6.97 (d, 2 H, 7.8 Hz), 7.06-7.16 (m, 8 H), 7.29 (t, 4 H), 7.36 (t, 2 H, 7.8 Hz), 7.56 (d, 2 H, 7.8 Hz), 7.57 (s, 2 H), 8.27 (d, 2 H, 7.8 Hz), 9.15 (s, 2 H), 11.47 (s, 2 H, imidazole N-H), 14.27 (s, 2 H, imidazole N-H). ^{13}C-NMR ($CDCl_3$, 31 of 40 expected signals were observed): δ 9.10, 11.98, 28.24, 28.51, 48.20, 48.94, 125.45, 125.53, 126.48, 127.33, 127.58, 127.70, 128.22, 128.51, 129.04, 129.42, 129.57, 129.78, 129.90, 130.55, 131.57, 132.75, 136.08, 136.51, 137.11, 142.35, 145.35, 146.62, 148.32, 189.75, 194.87. IR (cm^{-1}): 2950, 1680 (s), 1560 (w), 1465 (m). Anal. Calcd for $C_{88}H_{72}N_{16}O_4$: C, 74.55; H, 5.12. Found: C, 74.61; H, 5.12.

LiOH- and NaOH-Promoted N-Benzylation of 1 and 6a. 1 (5 mg, 7.05 μmol) is placed in a 5-mL flask with 1 mg of LiOH or NaOH and a spatula tip full of powdered 4A molecular sieves. A 150-μL portion of a 0.116 M benzyl bromide solution in DMF is added and the mixture stirred 1 h at room temperature. The temperature is then raised to 75 °C for 17 h. The mixture is diluted with $CHCl_3$ and filtered, and volatiles are removed in vacuo. TLC chromatography (alumina, 50:50 v/v $CHCl_3$, EtOAc; R_f = 0.16) provides 5.5 mg (88%) of **6a**, which crystallizes from $CHCl_3$/EtOAc as a white powder, mp > 310 °C. FAB-MS: m/z 889.6 (exact mass $C_{58}H_{48}N_8O_2 + H^+$ = 889.4). ^{1}H-NMR ($CDCl_3$): δ 2.59 (s, 12 H), 5.24 (s, 8 H), 7.08 (t, 2 H), 7.14 (d, 8 H, 7.21 Hz), 7.19 (d, 4 H), 7.33 (t, 4 H), 7.38 (d of d, 8 H), 8.93 (s, 2 H). ^{13}C-NMR ($CDCl_3$, the carbonyl signal was not observed): δ 10.36, 48.27, 125.77, 127.26, 127.34, 127.54, 127.83, 129.26, 131.48, 132.747, 136.16, 136.37, 146.44. IR (cm^{-1}): 1620, 1560, 1405, 880.

K_2CO_3-, Rb_2CO_3-, and Cs_2CO_3-Promoted N-Benzylation of 1 and 6b. 1 (3.4 mg, 4.8 μmol) is placed with 4 mg of cesium carbonate in a 5-mL flask, and 100 μL of a 0.116 M DMF solution of benzyl bromide is added. After stirring at room temperature for 1 h, the temperature is raised to 75 °C for 21 h. TLC chromatography (alumina, 20:20:1 v/v $CHCl_3$/EtOAc/EtOH; R_f = 0.16) gave 3.0 mg (70%) of **6b** as a solid residue. FAB-MS: m/z 889.6 ^{1}H-NMR ($CDCl_3$): δ 2.30 (s, 6 H), 2.54 (s, 6 H), 5.27 (s, 4 H), 5.67, 5.96 (AB quartet, 4 H), 6.50 (d, 4 H, 7.2 Hz), 6.98 (d of d, 4 H), 7.06 (d, 4 H, 6.3 Hz), 7.1-7.4 (m, 11 H), 7.65 (t, 1 H, 7.5 Hz), 7.99 (d, 2 H, 7.5 Hz), 8.06 (s, 1 H), 8.34 (s, 1 H). ^{13}C-NMR ($CDCl_3$) upfield region: δ 10.53, 14.55, 48.08, 52.17. IR (cm^{-1}): 1625, 1555, 890. Analogous reactions, employing excesses of either rubidium carbonate or potassium carbonate were performed as above. The reaction mixture was analyzed by TLC by comparison with authentic samples, showing **6b** as the sole product.

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Registry No. 1, 137389-54-9; **2a**, 138286-12-1; **2b**, 138286-13-2; **3**, 138286-14-3; **4**, 138286-15-4; **5**, 138286-16-5; **6a**, 138286-17-6; **6b**, 138286-18-7; *m*-xylylenediamine, 1477-55-0; 1,3-bis[1-benzyl-5-methyl-4-(2-(hydroxyimino)-1,3-dioxobutyl)imidazol-2-yl]benzene, 137389-52-7.

Supplementary Material Available: Complete crystal structure parameters for **6a**, including atomic positional and thermal parameters; ^{1}H-NMR and FAB-MS for compounds **2-6** (20 pages). Ordering information is given on any current masthead page.

5,6,11,12-Tetrahydrochrysenes: Synthesis of Rigid Stilbene Systems Designed To Be Fluorescent Ligands for the Estrogen Receptor

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We have prepared a series of tetrahydrochrysenes as fluorescent ligands for the estrogen receptor. The stilbene chromophore in this tetracyclic system is held rigid and is adorned with an electron-donating hydroxyl group at C-8 that corresponds to the phenolic hydroxyl of estrogens and an electron acceptor at C-2 to give a donor-acceptor fluorophore. Additional substituents at C-5 and C-11 provide additional bulk that improves receptor binding affinity without distorting the planar conjugated system. The tetrahydrochrysene core was prepared by an acyloin condensation of α-alkyl *m*-methoxyhydrocinnamate esters, followed by a double dehydrative cyclization. The cis and trans isomers of the alkyl substituted systems could be separated and their stereochemistry ascertained by X-ray crystallographic analysis; the trans isomer has the higher receptor binding affinity, and the derivative with ethyl substituents at C-5 and C-11 has the best affinity. The donor-acceptor systems were prepared by functional group manipulations on one of the aromatic methoxy groups: conversion to the trifluoromethanesulfonate was followed by a palladium-mediated carbonylation to give the acetyl derivative and methoxycarbonylation to give the ester. The ester was further elaborated to the amide and nitrile. The nitro compound was prepared by nitration of protio system, itself prepared by hydrogenolysis of the trifluoromethanesulfonate. As will be described later, these tetrahydrochrysenes provide a favorable combination of estrogen receptor binding affinity and long wavelength, high quantum yield fluorescence to make them useful as fluorescent ligands for the estrogen receptor.

Introduction

Fluorescent probes have proved to be widely useful in characterizing cellular binding sites, providing both quantitation and spacial resolution.[1] In this regard, there has been a longstanding interest in the development of fluorescence-based methods for detecting steroid receptors that might permit a cell-by-cell assay of receptor content in hormone responsive cells.[2-5] Of particular interest is

(1) (a) Lakowicz, J. R. *Principles of Fluorescence and Spectroscopy*; Plenum: New York, 1983 and references cited therein. (b) Taylor, O. L.; Waggoner, A. S.; Murphy, R. F.; Lanni, F.; Birge, R. R. *Applications of Fluorescence in the Biomedical Science*; Alan R. Liss: New York, 1985.

(2) For immunofluorescence methods, see: (a) Mercer, W. D.; Edward, D. P.; Chamness, G. C.; McGuire, W. L. *Cancer Res.* **1981**, 4644. (b) Stumpe, W. E.; Sar, M. *Acta Histochem. Cytochem.* **1982**, 560. (c) Pertschuk, L. P.; Tobin, E. H.; Carter, A. C.; Eisenberg, K. B.; Leo, V. C.; Gaetjens, E.; Bloom, N. D. *Breast Cancer Res. Treat.* **1981**, 297 (1) and references cited therein.

0022-3263/92/1957-1262$03.00/0

the use of such agents to assay the quantity and distribution of estrogen receptor in breast cancer cells, as both of these features have been used in predicting patient response to hormonal therapy.[6]

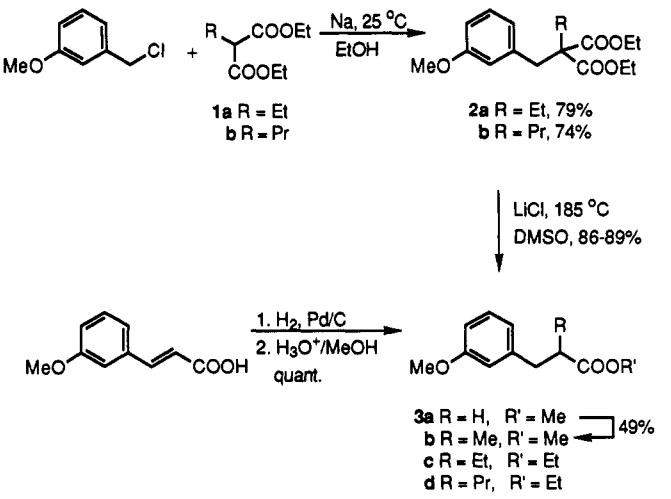
There are many reports of the preparation of fluorescent estrogens and their use in receptor assays.[3,4] Reagents of a conjugate design, with a ligand linked to a fluorophore, have generally shown low affinity for receptor and high nonspecific binding,[3] while inherently fluorescent ligands, in which the fluorochrome is built within the structure of the ligand, have usually suffered from suboptimal fluorescence or binding characteristics.[4] The stilbene-type nonsteroidal estrogenxs present an intriguing case in point, as optimal fluorescence characteristics require a planar stilbene chromophore, yet optimal binding requires a thickness that is generally achieved by bulky substituents that twist the aryl groups out of conjugation, reducing emission intensity and wavelength.[4,5]

Since the estrogen receptor is present at only trace levels in cells (ca. 15 000–50 000 sites per cell),[6b] its detection demands fluorescent ligands whose emission is both well red shifted (away from cellular autofluorescence) and whose quantum yield is high. In this report we present the synthesis of a series of 5,6,11,12-tetrahydrochrysenes as inherently fluorescent ligands for the estrogen receptor. The tetracyclic nature of the chrysene system keeps the stilbene chromophore planar, and the bulk or thickness required for higher receptor binding affinity is achieved, independent of chromophore twisting, by substitution at positions C-5 and C-11. Substitution with appropriate electron-donor and -acceptor systems at C-2 and C-8 provides functions for high receptor binding affinity and long wavelength fluorescence emission. Members of this tetrahydrochrysene system appear to be the first fluorescent ligands with which one can observe fluorescence from a receptor-bound ligand. The details of the receptor binding and fluorescence properties of these compounds systems will be published elsewhere (Hwang, K.-J.; Carlson, K. E.; Katzenellenbogen, J. A. Manuscript in preparation).

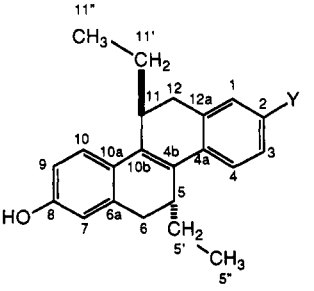
Results and Discussion

Tetrahydrochrysene Structures. The structures of the tetrahydrochrysene systems we have prepared are shown below. All of these systems bear a hydroxyl substituent at C-8. This hydroxyl is congruent with the C-3 phenolic hydroxyl group that is present in all steroidal estrogens; it affords, as well, an electron-donating character

Scheme I



essential for the long-wavelength stilbene fluorescence. The other aromatic substituent at C-2 is either hydroxyl (for binding affinity studies) or an electron-withdrawing group that is tolerated by the estrogen receptor and provides the acceptor part of the stilbene donor–acceptor fluorophore. In some cases these systems are embellished with alkyl substituents at positions C-5 and C-11. These are sites analogous to steroid positions 11β and 7α, positions where the receptor is known to tolerate and even prefer hydrophobic substituents.[7]



III

Y: **a** = OH, **b** = $COCH_3$, **c** = COOMe, **d** = $CONH_2$, **e** = CN, **f** = NO_2

Synthesis of the Symmetrical Dihydroxy Tetrahydrochrysene Derivatives Ia–IVa. The synthesis of the unsubstituted tetrahydrochrysene diol Ia has been carried out by various routes.[8,9] The acyloin condensation–double cyclization strategy described by Collins,[9] with minor modifications, was used to prepare the substituted dihydroxychrysene derivatives II–IVa (Schemes I and II).

