Antineoplastic Agents. 578. Synthesis of Stilstatins 1 and 2 and Their Water-Soluble Prodrugs[†]

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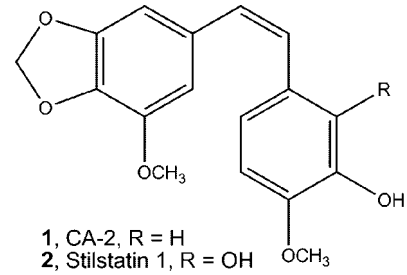
Efficient syntheses of 3,4-methylenedioxy-4′,5-dimethoxy-2′,3′-dihydroxy-*Z*-stilbene (stilstatin 1, **2**), 3,4,4′-trimethoxy-2′,3′,5-trihydroxy-*Z*-stilbene (stilstatin 2, **5**), and respective phosphate prodrugs have been summarized. Both **2** and **5** were accessed via a convergent step synthesis using phosphonium bromides **6** and **21** in Wittig reactions with 2,3-bis(*tert*-butyldimethylsilyloxy)-4′-methoxybenzaldehyde **14**. Deprotection of silyl ethers **15** and **26** with TBAF furnished **2** and **5**, respectively. Phosphorylation of **2** and **5** afforded the phosphoric acid intermediates **17** and **28** for prodrug development. These phosphoric acid precursors were employed in parallel series of reactions to produce a selection of metal cation prodrug candidates. The biological activities of stilstatins 1 (**2**) and 2 (**5**) and their respective prodrugs were evaluated against a panel of one murine (P388) and six human cancer cell lines. Compared to combretastatin A-2 (**1**), stilstatin 1 (**2**) has an additional vicinal hydroxy group on the B ring, the presence of which was detrimental to the cancer cell line potency; *in vivo*, however, compound **2** would be predicted to have greater anticancer activity resulting from the *o*-quinone mechanism of action analogous to that of combretastatin A-1 (**4**). The substitution of a hydroxy group for a methoxy group on the A ring of combretastatin A-1 (**4**), resulting in stilstatin 2 (**5**), gave rise to a modest level of inhibition consistent with that found for **4** against cancer cell lines.

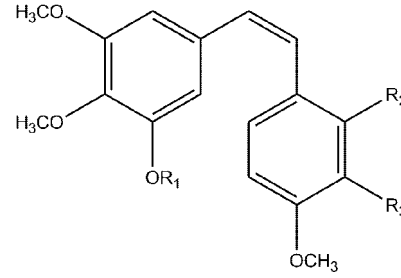
Some of the natural products we isolated from the African bush willow, *Combretum caffrum* (Combretaceae), are characterized by their remarkable biological activity. Our early research endeavor with *C. caffrum* included the isolation, structural elucidation, and synthesis of combretastatins A-2 (**1**), A-3, D-1, and D-2, as well as initial structural modifications.[1] Illustrative of the biological activity are the *Z*-stilbenes we designated combretastatins A-4 (**3**, CA4)[2] and A-1 (**4**, CA1).[3] Compounds **3** and **4**, along with their corresponding phosphate prodrug salts (CA4P[4] and CA1P,[5] respectively), are now of great therapeutic interest, and CA1P and CA4P are in phase I and III human cancer clinical trials, respectively.

A comparison of the vicinal diphenol CA1 (**4**)[3] with the monophenol counterpart CA4 (**3**)[2] revealed a very similar antimitotic activity (IC_{50} 2−3 μM) but much lower cancer cell growth inhibition for CA1 (**4**) compared to that of CA4 (**3**, ED_{50} ~0.0009 mg/mL, P388 leukemia cell line). Development of both CA1 and CA4 to the current human cancer clinical trials was accelerated following syntheses of the phosphate prodrugs and discovery of their very promising cancer vascular disrupting (VTA) and anti-angiogenesis effects, as well as aqueous solubility, e.g., for CA4P, 20 mg/mL of H_2O. Once administered, the phosphate prodrugs are converted into the parent drug via nonspecific phosphatases and then transported intracellularly.[4] Both CA1P and CA4P have been shown to selectively damage tumor neovasculature with induction of extensive blood flow shutdown in the metastatic tumor compared to normal tissues.[6−8] CA1P (aka Oxi-4503) has shown excellent potential in preclinical studies leading to tumor regression as a single agent that also attacks the remaining tumor rim cancer cells.[5,7] The mechanism of action (formation of an *o*-quinone intermediate *in vivo*)[1,9] of CA1P differs from that of CA4P.

We now report the syntheses of two new and closely related stilbenes, stilstatin 1 (**2**) and stilstatin 2 (**5**), and their phosphate prodrugs as a next step toward the objective of locating structural modifications with increased anticancer activity. Compounds **2** and **5** each have an additional phenolic hydroxy group compared to CA2 (**1**) and CA1 (**4**), respectively.



1, CA-2, R = H
2, Stilstatin 1, R = OH



3, CA-4, $R_1 = CH_3$, $R_2 = H$, $R_3 = OH$
4, CA-1, $R_1 = CH_3$, $R_2 = R_3 = OH$
5, Stilstatin 2, $R_1 = H$, $R_2 = R_3 = OH$

Figure 1. Structures of combretastatins A-2 (**1**), A-4 (**3**), and A-1 (**4**) and stilstatins 1 (**2**) and 2 (**5**).

Results and Discussion

In the syntheses of both stilstatin 1 (**2**) and stilstatin 2 (**5**), stilbene formation was based on a Wittig reaction sequence. Synthesis of the stilstatin 1 A-ring moiety is outlined in Scheme 1. Treatment of 5-bromovanillin (**7**) with NaOH furnished benzaldehyde **8**. Subsequent reaction with CH_2Br_2 gave access to the methylenedioxy derivative **9**. Reduction of **9** with $NaBH_4$ afforded alcohol **10**, which was treated consecutively with PBr_3 and triphenylphosphine to furnish phosphonium bromide **6**, the A-ring moiety of **2**. The bromide intermediate **11** was not characterized.

The synthesis of the B-ring moiety for both **2** and **5** is outlined in Scheme 2. Commercially available 2,3,4-trimethoxybenzaldehyde

[†] Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

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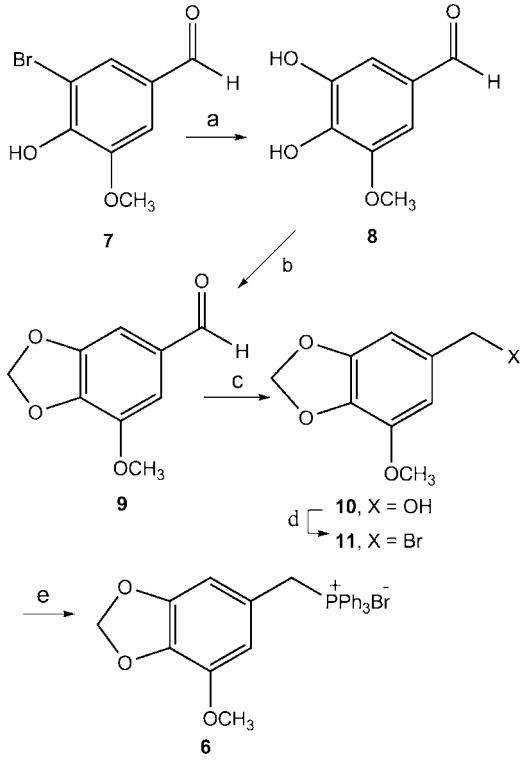
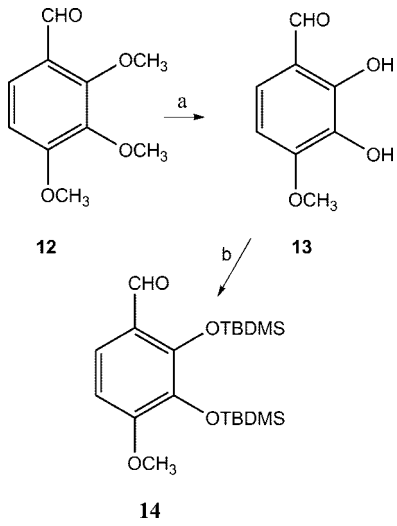
Scheme 1. Synthesis of Phosphonium Bromide **6**[a]



[a] Reagents and conditions: (a) NaOH, Cu^0, H_2O, Δ, 24 h, 97%; (b) CH_2Br_2, K_2CO_3, DMF, 100 °C, 2 h, 88%; (c) $NaBH_4$, EtOH, rt, 3 h, 97%; (d) DCM, 0 °C, PBr_3, 8 h to rt, quantitative; (e) toluene, PPh_3, Δ, 12 h, 74%.

