was adjusted to 50 g, which corresponded to 10 mm of recorder pen deflection. Rapid iv injection of 50 mL of 5% dextran and mechanical compression of the aorta demonstrated that contractility measurements were independent on changes in preload and afterload. Subcutaneous pin electrodes provided a lead II ECG. Increasing doses of test compounds were administered iv in volumes of 0.25–4.0 mL at 5-min intervals; no responses occurred with appropriate vehicle injections. ED_{50} values were determined by linear regression analysis and are reported as the mean ± SEM of experimental values. A different set of animals was used for each test compound.

Conscious Dog Studies. Male mongrel dogs weighing 15–36 kg were chronically instrumented to monitor $LVdP/dt_{60}$ (the first derivative of left ventricular pressure at 60 mmHg), peak systolic blood pressure, and heart rate. Under halothane–nitrous oxide anesthesia, a precalibrated Konigsberg P22 pressure transducer was implanted into the left ventricle through a stab wound at the apex. Dogs were allowed to recover from surgery a minimum of 2 weeks before use in a study. Animals were conditioned to the test laboratory and trained to lie quietly for 4-h periods. This conditioning was necessary to obtain stable, reproducible results. Dogs were fasted 18 h before an experiment and gross behavioral observations of animals were made throughout each study. Drugs or placebo (lactose) were administered in 000 gelatin capsules.

Preparation of Sarcoplasmic Reticulum Vesicles. Subfractions of sarcoplasmic reticulum (SR) vesicles, A–E, were prepared from ventricles of pentobarbital-anesthetized dogs as described by Jones and co-workers.[30,31] Fraction E, which originates from free SR vesicles,[32] was used in all inhibition studies. Aliquots of free SR vesicles were stored frozen at −80 °C until used. Under these conditions no loss of PDE activity was detected after 6 months of storage. Vesicle protein was determined by the method of Lowry.[33]

Enzyme Assays. Cyclic nucleotide phosphodiesterase was assayed by the two-step technique of Thompson et al.[25] PDE reactions were initiated by adding sufficient enzyme to hydrolyze less than 20% of the substrate (1 μM cAMP) in 60 min at room temperature (22 ± 2 °C). Reactions were terminated by placing tubes in boiling water for 45 s. PDE activity was linear vs. time and protein concentration; test compounds had no effect upon the snake venom (*Ophiophagus hannah*) used to convert [^{3}H]-AMP to [^{3}H]adenosine in the second step of the assay (data not shown). Dimethyl sulfoxide was utilized as solvent for PDE inhibitors. Solutions were prepared on the day of an experiment, and controls were run to ensure that carryover solvent (2.5%, v/v) did not affect assay results. IC_{50} values and 95% confidence limits were calculated as previously described.[34]

Acknowledgment. We thank Patsy Abbett for typing the manuscript.

Registry No. **2**, 103490-49-9; **3**, 41058-67-7; **4**, 103490-50-2; **5**, 59-48-3; **6**, 103490-47-7; **7**, 13861-75-1; **8**, 83419-51-6; **9**, 103490-56-8; **10**, 103490-44-4; **11**, 103515-48-6; **12**, 103490-46-6; **13**, 107081-83-4; **19**, 103490-51-3; **20**, 103490-52-4; **21**, 103490-53-5; **22**, 103490-48-8; **23**, 103490-54-6; CH_3CH_2COCl, 79-03-8; HCHO, 50-00-0; $\overline{CH_2CH_2CH_2}CHCOCl$, 5006-22-4; succinic anhydride, 108-30-5; 2′,8-dioxo-β-methylspiro[cyclopropane-1,3′-indol]-5′-butanoic acid, 103490-57-9.

(30) Jones, L. R.; Besch, H. R., Jr.; Fleming, J. W.; McConnaughey, M. M.; Watanabe, A. M. *J. Biol. Chem.* **1979**, *254*, 530.
(31) Jones, L. R.; Cala, S. E. *J. Biol. Chem.* **1981**, *256*, 11809.
(32) Seiler, S.; Wegener, A. D.; Whang, D. D.; Hathaway, D. R.; Jones, L. R. *J. Biol. Chem.* **1984**, *259*, 8550.
(33) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
(34) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculations*; Springer-Verlag: New York, 1981.

Estrogenic Affinity Labels: Synthesis, Irreversible Receptor Binding, and Bioactivity of Aziridine-Substituted Hexestrol Derivatives

Jeffery A. Zablocki,† John A. Katzenellenbogen,*† Kathryn E. Carlson,† M. J. Norman,‡ and Benita S. Katzenellenbogen‡

Department of Chemistry and Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.
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To develop an affinity label for the estrogen receptor that would be an estrogen agonist, rather than antagonist, we prepared several aziridine derivatives of the potent nonsteroidal estrogen hexestrol ((3*R**,4*S**)-3,4-bis(4-hydroxyphenyl)hexane) bearing an aziridine function on the side chain. Three functional groups link the hexestrol ligand and the aziridine: a carbonyl group (ketone or ester), a thioether, or a methylene chain. The apparent competitive binding affinity of these derivatives for the estrogen receptor ranges from 1.8% to 25% that of estradiol, and most of them bind in a time-dependent, irreversible manner with the receptor, although the rate and efficiency of this binding vary widely, often with relatively small changes in structure. This is consistent with the irreversible attachment requiring a precise alignment of activating and reacting residues in the binding site of the receptor. The estrogenic and antiestrogenic activity of these aziridine derivatives was investigated in MCF-7 human breast cancer cells. Most of the compounds are agonists, with one being an antagonist. The derivative (6*R**,7*S**)-1-*N*-aziridinyl-6,7-bis(4-hydroxyphenyl)-5-nonanone (keto-nonestrol aziridine **3**) appears to have the most ideal behavior of the estrogenic affinity labeling agents prepared: It is an agonist, and it binds to receptor irreversibly, efficiently, and quite rapidly.

The affinity labeling concept, whereby reversibly binding ligands are elaborated into chemically reactive derivatives capable of covalently labeling binding proteins, has been applied effectively to develop covalent labeling agents for steroid receptors.[1] Both photochemically reactive and electrophilic derivatives have been prepared, and labeling studies have been conducted with estrogen, androgen, progestin, and corticosteroid receptors.[1] In general, the affinity labeling agents have high covalent labeling selectivity, since they retain a good deal of the binding selectivity of the reversibly binding ligands.

We have found that an aziridine analogue of tamoxifen, tamoxifen aziridine (**1b**), is an effective affinity label for the estrogen receptor.[2] It is capable of covalent labeling

† Department of Chemistry.
‡ Department of Physiology and Biophysics.

(1) (a) *Methods Enzymol.* **1977**, *46*, entire volume. (b) Katzenellenbogen, J. A. In *Biochemical Actions of Hormones*; Litwack, G., Ed.; Academic: New York, 1977; Vol. IV, Chapter 1. (c) Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *J. Org. Chem.* **1984**, *49*, 1238–1246. (d) Gronemeyer, H.; Govindan, M. V. *Mol. Cell. Endocrinol.* **1986**, *46*, 1–19.

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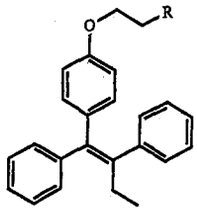
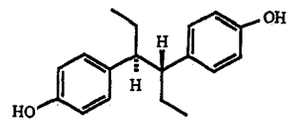
of estrogen receptor from rat and lamb uterus and from MCF-7 human breast cancer cells, and the labeling is notably efficient and selective. Structurally, tamoxifen



1a R = NMe_2 (RBA = 1.5)

1b R = Az (RBA = 23)

Az = -N

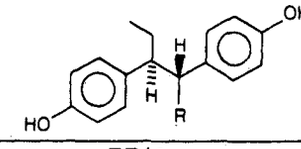


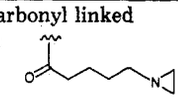
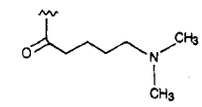
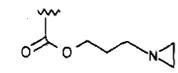
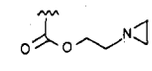
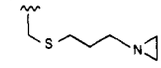
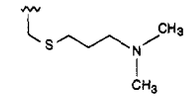
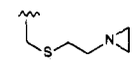
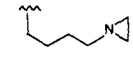
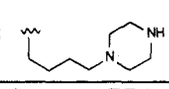
2 (RBA = 300)

aziridine is related to the antiestrogen tamoxifen (1a), and in biological characterization studies, it retains the antagonistic properties of tamoxifen: It suppresses estrogen-stimulated growth of MCF-7 cells and reduces the estrogen-induced increase in uterine weight in rats.[3]

In using affinity labeling agents to probe for gene interaction sites of the estrogen receptor that are related to estrogen-stimulated gene products, it is important to use an affinity labeling agent that is an estrogen, rather than an antiestrogen. Therefore, we undertook the development of an efficient and selective affinity labeling agent for the estrogen receptor that has *estrogenic* activity. On the basis of the high estrogenic potency of the nonsteroidal estrogen hexestrol (**2**)[4] and the high receptor binding affinity[5] of numerous hexestrol side-chain derivatives,[6] we have synthesized several hexestrol derivatives bearing an aziridine function on a side-chain appendage, and we have examined their binding and irreversible interaction with the estrogen receptor and their effect on the growth of MCF-7 cells. The apparent binding affinity, the rate and extent of the time-dependent, irreversible binding, and the agonist–antagonist activity of these derivatives vary widely, with major differences deriving from minor structural changes. One of these derivatives, (6*R**,7*S**)-1-*N*-aziridinyl-6,7-bis(4-hydroxyphenyl)-5-nonanone (keto-nonestrol aziridine **3**),[5] appears to be both an effective, irreversible ligand for the estrogen receptor and an estrogen agonist. The preparation of this compound in radiolabeled form and studies on its covalent attachment to the estrogen receptor will be described elsewhere.[7]

Table I. Receptor Binding and Biological Activity of Hexestrol Aziridine and Related Derivatives



no.	R	RBA[a] (apparent RBA)	irreversible[b] binding	agonist or antagonist character[c]
carbonyl linked				
3		8.3	yes (good)	agonist (10^{-8} M)
4		0.9		weak agonist (3×10^{-7} M)
5		3.2	yes (slow–weak)	potent agonist (10^{-9} M)
6		8.9	yes (slow–weak)	potent agonist (10^{-9} M)
7		25	no	potent agonist (10^{-9} M)
thioether linked				
8		1.8	yes (good)	agonist (3×10^{-8} M)
9		6.6		agonist (4×10^{-8} M)
10		5.8	no	agonist (4×10^{-8} M)
methylene linked				
11		14	yes (good)	weak antagonist (3×10^{-7} M)
12		53		very weak[d]

[a] Apparent RBA (relative binding affinity) is determined in a competitive radioreceptor assay, using [^{3}H]estradiol as tracer. See text and ref 17. [b] Determined in an inactivation-exchange assay. See Figure 1 and ref 2a and 18. Where there is no entry, compounds have no capacity for irreversible binding and were not tested. [c] Agonist denotes estrogenic; in parentheses is the concentration at which the compound stimulates the proliferation of MCF-7 cells at least 50% of that achieved with 10^{-9} M estradiol. Antagonist denotes a compound capable of suppressing estradiol stimulation of cell proliferation; in parentheses is stated the concentration at which the compound suppressed 50% of the stimulation of MCF-7 cell proliferation evoked by 10^{-9} M estradiol. [d] No estrogenic or antiestrogenic effect on MCF-7 cell proliferation at 3×10^{-7} M.

