1-Aryl-2-pyridyl-3,4-dihydronaphthalenes: Photofluorogenic Ligands for the Estrogen Receptor

Andrew W. Scribner, Serkos A. Haroutounian, Kathryn E. Carlson, and John A. Katzenellenbogen*

Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801

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Three 1,2-substituted-3,4-dihydronaphthalenes that are pyridine analogs of the antiestrogen desmethylnafoxidine were prepared and evaluated as fluorescent ligands for the estrogen receptor. These compounds represent a class of fluorescent probes that we term "photofluorogenic", denoting their ability to exist initially as a high affinity though weakly fluorescent stilbazole form which can be photocyclized−oxidized to a highly fluorescent though low affinity azaphenanthrenoid form. These probes also contain an aziridine function that provides a means for their permanent, covalent attachment to the receptor. The three dihydronaphthalene systems were prepared by efficient routes from α-(2-, 3-, and 4-pyridyl)acetophenone precursors. They demonstrate high apparent affinity for the estrogen receptor and show time-dependent irreversible inactivation, consistent with their covalent attachment to the receptor via the aziridine function. Each system is converted into an azaphenanthrene by photocyclization−oxidation of the *cis*-stilbazole unit. The absorbance and fluorescence emission spectra of the dihydronaphthalene precursors and azaphenanthrene products have been characterized, and they display marked sensitivity to both solvent polarity and pH. The azaphenanthrenoids derived from the 2- and 4-pyridyl isomers exhibit intense emission at wavelengths that exceed 500 nm under certain conditions and appear to be well suited as fluorescent probes for the estrogen receptor.

Introduction

We have been interested in the development of fluorescent probes for the estrogen receptor (ER) that would be useful in elucidating receptor structure and function and in predicting the hormonal responsiveness of breast cancer patients.[1,2] The latter application involves measuring the receptor content of individual cells using flow cytometry[3] or fluorescence microscopy[4] in order to distinguish ER-positive from ER-negative cells.[5] Such a fluorescent probe should demonstrate high relative binding affinity (RBA) for the ER and exhibit fluorescence at wavelengths greater than 500 nm, in order for the fluorophore emission to be distinguishable from the background of cell autofluorescence.[6]

In earlier studies, we have developed three major classes of fluorescent estrogenic probes (Figure 1): estrogen−fluorophore conjugates,[7] inherently fluorescent estrogens,[8] and photofluorogenic estrogens.[9] Estrogen−fluorophore conjugates link a fluorescent moiety such as a nitrobenzoxadiazole to an estrogen such as hexestrol *via* an alkyl spacer, as seen in **1**. These systems are bulky and generally have poor affinity for the estrogen receptor. Inherently fluorescent estrogens are fluores-

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(1) Lee, Y. J.; Notides, A. C.; Tsay, Y. G.; Kende, A. S. *Biochemistry* **1977**, *16*, 2896.

(2) (a) Thorpe, S. M. *Acta Oncol.* **1988**, *27*, 1. (b) Valavaara, R.; Touminen, J.; Johansson, R. *Cancer* **1990**, *66*, 2264. (c) Thompson, E. B.; Lippman, M. E. *Steroid Receptors and the Management of Cancer, vol. I*; CRC Press: Boca Raton, 1979. (d) DeSombre, E. R.; Green, G. L.; Jensen, E. V. *Hormones, Receptors, and Breast Cancer*; Raven Press: New York, 1978.

(3) (a) Van, N. T.; Raber, M.; Barrows, G. H.; Barlogie, B. *Science* **1984**, *224*, 876. (b) Kute, T. E.; Linville, C.; Barrows, G. *Cytometry* **1983**, *4*, 132. (c) Oxenhandler, R. W.; McvCune, R.; Subtelney, A.; Truelove, C.; Tyrer, H. W. *Cancer Res.* **1984**, *44*, 2516. (d) Benz, C.; Wizner, I.; Lee, S. H. *Localiztion of Putative Steroid Receptors, vol. I*; Pertschuk, P. L., Lee, S. H., Eds.; CRC Press: Boca Raton, 1985; pp 95−110.

(4) (a) Nenci, I. *Cancer Res.* **1978**, *38*, 4204. (b) Dandliker, W. B.; Brown, R. J.; Hsu, M. L.; Brown, P. N.; Levin, J.; Meyers, C. Y.; Kolb, V. M. *Cancer Res.* **1978**, *38*, 4212. (c) Pertschuk, L. P.; Tobin, E. H.; Gaetjens, E.; Carter, A. C.; Degenshein, G. A.; Bloom, N. D.; Brigati, D. J. *Cancer* **1980**, *46*, 2896. (d) Barrows, G. H.; Stroupe, S. B.; Rieh, M. J. D. *Am. J. Clin. Pathol.* **1980**, *73*, 330. (e) Joyce, B. D.; Nicholson, R. I.; Morton, M. S.; Griffiths, K. *Eur. J. Cancer Clin. Oncol.* **1982**, *18*, 1147. (f) Fisher, B.; Gunduz, N. Saffer, E. A. *Cancer Res.* **1983**, *43*, 5244. (g) Lee, S. H. *Am. J. Clin. Pathol.* **1983**, *79*, 484. (h) Pertschuk, L. P.; Tobin, E. H.; Tanapat, P.; Gaetjens, E.; Carter, A. C.; Bloom, N. D.; Macchia, R. J.; Eisenberg, K. B. *J. Histochem. Cytochem.* **1980**, *28*, 799. (i) Lee, S. H. *Cancer* **1979**, *44*, 1. (j) Martin, P. M.; Horwitz, K. B.; Ryan, D. S.; McGuire, W. L. *Endocrinology* **1978**, *103*, 1860. (k) Tobin, E. H.; Pertschuk, L. P.; Tanapat, P.; Brigati, D. J.; Azizi, F.; Bloom, D. D.; Degenshein, G. A. *Fed. Proc.* **1980**, *39*, 550. (l) Pertschuk, L. P.; Eisenberg, K. B.; Carter, A. C.; Feldman, J. G. *Breast Cancer Res. Treat.* **1985**, *5*, 137.

(5) (a) Silverswaard, C.; Skoog, L.; Humla, S.; Gustafsson, S. A.; Nordensjold, B. *Cancer* **1980**, *16*, 59. (b) McCarty, K. S., Jr.; Reintgen, D. S.; Seigler, H. F. *Br. Cancer Res. Treat.* **1982**, *1*, 315. (c) McCarty, K. S., Jr.; Lubahn, D. B.; McCarty, K. S., Sr. *Clin. Endocrinol. Metab.* **1983**, *12*, 133. (d) McGuire, W. L. *Estrogen Receptors in Human Breast Cancer*; Carbone, P., Vollmer, E. P., Eds.; Raven Press: New York, 1975.

(6) Waggoner, A. S. In *Applications of Fluorescence in the Biomedical Sciences*; Taylor, P. L., Waggoner, A. S., Murphy, R. F., Lanni, F., Birge, R. R., Eds.; Alan Liss: New York, 1986.

(7) For examples of estrogen−fluorophore conjugates, see: (a) Fevig, T. L.; Lloyd, J. E.; Zablocki, J. A.; Katzenellenbogen, J. A. *J. Med. Chem.* **1987**, *30*, 156. (b) Carlson, K. E.; Coppey, M.; Magdelenat, H.; Katzenellenbogen. J. A. *J. Steroid Biochem.* **1989**, *32*, 345. (c) French, A. N.; Wilson, S. R.; Welch, M. J.; Katzenellenbogen, J. A. *Steroids* **1993**, *58*, 157.

(8) For examples of inherently fluorescent estrogens, see: (a) Martin, P. M.; Magdelenat, H. P.; Benyahia, B.; Rigaud, O.; Katzenellenbogen, J. A. *Cancer Res.* **1983**, *43*, 4956. (b) Katzenellenbogen, J. A.; Carlson, K. E.; Bindal, R. D.; Neeley, R. L.; Martin, P. M.; Magdelenat, H. P. *Anal. Biochem.* **1986**, *159*, 336. (c) Anstead, G. M.; Altenbach, R. J.; Wilson, S. R.; Katzenellenbogen, J. A. *J. Med. Chem.* **1988**, *31*, 1316. (d) Anstead, G. M.; Katzenellenbogen, J. A. *J. Med. Chem.* **1988**, *31*, 1754. (e) Anstead, G. M.; Katzenellenbogen, J. A. *J. Phys. Chem.* **1988**, *92*, 6249. (f) Anstead, G. M.; Ensign, J. L.; Peterson, C. S.; Katzenellenbogen, J. A. *J. Org. Chem.* **1989**, *54*, 1485. (g) Anstead, G. M.; Wilson, S. R.; Katzenellenbogen, J. A. *J. Med. Chem.* **1989**, *32*, 2163. (h) Anstead, G. M.; Peterson, C. S.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1989**, *33*, 877. (k) Miksicek, R. J.; Carlson, K. E.; Hwang, K.-J.; Katzenellenbogen, J. A. *Mol. Endocrinol.* **1995**, *9*, 592.

(9) For examples of photofluorogenic estrogens, see: (a) Bindal, R. D.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1985**, *23*, 929. (b) Bindal, R. D.; Katzenellenbogen, J. A. *Photochem. Photobiol.* **1986**, *43*, 121.

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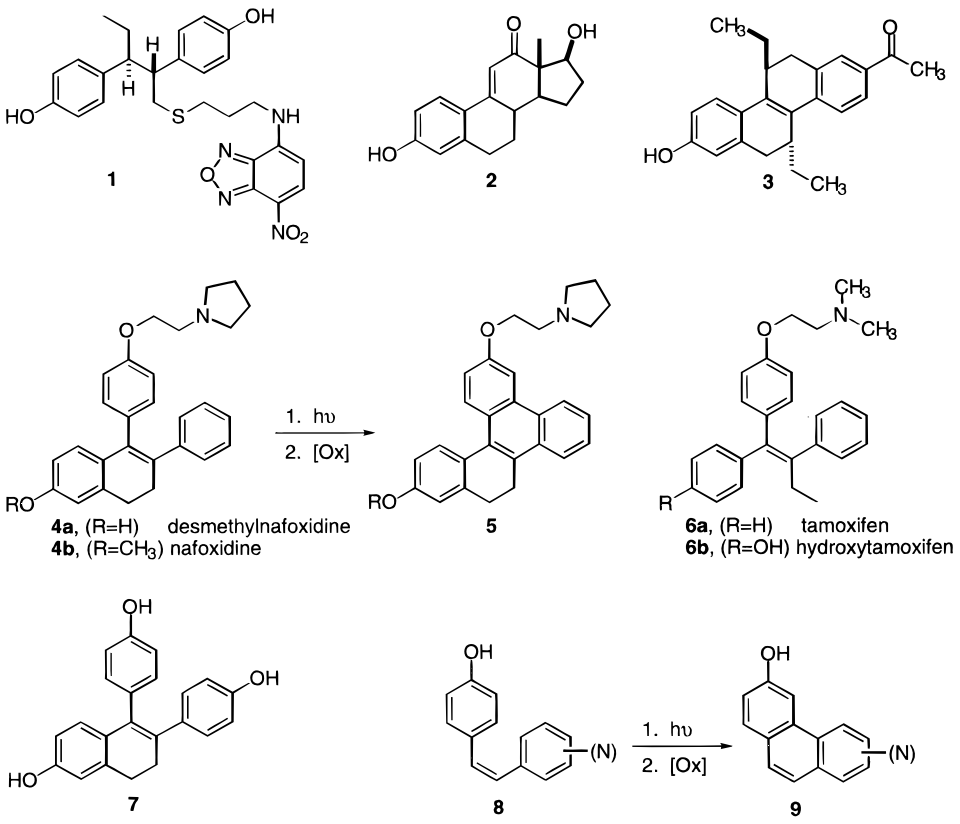


Figure 1. Examples of fluorescent estrogens.

cent by virtue of extended conjugation within the molecular confines of an estrogenic ligand, as exhibited by 12-oxo-9(11)-dehydroestradiol (**2**).[8a] Although the design of these systems can entail a compromise to achieve both good fluorescence and receptor binding within a single molecular entity, some compounds show promising characteristics, and we are currently continuing our investigation of tetrahydrochrysene derivatives in this class, such as the ketone **3**.[8i,j]

The work described herein involves fluorescent probes from the third class, which we have termed "photofluorogenic". As exemplified by the 1,2-diaryl-3,4-dihydronaphthalene system **4a**, an analog of the antiestrogen nafoxidine (**4b**), these molecules exist in two distinct forms: a parent form (**4a**) that is weakly fluorescent but has high affinity for the receptor, and a daughter form (**5**) which has lower binding affinity but is strongly fluorescent.[9] This first form can be converted into the second form upon photocyclization and oxidation. An attractive feature of photofluorogenic probes is their potential for achieving background correction, i.e., a cell background fluorescence image, captured prior to photocyclization, could be stored in digitized form and then subtracted from the fluorescence image obtained after photochemical generation of the fluorophore (effected by brief irradiation at a shorter wavelength and spontaneous oxidation).[10] For this purpose, we have considered the photocyclization of hydroxy-substituted stilbene moieties[11] (which are present in many potent estrogens, such as **4ab**) to their corresponding hydroxyphenanthrenes, such as **5**. The antiestrogen tamoxifen (**6a**) has been used in a photofluorogenic based HPLC assay;[12] this compound, however, has low affinity for the receptor. The behavior of its high affinity 4-hydroxy metabolite of tamoxifen (**6b**) is complicated by its facile *cis*−*trans* isomerization.[13] Thus, we chose to explore derivatives of the analogous but cyclic antiestrogen nafoxidine (**4b**), whose rigid dihydronaphthalene core is inert to such isomerization. We have reported preliminary spectroscopic studies on both desmethylnafoxidine (**4a**) and a related analog (**7**) and on the products of photocyclization, which exhibit long wavelength fluorescence emission.[9] In related work, we explored the photocyclization of hydroxystilbazoles (**8**) to hydroxyazaphenanthrenes (**9**) as model systems for photofluorogenic estrogens that give photoproducts with wavelength-extended emission that in some cases extends beyond 600 nm.[14]

In this report, we describe three new photofluorogenic estrogens in which the 2-phenyl ring of desmethylnafoxidine is replaced by a pyridine ring (Figure 2; **10**−**12**). This substitution leads, after photocyclization−oxidation, to four hydroxyazaphenanthrene photoproducts (**13**) that have a donor−acceptor character and show intense fluorescence that is very sensitive to solvent pH and polarity.[14]

A final consideration in the design of these photofluorogenic probes involves giving them the capacity to covalently attach to the receptor, to preclude their loss from the ER upon photocyclization or subsequent pH manipulation. We know from earlier studies that a suitably positioned aziridine function will undergo an efficient covalent reaction with a specific cysteine residue

(10) Katzenellenbogen, J. A. In *Trends in Drug Research*; Claassen, A., Ed.; Elsevier Science Publishers, B.V.: Amsterdam, 1993; p 243.

(11) Mallory, F. B.; Mallory, C. W. In *Organic Reactions*; Dauben, W. G., Ed.; John Wiley & Sons Inc.: New York, 1984; Vol. 30, p 1.