Scheme 2. Synthesis of Silyl Ether **14**[a]

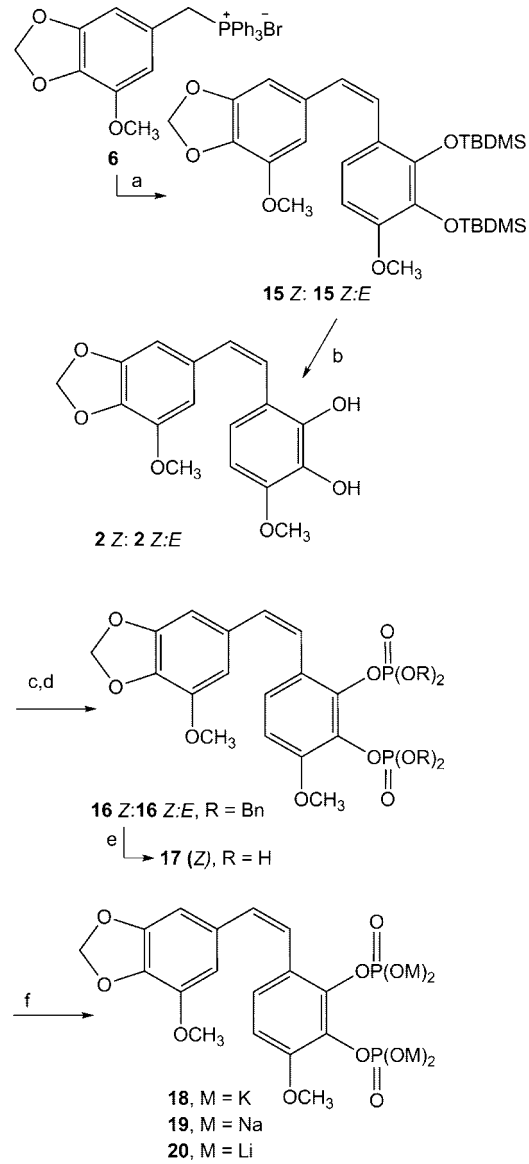


[a] Reagents and conditions: (a) BCl_3, DCM, 26 h, rt, 87%; (b) DMF, DIPEA, rt, TBDMSCl, 84%.

(**12**) was selectively demethylated at the *ortho* and *meta* positions with BCl_3 to yield the vicinal phenol **13**, which was protected as silyl ether **14**. The preceding reaction sequence was based on our earlier synthesis of combretastatin A-1.[3]

Next, phosphonium bromide **6** was treated with *n*-butyllithium to generate the phosphonium ylide at −10 °C (Scheme 3). Addition of aldehyde **14** at room temperature yielded stilbene **15** in a *Z*/*E* ratio of 5:1 on a small scale (less than 1.0 g) and 2:1 on larger scales. Geometrical isomers were determined by NMR spectroscopy. Separation of the *Z*- and *E*-stilbenes (**15**) was achieved with column chromatography, which gave the *Z*-isomer in 10% yield

Scheme 3. Synthesis of Stilstatin 1 (**2**) and Its Water-Soluble Prodrugs **18**, **19**, and **20**[a]



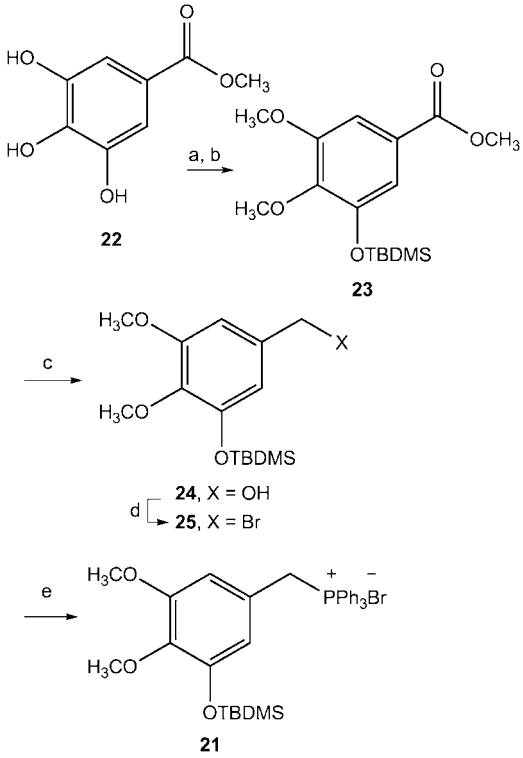
[a] Reagents and conditions: (a) (i) *n*-BuLi, THF, −10 °C to rt, 10 min; (ii) **14**, 30 min; (b) THF, 0 °C, TBAF, 2 h, 74%; (c) acetonitrile, CCl_4, −10 °C; (d) DIPEA, DMAP, 1 min, dibenzyl phosphite; (e) DCM, TMSBr, rt, 30 min, quantitative; (f) 1.0 N KOH in CH_3OH, 0 °C, 30 min, 57%.

and the *Z*/*E* mix in 50% yield. Further attempts at separation proved ineffective. Therefore, the isomeric mixture (**15**) was treated with TBAF at 0 °C to effect deprotection, yielding stilstatin 1 (*Z*-**2**) and its isomer. However, as was observed with **15**, separation of the isomers proved to be inefficient.

The key intermediate in the formation of the phosphate prodrugs was accessed by phosphorylation of **2** as the *Z*/*E* mixture, using dibenzyl phosphite for *in situ* generation of phosphoryl chloride. Column chromatography led to better separation of the *Z*- and *E*-isomers and provided *Z*-**16** in 31% yield. As expected, a *Z*/*E* mixture was also obtained (44%), along with the pure *E* (14%) product. Because the *Z*- and *E*-stilbenes had nearly identical R_f values by TLC, *Z*-**16** was analyzed by HPLC to establish its purity.

Next, removal of the benzyl groups from *Z*-**16** was carried out with TMSBr. The intermediate phosphoric acid **17** was not isolated, although FTIR analysis indicated the presence of the phosphoryl hydroxy groups. Immediate dissolution in CH_3OH and addition of 1.0 N KOH solution in CH_3OH gave access to the potassium

Scheme 4. Synthesis of Phosphonium Bromide **21**[a]



[a] Reagents and conditions: (a) DMF, TBDMSCl, rt, 2 h; (b) NaH, 30 min, CH_3I, rt, 45%; (c) $LiAlH_4$, THF, rt, 89%; (d) DCM, 0 °C, PBr_3, 10 min, quantitative; (e) PPh_3, toluene, rt (10 min), Δ (2 h), rt (24 h), 66%.

prodrug **18**. The sodium (**19**) and lithium (**20**) prodrugs were obtained from **18** via ion-exchange chromatography using DOWEX-50W. The resin was charged with the appropriate 1.0 N metal hydroxide solution, and the salts were eluted in H_2O.

In order to prepare stilstatin 2 (**5**), phosphonium bromide **21** was first synthesized (Scheme 4). Selective silyl protection of the C-3 hydroxy group of methyl gallate (**22**) followed by methylation of the 4,5-dihydroxy functionality with CH_3I furnished **23** as the major product (45% yield). Reduction of ester **23** with $LiAlH_4$ gave alcohol **24** in high yield, and reaction with PBr_3 gave the intermediate bromide **25**. Initial attempts to prepare the phosphonium bromide **21** in acceptable yield proved problematic owing to cleavage of the silyl ether unit under extended reflux conditions. It was found that a reflux period of 45 min followed by stirring with cooling over 24 h gave **21** in 45% yield. With 2 h at reflux and a 24 h period with stirring at room temperature, the yield increased to 66%. However, a reflux time of greater than 2 h resulted in loss of the silyl ether group and degradation of **21**.

Treatment of **21** with *n*-butyllithium at −10 °C yielded the ylide, which reacted with aldehyde **14** at room temperature to give an isomeric mixture of stilbenes (**26**, Scheme 5), separation of which was problematic. Initial attempts via column chromatography with gradient elutions failed to separate the *Z*- and *E*-isomers, and therefore **26** was subjected to medium-pressure liquid chromatography, with 4:5 DCM−hexanes as eluant. This technique led to *Z*-**26** (14%), a *Z*/*E* mixture (67%), and *E*-**26** (3%) in 84% total yield. Deprotection of each isomer with TBAF at 0 °C furnished **5** and its *E*-isomer in 76% and 71% yields, respectively. In another experiment, the stilbene mixture **26** was not separated but was deprotected as an isomeric mixture to give a mixture of **5** and its isomer, which was carried forward without separation and treated with dibenzyl phosphite and CCl_4 to give a mixture of tri- (**27a**,**c**) and diphosphorylated (**27b**,**d**) products. Chromatographic separation of this mixture (gradient elution) led to **27a** in 26% yield, along with a mixture of **27a** and **27b** (11%) and a mixture of **27c** and