Preparation of Hydrocinnamyl Esters 3. Alkyl-substituted esters **3b–d** could be generated by either alkylation of the hydrocinnamyl ester **3a** or dealkoxycarbonylation of the α-benzylmalonic diester **2**, as shown in Scheme I. The ethyl esters **3c** and **d** were prepared by the nucleophilic substitution of *m*-methoxybenzyl chloride with the anion derived from the diethyl alkylmalonates **1a** and **b**, followed by dealkoxycarbonylation (LiCl, DMSO), in 66–68% overall yield. Alternatively, the methyl-substituted ester **3b** was prepared in 47–51% yield by the

(3) For the fluorophore–ligand conjugates, see: (a) Fevig, T. L.; Lloyd, J. E.; Zablocki, J. A.; Katzenellenbogen, J. A. *J. Med. Chem.* **1987**, 156. (b) Carlson, K. E.; Coppey, M.; Magdelenat, H.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1989**, 345. (c) Rau, B. R.; Rry, C. G.; Hunt, S.; Kuhnel, R.; Dandliker, W. B. *Cancer* **1980**, 2902. (d) Lee, Y. J.; Notides, A. C.; Tsay, Y. G.; Kende, A. S. *Biochemistry* **1977**, 2896. (e) Lee, S. H. *Cancer* **1980**, 2825. (f) Fisher, B.; Gundez, N.; Zheng, S.; Saffer, E. A. *Cancer Res.* **1982**, 540. (g) Van, N. T.; Raber, M.; Barrows, G. H.; Barlogie, B. *Science* **1984**, 876 (224).

(4) For inherently fluorescent estrogens, see: (a) Martin, P. M.; Magdelenat, H. P.; Benyahia, B.; Rigaud, O.; Katzenellenbogen, J. A. *Cancer Res.* **1983**, *43*, 4956. (b) Anstead, G. M.; Altenbach, R. J.; Wilson, S. R.; Katzenellenbogen, J. A. *J. Med. Chem.* **1988**, 1316. (c) Katzenellenbogen, J. A.; Carlson, K. E.; Bindal, R. D.; Neeley, R. L.; Martin, P. M.; Magdelenat, H. P. *Anal. Biochem.* **1986**, *159*, 336. (d) Nelson, K.; Paulik, E. J.; Nagell, J. R., Jr.; Hanson, M. B.; Donaldson, E. S.; Flanigan, R. C. *Biochemistry* **1984**, 2565.

(5) For another approach using photofluorogenic ligands, see: (a) Bindal, R. D.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1985**, *23*, 929–937. (b) Bindal, R. D.; Katzenellenbogen, J. A. *Photochem. Photobiol.* **1986**, 121.

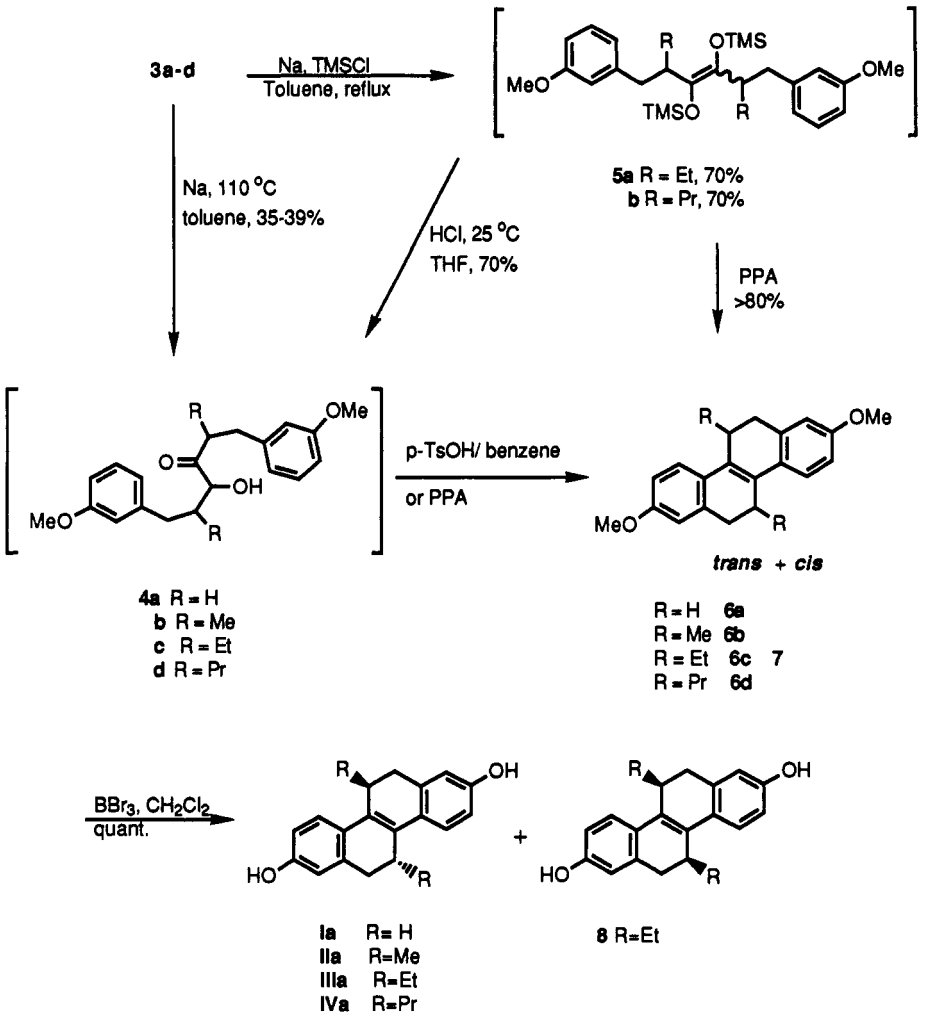
(6) (a) Thorpe, S. M. *Acta Oncol.* **1988**, *27*, 1. (b) Allegra, J. C.; Lippman, M. E.; Thompson, E. B.; Simon, R.; Barlock, A.; Green, L.; Huff, K. K.; Do, H. M. T.; Aitkin, S. C. *Cancer Res.* **1979**, *39*, 1447–54. (c) Valavaara, R.; Touminen, J.; Johansson, R. *Cancer* **1990**, *66*, 2264.

(7) (a) Gabbard, R. B.; Segaloff, A. *Steroids* **1983**, *41*, 791. (b) Bélanger, A.; Philibert, D.; Teutsch, G. *Steroids* **1981**, *37*, 361. (c) Bowler, J.; Lilley, T. J.; Pittam, J. D.; Wakeling, A. E. *Steroids* **1989**, *54*, 71–99.

(8) (a) Johnson, W. S.; Erickson, C. A.; Ackerman, J. *J. Am. Chem. Soc.* **1952**, *74*, 2251. (b) Salzer, W. Z. *Physiol. Chem.* **1942**, 274.

(9) (a) Collins, D. J.; Cullen, J. D. *Aus. J. Chem.* **1988**, *41*, 735. (b) Collins, D. J.; Matthews, W. A. *Aust. J. Chem.* **1979**, *32*, 1093.

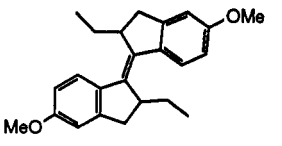
Scheme II



alkylation of the unsubstituted ester **3a** with $LiNiPr_2$ and CH_3I in THF/DMPU. The unsubstituted ester **3a** was obtained quantitatively from *m*-methoxycinnamic acid by hydrogenation and esterification. In practice, the isolation of the alkylated esters **3c–d** from the diesters **2a** and **b** was more convenient than that from the unsubstituted ester **3a**.

Acyloin Condensation. Classical acyloin condensation of esters **3a–c** with sodium in toluene gave acyloins **4a–c** in 35–39% yield (Scheme II). The major side reactions under these conditions were Claisen condensation and oxidation of the acyloin α-hydoxyl group to a carbonyl group. However, the addition of 2 equiv of TMSCl suppressed these side reactions completely,[10] so the reaction of esters **3c,d** with sodium in the presence of 2 equiv of TMSCl generated silyl ethers **5a** and **b** in high yield. Subsequent hydrolysis of silyl ether **5a** with aqueous acid (5 N HCl) in THF gave acyloin **4c** in 70% yield. By contrast, treatment of silyl ether **5a** with tetrabutylamonium fluoride or base (LiOH) failed to generate acyloin **4c**. No further optimization of the hydrolysis of silyl ethers **5a** and **b** was done, since we found that the silyl ethers themselves could be cyclized to the corresponding chrysenes **6** and **7** in the presence of polyphosphoric acid (PPA).

Dehydrocyclization. Chrysenes **6** and **7** were prepared quantitatively by cyclization of silyl ethers **5a** and **b** or acyloins **4a–d**, each as a mixture of diasteromers, in the presence of PPA. Acyloins **4a–d** could also be cyclized in the presence of *p*-toluenesulfonic acid in refluxing benzene, but yields (36–68%) were lower than those with PPA. In either case, the cyclization was regiospecific, resulting in the formation of the endocyclic tetrahydrochrysene derivatives **6** and **7**, without evidence of any exocyclization (5-membered ring formation). For comparison, an authentic sample of exoindene **9** was generated by McMurry coupling[11] of 5-methoxy-2-ethylindanone with $TiCl_4/Zn$ in THF.



exoindene **9**

The trans and cis stereoisomers of dimethoxychrysenes **6** and **7** were separated by repeated column chromatography and recrystallization. The ratio of these isomers formed by the cyclization process was dependent upon the alkyl substituents and cyclization conditions. With the methyl substituents, only trans isomer **6b** was isolated from the cyclization of acyloin **4b** under *p*-TsOH catalysis. In the ethyl-substituted case, trans and cis isomers **6c** and **7** were obtained as a mixture (trans/cis = 60/40) by the

(10) Rühlmann, K. *Synthesis* **1971**, *14*, 236.

(11) (a) McMurry, J. E.; Lectka, T.; Rico, J. G. *J. Org. Chem.* **1989**, *54*, 3748. (b) Coe, P. L.; Scriven, C. E. *J. Chem. Soc., Perkin Trans. 1* **1986**, 475.