(12) (a) Mendenhall, D. W.; Kobayashi, H.; Shih, F. M. L.; Sternson, L. A.; Higuchi, T.; Fabian, C. *Clin. Chem.* **1978**, *24*, 1518. (b) Brown, R. R.; Bain, R.; Jordan, V. C. *J. Chromatogr.* **1983**, *272*, 351.

(13) Katzenellenbogen, J. A.; Carlson, K. E.; Katzenellenbogen, B. S. *J. Steroid Biochem.* **1985**, *22*, 589.

(14) Haroutounian, S. A.; Katzenellenbogen, J. A. *Photochem. Photobiol.* **1988**, *47*, 503.

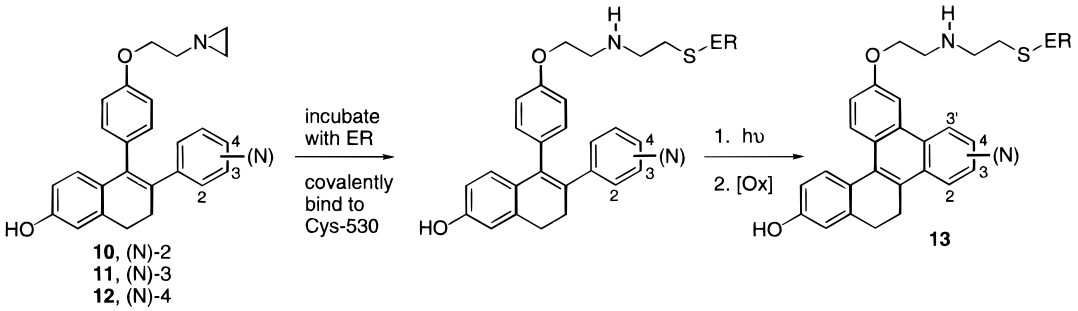


Figure 2. New photofluorogenic estrogen aziridines (**10**−**12**) and general strategy for covalent and fluorescent labeling of the estrogen receptor.

near the C-terminus of ER (C530 in human ER).[15] This aziridine function has been included in some of the pyridine-substituted desmethylnafoxidine photofluorogenic estrogens that we have prepared. Thus, the overall strategy for labeling ER-positive cells (Figure 2) would involve first the incubation of the high affinity stilbazole aziridine (**10**−**12**) to form a covalent bond with C530 of ER. The ER−ligand complex could then be irradiated to induce photocyclization of the stilbazole to the corresponding azaphenanthrenoid **13** (after spontaneous oxidation of a dihydro intermediate). The pH could then be manipulated in order to optimize fluorescence emission.

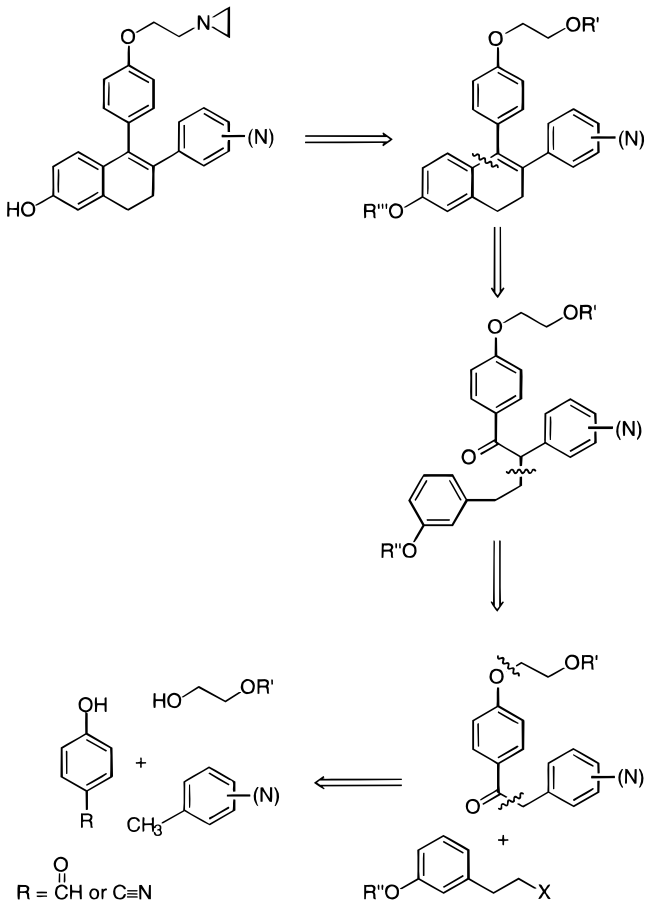
Therefore, in this report we present the syntheses of these three new photofluorogenic estrogen aziridines (**10**−**12**) from monocyclic precursors, accounting for each of the three possible sites of pyridyl nitrogen substitution, and we describe the photocyclization of their benzyl ether analogs to the azaphenanthrenoid products. We also report the ER binding affinities of these aziridines and various analogs and demonstrate the capacity of the aziridines for covalent attachment to ER. Lastly, we provide ultraviolet absorbance and fluorescence emission spectral data of benzyl ether-protected derivatives of each of the three stilbazoles and their four products of photocyclization.

Results and Discussion

Chemical Syntheses. The synthetic approach we have taken to the 1-aryl-2-pyridyl-3,4-dihydronaphthalene system parallels one we have used for the preparation of 1,2-diarylindenes.[8c] A retrosynthetic plan is given in Scheme 1. The route involves the synthesis of an α-pyridylacetophenone intermediate from a picoline anion, α-alkylation with an activated phenethyl species, and then Friedel−Crafts cyclodehydration to the dihydronaphthalene core structure. Functional group interchange, attachment of the aziridine function, and deprotection completed the synthesis.

The two phenyl ether precursors (**15** and **16**) were prepared (Scheme 2) by alkylation of the corresponding phenols with 2-(benzyloxy)ethyl methanesulfonate (mesylate) (**14**), which itself was prepared by activation of 2-(benzyloxy)ethanol. Deprotonation of 2-picoline with lithium diisopropylamide followed by addition of nitrile **15** yielded the corresponding imine, which was acidified

Scheme 1



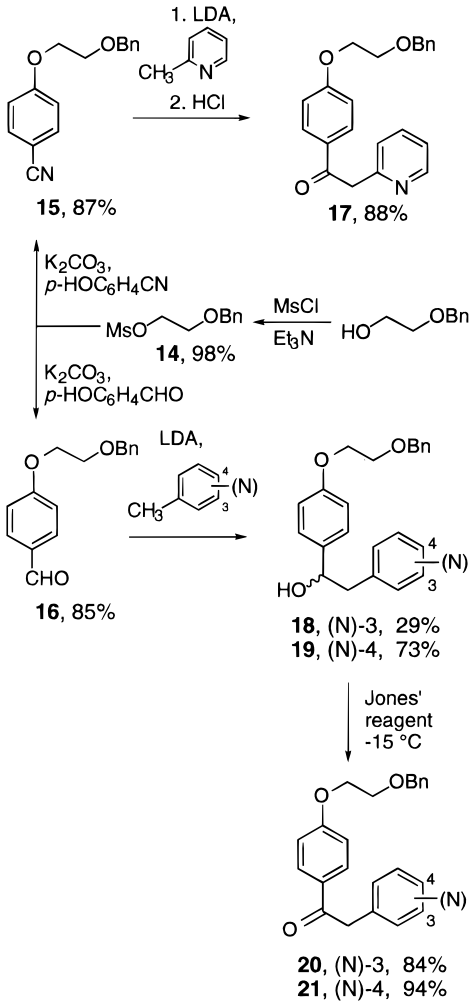
directly to give the ketone **17**,[16] found to exist as a mixture of keto and enol tautomers, the latter due to intramolecular hydrogen bonding.[16a] The anions of 3-picoline and 4-picoline, however, gave low yields in this transformation. As an alternative approach, the anions of these picoline isomers were added to aldehyde **16** to yield alcohols **18** and **19**, which were then oxidized by Jones reagent to afford ketones **20** and **21**, for which enol tautomers were not observed. The reduced yield for the sequence with 3-picoline is most likely due its weaker acidity ($pK_a = 37.7$) compared to either 2-picoline ($pK_a = 34$) or 4-picoline ($pK_a = 32.2$),[17] whose anions are more effectively resonance stabilized. We were unsuccessful in improving the yield of the 3-picoline sequence with polar additives (DMPU, HMPA), despite literature claims.[18]

(15) Harlow, K. W.; Smith, D. M.; Katzenellenbogen, J. A.; Greene, G. L.; Katzenellbogen, B. S. *J. Biol. Chem.* **1989**, *264*, 17476.

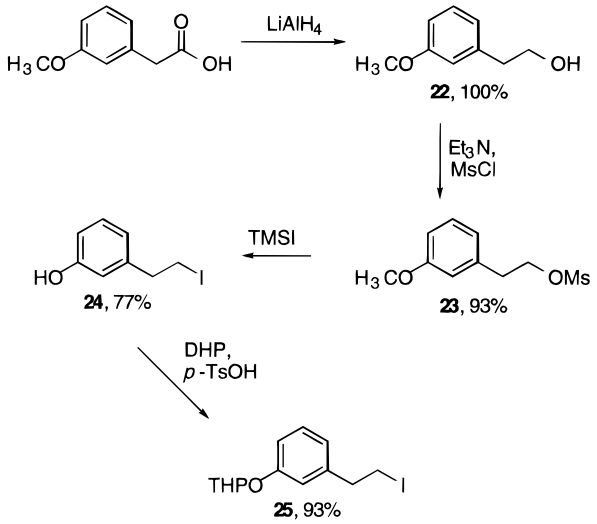
(16) (a) Konakahara, T.; Takagi, Y. *Heterocycles* **1980**, *14*, 393. (b) Konakahara, T.; Sato, K. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1241.

(17) Fraser, R. R.; Mansour, T. S.; Savard, S. *J. Org. Chem.* **1985**, *50*, 3232.

Scheme 2

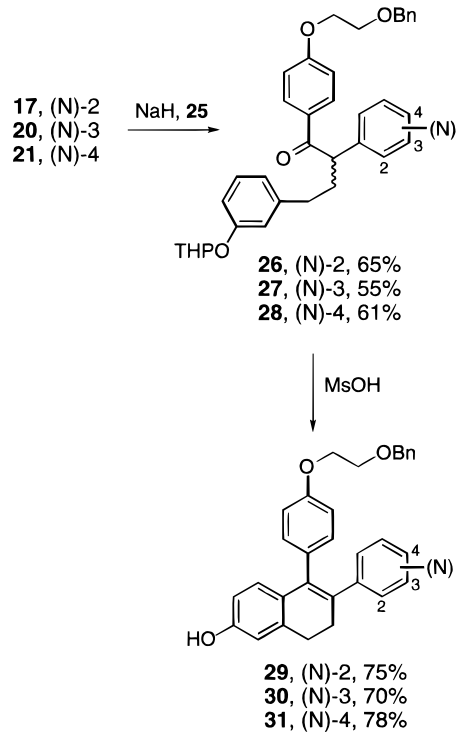


Scheme 3



To form the dihydronaphthalene skeleton, the three pyridyl acetophenones (**17**, **20**, and **21**) were alkylated with the phenylethyl iodide **25**. This agent **25** was prepared in four steps from commercially available (3-methoxyphenyl)acetic acid (Scheme 3).[19] Lithium aluminum hydride reduction afforded primary alcohol **22**, which was then activated to mesylate **23**.[19] Simultaneous iodination of the mesylate and cleavage of the methyl ether by iodotrimethylsilane yielded phenol **24**, which

Scheme 4



was then protected as tetrahydropyranyl (THP) ether **25** using dihydropyran and catalytic pyridinium *p*-toluenesulfonate.[20] The acid-labile THP group was used to protect the phenol, as it is then cleaved during the methanesulfonic acid cyclodehydration step without affecting the benzyl ether. Other protecting group strategies proved to be less efficient.[21]

The sodium salts of each of the three ketones (**17**, **20**, and **21**) were alkylated with iodide **25** to afford products **26**−**28** (Scheme 4). Methanesulfonic acid-catalyzed cyclodehydration of the α-phenethylacetophenones,[22] with concomitant THP ether cleavage, afforded dihydronaphthalenes **29**−**31**.

The remaining steps in the synthesis of the aziridines are shown in Scheme 5. First, the phenols were protected as aryl trifluoromethanesulfonates (triflates) **32**−**34**.[23] Benzyl ether cleavage with iodotrimethylsilane[24] proceeded efficiently in the presence of at least 1 equiv of thiophenol, which was needed to trap the benzyl iodide and avoid pyridyl *N*-benzylation. Although hydrogenolysis is generally a practical method for cleaving benzyl ethers, the double bond in the dihydronaphthalene system is prone to reduction under such conditions.[21]

The resulting primary alcohols **35**−**37** were converted to their corresponding mesylates **38**−**40**, which underwent nucleophilic displacement by ethylenimine to afford

(18) (a) Kaiser, E. M.; Petty, J. D. *Synthesis* **1975**, 705. (b) Davis, M. L.; Wakefield, B. J.; Wardell, J. A. *Tetrahedron* **1992**, *48*, 939. (c) Kaiser, E. M.; Petty, J. D. *J. Org. Chem.* **1976**, *41*, 716.

(19) Lednicer, D.; Lyster, S. C.; Aspergren, B. D.; Duncan, G. W. *J. Med. Chem.* **1966**, *9*, 172.

(20) Miyashita, N.; Yoshikoshi, A.; Grieco, P. A. *J. Org. Chem.* **1977**, *42*, 3772.

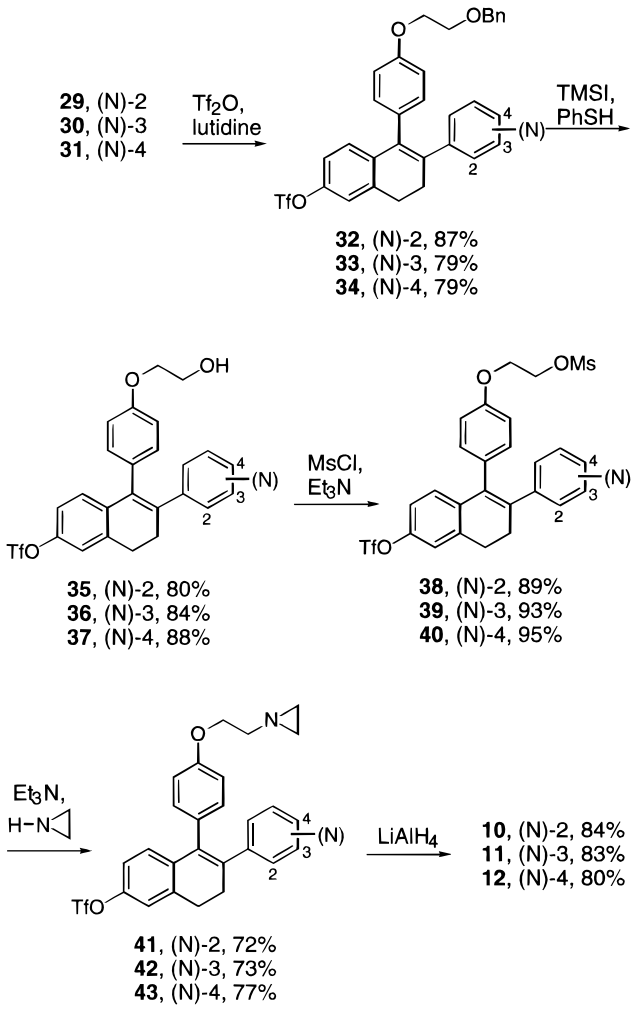
(21) Simpson, D. M. Ph.D. Thesis, University of Illinois at Urbana−Champaign, 1986.

