Antineoplastic Agents. 443. Synthesis of the Cancer Cell Growth Inhibitor Hydroxyphenstatin and Its Sodium Diphosphate Prodrug

George R. Pettit,*,† Matthew P. Grealish,† Delbert L. Herald,† Michael R. Boyd,‡ 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, and Laboratory of Drug Discovery Research and Development and Screening Technologies Branch, DTP/DCTD, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201

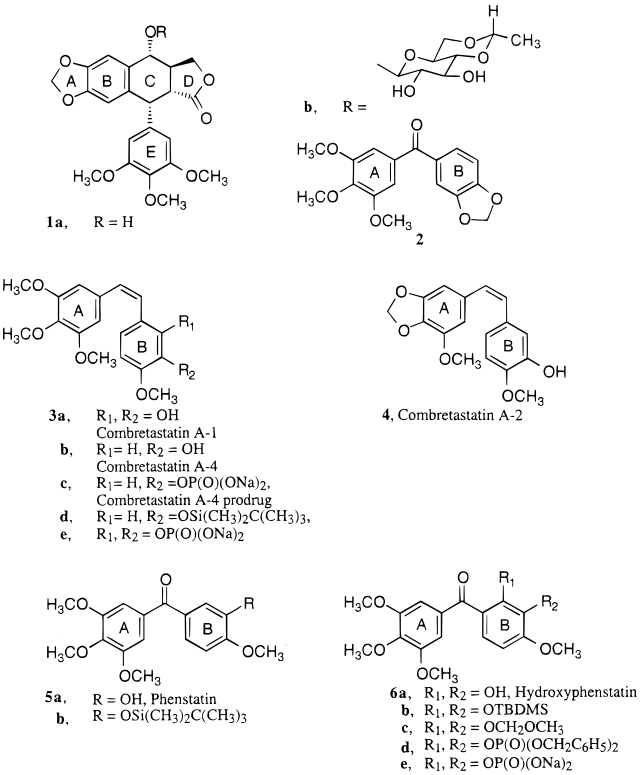
Received February 4, 2000

A structure−activity relationship (SAR) study of the South African willow tree (*Combretum caffrum*) antineoplastic constituent combretastatin A-4 (**3b**) led to the discovery of a potent cancer cell growth inhibitor designated phenstatin (**5a**). This benzophenone derivative of combretastatin A-4 showed remarkable antineoplastic activity, and the benzophenone derivative of combretastatin A-1 was therefore synthesized. The benzophenone, designated hydroxyphenstatin (**6a**), was synthesized by coupling of a protected bromobenzene and a benzaldehyde to give the benzhydrol with subsequent oxidation to the ketone. Hydroxyphenstatin was converted to the sodium phosphate prodrug (**6e**) by a dibenzyl phosphite phosphorylation and subsequent benzyl cleavage (**6a** → **6d** → **6e**). While hydroxyphenstatin (**6a**) was a potent inhibitor of tubulin polymerization with activity comparable to that of combretastatin A-1 (**3a**), the phosphorylated derivative (**6e**) was inactive.

Podophyllum, the roots and rhizomes of *Podophyllum* species such as *peltatum L.* (Podophyllaceae, May Apple), found important uses, including cancer and antiviral applications, in the traditional medicine of early Americans and in India.[2a] Indeed, it was an important component of the United States Pharma copoeia from 1820 to 1942[2b] (the derived resin has been found to contain up to 38% podophyllotoxin[2c] (**1a**, Chart 1)) and was the first terrestrial plant anticancer agent developed to clinical trials by the U.S. National Cancer Institute some 50 years ago.[2c] Subsequently, podophyllotoxin was converted to the glycoside derivative known as etoposide (**1b**), now widely used in human cancer treatment.[3a]

In 1958, we initiated a SAR investigation[3b,c] focused on the trimethoxy and methylenedioxy diarylmethylene unit of podophyllotoxin (**1a**). While not detected at the time,[3b,c] owing to limitations of the early antineoplastic evaluation options, we later found the diaryl ketone (**2**) to significantly inhibit growth of the P388 lymphocytic leukemia cell line with an ED_{50} value of 2.6 μg/mL. By 1978, we were investigating cancer cell growth inhibition by extracts of the African willow tree *Combretum caffrum* Kuntze (Combretaceae) and later discovered three potentially important constituents, designated combretastatins A-1 (**3a**), A-2 (**4**), and A-4 (**3b**).[4] Combretastatin A-4, as the water soluble prodrug (**3c**), has so far reached the most advanced stage of preclinical and clinical development. More recently, we found the diaryl ketone named phenstatin (**5a**),[5] structurally related to podophyllotoxin (**1a**) and combretastatin A-4 (**3b**), to be a very strong anticancer substance comparable to the stilbene **3b** (Table 1). These and other results[6] encouraged us to undertake synthesis and evaluation of diphenol **6a**.

Chart 1



The general procedure we reported[3c] in 1962 for obtaining ketone (**2**) was attempted first. Coupling reactions between 2,3-bis(*tert*-butyldimethylsilyloxy)-4-methoxybromobenzene (**7b**) and *N*-(3,4,5-trimethoxybenzoyl)morpholine (**8a**) (Scheme 1) utilizing either

* To whom correspondence should be addressed. Tel: 480-965-3351. Fax: 480-965-8558.
† Arizona State University.
‡ LDDRD, NCI.
§ STB, NCI.

10.1021/jm000045a CCC: $19.00
Published on Web 06/24/2000

Table 1. Evaluation of Hydroxyphenstatin (**6a**), the Sodium Diphosphate Prodrug (**6e**), Related Derivatives, and Synthetic Intermediates compared to Combretastatin A-1 (**3a**), A-4 (**3b**) and Phosphate Prodrugs (**3c** and **3e**) against a Series of Human Cancer Cell Lines and the Murine P388 Lymphocytic Leukemia Cell Line

		GI_{50},[a] μg/mL								
cell type	cell line	**6a**	**6c**	**6d**	**6e**	**13**	**3a**	**3b**	**3c**	**3e**
leukemia	P388	0.315	>10	2.55	0.0336	>10	0.3	0.0003	0.0004	<0.01
pancreas-a	BXPC-3	3.5	>10	>10	5.3	14.2	4.4	0.39	ND	1.5
melanoma	RPMI-7951	0.58	>10	>10	ND	ND	ND	ND	ND	ND
CNS	SF-295	0.04	>10	>10	0.23	23.1	ND	>0.001	0.036	0.036
lung-NSC	NCI-H460	0.21	>10	>10	0.35	19.0	0.74	0.0006	0.029	0.038
colon	KM20L2	ND[a]	ND	5.9	33.5	ND	0.061	0.34	0.53	
prostate	DU-145	0.048	>10	>10	0.3	32.5	0.17	0.0008	ND	0.034

[a] ND, no data available.

n-butyl- or *tert*-butyllithium were unsuccessful. Changing the acylating agent to a benzoyl chloride was also not productive. Presumably, the bulky TBDMS substituents caused enough steric hindrance to prevent nucleophilic attack of the lithium−benzene complex on the carbonyl group. Thus, we next chose the smaller methoxymethyl ether (MOM) protecting group.[7] However, formation of the benzophenone using the MOM-protected bromobenzene (**7c**) and either the morpholine amide (**8a**) or the benzoyl chloride (**8b**) met with limited success, affording 24% and 20% yields, respectively. Further attempts to prepare protected diphenol (**6c**) using Grignard reactions, Weinreb amides,[8] or dimethylamides also afforded low yields (14−44%). Application of organometallic reagents such as $La(OTf)_3$[9], Bu_3P,[10] and $Fe(acac)_3$[11] did not lead to improved yields of ketone (**6c**).

