Torsionally and Hydrophobically Modified 2,3-Diarylindenes as Estrogen Receptor Ligands

Gregory M. Anstead, Chad S. Peterson, Kevin G. Pinney, Scott R. Wilson, and John A. Katzenellenbogen*

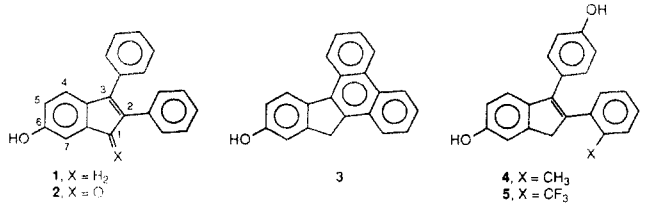
Department of Chemistry, University of Illinois, 1209 West California Street, Urbana, Illinois 61801.
Received February 12, 1990

2,3-Diarylindenes are ligands for the estrogen receptor which display intrinsic fluorescence. In order to optimize the receptor binding affinity of these compounds while preserving their desirable fluorescence properties, a series of torsionally modified analogues were prepared. A fluorine or methyl group was introduced on either of the two phenyl substituents ortho to their attachment site to the indene nucleus, in order to increase the out-of-plane twist of the appended rings. The analogues were prepared by the benzylation of appropriate deoxybenzoins, followed by Friedel-Crafts cyclic alkylation-dehydration. Comparison of the X-ray crystal structure of one analogue with unsubstituted analogues confirms the torsional perturbation effected by the ortho substituent. The torsional disposition of the C-2 aryl group in the substituted diphenylindenes is further investigated by UV (absorbance maxima and molar absorptivities), fluorescence (Stokes' shift), and NMR (chemical shifts). These spectroscopic measurements indicate increasing twisting between the C-2 aryl substituent and the indene system according to the following order: 3-ring *o*-Me-indene **9f** < diphenylindene **15** = 20° < 3-ring *o*-F-indene **9c** < 1-Me-indene **16** < 2-ring *o*-F-indene **9b** < 2-ring *o*-Me-indene **9e** = 63°. The binding affinity of these analogues to the estrogen receptor was evaluated by a competitive radiometric receptor binding assay. While *o*-fluoro or *o*-methyl substitution on the 3-ring increases binding only slightly, binding of the *o*-fluoro 2-ring analogue is increased ca. 6-fold and the *o*-methyl analogue 11-fold, giving, in the latter case, a compound with an affinity equivalent to that of estradiol. The increase in binding affinity afforded by ortho substitution correlates with the increase in the torsion angle of the C-2 aryl ring. A thermodynamic evaluation of the receptor fit (Andrews, P. R.; Craik, D. J.; Martin, J. L. *J. Med. Chem.* **1984**, *27*, 1648) indicates that, for the *o*-methyl 2-ring analogue, the effect of the ortho substitution on increasing receptor binding appears to be a combination of increased surface area due to the substituent itself, together with a change in surface area of the ligand that results from the increased torsion of the two aryl rings. An *o*-fluoro substituent on the 2-ring provides a compromise between the relative planarity required for high fluorescence intensity and the molecular shape needed for increased estrogen receptor binding affinity. *o*-Methyl, *o*-fluoro, and *p*-methyl substitution of the 3-ring have no value in the development of a fluorescent, higher affinity 2,3-diarylindene.

Introduction

A specific fluorophore for the quantitation of the estrogen receptor (ER) in individual cells may provide the clinician with information of prognostic value in selecting the most effective strategy for the treatment of breast cancer.[1] Unfortunately, the fluorescent ligands for the ER heretofore described do not meet the requisite biological (high receptor binding affinity) or spectroscopic (long emission wavelength and high fluorescence quantum yield) criteria.[2] The 2,3-diphenylindene **1** has a modest ER binding affinity (9% of estradiol), and its emission maximum (ca. 420 nm) lies in the range of cellular autofluorescence.[3] Substitution of the 2-aryl ring in the para position with a nitro group bathochromically shifted the emission into a useful wavelength range, but the receptor binding affinity (RBA) was greatly reduced.[4] Thus, structural alterations were sought to enhance binding affinity in the 2,3-diarylindene series.

The high RBA of the corresponding indenone **2** (59% that of estradiol) and the very low RBA of indeno[1]-phenanthrene **3** (0.01% of estradiol) lead to the hypothesis that RBA increases with increasing torsion between the two pendant aryl rings and the central double bond.[3] However, fluorescent estrogens based on the indenone system are not practical, because the carbonyl group decreases fluorescence by promoting intersystem crossing.[5] Ortho substitution of the 2-ring (as in **4** and **5**) also increased the RBA by a torsional effect, but the integrity of the *trans*-stilbene fluorophore was compromised by the severe twisting of the π-system.[4] Thus, a principal problem in the design of an integrated fluorescent estrogen[6] is that a compromise must be reached between the relative molecular planarity required for high fluorescence quantum yield and the precise three-dimensional topology necessary for high estrogen receptor binding affinity.[7]

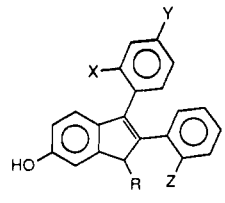


In this paper, the synthesis and estrogen receptor binding affinities of five new torsionally modified and one hydrophobically modified 2,3-diarylindenes (**6a-f**) are described. The effect of ortho substitution of the 2-ring and 3-ring on the molecular geometry, absorption, and fluorescence spectra, and RBA are compared. An X-ray crystal structure was obtained on the methyl ether of **6e**, the analogue with the highest ER binding affinity in this series. Ultraviolet and fluorescence spectroscopy is used to infer the geometry of the other analogues. The structure-binding affinity relationships are analyzed by molecular graphics and thermodynamic approaches.

* Address correspondence to: John A. Katzenellenbogen, 461 Roger Adams Laboratory, Box 37, 1209 W. California St., University of Illinois, Urbana, IL 61801.

(1) Fevig, T. L.; Zablocki, J. A.; Lloyd, J. E.; Katzenellenbogen, J. A. *J. Med. Chem.* **1987**, *30*, 156 and references cited therein.
(2) Carlson, K. E.; Coppey, M.; Magdelenat, H.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1989**, *32*, 345.
(3) Anstead, G. M.; Altenbach, R. J.; Wilson, S. R.; Katzenellenbogen, J. A. *J. Med. Chem.* **1988**, *31*, 1316.
(4) Anstead, G. M.; Katzenellenbogen, J. A. *J. Med. Chem.* **1988**, *31*, 1754.
(5) Turro, N. J. *Modern Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, CA, 1978.
(6) (a) Anstead, G. M.; Katzenellenbogen, J. A. *J. Phys. Chem.* **1990**, *94*, 1328. (b) Katzenellenbogen, J. A. *Cancer Treat. Rep.* **1978**, *62*, 1243. (c) Lee, Y. J.; Notides, A. C.; Tsay, Y.-G.; Kende, A. S. *Biochemistry* **1977**, *16*, 2896. (d) Katzenellenbogen, J. A.; Carlson, K. E.; Bindal, R. D.; Neeley, R. L.; Martin, P. M.; Magdelenat, H. P. *Anal. Biochem.* **1986**, *159*, 336.
(7) (a) Bindal, R. D.; Katzenellenbogen, J. A. *Photochem. Photobiol.* **1986**, *43*, 121. (b) Bindal, R. D.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1985**, *23*, 929.

0022-2623/90/1833-2726$02.50/0



6a–f

	X	Y	Z
a	F	OH	H
b	H	CH_3	H
c	F	H	H
d	H	H	F
e	H	H	CH_3
f	CH_3	H	H

Results and Discussion

Rationale. Substitution at one of four principal sites on the 2,3-diarylindenes may increase the torsion of the pendant rings with respect to the double bond (Figure 1, sites a–d). Due to the correlated nature of the ring rotation,[3] both rings may be affected. C-4 substitution of the indene (site a) may torsionally perturb the 3-ring in a manner similar to the bay-region steric effect in aromatic polycycles.[8] However, substitution of ER ligands at positions analogous to C-1 of estradiol (E_2) are not well-tolerated in terms of RBA.[9] Substitution at C-1 (site b) of the 2,3-diarylindene (roughly corresponding to C-6 of E_2) increases the torsion of the ring, but may interfere with the local receptor-essential volume.[4] Substitution at the ortho position of the 2-ring (site c) is known to produce a higher RBA;[4] ortho substitution of the 3-ring (side d) was unexplored.

In ortho-substituted stilbenes, the torsional angle is determined by both the steric and electronic nature of the group.[10] In our selection of ortho substituents, it was necessary to consider the possible photophysical and biological consequences of substitution. The low RBA generally produced by polar groups attached to ER ligands precluded their use.[11] Heavy halogens (Cl, Br, I) were undesirable because of the heavy atom quenching effect on fluorescence.[5] Thus, only a limited set of ortho-substituted analogues (F or CH_3 on each ring) fulfilled the selection criteria. The steric (E_s) parameters for F and CH_3 are −0.46 and −1.24, respectively, with E_s of H equal to 0.00.[12]

An alternate strategy to enhance the RBA was to incorporate a methyl group at the para position of the 3-ring (**6b**). Previously, placement of a hydroxyl group at this position produced a modest decrease in the RBA, presumably by an electronic effect.[13] Thus, it was hypothesized that a nonpolar substituent at the para position may increase RBA.

