Articles

Bis(bioreductive) Alkylating Agents: Synthesis and Biological Activity in a Nude Mouse Human Carcinoma Model[1]

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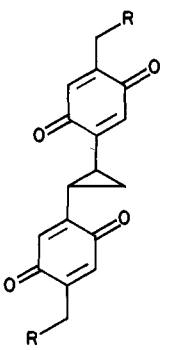
Chemical investigations leading to the construction of bis(bioreductive) alkylating agents having both conformationally restricted and mobile spacer regions are described. Two targets having the conformationally mobile ethylene spacer group, namely, 2,2′-ethylenebis[6-(hydroxymethyl)-*p*-benzoquinone] diacetate (**3b**) and 2,2′-ethylenebis[6-(bromomethyl)-*p*-benzoquinone] (**3c**), were studied in vivo and in vitro using an established epithelial/Burkitt lymphoma hybrid cell line (D98/HR1) previously shown to induce carcinomas in nude mice. Inactivity of both test compounds in vitro, the relative resistance of these cells to test drugs in vitro, and the selective antitumor properties of the bis(bromomethyl) analogue in vivo lead to the proposal that this compound undergoes bioreduction to an alkylating species in the hypoxic core of the tumor, thereby exerting its action.

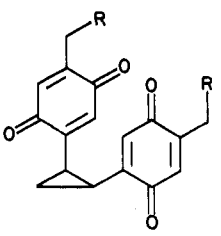
Several naturally occurring antitumor drugs having a quinone nucleus owe their antineoplastic properties to their ability to interact with DNA. The mechanism of action of antitumor mitomycins is thought to involve enzymatic reduction of the quinone ring in vivo leading to generation of reactive quinone methides, which subsequently alkylate DNA, RNA, and other biological macromolecules.[2,3] Some of these agents seem to act as bifunctional alkylators, which add across both strands of the DNA double helix to cause cross-linking.[4] Additionally, certain bis(alkylators) not requiring bioreductive activation are more effective than their monoalkylator counterparts.[5-7] Lin et al.[8-10] observed that certain bioreductive alkylating analogues exhibited varying degrees of antitumor activity. When two alkylating chains were bonded to the same quinone ring, no efficacy advantage was observed over the monoalkylating substrates.[8] However, bis(bioreductive) alkylators in which a spacer region is inserted between two quinone alkylating moieties have not been explored.

Targets **1–3** represent such bis(bioreductive) alkylating agents having both conformationally mobile and restricted spacer regions. Structural modifications defined by these compounds represent permutations of bond distances reflecting either 1,4- or 1,3-bonding of the spacer and alkylating groups to the quinone nucleus. Distance differences in the two alkylating sites could be critical for antitumor activity.

Previously, Glaser et al.[11] demonstrated the usefulness of a model system developed to study nasopharyngeal carcinoma (NPC) in vivo, using nude mice. By employing epithelial/Burkitt lympmoma hybrid cell lines (D98/HR1 and D98/Raji), these investigators were able to demonstrate differences in oncogenicity in nuce mice using parameters of appearance of tumor and mean tumor size over different periods of time. In this article we describe relevant synthetic efforts leading to the preparation of quinones **1a**, **3b**, and **3c**, as well as our initial biological findings with the latter two compounds in this well-characterized model. We simultaneously used the stem-cell assay to measure the activity of these drugs in vitro against the same cell line (D98/HR1) and compared these data with several other known drugs presently used in chemotherapy. Comparison of activity profiles in vitro and in vivo could suggest a bioreductive metabolic mechanism



1a, R = H
b, R = OAc
c, R = Br



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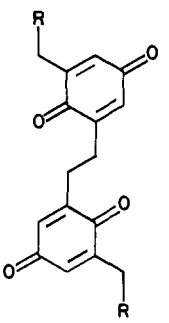

3a, R = H
b, R = OAc
c, R = Br

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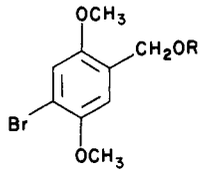
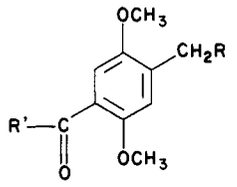
requirement in a solid tumor.

Chemistry. Oxidative demethylation[12-18] of hydroquinone dimethyl ethers represents a facile method for synthesizing quinones. Our approach to the preparation of bis(quinone)s **1–3**, therefore, involved prior synthesis of certain tetramethoxy aromatic compounds, **34–38**, derivable from stilbene precursors. Initial attempts to prepare intermediate stilbenes via Grignard reagents of protected bromobenzyl alcohols **5** or **6** derived from **4** failed



4, R = H
5, R = Si(Me$_2$)-*t*-Bu
6, R = THP

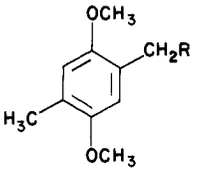


7, R = OSi(Me$_2$)-*t*-Bu; R′ = H
8, R = OSi(Me$_2$)-*t*-Bu; R′ = OH
9, R = R′ = H
10, R = Br; R′ = H

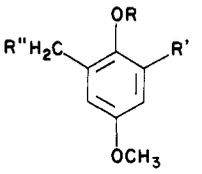
at the Grignard stage under a variety of conditions.[19-21] Although lithio derivatives of **5** and **6** presumably afforded corresponding stilbenes **26** and **27** when they underwent reaction with (*Z*)- and (*E*)-1,2-dichloroethylene, the method[22] was not explored further due to poor yields. Attempts to prepare **7** or **8** by reacting the lithio derivative of **5** with ethyl formate (or DMF) or CO_2, respectively, were unsuccessful. Bromination of aldehyde **9**[23] with NBS afforded unsatisfactory yields of **10**.

(*E*)-Stilbene **28** was obtained from aldehyde **9** under McMurry's[24] reductive coupling conditions (Li–$TiCl_3$). However, subsequent conversion of (*E*)-**28** to the bis[(acetyloxy)methyl] derivative **30** through benzylic bromination (NBS),[25] followed by displacement with NaOAc, took place only in 27% yield. Thus, this method was unsatifactory for the preparation of needed quantities of target quinones for biological studies. Alternatively, Wittig reaction of aldehyde **9** with phosphonium salt **13** derived from halide **13**[23] yielded a 65:35 mixture of (*Z*)- and (*E*)-**28**, which were separated by fractional crystallization.

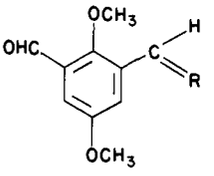
(*E*)-Stilbene **31**, a regiomer of **28**, was prepared from bis(hydroxymethyl) derivative **14**, which in turn was obtained from *p*-methoxyphenol under Lederer–Manasse



11, R = OH
12, R = Cl
13, R = PPh_3Cl



14, R = H; R′ = CH_2OH; R″ = OH
15, R = R′ = CH_3; R″ = OH
16, R = R′ = CH_3; R″ = Cl
17, R = R′ = CH_3; R″ = PPh_3Cl
18, R = CH_3; R′ = CHO; R″ = H
19, R = CH_3; R′ = CH_2OH; R″ = OH
20, R = CH_3; R′ = CHO; R″ = OH
21, R = CH_3; R′ = CHO; R″ = OAc
22, R = CH_3; R′ = CH_2OH; R″ = Cl
23, R = CH_3; R′ = CH_2OH; R″ = PPh_3Cl



24, R = O
25, R = $CHCOCH_3$

conditions.[26] Partial hydrogenolysis of **14**, followed by alkylation with MeI, yielded known **15**.[27] Aldehyde **18**,[28] obtained by oxidation of **15** with pyridinium chlorochromate,[29] underwent reaction with the phosphonium salt **17** to yield (*E*)-**31** in 76% yield. Conversion of (*E*)-**31** to the corresponding diacetate **33** was achieved in 20% overall yield utilizing NBS and NaOAc. The diacetate (*E*)-**33** could also be obtained in 73% yield by reductive coupling (Li–$TiCl_3$) of aldehyde **21** obtained from **20**. No *Z* isomer could be detected in the reaction mixture.

