# Antineoplastic Agents. 592. Highly Effective Cancer Cell Growth Inhibitory Structural Modifications of Dolastatin 10

George R. Pettit,* Fiona Hogan, and Steven Toms

Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 871604, Tempe, Arizona 85287-1604, United States

S *Supporting Information*

ABSTRACT: The dolastatin series of unique peptides, originally discovered as constituents of the sea hare *Dolabella auricularia*, is of increasing importance in providing biological leads, especially to new and useful anticancer drugs. Dolastatin 10 and three analogues, minor structural modifications designated auristatins, are currently in human cancer clinical trials. The present study was undertaken to explore delivery to the cancer sites by way of phosphate or quinoline modifications. The initial objectives, auristatin TP as sodium phosphate **3b** (GI$_{50}$ 10^{-2}–10^{-4} μg/mL), auristatin 2-AQ (**4**, GI$_{50}$ 10^{-2}–10^{-3} μg/mL), and auristatin 6-AQ (**5**, GI$_{50}$ 10^{-4} μg/mL), exhibited superior cancer cell growth inhibitory properties.
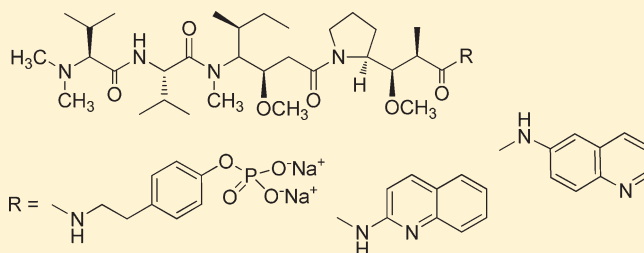
The remarkable anticancer properties of dolastatin 10 (**1**), a unique pentapeptide that we isolated from the sea hare *Dolabella auricularia*,[2a,b] has led to intense interest in closely related derivatives (auristatins) that are suitable for clinical trial. Such structural modifications have provided a number of potential clinical candidates with enhanced efficacy and pharmacological characteristics.[2c,d] Replacement of the dolaphenine (Doe) unit with phenethylamides, to give auristatins PE[2c,3,4] (**2a**), PHE[2d,3,5] (**2b**), and E,[3,6] and with pyridylethylamide (auristatin PYE, **2c**)[2d,3,7] has led to active analogues that are undergoing preclinical[7] and clinical development.[3] Dolastatin 10 and three of the auristatins are in human cancer clinical trials, ranging from phase I to phase III.
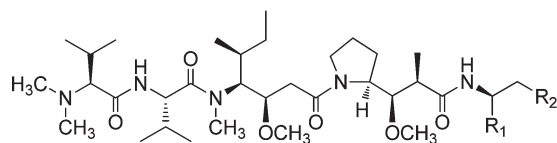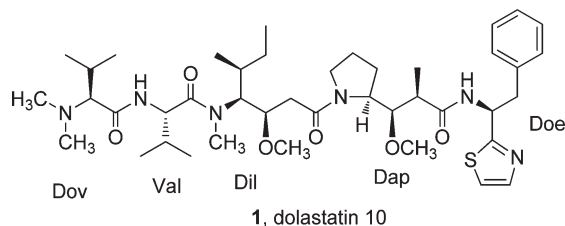
1, dolastatin 10

2a, R$_1$ = H, R$_2$ = Ph, auristatin PE
b, R$_1$ = CO$_2$CH$_3$, R$_2$ = Ph, auristatin PHE
c, R$_1$ = H, R$_2$ = 2-pyridyl, auristatin PYE

We now report the synthesis of auristatin TP (**3**), a tyramine phosphate modification of dolastatin 10 in the form of water-soluble salts. The synthesis of auristatins suitable for formulation of such salts is of considerable interest because the use of water-soluble phosphate derivatives has increased the bioavailability of a number of anticancer drugs, including combretastatins A-1[8] and A-4,[8b,9] pancratistatin,[10] taxol,[11] and etoposide.[12] The salts are dephosphorylated by serum phosphatases to yield the active drug, which is then transported intracellularly. We also report the syntheses of aminoquinoline (AQ) auristatin modifications **4** and **5**. A number of 4- and 8-AQ derivatives have been used historically as antimalarial agents,[13] and various biological activities have been reported for 2-AQ itself[14a] and for derivatives of the 3, 4-, 5-, 6-, and 8-AQ isomers.[14b−f] Use of the readily available isomers 2- and 6-AQ led to new auristatins **4** and **5**, respectively. Each of the new auristatins displayed very strong cancer cell growth inhibition against a panel of murine and human cancer cell lines.

■ RESULTS AND DISCUSSION

The synthesis of **3** was carried out as shown in Scheme 1. Reaction of the γ-amino acid Boc-Dap (**6**)[15] with tyramine in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl) and 1-hydroxybenzotriazole (HOBT) gave the protected amide **7a**. Removal of the Boc group with bromotrimethylsilane (TMSBr) yielded the hydrobromide salt (**7b**), which was coupled with Dov-Val-Dil.TFA (**8**)[16] in the presence of EDCI and HOBT to give the parent auristatin tyramide (**9**). The doubling of signals in the ^{1}H and ^{13}C NMR spectra of **9** indicated the presence of two isomers, a pattern similar to that of dolastatin 10 and due to conformational isomers arising from *cis*−*trans* isomerism at the Dil−Dap bond.[16]
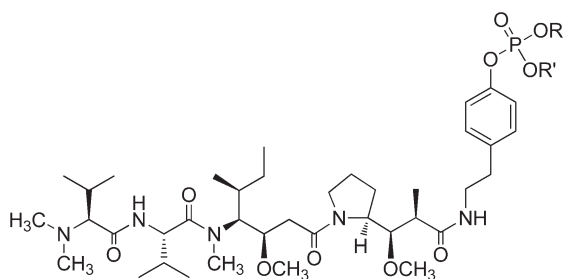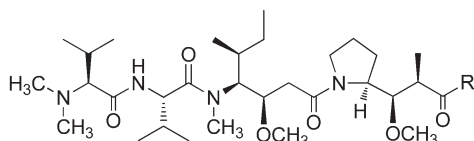
3, auristatin TP

a, R = R' = Li⁺	**e**, R = R' = H, quinine
b, R = R' = Na⁺	**f**, R = R' = H, TRIS
c, R = R' = K⁺	**g**, R = R' = H, serine
d, R = R' = H, morpholine	**h**, R = R' = H, nitroarginine

4, auristatin 2-AQ, R =

5, auristatin 6-AQ, R =

Scheme 1
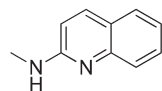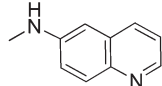
Formation of phosphate diester **10a** was achieved via in situ generation of dibenzyl chlorophosphate, from reaction of dibenzyl phosphite and carbon tetrachloride, and was followed by removal of the benzyl ester groups by hydrogenolysis to provide the free phosphoric acid **10b**. The pure **10b** was quite unstable but could be stored for short periods as a methanolic solution (<0.01 M at 4 °C) and was generally used immediately as follows to provide compounds **3a–h**. The passage of acid **10b** through a Dowex cation exchange resin (Na⁺ form) provided the sodium salt (**3b**), and compounds **3a,c,d** were similarly produced by ion exchange of the free acid or of either the sodium or potassium salts in the appropriate Dowex resin. The remaining salts (**3e–h**) were prepared directly from the free acid **10b** by treatment with the appropriate base or amino acid. The solubilities of each salt and of precursor **9** were measured in distilled water at room temperature. The most soluble were the sodium (**3b**) and potassium (**3c**) salts (Table 1).

A similar convergent synthesis was planned for the preparation of the auristatin aminoquinoline modifications (**4**, **5**), that is, formation of the Dap–aminoquinoline unit, followed by condensation with tripeptide **8**. As shown in Scheme 2, Boc-Dap (**6**) and 2-aminoquinoline (2-AQ) were condensed to give Boc-Dap-2-AQ (**11**), diethylcyanophosphonate (DEPC) being used as coupling reagent, followed by deprotection to give the amine TFA salt (**12**). Coupling of **12** and **8** with use of DEPC gave the desired auristatin 2-AQ (**4**). The doubling of signals in the ¹H NMR spectrum of **4** because of conformational changes was also noted.