Synthesis. The 2,3-diarylindenes were prepared by

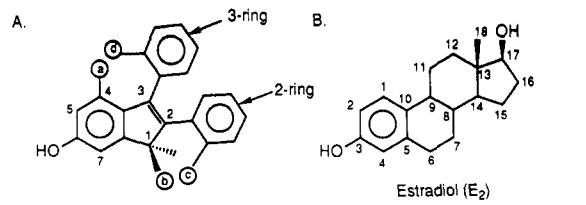
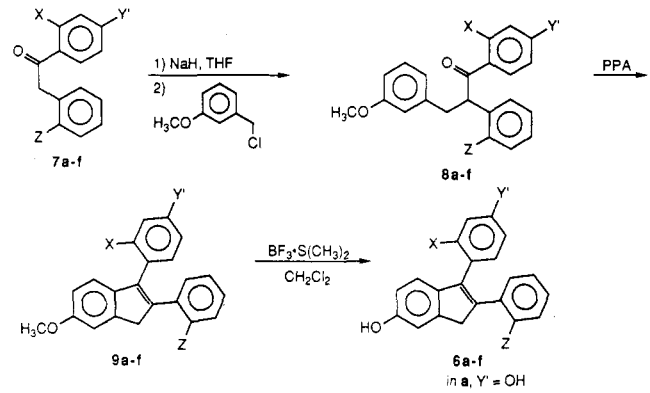


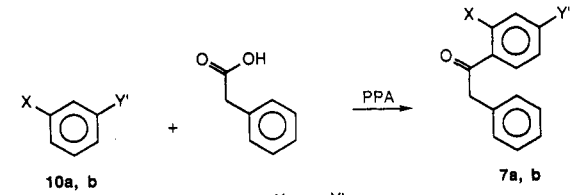
Figure 1. (A) The four positions of the 2,3-diarylindene system at which substitution may increase the torsion of the pendant rings. (B) Numbering system of estradiol.

Scheme I



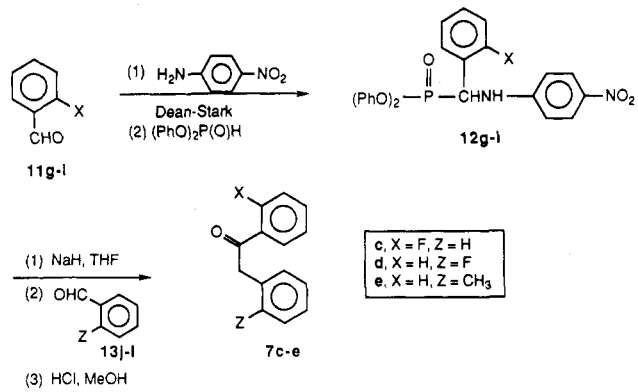
	X	Y'	Z		X	Y'	Z
(a)	F	OCH_3	H	(d)	H	H	F
(b)	H	CH_3	H	(e)	H	H	CH_3
(c)	F	H	H	(f)	CH_3	H	H

Scheme II



	X	Y'
(a)	F	OCH_3
(b)	H	CH_3

Scheme III



g, X = F	j, Z = H
h, X = H	k, Z = F
l, X = CH_3	l, Z = CH_3

12g + 13j ⟶ 7c

12h + 13k ⟶ 7d

12h + 13l ⟶ 7e

12l + 13j ⟶ no desired product

(8) Briant, C. E.; Jones, D. W.; Shaw, J. D. *J. Mol. Struct.* **1985**, *130*, 167.

(9) Anstead, G. M.; Wilson, S. R.; Katzenellenbogen, J. A. *J. Med. Chem.* **1989**, *32*, 2163 and references therein.

(10) Charton, M. *J. Org. Chem.* **1983**, *48*, 1011.

(11) (a) von Angerer, E.; Prekajac, J.; Strohmeier, J. *J. Med. Chem.* **1984**, *27*, 1439. (b) Zeelen, F. S.; Bergink, E. W. In *Cytotoxic Estrogens in Hormone Receptive Tumors*; Raus, J., Martens, H., LeClercq, G., Eds.; Academic Press: London, 1980; pp 39–48. (c) Fevig, T. L.; Mao, M. K.; Katzenellenbogen, J. A. *Steroids* **1988**, *51*, 471–497. (d) Katzenellenbogen, J. A. *Fed. Proc.* **1978**, *37*, 174.

(12) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.

(13) Anstead, G. M.; Peterson, C. S.; Katzenellenbogen, J. A. *J. Steroid Biochem.* **1989**, *33*, 877.

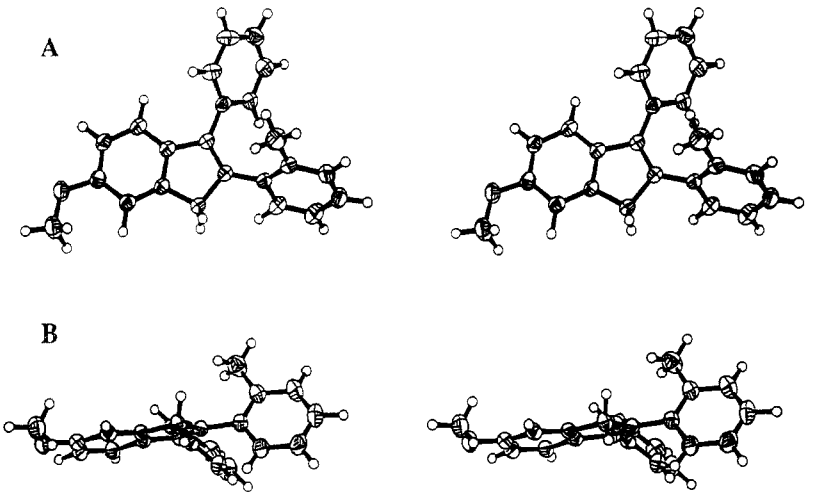
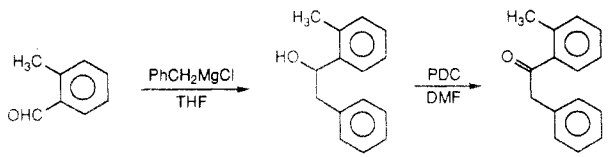


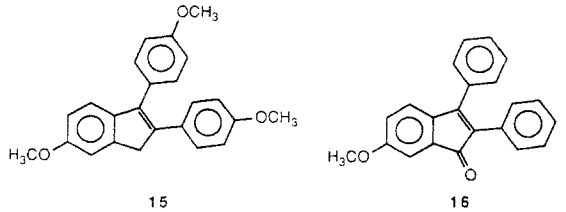
Figure 2. Stereoscopic thermal ellipsoid representations (35% probability): (A) compound **9e** plotted parallel to the best plane normal; (B) compound **9e** plotted perpendicular to the best plane normal.

Scheme IV



benzylation of a substituted deoxybenzoin, followed by Friedel–Crafts cyclic alkylation–dehydration, and methyl ether cleavage (Scheme I). The required deoxybenzoins were prepared in one of three ways. For deoxybenzoins, such as **7a** and **7b**, that are para-substituted with an activating substituent, Friedel–Crafts acylation of the activated arene sufficed (Scheme II). Phosphonate carbanion methodology[14] was fruitful for the synthesis of ortho-substituted deoxybenzoins **7c–e** (Scheme III). However, the condensation of *o*-methylphosphonate **12i** with benzaldehyde was not successful. Perhaps the phosphonate carbanion has certain steric limitations in the condensation with the aldehyde. A successful alternate route to **7f** was the addition of benzylmagnesium chloride to 2-tolualdehyde, providing alcohol **14**. Oxidation of **14** with pyridinium dichromate (PDC)[15] afforded the desired deoxybenzoin **7f** (Scheme IV).

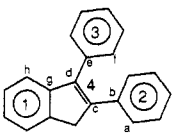
Molecular Structure. Single-crystal X-ray crystallography was performed on the methyl ether (**9e**) of the 2-ring *o*-methylindene **6e**, the compound with the highest RBA in this series (vide infra). Two perspectives on this molecule are shown in Figure 2. The torsion angle of the C-2 pendant ring exceeded that of the non-ortho-substituted dianisylindene **15** as well as that of diphenylindenone **16** (Table I), two compounds whose X-ray structures we have reported previously.[3,9] Due to the fusion of one aryl group to the five-membered ring, none of these compounds have the characteristic propeller conformation of typical triarylethylenes.[3,16]



15 16

(14) Zimmer, H.; Nene, D. M. *Chimia* **1977**, *31*, 330.
(15) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, *5*, 399.

Table I. Dihedral Angles (deg) between Selected Planes and Torsional Angles (deg) in Diarylindenes and Indenones[a]



dihedral angle	(torsional angle)	*o*-methylindene **9e**	diarylindene **15**[b]	diphenyl-indenone **16**[b]
1–4 [θ_1]	c–d–g–h	4 (−176)	1 (−179)	5 (+175)
2–4 [θ_2]	d–c–b–a	63 (−117)	20 (−160)	36 (+144)
3–4 [θ_3]	c–d–e–f	48 (+48)	51 (+51)	55 (+125)
sum of the dihedral angles[c]		115	72	96

[a] Dihedral angles are an index of planarity and thereby influence the extent of conjugation (ref 17). Torsional angles determine the ligand shape. [b] References 3 and 9. [c] Sum of the three prominent dihedral angles.

Table II. Absorption and Emission Maxima of Indene Methyl Ethers **9c–f** and Related Compounds[a]

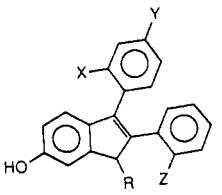
compound	λ_{abs}, nm	ϵ	λ_{em}, nm	Stokes' shift, cm^{-1}
diphenylindene **17**	317	19 000	418	7620
1-methylindene **18**[b]	312	17 200	416	8010
3-ring *o*-fluoroindene **9c**	314	17 700	421	8090
2-ring *o*-fluoroindene **9d**	306	15 400	422	8980
3-ring *o*-methylindene **9f**	319	23 000	410	6960
2-ring *o*-methylindene **9e**	290	11 600	423	10800
4-methoxy-*trans*-stilbene[c]	304	27 700	357	4890
4-methoxy-*cis*-stilbene[d,e]	280	10 600	*f*	*g*

[a] Spectra were recorded in EtOAc, unless otherwise indicated. [b] Reference 4. [c] Reference 22. [d] Reference 23. [e] Spectrum obtained in 95% ethanol. [f] No fluorescence observed in fluid solutions at 25 °C (ref 24). [g] Not applicable.

