Synthesis and Spectroscopic Characterization of Two Azatetrahydrochrysenes as Potential Fluorescent Ligands for the Estrogen Receptor

Corinne Mazur Bowen† and John A. Katzenellenbogen*

Department of Chemistry, University of Illinois, Urbana, Illinois 61801-3792

Received May 9, 1997[⊗]

In an effort to improve upon the spectroscopic characteristics of donor−acceptor tetrahydrochrysene systems that we have previously prepared as fluorescent ligands for the estrogen receptor, we have synthesized two aza analogs as receptor ligand prototypes and characterized their absorbance and fluorescence spectra. 1-Aza-9-hydroxy-5,6,11,12-tetrahydrochrysene (**4**) and 1,3-diaza-9-hydroxy-5,6,11,12-tetrahydrochrysene (**5**) were prepared by convenient heterocyclization reactions on a known tricyclic ketone (**6**). The UV spectra of these heterocycles were quite insensitive to solvent polarity but showed marked red shifts under acidic or basic conditions. These heterocycles displayed complex fluorescence spectra, with emission bands around 400 nm in aprotic medium that shifted to longer wavelength in protic solvents. In many cases, acid and base caused strong red shifts in the emission, with large alterations in quantum yield; in some cases emission bands were shifted as far as 600 nm and dual-emission peaks were observed. Thus, these two azatetrahydrochrysenes show greater environmental sensitivity—especially to changes in pH—than do our previously described donor−acceptor tetrahydrochrysenes. The binding affinity of these ligand prototypes for the estrogen receptor, however, is low, indicating that other structure modifications will be required to make them useful fluorescent ligands for this receptor.

Introduction

The estrogen receptor is a ligand-modulated transcription factor which is part of the nuclear receptor superfamily.[1] Estrogens, ligands for the estrogen receptor (ER), are known to act as mitogens in certain tissues, and they play a role in the growth of hormone-responsive breast cancer. In fact, analysis of estrogen receptor levels in breast tumors is commonly used to predict the likelihood that a breast cancer patient will have a favorable response to hormone therapy.[2] However, simply measuring the ER content of a tumor biopsy sample does not provide information on whether the tumor contains a mixture of ER-containing cells together with cells lacking ER or whether the ER distribution is uniform. It is thought that additional data regarding the distribution of the receptor within a tumor may lead to a better prediction of patient response to hormonal therapy.[2,3]

Recent technological advances in optical methods for biological sample analysis have increased the utility of fluorescence spectroscopy. Fluorescence microscopy has been used qualitatively to examine the spatial distribution of fluorescent probes in a biological tissue sample, and flow cytometry can determine quantitatively the number of fluorescent markers per cell. In the course of investigations toward the development of a fluorescence-based assay of the quantity and distribution of estrogen receptors in breast cancer cells, other workers have shown that in situ staining with coumestrol, an inherently fluorescent phytoestrogen, can be used to visualize ER expressed in cells at high levels following transfection.[4] The major limitation of this method is that fluorescence emission from coumestrol lies in the blue-violet region of the visible spectrum, the same region in which cellular fluorochromes such as the flavin and nicotinamide adenine dinucleotides show strong autofluorescence.[5] Recently, we have identified a group of tetrahydrochrysenes (THCs, **1**; Scheme 1) which show both good binding affinity for the estrogen receptor and desirable, long wavelength fluorescence emission properties.[6] Using epifluorescence microscopy, we have used these fluorescent THC estrogens to visualize the ER in cells in a model receptor expression system.[7]

To achieve a further improvement in the favorable emission properties of the THC estrogens, we wanted to build in greater environmental sensitivity by making THC-like probes which are sensitive to pH changes. This endeavor led us first to the study and optimization of a

* Corresponding author: Department of Chemistry, University of Illinois, 461 Roger Adams Laboratory, Box 37, 600 S. Mathews Ave., Urbana, IL 61801. Tel: (217) 333-6310. Fax: (217) 333-7325. E-mail: jkatzene@uiuc.edu.

† Current address: Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323.

⊗ Abstract published in *Advance ACS Abstracts,* October 1, 1997.

(1) (a) *Nuclear Hormone Receptors: Molecular Mechanisms, Cellular Function, Clinical Abnormalities*; Parker, M. G., Ed.; Academic Press: London, 1991. (b) Evans, K. M. *Science* **1988**, *240*, 889. (c) Beato, M. *Cell* **1988**, *56*, 335. (d) Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Chem. Biol.* **1996**, *3*, 529.

(2) Castles, C. G.; Fuqua, S. A. W. Alterations within the estrogen receptor in breast cancer. In *Hormone Dependent Breast Cancer*; Pasqualini, J. R., Katzenellenbogen, B. S., Eds.; Marcell Dekker: New York, 1996; Chapter 3, pp 81−105 and references cited therein.

(3) (a) Lippman, M. E.; Huff, K. K.; Jakesz, R.; Hecht, T.; Kasid, A.; Bates, S.; Dickson, R. In *Estrogen/Antiestrogen Action and Breast Cancer Therapy*; Jordan, V. C., Ed.; University of Wisconsin: Madison, 1986; p 237. (b) Silverswaard, C.; Skoog, L.; Humla, S.; Gustafsson, S. A.; Nordensjold, B. *Cancer* **1980**, *16*, 59. (c) McCarty, K. S., Jr.; Reintgen, D. S.; Seigler, H. F. *Br. Cancer Res. Treat.* **1982**, *1*, 315. (d) McCarty, K. S., Jr.; Lubahn, D. B.; McCarty, K. S., Sr. *Clin. Endocrinol. Metab.* **1983**, *12*, 133. (e) McGuire, W. L. In *Estrogen Receptors in Human Breast Cancer*; Carbone, P., Bollmer, E. P., Eds.; Raven Press: New York, 1975.

(4) Miksicek, R. J. *J. Histochem. Cytochem.* **1979**, *41*, 801.

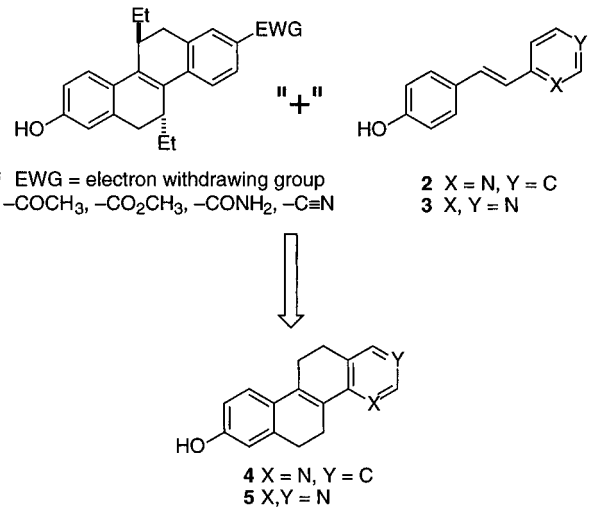
(5) (a) Aubin, J. E. *J. Histochem. Cytochem.* **1979**, *27*, 36−43. (b) Waggoner, A. S. In *Applications of Fluorescence in the Biomedical Sciences*; Taylor, R. L., Waggoner, A. S., Murphy, R. F., Lanni, F., Birge, R. R., Eds.; Alan R. Liss: New York, 1986.