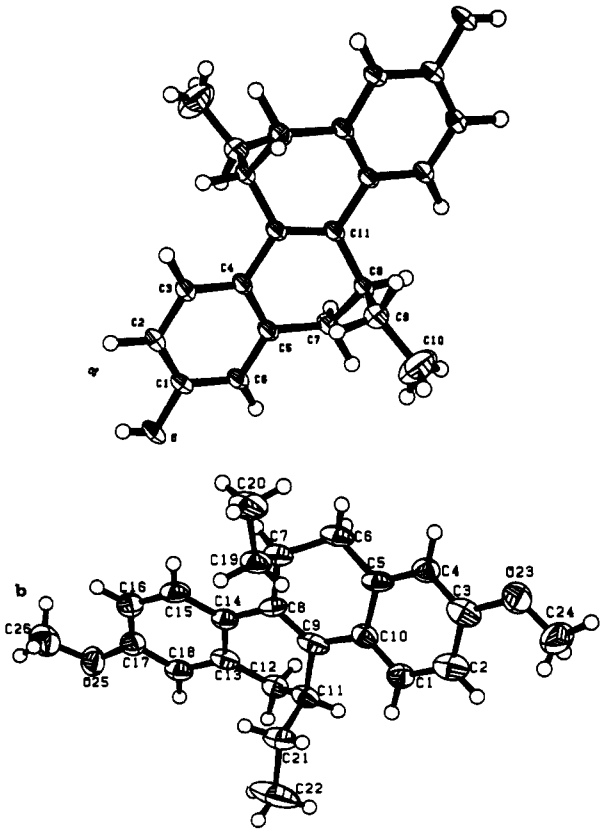


Figure 1. Thermal ellipsoid representation of 5,11-*trans*-diethyltetrahydrochryene-2,8-diol IIIa and 2,8-dimethoxy-5,11-*cis*-diethyltetrahydrochryene 7: (a) trans isomer IIIa; (b) cis isomer 7.

cyclization of silyl ether **5a** with PPA. A similar cyclization of the dipropyl acyloin[4d] **5** gave a high yield of the trans isomer **6d**. Finally, demethylation of tetrahydrochrysenes **6** and **7** with BBr_3 yielded the corresponding dihydroxy chrysenes I–IVa and **8** in quantitative yield.

Structure Analysis of Tetrahydrochrysene Derivatives. The stereochemistry of the trans and cis isomers was determined by X-ray crystallographic analysis of *trans*-diethylchrysene IIIa (a meso compound) and 2,8-dimethoxy-*cis*-diethylchrysene **7** (with C_2 symmetry). The crystals of chrysenes IIIa and **7** were obtained by repeated recrystallization from hexane, ethyl acetate, and ethanol. ORTEP plots are represented in Figure 1. The torsion angle between both phenyl groups through the central double bond of cis isomer **7** (θ = 5°) is larger than that of trans isomer IIIa (θ = 0°), a result of its more highly bent shape. However, the torsion angle between a phenyl ring and the double bond of cis isomer **7** (θ = 20°) is smaller than that of trans isomer IIIa (θ = 26°).

The stereochemistry of diethyl substituents at positions C-5 and C-11 on the dimethoxy diethyl chrysenes **6c** and **7** could be analyzed by NMR. The chemical shifts of benzylic protons Ht and Hc (δ = ~3.0, ~2.9 ppm) of both isomers **6c** and **7** are not greatly affected by the stereochemistry at the C-5 and C-11 positions on both isomers. In contrast, the chemical shift of the allylic protons H_β is dependent on the geometry at the 5 and 11 positions. In the case of cis isomer **7**, the chemical shift of H_β is more upfield (δ = 2.59 ppm) than that of the benzylic protons H_t and H_c (δ = 3.00, 2.81 ppm). The chemical shift of the allylic proton H_β in trans isomer **6c** shifted more downfield (δ = 2.92 ppm) than that of cis isomer **7**. The coupling constants of allylic and benzylic protons of both isomers **6c** and **7** are explained well by the different torsion angles

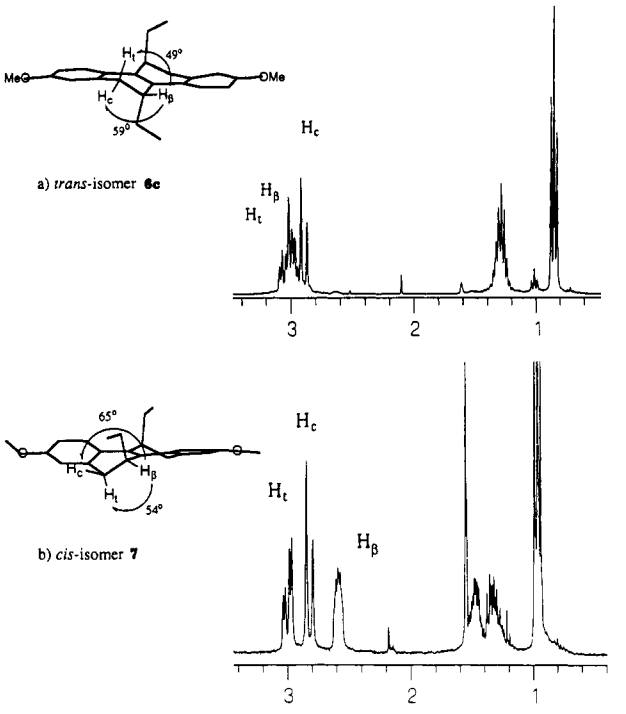
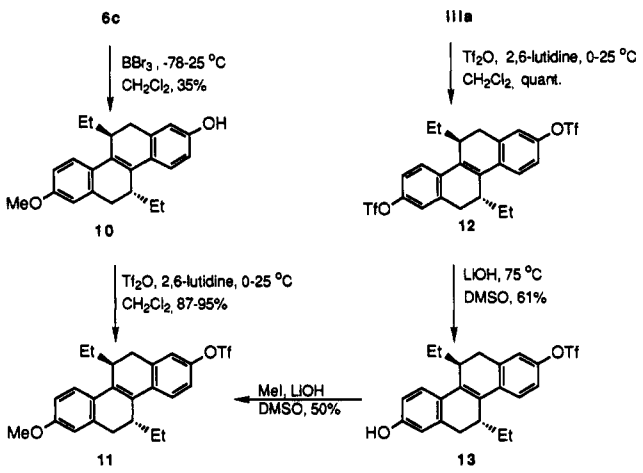


Figure 2. NMR spectra of *trans*- and *cis*-2,8-dimethoxy-5,11-diethyltetrahydrochrysene **6c** and **7**. The drawings in left side are obtained using the SYBYL molecular modeling program, based on the X-ray crystallographic data of IIIa and **7** and all the hydrogens are omitted for visual clarity.

Scheme III

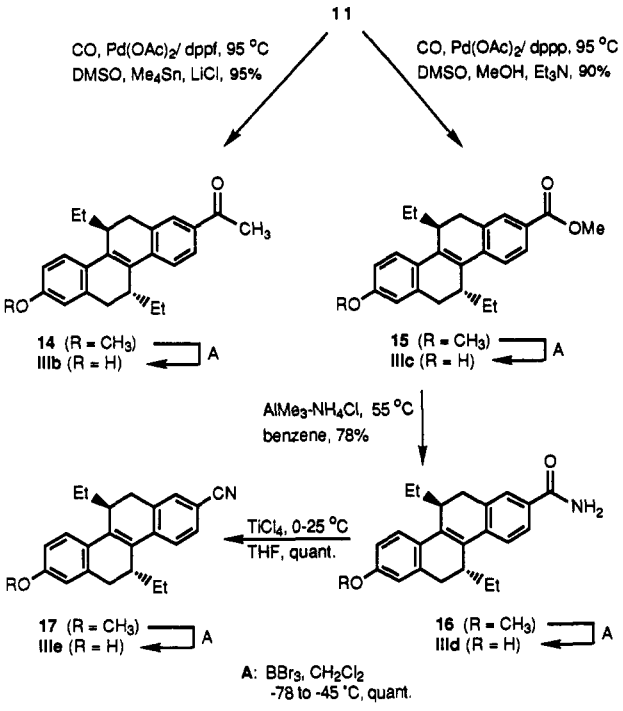


between H_t–H_β and H_c–H_β: $J_{H_t-H_\beta}$ (5.8 Hz) of cis isomer **7** is larger than $J_{H_c-H_\beta}$ (1.2 Hz), due to its smaller torsion angle 54° vs 65°, respectively.[12]

Synthesis of Unsymmetrical Chrysene Derivatives IIIb–f. Estrogen receptor binding assays of dihydroxychrysenes I–IVa showed that *trans*-diethylchrysenediol IIIa had the highest binding affinity for the estrogen receptor (Carlson, K. E.; Hwang, K.-J.; Katzenellenbogen, J. A. Unpublished results). However, its emission wavelength (382 nm in ethanol) was too short for it to be useful as a fluorescent probe for the estrogen receptor. In order to modify the spectroscopic properties of the chrysene derivatives so that they could emit at longer wavelengths, we prepared the chrysene derivatives IIIb–f containing donor–acceptor groups. Since electron-withdrawing groups deactivate the aromatic ring toward electrophilic substitution, the acyloin condensation/dehydrocyclization route

(12) Karplus, M., *J. Chem. Phys.* **1959**, *30*, 11.

Scheme IV



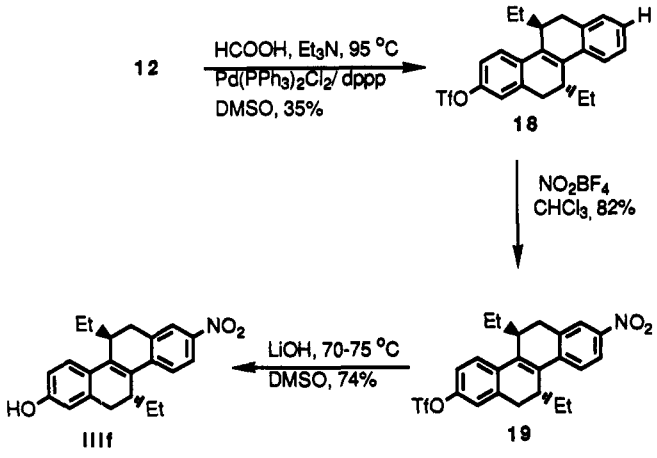
is not a favorable one to use to prepare these unsymmetrical chrysenes IIIb–f. Therefore, a hydroxyl group on diol IIIa was activated as its trifluoromethanesulfonate (triflate) 11 and then manipulated appropriately to give the unsymmetrical chrysene derivatives (Scheme IV). It is noteworthy that all the unsymmetrical diethylchrysene derivatives IIIb–f could be prepared from the same intermediate (11) by palladium-mediated alkoxy/methylcarbonylation (14, 15) or by hydrogenolysis (18) as a key step. Oxidative aromatization of the B and C rings of the unsymmetrical chrysenes was more facile than with the symmetrical chrysenes I–IVa. Therefore, extreme care was required in the handling of reaction intermediates and products to avoid this oxidation.

Intermediate 11 was prepared either from dimethoxy chrysene **6c** by monodemethylation (BBr_3, 35%) followed by triflation or from diol IIIa by triflation, monohydrolysis, and methylation (Scheme III). The yield of monodemethylation of **6c** and monohydrolysis of 12 was not high, since it was difficult to control the reaction to avoid double deprotection. Also, monophenols **10** and **13** are prone to decomposition, resulting in the low yields for those reactions.

Methyl ketone **14** was generated by a carbonylative coupling[13] of triflate 11 with tetramethyltin in the presence of palladium(II) acetate with 1,1′-bis(diphenylphosphino)ferrocene (dppf) as shown in Scheme IV. Originally, the carbonylative coupling of the aryl triflates was conducted under pressure (4 atm CO). However, the reaction with triflate 11 could be carried out under milder conditions (1 atm CO), presumably because of the high reactivity of the bulky aryl palladium intermediate.

Alkoxycarbonylation[14] of aryl triflate 11 in the presence of palladium generated aryl ester **15** in 90% yield. The same reaction on the corresponding hydroxy triflate **13**, however, gave hydroxy ester IIIc in low yield (18%), together with unidentified products. Silyl protection[14a] of the phenolic hydroxyl group as the *tert*-butyldimethylsilyl ether was not successful during the alkoxycarbonylation of triflate **23a**, as the desilylated alcohol **13** was obtained along with low yields of carboxylated products.