To determine if the protecting groups were interfering, ketone formation was evaluated starting with 2,3,4-trimethoxybromobenzene (**9**) and morpholine amide (**8a**). The resulting yields were found to range from 17% to 20%. These results indicated that the protecting groups used in the preceding reaction may not have significantly influenced the poor yields. However, we found that condensing the bromobenzene (**9**) with 3,4,5-trimethoxybenzaldehyde led to the formation of benzhydrol **14** in 86% yield. Subsequent oxidation with pyridinium dichromate (PDC) to benzophenone **13** was accomplished at 83% yield. Those favorable results led us to utilize the reaction between an efficient aldehyde and an organolithium reagent to prepare a benzhydrol derivative of ketone **6a**. That approach was realized when the lithium derivative of MOM-protected bromobenzene **7c** and 3,4,5-trimethoxybenzaldehyde were condensed to afford protected benzhydrol **15** in 92% yield. Oxidation by PDC produced protected hydroxyphenstatin (**6c**) in good yield (96%) and MOM cleavage (acidic) afforded hydroxyphenstatin (**6a**) in 97% yield.

Verification of the structure of hydroxyphenstatin (**6a**) was established via single-crystal X-ray crystallography. The unit cell contained four molecules of the parent compound, each asymmetric unit consisting of two independent molecules of hydroxyphenstatin. In addition, adjacent molecules of hydroxyphenstatin are linked via intermolecular hydrogen bonding between the O10 carbonyl and O7 hydroxyl group, as shown in Figure 1.

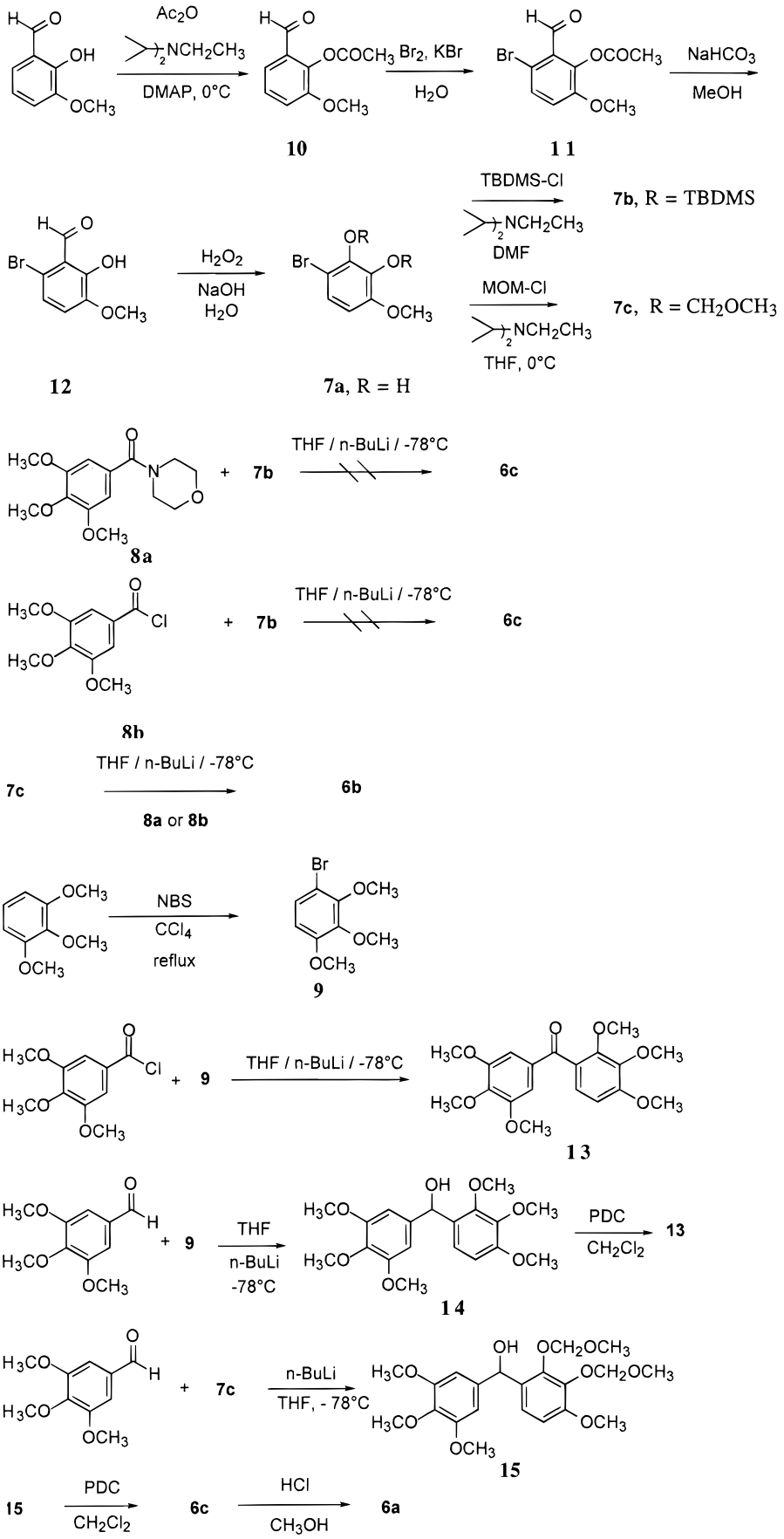
A comparative view of the solid-state conformations exhibited by hydroxyphenstatin (**6a**) and the combretastatin A-4 prodrug (**3c**) is shown in Figure 2. Noteworthy, is the distinct "cis" conformational arrangement of the two aromatic rings in both compounds. Even more striking is the obvious out-of-plane twist adopted by the lower ring in relation to the common trimethoxyphenyl ring of each compound. The conformations of these two compounds are strikingly similar, possibly explaining in part the similar antineoplastic activity exhibited by these substances.

Hydroxyphenstatin (**6a**) was found to potently inhibit tubulin polymerization, and its activity appeared to be somewhat greater than that of combretastatin A-1 (**3a**) (Table 2). Nevertheless, **6a** was somewhat less active than **3a** as an inhibitor of the binding of [^{3}H]colchicine to tubulin (Table 2). The reason for the apparent difference in relative activities between the catalytic assembly assay and the stoichiometric colchicine binding assay is not understood. However, it has been observed with other colchicine site drugs,[12] and an analogous pattern was also observed when phenstatin (**5a**) and combretastatin A-4 (**3b**) were compared.[5]

Due to the improved therapeutic properties of the combretastatin A-4 sodium phosphate prodrug (**3c**) vs the parent phenol (**3b**),[14] the corresponding hydroxyphenstatin prodrug (**6e**) was synthesized (**6a**−**e**, Scheme 2). The previous phosphorylation techniques[4c,d,15] we used for such syntheses, based on pentavalent and trivalent phosphorus precursors, were evaluated. They proved to be substantially less effective than employment of the dibenzyl phosphite approach.[4c,d,16] The prodrug was synthesized in three steps from hydroxyphenstatin by phosphorylation of phenol (**6a**) utilizing dibenzyl phosphite (under basic conditions in acetonitrile),[16] followed by cleavage of the benzyl groups (**6d**) with trimethylsilyl iodide (formed in situ) and reaction of the resulting phosphoric acid with sodium methoxide in ethanol to afford the sodium phosphate prodrug (**6e**) in 92% overall yield. As expected, **6e** was not active as an inhibitor of tubulin polymerization (IC_{50} > 40 μM; Table 2), as has been the case with other phosphorylated derivatives in the combretastatin series. However, its activity as an inhibitor of cancer cell growth (Table 1) was significant.