(22) Leon, A. A.; Daub, G.; Silverman, I. R. *J. Org. Chem.* **1984**, *49*, 4544.

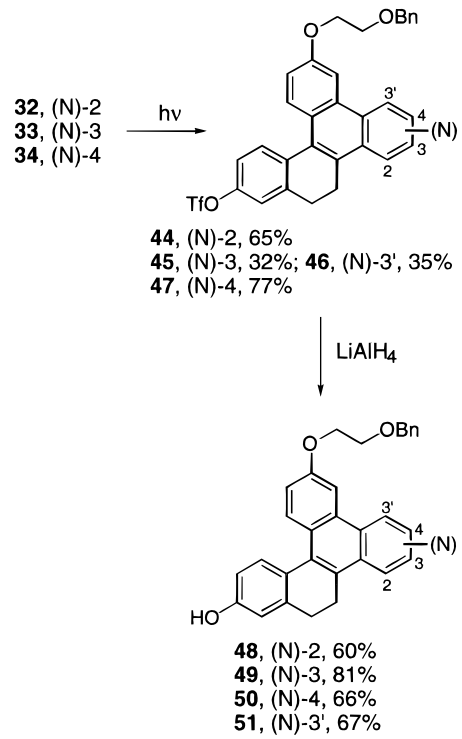
(23) Stang, P. J.; Hanack, M.; Subramanian, L. R. *Synthesis* **1982**, 85.

(24) (a) Jung, M. E.; Lyster, M. A. *J. Org. Chem.* **1977**, *42*, 3761. (b) Olah, G. A.; Narang, S. C.; Gupta, B. G. B.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 1247.

Scheme 5



Scheme 6

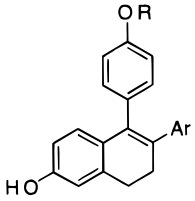


aziridines **41**–**43**.[21] Ethylenimine itself was prepared by flash distillation of 2-aminoethyl hydrogen sulfate in concentrated sodium hydroxide.[25] The triflates were then cleaved by lithium aluminum hydride treatment to produce the desired phenolic aziridines **10**–**12**.[26]

We prepared the four azaphenanthrenes (**48**–**51**) by photocyclization (Scheme 6). Cyclohexane was found to be the best solvent to carry out the photocyclizations of stilbazoles.[14] However, since phenols **29**–**31** exhibited poor solubility in cyclohexane, we found it to be more convenient to photocyclize aryl triflates **32**–**34** to their corresponding azaphenanthrenoid products (**44**–**47**) and then deprotect them to phenols **48**–**51**. The 2- and 4-pyridyl stilbazoles **32** and **34** each photocyclized to the single azaphenanthrenoid products **44** and **47**, respectively, whereas 3-pyridyl stilbazole **33** photocyclized to an approximately 1:1 mixture of the two azaphenanthrenoid isomers **45** and **46**, despite claims of other product ratios in related systems.[11] In the case of 2-pyridyl stilbazole **32**, no quaternary pyridinium salt was formed, which is consistent with previous studies.[11]

Biochemical Studies. Estrogen Receptor Relative Binding Affinities. The ER relative binding affinities (RBAs) of the three pyridyl aziridines, along with various deprotected intermediates and previously prepared analogs,[21] are shown in Table 1. These values were obtained from a competitive binding assay with [^{3}H]-estradiol as a tracer using dextran-coated charcoal to adsorb free ligand,[27] and they are expressed in percent,

Table 1. Relative Binding Affinities of Desmethylnafoxidine Analogs[a]



R	Ar	compound	RBA
H	phenyl	**52**	NA
H	2-pyridyl	**53**[b]	50
H	3-pyridyl	**54**	NA
H	4-pyridyl	**55**[b]	83
CH_2CH_2OBn	phenyl	**56**[21]	7
CH_2CH_2OBn	2-pyridyl	**29**	7.8
CH_2CH_2OBn	3-pyridyl	**30**	4.3
CH_2CH_2OBn	4-pyridyl	**31**	16.5
CH_2CH_2OH	phenyl	**57**[21]	82
CH_2CH_2OH	2-pyridyl	**58**[c]	6.4
CH_2CH_2OH	3-pyridyl	**59**[c]	3.5
CH_2CH_2OH	4-pyridyl	**60**[c]	17
$CH_2CH_2N(CH_2)_2$	phenyl	**61**[21]	79[d]
$CH_2CH_2N(CH_2)_2$	2-pyridyl	**10**	27[d]
$CH_2CH_2N(CH_2)_2$	3-pyridyl	**11**	47[d]
$CH_2CH_2N(CH_2)_2$	4-pyridyl	**12**	62[d]

[a] Determined by a radiometric competitive binding assay as previously described.[27] Values represent the mean of duplicate determinations in separate assays. The coefficient of variation of these values is 0.3. [b] Prepared by treating bis-aryl methyl ether precursors with boron trifluoride–dimethyl sulfide complex. [c] Prepared by treating compounds **35**–**37** with lithium aluminum hydride. [d] Apparent relative binding affinities (see text for explanation).

(25) Reeves, W.; Drake, G.; Hoffpauir, C. *J. Am. Chem. Soc.* **1951**, *73*, 3522.

(26) Hwang, K.-J. Ph.D. Thesis, University of Illinois at Urbana–Champaign, 1991.

(27) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. *Biochemistry* **1973**, *12*, 4085.

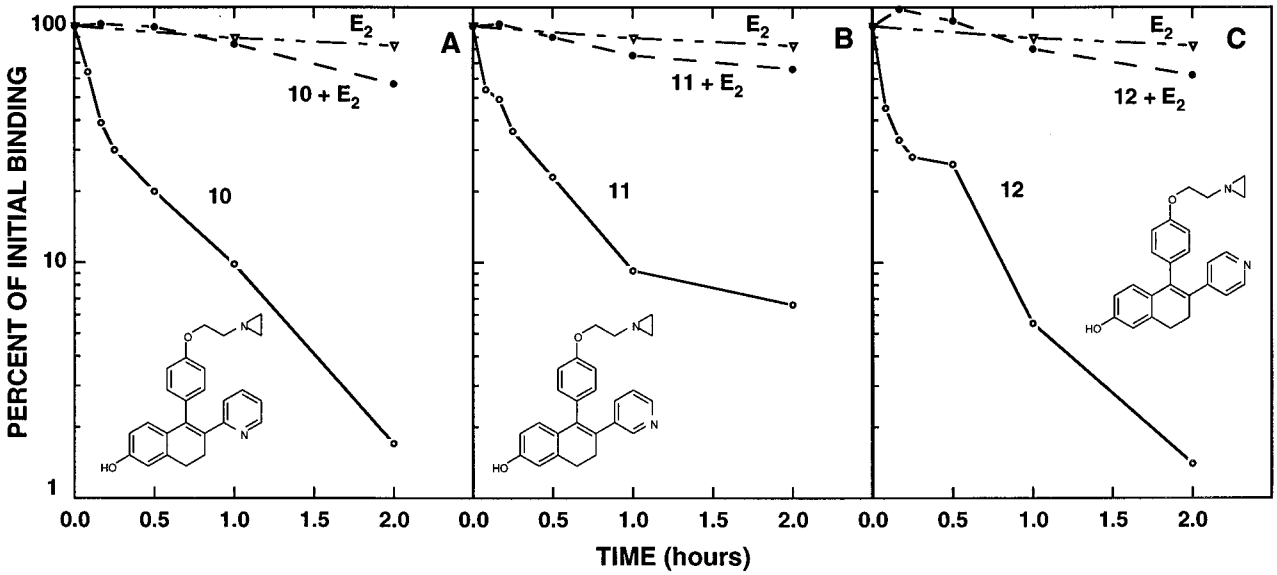
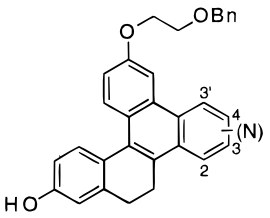


Figure 3. Rate of inactivation of estrogen receptor by aziridines **10**−**12**. Data are expressed as a percentage of initial estrogen-specific binding site concentration.[28] The dotted line represents incubation with estradiol alone, the dashed line incubation with estradiol plus aziridine, and the solid line aziridine alone. Panel A, aziridine **10**; panel B, aziridine **11**; panel C, aziridine **12**. For details, see Experimental Section and ref 28.

Table 2. Relative Binding Affinities of Photocyclized Desmethylnafoxidine Analogs[a]



N position	compound	RBA
2	**48**	0.040
3	**49**	0.042
4	**50**	0.26
3′	**51**	0.10

[a] Determined by a radiometric competitive binding assay as previously described.[27] Values represent the mean of duplicate determinations in separate assays. The coefficient of variation of these values is 0.3.

with estradiol having an affinity of 100%. All three aziridines (**10**−**12**) display appreciable apparent affinity for the estrogen receptor.[15] Although the values vary, it is difficult to conclude whether or not the location of the pyridyl nitrogen has an influence on receptor binding, as the coefficient of variation in RBA measurements is 0.3. Also, it should be emphasized that the aziridines bind irreversibly to the receptor; therefore, the measured affinity is not the result of a true competition and thus is termed "apparent".[15]

As for other intermediates, the bis-phenols **53** and **55** are among the highest affinity. In the hydroxyethyl ether series (**57**−**60**), the pyridyl-substituted desmethylafoxidine analogs (**58**−**60**) have considerably lower affinity than the phenyl-substituted one (**57**), whereas in the (benzyloxy)ethyl ether series (**29**−**31**, **56**), the binding affinities of the pyridyl (**29**−**31**) vs phenyl-substituted (**56**) analogs are more comparable. Within a given series, however, the 4-pyridyl species consistently has higher affinity than either its 2- or 3-pyridyl-substituted isomers (compare **31** vs **29** and **30**; **60** vs **58** and **59**).

Table 2 provides the RBAs of photocyclized desmethylnafoxidine analogs. Here it is seen that binding affinities of these azaphenanthrenoid species (**48**−**51**) are considerably lower than those of their stilbazole counterparts (**29**−**31**) shown in Table 1, again with the 4-pyridyl-derived isomer (**50**) having greater affinity than any of its isomers (**48**, **49**, and **51**).

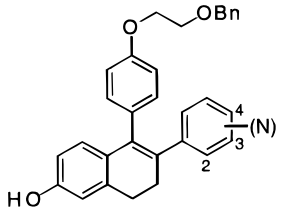
Estrogen Receptor Inactivation Assay. Each of the three aziridines (**10**−**12**) were tested for their ability to covalently attach to the ER by a time-dependent receptor inactivation assay.[28] This was done by incubating each compound with ER, then assaying remaining ER binding sites by an exchange assay with [^{3}H]estradiol at various time points. Data for compounds **10**−**12** are shown in Figures 3A−C, respectively. Although each aziridine did not necessarily behave identically, all three inactivated ER by over 90% within 1 h, and 95% within 2 h.

Spectroscopic Studies. Ultraviolet absorbance of the seven phenols **29**−**31** and **48**−**51** was measured in tetrahydrofuran, acetonitrile, ethanol, and water under neutral and acidic (0.1 N HCl) conditions. Spectra in the latter three solvents were also obtained under basic (0.1 N KOH) conditions; THF was excluded from studies in base due to solubility problems. The fluorescence emission of each compound was then obtained with the same four solvents and at the same three pHs, using the major long-wavelength absorbance maximum as the excitation wavelength.

Table 3 displays wavelengths of maximum absorbance of the three stilbazoles (**29**−**31**), and selected spectra are shown in Figure 4. Under neutral conditions, all three compounds have absorbance maxima at ∼330 nm, with the 4-pyridyl (∼335 nm) and 2-pyridyl (∼329 nm) isomers absorbing at somewhat longer wavelengths than the 3-pyridyl isomer (∼318 nm). Substantial bathochromic shifts are observed in both acid and base, particularly in organic solvents. In base, all three isomers displayed a pronounced bathochromic shift with the same relative order of wavelengths of maximum absorption among the

(28) Robertson, D. W.; Wei, L. L.; Hayes, J. R.; Carlson, K. E.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology* **1981**, *109*, 1298.

Table 3. Long Wavelength Maxima of Ultraviolet Absorbance of Desmethylnafoxidine Analogs



29, (N)-2
30, (N)-3
31, (N)-4

		absorbance λ_{max} (ϵ)			
compound	condition[a]	THF	CH_3CN	EtOH	H_2O
29	neutral	328 (21 000)	320 (17 900)	330 (17 000)	336 (15 200)
	acidic	390 (17 800)	388 (16 600)	396 (18 300)	376 (12 500)
	basic	–	386 (21 700)	366 (21 800)	344 (15 300)
30	neutral	316 (17 100)	312 (13 900)	320 (14 600)	324 (12 400)
	acidic	334 (12 900)	360 (19 400)	342 (12 200)	318 (10 200)
	basic	–	374 (17 500)	364 (18 800)	332 (14 200)
31	neutral	328 (16 500)	335 (15 000)	336 (15 700)	346 (13 300)
	acidic	396 (20 500)	402 (16 600)	412 (18 300)	388 (14 100)
	basic	–	394 (21 600)	380 (21 400)	356 (16 400)

[a] Acidic = 0.1 N HCl prepared by adding concd HCl; basic = 0.1 N KOH prepared by adding 6 N KOH.

three isomers (4-pyridyl ~387 nm, 2-pyridyl ~376 nm, 3-pyridyl ~369 nm); in acid, however, the 2- and 4-pyridyl isomers displayed a considerable bathochromic shift (to ~403 nm and ~391 nm, respectively), whereas the 3-pyridyl isomer (to ~345 nm) showed merely a broadening of the peak. The most discernible solvatochromic trend was a hypsochromic shift in base with an increase in solvent polarity. Shifts in absorbance maxima in neutral and acidic media, on the other hand, were relatively modest. Overall, the trends observed in these spectra correlated reasonably well with those of the unsubstituted stilbazoles previously reported.[14]

The wavelengths of maximum absorbance of the four azaphenanthrenes (**48**–**51**) are shown in Table 4, and selected spectra are shown in Figure 5. The absorbance spectra for these rigid compounds are considerably more complex than those of the corresponding stilbazoles; however, some trends are similar. Once again, the most striking observation is the bathochromic shift shown by all compounds in all solvents when going from neutral media (334–362 nm) to acidic (418–442 nm) or basic (356–406 nm) media. In base, all compounds exhibited a pronounced bathochromic shift that is most pronounced for the 4-pyridyl-derived system (**50**). In acid, the 2- and 4-pyridyl-derived isomers (**48** and **50**) exhibited substantial red-shifted absorbances with high molar absorptivities (ϵ = 10 100–18 300 M^{-1} cm^{-1}), whereas both 3-pyridyl-derived isomers (**49**–**51**) were also red-shifted, but exhibited rather weak absorbances in this range (ϵ = 2900–3900 M^{-1} cm^{-1}).