Preparation of auristatins from other aminoquinoline isomers proved more difficult. First, the coupling of 5-aminoquinoline (5-AQ) with compound **6** was attempted, but use of DEPC failed to give the desired product. The coupling agent PyBroP was next used under standard conditions, but only starting material (**6** and
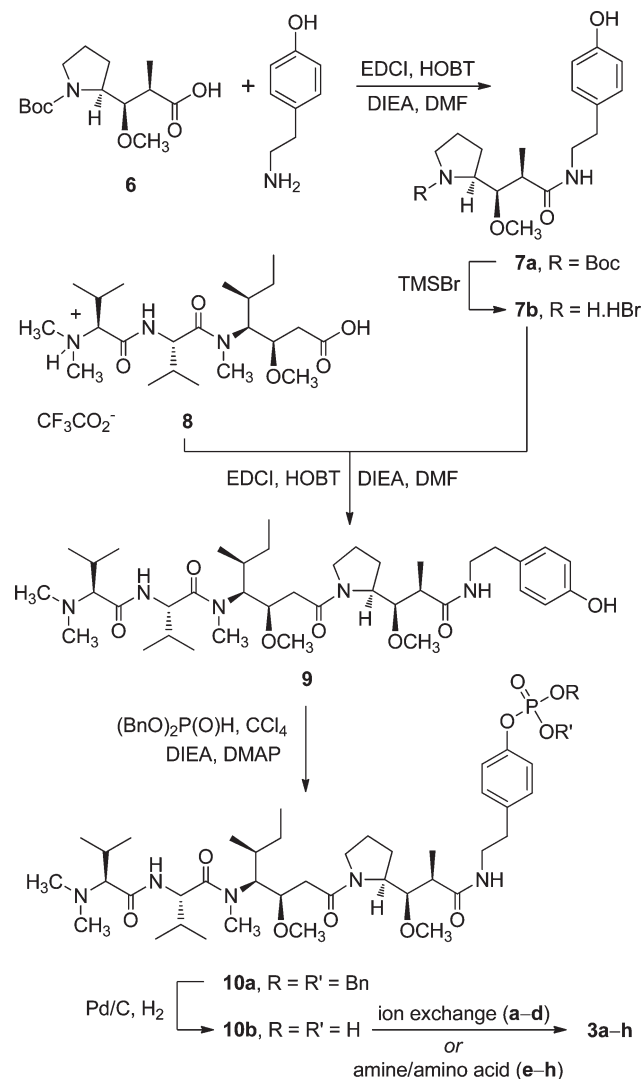
5-AQ) was detected after 100 h. The activity of the aminoquinolines varies with the position of the amino group,[13a,17] and they are in general poor nucleophiles. Therefore, we considered a route involving an activated intermediate preformed from the amino acid. Pozdnev[18a] used di-*tert*-butyl dicarbonate (Boc anhydride, Boc₂O), in the presence of pyridine, to form activated esters of a number of protected amino acid derivatives, which were then condensed successfully with 6-aminoquinoline (6-AQ). Use of this method to couple 5-AQ and compound **6** failed and was not further pursued, and the condensation of 6-aminoquinoline (6-AQ) with **6**, via mixed anhydride **13** (Scheme 3), was next attempted.

A mixture of Boc₂O and **6** in pyridine and dimethylformamide (DMF) was allowed to stir for 10 min, and 6-AQ was then added.[18a] After isolation of products, the reaction was found to have given the desired Boc-Dap-6-AQ (**14**), along with Boc-6-AQ (at least half of the 6-AQ was used in formation of this product). When **6** and Boc₂O were allowed to stir in base for an hour so that formation of ester **13** could go to completion (with evolution of CO₂) before addition of 6-AQ,[18b] formation of Boc-6-AQ was avoided, and in isolation of the desired product a

Table 1. Solubility of Compounds 3a–h and 10[a]

compound no.	mg/mL	compound no.	mg/mL
3a	>65	3f	>51
3b	>236	3g	7
3c	>120	3h	7
3d	>72	9	<1
3e	<5		

[a] In distilled water at 23 °C.

Scheme 2
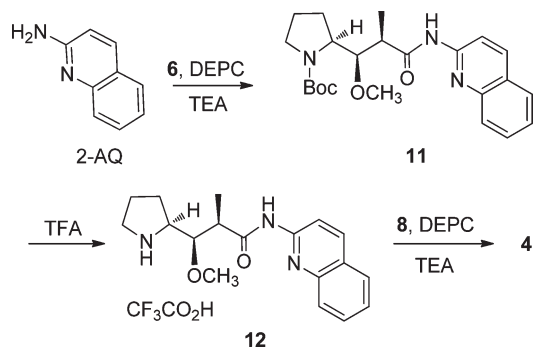
Scheme 3

citric acid wash was found useful for removal of unreacted aminoquinoline. However, the yield of product was still quite low, at 25%, and another method was sought.

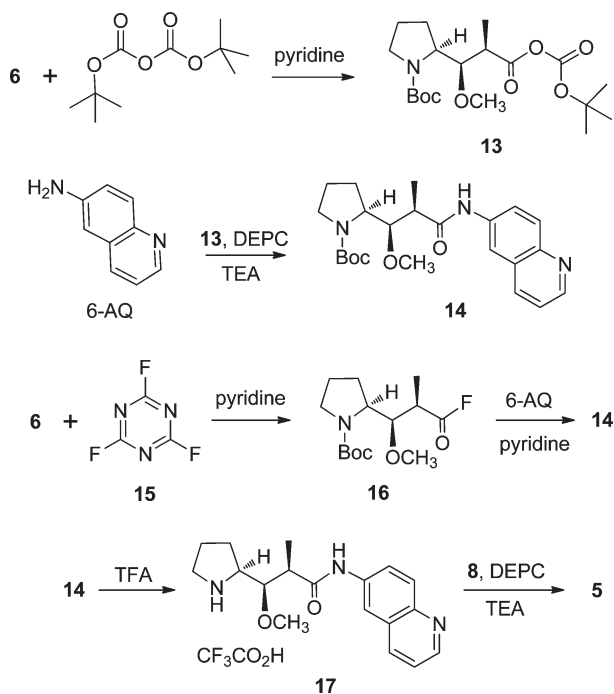Among the most reactive of the common activated intermediates are the amino acid fluorides,[19] which have been shown to be very efficient reagents for peptide bond formation.[20] With a sample of Boc-Dap-6-AQ (14) in hand for comparison, the condensation of the acid fluoride of 6 with 6-AQ was next

attempted (Scheme 3). Reaction of cyanuric fluoride (15) with Boc-Dap (6) to give Boc-Dap-C(O)F (16) was carried out under mild conditions, and the crude product was used immediately in a condensation reaction with 6-AQ, in the presence of pyridine. The reaction did not go to completion (there was no detectable reduction in the amounts of unreacted compounds from 6 to 20 h later), and the desired Boc-Dap-6-AQ (14) was isolated in 26% yield. Compound 14 was then treated with TFA to give the Dap-6-AQ·TFA salt (17), which was condensed with Dov-Val-Dil·TFA (8) to give auristatin-6-AQ (5).

In a repeat of the synthesis of acid fluoride 16, diisopropylethylamine (DIEA) was used as base, and purification of 16 was carried out on silica gel before condensation with 6-AQ, in the presence of DIEA, to give Boc-Dap-6-AQ (14). Reaction was slow, and at 44 h no change was apparent compared to the mixture at 32 h. The colorless oil that was isolated contained both product 14 and unreacted 16 (by TLC). According to the literature, the reaction of Fmoc amino acid fluorides with amines is often very slow and is not dependent on base[20b,c] (the presence of base can increase the reaction rate, but lack of it can result in a cleaner reaction). Of the two methods to synthesize Boc-Dap-6-AQ (5), use of Boc$_2$O to form active intermediate 13 led consistently to a yield of about 24%, whereas the yield from the Boc-Dap-C(O)F (16) method varied from 26% (using pyridine) to 6.6% (using DIEA and purifying the intermediate).