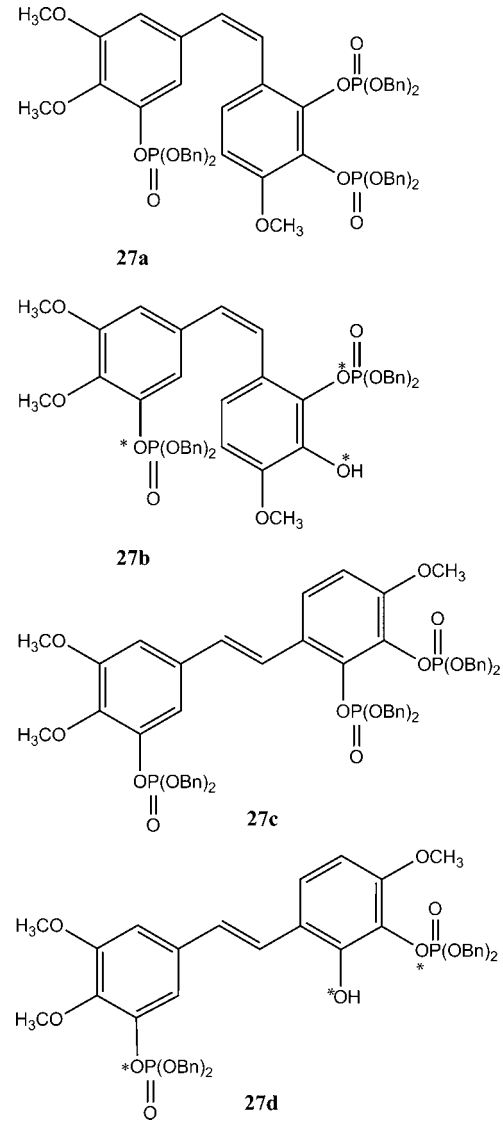


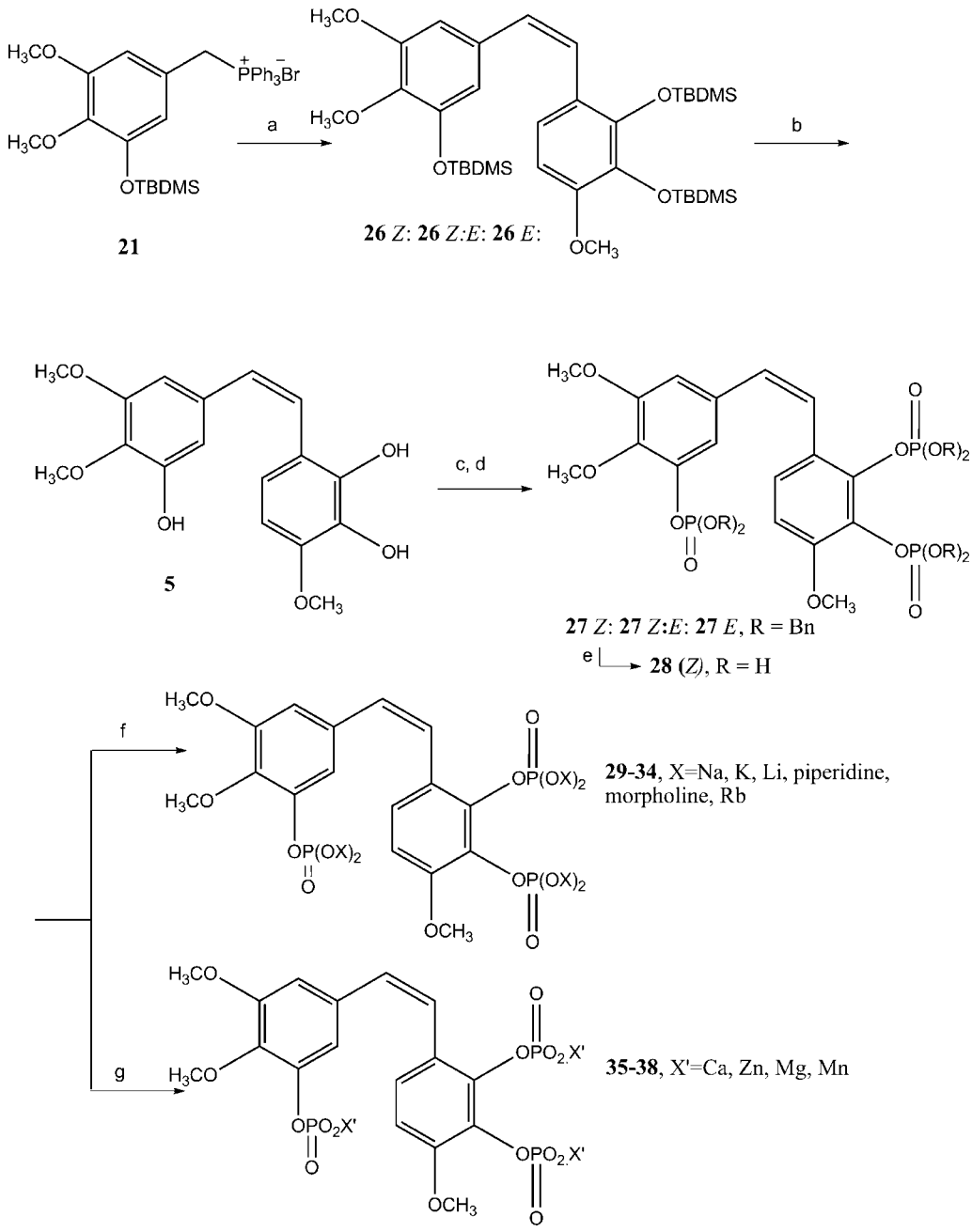
Figure 2. Phosphorylated products: * denotes interchangeable assignments.

27d (37%). Since **27a** was the necessary precursor for the target phosphate prodrug, it was apparent that the *Z*- and *E*-isomers (using careful chromatography) could be more conveniently separated at this stage.

Deprotection of **27a** with TMSBr provided the free phosphoric acid intermediate **28** in quantitative yield, and this was immediately treated with $NaOCH_3$ to give sodium prodrug **29**. Potassium (**30**) and lithium (**31**) prodrugs were similarly prepared using KOH and LiOH, respectively, and each was further purified on a DOWEX-50W ion-exchange column. The piperidine (**32**) and morpholine prodrugs (**33**) were obtained from **30** via an ion-exchange procedure. Treatment of phosphoric acid **28** with $RbHCO_3$ gave rubidium salt **34**, and calcium prodrug **35** was obtained using calcium hydroxide. The remaining divalent metal prodrugs (**36**−**38**) were synthesized via treatment with the corresponding metal acetate.

Biological Evaluation. The cancer cell growth inhibitory activities of stilstatin 1 (**2**), stilstatin 2 (**5**), related stilbenes, and phosphorylated prodrugs are summarized in Table 1. The additional vicinal hydroxy group on ring B of **2** proved to be detrimental, as the cancer cell line activity dropped some 100-fold in comparison to CA2 (**1**). Interestingly, the phosphate prodrugs (**18**−**20**) of **2** show improved activity (∼10-fold) over that of the parent compound, perhaps by increasing its bioavailability. The activity of stilstatin 2 (**5**) showed the presence of a hydroxy group on ring A

Scheme 5. Synthesis of Stilstatin 2 (**5**) and Its Water-Soluble Prodrugs **29**−**38**[a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, THF, −10 °C, **14**; (b) THF, TBAF (1.0 M in THF); (c) CCl_4, −10 °C, acetonitrile; (d) DIPEA, DMAP, dibenzyl phosphite; (e) TMSBr, DCM, rt, quantitative; (f) $NaOCH_3$/XOH/$RbHCO_3$, CH_3OH, rt, 30 min; (g) $Ca(OH)_2$ or $X'(OAc)_2$, CH_3OH, rt, 30 min.

to have a moderate effect, and the derived phosphate prodrugs again improved the activity, giving a similar overall 10-fold increase. Very importantly, both stilstatins 1 (**2**) and 2 (**5**) can be predicted to provide impressive anticancer activity *in vivo* owing to the *o*-quinone mechanism of action established recently for the analogous vicinal phenol system of combretastatin A-1 prodrug (CA1P, OXi-4503).[9]

Experimental Section

General Experimental Procedures. All solvents and reagents were obtained from Sigma-Aldrich (Milwaukee,WI) or Acros Organics (Fischer Scientific, Pittsburgh, PA) and used as purchased unless otherwise stated. Reactions were monitored by TLC using Analtech silica gel GHLF uniplates visualized under long-wave and short-wave UV irradiation, along with staining with phosphomolybdic acid/heat, I_2, or $KMnO_4$. Solvent extracts were dried over anhydrous $MgSO_4$ unless otherwise stated. When appropriate, crude products were separated by flash column chromatography (230−400 mesh ASTM silica from E. Merck). Ion-exchange column chromatography was carried out on Dowex ion-exchange resin (Dowex 50 × 4−400, Sigma).

Melting points are uncorrected and were determined with an electrothermal 9100 apparatus. The infrared spectra were obtained using a Thermo Nicolet AVATAR 360 system with a SMART MIRacle single reflection HATR. The ^1H and ^{13}C NMR spectra were recorded with Varian Gemini 300, Varian Unity 400, or Varian Unity 500 instruments using a deuterated solvent and were referenced to either TMS or the solvent. The reported figures are given as ppm. Phosphorus NMR spectra were referenced against a standard of 85% H_3PO_4. HRMS data were recorded with a Jeol LCmate mass spectrometer and Jeol GCmate. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

3,4-Dihydroxy-5-methoxybenzaldehyde (8). Preparation of compound **8** was repeated as originally described[10] from 5-bromovanillin

Table 1. Human Cancer Cell Line (GI_{50} μg/mL) and Murine P388 Lymphocytic Leukemia Cell Line Inhibitory Acitvity (ED_{50} μg/mL) of Stilbenes **1**−**5** and Their Phosphate Prodrugs **15**−**38**

compound	murine leukemia P388	pancreas BXPC-3	breast MCF-7	CNS SF-268	lung NCI-H460	colon KM2012	prostate DU-145	thyroid KAT-4	thyroid SW1736
3, CA4	0.0026	0.39		>0.01	0.0006	0.34	0.0008		
CA4P	0.0029	0.23			0.0004		0.0007		
4, CA1	0.3	4.4			0.74		0.17		
CA1P	0.3	>10			3.3	>10	2.7		
1, CA2	0.016	0.014	0.0042		0.043	0.47	0.0054		
CA2P	0.0250	2.1	0.045		0.41	3.8	0.053		
15	>10	>10	>10	>10	>10	>10	>10		
2	2.0	4.9	0.16	0.63	0.97	>10	>10		
18	0.17	5.3	0.39	0.65	1.4	>10	0.93	9.6	4.7
19	1.4	>10	3.4	2.8	2.8	>10	2.5		
20	0.3	>10	1.2	2.4	2.7	>10	2.1		
26	>100	>10	>10	>10	>10	>10	>10		
5	1.7	1.8	1.6	1.6	1.5	1.9	1.8		
29	0.14	1.2	0.3	0.34	0.30	3.1	0.26		
30	0.12	0.75	0.15	0.3	0.30	2.9	0.22		
31	0.028	5.8	0.36	0.76	0.35	6.1	0.82		
34	0.26	>10	3.9	4.3	3.3	>10	3.9		
35	0.14	3.6	0.38	0.46	0.35	5.2	0.37		
36	0.15	>10	3.5	4.5	3.5	>10	4.6	>10	>10
37	0.064	5.9	0.5	0.63	0.32	< 10	0.42	>10	>10
38	0.59	>10	>10	3.6	0.51	>10	>10	1.3	1.7

(**7**). Additional NMR and IR data are provided here: colorless solid that crystallized from toluene (97% yield); mp 131−133 °C [lit.[10] mp 132−133 °C]; R_f 0.5 (1:1 EtOAc−hexanes); IR (neat) ν_{max} 3305, 1674, 1587, 1434, 1289, 1156, 1045, 678 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.96 (3H, s, OCH_3), 5.49 (1 H, s, OH), 5.99 (1 H, s, OH), 7.08 (1 H, d, J = 1.5 Hz, ArH), 7.14 (1H, d, J = 1.5 Hz, ArH), 9.78 (1H, s, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.61, 107.99, 108.15, 130.01, 130.09, 147.65, 148.87, 189.67.

