL), H_2O (10 mL), 6% $NaHCO_3$ (2 × 25 mL), and 5 M NaCl (20 mL) and dried, and the solvent was evaporated. The residue was chromatographed (silica gel, 150 g; 4:1 hexane−EtOAc) to afford 5.06 g (87%) of **13** as a pale yellow oil that solidified on standing: mp 49−50 °C; R_f 0.66 (4:1 hexane−EtOAc); ^{1}H NMR δ 7.37 (2H, t, *J* = 7.5 Hz), 7.30 (1H, t, *J* = 7.2 Hz), 7.18 (2H, d, *J* = 7.6 Hz), 6.75 (1H, br s), 5.95 (1H, q, *J* = 6.9 Hz), 4.91 (2H, s), 4.06 (2H, d, *J* = 4.9 Hz), 1.66 (3H, d, *J* = 6.9 Hz); ^{13}C NMR δ 167.9, 156.7 (m), 137.3, 135.4, 135.4, 128.5, 128.4, 127.4, 116.9, 70.8, 41.4, 14.6; MS APCI$^+$ *m*/*z* 302.1026 $[M + H]^+$ (calcd for $C_{14}H_{15}F_3NO_3$, 302.1004); *anal.* C 55.73, H 4.93, N 4.67%, calcd for $C_{14}H_{14}F_3NO_3$, C 55.82, H 4.68, N 4.65%.

3-Methyl-4-phenyl-2-(2,2,2-trifluoroacetamido)-(2S,3R)-pent-4-enoic Acid (***14***). To a solution of hexamethyldisilazane (6.19 g, 8.0 mL,

38.5 mmol) in THF (20 mL at −20 °C under N_2) was added BuLi (1.6 M solution in hexane, 20 mL, 32 mmol). The solution was stirred at −20 °C for 20 min and added via cannula to a suspension of **13** (2.00 g, 6.64 mmol), quinidine (4.30 g, 13.27 mmol), and aluminum isopropoxide (2.04 g, 10.0 mmol) in THF (70 mL) at −78 °C. The solution was allowed to come to ambient temperature, and stirring was continued for 18 h. The mixture was next diluted with EtOAc (250 mL) and washed with 1 N HCl (3 × 75 mL). The combined washings were extracted with EtOAc (50 mL). The organic solutions were combined and extracted with 6% $NaHCO_3$ (7 × 50 mL). The aqueous extracts were combined, cooled in an ice bath, acidified (pH 1) with 6 N HCl, and extracted with EtOAc (4 × 50 mL). The organic extracts were combined, washed with 5 M NaCl (20 mL), dried, and evaporated to give 1.54 g (77%) of **14** as a pale yellow semisolid: R_f 0.76 (95:5:1 DCM−CH_3OH−HOAc); ^1H NMR δ 9.10 (1H, br), 7.33 (5H, m), 6.42 (1H, d, J = 8.2 Hz), 5.37 (1H, m), 5.15 (1H, s), 4.68 (1H, dd, J = 8.6 and 3.4 Hz), 3.57 (1H, m), 1.31 (3H, d, J = 7.2 Hz); ^{13}C NMR δ 174.8, 156.6 (m), 148.6, 140.8, 128.6, 128.2, 126.7, 115.1, 55.0, 39.5, 14.0; MS APCI$^+$ m/z 302.1010 $[M + H]^+$, (calcd for $C_{14}H_{15}F_3NO_3$, 302.1004).

*Methyl 3-Methyl-4-phenyl-2-(2,2,2-trifluoroacetamido)-(2S,3R)-pent-4-enoate (**15**).* Carboxylic acid **14** (544.4 mg, 1.81 mmol) was placed in 1:1 CH_3OH−toluene (6 mL) under N_2, and trimethylsilyldiazomethane (2 M solution in hexane, 4.0 mL, 8.0 mmol) was added. The solution was stirred at ambient temperature for 16 h. The solvent was evaporated, and the residue was chromatographed (silica gel, 17 g; 9:1 hexane−EtOAc) to yield 0.46 g (81%) of **15** as a colorless oil: R_f 0.54 (4:1 hexane−EtOAc); ^1H NMR δ 7.33 (5H, m), 6.48 (1H, br d), 5.34 (1H, s), 5.11 (1H, d, J = 0.8 Hz), 4.64 (1H, dd, J = 8.7 and 4.0 Hz), 3.76 (3H, s), 3.48 (1H, m), 1.26 (3H, d, J = 7.1 Hz).