(6) (a) Hwang, K. J.; O'Neil, J. P.; Katzenellenbogen, J. A. *J. Org. Chem.* **1992**, *57*, 1262. (b) Hwang, K.-J.; Carlson, K. E.; Anstead, G. M.; Katzenellenbogen, J. A. *Biochemistry* **1992**, *31*, 11536.

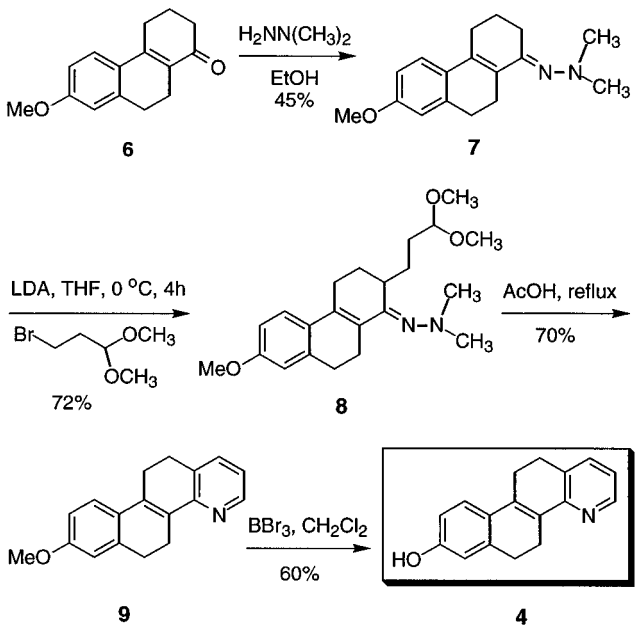
(7) Miksicek, R. J.; Carlson, K. E.; Hwang, K.-J.; Katzenellenbogen, J. A. *Mol. Endocrinol.* **1995**, *9*, 592.

S0022-3263(97)00840-2 CCC: $14.00

Scheme 1



Scheme 2



hydroxystilbene model system. We found that substitution of one phenyl ring of stilbene with a pyridine or pyrimidine ring significantly affected the photophysical and photochemical behavior of these systems, because of the involvement of the (n,π^*) state of these heterocycles.[8] Thus, the fluorescence emission observed from both (4-hydroxystyryl)pyridine **2** and (4-hydroxystyryl)pyrimidine **3** was found to have greater sensitivity to pH than 4,4′-dihydroxystilbene. These data provided the impetus for further development of heterocyclic fluorescent probes containing the styrylazine chromophore.

In this report, we will describe the synthesis and evaluation of the spectroscopic properties of two azatetrahydrochrysenes which result from incorporation of the (hydroxystyryl)pyridine and (hydroxystyryl)pyrimidine chromophores into a tetrahydrochrysene ring system. Substitution of the acceptor-substituted phenyl ring of our original donor−acceptor THCs **1** with the pyridine or pyrimidine rings of styrylazine **2** or **3**, respectively, affords 1-aza-9-hydroxy-5,6,11,12-tetrahydrochrysene (**4**) and 1,3-diaza-9-hydroxy-5,6,11,12-tetrahydrochrysene (**5**) (Scheme 1).

These novel ligand prototypes contain the rigid ring system of the THC series as well as a donor−heterocyclic acceptor chromophore. The fluorescence emission of these molecules was expected to be sensitive to changes in both solvent polarity and acidity, but we anticipated that further refinement in the structure of these ligand prototypes would be needed in order for them to have high binding affinity for the estrogen receptor.

Results and Discussion

Chemical Syntheses. Direct routes to pyridyl aza-THC **4** and pyrimidyl aza-THC **5** involved annulation of the desired heterocycle onto a tricyclic ketone precursor (**6**). A procedure involving the acid-catalyzed cyclization of appropriately substituted dimethylhydrazones to pyridines, reported by Gladiali and co-workers, was used in the synthesis of pyridyl aza-THC **4**[9] (Scheme 2). 3,4,9,10-Tetrahydro-1(2*H*)-phenanthrenone **6**, the ketone precursor required for this method, was prepared according to a procedure described by Stork[10] and was converted to the corresponding dimethylhydrazone **7**. Generation of the azaenolate from this hydrazone (**7**) required exposure to lithium diisopropylamide for 4 h, at 0 °C. Subsequent addition of 3-bromopropionaldehyde dimethyl acetal afforded the substituted hydrazone **8**. It has been reported that alkylation of the dimethylhydrazone of α-tetralone required similar conditions.[11] Finally, acid-catalyzed heterocyclization of **8** and deprotection of the resulting aryl methyl ether **9** afforded pyridyl aza-THC **4** in moderate yield.

In the synthesis of pyrimidines, annulation requires condensation of a bis-nucleophilic "N−C−N" equivalent reagent with an appropriate 1,3-dicarbonyl unit. Often, β-keto aldehyde equivalents such as formyl ketone dimethyl acetals and alkoxy methylene ketones are used. Applying a method reported by Mallamo and co-workers[12] to our synthesis of pyrimidyl aza-THC **5** (Scheme 3), we first formylated the tetrahydrophenanthrenone **6** to afford the β-hydroxyenone **10**, which was converted to its benzoyl ester **11**. Reaction of this 1,3-dicarbonyl equivalent with 2-(methylthio)pseudourea under basic conditions effected heterocyclization to 1,3-diaza-9-methoxy-3-(methylthio)-5,6,11,12-tetrahydrochrysene, **12a**. This desired product was accompanied by the corresponding 3-ethoxy analog **12b**, which resulted from displacement of the thiomethyl substituent of pyrimidine **12a** by ethoxide. The remaining material recovered from this cyclization reaction was the β-hydroxyenone **10**, resulting from debenzoylation of ester **11**.

(8) (a) Haroutounian, S. A.; Katzenellenbogen, J. A. *Photochem. Photobiol.* **1988**, *47*, 503. (b) Haroutounian, S. A.; Katzenellenbogen, J. A. *Tetrahedron* **1995**, *51*, 1585.

(9) Chelucci, G.; Gladiali, S.; Marchetti, M. *J. Heterocycl. Chem.* **1988**, *25*, 1761.

(10) Stork, G. *J. Am. Chem. Soc.* **1947**, *69*, 2936. The physical data for 3,4,9,10-tetrahydro-1(2*H*)-phenanthrenone are as follows: mp 78−79 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (m, 2H, -C(O)CH$_2$C*H*$_2$CH$_2$-), 2.51 (m, 4H, C(O)CH$_2$CH$_2$C*H*$_2$-, Ar-CH$_2$CH$_2$-), 2.73 (m, 4H, C(O)CH$_2$-C*H*$_2$CH$_2$, ArCH$_2$), 3.83 (s, 1H, HC(8)), 6.78 (dd, J = 3 Hz, 1H, HC(6)), 7.36 (d, J = 3 Hz, 1H, HC(5)); ^{13}C NMR (75.4 MHz) d 19.7, 22.3, 26.1, 28.1, 37.4, 55.2, 111.6, 113.4, 126.1, 127.1, 130.5, 140.5, 149.8, 160.6, 198.6; MS (70 eV) 228 (M^+, 100); TLC R_f = 0.35 (5% EtOAc/benzene). Anal. Calcd for $C_{15}H_{16}O_2$ (228.31): C, 78.91; H, 7.08. Found: C, 78.90; H, 7.07.

(11) Enders, D. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1984; Vol. 3.

