

Antineoplastic Agents. 515. Synthesis of Human Cancer Cell Growth Inhibitors Derived from 3,4-Methylenedioxy-5,4'-dimethoxy-3'-amino-Z-stilbene

George R. Pettit,^{*,†} Collin R. Anderson,[†] Eric J. Gapud,[‡] M. Katherine Jung,[§] John C. Knight,[†] Ernest Hamel,[‡] and Robin K. Pettit[†]

Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 872404, Tempe, Arizona 85287-2404, Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702-1201, and SAIC-Frederick, Inc., Frederick, Maryland 21702

Received March 4, 2005

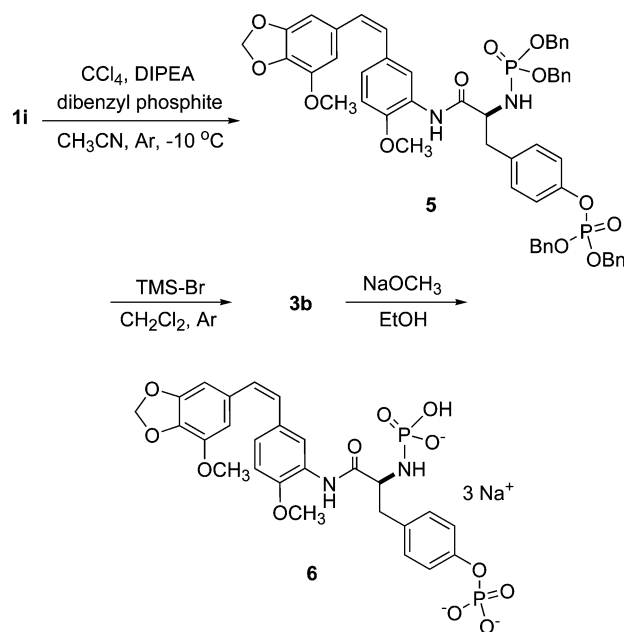
Further structure–activity relationship (SAR) exploration of 3,4-methylenedioxy-5,4'-dimethoxy-3'-amino-Z-stilbene (**1a**) derivatives resulted in the efficient synthesis of tyrosine amide hydrochloride **9**, two tyrosine amide phosphate prodrugs (**3a** and **6**), and sodium aspartate amide **11**. Two additional cancer cell growth inhibitors (**14** and **16**) were synthesized by employing peptide coupling between amine **1a** and the Dap unit of dolastatin 10 (**4a**) to yield amide **14** followed by Dov-Val-Dil (**15**) to yield peptide **16**. The latter represents a combination of stilbene **1a** with the des-Doe tetrapeptide unit of the powerful tubulin assembly inhibitor dolastatin 10. Peptide **16** was examined for potential binding to tubulin in the vinca and/or colchicine regions and found to perform primarily as a relative of dolastatin 10. Amide **14** had anticryptococcal and antibacterial activities.

We recently completed syntheses of 3'-amino derivatives (**1a,b**, **1d–j**) of combretastatin A-2 (**2c**),¹ encouraged by the remarkable success of the potent cancer vascular targeting^{2–4} that occurs with combretastatin A-4 phosphate prodrug (CA4P) **2h**.^{5–10} Subsequently, we undertook the synthesis and initial anticancer evaluation of two phosphate derivatives (**3a,b**) of tyrosine stilbene amide **1i**. Attachment of the phosphate group at the 3'-phenol position has been investigated in detail in the combretastatin A and B series.^{1,11–13} In the present study we chose to use the 4''-hydroxyl group of tyrosine amide **1i** for attachment of a phosphate group (**3a**) as well as obtaining a diphosphoryl derivative (**3b**) by phosphorylating tyrosine amide **1i** at both the amine¹⁴ and hydroxyl groups. A parallel important objective of this research involved employing a tetrapeptide segment of dolastatin 10 (**4a**) for bonding to the 3'-amino group (**1b**). Dolastatin 10 (D-10, **4a**), isolated^{15–17} from the Indian Ocean sea hare *Dolabella auricularia*, has continued to progress through preclinical¹⁸ and clinical development as an impressive antineoplastic agent.^{19–22} Dolastatin 10 (**4a**) and various synthetic derivatives also have specific antifungal activity against *Cryptococcus neoformans*.^{23,24} Previous SAR studies have confirmed that D-10 (**4a**) inhibits microtubule assembly by binding to the β -tubulin subunit near the vinca site. SAR studies have shown the des-Doe tetrapeptide unit (**4b**, Dov-Val-Dil-Dap) to be necessary for potent anticancer activity.^{25,26} We now also report synthesis of the amide representing coupling of amine **1a** to Dov-Val-Dil-Dap (**4b**).

Results and Discussion

Our synthesis of the tyrosine stilbene amide **1i**²⁷ presented the opportunity to develop two additional phosphate prodrugs in the combretastatin series. Accordingly, the 4''-phenol and α -amino groups were phosphorylated in one step using dibenzyl phosphite (**1i** $\rightarrow$ **5**) (Scheme 1). Debonylation of the resulting phosphate diesters (**5**) was achieved

Scheme 1



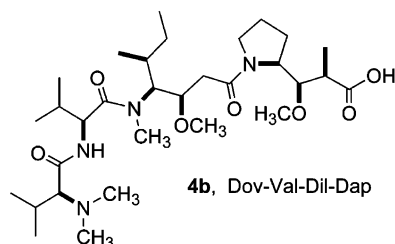
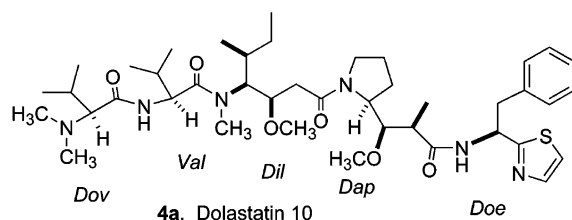
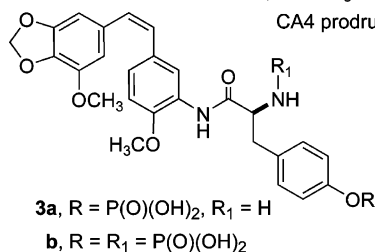
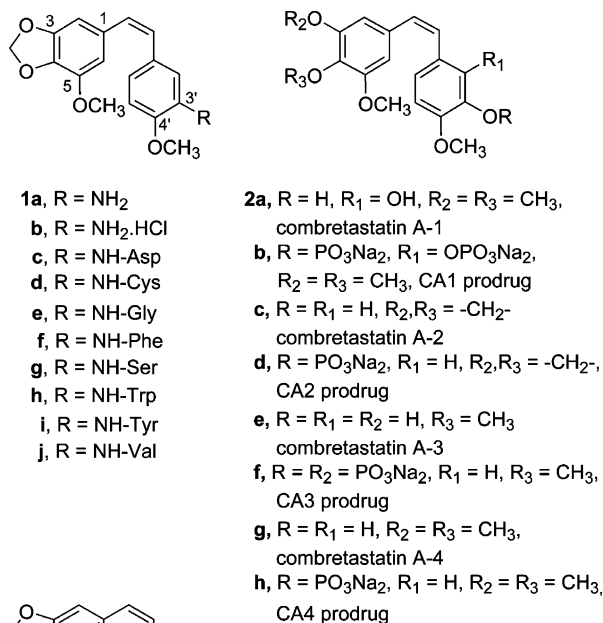
using bromotrimethylsilane to afford the free acid (**3b**), which was subsequently converted to sodium salt **6** using sodium methoxide in methanol.^{11,12} Phosphorylation solely at the 4''-hydroxyl position was accomplished via the coupling of the 3'-amino stilbene **1a** to *O*-*tert*-butyl-*N* $^\alpha$ -Z-L-Tyr using PyBroP as the coupling reagent to provide amide **7** (Scheme 2).²⁸ *tert*-Butyl removal using TFA in DCM followed by phosphorylation afforded the dibenzyl ester **8**. Debonylation at the ester and benzyloxycarbonyl removal at the amine were achieved simultaneously with the in situ generation of trimethylsilyl iodide (TMSI) from the reaction of sodium iodide and chlorotrimethylsilane in acetonitrile.^{29,30} This method resulted in the immediate precipitation of the phosphate prodrug **3a**. Surprisingly, both phosphoric acids and the corresponding sodium salts proved to be sparingly soluble in water. In retrospect, the physical properties of **6** might correspond better to a betaine structure arising from enolization of the amide group in **3b** with a concurrent shift of the adjacent

* To whom correspondence should be addressed. Phone: 480-965-3351. Fax: 480-965-8558. E-mail: bpettit@asu.edu.

