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Antineoplastic Agents. 599. Total Synthesis of Dolastatin 16

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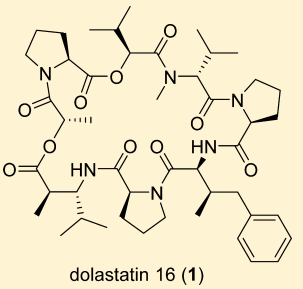
Supporting Information

ABSTRACT: The first 23-step total synthesis of the cyclodepsipeptide dolastatin 16 (**1**) has been achieved. Synthesis of the dolaphenvaline and dolamethylleuine amino acid units using simplified methods improved the overall efficiency. The formation of the 25-membered macrocycle employing lactonization with 2-methyl-6-nitrobenzoic anhydride completed a key step in the synthesis. Regrettably, the synthetic dolastatin 16 (**1**), while otherwise identical (by X-ray crystal structure and spectral analyses) with the natural product, did not reproduce the powerful (nanomolar) cancer cell growth inhibition displayed by the natural isolate. Presumably this result can be attributed to conformation(s) of the synthetic dolastatin 16 (**1**) or to a chemically undetected component isolated with the natural product.



dolastatin 16 (**1**)

The isolation of dolastatin 16 (**1**) from *Dolabella auricularia* and its impressive activity as an inhibitor (GI_{50} 10^{-3}–10^{-4} μg/mL) of cancer cell growth were reported in 1997.[1] The exceptional activity shown in cancer cell line biological assays made **1** an obvious candidate for further preclinical development, but this initiative was delayed by the need for unequivocal configurational and conformational assignments leading to a practical total synthesis. In 2011, we reported the X-ray crystal structure of dolastatin 16 (**1**) as well as the syntheses of synthons suitable for incorporation of the novel amino acid units dolamethylleuine (Dml) (**2**) and dolaphenvaline (Dpv) (**3**).[2] We now are pleased to report a successful and efficient total synthesis of dolastatin 16 (**1**).

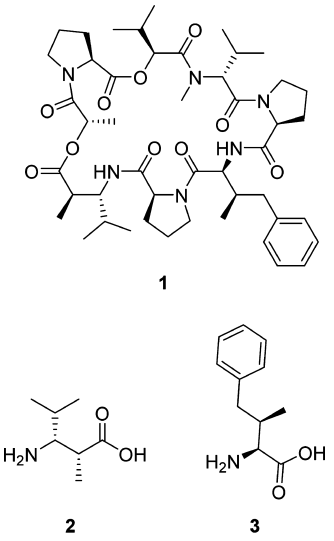


Figure 1. Structure of dolastatin 16 (**1**), dolamethylleuine (**2**), and dolaphenvaline (**3**).

RESULTS AND DISCUSSION

Inspection of the dolastatin 16 macrocycle revealed it to be composed of six known α-amino or α-hydroxy acids, which include three proline residues, one *N*-methylated valine, lactic acid, and 2-hydroxyisovaleric acid, and two novel amino acids, the β-amino acid residue dolamethylleuine (**2**) and the α-amino acid residue dolaphenvaline (**3**). A retrosynthetic analysis of our approach to dolastatin 16 is presented in Figure 2. In this approach we sought to generate **1** via macrolactonization of an acyclic precursor (**4**), which was to be prepared by a fragment condensation approach as illustrated in Figure 2.

Intermediate **7** was synthesized in five steps (Scheme 1). First, *N*-Cbz-*N*(Me)-D-valine was coupled to L-proline *tert*-butyl ester in the presence of the peptide-coupling reagent bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP) to obtain compound **11** in 89% yield. Deprotection of compound **11** using palladium-on-carbon in the presence of hydrogen afforded compound **12** in a 92% yield. Compound **13** was prepared in 78% yield by treating *S*-2-hydroxy-3-methylbutanoic acid (Hiv) with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) in the presence of imidazole. Compound **13** was activated with oxalyl chloride and then treated with compound **12** in the presence of *N*,*O*-bis(trimethylsily)acetamide (BSA) and triethylamine (TEA) to obtain compound **14** as a mixture of rotamers in 82% yield. Next, compound **14** was deprotected using tetrabutylammonium fluoride (TBAF) to obtain compound **7** as a mixture of rotamers in 73% yield.

Intermediate **5** was also synthesized in five steps using intermediate **7** (Scheme 2). First, compound **16** was prepared from methyl L-lactate according to the procedure of Qi and McIntosh.[3] Methyl L-lactate was treated with benzyl bromide in

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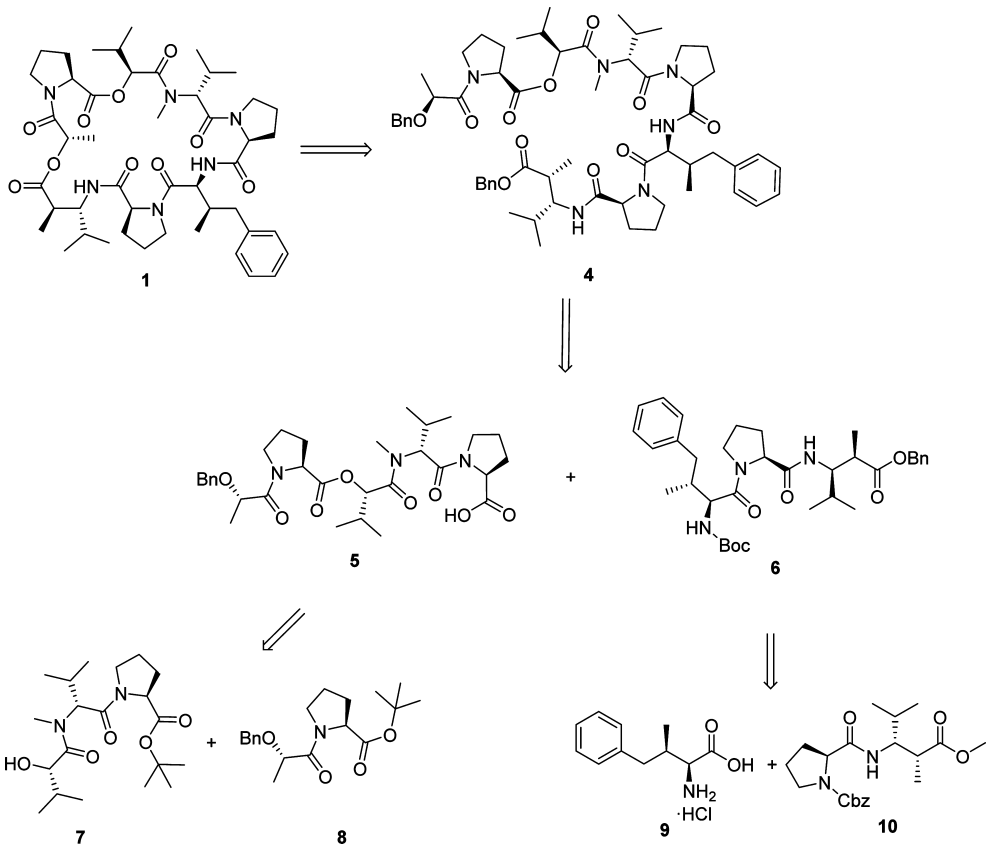
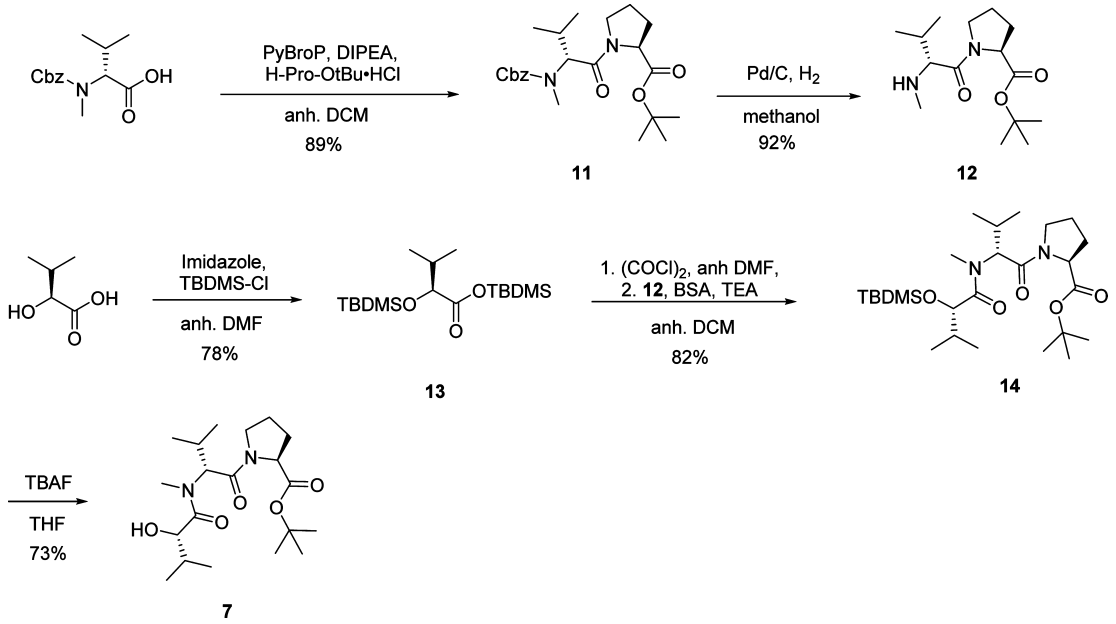


Figure 2. Retrosynthetic analysis for dolastatin 16 (**1**).