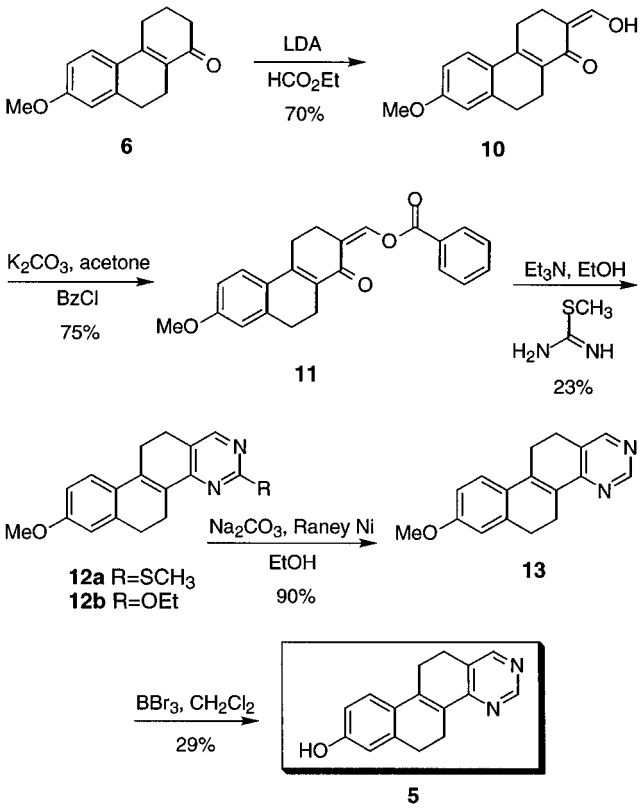
(12) Mallamo, J. P.; Pilling, G. M.; Wetzel, J. R.; Kowalczyk, P. J.; Bell, M. R.; Kullnig, R. K.; Batzold, F. H.; Juniewicz, P. E.; Winneker, R. C. *J. Med. Chem.* **1992**, *35*, 1663.

Table 1. Spectroscopic Characteristics of Pyridyl Aza-THC 4 and Pyrimidyl Aza-THC 5

compd	condition	*c*-C_6H_{12}	THF	EtOAc	CH_3COCH_3	CH_3CN	MeOH	H_2O
				UV Absorbance[a] δ_{max} (ϵ)				
4	neutral	340 (25 900)	342 (27 900)	340 (29 400)	340 (30 300)	340 (29 400)	346 (34 000)	344 (28 800)
	acidic[b]					398 (29 700)	404 (35 200)	390 (24 900)
	basic[c]					340 (30 000)	378 (36 600)	370 (28 500)
5	neutral	350 (14 100)	356 (14 600)	354 (17 900)	354 (18 300)	352 (17 400)	364 18 200)	360 (14 200)
	acidic					426 (19 700)	432 (20 800)	420 (15 800)
	basic					352 (17 500)	414 (19 700)	402 (18 900)
				Fluorescence Emission[d] λ_{max} (Φ_F)				
4	neutral	400 (0.50)	417 (0.47)	417 (0.41)	420 (0.23)	420 (0.24)	429, 482 (0.078)	500 (0.047)
	acidic[b]					504 (0.45)	504 (0.48)	503 (0.29)
	basic[c]					420 (0.21)	500, 599 (0.038)	505, 571 (0.024)
5	neutral	420 (0.10)	430 (0.062)	430 (0.053)	434 (0.041)	435 (0.038)	476 (0.030)	536 (0.097)
	acidic					531 (0.42)	527 (0.46)	530 (0.23)
	basic					438, 514 (0.043)	539 (0.68)	536 (0.61)

[a] UV spectra were obtained at ca. 10^{-5} M. [b] Acidic: enough concentrated hydrochloric acid was added to make a 0.1 N solution in the indicated solvent. [c] Basic: enough 12 N sodium hydroxide solution was added to make a 0.1 N solution in the indicated solvent. [d] Fluorescence spectra were obtained at ca. 10^{-6} M.

Scheme 3



Reductive removal of the thiomethyl substituent of pyrimidine **12a** proceeded quickly in the presence of Raney nickel. Finally, deprotection of aryl methyl ether **13** afforded pyrimidyl aza-THC **5**. This deprotection reaction required a large excess of the Lewis acid, due to complexation of the boron reagent by the pyrimidyl nitrogens.

Ultraviolet Absorbance Spectra. The ultraviolet/visible absorbance spectra of the pyridyl (**4**) and pyrimidyl (**5**) aza-THCs were measured in nonpolar and polar aprotic solvents, in protic solvents, and under neutral, and in some cases acidic and basic, conditions. The results of these measurements are summarized in Table 1 (top), and selected absorbance spectra in methanol under neutral, acidic, and basic conditions are shown in Figures 1 and 2. The most notable difference between these compounds was that in all cases pyrimidyl aza-THC **5** has an absorbance maximum at longer wavelength (5–30 nm) than does pyridyl aza-THC **4**. Aside from this difference, these molecules showed very similar trends in their absorbance behavior.

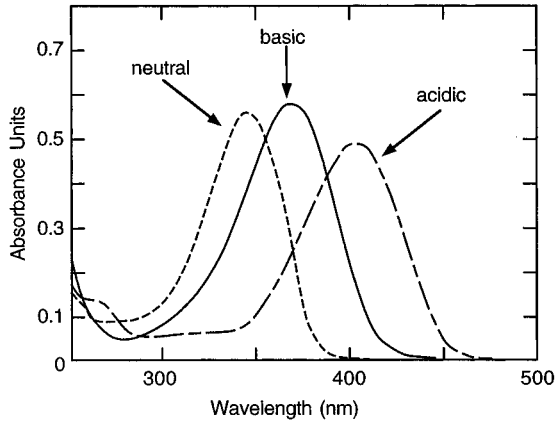


Figure 1. UV absorbance spectra for pyridyl aza-THC **4** in methanol (ca. 10^{-5} M) under neutral, acidic (0.1 N HCl), and basic (0.1 N NaOH) conditions.

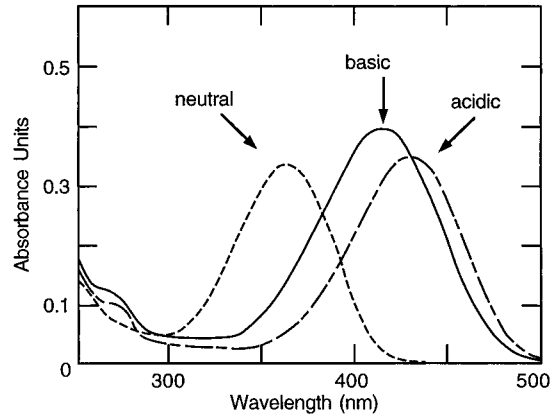
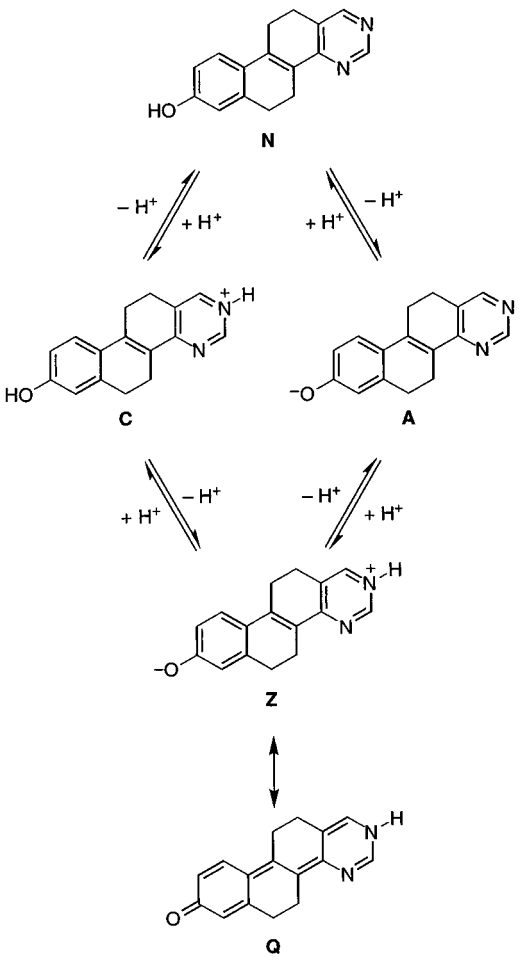


