Mono and Bis(bioreductive) Alkylating Agents: Synthesis and Antitumor Activities in a B16 Melanoma Model

Donald T. Witiak,* John T. Loper, Subramaniam Ananthan, Anna Maria Almerico, Vernon L. Verhoef, and Joyce A. Filppi

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, and Adria Laboratories, Division of Erbemont Inc., Columbus, Ohio 43216. *Received July 22, 1988*

Several potentially bis(alkylating) bis(quinones) (**3–5**) and 1,4- and 1,3-bis(alkylating) monoquinones (**6–13**) belonging to general structure 2,2′-ethylenebis[5-[(leaving group)methyl]-1,4-benzoquinone] (**3–5**) and 2,5- and 2,6-bis[(leaving group)methyl]-1,4-benzoquinone water-soluble and -insoluble classes were prepared by oxidative demethylation of the corresponding tetramethoxydiphenylethanes (**17–19**) and dimethoxybenzenes (**24, 27, 36–39**), respectively. Methods employed for the preparation of tetramethoxydiphenylethane intermediates involved (1) arylmethyl bromide coupling and (2) catalytic hydrogenation of stilbene intermediates derived via Wittig reaction of (arylmethyl)phosphonium salts with aryl aldehydes. However, in biological investigations using a subcutaneous B16 (hypoxic) melanoma tumor in BDF_1 hybrid mice with cyclophosphamide as positive control the most interesting series of structurally related analogues were the potentially monoalkylating monoquinones of the 2-[(leaving group)methyl]-1,4-benzoquinone type (i.e., **14** and **15**) having water-insoluble (acetoxy) and water-solubilizing (succinate) groups. Serial measurements of tumor size, and evaluation of increased life span, in response to drug treatment also revealed potentially 1,4-bis(alkylating) (bromomethyl)-1,4-quinone **7** and 1,3-bis(alkylating) (hydroxymethyl)-1,4-quinone **10** to have variable activity, but none of the potentially bis(alkylating) bis(quinones) showed antitumor properties in this model.

Selective bioreductive activation of the alkylating potential of certain synthetic and naturally occurring quinones has been implicated in the antitumor activity of such compounds.[1] This has generated considerable interest in the design of quinonoid targets as hypoxic cell selective antitumor agents. Lin et al.[2] have synthesized and evaluated a series of halomethyl and (acyloxy)methyl quinones. Our earlier work[3] in this area explored the design and synthesis of bis(quinones) as targets having the potential for bis(bioreductive) alkylation leading to interstrand cross-linking of DNA or intra- and interstrand cross-linking of macromolecules in general. Indeed, the bis(quinones) **1** and **2** exhibited selective antitumor properties in a nude mouse carcinoma model.

The present investigations were aimed at assessing the effect of certain structural modifications such as (a) the nature of the leaving group, (b) the water solubility of the molecule, (c) the 1,3 or 1,4 disposition of two alkylating functions on monoquinones, and (d) the 1,3 or 1,4 disposition of an alkylating function and ethane spacer group in bis(quinones), since changes in the structural and physicochemical parameters induced by such modifications could be critical for antitumor activity. The biological evaluation of the mono- and bis(quinones) was performed by using subcutaneous B16 melanoma in BDF_1 hybrid mice. This syngenic tumor model allows serial measurements of tumor size, as well as evaluation of increased life span, in response to drug treatment. Since the hypoxic fraction of B16 melanoma increases with tumor growth,[4] the mono- and bis(quinones) were administered only after the tumors had grown to a palpable size. This report details the synthesis of bis(alkylating) bis(quinone) targets **3–5**, bis- and monoalkylating monoquinones **6–16** along with our biological findings.

Chemistry

Synthetic strategies for the preparation of bis(alkylating) bis- (**3–5**) and mono- (**6–13**) quinone targets (see Chart I) utilized oxidative demethylation of the corresponding tetramethoxydiphenylethanes (**17–19**) (see Chart II) and dimethoxybenzenes (**24–27, 36–39**) (see Chart III), respectively. Two complementary approaches were developed for the synthesis of bis(alkylating) bis(quinone) targets via the tetramethoxydiphenylethane intermediates **18**, **20**, and **21**, the latter of which served as a precursor to **17** and **19**. One method employed coupling of an arylmethyl bromide, and a second employed catalytic hydrogenation of stilbene intermediates accessible through Wittig reaction of an (arylmethyl)phosphonium salt with an aryl aldehyde. The former approach provided facile access to key symmetric intermediates such as diarylethane **22**, easily convertible to chloromethyl (**20**) and hydroxymethyl (**21**) intermediates wherein each ring bears identical substituents. The second approach provided greater flexibility and is anticipated to be applicable for the synthesis of diarylethanes having different substituents and/or different substitution patterns. Reaction of 2,5-dimethoxybenzyl bromide with low-valent vanadium,[5] Mg in THF,[6] or MeMgBr[7] produced tetramethoxydiphenylethane (**22**)[6,8-12] in 61, 69, and 26% yields, respectively. Whereas

* Address correspondence to this author at The Ohio State University.

(1) (a) For a review of pioneering work in this area see: Sartorelli, A. C. *Cancer Res.* **1988**, *148*, 775–778. (b) Moore, H. W. *Science* **1977**, *197*, 527–532. (c) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249–280. (d) Moore, H. W.; Czerniak, R.; Hamdan, A. *Drugs Exp. Clin. Res.* **1986**, *12*, 475–494.

(2) (a) Lin, A. J.; Cosby, L. A.; Shansky, C. W.; Sartorelli, A. C. *J. Med. Chem.* **1972**, *15*, 1247–1252. (b) Antonini, I.; Lin, T. S.; Cosby, L. A.; Dai, Y. R.; Sartorelli, A. C. *J. Med. Chem.* **1982**, *25*, 730–735. (c) Lin, A. J.; Cosby, L. A.; Sartorelli, A. C. In *Cancer Chemotherapy*; Sartorelli, A. C., Ed.; ACS Symp. Ser. 30; American Chemical Society: Washington, DC, 1976; pp 71–86.

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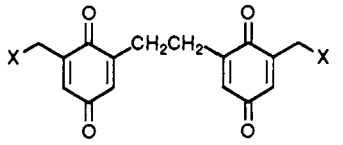
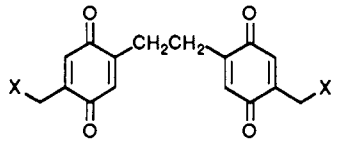
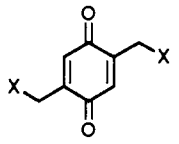
Chart I



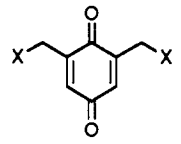
1: X = Br
2: X = OAc



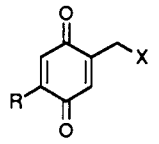
3: X = Br
4: X = OAc
5: X = $OCO(CH_2)_2CO_2H$