[†] Arizona State University.

[‡] Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick.

[§] SAIC-Frederick, Inc.



phosphate to give a phosphate ester (see Figure 1). This structure would account for the trisodium salt formation to give **6** and the lower aqueous solubility.

We further investigated the possibility of making a soluble amide derivative first by making the hydrochloride salt of **1i** (Scheme 3) and second by coupling amine **1a** to aspartic acid and converting to the β -aspartate sodium salt **11** (Scheme 4). Amide formation was accomplished using PyBroP followed by removal of the β -tert-butyl protecting group in the presence of triethylsilane.³¹ The resulting carboxylic acid was subsequently treated with sodium methoxide in methanol, yielding sodium salt **11**. Both the hydrochloride salt **9** and the sodium salt **11** were essentially insoluble (<0.1 mg/mL) in water. Although we found earlier that the combretastatin A-2 phosphate prodrug (**2d**) has reduced aqueous solubility when compared to its combretastatin A-1 (**2b**) and A-3 counterparts (**2f**),^{11,12} we were surprised to find that our new CA2 modifications

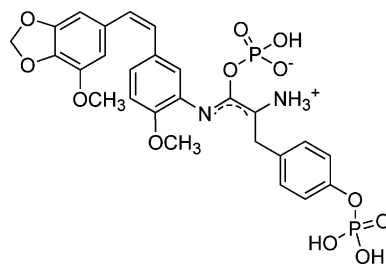
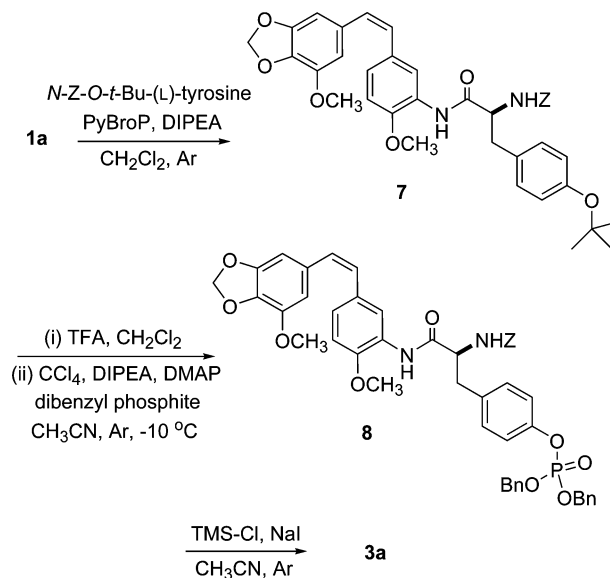
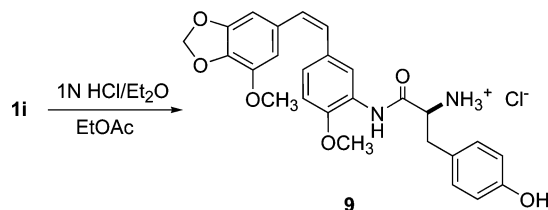


Figure 1.

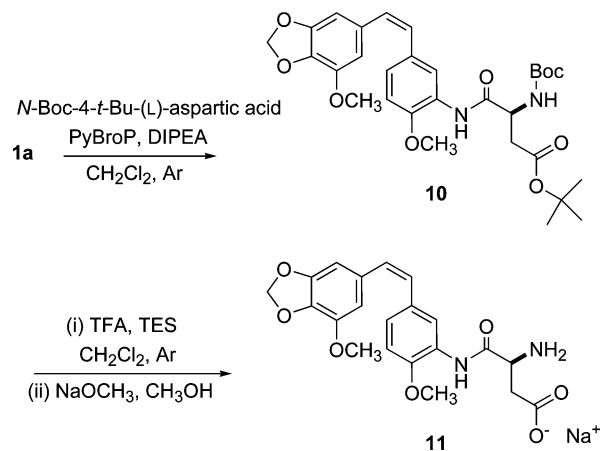
Scheme 2



Scheme 3



Scheme 4



(**1b**, **3a**, **6**, **9**, and **11**) exhibited only sparing water solubility, presumably owing to the hydrophobicity created by the stilbene. Interestingly, addition of the phosphate group to the tyrosine amide **1i** to yield **3a** resulted in decreased anticancer activity (Table 1). Since the cancer cell line results obtained for each of the substances recorded in Table 1 were obtained in solution, presumably the sparing

Table 1. Human Cancer Cell Line GI₅₀ (μg/mL) and Murine P388 Lymphocytic Leukemia Cell Line Inhibitory Activity ED₅₀ (μg/mL) of the 3'-Substituted Stilbenes

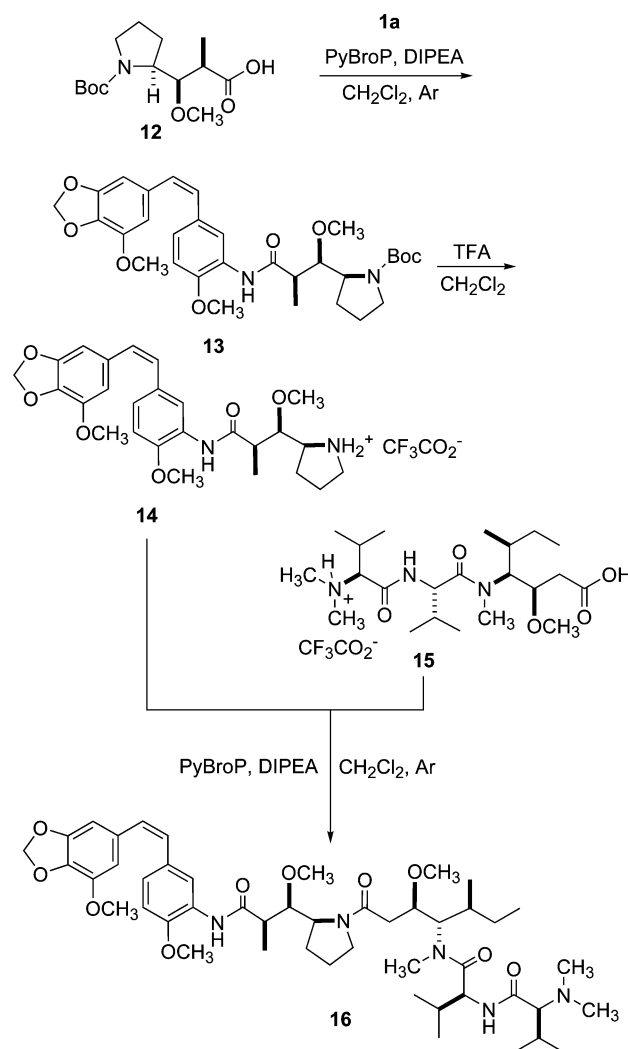
compound	leukemia P388	pancreas BXPC-3	breast MCF-7	CNS SF-295	lung-NSC NCI-H460	colon KM20L2	prostate DU145	mean
1a	0.00189	0.0071	0.0047	0.023	0.0050	0.022	0.028	0.013
3a	>10	>1	>1	>1	>1	>1	>1	
5	>10							
6	0.270	18.6	1.3	>1	>1	>1	>1	
7	0.380	0.049	0.36	0.44	0.41	1.8	0.39	0.61
8	0.133	>10	>10	>10	>10	>10	>10	
9	0.0329	0.064	0.041	0.064	0.096	0.034	0.080	0.059
10	0.385	3.4	2.4	1.7	3.8	2.3	3.0	2.4
11	0.361	0.38	0.51	0.25	0.30	0.19	0.33	0.33
13	0.225	0.58	0.53	0.75	0.52	1.9	0.38	0.70
14	0.0263	0.23	0.054	0.062	0.083	0.26	0.070	0.11
16	<0.01	0.048	0.0031	0.025	0.033	0.020	0.032	

water solubility did not significantly influence the cell line results. However, the reduced ability to hydrogen bond in vitro could be important.

