Antineoplastic Agents. 445. Synthesis and Evaluation of Structural Modifications of (*Z*)- and (*E*)-Combretastatin A-4[1]

George R. Pettit,*,† Monte R. Rhodes,† Delbert L. Herald,† Ernest Hamel,‡ Jean M. Schmidt,† and Robin K. Pettit†

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

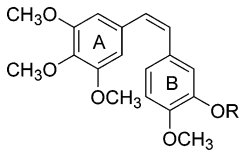
Received December 27, 2002

A series of *cis*- and *trans*-stilbenes related to combretastatin A-4 (**1a**), with a variety of substituents at the 3′-position of the aryl B-ring, were synthesized and evaluated for inhibitory activity employing six human cancer cell lines (NCI-H460 lung carcinoma, BXPC-3 pancreas, SK-N-SH neuroblastoma, SW1736 thyroid, DU-145 prostate, and FADU pharynx-squamous sarcoma) as well as the P-388 murine lymphocyte leukemia cell line. Several of the *cis*-stilbene derivatives were significantly inhibitory against all cell lines used, with potencies comparable to that of the parent **1a**. All were potent inhibitors of tubulin polymerization. The corresponding *trans*-stilbenes had little or no activity as tubulin polymerization inhibitors and were relatively inactive against the seven cancer cell lines. In terms of inhibition of both cancer cell growth and tubulin polymerization, the dimethylamino and bromo *cis*-stilbenes were the most potent of the new derivatives, the latter having biological activity approaching that of **1a**. As part of the present study, the X-ray crystal structure of the 3′-*O*-phosphate of combretastatin A-4 (**1b**) was successfully elucidated. Compound **1b** has been termed the "combretastatin A-4 prodrug", and it is currently undergoing clinical trials for the treatment of human cancer patients.

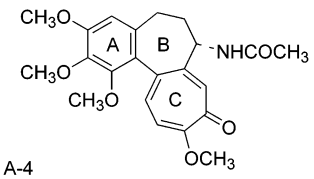
Introduction

Tubulin is one of the most useful and strategic molecular targets for anticancer drugs.[2] Ligands such as combretastatin A-4 (**1a**) that bind in the colchicine[3] (**2**) site of tubulin and inhibit cancer cell proliferation include certain other *cis*-stilbenes[4] (but not their trans isomers, for example, resveratrol **3**[5]), as well as podophyllotoxin[6] (**4**), steganacin[7] (**5**), and some of their synthetic analogues. The most promising of these compounds thus far is combretastatin A-4[4] (**1a**), which has been shown to cause mitotic arrest[4,8] in murine leukemia cells and human ovarian and colon cancer cell lines, exhibits activity against multi-drug-resistant (MDR) cancer cell lines[8a] and, most importantly, has demonstrated powerful cancer antiangiogenesis properties.[9] Currently, the sodium combretastatin A-4 3′-phosphate[4b,c] prodrug (**1b**) is undergoing human cancer clinical trials and shows significant promise.[4m,n]

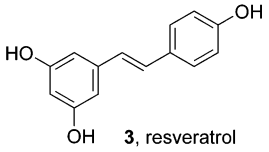
The relative molecular simplicity of combretastatin A-4 (**1a**) suggests a number of practical approaches to the design of new antineoplastic agents, and such efforts in recent years have been devoted to detailed studies of the structure−activity relationships (SAR) of variously substituted stilbenes[2a,10] as well as water-soluble prodrugs.[4b,c,9a] From these investigations, we noticed that (a) the (*Z*)-configuration of the olefin bridge is essential for biological activity and (b) a substituent at the 3′-position of the B-ring was almost always necessary for significant cytotoxic activity. The present report summarizes the synthesis of a selection of 3′-halide, -amine, and -*O*-alkylamine derivatives as potential cancer cell growth inhibitors and evaluation of these compounds for inhibition of tubulin polymerization and antimicrobial activity.



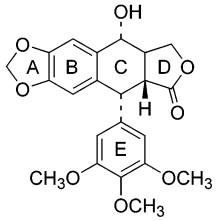
1a, R = OH, combretastatin A-4
b, R = OP(O)(ONa)$_2$
or
OP(O)(OH)ONa



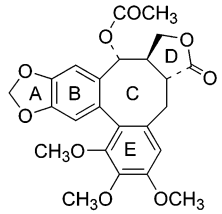
2, colchicine



3, resveratrol



4, podophyllotoxin



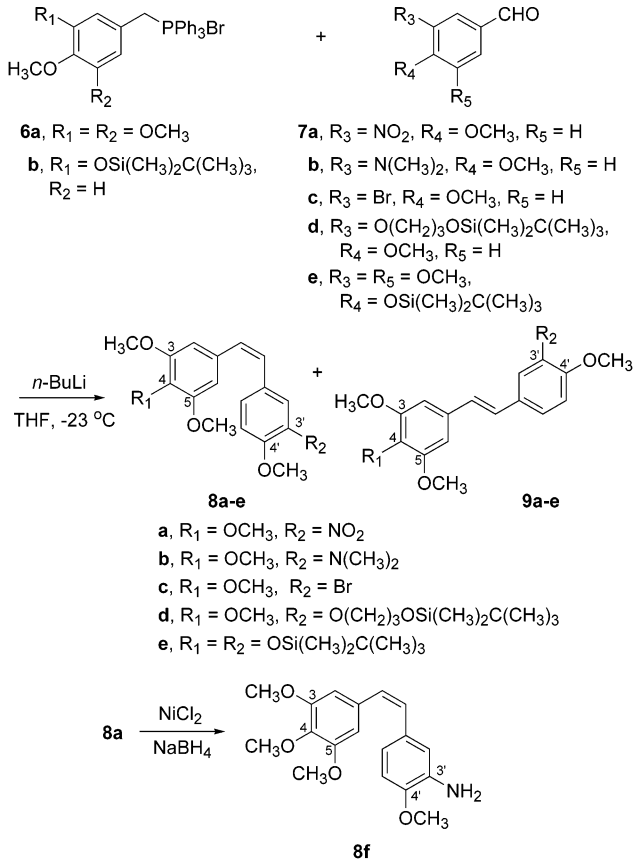
5, steganacin

Wittig reaction[4b,11] between phosphonium bromides **6a**[12] or **6b**[4d] with the aryl aldehydes **7a**−**e** in tetrahy-

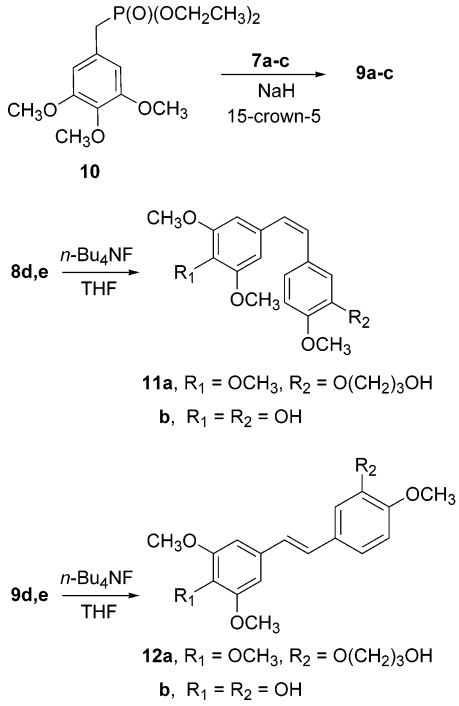
* To whom correspondence should be addressed. Tel.: (480) 965-3351. Fax: (480) 965-8558. E-mail: bpettit@asu.edu.
† Arizona State University.
‡ National Cancer Institute.

10.1021/jm0205797 CCC: $30.25
Published on Web 05/24/2005

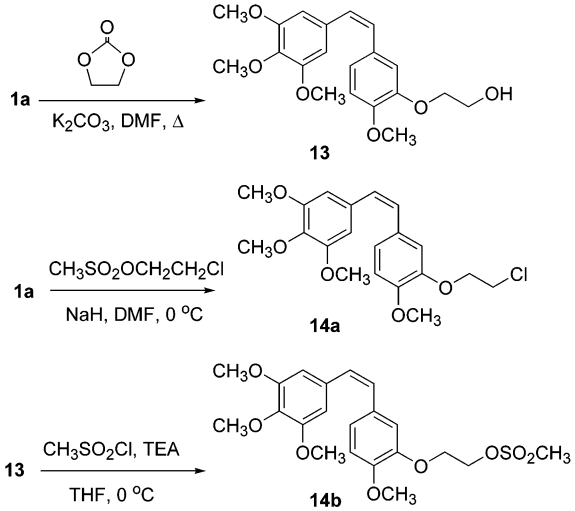
drofuran with *n*-butyllithium (sodium hydride may also be used) to generate the ylide, followed by separation using flash column chromatography, led to the corresponding *cis*-stilbenes **8a**−**e** and *trans*-stilbenes **9a**−**e**. Next, we completed a synthesis of the 3′-amino derivative **8f** of combretastatin A-4 by reducing the 3′-nitro precursor **8a**. While this synthesis seemed simple upon initial inspection, the presence of the ethylene bridge made selective reduction difficult. Initial attempts with aqueous $Na_2S_2O_4$/acetone,[13] Fe−ammonium chloride,[14] $NaBH_4/CuSO_4$ in ethanol,[15] and hydrazine/montmorillonite[16] in refluxing ethanol, while all superficially promising, failed to give the 3′-amine **8f**. In all instances, a complex mixture of products and/or isomerization of the product to the undesired (*E*)-olefin resulted. Turning our attention to several newer methodologies involving organometallics, we first tried sonication of the 3′-nitrostilbene with anhydrous $NiCl_2$,[17] but no reaction product was detected. Attempting a similar reaction utilizing $NiCl_2$ hexahydrate in combination with sodium borohydride[18] gave the 3′-amino derivative in yields greater than 50% following purification. Although this is a new route to amine **8f**, the compound has since been reported in the literature by several other groups using two different methods.[10e,19] Nevertheless, with such an efficient route in hand, the path has been opened to other potentially useful structural modifications of combretastatin A-4. Comparison of the biological activity of the 3′-amine **8f** with that of the 3′-amine derivatives already reported showed a good correlation.



By use of the chemistry described above, the cis isomers were the major products, and the trans isomers were isolated as the minor products. From aryl aldehydes **7a**−**c**, the cis isomers were obtained in high yields, whereas the trans isomers were barely detectable. Consequently, the *trans*-stilbenes **9a**−**c** were prepared by the Wittig−Horner reaction employing phosphonate ester **10**[20] with aryl aldehydes **7a**−**c** and 15-crown-5 as a cocatalyst.[21] Under these conditions the trans isomers were obtained exclusively. The 3′,4-dihydroxystilbenes **11b** and **12b** were prepared from the corresponding *O*-silyloxylated stilbenes **8e** and **9e** by deprotection using tetra-*n*-butylammonium fluoride in tetrahydrofuran. The 3′-hydroxypropyloxy derivatives **11a** and **12a** were prepared by an analogous route from **8d** and **9d**.

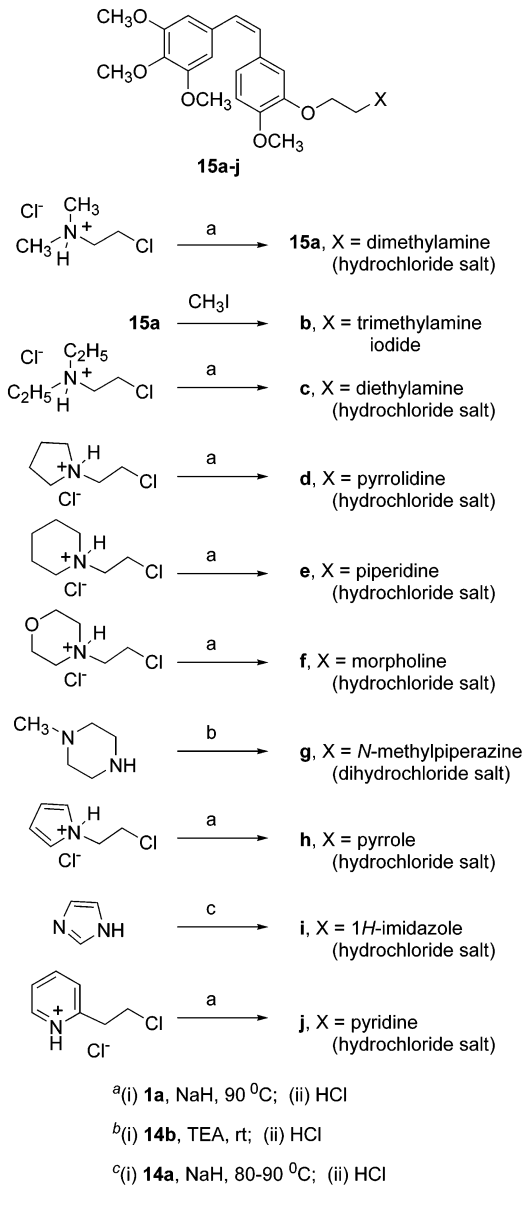


Preparation of the 3′-side-chain tertiary amine derivatives was accomplished by use of two separate approaches. The first involved synthesis of 2-hydroxyethoxy derivative **13** by reaction of combretastatin A-4 (**1a**) with ethylene carbonate and base. Treatment of **13**

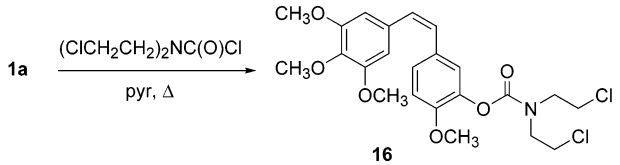


with methanesulfonyl chloride gave **14b**, and subsequent reaction of **14b** with the selected amine in acetonitrile containing triethylamine[22] gave products

15a,**c**−**g**. However, owing to the number of steps and less than desirable yields, most of these amines were made via a second route: reaction of **1a** itself with the appropriate aminoethyl halide and sodium hydride[23] afforded amines **15a**,**c**−**f**,**h**,**j** in yields of 70% or greater.



Compound **15i** was prepared from the ethoxy chloride analogue (**14a**) of **1a**, and methylation of **15a** gave **15b**. Treatment of **1a** with *N*,*N*-(2-chloroethyl)aminocarbonyl chloride in dry pyridine over 54 h gave the 3′-glycine mustard **16** in 72% yield. The cis or trans geometries



were established by their characteristic ^{1}H NMR coupling constants for the olefinic protons, consistent with this class of compounds of approximately 12 Hz for the cis and 16−17 Hz for the trans isomers.[24,4d] In some cases (**8a**,**b** and **9d**), the two olefinic protons gave singlets in the 300-MHz NMR spectrum. Consequently, those isomers were assigned relative to their corresponding geometric isomer by 500-MHz NMR spectroscopy, which gave distinct couplets with characteristic coupling constants, and by 2D NMR experiments.