Results

The structures of the side-chain aziridine-substituted hexestrols and of some related tertiary amine derivatives are shown in Table I. They represent three structural classes distinguished by the functional nature of the linkage between the hexestrol ligand and the aziridine. Compounds **3–7** have a carbonyl function (either as a ketone or an ester) at a position that corresponds to carbon 2 of hexestrol; compounds **8–10** have a thioether linkage, and compounds **11** and **12** have a methylene link. All three

(2) (a) Robertson, D. W.; Wei, L. L.; Hayes, J. R.; Carlson, K. E.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology (Baltimore)* **1981**, *109*, 1298–1300. (b) Katzenellenbogen, J. A.; Carlson, K. E.; Heiman, D. F.; Robertson, D. W.; Wei, L. L.; Katzenellenbogen, B. S. *J. Biol. Chem.* **1983**, *258*, 3487–3495. (c) Monsma, F. J.; Katzenellenbogen, B. S.; Miller, M. A.; Ziegler, Y. S.; Katzenellenbogen, J. A. *Endocrinology (Baltimore)* **1984**, *115*, 143–153.

(3) Wei, L. L.; Mangel, W. F.; Katzenellenbogen, B. S. *J. Steroid Biochem.* **1985**, *23*, 876–882.

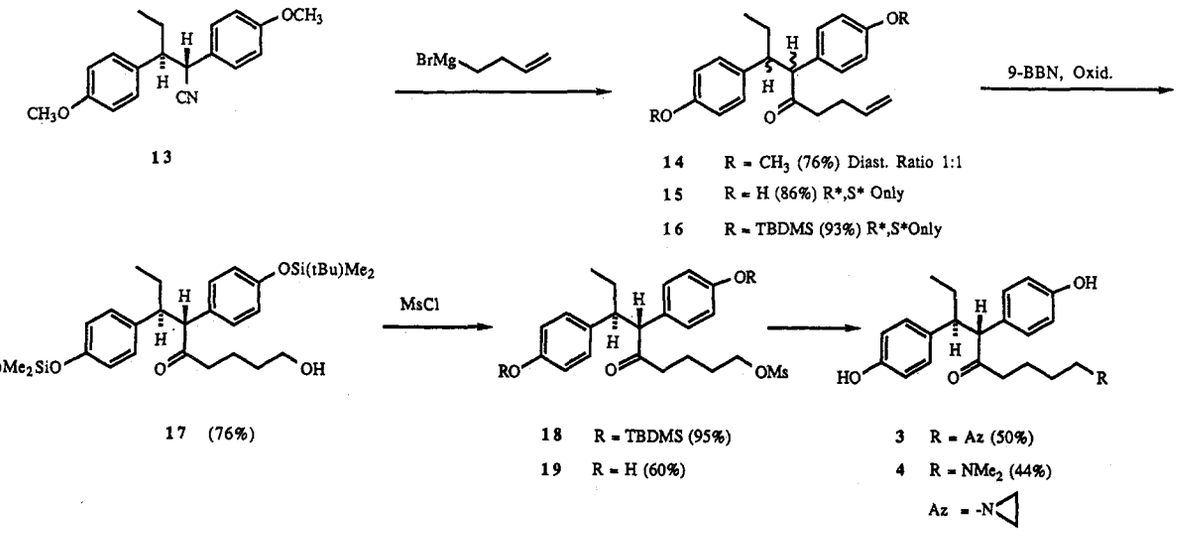
(4) Katzenellenbogen, J. A.; Hsiung, H. M.; Carlson, K. E.; McGuire, W. L.; Kraay, R. J.; Katzenellenbogen, B. S. *Biochemistry* **1975**, *14*, 1742–1750.

(5) (a) Binding affinities are generally expressed relative to that of a standard, estradiol, as a relative binding affinity (RBA) value (RBA for estradiol ≈ 100%). (b) The common name "keto-nonestrol aziridine" is used to describe compound **3**. The root "nonestrol" indicates that it is a homologue of hexestrol (**2**); the additional two functional groups are noted as prefix and suffix.

(6) (a) Katzenellenbogen, J. A.; Carlson, K. E.; Johnson, H. J.; Meyers, H. N. *J. Toxicol. Environ. Health, Suppl. 1* **1976**, 205. (b) Landvatter, S. W.; Katzenellenbogen, J. A. *J. Med. Chem.* **1982**, *25*, 1300. (c) Fevig, T. L.; Lloyd, J. E.; Zablocki, J. A.; Katzenellenbogen, J. A. *J. Med. Chem.* **1987**, *30*, 156.

(7) Elliston, J. F.; Zablocki, J. A.; Norman, M. J.; Katzenellenbogen, B. S.; Carlson, K. E.; Katzenellenbogen, J. A. *Endocrinology (Baltimore)*, in press.

Scheme I



13

14 R = CH_3 (76%) Diast. Ratio 1:1

15 R = H (86%) R*,S* Only

16 R = TBDMS (93%) R*,S*Only

17 (76%)

18 R = TBDMS (95%)

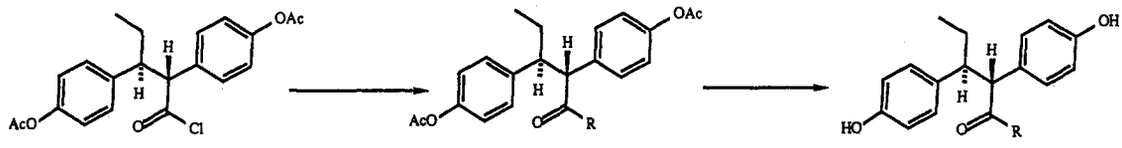
19 R = H (60%)

3 R = Az (50%)

4 R = NMe_2 (44%)

Az = -N

Scheme II



20

21 R = $OCH_2CH_2CH_2Br$, (33%)

22 R = OCH_2CH_2I (76%)

23 R = Az (98%)

Az = -N

24 R = $OCH_2CH_2CH_2Br$ (100%)

25 R = OCH_2CH_2I (100%)

5 R = $OCH_2CH_2CH_2Az$ (13%)

6 R = OCH_2CH_2Az (21%)

7 R = Az (90%)

classes are based on previously reported hexestrol derivatives with high binding affinity for the receptor.[6b,6c] In previous studies of side-chain-functionalized hexestrol derivatives, we have noted that both C_2 ketone and ester analogues of hexestrol demonstrate very good binding affinity for the estrogen receptor;[6] in preparing fluorescent conjugates of hexestrol analogues, we have also found that norhexestrol thioethers have substantial affinity for the estrogen receptor,[6c] and studies by Hemme, Kulikowski, and Krohn have shown that the simple alkyl-linked derivatives have substantial affinity for receptor, as well.[8]

Synthesis. Carbonyl-Linked Hexestrol Derivatives 3–7. The methanesulfonate precursor **19** of aziridine **3** was prepared in six steps (26% overall yield) from the known nitrile **13**[9] (Scheme I).[10] Addition of the Grignard of 4-bromo-1-butene to nitrile **13** afforded ketone **14** as a mixture of diastereomers (1:1). Methyl ether deprotection using lithium butanethiolate proceeded with concomitant epimerization that increased the diastereomer ratio from 1:1 to 5:1 in favor of the desired *R**,*S** diastereomer.[11] After the phenolic functions of alkene **15** were reprotected as *tert*-butyldimethylsilyl ethers, alkene **16** was submitted to hydroboration–oxidation conditions (9-borabicyclo-[3.3.1]nonane (9-BBN) and basic hydrogen peroxide) to afford the primary alcohol **17**. It is of note that the sterically hindered carbonyl group of **16** was not reduced even with an excess of 9-BBN. The primary methanesulfonate **18** was formed (methanesulfonyl chloride and triethylamine), and the silyl ethers were removed by treatment with toluenesulfonic acid monohydrate in ethyl acetate at 40 °C to afford methanesulfonate **19**, the precursor to aziridine **3**.[12]

Nucleophilic displacement of the methanesulfonate in **19** by ethylenimine (80 equiv, ethylenimine/triethylamine/acetonitrile, 1:1:1) afforded aziridine **3** in 50% yield. The large excess (80 equiv) and high concentration of ethylenimine eliminated the elevated temperatures and long reaction times (60 °C, 3–5 days, DMF) that were required for a similar displacement in the preparation of 11β-(aziridinylmethyl)-17β-estradiol.[13] For biological

(8) Krohn, K.; Hemme, C. *Justus Leibigs Ann. Chem.* **1978**, 726. (b) Kulikowski, K. Ph.D. Thesis, Technichen Universitat Carolo-Wilhelmina zu Braunschweig, West Germany, 1984.

(9) Niederl, J. B.; Ziering, A. *J. Am. Chem. Soc.* **1942**, *64*, 885.