In the crystal structure, indene **9e** had no intermolecular contacts less than 2.5 Å, so the observed conformation is probably near local energy minimum.[18] Furthermore, this conformation may represent a global energy minimum because there is only one unique molecule per asymmetric unit[19] and the intermolecular forces are minimal.[20]

(16) (a) Gilbert, J.; Miquel, J.-F.; Prêcigoux, G.; Hospital, M.; Raynaud, J.-P.; Michel, F.; Crastes de Paulet, A. *J. Med. Chem.* **1983**, *26*, 693. (b) Biali, S. E.; Kaftory, M.; Rappoport, Z. *J. Org. Chem.* **1989**, *54*, 4959.
(17) Dewar, M. J. S. *J. Am. Chem. Soc.* **1952**, *74*, 3345.
(18) McKinney, J. D.; Singh, P. *Acta Crystallogr.* **1988**, *C4*, 558.

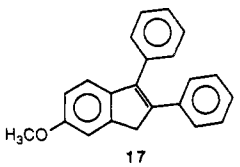
Table III. Estrogen Receptor Binding Affinities[a]



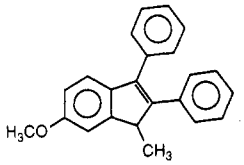
compd no.	R	X	Y	Z	RBA,[b] %
6a	H	F	OH	H	9.6
6b	H	H	CH_3	H	1.7
6c	H	F	H	H	12
6d	H	H	H	F	49
6e	H	H	H	CH_3	100
6f	H	CH_3	H	H	11
1[c]	H	H	H	H	8.9
18[d]	CH_3	H	H	H	12
19[d]	H	H	OH	CH_3	23
20[e]	H	H	OH	H	4.7

[a] Determined by competitive radiometric binding assay using rat uterine cytosol as a receptor source, [^{3}H]estradiol as tracer, and dextran-coated charcoal as adsorbant for free ligand (for details, see ref 26). [b] Binding affinities are expressed relative to estradiol = 100% (RBA = relative binding affinity) and are the average of duplicate determinations. The results are generally reproducible within ±30%. [c] Reference 3. [d] Reference 4. [e] Reference 13.

Absorption and Fluorescence Spectra. In order to determine the perturbation of the *trans*-stilbene fluorochrome by ortho substituents on the pendant rings, the absorption and emission spectra of **9c–f** were examined (Table II). Because these spectra are functions of the geometry of the π-network, it was intended to use this data in a comparative manner to assign the relative planarity of the *trans*-stilbene unit in the 2,3-diarylindenes. Data for 2,3-diphenylindene **17**, 1-methyl-2,3-diphenylindene **18**, and 4-methoxy-*cis*- and -*trans*-stilbene are included for comparison. Hypochromism, a hypsochromic shift in absorption maximum, and an increased Stokes' shift ($energy_{absorbance} - energy_{emission}$) are all indicative of steric hindrance to planarity in stilbenes.[21]



17



18

The magnitudes of the extinction coefficients, Stokes' shifts, and absorption maxima suggest the following order of decreasing planarity for θ_2: 3-ring *o*-Me-indene **9f** < diphenylindene **17** = 20° < 3-ring *o*-F-indene **9c** < 1-Me-indene **18** < 2-ring *o*-F-indene **9d** < 2-ring *o*-Me-indene **9e** = 63°. Thus, for a single methyl substituent, it appears that ortho substitution of the 3-ring increases the planarity of θ_2, whereas progressively greater distortions from planarity for θ_2 are achieved by substitution at C-1 and ortho substitution of the 2-ring. These simple experimental methods provide an index of the integrity of the *trans*-stilbene fluorochrome and a rank order of the planarity along the long axis of the various 2,3-diarylindenes. Unfortunately, for conjugated molecules, theoretical methods of structure assignment are of uncertain accuracy.[25]

Table IV. Binding Energies and AVERAGE Values (kcal/mol)

compound	$-\Delta G_{obsd}$[a]	ΔG_{av}[b]	difference
3-ring *o*-F-*p*-OH-indene **6a**	10.5	4.3	6.3
3-ring *p*-Me-indene **6b**	9.6	2.0	7.6
3-ring *o*-F-indene **6c**	10.7	2.5	8.2
2-ring *o*-F-indene **6d**	11.4	2.5	8.9
2-ring *o*-Me-indene **6e**	11.8	2.0	9.8
3-ring *o*-Me-indene **6f**	10.6	2.0	8.6
diphenylindene **1**	10.5	1.2	9.3
1-methylindene **16**	10.7	2.0	8.7
2-ring *o*-Me-3-ring-*p*-OH-indene **19**	11.2	3.8	7.4
3-ring *p*-OH-indene **20**	10.2	3.0	7.2
estradiol (E_2)	11.8	3.4	8.4

[a] Calculated from eqs 2 and 3. For the fit procedure, Andrews et al. (ref 27) converted ΔG_{obsd} to a positive value. [b] Calculated from eq 4.

Nuclear Magnetic Resonance Spectra. For the 2-ring *o*-methylindene **9e**, the severe torsion of θ_2 causes the shielding cone of the 2-ring to twist toward the C-1 methylene protons, which thereby experience an upfield shift in the proton nuclear magnetic resonance spectrum (δ 3.77). The other compounds in the series (**9c**, **9d**, **9f**, and **17**), with apparently lesser θ_2 torsion, do not differ much in the field position of the C-1 protons (δ 3.94, 3.92, 3.94, and 3.89, respectively).

Structure–Receptor Binding Affinity Relationships. The ER binding affinities (RBA) were determined in a competitive protein binding assay (Table III). Analogous compounds are included for comparison.

Hydroxylation of the para position of the 3-ring reduces RBA by 1.5–4-fold, regardless of substitution elsewhere (**6a** vs **6c**, **20** vs **1**, **19** vs **6e**). *o*-Fluoro- or -methyl substitution of the 3-ring (as in **6a** vs **20**, **6c** vs **1**, and **6f** vs **1**) produces only a small RBA increase. However, an ortho fluorine on the 2-ring causes a 5.5-fold increase in the binding affinity (**6d** compared to **1**). *o*-Methyl substitution on the 2-ring, as in **19** (vs **20**) and **6e** (vs **1**), increases the RBA 5- and 11-fold, respectively, with the affinity of the latter compound being comparable to E_2. Substitution at the para position of the 3-ring with either a polar (OH) or nonpolar (CH_3) group decreases the RBA.

Thermodynamic Evaluation of Receptor Fit. The complementarity of fit between a ligand and its receptor can be evaluated by a thermodynamic approach.[27] In this procedure, the observed free energy of binding of the compound (ΔG_{obsd}), calculated from the RBA, is compared to the sum of empirically derived binding energies (AVERAGE values, ΔG_{av}) for all the functional groups in

(19) Duax, W. L.; Griffith, J. F.; Weeks, C. M.; Korach, K. J. *Environ. Health Perspect.* **1985**, *61*, 111.
(20) DesJarlais, R. L.; Sheridan, R. P.; Seibel, G. L.; Dixon, J. S.; Kuntz, I. D.; Venkataraghaven, R. *J. Med. Chem.* **1988**, *31*, 722.
(21) (a) Fischer, G.; Seger, G.; Muszkat, K. A.; Fischer, E. *J. Chem. Soc., Perkin Trans. 2* **1975**, *1569* and references therein. (b) Suzuki, H. *Electronic Absorption Spectra and Geometry of Organic Molecules*; Academic Press: New York, 1967.
(22) Anstead, G. M.; Katzenellenbogen, J. A. *J. Phys. Chem.* **1988**, *92*, 6249.
(23) Shari, T.; Gazit, A.; Livshitz, T.; Biran, S. *J. Med. Chem.* **1985**, *28*, 1504.
(24) Gegiou, D.; Muszkat, K. A.; Fischer, E. *J. Am. Chem. Soc.* **1968**, *90*, 12, 3970.
(25) The AM1 and MNDO methods overestimate torsional angles in conjugated moleules; see: (a) Fabian, W. M. F. *J. Comput. Chem.* **1988**, *9*, 369. (b) Cioslowski, J. Z. *Naturforsch.* **1984**, *39a*, 1250. Molecular mechanics methods tend to underestimate the torsional angles in triarylethylene systems: (c) Kaftory, M.; Nugiel, D. A.; Biali, S. E.; Rappoport, Z. *J. Am. Chem. Soc.* **1989**, *111*, 8181. (d) Duax, W. L.; Griffith, J. F. *J. Steroid Biochem.* **1987**, *27*, 271.
(26) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. *Biochemistry* **1973**, *12*, 4085.
(27) Andrews, P. R.; Craik, D. J.; Martin, J. L. *J. Med. Chem.* **1984**, *27*, 1648.

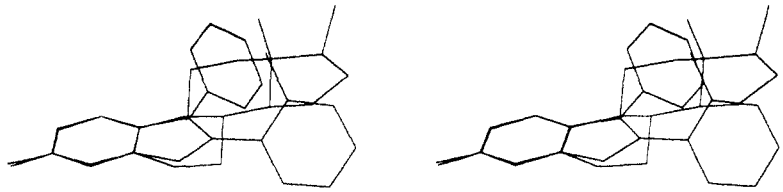


Figure 3. Relaxed stereoview of the superposition of estradiol hemihydrate[34] with the methyl ether (**9e**) of *o*-methylindene **6e**. The crystallographic coordinates were used in each case. The hydrogens of both molecules and the methyl ether carbon of **9e** were omitted for clarity. A rigid A-ring fit was adopted.

the molecule, with consideration of the loss of molecular degrees of freedom and overall translational/rotational entropy. If $\Delta G_{obsd} > \Delta G_{av}$, the compound matches its receptor well.