Further study of the 3′-*O*-phosphate sodium salt[4b,c] (**1b**) with respect to its crystal structure proved to be an especially useful avenue of the present investigation. A crystal of prodrug **1b** suitable for X-ray analysis (Figure 1) was grown from a mixture of water and acetone (1:2, v/v). On the basis of the X-ray crystal structure combined with the combustion analysis for sodium and results of a Karl Fisher water analysis, it appears that the crystalline combretastatin A-4 prodrug specimen selected for crystal structure determination corresponded on average to one sodium atom per molecule. Presumably the disodium, monosodium, and acid phosphates exist in equilibrium, and the relative amounts are a function of pH. For example, at pH near 9.5, the disodium salt should predominate, while at pH 4.5 the monosodium salt is the most stable form, and at pH <4 the phosphoric acid should be present.

The X-ray crystal structure of combretastatin A-4 prodrug (**1b**) also suggests that the conformation of this stilbene is not planar. The crystal structure reveals that the planes of the two phenyl rings are inclined to each other, suggesting a low-energy conformation that may be the one involved in binding at the tubulin receptor site. After our original isolation and initial structure determination of combretastatin A-4, we, and more recently others,[25] completed X-ray crystal structure elucidations of this natural product and found the same nonplanarity.[8d] The planar conformation of phosphate **1b** would be a high-energy species owing to a nonbonded interaction between the protons of the two aromatic rings.

The effects on cancer cell growth and tubulin polymerization of the 4 *trans*- and 20 *cis*-stilbenes have been summarized in Tables 1 and 2, respectively. Previous studies involving combretastatin A-4 (**1a**) have established that the positions of the methoxy groups in both the A- and B-rings determine the magnitude of cytotoxicity and antitumor activity.[10f,j] Replacement of the 4-methoxy group of the A-ring with a hydroxyl group (**11b** and **12b**) resulted in a significant reduction (about 100 times) in cancer cell growth inhibition with the cis isomer, whereas the trans isomer maintained activity comparable with that of *trans*-combretastatin A-4.[4a,d] The ability of **12b** to inhibit tubulin polymerization was greater than that of the parent *trans*-stilbene, but we were unable to evaluate the cis isomer **11b** since it was unstable and isomerized to *trans*-stilbene **12b** in 1 day at room temperature. This may also account for the reduced cytotoxicity observed with **11b**.

Replacement of the B-ring 3′-hydroxyl group of *cis*-stilbene **1a** with nitro, dimethylamino, or bromo groups (compounds **8a**−**c**) resulted in products with a decreased potency relative to the parent stilbene (**1a**), albeit still cytotoxic ($ED_{50} < 10^{-1}$ μM). These results suggest that the location of the four methoxy groups and the presence of a substituent at the 3′-position of the *cis*-stilbene are important features for pronounced inhibition of cancer cell growth.

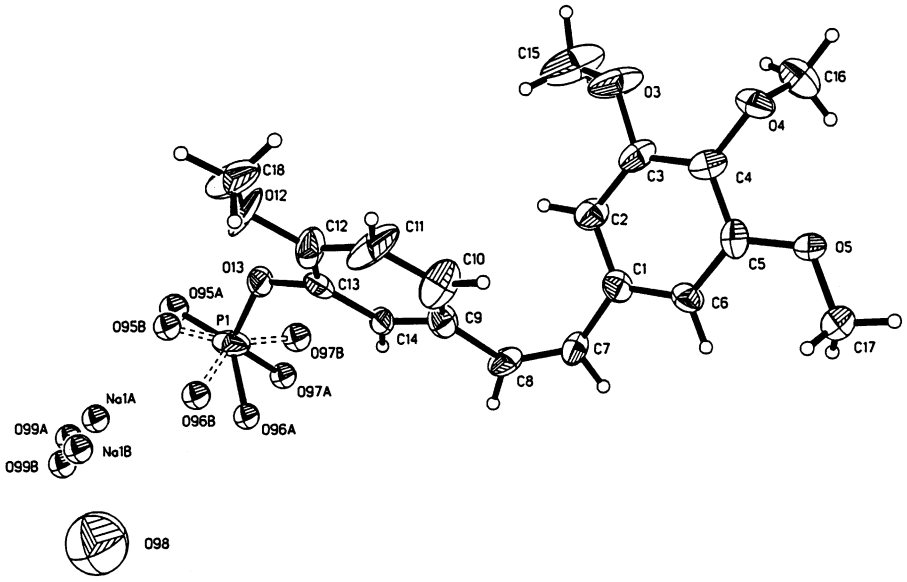


Figure 1. X-ray crystal structure of combretastatin A-4. Atoms are depicted as 50% thermal probability ellipsoids, and the dashed lines depict bonds to alternate disordered sites.

Table 1. Human Cancer Cell Line (GI_{50})[a] and Murine P-388 Lymphocytic Leukemia Inhibitory Activity (ED_{50})[a] of Combretastatins A-4 and A-4 Prodrug, and Synthetic Modifications

compd (μg/mL)	leukemia P-388	pancreas BXPC-3	neuroblast SK-N-SH	thyroid SW1736	lung-NSC NCI-H460	prostate DU-145	pharynx FADU
1a	0.0026	>0.1	0.00026	0.00026	0.00056	0.00076	0.00065
1b	0.0029	0.23	0.00025	0.00061	0.00035	0.00072	0.00045
8a	2.4	0.029	0.014	0.0067	0.0038	0.047	0.047
8b	0.9	0.015	0.010	0.80	0.4	0.11	0.090
8c	0.16	0.007	0.002	0.034	0.033	0.027	0.0058
8f	>0.010	0.00043	0.00023	0.00080	0.00033	0.00033	0.00053
9a	>10.0	>10.0	7.3	2.8	>0.0	>10.0	3.8
9b	–[b]	–	–	–	–	–	–
9c	3.08	0.34	0.40	10.8	0.36	0.46	11.4
11a	0.63	0.26	0.18	0.38	0.37	0.43	0.63
11b	0.25	>4.0	0.19	3.4	0.74	0.17	0.23
12a	6.8	>10.0	8.1	11.6	>10.0	4.5	2.3
12b	4.49	5.0	5.5	>10.0	4.9	6.3	3.2
13	0.195	0.045	0.028	0.28	0.14	0.32	0.069
14a	2.9	2.7	2.0	4.7	3.7	3.4	4.6
14b	2.87	2.7	3.0	1.0	0.42	0.59	0.41
15a	0.523	>10.0	0.21	0.64	0.34	0.38	0.47
15b	1.94	37.2	0.28	0.10	0.36	0.33	0.24
15c	0.255	>10.0	1.5	1.1	3.3	3.4	1.1
15d	0.421	2.0	0.22	0.60	0.34	0.35	0.49
15e	0.348	>10.0	1.6	5.1	3.3	3.4	4.2
15f	0.634	2.9	0.22	0.97	0.41	0.40	0.53
15g	1.60	>10.0	2.8	>10.0	3.4	4.1	2.9
15h	0.0232	2.3	0.0064	4.9	0.0033	0.56	0.0030
15i	19.0	0.82	0.31	1.5	0.56	1.5	0.67
15j	0.75	1.01	0.22	2.3	0.68	0.32	0.54
16	0.074	2.8	0.14	0.72	0.37	0.31	0.15

[a] GI_{50} (HTCL) and ED_{50} (P-388) refer to the drug dosages required to inhibit tumor cell growth by 50%. There is no mathematical difference between the two values which are both calculated identically; the only difference is historical usage. [b] Not tested owing to instability of the compound.

Among the structural features considered to be important in such structurally related anticancer agents as tamoxifen, clomiphene, and nafoxidine[26] is the amine side chain. This substituent has been reported to be an essential contributor to the anticancer properties of both tamoxifen and trioxifene.[27] Consequently, as an extension of our SAR efforts with combretastatin A-4, a selection of derivatives that include amine-containing (**15a**,**c**–**j** and **16**) side chains, as well as quaternary salt[28] **15b**, were prepared from the parent stilbene (**1a**). The result was a series of substances that are only weakly active against the cancer cell lines employed.

Consistent with earlier observations,[4a,d] all of the *trans*-stilbenes (**9a**,**c**, **12a**,**b**) were less potent than their corresponding cis isomers. Compound **9c** showed moderate cytotoxicity (1.0×10^{-1} μM range) in several of the cell lines tested, while the other *trans*-stilbenes proved to be less active.

The principal mechanism of action of the combretastatins has been shown to involve microtubules.[29] Accordingly, the newly synthesized compounds were evaluated for inhibitory effects on tubulin assembly in comparison with combretastatin A-4 (**1a**) and its trans isomer, when sufficient sample was available (Table 2).

Table 2. Effect of Newly Synthesized Combretastatin Analogues on Tubulin Polymerization[a]

compd	IC_{50} (μM) ± SD
combretastatin A-4 (**1a**)	1.2 ± 0.03
trans-combretastatin A-4	33 ± 7
8a	2.6 ± 0.5
8c	1.1 ± 0.02
9c	31 ± 1
11a	6.5 ± 0.03
12b	14 ± 4
13	2.8 ± 0.4
14a	>40
14b	>40
15a	>40
15c	>40
15d	>40
15e	>40
15f	>40
15g	>40
15h	20 ± 6
15i	7.6 ± 0.2
16	>40

[a] Reaction mixtures contained 0.8 M monosodium glutamate (pH 6.6, with HCl in 2 M stock solution), 10 μM (1.0 mg/mL) purified tubulin, 4% (v/v) dimethyl sulfoxide (drug solvent), and varying concentrations of compounds being evaluated. Reaction mixtures were incubated for 15 min at 37 °C and then chilled on ice. GTP (0.4 mM) was added, and reaction mixtures were transferred to cuvettes held at 0 °C. The temperature was raised to 30 °C, and tubulin assembly was followed for 20 min by turbidimetry at 350 nm in Beckman DU7400/7500 diode array spectrophotometers. IC_{50} values were determined by interpolation between experimental values. A minimum of two determinations was performed with each compound. SD, standard deviation.

In these studies we evaluated the ability of these compounds to inhibit the extent of assembly of 10 μM tubulin following an incubation of 30 °C for 20 min.

In previous work the amino derivative **8f** was shown to have inhibitory activity identical to that of combretastatin A-4 (**1a**).[30] In the present study, the bromo analogue **8c** also was as active as **1a** (IC_{50} values of 1.1 and 1.2 μM, respectively), while the nitro derivative **8a** and the hydroxyethyl derivative **13** were about half as active (IC_{50} values of 2.6 and 2.8 μM, respectively). This contrasts with the cytotoxicity evaluation presented in Table 1, where only **8f** had activity against cells comparable to the activity of **1a**, and **8a**,**c** and **13**, although similar to each other in overall activity, were substantially less active than **1a** in most cell lines examined. *cis*-Stilbenes with bulkier substituents at position C-3′ were invariably much less active as inhibitors of assembly, and in most cases (**14a**,**b**, **15a**,**c**−**g**, **16**) showed minimal or no inhibitory activity at 40 μM, the highest concentration evaluated. Nevertheless, the hydroxypropyl derivative **11a** and the imidazolylethyl derivative **15i** had IC_{50} values (6.5 and 7.6 μM, respectively) substoichiometric to the tubulin concentration of 10 μM, while the pyrrolloethyl derivative **15h** was weakly inhibitory, with an IC_{50} value of 20 μM. The activity of the latter two compounds is somewhat surprising, but it suggests that the aromatic substituent overcomes the presumably steric effect that interferes with the stilbene−tubulin interaction implied by the inactivity of **14a**,**b**, **15a**,**c**−**g**, and **16**. Curiously, the better tubulin inhibitor **15i** had little cytotoxicity, while the less effective **15h** was quite cytotoxic in four of the seven cell lines.

Two of the new *trans*-stilbenes were also evaluated for antitubulin activity. The bromo derivative **9c** had weak activity that was essentially identical to that of *trans*-combretastatin A-4 (IC_{50} values of 31 and 33 μM, respectively). Somewhat surprisingly, the C-4 demethyl analogue of *trans*-combretasatain A-4, compound **12b**, was over twice as active as the parent compound, with an IC_{50} value of 14 μM. This could be the result of some isomerization to the cis isomer, since rapid isomerization of the latter to **12b** had precluded its evaluation for inhibition of tubulin assembly (see above).

Combretastatin A-4, combretastatin A-4 prodrug, and several prodrug modifications were previously shown to have marginal antimicrobial activity.[4b] Of the combretastatin A-4-related stilbenes available for antimicrobial evaluation, compounds **11b**, **12b**, and **15b**,**c**,**d**,**i** had the most activity (Table 3). Compound **15c** was the most potent, with a minimum inhibitory concentration (MIC) of <6.25 μg/disk for *Micrococcus luteus*. Compound **12b** had MICs of 6.25−12.5 μg/disk for *Cryptococcus neoformans* and 12.5−25 μg/disk for *Neisseria gonorrhoeae*, while compound **11b** exhibited a MIC of 12.5−25 μg/disk against *N. gonorrhoeae*. Compounds **15b**,**d**,**i** had MICs of 12.5−25 μg/disk for *M. luteus*.

Table 3. Antimicrobial Activities [MIC (μg/disk)] of the Combretastatin A-4-Related Stilbenes in the Disk Diffusion Assay

compd	*Candida albicans*	*Cryptococcus neoformans*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Enterococcus faecalis*	*Micrococcus luteus*	*Stenotrophomonas maltophilia*	*Escherichia coli*	*Enterobacter cloacae*	*Neisseria gonorrhoeae*
8a	*	*	*	NT	*	*	NT	*	NT	*
8c	*	*	*	*	*	*	*	*	*	*
9a	*	*	*	*	*	*	*	*	*	*
9c	*	*	*	*	*	*	*	*	*	*
11a	*	*	*	NT	*	*	NT	*	NT	50−100
11b	*	*	*	NT	*	*	NT	*	NT	12.5−25
12b	50−100	6.25−12.5	*	*	*	*	*	*	*	12.5−25
13	*	*	*	NT	*	*	NT	*	NT	50−100
14a	*	*	*	*	*	*	*	*	*	*
14b	*	*	*	*	*	*	*	*	*	*
15a	*	*	*	*	*	*	*	*	*	*
15b	25−50	*	25−50	50−100	*	12.5−25	*	*	*	*
15c	*	*	25−50	NT	50−100	<6.25	NT	*	NT	50−100
15d	*	*	50−100	NT	*	12.5−25	NT	*	NT	*
15e	*	*	*	NT	*	*	NT	*	NT	*
15f	*	*	*	NT	*	*	NT	*	NT	50−100
15g	*	*	*	*	*	*	*	*	*	*
15h	*	*	*	*	*	*	*	*	*	*
15i	*	*	*	*	*	12.5−25	*	*	*	50−100
16	*	*	*	*	*	*	*	*	*	*

Experimental Section

Tetrahydrofuran (THF) was freshly distilled from sodium/benzoketal, dimethylformamide (DMF) from CaH_2, and triethylamine (TEA) from KOH. All other solvents were redistilled prior to use. Air-sensitive reactions were carried out under argon. Ether refers to diethyl ether. Organic extracts of aqueous solutions were dried over $MgSO_4$ unless specified otherwise.