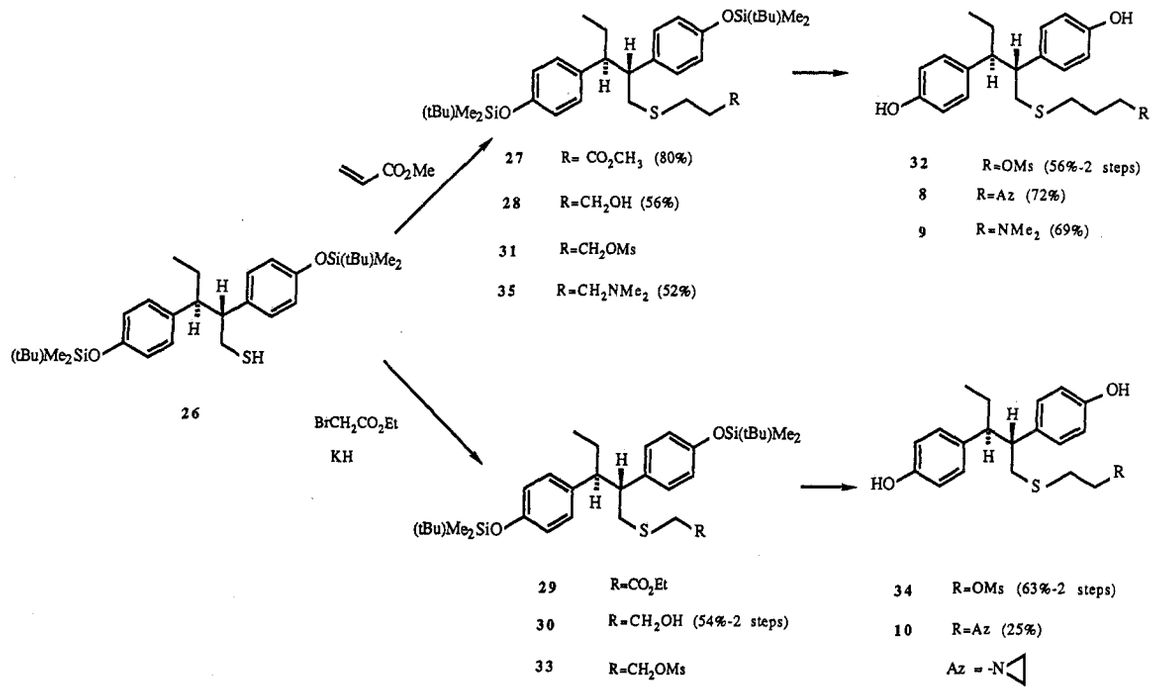
(10) The R*,S* system of designating relative stereochemistry (IUPAC 1968 Tentative Rules, Section E) is used to define unambiguously the appropriate diastereomer of each hexestrol and norhexestrol derivative. In this system, the R*,S* diastereomers correspond in each case to those referred to traditionally as the meso or erythro diastereomers. (In Schemes I–IV, only one enantiomer of each R*,S* diastereomeric pair is shown.) Members of the other diastereomeric series (R*,R*, dl or threo) have much lower binding affinity for the estrogen receptor (Kilbourn, M. R.; Arduengo, A. J.; Park, J. T.; Katzenellenbogen, J. A. *Mol. Pharmacol.* **1981**, *19*, 388).

(11) This diastereomeric enrichment is consistent with model studies: Landvatter, S. W. Ph.D. Dissertation, University of Illinois, 1982.

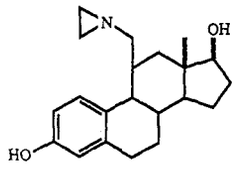
(12) Greene, T. W. *Protective Groups in Organic Synthesis*; Wiley: New York, 1981; p 309. This provides experimental proof to the conjecture that the *tert*-butyldimethylsilyl phenol protecting group is stable to toluenesulfonic acid at 0 °C but reactive at 80 °C.

(13) Schönemann, K. H.; Vliet, N. P. V.; Zeelen, F. J. *Eur. J. Med. Chem.—Chim. Ther.* **1980**, *15*, 333–335.

Scheme III



comparison, compound **4**, the dimethylamino analogue of aziridine **3**, was prepared from methanesulfonate **19** by displacement with dimethylamine.



Although nonfunctionalized aziridines, such as tamoxifen aziridine (**1b**), can be purified on silica gel with a basic eluent (triethylamine/benzene),[2a] the presence of the acidic phenol makes keto-nonestrol aziridine **3** unstable in this basic chromatography system. Purification of the phenolic aziridine **3**, however, was possible by using a buffered semipreparative reverse-phase HPLC system (pH 7.8), thereby minimizing ionization of both functional groups.

The halide precursors to aziridines **5** and **6** were prepared by reaction of 2-iodoethanol and 3-bromo-1-propanol with the known acid chloride **20**[5b] (Scheme II). After acetate hydrolysis, aziridination conditions described above afforded aziridines **5** and **6** in low yield (13% and 21%, respectively). These lower yields can be attributed to ester hydrolysis, which was an observed side reaction in the aziridination step. Acyl aziridine **7** was prepared by reaction of ethylenimine with acid chloride **20** in the presence of an excess of triethylamine (98%),[14] followed by acetate hydrolysis (90%) (Scheme II).

Thioether-Linked Hexestrol Derivatives 8–10. Scheme III shows the synthesis of the methanesulfonate precursors **32** and **34** of the thioether derivatives **8–10**; we have described the synthesis of thiol **26** previously.[5c] The synthesis of **32** began with the Michael addition of thiol **26** to methyl acrylate,[15] affording methyl ester **27**. Lithium aluminum hydride reduction, followed by methanesulfonate formation and phenol deprotection, afforded the desired methanesulfonate **32**. Methanesulfonate **34** was obtained by alkylation of thiol **26** with ethyl bromoacetate, followed by ester reduction (lithium aluminum hydride), methanesulfonate formation, and phenol deprotection. The aziridines **8** and **10** were formed from the corresponding methanesulfonates **32** and **34** as described previously.

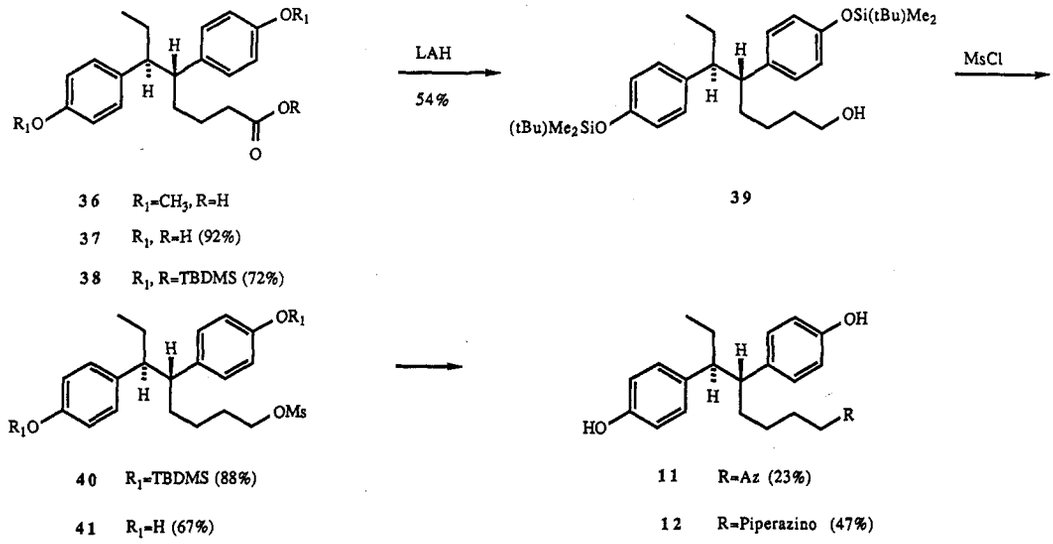
For comparative purposes, compound **9**, the dimethylamino analogue of aziridine **8**, was prepared from the protected methanesulfonate **31** in two steps: displacement (anhydrous dimethylamine) to give **35** and deprotection (potassium fluoride) to give **9**.

Methylene-Linked Hexestrol Derivatives 11 and 12. Octestrol aziridine **11**[6] was prepared in six steps from acid **36** (a gift from Krohn[16]) (Scheme IV). The methyl ether protecting groups on acid **36** were removed and replaced by the more easily removed *tert*-butyldimethylsilyl group; the latter reaction was accompanied by silyl ester formation. Reduction of the silyl ester (LAH) was followed by methanesulfonate formation, deprotection, and aziridination as described above to afford aziridine **11**. For comparative purposes, the piperazino analogue **12** was prepared directly from methanesulfonate **41** through a simple displacement.

Receptor Binding Affinity. The binding affinity of nonradiolabeled ligands for the estrogen receptor can be assayed conveniently by a competitive binding radiometric assay, using [^{3}H]estradiol as a tracer,[17] and is expressed as an RBA value.[5a] In principle, such an assay is valid only for reversibly binding ligands; however, we have found it useful in characterizing an "apparent RBA" for irreversibly binding ligands.[2a,18] The RBA values, or apparent RBA values (in the case of irreversible binding), of the hexestrol derivatives we have prepared are summarized in Table I.

(14) Brown, H. C.; Rei, M. *J. Am. Chem. Soc.* **1969**, *68*, 481.
(15) Rapoport, L.; Smith, A.; Newman, M. S. *J. Am. Chem. Soc.* **1969**, *68*, 481.
(16) For the preparation of unsaturated acid related to **36** (i.e., in the diethylstilbestrol series), see ref 8.
(17) (a) Berthois, Y.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 2496–2500. (b) Katzenellenbogen, J. A.; Johnson, H. J.; Meyers, H. N. *Biochemistry* **1973**, *12*, 4085.
(18) Katzenellenbogen, J. A.; McGorrin, R. J.; Tatee, T.; Kempton, R. J.; Carlson, K. E.; Kinder, D. H. *J. Med. Chem.* **1981**, *24*, 435–450.

Scheme IV



36 R_1=CH_3, R=H
37 R_1, R=H (92%)
38 R_1, R=TBDMS (72%)
39
40 R_1=TBDMS (88%)
41 R_1=H (67%)
11 R=Az (23%)
12 R=Piperazino (47%)

Of the carbonyl-linked analogues, the acyl aziridine **7** had the highest affinity. The C_2-linked ester **6** and C_4-linked ketone **3** have substantial affinities, with that of the C_3-linked ester **5** somewhat lower. Lowest in this series is the dimethylamino analogue of the C_4-linked ketone aziridine, compound **4**.

There is about a threefold difference in the apparent RBAs of the two thioether-linked aziridines **8** and **10**, and, in this series, the dimethylamino analogue **9** demonstrates higher affinity than the related aziridine **8**. The two methylene-linked analogues **11** and **12** demonstrate reasonably high affinity, with the reversibly binding piperazino analogue **12** showing higher affinity than the related aziridine **11**.

If one looks at the structure–binding affinity data simply in terms of the number of atoms intervening between the bibenzyl system of hexestrol and the aziridine function, somewhat higher affinities are associated with a four-atom linkage (9.6%, average of compounds **6**, **10**, and **11**) vs. a five-atom linkage (4.4%, average of compounds **3**, **5**, and **8**). It is difficult to make any more detailed comparisons between the RBA/apparent RBA values of these compounds, because the irreversible binding of some of the aziridines is, most likely, distorting the estimate of competitive binding affinity. While one might imagine that the covalent reaction of the aziridines with receptor would increase the apparent RBA of these compounds, covalent binding to other nonreceptor proteins might reduce it.

Time-Dependent Irreversible Binding to the Estrogen Receptor. Although it is not possible to demonstrate directly that nonradiolabeled ligands for the estrogen receptor are becoming covalently attached to the receptor, we can obtain an indirect indication of such a reaction by measuring the time-dependent irreversible binding of ligands to receptor. Soluble (cytosol) preparations of receptor are exposed to a saturating concentration of the aziridine (150–1700 nM, calculated to be equivalent to 30 nM estradiol), and aliquots, removed periodically and freed of excess aziridine by treatment with charcoal dextran, are then incubated with an excess of [^{3}H]estradiol to assay remaining binding activity.[2a] Control assays are performed by incubating cytosol with 30 nM estradiol to determine the stability of the receptor and with compound plus 3000 nM estradiol to indicate the level of nonspecific irreversible binding. The degree of specific irreversible binding is considered to be the difference between the total and nonspecific binding and is expressed as a percent of the estradiol control level. Irreversible binding assays were also performed in intact MCF-7 cells in culture, by using an analogous protocol. The results of these experiments in cytosol preparations are shown in Figure 1A and in MCF-7 cells in Figure 1B.