Compounds 3b, 3c, 4, 5, and 9 were evaluated against the murine P388 lymphocytic leukemia cell line and showed exceptional activity; auristatins 3b, 4, and 5 were also tested against a minipanel of human cancer cell lines in our laboratories, with similarly strong activity evident (Table 2), especially from compounds 3b and 5. These in vitro data are quite comparable to those of dolastatin 10 (1) and auristatin PE (2a), each of which had GI$_{50}$ values of 10^{-5}–10^{-6} μg/mL (10^{-2}–10^{-3} nM) against a similar minipanel of human cell lines.[2b,c,3] Further biological testing of the new auristatins is under way.

■ EXPERIMENTAL SECTION

General Experimental Procedures. *N*-Boc-Dolaproine and Dov-Val-Dil·TFA were synthesized as described earlier.[15,16] Reagents and anhydrous solvents were purchased from Acros Organics (Fisher Scientific), Sigma-Aldrich Chemical Co., and Lancaster Synthesis and were used as received. Diisopropylethylamine (DIEA) was redistilled over potassium hydroxide. Dibenzylphosphite was redistilled before use (bp 160 °C at 0.1 mmHg). For thin-layer chromatography, Analtech silica gel GHLF Uniplates were used and visualized with short-wave UV irradiation and use of a permanganate dip followed by heating. Solvent extracts of aqueous solutions were dried over magnesium sulfate. For column chromatography, silica gel (230–400 mesh ASTM) from E. Merck (Darmstadt, Germany) was used. For ion-exchange chromatography, Dowex 50W×8–400 hydrogen form resin (Sigma-Aldrich) was washed with MeOH, hydrochloric acid (1 M), and deionized H$_2$O before use. The cation forms of the resin were prepared by elution of an aqueous solution (1 M) of the corresponding base followed by deionized H$_2$O.

Melting points are uncorrected and were determined with a Fischer-Johns melting point apparatus. Optical rotations were measured by use of a Perkin-Elmer 241 polarimeter, and the [α]$_D$ values are given in 10^{-1} deg cm^2 g^{-1}. The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded using Varian Gemini 300 and Unity 400 and 500 instruments with deuterated solvents. The ^{31}P spectra were referenced to 80% phosphoric acid or to the corresponding ^{1}H spectra. High-resolution mass spectra were obtained with a Jeol JMS-LCmate mass spectrometer. Elemental analyses were determined by Galbraith Laboratories, Inc.

Table 2. Murine and Human Cancer Cell Line Results [ED$_{50}$ and GI$_{50}$, μg/mL (nM)]^a

compound no.	P388	NCI-H460	KM20L2	DU-145	BXPC-3	MCF-7	SF-268
3b	<0.001	0.00088	0.00061	0.00054	0.046	0.00068	0.00125
	(<1.2)	(1.05)	(0.72)	(0.64)	(54.6)	(0.81)	(1.48)
3c	0.0076						
	(8.7)						
4	0.031	0.016	0.0077	0.023	0.029	0.0046	0.029
	(42.8)	(22.1)	(10.6)	(31.8)	(40.1)	(6.35)	(40.1)
5	0.0026	0.00036	0.00025	0.00030	0.00031	0.00014	0.00016
	(3.59)	(0.50)	(0.35)	(0.41)	(0.43)	(0.19)	(0.22)
9	0.0036						
	(5.0)						

^{a} Cytotoxicity concentrations as nanomolar values are given in parentheses. ^{b} Cancer cell lines in order: murine lymphocytic leukemia (P388); lung (NCI-H460); colon (KM20L2); prostate (DU-145); pancreas (BXPC-3); breast (MCF-7); CNS (SF-268).

N-Boc-Dap-4-hydroxyphenethylamide (7a). To a solution of Boc-Dap[15] (**6**, 0.49 g, 1.71 mmol) in dry DMF (3 mL) that was stirring at 20 °C was added 1-hydroxybenzotriazole (HOBT, 0.37 g, 2.74 mmol). Diisopropylethylamine (DIEA, 0.95 mL, 5.48 mmol) was added, followed by N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI), and the reaction mixture was stirred for 10 min before the addition of tyramine (0.28 g, 2.05 mmol). The mixture was stirred at 20 °C for 16 h before termination of reaction by addition of saturated NaHCO$_3$ solution (5 mL) and extraction with EtOAc (4 × 5 mL). The combined organic extract was washed with brine (20 mL) and dried. Removal of solvent yielded a yellow oil (0.89 g), which was fractionated by column chromatography (eluent: 2.5−6.0% CH$_3$OH in CH$_2$Cl$_2$) to provide **7a** as a colorless oil (0.57 g, 82%) that crystallized from 1:1 CH$_2$Cl$_2$−hexane: mp 163−164 °C; [α]^{23}$_D$ −30.4 (c 1.9, CHCl$_3$); IR (neat) ν_{max} 3305, 2975, 2935, 1650, 1515, 1168, 755 cm^{-1}; ^{1}H NMR (CD$_3$OD, 300 MHz) δ 6.92 (d, J = 8.4 Hz, 2H), 6.57 (d, J = 8.4 Hz, 2H), 3.55 (br m, 1H), 3.49− 3.33 (m, 3H), 3.29 (s, 3H), 3.26−3.19 (m, 2H), 3.19−3.03 (m, 2H), 2.70−2.53 (m, 2H), 2.11 (m, 1H), 1.81−1.70 (m, 2H), 1.61−1.50 (m, 2H), 1.40 (br s, 9H), 1.05 (d, J = 6.3 Hz, 3H); ^{13}C NMR (CDCl$_3$, 100.5 MHz) (two conformers observed) δ 174.7, 174.1, 155.5, 155.3, 154.8, 154.4, 129.5, 129.3, 115.5, 115.4, 83.7, 82.0, 80.1, 79.5, 60.6, 59.1, 58.6, 46.9, 46.5, 44.2, 43.8, 40.9, 40.7, 34.3, 28.5, 28.4, 25.7, 25.1, 24.4, 24.0, 14.3, 14.0; HRMS (FAB) m/z 407.2565 [M + H]$^+$ (calcd for C$_{22}$H$_{35}$N$_2$O$_5$, 407.2546).

Dap-4-hydroxyphenethylamide Hydrobromide (7b). Bromotrimethylsilane (0.46 mL, 3.5 mmol) was added to a stirred solution of **7a** (0.57 g, 1.4 mmol) in dry CH$_2$Cl$_2$ at 20 °C, and stirring was continued for 18 h. Water (5 mL) was added, and the mixture was stirred vigorously for 30 min. The aqueous layer was removed, and the organic phase was extracted again with H$_2$O (5 mL). Freeze-drying of the combined aqueous phase provided the hydrobromide salt **7b** as a colorless solid (0.54 g, 99%), which was used without further purification. A sample was crystallized from CH$_2$Cl$_2$−hexane: mp 79−81 °C; IR (neat) ν_{max} 3275, 2980, 1640, 1515, 1235, 830 cm^{-1}; ^{1}H NMR (CD$_3$OD, 300 MHz) δ 6.95 (d, J = 8.4 Hz, 2H), 6.60 (d, J = 8.4 Hz, 2H), 3.47 (m, 1H), 3.41 (s, 3H), 3.25−3.02 (m, 5H), 2.72−2.62 (m, 2H), 2.33 (m, 1H), 1.88−1.76 (m, 2H), 1.72−1.64 (m, 2H), 1.13 (d, J = 7.2 Hz, 3H); ^{13}C NMR (CD$_3$OD, 125.5 MHz) δ 175.6, 157.0, 131.0, 130.8, 116.2, 81.6, 62.9, 61.8, 46.5, 45.5, 41.3, 35.2, 24.1, 23.9, 15.5; HRMS (APCI) m/z 307.2026 [(M − HBr) + H]$^+$ (calcd for C$_{17}$H$_{27}$N$_2$O$_3$, 307.2022).