3,4-Methylenedioxy-5-methoxybenzaldehyde (9).[11a,b] A solution of **8** (29.0 g, 0.17 mol), CH_2Br_2 (35.46 g, 0.20 mol, 14.55 mL), and K_2CO_3 (28.19 g, 0.204 mol) in anhydrous DMF (350 mL) was gradually heated to 100 °C and stirred for 2 h. After cooling to rt, H_2O (500 mL) was added, the reaction mixture was extracted with EtOAc (2 × 100 mL) and dried, and the solvent was removed *in vacuo* to furnish a brown oil. The crude product was purified by flash chromatography, eluting in 7:3 hexanes−EtOAc, to give the title compound in quantitative yield as a white solid that crystallized from 1:1 EtOAc−hexanes as a colorless solid (26.8 g, 88%): mp 132−133 °C [lit.[11c] mp 130−131 °C]; R_f 0.36 (7:3 hexanes−EtOAc); IR (neat) ν_{max} 3100, 2844, 1688, 1459, 1321, 1274, 1125, 1039, 970 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.96 (3H, s, OCH_3), 6.09 (2H, s, O−CH_2−O), 7.05 (1H, d, J = 2.3 Hz, ArH), 7.13 (1H, d, J = 2.3 Hz, ArH), 9.78 (1H, s, CHO); ^{13}C NMR (100 MHz $CDCl_3$) δ 56.23, 95.75, 103.63, 110.07, 133.13, 140.96, 147.76, 153.17, 189.73. HRMS (APCI+) *m*/*z* 181.0509 $[M + H]^+$ (calcd for $C_9H_9O_4$, 181.0508).

3,4-Methylenedioxy-5-methoxybenzyl Alcohol (10). A solution of **9** (3.0 g, 16.67 mmol) in EtOH (40.0 mL) was stirred for 10 min, and $NaBH_4$ (0.75 g, 20.0 mmol) was added in portions over 30 min.[12] After stirring for 3 h at rt, solid $NaHCO_3$ was added until the effervescing ceased. The solution was filtered, and the solvent was removed *in vacuo*, which furnished a white foam. The foam was redissolved in EtOAc and washed with H_2O (2 × 50 mL). The organic phase was dried, the solvent removed *in vacuo*, and the colorless solid crystallized from 1:1 hexanes−EtOAc to yield the title compound in 97% (2.9 g) yield: mp 64−66 °C [lit.[11b] mp 66−67 °C]; R_f 0.25 (7:3 hexanes−EtOAc); IR (neat) ν_{max} 3100, 2844, 1688, 1459, 1321, 1274, 1125, 1039, 970 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.40 (1H, brs, OH), 3.81 (3H, s, OCH_3), 4.44 (2H, s, CH_2OH), 5.87 (2H, s, O−CH_2−O), 6.44 (1H, s, ArH), 7.13 (1H, s, ArH); ^{13}C NMR (100 MHz $CDCl_3$) δ 56.44, 65.15, 101.21, 101.36, 106.34, 134.49, 135.52, 143.53, 148.80; GC MS (EI+) *m*/*z* 182.0593 $[M]^+$ (calcd for $C_9H_{10}O_4$ 182.0579).

3,4-Methylenedioxy-5-methoxybenzyltriphenylphosphonium Bromide (6). Preparation of the bromide **6** was repeated as originally described:[13] colorless solid, 74% yield; mp 241−243 °C [lit.[13] mp 241−243 °C].

2,3-Dihydroxy-4-methoxybenzaldehyde (13). A solution of 2,3,4-trimethoxybenzaldehyde (**12**, 39.2 g, 200 mmol) in anhydrous CH_2Cl_2 (1000 mL) was stirred at rt for 10 min, and then BCl_3 (200 mL, 200 mmol) was added via cannula. After 2 h the second equivalent of BCl_3 (200 mL, 200 mmol) was added and the dark reaction mixture was allowed to stir at rt for 24 h. The resultant mixture was carefully poured onto ice-cold 10% $NaHCO_3$ solution (1700 mL) and reacidified to pH 1 with concentrated HCl. The CH_2Cl_2 layer was separated and the aqueous phase extracted with EtOAc (3 × 500 mL). The combined organic phases were dried and the solvent was removed *in vacuo*. Purification was achieved with crystallization from 1:1 hexanes−EtOAc, which furnished the title compound as a colorless solid in 87% (28.9 g) yield: mp 115−116 °C [lit.[3] mp 116−117 °C, lit.[14] mp 118−119 °C]; R_f 0.22 (1:1 EtOAc −hexanes); IR (neat) ν_{max} 3365, 2944, 1645, 1507, 1453, 1277, 1104, 1025 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.98 (3H, s, OCH_3), 5.44 (1H, s, OH-3), 6.62 (1H, d, J = 9 Hz, ArH), 7.12 (1H, d, J = 9 Hz, ArH), 9.74 (1H, s, CHO), 11.09 (1H, s, OH-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.58, 103.86, 116.32, 126.28, 133.25, 149.29, 153.23, 195.38; HRMS (APCI+) *m*/*z* 169.0554 $[M + H]^+$ (calcd for $C_8H_9O_4$, 169.0500).

2,3-Bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzaldehyde (14). DIPEA (68.5 g, 0.53 mol, 92.3 mL) was added to a stirred solution of **13** (28.9 g, 0.17 mol) in anhydrous DMF (200 mL) at rt. *tert*-Butyldimethylsilyl chloride (57.04 g, 0.37 mol) was added (in portions) over 5 min, and the reaction mixture was allowed to stir at rt for 3 h. Water (300 mL) was added to terminate the reaction, and the mixture was extracted with EtOAc (3 × 300 mL). The organic phase was washed consecutively with 10% $NaHCO_3$ (200 mL) and H_2O (200 mL). The organic phase was dried and the solvent was removed *in vacuo*. Purification by crystallization from CH_3OH furnished **14** in 84% (57.4 g) yield as a colorless solid: mp 75−76 °C [lit.[3] mp 74.5−76 °C]; R_f 0.76 (1:1 hexanes−EtOAc); IR (neat) ν_{max} 2932, 2859, 1683, 1585, 1455, 1297, 1099 cm^{-1}; ^1H NMR ($CDCl_3$ 300 MHz) δ 0.12 (12H, s, 2 × $(CH_3)_2Si$)), 0.98 (9H, s, *t*-Bu), 1.02 (9H, s, *t*-Bu), 3.82 (3H, s, OCH_3), 6.61 (1H, d, J = 8.9 Hz, ArH), 7.47 (1H, d, J = 8.9 Hz, ArH), 10.21 (1H, s, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −3.92, −3.84, 18.55, 18.74, 26.02, 26.17, 55.17, 105.40, 115.21, 120.75, 121.37, 123.36, 136.80, 150.99, 157.53, 189.26; HRMS (APCI+) *m*/*z* 397.2234 $[M + H]^+$ (calcd for $C_{20}H_{37}O_4Si_2$, 397.2230).

3,4-Methylenedioxy-4′,5-dimethoxy-2′,3′-bis(*tert*-butyldimethylsilyloxy)-*E*- and -*Z*-stilbenes (15). *n*-Butyllithium (22.17 mmol, 8.87 mL, 2.5 M by titration) was added dropwise to a stirred suspension of **6** (13.19 g, 21.17 mmol) at −10 °C in anhydrous THF (300 mL). After 10 min at rt **14** (7.98 g, 20.16 mmol) was added and stirring continued at rt. TLC analysis (10% EtOAc−hexanes, stain 2,4-DNPH-PMA) was used to follow the reaction. After 30 min the reaction was terminated by the addition of ice water (200 mL). The mixture was extracted with Et_2O (3 × 150 mL). The ethereal phase was rewashed with H_2O and dried, and the solvent was removed (*in vacuo*) to furnish crude 2′,3′-bis(*tert*-butyldimethylsilyloxy)-*E*,*Z*-stilstatin 1 as a yellow oil. Separation was initially achieved by flash chromatography, eluting with 95:5

hexanes−EtOAc. Fractions 15−28 were combined and separated again with a gradient elution of hexane−EtOAc (99:1, 4000 mL; 97:3, 3000 mL; 95:5, 3000 mL). By this means, two main fractions were obtained: the pure *Z*-isomer (1.13 g, 10%) as a colorless solid (recrystallized from DCM−hexanes) and a *Z*/*E* mix (5.5 g, 50%) as a colorless solid.