The following compounds were prepared according to literature procedures: combretastatin A-4,[4d] 1-(2-chloroethyl)-1*H*-pyrrole,[31] 1-(2-chloroethyl)-1*H*-imidazole hydrochloride,[32] 2-(2-chloroethyl)pyridine,[33] 3-(2-chloroethyl)pyridine hydrochloride,[34] 2-diethylaminoethyl chloride hydrochloride,[35] 4-methoxy-3-nitrobenzaldehyde,[36] [(3,4,5-trimethoxyphenyl)methyl]-phosphoric acid diethyl ether,[20] 4-[(*tert*-butyldimethylsilyl)oxy]-3,5-dimethoxybenzaldehyde,[10j] 3-(3-hydroxypropoxy)-4-methoxybenzaldehyde,[37] [3-(*tert*-butyldimethylsilyl)oxy]-4-methoxybenzyltriphenylphosphonium bromide,[38] 3,4,5-trimethoxybenzyltriphenylphosphonium bromide,[12] *N*-(chloroformyl)bis-(2-chloroethyl)amine,[39] and 3-dimethylamino-4-methoxybenzaldehyde (**7**).[40] All other chemicals were purchased from either Acros Organics or Sigma-Aldrich Chemical Co.

Reaction progress and purity of products were checked by analytical TLC using Analtech (GHLF) silica-coated glass plates, with visualization by UV_{254} light, iodine, 0.3% ninhydrin in (97:3) *n*-butanol−HOAc, or 5% anisaldehyde in 95:5:1 ethanol−acetic acid−H_2SO_4. Separation of products was achieved by flash column chromatography on fine silica (EM Sciences, 230−400 mesh ASTM).

Melting points were determined in a capillary tube using an Electrothermal apparatus (Model IA9200) and are uncorrected. The ^1H and ^{13}C NMR spectra were determined with a Varian Gemini 300-MHz spectrometer, unless otherwise noted, with TMS as an internal standard in $CDCl_3$. The IR spectra were recorded by employing a Nicolet FTIR model MX-1 spectrophotometer, and the EIMS data were measured with a MAT 312 mass spectrometer. Microanalyses were performed at Galbraith Laboratories. X-ray crystal structure analysis was performed on a Bruker SMART 6000 diffractometer.

3-[(*tert*-Butyldimethylsilyloxy)propoxy]-4-methoxybenzaldehyde (7d). To a stirred solution of 3-(3-hydroxypropoxy)-4-methoxybenzaldehyde[37] (1.00 g, 4.8 mmol) in dry DMF (20 mL) was added imidazole (0.82 g, 12 mmol, 2.5 equiv), followed by *tert*-butyldimethylsilyl chloride (0.87 g, 5.76 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 4 h, and water (20 mL) was added, followed by another 10 min of stirring. Ether (25 mL) was added, followed by saturated sodium bicarbonate solution (25 mL), and stirring was continued for another 15 min. The ethereal phase was separated, and the aqueous phase was washed with ether (3 × 25 mL). The combined ether extract was washed with brine (50 mL) and water (50 mL), dried, and filtered, and solvent was removed in vacuo. Purification by flash chromatography gave a clear viscous oil (1.4 g, 98%): R_f 0.38 (hexanes−EtOAc, 9:1); IR (neat) ν_{max} 2955, 2856, cm^{-1}; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 2.05 (qn, J = 7.5, 2 Hz, 2H, $CH_2CH_2CH_2$), 3.81 (t, J = 6.0 Hz, 2H, CH_2OSi), 3.93 (s, 3H, OCH_3), 4.17 (t, J = 6.0 Hz, 2H, $OCH_2CH_2CH_2$), 6.86 (d, 1H, H-5), 7.20 (dd, J = 9.0, 2.4 Hz, 1H, H-6), 7.32 (d, J = 2.1 Hz, 1H, H-2), 9.79 (s, 1H, CHO); ^{13}C NMR (75 MHz) δ 0.00, 23.74, 31.35, 37.61, 61.53, 64.87, 71.16, 116.04, 131.89, 135.54, 154.56, 160.28, 196.37. Anal. Calcd for $C_{17}H_{28}O_4Si$: C, 62.9; H, 8.7. Found: C, 63.07; H, 8.76.

General Procedure for the Preparation of (*Z*)-Stilbenes 8a−d. A homogeneous suspension of phosphonium bromide **6a** (15.1 g, 28.9 mmol) in THF (900 mL) under argon was cooled and retained at −23 °C for 1 h. Butyllithium (11.6 mL, 28.9 mmol of a 2.5 M solution in hexanes) was added dropwise via syringe, and the resultant blood-red solution was stirred at −23 °C for 60 min, at which time the aldehyde (28.9 mmol) in 100 mL of dry THF was added (dropwise) from an addition funnel. Stirring was continued at −23 °C for 3 h and at room temperature for 18 h. By this time, the red color had completely disappeared. Ice−water (300 mL) was added to the mixture, and two phases separated. The aqueous phase was washed with ether (3 × 200 mL), and the ethereal solution was added to the THF layer from the reaction mixture. The combined organic phase was washed with water (3 × 200 mL) and dried. Removal of solvent in vacuo yielded a crude residue, which was subjected to flash chromatography (silica gel; eluent 5% EtOAc in hexane) to afford the pure isomeric stilbenes.

3′-Nitro-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (8a). Following flash column chromatography (eluent hexanes−ethyl acetate, 5:1), the product was obtained as a yellow solid that recrystallized from ethyl acetate−hexane as a pale yellow powder (2.2 g, 66%): mp 127−127.5 °C; IR (KBr) ν_{max} 2960 (aromatic) 2937, 2827, 1577, 1527, 1502 (assym. NO_2) 1464, 1413, 1365, 1288 (symm. NO_2), 1236, 1126, 1004, 856 (C−N), 781 cm^{-1}; EIMS *m/z* (relative intensity) 345 (M^+, 100), 330 (65), 315, 283, 270, 254, 240, 211, 195, 181, 165, 152, 139; ^1H NMR (500 MHz) δ 3.71 (s, 6H, 2 × OCH_3), 3.84 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.44 (d, J = 12.0 Hz, 1H, H-1a′), 6.46 (s, 2H, H-2, H-6), 6.58 (d, J = 12.1 Hz, 1H, H-1a), 6.93 (d, J = 8.1 Hz, 1H, H-5′), 7.42 (dd, J = 8.1, 2.1 Hz, 1H, H-6′), 7.79 (d, J = 2.1 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 56.95, 56.59, 60.99, 105.85, 113.10, 125.99, 126.87, 129.74, 131.32, 131.82, 134.64, 137.72, 139.48, 151.72, 153.2. Anal. Calcd for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; N, 4.06. Found: C, 62.74; H, 5.63; N, 3.92.

3′-Dimethylamino-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (8b). Flash column chromatography (eluent hexanes−EtOAc, 5:1) gave the product as a viscous, yellow oil (200 mg, 37%), which was found to be unstable at room temperature: R_f 0.30 (hexanes−ethyl acetate, 5:1); EIMS *m/z* (relative intensity) 343 (45), 315 (5), 300 (15), 100 (100); IR (neat) ν_{max} 2850, 2770, 1572, 1500, 1450, 1241, 1120, 1010, 870 cm^{-1}; ^1H NMR (500 MHz) δ 2.93 (s, 6H, $N(CH_3)_2$), 3.70 (s, 6H, 2 × OCH_3), 3.81 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.46 (d, J = 12.0 Hz, 1H, H-1a′), 6.49 (d, J = 12.0 Hz, 1H, H-1a), 6.50 (s, 2H, H-2, H-6), 6.77 (d, J = 8.1 Hz, 1H, H-5′), 6.86 (dd, J = 8.1, 1.6 Hz, 1H, H-6′), 7.23 (d, J = 1.8 Hz, 1H, H-2′).

3′-Bromo-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (8c). Recrystallization from ethyl acetate−hexane, following purification by flash column chromatography, gave prismatic crystals (4.4 g, 40%): mp 100−101 °C; R_f 0.30 (hexanes−ethyl acetate, 9:1); IR (KBr υ_{max} 2850, 2695, 1250, 1110, 1010, 870 cm^{-1}; EIMS *m/z* 379, (M^+, 100), 363 $(M - CH_3)^+$; ^1H NMR δ 3.71 (s, 6H, 2 × OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.42 (d, J = 12.0 Hz, 1H, H-1a′), 6.47 (d, J = 12.0 Hz, 1H, H-1a), 6.51 (s, 2H, H-2, H-6), 6.78 (d, J = 8.7 Hz, 1H, H-5′), 7.19 (dd, J = 8.7, 2.4 Hz, 1H, H-6′), 7.55 (d, J = 2.4 Hz, 1H, H-2′). Anal. Calcd for $C_{18}H_{19}BrO_4$: C, 57.01; H, 5.05; Br, 21.07. Found: C, 56.99; H, 5.1; Br, 21.00.

3′-[(*tert*-Butyldimethylsilyloxy)propoxy]-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (8d). Following purification by flash chromatography, the product was isolated as a clear oil (65%): R_f 0.45 (hexanes−ethyl acetate, 7:3); IR (neat) ν_{max} 3001, 2978, 2901, 1745, 1545, 1432, 1211, 1189, 1010, 854 cm^{-1}; EIMS *m/z* (relative intensity) 488 (M^+, 100), 473, 431, 398, 373, 358, 343, 163, 73; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 2.05 (q, 2H, $CH_2CH_2CH_2$−), 3.69 (s, 6H, 2 × OCH_3), 3.81 (t, J = 6.0 Hz, 2H, CH_2Si), 3.83 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.20 (t, J = 6.0 Hz, 2H, OCH_2), 6.43 (d, J = 12.3 Hz, 1H, H-1a′), 6.50 (d, J = 12.3 Hz, 1H, H-1a), 6.52 (s, 2H, H-2, H-6), 6.76 (d, J = 8.7 Hz, 1H, H-5′), 7.44 (dd, J = 8.7, 1.5 Hz, 1H, H-6′), 7.47 (d, J = 1.5 Hz, 1H, H-2′).

3′,4-Di[(*tert*-butyldimethylsilyl)oxy)]-3,4′,5-trimethoxy-(*Z*)-stilbene (8e). Wittig coupling of phosphonium salt **6b** with TBDMS aldehyde **7e** and separation (eluent hexanes−ethyl acetate, 50:1) led to a clear oil (1.3 g, 36%): EIMS *m/z* 530 (M^+, 70), 473 (75), 458 (45), 386 (100); ^1H NMR δ 0.086 (s, 6H, $Si(CH_3)_2$), 0.12 (s, 6H, $Si(CH_3)_2$), 0.95 (s, 9H, $C(CH_3)_3$), 1.00 (s, 9H, $C(CH_3)_3$), 3.62 (s, 6H, 2 × OCH_3), 3.77 (s, 3H, 4′-OCH_3), 6.41 (d, J = 12.0 Hz, 1H, H-1a′), 6.48 (d, J = 12.0 Hz, 1H, H-1a), 6.48 (s, 2H, H-2, H-6), 6.71 (d, J = 8.4 Hz, 1H, H-5′), 6.83 (m, 1H, H-6′), 7.01 (d, J = 2.0 Hz, 1H, H-2′). Anal. Calcd for $C_{29}H_{46}O_5Si_2$: C, 65.6; H, 8.7. Found: C, 65.98; H, 9.05.

3′-Amino-3,4,4′,5-tetramethoxy-(*Z*-)stilbene (8f). Nitrostilbene **8a** (50 mg, 0.8 mmol) and nickel(II) chloride hexahy-

drate (1.6 mmol, 2 equiv) were dissolved in dry methanol (5 mL). Sodium borohydride (0.12 g, 3.2 mmol, 4 equiv) was added (in portions with stirring under cooling) over the course of 30 min. The reaction mixture was then warmed to room temperature and stirred for 30 min. The solvent was removed (in vacuo), the resulting black, granular solid residue was redissolved in methanol, and the mixture was filtered through a 1-in. pad of silica gel. The filtrate (200 mL) was reduced in volume, adsorbed onto silica gel, and separated by flash column chromatography (hexanes−ethyl acetate, 5:1) to give the product as a pale yellow gum (25 mg, 63%) that was stored in an amber vial below 0 °C: R_f 0.38 (hexanes−ethyl acetate, 2:1); IR (neat) ν_{max} 3472 (N−H stretch), 3371 (N−H stretch), 3001 (aromatic C−H stretch), 2937, 2835, 1614, 1579 (aromatic C=C stretch), 1512, 1454, 1327, 1284 (C−N stretch), 1234, 1126, 1006, 875 cm^{-1}; EIMS *m/z* (relative intensity) 315 (M^+, 100), 300 ($M - NH)^+$, 285, 254, 240, 225, 152; ^1H NMR δ 3.70 (s, 6H, 2 × OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.37 (d, J = 12.6 Hz, 1H, H-1a′), 6.46 (d, J = 12.1 Hz, 1H, H-1a), 6.55 (s, 2H, H-2, H-6), 6.68 (s, 2H, H-5′, H-6′), 6.70 (d, J = 1.2 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 55.49, 55.90, 60.90, 106.04, 110.06, 115.23, 119.50, 128.36, 129.99, 132.96, 135.73, 137.03, 146.61, 152.79; HRFABMS $(M)^+$ calcd for $C_{18}H_{21}NO_4$, 315.1471, found 315.1465.

3′-[(*tert*-Butyldimethylsilyl)propoxy]-3,4,4′,5-tetramethoxy-(*E*)-stilbene (9d). The olefin recrystallized from ethyl acetate−heptane as colorless needles (35%): mp 116−117 °C; R_f 0.36 (hexanes−ethyl acetate, 7:3); IR (KBr) ν_{max} 2998, 2875, 1725, 1654, 1432, 1211, 1178, 1020, 854 cm^{-1}; EIMS *m/z* (relative intensity) 488 (M^+, 100), 473, 431, 398, 373, 358, 343, 163; ^1H NMR (500 MHz) δ 0.087 (s, 6H, $Si(CH_3)_2$), 1.01 (s, 9H, $C(CH_3)_3$), 2.10 (q, 2H, $CH_2CH_2CH_2$), 3.70 (s, 3H, OCH_3), 3.80 (t, J = 6.0 Hz, 2H, $-CH_2-Si$), 3.81 (s, 6H, 2 × OCH_3), 3.91 (s, 3H, OCH_3), 4.18 (t, J = 6.0 Hz, 2H, OCH_2), 6.56 (d, J = 16.3 Hz, 1H, H-1a′), 6.58 (s, 2H, H-2, H-6), 6.62 (d, J = 16.4 Hz, 1H, H-1a), 7.01 (d, J = 8.7 Hz, 1H, H-5′), 7.36 (s, 1H, H-6′), 7.50 (d, J = 1.2 Hz, 1H, H-2′). Anal. Calcd for $C_{27}H_{40}O_6Si$: C, 66.36; H, 8.25. Found: C, 66.35; H, 8.45.