In both uterine cytosol and MCF-7 cells, three aziridines (**3**, **8**, and **11**) show rapid irreversible binding that is maximal by 1–4 h and is comparable to that of tamoxifen aziridine (TAZ).[2a] Two aziridines (**5** and **6**) are slower and less complete inactivators, and two (**7** and **10**) show no significant inactivation at all.

There are a number of notable comparisons: The thioether-linked aziridine **10** shows no irreversible binding, yet its methylene isostere **11** and its homologue **8** are reactive. The acyl aziridine **7**, while nominally considered more reactive toward ring opening because of the negative charge stabilizing capacity of the *N*-acyl group,[19] is not an irreversible binder, despite its good RBA value.

Hydrolytic Stability. The hydrolytic stability of selected aziridines in tissue culture medium (IMEM) supplemented with 5% charcoal dextran treated calf serum at 37 °C was determined by HPLC analysis. The aziridines were relatively stable, giving half-lives that ranged from 13 h (for aziridine **8**) to 19–23 h (for aziridines **3**, **5**, and **6**). Thus, this hydrolysis process probably has little effect on the bioassay of these compounds in cell culture.

Estrogenic vs. Antiestrogenic Activity of Hexestrol Aziridines. The estrogen-responsive human breast cancer MCF-7 cell line provides a convenient system for assaying the estrogenic vs. antiestrogenic activity of the aziridine derivatives.[20] The growth rate of the cells over an 8-day period is measured while they are exposed to various concentrations of the aziridine: Agonist activity is measured as an increase in growth rate over that of control cells and is compared to the activity of the agonist standard, estradiol. Antagonist activity is measured as the suppression of estradiol-stimulated MCF-7 cell growth and is compared to that of the antiestrogen *trans*-hydroxytamoxifen.

An example of a cell-proliferation assay is shown in Figure 2. The proliferation of MCF-7 cells is stimulated many-fold by 10^{-9} M estradiol; *trans*-hydroxytamoxifen

(19) *Ethylenimine and Other Aziridines*; Dermer, O. C., Ham, G. E., Eds.; Academic: New York, 1969; pp 226–227.
(20) Katzenellenbogen, B. S.; Norman, M. J.; Eckert, R. E.; Peltz, S. W.; Mangel, W. F. *Cancer Res.* **1984**, *44*, 112–119.

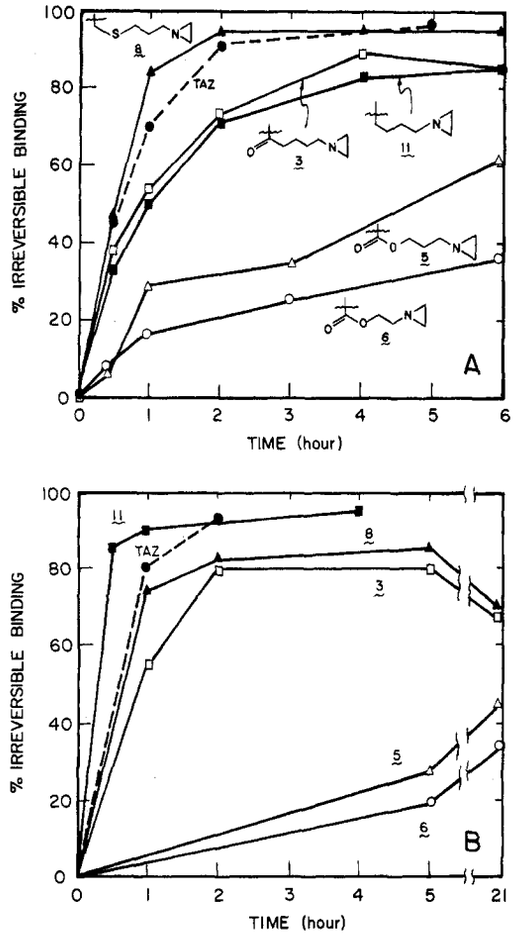


Figure 1. Time course of irreversible binding of hexestrol aziridine derivatives to estrogen receptor in uterine cytosol preparations and in MCF-7 cells. Panel A: Cytosol preparations from lamb uterus were incubated with the indicated aziridines at 25 °C in the presence of 5% dimethylformamide at a concentration equivalent to 30 nM estradiol (30/RBA × 100 nM) (360 nM **3**, 900 nM **5**, 340 nM **6**, 1700 nM **8**, 215 nm **11**, 150 nM TAZ). Aliquots were removed, treated with charcoal dextran to remove excess aziridine, and then incubated with 30 nM [^{3}H]-estradiol in the absence and presence of a 100-fold excess of unlabeled estradiol (to determine nonspecific binding) for 24 h at 0 °C. Binding after exchange was determined by charcoal adsorption. Control assays contained either 30 nM estradiol (receptor stability) or the aziridine plus 3000 nM estradiol (nonspecific irreversible binding). The percent irreversible binding is calculated as the specific irreversible binding (total minus nonspecific) and is corrected for receptor stability. Panel B: MCF-7 cells were treated at 37 °C with the indicated aziridines (400 nM). At the times indicated, cells were removed from the flasks, whole cell receptor extracts were prepared, and reversible estrogen receptor binding activity was assayed by incubation with 30 nM [^{3}H]estradiol (in the absence and presence of a 100-fold excess of unlabeled estradiol) for 20 h at 21 °C.

at 10^{-7} M causes only minimal stimulation and is effective in suppressing the stimulation by estradiol. The effects of two agonists, aziridines **3** and **8**, are shown: By themselves at 3×10^{-7} M, both compounds stimulate cell proliferation; aziridine **3** is more effective than aziridine **8**, but neither is as effective as estradiol. Neither compound blocks the response induced by estradiol, but both are suppressed by *trans*-hydroxytamoxifen. The activity of aziridine **11** is that of an antagonist; it causes no increase in cell proliferation, and it suppresses the stimulation by estradiol.

The agonist–antagonist activity of the hexestrol aziridines and related compounds is summarized in Table I,

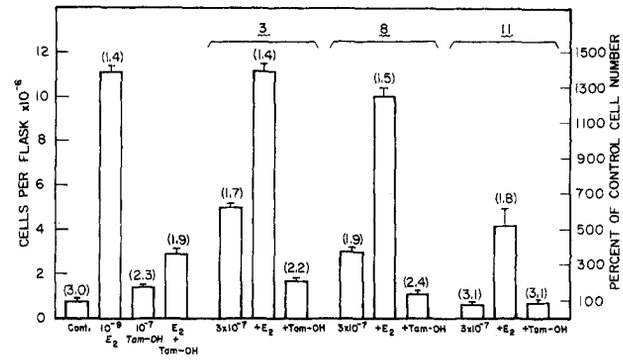


Figure 2. MCF-7 cell proliferation assays for evaluation of the estrogen agonist and antagonist activity of hexestrol aziridine derivatives. MCF-7 cells, grown in T-25 flasks, were treated every 48 h with 0.1% control vehicle (control) or with 10^{-9} M estradiol, 10^{-7} M *trans*-hydroxytamoxifen, or 3×10^{-7} M hexestrol aziridines **3**, **8**, and **11**, alone and in the combinations listed. The number of cells/flask were determined after 8 days of growth. Data are expressed as cells/flask and percent of control cell number; the number in parentheses is the cell-doubling time in days.

and an indication is made of their potency (see Table I, footnotes *c* and *d*). Except for compounds **11** and **12**, all the derivatives are agonists; however, their potencies vary widely. The most potent agonists appear to be the carbonyl-linked aziridines **5**–**7**. Keto-nonestrol aziridine **3** is nearly as potent an agonist and is somewhat more potent than the thioether-linked derivatives **8**–**10**. The dimethylamine **4** is the weakest agonist. The only compound to demonstrate antagonist activity is the methylene-linked aziridine **11**; its activity is weak, however. The piperazine analogue, compound **12**, had very little demonstrable agonist or antagonist activity.

Therefore, of the aziridine derivatives studied, keto-nonestrol aziridine **3** has the best combination of a good apparent RBA, effective irreversible binding, and substantial potency as an estrogen agonist. The next most suitable compound, the thioether **8**, is slightly less potent an agonist and has a lower apparent RBA.

Discussion

To date, there is only one efficient and selective covalent labeling agent for the estrogen receptor, tamoxifen aziridine,[2] but it is an estrogen antagonist, as is tamoxifen.[3] In this paper, we describe the synthesis of a number of analogues of the nonsteroidal estrogen hexestrol that bear an aziridine function on the side chain. We selected the nonsteroidal estrogen hexestrol as the basic ligand for these studies because hexestrol itself is a very potent estrogen and because the estrogen receptor binds a large number of substituted hexestrol derivatives with high affinity.[6] One of the compounds studied, keto-nonestrol aziridine **3**, is an efficient and rapid irreversible binder and an agonist.

While we have found that several of the side-chain-substituted hexestrol aziridines are capable of time-dependent irreversible binding to the estrogen receptor, this is not true in every case. In fact, the derivatives **7** and **10** appear to be quite ineffective as irreversible binders, and closely related analogues vary widely in effectiveness (e.g., **8** and **3** vs. **5**; **11** vs. **6**). In addition, two steroidal aziridine derivatives, prepared by the Organon company, bind reversibly, not irreversibly, to the estrogen receptor.[13] Thus, the capacity to bind irreversibly to the estrogen receptor requires more than simply the presence of an aziridine function on a reversibly binding ligand.

In our studies on tamoxifen aziridine, we speculated that the high covalent labeling efficiency and selectivity of this

compound and the ineffectiveness of external nucleophile scavengers suggested that the aziridine function was being "activated" upon binding to the receptor, perhaps through a specific interaction with an acidic residue that protonates it to form the highly reactive aziridinium species, which is the actual alkylating agent.[2b] If such a process were occurring, one could imagine that small structural differences, ones that might not affect the binding affinity in a major way, could still affect the proper alignment required for activation and alkylation and thus reduce the rate and efficiency of irreversible binding. This may well explain the widely differing effectiveness and rates of irreversible binding of compounds noted above.

The estrogen-responsive human breast cancer cell line (MCF-7) has been widely used to assess the estrogen agonist and antagonist activity and potency of many compounds.[20] In this assay system, the hexestrol aziridine derivatives we have prepared display varied activities: All the carbonyl-linked and thioether-linked derivatives are agonists, though their potency varies widely; the methylene-linked derivatives were either antagonistic or very weak altogether. Most potent estrogen *antagonists* are compounds having a *tri*aryl ethylene or ethane structure with a basic side chain.[21] It is of note that the derivatives we have prepared are *di*aryl ethane derivatives with a basic side chain, and all (save two) are *agonists*. This suggests that the presence of *three* aryl groups is an essential component to effective *antagonist* activity of compounds in the arylethylene/ethane class.