Z-15: mp 63−65 °C; R_f 0.25 (95:5 hexanes−EtOAc); IR (neat) ν_{max} 2931, 2857, 1592, 1492, 1442, 1310, 1250, 1101 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.00 (6H, s, $(CH_3)_2Si$), 0.06 (6H, s, $(CH_3)_2Si$), 0.88 (9H, s, *t*-Bu), 0.91 (9H, s, *t*-Bu), 3.60 (3H, s, OCH_3), 3.62 (3H, s, OCH_3), 5.80 (2H, s, O−CH_2−O), 6.23 (1H, d, J = 12.3 Hz, −CH═CH−), 6.27 (1H, d, J = 8.7 Hz, ArH), 6.40 (1H, s, ArH), 6.44 (1H, d, J = 12.6 Hz, −CH═CH−), 6.48 (1H, d, J = 1.2 Hz, ArH), 6.77 (1H, d, J = 8.7 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −3.89, −3.32, 18.52, 18.72, 26.12, 26.34, 54.90, 56.21, 101.27, 102.81, 104.40, 108.31, 121.83, 123.20, 126.76, 127.76, 132.01, 134.10, 136.86, 143.13, 146.04, 148.45, 151.64; HRMS (APCI+) *m*/*z* 545.2754 $[M + H]^+$ (calcd for $C_{29}H_{45}O_6Si_2$, 545.2759); *anal.* C 64.33%, H 8.34%, calcd for $C_{29}H_{44}O_6Si_2$, C 63.93%, H 8.14%.

Stilstatin 1 (2). To a solution of *Z*-**15** (0.87 g, 1.60 mmol) in anhydrous THF (8 mL) was added a solution of TBAF (3.52 mmol, 3.52 mL, 1.0 M solution in THF) at 0 °C. The reaction mixture was stirred with warming to ambient temperature over 2 h. The reaction was terminated by addition of ice-cold 6 N HCl (4.82 mL) and extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine (2 × 30 mL) and dried, and the solvent was removed (*in vacuo*) to yield a dark brown oil. Separation using flash chromatography and eluting with 1:1 hexanes−EtOAc furnished **2** as a tan oil in 74% (0.37 g) yield: R_f 0.24 (1:1 hexanes−EtOAc); IR (neat) ν_{max} 3487, 3006, 2900, 1624, 1508, 1290, 800, 736 cm^{-1}; ^{1}H NMR ($CDCl_3$,300 MHz) δ 3.76 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 5.45 (2H, brs, 2 × OH), 5.90 (2H, s, O−CH_2−O), 6.38 (1H, d, J = 8.1 Hz), 6.45−6.51 (4H, m, 2 × −CH═CH−, 2 × ArH), 6.74 (1H, d, J = 9.0 Hz, ArH); ^{13}C NMR ($CDCl_3$,100 MHz) δ 56.08, 56.22, 101.31, 102.77, 102.98, 108.41, 117.72, 120.18, 123.65, 130.04, 131.54, 132.57, 134.43, 141.52, 143.21, 146.28, 148.49; HRMS *m*/*z* 317.1025 $[M + H]^+$ (calcd for $C_{17}H_{17}O_6$, 317.1010).

3,4-Methylenedioxy-4′,5-dimethoxy-2′,3′-*O*-di[bis(benzyl)phosphory]-*E*- and -*Z*-stilbenes (16). A mixture of DIPEA (1.67 g, 12.95 mmol, 2.25 mL), DMAP (0.07 g, 0.63 mmol), and dibenzyl phosphite (2.51 g, 9.57 mmol, 2.12 mL, added dropwise over 5 min) was added to a cooled (−10 °C) solution prepared in order from acetonitrile (5 mL), *E,Z* -**2** (1.0 g, 3.19 mmol), and CCl_4 (4.91 g, 31.9 mmol, 3.08 mL) that had stirred for 10 min. After 3 h at −10 °C the reaction was treated with KH_2PO_4 (0.5 M, 50 mL), stirred for a further 10 min, and extracted with EtOAc (3 × 100 mL). The organic phase was dried and the solvent removed (*in vacuo*) to furnish a yellow oil. The products were separated by flash chromatography in hexanes−EtOAc (gradient elution: 3:1, 1000 mL; 7:3, 1000 mL; 1:1, 3000 mL). The result was three main fractions: pure *Z*-isomer (0.81 g, 31%) as a yellow oil; a *Z*/*E* mixture (1.16 g, 44%); and pure *E*-isomer (0.38 g, 14%) as a colorless solid.

Z-16: R_f 0.25 (1:1 EtOAc−hexanes); IR (neat) ν_{max} 2955, 1609, 1502, 1453, 1286, 1212, 1013, 739 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.65 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 5.06−5.09 (4H, m, 2 × CH_2Ph), 5.16−5.18 (4H, m, 2 × CH_2Ph), 5.87 (2H, s, O−CH_2−O), 6.44 (2H, s, ArH), 6.47 (1H, d, J = 11.6 Hz, −CH═CH-), 6.59 (1H, d, J = 11.6 Hz, −CH═CH−), 6.67 (1H, d, J = 8.7 Hz, ArH), 7.02 (1H, d, J = 9.0 Hz, ArH), 7.20−7.30 (20H, m, 2 × Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.21, 56.27, 69.65, 69.70, 69.86, 69.91, 101.29, 102.94, 108.52, 109.29, 123.75, 124.30, 126.61, 127.74−128.50, 131.17, 134.38, 135.57, 135.64, 135.84, 135.92, 143.28, 148.46, 151.45; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −4.91 (d, J = 3.2 Hz), −4.85 (d, J = 2.9 Hz); HRMS (APCI+) *m*/*z* 837.2228 $[M + H]^+$ (calcd for $C_{45}H_{43}O_{12}P_2$, 837.2230).

E-16: mp 145−146 °C (EtOAc−hexanes); R_f 0.20 (1:1 EtOAc−hexanes); IR (neat) ν_{max} 2895, 1610, 1507, 1454, 1293, 1041, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.80 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 5.04 (4H, m, CH_2Ph), 5.21 (4H, m, CH_2Ph), 5.97 (2H, s, O−CH_2−O), 6.59 (1H, d, J = 1.2 Hz, ArH), 6.66 (1H, d, J = 1.2 Hz, ArH), 6.84 (1H, d, J = 16.4 Hz, −CH═CH−), 6.84 (1H, d, J = 10.0 Hz, ArH), 7.16−7.31 (11H, m, 2 × Ph, −CH═CH−), 7.41 (1H, d, J = 8.8 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.34, 56.42, 69.75, 69.79, 70.09, 100.14, 101.47, 106.83, 109.82, 121.12, 122.11, 127.79−129.09, 132.47, 135.03, 135.45, 135.52, 135.88, 135.94, 143.56, 149.13; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −5.06 (d, J = 3.5 Hz), −4.87 (d, J = 2.4 Hz); HRMS (APCI+) *m*/*z* 837.2247 $[M + H]^+$ (calcd for $C_{45}H_{43}O_{12}P_2$, 837.2230); *anal.* C 63.34%, H 5.28%, calcd for $C_{45}H_{42}O_{12}P_2 \cdot CH_3CH_2OCOCH_3$, C 63.58%, H 5.40%.

Potassium Stilstatin 1 2′,3′-*O*-Diphosphate (18). TMSBr (152 mg, 0.99 mmol, 130 μL) was added (dropwise) to a solution of *Z*-**16** (208 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (16 mL), and the mixture stirred at rt for 30 min. The reaction was terminated by the addition of 10% $Na_2S_2O_7$ solution (10 mL) and extracted with *n*-butanol (3 × 10 mL), and the solvent was evaporated (*in vacuo*) to afford the phosphoric acid intermediate **17** as a colorless oil. Phosphoric acid **17** was dissolved in CH_3OH (10 mL) and cooled to 0 °C. KOH solution in CH_3OH (1.0 N, 5 mL) was added, and the reaction mixture was allowed to stir at 0 °C for 30 min. Et_2O (20 mL) was added at 0 °C to allow for precipitation of the solid salt, which was collected and washed with further portions of Et_2O (3 × 30 mL). After drying under reduced pressure, the off-white solid was reprecipitated with H_2O−acetone. The resultant phosphate prodrug was then crystallized from EtOH−H_2O to furnish a colorless solid in 57% (89 mg) yield: mp 152−154 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.75 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 5.78 (2H, s, O−CH_2−O), 6.47 (1H, d, J = 12.3 Hz, −CH═CH−), 6.48 (2H, s, ArH), 6.50 (1H, d, J = 12.3 Hz, −CH═CH−), 6.56 (1H, d, J = 8.4 Hz, ArH), 6.80 (1H, d, J = 8.4 Hz, ArH).

General Procedure for the Synthesis of Other Stilstatin 1 Prodrugs by Ion Exchange. The exchange resin Dowex-50W was washed consecutively with H_2O (200 mL), CH_3OH (200 mL), H_2O (200 mL), 1 N HCl (until pH 1), H_2O (until pH 7), and 1.0 M metal hydroxide (until pH 14) and rinsed with H_2O (until pH 7). The potassium stilstatin 1 2′,3′-*O*-phosphate salt (25 mg) was dissolved in H_2O (5 mL) and passed down the resin, eluting with H_2O (30 mL). Fractions were spotted onto TLC plates, and the product-containing fractions were visualized using both UV light and I_2 vapor. The pooled fractions were reduced to dryness via freeze-drying.

Sodium Stilstatin 1 2′,3′-*O*-Diphosphate (19). Recrystallization from H_2O−acetone and H_2O−EtOH yielded the sodium salt as a colorless solid (14.6 mg) in 58% yield: mp 176−177 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.57 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 5.77 (2H, s, O−CH_2−O), 6.41 (1H, s, ArH), 6.46 (1H, d, J = 12.3 Hz, −CH═CH−), 6.48 (1H, s, ArH), 6.50 (1H, d, J = 12.3 Hz, −CH═CH−), 6.56 (1H, d, J = 8.1 Hz, ArH), 6.79 (1H, d, J = 8.1 Hz, ArH).

Lithium Stilstatin 1 2′,3′-*O*-Diphosphate (20). Recrystallization from H_2O−acetone and H_2O−EtOH yielded the lithium salt as a colorless solid (16.0 mg) in 70% yield: mp 208−209 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.56 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 5.76 (2H, s, O−CH_2−O), 6.40 (1H, s, ArH) 6.46 (1H, s, ArH), 6.49 (1H, d, J = 10.4 Hz, −CH═CH−), 6.52 (1H, d, J = 10.4 Hz, −CH═CH−), 6.57 (1H, d, J = 7.2 Hz, ArH), 6.78 (1H, d, J = 8.4 Hz, ArH).