Aldehyde **20** was obtained from phenol **14** by alkylation (MeI) followed by partial oxidation of the resulting diol **19**. Use of active MnO_2,[30] even in large excess, provided only a 60% conversion to the aldehyde **20**, with no detectable amounts of dialdehyde. Alternatively, oxidation of **19** using 1 equiv of pyridinium chlorochromate afforded a mixture of aldehyde and dialdehyde, **20** (62%) and **24** (24%), and a minor product **25** (4%) when this reaction was performed in acetone. With dichloromethane as solvent, approximately equal amounts of **20** and **24** were obtained. This loss of selectivity may reflect the faster oxidation of benzylic hydroxyls in this solvent relative to acetone.[29]

Z-isomer **33** was obtained starting from diol **19**, which was treated with concentrated HCl at room temperature to obtain intermediate chloro derivative **22**. No dichloro compound could be detected even under prolonged reaction times. Phosphonium salt **23** derived from **22** was condensed with aldehyde **21**, affording isomeric stilbene diols **32**, which could not be isolated in a pure state. Acetylation of this mixture, followed by chromatography on silica gel using hexane–ethyl acetate as eluant, yielded the pure geometric isomers of **33**.

Reduction of olefinic bonds in (*Z*)- or (*E*)-stilbenes has been reported using $NaBH_4$ and Co(II).[31] However,

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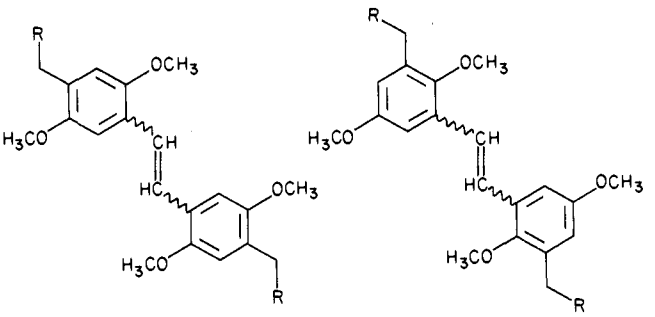
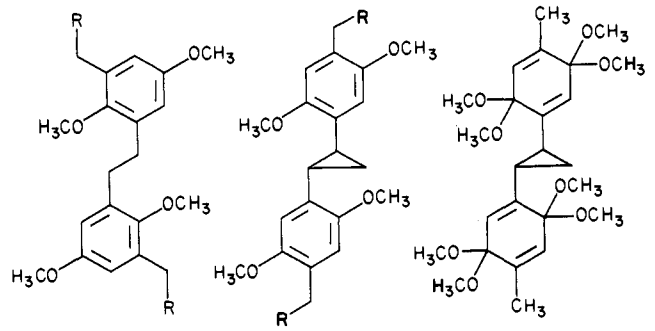


26, R = OSi(Me$_2$)-*t*-Bu
27, R = OTHP
28, R = H
29, R = Br
30, R = OAc

31, R = H
32, R = OH
33, R = OAc



34, R = OAc
35, R = OH
36, R = Br

37, R = H
38, R = OAc

39

(*E*)-stilbene **33** failed to undergo reduction under these conditions. Catlytic hydrogenation over Pd/C also failed to afford ethane **34**. Hydrogenation over PtO_2 in glacial HOAc afforded only 35% of **34**, which could not be separated from starting olefin. However, reduction of (*Z*)-**33** with $NaBH_4$–$CoCl_2$ proceeded smoothly to afford quantitative yields of **34**.

To obtain **37**, cyclopropanation of (*E*)-stilbene **28** was carried out under modified Simmons–Smith conditions[32] using diiodomethane and Zn–Cu couple in the presence of EtI. Attempts to prepare **37** in the absence of EtI failed. However, this procedure was unsuccessful in the cyclopropanation of (*E*)-**33**. Efforts to brominate the methyl groups in **37** under a variety of conditions using NBS, as well as 1,3-dibromo-5,5-dimethylhydantoin,[33] were unsuccessful. Reaction of **37** with sodium peroxydisulfate and copper(II) acetate in an attempt to obtain acetate **38** resulted in polymerization.[34]

In order to convert tetramethoxy substrates to their respective quinones, numerous oxidation procedures were attempted. Mild oxidizing conditions employing AgO in the presence of either weak organic[13] (pyrazine-2,3-dicarboxylic) or mineral[12] (phosphoric) acid failed to oxidize **34** to bis(quinone) **3b**. However, $Ce(NH_4)_2(NO_3)_6$[35] oxidation readily afforded **3b** in excellent yields. The corresponding bromo analogue **3c** was also prepared by $Ce(NH_4)_2(NO_3)_6$ oxidation of the bis(bromomethyl) compound **36** derived from diacetate **34** by saponification, followed by bromination with PBr_3. In the case of cyclopropane **37**, AgO, HNO_3[36] and $Ce(NH_4)_2(NO_3)_6$ failed to afford the corresponding quinone **1a**. A complex mixture of products was obtained when these reagents were employed for the oxidation of (*E*)-stilbene **28**. Various attempts to demethylate[37-39] **28**, as well as **37**, to their respective bis(hydroquinone)s did not yield well-defined products. However, quinone **1a** was obtained readily by use of electrochemical oxidation.[40] Electrolysis of **37** in methanolic KOH (Pt electrode) afforded quinone bis(ketal) **39**, which upon hydrolysis (HCl) afforded bis(quinone) **1a**. The bis(ketal) **39** was unstable on undergoing partial hydrolysis to the symmetrical bis(keto)–bis(dimethyl acetal) when chromatographed on neutral alumina using hexane–ethyl acetate as eluant.

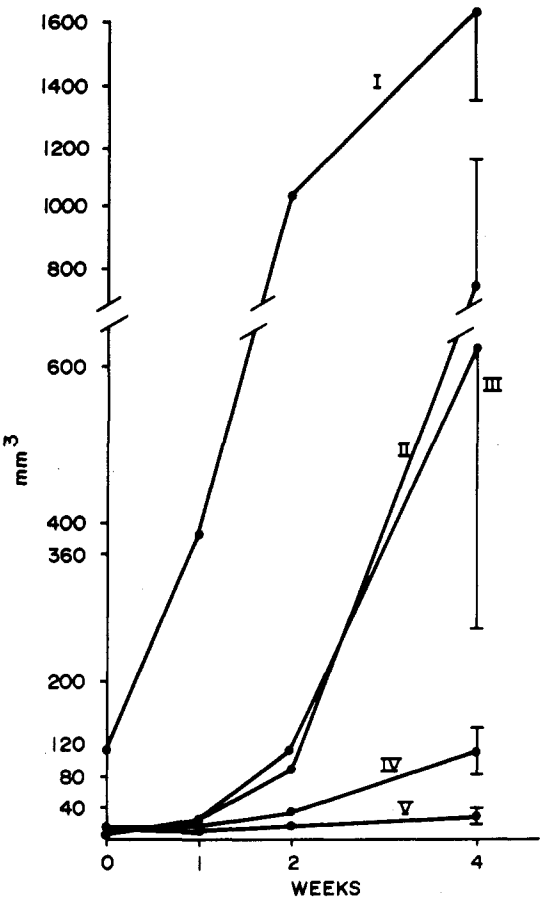


Figure 1. Mean volume (mm^3) of tumors induced in nude mice by D98/HR1 cells. The mice were inoculated with drugs **3b** and **3c** or with vehicle daily for 14 days starting on 0 week and then held for an additional 2 weeks: I = 5 mg/kg of **3b**; II = 20 mg/kg of **3b**; III = vehicle control; IV = 5 mg/kg of **3c**; V = 20 mg/kg of **3c**.

Biological Results and Discussion

Compounds **3b** and **3c** having bis[(acetyloxy)methyl] and bis(bromomethyl) functions, respectively, were administered to nude mice for 14 days subsequent to the appearance of tumors. Tumor measurements were taken on the 1st day of drug therapy and weekly thereafter for 4 weeks, and these results are summarized in Figure 1.