Scheme 1. Synthesis of Intermediate 7



the presence of silver(I) oxide in order to obtain compound **15** in 51% yield. Compound **15** was hydrolyzed in the presence of potassium hydroxide to obtain acid **16** in 88% yield. Then, compound **16** was treated with L-proline *tert*-butyl ester in the presence of the peptide coupling reagent PyBroP and diisopropylethylamine (DIPEA) to obtain compound **8** as a mixture of rotamers in 82% yield. Compound **8** was deprotected with trifluoroacetic acid and coupled to intermediate **7** using 2-methyl-6-nitrobenzoic anhydride (MNBA) in the presence of 4-dimethylaminopyridne (DMAP) and triethylamine to afford compound **17** as a complex mixture of rotamers in 81% yield. Finally, compound **17** was treated with trifluoroacetic acid (TFA) to obtain intermediate **5** again as a mixture of rotamers in quantitative yield.

The synthesis of the hydrochloric salt of dolaphenvaline was accomplished in six steps following a strategy developed by Li

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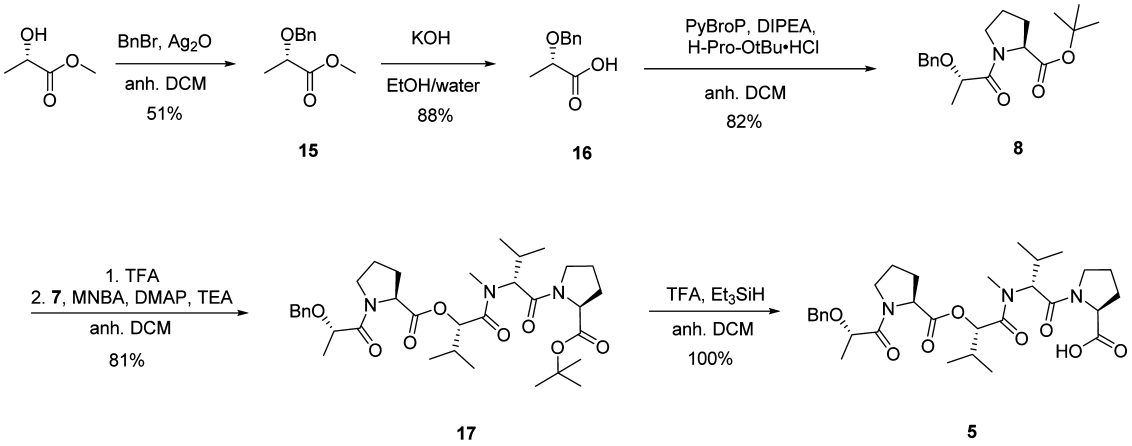
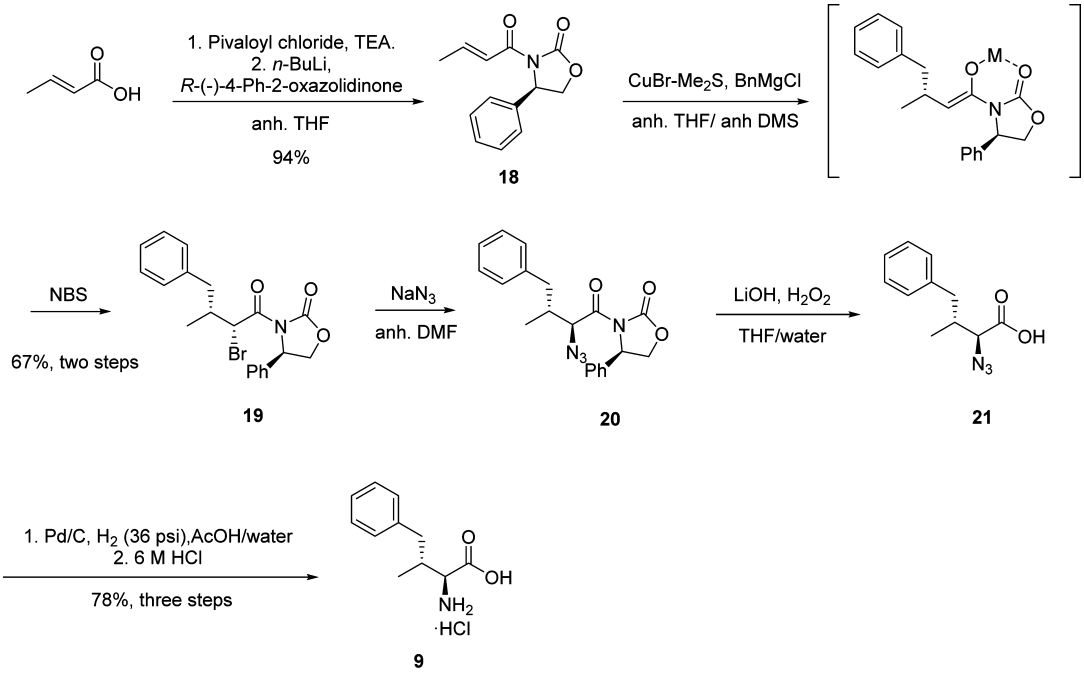
Scheme 2. Synthesis of Intermediate 5



Scheme 3. Synthesis of Dolaphenvaline·HCl (9)



et al. using an Evan's-type chiral auxiliary to control the stereochemistry (Scheme 3).[4,5] First, compound **18** was obtained in 94% yield by treating crotonic acid with pivaloyl chloride in the presence of triethylamine and then with a solution containing the lithium salt of *R*-4-phenyl-2-oxazolidinone. Compound **18** was treated with benzylmagnesium chloride in the presence of copper(I) bromide dimethyl sulfide complex followed by *N*-bromosuccinimide to obtain compound **19** in 67% yield over two steps. Compound **19** was treated with sodium azide in order to obtain compound **20**, which was treated as a crude with lithium hydroxide in the presence of hydrogen peroxide to obtain compound **21**. Lastly, crude compound **21** was treated with hydrogen in the presence of palladium-on-carbon and then with 6 N HCl to obtain dolaphenvaline hydrochloric salt (**9**) in 78% yields over three steps. The synthesis proposed here for dolaphenvaline is stereoselective as compared to the synthesis performed by Kimura et al.[6] and more efficient as compared to the synthesis reported earlier by us.[2]

Finally, dolastatin 16 (**1**) was synthesized in seven steps using intermediates **5** and **9** (Scheme 4). Compound **22** was obtained in 92% yield by treating *S*-homo-*β*-valine with thionyl chloride in anhydrous methanol. Compound **22** was alpha-methylated and N-protected by first treating it with LiHMDS and then with methyl iodide[7,8] followed by Cbz-Cl in the presence of potassium carbonate to obtain Cbz-protected dolamethylleuine methyl ester (**23**) in 58% yield. In this step the 2*S*,3*R*-Dml diastereomer was also obtained in about 20% yield. The strategy followed here for the synthesis of Dml is more efficient as compared to the synthesis proposed previously.[2] Compound **23** was treated with hydrogen in the presence of palladium-on-carbon to deprotect the amino group. The crude product was then treated with Cbz-L-proline in the presence of *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)-uronium hexafluorophosphate (HBTU) and triethylamine to obtain compound **10** in 73% yield over two steps. Compound **10** was subjected to the same series of reactions as for **23** using Boc-protected Dpv, which was prepared by treating **9** with Boc_2O in an aqueous solution of KOH, to obtain compound **24**