In conclusion, we have been successful in preparing a hexestrol aziridine derivative, keto-nonestrol aziridine **3**, that binds irreversibly to the estrogen receptor, rapidly and efficiently, and that is estrogenic. In work to be presented elsewhere, we have prepared this compound in high-specific-activity tritium-labeled form and have shown that it does label the estrogen receptor covalently.[7]

Experimental Section

Flash chromatography[22] was performed by using Woelm silica gel (32–64 μm). TLC and preparative TLC were performed on glass-backed silica gel plates (Merck, 0.25 and 2 mm, respectively). High-performance liquid chromatography (HPLC) was performed on either a Spectra-Physics 8700 solvent delivery system using a Beckman 153 ultraviolet detector (280 nm) or a Varian 5060 system using a Perkin-Elmer LC-75 spectrophotometric detector (275 nm). Columns and solvent systems used were, respectively: column A (0.4 × 30 cm Varian MCH-10 ODS), column B (0.9 × 50 cm Whatman ODS 10/50); solvent system A (CH_3CN/buffer (2% aqueous $HNEt_2$ titrated with phosphoric acid to pH 7.8), 63:37, 1 mL/min), solvent system B (methanol/buffer (2% aqueous $HNEt_2$ titrated with phosphoric acid to pH 8.3), 9:1, 1 mL/min). All compounds submitted for biological testing were purified by semipreparative HPLC and were determined to be ≥95% one component upon reinjection onto an analytical HPLC column (A).

Except where mentioned otherwise, a standard procedure was used for product isolation; this involved quenching by addition to water or an aqueous solution, exhaustive extraction with an organic solvent, washing the extracts with aqueous solutions if necessary, drying with $MgSO_4$ or Na_2SO_4, filtration, and evaporation of solvent under reduced pressure. The particular solvents and aqueous washes used are mentioned in parentheses after the phrase "product isolation".

The following NMR resonances characteristic of the norhexestrol backbone are described here and omitted in the experimental description of each compound: δ 0.52 (t, 3 H, J = 6 Hz, CH_2CH_3 R^*,S^* diastereomer), 0.70 (t, 3 H, J = 6 Hz, CH_2CH_3 R^*,R^* diastereomer), 1.10–1.80 ($(CH_2)_n$), 2.95–3.95 (m, benzylic protons), 6.50–7.61 (m, 8 H, Ar *H*). Elemental analyses were performed by the Microanalytical Services Laboratory of the University of Illinois School of Chemical Sciences.

Caution: Ethylenimine, used in the preparation of compounds **3**, **5–8**, **10**, and **11**, is a volatile, reactive, carcinogenic, and actively toxic substance.[19] All manipulations involved in its preparation, purification, and use should be performed in a well-ventilated fume hood, providing protection against explosion. A full-face gas mask with a fresh ammonia (silica gel) canister is recommended in the event of inadequate ventilation. The ammonia-like odor threshold is reported as 2 ppm, but a threshold limit of 0.5 ppm in air for continuous exposure has been set. Dry ethylenimine may be stored in the freezer, either neat (over potassium hydroxide pellets) or as a solution in an inert solvent (e.g., toluene) for several months without decomposition.

(6*R,7*S**)- and (6*R**,7*R**)-6,7-Bis(4-methoxyphenyl)-1-nonen-5-one (14).** To 3.254 g (135.6 mmol) of magnesium turnings in 250 mL of dry benzene and 4 mL of dry ether at room temperature under nitrogen was added dropwise over 0.5 h 18.30 g (135.6 mmol) of 4-bromo-1-butene in 50 mL of dry benzene. The mixture was brought to reflux for 2 h. In a separate reaction flask, 5.00 g (16.95 mmol) of nitrile **13**[9] was dissolved in 50 mL of dry benzene by heating to reflux. Once dissolution occurred, the hot solution of nitrile **13** was immediately transferred in one portion to the refluxing Grignard solution followed by continued reflux for 2 h. After being cooled to 0 °C, the solution was poured into ice/dilute HCl (300 g of ice, 50 mL of concentrated HCl) and stirred for 20 min. Product isolation (pentane, EtOAc, $NaHCO_3$, brine) and repeated flash chromatography (silica gel, ethyl acetate/hexane, 1:4) afforded 4.53 g (76%) of ketone **14** (both diastereomers, 1:1) as a white solid: mp 89–90 °C; ^1H NMR of both diastereomers ($CDCl_3$, 200 MHz) δ 3.65 and 3.80 (2 s, 6 H, OCH_3), 4.52–5.0 (m, 2 H, $RCH{=}CH_2$), 5.1–5.8 (m, 1 H, $CH{=}CH_2$); IR (Nujol mull) 1710 cm^{-1}; MS (10 eV), m/z (relative intensity) 204 (12), 149 (100). Anal. ($C_{23}H_{28}O_3$) C, H.

(6*R,7*S**)-6,7-Bis(4-hydroxyphenyl)-1-nonen-5-one (15).** *n*-Butyllithium (1.388 mL of a 2.05 M solution in hexane, 2.845 mmol) was added dropwise to *n*-butanethiol (307 mg, 3.409 mmol) in 10 mL of dry hexamethylphosphoramide (HMPA) at room temperature. This resulted in a rapid evolution of butane and the formation of a white precipitate. After the mixture was stirred for 10 min, 300 mg (0.852 mmol) of the bis(methyl ether) **14** in 2 mL of HMPA was added in one portion, and the reaction mixture was heated to 130 °C for 24 h. After being cooled to 25 °C, the reaction was quenched by the addition of 20 mL of 1 N HCl. Product isolation (EtOAc, H_2O, brine) and chromatography (ethyl acetate/hexane, 2:3) afforded 238 mg (86%, R_f 0.23) of the bisphenol **15** as a clear oil; trituration with hexane/chloroform (3:1, 2 mL) gave 184 mg (67%) of **15** as a white solid: mp 154–155 °C; ^1H NMR (acetone-d_6, 220 MHz) δ 1.85–2.47 (m, 4 H, CH_2CO and $CH_2CH{=}CH_2$), 4.68–4.79 (m, 2 H, $CH{=}CH_2$), 5.39–5.61 (m, 1 H, $CH{=}CH_2$); IR (KBr) 3600–3200, 1700 cm^{-1}; MS (70 eV), m/z (relative intensity) 324 (M^+, 5), 190 (59), 135 (100), 107 (66); exact mass calcd for $C_{21}H_{24}O_3$ 324.1726, found 324.1735. Anal. ($C_{21}H_{24}O_3$) C, H.

(6*R,7*S**)-6,7-Bis[4-(*tert*-butyldimethylsiloxy)phenyl]-1-nonen-5-one (16).** The general procedure of Corey and Venkateswarlu[23] was used to afford 540 mg (93%) of the bis-(*tert*-butyldimethylsilyl phenolic ether) **16** as a clear oil: ^1H NMR ($CDCl_3$, 220 MHz) δ 0.18 and 0.22 (2 s, 12 H, 2 $Si(CH_3)_2$), 1.00 (s, 18 H, 2 SiC $(CH_3)_3$), 1.84–2.43 (m, 4 H, CH_2CO and $CH_2CH{=}CH_2$), 4.68–4.83 (m, 2 H, $CH{=}CH_2$), 5.37–5.56 (m, 1 H, $CH{=}CH_2$); IR (neat) 1715, 1640, 1250 cm^{-1}; MS (70 eV), m/z (relative intensity) 552 (M^+, observable on offscale trace only), 469 (1), 249 (100); exact mass calcd for $C_{33}H_{52}O_3Si_2$ 552.3469, found 552.3462. Anal. ($C_{33}H_{52}O_3Si_2$) C, H.

(6*R,7*S**)-1-Hydroxy-6,7-bis[4-(*tert*-butyldimethylsiloxy)phenyl]-5-nonanone (17).** To a solution of 846.4 mg (1.533 mmol) of 6*R**,7*S** alkene **16** in 5 mL of tetrahydrofuran was added

(21) (a) Jordan, V. C. *Pharmacol. Rev.* **1984**, *36*, 245–276. (b) Crawley, G. C. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed.; Wiley: New York, 1980; Vol. 12, pp 658–691.

(22) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(23) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190–6191.

9-borabicyclo[3.3.1]nonane (9.20 mmol, 0.5 M solution in tetrahydrofuran) over 4 min at 60 °C under nitrogen. After 40 min, the solution was cooled to 0 °C; 3 mL of water was added, and stirring was continued for 5 min. Sodium hydroxide (3 mL of 3 N) was added, and after an additional 5 min, 3 mL of aqueous hydrogen peroxide (30%) was added dropwise. After 30 min, product isolation ($NaHCO_3$, EtOAc, brine) and chromatography (ethyl acetate/hexane, 2:3) afforded 667 mg (76%; R_f 0.50) of the primary alcohol **17** as a white solid: mp 75–77 °C; ^1H NMR ($CDCl_3$, 220 MHz) δ 0.18 and 0.22 (2 s, 12 H, 2 $Si(CH_3)_2$), 1.00 (s, 18 H, 2 $SiC(CH_3)_3$), 1.84–2.40 (m, 2 H, CH_2CO), 3.28 (m, 2 H, CH_2OH); IR (Nujol mull) 3330 (w), 1712 cm^{-1}; MS (70 eV), m/z (relative intensity) 570 (M^+, observable on offscale trace only), 303 (6), 249 (100), 73 (47); exact mass calcd for $C_{33}H_{54}O_4Si_2$ 570.3577, found 570.3569. Anal. ($C_{33}H_{54}O_4Si_2$) C, H.

(6*R,7*S**)-5-Oxo-6,7-bis[4-(*tert*-butyldimethylsiloxy)-phenyl]-1-nonanyl Methanesulfonate (18).** To a solution of 55 mg (0.0965 mmol) of 6*R**,7*S** primary alcohol **17** in 1 mL of tetrahydrofuran at room temperature under nitrogen were added 97 mg (0.965 mmol) of triethylamine and 55 mg (0.482 mmol) of methanesulfonyl chloride. A white precipitate formed immediately, and the solution was stirred for an additional 20 min. Product isolation ($NaHCO_3$, EtOAc, brine) and purification by passage through a silica gel pad (2 g, EtOAc/hexane, 1:9) afforded 59 mg (95%) of the primary mesylate **18** as a clear oil: ^1H NMR ($CDCl_3$, 220 MHz) δ 0.16 and 0.18 (2 s, 12 H, 2 $Si(CH_3)_2$), 0.95 (s, 18 H, 2 $SiC(CH_3)_3$), 1.82–2.38 (m, 2 H, CH_2CO), 2.94 (s, 3 H, CH_3SO_3); MS (70 eV), m/z (relative intensity) 523 (4), 304 (70), 249 (84), 153 (100).