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Table I. Effects of Quinone Analogues on Tumor Growth in the Carcinoma Nude Mouse Model

group (compd administered; dose, mg/kg)	sample size (n)	week 0 (base line)	week 1	week 2	week 4
I (3b; 5)	5	112.88 ± 40.43[a]	386.96 ± 149.51	892.20 ± 346.90	1647.52 ± 267.00
II (3b; 20)	4	7.00 ± 2.50	23.20 ± 8.04	90.78 ± 48.40	666.25 ± 523.32
III (Klucel control)	3	6.70 ± 5.13	24.60 ± 11.17	113.47 ± 87.64	619.17 ± 355.75
IV (3c; 5)	5	8.14 ± 1.73	16.62 ± 2.84	33.86 ± 6.69	122.02 ± 29.74
V (3c; 20)	3	11.83 ± 1.74	12.4 ± 1.96	17.07 ± 1.56	28.97 ± 2.99

[a] Means and standard error of the means, $\bar{X}$ ± SEM (in mm^3) for 4 weeks. The reasons for the difference between group I and the other groups at week 0 is due to the fact that one mouse in this group had a tumor that was considerably larger than any of the others.

Table II. Group Means Adjusted (via Analysis of Covariance) for Base Line Measurements for 3 Weeks

group (compd administered; dose, mg/kg)	sample size (n)	week 1	week 2	week 4
I (3b; 5)	5	69.66	162.76	358.79
II (3b; 20)	4	34.53	94.83	360.23
III (Klucel control)	3	53.89	151.58	921.26
IV (3c; 5)	5	22.32	43.20	137.03
V (3c; 20)	3	12.54	17.52	29.69

Marked tumor growth was observed for groups I and II (5 and 20 mg/kg of **3b**) and group III (control), whereas groups IV and V (5 and 20 mg/kg of **3c**) appeared to have little tumor growth at 2 weeks, with some acceleration following cessation of drug therapy.

Since there are five independent groups of mice with repeated measurements taken on each mouse at 0, 1, 2, and 4 weeks (Table I), the proper statistical model for analysis of the data is a two-factor analysis of variance with one factor repeated. We explored differences in groups as well as group by time interactions via a repeated measures analysis.[41] We checked the underlying assumptions in order to employ this model and found the variances for the various groups had wide disparity at the various weeks. For this reason, the analysis was performed on the logarithm of the observations. We also noted that group I had much higher base-line measurements than did the remaining four groups. The reason for this is that one mouse had an exceptionally large tumor relative to the other mice in group I. Consequently, an analysis of covariance with week 0 (base line) was employed as a single covariate. We followed up significant differences of group and group by time interactions found in the analysis of variance via a Newman–Keuls procedure.[42]

Upon performing a repeated measures analysis (two-factor design with one factor repeated) using BMDP2V[43] using week 0 as a covariate, we found group differences ($p = 0.0016$) and group by time differences ($p = 0.056$). The Newman–Keuls follow up testing at week 4 showed group V significantly different from groups I–III ($p = 0.05$). Groups IV and V were not significantly different from each other. Similarly, Groups I–IV were not significantly different. The logarithms of the group means were adjusted for differences in base-line measurement (Table II) in order to normalize for the variation of mean tumor size in each group at week 0. Statistical analysis, as previously described, implicates the bis(bromomethyl) analogue **3c** at 20 mg/kg as an inhibitor of tumor growth. Furthermore, this analysis indicates **3b** is not effective at either dose.

Table III. Effect of Antitumor Drugs on the EBV-Positive Nasopharyngeal Cell Line (D98/HR1)

antitumor drug	drug concn, μg/mL	survival[a]	% reduction
	Experiment A		
control	none	1.00 ± 0.27	0
velban	6.0	<0.01	>99[b,c]
DHAD	0.1	0.33 ± 0.03	67[d]
VP 16	3.0	0.30 ± 0.03	70[c]
methotrexate	100	<0.01	>99[b,c]
methotrexate	0.3	0.40 ± 0.10	60[d]
doxorubicin	0.04	0.37 ± 0.10	63[c]
cisplatin	10.0	0.03 ± 0.03	97[b,c]
cisplatin	0.2	0.40 ± 0.10	60[d]
	Experiment B		
vehicle control		1.00 ± 0.25	0
3b	2.68	1.35 ± 0.23	+35[e]
3b	1.34	1.19 ± 0.26	+19
3b	0.67	1.34 ± 0.27	+34
3b	0.067	0.70 ± 0.10	30[d]
3c	2.68	0.91 ± 0.21	9
3c	1.34	0.77 ± 0.25	23
3c	0.67	0.81 ± 0.31	19
3c	0.2	1.00 ± 0.48	0
doxorubicin	0.4	<0.1	>99[b,c]
doxorubicin	0.04	0.18 ± 0.09	82[c]
doxorubicin	0.004	0.69 ± 0.26	31[d]

[a] Colony counts obtained from the examination of uniform plate surface areas. Control colony counts were 300 ± 80 for experiment A and 373 ± 95 for experiment B. [b] Nearly total lack of cell growth. [c] Indicates significant colony reduction ($p < 0.001$). [d] Indicates significant colony reduction ($p < 0.01$). [e] Indicates significant colony increase ($p < 0.01$).

During the course of these studies neither drug caused toxicity, as determined by a loss in weight relative to controls (data not shown).

In vitro, employing the D98/HR1 cells[11] in a modified clonogenic assay procedure (see Experimental Section), significant but variable inhibition of colony formation was observed with all drugs tested (Table III, experiment A). Almost complete inhibition was noted with Velban, methotrexate (100 μg/mL), and cisplatin (10 μg/mL), whereas moderate colony reductions of 60–70% were noted with standard screening drugs. In a second experiment (Table III, experiment B) comparing the quinone compounds with doxorubicin, no predictably significant colony reduction was noted for either quinone compound at concentrations of 0.007–2.7 μg/mL. Drug clonogenic activity is assessed by colony reduction of at least 67%.[44] Significant inhibition of colony formation was observed for doxorubicin at 0.4 and 0.04 μg/mL. No significant inhibition was ob-

(41) Winer, B. J. "Statistical Principles in Experimental Design"; McGraw-Hill: New York, 1971; chapter 7.
(42) Reference 41; chapter 3.
(43) Department of Biomathematics, UCLA, "BMDP Statistical Software"; University of California Press: Los Angeles.
(44) Salmon, S. E.; Hamburger, A. W.; Sochnlen, D.; Durie, B.; Alberts, D.; Moon, T. E. *N. Engl. J. Med.* **1978**, *298*, 1321–1327.

served at concentrations of 0.004 μg/mL.

Failure of quinones **3b** or **3c**, even at very high concentrations, to evoke a significant reduction in clonogenic activity of the Epstein Barr virus genome (EBV) positive epithelial cell line likely reflects inability to undergo bioreduction in tissue culture. Generally, drug sensitivity in the clonogenic assay has shown promising correlations with clinical response.[44,45] However, this would not be the case for those drugs required reductive metabolic transformation within tumor cells. The apparently significant stimulation of cell growth in vitro by **3b** at high concentrations cannot be explained at this time.

Interestingly, only three drugs (Velban, methotrexate, and cisplatin) were highly effective in significantly reducing the clonogenicity of D98/HR1 cells at concentrations normally used in drug screening assays. Only moderate reductions were observed with DHAD, VP16, methotrexate (0.3 μg/mL), cisplatin (0.2 μg/mL), and doxorubicin. It is interesting that this cell line has some similarities to the drug-resistant NPC, which is an EBV-positive tumor (carcinoma). Although further work is necessary to establish possible in vitro–in vivo correlations with D98/HR1 cells, it is nonetheless significant that **3c** and not **3b** exhibits antitumor activity in vivo. The bromo group is a better leaving group than the acetyloxy function, and if bioreduction in vivo leads to an alkylating species in the hypoxic core of the tumor, it might be expected that **3c** rather than **3b** would be the more effective inhibitor of tumor growth.