The same solvatochromic trends were observed with these azaphenanthrenes as with the stilbazoles. As solvent polarity increased, a distinct hypsochromic shift was observed in base in all cases, while bathochromic shifts occurred in neutral conditions. The profound distinction between the 2- and 4-pyridyl-derived isomers from the two 3-pyridyl-derived isomers in acidic media with regard to intensity of longest wavelength of absorbance was not apparent in our previous studies of unsubstituted azaphenanthrenes.[14]

Fluorescence emission of each of the seven phenols was then measured in each of the aforementioned solvents and pH conditions; in each case, wavelengths of maximum emission were measured, along with quantum yields, which were calculated by the method of Olmsted, using Coumarin I in ethanol (320–410 nm), acridine yellow in methanol (410–480 nm), and quinine sulfate in 1.0 N H_2SO_4 (280–380 nm) as standards.[29] Table 5 shows the fluorescence data of the three stilbazoles (**29**–**31**). As expected, the fluorescence emission is extremely weak because the 1- and 2-aryl rings are twisted out of planarity from one another.

The fluorescence emission of azaphenanthrenes **48**–**51** are reported in Table 6, and selected spectra are illustrated in Figure 6. As with the absorbance spectra, bathochromic shifts occurred in both acid and base. In acid, the 4-pyridyl-derived isomer (**50**) was red-shifted the least of the four isomers (to 482–499 nm), but emitted with the greatest quantum yield (0.65–0.82), greater than what was observed in neutral conditions (0.42–0.69). Reasonably strong quantum yields (0.10–0.24) were observed for compound **50** in base as well, where wavelength maxima were red-shifted to 533–570 nm. The 2-pyridyl-derived isomer (**48**), on the other hand, was red-shifted up to 535–550 nm in acid, but emitted with weaker quantum yields (0.042–0.058) than those observed in neutral solvent (0.18–0.29). In addition, compound **48** displayed very weak fluorescence emission in base. The 3-pyridyl-derived isomers (**49** and **51**), however, displayed extremely weak fluorescence in both acid and base.

In addition to being responsive to pH, each compound displayed sensitivity to solvent polarity. In neutral solvents, a distinct bathochromic shift occurred with an increase in solvent polarity. It is difficult, however, to conclude what solvatochromic trends were exhibited in acid and base, due to the weak intensities of emission under these conditions. A final note concerns the profound quenching effect of water. Although some wavelength maxima appear considerably red-shifted in water, they are difficult to observe because of their weak intensities. The only exception to this is 4-pyridyl-

(29) Olmsted, J., III. *J. Phys. Chem.* **1979**, *83*, 2581.

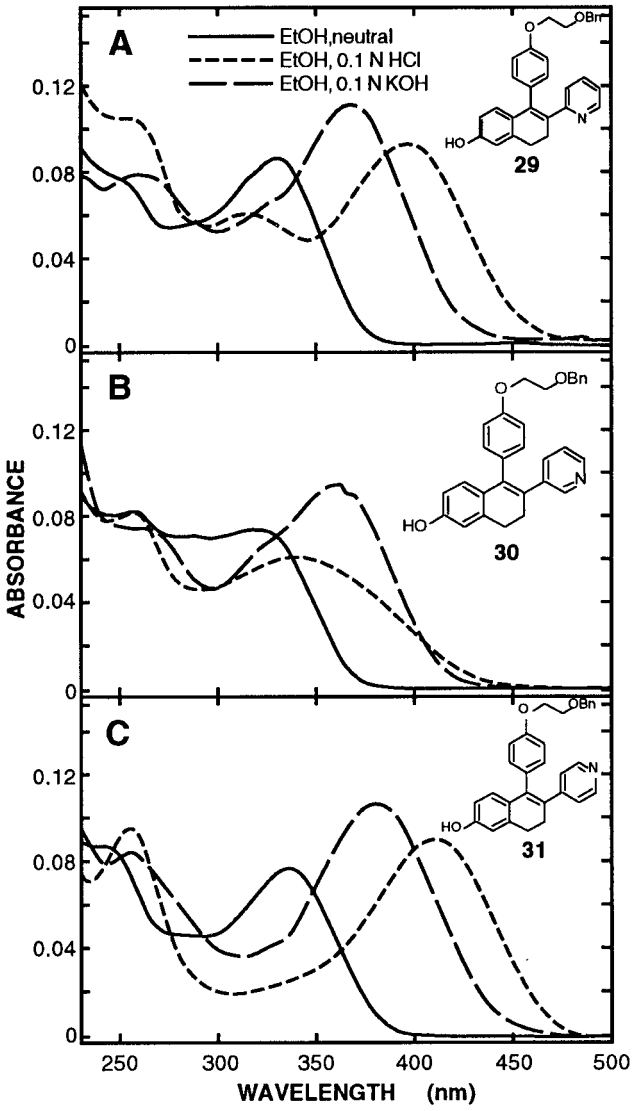


Figure 4. Ultraviolet spectra of dihydronaphthalenes **29**−**31** in EtOH. All spectra were obtained at the same concentraiton (5×10^{-6} M) in EtOH (neutral), or EtOH with 0.1 N HCl (acidic) or 0.1 N KOH (basic).

derived isomer **50**, which was the only isomer found to exhibit appreciable quantum yields in water, specifically, under both acidic and basic conditions (0.079 and 0.012, respectively).

In summary, the greatest distinction between the compounds studied with regard to both ultraviolet absorbance and fluorescence emission appears to exist between the resonance reinforced 2- and 4-pyridyl-derived isomers and the non-resonance-reinforced 3-pyridyl-derived isomers (Figure 7). Whereas one can draw resonance forms for the 2- and 4-pyridyl-derived isomers in which the positive charge on the phenolic ring oxygen can be compensated by negative charge localization on the nitrogen, one cannot draw such resonance forms with any of the 3-pyridyl stilbazoles or azaphenanthrenes. This electronic contribution to absorbance and emission appears to supersede all others, particularly for compound **50** in water.

A final comment is made concerning dual fluorescence. In our previous investigation,[14] we observed that the 4-pyridyl-derived azaphenanthrene exhibited dual fluorescence in protic solvent at any pH, presumably due to a zwitterionic phototautomer which can exist only in the excited state due to increased acidity and basicity of the phenol and pyridyl rings, respectively. This phenomenon, however, was not apparent in our present study.

Conclusion

We have prepared three isomeric pyridine-substituted analogs of desmethylnafoxidine as potential photofluorogenic probes for the estrogen receptor (ER). Each isomer possesses an aziridyl ring to covalently bind to ER, and a pyridyl ring within a stilbazole moiety, which can be photocyclized to one of four fluorescent azaphenanthrenoids after covalent attachment. All three aziridines prepared (**10**−**12**) were found to have high apparent binding affinity for ER, and were also shown to bind covalently to ER. Spectroscopic studies of intermediates (**29**−**31**, **48**−**51**) have shown that the 2- and 4-pyridyl isomers appear the most promising. While each of the four azaphenanthrenes (**48**−**51**) displayed strong sensitivity to both solvent polarity and pH, only the 2- and 4-pyridyl-derived isomers (**48** and **50**, respectively) exhibited appreciable quantum yields in acid and base, at wavelengths greater than or equal to 500 nm.

Future work entails optimizing the conditions for the photocyclization of these compounds once they are covalently bound to the estrogen receptor and studying their fluorescence properties in whole cells. Ultimately, we hope to use these compounds to aid both in ER structural elucidation and in breast tumor cell ER content measurements.

Experimental Section

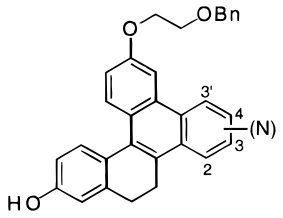
General. All reagents and solvents were obtained from Aldrich, Mallinckrodt, EM Science, and Burdick and Jackson. Tetrahydrofuran was distilled from sodium/benzophenone. Diisopropylamine, triethylamine, and methylene chloride were distilled from calcium hydride. Dimethylformamide was vacuum-distilled over magnesium sulfate and stored over 4 Å molecular sieves. Hexane was distilled prior to chromatographic use. *n*-Butyllithium (~1.6 M in hexane) was titrated with diphenylacetic acid. All reactions were performed under a dry N_2 atmosphere unless specified otherwise.[30] Reaction progress was monitored by analytical thin-layer chromatography, performed with 0.25 mm silica gel glass plates containing F-254 indicator (Merck). Visualization on TLC was achieved by either UV light (254 nm, 350 nm) or phosphomolybdic acid indicator. Flash chromatography[31] was performed with Woelm 32−63 μm silica gel packing. HPLC purification was performed on a Whatman Partisil M9 10/50 ODS-2 reverse phase column.

Fluorescence spectra were recorded by photon counting on a Spex Fluorolog 2 Spectrophotometer (Model FL111) with DM3000 software. Samples were prepared from stock solutions (~10^{-3} M) of the corresponding compounds in ethanol giving final concentrations of ~5×10^{-6} M. Acidic and basic solutions were prepared by addition of an amount of concentrated HCl or 6 N KOH solution in water, calculated to give a 0.1 N solution. All spectra were recorded at room temperature with 0.50 mm slits. All emission spectra are corrected, and solvent background is subtracted. Irradiations were conducted in a Rayonet photochemical reactor (RPR-100) fitted with 16 8 W lamps emitting 300 nm wavelength. Reactions took place in a water-cooled quartz vessel. Proton magnetic resonance spectra (200 MHz or 400 MHz) were recorded with chemical shifts reported as parts per million downfield from an internal tetramethylsilane standard.

Compounds **22** and **23** were prepared as described by Lednicer.[19] Ethyleneimine was prepared as described by

(30) Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, *41*, 1879.
(31) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Table 4. Long Wavelength Maxima of Ultraviolet Absorbance of Photocyclized Desmethylnafoxidine Analogs



48, (N)-2
49, (N)-3
50, (N)-4
51, (N)-3'

		absorbance λ_{max} (ϵ)			
compound	condition[a]	THF	CH_3CN	EtOH	H_2O
48	neutral	342 (17 700)	342 (15 200)	346 (14 900)	350 (10 600)
	acidic	430 (11 900)	432 (10 100)	440 (11 500)	424 (6 610)
	basic	–	396 (18 000)	370 (19 800)	360 (15 400)
49	neutral	344 (14 700)	334 (12 500)	342 (13 500)	346 (9 810)
	acidic	432 (3 900)	434 (2 830)	442 (3 350)	430 (2 460)
	basic	–	384 (31 000)	370 (17 000)	356 (13 600)
50	neutral	346 (14 700)	346 (12 400)	356 (13 400)	362 (9 310)
	acidic	418 (18 300)	420 (15 800)	426 (17 700)	412 (10 200)
	basic	–	406 (24 600)	396 (20 200)	382 (15 100)
51	neutral	340 (14 300)	340 (12 600)	346 (13 800)	348 (7 650)
	acidic	432 (2 900)	438 (3 480)	436 (2 840)	428 (1 730)
	basic	–	386 (27 800)	370 (18 100)	358 (14 500)

[a] Acidic = 0.1 N HCl prepared by adding concd HCl; basic = 0.1 N KOH prepared by adding 6 N KOH.

Reeves.[25] ***Cautionary Note:*** *Ethylenimine is volatile, reactive, carcinogenic, and acutely toxic.*[32] *All manipulations involved in its preparation, purification, and use should be performed in a well ventilated fume hood, providing protection against explosion. 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.*

In most cases, a general procedure for product isolation and purification was utilized that involved quenching the reaction in an aqueous solution, exhaustive extraction in an organic solvent, drying over an anhydrous salt, filtration, evaporation under reduced pressure, and flash chromatography. In the experimental descriptions this is indicated by the phrase "product isolation" (which is then followed, in parentheses, by a listing of quenching agent, extraction solvent, drying agent if not Na_2SO_4, which is not listed) and "purification" (which is followed, in parenthesis, by the elution solvent used in the flash chromatography).

2-(Benzyloxy)ethyl Methanesulfonate (14). To a solution of 2-(benzyloxy)ethanol (2.00 g, 13.1 mmol) dissolved in 100 mL of THF at 0 °C was added dropwise first Et_3N (1.99 g, 19.7 mmol) followed by MsCl (2.26 g, 19.7 mmol). The reaction mixture was warmed to room temperature and stirred for an additional 30 min. Product isolation (1 M $NaHCO_3$, EtOAc) and purification (1:1 EtOAc:hexane) yielded mesylate **14** as a colorless oil (2.98 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.04 (s, 3H), 3.75 (m, 2H, 4.40 (m, 2H), 4.58 (s, 2H), 7.34 (m, 5H); MS (EI, 70 eV) *m*/*z* (relative intensity) 230 (M^+, 3), 123 (3), 107 (37), 91 (100), 79 (12), 65 (15), 51 (5), 40 (7). Anal. Calcd for $C_{10}H_{14}O_4S$: C, 52.16; H, 6.13; S, 13.92. Found: C, 52.08; H, 6.19; S, 13.89.

General Procedure for Alkylation of Phenols. To a stirred solution of 1.0 equiv of phenol in 2-butanone was added 5.0 equiv of K_2CO_3 and 1.2 equiv of mesylate **14**. The reaction mixture was mechanically stirred and heated to 80 °C for 72 h. Product isolation (H_2O, EtOAc) and purification yielded the desired aryl ether.

4-[2-(Benzyloxy)ethoxy]benzonitrile (15). Product isolation and purification (1:4 EtOAc:hexane) afforded **15** as a colorless oil (370 mg, 87%), prepared from 4-cyanophenol (200 mg, 1.68 mmol), mesylate **14** (463 mg, 2.01 mmol), and K_2CO_3 (1.16 g, 8.39 mmol) in 100 mL of 2-butanone. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (m, 2H), 4.18 (m, 2H), 4.63 (s, 2H), 6.97 (d, J = 9.0 Hz, 2H), 7.36 (m, 5H), 7.54 (d, J = 9.0 Hz, 2H); MS (EI, 70 eV) *m*/*z* (relative intensity) 253 (M^+, 8), 119 (7), 105 (8), 91 (100), 77 (4), 70 (14), 61 (90), 51 (4), 43 (33). Anal. Calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.70; H, 5.94; N, 5.40.

4-[2-(Benzyloxy)ethoxy]benzaldehyde (16). Product isolation and purification (1:4 EtOAc:hexane) afforded **16** as a colorless oil (8.92 g, 85%), prepared from 4-hydroxybenzaldehyde (5.00 g, 40.9 mmol), mesylate **14** (9.72 g, 49.0 mmol), and K_2CO_3 (28.3 g, 205 mmol) in 250 mL of 2-butanone. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (m, 2H), 4.23 (m, 2H), 4.64 (s, 2H), 7.03 (d, J = 8.8 Hz, 2H), 7.36 (m, 5H), 7.83 (d, J = 8.8 Hz, 2H), 9.89 (s, 1H); MS (EI, 70 eV) *m*/*z* (relative intensity) 256 (M^+, 3), 150 (7), 122 (8), 105 (12), 91 (100), 77 (9), 65 (11), 51 (6), 40 (6). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.94; H, 6.35.