Figure 2. UV absorbance spectra for pyrimidyl aza-THC **5** in methanol (ca. 10^{-5} M) under neutral, acidic (0.1 N HCl), and basic (0.1 N NaOH) conditions.

In aprotic solvents under neutral conditions, the positions of the absorbance maxima of both the pyridyl aza-THC **4** and the pyrimidyl aza-THC **5** showed remarkably little dependence on solvent polarity.

The absorbance maxima of the aza-THC phenols **4** and **5** were strongly dependent on acidity. Both compounds showed a red shift of 46–68 nm upon addition of acid in acetonitrile, methanol, or water. Under basic conditions

Scheme 4

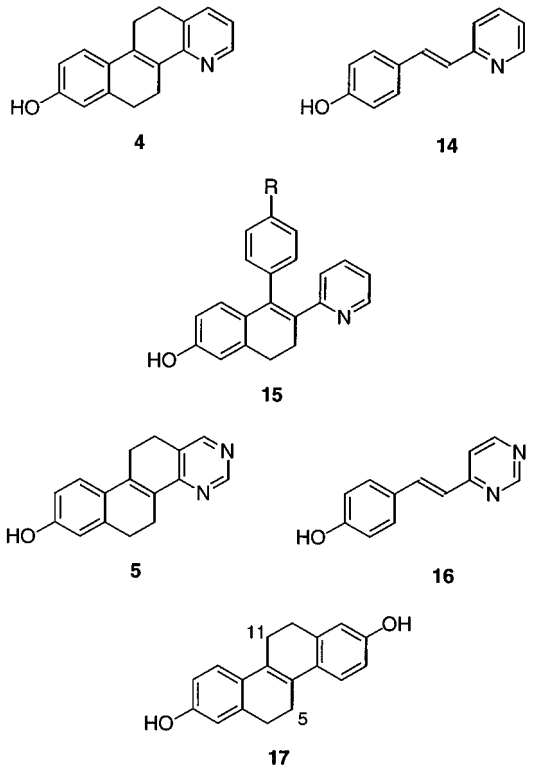


in the protic solvents, methanol and water, the absorbance maxima of **4** and **5** undergo red shifts of 32−50 nm. Slightly larger shifts were observed for the pyrimidyl aza-THC **5**. A significant red shift was observed in the absorbance maximum of either **4** or **5** in acetonitrile.

The observed dependence of absorbance wavelength of **4** and **5** on environmental acidity may be explained by considering a series of proton transfer reactions which are illustrated for pyrimidyl aza-THC **5** in Scheme 4. The corresponding reactions are also valid for the pyridyl aza-THC **4** and have been invoked in our earlier reports on the UV spectra of (*p*-hydroxystyryl)pyridines[8a] and -pyrimidines.[8b] Under acidic conditions, the pyrimidyl nitrogen(s) will be protonated and the diazinium cation **C** is generated. Under basic conditions, the phenolate anion **A** is formed. Formation of these ions accounts for the observed shifts of absorbance maxima with changes in pH. The effect of solvent polarity and proticity on the position of the longest wavelength absorbance band in these systems is complex, as it depends on the relative stability of species **N**, **A**, and **C** in the ground state as a function of solvent and acidity.

Under neutral and acidic conditions, the methoxy-substituted pyridyl and pyrimidyl THCs, **9** and **13**, respectively, generated absorbance spectra very similar to those of the corresponding hydroxy-substituted aza-THCs (data not shown), with their absorbance maxima at slightly shorter wavelengths (2−8 nm). As expected, these methyl ethers do not undergo deprotonation, so there is little change in their absorbance spectra under basic conditions. Their red shift in acid, however, was comparable to that of the aza-THC phenols.

The chromophores in the pyridyl aza-THC **4** and pyrimidinyl aza-THC **5** are the same as those we have studied earlier in the related 2-styrylpyridine and 2-styrylpyrimidine systems (**14** and **16**),[8ab] and the pyridyl aza-THC is also related to a 2-pyridyl-substituted dihydronaphthalene system (**15**) that we have prepared.[13] When the chromophore is situated within the rigid, planar tetrahydrochrysene skeleton, as in **4** and **5**, the absorbance peaks are shifted to red relative to the acyclic analogs **14** and **16** by ca. 15−30 nm. This bathochromic shift results from the four additional sites of alkyl substitution in **4** and **5** vs **14** and **16**. The dihydronaphthalene system **15** showed absorbance maxima that were blue-shifted relative to **4**, and its chromophore strength was also lower. These changes are expected because the chromophore in **15** is twisted out of planarity by interaction between the cis-situated phenyl and pyridine rings.



Fluorescence Spectra. The fluorescence emission spectra of pyridyl aza-THC **4**, and pyrimidyl aza-THC **5**, and their corresponding methyl ethers were measured in cyclohexane, tetrahydrofuran, ethyl acetate, acetone, acetonitrile, methanol, and water, under neutral, acidic (0.1 N HCl), and basic (0.1 N NaOH) conditions. Complete results are given in Table 1 (bottom), and selected spectra are shown in Figures 3−6.

A few generalizations can be made about the fluorescence emission of the hydroxy-substituted aza-THCs. First, the emission maxima, emission character (i.e., dual emission), and emission intensities in all cases show strong dependence upon the polarity and acidity of the medium. Under neutral conditions, normal fluorescence solvatochromism is observed, and there is a clear red shift in the emission maxima for aza-THCs **4** and **5** as the solvent polarity increases from cyclohexane to water (Figures 3 and 4). Second, the largest bathochromic shift

(13) Scribner, A. W.; Haroutounian, S. A.; Carlson, K. E.; Katzenellenbogen, J. A. *J. Org. Chem.* **1997**, *62*, 1043.

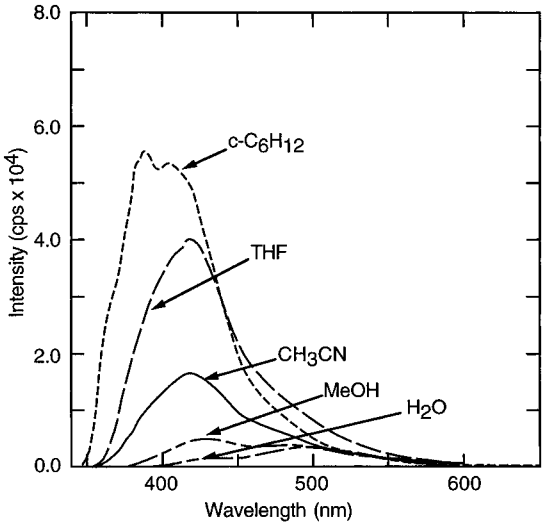


Figure 3. Fluorescence emission spectra for pyridyl aza-THC **4** in various solvents (ca. 10^{-5} M).