Experimental Section

Chemistry. Melting points are uncorrected and were determined on a Thomas-Hoover Uni-Melt apparatus. Infrared spectra were recorded on a Beckman 4230 spectrophotometer. NMR spectra were determined either on a Varian A-60A or Bruker HX-90E spectrometer operating in the pulse mode. Mass spectra were determined with a DuPont Model 21-491 mass spectrometer. Elemental analyses were obtained from Galbraith Laboratories, Inc., Knoxville, TN. In electrochemical experiments for electrolysis at constant potential, we employed a potentiostat constructed from a Kepco Model BOP72-5M biopolar operational amplifier capable of a maximum output of 5.5 A at 80 V in Professor John Swenton's laboratories at The Ohio State University. For constant-current experiments, the power supply Model QRD60-1.5/30-3 from Sorensen Power Supplies was employed.

4-Bromo-2,5-dimethoxybenzyl Alcohol (4). An aqueous solution of $NaBH_4$ (0.76 g, 0.02 mol) was added at room temperature to a stirred solution of 4-bromo-2,5-dimethoxybenzaldehyde[46] (10.0 g, 0.04 mol) in 200 mL of THF–MeOH (1:1). After 0.5 h, the solution was cooled (0 °C), acidified with dilute HCl solution, and extracted with EtOAc. The organic layer was washed twice with brine and dried (Na_2SO_4), and the solvent was removed in vacuo to yield 9.78 g (97%) of crude **4**. Recrystallization from CH_2Cl_2/pentane afforded colorless crystals: mp 108–110 °C; NMR ($CDCl_3$) δ 2.16 (t, 1 H, J = 6.2 Hz, OH), 3.79 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 4.61 (d, 2 H, J = 6.2 Hz, CH_2), 6.92 (s, 1 H, ArH), 7.03 (s, 1 H, Ar H). Anal. ($C_9H_{11}O_3Br$) C, H, Br.

1-Bromo-2,5-dimethoxy-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]benzene (5). To a solution of **4** (0.25 g, 1.0 mmol) and imidazole (0.17 g, 2.5 mmol) in 5 mL of DMF was added *tert*-butyldimethylsilyl chloride (0.22 g, 1.46 mmol). The mixture was left to stir at room temperature overnight. After the mixtured cooled (0–5 °C), 5% $NaHCO_3$ solution was added, and the precipitate was removed by extraction with Et_2O. The Et_2O solution was washed several times with H_2O and dried ($MgSO_4$). Solvent was removed in vacuo, and the residue was recrystallized from EtOH, affording 0.32 g (88%) of colorless crystals, mp 66–67 °C; NMR ($CDCl_3$) δ 0.11 (s, 6 H, CH_3), 0.96 (s, 9 H, *t*-Bu), 3.75 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 4.69 (s, 2 H, CH_2), 6.99 (s, 1 H, Ar H), 7.15 (br s, 1 H, Ar H). Anal. ($C_{15}H_{25}O_3SiBr$) C, H, Br.

1-Bromo-2,5-dimethoxy-4-[[(tetrahydropyran-2-yl)oxy]methyl]benzene (6). Dihydropyran (18.4 g, 0.22 mol) was added to a stirred solution of **4** (17.2 g, 0.07 mol) in 250 mL of CH_2Cl_2 containing 4 drops of concentrated HCl, and the mixture was left stirring at room temperature overnight. After the mixture was washed with 5% $NaHCO_3$ solution and H_2O, the organic layer was dried (Na_2SO_4). The oil obtained after removal of the solvent in vacuo was distilled under reduced pressure, affording 19.55 g (85%) of colorless liquid: bp 128–130 °C (0.25 mm); NMR ($CDCl_3$) δ 1.35–1.87 [br m, 6 H, $(CH_2)_3$], 3.78 (s, 6 H, OCH_3), 3.87 (s, 2 H, CH_2O), 4.59 (s, 1 H, OCHO), 4.67 (m, 2 H, Ar CH_2), 7.05 (s, 2 H, Ar H). Anal. ($C_{14}H_{19}O_4Br$) C, H, Br.

2,5-Dimethoxy-4-methylbenzaldehyde (9) was prepared according to the reported procedure,[23] mp 80–82 °C (lit.[23] mp 77–78 °C).

(2,5-Dimethoxy-4-methylbenzyl)triphenylphosphonium Chloride (13). A mixture of 2,5-dimethoxy-4-methylbenzyl chloride **12** (24 g, 0.12 mol; prepared from **9** via alcohol **11** according to a literature procedure[23]) and triphenylphosphine (37.65 g, 0.14 mol) in 200 mL of benzene was refluxed for 5 days. The white precipitate was filtered, washed with benzene, and air-dried, affording 53.0 g (96%) of **13**. Recrystallization from MeOH/acetone furnished colorless prisms, mp 237–239 °C. Anal. ($C_{28}H_{28}O_2PCl{\cdot}CH_3OH$) C, H, Cl.

2,6-Bis(hydroxymethyl)-4-methoxyphenol (14) was prepared following the procedure of Moran et al.,[26] mp 130–131 °C (lit.[26] mp 127–128 °C).

2,5-Dimethoxy-3-methylbenzyl alcohol (15) was prepared from **14** by the procedure of Nichols et al.,[27] bp 112–115 °C (0.65 mm) [lit.[27] bp 94–96 °C (0.1 mm)].

(2,5-Dimethoxy-3-methylbenzyl)triphenylphosphonium Chloride (17). A solution of 2,5-dimethoxy-3-methylbenzyl chloride (**16**; 9.1 g, 45.4 mmol; prepared from **15** by the literature procedure[23]) and triphenylphosphine (14.3 g, 54.5 mmol) in 100 mL of benzene was refluxed for 5 days. The white precipitate was filtered, washed with benzene, and air-dried, affording 16.3 g (78%) of **17**, mp 220–223 °C. Anal. ($C_{28}H_{28}O_2PCl$) C, H, Cl.

2,5-Dimethoxy-3-methylbenzaldehyde (18) was prepared by the procedure of Glennon et al.,[28] mp 39–41 °C (lit.[28] mp 42–44 °C).

1,3-Bis(hydroxymethyl)-2,5-dimethoxybenzene (19). A mixture of the phenol diol **14** (0.92 g, 5.0 mmol), K_2CO_3 (0.69 g, 5.0 mmol), and MeI (1 mL, 16.1 mmol) in 30 mL of acetone was refluxed for 2 days, during which time MeI (1 mL) was added three times. The reaction mixture was filtered, and the filtrate was evaporated in vacuo to dryness. Extraction with hot EtOAc, followed by filtration and solvent removal in vacuo afforded **19** (0.89 g, 90%) as a colorless solid. Crystallization from EtOAc/hexane afforded pure **19**, mp 106–108 °C; IR (KBr) 3200 (br) cm^{-1}; NMR ($CDCl_3$) δ 2.05 (br s, 2 H, OH, D_2O exchangeable), 3.80 (s, 6 H, OCH_3), 4.70 (br s, 4 H, CH_2), 6.88 (s, 2 H, Ar H); MS m/e 198 (M^+). Anal. ($C_{10}H_{14}O_4$) C, H.

2,5-Dimethoxy-3-(hydroxymethyl)benzaldehyde (20). Method A. To a stirred, ice-cold solution of diol **19** (10 g, 50.5 mmol) in 1 L of acetone was added solid pyridinium chlorochromate (10.9 g, 50.6 mmol). After 15 min, stirring was continued at room temperature for 2.5 h. Following the addition of 50 mL of H_2O, the solution was concentrated under reduced pressure to 150 mL and extracted with EtOAc. The EtOAc layer was washed with 2 × 250 mL of H_2O and saturated brine and dried (K_2CO_3). Removal of the solvent in vacuo afforded an orange solid (9.45 g). Chromatography over silica gel using hexane/EtOAc (95:5) as eluting solvent afforded 2.33 g (24%) of dialdehyde **24** (**2,5-dimethoxy-1,3-benzenedicarboxaldehyde**), crystallized from EtOAc as long colorless needles: mp 113–115 °C; IR (KBr) 1680, 1600, 1585 cm^{-1}; NMR ($CDCl_3$) δ 3.87 (s, 3 H, OCH_3), 4.04 (s, 3 H, OCH_3), 7.61 (s, 2 H, Ar H), 10.39 (s, 2 H, CHO); MS, m/e 194 (M^+). Anal. ($C_{10}H_{10}O_4$) C, H.