The des-Doe tetrapeptide unit (**4b**) of the potent anticancer drug dolastatin 10 (D-10, **4a**) isolated in 1984¹⁵ was chosen for coupling with amine **1a**. By replacing the Doe portion of D-10 with the amine **1a**, we planned to synthesize a stilbene peptide with the possibility of binding to the colchicine and/or vinca regions of tubulin. The tripeptide Dov-Val-Dil TFA salt (**15**) and Boc-Dap (**12**) were obtained via published methods.^{32,33} PyBroP coupling of amine **1a** to Boc-Dap (**12**) followed by Boc deprotection with TFA in DCM afforded compound **14** (Scheme 5). Further coupling resulted in des-Doe D-10 stilbene amide **16**. The cancer cell inhibition activity of the potentially dual functioning anticancer agent **16** was notable (Table 1), as was the broad spectrum antimicrobial action of amide **14**. The latter substance (**14**) inhibited the growth of the pathogenic fungus *Cryptococcus neoformans* ATCC 90112 (MIC = 64 μg/mL), the pathogenic bacterium *Neisseria gonorrhoeae* ATCC 49226 (MIC = 16 μg/mL), and the opportunistic bacteria *Streptococcus pneumoniae* ATCC 6303 (MIC = 8–16 μg/mL) and *Enterococcus faecalis* ATCC 29212 (MIC = 32–64 μg/mL). Prodrug **3a** inhibited *N. gonorrhoeae* (MIC = 0.125 μg/mL) and *E. faecalis* (MIC = 16 μg/mL), and prodrug **6** inhibited *N. gonorrhoeae* (MIC = 4 μg/mL). Stilbenes **8** and **13** inhibited *E. faecalis* (MIC = 64 μg/mL) or *C. neoformans* (MIC = 64 μg/mL), respectively.

Previous work showed that D-10 (**4a**) is an exceptionally cytotoxic antimitotic agent and that it inhibits tubulin assembly by binding tightly in the vinca domain of tubulin (its inhibition of vinblastine binding to tubulin, although extensive, is noncompetitive).^{34,35} In contrast, while combretastatin A-4 (**2g**) is quite potent for a colchicine site drug, it is about 100-fold less cytotoxic than D-10 (**4a**), and the stilbene inhibits tubulin assembly by binding avidly to the colchicine site in a competitive fashion.^{36,37} These findings were confirmed in the studies presented in Table 2. Note the similar inhibitory effects of **4a** and **2g** on tubulin assembly, their contrasting effects on inhibition of binding of radiolabeled vinblastine and colchicine, and the greater than 100-fold enhanced inhibition of growth of the MCF-7 cells by **4a** relative to **2g**.

Previous SAR studies with D-10²⁴ (**4a**) structural modifications had shown that loss of its C-terminal unit Doe had a significant effect on cell growth and a greater effect on inhibition of ligand binding to tubulin than on inhibition of tubulin assembly. Thus, Dov-Val-Dil-Dap (**4b**) is only 2-fold less active than **4a** as an assembly inhibitor, but at least 6-fold less active as an inhibitor of MCF-7 cell growth (Table 2). These SAR studies had also shown that replace-

Scheme 5

ment of the Doe residue with a variety of aromatic derivatives could restore some or all of the activity of D-10 (**4a**) in both the cytological and biochemical assays.^{23,24}

SAR studies with structural manipulation of combretastatin A4^{27,36} (**2g**) revealed that relatively minor effects on cytotoxicity and tubulin interactions were observed when a methylenedioxy bridge replaced two vicinal methoxy groups in the A ring or an amino group replaced the hydroxyl group in the B ring. These findings are demonstrated again in Table 2, where amine hydrochloride **1b**, incorporating both these changes, is compared with CA4 **2g**. In addition, we found that a variety of amino acid amides formed from amine **1a** had sharply reduced activity

Table 2. Effects of Peptide **16** and Related Compounds on Tubulin Assembly, Binding of Colchicine and Vinblastine to Tubulin, and the Growth and Mitotic Index of MCF-7 Breast Cancer Cells

compound	inhibition of tubulin assembly IC ₅₀ (μM) ± SD ^a	inhibition of colchicine binding % inhibition ± SD ^a		inhibition of vinblastine binding % inhibition ± SD ^a		inhibition of growth of MCF-7 breast cancer cells IC ₅₀ (nM) ± SD ^a	mitotic index of MCF-7 cells % mitoses ^b
		2 μM ^c	50 μM ^c	10 μM ^c	80 μM ^c		
16	1.3 ± 0.04		30 ± 2	48 ± 3	87 ± 2	17 ± 7	57
Dov-Val-Dil-Dap (4b)	1.4 ± 0.03		5.2 ± 3	16 ± 6	68 ± 0.4	36 ± 10	56
1b	3.4 ± 0.5 ^d	85 ± 3		0	0	68 ± 30	65
dolastatin 10 (4a)	0.70 ± 0.06	13 ± 5		96 ± 0.7		0.083 ± 0.06	39
4b + 1b ^e						14 ± 2	52
combreastatin A-4 (2g)	1.8 ± 0.3	98 ± 0.4		0	0	11 ± 10	46

^a SD, standard deviation. ^b The mitotic indices were determined following growth of the cells for 16 h at 10 times the IC₅₀ concentration. Without drug, 2.9% of the cells were in mitosis. ^c Inhibitor concentrations. ^d Compound **1a** had a nearly identical IC₅₀ value (4.3 ± 0.5 μM). ^e Equimolar concentrations of compounds **4b** and **1b** were present in all cell culture mixtures.

in the tubulin-based biochemical assays but retained the amine's cytotoxic activity.²⁷ Since these compounds caused a marked increase in the mitotic index of drug-treated cells, the most reasonable explanation was that the amine **1a** was regenerated by either extracellular or intracellular hydrolysis of the amides.

When the dolastatin 10-combreastatin amine hybrid **16** was evaluated, it was found to be active in all the biochemical assays (Table 2), although its effect on the binding of [³H]colchicine was relatively weak, concordant with the weak activities observed previously with the amino acid amides of **1a**.²⁷ In contrast, peptide **16** was about half as active as D-10 (**4a**) as an inhibitor of both tubulin assembly and [³H]vinblastine binding to tubulin. We therefore conclude that at the biochemical level peptide **16** is acting primarily as a D-10 (**4a**) analogue.