Dov-Val-Dil-Dap-4-hydroxyphenethylamide (9). To a solution of **8**[16] (0.78 g, 1.43 mmol) that was stirring in dry DMF (2 mL) at 20 °C was added HOBT (0.31 g, 2.29 mmol). Next was added DIEA (0.96 mL, 5.50 mmol), followed by EDCI (0.44 g, 2.29 mmol), and the reaction mixture was stirred for 15 min before the addition of a solution of **7b** (0.50 g, 1.30 mmol) in DMF (4 mL). The mixture was stirred at

20 °C for 6 h, and then reaction was terminated by addition of saturated NaHCO$_3$ solution (10 mL), followed by extraction of the mixture with EtOAc (4 × 10 mL). The combined organic extract was washed with brine (50 mL) and dried. Removal of solvent yielded a viscous, brown oil (0.83 g), which was fractionated by column chromatography (eluent: 5−10% MeOH in CH$_2$Cl$_2$) to provide **9** as a viscous, colorless oil (0.61 g, 65%): [α]^{23}$_D$ −44.0 (c 2.2, CHCl$_3$); IR (neat) ν_{max} 3295, 2965, 1620, 1515, 1100, 755 cm^{-1}; ^{1}H NMR (CD$_3$OD, 400 MHz) δ 7.25 (m, 1H), 7.05−7.01 (m, 4H), 6.68 (t, J = 8.5 Hz, 4H), 4.74 (d, J = 8.4 Hz, 1H), 4.71 (d, J = 8.4 Hz, 1H), 4.63 (d, J = 8.8 Hz, 1H), 4.15 (m, 1H), 4.07 (m, 1H), 3.90−3.83 (m, 2H), 3.78 (m, 1H), 3.68 (m, 1H), 3.57 (m, 6H), 3.40−3.32 (m, 2H), 3.38 (s, 3H), 3.36 (s, 3H), 3.29 (s, 6H), 3.26 (s, 3H), 3.13 (s, 3H), 2.81−2.68 (m, 4H), 2.65−2.62 (m, 2H), 2.48 (d, J = 6.6 Hz, 2H), 2.31 (s, 6H), 2.29 (s, 6H), 2.27−2.19 (m, 2H), 2.08−1.86 (m, 10H), 1.78−1.63 (m, 4H), 1.44−1.35 (m, 2H), 1.16 (t, J = 7.1 Hz, 6H), 1.05−0.95 (m, 28H), 0.90−0.84 (m, 12H); ^{13}C NMR (CD$_3$OD, 100.5 MHz) δ 176.5, 176.4, 175.3, 173.3, 173.2, 171.9, 157.0, 156.9, 136.5, 131.2, 130.9, 130.8, 130.7, 116.3, 116.2, 87.2, 83.6, 79.8, 76.0, 75.8, 62.1, 61.4, 61.0, 60.8, 58.6, 58.3, 57.8, 56.2, 56.0, 45.9, 45.7, 42.5, 42.4, 41.8, 41.4, 38.2, 35.3, 33.8, 33.1, 31.8, 31.7, 28.8, 27.0, 26.8, 25.8, 24.5, 20.2, 20.2, 19.9, 19.5, 19.3, 16.3, 16.0, 15.8, 15.1, 10.9, 10.8; HRMS (FAB) m/z 718.5084 [M + H]$^+$ (calcd for C$_{39}$H$_{68}$N$_5$O$_7$, 718.5119).

Dov-Val-Dil-Dap-4-(dibenzylphosphoryloxy)phenethylamide (10a). To a solution of **9** (0.51 g, 0.70 mmol) in dry CH$_3$CN (4 mL) at −15 °C (ice/salt) was added carbon tetrachloride (0.34 mL, 1.02 mmol), followed by DIEA (0.26 mL, 1.50 mmol) and 4-dimethylaminopyridine (9 mg, 0.07 mmol). Dibenzylphosphite (0.23 mL, 1.02 mmol) was next added over a 20 min period to the mixture, the temperature being maintained between −15 and −18 °C. After addition, the mixture was cooled to −20 °C and then allowed to warm to 5 °C over 90 min, and reaction was terminated by addition of saturated NaHCO$_3$ solution (10 mL). The mixture was extracted with EtOAc (3 × 10 mL), and the combined organic extract was washed with brine (50 mL) and dried. Removal of solvent yielded a viscous, pale yellow oil (0.60 g), which was fractionated by column chromatography (eluent: 5−10% MeOH in CH$_2$Cl$_2$) to provide **10a** as a colorless oil (0.34 g, 49%): IR (neat) ν_{max} 3305, 2965, 1620, 1455, 1215, 1015, 955 cm^{-1}; ^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.36−7.31 (m, 20H), 7.21 (d, J = 6.6 Hz, 2H), 7.20 (d, J = 6.6 Hz, 2H), 7.07 (d, J = 6.6 Hz, 2H), 7.03 (d, J = 6.6 Hz, 2H), 5.13−5.10 (m, 8H), 4.81−4.71 (m, 2H), 4.71 (d, J = 8.0 Hz, 1H), 4.62 (d, J = 8.0 Hz, 1H), 4.14 (m, 1H), 4.06 (m, 1H), 3.91−3.86 (m, 2H), 3.80 (m, 1H), 3.69 (m, 1H), 3.56−3.47 (m, 4H), 3.43−3.32 (m, 2H), 3.38 (s, 3H), 3.36 (s, 3H), 3.28 (s, 6H), 3.26 (s, 3H), 3.11 (s, 3H), 2.86−2.77 (m, 4H), 2.65−2.61 (m, 3H), 2.51 (m, 1H), 2.46 (d, J = 6.5 Hz, 2H), 2.30 (s, 6H), 2.29 (s, 6H), 2.28−2.18 (m, 2H), 2.08−1.86 (m, 9H), 1.76−1.64 (m, 5H), 1.43−1.36 (m, 2H), 1.16 (d, J = 7.5 Hz, 3H), 1.15 (d, J = 7.5 Hz, 3H),

1.02−0.92 (m, 28H), 0.87−0.81 (m, 12H); ^{13}C NMR (CD$_3$OD, 125.5 MHz) δ 175.2, 175.1, 171.9, 170.6, 149.0 (d, J_{C-P} = 7.0 Hz), 148.9 (d, J_{C-P} = 7.0 Hz), 136.6, 136.4, 129.9, 129.8, 128.5, 128.3, 127.9 (d, J_{C-P} = 2.6 Hz), 119.8 (d, J_{C-P} = 4.4 Hz), 119.6 (d, J_{C-P} = 4.4 Hz), 85.7, 82.2, 78.5, 78.4, 78.4, 74.5, 74.4, 70.1, 70.1, 60.7, 60.0, 59.6, 57.3, 56.9, 54.8, 54.6, 46.7, 44.5, 44.3, 41.1, 41.1, 40.0, 39.7, 36.8, 35.6, 34.1, 32.3, 32.2, 31.7, 30.4, 30.3, 27.4, 25.7, 25.5, 24.4 (d, J_{C-P} = 3.6 Hz), 23.1, 18.8 (d, J_{C-P} = 4.4 Hz), 18.5, 18.2, 18.0, 14.9, 14.6, 14.5, 13.7, 9.5; ^{31}P NMR (CD$_3$OD, 202.5 MHz) δ −6.51, −6.54; HRMS (FAB) m/z 978.5811 [M + H]$^+$ (calcd for C$_{53}$H$_{81}$N$_5$O$_{10}$P 978.5721).

Dov-Val-Dil-Dap-4-(dihydrophosphoryloxy)phenethyl-amide (10b). To a solution of dibenzyl phosphate **10a** (38 mg, 0.04 mmol) in MeOH (5 mL) was added palladium on activated carbon (10 wt % Pd, 10 mg), and hydrogen gas (balloon) was bubbled through the suspension for 1 h. The mixture was filtered through a plug of Celite, and the filter was washed with MeOH (2 × 5 mL). Removal of solvent from the filtrate yielded the free phosphoric acid **10b** as a glassy solid (32 mg, quantitative): mp 168−170 °C; IR (neat) ν_{max} 3400, 2970, 1635, 1460, 1095, 910 cm^{-1}; ^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.20−7.12 (m, 8H), 4.77−4.71 (m, 2H), 4.67 (d, J = 8.5 Hz, 1H), 4.62 (d, J = 9.0 Hz, 1H), 4.11 (m, 1H), 4.05 (m, 1H), 3.93−3.89 (m, 2H), 3.73−3.68 (m, 2H), 3.61−3.48 (m, 4H), 3.44−3.33 (m, 2H), 3.41 (s, 3H), 3.37 (s, 3H), 3.29 (s, 6H), 3.28 (s, 3H), 3.15 (s, 3H), 2.90 (s, 6H), 2.79−2.73 (m, 4H), 2.66−2.49 (m, 4H), 2.42−2.26 (m, 4H), 2.08−1.64 (m, 14H), 1.46−1.38 (m, 2H), 1.23 (d, J = 7.0 Hz, 3H), 1.17 (d, J = 7.0 Hz, 3H), 1.07−1.00 (m, 20H), 0.97−0.84 (m, 20H); ^{31}P NMR (CD$_3$OD, 202.5 MHz) δ −4.11.