Further elution with hexane/EtOAc (85:15) gave keto aldehyde **25 [2,5-dimethoxy-3-(3-oxo-1-butenyl)benzaldehyde]** (0.5 g, 4%). Recrystallization from EtOAc/hexane yielded **25** as colorless needles: mp 94–96 °C; IR (KBr) 1695, 1615, 1480 cm^{-1}; NMR ($CDCl_3$) δ 2.43 (s, 3 H, $COCH_3$), 3.86 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 6.76 (d, 1 H, J = 16.5 Hz, HCC=O), 7.38 (s, 2 H, Ar

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H), 7.78 (d, 1 H, J = 16.5 Hz, Ar H C=C), 10.37 (s, 1 H, CHO); MS, m/e 234 (M^+). Anal. ($C_{13}H_{14}O_4$) C, H.

Continued elution with hexane/EtOAc (85:15) afforded **20** (6.15 g, 62%), which crystallized from EtOAc/hexane, affording colorless needles: mp 74–76 °C; IR (KBr) 3260 (br), 1695, 1600, 1590, 1475 cm^{-1}; NMR ($CDCl_3$) δ 2.20 (t, 1 H, J = 4.8 Hz, OH, D_2O exchangeable), 3.83 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 4.73 (d, 2 H, J = 4.8 Hz, CH_2), 7.25 (s, 2 H, Ar H), 10.32 (s, 1 H, CHO); MS, m/e 196 (M^+). Anal. ($C_{10}H_{12}O_4$) C, H.

Method B. Active MnO_2 (1.09 g, 12.5 mmol) was added to a solution of **19** (1.0 g, 5.1 mmol) in 50 mL of acetone (or $CHCl_3$) and stirred at room temperature for 24 h. Filtration and evaporation of the filtrate on a rotary evaporator afforded a 60:40 mixture (NMR) of aldehyde **20** and starting compound **19**. Pure **20** was isolated by chromatography on silica gel using hexane/EtOAc (85:15) as the eluting solvent.

3-[(Acetyloxy)methyl]-2,5-dimethoxybenzaldehyde (21) was obtained in quantitative yield by stirring a solution of **20** (5.6 g) in 10 mL of dry pyridine and 5 mL of Ac_2O at room temperature overnight. Ether extraction and standard workup afforded a colorless oil: bp 140–144 °C (bath temperature, 0.5 mm); IR (CCl_4) 1750, 1700, 1610, 1595, 1485, 1225 cm^{-1}; NMR ($CDCl_3$) δ 2.13 (s, 3 H, $OCOCH_3$), 3.83 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 5.19 (s, 2 H, CH_2), 7.26 (dd, 2 H, J = 3.2 and 8.0 Hz, Ar H), 10.35 (s, 1 H, CHO). Anal. ($C_{12}H_{14}O_5$) C, H.

3-(Chloromethyl)-2,5-dimethoxybenzyl Alcohol (22). To a stirred suspension of **19** (7.1 g, 35.9 mmol) in 150 mL of benzene was added 20 mL of concentrated HCl. The resulting clear solution was stirred overnight at room temperature. The solution was washed with brine, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (K_2CO_3), and the solvent was removed in vacuo, affording 7.76 g (100%) of **22** as a light brown oil, which solidified on standing. Crystallization twice from EtOAc/hexane furnished long, colorless needles: mp 48–50 °C; IR (KBr) 3245, 3140, 1610, 1480 cm^{-1}; NMR ($CDCl_3$) δ 2.31 (br s, 1 H, OH, D_2O exchangeable), 3.79 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 4.62 (s, 2 H, CH_2Cl), 4.69 (s, 2 H, CH_2O), 6.89 (dd, 2 H, J = 3.2 and 6.0 Hz, Ar H); MS, m/e 216 (M^+). Anal. ($C_{10}H_{13}O_3Cl$) C, H, Cl.

[2,5-Dimethoxy-3-(hydroxymethyl)benzyl]triphenylphosphonium Chloride (23). A solution of chloro compound **22** (7.43 g, 34.3 mmol) and triphenylphosphine (10.8 g, 41.2 mmol) in 100 mL of benzene was stirred and refluxed for 18 h and cooled. The precipitate was filtered, washed with benzene, and dried under reduced pressure, yielding 13.89 g of white solid. Treatment of the filtrate with fresh triphenylphosphine (1.0 g) for 2 days afforded an additional 1.5 g of white solid. The combined solids represents a 94% yield. Recrystallization from MeOH/acetone gave colorless, transparent prisms, mp 230–232 °C. Anal. ($C_{28}H_{28}O_3PCl$) C, H, Cl.

(*E*)-1,2-Bis(2,5-dimethoxy-*p*-tolyl)ethene (28). To a slurry of $TiCl_3$ (4.2 g, 27.2 mmol) in 40 mL of freshly distilled dimethoxyethane (DME) was added Li wire (0.54 g, 0.078 gatom) under an argon atmosphere with stirring. After the addition, the mixture was stirred at reflux for 2 h. After the mixture was cooled, a solution of aldehyde **9** (1.8 g, 10.0 mmol) in 10 mL of DME was added, and the mixture was refluxed for 16 h. After cooling to room temperature, the mixture was diluted with an equal volume of petroleum ether (60–90 °C) and filtered through a pad of Florisil (60–100 mesh) on a sintered glass funnel. The residue was cautiously quenched by the slow addition of MeOH. The filtrate was concentrated in vacuo, and the crude product was recrystallized from cyclohexane, affording 0.55 g (34%) of (*E*)-**28** as yellow crystals: mp 160–161 °C; IR (KBr) 1510, 1465, 960 cm^{-1}; NMR ($CDCl_3$) δ 2.22 (s, 6 H, CH_3), 3.78 (s, 6 H, OCH_3), 3.82 (s, 6 H, OCH_3), 6.64 (s, 2 H, =CH), 7.02 (s, 2 H, Ar H), 7.30 (s, 2 H, Ar H); MS, m/e 328 (M^+). Anal. ($C_{20}H_{24}O_4$) C, H.

(*E*)- and (*Z*)-1,2-Bis(2,5-dimethoxy-*p*-tolyl)ethene (28). To a solution of bromobenzene (17 g, 0.11 mol) in 200 mL of anhydrous Et_2O under an argon atmosphere was added Li wire (1.69 g, 0.24 mol). The mixture was stirred for 0.5 h at room temperature and decanted. Phosphonium chloride **13** (50.0 g, 0.11 mol) was added to the decanted solution in small portions and stirred at room temperature for 4 h. Aldehyde **9** (19.0 g, 0.11 mol) in 300 mL of Et_2O was added, and the mixture was refluxed for 2 days. Excess phenyllithium was decomposed by the addition of H_2O. The mixture was filtered, the solids were washed with Et_2O, and the combined filtrates were dried (Na_2SO_4). Removal of the solvent gave a yellow solid (27.5 g), which consisted of (*Z*)- and (*E*)-**28** (65:35 by NMR). Separation of the isomers was accomplished by repeated crystallization from EtOAc, affording pure (*E*)-**28** (8.25 g, 24%), mp 159–161 °C, identical in all respects with previously described (*E*)-**28**. Pure (*Z*)-**28** was obtained by repeated recrystallization of the filtrate solids as colorless flakes (14.20 g, 41%), mp 114–115 °C, by recrystallization from ethyl acetate: IR (KBr) 1500, 1470 cm^{-1}; NMR ($CDCl_3$) δ 2.17 (s, 6 H, CH_3), 3.42 (s, 6 H, OCH_3), 3.79 (s, 6 H, OCH_3), 6.68 (s, 4 H, Ar H), 6.72 (s, 2 H, =CH); MS, m/e 328 (M^+). Anal. ($C_{20}H_{24}O_4$) C, H.