The cytotoxic properties of peptide **16**, however, are more difficult to unravel. Using MCF-7 cells for a detailed comparison, we found that peptide **16** was about twice as active as Dov-Val-Dil-Dap (**4b**) and 4 times as active as hydrochloride **1b**, but only 1/200th as active as D-10 (**4a**). Further, when **4b** and **1b** were mixed in equimolar amounts, as would occur if **16** were completely hydrolyzed, an IC₅₀ value for inhibition of the growth of the MCF-7 cells was obtained that was almost identical to that obtained with peptide **16** (14 vs 17 nM). Since the IC₅₀ values obtained for **16**, **4b**, and **1b** are in the same range, it is impossible to determine whether the cytotoxicity of **16** derives from its acting primarily as a relatively weak D-10 (**4a**) analogue or primarily as a relatively potent combreastatin A-4 (**2g**) analogue. The similarity in IC₅₀ values obtained with **16** and with the mixture of its components **4b** and **1b** indicates that it, like the previously studied amides,²⁷ undergoes extracellular or intracellular hydrolysis. Finally, the similarity in IC₅₀ values for **16**, **4b**, **1b**, and the **4b** + **1b** mixture indicates that there is little, if any, synergistic in vitro cytotoxic effect obtained from having **4b** and **1b** conjoined in a single molecule.

In conclusion, although initial attempts to synthesize a very water soluble derivative from the 3,4-methylenedioxy-5,4'-dimethoxy-3'-amido-Z-stilbene tyrosine amide and aspartate amide series proved unrewarding and will require a different approach, three new stilbene amides (**9**, **14**, and **16**) strongly inhibiting a minipanel of human cancer cell lines were discovered. Most importantly, the preliminary biological results from the potentially multitargeting compounds (amide **14** and peptide **16**) merit further investigation.

Experimental Section

Materials and Methods. Ether refers to diethyl ether and Ar to argon gas. Bromotrispyrrolidinophosphonium hexafluorophosphate (PyBroP), *O*^β-*tert*-butyl-*N*^α-Boc-L-aspartic acid,

and *O*-*tert*-butyl-*N*^α-Z-L-tyrosine were obtained from Calbiochem-Novabiochem Corporation (San Diego, CA). Diisopropylethylamine (DIPEA), anhydrous methanol, sodium methoxide, triethylsilane (TES), and trifluoroacetic acid (TFA) were obtained from Acros Organics (Fisher Scientific, Pittsburgh, PA). All other reagents were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI).

Reactions were monitored by TLC using Analtech silica gel GHLF Uniplates visualized under short-wave UV irradiation. Solvent extracts of aqueous solutions were dried over anhydrous MgSO₄ or Na₂SO₄. Where appropriate, the crude products were separated by column chromatography on flash (230–400 mesh ASTM) silica from E. Merck, gravity (70–230 mesh ASTM) silica from E. Merck, or Sephadex LH-20.

Melting points are uncorrected and were determined employing an Electrothermal 9100 apparatus. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. The [α]_D values are given in 10⁻¹ deg cm² g⁻¹. The ¹H and ¹³C NMR spectra were recorded employing Varian Gemini 300, Varian Unity 400, or Varian Unity 500 instruments using a deuterated solvent and were referenced to either TMS or the solvent. HRMS data were recorded with a JEOL LCmate mass spectrometer. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-[O,*N*^α-di(bis-benzylphosphoryl)-L-Tyr]-amido-Z-stilbene (5). To a stirred solution of amine **1i**²⁷ (71 mg, 0.15 mmol) in acetonitrile (1 mL) at -10 °C in an acetone/ice bath, under Ar, was added CCl₄ (0.15 mL, 1.6 mmol, 11 equiv). Ten minutes later DIPEA (0.12 mL, 0.66 mmol, 4.4 equiv) and 4-(dimethylamino)pyridine (3.8 mg, 0.031 mmol, 0.21 equiv) were added, followed 1 min later by the dropwise addition of dibenzyl phosphite (0.11 mL, 0.47 mmol, 3.1 equiv). After 1 h, the reaction was warmed to room temperature and aqueous 0.5 M KH₂PO₄ was added. The mixture was extracted with EtOAc (3 × 10 mL), and the combined extracts were washed with brine (15 mL) and H₂O (15 mL). Upon drying and condensing in vacuo, the product was separated by gravity column chromatography (4:1, DCM/EtOAc) to afford a colorless oil **5** (97 mg, 65%): *R*_f 0.12 (4:1, DCM/EtOAc); [α]_D²⁵ -45.1° (*c* 0.72, CHCl₃); P NMR (400 MHz, CDCl₃) δ 5.693, -8.113; ¹H NMR (400 MHz, CDCl₃) δ 2.89 (1H, m, -CH₂-), 3.18 (1H, m, -CH₂-), 3.56 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 4.05 (1H, m, α H), 4.96 (8H, m, 4 × -CH₂-), 5.87 (2H, s, -OCH₂O-), 6.39 (1H, d, *J* = 12.0 Hz, vinyl H), 6.45 (3H, m, vinyl H, 2 × ArH), 6.59 (1H, d, *J* = 8.4 Hz, ArH), 6.97 (1H, dd, *J* = 8.4, 1.6 Hz, ArH), 6.99 (2H, d, *J* = 8.4 Hz, 2 × ArH), 7.03 (2H, d, *J* = 8.8 Hz, 2 × ArH), 7.29 (20 H, m, 20 × ArH), 8.27 (1H, d, *J* = 1.6, ArH), 8.36 (1H, s); HRMS calcd for C₅₄H₅₃N₂O₁₂P₂ [M + H]⁺ 983.3074, found 983.3115; *anal.* calcd for C₅₄H₅₂N₂O₁₂P₂, C, 65.98; H, 5.33; N, 2.85; found, C, 65.51; H, 5.55; N, 2.84.

Sodium 3,4-Methylenedioxy-5,4'-dimethoxy-3'-(L-Tyr)-amido-Z-stilbene 3'-O,*N*^α-Diphosphate (6). To a stirred solution of benzyl phosphite **5** (76 mg, 0.077 mmol) in DCM (1 mL) at 0 °C, under Ar, was added bromotrimethylsilane (35 μL, 0.33 mmol, 4.3 equiv). The reaction mixture was stirred for 30 min and concentrated under vacuum. The residue was