3-*tert*-Butyldimethylsilyloxy-4,5-dimethoxybenzoate (23). A solution of anhydrous DMF (1000 mL), *t*-BDMSCl (72.0 g, 0.47 mol), and DIPEA (59.45 g, 0.46 mol, 80.0 mL) was stirred at rt for 10 min, and methyl gallate (**22**, 80.0 g, 0.43 mol) was added portionwise. After the mixture had stirred at rt for 2 h, NaH (60%, 56.0 g, 1.4 mol) was added, followed 30 min later by CI_4 (204.4 g, 1.44 mol, 90.0 mL). After 4 h the reaction was terminated by the addition of H_2O (500 mL) and extracted with hexanes (4 × 300 mL), the organic phase was dried, and the solvent was evaporated *in vacuo*. The residue was separated by flash chromatography with 98:2 hexanes−EtOAc (2000 mL) followed by 95:5 hexanes−EtOAc (4000 mL) as eluant to furnish the title compound in 45% (63.1 g) yield as a colorless oil: R_f 0.20 (95:5 hexanes−EtOAc); IR (neat) ν_{max} 2953, 2857, 1721, 1584, 1347, 1222, 1115, 834 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.15 (6H, s, $(CH_3)_2Si$), 0.96 (9H, s, *t*-Bu), 3.79 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 7.14 (1H, d, J = 3.0 Hz, ArH), 7.18 (1H, d, J = 3.0 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.50, 18.15, 25.82, 52.27, 56.23, 60.55, 106.94, 116.06, 125.28, 144.87, 149.31, 153.61, 166.87; GC MS (CI+) *m*/*z* 327.1654 $[M + H]^+$ (calcd for $C_{16}H_{27}O_5Si$, 327.1628).

3-*tert*-Butyldimethylsilyloxy-4,5-dimethoxybenzyl alcohol (24). A suspension of $LiAlH_4$ (4.44 g, 0.11 mol) in anhydrous THF (200 mL) was stirred at rt for 10 min. A solution of **23** (28.5 g, 0.09 mol) in anhydrous THF (20 mL) was next added dropwise over 30 min. TLC evaluation indicated the presence of starting material, and therefore a further portion of $LiAlH_4$ (2.0 g, 0.05 mol) was added and the mixture

stirred at rt for 12 h. The reaction mixture was cooled to 0 °C and terminated by careful addition of H_2O (20 mL) and brine (300 mL). The solvent and suspension was passed through a bed of Celite and the filtrate extracted with EtOAc (3 × 100 mL). The organic phase was dried, and the solvent was removed *in vacuo* to furnish the crude product. Purification was achieved by chromatography, and elution with 3:2 hexanes−EtOAc gave access to the title compound as a clear oil in 89% (23.9 g) yield: R_f 0.05 (9:1 hexanes−EtOAc); IR (neat) ν_{max} 3427, 2953, 2858, 1586, 1427, 1113 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.00 (6H, s, $(CH_3)_2Si$), 0.83 (9H, s, *t*-Bu), 1.5 (1H, s, OH), 3.58 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 4.39 (2H, s, CH_2OH), 6.32 (1H, d, J = 3.0 Hz, ArH), 6.43 (1H, d, J = 3.5 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.67, 18.28, 25.66, 55.91, 60.34, 65.30, 104.04, 112.59, 136.37, 139.71, 149.43, 153.82; HRMS (APCI+) *m/z* 281.1581 [M + H − H_2O]$^+$ (calcd for $C_{15}H_{25}O_3Si$, 281.1573).

3-*tert*-Butyldimethylsilyloxy-4,5-dimethoxybenzyltriphenylphosphonium Bromide (21). To a cooled (0 °C) solution of **24** (26.3 g, 88.22 mmol) in anhydrous CH_2Cl_2 (340 mL) was added dropwise a solution of PBr_3 (11.94 g, 44.11 mmol, 4.14 mL) in anhydrous CH_2Cl_2 (120 mL). After stirring at 0 °C for 10 min, the reaction was terminated by careful addition of saturated $NaHCO_3$ (20 mL). The organic phase was dried and the solvent evaporated (*in vacuo*). The bromide intermediate (**25**) was dissolved in toluene (370 mL), and a solution of Ph_3P (27.8 g, 105.86 mmol) in toluene (200 mL) was added. The reaction mixture was stirred at room temperature for 10 min, heated at reflux for 2 h, and then allowed to cool with stirring over 24 h. The resultant precipitate was collected via filtration, dried under reduced pressure, and crystallized from EtOH as a colorless solid in 66% (36.0 g) yield: mp 249−250 °C; R_f 0.59 (9:1 CH_2Cl_2−CH_3OH); IR (neat) ν_{max} 2955, 1585, 1435, 1111, 730 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.00 (6H, s, $(CH_3)_2Si$), 0.89 (9H, s, *t*-Bu), 3.73 (3H, s, OCH_3), 5.37 (2H, d, J = 14.1 Hz, CH_2P), 6.11 (1H, t, J = 2.1 Hz, ArH), 6.79 (1H, t, J = 2.1 Hz, ArH), 7.9 (15H, m, 3 × Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.97, 18.00, 25.39, 30.53 (d, J = 47.2 Hz), 56.16, 60.22, 109.98 (d, J = 6.1 Hz), 115.78 (d, J = 5.3 Hz), 117.23, 118.08, 122.20 (d, J = 9.2 Hz), 129.89−134.79, 140.22 (d, J = 4.5 Hz), 149.15 (d, J = 3.8 Hz), 153.67 (d, J = 3.0 Hz); ^{31}P NMR($CDCl_3$, 162 MHz) δ 23.62; HRMS (APCI+) *m/z* 543.2487 [M − Br]$^+$ (calcd for $C_{33}H_{40}O_3PSi$, 543.2484).

2′,3,3′-Tris(*tert*-butyldimethylsilyloxy)-4,4′,5-trimethoxy-*E*- and -*Z*-stilbenes (26). To a stirred suspension of **21** (37.24 g, 59.76 mmol) at −10 °C in anhydrous THF (1000 mL) was added (dropwise) *n*-BuLi (62.62 mmol, 25.04 mL, 2.5 M by titration). After 10 min, **14** (22.54 g, 56.92 mmol) was added to the red solution at rt. TLC analysis (1:3 EtOAc−hexanes, stain 2,4-DNPH/PMA) was used to follow the reaction, which was terminated (after 30 min) by the addition of ice water (1400 mL). The mixture was extracted with Et_2O (3 × 500 mL). The ethereal phase was rewashed with H_2O (2 × 250 mL) and dried and the solvent removed (*in vacuo*) to furnish crude 2′,3,3′-tris(*tert*-butyldimethylsilyloxy)-*E*- and -*Z*-stilstatin 2 as a yellow oil. Separation was done by flash chromatography using a gradient elution beginning with hexanes (2 L) and continuing with hexanes−EtOAc (97:3, 3 L; 95:5, 3 L). Fractions 13−19 were combined and further separated using medium-pressure liquid chromatography [LOBAR size B (310−25) Si60, 3 × 1-inch columns in series, 1.0 g injections]. By this procedure three main fractions were obtained: pure *Z* (5.45 g, 14%) as a colorless solid recrystallized from CH_2Cl_2−hexanes; a mixture of *Z* and *E* (25.12 g, 67%) as a colorless solid; and pure *E* (1.16 g, 3%) as a colorless solid.

***Z*-26:** mp 135−137 °C; R_f 0.45 (95:5 hexanes−EtOAc); IR (neat) ν_{max} 2931, 2857, 1574, 1497, 1449, 1250, 1103, 837 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.04 (12H, s, 2 × $(CH_3)_2Si$), 0.07 (6H, s, $(CH_3)_2Si$), 0.85 (9H, s, *t*-Bu), 0.88 (9H, s, *t*-Bu), 0.93 (9H, s, *t*-Bu), 3.53 (3H, s, OCH_3), 3.61 (3H, s, OCH_3), 3.65 (3H, s, OCH_3), 6.22 (1H, d, J = 12.3 Hz, −CH═CH−), 6.24 (1H, d, J = 8.4 Hz, ArH), 6.42 (2H, s, ArH), 6.47 (1H, d, J = 12.0 Hz, −CH═CH−), 6.80 (1H, d, J = 8.7 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.75, −3.94, −3.34, 18.18, 18.51, 18.68, 25.63, 26.08, 26.33, 54.86, 55.88, 60.33, 104.33, 105.93, 114.39, 122.01, 123.16, 126.90, 127.44, 132.78, 136.71, 139.24, 146.08, 148.97, 151.55, 153.03; HRMS (APCI+) *m/z* 661.3771 [M + H]$^+$ (calcd for $C_{35}H_{61}O_6Si_3$, 661.3776); *anal*. C 62.68%, H 9.69%, calcd for $C_{35}H_{60}O_6Si_3 \cdot 1/2H_2O$, C 62.78%, H 9.11%.