General Procedure for Picoline Additions. To a stirred solution of THF cooled to −78 °C was added 1.0−1.2 equiv of diisopropylamine, followed by the dropwise addition of 1.0−1.2 equiv of *n*-butyllithium (not exceeding the quantity of diisopropylamine). The solution was stirred for 10 min, allowed to warm to room temperature for 10 min, and then recooled to −78 °C for an additional 10 min. This was followed by the dropwise addition of 1.0−1.2 equiv of the appropriate picoline isomer. The reaction mixture was allowed to warm to room temperature while stirring continued for 1 h. Dropwise addition of 1.0 equiv of appropriate aldehyde then followed, and stirring was continued at room temperature for the specified time. Product isolation (1 M $NaHCO_3$, EtOAc) and purification yielded the desired product.

4′-[2-(Benzyloxy)ethoxy]-2-(2-pyridyl)acetophenone (17). Lithium diisopropylamide was prepared as described above from diisopropylamine (3.08 g, 30.5 mmol) and *n*-butyllithium (23.4 mL of 1.30 M in hexane, 30.5 mmol) in 250 mL of THF. The subsequent addition followed as described above using 2-picoline (2.84 g, 30.5 mmol) and nitrile **15** (6.43 g, 25.4 mmol), and the mixture was stirred for 3 h. Upon concentration of organic extracts, the resulting imine was hydrolyzed with 2 N HCl for 10 min. Product isolation (2 M NaOH, EtOAc) and purification (1:1 EtOAc:hexane) yielded a yellow solid (7.74 g, 88%) as a mixture of keto and enol tautomers. ^{1}H NMR of keto tautomer (400 MHz, $CDCl_3$) δ 3.85

(32) Dermer, O.; Ham, G. *Ethylenimine and Other Aziridines*; Academic Press: New York, 1969.

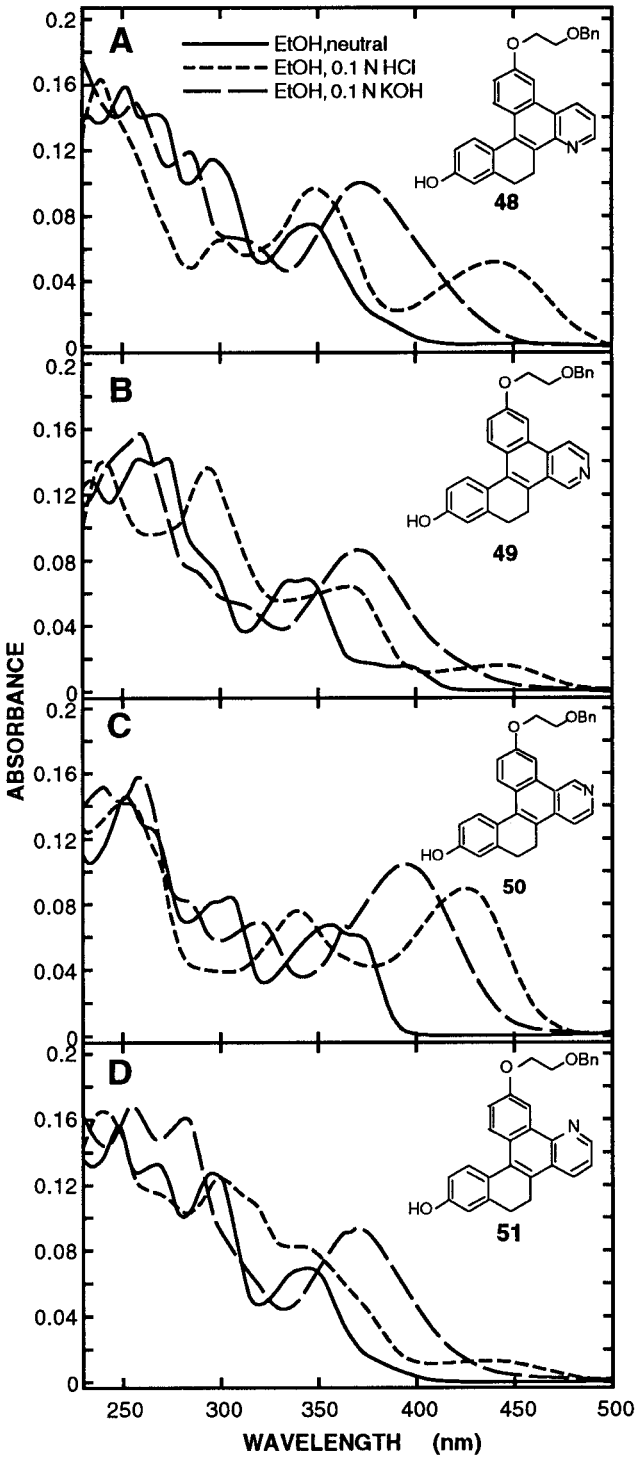


Figure 5. Ultraviolet spectra of azaphenanthrenoids **48**−**51**. All spectra were obtained at the same concentraiton (5×10^{-6} M) in EtOH (neutral), or EtOH with 0.1 N HCl (acidic) or 0.1 N KOH (basic).

(m, 2H), 4.20 (m, 2H), 4.45 (s, 2H), 4.63 (s, 2H), 6.95 (d, J = 9.0 Hz, 2H), 7.17 (m, 1H), 7.30−7.36 (m, 6H), 7.79 (d, J = 9.0 Hz, 1H), 8.04 (d, J = 9.0 Hz, 2H), 8.55 (m, 1H,); MS (EI, 70 eV) m/z (relative intensity) 347 (M^+, 2), 319 (4), 255 (14), 121 (5), 105 (7), 91 (100), 77 (4), 65 (11), 51 (3), 40 (5). Anal. Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 76.04; H, 6.10; N, 4.02.

Ethanol, 1-[4-[2-(Benzyloxy)ethoxy]phenyl]-2-(3-pyridyl)-, (*R*,*S*)- (18). Alcohol **18** was prepared from 2.05 g (20.3 mmol) of diisopropylamine, 13.4 mL of 1.41 M *n*-butyllithium, 1.76 g (18.9 mmol) of 3-picoline, and 4.85 g (18.9 mmol) of aldehyde **16** in 250 mL of THF. After stirring for 12 h, product isolation (1 M $NaHCO_3$, EtOAc) and purification (1:2 CH_2Cl_2) afforded **18** as a white solid (1.92 g, 29%). Mp 72−74 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.00 (m, 2H), 3.82 (m, 2H), 4.13 (m, 2H), 4.63 (s, 2H), 4.83 (dd, J = 7.6, 5.6 Hz, 1H), 6.88 (d, J = 8.7 Hz, 2H), 7.13 (m, 1H), 7.20 (d, J = 8.7 Hz, 2H), 7.30−7.37 (m, 5H), 7.42 (d, J = 7.8 Hz, 1H), 8.35 (s, 1H), 8.41 (m, 1H); MS (EI, 70 eV) m/z (relative intensity) 349 (M^+, off-scale), 257 (15), 123 (3), 105 (5), 91 (100), 77 (6), 65 (9), 39 (5). Anal. Calcd for $C_{22}H_{23}NO_3$: C, 75.62; H, 6.63; N, 4.01. Found: C, 75.65; H, 6.66; N, 4.01.

Ethanol, 1-[4-[2-(Benzyloxy)ethoxy]phenyl]-2-(4-pyridyl)-, (*R*,*S*)- (19). Alcohol **19** was prepared from 917 mg (9.06 mmol) of diisopropylamine, 6.60 mL of 1.30 M *n*-butyllithium (8.58 mmol), 799 mg (8.58 mmol) of 4-picoline, and 2.00 g (7.80 mmol) of aldehyde **16** in 100 mL of THF. After stirring for 12 h, product isolation (1 M $NaHCO_3$, EtOAc) and purification (1:2 acetone:methylene chloride) afforded alcohol **19** as a white solid (1.97 g, 73% yield). Mp 83−85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.03 (m, 2H), 3.83 (m, 2H), 4.15 (m, 2H), 4.64 (s, 2H), 4.90 (dd, J = 7.6, 5.4 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 7.13 (d, J = 5.9 Hz, 2H), 7.20 (d, J = 8.7 Hz, 2H), 7.30−7.37 (m, 5H), 8.48 (m, 2H); MS (EI, 70 eV) m/z (relative intensity) 349 (M^+, 2), 257 (17), 123 (3), 105 (4), 91 (100), 77 (5), 65 (9), 40 (4). Anal. Calcd for $C_{22}H_{23}NO_3$: C, 75.62; H, 6.63; N, 4.01. Found: C, 75.30; H, 6.71; N, 4.07.

General Procedure for Oxidation of Secondary Alcohols. To a solution of the alcohol in acetone at −15 °C (CCl_4/dry ice) was added Jones reagent (~10 mL per gram of alcohol). Jones reagent was prepared from 23.5 g of CrO_3 dissolved in 21 mL of concd H_2SO_4 with cooling and then diluted with distilled water to give a total volume of 175 mL. After 15 min, product isolation (1 M Na_2CO_3, EtOAc) and purification gave the desired ketone.

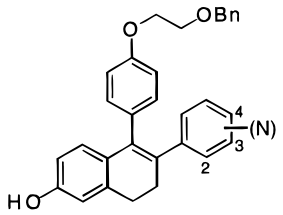
4′-[2-(Benzyloxy)ethoxy]-2-(3-pyridyl)acetophenone (20). Ketone **20** was prepared from 1.53 g (4.38 mmol) of alcohol **18** and 15 mL of Jones reagent in 150 mL of acetone. Product isolation and purification (1:2 acetone:CH_2Cl_2) afforded 1.27 g (84%) of ketone **20** as a white solid. Mp 49−51 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (m, 2H), 4.22 (m, 2H), 4.26 (s, 2H), 4.64 (s, 2H), 6.98 (d, J = 9.0 Hz, 2H), 7.35 (m, 6H), 7.64 (m, 1H), 7.99 (d, J = 9.0 Hz, 2H), 8.53 (m, 2H); MS (EI, 70 eV) m/z (relative intensity) 347 (M^+, off-scale), 319 (14), 255 (12), 150 (5), 122 (6), 105 (12), 91 (100), 77 (11), 65 (15), 57 (25), 43 (22). Anal. Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 75.86; H, 6.04; N, 4.04.

4′-[2-(Benzyloxy)ethoxy]-2-(4-pyridyl)acetophenone (21). Ketone **21** was prepared from 1.00 g (2.86 mmol) of alcohol **19** and 10 mL of Jones reagent in 100 mL of acetone. Product isolation and purification (1:2 acetone:CH_2Cl_2) afforded 932 mg (94%) of ketone **21** as a white solid. Mp 68−70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (m, 2H), 4.22 (m, 2H), 4.25 (s, 2H), 4.64 (s, 2H), 6.98 (d, J = 9.0 Hz, 2H), 7.24 (d, J = 5.1 Hz, 2H), 7.36 (m, 5H), 7.97 (d, J = 9.0 Hz, 2H), 8.57 (m, 2H); MS (EI, 70 eV) m/z (relative intensity) 347 (M^+, off-scale), 269 (1), 255 (36), 149 (11), 121 (9), 105 (9), 91 (100), 77 (6), 65 (10), 51 (3), 40 (5). Anal. Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 75.86; H, 6.18; N, 4.00.

3-Hydroxyphenethyl Iodide (24). To a solution of mesylate **23** (2.06 g, 8.93 mmol) in 25 mL of CH_2Cl_2 was added iodotrimethylsilane (5.57 g, 35.7 mmol). After stirring for 20 h at room temperature, product isolation (saturated $Na_2S_2O_3$, CH_2Cl_2) and purification (1:4 EtOAc:hexane) provided phenolic iodide **24** as a white solid (1.70 g, 77%). Mp 54−56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.13 (t, J = 7.8 Hz, 2H), 3.34 (t, J = 7.8 Hz, 2H), 4.76 (s, 1H, -O*H*), 6.68 (s, 1H), 6.75 (m, 2H), 7.18 (t, J = 7.8 Hz, 1H); MS (EI, 70 eV) m/z (relative intensity) 248 (M^+, 12), 121 (100), 103 (21), 91 (22), 77 (30), 65 (12), 51 (6), 43 (41). Anal. Calcd for C_8H_9IO: C, 38.74; H, 3.66; I, 51.16. Found: C, 38.70; H, 3.67; I, 51.01.

3-(Tetrahydropyran-2-yloxy)phenethyl Iodide (25). To a solution of phenol **24** (1.38 g, 5.58 mmol) dissolved in 5 mL of CH_2Cl_2 at 0 °C were added dihydropyran (0.939 g, 11.2 mmol) and a catalytic amount of pyridinium *p*-toluenesulfonate. The reaction was allowed to warm to room temperature and stir for 2 h. Product isolation (1 M $NaHCO_3$, CH_2Cl_2) and purification (1:4 EtOAc:hexane) yielded tetrahy-

Table 5. Fluorescence Emission Maxima of Desmethylnafoxidine Analogs

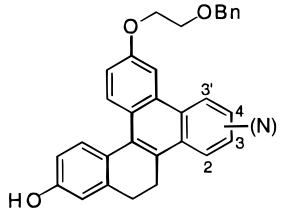


29, (N)-2
30, (N)-3
31, (N)-4

		emission λ_{max} (Φ_F)			
compound	condition[a]	THF	CH_3CN	EtOH	H_2O
29	neutral	425 (0.0037)	423 (0.0009)	426 (0.0009)	432 (0.0015)
	acidic	516 (0.0006)	516 (0.0005)	508 (0.0009)	517 (0.0006)
	basic	–	520 (0.0003)	490 (0.0013)	502 (0.0006)
30	neutral	425 (0.0021)	427 (0.0007)	498 (0.0027)	431 (0.0018)
	acidic	518 (0.0038)	563 (0.0001)	557 (0.0003)	425 (0.0005)
	basic	–	503 (0.0003)	486 (0.0001)	485 (0.0004)
31	neutral	404 (0.0059)	430 (0.0005)	428 (0.0012)	498 (0.0004)
	acidic	501 (0.0017)	533 (0.0008)	530 (0.0012)	525 (0.0009)
	basic	–	541 (0.0005)	515 (0.0013)	539 (0.0008)

[a] Acidic = 0.1 N HCl prepared by adding concd HCl; basic = 0.1 N KOH prepared by adding 6 N KOH. [b] Φ_F Calculated using Coumarin I in ethanol, quinine sulfate in 1.0 N H_2SO_4, or acridine yellow in methanol as standards.