6: X = Cl
7: X = Br
8: X = OAc
9: X = $OCO(CH_2)_2CO_2H$

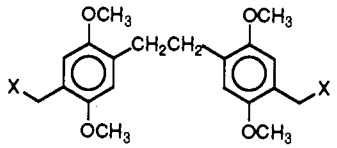


10: X = OH
11: X = Br
12: X = OAc
13: X = $OCO(CH_2)_2CO_2H$

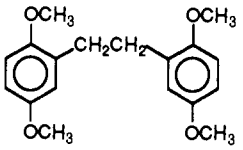


14: R = H; X = OAc
15: R = H; X = $OCO(CH_2)_2CO_2H$
16: R = CH_3O; X = OAc

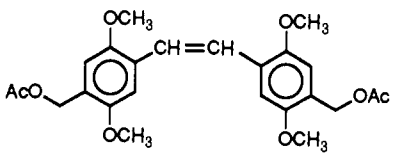
Chart II



17: X = Br
18: X = OAc
19: X = $OCO(CH_2)_2CO_2H$
20: X = Cl
21: X = OH

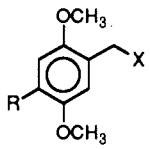


22

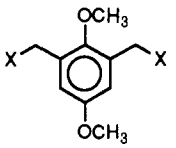


23

Chart III



24: R = CH_2Cl; X = Cl
25: R = CH_2Br; X = Br
26: R = CH_2OAc; X = OAc
27: R = $CH_2OCO(CH_2)_2CO_2H$; X = $OCO(CH_2)_2CO_2H$
28: R = CH_2OH; X = OH
29: R = CHO; X = OH
30: R = CHO; X = OAc
31: R = CH_2Cl; X = PPh_3Cl
32: R = CH_2OAc; X = PPh_3Cl
33: R = H; X = $OCO(CH_2)_2CO_2H$
34: R = CH_3O; X = OH
35: R = CH_3O; X = OAc



36: X = OH
37: X = Br
38: X = OAc
39: X = $OCO(CH_2)_2CO_2H$

$TiCl_4$-catalyzed chloromethylation of **22** with $ClCH_2OCH_3$[7] failed, reaction with formalin and HCl in dioxane[13] yielded bis(chloromethyl) derivative **20** (93%). Hydrolysis (aqueous Na_2CO_3) afforded bis(alcohol) **21** (81%), and conversion with PBr_3 produced bis(bromomethyl) compound **17** (93%). Acylation [succinic anhydride/Et_3N/4-(dimethylamino)pyridine (DMAP)] yielded bis(succinate) **19** (87%).

Alternatively, known[13] bis(chloromethyl) derivative **24** underwent reaction with NaOAc in refluxing AcOH, producing bis(acetoxymethyl) intermediate **26** (95%). Hydrolysis afforded bis(alcohol) **28** (94%), and controlled oxidation [2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)/CH_2Cl_2] at −5 to 0 °C gave monoaldehyde **29** (83%). At higher temperatures or with use of different oxidizing agents (MnO_2 or pyridinium dichromate) mixtures of mono- and bis(aldehydes) were obtained. Acetylation of **29** afforded **30** (91%), which was coupled with the ylide derived from monophosphonium salt **31** prepared in 94% yield from bis(chloromethyl) compound **24**. Insolubility of the monophosphonium salt in the reaction medium likely prevents formation of the undesired bis(phosphonium) derivative. The mixture of (*Z*)- and (*E*)-stilbenes (**23**, 54%) was separated by column chromatography (silica gel/EtOAc–hexane), and the *Z* isomer [UV (MeOH) λ_{max} 330 nm], isolated in 44% yield, but not the *E* isomer [UV (MeOH) λ_{max} 360 nm], produced the desired bis(acetoxymethyl) intermediate **18** (83%) upon catalytic hydrogenation (PtO_2/AcOH/H_2/40 psi). Acetate hydrolysis yielded bis(alcohol) **21** identical in all respects with the previously prepared material.

For dimethoxybenzene precursors (**24–27** and **36–39**), to the 1,4-bis(alkylating) and 1,3-bis(alkylating) monoquinones (**6–9** and **10–13**, respectively), 1,4-dimethoxybenzene and *p*-methoxyphenol, respectively, served as starting materials. In the 1,4-bis(alkylating) series bis(hydroxymethyl) compound **28** (94%) was treated with PBr_3 to yield the bis(bromomethyl) intermediate **25** (82%). Acylation of **28** with succinic anhydride produced the alkali-soluble bis[(succinyloxy)methyl] derivative **27** (90%). For the 1,3-bis(alkylating) series, hydroxymethylation of *p*-methoxyphenol followed by methylation produced the known[3] bis(hydroxymethyl) species **36**. Treatment with PBr_3, Ac_2O, or succinic anhydride yielded bis(bromomethyl), bis(acetoxymethyl), or bis[(succinyloxy)methyl] species **37** (56%), **38** (74%), or **39** (94%), respectively.

Tetramethoxy (**17–19**) and dimethoxy (**24–27** and **36–39**) arenes were oxidized to bis- (**3–5**) and mono- (**6–13**) quinone bis(alkylating) targets by using either $Ce(NH_4)_2(NO_3)_6$ in aqueous MeCN or HNO_3 in AcOH in 25–80% yields. Monoalkylating (acetoxymethyl)quinones 14[2a] and **16** and [(succinyloxy)methyl]quinone **15** were prepared for comparative biological studies. Quinone **15** was obtained from 2,4-dimethoxybenzyl alcohol in 57% overall yield, and

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Table I. Biological Data in B16 Melanoma Mice

no.	max dose of test compd, mg/kg	antitumor efficacy at opt dose vs B16 melanoma in mice[a]			
		% T/C, mg/kg		% tumor growth inhibition, mg/kg	
		test compd	cyclophosphamide	test compd	cyclophosphamide
3	10	102 (10)	123 (10)	16 (10)	50 (10)
4	10	129 (5)	148 (10)	16 (5)	34 (10)
5	10	123 (10)	123 (10)	39 (10)	50 (10)
	10	106 (10)	115 (20)	6 (2.5)	43 (5)
6	10	104 (5)	149 (5)	4 (10)	65 (10)
7	10	140 (5)	148 (10)	20 (5)	34 (10)
	10	118 (5)	129 (20)	30 (2.5)	47 (5)
9	10	115 (2.5)	123 (10)	38 (2.5)	50 (10)
	10	111 (10)	115 (20)	19 (2.5)	43 (5)
10	20	171 (10)	182 (5)	36 (20)	24 (20)
	10	90 (2.5)	130 (20)	19 (2.5)	23 (10)
11	10	105 (2.5)	197 (20)	24 (2.5)	57 (20)
12	10	100 (2.5)	149 (5)	13 (10)	65 (10)
13	20[b]	110 (5)	131 (30)	23 (10)	33 (30)
	20	130 (20)	130 (20)	29 (20)	23 (10)
14	20[c]	142 (2.5)	182 (5)	63 (2.5)	24 (20)
	4	144 (2)	129 (20)	31 (2)	47 (5)
15	20[b]	115 (10)	131 (30)	19 (10)	33 (30)
	10	164 (2.5)	197 (20)	27 (2.5)	57 (20)
	10	130 (10)	130 (20)	32 (10)	23 (10)
16	10[d]	110 (2.5)	148 (10)	0 (5)	34 (10)

[a] Mice were injected sc with 0.5 mL tumor brei (1:10 dilution, w/v) and then treated ip for 9 consecutive days after the tumor grew to a palpable size. T/C = median survival time of treated mice/control mice = antitumor efficacy and must be ≥140 for an active result. Percent tumor growth inhibition = 100 − [(change in average tumor diameter treated mice/control mice) × 100] = antitumor efficicay and must be ≥25% for an active result. [b] Compound was injected iv rather than ip in this study. [c] Compound 14 was toxic ($T/C \leq 85\%$) at doses ranging from 5 to 20 mg/kg. [d] Compound 16 was toxic ($T/C \leq 85\%$) at 10 mg/kg.