The observed free energy of binding is given by eq 1.

$$\Delta G_{obsd} = RT \ln K_d \quad (1)$$

The dissociation constant K_d can be obtained from the association constant K_a, which is calculated from the RBA value of the compound and a known K_a for E_2. The ratio of association constants (RAC) is given by

$$\text{RAC} = \frac{K_a^{\text{competitor}}}{K_a^{\text{estradiol}}} = \frac{(R)\text{RBA}}{R + 1 - \text{RBA}} \quad (2)$$

where R is the ratio of free to bound [^{3}H]estradiol at half-saturation.[28] The K_a of E_2 under the assay conditions is 3×10^9 M^{-1}.[29]

The AVERAGE values were calculated by eq 3,[27]

$$\Delta G_{av} = -14 - 0.7_{n_{DOF}} + 0.7_{n_{Csp^2}} + 0.8_{n_{Csp^3}} + 2.5_{n_{OH}} + 1.3_{n_{Hal}} \quad (3)$$

where −14 is a standard value for the loss of translational and rotational entropy and n is the number of the indicated functional groups or degrees of freedom (DOF) in the molecule. The values of ΔG_{obsd} and ΔG_{av} are summarized in Table IV.

The greater difference value of 2-ring *o*-methylindene **6e** as compared to 2,3-diarylindene **1** in the thermodynamic fit procedure indicates that *o*-methyl substitution of the 2-ring produces an increase in binding affinity above that expected from methyl substitution alone. Thus, the change in the torsion angles of the pendant rings results in improved fit of **6e** in the ER binding site. The thermodynamic fit procedure quantifies the oft-cited relationship between the steric profile ("thickness") of an ER ligand and its binding affinity.[3,4,7,9,30,31] None of the other diarylindenes showed an increased difference value compared to diphenylindene **1**. However, compounds **6d**, **6f**, and **16** also have difference values greater than that of E_2, suggesting that these compounds utilize their functionality and molecular bulk more effectively than E_2 in ER binding.

The relatively high difference values obtained for diphenylindene **1** and 2-ring *o*-methylindene **6e** support the assumption that these compounds bind to the ER in a low-energy conformation (i.e., similar to the X-ray crystallographic structure);[20] binding in a strained geometry would reduce ΔG_{obsd} and the difference value.[27,32]

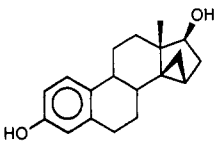
Contribution to Binding Free Energy by *o*-Methyl Group. The contribution of the 2-ring *o*-methyl group to the binding free energy (1.3 kcal/mol) can be obtained by subtracting ΔG_{obsd} of **1** (10.5 kcal/mol) from that of **6e** (11.8 kcal/mol). An *o*-methyl group may affect the binding in two ways: (1) by local interactions with the ER in the vicinity of the methyl group, and (2) by altering the torsion of the C-2 and C-3 aryl substituents, overall fit of the ligand within the receptor may be changed.

Under certain conditions, a methylene group (a methyl group minus the original hydrogen) may locally contribute 3.1 kcal/mol to the free energy of binding of a ligand and a protein.[33] In the case of 2-ring *o*-methylindene **6e**, the free energy contribution of the methylene group (1.3 kcal/mol) is well below this optimal value. On the other hand, the thermodynamic fit procedure[27] uses 0.8 kcal/mol for the typical contribution of a Csp^3 group to the binding free energy. Since in the case of **6e**, the free energy increase due to the methylene group falls between the typical and optimal values, it is impossible to be certain that the 2-ring *o*-methyl group is improving the overall fit of the 2,3-diarylindene in the ER binding site.

Receptor Orientation. In analogy to other 2,3-diarylindenes,[9] the indene nuclei of compounds **6a–f** are predicted to be the mimics of the AB rings of E_2. The suggested orientation of the high-affinity ortho analogue **6e** relative to E_2 in the ER binding site is shown in Figure 3. In this orientation, the *o*-methyl carbon lies 1.54 Å from the 13β-methyl carbon of E_2. The *o*-methyl group of **6e** is very well tolerated in terms of RBA, and thus may partially occupy a hydrophobic pocket. This is supported by the finding that estradiol analogue **21**, with a nonpolar substituent near the β-C-14 region, has a high RBA, despite its unnatural configuration at C-14.[35] However, the size of this putative hydrophobic pocket is limited, because the 13β-methyl group in estradiol does not contribute to the binding affinity,[36] and a C-15 β-methyl group decreases it.[11b]

Conclusions. Ortho substitution of the 2-ring by a methyl group or fluorine disrupts the *trans*-stilbene fluorochrome of the 2,3-diarylindenes and increases the RBA 5.5- and 11-fold, respectively, compared to the parent ligand. Ortho substitution of the 3-ring by fluorine causes

(28) Korenman, S. G. *Endocrinology* **1970**, *87*, 1119.
(29) Katzellenbogen, J. A.; Heiman, D. F.; Carlson, K. E.; Lloyd, J. E. In Receptor Binding Radiotracers; Eckelman, W. C., Volume Ed.; CRC Press: Boca Raton, FL, 1982; Vol. 1, Chapter 6, pp 93–126.
(30) McCague, R.; Kuroda, R.; LeClercq, G.; Stoessel, S. *J. Med. Chem.* **1986**, *29*, 2053.
(31) A correlation has also been made between molecular thickness and estrogenicity; see: (a) Oki, M.; Urushibara, Y. *Bull. Chem. Soc. Jpn.* **1952**, *25*, 109. (b) Weeks, C. M.; Cooper, A.; Norton, D. A. *Acta Crystallogr.* **1970**, *B26*, 429.
(32) (a) Crosby, G. A.; Dubois, G. E.; Wingard, R. E., Jr. *Drug Des.* **1979**, *8*, 215. (b) Duax, W. L.; Griffin, J. F.; Weeks, C. M. In *Interaction of Steroid Hormone Receptors with DNA*; Sluyser, M., Volume Ed.; VCH Publishers: New York, 1985; Chapter 3, pp 83–106.
(33) Fersht, A. R.; Shindler, J. S.; Tsui, W.-C. *Biochemistry* **1980**, *19*, 5520.
(34) Busetta, B.; Hospital, M. *Acta Crystallogr.* **1972**, *B28*, 560.
(35) Bohl, M.; Schubert, G.; Koch, M.; Reck, G.; Strecke, J.; Wunderwald, M.; Prousa, R.; Ponsold, K. *J. Steroid Biochem.* **1987**, *26*, 589.
(36) Hähnel, R.; Twaddle, E.; Ratajczak, T. *J. Steroid Biochem.* **1973**, *4*, 21.



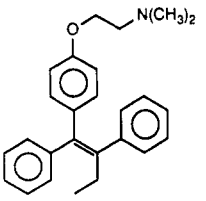
21

little perturbation of the *trans*-stilbene unit, and an *o*-methyl group on this ring may actually increase the planarity of the *trans*-stilbene. These 3-ring substituents produce little change in the RBA. A methyl group at the para position of the 3-ring decreases the RBA 5-fold.

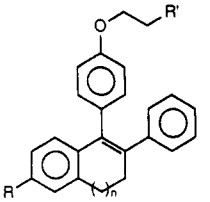
A thermodynamic fit procedure indicates that the RBA enhancement produced by the 2-ring *o*-methyl group is due to improved fit within the ER binding site. The 2-ring ortho substituents change ligand shape and may alter accessible surface area[37] or rotational barriers such that rotamers with poor RBA are less abundant.[38] Our results indicate that in 2,3-diarylindenes, as in other ligand systems, disrupting the extended, planar π-network increases the RBA.[3,7,30,39] This suggests that dispersive (stacking) forces between the ER and the ligand are not important in the binding interactions,[40] except perhaps in the region of the A ring[9b,41] or its mimics. Fluorine is known to shorten neighboring bonds,[42] and this may influence the molecular geometry and RBA of **6a**, **6c**, and **6d**.

There is great interest in the development of analogues of the antiestrogen tamoxifen (**22**)[43] that do not isomerize to the estrogenic *E* form.[44] Elimination of isomerization has been achieved by bridging of the aromatic rings or the double bond, as in **23**–**26**, or by ortho-methylation, as in **27**.

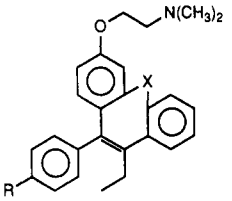
Compounds **23** and **26** are less active than tamoxifen,[45,47] and **24** and **26** have undesirable side effects.[47,49] Compounds **25** and **27** appear more promising and are under investigation.[30,48] The differences in bioactivity and ER binding affinity between the various bridged analogues has been ascribed to the change in the torsional angles of the appended rings with respect to the double bond.[30] However, bridging also alters the bond angles at the double bond, thereby changing the relative ring positions.[3] Thus, it is difficult to determine the relative torsional and positional contributions to the RBA/bioactivity.