Comparative testing of **6a** and **6e** in the NCI 60-cell screen revealed a differential cytotoxicity profile and potency (e.g. mean-panel $GI_{50} = 1.67 \pm 0.24 \times 10^{-7}$ M) that were essentially indistinguishable from each other or from those of combretastatin A-4. The antimicrobial activities of the *Combretum caffrum* constituents combretastatins A-1 and A-4 have been reported.[13] While several precursors to the related compound sodium hydroxyphenstatin diphosphate (**6e**) exhibited antifungal and/or antibacterial action (Table 3), the prodrug

Scheme 1



(**6e**) did not. Compounds **6a**, **6c**, **7b**, **7c, 8a**, **13**, **14**, and **15** were also available in sufficient quantity for antibiotic screening. At 100 μg/disk, none of these compounds inhibited growth of the two fungal and eight bacterial

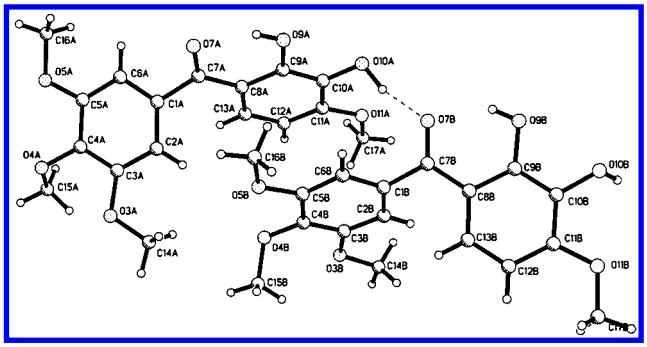


Figure 1. Crystal structure of hydroxyphenstatin (**6a**), showing intermolecular H-bonding between the carbonyl oxygen O7 and the hydroxyl hydrogen on O10.

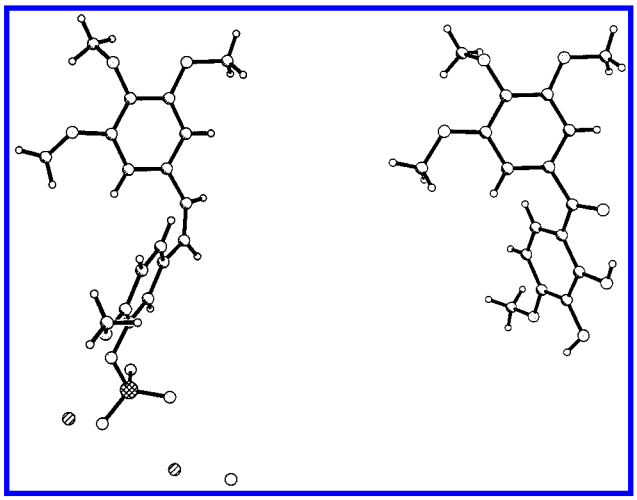


Figure 2. Comparative view of the solid-state conformations of combretastatin A-4 prodrug **3c** (left) and hydroxyphenstatin (**6a**) (right).

Table 2. Interactions with Tubulin of Hydroxyphenstatin (**6a**), Its Diphosphate Derivative (**6e**), Combretastatin A-1 (**3a**), Combretastatin A-4 (**3b**), and Its Prodrug (**3c**)

compd	inhibition of tubulin polymerization ($IC_{50} \pm$ SD, μM)	% inhibition of colchicine binding ± SD
6a	0.82 ± 0.2	77 ± 4
6e	>40	ND[a]
3a	1.1 ± 0.07	99.6 ± 0.7
3b	1.0 ± 0.05	98 ± 1
3c	>40	ND

[a] Not done.

strains tested. Further biological evaluations, including animal studies, of the hydroxyphenstatin series are planned including the sodium (**6e**), lithium (**6f**), potassium (**g**), calcium (**h**) and ammonium cation prodrugs.

Experimental Section

All solvents were redistilled. Both the course of and products from reactions were monitored by thin-layer chromatography using Analtech silica gel GHLF uniplates. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate unless otherwise noted. Flash column chromatography was performed using silica gel (230−400 mesh ASTM).

Melting points were recorded employing an Electrothermal 9100 digital melting point apparatus and are uncorrected. The IR spectra were obtained using a Mattson FTIR model 2020 instrument. Low resolution mass spectral data were collected using a Varian MAT 312 instrument (EIMS). The high-resolution FAB spectra were obtained at the Midwest Center for Mass Spectrometry employing a Kratos MS-50 mass spectrometer, University of Nebraska, Lincoln NE. All ^{1}H and

Scheme 2. Synthesis of Hydroxyphenstatin Prodrug (**6e**)

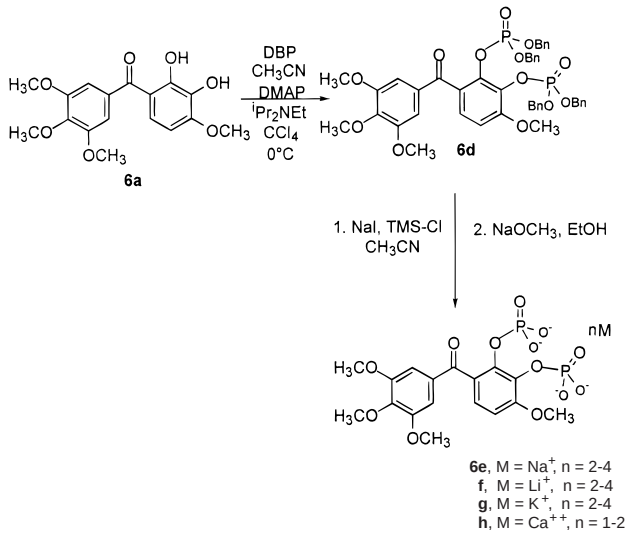


Table 3. Antimicrobial Activities of Sodium Hydroxyphenstatin Phosphate Synthetic Intermediates

compd	microbe(s) inhibited	minimum inhibitory concentration (μg/disk)
6d	*Micrococcus luteus*	50−100
10	*Cryptococcus neoformans*	25−50
	Stenotrophomonas maltophilia	50−100
11	*C. neoformans*	6.25−12.5
	Candida albicans	12.5−25
	Escherichia coli	50−100
	Neisseria gonorrhoeae	25−50
12	*C. neoformans*	12.5−25
	C. albicans	50−100
	N. gonorrheae	12.5−25
7a	*S. maltophilia*	25−50
	E. coli	50−100
	Staphylococcus aureus	25−50
	N. gonorrhoeae	<6.25

^{13}C NMR spectra were determined using a Varian Gemini 300 MHz instrument with $CDCl_3$ (TMS internal reference) as solvent, unless otherwise noted. The ^{31}P NMR spectra were measured in $CDCl_3$ with 85% H_3PO_4 as an external standard employing a Varian Unity 500 MHz instrument. The X-ray crystal structure data collection was performed on an Enraf-Nonius CAD4 diffractometer. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

2-Acetoxy-3-methoxybenzaldehyde (10). To a solution of *o*-vanillin (10.1 g) and a catalytic quantity (0.8 g) of (dimethylamino)pyridine in *N,N*-diisopropylethylamine (23 mL) at 0 °C was added acetic anhydride (8 mL). The solution was stirred overnight, poured into 2 N hydrochloric acid (100 mL), and extracted with dichloromethane, and the solvent was removed in vacuo to afford a yellow solid. Recrystallization from ethanol yielded yellow crystals (10.9 g, 85%): mp 75.4−76.2 °C, lit.[17] mp 76 °C; EIMS *m/z* 194 (M^+), 152, 106, 43.