Scheme V



Amination[15] of ester **15** with $AlMe_3$–NH_4Cl generated amide **16** in 78% yield (Scheme IV). Subsequent dehydration[16] of amide **16** with $TiCl_4$ generated aryl cyanide **17** quantitatively. Finally, hydroxychrysenes IIIb–e were obtained quantitatively from the corresponding methoxychrysenes **14**–**17** by treatment with BBr_3 (Scheme IV).

Nitrochrysene IIIf was prepared by the electrophilic nitration of monotriflate **18** as the key step (Scheme V). Monotriflated chrysene **18** was prepared from bistriflate 12 by palladium-mediated selective hydrogenolysis in 35% yield.[17] Alternatively, monotriflate **18** could be obtained from triflate 11 by palladium-mediated hydrogenolysis,[18] followed by demethylation (BBr_3) and trifluoromethanesulfonylation in 46% yield overall. The treatment of this monotriflate **18** with nitronium tetrafluoroborate in chloroform[19] gave nitrotriflate **19** in 82% yield. Subsequent hydrolysis of nitrotriflate **19** with LiOH generated nitrochrysene IIIf. The yield of electrophilic nitration of monotriflate **18** was affected by solvent and the source of nucleophiles. The reaction of monotriflate **18** with copper(II) nitrate in trifluoroacetic anhydride did not give nitrotriflate **19**.[20]

Conclusion

We have devised efficient synthetic routes to a series of tetrahydrochrysenes, designed to be fluorescent ligands for the estrogen receptor. These compounds have the stilbene chromophore rigidified by the tetracyclic nature of the chrysene system, and they are adorned with aromatic electron donor and acceptor groups to enhance the long wavelength fluorescence of the stilbene system. Additional

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substituents are placed strategically to increase binding affinity to the estrogen receptor without interfering with the planar conjugated π system of the fluorophore. The estrogen receptor binding properties and fluorescence characteristics of these compounds will be described elsewhere.

Experimental Section

General Methods. All the reactions were run under a dry N_2 pressure unless specified otherwise. Reaction progress was followed by analytical thin-layer chromatography, performed with 0.25-mm silica gel glass-backed plates with F-254 indicator (Merck) or gas chromatography using a Hewlett-Packard Ultra 1 fused silica microcapillary column. Visualization on TLC was done by UV light (254, 350 nm). The fluorescence from the chrysene compounds could be observed under long-wavelength UV irradiation. Flash chromatography[21] was performed using ~15 cm of Woelm 32–63-μm silica gel packing. Samples were loaded either in solution or in the solid state after being absorbed to silica. HPLC analysis was performed on IBM silica (normal phase), Partisil M9 DDS-2, or Micropak C_{18} (Varian) reversed-phase columns. Solvents and reagents were purified by distillation from the indicated drying agent. From calcium hydride: DMSO, DMF, methylene chloride, carbon tetrachloride, chloroform, toluene, benzene, triethylamine, and diisopropylamine. From sodium benzophenone: tetrahydrofuran. Proton nuclear magnetic resonance spectra were recorded at 200, 300, or 500 MHz; carbon NMR were recorded 75.6 or 100.6 MHz. Electron impact (EI) mass spectra were recorded at 70 or 10 eV. The corrected fluorescence emission spectra were recorded on a Spex Fluorolog III fluorometer. Melting points are uncorrected. X-ray crystallographic data are in the supplementary material.

Diethyl Ethyl(*m*-methoxybenzyl)malonate (2a). Sodium (4.388 g, 0.191 mol) was added to EtOH (250 mL) at 0 °C, and then the mixture was stirred at room temperature until all the sodium disappeared. After addition of diethyl ethylmalonate (**1a**, 30 mL, 0.159 mol), the mixture was stirred for 1 h, then *m*-methoxybenzyl chloride (23.1 mL, 0.159 mol) was added at room temperature, and then the mixture was stirred for 6 h. The reaction mixture was filtered to remove precipitates and concentrated, and then ether was added. The organic phase was washed with water and brine twice and then concentrated. Bulb to bulb distillation (170 °C (0.04 mmHg)) gave diester **2a** (38.7 g, 79%) as clear liquid: ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.6 Hz, 3 H, CCH_2CH_3), 1.25 (t, J = 7.1 Hz, 6 H, OCH_2CH_3), 1.83 (q, J = 7.6 Hz, 2 H, CH_2CH_3), 3.20 (s, 2 H, $ArCH_2$), 3.76 (s, 3 H, OCH_3), 4.14–4.23 (m, 4 H, OCH_2CH_3), 6.63–6.68 (m, 2 H, ArH ortho to OMe), 6.75 (dd, J_1 = 8.3 Hz, J_2 = 2.4 Hz, 1 H, ArH para to OMe), 7.16 (m, 1 H, ArH meta to OMe); MS m/z (relative intensity) 308 (M^+, 100), 234 (51), 138 (97); HRMS exact mass calcd for $C_{24}H_{27}NO_2$ 308.1625, found 308.1624.

Diethyl propyl(*m*-methoxybenzyl)malonate (2b) was prepared from diester **1b** (8.5 mL, 41.3 mmol) according to the procedure described above. Bulb to bulb distillation (220 °C (0.05 mmHg)) gave 9.92 g of malonate **2b** (74%) as clear liquid: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 7.0 Hz, 3 H, $CH_2CH_2CH_3$), 1.14–1.30 (m, 8 H, OCH_2CH_3 and $CH_2CH_2CH_3$), 1.64–1.73 (m, 2 H, $CH_2CH_2CH_3$), 3.14 (s, 2 H, $ArCH_2$), 3.69 (s, 3 H, OCH_3), 4.05–4.17 (m, 4 H, OCH_2), 6.55–6.72 (m, 3 H, ArH ortho or para to OMe), 7.05–7.13 (m, 1 H, ArH meta to OMe); MS m/z (relative intensity) 322 (M^+, 100), 248 (37), 202 (22); HRMS exact mass calcd for $C_{18}H_{26}O_5$ 322.1784, found 322.1780.

Methyl 3-(*m*-Methoxyphenyl)propionate (3a). A solution of *m*-methoxycinnamic acid (9.5 g, 53 mmol) and palladium hydroxide on carbon (Pearlman's catalyst, 500 mg) in EtOH (50 mL) and THF (20 mL) was stirred at room temperature under H_2 (1 atm) until no starting material was observed by GC analysis. After removal of solids by filtration, the solution was concentrated to give crude 3-(*m*-methoxyphenyl)propionic acid (9.45 g, 99%): mp 44–46 °C (lit.[9b] mp 43–45 °C); ^{1}H NMR ($CDCl_3$) δ 2.69 (t, J = 7.8 Hz, 2 H, CH_2COO), 2.94 (t, J = 7.8 Hz, 2 H, benzylic H), 3.80 (s, 3 H, OCH_3), 6.76–6.82 (m, 3 H, ArH ortho and para to OMe), 7.19–7.25 (m, 1 H, ArH meta to OMe).

The crude propionic acid prepared as above was dissolved with concd H_2SO_4 (13 N, 538 μL) in MeOH (100 ML) and refluxed for 48 h at 80 °C. The solution was concentrated and then poured into water–EtOAc. The organic layer was washed with $NaHCO_3$ solution (sat'd) and brine, dried ($MgSO_4$), and concentrated. Bulb to bulb distillation [lit.[9b] bp 82 °C (0.2 mmHg)] and flash chromatography (5% ether/hexane) afforded methyl ester **3a** (27.4 g) quantitatively from *m*-methoxycinnamic acid: ^{1}H NMR ($CDCl_3$) δ 2.61 (t, J = 7.4 Hz, 2 H, CH_2COO), 2.92 (t, J = 7.4 Hz, 2 H, $ArCH_2$), 3.69 (s, 3 H, $COOCH_3$), 3.78 (s, 3 H, $ArOCH_3$), 6.77–6.81 (m, 3 H, ArH ortho and para to OMe), 7.18–7.23 (m, 1 H, ArH meta to OMe).

Methyl 3-(*m*-Methoxyphenyl)-2-methylpropionate (3b). To a solution of diisopropylamine (3.8 mL, 27 mmol) in THF (10 mL) was added 0.97 M *n*-BuLi/*n*-hexane (27.8 mL, 27 mmol) over a 10-min period at 0 °C. After being stirred for 10–15 min, the reaction was cooled to −78 °C. Methyl ester **3a** (5 g, 25.7 mmol) in THF (10 mL) was added slowly at −78 °C and stirring continued for 15 min. This enolate was transferred slowly to MeI in THF (30 mL) and *N,N'*-dimethylpropyleneurea (5 mL) at −78 °C and then stirred at 10 °C until no starting material was observed by TLC. Ethyl ether (wet) and water were added sequentially, and the organic layer was washed (brine, twice), dried ($MgSO_4$), and concentrated. Flash chromatography (silica, 5–10% ether/hexane) gave methyl-substituted ester **3b** (2.5 g, 47%) as a clear liquid: ^{1}H NMR ($CDCl_3$) δ 1.14 (d, J = 6.6 Hz, 3 H, $CHCH_3$), 2.58–2.65 (dd, J_1 = 12.9 Hz, J_2 = 7.8 Hz, 1 H, benzylic H), 2.73 (q, J = 7.0 Hz, 1 H, $CHCH_3$), 2.97–3.03 (dd, J_1 = 12.9 Hz, J_2 = 6.4 Hz, 1 H, benzylic H), 3.64 (s, 3 H, $COOCH_3$), 3.78 (s, 3 H, $ArOCH_3$), 6.70–6.75 (m, 3 H, ArH ortho and para to OMe), 7.16–7.21 (m, 1 H, ArH meta to OMe); MS m/z (relative intensity) 208 (M^+, 32), 148 (41), 121 (100); HRMS exact mass calcd for $C_{12}H_{16}O_3$ 208.1097, found 208.1099.

Ethyl 2-(*m*-Methoxybenzyl)butyrate (3c). A heterogeneous mixture of diester **2a** (14.322 g, 46.5 mmol), LiCl (1.971 g, 46.5 mmol), and water (1.674 mL, 93 mmol) in DMSO (40 mL) was refluxed at 180 °C until no starting material was observed by TLC. After the mixture was cooled to room temperature, water was added, and then the organic materials were extracted with ether. The organic layer was washed with brine twice, dried over $MgSO_4$, then concentrated. Flash chromatography (5% ether/hexane) and the bulb to bulb distillation (150 °C (0.04 mmHg)] gave 9.2 g of ester **3c** in 84% yield as a clear liquid: ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.3 Hz, 3 H, $CHCH_2CH_3$), 1.16 (t, J = 7.5 Hz, 3 H, OCH_2CH_3), 1.16–1.54 (m, 2 H, $CHCH_2CH_3$), 2.53–2.63 (m, 1 H, $CHCOO$), 2.65–2.75 and 2.86–2.97 (m, 2 H, $ArCH_2$), 3.78 (s, 3 H, OCH_3), 4.08 (q, J = 7.5 Hz, 2 H, OCH_2), 6.71–6.76 (m, 3 H, ArH ortho or para to OMe), 7.14–7.21 (m, 1 H, ArH meta to OMe); MS m/z (relative intensity) 236 (M^+, 31), 162 (53), 121 (100); HRMS exact mass calcd for $C_{14}H_{20}O_3$ 236.1410, found 236.1412.