3′,4-Di((*tert*-butyldimethylsilyl)oxy)-3,4′,5-trimethoxy-(*E*)-stilbene (9e). Following the Wittig reaction and purification as described above (see **8e**), the solid product recrystallized from ethyl acetate−hexane as prisms (1.4 g, 37%): mp 85−86 °C; EIMS *m/z* (relative intensity) 530 (M^+, 70), 473 (75), 458 (45), 386 (100); ^1H NMR δ 0.139 (s, 6H, $Si(CH_3)_2$), 0.181 (s, 6H, $Si(CH_3)_2$), 1.02 (s, 9H, $C(CH_3)_3$), 1.16 (s, 9H, $C(CH_3)_3$), 3.82 (s, 3H, 4′-OCH_3), 3.84 (s, 6H, 2 × OCH_3), 6.69 (s, 2H, H-2, H-6), 6.80 (d, J = 16.5 Hz, 1H, H-1a′), 6.85 (s, 1H, H-5′), 6.88 (d, J = 16.5 Hz, 1H, H-1a), 7.02 (s, 1H, H-6′), 7.05 (d, J = 1.8 Hz, 1H, H-2′). Anal. Calcd for $C_{29}H_{46}O_5Si_2$: C, 65.6; H, 8.7. Found: C, 65.68; H, 9.00.

General Procedure for Preparation of (*E*)-Stilbenes (9a−c). A solution of the aldehyde (5 mmol) and the diethyl phosphonate (**10**, 5 mmol) in dry THF (10 mL) was added to a stirred slurry of sodium hydride (NaH) (5 mmol) in THF (10 mL) containing 15-crown-5 (0.15 mmol) at 0 °C (under argon). A rapid evolution of H_2 was observed, and the solution developed a gelatinous, orange/red appearance. Upon completion of addition (ca. 15 min), the reaction mixture was warmed to room temperature and stirred for 12 h. The mixture was poured into water (15 mL), transferred to a separatory funnel, and extracted with ether (3 × 20 mL). The combined extract was washed with 10% $NaHSO_3$ (2 × 10 mL) and saturated NaCl (2 × 10 mL) and dried. Evaporation of solvent followed by flash chromatographic separation gave the desired product.

3′-Nitro-3,4,4′,5-tetramethoxy-(*E*)-stilbene (9a). Following purification by column chromatography, the resultant yellow solid recrystallized slowly from ethyl acetate−hexane as pale yellow needles (1.0 g, 64%): mp 159−160 °C; R_f 0.20 (hexanes−ethyl acetate, 3:1); EIMS *m/z* 345 (M^+), 330, 315, 270, 254, 240, 195, 181 (base); ^1H NMR δ 3.89 (s, 3H, OCH_3), 3.92 (s, 6H, 2 × OCH_3), 3.99 (s, 3H, OCH_3), 6.73 (s, 2H, H-2, H-6), 6.93 (d, J = 16.5 Hz, 1H, H-1a′), 7.00 (d, J = 16.5 Hz, 1H, H-1a), 7.09 (d, J = 8.7 Hz, 1H, H-5′), 7.65 (dd, J = 8.7, 2.1 Hz, 1H, H-6′), 8.01 (d, J = 2.4 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 56.50, 60.85, 63.33, 103.58, 122.95, 123.93, 125.07, 129.34, 131.80, 132.32, 132.63, 133.48, 134.62, 152.09, 153.31. Anal. Calcd for $C_{18}H_{19}NO_6$: C, 62.6; H, 5.55; N, 4.05. Found: C, 61.6; H, 5.49; N, 3.88.

3′-Dimethylamino-3,4,4′,5-tetramethoxy-(*E*)-stilbene (9b). Following purification by flash column chromatography, the resultant pale yellow oil (0.5 g, 29%) was stored at low temperature owing to thermal and UV instability: R_f 0.26 (hexanes−ethyl acetate, 4:1); EIMS *m/z* 343 (M^+), 328 ($M^+ - CH_3$), 315, 270, 181 (bases); IR (neat) ν_{max} 2930, 2850, 2780, 1735, 1580, 1126, 1000 cm^{-1}; ^1H NMR (400 MHz) δ 2.90 (s, 6H, $N(CH_3)_2$), 3.90 (s, 3H, OCH_3), 3.91 (s, 6H, 2 × OCH_3), 3.98 (s, 3H, OCH_3), 6.73 (s, 2H, H-2, H-6), 6.96 (d, J = 17.0 Hz, 1H, H-1a′), 7.01 (d, J = 17.1 Hz, 1H, H-1a), 7.10 (d, J = 8.5 Hz, 1H, H-5′), 7.67 (dd, J = 8.6, 2.2 Hz, 1H, H-6′), 8.03 (d, J = 2.4 Hz, 1H, H-2′); ^{13}C NMR (100 MHz) δ 40.6 ($N(CH_3)_2$), 56.00, 60.98, 64.01, 103.66, 122.90, 124.01, 125.21, 129.66, 132.00, 132.56, 132.72, 133.59, 134.83, 152.11, 151.6 (C-3′).

3′-Bromo-3,4,4′,5-tetramethoxy-(*E*)-stilbene (9c). After purification by column chromatography, the solid was recrystallized from ethanol as needles (0.56 g, 65%): mp 142−144 °C; R_f 0.23 (hexanes−ethyl acetate, 9:1); EIMS *m/z* (relative intensity) 379 (M^+, 100), 363, 302, 256, 241, 225, 211, 198; ^1H NMR δ 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.91 (s, 6H, 2 × OCH_3), 6.71 (s, 2H, H-1a′, H-1a), 6.88 (d, J = 8.4 Hz, 1H, H-5′), 6.89 (s, 2H, H-2, H-6), 7.38 (dd, J = 8.7, 2.1 Hz, 1H, H-6′), 7.89 (d, J = 2.2 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 56.06, 56.27, 60.93, 103.43, 111.89, 126.187, 126.75, 127.84, 130.84, 131.44, 132.89, 137.88, 153.36. Anal. Calcd for $C_{18}H_{19}BrO_4$: C, 57.01; H, 5.05; Br, 21.07. Found: C, 56.34; H, 5.04; Br, 21.00.

General Method for the Preparation of Stilbenes 11a,b and 12a,b. A solution of tetra-*n*-butylammonium fluoride in THF (1.0 M, 2 mL, 2 mmol) was added to a solution of the stilbene (**8d**,**e**; **9d**,**e**, 1 mmol) in THF (5 mL), and the mixture was stirred at room temperature. After 30 min, ice (1 g) was added, followed by ether (10 mL). The organic layer was washed with water (2 × 10 mL), dried, and filtered. Evaporation of the solvent and separation of the crude product by flash chromatography (40% ethyl acetate in hexanes) afforded the product.

3′-*O*-(3″-Hydroxypropyl)-combretastatin A-4 (11a). Deprotection as described above, followed by recrystallization from ethyl acetate−hexane, afforded colorless needles (0.98 g, 89%): mp 48−50 °C; R_f 0.25 (hexanes−ethyl acetate, 3:2); IR (neat) ν_{max} 3533 (OH), 2999, 2937, 2837, 1736, 1579, 1126, 1004, 869 (cis C=C) cm^{-1}; EIMS *m/z* (relative intensity) 374 (M^+, 100), 359 (35), 316 (5), 301 (10), 241 (5); ^1H NMR δ 1.88 (q, 2H, $CH_2CH_2CH_2$), 2.84 (bs, 1H, −OH), 3.63 (s, 6H, 2 × OCH_3), 3.70 (t, J = 6.0 Hz, 2H, $ArOCH_2$), 3.74 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.87 (t, J = 6.0 Hz, 2H, CH_2CH_2OH), 6.37 (d, J = 12.0 Hz, 1H, H-1a′), 6.42 (d, J = 12.0 Hz, 1H, H-1a), 6.45 (s, 2H, H-2, H-6), 6.68 (d, J = 8.1 Hz, 1H, H-5′), 6.76 (dd, J = 8.1, 1.5 Hz, 1H, H-6′), 6.80 (d, J = 1.5 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 31.69, 55.92, 60.84, 67.84, 105.96, 111.08, 113.59, 122.37, 128.79, 129.69, 129.80, 133.09, 136.99, 147.58, 148.59, 152.97. Anal. Calcd for $C_{21}H_{26}O_6$: C, 62.9; H, 8.7. Found: C, 63.07; H, 8.76.

3′,4-Dihydroxy-3,4′,5-trimethoxy-(*Z*)-stilbene (11b). Deprotection as described above yielded a clear oil that solidified upon standing. Recrystallization from ethyl acetate−hexane afforded pale yellow needles (0.15 g, 80%): mp 104−106 °C; R_f 0.38 (hexane−acetone, 3:2); EIMS *m/z* (relative intensity) 302 (M^+, 100), 287, 269, 256, 227, 213, 199, 181; IR (neat) ν_{max} 3005 (OH), 2998 (aromatic), 2920, 2840, 1641, 1573, 1500, 1454, 1325, 1242, 1121, 870 (cis C=C) cm^{-1}; ^1H NMR δ 3.65 (s, 6H, 2 × OCH_3), 3.75 (s, 3H, 4′-OCH_3), 4.98 (bs, 2H, 2 × OH), 6.51 (d, J = 12.1 Hz, 1H, H-1a′), 6.58 (d, J = 12.0 Hz, 1H, H-1a), 6.59 (s, 2H, H-2, H-6), 6.71 (d, J = 8.3 Hz, 1H, H-5′), 6.80 (dd, J = 8.4, 2.1 Hz, 1H, H-6′), 6.83 (d, J = 1.8 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 56.01 (OCH_3), 56.11 (4′-OCH_3), 105.20 (C-2, C-6), 111.52 (C-5′), 117.08 (C-2′), 121.22 (C-6′), 129.01 (C-1a′), 129.51 (C-1a), 130.81 (C-1′), 132.48 (C-1), 137.54

(C-4), 145.21 (C-4′), 145.67 (C-4′), 146.02 (C-3′), 152.67 (C-3, C-5). Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.5; H, 6.0. Found: C, 67.2; H, 6.12.

3′-*O*-(3″-Hydroxypropyl)-3,4,4′,5-tetramethoxy-(*E*)-stilbene (12a). Deprotection by the general procedure gave a colorless solid that was crystallized from ethanol−pentane as colorless prisms (0.12 g, 90%): mp 121−123 °C; R_f 0.20 (hexanes−ethyl acetate, 3:12); EIMS *m/z* (relative intensity) 374 (M^+, 83), 359 (20), 316 (8), 301 (10), 241 (7); IR (KBr) ν_{max} 3501 (OH), 2989 (aromatic), 2934, 1585, 1501, 1460, 1450, 1404, 1323, 1270, 1262, 1253, 1125 cm^{-1}; ^1H NMR δ 1.81 (q, 2H, $CH_2CH_2CH_2OH$), 2.75 (bs, 1H, OH), 3.71 (t, J = 6.5 Hz, 2H, $ArOCH_2$), 3.85 (s, 3H, OCH_3), 3.88 (t, J = 6.5 Hz, 2H, CH_2OH), 3.91 (s, 3H, OCH_3), 3.92 (s, 6H, 2 × OCH_3), 6.72 (s, 2H, H-2, H-6), 6.85 (d, J = 8.5 Hz, 1H, H-5′), 6.92 (d, J = 16.1 Hz, 1H, H-1a′), 6.93 (d, J = 16.3 Hz, 1H, H-1a), 7.01 (dd, J = 8.5, 2.0 Hz, 1H, H-6′), 7.23 (d, J = 2.3 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 32.10 (CH_2), 56.01 (OCH_3), 60.66 (4-OCH_3), 60.84 (CH_2OH), 67.84 ($ArOCH_2$−), 105.96 (C-6, C-2′), 111.08 (C-2′), 113.59 (C-5′), 122.37 (C-6′), 128.79 (C-1a′), 129.69 (C-1a), 129.80 (C-1′), 133.09 (C-1), 136.99 (C-4), 147.58 (C-4′), 148.59 (C-3′), 152.97 (C-3, C-5). Anal. Calcd for $C_{21}H_{26}O_6$: C, 62.9; H, 8.7. Found: 62.5; H, 8.5.

3′,4-Dihydroxy-3,4′,5-trimethoxy-(*E*)-stilbene (12b). Deprotection followed by recrystallization from ethyl acetate−hexane gave colorless opalescent flakes (0.11 g, 98%): mp 150−152 °C; R_f 0.40 (hexane−acetone, 3:2); EIMS *m/z* 302 (M^+, 100), 287, 269, 256, 181; IR (KBr) ν_{max} 3010 (OH), 2998, 1560, 1500, 1463, 1271, 1010 cm^1; ^1H NMR δ 3.81 (s, 3H, OCH_3), 3.92 (s, 6H, 2 × OCH_3), 5.03 (bs, 1H, OH), 6.68 (s, 2H, H-2, H-6), 6.71 (d, J = 8.4 Hz, 1H, H-5′), 6.86 (d, J = 16.0 Hz, 1H, H-1a′), 6.88 (d, J = 16.2 Hz, 1H, H-1a), 7.10 (dd, J = 8.5, 1.2 Hz, 1H, H-6′), 7.18 (d, J = 1.2 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 55.98 (OCH_3), 56.10 (OCH_3), 60.83 (4-OCH_3), 103.41 (C-2, C-6), 110.61 (C-5′), 111.62 (C-2′), 120.01 (C-6′), 126.99 (C-1a′), 127.81 (C-1a), 130.90 (C-1′), 133.31 (C-1), 137.59 (C-4), 145.75 (C-4′), 146.52 (C-3′), 153.32 (C-3, C-5). Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.5; H, 6.0. Found: C, 66.90; H, 6.05.

3′-*O*-(2″-Hydroxyethyl)-combretastatin A-4 (13). A mixture of the phenol **1a** (4.0 g, 12.6 mmol), ethylene carbonate (3.7 g, 42 mmol, 3.3 equiv), and potassium carbonate (5.22 g, 37.8 mmol, 3 equiv) in anhydrous DMF (15 mL) was heated for 24 h at 100 °C under argon, whereupon TLC analysis showed that the reaction was complete. After cooling, water (25 mL) was added, followed by dichloromethane (30 mL). The organic layer was removed, washed with 3 N NaOH (3 × 25 mL) and brine (25 mL), dried, filtered, and evaporated under reduced pressure. Separation by flash column chromatography (hexane−acetone, 2:1) gave a colorless oil that solidified upon cooling. Recrystallization from ethyl acetate−hexane gave colorless crystals (4.1 g, 90%): mp 89 °C; R_f 0.24 (hexane−acetone, 2:1); EIMS *m/z* (relative intensity) 360 (100), 345 (46), 301 (4), 268 (2), 241 (4), 181 (3), 142 (4), 115 (4); IR (neat) ν_{max} 3418, 2937, 1641, 1512, 1413, 1238, 1188, 1016, 868, 775 cm^{-1}; ^1H NMR δ 2.44 (bs, 1H, OH), 3.70 (s, 6H, 2 × OCH_3), 3.83 (t, J = 3.9 Hz, 2H, CH_2OH), 3.84 (s, 3H, 4-OCH_3), 3.85 (s, 3H, 4′-OCH_3), 3.93 (t, J = 3.9 Hz, 2H, CH_2CH_2), 6.43 (d, J = 12.0 Hz, 1H, H-1a′), 6.49 (d, J = 12.0 Hz, 1H, H-1a), 6.51 (s, 2H, H-2, H-6), 6.78 (d, J = 8.4 Hz, 1H, H-5′), 6.88 (dd, J = 8.4, 2.1 Hz, 1H, H-6′), 6.92 (d, J = 2.1 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 55.89 (OCH_3), 56.00 (OCH_3), 60.89 (4-OCH_3), 61.09 (CH_2CH_2), 71.19 (CH_2CH_2), 106.01 (C-2), 160.28, 196.37. Anal. Calcd for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.63; H, 6.78.