dissolved in EtOH (1 mL), and NaOMe (20 mg, 0.36 mmol, 4.7 equiv) was added, yielding a precipitate that was collected and washed with EtOAc and ether. The product (**6**) was obtained as a colorless powder (52 mg, 95%); mp (dec) 188–190 °C; $[\alpha]_D^{24}$ –30.6° (*c* 0.72, DMSO); P NMR (400 MHz, DMSO) δ 6.262, 0.585; ^{13}C NMR (300 MHz, DMSO) δ 143.8, 132.3, 130.4, 130.2, 129.7, 129.4, 127.4, 127.2, 126.3, 122.1, 119.7, 116.5, 115.1, 111.0, 106.8, 101.2, 99.5, 56.6, 56.2, 55.9; ^1H NMR (400 MHz, DMSO) δ 2.63 (1H, m, $-\text{CH}_2-$), 3.10 (1H, m, $-\text{CH}_2-$), 3.30 (1H, d, J = 8.8 Hz), 3.56 (1H, m), 3.86 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 6.00 (2H, s, $-\text{OCH}_2\text{O}-$), 6.55 (1H, d, J = 0.8 Hz, ArH), 6.99 (8H, m, 2 $\times$ vinyl H, 6 $\times$ ArH), 7.11 (1H, d, J = 8.4 Hz, ArH), 7.23 (1H, dd, J = 6.8, 1.6 Hz, ArH), 10.12 (1H, br s); *anal.* calcd for C₂₆H₂₅N₂Na₃O₁₂P₂·3H₂O, C, 42.06; H, 4.21; N, 3.77; found, C, 41.68; H, 4.65; N, 3.45.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*O*-*tert*-butyl-*N*^α-Z-L-Tyr)-amido-Z-stilbene (7). To a stirred mixture of amine **1a** (0.19 g, 0.63 mmol), *O*-*tert*-butyl-*N*^α-Z-L-Tyr (0.45 g, 1.2 mmol, 1.9 equiv), and PyBroP (0.57 g, 1.2 mmol, 1.9 equiv) in DCM (2 mL) at 0 °C under Ar was added DIPEA (0.55 mL, 3.2 mmol, 5.1 equiv). The reaction mixture was stirred for 45 min at room temperature and concentrated under vacuum. The product was obtained by gravity column chromatography (4:1, DCM/EtOAc) as a colorless oil (**7**, 0.38 g, 93%); *R*_f 0.74 (4:1, DCM/EtOAc); $[\alpha]_D^{24}$ +1.9° (*c* 1.04, CHCl₃); ^1H NMR (300 MHz, CDCl₃) δ 1.30 (9H, s, *t*Bu), 3.05 (2H, m, $-\text{CH}_2-$), 3.63 (3H, s, OCH₃), 3.68 (3H, s, OCH₃), 4.47 (1H, m, alpha H), 5.05 (2H, d, J = 2.4 Hz, $-\text{CH}_2-$), 5.60 (1H, br), 5.85 (2H, s, $-\text{OCH}_2\text{O}-$), 6.33 (1H, d, J = 12.0 Hz, vinyl H), 6.40 (3H, m, vinyl H, 2 $\times$ ArH), 6.59 (1H, d, J = 9.0 Hz, ArH), 6.83 (2H, m, ArH), 6.91 (1H, dd, J = 8.1, 1.5 Hz, ArH), 7.05 (2H, d, J = 8.4 Hz, 2 $\times$ ArH), 7.26 (5H, m, 5 $\times$ ArH), 7.92 (1H, s), 8.22 (1H, d, J = 2.1 Hz, ArH); HRMS calcd for C₃₈H₄₁O₈N₂ [M + H]⁺ 653.2863, found 653.2876; *anal.* calcd for C₃₈H₄₀N₂O₈, C, 69.92; H, 6.18; N, 4.29; found, C, 69.45; H, 6.37; N, 4.21.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*O*-bisbenzylphosphoryl-*N*^α-Z-L-Tyr)-amido-Z-stilbene (8). To a stirred solution of amide **7** (0.26 g, 0.40 mmol) in DCM (2 mL) was added TFA (2 mL). The reaction mixture was stirred for 20 min, the solution was concentrated under vacuum, and the free phenol obtained by gravity column chromatography (4:1, DCM/EtOAc) was placed in acetonitrile (4 mL) under Ar. The mixture was cooled to –10 °C, and CCl₄ (0.21 mL, 2.1 mmol, 5.3 equiv) was added. Ten minutes later DIPEA (0.16 mL, 0.89 mmol, 2.2 equiv) and 4-(dimethylamino)pyridine (5 mg, 0.041 mmol, 0.10 equiv) were added, followed 1 min later by the dropwise addition of dibenzyl phosphite (0.15 mL, 0.65 mmol, 1.6 equiv). After 2 h the reaction mixture was warmed to room temperature, and aqueous 0.5 M KH₂PO₄ (16 mL) added. The same procedure given for phosphate **5** was followed to obtain the product **8** as a colorless oil (0.24 g, 71%); *R*_f 0.60 (4:1, DCM/EtOAc); $[\alpha]_D^{23}$ –5.1° (*c* 0.25, CHCl₃); P NMR (400 MHz, CDCl₃) δ –8.095; ^1H NMR (MHz, CDCl₃) δ 3.08 (2H, m, $-\text{CH}_2-$), 3.66 (1H, s, OCH₃), 3.72 (1H, s, OCH₃), 4.51 (1H, m, alpha H), 5.08 (2H, d, J = 0.9 Hz, $-\text{CH}_2-$), 5.11 (4H, m, 2 $\times$ $-\text{CH}_2-$), 5.44 (1H, br d, J = 7.2 Hz), 5.90 (2H, s, $-\text{OCH}_2\text{O}-$), 6.39 (1H, d, J = 12.0 Hz, vinyl H), 6.46 (3H, m, vinyl H, 2 $\times$ ArH), 6.62 (1H, d, J = 8.1 Hz, ArH), 6.98 (1H, dd, J = 8.4, 1.8 Hz, ArH), 7.04 (2H, d, J = 8.1 Hz, 2 $\times$ ArH), 7.14 (2H, d, J = 8.1 Hz, 2 $\times$ ArH), 7.31 (15H, m, 15 $\times$ ArH), 7.90 (1H, s), 8.24 (1H, d, J = 2.4 Hz, ArH); HRMS calcd for C₄₈H₄₆N₂O₁₁P [M + H]⁺ 857.2840, found 857.2853; *anal.* calcd for C₄₈H₄₅N₂O₁₁P, C, 67.28; H, 5.29; N, 3.27; found, C, 66.89; H, 5.27; N, 3.17.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*O*-phosphoryl-*N*^α-L-Tyr)-amido-Z-stilbene (3a). To a stirred solution of benzyl ester **8** (98 mg, 0.11 mmol) in acetonitrile under Ar was added sodium iodide (60 mg, 0.40 mmol, 3.6 equiv), followed by chlorotrimethylsilane (51 μL , 0.41 mmol, 3.7 equiv). A white precipitate formed while the reaction mixture was stirred for 20 min. Aqueous (1%) sodium thiosulfate (0.5 mL) was added to the mixture before the precipitate was collected and washed with ethyl acetate, H₂O, and acetone. The product was obtained as a colorless amorphous solid (**3a**, 35 mg, 58%); mp (dec) 175–177 °C; $[\alpha]_D^{25}$ –6.3° (*c* 0.35, DMSO); P NMR (400 MHz, DMSO) δ –2.043; ^{13}C NMR (400 MHz, DMSO) δ 170.6,

156.1, 149.0, 148.8, 143.3, 136.9, 134.2, 132.3, 129.7, 129.6, 128.9, 128.3, 127.7, 127.2, 127.0, 126.4, 122.8, 119.5, 118.9, 111.3, 106.8, 101.2, 99.5, 65.4, 57.0, 56.2, 36.4; ^1H NMR (400 MHz, DMSO) δ 2.78 (2H, m, $-\text{CH}_2-$), 3.06 (1H, m, $-\text{CH}_2-$), 3.82 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 4.49 (1H, m, alpha H), 5.98 (2H, s, $-\text{OCH}_2\text{O}-$), 6.89 (1H, d, J = 12.8 Hz, vinyl H), 6.94 (1H, s, ArH), 7.07 (2H, m, vinyl H, ArH), 7.22 (1H, d, J = 6.4 Hz, ArH), 7.28 (2H, d, J = 5.6, 2 $\times$ ArH), 7.32 (2H, d, J = 5.6, 2 $\times$ ArH), 7.74 (1H, d, J = 6.4, ArH), 8.22 (1H, s), 9.25 (1H, s).