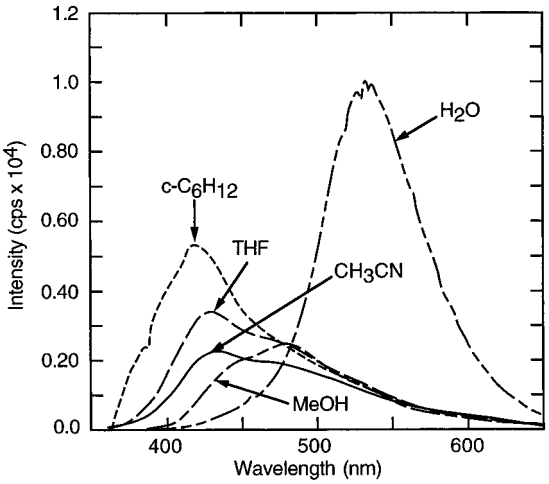


Figure 4. Fluorescence emission spectra for pyrimidyl aza-THC **5** in various solvents (ca. 10^{-5} M).

occurs upon changing the protic character of the solvent; the emission maxima of both **4** and **5** are at much longer wavelengths in methanol, a protic solvent, than in acetonitrile, an aprotic solvent.

Finally, we observed that the pH of a solution of each aza-THC strongly influences the emission wavelength and intensity. Upon addition of acid to aza-THC **4** or **5** in either acetonitrile or methanol, a strong red shift in emission occurs (Figures 5 and 6). Aqueous solutions of **4** and **5** do not behave in this way; their emission maxima in water are already at rather long wavelength and do not shift further upon addition of acid.

While the behavior of **4** and **5** under acidic conditions is similar, their behavior under basic conditions is quite different. The emission band of pyridyl aza-THC **4** does not undergo a red shift in acetonitrile at high pH but does in methanol and aqueous sodium hydroxide solution. On the other hand, **5** *does* undergo a red shift in basic acetonitrile and methanol but not in basic aqueous solution.

Interpretation of the fluorescence properties of ionizable molecules such as the azatetrahydrochrysenes is complicated by the fact that proton transfer in the excited state can result in formation of not only the anion (**A**) and cation (**C**) forms but also the zwitterionic/quinoid (**Z**/**Q**) forms (cf. Scheme 4). The existence of two ionizable functionalities [the aromatic nitrogen(s) and the phenolic hydroxyl] can account for proton transfer which ultimately leads to the formation of another emissive species. Protic conditions enhance this proton transfer. Since the acidity of the phenolic oxygen and the basicity of the aromatic nitrogen(s) increase in the excited state,[14] there is a driving force for formation of this **Z**/**Q** species in the excited state. However, this **Z**/**Q** form will be formed only if proton transfer occurs during the lifetime of the excited state. Emission from the zwitterionic/quinoid form would be expected to be at a longer wavelength than from the other three forms, and its presence can explain the very long wavelength emissions that are observed in protic media for the pyridyl and pyrimidyl aza-THCs **4** (at ca. 600 nm) and **5** (at ca. 540 nm).

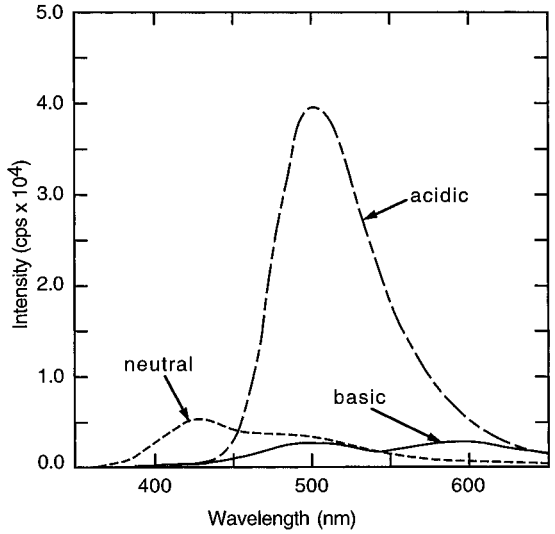


Figure 5. Fluorescence emission spectra for pyridyl aza-THC **4** in methanol (ca. 10−5 M) under neutral, acidic (0.1 N HCl), and basic (0.1 N NaOH) conditions.

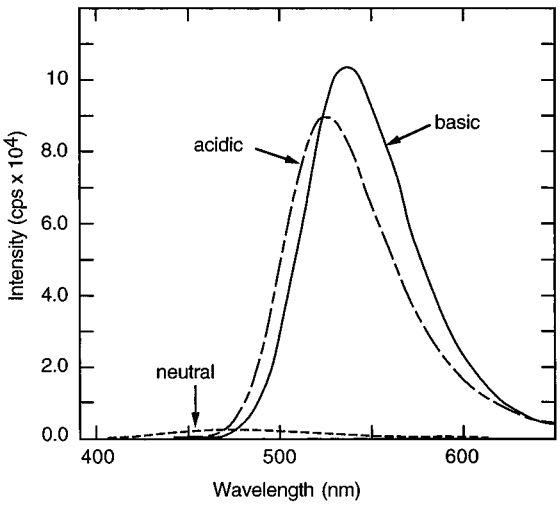


Figure 6. Fluorescence emission spectra for pyrimidyl aza-THC **5** in methanol (ca. 10^{-5} M) under neutral, acidic (0.1 N HCl), basic (0.1 N NaOH) conditions.

Dual emission was observed for both aza-THCs **4** and **5** under certain conditions. In earlier studies, similar dual emission was also observed in the cases of styryl-

(14) Ireland, J. F.; Wyatt, A. H. *Adv. Phys. Org. Chem.* **1976**, *12*, 131.

pyridines and styryldiazines.[8] Partial formation of the zwitterionic/quinoid form, with persistence of some of the other forms, can account for dual fluorescence emission. Although both aza-THCs exhibit dual emission, a closer inspection of their emissive behavior reveals important differences. In the case of pyridyl aza-THC **4** under neutral conditions, the longer wavelength shoulder became more distinct in more polar solvents (Figure 3). This longer wavelength band was generally more pronounced in the case of pyrimidyl aza-THC **5** (Figure 4). In fact, whereas dual emission was observed for pyridyl aza-THC **4** in protic solvents, emission from pyrimidyl aza-THC **5** in protic solvents was clearly a single band. Most interestingly, this single emission band observed for pyrimidyl aza-THC **5** in neutral water was strongly red-shifted, relative to methanol (60 nm shift), and is 3 times as intense (Figure 5); it is 2 times as intense as in cyclohexane. This behavior was unexpected. On the other hand, pyridyl aza-THC **4** exhibits the expected decrease in emission intensity in protic solvents (Table 1).