(*E*)-1,2-Bis[4-[(acetyloxy)methyl]-2,5-dimethoxyphenyl]ethene (30). To a solution of (*E*)-**28** (0.30 g, 0.9 mmol) in 25 mL of CCl_4 was added *N*-bromosuccinimide (0.32 g, 1.8 mmol) and a catalytic amount of 2,2′-azobis[2-methylpropionitrile]. The mixture was refluxed for 45 min, cooled, and filtered. Bromo compound **29**, obtained upon evaporation of the filtrate and used without purification, was refluxed in 20 mL of HOAc with anhydrous KOAc (0.35 g, 3.6 mmol) for 3 h. The HOAc was removed in vacuo, and the resultant oil was combined with 20 mL of H_2O and extracted twice with CH_2Cl_2. The combined organic extracts were washed with 5% $NaHCO_3$ solution and H_2O and dried ($MgSO_4$). The product obtained, upon removal of solvent, was crystallized from MeOH/H_2O, affording 0.10 g (27%) of **30** as yellow crystals: mp 165–167 °C; IR (KBr) 1740 cm^{-1}; NMR ($CDCl_3$) δ 2.12 (s, 6 H, $OCOCH_3$), 3.84 (s, 6 H, OCH_3), 3.88 (s, 6 H, OCH_3), 5.16 (s, 4 H, CH_2), 6.91 (s, 2 H, =CH), 7.15 (s, 2 H, Ar H), 7.42 (s, 2 H, Ar H). Anal. ($C_{24}H_{28}O_8$) C, H.

(*E*)-1,2-Bis(2,5-dimethoxy-*m*-tolyl)ethene (31). To a solution of bromobenzene (3.49 g, 22 mmol) in 200 mL of anhydrous Et_2O under an argon atmosphere was added Li wire (0.35 g, 0.05 mol). The mixture was stirred at room temperature for 30 min and decanted. Phosphonium chloride **17** (10.23 g, 22 mmol) was added to the decanted solution in small portions, and the mixture was stirred for 4 h at room temperature. Aldehyde **18** (4.0 g, 22 mmol) in 200 mL of Et_2O was added to the stirred mixture. After the mixture was refluxed for 2 days, excess phenyllithium was decomposed by the addition of H_2O. The mixture was filtered, the precipitate was washed with Et_2O, and the combined filtrates were dried (Na_2SO_4). Removal of solvent and crystallization of the resulting white solid from cyclohexane afforded 5.5 g (76%) of **31**: mp 138–140 °C; IR ($CHCl_3$) 1590, 1465, 970 cm^{-1}; NMR ($CDCl_3$) δ 2.30 (s, 6 H, CH_3), 3.72 (s, 6 H, OCH_3), 3.82 (s, 6 H, OCH_3), 6.68 (d, 2 H, J = 2.9 Hz, Ar H), 7.02 (d, 2 H, J = 3.2 Hz, Ar H), 7.36 (s, 2 H, =CH); MS, m/e 328 (M^+).

(*E*)-1,2-Bis[3-[(acetyloxy)methyl]-2,5-dimethoxyphenyl]ethene (33). Method A. A solution of **31** (0.7 g, 2.13 mmol), *N*-bromosuccinimide (0.76 g, 4.27 mmol), and a catalytic amount of benzoyl peroxide in 100 mL of CCl_4 was refluxed for 1 h, cooled, and filtered. The precipitate was washed with CCl_4, and the combined filtrates were evaporated to dryness in vacuo. The pale yellow solid was dissolved in 50 mL of HOAc containing NaOAc (0.33 g, 4.02 mmol) and refluxed for 3 h. The solvent was removed in vacuo, and the residue was chromatographed on silica gel using CH_2Cl_2 as eluant. Colorless solid (0.19 g, 22%) thus obtained crystallized from EtOAc as white needles: mp 149–151 °C; IR (KBr) 1730, 1610, 1595, 1485, 955 cm^{-1}; NMR ($CDCl_3$) δ 2.13 (s, 6 H, $OCOCH_3$), 3.77 (s, 6 H, OCH_3), 3.84 (s, 6 H, OCH_3), 5.18 (s, 4 H, CH_2), 6.87 (d, 2 H, J = 3.1 Hz, Ar H), 7.15 (d, 2 H, J = 2.9 Hz, Ar H), 7.35 (s, 2 H, =CH). Anal. ($C_{24}H_{28}O_8$) C, H.

Method B. Li wire (1.75 g, 0.25 mol) was added to a stirred slurry of $TiCl_3$ (12.96 g, 84 mmol) in 150 mL of freshly distilled dimethoxyethane (DME) under N_2 and refluxed for 2 h. After the mixture was cooled, aldehyde **21** (5.0 g, 21 mmol) in 10 mL of DME was added. The mixture was refluxed for 16 h and cooled, and most of the unreacted Li was removed by using tweezers. The mixture was filtered, the solids were washed thoroughly with DME, and the combined filtrates were concentrated in vacuo to approximately 50 mL. The concentrate was diluted with an equal volume of H_2O and extracted thoroughly with CH_2Cl_2. The organic phase was washed with H_2O and saturated brine and dried (Na_2SO_4). Evaporation of the dried extract afforded pale yellow, crystalline (*E*)-**33** (3.4 g, 73%), having physicochemical properties identical with compound (*E*)-**33** obtained from method A.

(*Z*)- and (*E*)-1,2-Bis[3-[(acetyloxy)methyl]-2,5-dimethoxyphenyl]ethene (33). NaH (5.56 g, 50% slurry, 116 mmol) was added to a stirred suspension of the phosphonium salt **23** (13.89 g, 29 mmol) in 100 mL of benzene at room temperature under a N_2 atmosphere. The resulting yellow mixture was refluxed for 2 h and cooled, and aldehyde **21** (6.9 g, 29 mmol) was added. Stirring and refluxing was continued overnight. After the mixture was cooled, excess NaH was decomposed by the addition of MeOH, and the mixture was poured over crushed ice and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried (Na_2SO_4). Concentration under reduced pressure gave a light brown oil (18.5 g). The oil was dissolved in 25 mL of pyridine, to which 20 mL of Ac_2O was added. The mixture was allowed to stir at room temperature overnight, poured over ice–H_2O, and extracted with CH_2Cl_2. The organic phase was washed with H_2O and dried (Na_2SO_4). The crude acetylated product (20.5 g) obtained upon solvent removal was chromatographed over silica gel. The paraffin oil present in the product (carried from the original unwashed NaH slurry) was removed by eluting with hexane. Elution with EtOAc/hexane (12:88) afforded (*Z*)-**33** as a colorless liquid (7.20 g, 56%), which analyzed correctly in the absence of distillation. Further elution yielded a mixture of (*Z*)- and (*E*)-olefins (1.80 g) in which the *Z* isomer predominated. *Z* isomer: IR (CCl_4) 1750, 1610, 1475, 1230 cm^{-1}; NMR ($CDCl_3$) δ 2.12 (s, 6 H, $OCOCH_3$), 3.55 (s, 6 H, OCH_3), 3.80 (s, 6 H, OCH_3), 5.14 (s, 4 H, CH_2), 6.69 (d, 2 H, J = 2.9 Hz, Ar H), 6.79 (d, 4 H, J = 2.9 Hz, Ar H and =CH); MS, m/e 444 (M^+). Anal. ($C_{24}H_{28}O_8$) C, H. Pure (*E*)-**33** (0.63 g, 5%) was eluted last with EtOAc/hexane (13:87) and was identical with previously described material. Chromatographic mixtures of (*Z*)- and (*E*)-**33** when triturated with benzene–hexane (1:1) yielded (*Z*)-**33** in solution and (*E*)-**33** as a crystalline solid.