16 was prepared from alcohol **34**[14] via acetate **35** in 58% overall yield.

Biological Results and Discussion

Antitumor screening results for the mono- and bis(quinone) compounds **3–16** against sc implanted B16 melanoma in mice are compared in Table I. Two parameters were used to monitor drug efficacy—median life span (% T/C) and percent tumor growth inhibition.

Although none of the test compounds were as active as cyclophosphamide against sc B16 melanoma, several showed modest activity. The most interesting series of structurally related compounds were the potentially monoalkylating monoquinones **14–16**. Acetoxy analogue **14** was active (maximum % T/C = 142–144 and maximum tumor growth inhibition = 31–63%) in duplicate tests when administered ip in nine daily doses at 2 or 2.5 mg/kg. Compound **15**, a water-soluble succinate analogue of acetate ester **14**, also showed ip activity (% T/C = 164 at 2.5 mg/kg) but was not active when administered by the iv route. In contrast, methoxy-substituted species **16** showed no antitumor activity. At a high dose (10 mg/kg) acetoxy ether **16** was toxic. Analogue **16** differs from compound **14** by addition of a methoxy group at the 4-position of the quinone, and this simple substitution selectively interferes with the antitumor activity of this series.

The potentially bis(alkylating) monoquinones **7** and **10** showed variable activity. Intrinsic variability was also observed with cyclophosphamide in this model; however, this variability was always taken into account by the inclusion of the positive control. In single tests each drug (**7** or **10**) significantly increased the life span (% $T/C \geq 140$) of tumor-bearing animals (Table I). However, in view of the lack of activity of these compounds in a duplicate test and the lack of activity of their structurally related water-insoluble and -soluble analogues with varying leaving groups (compounds **6**, **9** and **11–13**), it would seem that further work in these series likely will not produce compounds of therapeutic value in the B16 melanoma model. The potentially bis(alkylating) water-insoluble and -soluble types of bis(quinones) **3–5** also showed no antitumor activity in this model.

Compound **14** was further tested in a murine leukemia model P388 on a day 1 ip schedule. This potentially monoalkylating monoquinone, although active against B16 melanoma (Table I), did not significantly increase the life span of P388-bearing mice at doses of 5 or 10 mg/kg. High doses of compound **14** (20 and 40 mg/kg) showed marked toxicity including weight loss and early deaths compared to untreated leukemic mice (data not shown). The reason for the lack of activity for compound **14** against P388 leukemia is not clear but could be related to dosing schedule or to the intrinsic resistance of murine leukemic cells to this drug. One possibility involves the relatively high oxygen tension that likely exists in ascitic leukemia P388 cells versus B16 melanoma solid tumor. It is known that mitomycin C, a well-characterized compound that seems to require bioreductive activation, can preferentially kill hypoxic tumor cells versus oxygenated cells.[15] The mono- and bis(quinones) may require a similar hypoxic environment for bioactivation, but structure–activity relationships are not straightforward. Thus, in a mouse human carcinoma model potentially bis(alkylating) bis(quinones) showed activity when the leaving groups were bromide, but not with acetoxy functions.[3] Further studies are under way to evaluate the effects of hypoxia on the cytocidal activity of potentially monoalkylating acetoxy quinone **14** and related quinone analogues.

Experimental Section

Chemistry. Melting points were determined in open capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected.

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Infrared spectra were recorded with a Beckman Model 4230 spectrophotometer. Proton magnetic resonance spectra were obtained on a Bruker HX-90E spectrometer or IBM AF-270 instrument. Unless otherwise specified, the spectra were obtained at 90 MHz. ^{13}C spectra were taken at 67.925 MHz on an IBM AF-270 instrument. Chemical shifts are reported in δ units relative to tetramethylsilane in $CDCl_3$. Mass spectra were recorded at 70 eV on a Kratos MS-30 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

2,2′-Ethylenebis[5-(bromomethyl)-1,4-benzoquinone] (3). To a stirred suspension of **17** (1.5 g, 0.31 mmol) in glacial AcOH (15 mL) was added dropwise concentrated HNO_3 (2.5 mL). The mixture was stirred for 1 h and diluted with H_2O. The precipitate was filtered and recrystallized from EtOAc/hexane to yield 0.06 g (48%) of **3** as a yellow solid: mp 179–182 °C; IR (KBr) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.67 (s, 4 H, CH_2CH_2), 4.23 (d, 4 H, CH_2Br, J = 1.3 Hz), 6.62 (s, 2 H, =CH), 6.88 (t, 2 H, =CH, J = 1.3 Hz). Anal. ($C_{16}H_{12}Br_2O_4$) C, H, Br.

General Procedure for the Preparation of Quinones by Oxidative Demethylation with Ceric Ammonium Nitrate. The procedure described for the preparation of **4** is typical of the method employed for the oxidative demethylations.

2,2′-Ethylenebis[5-(hydroxymethyl)-1,4-benzoquinone] Diacetate (4). To a stirred solution of **18** (1.75 g, 3.92 mmol) in CH_3CN (150 mL) was added dropwise a solution of $Ce(NH_4)_2(NO_3)_6$ (14.6 g, 26.6 mmol) in H_2O (50 mL). The mixture was stirred for an additional 10 min at room temperature. The mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4) and concentrated under reduced pressure. Recrystallization of the residue from CH_2Cl_2 afforded 0.97 g (64%) of **4** as tan flakes: mp 208–210 °C; IR (KBr) 1745, 1635, 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.16 (s, 6 H, $COCH_3$), 2.66 (s, 4 H, CH_2CH_2), 4.98 (d, 4 H, CH_2O, J = 1.9 Hz), 6.58 (s, 2 H, =CH), 6.68 (t, 2 H, =C—CH_2, J = 1.9 Hz). Anal. ($C_{20}H_{18}O_8$) C, H.

2,2′-Ethylenebis[5-(hydroxymethyl)-1,4-benzoquinone] Bis(hydrogen succinate) (5). The tetramethoxy compound **19** (1.23 g, 2.19 mmol) was dissolved in boiling CH_3CN (6.4 mL). The solution was rapidly cooled to room temperature and prior to crystallization of starting material was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (7.2 g, 13 mmol) in H_2O (3.2 mL). The reaction mixture was stirred for an additional 25 min and diluted with H_2O (100 mL). The precipitate was filtered, dried, and crystallized from AcOH to afford 0.75 g (68%) of **5** as a yellow crystalline solid: mp >200 °C dec; IR (KBr) 3440 (br), 3060–2920, 1745, 1710, 1335, 1155, 795 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.58 (s, 12 H, $COCH_2CH_2CO$ + $ArCH_2CH_2Ar$), 4.90 (d, 4 H, CH_2O), 6.65–6.80 (m, 4 H, =CH), 12.25 (br s, 2 H, OH); MS, m/e 384 (M^+ - 118). Anal. ($C_{24}H_{22}O_{12}$) C, H.