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*N*^α-L-Tyr)-amido-Z-stilbene Hydrochloride (9). To a stirred solution of amine **1i** (27 mg, mmol) in ethyl acetate (1 mL) was added ethereal HCl (1 M) in excess. A white solid immediately formed, the solvent was removed in vacuo, and the resulting solid was recrystallized from ethanol/ethyl acetate to yield an off-white powder (**9**, 29 mg, quantitative); mp 169–170.5 °C; $[\alpha]_D^{25}$ 82.5° (*c* 0.73, CH₃OH); ^1H NMR (300 MHz, CDCl₃) δ 3.07 (2H, m, $-\text{CH}_2-$), 3.70 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 4.27 (1H, m, alpha H), 5.93 (2H, s, $-\text{OCH}_2\text{O}-$), 6.37 (1H, d, J = 1.2 Hz, ArH), 6.42 (1H, d, J = 12.0 Hz, vinyl H), 6.46 (1H, d, J = 12.6 Hz, vinyl H), 6.49 (1H, d, J = 1.2 Hz, ArH), 6.75 (2H, d, J = 8.4 Hz, 2 $\times$ ArH), 6.90 (1H, d, J = 8.1 Hz, ArH), 7.03 (1H, dd, J = 8.1, 2.1 Hz, ArH), 7.09 (2H, d, J = 8.7 Hz, 2 $\times$ ArH), 7.92 (1H, d, J = 1.5 Hz, ArH); *anal.* calcd for C₂₆H₂₆ClN₂O₆, C, 59.32; H, 5.75; N, 5.33; found, C, 59.53; H, 5.56; N, 5.26.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*O*^β-*tert*-butyl-*N*^α-Boc-L-Asp)-amido-Z-stilbene (10). To a stirred mixture of amine **1a** (0.13 g, 0.43 mmol), *O*^β-*tert*-butyl-*N*^α-Boc-L-Asp (0.22 g, 0.76 mmol, 1.8 equiv), and PyBroP (0.35 g, 0.76 mmol, 1.8 equiv) in DCM (3 mL) at 0 °C under Ar was added DIPEA (0.21 mL, 1.2 mmol, 2.8 equiv). The reaction mixture was stirred for 75 min at room temperature, and the solvent was removed in vacuo. The product was obtained by flash column chromatography (1:1, *n*-hexane/acetone) as an oil (**10**, 0.22 g, 88%); *R*_f 0.64 (1:1, *n*-hexane/acetone); $[\alpha]_D^{24}$ –13.0° (*c* 1.42, CHCl₃); ^1H NMR (300 MHz, CDCl₃) δ 1.44 (9H, s, *t*Bu), 1.49 (9H, s, *t*Bu), 2.88 (2H, m, $-\text{CH}_2-$), 3.73 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 4.59 (1H, m, alpha H), 5.81 (1H, br), 5.92 (2H, s, $-\text{OCH}_2\text{O}-$), 6.38 (1H, d, J = 12.6 Hz, vinyl H), 6.44 (1H, s, ArH), 6.46 (1H, d, J = 12.6 Hz, vinyl H), 6.47 (1H, s, ArH), 6.70 (1H, d, J = 8.1 Hz, ArH), 6.97 (1H, dd, J = 8.4, 1.8 Hz, ArH), 8.28 (1H, d, J = 2.4 Hz, ArH), 8.76 (1H, s); HRMS calcd for C₃₀H₃₉N₂O₉ [M + H]⁺ 571.2655, found 571.2617; *anal.* calcd for C₃₀H₃₈N₂O₉, C, 63.14; H, 6.71; N, 4.91; found, C, 62.64; H, 7.00; N, 4.89.

Sodium 3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*N*^α-L-Asp)-amido-Z-stilbene (11). To *tert*-butyl ester **10** (0.13 g, 0.23 mmol) in DCM (1.5 mL) was added a mixture of TFA (0.71 mL, 9.6 mmol, 42 equiv) and TES (0.30 mL, 1.8 mmol, 7.8 equiv), and stirring was continued for 4 h under Ar. The solvents were removed in vacuo, and the TFA salt, obtained by Sephadex LH-20 column chromatography (solvent, MeOH), was placed in methanol (1 mL). Sodium methoxide (0.22 mg, 0.41 mmol, 1.8 equiv) was added to the reaction mixture, and the white precipitate that formed was collected and reprecipitated from DCM/CH₃OH to give the product **11** as a colorless solid (62 mg, 62%); mp 168–170 °C; $[\alpha]_D^{25}$ +14.0° (*c* 1.18, CH₃OH); ^1H NMR (300 MHz, CD₃OD) δ 2.62 (1H, m, $-\text{CH}_2-$), 2.77 (1H, m, $-\text{CH}_2-$), 2.78 (2H, br), 3.69 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 4.29 (1H, m, alpha H), 5.87 (2H, s, $-\text{OCH}_2\text{O}-$) 6.36 (1H, d, J = 1.2 Hz, ArH), 6.40 (1H, d, J = 12.0 Hz, vinyl H), 6.45 (1H, d, J = 12.3 Hz, vinyl H), 6.50 (1H, d, J = 0.9 Hz, ArH), 6.91 (1H, d, J = 8.4 Hz, ArH), 7.02 (1H, dd, J = 8.4, 1.8 Hz, ArH), 7.99 (1H, d, J = 2.4 Hz, ArH), 8.52 (1H, s); *anal.* calcd for C₂₁H₂₁N₂NaO₇·H₂O, C, 55.51; H, 5.10; N, 6.16; found, C, 55.81; H, 5.70; N, 6.16.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*N*^α-Boc-L-Dap)-amido-Z-stilbene (13). To a stirred mixture of amine **1a** (51 mg, 0.17 mmol), *N*^α-Boc-L-Dap **12** (52 mg, 0.18 mmol, 1.1 equiv), ^{32,33} and PyBroP (87 mg, 0.19 mmol, 1.1 equiv) at 0 °C under Ar was added DIPEA (65 μL , 0.37 mmol, 2.2 equiv). The reaction mixture was stirred for 1.5 h at room temperature. DCM (10 mL) was added, and the mixture was washed with aqueous citric acid (10% by wt, 10 mL). The organic layer was

dried with MgSO_4 and concentrated in vacuo, and the residue was subjected to gravity column chromatography (8:1, DCM/EtOAc), resulting in the product **13** as a colorless oil (40 mg, 41%): R_f 0.24 (8:1, DCM/EtOAc); $[\alpha]_D^{24}$ -48.5° (c 1.2, CHCl_3); ^{13}C NMR (500 MHz, CDCl_3) δ 148.5, 146.9, 143.3, 134.2, 131.9, 130.1, 129.5, 128.8, 127.6, 123.8, 120.8, 109.6, 108.5, 103.0, 101.3, 58.7, 56.3, 55.8, 28.5; ^1H NMR (300 MHz, CDCl_3) δ 1.30 (3H, d, J = 6.9 Hz, CH_3), 1.46 (9H, s, Boc), 1.72 (1H, m, Pro), 1.90 (3H, m, Pro), 2.62 (1H, m), 3.23 (1H, m), 3.44 (1H, m), 3.49 (3H, s, OCH_3), 3.58 (1H, m), 3.74 (3H, s, OCH_3) 3.85 (3H, s, OCH_3), 3.90 (1H, m), 5.92 (2H, s, $-\text{OCH}_2-$), 6.38 (1H, d, J = 12.3 Hz, vinyl H), 6.45 (1H, s, ArH), 6.46 (1H, d, J = 12.0 Hz, vinyl H), 6.47 (1H, s, ArH), 6.69 (1H, d, J = 8.7, ArH), 6.96 (1H, dd, J = 8.4, 1.8 Hz, ArH), 8.30 (1H, d, J = 2.4, ArH), 8.41 (1H, br); HRMS calcd for $\text{C}_{31}\text{H}_{41}\text{N}_2\text{O}_8$ $[\text{M} + \text{H}]^+$ 569.2863, found 569.2884; *anal.* calcd for $\text{C}_{31}\text{H}_{40}\text{N}_2\text{O}_8$, C, 65.48; H, 7.09; N, 4.93; found, C, 64.92; H, 7.42; N, 4.97.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(*N*^α-L-Dap)-amido-Z-stilbene TFA Salt (14). To a stirred solution of amide **13** (0.15 g, 0.26 mmol) in DCM (0.65 mL) at 0°C was added TFA (0.20 mL, 2.6 mmol, 10 equiv). The reaction mixture was stirred for 20 min at room temperature. The product was obtained by gravity column chromatography (20:1, DCM/MeOH) as an oil (**14**, 69 mg, 46%): R_f 0.54 (8:1, DCM/MeOH); $[\alpha]_D^{24}$ -61.9° (c 0.88, CHCl_3); ^{13}C NMR (300 MHz, CDCl_3) δ 170.5, 148.1, 146.9, 142.8, 131.3, 129.6, 128.6, 126.3, 124.3, 120.5, 109.3, 108.1, 102.4, 100.8, 100.3, 80.2, 60.5, 58.6, 55.8, 55.3, 44.8, 41.5, 24.1, 23.4, 12.1; ^1H NMR (300 MHz, CDCl_3) δ 1.27 (3H, d, J = 6.9, CH_3), 1.98 (4H, m), 2.94 (1H, m), 3.33 (2H, m), 3.57 (3H, s, OCH_3), 3.73 (1H, s, OCH_3), 3.78 (1H, m), 3.85 (3H, s, OCH_3), 3.89 (1H, m), 5.91 (2H, s, $-\text{OCH}_2\text{O}-$), 6.38 (1H, d, J = 12.6, vinyl H), 6.43 (3H, m, vinyl H, $2 \times \text{ArH}$), 6.73 (1H, d, J = 9.0, ArH), 7.00 (1H, dd, J = 8.4, 1.8 Hz, ArH), 8.15 (1H, d, J = 1.8 Hz, ArH), 8.35 (1H, s); HRMS calcd for $\text{C}_{26}\text{H}_{33}\text{N}_2\text{O}_6$ $[\text{M} + \text{H}]^+$ 469.2339, found 469.2374; *anal.* calcd for $\text{C}_{30}\text{H}_{34}\text{F}_6\text{N}_2\text{O}_{10} \cdot 2\text{H}_2\text{O}$, C, 49.18; H, 5.23; N, 3.82; found, C, 49.48; H, 5.23; N, 4.09.