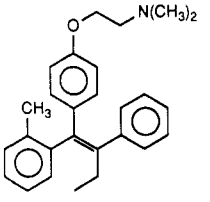
22[43]



23[45] n = 0; R = OCH_3; R' = c-NC_4H_8
24[46] n = 1; R = OCH_3; R' = c-NC_4H_8
25[32c] n = 2; R = H; R' = $N(CH_3)_2$



26[47] R = H, OH
X = O, S, CH_2CH_2, SCH_2



27[48]

In our study, we have used ortho substituents in the 2,3-diarylindene system to alter the ring torsion without affecting the relative ring positions. The 2-ring *o*-methyl indene **6e** is the first high-affinity triarylethylene which does not have the typical propeller conformation.[16] Such 2-ring ortho-substituted indenes may have an advantage over compounds such as **25** and **27** in that fewer hydrocarbon units (CH_2, CH_3) are needed to construct the high-affinity framework. Thus, there may be less nonspecific binding.[29]

In the design of a fluorescent estrogen based on the 2,3-diarylindene system, a 2-ring *o*-fluoro group provides a marked enhancement of the RBA without total disruption of the *trans*-stilbene fluorochrome, as occurs in the *o*-methyl analogue. Thus, 2-ring *o*-fluoro substitution of the 2,3-diarylindenes may offer a suitable compromise between the planarity typically needed for a high fluorescent quantum yield and the precise three-dimensional topology required for high estrogen receptor binding affinity. Fluorine is also electronically compatible with the development of charge transfer in the excited state of a donor/acceptor fluorescent estrogen.[4,9,22]

Experimental Section

General. Melting points (uncorrected) were determined on a Thomas-Hoover apparatus. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254 glass-backed plates. Flash chromatography[50] was done using Woelm 32-63-mm silica gel.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Varian XL-200 (200 MHz) or a General Electric QE-300 (300 MHz) spectrometer; chemical shifts are reported downfield from a tetramethylsilane internal standard (δ scale). Ultraviolet (UV) spectra were determined with a Hewlett-Packard 8451A spectrophotometer. Low-resolution mass spectra (MS) were done in the electron-impact mode on the Varian CH-5 spectrometer. The reported data are for an electron energy of 70 eV (unless otherwise noted) and follows the form of m/z (intensity relative to base peak = 100). High-resolution mass spectra (HRMS) were obtained in the electron-impact mode on a Varian MAT-371 spectrometer. The corrected fluorescence emission spectra were acquired on a Spex Fluorolog 2 (Model IIIC) instrument. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois.

Molecular superpositions were performed with the SYBYL Molecular Modeling System (Version 3.4; Tripos Associates, St. Louis, MO). Crystallographic coordinates were inputted directly

(37) (a) Richards, F. M. *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 151. (b) Pearlman, R. S. In *Physical Chemical Properties of Drugs*; Yalkowsky, S. H., Sinkula, A. A., Valvani, S. C., Eds.; Marcel Dekker: New York, 1980; pp 321–347.
(38) McKinney, J. D.; Gottschalk, K. E.; Pedersen, L. *J. Mol. Struct.* **1983**, *104*, 445.
(39) Equilenin has a much lower RBA than estradiol; see ref 36.
(40) (a) Singh, P.; Pedersen, L. G.; McKinney, J. D. *Acta Crystallogr.* **1986**, *C42*, 1172. (b) McKinney, J. D.; Darden, T.; Lyerly, M. A.; Pedersen, L. G. *Quant. Struct.-Act. Relat.* **1985**, *4*, 166.
(41) Duax, W. L.; Griffin, J. F.; Rohrer, D. C.; Swenson, D. C.; Weeks, C. M. *J. Steroid Biochem.* **1981**, *15*, 41.
(42) Singh, P.; Posner, H.; McKinney, J. *Acta Crystallogr.* **1987**, *C43*, 106.
(43) (a) Harper, M. J. K.; Walpole, A. L. *J. Reprod. Fertil.* **1967**, *13*, 101. (b) Furr, B. J. A.; Jordan, V. C. *Pharmacol. Ther.* **1984**, *25*, 127.
(44) Robertson, D. W.; Katzenellenbogen, J. A.; Long, D. J.; Rorke, E. A.; Katzenellenbogen, B. S. *J. Steroid Biochem.* **1982**, *16*, 1.
(45) Lednicer, D.; Babcock, J. C.; Marlatt, P. E.; Lyster, S. C.; Duncan, G. W. *J. Med. Chem.* **1965**, *8*, 52.
(46) Duncan, G. W.; Lyster, S. C.; Clark, J. J.; Lednicer, D. *Proc. Soc. Exp. Biol. Med.* **1963**, *112*, 439.
(47) Acton, D.; Hill, G.; Tait, B. S. *J. Med. Chem.* **1983**, *26*, 1131.
(48) Foster, A. B.; Jarman, M.; Leung, O.-T.; McCague, R.; Leclercq, G.; Devleeschouwer, N. *J. Med. Chem.* **1985**, *28*, 1491.
(49) (a) Clark, J. H.; McCormack, S. *Science* **1977**, *197*, 164. (b) Legha, S. S.; Slavik, M.; Carter, S. K. *Cancer* **1976**, *38*, 1535.
(50) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

or were obtained from the Cambridge Structural Database via SYBYL.

Unless otherwise noted, a standard sequence for product isolation was used: quenching by addition of water or an aqueous solution, extraction with an organic solvent, washing the extracts, drying ($MgSO_4$), and solvent evaporation under reduced pressure. The quenching media, extraction solvents, and aqueous washes are noted after "product isolation". Unless stated otherwise, ether refers to diethyl ether.

General Method for Preparation of 4-Substituted Aryl Benzyl Ketones 7a and 7b. Phenylacetic acid (16.5 mmol) and the arene (12.5 mmol; 2-fluoroanisole for **7a**, toluene for **7b**) were added to polyphosphoric acid (PPA, 10× the weight of the acid). The mixture was stirred at 40–70 °C and monitored by TLC. The reaction was complete in 0.5–5 h. Product isolation (ice water, EtOAc, saturated $NaHCO_3$) was followed by purification as described.

1-(2-Fluoro-4-methoxyphenyl)-2-phenylethanone (7a). This compound was recrystallized from ether at −30 °C. Three crops of white needles were obtained (4.62 g, 64%): mp 81–82.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (t, 1 H, J = 9 H, Ar*H* ortho to CO), 7.39–7.21 (m, 5 H, Ph*H*), 6.74 (dd, 1 H, J = 9, 2 Hz, Ar*H* ortho to F), 6.60 (dd, 1 H, J = 13, 2 Hz, Ar*H* ortho to OCH_3), 4.24 (d, 2 H, J = 3 Hz, CH_2 (split by F)), 3.84 (s, 3 H, OCH_3); MS (10 eV) m/z 244 (2, M^+), 153 (100). Anal. ($C_{15}H_{13}FO_2$) C, H, F.

1-(4-Methylphenyl)-2-phenylethanone (7b). This compound was recrystallized from methanol at −30 °C. An off-white solid was obtained (0.86 g, 24%): mp 106–108 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 2 H, J = 9 Hz, Ar*H* ortho to CO), 7.39–7.19 (m, 7 H, Ar*H* ortho to CH_3; Ph*H*), 4.25 (s, 2 H, CH_2), 2.40 (s, 3 H, $ArCH_3$); MS m/z 210 (3, M^+), 119 (100), 65 (15). Anal. ($C_{15}H_{14}O$) C, H.

General Procedure for the Preparation of Phosphonates 12g–i. 4-Nitroaniline (0.1 mol) and the appropriate aldehyde (0.1 mol; 2-fluorobenzaldehyde for **12g**, benzaldehyde for **12h**, and 2-tolualdehyde for **12i**) were dissolved in benzene and heated at reflux for 24 h. A Dean–Stark trap was used to remove water and wet benzene; fresh benzene was added. Diphenyl phosphite (0.1 mol) was added and the solution was heated at reflux for 12 h, with intermittent water/wet benzene removal and benzene replenishment. The solvent was evaporated and the residue was purified as described. The characterization of **12h** is published elsewhere.[6a]

Diphenyl [(4-Nitrophenyl)amino](2-fluorophenyl)-methanephosphonate (12g). The residue was triturated with 1:1 EtOAc–ether, affording a pale yellow solid (30.6 g, 82%): mp 136–137 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 2 H, J = 9 Hz, Ar*H* ortho to NO_2), 7.56 (t, 1 H, J = 8 Hz, Ar*H* ortho to F), 7.35–7.07 (m, 12 H, Ar*H*), 6.80 (d, 1 H, J = 8 Hz, Ar*H*), 6.62 (d, 2 H, J = 9 Hz, Ar*H* ortho to NH), 5.87 (t, 1 H, J = 9 Hz, N*H*), 5.68 (dd, 1 H, J = 25, 9 Hz, C*H*); MS (10 eV) m/z 478 (2, M^+), 245 (100), 234 (32). Anal. ($C_{25}H_{20}FN_2O_5P$) C, H, F, N, P.

Diphenyl [(4-Nitrophenyl)amino](2-methylphenyl)-methanephosphonate (12i). A pale yellow solid was obtained after trituration with 1:1 EtOAc–ether (41.8 g, 88%): mp 149–151 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 2 H, J = 9 Hz, Ar*H* ortho to NO_2), 7.64 (dd, 1 H, J = 8, 2, Hz, Ar*H* ortho to CH_3), 7.35–6.90 (m, 12 H, Ar*H*), 6.59 (d, 1 H, J = 8 Hz, Ar*H*), 6.44 (d, 2 H, J = 9 Hz, Ar*H* ortho to NH), 5.46 (d, 1 H, J = 24 Hz, C*H*), 2.56 (s, 3 H, CH_3); MS m/z 474 (2, M^+), 241 (100), 234 (25), 223 (12), 193 (35), 94 (73). Anal. ($C_{26}H_{23}N_2O_5P$) C, H, N, P.