*tert-Butyl 3-Methyl-4-phenyl-2-(2,2,2-trifluoroacetamido)-(2S,3R)-pent-4-enoate (**16**).* To carboxylic acid **14** (1.54 g, 5.12 mmol) in a 50 mL round-bottom flask were added *tert*-butyl acetoacetate (5.8 mL, 5.53 g, 34.97 mmol) and H_2SO_4 (43.1 mg, 0.44 mmol). The flask was tightly stoppered, and the solution was stirred at ambient temperature under N_2 for 20 h. The mixture was cooled (ice) before dilution with EtOAc (100 mL). The organic solution was washed with 6% $NaHCO_3$ (4 × 20 mL) and 5 M NaCl (10 mL) and dried, and the solvent was evaporated. The $NaHCO_3$ washings were combined, acidified (pH 1) with 6 N HCl, and extracted with EtOAc (3 × 15 mL). The extracts were combined, washed with 5 M NaCl (10 mL), and dried, and the solvent was evaporated to afford 0.65 g (42%) of **14**. The neutral residue was chromatographed (silica gel, 30 g, 95:5 hexane−EtOAc) and led to 1.06 g (58%, 100% based on recovered starting material) of **16** as a colorless solid: mp 108 °C; R_f 0.50 (95:5 hexane−EtOAc); $[\alpha]^{23}_D$ 25.2 (c 1.04, CH_3OH); ^1H NMR δ 7.34 (5H, m), 6.48 (1H, br d, J = 6.8 Hz), 5.33 (1H, s), 5.10 (1H, s), 4.52 (1H, dd, J = 8.4 and 3.5 Hz), 3.47 (1H, m), 1.49 (9H, s), 1.27 (3H, d, J = 7.0 Hz); ^{13}C NMR δ 168.8, 156.5 (m), 149.0, 141.3, 128.5, 127.9, 126.8, 114.7, 83.3, 55.6, 39.9, 28.0, 14.3; MS APCI$^+$ m/z 358.1681 (0.6) $[M + H]^+$ (calcd for $C_{18}H_{23}F_3NO_3$, 358.1630), 302.0990 (100) $[M + H - C_4H_8]^+$ (calcd for $C_{14}H_{15}F_3NO_3$, 302.1004); *anal.* C 60.14, H 6.41, N 3.96%, calcd for $C_{18}H_{22}F_3NO_3$, C 60.50, H 6.21, N 3.92%.

*tert-Butyl 3-Methyl-4-oxo-4-phenyl-2-(2,2,2-trifluoroacetamido)-(2S,3R)-butanoate (**18**).* To olefin **16** (903.4 mg, 2.53 mmol) in THF (25 mL) under N_2 were added NMO (60 wt % solution in H_2O, 0.90 mL, 5.06 mmol) and OsO_4 (4 wt % solution in H_2O, 1.50 mL, 0.25 mmol). The solution was stirred at ambient temperature for 16 h, and $NaIO_4$ (2.16 g, 10.12 mmol) was then added, followed by H_2O (2.7 mL). Stirring was continued for 4 h, and the reaction mixture was then diluted with EtOAc (200 mL) and washed with 10% $Na_2S_2O_3$ (4 × 50 mL). The combined washings were extracted with EtOAc (50 mL). The organic solutions were combined and washed with 5 M NaCl (20 mL) and dried, and the solvent was evaporated. The residue was separated by chromatography (silica gel, 30 g; 9:1 hexane−EtOAc) and led to 0.632 g (70%) of **18** as a colorless solid: mp 127−128 °C; R_f 0.19 (95:5 hexane−EtOAc); $[\alpha]^{23}_D$ −39.0 (c 1.05, CH_3OH); ^1H NMR δ 7.92 (2H, dd, J = 7.5 and 1.3 Hz), 7.60 (1H, tt, J = 7.5 and 1.3 Hz), 7.49 (2H, t, J = 7.6 Hz), 7.03 (1H, br d, J = 6.7 Hz), 4.72 (1H, dd, J = 7.4 and 4.8 Hz), 4.13 (1H, m), 1.49 (9H, s), 1.36 (3H, d, J = 7.2 Hz); ^{13}C NMR δ 200.0, 168.3, 156.9 (q), 135.5, 133.6, 128.8, 128.4, 83.8, 55.0, 42.9, 27.8, 14.2; MS APCI$^+$ m/z 360.1417 $[M + H]^+$ (calcd for $C_{17}H_{21}F_3NO_4$, 360.1423); *anal.* C 56.39, H 5.60, N 3.96%, calcd for $C_{17}H_{20}F_3NO_4$, C 56.82, H 5.61, N 3.90%.