2,5-Bis(chloromethyl)-1,4-benzoquinone (6). A cold solution of **24** (1.0 g, 4.25 mmol) in CH_3CN (200 mL) was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (4.7 g, 8.5 mmol) in H_2O (20 mL). The mixture was stirred at room temperature for 30 min. Workup as described for **4** and purification of the crude product by column chromatography over silica gel (elution with CH_2Cl_2/hexane 9:5) followed by crystallization from CH_2Cl_2/hexane afforded 0.22 g (25%) of **6**: mp 97–99 °C (lit.[16] mp 102–104 °C); ^1H NMR ($CDCl_3$) δ 4.42 (d, 4 H, CH_2Cl, J = 1.6 Hz). Anal. ($C_8H_6Cl_2O_2$) C, H; Cl: calcd, 34.58; found, 34.05.

2,5-Bis(bromomethyl)-1,4-benzoquinone (7). Method I. A solution of **25** (0.1 g, 0.31 mmol) in CH_3CN (30 mL) was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (0.34 g, 0.62 mmol) in H_2O (2 mL). The mixture was stirred at room temperature for 30 min. Workup as described for **4** and purification of the crude product by column chromatography over silica gel (elution with CH_2Cl_2/hexane 3:2) afforded 0.03 g (30%) of **7** as a yellow solid: mp 123–126 °C; ^1H NMR ($CDCl_3$) δ 4.25 (d, 4 H, CH_2Br, J = 1.0 Hz), 6.93 (t, 2 H, =CH, J = 1.0 Hz). Anal. ($C_8H_6Br_2O_2$) C, H, Br.

Method II. To a stirred suspension of **25** (0.25 g, 0.77 mmol) in glacial AcOH (8.0 mL) was added concentrated HNO_3 (3.5 mL). The mixture was stirred for 1 h and then diluted with H_2O. The precipitate was collected by filtration and recrystallized from EtOAc/hexane to yield 0.12 g (53%) of **7**, identical in all respects with the product obtained by Method I.

2,5-Bis(acetoxymethyl)-1,4-benzoquinone (8). A cold solution of **26** (1.0 g, 3.54 mmol) in CH_3CN (25 mL) was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (7.8 g, 14.2 mmol) in H_2O (10 mL). The mixture was stirred at room temperature for 30 min. Workup as described for **4** and purification of the crude product by crystallization from EtOAc/hexane afforded 0.46 (52%) of **8**: mp 132–134.5 °C (lit.[17] mp 134 °C); ^1H NMR ($CDCl_3$) δ 2.17 (s, 6 H, $COCH_3$), 4.99 (d, 4 H, CH_2O, J = 1.9 Hz), 6.69 (t, 2 H, =CH, J = 1.9 Hz). Anal. ($C_{12}H_{12}O_6$) C, H.

2,5-Bis(hydroxymethyl)-1,4-benzoquinone Bis(hydrogen succinate) (9). To a solution of **27** (1.99 g, 5 mmol) in CH_3CN (20 mL) was added, dropwise and with stirring, a solution of $Ce(NH_4)_2(NO_3)_6$ (5.5 g, 10 mmol) in H_2O (20 mL). After stirring for 30 min at room temperature, the yellow solid was filtered, air-dried, and recrystallized from EtOAc to give 1.47 g (80%) of **9**: mp 187–188 °C; IR ($CHBr_3$) 2930 (br), 1750, 1700 (br), 1645 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.55 (m, 4 H, CH_2), 2.65 (m, 4 H, CH_2), 4.94 (d, 4 H, CH_2, J = 1.7 Hz), 6.74 (t, 2 H, =CH, J = 1.7 Hz), 12.33 (br s, 2 H, OH, D_2O exchangeable); ^{13}C NMR (Me_2SO-d_6) δ 28.58, 28.62, 59.06, 130.97, 143.04, 171.60, 173.28, 185.76 (2C); MS, m/e 350 (M^+ - 18). Anal. ($C_{16}H_{16}O_{10}$) C, H.

2,6-Bis(hydroxymethyl)-1,4-benzoquinone (10). To an ice-cold solution of **36** (1.98 g, 10 mmol) in CH_3CN (60 mL) was added, dropwise and with stirring, a solution of $Ce(NH_4)_2(NO_3)_6$ (10.96 g, 20 mmol) in H_2O (60 mL). After being stirred for 30 min at room temperature, the reaction mixture was extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated on a rotary evaporator to give 1.35 g (80%) of **10**: mp (EtOAc) 151–152 °C dec; IR ($CHBr_3$) 3460 (br), 3280 (br), 1660, 1620 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.34 (br s, 2 H, OH), 4.30 (br s, 4 H, CH_2), 6.61 (s, 2 H, =CH); ^{13}C NMR (Me_2SO-d_6) δ 56.88, 129.52, 149.21, 186.86, 187.50; MS, m/e 168 (M^+). Anal. ($C_8H_8O_4$) C, H.

2,6-Bis(bromomethyl)-1,4-benzoquinone (11). A solution of **37** (0.1 g, 0.31 mmol) in CH_3CN (30 mL) was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (0.69 g, 1.24 mmol) in H_2O (3 mL). The mixture was stirred at room temperature for 30 min. Workup as described for **4** and purification of the crude product by column chromatography over silica gel (elution with CH_2Cl_2/hexane 3:2) afforded 0.06 g (70%) of **11**: mp 55–58 °C; ^1H NMR ($CDCl_3$) δ 4.28 (s, 4 H, CH_2Br), 6.91 (s, 2 H, =CH). Anal. ($C_8H_6Br_2O_2$) C, H.

2,6-Bis(acetoxymethyl)-1,4-benzoquinone (12). A solution of **38** (3.0 g, 10.63 mmol) in CH_3CN (100 mL) was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (17.5 g, 31.9 mmol) in H_2O (75 mL). The mixture was stirred at room temperature for 10 min. Workup as described for **4** and crystallization from EtOAc/hexane yielded 1.75 g (65%) of **12**: mp 117.5–119.5 °C; IR (KBr) 1735, 1640, 1225 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.17 (s, 6 H, CH_3), 4.98 (d, 4 H, CH_2O), 6.68 (t, 2 H, =CH). Anal. ($C_{12}H_{12}O_6$) C, H.

2,6-Bis(hydroxymethyl)-1,4-benzoquinone Bis(hydrogen succinate) (13). To an ice-cold solution of **39** (2.0 g, 5 mmol) in CH_3CN (15 mL) was added dropwise, and with stirring, a solution of $Ce(NH_4)_2(NO_3)_6$ (5.5 g, 10 mmol) in H_2O (15 mL). After the solution was stirred for 30 min at room temperature, the yellow solid was filtered, air-dried, and recrystallized to give 1.2 g (65%) of **13**: mp (EtOAc) 174–175 °C; IR ($CHBr_3$) 2920 (br), 1750, 1700 (br), 1650 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.52 (m, 4 H, CH_2), 2.65 (m, 4 H, CH_2), 4.94 (s, 4 H, CH_2), 6.70 (s, 2 H, =CH), 12.32 (br s, 2 H, OH, D_2O exchangeable); ^{13}C NMR (Me_2SO-d_6) δ 28.51 (2C), 59.07, 130.80, 143.05, 171.51, 173.15, 184.17, 186.51; MS, m/e 368 (M^+). Anal. ($C_{16}H_{16}O_{10}$) H; C: calcd, 52.18; found, 51.53.