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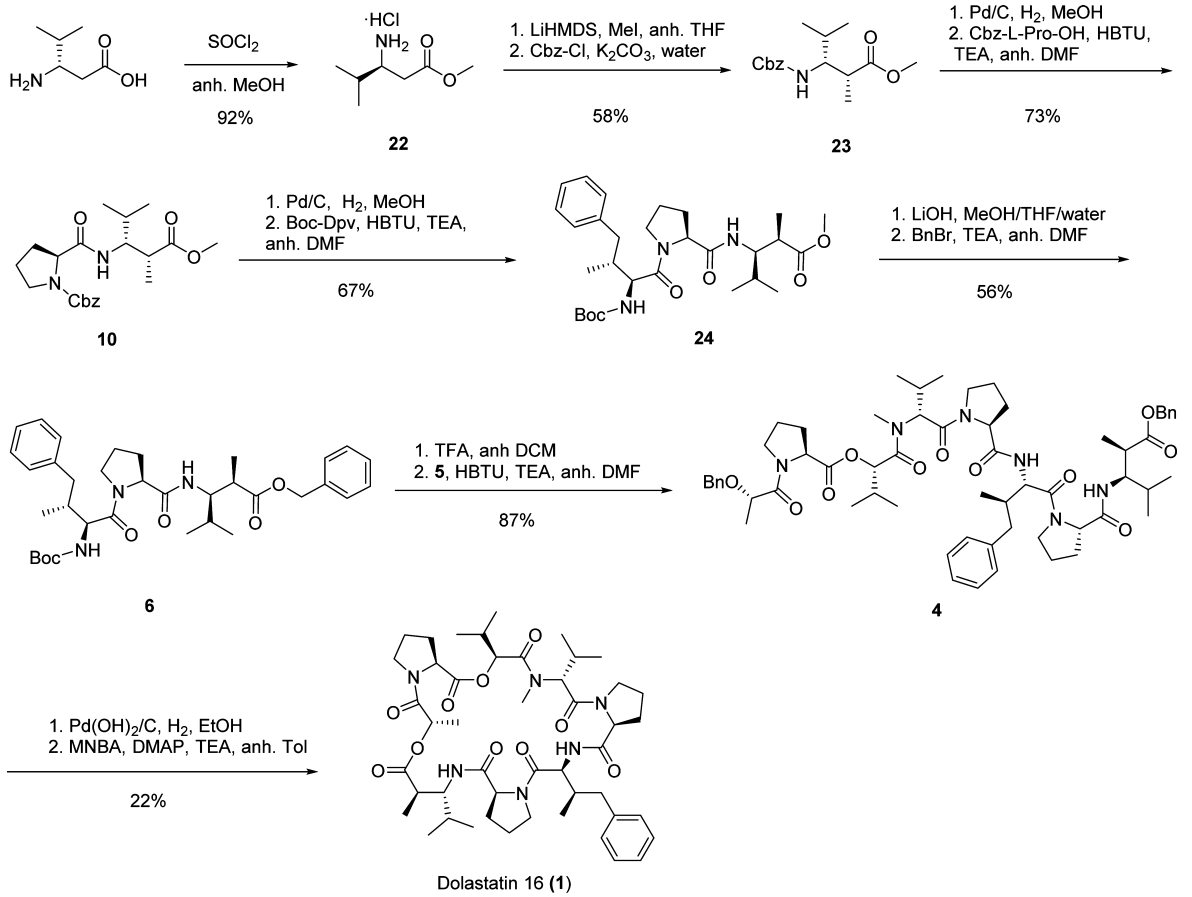
Scheme 4. Synthesis of Dolastatin 16



in 67% yield. Compound **24** was then hydrolyzed using LiOH followed by treatment with benzyl bromide in the presence of triethylamine to obtain compound **6** in 56% yield. Then, compound **6** was treated with TFA to deprotect the amino group. The crude product was next treated with compound **5** in the presence of HBTU and triethylamine to obtain compound **4** as a mixture of rotamers in 87% yield over two steps. For the last step, compound **4** was subjected to hydrogenolysis to remove the two benzyl groups and then treated with MNBA in the presence of DMAP and triethylamine under high dilution to afford dolastatin 16 (**1**) in 22% yield.

The synthetic dolastatin 16 was found to be identical to the natural product as compared by HPLC, NMR (400 MHz), optical rotation, HRMS, and X-ray crystallography data (Figure 3). The X-ray crystal structure observed for the synthetic dolastatin 16 showed the same stereochemistry as the natural dolastatin 16; however, there are small differences in bond angles due to the solvent used for crystallization.

Biological evaluation of the synthetic dolastatin 16 against a small panel of cancer cell lines showed a surprising lack of cancer cell growth inhibition (GI_{50} > 10 μg/mL) as compared to the natural counterpart, which consistently led to GI_{50} 0.0012−0.000 96 μg/mL cancer cell growth inhibition against a minipanel of human cancer cell lines.[1] The results of this analysis suggest a conformational change in the synthetic specimen or presence of a chemically undetected compound in the sample that was isolated from the natural source in 1997. Previously, it was observed that certain cyclic depsipeptides could carry traces of compounds too small to be detected by NMR or chromatographic techniques responsible for the biological activity.[9]

In 2011, dolastatin 16 was also isolated from the cyanobacterium *Symploca* cf. *hydnoides* by Luesch and colleagues.[10] The activity of dolastatin 16 isolated from this particular organism was greatly lower (IC_{50}'s of 69 and 51 μg/mL for the HT-29 and HeLa cell lines, respectively) as compared to the specimen isolated from *D. auricularia*.

Recently, it was shown that the activity of phakellistatin 2, a cyclic peptide also containing proline residues in its sequence, exhibited different cancer cell growth inhibition depending on if methanol or dimethyl sulfoxide was used for the bioassay.[11] These findings suggest that conformational changes, due to the solvent used, can have a big impact on the biological activity of certain macrocycles containing proline residues and possibly N-alkylated amino acids. In fact, NMR data (not shown) were also collected in deuterated solvents such as methanol and dimethyl sulfoxide, and the presence of two or more conformers was observed. Following the logic in the findings mentioned above, the synthetic dolastatin 16 was also evaluated in methanol; unfortunately, no activity was observed (Table 1). The results shown for the natural sample of dolastatin 16 require some background. Table 1 data (current, 2014) for the natural dolastatin 16 were on a very small sample from the remaining ~100 μg from the original isolation (3.1 mg, 10^{-7} % yield) from 1000 kg (wet weight) of the sea hare *D. auricularia* that we collected in Papua New Guinea in 1983 and reported following 14 years of research by 1997.[1] Before we used this sample, it was repurified by reversed-phase HPLC using the same conditions as for the purification of the synthetic dolastatin

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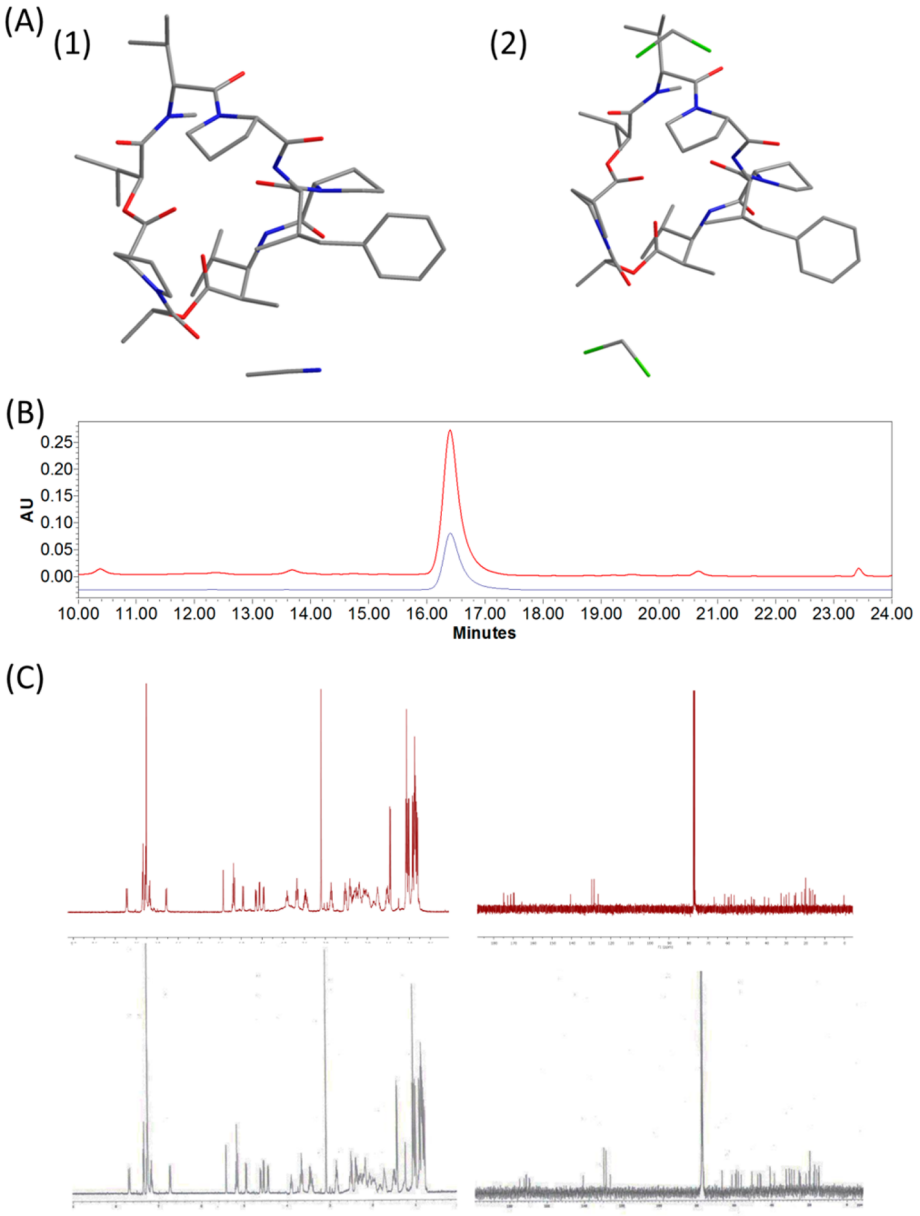