*3-N-(2′,2′,2′-Trifluoroacetamido)-4-methyl-2-oxo-5-phenyl-(3S,4S)-tetrahydrofuran (**19**).* To ketone **18** (331.2 mg, 0.92 mmol) in 1,2-dichloroethane (5.0 mL) under N_2 were added ZnI_2 (440.2 mg, 1.38 mmol) and $NaCNBH_3$ (434.7 mg, 6.90 mmol). The mixture was stirred at 75 °C for 16 h, quenched with 9:1 saturated NH_4Cl−6 N HCl (20 mL), and extracted with EtOAc (3 × 15 mL). The extracts were combined, washed successively with 6% $NaHCO_3$ (2 × 15 mL) and 5 M NaCl (10 mL), and dried. After evaporation of solvent, the residue was chromatographed (silica gel, 10 g; 4:1 hexane−EtOAc) to afford 0.123 g (47%) of **19** as a white, waxy solid: R_f 0.32 (4:1 hexane−EtOAc); ^1H NMR δ 7.37 (5H, m), 7.14 (1H, br d), 5.61 (0.33H, d, J = 8.4 Hz), 4.99 (0.67H, d, J = 10.2 Hz), 4.68 (1H, m), 2.99 (0.33 H, m), 2.59 (0.67H, m), 1.22 (0.67H, d, J = 6.6 Hz), 0.87 (0.33H, d, J = 6.9 Hz); HRMS (APCI$^+$) m/z 288.0851 $[M + H]^+$ (calcd for $C_{13}H_{13}F_3NO_3$, 288.0848).

*(2S,3R)-2-(2,2,2-Trifluoroacetamido)-3-methyl-4-phenyl-2-butanoic Acid (**20**).* To lactone **19** (74.7 mg, 0.26 mmol) in CH_3OH (2.0 mL) under N_2 (cooled to 0 °C) was added 10% Pd/C (75 mg), followed by 1,4-cyclohexadiene (0.21 g, 2.60 mmol), and the mixture was stirred at ambient temperature under N_2 for 4 h. The reaction mixture was diluted with CH_3OH (10 mL), and the solution was filtered through Celite. The filter cake was washed with CH_3OH (10 mL). The solvent was evaporated from the combined filtrate and washings, and the residue was dissolved in EtOAc (20 mL) and extracted with 6% $NaHCO_3$ (3 × 15 mL). The organic phase was washed with 5 M NaCl (10 mL) and dried, and removal of solvent gave 48.5 mg of recovered lactone **19**. The $NaHCO_3$ extracts were combined, acidified (pH 1) with 6 N HCl, and extracted with EtOAc (3 × 15 mL). The extracts were combined, washed with 5 M NaCl (10 mL), and dried, and the solvent was evaporated to afford 22.7 mg (86% based on recovered starting material) of **20** as a white solid: mp 122 °C; R_f 0.38 (97.5:2.5:0.5 DCM−CH_3OH−HOAc); $[\alpha]^{25}_D$ +24.2 (c 1.49, CH_3OH); ^1H NMR δ 9.78 (1H, br), 7.31 (2H, t, J = 7.3 Hz), 7.23 (1H, t, J = 7.3 Hz), 7.17 (2H, d, J = 7.1 Hz), 6.64 (1H, d, J = 8.4 Hz), 4.75 (1H, dd, J = 8.8 and 3.2 Hz), 2.76 (1H, dd, J = 12.9 and 6.0 Hz), 2.55 (1H, m), 2.49 (1H, dd, J = 13.1 and 8.0 Hz), 0.99 (3H, d, J = 6.6 Hz); ^{13}C NMR δ 175.23, 157.17 (m), 138.58, 128.96, 128.64, 126.71, 115.60 (m), 55.73, 39.64, 37.85, 14.84; MS APCI$^-$ m/z 288.0835 $[M - H]^-$ (calcd for $C_{13}H_{13}F_3NO_3$, 288.0845); *anal.* C 53.15, H 4.69, N 4.70%, calcd for $C_{13}H_{14}F_3NO_3 \cdot 0.2H_2O$, C 53.32, H 4.96, N 4.78%.

■ ASSOCIATED CONTENT

Supporting Information. X-ray crystal structure data for dolastatin 16 (**1**), *Z*-Dml (**8**), and trifluoroacetyl-Dpv (**20**). This material is available free of charge via the Internet at http://pubs.acs.org.

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DEDICATION

[†]In memory of Academician Georgy B. Elyakov (1929−2005), a pioneering expert in the chemistry of marine organism constituents, who is deeply missed.

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(17) CCDC 801992 (**1**), 801990 (**8**), and 801991 (**20**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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