Ethyl 2-(*m*-methoxybenzyl)pentanoate (3d) was prepared from diester **2b** (9.83 g, 30.5 mmol) by the procedure described above. Bulb to bulb distillation (163–173 °C, (0.1 mmHg)] and column chromatography on silica (5% EtOAc/hexane) yielded 6.77 g of ester **3d** (89%): ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.3 Hz, 3 H, $CH_2CH_2CH_3$), 1.16 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 1.27–1.35 (m, 2 H, $CH_2CH_2CH_3$), 1.43–1.53 and 1.58–1.69 (m, 2 H, $CH_2CH_2CH_3$), 2.60–2.70 (m, 1 H, $CHCOO$), 2.67–2.74 and 2.87–2.91 (m, 2 H, $ArCH_2$), 3.78 (s, 3 H, OCH_3), 4.07 (q, J = 7.1 Hz, 2 H, OCH_2), 6.71–6.77 (m, 3 H, ArH ortho or para to OMe), 7.15–7.21 (m, 1 H, ArH meta to OMe); MS m/z (relative intensity) 250 (M^+, 32), 176 (38), 121 (100); HRMS exact mass calcd for $C_{15}H_{22}O_3$ 250.1571, found 250.1569.

4,5-Bis[(trimethylsilyl)oxy]-3,6-bis(*m*-methoxybenzyl)-4-octene (5a). A solution of sodium (1.550 g, 67.4 mmol) in anhydrous toluene (130 mL) was stirred at reflux under nitrogen to form sodium sand. After the solution was cooled to 75 °C with continued stirring, ester **3c** (5.31 g, 22.5 mmol) and TMSCl (10 mL, 67.4 mmol) in toluene (10 mL) were added. The mixture was refluxed for 9 h at 110 °C and then cooled to room temperature. Diethyl ether was added, and the mixture was filtered to remove NaCl and unreacted sodium. The filtrate was washed with 1 N HCl and brine, dried over $MgSO_4$, then concentrated. Flash chromatography (10% ether/hexane) gave silyl enol ether **5a** (4.584 g, 77%) as a viscous liquid: ^{1}H NMR ($CDCl_3$) δ 0.22

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(t, J = 7.7 Hz, 18 H, $SiCH_3$) 0.71 (t, J = 7.4 Hz, 3 H, CH_2CH_3), 0.83 (t, J = 7.3 Hz, 3 H, CH_2CH_3) 1.22–1.34 (m, 4 H, CH_2CH_3), 2.46 (m, 2 H, allylic protons), 2.52–2.57 and 2.67–2.73 (m, 4 H, $ArCH_2$), 3.76 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 6.68–6.75 (m, 6 H, ArH ortho or para to OMe), 7.13–7.18 (m, 2 H, ArH meta to OMe); MS m/z (relative intensity) 528 (M^+, 100), 407 (83), 319 (84), 259 ($M^+/2$, 44); HRMS exact mass calcd for $C_{30}H_{48}Si_2O_4$ 528.3091, found 528.3089.

2,8-Dimethoxy-5,6,11,12-tetrahydrochrysene (6a). This material was prepared by a modification of a literature procedure.[9b] A solution of sodium (1.987 g, 86.4 mmol) in anhydrous toluene (150 mL) was stirred at reflux to disperse sodium. After the mixture was cooled to ~65 °C with stirring, ester **3a** (6.993 g, 36 mmol) was added, and reflux was resumed overnight at 110 °C. MeOH (6 mL) and ether were added, and the mixture was filtered to remove precipitates. The filtrate was washed with water and brine, dried over $MgSO_4$, and then concentrated. Flash chromatography (silica, 10–20% EtOAc in hexane) generated yellow acyloin **4a** (1.83 g, 31%). This acyloin **4a** (1.42 g, 4.32 mmol) in benzene was refluxed with *p*-toluenesulfonic acid monohydrate (71 mg, 0.373 mmol) for 9 h at 80 °C. The organic materials were extracted with EtOAc, washed with $NaHCO_3$ and brine, dried (Na_2SO_4), and then concentrated. Flash chromatography on silica (10% EtOAc in hexane) and recrystallization (EtOAc) gave protiochrysene **6a** (442 mg, 35% yield) as a white solid with mp 162–164 °C (lit.[9a] mp 165–166 °C): ^1H NMR ($CDCl_3$) δ 2.61–2.66 (m, 4 H, allylic protons), 2.85–2.90 (m, 4 H, $ArCH_2$), 3.82 (s, 6 H, OCH_3), 6.73–6.74 (m, 4 H, ArH ortho to OMe), 7.25 (d, J = 9.2 Hz, 2 H, ArH meta to OMe); MS m/z (relative intensity) 292 (M^+, 100), 277 (M – CH_3, 41); HRMS exact mass calcd for $C_{20}H_{20}O_2$ 292.1463, found 292.1466.

2,8-Dimethoxy-5,11-*trans*-dimethyl-5,6,11,12-tetrahydrochrysene (6b) was prepared from ester **3b** (2.433 g, 11.7 mmol) in two steps. First, the procedure described for the preparation of **5a** (except different amounts of Na (3 equiv) and TMSCl (4 equiv)) was used to give acyloin **4b** (1.04 g, 50%), R_f 0.25–0.29 (30% EtOAc/hexane). Then, according to the method described for the preparation of chrysene **6c**, this acyloin **4b** (1.039 g, 2.9 mmol) was converted to dimethylchrysene **6b** (335 mg, 36%), a white solid with mp 178–182 °C: ^1H NMR ($CDCl_3$) δ 1.05 (d, J = 6.9 Hz, 6 H, $CHCH_3$), 2.58 (dd, J_1 = 15.3 Hz, J_2 = 2.5 Hz, 2 H, $ArCH_2$ syn to Me), 2.90 (m, 2 H, allylic protons), 3.13 (dd, J_1 = 15.3 Hz, J_2 = 6.2 Hz, 2 H, $ArCH_2$ anti to Me), 3.83 (s, 6 H, OCH_3), 6.74–6.78 (m, 4 H, ArH ortho to OMe), 7.30 (d, J = 9 Hz, 2 H, ArH meta to OMe); MS m/z (relative intensity) 320.1 (M^+, 100), 305.1 (M – CH_3, 45), 245.0 (12). Anal. Calcd for $C_{22}H_{24}O_2$: C, 82.46; H, 7.50. Found: C, 82.51; H, 7.55.

2,8-Dimethoxy-5,11-diethyl-5,6,11,12-tetrahydrochrysene (6c, 7). A mixture of crude silyl ether **5a** (3.455 g, 6.53 mmol) and polyphosphoric acid (PPA, 29 g) was stirred mechanically for 3 h at room temperature to give a red tar. The reaction mixture was diluted with H_2O (75 mL) and EtOAc (150 mL), then stirred until all materials dissolved. The organic layer was washed with $NaHCO_3$, brine (saturated), dried over $MgSO_4$, and then concentrated. Flash chromatography on silica (5% EtOAc/hexane) gave 1.972 g of chrysenes **6c** and **7** (**6c**/**7** = ~2/3) in 87% yield. Repeated flash chromatography and recrystallization from hexane–EtOAc separated trans and cis isomers.

Trans isomer 6c: white solid, mp 168–170 °C; ^1H NMR ($CDCl_3$) δ 0.79 (t, J = 7.4 Hz, 6 H, CH_2CH_3), 1.20–1.27 (m, 4 H, CH_2CH_3), 2.84 (d, J = 14.7 Hz, 2 H, $ArCH_2$ syn to Et), 2.91–3.01 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 3.83 (s, 6 H, OCH_3), 6.74 (m, 4 H, ArH ortho to OMe), 7.33 (d, J = 9 Hz, 2 H, ArH meta to OMe); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 12.4, 24.4, 33.1, 33.2, 55.2 (2 C), 110.7, 114.7, 124.0, 127.9, 131.8, 136.8, 158.1; MS m/z (relative intensity) 348 (M^+, 100), 319 (42), 279 (13). Anal. Calcd for $C_{24}H_{28}O_2$: C, 82.72; H, 8.10. Found: C, 82.41; H, 8.11.

Cis isomer 7: white solid, mp 104–107 °C; ^1H NMR ($CDCl_3$) δ 0.96 (t, J = 7.4 Hz, 6 H, CH_2CH_3), 1.29–1.38 and 1.44–1.55 (m, 4 H, CH_2CH_3), 2.56–2.60 (m, 2 H, allylic protons), 2.81 (dd, 2 H, J = 15.5 and 1.2 Hz, $ArCH_2$ syn to Et), 3.00 (dd, 2 H, J = 15.5 and 5.8 Hz, $ArCH_2$ anti to Et), 3.83 (s, 6 H, OCH_3), 6.74–6.77 (m, 4 H, ArH ortho to OMe), 7.21–7.25 (m, 2 H, ArH meta to OMe); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 12.1, 23.2, 32.1, 35.8, 55.3, 110.9, 114.7, 122.9, 128.3, 131.7, 136.2, 158.1; MS m/z (relative intensity) 348 (M^+, 97), 319 (M – Et, 70), 159 (100). Anal. Calcd for $C_{24}H_{28}O_2$: C, 82.72; H, 8.10. Found: C, 82.37; H, 8.21.

2,8-Dimethoxy-5,11-*trans*-dipropyl-5,6,11,12-tetrahydrochrysene (6d). The procedure, described in the preparation of compound **5a**, was applied to convert ester **3d** (5.442 g, 21.7 mmol) to silyl ether **5b** (4.382 g, 73%). Without isolation of the trans and cis isomers, this disilyl ether **5b** (4.11 g, 7.39 mmol) was dissolved in 5 N HCl (500 μL) in THF (40 mL) and stirred at room temperature for 20 h. The organic materials were extracted with ethyl ether, washed with $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. Flash silica chromatography (10% EtOAc in hexane) gave acyloin **4d** (2.136 g, 70%) as a colorless liquid: MS m/z (relative intensity) 412.3 (M^+, 100), 291.2 (34), 206.1 ($M^+/2$, 44); HRMS exact mass calcd for $C_{26}H_{36}O_4$ 361.2042, found 361.2038. The same procedure in the preparation of chrysene **6c** gave propylchrysene **6d** (315 mg, 84%) from acyloin **4d** (436 mg, 1.058 mmol) as a white solid with mp 130–133 °C: MS m/z (relative intensity) 376 (M^+, 77), 333 (M – Pr, 100), 277 (37); HRMS exact mass calcd for $C_{26}H_{32}O_2$ 376.2402, found 376.2401.

5,6,11,12-Tetrahydrochrysene-2,8-diol (Ia). The mixture of methyl ether **6a** (220 mg, 0.752 mmol) and TMSI (535 μL, 3.762 mmol) in CH_3CN (8 mL) was refluxed for 12 h at 80 °C. The cooled reaction mixture was acidified with 0.1 N HCl, and then organic materials were extracted with ethyl acetate. The organic layer was washed with brine twice and NaS_2O_3 (saturated) and then concentrated. Flash chromatography on silica (20% EtOAc in hexane) and recrystallization from ethyl acetate generated diol Ia (109 mg, 55%): IR (KBr) 3480, 2970, 2897, 1602, 1498, 1255 cm^{-1}; MS m/z (relative intensity) 264.2 (M^+, 25), 262.1 (100); HRMS exact mass calcd for $C_{18}H_{16}O_2$ 264.1152, found 264.1150.

5,11-*trans*-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (IIa). The same procedure as described in the preparation of IIIa was used to convert methyl ether **6b** to diol **IIa** (35 mg, 20%) and monophenol (110 mg, 59%). Diol IIa: white solid, mp 266–268 °C; IR (KBr) 3414, 1606, 1496, 1456, 1232 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (d, J = 5.6 Hz, 6 H, CH_3), 2.53–2.59 (m, 2 H, $ArCH_2$ syn to Me), 2.87–2.93 (m, 2 H, allylic protons), 3.02–3.09 (m, 2 H, $ArCH_2$ anti to Me), 6.71 (m, 4 H, ArH ortho to OH), 7.28 (m, 2 H, ArH meta to OH); MS m/z (relative intensity) 292 (M^+, 100), 277 (70), 248 (39); HRMS exact mass calcd for $C_{20}H_{20}O_2$ 292.1463, found 292.1465.