Figure 3. (A) Crystal structure of natural dolastatin 16 (1) and synthetic dolastatin 16 (2). Crystal structures were created using the Chem3D program from CIF files. (B) HPLC trace comparison of synthetic (blue) and natural (red) dolastatin 16. (C) NMR (400 MHz) comparison of synthetic (red) and natural (black) dolastatin 16.

Table 1. Human Cancer Cell Growth Evaluation of Natural and Synthetic Dolastatin 16, GI_{50} (μg/mL)

		cell line[a]					
compound	solvent	BXPC-3	MCF-7	SF-268	NCI-H460	KM20L2	DU-145
dolastatin 16 (natural)	DMSO	0.050	0.027	0.016	0.270	0.013	0.009
dolastatin 16 (synthetic)	DMSO	>10	>10	>10	>10	>10	>10
	MeOH	>10	>10	>10	>10	>10	>10

[a]Cancer cell lines in order: pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); lung (NCI-H460); colon (KM20L2); prostate (DU-145).

16. After purification, a reduced activity was observed in some cancer cell lines as growth inhibitor, except for DU-145 (prostate), as compared to the previous results obtained for the original dolastain 16.

Although the utility of the synthetic dolastatin 16 for cancer cell growth inhibition is disappointing, we will begin an evaluation against other medical indications and the potential of SAR modifications especially involving the proline unit.[12]

In recent years other proline-rich cyclodepsipeptides, analogues of dolastatin 16, have been isolated from different organisms. Kulokekahilide-1[6] was isolated from the cephalaspidean mollusk *Philinopsis speciosa* collected from Shark's cove, Pupukea, O'ahu, in 2002. Homodolastatin 16[13] and pitiprolamide[14] were later isolated by different research groups (in 2003 and 2011, respectively) from the marine cyanobacterium *Lyngbya majuscula* collected from different parts of the

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world. These analogues of dolastatin 16 showed low activity as cancer cell growth inhibitors, with GI_{50}'s ranging from 2 to 29 μg/mL against different cancer cell lines.

Taking into account the reduced activity observed for the repurified sample of the natural dolastatin 16 isolated from *D. auricularia*, a likely explanation for the superior activity as cancer cell growth inhibitor of the natural dolastatin 16 as compared to our synthetic dolastatin 16 and to the one isolated from the cyanobacterium *Symploca* cf. *hydnoides* by Luesch and colleagues resides in the presence of a highly active untraceable impurity in the natural sample.[14a]

EXPERIMENTAL SECTION

General Experimental Procedures. Reagents and anhydrous solvents were purchased from Sigma-Aldrich Chemical Co. and Alfa-Aesar Inc. and were used as received. The reactions were carried out under an atmosphere of nitrogen unless specified. Column chromatography was conducted using silica gel (E. Merck 60 Å, 230−400 mesh), applying a low-pressure stream of nitrogen. Analytical thin-layer chromatography separations were carried out on glass plates coated with silica gel (Analtech, GHLF uniplates). The TLC chromatograms were visualized using UV (short-wave) lamp irradiation or by immersing the plates in 2.5% potassium permanganate in water followed by heating with a heat gun. Melting points are uncorrected and were determined with a Fisher-Johns melting point apparatus. Optical rotations were measured by use of a Perkin-Elmer 241 polarimeter, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity INOVA 400 and 500 instruments with deuterated solvents. ^{1}H NMR chemical shifts were recorded relative to residual $CHCl_3$ at 7.26 ppm, MeOH at 3.31 ppm, DMSO at 2.50 ppm, or H_2O at 4.87 ppm. ^{13}C NMR chemical shifts were reported relative to residual $CHCl_3$ at 77.16 ppm, MeOH at 49.00 ppm, or DMSO at 39.52 ppm. High-resolution mass spectra were obtained with a JEOL JMS-LCmate mass spectrometer. Elemental analyses were determined by Galbraith Laboratories, Inc. The X-ray crystal structure data were obtained on a Bruker APEX2 CCD diffractometer using Mo Kα (0.710 73 Å) radiation.

Cbz-D-*N*(Me)Val-Pro-O*t*Bu (11). To a stirred solution of Cbz-D-*N*(Me)Val (3.85 g, 15.0 mmol), L-Pro-O*t*Bu·HCl (3.43 g, 16.5 mmol), and PyBrop (10.5 g, 22.5 mmol) in anhydrous DCM (60 mL) at 0 °C was added DIPEA (4.01 mL, 2.97 g, 23.0 mmol). The solution was then stirred at 23 °C for 3.5 h and then concentrated under diminished pressure. The residue was dissolved in EtOAc (100 mL) and washed with 80 mL of 10% aqueous citric acid, 80 mL of 6% $NaHCO_3$, and 70 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 4:1 hexanes−EtOAc gave the product as a colorless oil: yield 5.56 g (89%); $[\alpha]^{24}_D$ +41.8 (*c* 1.0, CH_3OH); TLC R_f 0.50 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (5H, m), 5.14 (2H, m), 4.56 (1H, d, *J* = 8.7 Hz), 4.33 (1H, m), 3.60 (2H, t, *J* = 6.4 Hz), 2.88 (3H, s), 2.35 (1H, m), 2.18 (1H, m), 1.90 (3H, m), 1.43 (9H, s), 0.93 (3H, d, *J* = 6.4 Hz), and 0.85 (3H, d, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.4, 168.3, 156.9, 136.7, 128.4, 127.8, 127.5, 80.9, 67.3, 62.1, 59.7, 46.9, 29.2, 28.9, 27.9, 26.6, 24.9, 19.8, and 17.9; HRFABMS *m*/*z* 419.2537 $[M + H]^+$ (calcd for $C_{23}H_{35}N_2O_5$, 419.2546); anal. C 65.89, H 8.28, N 6.78%, calcd for $C_{23}H_{34}N_2O_5$, C 66.00, H 8.19, N 6.69%.

H-D-*N*(Me)Val-Pro-O*t*Bu (12). To a stirred solution of Cbz-D-*N*(Me)Val-L-Pro-O*t*Bu (**11**) (5.19 g, 12.4 mmol) in methanol (60 mL) was added 10% palladium-on-carbon (500 mg). Then, the mixture was stirred under a hydrogen atmosphere (1 atm) at 23 °C for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under diminished pressure to afford the product as a colorless oil: yield 3.23 g (92%); TLC R_f 0.53 (95:5 DCM−MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.40 (1H, dd, *J* = 12, 4 Hz), 3.74 (1H, m), 3.56 (1H, m), 3.03 (1H, d, *J* = 6.6 Hz), 2.38 (3H, s), 2.04−2.22 (2H, m), 1.92−1.99 (2H, m), 1.81 (1H, m), 1.47 (9H, s), 0.99 (3H, d, *J* = 6.8 Hz), and 0.96 (3H, d, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.7, 171.3, 80.9, 67.1, 59.7, 46.9, 34.8, 31.1, 28.9, 27.8, 24.6, 19.6, and 18.3.