Table 6. Fluorescence Emission Maxima of Photocyclized Desmethylnafoxidine Analogs



48, (N)-2
49, (N)-3
50, (N)-4
51, (N)-3'

		emission λ_{max} (Φ_F)			
compound	condition[a]	THF	CH_3CN	EtOH	H_2O
48	neutral	419 (0.2311)	422 (0.1800)	430 (0.2889)	433 (0.0009)
	acidic	535 (0.0578)	550 (0.0599)	550 (0.0423)	558 (0.0029)
	basic	–	628 (0.0003)	573 (0.0015)	560 (0.0002)
49	neutral	419 (0.3217)	422 (0.3533)	426 (0.3555)	490 (0.0006)
	acidic	524 (0.0150)	548 (0.0058)	527 (0.0022)	516 (0.0007)
	basic	–	573 (0.0001)	514 (0.0004)	527 (0.0001)
50	neutral	402 (0.4199)	406 (0.4415)	420 (0.6851)	500 (0.0003)
	acidic	482 (0.7046)	499 (0.6469)	498 (0.8227)	500 (0.0782)
	basic	–	570 (0.0967)	533 (0.2377)	599 (0.0123)
51	neutral	420 (0.2817)	423 (0.2456)	432 (0.1971)	481 (0.0023)
	acidic	548 (0.0143)	574 (0.0022)	551 (0.0024)	503 (0.0005)
	basic	–	545 (0.0010)	506 (0.0032)	505 (0.0014)

[a] Acidic = 0.1 N HCl prepared by adding concd HCl; basic = 0.1 N KOH prepared by adding 6 N KOH. [b] Φ_F Calculated using Coumarin I in ethanol, quinine sulfate in 1.0 N H_2SO_4, or acridine yellow in methanol as standards.

dropyranyl ether **25** as a colorless oil (1.82 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54−2.03 (m, 6H), 3.15 (m, 2H), 3.34 (m, 2H), 3.62 (m, 1H), 3.91 (m, 1H), 5.42 (t, J = 3.3 Hz, 1H), 6.81 (m, 1H), 6.89 (s, 1H), 6.96 (m, 1H), 7.22 (t, J = 7.9 Hz, 1H); MS (EI, 70 eV) m/z (relative intensity) 332 (M^+, off-scale), 248 (7), 121 (13), 85 (100), 77 (3), 67 (16), 57 (18), 43 (19). Anal. Calcd for $C_{13}H_{17}IO_2$: C, 47.01; H, 5.16; I, 38.20. Found: C, 47.10; H, 5.18; I, 38.02.

General Procedure for Alkylation of Ketones. To a stirred suspension of sodium hydride (1.0−1.1 equiv of a 60% w/w oil dispersion) in DMF at 0 °C was added dropwise a solution of the ketone dissolved in DMF. After stirring at room temperature for 4 h, a solution of 1.0−1.1 equiv of iodide **25** dissolved in DMF was then added dropwise. After stirring for 48 h, product isolation (saturated NH_4Cl solution, EtOAc) and purification afforded the alkylated ketones.

4′-[2-Benzyloxy)ethoxy]-2-[3-(tetrahydropyran-2-yloxy)phenyl]-2-(2-pyridyl)acetophenone (26). Product isolation and purification (1:1 EtOAc:hexane) afforded the mixture of diastereomers **26** as a colorless oil (346 mg, 65% yield) from ketone **17** (335 mg, 0.962 mmol), NaH dispersion (40 mg, 1.01 mmol), and iodide **25** (336 mg, 1.01 mmol) in 3 mL of DMF. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56−2.05 (m, 6H), 2.22 (m, 1H), 2.52−2.67 (m, 3H), 3.54 (m, 1H), 3.81 (m, 2H), 3.89 (m, 1H), 4.16 (m, 2H), 4.61 (s, 2H), 4.85 (m, 1H), 5.36 (dt, J = 14.9, 3.2 Hz, 1H), 6.70−6.77 (m, 3H), 6.88 (d, J = 8.5 Hz, 2H), 7.10−7.18 (m, 2H), 7.26−7.36 (m, 6H), 7.60 (m, 1H), 8.00 (m, 2H), 8.54 (m, 1H); MS (EI, 70 eV) m/z (relative intensity) 551 (M^+, off-scale), 467 (2), 446 (2), 347 (23), 255 (28), 211 (4), 121 (8), 105 (5), 91 (100), 84 (29), 77 (5), 65 (8), 55 (34), 41 (20). Anal. Calcd for $C_{35}H_{37}NO_5$: C, 76.20; H, 6.76; N, 2.54. Found: C, 75.96; H, 6.78; N, 2.52.

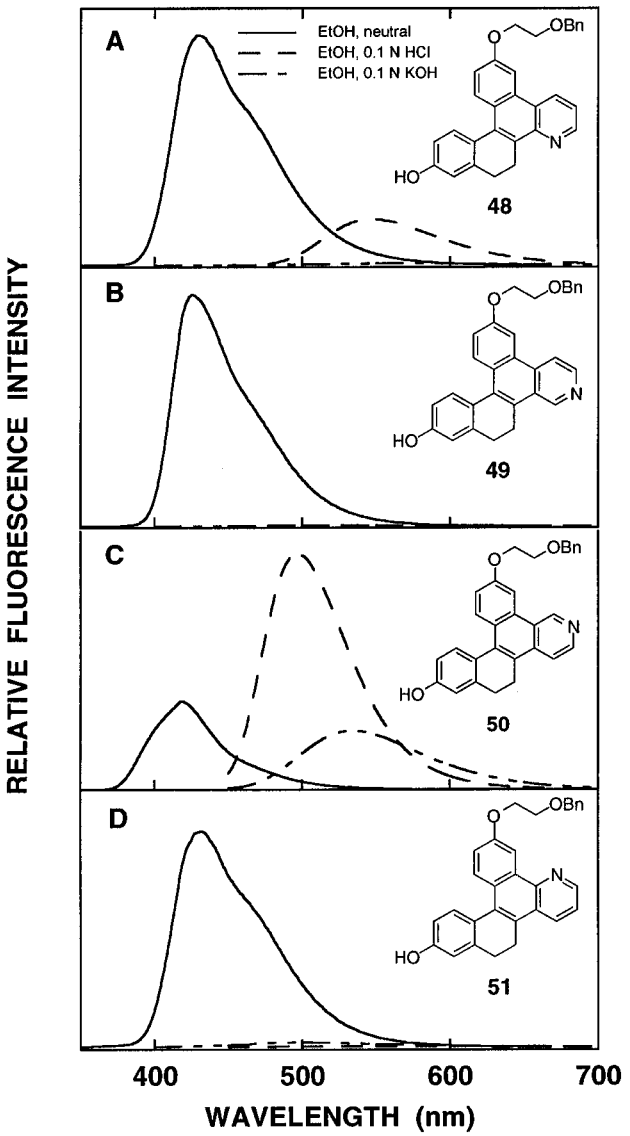


Figure 6. Fluorescence emission spectra of azaphenanthrenoids **48**−**51**. All spectra were obtained at the same concentration (5×10^{-6} M) and under the same instrumental conditions in EtOH (neutral) or EtOH with 0.1 N HCl (acidic) or 0.1 N KOH (basic). Excitation was done at the maximum absorbance of the longest wavelength ultraviolet absorption peak under the same conditions (see Figure 5 or Table 4).

4′-[2-(Benzyloxy)ethoxy]-2-[3-(tetrahydropyran-2-yloxy)phenyl]-2-(3-pyridyl)acetophenone (27). Product isolation and purification (1:19 acetone:CH_2Cl_2) afforded the mixture of diastereomers **27** as a colorless oil (320 mg, 55% yield) from ketone **20** (366 mg, 1.05 mmol), NaH dispersion (42 mg, 1.05 mmol), and iodide **25** (349 mg, 1.05 mmol) in 2 mL of DMF. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57−2.01 (m, 6H), 2.11−2.16 (m, 1H), 2.49−2.59 (m, 3H), 3.56−3.61 (m, 1H), 3.82 (m, 2H), 3.84−3.92 (m, 1H), 4.17 (m, 2H), 4.52−4.56 (m, 1H), 4.62 (s, 2H), 5.37 (dt, J = 11.7, 3.3 Hz, 1H), 6.74−6.94 (m, 3H), 6.89 (m, 2H), 7.17−7.25 (m, 2H), 7.26−7.38 (m, 6H), 7.65−7.69 (m, 1H), 7.84−7.88 (m, 2H), 8.48 (m, 1H), 8.57 (m, 1H); MS (EI, 70 eV) m/z (relative intensity) 551 (M^+, off-scale), 467 (1), 347 (4), 255 (27), 243 (20), 213 (6), 120 (38), 106 (20), 91 (100), 85 (95), 78 (20), 67 (19), 57 (21), 51 (9), 43 (23). Anal. Calcd for $C_{35}H_{37}NO_5$: C, 76.20; H, 6.76; N, 2.54. Found: C, 76.21; H, 6.80; N, 2.50.

4′-[2-(Benzyloxy)ethoxy]-2-[3-(tetrahydropyran-2-yloxy)phenyl]-2-(4-pyridyl)acetophenone (28). Product isolation and purification (1:19 acetone:CH_2Cl_2) afforded the mixture of diastereomers **28** as a colorless oil (969 mg, 61% yield) from ketone **21** (1.02 g, 2.93 mmol), NaH dispersion (123 mg, 3.08 mmol), and iodide **25** (1.02 g, 3.08 mmol) in 3 mL of DMF. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56−2.01 (m, 6H), 2.13 (m, 1H), 2.48−2.59 (m, 3H), 3.55−3.61 (m, 1H), 3.82 (m, 2H), 3.89 (m, 1H), 4.16−4.19 (m, 2H), 4.48−4.53 (m, 1H), 4.62 (s, 2H), 5.34−5.39 (m, 1H), 6.73−6.92 (m, 5H), 7.17−7.38 (m, 8H), 7.81−7.86 (m, 2H), 8.00−8.54 (m, 2H); MS (EI, 70 eV) m/z (relative intensity) 551 (M^+, off-scale), 467 (6), 347 (19), 255 (35), 121 (9), 106 (11), 91 (100), 84 (63), 77 (6), 69 (11), 55 (78), 43 (21). Anal. Calcd for $C_{35}H_{37}NO_5$: C, 76.20; H, 6.76; N, 2.54. Found: C, 76.26; H, 6.69; N, 2.57.

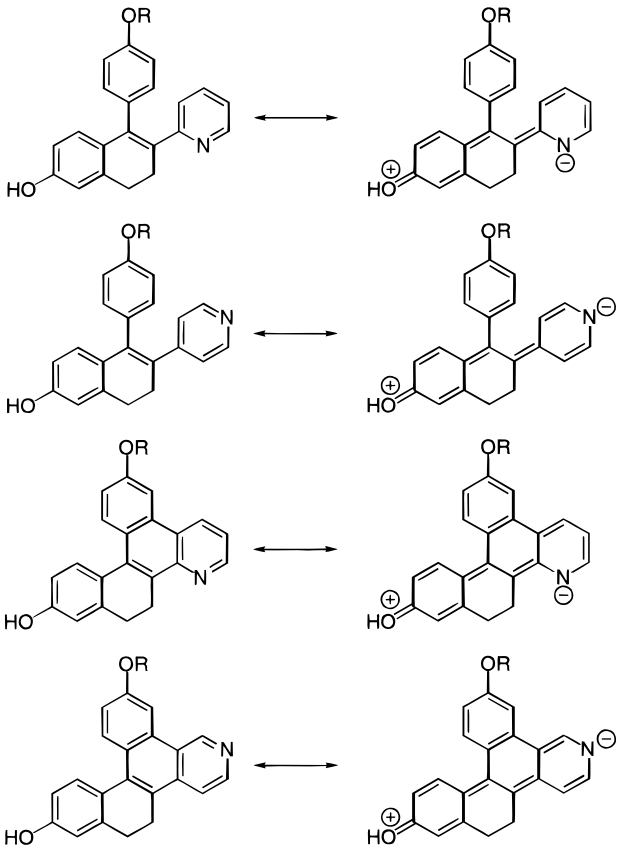


Figure 7. Resonance structures of the 2- and 4-pyridyl dihydronaphthalenes and their related azaphenanthrenes.

General Procedure for Cyclization of Ketones. To a solution of ketone in CH_2Cl_2 was added dropwise 5 equiv of methanesulfonic acid. The olive green solution was allowed to stir at room temperature for 24 h. Product isolation (H_2O, CH_2Cl_2) and purification yielded the cyclized phenol.

1-[4-[2-(Benzyloxy)ethoxy]phenyl]-6-hydroxy-2-(2-pyridyl)-3,4-dihydronaphthalene (29). The cyclized phenol was obtained as a clear oil (1.86 g, 75%) from ketone **26** (3.06 g, 5.54 mmol) and methanesulfonic acid (2.66 g, 27.7 mmol) in 90 mL of CH_2Cl_2, after product isolation and purification (1:1 EtOAc:hexane). An analytically pure sample was obtained as a white solid by recrystallization from EtOAc/hexane. Mp 146−148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 (m, 4H), 3.83 (m, 2H), 4.13 (m, 2H), 4.65 (s, 2H, 6.55 (dd, J = 8.5, 2.7 Hz, 1H), 6.66 (d, J = 8.5 Hz, 1H), 6.72 (d, J = 8.1 Hz, 1H), 6.75 (m, 1H), 6.81 (d, J = 8.7 Hz, 2H), 6.97 (m, 1H), 6.98 (d, J = 8.7 Hz, 2H), 7.28−7.39 (m, 6H), 8.53 (m, 1H); MS (FAB) m/z 450 ($M^+ + 1$), 279, 223, 205, 195. Anal. Calcd for $C_{30}H_{27}NO_3$: C, 80.15; H, 6.05; N, 3.12. Found: C, 80.17; H, 6.06; N, 3.12.

1-[4-[2-(Benzyloxy)ethoxy]phenyl]-6-hydroxy-2-(3-pyridyl)-3,4-dihydronaphthalene (30). The cyclized phenol was obtained as a clear oil (555 mg, 70%) was prepared from ketone **27** (972 mg, 1.76 mmol) and methanesulfonic acid (847 mg, 8.81 mmol) in 30 mL of CH_2Cl_2, after product isolation and purification (1:4 acetone:CH_2Cl_2). An analytically pure sample was obtained as a white solid by recrystallization from EtOAc/hexane. Mp = 175−177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.69 (m, 2H, 2.93 (m, 2H), 3.83 (m, 2H), 4.12 (m, 2H), 4.64

(s, 2H), 6.58 (dd, J = 8.5, 2.5 Hz, 1H), 6.67 (d, J = 8.3 Hz, 1H), 6.74 (d, J = 2.5 Hz, 1H), 6.79 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 7.14 (m, 1H), 7.27−7.40 (m, 6H), 8.29 (m, 2H); MS (FAB) m/z 450 ($M^+ + 1$), 223, 195. HRMS calcd for $C_{20}H_{28}NO_3$ $(M + 1)^+$ 450.2069, found 450.2075.

1-[4-[2-(Benzyloxy)ethoxy]phenyl]-6-hydroxy-2-(4-pyridyl)-3,4-dihydronaphthalene (31). The cyclized phenol was obtained as a clear oil (1.27 g, 78%) from ketone **28** (2.00 g, 3.64 mmol) and methanesulfonic acid (1.75 g, 18.2 mmol) in 60 mL of CH_2Cl_2, after product isolation and purification (1:4 acetone:CH_2Cl_2). An analytically pure sample was obtained as a yellow solid by recrystallization from EtOAc/hexane. Mp 168−170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 (m, 2H), 2.92 (m, 2H), 3.83 (m, 2H), 4.13 (m, 2H), 4.64 (s, 2H), 6.55 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 2.5 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 6.3 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 7.26−7.41 (m, 5H), 8.29 (d, J = 6.3 Hz, 2H); MS (FAB) m/z 450 ($M^+ + 1$), 279, 223, 169. Anal. Calcd for $C_{30}H_{27}NO_3$: C, 80.15; H, 6.05; N, 3.12. Found: C, 79.78; H, 6.09; N, 3.05.