5,11-*trans*-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (IIIa). To a solution of dimethoxychrysene **6c** (569 mg, 1.633 mmol) in CH_2Cl_2 (15 mL) was added BBr_3 (315 μL, 3.266 mmol) at −78 °C. After the mixture was stirred for 16 h at room temperature, EtOAc and 5.1 N HCl (~0.5 mL) were added, respectively. The organic layer was washed with $Na_2S_2O_3$ and brine and filtered through $MgSO_4$ and activated carbon. After concentration, flash silica chromatography (5–20% EtOAc/hexane) gave diol IIIa (323 mg, 91%) and monophenol **10** (12 mg, 2%; see below for the characterization). Diol IIIa: mp 243 °C dec; IR (KBr) 3386, 2955, 1583, 1496, 1256 cm^{-1}; ^1H NMR (acetone-d_6) δ 0.78 (t, J = 7.5 Hz, 6 H, CH_2CH_3), 1.20 (m, 4 H, CH_2CH_3), 2.84–2.95 (m, 6 H, allylic and benzylic), 6.66–6.70 (m, 4 H, ArH ortho to OH), 7.29 (d, J = 8.3 Hz, 2 H, ArH meta to OH); MS m/z (relative intensity) 320 (M^+, 81), 291 (M – Et, 100), 249 (48). Anal. Calcd for $C_{22}H_{24}O_2$: C, 82.46; H, 7.55. Found: C, 82.22; H, 7.61.

5,11-*trans*-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol (IVa). The same procedure as in the preparation of diol IIIa was used to convert dimethyl ether **6d** (268 mg, 0.713 mmol) into 239 mg of propylchrysenediol IVa (96%), which was obtained as a white solid, mp 235–236 °C: ^1H NMR ($CDCl_3$) δ 0.72–0.77 (m, 6 H, CH_2CH_3), 1.10–1.28 (m, 8 H, $CH_2CH_2CH_3$), 2.79–2.86 (m, 4 H, allylic and benzylic syn to Pr), 3.05 (m, 2 H, benzylic anti to Pr), 6.65–6.69 (m, 4 H, ArH ortho to OH), 7.30 (br m 2 H, ArH meta to OH), 8.20 (s, OH); MS m/z (relative intensity) 348 (M^+, 78), 305 (M – Pr, 100), 249 (63); HRMS exact mass calcd for $C_{24}H_{28}O_2$ 348.2089, found 348.2091.

5,11-*cis*-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (8). The same procedure used for the preparation of *trans*-diethylchrysene IIIa was used to convert *cis*-diethylchrysene **7** to diol **8**, which was obtained quantitatively as a white solid with mp 248 °C dec: ^1H NMR (acetone-d_6) δ 0.90 (t, J = 7.4 Hz, 6 H, CH_3), 1.18–1.30 and 1.33–1.47 (m, 4 H, CH_2CH_3), 2.50 (m, 2 H, allylic protons), 2.76 (d, J = 15.8 Hz, 2 H, $ArCH_2$ syn to Et), 2.84–2.94 (m, 2 H, $ArCH_2$ anti to Et), 6.66 (m, 4 H, ArH ortho to OH), 7.09

(d, J = 7.5 Hz, 2 H, ArH meta to OH); MS (70 eV) m/z (relative intensity) 320 (M^+, 100), 291 (M – Et, 95), 249 (45); HRMS exact mass calcd for $C_{22}H_{24}O_2$ 320.1776, found: 320.1770.

(*E*)-1-(2-Ethyl-5-methoxy-1-indanylidene)-2-ethyl-5-methoxyindan (9). To a solution of acid-treated zinc (517 mg, 7.906 mmol) in THF (20 mL) was added $TiCl_4$ (433 mL, 3.953 mmol) for 10 min at –78 °C to give a yellow solution which was then stirred for 1 h at room temperature. After addition of more THF (20 mL), the gray heterogeneous solution was refluxed for 1 h at 55 °C. A sample of 2-ethyl-5-methoxyindanone (376 mg, 1.976 mmol) in THF (15 mL) was added at room temperature, and reflux was resumed for 1 h at 55 °C. After being cooled to room temperature, the reaction mixture was poured into K_2CO_3 solution and the organic materials were extracted with ether. The fractional crystallization (acetone) and flash chromatography (10% EtOAc/hexane) generated indan **9** (306 mg, 89%) as a white solid with mp 189–191 °C: ^1H NMR ($CDCl_3$) δ 0.97 (t, J = 7.3 Hz, 6 H, CH_2CH_3), 1.15–1.29 and 1.60–1.71 (m, 4 H, CH_2CH_3), 2.78 (d, J = 16.5 Hz, 2 H, $ArCH_2$ syn to Et), 3.18 (dd, J_1 = 8.0, J_2 = 2.0 Hz, 2 H, $ArCH_2$ anti to Et), 3.57 (m, 2 H, allylic protons), 3.84 (s, 6 H, OCH_3), 6.78–6.83 (m, 4 H, ArH ortho to OMe), 7.51 (d, J = 8.3 Hz, 2 H, ArH meta to OMe); MS m/z (relative intensity) 348 (M^+, 100), 320 (15), 319 (61); HRMS exact mass calcd for $C_{24}H_{28}O_2$ 348.2094, found 348.2089.

8-Methoxy-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysen-2-ol (10), prepared by the method used in the preparation of chrysene IIIa, was obtained as a pale purple solid: ^1H NMR ($CDCl_3$) δ 0.79 (t, J = 7.4 Hz, 6 H, CH_2CH_3), 1.20–1.27 (m, 4 H, CH_2CH_3), 2.84–2.95 (m, 6 H, allylic and benzylic), 3.83 (s, 3 H, OCH_3), 4.65 (s, 1 H, OH), 6.66–6.76 (m, 4 H, ArH ortho to OR), 7.15–7.35 (m, 2 H, ArH meta to OR); MS (70 eV) m/z (relative intensity) 334 (M^+, 87), 305 (M – Et, 100); HRMS-EI exact mass calcd for $C_{23}H_{26}O_2$ 334.1933, found 334.1934.

5,11-*trans*-Diethyl-2-[(trifluoromethanesulfonyl)oxy]-8-methoxy-5,6,11,12-tetrahydrochrysene (11). (Method A). To a solution of monophenol **10** (98 mg, 0.293 mmol) and 2,6-lutidine (41 μL, 0.352 mmol) in methylene chloride (4 mL) was added trifluoromethanesulfonic anhydride (58 μL, 0.352 mmol) at 0 °C. Stirring was continued for 35 min to give a red solution. EtOAc and water were added, and the organic layer was washed with $NaHCO_3$ and brine and then dried ($MgSO_4$) and concentrated. Flash chromatography on silica (5% ether/hexane) gave triflate **11** (137 mg) in quantitative yield as a white solid with mp 113–115 °C: ^1H NMR ($CDCl_3$) δ 0.80 (t, J = 7.4 Hz, 6 H, CH_2CH_3), 1.20–1.28 (m, 4 H, CH_2CH_3), 2.84–3.06 (m, 6 H, allylic protons and $ArCH_2$), 3.84 (s, 3 H, OCH_3), 6.76–6.78 (m, 2 H, ArH ortho to OMe), 7.11–7.13 (m, 2 H, ArH ortho to OTf), 7.37 (d, J = 9.4 Hz, 1 H, ArH meta to OMe), 7.44 (d, J = 8.2 Hz, 1 H, ArH meta to OTf); MS m/z (relative intensity) 466 (M^+, 39), 437 (M – Et, 23), 333 (M – SO_2CF_3, 100); HRMS exact mass calcd for $C_{24}H_{25}SO_2F_3$ 466.1426, found 466.1430.

Method B. To a solution of monotriflate **13** (2.134 g, 4.72 mmol) and $LiOH{\cdot}H_2O$ (396 mg, 9.44 mmol) in DMSO (40 mL) was added methyl iodide (5.8 mL, 94 mmol) at room temperature. After being stirred for 8 h, the reaction mixture was poured into ice–HCl (5 N) solution. The organic materials were extracted with ethyl acetate, washed with $NaHCO_3$ and brine, and then dried over $MgSO_4$. Flash chromatography on silica (10–20% ethyl ether in hexane) afforded triflate **11** (883 mg, 50%) as a foam.

5,11-*trans*-Diethyl-2,8-bis[(trifluoromethanesulfonyl)oxy]-5,6,11,12-tetrahydrochrysene (12). The same procedure described for the preparation of **11** (method A) was used to convert diol IIIa (108 mg, 0.337 mmol) to bis-triflate **12** (193 mg, 98%), which was obtained as a white solid with mp 139–141 °C: ^1H NMR ($CDCl_3$) δ 0.78 (m, 6 H, CH_3), 1.25 (m, 4 H, CH_2CH_3), 2.90–3.05 (m, 6 H, allylic and benzylic protons), 6.97 (m, 2 H, ArH_1, ArH_7), 7.11 (m, 2 H, ArH_3, ArH_9), 7.45 (m, 2 H, ArH meta to OTf); MS m/z (relative intensity) 584 (M^+, 14), 555 (M – Et, 7), 494 (45), 319 (100); HRMS exact mass calcd for $C_{24}H_{22}F_6O_6S_2$ 584.0762, found 584.0759.

5,11-*trans*-Diethyl-8-[(trifluoromethanesulfonyl)oxy]-5,6,11,12-tetrahydrochrysen-2-ol (13). The same method described for the preparation of nitrochrysene IIIf was applied to convert bis-triflate **12** (4.504 mg, 7.71 mmol) into monotriflate **13** (2.134 mg, 61%), which was obtained as a white solid with mp 67–69 °C: IR (KBr) 3360, 2961, 1425, 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.79 (t, J = 7.4 Hz, 6 H, CH_3), 1.21–1.29 (m, 4 H, CH_2CH_3), 2.81–3.00 (m, 6 H, allylic and benzylic protons), 4.87 (s, 1 H, OH), 6.69–6.71 (m, 2 H, ArH ortho to OH), 7.08–7.12 (m, 2 H, ArH ortho to OTf), 7.32 (d, J = 9.2 Hz, 1 H, ArH meta to OH), (d, J = 8.3 Hz, 1 H, ArH meta to OTf); MS m/z (relative intensity) 452 (M^+, 49), 423 (M – Et, 27), 319 (M – OTf, 100); HRMS exact mass calcd for $C_{23}H_{23}F_3SO_4$ 452.1264, found 452.1269.

2-(Methylcarbonyl)-8-methoxy-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysene (14). To a mixture of chrysene triflate **11** (64 mg, 0.137 mmol), palladium(II) acetate (3.0 mg, 0.014 mmol), 1,1′-bis(diphenylphosphino)ferrocene (7.8 mg, 0.014 mmol), LiCl (17.4 mg, 0.411 mmol), and a few crystals of 2,6-di-*tert*-butyl-4-methylphenol in anhydrous DMSO (5 mL) was added tetramethyltin (68 μL, 0.491 mmol) at room temperature. After being purged with carbon monoxide several times, the mixture was refluxed for 7 h at 90 °C. Ethyl ether and water were added to the reaction mixture, and the organic layer was washed with brine and dried over $MgSO_4$. Flash chromatography on silica gel (10% ether/hexane) and recrystallization over ether/hexane afforded methyl ketone **14** (47 mg, 95%) as a yellow solid with mp 108–110 °C: IR (KBr) 2933, 1673, 1595, 1235 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76–0.83 (m, 6 H, CH_2CH_3), 1.18–1.29 (m, 4 H, CH_2CH_3), 2.61 (s, 3 H, COMe), 2.87 (d, J = 13.7 Hz, 2 H, $ArCH_2$ syn to Et), 2.97–3.07 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 3.85 (s, 3 H, OCH_3), 6.76–6.79 (m, 2 H, ArH ortho to OMe), 7.40 (d, J = 9.4 Hz, 1 H, ArH meta to OMe), 7.48 (d, J = 8.2 Hz, 1 H, ArH meta to COMe), 7.78–7.83 (m, 2 H, ArH ortho to COMe); MS m/z (relative intensity) 360 (M^+, 46), 331 (M – Et, 53), 289 (15); HRMS exact mass calcd for $C_{25}H_{28}O_2$ 360.2089, found 360.2091.