3′-*O*-(2″-Chloroethyl)-combretastatin A-4 (14a). A 60% dispersion of NaH in mineral oil (25 mg, 0.63 mmol) was added in portions to a stirred solution of phenol **1a** (0.20 g, 0.63 mmol) in dry DMF (10 mL). The mixture was stirred for 30 min and cooled to 0 °C before the addition of 2-chloroethyl methanesulfonate (120 mg, 0.76 mmol, 1.2 equiv). Stirring was continued at 65 °C for 2 h, whereupon the reaction mixture was poured into water and extracted with ethyl acetate (3 × 20 mL). Drying, filtration, and removal of solvent in vacuo, followed by purification by flash chromatography (eluent hexanes−ethyl acetate, 2:1), gave the product as a colorless oil (0.16 g, 65%): R_f 0.63 (hexanes−ethyl acetate, 2:1); EIMS *m/z* (relative intensity) 378 (M^+, 100), 363 (58), 328 (3), 313 (5), 284 (7), 269 (7), 252 (10), 241 (13), 225 (10), 210 (8), 197 (12), 155 (12), 115 (12), 64 (20), 27 (22); ^1H NMR δ 3.67 (s, 6H, 2 × OCH_3), 3.68 (t, J = 6.0 Hz, 2H, $-CH_2CH_2Cl$), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.04 (t, J = 6.0 Hz, 2H, OCH_2CH_2Cl), 6.42 (d, J = 12.6 Hz, 1H, H-1a′), 6.46 (d, J = 12.6 Hz, 1H, H-1a), 6.47 (s, 2H, H-2, H-6), 6.76 (d, J = 8.7 Hz, 1H, H-5′), 6.81 (d, J = 2.4 Hz, 1H, H-2′), 6.88 (dd, J = 8.7, 2.1 Hz, 1H, H-6′); ^{13}C NMR (75 MHz) δ 41.42 (CH_2Cl), 55.89 (3 × OCH_3), 60.75 (4-OCH_3), 68.96 ($-OCH_2CH_2$), 105.80 (C-2), 111.65 (C-5′), 114.83 (C-2′), 123.03 (C-6′), 128.97 (C-1a′), 129.26 (C-1a), 129.83 (C-1′), 132.73 (C-1), 137.00 (C-4), 146.90 (C-4′), 148.82 (C-3′), 152.85 (C-5,3).

Note: Ether **14a** proved to be thermally unstable and decomposed readily at room temperature. Although it is stable in the refrigerator under an inert atmosphere for approximately 1 day, it should be used immediately following purification.

3′-*O*-[2″-(Methanesulfonyl)ethyl]-combretastatin A-4 (14b). Freshly distilled triethylamine (0.791 mL, 5.65 mmol, 4 equiv) was slowly added (dropwise) to a stirring solution of alcohol **13** (0.51 g, 1.4 mmol) and methanesulfonyl chloride (0.44 mL, 5.65 mmol, 4 equiv) in THF (10 mL) at 0 °C. After 0.5 h, water (10 mL) was added to the reaction mixture, and the aqueous phase was removed and extracted with ethyl acetate (3 × 10 mL). The combined organic extract was dried, filtered, and concentrated in vacuo, resulting in a pale yellow oil. This was subjected to flash column chromatography (eluent hexane−acetone, 2:1) to yield the product as a clear oil (0.66 g, 95%): EIMS *m/z* (relative intensity) 438 (M^+, 100), 423 (38), 327 (6), 269 (4), 241 (6), 197 (4), 155 (4), 123 (25), 79 (28); IR (neat) ν_{max} 2937 (aromatic), 2839, 1736, 1579, 1512, 1356 ($S(=O)_2$ stretch), 1242 (C−O−C), 1177 (asymm. $S(=O)_2$ stretch), 1128, 925 cm^{-1}; ^1H NMR δ 3.10 (s, 3H, SO_2CH_3), 3.70 (s, 6H, 2 × OCH_3), 3.81 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.05 (t, J = 4.5 Hz, 2H, CH_2OSO_2), 4.49 (t, J = 4.5 Hz, 2H, $ArOCH_2$−), 6.46 (s, 2H, H-1a′, H-1a), 6.51 (s, 2H, H-2, H-6), 6.76 (d, J = 8.4 Hz, 1H, H-5′), 6.79 (d, J = 1.8 Hz, 1H, H-2′), 6.90 (dd, J = 8.3, 1.8 Hz, 1H, H-6′); ^{13}C NMR (75 MHz) δ 37.59, 55.82, 55.97, 60.84, 66.95, 68.40, 106.00, 111.70, 114.81, 123.33, 129.15, 129.31, 129.93, 132.88, 137.18, 146.93, 148.90, 152.99. Anal. Calcd for $C_{21}H_{26}O_8S$: C, 57.5; H, 6.0. Found: C, 56.8; H, 5.7.

General Procedures for the Preparation of Combretastatin A-4 3′-Derivatives 15a,c−j. Method A. 3′-*O*-[2″-(*N*-Pyrrolidinyl)ethyl]-combretastatin A-4 (15d). To a stirred solution of phenol **1a** (0.5 g, 1.6 mmol) in DMF (10 mL) under argon was added NaH (0.38 g of a 60% dispersion, 9.5 mmol, 6 equiv). The mixture was stirred at room temperature for 30 min, by which point H_2 evolution had ceased. The reaction mixture was heated to 90 °C (oil bath), and 1-(2-chloroethyl)pyrrolidine hydrochloride (0.61 g, 3.6 mmol, 2.25 equiv) was added in portions over 30 min. The reaction mixture was stirred at 90 °C for another 12 h, whereupon TLC analysis (hexanes−ethyl acetate, 3:2) showed the reaction to be complete. The mixture was cooled to ambient temperature, and isopropyl alcohol (3 mL) was added to destroy excess NaH. The slurry was partitioned between water (50 mL) and ether (50 mL), and the water was extracted with ether (3 × 20 mL). The combined ethereal extract was dried (Na_2SO_4), filtered, and concentrated in vacuo to yield a brown oil. Chromatographic separation (hexanes−ethyl acetate−triethylamine, 6:4:1) gave the product as a pale yellow oil (0.60 g, 90%). The oily product was dissolved in ether and cooled in an ice bath, and 1.0 equiv of 1.0 N HCl in ether was added dropwise until a precipitate formed. The solvent was removed in vacuo, and the colorless solid was recrystallized (3×) from 2-propanol−ether to give the hydrochloride salt as colorless needles: mp 131−132 °C; R_f 0.47 (hexanes−ethyl acetate−triethylamine, 6:4:1); IR (neat) ν_{max} 2957, 2833, 2783, 1676, 1579, 1512, 1462, 1327, 1238, 1128, 1028, 869, 776 cm^{-1}; EIMS *m/z* 413 (M^+),

382, 316, 241, 211, 165, 115, 98, 84 (base); ^{1}H NMR δ 1.78 (m, 4H, pyrrolidine 3,4-H), 2.58 (m, 4H, pyrrolidine 2,5-H), 2.86 (t, J = 6.6 Hz, 2H), 3.34 (s, 3H, OCH_3), 3.69 (s, 6H, 2 × OCH_3), 3.83 (s, 3H, OCH_3), 3.98 (t, J = 6.6 Hz, 2H), 6.43 (d, J = 12.0 Hz, 1H, H-1a′), 6.48 (d, J = 12.0 Hz, 1H, H-1a), 6.51 (s, 2H, H-2, H-6), 6.76 (d, J = 8.7 Hz, 1H, H-5′), 6.86 (dd, J = 4.0 Hz, 1.8, 1H, H-6′), 6.88 (bd, J = 1.8 Hz, 1H, H-2′). Anal. Calcd for $C_{24}H_{32}ClNO_5$: C, 64.06; H, 7.17; N, 3.11. Found: C, 64.00; H, 7.17; N, 3.00.

3′-*O*-[2″-(*N*,*N*-Dimethylamino)ethyl]-combretastatin A-4 (15a). Ether formation via method A gave a pale yellow oil (0.52 g, 85%) following flash column chromatography (hexanes−ethyl acetate−triethylamine, 6:4:1). The hydrochloride salt was prepared as described and crystallized from 2-propanol−ether as colorless needles: mp 125−126 °C; R_f 0.27 (hexanes−ethyl acetate−triethylamine, 6:4:1); EIMS *m/z* (relative intensity) 387 (M^+, 30), 356 ($M^+ - Me_2$, 1), 316 (1), 241 (1), 195 (1), 165 (2), 126 (1), 98 (2), 86 (2), 72 (28), 58 (100); IR (neat) ν_{max} 2939, 2831, 2771, 1579, 1512, 1462, 1427, 1327, 1238, 1128, 1028, 869 cm^{-1}; ^{1}H NMR δ 2.30 (s, 6H, $N(CH_3)_2$), 2.70 (t, 2H, J = 6.0 Hz, CH_2CH_2N), 3.70 (s, 6H, 3,5-OCH_3), 3.83 (s, 3H, 4-OCH_3), 3.84 (s, 3H, 4′-OCH_3), 3.92 (t, 2H, J = 6.0 Hz, OCH_2CH_2), 6.44 (d, 1H, J = 12.0 Hz, H-1a′), 6.50 (d, 1H, J = 12.0 Hz, H-1a), 6.52 (s, 2H, H-2, H-6), 6.77 (d, 1H, J = 7.5 Hz, H-5′), 6.86 (s, 1H, H-6′), 6.89 (d, 1H, J = 1.8 Hz, H-2′). Anal. Calcd for $C_{22}H_{30}ClNO_5$: C, 62.33; H, 7.13; N, 3.30. Found: C, 62.14; H, 7.08; N, 3.23.

3′-*O*-[2″-(*N*,*N*-Diethylamino)ethyl]-combretastatin A-4 (15c). Use of method A led to a pale yellow oil (0.26 g, 98%) following purification by flash column chromatography (hexanes−ethyl acetate−triethylamine, 6:4:1). The hydrochloride salt (prepared as described above, see **15d**) crystallized from 2-propanol−ether as colorless needles: mp 113−114 °C; R_f 0.60 (hexanes−ethyl acetate−triethylamine, 6:4:1); EIMS *m/z* (relative intensity) 415 (M^+, 40), 400 ($M^+ - CH_3$, 7), 384 (12), 342 (3), 316 (10), 100 (40), 86 (100); IR (neat) ν_{max} 2966, 2833, 2771, 1579, 1512, 1483, 1427, 1327, 1238, 1128, 1026, 871, 763 cm^{-1}; ^{1}H NMR δ 1.01 (t, J = 7.2 Hz, 6H, $N(CH_2CH_3)_2$), 2.56 (q, J = 7.2 Hz, 4H, $N(CH_2CH_3)_2$), 2.83 (t, J = 6.6 Hz, 2H, CH_2N), 3.68 (s, 6H, 3, 5-OCH_3), 3.82 (s, 3H, 4-OCH_3), 3.83 (s, 3H, 4′-OCH_3), 3.90 (t, J = 6.6 Hz, 2H, OCH_2), 6.42 (d, J = 12.0 Hz, 1H, H-1a′), 6.49 (d, J = 12.0 Hz, 1H, H-1a), 6.51 (s, 2H, H-2, H-6), 6.75 (d, J = 8.7 Hz, 1H, H-5′), 6.85 (dd, J = 4.2, 1.8 Hz, 1H, H-6′), 6.87 (d, J = 1.8 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 11.78 ($N(CH_2CH_3)_2$), 47.74 ($N(CH_2CH_3)_2$), 51.50 (CH_2N), 55.83 (3 × OCH_3), 60.81 (4-OCH_3), 67.20 (OCH_2CH_2), 106.02 (C-2, C-6), 111.35 (C-5′), 113.66 (C-2′), 122.00 (C-6′), 128.91 (C-1a′), 129.72 (C-1a), 130.01 (C-1′), 132.86 (C-1), 137.23 (C-4), 148.01 (C-4′), 148.70 (C-3′), 152.99 (C-3, C-5). Anal. Calcd for $C_{24}H_{34}C1NO_5$: C, 63.78; H, 7.58; N, 3.10. Found: C, 63.47; H, 7.94; N, 3.19.

3′-*O*-[2″-(*N*-Piperidinyl)ethyl]-combretastatin A-4 (15e). Method A led to 0.53 g (78% yield) of amine **15e**. The hydrochloride salt crystallized from 2-propanol−ether as colorless needles: mp 144−145 °C; R_f 0.49 (hexanes−ethyl acetate−triethylamine, 6:4:1); EIMS *m/z* (relative intensity) 427 (M^+, 20), 396 (7), 316 (5), 112 (20), 98 (100); IR (neat) ν_{max} 3474, 3030, 3003, 2938, 2837, 1579, 1512, 1454, 1327, 1280, 1128, 1010, 889 cm^{-1}; ^{1}H NMR δ 1.43 (m, 2H, γ-CH_2), 1.56 (m, 4H, β-CH_2), 2.44 (t, J = 4.8 Hz, 4H, α-CH_2), 2.71 (t, J = 6.6 Hz, 2H, OCH_2CH_2N), 3.69 (s, 6H, 2 × OCH_3), 3.83 (s, 6H, 2 × OCH_3), 3.96 (t, J = 6.6 Hz, 2H, OCH_2CH_2N), 6.43 (d, J = 12.0 Hz, 1H, H-1a′), 6.50 (d, J = 12.0 Hz, 1H, H-1a), 6.51 (s, 2H, H-2, H-6), 6.76 (dd, J = 6.6, 2.1 Hz, 1H, H-5′), 6.86 (dd, J = 6.6, 1.8 Hz, 2H, H-6′, H-2′); ^{13}C NMR (75 MHz) δ 24.14 (γ-CH_2), 25.88 (β-CH_2), 54.92 (OCH_3), 55.85 (OCH_3), 57.59 (α-CH_2), 60.79 (4-OCH_3), 66.65 (OCH_2CH_2), 106.06 (C-2), 111.44 (C-5′), 114.00 (C-2′), 122.08 (C-6′), 128.89 (C-1a′), 129.67 (C-1a), 130.00 (C-1′), 132.83 (C-1), 137.20 (C-4), 147.92 (C-4′), 148.72 (C-3′), 152.94 (C-5,3). Anal. Calcd for $C_{25}H_{34}C1NO_5$: C, 64.71; H, 7.39; N, 3.02. Found: C, 65.00; H, 7.86; N, 3.00.