3,4-Methylenedioxy-5,4'-dimethoxy-3'-(Dov-Val-Dil-Dap)-amido-Z-stilbene (16). To a stirred mixture of amide **14** (41 mg, 0.070 mmol), Dov-Val-Dil TFA salt **15** (62 mg, 0.11 mmol, 1.6 equiv), and PyBroP (50 mg, 0.11 mmol, 1.6 equiv) in DCM (0.5 mL) at 0°C under Ar was added DIPEA (91 μL , 0.52 mmol, 7.4 equiv). The reaction mixture was stirred 14 h at room temperature, and the solvent was removed in vacuo. The product was obtained by gravity column chromatography (1:1, *n*-hexane/acetone) as a colorless oil (**16**, 30 mg, 48%): R_f 0.34 (1:1, *n*-hexane/acetone); $[\alpha]_D^{23}$ -62° (c 1.05, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 0.79 (3H, t, J = 6.9 Hz, CH_3), 0.91 (3H, d, J = 6.6 Hz, CH_3), 0.94 (3H, d, J = 6.6 Hz), 0.94 (3H, d, J = 6.3 Hz, CH_3), 1.00 (3H, d, J = 6.6 Hz, CH_3), 1.01 (3H, d, J = 6.3 Hz, CH_3), 1.32 (3H, d, J = 6.9 Hz, CH_3), 1.35 (2H, m), 1.80 (1H, m), 2.0 (3H, m), 2.31 (6H, s, $2 \times \text{NCH}_3$), 2.34 (4H, m), 2.62 (1H, m), 3.00 (3H, s, NCH_3), 3.05 (1H, m) 3.08 (1H, s), 3.20 (1H, m), 3.29 (1H, m), 3.32 (3H, OCH_3), 3.34 (1H, m), 3.44 (1H, m), 3.45 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.06 (1H, m), 4.12 (1H, m), 4.20 (1H, m), 4.79 (1H, m), 5.90 (2H, s, $-\text{OCH}_2\text{O}-$), 6.36 (1H, d, J = 12.0 Hz, vinyl H), 6.44 (3H, m, vinyl H, $2 \times \text{ArH}$), 6.68 (1H, d, J = 8.7 Hz, ArH), 6.94 (1H, dd, J = 8.1, 1.5 Hz, ArH), 8.27 (1H, d, J = 1.8 Hz), 8.33 (1H, s); HRMS calcd for $\text{C}_{48}\text{H}_{74}\text{N}_5\text{O}_{10}$ $[\text{M} + \text{H}]^+$ 880.5435, found 880.5426; *anal.* calcd for $\text{C}_{48}\text{H}_{73}\text{N}_5\text{O}_{10}$, C, 65.50; H, 8.36; N, 7.96; found, C, 65.42; H, 8.74; N, 7.59.

Tubulin and Cancer Cell Procedures. Bovine brain tubulin was prepared³⁸ and the tubulin assembly³⁹ and colchicine binding⁴⁰ assays were performed as described previously. In the assembly assay, reaction mixtures contained 1.0 mg/mL (10 μM) tubulin and varying concentrations of potential inhibitors. In the colchicine binding assay reaction mixtures contained 0.1 mg/mL (1.0 μM) tubulin, 5.0 μM [^3H]colchicine, and potential inhibitor as indicated. The vinblastine binding assay was performed as described previously for GTP binding⁴¹ by centrifugal gel filtration, except that a Beckman Allegra 6KR centrifuge equipped with a GH-3.8A swinging bucket rotor was used and the syringe-columns were centrifuged at 2000 rpm. Reaction mixtures contained 0.5 mg/mL (5.0 μM)

tubulin, 5 μM [^3H]vinblastine, potential inhibitor as indicated, 0.5 mM MgCl_2 , 0.1 M 4-morpholineethanesulfonate (1.0 M stock solution adjusted to pH 6.9 with NaOH), and 4% (v/v) dimethyl sulfoxide. Incubation (30 min) and centrifugal gel filtration were performed at room temperature ($20-22^\circ\text{C}$).

Cytotoxicity assays were performed by the sulforhodamine B method, in which inhibition of formation of cellular protein is measured.⁴² The mitotic index of MCF-7 breast cancer cells was determined as described previously,²⁷ except that cells were incubated in the presence of drug for 16 h prior to addition of the DNA stain.

Antimicrobial Evaluation. Compounds were screened in broth microdilution assays according to National Committee for Clinical Laboratory Standards.^{43,44} Assays were repeated at least twice on separate days.

Acknowledgment. We are pleased to thank the following for financial assistance: Outstanding Investigator Grant CA44344-05-12 and RO1 CA90441-01-03 awarded by the Division of Cancer Treatment and Diagnosis, National Cancer Institute, DHHS; the Arizona Disease Control Research Commission; the Caitlin Robb Foundation; Dr. Alec D. Keith and Mrs. Kay M. Keith; the J. W. Kieckhefer Foundation; the Margaret T. Morris Foundation; Rod J. and Hazel V. McMullin; Gary L. and Diane Tooker; Polly J. Trautman; John and Edie Reyno; Dr. John C. Budzinski; the Ladies Auxiliary to the Veterans of Foreign Wars; and the Robert B. Dalton Endowment Fund. In addition, we would like to thank Dr. G. Boland for conducting some preliminary experiments. Other very helpful assistance was provided by Drs. F. Hogan, J. M. Schmidt, and J.-C. Chapuis, and by Ms. B. Fakoury and Mr. M. J. Dodson. This work was supported in part by NCI, National Institutes of Health Contract NO1-CO-12400.