1,2-Bis[3-[(acetyloxy)methyl]-2,5-dimethoxyphenyl]ethane (34). To a solution of (*Z*)-olefin **33** (5.0 g, 11.2 mmol) in 40 mL of anhydrous THF and 80 mL of absolute EtOH at 0 °C was added anhydrous $CoCl_2$ (1.45 g, 11.2 mmol). Solid $NaBH_4$ (1.07 g, 28.3 mmol) was added to the stirred mixture under N_2. After 10 min, the ice bath was removed, and the reaction was stirred at room temperature overnight and poured over 100 mL of cold 3 N HCl solution. The pink solution was extracted with CH_2Cl_2, which was washed with H_2O and dried (Na_2SO_4). Concentration under reduced pressure afforded 5.0 g (100%) of **34** as a colorless solid, which was crystallized from EtOAc/hexane, affording needles: mp 100–102 °C; IR (KBr) 1730, 1615, 1600, 1480 cm^{-1}; NMR ($CDCl_3$) δ 2.12 (s, 6 H, $OCOCH_3$), 2.92 (s, 4 H, CH_2), 3.76 and 3.77 (2 overlapping s, 12 H, OCH_3), 5.16 (s, 4 H, OCH_2), 6.76 (s, 4 H, Ar H); MS, m/e 446 (M^+). Anal. ($C_{24}H_{30}O_8$) C, H.

1,2-Bis[3-(hydroxymethyl)-2,5-dimethoxyphenyl]ethane (35). To an ice-cold and stirred suspension of diacetate **34** (2.0 g, 4.5 mmol) in 50 mL of MeOH was added dropwise a solution of NaOH (0.4 g, 10 mmol), H_2O (1 mL), and MeOH (10 mL). The resulting solution was stirred at room temperature overnight, concentrated in vacuo, and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated in vacuo, affording 1.55 g (96%) of colorless solid. Crystallization from EtOAc/hexane gave light needles: mp 112–114 °C; IR (KBr) 3300 (br), 3210 (br), 1605, 1470 cm^{-1}; NMR ($CDCl_3$) δ 2.05–2.32 (br m, 2 H, OH, D_2O exchangeable), 2.90 (s, 4 H, CH_2), 3.76 and 3.77 (2 overlapping s, 12 H, OCH_3), 4.72 (s, 4 H, OCH_2), 6.70 (d, 2 H, J = 3.2 Hz, Ar H), 6.79 (d, 2 H, J = 2.9 Hz, Ar H); MS, m/e 362 (M^+). Anal. ($C_{20}H_{26}O_6$) C, H.

1,2-Bis[3-(bromomethyl)-2,5-dimethoxyphenyl]ethane (36). PBr_3 (0.77 g, 2.8 mmol) was added dropwise to a cooled (0 °C) and stirred solution of **35** (1.27 g, 3.5 mmol) in 25 mL of anhydrous THF. After 10 min, the reaction was allowed to warm to room temperature and stirred overnight. Standard workup with CH_2Cl_2 and solvent removal in vacuo afforded crude **36** as a sticky colorless solid. Chromatography over silica gel with CH_2Cl_2 as the eluting solvent gave pure **36** (1.45 g, 85%), which crystallized as light flakes from EtOAc: mp 158–161 °C; IR (KBr) 1610, 1590, 1480 cm^{-1}; NMR ($CDCl_3$) δ 2.90 (s, 4 H, CH_2), 3.76 (s, 6 H, OCH_3), 3.85 (s, 6 H, OCH_3), 4.56 (s, 4 H, CH_2Br), 6.71 (d, 2 H, J = 3.2 Hz, Ar H), 6.79 (d, 2 H, J = 2.9 Hz, Ar H); MS, m/e 488 (M^+). Anal. ($C_{20}H_{24}O_4Br_2$) C, H, Br.

***trans*-1,2-Bis(2,5-dimethoxy-*p*-tolyl)cyclopropane (37).** A mixture of freshly distilled EtI (52.0 g, 0.33 mol) and Zn–Cu[47] (23.3 g, 0.33 mol) in 300 mL of anhydrous THF was stirred at room temperature overnight. The supernatant was decanted, and to this clear liquid was added an I_2 crystal and CH_2I_2 (70.0 g, 0.26 mol) freshly distilled over Cu wire. The mixture was refluxed for 1 h and cooled to room temperature, and a solution of (*E*)-**28** (7.3 g, 0.022 mol) in 50 mL of dry THF was added. The mixture was refluxed for 48 h, cooled, and filtered. The solids were washed with THF, and the combined filtrates were concentrated in vacuo to dryness. Et_2O (250 mL) was added, and the resulting solution was washed successively with dilute HCl, 5% $NaHCO_3$, 10% $Na_2S_2O_3$, and brine solutions. Following drying ($MgSO_4$), the solution was concentrated in vacuo, and the oily product was distilled (Kugelrohr) to remove EtI. The yellow residue solidified and was crystallized from cyclohexane, affording 3.5 g (46%) of *trans*-**37** as colorless crystals: mp 127–129 °C; IR ($CHCl_3$) 3030, 1515, 1475 cm^{-1}; NMR ($CDCl_3$) δ 1.29 (m, 2 H, CH_2), 2.21 (s, 6 H, CH_3), 2.39 (m, 2 H, CH), 3.79 (s, 6 H, OCH_3), 3.80 (s, 6 H, OCH_3), 6.58 (s, 2 H, Ar H), 6.68 (s, 2 H, Ar H); MS, m/e 342 (M^+). Anal. ($C_{21}H_{26}O_4$) C, H.

2,2′-Ethylenebis[6-(hydroxymethyl)-*p*-benzoquinone] Diacetate (3b). Ceric ammonium nitrate (2.7 g, 4.92 mmol) in 5 mL of H_2O was added dropwise to an ice-cold, stirred solution of **34** (0.5 g, 1.12 mmol) in 15 mL of acetonitrile. The resulting reddish solution was stirred at room temperature for 30 min and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated in vacuo, affording 0.43 g (99%) of yellow solid, which crystallized from EtOAc, affording yellow needles: mp 179–181 °C dec; IR (KBr) 1745, 1665, 1615 cm^{-1}; NMR ($CDCl_3$) δ 2.17 (s, 6 H, $OCOCH_3$), 2.67 s, 4 H, CH_2), 4.99 (d, 4 H, J = 1.6 Hz, OCH_2), 6.58 (d, 2 H, J = 2.5 Hz, =CH), 6.66 (dd, 2 H, J = 4.5 and 1.9 Hz, =CH); MS, m/e 326 (M - 60). Anal. ($C_{20}H_{18}O_8$) C, H.

2,2′-Ethylenebis[6-(bromomethyl)-*p*-benzoquinone] (3c). Bromo compound **36** (0.85 g, 1.7 mmol) was dissolved in 50 mL of hot acetonitrile. Following cooling to the temperature prior to cloudiness, $Ce(NH_4)_2(NO_3)_6$ (6.37 g, 11.6 mmol) in 8 mL of H_2O was added dropwise. Stirring was continued for 10 min after the addition was complete. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic extract was washed with H_2O and dried (Na_2SO_4), and the solvent was removed in vacuo, affording a dark brown solid (0.75 g). Trituration with a small volume of EtOAc afforded **3c** as a yellow-brown solid (0.62 g, 83%). Crystallization from EtOAc/hexane gave yellow-brown needles: mp 146–147 °C; IR (KBr) 1650, 1635, 1615 cm^{-1}; NMR ($CDCl_3$) δ 2.72 (s, 4 H, CH_2), 4.25 (d, 4 H, J = 1.0 Hz, CH_2Br), 6.59 (d, 2 H, J = 2.2 Hz, =CH), 6.85 (m, 2 H, =CH); MS, m/e 348 (M - 80). Anal. ($C_{16}H_{12}O_4Br_2$) C, H, Br.