Sodium Auristatin TP (3b). Ion-exchange chromatography of free acid **10b** (32 mg) with aqueous NaOH led to **3b** as a colorless solid (24 mg, 71%): mp 170−171 °C; IR (neat) ν_{max} 3305, 2965, 1625, 1510, 1105, 990 cm^{-1}; ^{1}H NMR (CD$_3$OD, 400 MHz) δ 7.17−7.15 (m, 4H), 7.09 (d, J = 8.0 Hz, 4H), 4.78−4.72 (m, 2H), 4.72 (d, J = 8.0 Hz, 1H), 4.64 (d, J = 8.4 Hz, 1H), 4.12 (m, 1H), 4.07 (m, 1H), 3.98−3.94 (m, 2H), 3.91 (dd, J = 9.1, 2.3 Hz, 2H), 3.70 (m, 1H), 3.59 (m, 1H), 3.51−3.41 (m, 4H), 3.39 (s, 3H), 3.37 (s, 3H), 3.36−3.32 (m, 2H), 3.30 (s, 6H), 3.27 (s, 3H), 3.14 (s, 3H), 2.81−2.70 (m, 4H), 2.65 (d, J = 7.2 Hz, 1H), 2.63 (d, J = 7.2 Hz, 1H), 2.49 (d, J = 6.4 Hz, 2H), 2.31 (s, 6H), 2.29 (s, 6H), 2.29−2.22 (m, 2H), 2.08−1.87 (m, 9H), 1.81−1.68 (m, 5H), 1.43−1.36 (m, 2H), 1.17 (t, J = 5.3 Hz, 6H), 1.03−0.95 (m, 28H), 0.85 (q, J = 7.2 Hz, 12H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −3.42.

Lithium Auristatin TP (3a). Ion-exchange chromatography of sodium salt **3b** (12 mg, 0.014 mmol) with aqueous LiOH led to **3a** as a colorless solid (11 mg, 96%): mp 263 °C (dec); IR (neat) ν_{max} 3315, 2965, 1630, 1105, 1005, 920 cm^{-1}; ^{1}H NMR (CD$_3$OD, 400 MHz) δ 7.20 (d, J = 8.0 Hz, 2H), 7.18 (d, J = 8.0 Hz, 2H), 7.04 (d, J = 8.0 Hz, 4H), 4.74−4.70 (m, 2H), 4.72 (d, J = 8.0 Hz, 1H), 4.64 (d, J = 8.8 Hz, 1H), 4.14−4.00 (m, 4H), 3.95 (m, 1H), 3.91 (dd, J = 9.0, 2.2 Hz, 1H), 3.71 (m, 1H), 3.58 (m, 1H), 3.52−3.34 (m, 6H), 3.39 (s, 3H), 3.38 (s, 3H), 3.30 (s, 6H), 3.27 (s, 6H), 3.13 (s, 3H), 2.75−2.68 (m, 4H), 2.64 (d, J = 4.8 Hz, 1H), 2.63 (d, J = 4.8 Hz, 1H), 2.51 (d, J = 6.4 Hz, 2H), 2.30 (s, 6H), 2.29 (s, 6H), 2.27−2.23 (m, 2H), 2.07−1.94 (m, 9H), 1.82−1.71 (m, 5H), 1.45−1.37 (m, 2H), 1.18 (t, J = 6.2 Hz, 6H), 1.03−0.95 (m, 28H), 0.85 (q, J = 6.9 Hz, 12H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −0.58.

Potassium Auristatin TP (3c). Ion-exchange chromatography of acid **10b** with aqueous KOH led to **3c** as a colorless solid (4 mg, 64%): mp 198 °C; IR (neat) ν_{max} 3230, 2965, 1620, 1100, 980, 885 cm^{-1}; ^{1}H NMR (CD$_3$OD, 400 MHz) δ 7.21 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.2 Hz, 2H), 7.04 (d, J = 8.2 Hz, 4H), 4.75−4.72 (m, 2H), 4.72 (d, J = 8.0 Hz, 1H), 4.64 (d, J = 6.6 Hz, 1H), 4.14−4.01 (m, 4H), 3.95 (m, 1H), 3.91 (dd, J = 8.8, 2.4 Hz, 1H), 3.71 (m, 1H), 3.58 (m, 1H), 3.52−3.35 (m, 6H), 3.39 (s, 3H), 3.38 (s, 3H), 3.30 (s, 6H), 3.26 (s, 3H), 3.13 (s, 3H), 2.75−2.68 (m, 4H), 2.64 (d, J = 8.8 Hz, 1H), 2.63 (d, J = 9.2 Hz, 1H), 2.49 (d, J = 5.6 Hz, 2H), 2.30 (s, 6H), 2.29 (s, 6H), 2.27−2.23 (m, 2H), 2.07−1.94 (m, 9H), 1.83−1.72 (m, 5H), 1.45−1.37 (m, 2H), 1.18

(t, J = 6.2 Hz, 6H), 1.03−0.95 (m, 28H), 0.84 (q, J = 6.9 Hz, 12H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −0.42.

Morpholine Auristatin TP (3d). Ion-exchange chromatography of potassium salt **3c** with aqueous morpholine led to **3d** as a colorless solid: mp 148−150 °C; IR (neat) ν_{max} 3295, 2965, 1620, 1455, 1105, 880 cm^{-1}; ^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.18−7.11 (m, 8H), 4.82−4.74 (m, 2H), 4.71 (d, J = 8.5 Hz, 1H), 4.65 (d, J = 8.5 Hz, 1H), 4.13 (m, 1H), 4.07 (m, 1H), 3.97 (m, 1H), 3.91 (dd, J = 9.3, 2.3 Hz, 1H), 3.80 (br s, 16H), 3.71 (m, 1H), 3.60 (m, 1H), 3.52 (d, J = 8.5 Hz, 1H), 3.49−3.43 (m, 3H), 3.40 (s, 3H), 3.39 (s, 3H), 3.37−3.34 (m, 2H), 3.31 (s, 6H), 3.28 (s, 3H), 3.15 (s, 3H), 3.06 (br s, 16H), 2.82 (m, 1H), 2.77 (q, J = 7.2 Hz, 4H), 2.69 (d, J = 8.5 Hz, 1H), 2.67 (m, 1H), 2.54 (d, J = 9.0 Hz, 1H), 2.50 (d, J = 6.0 Hz, 2H), 2.42 (s, 6H), 2.34 (s, 6H), 2.32−2.14 (m, 2H), 2.13−1.88 (m, 9H), 1.81−1.71 (m, 5H), 1.46−1.38 (m, 2H), 1.20 (d, J = 6.5 Hz, 3H), 1.18 (d, J = 7.5 Hz, 3H), 1.04−0.95 (m, 28H), 0.91−0.87 (m, 12H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −3.43.

General Procedure for the Synthesis of 3e−h. The amine or amino acid (25.0 μmol) was added to a stirred solution of acid **10b** (10 mg, 12.5 μmol) in either MeOH (300 μL) or deionized H$_2$O (for **3h**), and the mixture was stirred for 15 h. Removal of solvent yielded the desired salt.