E-26: mp 147−149 °C; R_f 0.44 (95:5 hexanes−EtOAc); IR (neat) ν_{max} 2931, 2857, 1574, 1497, 1449, 1331, 1250, 1103, 837 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.02 (6H, s, $(CH_3)_2Si$), 0.04 (6H, s, $(CH_3)_2Si$), 0.10 (6H, s, $(CH_3)_2Si$), 0.90 (9H, s, *t*-Bu), 0.93 (9H, s, *t*-Bu), 0.99 (9H, s, *t*-Bu), 3.69 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 6.46 (1H, d, J = 8.1 Hz, ArH), 6.51 (1H, d, J = 16.5 Hz, −CH═CH−), 6.62 (1H, s, ArH), 6.68 (1H, s, ArH), 7.10 (1H, d, J = 8.7 Hz, ArH), 7.17 (1H, d, J = 16.50 Hz, −CH═CH−); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.67, −3.85, −3.52, 18.30, 18.61, 18.75, 25.70, 26.11, 26.46, 54.96, 55.75, 60.42, 102.64, 105.12, 112.73, 117.38, 123.81, 123.89, 126.06, 133.63, 136.89, 139.60, 145.43, 149.39, 151.72, 153.68; HRMS (APCI+) *m/z* 661.3771 [M + H]$^+$ (calcd for $C_{35}H_{61}O_6Si_3$, 661.3776); *anal*. C 63.35%, H 9.77%, calcd for $C_{35}H_{60}O_6Si_3$, C 63.59%, H 9.15%.

***Z*-Stilstatin 2 (*Z*-5).** To a cooled (0 °C) solution of *Z*-**26** (2.0 g, 3.03 mmol) in anhydrous THF (10 mL) was added TBAF (9.99 mL, 9.99 mmol, 1.0 M solution in THF), and the reaction mixture was stirred at 0 °C for 90 min. Ice-cold 6 N HCl (13.6 mL) was added, and the mixture was extracted with EtOAc (4 × 50 m). The organic phase was dried and the solvent removed (*in vacuo*) to afford a brown oil. Separation via column chromatography with 1:1 acetone−hexanes as eluant provided the title compound, which crystallized from EtOAc−hexanes in 76% yield (0.73 g): mp 147−148 °C; R_f 0.46 (49.5:49.5:1 EtOAc−hexanes−HOAc); IR (neat) ν_{max} 3434, 2933, 1625, 1583, 1508, 1464, 1288, 1091, 997, 912, 731 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.62 (3H, s, OCH_3), 3.87 (6H, s, 2 × OCH_3), 5.34 (2H, brs, 2 × OH), 5.67 (1H, s, OH), 6.39 (1H, d, J = 9 Hz, ArH), 6.41 (1H, s, ArH), 6.51 (1H, d, J = 12.0 Hz, −CH═CH−), 6.54 (1H, s, ArH), 6.58 (1H, d, J = 12.6 Hz, −CH═CH−), 6.76 (1H, d, J = 9 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.54, 56.09, 60.92, 102.94, 104.72, 108.71, 117.74, 120.28, 124.17, 130.00, 132.57, 133.01, 134.73, 141.58, 146.32, 148.83, 151.80; HRMS (APCI+) *m/z* 319.1238 [M + H]$^+$ (calcd for $C_{17}H_{19}O_6$, 319.1181); *anal*. C 63.25%, H 5.83%, calcd for $C_{17}H_{18}O_6 \cdot 1/2CH_3CH_2OCOCH_3$, C 62.97%, H 6.12%.

***E*-Stilstatin 2 (*E*-5).** To a cooled (0 °C) solution of *E*-**26** (1.0 g, 1.51 mmol) in anhydrous THF (5 mL) was added TBAF (4.98 mmol, 4.98 mL, 1.0 M solution in THF). After 90 min at 0 °C, ice-cold 6 N HCl (6.77 mL) was added, and the mixture was extracted with EtOAc (4 × 50 mL). The dried extract was evaporated (*in vacuo*) to a brown oil. Separation of the oil using column chromatography and elution with 1:1 hexanes−EtOAc gave a solid, which crystallized from EtOAc−hexanes in 71% (0.34 g) yield: mp 178−180 °C; R_f 0.35 (1:1 EtOAc−hexanes); IR (neat) ν_{max} 3445, 1585, 1511, 1291, 1096, 910, 734 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.81 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 5.62 (1H, brs, OH), 5.79 (1H, s, OH), 5.85 (1H, s, OH), 6.42 (1H, d, J = 9.0 Hz, ArH), 6.57 (1H, d, J = 1.8 Hz, ArH), 6.71 (1H, d, J = 1.8 Hz, ArH), 6.94 (1H, d, J = 16.5 Hz, −CH═CH−), 6.97 (1H, d, J = 8.7 Hz, ArH), 7.17 (1H, d, J = 16.5 Hz, −CH═CH−); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.88, 56.16, 61.05, 102.29, 103.04, 106.05, 117.76, 118.34, 122.98, 127.59, 132.25, 134.46, 134.95, 142.08, 146.17, 149.27, 152.36; HRMS (APCI+) *m/z* 319.1238 [M + H]$^+$ (calcd for $C_{17}H_{19}O_6$, 319.1181).

2′,3,3′-*O*-Tri[bis(benzyl)phosphoryl]-4,4′,5-trimethoxy-*E*- and -*Z*-stilbenes (27a,c) and 2′,3-*O*-Di[bis(benzyl)phosphoryl]-3′-hydroxy-4,4′,5-trimethoxy-*E*- and -*Z*-stilbenes (27b,d). A solution prepared from DIPEA (6.32 g, 48.92 mmol, 8.52 mL), DMAP (0.30 g, 2.40 mmol), and dibenzyl phosphite (9.68 g, 36.92 mmol, 8.15 mL) in that order was added (dropwise) over 5 min to a cooled (−10 °C) solution prepared from acetonitrile (13 mL), *Z*-, *E*-stilstatin 2 (*Z*-, *E*-**5**, 2.54 g, 8.00 mmol), and CCl_4 (18.46 g, 120.0 mmol, 11.59 mL) in that order and stirred for 10 min. After 3 h at −10 °C the mixture was treated with KH_2PO_4 (0.5 M, 100 mL), stirred for a further 10 min, and extracted with EtOAc (3 × 150 mL). The organic phase was dried and the solvent evaporated (*in vacuo*) to a yellow oil. The products were separated by flash chromatography using a gradient elution sequence of 1:1 (4000 mL) to 1:3 (1400 mL) to 1:4 (2000 mL) hexanes−EtOAc, resulting in three main fractions: a mixture of the di- (**27b**) and triphosphorylated (**27a**) *Z*-isomers (0.94 g, 11%) as a yellow oil, pure **27a** (2.52 g, 26%) as a yellow oil, and a mixture of the di- (**27d**) and triphosphorylated (**27c**) *E*-isomers (3.31 g, 37%) as a white solid.

2′,3,3′-*O*-Tri[bis(benzyl)phosphoryl]-4,4′,5-trimethoxy-*Z*-stilbene (27a): R_f 0.18 (7:3 EtOAc−hexanes); IR (neat) ν_{max} 3050, 2923, 2852, 1502, 1453, 1281, 1100, 999, 735 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.50 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.81 (3H, s,

OCH_3), 5.06−5.19 (12H, m, CH_2Ph), 6.42 (1H, d, J = 11.4 Hz, −CH=CH−), 6.64 (3H, d, J = 9.9 Hz, 2 × ArH, −CH=CH−), 6.76 (1H, s, ArH), 6.98 (1H, d, J = 8.7 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.91, 56.36, 61.08, 69.69, 69.73, 69.77, 69.82, 69.91, 69.96, 109.36, 109.83, 114.57, 124.17, 124.93, 126.87, 130.56, 132.11, 135.54−135.87, 139.52, 143.80, 149.32, 151.57, 152.37, 153.15; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −5.68, −5.04 (d, J = 3.7 Hz), −4.76 (d, J = 2.4 Hz); HRMS (APCI+) m/z 1099.2961 $[M + H]^+$ (calcd for $C_{59}H_{58}O_{15}P_3$, 1099.2988).

2′,3,3′-*O*-Tri[bis(benzyl)phosphoryl]-4,4′,5-trimethoxy-*E*-stilbene (27c): R_f 0.13 (7:3 EtOAc−hexanes); IR (neat) ν_{max} 3033, 2839, 1578, 1504, 1453, 1280, 1100, 999, 731 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.70 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 5.00−5.10 (4H, m, CH_2Ph), 5.15−5.21 (8H, m, CH_2Ph), 6.78 (1H, d, J = 16.0 Hz, −CH=CH−), 6.84 (1H, d, J = 9.5 Hz, ArH), 6.84 (1H, s, ArH), 6.92 (1H, s, ArH), 7.13−7.35 (31H, m, 6 × Ph, 1 × ArH), 7.40 (1H, d, J = 9.0 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.85, 56.28, 61.12, 69.72, 69.76, 69.86, 69.91, 70.05, 70.09, 106.11, 109.80, 112.83, 112.85, 122.18, 122.40, 124.08, 127.74, 127.85, 127.96, 128.24, 128.31, 128.36, 128.47, 132.92, 133.18, 135.36, 135.41, 135.50, 135.56, 135.79, 135.85, 139.92, 139.97, 143.96, 144.02, 151.70, 153.72; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −5.63, −5.12 (d, J = 2.7 Hz), −4.91 (d, J = 2.7 Hz); HRMS APCI$^+$ m/z 1099.2961 $[M + H]^+$ (calcd for $C_{59}H_{58}O_{15}P_3$, 1099.2988).

Sodium Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (29). To a solution of **27a** (300 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (15 mL) was added TMSBr (267 mg, 1.74 mmol, 230 μL), and the reaction mixture stirred at rt for 30 min. After addition of H_2O (10 mL), the phases were separated and the aqueous layer extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried and concentrated (*in vacuo*) to provide the phosphoric acid intermediate (**28**). The resulting oil was dissolved in EtOH (10 mL), and NaOMe (94.0 mg, 1.74 mmol) was added. An immediate precipitate formed and after 30 min was collected and washed with EtOAc (3 × 20 mL) and hexanes (3 × 20 mL). After drying under reduced pressure the off-white solid was reprecipitated with H_2O−acetone. The resultant solid crystallized from EtOH−H_2O as an off-white solid (134 mg, 71% yield): mp 185−187 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.44 (3H, s, OCH_3), 3.64 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 6.51 (1H, d, J = 11.9 Hz, −CH=CH−), 6.58 (1H, d, J = 10 Hz, ArH), 6.62 (1H, d, J = 11.9 Hz, −CH=CH−), 6.62 (1H, s, ArH), 6.81 (1H, s, ArH), 6.84 (1H, d, J = 10 Hz, ArH).