(6*R,7*S**)-5-Oxo-6,7-bis(4-hydroxyphenyl)-1-nonanyl Methanesulfonate (19).** To a solution of 231 mg (0.356 mmol) of bis(*tert*-butyldimethylsilyl ether) **18** in 1 mL of ethyl acetate was added 300 mg (1.577 mmol) of *p*-toluenesulfonic acid monohydrate. After 20 min at 40 °C, product isolation (H_2O, EtOAc) and trituration with methylene chloride afforded 90 mg (60%) of the bisphenolic primary mesylate **19** as a white solid: mp 128–129 °C; ^1H NMR (acetone-d_6, 220 MHz) δ 2.00–2.42 (m, 2 H, $COCH_2$), 3.03 (s, 3 H, CH_3SO_3), 3.97–4.04 (m, 2 H, CH_2OMs); IR (neat) 3550–3150, 1700 cm^{-1}; MS (FAB), m/z 443 (M + Na), 421 (M + 1), 295, 134; exact mass calcd for $C_{22}H_{29}O_6S$ 421.1685, found 421.1689. Anal. ($C_{22}H_{28}O_6S$) C, H, S.

Ethylenimine. (See cautioning note at the start of the Experimental Section.) The flash distillation procedure of Reeves et al.[24] was followed, with 200 g (1.56 mol) of aminoethyl hydrogen sulfate. The authors report an 83% yield, but this is a crude yield with no drying. In our case, 26.80 g (40%) of pure ethylenimine was obtained after one distillation from sodium hydroxide pellets: bp 56–58 °C (lit.[24] bp 56 °C); ^1H NMR ($CDCl_3$, 90 MHz) δ 1.26 (s, CH_2). Ethylenimine is stable for months when stored over KOH in the freezer to prevent polymerization.

(6*R,7*S**)-1-*N*-Aziridinyl-6,7-bis(hydroxyphenyl)-5-nonanone (3).** To a solution of 63 mg (0.15 mmol) of primary methanesulfonate **19** in acetonitrile/triethylamine (0.60 mL, 1:1) was added 311 μL (258 mg, 6 mmol) of ethylenimine at room temperature. The reaction was monitored by TLC analysis (silica gel, ethanol/chloroform, 1:9), showing the complete conversion of primary mesylate (R_f 0.40) to aziridine compound (R_f 0.19) after 20 h (polymer at origin). The solution was diluted with ethanol/chloroform (5 mL, 1:9) and passed through a pad of silica gel (2 g) to afford **3** as a pale yellow solid (30 mg, 54%), mp 140–144 °C. Further purification prior to biological testing was effected by semipreparative HPLC (column B, system A, 3 mL/min) to afford 25.5 mg (46%) of aziridine **3**: ^1H NMR (acetone-d_6, 200 MHz) δ 0.91–0.93 (m, 2 H, aziridine protons anti to lone pair), 1.47–1.49 (m, 2 H, aziridine protons syn to lone pair), 1.89 (t, 2 H, J = 7 Hz, CH_2N), 1.95–2.42 (m, 2 H, CH_2CO); IR (neat) 1705 cm^{-1}; MS (FAB), m/z 368 (M + 1), 134; exact mass calcd for $C_{23}H_{30}NO_3$ 368.2226, found 368.2213.

(6*R,7*S**)-*N*,*N*-Dimethyl-5-oxo-6,7-bis(4-hydroxyphenyl)-1-nonanamine (4).** To a solution of 54 mg (0.129 mmol) of primary methanesulfonate **19** in acetonitrile/triethylamine (2 mL, 1:1) was added 1 mL (0.68 g, 15.1 mmol) of anhydrous dimethylamine at 25 °C. After 24 h, purification by preparative TLC (ethanol/chloroform, 1:1) afforded 20.9 mg (44%) of **4** as a white solid (>98% pure by HPLC, column A, system B): mp 185–187 °C dec; ^1H NMR (CD_3OD, 300 MHz) δ 2.01 (t, 2 H, J = 7 Hz, $COCH_2$), 2.08 (s, 6 H, $N(CH_3)_2$), 3.29–3.34 (m, 2 H, CH_2N); IR (KBr) 3550–3300, 1700, 1610 cm^{-1}; MS (FAB), m/z (relative intensity) 370 (M + 1, 53), 135 (57), 119 (100); exact mass calcd for $C_{23}H_{32}NO_3$ 370.2408, found 370.2395.

3-Bromopropyl (2*R,3*S**)-2,3-Bis(4-acetoxyphenyl)pentanoate (21).** To a solution of acid chloride **20**[6b] (300 mg, 0.773 mmol) in 10 mL of dry THF at room temperature under nitrogen was added triethylamine (114.5 mg, 1.134 mmol) followed by 3-bromo-1-propanol (430 mg, 3.092 mmol). After 2 h, 3 mL of ethyl acetate was added, and the reaction mixture was purified directly by flash chromatography (ethyl acetate/hexane, 1:4) to afford primary bromide **21** (50.0 mg, 33%) as a white solid: mp 97–98 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.29 and 2.31 (2 s, 6 H, CH_3CO), 3.04 (t, 2 H, J = 7 Hz, CH_2Br), 3.75–4.08 (m, 2 H, $RCO_2CH_2CH_2$); IR (KBr) 1755, 1730 cm^{-1}; MS (FAB), m/z (relative intensity) 493 (M + 3, 29), 491 (M + 1, 38), 413 (15), 135 (100). Anal. ($C_{24}H_{27}O_6Br$) C, H, Br.

2-Iodoethyl (2*R,3*S**)-2,3-Bis(4-acetoxyphenyl)pentanoate (22).** Ester **22** was obtained as a white solid from acid chloride **20** in 76% yield (225 mg) by the procedure described for compound **21**: mp 106–107 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.29 and 2.31 (2 s, 6 H, CH_3CO), 2.84 (dt, 2 H, J = 2 and 7 Hz, $CO_2CH_2CH_2I$), 3.91 (dt, 2 H, J = 2 and 7 Hz, $COCH_2CH_2I$); IR ($CHCl_3$) 1780–1700 (br C=O) cm^{-1}; MS (FAB), m/z 369, 325, 127. Anal. ($C_{23}H_{25}O_6I$) C, H.

(2*R,3*S**)-1-[2,3-Bis(4-acetoxyphenyl)-1-pentanoyl]aziridine (23).** 2*R**,3*S** Acid chloride **20**[6b] (0.154 mmol) was added as a solid to 66.5 mg (1.54 mmol, 80 μL) of ethylenimine (see cautioning note at the start of the Experimental Section) and 155 mg (1.54 mmol, 216 μL) of triethylamine in 2 mL of ether at −4 °C. After being stirred for 20 min at −4 °C, the reaction mixture was allowed to warm to room temperature and was stirred for an additional hour. After the addition of 5 mL of ethyl acetate, the amine hydrochloride was filtered and washed with an additional 5 mL of ethyl acetate. The solvent was removed in vacuo from the collected filtrate to afford 58.6 mg (98%) of acyl aziridine **23** as a white solid: mp 138–139 °C; ^1H NMR (acetone-d_6, 220 MHz) δ 1.59–1.61 (m, 2 H, aziridine protons), 1.74–1.76 (m, 2 H, aziridine protons), 2.28 and 2.31 (2 s, 6 H, CH_3CO); IR (KBr) 1762, 1680 cm^{-1}; MS (70 eV), m/z (relative intensity) 396 (M^+ + 1, 1), 395 (M^+, 4), 135 (100); exact mass calcd for $C_{23}H_{25}NO_5$ 395.1732, found 395.1712.

3-Bromopropyl (2*R,3*S**)-2,3-Bis(4-hydroxyphenyl)pentanoate (24).** To a solution of bis(acetoxyphenol) compound **21** (136.0 mg, 0.277 mmol) in 2 mL of a methanol/tetrahydrofuran (1:1) solution was added 1 mL of saturated aqueous potassium carbonate at room temperature. After 30 min, product isolation (buffer pH 7.8, EtOAc) and purification by flash chromatography (ethyl acetate/hexane, 2:3) afforded 108 mg (96%) of bromide **24** as a pale yellow solid: mp 60–61.5 °C; ^1H NMR (acetone-d_6, 200 MHz) δ 3.04 (t, 2 H, J = 7 Hz, CH_2Br), 3.62–4.15 (m, 2 H, RCO_2CH_2), 7.91 and 8.09 (2 s, 2 H, Ar OH); IR (KBr) 3550–3330, 1710, 1610 cm^{-1}; MS (FAB), m/z (relative intensity) 409 (M + 3, 58), 407 (M + 1, 63), 285 (63), 135 (100); exact mass calcd for $C_{20}H_{24}O_4{}^{79}Br$ 407.0858, found 407.0840.

2-Iodoethyl (2*R,3*S**)-2,3-Bis(4-hydroxyphenyl)pentanoate (25).** See procedure for compound **24**; 81 mg (96%) of iodide **25** as a pale yellow solid: mp 49–50 °C; ^1H NMR (acetone-d_6, 200 MHz) δ 2.98 (dt, 2 H, J = 2 and 7 Hz, $CO_2CH_2CH_2I$), 3.91 (dt, J = 2 and 7 Hz, $CO_2CH_2CH_2I$), 8.30 (br s, 2 H, Ar OH); IR (KBr) 3600–3330, 1730–1710 cm^{-1}; MS (70 eV), m/z (relative intensity) no molecular ion peak, 249 (98), 241 (46), 177 (52), 135 (100).

3-*N*-Aziridinylpropyl (2*R,3*S**)-2,3-Bis(4-hydroxyphenyl)pentanoate (5).** See procedure for compound **3**. Aziridine **5** was obtained in 13% yield (5.0 mg) after reversed-phase-HPLC purification (column B, system A, 3 mL/min) as a white solid: mp 110–111 °C; ^1H NMR (acetone-d_6, 200 MHz) δ 0.87–0.89 (m, 2 H, aziridine protons anti to lone pair), 1.47–1.49 (m, 2 H, aziridine protons syn to lone pair), 1.95–2.10 (m, 2 H, CH_2N overlapped by acetone-d_6), 3.75–3.95 (m, 2 H, $RCO_2CH_2CH_2$); IR (KBr) 3550–3300, 1730–1700, 1610 cm^{-1}; MS

(24) Reeves, W. A.; Drake, G. L., Jr.; Hoffpavir, C. L. *J. Am. Chem. Soc.* **1951**, *73*, 3522.

(FAB), m/z 370 (M + 1), 135; exact mass calcd for $C_{22}H_{28}NO_4$ 370.2018, found 370.2029.