2-Acetoxy-3-methoxy-6-bromobenzaldehyde (11). To a solution of potassium bromide (40 g) in H_2O (250 mL) was added bromine (6.8 mL). To the dark red solution was added aldehyde **10** (20.2 g). The turbid orange solution was stirred overnight, filtered, rinsed with ethyl acetate, and recrystallized from EtOAc/hexane to afford yellow crystals (22.4 g, 79%): mp 121.6−123.4 °C, lit.[18] mp 119−120 °C; EIMS *m/z* 274 (M^+, ^{81}Br), 272 (M^+, ^{79}Br), 232, 230, 186, 184, 43; ^{1}H NMR δ 10.26 (1H, s, CHO), 7.51 (1H, d, J = 9.0 Hz, H_5), 7.05 (1H, d, J = 9.0 Hz, H_4), 3.85 (3H, s, OCH_3), 2.38 (3H, s, $COCH_3$); ^{13}C NMR (75.5 MHz) δ 190.38, 168.71, 151.78, 140.37, 131.41, 126.49, 117.69, 116.35, 56.40, 20.44. Anal. ($C_{10}H_9O_4Br$) C,H.

2-Hydroxy-3-methoxy-6-bromobenzaldehyde (12). To the aldehyde **11** (17.7 g) in aqueous methanol (125 mL) was added sodium bicarbonate (7.6 g, 1.1 equiv), and the turbid bright yellow solution was stirred for 2 h. The solution was

acidified and extracted with dichloromethane and the solvent removed in vacuo to afford a yellow solid. The product was recrystallized from ethyl acetate/hexane to afford yellow crystals (14.7 g, 98%): mp 105.6−106.4 °C, lit.[19] mp 102−103 °C; EIMS *m/z* 232 (M^+, ^{81}Br), 230 (M^+, ^{79}Br), 186, 107, 79, 54, 32.

1-Bromo-2,3-dihydroxy-4-methoxybenzene (7a). Aldehyde **12** (23 g) was suspended in 2% sodium hydroxide (300 mL), and a solution of 30% hydrogen peroxide (15.8 g, 1.4 equiv) was added. After 2 h, another portion of 30% hydrogen peroxide (1.4 equiv) was added and the solution stirred overnight. The reaction mixture was acidified, extracted with dichloromethane, and washed with sodium thiosulfate, and the solvent was removed in vacuo to afford a tan solid. The solid was recrystallized from methanol to afford colorless crystals (14 g, 64%): mp 122−124 °C, lit.[20] mp 124−126 °C; EIMS *m/z* 220 (M^+, ^{81}Br), 218 (M^+, ^{79}Br), 205, 203, 177, 175, 95; ^1H NMR δ 6.99 (1H, d, J = 9.0 Hz, H_5), 6.42 (1H, d, J = 9.0 Hz, H_4), 5.56 (1H, s, OH), 5.51 (1H, s, OH), 3.88 (3H, s, OCH_3); ^{13}C NMR (75.5 MHz) δ 146.59, 140.95, 133.47, 122.21, 104.35, 101.55, 56.31.

1-Bromo-2,3-bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzene (7b). To a solution of diphenol **7a** (0.51 g) in dry dimethylformamide (10 mL) was added successively diisopropylethylamine (1.25 mL, 3.1 equiv) and *tert*-butyldimethylsilyl chloride (0.78 g, 2.2 equiv), and the mixture was stirred at room temperature under argon for 3 h. (HCl evolution was noted.) The reaction was terminated by adding ice. After extraction with DCM, the combined solvent was washed with water, saturated sodium bicarbonate, and water, and dried. Removal of solvent gave an oil which solidified on trituration with ether. The solid was recrystallized from methanol and afforded colorless crystals (0.92 g, 90%): mp 68.9−69.6 °C; EIMS *m/z* 448 (M^+, ^{81}Br), 446 (M^+, ^{79}Br), 443, 431, 391, 389, 167; IR (Kbr, cm^{-1}) ν_{max} 2934, 2859, 1576, 1472, 1254, 1092, 845, 671; ^1H NMR δ 7.06 (1H, d, J = 8.7 Hz, H_6), 6.42 (1H, d, J = 8.7 Hz, H_5), 3.75 (3H, s, OCH_3), 1.06 (9H, s, $C(CH_3)_3$), 0.99 (9H, s, $C(CH_3)_3$), 0.19 (6H, s, $Si(CH_3)_2$), 0-10 (6H, s, $Si(CH_3)_2$); ^{13}C NMR 75.5 MHz) δ 151.74, 145.32, 138.06, 124.33, 108.36, 105.64, 55.04, 26.46, 26.12, 18.75, 18.73, −3.10, −3.85. Anal. ($C_{19}H_{35}BrO_3Si_2$) C, H.

1-Bromo-2,3-bis(methoxymethyloxy)-4-methoxybenzene (7c). To a solution of 1-bromo-2,3-dihydroxy-4-methoxybenzene (5.0 g) and anhydrous THF (20 mL) at 0 °C under argon was added diisopropylethylamine (8.0 mL). The solution was stirred for 15 min, methyloxymethyl chloride (3.5 mL) was added, and the reaction mixture stirred for 3 h. The solution was poured into water (250 mL) and extracted with DCM and the solvent removed in vacuo to provide an orange oil. The oil was purified by flash column chromatography (hexane/EtOAc 2:1) to yield a clear oil (6.4 g, 91%): EIMS *m/z* 308 (M^+, ^{81}Br), 306 (M^+, ^{79}Br), 232, 230, 45; IR (neat, cm^{-1}) ν_{max} 2963, 2836, 1221, 1159, 1084, 966; ^1H NMR δ 7.25 (1H, d, J = 9.0 Hz, H_6), 6.61 (1H, d, J = 9.0 Hz, H_5), 5.20 (2H, s, OCH_2), 5.13 (2H, s, OCH_2), 3.84 (3H, s, OCH_3), 3.66 (3H, s, OCH_3), 3.59 (3H, s, OCH_3); ^{13}C NMR (75.5 MHz) δ 153.31, 148.24, 140.05, 127.49, 108.94, 108.73, 99.39, 98.73, 58.14, 57.46, 56.11. Anal. ($C_{11}H_{15}BrO_5$) C, H.