***trans*-2,2′-Cyclopropylenebis[5-methyl-*p*-benzoquinone] Tetrakis(dimethyl acetal) (39).** To a solution of **37** (0.1 g, 0.29 mmol) in 10 mL of THF held in a jacketed beaker was added 50 mL of 1% KOH in MeOH. The stirred solution was electrolyzed [33 (diameter) × 28 mm (high) Pt gauze anode and 8 × 8 mm Pt sheet cathode] at 1.0–1.6 V relative to a Pt electrode. The temperature was held below 30 °C by circulating cold H_2O through the jacket. The current ranged from an initial maximum of 1.0 A to a minimum of 0.07 A toward the conclusion of the electrolysis. The reaction was monitored by the disappearance of UV absorption at 297 nm and found to be complete within approximately 40 min (71% current efficiency; electrolysis could also be performed at constant current with equally good results). The pale yellow solution was concentrated in vacuo, diluted with H_2O, and extracted with Et_2O. The nearly colorless organic layer was washed with H_2O and dried (Na_2SO_4). Removal of solvent under reduced pressure resulted in virtually quantitative yield of the quinone bis(ketal) **39** (0.14 g) as a viscous pale yellow liquid: NMR ($CDCl_3$) δ 1.13 (m, 2 H, CH_2), 1.81 (a doublet overlapping a multiplet, 8 H, $J_{doublet}$ = 1.3 Hz, CH_3 and CH), 3.16, 3.19, and 3.24 (s, 24 H, OCH_3), 5.60 (s, 2 H, =CH), 5.81 (d, 2 H, J = 1.3 Hz, =CH).

Bis(ketal) **39** could not be purified further due to instability under chromatographic conditions. For example, following chromatography of **39** on neutral alumina using EtOAc/hexane

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(5:95), only the symmetrical bis(keto)–bis(dimethyl acetal) was obtained. Recrystallization of this partially hydrolyzed material from EtOAc afforded pale yellow prisms: mp 176–179 °C; IR (KBr) 1680, 1640 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (m, 2 H, CH_2), 1.95 (d, 6 H, J = 1.6 Hz, CH_3), 2.09 (m, 2 H, CH), 3.21 (s, 6 H, OCH_3), 3.26 (s, 6 H, OCH_3), 5.91 (s, 2 H, =CH), 6.50 (partly resolved q, 2 H, J = 1.6 Hz, =CH). Anal. ($C_{21}H_{26}O_6$) C, H.

***trans*-2,2′-Cyclopropylenebis[5-methyl-*p*-benzoquinone] (1a).** Octamethoxy compound **39** (0.25 g, 0.54 mmol) in 10 mL of acetone was treated with 1 mL of 0.5 N HCl solution at room temperature. After stirring for 10 min, the yellow solution was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated in vacuo, affording 0.17 g of brown solid. Trituration several times with EtOAc provided 0.05 g (34%) of pure **1a** as a bright yellow solid. Crystallization (EtOAc) furnished microcrystals: mp 159–160 °C dec; IR (KBr) 1650 (multiple shoulders), 1630, 1610 cm^{-1}; NMR ($CDCl_3$) δ 1.51 (m, 2 H, CH_2), 2.05 (d, 6 H, J = 1.6 Hz, CH_3), 2.30 (m, 2 H, CH), 6.31 (s, 2 H, =CH), 6.62 (partly resolved q, 2 H, J = 1.6 Hz, =CH); MS, m/e 282 (M^+). Anal. ($C_{17}H_{14}O_4$) C, H.

Biology. Studies with Nude Mice. Four-week-old, random-mated, homozygous, nude (NU/NU), female, athymic mice were obtained from the National Cancer Institute. Each mouse received 0.5 mL of rabbit anti-mouse thymocyte serum injected subcutaneously. Seven days later the mice were inoculated subcutaneously with 1×10^7 D98/HR1 cells in phosphate-buffered saline. Two weeks later, a second injection of 0.5 mL of rabbit anti-mouse thymocyte serum was given. Administration of drug began when the tumors were approximately 2–3 mm in diameter or when the number of mice was sufficient to establish groups, and administration continued for 14 days. The drug dosage was based on milligrams per kilogram of body weight of each mouse, determined by individual weighing every day. Drugs were administered intraperitoneally as a suspension in 0.3% Klucel at a volume of 0.01 mL/g of body weight. One group of mice studied received only the Klucel. Tumor measurements were taken the 1st day of drug therapy and weekly thereafter for 4 weeks. Tumor mass was obtained by measuring the tumor along the longitudinal and transverse axis of the animal and the height of the tumor above the back. Mean tumor size was determined for each group and statistically evaluated over time.

The mice were divided into five groups: group I (n = 5) were administered **3b**, 5 mg/kg; group II (n = 4) were administered **3b**, 20 mg/kg; group III (n = 3) were injected with Klucel as control; group IV (n = 5) were injected with **3c**, 5 mg/kg; and group V (n = 3) were injected with **3c**, 20 mg/kg. Results of drug treatment are shown in Figure 1 and Table I.

Investigations in Vitro. Determinations in vitro were conducted with the D98/HR1 cell line.[11,48,49] The clonogenic assay system was a modified procedure of that used by the Southwest Oncology Group for primary tumor tissue.[44,50] Drug preparations (except quinones **3b,c**) at appropriate stock concentrations were made in sterile distilled water and stored at −70 °C in 0.2-mL aliquots. Quinones were first dissolved in dichloromethane (2.5 μg/mL), diluted further in 95% ethanol, and finally diluted to the appropriate concentration in warm (37 °C) McCoy's 5A medium. Indicator cells (D98/HR1) were grown as monolayers as reported earlier,[49] and cell suspensions were prepared from 3–4-day-old cultures. The cell concentration was adjusted to 4.0×10^5/mL and exposed to antitumor drugs for 1 h at 37 °C. Following incubation, the treated cell suspensions were washed twice with Eagles spinner medium containing 10% fetal bovine serum, resuspended in plating medium, and overlaid on a 3-mL agar base in 60-mm plastic petri dishes (6.7×10^4 cells/plate).

The agar base contained 5% horse serum, 10% fetal calf serum, 0.22 mg/mL of sodium pyruvate, 42 μg/mL of L-serine, 2 mM glutamine, 50 μg/mL of Gentamycin, and 0.5% washed agar in McCoy's 5A medium. Plating medium (1.5 mL/dish) contained 10% horse serum, 1.5 U/mL of insulin, 0.2 mM ascorbic acid, 1.5 mM glutamine, 50 μg/mL of asparagine, 200 μg/mL of DEAE-dextran, and 50 μg/mL of Gentamycin in CMRL 1066 medium with 0.3% washed agar. Plates were incubated at 37 °C in 4.0% CO_2 in air for 10 days and examined for colony formation.

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Registry No. *trans*-**1a**, 87050-85-9; **3b**, 87050-82-6; **3c**, 87050-83-7; **4**, 87050-61-1; **5**, 87050-62-2; **6**, 87050-63-3; **9**, 4925-88-6; **12**, 32378-21-5; **13**, 25224-33-3; **14**, 21893-97-0; **15**, 5600-82-8; **16**, 87050-64-4; **17**, 87050-65-5; **18**, 5548-30-1; **19**, 78840-04-7; **20**, 87050-66-6; **21**, 87050-68-8; **22**, 87050-69-9; **23**, 87050-70-2; **24**, 25224-72-0; **25**, 87050-67-7; (*E*)-**28**, 21071-42-1; (*Z*)-**28**, 87050-71-3; (*E*)-**29**, 87050-72-4; (*E*)-**30**, 87050-73-5; (*E*)-**31**, 87050-74-6; (*E*)-**31** brominated, 87050-75-7; (*E*)-**33**, 87050-76-8; (*Z*)-**33**, 87050-77-9; **34**, 87050-78-0; **35**, 87050-79-1; **36**, 87050-80-4; *trans*-**37**, 87050-81-5; *trans*-**39**, 87050-84-8; 4-bromo-2,5-dimethoxybenzaldehyde, 31558-41-5.

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