Quinine Auristatin TP (3e): colorless solid; mp 118−120 °C; ^{1}H NMR (CD$_3$OD, 500 MHz) δ 8.67 (d, J = 4.8 Hz, 4H), 7.93 (d, J = 9.3 Hz, 4H), 7.72 (d, J = 4.8 Hz, 4H), 7.43 (d, J = 2.3 Hz, 4H), 7.40 (dd, J = 9.3, 2.3 Hz, 4H), 7.17 (t, J = 7.3 Hz, 4H), 7.03 (d, J = 7.3 Hz, 4H), 5.93 (s, 4H), 5.73 (m, 4H), 5.05 (d, J = 17.5 Hz, 4H), 4.95 (d, J = 11 Hz, 4H), 4.82−4.71 (m, 2H), 4.72 (d, J = 8.0 Hz, 1H), 4.65 (d, J = 8.5 Hz, 1H), 4.12 (m, 1H), 4.07 (m, 1H), 3.98 (s, 12H), 3.91 (d, J = 2.0 Hz, 1H), 3.89 (d, J = 2.0 Hz, 1H), 3.71−3.65 (m, 2H), 3.56 (m, 1H), 3.50 (d, J = 10.0 Hz, 1H), 3.45−3.23 (m, 12H), 3.39 (s, 3H), 3.38 (s, 3H), 3.30 (s, 3H), 3.29 (s, 3H), 3.27 (s, 3H), 3.14 (s, 3H), 3.00−2.92 (m, 8H), 2.71−2.65 (m, 6H), 2.56−2.48 (m, 8H), 2.34 (s, 6H), 2.31 (s, 6H), 2.30−2.21 (m, 2H), 2.08−1.90 (m, 24H), 1.78−1.71 (m, 10H), 1.45−1.40 (m, 6H), 1.17 (t, J = 7.0 Hz, 6H), 1.04−0.95 (m, 28H), 0.98−0.80 (m, 12H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −1.82.

TRIS Auristatin TP (3f): colorless solid; mp 122−123 °C; ^{1}H NMR (D$_2$O, 500 MHz) δ 7.21−7.12 (m, 1H), 4.73−4.64 (m, 4H), 4.17 (m, 1H), 4.11 (m, 1H), 3.92−3.86 (m, 2H), 3.74−3.62 (m, 2H), 3.67 (s, 24H), 3.59−3.38 (m, 6H), 3.44 (s, 3H), 3.42 (s, 3H), 3.33 (s, 3H), 3.33 (s, 3H), 3.26 (s, 3H), 3.18 (s, 3H), 3.12 (d, J = 8.5 Hz, 3H), 3.02 (d, J = 9.5 Hz, 1H), 2.87−2.75 (m, 2H), 2.67−2.62 (m, 2H), 2.58−2.54 (m, 2H), 2.52 (s, 6H), 2.45 (s, 6H), 2.36−2.27 (m, 2H), 2.22−2.09 (m, 2H), 2.08−2.01 (m, 2H), 1.99−1.72 (m, 9H), 1.68−1.61 (m, 1H), 1.39−1.30 (m, 2H), 1.20 (d, J = 6.5 Hz, 3H), 1.16 (d, J = 7.0 Hz, 3H), 1.05−0.97 (m, 28H), 0.88−0.84 (m, 12H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −0.01.

Serine Auristatin TP (3g): colorless solid; mp 158 °C (dec); ^{1}H NMR (D$_2$O, 500 MHz) δ 7.26 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 4.75 (d, J = 9.5 Hz, 1H), 4.73−4.68 (m, 2H), 4.66 (d, J = 9.5 Hz, 1H), 4.18 (m, 1H), 4.11 (m, 1H), 4.01−3.93 (m, 10H), 3.86−3.83 (m, 6H), 3.79 (t, J = 5.8 Hz, 2H), 3.74−3.68 (m, 2H), 3.62−3.51 (m, 4H), 3.47−3.36 (m, 4H), 3.44 (s, 3H), 3.39 (s, 3H), 3.33 (s, 3H), 3.32 (s, 3H), 3.24 (s, 3H), 3.18 (s, 3H), 2.97 (s, 6H), 2.95 (s, 6H), 2.92−2.79 (m, 4H), 2.67−2.44 (m, 6H), 2.33 (m, 1H), 2.24 (m, 1H), 2.12−2.02 (m, 2H), 1.97−1.66 (m, 9H), 1.51 (m, 1H), 1.38−1.32 (m, 2H), 1.20 (d, J = 7.0 Hz, 3H), 1.14 (d, J = 7.0 Hz, 3H), 1.05−0.95 (m, 30H), 0.92−0.82 (m, 10H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −4.07.

Nitroarginine Auristatin TP (3h): colorless solid; mp 157−158 °C (dec); IR (neat) ν_{max} 3295, 2965, 1625, 1360, 1270, 1095 cm^{-1}; ^{1}H NMR (D$_2$O, 500 MHz) δ 7.21 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.10 (d, J = 7.8 Hz, 2H), 7.05 (d, J = 7.8 Hz, 2H), 4.71 (d, J = 9.0 Hz, 1H), 4.70−4.64 (m, 2H), 4.62 (d, J = 8.5 Hz, 1H), 4.14 (m, 1H), 4.06 (m, 1H), 3.81−3.72 (m, 4H), 3.74 (t, J = 6.5 Hz, 4H), 3.69−3.63 (m, 2H),

3.59−3.45 (m, 4H), 3.43−3.33 (m, 2H), 3.40 (s, 3H), 3.35 (s, 3H), 3.30 (t, J = 6.5 Hz, 8H), 3.29 (s, 3H), 3.28 (s, 3H), 3.20 (s, 3H), 3.14 (s, 3H), 2.93 (s, 6H), 2.90 (s, 6H), 2.88−2.75 (m, 4H), 2.63−2.48 (m, 5H), 2.44−2.39 (m, 1H), 2.29 (m, 1H), 2.20 (m, 1H), 2.08−1.97 (m, 2H), 1.95−1.61 (m, 25H), 1.47 (m, 1H), 1.34−1.27 (m, 2H), 1.16 (d, J = 6.5 Hz, 3H), 1.10 (d, J = 7.0 Hz, 3H), 1.01−0.96 (m, 18H), 0.93−0.90 (m, 12H), 0.86−0.81 (m, 10H); ^{31}P NMR (CD$_3$OD, 162.0 MHz) δ −3.56.

***N*-Boc-Dap-2-aminoquinoline (11).** To a solution of Boc-Dap[15] (**6**, 0.172 g; 0.6 mmol) in CH$_2$Cl$_2$ (3 mL) was added 2-amino-quinoline (82.8 mg; 0.57 mmol), and the mixture was stirred and cooled to 0 °C under argon. Triethylamine (TEA, 0.3 mL; 2.1 mmol) and diethylcyanophosphonate (DEPC; 0.2 mL; 1.2 mmol) were added, and the resultant yellow solution was allowed to warm to room temperature (rt) and was stirred under argon for 6 h. Removal of solvent yielded a dark orange-brown residue, which was fractioned under pressure on silica gel [eluent: hexane−acetone (7:2 to 2:3)] to give the product as a colorless solid (90.8 mg, 0.22 mmol, 36.6%, based on recovery of starting material): ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.43 (1H, dd, J = 8.7. 1.5 Hz), 8.16 (1H, d, J = 8.7 Hz), 7.83 (1H, d, J = 8.7 Hz), 7.72 (1H, d, J = 8.4 Hz), 7.66 (1H, t, J = 7.5 Hz), 7.44 (1H, t, J = 7.5 Hz), 4.05−3.92 (2H, m, NCH, OCH), 3.53 (3H, s, OCH$_3$), 3.44 (2H, br d, J = 13 Hz, NCH$_2$), 2.60−2.80 (1H, m, C*H*CH$_3$), 1.74−1.98 (4H, m, 2 × CH$_2$), 1.52 (9H, s, C(CH$_3$)$_3$), 1.45 (3H, d, J = 9.3 Hz, CHC*H*$_3$)); MS (APCI+) m/z 414.2373 [M + H]$^+$ (calcd for C$_{23}$H$_{32}$N$_3$O$_4$, 414.2393).

Dap-2-aminoquinoline Trifluoroacetate (12). To a solution of *N*-Boc-Dap-2-AQ (**11**, 68.0 mg, 0.16 mmol) in CH$_2$Cl$_2$ (4 mL) that was stirring at 0 °C under argon was added trifluoroacetic acid (TFA, 2 mL), and stirring was continued for 2 h with warming to rt. The solvent was removed under vacuum, toluene being used to form an azeotrope with the remaining TFA. The residue, a yellow oil, was allowed to stand under diethyl ether for 1 h. Removal of the ether left a yellowish oily solid, to which hexane was added and removed under vacuum until a constant weight was reached (99.4 mg; quantitative), and this material was used immediately in the next reaction.