***N*-(3,4,5-Trimethoxybenzoyl)morpholine (8a).** Morpholine (0.8 mL) was slowly added to a solution composed of toluene (10 mL) and 3,4,5-trimethoxybenzoyl chloride (1.1 g). The reaction was accompanied by evolution of heat and precipitation of morpholine hydrochloride. After a 3 h period, the solution was filtered and concentrated in vacuo to afford a white solid which was recrystallized from ethanol to afford colorless needles (1.2 g, 86%): mp 119.8−120.7 °C, lit.[3c] mp 120−121 °C; EIMS *m/z* 281 (M^+), 266, 195; ^1H NMR δ 6.63 (2H, s, $H_{2,6}$), 3.87 (6H, s, $OCH_3 \times 2$), 3.86 (3H, s, OCH_3), 3.70 (8H, bs, $CH_2 \times 4$).

1-Bromo-2,3,4-trimethoxybenzene (9). Pyrogallol trimethyl ether (5.1 g) was suspended in CCl_4 (60 mL), and *N*-bromosuccinimide (6.5 g, 1.2 equiv) was added. The reaction mixture was heated at reflux for 20 h. The succinimide was collected and the filtrate concentrated in vacuo to a brown oil. The oil was separated by gravity column chromatography (hexanes/ethyl acetate 19:1) and yielded the title compound[21] as a yellow oil (5.9 g, 78%): EIMS *m/z* 234 (($M^+ - CH_3$, ^{81}Br), 232 ($M^+ - CH_3$, ^{79}Br), 107, 95, 69, 58, 44; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.21 (1H, d, J = 9.0 Hz, H_6), 6.58 (1H, d, J = 9.0 Hz, H_5), 3.91 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.85 (3H, s, OCH_3).

2′,3,3′,4,4′,5-Hexamethoxybenzophenone (13). To a solution of bromobenzene **9** (0.21 g) in dry THF (5 mL) cooled to −78 °C was added *n*-butyllithium (0.38 mL, 2.5 M, 1.1 equiv). The solution was stirred for 30 min, 3,4,5-trimethoxybenzoyl chloride (0.2 g) in anhydrous tetrahydrofuran was added, and the solution was stirred for 28 h. The reaction was stopped with water and extracted with ethyl acetate and the solvent removed (in vacuo) to give a yellow oil. Separation by flash column chromatography (hexanes/ethyl acetate 3:1) afforded a colorless solid (0.06 g, 20.5%). The solid was recrystallized twice from ethyl acetate/hexane: mp 124.6−125.9 °C, lit.[22] mp 121 °C; EIMS *m/z* 362 (M^+), 345, 317, 181, 169, 151; ^1H NMR δ 7.11 (1H, d, J = 9.0 Hz, $H_{6'}$), 7.08 (2H, s, $H_{2,6}$), 6.72 (1H, d, J = 9.0 Hz, $H_{5'}$), 3.94 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.85 (6H, s, $OCH_{3\ 3,5}$), 3.80 (3H, s, OCH_3).

2′,3,3′,4,4′,5-Hexamethoxydiphenylcarbinol (14). The preceding experiment (cf. **10**) was repeated using bromobenzene **9** (0.55 g), anhydrous tetrahydrofuran (15 mL), and *n*-butyllithium (0.93 mL, 2.5 M, 1.05 equiv). A solution of 3,4,5-trimethoxybenzaldehyde (0.44 g) was added and the solution stirred for 16 h. The resulting oily product was separated by flash column chromatography (hexanes/ethyl acetate 9:1) to give a clear oil (0.70 g, 86%): EIMS *m/z* 364 (M^+), 331, 315, 195, 181, 169; IR (neat, cm^{-1}) ν_{max} 3462, 2940, 2837, 1593, 1464, 1234, 1127, 1015; ^1H NMR δ 6.90 (1H, d, J = 8.7 Hz, $H_{6'}$), 6.64 (1H, d, J = 8.7 Hz, $H_{5'}$), 6.61 (2H, s, $H_{2,6}$), 5.88 (1H, d, J = 3.9 Hz, CH), 3.86 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.82 (6H, s, $OCH_3 \times 2$), 3.76 (3H, s, OCH_3); ^{13}C NMR (75.5 MHz) δ 153.37, 152.98, 151.20, 142.03, 139.55, 136.91, 129.63, 122.15, 106.98, 103.46, 72.03, 60.85, 60.74, 60.61, 56.01, 55.88. Anal. ($C_{19}H_{24}O_7$) C, H.

2′,3′-Bis(methoxymethyloxy)-3,4,4′,5-tetramethoxydiphenylcarbinol (15). To a solution of protected bromobenzene **7c** (0.91 g, 2.95 mmol) in anhydrous tetrahydrofuran (5.0 mL) cooled to −78 °C was added *n*-butyllithium (1.21 mL, 2.44 M, 2.95 mmol). The solution was stirred for 1 h, 3,4,5-triethoxybenzyaldehyde (0.58 g, 2.95 mmol) was added, and the solution was stirred for 4 h. The reaction was ended by adding water, and the mixture was extracted with ethyl acetate. Removal of solvent (in vacuo) led to a yellow oil that was separated by flash column chromatography (hexanes/ethyl acetate 9:1) to afford a clear oil that solidified upon standing (1.15 g, 92%). The solid was recrystallized from methanol and yielded colorless plates: mp 79.9−81.8 °C; HRMS 424.1749 $C_{21}H_{28}O_9$; EIMS *m/z* 424 (M^+), 362, 347, 331, 317, 289, 181; IR (KBr, cm^{-1}) ν_{max} 3407, 3001, 2942, 2836, 1236, 1155, 1123, 1063; ^1H NMR δ 6.71 (1H, d, J = 9.0 Hz, $H_{6'}$), 6.68 (2H, s, $H_{2,6}$), 6.63 (1H, d, J = 9.0 Hz, $H_{5'}$), 6.09 (1H, d, J = 3.3 Hz, CH), 5.20 (2H, dd, J = 6.0, 10.5 Hz, OCH_2), 5.13 (2H, s, OCH_2), 3.85 (3H, s, OCH_3), 3.84 (6H, s, $OCH_3 \times 2$), 3.82 (3H, s, OCH_3), 3.61 (3H, s, OCH_3), 3.58 (3H, s, OCH_3); ^{13}C NMR (75.5 MHz) δ 153.23, 153.05, 149.73, 138.17, 138.12, 136.80, 131.16, 123.52, 108.04, 104.32, 103.53, 100.03, 98.59, 69.79, 60.85, 57.74, 57.37, 56.07, 55.93. Anal. ($C_{21}H_{28}O_9$) C, H.

2′,3′-Bis(methoxymethyloxy)-3,4,4′,5-tetramethoxybenzophenone (6c). To a stirred solution of diphenylcarbinol **15** (6.85 g) in dichloromethane (250 mL) was added 4 Å molecular sieves (9 g) and pyridinium dichromate (9.1 g, 1.5 equiv). The black solution was stirred overnight, filtered through Celite, rinsed with methanol and the solvent removed in vacuo to afford a black residue. The mixture was separated by flash column chromatography (hexanes−ethyl acetate, 4:1) to provide a clear oil that solidified upon standing (6.5 g, 96%). The solid was recrystallized twice from ethyl acetate−hexane to afford colorless crystals: mp 70.2−71.7 °C; HRMS 422.1577 $C_{21}H_{26}O_9$; EIMS *m/z* 422 (M^+), 346, 195, 181; IR (KBr, cm^{-1}) ν_{max} 3005, 2944, 2845, 1649, 1583, 1231, 1125, 1071; ^1H NMR δ 7.16 (1H, d, J = 8.5 Hz, $H_{6'}$), 7.12 (2H, s, $H_{2,6}$), 6.78 (1H, d,

J = 8.5 Hz, $H_{5'}$), 5.18 (2H, s, OCH_2 2′), 5.01 (2H, s, OCH_2), 3.93 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.85 (6H, s, $OCH_{3\ 3,5}$), 3.62 (3H, s, OCH_3), 3.26 (3H, s, OCH_3); ^{13}C NMR (75.5 MHz) δ 193.87, 155.93, 152.77, 149.48, 142.42, 138.80, 133.07, 127.35, 125.48, 107.64, 107.36, 99.76, 98.66, 60.92, 57.41, 57.32, 56.22, 56.05. Anal. ($C_{21}H_{26}O_9$) C, H.