Preparation of Deoxybenzoins 7c, 7d, and 7e from Phosphonates. Sodium hydride (36 mmol) was washed with hexane and suspended in THF (40 mL). The appropriate phosphonate (33 mmol) was dissolved in THF (35 mL) and added dropwise over 1 h. After 2.5 h, the requisite aldehyde (33 mmol), dissolved in THF (15 mL), was added over 30 min. After 20 h, the solvent was evaporated and the residue was treated with methanol (80 mL) and concentrated HCl (10 mL). The solution was heated at reflux for 5–8 h. Product isolation (ice water, EtOAc, brine) was followed by purification as described.

1-(2-Fluorophenyl)-2-phenylethanone (7c). This compound was prepared from phosphonate **12g** and benzaldehyde. Purification was achieved by flash chromatography (two runs: 4:1 hexane–EtOAc, 97:3 hexane–EtOAc). A clear oil (2.4 g, 33%) was obtained, which solidified on standing: mp 101–103 °C; ^{1}H NMR ($CDCl_3$) δ 8.17–8.02 (m, 3 H, Ar*H*), 7.68–7.46 (m, 4 H, Ar*H*), 7.31–7.15 (m, 2 H, Ar*H*), 4.31 (d, 2 H, J = 3 Hz, CH_2); MS m/z 214 (6, M^+), 123 (100). Anal. ($C_{14}H_{11}FO$) C, H, F.

1-Phenyl-2-(2-fluorophenyl)ethanone (7d). This compound was prepared from phosphonate **12h** and 2-fluorobenzaldehyde (**13k**). Flash chromatography (4:1 hexane–EtOAc) yielded white crystals (1.90 g, 34%): mp 75–76 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (dt, 2 H, J = 7, 2 Hz, Ar*H* ortho to CO), 7.59–7.44 (m, 3 H, Ar*H*), 7.29–7.04 (m, 4 H, Ar*H*), 4.33 (s, 2 H, CH_2); MS m/z 214 (2, M^+), 105 (100). Anal. ($C_{14}H_{11}FO$) C, H, F.

1-Phenyl-2-(2-methylphenyl)ethanone (7e). This compound was prepared from phosphonate **12h** and 2-tolualdehyde (**13l**). Flash chromatography (two runs: 4:1 hexane–EtOAc, 96:4 hexane–EtOAc) and recrystallization (pentane, −30 °C) provided white crystals (0.89 g, 36%): mp 69–70 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (dd, 2 H, J = 8, 2 Hz, Ar*H* ortho to CO), 7.59–7.44 (m, 3 H, Ar*H*), 7.23–7.11 (m, 4 H, Ar*H*), 4.31 (s, 2 H, CH_2), 2.26 (s, 3 H, CH_3); MS m/z 210 (7, M^+), 105 (100), 77 (6). Anal. ($C_{15}H_{14}O$) C, H.

1-(2-Methylphenyl)-2-phenylethanol (14). To a stirred solution of benzylmagnesium chloride (9.05 g, 60.0 mmol, 1.0 M solution in Et_2O) at 0 °C under N_2 was slowly added 2-tolualdehyde (7.93 g, 66.0 mmol) over 30 min. The reaction was complete after 3 h. Product isolation (water, EtOAc, brine), followed by crystallization from hexane, afforded alcohol **14** (7.48 g, 59%): mp 43–46 °C (lit.[51] mp 48.0–49.5 °C); ^{1}H NMR ($CDCl_3$) δ 7.56 (d, 1 H, J = 8 Hz, Ar*H* ortho to OH), 7.35–7.10 (m, 8 H, Ph*H*), 5.18–5.08 (m, 1 H, C*H*), 3.01 (dd, 1 H, J = 14, 4 Hz, CH_2), 2.91 (dd, 1 H, J = 14, 9 Hz, CH_2), 2.28 (s, 3 H, CH_3), 1.87 (d, 1 H, J = 2 Hz, O*H*); MS m/z 212 (3, M^+), 121 (100), 91 (14), 77 (6). Anal. ($C_{15}H_{16}O$) C, H.

1-(2-Methylphenyl)-2-phenylethanone (7f). A solution of benzyl alcohol **14** (1.80 g, 8.50 mmol) was dissolved in DMF (30 mL, distilled from $MgSO_4$) at 0 °C under N_2. Pyridinium dichromate (5.44 g, 14.45 mmol) was added to the solution in one portion. The reaction was complete after 3 h. Product isolation (water, EtOAc, brine), followed by vacuum distillation (125 °C, 0.1 Torr) gave ketone **7f**[52] (1.16 g, 65%) as a clear liquid; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 1 H, J = 8 Hz, Ar*H* ortho to CO), 7.20–7.08 (m, 8 H, Ar*H*), 4.21 (s, 2 H, CH_2), 2.44 (s, 3 H, CH_3); MS m/z 210 (1, M^+), 119 (100), 91 (50), 65 (19). Anal. ($C_{15}H_{14}O$) C, H.

3-Methoxybenzylation of Deoxybenzoins. This method was used for the synthesis of triaryl ketones **8a–f**. Sodium hydride (8.6 mmol, 50% dispersion in oil) was rinsed with hexane and suspended in THF (5 mL). The substituted deoxybenzoin (**7a–f**; 6.9 mmol), dissolved in THF (30 mL), was added dropwise over 1 h. After enolate formation was complete (ca. 2 h), the enolate solution was transferred by cannula into a solution of 3-methoxybenzyl chloride (14 mmol) in THF (5 mL). The solution was stirred at 25 °C until the reaction was complete (up to 48 h), as determined by TLC. The 3-methoxybenzylated deoxybenzoins produce a characteristic golden yellow color on TLC plates after treatment with saturated $CeSO_4$ in 65% H_2SO_4 (with heat). The deoxybenzoins typically produce brown spots under these conditions. Product isolation (5% HCl, EtOAc, brine) was followed by purification as described.

1-(2-Fluoro-4-methoxyphenyl)-2-phenyl-3-(3-methoxyphenyl)-1-propanone (8a). The compound was prepared from **7a**. Flash chromatography (9:1 pentane–THF) provided a clear oil (2.6 g, 86%): ^{1}H NMR ($CDCl_3$) δ 7.78 (t, 1 H, J = 9 Hz, Ar*H* meta to CH_2), 7.23–7.04 (m, 5 H, Ar*H*), 6.75–6.60 (m, 5 H, Ar*H*), 6.45 (dd, 1 H, J = 14, 2 Hz, Ar*H* para to F), 4.76 (t, 1 H, J = 7 Hz, C*H*CO), 3.71 (s, 3 H, OCH_3 para to CO), 3.67 (s, 3 H, OCH_3), 3.53 (dd, 1 H, J = 14, 7 Hz, CH_2), 3.00 (dd, 1 H, J = 14, 7 Hz, CH_2); MS m/z 364 (5, M^+), 205 (3), 153 (100), 122 (9), 110 (6); HRMS, calcd/found ($C_{23}H_{21}FO_3$) 364.1469/364.1477.

1-(4-Methylphenyl)-2-phenyl-3-(3-methoxyphenyl)-1-propanone (8b). This compound was prepared from **7b**. Flash chromatography (9:1 hexane–EtOAc) yielded a clear oil (1.1 g, 86%): ^{1}H NMR ($CDCl_3$) δ 7.82 (d, 2 H, J = 8 Hz, Ar*H* ortho to CO), 7.24–7.08 (m, 7 H, Ar*H*), 6.70–6.55 (m, 3 H, Ar*H* ortho and meta to OCH_3), 4.78 (t, 1 H, J = 7 Hz, C*H*), 3.68 (s, 3 H, OCH_3), 3.54 (dd, 1 H, J = 14, 7 Hz, CH_2), 3.03 (dd, 1 H, J = 14, 7 Hz,

(51) Loudon, G. M.; Noyce, D. S. *J. Am. Chem. Soc.* **1969**, *91*, 1433.
(52) (a) Craig, J. T.; McNatty, K. P. *Aust. J. Chem.* **1967**, *20*, 1921. (b) Teiti, T. *Aust. J. Chem.* **1980**, *33*, 605.

CH_2), 2.32 (s, 3 H, CH_3); MS m/z 330 (8, M^+), 211 (2), 210 (2), 165 (3), 119 (100), 91 (25), 43 (44); HRMS, calcd/found ($C_{23}H_{22}O_2$) 330.1614/330.1623.

1-(2-Fluorophenyl)-2-phenyl-3-(3-methoxyphenyl)-1-propanone (8c). This compound was prepared from **7c**. Flash chromatography (9:1 pentane–THF) afforded a clear oil (1.2 g, 76%): ^1H NMR ($CDCl_3$) δ 7.70 (td, 1 H, J = 8, 2 Hz, Ar*H* para to CO), 7.48–7.36 (m, 1 H, Ar*H*), 7.25–6.95 (m, 8 H, Ar*H*), 6.75–6.55 (m, 3 H, Ar*H* ortho and para to OCH_3), 4.78 (t, 1 H, J = 7 Hz, C*H*CO), 3.70 (s, 3 H, OCH_3), 3.54 (dd, 1 H, J = 14, 7 Hz, CH_2), 3.02 (dd, 1 H, J = 14, 7 Hz, CH_2); MS (10 eV) m/z 334 (53, M^+), 211 (72), 123 (100). Anal. ($C_{22}H_{19}FO_2$) C, H, F.

1-Phenyl-2-(2-fluorophenyl)-3-(3-methoxyphenyl)-1-propanone (8d). This compound was synthesized from **7d**. Flash chromatography (19:1 hexane–EtOAc) provided a colorless oil (0.79 g, 62%): ^1H NMR ($CDCl_3$) δ 7.93 (d, 2 H, J = 7 Hz, Ar*H* ortho to CO), 7.55–6.95 (m, 8 H, Ar*H*), 6.79–6.65 (m, 3 H, Ar*H*), 5.24 (t, 1 H, J = 7 Hz, C*H*CO), 3.70 (s, 3 H, OCH_3), 3.53 (dd, 1 H, J = 14, 7 Hz, CH_2), 3.04 (dd, 1 H, J = 14, 7 Hz, CH_2); MS m/z 334 (53, M^+), 229 (27), 121 (28), 105 (100). Anal. ($C_{22}H_{19}FO_2$) C, H, F.