References and Notes

- Pettit, G. R.; Moser, B. R.; Boyd, M. R.; Schmidt, J. M.; Pettit, R. K.; Chapuis, J. C. *Anti-Cancer Drug Des.* **2002**, *16*, 185–194.
- Kanthou, C.; Tozer, G. M. *Blood* **2002**, *99*, 2060–2069.
- Beauregard, D. A.; Pedley, R. B.; Hill, S. A.; Brindle, K. M. *NMR in Biomed.* **2002**, *15*, 99–105.
- Eikesdal, H. P.; Landuyt, W.; Dahl, O. *Cancer Lett.* **2002**, *178*, 209–217.
- Li, L.; Rojiani, A. M.; Siemann, D. W. *Acta Oncol.* **2002**, *41*, 91–97.
- Boehle, A. S.; Sipos, B.; Kliche, U.; Kalthoff, H.; Dohrmann, P. *Ann. Thoracic Surg.* **2001**, *71*, 1657–1665.
- Dowlati, A.; Robertson, K.; Cooney, M.; Petros, W. P.; Stratford, M.; Jesberger, J.; Rafie, N.; Overmoyer, B.; Makkar, V.; Stambler, B.; Taylor, A.; Waas, J.; Lewin, J. S.; McCrae, K. R.; Remick, S. C. *A Cancer Res.* **2002**, *62*, 3408–3416.
- Remick, S. C.; Dowlati, A.; Robertson, K.; Spiro, T.; Connell, C.; Levitan, N.; Stratford, M. Phase I Pharmacokinetics Study of Single Dose Intravenous (IV) Combretastatin A-4 Prodrug (CA4P) in Patients (Pts) with Advanced Cancer. In *Molecular Targets and Cancer Therapeutics Discovery, Development, and Clinical Validation. Proceedings of the AACR-NCI-EORTC International Congress*, Washington, DC, 1999; p 4.
- Rustin, G. J. S.; Galtraith, S. M.; Taylor, N. J.; Maxwell, R.; Tozer, G.; Baddeley, H.; Wilson, L.; Prise V. Combretastatin A-4 Phosphate (CA4P) Selectively Targets Vasculature in Animal and Human Tumors. In *Molecular Targets and Cancer Therapeutics Discovery, Development, and Clinical Validation. Proceedings of the AACR-NCI-EORTC International Congress*, Washington, DC, 1999; p 4.
- Pettit, G. R.; Temple, C.; Narayanan, V. L.; Varma, R.; Simpson, M. M.; Boyd, M. R.; Renner, G. A.; Bansal, N. *Anti-Cancer Drug Des.* **1995**, *10*, 299–309.
- Pettit, G. R.; Minardi, M. D.; Boyd, M. R.; Pettit, R. K. *Anti-Cancer Drug Des.* **2000**, *15*, 397–404.
- Pettit, G. R.; Lippert, J. W. *Anti-Cancer Drug Des.* **2000**, *15*, 203–216.
- Pettit, G. R.; Rhodes, M. R. *Anti-Cancer Drug Des.* **1998**, *13*, 183–191.
- Chakravarty, P. K.; Greenlee, W. J.; Parsons, W. H.; Patchett, A. A.; Combs, P.; Roth, A.; Busch, R. D.; Mellin, T. N. *J. Med. Chem.* **1989**, *32*, 1886–1890.
- Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynsky, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883–6885.
- Pettit, G. R. In *The Dolastatins*; Herz, W.; Kirby, G. W.; Moore, R. E.; Stiglich, W.; Tamm, Ch., Eds.; Progress in the Chemistry of Organic Natural Products 70; Springer: Vienna, 1997; pp 1–79.
- Pettit, G. R. In *Evolutionary Biosynthesis of Anticancer Drugs*; Ojima, I.; Vite, G. D.; Altmann, K.-H., Eds.; Anticancer Agents, Frontiers in Cancer Chemotherapy; American Chemical Society: Washington, DC, 2001; pp 16–42.

- (18) Dang, L. H.; Bettegowda, C.; Huso, D. L.; Kinzler, K. W.; Vogelstein, B. *Prod. Natl. Acad. Sci.* **2001**, *98*, 15155–15160.
- (19) Thamm, D. H.; MacEwen, E. G.; Phillips, B. S.; Hershey, A. E.; Burgess, K. M.; Pettit, G. R.; Vail D. M. *Cancer Chemother. Pharmacol.* **2002**, *49*, 251–255.
- (20) Margolin, K.; Longmate, J.; Synold, T. W.; Gandara, D. R.; Weber, J.; Gonzalez, R.; Johansen, M. J.; Newman, R.; Baratta, T.; Doroshow, J. H. *Invest. New Drugs* **2001**, *19*, 335–340.
- (21) Vaishampayan, U.; Glode, M.; Du, W.; Kraft, A.; Hudes, G.; Wright, J.; Hussain, M. *Clin. Cancer Res.* **2000**, *6*, 4205–4208.
- (22) Krug, L. M.; Miller, V. A.; Kalemkerian, G. P.; Kraut, M. J.; Ng, K. K.; Heelan, R. T.; Pizzo, B. S.; Perez, W.; McClean, N.; Kris, M. G. *Ann. Oncol.* **2000**, *11*, 227–228.
- (23) Pettit, R. K.; Pettit, G. R.; Hazen, K. C. *Antimicrob. Agents Chemother.* **1998**, *42*, 2961–2965.
- (24) Woyke, T.; Pettit, G. R.; Winkelmann, G.; Pettit, R. K. *Antimicrob. Agents Chemother.* **2001**, *45*, 3580–3584.
- (25) 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. C.; Schmidt, J. M. *Anti-Cancer Drug Des.* **1998**, *13*, 243–277.
- (26) Pettit, G. R.; Srirangam, J. K.; Barkoczy, J.; Williams, M. D.; Durkin, K. P. M.; Boyd, M. R.; Bai, R. L.; Hamel, E.; Schmidt, J. M.; Chapuis, J. C. *Anti-Cancer Drug Des.* **1995**, *10*, 529–544.
- (27) Pettit, G. R.; Anderson, C. R.; Herald, D. L.; Jung, M. K.; Lee, D. J.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2003**, *46*, 525–531.
- (28) Coste, J.; Dufour, M.-N.; Pantaloni, A.; Bertrand, C. *Tetrahedron Lett.* **1990**, *31*, 669–672.
- (29) Lott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495–496.
- (30) Ho, T.-L.; Olah, G. A. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 774–775.
- (31) Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T. *Tetrahedron Lett.* **1992**, *33*, 5441–5444.
- (32) Pettit, G. R.; Srirangam, J. K.; Singh, S. B.; Williams, M. D.; Herald, D. L.; Barkoczy, J.; Kantoci, D.; Hogan, F. *J. Chem. Soc., Perkin Trans. 1* **1996**, 859–863.
- (33) Pettit, G. R.; Burkett, D. D.; Barkoczy, J.; Breneman, G. L.; Pettit, W. E. *Synthesis* **1996**, 719–725.
- (34) Bai, R.; Pettit, G. R.; Hamel, E. *Biochem. Pharmacol.* **1990**, *39*, 1941–1949.
- (35) Bai, R.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1990**, *265*, 17141–17149.
- (36) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200–208.
- (37) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984–6991.
- (38) Hamel, E.; Lin, C. M. *Biochemistry* **1984**, *23*, 4173–4184.
- (39) Hamel, E. *Cell Biochem. Biophys.* **2003**, *38*, 1–21.
- (40) Verdier-Pinard, P.; Lai, J.-Y.; Yoo, H.-D.; Yu, J.; Marquez, B.; Nagle, D. G.; Nambu, M.; White, J. D.; Falck, J. R.; Gerwick, W. H.; Day, B. W.; Hamel, E. *Mol. Pharmacol.* **1998**, *53*, 62–76.
- (41) Hamel, E.; Lin, C. M. *J. Biol. Chem.* **1984**, *259*, 11060–11069.
- (42) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
- (43) NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Fifth Edition; NCCLS Document M7-A5; 2000.
- (44) NCCLS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Second Edition; NCCLS Document M27-A2; 2002.

NP058033L