3′-*O*-[2″-(*N*-Morpholino)ethoxy]-combretastatin A-4 (15f). Use of method A led to the hydrochloride, which crystallized from 2-propanol−ether as colorless needles: mp 119−120 °C; R_f 0.29 (hexanes−ethyl acetate-triethylamine, 6:4:1); EIMS *m/z* (relative intensity) 429 (M^+, 22), 398 (1), 316 (2), 114 (32), 100 (100); IR (neat) ν_{max} 2999, 2953, 2835, 1579, 1512, 1454, 1327, 1126, 1026, 858 cm^{-1}; ^{1}H NMR δ 2.53 (t, J = 4.5 Hz, 4H, α-CH_2), 2.74 (t, J = 6.0 Hz, 2H, OCH_2CH_2N), 3.70 (s, 6H, 2 × OCH_3), 3.71 (t, J = 4.5 Hz, 4H, β-CH_2), 3.83 (s, 3H, 4′-OCH_3), 3.84 (s, 3H, 4-OCH_3), 3.95 (t, J = 6.0 Hz, 2H, OCH_2CH_2), 6.44 (d, J = 12 Hz, 1H, H-1a′), 6.49 (d, J = 12 Hz, 1H, H-1a), 6.52 (s, 2H, H-2, H-6), 6.77 (d, J = 8.1 Hz, 1H, H-5′), 6.86 (dd, J = 8.1 Hz, 1.5, 1H, H-6′), 6.89 (d, J = 1.5 Hz, 1H, H-2′); ^{13}C NMR (75 MHz) δ 53.55 (α-CH_2), 54.00 (OCH_3), 55.95 (OCH_3), 57.39 (OCH_2CH_2N), 60.89 (4-OCH_3), 66.61 ($-OCH_2CH_2N$), 66.90 (β-CH_2), 106.00 (C-2), 111.44 (C-5′), 114.19 (C-2′), 122.45 (C-6′), 128.89 (C-1a′), 129.62 (C-1a), 129.93 (C-1′), 132.90 (C-1), 137.15 (C-4), 147.68 (C-4′), 148.79 (C-3′), 152.97 (C-5,3). Anal. Calcd for $C_{24}H_{32}ClNO_6$: C, 61.86; H, 6.92; N, 3.01. Found: C, 61.96; H, 7.67; N, 3.00.

3′-*O*-[2″-(*N*-Pyrrollyl)ethyl]-combretastatin A-4 (15h). Use of method A led to 0.43 g (67% yield) of amine **15h** as a deep yellow−brown gum. Subsequent conversion to the hydrochloride salt yielded a solid that crystallized from ethanol−ether to afford colorless needles: mp 155−157 °C; R_f 0.25 (acetone−hexane, 3:1); EIMS *m/z* (relative intensity) 409 (M^+, 25), 329 (11), 316 (5), 100 (100); IR (neat) ν_{max} 2870, 2710, 1580, 1512, 1455, 1327, 1127, 1050, 825 cm^{-1}; ^{1}H NMR δ 3.12 (t, J = 6.6 Hz, 2H, CH_2CH_2N), 3.70 (s, 6H, 2 × OCH_3), 3.84 (s, 3H, 4′-OCH_3), 3.85 (s, 3H, 4-OCH_3), 3.95 (t, J = 6.6 Hz, 2H, OCH_2-CH_2), 6.38 (m, ^3J = 3.5, 2.6, 1.3 Hz, 2H, $$4,4′-CH), 6.45 (d, J = 12.0 Hz, 1H, H-1a′), 6.49 (d, J = 12.1 Hz, 1H, H-1a), 6.51 (s, 2H, H-2, H-6), 6.76 (d, J = 8.1 Hz, 1H, H-5′), 6.86 (dd, J = 8.1, 1.5 Hz, 1H, H-6′), 6.89 (d, J = 1.5 Hz, 1H, H-2′), 6.94 (m, ^3J = 3.5, 2.6, 1.2 Hz, 2H, α,α′-CH). Anal. Calcd for $C_{24}H_{28}$-$ClNO_5$: C, 64.64; H, 6.33; N, 3.14. Found: C, 64.22; H, 6.30; N, 3.11.

3′-[2″-(2″Pyridyl)ethy]-combretastatin A-4 (15j). Method A led to a pale yellow−brown oil (0.26 g, 39%), following purification by flash column chromatography (hexanes−ethyl acetate−triethylamine, 4:4:2). The hydrochloride salt (prepared as described above, see **15d**) crystallized from 2-propanol−ether as colorless needles: mp 178−180 °C; R_f 0.30; EIMS *m/z* (relative intensity) 421 (M^+, 10), 316 (11), 100 (100); IR (neat) ν_{max} 2980 cm^{-1}; ^{1}H NMR δ 3.16 (t, J = 6.0 Hz, 2H, CH_2CH_2), 3.70 (s, 6H, 2 × OCH_3), 3.83 (s, 6H, 2 × OCH_3), 4.08 (t, J = 6.1 Hz, 2H, OCH_2CH_2), 6.46 (d, J = 12.2 Hz, 1H, H-1a′), 6.48 (d, J = 12.1 Hz, 1H, H-1a), 6.50 (s, 2H, H-2, H-6), 6.76 (d, J = 8.0 Hz, 1H, H-5′), 6.88 (dd, J = 8.1, 2.1 Hz, 1H, H-6′), 6.90 (d, J = 2.1 Hz, 1H, H-2′), 7.19 (d, J = 7.6 Hz, 1H, β-CH), 7.34 (m, 1H, δ-CH), 7.79 (m, 1H, (−CH), 8.66 (d, J = 5.5 Hz, 1H, −CH). Anal. Calcd for $C_{25}H_{28}ClNO_5$; C, 65.57; H, 6.16; N, 3.06. Found: C, 65.76; H, 6.30; N, 3.30.

Method B. 3′-*O*-[2″-(*N*-Methylpiperazinyl)ethyl]-combretastatin A-4 (15g). Both *N*-methylpiperazine (10 mL) and triethylamine (10 mL) were added to a solution of methanesufonate **14b** (0.66 g) in 15 mL of dry acetonitrile. The solution was stirred (under argon) for 48 h at room temperature, and the solvent was then evaporated to yield a brown oil that was dissolved in ether (25 mL). After extraction with 10% hydrochloric acid (3 × 10 mL), the pH of the combined aqueous extract was raised to 11 with 20% sodium hydroxide, and the solution was extracted with ether (3 × 10 mL). The combined ethereal extract was washed with water (10 mL), dried, and concentrated (reduced pressure) to yield, following chromatography (hexanes−ethyl acetate−triethylamine, 6:4:1 as eluent), a yellow oil (0.51 g, 76%). The dihydrochloride salt was prepared as described above as a colorless solid, which recrystallized from 2-propanol−ether as colorless needles: mp 190−191 °C; R_f 0.28 (hexanes−ethyl acetate-triethylamine, 6:4:1); EIMS *m/z* (relative intensity) 442 (M^+, 10) 316 (2), 114 (25), 100 (100); IR (neat) ν_{max} 2940, 2880, 1580, 1510, 1325, 1128, 1010, 869 cm^{-1}; ^{1}H NMR δ 2.28 (s, 3H, N-CH_3), 2.45 (m, 4H, α-CH_2), 2.57 (m, 4H, β-CH_2), 2.77 (t, J = 6.0 Hz, 2H, OCH_2CH_2N), 3.70 (s, 6H, 2 × OCH_3), 3.79 (t, J = 6.0 Hz, 2H, OCH_2CH_2N), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.43 (d, J = 12.0 Hz, 1H, H-1a′), 6.50 (d, J = 12.0 Hz, 1H, H-1a), 6.52

(s, 2H, H-2, H-6), 6.77 (d, J = 8.7 Hz, 1H, H-5′), 6.86 (s, 1H, H-6′), 6.88 (d, J = 2.1 Hz, 1H, H-2′). Anal. Calcd for $C_{25}H_{36}Cl_2N_2O_5$ (di-HCl salt): C, 58.2; H, 7.1; N, 5.4. Found: C, 58.29; H, 7.51; N, 5.15.

Method C. 3′*O*-[2″-(1*H*-Imidazol-1-yl)ethyl-combretastatin A-4 (15i). Sodium hydride (60% dispersion in mineral oil, 18 mg, 0.45 mmol, 1.1 equiv) was added in portions to a stirred solution of imidazole (28 mg, 0.41 mmol) in dry DMF (4 mL). The mixture was stirred for 30 min, and 2-chloroethyl ether **14a** (0.16 g, 0.41 mmol) was added. The solution was heated for 6 h at 80−90 °C and then poured into water. The aqueous phase was extracted with ethyl acetate (3 × 15 mL), and the combined extract was dried and concentrated. Separation by flash chromatography (eluent acetone−hexane, 2:1) gave the product as a colorless oil that was then treated with ethereal hydrogen chloride (see method a) to give the hydrochloride salt, which crystallized from 2-propanol−ether as nearly colorless needles (0.12 g, 71%): mp 133−135 °C; R_f 0.35 (acetone−hexane, 2:1); EIMS *m/z* (relative intensity) 410 (M^+, 100), 395 (95), 379 (15), 363 (12), 349 (5), 312 (8), 300 (10), 241 (8), 205 (7), 128 (5), 115 (7), 110 (15), 96 (60), 82 (16), 68 (50), 55 (7), 42 (25); IR (neat) ν_{max} 3396, 2937, 2839, 2700, 2609, 1581, 1512, 1454, 1327, 1236, 1126, 1062, 1006, 825, 750 cm^{-1}; ^1H NMR δ 3.68 (s, 6H, 2 × OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.02 (t, J = 5.1 Hz, 2H, OCH_2CH_2N), 4.25 (t, J = 5.1 Hz, 2H, OCH_2CH_2), 6.46 (d, J = 12.0 Hz, 1H, H-1a′), 6.48 (d, J = 12.0 Hz, 1H, H-1a), 6.50 (s, 2H, H-2, H-6), 6.73 (d, J = 1.8 Hz, 1H, H-2′), 6.78 (d, J = 8.4 Hz, 1H, H-5′), 6.91 (dd, J = 8.4, 1.8 Hz, 1H, H-6′), 7.04 (d, J = 1.2 Hz, 2H, H-δ_4, H-δ_5), 7.60 (s, 1H, H-δ_2); ^{13}C NMR (75 MHz) δ 46.27, 55.88 (3 × OCH_3), 60.80 (4-OCH_3), 68.33 (−OCH_2-CH_2), 105.84 (C-2, C-6), 111.56 (C-5′), 114.38 (C-2′), 119.40 (C-6′), 123.15 (C-δ_4,δ_5), 128.93 (C-1a′), 129.19 (C-δ_2), 129.26 (C-1a), 129.76 (C-1′), 132.81 (C-1), 137.57 (C-4), 146.89 (C-4′), 148.83 (C-3′), 152.90 (C-5,3). Anal. Calcd for $C_{23}H_{27}ClN_2O_5$: C, 61.81; H, 6.09; N, 6.27. Found: C, 61.93; H, 6.36; N, 6.00.

3′-*O*-[2″-(Trimethylamino)ethyl]-combretastatin A-4 iodide (15b). Method A. A solution of dimethylamine **15a** (0.30 g, 0.77 mmol) in methyl iodide (1 mL) was stirred under argon for 30 min at room temperature. The solution was concentrated in vacuo to give a yellow gum that was dissolved in boiling methanol. Cooling afforded crystals (46% yield) of the iodide: mp 201−205 °C; identical spectroscopically with the sample prepared by method B.

Method B. A solution of amine **15a** (0.40 g, 1 mmol) in a mixture of methyl iodide (4 mL) and dry methanol (10 mL) was stirred in the dark for 16 h at room temperature and then concentrated in vacuo to an oily residue that crystallized from ethanol (25%): mp 202−204 °C; ^1H NMR δ 1.90 (s, 9H, $(CH_3)_3$), 2.70 (t, J = 6.0 Hz, 2H, CH_2N), 3.60 (s, 6H, 2 × OCH_3), 3.78 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.15 (t, J = 6.0 Hz, 2H, OCH_2), 6.46 (d, J = 12.0 Hz, 1H, H-1a′), 6.49 (d, J = 12.0 Hz, 1H, H-1a), 6.51 (s, 2H, H-2, H-6), 6.78 (d, J = 8.1 Hz, 1H, H-5′), 6.84 (dd, J = 8.1, 1.2 Hz, 1H, H-6′), 6.90 (d, J = 1.2 Hz, 1H, H-2′); HRFABMS M^+ calcd for $C_{23}H_{32}INO_5$ 529.1325, found 529.2318.

3′-*O*-[Bis(2-chloroethyl)carbamoyl]-combretastatin A-4 (16). A solution of *N*-(chloroformyl)-bis(2-chloroethyl)amine (0.15 g, 0.76 mmol, 1.2 equiv) was added to a solution of phenol **1a** (0.2 g, 0.63 mmol) in dry pyridine (5 mL) at 0 °C. The mixture was stirred at room temperature for 4 h, then at 50−60 °C for 12 h. The mixture was allowed to cool, and ice was added. The product was extracted with ethyl acetate (3 × 15 mL). Drying of the combined extract, filtration, and solvent removal followed by flash chromatography (hexanes−ethyl acetate, 3:1) gave the hydrochloride salt as a clear gum (0.27 g, 88%): EIMS *m/z* (relative intensity) 483 (M^+, 50), 316 (45); IR (neat) ν_{max} 1730 (C=O) cm^{-1}; ^1H NMR δ 3.72 (t, J = 4.2 Hz, 4H, 2 × CH_2), 3.75 (t, J = 4.2 Hz, 4H, 2 × CH_2), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.84 (s, 6H, 2 × OCH_3), 6.45 (s, 2H, H-1a, H-1a′), 6.53 (s, 2H, H-2, H-5), 6.83 (d, J = 9.0 Hz, 1H, H-5′), 7.10 (dd, J = 7.8, 2.1 Hz, 1H, H-6′), 7.14 (d, J = 2.1 Hz, 1H, H-2′). Anal. Calcd for $C_{23}H_{27}Cl_2NO_6$·HCl: C, 53.0; H, 5.4; N, 2.7. Found: C, 53.19; H, 5.6; N, 3.3.

X-ray Crystal Structure of Sodium Combretastatin A-4 Phosphate (1b). (a) X-ray Crystal Structure Determination. For sodium combretastatin A-4 phosphate prodrug hydrate (**1b**), a thin plate (∼0.18 mm × 0.24 mm × 0.36 mm), grown from an acetone−water solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 165 ± 1 K with a Bruker SMART 6000 diffractometer system using Mo Kα radiation. A sphere of reciprocal space was covered using the MULTIRUN technique.[41] Thus, six sets of frames of data were collected with 0.30° steps in ω, and a last set of frames with 0.30° steps in φ, such that 86.6% coverage of all unique reflections to a resolution of 0.84 Å was accomplished.