1-Phenyl-2-(2-methylphenyl)-3-(3-methoxyphenyl)-1-propanone (8e). The target was prepared from **7e**. Flash chromatography (19:1 hexane–EtOAc) afforded a clear oil (1.0 g, 82%): ^1H NMR ($CDCl_3$) δ 7.77 (dd, 2 H, J = 8, 2 Hz, Ar*H* ortho to CO), 7.45–7.06 (m, 8 H, Ar*H*), 6.70 (m, 2 H, Ar*H* ortho and para to OCH_3), 6.55 (s, 1 H, Ar*H* ortho to OCH_3, CH_2), 4.93 (t, 1 H, J = 7 Hz, C*H*CO), 3.67 (s, 3 H, OCH_3), 3.51 (dd, 1 H, J = 14, 7 Hz, CH_2), 2.92 (dd, 1 H, J = 14, 7 Hz, CH_2); MS m/z 330 (48, M^+), 225 (30), 105 (100); HRMS, calcd/found ($C_{23}H_{22}O_2$) 330.1614/330.1619.

1-(2-Methylphenyl)-2-phenyl-3-(3-methoxyphenyl)-1-propanone (8f). This compound was prepared from **7f** with catalysis by NaI (0.3 equiv). Heating at 60 °C for 2 h was required for complete reaction. Flash chromatography (19:1 hexane–EtOAc) provided a pale yellow oil (0.41 g, 33%): ^1H NMR ($CDCl_3$) δ 7.34 (d, 1 H, J = 8 Hz, Ar*H* ortho to CO), 7.29–7.22 (m, 6 H, Ar*H*), 7.20–7.07 (m, 3 H, Ar*H*), 6.76–6.66 (m, 3 H, Ar*H* ortho and para to OCH_3), 4.65 (dd, J = 8, 7 Hz, C*H*), 3.71 (s, 3 H, OCH_3), 3.58 (dd, 1 H, J = 14, 8 Hz, CH_2), 3.03 (dd, 1 H, J = 14, 6 Hz, CH_2), 2.22 (s, 3 H, $ArCH_3$); MS m/z 330 (4, M^+), 119 (100), 91 (28). Anal. ($C_{23}H_{22}O_2$) C, H.

Procedure for Polyphosphoric Acid Cyclizations. This method was used for the synthesis of indenes **9a–f**, from triaryl ketones **8a–f**, respectively, in the manner previously described.[3,4,13] Purification is described below.

2-Phenyl-3-(2-fluoro-4-methoxyphenyl)-6-methoxyindene (9a). Recrystallization (MeOH, –30 °C) gave a pinkish powder (0.43 g, 45%): mp 100–104 °C; ^1H NMR ($CDCl_3$) δ 7.35–6.48 (m, 11 H, Ar*H*), 3.91 (s, 2 H, CH_2), 3.86 (br s, 6 H, 2 OCH_3); MS m/z 346 (100, M^+), 331 (16), 303 (7), 173 (10); HRMS, calcd/found ($C_{23}H_{19}FO_2$) 346.1369/346.1365.

2-Phenyl-3-(4-methylphenyl)-6-methoxyindene (9b). Recrystallization from MeOH at –30 °C afforded off-white crystals (0.72 g, 76%): mp 159–160.5 °C; ^1H NMR ($CDCl_3$) δ 7.23 (s, 5 H, Ph*H*), 7.20–7.10 (m, 6 H, Ar*H*), 6.82 (dd, 1 H, J = 8, 2 Hz, Ar*H* para to CH_2), 3.87 (s, 2 H, CH_2), 3.85 (s, 3 H, OCH_3), 2.41 (s, 3 H, CH_3); MS m/z 312 (100, M^+), 297 (21), 281 (12), 126 (12). Anal. ($C_{23}H_{20}O$) C, H.

2-Phenyl-3-(2-fluorophenyl)-6-methoxyindene (9c). Flash chromatography (two runs: 4:1 hexane–EtOAc, 9:1 hexane–EtOAc) provided a white solid (0.52 g, 55%): mp 109–110 °C; ^1H NMR ($CDCl_3$) δ 7.44–7.18 (m, 9 H, Ar*H*), 7.13 (d, 1 H, J = 2 Hz, Ar*H* ortho to CH_2), 7.01 (dd, 1 H, J = 8, 1 Hz, Ar*H* meta to CH_2), 6.83 (dd, 1 H, J = 9, 2 Hz, Ar*H* para to CH_2), 3.93 (s, 2 H, CH_2), 3.85 (s, 3 H, OCH_3); MS m/z 316 (100, M^+), 301 (6), 273 (3). Anal. ($C_{22}H_{17}FO$) C, H, F.

2-(2-Fluorophenyl)-3-phenyl-6-methoxyindene (9d). Trituration (ether–pentane) gave a pale yellow solid (0.32 g, 54%): mp 106–107.5 °C; ^1H NMR ($CDCl_3$) δ 7.33 (s, 5 H, Ph*H*), 7.31–6.92 (m, 5 H, Ar*H*), 6.88 (dd, 1 H, J = 8, 2 Hz, Ar*H* para to CH_2), 3.91 (s, 2 H, CH_2), 3.86 (s, 3 H, OCH_3); MS m/z 316 (83, M^+), 301 (20), 142 (16), 58 (100). Anal. ($C_{22}H_{17}FO$) C, H, F.

2-(2-Methylphenyl)-3-phenyl-6-methoxyindene (9e). Flash chromatography (4:1 hexane–EtOAc) yielded a white solid (0.34 g, 52%): mp 117–119 °C; ^1H NMR ($CDCl_3$) δ 7.38 (d, 1 H, J = 8 Hz, Ar*H* meta to OCH_3), 7.29–7.06 (m, 9 H, Ar*H*), 6.88 (dd, J = 8, 2 Hz, Ar*H* para to CH_2), 3.87 (s, 3 H, OCH_3), 3.77 (s, 2 H, CH_2), 1.97 (s, 3 H, CH_3); MS m/z 312 (100, M^+), 297 (5), 281 (4). Anal. ($C_{23}H_{20}O$) C, H.

2-Phenyl-3-(2-methylphenyl)-6-methoxyindene (9f). Flash chrmoatography (9:1 hexane–EtOAc), followed by crystallization (MeOH, –30 °C), afforded a white solid (0.162 g, 57%): mp 96–97 °C; ^1H NMR ($CDCl_3$) δ 7.32–7.10 (m, 10 H, Ar*H*), 6.82 (d, 1 H, J = 8 Hz, Ar*H* at C-4), 6.78 (dd, 1 H, J = 8, 2 Hz, Ar*H* at C-5), 3.94 (s, 2 H, CH_2), 3.85 (s, 3 H, OCH_3), 2.07 (s, 3 H, CH_3); MS m/z 312 (100, M^+), 297 (9), 281 (10), 203 (8). Anal. ($C_{23}H_{20}O$), C, H.

Preparation of Hydroxyindenes 6a–f. Demethylation of methoxyindenes **9a–f** was achieved with boron trifluoride–dimethyl sulfide,[53] as previously described,[4,54] providing hydroxyindenes **6a–f**, respectively.

2-Phenyl-3-(2-fluoro-4-hydroxyphenyl)-6-hydroxyindene (6a). This compound was purified by preparative TLC (two developments: 7:3 hexane–EtOAc), followed by recrystallization from diisopropyl ether/hexane at –30 °C. A tan solid was obtained (61 mg, 55%): mp 176 °C dec; ^1H NMR (acetone-d_6) δ 7.32 (d, 2 H, J = 7 Hz, Ph*H* ortho to CC=C), 7.25–6.81 (m, 8 H, Ar*H*), 6.68 (d, 1 H, J = 2 Hz, Ar*H* ortho to CH_2), 3.88 (s, 2 H, CH_2); MS m/z 318 (100, M^+), 301 (3), 241 (3). Anal. ($C_{21}H_{15}FO_2$) C, H, F.

2-Phenyl-3-(4-methylphenyl)-6-hydroxyindene (6b). Flash chromatography (3:1 hexane:EtOAc) and recrystallization from ether/pentane at –30 °C, provided a pale yellow powder (25 mg, 22%): mp 148.5–150 °C; ^1H NMR ($CDCl_3$) δ 7.25 (s, 5 H, Ph*H*), 7.20–7.03 (m, 6 H, Ar*H*), 6.74 (dd, 1 H, J = 8, 2 Hz, Ar*H* para to CH_2), 3.86 (s, 2 H, CH_2), 2.41 (s, 3 H, CH_3); MS m/z 298 (100, M^+), 283 (12), 265 (4), 252 (4). Anal. ($C_{22}H_{18}O$) C, H.

2-Phenyl-3-(2-fluorophenyl)-6-hydroxyindene (6c). This compound was purified by flash chromatography (4:1 hexane–EtOAc), followed by trituration (ether–pentane). A white solid was secured (68 mg, 59%): mp 140–141 °C; ^1H NMR ($CDCl_3$) δ 7.41–7.14 (m, 8 H, Ar*H*), 7.05 (d, 1 H, J = 2 Hz, Ar*H* ortho to CH_2), 6.96 (dd, 1 H, J = 9, 1 Hz, Ar*H* ortho to F), 6.74 (dd, 1 H, J = 8, 2 Hz, Ar*H* para to CH_2), 4.64 (s, 1 H, ArO*H*), 3.92 (s, 2 H, CH_2); MS m/z 302 (100, M^+), 207 (2). Anal. ($C_{21}H_{15}FO$) C, H, F.