General Procedure for the Synthesis of Stilstatin 2 Phosphate Prodrugs. Each of the metal cation-containing salts was obtained by the procedure outlined above for the preparation of the sodium salt. The metal counterions were introduced by treatment of the phosphoric acid intermediate using the corresponding hydroxide (Li, K, and Ca), hydrogen carbonate (Rb), or acetate (Zn, Mg, and Mn). The alkali metal salts were further purified by ion-exchange chromatography as follows. Dowex-50W was treated in succession with H_2O (200 mL), CH_3OH (200 mL), H_2O (200 mL), 1 N HCl (until pH 1), H_2O (until pH 7), and 1.0 M metal hydroxide (until pH 14) and rinsed with H_2O (until pH 7). The phosphate prodrug (25 mg) was dissolved in H_2O (5 mL) and passed down the resin, eluting in H_2O (30 mL). Fractions were spotted onto TLC plates, and the product-containing fractions were visualized using both UV light and I_2 vapor. The pooled fractions were reduced to dryness via freeze-drying.

Potassium Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (30). Precipitation from H_2O−acetone followed by H_2O−EtOH provided the potassium salt as a colorless solid (68 mg, 74%): mp 193−194 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.41 (3H, s, OCH_3), 3.67 (6H, s, 2 × OCH_3), 6.48 (1H, d, J = 12.8 Hz, −CH=CH−), 6.55 (1H, s, ArH), 6.58 (1H, d, J = 7.5 Hz, ArH), 6.83 (1H, d, J = 7.5 Hz, ArH), 6.83 (1H, d, J = 12.8 Hz, −CH=CH−), 7.01 (1H, s, ArH).

Lithium Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (31). Precipitation (2×) from H_2O−acetone and H_2O−EtOH provided the lithium salt as a yellow solid (63 mg, 39%): mp 174−176 °C; IR (solid) ν_{max} 3712, 3058, 2949, 2850, 1752, 1576, 1437, 1342, 1108, 609 cm^{-1}.

Piperidine Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (32). The piperidine prodrug was obtained via ion exchange from the potassium prodrug (32.9 mg), the resin being loaded with piperidine. After eluting in H_2O (30 mL), followed by freeze-drying and reprecipitation from H_2O−acetone, the prodrug was obtained as a tan solid (25 mg, 75%): mp 145−147 °C; ^{1}H NMR (D_2O, 300 MHz) δ 1.51 (12H, m, $-CH_2CH_2CH_2-$), 1.62 (24H, m, $CH_2CH_2CH_2$), 3.00 (24H, t, J = 5.5 Hz, $-CH_2N-$), 3.67 (6H, s, 2 × OCH_3), 3.77 (3H, s, OCH_3), 6.46 (1H, d, J = 11.8 Hz, −CH=CH−), 6.57 (1H, d, J = 9.0 Hz, ArH), 6.57 (1H, s, ArH), 6.60 (1H, d, J = 11.8 Hz, −CH=CH−), 6.81 (1H, d, J = 8.5 Hz, ArH), 6.83 (1H, s, ArH).

Morpholine Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (33). The morpholine prodrug was obtained from the potassium prodrug (30 mg) via ion exchange on morpholine-loaded resin. After eluting in H_2O (30 mL), the prodrug was freeze-dried and precipitated from H_2O−acetone as an off-white solid (25 mg, 73%): mp 148−149 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.14 (24H, t, J = 4.8 Hz, 6 × $(CH_2)_2$) 3.65 (3H, s, OCH_3), 3.66 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 3.79 (24H, t, J = 5.2 Hz, 6 × $(CH_2)_2$), 6.45 (1H, d, J = 12.4 Hz, −CH=CH−), 6.55 (1H, d, J = 8.8 Hz, ArH), 6.64 (1H, d, J = 12.0 Hz, −CH=CH−), 6.76 (1H, brs, ArH), 6.85 (1H, d, J = 9.2 Hz, ArH) 6.90 (1H, brs, ArH).

Rubidium Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (34). The rubidium prodrug was precipitated from H_2O−acetone, collected, and dried under vacuum. The procedure was repeated three times. The solid was reprecipitated from H_2O−EtOH as a tan solid (195 mg, 83%): mp 188−190 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.42 (3H, s, OCH_3), 3.63 (3H, s, OCH_3), 3.68 (3H, s, OCH3), 6.40 (1H, d, J = 12.4 Hz, −CH=CH−), 6.46 (1H, d, J = 8.8 Hz, ArH), 6.61 (1H, s ArH), 6.75 (1H, d, J = 12.4 Hz, −CH=CH−), 6.86 (1H, d, J = 8.8 Hz, ArH), 6.93 (1H, s, ArH).

Calcium Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (35). The solid that separated over 30 min was collected and washed successively with hexanes (3 × 30 mL) and Et_2O (3 × 30 mL). Reprecipitation from H_2O−acetone led to an off-white solid (55 mg, 36%): mp 201−203 °C; IR (solid) ν_{max} 3630, 3362, 3005, 1680, 1615, 1405, 1107, 873, 621 cm^{-1}.

Zinc Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (36). The mixture was stirred for 30 min, and the resulting solution was evaporated to dryness. The solid residue was washed with hexanes (3 × 30 mL), Et_2O (3 × 30 mL), and hexanes (3 × 30 mL) to afford a tan solid (134 mg) in 72% yield: mp 185−187 °C; IR (solid) ν_{max} 3594, 2955, 1541, 1455, 1263, 1105, 1024 cm^{-1}.

Magnesium Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (37). After the mixture was stirred for 30 min, the solvent was evaporated to dryness. The resulting solid was washed with hexanes (3 × 30 mL), Et_2O (3 × 30 mL), and hexanes (3 × 30 mL). This method gave an off-white solid (125 mg) in 92% yield: mp 157−158 °C; IR (solid) ν_{max} 3447, 3326, 2961, 1543, 1418, 1262, 1102, 806 cm^{-1}.

Manganese Stilstatin 2 2′,3,3′-*O*-Triphosphate Prodrug (38). The manganese prodrug was prepared as summarized above for the magnesium salt and provided an off-white solid (100 mg) in 72% yield: mp 156−157 °C; IR (solid) ν_{max} 3474, 2960, 2921, 1550, 1395, 1262, 1101, 1022, 803 cm^{-1}.

Cancer Cell Line Bioassay Evaluations. Cancer cell growth inhibitory data were obtained using the National Cancer Institute's standard sulforhodamine B assay as described earlier.[15,16]

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References and Notes

(1) Pinney, K. G.; Edvardsen, J. K.; Chaplin, D. J.; Pettit, G. R. In *Anticancer Agents from Natural Products*; Cragg, G. M., Kingston, G. I., Newman, D. J., Eds.; Talyor and Francis: Boca Raton, FL, 2005; pp 26−46.
(2) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209–211.
(3) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119–131.
(4) Pettit, G. R.; Rhodes, M. *Anti-Cancer Drug Des.* **1998**, *13*, 183–191.

(5) Pettit, G. R.; Lippert, J. W., III *Anti-Cancer Drug Des.* **2000**, *15*, 203–216.
(6) Lippert, J. W., III *Bioorg. Med. Chem.* **2007**, *15*, 605–615.
(7) Sheng, Y.; Hua, J.; Pinney, K. G.; Garner, C. M.; Kane, R. R.; Prezio, J. A.; Chaplin, D. J.; Edvardsen, K. *Int. J. Cancer* **2004**, *111*, 604–610.
(8) Salmon, H. W.; Siemann, D. W. *Clin. Cancer Res.* **2006**, *12*, 4090–4094.
(9) (a) Pettit, G. R.; Thornhill, A. J.; Moser, B. R.; Hogan, F. *J. Nat. Prod.* **2008**, *71*, 1561–1563. (b) Folkes, L. K.; Christlieb, M.; Madej, E.; Stratford, M. R. L.; Wardman, P. *Chem. Res. Toxicol.* **2007**, *20*, 1885–1894.
(10) Ellis, J. E.; Lenger, S. R. *Synth. Commun.* **1998**, *28*, 1517–1524.
(11) (a) Dallacker, F.; Sanders, J. *Chem. Ztg.* **1984**, *108*, 186–187. (b) Shirasaka, T.; Takuma, Y.; Imaki, N. *Synth. Commun.* **1990**, *20*, 1223–1232. (c) Pettit, G. R.; Singh, S. B. *Can. J. Chem.* **1987**, *65*, 2390–2396.
(12) Pettit, G. R.; Minardi, M. D.; Boyd, M. R.; Pettit, R. K. *Anti-Cancer Drug Des.* **2000**, *15*, 397–403.
(13) Pettit, G. R.; Moser, B. R.; Boyd, M. R.; Schmidt, J. M.; Pettit, R. K.; Chapuis, J.-C. *Anti-Cancer Drug Des.* **2001**, *16*, 185–193.
(14) Baker, W.; Evans, C. *J. Chem. Soc.* **1938**, 372–375.
(15) Pettit, G. R.; Tan, R.; Pettit, R. K.; Smith, T. H.; Feng, S.; Doubek, D. L.; Richert, L.; Hamblin, J.; Weber, C.; Chapuis, J.-C. *J. Nat. Prod.* **2007**, *70*, 1069–1072.
(16) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.

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