Dov-Val-Dil-Dap-2-aminoquinoline (Auristatin 2-AQ, 4). The Dap-2-AQ salt **12** and Dov-Val-Dil·TFA[16] (**8**, 87.0 mg; 0.16 mmol) were dissolved in CH$_2$Cl$_2$ (5 mL), and the solution was stirred under argon and cooled to 0 °C. Next, TEA (0.12 mL; 0.86 mmol) and DEPC (0.035 mL; 0.21 mmol) were added, and the mixture was stirred under argon for 7 h with warming to rt. Removal of solvent yielded a yellowish oil (310 mg), which was separated on silica gel under pressure [eluent: hexane−acetone (5:2 to 3:2)] to give the product as a colorless glass (powder when scratched) (64 mg; 0.09 mmol): ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.43 (1H, dd, J = 8.7. 1.5 Hz), 8.14 (1H, d, J = 8.7 Hz), 7.80−7.41 (4H, m), 6.90 (1H, t, J = 9.3 Hz), 6.73 (1H, d, J = 9.0 Hz), 4.86 (1H, m), 4.75 (1H, m), 4.26 (1H, m), 4.14 (1H, m), 4.04 (1H, m), 3.51 and 3.44 (3H, s), 3.35 and 3.32 (3H, s), 3.38−3.19 (2H, m), 3.02 (3H, s), 2.42 (3H, m), 2.23 (6H, s), 2.23 (1H, m), 2.08−1.98 (5H, m), 1.95−1.74 (1H, m), 1.43−1.33 (2H, m), 0.80−1.06 (22H, m); MS (APCI+) m/z 725.4997 [M + H]$^+$ (calcd for C$_{40}$H$_{65}$N$_6$O$_6$, 725.4966).

***N*-Boc-Dap-6-aminoquinoline (14).** *Method A.* To a stirring solution of Boc-Dap[15] (**6**, 87.2 mg; 0.3 mmol) in DMF (2 mL) and pyridine (0.1 mL) was added Boc$_2$O (0.183 g; 0.84 mmol). After 10 min, 6-aminoquinoline (6-AQ; 50.4 mg; 0.35 mmol) was added to the solution, and stirring was continued for 64 h, at which time starting material was still present. Solvent was removed from the mixture, and the residue was fractionated by column chromatography in hexane−acetone (5:1 to 2:1 gradient). The first fractions to elute contained Boc-6-AQ (35 mg): ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.79 (1H, dd, J = 4.5, 1.5 Hz), 8.01−8.12 (3H, m), 7.48 (1H, dd, J = 9, 2.7 Hz), 7.36 (1H, dd, J = 7.1, 4.2 Hz), 7.03 (1H, br s), 1.55 (9H, s).

Following the elution of the remaining Boc-Dap (**6**), compound **14** (29.7 mg, 0.07 mmol, 23% yield, or 28% based on 14.9 mg recovery of 6-AQ) was collected: ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.82 (2H, m), 8.45

(1H, br s), 8.11 (1H, d, J = 8.1 Hz), 8.04 (1H, d, J = 9.3 Hz), 7.72 (1H, br), 7.37 (1H, dd, J = 8.4, 4.2 Hz), 4.15−3.90 (2H, m, NCH, OCH), 3.55 (3H, s, OCH$_3$), 3.43 (m, 1H), 3.27 (m, 1H), 2.72 (1H, m), 2.06−1.76 (4H, m), 1.50 (9H, s), 1.39 (3H, m); MS (APCI+) m/z 414.2408 [M + H]$^+$ (calcd for C$_{23}$H$_{32}$N$_3$O$_4$, 414.2393).

Method B. Intermediate acid fluoride **16** was first prepared by successive addition of pyridine (0.05 mL) and cyanuric fluoride (**15**, 0.15 mL, 1.75 mmol) to a solution of Boc-Dap (**6**, 76.3 mg, 0.27 mmol) that was stirring under argon at 0 °C, with continued stirring for 20 h and warming to rt. Next, CH$_2$Cl$_2$ (10 mL) and ice were added, followed by cold H$_2$O. The organic phase was removed and the aqueous layer was further extracted with CH$_2$Cl$_2$. The combined organic extract was washed with cold H$_2$O and dried to give a dark orange, oily solid (65.6 mg) that by TLC comprised product **16** and a trace of Boc-Dap (**6**). Without further purification, the crude product was dissolved in CH$_2$Cl$_2$ and was treated with pyridine (0.1 mL) followed by 6-AQ (34.8 mg, 0.24 mmol). The mixture was stirred for 21 h and was then extracted with CH$_2$Cl$_2$ (10 mL). The solution was washed with 10% citric acid solution, followed by H$_2$O. Drying over Na$_2$SO$_4$ and removal of solvent yielded a pale brown oil (52.8 mg), which was separated by column chromatography [eluent: toluene−acetone (2:1)] to give product **14** (30.3 mg, 0.07 mmol, 26%).

Dap-6-aminoquinoline Trifluoroacetate (17). To a solution of *N*-Boc-Dap-6-AQ (**14**, 49.3 mg, 0.12 mmol) in CH$_2$Cl$_2$ (2 mL) that was stirring at 0 °C under argon was added trifluoroacetic acid (TFA, 2 mL). Stirring was continued for 3 h with warming to rt. The solvent was removed under vacuum, toluene being used to form an azeotrope with the remaining TFA, to give a green-tinged oily solid (**17**; quantitative), which was used immediately in the next reaction.

Dov-Val-Dil-Dap-6-aminoquinoline (Auristatin 6-AQ, 5). Dap-6-AQ salt **17** (0.12 mmol) and Dov-Val-Dil·TFA[16] (**8**, 70.0 mg; 0.13 mmol) were dissolved in CH$_2$Cl$_2$ (2 mL), and the solution was stirred under argon and cooled to 0 °C. Next were added TEA (0.11 mL; 0.79 mmol) and DEPC (0.03 mL; 0.18 mmol), and the mixture was stirred under argon for 18 h with warming to rt. Removal of solvent and separation on silica gel under pressure [eluent: hexane−acetone (5:2 to 2:3)] gave the crude product **5** (48.6 mg), of which a 19.1 mg sample was further purified by column chromatography in CH$_2$Cl$_2$−MeOH (19:1) to give auristatin 6-AQ (**5**) as a pale yellow, glassy oil (powder when scratched): ^{1}H NMR (CDCl$_3$, 300 MHz) δ 9.04 (1H, br s), 8.81 (1H, br d, J = 3 Hz), 8.47 (1H, s), 8.11 (1H, d, J = 8.4 Hz), 8.02 (1H, d, J = 9.3 Hz), 7.76 (1H, br d, J = 8.7 Hz), 7.36 (1H, dd, J = 8.4, 3.8 Hz), 6.96 (1H, br), 4.79 (2H, m), 4.30−4.07 (3H, m), 3.51 (3H, s), 3.50−3.26 (2H, m), 3.35 (3H, s), 3.05 (s, 3H), 2.71 (1H, m), 2.54−2.42 (2H, m), 2.32−2.22 (1H, m), 2.28 (6H, s), 2.12−2.05 (2H, m), 1.82 (2H, m), 1.42−1.26 (5H, m), 1.08−0.80 (21H, m); MS (APCI+) m/z 725.4907 [M + H]$^+$ (calcd for C$_{40}$H$_{65}$N$_6$O$_6$, 725.4966).

■ ASSOCIATED CONTENT

ⓢ Supporting Information. ^{1}H NMR spectra of compounds **3b**, **4**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Tel: (480) 965-3351. Fax: (480) 965-2747. E-mail: bpettit@asu.edu.

■ ACKNOWLEDGMENT

■ REFERENCES

(1) For Antineoplastic Agents Part 591 refer to: Bai, R.; Nguyen, T. L.; Burnett, J. C.; Atasoylu, O.; Munro, M. H. G.; Pettit, G. R.; Smith, A. B., III; Gussio, R.; Hamel, E. *J. Chem. Inf. Model.*, submitted.

(2) (a) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883–6885. (b) Pettit, G. R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W.; Kirby, G. W.; Moore, R. E.; Steglich, W.; Tamm, C., Eds.; Springer: Vienna, 1997; Vol. 70, pp 1−79. (c) Pettit, G. R.; Srirangam, J. K.; Barkoczy, J.; Williams, M. D.; Durkin, K. P. M.; Boyd, M. R.; Bai, R.; Hamel, E.; Schmidt, J. M.; Chapuis, J.-C. *Anti-Cancer Drug Des.* **1995**, *10*, 529–544. (d) Pettit, G. R.; Srirangam, J. K.; Barkoczy, J.; Williams, M. D.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Hogan, F.; Bai, R.; Chapuis, J.-C.; McAllister, S.; Schmidt, J. M. *Anti-Cancer Drug Des.* **1998**, *13*, 243–277.