***tert*-Butyldimethylsilyl 2-(*S*)-*tert*-Butyldimethylsilyloxy-3-methylbutyrate (13).** To a stirred solution of (*S*)-2-hydroxy-3-methylbutanoic acid (3.00 g, 25.4 mmol) and TBDMSCl (9.96 g, 66.0 mmol) in anhydrous DMF (15 mL) was added imidazole (8.99 g, 132 mmol). The reaction mixture was stirred at 40 °C for 20 h and then partitioned between 150 mL of EtOAc and 50 mL of H_2O. The organic phase was separated and washed with 80 mL of 10% citric acid, 80 mL of 6% $NaHCO_3$, and 80 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 19:1 hexanes−ethyl acetate gave the product as a colorless oil: yield 6.48 g (78%); $[\alpha]^{24}_D$ −31.5 (*c* 1.2, $CHCl_3$); TLC R_f 0.63 (19:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.91 (1H, d, *J* = 4.5 Hz), 2.03 (1H, m), 0.94−0.84 (24H, m), 0.28 (3H, s), 0.27 (3H, s), 0.06 (3H, s), and 0.03 (3H, s); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.6, 32.7, 25.7, 25.5, 19.1, 18.2, 17.6, 16.8, −4.9, −5.0, and −5.5; HRAPCIM *m*/*z* 347.2444 $[M + H]^+$ (calcd for $C_{17}H_{39}O_3Si_2$, 347.2432); anal. C 55.55, H 10.85%, calcd for $C_{17}H_{38}O_3Si_2·H_2O$, C 55.99, H 11.06%.

(TBDMSO)Hiv-D-*N*(Me)Val-Pro-O*t*Bu (14). To a stirred solution of compound **13** (5.00 g, 14.4 mmol) and DMF (1.07 mL, 1.02 g, 13.9 mmol) in anhydrous DCM (60 mL) at 0 °C was added a 2 M solution of oxalyl chloride in DCM (9.45 mL, 18.9 mmol), dropwise (gas evolution). The solution was stirred at 23 °C for 3 h and then concentrated under diminished pressure, and the residue was dissolved in anhydrous DCM (60 mL). The mixture was cooled to 0 °C, and a solution of compound **12** (3.15 g, 11.1 mmol) and BSA (5.71 mL, 4.74 g, 23.3 mmol) in anhydrous DCM (60 mL) at 0 °C was added via cannula. Next, triethylamine (5.23 mL, 3.85 g, 37.7 mmol) was added, and the solution was stirred at 23 °C for 16 h. The mixture was concentrated under diminished pressure, and the residue was dissolved in ethyl acetate (150 mL). The organic solution was washed with 80 mL of 10% aqueous citric acid, 80 mL of 6% aqueous $NaHCO_3$, and 80 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 4:1 hexanes−ethyl acetate gave the product as a colorless oil (two conformers ∼2:1): yield 4.55 g (82%); $[\alpha]^{24}_D$ +32 (*c* 1.14, $CHCl_3$); TLC R_f 0.35 (4:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) major conformer δ 5.07 (1H, d, *J* = 10.8 Hz), 4.32 (1H, dd, *J* = 8.5 and 3.2 Hz), 4.21 (1H, d, *J* = 5.5 Hz), 3.78 (1H, m), 3.56 (1H, m), 3.03 (3H, s), 2.36 (1H, m), 2.14 (1H, m), 1.82−2.04 (4H, m), 1.44 (9H, s), 0.98 (6H, d, *J* = 6.7 Hz), 0.93 (9H, s), 0.83 (6H, d, *J* = 7.6 Hz), and 0.07 (6H, s); ^{13}C NMR ($CDCl_3$, 101 MHz) major conformer δ 172.7, 171.1, 168.6, 80.8, 59.9, 59.6, 47.3, 31.7, 30.1, 29.1, 28.0, 26.5, 25.9, 25.8, 24.8, 19.9, 19.7, 18.3, 18.2, 17.5, −3.5, −4.5, and −5.1; HRAPCIMS *m*/*z* 499.3558 $[M + H]^+$ (calcd for $C_{26}H_{51}N_2O_5Si$, 499.3567); anal. C 62.19, H 10.14, N 5.54%, calcd for $C_{26}H_{50}N_2O_5Si$, C 62.61, H 10.10, N 5.62%.

H-Hiv-D-*N*(Me)Val-Pro-O*t*Bu (7). To a stirred solution of compound **14** (1.47 g, 2.96 mmol) in THF (15 mL) at 0 °C was added 80 μL of water followed by a 1 M solution of TBAF in THF (7.70 mL, 7.70 mmol). The solution was stirred at 0 °C for 4 h. The reaction was terminated with 100 mL of water and extracted with two 100 mL portions of EtOAc. The combined organic solution was washed with 50 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 3:2 hexanes−EtOAc gave the product as a colorless oil (two conformers ∼5:1): yield 834 mg (73%); $[\alpha]^{24}_D$ +73 (*c* 0.86, $CHCl_3$); TLC R_f 0.25 (3:2 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) major conformer δ 5.01 (1H, d, *J* = 11.0 Hz), 4.34 (2H, m), 3.60 (2H, t, *J* = 6.2 Hz), 3.50 (1H, d, *J* = 7.8 Hz), 2.95 (3H, s), 2.38 (1H, m), 2.17 (1H, m), 1.90 (4H, m), 1.43 (9H, s), 1.11 (3H, d, *J* = 6.8 Hz), 0.95 (3H, d, *J* = 6.5 Hz), and 0.82 (6H, d, *J* = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 174.3, 171.2, 167.7, 81.1, 72.4, 60.6, 59.7, 47.2, 30.5, 29.9, 28.9, 27.9, 26.4, 24.9, 20.2, 19.8, 18.0, and 14.4; HRFABMS, *m*/*z* 385.2718 $[M + H]^+$ (calcd

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for $C_{20}H_{37}N_2O_5$, 385.2703); anal. C 61.66, H 9.69, N 7.16%, calcd for $C_{20}H_{36}N_2O_5 \cdot 0.25\ H_2O$, C 61.75, H 9.46, N 7.20%.

Methyl (*S*)-2-(Benzyloxy)propionate (15). To a stirred solution of methyl L-lactate (1.00 mL, 1.09 g, 10.5 mmol) in DCM (40 mL) was added benzyl bromide (1.37 mL, 1.97g, 12.6 mmol) followed by Ag_2O (2.92 g, 12.6 mmol). The reaction mixture was stirred in the dark at 23 °C for 60 h followed by passage through Celite, and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 4:1 hexanes−ethyl acetate gave the product as a colorless oil: yield 1.03 g (51%); TLC R_f 0.50 (4:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (5H, m), 4.71 (1H, d, J = 11.6 Hz), 4.45 (1H, d, J = 11.6 Hz), 4.07 (1H, q, J = 6.9 Hz), 3.75 (3H, s), and 1.54 (3H, d, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.7, 137.6, 128.5, 128.0, 127.9, 74.0, 72.1, 51.9, and 18.8.

(*S*)-2-(Benzyloxy)propanoic Acid (16). To a stirred solution of methyl (*S*)-2-(benzyloxy)propionate (**15**) (1.03 g, 5.30 mmol) in EtOH (8 mL) at 0 °C was added a solution of KOH (327 mg, 5.83 mmol) in water (8 mL), and the mixture stirred at 0 °C for 60 min. The mixture was diluted with 10 mL of water and washed with 20 mL of ethyl acetate. The aqueous phase was set to pH ≤ 3 with 6 M HCl and extracted with two 20 mL portions of ethyl acetate. The combined organic solution was washed with 25 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure to afford the product as a colorless oil: yield 833 mg (88%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.30 (1H, br s), 7.36 (5H, m), 4.70 (1H, d, J = 11.6 Hz), 4.56 (1H, d, J = 11.6 Hz), 4.12 (1H, q, J = 6.9 Hz), and 1.50 (3H, d, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 178.9, 137.1, 128.5, 128.0, 128.0, 73.5, 72.1, and 18.4.

(OBn)Lac-Pro-O*t*Bu (8). To a stirred solution of (*S*)-2-(benzyloxy)propanoic acid (**16**) (2.70 g, 15.0 mmol), L-Pro-O*t*Bu·HCl (3.43 g, 16.5 mmol), and PyBroP (10.5 g, 22.5 mmol) in anhydrous DCM (60 mL) at 0 °C was added DIPEA (7.86 mL, 5.88 g, 45.0 mmol). The solution was stirred at 23 °C for 5 h and then concentrated under diminished pressure. The residue was dissolved in EtOAc (100 mL) and washed with 80 mL of 10% aqueous citric acid, 80 mL of 6% $NaHCO_3$, and 70 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 1:1 EtOAc−hexanes gave the product as a colorless oil (two conformers ~4:1): yield 4.11 g (82%); $[\alpha]^{24}_D$ −110 (c 2.04, $CHCl_3$); TLC R_f 0.48 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) major conformer δ 7.36 (5H, m), 4.71 (1H, m), 4.43 (2H, m), 4.21 (1H, q, J = 6.7 Hz), 3.60 (2H, m), 2.17 (1H, m), 2.02 (1H, m), 1.90 (2H, m), 1.47 (9H, s), and 1.44 (3H, d, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.2, 171.0, 137.7, 128.3, 127.9, 127.6, 81.7, 74.6, 71.0, 60.0, 46.5, 28.5, 27.9, 25.2, and 17.4; HRFABMS m/z 334.2028 $[M + H]^+$ (calcd for $C_{19}H_{28}NO_4$, 334.2018); anal. C 68.44, H 8.36, N 4.44%, calcd for $C_{19}H_{27}NO_4$, C 68.44, H 8.16, N 4.20%.