(b) Crystal data. $C_{18}H_{20}NaO_8P·2H_2O$ (hydrate), FW = 454.33, monoclinic, $C2/c$, a = 61.421(12) Å, b = 4.5533(9) Å, c = 14.558(3) Å, β = 100.44(3)°, V = 4004.1(14) $Å^3$, Z = 8, ρ_c = 1.507 Mg/m^3, μ(Mo Kα) = 0.214 mm^{-1}, λ= 0.71073 Å, F(000) = 1904.

A total of 2028 reflections were collected, of which 1503 reflections were independent reflections (R^{int} = 0.0310). Subsequent statistical analysis of the data set with the XPREP[42] program indicated the space group was $C2/c$. Final cell constants were determined from the set of the 1089 observed ($>2\sigma(I)$) reflections which were used in structure solution and refinement. An absorption correction was applied to the data with SADABS.[43] Structure determination and refinement was readily accomplished with the direct-methods program SHELXTL.[44] All non-hydrogen atom coordinates were located in a routine run using default values for that program. Surprisingly, the structure proved to be the monosodium phosphate derivative, rather than the disodium salt initially anticipated. In addition to the parent molecule of the A-4 monosodium salt, two molecules of water were present in each asymmetric unit of the cell. For subsequent refinement, the remaining H atom coordinates were calculated at optimum positions. Considerable disorder was noted for the phosphate moiety, with two equally occupied sites being noted for each of the phosphate oxygen atoms (O95, O96, and O97), as well as the sodium cation. In addition, one of the water molecules was disordered over two sites. The final model for **1b** is shown in Figure 1, in which the alternate disordered sites are shown with dashed lines. All non-hydrogen atoms were refined anisotropically (with the exception of the disordered atoms) in a full-matrix least-squares refinement procedure. The H atoms were included; their U_{iso} thermal parameters were fixed at either 1.2 or 1.5 (depending on atom type), the value 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 **1b** was 0.0771 for observed data and 0.0941 for all data. The goodness-of-fit on F^2 was 0.967. The corresponding Sheldrick R values were wR_2 = 0.2028 and 0.2182, respectively. A final difference Fourier map showed minimal residual electron density, the largest difference peak and hole being 0.282 and −0.783 e/$Å^3$, respectively. Final bond distances and angles were all within expected and acceptable limits.

Disk Diffusion Susceptibility Testing. Antimicrobial activity was assayed by the National Committee for Clinical Laboratory Standards (NCCLS) disk susceptibility test.[45] Compounds were reconstituted in a small volume of sterile dimethyl sulfoxide immediately prior to susceptibility experiments. Mueller-Hinton agar supplemented with 5% sheep blood was used for *Streptococcus pneumoniae*, gonococcal typing agar for *Neisseria gonorrhoeae*, and Mueller-Hinton agar for all other bacteria. *Candida albicans* was tested on Sabouraud Dextrose Agar and *Cryptococcus neoformans* on Yeast Morphology agar. The MIC was defined as the lowest drug concentration resulting in a clear zone of growth inhibition.

Acknowledgment. We are pleased to thank the following for the very necessary financial assistance: Outstanding Investigator Grants CA44344-06-12 and RO1CA90441-01 awarded by the Division of Cancer Treatment and Diagnosis, National Cancer Institute,

DHHS; the Arizona Disease Control Research Commission; the Robert B. Dalton Endowment Fund; the Caitlin Robb Foundation; Gary L. and Diane R. Tooker; Lottie Flugel; the Eagles Art Ehrmann Cancer Fund; and the Ladies Auxiliary to the Veterans of Foreign Wars. For other helpful assistance we thank Drs. Fiona Hogan and Michael Williams.

Supporting Information Available: X-ray data for combretastatin A-4 phosphate prodrug. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For contribution no. 444, refer to: Pettit, G. R.; Lippert, J. W., III; Herald, D. L. A Pinacol Rearrangement/Oxidation Synthetic Route to Hydroxyphenstatin. *J. Org. Chem.* **2000**, *65*, 7438−7444.

(2) (a) Pinney, K. G.; Bounds, A. D.; Dingeman, K. M.; Mocharla, V. P.; Pettit, G. R.; Bai, R.; Hamel, E. A New Anti-tubulin Agent Containing the Benzo(*b*)thiophene Ring System. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1081−1086. (b) Miller, T. A.; Bulman, A. L.; Thompson, C. D.; Macdonald, T. L. The Synthesis and Evaluation of Temperature Sensitive Tubulin Toxins. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 407−412. (c) Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. Tubulin as a Target for Anticancer Drugs: Agents Which Interact with the Mitotic Spindle. *Med. Res. Rev.* **1998**, *18*, 259−296. (d) Chaudhuri, A. R.; Seetharamalu, P.; Schwarz, P. M.; Hausheer, F. H.; Ludueña, R. F. The Interaction of the B-ring of Colchicine with α-Tubulin: A Novel Footprinting Approach. *J. Mol. Biol.* **2000**, *303*, 679−692. (e) ter Haar, E.; Rosenkranz, H. S.; Hamel, E.; Day, B. W. Computational and Molecular Modeling Evaluation of the Structural Basis for Tubulin Polymerization Inhibition by Colchicine Site Agents. *Bioorg. Med. Chem.* **1996**, *4*, 1659−1671.

(3) (a) DeVincenzo, R.; Scambia, G.; Ferlini, C.; Distefano, M.; Filippini, P.; Riva, A.; Bombardelli, E.; Pocar, D.; Gelmi, M. L.; Benedetti-Panici, P.; Mancuso, S. Antiproliferative Activity of Colchicine Analogues on MDR-Positive and MDR-Negative Human Cancer Cell Lines. *Anti-Cancer Drug Des.* **1998**, *13*, 19−33. (b) Shi, Q.; Verdier-Pinard, P.; Brossi, A.; Hamel, E.; McPhail, A. T.; Lee, K.-H. Antitumor Agents. 172. Synthesis and Biological Evaluation of Novel Deacetamidothiocolchicine-7-ols and Ester Analogs as Antitubulin Agents. *J. Med. Chem.* **1997**, *40*, 961−966. (c) Ducray, P.; Lebeau, L.; Mioskowski, C. Synthesis of Lipid Derivatives of Colchicine. *Helv. Chim. Acta* **1996**, *79*, 2346−2352. (d) Boye, O.; Brossi, A. Tropolonic Colchicum Alkaloids and Allo Congeners. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1992; Vol. 41, pp 125−176. (e) Muzaffar, A.; Brossi, A.; Lin, C. M.; Hamel, E. Antitubulin Effects of Derivatives of 3′-Dimethylthiocolchine, Methylthio Ethers of Natural Colchicinoids, and Thioketones Derived from Thiocolchicine. Comparison with Colchicinoids. *J. Med. Chem.* **1990**, *33*, 567−571.

(4) (a) Pettit, G. R.; Toki, B. E.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Chapuis, J. C. Antineoplastic Agents 410. Asymmetric Hydroxylation of *trans*-Combretastatin A-4. *J. Med. Chem.* **1999**, *42*, 1459−1465. (b) Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Chaplin, D. J.; Stratford, M. R. L.; Hamel, E.; Pettit, R. K.; Chapuis, J.-C.; Oliva, D. Antineoplastic Agents 393. Synthesis of the *trans*-isomer of Combretastatin A-4 Prodrug. *Anti-Cancer Drug Des.* **1998**, *13*, 981−993. (c) Pettit, G. R.; Rhodes, M. R. Antineoplastic Agents 389. New Syntheses of the Combretastatin A-4 Prodrug. *Anti-Cancer Drug Des.* **1998**, *13*, 183−191. (d) 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. (e) 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. (f) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. Interactions of Tubulin with Potent Natural and Synthetic Analogs of the Antimitotic Agent Combretastatin: A Structure−Activity Study. *Mol. Pharmacol.* **1988**, *34*, 200−208. (g) Pettit, G. R.; Singh, S. B.; Schmidt, J. M.; Niven, M. L.; Hamel, E.; Lin, C. M. Isolation, Structure, Synthesis and Antimitotic Properties of Combretastatins B-3 and B-4 from *Combretum caffrum*. *J. Nat. Prod.* **1988**, *51*, 517−527. (h) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. Isolation, Structure and Synthesis of Combretastatins A-1 and B-1, Potent New Inhibitors of Microtubule Assembly, Derived from *Combretum caffrum*. *J. Nat. Prod.* **1987**, *50*, 119−131. (i) Pettit, G. R.; Cragg, G. M.; Singh, S. B. Antineoplastic Agents 122. Constituents of *Combretum caffrum*. *J. Nat. Prod.* **1987**, *50*, 386−391. (j) Pettit, G. R.; Singh, S. B. Isolation, Structure, and Synthesis of Combretastatin A-2, A-3, and B-2. *Can. J. Chem.* **1987**, *65*, 2390−2396. (k) Pettit, G. R.; Singh, S. B.; Cragg, G. M. Synthesis of Natural (−)-Combretastatin. *J. Org. Chem.* **1985**, *50*, 3404−3406. (l) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. Isolation and Structure of Combretastatin. *Can. J. Chem.* **1982**, *60*, 1374−1376. (m) Nabha, S. M.; Mohammad, R. M.; Wall, N. R.; Dutcher, J. A.; Salkini, B. M.; Pettit, G. R.; Al-Katib, A. M. Evaluation of Combretastatin A-4 Prodrug in a Non-Hodgkin's Lymphoma Xenograft Model: Preclinical Efficacy. *Anti-Cancer Drugs* **2001**, *12*, 57−63. (n) Nabha, S. M.; Wall, N. R.; Mohammad, R. M.; Pettit, G. R.; Al-Katib, A. M. Effects of Combretastatin A-4 Prodrug against a Panel of Malignant Human β-Lymphoid Cell Lines. *Anti-Cancer Drugs* **2000**, *11*, 385−392.

(5) (a) Mannila, E.; Talvitie, A.; Kolehmainen, E. Antileukemic Compounds Derived from Stilbenes in *Picea abies* Bark. *Phytochemistry* **1993**, *33*, 813−816. (b) Schmitt, E.; Lehmann, L.; Metzler, M.; Stopper, H. Hormonal and Genotoxic Activity of Resveratrol. *Toxicol. Lett.* **2002**, *136*, 133−142. (c) Matsuoka, A.; Takeshita, K.; Furuta, A.; Ozaki, M.; Fukuhara, K.; Miyata, N. The 4′-Hydroxy Group is Responsible for the *in vitro* Cytogenetic Activity of Resveratrol. *Mutat. Res.* **2002**, *521*, 29−35. (d) Arichi, H.; Kimura, Y.; Okuda, H.; Baba, H.; Kozawa, K.; Arichi, S. Effects of Stilbene Components of the Roots of *Polygonum cuspidatum* SIEB ET ZUCC on Lipid-metabolism. *Chem. Pharm. Bull.* **1982**, *30*, 1766−1770. (e) Ingham, J. L. 3,5,4′-Trihydroxystilbene as a Phytoalexin from Groundnuts (*Arachis hypogeae*). *Phytochemistry* **1976**, *15*, 1791−1793. (f) Pettit, G. R.; Grealish, M. P.; Jung, M. K.; Hamel, E.; Pettit, R. K.; Chapuis, J.-C.; Schmidt, J. M. Antineoplastic Agents 465. Structural Modification of Resveratrol: Sodium Resverastatin Phosphate. *J. Med. Chem.* **2002**, *45*, 2534−2542.

(6) (a) Gordaliza, M.; Castro, M. A.; del Corral, J. M. M.; Lopez-Vazquez, M. L.; Garcia, P. A.; San Feliciano, A.; Garavia-Gravalos, M. D.; Broughton, H. Preparation and Cytotoxicity of Podophyllotoxin Derivatives Lacking the Lactone Ring. *Tetrahedron* **1997**, *53*, 15742−15760. (b) Utsugi, T.; Shibata, J.; Sugimoto, Y.; Aoyagi, K.; Wierzba, K.; Kobunai, T.; Terada, T.; Oh-hara, T.; Tsuruo, T.; Yamada, Y. Antitumor Activity of a Novel Podophyllotoxin Derivative (TOP-53) against Lung Cancer and Lung Metastatic Cancer. *Cancer Res.* **1996**, *56*, 2809−2814. (c) Kamal, A.; Atchison, K.; Daneshtalab, M.; Micetich, R. G. Synthesis of Podophyllotoxin Congeners as Potential DNA Topoisomerase II Inhibitors. *Anti-Cancer Drug Des.* **1995**, *10*, 545−554. (d) Lee, K.-H.; Imakura, Y.; Haruna, M.; Beeers, S. A.; Thurston, L. S.; Dai, H.-J.; Chen, C.-H.; Liu, S.-Y.; Cheng, Y.-C.; Antitumor Agents. 107. New Cytotoxic 4-Alkylamino Analogs of 4′-Demethyl-Epipodophyllotoxin as Inhibitors of Human DNA Topoisomerase II. *J. Nat. Prod.* **1989**, *52*, 606−613.

(7) (a) Wickdramaratne, D. B. M.; Pengsuparp, T.; Mar, W.; Chai, H.-B.; Chagwedera, T. E.; Beecher, C. W. W.; Farnsworth, N. R.; Kinghorn, A. D.; Pezzuto, J. M.; Cordell, G. A. Novel Antimitotic Dibenzocyclo-octadiene Lignan Constituents of the Stem Bark of *Steganotaenia araliacea*. *J. Nat. Prod.* **1993**, *56*, 2083−2090. (b) Tomioka, K.; Ishiguro, T.; Mizuguchi, H.; Komeshima, N.; Koga, K.; Tsukagoshi, S.; Tsuruo, T.; Tashiro, T.; Tanida, S.; Kishi, T. Absolute Structure−Cytotoxic Activity Relationships of Steganacin Congeners and Analogues. *J. Med. Chem.* **1991**, *34*, 54−57. (c) Schiff, P. B.; Kende, A. S.; Horwitz, S. B. An Inhibitor of Hela Cell Growth and Microtubule Assembly *in vitro*. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 737−746. (d) Wang, R. W.-J.; Rebhun, L. I.; Kupchan, S. M. Antimitotic and Antitubulin Activity of the Tumor Inhibitor Steganacin. *Cancer Res.* **1977**, *37*, 3071−3079.

(8) (a) McGown, A. T.; Fox, B. W. Differential Cytotoxicity of Combretastatins A-1 and A-4 in Two Daunorubicin-Resistant P388 Cell Lines. *Cancer Chemother. Pharmacol.* **1990**, *26*, 79−81. (b) Jiang, J. B.; Hesson, D. P.; Dusak, B. A.; Dexter, D. L.; Kang, G. L.; Hamel, E. Synthesis/Biological Evaluation of 2-Styrylquinazolin-4(*3H*)-ones, a New Class of Antimitotic Anticancer Agents Which Inhibit Tubulin Polymerization. *J. Med. Chem.* **1990**, *33*, 1721−1728. (c) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. Antimitotic Natural Products Combretastatin A-4 and Combretastatin A-2: Studies on the Mechanism of Their Inhibition of the Binding of Colchicine to Tubulin. *Biochemistry* **1989**, *28*, 6984−6991. (d) McGown, A. T.; Fox, B. W. Structural and Biochemical comparison of the Antimitotic Agents Colchicine, Combretastatin A-4 and Amphethinile. *Anti-Cancer Drug Des.* **1989**, *3*, 249−254.