The fluorescence quantum yields of pyridyl aza-THC **4** and pyrimidyl aza-THC **5** were measured by a relative method using either coumarin I or acridine yellow G as a standard (Table 1).[15] Two trends in the quantum yields were observed as follows: (a) the quantum yield of fluorescence emission of **4** is generally greater than that of **5** under the same conditions, and (b) under neutral conditions, the quantum yields of both aza-THCs decreased as solvent polarity increased. A decrease in quantum yields with increasing solvent polarity can be explained by an enhancement of radiationless decay in polar solvents, resulting in lower quantum efficiencies of fluorescence.[16] One important exception exists to the general trends in quantum yields mentioned above. In aqueous solution the quantum yield of **5** is higher than that of **4** and also 3 times that measured in methanol.

Changes in the acidity of the solvent result in significant differences in the quantum yield of both aza-THCs. In protic solvents under acidic conditions, the quantum yields of both pyridyl aza-THC **4** and pyrimidyl aza-THC **5** increased dramatically (cf. Figures 5 and 6). Under basic conditions, quantum yields varied with aza-THC and solvent (Table 1). Yields of emission from the pyridyl aza-THC **4** in basic methanol or water were only about one-half those observed under neutral conditions. On the other hand, the quantum yield of the pyrimidyl aza-THC **5** in methanol increased from 0.030 under neutral conditions to 0.68 under basic conditions (cf. Figure 6). A similar increase was observed for **5** in water (from 0.024 to 0.61). In fact, in basic protic conditions the quantum yield of fluorescence emission of **5** is ca. 20 times that of **4**. These dramatic changes in quantum yield with changes in pH can be explained by recalling that the zwitterionic/quinoid form may actually be the predominant species in solution. The existence of this fully conjugated tautomer may also explain the bathochromic shift of emission.

Estrogen Receptor Binding Affinity. In competitive radioligand binding assays,[17] the two aza-THC ligand prototypes showed very low binding affinity for the estrogen receptor: the affinity of the pyridyl aza-THC **4** was 0.028 ± 0.016% and that of the pyrimidyl aza-THC **5** was 0.0051 ± 0.0019% that of estradiol. Although we have shown that, in the basic tetrahydrochrysene system, high affinity for the estrogen receptor is engendered by alkyl substituents at the 5 and 11 positions, the unsubstituted THC diol **17** still bound to the estrogen receptor with an affinity ca. 8% that of estradiol. Therefore, it appears that the heterocyclic component of compounds **4** and **5** is poorly tolerated by the estrogen receptor and that further structural refinements beyond simply the addition of alkyl substituents at the 5 and 11 positions will be required to raise the binding affinity of these ligand prototypes to useful levels.

Conclusions

We have described the synthesis of two novel aza-THCs and presented an investigation of their spectroscopic properties. Their absorbance and fluorescence properties show strong dependence on solvent polarity and pH. This behavior is due in part to the ionizable functionalities (e.g., the acidic phenolic hydroxyl and the basic heterocyclic nitrogens) of aza-THCs **4** and **5**. Although both systems show potential for use as spectroscopic probes in biological systems, the pyrimidyl aza-THC **5** appears most interesting because of its strong and long wavelength emissive behavior in aqueous conditions. Further work is necessary to modify these ligand prototype fluorophores to obtain practical probes which have high affinity for the estrogen receptor.

Experimental Section

General. All reagents and solvents were obtained from Aldrich, Eastman, Fisher, or Sigma. Tetrahydrofuran was distilled from sodium/benzophenone. Diisopropylamine and toluene were distilled from calcium hydride. *n*-Butyllithium was standardized against dry diphenylacetic acid.[18] All reactions were performed under a dry nitrogen atmosphere unless specified otherwise. Reaction progress was monitored by analytical thin-layer chromatography (TLC), performed with 0.25 mm silica gel glass plates containing F-254 indicator. Visualization on TLC was achieved by either UV light (254 nm), phosphomolybdic acid indicator, or potassium permanganate indicator. Flash chromatography was performed with 32−63 μm silica gel packing.[19]

Samples for ultraviolet spectra were prepared from $\sim 10^{-3}$ M stock solutions in ethyl acetate to give final concentrations of $\sim 10^{-5}$ M. A 10-fold dilution in the test solvent was then prepared for fluorescence quantum yield measurement. Solutions of coumarin I (Eastman Kodak laser grade) or acridine yellow G (Aldrich) in ethanol were used as standards. Fluorescence spectra were recorded by photon counting on a Spex Fluorolog 2 spectrometer (Model IIIC) with a Datamate processor. All spectra were recorded at room temperature with four 0.50 mm slits. All emission spectra were corrected, and solvent background was subtracted.

Proton magnetic resonance spectra were recorded at 300 MHz, with chemical shifts reported as parts per million downfield from an internal tetramethylsilane standard. Carbon spectra were recorded at 75 or 125 MHz, using either $CHCl_3$ (^1H δ 7.26, ^{13}C δ 77.1) or $(CH_3)_2CO$ (^1H δ 2.04, ^{13}C δ 205.7, 29.8) as an internal standard. Low-resolution electron impact mass spectra were obtained on a Finnigan MAT CH-5 spectrometer. High-resolution mass spectra were obtained on

(15) Olmsted, J., III. *J. Phys. Chem.* **1979**, *83*, 2581.
(16) Anstead, G. M.; Hwang, K. J.; Katzenellenbogen, J. A. *Photochem. Photobiol.* **1993**, *57*, 616.
(17) These assays were performed using an estrogen receptor preparation from lamb uterus, with tritium-labeled estradiol as tracer and dextran-charcoal as an adsorbant for free ligand. The values are the average and range of repeat assays. This assay has been described in detail: Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. *Biochemistry* **1973**, *12*, 4085.
(18) Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, *41*, 1879.
(19) Still, W. C.; Kahn, M.; MItra, A. *J. Org. Chem.* **1978**, *43*, 2923.

a Varian MAT 731 spectrometer. Chemical ionization mass spectra were obtained on a VG 70-VSE spectrometer. Elemental analyses were performed by the Microanalytical Service of the University of Illinois.

Compounds used in spectroscopic studies were purified by preparative high-pressure liquid chromatography (HPLC) with ultraviolet detection at 254 nm. The column used was a preparative Whatman Partisil M9 10 mm × 50 cm.

7-Methoxy-3,4,9,10-tetrahydro-1(2*H*)-phenanthrenone Dimethylhydrazone (7). 3,4,9,10-Tetrahydro-1(2*H*)-phenanthrenone (**6**)[10] (1.81 g, 7.94 mmol) and anhydrous 1,1-dimethylhydrazine (6.0 mL, 79.4 mmol) in anhydrous ethanol (30 mL) were refluxed for 24 h. Excess dimethylhydrazine and ethanol were removed under reduced pressure. Purification by flash chromatography (20% EtOAc/hexane) and recrystallization from ethanol yielded 0.308 g of yellow crystalline hydrazone **7** (41%): mp 78−79 °C; ^{1}H NMR (300 MHz) δ 7.23 (d, J = 1.8 Hz, 1H), 6.74 (d, J = 2.7 Hz, 1H), 6.73 (s, 1H), 3.81 (s, 3H), 2.65 (m, 8H), 2.53 (s, 6H), 1.90 (m, 2H); ^{13}C NMR (75.4 MHz) δ 21.4, 21.9, 26.1, 26.2, 28.5, 47.2, 55.1, 111.1, 113.1, 124.2, 129.0, 129.7, 137.1, 139.2, 158.8, 162.4; MS (70 eV) 270 (M^+, 100); exact mass calcd for $C_{17}H_{22}N_2O$ m/z 270.1732, found m/z 270.1731; TLC R_f = 0.40 (20% EtOAc/hexane). Anal. Calcd for $C_{17}H_{22}N_2O$ (270.17): C, 75.51; H, 8.22; N, 10.36. Found: C, 75.22; H, 8.28; N, 10.26.