2-(Methoxycarbonyl)-8-methoxy-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysene (15). To a yellow solution of dppp (35 mg, 0.086 mmol) and palladium acetate (21 mg, 0.086 mmol) in MeOH (18 mL) and DMSO (25 mL) was added triflate **11** (614 mg, 1.316 mmol) and triethylamine (401 μL, 2.90 mmol). The system was then purged with carbon monoxide several times. This mixture was refluxed for 15 h at 90 °C to give a fluorescent gray solution that was cooled to room temperature. EtOAc and water were added, and the organic layer was washed with water and brine and then dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel (5–30% ether in hexane) afforded methyl ester **15** (444 mg, 90%) as a white solid with mp 111–112 °C: IR (KBr) 2955, 1715, 1435, 1250, 1285 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.88 (m, 6 H, CH_2CH_3), 1.18–1.28 (m, 4 H, CH_2CH_3), 2.87 (d, J = 13.7 Hz, 2 H, $ArCH_2$ syn to Et), 2.98–3.06 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 3.84 (s, 3 H, $ArOCH_3$), 3.91 (s, 3 H, $COOCH_3$), 6.75–6.78 (m, 2 H, ArH ortho to OMe), 7.39 (d, J = 9.4 Hz, 1 H, ArH meta to OMe), 7.46 (d, J = 8.2 Hz, 1 H, ArH meta to COOMe), 7.84–7.90 (m, 2 H, ArH ortho to COOMe); MS m/z (relative intensity) 376 (M^+, 73), 347 (M – Et, 100), 246 (21), 215 (25); HRMS exact mass calcd for $C_{25}H_{28}O_3$ 376.2038, found 376.2037.

2-(Aminocarbonyl)-5,11-*trans*-diethyl-8-methoxy-5,6,11,12-tetrahydrochrysene (16). To a solution of NH_4Cl (858 mg, 16 mmol) in anhydrous benzene (16 mL) was added 2 M solution (8 mL) of trimethylaluminum for 4 min at 0 °C. After removal of the ice bath, the reaction mixture was stirred for 1.5 h until no gas evolution was observed. To this aluminum reagent was added a solution of ester **15** (302 mg, 0.802 mmol) in benzene (16 mL) at room temperature to give a yellow fluorescent solution which was then stirred for 1.5 h at 60 °C. After the reaction mixture was cooled to room temperature, ether and water were added, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give crude amide **16**. Repeated recrystallization from ether gave amide **16** (226 mg, 78%) as a white solid with mp 194–197 °C: IR (KBr) 3424, 3204, 2926, 1651, 1612 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.83 (m, 6 H, CH_2CH_3), 1.21–1.28 (m, 4 H, CH_2CH_3), 2.87 (d, J = 14.1 Hz, 2 H, $ArCH_2$ syn to Et), 2.99–3.01 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 3.84 (s, 3 H, OCH_3), 6.07 (br, 2 H, NH_2), 6.76–6.78 (m, 2 H, ArH ortho to OMe), 7.39 (d, J = 9.3 Hz, 1 H, ArH meta to OMe), 7.48 (d, J = 8.6 Hz, 1 H, ArH meta to $CONH_2$), 7.64–7.66 (m, 2 H, ArH ortho to $CONH_2$); MS m/z (relative intensity) 361 (M^+, 99), 332 (M – Et, 100), 289 (31), 247 (39); HRMS exact mass calcd for $C_{24}H_{27}NO_2$ 361.2042, found 361.2038.

2-Cyano-8-methoxy-5,11-*trans*-diethyl-5,6,11,12-tetra-

hydrochrysene (17). To a solution of CCl_4 (110 μL, 1.17 mmol) and THF (6 mL) at 0 °C was added $TiCl_4$ (58 μL, 0.52 mmol). After 5 min, amide **16** (47 mg, 0.13 mmol) in THF (14 mL) and Et_3N (72 μL, 0.52 mmol) were added to this yellow heterogeneous solution, and stirring was continued at room temperature until no starting material remained. Diethyl ether and water were added, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Repeated recrystallization from diethyl ether gave cyanide **17** (45 mg, 99%) as a white solid with mp 137–139 °C: IR (KBr) 2928, 2217, 1593, 1491, 1227 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74–0.85 (m, 6 H, CH_2CH_3), 1.21–1.30 (m, 4 H, CH_2CH_3), 2.89–3.03 (m, 6 H, allylic and benzylic), 3.84 (s, 3 H, OCH_3), 6.76–6.78 (m, 2 H, ArH ortho to OMe), 7.38–7.49 (m, 4 H, ArHCN and ArH meta to OMe); MS *m/z* (relative intensity) 343 (M^+, 41), 314 (89), 55 (59); HRMS exact mass calcd for $C_{24}H_{25}NO$ 343.1936, found 343.1937.

2-(Methylcarbonyl)-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysen-8-ol (IIIb). To a solution of ketone **14** (32 mg, 0.089 mmol) in CH_2Cl_2 (3 mL) was added BBr_3 (17 μL, 0.178 mmol) at −78 °C under nitrogen. Stirring was continued for 9 h at room temperature; ether and 5 N HCl were added, and the organic layer was separated, washed with $NaHCO_3$ and brine, then dried over $MgSO_4$, and concentrated. Flash chromatography on silica gel (10–20% ether/hexane) gave phenol **IIIb** (27 mg, 88%) as a yellow solid with mp 85–86 °C: IR (KBr) 3418, 2924, 1670, 1599, 1287 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.83 (m, 6 H, CH_2CH_3), 1.20–1.31 (m, 4 H, CH_2CH_3), 2.62 (s, 3 H, COMe), 2.84 (d, *J* = 13.6 Hz, 2 H, $ArCH_2$ syn to Et), 2.98–3.04 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 6.69–6.72 (m, 2 H, ArH ortho to OH), 7.35 (d, *J* = 9.0 Hz, 1 H, ArH meta to OH), 7.48 (d, *J* = 8.2 Hz, 1 H, ArH meta to COMe), 7.78–7.83 (m, 2 H, ArH ortho to COMe); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 197.9, 155.5, 139.9, 137.8, 137.6, 134.9, 134.5, 131.2, 128.1, 127.1, 126.9, 125.1, 122.7, 116.0, 112.9, 33.3, 33.1, 32.7, 32.5, 26.5, 24.7, 24.4, 12.3, 12.3; MS *m/z* (relative intensity) 346 (M^+, 17), 317 (M − Et, 17), 43 (100); HRMS exact mass calcd for $C_{24}H_{26}O_2$ 346.1933, found 346.1934.

2-(Methoxycarbonyl)-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysen-8-ol (IIIc). The procedure used to prepare **IIIb** converted methyl ether **15** (20 mg, 0.053 mmol) into ester **IIIc** (18 mg, 95%), which was obtained as a white solid with mp 75–77 °C: IR (KBr) 3411, 2928, 1714, 1692, 1289 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.82 (m, 6 H, CH_2CH_3), 1.18–1.28 (m, 4 H,CH_2CH_3), 2.84 (d, *J* = 13.8 Hz, 2 H, $ArCH_2$ syn to Et), 2.94–3.04 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 3.92 (s, 3 H, $COOCH_3$), 4.97 (br, 1 H, OH), 6.69–6.72 (m, 2 H, ArH ortho to OH), 7.34 (d, *J* = 9.1 Hz, 1 H, ArH meta to OH), 7.46 (d, *J* = 8.2 Hz, 1 H, ArH meta to COOMe), 7.84–7.91 (m, 2 H, ArH ortho to COOMe); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 167.3, 155.0, 139.6, 137.8, 137.1, 134.8, 131.4, 129.3, 127.9, 127.3, 127.3, 125.1, 122.6, 115.9, 112.8, 52.0, 33.2, 33.1, 32.7, 32.5, 24.6, 24.3, 12.3, 12.2; MS *m/z* (relative intensity) 361.5 (M^+, 100), 332.6 (M − Et, 90), 230.7 (39); HRMS exact mass calcd for $C_{24}H_{26}O_3$ 362.1882, found 362.1882.

2-(Aminocarbonyl)-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysen-8-ol (IIId). The procedure used to prepare **IIIb** converted methyl ether **16** (40 mg, 0.111 mmol) into amide **IIId** (36 mg, 94%), which was obtained as a white solid: IR (KBr) 3389, 3190, 2927, 1651, 1608 cm^{-1}; ^1H NMR (acetone-d_6) δ 0.75–0.89 (m, 6 H, CH_3), 1.21–1.36 (m, 4 H, CH_2CH_3), 2.88–2.95 (m, 2 H, $ArCH_2$ syn to Et), 2.98–2.99 and 3.03–3.14 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 6.70–6.75 (m, 2 H, ArH ortho to OH), 7.40 (d, *J* = 7.3 Hz, 1 H, ArH meta to OH), 7.54 (d, *J* = 8.4 Hz, 1 H, ArH meta to $CONH_2$), 7.78 (m, 2 H, ArH ortho to $CONH_2$); ^{13}C NMR (acetone-d_6; 75.6 MHz) δ 168.8, 157.8, 138.8, 138.1, 137.3, 135.2, 132.5, 131.6, 128.4, 126.8, 126.6, 125.9, 123.4, 116.7, 113.7, 33.8, 33.8, 33.3, 33.2, 25.4, 25.1, 12.5, 12.4; MS *m/z* (relative intensity) 347 (M^+, 100), 345 (M − 2, 47), 318 (M − Et, 99), 233 (69); HRMS exact mass calcd for $C_{23}H_{25}NO_2$ 347.1880, found 347.1885.

2-Cyano-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysen-8-ol (IIIe). The procedure used to prepare **IIIb** converted methyl ether **17** (32 mg, 0.093 mmol) into nitrile **IIIe** (30 mg, 98%), which was obtained as a white solid with mp 66–71 °C: IR (KBr) 3378, 2928, 2228, 1568, 1223 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.80 (m, 6 H, CH_3), 1.21–1.27 (m, 4 H, CH_2CH_3), 2.83 (d, *J* = 14.4 Hz, 2 H, $ArCH_2$ syn to Et), 2.93–2.98 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 6.70–6.72 (m, 2 H, ArH ortho to OH), 7.34 (d, *J* = 9.3 Hz, 1 H, ArH meta to OH), 7.43–7.50 (m, 3 H, ArH ortho and meta to CN); ^{13}C NMR ($CDCl_3$; 100.6 MHz) δ 155.4, 139.6, 138.1, 137.8, 135.7, 131.4, 130.6, 130.5, 126.8, 125.2, 123.1, 119.5, 116.0, 113.0, 108.6, 33.0, 33.0, 32.6, 32.3, 24.6, 24.4, 12.3, 12.2; MS *m/z* (relative intensity) 329 (M^+, 49), 300 (M − Et, 100), 258 (60); HRMS exact mass calcd for $C_{23}H_{23}NO$ 329.1780, found 329.1776.