(9) (a) Chaplin, D. J.; Pettit, G. R.; Hill, S. A. Anti-Vascular Approaches to Solid Tumour Therapy: Evaluation of Combretastatin A4 Phosphate. *Anticancer Res.* **1999**, *19*, 189−196. (b) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. Combretastatin A-4, an Agent That Displays Potent and Selective Toxicity toward Tumor Vasculature. *Cancer Res.* **1997**, *57*, 1829−1834.

(10) (a) Aleksandrzak, K.; McGown, A. T.; Hadfield, J. A. Antimitotic Activity of Diaryl Compounds with Structural Features Resembling Combretastatin A-4. *Anti-Cancer Drugs* **1998**, *9*, 545−550. (b) Hatanaka, T.; Fujita, K.; Obsumi, K.; Nakagawa, R.; Fukuda, Y.; Nihei, Y.; Suga, Y.; Akiyama, Y.; Tsuji, T. Novel B-Ring Modified Combretastatin Analogues: Syntheses and Antineoplastic Activity. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3371−3374. (c) Ohsumi, J.; Hatanaka, T.; Fujita, K.; Nakagawa, R.; Fukuda, Y.; Hihei, Y.; Suga, Y.; Morinaga, Y.; Akiyama, Y.; Tsuji, T. Syntheses and Antitumor Activity of cis-Restricted Combretastatins: 5-membered Heterocyclic Analogues. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3153−3158. (d) Medarde, M.; Ramos, A.; Caballero, E.; Peláez-Lamamié de Clairac, R.; López, L.; Grávalos, D. G.; Feliciano, A. S. Synthesis and Antineoplastic Activity of Combretastatin Analogues: Heterocombretastatins. *Eur. J. Med. Chem.* **1998**, *33*, 71−77. (e) Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. Novel Combretastatin Analogues Effective against Murine Solid Tumors: Design and Structure−Activity Relationships. *J. Med. Chem.* **1998**, *41*, 3022−3032. (f) Woods, J. A.; Hadfield, J. A.; Pettit, G. R.; Fox, B. W.; McGown, A. T. The Interaction with Tubulin of a Series of Stilbenes Based on Combretastatin A-4. *Br. J. Cancer* **1995**, *71*, 705−711. (g) Gwaltney, S. L., II; Imade, H. M.; Barr, K. J.; Li, Q.; Gehrke, L.; Credo, R. B.; Warner, R. B.; Lee, J. Y.; Kovar, P.; Wang, J.; Nukkala, M. A.; Zielinski, N. A.; Frost, D.; Ng, S.-C.; Sham, H. L. Novel Sulfonate Analogues of Combretastatin A-4: Potent Antimitotic Agents. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 871−874. (h) Lawrence, N. J.; Rennison, D.; Woo, M.; McGown, A. T.; Hadfield, J. A. Antimitotic and Cell Growth Inhibitory Properties of Combretastatin A-4 Like Ethers. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 51−54. (i) Ohsumi, K.; Hatanaka, T.; Nakagawa, R.; Fukuda, Y.; Morinaga, Y.; Suga, Y.; Nihei, Y.; Ohishi, K.; Akiyama, Y.; Tsuji, T. Synthesis and Antitumor Activities of Amino Acid Prodrugs of Amino-Combretastatins. *Anti-Cancer Drug Des.* **1999**, *14*, 539−548. (j) Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.-M.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as Potential Cytotoxic and Antimitotic Agents. *J. Med. Chem.* **1992**, *35*, 2293−2306.

(11) Vedejs, E.; Peterson, M. J. In *Topics in Stereochemistry*; Eliel, E. L., Wilen, S. H., Eds.; John Wiley & Sons: New York, 1994; Vol. 21, pp 1−125.

(12) Asakawa, Y.; Tanikawa, K.; Aratani, T. New Substituted Bibenzyls of *Frullania brittoniae* subsp. *truncatifolia*. *Phytochemistry* **1976**, *15*, 1057−1059.

(13) (a) Fieser, L. F. 1,2-Aminonaphthol Hydrochloride (2-Naphthol, 1-Amino-, Hydrochloride). *Org. Synth.* **1943**, 35−39. (b) Fieser, L. F. 1,4-Aminonaphthol Hydrochloride (1-Naphthol, 4-Amino-, Hydrochloride). *Org. Synth.* **1943**, *Collect. Vol. 2*, 39−42.

(14) Ramadas, K.; Srinivasan, N. Iron-Ammonium Chloride−a Convenient and Inexpensive Reductant. *Synth. Commun.* **1992**, *22*, 3189−3195.

(15) Yoo, S.; Lee, S.-L. Reduction of Organic Compounds with Sodium Borohydride−Copper (II) Sulfate System. *Synth. Lett.* **1990**, 419−420.

(16) Han, B. H.; Jang, D. G. Montmorillonite Catalyzed Reduction of Nitroarenes with Hydrazine. *Tetrahedron Lett.* **1990**, *31*, 1181−1182.

(17) Li, H.; Zhang, R.; Wang, H.; Pan, Y.; Shi, Y. A Convenient and Mild Synthetic Route to Aminoarenes by Reduction of Nitroarenes with Activated Nickel and Hydrazine Hydrate. *Synth. Commun.* **1997**, *27*, 3047−3052.

(18) Nose, A.; Kudo, T. Reduction with Sodium Borohydride−Transition Metal Salt Systems. I. Reduction of Aromatic Nitro Compounds with the Sodium Borohydride−Nickelous Chloride System. *Chem. Pharm. Bull.* **1981**, *29*, 1159−1161.

(19) (a) Pinney, K. L., personal communication. (b) Ohsumi, K.; Tsuji, T.; Morinaga, Y.; Ohishi, K. Stilbene Derivatives and Pharmaceutical Compositions Containing Them. U.S. Patent No. 5,525,-632, 1996; 7 pp. (c) Hatanaka, T.; Ohsumi, K.; Tsuji, T.; Nihei, Y.; Nakagawa, R.; Ohishi, K. Stilbene Compounds and Pharmaceutical Compositions Containing Them. U.S. Patent No. 5,-674,906, 1997; 13 pp.

(20) Bachelor, F. W.; Loman, A. A.; Snowdon, L. R. Synthesis of Pinosylvin and Related Hardwood Stilbenes. *Can. J. Chem.* **1970**, *48*, 1554−1557.

(21) (a) Baker, R.; Sims, R. J. A Convenient Modification of the Wadsworth-Emmons Method of Olefin Formation Using a Crown Ether. *Synthesis* **1981**, 117. (b) Baker, R.; Sims, R. J. Synthesis of Pallescensin-E: Use of a Crown Ether in the Wadsworth Procedure for Olefin Formation. *Tetrahedron Lett.* **1981**, *22*, 161−162.

(22) Magarian, R. A.; Avor, K. S.; Overacre, L. B.; Kunchandy, J.; Paxton, T. R. Synthesis and Biological Evaluation of Basic Side Chain Derivatives of Analog II as Pure Antiestrogens and Antitumor Agents. *Anti-Cancer Drug Des.* **1995**, *10*, 311−331.

(23) Qian, X.; Abul-Hajj, Y. J. Synthesis and Biological Activity of 17β-Substituted Estradiol. *J. Steroid Biochem.* **1988**, *28*, 657−664.

(24) Günther, H. In *NMR Spectroscopy*: *Basic Principles*, *Concepts*, *and Applications in Chemistry*, 2nd ed.; Wiley: West Sussex, England, 1995; pp 15−52.

(25) (a) Monaco, R. R.; Gardiner, W. C.; Kirschner, S. Semiempirical Studies of Ring Twisting in *cis*-Stilbene and Related Biomolecules. *Int. J. Quantum Chem.* **1999**, *71*, 57−62. (b) Nandy, P.; Banerjee, S.; Gao, H.; Hui, M. B. V.; Lien, E. J. Quantitative Structure−Activity Relationship Analysis of Combretastatins: A Class of Novel Antimitotic Agents. *Pharm. Res.* **1991**, *8*, 776−781.

(26) (a) Sato, M.; Rippy, M. K.; Bryant, H. U. Raloxifene, Tamoxifen, Nafoxidine, or Estrogen Effects on Reproductive and Nonreproductive Tissues in Ovariectomized Rats. *FASEB J.* **1996**, *10*, 905−912. (b) Horwitz, K. B.; McGuire, W. L. Nuclear Mechanism of Estrogen Action. Effect of Estradiol and Antiestrogens on Estrogen Receptors and Nuclear Receptor Processing. *J. Biol. Chem.* **1978**, *253*, 8185−8191.

(27) (a) Somjen, D.; Waisman, A.; Kaye, A. M. Tissue Selective Action of Tamoxifen Methiodide, Raloxifene and Tamoxifen on Creatine Kinase B Activity *in vitro* and *in vivo*. *J. Steroid Biochem. Mol. Biol.* **1996**, *59*, 389−396. (b) Jordan, V. C.; Gosden, B. Importance of the Alkylaminoethoxy Side-Chain for the Estrogenic and Antiestrogenic Actions of Tamoxifen and Trioxifene in the Immature Rat Uterus. *Mol. Cell. Endocrinol.* **1982**, *27*, 291−299. (c) Stauffer, S. R.; Huang, Y. R.; Aron, Z. D.; Coletta, C. J.; Sun, J.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Triarylpyrazoles with Basic Side Chains: Development of Pyrazole-Based Estrogen Receptor Antagonists. *Bioorg. Med. Chem.* **2001**, *9*, 151−161.

(28) (a) Jarman, M.; Leung, O.-T.; Leclercq, G.; Devleeschouwer, N.; Stoessel, S.; Coombes, R. C.; Skilton, R. A. Analogues of Tamoxifen: The Role of the Basic Sidechain. Application of a Whole-Cell Oestrogen-Receptor Binding Assay to *N*-Oxides and Quaternary Salts. *Anti-Cancer Drug Des.* **1986**, *1*, 259−268. (b) Foster, A. B.; McCague, R.; Seago, A.; Leclercq, G.; Stoessel, S.; Roy, F. Modification of the Basic Side Chain in Tamoxifen: Effects on Microsomal Metabolism and *in vitro* Biological Activity. *Anti-Cancer Drug Des.* **1986**, *1*, 245−257.

(29) Hamel, E. Antimitotic Natural Products and Their Interactions with Tubulin. *Med. Res. Rev.* **1996**, *16*, 207−231.

(30) Pinney, K. G.; Mejia, M. P.; Villalobos, V. M.; Rosenquist, B. E.; Pettit, G. R.; Verdier-Pinard, P.; Hamel, E. Synthesis and Biological Evaluation of Aryl Azide Derivatives of Combretastatin A-4 as Molecular Probes for Tubulin. *Bioorg. Med. Chem. Lett.* **2000**, *8*, 2417−2425.

(31) Carpio, H.; Galeazzi, E.; Greenhouse, R.; Guzman, A.; Valarde, E.; Antonio, Y.; Franco, F.; Leon, A.; Perez, V.; Salas, R.; Valdes, D.; Ackrell, J.; Cho, D.; Gallegra, P.; Halpern, O.; Koehler, R.; Maddox, M. L.; Muchowski, J. M.; Prince, A.; Tegg, D.; Thurber, T. C.; Van Horn, A. R.; Wren, D. Synthesis of 1,2-Dihydro-3*H*-pyrrolo[1,2-a]pyrrole-1-carboxylic Acids and Homologous Pyridine and Azepine Analogues Thereof. *Can. J. Chem.* **1982**, *60*, 2295−2312.

(32) (a) Montginoul, C.; Richard, M. J.; Vigne, C.; Giral, L. Synthesis of Aminoalkylbenzyltertiobutylketones. *Ann. Pharm. Fr.* **1985**, *43*, 249−255. (b) Bandi, A.; Sala, A.; Angelo Soresinetti, P. Synthesis of New Imidazole Derivatives as Potential Inhibitors of Thromboxane Synthetase. *J. Heterocycl. Chem.* **1990**, *27*, 215−219.

(33) (a) Gump, W. S.; Nikawitz, E. J. U.S. Patent 2 533 243, 1950; *Chem. Abstr.* **1950**, *45*, 4271c. (b) Bagley, J. R.; Thomas, S. A.; Rudo, F. G.; Spencer, H. K.; Doorley, B. M.; Ossipov, M. H.; Jerussi, T. P.; Benvenga, M. J.; Spaulding, T. New 1-(Heterocyclylalkyl)-4-(propionanilido)-4-piperidinyl Methyl Ester and Methylene Methyl Ether Analgesics. *J. Med. Chem.* **1991**, *34*, 827−841.

(34) Hawes, E. M.; Davis, H. L. Intramolecular Nucleophilic Cyclization of 3-Substituted Pyridylalkylamines onto the 2-Position of the Pyridine Ring. *J. Heterocycl. Chem.* **1973**, *10*, 39−42.

(35) Gough, G. A. C.; King, H. Trypanocidal Action and Chemical Constitution. Part VIII. Derivatives of β-Aminoethyl and γ-Aminopropyl-arsinic Acids. *J. Chem. Soc.* **1928**, 2426−2447.

(36) Johnson, T. B.; Bengls, R. Hydantoins: Synthesis of the Hydantoin of 3-Amino-Tyrosine. *J. Am. Chem. Soc.* **1912**, *34*, 1054−1061.

(37) Green, G. D.; Weinscherik, J. I., III; Mulvaney, J. E.; Hall, H. K., Jr. Synthesis of Polyesters Containing a Nonrandomly Placed Highly Polar Repeating Unit. *Macromolecules* **1987**, *20*, 722−726.

(38) Singh, S. B.; Pettit, G. R. Isolation, Structure, and Synthesis of Combretastatin C-1. *J. Org. Chem.* **1987**, *54*, 4105−4114.

(39) Mann, F. G. Hofmann's Ethylene Bases. Synthesis of β,β'-Diaminodiethylamine and of β,β'-Diaminodiethylmethylamine. *J. Chem. Soc.* **1934**, 461−466.

(40) Dave, M. A.; Kulkami, A. B. Synthesis of Dimethylamino Aromatic Ketones and Aldehydes. II. *Indian J. Chem.* **1975**, *13*, 33−34.
(41) *SMART* for Windows NT, v. 5.605; Bruker AXS Inc.: Madison, WI, 2000.
(42) XPREP−The automatic space group determination program in the SHELXTL (se ref 44).
(43) Blessing, R. H. *Acta Crystallogr.* **1995**, *A51*, 33−38.
(44) *SHELXTL-NT*, Version 5.10; Bruker AXS Inc.: Madison, WI, 1997. An integrated suite of programs for the determination of crystal structures from diffraction data. This package includes, among other, XPREP (an automatic space group determination program), SHELXS (a structure solution program via Patterson or direct methods), and SHELXL (structure refinement software).
(45) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests; Approved standard M2-A6; NCCLS: Wayne, PA, 1997.

JM0205797