(OBn)Lac-Pro-*O*-Hiv-D-*N*(Me)Val-Pro-O*t*Bu (17). To a stirred solution of compound **8** (535 mg, 1.60 mmol) in anhydrous DCM (4 mL) was added 2 mL of TFA. The reaction mixture was then stirred at 23 °C for 4 h and then concentrated under diminished pressure. The residual TFA was coevaporated with toluene. The residue was dissolved in anhydrous DCM (13 mL), and compound **7** (576 mg, 1.50 mmol) was added followed by MNBA (661 mg, 1.92 mmol), DMAP (78 mg, 0.64 mmol), and TEA (665 μL, 486 mg, 4.80 mmol). The reaction mixture was stirred at 23 °C for 5 h. The mixture was concentrated under diminished pressure. The residue was dissolved in EtOAc (80 mL) and washed with 40 mL of 10% aqueous citric acid, 40 mL of 6% $NaHCO_3$, and 40 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 4:1 EtOAc−hexanes gave the product as a colorless solid (mixture of conformers): yield 776 mg (81%); mp 53−57 °C; $[\alpha]^{24}_D$ −19 (c 1.00, $CHCl_3$); TLC R_f 0.50 and 0.15 (4:1 EtOAc−hexanes); ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 101 MHz) show a complex mixture; HRAPCIMS m/z 644.3946 $[M + H]^+$ (calcd for $C_{35}H_{54}N_3O_8$, 644.3911); anal. C 64.55, H 8.34, N 6.02%, calcd for $C_{35}H_{53}N_3O_8 \cdot 0.5\ H_2O$, C 64.39, H 9.46, N 6.44%.

(OBn)Lac-Pro-*O*-Hiv-D-(NMe)Val-Pro-OH (5). To a stirred solution of compound **17** (950 mg, 1.48 mmol) in anhydrous DCM (10 mL) was added triethylsilane (3.54 mL, 2.58 g, 22.2 mmol) followed by TFA (3.40 mL, 5.06 g, 44.4 mmol). The solution was stirred at 23 °C for 4 h. The mixture was concentrated under diminished pressure, and the residual TFA coevaorated several times with toluene and finally with diethyl ether to afford the product as a colorless solid (mixture of conformers): yield 869 mg (100%); mp 48−55 °C; $[\alpha]^{24}_D$ +3.8 (c 0.31, EtOAc); ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 101 MHz) showed a complex mixture; HRAPCIMS m/z 588.3278 $[M + H]^+$ (calcd for $C_{31}H_{46}N_3O_8$, 588.3285).

(*R*,*E*)-3-(But-2-enoyl)-4-phenyloxazolidin-2-one (18). To a stirred solution of crotonic acid (2.00 g, 23.2 mmol) in anhydrous THF (60 mL) at −78 °C was added TEA (3.22 mL, 2.34 g, 23.2 mmol) followed by trimethylacetyl chloride (2.86 mL, 2.80 g, 23.2 mmol). The reaction mixture was stirred at 0 °C for 60 min and then cooled to −78 °C. Separately, to a stirred solution of *R*-(−)-4-phenyl-2-oxazolidinone (3.78 g, 27.8 mmol) in anhydrous THF (80 mL) at −78 °C was added *n*-BuLi (2.5 M in hexanes) (11.1 mL, 27.8 mmol). The reaction mixture was stirred at −78 °C for 30 min and then transferred via cannula to the mixture previously prepared (vide supra). The reaction mixture was stirred at 23 °C for 16 h. The mixture was then diluted with 250 mL of EtOAc and washed with 100 mL of 10% aqueous citric acid, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 4:1 hexanes−ethyl acetate gave the product as an off-white solid: yield 5.03 g (94%); TLC R_f 0.35 (4:1 hexanes−EtOAc); mp 73−74 °C; $[\alpha]^{24}_D$ −123.2 (c 0.93, acetone); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44−7.27 (6H, m), 7.17−7.03 (1H, m), 5.48 (1H, dd, J = 8.7, 3.9 Hz), 4.70 (1H, t, J = 8.8 Hz), 4.27 (1H, dd, J = 8.8, 3.9 Hz), and 1.97−1.90 (3H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 164.6, 153.9, 147.4, 139.2, 129.3, 128.8, 126.0, 121.9, 70.1, 57.9, and 18.7; HRFABMS m/z 232.09770 $[M + H]^+$ (calcd for $C_{13}H_{14}NO_3$, 232.09737).

(*R*)-3-[(2*R*,3*R*)-2-Bromo-3-methyl-4-phenylbutanoyl]-4-phenyloxazolidin-2-one (19). To a stirred mixture of copper(I) bromide dimethyl sulfide complex (4.93 g, 24.0 mmol) in anhydrous THF (54 mL) at −78 °C was added 54 mL of anhydrous dimethyl sulfide. The mixture was stirred for 5 min at −78 °C, and benzylmagnesium chloride (2 M in THF) (24.0 mL, 48.0 mmol) was added dropwise. The reaction mixture was stirred at −78 °C for 45 min, and a solution of compound **18** (5.03 g, 21.8 mmol) in anhydrous THF (54 mL) at −78 °C was added via cannula. The mixture was stirred at −78 °C for 90 min and then at 0 °C for 30 min (formation of a gray-black precipitate). The mixture was cooled to −78 °C, and a solution of NBS (12.8 g, 71.9 mmol) in anhydrous THF (150 mL) at −78 °C was added via cannula. The reaction mixture was stirred at −78 °C for 2 h. The reaction was terminated with 300 mL of saturated aqueous NH_4Cl and extracted with 300 mL of EtOAc. The organic solution was washed with 300 mL of 10% aqueous $Na_2S_2O_4$ and 300 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 5:4:1 hexanes−toluene−ethyl acetate gave the product as an off-white solid: yield 5.99 g (67%); TLC R_f 0.25 (5:4:1 hexanes−toluene−EtOAc); mp 112−113 °C; $[\alpha]^{24}_D$ −75.3 (c 0.43, acetone); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53−6.98 (10H, m), 5.64 (1H, d, J = 8.6 Hz), 5.41 (1H, dd, J = 8.8, 4.5 Hz), 4.67 (1H, t, J = 8.9 Hz), 4.24 (1H, dd, J = 8.9, 4.5 Hz), 3.26 (1H, d, J = 10.4 Hz), 2.52−2.31 (2H, m), and 0.91 (3H, d, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 167.9, 152.8, 139.5, 137.7, 129.3, 129.2, 128.9, 128.4, 126.3, 125.8, 69.9, 57.8, 50.3, 40.1, 37.7, and 16.8; HRAPCIMS m/z 404.0683 $[M + H]^+$ (calcd for $C_{20}H_{21}NO_3{}^{81}Br$, 404.0684).

Dolaphenvaline·HCl (9). To a stirred mixture of compound **19** (5.99 g, 14.9 mmol) in anhydrous DMF (70 mL) at 0 °C was added sodium azide (2.91 g, 44.7 mmol). The reaction mixture was stirred at 0 °C for 3 h. The solution was diluted with 300 mL of EtOAc and washed with 300 mL of water followed by three 300 mL portions of

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brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was dissolved in 3:1 THF−water (180 mL) and cooled to 0 °C, and 35% aqueous H_2O_2 (8.69 mL, 89.4 mmol) was added dropwise followed by 1 M aqueous LiOH (29.8 mL, 29.8 mmol). The reaction mixture was stirred at 0 °C for 3 h. Then, the reaction was terminated with 129 mL of 1.3 M aqueous Na_2SO_3 and stirred at 23 °C for 30 min. The reaction mixture was concentrated under diminished pressure, and the aqueous residue was washed with three 100 mL portions of DCM. The aqueous phase was cooled to 0 °C, brought to pH ∼1.5 with 6 N HCl, and extracted with three 100 mL portions of DCM. The combined organic solution was dried over $MgSO_4$ and concentrated under diminished pressure to give the crude product **21** as a colorless oil: yield 2.56 g (78%); $[\alpha]^{24}_D$ −89.4 (*c* 0.45, acetone); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.29 (1H, s), 7.37−7.10 (5H, m), 3.92 (1H, d, *J* = 3.6 Hz), 2.74−2.59 (2H, m), 2.49−2.38 (1H, m), and 0.98 (3H, d, *J* = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 176.46, 139.12, 129.31, 129.05, 128.64, 128.36, 126.52, 64.81, 39.90, 37.72, and 14.78.