2-(Hydroxymethyl)-1,4-benzoquinone Hydrogen Succinate (15). A solution of **33** (0.086 g, 0.3 mmol) in CH_3CN (1.5 mL) was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (0.5 g, 0.9 mmol) in H_2O (1.5 mL). Workup as described for the preparation of **4** followed by crystallization of the crude product from CH_2Cl_2/hexane yielded 0.05 g (66%) of **15** as pale yellow crystals: mp 124–125 °C dec; IR (KBr) 3060, 2950, 2750–2540, 1755, 1745, 1705, 1655, 1330, 1165, 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.72 (s, 4 H,

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CH_2CH_2), 5.02 (d, 2 H, CH_2O), 6.65–6.8 (m, 3 H, =CH); MS, *m/e* 238 (M^+). Anal. ($C_{11}H_{10}O_6$) C, H.

2-(Acetoxymethyl)-5-methoxy-1,4-benzoquinone (16). A solution of **35** (1.0 g, 4.1 mmol) in CH_3CN (50 mL) was treated with a solution of $Ce(NH_4)_2(NO_3)_6$ (4.56 g, 8.3 mmol) in H_2O (6 mL). The mixture was stirred at room temperature for 30 min. Workup as described for **4** and crystallization from EtOAc/hexane afforded 0.58 g (66%) of **16**: mp 157–159.5 °C; IR (KBr) 1740, 1600, 1360, 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.16 (s, 3 H, $COCH_3$), 3.84 (s, 3 H, OCH_3), 5.01 (d, 2 H, CH_2O, J = 1.9 Hz), 6.59 (s, 1 H, =CH), 6.63 (t, 1 H, =CH, J = 1.9 Hz). Anal. ($C_{10}H_{10}O_5$) C, H.

1,2-Bis[4-(bromomethyl)-2,5-dimethoxyphenyl]ethane (17). Phosphorous tribromide (0.09 mL, 0.93 mmol) was added to a cooled (0 °C) and stirred solution of **21** (0.51 g, 1.4 mmol) in anhydrous THF (20 mL). The mixture was stirred for 4 h at room temperature and partitioned between CH_2Cl_2 and H_2O. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Recrystallization from EtOAc/hexane afforded 0.64 g (93%) of **17** as white flakes: mp 160–162 °C; ^1H NMR ($CDCl_3$) δ 2.85 (s, 4 H, $ArCH_2CH_2Ar$), 3.76 (s, 12 H, OCH_3), 4.56 (s, 4 H, CH_2Br), 6.51 (s, 2 H, ArH), 6.81 (s, 2 H, ArH). Anal. ($C_{20}H_{24}Br_2O_4$) C, H, Br.

1,2-Bis[4-(acetoxymethyl)-2,5-dimethoxyphenyl]ethane (18). To a solution of (*Z*)-**23** (1.90 g, 4.28 mmol) in glacial AcOH (100 mL) was added Pt_2O (97 mg, 0.43 mmol). This mixture was hydrogenated (Parr shaker) at 40 psi for 6 h. The colorless reaction mixture was filtered and concentrated under reduced pressure. The resulting solid was dissolved in CH_2Cl_2 and extracted with H_2O. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Recrystallization of the white residue from EtOAc/hexane afforded 1.65 g (87%) of **17** as white flakes: mp 120–123 °C; ^1H NMR ($CDCl_3$) δ 2.09 (s, 6 H, $COCH_3$), 2.87 (s, 4 H, CH_2CH_2), 3.73 (s, 6 H, OCH_3), 3.78 (s, 6 H, OCH_3), 5.12 (s, 4 H, CH_2O), 6.61 (s, 2 H, ArH), 6.85 (s, 2 H, ArH). Anal. ($C_{24}H_{30}O_8$) C, H.

2,2′-Ethylenebis[2,5-dimethoxybenzyl alcohol] Bis(hydrogen succinate) (19). To a solution of **21** (0.362 g, 1.0 mmol) in dioxane (1 mL) were added succinic anhydride (0.24 g, 2.4 mmol), Et_3N (2.0 mL, 14.4 mmol), and 4-(dimethylamino)pyridine (DMAP) (0.02 g, 0.2 mmol), and the mixture was stirred at room temperature for 24 h. Dilution of the reaction mixture was 5% aqueous HCl yielded the product as a solid material which was filtered, dried, and recrystallized from CH_3CN to obtain 0.49 g (87%) of **19** as colorless crystals: mp 145–147 °C; IR (KBr) 3500–3300, 2940, 1725, 1710, 1220, 1160, 1045 cm^{-1}; ^1H NMR ($CDCl_3$ + Me_2SO-d_6) δ 2.65 (s, 8 H, $COCH_2CH_2CO$), 2.85 (s, 4 H, CH_2CH_2), 3.72 (s, 6 H, OCH_3), 3.78 (s, 6 H, OCH_3), 5.14 (s, 4 H, CH_2O), 6.58 (s, 2 H, ArH), 6.84 (s, 2 H, ArH); MS, *m/e* 444 (M^+ – 118). Anal. ($C_{28}H_{34}O_{12}$) C, H.

1,2-Bis[4-(chloromethyl)-2,5-dimethoxyphenyl]ethane (20). A stream of HCl gas was bubbled through a stirred mixture of **22** (0.61 g, 2.0 mmol), dioxane (1.6 mL), concentrated HCl (0.26 mL, 8.6 mmol), and formalin (37% aqueous solution, 0.44 mL, 5.9 mmol). A colorless solid separates out of the reaction mixture in about 15 min. HCl gas was passed through the mixture for an additional 1 h. Dilution with concentrated HCl, filtration, and drying afforded 0.74 g (93%) of **20**. Crystallization from Me_2CO or CH_2Cl_2 yielded colorless crystals: mp 136–137 °C; IR (KBr) 2960, 2840, 1510, 1400, 1225, 1045, 665 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.86 (s, 4 H, CH_2CH_2), 3.75 (s, 6 H, OCH_3), 3.78 (s, 6 H, OCH_3), 4.64 (s, 4 H, CH_2Cl), 6.55 (s, 1 H, ArH), 6.84 (s, 1 H, ArH); MS, *m/e* calcd (M^+) 398.1053, obsd 398.1059. Anal. ($C_{20}H_{24}Cl_2O_4$) C, H; Cl: calcd, 17.76; found, 16.87.

1,2-Bis[4-(hydroxymethyl)-2,5-dimethoxyphenyl]ethane (21). Method I. A solution of **20** (0.2 g, 0.5 mmol) in dioxane (10 mL) was treated with a solution of Na_2CO_3 (0.53 g, 5.0 mmol) in H_2O (5 mL). The mixture was refluxed gently for 8 h, cooled to room temperature, and concentrated under reduced pressure. The residue was partitioned between $CHCl_3$ and H_2O. The aqueous layer was separated and extracted twice with $CHCl_3$, and the combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The solid residue thus obtained was chromatographed over silica gel (elution with EtOAc/petroleum ether 2:1) to afford 0.15 g (81%) of **21**. Recrystallization from EtOAc yielded colorless crystals: mp 143–144 °C; IR (KBr) 3300 (br), 2930, 2830, 1505, 1405, 1200, 1045, 860 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.30 (t, 2 H, OH), 2.86 (s, 4 H, CH_2CH_2), 3.76 (s, 6 H, OCH_3), 3.80 (s, 6 H, OCH_3), 4.65 (d, 4 H, CH_2O), 6.61 (s, 2 H, ArH), 6.82 (s, 2 H, ArH); MS, *m/e* 362 (M^+). Anal. ($C_{20}H_{26}O_6$) C, H.