General Procedure for Triflation of Phenols. Trifluoromethanesulfonic anhydride (1.5 equiv) was added to a solution of the phenol and 2,6-lutidine (2.0 equiv) in CH_2Cl_2 at 0 °C. After stirring for 1 h while warming to room temperature, product isolation (H_2O, CH_2Cl_2) and purification afforded the aryl triflate.

1-[4-[2-(Benzyloxy)ethoxy]phenyl]-2-(2-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (32). The product was obtained as a colorless oil (56 mg, 87% yield) from phenol **29** (50 mg, 0.11 mmol), triflic anhydride (48 mg, 0.17 mmol), and 2,6-lutidine (24 mg, 0.22 mmol) in 10 mL of CH_2Cl_2, after product isolation and purification (1:2 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96 (m, 4H), 3.84 (m, 2H, 4.14 (m, 2H), 4.65 (s, 2H), 6.71 (d, J = 8.1 Hz, 1H), 6.82 (d, J = 8.3 Hz, 2H), 6.88 (d, J = 8.6 Hz, 1H), 6.95 (dd, J = 8.6, 2.7 Hz, 1H), 6.97 (d, J = 8.3 Hz, 2H), 6.98 (m, 1H), 7.13 (d, J = 2.7 Hz, 1H), 7.22−7.40 (m, 6H), 8.55 (m, 1H); MS (FAB) m/z 582 ($M^+ + 1$). Anal. Calcd for $C_{31}H_{26}F_3NO_5S$: C, 64.02; H, 4.51; F, 9.80; N, 2.41; S, 5.51. Found: C, 63.97; H, 4.50; F, 9.72; N, 2.40; S, 5.47.

1-[4-[2-(Benzyloxy)ethoxy]phenyl]-2-(3-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (33). The product was obtained as a colorless oil (78 mg, 79% yield) from phenol **30** (76 mg, 0.17 mmol), triflic anhydride (72 mg, 0.25 mmol), and 2,6-lutidine (36 mg, 0.34 mmol) in 5 mL of CH_2Cl_2, after product isolation and purification (1:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.82 (m, 2H), 3.03 (m, 2H 3.83 (m, 2H), 4.12 (m, 2H), 4.64 (s, 2H), 6.81 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.7 Hz, 1H), 6.92 (d, J = 8.5 Hz, 2H), 6.95 (dd, J = 8.7, 2.5 Hz, 1H), 7.12 (d, J = 2.5 Hz, 1H), 7.28−7.40 (m, 6H), 8.35 (m, 2H); MS (FAB) m/z 582 ($M^+ + 1$), 447, 279. Anal. Calcd for $C_{31}H_{26}F_3NO_5S$: C, 64.02; H, 4.51; F, 9.80; N, 2.41; S, 5.51. Found: C, 64.14; H, 4.54; F, 9.80; N, 2.41; S, 5.40.

1-[4-[2-(Benzyloxy)ethoxy]phenyl]-2-(4-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (34). The product was obtained as a colorless oil (314 mg, 79% yield) from phenol **31** (307 mg, 0.682 mmol), triflic anhydride (288 mg, 1.02 mmol), and 2,6-lutidine (146 mg, 1.36 mmol) in 50 mL of CH_2Cl_2, after product isolation and purification (1:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (m, 2H, 3.02 (m, 2H), 3.84 (m, 2H), 4.13 (m, 2H), 4.65 (s, 2H), 6.82 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.5 Hz, 1H), 6.91 (d, J = 8.6 Hz, 2H), 6.92 (m, 2H), 6.96 (dd, J = 8.5, 2.5 Hz, 1H), 7.13 (d, J = 2.5 Hz, 1H), 7.28−7.40 (m, 5H), 8.36 (m, 2H); MS (FAB) m/z 582 ($M^+ + 1$), 449. Anal. Calcd for $C_{31}H_{26}F_3NO_5S$: C, 64.02; H, 4.51; F, 9.80; N, 2.41; S, 5.51. Found: C, 63.97; H, 4.49; F, 9.75; N, 2.40; S, 5.52.

General Procedure for Cleavage of Benzyl Ethers. Iodotrimethylsilane (4.0−5.0 equiv) was added to a solution of the benzyl ether (1.0 equiv) and thiophenol (1.0 equiv) in CH_2Cl_2. An additional 4.0−5.0 equiv of iodotrimethylsilane was added after 12 h of stirring at room temperature. After stirring for another 12 h, product isolation (5% aqueous $Na_2S_2O_3$, CH_2Cl_2) and purification afforded the desired alcohol.

1-[4-(2-Hydroxyethoxy)phenyl]-2-(2-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (35). The product was obtained as a white foam (38 mg, 80%) from ether **32** (56 mg, 0.096 mmol), iodotrimethylsilane (173 mg, 0.867 mmol), and thiophenol (12 mg, 0.11 mmol) in 2 mL of CH_2Cl_2, after product isolation and purification (1:49 MeOH:CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.97 (m, 4H), 3.98 (m, 2H), 4.08 (m 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.83 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.5 Hz, 1H), 6.97 (dd, J = 8.5, 2.7 Hz, 1H), 6.98 (m, 1H), 6.99 (d, J = 8.8 Hz, 2H), 7.13 (d, J = 2.7 Hz, 1H), 7.30 (m, 1H), 8.55 (m, 1H); MS (FAB) m/z 492 ($M^+ + 1$). Anal. Calcd for $C_{24}H_{20}F_3NO_5S$: C, 58.65; H, 4.10; F, 11.60; N, 2.85; S, 6.52. Found: C, 58.42; H, 4.32; F, 11.44; N, 2.73; S, 6.36.

1-[4-(2-Hydroxyethoxy)phenyl]-2-(3-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (36). The product was obtained as a white foam (37 mg, 84%) from ether **33** (52 mg, 0.089 mmol), iodotrimethylsilane (180 mg, 0.89 mmol), and thiophenol (9.8 mg, 0.089 mmol) in 2 mL of CH_2Cl_2, after product isolation and purification (1:19 MeOH:CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 (m, 2H), 3.05 (m, 2H), 3.97 (m, 2H), 4.06 (m, 2H), 6.81 (d, J = 8.8 Hz, 1H), 6.85 (d, J = 8.6 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.96 (dd, J = 8.6, 2.5 Hz, 2H), 7.09 (dd, J = 7.9, 4.8 Hz, 1H), 7.13 (d, J = 2.5 Hz, 1H), 7.33 (dt, J = 7.9, 2.0 Hz, 1H), 8.35 (m, 2H); MS (FAB) m/z 492 ($M^+ + 1$). Anal. Calcd for $C_{24}H_{20}F_3NO_5S$: C, 58.65; H, 4.10; F, 11.60; N, 2.85; S, 6.52. Found: C, 58.91; H, 4.18; F, 11.37; N, 2.82; S, 6.41.

1-[4-(2-Hydroxyethoxy)phenyl]-2-(4-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (37). The product was obtained as a yellow foam (145 mg, 88%) from ether **34** (194 mg, 0.332 mmol), iodotrimethylsilane (666 mg, 3.33 mmol), and thiophenol (40 mg, 0.37 mmol) in 8 mL of CH_2Cl_2, after product isolation and purification (1:19 MeOH:CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83 (m, 2H), 3.03 (m, 2H), 3.98 (m, 2H), 4.06 (m, 2H), 6.82 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.8 Hz, 1H), 6.92 (m, 2H), 6.93 (d, J = 8.5 Hz, 2H), 6.95 (m, 1H), 7.13 (d, J = 2.5 Hz, 1H), 8.36 (m, 2H); MS (FAB) m/z 492 ($M^+ + 1$), 359. Anal. Calcd for $C_{24}H_{20}F_3NO_5S$: C, 58.65; H, 4.10; F, 11.60; N, 2.85; S, 6.52. Found: C, 58.56; H, 4.13; F, 11.52; N, 2.81; S, 6.48.

General Procedure for Mesylation of Primary Alcohols. Methanesulfonyl chloride (MsCl) (3 equiv) was added to a solution containing the alcohol (1 equiv) and Et_3N (3 equiv) in THF at 0 °C. The reaction was allowed to warm to room temperature as it stirred for 1 h, and product isolation (H_2O, EtOAc) and purification afforded the mesylate.

1-[4-[2-[(Methanesulfonyl)oxy]ethoxy]phenyl]-2-(2-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (38). The product was obtained as a white foam (321 mg, 89%) from alcohol **35** (312 mg, 0.635 mmol), MsCl (218 mg, 1.90 mmol), and Et_3N (192 mg, 1.90 mmol) in 10 mL of THF, after product isolation and purification (2:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.98 (m, 4H), 3.11 (s, 3H), 4.24 (m, 2H), 4.58 (m, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.5 Hz, 1H), 6.95 (dd, J = 8.5, 2.4 Hz, 1H), 7.01 (d, J = 8.8 Hz, 2H), 7.02 (m, 1H), 7.14 (d, J = 2.4 Hz, 1H), 7.31 (t, J = 8.1 Hz, 1H), 8.55 (m, 1H); MS (FAB) m/z 570 ($M^+ + 1$). Anal. Calcd for $C_{25}H_{22}F_3NO_7S_2$: C, 52.72; H, 3.89; F, 10.01; N, 2.46; S, 11.26. Found: C, 52.35; H, 4.05; F, 9.83; N, 2.33; S, 11.15.

1-[4-[2-[(Methanesulfonyl)oxy]ethoxy]phenyl]-2-(3-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (39). The product, was obtained as a white foam (67 mg, 93%) from alcohol **36** (63 mg, 0.13 mmol), MsCl (44 mg, 0.38 mmol), and Et_3N (39 mg, 0.38 mmol) in 10 mL of THF, after product isolation and purification (1:19 MeOH:CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.82 (m, 2H), 3.03 (m, 2H), 3.10 (s, 3H), 4.22 (m, 2H), 4.56 (m, 2H), 6.80 (d, J = 8.1 Hz, 2H), 6.83 (d, J = 8.8 Hz, 1H), 6.95 (d, J = 8.1 Hz, 2H), 6.96 (m, 1H), 7.13 (d, J = 2.4 Hz, 1H), 7.19 (dd, J = 7.8, 4.9 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 8.29 (s, 1H), 8.35 (d, J = 4.9 Hz, 1H); MS (FAB) m/z 570 ($M^+ + 1$). HRMS calcd for $C_{25}H_{23}F_3NO_7S_2$ (M + 1) 570.0868, found 570.0860.

1-[4-[2-[(Methanesulfonyl)oxy]ethoxy]phenyl]-2-(4-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaph-

thalene (40). The product was obtained as a yellow foam (240 mg, 95%) from alcohol **37** (217 mg, 0.442 mmol), MsCl (151 mg, 1.32 mmol), and Et_3N (134 mg, 1.32 mmol) in 15 mL of THF, after product isolation and purification (1:19 MeOH:CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.82 (m, 2H), 3.03 (m, 2H), 3.11 (s, 3H), 4.24 (m, 2H), 4.58 (m, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 6.97 (m, 3H), 7.14 (d, J = 2.5 Hz, 1H), 8.38 (m, 2H); MS (FAB) m/z 570 ($M^+ + 1$). Anal. Calcd for $C_{25}H_{22}F_3NO_7S_2$: C, 52.72; H, 3.89; F, 10.01; N, 2.46; S, 11.26. Found: C, 52.62; H, 3.94; F, 9.84; N, 2.41; S, 11.19.

General Procedure for Aziridination of Methanesulfonates.[32] Ethylenimine[34] (50 equiv) was added to a solution of the methanesulfonate (1 equiv) in 1:1 CH_3CN:Et_3N and stirred for 12 h at room temperature, after which an additional 50 equiv of ethylenimine were added. After 24 h total reaction time, the reaction mixture was concentrated under a stream of nitrogen. The crude mixture was then dissolved in the appropriate chromatography solvent (a small amount of CH_2Cl_2 was added to dissolve if necessary) and then loaded directly onto a silica column and flash chromatographed.

1-[4-(2-Aziridinylethoxy)phenyl]-2-(2-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (41). The product was obtained as a yellow oil (59 mg, 72%) from mesylate **38** (90 mg, 0.16 mmol) and ethylenimine[34] (677 mg, 1.57 mmol) in 1 mL of CH_3CN:Et_3N, after product isolation and purification (1:19 Et_3N:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (m, 2H), 1.80 (m, 2H), 2.61 (m, 2H), 2.93 (m, 4H), 4.11 (m, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 1H), 6.94 (m, 1H), 6.96 (d, J = 8.5 Hz, 2H), 6.97 (m, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.26 (m, 1H), 8.53 (m, 1H); MS (FAB) m/z 517 ($M^+ + 1$). Anal. Calcd for $C_{26}H_{23}F_3N_2O_4S$: C, 60.46; H, 4.49; F, 11.03; N, 5.42; S, 6.21. Found: C, 60.48; H, 4.48; F, 10.93; N, 5.42; S, 6.20.

1-[4-(2-Aziridinylethoxy)phenyl]-2-(3-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (42). The product was obtained as a yellow oil (25 mg, 73%) from mesylate **39** (37 mg, 0.065 mmol) and ethylenimine[34] (280 mg, 6.50 mmol) in 1 mL of CH_3CN:Et_3N, after product isolation and purification (1:9 Et_3N:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (m, 2H), 1.81 (m, 2H), 2.61 (m, 2H), 2.82 (m, 2H), 3.02 (m, 2H), 4.10 (m, 2H), 6.81 (d, J = 8.5 Hz, 2H, 6.86 (d, J = 8.8 Hz, 1H), 6.89 (m, 1H), 6.92 (d, J = 8.5 Hz, 2H), 7.05 (m, 1H), 7.12 (d, J = 2.5 Hz, 1H), 7.29 (m, 1H), 8.30 (m, 2H); MS (FAB) m/z 517 ($M^+ + 1$). HRMS calcd for $C_{26}H_{24}F_3N_2O_4S$ ($M + 1$)$^+$ 517.1409, found 517.1411.

1-[4-(2-Aziridinylethoxy)phenyl]-2-(4-pyridyl)-6-[(trifluoromethanesulfonyl)oxy]-3,4-dihydronaphthalene (43). The product was obtained as a yellow oil (20 mg, 77%) from mesylate **40** (28 mg, 0.049 mmol) and ethylenimine[34] (212 mg, 4.80 mmol) in 1 mL of CH_3CN:Et_3N, after product isolation and purification (1:9 Et_3N:EtOAc). ^{1}H NMR (400 MHz) δ 1.25 (m, 2H), 1.81 (m, 2H), 2.62 (m, 2H), 2.80 (m, 2H, 3.01 (m, 2H), 4.12 (m, 2H), 6.83 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 8.8 Hz, 2H), 6.93 (m, 3H), 7.12 (d, J = 2.5 Hz, 1H), 8.34 (m, 2H); MS (FAB) m/z 517 ($M^+ + 1$). Anal. Calcd for $C_{26}H_{23}F_3N_2O_4S$: C, 60.46; H, 4.49; F, 11.03; N, 5.42; S, 6.21. Found: C, 60.54; H, 4.79; F, 10.91; N, 5.39; S, 6.14.