2-(3′,3′-Dimethoxypropyl)-7-methoxy-3,4,9,10-tetrahydro-1-phenanthrenone Dimethylhydrazone (8). To diisopropylamine (0.217 mL, 1.86 mmol) in 25 mL of THF at 0 °C was slowly added *n*-butyllithium (1.3 M) (1.43 mL, 1.86 mmol). The solution was allowed to stir for 15 min. Dimethylhydrazone **7** (0.420 g, 1.55 mmol) was dissolved in THF (3 mL) and then added slowly to the lithium diisopropylamide. The reaction mixture was stirred at 0 °C for 3 h, and the reddish solution was then cooled to −78 °C. A mixture of 3-bromopropionaldehyde dimethyl acetal (0.212 mL, 1.55 mmol) and 2 mL of DMPU was added dropwise. The resulting mixture was stirred for 1 h at −78 °C and then slowly warmed to rt. After 2 h, the THF was removed under reduced pressure. Water was added to the residue and the mixture extracted with Et_2O. The combined organics were dried over $MgSO_4$, evaporated, and purified by flash chromatography (15% EtOAc/hexane) to afford alkylated hydrazone **8** (65%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (d, J = 2.7 Hz, 1H), 6.76 (d, J = 2.7 Hz, 1H), 6.73 (s, 1H), 4.39 (t, J = 3.4 Hz, 1H), 3.82 (s, 3H), 3.42 (m, 1H), 3.31 (s, 3H), 3.29 (s, 3H), 2.92 (m, 1H), 2.73 (t, J = 2.2 Hz, 2H), 2.46 (s, 6H), 2.37 (m, 2H), 1.63−1.84 (m, 3H), 1.47 (m, 2H), 0.87 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.6, 21.8, 25.0, 25.2, 28.6, 30.4, 32.8, 47.9, 52.3, 53.0, 55.3, 104.5, 111.1, 113.3, 124.4, 128.3, 128.6, 136.1, 139.6, 159.0, 169.2; MS (10 eV) 372 (M^+, 100); exact mass calcd for $C_{22}H_{32}N_2O_3$ m/z 372.2413, found m/z 372.2411; TLC R_f = 0.35 (15% EtOAc/hexane). Anal. Calcd for $C_{22}H_{32}N_2O_3$ (372.24): C, 71.31; H, 8.18; N, 7.56. Found: C, 71.26; H, 8.20; N, 7.51.

1-Aza-9-methoxy-5,6,11,12-tetrahydrochrysene (9). A solution of alkylated hydrazone **8** (0.374 g, 1.00 mmol) in glacial acetic acid (3 mL) was refluxed for 4 h under N_2. Most of the acetic acid was removed under reduced pressure. The residue was taken up in 10% aq HCl and ether. The aqueous layer was separated, made alkaline with 10% aq NaOH, extracted with Et_2O, and dried over $MgSO_4$. Purification by flash chromatography (30% EtOAc/hexane) afforded pyridinyl methyl ether **9** (0.184 g, 0.70 mmol) in 70% yield. Further purification was done by HPLC, using a Whatman Partisil 10 preparatory column [mobile phase: 25% (5% IPA/CH_2Cl_2)/75% hexane]: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.41 (d, J = 4.3 Hz, 1H), 7.38 (d, J = 7.3 Hz, 1H), 7.27 (m, J = 8.4 Hz, 1H), 6.99 (dd, J = 7.4, 5.0 Hz, 1H), 6.73−6.79 (m, 2H), 3.82 (s, 3H), 2.68−2.94 (m, 8H); MS (10 eV) 263 (M^+, 100); TLC R_f = 0.35 (30% EtOAc/hexane); exact mass calcd for $C_{18}H_{17}NO$ m/z 263.1310, found m/z 263.1309.

1-Aza-9-hydroxy-5,6,11,12-tetrahydrochrysene (4). To a solution of pyridinyl methyl ether **9** (59 mg, 0.22 mmol) in 3 mL of CH_2Cl_2, at −78 °C, was added BBr_3 (0.12 mL, 1.12 mmol). The reaction mixture was warmed to 0 °C, allowed to stir for 30 min, and then warmed to rt. After 30 min, the reaction mixture was cooled to 0 °C, and water-saturated ethyl ether was added dropwise to quench the excess BBr_3. The reaction mixture was then washed with a 1% aqueous solution of $NaHSO_3$. The resulting yellow precipitate was dissolved in 2 N NaOH and extracted with ether. The combined organics were washed with brine and water and then dried over Na_2SO_4. Purification by reversed phase HPLC (80% MeOH/20% H_2O) afforded the phenol **4** in 60% yield (33.0 mg) as a yellow powder: mp 211.5−212.5 °C; ^{1}H NMR (10:1 $(CD_3)_2CO$:CD_3OD, 300 MHz) δ 8.31 (dd, J = 5.0, 1.0 Hz, 1H), 7.48 (d, J = 6.8 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.04 (dd, J = 7.4, 4.9 Hz, 1H), 6.64−6.69 (m, 2H), 2.65−2.93 (m, 8H); ^{13}C NMR (20:1 $CDCl_3$:DMSO-d_6, 75 MHz) δ 22.5, 23.6, 27.1, 28.4, 30.8, 112.8, 114.7, 120.5, 123.7, 126.8, 129.6, 130.1, 133.7, 135.5, 138.5, 146.6, 156.8; TLC R_f = 0.35 (5% MeOH/CH_2Cl_2); exact mass calcd for $C_{17}H_{15}NO$ m/z 249.1153, found m/z 249.1142.

2-Formyl-7-methoxy-3,4,9,10-tetrahydro-1-phenanthrenone (10). To a solution of diisopropylamine (0.277 mL, 1.97 mmol) in 5 mL of THF at 0 °C was added *n*-butyllithium (1.76 mL of 1.34 M solution in hexanes, 2.36 mmol) dropwise. This solution was allowed to stir at 0 °C for 15 min as a solution (450 mg, 1.97 mmol) of tricyclic ketone **6**,[10] in 1 mL of THF, was added. The reaction mixture was stirred at 0 °C for 45 min. The reaction flask was then cooled to −78 °C, and ethyl formate (0.239 mL, 2.96 mmol) was added dropwise. Stirring was continued at 0 °C for 30 min, and then the mixture was warmed to rt and allowed to stir for 3 h. The solvent was removed in vacuo and the dark yellow residue taken up in 20 mL of EtOAc. This organic solution was shaken with 25 mL of H_2O and 2 mL of 1 N HCl. The layers were separated. The organic layer was washed twice with brine (20 mL). The aqueous layer was extracted twice with 15 mL of EtOAc. The organics were combined, dried over $MgSO_4$, and concentrated. Purification was carried out by flash chromatography over silica gel (20% EtOAc/hexane) to afford 353 mg (1.38 mmol) of keto aldehyde **7** (70%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (s, 1H), 7.38 (d, J = 3.0 Hz, 1H), 6.79 (d, J = 3.0 Hz, 1H), 6.77 (s, 1H), 3.83 (s, 3H), 2.50−2.82 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.4, 22.8, 25.5, 28.2, 55.3, 107.6, 111.7, 113.7, 126.2, 126.9, 130.2, 140.5, 148.4, 160.8, 163.6, 190.0; CIMS (NH_3) 257 (M^+ , 100); TLC R_f = 0.32 (20% EtOAc/hexane); HRMS calcd for $C_{16}H_{16}O_3$ 256.1098, found 256.1098.