Hydroxyphenstatin [(2′,3′-Dihydroxy-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone] (6a). To a stirred solution of MOM-protected hydroxyphenstatin **6c** (0.120 g, 0.284 mmol) in methanol (10.0 mL) was added 1 N HCl (0.57 mL) and the solution was stirred for 2 h. The reaction mixture was poured into water and, extracted with dichloromethane and the solvent evaporated in vacuo to yield a yellow solid (0.09 g, 97%). The solid was recrystallized (twice) from methanol: mp 171.1−171.9 °C; HRMS 334.1052 $C_{17}H_{18}O_7$; EIMS m/z 334 (M^+), 303, 195, 168, 153; IR (KBr, cm^{-1}) ν_{max} 3273, 3100, 3001, 2944, 1636, 1574, 1121, 1063; ^{1}H NMR δ 12.23 (1H, s, OH), 7.27 (1H, d, J = 8.7 Hz, $H_{6'}$), 6.92 (2H, s, $H_{2,6}$), 6.51 (1H, d, J = 8.7 Hz, $H_{5'}$), 5.57 (1H, s, OH), 3.99 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.90 (6H, s, OCH_3 × 2); ^{13}C NMR (75.5 MHz) δ 199.58, 152.93, 152.08, 150.94, 141.33, 133.67, 133.13, 125.59, 113.99, 106.80, 102.53, 60.98, 56.32, 56.24. Anal. ($C_{17}H_{18}O_7$) C, H.

X-ray Crystal Structure Determination. Hydroxyphenstatin, 6a. A thick, plate-shaped X-ray sample (∼0.38 × 0.36 × 0.08 mm), grown from methanol solution, was mounted on the tip of a glass fiber with Super-Glue. Data collection was performed at 301 ± 1° K. Accurate cell dimensions were determined by least-squares fitting of 25 carefully centered reflections in the range of 35° < θ < 40° using Cu Kα radiation.

Crystal Data: $C_{17}H_{18}O_7$, FW = 334.31, monoclinic, space-group P_c, a = 10.423(2) Å, b = 11.297(2) Å, c = 14.173(2) Å, β = 111.02(3)°, V = 1557.8(5) Å^3, Z = 4, ρ_c = 1.425 Mg/m^3, μ(Cu Kα) = 0.942 mm^{-1}, λ = 1.54178 Å.

All reflections corresponding to a complete quadrant ($0 \le h \le 12$, $0 \le k \le 13$, $-16 \le l \le 15$) were collected over the range of $0 < 2\theta < 130°$ using the $\omega/2\theta$ scan technique. Three intensity control reflections were also measured for every 60 min of X-ray exposure time and showed a maximum variation of −0.1% over the course of the collection. A total of 6255 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[23] program, verified that the space group was P_c. After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic absences, 2795 unique reflections remained, of which 2492 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of ψ-scans).[24] Structure determination was readily accomplished with the direct-methods program SHELXS.[25] All non-hydrogen atom coordinates were located in a routine run using default values in that program. The remaining H atom coordinates were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement using SHELXL.[25] The H atoms were included, their U_{iso} thermal parameters fixed at either 1.2 or 1.5 (depending upon atom type) of the U_{iso} of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for **6a** was 0.0661 for observed data and 0.0701 for all data. The goodness-of-fit on F^2 was 1.058. The corresponding Sheldrick R values were wR_2 of 0.1689 and 0.1771, respectively. A final difference Fourier map showed minimal residual electron density; the largest difference peak and hole being 0.422 and −0.294 e/Å^3, respectively. Final bond distances and angles were all within expected and acceptable limits. Intermolecular hydrogen bonding was observed between the carbonyl oxygen (O7) of one molecule and the m-hydroxyl group (O10) of an adjacent molecule.

2′,3′-*O*-Bis(benzylphosphoryl)hydroxyphenstatin (6d). To a solution of hydroxyphenstatin **6a** (4 g) in dry acetonitrile (100 mL) and carbon tetrachloride (11.4 mL, 10 equiv) were added DMAP (0.14 g, 0.1 equiv) and diisopropylethylamine (8.7 mL, 4.2 equiv). After cooling to −10 °C, dibenzyl phosphite (7.8 mL, 3.0 equiv) was added and the solution was stirred for 16 h under argon at −10 °C and then brought to room temperature. The reaction was terminated with 0.5 M KH_2PO_4 and extracted with ethyl acetate, and the combined solvent was washed with brine and dried. Removal of solvent (in vacuo) afforded an orange oil which was separated by flash column chromatography (hexanes/ethyl acetate 1:1 to 0:1) to provide a white solid (9.7 g, 96%). The solid was recrystallized twice from ethyl acetate/hexane: mp 86.9−87.4 °C; EIMS m/z 854 (M^+), 656, 576, 514, 486, 91; IR (KBr, cm^{-1}) ν_{max} 2967, 2945, 2841, 1659, 1298, 1020, 951; ^{1}H NMR δ 7.44 (1H, d, J = 9.0 Hz, $H_{6'}$), 7.27 (18H, m, Ar−H), 7.11 (2H, s, $H_{2,6}$), 7.08 (2H, m, Ar−H), 6.92 (1H, d, J = 9.0 Hz, $H_{5'}$), 5.23 (2H, s, CH_2Bn), 5.21 (2H, s, CH_2Bn), 4.77 (2H, dd, J = 4.5, 6.9 Hz, CH_2Bn), 4.64 (2H, dd, J = 4.5, 6.9 Hz, CH_2Bn), 3.84 (3H, s, OCH_3), 3.80 (6H, s, $OCH_{3\ 3,5}$), 3.77 (3H, s, OCH_3); ^{13}C NMR (75.5 MHz) δ 191.73, 171.11, 154.84, 152.84, 142.20, 135.81, 135.74, 135.24, 135.18, 132.77, 128.42, 128.37, 128.29, 127.96, 127.67, 127.58, 125.90, 109.19, 107.55, 69.98, 69.93, 69.87, 69.82, 60.80, 60.36, 56.30, 56.23; ^{31}P NMR (DMSO, decoupled, −202.35 MHz) δ −5.01, −5.79. Anal. Calcd for $C_{45}H_{44}O_{13}P_2$: C, 63.23; H, 5.19. Found: C, 62.81; H, 5.58.