Method II. To a solution of **18** (1.65 g, 3.7 mmol) in MeOH/THF (2:1) (60 mL) was added a solution of NaOH (0.37 g, 9.3 mmol) in H_2O (3 mL). The reaction mixture was stirred at room temperature overnight and concentrated under reduced pressure, and the residue was dissolved in EtOAc. The organic layer was extracted with H_2O, washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Recrystallization from CH_2Cl_2 afforded 1.1 g (82%) of **21**, identical in all respects with the sample obtained by Method I.

1,2-Bis(2,5-dimethoxyphenyl)ethane (22). Method I. In a dry flask equipped with a reflux condensor and a dropping funnel were placed Mg chips (0.24 g, 0.01 mol) under argon. Dry THF (1 mL) and a crystal of I_2 were added. A few drops of a solution of 2,5-dimethoxybenzyl bromide (2.31 g, 0.01 mol) in THF (5 mL) was added. After the exothermic reaction began, benzyl bromide solution was added at a rate to maintain gentle reflux. The reaction mixture was refluxed overnight, cooled to room temperature, and cautiously decomposed by dropwise addition of 10% aqueous HCl solution. The mixture was diluted with Et_2O (50 mL) and the organic layer separated, washed with H_2O (50 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave an oil which was chromatographed over silica gel (elution with EtOAc/petroleum ether 1:9) to yield 1.05 g (69%) of **22**. Recrystallization from EtOAc gave colorless crystals: mp 75–77 °C (lit.[8] mp 72 °C); IR (KBr) 2930, 1505, 1230, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.85 (s, 4 H, CH_2CH_2), 3.73 (s, 6 H, OCH_3), 3.77 (s, 3 H, OCH_3), 6.69–6.80 (m, 6 H, ArH). Anal. ($C_{18}H_{22}O_4$) C, H.

Method II. An Et_2O solution of MeMgBr (4.46 mL of 2.8 M solution, 12.5 mmol) was introduced into a dry argon-flushed reaction vessel fitted with a reflux condenser and a dropping funnel. To the stirred solution was added dropwise a solution of 2,5-dimethoxybenzyl bromide (1.16 g, 5.0 mmol) in Et_2O (10 mL). Following the addition, the mixture was refluxed for 2 h, cooled to room temperature, and cautiously decomposed by the dropwise addition of 10% aqueous HCl. Workup of the reaction mixture as described above afforded 0.20 g (26%) of **22** and 0.22 g (27%) of 2,5-dimethoxyethylbenzene as an oil: ^1H NMR ($CDCl_3$) δ 1.18 (t, 3 H, CH_3), 2.61 (q, 2 H, CH_2), 6.7–6.8 (m, 3 H, ArH).

Method III. In a dry two-necked flask equipped with a magnetic stirrer and a reflux condensor connected to an argon purge was placed VCl_3 (2.36 g, 15 mmol) and dry THF (20 mL). To the stirred suspension of VCl_3 was added LAH (0.19 g, 5.0 mmol), and after 5 min a solution of 2,5-dimethoxybenzyl bromide in THF (5 mL) was added dropwise. The mixture was heated under reflux and stirred overnight. The reaction mixture was cooled to room temperature, diluted with H_2O (80 mL), and extracted with benzene (2 × 100 mL). The organic extract was dried (Na_2SO_4) and concentrated under reduced pressure, and the residue obtained was chromatographed as described above to afford 0.50 g (66%) of **22**.

(*Z*)- and (*E*)-1,2-Bis[4-(acetoxymethyl)-2,5-dimethoxyphenyl]ethene (23). NaH (0.61 g, 50% slurry, 1.27 mmol) was added to a stirred suspension of **31** (0.22 g, 0.42 mmol) in benzene (30 mL) at room temperature under N_2 atmosphere. The resulting yellow mixture was heated at reflux for 2 h and cooled and aldehyde **30** (0.10 g, 0.42 mmol) added. The reaction mixture was stirred and heated at reflux overnight. After cooling, excess NaH was decomposed by the addition of MeOH. The mixture was poured over crushed ice and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure, affording a yellow oil. The oil was washed with hexane and chromatographed over silica gel/EtOAc–hexane, affording a mixture of (*Z*)- and (*E*)-**23**. Recrystallization from EtOAc/hexane afforded 0.08 g (44%) of (*Z*)-**23**: mp 100–102 °C; UV (MeOH) λ_{max} 330 nm; ^1H NMR ($CDCl_3$) δ 2.09 (s, 6 H, $COCH_3$), 3.43 (s, 6 H, OCH_3), 3.81 (s, 6 H, OCH_3), 5.09 (s, 4 H, CH_2OAc), 6.68 (s, 2 H, ArH), 6.78 (s, 2 H, =CH), 6.87 (s, 2 H, ArH). Anal. ($C_{24}H_{28}O_8$) C, H: calcd, 5.44; found, 6.18. For (*E*)-**23** (oil): UV (MeOH) λ_{max} 360 nm; ^1H NMR ($CDCl_3$) δ 2.12 (s, 6 H, $COCH_3$), 3.86 (s, 6 H, OCH_3), 5.16 (s, 4 H, CH_2OAc), 6.91, 7.15, 7.42 (3 s, 6 H, ArH + =CH).

1,4-Bis(bromomethyl)-2,5-dimethoxybenzene (25). Phosphorus tribromide (1.58 mL, 16.8 mmol) was added dropwise to a cooled (0 °C) and stirred solution of **28** (5.0 g, 25.2 mmol) in anhydrous THF (200 mL). The reaction mixture was stirred for 10 min at 0 °C and for 4 h at room temperature. The mixture was concentrated under reduced pressure and recrystallized from EtOAc/hexane to yield 6.72 g (82%) of **25** as a white solid: mp 200–202 °C; ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 6 H, OCH_3), 4.53 (s, 4 H, CH_2Br), 6.87 (s, 2 H, ArH). Anal. ($C_{10}H_{12}Br_2O_2$) C, H, Br.

1,4-Bis(acetoxymethyl)-2,5-dimethoxybenzene (26). A stirred suspension of **24** (1.0 g, 4.25 mmol) and NaOAc (0.87 g, 10.6 mmol) in glacial AcOH (30 mL) was heated at reflux overnight. The reaction mixture was filtered and concentrated under reduced pressure, and the residue was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure to yield 1.14 g (95%) of **26** as a tan solid. Crystallization from EtOAc/hexane gave white flakes: mp 120–122.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6 H, $COCH_3$), 3.82 (s, 6 H, OCH_3), 5.14 (s, 4 H, CH_2O), 6.91 (s, 2 H, ArH); MS, *m/e* 282 (M^+). Anal. ($C_{14}H_{18}O_6$) C, H: calcd, 6.43; found, 5.98.