2-[(Trifluoromethanesulfonyl)oxy]-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysene (18). To a solution of bistriflate **12** (832 mg, 1.425 mmol) and bis(triphenylphosphine)palladium(II) chloride (50 mg, 0.071 mmol) in DMSO (20 mL) were added triethylamine (394 μL, 2.85 mmol) and formic acid (54 μL, 1.425 mmol), respectively, at room temperature. The heterogeneous mixture was refluxed for 1 h at 90 °C to give a dark red solution. The organic material was extracted with ethyl ether, washed with water and brine, and then dried over $MgSO_4$. Flash chromatography (silica, 5% ether/hexane) gave monotriflate **18** (223 mg, 36%) as a white semisolid: ^1H NMR ($CDCl_3$) δ 0.80 (t, *J* = 7.4 Hz, 6 H, CH_3), 1.20–1.29 (m, 4 H, CH_2CH_3), 2.90–3.10 (m, 6 H, allylic and benzylic protons), 7.11–7.14 (m, 2 H, ArH ortho to OTf), 7.20–7.26 (m, 2 H, ArH_7 and ArH_9), 7.44–7.49 (m, 3 H, ArH meta to OTf and ArH_8, ArH_{10}); MS *m/z* (relative intensity) 436 (M^+, 75) 407 (M − Et, 55) 303 (M − SO_2CF_3, 100); HRMS exact mass calcd for $C_{23}H_{23}F_3O_3S$ 436.1320, found 436.1320.

2-Nitro-8-[(trifluoromethanesulfonyl)oxy]-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysene (19). To a solution of BF_4NO_2 (92 mg, 0.688 mmol) in $CHCl_3$ (5 mL) was added triflate **18** (60 mg, 0.138 mmol) in $CHCl_3$ (4 mL) at −78 °C. The reaction mixture was subsequently warmed to room temperature then refluxed for 5 h at 45 °C. A black solution resulted from the addition of ether and $NaHCO_3$. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated. Flash chromatography (silica, 5% ether in hexane) gave nitrochrysene **19** as a yellow solid (54 mg, 82%) with mp 118–120 °C: ^1H NMR ($CDCl_3$) δ 0.88–1.02 (m, 6 H, CH_3), 1.18–1.30 (m, 4 H, CH_2CH_3), 2.98–3.12 (m, 6 H, allylic and $ArCH_2$), 7.12–7.44 (m, 3 H, TfOArH), 7.72–8.21 (m, 3 H, $Ar(NO_2)H$); MS *m/z* (relative intensity) 481 (M^+, 100), 452 (M − Et, 70) 348 (M − CF_3SO_2, 35); FABHRMS exact mass calcd for $C_{23}H_{22}F_3NO_5S$ 481.1165, found 481.1171.

2-Nitro-5,11-*trans*-diethyl-5,6,11,12-tetrahydrochrysen-8-ol (IIIf). A mixture of triflate **19** (47 mg, 0.098 mmol) and $LiOH·H_2O$ (41 mg, 0.976 mmol) in DMSO (10 mL) was refluxed for 2.5 h at 70 °C and then cooled to room temperature. Water, EtOAc, then 5 N HCl were added to the reaction mixture. The organic layer was washed with $NaHCO_3$ (saturated) and brine and then dried over $MgSO_4$. Flash chromatography (silica, 20% ether in hexane) afforded nitrochrysene **IIIf** as a red foam (25 mg, 74%). Preparative HPLC (acetonitrile–water) gave further purified **IIIf** for the receptor assay as a red powder: dec at 90 °C; IR (KBr) 3441, 1633, 1237, 1073 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.83 (m, 6 H, CH_3), 1.17–1.59 (m, 4 H, CH_2CH_3), 2.83–2.91 (m, 2 H, $ArCH_2$ syn to Et), 2.99–3.04 (m, 4 H, allylic protons and $ArCH_2$ anti to Et), 6.71–6.73 (m, 2 H, ArH ortho to OH), 7.37 (d, *J* = 9.3 Hz, 1 H, ArH meta to OH), 7.51 (d, *J* = 8.7 Hz, 1 H, ArH meta to NO_2), 8.04 (s, 1 H, ArH ortho to NO_2; ArH_1), 8.08 (d, *J* = 8.7 Hz, 1 H, ArH ortho to NO_2; ArH_3); ^{13}C NMR ($CDCl_3$; 1.006 Hz) δ 155.4, 145.4, 141.6, 139.2, 138.0, 135.9, 130.8, 126.8, 125.5, 123.2, 123.1, 122.1, 116.0, 113.0, 33.1, 33.0, 32.8, 32.4, 24.7, 24.4, 12.3, 12.2; FABMS *m/z* 350 (M + 1), 279; HRMS (FAB) exact mass calcd for $C_{22}H_{24}NO_3$ (M + 1) 350.1756, found 350.1756.

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Registry No. **1a**, 133-13-1; **1b**, 2163-48-6; **2a**, 138889-93-7; **2b**, 138089-94-8; **3a**, 50704-52-4; **3b**, 62007-42-5; **3c**, 138089-95-9; **3d**, 138089-96-0; **4a**, 71505-81-2; **4b**, 138089-97-1; **4c**, 138089-98-2; **4d**, 138089-99-3; **5a**, 138090-00-3; **5b**, 138090-01-4; **6a**, 18930-99-9; **6b**, 138090-02-5; **6c**, 138090-03-6; **6d**, 138090-04-7; **7**, 138090-05-8; **8**, 138090-06-9; **9**, 138090-07-0; **10**, 138090-08-1; **11**, 138090-09-2; **12**, 138090-10-5; **13**, 138090-11-6; **14**, 138090-12-7; **15**, 138090-13-8; **16**, 138090-14-9; **17**, 138090-15-0; **18**, 138090-16-1; **19**, 138128-48-0; **Ia**, 138090-17-2; **IIa**, 138090-18-3; **IIIa**, 138090-19-4; **IVa**, 138090-20-7; **IIIb**, 138090-21-8; **IIIc**, 138090-22-9; **IIId**, 138090-23-0; **IIIe**, 138090-24-1; **IIIf**, 138090-25-2; *m*-methoxybenzyl

chloride, 824-98-6; *m*-methoxycinnamic acid, 6099-04-3; 3-(*m*-methoxyphenyl)propionic acid, 10516-71-9; 2-ethyl-5-methoxyindanone, 138090-26-3.

Supplementary Material Available: Atomic numbering schemes and tables of atomic coordinates, thermal parameters, bond lengths, and bond angles for compounds IIIa and 7; ^{1}H NMR spectra of compounds **2a**, **3c**, **6a–c**, **7**, **9**, **11–17**, IIIa,c,e,f, IVa; ^{13}C NMR spectra of compounds **6c**, **7**, and IIIb–f (40 pages). Ordering information is given on any current masthead page.

Biosynthesis of Purpactin A†

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The biosynthetic origin of purpactin A (1) was studied by feeding sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates, D,L-[2-^{13}C]mevalonolactone, and L-[methyl-^{13}C]methionine to the producing organism *Penicillium purpurogenum* FO-608. ^{13}C NMR spectroscopy established that 1 is derived from one mevalonate, one methionine, and nine acetate units. In the biosynthetic scheme for 1 it is proposed that (1) the tricyclic skeleton of purpactin B (**2**) is produced first from a single octaketide chain condensed in a "head-to-tail" fashion, which involves decarboxylation of the "tail" carboxylic acid group to form the carbocyclic intermediate **5**, an oxidative cleavage of the B ring of **5** to form benzophenone intermediates (rotation between **6a** and **6b**), and recyclization (phenol oxidative coupling), and then (2) a methyl residue from methionine, a C-5 unit from mevalonate, and an acetate are introduced into the isogrisan skeleton **7** to yield **2**, and (3) finally **2** is nonenzymatically converted to 1.

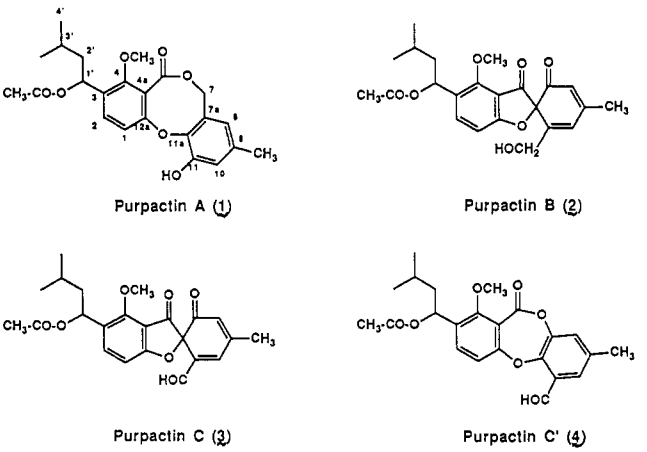
Introduction

Purpactins A (1), B (2), and C (3), new acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors, have been found in the cultured broth of *Penicillium purpurogenum* FO-608.[1,2] They showed ACAT inhibitory activity in both the enzyme assay using rat liver microsomes and the cell assay using J774 macrophages. It seems that purpactins A and C′ (4) (Chart I), having similar tricyclic skeletons, are nonenzymatically derived from purpactins B and C, respectively.[2] Purpactin A is structurally related to penicillide, which was reported as a root-growth stimulant by Sassa et al.,[3] but the biosynthetic study of penicillide has not been carried out in detail. Of particular interest are the decarboxylation, oxidative cleavage, and cyclization of a carbocyclic intermediate derived from a single octaketide chain and the conversion of 2 into 1. To clarify the biosynthetic pathway to 1, we obtained ^{13}C-enriched samples of 1 through feeding ^{13}C-precursors to the growing culture of *P. purpurogenum* FO-608.

Experimental Section

Incorporation Experiments. The inoculum for the fermentation of *P. purparogenum* FO-608 was built up in two stages on a slant culture in a seed medium consisting of glucose (2%, w/v), yeast extract (0.2%), $MgSO_4 \cdot 7H_2O$ (0.05%), polypeptone (0.5%), KH_2PO_4 (0.1%), and agar (0.1%) for 2 days at 28 °C. The second-stage seed culture was used to inoculate a fermentation medium (100 mL in a 500-mL flask). The fermentation medium consisted of glycerol (2.5%, w/v), glucose (0.5%), peptone (0.5%), NaCl (0.2%), and agar (0.1%). At 24 h following inoculation, the labeled compounds were added in equal portions. Single-labeled acetates (sodium [1-^{13}C]- and [2-^{13}C]acetates, ISOTEC Inc., minimum 99 atom % ^{13}C) were included at a final concentration of 0.1%; double-labeled acetate (sodium [1,2-$^{13}C_2$]acetate, ISOTEC Inc., minimum 99 atom % ^{13}C) was added at a final concentration of 0.01%; and the labeled mevalonolactone and methionine (D,L-[2-^{13}C]mevalonolactone and L-[methyl-^{13}C]methionine, ISOTEC Inc., minimum 99 atom % ^{13}C) were both at 0.1%.

Chart I. Structures of Purpactins A, B, C, and C′



Isolation Procedure. Each fermentation broth was extracted twice with the same volume of EtOAc. The extracts were filtered through a phase separator (Whatman 1PS) and concentrated under reduced pressure to an oily residue. The residue was dissolved in CH_3CN and applied to an ODS column (YMC pack, 5-ODS, AM 324, 10 × 300 mm). The mobile phase consisted of 60% aqueous CH_3CN. The flow rate was maintained at 8 mL/min, and the column effluent was monitored by UV absorbance at 280 nm. The yields of the purified ^{13}C-labeled 1 were as follows: [1-^{13}C]acetate-labeled 1 (1 mg) from 200 mL of the fermentation broth, [2-^{13}C]acetate-labeled 1 (0.9 mg) from 200 mL of the fermentation broth, [1,2-$^{13}C_2$]acetate-labeled 1 (3.8 mg) from 500 mL of the fermentation broth, [2-^{13}C]mevalonolactone-labeled 1 (1 mg) from 200 mL of the fermentation broth,

† This manuscript is dedicated to the late Dr. Shigenobu Okuda.

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