General Procedure for Deprotection of Phenols. To a solution of the aryl triflate in THF was added $LiAlH_4$ (5 equiv) at room temperature. An additional 5 equiv was added after 1 h of stirring. After a total reaction time of 2 h, the reaction mixture was poured into a flask containing diethyl ether and 1:1 (v/v) Celite:$Na_2SO_4 \cdot 10H_2O$. The mixture was filtered, and the filtrate was dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude phenol, which was then purified by reverse phase HPLC (1:9 H_2O:MeOH).

1-[4-(2-Aziridinylethoxy)phenyl]-6-hydroxy-2-(2-pyridyl)-3,4-dihydronaphthalene (10). The product, isolated as a yellow oil (11.3 mg, 84%), was prepared from aryl triflate **41** (18 mg, 0.035 mmol) and $LiAlH_4$ (13.3 mg, 0.350 mmol) in 2 mL of THF. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (m, 2H), 1.90 (m, 2H), 2.68 (m, 2H), 2.89 (m, 4H), 4.13 (m, 2H), 6.54 (d, J = 8.5 Hz, 1H), 6.66 (d, J = 8.5 Hz, 1H), 6.71 (m, 1H), 6.79 (m, 1H), 6.80 (d, J = 8.7 Hz, 2H), 6.96 (m, 1H), 6.97 (d, J = 8.7 Hz, 2H), 7.27 (m, 1H), 8.52 (m, 1H); MS (FAB) m/z 385 ($M^+ + 1$). HRMS calcd for $C_{25}H_{25}N_2O_2$ ($M + 1$) 385.1916, found 385.1920.

1-[4-(2-Aziridinylethoxy)phenyl]-6-hydroxy-2-(3-pyridyl)-3,4-dihydronaphthalene (11). The product, isolated as a yellow oil (6.5 mg, 83%), was prepared from aryl triflate **42** (10.5 mg, 0.020 mmol) and $LiAlH_4$ (7.8 mg, 0.20 mmol) in 1 mL of THF. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (m, 2H), 1.85 (m, 2H), 2.65 (m, 2H), 2.75 (m, 2H), 2.92 (m, 2H), 4.11 (m, 2H), 6.52 (dd, J = 8.3, 2.5 Hz, 1H), 6.64 (d, J = 8.3 Hz, 1H), 6.70 (m, 1H), 6.75 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 7.01 (dd, J = 7.0, 4.8 Hz), 7.25 (m, 1H), 8.24 (m, 2H); MS (FAB) m/z 385 ($M^+ + 1$). HRMS calcd for $C_{25}H_{25}N_2O_2$ ($M + 1$) 385.1916, found 385.1917.

1-[4-(2-Aziridinylethoxy)phenyl]-6-hydroxy-2-(4-pyridyl)-3,4-dihydronaphthalene (12). The product, isolated as a yellow oil (11.6 mg, 80%), was prepared from aryl triflate **43** (19.5 mg, 0.039 mmol) and $LiAlH_4$ (14 mg, 0.38 mmol) in 2 mL of THF. ^{1}H NMR (400 MHz) δ 1.25 (m, 2H), 1.80 (m, 2H), 2.61 (m, 2H), 2.81 (m, 2H), 2.96 (m, 2H), 4.11 (m, 2H), 6.55 (d, J = 8.5 Hz, 1H), 6.63 (d, J = 8.5 Hz, 1H), 6.70 (m, 1H), 6.81 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 8.7 Hz), 6.92 (m, 2H), 8.34 (m, 2H); MS (FAB) m/z 385 ($M^+ + 1$). HRMS calcd for $C_{25}H_{25}N_2O_2$ ($M + 1$) 385.1916, found 385.1909.

General Procedure for Photocyclization of Stilbazoles to Azaphenanthrenes. A solution of the stilbazole in cyclohexane (0.6 M) in a water cooled quartz vessel was irradiated for 2 h by 16 bulbs emitting light at 300 nm inside a Rayonet photoreactor (see General section). The reaction mixture was then concentrated under reduced pressure and purified by flash chromatography.

12-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-[(trifluoromethanesulfonyl)oxy]-13,14-dihydrochrysene (44). The product was isolated as a yellow oil (24 mg, 65%) from stilbazole **32** (37 mg, 0.063 mmol) following flash chromatography (1:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.97 (m, 2H), 3.49 (m, 2H), 3.96 (m, 2H, 4.40 (m, 2H), 4.70 (s, 2H), 7.25 (dd, J = 8.5, 2.7 Hz, 1H), 7.29−7.43 (m, 7H), 7.53 (dd, J = 8.4, 4.4 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 8.09 (d, J = 2.7 Hz, 1H), 8.38 (d, J = 9.3 Hz, 1H), 8.84 (dd, J = 8.4, 1.5 Hz, 1H), 8.98 (d, J = 4.4, 1.5 Hz, 1H); MS (EI, 70 eV) m/z (relative intensity) 579 (M^+, 100), 446 (84), 418 (61), 311 (8), 296 (5), 284 (15), 267 (7), 255 (25), 240 (4), 153 (6), 121 (5), 105 (7), 91 (87), 84 (4), 77 (6), 65 (6), 55 (4), 44 (18), 39 (4). Anal. Calcd for $C_{31}H_{24}F_3NO_5S$: C, 64.21; H, 4.17; F, 9.83; N, 2.42; S, 5.53. Found: C, 64.26; H, 4.18; F, 9.86; N, 2.45; S, 5.52.

11-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-[(trifluoromethanesulfonyl)oxy]-13,14-dihydrochrysene (45). The product was isolated as a yellow oil (11 mg, 32%) from stilbazole **33** (35 mg, 0.059 mmol) following flash chromatography (pure EtOAc after 1:1 EtOAc:hexane finished eluting **46**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.98 (m, 2H), 3.27 (m, 2H), 3.97 (m, 2H), 4.41 (m, 2H), 4.70 (s, 2H), 7.24 (dd, J = 8.5, 2.4 Hz, 1H), 7.29−7.43 (m, 7H), 7.80 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 2.4 Hz, 1H), 8.30 (d, J = 5.6 Hz, 1H), 8.36 (d, J = 9.0 Hz, 1H), 8.73 (d, J = 5.6 Hz, 1H), 9.52 (s, 1H). MS (EI, 70 eV) m/z (relative intensity) 579 (M^+, 6), 446 (5), 312 (1), 295 (4), 283 (4), 266 (7), 254 (11), 240 (5), 226 (4), 213 (1), 105 (4), 91 (100), 79 (13), 77 (9), 55 (4). Anal. Calcd for $C_{31}H_{24}F_3NO_5S$: C, 64.21; H, 4.17; F, 9.83; N, 2.42; S, 5.53. Found: C, 64.10; H, 4.19; F, 9.80; N, 2.44; S, 5.49.

9-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-[(trifluoromethanesulfonyl)oxy]-13,14-dihydrochrysene (46). The product was isolated as a yellow oil (12 mg, 35%) from stilbazole **33** (35 mg, 0.059 mmol) following flash chromatography (1:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.98 (m, 2H), 3.17 (m, 2H), 3.97 (m, 2H), 4.49 (m, 2H), 4.70 (s, 2H), 7.25 (dd, J = 8.5, 2.7 Hz, 1H), 7.27−7.43 (m, 7H), 7.57 (dd, J = 8.4, 4.4 Hz, 1H), 7.89 (d, J = 8.5 Hz, 1H), 8.32 (d, J = 9.0 Hz, 1H), 8.44 (d, J = 8.4, 1.5 Hz, 1H), 8.82 (d, J = 2.7 Hz,

(33) Simpson, D. M.; Elliston, J. F.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1987**, *28*, 233.

(34) See cautionary note at the end of the General section at the beginning of the Experimental Section.

1H), 8.97 (dd, J = 4.4, 1.5 Hz, 1H); MS (EI, 70 eV) m/z (relative intensity) 579 (M^+, off-scale), 446 (3), 312 (1), 284 (1), 256 (2), 236 (3), 213 (1), 198 (3), 177 (5), 167 (5), 156 (100), 112 (36), 91 (26), 77 (5), 55 (24). Anal. Calcd for $C_{31}H_{24}F_3NO_5S$: C, 64.21; H, 4.17; F, 9.83; N, 2.42; S, 5.53. Found: C, 64.24; H, 4.30; F, 9.76; N, 2.27; S, 5.49.

10-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-[(trifluoromethanesulfonyl)oxy]-13,14-dihydrochrysene (47). The product was isolated as a yellow oil (70 mg, 77%) from stilbazole **34** (92 mg, 0.16 mmol) following flash chromatography (2:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99 (m, 2H), 3.15 (m, 2H), 3.97 (m, 2H), 4.42 (m, 2H), 4.71 (s, 2H), 7.26 (dd, J = 8.6, 2.5 Hz, 1H), 7.28−7.43 (m, 7H), 7.84 (d, J = 8.6 Hz, 1H), 7.89 (d, J = 5.7 Hz, 1H), 8.24 (d, J = 2.5 Hz, 1H), 8.35 (d, J = 9.0 Hz, 1H), 8.73 (d, J = 5.7 Hz, 1H), 9.95 (s, 1H). MS (EI, 70 eV) m/z (relative intensity) 579 (M^+, 25), 446 (14), 312 (5), 254 (4), 105 (4), 91 (100), 65 (4). HRMS calcd for $C_{31}H_{24}F_3NO_5S$ 579.1327, found 579.1318.

General Procedure for Deprotection of Phenols. The same procedure was used here as for the deprotection of aziridinyl phenols **10**−**12**. The products were flash chromatographed after workup.

12-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-hydroxy-13,14-dihydrochrysene (48). The product, isolated as a yellow oil (7 mg, 60%), was prepared from aryl triflate **44** (15 mg, 0.026 mmol) and $LiAlH_4$ (9.9 mg, 0.26 mmol) in 2 mL of THF and flash chromatographed (1:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89 (m, 2H), 3.44 (m, 2H, 3.96 (m, 2H), 4.40 (m, 2H), 4.70 (s, 2H), 6.83 (dd, J = 8.3, 2.7 Hz, 1H), 6.92 (d, J = 2.7 Hz, 1H), 7.27−7.42 (m, 6H), 7.50 (dd, J = 8.3, 4.2 Hz, 1H), 7.69 (d, J = 9.3 Hz, 1H), 8.08 (d, J = 2.4 Hz, 1H), 8.45 (d, J = 8.3 Hz, 1H), 8.85 (dd, J = 8.3, 1.6 Hz, 1H), 8.96 (dd, J = 4.2, 1.6 Hz, 1H); MS (EI, 70 eV) m/z (relative intensity) 447 (M^+, 100), 312 (29), 295 (5), 283 (19), 254 (4), 91 (53), 73 (6), 57 (8), 43 (12). HRMS calcd for $C_{30}H_{25}NO_3$ 447.1834, found 447.1830.

11-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-hydroxy-13,14-dihydrochrysene (49). The product, isolated as a yellow oil (5.0 mg, 81%), was prepared from aryl triflate **45** (8.0 mg, 0.013 mmol) and $LiAlH_4$ (4.9 mg, 0.13 mmol) in 2 mL of THF and flash chromatographed (2:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89 (m, 2H), 3.23 (m, 2H, 3.96 (m, 2H), 4.40 (m, 2H), 4.69 (s, 2H), 6.86 (dd, J = 8.2, 2.5 Hz, 1H), 6.93 (d, J = 2.5 Hz, 1H), 7.32−7.39 (m, 6H), 7.61 (d, J = 9.3 Hz, 1H), 8.11 (d, J = 2.5 Hz, 1H), 8.32 (d, J = 5.7 Hz, 1H), 8.45 (d, J = 8.2 Hz, 1H), 8.68 (d, J = 5.7 Hz, 1H), 9.51 (s, 1H); MS (EI, 70 eV) m/z (relative intensity) 447 (M^+, 10), 312 (10), 296 (8), 284 (12), 266 (7), 254 (8), 239 (6), 226 (6), 213 (3), 200 (2), 115 (3), 105 (7), 91 (100), 77 (18), 65 (18). HRMS calcd for $C_{30}H_{25}NO_3$ 447.1834, found 447.1830.

10-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-hydroxy-13,14-dihydrochrysene (50). The product, isolated as a yellow oil (5.5 mg, 66%), was prepared from aryl triflate **47** (10.8 mg, 0.019 mmol) and $LiAlH_4$ (7.1 mg, 0.19 mmol) in 2 mL of THF and flash chromatographed (2:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.93 (m, 2H), 3.12 (m, 2H, 3.97 (m, 2H), 4.42 (m, 2H), 4.70 (s, 2H), 6.83 (dd, J = 8.5, 2.4 Hz, 1H), 6.92 (d, J = 2.4 Hz, 1H), 7.22−7.44 (m, 6H), 7.65 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 4.9 Hz, 1H), 8.24 (d, J = 2.2 Hz, 1H), 8.42 (d, J = 8.5 Hz, 1H), 8.68 (d, J = 4.9 Hz, 1H), 9.92 (s, 1H); MS (EI, 70 eV) m/z (relative intensity) 447 (M^+, 4), 296 (3), 284 (4), 265 (1), 254 (2), 239 (1), 226 (2), 213 (1), 105 (4), 91 (100), 77 (10), 65 (11). HRMS calcd for $C_{30}H_{25}NO_3$ 447.1834, found 447.1826.

9-Aza-7-[2-(benzyloxy)ethoxy]benzo[*g*]-2-hydroxy-13,14-dihydrochrysene (51). The product, isolated as a yellow oil (4 mg, 67%), was prepared from aryl triflate **46** (8.5 mg, 0.015 mmol) and $LiAlH_4$ (5.6 mg, 0.15 mmol) in 2 mL of THF and flash chromatographed (1:1 EtOAc:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89 (m, 2H), 3.11 (m, 2H), 3.96 (m, 2H), 4.49 (m, 2H), 4.70 (s, 2H), 6.83 (dd, J = 8.7, 2.7 Hz, 1H), 6.90 (d, J = 2.7 Hz, 1H), 7.21−7.42 (m, 6H), 7.54 (dd, J = 8.2, 4.0 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 8.38 (d, J = 8.7 Hz, 1H), 8.39 (m, 1H), 8.80 (d, J = 2.9 Hz, 1H), 8.92 (dd, J = 4.0, 1.5 Hz, 1H); MS (EI, 70 eV) m/z (relative intensity) 447 (M^+, 14), 326 (6), 312 (9), 296 (12), 284 (24), 266 (10), 254 (10), 240 (5), 226 (5), 213 (3), 189 (1), 105 (5), 91 (100), 77 (12), 65 (15). HRMS calcd for $C_{30}H_{25}NO_3$ 447.1834, found 447.1817.

Biological Procedures. Relative Binding Affinity for the Estrogen Receptor. Radiometric competitive binding assays were performed as previously reported,[27] using lamb uterine cytosol diluted to approximately 1.5 nM of receptor, which was incubated with buffer or several concentrations of unlabeled competitor together with 10 nM [^{3}H]estradiol for 18−24 h. Free ligand was removed by adsorption to dextran-coated charcoal. Unlabeled competitors were prepared and serially diluted in 1:1 DMF:buffer (10 mM Tris, 1.5 mM EDTA, 3 mM sodium azide, pH 7.4) to ensure solubility.

Estrogen Receptor Inactivation. Time-dependent assays for the inactivation of the estrogen receptor were performed as previously reported,[28] using lamb uterine cytosol.

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