7-Methoxy-2-[(benzoyloxy)methylene]-3,4,9,10-tetrahydro-1-phenanthrenone (11). To a heterogeneous mixture of the enol **10** (0.35 g, 1.38 mmol) and potassium carbonate (0.23 g, 1.65 mmol) in 20 mL of anhydrous acetone was added benzoyl chloride (0.48 mL, 4.14 mmol), over 30 min. The suspension was stirred at rt overnight. The reaction mixture was filtered to remove K_2CO_3, and the solvent was removed in vacuo. The residue was taken up in EtOAc and poured over ice (10 mL). The layers were separated, and the organic layer was washed with 10% $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. Flash chromatography over silica gel (20% EtOAc/hexane) afforded 0.37 g (1.04 mmol) of benzoyl ester **11** (75%) as a yellow solid that was recrystallized from toluene: mp 150−153 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.21 (m, 1H), 8.18 (d, 2H, J = 1.4 Hz), 7.75 (t, 1H), 7.61 (m, 1H), 7.48 (d, J = 9.4 Hz, 1H), 6.86 (m, 2H), 3.84 (s, 3H, $ArOCH_3$), 2.5−3.2 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.6, 22.5, 24.7, 28.2, 55.3, 111.9, 113.5, 120.1, 126.2, 127.1, 128.5, 128.7, 130.1, 131.9, 134.0, 140.3, 140.7, 149.1, 160.9, 162.8, 187.2; HRMS calcd for $C_{23}H_{20}O_4$ 360.1362, found 360.1362.

1,3-Diaza-9-methoxy-2-(methylthio)-5,6,11,12-tetrahydrochrysene (12a). To a solution of triethylamine (0.49 mL, 3.50 mmol) and 2-(methylthio)pseudourea (H_2SO_4 salt) (0.97 g, 3.50 mmol) in 20 mL of EtOH was added benzoyl ester **11** (1.05 g, 2.92 mmol). The heterogeneous mixture was refluxed for 24 h under N_2. The solvent was removed in vacuo, and the orangish residue was taken up in CH_2Cl_2. This solution was washed three times with 10% $NaHCO_3$ and once with brine. The first aqueous layer was extracted once with additional CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, concentrated, and flash chromatographed over silica gel (20% EtOAc/hexane) and then again using 10% EtOAc/toluene affording 0.21 g (0.67 mmol) of (methylthio)pyrimidine **12a** (23%) as a yellow powder: mp 121.5−122.5 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (s, 1H), 7.33 (d, J = 7.48

Hz, 1H), 6.77 (m, 2H), 3.84 (s, 3H), 2.58−2.88 (m, 8H), 2.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.2, 21.7, 23.1, 23.7, 28.5, 55.3, 111.4, 113.7, 121.4, 124.7, 127.5, 129.1, 139.7, 140.8, 153.3, 159.9, 161.2, 169.8; EIMS (10 eV) 310 (M^+, 100); TLC R_f = 0.50 (20% EtOAc/hexane); HRMS calcd for $C_{18}H_{18}N_2OS$ 310.1140, found 310.1123.

1,3-Diaza-9-methoxy-5,6,11,12-tetrahydrochrysene (13). A heterogeneous mixture of (methylthio)pyrimidine **12a** (0.16 g, 0.52 mmol), Raney nickel (1.80 g, pore size 50 μ, s.a. = 80−100 m^2/g, pH > 9, 50% in H_2O), and sodium carbonate (0.24 g, 2.26 mmol) in 15 mL of EtOH was refluxed for 30 min or until no starting material was observable by TLC. The reaction mixture was filtered and the filtrate concentrated. Flash chromatography over silica gel (30−100% EtOAc/hexane) afforded 0.12 g (0.07 mmol) of the demethylthiolated product **13** (90%) as a light yellow solid: mp 108.5−109.5 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.00 (s, 1H), 8.39 (s, 1H), 7.33 (d, J = 8.6 Hz, 1H), 6.76−6.80 (m, 2H), 3.84 (s, 3H), 2.77−2.94 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.9, 23.6, 23.7, 28.5, 55.3, 111.5, 113.7, 124.6, 126.5, 127.5, 129.0, 139.6, 140.8, 153.0, 157.3, 159.9, 161.0; CIMS (CH_4) 265 (M^+, 100); HRMS calcd for $C_{17}H_{16}N_2O$ 264.1254, found 264.1254.

1,3-Diaza-9-hydroxy-5,6,11,12-tetrahydrochrysene (5). To a solution of pyrimidinyl methyl ether **13** (18.0 mg, 0.07 mmol) in 2 mL of CH_2Cl_2 at −78 °C was added BBr_3 (0.26 mL, 2.72 mmol) dropwise. After 5 min, the reaction mixture was warmed to 0 °C for 30 min and then to rt and stirred for 1 h. The reaction mixture was cooled to 0 °C, then the reaction was quenched with water-saturated Et_2O (5 mL), and the mixture was washed with 1% $NaHSO_3$. The layers were separated, and the organic layer was extracted with 2 N NaOH. The pH of this aqueous layer was adjusted to pH 6.8 with 1.5 M KH_2PO_4. This neutralized aqueous solution was then extracted three times with EtOAc. These organics were combined and dried over $MgSO_4$. Most of the product was isolated in this extraction. The original aqueous layer (obtained upon quenching the reaction) was brought to pH 5.5−6.0 with 1.5 M K_2HPO_4. This aqueous solution was extracted three times with EtOAc. The organics were combined, dried, filtered, concentrated, and purified by flash chromatography over silica gel (40% EtOAc/hexane) to afford 5.0 mg (0.02 mmol) of **5** (29%) as a yellow powder: mp ca. 220 °C dec; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.00 (s, 1H), 8.39 (s, 1H), 7.30 (d, J = 9.1 Hz, 1H), 6.71−6.74 (m, 2H), 5.53 (bs, 1H), 2.78−2.95 (m, 8H); ^{13}C NMR ($(CD_3)_2CO$, 125 MHz) δ 22.5, 24.1, 28.9, 113.9, 115.6, 125.9, 127.4(2), 128.9, 140.5, 141.6, 153.9, 157.9, 158.8, 161.4; TLC R_f = 0.50 (40% EtOAc/hexane); HRMS calcd for $C_{16}H_{14}N_2O$ 250.1092, found 250.1106.

Acknowledgment. We are grateful for support of this research through a grant from the National Institutes of Health (PHS 5R37 DK15556). Estrogen receptor binding measurements were performed by Kathryn Carlson. We thank Marvin Meyers for help with physical measurements.

Supporting Information Available: ^{1}H NMR spectra of compounds **4**, **5**, **9**, **10**, **12**, **12a**, and **13** and ^{13}C NMR spectra of compounds **5**, **11**, and **13** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9708405