Without further purification, crude product **21** (2.50 g, 11.4 mmol) was dissolved in 2:1 AcOH−water (150 mL), and 10% palladium-on-carbon (250 mg) was added. The reaction mixture was purged of air and charged with hydrogen (36 psi). The reaction mixture was shaken at 23 °C for 24 h in a Parr hydrogenator. The reaction was filtered through Celite, and the filtrate concentrated under diminished pressure. The residue was dissolved in 20 mL of 6 N HCl and concentrated under diminished pressure. The residual water was coevaporated with toluene several times, and finally the residue was triturated with ether. The precipitate was filtered and dried to afford the product (compound **9**) as an off-white solid: yield 2.60 g (100%); mp 249−250 °C (dec); $[\alpha]^{24}_D$ +35.7 (*c* 0.28, MeOH); ^{1}H NMR (D_2O, 400 MHz) δ 7.35−7.11 (5H, m), 3.85 (1H, d, *J* = 2.7 Hz), 2.77−2.65 (1H, m), 2.55−2.37 (2H, m), and 0.81 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (D_2O, 101 MHz) δ 171.9, 138.9, 129.0, 128.7, 126.7, 56.9, 38.1, 35.7, and 13.2; HRAPCIMS *m*/*z* 194.1183 $[M + H]^+$ (calcd for $C_{11}H_{16}NO_2$, 194.1181).

(*S*)-Homo-*β*-Val-OMe·HCl (22). To 2.29 mL of anhydrous methanol at 0 °C was added very slowly thionyl chloride (498 μL, 817 mg, 6.87 mmol). The reaction mixture was stirred for 10 min before adding (*S*)-homo-*β*-Val (300 mg, 2.29 mmol). The reaction mixture was stirred at 23 °C for 16 h and diluted with 60 mL of diethyl ether. The precipitate was collected and dried under diminished pressure to afford the product as a colorless solid: yield 383 mg (92%); mp 133−134 °C; $[\alpha]^{24}_D$ −41.1 (*c* 0.13, MeOH); ^{1}H NMR (D_2O, 400 MHz) δ 3.62 (3H, s), 3.44−3.34 (1H, m), 2.65 (2H, m), 1.88 (1H, m), and 0.86 (6H, m); ^{13}C NMR (D_2O, 101 MHz) δ 173.2, 53.4, 52.5, 33.4, 29.8, 17.2, and 16.7; HRAPCIMS *m*/*z* 146.1178 $[M + H]^+$ (calcd for $C_7H_{16}NO_2$, 146.1181).

Cbz-Dml-OMe (23). To a stirred solution of compound **22** (801 mg, 4.67 mmol) in anhydrous THF (14 mL) at −10 °C was added a 1 M solution of LiHMDS in toluene (10.3 mL, 10.3 mmol). The reaction was stirred for 10 min at −10 °C before adding (dropwise) methyl iodide (436 μL, 995 mg, 7.01 mmol). The reaction mixture was stirred at 23 °C for 2 h prior to adding 15 mL of 10% aqueous K_2CO_3 followed by benzyl chloroformate (4.01 mL, 4.78 g, 28.0 mmol). The resulting mixture was stirred at 23 °C for 2 h, diluted with 50 mL of ethyl acetate, washed with 40 mL of 10% aqueous citric acid and 40 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 9:1 hexanes−acetone gave the product as a colorless oil: yield 788 mg (58%); TLC R_f 0.30 (9:1 hexane−acetone); $[\alpha]^{24}_D$ +16.7 (*c* 1.90, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42−7.21 (5H, m), 5.54 (1H, d, *J* = 10.2 Hz), 5.11 (2H, s), 3.64 (3H, s), 3.56−3.40 (1H, m), 2.81 (1H, dd, *J* = 6.9, 4.5 Hz), 1.69 (1H, m), 1.21 (3H, d, *J* = 7.1 Hz), and 0.93 (6H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 176.0, 156.9, 136.9, 128.5, 128.0, 127.9, 66.6, 59.4, 51.7, 40.4, 31.8, 19.9, 19.2, and 15.8; HRAPCIMS *m*/*z* 294.1701 $[M + H]^+$ (calcd for $C_{16}H_{24}NO_4$, 294.1705).

Cbz-Pro-Dml-OMe (10). To a stirred solution of compound **23** (928 mg, 3.16 mmol) in methanol (15 mL) was added 10% palladium over activated carbon (98 mg). Then, the mixture was stirred under a hydrogen atmosphere (1 atm) at 23 °C for 1 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under diminished pressure. The residual methanol was coevaporated with toluene twice, and the residue was dissolved in anhydrous DMF (15 mL). The mixture was cooled to 0 °C before adding Cbz-L-Pro-OH (944 mg, 3.79 mmol) followed by TEA (1.31 mL, 959 mg, 9.48 mmol) and HBTU (1.50 g, 3.95 mmol). The resulting reaction mixture was stirred at 23 °C for 16 h, diluted with 100 mL of ethyl acetate, and washed with 80 mL of 0.5 N HCl, 80 mL of saturated aqueous $NaHCO_3$, and 80 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 1:1 hexanes−ethyl acetate gave the product as a colorless oil (mixture of conformers ∼1:1): yield 900 mg (73%); TLC R_f 0.12 (2:1 hexane−ethyl acetate); $[\alpha]^{24}_D$ −33.7 (*c* 0.38, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) one conformer δ 7.47−7.16 (6H, m), 5.04 (2H, s), 4.22 (1H, d, *J* = 8.9 Hz), 3.79−3.63 (1H, m), 3.50 (3H, s), 3.45−3.31 (2H, m), 2.72−2.56 (1H, m), 2.22−2.00 (1H, m), 1.84−1.60 (4H, m), 1.01 (3H, d, *J* = 6.8 Hz), and 0.82−0.63 (6H, m); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 174.6, 171.6, 154.1, 136.9, 128.2, 127.7, 127.3, 65.8, 59.8, 55.7, 51.3, 47.1, 40.8, 31.5, 29.4, 22.9, 19.8, 17.5, and 14.0; HRAPCIMS *m*/*z* 391.2236 $[M + H]^+$ (calcd for $C_{21}H_{31}N_2O_5$, 391.2233).

Boc-Dpv-Pro-Dml-OMe (24). To a stirred solution of compound **10** (480 mg, 1.23 mmol) in methanol (10 mL) was added 10% palladium over activated carbon (48 mg). Then, the mixture was stirred under a hydrogen atmosphere (1 atm) at 23 °C for 2.5 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under diminished pressure. The residual methanol was coevaporated with toluene (twice), and the residue was dissolved in anhydrous DMF (10 mL). The mixture was cooled to 0 °C before adding Boc-Dpv-OH (410 mg, 1.40 mmol) followed by TEA (597 μL, 436 mg, 4.31 mmol) and HBTU (536 mg, 2.15 mmol). The resulting reaction mixture was stirred at 23 °C for 16 h, diluted with 80 mL of ethyl acetate, and washed with 80 mL of 10% aqueous citric acid, 80 mL of saturated aqueous $NaHCO_3$, and 80 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 1:1 hexanes−ethyl acetate gave the product as a colorless foam: yield 439 mg (67%); TLC R_f 0.25 (1:1 hexane−ethyl acetate); $[\alpha]^{24}_D$ −6.9 (*c* 0.14, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28−7.08 (5H, m), 6.83 (1H, d, *J* = 10.2 Hz), 5.42 (1H, d, *J* = 9.3 Hz), 4.55−4.42 (2H, m), 3.64 (1H, tt, *J* = 17.8, 8.8 Hz), 3.54 (3H, s), 3.38−3.20 (2H, m), 2.82−2.67 (2H, m), 2.45 (1H, dt, *J* = 22.1, 11.0 Hz), 2.27−2.17 (1H, m), 2.15−1.74 (4H, m), 1.52−1.36 (10H, m), 1.12 (3H, d, *J* = 7.1 Hz), and 0.89−0.79 (9H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 176.5, 172.1, 171.6, 156.0, 140.4, 129.5, 128.3, 126.1, 79.6, 60.7, 57.1, 54.0, 51.7, 46.8, 40.5, 39.7, 38.3, 31.8, 29.1, 28.4, 24.9, 19.9, 19.4, 15.9, and 14.1; HRAPCIMS *m*/*z* 532.3383 $[M + H]^+$ (calcd for $C_{29}H_{46}N_3O_6$, 532.3387).