2-*N*-Aziridinylethyl (2*R,3*S**)-2,3-Bis(4-hydroxyphenyl)pentanoate (6).** See procedure for compound **3**. Aziridine **6** was obtained in 21% yield (4.5 mg) after reversed-phase-HPLC purification (column B, system A, 3 mL/min) as a white solid: mp 118–119 °C; ^{1}H NMR (acetone-d_6, 200 MHz) δ 0.92–0.94 (m, 2 H, aziridine protons anti to lone pair), 1.45–1.47 (m, 2 H, aziridine protons syn to lone pair), 1.95–2.10 (m, 2 H, overlapped by acetone-d_6, $CO_2CH_2CH_2N$), 3.84 (dt, 2 H, J = 6 and 3 Hz, $RCO_2CH_2CH_2NR_2$); IR (KBr) 3600–3330, 1705 cm^{-1}; MS (FAB), m/z 356 (M + 1), 279; exact mass calcd for $C_{21}H_{26}NO_4$ 356.1862, found 356.1857.

(2*R,3*S**)-1-[2,3-Bis(4-hydroxyphenyl)-1-pentanoyl]aziridine (7).** Acyl aziridine **23** (15 mg, 0.038 mmol) was dissolved in 2 mL of methanol, and 1 mL of saturated aqueous K_2CO_3 was added. The reaction mixture was stirred for 1 h at room temperature, at which point the solution was neutralized (pH ~8.2) with a phosphate buffer, and the methanol was removed in vacuo. After the addition of 15 mL of water to the buffered solution, product isolation (EtOAc, brine) afforded 10.7 mg (90%) of **7** as a white solid: mp 216–217 °C; ^{1}H NMR (acetone-d_6, 220 MHz) δ 1.55–1.57 (m, 2 H, aziridine protons), 1.76–1.78 (m, 2 H, aziridine protons); IR (KBr) 3500–3150, 1635, 1610 cm^{-1}; MS (70 eV), m/z (relative intensity) 311 (M$^+$, 1), 268 (1), 107 (100); exact mass calcd for $C_{19}H_{21}NO_3$ 311.1521, found 311.1515.

(2*R,3*S**)-2,3-Bis[4-(*tert*-butyldimethylsiloxy)phenyl]pentyl 2-Carbomethoxyethyl Sulfide (27).** To a solution of thiol **26**[6c] (800 mg, 1.55 mmol) in 5 mL of anhydrous dioxane under nitrogen at 45 °C were added methyl acrylate (666 mg, 7.75 mmol) and solid sodium methoxide (6.0 mg, 0.11 mmol). The reaction mixture was stirred for 1 h at 45 °C, cooled to room temperature, and purified directly by flash chromatography (ethyl acetate/hexane, 1:9) to afford methyl ester **27** as a clear oil (741 mg, 79.4%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.20 (s, 12 H, 2 Si(CH_3)$_2$), 0.99 (s, 18 H, 2 SiC(CH_3)$_3$), 2.31 (t, 2 H, J = 6 Hz, CH_2CO_2Et), 2.35 (t, 2 H, J = 6 Hz, SCH_2), 2.48 (distorted d, 2 H, J = 7 Hz, $CHCH_2S$), 3.61 (s, 3 H, CO_2CH_3); IR ($CHCl_3$) 1735, 1260, 840 cm^{-1}; MS (FAB), m/z (relative intensity) 587 (12), 545 (48), 249 (100).

(2*R,3*S**)-2,3-Bis[4-(*tert*-butyldimethylsiloxy)phenyl]pentyl 3-Hydroxypropyl Sulfide (28).** To a cold (0 °C) solution of methyl ester **27** (1.02 g, 1.69 mmol) in 20 mL of dry tetrahydrofuran under nitrogen was added lithium aluminum hydride (5.1 mL of a 0.4 M solution in tetrahydrofuran, filtered through dry Celite under nitrogen) in one portion. After 20 min, product isolation (5% Na_2HPO_4, EtOAc, brine) and purification by flash chromatography (ethyl acetate/hexane, 1:4) afforded primary alcohol **28** (590 mg, 60.6%) as a white solid: mp 86–87 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.20 (s, 12 H, 2 Si(CH_3)$_2$), 0.99 (s, 18 H, 2 SiC(CH_3)$_3$), 2.23 (t, 2 H, J = 7 Hz, SCH_2CH_2), 2.48 (distorted d, 2 H, J = 7 Hz, $CHCH_2S$), 3.54 (t, 2 H, J = 6 Hz, CH_2OH); IR ($CHCl_3$) 3605, 3520–3320, 1600 cm^{-1}; MS (FAB), m/z 575 (M + 1), 483, 325, 249. Anal. ($C_{32}H_{54}O_3SSi_2$) C, H, S.

(2*R,3*S**)-2,3-Bis[4-(*tert*-butyldimethylsiloxy)phenyl]pentyl Carbethoxymethyl Sulfide (29).** To a cold (−78 °C) solution of thiol **26**[5c] (350 mg, 0.678 mmol) in 5 mL of dry tetrahydrofuran under nitrogen were added ethyl bromoacetate (1.133 g, 6.78 mmol) and a subsequent excess of potassium hydride (~100 mg, 2.50 mmol, rinsed with THF). After 15 min at −78 °C, the solution was stirred for 1 h while being allowed to warm slowly to room temperature. Product isolation (5% Na_2HPO_4, EtOAc, brine) and purification by preparative TLC (ethyl acetate/hexane, 1:9, two plates) afforded 233 mg (76% based on starting material consumed) of ester **29** as a clear oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.20 (s, 12 H, 2 Si(CH_3)$_2$), 0.99 (s, 18 H, 2 SiC(CH_3)$_3$), 1.18 (t, 3 H, J = 7 Hz, OCH_2CH_3), 2.66 (d, 1 H, J = 7 Hz, $CHCH_2S$), 2.77 (s, 1 H, $SCHHCO_2Et$), 2.82 (s, 1 H, $SCHHCO_2Et$), 4.04 (q, 2 H, J = 7 Hz, OCH_2CH_3); IR ($CHCl_3$) 1728, 1264, 841 cm^{-1}; MS (FAB), m/z no molecular ion, 545 (M − *tert*-butyl), 249, 135. Anal. ($C_{33}H_{54}O_4SSi_2$) C, H, S.

(2*R,3*S**)-2,3-Bis[4-(*tert*-butyldimethylsiloxy)phenyl]pentyl 2-Hydroxyethyl Sulfide (30).** To a solution (25 °C) of ester **29** (150 mg, 0.249 mmol) in 5 mL of dry tetrahydrofuran was added lithium aluminum hydride (10.0 mL of a 0.05 M tetrahydrofuran solution, filtered through dry Celite under nitrogen) in one portion. After 20 min, product isolation (5% Na_2HPO_4, EtOAc, brine) and purification by preparative TLC (ethyl acetate/hexane, 3:7) afforded alcohol **30** (99 mg, 71%) as a thick oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.20 (s, 12 H, 2 Si(CH_3)$_2$), 1.00 (s, 18 H, 2 SiC(CH_3)$_3$), 2.33 (t, 2 H, J = 6 Hz, $RSCH_2CH_2OH$), 2.55 (d, 2 H, J = 7 Hz, $CHCH_2SR$), 3.36 (t, 2 H, J = 6 Hz, $RSCH_2CH_2OH$); IR ($CHCl_3$) 3600, 3500–3200 cm^{-1}; MS (FAB), m/z (relative intensity) 561 (M + 1, 8), 545 (8), 249 (100). Anal. ($C_{31}H_{52}O_3SSi_2$) C, H, S.

(2*R,3*S**)-2,3-Bis(4-hydroxyphenyl)pentyl 3-*N*-Aziridinylpropyl Sulfide (8).** Methanesulfonate **31** was obtained in 93% yield (190 mg; see compound **18**): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.92 (s, 3 H, CH_3SO_3R), 4.14 (t, 2 H, J = 6 Hz, CH_2OMs). Bisphenolic methanesulfonate **32** was obtained in 60% yield (74 mg; see compound **19**): ^{1}H NMR (acetone-d_6, 200 MHz) δ 7.77 (br s, 2 H, Ar O*H*); IR (KBr) 3500–3200 cm^{-1}. For aziridine formation, see compound **3**. The desired aziridine **8** (22 mg, 72%) was obtained as a thick oil: ^{1}H NMR (acetone-d_6, 200 MHz) δ 0.97–0.99 (m, 2 H, aziridine protons anti to lone pair), 1.51–1.53 (m, 2 H, aziridine protons syn to lone pair), 1.98–2.09 (m, 2 H, CH_2CH_2N obscured by acetone-d_6), 2.24 (t, 2 H, J = 7 Hz, SCH_2CH_2), 2.50 (distorted d, 2 H, $CHCH_2S$); IR (KBr) 3550–3330, 1610 cm^{-1}; MS (FAB), m/z (relative intensity) 372 (M + 1, 28), 135 (88), 119 (100); exact mass calcd for $C_{22}H_{30}NO_2S$ 372.1997, found 372.2008.

(2*R,3*S**)-2,3-Bis(4-hydroxyphenyl)pentyl 3-(*N*,*N*-Dimethylamino)propyl Sulfide (9).** Alcohol **28** was converted to methanesulfonate **31** as described above, followed by reaction with anhydrous dimethylamine (200 equiv) in dry tetrahydrofuran at reflux for 20 h; product isolation ($NaHCO_3$, EtOAc, brine) and purification by preparative TLC (ethyl acetate/hexane/triethylamine, 3:3:1) afforded 91.4 mg (52.5%) of dimethylamino derivative **35** as a clear oil: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.09 (2 s, 6 H, N(CH_3)$_2$), 2.51–2.61 (m, 2 H, CH_2N). Deprotection: To a solution of 31.4 mg (0.0522 mmol) of dimethylamino derivative **35** in 2 mL of dry tetrahydrofuran were added potassium fluoride (0.25 mmol, 0.5 M solution in methanol) and 18-crown-6 (0.50 mmol, 1.0 M solution in methanol) at 25 °C. After 30 min, product isolation (brine, EtOAc, brine) afforded 13.3 mg (68.5%) of the desired dimethylamino derivative **9** (>96% pure by HPLC, column A, system A): ^{1}H NMR (acetone-d_6, 200 MHz) δ 2.05–2.22 (m, 10 H, N(CH_3)$_2$, SCH_2CH_2, and CH_2N), 2.52 (d, 2 H, J = 7 Hz, $CHCH_2S$); IR (KBr) 3550–3330, 1610 cm^{-1}; MS (FAB), m/z (relative intensity) 374 (M + 1, 100), 135 (25); exact mass calcd for $C_{22}H_{32}NO_2S$ 374.2146, found 374.2150.