Sodium Hydroxyphenstatin Diphosphate (6e). A mixture of the phosphorylated hydroxyphenstatin **6d** (9.0 g) and sodium iodide (6.3 g, 4.0 equiv) in anhydrous acetonitrile (30 mL) was stirred (under argon) and trimethylsilyl chloride (5.4 mL, 4.0 equiv) was added. The solution was stirred for 2 h and the reaction was stopped with water. After extraction with ethyl acetate, the aqueous layer was concentrated to a light brown foam. To the residue in ethanol (75 mL) was added sodium methoxide (2.3 g, 4.0 equiv) and the solution stirred for 12 h. The reaction mixture was concentrated and the residue crystallized from water/acetone to yield an amphorous solid (5.6 g, 92%) which was recrystallized (three times) from water/acetone: mp 145−147; LRFAB m/z 583.1 (M + H^+), calcd 583.2; IR (KBr, cm^{-1}) ν_{max} 3009, 2947, 2843, 1643, 1343, 1289, 1182, 1123, 990; ^{1}H NMR (D_2O, 500 MHz) δ 7.07 (2H, s, $H_{2,6}$), 6.98 (1H, d, J = 5.1 Hz, $H_{6'}$), 6.75 (1H, d, J = 5.1 Hz, $H_{5'}$), 3.80 (3H, s, OCH_3), 3.75 (6H, s, OCH_3 × 2), 3.74 (3H, s, OCH_3); ^{13}C NMR (D_2O, reference to $CDCl_3$, 75.5 MHz) δ 197.15, 156.15, 152.24, 145.03, 141.38, 135.70, 133.99, 126.37, 125.49, 108.72, 106.95, 61.16, 56.40, 56.30; ^{31}P NMR (D_2O, decoupled, −202.35 MHz) δ 0.05, −1.49. The solubility of sodium hydroxyphenstatin diphosphate was found to be 100 mg/mL in distilled water at 25 °C.

Lithium Hydroxyphenstatin Diphosphate (6f). To the light brown foam in methanol (10 mL) was added lithium hydroxide (0.049 g, 4.0 equiv) and the solution stirred for 12 h. The reaction mixture was concentrated and the residue crystallized from water/acetone to yield an amphorous solid (0.11 g, 74%) which was recrystallized from water/acetone: mp 174−176 °C (dec); LRFAB m/z 511 (M^+), 505 (M^+ − Li), 499 (M^+ − 2Li), 493 (M^+ − 3Li), 435, 413, 199; IR (Kbr, cm^{-1}) ν_{max} 3011, 2945, 2845, 1632, 1339, 1283, 1187, 1127, 1003; ^{1}H NMR (D_2O, 300 MHz) δ 7.13 (2H, s, $H_{2,6}$), 6.96 (1H, d, J = 7.5 Hz, $H_{6'}$), 6.69 (1H, d, J = 7.5 Hz, $H_{5'}$), 3.77 (6H, s, OCH_3 × 2), 3.76 (3H, s, OCH_3), 3.75 (3H, s, OCH_3). The solubility of lithium hydroxyphenstatin diphosphate was found to be 25 mg/mL in distilled water at 25 °C.

Potassium Hydroxyphenstatin Diphosphate (6g). To the light brown foam in methanol (10 mL) was added potassium hydroxide (0.065 g, 4.0 equiv) in water (5 mL) and the solution stirred for 12 h. The reaction mixture was concentrated and the yellow solid crystallized from water/acetone to yield an amphorous solid (0.161 g, 86%) which was recrystallized from water/acetone: mp 141−143 °C (dec); LRFAB m/z 647 (M^+ − K), 545, 395, 333, 181; IR (Kbr, cm^{-1}) ν_{max} 3010, 2946, 2843, 1640, 1335, 1269, 1169, 1123, 988; ^{1}H NMR (D_2O, 300 MHz) δ 7.14 (2H, s, $H_{2,6}$), 6.96 (1H, d, J = 8.1 Hz, $H_{6'}$), 6.69 (1H, d, 8.1 Hz, $H_{5'}$), 3.77 (6H, s, OCH_3 × 2), 3.76 (3H, s, OCH_3), 3.75 (3H, s, OCH_3). The solubility of potassium hydroxyphenstatin diphosphate was found to be >100 mg/mL in distilled water at 25 °C.

Calcium Hydroxyphenstatin Diphosphate (6h). To the

light brown foam in methanol (10 mL) was added calcium acetate (0.102 g, 2.0 equiv) and the solution stirred for 12 h. The reaction mixture was concentrated and the residue crystallized from water/acetone to yield an amphorous solid (0.159 g, 79%) which was recrystallized from water/acetone: mp 186−188 °C (dec); LRFAB *m/z* 531 (M^+), 493, 413, 395, 277; IR (KBr, cm^{-1}) ν_{max} 3011, 2940, 2847, 1638, 1337, 1296, 1182, 1127, 964; ^1H NMR (D_2O, 300 MHz) δ 7.13 (1H, d, J = 8.4 Hz, $H_{6'}$), 7.07 (2H, s, $H_{2,6}$), 6.84 (1H, d, J = 8.4 Hz, $H_{5'}$), 3.82 (3H, s, OCH_3), 3.76 (6H, s, $OCH_3 \times 2$), 3.75 (3H, s, OCH_3); The solubility of calcium hydroxyphenstatin diphosphate was found to be <1 mg/mL in distilled water at 25 °C.

Antimicrobial Susceptibility Testing. The new substances were screened against the bacteria *Stenotrophomonas maltophilia*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, and *Neisseria gonorrhoeae* and the fungi *Candida albicans* and *Cryptococcus neoformans*, according to established disk susceptibility testing protocols.[26]

Tubulin Assays. The tubulin polymerization and colchicine binding assays were performed as described previously,[12] except that Beckman DU7400/7500 spectrophotometers equipped with "high performance" temperature controllers were used in the former assay. Unlike the manual control possible with the previously used Gilford spectrophotometers, the polymerization assays required use of programs provided by MDB analytical Associates, South Plainfield, NJ, since the Beckman instruments are microprocessor controlled. The Beckman instruments were unable to maintain 0 °C, and the lower temperature in the assays fluctuated between 2 and 4 °C. Temperature changes were, however, more rapid than in the Gilford instruments with the jump from the lower temperature to 30 °C taking about 20 s and the reverse jump about 100 s.

Acknowledgment. We are pleased to thank for support of this research the Outstanding Investigator Grant CA 44344-06-12 with the Division of Cancer Treatment and Diagnosis, NCI, DHHS, the Arizona Disease Control Research Commission, Gary L. and Diane R. Tooker, Captain and Mrs. George M. Brereton, the Caitlin Robb Foundation, Dr. John C. Budzinski, Robert and Sharon Witzer, Salley Schloegel, and the Robert B. Dalton Endowment. For other assistance, we thank Drs. Cherry L. Herald, Fiona Hogan, Michael D. Williams, Jean M. Schmidt, David Molloy, and Jean-Charles Chapuis, as well as Laura Crews and Lee Williams.

Supporting Information Available: X-ray crystallographic tables of atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for hydroxyphenstatin (**6a**). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For contribution 442, see: Pettit, G. R.; Lippert, J. W., III.; Boyd, M. R.; Hamel, E.; Pettit, R. K. Antineoplastic Agents 442. The Remarkable Antitubulin Assembly and Cancer Cell Growth Inhibitor Dioxostatin. *J. Med. Chem*. Submitted.