1,4-Bis(hydroxymethyl)-2,5-dimethoxybenzene Bis(hydrogen succinate) (27). To a solution of **28** (2.0 g, 10 mmol) and succinic anhydride (2.0 g, 20 mmol) in dioxane was added Et_3N (5.0 mL). The reactants were stirred at room temperature for 24 h. The solution was poured onto ice–water and extracted with EtOAc. The aqueous solution was acidified with dilute HCl. The white solid was filtered, air-dried, and recrystallized to give **26** (90): mp (EtOAc) 118–120 °C; IR ($CHBr_3$) 2950, 2850, 1710 (br) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.55 (m, 8 H, CH_2), 3.77 (s, 6 H, CH_3), 5.07 (s, 4 H, CH_2), 6.98 (s, 2 H, ArH); ^{13}C NMR (Me_2SO-d_6) δ 28.70, 28.76, 56.11 (2C), 60.76 (2C), 112.27, 124.54, 150.69, 171.91, 173.24; MS, *m/e* 398 (M^+). The crude product was used without further purification to produce **9**.

1,4-Bis(hydroxymethyl)-2,5-dimethoxybenzene (28). To a solution of **26** (10.4 g, 37 mmol) in a 1:1 mixture of THF and MeOH (100 mL) was added dropwise a solution of NaOH (5.3 g, 133 mmol) and H_2O (10 mL). The reaction mixture was heated at reflux overnight, concentrated under reduced pressure, and extracted with EtOAc. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. Recrystallization from EtOAc/hexane afforded 6.87 g (94%) of **28**: mp 162–164 °C; ^{1}H NMR ($CDCl_3$) δ 2.26 (br t, 2 H, OH), 3.84 (s, 6 H, OCH_3), 4.68 (br d, 4 H, CH_2, J = 5.4 Hz), 6.88 (s, 2 H, ArH). Anal. ($C_{10}H_{14}O_4$) C, H.

2,5-Dimethoxy-4-(hydroxymethyl)benzaldehyde (29). To a stirred, cooled (−5 to 0 °C) solution of **28** (5.0 g, 25 mmol) in CH_2Cl_2 (1.3 mL) was added dropwise a solution of DDQ (5.37 g, 25 mmol) in CH_2Cl_2 (700 mL). The reaction mixture was stirred at room temperature overnight. Filtration, concentration under reduced pressure, and recrystallization from CH_2Cl_2 afforded 4.1 g (83%) of **29**: mp 139–141 °C; IR (KBr) 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.34 (br s, 1 H, OH), 3.85 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 4.74 (br s, 2 H, CH_2O), 7.07 (s, 1 H, ArH), 7.30 (s, 1 H, ArH), 10.43 (s, 1 H, CHO). Anal. ($C_{10}H_{12}O_4$) C, H.

4-(Acetoxymethyl)-2,5-dimethoxybenzaldehyde (30). To a solution of **29** (2.5 g, 12.7 mmol) in pyridine (15 mL) was added Ac_2O (2.4 mL, 25 mmol). The reaction mixture was stirred for 6 h and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, and recrystallization from CH_2Cl_2/hexane, affording 2.77 g (91%) of **30** as tan needles: mp 89–91 °C; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 3 H, $COCH_3$), 3.85 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 5.18 (s, 2 H, CH_2OAc), 7.02 (s, 1 H, ArH), 7.31 (s, 1 H, ArH), 10.43 (s, 1 H, CHO); MS, *m/e* 238 (M^+). Anal. ($C_{12}H_{14}O_5$) C, H.

[4-(Chloromethyl)-2,5-dimethoxybenzyl]triphenylphosphonium Chloride (31). A solution of **24** (23.8 g, 0.1 mol) and Ph_3P (26.6 g, 0.1 mol) in benzene (200 mL) was stirred at reflux for 18 h and cooled. The solid was removed by filtration, washed with benzene, and dried under reduced pressure. Treatment of the filtrate with fresh Ph_3P (7.40 g) afforded additional solid, providing a combined yield of 47.6 g (94%) of **31**: mp 223–225 °C dec; ^{1}H NMR ($CDCl_3$) δ 3.18 (s, 3 H, OCH_3), 3.60 (s, 3 H, OCH_3), 4.56 (d, 2 H, CH_2Cl), 5.33 (d, 2 H, CH_2P, J = 14.0 Hz), 6.62 (s, 1 H, ArH), 7.23 (d, 1 H, ArH), 7.64–7.83 (m, 15 H, ArH). Anal. ($C_{28}H_{27}Cl_2O_2P$) C, H, P.

[4-(Acetoxymethyl)-2,5-dimethoxybenzyl]triphenylphosphonium Chloride (32). A stirred suspension of **31** (1.0 g, 2.0 mmol) and NaOAc (0.18 g, 2.1 mmol) in glacial AcOH (10 mL) was heated at reflux overnight and cooled. Filtration and concentration of the filtrate under reduced pressure afforded 0.99 g (90%) of **32** as a tan solid: mp 201–203 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3 H, $COCH_3$), 3.18 (s, 3 H, OCH_3), 3.57 (s, 3 H, OCH_3), 5.05 (d, 2 H, CH_2O), 5.32 (d, 2 H, CH_2P, J = 14.3 Hz), 6.60 (s, 1 H, ArH), 7.61–7.83 (m, 15 H, ArH). Anal. ($C_{30}H_{30}ClO_4P$) C, H, Cl, P.

2-(Hydroxymethyl)-1,4-dimethoxybenzene Hydrogen Succinate (33). To a stirred solution of 2,5-dimethoxybenzyl alcohol (3.36 g, 0.02 mol) in dioxane (4 mL) was added succinic anhydride (2.4 g, 0.02 mol), Et_3N (5 mL, excess), and DMAP (0.24 g, 0.002 mol). The mixture was stirred at room temperature for 24 h and poured into a mixture of ice (100 g) and 10% aqueous HCl (100 mL). The solid that separates was filtered, dried, and crystallized from $CHCl_3$/hexane to yield 4.68 g (87%) of **33** as colorless crystals: mp 81–82 °C; IR (KBr) 3200–2820, 2700–2500, 1735, 1715, 1510, 1240, 1195, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 4 H, CH_2CH_2), 3.77 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 5.17 (s, 2 H, CH_2O), 6.80–6.92 (m, 3 H, ArH); MS, *m/e* 268 (M^+). Anal. ($C_{13}H_{16}O_6$) C, H.

2,4,5-Trimethoxybenzyl Alcohol (34). Sodium borohydride (0.97 g, 25.7 mmol) was added to a stirred solution of 2,4,5-trimethoxybenzaldehyde (5.0 g, 25.5 mmol) in MeOH (150 mL). The mixture was stirred for 30 min and concentrated under reduced pressure, and the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was recrystallized from EtOAc/hexane to yield 4.24 g (84%) of **34** as colorless crystals: mp 67–69 °C (lit.[14] mp 70 °C); ^{1}H NMR ($CDCl_3$) δ 2.17 (t, 1 H, OH, J = 6.0 Hz), 3.84 (s, 6 H, OCH_3), 3.88 (s, 3 H, OCH_3), 4.62 (d, 2 H, CH_2, J = 6.0 Hz), 6.53 (s, 1 H, ArH), 6.85 (s, 1 H, ArH).