(2*R,3*S**)-2,3-Bis(4-hydroxyphenyl)pentyl 2-*N*-Aziridinylethyl Sulfide (10).** The desired methanesulfonate **34** (23 mg, 66%) was obtained as a clear oil by following the procedures described for compounds **18** and **19**. The desired aziridine **10** (5 mg, 25%) was obtained as a thick oil by following the procedure for compound **3**: ^{1}H NMR (acetone-d_6, 200 MHz) δ 0.94–0.96 (m, 2 H, aziridine protons anti to lone pair), 1.45–1.47 (m, 2 H, aziridine protons syn to lone pair), 1.98–2.09 (m, 2 H, CH_2N obscured by acetone-d_6), 2.29 (m, 2 H, J = 7 Hz, SCH_2CH_2N), 2.53 (distorted d, 2 H, $CHCH_2S$); IR (KBr) 3550–3330, 1610 cm^{-1}; MS (FAB), m/z 358 (M + 1), 135; exact mass calcd for $C_{21}H_{28}NO_2S$ 358.1841, found 358.1841.

***tert*-Butyldimethylsilyl (5*R**,6*S**)-5,6-Bis[4-(*tert*-butyldimethylsiloxy)phenyl]octanoate (38).** To a cold (−78 °C) solution of boron tribromide (22.47 mmol) in 2 mL of chloroform was added 1.00 g (22.47 mmol) of the protected bisphenolic acid **36**[16] in 6 mL of chloroform over 5 min. After being stirred for 15 min at −78 °C, the solution was allowed to warm up to 0 °C and was stirred for an additional 8 h. The reaction was quenched at 0 °C by the careful addition of 10 mL of ice/water. The chloroform was removed in vacuo, and product isolation (H_2O, EtOAc, brine) afforded 848 mg (92%) of the crude bisphenol **37**. The crude bisphenol compound (848 mg, 2.58 mmol) was converted to the *tert*-butyldimethylsilyl ether by the method of Corey and Venkateswarlu.[23] Purification by flash chromatography (ether/hexane, 1:9) afforded 1.254 g (72.4%) of compound **38** as a white solid: mp 69–70.5 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.10 (s, 3 H, ester SiCH_3), 0.16 (s, 3 H, ester SiCH_3), 0.20 (s, 12 H, 2 Si(CH_3)$_2$), 0.85 (s, 9 H, ester SiC(CH_3)$_3$), 0.91 (s, 18 H, 2 SiC(CH_3)$_3$), 2.00–2.11 (m, 2 H, CH_2CO_2SiR); IR (KBr) 1720, 1610, 1260, 840 cm^{-1}; MS (FAB), m/z (relative intensity) 613 (57), 421 (20), 249 (100).

(5*R,6*S**)-5,6-Bis[4-(*tert*-butyldimethylsiloxy)phenyl]-1-octanol (39).** The desired alcohol **39** (130 mg, 53.5%) was obtained as a white solid by following the procedure described for compound **28** and purification by flash chromatography (ethyl acetate/hexane, 1:3): mp 74–75 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.20 (s, 12 H, 2 Si(CH_3)$_2$), 0.99 (s, 18 H, 2 SiC(CH_3)$_3$), 3.39 (t, 3 H, J = 6 Hz, RCH_2OH); IR (KBr) 3600, 3200, 1605, 1255, 840 cm^{-1}; MS (FAB), m/z (relative intensity) 541 (M − 1, 38), 525 (13), 469 (42), 293 (57), 249 (100). Anal. ($C_{32}H_{54}O_3Si_2$) C, H.

(5*R,6*S**)-1-[5,6-Bis(4-hydroxyphenyl)-1-octyl]aziridine (11).** Alcohol **39** was converted to methanesulfonate **40** (107 mg, 88%) as described above (compound **18**): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.85 (s, 3 H, ROSO$_2$CH_3), 3.98 (t, 2 H, J = 6 Hz, CH_2OMs). Deprotection (see compound **19**) afforded 45 mg (66%) of bisphenolic methanesulfonate **41** as a thick oil: IR (KBr) 3550–3300 cm^{-1}.

The desired aziridine **11** (7 mg, 23%) was obtained as a white solid by following the procedure for compound **3**: mp 120–122 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90–0.92 (m, 2 H, aziridine protons anti to lone pair), 1.44–1.46 (m, 2 H, aziridine protons syn to lone pair), 1.91 (t, 2 H, J = 7 Hz, RCH_2N); IR (KBr) 3550–3330, 1610 cm^{-1}; MS (FAB), m/z (relative intensity) 340 (M + 1, 73), 204 (20), 135 (58), 119 (62); exact mass calcd for $C_{22}H_{30}NO_2$ 340.2279, found 340.2278.

(5*R,6*S**)-*N*-[5,6-Bis(4-hydroxyphenyl)-1-octyl]piperazine (12).** To a solution of 40 mg (0.102 mmol) of primary methanesulfonate **41** in 2 mL of acetonitrile/triethylamine (1:1) was added 175.5 mg (2.04 mmol) of piperazine at 25 °C. After 20 h, the reaction mixture was subjected directly to reversed-phase semipreparative HPLC (column B, methanol/water, 3:2, 3 mL/min). The desired compound **12** was completely retained on the column, and piperazine was eluted. The piperazine compound **12** was recovered by elution with 100% methanol. Further purification by reversed-phase semipreparative HPLC (column B, system B, 6 mL/min, t_R = 13 min) afforded 18.3 mg (47%) of **12** as a thick oil: ^{1}H NMR (CD_3OD, 300 MHz) δ 2.04 (br t, 2 H, J = 7 Hz, CH_2N), 2.22–2.36 (m, 4 H, ring (CH_2)$_2$NR), 2.72–2.76 (m, 4 H, ring (CH_2)$_2$NH); IR (KBr) 3550–3300, 1610 cm^{-1}; MS (FAB), m/z (relative intensity) 383 (M + H, 90), 247 (8), 135 (58). Exact mass calcd for $C_{24}H_{35}N_2O_2$: 383.2741. Found: 383.2720.

Cell Culture. MCF-7 cells, obtained originally from Dr. M. E. Lippman (NIH, Bethesda, MD), were grown in plastic T-150 flasks in improved minimal essential medium (IMEM) containing 10 mM HEPES buffer, gentamicin (50 μg/mL), penicillin (100 units/mL), streptomycin (0.1 mg/mL), hydrocortisone (3.75 ng/mL), insulin (Lilly, 0.02 unit/mL), and 5% calf serum.[20]

Cell Proliferation Experiments. To determine the effect of hexestrol aziridines and related derivatives on cell proliferation, MCF-7 cells were grown for 1 week before experiments in phenol red free IMEM supplemented as described above and containing 5% charcoal dextran treated calf serum (calf serum that had been treated with dextran-coated charcoal for 45 min at 55 °C to remove endogenous steroids[17a,20]) and seeded into T-25 flasks (ca. 0.5 × 10^5 cells/flask). The following day, cells from three flasks were harvested and counted with a Coulter counter. The medium was then changed to phenol red free and insulin-free IMEM that contained 5% charcoal dextran treated calf serum and hexestrol aziridine derivatives, hydroxytamoxifen, estradiol, or control ethanol vehicle (0.1%), and cell number was monitored as a function of time.

Competitive Binding Assays. Uterine tissue from immature lambs was homogenized at 4 °C in 0.01 M Tris/1.5 mM EDTA/0.02% sodium azide, pH 7.4 buffer, and the homogenate was centrifuged at 180000*g* for 60 min. The supernatant was carefully removed, and this cytosol was diluted to a concentration of 3 mg of protein/mL. This cytosol was incubated with various concentrations (10^{-11}–10^{-5} M) of radioinert estradiol, hexestrol aziridine, or related derivative and with 2.5×10^{-9} M [^{3}H]estradiol and was analyzed after charcoal dextran treatment. The relative binding affinity of each competitor is taken as the ratio of the concentrations of radioinert estradiol/competitor required to inhibit one-half of the specific [^{3}H]estradiol binding, with the affinity of estradiol being set at 100%.[17]

Time-Dependent Irreversible Binding Assays. Fresh solutions of hexestrol aziridine compounds or estradiol were added to uterine cytosol to give the concentrations of compounds indicated with a final concentration of 5% DMF. To protect the binding sites, additional incubations were performed with an excess of estradiol for 1 h at 4 °C prior to the addition of hexestrol aziridine compound. The mixture was incubated at 21 °C, and aliquots were removed at different times and assayed after charcoal dextran treatment for surviving reversible estrogen binding activity by exchange with 30 nM [^{3}H]estradiol in the presence and absence of 100-fold excess estradiol for 20 h at 21 °C, as detailed previously.[2a,18]

Intact monolayer cultures of MCF-7 cells were also incubated with hexestrol aziridine compounds for varying time periods at 37 °C in tissue culture medium containing 5% charcoal-treated calf serum. After indicated times of incubation, cells were harvested from the flasks and homogenized as described previously[2a,25] to prepare a total cell receptor extract that was centrifuged at 180000*g* for 30 min and treated with charcoal dextran. Receptor extracts were then assayed for surviving reversible estrogen binding activity as described above.

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Registry No. **3**, 107036-07-7; **4**, 107036-08-8; **5**, 107036-09-9; **6**, 107036-10-2; **7**, 107036-11-3; **8**, 107036-12-4; **9**, 107036-13-5; **10**, 107036-14-6; **11**, 107036-15-7; **12**, 107036-16-8; **13**, 79568-15-3; **14** (isomer 1), 107036-17-9; **14** (isomer 2), 107036-44-2; **15**, 107036-18-0; **16**, 107036-19-1; **17**, 107036-20-4; **18**, 107036-21-5; **19**, 107036-22-6; **20**, 107036-23-7; **21**, 107036-24-8; **22**, 107036-25-9; **23**, 107036-26-0; **24**, 107036-27-1; **25**, 107036-28-2; **26**, 104946-98-7; **27**, 107036-29-3; **28**, 107036-30-6; **29**, 107036-31-7; **30**, 107036-32-8; **31**, 107036-33-9; **32**, 107036-34-0; **33**, 107036-35-1; **34**, 107036-36-2; **35**, 107036-37-3; **36**, 107036-38-4; **37**, 107036-39-5; **38**, 107036-40-8; **39**, 107036-41-9; **40**, 107036-42-0; **41**, 107036-43-1; CH_2=$CH(CH_2)_2Br$, 5162-44-7; $H_2NCH_2CH_2OSO_3H$, 926-39-6; HNCH_2CH_2, 151-56-4; Me_2NH, 124-40-3; $HO(CH_2)_3Br$, 627-18-9; $HO(CH_2)_2I$, 624-76-0; CH_2=$CHCO_2Me$, 96-33-3; $BrCH_2CO_2Et$, 105-36-2; piperazine, 110-85-0.

(25) Reiner, G. C. A.; Katzenellenbogen, B. S.; Bindal, R. D.; Katzenellenbogen, J. A. *Cancer Res.* **1984**, *44*, 2302–2308.