(2) (a) Morton, J. F. *Major Medicinal Plants*, C. G. Thomas, Springfield, IL, 1997; p. 87−90. (b) Hartwell, J. L.; Schrecker, A. W. *Fortsch. Chem. Org. Nat.* **1958**, 83. (c) Pettit, G. R. The Scientific Contributions of Jonathan L. Hartwell, Ph.D. *J. Nat. Prod*. **1995**, *58*, 359−364.

(3) (a) Cragg, G. M.; Newman, D. J. Discovery and development of Antineoplastic Agents from Natural Sources. *Cancer Investigation* **1999**, *17*, 153−163. (b) Pettit, G. R.; Alkalay, D. S. Potential Cancerocidal Agents I. The Aromatic System of Podophyllotoxin. (Part A). *J. Org. Chem*. **1960**, *25*, 1363−1365. (c) Pettit, G. R.; Baumann, M. F.; Rangammal, K. N. Antineoplastic Agents V. The Aromatic System of Podophyllotoxin (Part B). *J. Med. Pharm. Chem*. **1962**, *5*, 800−808.

(4) (a) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. Antineoplastic Agents 291. Isolation and Synthesis of Combretastatins A-4, A-5, and A-6. *J. Med. Chem*. **1995**, *38*, 1666−1672. (b) Hamel, E. Antimitotic Natural Products and their Interactions with Tubulin. *Med. Res. Rev*. **1996**, *16*, 207−231. (c) Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Chaplin, D. J.; Stratford, M.; Pettit, R. K.; Chapuis, J.-C.; Oliva, D. Antineoplastic Agents 393. Synthesis of the *trans*-isomer of Combretastatin A-4 Prodrug. *Anti-Cancer Drug Design* **1998**, *13*, 981−994. (d) Pettit, G. R.; Rhodes, M. R. Antineoplastic Agents 389. New Syntheses of the Combretastatin A-4 Prodrug. *Anti-Cancer Drug Design* **1998**, *13*, 183−191.

(5) Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. K. Antineoplastic Agents 379. Synthesis of Phenstatin Phosphate. *J. Med. Chem*. **1998**, *41*, 1688−1695.

(6) Chaplin, D. J.; Pettit, G. R.; Hill, S. A. Antivascular Approaches to Solid Tumor Therapy: Evaluation of Combretastatin A-4 Phosphate. *Anticancer Res*. **1999**, *19*, 189−195.

(7) Greene, T. W.; Wutz, P. G. M. *Protective Groups in Organic Synthesis*. J. Wiley & Sons: New York, 1999; pp 27−33.

(8) Nahm, S.; Weinreb, S. M. *N*-Methoxy-*N*-methylamides as Effective Acylating Agents. *Tetrahedron Lett*. **1981**, *22*, 3815−3818.

(9) Collins, S.; Hang, Y. Alkyl and Aryllanthanum Triflates: New Reagents for the Conversion of Tertiary Amides to Ketones. *Tetrahedron Lett*. **1987**, *28*, 4391−4394.

(10) Maeda, H.; Okamoto, J.; Ohmori, H. Reactions of In Situ Formed Acyl Tributylphosphonium Ions with Grignard Reagents as an Effective Route to Ketones from Acid Chlorides. *Tetrahedron Lett*. **1996**, *37*, 5381−5384.

(11) Cardellicchio, C.; Fiandanese, V.; Marchese, G.; Ronzini, L. Functionalized Ketones by Iron Mediated Reaction of Grignard Reagents with Acyl Chlorides. *Tetrahedron Lett*. **1987**, *28*, 2053−2056.

(12) 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. Structure−activity Analysis of the Interaction of Curacin A, the Potent Colchicine Site Antimitotic Agent, with Tubulin and Effects of Analogues on the Growth of MCF-7 Breast Cancer Cells. *Mol. Pharmacol*. **1998**, *53*, 62−76.

(13) Pettit, G. R.; Lippert, J. W., III, Herald, D. L.; Pettit, R. K.; Hamel, E. Antineoplastic Agents 440. Asymmetric Synthesis and Evaluation of the Combretastatin A-1 SAR Probes (1*S*,2*S*) and (1*R*,2*R*)-1,2-Dihydroxy-1-(2′,3′-dihydroxy-4′-methoxyphenyl)-2-(3′,4′,5′-trimethoxyphenyl)-ethane. *J. Nat. Prod*. In press.

(14) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. Isolation and Structure of the Strong Cell Growth and Tubulin Inhibitor Combretastatin A-4. *Experientia* **1989**, *45*, 209−211.

(15) Pettit, G. R.; Freeman, S.; Simpson, M. J.; Thompson, M. A.; Boyd, M. R.; Williams, M. D.; Pettit, G. R. III; Doubek, D. L. Antineoplastic Agents 320. Synthesis of a Practical Pancratistatin Prodrug. *Anticancer Drug Design* **1995**, *10*, 243−250.

(16) Silverberg, L. J.; Dillon, J. L.; Vemishetti, P. A Simple, Rapid and Efficient Protocol for the Selective Phosphorylation of Phenols with Dibenzyl Phosphite. *Tetrahedron Lett*. **1996**, *37*, 771−774.

(17) Lambooy, J. P. The Syntheses, Paper Chromatography and Substrate Specificity for Tyrosinase of 2,3-,2,4-,2,5-,2,6- and 3,5-Dihydroxyphenylalanines. *J. Am. Chem. Soc*. **1954**, *76*, 133−138.

(18) Simig, G.; Schlosser, M. The Synthesis of 6-Methoxy-7,8-methylenedioxyisoquinoline Derivatives. *ACH−Models Chem*. **1994**, *131*, 217−228.

(19) Brink, M. Bromo Derivatives of *o*-Vanillin and *o*-Bourbonal. *Chem. Abstr*. **1968**, *69*, 76823.

(20) Cleaver, L.; Nimgirawath, S.; Ritchie, E.; Taylor, W. C. The Alkaloids of *elmerrilla papuana* (Magnoliaceae): Structure and Synthesis of Elmerrillicine. *Aust. J. Chem*. **1976**, *29*, 2003−2021.

(21) Friedman, D.; Ginsburg, D. Halogenation of Pyrogallol Trimethyl Ether and Similar systems. *J. Org. Chem*. **1958**, *23*, 16−17.

(22) Harding, V. J. 2-Hydroxy-3:4:3′:4′:5′Pentamethoxybenzophenone and its Derivatives. *J. Chem. Soc*. **1906**, *89*, 1665.

(23) XPREP−The automatic space group determination program in the SHELXTL. (see ref 25).

(24) North, A. C. T.; Phillips, D. C.; Mathews, F. S. A Semiempirical Method of Absorption Correction. *Acta Crystallogr*. **1968**, A2, 351−359.

(25) *SHELXTL-PC Version 5.1*, 1997, an integrated software system for the determination of crystal structures from diffraction data, Bruker Analytical X-ray Systems, Inc., Madison, WI 53719. This package includes, among others: XPREP, an automatic space group determination program; XS, the Bruker SHELXS module for the solution of X-ray crystal structures from diffraction data; XL, the Bruker SHELXL module for structure refinement; XP, the Bruker interactive graphics display module for crystal structures.

(26) *National Committee for Clinical Laboratory Standards 1997. Performance standards for antimicrobial disk susceptibility tests*, 6th ed.; Approved Standard M2-A6. NCCLS: Wayne, PA, 1997.

JM000045A