(3) Pettit, G. R. In *International Oncology Updates: Marine Anticancer Compounds in the Era of Targeted Therapies*; Chabner, B.; Cortés-Funes, H., Eds.; Permanyer Publications: Barcelona, 2009.

(4) (a) Kobayashi, M.; Natsume, T.; Tamaoki, S.; Watanabe, J.; Asano, H.; Mikami, T.; Miyasaka, K.; Miyazaki, K.; Gondo, M.; Sakakibara, K.; Tsukagoshi, S. *Jpn. J. Cancer Res.* **1997**, *88*, 316–327. (b) Fennell, B.; Carolan, S.; Pettit, G. R.; Bell, A. *J. Antimicrob. Chemother.* **2003**, *51*, 833–841. (c) Watanabe, J.; Minami, M.; Kobayashi, M. *Anticancer Res.* **2006**, *26*, 1973–1981. (d) Horti, J.; Juhasz, E.; Monostori, Z.; Maeda, K.; Eckhardt, S.; Bodrogi, I. *Cancer Chemother. Pharmacol.* **2008**, *62*, 173–180. (e) Banerjee, S.; Wang, Z.; Mohammad, M.; Sarkar, F. H.; Mohammad, R. M. *J. Nat. Prod.* **2008**, *71*, 492–496.

(5) Woyke, T.; Berens, M. E.; Hoelzinger, D. B.; Pettit, G. R.; Winkelmann, G.; Pettit, R. K. *Antimicrob. Agents Chemother.* **2004**, *48*, 561–567.

(6) (a) Doronina, S. O.; Toki, B. E.; Torgov, M. Y.; Mendelsohn, B. A.; Cerveny, C. G.; Chace, D. F.; DeBlanc, R. L.; Gearing, R. P.; Bovee, T. D.; Siegall, C. B.; Francisco, J. A.; Wahl, A. F.; Meyer, D. L.; Senter, P. D. *Nat. Biotechnol.* **2003**, *21*, 778–784. (b) Temming, K.; Mayer, D. L.; Zabinski, R.; Dijkers, E. C. F.; Poelstra, K.; Molema, G.; Kok, R. J. *Bioconjugate Chem.* **2006**, *17*, 1385–1394. (c) Temming, K.; Meyer, D. L.; Zabinski, R.; Senter, P. D.; Poelstra, K.; Molema, G.; Kok, R. J. *Mol. Pharmaceut.* **2007**, *4*, 686–694.

(7) Shnyder, S. D.; Cooper, P. A.; Millington, N. J.; Pettit, G. R.; Bibby, M. C. *Int. J. Oncol.* **2007**, *31*, 353–360.

(8) (a) Pettit, G. R.; Lippert, J. W., III. *Anti-Cancer Drug Des.* **2000**, *15*, 203–216. (b) Kirwan, I. G.; Loadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R.; Lippert, J. W., III; Shnyder, S. D.; Cooper, P. A.; Bibby, M. C. *Clin. Cancer Res.* **2004**, *10*, 1446–1453. (c) Hokland, S. L.; Horsman, M. R. *Int. J. Hypothermia* **2007**, *23*, 599–606. (d) Dalal, S.; Burchill, S. A. *Eur. J. Cancer* **2009**, *45*, 713–722.

(9) (a) Pettit, G. R.; Rhodes, M. R. *Anti-Cancer Drug Des.* **1998**, *13*, 183–191. (b) Patterson, D. M.; Rustin, G. J. S.; Serradell, N.; Rosa, E.; Bolos, J. *Drugs Future* **2007**, *32*, 1025–1032.

(10) Pettit, G. R.; Melody, N.; Herald, D. L. *J. Nat. Prod.* **2004**, *67*, 322–327.

(11) Mamber, S. W.; Mikkilineni, A. B.; Pack, E. J.; Rosser, M. P.; Wong, H.; Ueda, Y.; Forenza, S. *J. Pharm. Exp. Ther.* **1995**, *274*, 877–883.

(12) Saulnier, M. G.; Langley, D. R.; Kadow, J. F.; Senter, P. D.; Knipe, J. O.; Tun, M. M.; Vyas, D. M.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2567–2572.

(13) (a) Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. *J. Med. Chem.* **2000**, *43*, 283–291. (b) Nanayakkara, N. P. D.; Ager, A. L., Jr.; Bartlett, M. S.; Yardley, V.; Croft, S. L.; Khan, I. A.; McChesney, J. D.; Walker, L. A. *Antimicrob. Agents Chemother.* **2008**, *52*, 2130–2137. (c) Vale, N.; Moreira, R.; Gomes, P. *Eur. J. Med. Chem.* **2009**,

44, 937–953. (d) Kouznetsov, V. V.; Gómez-Barrio, A. *Eur. J. Med. Chem.* **2009**, *44*, 3091–3113.

(14) (a) Pfister, J. R. *J. Nat. Prod.* **1988**, *51*, 969–970. (b) Sheridan, R. E.; Deshpande, S. S.; Nicholson, J. D.; Adler, M. *Toxicon* **1997**, *35*, 1439–1451. (c) Beresnevicius, Z. J.; Viliunas, V.; Kantminiene, K. *Chem. Heterocycl. Compd. (Khim. Geterotsikl. Soedin.)* **2000**, *36*, 432–438. (d) Goda, F. E.; Abdel-Aziz, A. A.-M.; Ghoneim, H. A. *Bioorg. Med. Chem.* **2005**, *13*, 3175–3183. (e) Shao, Y.; Zhang, J.; Tu, C.; Dai, C.; Xu, Q.; Guo, Z. *J. Inorg. Chem.* **2005**, *99*, 1490–1496. (f) DeVita, R. J. *Curr. Top. Med. Chem.* **2007**, *7*, 1433–1439.

(15) (a) Pettit, G. R.; Singh, S. B.; Herald, D. L.; Lloyd-Williams, P.; Kantoci, D.; Burkett, D. D.; Barkóczy, J.; Hogan, F.; Wardlaw, T. R. *J. Org. Chem.* **1994**, *59*, 6287–6295. (b) Pettit, G. R.; Grealish, M. P. *J. Org. Chem.* **2001**, *66*, 8640–8642. (c) Mordant, C.; Reymond, S.; Tone, H.; Lavergne, D.; Touati, R.; Ben Hassine, B.; Ratovelomanana-Vidal, V; Genet, J.-P. *Tetrahedron* **2007**, *63*, 6115–6123.

(16) Pettit, G. R.; Srirangam, J. K.; Singh, S. B.; Williams, M. D.; Herald, D. L.; Barkóczy, J.; Kantoci, D.; Hogan, F. *J. Chem. Soc., Perkin Trans. 1* **1996**, 859–863.

(17) (a) Schulman, S. G.; Abate, K.; Kovi, P. J.; Capomacchia, A. C.; Jackman, D. *Anal. Chim. Acta* **1973**, *65*, 59–67. (b) Abernethy, J. L.; Kilday, W. *J. Org. Chem.* **1960**, *25*, 1924–1928.

(18) (a) Pozdnev, V. F. *Int. J. Peptide Protein Res.* **1994**, 36–48. (b) Furlong, S. T.; Mauger, R. C.; Strimpler, A. M.; Liu, Y.-P.; Morris, F. X.; Edwards, P. D. *Bioorg. Med. Chem.* **2002**, *10*, 3637–3647.

(19) (a) Olah, G. A.; Nojima, M.; Kerekes, I. *Synthesis* **1973**, 487–488. (b) Bertho, J.-N.; Loffet, A.; Pinel, C.; Reuther, F.; Sennyey, G. *Tetrahedron Lett.* **1991**, *32*, 1303–1306.

(20) (a) Carpino, L. A.; Mansour, E.-S. M. E.; Sadat-Aalee, D. *J. Org. Chem.* **1991**, *56*, 2611–2614. (b) Wenschuh, H.; Beyermann, M.; El-Faham, A.; Ghassemi, S.; Carpino, L. A.; Bienert, M. *J. Chem. Soc., Chem. Commun.* **1995**, 669–670. (c) Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29*, 268–274.