Boc-Dpv-Pro-Dml-OBn (6). To a stirred solution of compound **24** (200 mg, 0.38 mmol) in 2:1:1 MeOH−THF−water (1.2 mL) was added LiOH·H_2O (159 mg, 3.80 mmol). The reaction mixture was stirred at 23 °C for 18 h and then concentrated under diminished pressure. The residue was dissolved in 5 mL of water, and the pH was adjusted to ≤3. The mixture was then extracted with three 10 mL portions of ethyl acetate. The combined organic solution was washed with 10 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was dissolved in anhydrous DMF (1 mL), and TEA (316 μL, 231 mg, 2.28 mmol) was added followed by benzyl bromide (135 μL, 195 mg, 1.14 mmol). The reaction mixture was stirred at 23 °C for 6 h and then diluted with 50 mL of ethyl acetate. The organic mixture was washed with 10 mL of 10% aqueous citric acid, 10 mL of saturated aqueous sodium bicarbonate, 10 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 1:1 hexanes−ethyl acetate gave the product as a colorless foam: yield 129 mg (56%); TLC R_f 0.26 (1:1 hexane−ethyl acetate); $[\alpha]^{24}_D$ −2.1 (*c* 0.19, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43−7.22 (9H, m), 7.21−7.08 (1H, m), 6.84 (1H, d, *J* = 10.2 Hz), 5.38

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(1H, d, J = 9.2 Hz), 5.13−4.87 (2H, m), 4.58−4.45 (2H, m), 3.67 (1H, dt, J = 17.1, 10.0 Hz), 3.40−3.21 (2H, m), 2.91−2.71 (2H, m), 2.49 (1H, dd, J = 13.3, 7.0 Hz), 2.31−2.20 (1H, m), 2.12−1.82 (4H, m), 1.55−1.42 (10H, m), 1.18 (3H, d, J = 7.1 Hz), and 0.92−0.82 (9H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 175.9, 172.0, 171.6, 156.0, 140.4, 135.7, 129.6, 128.6, 128.4, 128.3, 128.1, 126.1, 79.6, 66.3, 60.7, 57.1, 54.0, 46.8, 40.6, 39.8, 38.4, 31.9, 29.2, 28.4, 25.0, 19.9, 19.6, 16.0, and 14.1; HRAPCIMS m/z 608.3694 $[M + H]^+$ (calcd for $C_{35}H_{50}N_3O_6$, 608.3700).

(OBn)Lac-Pro-*O*-Hiv-D-*N*(Me)Val-Pro-Dpv-Pro-Dml-OBn (4). To a stirred solution of compound **6** (140 mg, 0.23 mmol) in anhydrous DCM (2 mL) was added 2 mL of TFA. The reaction mixture was stirred at 23 °C for 4 h and concentrated under diminished pressure. The residual TFA was coevaporated with toluene. The residue was dissolved in anhydrous DMF (3 mL), and compound **5** (135 mg, 0.23 mmol) was added followed by TEA (97 μL, 70 mg, 0.69 mmol) and HBTU (86 mg, 0.35 mmol). The mixture was stirred at 23 °C for 16 h. The mixture was then diluted with 80 mL of ethyl acetate and washed with two 40 mL portions of brine. The organic solution was dried over $MgSO_4$ and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 1:1 hexanes−acetone gave the product as a colorless foam (mixture of conformers): yield 216 mg (87%); TLC R_f 0.3 and 0.4 (1:1 hexane−acetone); $[\alpha]^{24}_D$ +5.0 (c 0.06, EtOAc); ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 101 MHz) showed a complex mixture; HRAPCIMS m/z 1077.631 $[M + H]^+$ (calcd for $C_{61}H_{85}N_6O_{11}$, 1077.628).

Dolastatin 16 (1). To a stirred solution of compound **4** (204 mg, 0.19 mmol) in ethanol (3 mL) was added 20% $Pd(OH)_2$ on carbon (41 mg). The mixture was stirred under a hydrogen atmosphere (1 atm) at 23 °C for 4 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under diminished pressure. The residue was dissolved in 5 mL of anhydrous toluene containing TEA (26 μL, 19 mg, 0.19 mmol) and added (at 0.25 mL/h, using a syringe pump) to a solution containing MNBA (327 mg, 0.95 mmol) and DMAP (232 mg, 1.90 mmol) in anhydrous toluene (126 mL). After the addition was complete the reaction mixture was stirred at 23 °C for 16 h. The reaction mixture was concentrated under diminished pressure, and the residue was dissolved in ethyl acetate (100 mL). The organic solution was washed with 50 mL of 1 N HCl, 50 mL of saturated aqueous sodium bicarbonate, and 50 mL of brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 2:3 hexanes−acetone gave dolastatin 16 as an off-white solid: yield 36 mg (22%); TLC R_f 0.55 (2:3 hexanes−acetone). An analytical sample was purified by reversed-phase HPLC (Zorbax-SB C-18 column, 250 × 9.4 mm, flow rate 3.5 mL/min, elution gradient 40% ACN in water to 99% ACN in water in 20 min, retention time 16.4 min); mp 199−201 °C; $[\alpha]^{24}_D$ +14.5 (c 0.20, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (1H, d, J = 10.1 Hz), 7.35 (2H, d, J = 7.4 Hz), 7.29 (2H, d, J = 7.4 Hz), 7.22−7.13 (1H, m), 6.79 (1H, d, J = 8.8 Hz), 5.43 (1H, d, J = 2.9 Hz), 5.19 (2H, m), 4.96 (1H, d, J = 8.8 Hz), 4.65 (1H, dd, J = 8.7, 2.1 Hz), 4.57 (1H, d, J = 7.5 Hz), 4.47 (1H, d, J = 6.7 Hz), 3.96−3.88 (1H, m), 3.73−3.61 (2H, m), 3.47 (2H, m), 3.10 (3H, s), 2.90−2.81 (2H, m), 2.56−2.48 (2H, m), 2.46−1.69 (15H, m), 1.60−1.48 (2H, m), 1.46 (3H, d, J = 6.8 Hz), 1.13−0.97 (9H, m), and 0.97−0.77 (15H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 174.8, 172.6, 171.4, 171.1, 171.0, 169.7, 169.3, 169.2, 140.6, 129.6, 128.4, 126.3, 76.5, 66.7, 61.3, 59.6, 58.9, 57.9, 56.5, 50.6, 47.6, 46.5, 46.0, 41.0, 40.9, 38.7, 32.4, 31.0, 30.8, 29.8, 28.3, 25.6, 25.5, 25.0, 24.8, 21.8, 20.3, 19.74, 19.73, 19.72, 17.9, 17.2, 16.1, 15.1, and 14.9; HRESIMS m/z 879.5225 $[M + H]^+$ (calcd for $C_{47}H_{71}N_6O_{10}$, 879.5231).

X-ray Crystal Structure of Synthetic Dolastatin 16 (1). A plate-like specimen of $C_{47}H_{70}N_6O_{10}\cdot1.62(CH_2Cl_2)$, approximate dimensions 0.095 mm × 0.289 mm × 0.435 mm, was obtained from dichloromethane−hexanes and used for X-ray crystallographic analysis. The X-ray intensity data were measured. A total of 1092 frames were collected. The total exposure time was 18.20 h. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using an orthorhombic unit cell yielded a total of 45 655 reflections to a maximum θ angle of 25.48° (0.83 Å resolution), of which 10 048 were independent (average redundancy 4.544, completeness 99.6%, R_{int} = 6.61%) and 8727 (86.85%) were greater than $2\sigma(F^2)$. The final cell constants of a = 10.3987(10) Å, b = 18.7734(18) Å, c = 27.849(3) Å, volume = 5436.7(9) Å^3, are based upon the refinement of the XYZ-centroids of 9674 reflections above $20\sigma(I)$ with 4.339° < 2θ < 45.71°. Data were corrected for absorption effects using the multiscan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.708. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9030 and 0.9780.

The final anisotropic full-matrix least-squares refinement on F^2 with 633 variables converged at R1 = 6.01% for the observed data and wR2 = 16.78% for all data. The goodness-of-fit was 1.085. The largest peak in the final difference electron density synthesis was 1.501 e$^-$/Å^3, and the largest hole was −0.518 e$^-$/Å^3 with an RMS deviation of 0.074 e$^-$/Å^3. On the basis of the final model, the calculated density is 1.242 g/cm^3 and $F(000)$ is 2168 e$^-$.

Cancer Cell Line Procedures. Inhibition of human cancer cell growth was assessed using the National Cancer Institute's standard sulforhodamine B assay as previously described.[15] Briefly, cells in a 5% fetal bovine serum/RPMI1640 medium were inoculated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50}, or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray crystallographic data for **1** as well as copies of the ^{1}H and ^{13}C NMR spectra of compounds **1**, **4**−**19**, and **22**−**24**. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data have been deposited with Cambridge Crystallographic Data Center as supplementary publication no. CCDC 1035008. This can be obtained free of charge on application to the Cambridge Crystallographic Data Center, 2 Union Rd, Cambridge CBZ 1EZ, UK [fax: (+44) 1223-336 033; e-mail: deposit@ccdc.cam.ac.uk].

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

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