2,4,5-Trimethoxybenzyl Acetate (35). To a stirred solution of **34** (2.0 g, 10 mmol) in pyridine (20 mL) was added Ac_2O (3.8 mL, 40 mmol). The mixture was stirred for 4 h and partitioned between CH_2Cl_2 and H_2O. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure, and the residue was recrystallized from EtOAc/hexane to yield 2.14 g (88%) of **35** as colorless crystals: mp 62–63.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3 H, $COCH_3$), 3.82 (s, 6 H, OCH_3), 3.88 (s, 3 H, OCH_3), 5.08 (s, 2 H, CH_2O), 6.52 (s, 1 H, ArH), 6.87 (s, 1 H, ArH). Anal. ($C_{12}H_{16}O_5$) C, H.

1,3-Bis(bromomethyl)-2,5-dimethoxybenzene (37). Phosphorus tribromide (0.95 mL, 10 mmol) was added dropwise to a cooled (0 °C) and stirred solution of **36** (3.0 g, 15 mmol) in 150 mL of THF. The reaction mixture was stirred for 10 min at 0 °C and for 6 h at room temperature. The mixture was concentrated under reduced pressure and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to obtain off-white solid. Recrystallization from hexane afforded 2.72 g (56%) of **37** as a white solid: mp 90–92.5 °C; ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3 H, OCH_3) 3.97 (s, 3 H, OCH_3), 4.52 (s, 4 H, CH_2Br), 6.89 (s, 2 H, ArH). Anal. ($C_{10}H_{12}Br_2O_2$) C, H, Br.

1,3-Bis(acetoxymethyl)-2,5-dimethoxybenzene (38). To a stirred solution of **36** (5.0 g, 25 mmol) in pyridine (20 mL) was added Ac_2O (6.0 mL, 63.5 mmol). The reaction mixture was stirred for 4 h, diluted with H_2O, and extracted with CH_2Cl_2. The organic layer was dried and concentrated under reduced pressure, affording 5.28 g (74%) of **38** as a colorless liquid: bp 138–140 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 2.12 (s, 6 H, $COCH_3$), 3.80 (s, 6 H, OCH_3), 5.16 (s, 4 H, CH_2OAc), 6.91 (s, 2 H, ArH). Anal. ($C_{14}H_{18}O_6$) C, H.

1,3-Bis(hydroxymethyl)-2,5-dimethoxybenzene Bis(hydrogen succinate) (39). To a solution of **36** (1.98 g, 10 mmol) and succinic anhydride (2.0 g, 20 mmol) in dioxane was added Et_3N (5 mL). The reactants were stirred at room temperature for 24 h. The solution was poured onto ice–water and extracted with EtOAc. The aqueous solution was acidified with dilute aqueous HCl and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give **39** as a colorless oil (94%): IR ($CHBr_3$) 2940 (br), 1710 (br) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.69 (s, 8 H, CH_2), 3.78 (s, 6 H, CH_3), 5.19 (s, 4 H, CH_2), 6.90 (s, 2 H, ArH), 10.43 (s, 2 H, OH, D_2O ex-

changeable); MS, m/e 398 (M^+). Anal. ($C_{18}H_{22}O_{10}$) H; C: calcd, 54.27; found, 53.74.

Biology. Female BDF_1 and C57BL/6 mice were received from Harlan Industries, Indianapolis, IN. All animals were quarantined for 5 days before being released for use in studies. Prior to study, animals were housed five per cage in suspended gang stainless steel cages. During study, animals were housed individually in suspended stainless steel cages with wire mesh fronts and bottoms. Water and food (Purina Mouse Chow No. 5015) were available ad libitum to all animals at all times.

Tumor. B16 tumor was maintained twice per month by subcutaneous passage in syngenic C57BL/6 mice. On day 0, solid tumor was removed from a donor mouse. Tumor brei was prepared by making a 1:10 dilution (w/v) with HBSS (Hank's balanced salt solution) and then implanted (0.5 mL each) subcutaneously into a shaved dorsoscapular area of BDF_1 mice.

Drug Administration. Drugs were prepared in either normal saline, 0.3% Klucel, or 0.33% Klucel/$NaHCO_3$. Animals were injected for 9 consecutive days after observation of measurable tumor with doses usually ranging from 2.5 to 20 mg/kg injection.

Observations and Calculations. Drug-treated mice were observed daily for no longer than 120 days. Tumor measurements (diameter) and body weights were recorded twice per week. Tumor diameter was calculated in millimeters by using Vernier calipers. Diameters were taken by measuring the long axis (length) and the two short axes (width and depth). Tumor size was expressed as an average of the three diameters.

Median survival time and percent T/C were calculated according to Instruction 14 of the National Cancer Institute (Bethesda, MD). Tumor growth inhibition was calculated as

$$\text{TGI} = 100 - \frac{(\text{change in tumor diameter treated})(100)}{\text{change in tumor diameter control}}$$

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17-Desoxy Estrogen Analogues

Richard H. Peters, David F. Crowe, Mitchell A. Avery, Wesley K. M. Chong, and Masato Tanabe*

Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025. *Received March 21, 1988*

A series of 17-substituted, 17-desoxyestratrienes have been synthesized and tested as potential postcoital antifertility agents. Estrogen-relative binding affinities were determined, in vivo assays for estrogenic and postcoital antifertility activity were conducted in rats, and selected candidate compounds were further tested for estrogenic activity in monkeys. In the rat, the 17-desoxyestratriene derivatives **8a**, **8b**, and **30** have shown low estrogenic activity while retaining potent antifertility activity. Structural modifications at the outset included a variety of 17-substituents and an omission of the 17-oxygen functionality, which was previously thought to be necessary for potent activity. The 17β-ethyl side chain exhibited the greatest antifertility activity with the largest separation ratio to estrogenicity. Nuclear modification of 17-desoxyethylestrane derivatives at positions 7 and 11 further increased the desired separation of activity, with the 11-hydroxy moiety enhancing separation more than other features.

For a number of years we have been engaged in an ongoing program for the design of improved antifertility agents. Despite the wide variety of structural modifications that have been realized in the evolution of steroidal drugs, we felt that the role of the C17 oxygen functionality of estrogens had not been fully evaluated. In this regard, we describe herein our examination of structure–activity relationships (SAR) among various 17-desoxy estrogens.

The arrangement of oxygen substituents on a steroid nucleus is well recognized as a key determinant among the variety of biological activities that are modulated by steroids. As each of the specific receptors for progestins, androgens, and estrogens have become better characterized and available in pure form, the direct relationships between binding affinity and the resultant hormonal activities have been verified,[1] although the exact mechanistic details remain obscure. Therefore, it is easy to visualize how the site and geometrical orientation of oxygens on a steroid skeleton can affect its binding affinity for a receptor, and thus the degree of activity.

The estrogen receptor, which elicits a uterotrophic response, has been isolated from rat uterine tissue and used in studies that have defined the connection between binding affinities, structures, and hormonal activity.[2] These studies have suggested that estrogens must have a 17β-hydroxyl and a phenolic 3-hydroxyl in order to have a high binding affinity and, consequently, potent hormonal activity. Different descriptions of the requisite disposition of these two oxygens have been reported;[3a-c] among these are a critical intramolecular distance of 11 Å proposed by Weber and Galantay[4] and an angular dependence examined by Raynaud and co-workers.[3d,e] However, the effects of different numbers of oxygens—either more or fewer—

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