2-(2-Fluorophenyl)-3-phenyl-6-hydroxyindene (6d). Flash chromatography (4:1 hexane–EtOAc) and recrystallization (hexane–2-propanol, –30 °C) afforded an off-white solid (66 mg, 64%): mp 132–133 °C; ^1H NMR ($CDCl_3$) δ 7.32 (s, 5 H, Ph*H*), 7.30–6.87 (m, 6 H, Ar*H*), 6.77 (dd, 1 H, J = 8, 2 Hz, Ar*H* para to CH_2), 4.70 (br s, 1 H, ArO*H*), 3.89 (s, 2 H, CH_2); MS m/z 302 (100, M^+), 224 (2). Anal. ($C_{21}H_{13}FO$) C, H, F.

2-(2-Methylphenyl)-3-phenyl-6-hydroxyindene (6e). Crystallization (pentane–ether) provided a white solid (124 mg, 43%): mp 159–160.5 °C; ^1H NMR ($CDCl_3$) δ 7.33 (d, 1 H, J = 8 Hz, Ar*H* meta to OH), 7.53 (s, 5 H, Ph*H*), 7.22–7.09 (m, 4 H, Ar*H*), 7.06 (d, 1 H, J = 2 Hz, Ar*H* ortho to CH_2), 6.81 (dd, 1 H, J = 8, 2 Hz, Ar*H* para to CH_2), 4.65 (s, 1 H, ArO*H*), 3.75 (s, 2 H, CH_2), 1.97 (s, 3 H, CH_3); MS m/z 298 (100, M^+), 283 (10), 207 (16). Anal. ($C_{22}H_{18}O$) C, H.

2-Phenyl-3-(2-methylphenyl)-6-hydroxyindene (6f). Purification was achieved by flash chromatography (3:2 hexane–EtOAc), followed by crystallization from hexane/Et_2O at –30 °C. Hydroxyindene **6f** was obtained as white crystals (49.6 mg, 52%): mp 75–77 °C; ^1H NMR ($CDCl_3$) δ 7.34–7.05 (m, 10 H, Ar*H*), 6.77 (d, 1 H, J = 8 Hz, Ar*H* at C-4), 6.71 (dd, 1 H, J = 8, 2 Hz, Ar*H* at C-5), 4.67 (s, 1 H, O*H*), 3.93 (s, 2 H, CH_2), 2.07 (s, 3 H, CH_3); MS m/z 298 (100, M^+), 281 (8), 207 (13). Anal. ($C_{22}H_{18}O$) C, H.

Biochemical Methods. Experimental details for the relative binding affinity determinations are found in ref 26. A synopsis is given in the footnotes of Table III.

X-ray Crystallography. Crystals of **9e** were obtained by rotary evaporation of a hexane–ethyl acetate solution. Diffraction data were measured at room temperature using an Enraf-Nonius diffractometer equipped with monochromated Mo radiation [λ(Kα) = 0.710 73 Å]. Final cell dimensions were obtained by a

(53) Brown, H. C.; Ravindran, N. *Inorg. Chem.* **1977**, *21*, 2938.
(54) Williard, P. G.; Fryhle, C. B. *Tetrahedron Lett.* **1980**, *21*, 3731.

Table V. Crystal Data for 9e

formula	$C_{23}H_{20}O$
crystal system	triclinic
space group	$P\bar{1}$
a, Å	10.034 (2)
b, Å	12.474 (2)
c, Å	7.604 (3)
α, deg	107.55 (2)
β, deg	106.00 (2)
γ, deg	78.75 (2)
V, Å^3	865.8 (8)
Z	2
density calcd, g/cm^3	1.198
crystallizing solvent	hexane–ethyl acetate
crystal habit	tabular (white)
crystal dimensions, mm	0.2 × 0.5 × 0.6
μ, cm^{-1}	0.67
transmission factor range	not applied
extinction	not applied
2θ limit, deg (octants)	50.5 ($\pm h \pm k + l$)
intensities (unique, R_i)	3436 (3033, 0.015)
intensities > $1.96\sigma(I)$	1809
R (observed intensities)	0.048
R_w [for $w = 1/\sigma^2(F_o) + pF_o^2$]	0.055 (p = 0.020)
max density in ΔF map, e/Å^3	0.16

least-squares fit to the automatically centered settings for 25 reflections ($2\theta > 20°$). Three reference reflections monitored during the experiment showed no significant variation. Intensity data were corrected for Lorentz–polarization effects. Crystal data are listed in Table V. Space group assignment was suggested by cell geometry and average values of the normalized structure factors; the choice was confirmed by successful refinement.

The structure was solved by direct methods (SHELX[55]); correct positions for all non-hydrogen atoms were deduced from E maps. Difference Fourier electron density maps revealed positions for all hydrogen atoms, and the final least squares refinement cycle (SHELX) included independent parameters for all positions, anisotropic thermal coefficients for all nonhydrogen atoms, and isotropic thermal parameters for hydrogen atoms. The final difference Fourier map had no significant features. Atomic scattering factors, mass attenuation coefficients, and anomalous dispersion corrections were taken from ref 56.

Acknowledgment. We are grateful for support of this research through a grant from the National Institutes of Health (Grant PHS 2R37 DK 15556). High-field NMR spectra and high-resolution mass spectra were obtained on instruments supported by grants from the National Institutes of Health (Grants RR 02299 and GM 27029, respectively). We are thankful to Kathryn E. Carlson for performing the estrogen receptor binding assays.

Supplementary Material Available: Atomic numbering scheme, tables of atomic coordinates, thermal parameters, bond lengths, and bond angles for compound **9e** (4 pages). Ordering information is given on any current masthead page.

(55) Sheldrick, G. M. SHELX-76, a program for crystal structure determination, University Chemical Laboratory, Cambridge, England, 1976.
(56) Ibers, J. A.; Hamilton, W. C., Eds. *International Tables for X-Ray Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV.

Synthesis of Acyclic and Dehydroaspartic Acid Analogues of Ac-Asp-Glu-OH and Their Inhibition of Rat Brain N-Acetylated α-Linked Acidic Dipeptidase (NAALA Dipeptidase)

Nalin Subasinghe,[†] Marvin Schulte,[‡] Michael Y.-M. Chan,[†] Robert J. Roon,[‡] James F. Koerner,[‡] and Rodney L. Johnson*[,†]

Department of Medicinal Chemistry, College of Pharmacy, and Department of Biochemistry, Medical School, University of Minnesota, Minneapolis, Minnesota 55455. *Received February 27, 1990*

The following structural and conformationally constrained analogues of Ac-Asp-Glu-OH (1) were synthesized: Ac-Glu-Glu-OH (2), Ac-D-Asp-Glu-OH (3), Ac-Glu-Asp-OH (4), Ac-Asp-Asp-OH (5), Ac-Asp-3-aminohexanedioic acid (6), Ac-3-amino-3-(carboxymethyl)propanoyl-Glu-OH (7), *N*-succinyl-Glu-OH (8), *N*-maleyl-Glu-OH (9), *N*-fumaryl-Glu-OH (10), and Ac-Δ^ZAsp-Glu-OH (11). These analogues were evaluated for their ability to inhibit the hydrolysis of Ac-Asp-[3,4-^3H]-Glu-OH by N-acetylated α-linked acidic dipeptidase (NAALA dipeptidase) in order to gain some insight into the structural requirements for the inhibition of this enzyme. Analogues **4**–**6** and **9** were very weak inhibitors of NAALA dipeptidase (K_i > 40 μM), while **2**, **3**, and **7** with K_i values ranging from 3.2–8.5 μM showed intermediate inhibitory activity. The most active inhibitors of NAALA dipeptidase were compounds **8**, **10**, and **11** with K_i values of 0.9, 0.4, and 1.4 μM, respectively. These results suggest that the relative spacing between the side chain carboxyl and the α-carboxyl group of the C-terminal residue may be important for binding to the active site of the enzyme. They also indicate that the χ_1 torsional angle for the aspartyl residue is in the vicinity of 0°.

Localization[1-3] and release studies[4,5] suggest a role for the acidic dipeptide Ac-Asp-Glu-OH (1) in synaptic processes. Binding studies[6] which demonstrated that Ac-Asp-Glu-OH was capable of displacing radiolabeled glutamic acid from synaptic plasma membranes and electrophysiological studies[7-13] that reported that Ac-Asp-Glu-OH exhibited excitatory effects when injected into a rat brain led many researchers to suggest a neurotransmitter role for 1. The identification of an N-acetylated α-linked acidic dipeptidase (NAALA dipeptidase) capable of degrading Ac-Asp-Glu-OH into Ac-Asp-OH and Glu-OH

[†] Department of Medicinal Chemistry.
[‡] Department of Biochemistry.

(1) Guarda, A. S.; Robinson, M. B.; Ory-Lavolle'e, L.; Forloni, G. L.; Blakely, R. D.; Coyle, J. T. *Mol. Brain Res.* **1988**, *3*, 223.
(2) Reichelt, K. L.; Fonnum, F. *J. Neurochem.* **1969**, *16*, 1409.
(3) Williamson, L. C.; Neale, J. H. *Brain Res.* **1988**, *456*, 375.
(4) Tsai, G.; Forloni, G.; Robinson, M. B.; Stauch, B. L.; Coyle, J. T. *J. Neurochem.* **1988**, *51*, 1956.
(5) Williamson, L. C.; Neale, J. H. *Brain Res.* **1988**, *475*, 151.
(6) Koller, K. J.; Coyle, J. T. *Eur. J. Pharmacol.* **1984**, *98*, 193.
(7) Avoli, M.; Bara, P. F. A.; Brancati, A.; Cecchi, L.; Deodati, M. *Boll.—Soc. It. Biol. Sper.* **1976**, *52*, 1525.

0022-2623/90/1833-2734$02.50/0